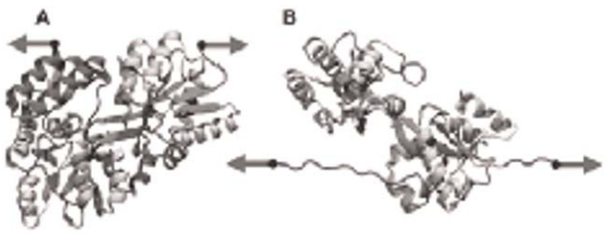
onto which several α-helices are finally wrapped. Interactions with SecB completely prevent stable tertiary contacts in the core structure, but have no detectable effect on the folding of the external α-helices. It appears that SecB only binds to the extended or molten globule-like structure and retains MBP in this latter state. Thus during MBP translocation, no energy is required to disrupt stable tertiary interactions.



2659-Minisymp The Conformational Diffusion Rate of Unfolded Protein Chains: Evidence from Cytochrome *c* Supports the Generality of k_{diff} as a Prefactor for Transition State Expressions for Folding Rates

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The nature of the prefactor in Arrhenius or transition state theory expressions for protein folding rates and the speed with which the conformers of unfolded protein chains interconvert are fundamentally related questions. We have measured the time constant for interconversion of disparate unfolded chain conformations of a small globular protein, cytochrome *c* (Abel et al., 2007, *Biochemistry*, *46*:4090–4099). The axial binding reactions of histidine and methionine residues with the Fe(II) heme cofactor were monitored with time-resolved magnetic circular dichroism (TRMCD) spectroscopy after photodissociation of the CO complexes of denaturant-unfolded protein obtained from horse and tuna, as well as from several histidine mutants of the horse protein. Using a kinetic model that fit both the reaction rate constants and spectra of the intermediates, we obtained an estimate of the conformational diffusion time (approximated as a first-order time constant for exchange between conformational subensembles presenting either a methionine or a histidine residue to the heme iron for facile binding) of 3 μs for these sequences, very close to the ~1 μs value often cited as the folding "speed limit". Expanding on the proposal that the conformational diffusion rate constant is the prefactor for folding (W. Y. Yang & M. Gruebele, 2003, *Nature*, *423*:193–197), the convergence of our result with similar values for other folding and nonfolding sequences of similar length suggests that k_{diff} may provide a general, approximately sequence-identity-independent prefactor for the transition state theory of folding.

Platform BD: Imaging and Optical Miscroscopy

2660-Plat Simultaneous Transport of Different Localized mRNA Species Revealed by 3D Single-Particle Tracking

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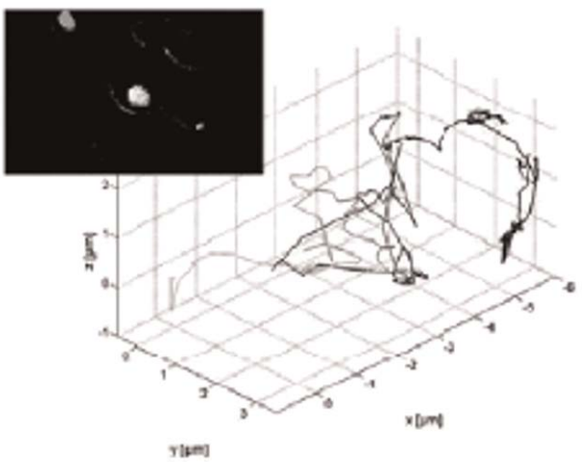
Intracellular mRNA localization is a common mechanism for creating asymmetric distributions of proteins in live cells. Previous studies have revealed that, in a number of cell types, different mRNA species are localized by the same transport machinery. However, it has been unclear if these individual mRNA species are specifically sorted into different or common ribonucleoprotein particles before or during transport. Using yeast as a model system, we analyzed the intracellular movement of individual pairs of localized mRNA in live cells using 3D particle tracking with dual-color detection. For this purpose, mRNAs were tagged with tandem repeats of either bacteriophage MS2 or lambda boxB RNA sequences and fluorescently labeled by fusion protein constructs that bind to the RNA tag sequences. We have tracked the transport of two different localized mRNA species in live cells. Our observations demonstrate that different localized mRNAs are co-assembled into common ribonucleoprotein particles and co-transported in a directional manner to the target site. Non-localized mRNAs or mutant mRNAs that lack functional localization signals form separate particles that are not transported to the bud.



