

Center for Optical Technologies Biophotonics Group Research Activities

Depletion-enhanced optical trapping in nanoparticle suspensions: J. Junio and H.D. Ou-Yang (hdo0@lehigh.edu), *Lehigh University*. With the right proportions, a binary suspension of different sized particles may be subject to entropic effects that can generate a depletion-induced attraction between large particles. A manifestation of the induced attraction is the enhanced osmotic compressibility of the larger species in the presence of the smaller species. Using the pressure generated by the optical gradient force, we measured the enhanced compressibility of a colloid polymer mixture.

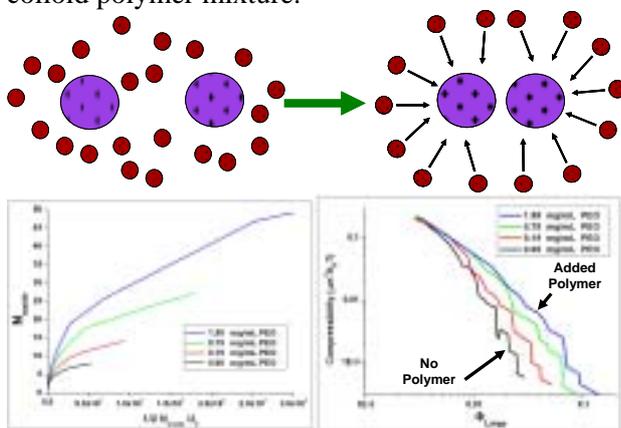


Fig. (Upper) In a mixture, the small particles will exert an unbalanced osmotic pressure on the large particles, forcing them together. This induces an additional attraction between the large, making them more compressible, and possibly causes the system to separate (right). (Lower) The number density over the same range of laser powers is greatly increased, with added polymer signifying an increased large particle compressibility. This enhancement increases with increasing polymer concentration, and is more pronounced at high particle concentrations and is not found to be appreciable at low particle concentrations.

Measurement of optical binding force between two colloidal particles: Ming-Tzo Wei, J. Ng, C. T. Chan, and H. Daniel Ou-Yang (hdo0@lehigh.edu), *Lehigh University*. Optical binding has been proposed to be responsible for the cluster formation of micron size dielectric spheres in coherent light fields. However, a direct measurement of the forces involved in binding is missing. We report an experimental study of optical binding forces between two optically trapped dielectric spheres. Results for optical

forces are presented as a function of three parameters: interparticle separation, particle size, and respective polarizations. A comprehensive calculation based on the generalized Mie scattering theory for the experiment has also been conducted. We suggested that the oscillatory optical forces as a function of the particle separation are not due solely to simple dipole forces, as the quadrupole and higher order polarizations played an important role in this study.

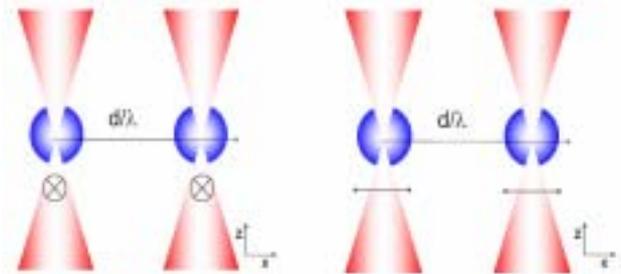


Fig. A schematic illustration of the experimental procedure. The directions of the electric field polarization are in the direction perpendicular (the Y-Y configuration) shown in the left or parallel (the X-X configuration) to the direction along the x axis which is the direction of the separation of the two particles.

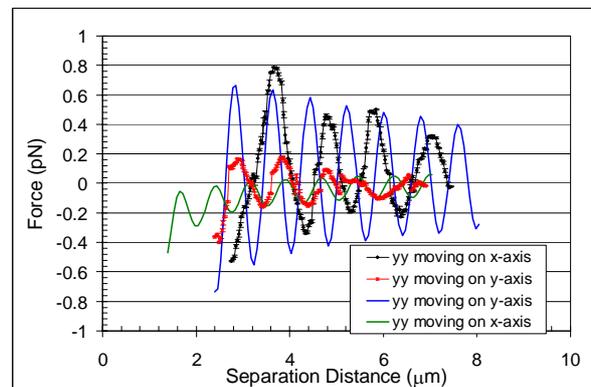


Fig. The optical binding force data shows the force of optical binding between two $1.5\mu\text{m}$ polystyrene particles trapped in separate quadratic potential wells each made from polarized s-wave 72mW 1064nm laser beams, as function of the interparticle separation. The experiments and simulations for two particles moved along the perpendicular direction with the laser beam polarization were shown in black and in green, respectively; along the direction parallel to the laser beam polarization were shown in red and in blue, respectively.

