S. JUNTTILA

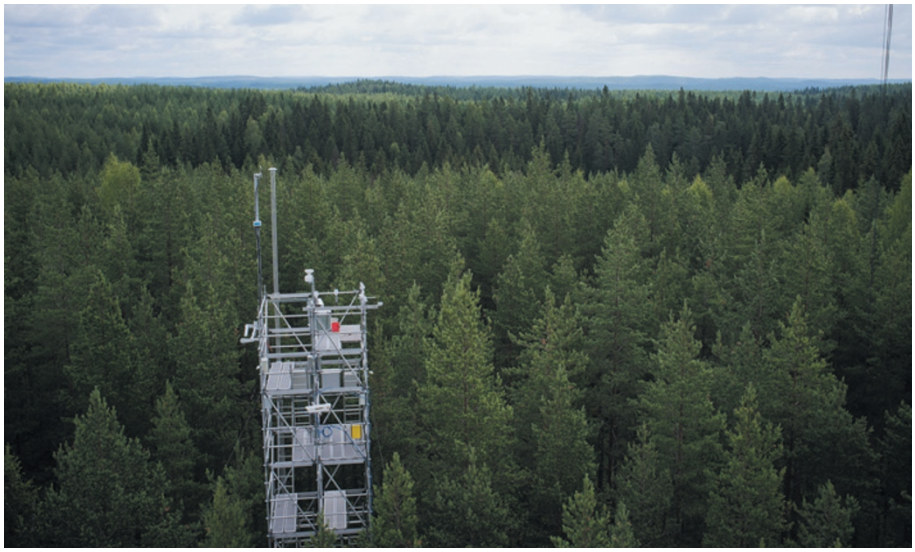

Figure 1 | Something in the air. This tower at the SMEAR II field station, Finland, supports several instruments that are used to analyse the atmosphere above the forest canopy. Mauldin *et al.*[1] have detected a new oxidant in the atmosphere above the station.

the atmosphere that was not derived from OH. When they added this value to the calculated concentration of acid that was derived from OH, they found that the total agreed well with the observed atmospheric concentration of the acid in the forest.

The technique[7] used by Mauldin and colleagues to measure OH is known as chemical ionization mass spectroscopy (CIMS), and it has been used in a range of environments. It is therefore surprising that the significance of background signals has not been recognized in previous studies. That said, the forested environment studied by the authors produces large quantities of alkene emissions, and so provides ideal conditions for the formation of X. Measurements of X are now needed in other environments, to determine its global impact on the production of atmospheric sulphuric acid.

Mauldin *et al.* propose that X converts sulphur dioxide to sulphur trioxide (SO_3), which then reacts with water vapour to form sulphuric acid (see Fig. 3 of the paper[1]). But sulphur trioxide might not be the only product of sulphur dioxide's reaction with X, and the authors do not determine the — possibly multistep — reaction mechanism for this transformation. Indeed, X might not be a Criegee intermediate at all; perhaps a derivative of it, or another compound, reacts with sulphur dioxide[8]. Direct identification and field measurements of X are necessary to resolve this issue.

Furthermore, the authors evaluate only the role of X in oxidizing sulphur dioxide to sulphuric acid. Until the rate constants for reactions of X with a wide range of atmospheric species have been determined, its overall importance for atmospheric chemistry relative to OH will remain unknown — even though its concentration in Mauldin and colleagues' study exceeds that of OH. A more practical issue is that, if X's contribution to the production of sulphuric acid is greater than that of OH, it will make the measurement of OH by CIMS more challenging, because of the need to subtract a background signal larger than the OH signal.

