DOI: 10.1478/AAPP.99S1A32

AAPP | Atti della Accademia Peloritana dei Pericolanti
Classe di Scienze Fisiche, Matematiche e Naturali
ISSN 1825-1242

Vol. 99, No. S1, A32 (2021)

TRANSDISCIPLINARY METHODS IN THE STUDY OF BIOLOGICAL MEMBRANES: LABORATORY LEARNING BY DOING AND IMPLICATIONS FOR RESEARCH AND EDUCATION

DOMENICO LOMBARDO *

ABSTRACT. The investigation of the structural features in biological membranes represents a central topic in many aspects of biological science. It involves the study of the collective behavior of a great number of interacting macromolecules, while the study of the structure-function relationship requires the observation and calculation of a large number of key factors. The self-assembly processes involved in biomembranes represent the cornerstone of the biological systems functioning, due to the special role of the complex macromolecular assemblies containing lipids, proteins, carbohydrates, nucleic acids, and other active components. In this article, we describe the main techniques and approaches employed for the investigation of biological membranes under a multidisciplinary point of view. A special focus will be put on the future challenges in this academic research field and the related academic teaching programs, that must provide the integration of the different research approaches and protocols into a common scientific background based on multi- and trans-disciplinary methods that combine the expertise coming from the different disciplines. In this respect, the laboratory learning by doing can have strong implications for research and education activities, and stimulate both the scientific research community and academia to develop their skills to face the interdisciplinary challenges of modern science.

1. Introduction

Lipids are fundamental basic components of cellular membranes, that play several crucial roles in many cellular functions, including inter- and intra-cellular barriers, biomembranes matrices, signalling, selective transport, cell adhesion communication and recognition (Sackmann 1995; Goni 2014). A large variety of (cellular) lipids molecular species form complex dynamic nanostructures, that are characterised by a self-assembly process that change constantly with (physiological or pathological) environmental conditions (Katsaras and Gutberlet 2001; Kiselev *et al.* 2013; Tao *et al.* 2015), while many fundamental functions of biological membranes involve the collective behavior of numerous interacting macromolecules (including lipids, proteins and carbohydrates) that take place in a wide range of time and size scales. Moreover, the investigation of the interaction of nanoparticles (and biomolecules) with biological membranes evidences the fundamental role of the lipid-based biomembranes as they strongly influences the drug biodistribution at the target

sites within the tissues and organisms (Allen and Cullis 2012; Bourgaux and Couvreur 2014; Bozzuto and Molinari 2015; Lombardo *et al.* 2019; Caccamo *et al.* 2020; Lombardo *et al.* 2020b). Therefore, the investigation of the structural processes occurring on the cellular and model membranes, as well as their interaction with specific biomolecules in the physiological (or pathological) situations, is crucial for the development of novel therapeutic potential strategies (Blanco *et al.* 2015; Chen *et al.* 2016; Lombardo *et al.* 2016; Wilhelm *et al.* 2016; Dai *et al.* 2017; Pasqua *et al.* 2019). For those reasons, the investigation of the self-assembly properties of lipid biomembranes has been the central topic of an intensive experimental and theoretical research, at the crossroad of different research fields, including physics, chemical engeneering, biochemistry, biotechnology and nano-medicine (Ishida *et al.* 2002; Kiselev *et al.* 2008; Allen and Cullis 2012; Xing *et al.* 2016).

Herein, we propose a conceptual organization of the research methods concerning the study of the biological membranes in order to support the conceptual understanding and to establish fruitful cross-disciplinary connections between different research fields, in view of the development of modern teaching approaches. We shortly introduce some of the major approach commonly used for studying lipid (bio-)membranes by highlighting the synergistic relation and the conceptual interconnection between different fields. The rational organization of all the current approaches may stimulate the exploration of new multi- and trans-disciplinary integration of knowledge and skills that students are required nowadays to have.

2. Central role of biomembranes in biology and medical science academic curricula

The biomembranes of living organisms are permeable protective barrier of the cells, which are mainly composed of lipids, proteins and carbohydrates. They are involved in many crucial functions of the cells such as sensing, transport of drug components, adhesion and recognition processes (Gennis 2008). More specifically, the biomembranes of living systems look like a mosaic structure of (fluid and flexible) lipids bilayers containing *integral, peripheral and transmembrane proteins* that favor the interaction of the cells with its (bio-)environment (Singer and Nicolson 1972). Furthermore, they facilitate the carrying out of active (drugs or nutrients) components across the biomembranes, and favor the transmission/translation of chemical signals from outside the cells (Singer and Nicolson 1972; Koshy and Ziegler 2014).

The presence of some specialized sub-domains of bio-membranes called *lipid rafts* were proposed in order to explain the protein role in some specific cellular membrane processes (Gennis 2008; Kiselev *et al.* 2008; Xing *et al.* 2016). Lipid rafts are (tightly packed) liquid-ordered phases composed of cholesterol and sphingolipids in the outer exoplasmic leaflet, that are connected to cholesterol and phospholipids (such as phosphatidylcholine, phosphatidylethanolamine, and phosphatidylserine) in the inner cytoplasmic leaflet of the lipid bilayer (Singer and Nicolson 1972; Gennis 2008; Koshy and Ziegler 2014).

The presence of some specialized and more ordered sub-domains of bio-membranes, composed of cholesterol and sphingolipids (called *lipid rafts*) are presumed to favorite the proteins in performing highly specific cellular membrane functional tasks (such as signaling, processing, and transport processes), while anomalies in raft composition are supposed to be associated with various diseases (Jacobson *et al.* 2007). Finally, despite the raft hypothesis

is a subject of some controversy, the self-assembly process involving lipid rafts represent an interesting interdisciplinary research topic in the fields of the human physiology (and physiopathology), biomembrane science and nanomedicine (Lingwood and Simons 2010; Sevcsik and Schütz 2015).

Apart from the lipid rafts, most of the assembly ability and specific biophysical and biochemical interaction of biologica membranes depend on the particular lipid composition, chemical structure and morphology. As evidence by various investigations a special role is also played by the interactions of water molecules adsorbed on surfaces of lipids and proteins, as their biomolecular activity is involved in protein and biomembrane structures, stability and dynamics (Israelachvili and Wennerström 1996a; Branca *et al.* 2002; Magazù *et al.* 2012; Disalvo 2015). Several studies evidence the important role of the interaction at the complex interface between the biomolecular systems (such as proteins or bio-membranes) and water molecules, in realizing a functional network in which the biological systems may be considered as a complex structure stabilized by water (Franks 1972; Minutoli *et al.* 2008; Kumar and Keyes 2012; Fenimore *et al.* 2013). In this case the hydrogen bonds and hydrophobic effect play a crucial role for the establishment of the protein structure and stability within the bio-membrane environment. More specifically, the investigation of the properties of the *structural water*, confined at the hydration sites, and the *functional water*, which is located around the lipids headgroups, represents a crucial step for the understanding of complex bio-processes in biomembranes at the level of tissues and cells (Disalvo *et al.* 2008; Caccamo and Magazù 2016; Roy *et al.* 2016; Caccamo and Magazù 2017b). For example, disaccharides (such as trehalose, maltose, or sucrose) have received considerable interest over the past decades for their preservation properties toward cells, or therapeutic proteins, as they can be added to biologically active solutions to overcome the limited stability of proteins (in pH, temperature, salt concentration, etc.) and to prevent the partial (or total) degradation of biological molecules caused by the dehydration or thermal stresses (Israelachvili and Wennerström 1996b; Kiselev *et al.* 2001a,b; Moiset *et al.* 2014; Caccamo and Magazù 2017a; Magazù *et al.* 2018).

In basic research, the investigation concerning model and biological membranes are also important for the study involving their interaction with active components (such as food, drugs, enzymes and genetic material) and their drug delivery processes through the living cell membranes and other biological barriers (Contini *et al.* 2018). For this reason the biological membranes can be considered the most multifunctional and performant cellular structure, while its study represents a central topic at the crossroad of different disciplines including biochemistry, food industry, biotechnology and nano-medicine.

