

scientific reports



nature research

Editorial Board Highlights

Interviews

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Dr Carlo Cannistraci



Dr Carlo Cannistraci is a Theoretical Engineer and Editorial Board Member for Scientific Reports.

1. What is your current research focused on?

I am a Theoretical Engineer; my research interests include subjects at the interface between physics of complex systems, complex networks and machine intelligence, with particular interest in brain/bio-inspired computing for Big Data analysis, and applications in precision biomedicine and neuroscience.

2. What has been your biggest challenge and your greatest achievement in your career so far?

Mapping complex networks to their latent geometric spaces helps to investigate, understand and predict the structure and function of complex systems. My biggest challenge and greatest achievement was to recently propose a class of intelligent machines for efficient embedding of large real networks to the hyperbolic space, with future impact on big-network-data analysis in biology, medicine and social science. This work was proposed in the article: Machine learning meets complex networks via coalescent embedding in the hyperbolic space, A Muscoloni, JM Thomas, S Ciucci, G Bianconi, CV Cannistraci. Nature Communications 8 (1), 1, 2017.

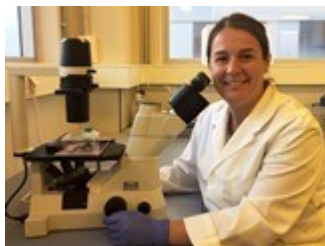
3. Why did you decide to become a board member?

I love to support other colleagues to improve their studies and to achieve high standards in their publications. This is the spirit of the review process: to offer feedback that improves science and its dissemination with a clear benefit for all of the scientific community.

4. What do you like most about being a board member for *Scientific Reports*?

Being an Editor for *Scientific Reports* for me is something more than being a normal Editor. The spirit of *Scientific Reports* is the spirit of 'freedom and equal opportunity' in science. I like the fact that *Scientific Reports* accept articles according to the only requirement that they should be technically correct. This ensures that the article selection is not biased by the opinion of a 'group of experts' that, in my opinion, can be also risky, because very innovative ideas that are against the mainstream in science might be rejected. I feel that being an editor for *Scientific Reports* allows me to sponsor the freedom to publish new scientific ideas which are technically correct but might not be recognised by a conservative establishment of experts.

Dr Joana Maria Ramis



Dr Joana Maria Ramis is a Miguel Servet Researcher at the Balearic Islands Health Research Institute (IdISBa), as well as Adjunct Lecturer at the University of the Balearic Islands, Spain.

1. What is your current research focused on?

My research is focused on the development of new therapies and biomaterials for restorative and regenerative medicine and its translation to clinical practice. My newest research line focus on the approach to cell-free regenerative medicine through the use of extracellular vesicles derived from different cells types.

2. What has been your biggest challenge and your greatest achievement in your career so far?

My biggest challenge and greatest achievement has been, and keeps being, reconciling family life and my research career.

3. Why did you decide to become a board member?

I considered the offer to become a board member as a great opportunity to deepen in the knowledge of the review process and to be an active part of it. Publication of our results is an important part of our work as researchers, and before becoming a board member for *Scientific Reports*, I have only acted as author or as reviewer, thus, to act as editorial board member was a role I was interested in exploring.

4. What do you like most about being a board member for *Scientific Reports*?

Being a board member for *Scientific Reports* allows me to be updated in the ongoing research in my areas of expertise and to really deepen in the technical aspects of the manuscripts I handle. On top of that, the most positive aspect of being a board member is how much I learn from the interaction with the reviewers and the authors and how manuscripts improve from it.

5. You are leading one of our Guest Edited Collections. What interested you about becoming a Guest Editor? What is your Collection focused on?

Yes, I am leading the Special Collection entitled "[Extracellular vesicles in cell biology and medicine](#)". The collection is focused on extracellular vesicles (EV), cell-derived membranous structures known as intercellular communicators exerting their function by exchanging their cargo. EV research is a burgeoning field with a high number of researchers from different disciplines working in this field. This Special Collection intends to deliver an up-to-date overview on some of the current developments in the field. To increase my interaction with other researchers of the field is what most interested me about becoming a Guest Editor for the Collection.

6. Which is your favourite *Scientific Reports* paper?

It is really difficult to select one single paper! I will list you some:

[Cells release subpopulations of exosomes with distinct molecular and biological properties.](#)

Willms, E.; Johansson, H. J.; Mäger, I.; Lee, Y.; Blomberg, K. E. M.; Sadik, M.; Alaarg, A.; Smith, C. I. E.; Lehtiö, J.; El Andaloussi, S.; Wood, M. J. A.; Vader, P.

[Size-Exclusion Chromatography-based isolation minimally alters Extracellular Vesicles' characteristics compared to precipitating agents.](#)

Gámez-Valero, A.; Monguió-Tortajada, M.; Carreras-Planella, L.; Franquesa, M. I.; Beyer, K.; Borràs, F. E.

[Labeling Extracellular Vesicles for Nanoscale Flow Cytometry.](#)

Morales-Kastresana, A.; Telford, B.; Musich, T. A.; McKinnon, K.; Clayborne, C.; Braig, Z.;

Rosner, A.; Demberg, T.; Watson, D. C.; Karpova, T. S.; Freeman, G. J.; DeKruyff, R. H.; Pavlakis, G. N.; Terabe, M.; Robert-Guroff, M.; Berzofsky, J. A.; Jones, J. C.

Bone marrow stromal/stem cell-derived extracellular vesicles regulate osteoblast activity and differentiation *in vitro* and promote bone regeneration *in vivo*.

Qin, Y.; Wang, L.; Gao, Z.; Chen, G.; Zhang, C.

Exosomes derived from human adipose mesenchymal stem cells accelerates cutaneous wound healing via optimizing the characteristics of fibroblasts.

Hu, L.; Wang, J.; Zhou, X.; Xiong, Z.; Zhao, J.; Yu, R.; Huang, F.; Zhang, H.; Chen, L.

Professor Xiaochun Li



Professor Xiaochun Li is the Raytheon Endowed Chair in Manufacturing Engineering at the Departments of Mechanical and Aerospace Engineering & Materials Science and Engineering at UCLA, USA. He is also a Guest Editor for the [Nanotechnology enabled metallurgy Collection](#), which is currently welcoming submissions.

1. What has been your biggest challenge and your greatest achievement in your career so far?

The biggest challenge has been to bridge science and manufacturing for long term impact. The greatest achievement in my career so far is the discovery of the nano-particle self-dispersion and stabilisation mechanism in molten metals, which establishes a scientific foundation for nanotechnology enabled metallurgy.

2. Why did you decide to become a board member?

Being a board member is a good opportunity to provide a valuable service to the technical community.

3. What do you like most about being a board member for *Scientific Reports*?

My greatest pleasure is to help make the review process better, while having the opportunity to Guest Edit a special Collection.

4. You are leading one of our Guest Edited Collections. What interested you about becoming a Guest Editor? What is your Collection focused on?

I really like the opportunity to promote an important emerging field, Nanotechnology enabled metallurgy.

5. Which is your favourite *Scientific Reports* paper?

[Core-shell nanoparticle arrays double the strength of steel](#)

J.-B. Seol et al.

Professor Luciano Bosso



Professor Luciano Bosso is an Assistant Professor of Ecology at the University of Naples Federico II.

1. What is your current research focused on?

I am an ecologist expert in ecological modelling and GIS analysis. My main research interests include conservation biology, global change ecology, biogeography and invasion ecology and the application of species distribution models, niche analysis, risk mapping, conservation gap analysis, landscape ecology, spatial analysis, species connectivity and corridor network simulation.

2. What have been your biggest challenge and your greatest achievement in your career so far?

My biggest challenge is to make scientific research more accessible and comprehensible to a wider non-scientific audience. I am particularly devoted to increasing the ecological knowledge of non-experts. Several of my studies have informed policy makers about best management strategies of protected areas in Italy and Europe, and I consider this use of my research as my greatest achievement.

3. Why is *Scientific Reports* one of your favourite journals?

Scientific Reports is a multidisciplinary journal that publishes scientifically valid primary research from all areas of the natural sciences and beyond. I have always admired the quality and rigour of the scientific studies published in this prestigious journal.

4. Why did you decide to become a board member?

Considering the crucial role that editors have in scientific communication, I was looking for an opportunity where I can apply and especially improve/broaden my editorial skills as well as find professional and personal satisfaction both as an editor and a scientist. I am very grateful to *Scientific Reports* for this exciting opportunity.

5. What do you like most about being a board member for *Scientific Reports*?

I am particularly satisfied by the opportunity to enrich my background, collaborate in an international context, and contribute to the journal's high-quality publication standards.

6. Which is your favourite *Scientific Reports* paper?

It is really difficult to select a single paper! I was really impressed by these recent publications:

[Integrating experimental and distribution data to predict future species patterns.](#)
Kotta et al. (2019).

[Risk of biodiversity collapse under climate change in the Afro-Arabian region.](#)
Soultan et al. (2019).

[Climate change-driven range losses among bumblebee species are poised to accelerate.](#)
Sirois-Delisle and Kerr (2019).

[Modeling the distributions of tegu lizards in native and potential invasive ranges.](#)
Jarnevich et al. (2018).

[Assessment of the effect of climate changes in the Late Pleistocene and Holocene on niche conservatism of an arvicolid specialist.](#)
Castellanos-Frías et al. (2018).

[Can Niche Modeling and Geometric Morphometrics Document Competitive Exclusion in a Pair of Subterranean Rodents \(Genus *Ctenomys*\) with Tiny Parapatric Distributions?](#)
Kubiak et al. (2017).

Dr Ryoung Shin

Dr. Ryoung Shin is a Unit Leader at RIKEN Center for Sustainable Resource Science.

1. What is your current research focused on?

Potassium is one of major nutrients for plant growth, and lack of it in the soil environments has led to the increased use of fertilizers. However, such increased fertilizer usage does not necessarily result in a comparable production increase, and excess fertilizer run-off creates soil pollution. To address these issues, we elucidate the components of plant potassium sensing and deficiency signaling in plants using various approaches. In parallel, we are also using a marine red macroalgae *Pyropia yezoensis* in order to understand the mechanisms that enable seaweeds to survive in high salt conditions and to compare these mechanisms with those of the land plants in terms of Na^+/K^+ homeostasis. In addition, we identify and characterize chemicals which affect cesium and heavy metals uptake in plants to establish new methods of phytoremediation.

2. What has been your biggest challenge and your greatest achievement in your career so far?

My biggest challenge was starting the radiocesium phytoremediation research after the accident of Fukushima nuclear power plants following the big earthquake in Japan. It was a very challenging project (see [here](#) and [here](#)); and we are still actively working on this. One of my greatest achievements was that I have found and proven for the first time that [Reactive Oxygen Species \(ROS\) is a key signal molecule of potassium-deficient signal transduction in plant roots](#).

3. Why did you decide to become a Board Member?

When I got the offer of being a Board Member, I recalled the time when we published two papers in *Scientific Reports*. It was an interesting and unique experience. *Scientific Reports* publishes articles from a variety of scientific backgrounds, including interdisciplinary research. Additionally, the editorial criteria for decision are different from other journals. I wanted to know more about this, and to be involved in *Scientific Reports* as a Board Member.

4. What do you like most about being a Board Member for *Scientific Reports*?

When I review the manuscript as a reviewer or handle the manuscripts as an editor, the manuscripts are usually from the specialised fields. At *Scientific Reports* I have really

enjoyed reading the interdisciplinary manuscripts. These are not easy because I should know many different fields to handle these manuscripts, but handling these papers gives me opportunity to learn about the various aspects and I can then see and appreciate the different angles.

5. Which is your favourite *Scientific Reports* paper?

One of my favourite *Scientific Reports* paper is "[The antifungal plant defensin AtPDF2.3 from *Arabidopsis thaliana* blocks potassium channels](#)" by Virens et al. Previously we found the link between Jasmonic acid signalling, including PDF genes and potassium/cesium signalling. This particular paper provides much more detailed insights into the relationship between PDFs and potassium channels. I really enjoyed reading this article. Recently, I have also had a pleasure of reading and handling a later published article "[Effects of green seaweed extract on *Arabidopsis* early development suggest roles for hormone signalling in plant response to algal fertilisers](#)" by Ghaderiardakani et al. This one was well balanced between the practical and basic scientific approaches using seaweeds. Ideas are attractive and the logical flow through was clear.

Dr. Alberto G. Fairén

Alberto G. Fairén is an Astrobiologist at the Centro de Astrobiología in Madrid, Spain, a Visiting Scientist at the Department of Astronomy, Cornell University, and Editorial Board Member for *Scientific Reports*.



1. What is your current research focused on?

My research is focused on helping to understand the nature of the early Martian environments using a variety of tools from different disciplines: geomorphology, sedimentology, paleohydrology, geochemistry, aqueous mineralogy, and environmental microbiology.

My research interests in Martian science are broad, spanning from surface evolution and habitability, to robotic exploration and mission design and operation. My research activities include a combination of theoretical modeling, laboratory experiments, field work and spacecraft data analysis.

2. What has been your biggest challenge and your greatest achievement in your career so far?

The biggest challenge has been keeping focus while moving among institutions, countries and continents, during my postdoc years. As soon as I got my PhD in Spain, I moved to NASA Ames in California for my postdoc, and later I worked for the SETI Institute also in California, and Cornell University in New York. After 8 years in the US, I returned to my home country and joined Centro de Astrobiología, where I have recently got tenure. The journey has been challenging, but it also had its rewards, because the interaction with a wide variety of colleagues with different expertise and backgrounds allowed me to significantly grow in my career, and to reach my greatest achievements in publications, grants and awards.

3. Why did you decide to become a board member?

One of the most important tasks for a scientist is communicating the results of your investigations to the community. We, as researchers, should help to disseminate the scientific outcomes of our work. These responsibilities are essential for authors and reviewers indeed, but also for editors contributing to achieve high standards in publication quality, ethics, and fairness.

4. What do you like most about being a board member for *Scientific Reports*?

Being a board member for *Scientific Reports* is a good opportunity to provide a valuable service to the Mars community, helping to improve the review process facilitating the interactions between authors and reviewers, while contributing to *Scientific Reports*'s high-quality publication standards. In addition, the only requirement for the publication of a paper in *Scientific Reports* is that they must be "technically correct original contributions", and they don't need to have a subjective significance as perceived by the editors, which may very well be erroneous and most likely biased.

5. Which is your favourite *Scientific Reports* paper?

It is very difficult to single one paper out, but I was really impressed by "[Perchlorates on Mars enhance the bacteriocidal effects of UV light](#)", by Wadsworth and Cockell, because of its significant implications to our current search for life on Mars. In addition, I recently had the pleasure of handling "[The fate of lipid biosignatures in a Mars-analogue sulfur stream](#)", by Tan et al., "[A record of igneous evolution in Elysium, a major martian volcanic](#)

province”, by Susko et al., and “[Seasonal deposition and lifting of dust on Mars as observed by the Curiosity Rover](#)”, by Vicente-Retortillo et al., three very impressive papers dealing with different aspects of Mars exploration.

Professor Xuyang Lu



Professor Xuyang Lu is an Associate Professor at the Institute of Mountain Hazards and Environment at the Chinese Academy of Sciences. He is an Editorial Board Member for Scientific Reports and is currently Guest Editing a Collection on [Mountain surface processes and regulation](#), which is currently welcoming submissions until the end of September 2019.

1. What is your current research focused on?

My academic research interests focus on the biogeochemical cycle and its microbial mechanism in mountain ecosystems. My recent work focuses on the effect of litter and soil organic matter chemistry on soil N transformation and its microbial function molecular ecological networks by comparing the China (Tibetan Plateau) and U.S. (Rocky Mountains) alpine ecosystems.

2. What has been your biggest challenge and your greatest achievement in your career so far?

I think my biggest challenge is the collection of soil and plant samples from alpine ecosystems, especially in the Tibetan Plateau. The plateau is considered to be the third “pole” of the world with an average elevation of 4 km above sea level. The natural environment is extremely harsh which characterized by high altitude, strong solar radiation, low temperatures and thin air. In this extreme environment, human survival is a challenge in itself, not to mention taking samples. In present, the greatest achievement in my career was that I discovered the influence extent and pattern of litter chemical composition and diversity on soil C, N transformation, which the related researches have

been published in *Scientific Reports*, *Soil Biology & Biochemistry*, *Biology and Fertility of Soils*.

3. Why did you decide to become a board member?

The first motivation is to learn how to become a qualified editor the academic journals. I feel a sense of accomplishment when a paper could reach the high-quality publication standard for *Scientific Reports* through the process of peer review and feedback with the authors.

4. What do you like most about being a board member for *Scientific Reports*?

I particularly like the criteria for publication in *Scientific Reports*, assessing an original article on the basis that they are technically sound and scientifically valid rather than innovation and importance, which provides the opportunity for more research works to be published. In addition, the Guest Edited Collection "[Mountain surface processes and regulation](#)", which I served as Guest Editor, supplies a high-quality publication platform for interdisciplinary studies of mountain surface processes and their responses to climate change and human activities. I sincerely appreciate *Scientific Reports* for this exciting opportunity.

5. Which is your favourite *Scientific Reports* paper?

Here I list three of my favourite *Scientific Reports* papers:

["A transnational perspective of global and regional ecosystem service flows from and to mountain regions"](#), by Schirpke et al. (Article number: 6678, 2019).

["Altitudinal, temporal and trophic partitioning of flower-visitors in Alpine communities"](#), by Lefebvre et al. (Article number: 4706, 2018).

["Planting increases the abundance and structure complexity of soil core functional genes relevant to carbon and nitrogen cycling"](#), by Wang et al. (Article number: 14345, 2015).

Professor Matjaž Perc

Matjaž Perc is a Professor of Physics at the University of Maribor, Slovenia and Editorial Board Member for Scientific Reports.

1. What is your current research focused on?



Climate inaction, the overexploitation of natural resources, and a harrowing gap between rich and poor are some of the most pressing issues of our time. Since these problems can't even be fully understood, let alone solved, from a perspective of a single scientific discipline, the future prospects of our societies will be determined by interdisciplinary and cross disciplinary research that cuts across different domains of science. We are working hard to make physics, particularly methods of statistical physics, together with network and data science, a key piece of this puzzle. Social physics enables us to theoretically describe and understand collective social phenomena that are due to the interactions among individuals, groups, and governments. From the responsible use of antibiotics and vaccination, to the mitigation of social crisis and inequalities, we aim to develop better social systems and more efficient policies for a sustainable and better future by synergizing physics with the social sciences.

2. What has been your biggest challenge and your greatest achievement in your career so far?

One of my career goals has always been to stay at my home university, and ultimately to get a permanent position there. This has turned out to be the biggest challenge, as well as the greatest achievement of my career. Slovenia is a small country. Research funding is scarce, and vacant tenure track positions are basically nonexistent. A consistently impressive research output is needed, along with some luck and sponsorship from senior colleagues, to finally make it. For me it worked out great, but for many talented researchers in Slovenia this reality means being forced to go work abroad.

3. Why did you decide to become a board member?

Being a *Scientific Reports* board member is for me a way to give back to the research community, and to promote and support the research field in which I am active. *Scientific Reports* always stood for high quality, but also for freedom and opportunity, which together with the open access policy make it a perfect outlet for the most innovative research. Such research is often difficult to publish in journals that judge impact and novelty because it often challenges the mainstream in science.

4. What do you like most about being a board member for *Scientific Reports*?

The role puts you right in the center of research that is close to your heart. It feels a little bit like having reserved the finest seats in the theater of science, getting to learn about fascinating new discoveries before they are officially published. I also very much enjoy good cover letters, where authors usually explain the gist of their research in simpler terms, discussing its broader implications, and pondering about potential impact and directions for the future.

5. Which is your favourite *Scientific Reports* paper?

My favorite *Scientific Reports* paper remains to be the first paper I have handled after joining the editorial board, titled "[Flavor network and the principles of food pairing](#)" published in 2011. The paper generated a lot of attention at the time, both in the scientific community and in the media, and it stands precisely for the type of avant-garde research I was hoping *Scientific Reports* would encourage. In this particular case opening new avenues towards a systematic understanding of culinary practice across the world. From my own publications in *Scientific Reports* my favorite is "[Self-organization of progress across the century of physics](#)" published in 2013, for its collaboration with the American Physical Society and the resulting n-gram viewer for physics that is akin to Google's version for books.

Professor Yu Xin Zhang



Professor Yu Xin Zhang is a Professor at the College of Materials Science and Engineering, Chongqing University, China. He is also a Scientific Reports Editorial Board Member and a Guest Editor for the [Nanomaterials for energy and environmental applications Collection](#).

1. What is your current research focused on?

My current research is mainly focused on the self-assembly of nanostructures with various morphologies, involving MnO₂ and diatomite-based nanocomposites, and their applications in energy storage and environmental fields.

2. What has been your biggest challenge and your greatest achievement in your career so far?

In my career, the biggest challenge was teaching Chinese students Technical Writing using English, which is my second language, while striving in materials science and engineering in Chongqing University. Indeed, it is highly important that teachers keep an eye on the learning process and abilities of students. Fortunately, my advisor, Prof. Hua Chun Zeng gave me a strict supervision in National University of Singapore, molding me to be a well-trained researcher and independent lecturer. In addition to being the biggest challenge in my career, teaching this course is also my greatest achievement. More than 5000 students who have had a direct or indirect contact with this course through me, have been able to improve their writing ability and some of them have even published their first article, which is a great motivation and confidence booster in scientific research, and nudged them to embark on the path of scientific research. I take pride in this.

3. Why did you decide to become a board member?

Becoming a board member is not only an endorsement of oneself, but also a means of staying abreast of the latest developments in scientific research, and actively communicating with international counterparts. Being a board member avails me these opportunities.

4. What do you like most about being a board member for *Scientific Reports*?

Scientific Reports is an outstanding journal, which publishes high quality articles with enormous future development prospects. I enjoy being able to make profound suggestions for the development of the journal, accepting excellent articles, and being able to learn substantially, even while doing something I love, and this is possible for me as a board member for *Scientific reports*. I like the rate at which the journal is developing in all aspects, it gets better and better.

Professor Z. Altounian



Professor Z. Altounian is a Professor in the Physics Department at McGill University, Montreal, Canada and Editorial Board Member for Scientific Reports.

1. What is your current research focused on?

My current research is mainly focused on studies of magnetic properties of materials.

2. What has been your biggest challenge and your greatest achievement in your career so far?

Challenges always occur if one enters a new field of study. My first challenge started in the winter of 1979 when I got interested in metallic glasses. This was also my greatest achievement as our group at McGill University became a world famous centre in this field. I still contribute to this field, but mainly through editorial/reviewing responsibilities for journals and conferences. My second, and more recent challenge occurred when I got involved in the studies of magnetic properties of hard magnetic materials. It is worth to mention that there was a very close relationship between my two challenges as we used the technique of rapid solidification, which is commonly used to prepare metallic glasses, to manufacture rare-earth based magnets. This also brought us to worldwide recognition in this particular field.

3. Why did you decide to become a board member?

One of the aspects that drew me to become an EBM was the problems that I had encountered while reviewing manuscripts. Mostly the poor quality of some of the reviewers which in many cases did not agree on the evaluation of the same submission. In those cases, I was asked to make the final decision. As an EBM, I hoped to improve the reviewing protocol.

4. What do you like most about being a board member for *Scientific Reports*?

Prior to becoming an EBM, I was a very active reviewer for a number of publications in the above-mentioned fields. When *Scientific Reports* invited me to join their editorial team, I accepted the offer as I had always enjoyed reviewing journal submissions.

5. Which is your favourite *Scientific Reports* paper?

My favorite paper is SR 7, 646 (2017), "[The magnetic and crystal structure of \$Mn_xGa\$ \(\$1.15 \leq x \leq 1.8\$ \) Alloys](#)". This was our first publication in *Scientific Reports* as well as the first introduction to this fascinating material. Even-though we have been studying magnetic materials for more than two decades, this publication finally put us on the world map in this field.

Dr Lena Ciric



Lena Ciric is Associate Professor in Environmental Engineering at the Department of Civil, Environmental and Geomatic Engineering of University College London, UK. She is also a Scientific Reports Editorial Board Member and a Guest Editor for the [Microbes in the built environment Collection](#).

1. What is your current research focused on?

I am an environmental microbiologist. The environment I study is the built environment. My group works on two themes. The first is how microbes behave in the built environment. We have been studying the microbial communities and the horizontal gene transfer of antibiotic resistance genes in slow sand filtration, a commonly used drinking water treatment process. The other theme we study is how we can use engineering solutions to reduce the numbers of harmful microbes in our environment. We have been manufacturing [water and air filters with antimicrobial nanoparticles](#) embedded in them which are capable of killing bacterial and viral pathogens.

2. What has been your biggest challenge and your greatest achievement in your career so far?

I would say being an academic is both the biggest challenge and the biggest achievement of my career. The job can be extremely challenging as there are huge demands on your time. There is frequent failure – rejected grant applications, experiments that don't work, papers that are difficult to publish. But these challenges are also what motivates me. The job of an academic is so varied. We have the freedom to pursue our research ideas, we get to write, we get to read, collaborate, travel, interact with young people through teaching and speak to the public about our work. All of these experiences are extremely rewarding.

3. Why did you decide to become a board member?

I am naturally nosy, so I was curious to see what research is going on out there. As an editorial member you have the opportunity to reach out to experts in the field to help you scrutinise manuscripts and help the authors improve their work.

4. What do you like most about being a board member for *Scientific Reports*?

Scientific Reports has a novel approach. Instead of looking for the next big thing, it is happy to publish any scientific work that is technically sound. This is extremely useful to the scientific community. It means that we have a less biased view of the research going on around the globe.

5. Which is your favourite *Scientific Reports* paper?

[Patterns in the skin microbiota differ in children and teenagers between rural and urban environments](#)

I am fascinated by how the microbes that live on and in our bodies shape us physically and, some studies say, psychologically. I think it's wonderful to know that our body as an ecosystem rather than just one organism.

Professor Kah-Wee Ang



Prof. Kah-Wee Ang is Associate Professor in the Department of Electrical and Computer Engineering at the National University of Singapore.

1. What is your current research focused on?

I am an experimentalist and my current research focuses on the development of both electronic and photonic devices based on two-dimensional (2D) materials, which include field-effect transistor and integrated circuit, non-volatile memory, artificial intelligence synapse for neuro-inspired computing, as well as electro-optic modulator and photodetector for mid-infrared sensing applications.

2. What has been your biggest challenge and your greatest achievement in your career so far?

I started off my research career exploring the use of beneficial strain effect to modify the carrier transport properties in the conduction channel for enhancing the mobility and drive current performance of nanoscale field-effect transistors (FETs). Specifically, I pioneered a novel concept in employing lattice-mismatched stressor made of silicon-carbon (Si:C) alloy in the source and drain regions of metal-oxide-semiconductor FETs,

which was widely evaluated by major semiconductor foundries worldwide. The technology was eventually adopted in high volume manufacturing, which gave me a sense of satisfaction and achievement.

Since five years ago, I started investigating two-dimensional materials for electronic and photonic device applications. As a new comer to this emerging field, many technical challenges were encountered which spanned across material synthesis, device fabrication to large-scale circuit and system integration. Through conscientious efforts, several advancements have been made in addressing module and process integration issues on flexible substrates, which allow energy-efficient logic gates, memories and sensors to be realized. Going forward, we aim to demonstrate a transformative 3D monolithic system via van der Waals integration of new logic and memory devices built on atomic-scale 2D materials, which can deliver a remarkable improvement in computational energy efficiency over traditional system architecture.

3. Why did you decide to become a board member?

Today the research landscape is dynamically changing where new exciting fields are emerging rapidly. Being an editorial board member allows me to not just keep up with the latest progress made in my field, but also gives me the opportunity to keep abreast of the new development that takes place across multidisciplinary fields. Getting to know the experts often helps me in assigning appropriate reviewers to assess the submitted manuscripts, and make constructive recommendations to improve the quality.

4. What do you like most about being a board member for *Scientific Reports*?

Scientific Reports' approach in publishing technically sound work and let the wider scientific community determine the impact of the work is well received by like-minded researchers including myself. This allows board member to avoid making subjective decisions on rejecting manuscripts which are perceived with little immediate interests but may grow in importance over time.

Dr Pedro Oliveira



Dr Pedro Oliveira is a Senior Scientist at Mount Sinai School of Medicine, USA.

1. What is your current research focused on?

My current interests are on identifying emerging challenges in the fast-evolving research field of epigenomics, and on better understanding the epigenetic regulation mechanisms affecting gene expression and cellular processes in Bacteria. On a broader perspective, I seek to elucidate key biological insights that can translate into more accurate disease diagnosis and more effective treatment for certain bacterial pathogens.

2. What has been your biggest challenge and your greatest achievement in your career so far?

My biggest challenge has been in embracing some of the downsides of a career in academia, such as frequent failure and rejection, and being able to transform such missteps and roadblocks into an opportunity for something new. My greatest achievement as a researcher has been in help propelling the field of bacterial epigenomics. Such field has been attracting increased attention for its exciting potential to transform our knowledge on gene regulation, virulence, and adaptation. As an example, we recently performed the first large-scale comprehensive epigenomic analysis in *Clostridioides difficile*, known to be one of the leading causes of nosocomial antibiotic-associated disease in the developed world. We found evidence for epigenetic regulation associated with sporulation (a key step in *C. difficile* disease transmission), cell length, biofilm formation, and host colonization. For more information, please check: Oliveira *et al.* [Epigenomic characterization of *Clostridioides difficile* finds a conserved DNA methyltransferase that mediates sporulation and pathogenesis](#). *Nature Microbiology*.

3. Why did you decide to become a board member?

Essentially, it was a great opportunity to become an active part of the decision-making process, while simultaneously helping the authors to achieve a higher quality standard in their publications. I am very thankful to *Scientific Reports* for this opportunity.

4. What do you like most about being a board member for *Scientific Reports*?

I particularly enjoy contributing to a journal that seeks high quality standards in its publications, focusing primarily on research robustness and validity, and less on subjective

editorial decisions. In this sense, *Scientific Reports* makes a distinctive contribution when compared with other journals. Furthermore, being a Board Member has given me the opportunity to learn and keep myself up to date on the latest research in my areas of expertise, while simultaneously delving into the multiple aspects of the manuscripts I handle.

5. Which is your favourite *Scientific Reports* paper?

It's difficult to highlight one single paper. I particularly enjoyed reading a recent publication describing a [portable epigenetic switch for bistable gene expression in bacteria](#) based on the *opvAB* operon of *Salmonella enterica*.

Dr Ciria C. Hernandez



Dr. Ciria C. Hernandez is an Assistant Research Scientist at the Life Sciences Institute, University of Michigan, USA. She is an Editorial Board Member for Scientific Reports and currently Guest Editing a Collection on [Channelopathies](#), which is welcoming submissions on a rolling basis.

1. What is your current research focused on?

The focus of my research has always been to understand the fine regulation of ion channels in physiological and pathological states. Thus, I have been fully immersed in the study of the molecular determinants of the interaction of small peptides, G-protein coupled receptors (GPCRs) and both voltage and ligand gated ion channels, whether they are defective or not, by carrying mutations in their structure that prevents them from normal function. Most recently, I have been working on the elucidation of the structural determinants of the interaction of GPCRs that mediate a variety of physiological processes critical for energy homeostasis, and potassium channels known to be involved in a novel G protein independent signaling pathway in a brain region that controls hunger. We are tackling the idea of bias agonism as a therapeutic approach to overcome defective GPCR-channel coupling in conditions where the rheostats of energy homeostasis are defective.

2. What has been your biggest challenge and your greatest achievement in your career so far?

My biggest challenge and my greatest achievement merge at the same point. Be competitive and remain relevant in the face of the unstoppable and continuing flow of new technologies. While this is true for any electrophysiologist, as a woman, coming from a developing country, there is a bigger challenge considering the general perception that you have about making a career in science, and still being able to respond to the demands of academia. Scientifically perhaps the biggest challenge is to understand how to fit every discovery I make, however small and trivial it may seem, in the greater puzzle we are helping to complete. We merge these pieces when we write the discoveries we made in the scientific articles, when we guide the youngest, and when we collaborate with peers from different fields. This is at the essence of science and my career has fundamentally been learning how to “put the pieces together” ever since I started my career. Although I have managed to break many barriers and be in a privileged position, I have not stopped learning and growing scientifically, and honestly, I will never stop doing it. This is my greatest achievement as a scientist.

3. Why did you decide to become a board member?

Being part of the editorial board of *Scientific Reports* implies transforming and transferring knowledge beyond critical thinking. We have to be not only the best scientists, but the best communicators. Being a board member of *Scientific Reports* made me realize how to communicate the passion that we scientists have for discovering the smallest molecular interactions and chats between receptors, channels, proteins, and bring them to public light. My passive voice became a strong megaphone through the publications of all the authors who contribute to making the best science in the world. By realizing that through my contribution, I am shaping the science that is read and at the same time inspiring me to be the best scientific communicator.

4. What do you like most about being a board member for *Scientific Reports*?

Each manuscript submitted is a challenge of new knowledge and unexpected creativity. The diversity of topics within our area of expertise is tremendous. It shows the high quality of science that is translated from the bench and witnessing that is unmatched and novel in many ways. At the same time, we are the role models for the challenges imposed in the current era, where the disparity of world economies challenges science in the quest for new discoveries.

5. Which is your favourite *Scientific Reports* paper?

Without reservations I invite you to read part of my work published in 2017 describing the [defects on GABAA receptor function caused by deleterious mutations in cases with catastrophic epileptic encephalopathies](#), and many other fascinating studies on the special Collection dedicated to [Channelopathies](#).

Professor Claudia RC Moreno



Prof. Claudia RC Moreno is Head of Department at the School of Public Health, University of Sao Paulo, Brazil and is currently an Editorial Board Member for Scientific Reports.

1. What is your current research focused on?

The process of urbanization, as well as the use of electricity and its technology, has changed the way of life of society. The possibility of extending the waking period through the use of electric light changed working, sleeping, eating and leisure hours. I have been studying communities with and without access to electricity with the focus on lifestyle, working hours, sleep and eating behavior.

2. What has been your biggest challenge and your greatest achievement in your career so far?

The biggest challenge was to run a study in the Amazon forest, collecting data from rubber tappers and having to freeze saliva samples to send to Sao Paulo, many kilometers away. It is hard to say my greatest achievement, but there are two things that I would like to highlight, first, the success of my former PhD students, and second, my participation on a monograph at the IARC-WHO regarding night shift work and cancer.

3. Why did you decide to become a board member?

Scientific Reports is a quite interesting journal due to its policy to evaluate papers technically sound. Moreover, I think it is important to have board members from Latin America.

4. What do you like most about being a board member for *Scientific Reports*?

It is a very dynamic task, and help me to keep myself updated.

5. Which is your favourite *Scientific Reports* paper?

My favorite paper is from an international group of researchers with focus on sleep in a rural cohort study. [Timing and quality of sleep in a rural Brazilian family-based cohort, the Baependi Heart Study](#)

Professor Jimin Zhao



Professor Jimin Zhao currently works in the Institute of Physics at the Chinese Academy of Sciences (CAS), Beijing, and is an Editorial Board Member for Scientific Reports.

1. What is your current research focused on?

I currently work on ultrafast spectroscopy and ultrafast dynamics of correlated quantum materials.

2. What has been your biggest challenge and your greatest achievement in your career so far?

The biggest challenge in my career so far is to convince my colleagues outside of my specific area that what I have done is reliable. This is especially challenging when our manuscripts are being reviewed, whereby very simple questions and unreasonable doubts come in by renowned yet non-experts in the field. I have investigated the ultrafast dynamics in novel iron-based superconductors and Weyl semimetals, I have also achieved SSPM and all-optical switching in 2D materials. I am currently a full professor at IOP-CAS and I am also an OSA senior member.

