

Institution of Washington; P. Olson, Johns Hopkins University) allows the subducted plates to remove heat from the lower mantle but results in little net mass transfer between upper and lower mantle.

The return of subducted plates into the upper mantle is expected to be concentrated in areas of convective upwelling in the lower mantle. These areas, identified by their relatively slow seismic velocities on global tomographic maps of the lower

mantle (Dziewonski) do seem to coincide with areas where young oceanic basalts have anomalous isotope compositions (P.R. Castillo, Carnegie Institution of Washington) suggesting the first direct correlation between features of the lower mantle and the geochemical properties of the Earth's surface. □

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## Protein structure

# What's left out tells the story

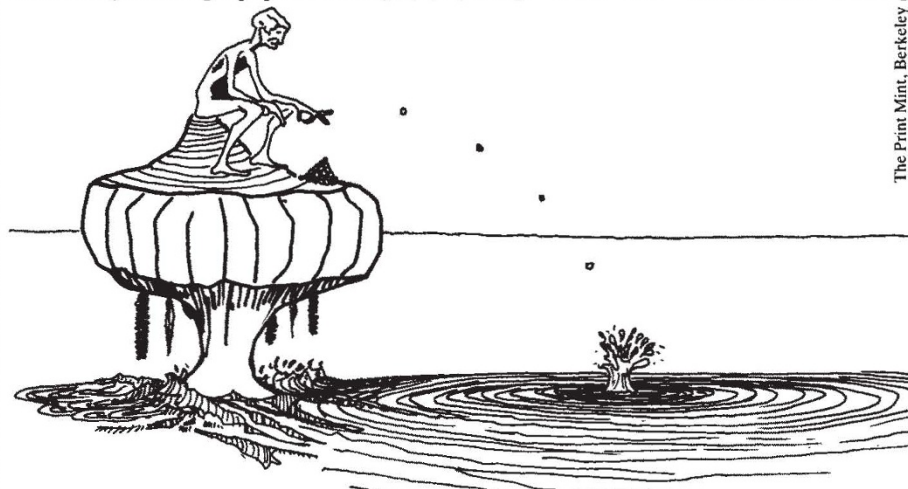
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"RUBIK's nightmare" is a three-dimensional jigsaw puzzle having hundreds of irregular, slightly sticky, solid pieces strung out at precise intervals along a semi-flexible chain. For some, it is a two-person underwater game, where one player tries to disassemble what the other is piecing together<sup>1</sup>. The essence of the problem is that the chain cannot acquire strain as the pieces snap neatly into place without leaving interior voids, much like those neat little packages we call proteins. Two recent papers, one by Alan Fersht and colleagues<sup>2</sup> and the other by Brian Matthews and colleagues on page 406 of this issue<sup>3</sup> chip away at the solution, methyl group by methyl group, by providing direct proof that introducing cavities into proteins leads to less stable structures.

Pauling deduced from the planarity of the peptide bond and the precise geometry of hydrogen-bond donor-acceptor pairs that backbones of proteins could form regular, unstrained helices and sheets despite the chemical diversity of amino-acid side chains — "the law of the chain". Left unexamined, our human tendency would be to read into the appearance of such regularity an underlying basis for stability. In fact, short isolated  $\alpha$ -helices and  $\beta$ -sheets are, with few exceptions, not very stable because solvent water molecules can form hydrogen bonds with the backbone with equal ease<sup>4</sup>. What stabilizes globular proteins in aqueous solution is the net entropy gained as aliphatic side chains are buried in the hydrophobic core<sup>5</sup>. Completing the "law of side chains" is F.M. Richards' canonical dictum, "eschew voids", a compact summary of observations made on many proteins that interior hydrophobic residues are nearly close packed.

Like Newton's laws it is not at once obvious how one should apply these principles to solve specific molecular-design problems which involve, among others, tradeoffs between backbone distortions and solvent entropy. What has hampered attempts to develop a precise analytical

theory of protein stability is the very small free-energy difference between the native and unfolded states, something of the order of only 10 kcal mole<sup>-1</sup> (ref. 6). From the perspective of our main-chain-side chain balancing act this represents approximately the energy required to isomerize just a single peptide bond<sup>7</sup>, or to



*An epitome of the protein stability problem. Exposure of methyl groups, represented as small stones, to solvent leads to instability of the whole structure. Eventually the seated crystallographer is undermined. From an original poster by Hey Mack.*

create a void by exposing a single large hydrophobic group to solvents. A useful notion is that of solvent-accessible surface area, which prescribes a free-energy gain of approximately 20 cal mole<sup>-1</sup> for each square angstrom of buried hydrocarbon surface<sup>8</sup>. Although many tests of this concept have been proposed, including a bold application to a *de novo* protein, each in some way relies on indirect correlation with measurements on the free energy of transfer of amino acids from organic to aqueous solvents<sup>9</sup>.

The new work of Fersht's<sup>2</sup> and Matthews's<sup>3</sup> groups provides some data that bear on the problem. In the experiments of both groups, single-site mutations were made at known positions in proteins resolved at high resolutions so that cavity geometry could be calculated. These values are compared directly with

measurements of free energy obtained by thermal or urea denaturation. In the case of barnase, the protein alterations are as small as possible, removal of just a single methyl group, introducing only the merest complications into the interpretation of the urea denaturation experiments. This is an extremely important point that has been discussed recently in the News and Views article<sup>10</sup> by Walter Kauzmann. (For lysozyme, the changed residue is part of the amino-terminal surface-bound chain.) Given that the chain may play according to its own rules, the mutants were subjected to high-resolution X-ray crystallography to verify residue positions. It is intriguing that the only buried mutant residue (Ile → Val) that crystallizes is the same truncated, or minimal, mutation studied for barnase. Clearly, more analysis of molecular contacts in the crystal are required and perhaps even crystal-melting data will be needed to balance the 'nooks' against the 'crooks'.

Finally, paraphrasing Hesse's *Magister Ludi*, perhaps our "bead game" is moving away from the chain-based folding

problem, maybe only an epiphenomenon, to anticipating all possible side-chain packing combinations and an answer to the question of why nature needs three isochothic side chains<sup>11</sup>. □

- Harrison, S.C. & Durbin, R. *Proc. natn. Acad. Sci. U.S.A.* **82**, 4028–4030 (1985).
- Kellis Jr, J.T., Nyberg, K., Šali, D. & Fersht, A.R. *Nature* **333**, 784–786 (1988).
- Matsumura, M., Becktel, W.J. & Matthews, B.W. *Nature* **334**, 406–410 (1988).
- Shoemaker, K.R. *et al. Proc. natn. Acad. Sci. U.S.A.* **82**, 2349–2353 (1985).
- Kauzmann, W. *Adv. Prot. Chem.* **14**, 1–63 (1959).
- Pace, C.N. *Meth. Enzym.* **131**, 266–279 (1986).
- Winkler, F.K. & Dunitz, J.D. *J. molec. Biol.* **59**, 169–182 (1971).
- Lee, B. & Richards, F.M. *J. molec. Biol.* **55**, 379 (1971).
- Eisenberg, D. & McLachlan, A.D. *Nature* **319**, 199 (1986).
- Kauzmann, W. *Nature* **325**, 763–764 (1987).
- Ponder, J.W. & Richards, F.M. *J. molec. Biol.* **193**, 775–791 (1987).

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