3. Experimental approaches for the study of biomembranes

The structural properties of biomembranes in living systems, which encompasses rich variety of different nanostructures and morphologies, require the combination of different techniques in order to identify the basic properties of the involved association processes as well as the underling (collective) molecular phenomena involved in the resulting biological functions. One of the main research challenges in biomembranes science is to decipher the intimate relation between the structural configuration of its main components (*i.e.*, lipids,

proteins and carbohydrates) and the interactions (at the molecular level) within the micro-environment of the biological system under study. This allows to connect the self-assembly properties (that regulate the composition-structure relationship) to the underlying biological functions of the bio-membranes. In order to characterize the structure and the interactions of drugs with (model) biomembrane, from both a quantitative and qualitative point of view, the combination of complementary, experimental methods are necessary.

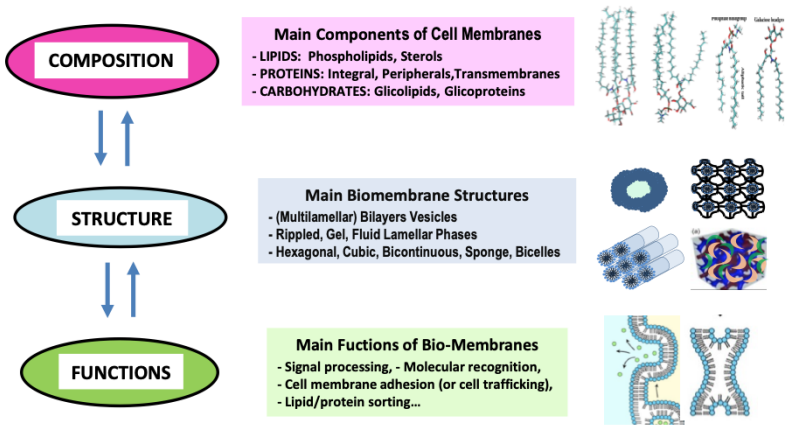

FIGURE 1. Schematic representation of the synergistic relationship between composition, structure and functions in a biological membrane system.

As reported in Figure 1, it possible to identify a relationship between the composition and structure and between the structure and functions of the biomembranes under investigation. According to this conceptual map the experimental characterization methods can be grouped into three main categories. The *first stage* of the experimental investigation consists in the *composition analysis* of the (model or real) biological membranes, that can be performed by means of the high-resolution mass spectroscopy techniques (Mashaghi *et al.* 2013). Detailed information about the population distribution of the bio-membrane components is also obtained by using the size-exclusion chromatography (SEC) (Grabielle-Madelmont *et al.* 2003), that allow to separate (and quantify) the liposome size distribution by exploiting the separation process based on the compound hydrodynamic size.

The *second stage* consists in the *structural characterization methods*. The X-rays and Neutrons small angle scattering (SAXS and SANS) and diffraction methods are among the most employed (non-destructive) experimental methods for the structural investigation of the biological membranes in complex biological systems (Nagle and Tristram-Nagle 2000; Harroun *et al.* 2006; Kiselev and Lombardo 2017). Those methods efficiently evidence the biomembrane structures and phase transitions in many different conditions (Lesieur *et al.* 2000; Di Cola *et al.* 2016; Eicher *et al.* 2017). Moreover, a variety other complementary experimental methods can be used for biological membranes characterization, including the dynamic light scattering (DLS), FT- infrared (IR), ultraviolet (UV), electron spin resonance (ESR), electron paramagnetic resonance (EPR), circular dichroism, nuclear

magnetic resonance (NMR) (Buffy *et al.* 2004; Bowerman *et al.* 2019). Furthermore, the use of complementary biophysical techniques, such as the differential scanning calorimetry (DSC), isothermal titration calorimetry (ITC), surface pressure changes and potentiometric measurements allow to obtain some complementary useful thermodynamic information on the investigated systems (Pignatello *et al.* 2011). Finally, (cryo-)electron-microscopy (EM) experiments provide detailed insights into biomembrane packing and phase states, as well as protein conformations and folding, and drug inclusion processes (Almgren *et al.* 2000; Helvig *et al.* 2015).

Finally, the *third stage* is focused on the investigation of the main features of biomembranes functions. The experimental investigation of biomembranes functions within the complex environment of living cells is a complex task. Given the nanoscale dimensions and the dynamic nature of these membrane nanodomains and compartments, highly performing methods that allow the observation of lipid and protein structure and dynamics at high spatial and temporal resolution are needed. Among the various experimental approaches, the *optical far-field microscopy* is a method of choice (Van Zanten *et al.* 2010), since it facilitate the structural and dynamics investigation in an (almost) non-invasive way, by fluorescence labeling of the macromolecular components under observation. More specifically, high-resolution fluorescence microscopy technique provides excellent spectral contrast and, in combination with sensitive detectors, allows the detection of individual molecules and live cell imaging. Recent progresses enhanced the optical microscopy resolution up to the molecular level range of the living cells (in a non-invasive way) by means of *fluorescence correlation spectroscopy* (FCS) (Elson 2011). This technique allows the observation of the interaction dynamics and molecular diffusion in the millisecond time scale resolution.

4. From lipid composition to biomembrane structure: elaboration of predictive models

Despite the progress made with the experimental investigations of biomembrane structure and dynamics, the study of the self-assembly processes in biomembranes is limited by the space-time resolution of the experimental methods. Moreover, the natural biological membranes present a great complexity of structures, with a large variety of cross-connections and functionality. For this reason the investigation of simplified (artificial) model systems, greatly helps the scientists to understanding of the complex interactions that a biomolecule can develop toward biological membranes, as well as the effects of biomembrane lipids in many processes (including drug transport and uptake into cells). As bio-membranes functions emerge at multi-scale hierarchical levels across a wide ranges of space and time domains, the self-assembly morphology (ordered/disordered, ripple phase, liquid-crystal) and their pattern aggregation (bilayer, multi-lamellar, hexagonal/cubic phases) is determined by the combination of soft interactions (such as electrostatic forces, H-bonds, steric hindrance and hydrophobic effects). Those interactions regulate the formation of energetic barriers controlling the thermally activated (diffusional, rotational and vibrational) molecular dynamics of the building-block molecules forming the biomembranes aggregates.

In this respect, it is possible to assume artificial membrane models, such as monolayers, vesicle-forming bilayers (liposomes) and supported bilayers, that allow to perform specific investigation in well-defined experimental conditions (such as concentration, temperature, or

ionic strength). The, simplified, version of model biomembranes allow to better reproduce the complex phenomena, although some part of the cell membrane properties are obviously lost, and may not reproduce exactly all the aspects of the biomembrane environment.

The structural properties of lipids are regulated by the general self-assembly properties of amphiphilic systems (Hunter 1986; Fuhrhop and Koning 1994; Calandra *et al.* 2000, 2012; Sorrenti *et al.* 2013; Lombardo *et al.* 2020a). Amphiphiles that self-assemble in complex organized nanostructures are driven by the hydrophobic effect and other structure directing interactions like acid-base interactions and H-bonds which dictate the aggregation pattern and the energetic barriers to overcome (Glotzer and Solomon 2007; Calandra *et al.* 2013b, 2015a; Liveri *et al.* 2018). In this case, both the molecular structure of the amphiphile (nature of the hydrophilic head and hydrophobic tail, stereochemistry, etc.) and the experimental conditions have a fundamental role in determining the outcome of the aggregation (Holmberg *et al.* 2003; Calandra *et al.* 2013a, 2015b) and the solubilizing/encapsulating properties toward a wide range of materials (Malmsten 2002; Calandra *et al.* 2014). Moreover, the possibility to finely tune the aggregate morphology is crucial for their application in materials science and biotechnology (Calandra *et al.* 2010; Pochylski *et al.* 2016; Grzelczak *et al.* 2019; Pochylski *et al.* 2019; Calandra 2020). In many cases, the aspect ratio of amphiphile aggregates have served as templates for mesoporous silica materials and zeolites (Wan and Zhao 2007; Bonaccorsi *et al.* 2013a,b; Lan *et al.* 2018).

