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REVIEW

Recent developments in methodology employed to study the interactions between nanomaterials and model lipid membranes

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Abstract With the boom of nanotechnology, nanomaterials (NMs) have been widely utilized in diverse applications, especially in biological and biomedical fields. Understanding how NMs interact with biomolecules, including proteins, DNA, and lipids, is of great importance for revealing the limitations posed and opportunities offered. Model lipid membrane, as a simplified cell membrane model, has been widely used to study the nanomaterial–lipid membrane interactions. In this article, current and emerging techniques, both experimental and theoretical, to investigate the interactions between NMs and model lipid membrane are summarized with each tool's capacities and limitations, along with future directions and challenges in this exciting area. This critical information will provide methodological guidance for researchers in this field.

Keywords Model lipid membrane · Interactions · Nanomaterial · Nano-bio interface

Introduction

Advances in nanotechnology have accelerated the translocation of engineered nanomaterials (NMs) into many fields, including cosmetics, textiles, paints, and nanomedicine [1, 2]. Accordingly, release of these NMs into the environment will undoubtedly increase potential occupational and public exposure to manufactured NMs. To address their safety issue, evaluations of nano-bio effects are crucial and urgently needed [3–6]. Biomembrane is the first barrier NMs encounter when presented in a biological environment. Therefore, understanding the nature of NM–biomembrane interactions is crucial for evaluation of nano-bio effect, and revealing the underlying mechanism of NM–biomembrane interactions is important for improvement of NMs design.

Biomembrane is a dynamic complex system that contains multiple components both within the membranes and on the membrane surface (such as distinct spatial localized phospholipid, cholesterol, proteins, peptidoglycans, and polysaccharides) [7]. It is advantageous to employ simplified biomimetic membrane (model lipid membrane) of known compositions to systematically investigate the fundamental physical and chemical properties in NM–biomembrane interactions [8]. Using model lipid membrane, the interactions between NMs and individual components of cell membrane could be assessed. By applying model lipid membrane of various compositions, fundamental insights into complex process of NM–biomembrane interactions may be probed. The aim of the introduction of model lipid membrane into NM–biomembrane interactions is not only to establish an in vitro model but, more importantly, also to provide molecular details of interactions between NMs and each component of cell membrane, based on which the nature of such complex NM–biomembrane interactions could be revealed from simple systems to complex systems.

Up to date, various model lipid membranes have been employed in NM–biomembrane interaction studies, such as supported lipid bilayer (SLB), hybrid bilayer membrane (HBM), tethered bilayer lipid membrane (t-BLM), Langmuir-Blodgett lipid monolayer (LB monolayer), lipid

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vesicles, lipid monolayer on hanging mercury drop electrode, and black lipid membrane (BLM) (shown in Fig. 1). Numerous techniques are adapted to investigate the interactions between NMs and various model lipid membranes, both experimentally and theoretically. In this article, we present state of the art techniques applied in this area and highlight each tool's capacities and limitations. Since the area of NM–biomembrane interactions is still a new frontier, challenges related to these types of measurements and opportunities to further this field of study are also discussed.

Methods based on advanced microscopic techniques

When NMs "meet" model lipid membranes, the adsorption of NMs will induce morphologic changes of membrane, varying from NM to NM, because of the different properties (shape, size, charge, etc.) of NMs. NM-induced morphologic change of model membranes could reflect the process and consequence of their interaction. Thus, studying the morphologic change of model lipid membranes is just about the most straight forward and effective method of probing their interactions. Atomic force microscopy (AFM) and confocal fluorescence microscope-based methods are the most adopted techniques to characterize morphologic change of model lipid membranes induced by NMs.

AFM-based techniques

AFM is a surface analysis technique that scans across the surface of a sample with a sharp cantilever tip to record the morphologies and physical properties of a sample based on the change of interaction force between tip and sample surface. The ability to be performed in aqueous solutions under physiological conditions, in real time, and with (sub)nanometer resolution makes it particularly suitable for the characterization of biological samples [9], especially for model lipid membranes [10]. The NM-induced morphologic changes observed by AFM are mainly based on SLBs and transferred LB monolayers. Various morphologic changes of membrane were observed by AFM, including pore formation [11, 12], phase and thickness change [12, 13], pealing and bending [14], and NMs adsorption, insertion, and envelopment by membrane [15–17]. For instance, low generation (G3) PMAMA dendrimers adsorbed preferentially to bilayer edges, but PAMAM dendrimers of higher generation behave differently (Fig. 2) [12, 18–20]. G5- and G7-PAMAM dendrimers were found to cause pore formation in SLBs with a different mechanism. G7-PAMAM with amine or carboxyl groups is capable of initiating pore formation in previously intact bilayers, whereas G5-PAMAM only expands holes at an existing defect. For multicomponent phase-separate SLBs, preferential interaction with liquid phase domain (Lα region) of G7- and G5-PAMAM was observed [21, 22]. AFM dynamic imaging was also used to directly observe the interaction between SLBs and graphene oxide (GO) [23]. LB monolayer-based AFM image was also adapted to characterize the adsorption and/or incorporation of NMs [24].

By combination with other techniques, more information of NM–model lipid membrane interaction could be provided. Parimi et al. combined AFM with optical waveguide light mode spectroscopy (OWLS), and concentration dependence of adsorption and/or disruption of model membrane by PAMAMs was found and rate constants of bilayer removal and PAMAMs adsorption were obtained [25]. Taking advantage of force modulation imaging with AFM, which could reveal information about a sample's mechanical properties, Sachan et al. confirmed the presence of hydrophobic polyorganosiloxane nanoparticle (POS NP) clusters in

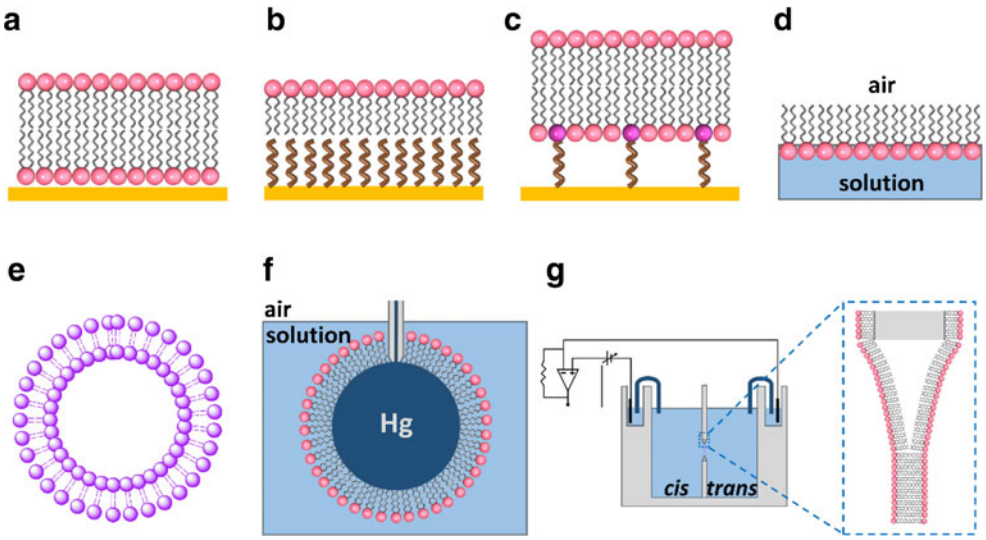

Fig. 1 Model lipid membranes used in NM-model lipid membrane interactions. (**a**) Supported lipid bilayer. (**b**) Hybrid bilayer membrane. (**c**) Tethered bilayer lipid membrane. (**d**) Langmuir-Blodgett lipid monolayer. (**e**) Lipid vesicles. (**f**) Lipid monolayer on hanging mercury drop electrode. (**g**) Black lipid membrane