Trapping energy of nanoparticles: optical trapping combined with fluorescence correlation spectroscopy: Yi Hu, Xuanhong Cheng and H. Daniel Ou-Yang (hdo0@lehigh.edu), *Lehigh University*. The fluorescence correlation spectroscopic (FCS) technique can be used to determine the number density and diffusion of a nanoparticle suspension. By combining optical trapping with FCS, we found that the trapping energy could be measured for sub-nanomolar concentrations of fluorescently labeled 110nm polystyrene particles. Moreover, we showed that by enhancing the concentration of the suspension with optical trapping, this technique may be useful as a way to detect extremely small amounts of nano-sized suspended particles in solution, without the further filtration steps usually associated with FCS experiments.

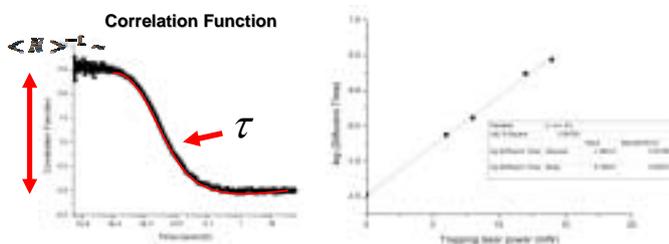


Fig. (Left) The $G(0)$ is proportional to the number of particles in the illuminated volume, while a fit of the curve itself also yields the diffusion time. (Right) Fitting the diffusion time vs. trapping power graph in log scale, it is shown that at 1 mW of trapping laser power, the trapping energy is $0.14 k_B T$ for 110nm diameter particles.

Direct measurements of the frequency dependent dielectrophoresis force: Ming-Tzo Wei, J. Junio, J. Wang, and H. Daniel Ou-Yang (hdo0@lehigh.edu), *Lehigh University*. Dielectrophoresis (DEP), the phenomenon of directed motion of electrically polarizable particles in a non-uniform electric field, is promising for applications in biochemical separation and filtration. The DEP force exerted on the particles is dependent on the electric field frequency in both magnitude and direction. Thus, by accurately measuring the force as a function of frequency, DEP can be used as a powerful technique to preferentially manipulate or even sort a wide variety of suspended particles i.e. (cells, bacteria, viruses, DNA and proteins, etc.)

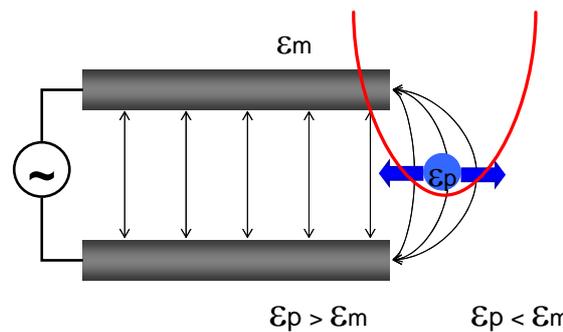


Fig. As the frequency of the electric field is varied, the polarizability of the particle relative to the surrounding medium determines the magnitude and direction of the DEP force. By holding the particle in the optical tweezers (red line), we can accurately measure the frequency-dependent DEP force.

The usefulness of this technique resides in the accurate quantification of the pico-Newton level of forces involved, which up until now have been lacking experimentally. By making use of optical tweezers as a force transducer coupled with lock-in phase sensitive detection, we report the quantification of the frequency-dependent DEP force for a variety of different sized suspended colloidal particles.