Predictive models of complex multicomponent lipid systems can be addressed by suitable *theoretical approaches* that make use of the concepts of the thermodynamic and statistical physics approaches. Among them, the mean-field approximation (MFA) and the Ornstein-Zernike (O. Z.) integral equation methods are widely employed in the field of soft matter and bio-nanomaterials science (Belloni 1991; Hamley 2003). Those approaches has been employed to study the range and strength of a large variety of soft interactions usually encountered in biological and nanostructured systems, including proteins (Berezovsky and Bastolla 2017), dendrimers (Lombardo 2009; Porcar *et al.* 2010; Lombardo 2014), and model biomembranes (Lombardo *et al.* 2018).

The progress of *molecular dynamics* (MD) simulations methods offers the opportunity to study the collective behavior of multicomponent systems for a wide range of biophysical processes, in great space-time resolution (Sundararajan 2008; Deserno *et al.* 2013). Computer simulations approach can track the system complex behavior across a vast space-time scales ranges, otherwise inaccessible with traditional experiments. This comes at the cost of the simulated system dimension (*i.e.*, limited to thousands of molecules) and only for short time periods (of the order of microseconds). In this ambit, even ab-initio approaches have been proved to furnish useful information and data in the modeling of complex systems made of amphiphiles and membrane constituents (Sundararajan 2008; Deserno *et al.* 2013).

Recently, a *machine learning* approach has been proposed to investigate the self-assembly of lipid-based system (Cho *et al.* 2012; Mitra *et al.* 2018). Machine learning is an approach for mining dataset by using example input-output pairs to train a specific algorithm. The dataset is usually split into *training*, *cross-validation*, and *testing* categories. The training dataset is used to train a predictive model, validating dataset helps improve the model during training, and finally, the testing dataset allow to evaluate the performance of the adopted predictive model (Figure 2). This novel approach could help to identify lipids phase

diagrams without the need for (time-consuming) experimental techniques, thus enhancing the current understanding of lipid-based biomembranes phase behaviour.

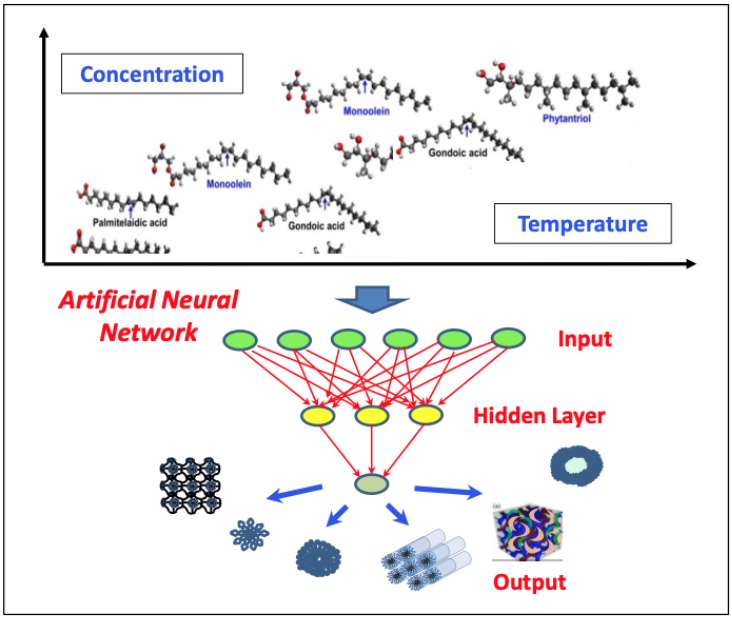

FIGURE 2. Main features of the machine learning model approach for the study of biological membranes.

Machine learning has found applications in the characterization of lipid-protein interactions in biological membranes (Cho *et al.* 2012). Recently a three-component phase diagrams, composed of a high melting temperature (Tm) lipid, cholesterol, and a low Tm lipid have been proposed as a valuable tool in the investigation of lipid phase behaviour (Mitra *et al.* 2018). The artificial neural network (ANN) was trained using available Tm data and was able to predict the Tm and generate phase diagrams for different lipid mixtures. Moreover, a machine learning approach has been used to predict the self-assembly of liquid crystalline nanostructures (LCNs) consisting of a base lipid, (composed of monoolein, or phytantriol) and a variety of (10 different types) saturated and of (20 different types) unsaturated fatty acids at different concentrations (fatty acid/lipid ratios), and temperatures (Le and Tran 2019). The experimental data, acquired by high throughput characterization techniques, were used to train two separate models, *i.e.*, multiple linear regression (MLR) and Bayesian regularized artificial neural networks (ANNs). The models were capable to extrapolate the data for new fatty acid structures, thus allowing the description of the complex phase behavior under different concentrations and temperatures, with high accuracy (ranging from 66% to 96%) (Le and Tran 2019).

5. From lipid composition to biomembrane function: system biology and lipidomic approach

Lipidomics is a new branch of the system biology approach and allow the discovery of biomarkers and identification of mechanisms underlying various diseases, through a large-scale analysis of lipids in a biological system (cells or tissues). The *systems biology* approach (Hwang *et al.* 2005) represents a modern predictive method in biotechnology, based on the so called -*"omics" technologies*, (such as the metabolomics, proteomics and genomics approaches) (Yang and Han 2016). Based on the collection of data from many components in parallel this approach aims to study the complex interactions and functioning of the living cells at various levels (such as a cell, tissue, or organism), by inferring the complex pathways that regulate the biological physiological (or pathological) process.

More specifically, the lipidomics method investigate the pathways (and networks) of cellular lipids that attempt a large-scale mapping of the complete lipid profile within a variety of biological systems (such as a cell, tissue, or organism). Despite, lipids play a crucial role in cell organization and structure, signaling, trafficking events, and sorting of macromolecules, the study of the precise role of lipids is complicated by their chemical diversity and by some technical challenges associated with distinguishing pathogenic/non-pathogenic lipid species within samples that contain several thousand lipid isoforms. The dynamics profiles of cellular lipids and the changes that occur after the perturbation of the cell system, are examined in order to identify biomarkers and elucidate the main changes in its physiological or pathological state (Yang and Han 2016).

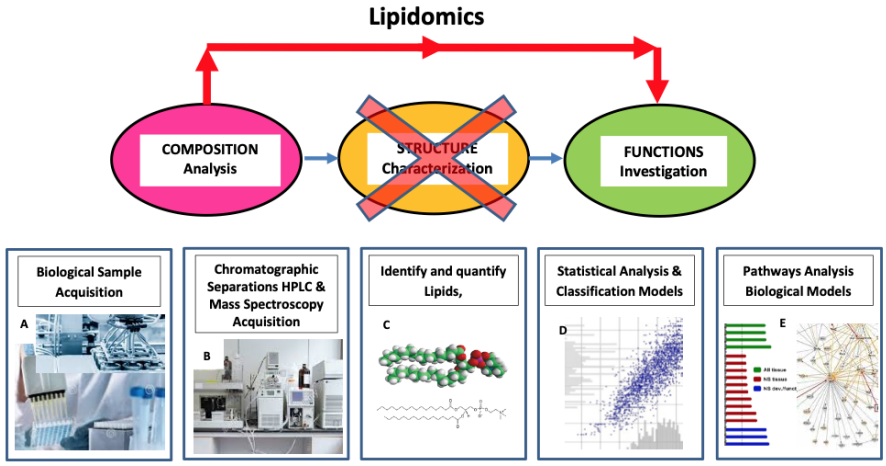

FIGURE 3. Main stages of a lipidomic workflow. The biological sample (from blood, urine, saliva, tissue, or cell culture) of an organism (A), is analyzed by means of a chromatographic separations (HPLC) instrument connected to a high resolution mass spectrometer. After the identification (and quantification) of the lipid profiles (C) relevant to a specific biological (or pathological) condition, the analysis continues with a large-scale mapping and a combined statistical analysis and classification methods (D). The resulting pathway and networks analysis allow to identify the relevant biomarkers associated with the lipidomic analysis (E).

