with experimental data in all scenarios. Of particular biological significance, our model predicts a threshold σ_s value below which the NC binding affinities reduce drastically and drop lower than that of single anti-ICAM-1 molecule to ICAM-1; our results reveal that this is due to a change in the multivalency (or number of bonds formed per NC). The trend and threshold values are exactly recovered by the *in vivo* measurements of the endothelium targeting of NCs in the pulmonary vascular in mice [Liu *et al*. *PNAS* 107: 16530-16535 (2010)]. Increasing the shear flow rate enhances the NC binding affinities till a threshold value is reached; this quantitatively agrees with existing experiments and a novel mechanism is revealed based on our model results. On this basis, our computational protocol represents a quantitative and predictive approach for model driven design and optimization of functionalized nanocarriers in targeted vascular drug delivery.

This work is supported by NIH Grants: R01-EB006818 (D.M.E.), R01-HL087036 (V.R.M.), and NSF Grant: CBET-0853389 (R.R.).

Imaging and Optical Microscopy III

3328-Pos Board B433

Characterization of Binding Affinity and Epitope Dynamics of Anti-HIV-1 Antibodies

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A preventive vaccine is potentially the most effective way to control the HIV pandemic. Such a vaccine needs to successfully harness humoral immunity and produce cross-reactive anti-envelope antibodies that mediate direct virus neutralization and/or Fc receptor-dependent killing. For these antibodies to carry out their functions in clearing HIV infection, they must bind the virus and prevent it from infecting target CD4+ cells. The capacity of an antibody to do this is dependent on the timing, duration and extent of cognate epitope exposure before and during the attachment and entry processes. The goal of this study was (i) to quantify antibody binding to HIV, and (ii) to characterize when and for how long antibody epitopes are exposed before and during virus-cell fusion. We studied the binding properties and epitope dynamics of antibodies against HIV envelope gp120 [b12 (CD4 binding site), 2G12 (carbohydrate clusters), A32 (C1, C4, & C5 domains)], CD4-induced epitope of gp120 (17b & 19e), and the membrane-proximal external region (MPER) of gp41 (4E10). To directly quantify antibody binding to virus in solution, we developed a fluorescence correlation spectroscopy (FCS) methodology that uses fluctuations in fluorescent signals to measure diffusion and reaction kinetics of fluorescently-labeled anti-envelope Mabs as they attach to HIV-1-JRFL, HIV-1-Bal, and HIV-1-NL4-3 pseudoviruses and infectious molecular clones. We have also developed methods to visualize the temporal appearance and disappearance of cognate epitopes during virus-cell fusion using immunofluorescence and live-cell imaging techniques. In this case, viral particles were labeled with a novel SNAP-tag technology that permits tracking of particles during different stages of fusion with CD4+ target cells, and concurrent imaging of epitopes that become exposed on the HIV envelope.

3329-Pos Board B434

An Improved Method for Studying Single Proteins Trapped in Lipid Vesicles

Claudiu C. Gradinaru.

We report on an improved method to encapsulate proteins inside surface-tethered liposomes in order to reduce or eliminate environmental interference for single-molecule investigations. The liposomes are large enough for the molecule to experience free diffusion, but sufficiently small so that the molecule appears effectively immobile for fluorescence imaging. Various single-molecule fluorescence experiments were performed to fully characterize this anchoring method relative to direct immobilization via biotin-streptavidin linkers. Multidimensional histograms of intensity, polarization and lifetime reveal that molecules trapped in liposomes display a narrow distribution around a single peak, while the molecules directly immobilized on surface show highly dispersed values for all parameters. For instance, when TMR-labelled molecules were immobilized directly on surface, we recorded large intensity fluctuations (6.30 ± 4.91 emission states/molecule), whereas the fluctuations were much smaller for the vesicle-trapped molecules (1.37 ± 0.71 emission states/molecule). During sample preparation, by hydrating the lipid film at low volumes, high encapsulation efficiencies can be achieved with ~10 times less biological material than previous protocols. By measuring directly the vesicle size distribution, we found no significant advantage for using freeze-thaw cycles during vesicle preparation. On the contrary, the temperature jump can induce irreversible damage of fluorophores and it reduces significantly the functionality of proteins, as demonstrated on single-molecule binding experiments involving a peptidic inhibitor for the oncogenic protein STAT3. Our improved and biologically gentle molecule encapsulation protocol has a great potential for widespread applications in single-molecule fluorescence spectroscopy.