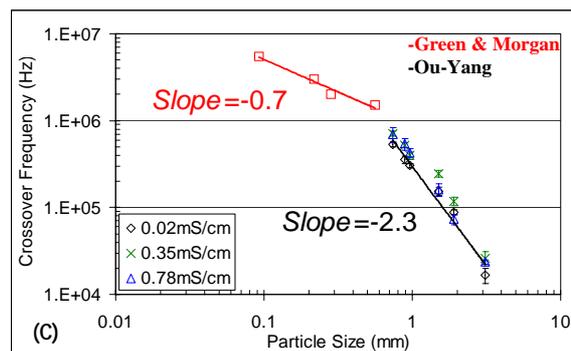
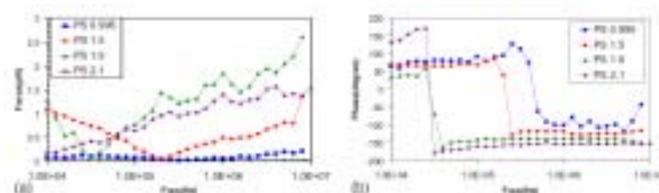


Fig. The DEP force (A) and displacement phase (B) as a function of frequency for four different sizes of polystyrene particles with diameters ranging from 0.998 to 2.1 μ m. Crossover frequencies as a function of particle size data (C).

Three-dimensional mapping of dielectrophoresis force and AC electroosmosis flow: Jingyu Wang and H. Daniel Ou-Yang (hdo0@lehigh.edu), *Lehigh University*. Forces experienced by colloidal particles in an electric field such as dielectrophoresis (DEP) and AC electro-osmosis (ACEO) have been widely investigated for the transportation, sorting, and mixing of samples in microfluidic devices. However, these forces are not well characterized. To provide a more complete theoretical basis for such AC electrokinetic mechanisms, we quantified the forces of DEP and ACEO exerted on individual particles. We hold a single particle in an AC electric field with optical tweezers, which serves as a force transducer to accurately quantify the frequency-dependent DEP and ACEO forces felt by the particle. Lock-in phase sensitive detection allowed the isolation of the DEP and ACEO forces from the electrophoresis and heat-induced convection forces omnipresent in the colloidal system. Comprehensive 3-D mapping of the DEP and ACEO forces involved will lead to a more complete understanding in a design of an implementation of micro-scale fluidic devices to exploit these electrokinetic phenomena.

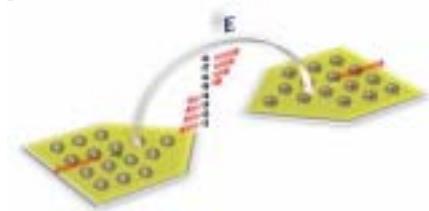


Fig. Particle suspended above the electrode surface subject to ACEO flow (red line) in the half circle of the AC electric field (gray line). Magnitude and direction of the force both vary with the position of the particle in the chamber.

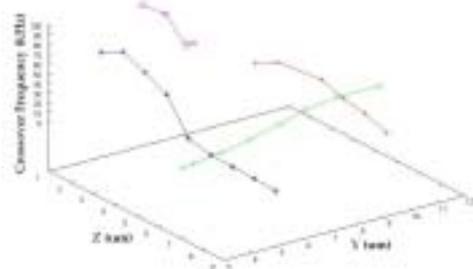


Fig. ACEO-influenced DEP crossover frequencies tend to reach a stable value when the particle is placed away from electrode surface where ACEO is the weakest and DEP is the dominant force on particle.

Probing the dynamic differential stiffness of dsDNA interacting with RecA in the enthalpic regime: Chia-Hui Lien, Ming-Tzo Wei, H. Daniel Ou-Yang, and Arthur Chiou (hdo0@lehigh.edu), *Lehigh University*. RecA plays a central role in homologous recombination of DNA. When RecA combines with dsDNA to form RecA-dsDNA nucleofilament, it unwinds dsDNA and changes its structure. The unwinding length extension of a DNA segment interacting with RecA has been studied by various techniques, but the dynamic differential stiffness of dsDNA conjugating with RecA has not been well characterized. We applied oscillatory optical tweezers to measure the differential stiffness of dsDNA molecules, interacting with RecA, as a function of time at a constant stretching force of 33.6pN. The values of the differential stiffness of DNA (for stretching force in the range of 20.0pN to 33.6pN) measured by oscillatory optical tweezers, both before and after its interaction with RecA, are consistent with those measured by stationary optical tweezers. In the dynamic measurement, we have shown that the association (or binding) rate increases with higher concentration of RecA; besides, we have also monitored in real-time the dissociation of RecA from the stretched RecA-dsDNA filament as ATP S was washed off from the sample chamber. Finally, we verified that RecA (I26C), a form of RecA mutant, does not affect the differential stiffness of the stretched DNA sample. It implies that mutant RecA (I26C) does not bind to the DNA, which is consistent with the result obtained by conventional biochemical approach.