As lipids play an important role in many metabolic diseases (such as diabetes, obesity, atherosclerosis and hypertension) the lipidomics approach is considered as a subset of the more general field of "metabolomics" which includes three other major classes of biological molecules (proteins/amino-acids, sugars and nucleic acids). Lipids bilayers biomembranes also regulate protein functions and gene transcription, and for this reasons they are also considered as a part of a dynamic *"interactome"* within the cell (Yang and Han 2016). In clinical use the current plasma lipid analysis include total triglycerides, total cholesterol, high-density and low-density lipoprotein (HDL) cholesterol, which provide a very narrow snapshot of lipid metabolism. Current methods for lipid analysis are based on the combination of the chromatographic separation methods with the mass spectrometry (MS) technique. In lipidomics investigations typically membrane lipids (phospholipids, sphingolipids) and neutral lipids such as triacylglycerols (TGs) and cholesterol esters are analyzed. Other lipid classes, such as bile acids, steroid and lipids involved in signaling and immune system regulation require precise targeted methods, due to specific physicochemical features that require some analytical procedures.

The integration of lipidomics with genomics, proteomics and metabolomics provide a powerful approach to interpret the molecular mechanisms of lipid-associated disorders, and to identify both biomarkers and novel therapeutic targets. Those rapidly expanding fields complement the huge progress made in proteomics and genomics, within the large family

of systems biology. Finally, this novel conceptual organization of disciplines facilitates the framing of the study of biomembrane within a true interdisciplinary approach at the crossroad between chemistry, informatics, mathematics, biology and medicine.

6. Strengthening the interdisciplinary and transdisciplinary study in research and teaching programs

Biomembranes in living organisms, which have the prominent role of tissues and cells protection from foreign molecules, allow the exchange of information between extra- and intra-cellular environments due to its biochemical-active surface. A The study of the *composition-structure* and *structure-functions* relationship of biological membranes is crucial for the understanding of the effects that are at the origin of many physiological and pathological processes involving biomembranes. In this respect research and academic scientific programs should include the study of the molecular events occurring on cell membranes (self-assembly), as well as the multiplicity of interactions that involve bioactive compounds in either physiological or pathological situations. This approach requests then an interdisciplinary effort of many different field of science (Figure 4), and involves the investigation of the structural and dynamic processes of the relevant nanostructured assemblies over a wide range of relevant time scales and distances, starting from the analysis of *molecular interactions* ($\sim 10nm$), to the assemblies of lipid-protein complexes ($\sim 100nm$) up to the meso-scale ($\sim 1\mu m$) and macro-scale (tissues and cells).

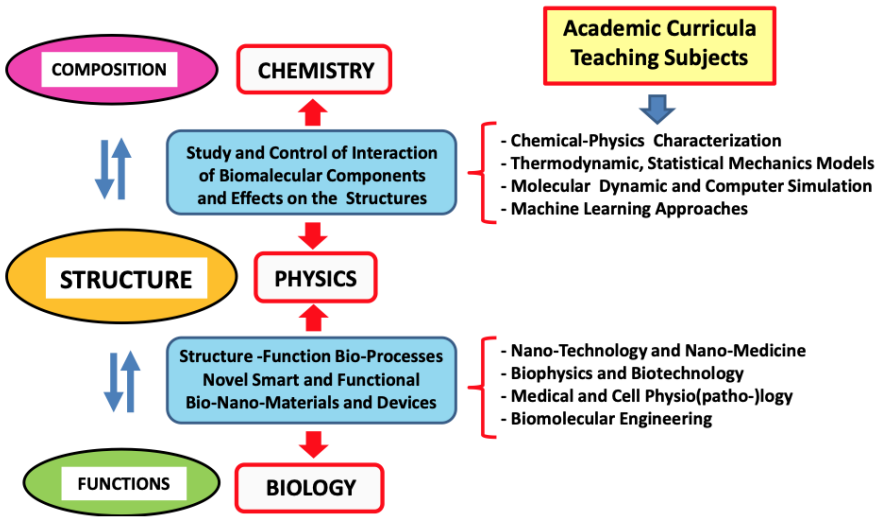

FIGURE 4. A conceptual map for the description of the main stages involved in the interdisciplinary study of biological membranes.

In Figure 4 we report a conceptual map for the description of the main stages involved in the study of biological membranes. The proposed scheme highlights some of the key disciplines of prominent expertise and technology that will need to be incorporated into tran-disciplinary and inter-disciplinary organizations and institutes.

A novel conceptual framework towards an interdisciplinary approach is therefore of paramount importance, to enlarge our knowledge of many diseases and to identify further potential therapeutic targets.

In this respect, the single experimental (or theoretical) method alone is not sufficient to describe the complex synergist relations between components, structure and function of biomembranes. The integration and use of different computational (or theoretical) methods with experimental techniques will provided valuable opportunities to extend the domain of applicability of each methods. The choice of the appropriate combination of experimental (and/or theoretical) approach(es) depend on the (complex) processes under study and on the corresponding information already available (in the literature or in public databases.

Moreover, the study of a multiplicity of simultaneous factors and biological functionality may be substituted with the investigation of the effect of a few parameters at a time. For biological membranes, some of those factors are the surface charge density, nanoparticles size/topology/chemistry, solution conditions (pH, ionic strength). The choice of a simplified version of the system allow to identify the key factors for the design of novel nanostructured systems, and represents then the fundamental (initial) step to decipher the complexity involved in complex biological processes. Future efforts should be directed toward the creation of suitable links between experiment and theory, which are able to identify the key factors for the design of novel nanostructured systems, through the choice of a simplified (or approximated) version of the (in vivo/in vitro) system to investigate. This effort requires an integration of multi- and interdisciplinary methods that combine results coming from a wide range of different sub-disciplines and research fields. Both the scientific research community and academia must develop their skills to face the interdisciplinary challenges of modern science. Those links may help to develop a network-based method built upon the synergistic connections between components, structure and functions of biological membranes that will likely provide a better integration of multidisciplinary research and education work.

7. Laboratory learning by doing: implications for research and education

Laboratory learning methods have a central role in the modern science education. Contrary to the traditional lectures, the method of laboratory learning allows students (either individually or in groups) to be actively engaged in their learning process. Students learn by doing, and then drawing understanding from lab experiences by reinforcing the theoretical concepts taught in class. Those abilities, that cannot be developed through only lectures and tutorials, allow the development of higher-level cognitive skills. Moreover, laboratory sessions can stimulate an increasing students' interests in their academic discipline, and motivate the students to participate in the process of the scientific investigation and discovery.

In view of developing a research and education transdisciplinary approach in the study of biological membranes, the development of laboratory activities may follow the logical

sequence of the conceptual map reported in Figure 1. Cognitive researchers evidenced that organizing knowledge (and skills) in schemas (onceptual maps) facilitates the use of material from long-term memory and a meaningful learning in complex cognitive domains (Goldman *et al.* 2016; You 2017). Moreover, the student's learning process is enhanced when they have a cognitive map that explain the relationships among concepts within a scientific domain, and that reveal how their basic principles are (inter-)connected with one another (You 2017). The laboratory activities in the field of biomembranes can be organized according to three *stages* that investigate the *composition* (through chemistry and biochemistry classes), *structure* (through chemical-physics structural characterization methods) and *functions* (through biology, biotechnology and nano-medicine classes). In this respect, both theoretical and experimental learning approaches require considerable re-structuring of teaching. extensive re-training of faculty, and a careful preparation and orientation of students in view of the interdisciplinary and transdisciplinary content of the proposed curricula (You 2017; Orhan and Sahin 2018).

