Pore Formation and pH-Dependent Behavior of Lipid Bilayer Coatings

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Abstract

The Sandia-developed protocell is a drug delivery vehicle consisting of a mesoporous silica nanoparticle (MSNP) coated with a lipid bilayer. The high pore volume of the MSNP allows for uptake of large quantities of drug cargo while the lipid bilayer encapsulates and protects the cargo from the environment. Although the bilayer coating serves the role of protective barrier it must also provide a pathway for release of the drug cargo at the appropriate site. In the current work we are studying how the lipid bilayer can release the molecular cargo using a change in pH as the trigger. We have found that supported lipid bilayers on glass form large pores in response to a drop in pH from 7.4 to 5.0, as a consequence of membrane curvature effects.

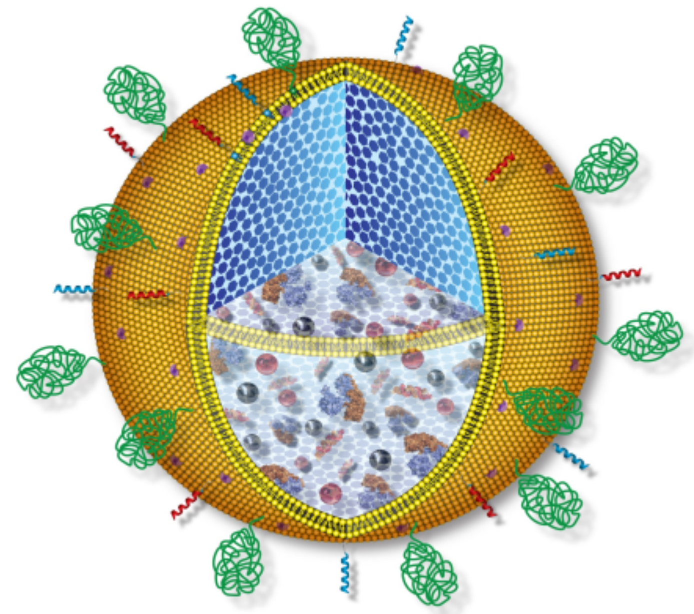


Figure 1: Protocell Delivery Vehicle, MSNP with lipid coating (yellow)

