Creating Functional Nano-Environments by Controlled Self-Assembly

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Project Objectives: Defining regions of space, with delineated boundaries and controllable mechanisms for entrance and exit, will be essential to making all but the most primitive functional nanostructures. Specific examples include miniaturized systems for chemical/drug encapsulation and controlled release, sensors, separations, and nano- and micro- scale wires and switches for electronic and magnetic applications. Nanoscale components are organized into functional systems in which different functions are carried out in different locations within the overall system. An important challenge to the advancement in all areas of nanoscale technology, in general, and the construction of nanoscale apparatus, instrumentation and machinery, in particular, is the ability to construct compartments, packages and to divide space into subunits to increase or to separate functionality. Traditional ways of doing this are "top-down", subtractive processes such as etching and lithography. For systems made of, mimicking or designed to interact with biological or other soft materials, "bottom-up" processes of self-assembly are more appropriate but, at the same time, less well-developed. This project addresses biosystems at the nanoscale. The proposal progresses from biological and bio-inspired structures to more synthetic physical systems with hybrid character. In all of the work, the focus is on spontaneous assembly to form controllable regions of space that have well-defined boundaries and functionality.

Self-Assembled, Multicompartment Lipid Nanostructures

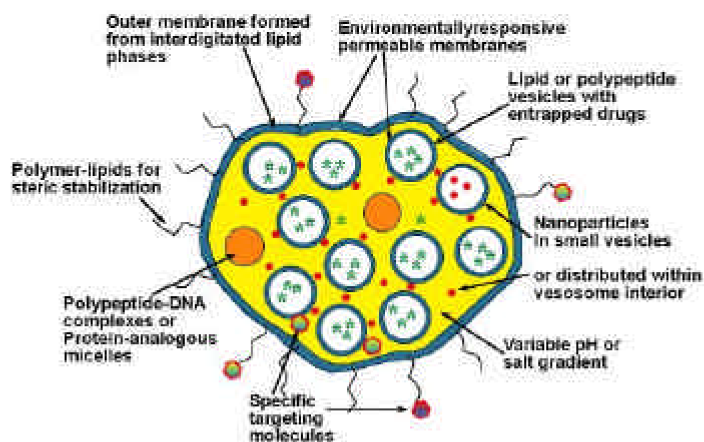


Figure 1. **Schematic of vesosome**

Nature optimizes transport and reaction conditions through controlling micro to nano-environments by encapsulating specific volumes and materials within lipid membranes. The lipid membranes divide space into a distinct inside and outside, and the properties of the membrane cause chemical species to cross this barrier at widely different rates. Small neutral molecules such as water or ethanol cross this barrier readily, while charged species or larger molecules such as polymers or proteins, are effectively trapped within the membrane. However, it is difficult to control the nano-environment adequately using a single membrane. Nature typically creates nested series of membranes to perform complex chemical tasks. Duplicating this level of hierarchical encapsulation requires a more sophisticated multi-layer capsule than unilamellar vesicles – a nanostructure we call the vesosome[1]. The vesosome is constructed by simple, self-assembly steps that rely on novel lipid phase behavior.

One motivation for developing the vesosome is that for a limited class of drugs, unilamellar liposome are a *proven* clinical success. The use of PEG polymer-lipids on the liposomes provides extended intravenous lifetimes.

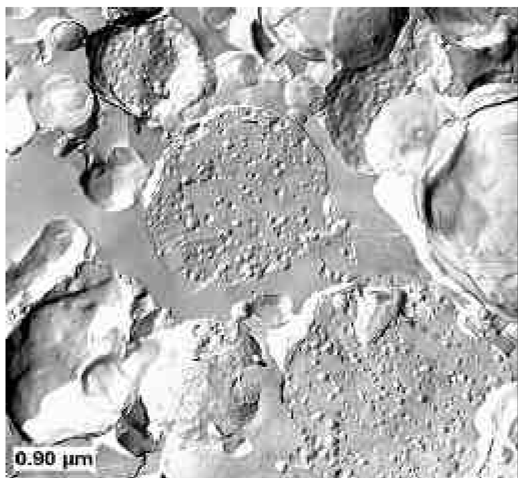
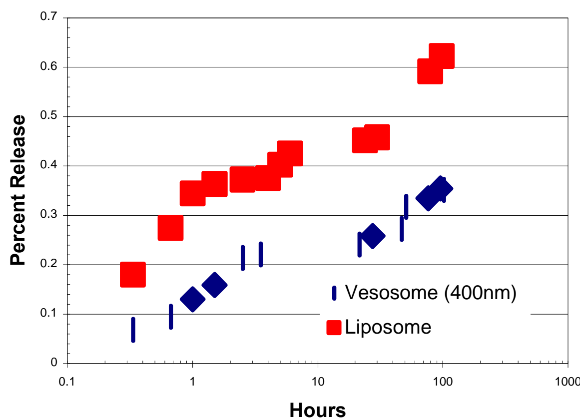


Figure 2**. Freeze-fracture TEM images of pelleted vesosomes. Compare this to the schematic structure in Figure 1a. Small Vesicles are contained within a limiting membrane containing PEG lipids.**

What limits the applications of conventional liposomes is that many important drugs are released faster than optimal *in vivo*. This problem is significantly addressed by the vesosome: while small molecules are released from unilamellar liposomes in minutes, they are retained in vesosomes from hours to days, even though the liposomes and vesosomes have the same bilayer composition and size.