3. Why did you decide to become a board member?

Being a board member will open a new venue that I can do service to the community. Optical spectroscopy of solids is not a big area, and I feel my colleagues may need me to objectively judge on works using sophisticated optical means to investigate condensed matter physics.

4. What do you like most about being a board member for *Scientific Reports*?

Handling works in slightly different areas is interesting. My own experience can make a decisive contribution, whereas I can also enlarge my horizon through the handling process.

5. Which is your favourite *Scientific Reports* paper?

There are many. I remember one very good theoretical paper called [Anomalous isotope effect in iron-based superconductors](#). I should say another one is one of our works. Out of the 6 papers I have published in *Scientific Reports*, our work [Coherent Generation of Photo-Thermo-Acoustic Wave from Graphene Sheets](#), *Scientific Reports* 5, 10582 (2015) is my most favorite one. This work is highlighted in Chinese magazine *Physics*. It is a phenomenon parallel to photoluminescence. After we publish, I got to know that Alexander Graham Bell also worked on this topic and had filed a patent, and he thought it was one of his greatest achievements. Dr. Bell was a great scientist, and knowing this is wonderful. Nonetheless, before us, there are no serious and solid experimental proofs that cleanly removed other very possible mechanisms. We have also achieved remote coherent control of acoustic sound.

Professor Amy Peterson



Professor Amy Peterson is Associate Professor of Plastics Engineering at the University of Massachusetts Lowell, USA and is an Editorial Board Member for Scientific Reports.

1. What is your current research focused on?

My current research is focused on multilayered polymer structures – these range from nanometer-scale polyelectrolyte multilayers for cell culture coatings, to packaging materials, to additively manufactured structures. As engineers, we use experimental and computational approaches to probe what happens at interfaces and surfaces in these systems, and use this knowledge to guide creation/selection of improved materials and processes.

2. What has been your biggest challenge and your greatest achievement in your career so far?

This past week has been the most intensely challenging of my career. It ended with shutting down our laboratory on Saturday, but the lead up to that final act included figuring out what critical experiments were needed for each group member, attempting to get them approved as access to labs became increasingly restricted by my university, trying to keep my group up to date on the status of their possible experimental work, and doing all of this in a dynamic situation via phone, email, and videoconference. I feel fortunate because we have substantial modelling efforts as well as experimental ones, so we'll be able to continue to make progress on our research. However, my group meets weekly in person for group as well as one-on-one meetings, and I am concerned about how we will all fare being isolated in our homes.

My proudest moments are when I see my students succeed, whether it's at graduate, a defense, an award, a paper, or a presentation.

3. Why did you decide to become a board member?

Being a board member gives me an opportunity to give back to my broader research community and learn at the same time.

4. What do you like most about being a board member for *Scientific Reports*?

The same reasons I became a board member.

5. Which is your favourite *Scientific Reports* paper?

[Clogging transition of many-particle systems flowing through bottlenecks](#) – I like how it makes connections between systems of large numbers of people, flocks of sheep, and colloidal suspensions.

Dr Jagadeesh Bayry

Dr. Jagadeesh Bayry is Director of Research at the Institut National de la Santé et de la Recherche Médicale, Centre de Recherche des Cordeliers, Paris, France and is an Editorial Board Member for Scientific Reports.



1. What is your current research focused on?

Our current research is focused on the mechanisms by which regulatory T cells and circulating immunoglobulins maintain immune homeostasis and deciphering the host-pathogen interaction.

2. What has been your biggest challenge and your greatest achievement in your career so far?

Though we identify mechanisms of various biological processes in the experimental systems, translation of these results to humans is always challenging. For that matter, I am proud of many achievements of my team and myself that include:

- Demonstration that regulatory T cells in contrast to central dogma on them as universal immunosuppressor cells, induce activation of human basophils (Science Immunology 2018. doi: 10.1126/sciimmunol.aan0829)
- Demonstration that surface hydrophobin protein layer prevents immune recognition of airborne fungal spores (Nature 2009. doi: 10.1038/nature08264)
- Demonstration of molecular mechanisms by which therapeutic normal immunoglobulin G expands regulatory T cells (Blood 2013. doi: 10.1182/blood-2012-11-468264) and induces induction of IL-4 in human basophils (J Allergy Clin Immunology 2019. doi: 10.1016/j.jaci.2018.10.064)
- Identification and validation of small molecule antagonists to CCR4 that function as molecular adjuvants in vaccination by transiently inhibiting the migration of regulatory T cells (Proc Natl Acad Sci USA 2008. doi: 10.1073/pnas.0803453105)
- Activation signal-dependent regulation of human dendritic cell functions by B lymphocytes (Nature Communications 2014. doi: 10.1038/ncomms5092)

3. Why did you decide to become a board member?

For me, it is a service to scientific community. Though it demands my time, I feel that it is a highly privileged position. In addition, it gives visibility to myself, team and institute.

4. What do you like most about being a board member for *Scientific Reports*?

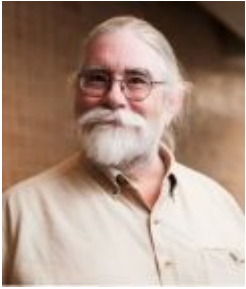
'Open Access' journals have been misinterpreted by some people as 'journals that can publish anything'. As an editorial board member, I ensure that low-quality studies are not published in Scientific Reports. One should not forget that currently there is huge demand

for external reviewers. Therefore, being a board member of journal, I can allow only original and technically sound articles for peer review. It will reduce burden on the reviewers.

5. Which is your favourite *Scientific Reports* paper?

It is difficult to pick a single paper. I have many favourite *Scientific Reports* papers.

Professor Timothy Geary



Prof. Timothy Geary is a Professor in the Institute of Parasitology in the Faculty of Agricultural and Environmental Sciences at McGill and is an Editorial Board Member for Scientific Reports.

1. What is your current research focused on?

I do research in two areas: the pharmacology of antiparasitic drugs (including drug target identification, mechanisms of action and resistance, and development), and understanding the molecular language of the host-parasite interface, with an emphasis on nematodes. The primary focus of this work is the identification and characterization of parasite-derived proteins, metabolites and miRNAs that have immunomodulatory activity in the mammalian host, enabling the establishment of chronic infections.

2. What has been your biggest challenge and your greatest achievement in your career so far?

It's always hard to rank an event as #1 in these areas. Certainly, a major challenge was transitioning from a 20-year career in the pharmaceutical industry to a 15-year career in academia at McGill and now also QUB. The switch was made possible by the Canada Research Chairs programme, which enabled me to get started at McGill, and by support from the Bill and Melinda Gates Foundation, which enabled me to use some of the technology I had developed in the industry to conduct an innovative drug discovery program with partners in Africa. The greatest achievement may have been research that provided a plausible explanation for how the anthelmintic ivermectin removes larval stages of filariid nematodes (microfilariae) from hosts, a key action that makes the drug of value in filariasis control programmes. In another sense, my greatest achievement has

been to work with enormously talented colleagues around the world in fascinating areas of research.

3. Why did you decide to become a board member?

It is an honour to be asked to contribute to the success of one of the Nature family of journals, and I was flattered to be invited. I am on the editorial boards of a number of journals in parasitology, and am glad to serve in that capacity, but *Scientific Reports* addresses broader topics; I wanted to make sure that parasite-related manuscripts submitted here would be fairly and rigorously evaluated.

4. What do you like most about being a board member for *Scientific Reports*?

I enjoy the breadth of manuscripts sent to me to consider as board member. Also – and importantly – the staff assistance is exceptional and makes the tasks associated with securing reviews much easier. It is a pleasure to work with them.

5. Which is your favourite *Scientific Reports* paper?

This I cannot answer....there have been too many to rank. I am confident that they have all been valuable additions to the literature.

Dr Leyla Soleymani



Dr. Leyla Soleymani is a Professor and Canada Research Chair at McMaster University and an Editorial Board Member for Scientific Reports.

1. What is your current research focused on?

My current research is focused on creating new hierarchical materials and applying these to biosensors and antimicrobial surfaces.

2. What has been your biggest challenge and your greatest achievement in your career so far?

I guess my greatest achievements and greatest challenges are related. I have found it challenging to balance the needs of an industry relevant product with the required novelty

for successful scientific publication. Some of my proudest achievements have been on technology development activities that are in the process of being translated to knowledge users.

3. Why did you decide to become a board member?

I highly respect the peer-review process and I find it critically important for advancing science. Being an editorial board member at *Scientific Reports* allows me to take part in overseeing the peer review process and work in ensuring that a fair and transparent process is followed, to get the first scoop on new and exciting papers, and to contribute to the publication of high quality papers, not just as an author or a peer reviewer but also as an editorial board member.

4. What do you like most about being a board member for *Scientific Reports*?

I get to read new papers in more diverse areas that I would normally read, which gives me inspiration and insight.

5. Which is your favourite *Scientific Reports* paper?

It is tough to choose an all time favourite, but recently I came across this paper "[Logistic growth of a surface contamination network and its role in disease spread](#)" and found it eye-opening in the context of the COVID-19 pandemic.

Dr Ruth Blasco



Dr Ruth Blasco is Taphonomy researcher at Centro Nacional de Investigación sobre la Evolución Humana (CENIEH), Burgos, Spain and is an Editorial Board Member for Scientific Reports.

1. What is your current research focused on?

My current research explores human subsistence strategies during the Pleistocene using a taphonomic approach. I am trying to determine several aspects of human behaviour from the faunal record and contribute to evolutionary anthropology by developing several research sub-areas, such as butchery patterns and hominid–carnivore interactions.

2. What has been your biggest challenge and your greatest achievement in your career so far?

Documenting an intentional delayed consumption of some animal resources (specifically bone marrow from fallow deer metapodials) with implications that affect the subsistence systems of more than 300 thousand years ago.

Food storage is considered a “risk-reducing mechanism” designed to offset downturns in resource availability. The deliberate accumulation of some bones for later marrow consumption implies an anticipation of and concern for future needs. This fact marks a threshold for new modes of Palaeolithic adaptation because the foresight capacity surpasses the “here and now” as a means of subsistence. This study was published last year in *Science Advances* [Blasco, R., Rosell, J., Arilla, M., Margalida, A., Villalba, D., Gopher, A., Barkai, R., [Bone marrow storage and delayed consumption at Middle Pleistocene Qesem Cave, Israel \(420 to 200 ka\)](#). *Sci. Adv.* 5, eaav9822 (2019)].

3. Why did you decide to become a board member?

I think that the peer-review process is a fundamental tool for generating quality scientific production. Participating in this process not only as an author or a reviewer but also as an editorial board member allows me to ensure that these processes are objective and transparent and that rigorous, novel and exciting studies are released. I consider it to be a highly privileged position as well as a way of serving the scientific community.

4. What do you like most about being a board member for *Scientific Reports*?

Beyond reading papers from very diverse areas that continuously broaden my knowledge and inspire my future work, being a board member for *Scientific Reports* has allowed me to lead and handle a special collection on “Quaternary Taphonomy” that involves trans- to pluri-disciplinary approaches, such as archaeology, palaeontology, geology, biology and ecology, with a wide array of methodologies. The number of researchers working on taphonomy is steadily increasing, and their contributing to high-ranked scientific journals is becoming a regular trend. This collection is gathering some of the most relevant current studies within this field in the attempt to become a reference source for further studies.

5. Which is your favourite *Scientific Reports* paper?

It is difficult to choose a specific paper, but I will highlight [“Deep learning and taphonomy: high accuracy in the classification of cut marks made on fleshed and defleshed bones](#)

using convolutional neural networks”, which is a clear example of how the recent application of machine learning algorithms to the taphonomic discipline is achieving higher accuracy in the identification of bone surface modifications and the agents and/or processes that produce them.

Dr. Feng Gao



Dr. Feng Gao is a Professor in the Department of Physics, School of Science, at Tianjin University, Tianjin, China and an Editorial Board Member for Scientific Reports.

1. What is your current research focused on?

I am engaged in bioinformatics research, currently focusing on microbial replication origins.

2. What has been your biggest challenge and your greatest achievement in your career so far?

In the last semester, how to balance teaching, research, editorial activities, and family life has been my biggest challenge, especially during the COVID-19 pandemic. The greatest achievement in my career is the systematic prediction of microbial replication origins using bioinformatics methods, some of which have been confirmed experimentally. The related web server Ori-Finder and online database DoriC have been gradually recognized by researchers in this field.

3. Why did you decide to become a board member?

A Nature Research journal launched in 2011, *Scientific Reports* is really a rising star with an experienced Chief Editor and renowned Editorial Board Members, and an editorial ethos unlike those of other Nature Research journals. This attracted me to become a board member in 2013.

4. What do you like most about being a board member for *Scientific Reports*?

I really enjoy the process of handling manuscripts from all over the world, which report the latest findings from the scientific community. I pick qualified manuscripts using rapid,

high-quality peer review, and present them to the readers in a timely manner. It's an excellent experience to see that the handled manuscripts were well-received by a wide audience or highly cited, which gives me a great sense of achievement.

5. What is your favourite *Scientific Reports* paper?

My favourite *Scientific Reports* paper is: '[RASTtk: A modular and extensible implementation of the RAST algorithm for building custom annotation pipelines and annotating batches of genomes](#)', which has already been cited 570 times according to Web of Science. In fact, I often use the RAST server, which is a very popular genome annotation tool, to annotate our newly sequenced genomes. I was very glad to handle this manuscript and accept it for publication in *Scientific Reports* as quickly as possible.

Professor Kenji Kansaku



Professor Kenji Kansaku is a Professor and Chair at the Department of Physiology, Dokkyo Medical University School of Medicine, Japan. He is also an Editorial Board Member for Scientific Reports and Guest Editor for the '[Neuroprosthetics in systems neuroscience and medicine](#)' Collection.

1. What is your current research focused on?

I am a Systems Neuroscientist. I began my career as a neurosurgeon and, therefore, my research interests include both understanding brain functions and applying neuroscientific knowledge to medical practice. I am more specifically focused on practical neuroprosthetics, as well as neural mechanisms of body image and sense of self.

2. What has been your biggest challenge and your greatest achievement in your career so far?

Over the past 15 years, I have mainly focused on the brain–machine interface, which is a neuroprosthetic for patients with neurological disorders. Five years ago, I co-edited a book entitled “Clinical Systems Neuroscience” (Springer), which gathered chapters authored by researchers from various fields, including systems neuroscience, rehabilitation, neurology, psychology, and engineering.

3. Why did you decide to become a board member?

I felt that it would be an opportunity to contribute to various research fields worldwide.

4. What do you like most about being a board member for *Scientific Reports*?

Scientific Reports is unique in that it accepts articles with the only requirements being that they should be technically sound original contributions, and there is no focus on perceived importance and significance. Although the strategy may make it difficult for the journal to set research trends, it provides fair opportunities for papers that would otherwise potentially remain hidden for various reasons.

5. You are leading one of our Guest Edited Collections. What interested you about becoming a Guest Editor? What is your Collection focused on?

I am editing the Guest Edited Collection: [Neuroprosthetics in Systems Neuroscience and Medicine](#). This Collection provides a platform for interdisciplinary research in neuroprosthetics, and gathers studies investigating medical applications of systems neuroscience, informatics, and engineering in the development of neuroprosthetic devices. As mentioned above, I edited a book 5 years ago entitled "Clinical Systems Neuroscience," in which all authors of the provided chapters were assigned by the editors. Here, the free submission style of the Collection has successfully gathered wonderful papers from various research fields. I would be happy for it to contribute to the development of a new special interest group worldwide.

Scientific Reports (*Sci Rep*) | ISSN 2045-2322 (online)

nature > scientific reports > research articles

Research articles

Article Type

Article (5496)

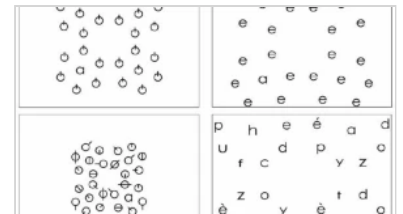
Year

2023 (5496)

Enhancing reading accuracy through visual search training using symbols

Audrey Vialatte, Pierre-Emmanuel Aguera ... Laure Pisella

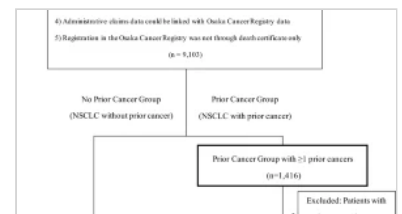
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Survival in non-small cell lung cancer patients with versus without prior cancer

Akira Sato, Toshitaka Morishima ... Isao Miyashiro

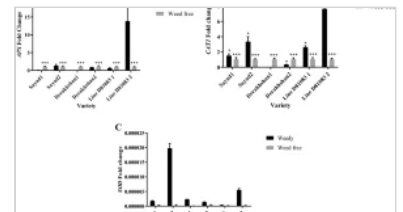
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Effect of redroot pigweed interference on antioxidant enzyme and light response of common bean (*Phaseolus vulgaris* L.) depends on cultivars and growth stages

Seyede Zahra Tabatabaiepour, Zahra Tahmasebi ... Sajad Rashidi-Monfared

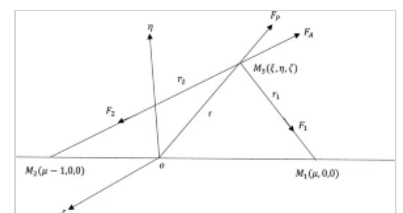
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Albedo effects in the ER3BP with an oblate primary, a triaxial secondary and a potential due to belt

Jagadish Singh & Tyokyaa K. Richard

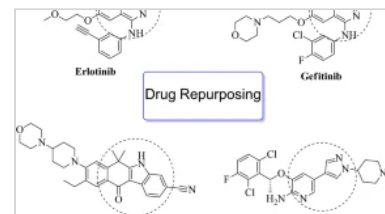
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Revisiting the inhibitory potential of protein kinase inhibitors against NEK7 protein via comprehensive computational investigations

Syeda Abida Ejaz, Mubashir Aziz ... Hanan A. Ogaly

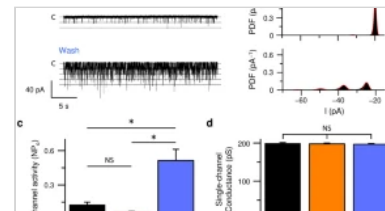
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Polycystic kidney disease 2-like 1 channel contributes to the bitter aftertaste perception of quinine

Takahiro Shimizu, Takuto Fujii ... Hideki Sakai

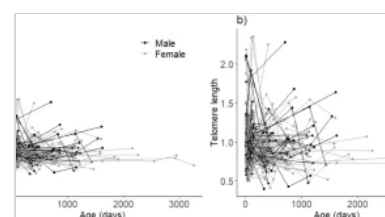
Article | [Open Access](#) | 15 Mar 2023



Longitudinal telomere dynamics within natural lifespans of a wild bird

Michael Le Pepke, Thomas Kvalnes ... Thor Harald Ringsby

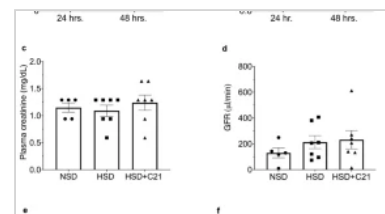
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Angiotensin II type 2 receptor activation preserves megalin in the kidney and prevents proteinuria in high salt diet fed rats

Kalyani Kulkarni, Sanket Patel ... Tahir Hussain

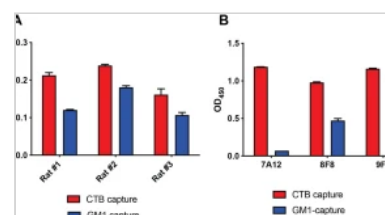
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Characterization and utility of two monoclonal antibodies to cholera toxin B subunit

Noel Verjan Garcia, Ian Carlosalberto Santisteban Celis ... Nobuyuki Matoba

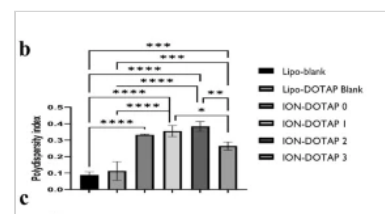
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The effect of 1,2-dioleoyl-3-trimethylammonium propane (DOTAP) Addition on the physical characteristics of β-ionone liposomes

Andang Miatmoko, Febe Harum Asmoro ... Chin-Shiu Huang

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OPEN The effect of 1,2-dioleoyl-3-trimethylammonium propane (DOTAP) Addition on the physical characteristics of β -ionone liposomes

Andang Miatmoko^{1,2✉}, Febe Harum Asmoro¹, Andre Alwi Azhari¹, Noorma Rosita¹ & Chin-Shiu Huang³

β -ionone (ION) is a cyclic terpenoid compound that demonstrates considerable potential for the prevention and treatment of cancer. However, the water solubility of β -ionone is poor and the compound demonstrates low permeability. Liposomes have been reported as increasing both qualities. In this study, the development of β -ionone liposomes was initiated by adding 1,2-dioleoyl-3-trimethylammonium propane (DOTAP) to produce cationic liposomes as a means of enhancing binding to cancer cells. Liposomes composed of β -ionone, HSPC, cholesterol, and DSPE-mPEG₂₀₀₀ were prepared using the thin layer hydration method. Cellular uptake studies were carried out with HeLa cells incubated with β -ionone liposomes for two hours. The results indicated that the addition of DOTAP increased particle size and affected the spectroscopical and thermogram profiles of the liposomes, thereby confirming reduction in liposome crystallinity, while the zeta potential became positive. Moreover, the calcein release profile further showed that additional DOTAP increased both membrane fluidity and cellular uptake in HeLa cells. In conclusion, adding DOTAP affected the physicochemical cationic properties of liposome and improved cellular uptake in HeLa cells.

β -ionone is a cyclic terpenoid compound with a basic core structure of retinoic acid, retinol, -carotene, and vitamin A which is found in many fruits, vegetables, and grains such as carrots, raspberries, almonds, tea, and tomatoes¹. Research has shown that β -ionone has the potential to both prevent and treat several types of cancer, such as breast, colon, lung, and liver². The anticancer activity involves several mechanisms, including antiproliferation, where β -ionone inhibits the proliferation of lung cancer cells (A549) by inducing apoptosis as seen from the increased expression of the Bax gene and reduced expression of the Bcl-2 gene which suppresses the expression of cPLA2 and COX-2 proteins³. β -ionone causes apoptosis, as seen in lung cancer (A549) cell death, (A549) through loss of mitochondrial membrane potential as measured by staining using rhodamine-123⁴. Besides that, β -Ionone can inhibit the in vitro proliferation of breast cancer cells. One of the mechanisms for inhibiting MCF-7 cell proliferation is reducing cyclooxygenase (COX-2) activity in the induction of apoptosis of MCF-7 cells. β -Ionone at a concentration of less than 50 μ mol/L can reduce the expression of COX-2 protein in mice tumor tissue, which is considered a target for cancer molecules⁵.

Dissolving β -ionone in water at a level of 0.169 g/L is extremely difficult. β -ionone also has low permeability with log $P = 4$ ^{6,7} and is classified as falling within Biopharmaceuticals Classification System (BCS) class IV. Numerous studies have shown that using liposomes can improve the solubility and permeability of compounds belonging to class IV BCS⁸.

Liposomes are lipid vesicles consisting of an aqueous phase core surrounded by one or more phospholipid bilayers⁹. Liposome membranes are composed of natural and/or synthetic phospholipids which are relatively biocompatible, biodegradable, and non-immunogenic. Due to the unique bilayer structure, liposomes act as

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carriers for both lipophilic and hydrophilic materials. The latter materials are in the aqueous phase, while the former are trapped in the lipid bilayer¹⁰.

The liposome delivery system is reported as overcoming Lopinavir's low solubility and bioavailability to increase therapeutic efficacy¹¹. The use of liposomes can also increase drug permeability. The particle size of liposomes allows higher intracellular uptake than other systems, thereby increasing drug bioavailability. Previous research has indicated that liposome encapsulation can significantly increase macrovascular permeability after administration of Paclitaxel liposomes¹², where Paclitaxel is also classified as the compound in BCS class 4, like β -ionone.

The nature and charge density of liposomes affect the stability, kinetics, and extent of biodistribution and interactions with liposome uptake by target cells¹³. Cationic liposomes have the advantage of promoting higher therapeutic efficacy against cancer cells⁹, where they can bind to negatively charged cell membranes. Other studies have shown that they accumulate selectively in tumor angiogenic endothelial cells and are superior to anionic or neutral liposomes^{14,15}. The research results obtained by Peters et al. (2015) showed that the cell uptake of 1,2-Dioleoyl-3-trimethylammonium propane (DOTAP)-liposomes was higher than that of the 1,2-dioleoyl-L-a-glycero-3-phosphatidylethanolamine (DOPE)-liposomal formulation. Another study using DOTAP as lipid composition demonstrated that transgene expression was increased in tumor cells due to the uptake of liposome-DNA complexes by tumor cell phagocytosis *in vitro*¹⁶. Research conducted on the cytotoxicity test confirmed that adding DOTAP to liposomes increased the cytotoxicity of HepG2 cancer therapy¹⁷.

Cationic lipid¹⁷ strongly influences biodistribution and drug efficiency^{17,18}. DOTAP is a phospholipid type frequently employed as a component of cationic liposomes. DOTAP contains an NH^{4+} group which renders it positively charged and is low in toxicity compared to other cationic lipids¹⁹. Based on the research of Chang et al. (2018), the addition of DOTAP to the liposome formulation causes a change in size²⁰. The liposomes prepared with PC only have a larger size than those prepared with a 1:1 ratio of PC and DOTAP (PC/DOTAP liposomes) with the same amount of lipids. This is because by accumulating the same amount of lipids, DOTAP, which has a shorter lipid chain, reduces the size of the liposome particle²¹. In the DOTAP-DNA complexes, the charge ratio of lipid/DNA highly affects the physical properties of the liposome. The addition of low-concentration DOTAP produced a larger size, namely 190 ± 8.4 nm, compared to liposomes with a high concentration of DOTAP, which was 140 ± 3.8 nm²². DOTAP-induced changes at the membrane interface may facilitate electrostatic interactions in the polar head region. This physical interaction can decrease the particle size by reducing the radius of bilayer curvature²³. In addition, the low lipid/DNA charge ratio enhanced the repulsion of excess negative charges, resulting in small particle sizes.

The relationship between liposome vesicle size and cell interactions was observed by means of the uptake from bone marrow-derived macrophages (BMDM). The absorption of BMDM from small DOTAP:DOPE liposomes was superior to that of their larger counterparts. Small cationic liposomes tend to be distributed to lymph nodes more rapidly from the injection site than larger ones. The liposome charge was also observed where the addition of DOTAP as a cationic lipid resulted in an extremely positive zeta potential of approximately +40 mV to +60 mV²⁴.

It was additionally reported that the dispersibility of liposomes following the addition of DOTAP was more effective than without DOTAP due to the presence of electrostatic forces²⁵. Furthermore, another study stated that adding DOTAP can render liposomes spherical with an approximate size of 100 nm¹⁵. On the other hand, adding DOTAP can increase the fluidity of the bilayer membrane. This is evidenced by the research of Takechi-Haraya et al. (2016), where DOTAP/Chol (90/10) has a liquid disorder arrangement, while DOTAP/Chol (50/50) has a liquid order arrangement which was observed using the Atomic Force Microscope (AFM). It is also proven by the deformation force curve where DOTAP/Chol (50/50) is steeper than DOTAP/Chol (90/10)²⁶.

Lipophilic compounds such as β -ionone will be entrapped in the liposome membrane which will, in turn, affect its fluidity. The results showed that the greater the amount of β -ionone trapped, the faster the release rate²⁷. Previous studies have also reported that drug compounds can interact with phospholipids, thereby affecting membrane fluidity^{28–32}. Therefore, the membrane's fluidity will affect the stability of the drug entrapment in liposomes and the drug released during circulation, thus determining the effectiveness of the treatment²⁸. Therefore, it is necessary to test the fluidity of the membrane, one method being calcein release. Calcein is a water-soluble, quenching, and fluorescent substance widely used in cell viability studies³³. Calcein release studies can be undertaken to determine membrane stability related to the presence or absence of liposome leakage measured using a spectrofluorometer³⁴.

In this study, the effect of the addition of different levels of DOTAP on the membrane's physical characteristics and the conducting of a fluidity test were evaluated. The data obtained may yield information on the effect of adding DOTAP to β -ionone liposomes and the development of further liposome formulations for inclusion in anticancer therapy.

Result

The particle size and zeta potential of β -ionone liposomes. The addition of DOTAP concentration tended to increase particle size. The largest liposome with an average size of 191.27 ± 4.20 nm was achieved by lipo-DOTAP blank. However, the size increased with the addition of DOTAP to the formula, as shown in Fig. 1a. PDI measurements were carried out to determine the homogeneity of particle size. Particles with smaller PDI values indicate a system with a homogeneous size distribution. PDI values > 0.7 indicate that the preparation has an extensive distribution of particle sizes³⁵. The lowest PDI value was obtained by the Liposome Blank formula, as shown in Fig. 1b.

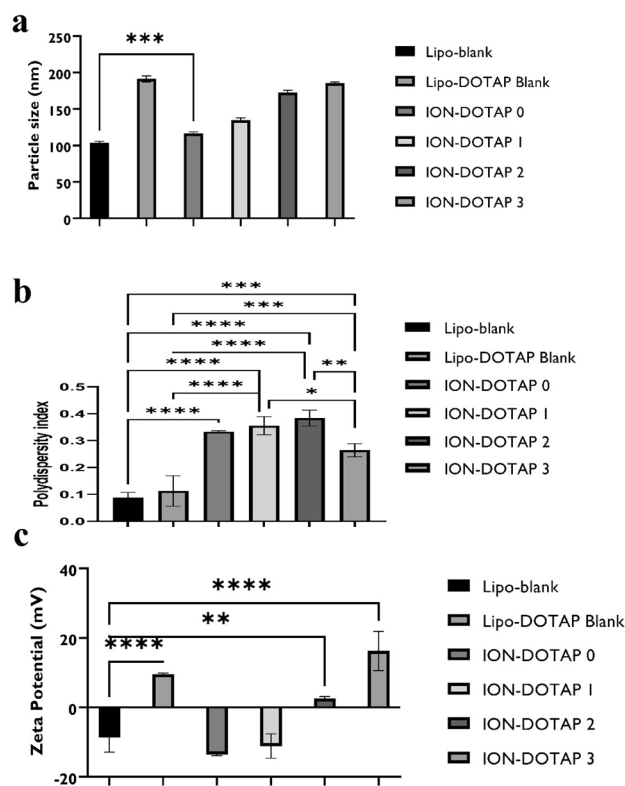


Figure 1. The bar charts of average (a) particle size value, (b) polydispersity index and (c) zeta potential of β -ionone liposomes prepared with various concentrations of DOTAP. Lipo-Blank contained no DOTAP or β -ionone, while Lipo-DOTAP Blank contained DOTAP at the highest concentration without the addition of β -ionone, $*p < 0.5$, $**p < 0.01$, $***p < 0.001$, compared to Lipo-Blank.

For the zeta potential value, the results indicated that the Lipo-blank, ION-DOTAP-2, and ION-DOTAP-3 liposomes differed significantly from liposomes ION-DOTAP-0, while Lipo-blank and ION-DOTAP-1 did not show any difference with ION-DOTAP-0 as shown in Fig. 1c.

The vesicle morphology of β -ionone liposomes analyzed by transmission electron microscopy (TEM). From the results of the vesicle morphology analysis with TEM contained in Fig. 2, it can be observed that the vesicles of ION-DOTAP-0 liposomes appear spherical with unilamellar membrane formation. In con-

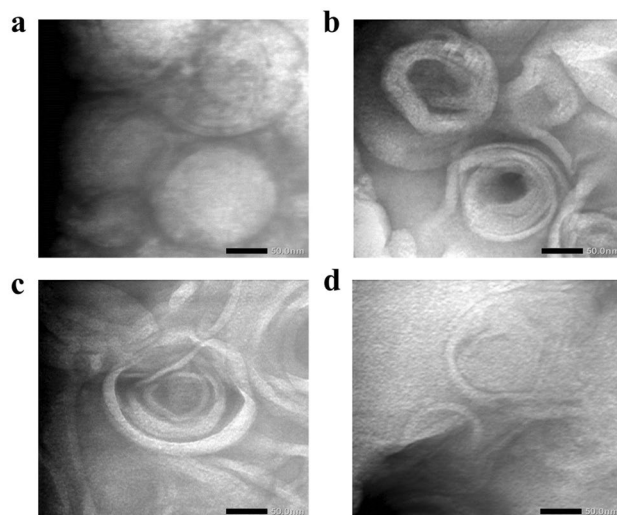


Figure 2. Morphological observations of (a) ION-DOTAP-0, (b) ION-DOTAP-1, (c) ION-DOTAP-2, and (d) ION-DOTAP-3 liposomes were analyzed using Transmission Electron Microscopy (scale bar: 50 nm).

trast, the formula, while also apparently spherical in shape, has a layered membrane due to the addition of DOTAP.

The spectra profiles of fourier transform infrared spectroscopy (ftir) of β -ionone liposomes. Identification of the compound's structure was completed using the FTIR Alpha II Spectrophotometer within the range of wave numbers 4000–600 cm^{-1} . The spectra of liposome constituents can be seen in Fig. 3, while those of the liposome formula produced can be seen in Fig. 4.

In the FTIR analysis, the Lipo-blank liposome spectra showed absorption bands identical to those in the spectra of the liposome constituents, as shown in Fig. 3. However, a decrease in the intensity of the absorption band after liposome formation occurred. The observed bands of the N–H functional group (3300–3500 cm^{-1}) were identical to the DOTAP absorption band, the C=C group band (1640–1680 cm^{-1}) was identical to the β -ionone absorption band, and the C–O group band (1050–1150 cm^{-1}) was identical to the absorption band of β -ionone, DSPE-mPEG₂₀₀₀, and cholesterol. The CH₃–CH₂–CH group (2850–3000 cm^{-1}) was identical to the absorption band of all the constituents of liposomes.

According to the overlaid spectra of β -ionone liposomes prepared with various concentration of DOTAP shown in Fig. 4, there were observed bands of N–H, CH₃–CH₂–CH, C=C (alkene), dan C–O functional groups for all formula. There are disappearances of some identical peaks of β -ionone and DOTAP observed for β -ionone liposomes with DOTAP addition. The spectra profiles of β -ionone liposomes show increasing the intensity and widening of absorption band of N–H groups that represent the DOTAP. However, spectra shown for each β -ionone liposomes showed no significant differences in the intensity of same type of absorption band.

Differential thermal analysis (DTA) of β -ionone liposomes. The thermal analysis results produced using differential thermal analysis (DTA) on each liposome component and liposome formula can be seen in the thermogram shown in Fig. 5. The results show that the DTA thermogram of liposome constituents, i.e., DOTAP, experienced endothermic peaks at 86.0 $^{\circ}\text{C}$ and 200.8 $^{\circ}\text{C}$, while the HSPC thermogram registered two endothermic peaks at 83.6 $^{\circ}\text{C}$ and 182 $^{\circ}\text{C}$. On the other hand, the DSPE-mPEG2000 thermogram recorded endothermic peaks at 59.2 $^{\circ}\text{C}$ and 249.2 $^{\circ}\text{C}$, and cholesterol at 50.9 $^{\circ}\text{C}$ and 152.5 $^{\circ}\text{C}$. The Lipo-blank, which consisted of a mixture of cholesterol, HSPC, and DSPE-mPEG2000, registered two endothermic peaks at 139.2 $^{\circ}\text{C}$ and 214.0 $^{\circ}\text{C}$. The formation of liposomes produced endothermic peak shifts.

