



Figure 1 | Plots of isotropic B factors calculated for protein structures solved in both the native and methylated states. (a) Putative HopJ protein VP0580 (Protein Data Bank (PDB) identifiers 2QHQ (methylated) and 2QM2 (native)). (b) Flavodoxin FldA (PDB identifier 2ARK (methylated)). (c) Gfo/Idh/MocA family oxidoreductase SP1482 (PDB identifier 2H03 (methylated) and 2H05 (native)). (d) Extracellular domain of arabinofuranosyltransferase DIP0159 (PDB identifier 2IDL (methylated)).

Note: Supplementary information is available on the Nature Methods website.

ACKNOWLEDGMENTS

We thank all members of the Structural Biology Center and Midwest Center for Structural Genomics at Argonne National Laboratory for their help in conducting these experiments, S. Li, C.B. Lindberg and C. Giometti for running mass spectrometry experiments, A. Edwards for reading and commenting on the drafts, and L. Butler for preparing the manuscript. This work was supported by US National Institutes of Health (GM62414, GM074942) and by the US Department of Energy, Office of Biological and Environmental Research, under contract DE-AC02-06CH11357.

Youngchang Kim¹, Pearl Quartey¹, Hui Li¹, Lour Volkart¹, Catherine Hatzos¹, Changsoo Chang¹, Boguslaw Nocek¹, Marianne Cuff¹, Jerzy Osipiuk¹, Kemin Tan¹, Yao Fan¹, Lance Bigelow², Natalia Maltseva¹, Ruiying Wu¹, Maria Borovilos², Erika Duggan², Min Zhou¹, T Andrew Binkowski¹, Rong-guang Zhang¹ & Andrzej Joachimiak¹

¹Midwest Center for Structural Genomics, Biosciences Division, Argonne National Laboratory, 9700 S. Cass Ave., Argonne, Illinois 60439, USA. ²Midwest Center for Structural Genomics, On Assignment, Labsupport Services, 9450 W. Bryn Mawr Avenue, Rosemont, Illinois 60018, USA. e-mail: andrzej@anl.gov

- D'Arcy, A., Stihle, M., Kostrewa, D. & Dale, G. *Acta Crystallogr. D Biol. Crystallogr.* **55**, 1623–1625 (1999).
- Rypniewski, W.R., Holden, H.M. & Rayment, I. *Biochemistry* **32**, 9851–9858 (1993).
- Walter, T.S. *et al. Structure* **14**, 1617–1622 (2006).
- Shaw, N. *et al. BMC Struct. Biol.* **7**, 46 (2007).
- Means, G.E. *Methods Enzymol.* **47**, 469–478 (1977).
- Means, G.E. & Feeney, R.E. *Anal. Biochem.* **224**, 1–16 (1995).
- Canaves, J.M., Pagea, R., Wilson, I.A. & Stevens, R.C. *J. Mol. Biol.* **344**, 977–991 (2004).
- Kyte, J. & Doolittle, R.F. *J. Mol. Biol.* **157**, 105–132 (1982).
- Kobayashi, M., Kubota, M. & Matsuura, Y. *Acta Crystallogr. D Biol. Crystallogr.* **55**, 931–933 (1999).
- Schubot, F.D. & Waugh, D.S. *Acta Crystallogr. D Biol. Crystallogr.* **60**, 1981–1986 (2004).
- Means, G.E. & Feeney, R.E. *Bioconjug. Chem.* **1**, 2–12 (1990).
- Kim, Y. *et al. J. Struct. Funct. Genomics* **5**, 111–118 (2004).
- Magalhaes, A. *et al. J. Protein Chem.* **13**, 195–215 (1994).

- Anashkina, A., Kuznetso, E., Esipova, N. & Tumanyan, V. *Proteins* **67**, 1060–1077 (2007).
- Glaser, F., Steinberg, D.M., Vakser, I.A. & Ben-Tal, N. *Proteins* **43**, 89–102 (2001).
- Juers, D.H. & Matthews, B.W. *J. Mol. Biol.* **311**, 851–862 (2001).

A pilot project to generate affinity reagents to human proteins

To the editor: There is a great need to generate protein-specific affinity reagents to explore the human proteome. High-throughput methods to generate renewable antibodies or other affinity reagents are still unproven, and therefore there is a need to explore systematic production of well-characterized sets of protein binders, to ultimately generate antibodies or other affinity reagents to all human proteins as a sustainable resource.

This was the background for a workshop organized by the Structural Genomics Consortium¹, the Human Protein Atlas program² and the EU ProteomeBinders network program³, and held in Stockholm in March 2008. Representatives of scientists and funders from more than 20 countries met to discuss demonstration projects to investigate the possibility of systematically generating renewable protein binders. The workshop participants agreed to launch a pilot study to generate—in a decentralized and coordinated manner—protein binders to the human SH2-containing proteins, taking advantage of a strong biological community with a commitment to take part in the binder evaluation. The first objective was to generate antigens to 22 of the human SH2-containing proteins based on the availability of both soluble protein domains¹ and partially unfolded recombinant protein fragments (protein epitope signature tags or PrESTs) selected because of their low homology to other human proteins². In this way, it will be possible to compare the

generation of renewable affinity reagents using alternative antigens and different approaches. At present, soluble domains have been generated to 20 and PrEST fragments to 11 of the protein targets (Supplementary Table 1 online). These purified protein antigens, now available in milligram quantities, have been distributed to research groups willing to generate affinity reagents within the framework of the pilot project. These affinity reagents will subsequently be validated and compared in a centralized manner using, for example, protein arrays, western blots and immunohistochemistry analyses.

To date, seven research groups in Europe, Asia, North America and Australia, working with different technologies to generate renewable affinity reagents, spanning from hybridoma technology to recombinant protein scaffolds, have joined the project. A second workshop will be held in March 2009 to summarize and evaluate the first phase of the pilot project. All research groups interested in participating either in the binder generation or in

the validation phase, or who want to be informed about the progress of the study can contact us or any of the 17 members of the steering committee (Supplementary Table 2 online).

Note: Supplementary information is available on the Nature Methods website.

Mathias Uhlen¹, Susanne Gräslund² & Michael Sundström³

¹School of Biotechnology, Royal Institute of Technology, AlbaNova University Center, SE-10691 Stockholm, Sweden. ²The Structural Genomics Consortium, Schéeles väg 2, Karolinska Institute, SE-171 77 Stockholm, Sweden. ³The Novo Nordisk Foundation Center for Protein Research, University of Copenhagen, Blegdamsvej 3B, DK-2200 Copenhagen, Denmark.
e-mail: mathias@biotech.kth.se or susanne.graslund@ki.se or michael.sundstrom@cpr.ku.dk

1. Gileadi, O. *et al. J. Struct. Funct. Genomics* **8**, 107–119 (2007).
2. Berglund, L. *et al. Mol. Cell. Proteomics*, published online 31 July 2008 (doi:10.1074/mcp.R800013-MCP200).
3. Taussig, M.J. *et al. Nat. Methods* **4**, 13–17 (2007).