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Optical Broadband Spectroscopy with Femtosecond Time Resolution

When a dye molecule absorbs a photon from the visible or UV spectral region, then its structure and properties are changed almost instantaneously. The microscopic surrounding (for instance a biopolymer) is thus perturbed and relaxes towards a new equilibrium. The relaxation, which takes about 10^{-12} s for the systems studied, causes a shift of the fluorescence spectrum over time. By monitoring the fluorescence shift, we measure the ultrafast motion of the surrounding and thereby hope to gain insight into the functioning of the biopolymer.

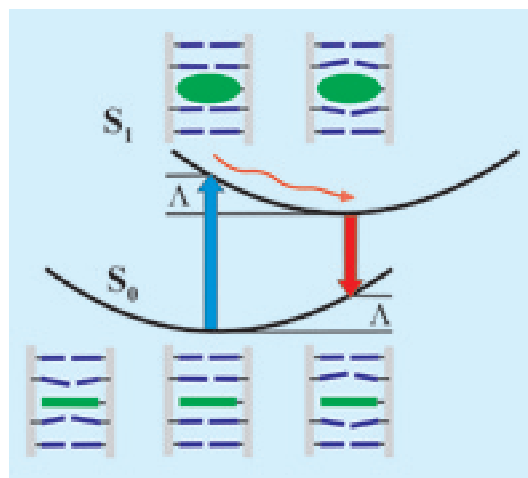


Fig. 1
To illustrate basic concepts, a dye molecule (green) is placed inside duplex DNA. The chromophore is assumed to be flat in the electronic ground state S_0. Thermal deviations of the biopolymer from the optimal geometry correspond to higher potential energy (parabolic curve for S_0). By absorption of a UV/vis photon (blue arrow) the shape of the chromophore is suddenly changed to ellipsoidal. Structural relaxation of the surrounding (wavy line) takes place on a picosecond (10^{-12} s) time scale. Following this, fluorescence (downward arrow) occurs mainly from the excited-state minimum over nanoseconds (10^{-9} s). The fluorescence spectrum is separated from the absorption by twice the structural reorganisation energy Λ.

Color characteristics of dyes reflect the microscopic environment

The green color of leaves, the turquoise of tropical saltwater lagoons, the brilliant yellow of dandelion petals – they are beautiful signs that sunlight is being harnessed for biological purpose. At the microscopic level, molecules with conjugated π-electron systems (the »chromophores«) absorb light, whereby an electron is promoted and a new electronic state is created. The properties (structure, charge distribution) of the chromophore and the arrangement of the surrounding are usually different in the electronic ground state S_0 and in the excited state S_1.

This is shown schematically in Fig. 1 where the chromophore, for example, is a special nucleobase surrogate inside duplex DNA strands [1, 2] and we focus on its molecular shape.

The chromophore is assumed to be essentially flat before light absorption, *i.e.* in S_0, and stacks nicely into its site in the biopolymer. During thermal motion the latter will of course also attain structures where the long axis of the chromophore is slightly squeezed while adjacent members of the stack are placed at larger distance. In this case the chromophore does not fit as well and the system (chromophore in S_0 + biopolymer) has higher energy. Therefore, when the potential energy of the system is drawn against a coordinate which measures the »shape« of the cavity provided by the surrounding, then one obtains essentially a parabola with minimum for flat shape. – Now consider the electronically excited state S_1 and assume that in this case the chromophore has an ellipsoidal form. The absorption of a photon (upward arrow in the figure) abruptly alters the shape of the chromophore. But the biopolymer can not follow immediately and still retains the structure which was best for the ground state, *i.e.* the site is still flat. The ellipsoidal chromophore does not fit well now, which means that the energy of the system is relatively high and forces are exerted on the surrounding. Consequently the outer structure reorganizes and the excess energy dissipates. Thus the excited system relaxes until the optimal shape for the site is attained. For most systems studied by us, structural relaxation takes only a few picoseconds (ps = 10^{-12} s). – Finally we discuss fluorescence which may lasts up to 10 nanoseconds (ns = 10^{-9} s) and therefore occurs mainly from the relaxed excited state, *i.e.* from the minimum of the S_1 energy curve. Fluorescence corresponds to the spontaneous emission of a photon while the chromophore drops back (downward arrow) to the ground state S_0. In this way the flat form of the chromophore is recreated quite suddenly (if we ignore internal processes) but in the extended cavity of the relaxed S_1 state and therefore with excess energy again. It is easily seen that the photon energy (arrow length) is lower for fluorescence, compared with absorption, by twice the structural reorganisation energy Λ. The energy difference between absorption and emission is called »Stokes shift« [3].