Figure 5 also contains the results of the overlaid DTA thermograms of the liposomes, namely: DOTAP, Lipo-blank, Lipo-DOTAP Blank, ION-DOTAP-0, ION-DOTAP-1, ION-DOTAP-2, and ION-DOTAP-3. Each formula had two endothermic peaks. For example, thermograms on Lipo-DOTAP Blank, ION-DOTAP-0, and ION-DOTAP-1 liposomes produced endothermic peaks of approximately 140.2–140.8 $^{\circ}\text{C}$ and 213.2–214.2 $^{\circ}\text{C}$. The endothermic peak formed was identical to the peaks of its constituents, namely: cholesterol and DOTAP.

Furthermore, the thermogram of ION-DOTAP 2 liposomes underwent a shift with endothermic peaks at temperatures of 176 $^{\circ}\text{C}$ and 114 $^{\circ}\text{C}$. Meanwhile, the ION-DOTAP-3 liposomes, which contain the highest

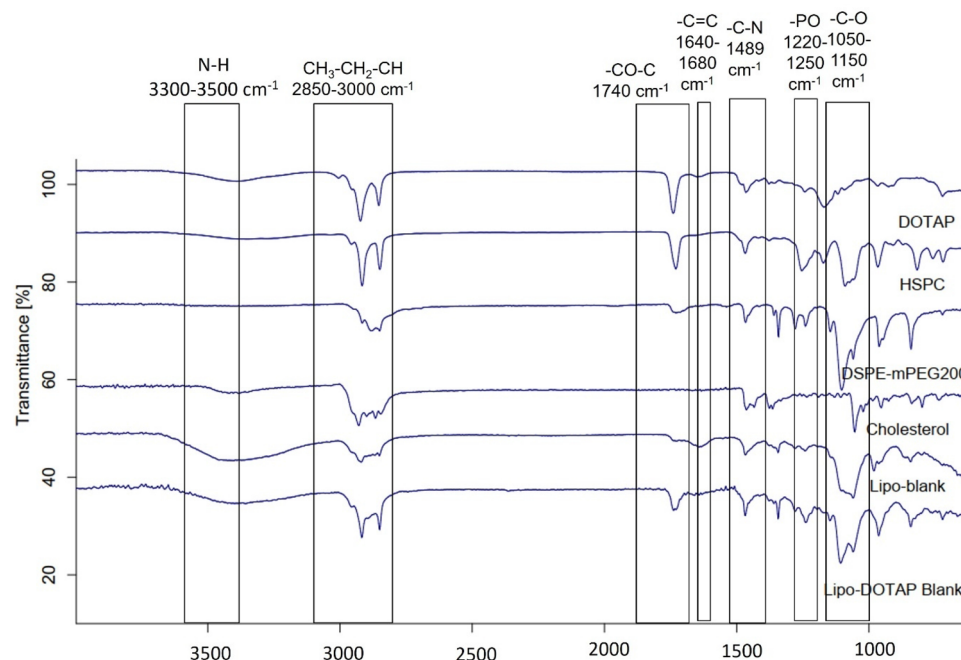


Figure 3. FTIR spectra of β -ionone, DOTAP, HSPC, DSPE-mPEG2000, cholesterol, Lipo-Blank, and Lipo-DOTAP Blank. Lipo-Blank contained no DOTAP or β -ionone, while Lipo-DOTAP Blank contained DOTAP at the highest concentration without the addition of β -ionone.

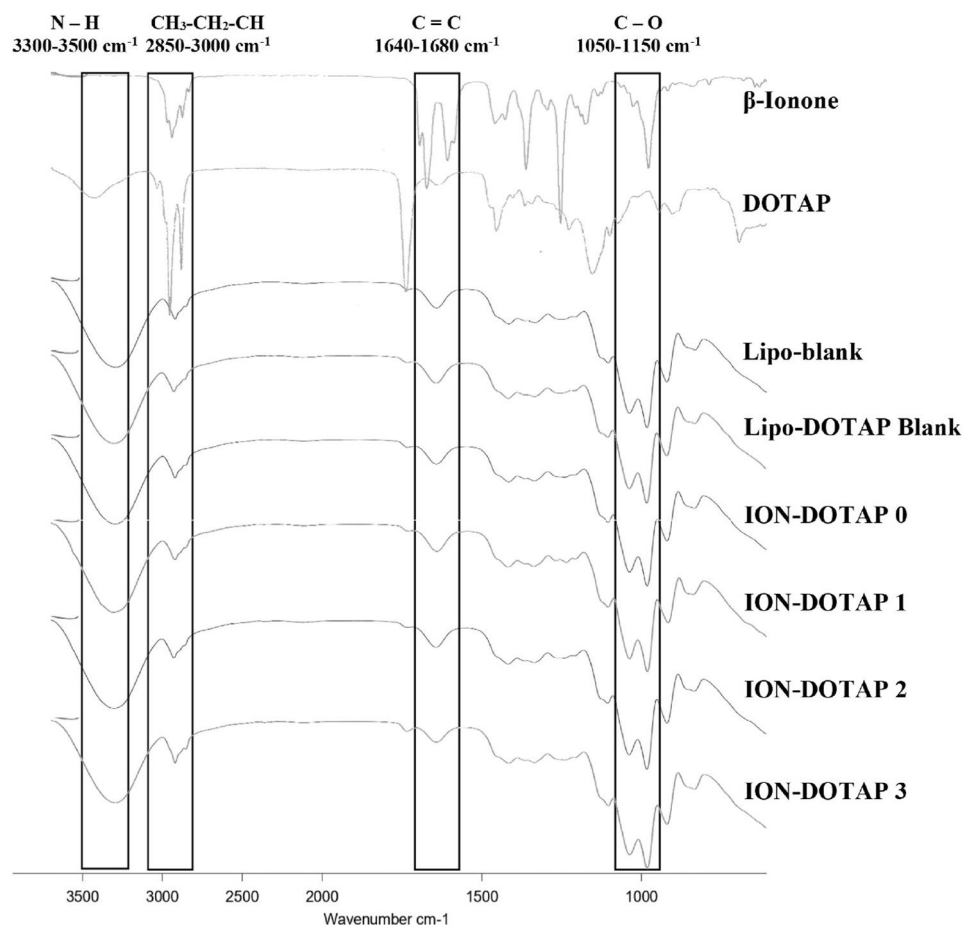


Figure 4. FTIR spectra of β -ionone, DOTAP, ION-DOTAP liposomes, and Lipo-DOTAP Blank, ION-DOTAP 1, ION DOTAP 2, ION DOTAP 3 Lipo-Blank contained no DOTAP or β -ionone, while Lipo-DOTAP 3 contained DOTAP at the highest concentration without the addition of β -ionone.

concentration of DOTAP, experienced a significant shift of endothermic peaks at 81.6 °C and 214.1 °C, with relatively wide peaks when compared to the those of the other liposomes. It also resulted in the loss of the endothermic peak of 152.5 °C, which is identical to that of cholesterol. This result confirms that the ION-DOTAP-3 thermogram experienced an endothermic peak increasingly similar to that of DOTAP.

The calcein released from β -ionone liposomes. Calculation of the cumulative percentage of the calcein released from the liposomes indicates that the smallest percentage of the calcein released was in the ION-DOTAP-0, indicating that this liposome has the most rigid structure compared to those of other formulas as presented in Fig. 6. Meanwhile, the highest percentage of released calcein was observed in ION-DOTAP 3 liposomes.

In addition, the release flux value obtained from this study reveals that the liposome ION-DOTAP-3 had the largest flux value (6.7074 ± 1.8650), followed by ION-DOTAP-2 (2.8357 ± 0.7453), ION-DOTAP 1 (1.6169 ± 0.4700) and ION-DOTAP-0 (0.2674 ± 0.0545) as shown in Table 1.

The cellular uptake of β -ionone liposomes in HeLa cells. In Fig. 7, cells in the LIPO-blank treatment can be observed where the fluorescence intensity of coumarin-6 loaded into liposomes was relatively low. The liposomes containing coumarin-6 with high DOTAP levels increased the fluorescence intensity. Figure 7 shows that ION-DOTAP-0 liposomes containing coumarin-6 infiltrated the HeLa cells indicated by green fluorescence, while ION-DOTAP-1 liposomes were only slightly fluorescent. However, they were less visible and the fluorescence of the ION-DOTAP-2 liposomes was relatively weak in intensity as shown in the cell membrane and cytoplasm.

Meanwhile, as shown in Fig. 8, the ION-DOTAP-3 liposome with the highest DOTAP content increased the fluorescence intensity and produced the highest cellular coumarin-6 levels than other formulas.

In vitro cytotoxicity evaluation of β -ionone liposomes. The IC_{50} cytotoxicity test on HeLa cells aims to determine the effect of adding DOTAP to β -ionone liposomes in cancer therapy. The cells incubated with

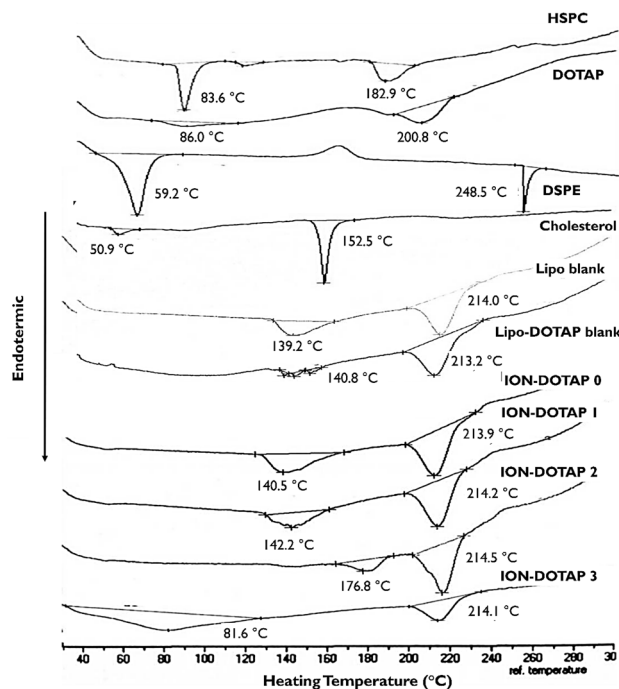


Figure 5. Thermogram profile of HSPC, DOTAP, DSPE-mPEG2000, Cholesterol, Lipo-Blank, Lipo-DOTAP Blank, ION-DOTAP-0, ION-DOTAP-1, ION-DOTAP-2, and ION-DOTAP-3.

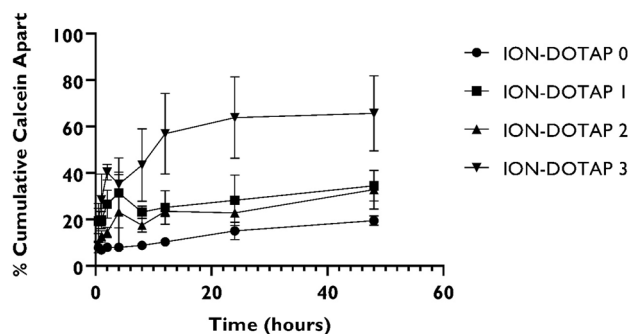


Figure 6. The calcein release profile of ION-DOTAP-0, ION-DOTAP-1, ION-DOTAP-2, and ION-DOTAP-3 liposomes in PBS pH 7.4 as the release media at a temperature of 37 °C.

Formula	Calcein release flux ($\mu\text{g}/\text{cm}^2/\text{h}$, n = 3)
ION-DOTAP 0	0.2674 ± 0.0545
ION-DOTAP 1	1.6169 ± 0.4700
ION-DOTAP 2	2.8357 ± 0.7453
ION-DOTAP 3	6.7074 ± 1.8650

Table 1. The release flux value of calcein released from β -ionone liposomes prepared with various concentrations of added DOTAP.

β -ionone liposomes for 48 h resulted in the percentage of cell viability shown in Fig. 9A. It can be seen that HeLa cells were not sensitive to β -ionone liposomes, and all treatments resulted in high cell viability.

To further evaluate the cytotoxic effects on these cells, DOXO was co-loaded into β -ionone liposomes. As shown in Fig. 9B, an increase in the cytotoxic effect also occurred in T47D cells, where the ION-DOXO-DOTAP-2 formula gave the lowest IC_{50} value compared to other liposome formulas, indicating its high cytotoxicity. On the other hand, ION-DOXO-DOTAP-0, which does not contain DOTAP, produces the smallest IC_{50}

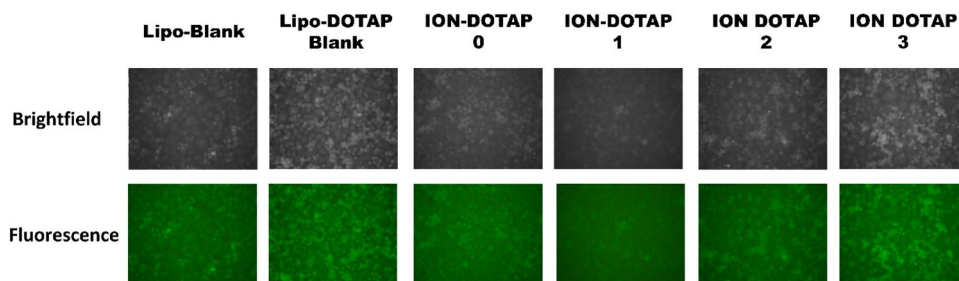


Figure 7. Photomicroscopy of cellular uptake of HeLa cells after incubation with β -ionone liposomes containing Coumarin-6 (in green color) prepared with various concentrations of DOTAP for two hours at $20\times$ magnification under a fluorescence microscope.

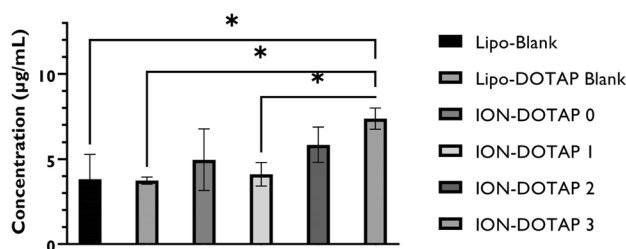


Figure 8. Coumarin-6 levels in HeLa cells post-incubation with β -ionone liposomes containing Coumarin-6 prepared with various concentrations of DOTAP for two hours.

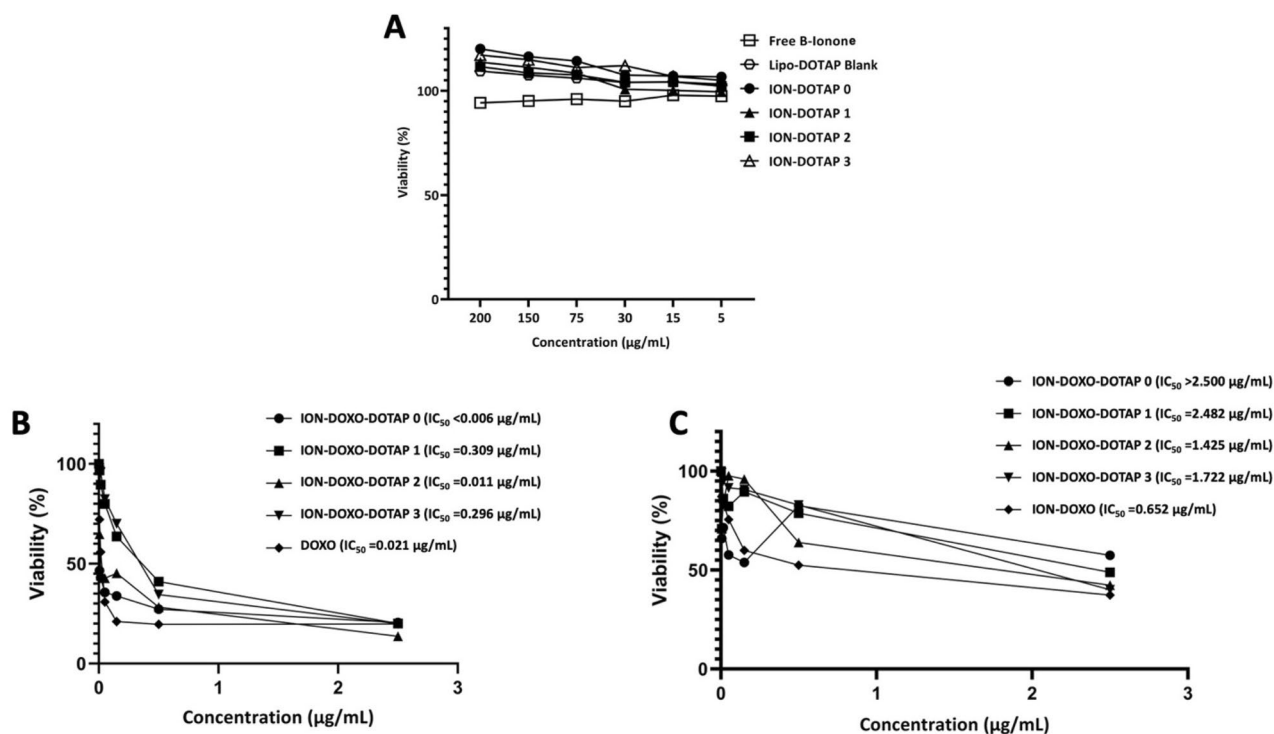


Figure 9. The viability of (A) HeLa cells after incubation of β -ionone liposomes and (B) T47D and (C) HeLa cells after incubation with β -ionone liposomes co-loaded with doxorubicin for 48 h.

value. The results also showed that there is an increase in cytotoxicity of the ION-DOXO-DOTAP-2 formula in HeLa cells; however, ION-DOXO-DOTAP-0 produces the highest IC_{50} value (Fig. 9C).

Discussion

In this study, liposomes were prepared to deliver β -ionone, a low water soluble and poorly permeable drug, used in anticancer treatment^{6,7}. However, the composition of phospholipids significantly affects liposomes' characteristics which may relate to their pharmacological activity. Therefore, this study evaluated the effect of the addition of DOTAP on the physical characteristics of β -ionone-loaded liposomes, including: particle size, zeta potential, morphology, infrared (IR) spectroscopic profiles, thermal analysis, and calcein release.

The addition of DOTAP affects the physicochemical characteristics of the liposomes, with increased DOTAP concentration resulting in larger particle size. This is probably due to the HSPC, cholesterol, and DSPE-mPEG2000 components which have ordered molecule arrangements producing a rigid membrane structure³⁶. Moreover, the possibility exists of interaction between the polar groups of DOTAP and the choline groups of HSPC, both of which are positively charged and therefore repel each other, leading to a widening of the bilayer membrane's space.

The spectra of liposomes loaded β -ionone with DOTAP addition experienced a reduction in the intensity and widening of the absorption band in the N–H group which is characteristic of DOTAP. The N–H group in DOTAP is the polar section of the phospholipid head³⁷. Furthermore, the analysis of the absorption band on the alkyl group (CH_2) can be used as an indicator of the regularity of the lipid chain arrangement. The reduced intensity and widening of the absorption band indicate an increase in the gauche conformation³⁸. The spectra of liposomes formed showed a decrease in the intensity of the absorption band on the alkyl group, indicating a potential increase in the gauche conformation, which changed the composition of the lipid chain thereby rendering it more fluid.

Several identical peaks in DOTAP and β -ionone were not visible in the spectra of the liposomes loaded β -ionone with DOTAP addition, indicating that there could be physical interaction³⁹. Meanwhile, the spectra shown for each liposome formula did not indicate a significant difference in the intensity of the absorption bands.

FTIR analysis, in determining the interaction of DOTAP with liposome membranes, can be performed by observing the absorption band on certain functional groups, namely: the phosphate group ($R-PO_2-R'$) and the ester group ($-CO-O$) present on the head of HSPC which is polar⁴⁰. Neither the Lipo-blank FTIR spectra nor the blank Lipo-DOTAP showed any absorption bands in the two functional groups. Therefore, it is thought that hydrogen bonding occurs in both the phosphate and phospholipid ester groups due to stretching of the absorption band⁴¹. Lipo-blank and Lipo-DOTAP Blank liposomes did not show a significant shift in the absorption band and no new band formation, leading to the conclusion that only chemical interaction and no physical interaction occurs. Moreover, the ION-DOTAP-3 thermogram shows an endothermic peak that is increasingly similar to that of DOTAP. The widening and disappearance of the endothermic peaks on the thermogram indicate a change in the regularity of the lipid structure lipid⁴². In other words, adding DOTAP can reduce the rigidity of the membrane, thereby rendering it more fluid.

The calcein release test showed that the liposome ION-DOTAP-3 experienced the highest calcein release compared to the other formulas. The release of calcein from the liposomes increased in line with that of membrane fluidity³³. It can therefore be concluded that ION-DOTAP 3 demonstrates the highest membrane fluidity. Based on the results of the analysis conducted to determine the physical characteristics of β -ionone liposomes, the addition of DOTAP can increase the particle size, rendering the zeta potential positive with the morphology of liposomes appearing spherical with a layered membrane. Furthermore, the results of thermogram data and spectroscopic profiles, show that the addition of DOTAP can cause the composition of the bilayer membrane to become more fluid. This result is in line with the calcein release profile which indicates that adding DOTAP can increase the fluidity of the liposome membrane.

With regard to the surface charge of liposomes, the liposome constituents such as HSPC and DSPE-mPEG₂₀₀₀ have a neutral charge, while β -ionone and cholesterol have no charge or are non-ionic⁴³. Meanwhile, DOTAP is a positively charged phospholipid with an NH^{4+} group on the head of the phospholipid¹⁹. Consequently, adding DOTAP to liposomes increased the positivity of the potential charge. Increasing the concentration of DOTAP heightened the positive charge of the liposomes.

A cellular uptake test was carried out to determine the ability of β -ionone liposomes to infiltrate the HeLa cells. The levels of liposomes entering HeLa cells were calculated as coumarin-6 levels. Coumarin-6 is a hydrophobic compound trapped in the bilayer membrane and the measured coumarin-6 level indicates how many liposomes enter the cell. The green fluorescence indicates that β -ionone liposomes containing coumarin-6 are present in the cells⁴⁴. The more intense the fluorescence color, the greater the amount of coumarin-6 absorbed⁴⁵. Based on the findings of the quantification analysis of coumarin-6 levels, the ION-DOTAP-3 formula produced higher levels than other formulas. It is because the ION-DOTAP-3 liposomes contain cationic lipid (DOTAP) at a higher concentration than other formulas, resulting in a significant ionic interaction between negatively charged cancer cells and β -ionone liposomes containing DOTAP (cationic lipid)⁴⁶.

It was also reported that particle size affects the endocytosis pathway of liposomes into cells. In this study, rising levels of DOTAP increased the particle size of liposomes, although this was in the range of < 200 nm which was still relatively modest. Consequently, no significant effect on cellular uptake was regarded as having occurred. The entry of β -ionone liposomes was due to the addition of DOTAP to cells through a process of endocytosis which requires energy to introduce foreign particles into the cell. The results demonstrated a significant difference between the ION-DOTAP-3 and the blank lipid formula, blank ION-DOTAP, and ION-DOTAP-1. In this study, it can be seen that, with the addition of higher DOTAP levels, β -ionone liposomes can increase cell uptake

in HeLa cells. This greater cell uptake leading to increased coumarin-6 levels and microscopic observations of HeLa cells showed that the intensity of coumarin-6 rose with the occurrence of higher DOTAP levels.

The IC₅₀ cytotoxicity test was carried out on HeLa cells to determine the effect of adding DOTAP to β -ionone liposomes in cancer therapy. Unfortunately, β -ionone liposomes had no cytotoxic effects even though the cellular uptake test results show that liposomes entered the cells. This result indicates that it is possible that these cells are not sensitive to β -ionone, which becomes the limitation of our study. Therefore, a cytotoxicity test was carried out by co-encapsulating DOXO into β -ionone liposomes (ION-DOXO-DOTAP liposomes). DOXO has been known as an anticancer compound sensitive to HeLa cells and T47D cells. The results showed that the ION-DOXO-DOTAP liposomes had toxicity to both cancer cells. The ION-DOXO-DOTAP-2 had the highest cytotoxicity. Compared to the ION-DOXO-DOTAP-0, which had no DOTAP addition, the toxicity is relatively similar to that of DOXO. This is related to liposome formulations with the addition of DOTAP providing better retention than liposomes without DOTAP. The increased drug release was proportional to the cell uptake, which also increased with the addition of DOTAP. This result is in line with the study of Lei et al. (2011), where it was reported that cytotoxicity results were associated with cellular uptake. Therefore, high cellular uptake will give an increased cytotoxicity value⁴⁷.

Conclusions

The results of this study indicate that the addition of DOTAP to β -ionone liposomes increased the particle size slightly and rendered the charge of the liposomes positive. In addition, the higher the DOTAP concentration added, the more fluid the liposomal membrane as indicated by the reduction in membrane crystallinity which supported the higher calcein release from the liposomes. The introduction of DOTAP into liposomes highly affects the physical properties of β -ionone liposomes, thereby increasing the cellular uptake and cytotoxicity. Proposing the incorporation of DOTAP into β -ionone liposomes requires further studies on its anticancer efficacy. From the results of this analysis, although the addition of DOTAP renders the liposomes more fluid, its ability to enhance the cellular uptake provides potential benefits when used as a delivery system for cancer therapy.

Materials and methods

Materials. In this study, β -ionone was purchased from Sigma-Aldrich Inc. (Saint Louis, USA). The liposome component was 1,2-dioleoyl-3-trimethylammonium propane (DOTAP), Hydrogenated Soybean Phosphatidylcholine (HSPC), and Distearoyl-glycerophosphoethanolamine-N-(Carbonyl-methoxy polyethylene glycol 2000 (DSPE-mPEG₂₀₀₀), which were products of Nof Corporation (Tokyo, Japan). Cholesterol was obtained from Wako Inc., Ltd. (Osaka, Japan), while calcein (Fluorexone[®]) was a product of Nacalai Tesque Inc. (Kyoto, Japan). The buffer components include phosphate buffer pH 7.4, KH₂PO₄, Na₂HPO₄, NaCl, and KCl were purchased from Merck Inc. (Darmstadt, Germany). Chloroform and methanol were products of Merck Inc. (Darmstadt, Germany). Other reagents and materials used were of non-technical grade.

Preparation of β -ionone liposomes. β -ionone liposomes were prepared by the thin film hydration method. First, β -ionone, DOTAP, HSPC, DSPE-mPEG₂₀₀₀, and cholesterol were dissolved in chloroform. As shown in Table 2, appropriate amounts of each solution were, mixed in a round bottom flask. The chloroform was then evaporated and completely removed using a rotary evaporator at 55 °C to form a thin film layer on the flask wall. The thin film formed was hydrated with phosphate-buffered saline (PBS) pH 7.4 and sonicated using a sonicator at 55 °C for 10 min²⁷. Extrusion was subsequently carried out with an extruder (Avanti[®], Mini Extruder, Avanti Polar Lipid Inc., Alabama, USA) by passing liposomes through a polycarbonate membrane with various pore sizes, i.e., 400, 200, and 100 nm, with a heating block at a temperature of 55–60 °C to produce liposomes approximately 100 nm in size⁴².

Evaluation of physical characteristics of β -ionone liposomes. The particle size of β -ionone liposomes were determined by the dynamic light scattering method using a Delsa Nano C Particle Analyzer at 25 °C. Meanwhile, the zeta potential was measured using the electrophoresis light scattering method using the Malvern Zetasizer Nano Series Analyzer at 25 °C at the Sepuluh Nopember Institute of Technology (ITS), Surabaya, Indonesia. The liposomes were diluted with demineralized water, placed in a cuvette, and measured for particle size and zeta potential²⁷.

Components	Composition (mole ratio)					
	Lipo-blank	Lipo-DOTAP blank	ION-DOTAP-0	ION-DOTAP-1	ION-DOTAP-2	ION-DOTAP-3
β -ionone	–	–	30	30	30	30
DOTAP	–	8	0	2.5	5	8
HSPC	35	35	35	35	35	35
Cholesterol	25	25	25	25	25	25
DSPE-mPEG ₂₀₀₀	10	10	10	10	10	10
Hydrating buffer	Phosphate buffered saline (PBS) pH 7.4					

Table 2. Composition of β -ionone liposomes.

Evaluation of vesicle morphology of β -ionone liposomes using transmission electron microscopy (TEM). Liposome morphology, determined using the JEOL JEM-1400 TEM (Jeol Inc., USA) instrument, was carried out at the Laboratory of Chemistry of Gadjah Mada University (UGM), Yogyakarta, Indonesia. Liposomes were first diluted using demineralized water at a volume ratio of 1:5 of liposome and water respectively. The sample was subsequently stained with 4% phosphotungstic acid at a ratio of 1:1. Samples were placed on a copper grid, and images taken at various magnification sizes¹⁵.

Fourier-transform infrared spectroscopy (FTIR) Analysis of β -ionone Liposomes. FTIR evaluation was carried out to determine molecular interactions within the liposomes. Before the analysis, each β -ionone liposome was hydrated with PBS pH 7.4 containing 7% sucrose and freeze dried. FTIR analysis was then conducted using an FTIR Alpha II Spectrophotometer (Bruker, Ettlingen, Germany). The sample was put into the sample container and the spectra measured at wave numbers of 4000–600 cm^{-1} .

Differential thermal analysis (DTA) of β -ionone liposomes. DTA is used to evaluate changes in sample properties evident from the endothermic and exothermic peaks on the DTA thermogram when heat is applied. The tests were carried out using a DTA instrument (Mettler Toledo FP90, Switzerland). Each β -ionone liposome was hydrated with PBS pH 7.4 containing 7% sucrose and freeze-dried before analysis. The freeze-dried samples were placed in aluminum crucibles and heated from 30 to 300 °C at the rate of 10 °C/min²⁷.

Evaluation of calcein release. *Preparation of β -ionone liposomes containing calcein for release study.* The β -ionone liposomes were prepared on the basis of the above method. The thin lipid film formed was then hydrated with 1 mL of PBS pH 5.0 containing 3.4 mM calcein (Nacalai Tesque Inc., Kyoto, Japan). Extrusion was subsequently carried out with an extruder (Avanti[®], Mini Extruder, Avanti Polar Lipid Inc., Alabama, USA) by passing liposomes through a polycarbonate membrane with various pore sizes of 400, 200, and 100 nm with a heating block at 55–60 °C⁴². The free calcein was subsequently separated from the liposomes by placing them into the Amicon[®] Ultra-4 Centrifugal Filter Unit tube with a molecular weight cut-off (MWCO) of 10,000 Da (Merck Millipore, Darmstadt, Germany). The tube was centrifuged using a Hettich Rotofix 32 Benchtop Centrifuge (Hettich Lab., Tuttlingen, Germany) at 3500 rpm in order to remove the untrapped or free calcein.

Calcein release assay from β -ionone liposomes. A calcein release study was conducted by dialysis. The liposomes were placed in the Spectra/Por[®]7 dialysis membrane with MWCO (Spectrum Laboratories, USA) of 3500 Da. 50 mL of PBS pH 7.4 was employed as a release medium with a stirring speed of 350 rpm at 37 °C. Sampling was performed at 0.5, 1, 2, 4, 8, 12, 24, and 48 h by replacing some samples with the same volume of fresh PBS pH 7.4. The calcein fluorescence intensity was subsequently measured using the GloMax[®]-Multi Detection System (Promega Corporation, Madison, USA) microplate reader at $\lambda_{\text{ex}} = 475 \text{ nm}$ and $\lambda_{\text{em}} = 500\text{--}550 \text{ nm}$.

The cellular uptake assay of β -ionone liposomes. *Preparation of β -ionone liposomes containing coumarin-6 as the fluorescent labeling agent.* The first procedure involved dissolving each liposome component in chloroform. Liposomes were prepared according to the formula in the Table 2 by adding 0.3 mg Coumarin-6 to each mL of liposomes containing 9.7 mg total lipid of HSPC, Cholesterol, and DSPE-mPEG₂₀₀₀. The amount of coumarin-6 added was in accordance with the previous study of Miatmoko et al. (2021), which successfully represented liposome uptake in cancer cells⁴⁸. Each component solution was appropriately mixed in a round bottom flask covered with aluminum foil. The organic solvent was then placed in a rotary evaporator at a temperature of 55 °C until all organic solvents were removed and formed a thin film which was hydrated using 1 mL of PBS pH 7.4, vortexed, and sonicated for 10 mins at 55 °C. Liposomes were subsequently extruded using polycarbonate membranes of sizes from 400, 200, to 100 nm to produce liposomes with a size of about 100 nm.

The cellular uptake assay. Initially, the HeLa cells, which were obtained from Stem Cell Research and Development Center, Universitas Airlangga, were suspended in RPMI 1640 medium containing 10% Fetal Bovine Serum (FBS) and 1% Penicillin–Streptomycin antibiotic at a concentration of 1×10^6 cells/mL, placed into a 6-well plate, and incubated at 37 °C with 5% CO₂ environment for 24 h. Cells can be used for cellular uptake assay if they have a confluence of 70–80%⁴⁴. The medium was replaced with β -ionone liposomes at a coumarin-6 concentration of 10 $\mu\text{g/mL}$ and incubated for two hours, with the media being disposed of. The cells were first twice washed with PBS pH 7.4 and then added to 1 mL of methanol and left for 5–10 mins. After the methanol had been discarded, the cells were washed twice with PBS pH 7.4, containing 0.5% Tween 80 and approximately 1 mL of PBS pH 7.4 containing 1% FBS was added and left for 15 min. The cells were then washed twice with PBS solution pH 7.4. The final step was to observe the cells by means of a fluorescence microscope with an FITC filter⁴⁴.

Measurement of coumarin-6 levels in HeLa cells. In the first step, the cells were prepared with the same procedure as that for the cellular uptake assay and the samples were incubated for two hours. The medium was then discarded and the cells washed twice with PBS pH 7.4. In order to measure the amount of intra-cellular coumarin-6, the cells were lysed with 100 μL of lysis buffer containing 10 mM NaCl, 1 mM Ethylenediaminetetraacetic acid (EDTA), and 0.5% Triton X-100 by means of pipetting and added to 900 μL PBS pH 7.4 to enable collection of the samples. The cell suspension was vortexed and centrifuged at 12,000 rpm for ten minutes. After centrifugation, the supernatant's fluorescence intensity was measured using GloMax[®]-Multi Detection System (Promega Corporation, Madison, USA) microplate reader at $\lambda_{\text{ex}} = 475 \text{ nm}$ and $\lambda_{\text{em}} = 500\text{--}550 \text{ nm}$ ⁴⁴.

In vitro cytotoxicity assay. Preparation of liposomes containing doxorubicin. In addition to β -ionone liposomes, we prepared β -ionone liposomes co-loaded with Doxorubicin (DOXO) to produce ION-DOXO-DOTAP liposomes and evaluate their cytotoxicities on T47D and HeLa cells. β -ionone liposomes co-loaded with DOXO were prepared by the same formula and methods as β -ionone liposomes; however, the 0.65 M Triethylamine was used as the hydrating solution. The liposomes were then eluted through a column packed with Sephadex G50 using PBS pH 7.4. DOXO was added at DOX/total lipid ratio of 1:5, respectively, and the mixture was then incubated in a water bath at 60 °C for 10 min. The liposomes were further separated from the free DOXO by elution through the Sephadex G50 gel column.

WST assay. The in vitro cytotoxicity test procedure was evaluated for T47D cells and HeLa cells using the WST-1 assay method. The T47D and HeLa cell were obtained from Stem Cell Research and Development Center, Universitas Airlangga, and then cultured in Roswell Park Memorial Institute (RPMI)-1640 medium containing 10% Fetal Bovine serum and 1% Penicillin Streptomycin in a humidified atmosphere at 37 °C with 5% CO₂ air supply. For the assay, T47D and HeLa cells with a concentration of 5×10^3 cells were added to each well of 96-well plates and incubated for 24 h before treatment.

