Program Title: Miniaturized Hybrid Materials Inspired by Nature

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Program Scope

The goals of the program are to develop a fundamental understanding of the mechanisms underlying lipid and protein based nanometer scale assembly. The understanding should lead to the development of nanometer scale materials, which are scientifically and technologically important. Nanometer scale tubes and rods and their assemblies are of interest as miniaturized materials with diverse applications as circuitry components, enzyme encapsulation systems and biosensors, vehicles for chemical delivery, and as templates for hierarchical nanostructures.

The main strategy that we use to achieve our goals consists of learning from, and building upon, the many illuminating examples of assembly occurring in vivo far from equilibrium. For example, the nerve cell cytoskeleton provides a rich variety of assembled bundles and networks of filamentous actin, microtubules (MT), and neurofilaments, where the nature of their interactions leading to their hierarchical structures, and the structure-function correlations are not understood [1-4,6]. In earlier work (including DOE supported work dating back to 2007) in far simpler protein systems (using a combination of synchrotron x-ray diffraction, electron microscopy, and optical imaging data), we have demonstrated how (i) MTs (model nanotubes) may be assembled in distinct structures (e.g. 2D versus 3D bundles) through a competition between short-range attractions and longer-ranged repulsions, and (ii) isolated MTs may be used as templates to form lipid-protein nanotubes with open and closed ends and varying inner diameters [2]. In more recent work we have discovered an entirely new mechanism, using custom synthesized curvature stabilizing lipids, to develop lipid bio-nanotubes and bio- nanorods. The nanorods and nanotubes are a result of the formation of block liposomes [5]; a new class of liposomes, with the blocks consisting of distinctly shaped nanoscale spheres, tubes, or rods. Indeed, similar membrane shape changes, occurring in vivo for the purpose of specific cellular functions, are often induced by curvature generating/stabilizing proteins through their interactions with cellular membranes [7]. For example, in endocytosis, which requires vesicle budding, the (curvature stabilizing) protein dynamin is recruited and assembled into stacks of rings in the negative curvature region of the invaginated membrane vesicle forcing dynamical lipid shape changes where a transient tubular neck formation is followed by membrane fission.

The projects involve both custom synthesis of novel lipids, peptide-lipids, and PEG-lipids with or without functional end groups, and purification of biological molecules [8, 9]. In some instances the enhanced stability of the lipid assemblies will be achieved by lipids with polymerizable chains. The higher order assembly of the building blocks will be achieved via competing interactions, where end-functionalized PEG-lipids may play an important role.

The projects utilize the broad spectrum of expertise of the group members in biomolecular self- assembling methods, synchrotron x-ray scattering and diffraction, electron and optical microscopy characterization techniques.

Recent Progress

Block Liposomes consisting of Distinctly Shaped and Connected Nanotubes, -rods or –spheres: Membrane Shape Evolution in Response to Incorporation of Curvature Generating Charged Lipids [Reference 5].

BACKGROUND

Lipids – one of the main building blocks of life – and their assemblies (e.g. membranes and liposomes) play a major role in numerous cellular processes including compartmentalization, macromolecular transport, and signal/energy transduction. A distinguishing feature of membranes in vivo, *enabling their function*, is the evolution in their shapes. Thus, understanding the mechanisms by which membranes undergo shape changes remains a major scientific challenge. Indeed, much effort has been expended to elucidate membrane shapes in the context of their interactions with membrane-associated, curvature generating proteins. Such curvature generating proteins play a key role in vesicle budding, which is required in receptor- mediated endocytosis, in inter-organelle trafficking and at synaptic junctions recycling vesicles. In our work described below we are interested in elucidating the key parameters; for example, charge and steric shape of the curvature generating lipid molecule, involved in membrane shape evolution in relatively simple model systems.

Discussion of Findings In exploring the properties of curvature forming/stabilizing lipids on membrane shape evolution (a simple mimic of the more complicated process occurring *in vivo* via curvature stabilizing proteins) we have discovered *block liposomes*, an entirely new class of liposomes, with the blocks consisting of distinctly shaped nanoscale spheres, tubes, or rods (see Figs. 1 and 2) [5]. The block liposomes resulted from membrane shape evolution in response to the incorporation of a custom synthesized charged lipid (the cone-shaped MVLBG2, shown schematically in FIG. 2) to giant vesicles with:: zero curvature. Cryogenic- TEM revealed the blocks to consist of distinctly shaped nanoscale spheres, tubes, or rods. Diblock (sphere-rod) liposomes were found to contain micellar nanorods :: 4 nm in diameter and several µm in length, analogous to cytoskeletal filaments of eukaryotic cells (FIG. 1).

