# From Membrane Assembly and Dynamics to a Model Cell

**Research Summary and Proposal – Martin M. Hanczyc**

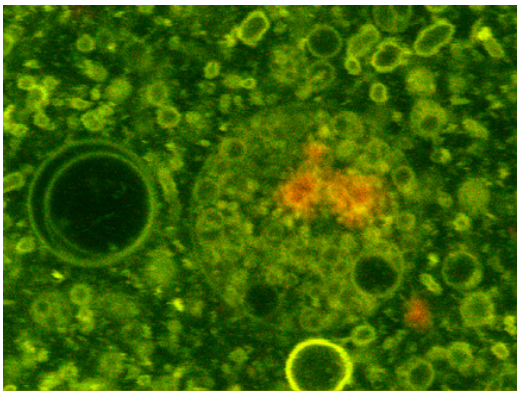
## Background

Primitive cells, lacking the complex bio-machinery present in modern cells, would have had to rely on the self-organizing properties of their components and on interactions with their environment to achieve basic cellular functions such as growth and division. Many bilayer-membrane vesicles, depending on their composition and environment, can exhibit complex morphological changes such as growth, fusion, fission, budding, internal vesicle assembly and vesicle-surface interactions [1]. These vesicles, with their rich dynamic properties, may serve as interesting models of how primitive cellular organization and replication might have initially occurred in response to purely physical and chemical forces.

## Postdoctoral Research Summary

My goal was to engineer the first experimental model system of cellular growth and division [2]. The model 'cells' consisted of vesicles with bilayer membranes composed of simple fatty acids. Growth was achieved by the slow and regulated input of additional fatty acids of dilute concentration to a population of vesicles. I was able to demonstrate efficient growth (incorporation of 90% of the incoming lipid) by identifying and then optimizing several critical experimental parameters. Division was produced by extrusion of the vesicle population through small pores. Significantly, the vesicles were propagated through many consecutive cycles of growth and division with retention of the original contents. The retention of internal contents throughout the dynamic growth and division cycles is a necessary element for the construction of a synthetic cell and is pertinent for nanotechnology applications.

Considering the organization of biological structure at the origin of life, I began to explore what 'non-living' agents could organize membranes. I developed a simple assay where fatty acids, in the form of micelles, would slowly convert into bilayer vesicles. A most significant discovery in my research is that a small amount of the clay, montmorillonite, greatly accelerates the rate of this conversion. Montmorillonite is notable for being able to catalyze the polymerization of RNA on its surface. Further, I was able to show that RNA (red in the adjacent micrograph) adsorbed to the surface of the clay becomes encapsulated in the resulting vesicles (green). My discovery suggests that a direct path could have existed that could bring together a catalytically active surface (clay) and a potentially genetic polymer (RNA) within the same membrane compartment [2]. This result lends credence to the hypothesis that RNA could have been one of the first genetic molecules. By extension, this transference of order from minerals



to biologically relevant molecules as described here could have played a key step in the evolution of the first cells.

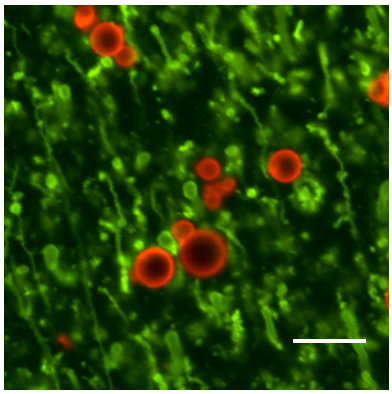
**Proposed research: short-term goals**

**A) The mechanism of mineral-catalyzed vesicle formation and encapsulation**

This project is a continuation of my work investigating the interaction of amphiphiles with mineral surfaces that results in the organization and production of vesicles. Apart from vesicle production, catalytically active surfaces become encapsulated as a product of the reaction. The aim is to gain a more detailed understanding of this mechanism, which may lead to the *preferential* encapsulation of surfaces. This approach would then be capable of providing encapsulated surfaces for drug delivery and nanoscale applications.

1. **Varying mineral surfaces.** How does vesicle formation occur in the presence of a mineral surface? I will test many mineral surfaces to determine the surface parameters necessary to effect vesicle formation. Although previous experiments with alumino-silicates implicated surface charge density as a parameter in the reaction, the necessary character of the surface appears to be more complicated. More detailed analysis of the necessary surface parameters will entail derivatized surfaces, varying mono- and divalent homoionic surfaces, and synthetic surfaces of varying size and shape. The data so far, albeit preliminary, show that the conditions necessary for catalyzed vesicle formation are general, and therefore this system may be useful for the encapsulation of many different types of surfaces.

2. **Direct visualization.** How do bilayers form on or proximal to the mineral surface? By coupling fluorescent dyes and microscopic analysis, the mechanism can be dissected as shown in the adjacent micrograph. Amphiphiles (red) adsorbed directly to the silica sphere surface do not mix with added amphiphiles (green) that are forming vesicles. Since the coated spheres still catalyze vesicle formation, vesicle assembly likely occurs proximal to the particle surface rather than directly on the surface. Further analysis of vesicle assembly in the presence of various surfaces will be investigated using microfluidics to control reaction conditions and optical tweezers to isolate individual mineral particles.



**B) Dynamic vesicle growth and division**

My system of vesicle growth and division is particularly applicable to encapsulation of reactions involving self-reproduction and is ideally suited toward the synthesis of a model cell. The aim is to engineer a system of compartment growth and division that would be suitable for a wider range of applications than my current system will allow. Although this plan will involve the use of different membrane compositions, the techniques used

for evaluation and characterization of supramolecular structures will be the same as those I have been using.