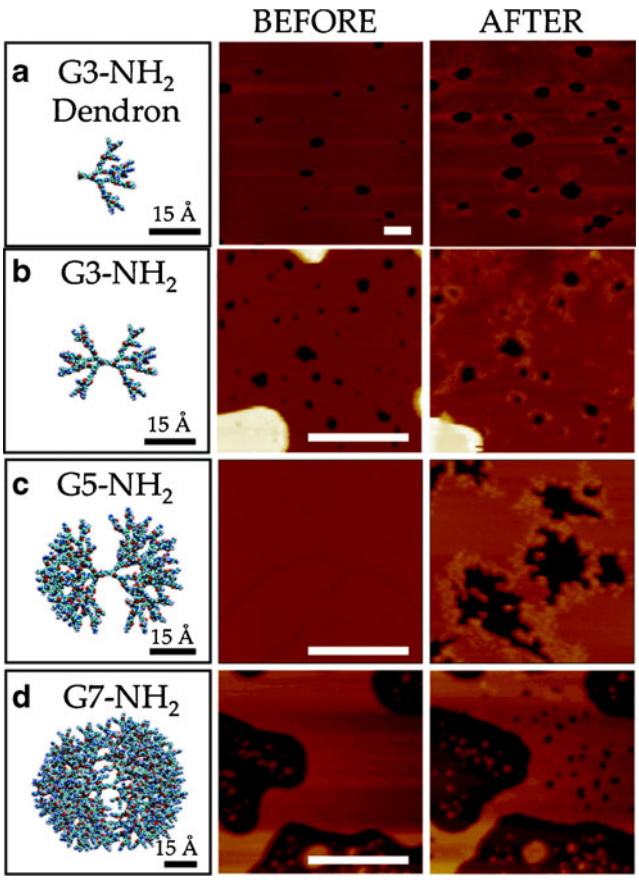

Fig. 2 AFM height images of SLBs before and after interaction with dendrimers of various generations. (**a**) G3-NH_2 dendron (16 e^+) expanded pre-existing defects and accumulated around the edges. (**b**) G3-NH_2 (32 e^+) accumulated around the edges of pre-existing SLB defects. (**c**) G5-NH_2 (128 e^+) expanded preexisting line defects and accumulated around the edges. (**d**) G7-NH_2 (512 e^+) primarily induced the formation of new defects on SLB. Scale bars are 500 nm. (Reprinted from [12] with permission from the American Chemical Society)

nanoscale globular structures in compressed model pulmonary surfactant monolayer [26]. They also employed a quantitative statistical analysis based on AFM topographic image to characterize the influence of NMs on multilayer protrusions of pulmonary surfactant monolayer.

Although AFM is particularly suitable for image model lipid membrane, slow scan rate and limited image area are the main disadvantages in studies of NM–model lipid membrane interactions. As a morphologic characterization technique, AFM needs to combine with other methods to provide more information of NM–model lipid membrane interactions.

Confocal fluorescence microscope-based techniques

Confocal laser scanning microscope (CLSM) is an advanced fluorescence microscope design equipped with fluorescence microscope with laser light source, scanning, and focusing system for obtaining high-resolution optical image with depth selectivity. By optical sectioning, high-resolution 3D image of specimen could be obtained by computer reconstruction (Fig. 3). By imaging the fluorescent signal of NMs and model lipid membranes, their interaction could be visualized to reveal NM-induced changes of the properties of lipid membrane, including morphology [27, 28], permeability [29], and integrity [28, 30]. For instance, adsorption of citrate capped 15 nm Au NPs and the same NPs coated with a corona of serum proteins induced marked difference in GUVs morphologic and microstructural changes [31]. To identify if the morphologic change of NM-treated membrane is accompanied by the change in permeability, we can monitor NM-induced fluorescent dye leakage from GUVs [32].

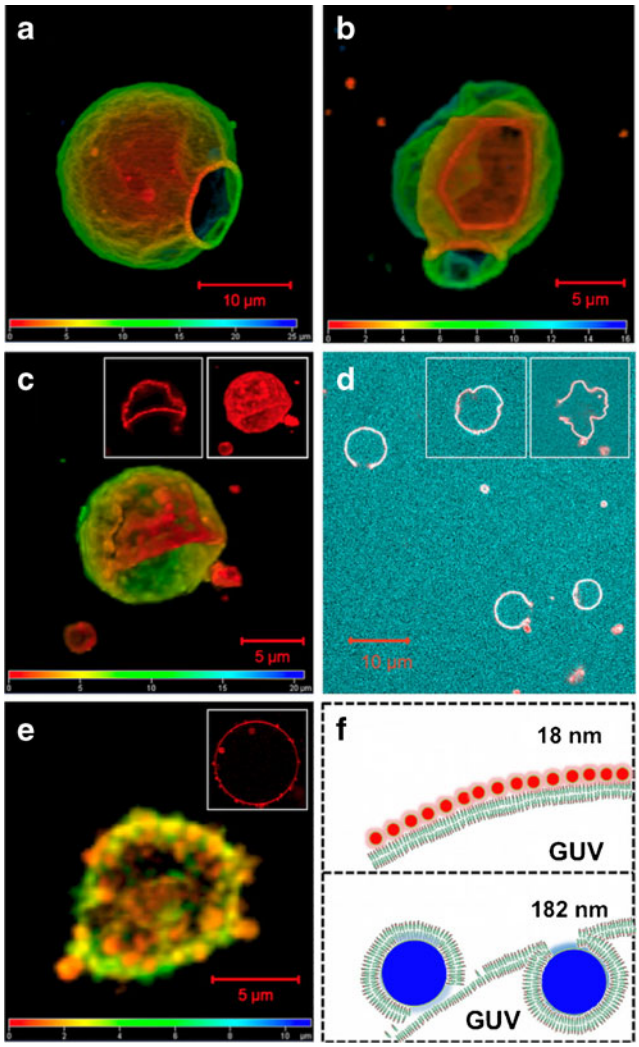

Fig. 3 CLSM of DOPC GUVs after NP interactions. (**a**), (**b**) Reconstituted 3D images of GUVs with unusual curvature and stabilized holes. (**c**) A helmet-shaped GUV. (**d**) 2D confocal image of deformed GUVs and dextran fluorescence leakage after interaction with 18 nm SiO_2 NPs. (**e**) 3D reconstruction of a GUV interacting with 182 nm SiO_2 NPs. (**f**) Schematic view of the effect of sizes on the NP-membrane interactions. (Reprinted from [33] with permission from the American Chemical Society)

Furthermore, the change of membrane integrity could be observed by characterization of translocation of fluorescent labeled NMs [32]. Similarly, the pore formation of membrane could be identified by the leakage of fluorescent dextran preencapsulated inside GUVs, and the size of pore could be characterized by encapsulation of fluorescent dextran with different molecular weights [28]. It is worth noting that Zhang et al. clearly distinguished smaller SiO_2 NP-induced hole and larger SiO_2 NP-induced collapse of vesicle by reconstituting of CLSM images to 3D images of GUVs by computer (Fig. 3) [33]. Some nondisruptive adsorption or penetration of cationic fluorescein-5-iso-thiocyanate (FITC) labeled core-shell NPs [34], anionic monolayer-protected Au NPs [35], and amphiphilic QDs [29] on or through GUVs membrane were also observed by CLSM. In addition to GUV-based model, SLBs were also used as model to characterize the disruption of lipid membrane by polystyrene NPs, the disruption kinetic process could be obtained based on semiquantified data (Fig. 4) [30, 36].

Compared with AFM-based technique, fast imaging speed and large image area make CLSM more suitable to monitor the dynamic process of NM–model lipid membrane interactions in real time. Semiquantified and even quantified data could be obtained based on fluorescence intensity. However, low spatial resolution and sensitivity are the main limitation in traditional CLSM. Total internal reflection fluorescence microscopy (TIRFM), which enables selective visualization of surface region [37], was applied to observe the interaction between lipid vesicle and surface-immobilized NPs at the single nanoparticle level [38]. The dynamics of membrane deformation for a lipid membrane in contact with NPs coated with a conjugated polyelectrolyte was observed. Despite such progress, the introduced fluorescent label on NMs or model lipid membranes and the labeling process may introduce unknown influence on their interaction process.