To evaluate the cytotoxicity, β -ionone liposomes and β -ionone liposomes co-loaded with DOXO were prepared at various concentrations. These samples were added to each well containing the cells and incubated for 48 h. After the treatment, the cell number was determined using WST-1 assay. The cell viability was determined as the relative measurement to the untreated cells by evaluating the absorbance of each well at 440 nm using a microplate reader. The IC₅₀ values of the compounds were calculated using the percentage of living cells and analyzed using SPSS (probit) software.

Data analysis. The data for analysis consisted of the average \pm standard deviation measured in replications. First, a Kolmogorov–Smirnov test was used to analyze the numerical data for normal distribution. If the data was normal (P value ≥ 0.05), the quantitative analysis was analyzed using One Way Analysis of Variance. If the P value < 0.05 , the data analysis was followed by a post hoc test incorporating the use of Honestly Significant Difference (HSD). The analysis was conducted using the IBM SPSS Statistics software version 24.

Data availability

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

Received: 27 September 2022; Accepted: 14 March 2023

Published online: 15 March 2023

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Acknowledgements

The authors would like to thank Ms. Berlian Sarasitha Hariawan and Ms. Devy Maulidya Cahyani for kind assistantship for manuscript preparation.

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A.M.: (1) conception and design of the work, data acquisition, data analysis and interpretation; (2) critically revising the article for important intellectual content; (3) Final approval of the version to be published; (4) Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of the work are appropriately investigated and resolved. F.H.A.: (1) conception and design of the work, data acquisition, data analysis and interpretation; (2) Drafting the article; (3) Final approval of the version to be published; (4) Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of the work are appropriately investigated and resolved. A.A.A.: (1) conception and design of the work, data acquisition, data analysis and interpretation; (2) Drafting the article; (3) Final approval of the version to be published; (4) Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of the work are appropriately investigated and resolved. N.R.: (1) data analysis and interpretation; (2) critically revising the article for important intellectual content; (3) Final approval of the version to be published; (4) Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of the work are appropriately investigated and resolved. C.-S.H.: (1) data analysis and interpretation; (2) Final approval of the version to be published; (3) Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of the work are appropriately investigated and resolved.

Funding

This study was financially supported by Research Collaboration Funding between Universitas Airlangga, Indonesia and Asia University, Taiwan.

Competing interests

The authors declare no competing interests.

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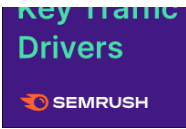
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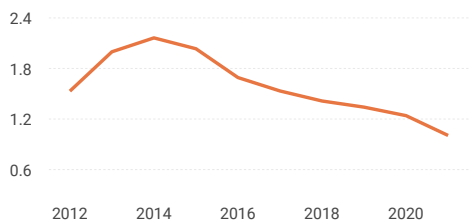
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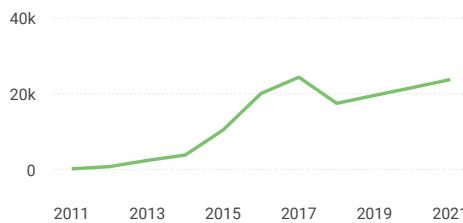
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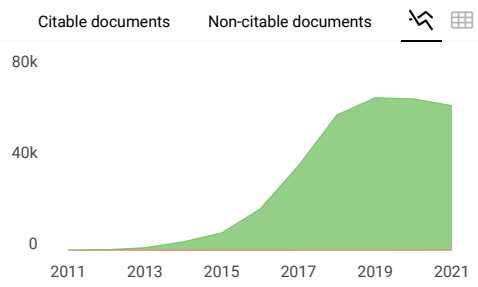
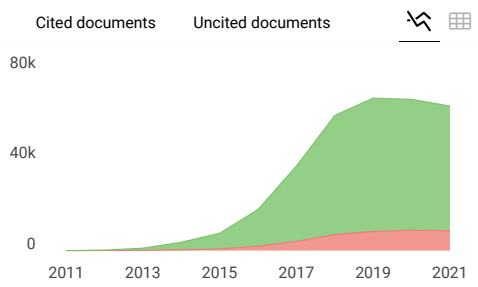
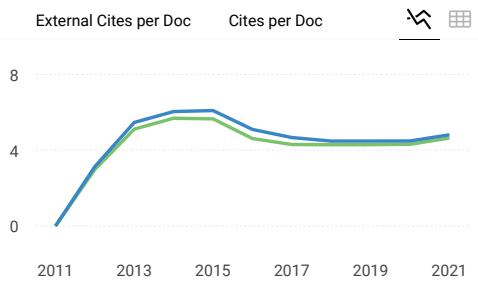
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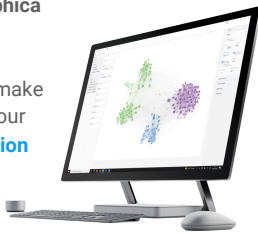
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Dr Carlo Cannistraci



Dr Carlo Cannistraci is a Theoretical Engineer and Editorial Board Member for Scientific Reports.

1. What is your current research focused on?

I am a Theoretical Engineer; my research interests include subjects at the interface between physics of complex systems, complex networks and machine intelligence, with particular interest in brain/bio-inspired computing for Big Data analysis, and applications in precision biomedicine and neuroscience.

2. What has been your biggest challenge and your greatest achievement in your career so far?

Mapping complex networks to their latent geometric spaces helps to investigate, understand and predict the structure and function of complex systems. My biggest challenge and greatest achievement was to recently propose a class of intelligent machines for efficient embedding of large real networks to the hyperbolic space, with future impact on big-network-data analysis in biology, medicine and social science. This work was proposed in the article: Machine learning meets complex networks via coalescent embedding in the hyperbolic space, A Muscoloni, JM Thomas, S Ciucci, G Bianconi, CV Cannistraci. Nature Communications 8 (1), 1, 2017.

3. Why did you decide to become a board member?

I love to support other colleagues to improve their studies and to achieve high standards in their publications. This is the spirit of the review process: to offer feedback that improves science and its dissemination with a clear benefit for all of the scientific community.

4. What do you like most about being a board member for *Scientific Reports*?

Being an Editor for *Scientific Reports* for me is something more than being a normal Editor. The spirit of *Scientific Reports* is the spirit of 'freedom and equal opportunity' in science. I like the fact that *Scientific Reports* accept articles according to the only requirement that they should be technically correct. This ensures that the article selection is not biased by the opinion of a 'group of experts' that, in my opinion, can be also risky, because very innovative ideas that are against the mainstream in science might be rejected. I feel that being an editor for *Scientific Reports* allows me to sponsor the freedom to publish new scientific ideas which are technically correct but might not be recognised by a conservative establishment of experts.

Dr Joana Maria Ramis



Dr Joana Maria Ramis is a Miguel Servet Researcher at the Balearic Islands Health Research Institute (IdISBa), as well as Adjunct Lecturer at the University of the Balearic Islands, Spain.

1. What is your current research focused on?

My research is focused on the development of new therapies and biomaterials for restorative and regenerative medicine and its translation to clinical practice. My newest research line focus on the approach to cell-free regenerative medicine through the use of extracellular vesicles derived from different cells types.

2. What has been your biggest challenge and your greatest achievement in your career so far?

My biggest challenge and greatest achievement has been, and keeps being, reconciling family life and my research career.

3. Why did you decide to become a board member?

I considered the offer to become a board member as a great opportunity to deepen in the knowledge of the review process and to be an active part of it. Publication of our results is an important part of our work as researchers, and before becoming a board member for *Scientific Reports*, I have only acted as author or as reviewer, thus, to act as editorial board member was a role I was interested in exploring.

4. What do you like most about being a board member for *Scientific Reports*?

Being a board member for *Scientific Reports* allows me to be updated in the ongoing research in my areas of expertise and to really deepen in the technical aspects of the manuscripts I handle. On top of that, the most positive aspect of being a board member is how much I learn from the interaction with the reviewers and the authors and how manuscripts improve from it.

5. You are leading one of our Guest Edited Collections. What interested you about becoming a Guest Editor? What is your Collection focused on?

Yes, I am leading the Special Collection entitled "[Extracellular vesicles in cell biology and medicine](#)". The collection is focused on extracellular vesicles (EV), cell-derived membranous structures known as intercellular communicators exerting their function by exchanging their cargo. EV research is a burgeoning field with a high number of researchers from different disciplines working in this field. This Special Collection intends to deliver an up-to-date overview on some of the current developments in the field. To increase my interaction with other researchers of the field is what most interested me about becoming a Guest Editor for the Collection.

6. Which is your favourite *Scientific Reports* paper?

It is really difficult to select one single paper! I will list you some:

[Cells release subpopulations of exosomes with distinct molecular and biological properties.](#)

Willms, E.; Johansson, H. J.; Mäger, I.; Lee, Y.; Blomberg, K. E. M.; Sadik, M.; Alaarg, A.; Smith, C. I. E.; Lehtiö, J.; El Andaloussi, S.; Wood, M. J. A.; Vader, P.

[Size-Exclusion Chromatography-based isolation minimally alters Extracellular Vesicles' characteristics compared to precipitating agents.](#)

Gámez-Valero, A.; Monguió-Tortajada, M.; Carreras-Planella, L.; Franquesa, M. I.; Beyer, K.; Borràs, F. E.

[Labeling Extracellular Vesicles for Nanoscale Flow Cytometry.](#)

Morales-Kastresana, A.; Telford, B.; Musich, T. A.; McKinnon, K.; Clayborne, C.; Braig, Z.;

Rosner, A.; Demberg, T.; Watson, D. C.; Karpova, T. S.; Freeman, G. J.; DeKruyff, R. H.; Pavlakis, G. N.; Terabe, M.; Robert-Guroff, M.; Berzofsky, J. A.; Jones, J. C.

Bone marrow stromal/stem cell-derived extracellular vesicles regulate osteoblast activity and differentiation *in vitro* and promote bone regeneration *in vivo*.

Qin, Y.; Wang, L.; Gao, Z.; Chen, G.; Zhang, C.

Exosomes derived from human adipose mesenchymal stem cells accelerates cutaneous wound healing via optimizing the characteristics of fibroblasts.

Hu, L.; Wang, J.; Zhou, X.; Xiong, Z.; Zhao, J.; Yu, R.; Huang, F.; Zhang, H.; Chen, L.

Professor Xiaochun Li



Professor Xiaochun Li is the Raytheon Endowed Chair in Manufacturing Engineering at the Departments of Mechanical and Aerospace Engineering & Materials Science and Engineering at UCLA, USA. He is also a Guest Editor for the [Nanotechnology enabled metallurgy Collection](#), which is currently welcoming submissions.

1. What has been your biggest challenge and your greatest achievement in your career so far?

The biggest challenge has been to bridge science and manufacturing for long term impact. The greatest achievement in my career so far is the discovery of the nano-particle self-dispersion and stabilisation mechanism in molten metals, which establishes a scientific foundation for nanotechnology enabled metallurgy.

2. Why did you decide to become a board member?

Being a board member is a good opportunity to provide a valuable service to the technical community.

3. What do you like most about being a board member for *Scientific Reports*?

My greatest pleasure is to help make the review process better, while having the opportunity to Guest Edit a special Collection.

4. You are leading one of our Guest Edited Collections. What interested you about becoming a Guest Editor? What is your Collection focused on?

I really like the opportunity to promote an important emerging field, Nanotechnology enabled metallurgy.

5. Which is your favourite *Scientific Reports* paper?

[Core-shell nanoparticle arrays double the strength of steel](#)

J.-B. Seol et al.

Professor Luciano Bosso



Professor Luciano Bosso is an Assistant Professor of Ecology at the University of Naples Federico II.

1. What is your current research focused on?

I am an ecologist expert in ecological modelling and GIS analysis. My main research interests include conservation biology, global change ecology, biogeography and invasion ecology and the application of species distribution models, niche analysis, risk mapping, conservation gap analysis, landscape ecology, spatial analysis, species connectivity and corridor network simulation.

2. What have been your biggest challenge and your greatest achievement in your career so far?

My biggest challenge is to make scientific research more accessible and comprehensible to a wider non-scientific audience. I am particularly devoted to increasing the ecological knowledge of non-experts. Several of my studies have informed policy makers about best management strategies of protected areas in Italy and Europe, and I consider this use of my research as my greatest achievement.

3. Why is *Scientific Reports* one of your favourite journals?

Scientific Reports is a multidisciplinary journal that publishes scientifically valid primary research from all areas of the natural sciences and beyond. I have always admired the quality and rigour of the scientific studies published in this prestigious journal.

4. Why did you decide to become a board member?

Considering the crucial role that editors have in scientific communication, I was looking for an opportunity where I can apply and especially improve/broaden my editorial skills as well as find professional and personal satisfaction both as an editor and a scientist. I am very grateful to *Scientific Reports* for this exciting opportunity.

5. What do you like most about being a board member for *Scientific Reports*?

I am particularly satisfied by the opportunity to enrich my background, collaborate in an international context, and contribute to the journal's high-quality publication standards.

6. Which is your favourite *Scientific Reports* paper?

It is really difficult to select a single paper! I was really impressed by these recent publications:

[Integrating experimental and distribution data to predict future species patterns.](#)
Kotta et al. (2019).

[Risk of biodiversity collapse under climate change in the Afro-Arabian region.](#)
Soultan et al. (2019).

[Climate change-driven range losses among bumblebee species are poised to accelerate.](#)
Sirois-Delisle and Kerr (2019).

[Modeling the distributions of tegu lizards in native and potential invasive ranges.](#)
Jarnevich et al. (2018).

[Assessment of the effect of climate changes in the Late Pleistocene and Holocene on niche conservatism of an arvicolid specialist.](#)
Castellanos-Frías et al. (2018).

[Can Niche Modeling and Geometric Morphometrics Document Competitive Exclusion in a Pair of Subterranean Rodents \(Genus *Ctenomys*\) with Tiny Parapatric Distributions?](#)
Kubiak et al. (2017).

Dr Ryoung Shin

Dr. Ryoung Shin is a Unit Leader at RIKEN Center for Sustainable Resource Science.

1. What is your current research focused on?

Potassium is one of major nutrients for plant growth, and lack of it in the soil environments has led to the increased use of fertilizers. However, such increased fertilizer usage does not necessarily result in a comparable production increase, and excess fertilizer run-off creates soil pollution. To address these issues, we elucidate the components of plant potassium sensing and deficiency signaling in plants using various approaches. In parallel, we are also using a marine red macroalgae *Pyropia yezoensis* in order to understand the mechanisms that enable seaweeds to survive in high salt conditions and to compare these mechanisms with those of the land plants in terms of Na^+/K^+ homeostasis. In addition, we identify and characterize chemicals which affect cesium and heavy metals uptake in plants to establish new methods of phytoremediation.

2. What has been your biggest challenge and your greatest achievement in your career so far?

My biggest challenge was starting the radiocesium phytoremediation research after the accident of Fukushima nuclear power plants following the big earthquake in Japan. It was a very challenging project (see [here](#) and [here](#)); and we are still actively working on this. One of my greatest achievements was that I have found and proven for the first time that [Reactive Oxygen Species \(ROS\) is a key signal molecule of potassium-deficient signal transduction in plant roots](#).

3. Why did you decide to become a Board Member?

When I got the offer of being a Board Member, I recalled the time when we published two papers in *Scientific Reports*. It was an interesting and unique experience. *Scientific Reports* publishes articles from a variety of scientific backgrounds, including interdisciplinary research. Additionally, the editorial criteria for decision are different from other journals. I wanted to know more about this, and to be involved in *Scientific Reports* as a Board Member.

4. What do you like most about being a Board Member for *Scientific Reports*?

When I review the manuscript as a reviewer or handle the manuscripts as an editor, the manuscripts are usually from the specialised fields. At *Scientific Reports* I have really

enjoyed reading the interdisciplinary manuscripts. These are not easy because I should know many different fields to handle these manuscripts, but handling these papers gives me opportunity to learn about the various aspects and I can then see and appreciate the different angles.

5. Which is your favourite *Scientific Reports* paper?

One of my favourite *Scientific Reports* paper is "[The antifungal plant defensin AtPDF2.3 from *Arabidopsis thaliana* blocks potassium channels](#)" by Virens et al. Previously we found the link between Jasmonic acid signalling, including PDF genes and potassium/cesium signalling. This particular paper provides much more detailed insights into the relationship between PDFs and potassium channels. I really enjoyed reading this article. Recently, I have also had a pleasure of reading and handling a later published article "[Effects of green seaweed extract on *Arabidopsis* early development suggest roles for hormone signalling in plant response to algal fertilisers](#)" by Ghaderiardakani et al. This one was well balanced between the practical and basic scientific approaches using seaweeds. Ideas are attractive and the logical flow through was clear.

Dr. Alberto G. Fairén

Alberto G. Fairén is an Astrobiologist at the Centro de Astrobiología in Madrid, Spain, a Visiting Scientist at the Department of Astronomy, Cornell University, and Editorial Board Member for *Scientific Reports*.



1. What is your current research focused on?

My research is focused on helping to understand the nature of the early Martian environments using a variety of tools from different disciplines: geomorphology, sedimentology, paleohydrology, geochemistry, aqueous mineralogy, and environmental microbiology.

My research interests in Martian science are broad, spanning from surface evolution and habitability, to robotic exploration and mission design and operation. My research activities include a combination of theoretical modeling, laboratory experiments, field work and spacecraft data analysis.

2. What has been your biggest challenge and your greatest achievement in your career so far?

The biggest challenge has been keeping focus while moving among institutions, countries and continents, during my postdoc years. As soon as I got my PhD in Spain, I moved to NASA Ames in California for my postdoc, and later I worked for the SETI Institute also in California, and Cornell University in New York. After 8 years in the US, I returned to my home country and joined Centro de Astrobiología, where I have recently got tenure. The journey has been challenging, but it also had its rewards, because the interaction with a wide variety of colleagues with different expertise and backgrounds allowed me to significantly grow in my career, and to reach my greatest achievements in publications, grants and awards.

3. Why did you decide to become a board member?

One of the most important tasks for a scientist is communicating the results of your investigations to the community. We, as researchers, should help to disseminate the scientific outcomes of our work. These responsibilities are essential for authors and reviewers indeed, but also for editors contributing to achieve high standards in publication quality, ethics, and fairness.

4. What do you like most about being a board member for *Scientific Reports*?

Being a board member for *Scientific Reports* is a good opportunity to provide a valuable service to the Mars community, helping to improve the review process facilitating the interactions between authors and reviewers, while contributing to *Scientific Reports*'s high-quality publication standards. In addition, the only requirement for the publication of a paper in *Scientific Reports* is that they must be "technically correct original contributions", and they don't need to have a subjective significance as perceived by the editors, which may very well be erroneous and most likely biased.

5. Which is your favourite *Scientific Reports* paper?

It is very difficult to single one paper out, but I was really impressed by "[Perchlorates on Mars enhance the bacteriocidal effects of UV light](#)", by Wadsworth and Cockell, because of its significant implications to our current search for life on Mars. In addition, I recently had the pleasure of handling "[The fate of lipid biosignatures in a Mars-analogue sulfur stream](#)", by Tan et al., "[A record of igneous evolution in Elysium, a major martian volcanic](#)

province”, by Susko et al., and “[Seasonal deposition and lifting of dust on Mars as observed by the Curiosity Rover](#)”, by Vicente-Retortillo et al., three very impressive papers dealing with different aspects of Mars exploration.

Professor Xuyang Lu



Professor Xuyang Lu is an Associate Professor at the Institute of Mountain Hazards and Environment at the Chinese Academy of Sciences. He is an Editorial Board Member for Scientific Reports and is currently Guest Editing a Collection on [Mountain surface processes and regulation](#), which is currently welcoming submissions until the end of September 2019.

1. What is your current research focused on?

My academic research interests focus on the biogeochemical cycle and its microbial mechanism in mountain ecosystems. My recent work focuses on the effect of litter and soil organic matter chemistry on soil N transformation and its microbial function molecular ecological networks by comparing the China (Tibetan Plateau) and U.S. (Rocky Mountains) alpine ecosystems.

2. What has been your biggest challenge and your greatest achievement in your career so far?

I think my biggest challenge is the collection of soil and plant samples from alpine ecosystems, especially in the Tibetan Plateau. The plateau is considered to be the third “pole” of the world with an average elevation of 4 km above sea level. The natural environment is extremely harsh which characterized by high altitude, strong solar radiation, low temperatures and thin air. In this extreme environment, human survival is a challenge in itself, not to mention taking samples. In present, the greatest achievement in my career was that I discovered the influence extent and pattern of litter chemical composition and diversity on soil C, N transformation, which the related researches have

been published in *Scientific Reports*, *Soil Biology & Biochemistry*, *Biology and Fertility of Soils*.

3. Why did you decide to become a board member?

The first motivation is to learn how to become a qualified editor the academic journals. I feel a sense of accomplishment when a paper could reach the high-quality publication standard for *Scientific Reports* through the process of peer review and feedback with the authors.

4. What do you like most about being a board member for *Scientific Reports*?

I particularly like the criteria for publication in *Scientific Reports*, assessing an original article on the basis that they are technically sound and scientifically valid rather than innovation and importance, which provides the opportunity for more research works to be published. In addition, the Guest Edited Collection "[Mountain surface processes and regulation](#)", which I served as Guest Editor, supplies a high-quality publication platform for interdisciplinary studies of mountain surface processes and their responses to climate change and human activities. I sincerely appreciate *Scientific Reports* for this exciting opportunity.

5. Which is your favourite *Scientific Reports* paper?

Here I list three of my favourite *Scientific Reports* papers:

["A transnational perspective of global and regional ecosystem service flows from and to mountain regions"](#), by Schirpke et al. (Article number: 6678, 2019).

["Altitudinal, temporal and trophic partitioning of flower-visitors in Alpine communities"](#), by Lefebvre et al. (Article number: 4706, 2018).

["Planting increases the abundance and structure complexity of soil core functional genes relevant to carbon and nitrogen cycling"](#), by Wang et al. (Article number: 14345, 2015).

Professor Matjaž Perc

Matjaž Perc is a Professor of Physics at the University of Maribor, Slovenia and Editorial Board Member for Scientific Reports.

1. What is your current research focused on?



Climate inaction, the overexploitation of natural resources, and a harrowing gap between rich and poor are some of the most pressing issues of our time. Since these problems can't even be fully understood, let alone solved, from a perspective of a single scientific discipline, the future prospects of our societies will be determined by interdisciplinary and cross disciplinary research that cuts across different domains of science. We are working hard to make physics, particularly methods of statistical physics, together with network and data science, a key piece of this puzzle. Social physics enables us to theoretically describe and understand collective social phenomena that are due to the interactions among individuals, groups, and governments. From the responsible use of antibiotics and vaccination, to the mitigation of social crisis and inequalities, we aim to develop better social systems and more efficient policies for a sustainable and better future by synergizing physics with the social sciences.

2. What has been your biggest challenge and your greatest achievement in your career so far?

One of my career goals has always been to stay at my home university, and ultimately to get a permanent position there. This has turned out to be the biggest challenge, as well as the greatest achievement of my career. Slovenia is a small country. Research funding is scarce, and vacant tenure track positions are basically nonexistent. A consistently impressive research output is needed, along with some luck and sponsorship from senior colleagues, to finally make it. For me it worked out great, but for many talented researchers in Slovenia this reality means being forced to go work abroad.

3. Why did you decide to become a board member?

Being a *Scientific Reports* board member is for me a way to give back to the research community, and to promote and support the research field in which I am active. *Scientific Reports* always stood for high quality, but also for freedom and opportunity, which together with the open access policy make it a perfect outlet for the most innovative research. Such research is often difficult to publish in journals that judge impact and novelty because it often challenges the mainstream in science.

4. What do you like most about being a board member for *Scientific Reports*?

The role puts you right in the center of research that is close to your heart. It feels a little bit like having reserved the finest seats in the theater of science, getting to learn about fascinating new discoveries before they are officially published. I also very much enjoy good cover letters, where authors usually explain the gist of their research in simpler terms, discussing its broader implications, and pondering about potential impact and directions for the future.

5. Which is your favourite *Scientific Reports* paper?

My favorite *Scientific Reports* paper remains to be the first paper I have handled after joining the editorial board, titled "[Flavor network and the principles of food pairing](#)" published in 2011. The paper generated a lot of attention at the time, both in the scientific community and in the media, and it stands precisely for the type of avant-garde research I was hoping *Scientific Reports* would encourage. In this particular case opening new avenues towards a systematic understanding of culinary practice across the world. From my own publications in *Scientific Reports* my favorite is "[Self-organization of progress across the century of physics](#)" published in 2013, for its collaboration with the American Physical Society and the resulting n-gram viewer for physics that is akin to Google's version for books.

Professor Yu Xin Zhang



Professor Yu Xin Zhang is a Professor at the College of Materials Science and Engineering, Chongqing University, China. He is also a Scientific Reports Editorial Board Member and a Guest Editor for the [Nanomaterials for energy and environmental applications Collection](#).

1. What is your current research focused on?

My current research is mainly focused on the self-assembly of nanostructures with various morphologies, involving MnO_2 and diatomite-based nanocomposites, and their applications in energy storage and environmental fields.

2. What has been your biggest challenge and your greatest achievement in your career so far?

In my career, the biggest challenge was teaching Chinese students Technical Writing using English, which is my second language, while striving in materials science and engineering in Chongqing University. Indeed, it is highly important that teachers keep an eye on the learning process and abilities of students. Fortunately, my advisor, Prof. Hua Chun Zeng gave me a strict supervision in National University of Singapore, molding me to be a well-trained researcher and independent lecturer. In addition to being the biggest challenge in my career, teaching this course is also my greatest achievement. More than 5000 students who have had a direct or indirect contact with this course through me, have been able to improve their writing ability and some of them have even published their first article, which is a great motivation and confidence booster in scientific research, and nudged them to embark on the path of scientific research. I take pride in this.

3. Why did you decide to become a board member?

Becoming a board member is not only an endorsement of oneself, but also a means of staying abreast of the latest developments in scientific research, and actively communicating with international counterparts. Being a board member avails me these opportunities.

4. What do you like most about being a board member for *Scientific Reports*?

Scientific Reports is an outstanding journal, which publishes high quality articles with enormous future development prospects. I enjoy being able to make profound suggestions for the development of the journal, accepting excellent articles, and being able to learn substantially, even while doing something I love, and this is possible for me as a board member for *Scientific reports*. I like the rate at which the journal is developing in all aspects, it gets better and better.

Professor Z. Altounian



Professor Z. Altounian is a Professor in the Physics Department at McGill University, Montreal, Canada and Editorial Board Member for Scientific Reports.

1. What is your current research focused on?

My current research is mainly focused on studies of magnetic properties of materials.

2. What has been your biggest challenge and your greatest achievement in your career so far?

Challenges always occur if one enters a new field of study. My first challenge started in the winter of 1979 when I got interested in metallic glasses. This was also my greatest achievement as our group at McGill University became a world famous centre in this field. I still contribute to this field, but mainly through editorial/reviewing responsibilities for journals and conferences. My second, and more recent challenge occurred when I got involved in the studies of magnetic properties of hard magnetic materials. It is worth to mention that there was a very close relationship between my two challenges as we used the technique of rapid solidification, which is commonly used to prepare metallic glasses, to manufacture rare-earth based magnets. This also brought us to worldwide recognition in this particular field.

3. Why did you decide to become a board member?

One of the aspects that drew me to become an EBM was the problems that I had encountered while reviewing manuscripts. Mostly the poor quality of some of the reviewers which in many cases did not agree on the evaluation of the same submission. In those cases, I was asked to make the final decision. As an EBM, I hoped to improve the reviewing protocol.

4. What do you like most about being a board member for *Scientific Reports*?

Prior to becoming an EBM, I was a very active reviewer for a number of publications in the above-mentioned fields. When *Scientific Reports* invited me to join their editorial team, I accepted the offer as I had always enjoyed reviewing journal submissions.

5. Which is your favourite *Scientific Reports* paper?

My favorite paper is SR 7, 646 (2017), "[The magnetic and crystal structure of \$Mn_xGa\$ \(\$1.15 \leq x \leq 1.8\$ \) Alloys](#)". This was our first publication in *Scientific Reports* as well as the first introduction to this fascinating material. Even-though we have been studying magnetic materials for more than two decades, this publication finally put us on the world map in this field.

Dr Lena Ciric



Lena Ciric is Associate Professor in Environmental Engineering at the Department of Civil, Environmental and Geomatic Engineering of University College London, UK. She is also a Scientific Reports Editorial Board Member and a Guest Editor for the [Microbes in the built environment Collection](#).

1. What is your current research focused on?

I am an environmental microbiologist. The environment I study is the built environment. My group works on two themes. The first is how microbes behave in the built environment. We have been studying the microbial communities and the horizontal gene transfer of antibiotic resistance genes in slow sand filtration, a commonly used drinking water treatment process. The other theme we study is how we can use engineering solutions to reduce the numbers of harmful microbes in our environment. We have been manufacturing [water and air filters with antimicrobial nanoparticles](#) embedded in them which are capable of killing bacterial and viral pathogens.

2. What has been your biggest challenge and your greatest achievement in your career so far?

I would say being an academic is both the biggest challenge and the biggest achievement of my career. The job can be extremely challenging as there are huge demands on your time. There is frequent failure – rejected grant applications, experiments that don't work, papers that are difficult to publish. But these challenges are also what motivates me. The job of an academic is so varied. We have the freedom to pursue our research ideas, we get to write, we get to read, collaborate, travel, interact with young people through teaching and speak to the public about our work. All of these experiences are extremely rewarding.

3. Why did you decide to become a board member?

I am naturally nosy, so I was curious to see what research is going on out there. As an editorial member you have the opportunity to reach out to experts in the field to help you scrutinise manuscripts and help the authors improve their work.

4. What do you like most about being a board member for *Scientific Reports*?

Scientific Reports has a novel approach. Instead of looking for the next big thing, it is happy to publish any scientific work that is technically sound. This is extremely useful to the scientific community. It means that we have a less biased view of the research going on around the globe.

5. Which is your favourite *Scientific Reports* paper?

[Patterns in the skin microbiota differ in children and teenagers between rural and urban environments](#)

I am fascinated by how the microbes that live on and in our bodies shape us physically and, some studies say, psychologically. I think it's wonderful to know that our body as an ecosystem rather than just one organism.

Professor Kah-Wee Ang



Prof. Kah-Wee Ang is Associate Professor in the Department of Electrical and Computer Engineering at the National University of Singapore.

1. What is your current research focused on?

I am an experimentalist and my current research focuses on the development of both electronic and photonic devices based on two-dimensional (2D) materials, which include field-effect transistor and integrated circuit, non-volatile memory, artificial intelligence synapse for neuro-inspired computing, as well as electro-optic modulator and photodetector for mid-infrared sensing applications.

2. What has been your biggest challenge and your greatest achievement in your career so far?

I started off my research career exploring the use of beneficial strain effect to modify the carrier transport properties in the conduction channel for enhancing the mobility and drive current performance of nanoscale field-effect transistors (FETs). Specifically, I pioneered a novel concept in employing lattice-mismatched stressor made of silicon-carbon (Si:C) alloy in the source and drain regions of metal-oxide-semiconductor FETs,

which was widely evaluated by major semiconductor foundries worldwide. The technology was eventually adopted in high volume manufacturing, which gave me a sense of satisfaction and achievement.

Since five years ago, I started investigating two-dimensional materials for electronic and photonic device applications. As a new comer to this emerging field, many technical challenges were encountered which spanned across material synthesis, device fabrication to large-scale circuit and system integration. Through conscientious efforts, several advancements have been made in addressing module and process integration issues on flexible substrates, which allow energy-efficient logic gates, memories and sensors to be realized. Going forward, we aim to demonstrate a transformative 3D monolithic system via van der Waals integration of new logic and memory devices built on atomic-scale 2D materials, which can deliver a remarkable improvement in computational energy efficiency over traditional system architecture.

3. Why did you decide to become a board member?

Today the research landscape is dynamically changing where new exciting fields are emerging rapidly. Being an editorial board member allows me to not just keep up with the latest progress made in my field, but also gives me the opportunity to keep abreast of the new development that takes place across multidisciplinary fields. Getting to know the experts often helps me in assigning appropriate reviewers to assess the submitted manuscripts, and make constructive recommendations to improve the quality.

4. What do you like most about being a board member for *Scientific Reports*?

Scientific Reports' approach in publishing technically sound work and let the wider scientific community determine the impact of the work is well received by like-minded researchers including myself. This allows board member to avoid making subjective decisions on rejecting manuscripts which are perceived with little immediate interests but may grow in importance over time.

Dr Pedro Oliveira



Dr Pedro Oliveira is a Senior Scientist at Mount Sinai School of Medicine, USA.

1. What is your current research focused on?

My current interests are on identifying emerging challenges in the fast-evolving research field of epigenomics, and on better understanding the epigenetic regulation mechanisms affecting gene expression and cellular processes in Bacteria. On a broader perspective, I seek to elucidate key biological insights that can translate into more accurate disease diagnosis and more effective treatment for certain bacterial pathogens.

2. What has been your biggest challenge and your greatest achievement in your career so far?

My biggest challenge has been in embracing some of the downsides of a career in academia, such as frequent failure and rejection, and being able to transform such missteps and roadblocks into an opportunity for something new. My greatest achievement as a researcher has been in help propelling the field of bacterial epigenomics. Such field has been attracting increased attention for its exciting potential to transform our knowledge on gene regulation, virulence, and adaptation. As an example, we recently performed the first large-scale comprehensive epigenomic analysis in *Clostridioides difficile*, known to be one of the leading causes of nosocomial antibiotic-associated disease in the developed world. We found evidence for epigenetic regulation associated with sporulation (a key step in *C. difficile* disease transmission), cell length, biofilm formation, and host colonization. For more information, please check: Oliveira *et al.* [Epigenomic characterization of *Clostridioides difficile* finds a conserved DNA methyltransferase that mediates sporulation and pathogenesis](#). *Nature Microbiology*.

3. Why did you decide to become a board member?

Essentially, it was a great opportunity to become an active part of the decision-making process, while simultaneously helping the authors to achieve a higher quality standard in their publications. I am very thankful to *Scientific Reports* for this opportunity.

4. What do you like most about being a board member for *Scientific Reports*?

I particularly enjoy contributing to a journal that seeks high quality standards in its publications, focusing primarily on research robustness and validity, and less on subjective

editorial decisions. In this sense, *Scientific Reports* makes a distinctive contribution when compared with other journals. Furthermore, being a Board Member has given me the opportunity to learn and keep myself up to date on the latest research in my areas of expertise, while simultaneously delving into the multiple aspects of the manuscripts I handle.

5. Which is your favourite *Scientific Reports* paper?

It's difficult to highlight one single paper. I particularly enjoyed reading a recent publication describing a [portable epigenetic switch for bistable gene expression in bacteria](#) based on the *opvAB* operon of *Salmonella enterica*.

Dr Ciria C. Hernandez



Dr. Ciria C. Hernandez is an Assistant Research Scientist at the Life Sciences Institute, University of Michigan, USA. She is an Editorial Board Member for Scientific Reports and currently Guest Editing a Collection on [Channelopathies](#), which is welcoming submissions on a rolling basis.

1. What is your current research focused on?

The focus of my research has always been to understand the fine regulation of ion channels in physiological and pathological states. Thus, I have been fully immersed in the study of the molecular determinants of the interaction of small peptides, G-protein coupled receptors (GPCRs) and both voltage and ligand gated ion channels, whether they are defective or not, by carrying mutations in their structure that prevents them from normal function. Most recently, I have been working on the elucidation of the structural determinants of the interaction of GPCRs that mediate a variety of physiological processes critical for energy homeostasis, and potassium channels known to be involved in a novel G protein independent signaling pathway in a brain region that controls hunger. We are tackling the idea of bias agonism as a therapeutic approach to overcome defective GPCR-channel coupling in conditions where the rheostats of energy homeostasis are defective.

