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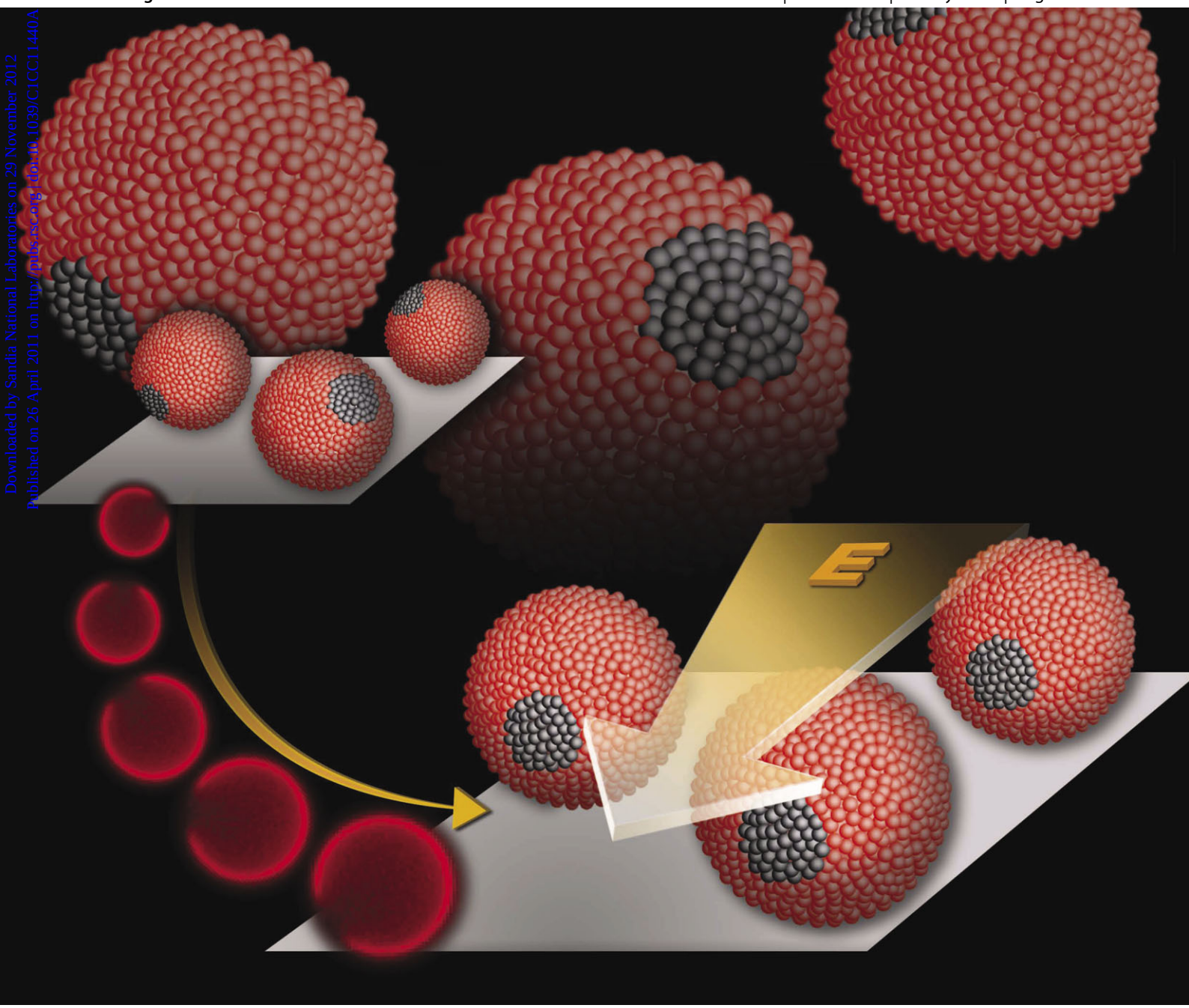

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COMMUNICATION

Orienting lipid domains in giant vesicles using an electric field†‡

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Directing the orientation of molecular assemblies is a key step toward creating complex hierarchical structures that yield higher order functional materials. Here, we demonstrate the directed orientation of functionalized lipid domains and protein–membrane assemblies, using an electric field.