Methods based on spectroscopic techniques

The interactions between NMs and mode lipid membranes not only cause morphologic changes of membrane but also may induce physicochemical property changes. Several spectroscopic techniques are applied to characterize these physicochemical changes, which could provide information about both interaction mechanism and potential influence. In this section, we summarize these spectroscopic techniques adopted to study NM–mode lipid membrane interactions: fluorescence-based spectroscopies and vibration spectroscopy-based techniques.

Fluorescence-based spectroscopic techniques

Fluorescence-based spectroscopies are mainly performed to characterize the NM-induced phase, permeability, and mobility change of model lipid membrane, but not limited to such information. At present, four main fluorescence-based techniques, fluorescence spectroscopy, fluorescence resonance energy transfer measurement (FRET), fluorescent anisotropy measurement, and fluorescence correlation spectroscopy (FCS), have been performed in studies of NM–model lipid membrane interactions.

Fluorescence spectroscopy-based dye leakage assay has been widely used to characterize the NM-induced permeability change of lipid vesicles by measuring the ratio of fluorescent intensity of dye molecule leaked from lipid vesicle and total fluorescent intensity of dye molecule inside lipid vesicle [39–41]. Furthermore, through mathematic modeling of the leakage kinetics, single NP-induced defect on a lipid vesicle could be revealed [42]. Fluorescence quenching measurement was applied to assess the solvent accessibility of NM-conjugated fluorophores, based on which the NM-induced

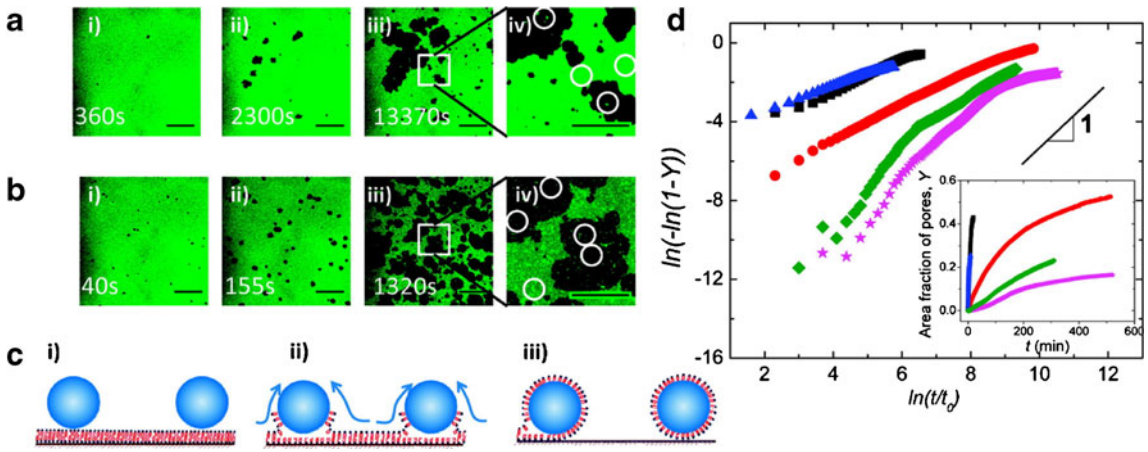

Fig. 4 Morphologic evolution of SLBs after adding polystyrene NPs in (**a**) 0.2× PBS and (**b**) 0.6× PBS buffer solutions. (**c**) Schematic of the morphologic evolution of SLBs upon the adsorption of polystyrene NPs in PBS solutions. (**d**) Kinetics of the space-spanning of lipid-poor regions on SLBs in varied PBS volume fraction, 1× (triangles), 0.8× (squares), 0.6× (circles), 0.4× (diamonds), and 0.2× (stars). Scale bars are 20 μm. (Reprinted from [30] with permission from the American Chemical Society)

aggregation of lipid vesicles was revealed [40]. Through comparing the fluorescence quenching assay of external fluorescence probe before and after interacting with dendrimer NPs, one can conclude whether the internalization takes place [41]. Moreover, the fluidity and phase change of membrane could be monitored by excimer/monomer ratio of pyrene-containing fluorescent probe [41]. Furthermore, comparing the emission intensity at the wavelengths of peak emission intensity for bilayers in pure fluid and gel phases, quantified fluid-gel phase conversion could be obtained [43].

FRET measurement was also used to monitor the NM-induced phase change by observing FRET efficiency change between two phase-specific probe labeled lipids before and after interacting with NMs [43]. In addition, FRET measurement was also performed to characterize whether NMs interact with lipid vesicles by monitoring the FRET efficiency increase between fluorescent dye labeled lipids and fluorescent NPs [40, 44], or decrease between two fluorescent dyes both entrapped inside lipid-based liquid crystalline nanoparticles (LCNPs) because of the diluting effect caused by fusion of lipids [45]. Also, FRET could be used to determine the efficiency of NM-induced lipid mixing by observing FRET of two fluorescent dyes labeled on one lipid vesicle that was intermixed with unlabeled lipid vesicles in the presence of NMs [41].

Fluorescent anisotropy measurement, which uses a polarized light to excite fluorescent molecules and measures the anisotropy of emitted light, provides information of scrambling of fluorescent molecule orientations, and thus could be used to characterize the mobility of model lipid membranes. Wrobel et al. measured fluorescent anisotropy of two fluorescent probes incorporated into the hydrophobic and hydrophilic regions of the bilayer membrane in lipid vesicles to reveal mobility change of membrane and interacting region and strength in membrane [46, 47]. Apparent dissociation constants (K_d) of NMs adsorption on lipid vesicles could also be obtained by fluorescence anisotropy measurement [40].

FCS is a powerful technique to measure concentration, mobility, equilibrium, and rate constants for the interactions and internal dynamics of biomolecules in various biological systems [48], and has been used to determine various parameters in NM–model lipid membrane interactions, for instance, the anionic NPs adsorption-induced shrinkage of initially fluid lipid vesicle [43] and NM-induced mobility change of SLBs characterized by diffusion coefficient (D) [30, 49]. Taking advantage of the property that FCS measurements based on statistical analysis of intensity fluctuations in very small ensembles, Zhang et al. found that the "slaved" diffusion of phospholipid in SLBs induced by adsorption of polyelectrolyte are localized [50]. Moreover, the molecular trafficking through the membrane of GUVs could be investigated by FCS (Fig. 5). The permeation of fluorescent dye (Alexa) was evidenced by monitoring the concentration of the dye inside and/or outside GUVs, whilst the fluorescent dye status, free molecule or adsorbed on NMs, was confirmed by decay times of the autocorrelation function [31].

Fluorescence-based spectroscopies could provide valuable physical information of model lipid membrane (especially mobility and phase changes) and ultra-high sensitivity. However, in most case, a suitable fluorescent label is required.

Vibrational spectroscopy-based techniques

Vibrational spectroscopy is a valuable tool for elucidation of molecular structure. It often provides a fingerprint by which molecules can be identified. As far as we know, three vibrational spectroscopies have been used to study the interactions between NMs and model lipid membranes, including Fourier transform infrared (FTIR), Raman, and sum-frequency generation (SFG) spectroscopy.

FTIR spectroscopy was applied to characterize the fullerenol (FulOH)-induced disordering of lipid acyl chain and decreased phase transition temperature of lipid vesicles by monitoring peak shifts of CH_2 and CD_2 asymmetric stretch modes [51]. The intensity ratios and the changes of frequencies of Raman spectroscopy indicated that the incorporation of the G4- and G3.5-PAMAM dendrimers into DPPC lipid vesicles causes a concentration-dependent increase of the membrane fluidity [52]. SFG spectroscopy was also applied to verify the NM-induced orientation and molecular conformation changes of LB membranes [53], and the lipid flip-flop rate of SLBs [54]. Through comparing the intensity ratio of methyl antisymmetric stretch to methyl symmetric stretch modes [ν_{as} (CH_3)/ν_s (CH_3)] and the intensity ratio of methylene to methyl symmetric stretch modes [ν_s (CH_2)/ν_s (CH_3)], the changes in tilt angle and the conformational and orientational order of lipid alkyl chains in LB monolayer and SLBs caused by interacting with NMs could be obtained [55–57]. By phase-sensitive SFG spectroscopy, the orientation change of interfacial water molecules associated with LB monolayer in the presence of NMs can be identified [56].