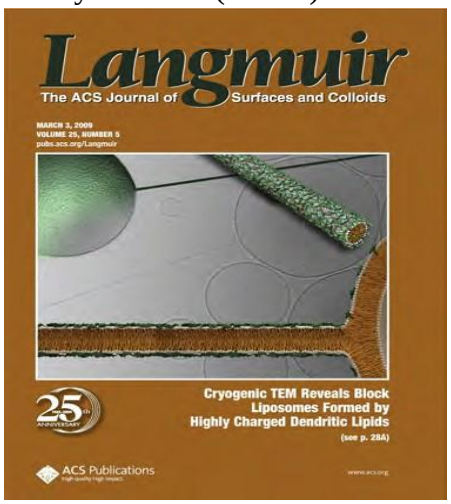

FIG. 1 An example of a block liposome induced in response to the incorporation of a highly charged curvature-generating dendritic lipid MVLBG2 to a vesicle of neutral lipid DOPC as revealed by cryogenic TEM. Block liposomes (BL) are a new class of liposomes, consisting of distinctly shaped, yet connected, nanoscale spheres, tubes, or rods. Within a BL shapes are separated on the nanoscale. The image shows a diblock (sphere-rod) consisting of a spherical vesicle connected to a cylindrical micelle (rod). The rod diameter is the thickness of a lipid bilayer (4 nm, the hydrophobic core with high contrast in cryo-TEM). The schematic illustrates an arrangement of lipid molecules within this diblock (sphere-rod) with a higher concentration of the curvature-generating MVLBG2 (green) in the high curvature micellar region as opposed to the spherical part where more DOPC (white) resides. See reference 5 for more detail (A. Zidovska et al, Langmuir 2009, 25, 2979.)

Coexsisting with sphere-rod block liposomes are diblock (sphere-tube) and triblock (sphere-tube-sphere) liposomes, which contain nanotubes with inner lumen diameter in the range between 10-50 nm (FIG. 2).

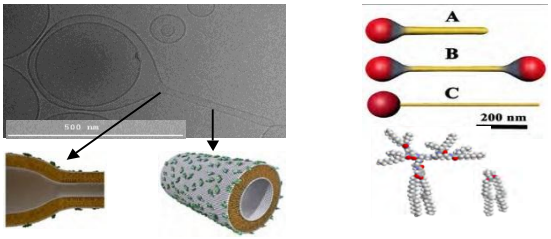

FIG.2 **(LEFT)** Example of a diblock (sphere-tube) liposome as seen by cryogenic TEM. Bottom schematics showing the intersection region between the sphere and tube and a section of the tube. The schematic shows an arrangement of lipid molecules with a higher concentration of the curvature- stabilizing MVLBG2 (green) in the outer monolayer as opposed to the inner monolayer, which has a higher concentration of neutral DOPC (white) lipids. **(RIGHT)** Top: schematics of the different nanoscale Block Liposomes revealed by Cryo-TEM: (**A**) diblock (sphere–tube) liposome seen in the left TEM image, (**B**) triblock (sphere–tube–sphere) and (**C**) diblock (sphere–rod) liposome seen in FIG. 1. The color coding represents different membrane Gaussian curvature: positive (red), negative (blue) and zero (yellow). Botttom: Molecular models of neutral DOPC (right) and hexadecavalent MVLBG2 (left). (Adapted from reference 5, A. Zidovska; et al. Langmuir 2009, 25, 2979-2985.)

Importance of findings both from experimental and theoretical perspectives: While a large variety of micron scale vesicle shapes including spheres, ellipsoids and oblates, tori, and discocytes has been described previously, the discovery of block liposomes presents a major challenge to the current understanding of membrane shapes at equilibrium. Current theories, based on the widely accepted Helfrich elastic free energy of membranes, predict macroscopically phase separated distinctly shaped liposomes. In contrast, shapes are connected and separated on the nanometer scale in block liposomes (see Figures 1 and 2). New theories of charged membranes are required to account for block liposomes. Broadly speaking, nano-tubes and nano-rods are indispensable components in the development of future miniaturized materials. They have applications in diverse areas from uses as chemical encapsulation vehicles, to biosensors and circuitry components. Thus, an area of intense current research in nanoscience is in elucidating the key parameters, which control the self- assembling properties of nanometer scale tubes and rods. Block liposomes may find a range of applications in chemical and nucleic acid delivery and as building blocks in the design of templates for hierarchical structures and for nanostructures such as wires or needles.

Future Plans

(1)Towards the Development of Sterically Stabilized Block Liposomes

To date, we have only observed block liposome formation in charged membrane systems. We have planned a series of experiments to address the important question of whether longe-range electrostatic interactions are needed for BL formation or is the cone-shape of the lipid (which may be achieved through steric considerations alone) sufficient for BL formation. The systematic experiments should differentiate between the separate contributions of steric shape and charge of the lipid molecules to block liposome (BL) formation. In one set of pH studies we will explore whether very weakly charged lipids (e.g. at sufficiently high pH:: pKa values of primary/secondary amines of MVLBG2) might still form BLs entirely due to the fact that their relatively large steric lipid headgroups would still result in a cone-shaped lipid. In another set of experiments we plan to selectively neutralize the membrane charge by replacing cationic sections of MVLBG2's headgroup (see Fig. 2) with uncharged short-PEG units. This will allows us to decrease the

charge to zero while simultaneously maintaining the large lipid headgroup entirely through steric interactions (e.g. by increasing the number of ethylene-oxide units). These planned experiments, which involve new synthesis should enable us to develop sterically stabilized block liposomes, which would inherently be more stable than their charged counter- parts and thus more suitable in future applications. The studies involving systematic variations in the shape, size and charge of the curvature-stabilizing lipid should lead to optimal control of the tubule diameter distribution.

(2)Nanotubes from Proteins undergoing Conformational Transitions

We have recently discovered a novel biomolecule, which acts as a switch between two configurational states of tubulin oligomers (which are used in assembling microtubules). This conformation transition is observed to "effectively" invert one nanotube state into another. We are currently studying the relevant parameters, which (i) control the rate of transitions between the nanotube structures and (ii) control the reversibility of the switching between the nanostructures.

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