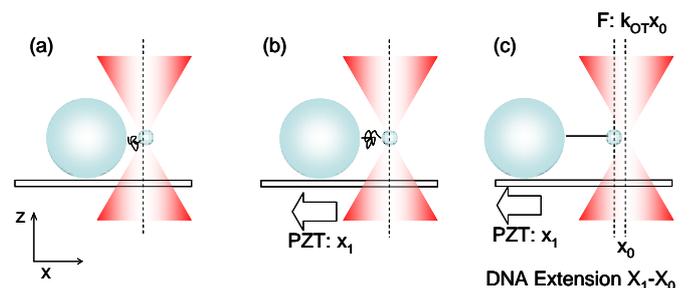


Fig. (a)~(c) A schematic illustration of the gradual stretching of a DNA sample by displacing one particle attached to one of its end while holding another particle attached to its other end via an optical tweezers.

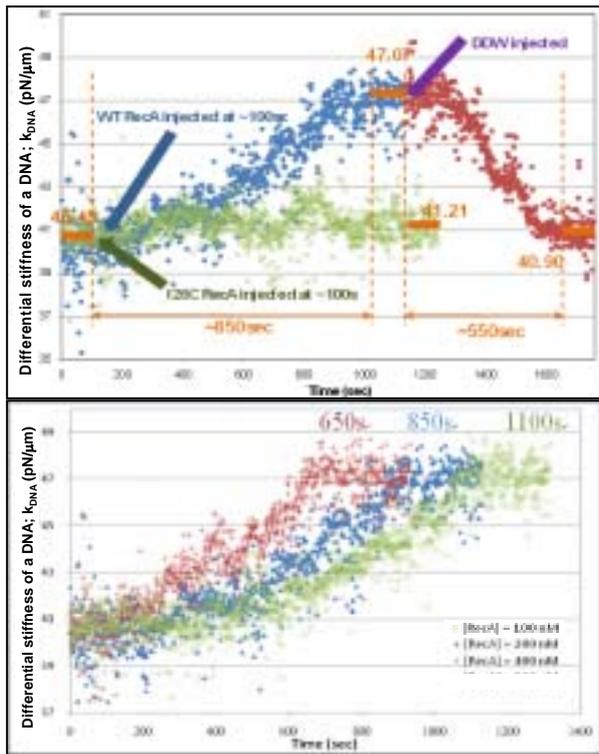


Fig. (a) The differential stiffness of the dsDNA sample stretched at a constant stretching force of 33.6pN as a function of time. From $t = 100$ sec. to $t = 1180$ sec., the measurement was taken as RecA was injected into the sample chamber to bind to the stretched dsDNA; from $t = 1180$ sec. to $t = 1750$ sec. the measurement was taken as de-ionized distilled water was injected into the sample chamber to dilute the concentration of ATP S to dissociate RecA from the stretched DNA; (b) The differential stiffness (k_{DNA}) of the dsDNA sample stretched at a constant stretching force of 33.6pN as a function of time when RecA was injected with different concentration.

Influence of cytoskeletal structure and mechanics on epithelial cell injury during cyclic airway reopening: H. C. Yalcin, K.M. Hallow, J. Wang, M.T. Wei, H.D. Ou-Yang and S. N. Ghadiali (hdo0@lehigh.edu), *Lehigh University*. Although patients with acute respiratory distress syndrome (ARDS) require mechanical ventilation, these ventilators often exacerbate the existing lung injury. For example, the cyclic closure and reopening of fluid-filled airways during ventilation can cause epithelial cell (EpC) necrosis and barrier disruption. Although much work has focused on minimizing the injurious mechanical forces generated during ventilation, an alternative approach is to make the EpC less susceptible to injury by altering the cell's intrinsic biomechanical and biostructural properties. In this study, we hypothesized that

alterations in cytoskeletal structure and mechanics can be used to reduce the cell's susceptibility to injury during airway reopening. EpC were treated with Jasplakinolide to stabilize actin filaments or Latrunculin A to depolymerize actin and then exposed to cyclic airway reopening conditions at room temperature using a previously developed *in-vitro* cell culture model. Actin stabilization did not affect cell viability, but significantly improved cell adhesion primarily due to the development of more numerous focal adhesions. Surprisingly, actin depolymerization significantly improved both cell viability and cell adhesion, but weakened focal adhesions.