It is clear by now that one can study the microscopic surrounding by measuring the absorption- and emission spectra of a chromophore. The strength of the

Ultrakurzzeitspektroskopie

Wenn ein Farbstoffmolekül ein Photon aus dem sichtbaren oder UV-Spektralbereich absorbiert, so werden seine Struktur und Eigenschaften plötzlich geändert. Dadurch wird die mikroskopische Umgebung (z.B. ein Biopolymer) gestört, und sie relaxiert für die betrachteten Systeme innerhalb 10^{-12} s in ein neues Gleichgewicht. Dieser Prozess wird über die Verschiebung der Fluoreszenz des Farbstoffs zeitlich verfolgt. So kann man die Bewegung der Umgebung messen und erhält z.B. Einsicht in die Funktion eines Biopolymers.

interaction is reflected by the spectral positions of absorption and fluorescence or by the Stokes shift. This is shown schematically in Fig. 2. The upper panel treats the isolated chromophore, as in the gas phase: its absorption and fluorescence spectra are highly structured because of an intramolecular stretching vibration and there is no Stokes shift because no energy is required to push the (nonexistent) surrounding away. For the middle and lower panels, the rigidity of the biopolymer (parabolic curvatures) or the reorganization energy Λ has been increased: the absorption spectrum shifts to the blue and the fluorescence to the red while the vibrational structure becomes blurred.

Artificial chromophores are commonly called »dyes«, and special dyes have been synthesized for a great variety of biological and biochemical diagnostics. Their optical spectra are typically measured under steady-state conditions for the excited state, *i.e.* with continuous illumination and without time resolution. In this case one can distinguish different environments, for example inside *vs.* outside the DNA duplex, or bound *vs.* unbound states of external ligands which are labelled with fluorescent markers.

Femtosecond color changes report microscopic environmental motion

In contrast to the stationary view formulated above, we concentrate on the initial picosecond or so after optical excitation of a chromophore. Look again at Fig.1 but now consider the process in the excited state which is indicated by a wavy red arrow. During this process the near environment changes from the equilibrium structure in S_0, *i.e.* before optical excitation, to the equilibrium structure in S_1. How fast can the surrounding be accelerated initially, which characteristic oscillations appear on the way, which exponential relaxation times are characteristic later on? If these questions are answered by experiments, then one can build a dynamic model which is consistent with the experimental results, and thus we obtain insight into the motion – or molecular mechanics – of the biopolymer. In essence this is the program which the *Femtosecond Spectroscopy Group* is presently advancing.

