nutrient niches that would have favoured the coexistence of plants with distinct abilities to capture particular limiting nutrients. So, although changes in plant competition for light can explain some of the effects of fertilization on diversity, other mechanisms must have a role, too. The experiment raises the interesting possibility that the relative contribution of light limitation on biodiversity varies over time, or changes as communities reassemble.

Eskelinen and colleagues' study is extremely valuable because it experimentally manipulates light in the field and clearly shows that changes in light levels explain the effect that herbivore grazing has on diversity. The work also raises many questions. It is unclear why light addition was unable to boost diversity on fertilized plots in the later years of the study. Is it the case that climate-induced variation in plant biomass production shifts the relative strength of competition for light from year to year? However, without multi-year data sets and information on plant productivity, it is not possible to test this idea. Alternatively, does fertilization result in reduced light levels at ground level only immediately after herbivores are removed, after which light limitation becomes less central for explaining the effects of fertilizer addition? At some point after the first year, the effects of sheep grazing on light availability became so strong that adding fertilizer did not alter light levels for low-growing plants. Unfortunately, data were not collected during the second year of the experiment, so the authors could not examine at what point grazer removal overwhelms the effect of fertilizer on light in the understorey.

Questions aside, the study's results highlight the value and power of this type of field experiment, in which several treatments are crossed with each other. The dominant effect of the grazers on diversity and on the availability of light underlines the crucial role of herbivores in structuring plant communities and maintaining diversity, especially in productive areas such as fertilized fields. However, animals might have less-positive effects in areas with naturally shorter vegetation, where grazers are less likely to drive an increase in light levels.

It would be interesting to extend the ideas explored here, by testing whether invertebrate herbivores or disease-causing fungi might also promote diversity[11] by causing a rise in light levels. Eskelinen and colleagues' study demonstrates how a simple experimental set-up can be used to pinpoint mechanisms underlying declines in diversity. More such investigations are needed to reveal fundamental mechanisms underlying plant coexistence and to improve our ability to predict how global change will alter biodiversity.

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Structural biology

Catching actin proteins in action

Pilar Cossio & Glen M. Hocky

Two groups have visualized actin — the protein polymer that gives cells their shape — at high resolution. The structures provide in-depth views of the polymer as it adopts fleeting states and undergoes conformational changes. **See p.374 & p.380**

Actin is the most abundant protein in our cells. When assembled into polymers called actin filaments, it has key roles as a structural support system (forming a cytoskeleton that gives cells their shape), as a network for transporting cellular cargo, and as a power system for cellular motion, division and adhesion[1]. Reynolds *et al.*[2] (page 380)and Oosterheert *et al.*[3] (page 374) describe complementary high-resolution structures for actin filaments, which they resolved thanks to advances in tools for the analysis of cryo-electron microscopy data. Their work deepens our understanding of how actin filaments that are undergoing assembly and maturation are recognized by binding proteins.

Actin filaments are semi-flexible polymers that are naturally straight, but show some degree of curvature owing to random forces in their surroundings (called thermal fluctuations). Further bending can be produced by the action of molecular motors, or through shear forces produced by fluid flow in *in vitro* experiments[4,5]. The level of flexibility depends in part on which of three nucleotide-bound states a filament is in. Shortly after the filaments form, actin undergoes a process dubbed ageing, in which a bound nucleotide called adenosine triphosphate (ATP) is hydrolysed, producing an intermediate nucleotide, ADP-P_i. Free phosphate (P_i) is then released, leaving a bound ADP molecule[1,5] (Fig. 1). ADP-bound actin is about twice as flexible as the ATP-bound filament[4], and the polymers' affinity for binding proteins can differ[1,5].

So far, our only knowledge of how bending affects local contacts between individual actin proteins in filaments comes from modelling[4,6]. But a detailed knowledge of the structural changes that actin undergoes as it bends is essential for understanding how actin-binding proteins regulate the actin cytoskeleton[5]. For example, why does the protein cofilin, which modulates filament depolymerization, preferentially bind to ADP-bound filaments[5,6]? The structure of an actin filament would be expected to differ substantially depending on whether it is bound by ATP, ADP-P_i or ADP. But previous studies could not determine whether there are, in fact, differences.

