determining a point in energy parameter space usually referred to as the quasi-chemical method [12]. Other approaches are also possible [7–15]. For example, given that the physical interpretation of the effective energy parameters is not straightforward, one can, at least temporarily, avoid being concerned about it and ask whether there is at all a set of energy parameters such that the experimental stability changes owing to mutations are exactly equal to the calculated ones. If such a set exists then the road is paved and one can hope to generalize the result to compute stabilities that have not yet been measured. Carter *et al.* [11] have shown that four-body energetics [16] is a promising improvement over two-body energetics and that further study on this route is likely to bear fruit.

So, can we predict the effect of a small perturbation on an enzymatic process? This question is among the most fascinating in protein science [17]. For example, altering a single group can enhance the binding affinity of an enzyme to its substrate, reduce it or sometimes even nullify it altogether [18]. Our increasing ability to accurately predict such outcome is opening the way to the systematic improvement of existing enzymes by computational protein engineering, for drug manufacturing and biocatalysis.

References

1 Kuchner, O. and Arnold F.H. (1997) Directed evolution of enzyme catalysis. *Trends Biotechnol.* 15, 523–530
2 Dahiyat, B.I. and Mayo, S.L. (1997) *De novo* protein design: fully automated sequence selection. *Science* 278, 82–87
3 Lazaridis, T. and Karplus, M. (1997) 'New view' of protein folding reconciled with the old though multiple unfolding simulations. *Science* 278, 1928–1931
4 Wang, W. *et al.* (2001) Biomolecular simulations: recent developments in force fields, simulations of enzyme catalysis, protein–ligand, protein–protein and protein–nucleic acids noncovalent interactions. *Annu. Rev. Biop. Biomol. Struct.* 30, 211–243
5 Guerois, R. and Serrano, L. (2000) The SH3-fold family: experimental evidence and prediction of variations in the folding pathways. *J. Mol. Biol.* 304, 967–982
6 Wlodawer, A. and Vondrasek, J. (1998) Inhibitors of HIV-1 protease: a major success of structure-assisted drug design. *Annu. Rev. Biophys. Biomol. Struct.* 27, 249–284
7 Cota, E. *et al.* (2000) Two proteins with the same structure respond very differently to mutation: the role of plasticity in protein stability. *J. Mol. Biol.* 302, 713–725
8 Vendruscolo, M. and Domany, E. (1998) Pairwise contact potentials are unsuitable for protein folding. *J. Chem. Phys.* 109, 11101–11108
9 Park, K. *et al.* (2000) Towards an energy function for the contact map representation of proteins. *Proteins* 40, 237–248
10 Banavar, J.R. and Maritan, A. (2001) Computational approach to the protein-folding problem. *Proteins* 42, 433–435
11 Carter, C.W. *et al.* (2001) Four-body potentials reveal protein-specific correlations to stability changes caused by hydrophobic core mutations. *J. Mol. Biol.* 311, 625–638
12 Lu, H. and Skolnick, J. (2001) A distance-dependent atomic knowledge-based potential for improved protein structure selection. *Proteins* 44, 223–232
13 Xia, Y. and Levitt, M. (2001) Extracting knowledge-based energy functions from protein structures by error rate minimization: comparison of methods using lattice model. *J. Chem. Phys.* 113, 9318–9330
14 Koretke, K.K. *et al.* (1998) Self-consistently optimized energy functions for protein structure prediction using molecular dynamics. *Proc. Natl. Acad. Sci. U. S. A.* 96, 2332–2337
15 Mirny, L.A. and Shakhnovich, E.I. (1996) How to derive a protein folding potential? A new approach to an old problem. *J. Mol. Biol.* 264, 1164–1169
16 Liwo, A. *et al.* (2001) Cumulant-based expression for the multibody terms for the correlation between local and electrostatic interactions in the united-residue force field. *J. Chem. Phys.* 115, 2323–2347
17 Fersht, A.R. (1999) *Structure and mechanism in protein science: A guide to enzyme catalysis and protein folding.* W.H. Freeman & Co. New York
18 Wen, J. *et al.* (1996) Exploring the allowed sequence space of a membrane protein. *Nat. Struct. Biol.* 3, 141–148

Michele Vendruscolo
University Chemical Laboratory, University of Cambridge, Lensfield Road, Cambridge, UK CB2 1EW.
e-mail: mv245@cus.cam.ac.uk

Energetics of enzyme stability

Response from Carter, Tropsha and Edgell

We welcome the 'Research News' article about our recent paper [1] establishing a connection between four-body database-derived likelihood potentials and experimental mutational free energy changes.