Phase separation in lipid membranes provides a route towards generating defined domains enriched in specific lipid species that can act as self-assembled sites for chemical interaction and structural transformation. In cellular membranes, molecular assemblies, such as those involved in lipid rafts,[1] are believed to mediate cellular processes such as biomaterial uptake and signal response.[2] Analogously, phase separation of synthetic bilayer membranes has enabled the preparation of novel materials for sensing,[3] delivery systems,[4] and structural assembly.[5]

A key step toward using membrane phase separation to assemble ordered structures is to control the orientation of phase-separated regions relative to the environment and each other. Application of external fields has been used to apply forces to lipid domains. For example, electric fields have been shown to organize and concentrate lipid molecules and lipid domains in Langmuir monolayer films,[6] supported bilayers,[7] and cell membranes.[8] Transport was shown to be influenced by the frictional force from electroosmotic flow as well as electrophoretic effects. In monolayers, domains of phosphocholine lipids in the gel and liquid ordered (L_o) state were found to be attracted to the negative electrode, and repelled by the positive electrode using potentials of 100–300 V applied in the air above the monolayer.[9] This attractive/repulsive interaction with zwitterionic phosphocholine lipids was believed to be due to the dipole moment created in the domain by the ordering of the molecular dipoles. In supported bilayers, electrophoresis has been reported for charged lipids that migrate towards electrodes of opposite sign, creating chemical gradients.[7] In these studies, separation of negatively charged lipids, such as phosphatidylserine, from charge neutral phosphocholine lipids was attained under electric fields of 1–270 V cm^{-1}.

Here, we demonstrate the use of electric fields to orient self-assembled membrane domains consisting of lipids and lipid–protein complexes in giant unilamellar vesicles (GUVs). This approach provides a simple and potentially useful step towards the construction of ordered bilayer architectures with specific protein attachment and functionality.

Fig. 1 illustrates the orientation of the lipid domains induced by the electric field. In particular, we have found that gel phase domains of 1,2-distearyl-*rac*-*glycero*-3-triethyleneglycylimino diacetic acid (DSIDA)[10] in GUVs of fluid phase 1,2-diphytanoyl-*sn*-*glycero*-3-phosphocholine (DPhPC) rapidly oriented towards the positive electrode with an applied field (Scheme 1). We also show that through the addition of $CuCl_2$ to solution, which enables the formation of Cu^{2+}–IDA complex on the domain, his-tagged proteins could be bound and the protein-enriched domains oriented with the field in a similar manner.

The domain orientation studies were performed using commercial (ibidi GmbH) and homebuilt microfluidic devices that were outfitted with electrodes and fluid inlet/outlet ports.

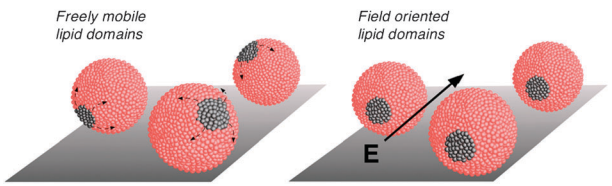
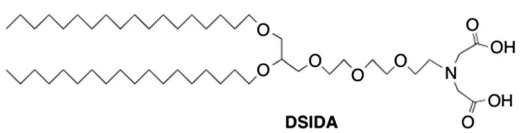
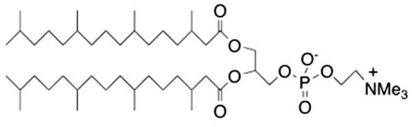


Fig. 1 Lipid domains of DSIDA (gray) in giant vesicles of DPhPC (red) are freely mobile in the absence of a field (left). Upon application of voltage the domains orient in the opposite direction to the field (right).





Scheme 1 Structures of DSIDA and DPhPC.

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‡ Electronic supplementary information (ESI) available: Full experimental procedures, supplemental figures and movies of domains and nanotubes oriented by an electric field. See DOI: 10.1039/c1cc11440a

These devices consisted of a three-port glass slide manifold and a glass cover slip that sandwiched a 250 μm thick silicone film with a slot that defined the fluidic channel. The vesicle orientation process in all devices was examined using an inverted fluorescence microscope. Platinum electrodes were inserted into two of the ports spaced 1.5 cm apart, and the third port served as a fluidic outlet. GUVs were prepared by electroformation in sucrose solution (350 mOsm) using a standard procedure.[11] Prior to the introduction of the vesicles, the microfluidic chamber was hydrated with 10 mM Tris–HCl buffer solution with 500 μM ethylenediaminetetraacetic acid (EDTA) at ~350 mOsm (adjusted with 2 M glucose solution) and pH 7.4. The conductivity of the buffer was 847 μS cm^{-1}. GUVs were then added into the channel by flowing solution through at a gentle rate of 5 μL min^{-1} for 20 minutes to minimize vesicle deformation.