2. What has been your biggest challenge and your greatest achievement in your career so far?

My biggest challenge and my greatest achievement merge at the same point. Be competitive and remain relevant in the face of the unstoppable and continuing flow of new technologies. While this is true for any electrophysiologist, as a woman, coming from a developing country, there is a bigger challenge considering the general perception that you have about making a career in science, and still being able to respond to the demands of academia. Scientifically perhaps the biggest challenge is to understand how to fit every discovery I make, however small and trivial it may seem, in the greater puzzle we are helping to complete. We merge these pieces when we write the discoveries we made in the scientific articles, when we guide the youngest, and when we collaborate with peers from different fields. This is at the essence of science and my career has fundamentally been learning how to “put the pieces together” ever since I started my career. Although I have managed to break many barriers and be in a privileged position, I have not stopped learning and growing scientifically, and honestly, I will never stop doing it. This is my greatest achievement as a scientist.

3. Why did you decide to become a board member?

Being part of the editorial board of *Scientific Reports* implies transforming and transferring knowledge beyond critical thinking. We have to be not only the best scientists, but the best communicators. Being a board member of *Scientific Reports* made me realize how to communicate the passion that we scientists have for discovering the smallest molecular interactions and chats between receptors, channels, proteins, and bring them to public light. My passive voice became a strong megaphone through the publications of all the authors who contribute to making the best science in the world. By realizing that through my contribution, I am shaping the science that is read and at the same time inspiring me to be the best scientific communicator.

4. What do you like most about being a board member for *Scientific Reports*?

Each manuscript submitted is a challenge of new knowledge and unexpected creativity. The diversity of topics within our area of expertise is tremendous. It shows the high quality of science that is translated from the bench and witnessing that is unmatched and novel in many ways. At the same time, we are the role models for the challenges imposed in the current era, where the disparity of world economies challenges science in the quest for new discoveries.

5. Which is your favourite *Scientific Reports* paper?

Without reservations I invite you to read part of my work published in 2017 describing the [defects on GABAA receptor function caused by deleterious mutations in cases with catastrophic epileptic encephalopathies](#), and many other fascinating studies on the special Collection dedicated to [Channelopathies](#).

Professor Claudia RC Moreno



Prof. Claudia RC Moreno is Head of Department at the School of Public Health, University of Sao Paulo, Brazil and is currently an Editorial Board Member for Scientific Reports.

1. What is your current research focused on?

The process of urbanization, as well as the use of electricity and its technology, has changed the way of life of society. The possibility of extending the waking period through the use of electric light changed working, sleeping, eating and leisure hours. I have been studying communities with and without access to electricity with the focus on lifestyle, working hours, sleep and eating behavior.

2. What has been your biggest challenge and your greatest achievement in your career so far?

The biggest challenge was to run a study in the Amazon forest, collecting data from rubber tappers and having to freeze saliva samples to send to Sao Paulo, many kilometers away. It is hard to say my greatest achievement, but there are two things that I would like to highlight, first, the success of my former PhD students, and second, my participation on a monograph at the IARC-WHO regarding night shift work and cancer.

3. Why did you decide to become a board member?

Scientific Reports is a quite interesting journal due to its policy to evaluate papers technically sound. Moreover, I think it is important to have board members from Latin America.

4. What do you like most about being a board member for *Scientific Reports*?

It is a very dynamic task, and help me to keep myself updated.

5. Which is your favourite *Scientific Reports* paper?

My favorite paper is from an international group of researchers with focus on sleep in a rural cohort study. [Timing and quality of sleep in a rural Brazilian family-based cohort, the Baependi Heart Study](#)

Professor Jimin Zhao



Professor Jimin Zhao currently works in the Institute of Physics at the Chinese Academy of Sciences (CAS), Beijing, and is an Editorial Board Member for Scientific Reports.

1. What is your current research focused on?

I currently work on ultrafast spectroscopy and ultrafast dynamics of correlated quantum materials.

2. What has been your biggest challenge and your greatest achievement in your career so far?

The biggest challenge in my career so far is to convince my colleagues outside of my specific area that what I have done is reliable. This is especially challenging when our manuscripts are being reviewed, whereby very simple questions and unreasonable doubts come in by renowned yet non-experts in the field. I have investigated the ultrafast dynamics in novel iron-based superconductors and Weyl semimetals, I have also achieved SSPM and all-optical switching in 2D materials. I am currently a full professor at IOP-CAS and I am also an OSA senior member.

3. Why did you decide to become a board member?

Being a board member will open a new venue that I can do service to the community. Optical spectroscopy of solids is not a big area, and I feel my colleagues may need me to objectively judge on works using sophisticated optical means to investigate condensed matter physics.

4. What do you like most about being a board member for *Scientific Reports*?

Handling works in slightly different areas is interesting. My own experience can make a decisive contribution, whereas I can also enlarge my horizon through the handling process.

5. Which is your favourite *Scientific Reports* paper?

There are many. I remember one very good theoretical paper called [Anomalous isotope effect in iron-based superconductors](#). I should say another one is one of our works. Out of the 6 papers I have published in *Scientific Reports*, our work [Coherent Generation of Photo-Thermo-Acoustic Wave from Graphene Sheets](#), *Scientific Reports* 5, 10582 (2015) is my most favorite one. This work is highlighted in Chinese magazine *Physics*. It is a phenomenon parallel to photoluminescence. After we publish, I got to know that Alexander Graham Bell also worked on this topic and had filed a patent, and he thought it was one of his greatest achievements. Dr. Bell was a great scientist, and knowing this is wonderful. Nonetheless, before us, there are no serious and solid experimental proofs that cleanly removed other very possible mechanisms. We have also achieved remote coherent control of acoustic sound.

Professor Amy Peterson



Professor Amy Peterson is Associate Professor of Plastics Engineering at the University of Massachusetts Lowell, USA and is an Editorial Board Member for Scientific Reports.

1. What is your current research focused on?

My current research is focused on multilayered polymer structures – these range from nanometer-scale polyelectrolyte multilayers for cell culture coatings, to packaging materials, to additively manufactured structures. As engineers, we use experimental and computational approaches to probe what happens at interfaces and surfaces in these systems, and use this knowledge to guide creation/selection of improved materials and processes.

2. What has been your biggest challenge and your greatest achievement in your career so far?

This past week has been the most intensely challenging of my career. It ended with shutting down our laboratory on Saturday, but the lead up to that final act included figuring out what critical experiments were needed for each group member, attempting to get them approved as access to labs became increasingly restricted by my university, trying to keep my group up to date on the status of their possible experimental work, and doing all of this in a dynamic situation via phone, email, and videoconference. I feel fortunate because we have substantial modelling efforts as well as experimental ones, so we'll be able to continue to make progress on our research. However, my group meets weekly in person for group as well as one-on-one meetings, and I am concerned about how we will all fare being isolated in our homes.

My proudest moments are when I see my students succeed, whether it's at graduate, a defense, an award, a paper, or a presentation.

3. Why did you decide to become a board member?

Being a board member gives me an opportunity to give back to my broader research community and learn at the same time.

4. What do you like most about being a board member for *Scientific Reports*?

The same reasons I became a board member.

5. Which is your favourite *Scientific Reports* paper?

[Clogging transition of many-particle systems flowing through bottlenecks](#) – I like how it makes connections between systems of large numbers of people, flocks of sheep, and colloidal suspensions.

Dr Jagadeesh Bayry

Dr. Jagadeesh Bayry is Director of Research at the Institut National de la Santé et de la Recherche Médicale, Centre de Recherche des Cordeliers, Paris, France and is an Editorial Board Member for Scientific Reports.



1. What is your current research focused on?

Our current research is focused on the mechanisms by which regulatory T cells and circulating immunoglobulins maintain immune homeostasis and deciphering the host-pathogen interaction.

2. What has been your biggest challenge and your greatest achievement in your career so far?

Though we identify mechanisms of various biological processes in the experimental systems, translation of these results to humans is always challenging. For that matter, I am proud of many achievements of my team and myself that include:

- Demonstration that regulatory T cells in contrast to central dogma on them as universal immunosuppressor cells, induce activation of human basophils (Science Immunology 2018. doi: 10.1126/sciimmunol.aan0829)
- Demonstration that surface hydrophobin protein layer prevents immune recognition of airborne fungal spores (Nature 2009. doi: 10.1038/nature08264)
- Demonstration of molecular mechanisms by which therapeutic normal immunoglobulin G expands regulatory T cells (Blood 2013. doi: 10.1182/blood-2012-11-468264) and induces induction of IL-4 in human basophils (J Allergy Clin Immunology 2019. doi: 10.1016/j.jaci.2018.10.064)
- Identification and validation of small molecule antagonists to CCR4 that function as molecular adjuvants in vaccination by transiently inhibiting the migration of regulatory T cells (Proc Natl Acad Sci USA 2008. doi: 10.1073/pnas.0803453105)
- Activation signal-dependent regulation of human dendritic cell functions by B lymphocytes (Nature Communications 2014. doi: 10.1038/ncomms5092)

3. Why did you decide to become a board member?

For me, it is a service to scientific community. Though it demands my time, I feel that it is a highly privileged position. In addition, it gives visibility to myself, team and institute.

4. What do you like most about being a board member for *Scientific Reports*?

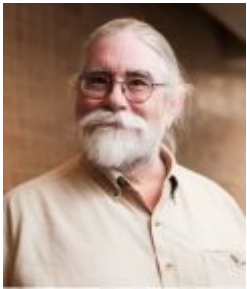
'Open Access' journals have been misinterpreted by some people as 'journals that can publish anything'. As an editorial board member, I ensure that low-quality studies are not published in Scientific Reports. One should not forget that currently there is huge demand

for external reviewers. Therefore, being a board member of journal, I can allow only original and technically sound articles for peer review. It will reduce burden on the reviewers.

5. Which is your favourite *Scientific Reports* paper?

It is difficult to pick a single paper. I have many favourite *Scientific Reports* papers.

Professor Timothy Geary



Prof. Timothy Geary is a Professor in the Institute of Parasitology in the Faculty of Agricultural and Environmental Sciences at McGill and is an Editorial Board Member for Scientific Reports.

1. What is your current research focused on?

I do research in two areas: the pharmacology of antiparasitic drugs (including drug target identification, mechanisms of action and resistance, and development), and understanding the molecular language of the host-parasite interface, with an emphasis on nematodes. The primary focus of this work is the identification and characterization of parasite-derived proteins, metabolites and miRNAs that have immunomodulatory activity in the mammalian host, enabling the establishment of chronic infections.

2. What has been your biggest challenge and your greatest achievement in your career so far?

It's always hard to rank an event as #1 in these areas. Certainly, a major challenge was transitioning from a 20-year career in the pharmaceutical industry to a 15-year career in academia at McGill and now also QUB. The switch was made possible by the Canada Research Chairs programme, which enabled me to get started at McGill, and by support from the Bill and Melinda Gates Foundation, which enabled me to use some of the technology I had developed in the industry to conduct an innovative drug discovery program with partners in Africa. The greatest achievement may have been research that provided a plausible explanation for how the anthelmintic ivermectin removes larval stages of filariid nematodes (microfilariae) from hosts, a key action that makes the drug of value in filariasis control programmes. In another sense, my greatest achievement has

been to work with enormously talented colleagues around the world in fascinating areas of research.

3. Why did you decide to become a board member?

It is an honour to be asked to contribute to the success of one of the Nature family of journals, and I was flattered to be invited. I am on the editorial boards of a number of journals in parasitology, and am glad to serve in that capacity, but *Scientific Reports* addresses broader topics; I wanted to make sure that parasite-related manuscripts submitted here would be fairly and rigorously evaluated.

4. What do you like most about being a board member for *Scientific Reports*?

I enjoy the breadth of manuscripts sent to me to consider as board member. Also – and importantly – the staff assistance is exceptional and makes the tasks associated with securing reviews much easier. It is a pleasure to work with them.

5. Which is your favourite *Scientific Reports* paper?

This I cannot answer....there have been too many to rank. I am confident that they have all been valuable additions to the literature.

Dr Leyla Soleymani



Dr. Leyla Soleymani is a Professor and Canada Research Chair at McMaster University and an Editorial Board Member for Scientific Reports.

1. What is your current research focused on?

My current research is focused on creating new hierarchical materials and applying these to biosensors and antimicrobial surfaces.

2. What has been your biggest challenge and your greatest achievement in your career so far?

I guess my greatest achievements and greatest challenges are related. I have found it challenging to balance the needs of an industry relevant product with the required novelty

for successful scientific publication. Some of my proudest achievements have been on technology development activities that are in the process of being translated to knowledge users.

3. Why did you decide to become a board member?

I highly respect the peer-review process and I find it critically important for advancing science. Being an editorial board member at *Scientific Reports* allows me to take part in overseeing the peer review process and work in ensuring that a fair and transparent process is followed, to get the first scoop on new and exciting papers, and to contribute to the publication of high quality papers, not just as an author or a peer reviewer but also as an editorial board member.

4. What do you like most about being a board member for *Scientific Reports*?

I get to read new papers in more diverse areas that I would normally read, which gives me inspiration and insight.

5. Which is your favourite *Scientific Reports* paper?

It is tough to choose an all time favourite, but recently I came across this paper "[Logistic growth of a surface contamination network and its role in disease spread](#)" and found it eye-opening in the context of the COVID-19 pandemic.

Dr Ruth Blasco



Dr Ruth Blasco is Taphonomy researcher at Centro Nacional de Investigación sobre la Evolución Humana (CENIEH), Burgos, Spain and is an Editorial Board Member for Scientific Reports.

1. What is your current research focused on?

My current research explores human subsistence strategies during the Pleistocene using a taphonomic approach. I am trying to determine several aspects of human behaviour from the faunal record and contribute to evolutionary anthropology by developing several research sub-areas, such as butchery patterns and hominid–carnivore interactions.

2. What has been your biggest challenge and your greatest achievement in your career so far?

Documenting an intentional delayed consumption of some animal resources (specifically bone marrow from fallow deer metapodials) with implications that affect the subsistence systems of more than 300 thousand years ago.

Food storage is considered a "risk-reducing mechanism" designed to offset downturns in resource availability. The deliberate accumulation of some bones for later marrow consumption implies an anticipation of and concern for future needs. This fact marks a threshold for new modes of Palaeolithic adaptation because the foresight capacity surpasses the "here and now" as a means of subsistence. This study was published last year in *Science Advances* [Blasco, R., Rosell, J., Arilla, M., Margalida, A., Villalba, D., Gopher, A., Barkai, R., [Bone marrow storage and delayed consumption at Middle Pleistocene Qesem Cave, Israel \(420 to 200 ka\)](#). *Sci. Adv.* 5, eaav9822 (2019)].

3. Why did you decide to become a board member?

I think that the peer-review process is a fundamental tool for generating quality scientific production. Participating in this process not only as an author or a reviewer but also as an editorial board member allows me to ensure that these processes are objective and transparent and that rigorous, novel and exciting studies are released. I consider it to be a highly privileged position as well as a way of serving the scientific community.

4. What do you like most about being a board member for *Scientific Reports*?

Beyond reading papers from very diverse areas that continuously broaden my knowledge and inspire my future work, being a board member for *Scientific Reports* has allowed me to lead and handle a special collection on "Quaternary Taphonomy" that involves trans- to pluri-disciplinary approaches, such as archaeology, palaeontology, geology, biology and ecology, with a wide array of methodologies. The number of researchers working on taphonomy is steadily increasing, and their contributing to high-ranked scientific journals is becoming a regular trend. This collection is gathering some of the most relevant current studies within this field in the attempt to become a reference source for further studies.

5. Which is your favourite *Scientific Reports* paper?

It is difficult to choose a specific paper, but I will highlight "[Deep learning and taphonomy: high accuracy in the classification of cut marks made on fleshed and defleshed bones](#)

using convolutional neural networks”, which is a clear example of how the recent application of machine learning algorithms to the taphonomic discipline is achieving higher accuracy in the identification of bone surface modifications and the agents and/or processes that produce them.

Dr. Feng Gao



Dr. Feng Gao is a Professor in the Department of Physics, School of Science, at Tianjin University, Tianjin, China and an Editorial Board Member for Scientific Reports.

1. What is your current research focused on?

I am engaged in bioinformatics research, currently focusing on microbial replication origins.

2. What has been your biggest challenge and your greatest achievement in your career so far?

In the last semester, how to balance teaching, research, editorial activities, and family life has been my biggest challenge, especially during the COVID-19 pandemic. The greatest achievement in my career is the systematic prediction of microbial replication origins using bioinformatics methods, some of which have been confirmed experimentally. The related web server Ori-Finder and online database DoriC have been gradually recognized by researchers in this field.

3. Why did you decide to become a board member?

A Nature Research journal launched in 2011, *Scientific Reports* is really a rising star with an experienced Chief Editor and renowned Editorial Board Members, and an editorial ethos unlike those of other Nature Research journals. This attracted me to become a board member in 2013.

4. What do you like most about being a board member for *Scientific Reports*?

I really enjoy the process of handling manuscripts from all over the world, which report the latest findings from the scientific community. I pick qualified manuscripts using rapid,

high-quality peer review, and present them to the readers in a timely manner. It's an excellent experience to see that the handled manuscripts were well-received by a wide audience or highly cited, which gives me a great sense of achievement.

5. What is your favourite *Scientific Reports* paper?

My favourite *Scientific Reports* paper is: '[RASTtk: A modular and extensible implementation of the RAST algorithm for building custom annotation pipelines and annotating batches of genomes](#)', which has already been cited 570 times according to Web of Science. In fact, I often use the RAST server, which is a very popular genome annotation tool, to annotate our newly sequenced genomes. I was very glad to handle this manuscript and accept it for publication in *Scientific Reports* as quickly as possible.

Professor Kenji Kansaku



Professor Kenji Kansaku is a Professor and Chair at the Department of Physiology, Dokkyo Medical University School of Medicine, Japan. He is also an Editorial Board Member for Scientific Reports and Guest Editor for the 'Neuroprosthetics in systems neuroscience and medicine' Collection.

1. What is your current research focused on?

I am a Systems Neuroscientist. I began my career as a neurosurgeon and, therefore, my research interests include both understanding brain functions and applying neuroscientific knowledge to medical practice. I am more specifically focused on practical neuroprosthetics, as well as neural mechanisms of body image and sense of self.

2. What has been your biggest challenge and your greatest achievement in your career so far?

Over the past 15 years, I have mainly focused on the brain-machine interface, which is a neuroprosthetic for patients with neurological disorders. Five years ago, I co-edited a book entitled "Clinical Systems Neuroscience" (Springer), which gathered chapters authored by researchers from various fields, including systems neuroscience, rehabilitation, neurology, psychology, and engineering.

3. Why did you decide to become a board member?

I felt that it would be an opportunity to contribute to various research fields worldwide.

4. What do you like most about being a board member for *Scientific Reports*?

Scientific Reports is unique in that it accepts articles with the only requirements being that they should be technically sound original contributions, and there is no focus on perceived importance and significance. Although the strategy may make it difficult for the journal to set research trends, it provides fair opportunities for papers that would otherwise potentially remain hidden for various reasons.

5. You are leading one of our Guest Edited Collections. What interested you about becoming a Guest Editor? What is your Collection focused on?

I am editing the Guest Edited Collection: [Neuroprosthetics in Systems Neuroscience and Medicine](#). This Collection provides a platform for interdisciplinary research in neuroprosthetics, and gathers studies investigating medical applications of systems neuroscience, informatics, and engineering in the development of neuroprosthetic devices. As mentioned above, I edited a book 5 years ago entitled "Clinical Systems Neuroscience," in which all authors of the provided chapters were assigned by the editors. Here, the free submission style of the Collection has successfully gathered wonderful papers from various research fields. I would be happy for it to contribute to the development of a new special interest group worldwide.

Scientific Reports (*Sci Rep*) | ISSN 2045-2322 (online)

nature > scientific reports > research articles

Research articles

Article Type

Article (5496)

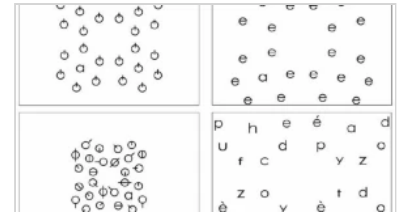
Year

2023 (5496)

Enhancing reading accuracy through visual search training using symbols

Audrey Vialatte, Pierre-Emmanuel Aguera ... Laure Pisella

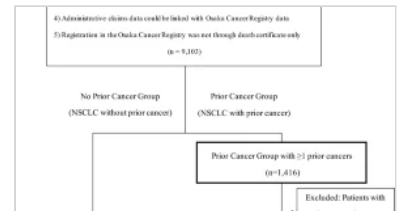
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Survival in non-small cell lung cancer patients with versus without prior cancer

Akira Sato, Toshitaka Morishima ... Isao Miyashiro

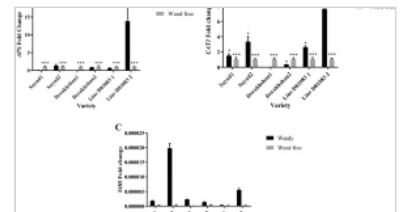
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Effect of redroot pigweed interference on antioxidant enzyme and light response of common bean (*Phaseolus vulgaris* L.) depends on cultivars and growth stages

Seyede Zahra Tabatabaiepour, Zahra Tahmasebi ... Sajad Rashidi-Monfared

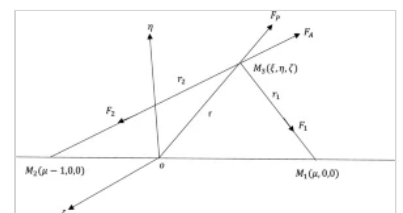
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Albedo effects in the ER3BP with an oblate primary, a triaxial secondary and a potential due to belt

Jagadish Singh & Tyokyaa K. Richard

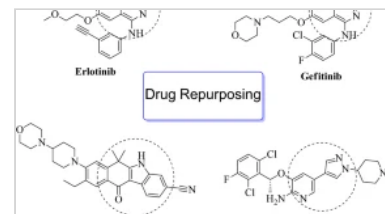
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Revisiting the inhibitory potential of protein kinase inhibitors against NEK7 protein via comprehensive computational investigations

Syeda Abida Ejaz, Mubashir Aziz ... Hanan A. Ogaly

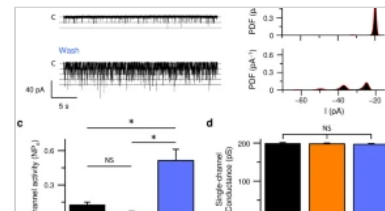
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Polycystic kidney disease 2-like 1 channel contributes to the bitter aftertaste perception of quinine

Takahiro Shimizu, Takuto Fujii ... Hideki Sakai

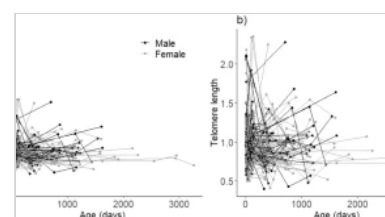
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Longitudinal telomere dynamics within natural lifespans of a wild bird

Michael Le Pepke, Thomas Kvalnes ... Thor Harald Ringsby

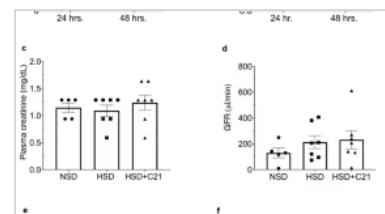
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Angiotensin II type 2 receptor activation preserves megalin in the kidney and prevents proteinuria in high salt diet fed rats

Kalyani Kulkarni, Sanket Patel ... Tahir Hussain

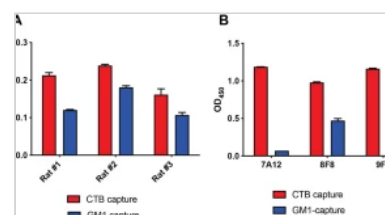
Article | [Open Access](#) | 15 Mar 2023



Characterization and utility of two monoclonal antibodies to cholera toxin B subunit

Noel Verjan Garcia, Ian Carlosalberto Santisteban Celis ... Nobuyuki Matoba

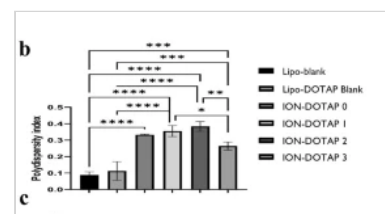
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The effect of 1,2-dioleoyl-3-trimethylammonium propane (DOTAP) Addition on the physical characteristics of β-ionone liposomes

Andang Miatmoko, Febe Harum Asmoro ... Chin-Shiu Huang

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OPEN The effect of 1,2-dioleoyl-3-trimethylammonium propane (DOTAP) Addition on the physical characteristics of β -ionone liposomes

Andang Miatmoko^{1,2✉}, Febe Harum Asmoro¹, Andre Alwi Azhari¹, Noorma Rosita¹ & Chin-Shiu Huang³

β -ionone (ION) is a cyclic terpenoid compound that demonstrates considerable potential for the prevention and treatment of cancer. However, the water solubility of β -ionone is poor and the compound demonstrates low permeability. Liposomes have been reported as increasing both qualities. In this study, the development of β -ionone liposomes was initiated by adding 1,2-dioleoyl-3-trimethylammonium propane (DOTAP) to produce cationic liposomes as a means of enhancing binding to cancer cells. Liposomes composed of β -ionone, HSPC, cholesterol, and DSPE-mPEG₂₀₀₀ were prepared using the thin layer hydration method. Cellular uptake studies were carried out with HeLa cells incubated with β -ionone liposomes for two hours. The results indicated that the addition of DOTAP increased particle size and affected the spectroscopical and thermogram profiles of the liposomes, thereby confirming reduction in liposome crystallinity, while the zeta potential became positive. Moreover, the calcein release profile further showed that additional DOTAP increased both membrane fluidity and cellular uptake in HeLa cells. In conclusion, adding DOTAP affected the physicochemical cationic properties of liposome and improved cellular uptake in HeLa cells.

β -ionone is a cyclic terpenoid compound with a basic core structure of retinoic acid, retinol, -carotene, and vitamin A which is found in many fruits, vegetables, and grains such as carrots, raspberries, almonds, tea, and tomatoes¹. Research has shown that β -ionone has the potential to both prevent and treat several types of cancer, such as breast, colon, lung, and liver². The anticancer activity involves several mechanisms, including antiproliferation, where β -ionone inhibits the proliferation of lung cancer cells (A549) by inducing apoptosis as seen from the increased expression of the Bax gene and reduced expression of the Bcl-2 gene which suppresses the expression of cPLA2 and COX-2 proteins³. β -ionone causes apoptosis, as seen in lung cancer (A549) cell death, (A549) through loss of mitochondrial membrane potential as measured by staining using rhodamine-123⁴. Besides that, β -Ionone can inhibit the in vitro proliferation of breast cancer cells. One of the mechanisms for inhibiting MCF-7 cell proliferation is reducing cyclooxygenase (COX-2) activity in the induction of apoptosis of MCF-7 cells. β -Ionone at a concentration of less than 50 μ mol/L can reduce the expression of COX-2 protein in mice tumor tissue, which is considered a target for cancer molecules⁵.

Dissolving β -ionone in water at a level of 0.169 g/L is extremely difficult. β -ionone also has low permeability with log $P = 4$ ^{6,7} and is classified as falling within Biopharmaceuticals Classification System (BCS) class IV. Numerous studies have shown that using liposomes can improve the solubility and permeability of compounds belonging to class IV BCS⁸.

Liposomes are lipid vesicles consisting of an aqueous phase core surrounded by one or more phospholipid bilayers⁹. Liposome membranes are composed of natural and/or synthetic phospholipids which are relatively biocompatible, biodegradable, and non-immunogenic. Due to the unique bilayer structure, liposomes act as

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carriers for both lipophilic and hydrophilic materials. The latter materials are in the aqueous phase, while the former are trapped in the lipid bilayer¹⁰.

The liposome delivery system is reported as overcoming Lopinavir's low solubility and bioavailability to increase therapeutic efficacy¹¹. The use of liposomes can also increase drug permeability. The particle size of liposomes allows higher intracellular uptake than other systems, thereby increasing drug bioavailability. Previous research has indicated that liposome encapsulation can significantly increase macrovascular permeability after administration of Paclitaxel liposomes¹², where Paclitaxel is also classified as the compound in BCS class 4, like β -ionone.

The nature and charge density of liposomes affect the stability, kinetics, and extent of biodistribution and interactions with liposome uptake by target cells¹³. Cationic liposomes have the advantage of promoting higher therapeutic efficacy against cancer cells⁹, where they can bind to negatively charged cell membranes. Other studies have shown that they accumulate selectively in tumor angiogenic endothelial cells and are superior to anionic or neutral liposomes^{14,15}. The research results obtained by Peters et al. (2015) showed that the cell uptake of 1,2-Dioleoyl-3-trimethylammonium propane (DOTAP)-liposomes was higher than that of the 1,2-dioleoyl-L- α -glycero-3-phosphatidylethanolamine (DOPE)-liposomal formulation. Another study using DOTAP as lipid composition demonstrated that transgene expression was increased in tumor cells due to the uptake of liposome-DNA complexes by tumor cell phagocytosis *in vitro*¹⁶. Research conducted on the cytotoxicity test confirmed that adding DOTAP to liposomes increased the cytotoxicity of HepG2 cancer therapy¹⁷.

Cationic lipid¹⁷ strongly influences biodistribution and drug efficiency^{17,18}. DOTAP is a phospholipid type frequently employed as a component of cationic liposomes. DOTAP contains an NH^{4+} group which renders it positively charged and is low in toxicity compared to other cationic lipids¹⁹. Based on the research of Chang et al. (2018), the addition of DOTAP to the liposome formulation causes a change in size²⁰. The liposomes prepared with PC only have a larger size than those prepared with a 1:1 ratio of PC and DOTAP (PC/DOTAP liposomes) with the same amount of lipids. This is because by accumulating the same amount of lipids, DOTAP, which has a shorter lipid chain, reduces the size of the liposome particle²¹. In the DOTAP-DNA complexes, the charge ratio of lipid/DNA highly affects the physical properties of the liposome. The addition of low-concentration DOTAP produced a larger size, namely 190 ± 8.4 nm, compared to liposomes with a high concentration of DOTAP, which was 140 ± 3.8 nm²². DOTAP-induced changes at the membrane interface may facilitate electrostatic interactions in the polar head region. This physical interaction can decrease the particle size by reducing the radius of bilayer curvature²³. In addition, the low lipid/DNA charge ratio enhanced the repulsion of excess negative charges, resulting in small particle sizes.

The relationship between liposome vesicle size and cell interactions was observed by means of the uptake from bone marrow-derived macrophages (BMDM). The absorption of BMDM from small DOTAP:DOPE liposomes was superior to that of their larger counterparts. Small cationic liposomes tend to be distributed to lymph nodes more rapidly from the injection site than larger ones. The liposome charge was also observed where the addition of DOTAP as a cationic lipid resulted in an extremely positive zeta potential of approximately +40 mV to +60 mV²⁴.

It was additionally reported that the dispersibility of liposomes following the addition of DOTAP was more effective than without DOTAP due to the presence of electrostatic forces²⁵. Furthermore, another study stated that adding DOTAP can render liposomes spherical with an approximate size of 100 nm¹⁵. On the other hand, adding DOTAP can increase the fluidity of the bilayer membrane. This is evidenced by the research of Takechi-Haraya et al. (2016), where DOTAP/Chol (90/10) has a liquid disorder arrangement, while DOTAP/Chol (50/50) has a liquid order arrangement which was observed using the Atomic Force Microscope (AFM). It is also proven by the deformation force curve where DOTAP/Chol (50/50) is steeper than DOTAP/Chol (90/10)²⁶.

Lipophilic compounds such as β -ionone will be entrapped in the liposome membrane which will, in turn, affect its fluidity. The results showed that the greater the amount of β -ionone trapped, the faster the release rate²⁷. Previous studies have also reported that drug compounds can interact with phospholipids, thereby affecting membrane fluidity^{28–32}. Therefore, the membrane's fluidity will affect the stability of the drug entrapment in liposomes and the drug released during circulation, thus determining the effectiveness of the treatment²⁸. Therefore, it is necessary to test the fluidity of the membrane, one method being calcein release. Calcein is a water-soluble, quenching, and fluorescent substance widely used in cell viability studies³³. Calcein release studies can be undertaken to determine membrane stability related to the presence or absence of liposome leakage measured using a spectrofluorometer³⁴.

In this study, the effect of the addition of different levels of DOTAP on the membrane's physical characteristics and the conducting of a fluidity test were evaluated. The data obtained may yield information on the effect of adding DOTAP to β -ionone liposomes and the development of further liposome formulations for inclusion in anticancer therapy.

Result

The particle size and zeta potential of β -ionone liposomes. The addition of DOTAP concentration tended to increase particle size. The largest liposome with an average size of 191.27 ± 4.20 nm was achieved by lipo-DOTAP blank. However, the size increased with the addition of DOTAP to the formula, as shown in Fig. 1a. PDI measurements were carried out to determine the homogeneity of particle size. Particles with smaller PDI values indicate a system with a homogeneous size distribution. PDI values > 0.7 indicate that the preparation has an extensive distribution of particle sizes³⁵. The lowest PDI value was obtained by the Liposome Blank formula, as shown in Fig. 1b.

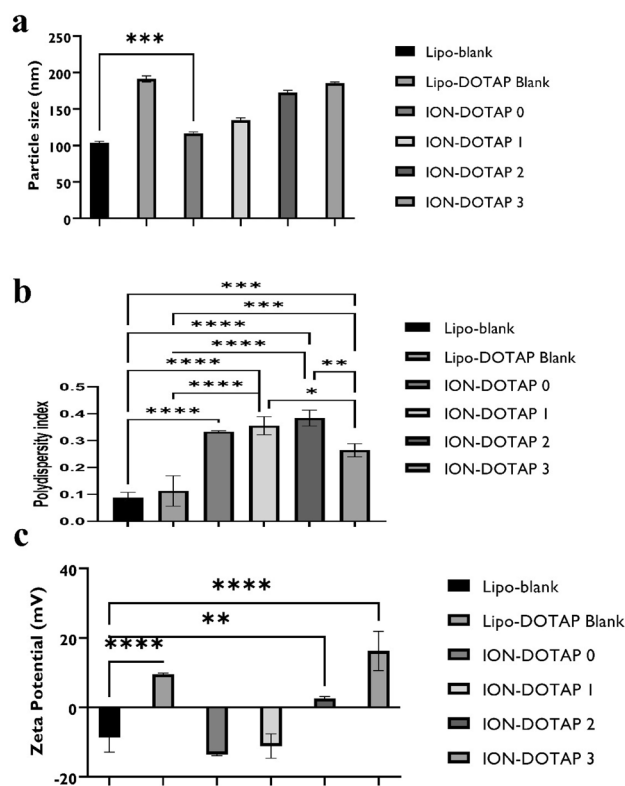


Figure 1. The bar charts of average (a) particle size value, (b) polydispersity index and (c) zeta potential of β -ionone liposomes prepared with various concentrations of DOTAP. Lipo-Blank contained no DOTAP or β -ionone, while Lipo-DOTAP Blank contained DOTAP at the highest concentration without the addition of β -ionone, $*p < 0.5$, $**p < 0.01$, $***p < 0.001$, compared to Lipo-Blank.

For the zeta potential value, the results indicated that the Lipo-blank, ION-DOTAP-2, and ION-DOTAP-3 liposomes differed significantly from liposomes ION-DOTAP-0, while Lipo-blank and ION-DOTAP-1 did not show any difference with ION-DOTAP-0 as shown in Fig. 1c.

