evidence that PFL3 and PFL2 neurons can generate goal-directed steering, as predicted by theoretical models — but they stop short of showing that these neurons are required for all goal-directed steering. Mussells Pires *et al.* investigate the effects of silencing PFL3 neurons in a task designed to assess memory of wind direction, which the authors show is dependent on the compass network. However, the effects of silencing PFL3 neurons are only modest. This might be because the genetic line used by the authors labels, and therefore silences, only a subset of neurons. Future studies will be needed to determine how PFL neurons as a population contribute to goal orientation during complex behaviours.

Although the current studies focused on flies, internal maps of the environment are found in the brains of many animals, including humans. In vertebrates, navigational abilities are strongly linked to the hippocampus, which forms maps of both real and abstract environments. How these maps are translated into locomotor commands remains unclear. A study in Egyptian fruit bats (*Rousettus aegyptiacus*) found that a subset of neurons in the hippocampus is tuned to both the direction and distance (the vector) between the animal and the location of a hidden goal platform[9]. Another study found that place cells (neurons that fire when an animal is in a particular location in its environment) show directional tuning towards a goal when rats navigate a series of moving platforms[10].

Both of these coding schemes are reminiscent of the fly brain, in which the direction of a goal is represented by the pattern of activity across an array of neurons. Defining the precise neural architectures that allow insects to convert such maps of the environment into steering commands for the body might therefore help to reveal how human brains navigate both real and imaginary spaces.

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Forum: Structural biology

Energetic laser pulses alter outcomes of X-ray studies

Cutting-edge X-ray sources have enabled the structural dynamics of proteins to be tracked during biochemical processes, but the findings have been questioned. Two experts discuss the implications of a study that digs into this issue. **See p.905**

The paper in brief

- **Ultrashort, intense X-ray pulses generated at facilities known as X-ray free-electron lasers (XFELs) have been used to probe light-induced structural changes in proteins.**
- **Light-responsive proteins typically absorb one optical or ultraviolet photon in natural settings, but could absorb more from the intense 'pump' lasers used to induce structural changes in these studies.**
- **Such unnatural absorption of multiple pump photons might force proteins to behave in ways that are not biologically relevant.**
- **Questions have therefore been raised about how these studies should be interpreted.**
- **Barends *et al.*[1] now show that the structure of a model protein changes in different ways depending on whether single or multiple photons are absorbed.**

Richard Neutze

Imperfect experiments can be informative

Structural changes that occur in proteins during biochemical reactions can be measured using a technique called time-resolved X-ray diffraction (TR-XRD). In this method, reactions are initiated in protein crystals, and X-ray pulses are used to record X-ray diffraction data at selected times after initiation. TR-XRD has produced structural insights into the pathways of diverse biological processes[2], including photosynthesis, sensory signalling, ion transport and photodissociation — the light-induced breakage of bonds between proteins and their ligand molecules.

For light-sensitive proteins, a pump laser pulse is used to initiate the reaction of interest. All molecules probed in a crystal contribute to the measured X-ray diffraction pattern, yet typically only a subpopulation is activated by the pump laser. A quantity known as the crystallographic occupancy estimates the fraction of molecules in a crystal that are activated. Raising the pump-laser fluence — the energy delivered per unit area by the pump laser onto a crystal — can increase the crystallographic occupancy, but more than one photon can be absorbed by the protein at high laser fluences[3,4].

Barends *et al.* studied structural changes that occur in the carbon monoxide complex of the protein myoglobin (MbCO) after pump-laser-induced photodissociation of CO from the iron atom of a haem group (Fig. 1). This process was previously studied using TR-XRD at time resolutions of 7.5 nanoseconds (ref. 5) and 150 picoseconds (1 ps is 10^{-12} seconds; ref. 6) using relatively large protein crystals (dimensions in the range of about 0.1 to 0.3 millimetres) and X-ray pulses from a synchrotron facility, which is a less intense X-ray source than an XFEL.