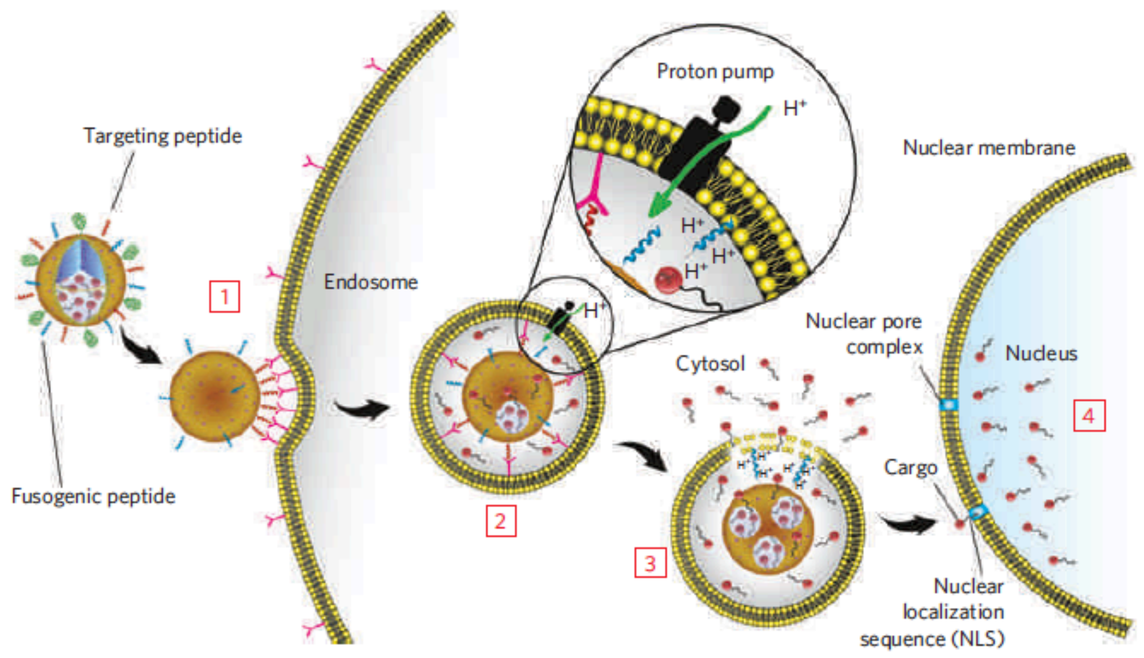


Figure 2: Diagram of how the protocol delivers drug cargo into cells

Background

A route for protocell uptake into cells involves the endosomal pathway. The protocell nanoparticle first attaches to the cell membrane surface then is endocytosed into an uptake vesicle (endosome). As the endosome progresses from early to late stage proton pumps drive the pH down from neutral (7.4) to acidic (~5.5 – 4.5). It has been previously found that small molecule cargo releases in this process but the mechanism is unknown. Using supported lipid bilayers as a model of the bilayer coating on the MSNP we can evaluate the effect of such a pH change on lipid membrane structure. To evaluate those changes we use epifluorescence microscopy, confocal imaging, and image analysis to determine structural change in both the lateral and vertical dimensions.

Results

Using fluorescence microscopy we find that the supported membrane structure changes dramatically with a pH change from 7.4 to 5.0. Figure 3 shows on the left an initially homogeneous coverage of the glass surface with the supported membrane. Upon addition of 1N HCl in citrate-phosphate buffer, however, large pores (dark regions) and smaller (micron size) bright circles form within seconds (right image). It is curious as to how these structures form as the pH change is too small to cause chemical degradation of the phospholipids. So how do the pores form if no lipid is lost? One possibility is that the membrane is undergoing a deformation where the initially two-dimensional membrane transforms into third-dimensional space through the formation of blister-like structures. Figure 4 shows an 3D image at an oblique angle of the same supported bilayer revealing the fluorescently bright blisters.

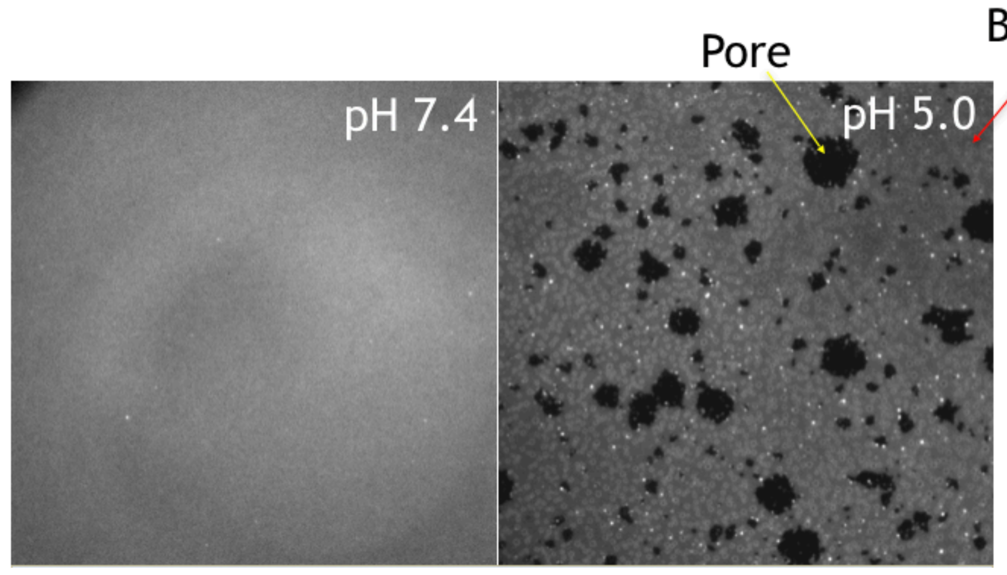


Figure 3: Supported bilayer at pH 7.4 (left) and 5.0 (right)