2661-Plat High-Contrast Single-Molecule Microscopy by Selective Focal Plane Illumination

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Wide-field single molecule fluorescence microscopy is a versatile tool for analyzing interactions in biophysical and biological systems in three dimensions without averaging over molecular ensembles. Known drawbacks of the technique are a high fluorescence background due to excitation of out-of-focus molecules, a limited axial resolution, and photobleaching. To overcome these problems we used a selective focal plane illumination instead of the conventional epi-illumination. A custom-designed objective lens (NA 0.33) was used to create a light sheet inside a water chamber with a thickness of 1.7 μm (FWHM at excitation with 543 nm light) perpendicular to the detection axis. Fluorescence light was collected by a water-dipping objective lens (60x, NA 1.0) and imaged onto an EMCCD-camera. By this means we constructed a simple optical sectioning microscope, which combines the contrast and high resolution of a confocal microscope with the speed and sensitivity of a video microscope. The lateral and axial resolution were 350 nm and 1.37μm, respectively (excitation 633 nm, emission 680 nm), similar as in confocal microscopy. In comparison to a conventional illumination we achieved a significantly improved signal-to-noise-ratio of $> 110\%$. The optical sectioning capability of the instrument was demonstrated by acquisition of high-resolution 3D image stacks of polytene chromosomes in nuclei of *C. tentans* salivary gland cells. For demonstration of the new instrument's suitability to perform fluorescence experiments on the millisecond time scale we measured the diffusion coefficient of quantum dots (QD655) in buffer solution correctly to D = 17 $\mu m^2/s$. The new experimental setup eliminated major problems of single molecule microscopy in three-dimensionally extended geometries.

2662-Plat Multicolor Super-Resolution Imaging with Photo-Switchable Fluorescent Probes

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Recent advances in far-field optical microscopy have enabled fluorescence imaging with a spatial resolution of 20 to 50 nanometers. Multicolor super-resolution imaging, however, remains a challenge. Here, we introduce a family of photo-switchable fluorescent probes and demonstrate multicolor stochastic optical reconstruction microscopy (STORM). Each probe consists of two fluorophores: a photo-switchable "reporter" that can be cycled between fluorescent and dark states, and an "activator" that facilitates photoactivation of the reporter. Combinatorial pairing of three reporters (Cy5, Cy5.5, and Cy7) and three activators (Alexa 405, Cy2, and Cy3) allows the creation of probes with up to nine independently distinguishable colors. Iterative, color-specific activation of sparse subsets of these probes allows their localization with nanometer accuracy, enabling the construction of a super-resolution image. Using this approach, we have demonstrated multicolor imaging of mammalian cells with 20- to 30-nanometer resolution - a tenfold improvement over conventional imaging techniques [1]. Our combinatorial scheme allows multicolor imaging with an unprecedented number of colors at sub-diffraction limit resolutions, allowing the direct visualization of interactions among several proteins simultaneously within a cell at a much finer scale than previously possible.

References

1. M. Bates, B. Huang, G. T. Dempsey and X. Zhuang, Science ***317***, 1749 (2007)

2663-Plat Live cell Photoactivated Localization Microscopy (PALM) for the spatio-temporal mapping of single molecule diffusion in the plasma membrane

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Cell membranes are characteristically heterogeneous, both structurally and dynamically. However, the study of membrane protein organization and cooperative dynamics has been limited by a scarcity of experimental methods capable of accessing information on multiple proteins simultaneously, at the single-molecule level. We present a new imaging technique that promises to address this by measuring the dynamics of many individual proteins within dense ensembles. This technique, called single particle tracking photoactivated localization microscopy (sptPALM), has significant advantages over previously developed single molecule tracking, in that many sets of molecules can be activated, tracked, and bleached. As a result, molecularly dense regions can be probed, allowing a more representative set of molecules to be tracked than is possible with standard techniques. We explore the capabilities of this method by imaging the membrane proteins Gag and VSVG, each labeled with the photoswitchable Eos fluorophore. We obtain up to thousands of tracks per cell, several orders of magnitude more than enabled by traditional single particle tracking, and analyze each track to extract spatially resolved single-molecule diffusion coefficients over large regions of the cell membrane. The mean diffusion coefficients of mobile molecules are consistent with those measured using fluorescence recovery after photobleaching (FRAP). We identify immobile and mobile fractions of molecules, and examine their spatial organization. Intriguingly, these immobile molecules can be used to reveal and characterize the morphology of dynamically heterogeneous local environments in individual cells.