The vesicle morphology of β -ionone liposomes analyzed by transmission electron microscopy (TEM). From the results of the vesicle morphology analysis with TEM contained in Fig. 2, it can be observed that the vesicles of ION-DOTAP-0 liposomes appear spherical with unilamellar membrane formation. In con-

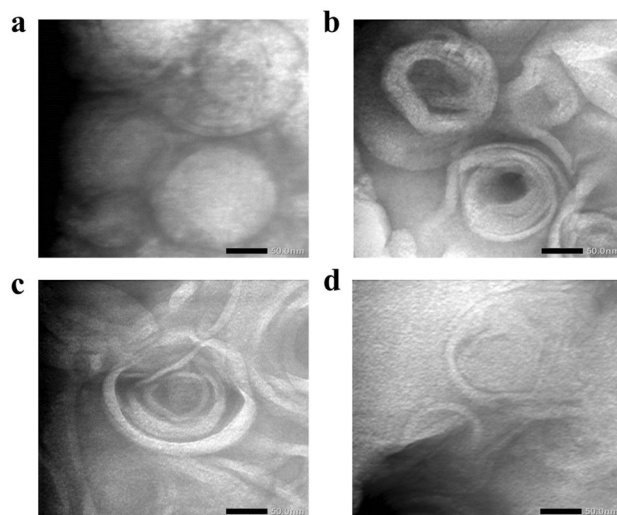


Figure 2. Morphological observations of (a) ION-DOTAP-0, (b) ION-DOTAP-1, (c) ION-DOTAP-2, and (d) ION-DOTAP-3 liposomes were analyzed using Transmission Electron Microscopy (scale bar: 50 nm).

trast, the formula, while also apparently spherical in shape, has a layered membrane due to the addition of DOTAP.

The spectra profiles of fourier transform infrared spectroscopy (ftir) of β -ionone liposomes. Identification of the compound's structure was completed using the FTIR Alpha II Spectrophotometer within the range of wave numbers 4000–600 cm^{-1} . The spectra of liposome constituents can be seen in Fig. 3, while those of the liposome formula produced can be seen in Fig. 4.

In the FTIR analysis, the Lipo-blank liposome spectra showed absorption bands identical to those in the spectra of the liposome constituents, as shown in Fig. 3. However, a decrease in the intensity of the absorption band after liposome formation occurred. The observed bands of the N–H functional group (3300–3500 cm^{-1}) were identical to the DOTAP absorption band, the C=C group band (1640–1680 cm^{-1}) was identical to the β -ionone absorption band, and the C–O group band (1050–1150 cm^{-1}) was identical to the absorption band of β -ionone, DSPE-mPEG₂₀₀₀, and cholesterol. The CH₃–CH₂–CH group (2850–3000 cm^{-1}) was identical to the absorption band of all the constituents of liposomes.

According to the overlaid spectra of β -ionone liposomes prepared with various concentration of DOTAP shown in Fig. 4, there were observed bands of N–H, CH₃–CH₂–CH, C=C (alkene), dan C–O functional groups for all formula. There are disappearances of some identical peaks of β -ionone and DOTAP observed for β -ionone liposomes with DOTAP addition. The spectra profiles of β -ionone liposomes show increasing the intensity and widening of absorption band of N–H groups that represent the DOTAP. However, spectra shown for each β -ionone liposomes showed no significant differences in the intensity of same type of absorption band.

Differential thermal analysis (DTA) of β -ionone liposomes. The thermal analysis results produced using differential thermal analysis (DTA) on each liposome component and liposome formula can be seen in the thermogram shown in Fig. 5. The results show that the DTA thermogram of liposome constituents, i.e., DOTAP, experienced endothermic peaks at 86.0 °C and 200.8 °C, while the HSPC thermogram registered two endothermic peaks at 83.6 °C and 182 °C. On the other hand, the DSPE-mPEG2000 thermogram recorded endothermic peaks at 59.2 °C and 249.2 °C, and cholesterol at 50.9 °C and 152.5 °C. The Lipo-blank, which consisted of a mixture of cholesterol, HSPC, and DSPE-mPEG2000, registered two endothermic peaks at 139.2 °C and 214.0 °C. The formation of liposomes produced endothermic peak shifts.

Figure 5 also contains the results of the overlaid DTA thermograms of the liposomes, namely: DOTAP, Lipo-blank, Lipo-DOTAP Blank, ION-DOTAP-0, ION-DOTAP-1, ION-DOTAP-2, and ION-DOTAP-3. Each formula had two endothermic peaks. For example, thermograms on Lipo-DOTAP Blank, ION-DOTAP-0, and ION-DOTAP-1 liposomes produced endothermic peaks of approximately 140.2–140.8 °C and 213.2–214.2 °C. The endothermic peak formed was identical to the peaks of its constituents, namely: cholesterol and DOTAP.

Furthermore, the thermogram of ION-DOTAP 2 liposomes underwent a shift with endothermic peaks at temperatures of 176 °C and 114 °C. Meanwhile, the ION-DOTAP-3 liposomes, which contain the highest

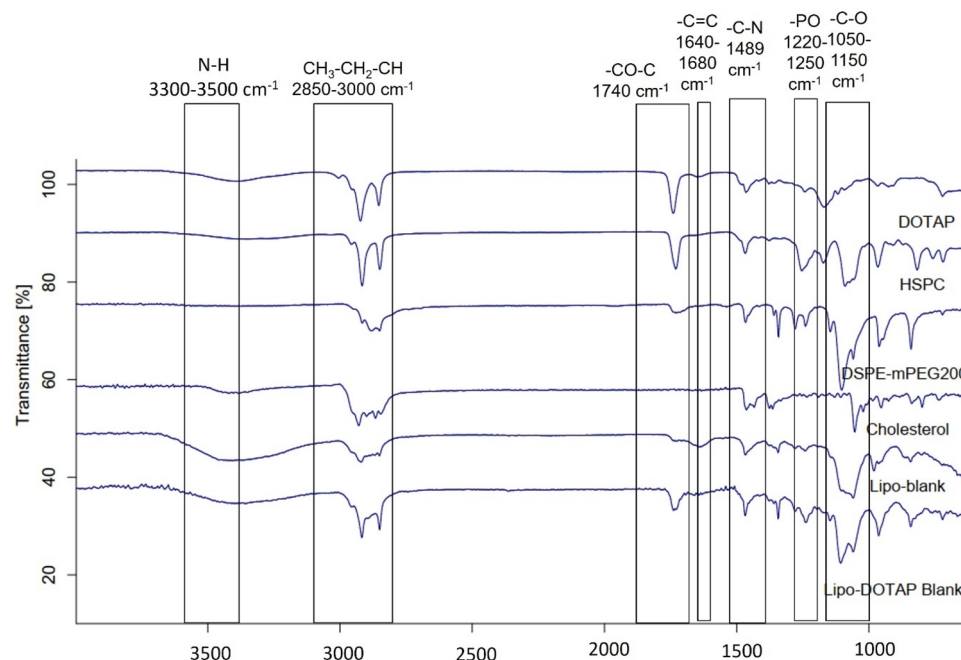


Figure 3. FTIR spectra of β -ionone, DOTAP, HSPC, DSPE-mPEG2000, cholesterol, Lipo-Blank, and Lipo-DOTAP Blank. Lipo-Blank contained no DOTAP or β -ionone, while Lipo-DOTAP Blank contained DOTAP at the highest concentration without the addition of β -ionone.

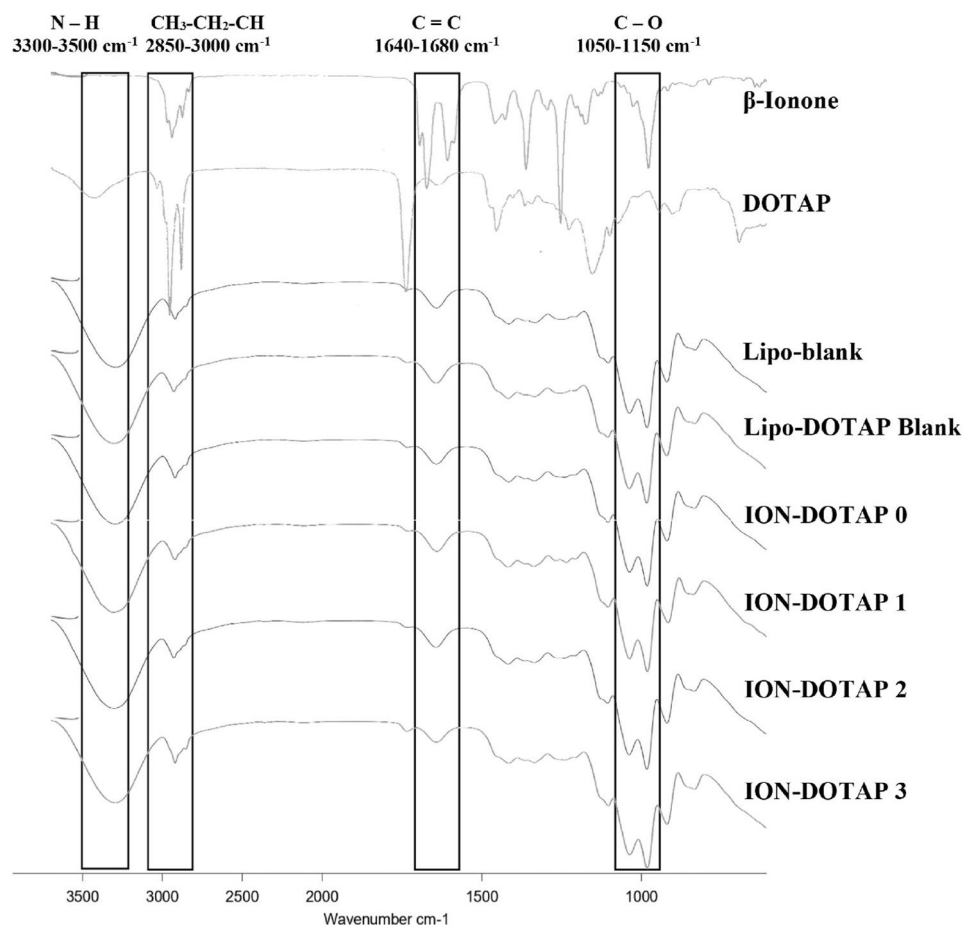


Figure 4. FTIR spectra of β -ionone, DOTAP, ION-DOTAP liposomes, and Lipo-DOTAP Blank, ION-DOTAP 1, ION DOTAP 2, ION DOTAP 3 Lipo-Blank contained no DOTAP or β -ionone, while Lipo-DOTAP 3 contained DOTAP at the highest concentration without the addition of β -ionone.

concentration of DOTAP, experienced a significant shift of endothermic peaks at 81.6 °C and 214.1 °C, with relatively wide peaks when compared to the those of the other liposomes. It also resulted in the loss of the endothermic peak of 152.5 °C, which is identical to that of cholesterol. This result confirms that the ION-DOTAP-3 thermogram experienced an endothermic peak increasingly similar to that of DOTAP.

The calcein released from β -ionone liposomes. Calculation of the cumulative percentage of the calcein released from the liposomes indicates that the smallest percentage of the calcein released was in the ION-DOTAP-0, indicating that this liposome has the most rigid structure compared to those of other formulas as presented in Fig. 6. Meanwhile, the highest percentage of released calcein was observed in ION-DOTAP 3 liposomes.

In addition, the release flux value obtained from this study reveals that the liposome ION-DOTAP-3 had the largest flux value (6.7074 ± 1.8650), followed by ION-DOTAP-2 (2.8357 ± 0.7453), ION-DOTAP 1 (1.6169 ± 0.4700) and ION-DOTAP-0 (0.2674 ± 0.0545) as shown in Table 1.

The cellular uptake of β -ionone liposomes in HeLa cells. In Fig. 7, cells in the LIPO-blank treatment can be observed where the fluorescence intensity of coumarin-6 loaded into liposomes was relatively low. The liposomes containing coumarin-6 with high DOTAP levels increased the fluorescence intensity. Figure 7 shows that ION-DOTAP-0 liposomes containing coumarin-6 infiltrated the HeLa cells indicated by green fluorescence, while ION-DOTAP-1 liposomes were only slightly fluorescent. However, they were less visible and the fluorescence of the ION-DOTAP-2 liposomes was relatively weak in intensity as shown in the cell membrane and cytoplasm.

Meanwhile, as shown in Fig. 8, the ION-DOTAP-3 liposome with the highest DOTAP content increased the fluorescence intensity and produced the highest cellular coumarin-6 levels than other formulas.

In vitro cytotoxicity evaluation of β -ionone liposomes. The IC_{50} cytotoxicity test on HeLa cells aims to determine the effect of adding DOTAP to β -ionone liposomes in cancer therapy. The cells incubated with

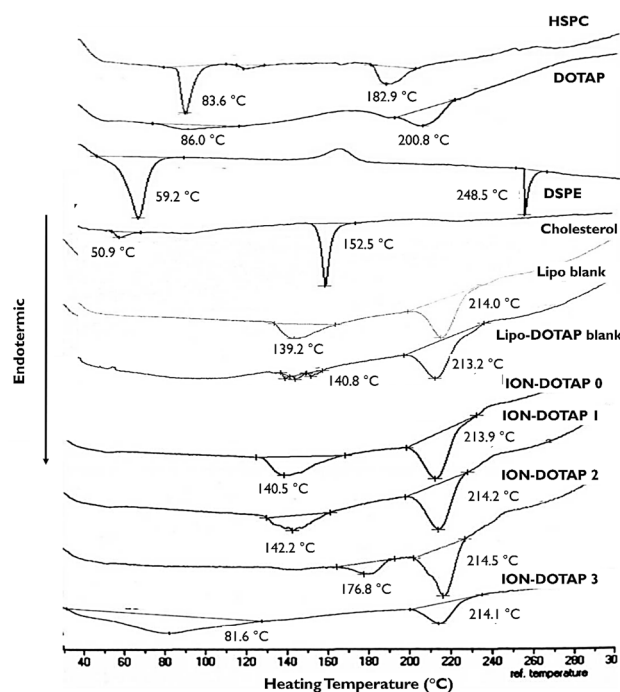


Figure 5. Thermogram profile of HSPC, DOTAP, DSPE-mPEG2000, Cholesterol, Lipo-Blank, Lipo-DOTAP Blank, ION-DOTAP-0, ION-DOTAP-1, ION-DOTAP-2, and ION-DOTAP-3.

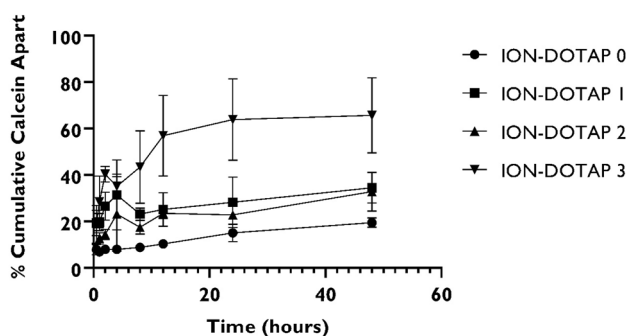


Figure 6. The calcein release profile of ION-DOTAP-0, ION-DOTAP-1, ION-DOTAP-2, and ION-DOTAP-3 liposomes in PBS pH 7.4 as the release media at a temperature of 37 °C.

Formula	Calcein release flux ($\mu\text{g}/\text{cm}^2/\text{h}$, n = 3)
ION-DOTAP 0	0.2674 ± 0.0545
ION-DOTAP 1	1.6169 ± 0.4700
ION-DOTAP 2	2.8357 ± 0.7453
ION-DOTAP 3	6.7074 ± 1.8650

Table 1. The release flux value of calcein released from β -ionone liposomes prepared with various concentrations of added DOTAP.

β -ionone liposomes for 48 h resulted in the percentage of cell viability shown in Fig. 9A. It can be seen that HeLa cells were not sensitive to β -ionone liposomes, and all treatments resulted in high cell viability.

To further evaluate the cytotoxic effects on these cells, DOXO was co-loaded into β -ionone liposomes. As shown in Fig. 9B, an increase in the cytotoxic effect also occurred in T47D cells, where the ION-DOXO-DOTAP-2 formula gave the lowest IC_{50} value compared to other liposome formulas, indicating its high cytotoxicity. On the other hand, ION-DOXO-DOTAP-0, which does not contain DOTAP, produces the smallest IC_{50}

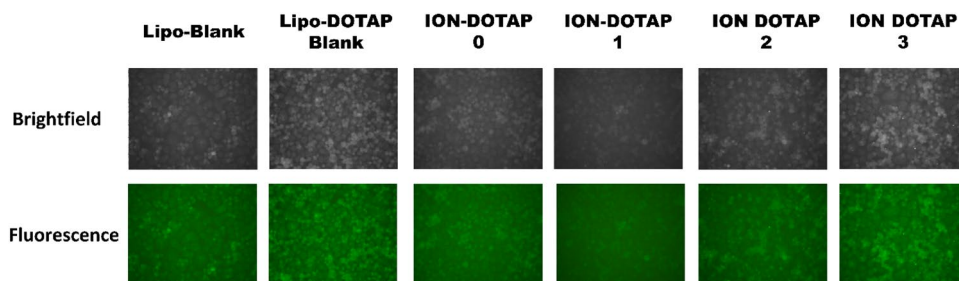


Figure 7. Photomicroscopy of cellular uptake of HeLa cells after incubation with β -ionone liposomes containing Coumarin-6 (in green color) prepared with various concentrations of DOTAP for two hours at $20\times$ magnification under a fluorescence microscope.

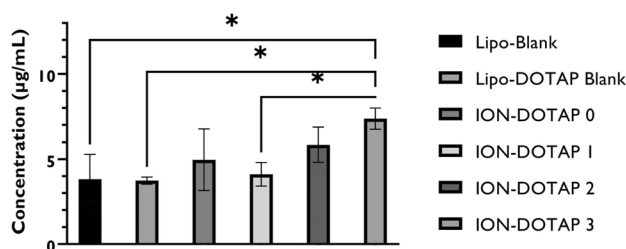


Figure 8. Coumarin-6 levels in HeLa cells post-incubation with β -ionone liposomes containing Coumarin-6 prepared with various concentrations of DOTAP for two hours.

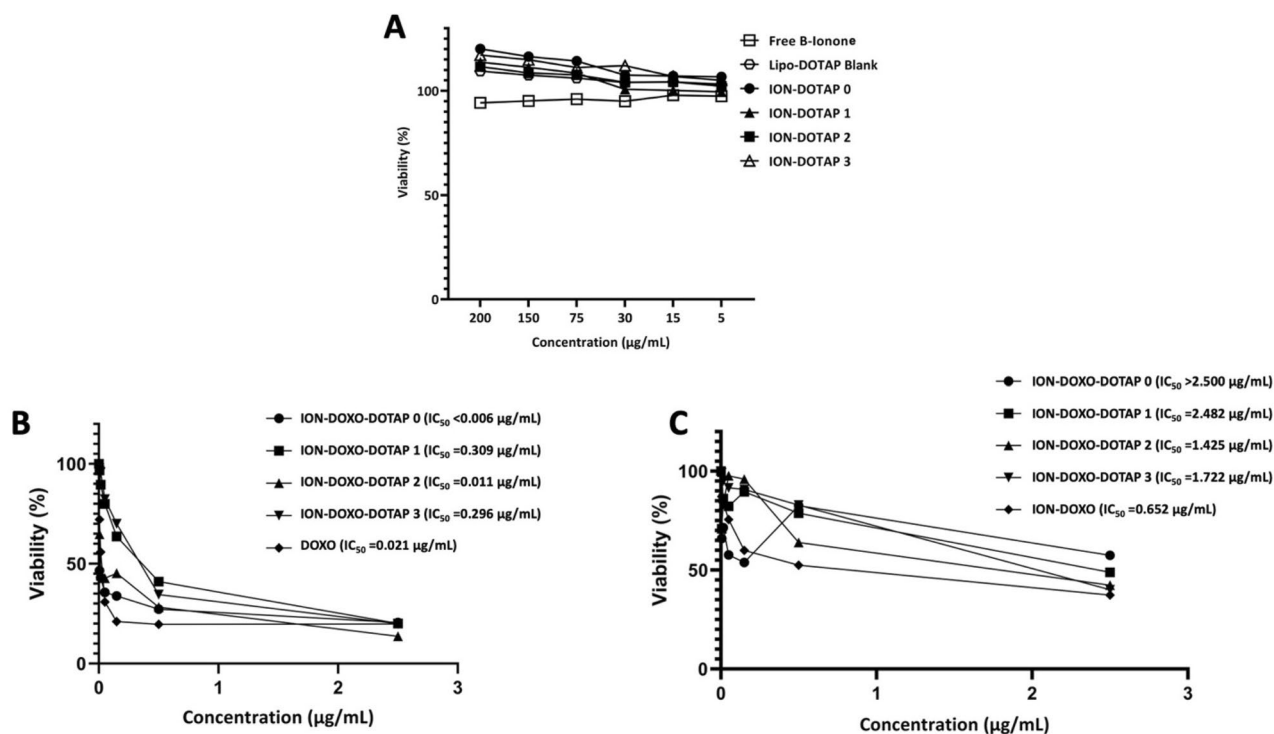


Figure 9. The viability of (A) HeLa cells after incubation of β -ionone liposomes and (B) T47D and (C) HeLa cells after incubation with β -ionone liposomes co-loaded with doxorubicin for 48 h.

value. The results also showed that there is an increase in cytotoxicity of the ION-DOXO-DOTAP-2 formula in HeLa cells; however, ION-DOXO-DOTAP-0 produces the highest IC₅₀ value (Fig. 9C).

Discussion

In this study, liposomes were prepared to deliver β -ionone, a low water soluble and poorly permeable drug, used in anticancer treatment^{6,7}. However, the composition of phospholipids significantly affects liposomes' characteristics which may relate to their pharmacological activity. Therefore, this study evaluated the effect of the addition of DOTAP on the physical characteristics of β -ionone-loaded liposomes, including: particle size, zeta potential, morphology, infrared (IR) spectroscopic profiles, thermal analysis, and calcein release.

The addition of DOTAP affects the physicochemical characteristics of the liposomes, with increased DOTAP concentration resulting in larger particle size. This is probably due to the HSPC, cholesterol, and DSPE-mPEG2000 components which have ordered molecule arrangements producing a rigid membrane structure³⁶. Moreover, the possibility exists of interaction between the polar groups of DOTAP and the choline groups of HSPC, both of which are positively charged and therefore repel each other, leading to a widening of the bilayer membrane's space.

The spectra of liposomes loaded β -ionone with DOTAP addition experienced a reduction in the intensity and widening of the absorption band in the N–H group which is characteristic of DOTAP. The N–H group in DOTAP is the polar section of the phospholipid head³⁷. Furthermore, the analysis of the absorption band on the alkyl group (CH₂) can be used as an indicator of the regularity of the lipid chain arrangement. The reduced intensity and widening of the absorption band indicate an increase in the gauche conformation³⁸. The spectra of liposomes formed showed a decrease in the intensity of the absorption band on the alkyl group, indicating a potential increase in the gauche conformation, which changed the composition of the lipid chain thereby rendering it more fluid.

Several identical peaks in DOTAP and β -ionone were not visible in the spectra of the liposomes loaded β -ionone with DOTAP addition, indicating that there could be physical interaction³⁹. Meanwhile, the spectra shown for each liposome formula did not indicate a significant difference in the intensity of the absorption bands.

FTIR analysis, in determining the interaction of DOTAP with liposome membranes, can be performed by observing the absorption band on certain functional groups, namely: the phosphate group (R–PO₂–R') and the ester group (–CO–O) present on the head of HSPC which is polar⁴⁰. Neither the Lipo-blank FTIR spectra nor the blank Lipo-DOTAP showed any absorption bands in the two functional groups. Therefore, it is thought that hydrogen bonding occurs in both the phosphate and phospholipid ester groups due to stretching of the absorption band⁴¹. Lipo-blank and Lipo-DOTAP Blank liposomes did not show a significant shift in the absorption band and no new band formation, leading to the conclusion that only chemical interaction and no physical interaction occurs. Moreover, the ION-DOTAP-3 thermogram shows an endothermic peak that is increasingly similar to that of DOTAP. The widening and disappearance of the endothermic peaks on the thermogram indicate a change in the regularity of the lipid structure lipid⁴². In other words, adding DOTAP can reduce the rigidity of the membrane, thereby rendering it more fluid.

The calcein release test showed that the liposome ION-DOTAP-3 experienced the highest calcein release compared to the other formulas. The release of calcein from the liposomes increased in line with that of membrane fluidity³³. It can therefore be concluded that ION-DOTAP 3 demonstrates the highest membrane fluidity. Based on the results of the analysis conducted to determine the physical characteristics of β -ionone liposomes, the addition of DOTAP can increase the particle size, rendering the zeta potential positive with the morphology of liposomes appearing spherical with a layered membrane. Furthermore, the results of thermogram data and spectroscopic profiles, show that the addition of DOTAP can cause the composition of the bilayer membrane to become more fluid. This result is in line with the calcein release profile which indicates that adding DOTAP can increase the fluidity of the liposome membrane.

With regard to the surface charge of liposomes, the liposome constituents such as HSPC and DSPE-mPEG₂₀₀₀ have a neutral charge, while β -ionone and cholesterol have no charge or are non-ionic⁴³. Meanwhile, DOTAP is a positively charged phospholipid with an NH⁴⁺ group on the head of the phospholipid¹⁹. Consequently, adding DOTAP to liposomes increased the positivity of the potential charge. Increasing the concentration of DOTAP heightened the positive charge of the liposomes.

A cellular uptake test was carried out to determine the ability of β -ionone liposomes to infiltrate the HeLa cells. The levels of liposomes entering HeLa cells were calculated as coumarin-6 levels. Coumarin-6 is a hydrophobic compound trapped in the bilayer membrane and the measured coumarin-6 level indicates how many liposomes enter the cell. The green fluorescence indicates that β -ionone liposomes containing coumarin-6 are present in the cells⁴⁴. The more intense the fluorescence color, the greater the amount of coumarin-6 absorbed⁴⁵. Based on the findings of the quantification analysis of coumarin-6 levels, the ION-DOTAP-3 formula produced higher levels than other formulas. It is because the ION-DOTAP-3 liposomes contain cationic lipid (DOTAP) at a higher concentration than other formulas, resulting in a significant ionic interaction between negatively charged cancer cells and β -ionone liposomes containing DOTAP (cationic lipid)⁴⁶.

It was also reported that particle size affects the endocytosis pathway of liposomes into cells. In this study, rising levels of DOTAP increased the particle size of liposomes, although this was in the range of < 200 nm which was still relatively modest. Consequently, no significant effect on cellular uptake was regarded as having occurred. The entry of β -ionone liposomes was due to the addition of DOTAP to cells through a process of endocytosis which requires energy to introduce foreign particles into the cell. The results demonstrated a significant difference between the ION-DOTAP-3 and the blank lipid formula, blank ION-DOTAP, and ION-DOTAP-1. In this study, it can be seen that, with the addition of higher DOTAP levels, β -ionone liposomes can increase cell uptake

in HeLa cells. This greater cell uptake leading to increased coumarin-6 levels and microscopic observations of HeLa cells showed that the intensity of coumarin-6 rose with the occurrence of higher DOTAP levels.

The IC₅₀ cytotoxicity test was carried out on HeLa cells to determine the effect of adding DOTAP to β -ionone liposomes in cancer therapy. Unfortunately, β -ionone liposomes had no cytotoxic effects even though the cellular uptake test results show that liposomes entered the cells. This result indicates that it is possible that these cells are not sensitive to β -ionone, which becomes the limitation of our study. Therefore, a cytotoxicity test was carried out by co-encapsulating DOXO into β -ionone liposomes (ION-DOXO-DOTAP liposomes). DOXO has been known as an anticancer compound sensitive to HeLa cells and T47D cells. The results showed that the ION-DOXO-DOTAP liposomes had toxicity to both cancer cells. The ION-DOXO-DOTAP-2 had the highest cytotoxicity. Compared to the ION-DOXO-DOTAP-0, which had no DOTAP addition, the toxicity is relatively similar to that of DOXO. This is related to liposome formulations with the addition of DOTAP providing better retention than liposomes without DOTAP. The increased drug release was proportional to the cell uptake, which also increased with the addition of DOTAP. This result is in line with the study of Lei et al. (2011), where it was reported that cytotoxicity results were associated with cellular uptake. Therefore, high cellular uptake will give an increased cytotoxicity value⁴⁷.

Conclusions

The results of this study indicate that the addition of DOTAP to β -ionone liposomes increased the particle size slightly and rendered the charge of the liposomes positive. In addition, the higher the DOTAP concentration added, the more fluid the liposomal membrane as indicated by the reduction in membrane crystallinity which supported the higher calcein release from the liposomes. The introduction of DOTAP into liposomes highly affects the physical properties of β -ionone liposomes, thereby increasing the cellular uptake and cytotoxicity. Proposing the incorporation of DOTAP into β -ionone liposomes requires further studies on its anticancer efficacy. From the results of this analysis, although the addition of DOTAP renders the liposomes more fluid, its ability to enhance the cellular uptake provides potential benefits when used as a delivery system for cancer therapy.

Materials and methods

Materials. In this study, β -ionone was purchased from Sigma-Aldrich Inc. (Saint Louis, USA). The liposome component was 1,2-dioleoyl-3-trimethylammonium propane (DOTAP), Hydrogenated Soybean Phosphatidylcholine (HSPC), and Distearoyl-glycerophosphoethanolamine-N-(Carbonyl-methoxy polyethylene glycol 2000 (DSPE-mPEG₂₀₀₀), which were products of Nof Corporation (Tokyo, Japan). Cholesterol was obtained from Wako Inc., Ltd. (Osaka, Japan), while calcein (Fluorexone[®]) was a product of Nacalai Tesque Inc. (Kyoto, Japan). The buffer components include phosphate buffer pH 7.4, KH₂PO₄, Na₂HPO₄, NaCl, and KCl were purchased from Merck Inc. (Darmstadt, Germany). Chloroform and methanol were products of Merck Inc. (Darmstadt, Germany). Other reagents and materials used were of non-technical grade.

Preparation of β -ionone liposomes. β -ionone liposomes were prepared by the thin film hydration method. First, β -ionone, DOTAP, HSPC, DSPE-mPEG₂₀₀₀, and cholesterol were dissolved in chloroform. As shown in Table 2, appropriate amounts of each solution were, mixed in a round bottom flask. The chloroform was then evaporated and completely removed using a rotary evaporator at 55 °C to form a thin film layer on the flask wall. The thin film formed was hydrated with phosphate-buffered saline (PBS) pH 7.4 and sonicated using a sonicator at 55 °C for 10 min²⁷. Extrusion was subsequently carried out with an extruder (Avanti[®], Mini Extruder, Avanti Polar Lipid Inc., Alabama, USA) by passing liposomes through a polycarbonate membrane with various pore sizes, i.e., 400, 200, and 100 nm, with a heating block at a temperature of 55–60 °C to produce liposomes approximately 100 nm in size⁴².

Evaluation of physical characteristics of β -ionone liposomes. The particle size of β -ionone liposomes were determined by the dynamic light scattering method using a Delsa Nano C Particle Analyzer at 25 °C. Meanwhile, the zeta potential was measured using the electrophoresis light scattering method using the Malvern Zetasizer Nano Series Analyzer at 25 °C at the Sepuluh Nopember Institute of Technology (ITS), Surabaya, Indonesia. The liposomes were diluted with demineralized water, placed in a cuvette, and measured for particle size and zeta potential²⁷.

Components	Composition (mole ratio)					
	Lipo-blank	Lipo-DOTAP blank	ION-DOTAP-0	ION-DOTAP-1	ION-DOTAP-2	ION-DOTAP-3
β -ionone	–	–	30	30	30	30
DOTAP	–	8	0	2.5	5	8
HSPC	35	35	35	35	35	35
Cholesterol	25	25	25	25	25	25
DSPE-mPEG ₂₀₀₀	10	10	10	10	10	10
Hydrating buffer	Phosphate buffered saline (PBS) pH 7.4					

Table 2. Composition of β -ionone liposomes.

Evaluation of vesicle morphology of β -ionone liposomes using transmission electron microscopy (TEM). Liposome morphology, determined using the JEOL JEM-1400 TEM (Jeol Inc., USA) instrument, was carried out at the Laboratory of Chemistry of Gadjah Mada University (UGM), Yogyakarta, Indonesia. Liposomes were first diluted using demineralized water at a volume ratio of 1:5 of liposome and water respectively. The sample was subsequently stained with 4% phosphotungstic acid at a ratio of 1:1. Samples were placed on a copper grid, and images taken at various magnification sizes¹⁵.

Fourier-transform infrared spectroscopy (FTIR) Analysis of β -ionone Liposomes. FTIR evaluation was carried out to determine molecular interactions within the liposomes. Before the analysis, each β -ionone liposome was hydrated with PBS pH 7.4 containing 7% sucrose and freeze dried. FTIR analysis was then conducted using an FTIR Alpha II Spectrophotometer (Bruker, Ettlingen, Germany). The sample was put into the sample container and the spectra measured at wave numbers of 4000–600 cm^{-1} .

Differential thermal analysis (DTA) of β -ionone liposomes. DTA is used to evaluate changes in sample properties evident from the endothermic and exothermic peaks on the DTA thermogram when heat is applied. The tests were carried out using a DTA instrument (Mettler Toledo FP90, Switzerland). Each β -ionone liposome was hydrated with PBS pH 7.4 containing 7% sucrose and freeze-dried before analysis. The freeze-dried samples were placed in aluminum crucibles and heated from 30 to 300 °C at the rate of 10 °C/min²⁷.

Evaluation of calcein release. *Preparation of β -ionone liposomes containing calcein for release study.* The β -ionone liposomes were prepared on the basis of the above method. The thin lipid film formed was then hydrated with 1 mL of PBS pH 5.0 containing 3.4 mM calcein (Nacalai Tesque Inc., Kyoto, Japan). Extrusion was subsequently carried out with an extruder (Avanti[®], Mini Extruder, Avanti Polar Lipid Inc., Alabama, USA) by passing liposomes through a polycarbonate membrane with various pore sizes of 400, 200, and 100 nm with a heating block at 55–60 °C⁴². The free calcein was subsequently separated from the liposomes by placing them into the Amicon[®] Ultra-4 Centrifugal Filter Unit tube with a molecular weight cut-off (MWCO) of 10,000 Da (Merck Millipore, Darmstadt, Germany). The tube was centrifuged using a Hettich Rotofix 32 Benchtop Centrifuge (Hettich Lab., Tuttlingen, Germany) at 3500 rpm in order to remove the untrapped or free calcein.

Calcein release assay from β -ionone liposomes. A calcein release study was conducted by dialysis. The liposomes were placed in the Spectra/Por[®]7 dialysis membrane with MWCO (Spectrum Laboratories, USA) of 3500 Da. 50 mL of PBS pH 7.4 was employed as a release medium with a stirring speed of 350 rpm at 37 °C. Sampling was performed at 0.5, 1, 2, 4, 8, 12, 24, and 48 h by replacing some samples with the same volume of fresh PBS pH 7.4. The calcein fluorescence intensity was subsequently measured using the GloMax[®]-Multi Detection System (Promega Corporation, Madison, USA) microplate reader at $\lambda_{\text{ex}} = 475 \text{ nm}$ and $\lambda_{\text{em}} = 500\text{--}550 \text{ nm}$.

