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Live Cell Imaging with Rab-Gtpases Elucidates Intracellular Pathways of RGD and iRGD Tagged Cationic Lipid-DNA Nanoparticles

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Steric stabilization of cationic lipid-DNA (CL-DNA) complexes is required for their use in vivo, but PEGylation (PEG; polyethylene glycol) of CL-DNA complexes reduces their efficacy as gene delivery vectors in vitro [1]. One approach to improving gene delivery with PEGylated CL-DNA nanoparticles is to covalently attach a targeting peptide at the distal end of PEG. We have developed PEGylated CL-DNA nanoparticles with an RGD or iRGD motif present at the distal end of PEG2000 and studied their efficacy and mechanism of entry in vitro. Studies have shown that nanoparticles exposing an RGD peptide are capable of targeting tumors in vivo via a specific interaction with the membrane bound receptor integrin. Recently, the next generation of RGD peptides, internalizing RGD (iRGD) has been shown to target and penetrate tumors in vivo [2]. Although neuropillin-1 is known to play a role in the tumor penetration properties of iRGD, the intracellular mechanism which allows for tumor penetration is unknown. In order to investigate how iRGD promotes tumor penetration, we performed live cell imaging of fluorescently labeled RGD and iRGD-tagged CL-DNA nanoparticles using GFP-Rab-GTPases, an endosomal membrane bound protein which allows for spatio-temporal tracking of endosomal cargo.
We present quantitative analysis using GFP-Rab-(5, 7 and 9) which label early and late endosomes and compare the colocalization of RGD and iRGD in these endosomes as a function of time. Finally, we investigated the gene delivery properties of RGD and iRGD-tagged lipid-DNA nanoparticles through the use of gene expression assays. A thorough understanding of the intracellular fate of CL-DNA nanoparticles will allow for optimization of gene delivery vectors. Funded by NIH-GM59288.
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Biomimetic Light Harvesting in Nanoporous Metal Organic Materials

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Metal organic framework materials (MOFs) represent a class of solid state materials with a number of properties advantageous for numerous applications ranging from gas storage and separation, heterogeneous catalysis, and controlled drug release to name only a few. Of the plethora of unexplored applications of MOFs, frameworks that can facilitate biomimetic solar photochemistry are of significant interest. Solar photochemistry applications rely on the ability of the MOF material to undergo facile and directional photoinduced electron transfer, much like biological light harvesting systems. One strategy is to utilize the nanoscale cavities within the MOF to encapsulate photoactive guests that can participate in directional photo-induced electron transfer reminiscent of biological electron transport chains. Here we discuss two such light harvesting systems. First, photoexcitation of a Zn(II)-trimesic acid based metal organic framework containing co-encapsulated Ru(II)tris(2,2'-bipyridine) (RuBpy) and Co(II)tris(2,2'-bipyridine) (CoBpy) results in inter-molecular electron transfer (ET) between the excited state of RuBpy (^{3}MLCT) and the ground state CoBpy The rate of inter-cavity ET, k_{ET}, is found to be $3.7x10^6$ s^{-1}. Using the semi-classical Marcus equation and the observed rate constant, it is determined that ET occurs between RuBpy and CoBpy complexes located in adjacent cavities (~19.6 Å). We have also previously demonstrated the ability to specifically encapsulate metall-porphyrins within the octahemioctahedral cavites of both Cu and Zn HKUST-1. Here the simultaneous encapsulation of both Zn(II) tetrakis(tetra 4-sulphonatophenyl)porphyrin (Zn4SP) and Fe(III)tetrakis (tetra 4-sulphonatophenyl)porphyrin (Fe4SP) into a Zn(II)-HKUST-1 metal organic framework is demonstrated. Photo-excitation of the Zn4SP results in inter-molecular electron transfer (ET) between the encapsulated ^{3}Zn4SP and the Fe(III)4SP sites as evident by the reduction in ^{3}Zn4SP lifetime from 370 μs (k_{obs} $2.7x10^3$ s^{-1}) to 83 μs (k_{obs} = $1.2x10^4$ s^{-1}) in the presence of Fe4SP giving a k_{ET} ~ $9.xx10^3$ s^{-1}.

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Superresolution Imaging of the Enthesis Under Mechanical Load

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The enthesis is the anatomical site where bone and tendon join. At some locations, such as the Achilles' tendon insertion in the heel, forces transferred from muscle through tendon can exceed multiples of the body weight. Additional stress results from continuous changes in tendon orientation.
Interfaces between materials with very different mechanical properties are known to be subject to early rupture caused by poor stress distribution, but the enthesis has an impressive durability. There currently only exists a limited understanding of the micromechanical and structural reasons behind this.
We image the enthesis under mechanical stresses typical of physiological loads. To this end we designed a micromechanical load-chamber. This is combined with confocal and STED microscopies to investigate rat and pig samples. Images taken with 60 nm resoltuion were stitched together to completely cover millimeter sized samples, allowing us to monitor the nanoscopic displacement of the collagen fibril and the strain fields on the scale of the whole sample, simultaneously.

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Investigation of Nanolipoprotein Particles Entrapped Within Nanoporous Silica: A Novel Platform for Immobilization of Integral Membrane Proteins

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Immobilization of integral membrane proteins (IMPs) in transparent, nanoporous silica gels has proven to be a challenge, as current and previous techniques utilize liposomes as biological membrane hosts. The instability of liposomes in nanoporous gels is attributed by their size (~150 nm) and altered structure and lipid dynamics upon entrapment within the nanometer scale pores (5-50 nm) of silica gel. This ultimately results in disruption of protein activity. We intend to overcome these barriers by using nanolipoprotein particles (NLPs) as biomembrane hosts. NLPs are discoidal patches of lipid bilayer that are belted by amphiphilic scaffold proteins and have an average thickness of 5 nm, with diameters ranging from 10-15 nm. The IMP-NLP complexes are synthesized in a cell-free environment, which circumvents traditional protein reconstitution in membranes. Bacteriorhodopsin - a robust IMP protein that indicates its proper conformation via distinct purple coloration - will serve as a model IMP for this system. The spectral and physical properties of bacteriorhodopsin-NLPs entrapped within the gel are examined, as well as the phase behavior of the lipids within the NLP, to ensure proper functionality of the system. This bio-inorganic hybrid nanomaterial possesses a variety of viable applications. The success of this work could lead to the development of novel platforms in several areas, including high-throughput drug screening, chromatography, and biosensors.

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High Speed All Optical Logic Operations Utilizing the Protein Bacteriorhodopsin

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In data-processing applications requiring high speed and wide bandwidth, photonic devices - where logic operations are processed on an all-optical basis - represent a promising alternative of their electronic counterparts. Besides in/organic active optical crystals, dyes and polymers, molecules of biological origin with suitable nonlinear optical properties can also find applications in integrated optical - biophotonic - devices.
The principle of all-optical logical operations utilizing the unique nonlinear optical properties of a protein was demonstrated by a logic gate constructed from an integrated optical Mach-Zehnder interferometer as a passive structure, covered by a bacteriorhodopsin (bR) adlayer as the active element. Logical operations were based on a reversible change of the refractive index of the bR adlayer over one or both arms of the interferometer. Depending on the operating point of the interferometer, we demonstrated binary and ternary logical modes of operation. Using an ultrafast transition of the bR photocycle (BR-K), we achieved high-speed (nanosecond) logical switching. This is the fastest operation of a protein-based integrated optical logic gate that has been demonstrated so far. The results are expected to have important implications for finding novel, alternative solutions in all-optical data processing research.