An important pedagogical value of the *laboratory learning by doing* approach consists in enabling the students to move *from the concrete* (specific observed phenomena) *to the abstract* (understanding the basic principles or theories). The main objectives of the laboratory experiments usually include the students collecting data from measurements, the testing of the hypotheses, and the analysing and interpreting the results. Moreover, the learning by doing approach stimulate the students to a critical cultural aspect of science, based on the circumstance that all scientific ideas and hypothesis need to be tested in a rigorous manner before to be considered valid. Historically the primary aim of laboratory learning was to develop the practical competence within specific area of specialization. However, by the twentieth century, laboratory learning by doing is no longer merely aimed at some specific (and focussed) practical competence, but has shifted to additional new educational outcomes that include collaboration, communication skills, creative thinking and problem solving. More specifically, working in small teams promotes the cooperative learning of students that are collectively engaged to collaborate in inquiry. All these learning outcomes constitute important parts in the research (and education) cycle for scientific inquiries, and provides the empirical (and experimental) basis to test and refine the scientific theories.

Together with these numerous advantages, some critical factors still inhibit the laboratory learning by doing method as an efficient approach in science education. Critical issues include the insufficient laboratory hours, poor assessment, and experiment design. Moreover, as equipment becomes more sophisticated and expensive, it becomes increasingly difficult to provide students, even in university, to easily access to such experimental equipments. Examples of these difficulties are the access to neutron and synchrotron radiation facilities, or nanotechnology advanced labs. However, recent advances in technologies, allow a new mode to preserve the laboratory experience known as *"Virtual Laboratory"*. A virtual laboratory represents an environment for experiments which is conducted (or controlled) locally (or remotely) through computer operation, simulation, and/or animation via the internet. Those systems (where students can remotely observe the experiments through live-video setup) has the advantages of accessing the main functions of a laboratory, even in those situations of restricted access due to safety reasons, available time, and distances issues.

8. Conclusion

The ultimate goal for modern bio-nanotechnology is to formulate our understanding of cellular, subcellular, and molecular systems in terms of quantitative models that are supported by the rigorous basic principles of the chemistry, physics, and biology sciences. This can be achieved through trans-disciplinary and inter-disciplinary researches that draws on the expertise of a broad range of different disciplines and technologies that includes the biophysics, biotechnology, medical and cell physio(patho-)logy and biomolecular engineering, just to name a few. The scientific investigation of biological membranes involves the collective behavior of numerous interacting (macro-)molecules, while their complete structural description requires the simultaneous calculation (and simulation) of a large number of parameters. The future challenge must provide an efficient integration of the different teaching approaches and research models into a common background, in order to stimulate new discoveries and new ways of operating. To achieve this objective, we need to improve the resolution of experimental approaches, together with the improvement of the theoretical models and computational efforts, and integrate them into a multi-scale description of the biological membranes systems. In this respect, the laboratory learning by doing can have strong implications for research and education activities. The method of laboratory learning allows students (either individually or in groups) to be actively engaged in their learning process. Thus allowing the development of higher-level cognitive skills. All those effort require an integration of multi- and interdisciplinary approaches that combine results coming from a wide range of sub-disciplines.

References

Allen, T. and Cullis, P. (2012). "Liposomal Drug Delivery Systems: From Concept to Clinical Applications." *Advanced drug delivery reviews* **65**(1), 36–48. DOI: 10.1016/j.addr.2012.09.037.

Almgren, M., Edwards, K., and Karlsson, G. (2000). "Cryo transmission electron microscopy of liposomes and related structures". *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **174**, 3–21. DOI: 10.1016/S0927-7757(00)00516-1.

Belloni, L. (1991). "Interacting monodisperse and polydisperse spheres". In: *Neutron X-ray and Light Scattering*. Ed. by P. Lindner and T. Zemb. New York: Elsevier Science Publishers B. V.

Berezovsky, I. and Bastolla, U. (2017). "Proteins: bridging theory and experiment". *Current Opinion in Structural Biology* **42**, 1–168. DOI: 10.1016/j.sbi.2016.12.013.

Blanco, E., Shen, H., and Ferrari, M. (2015). "Principles of nanoparticle design for overcoming biological barriers to drug delivery". *Nature biotechnology* **33**, 941–951. DOI: 10.1038/nbt.3330.

Bonaccorsi, L., Calandra, P., Amenitsch, H., Proverbio, E., and Lombardo, D. (2013a). "Growth of fractal aggregates during template directed SAPO-34 zeolite formation". *Microporous and Mesoporous Materials* **167**, 3–9. DOI: 10.1016/j.micromeso.2012.10.024.

Bonaccorsi, L., Calandra, P., Kiselev, M., Amenitsch, H., Proverbio, E., and Lombardo, D. (2013b). "Self-Assembly in Poly(dimethylsiloxane)–Poly(ethylene oxide) Block Copolymer Template Directed Synthesis of Linde Type A Zeolite". *Langmuir* **29**(23), 7079–7086. DOI: 10.1021/la400951s.

Bourgaux, C. and Couvreur, P. (2014). "Interactions of anticancer drugs with biomembranes: What can we learn from model membranes?" *Journal of controlled release : official journal of the Controlled Release Society* **190**, 127–138. DOI: 10.1016/j.jconrel.2014.05.012.

Bowerman, C., Bozelli Junior, J., Brown, M., Butler, P., Chan, C., and Cheng, X. (2019). "Characterization of Biological Membranes: Structure and Dynamics". In: ed. by F. Nieh M.P. Heberle and J. Katsaras. 07. DOI: 10.1515/9783110544657.

Bozzuto, G. and Molinari, A. (2015). "Liposomes as nanomedical devices". *International Journal of Nanomedicine* **10**, 975–999. DOI: 10.2147/IJN.S68861.

Branca, C., Magazù, S., Migliardo, F., and Migliardo, P. (2002). "Destructuring effect of trehalose on the tetrahedral network of water: A Raman and neutron diffraction comparison". *Physica A: Statistical Mechanics and its Applications* **304**, 314–318. DOI: 10.1016/S0378-4371(01)00517-9.

Buffy, J., McCormick, M., Wi, S., Waring, A., Lehrer, R., and Hong, M. (2004). "Solid-State NMR Investigation of the Selective Perturbation of Lipid Bilayers by the Cyclic Antimicrobial Peptide RTD-1". *Biochemistry* **43**, 9800–9812. DOI: 10.1021/bi036243w.

Caccamo, M. T., Mavilia, G., Mavilia, L., Calandra, P., Lombardo, D., and Magazù, S. (2020). "Thermal investigation of montmorillonite/BSA by fourier transform infrared spectroscopy measurements". *AMS Biophysics* **7**(4), 436–451. DOI: 10.3934/biophy.2020030.

Caccamo, M. T. and Magazù, S. (2016). "Tagging the oligomer-to-polymer crossover on EG and PEGs by infrared and Raman spectroscopies and by wavelet cross-correlation spectral analysis". *Vibrational Spectroscopy* **85**, 222–227. DOI: 10.1016/j.vibspec.2016.04.017.

Caccamo, M. T. and Magazù, S. (2017a). "Multiscaling wavelet analysis of infrared and Raman data on polyethylene glycol 1000 aqueous solutions". *Spectroscopy Letters* **50**(3), 130–136. DOI: 10.1080/00387010.2017.1291524.

Caccamo, M. T. and Magazù, S. (2017b). "Thermal restraint on PEG-EG mixtures by FTIR investigations and wavelet cross-correlation analysis". *Polymer Testing* **62**, 311–318. DOI: 10.1016/j.polymertesting.2017.07.008.

Calandra, P. (2020). "On the physico-chemical basis of self-nanosegregation giving magnetically-induced birefringence in dibutyl phosphate/bis(2-ethylhexyl) amine systems". *Journal of Molecular Liquids* **310**, 113186. DOI: 10.1016/j.molliq.2020.113186.