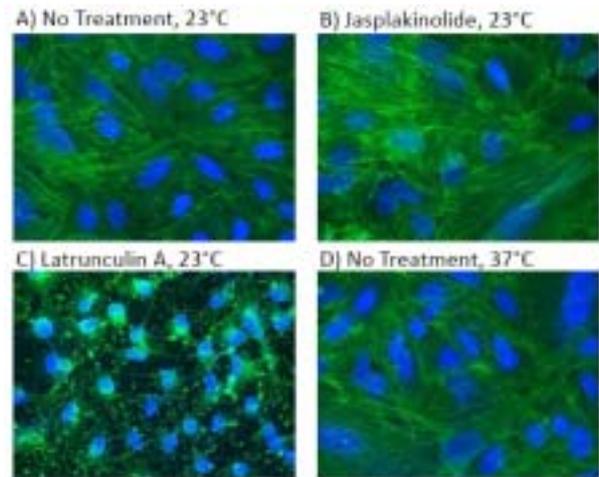


Fig. Actin staining of A549 epithelial cells with A) no treatment at 23°C, B) Jasplakinolide treatment at 23°C, C) Latrunculin A treatment at 23°C, and D) no treatment at 37°C. Images were obtained using a 40X objective.

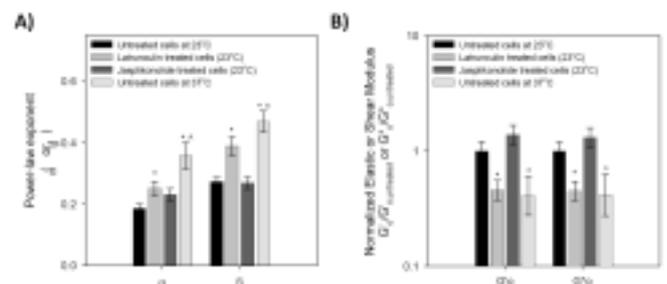


Fig. A) Effect of Latrunculin treatment, Jasplakinolide treatment and 37°C temperature on average power-law exponents. B) Effect of Latrunculin treatment, Jasplakinolide treatment and 37°C temperature on normalized elastic and shear modulus. Data are means \pm SE. ^ indicates significant difference compared with Latrunculin treated cells ($p < 0.05$) and * indicates significant difference compared with untreated cells at 23 ($p < 0.05$).

Optical tweezers based measurements of the EpC's micro-mechanical properties indicate that although Latrunculin-treated cells are softer, they also have increased viscous damping properties. To further investigate the effect of "fluidization" on cell injury, experiments were conducted at 37°C. Although cells held at 37°C exhibited minimal changes in cytoskeletal structure, they did exhibit increased viscous damping properties and improved cell viability. We conclude that fluidization of the actin cytoskeleton makes the EpC less susceptible to the injurious mechanical forces generated during cyclic airway reopening.

Characterization of bone cell proliferation and attachment on nano-macro porous glass scaffolds:

Himanshu Jain (h.jain@lehigh.edu), Matthias Falk, Department of Biological Sciences (mmf4@lehigh.edu) *Lehigh University*. One of the key challenges in today's medicine is the treatment of bone loss. We have recently developed two novel methods for fabricating bioactive glasses with dual nano-macro porosity based on (I) a conventional Melt-Quench method followed by selective heating and etching and (II) a novel Sol-Gel procedure with polymerization-induced phase separation. A macro porous structure of the glass scaffolds is necessary to obtain good implant incorporation through rapid vascularization and bone ingrowth, while the nanopores simulate the natural extracellular environment. We have fabricated such nano-macro dual-porous glass bone-replacement scaffolds and then tested their biocompatibility and bioactivity using immunofluorescence and confocal microscopy techniques. Specifically, the colonization and growth of MC3T3 and MG63 cells on the scaffolds have been monitored for cell attachment, proliferation, and differentiation using appropriate fluorescence-based cell detection methods. The results are helping us determine the most promising scaffolds and are thus expected to provide basis for further *in vivo* testing by our partners at Tissue Engineering Laboratory, Faculty of Dentistry, Alexandria University, Egypt.