3330-Pos Board B435

In Vivo Structure of the E. coli FtsZ-Ring Revealed by Photoactivated Localization Microscopy (PALM)

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The FtsZ protein, a tubulin-like GTPase, plays a pivotal role in prokaryotic cell division. In vivo it localizes to the midcell and assembles into a ring-like structure-the Z-ring. The Z-ring serves as an essential scaffold to recruit all other division proteins and generates contractile force for cytokinesis, but its supramolecular structure remains unknown. Electron microscopy (EM) has been unsuccessful in detecting the Z-ring due to the dense cytoplasm of bacterial cells, and conventional fluorescence light microscopy (FLM) has only provided images with limited spatial resolution (200-300 nm) due to the diffraction of light. Hence, given the small sizes of bacteria cells, identifying the in vivo structure of the Z-ring presents a substantial challenge. Here, we used photoactivated localization microscopy (PALM), a single molecule-based super-resolution imaging technique, to characterize the in vivo structure of the Z-ring in E. coli. We achieved a spatial resolution of ,35 nm and discovered that in addition to the expected ring-like conformation, the Z-ring of E. coli adopts a novel compressed helical conformation with variable helical length and pitch. We measured the thickness of the Z-ring to be ,110 nm and the packing density of FtsZ molecules inside the Z-ring to be greater than what is expected for a single-layered flat ribbon configuration. Our results strongly suggest that the Z-ring is composed of a loose bundle of FtsZ protofilaments that randomly overlap with each other in both longitudinal and radial directions of the cell. Our results provide significant insight into the spatial organization of the Z-ring and open the door for further investigations of structure-function relationships and cell cycle-dependent regulation of the Z-ring.

3331-Pos Board B436

Assessing the Cellular Uptake Pathway for Poly-Lysine Analogues using Triplet Lifetime Imaging

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Research on synthetic delivery vectors is of major interest for cell imaging and manipulation, as they allow an efficient transfer of nucleic acids, therapeutic proteins or small drugs into the cells. We have developed a library of L-lysine analogues that allow for highly efficient gene delivery with low cytotoxicity. However little is known on the exact mechanism of uptake and the final intracellular destination of the synthetic carriers. Therefore we have developed a novel optical technique based on a modulated excitation allowing for intracellular imaging of the triplet-lifetime and -yield of fluorophores attached to the delivery vector. Both these parameters are highly dependant on the intracellular environment thus provide insight into the subcellular localization of the labelled carrier. The method combines high temporal and spatial resolution and is compatible with a multiplicity of fluorophores.

We performed series of model experiments to compare the triplet lifetime and triplet yield behaviour during the natural uptake mechanism to a series of controlled conditions. The latter include microinjection of fluorescently labelled carriers directly into the cytoplasm and cell nucleus as well as in vitro measurements under conditions mimicking physiological, acidic, or DNA rich environments. To validate our technique the results from the triplet imaging were compared with two complementary methods: carrier localization by subcellular fractionation and confocal laser scanning microscopy.

— Reference —

Geissbuehler et al. Triplet imaging of Oxygen consumption during the contraction of a single smooth muscle cell (A7r5). Biophysical Journal (2010) vol. 98 (2) pp. 339-349

3332-Pos Board B437

Correlative EFTEM, Stem and Fluorescence Microscopy as a Tool for Chromatin Biology

Maria A. Aronova, Alioscka A. Sousa, Guofeng Zhang, Michael J. Kruhlak, Elissa P. Lei, Richard D. Leapman.

In eukaryotes, the highly coordinated gene expressions require sophisticated levels of regulation. One such mechanism regulates the spatial and temporal organization of genes and their associated sequences in higher-order chromatin domains. Chromatin insulators, specific gene regulatory assemblies, form large nucleoprotein complexes known as insulator bodies and are thought to influence the organization of higher-order chromatin domains. In order to test current models of insulator function and provide ultrastructural information about these chromatin based domains, we use a correlative microscopy approach based on light microscopy and electron microscopy operating in the mode of energy filtered transmission electron microscopy (EFTEM) and scanning transmission electron microscopy (STEM).