The cellular uptake assay of β -ionone liposomes. *Preparation of β -ionone liposomes containing coumarin-6 as the fluorescent labeling agent.* The first procedure involved dissolving each liposome component in chloroform. Liposomes were prepared according to the formula in the Table 2 by adding 0.3 mg Coumarin-6 to each mL of liposomes containing 9.7 mg total lipid of HSPC, Cholesterol, and DSPE-mPEG₂₀₀₀. The amount of coumarin-6 added was in accordance with the previous study of Miatmoko et al. (2021), which successfully represented liposome uptake in cancer cells⁴⁸. Each component solution was appropriately mixed in a round bottom flask covered with aluminum foil. The organic solvent was then placed in a rotary evaporator at a temperature of 55 °C until all organic solvents were removed and formed a thin film which was hydrated using 1 mL of PBS pH 7.4, vortexed, and sonicated for 10 mins at 55 °C. Liposomes were subsequently extruded using polycarbonate membranes of sizes from 400, 200, to 100 nm to produce liposomes with a size of about 100 nm.

The cellular uptake assay. Initially, the HeLa cells, which were obtained from Stem Cell Research and Development Center, Universitas Airlangga, were suspended in RPMI 1640 medium containing 10% Fetal Bovine Serum (FBS) and 1% Penicillin–Streptomycin antibiotic at a concentration of 1×10^6 cells/mL, placed into a 6-well plate, and incubated at 37 °C with 5% CO₂ environment for 24 h. Cells can be used for cellular uptake assay if they have a confluence of 70–80%⁴⁴. The medium was replaced with β -ionone liposomes at a coumarin-6 concentration of 10 $\mu\text{g}/\text{mL}$ and incubated for two hours, with the media being disposed of. The cells were first twice washed with PBS pH 7.4 and then added to 1 mL of methanol and left for 5–10 mins. After the methanol had been discarded, the cells were washed twice with PBS pH 7.4, containing 0.5% Tween 80 and approximately 1 mL of PBS pH 7.4 containing 1% FBS was added and left for 15 min. The cells were then washed twice with PBS solution pH 7.4. The final step was to observe the cells by means of a fluorescence microscope with an FITC filter⁴⁴.

Measurement of coumarin-6 levels in HeLa cells. In the first step, the cells were prepared with the same procedure as that for the cellular uptake assay and the samples were incubated for two hours. The medium was then discarded and the cells washed twice with PBS pH 7.4. In order to measure the amount of intra-cellular coumarin-6, the cells were lysed with 100 μL of lysis buffer containing 10 mM NaCl, 1 mM Ethylenediaminetetraacetic acid (EDTA), and 0.5% Triton X-100 by means of pipetting and added to 900 μL PBS pH 7.4 to enable collection of the samples. The cell suspension was vortexed and centrifuged at 12,000 rpm for ten minutes. After centrifugation, the supernatant's fluorescence intensity was measured using GloMax[®]-Multi Detection System (Promega Corporation, Madison, USA) microplate reader at $\lambda_{\text{ex}} = 475 \text{ nm}$ and $\lambda_{\text{em}} = 500\text{--}550 \text{ nm}$ ⁴⁴.

In vitro cytotoxicity assay. Preparation of liposomes containing doxorubicin. In addition to β -ionone liposomes, we prepared β -ionone liposomes co-loaded with Doxorubicin (DOXO) to produce ION-DOXO-DOTAP liposomes and evaluate their cytotoxicities on T47D and HeLa cells. β -ionone liposomes co-loaded with DOXO were prepared by the same formula and methods as β -ionone liposomes; however, the 0.65 M Triethylamine was used as the hydrating solution. The liposomes were then eluted through a column packed with Sephadex G50 using PBS pH 7.4. DOXO was added at DOX/total lipid ratio of 1:5, respectively, and the mixture was then incubated in a water bath at 60 °C for 10 min. The liposomes were further separated from the free DOXO by elution through the Sephadex G50 gel column.

WST assay. The in vitro cytotoxicity test procedure was evaluated for T47D cells and HeLa cells using the WST-1 assay method. The T47D and HeLa cell were obtained from Stem Cell Research and Development Center, Universitas Airlangga, and then cultured in Roswell Park Memorial Institute (RPMI)-1640 medium containing 10% Fetal Bovine serum and 1% Penicillin Streptomycin in a humidified atmosphere at 37 °C with 5% CO₂ air supply. For the assay, T47D and HeLa cells with a concentration of 5×10^3 cells were added to each well of 96-well plates and incubated for 24 h before treatment.

To evaluate the cytotoxicity, β -ionone liposomes and β -ionone liposomes co-loaded with DOXO were prepared at various concentrations. These samples were added to each well containing the cells and incubated for 48 h. After the treatment, the cell number was determined using WST-1 assay. The cell viability was determined as the relative measurement to the untreated cells by evaluating the absorbance of each well at 440 nm using a microplate reader. The IC₅₀ values of the compounds were calculated using the percentage of living cells and analyzed using SPSS (probit) software.

Data analysis. The data for analysis consisted of the average \pm standard deviation measured in replications. First, a Kolmogorov–Smirnov test was used to analyze the numerical data for normal distribution. If the data was normal (P value ≥ 0.05), the quantitative analysis was analyzed using One Way Analysis of Variance. If the P value < 0.05 , the data analysis was followed by a post hoc test incorporating the use of Honestly Significant Difference (HSD). The analysis was conducted using the IBM SPSS Statistics software version 24.

Data availability

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

Received: 27 September 2022; Accepted: 14 March 2023

Published online: 15 March 2023

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Acknowledgements

The authors would like to thank Ms. Berlian Sarasitha Hariawan and Ms. Devy Maulidya Cahyani for kind assistantship for manuscript preparation.

Author contributions

A.M.: (1) conception and design of the work, data acquisition, data analysis and interpretation; (2) critically revising the article for important intellectual content; (3) Final approval of the version to be published; (4) Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of the work are appropriately investigated and resolved. F.H.A.: (1) conception and design of the work, data acquisition, data analysis and interpretation; (2) Drafting the article; (3) Final approval of the version to be published; (4) Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of the work are appropriately investigated and resolved. A.A.A.: (1) conception and design of the work, data acquisition, data analysis and interpretation; (2) Drafting the article; (3) Final approval of the version to be published; (4) Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of the work are appropriately investigated and resolved. N.R.: (1) data analysis and interpretation; (2) critically revising the article for important intellectual content; (3) Final approval of the version to be published; (4) Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of the work are appropriately investigated and resolved. C.-S.H.: (1) data analysis and interpretation; (2) Final approval of the version to be published; (3) Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of the work are appropriately investigated and resolved.

Funding

This study was financially supported by Research Collaboration Funding between Universitas Airlangga, Indonesia and Asia University, Taiwan.

Competing interests

The authors declare no competing interests.

Additional information

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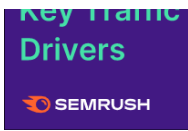
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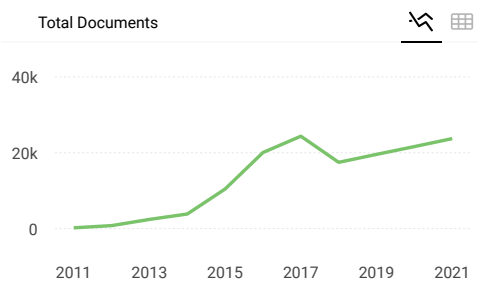
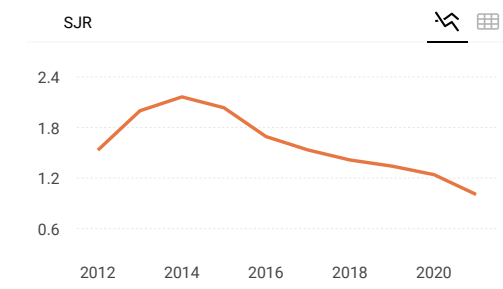
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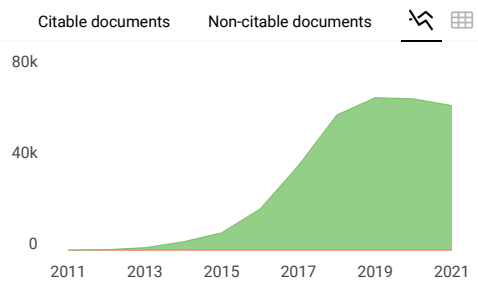
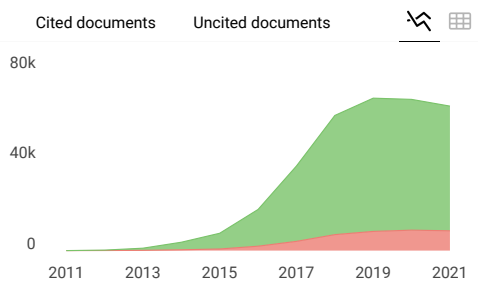
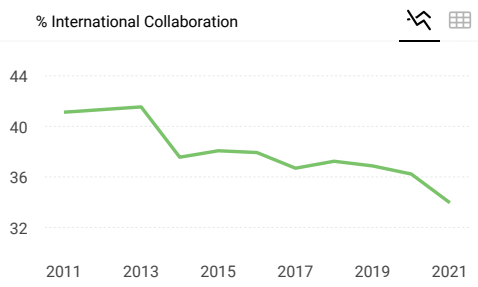
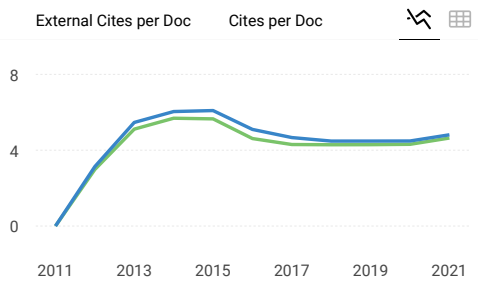
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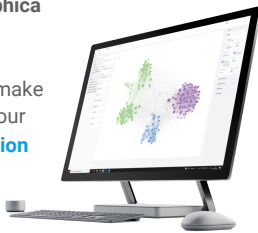
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Reviewer Comments:

Reviewer 1

1. In the introduction, before doing the literature survey of DOTAP, introduce what DOTAP is in one to two lines. Also, it is not required to add this much of literature survey for DOTAP, but make it more concise.
2. Line 81, Cationic lipid 17, 17 should be a superscript.
3. Line 123 the effect of the addition of different levels of DOTA, (typo, should be DOTAP).
4. Lines 89-96 the authors mentioned that in the literature the sizes reduced with increasing the concentration of DOTAP. while in their work the addition of DOTAP increases the size of liposomes (line 130), why is it contradicting to the literature?
5. Lipo-DOTAP blank and ION-DOTAP 3 are having the same concentration of DOTAP, and their sizes are similar, so the statement "However, the size decreased with the addition of β -ionone to the formula, as shown in Figure 1A." (line131-132), is not true. The size reduced because of the low concentration of DOTAP and not due to the addition of β -ionone.
6. Figure 1 is not a histogram, but a bar graph. The comparison is between Lipo-blank to the others.
7. Figure 3 is spectra is not properly visible.
8. Line 209, it is 214 C and not 124 C
9. From Calcein release studies - Increasing the DOTAP concentration reduced the stability of liposomes, which is not a positive feature.
10. The fluorescent images are not at the acceptable quality. Although the authors mentioned an increase in fluorescence, it appears that it is due to the increase in exposure time, the same increase in brightness can be seen in the bright field images as well for the Lipo-DOTAP-Blank and DOTAP-3. From figure 8, Lipo-DOTAP-Blank has the least amount of Coumarin-6, since both Lipo-DOTAP-Blank and ION-DOTAP-3 has the same amount of DOTAP, why is there a difference in Coumarin-6 intake by the cells, also this result contradicts to the fluorescent image in the figure 7.
11. "Preparation Of β -Ionone Liposomes Containing Coumarin-6 as the Fluorescent Labelling Agent" The component ratio of the liposome prepared is entirely different from what is mentioned in table 2. The mole ratio between HSPC: β -Ionone:Cholesterol:DSPE-PEG:DOTAP is 35:140:70:5:6. Again, the concentrations of lipids are too high, which might be the reason why they obtained multilamellar liposomes.
12. In the conclusion, the authors said that incorporation of DOTAP improved the liposome formulation for cancer therapy, but, it is not convincing from the results. Although β -Ionone was encapsulated, no studies were done to signify its effects, no cell viability studies, encapsulation efficiency etc. Furthermore, addition of DOTAP made the liposomes multilamellar, reduced the stability and it does not appear to have improved the cellular intake.

Reviewer 2

Comments to the Authors

This manuscript investigates that the effects of DOTAP addition on the physical characteristics of β -Ionone liposomes. This work reported that the addition of DOTAP to β -ionone liposomes made the liposomes positively charged and increased particle size, membrane fluidity, and intracellular uptake. The experiments are well designed and results are mostly credible. However, there are still some problems that need to be clarified.

1. The author found that DOTAP, as one of the widely used cationic liposome materials in recent years, can reduce particle size, increase membrane fluidity and increase cell uptake through literature research. So what is the innovation of this study?
2. The conclusions obtained in this paper are the same as the results of the literature survey. Is your research meaningful?
3. The notes in Figure 4 are the same as those in Figure 3. Please check them carefully.
4. Does line 238 refer to Table 2? Please confirm.
5. The author mentioned that β -ionone and DOTAP have certain effects on lung. Why did HeLa cells be selected as the research object?
6. In order to make the results more convincing, it is suggested to increase the quantitative analysis of fluorescence intensity in the cellular uptake of β -ionone liposomes.
7. The discussion section should be more in-depth.
8. It is mentioned in the experimental method that the polydispersion index is measured, but there is no result of this

part. Please explain.

9. In the experimental methods section, evaluation of vesicle morphology of β -Ionone liposomes using TEM was repeated. Please check it carefully.

Reviewer 3

The article is interesting, and deserves publication in the journal after some revision:

1. Table 2. from MATERIALS AND METHODS should be moved to RESULTS because it is not clear which liposomes are being discussed.
2. What the hydrodynamic diameter is given in fig. 1? Averaged over intensity or number of particles?
3. Why is there a slight decrease after the maximum in the calcein release curves in Fig. 6? For example, at 8 hours for ION-DOTAP 1 and ION-DOTAP 2 systems, at 4 hours - for ION-DOTAP 3.
4. What is the encapsulation efficiency of calcein? Could this have affected the release profiles?
5. For publication in such a journal, it is not enough to conduct research on a model substrate. It is necessary to load an anticancer drug, for example, doxorubicin, into the developed systems and study the change in its cytotoxicity when encapsulated in liposomes.
6. Why was this cell line chosen?
7. Why is this β -ionone molar ratio chosen? Have other molar ratios been tested?

Lots of misprints. Authors should carefully read their manuscript. For example:

1. Lines 49-50: «in lung cancer cell death, (A549) through» replace with «in lung cancer cell (A549) death, through»;
2. Line 79: «Cationic lipid 17» replace with «Cationic lipid17»;
3. Line 103: link is incorrectly formatted «(Wang et al., 2012)»;
4. Line 120: «DOTA» replace with «DOTAP»;
5. Line 215: the section name is given twice;
6. Lines 266-267: «DOTAP, a cationic phospholipid containing an NH₄⁺ group, is low in toxicity compared to other cationic lipids¹⁸.». Exactly the same sentence was given in the introduction (lines 80-82). Remove duplication;
7. Calcein sometimes writes with a small letter, sometimes with a capital letter. Bring everything to the same view.

Andang MIATMOKO <andang-m@ff.unair.ac.id>
To: Scientific Reports <srep@nature.com>

Thu, Jan 12, 2023 at 5:47 PM

Dear Editor,

Could you please give us an extension to submit the revision for about 2-3 weeks? Since there was an end of year holiday, we needed to arrange the revision appropriately.

Many thanks

[Quoted text hidden]

--

Salam,

[Andang Miatmoko, PhD., Apt.](#)

[Department of Pharmaceutical Sciences](#)
[Faculty of Pharmacy, Airlangga University](#)
[Nanizar Zaman Joenoes Building](#)
[Campus C Airlangga University, Mulyorejo, 60115](#)
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Pooja Bisht <srep@nature.com>
Reply-To: Pooja Bisht <srep@nature.com>
To: andang-m@ff.unair.ac.id

Fri, Jan 13, 2023 at 12:15 PM

Dear Dr. Miatmoko,

Thank you for your email.

This will not be a problem – we do appreciate that some revisions do take longer than others and we would be more than happy to accommodate an extension for you till 31 Jan 2023.

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Best Regards,

Pooja BishtEditorial Support at [Scientific Reports](#)

On Thu, 12 Jan at 10:47 AM , Andang-m <andang-m@ff.unair.ac.id> wrote:

[External - Use Caution]

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Pooja Bisht <srep@nature.com>
Reply-To: Pooja Bisht <srep@nature.com>
To: andang-m@ff.unair.ac.id

Fri, Feb 10, 2023 at 11:35 PM

Dear Dr. Miatmoko,

Hope this email finds you well.

As per your request we grant you further extension till 24 Feb 2023.

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This will not be a problem – we do appreciate that some revisions do take longer than others and we would be more than happy to accommodate an extension for you till 31 Jan 2023.

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andang miatmoko <andang-m@ff.unair.ac.id>

Scientific Reports: Decision on your manuscript

1 message

Scientific Reports <srep@nature.com>
To: andang-m@ff.unair.ac.id

Fri, Mar 3, 2023 at 9:07 PM

Ref: Submission ID cdf48bd0-0dc3-4db5-96f2-4bfd93589444

Dear Dr Miatmoko,

Re: "The Effect of 1,2-Dioleoyl-3-Trimethylammonium Propane (DOTAP) Addition on the Physical Characteristics of β -Ionone Liposomes"

We are pleased to let you know that your manuscript has now passed through the review stage and is ready for revision. Many manuscripts require a round of revisions, so this is a normal but important stage of the editorial process.

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Kind regards,

Arezoo Sodagar Taleghani
Editorial Board Member
Scientific Reports

Reviewer Comments:

Reviewer 3

I am satisfied with the work that the authors have done to correct all my comments and answer my questions.

Reviewer 1

There are some concerns in the revision

Line 18 efficiency1817 , check the citation style

Line 470 Liposomes were prepared according to the formula in the Table 2 by adding Coumarin-6 to each mL of liposomes. Please mention the amount of Coumarin-6 added.

Line 482 1x 10⁷ cells per mL is too high for a 6 well plate.

Line 484-485 The medium was replaced with β -ionone liposomes at a coumarin-6 concentration of 10g/mL. The concentration 10g/ml, is that correct?

Line 132 "However, the size decreased with the addition of DOTAP to the formula, as shown in Figure 1.A", From figure 1 A, the size increased with the addition of DOTAP.

For the comment no 10, "Since both Lipo-DOTAP-Blank and ION-DOTAP-3 have the same amount of DOTAP, why is there a difference in Coumarin-6 intake by the cells"

The response was. "LIPO-DOTAP-Blank has a high deviation, probably because the presence of beta-ionone affects it." However, from the figure, the deviation is not high and LIPO-DOTAP-Blank does not have beta-ionone. LIPO-DOTAP-Blank is the second bar in the graph, and it has a low error bar.

Line 274-275 The results showed that there is an increase in cytotoxicity of the ION-DOXO-DOTAP-2 formula in T47D cells (Fig. 9B). There is a mismatch between the statement and the figure 9 description where its written 9.B is HeLa and 9C is T47D.

The IC 50 values from 9.C should be much less than 1 μ g/ml and ION-DOXO-DOTAP 0 and ION-DOXO- DOTAP 2 should have similar IC 50 values.

Reviewer 2

The authors have responded well to the reviewers' comments, and the manuscript is ready for publication



andang miatmoko <andang-m@ff.unair.ac.id>

Scientific Reports: Decision on your manuscript

1 message

Scientific Reports <srep@nature.com>
To: andang-m@ff.unair.ac.id

Tue, Mar 14, 2023 at 1:11 PM

Ref: Submission ID cdf48bd0-0dc3-4db5-96f2-4bfd93589444

Dear Dr Miatmoko,

Re: "The Effect of 1,2-Dioleoyl-3-Trimethylammonium Propane (DOTAP) Addition on the Physical Characteristics of β -Isonone Liposomes"

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Reviewer Comments:

Reviewer 1

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1. Publishing and rights

Submission status	Date
Publishing and rights complete	14 Mar 2023
Submission is in publishing and rights	14 Mar 2023

2. Peer review

Submission status	Date
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Submission accepted	14 Mar 2023
Submission under peer review	09 Mar 2023
Submission passed technical check	09 Mar 2023
Revision received	07 Mar 2023
Submission under peer review	15 Feb 2023
Submission passed technical check	15 Feb 2023
Amendment received	14 Feb 2023
Revision received	13 Feb 2023
Submission under peer review	28 Sep 2022

3. Technical check

Submission status	Date
Submission passed technical check	28 Sep 2022
Amendment received	28 Sep 2022
Submission is under technical check	27 Sep 2022

4. Submission received

Submission status	Date
Submission received	27 Sep 2022

The effect of 1,2-dioleoyl-3-trimethylammonium propane (DOTAP) Addition on the physical characteristics of β -ionone liposomes

by Andang Miatmoko

Submission date: 30-Mar-2023 09:45AM (UTC+0800)

Submission ID: 2050480293

File name: s41598-023-31560-5.pdf (2.27M)

Word count: 8210

Character count: 45465



OPEN The effect of 1,2-dioleoyl-3-trimethylammonium propane (DOTAP) Addition on the physical characteristics of β -ionone liposomes

Andang Miatmoko^{1,2,✉}, Febe Harum Asmoro¹, Andre Alwi Azhari¹, Noorma Rosita¹ & Chin-Shiu Huang³

β -ionone (ION) is a cyclic terpenoid compound that demonstrates considerable potential for the prevention and treatment of cancer. However, the water solubility of β -ionone is poor and the compound demonstrates low permeability. Liposomes have been reported as increasing both qualities. In this study, the development of β -ionone liposomes was initiated by adding 1,2-dioleoyl-3-trimethylammonium propane (DOTAP) to produce cationic liposomes as a means of enhancing binding to cancer cells. Liposomes composed of β -ionone, HSPC, cholesterol, and DSPE-mPEG₂₀₀₀ were prepared using the thin layer hydration method. Cellular uptake studies were carried out with HeLa cells incubated with β -ionone liposomes for two hours. The results indicated that the addition of DOTAP increased particle size and affected the spectroscopical and thermogram profiles of the liposomes, thereby confirming reduction in liposome crystallinity, while the zeta potential became positive. Moreover, the calcein release profile further showed that additional DOTAP increased both membrane fluidity and cellular uptake in HeLa cells. In conclusion, adding DOTAP affected the physicochemical cationic properties of liposome and improved cellular uptake in HeLa cells.

β -ionone is a cyclic terpenoid compound with a basic core structure of retinoic acid, retinol, -carotene, and vitamin A which is found in many fruits, vegetables, and grains such as carrots, raspberries, almonds, tea, and tomatoes¹. Research has shown that β -ionone has the potential to both prevent and treat several types of cancer, such as breast, colon, lung, and liver². The anticancer activity involves several mechanisms, including antiproliferation, where β -ionone inhibits the proliferation of lung cancer cells (A549) by inducing apoptosis as seen from the increased expression of the Bax gene and reduced expression of the Bcl-2 gene which suppresses the expression of cPLA2 and COX-2 proteins³. β -ionone causes apoptosis, as seen in lung cancer (A549) cell death, (A549) through loss of mitochondrial membrane potential as measured by staining using rhodamine-123⁴. Besides that, β -Ionone can inhibit the in vitro proliferation of breast cancer cells. One of the mechanisms for inhibiting MCF-7 cell proliferation is reducing cyclooxygenase (COX-2) activity in the induction of apoptosis of MCF-7 cells. β -Ionone at a concentration of less than 50 $\mu\text{mol/L}$ can reduce the expression of COX-2 protein in mice tumor tissue, which is considered a target for cancer molecules⁵.

Dissolving β -ionone in water at a level of 0.169 g/L is extremely difficult. β -ionone also has low permeability with $\log P = 4.67$ and is classified as falling within Biopharmaceuticals Classification System (BCS) class IV. Numerous studies have shown that using liposomes can improve the solubility and permeability of compounds belonging to class IV BCS⁶.

Liposomes are lipid vesicles consisting of an aqueous phase core surrounded by one or more phospholipid bilayers⁹. Liposome membranes are composed of natural and/or synthetic phospholipids which are relatively biocompatible, biodegradable, and non-immunogenic. Due to the unique bilayer structure, liposomes act as

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carriers for both lipophilic and hydrophilic materials. The latter materials are in the aqueous phase, while the former are trapped in the lipid bilayer¹⁰.

The liposome delivery system is reported as overcoming Lopinavir's low solubility and bioavailability to increase therapeutic efficacy¹¹. The use of liposomes can also increase drug permeability. The particle size of liposomes allows higher intracellular uptake than other systems, thereby increasing drug bioavailability. Previous research has indicated that liposome encapsulation can significantly increase macrovascular permeability after administration of Paclitaxel liposomes¹², where Paclitaxel is also classified as the compound in BCS class 4, like β -ionone.

The nature and charge density of liposomes affect the stability, kinetics, and extent of biodistribution and interactions with liposome uptake by target cells¹³. Cationic liposomes have the advantage of promoting higher therapeutic efficacy against cancer cells⁹, where they can bind to negatively charged cell membranes. Other studies have shown that they accumulate selectively in tumor angiogenic endothelial cells and are superior to anionic or neutral liposomes^{14,15}. The research results obtained by Peters et al. (2015) showed that the cell uptake of 1,2-Dioleoyl-3-trimethylammonium propane (DOTAP)-liposomes was higher than that of the 1,2-dioleoyl-L- α -glycero-3-phosphatidylethanolamine (DOPE)-liposomal formulation. Another study using DOTAP as lipid composition demonstrated that transgene expression was increased in tumor cells due to the uptake of liposome-DNA complexes by tumor cell phagocytosis in vitro¹⁶. Research conducted on the cytotoxicity test confirmed that adding DOTAP to liposomes increased the cytotoxicity of HepG2 cancer therapy¹⁷.

Cationic lipid¹⁷ strongly influences biodistribution and drug efficiency^{17,18}. DOTAP is a phospholipid type frequently employed as a component of cationic liposomes. DOTAP contains an NH^{4+} group which renders it positively charged and is low in toxicity compared to other cationic lipids¹⁹. Based on the research of Chang et al. (2018), the addition of DOTAP to the liposome formulation causes a change in size²⁰. The liposomes prepared with PC only have a larger size than those prepared with a 1:1 ratio of PC and DOTAP (PC/DOTAP liposomes) with the same amount of lipids. This is because by accumulating the same amount of lipids, DOTAP, which has a shorter lipid chain, reduces the size of the liposome particle²¹. In the DOTAP-DNA complexes, the charge ratio of lipid/DNA highly affects the physical properties of the liposome. The addition of low-concentration DOTAP produced a larger size, namely 190 ± 8.4 nm, compared to liposomes with a high concentration of DOTAP, which was 140 ± 3.8 nm²². DOTAP-induced changes at the membrane interface may facilitate electrostatic interactions in the polar head region. This physical interaction can decrease the particle size by reducing the radius of bilayer curvature²³. In addition, the low lipid/DNA charge ratio enhanced the repulsion of excess negative charges, resulting in small particle sizes.

The relationship between liposome vesicle size and cell interactions was observed by means of the uptake from bone marrow-derived macrophages (BMDM). The absorption of BMDM from small DOTAP:DOPE liposomes was superior to that of their larger counterparts. Small cationic liposomes tend to be distributed to lymph nodes more rapidly from the injection site than larger ones. The liposome charge was also observed where the addition of DOTAP as a cationic lipid resulted in an extremely positive zeta potential of approximately $+40$ mV to $+60$ mV²⁴.

It was additionally reported that the dispersibility of liposomes following the addition of DOTAP was more effective than without DOTAP due to the presence of electrostatic forces²⁵. Furthermore, another study stated that adding DOTAP can render liposomes spherical with an approximate size of 100 nm¹⁵. On the other hand, adding DOTAP can increase the fluidity of the bilayer membrane. This is evidenced by the research of Takechi-Haraya et al. (2016), where DOTAP/Chol (90/10) has a liquid disorder arrangement, while DOTAP/Chol (50/50) has a liquid order arrangement which was observed using the Atomic Force Microscope (AFM). It is also proven by the deformation force curve where DOTAP/Chol (50/50) is steeper than DOTAP/Chol (90/10)²⁶.

Lipophilic compounds such as β -ionone will be entrapped in the liposome membrane which will, in turn, affect its fluidity. The results showed that the greater the amount of β -ionone trapped, the faster the release rate²⁷. Previous studies have also reported that drug compounds can interact with phospholipids, thereby affecting membrane fluidity^{28–32}. Therefore, the membrane's fluidity will affect the stability of the drug entrapment in liposomes and the drug released during circulation, thus determining the effectiveness of the treatment²⁸. Therefore, it is necessary to test the fluidity of the membrane, one method being calcein release. Calcein is a water-soluble, quenching, and fluorescent substance widely used in cell viability studies³³. Calcein release studies can be undertaken to determine membrane stability related to the presence or absence of liposome leakage measured using a spectrofluorometer³⁴.

In this study, the effect of the addition of different levels of DOTAP on the membrane's physical characteristics and the conducting of a fluidity test were evaluated. The data obtained may yield information on the effect of adding DOTAP to β -ionone liposomes and the development of further liposome formulations for inclusion in anticancer therapy.

Result

The particle size and zeta potential of β -ionone liposomes. The addition of DOTAP concentration tended to increase particle size. The largest liposome with an average size of 191.27 ± 4.20 nm was achieved by lipo-DOTAP blank. However, the size increased with the addition of DOTAP to the formula, as shown in Fig. 1a. PDI measurements were carried out to determine the homogeneity of particle size. Particles with smaller PDI values indicate a system with a homogeneous size distribution. PDI values > 0.7 indicate that the preparation has an extensive distribution of particle sizes³⁵. The lowest PDI value was obtained by the Liposome Blank formula, as shown in Fig. 1b.

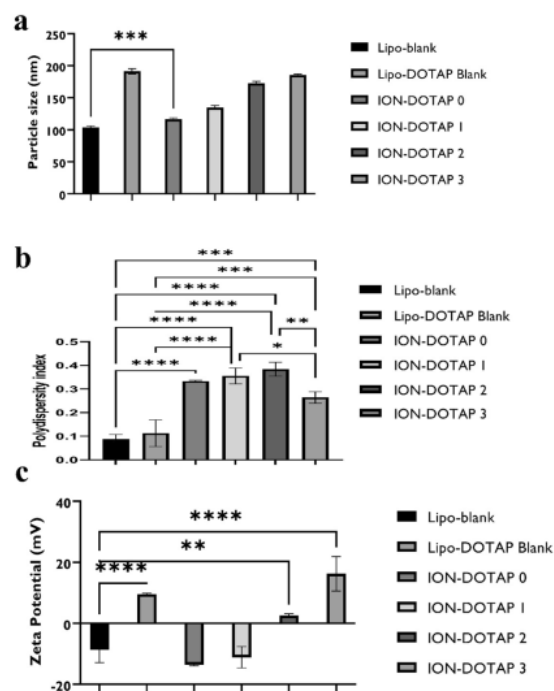


Figure 1. The bar charts of average (a) particle size value, (b) polydispersity index and (c) zeta potential of β -ionone liposomes prepared with various concentrations of DOTAP. Lipo-Blank contained no DOTAP or β -ionone, while Lipo-DOTAP Blank contained DOTAP at the highest concentration without the addition of β -ionone, * $p < 0.5$, ** $p < 0.01$, *** $p < 0.001$, compared to Lipo-Blank.

For the zeta potential value, the results indicated that the Lipo-blank, ION-DOTAP-2, and ION-DOTAP-3 liposomes differed significantly from liposomes ION-DOTAP-0, while Lipo-blank and ION-DOTAP-1 did not show any difference with ION-DOTAP-0 as shown in Fig. 1c.

The vesicle morphology of β -ionone liposomes analyzed by transmission electron microscopy (TEM). From the results of the vesicle morphology analysis with TEM contained in Fig. 2, it can be observed that the vesicles of ION-DOTAP-0 liposomes appear spherical with unilamellar membrane formation. In con-

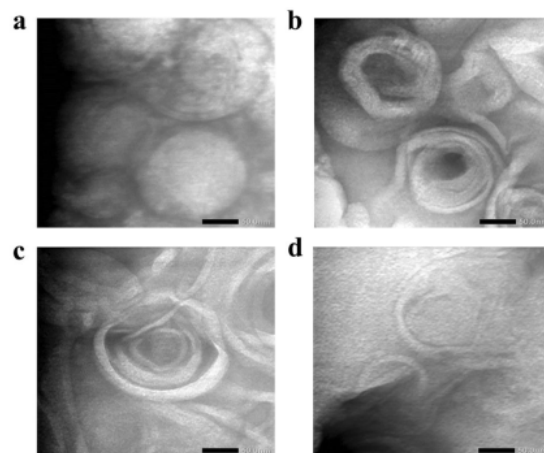


Figure 2. Morphological observations of (a) ION-DOTAP-0, (b) ION-DOTAP-1, (c) ION-DOTAP-2, and (d) ION-DOTAP-3 liposomes were analyzed using Transmission Electron Microscopy (scale bar: 50 nm).

trast, the formula, while also apparently spherical in shape, has a layered membrane due to the addition of DOTAP.

The spectra profiles of fourier transform infrared spectroscopy (ftir) of β -ionone liposomes. Identification of the compound's structure was completed using the FTIR Alpha II Spectrophotometer within the range of wave numbers $4000\text{--}600\text{ cm}^{-1}$. The spectra of liposome constituents can be seen in Fig. 3, while those of the liposome formula produced can be seen in Fig. 4.

In the FTIR analysis, the Lipo-blank liposome spectra showed absorption bands identical to those in the spectra of the liposome constituents, as shown in Fig. 3. However, a decrease in the intensity of the absorption band after liposome formation occurred. The observed bands of the N-H functional group ($3300\text{--}3500\text{ cm}^{-1}$) were identical to the DOTAP absorption band, the C=C group band ($1640\text{--}1680\text{ cm}^{-1}$) was identical to the β -ionone absorption band, and the C-O group band ($1050\text{--}1150\text{ cm}^{-1}$) was identical to the absorption band of β -ionone, DSPE-mPEG₂₀₀₀, and cholesterol. The CH₃-CH₂-CH group ($2850\text{--}3000\text{ cm}^{-1}$) was identical to the absorption band of all the constituents of liposomes.

According to the overlaid spectra of β -ionone liposomes prepared with various concentration of DOTAP shown in Fig. 4, there were observed bands of N-H, CH₃-CH₂-CH, C=C (alkene), dan C-O functional groups for all formula. There are disappearances of some identical peaks of β -ionone and DOTAP observed for β -ionone liposomes with DOTAP addition. The spectra profiles of β -ionone liposomes show increasing the intensity and widening of absorption band of N-H groups that represent the DOTAP. However, spectra shown for each β -ionone liposomes showed no significant differences in the intensity of same type of absorption band.

Differential thermal analysis (DTA) of β -ionone liposomes. The thermal analysis results produced using differential thermal analysis (DTA) on each liposome component and liposome formula can be seen in the thermogram shown in Fig. 5. The results show that the DTA thermogram of liposome constituents, i.e., DOTAP, experienced endothermic peaks at $86.0\text{ }^{\circ}\text{C}$ and $200.8\text{ }^{\circ}\text{C}$, while the HSPC thermogram registered two endothermic peaks at $83.6\text{ }^{\circ}\text{C}$ and $182\text{ }^{\circ}\text{C}$. On the other hand, the DSPE-mPEG2000 thermogram recorded endothermic peaks at $59.2\text{ }^{\circ}\text{C}$ and $249.2\text{ }^{\circ}\text{C}$, and cholesterol at $50.9\text{ }^{\circ}\text{C}$ and $152.5\text{ }^{\circ}\text{C}$. The Lipo-blank, which consisted of a mixture of cholesterol, HSPC, and DSPE-mPEG2000, registered two endothermic peaks at $139.2\text{ }^{\circ}\text{C}$ and $214.0\text{ }^{\circ}\text{C}$. The formation of liposomes produced endothermic peak shifts.

