

only 9% of the (*R/S*)-caDBA added to the aDBA in solution was sufficient to direct the self-assembly process to only clockwise-type interdigitation (Fig. 1b). Furthermore, it was not necessary for (*R/S*)-caDBA to be evenly distributed to exert control over chirality, suggesting that handedness was controlled by the overall hierarchical order rather than just locally via neighbouring molecules. It is also noteworthy that the assembly occurs on an achiral substrate (graphite) of the sort that would be in ample supply in prebiotic conditions, and that the mere presence of adsorption favours the expression of chirality of the achiral molecules.

This mechanism — a hierarchical recognition process through interdigitation interactions of the alkyl chains — was demonstrated using scanning tunnelling microscopy (STM). In contrast to X-ray diffraction, used for 3D structural analysis,

STM does not rely on ensemble averaging to extract periodic features, and therefore does not require high levels of order to produce high-resolution images. This made STM a very useful technique for analysing the 2D porous network formed by the self-assembly of aDBA from solution onto a graphite substrate.

The findings of De Feyter, Tobe and colleagues provide an excellent example of how chiral amplification using a chiral modifier can lead to controlled formation of a homochiral phase from achiral molecules. Beyond the potential implications of such work for understanding issues related to the origins of chirality — and in turn the origins of life — there is a shorter-term potential in separation processes, in which chiral pores can selectively host enantiomers from racemic mixtures. In this context it is also exciting to note that DBAs can coordinate with catalytic metals<sup>9</sup>, which means that

enantioselective catalysis, wherein high enantiomeric excess of the catalyst is achieved by using a dilute chiral ‘sergeant’ is within reach. □

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## PROTEIN DYNAMICS

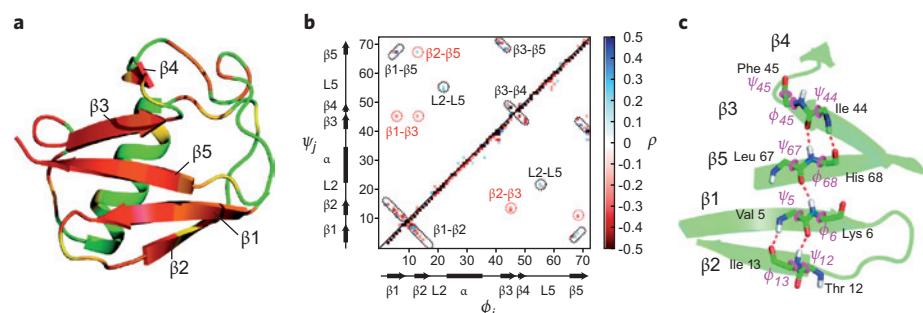
# Whispering within

Dynamic communication between atoms within folded proteins is potentially important for function, but its measurement has been a challenge. Now, a combined NMR and modelling study provides insights on the presence and strengths of such correlations.

Rafael Brüschweiler

Over recent years it has become increasingly clear that under physiological conditions proteins are intrinsically dynamic, and many believe that such internal dynamics can play a key role in their biological function in processes ranging from protein–protein recognition to enzymatic activity. Experimental biophysical techniques, such as NMR spectroscopy<sup>1</sup>, are typically used to study the mobility of individual atoms in a protein with respect to their surroundings, but they tell us little about whether and how atoms dynamically communicate with each other: are the motions of different atoms independent from each other or do they move in an orchestrated (correlated) manner? Writing in the *Journal of the American Chemical Society*, Christian Griesinger, Xavier Salvatella and colleagues<sup>2</sup> put forward an innovative method that combines experimental NMR with a new modelling approach, giving us a glimpse of the correlated atomic motions inside the globular protein ubiquitin at thermal equilibrium.

The 76-amino acid single-domain protein ubiquitin has served as a model system for



**Figure 1** | Dynamic dihedral angle correlations in ubiquitin<sup>2</sup>. **a**, Ribbon diagram of ubiquitin with definition of  $\beta$ -strands, which are coloured with respect to their correlation behaviour — green indicates little correlation ( $\rho \approx 0$ ) and red indicates a correlation with absolute value greater than 0.5 ( $\rho > |0.5|$ ) to a non-sequential residue. **b**, Correlation map of backbone dihedral angle pairs (black ellipses indicate hydrogen-bonded residue pairs; red circles indicate residues with long-range correlations). **c**, Hydrogen-bond network within ubiquitin showing the dynamically correlated dihedral angle pairs. Figure reproduced with permission from ref. 2, © 2011 ACS.

over 25 years for studying protein dynamics by NMR spectroscopy. In principle, certain types of correlated motion can be directly studied using NMR cross-correlated spin-relaxation experiments between pairs of magnetic dipole–dipole interactions, for

example, between two backbone amide N–H bonds<sup>3</sup>. However, because of the short-range nature of this nuclear spin–spin interaction, such effects cannot be observed beyond neighbouring residues. To access a greater range, Griesinger, Salvatella and

colleagues chose an alternative and less direct route. They collated a large body of experimental structural dynamics data on ubiquitin in solution, and used them as input in a computational modelling protocol that they specifically designed to create a set of possible conformations available to the protein. This 640-member ensemble, termed ERNST (for ‘ensemble refinement for native proteins using a single-alignment tensor’), was then rigorously cross-validated against other NMR data that were not used during ensemble generation, indicating that ERNST reproduces the experimental data accurately.