'...a decisive new coherence to side-chain packing analysis.'

The Delaunay tetrahedron is the simplest three-dimensional packing motif; we noted in our article, and presented more fully elsewhere [2] that, unlike potentials of smaller dimensionality, joint consideration of four interacting side chains achieves excellent proportionality between statistical and thermodynamic four-body potentials (calculated by summing the transfer free energies, or hydrophobicities, of the four interacting side chains). Decomposing tertiary structure into elementary three-dimensional simplices therefore appears to afford a decisive new coherence to side-chain packing analysis.

Practical use of higher-dimensional potentials may also benefit from our observation that mutational $\Delta(\Delta G_{unfold})$ values for different proteins scale differently to the four-body potentials. This seemingly counter-intuitive result reflects the fact that different proteins employ hydrophobic cores with different numbers of contributors, on average, per residue. Thus, even if two hydrophobic core mutations have similar contexts in different proteins, the observed free energies are often different, owing to different proportionate changes in the overall contributions made by hydrophobic bonding to stability. In Fig. 1, this effect is illustrated graphically for two valine to alanine mutations in staphylococcal nuclease (STN; blue) and chymotrypsin inhibitor 2 (CI2; gold). Side chains surrounding the mutated residues are similar enough in the two proteins that the predicted likelihood potentials are