Figure 5 also contains the results of the overlaid DTA thermograms of the liposomes, namely: DOTAP, Lipo-blank, Lipo-DOTAP Blank, ION-DOTAP-0, ION-DOTAP-1, ION-DOTAP-2, and ION-DOTAP-3. Each formula had two endothermic peaks. For example, thermograms on Lipo-DOTAP Blank, ION-DOTAP-0, and ION-DOTAP-1 liposomes produced endothermic peaks of approximately $140.2\text{--}140.8\text{ }^{\circ}\text{C}$ and $213.2\text{--}214.2\text{ }^{\circ}\text{C}$. The endothermic peak formed was identical to the peaks of its constituents, namely: cholesterol and DOTAP.

Furthermore, the thermogram of ION-DOTAP 2 liposomes underwent a shift with endothermic peaks at temperatures of $176\text{ }^{\circ}\text{C}$ and $114\text{ }^{\circ}\text{C}$. Meanwhile, the ION-DOTAP-3 liposomes, which contain the highest

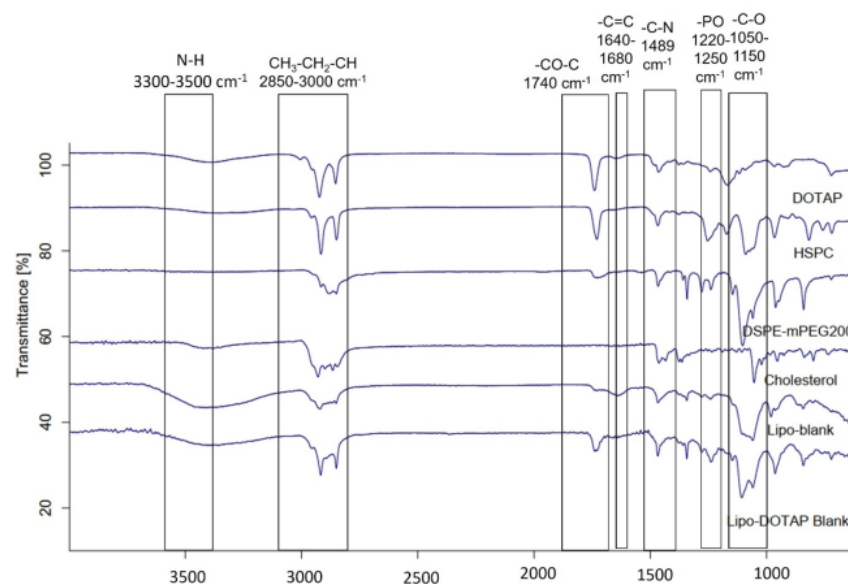


Figure 3. FTIR spectra of β -ionone, DOTAP, HSPC, DSPE-mPEG2000, cholesterol, Lipo-Blank, and Lipo-DOTAP Blank. Lipo-Blank contained no DOTAP or β -ionone, while Lipo-DOTAP Blank contained DOTAP at the highest concentration without the addition of β -ionone.

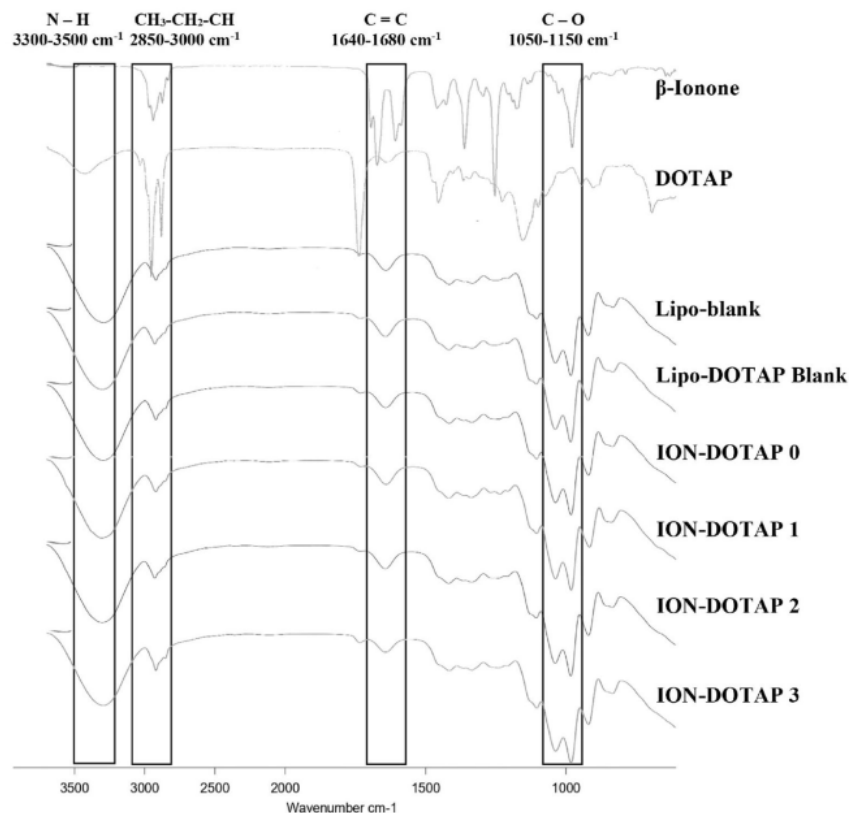


Figure 4. FTIR spectra of β -ionone, DOTAP, ION-DOTAP liposomes, and Lipo-DOTAP Blank, ION-DOTAP 1, ION-DOTAP 2, ION-DOTAP 3. Lipo-Blank contained no DOTAP or β -ionone, while Lipo-DOTAP 3 contained DOTAP at the highest concentration without the addition of β -ionone.

concentration of DOTAP, experienced a significant shift of endothermic peaks at 81.6 °C and 214.1 °C, with relatively wide peaks when compared to the those of the other liposomes. It also resulted in the loss of the endothermic peak of 152.5 °C, which is identical to that of cholesterol. This result confirms that the ION-DOTAP-3 thermogram experienced an endothermic peak increasingly similar to that of DOTAP.

The calcein released from β -ionone liposomes. Calculation of the cumulative percentage of the calcein released from the liposomes indicates that the smallest percentage of the calcein released was in the ION-DOTAP-0, indicating that this liposome has the most rigid structure compared to those of other formulas as presented in Fig. 6. Meanwhile, the highest percentage of released calcein was observed in ION-DOTAP 3 liposomes.

In addition, the release flux value obtained from this study reveals that the liposome ION-DOTAP-3 had the largest flux value (6.7074 ± 1.8650), followed by ION-DOTAP-2 (2.8357 ± 0.7453), ION-DOTAP 1 (1.6169 ± 0.4700) and ION-DOTAP-0 (0.2674 ± 0.0545) as shown in Table 1.

The cellular uptake of β -ionone liposomes in HeLa cells. In Fig. 7, cells in the LIPO-blank treatment can be observed where the fluorescence intensity of coumarin-6 loaded into liposomes was relatively low. The liposomes containing coumarin-6 with high DOTAP levels increased the fluorescence intensity. Figure 7 shows that ION-DOTAP-0 liposomes containing coumarin-6 infiltrated the HeLa cells indicated by green fluorescence, while ION-DOTAP-1 liposomes were only slightly fluorescent. However, they were less visible and the fluorescence of the ION-DOTAP-2 liposomes was relatively weak in intensity as shown in the cell membrane and cytoplasm.

Meanwhile, as shown in Fig. 8, the ION-DOTAP-3 liposome with the highest DOTAP content increased the fluorescence intensity and produced the highest cellular coumarin-6 levels than other formulas.

In vitro cytotoxicity evaluation of β -ionone liposomes. The IC_{50} cytotoxicity test on HeLa cells aims to determine the effect of adding DOTAP to β -ionone liposomes in cancer therapy. The cells incubated with

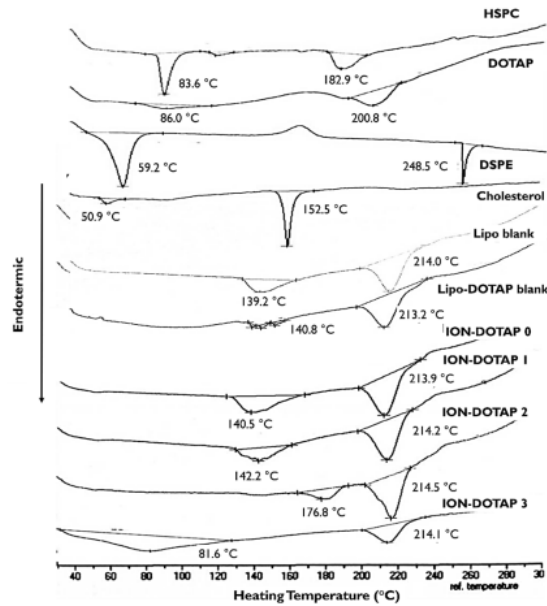


Figure 5. Thermogram profile of HSPC, DOTAP, DSPE-mPEG2000, Cholesterol, Lipo-Blank, Lipo-DOTAP Blank, ION-DOTAP-0, ION-DOTAP-1, ION-DOTAP-2, and ION-DOTAP-3.

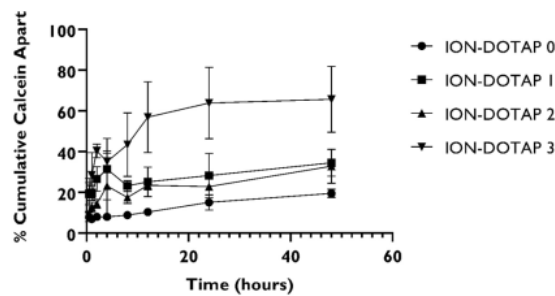


Figure 6. The calcein release profile of ION-DOTAP-0, ION-DOTAP-1, ION-DOTAP-2, and ION-DOTAP-3 liposomes in PBS pH 7.4 as the release media at a temperature of 37 °C.

Formula	Calcein release flux ($\mu\text{g}/\text{cm}^2/\text{h}$, n = 3)
ION-DOTAP 0	0.2674 ± 0.0545
ION-DOTAP 1	1.6169 ± 0.4700
ION-DOTAP 2	2.8357 ± 0.7453
ION-DOTAP 3	6.7074 ± 1.8650

Table 1. The release flux value of calcein released from β -ionone liposomes prepared with various concentrations of added DOTAP.

β -ionone liposomes for 48 h resulted in the percentage of cell viability shown in Fig. 9A. It can be seen that HeLa cells were not sensitive to β -ionone liposomes, and all treatments resulted in high cell viability.

To further evaluate the cytotoxic effects on these cells, DOXO was co-loaded into β -ionone liposomes. As shown in Fig. 9B, an increase in the cytotoxic effect also occurred in T47D cells, where the ION-DOXO-DOTAP-2 formula gave the lowest IC_{50} value compared to other liposome formulas, indicating its high cytotoxicity. On the other hand, ION-DOXO-DOTAP-0, which does not contain DOTAP, produces the smallest IC_{50}

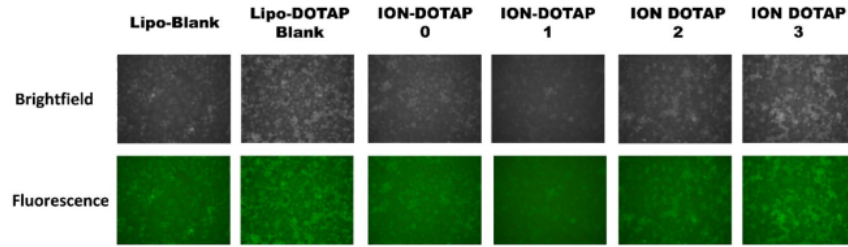


Figure 7. Photomicroscopy of cellular uptake of HeLa cells after incubation with β -ionone liposomes containing Coumarin-6 (in green color) prepared with various concentrations of DOTAP for two hours at 20 \times magnification under a fluorescence microscope.

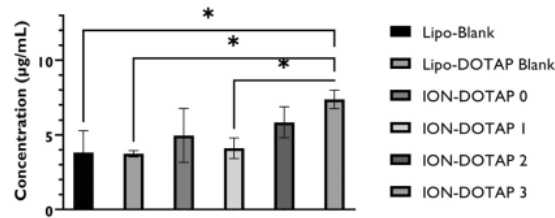


Figure 8. Coumarin-6 levels in HeLa cells post-incubation with β -ionone liposomes containing Coumarin-6 prepared with various concentrations of DOTAP for two hours.

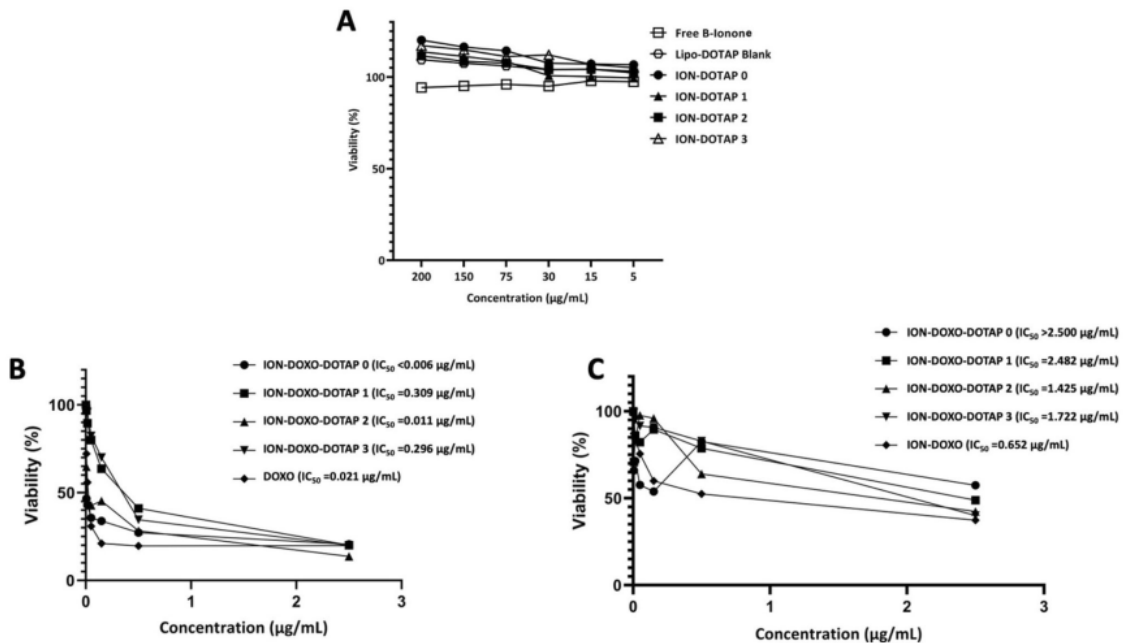


Figure 9. The viability of (A) HeLa cells after incubation of β -ionone liposomes and (B) T47D and (C) HeLa cells after incubation with β -ionone liposomes co-loaded with doxorubicin for 48 h.

value. The results also showed that there is an increase in cytotoxicity of the ION-DOXO-DOTAP-2 formula in HeLa cells; however, ION-DOXO-DOTAP-0 produces the highest IC₅₀ value (Fig. 9C).

Discussion

In this study, liposomes were prepared to deliver β -ionone, a low water soluble and poorly permeable drug, used in anticancer treatment^{6,7}. However, the composition of phospholipids significantly affects liposomes' characteristics which may relate to their pharmacological activity. Therefore, this study evaluated the effect of the addition of DOTAP on the physical characteristics of β -ionone-loaded liposomes, including: particle size, zeta potential, morphology, infrared (IR) spectroscopic profiles, thermal analysis, and calcein release.

The addition of DOTAP affects the physicochemical characteristics of the liposomes, with increased DOTAP concentration resulting in larger particle size. This is probably due to the HSPC, cholesterol, and DSPE-mPEG2000 components which have ordered molecule arrangements producing a rigid membrane structure³⁶. Moreover, the possibility exists of interaction between the polar groups of DOTAP and the choline groups of HSPC, both of which are positively charged and therefore repel each other, leading to a widening of the bilayer membrane's space.

The spectra of liposomes loaded β -ionone with DOTAP addition experienced a reduction in the intensity and widening of the absorption band in the N-H group which is characteristic of DOTAP. The N-H group in DOTAP is the polar section of the phospholipid head³⁷. Furthermore, the analysis of the absorption band on the alkyl group (CH₂) can be used as an indicator of the regularity of the lipid chain arrangement. The reduced intensity and widening of the absorption band indicate an increase in the gauche conformation³⁸. The spectra of liposomes formed showed a decrease in the intensity of the absorption band on the alkyl group, indicating a potential increase in the gauche conformation, which changed the composition of the lipid chain thereby rendering it more fluid.

Several identical peaks in DOTAP and β -ionone were not visible in the spectra of the liposomes loaded β -ionone with DOTAP addition, indicating that there could be physical interaction³⁹. Meanwhile, the spectra shown for each liposome formula did not indicate a significant difference in the intensity of the absorption bands.

FTIR analysis, in determining the interaction of DOTAP with liposome membranes, can be performed by observing the absorption band on certain functional groups, namely: the phosphate group (R-PO₂-R') and the ester group (-CO-O) present on the head of HSPC which is polar⁴⁰. Neither the Lipo-blank FTIR spectra nor the blank Lipo-DOTAP showed any absorption bands in the two functional groups. Therefore, it is thought that hydrogen bonding occurs in both the phosphate and phospholipid ester groups due to stretching of the absorption band⁴¹. Lipo-blank and Lipo-DOTAP Blank liposomes did not show a significant shift in the absorption band and no new band formation, leading to the conclusion that only chemical interaction and no physical interaction occurs. Moreover, the ION-DOTAP-3 thermogram shows an endothermic peak that is increasingly similar to that of DOTAP. The widening and disappearance of the endothermic peaks on the thermogram indicate a change in the regularity of the lipid structure lipid⁴². In other words, adding DOTAP can reduce the rigidity of the membrane, thereby rendering it more fluid.

The calcein release test showed that the liposome ION-DOTAP-3 experienced the highest calcein release compared to the other formulas. The release of calcein from the liposomes increased in line with that of membrane fluidity³³. It can therefore be concluded that ION-DOTAP 3 demonstrates the highest membrane fluidity. Based on the results of the analysis conducted to determine the physical characteristics of β -ionone liposomes, the addition of DOTAP can increase the particle size, rendering the zeta potential positive with the morphology of liposomes appearing spherical with a layered membrane. Furthermore, the results of thermogram data and spectroscopic profiles, show that the addition of DOTAP can cause the composition of the bilayer membrane to become more fluid. This result is in line with the calcein release profile which indicates that adding DOTAP can increase the fluidity of the liposome membrane.

With regard to the surface charge of liposomes, the liposome constituents such as HSPC and DSPE-mPEG₂₀₀₀ have a neutral charge, while β -ionone and cholesterol have no charge or are non-ionic⁴³. Meanwhile, DOTAP is a positively charged phospholipid with an NH⁴⁺ group on the head of the phospholipid¹⁹. Consequently, adding DOTAP to liposomes increased the positivity of the potential charge. Increasing the concentration of DOTAP heightened the positive charge of the liposomes.

A cellular uptake test was carried out to determine the ability of β -ionone liposomes to infiltrate the HeLa cells. The levels of liposomes entering HeLa cells were calculated as coumarin-6 levels. Coumarin-6 is a hydrophobic compound trapped in the bilayer membrane and the measured coumarin-6 level indicates how many liposomes enter the cell. The green fluorescence indicates that β -ionone liposomes containing coumarin-6 are present in the cells⁴⁴. The more intense the fluorescence color, the greater the amount of coumarin-6 absorbed⁴⁵. Based on the findings of the quantification analysis of coumarin-6 levels, the ION-DOTAP-3 formula produced higher levels than other formulas. It is because the ION-DOTAP-3 liposomes contain cationic lipid (DOTAP) at a higher concentration than other formulas, resulting in a significant ionic interaction between negatively charged cancer cells and β -ionone liposomes containing DOTAP (cationic lipid)⁴⁶.

It was also reported that particle size affects the endocytosis pathway of liposomes into cells. In this study, rising levels of DOTAP increased the particle size of liposomes, although this was in the range of < 200 nm which was still relatively modest. Consequently, no significant effect on cellular uptake was regarded as having occurred. The entry of β -ionone liposomes was due to the addition of DOTAP to cells through a process of endocytosis which requires energy to introduce foreign particles into the cell. The results demonstrated a significant difference between the ION-DOTAP-3 and the blank lipid formula, blank ION-DOTAP, and ION-DOTAP-1. In this study, it can be seen that, with the addition of higher DOTAP levels, β -ionone liposomes can increase cell uptake

in HeLa cells. This greater cell uptake leading to increased coumarin-6 levels and microscopic observations of HeLa cells showed that the intensity of coumarin-6 rose with the occurrence of higher DOTAP levels.

The IC₅₀ cytotoxicity test was carried out on HeLa cells to determine the effect of adding DOTAP to β -ionone liposomes in cancer therapy. Unfortunately, β -ionone liposomes had no cytotoxic effects even though the cellular uptake test results show that liposomes entered the cells. This result indicates that it is possible that these cells are not sensitive to β -ionone, which becomes the limitation of our study. Therefore, a cytotoxicity test was carried out by co-encapsulating DOXO into β -ionone liposomes (ION-DOXO-DOTAP liposomes). DOXO has been known as an anticancer compound sensitive to HeLa cells and T47D cells. The results showed that the ION-DOXO-DOTAP liposomes had toxicity to both cancer cells. The ION-DOXO-DOTAP-2 had the highest cytotoxicity. Compared to the ION-DOXO-DOTAP-0, which had no DOTAP addition, the toxicity is relatively similar to that of DOXO. This is related to liposome formulations with the addition of DOTAP providing better retention than liposomes without DOTAP. The increased drug release was proportional to the cell uptake, which also increased with the addition of DOTAP. This result is in line with the study of Lei et al. (2011), where it was reported that cytotoxicity results were associated with cellular uptake. Therefore, high cellular uptake will give an increased cytotoxicity value⁴⁷.

Conclusions

The results of this study indicate that the addition of DOTAP to β -ionone liposomes increased the particle size slightly and rendered the charge of the liposomes positive. In addition, the higher the DOTAP concentration added, the more fluid the liposomal membrane as indicated by the reduction in membrane crystallinity which supported the higher calcein release from the liposomes. The introduction of DOTAP into liposomes highly affects the physical properties of β -ionone liposomes, thereby increasing the cellular uptake and cytotoxicity. Proposing the incorporation of DOTAP into β -ionone liposomes requires further studies on its anticancer efficacy. From the results of this analysis, although the addition of DOTAP renders the liposomes more fluid, its ability to enhance the cellular uptake provides potential benefits when used as a delivery system for cancer therapy.

Materials and methods

Materials. In this study, β -ionone was purchased from Sigma-Aldrich Inc. (Saint Louis, USA). The liposome component was 1,2-dioleoyl-3-trimethylammonium propane (DOTAP), Hydrogenated Soybean Phosphatidylcholine (HSPC), and Distearoyl-glycerophosphoethanolamine-N-(Carbonyl-methoxy polyethylene glycol 2000 (DSPE-mPEG₂₀₀₀), which were products of Nof Corporation (Tokyo, Japan). Cholesterol was obtained from Wako Inc., Ltd. (Osaka, Japan), while calcein (Fluorexone[®]) was a product of Nacalai Tesque Inc. (Kyoto, Japan). The buffer components include phosphate buffer pH 7.4, KH₂PO₄, Na₂HPO₄, NaCl, and KCl were purchased from Merck Inc. (Darmstadt, Germany). Chloroform and methanol were products of Merck Inc. (Darmstadt, Germany). Other reagents and materials used were of non-technical grade.

Preparation of β -ionone liposomes. β -ionone liposomes were prepared by the thin film hydration method. First, β -ionone, DOTAP, HSPC, DSPE-mPEG₂₀₀₀, and cholesterol were dissolved in chloroform. As shown in Table 2, appropriate amounts of each solution were, mixed in a round bottom flask. The chloroform was then evaporated and completely removed using a rotary evaporator at 55 °C to form a thin film layer on the flask wall. The thin film formed was hydrated with phosphate-buffered saline (PBS) pH 7.4 and sonicated using a sonicator at 55 °C for 10 min²⁷. Extrusion was subsequently carried out with an extruder (Avanti[®], Mini Extruder, Avanti Polar Lipid Inc., Alabama, USA) by passing liposomes through a polycarbonate membrane with various pore sizes, i.e., 400, 200, and 100 nm, with a heating block at a temperature of 55–60 °C to produce liposomes approximately 100 nm in size⁴².

Evaluation of physical characteristics of β -ionone liposomes. The particle size of β -ionone liposomes were determined by the dynamic light scattering method using a Delsa Nano C Particle Analyzer at 25 °C. Meanwhile, the zeta potential was measured using the electrophoresis light scattering method using the Malvern Zetasizer Nano Series Analyzer at 25 °C at the Sepuluh Nopember Institute of Technology (ITS), Surabaya, Indonesia. The liposomes were diluted with demineralized water, placed in a cuvette, and measured for particle size and zeta potential²⁷.

Components	Composition (mole ratio)					
	Lipo-blank	Lipo-DOTAP blank	ION-DOTAP-0	ION-DOTAP-1	ION-DOTAP-2	ION-DOTAP-3
β -ionone	–	–	30	30	30	30
DOTAP	–	8	0	2.5	5	8
HSPC	35	35	35	35	35	35
Cholesterol	25	25	25	25	25	25
DSPE-mPEG ₂₀₀₀	10	10	10	10	10	10
Hydrating buffer	Phosphate buffered saline (PBS) pH 7.4					

Table 2. Composition of β -ionone liposomes.

Evaluation of vesicle morphology of β -ionone liposomes using transmission electron microscopy (TEM).

Liposome morphology, determined using the JEOL JEM-1400 TEM (Jeol Inc., USA) instrument, was carried out at the Laboratory of Chemistry of Gadjah Mada University (UGM), Yogyakarta, Indonesia. Liposomes were first diluted using demineralized water at a volume ratio of 1:5 of liposome and water respectively. The sample was subsequently stained with 4% phosphotungstic acid at a ratio of 1:1. Samples were placed on a copper grid, and images taken at various magnification sizes⁵.

Fourier-transform infrared spectroscopy (FTIR) Analysis of β -ionone Liposomes.

FTIR evaluation was carried out to determine molecular interactions within the liposomes. Before the analysis, each β -ionone liposome was hydrated with PBS pH 7.4 containing 7% sucrose and freeze dried. FTIR analysis was then conducted using an FTIR Alpha II Spectrophotometer (Bruker, Ettlingen, Germany). The sample was put into the sample container and the spectra measured at wave numbers of 4000–600 cm^{-1} .

Differential thermal analysis (DTA) of β -ionone liposomes.

DTA is used to evaluate changes in sample properties evident from the endothermic and exothermic peaks on the DTA thermogram when heat is applied. The tests were carried out using a DTA instrument (Mettler Toledo FP90, Switzerland). Each β -ionone liposome was hydrated with PBS pH 7.4 containing 7% sucrose and freeze-dried before analysis. The freeze-dried samples were placed in aluminum crucibles and heated from 30 to 300 °C at the rate of 10 °C/min³⁷.

Evaluation of calcein release. Preparation of β -ionone liposomes containing calcein for release study.

The β -ionone liposomes were prepared on the basis of the above method. The thin lipid film formed was then hydrated with 1 mL of PBS pH 5.0 containing 3.4 mM calcein (Nacalai Tesque Inc., Kyoto, Japan). Extrusion was subsequently carried out with an extruder (Avanti[®], Mini Extruder, Avanti Polar Lipid Inc., Alabama, USA) by passing liposomes through a polycarbonate membrane with various pore sizes of 400, 200, and 100 nm with a heating block at 55–60 °C³². The free calcein was subsequently separated from the liposomes by placing them into the Amicon[®] Ultra-4 Centrifugal Filter Unit tube with a molecular weight cut-off (MWCO) of 10,000 Da (Merck Millipore, Darmstadt, Germany). The tube was centrifuged using a Hettich Rotofix 32 Benchtop Centrifuge (Hettich Lab., Tuttlingen, Germany) at 3500 rpm in order to remove the untrapped or free calcein.

Calcein release assay from β -ionone liposomes.

A calcein release study was conducted by dialysis. The liposomes were placed in the Spectra/Por[®] 7 dialysis membrane with MWCO (Spectrum Laboratories, USA) of 3500 Da. 50 mL of PBS pH 7.4 was employed as a release medium with a stirring speed of 350 rpm at 37 °C. Sampling was performed at 0.5, 1, 2, 4, 8, 12, 24, and 48 h by replacing some samples with the same volume of fresh PBS pH 7.4. The calcein fluorescence intensity was subsequently measured using the GloMax[®]-Multi Detection System (Promega Corporation, Madison, USA) microplate reader at $\lambda_{\text{ex}} = 475 \text{ nm}$ and $\lambda_{\text{em}} = 500\text{--}550 \text{ nm}$.

The cellular uptake assay of β -ionone liposomes. Preparation of β -ionone liposomes containing coumarin-6 as the fluorescent labeling agent.

The first procedure involved dissolving each liposome component in chloroform. Liposomes were prepared according to the formula in the Table 2 by adding 0.3 mg Coumarin-6 to each mL of liposomes containing 9.7 mg total lipid of HSPC, Cholesterol, and DSPE-mPEG₂₀₀₀. The amount of coumarin-6 added was in accordance with the previous study of Miatmoko et al. (2021), which successfully represented liposome uptake in cancer cells⁴⁸. Each component solution was appropriately mixed in a round bottom flask covered with aluminum foil. The organic solvent was then placed in a rotary evaporator at a temperature of 55 °C until all organic solvents were removed and formed a thin film which was hydrated using 1 mL of PBS pH 7.4, vortexed, and sonicated for 10 mins at 55 °C. Liposomes were subsequently extruded using polycarbonate membranes of sizes from 400, 200, to 100 nm to produce liposomes with a size of about 100 nm.

The cellular uptake assay.

Initially, the HeLa cells, which were obtained from Stem Cell Research and Development Center, Universitas Airlangga, were suspended in RPMI 1640 medium containing 10% Fetal Bovine Serum (FBS) and 1% Penicillin–Streptomycin antibiotic at a concentration of 1×10^6 cells/mL, placed into a 6-well plate, and incubated at 37 °C with 5% CO₂ environment for 24 h. Cells can be used for cellular uptake assay if they have a confluence of 70–80%⁴⁴. The medium was replaced with β -ionone liposomes at a coumarin-6 concentration of 10 $\mu\text{g/mL}$ and incubated for two hours, with the media being disposed of. The cells were first twice washed with PBS pH 7.4 and then added to 1 mL of methanol and left for 5–10 mins. After the methanol had been discarded, the cells were washed twice with PBS pH 7.4, containing 0.5% Tween 80 and approximately 1 mL of PBS pH 7.4 containing 1% FBS was added and left for 15 min. The cells were then washed twice with PBS solution pH 7.4. The final step was to observe the cells by means of a fluorescence microscope with an FITC filter⁴⁴.

Measurement of coumarin-6 levels in HeLa cells.

In the first step, the cells were prepared with the same procedure as that for the cellular uptake assay and the samples were incubated for two hours. The medium was then discarded and the cells washed twice with PBS pH 7.4. In order to measure the amount of intra-cellular coumarin-6, the cells were lysed with 100 μL of lysis buffer containing 10 mM NaCl, 1 mM Ethylenediaminetetraacetic acid (EDTA), and 0.5% Triton X-100 by means of pipetting and added to 900 μL PBS pH 7.4 to enable collection of the samples. The cell suspension was vortexed and centrifuged at 12,000 rpm for ten minutes. After centrifugation, the supernatant's fluorescence intensity was measured using GloMax[®]-Multi Detection System (Promega Corporation, Madison, USA) microplate reader at $\lambda_{\text{ex}} = 475 \text{ nm}$ and $\lambda_{\text{em}} = 500\text{--}550 \text{ nm}$ ⁴⁴.

In vitro cytotoxicity assay. Preparation of liposomes containing doxorubicin. In addition to β -ionone liposomes, we prepared β -ionone liposomes co-loaded with Doxorubicin (DOXO) to produce ION-DOXO-DOTAP liposomes and evaluate their cytotoxicities on T47D and HeLa cells. β -ionone liposomes co-loaded with DOXO were prepared by the same formula and methods as β -ionone liposomes; however, the 0.65 M Triethylamine was used as the hydrating solution. The liposomes were then eluted through a column packed with Sephadex G50 using PBS pH 7.4. DOXO was added at DOXO/total lipid ratio of 1:5, respectively, and the mixture was then incubated in a water bath at 60 °C for 10 min. The liposomes were further separated from the free DOXO by elution through the Sephadex G50 gel column.

WST assay. The in vitro cytotoxicity test procedure was evaluated for T47D cells and HeLa cells using the WST-1 assay method. The T47D and HeLa cell were obtained from Stem Cell Research and Development Center, Universitas Airlangga, and then cultured in Roswell Park Memorial Institute (RPMI)-1640 medium containing 10% Fetal Bovine serum and 1% Penicillin Streptomycin in a humidified atmosphere at 37 °C with 5% CO₂ air supply. For the assay, T47D and HeLa cells with a concentration of 5×10^3 cells were added to each well of 96-well plates and incubated for 24 h before treatment.

To evaluate the cytotoxicity, β -ionone liposomes and β -ionone liposomes co-loaded with DOXO were prepared at various concentrations. These samples were added to each well containing the cells and incubated for 48 h. After the treatment, the cell number was determined using WST-1 assay. The cell viability was determined as the relative measurement to the untreated cells by evaluating the absorbance of each well at 440 nm using a microplate reader. The IC₅₀ values of the compounds were calculated using the percentage of living cells and analyzed using SPSS (probit) software.

Data analysis. The data for analysis consisted of the average \pm standard deviation measured in replications. First, a Kolmogorov–Smirnov test was used to analyze the numerical data for normal distribution. If the data was normal (P value ≥ 0.05), the quantitative analysis was analyzed using One Way Analysis of Variance. If the P value < 0.05 , the data analysis was followed by a post hoc test incorporating the use of Honestly Significant Difference (HSD). The analysis was conducted using the IBM SPSS Statistics software version 24.

Data availability

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

Received: 27 September 2022; Accepted: 14 March 2023

Published online: 15 March 2023

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Acknowledgements

The authors would like to thank Ms. Berlian Sarasitha Hariawan and Ms. Devy Maulidya Cahyani for kind assistantship for manuscript preparation.

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A.M.: (1) conception and design of the work, data acquisition, data analysis and interpretation; (2) critically revising the article for important intellectual content; (3) Final approval of the version to be published; (4) Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of the work are appropriately investigated and resolved. F.H.A.: (1) conception and design of the work, data acquisition, data analysis and interpretation; (2) Drafting the article; (3) Final approval of the version to be published; (4) Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of the work are appropriately investigated and resolved. A.A.A.: (1) conception and design of the work, data acquisition, data analysis and interpretation; (2) Drafting the article; (3) Final approval of the version to be published; (4) Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of the work are appropriately investigated and resolved. N.R.: (1) data analysis and interpretation; (2) critically revising the article for important intellectual content; (3) Final approval of the version to be published; (4) Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of the work are appropriately investigated and resolved. C.-S.H.: (1) data analysis and interpretation; (2) Final approval of the version to be published; (3) Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of the work are appropriately investigated and resolved.

Funding

This study was financially supported by Research Collaboration Funding between Universitas Airlangga, Indonesia and Asia University, Taiwan.

Competing interests

The authors declare no competing interests.

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