In the current studies, the groups used optimized cryo-electron microscopy (cryo-EM) sample preparation and work flows to obtain cryo-EM density maps for straight actin filaments in the different nucleotide states at unprecedented resolution (down to 2.2 ångströms). Reynolds *et al.* resolved structures for ADP-P_i- and ADP-bound actin, and Oosterheert *et al.* resolved these as well as a filament bound to an ATP analogue. The groups found that the average maps of ATP-, ADP-P_i- and ADP-bound actin are extremely similar in terms of the protein structure and the way in which subunits are arranged into a helical lattice, confirming the results of a previous, lower-resolution study[7].

But the groups' high-resolution maps also reveal how the detailed positioning of protein side chains, water molecules and magnesium (Mg^{2+}) or calcium (Ca^{2+}) ions (which associate with the bound nucleotide) differs as actin ages. It is well established[1] that actin monomers change shape as they polymerize, switching from a twisted, open configuration to a closed, flattened overall shape. Oosterheert and colleagues provide evidence for how this conformational shift alters the positioning of a specific water molecule, bringing it

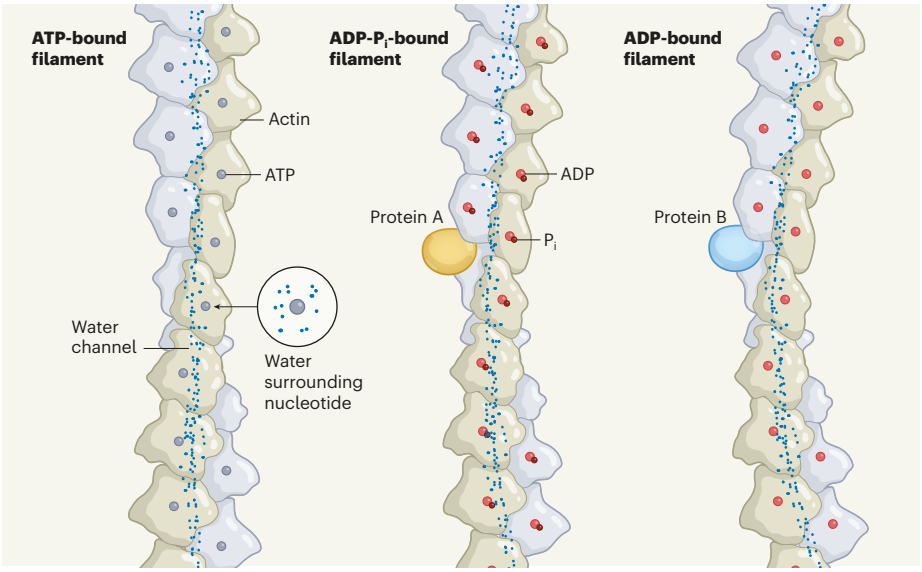

Figure 1 | Actin filaments in close-up. Shortly after actin proteins polymerize into filaments, they undergo a process dubbed ageing, in which a bound nucleotide (ATP) undergoes hydrolysis, forming an intermediate dubbed ADP-P_i, before releasing a phosphate group (P_i) to leave bound ADP. Two groups[2,3] have resolved high-resolution structures for actin filaments in different nucleotide-bound states, and find that the structures are almost identical. Reynolds *et al.*[2] confirm that ADP-bound filaments can bend more than can those bound by ADP-P_i, and find that each filament has a channel of water running down its centre. Oosterheert *et al.*[3] find changes in the positioning of water molecules, ions and associated atoms that could alter local flexibility (not shown). Both groups highlight the importance of water molecules around the nucleotide, and propose that changes in flexibility might allow binding of different proteins (here, protein A and protein B) by the filament in each state.

into a position that enables ATP hydrolysis. They found that, for Ca^{2+}-bound actin, the shift requires changes in the water molecules that surround the ion, making polymerization slower than for Mg^{2+}-bound actin. The slower ATP hydrolysis rate of Ca^{2+}-bound actin is also attributable to unfavourable positioning of water molecules. Reynolds and co-workers highlight a channel of water running down the filament centre. They propose that the water molecules in this channel mediate interactions between the amino-acid residues that bridge the actin-subunit interfaces.

Given the overall structural similarities between the nucleotide-bound states, how can the states be differentially recognized by binding proteins? Reynolds and colleagues reasoned that the answer might lie in differences in bent filaments. Straight filaments dominate in samples prepared for cryo-EM, so the authors developed a machine-learning algorithm, trained using simulated images, to pick out bent filaments in cryo-EM micrographs and identify their curvature. This enabled, for the first time, an analysis of bent particles, which would typically be discarded.