In past years, our group explored surface-enhanced infrared absorption (SEIRA) spectroscopy to study the interactions between NMs and model lipid membrane [58–61]. SEIRA spectroscopy, which uses nanostructured metal film as enhancement substrate, could enhance the vibrational bands of adsorbed molecule by a factor of 10–1000 [62]. Taking advantage of rapidly decayed enhancement with the distance from the substrate surface, contribution of bulk solution is eliminated, achieving selective detection of signals from the adsorbed molecule even when the surface is immersed in water [63–65]. This property overcomes the limitation of traditional attenuated total refection Fourier transform infrared (ATR-FTIR) spectroscopy in which water represents a major obstacle, endowing SEIRA spectroscopy with the ability to investigate biological samples in aqueous environments.

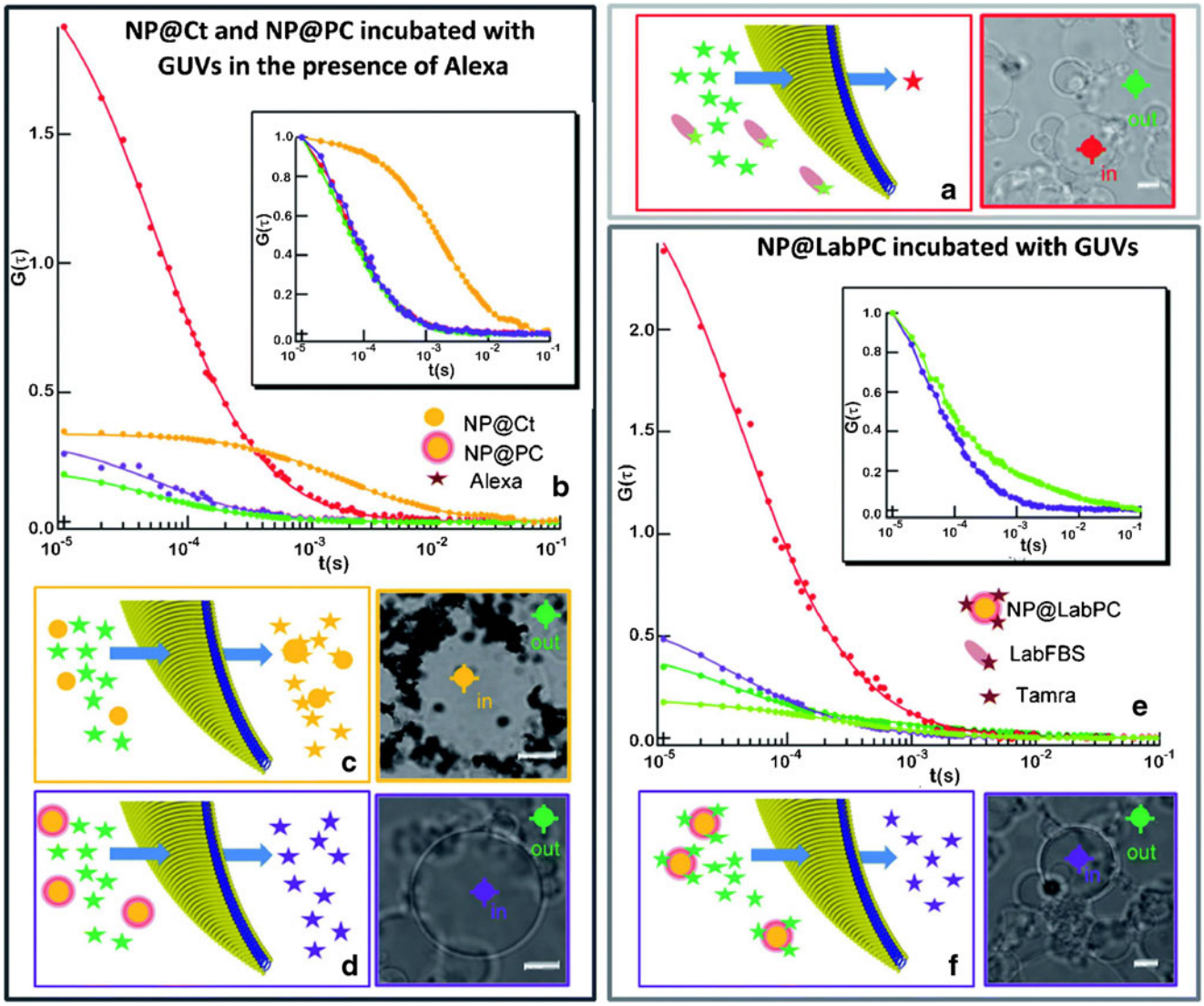

Fig. 5 Bilayer permeability upon interaction with NPs. (**a**) Scheme of the control experiment: membrane permeability to externally added LabFBS and Alexa in the absence of NPs. (**b**) Representative FCS profiles of the dye monitored outside (green) and inside the GUVs in the absence of NPs (red) and in the presence of NP@Ct (yellow) and NP@PC (purple); (**b**, *inset*) the FCS curves are normalized to compare the decay times: for NP@Ct, whole NP–dye aggregates are present inside the GUVs after incubation (**c**), whereas for NP@PC, only the diffusion of the free dye is detected (**d**). (**e**) Control FCS curves monitored outside (light green) and inside (red) the GUVs in the presence of LabFBS, compared with the FCS monitored outside (dark green) and inside (purple) the GUVs' lumen after incubation with NP@LabPC; (**e**, *inset*) normalized FCS curves: the incubation with NP@LabPC enhances permeability of molecular species but does not allow internalization of nanoparticle constructs as the corresponding scheme (**f**) sketches. Scale bars are 10 μm. (Reprinted from [31] with permission from the Royal Society of Chemistry)

HBMs could be constructed on SEIRA substrate by vesicle spreading and fusion, and NM-induced difference spectra could be obtained by taking HBM system in the absence of NMs as reference spectrum, as shown in Fig. 6. First, we applied this strategy to study the interaction of D-penicillamine coated CdSe/ZnS QDs with HBMs that mimic the inner and outer leaflet of red blood cell (RBC) membranes [58]. Based on the presence of molecular "finger print" vibration mode in SEIRA difference spectra, we identified that the zwitterionic ligand coated on QDs are still in their zwitterionic form when interacting with the lipid membrane. QDs interacting with either side of the lipid membrane of the RBC membranes soften the lipid structure indicated by the observation of blue shift of ν_{as} (CH_2) and ν_s (CH_2) peaks of HBMs, which might facilitate the penetration of zwitterionic QDs into lipid bilayer. Based on this model, SEIRA spectroscopy was further performed to reveal toxicity mechanism of GO, nitrogen-doped graphene quantum dots (N-GQDs), and mercaptosuccinic acid-capped CdTe QDs with different sizes in RBCs [59, 61]. Detailed analysis of the different spectra revealed that the adsorption of GO destroys the integrity of membrane by extracting the lipids from membrane, resulting in hemolysis, while coordination of CdTe QDs with diameter of 5 nm with phosphate will break the phosphodiester bond and induce