Calandra, P., Caponetti, E., Chillura Martino, D., D'Angelo, P., Minore, A., and Liveri, V. (2000). "FT-IR and dielectric study of water/AOT liquid crystals". *Journal of Molecular Structure* **522**, 165–178. DOI: 10.1016/S0022-2860(99)00351-8.

Calandra, P., Caschera, D., Liveri, V., and Lombardo, D. (2015a). "How self-assembly of amphiphilic molecules can generate complexity in the nanoscale". *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **484**, 164–183. DOI: 10.1016/j.colsurfa.2015.07.058.

Calandra, P., De caro, T., Caschera, D., Lombardo, D., Todaro, L., and Liveri, V. (2014). "Spectroscopic and structural characterization of pure and FeCl3-containing tri-n-butyl phosphate". *Colloid and Polymer Science* **293**, 597–603. DOI: 10.1007/s00396-014-3439-x.

Calandra, P., Liveri, V., Riello, P., Freris, I., and Mandanici, A. (2012). "Self-assembly in surfactant-based liquid mixtures: Octanoic acid/Bis(2-ethylhexyl)amine systems". *Journal of colloid and interface science* **367**, 280–285. DOI: 10.1016/j.jcis.2011.10.015.

Calandra, P., Liveri, V., Ruggirello, A., Licciardi, M., Lombardo, D., and Mandanici, A. (2015b). "Anti-Arrhenian behaviour of conductivity in Octanoic acid/bis(2-ethylhexyl) amine systems: a physico-chemical study". *Journal of Materials Chemistry C* **3**, 3198–3210. DOI: 10.1039/C4TC02500H.

Calandra, P., Mandanici, A., and Liveri, V. (2013a). "Self-assembly in surfactant-based mixtures driven by acid-base reactions: Bis(2-ethylhexyl) phosphoric acid-n-octylamine systems". *RSC Adv.* **3**, 5148–5155. DOI: 10.1039/C3RA23295F.

Calandra, P., Nicotera, I., Oliviero, C., and Liveri, V. (2013b). "Dynamical Properties of Self-Assembled Surfactant-Based Mixtures: Triggering of One-Dimensional Anomalous Diffusion in Bis(2-ethylhexyl)phosphoric Acid/n-Octylamine Systems". *Langmuir* **29**, 14848–14854. DOI: 10.1021/la403522q.

Calandra, P., Ruggirello, A., Pistone, A., and Liveri, V. (2010). "Structural and Optical Properties of Novel Surfactant Coated TiO2–Ag Based Nanoparticles". *Journal of Cluster Science* **21**(4), 767–778. DOI: 10.1007/s10876-010-0330-x.

Chen, G., Roy, I., Yang, C., and Prasad, P. (2016). "Nanochemistry and Nanomedicine for Nanoparticle-based Diagnostics and Therapy". *Chemical reviews* **116**(5), 2826–2885. DOI: 10.1021/acs.chemrev.5b00148.

Cho, H., Wu, M., Bilgin, B., Walton, S., and Chan, C. (2012). "Latest developments in experimental and computational approaches to characterize protein-lipid interactions". *Proteomics* **12**, 3273–3285. DOI: 10.1002/pmic.201200255.

Contini, C., Schneemilch, M., Gaisford, S., and Quirke, N. (2018). "Nanoparticle–membrane interactions". *Journal of Experimental Nanoscience* **13**(1), 62–81. DOI: 10.1080/17458080.2017.1413253.

Dai, Y., Xu, C., Sun, X., and Chen, X. (2017). "Nanoparticle design strategies for enhanced anticancer therapy by exploiting the tumour microenvironment". *Chemical Society reviews* **46**(12). DOI: 10.1039/c6cs00592f.

Deserno, M., Kremer, K., Paulsen, H., Peter-Tittelbach, C., and Schmid, F. (2013). "Computational Studies of Biomembrane Systems : Theoretical Considerations, Simulation Models, and Applications". **260**.

Di Cola, E., Grillo, I., and Ristori, S. (2016). "Small Angle X-ray and Neutron Scattering: Powerful Tools for Studying the Structure of Drug-Loaded Liposomes". *Pharmaceutics* **8**(2), 10 [16 pages]. DOI: 10.3390/pharmaceutics8020010.

Disalvo, E. (2015). *Membrane Hydration: The Role of Water in the Structure and Function of Biological Membranes*. DOI: 10.1007/978-3-319-19060-0.

Disalvo, E., Lairion, F., Martini, M., Tymczyszyn, E., Frías, M., Almaleck, H., and Gordillo, G. (2008). "Structural and functional properties of hydration and confined water in membrane interfaces". *Biochimica et biophysica acta* **1778**(12), 2655–70. DOI: 10.1016/j.bbamem.2008.08.025.

Eicher, B., Heberle, F., Marquardt, D., Rechberger, G., Katsaras, J., and Pabst, G. (2017). "Joint small-angle X-ray and neutron scattering data analysis of asymmetric lipid vesicles". *Journal of Applied Crystallography* **50**, 419–429. DOI: 10.1107/S1600576717000656.

Elson, E. (2011). "Fluorescence Correlation Spectroscopy: Past, Present, Future". *Biophysical journal* **101**(12), 2855–70. DOI: 10.1016/j.bpj.2011.11.012.

Fenimore, P., Frauenfelder, H., Magazù, S., McMahon, B., Mezei, F., Migliardo, F., Young, R., and Stroe, I. (2013). "Concepts and problems in protein dynamics". *Chemical Physics* **424**, 2–6. DOI: 10.1016/j.chemphys.2013.06.023.

Franks, F. (1972). *Water, a Comprehensive Treatise: The physics and physical chemistry of water*. Water, a Comprehensive Treatise 7. Plenum Press.

Fuhrhop, J.-H. and Koning, J. (1994). *Membranes and Molecular Assemblies. The Synkinetic Approach*. Monographs in Supramolecular Chemistry. The Royal Society of Chemistry. DOI: 10.1039/9781847551368.

Gennis, R. (2008). "Molecular structure and Function".

Glotzer, S. and Solomon, M. (2007). "Anisotropy of Building Blocks and Their Assembly Into Complex Structures". *Nature materials* **6**, 557–562. DOI: 10.1038/nmat1949.

Goldman, S., Britt, M., Brown, W., Cribb, G., George, M., Greenleaf, C., Lee, C., Shanahan, C., and READI, P. (2016). "Disciplinary Literacies and Learning to Read for Understanding: A Conceptual Framework for Disciplinary Literacy". *Educational Psychologist* **51**(2), 219–246. DOI: 10.1080/00461520.2016.1168741.

Goni, F. (2014). "The basic structure and dynamics of cell membranes: An update of the Singer-Nicolson model". *Biochimica et Biophysica Acta* **1838**(6), 1467–1476. DOI: 10.1016/j.bbamem.2014.01.006.

Grabielle-Madelmont, C., Lesieur, S., and Ollivon, M. (2003). "Characterization of loaded liposomes by size exclusion chromatography". *Journal of biochemical and biophysical methods* **56**(1–3), 189–217. DOI: 10.1016/S0165-022X(03)00059-9.

Grzelczak, M., Liz-Marzán, L., and Klajn, R. (2019). "Stimuli-responsive self-assembly of nanoparticles". *Chemical Society Reviews* **48**, 1342–1361. DOI: 10.1039/C8CS00787J.

Hamley, I. (2003). "Nanotechnology with Soft Materials". *Angewandte Chemie (International ed. in English)* **42**, 1692–1712. DOI: 10.1002/anie.200200546.

Harroun, T., Wignall, G., Fitter, J., Gutberlet, T., and Katsaras, J. (2006). "Neutron Scattering for Biology". In: pp. 1–18. DOI: 10.1007/3-540-29111-3_1.

Helvig, S., Azmi, I., Moghimi, S., and Yaghmur, A. (2015). "Recent Advances in Cryo-TEM Imaging of Soft Lipid Nanoparticles". *AIMS Biophysics* **2**(2), 116–130. DOI: 10.3934/biophy.2015.2.116.

