

# Optical Spectroscopy of Biomolecular Dynamics

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After the conclusion of the Human Genome Project it has become clear that the next stage towards understanding the machinery of life is the identification of all its molecular components and assignment of function to each of them. Concurrently, the realization that the dynamics of biomolecules may be intimately tied to their function has gained some ground. Optical spectroscopic techniques play a pivotal part in the elucidation of this connection. Optical techniques are unique in their ability to probe biomolecules over a broad range of both temporal and spatial scales, with minimal perturbation to the function of the biomolecules. It is thus not surprising at all that recent years have seen a huge surge in the application of sophisticated optical spectroscopies, which were first developed by physicists and physical chemists, to biological problems.

Biomolecular dynamics cover a grand spectrum of timescales, from vibrational motion on the femtosecond domain to large-amplitude conformational changes on time periods as long as seconds. Optical spectroscopy can approach this whole range, by using a variety of methods. A few typical examples might be ultrafast pump-probe spectroscopy on the femtosecond timescale, time-resolved single-photon counting for the resolution of picosecond to nanosecond dynamics, fluorescence correlation spectroscopy on the microsecond time domain and single-molecule fluorescence spectroscopy on time windows ranging between milliseconds and seconds. Inter-

estingly, a very large range of the "optical" spectrum is covered by these and complementary techniques, from ultraviolet to far infrared.

Since the scientists practicing different types of optical spectroscopy come from many scientific disciplines, they rarely meet together to discuss the common ground of their methodologies. A symposium was recently held that assembled, in one room, scientists from the whole range of areas of optical spectroscopy and allowed discussion and exchange of ideas to fertilize possible cooperation and collaborations. This is a report on the "Minerva-Gentner Symposium on Optical Spectroscopy of Biomolecular Dynamics (OSBD)", which took place between 21st and 25th March 2004 in the Franconian cloister Banz near Nürnberg, Germany. It was primarily funded by the Minerva foundation of the Max-Planck Society, which promotes collaboration between German and Israeli Scientists. The meeting assembled around 100 participants, many from Germany and Israel, but more than one third of the participants came from other countries, most notably from North America and western Europe.

The six topical sessions of the meeting were organized mostly by biological subject rather than physical technique, and further discussions were held during two poster sessions. A particular emphasis of the program was on allowing researchers who study single molecules to compare their methodologies and results to those of researchers who study ensembles of molecules. In this short report, there is simply not enough space to discuss all the exciting science that was presented in the meeting. Therefore, we try to exemplify the richness of the symposium by focusing here on only a few of its topics. We apologize to those participants whose work we do not mention explicitly here.

## Fluorescent Proteins

Proteins with built-in chromophores that can emit light in the visible range of the spectrum, the group of so-called fluorescent proteins, have revolutionized cell biology.<sup>[1]</sup> In combination with novel imaging techniques, they are nowadays routinely used as protein labels, gene-expression markers, and reporters of cellular signals. It is essential to fully understand their intrinsic spectrochemical properties to judiciously interpret experimental microscopy data. Lectures in the meeting emphasized mainly the exciting and novel information that can be gleaned by looking at the photochemistry and photophysics of the fluorescent proteins. Silvia Völker (Leiden, The Netherlands) showed how hole-burning optical spectroscopy at very low temperatures can disentangle the complex electronic structure of such proteins by establishing a complete set of their vibrationless 0–0 transitions. Green fluorescent proteins in general turn out to exist in three different conformations, in two of which their chromophore is deprotonated. These conformations can interconvert in both the electronic ground and excited states.<sup>[2]</sup> The kinetics of the proton transfer in the excited state was at the center of a lecture given by Dan Huppert (Tel Aviv, Israel). Huppert performed time-resolved fluorescence measurements to elucidate, in particular, the mechanism of geminate recombination of the ejected protons with the remaining anionic chromophore following an initial optical excitation of the green fluorescent protein (GFP). He found that, rather than being localized on a specific side group, the proton randomly walks in the interior of the  $\beta$ -barrel protein, before finally "finding its way back home" to the chromophore.<sup>[3]</sup> Ulrich Nienhaus discussed properties of red emitting proteins that were recently proposed as novel fluorescence probes by

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his research group from the University of Ulm (Germany).<sup>[4]</sup> These oligomeric variants of GFPs with emission spectra distributed across the visible spectrum carry the great promise of further expanding the powerful toolbox of auto-fluorescent protein probes available to biologists. He particularly emphasized the utility of fluorescence correlation spectroscopy and single-molecule spectroscopy in looking at fast blinking dynamics of these proteins.