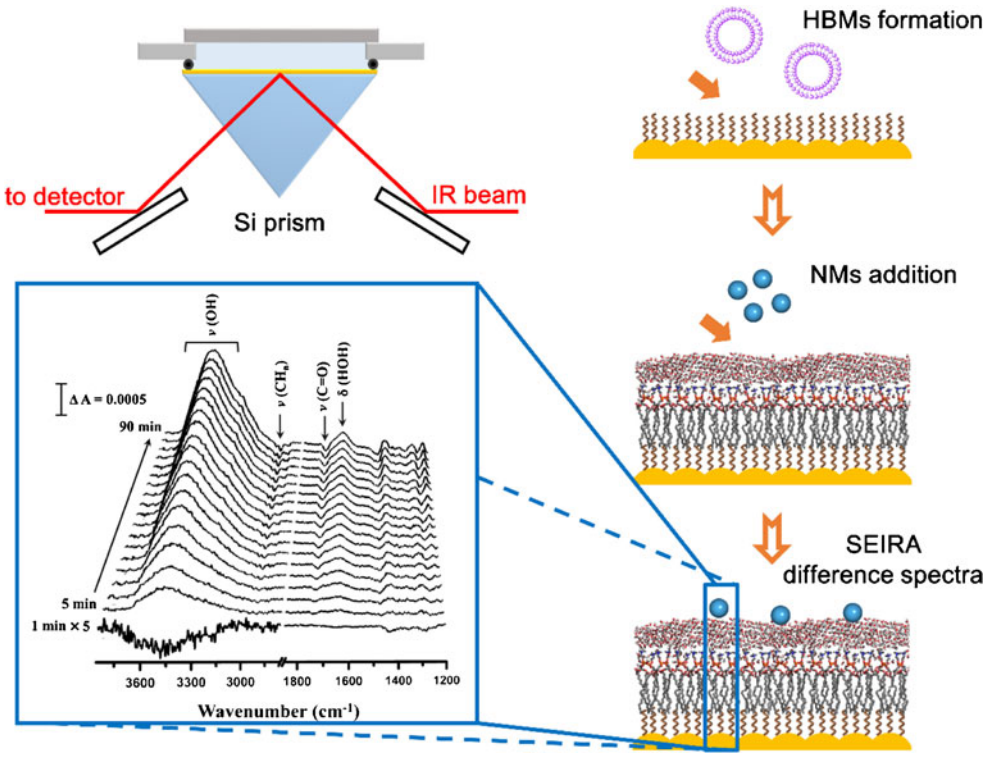

Fig. 6 Schematic of the procedure of applying SEIRA spectroscopy to investigate NM–model lipid membrane interactions. Hybrid bilayer membrane is formed by hydrophobic interactions between lipid vesicles and hydrophobic thio-alcohol monolayers self-assembled on a gold substrate. NM-induced difference spectra are acquired by taking lipid membrane immersed in solution as reference spectrum

hemolysis. N-GQDs and CdTe QDs with diameter of less than 5 nm just disturbs the structure and conformation of the lipid membrane. Recently, we successfully probed the interaction forces between GO and lipid membrane by combining the advantage of extremely large extinction coefficient of water molecule in the mid-infrared region and strict surface sensitivity of SEIRA spectroscopy [60]. Through observing the initial interfacial water change and time-dependent difference spectra of lipid membrane (positive peak, negative peak, and shift of peak position) induced by GO in the absence and/or presence of different interaction force probes in situ and in real-time, four kinds of interaction forces, electrostatic attraction, electrostatic repulsion, hydrogen bonding, and hydrophobic interaction, were identified in the interaction between GO and lipid membrane.

Vibration spectroscopy is a powerful, informative, and label-free technique to investigate the NM–lipid membrane interactions in situ. With surface-enhanced technique, minor spectra changes of monolayer lipid membrane could be obtained in situ and in real-time. However, the poor ability to distinguish same chemical bond in different molecules limits its application in more complex system.

Methods based on electrochemical techniques

Lipid membranes, composed of two layers of amphiphilic lipids with hydrophilic head groups oriented outward to an aqueous solution and long hydrophobic aliphatic tails positioned inward toward each other, are highly resistant and impermeable to hydrated ions. Thus, lipid membrane acts as an insulator, containing resistance and capacitance. The variation of bilayer lipid membranes' electrical properties in response to NMs could be exploited by electrochemical methods, including cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS), and electrophysiology.

CV technique

CV technique, which records the electrode current during the electrode potential ramping cyclically between a lower potential and a higher potential, has been applied to detect NM-induced membrane defect/pore formation by CV responses of redox probes such as $[Ru(NH_3)_6]^{3+}$ and $[Fe(CN)_6]^{3-}$ because of the enhanced electrode surface accessibility of redox probes through NM-induced defect/pore on membrane [58, 66]. Another CV-based electrochemical model system uses lipid monolayer adsorbed to the surface of a mercury electrode (Fig. 1f), such as hanging mercury drop electrode (HMDE) and mercury film electrode as a model lipid membrane. Lipid monolayer on mercury electrode exhibits two characteristic current peaks attributable to the phase transitions in the lipid monolayer corresponding to the successive ingress of electrolyte into the layer and the associated reorientation of the layer in response to the increasing applied negative potential (Fig.7a). Any modification to the phospholipid monolayer's organization and fluidity will result in alterations in the two

current peaks. This has been used to detect the silica NP–membrane interactions in an empirical semiquantitative manner [67]. Furthermore, lipid monolayer on HMDE was used to detect the penetration of small Au NPs through the membrane, taking the hydrogen redox-chemistry characteristic of ligand-stabilized Au NPs in molecularly close contact with a mercury electrode as an indicator (Fig. 7b) [68]. The ability of small Au NPs to spontaneously penetrate biological membranes can be evaluated using such established electrochemical membrane model. Furthermore, by calculating the charge transferred during hydrogen redox process, the electrode surface coverage with NPs inserted into the lipid monolayer, as well as the insertion rate, could be obtained quantitatively. The main drawback of CV is the limited information provided; no structure and detailed interaction mechanism could be obtained.

EIS technique

EIS is a well-established noninvasive yet extremely sensitive method to study the surface electro-dynamic properties of membranes and their changes upon interaction with NMs. By fitting the EIS spectrum with a suitable equivalent circuit, the membrane resistance (R_m) and membrane capacitance (C_m) could be obtained. EIS was used to investigate the perturbation and defect/pore formation induced by NMs [66, 69]. Furthermore, exponential rate constant and fractional loss of R_m could be obtained by fitting the time-dependent changes in R_m with an exponential-decay model, and NMs with difference in both size and surface functional group could be distinguished by statistical hierarchical clustering analysis of these two model parameters [70, 71]. Physical parameters, such as resistance and differential capacitance in the electrochemical system may be directly obtained from the EIS. The detailed physical information about the properties of membrane, however, is not directly accessible without modeling. The modeling process is rather complex in selecting or constructing the suitable equivalent circuit model and interpreting the physical meaning of every element.

Electrophysiology technique

Electrophysiology, the most sensitive method to evaluate membrane permeability, provides detailed current-time "fingerprints" with single-pore sensitivity and rapid dynamics, yielding a rich source of information about the underlying molecular interactions. In the past few years, it was applied to study the interactions between NMs and BLMs. The NM-induced membrane permeability change such as pore formation and membrane breakdown could be indicated by spike-like current burst in current-time curves [72–75] and onset changes of I_{max} in volt-ampere curves [76] even at femtomolar concentration [77]. An electrophysiology-based cyclable parallel lipid bilayer arrays chip was constructed, achieving greatly improved throughput (Fig. 8a) [78]. Furthermore, Carney et al. improved traditional electrophysiology measurement to monitor the capacitance change of membrane in the process of