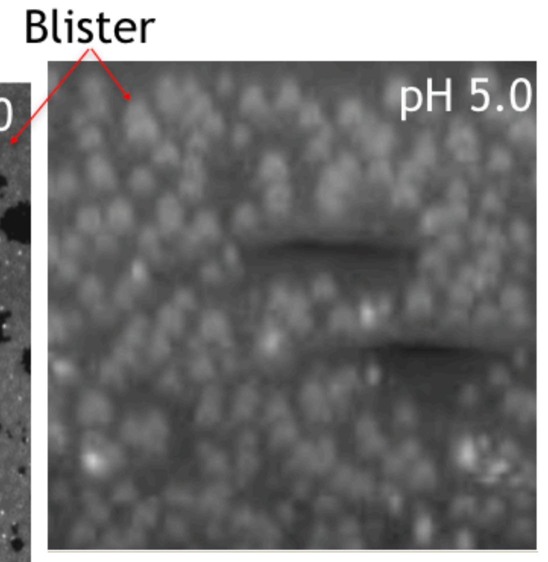
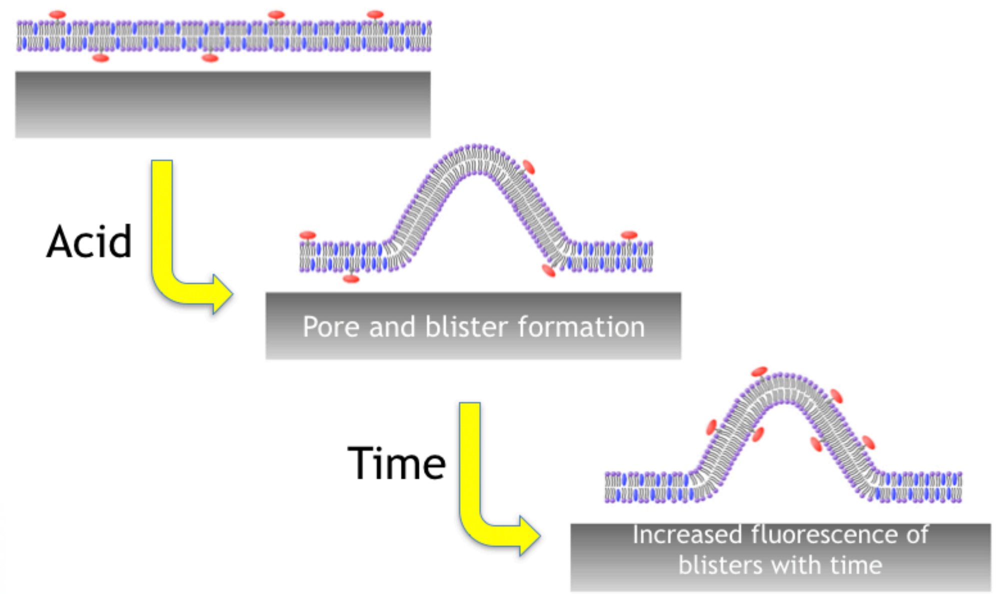


Figure 4: 3D Image showing membrane blisters at oblique angle.

Figure 5 (right): Explanation of what causes blister formation and appearance on imaging



Discussion

The membrane blister is slightly below microscopic resolution (300 nm), so to estimate their structure we tested some models to see if the blister deformation is the cause of pore formation. Our initial estimation of a hemispherical structure overestimated the membrane area deformation compared to the pore area. We are currently examining other blister shapes, such as a 2D Gaussian function. Figure 2 does appear to suggest that the shape of the blisters are more similar to a Gaussian function than a hemisphere. On another note, we found that with time the blisters become increasingly bright indicating an increases of fluorophores in the these regions. This is an interesting consequence of this membrane deformation – the highly curved blisters selectively exclude cholesterol and attract membrane fluorophore suggesting a highly fluid membrane region. This may have consequences for other possibilities of triggered membrane release for delivery vehicles.

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