### Time-Resolved Infrared Spectroscopy

Because of its ability to probe specific groups within large molecules and to provide detailed information about structure and dynamics, infrared (IR) spectroscopy has traditionally held a strong position among the various optical techniques applied to biomolecules. Recent years have seen a dramatic surge in the application of time-resolved IR techniques, and some of this excitement was represented in lectures in the conference. Klaus Gerwert (Bochum, Germany) showed how time-resolved IR difference spectroscopy can be used to study intraprotein proton transfer via a hydrogen-bonded network of internal water molecules.<sup>[5]</sup> He also demonstrated how caged molecules can be used to initiate fast reactions in proteins; the reactions are then followed by time-resolved IR spectroscopy. Friedrich Siebert (Freiburg, Germany) focused his attention on rhodopsin, providing a detailed picture of conformational changes accompanying light-induced activation of the protein.<sup>[6]</sup> Thomas Elsaesser from the Max-Born-Institute (Berlin, Germany) pointed his femtosecond IR lasers at the very basic problem of hydrogen-bond dynamics, by employing simple dimers of acetic acid as model systems for hydrogen-bonded base pairs in nucleic acids.<sup>[7]</sup> Using nonlinear optical techniques, he was able to measure coherent vibrational motion along the hydrogen-bond coordinate, as well as to measure the lifetimes of the OH stretching and bending modes. A few years ago, Robin Hochstrasser pioneered 2D infrared spectroscopy, a sophisticated ultrafast nonlinear method which is the vibrational corollary of the

familiar 2D NMR spectroscopy, and in Banz he discussed new developments in the technique, striving to make it ever more powerful. Hochstrasser also showed how 2D IR methodology can yield novel information on vibrational dynamics and structure in short peptides.<sup>[8]</sup> In a combined effort, Peter Hamm (Zürich, Switzerland) and Josef Wachtveitl (Frankfurt, Germany) applied time-resolved IR spectroscopies on time-scales from femtoseconds to milliseconds to follow the structural equilibration of small cyclic peptides, in which an ultrafast conformational transition is initiated by optically triggering a *cis-trans* isomerization of an azobenzene group integrated into the peptide backbone.<sup>[9]</sup>

### Protein Folding Dynamics

The field of protein folding has been burgeoning in recent years, much due to the development of a series of new experimental techniques that have provided a wealth of novel information on the basic events occurring during the folding process. Indeed, the complex energy landscape of proteins requires probing with a spectrum of methods to obtain proper understanding of structure and dynamics. Elisha Haas of Bar-Ilan University (Ramat Gan, Israel) gave a broad overview of the application of time-resolved fluorescence resonance energy transfer (FRET) spectroscopy to characterize conformational distributions of proteins in folded and unfolded states. In particular, he showed how different parts of adenylate kinase organize on different timescales during folding.<sup>[10]</sup> The FRET methodology for elucidating folding dynamics becomes particularly useful when it is applied to individual proteins as shown by Ben Schuler (Potsdam, Germany). Schuler pointed out that the widths of FRET efficiency distributions of proteins at various stages of the folding process contain dynamic information pertaining to the reconfiguration time of the protein chain in the unfolded state.<sup>[11]</sup> Further information can be gleaned by immobilizing proteins and measuring long time trajectories as they fold and unfold. Two lecturers discussed the effect of pressure on the thermodynamics of folding. Joseph Friedrich from

the Technical University in Munich (Germany) elaborated on the fascinating elliptic form of the phase diagram of proteins in the temperature-pressure plane and showed how solvent interactions change with pressure, as reflected in the spectra of the chromophore of Zn-cytochrome c.<sup>[12]</sup> Roland Winter (Dortmund, Germany) showed that temperature-pressure studies, combined with a variety of spectroscopic methods, can be used to parameterize an empirical energy landscape for a folding protein.<sup>[13]</sup> He also discussed a sophisticated pressure-jump spectrometer and its application to folding.