A 2015 study by some of the same researchers as Barends *et al.* used extremely short, intense XFEL pulses to record TR-XRD data from tens of thousands of much smaller MbCO crystals (average size 15 micrometres × 5 μm × 3 μm). This thereby achieved a time resolution of 250 femtoseconds (1 fs is 10^{-15} s) and revealed ultrafast conformational changes of the protein as photodissociation occurs[7]. But because those experiments used a high pump-laser fluence, Barends *et al.* have now repeated their study using a lower fluence that ensures single-photon excitation of MbCO.

The authors used their TR-XRD data to determine difference Fourier maps, which show differences in electron density in MbCO before and after activation. Barends *et al.* found that lower pump-laser fluences yield lower crystallographic occupancies in maps produced 10 ps after protein activation. For this time delay, differences between structural

changes induced by single-photon and multiphoton excitation are difficult to see from the difference Fourier maps, but the authors argue that some effects can be determined from careful analysis and structural modelling. A caveat is that structural modelling incurs larger errors when the crystallographic occupancy is low.

For sub-picosecond time delays, the authors' analysis shows that the protein responds unexpectedly rapidly to the highest pump-laser fluence, and that CO swiftly populates a new location near the haem (Fig. 1). By contrast, it takes longer for the protein to respond and for this CO site to be populated after single-photon activation.

Barends and colleagues' recording of high-quality maps from very small crystals of MbCO after single-photon excitation is impressive. Their study will motivate other researchers to recover high-quality difference Fourier maps using single-photon excitation. However, this might not be possible for many biological systems. Crucially, the structural perturbations of MbCO induced by single photons are in keeping with findings from earlier work[5–7], illustrating that useful insight does emerge from imperfect experiments. Historically, the field has chosen not to let the perfect be the enemy of the good. I contend that such pragmatism should continue, so that the diversity of biological systems studied by TR-XRD continues to grow[2].

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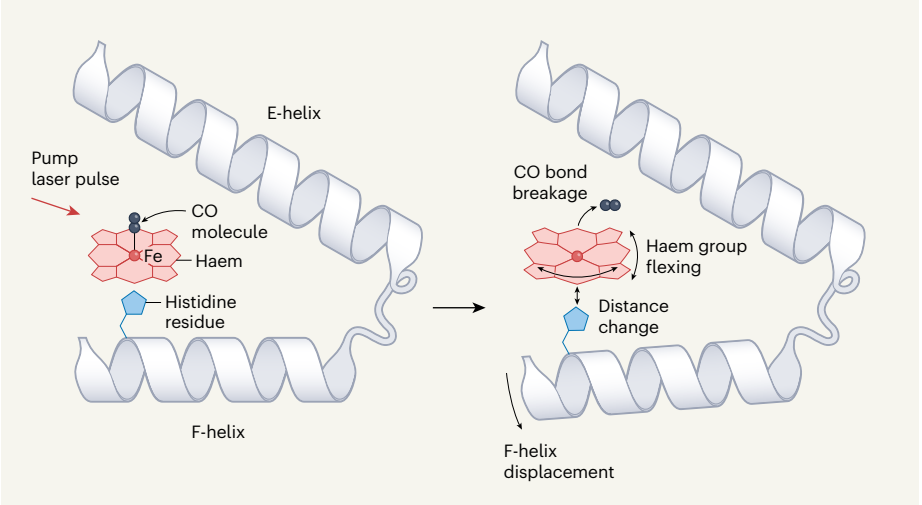

Figure 1 | Structural dynamics of a model protein system. Barends *et al.*[1] used a method called time-resolved X-ray diffraction (TR-XRD) to study crystals of the carbon monoxide complex of the protein myoglobin (MbCO; only part of the complex is shown). They used a 'pump' laser pulse to induce photodissociation (breakage of the bond between CO and the iron (Fe) atom of a haem group in the protein), and then used ultrashort, intense X-ray pulses to obtain X-ray structures of the protein over time. Three structural changes crucial to photodissociation are shown (right): flexing of the haem group; changes in the distance between the haem and a histidine amino-acid residue in the F α-helix of the protein; and displacement of the F-helix. The authors observed that the fluence of the pump laser (the energy delivered per unit area by the pump laser onto a crystal) alters the amplitude and timing of these motions, and also the motion of the CO away from the haem after photodissociation – suggesting that lower fluences are needed to observe structural changes that occur in natural settings.