Vesicles were allowed to settle onto the bottom glass coverslip. Prior to assembly of the microfluidic devices, the coverslips were cleaned with Piranha solution (25% H_2O_2/H_2SO_4) and either used as is (*i.e.*, untreated) or functionalized with cholesterol-tethered molecules (see ESI‡). The untreated glass gave inconsistent performance with regard to vesicle capture, sometimes permitting facile capture but more often inducing vesicle rupture. The cholesterol-tethered surface provided more consistent vesicle capture. Vesicles captured on either surface responded identically to the electric field. Following a settling time of fifteen minutes the liposome solution was flushed out with fresh buffer (5 μL min^{-1}) to remove unbound liposomes.

GUVs (~10 μm dia.) of 15% DSIDA/DPhPC, containing 0.3% β-BODIPY 530/550 C_5-HPC (Invitrogen) as a fluorescent label, are shown in Fig. 2 as they respond to an applied electric field. Dark regions of the membrane can be seen orienting perpendicular to the field. The domains are made visible using a BODIPY probe that is miscible with the fluid phase DPhPC membrane but is excluded from the gel phase domain rich in DSIDA, rendering the region dark.[12] In the absence of an electric field, phase separated islands of DSIDA randomly move about the three-dimensional surface of the vesicle. Application of an electric field of 1.6 V cm^{-1} across the fluidic channel caused the domain to orient toward the positive electrode within tens of seconds. In Fig. 2, the field was first held in one direction and then switched to the other direction to show that the domain responds to the field orientation (movie of the orientation found in ESI‡).

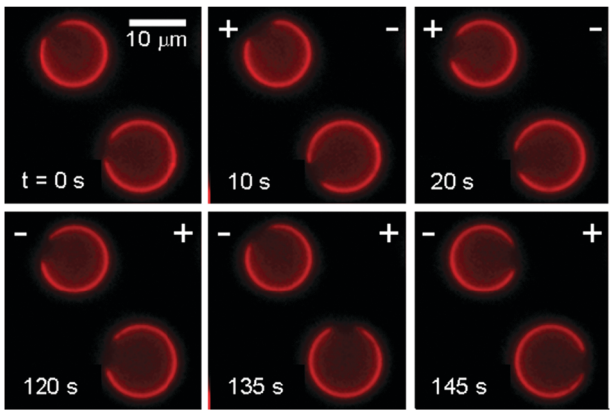

Fig. 2 Fluorescence images of 15% DSIDA/DPhPC GUVs labeled with 0.3% BODIPY 530/550 HPC showing the gel phase domain enriched in DSIDA (dark region) orienting towards the positive electrode in one direction (above) and in the other when bias was switched (below).

When an external electric field is applied across the GUVs, the charged domains in the buffer solution experience an electrostatic Coulomb force similar to that on a charged particle during electrophoresis.[13] This force causes the domain to align towards the electrode of opposite charge. The iminodiacetic acid lipid used in this study is known to carry a negative charge at neutral pH,[14] thus attraction towards the positive electrode correlates with the domain having a negative surface charge. As a control, we found that a vesicle having a domain of charge neutral lipids (15% distearoylphosphatidylcholine/DPhPC) was uninfluenced by fields as high as 10 V cm^{-1}. On the other hand, with giant vesicles containing another negatively charged lipid, dipalmitoyl phosphatidyl glycerol (DPPG) [15% DPPG/DPhPC/0.3% β-BODIPY 530/550], domain orientation towards the positive electrode was also observed.

To further examine the role of electrostatic charge in the observed field effects, as opposed to other possible contributions such as fluid flow in the microchannel, we conducted metal–ion binding experiments with the DSIDA/DPhPC vesicles. Previously, it has been found through surface force measurements that the charge density of a DSIDA film decreases with increasing concentration of $CuCl_2$, as the charge neutral Cu^{2+}–IDA complex forms.[14] GUVs composed of 15% DSIDA/DPhPC were examined under a field of 1.6 V cm^{-1} with increasing concentration of $CuCl_2$ in Tris–HCl buffer. Using the microfluidic device to exchange the bulk solution we increased the concentration of $CuCl_2$ in the channel. For concentrations up to 20 μM $CuCl_2$, a field of 1.6 V cm^{-1} was still effective for domain alignment. However, at higher concentrations (~70 μM) domain orientation became muted to the effects of the field (tested to 10 V cm^{-1}). Flushing of the fluidic channel with EDTA (500 μM) in Tris–HCl buffer solution to remove the complexed metal restored the field-driven orientation of the domain. This result further suggests that electrostatics control domain orientation.