### Fluorescence Correlation Spectroscopy (FCS)

FCS probes fluctuations in fluorescent signals as a means to measure diffusion coefficients of macromolecules, co-localize cellular components or study conformational dynamics. It has become the method of choice for studying equilibrium dynamics of biomolecules on the microsecond-to-millisecond timescale. The utility of FCS for measuring diffusion coefficients of biomolecules relies on careful examination of the various parameters involved in data fitting, mainly the geometry of the excitation volume probed. Indeed, Jörg Enderlein (Jülich, Germany) presented some detailed calculations and measurements that highlighted the limits of the technique and underscored pitfalls and artifacts inherent in its application.<sup>[14]</sup> Oleg Krichevsky (Beer Sheva, Israel) described the application of FCS to study monomer dynamics in large DNA molecules, using polymer theory to obtain detailed understanding of the motions probed.<sup>[15]</sup> Petra Schwille (Dresden, Germany) presented some new and clever multicolor versions of FCS including fluorescence cross-correlation spectroscopy which prove to be highly informative with regard to molecular interactions within live cells.<sup>[16]</sup>

The frontiers of optical spectroscopy have been pushed to previously unimaginable extremes: The ultimate sensitivity limit of individual molecules has finally been reached by methods that probe matter with light. Likewise, ultrafast lasers from the far-UV to the far-IR are

now able to “see” molecules as they vibrate and tumble in “real time”. Transient electronic and vibrational spectra recorded on time scales between milliseconds and femtoseconds can reliably be decomposed into kinetic schemes of utmost complexity using sophisticated numerical simulation techniques. It was exhilarating to experience how all these individual advances are nowadays being used to unravel ever finer details of biomolecular dynamics. Indeed, the enormous power and utility of optical spectroscopies (at the level of both ensembles and single molecules) to experimentally uncover this fascinating intricacy of molecular dynamics and biological function was collectively acknowledged by an overwhelming majority of participants. Since nearly everyone expressed their great interest in a regular forum for

“Optical Spectroscopy of Biomolecular Dynamics”, a committee was put together that will initiate the organization of a next meeting, OSBD II, to be held somewhere in Israel.

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- [1] *Methods in Enzymology*, Vol. 302, (Ed.: P. M. Conn), Academic Press, San Diego, 1999.
- [2] T. M. H. Creemers, A. J. Lock, V. Subramaniam, T. M. Jovin, S. Volker, *Proc. Natl. Acad. Sci. USA* **2000**, 97, 2974.
- [3] P. Leiderman, M. Ben-Ziv, L. Genosar, D. Huppert, K. M. Solntsev, L. M. Tolbert, *J. Phys. Chem. B* **2004**, 108, 8043.
- [4] J. Wiedenmann, A. Schenk, C. Rocker, A. Girod, K. D. Spindler, G. U. Nienhaus, *Proc. Natl. Acad. Sci. USA* **2002**, 99, 11 646.
- [5] A. Remy, K. Gerwert, *Nature Struct. Biol.* **2003**, 10, 637.
- [6] R. Vogel, F. Siebert, *Biopolymers* **2003**, 72, 133.
- [7] N. Huse, K. Heyne, J. Dreyer, E. T. Nibbering, T. Elsaesser, *Phys. Rev. Lett.* **2003**, 91, 197401.
- [8] I. V. Rubtsov, J. Wang, R. M. Hochstrasser, *Proc. Natl. Acad. Sci. USA* **2003**, 100, 5601.
- [9] J. Bredenbeck, J. Helbing, A. Sieg, T. Schrader, W. Zinth, C. Renner, R. Behrendt, L. Moroder, J. Wachtveitl, P. Hamm, *Proc. Natl. Acad. Sci. USA* **2003**, 100, 6452.
- [10] V. Ratner, E. Kahana, E. Haas, *J. Mol. Biol.* **2002**, 320, 1135.
- [11] B. Schuler, E. A. Lipman, W. A. Eaton, *Nature* **2002**, 419, 743.
- [12] H. Lesch, J. Schlichter, J. Friedrich, J. M. Vanderkooi, *Biophys. J.* **2004**, 86, 467.
- [13] R. Ravindra, R. Winter, *ChemPhysChem* **2003**, 4, 359.
- [14] J. Enderlein, I. Gregor, D. Patra, J. Fitter, *Curr. Pharm. Biotechnol.* **2004**, 5, 155.
- [15] R. Shusterman, S. Alon, T. Gavriyov, O. Krichinsky, *Phys. Rev. Lett.* **2004**, 92, 048303.
- [16] K. Bacia, P. Schwille, *Methods* **2003**, 29, 74.

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