

promiscuous ligand-binding site and can potentially be activated by many naturally occurring dietary and endogenous compounds<sup>6</sup>. Furthermore, the characteristics of AhR-deficient mice suggest a role for this receptor in the immune and vascular systems, the liver, ovaries and other organs<sup>5,12,13</sup>. Together, these observations raise the possibility that the activation of AhR by natural ligands, and its interaction with oestrogen receptors<sup>3</sup>, may be important during development and perhaps in adult life.

Clearly, Ohtake and colleagues' findings also provide a mechanism whereby exposure to chemicals can disrupt normal oestrogen-induced signalling events. Dioxins, the by-products of many industrial and combustion processes, have been detected in the blood of virtually every person tested<sup>14</sup>. They are present in the fat of breast milk, and can cross the placenta from mother to fetus. Toxicology studies indicate that exposure to low levels of toxins during critical developmental periods can have permanent adverse effects on health. To make matters worse, the current burden of dioxins and other chemicals in certain human populations has been reported to be near the range at which adverse effects occur in laboratory animals<sup>2,14</sup>.

Given these facts, why is it so difficult to establish an unequivocal causal link between dioxins and human disease in the general population? Part of the problem is that many

of the toxic effects attributed to dioxins — such as impaired immunity, fertility and cognition — are relatively subtle, multifactorial, difficult to diagnose and quantify, and often take years to appear. Resolving these issues will depend on a combination of improved diagnostic tests, large epidemiological studies, and the development of genetically manipulated animal models in which the role of crosstalk between the AhR and oestrogen-receptor signalling cascades can be distinguished from the role of their individual pathways. ■

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to produce HD; if instead the O–H bond was excited, H<sub>2</sub> was produced.

But similar experiments in reaction control are rarely successful in condensed phases (liquids, glasses and solids) because a specific vibrational excitation becomes redistributed rapidly over other modes within the molecule or among its neighbours. To decipher the effect that the local environment exerts on an individual molecule, investigators turned their attention to experiments on single molecules. For example, Xie and Dunn<sup>3</sup> recorded the fluorescence spectrum of a single molecule on a glass surface, and were able to discern fluctuations in the molecule's local environment that could not be seen in the data recorded for an analogous bulk sample.

Another line of approach in single-molecule spectroscopy involves the scanning tunnelling microscope (STM), invented in the 1980s by Binnig and Rohrer. An immense collection of solid-surface images has since been produced by STMs, with sufficient resolution to reveal the atomic and molecular adsorbates that might decorate a surface. Then in 1998, Stipe *et al.*<sup>4</sup> made the remarkable discovery that the current flowing from the tip of an STM can selectively excite different modes of vibration in a lone molecule that is bonded onto a surface.

Other investigations have shown that an STM tip can be manipulated to translate or rotate<sup>5</sup>, or fragment<sup>6</sup>, an atom or molecule bonded to a surface — or even to eject it from the surface<sup>7</sup> (desorption). An STM tip can be used to force two molecules to dissociate, and the resulting fragments can be rearranged and fused together to form a new product molecule<sup>8</sup>. In each of these cases, however, the STM tip has served either as an atomic-scale poker to push a molecule physically across the surface or as a localized heater (through electron bombardment) for inducing non-selective thermal excitations.

Pascual *et al.*<sup>1</sup> now demonstrate another way in which the STM can control the behaviour of a molecule on a surface. They use an STM tip to selectively excite vibrational modes in an ammonia molecule (NH<sub>3</sub>) as a way to sever the chemical bond between NH<sub>3</sub> and a copper surface, or alternatively to induce the molecule to move laterally across the surface (Fig. 1). The reaction outcome is controlled simply by selecting the tip voltage and thereby setting the energy of the tunnelling electrons to a value that will resonantly excite the preferred vibrational mode. Of the two modes investigated, the first is a stretching, or 'breathing', mode in which all three N–H bonds are symmetrically stretched and compressed, the second a bending mode that resembles an umbrella inverting on a windy day. Most of the excited molecules relax quickly by transferring their energy to the copper lattice, but some convert their vibrational motion into translational motion. Specifically, excitation of the