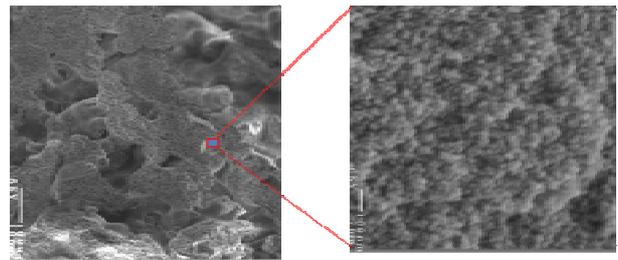


Fig. Macro (left) and nano (right) pores in glass developed at Lehigh for bone scaffold applications.

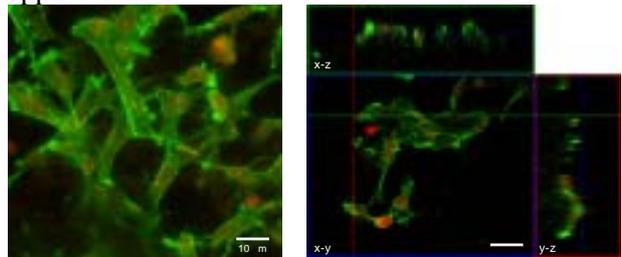
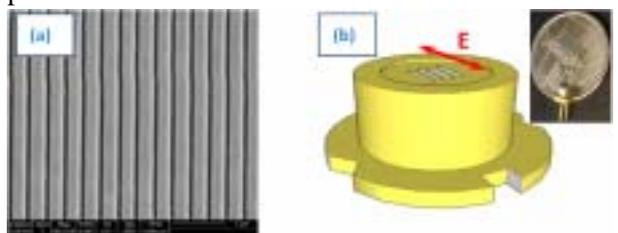


Fig. Confocal fluorescence microscopy of bone forming cells showing actin fibers and nuclei. The picture on the right shows three orthogonal views of the cells that had migrated into the pores of a nano-macro porous glass prepared by modified sol-gel method. F-actin was stained with Phalloidin Alexa 488 (green) and nuclei were stained with Propidium Iodide (red).

Nano Plasmonic Structures for compact sensing applications:

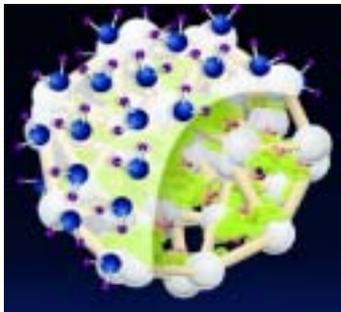
Fil Bartoli (fjb205@lehigh.edu) *Lehigh University*. Plasmonic grating structures were integrated into a semiconductor laser diode package to realize a prototype of a miniaturized chemical/bio-sensor¹. The advantages of such a device include compact size, low cost, energy efficiency, long device lifetime, stable performance and label-free sensing, which should have commercialization potential.



References:

[1] Q. Gan and F. J. Bartoli. Surface Plasmon-Coupled Semiconductor Diode Laser Package for Refractive Index Sensing, *Optics Lett.* **34**, 2180 (2009).

Bright and Photostable Cy3-Encapsulated Calcium Phosphate Nanoparticles for Bioimaging: Hari S. Muddana, Thomas T. Morgan, Tristan Tabouillot, James H. Adair, Peter J. Butler, Departments of Bioengineering and Materials Science, (pjbbio@engr.psu.edu) Penn State University.