**Mixed amphiphile membranes.** How can the diversification of membrane components improve the robustness and versatility of my vesicle growth and division system? The integrity of this system in its current form is susceptible to disruption with the introduction of millimolar amounts of divalent cations. Such concentrations of cations are necessary for the activity of many biological and chemical reactions that would be interesting to encapsulate [3]. It has been shown that fatty acid membranes mixed with glycerol ester alcohols can help the membrane tolerate higher concentrations of magnesium due to the stable presence of a hydrogen bond donor [4]. This suggests that the utility of mixed amphiphile membranes can be explored more fully by taking a combinatorial approach. Indeed the stability, sensitivity, selectivity and dynamics of natural cell membranes can be attributed to mixed compositions of membrane components. I am interested in forming membranes with fatty acids as before but with a percentage of similar and disparate chain length fatty acids and other membrane components such as long chain alcohols, cholesterol, squalene and polyaromatic hydrocarbons. The mixed membrane vesicles will be tested for stability and the ability to grow and divide in the presence of divalent cations. The properties of vesicles composed of short amphiphilic peptides will also be explored. It will be demonstrated how membrane compartments due to their compositional differences can be customized toward different specific functions.

**Proposed research: long-term goals**

**A) Clay-catalyzed RNA synthesis within vesicles.** Ferris and Ertem [5] demonstrated that RNA can be polymerized from activated monomers on the surface of montmorillonite clay. I have shown that RNA adsorbed to a clay particle becomes encapsulated. My aim is to combine these two findings and engineer vesicles that encapsulate and promote the polymerization of RNA on clay. The conditions that favor the diffusion of substrates into the vesicles, the polymerization of RNA adsorbed to the clay, the retention of the polymer product, and the stability of the vesicle compartment will be determined. This will constitute a demonstration of encapsulated genetic polymer synthesis on a mineral surface and may serve as the basis for *in vitro* evolution experiments.

**B) Synthesis of a model cell.** In conjunction with the above aims of preferential encapsulation of catalytic minerals, internal polymerization of RNA, and improved tolerances of the growing and dividing compartment, the long–term goal of this research program is to develop an experimental model of a primitive cell. This model would possess a catalytic agent (clay or RNA), information (RNA), and means of replication. An emergent property of this system would include the ability to evolve. Such a system would be selected to reproduce in varied environments and would be amenable to description at both the systems level and at the level of composition and information.

1. Hanczyc MM, Szostak JW: **Replicating vesicles as models of primitive cell growth and division**. *Current Opinion in Chemical Biology* 2004, **8**:*in press*.
2. Hanczyc MM, Fujikawa SM, Szostak JW: **Experimental models of primitive cellular compartments: encapsulation, growth, and division**. *Science* 2003, **302**:618-622.
3. Hanczyc MM, Dorit RL: **Replicability and recurrence in the experimental evolution of a group I ribozyme**. *Mol Biol Evol* 2000, **17**:1050-1060.
4. Monnard PA, Apel CL, Kanavarioti A, Deamer DW: **Influence of ionic inorganic solutes on self-assembly and polymerization processes related to early forms of life: implications for a prebiotic aqueous medium**. *Astrobiology* 2002, **2**:139-152.
5. Ferris JP EG: **Oligomerization of ribonucleotides on montmorillonite: reaction of the 5'-phosphorimidazolide of adenosine.** *Science* 1992, **257**:1387-1389.

# Teaching interests – Martin M. Hanczyc

As an undergraduate at Penn State and graduate student at Yale, I have had first hand experiences of various teaching methods successfully employed at different levels in the educational process: from large introductory courses to small advanced seminar courses to laboratory investigations. I have also had the experience of teaching a class of medical school students and a few classes of undergraduates. Recently I was invited to give two lectures at Rice University, which were broadcast worldwide. Outside of the classroom I have had the pleasure to mentor five undergraduate students on research projects.

On the undergraduate level, I would like to teach basic or advanced courses in Biology, Biochemistry, or Molecular Biology. Such topics can be presented broadly and tailored as an introductory class or presented in a more technical and specific manner as an advanced class. I believe that such disciplines should be introduced with a historical context and include the latest developments (e.g. genomics and proteomics). Students should have access to additional materials made available on the web, review sessions and office hours to reinforce the information and concepts presented in the classroom and to resolve questions.

In addition I would like to develop a small seminar/discussion course for seniors and graduate students on topics in Chemical Evolution/Astrobiology. This course would cover many topics in this area and draw heavily from the original scientific literature. Some of the main points of focus would be the search for biosignatures in the universe, detection of the earliest traces of life on Earth, the elucidation of early Earth conditions, and strategies of synthesizing biologically relevant compounds in the laboratory. In this class the limits of our understanding will be highlighted and questions to address in future research will be proposed. There will also be room for students to suggest specific topics in this broad area that capture their interest.

The research program I will pursue as an independent investigator will produce many research projects suitable for graduate students as well as undergraduates. Personally, I spent three years of my undergraduate education in the research team lead by Dr. Andy Clark. This experience was critical for my development as a scientist, and since then I have enjoyed mentoring students in their scientific pursuits. Apart from weekly group meetings I feel that one on one time between the principal investigator and the student is essential for the development of both the student and the research plan. In addition, I would encourage interaction among lab members. Particular attention will be placed on fostering independent thinking in students.