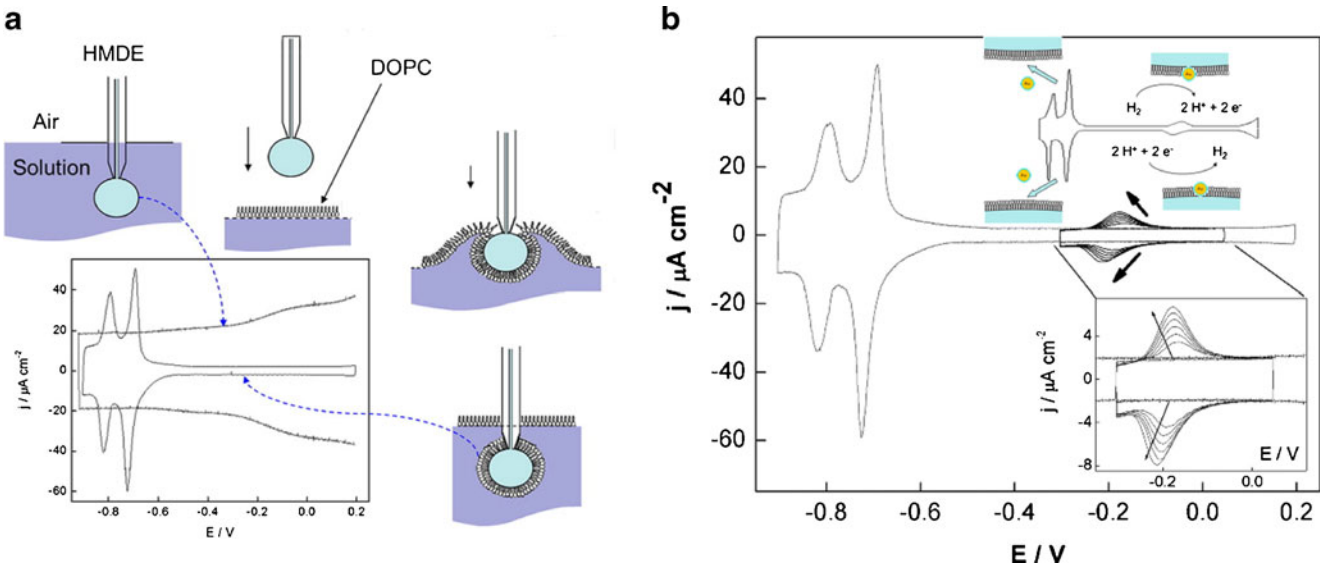

Fig. 7 (**a**) Schematic representation of lipid monolayer formation on HMDE. The electrode is passing through a compact lipid at the water/air interface to enable the formation of lipid monolayer on electrode surface. Significant drop in capacitance and the appearance of the characteristic reorganization peaks in the CV are observed after formation of the monolayer. (**b**) Spontaneous penetration of the compact lipid monolayer by 2 nm Au NPs. The inset shows the gradual development of the characteristic hydrogen redox peaks of Au NPs in contact with the mercury electrode in detail, indicating continuing insertion of NPs into the lipid monolayer. (Modified from [68] with permission from the American Chemical Society)

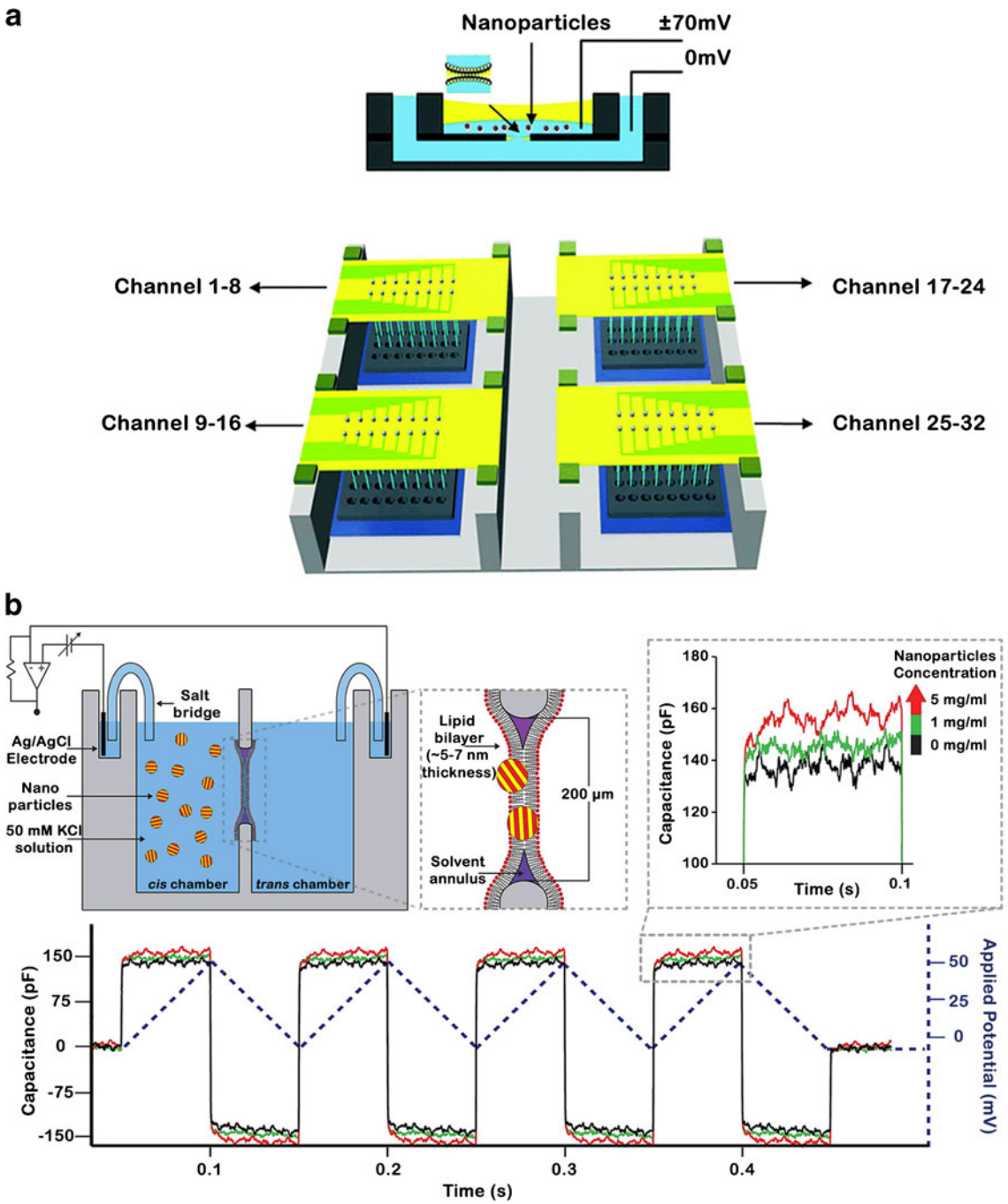

Fig. 8 (**a**) Schematic representation of electrophysiology based parallel lipid bilayer arrays chip. NMs are added to the central well after bilayer formation, and NM–model lipid membrane interactions are measured electrophysiologically. (Reprinted from [78] with permission from the Royal Society of Chemistry). (**b**) The schematic of experimental system of electrophysiology method for quantifying interaction between BLMs and "striped" mixed-monolayer-coated Au NPs. *Cis* and *trans* chambers filled with electrolyte and connected to electrodes via salt bridges are separated by a lipid bilayer. With a ramping transmembrane potential (at a constant rate of ±1 V/s) applied, NPs with increasing concentrations are then added to the *cis* compartment, and the current/capacitance is recorded by stepped magnitude increases of the square wave attributable to the insertion of individual NPs into the membrane. (Modified from [79] with permission from the American Chemical Society)

NMs absorption by ramping the transmembrane potential at a constant rate of ±1 V/s, under which the magnitude of the square-wave current measured is equal to the capacitance (Fig. 8b) [79]. By fitting the NPs concentration-dependent capacitance changes with a modified Langmuir isotherm model, quantified thermodynamic parameter, such as surface coverage, partition coefficient K, and the standard free energy change could be obtained.

Although electrophysiology measurement provides the most sensitive method to study NM–model lipid membrane interactions both qualitatively and quantitatively, the main restrictions are residual solvent and limited lifetime of BLMs.

Methods based on electromagnetic techniques

The structure variation of bilayer lipid membranes during NM–membrane interactions could be characterized based on the changes of the electromagnetic properties of the system. Nuclear magnetic resonance (NMR), X-ray reflectivity (XRR), neutron reflectivity (NR), small angle scattering (SAS), and electron paramagnetic resonance (EPR) are performed to investigate the interactions between NMs and model lipid membrane.

NMR is a physical phenomenon in which nuclei in a magnetic field absorb and re-emit electromagnetic radiation at a specific resonance frequency, which depends on the strength of the magnetic field and the intrinsic magnetic properties of the isotope of the atoms. Thus, the dynamic and structure details of NM–model lipid membrane interactions could be investigated by NMR technique through characterizing systems' isotopes. For instance, ^{31}P, ^{15}N, and ^{2}H NMR are performed to investigate the NM-induced structure change of lipid membrane: ^{31}P static NMR spectra reveal average orientation of the phosphate group and ^{31}P NMR magic angle spinning (MAS) reveals electronic environment and changes in phosphate group dynamics [19, 51, 80]; ^{14}N NMR reveals the electronic environment and tilt of the lipid headgroup [80]; and ^{2}H NMR reveals the perturbation of the tail group in membrane [51]. Furthermore, 2D NMR, such as ^{1}H radio frequency driven dipolar recoupling (RFDR), proton-detected local field with R-type recoupling (R-PDLF), and ^{1}H MAS nuclear Overhauser effect spectroscopy (NOESY) techniques, could provide more detailed molecular interactions [80].