Holmberg, K., Jönsson, B., Kronberg, B., and Lindman, B. (2003). "Surfactants and Polymers in Aqueous Solution, Second Edition". In: pp. 39–66. DOI: 10.1002/0470856424.ch2.

Hunter, R. J. (1986). *Foundations of Colloid Science*. Oxford, University Press.

Hwang, D., Smith, J., Leslie, D., Weston, A., Rust, A., Ramsey, S., Atauri, P. de, Siegel, A., Bolouri, H., Aitchison, J., and Hood, L. (2005). "A Data Integration Methodology for Systems Biology: Experimental Verification". *Proceedings of the National Academy of Sciences of the United States of America* **102**(48), 17302–7. DOI: 10.1073/pnas.0508649102.

Ishida, T., Harashima, H., and Kiwada, H. (2002). "Liposome Clearance". *Bioscience reports* **22**, 197–224. DOI: 10.1023/A:1020134521778.

Israelachvili, J. and Wennerström, H. (1996a). "Role of hydration and water structure in biological and colloidal interactions". *Nature* **379**(6562), 219–225. DOI: 10.1038/379219a0.

Israelachvili, J. and Wennerström, H. (1996b). "Role of hydration and water structure in biological and colloidal interactions". *Nature* **379**(6562), 219–225. DOI: 10.1038/379219a0.

Jacobson, K., Mouritsen, O., and Anderson, R. (2007). "Jacobson K, Mouritsen OG, Anderson RGLipid rafts: at a crossroad between cell biology and physics. Nat Cell Biol 9: 7-14". *Nature cell biology* **9**(1), 7–14. DOI: 10.1038/ncb0107-7.

Katsaras, J. and Gutberlet, T. (2001). *Lipid Bilayers*. Springer-Verlag Berlin Heidelberg. DOI: 10.1007/978-3-662-04496-4.

Kiselev, M., Janich, M., Hildebrand, A., Strunz, P., Neubert, R., and Lombardo, D. (2013). "Structural transition in aqueous lipid/bile salt [DPPC/NaDC] supramolecular aggregates: SANS and DLS study". *Chemical Physics* **424**, 93–99. DOI: 10.1016/j.chemphys.2013.05.014.

Kiselev, M., Lesieur, P., Kisselev, A., Lombardo, D., Killany, M., and Lesieur, S. (2001a). "Sucrose Solutions as Prospective Medium to Study the Vesicle Structure: SAXS and SANS study". *Journal of Alloys and Compounds* **328**(1–2), 71–76. DOI: 10.1016/S0925-8388(01)01348-2.

Kiselev, M., Lesieur, P., Kisselev, A., Lombardo, D., Killany, M., Lesieur, S., and Ollivon, M. (2001b). "A sucrose solutions application to the study of model biological membranes". *Nuclear Instruments & Methods in Physics Research Section A-accelerators Spectrometers Detectors and Associated Equipment* **470**, 409–416. DOI: 10.1016/S0168-9002(01)01087-7.

Kiselev, M. and Lombardo, D. (2017). "Structural Characterization in Mixed Lipid Membrane Systems by Neutron and X-ray scattering". *Biochimica et Biophysica Acta (BBA) - General Subjects* **1861**, 3700–3717. DOI: 10.1016/j.bbagen.2016.04.022.

Kiselev, M., Lombardo, D., Lesieur, P., Kisselev, A., Borbely, S., Simonova, T., and Barsukov, L. (2008). "Membrane self assembly in mixed DMPC/NaC systems by SANS". *Chemical Physics* **345**(2–3), 173–180. DOI: 10.1016/j.chemphys.2007.09.034.

Koshy, C. and Ziegler, C. (2014). "Structural insights into functional lipid–protein interactions in secondary transporters". *Biochimica et Biophysica Acta* **1850**(3), 476–487. DOI: 10.1016/j.bbagen.2014.05.010.

Kumar, R. and Keyes, T. (2012). "The relation between the structure of the first solvation shell and the IR spectra of aqueous solutions". *Journal of biological physics* **38**(1), 75–83. DOI: 10.1007/s10867-011-9243-7.

Lan, Y., Wang, X., Wang, C., and Zebarjadi, M. (2018). "Organic/Inorganic Hybrid Nanostructured Materials for Thermoelectric Energy Conversion". In: pp. 445–484. DOI: 10.1002/9783527807369.ch12.

Le, T. and Tran, N. (2019). "Using Machine Learning To Predict the Self-Assembled Nanostructures of Monoolein and Phytantriol as a Function of Temperature and Fatty Acid Additives for Effective Lipid-Based Delivery Systems". *ACS Applied Nano Materials* **2**, 1637–1647. DOI: 10.1021/acsanm.9b00075.

Lesieur, P., Kiselev, M., Barsukov, L., and Lombardo, D. (2000). "Temperature-induced micelle to vesicle transition: Kinetic effects in the DMPC/NaC system". *Journal of Applied Crystallography* **33**(3), 623–627. DOI: 10.1107/S0021889899012765.

Lingwood, D. and Simons, K. (2010). "Lipid Rafts As a Membrane-Organizing Principle". *Science* **327**, 46–50. DOI: 10.1126/science.1174621.

Liveri, V., Lombardo, D., Pochylski, M., and Calandra, P. (2018). "Molecular association of small amphiphiles: Origin of ionic liquid properties in dibutyl phosphate/propylamine binary mixtures". *Journal of Molecular Liquids* **263**, 274–281. DOI: 10.1016/j.molliq.2018.05.003.

Lombardo, D. (2009). "Liquid-like Ordering of Negatively Charged Poly(amidoamine) (PAMAM) Dendrimers in Solution". *Langmuir* **25**(5), 3271–3275. DOI: 10.1021/la804234p.

Lombardo, D. (2014). "Modeling Dendrimers Charge Interaction in Solution: Relevance in Biosystems". *Biochemistry research international* **2014**, 837651 [10 pages]. DOI: 10.1155/2014/837651.

Lombardo, D., Calandra, P., Bellocco, E., Lagana, G., Barreca, D., Magazù, S., Wanderlingh, U., and Kiselev, M. (2016). "Effect of anionic and cationic polyamidoamine (PAMAM) dendrimers on a model lipid membrane". *Biochimica et Biophysica Acta (BBA) - Biomembranes* **1858**(11), 2769–2777. DOI: 10.1016/j.bbamem.2016.08.001.

Lombardo, D., Calandra, P., Magazù, S., Wanderlingh, U., Barreca, D., and Kiselev, M. (2018). "Soft nanoparticles charge expression within lipid membranes: The case of amino terminated dendrimers in bilayers vesicles". *Colloids and Surfaces B: Biointerfaces* **170**, 609–616. DOI: 10.1016/j.colsurfb.2018.06.031.

Lombardo, D., Calandra, P., Pasqua, L., and Magazù, S. (2020a). "Self-Assembly of Organic Nanomaterials and Biomaterials: The Bottom-Up Approach for Functional Nanostructures Formation and Advanced Applications". *Materials* **13**(5), 1048. DOI: 10.3390/ma13051048.

Lombardo, D., Calandra, P., T., C. M., Magazù, S., and Kiselev, M. A. (2019). "Colloidal stability of liposomes". *AIMS Materials Science* **6**(2), 200–213. DOI: 10.3934/matersci.2019.2.200.

Lombardo, D., Calandra, P., T., C. M., Magazù, S., Pasqua, L., and Kiselev, M. A. (2020b). "Interdisciplinary approaches to the study of biological membranes". *AIMS Biophysics* **7**(4), 267–290. DOI: 10.3934/biophy.2020020.

Magazù, S., Calabrò, E., and Caccamo, M. T. (2018). "Experimental study of thermal restraint in bio-protectant disaccharides by FTIR spectroscopy". *Open Biotechnology Journal* **12**, 123–133. DOI: 10.2174/1874070701812010123.