The atmospheric oxidation of sulphur dioxide is closely associated with the rate of aerosol-particle formation and growth, and with the production of cloud condensation nuclei[9] — microscopic particles around which cloud droplets coalesce. In calculations predicting regional and global temperature rises caused by human activities, the largest uncertainties are associated with aerosols and clouds[10]. Until now, OH has been assumed to be the only oxidizer that converts sulphur dioxide to sulphuric acid. Mauldin and colleagues' findings will therefore help to reduce the uncertainties in climate predictions that aim to take into account future changes in man-made sulphur dioxide emissions and in natural hydrocarbon emissions from plants. ■

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STRUCTURAL BIOLOGY

Dynamic binding

Nuclear magnetic resonance spectroscopy has been used to establish a vital role for protein motion in the formation of a protein–DNA complex. The finding potentially opens up fresh approaches for modifying protein function. SEE LETTER P.236

ANDREW J. BALDWIN & LEWIS E. KAY

The continuing development of the tools of structural biology and their increasingly sophisticated application to studies of a wide range of biological molecules are some of the most noteworthy accomplishments of biophysics. The structures obtained have been used to explain molecular function, to design and modify proteins so as to engineer new biological properties, and in the rational generation of pharmaceuticals. Yet despite the well-documented successes, there have also been many cases in which the beautiful, high-resolution pictures produced leave many questions unanswered. Part of the reason is that the biological function of a molecule is driven by free energy changes that depend on contributions from both static (enthalpic) and dynamic (entropic) interactions[1]. Although static structures can provide atomic-resolution information about enthalpy, describing entropy at a similar level of detail has been far more difficult.

On page 236 of this issue, Tzeng and Kalodimos[2] quantify the role of dynamics in their study of the catabolite activator protein, CAP. They have used nuclear magnetic resonance (NMR) spectroscopy to demonstrate that the binding activity of CAP can be regulated by conformational entropy — the entropy component associated with the number of conformational states that the protein samples.

CAP acts as a transcriptional activator — it binds to DNA to increase gene transcription. It is also an allosteric protein: binding of a ligand molecule at one site can introduce changes in both the structure[3,4] and the dynamics[5] of distal sites. More specifically, binding of a small nucleotide molecule, cyclic AMP (cAMP),

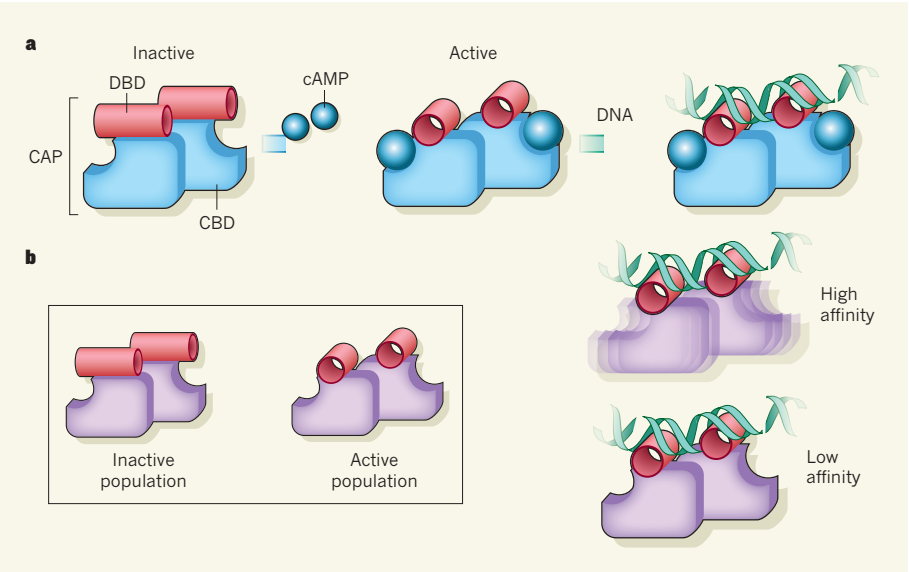

Figure 1 | Conformational entropy can modulate protein–DNA binding. **a**, When cAMP molecules bind to the inactive state of the dimeric catabolite activator protein (CAP) at specific sites in the cAMP-binding domains (CBDs), DNA-binding domains (DBDs) in the protein alter their orientation. This activates CAP so that it can bind with high affinity to DNA. **b**, Tzeng and Kalodimos[2] studied DNA binding to several engineered mutants of CAP, and found that conformational entropy can drive binding. In the CBD variant shown (purple), the equilibrium between inactive and active conformations is highly skewed towards the inactive conformation. Neglecting other factors, a higher affinity for DNA binding is realized if the dynamics of the CAP–DNA complex are increased (that is, if the complex has a large conformational entropy) relative to a complex that shows little change in motion upon binding.

to the cAMP-binding domain of CAP leads to substantial structural rearrangements in distal DNA-binding domains, priming the protein for DNA binding (Fig. 1a). Tzeng and Kalodimos studied CAP in the unbound and DNA-bound states.

