Tunable, Bio-Inspired Materials for Membrane Protein Storage and Transport

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Statement of Purpose: Membrane proteins represent an extremely important class of biomolecules whose functions are vital to human health. As such, they comprise the vast majority of drug targets being pursued at present. In contrast to soluble proteins, much less is known about them, primarily due to the difficulty of producing sizeable quantities of membrane proteins in natively-folded, functional, and relatively pure form.

It is well established that the function of most membrane proteins is short-lived, sometimes just hours, when removed from their native lipid bilayer. Consequently, methods are highly sought to preserve these proteins in a native state to facilitate further characterization of their structures and functions (1).

To this end, we have recently examined the use of a polymer / lipid-based complex fluid for the purpose of storing and transporting membrane protein samples. These complex fluids are comprised of a saturated phospholipid, a polymer, and a co-surfactant (2). When combined in water, these components possess an inverted phase transition, existing in a liquid-crystalline gel phase at temperatures above ~ 20 ºC and in a low viscosity, micellar state at reduced temperatures. Thus, the physicochemical and structural properties of this material are tunable. The gel phase mimics a biological membrane and can so be used to stabilize reconstituted integral membrane proteins for long-term storage or for transport between site of purification and site of characterization. The liquid state at lower temperatures serves as the phase in which membrane proteins can be introduced to the materials, and in which some membrane protein structural assays, mainly those based upon spectroscopy, can be conducted. Proteins can be extracted readily from the complex fluid by simple detergent exchange at 4 ºC.

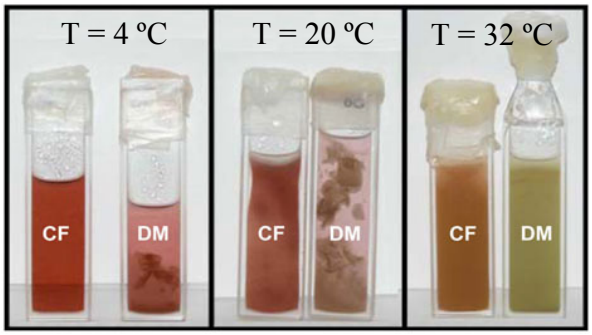
Methods: The stability of benchmark membrane proteins, the bacterial reaction centers from two species of purple bacteria – *Rhodobacter sphaeroides* and *Rhodobacter capsulatus* – was studied in buffered detergent micelles (10mM Tris pH 7.8, 0.05% LDAO) or complex fluid. Proteocomplex fluids were prepared as previously described (3). These experiments exploited the unique spectral signatures of these proteins in native or denatured states. Samples were incubated in 1-mm-pathlength cuvettes at 4, 20 or 32 degrees Celsius and regularly monitored visually for gross color changes or aggregation (e.g., Figure 1). Sample integrity as a function of time was quantitated through steady-state, UV-Vis-NIR spectroscopy.

Results / Discussion: In order for membrane proteins to subsist in the aqueous environments required for most chromatographies, they must first be removed from their native lipid bilayer, usually through the use of detergents, and remain soluble in detergent micelles.

Membrane protein-detergent complexes, upon purification, are most frequently preserved either through flash freezing or by reconstitution of samples into liposomes. Although flash freezing works successfully for some protein and detergent combinations, it is hardly a universal tool. Cycling across the liquid-solid transition has deleterious effects on the longevity of samples. Liposomes represent a better alternative in many cases. However, membrane proteins reconstituted into liposomes are not amenable to many assays commonly employed to probe structure and function. Furthermore, to remove membrane proteins from liposomes, one must reisolate protein components by reintroducing a detergent into the sample.

Our UV-Vis-NIR spectroscopic results show that the longevity of samples in complex fluid was increased by up to an order of magnitude (from weeks to days, depending upon the temperature assayed) compared with membrane proteins suspended in detergent micelles. This increase was most pronounced in samples incubated at temperatures above the phase transition of the fluid. Differences in relative stability extended to visually-discernable traits (e.g., aggregation and color; Figure 1).

Figure 1. Photos of purified samples of reaction centers incubated at three different temperatures for an extended period of time (day 40 shown) in complex fluids (CF) or an aqueous solution of detergent micelles (DM). The temperatures surveyed span all phases of the complex fluid: low viscosity (4 ºC), transition (20 ºC), and gel (32 ºC). The surfactant used for the detergent micelles, lauryldimethyl-amine-N-oxide (LDAO), was also part of the complex fluid formulation.



Conclusions: The stability of purified membrane proteins was markedly improved by incorporation into a polymer / lipid-based complex fluid. The tunability of this material allows for (i) ease of incorporation and recovery of the guest molecules and (ii) use of these samples in a wide range of spectroscopies.

References:

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