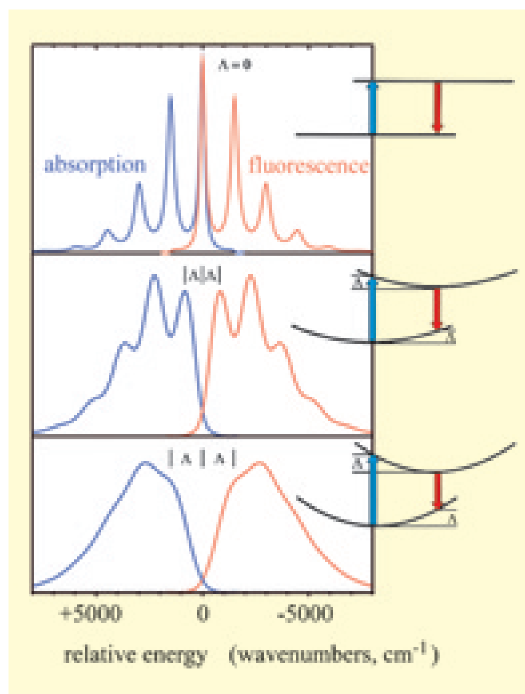


Fig.. 2
UV/vis absorption (blue) and fluorescence (red) spectra are simulated for a chromophore in several environments. The chromophore has an internal vibration which is »optically active«, i.e. *shows up as a series of vibrational bands in the electronic spectra.*
a) In the gas phase, emission occurs from the lowest vibrational level in the excited state and the same level is also seen by absorption. This is why the two spectra overlap and there is no Stokes shift.
b) In the condensed phase, absorption of a photon creates the excited chromophore in an environment which is not yet adapted to the excited-state properties. Therefore the excited system has higher energy (by Λ) as compared to the situation when the chromophore is excited in the gas phase. Similar arguments apply to fluorescence which has lower energy compared with the gas phase. The spectral shape is determined by the structural difference between ground- and excited-state environments together with the ultrafast dynamics of structural relaxation.
c) as in b) but with larger reorganisation energy Λ, which can be obtained from the Stokes shift = 2Λ.

As example consider the dye amino-nitro-fluorene (see Fig. 3) which is chemically linked into duplex DNA where it occupies an abasic site. Upon optical excitation $S_0 \rightarrow S_1$ this dye does not change its shape much (as was argued in the preceeding section just for the sake of illustration) but instead its charge distribution is suddenly altered. The adjacent nucleobases and the DNA backbone respond by ultrafast structural change.

The relaxation is followed in real time by monitoring the Stokes-shift of fluorescence with the so-called pump-probe method. An optical pump pulse at 400 nm with 30 fs duration excites the dye at time t=0. Then one waits for a delay t, by which time a white probe pulse (also lasting only 30 fs) is sent through the excited sample region. The transmission of this light is

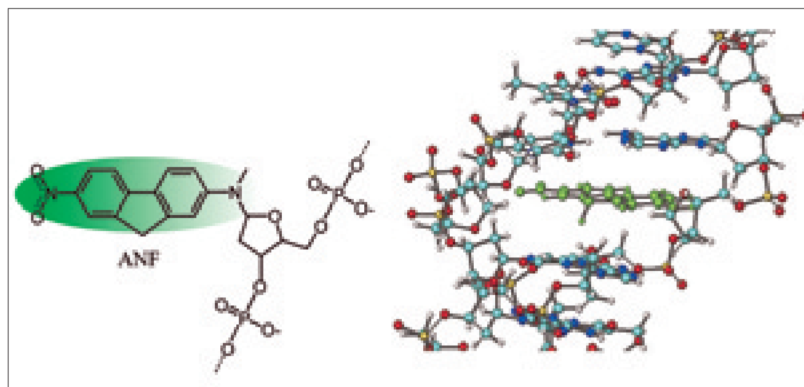
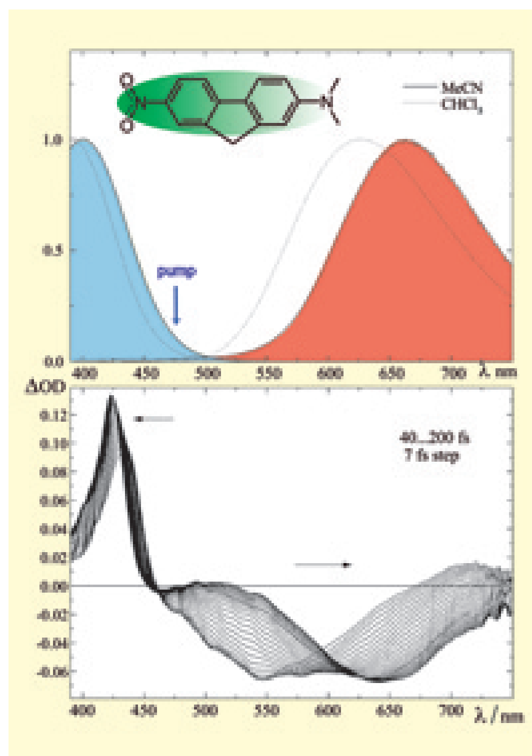


Fig. 3
The chromophore 7-Amino-2-nitro-fluorene (ANF, marked green) is sensitive to the polarity of the environment, i.e. its absorption and emission spectrum depend on the distribution of partial charges around it and thus on the surrounding structure. The molecule fits well into duplex DNA (right) where it is linked into an abasic site. The best linkage is found by molecular modelling. Synthesis and linkage are performed by organic chemists of the department.

