tionation approach. Sinha and colleagues[5] established that the β-secretase is a membrane-associated aspartyl protease with an optimal pH of 5.5. They then observed, like the other groups[2,4], that this presumably aspartyl-like activity is unusual in that it is not inhibited by pepstatin, a typical aspartyl-protease inhibitor. But to purify BACE, Sinha and colleagues needed a molecular hook. They designed several variants of the APP sequence, spanning the β-site, including so-called transition-state analogues. Such analogues 'freeze' a bound protease in the act of cleaving the substrate. Sinha *et al.* then tested their analogues against crude preparations of human brain containing abundant BACE activity. They found that one amino-acid substitution, from aspartate to valine with a statine analogue, at position +1 (that is, on the carboxy-terminal side of the cleavage site) resulted in a potent inhibitor with a half-maximum inhibition at 30 nM. Using this molecular hook, Sinha and colleagues pulled out their candidate protease from human brain extracts, with a 300,000-fold enrichment.

Finally Hussain and colleagues[3], like Yan *et al.*, named their β-secretase Asp2 — suggesting they have more than one candidate protease. However, Hussain *et al.* did not reveal in their paper how they obtained their cDNA clone from their proprietary expressed sequence tag (EST) database. They did show, however, that point mutations in Asp2 (or BACE) at both of its two active sites (the aspartic acid–serine/threonine–glycine sequence) mean that it can no longer process APP to Aβ.

Ever since it became clear that proteases chop APP down to Aβ, these proteases have been prime targets for drug discovery. In the absence of molecular targets, cellular reporter systems have been used to develop compounds that reduce the amount of Aβ produced, and we may soon see the first clinical trials of these drugs. But the isolation of BACE means we can now screen for drugs that act directly on the target protease. Future structural information from X-ray diffraction studies of BACE with a bound inhibitor might give valuable insights into the design of new structural classes of inhibitors.

Several challenges remain, however. The BACE and its homologue BACE-2 belong to a new class of membrane-bound aspartyl proteases. Are there other BACE homologues? And, if so, will these have to be considered for selectivity screens in drug-optimization studies? We also do not know which other precursor molecules or cellular processes depend on proper BACE activity. Transgenic mice with these genes knocked out, either conditionally or totally, will be very useful for resolving such questions. Another problem is the subcellular location of BACE, in the lumen of the Golgi body and endosomes (Fig. 1). This means that inhibitors will have to cross at least two lipid bilayers — a formidable penetration hurdle for even small-molecular-weight compounds. Moreover, any BACE inhibitor has to pass the blood–brain barrier to find its target in neurons. New compounds will therefore need to have excellent pharmacokinetic properties.

Despite all of this, the identification of the β-secretase means that the path towards specific inhibitors is now set, and it is time not only to test the amyloid hypothesis ('*in vivo veritas*'), but to find a way of halting this dreadful disease. ■

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Chemical physics

Ultrafast relaxation in water

Abraham Nitzan

When a molecule is excited, where and how fast does its excess energy go? The answer to this question is a prerequisite for understanding and predicting the course of many chemical and biological processes. Chemical reactions can take place only when enough internal energy has accumulated in the molecule. The study of chemical reactions is therefore intimately connected with the study of energy-relaxation processes that compete with the chemical reaction channel. Intermolecular excitation transfer is one such process that has been studied for more than half a century. A paper by Woutersen and Bakker on page 507 of this issue[1] may force us to re-examine some of our notions about this important relaxation pathway.

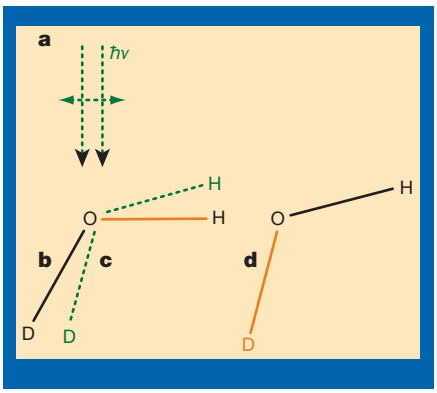

Figure 1 **Intermolecular energy transfer. Woutersen and Bakker[1] measure energy transfer in pure H_2O and in mixtures of HDO dissolved in D_2O using two infrared pulses. A pump pulse, polarized as shown in** a**, excites the OH bond of the HDO molecule,** b**. The induced rotational anisotropy can relax either by rotation of this molecule to configuration** c**, or by energy transfer to another molecule,** d**. Averaging over all final orientations amounts to loss of anisotropy, which is monitored by the probe pulse (not shown).**

When an isolated atom is optically excited it can relax to the ground state only by emitting radiation. In large molecular systems many degrees of freedom compete for the excitation energy and the winner rarely takes all. After a molecule is excited in solution, much of its energy usually ends up as increased solvent thermal motion, and can be regarded as wasted. Chemical interest often lies in other relaxation channels, for example electron transfer or chemical bond breaking. But even pathways that eventually lead to wasted thermal energy, such as intermolecular excitation transfer, can be of great interest at intermediate timescales. Knowing how energy flows between different molecular modes *en route* to complete relaxation can suggest ways to direct it to more useful channels, similar to harnessing the water flow in a river for useful work.