The authors found that curvature distributions showed statistically significant differences between states, with ADP-bound states adopting sharper curvatures, as expected[4]. However, the sharper curvatures that the authors observed exceed those that would occur owing to thermal fluctuations. The group concludes that the most-bent filaments are induced by the freezing process that is required for cryo-EM, and speculate that these filaments might mimic the structures adopted by filaments experiencing actively induced deformations in cells.

Reynolds *et al.* used a machine-learning algorithm[8], along with state-of-the-art cryo-EM analysis tools[9,10], to generate molecular models of the structure of large-curvature filaments, providing new details with which to flesh out previous models[4] of how the twisting and bending motion of filaments is coupled. The authors' molecular models reveal changes at the interfaces between actin subunits that depend on the bending state, highlighting those changes in the flexible ADP-bound state that are most likely to be sensed by actin-binding proteins. These models should help researchers to generate hypotheses for how actin-binding proteins recognize bent filaments.

Nevertheless, questions remain over how actin-binding proteins can recognize different nucleotide states in straight filaments[11]. The cryo-EM high-resolution structures represent the most probable configuration for each state, but do not reflect the entire range of conformations adopted (even for straight filaments). Oosterheert *et al.* observe state-dependent changes in interactions between actin's carboxy-terminal tail and the 'D-loop' — two regions that make contact between subunits, mediating lateral interactions along the filament. However, the authors find that this conformational variation is not enough to explain the difference in binding affinity of cofilin between new and aged filaments. Instead, they suggest, like Reynold and colleagues, that cofilin binding must be affected by an increase in flexibility.

Together, the two articles provide the strongest evidence yet that the change in nucleotide state does not produce any large-scale subunit rearrangement in actin filaments. Rather, the behavioural differences between new and aged filaments must be ascribed to minute differences in contacts that change flexibility. A remaining challenge is to determine to what degree conformational fluctuations modulate the interactions between specific actin-binding partners, and to work out whether some of these functional but transient states exist in the many particles that have been discarded in the cryo-EM analysis.

Nearly any biology textbook will tell you that the structure of a protein determines its function. But the current work suggests that it is not only the average structure, but also the full conformational ensemble of a biomolecule, that governs its behaviour. Even with the strides in experimental techniques that enabled the current work, our ability to capture and analyse rare conformational fluctuations is limited. Computational-modelling techniques will be invaluable for predicting the full range of large-scale conformational changes that a protein can adopt, and the timescales on which they occur, as well as for predicting the effect of non-equilibrium forces (such as those produced when polymerization occurs perpendicularly to a cell membrane, or owing to molecular motors) on biomolecular assemblies[12]. By matching structures from these simulations with cryo-EM data[13], researchers will be able to extract transient states and thermodynamic properties from the experimental data, arriving at ever more accurate and quantitative descriptions of biomolecules in action.

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Materials chemistry

A molecular flip-flop for separating heavy water

Thomas Heine & Randall Q. Snurr

Molecules of heavy water contain the deuterium isotope of hydrogen and have been impossible to separate from ordinary water. Nanoporous materials with flexible apertures in their structures point the way to a solution. **See p.289**

Isotopes are atoms that have the same atomic number but different numbers of neutrons in their nuclei. For example, protium (H) and deuterium (D) are isotopes of hydrogen that have 0 and 1 neutrons, respectively. Water molecules that contain deuterium atoms instead of protium atoms are known as heavy water. The physical properties of heavy water differ only slightly from those of ordinary water, which makes it extremely difficult to separate D_2O from H_2O. On page 289, Su *et al.*[1] report two nanoporous materials that take up H_2O in preference to D_2O — a property that can be used to separate these highly similar molecules.

Deuterium was discovered by the physical chemist Harold Urey in 1931, after which heavy water quickly became an essential material for nuclear research. Indeed, during the Second World War, the Allies sabotaged and eventually destroyed the first sizeable facility for producing heavy water after it was seized by German troops, to slow down the German development of nuclear weapons[2]. Today, besides its applications in the nuclear industry, heavy water is used in isotopic labelling techniques (such as those used to work out chemical-reaction mechanisms) and as a source of deuterium for making advanced drug candidates[3] (see also go.nature.com/3gwsvbz).

An obstacle for any direct separation of heavy water from ordinary water, apart from the similar physico-chemical properties of these compounds, is that D_2O quickly transforms into HDO when mixed with H_2O, owing to rapid exchange of hydrogen atoms between molecules. This is a problem because HDO is even more similar to H_2O than is D_2O.