The authors exploited CAP's allostery by engineering mutations in the protein at sites remote from the DNA-binding interface, but which nevertheless modulate DNA binding. By using NMR to study the derived mutants, as well as different nucleotide-bound forms of the protein, the authors established that the protein interconverts between inactive states that cannot bind DNA and active states that can, and that, for the mutants examined, the relative populations of these states can be very different.

In the simplest of binding models for CAP, the affinity of the protein for its target DNA is directly proportional to the fraction of molecules in the active conformation, as has been seen previously in different contexts for other systems[6–9]. But Tzeng and Kalodimos observed little such correlation. Indeed, the affinities of some of the CAP mutants for DNA are 50-fold greater, and others are 25-fold lower, than would have been predicted on the basis of the numbers of molecules populating the active state. This clearly indicates that static structures alone cannot explain CAP's behaviour.

The authors therefore went on to measure the enthalpic and entropic contributions to the CAP mutants' DNA-binding affinities using a calorimetric technique. Although the resulting data are informative, they are not at atomic resolution and they lump together contributions from a variety of terms. Of these contributions, one of the most useful to evaluate is conformational entropy, which counts the number of states adopted by bonds in CAP's 'backbone' and amino-acid side chains.

To gain more insight into the conformational entropy of the CAP mutants, Tzeng and Kalodimos carried out NMR experiments to quantify the amplitudes of motion of methyl groups in side chains, at the picosecond-to-nanosecond timescale (one picosecond is 10^{-12} seconds). They found that, on DNA binding, some of the mutants undergo very large net changes in conformational entropy that significantly increase the strength of association (Fig. 1b). In other cases, they observed that enthalpy changes drive binding, and that entropy changes oppose it.

Notably, the authors obtained an atomic-level description of how the picosecond–nanosecond dynamics of CAP respond to formation of the CAP–DNA complex from which the conformational entropy change for CAP–DNA binding was established. The pattern that emerges is not simple. For example, large changes in amplitudes of motion might have been expected only at regions close to the binding interface, but such changes extend much farther away, involving methyl groups more than 50 ångströms from the interface. Remarkably, the authors found a strong linear correlation between NMR-derived measures of conformational entropy change and the total change in entropy measured by calorimetry. Such a correlation has previously been observed[10] in the binding of the calmodulin protein to its target peptides.

Tzeng and Kalodimos's findings show that, although entropy changes are crucial for CAP's function, there are other ways of modulating the strength of DNA binding to the protein. Even though the correlation between the fractional population of CAP molecules in the active conformation and DNA-binding affinity is poor, increasing the population that is in the active state still remains one avenue for increasing affinity. The authors' NMR experiments revealed that, of the nine CAP mutants that occupy predominantly inactive structures, two interchange with active conformations on the millisecond timescale; approximately 2% of the molecules are in the active conformation for one of these mutants, and 7% for the other. Unlike the other seven variants, for which active populations could not be detected, these two mutants bound DNA.

Most interestingly, Tzeng and Kalodimos's work suggests an approach for manipulating protein function through allostery. For example, one could imagine targeting a drug to a site in an enzyme that is far removed from the active site, in order to modify the enzyme's function. By combining allosteric drugs of this sort with more traditional pharmaceuticals that bind to active sites, it may be possible to moderate the drug resistance that so often plagues conventional therapies. Understanding the fundamental roles of dynamics in protein function will also facilitate new ways of exploiting proteins and of modifying their activities. Tzeng and Kalodimos's work takes an important step in this direction. ■

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