As confirmation of the role of domain surface charge in electric field-directed orientation, we also investigated GUVs containing positively charged lipid domains. For this system, the domains are expected to orient in the direction opposite to that observed for the negatively charged domains (*i.e.*, toward the negative electrode). Vesicles were prepared by mixing the cationic lipid 1,2-distearoyl-3-dimethylammonium-propane (DSDAP) with 1,2-distearoyl-*sn-glycero*-3-phosphocholine (DSPC) and 1-palmitoyl-2-oleoyl-*sn-glycero*-3-phosphocholine (POPC), allowing the formation of gel phase domains containing DSDAP and DSPC in a fluid phase bilayer matrix of POPC. The GUVs in Tris–HCl buffer were syringe loaded into the microfluidic device and platinum electrodes were placed in each well, spaced 1.7 cm apart. A GUV (~16 μm dia.) of 10% DSDAP/10% DSPC/POPC containing 0.3% β-BODIPY 530/550 C_5-HPC is shown in Fig. 3. Upon application of an electric field of 1.6 V cm^{-1} across the channel, the domain was observed to orient toward the negative electrode, and to switch

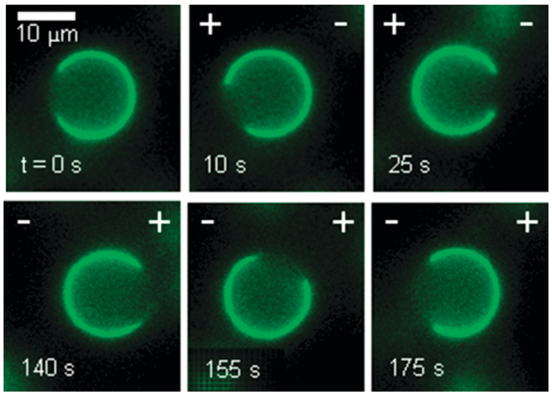

Fig. 3 Fluorescence images of a GUV composed of 10% DSDAP/10% DSPC/POPC/0.3% BODIPY 530/550 HPC that show a gel phase domain enriched in DSDAP/DSPC (dark region) orienting towards the negative electrode in one direction (above) and in the other direction when the bias was switched (below).

orientation when the electric field was reversed. This study further demonstrates that domain surface charge underlies the capacity of domains to orient relative to electric fields.

Enhanced levels of functionality of the membrane assemblies can be effected by complexation of proteins to the lipid domains. Protein adsorption can lead to transformation of membrane structure and function, such as the formation of lipid tubules[12] or chemical sensing schemes.[15] Previously, we have shown that Cu^{2+}–IDA functionalized domains in lipid membranes can selectively bind his-tagged proteins with high affinity.[16] In Fig. 4, we show a GUV composed of 10% DSIDA/DPhPC loaded with Cu^{2+} ions and bound with his-tagged green fluorescent protein (his6-GFP). In the presence of a 4 V cm^{-1} field, the domain rapidly orients towards the positive electrode (movie of the orientation found in ESI‡). GFP has an isoelectric point at pH 5.1, and a charge of −7 at neutral pH,[17] so its attachment to the domain is expected to add negative charge to the domain.

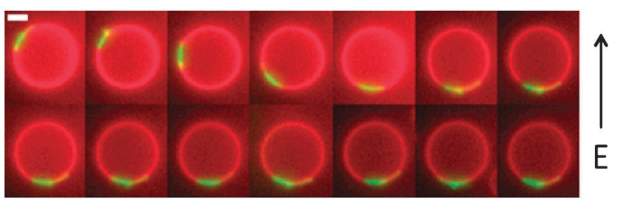

Fig. 4 Sequence of fluorescence images of a his6-GFP (green) bound lipid domain (10% DSIDA/DPhPC/0.3% BODIPY 530/550 HPC) orienting in an electric field of 4 V cm^{-1}. DSIDA domains were exposed to $CuCl_2$ solution (200 μM), followed by addition of GFP (2 μM). In the absence of any field the protein bound domain was observed to freely rotate in the membrane (top left). Upon application of the field the domain rapidly orients in the direction of the positive electrode, as is observed in the series of images from top left to bottom right taken with a two second interval between each image. Scale bar is 10 μm.

In conclusion, we have shown that rapid orientation of supramolecular assemblies in a lipid membrane can be readily achieved using electric fields. Lipid domains composed of charged species as well as domains with adsorbed proteins were oriented within seconds by the field. The results suggest that surface charge density underlies the observed orientation behavior. This work demonstrates a simple technique to direct the orientation of supramolecular assemblies that may aid in the development of hierarchical nano- to micro-structured materials with biochemical functionality.

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