Methods for producing heavy water therefore often involve separations of other small molecules that contain different hydrogen isotopes. The state-of-the-art techniques for D_2O production are the Girdler sulfide process[4], which involves the exchange of hydrogen isotopes between water and hydrogen sulfide (H_2S); and the oxidation of D_2 that has been separated by cryogenic distillation from a liquefied mixture of isotopic analogues of dihydrogen (H_2, HD and D_2). But these separation procedures suffer from low selectivity and have high energy demands.

Nanotechnology offers options that go beyond these conventional approaches. In 1995, it was suggested[5] that quantum effects at about 77 kelvin (the boiling temperature of liquid nitrogen at ambient pressure) would allow heavier D_2 molecules to diffuse more easily through subnanometre-scale pores than can lighter H_2 molecules. Various porous materials have since been shown[6] to effect such 'quantum sieving', including compounds known as metal–organic frameworks (MOFs; also called porous coordination polymers). An extension of this separation strategy, called chemical-affinity quantum sieving, has also been reported[6], in which D_2 is adsorbed by porous materials preferentially to H_2. A breakthrough[7] in this area, reported this year, is a process involving the selective pore opening of a flexible MOF. This material remains in a stable, closed-pore form when exposed to H_2, but adopts an open-pore structure in the presence of D_2.

A method has also been developed for separating mixtures of protons (H^+ ions) and deuterons (D^+ ions). This is achieved by electrochemically pumping these ions through 2D crystals[8] — the separation occurs as a result of selective diffusion of the protons through the crystals[9]. It has also been reported[10] that deuterons, rather than protons, can be selectively transported between the sheets of atoms in layered materials, because the energetic barrier to entering the spaces between the sheets is lower for deuterons.

Su *et al.* report two new MOFs that selectively adsorb H_2O over D_2O (Fig. 1). The nanoporous crystalline structures of MOFs consist of organic molecules stitched together with strong bonds at metal nodes. Su and colleagues' MOFs both have copper nodes but have different organic linkers, and form molecule-sized cages connected by

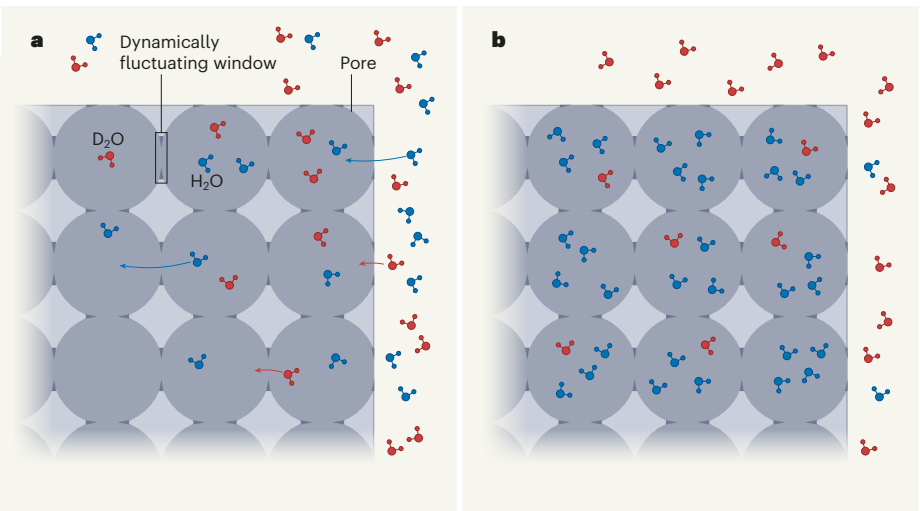

Figure 1 | A porous solid that selectively adsorbs one isotopic analogue of water. a, Su *et al.*[1] prepared a porous material whose cage-like molecular structure consists of pores connected by locally flexible windows. When immersed in a mixture of ordinary water and heavy water (the molecules of which contain deuterium, an isotope of hydrogen), dynamic fluctuations in the window sizes cause ordinary water to diffuse more rapidly through the material than does heavy water (indicated by longer arrows). **b**, After seven days, the difference in diffusion rates causes the liquid trapped in the pores to be enriched in ordinary water, compared with the original composition. This might lead to the development of methods for separating heavy water from ordinary water, for use in applications such as some in the nuclear industry.