862-Pos Photocromic Intrinsic Fluorescent Proteins: a Raman Study of the Cromophore States

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Intrinsically fluorescent proteins (IFPs) of the green fluorescent protein family are extensively used in molecular and cellular biology as genetically encoded fluorescent markers for monitoring protein dynamics and interactions. Specific mutations make it possible to tailor the protein structure and consequently their chemical and photophysical properties[1–3]. Raman spectroscopy is a powerful method to investigate selectively conformational changes in active domains of these proteins. Indeed by exciting under pre-resonance conditions it is possible to measure the vibrational spectrum of the chromophore without the need of crystallization. This is extremely helpful to enable rational protein engineering. Moreover, Raman is a non-destructive technique that allows one to monitor on-the-flow the products of photoconversion.

We will discuss the use of this technique for studying photochromism in two cases: a blue variant with highly-stable states, and a green mutant whose photochromism is fully reversible with negligible loss of active protein. Theoretical and experimental results on chemically synthesized model chromophores under different protonation and/or isomerization states will be presented: a very good agreement with calculations based on time-dependent density functional theory will be shown. This will allow us to clarify the nature of the detected vibrational modes and to link the latter to the different ground state configurations. Based on this knowledge, we shall discuss the chromophore state when in the protein. These results allow us to discriminate between the effect of *cis-trans* isomerization and of different protonation of the chromophore in the photoproducts of these proteins.

References

References:[1]. R. Bizzarri *et al.*, *Biochemistry* ***46***, 5494 (2007).
[2]. D. Arosio *et al.*, *Biophys. J.*, ***93***, 232 (2007).
[3]. S. Habuchi *et al.*, *J. Am. Chem. Soc.* ***127***, 8977 (2005)

863-Pos Robust measurements of water using Wavelength Modulation Spectroscopy(WMS)

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Based on the Wavelength Modulation spectroscopy(WMS) and direct detection schemes, we developed a robust measurement scheme which can precisely detect water vapor concentration independent of the scattering due to the dust inside the observing humidity chamber. The relative water vapor concentration was measured by use of 938nm Distributed Feed-Back Diode Laser at different temperature using wavelength modulation technique. These relative water vapor concentration is calibrated to absolute water vapor concentration and we confirmed that the experimental results are consistent for those of different temperature. Our measurement system gives the same water concentration in any case of high or low scattering due to the non-water dust inside the observing humidity chamber.

864-Pos Vibrational Dynamics, Mode Coupling, and Anisotropy of Tyrosine Side Chains in Peptides

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Through noncovalent interactions, aromatic side chains of Phe, Trp and Tyr play an important role in many biophysical processes such as molecular recognition, and folding and stabilization of proteins. Detailed knowledge of ultrafast side chain dynamics and interactions will contribute to fundamental understandings of these biophysical and chemical processes. To this end, we have investigated vibrational energy relaxation, mode coupling, and anisotropy of the tyrosine side chains in tyrosine and tyrosine-containing peptides using femtosecond infrared transient grating and two-dimensional infrared (2D IR) spectroscopy. Two tyrosine ring modes (mostly C=C stretch motion localized on the phenol ring) around 1515 and 1615 cm^{-1} as well as amide-I and -II modes are examined. The lower frequency ring mode is used to measure the anisotropy of the phenol ring, which reflects overall rotational diffusion of the tyrosine side chain and fast orientational fluctuations, on a picosecond time scale. Vibrational mode coupling between two tyrosine ring modes is clearly seen in the 2D IR spectra. Intramolecular vibrational energy transfer among tyrosine ring modes and amide-I modes is also observed in the 2D IR spectra with different waiting times. Anisotropy, mode coupling and energy transfer measured for different tyrosine peptides are compared. Spectral diffusion of tyrosine ring modes is investigated in neat solvents and membranes to reveal the influence of local environments on side chain conformational fluctuations.

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Symposium 7: Translation and the Translocon

865-Symp Sec61-Mediated Membrane Protein Integration

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Aquaporin water channels comprise a conserved family of six-spanning homotetrameric membrane proteins that utilize at least two distinct pathways for ER membrane insertion. Protease protec-