Intermolecular excitation energy transfer is the process by which one excited molecule, a donor, transfers its excess energy to another, an acceptor, leaving the latter in an excited state. This process continues until terminated by photon emission, chemical reaction or thermal relaxation. The intermolecular excitation pathway can be desirable or not, depending on your objective: it may obstruct an attempt to bring a molecule into higher excited states and it will destroy coherence that may otherwise help control a photochemical reaction. On the other hand it can provide ways to 'conduct' energy to where it is needed; an example is the use of sensitizers in photographic films in order to activate photoreactions in species that do not absorb natural light. Nature has also learned how to use these processes, for example in the light-harvesting complexes of photosynthetic systems[2].

The theory of such energy-transfer processes goes back to the well-known works of Förster[3] and Dexter[4]. The simplest Förster transfer mechanism is similar to the interaction between two electric dipoles. The rate of energy transfer, k, is described by $k = T^{-1}(r_o/r)^6$, where T is the lifetime of the excited state, r is the distance between the donor and acceptor, and r_o is a parameter called the Förster radius. This equation tells us that the rate of dipolar energy transfer behaves like r^{-6}. With increasing r, higher-order interactions (such as dipole–quadrupole, quadrupole–quadrupole and exchange interactions) decay much more rapidly than the dipole–dipole interaction, and are effective only at very small intermolecular distances.

How important is this mode of energy flow? It is significant only when its rate is comparable to or faster than other relaxation processes. The most important competing processes are intramolecular vibrational relaxation, where vibrationally excited molecules relax by transfer of energy within the molecule itself, and vibrational energy relaxation, where vibrational energy is transformed into solvent thermal energy. These processes are fast; relaxation of polyatomic molecules in condensed phases at room temperature occurs over a few picoseconds or less.

In contrast, vibrational energy transfer between molecules is generally believed to be too slow to be important. It is only expected to play a significant role for diatomic molecules or at cryogenic temperatures, where vibrational relaxation is relatively slow. Indeed, these are the conditions under which such processes have been observed in the past[5,6]. Contrary to such expectations, the experiment by Woutersen and Bakker[1] shows that resonant intermolecular energy transfer between OH bonds in liquid water is extremely fast. Moreover, it appears to be much faster than the vibrational energy relaxation of the OH group, which has recently been shown to have a short lifetime of 740 fs (femtoseconds)[7].

In their experiment, Woutersen and Bakker use two 200-fs infrared pulses: one relatively strong, linearly polarized 'pump' pulse to excite the OH groups and another, low-intensity pulse to probe this excitation. They use thin-layer samples of either pure water (liquid H_2O), or a mixture of HDO and D_2O (D is deuterium, a heavy isotope of hydrogen). The mixed samples make it possible to measure the dependence of the energy transfer rate on the OH concentration. Woutersen and Bakker measure the rotational anisotropy of the molecules as a function of the time delay between pump and probe. Rotational anisotropy is induced by the pump pulse, which excites molecules with specific orientations. This vibrational excitation can then relax either by rotational motion of the excited molecules or by energy transfer between molecules of different orientations (Fig. 1).

These two relaxation modes can be distinguished from each other for the low OH mixtures (that is, HDO dissolved in D_2O): energy transfer between HDO molecules depends on the concentration of this species, whereas their rotation does not. Therefore measuring the rotational anisotropy at different delay times as a function of HDO concentration yields the characteristic time for molecular rotation (four picoseconds), and more importantly the rate of intermolecular vibrational energy transfer between the OH groups. These results show that the Förster theory accounts well for the observed intermolecular vibrational energy transfer in HDO–D_2O mixtures and that the corresponding transfer rate is quite fast — in the range of a few picoseconds for molar concentrations of OH. With Woutersen and Bakker's technique, the transfer rate is measurable even though the competing processes of energy relaxation are very fast.