Organic fluorescent dyes are used extensively in the fields of bioimaging and bioassays. Cyanine dyes, in particular, have wide-spread application in microarray-based

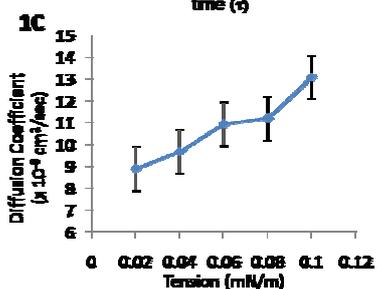
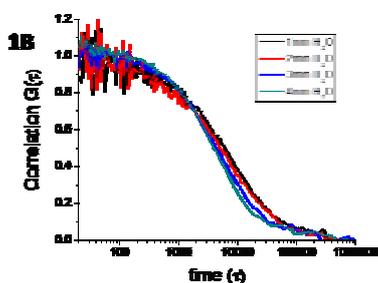
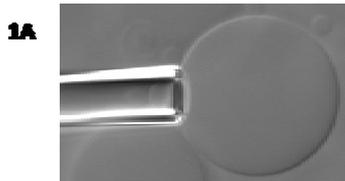
gene detection, bioconjugation, membrane probing, and as photosensitizers in photodynamic therapy. However, these dyes have low quantum yield, rapid photobleaching, random on/off blinking, and form non-fluorescent aggregates largely due to solvent interaction with the fluorophore. To ameliorate these solvent effects, organic dyes can be encapsulated in silica or polymer-based nanoparticles, but since these systems are non-bioresorbable, they have limited in vivo utility and do not permit delivery of fluorescence molecules to cells. The Adair group at Penn State has developed a novel method for synthesizing dye-encapsulated calcium phosphate (CP) nanoparticles based on a double-microemulsion method. In this study we used time-resolved fluorescence spectroscopy of cy3 to assess the changes in photophysics during encapsulation and after dissolution of the CP. Particle sizes measured using fluorescence correlation spectroscopy (FCS) and transmission electron microscopy (TEM) confirmed the presence of ~20 nm particles which were mono-dispersed. Brightness measurements determined using the photon counting histogram (PCH) demonstrates that cy3 encapsulated in CP is ~15 times brighter than free dye either due to increased dye photostability or increased number of dye molecules. Photostability as measured through photobleaching experiments showed that nanoparticles were ~50 times more stable than the free dye. Fluorescence lifetime of the dye in nanoparticles was found to be independent of solvent effects (hydrogen bonding ability,

viscosity), suggesting that the dye was well protected from the solvent. Furthermore, increased lifetime observed in nanoparticles in comparison to free cy3 suggests that the photoisomerization of cy3 was inhibited by rigid association with calcium phosphate. Thus detailed photophysical characterization of organic dyes demonstrates advantages of calcium phosphate encapsulation and may lead to more intelligent design of new probes for biological, diagnostic, and therapeutic imaging.

Tension Induces Changes In Lipid Lateral Diffusion In Model Fluid-Phase Membranes:

Hari S. Muddana, Ramachandra R. Gullapalli, Peter J. Butler, Department of Bioengineering, (pjbbio@engr.psu.edu) Penn State University. Shear stress due to blood flow on endothelial cells elicits numerous responses including G-protein coupled receptor activation and integrin-mediated signaling. Shear-induced change in membrane fluidity has been suggested to be one of the earliest mechanosensing mechanism involved in these processes. Alternatively, it has been suggested that shear forces are transduced through glycocalyx directly to transmembrane proteins and cytoskeleton, with very little shear force sensed by the membrane. It is not yet clear whether physiological tensions can alter membrane fluidity significantly.

In this study, we investigated the role of membrane tension in lipid diffusion using micropipette-aspirated model membranes. Giant unilamellar vesicles composed of DOPC (fluid-phase at room temperature) were prepared using electroformation method and stained with DiI₁₂. A series of isotropic membrane tensions in the range of 0.02 - 0.1 mN/m were applied on these giant vesicles using micropipette aspiration. Fluorescence correlation spectroscopy using time-correlated single photon counting was used to measure the 2D diffusion coefficients of the dye. To ensure that the dye behavior reflects the dynamics of lipids, we have conducted molecular dynamics simulations of a fluid-phase DPPC bilayer with dye incorporated in it. Our results suggest that physiological tensions can alter lipid diffusion (or membrane fluidity) significantly. Diffusion coefficients of the dye measured experimentally matched well with the molecular dynamics (MD) simulations.



(1A) Image depicting micropipette aspiration of giant unilamellar vesicles. (1B) Correlation curves from fluorescence correlation spectroscopy data. (1C) Diffusion coefficients of the dye measured as a function of applied membrane tension

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