R. J. Dwayne Miller
Protein 'music' must not be distorted

Biological processes are driven by chemistry. Chemistry is dynamic, with all the interconnecting atoms in molecules jiggling around, vying for right of way to take part in reaction pathways. In proteins, chemical transformations occur at an active or binding site. These processes involve the coupling of reaction forces such that some 10–100 atoms at the active site direct the motions of the 1,000–10,000 (or more) atoms of the surrounding protein. An astronomical number of possible conformational pathways (sequences of molecular structures) can occur, but only one or a few motions at the active site – known as reaction modes – direct biological function.

How can such a small number of reaction modes preferentially direct protein functions within the ocean of alternative conformational pathways? This apparent paradox is solved by considering spatially correlated motions – these arise when forces imprinted in the protein structure cause atoms to move together, rather than randomly and independently. By directly observing atomic motions during the defining moments of a chemical reaction, the initial distillation of all possible pathways down to a few reaction modes can be seen[8]. Such observations allow relationships between protein structure and function to be determined, but only if the reaction is initiated correctly. The whole point of such studies is that we don't know the length and timescales of protein responses to the chemical driving force.

To help make sense of this issue, consider the fluid that forms by mixing cornflour and water. If you dip your finger slowly into this mixture, the fluid flows around it like a liquid. But if you rapidly poke the mixture, the material responds as an elastic solid. Similarly, the spatially varying barriers to motions in proteins mean that protein responses to forces depend on both the time and the length scales of the applied force. Given that protein structures are highly anisotropic (different in different directions), the response will also depend on the spatial location of the induced force.

Until the work of Barends *et al.*, femtosecond TR-XRD studies had used extremely high pump fluences to ensure that structural changes in proteins could be observed. Because the laser pulses are so short, this came at the cost of inducing multiphoton protein activation that produces initial atomic displacements different to those arising from single-photon activation, and at higher energies, with the atoms being displaced farther from their equilibrium positions[4]. The driving forces for the observed structural changes were therefore both spatially different and significantly larger than those of the biological pathway of interest[4].

For the model MbCO system, there are three biologically crucial and spatially coupled motions (Fig. 1): the haem doming coordinate (which describes the flexing of the protein's haem group); movement of a histidine amino-acid residue positioned close to the haem; and the displacement of α-helices resulting from the transfer of driving forces from the first two motions. Barends *et al.* observed that multiphoton activation resulted in much faster displacement of the histidine than did single-photon activation, and led to similarly faster motions of one of the helices. Moreover, the spatially correlated helical motions extended over different distances along the helix than did those induced by single-photon activation, and subsequently dissipated to different degrees.

The impulsive nature of the force produced by multiphoton activation on the initial histidine motion, and the temporal evolution of the positioning of the CO molecule in the protein pocket that contains the haem, are evidence of a different reaction pathway to the one produced by single-photon activation. There are significant differences in the magnitude, temporal evolution and pathway of the structural dynamics throughout the protein.

Given the importance of energetics and

spatially correlated motions for understanding the structural changes that underpin protein function, we need to get this right. Biologically relevant protein motions are like music, albeit playing at frequencies we can't hear. We need to observe the motions that correspond to certain frequencies, and discern how those motions are damped to produce net displacements. Imagine listening to music in which violin strings are struck too strongly, causing the accidental playing of different chords together. You would not hear the music as written, but something else entirely. To understand how nature works, we need to listen to the molecular music as nature wrote it. Barends *et al.* have done just that.