tion and site-specific photocrosslinking studies have revealed that AQP4 TMs insert cotranslationally into and accumulate within the Sec61 translocon as the nascent polypeptide exits the ribosome. In contrast, two polar residues in AQP1 (Asn49 and Lys51) prevent TM2 from cotranslationally terminating translocation, and as a result, AQP1 acquires its proper transmembrane topology only after synthesis of TMs 5&6 is complete. We now show that Asn49 and Lys51 functionally interact with Asp185 at the C-terminus TM5 to form a nonlinear quaternary structural motif that influences multiple steps of the AQP folding pathway. Asn49 forms a critical intramolecular hydrogen bond with Asp185 that is required for proper helical packing, monomer formation and water channel function. In contrast, Lys51 forms an intermolecular ionic bond with Asp185 on an adjacent monomer that stabilizes the AQP1 tetramer. Although residues in this motif are highly divergent among AQP family members, they share a conserved quaternary architecture whose functional properties can be transferred en block to other family members. These findings suggest a general mechanism by which evolutionary divergence of closely related membrane proteins can confer new functional properties through alternate folding pathways that give rise to a common final structure. We propose that this sequence diversity contributes to the stability and selectivity of AQP homotetramerization in native tissues.

866-Symp Observing Protein Translocation in SecY and a SecY-Ribosome Complex Through Molecular Dynamics Simulations

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The protein-conducting channel, known as the SecY or Sec61 complex, is a unique membrane-bound protein, assisting both newly formed soluble proteins to cross the endoplasmatic reticulum (ER) membrane and membrane proteins to insert into the ER bilayer. These functions are often performed in tandem with the ribosome, which binds to the channel and feeds the protein in co-translationally. The resolution of a crystal structure of the core monomeric SecY complex from Methanococcus jannaschii four years ago has resulted in almost as many questions as it initially answered. For example, different structural elements such as a 'plug' and a 'pore ring' were both discovered, but their independent roles were still unclear. Additionally, the functional state of the channel, including its oligomeric arrangement, have become contentious issues. We have attempted to address many of these issues with molecular dynamics simulations of the channel, both alone and during translocation of small polypeptides. The flexibility and dynamic nature of both the plug and pore ring were observed, and recent results have clarified their individual roles in blocking the channel. Simulated opening of the lateral gate and subsequent relaxation indicated that the accessory protein SecE may not be very important in holding the gate closed. Simulations of a ribosome-channel complex have also been performed, illustrating in atomic detail how ribosome and channel work together. So far, all results have supported the idea that a SecY monomer forms the active channel.

Symposium 8: Imaging and Controlling Cellular Dynamics in vivo Using Light

867-Symp New Optical Tools For Controlling Neuronal Activity

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Correlating the activity of neurons with behaviour is of fundamental interest in neuroscience. Thus, acutely activating or inhibiting neurons in live and behaving animals is a key approach. With our collaborators G.Nagel, E.Bamberg (MPI Biophysics) and K.Deisseroth (Stanford), we established microbial-type rhodopsins as optical activators or inhibitors of neural function, both in rodent brains and live *Caenorhabditis elegans* nematodes. Channelrhodopsin-2 (ChR2) is a blue-light gated, algal cation-channel, while archaeal Halorhodopsin (NpHR) is a yellow-light driven Cl^--pump.

Depending on the promoters used, ChR2 facilitates rapid photodepolarization and thus activation of different types of *C. elegans* neurons. This allows triggering release of the neurotransmitters GABA or Acetylcholine at neuromuscular junctions, which is accompanied by complete relaxation of muscles (blocking locomotion), or muscle-hypercontraction, respectively. Combined with patch-clamp recording post-synaptic muscles, we now characterize mutations affecting neurotransmission.

Photoactivation of NpHR in cholinergic neurons caused an immediate arrest of locomotion. Since the action spectrum of NpHR is red-shifted relative to ChR2, combining both proteins in the same cell allows for bidirectional optical modulation of neural activity. Indeed, when co-expressed in cholinergic neurons, ChR2 and NpHR could be activated independently and concurrently by blue and yellow light, and NpHR could rapidly and reversibly counteract contractions observed during activation of ChR2 alone.

Towards a functional analysis of the 302 neurons in the *C. elegans* nervous system, we placed ChR2 in mechanosensory and proprioceptive neurons, command neurons that trigger backward locomotion, and dopaminergic cells that normally signal the presence of food. In all of these cells, photoactivation evoked characteristic behaviours.

In sum, ChR2 and NpHR enable repeated and reversible stimulation or inhibition of neurons in live animals with high temporal precision. These optogenetic tools will be instrumental to dissect the neural circuits controlling the numerous behaviours of *C. elegans*.

868-Symp Control of GPCR Pathways and Neuronal Circuits by Light

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A major challenge in understanding the relationship between neural activity and development, and ultimately behavior, is to control simultaneously the activity and intracellular signaling of either many neurons or various subcellular regions within individual neurons. Furthermore, changes of intracellular signaling pathways and changes of ion fluxes over cell membranes of any cell type are