Fig. 4
Transient absorption spectra of 7-Amino-2-nitro-fluorene in acetonitrile (lower panel). Spectra are shown every 7 fs. Negative optical density ΔOD correspond to bleach and stimulated emission which is strictly related to fluorescence. Therefore the fluorescence spectrum is seen to shift to lower energy within 200 fs. Positive ΔOD indicates excited-state absorption $S_1 \rightarrow S_n$.



converted into a transient absorption spectrum spectrum at time t. Every few fs such a transient absorption spectrum is recorded and a »spectral movie« is made from the results. Such a movie is shown (at least partially) in Fig. 4 where the induced optical density ΔOD is plotted against wavelength in the lower panel [4].

Here the chromophore is examined in solution of liquid acetonitrile because the motion of this liquid is well understood. The upper panel of Fig. 4 shows the stationary absorption and emission spectra in acetonitrile. The emission is shifted far from the absorption indicating a reorganisation energy of $\Lambda \approx 5000$ cm^{-1}. In the lower panel we see negative optical density in this region. In fact the negative-going part of the transient absorption spectrum may largely be identified with the fluorescence spectrum at the corresponding delay time. Altogether we see that the fluorescence band shifts to the red within the very short time of 200 fs! Just to illustrate: during this time interval, light propagates only 60 µm equivalent to the thickness of a single hair, and acetonitrile motion is again in equilibrium after such a short time. The spectral evolution is seen even better in the false-color representation of Fig. 5 where dark blue indicates ΔOD < 0. Time ranges to 1 ps from left to right and the photon energy (in wavenumbers / cm) from bottom to top. The white line presents the peak of the instantaneous fluorescence band evolving in time. Its form completely reflects the (known) librational motion of acetonitrile solvent molecules. We conclude that a large portion of the total reorganisation energy is due to dielectric interaction with the environment, and that the dynamics of the environment may indeed be measured in this way.

The experiment and results described above may serve as an introduction to the molecular interests, the experimental approach, and results of the *Femtosecond Spectroscopy Group*. Other studies concern charge transfer [5] (behind the green color of leaves), proton transfer [6] (which helps dandelion petals to their brilliant yellow) or internal conversion [7] (on which sun blockers rely).

Measurement of optical spectra with femtosecond resolution has many uses

To follow molecular band shapes, obviously entire optical spectra must be recorded at a time, and this requires methodological development. As a result, the group is presently leading in femtosecond technology which measures simultaneously at all relevant UV/vis wavelengths. Because dyes and optical detection are so prominent in biomolecular diagnostics, there is a tremendous need to understand how a given chromo-