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Photonics

Nanotraps boost light intensity for future optics

Kirill Koshelev

A method for configuring light-trapping devices promises better optical nanodevices by amplifying light and enhancing the emission efficiency of luminescent nanomaterials — without the need for complex technology upgrades. **See p.765**

Intense light beams are crucial for myriad applications, ranging from medicine to electronics, but they are challenging to produce with everyday light sources. They can, however, be generated by lasers. Lasers work by trapping light in a cavity, called an optical resonator, in which reflected light waves interfere constructively to amplify the light's intensity through a phenomenon known as optical resonance. But light can be emitted, scattered or absorbed by the resonator material, limiting the extent to which its intensity can be enhanced — especially in devices that operate on a nanometre scale, such as ultraprecise sensors. On page 765, Schiattarella *et al.*[1] report a smart way of balancing the possibilities for light to escape a nanoresonator, and therefore increase light intensity by a factor of up to 36,000.

In the past two decades, advances in nanoscale materials have enabled researchers to engineer visible and infrared light resonators that are no thicker than a human hair[2]. However, decreasing the size of a resonator inevitably leads to an increase in light emission. One way around this involves a special optical resonance called a bound state in the continuum, also known as a dark mode. This mode amplifies light intensity with very small losses[3]. Dark modes are produced by carefully tuning the properties of a resonator to induce destructive interference between two or more 'bright' waves, which are formed through constructive interference.

Confining light with dark modes might limit unwanted emission, but it doesn't overcome the challenges posed by absorption and by fabrication defects that lead to light being scattered. Optimal light intensity is usually achieved by satisfying the critical coupling condition[4], in which the escape rates of light through emission, scattering and absorption are perfectly matched. But Schiattarella and colleagues showed that they could enhance light confinement beyond the range of conventional critical coupling by tailoring the exchange of energy between a dark mode and a bright mode. In doing so, they achieved 'supercritical' coupling.

> **"This approach could benefit biosensing by enhancing sensitivity to small sample volumes."**

The authors investigated a resonator consisting of a 130-nm-thick slab of silicon nitride that was patterned with a square lattice of circular holes and placed on a silicon dioxide substrate of 0.1–1 millimetres in length (Fig. 1). They first calculated how the optical resonances of the slab could be optimized by adjusting various structural parameters of the slab, including its crystal-lattice spacing and thickness, as well as the diameter of the holes. They then used this information to create a dark mode and a bright mode with similar frequencies and waveforms.

By illuminating the centre of the slab with light that has the same frequency as the dark mode, the authors showed that they could induce conventional critical coupling. This offered moderate intensity enhancement that was limited by imperfections in the surface of the silicon nitride slab. They then showed that illuminating the edge of the slab had the effect of inducing a specific energy-exchange rate between the dark and bright waves, which modified the critical coupling. The authors' calculations suggested that incorporating this exchange into the usual loss-balancing equation could lead to the fulfilment of a supercritical coupling condition that would substantially improve the enhancement of the light intensity.

Schiattarella *et al.* used a process known as upconversion to demonstrate that their resonator could achieve the predicted supercritical coupling. Upconversion involves two or more photons combining and being absorbed to generate one higher-energy photon. It occurs, for example, when nanoparticles fabricated from the lanthanide series of elements absorb infrared light and convert it into visible light. These nanoparticles upconvert with increased efficiency when they are integrated with nanoresonators[5].

The authors covered their silicon nitride slabs uniformly with two layers of upconverting nanoparticles: one layer contained a compound that emits green light when excited by infrared light, whereas the particles in the other layer emitted red light. Using a laser producing extremely short pulses of light, they measured the change in luminescence as a result of upconversion, and found that it was substantially more enhanced at the edge of the resonator than it was at the centre. This is consistent with the authors' model predictions, which suggest that emission from the edge of the nanoparticle–resonator system should be up to 36,000 times higher than that from a thick bulk layer of these nanoparticles.

As well as being brighter, the luminescence from the edge was also more precisely focused than that from the centre — emerging from the side of the device as a beam that remained collimated (that is, its rays were parallel) for several millimetres. Compared with emission from the bulk, the directionality of this beam further enhanced the emission — by a factor of more than 100 million, in the authors' estimation. Schiattarella *et al.* also showed that the direction of emission could be gradually swapped by changing the direction in which the incoming laser light was polarized (the