 0167-7799/02/$ – see front matter PII: S0167-7799(01)01872-8

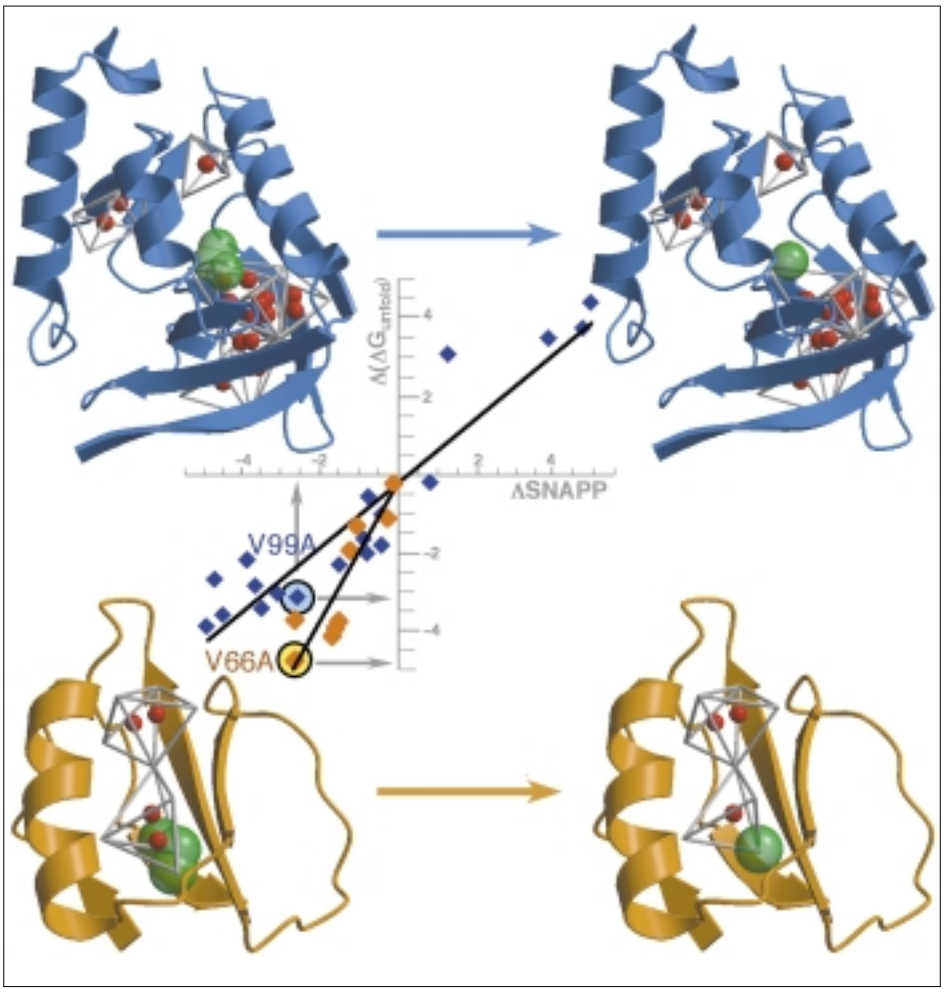

Fig. 1. Identical valine (V) to alanine (A) hydrophobic core mutations (green spheres) change the experimental free energy of unfolding [$\Delta(\Delta G_{unfold}$]; vertical axis) by different amounts at residue 66 of chymotrypsin inhibitor-2 (V66A; gold) and residue 99 of staphylococcal nuclease (V99A; blue). Quantitative analysis of the same mutants using four-body statistical potentials (ΔSNAPP; horizontal axis) suggests that the two mutations should induce the same degree of destabilization. The observed difference is a scaling effect arising systematically from differences in the proportionate impact of mutation indicated by the two slopes. The slopes, in turn, are inversely related to the relative contribution of hydrophobic bonding to stability in the two proteins, suggested graphically here by the density of grey cages and red spheres.

the same. The observed values, however, are different because that in CI2 represents a larger proportionate change than does the same mutation in STN. The differences are reflected in the two slopes relating mutant free-energy changes in the two different proteins to the statistical potential, showing that the two mutations are drawn from systematically distinct populations.

References

1 Carter, C.W. *et al.* (2001) Four-body potentials reveal protein-specific correlations to stability changes caused by hydrophobic core mutations. *J. Mol. Biol.* 311, 625–638

2 Cammer, S. *et al.* Identification of sequence-specific tertiary packing motifs in protein structures using Delaunay tessellation. In *Lecture Notes in Computational Science and Engineering* (Schlick, T., ed). Springer-Verlag, New York (in press)

Charles W. Carter, Jr.*
Dept of Biochemistry and Biophysics, CB 7260, University of North Carolina, Chapel Hill, NC 27599-7260, USA.
*e-mail: carter@med.unc.edu

Alex Tropsha
Laboratory for Molecular Modeling, CB 7360, University of North Carolina, Chapel Hill, NC 27599-7360, USA.

Marshall Edgell
Dept of Microbiology and Immunology, CB 7290, University of North Carolina, Chapel Hill, NC 27599-2790, USA.

Meeting Report

Honey I've shrunk biomedical technology!

Suzanne Berry

The Second Annual BioMEMS and Biomedical Nanotechnology World 2001 conference was held 22–25 September in Columbus, Ohio, USA. The conference was organised by Mauro Ferrari and the Cambridge Healthtech Institute.

Biomedical devices are getting smaller and the time when tiny machines will be flowing through our bloodstream targeting cells for treatment is not as far away as we might think. Neither are point-of-care diagnostic and analytical devices. Bio-microelectromechanical systems (bioMEMS) usually contain sensors, actuators, mechanical structures and electronics and are, in general, made from silicon. Such systems are being developed as diagnostic and analytical devices at an incredibly rapid speed. BioMEMS sensors and tools such as lab-on-a-chip will not only lead to 'point-of-care' assessments but also will take diagnosis out of the doctor's hands and into the hands of the patient.

This conference covered topics including micro- and nanotechnologies for drug discovery, tissue engineering, proteomics, microfluidics, biosensors, molecular assembly and integrated systems.

Bioassays and chips of the future

Many technologies from the field of electronics have not been exploited in biomedical fields, but bioassays of the future will integrate fluid, electronic and optical tools. Researchers are now moving from conventional microfluidics to polymer

 0167-7799/02/$ – see front matter PII: S0167-7799(01)01901-1