The real surprise comes from similar measurements in pure H_2O. Using the Förster results from mixtures of HDO in D_2O ($r_o = 2.1$ Å) to extrapolate to the intermolecular distance in pure water (2.8 Å) predicts an energy-transfer time in the range of a few hundred femtoseconds. But the observed intermolecular energy transfer in pure water takes place even faster than the experimental time resolution of $\sim$100 fs. This is considerably faster than the 740-fs lifetime of the excited OH population, and makes intermolecular vibrational energy transfer one of the fastest relaxation processes ever recorded in water. This means that vibrational energy cannot be localized in water long enough to affect most chemical reactions. On the other hand it implies that water is an extremely good conductor of vibrational energy through its OH groups. It is even possible that this energy-transfer process could involve other molecules containing OH groups, so water may play an important role in protein dynamics when energy is transported between different molecules.

The failure of the dipolar Förster theory in H_2O is not unexpected because the OH groups are so close to each other that higher-order interactions come into play. But the observation that intermolecular vibrational energy transfer in water is so amazingly fast calls for a reassessment of vibrational energy transfer and relaxation in condensed phases. Many questions remain. For example, what is the actual rate of vibrational energy transfer in water? Is this behaviour peculiar to this liquid (perhaps it is associated with its special structural properties)? Previous studies from the same laboratory[7] have suggested that the fast vibrational energy relaxation of the OH bond in water is possibly associated

100 YEARS AGO

Two or three months ago reports were published in the daily press of the discovery of an electrical method of giving sight to the blind. It was alleged that Mr. Stiens had succeeded in constructing an electrical apparatus which performed all the functions of the eye and was an efficient substitute for it. Like many other newspaper reports of so-called scientific discoveries, this has proved to be without sound foundation. Mr. G. H. Robertson, who is himself afflicted with blindness, describes in the *Electrician* the results of personal inquiries into the matter with a member of the staff of our contemporary. In spite of several visits to Mr. Stiens, no experimental proof in substantiation of the claims which were put forward on his behalf was obtained, and the conclusion arrived at is that these claims are foundless. Life is so short and crowded with so many important duties that it is impossible to investigate the many sensational statements made by irresponsible interviewers, but we are grateful to any one who will take the trouble to examine some of the rumours which are put forward in the name of science.

From *Nature* 30 November 1899.

50 YEARS AGO

In British Astronomical Association Circular No. 312 some details are given regarding the two newly discovered satellites of Uranus and Neptune, respectively. Both were discovered by Gerard P. Kuiper during his search for new satellites with the 82-in. reflector of the McDonald Observatory, University of Texas. The new satellite of Uranus, now named Miranda, was discovered on February 16, 1948, magnitude 17, and is now known to have a period of about 33h. 56m. The motion is approximately circular and in the plane of the other four satellites. Neptune ii, for which the name Nereid has been proposed by the discoverer, was found on May 1, 1949, on plates exposed for forty minutes at the prime focus, with the mirror stopped down to sixty-six inches (*f*/5). Its magnitude was estimated to be 19.5, and later observations show that its period is about two years and that the plane of its orbit is within six degrees of the ecliptic. Kuiper says that, as Neptune could retain satellites nearly ten times as far away as Nereid, with periods up to about fifty years, further work is planned to cover the outer regions of the system.

From *Nature* 3 December 1949.

with its coupling to the nearest hydrogen bond. Is there a link between that and the present observations? If not, what is the origin of this extremely fast energy-transfer process? The need to answer these questions is our next challenge. ■

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Neurobiology

Derailed axons get on track

Kai Zinn and Aloisia Schmid

How do growing axons in the central nervous system navigate through the dense jungle of cells and processes that they encounter on the way to their targets? On page 540 of this issue, Bonkowsky *et al.*[1] show that the choice of pathways for growth cones (the leading edges of growing axons) in the fruit fly *Drosophila melanogaster* is regulated by a receptor tyrosine kinase known as Derailed (Drl). These results further indicate that, rather than attracting growth cones to the right pathways, Drl causes them to be repelled from the wrong ones.

The array of axons in the embryonic *Drosophila* central nervous system has a ladder-like structure (Fig. 1). Anterior and posterior commissural tracts cross the midline in each body segment, and two longitudinal tracts extend the length of the embryo. Each of the roughly 300 neurons within a unit of this structure is thought to extend its axon along a genetically determined pathway. Most interneurons (neurons that synapse with other neurons) extend axons across the midline of the central nervous system, and attractive and repulsive factors that regulate this fundamental crossing decision have been identified[2,3]. But axon guidance at the midline involves more than just the decision whether or not to cross. Each commissure contains many distinct pathways, and the growth cone of a particular neuron always follows the same one. The unique sequence of navigational decisions made by its growth cone determines the complex and invariant shape of each axon in the central nervous system[4].