With a conformational ensemble at hand that is consistent with experiments, the authors looked at correlation properties of the ERNST ensemble, including ones that cannot be directly accessed by experiment. For this purpose, they analysed the degree of correlation between different protein parts (Fig. 1a) by determining a circular correlation coefficient,  $\rho$ , for all pairs of backbone  $\varphi, \psi$  dihedral angles. This coefficient can take a value between 1 (fully correlated) and -1 (fully anti-correlated), where  $\rho = 0$  indicates no correlation. Moderately strong correlations ( $\rho \approx -0.5$ ) were found between most consecutive dihedral angles, which include the well-known crankshaft motions between  $\varphi_i$  and  $\psi_{i-1}$  of the amide bonds (Fig. 1b). In addition, a set of weaker correlations ( $\rho \approx -0.3$ ) exist between non-sequential dihedral angles that belong to hydrogen-bonded residues of neighbouring strands in ubiquitin’s five-stranded  $\beta$ -sheet, reminiscent of the behaviour of protein G (ref. 4). Remarkably, a handful of additional long-range correlations could also be identified that bridge non-neighbouring  $\beta$ -strands, such as  $\beta_1-\beta_3$ ,  $\beta_2-\beta_3$  and  $\beta_2-\beta_5$  — indicating that amino acids can dynamically communicate with each other up to a distance of 15 Å, as is the case for Ile13 and Phe45 with  $\rho = -0.2$  (Fig. 1c). Residue Ile44, and other residues involved in these long-range correlations, lie at the binding

interface of ubiquitin when complexed with most ubiquitin-binding domains. Therefore, Griesinger, Salvatella and colleagues hypothesize that the long-range correlations could have a direct functional role during protein recognition through a conformational selection mechanism.

The detection of long-range motional correlations at atomic resolution in a globular protein has been a long-standing challenge. Therefore, the work by Griesinger, Salvatella and colleagues provides important new information about the presence and the strengths of such effects across the single  $\beta$ -sheet of ubiquitin. The reported correlations in which  $|\rho| < 0.3$ , which are small but statistically significant, imply quite ‘noisy’ long-range communication between dihedral angles imposing only weak mutual restrictions. Is the reduction in the conformational search space caused by these correlations biologically relevant during molecular recognition or merely a by-product of the interactions that stabilize the  $\beta$ -sheet? Useful clues can be gleaned from thermodynamic considerations because these correlations alone lower the configurational entropy, independent of the motional amplitudes: for a dihedral angle pair with  $\rho = -0.3$ , the entropy reduction is  $\Delta S = 0.5k_B \log(1-\rho^2) = -0.047k_B$  ( $k_B$  is Boltzmann’s constant), which at physiological temperatures contributes only 0.03 kcal mol<sup>-1</sup> to the absolute free energy. Dihedral angle correlations tend to be conserved during protein binding<sup>5</sup>, therefore their net effect on the binding-free-energy difference should be even smaller. Almost as puzzling as the observed correlations is the paucity of correlation effects (white areas in Fig. 1b) between most of the non-sequential amino acids including amino acids that are in direct contact with each other.

How can the findings of this study<sup>2</sup> be tested? On account of ubiquitin’s size, long all-atom molecular dynamics (MD) computer simulations represent an attractive choice. Interestingly, a  $7 \times 10^5$ -member

ensemble of ubiquitin from a 0.7 μs MD simulation in explicit solvent revealed<sup>6</sup> a pattern of correlated motions between next-neighbour  $\beta$ -strands similar to that of Fig. 1. However, relay correlation effects across the  $\beta_1-\beta_5-\beta_3$  sheet diminish rapidly in the simulation, which leads to the absence of sizable correlations for larger distances (>7 Å). The reason for this discrepancy between MD and ERNST is not clear and it deserves close monitoring — MD simulations are making rapid progress<sup>7</sup> and the ERNST ensemble and related approaches<sup>8–10</sup> are being applied to more proteins. Particularly intriguing is the application of these methods to proteins with allosteric binding properties<sup>11</sup> for which correlation effects should be more pronounced and extend over larger distances. The framework introduced by Griesinger, Salvatella and colleagues represents an important stepping stone that deepens the understanding of intra-protein communication inherent to protein–protein and protein–ligand interaction processes. □

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