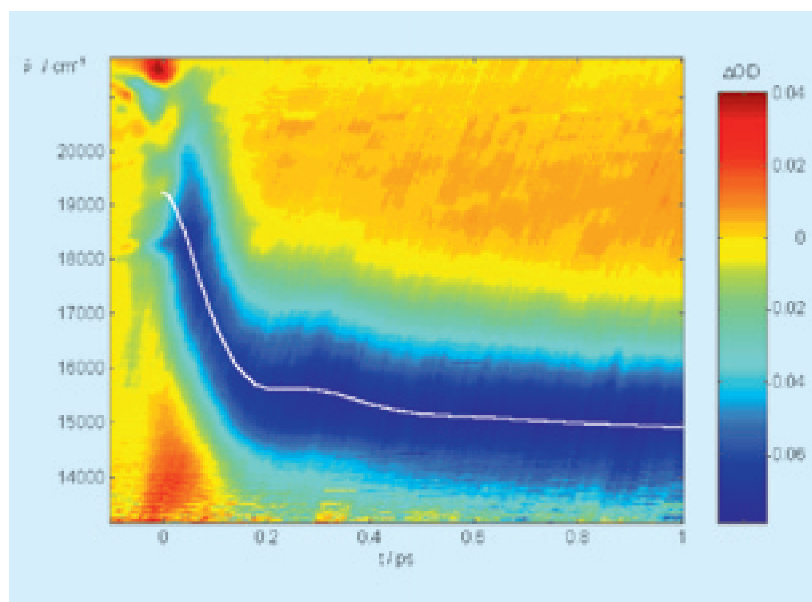


Fig. 5
Transient absorption spectra of Fig. 4 in a color-coded representation where dark blue indicates ΔOD < 0 or stimulated emission. Time ranges to 1 ps from left to right. The white line presents the peak of the stimulated emission band evolving in time. Its form accurately reflects the librational motion of acetonitrile solvent molecules.

phore interacts with its biophysical environment. This is why time-resolved absorption [8] and, most promising, femtosecond time resolved fluorescence [9] are much in demand. Fig. 6 shows our setup for transient absorption with supercontinuum (= white light) probing. Typically the optical properties of a new design are calculated just as with microscopes or astronomical telescopes, the elements are developed in cooperation with optical workshops, and a prototype device is built in the department. Extensive use with our own photochemistry results in further improvements, after which the device is available to laboratories around the world.

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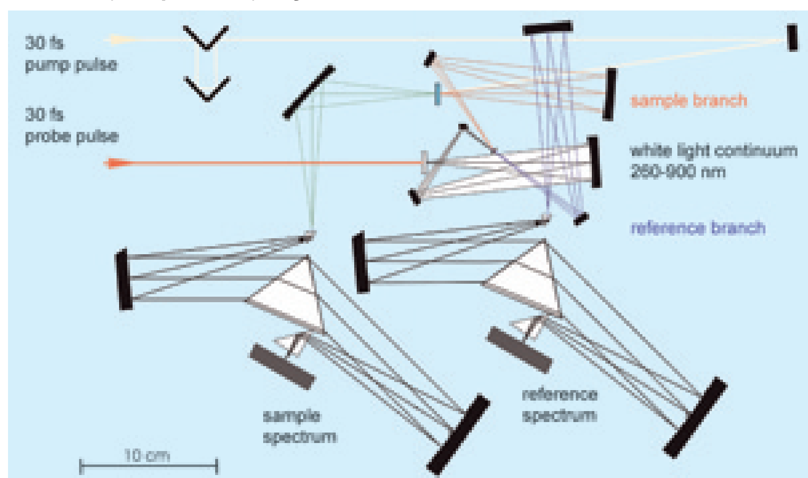
Fig. 6
Experimental setup for femtosecond transient absorption with supercontinuum (= white light) probing. Two spectrographs register the transmission of the 30 fs supercontinuum pulse through the sample (blue cell) which has been excited by a 30 fs pump pulse (along yellow beam). White light is generated by focussing a short pulse (along red beam) into a CaF_2 crystal. The timing between pump and probe pulses is controlled by an optical delay stage.



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Born 1950. He studied chemistry in Münster and theoretical chemistry in Dundee, U.K. (with Prof. A.D. Walsh) and in Bonn (with Prof. S. D. Peyerimhoff) where he graduated in 1976. He received his PhD in 1979 from the Heriot-Watt University, Edinburgh, for work on molecular spectroscopy of C-nitroso compounds (under the guidance of Prof. J. Pfab). Then followed two years of postdoctoral work on NO_2 laser spectroscopy with Prof. J.C.D. Brand at the Univerity of Western Ontario, Canada. In 1981 he became scientific coworker of Prof. F.P. Schäfer in the department of laser physics at the MPI for biophysical chemistry in Göttingen. Habilitation 1991 in physical chemistry at Göttingen University, and since 1993 chair of physical and theoretical chemistry at the Humboldt-Universität. Recipient of the Leibniz-prize 1998 of the Deutsche Forschungsgemeinschaft (together with Klaus Rademann).

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