Although we are far from understanding how complete axon trajectories (such as those in Fig. 1) are determined, the results of Bonkowsky *et al.*[1] are an important step forward. These authors define how axons choose between the two major subdivisions of the crossing pathways — the anterior and posterior commissures. They show that Drl is normally expressed on axons that follow the anterior commissure, but not on those that take the posterior route[1,5]. Moreover, forced expression of Drl on specific axons that normally take the posterior commissure causes them to choose the anterior tract instead (Fig. 2).

Can Drl redirect any crossing axon into the anterior commissure? To address this question, Bonkowsky *et al.* simultaneously misexpressed the Commissureless (Comm) protein and Drl on a set of axons that normally never cross the midline (the thoracic Ap axons). Expression of Comm in neurons downregulates a repulsive signal from the midline that is transduced by the Roundabout (Robo) receptor. So, non-crossing axons that express Comm are diverted into pathways that cross the midline[6]. The authors found that when the thoracic Ap axons expressed only Comm, they crossed in the posterior commissure. But when they

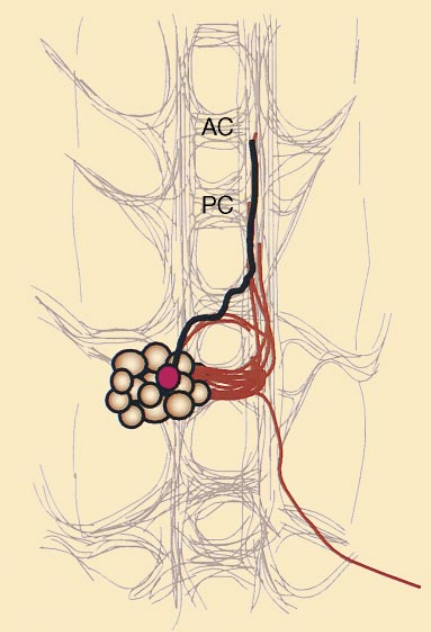

Figure 1 **Individual axons follow complex trajectories within the central nervous system (CNS). The axon scaffold of the fly CNS is drawn in grey. A specific neural lineage (from neuroblast 5–2) is indicated in brown; the lineage includes neurons that project in both the anterior commissure (AC) and the posterior commissure (PC). One AC neuron (red) — presumably a cell that expresses Drl — extends its axon (black) along the posterior edge of the AC. At the midline the axon veers anteriorly, grows along a bundle at the anterior edge of the AC, then turns into the longitudinal tract. (Modified from ref. 4.)**

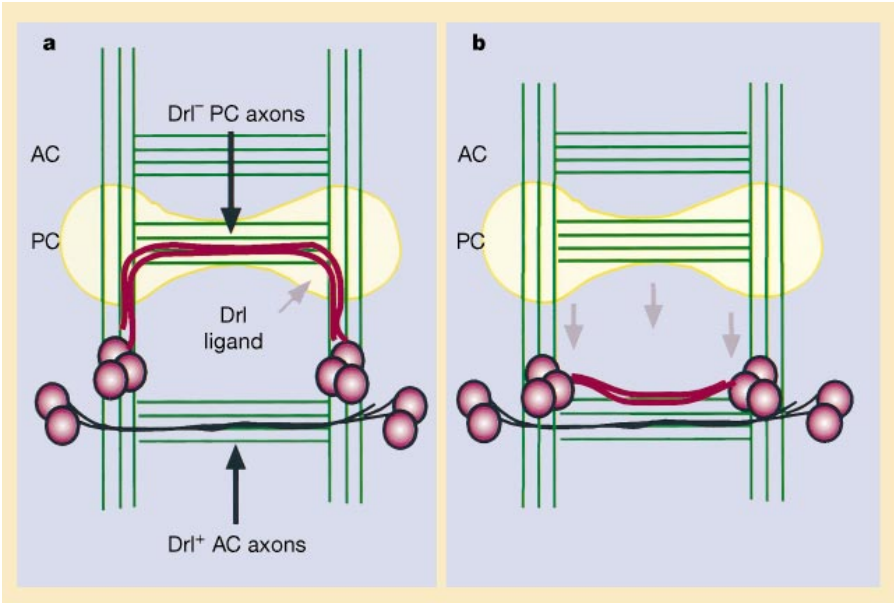

Figure 2 **Effects of Drl expression on axon guidance. Bonkowsky *et al.*[1] have found that expression of a protein called Drl controls pathfinding by causing growth cones to be repelled from the wrong pathway.** a, **Wild-type embryos. The AC (black) and PC (red) Eg axons cross the midline. The distribution of the putative Drl ligand is indicated by yellow shading, and the axon scaffold is in green.** b, **Forced expression of Drl in the PC Eg axons[1]. These axons are repelled by Drl ligand (grey arrows) and instead project across the AC.**