2664-Plat Imaging Single Transcription Factors with Two-Photon Fluorescence Microscopy in a Living Cell

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Our group recently demonstrated direct observation of protein expression, one molecule at a time (1), and the binding and unbinding of single molecules of transcription factor -YFP fusion proteins on DNA in a living bacterial cell (2), both with wide-field fluorescence microscopy. It is difficult to carry out similar experiments in a yeast or mammalian cell because of the larger cell volume in which strong autofluorescence out of the focal plane overwhelms

the single-molecule signal. While confocal fluorescence microscopy with one-photon excitation could be used, it causes photo-bleaching out of the focal plane. Two-photon fluorescence microscopy allows localized excitation only at the laser focus, significantly reducing photo-bleaching while providing 3D sectioning in living eukaryotic cells. We have demonstrated two-photon imaging of single transcription factors in living cells. We will report our study of transcription dynamics in yeast cells. In general, this opens up the possibility to study dynamics of protein-DNA interactions in living eukaryotic cells.

References

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2. J. Elf, G.-W. Li, and X. S. Xie, Science *316*, 1191 (2007)

2665-Plat Optical Lock-in Detection (OLID) Imaging Microscopy using synthetic and genetically-encoded optical switches

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One of the limitations on imaging of the distribution of fluorescent proteins inside live cells is that they are usually present in small numbers that need to be detected over a large quantity of fluorescent background. We have developed the means to separate the signal from these proteins from the background by modulating the fluorescence intensity of "optical switch probes," through deterministic optical control of their fluorescent and non-fluorescent states. Lock-in detection and fitting of the fluorescence signal on a pixel-by-pixel basis enables correlation with the optical stimulation and extraction of the modulated or "AC" component of signal, excluding other sources of fluorescence, even when they represent the bulk of the emission intensity. We use optical lock-in detection (OLID) microscopy within living cells in culture and for intravital imaging in the presence of significant and time-varying background to generate high-contrast images using two classes of optical switch probes, the synthetic switch nitrospirobenzopyran and the genetically-encoded optical switch Dronpa, which we fuse to β-actin, controlled by 1- and 2-photon excitation.

2666-Plat *In Vivo* multi-photon nanosurgery on cortical neurons

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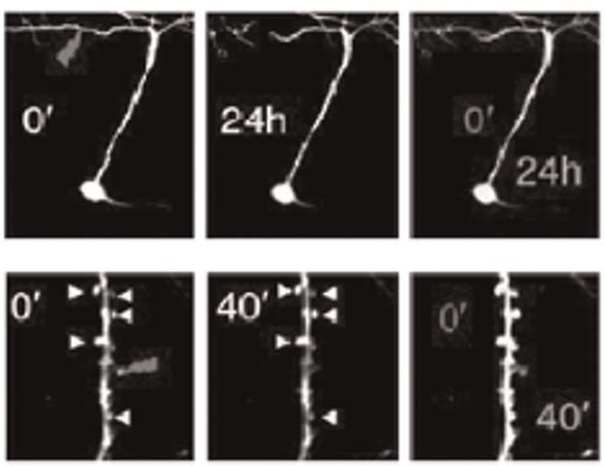
[2] *University of Verona, Verona, Italy.*

Two-photon microscopy has been used to perform high spatial resolution imaging of spine plasticity in the intact neocortex of living mice. Multi-photon absorption has also been used as a tool for the selective disruption of cellular structures in living cells and simple organisms. In this work we exploit the spatial localization of multi-photon excitation to perform selective lesions on the neuronal processes of cortical neurons in living mice expressing fluorescent proteins. Neurons were irradiated with a focused, controlled dose of femtosecond laser energy delivered through cranial optical windows. The morphological consequences were then characterized with time lapse 3D two-photon imaging over a period of minutes to days after the procedure. This methodology was applied to dissect single dendrites with sub-micrometric precision without causing any visible collateral damage to the surrounding neuronal structures. The spatial precision of this method was demonstrated by ablating individual dendritic spines, while sparing the adjacent spines and the structural integrity of the dendrite. The combination of multi-photon nanosurgery and *in vivo* imaging in mammals represents a promising tool for neurobiology and neuropharmacology research.



2667-Plat Imaging Mitochondria Dynamics In Motor Neurons In Vivo

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Neurons rely critically on microtubule-based transport to traffic nutrients and organelles to distant sites. Although defects in microtubule transport have been implicated in many neurodegenerative diseases, little is known about how these transport mechanisms function *in vivo*, particularly during development. New transgenic tools and imaging techniques are essential for studying the role of axonal transport *in vivo*.