Magazù, S., Migliardo, F., and Caccamo, M. T. (2012). "Innovative wavelet protocols in analyzing elastic incoherent neutron scattering". *Journal of Physical Chemistry B* **116**(31), 9417–9423. DOI: 10.1021/jp3060087.

Malmsten, M. (2002). *Surfactants and polymers in drug delivery*, pp. 1–349.

Mashaghi, A., Mashaghi, S., Reviakine, I., Heeren, R., Sandoghdar, V., and Bonn, M. (2013). "Label-Free Characterization of Biomembranes: From Structure to Dynamics". *Chemical Society reviews* **43**, 887–900. DOI: 10.1039/c3cs60243e.

Minutoli, L., Altavilla, D., Bitto, A., Polito, F., Bellocco, E., Lagana, G., Fiumara, T., Magazù, S., Migliardo, F., Venuti, F., and Squadrito, F. (2008). "Trehalose: A biophysics approach to modulate the inflammatory response during endotoxic shock". *European journal of pharmacology* **589**, 272–280. DOI: 10.1016/j.ejphar.2008.04.005.

Mitra, E., Whitehead, S., Holowka, D., Baird, B., and Sethna, J. (2018). "Computation of a Theoretical Membrane Phase Diagram, and the Role of Phase in Lipid Raft-Mediated Protein Organization". *The Journal of Physical Chemistry B* **122**(13), 3500–3513. DOI: 10.1021/acs.jpcb.7b10695.

Moiset, G., López, C., Bartelds, R., Syga, L., Rijpkema, E., Cukkemane, A., Baldus, M., Poolman, B., and Marrink, S. (2014). "Disaccharides Impact the Lateral Organization of Lipid Membranes". *Journal of the American Chemical Society* **136**, 16167–16175. DOI: 10.1021/ja505476c.

Nagle, J. and Tristram-Nagle, S. (2000). "Structure of lipid bilayer". *Biochimica et Biophysica Acta (BBA) - Reviews on Biomembranes* **1469**(3), 159–195. DOI: 10.1016/S0304-4157(00)00016-2.

Orhan, T. and Sahin, N. (2018). "The Impact of Innovative Teaching Approaches on Biotechnology Knowledge and Laboratory Experiences of Science Teachers". *Education Sciences* **8**(4), 213. DOI: 10.3390/educsci8040213.

Pasqua, L., De Napoli, I. E., Santo, M., Greco, M., Catizzone, E., Lombardo, D., Nigro, A., Montera, G., Comandè, A., Morelli, C., and Leggio, A. (2019). "Mesoporous silica-based hybrid materials for bone-specific drug delivery". *Nanoscale Advances* **1**, 3269–3278. DOI: 10.1039/C9NA00249A.

Pignatello, R., Musumeci, T., Basile, L., Carbone, C., and Puglisi, G. (2011). "Biomembrane models and drug-biomembrane interaction studies: Involvement in drug design and development". *Journal of pharmacy & bioallied sciences* **3**(1), 4–14. DOI: 10.4103/0975-7406.76461.

Pochylski, M., Lombardo, D., and Calandra, P. (2019). "Optical Birefringence Growth Driven by Magnetic Field in Liquids: The Case of Dibutyl Phosphate/Propylamine System". *Applied Sciences* **10**, 164 [14 pages]. DOI: 10.3390/app10010164.

Pochylski, M., Oliviero, C., Nicotera, I., Liveri, V., and Calandra, P. (2016). "Nano-demixing as a novel strategy for magnetic field responsive systems: the case of Dibutyl phosphate/bis(2-ethylhexyl) amine systems". *RSC Adv.* **6**, 26696–26708. DOI: 10.1039/C6RA02386J.

Porcar, L., Hong, K., Butler, P., Herwig, K., Smith, G., Liu, Y., and Chen, W.-R. (2010). "Intramolecular structural change of PAMAM dendrimers in aqueous solutions revealed by small-angle neutron scattering". *Journal of Physical Chemistry B* **114**(5), 1751–1756. DOI: 10.1021/jp9064455.

Roy, A., Dutta, R., Kundu, N., Banik, D., and Sarkar, N. (2016). "A Comparative Study of the Influence of Sugars Sucrose, Trehalose and Maltose on the Hydration and Diffusion of DMPC Lipid Bilayer at Complete Hydration: Investigation of Structural and Spectroscopic Aspect of Lipid-Sugar Interaction". *Langmuir* **32**(20), 5124–5134. DOI: 10.1021/acs.langmuir.6b01115.

Sackmann, E. (1995). "Physical Basis of Self-Organization and Function of Membranes: Physics of Vesicles". *Handbook of Biological Physics* **1**, 212–303. DOI: 10.1016/S1383-8121(06)80022-9.

Sevcsik, E. and Schütz, G. (2015). "With or without rafts? Alternative views on cell membranes". *BioEssays* **38**(2), 129–139. DOI: 10.1002/bies.201500150.

Singer, S. and Nicolson, G. (1972). "The Fluid Mosaic Model of the Structure of Cell Membranes". *Science* **175**, 720–731. DOI: 10.1126/science.175.4023.720.

Sorrenti, A., Illa, O., and Ortuno, R. (2013). "Amphiphiles in aqueous solution: Well beyond a soap bubble". *Chemical Society reviews* **42**(21), 8200–8219. DOI: 10.1039/c3cs60151j.

Sundararajan, V. (2008). *Computational Modeling of Membrane Bilayers*. Ed. by S. E. Feller. Elsevier Academic Press.

Tao, X., Jin, S., Wu, D., Ling, K., Yuan, L., Lin, P., Xie, Y., and Yang, X. (2015). "Effects of Particle Hydrophobicity, Surface Charge, Media pH Value and Complexation with Human Serum Albumin on Drug Release Behavior of Mitoxantrone-Loaded Pullulan Nanoparticles". *Nanomaterials* **6**(1), 2 [14 pages]. DOI: 10.3390/nano6010002.

Van Zanten, T., Cambi, A., and Parajo, M. (2010). "A nanometer scale optical view on the compartmentalization of cell membranes". *Biochimica et Biophysica Acta (BBA) - Biomembranes* **1798**(04), 777–787. DOI: 10.1016/j.bbamem.2009.09.012.

Wan, Y. and Zhao, D. (2007). "On the Controllable Soft-Templating Approach to Mesoporous Silicates". *Chemical reviews* **107**, 2821–60. DOI: 10.1021/cr068020s.

Wilhelm, S., Tavares, A., Dai, Q., Ohta, S., Audet, J., Dvorak, H., and Chan, W. (2016). "Analysis of nanoparticle delivery to tumours". *Nature Reviews Materials* **1**, 16014 [35 pages]. DOI: 10.1038/natrevmats.2016.14.

Xing, H., Hwang, K., and Lu, Y. (2016). "Recent Developments of Liposomes as Nanocarriers for Theranostic Applications". *Theranostics* **6**, 1336–1352. DOI: 10.7150/thno.15464.

Yang, K. and Han, X. (2016). "Lipidomics: Techniques, Applications, and Outcomes Related to Biomedical Sciences". *Trends in Biochemical Sciences* **41**(11), 954–969. DOI: 10.1016/j.tibs.2016.08.010.

You, H. (2017). "Why Teach Science with an Interdisciplinary Approach: History, Trends, and Conceptual Frameworks". *Journal of Education and Learning* **6**, 66–77. DOI: 10.5539/jel.v6n4p66.

---

* Consiglio Nazionale delle Ricerche (CNR)
Istituto per i Processi Chimico-Fisici (IPCF)
Viale F. Stagno d'Alcontres, 98158 Messina, Italy

Email: lombardo@ipcf.cnr.it

Paper contributed to the international workshop entitled "New Horizons in Teaching Science", which was held in Messina, Italy (18–19 november 2018), under the patronage of the *Accademia Peloritana dei Pericolanti*
Manuscript received 02 June 2020; published online 30 September 2021