XRR and NR are surface-sensitive analytical techniques to characterize interfaces, thin films and multilayers through recording intensity of reflected radiation. The exact shape of the reflectivity profile provides detailed information about the structure of the surface, including the thickness, density, and roughness. XRR is used to determine the NM-induced thickness and density change of lipid monolayer [56] and lipid packing-dependent absorption and penetration of cationic QDs [81]. Compared with XRR, NR has better sensitivity of light element (quiet important for lipid membrane), enhanced contrast through isotopes substitution, highly non-perturbing penetrating, which makes it more suitable for lipid membrane system to probe the interaction model [82–84], and the mass-exchange between NMs and model lipid membrane [85, 86].

SAS, including small-angle X-ray scattering (SAXS) and small-angle neutron scattering (SANS), is a scattering technique based on deflection of collimated radiation away from the straight trajectory after it interacts with structures that are much larger than the wavelength of the radiation. SAS has been used to characterize the NM-induced membrane layer structure and phase changes [57, 87, 88]. By combining with freeze-fracture or cryo-TEM, NM-induced formation of lamellar hybrid condensed phase were evidenced [44, 89]. Besides, EPR was also applied to investigate the interaction between starburst dendrimers and lipid vesicles by labeling a nitroxide radical to NMs [90, 91] or lipid vesicles [92]. Electromagnetic property-based techniques are capable of providing detailed information of NM–model membrane interactions at molecular level, but isotopes are necessary in most case. Furthermore, solvents effect should be considered in these measurements.

Methods based on thermodynamic techniques

Differential scanning calorimetry (DSC), isothermal titration calorimetry (ITC), and surface pressure-area isotherm (π-A isotherm) are performed to investigate the interactions between NMs and model lipid membrane based on the changes of thermodynamics of the interaction system.

DSC is a thermo-analytical technique in which the difference in the amount of heat required to increase the temperature of a sample and reference is measured as a function of temperature by maintaining the same temperature conditions for the sample and reference. NM-induced changes of lipid membrane in thermotropic properties, including enthalpy (ΔH), T_{onset}, T_m, and $T_{1/2}$ of pre-transition and main-transition could be monitored to characterize the interactions [47, 52, 93, 94]. The release of loaded cargo could be identified by the disappearance of a specific peak assigned to NPs loaded cargo molecule in calorimetric curve [95].

ITC is a quantitative thermodynamic technique that could be used to determine the thermodynamic parameters of the interactions between NMs and lipid vesicles in solution, such as enthalpy change (ΔH), binding affinity (K_a), binding stoichiometry (n), Gibbs energy change (ΔG), and entropy change (ΔS) [14, 43, 96]. For instance, the interaction between polyoxometalate macro ion {Mo176} and PC liposomes was investigated by ITC technique (Fig. 9) [14]. The total enthalpy change (ΔH_T) of 358 k_BT per {Mo176} was obtained by integrating ΔH normalized by {Mo176} concentration, while fitting this curve yielded the binding constant K_a of {Mo176} on PC liposome to be 6.5×10^6 M^{-1} indicating a free energy change (ΔG) of $-15.7k_BT$ per {Mo176}. The drastic difference between ΔH_T and ΔG clearly reveals a huge entropic change (ΔS) that mostly may arises from the loss of entropy in the gelled lipids. ITC is the only technique that can simultaneously determine all binding parameters in a single experiment with no modification of test system. All measurements are carried out in their native state in solution

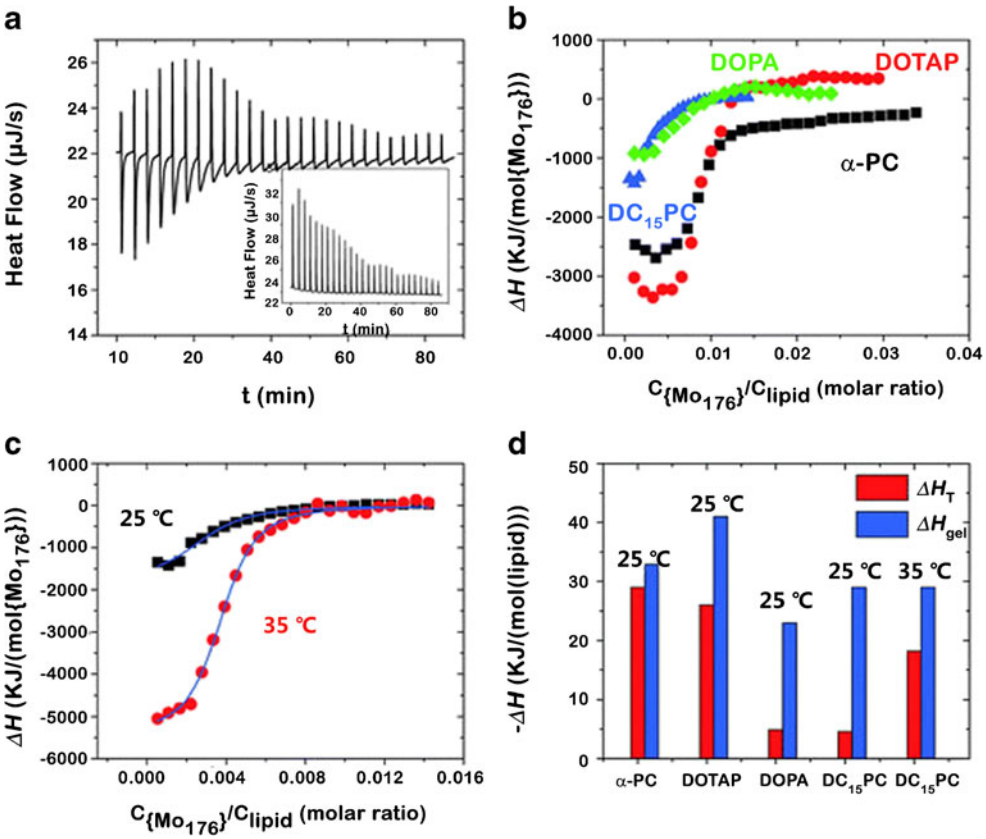

Fig. 9 (**a**) Heat flow change of PC liposome-added buffer suspension after adding {Mo176}. Inset: heat flow change of liposome-free buffer solution after adding {Mo176}. (**b**) Enthalpy change ΔH normalized by {Mo176} concentration against the molar ratio of {Mo176} to lipid. (**c**) Enthalpy change ΔH normalized by {Mo176} concentration against the molar ratio of {Mo176} to lipid in gel phase (black squares) and fluid phase (red circles). (**d**) The comparison of ΔH_T after adding {Mo176} to their reported fluid-to-gel phase transition enthalpy change, ΔH_{gel}. (Reprinted from [14] with permission from the Royal Society of Chemistry)

without labeling. It is a very valuable tool for studying NM–lipid membrane interactions.

π-A isotherm measures surface pressure at constant temperature as a function of the available area for each molecule in a LB monolayer. Holding the area of the lipid monolayer at the air/aqueous interface constant, an increase in surface pressure indicates penetration of the injected molecules into the membrane or by their electrostatic interactions with the phospholipid head groups, while a decrease in surface pressure indicates the loss of the membrane's phospholipids from the surface into the subphase. Various NMs penetrating into lipid monolayer have been observed by π-A isotherm measurements [13, 24, 53, 97, 98]. For instance, GO was found to incorporate or be adsorbed into the monolayer of positively charged lipid but not neutrally or negatively charged lipid [24]. Further analysis of GO-induced surface pressure and surface potential changes indicates a different "edge-in" and "face-in" model for GO adsorption. π-A isotherm measures are limited to LB monolayers, and no structure information could be obtained. Thus, it usually combines with other techniques, such as SFG and AFM.