## Chemistry

# Tips for moving single molecules

Dennis C. Jacobs

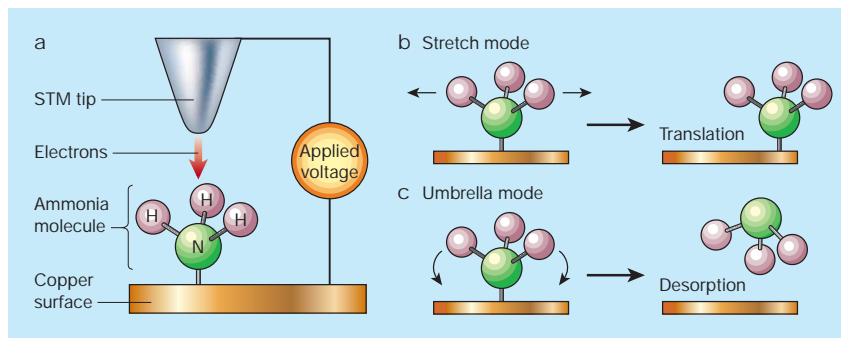
Scanning tunnelling microscopes provide a unique perspective on chemistry at the level of single molecules. Now there is a new way of using the tip of such a microscope to manipulate a single molecule.

In nature, reactants can be transformed into products through a vast array of mechanisms. In synthetic chemistry, progress hinges on finding a way to maximize the yield of the desired target molecule while minimizing the generation of unwanted by-products. In the same way that a golfer anticipates the terrain of the green when putting, chemists aim to control the chemical dynamics of a reaction, guiding the participating molecules along a specific path. On page 525 of this issue, Pascual *et al.*<sup>1</sup> demonstrate how a scanning tunnelling microscope can be used to control the excitations of a single molecule and achieve a desired reaction path.

Most chemical reactions require that some of the existing bonds within the reactants are weakened or broken before new bonds can form. This means that the reactants must receive a critical amount of energy from their surroundings to begin

their metamorphosis. Raising the temperature of the sample is a relatively inefficient way of doing this, because fluctuations due to thermal energy are typically ten to a hundred times smaller than the activation energy for a reaction. Furthermore, under thermal conditions, the energy is distributed over many types of molecular motion — translational, rotational or vibrational, for example — whereas usually only one specific type of motion (such as a vibrational stretch) is associated with crossing over the reaction barrier.

So a more controlled approach is needed. In 1991, Bronikowski *et al.*<sup>2</sup> showed that stretching vibrations could be excited between the atoms in molecules of deuterated water vapour (HOD instead of H<sub>2</sub>O) by laser radiation. If the stretch mode of the O–D bond was excited, they found that an approaching hydrogen atom preferentially abstracted the deuterium atom from HOD



**Figure 1 Moving molecules.** Energetic electrons emerging (a) from the tip of a scanning tunnelling microscope (STM) can selectively excite a specific vibrational mode within a molecule. For an ammonia molecule (NH<sub>3</sub>) adsorbed on a copper surface, electrons with an energy of 420 millielectronvolts excite a 'stretch' mode (b), causing the molecule to be translated along the surface. If the electron energy is 320 millielectronvolts, the 'umbrella' mode (c) is excited instead, and this flexing of the hydrogen–nitrogen bonds — similar to an umbrella turning inside out — results in desorption of the molecule from the copper surface.

umbrella mode tends to desorb the molecule, intact, from the surface; the symmetric breathing mode preferentially induces lateral translation of the molecule across the surface.

This pioneering experiment not only reveals how competing reaction mechanisms can be selectively activated within a single molecule, but also demonstrates a new approach for identifying reaction pathways in complex environments. Studies such as this expand the range of synthetic tools available for fabricating the next generation of molecular-scale nanostructures. ■

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## Evolutionary biology

# Fractional phylogenies

Thomas D. Kocher

Speciation has been unusually fast among the cichlid fishes of Lake Victoria. An unexpectedly distant ancestor, which perhaps already had a predisposition for rapid speciation, may have seeded this 'species flock'.

