A Novel Free Standing Lipid Membrane Model Designed for Dark Field Microscopy

Tianhong Chen, Bjoern Reinhard

Boston University, Department of Chemistry
02115 Boston, USA, E-Mail: thchen@bu.edu

1. Introduction

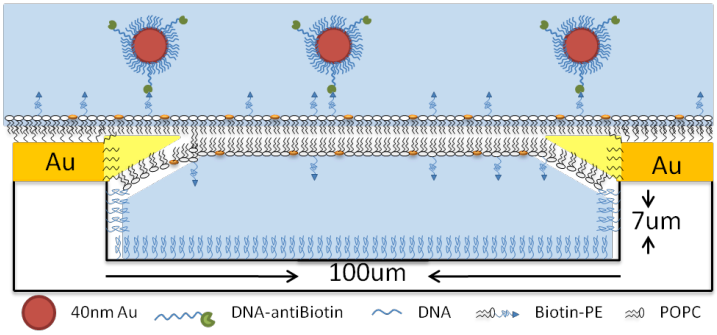

Fig. 1: Schematic diagram of the freestanding lipid membrane model.

Black Lipid Membranes(BLMs) are a widely used model system for study of cell-membrane properties and membrane-protein interactions. The traditional Mueller-Rudin method and Monte-Mueller method usually form vertical membranes soaked in a reservoir which is difficult to investigate with conventional microscopy. Our goal was to take advantage of recent advances in BLM architectures, particularly chip-based architectures, to engineer a BLM system that can be investigated with optical microscopy.

We are particularly interested in using dark field microscopy to study basic lipid-bilayer properties including its diffusion properties. Dark field microscopy of noble metal nanoparticles has several advantages over commonly used quantum dots and organic dye in tracking studies as noble metal nanoparticles have much larger optical cross-sections than both and do not bleach or blink. The superb photostability of the probes enables to improve time resolution and the total time of tracking. The size of noble metal nanoparticles can also be tuned in a straight forward fashion providing a convenient way for studying the influence of particle size on the diffusion behaviour.

Inspired by the work of Boxer etc. [1], we managed to design and build a black lipid membrane model (Fig.1) based on micro fabrication technique. This system is especially suitable for dark field microscopy and makes noble metal nanoparticle tracking studies under various conditions on a solvent free artificial membrane amenable.

2. *Preliminary Results*

Both Fluorescence Recovery After Photobleaching (FRAP) and Single Particle Tracking (SPT) experiments were performed with the implemented membrane model. FRAP data on 93%POPC, 5% Biotin-PE, 2%NBD-PE membrane gave an average diffusion coefficient of 12μm^2/s (Fig.3), which is in agreement with the literature value [2,3]. Preliminary single particle tracking experiments were performed with 40nm gold particle cross linked with anti-biotin antibody. Control experiments verified that the particles bound selectively to biotinylated lipids in the membrane surface by means of an anti-biotin antibody covalently attached to the nanoparticle. We were able to track these particles at a temporal resolution of 200Hz with a signal ratio around 10. The measured diffusion coefficients were found to vary between 1~6μm^2/s under different conditions. Further experiments are currently being performed to characterize the influence of various factors on particle diffusion.

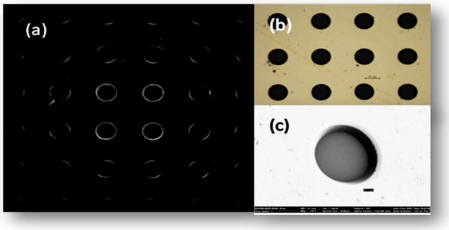

Fig. 2: Microscope Images of the membrane substrate with 50μm well: (a) Dark Field Microscope; (b) Bright Field Microscope; (c) SEM Image.

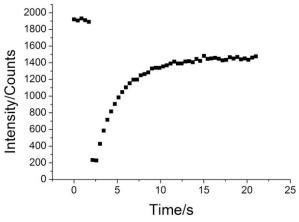

Fig. 3: Fluorescence recovery curve after photobleaching.

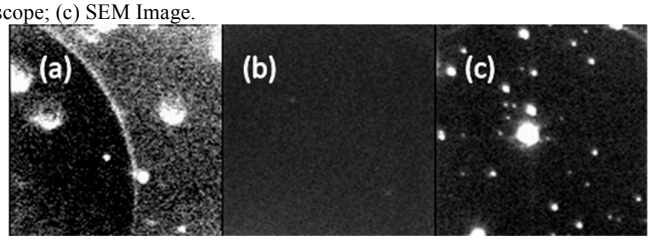

Fig. 4: (a) Membrane double layer forming on the left dark side. Specific Binding of Anti-Biotin cross linked gold nanoparticle on membrane (b) before flushing in particle; (c) after flushing in particle. Time Resolution used here is 200Hz. Control experiment was done with particle linked to an IGG secondary antibody which shows zero binding (data not shown).

3. Conclusion

We designed a free standing lipid membrane model which is suitable for microscopic imaging, especially for dark field imaging. We were able to integrate the model into a flow chamber which allows us to monitor the binding and dissociation of single particle. The FRAP data measured using this model was in agreement with the literature value. Single particle tracking experiments using gold nanoparticle were performed and

revealed great potential for a systematic characterization of the influence of multi-valency, particle size, viscosity of solution, and signal-noise ratio on the diffusion behaviour.

The designed membrane system of the substrate offers many opportunities for diffusion studies. The diameter and geometry of the wells can be easily tuned. Theoretically, a underlying electrical circular can also be incorporated to substrate and make electrical measurement possible.

References

[1] P. Ganesan andS. Boxer, PNAS 106 (2009) 5627–5632

[2] A. Sonnleitner, G. J. Schutz, and Th. Schmidt, Biophysical Journal 77 (1999) 2638–2642

[3] Z.I. Lalchev, A.R. Mackie, Colloids and Surfaces B: Biointerfaces 15 (1999) 147–160