Methods based on theoretical arithmetic

Due to the technological limitations of experimental methods, it is still quite a challenge to study NM–model lipid membrane interactions with high spatial scales and quick temporal scales with high resolution. Computational techniques could provide fundamental insights into dynamic processes of the system at nanoscale, as well as conditions that are inaccessible to experimental methods.

Molecular dynamic (MD) simulation simulates physical movements of atoms and molecules by numerically solving Newton's equations of motion for a system of interacting particles. The trajectories of atoms or molecules and dynamic processes of interactions could be described by MD simulations. Two types of dynamic model are adapted in the computer simulation of NM–model lipid membrane interactions: the atomistic model and coarseg rained (CG) model. The main difference between the two models is the simulation units adopted: atomistic models employ a single atom as simulation unit, and detailed atom–atom interactions could be determined; CG models combine a cluster of atoms, molecules, or chemical groups as a "bead," dramatically reducing the computation consumption, and huge complex system on long time scale simulations inaccessible to atomistic models could be achieved. Numerous studies involving the MD method have been done to determine the interactions between the lipid membrane and NMs. Take graphene as an example; it was found that small graphene and few-layer graphene (FLG) nanosheets form stable sandwiched graphene-lipid hybrid structures [99], but large graphene and FLG microsheets spontaneously pierced into the bilayer at their edge asperities and

corner sites [100]. Tu et al. showed that graphene nanosheets can penetrate into and extract large amounts of phospholipids from the cell membranes because of the strong dispersion interactions between graphene and lipid molecules (Fig. 10a) [101]. Through unbiased atomistic simulations, Van Lehn et al. identified the transition state of spontaneous insertion for amphiphilic organic monolayer-protected Au NPs (Fig. 10b) [16]. In addition, mathematical modeling has also been developed to investigate the adsorption/disruption and interaction model of NMs with lipid membrane [102, 103].

Other methods

In addition to the above discussed methods, mass sensor-based techniques are also performed to investigate the model lipid membrane exposed to NMs. Quartz crystal microbalance with dissipation (QCM-D) is an acoustic technique that could detect small mass changes on the sensor surface and to the visco-elastic (nanomechanical) properties of the adsorbed material. In NM–model lipid membrane interaction system, the change of resonance frequency (Δf) could reflect NMs absorption or lipid loss of membrane, while the shift in dissipation (ΔD) reflects the visco-elastic change at the interface. For instance, nonspecific adsorption of charged QDs on zwitterionic SLBs was monitored by QCM-D [104]. Results show that the adsorption behavior of charged QDs is dominated by electrostatic interaction characterized by an "adsorption window," which could be broadened by exploiting the bridging role of the Ca^{2+} ions. Upon combining QCM-D with AFM, the formation of a multilayer nano-composite consisting of alternating GO monolayers and lipid membranes was observed [105]. QCM-D could monitor the adsorption/desorption and binding kinetics of NMs in real time without labeling. However, QCM-D is a "wet mass" sensor that not only senses the adsorbed material but also acoustically coupled solvent (e.g., water). Thus, optical technique, such as surface plasmon resonance (SPR) spectroscopy [106], optical waveguide lightmode spectroscopy (OWLS) [25], dual polarization interferometry (DPI) [105], and ellipsometry [83], where the solvent associated with the sensor surface does not generate a response are also applied in this field.

Summary and perspectives

Great progress has been achieved by characterizing the NM-model lipid membrane system by advanced microscopic techniques, spectroscopic techniques, electrochemical techniques, electromagnetic techniques, thermodynamic techniques, as well as theoretical arithmetic. However, there are still many challenges and opportunities in this new frontier.

Simplified model membrane is good to investigate the fundamental biophysical phenomena, but limited in the complex structure of cellular membrane. Therefore, constructing a more "real" model membrane is crucial to improving our understanding. This may be achieved by (1) using multi-component membranes (phospholipids, sphingolipids,

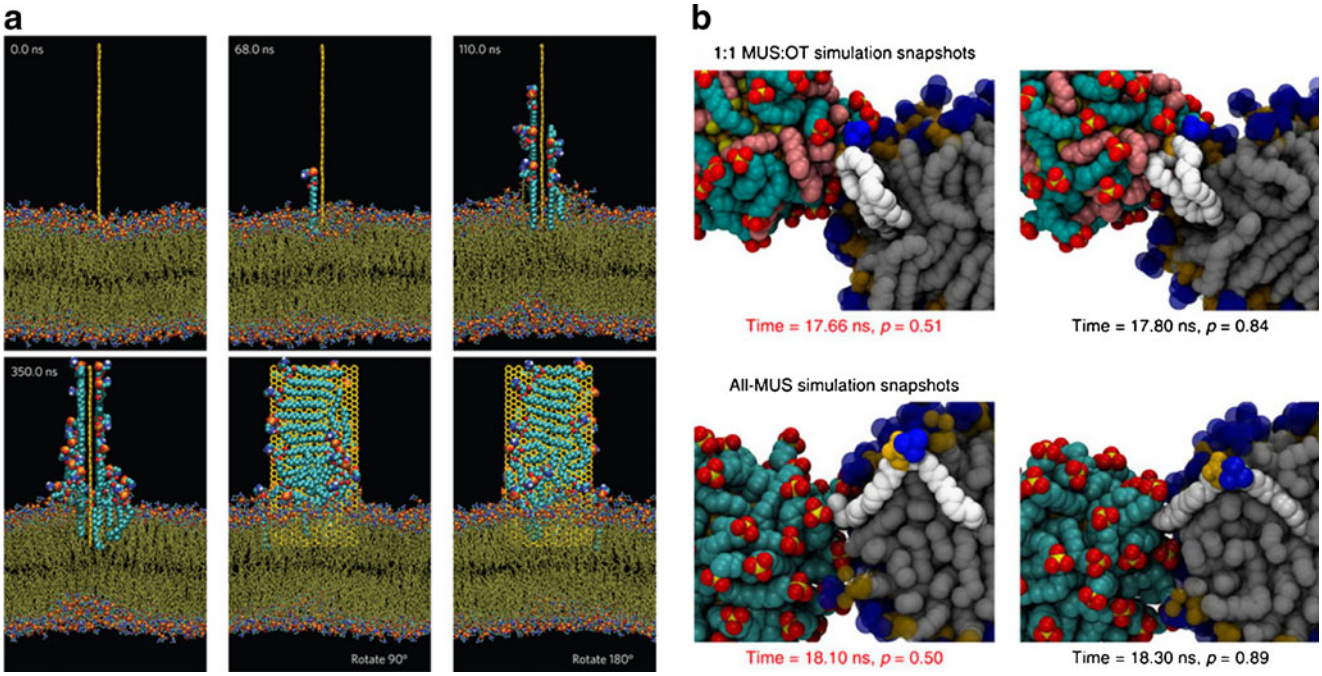

Fig. 10 MD simulation of NM–model lipid membrane interactions. (**a**) Graphene nanosheet insertion and lipid extraction (Reprinted from [101] with permission from the Nature Publishing Group). (**b**) Snapshots during and immediately after the transition state. (Reprinted from [16] with permission from the Nature Publishing Group)

cholesterol, etc.); (2) using peptidoglycan and polysaccharide incorporated membranes; (3) using membranes reconstituted from real cell membranes. The last one could be an ideal platform.

Secondly, performing studies in "real" physiological environments. This requires an environmentally relevant medium containing proteins, ions, and/or organic biomolecules. It is not just the "corona" that alters NMs surface chemistry and aggregation state, but also the dynamic nature of this coating process may influence the NM–biomembrane interactions.

Thirdly, developing experimental techniques that could provide structural information with high resolution of both spatial scales and temporal scales. Such techniques could overcome average effects, investigating of the spatial and temporal heterogeneity of such interactions would be possible, which is very important as it is nanoscale property that matters for nano–bio interface. Moreover, in situ label-free analytical techniques are urgently needed.

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Compliance with ethical standard

Conflicts of interest The authors declare that they have no competing interests.

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