Carboxyfluorescein (CF) release from vesosomes and conventional unilamellar liposomes in serum**. About 35% of CF is released within 1 hour from the liposomes compared to almost 100 hours from vesosomes.**

Diblock Copolypeptide Vesicles

As an alternative to lipid membranes, we have prepared diblock copolypeptides (See Fig. 3) containing hydrophilic and hydrophobic domains that self-assemble into vesicles whose size and structure is dictated by the sequence, chain length, and the conformation of the polymer chains. Polymeric vesicles offer advantages over lipid vesicles (e.g. increased stability) for applications such as drug delivery. The ethylene glycol sheath coating these vesicles should give them good biocom-patibility, similar to PEG, so they are expected to have a high circulation lifetime in the bloodstream. Vesicles incorporating amino-terminated lysine residues in the hydrophobic domains were prepared to render the vesicles sensitive to changes in pH, as would be desirable for endosomal release within a cell. Vesicles formed by dissolving the copolymer in water in the presence of fluorescent dye Fura-2 at pH 10.6, followed by sonication, give dye containing vesicles about 200 nm in diameter. Under these conditions, the fluorescence intensity of vesicle-encapsulated dye in the presence of calcium solution was found to be constant for several days. When the pH was lowered by addition of HCl, the absorbance spectrum of the dye was shifted within seconds, indicating near instantaneous disruption of the vesicle membranes and complexation of the calcium by Fura-2.

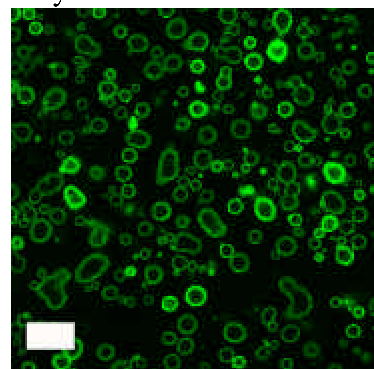


Fig. 3. Laser scanning confocal image of diblock polypeptide vesicles consisting of lysine functionalized with trimers of PEG on each side chain, coupled to leucine. The entire molecule is helical/rigid and readily forms vesicles.

Protein Analogous Micelles

Conjugating synthetic lipid tails to peptides confers some self-assembly character that the peptide alone does not possess. We have found that when C12 - C18 tails are conjugated to peptides derived from helical extracellular matrix or DNA-binding proteins, the conjugates form micelles. Furthermore, in the micelle the peptides adopt a helical secondary structure similar to that in the whole protein but absent in the peptide alone. We term these micelles, *protein analogous micelles*, since they have hydrophobic interiors, hydrophilic, biofunctional exteriors, and secondary structures constrained by the artificial tertiary structure produced by micellization. The connectivity of primary structure can be recreated by polymerization of the lipid tails.

CD spectra show that the helical DNA binding peptide, a variety of the "leucine zipper" class, becomes more helical in the presence of DNA, indicating an interaction with DNA that stabilizes the peptide helix further. Electrophoresis shows the mobility of fluoresecently labelled DNA in the presence of increasing concentration of leucine zipper peptide-lipid conjugate. The DNA mobility goes to zero at the CMC of the peptide amphiphiles, again indicating a DNA-peptide interaction, and that the interaction depends on the peptide being part of a protein analogous micelle.

Natural Amphiphilic Peptides

Marine bacteria have also adopted amphiphilic peptides as a strategy to acquire iron. Virtually all bacteria require iron for growth, yet iron is usually present as insoluble oxides in the environment. Aerobic bacteria produce siderophores (Greek for iron carrier) to solubilize colloidal iron(III). The iron(III)-siderophore complex is recognized by a specific iron-siderophore receptor protein on the outer membrane of the bacterium. Marine bacteria, in particular, seem to have adopted the production of amphiphilic peptides as their strategy to acquire iron (e.g., the Marinobactins and Aquachelins) [2,3]. These siderophores are characterized by a peptide head group that coordinates Fe(III) with high affinity through the two hydroxamate groups and the alpha-hydroxy acid group, and one of a series of fatty acid appendages ranging from C_{12} to C_{16}. The peptides are comprised of seven or six amino acids as shown for the aquachelins and marinobactins respectively, although recently we have completed the structure determination of other amphiphilic siderophores composed of four amino acids and one of a series of fatty acid tails ranging from C_{10}-C_{18}. Unlike the aquachelins and marinobactins which can be released from the bacterium upon centrifugation, this newest group of siderophores remains associated with the bacterium and until the bacterial cell culture is extracted with ethanol.

Developing Micro-Array based methods for probing Protein-Protein interactions

The completion of the Human Genome Project has ushered in the era of proteomics, which aims to understand how gene products, namely proteins, interact with each other for the purpose of performing a distinct biological function; for example, protein-protein interactions in gene regulation or those responsible for cell division and growth. They will train the students in state-of the-art techniques including, micro-electro-mechanical systems (MEMS) methods to fabricate small-scale micro-arrays containing highly aligned and interacting proteins. The students will also be trained in modern purification methods to develop cell-free models of protein-protein interactions derived from cytoskeletal proteins. These micro-arrays will be scanned and detected with unprecedented precision by high intensity x-rays emanating from the national synchrotron facilities at Stanford and the Lawrence Berkeley laboratory. Our research program will enable the students to acquire skills that are currently in great demand in the nanotechnology and biotechnology sectors.

References

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