The method involves using antibody functionalized magnetic beads to target CD3, a subunit of the TCR complex to induce TCR clustering. using magnetic tweezers, we move the magnetic beads through the cell membrane and simultaneously measure the trafficking of co-receptors and involved proteins. One of these is CD6 co-receptor, which is considered a co-stimulator for cell activation. We study CD6 during cluster formation.
We introduce αCD3 coated magnetic beads of sizes 1μm and 4.5μm respectively to CD6-GFP transfected Jurkat T-Cells. using confocal fluorescence microscopy, we measure recruitment of CD6 and co-localization with proteins involved in cell activation pathway as the bead is pulled through the membrane. First experiments suggest that while CD6 is not physically associated with TCR complex, it becomes actively recruited into the contact site mimicking the IS, and moves along with the IS as it translocates.
We will downscale this method by using functionalized magnetic nanoparticles in order to address individual T-cell receptors. using magnetic fields this allows to actively induce nanoscale TCR clusters of predefined size. This will allow us to study the importance of TCR cluster size on cell activation.

2562-Pos Board B581
Near-Field Optical Immobilization of Antibodies for Novel Fluorescent Bioassays
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Optical manipulation techniques have enabled the study of single molecules in biophysics. Optical tweezers have allowed force spectroscopic measurements of large biopolymers bond to microspheres. A newer generation of optical traps, based on near field optics, allow trapping of smaller molecules with the hope to manipulate directly the biomolecules rather than through microspheres. We previously developed a photonic crystal trap able to capture small biomolecules, such as Wilson disease molecule, while observing them with fluorescent microscopy. The technique has been shown to be compatible with biophysical experiments as it can be performed in the biological buffer without significant temperature increase. Although these techniques have been developed with the idea of trapping single molecules, in this presentation, we steer away from the single molecule focus. A novel bioassay platform is presented that takes advantage of the optical forces exerted on molecules near the photonic crystal. The aim of this method is to replace the chemical immobilization on a surface by optical immobilization because molecules optically attracted to the optically active surface can freely rotate on it and sample more orientations than molecules bond to a surface. On this platform, using fluorescent microscopy, we observe the aggregation of antibodies in the optical trap and measure their binding rates to antigens at various temperatures. Because of the optical and hydrodynamic forces at play, we expect to observe higher binding rates than in mass-transport limited equivalent systems.

2563-Pos Board B582
Singlet Oxygen Triplet Energy Transfer (STET) Based Imaging Technologies for Mapping Protein-Protein Proximity and Interactions in Intact Cells
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Forster resonance energy transfer (FRET) has been widely used for the detection of protein-protein interactions but its detection distance is <10 nm. To overcome the short detection range of FRET, we have developed a new concept for molecular imaging: Singlet oxygen Triplet Energy Transfer (STET). We have engineered genetically encoded singlet oxygen generator (SOG) and singlet oxygen sensor (SOS). The proximity of protein X to protein Y can be assayed by fusing X to SOS and Y to SOG. Upon illumination, SOG is excited and converts molecular oxygen into singlet oxygen through triplet energy transfer. The singlet oxygen transfers to SOS through diffusion and chemically reacts with the sensor. The closer X and Y are, the faster SOS will respond to illumination of SOG. STET has a distance range (~50-100 nm) an order of magnitude greater than that of FRET and does not depend on the orientation of chromophores. The utility of STET in mapping proximity is illustrated in a three-membrane-spanning protein complex that physically tethers ER and mitochondria in yeast. In fixed yeast cells, STET can detect any pair-wise protein proximity spanning ER, mitochondrial outer and inner membranes, and mitochondrial matrix (i.e. a detection range of >40nm). The strength of STET robustly reflects the relative distances between the protein pairs. In a separate set of experiments, we have demonstrated that STET can monitor the sub-second time course of the association of two proteins upon ligand induction inside live cells, highlighting the temporal resolution and single-molecule sensitivity of STET. Taken together, STET will find broad applications in (1) estimating the topology of a stable protein complex and (2) identifying and monitoring transient protein-protein interactions.

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Characterization of Nanoparticles for Therapeutics, Diagnostics and Imaging by Means of Second Harmonic Scattering
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A new field of study, called nano-bio interactions, investigates how nanomaterials interact with biological systems. This is an important issue because, in order to be able to engineer nanostructures capable of traveling in the body and interacting with cells, we need to develop an understanding of how the physicochemical properties of nanoparticles (NPs) affect the way these objects interact with biological systems. For instance, when considering a cancer nanomedicine different parameters have to be taken into consideration [1]. An understanding of how the NP size, shape, composition, structure, charge and surface chemistry influence transport to the tumor site [2] can help to maximize the efficiency of delivery of the drugs or contrast agents for imaging as well as the effectiveness of photothermal therapy [3].
Second harmonic scattering (SHS) is a coherent second-order optical technique that is specifically surface sensitive and can be performed in-situ [4]. It has also been recently shown to be sensitive to size, shape and composition of metallic and dielectric nano and microparticles with or without adsorbed molecular monolayers [5], presenting a huge potential in the characterization of nano-bio interactions as well as for use in imaging and photothermal therapy.
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2565-Pos Board B584
Spherical and Cubic Magnetic Colloids to Decipher the Mechanics of the Cytoskeleton
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We developed a new method of mechanical measurement, based on the dipolar attraction between magnetic colloids. A spherical gel formed around magnetic colloids is progressively deformed between two surfaces as the dipolar attraction between the colloids increase. Applied force is controlled by the external magnetic field, while the gel deformation is monitored by video-microscopy. Elastic modulus of the gel material is extracted by the fit of the force-deformation curve. This method was used on in vitro dense branched networks formed by the Arp2/3-complex nucleator, which are crucial for cell migration. The high throughput of this method allowed the probing of thousands of gels, orders of magnitude more than previous experiments done with demanding techniques such as atomic force microscopy. By changing the concentration of proteins used during the growth of the gel, we changed the proportion of branches and the length of the filaments, and conducted the first systematic study linking the architecture of the actin networks to their architecture. A limitation of this technique is the requirement of sphericity to extract the elastic modulus from force-deformation curves. We solved this issue by developing new magnetic colloids of arbitrary shapes (cubes, cylinders, disks, etc.). This allows the deformation of objects between two flat surfaces while retaining the high throughput of this method. The technique now permits the measurement of non-linear elasticity, viscous modulus and speed of polymerization versus applied force for dense branched actin gels. Micro-objects of different shapes can be probed, and development is underway to use this technique for the measurement of adherent cells' mechanical properties. This technique could then be used as a diagnosis tool by probing metastatic cancer cells with a great throughput.

2566-Pos Board B585
Novel Approaches for Identifying Phosphorus Species in Terrestrial and Aquatic Ecosystems with ^{31}P NMR
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Phosphorus (P) limits plant growth especially where agriculture is performed on older soils. Predictions indicate that rock-P mining peaks within 35 years with severe impacts on global food production[1]. Several studies have been made to completely understand fate and cycle of P in soils but knowledge in this field remains limited. Therefore we aim in our physicochemical/ecological