More than half of all living species of vertebrates are 'bony' fishes. Over the past 250 million years they have evolved into more than 25,000 species that span habitats from the highest mountains to the deepest oceans. Nowhere has their evolutionary radiation been more rapid than in the great lakes of East Africa. Several hundred species of cichlid fishes swim in the waters of Lake Victoria, and it has been assumed that this 'species flock' arose within the lake from one or a few common ancestors since the lake last dried up around 15,000 years ago<sup>1</sup>. In a paper in *Science*, Verheyen and colleagues<sup>2</sup> cast doubt on this assumption, and instead suggest that Lake Victoria was colonized several times, beginning more than 100,000 years ago, by fish from Lake Kivu, which lies 300 km to the west.

The very recent origin of the Lake

Victoria flock poses two challenges for those wishing to reconstruct the historical relationships among species. First, there has been little time for mutation to alter the DNA sequence of each species. This means that there are precious few sequence characters on which to apply phylogenetic analysis. Second, there has not been enough time for new variant genes to become fixed between instances of speciation<sup>3</sup>, a problem known as 'incomplete lineage sorting'. This means that although phylogenetic trees derived from DNA sequences accurately represent the history of genes, they do not necessarily reflect the history of the populations in which the variants are found (Fig. 1, overleaf).

The problem of too few mutations is usually addressed by focusing on rapidly evolving gene sequences. DNA in the cell's mitochondrion has been a particular

favourite for such studies, because it evolves roughly ten times faster than the average gene in the nucleus of the cell. An earlier study of the Lake Victoria flock<sup>4</sup> found considerably more diversity of mitochondrial DNA than could have arisen since the lake last dried out 15,000 years ago. This means that the most recent radiation in Lake Victoria was seeded by several lineages, in which genes for important morphological and behavioural traits were already polymorphic.

The bit added by Verheyen and colleagues<sup>2</sup> is data on the mitochondrial DNA sequences of cichlids native to Lake Kivu, which lies quite far to the west of Lake Victoria. They construct a 'haplotype network' in which each sequence is joined to the next in a way that explains the sequence differences with a minimum of mutational events. Then they consider the frequency and geographical distribution of these haplotypes so as to reconstruct a plausible biogeographical scenario for the evolution of the fishes. Their analysis identifies the Lake Kivu species *Haplochromis gracilior* as the closest relative of the Lake Victoria superflock, and suggests that there has been a complex pattern of faunal exchange through the region.

Neither of these studies<sup>2,4</sup> overcomes the problem of incomplete lineage sorting, and so the gene trees must be interpreted with caution. An alternative is to score DNA polymorphisms at thousands of independent gene loci from the cell nucleus<sup>5</sup>. The average of these many gene trees should be an accurate estimate of the history of the populations in which the genes are segregating. Seehausen and colleagues<sup>6</sup> have used this second approach to analyse a number of potential riverine ancestors, and have identified several species of the genus *Thoracochromis* as the closest relatives of the Lake Victoria superflock.

The nuclear and mitochondrial studies provide complementary perspectives, but it is difficult to combine their results into a coherent whole because the various data sets do not overlap for certain key species. Even if complete data were available, it is not clear that convincing statistical support would emerge for either phylogenetic tree because of the short time-frames involved. Verheyen *et al.* report a Bayesian posterior probability of 80% in support of their main conclusion — the linkage of *H. gracilior* to the Lake Victoria superflock — but such probabilities have been shown to be excessively liberal<sup>7</sup>. Seehausen *et al.* make the uncomfortable suggestion that the short branches in their nuclear gene tree may reflect a period of extensive hybridization among species early in the history of the flock. In that case it may never be possible to completely reconstruct the relationships among these species.

Regardless of the details, none of these analyses refutes the conclusion that most of the current species diversity has arisen within