Mitochondria are arguably the most important organelle transported along axons. They provide energy for the synaptic vesicle cycle and neurotransmitter release, and interference with mitochondrial function causes rapid neuronal death. In order to study the *in vivo* axonal transport of mitochondria, we developed new transgenic mice that express the photo-convertible fluorescent protein Kaede in the mitochondria of their motor neurons. We were able to specifi-

cally photo-convert mitochondria inside axons and neuromuscular junctions from green to red fluorescence *in vivo*. Importantly, the amount of UV light required for photo-conversion did not damage the tissue, the nerve or the mitochondria. Moreover, photo-converted mitochondria maintained their red fluorescence for remarkably long periods. These mitochondria remained in the junctions 48 hours following the initial photo-conversion. It was possible to photo-convert mitochondria in a small sub-region of a single neuromuscular junction from green to red. Time lapse imaging showed the turnover of mitochondria as some of the red mitochondria were replaced by new incoming green mitochondria two days post photo-conversion.

These experiments provide the first *in vivo* observations of the axonal transport and dynamics of a critical neuronal organelle and will be useful for studying how axonal transport may be distorted in mouse models of neurodegenerative diseases.

Platform BE: Peptide & Toxin Ion Channels

2668-Plat Electron Density Image of Alamethicin Pore: Constructed by X-Ray Anomalous Diffraction

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We report the first attempt to reconstruct the electron density of a peptide-induced transmebrane pore by x-ray diffraction. Lipidic structures can be long-ranged correlated into periodically ordered lattices that are amenable to diffraction analysis. We used a brominated lipid, di18:0(9,10Br)PC and the multiwavelength anomalous diffraction (MAD) method (BJ *91*, 736, 2006) in order to solve the phase problem. The same diffraction patterns were obtained from samples prepared in different peptide-lipid ratios. This implies that the pore size is determined by the energy of pore formation, not by the peptide-lipid ratios in the samples. We first extracted the diffraction intensities belonging to bromines alone. The Patterson function strongly indicates a barrel-stave model and rules out the toroidal model. Accordingly we modeled the distribution of bromines to obtain the phases, which were then used to construct the experimental electron density. Unlike protein crystals, lipid structures are described by the molecular distribution, rather than by atomic positions. This intrinsic feature corresponds to low resolution diffraction. Only the high values of the electron density are clearly revealed by such low resolution diffraction. The clearly visible regions include the distribution of phosphate groups of the lipids and the distribution of heavy atoms bromines that are bound to carbon 9 and 10 of each hydrocarbon chain. The headgroup and the chain distributions completely defined the lipid assembly. Unfortunately the peptide alamethicin does not possess high electron density; therefore their positions are not visible. Nevertheless the lipid assembly for the alamethicin pore is unmistakably that of a barrel-stave construction. The radius of the lumen is 17.8 A, which is consistent with a barrel-stave pore formed by 8 alamethicin peptides.

2669-Plat Characterizing Ion Channels with Membrane-Immobilized Polymers

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Nonelectrolyte polymers are commonly used to estimate the limiting aperture of ion channels. The pore diameter is deduced from the hydrodynamic radius of the critical polymer mass capable of inhibiting ion flux. The excessive polymer concentrations required for these measurements produce potentially detrimental solution and osmotic conditions which could perturb the ion channel structure. Here, we demonstrate how membrane immobilized polymers can be used as "molecular rulers" to probe channel features without requiring high concentrations of aqueous polymer.

2670-Plat Probing Single-molecule Ion Channel Dynamics By Combined Patch-clamp Single-molecule Imaging Microscopy

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The kinetic behavior of ion channel proteins is regulated by subtle conformational changes that are often difficult to characterize by conventional ensemble-averaged static structure analysis and by interpretations of ion-channel electrophysiological measurements. By combining real-time single-molecule fluorescence imaging measurements with real-time single-channel electric current measurements in artificail lipid bilayers and in living cell membranes, we were able to probe single ion-channel-protein conformational changes simultaneously, and thus providing an understanding the dynamics and mechanism of ion-channel proteins at the molecular level. Subtle structural dynamics of ion channels play an important role in regulating channel function and selectivity. This technical innovation has been used to gain an understanding of how ion-channel activities are regulated by the conformational change dynamics of the dye-labeled colicin channels. We were able to probe fluctuating polypeptide-domain diffusional motions of single-molecule colicin channels across a lipid bilayer. A new solvation-desolvation model for colicin ion-channel dynamics has been postulated based on our experimental results.

2671-Plat Imaging the mechanisms of Aβ42 peptide toxicity

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UCI, Irvine, CA, USA.

Converging evidence points to the oligomeric form of Aβ42-peptide as the primary toxic species in Alzheimer's disease via an uncontrolled elevation of intracellular free-Ca^{2+} [1]. However, many questions remain about the molecular mechanisms and sites of action by which Aβ42 exerts this toxicity. Here we use *Xenopus* oocytes as a model cell system together with advanced fluorescence