

## Community Page

# Putting the Pieces Together: Integrative Modeling Platform Software for Structure Determination of Macromolecular Assemblies

Daniel Russel<sup>1</sup>, Keren Lasker<sup>1,2</sup>, Ben Webb<sup>1</sup>, Javier Velázquez-Muriel<sup>1</sup>, Elina Tjioe<sup>1</sup>, Dina Schneidman-Duhovny<sup>1</sup>, Bret Peterson<sup>3</sup>, Andrej Sali<sup>1\*</sup>

**1** Department of Bioengineering and Therapeutic Sciences, Department of Pharmaceutical Chemistry, California Institute for Quantitative Biosciences (QB3), University of California, San Francisco, San Francisco, California, United States of America, **2** Raymond and Beverly Sackler Faculty of Exact Sciences, Blavatnik School of Computer Science, Tel Aviv University, Tel-Aviv, Israel, **3** Google, Mountain View, California, United States of America

## Introduction

Building models of a biological system that are consistent with the myriad data available is one of the key challenges in biology. Modeling the structure and dynamics of macromolecular assemblies, for example, can give insights into how biological systems work, evolved, might be controlled, and even designed. Modeling can also suggest future experiments. Unfortunately, current publishing norms make it hard to build on published models, because such models are often not available in usable form and because it is hard to publish refinements of others' models. Here, we present steps towards a future in which a scientist can read a paper, download a script, add new data, and see how the new data improve the published model. Integrative structure modeling casts the building of structural models as a computational optimization problem, for which information about the assembly is encoded into a scoring function that evaluates candidate models. We describe our software suite, Integrated Modeling Platform, and invite members of the scientific community to use it, improve on it, and apply it to their own scientific problems of interest.

Numerous structures have to date been solved by using an integrative structural modeling approach. The structure of the 26S proteasome was determined from an electron microscopy (EM) map of the whole assembly, proteomics data about its subunit composition, and comparative protein structure models of the component proteins [1]. The structure of the bacterial type II pilus was assembled from sparse

nuclear magnetic resonance (NMR) data and X-ray crystallographic structures of constituent proteins [2]. The structure of chromatin around the alpha-globin gene was assembled from so-called 5C data (chromosome conformation capture carbon copy) [3]. The value of integrative modeling is illustrated by its application to the yeast nuclear pore complex (NPC) [4,5]. The sheer size and flexibility of the NPC makes it all but impossible to solve its molecular architecture by conventional atomic resolution techniques, such as X-ray crystallography. However, integrating information from multiple sources, including stoichiometry from protein quantification, protein proximities from subcomplex purification, protein positions from immuno-EM, sedimentation analysis that sheds light on protein and subcomplex shapes, and the overall NPC shape from EM, resulted in an ensemble of medium-resolution models. The models were summarized by a 3-D probability map, resembling an EM map and localizing the 456 constituent proteins with an average precision of approximately 5 nm. This map has revealed fundamental new insights into the function of the NPC as a gatekeeper controlling the entry into and

exit from the nucleus of macromolecules, and also shed light on its evolution [4,6–8].

Integrative modeling entails a computational encoding of the standard scientific cycle of gathering data, proposing hypotheses, and then gathering more data to test and refine those hypotheses. It proceeds through repeated iterations of the stages of gathering information, choosing how to represent and evaluate models, finding models that score well, and analyzing the models and information (Figure 1; Box 1). The cycle terminates when a convergent ensemble of models is found fitting the current information and the models have been judged to be satisfactory [9]. When new information is gathered, whether by other scientists or other techniques, the cycle is resumed.

The integrative approach has a number of advantages over informal or partial consideration of available information (Box 2). Fully realizing these advantages requires encoding modeling efforts into integrative modeling applications that consist of the scripts and the associated information. Adoption of integrative modeling can occur through a tight collaboration between a computational lab and an

---

**Citation:** Russel D, Lasker K, Webb B, Velázquez-Muriel J, Tjioe E, et al. (2012) Putting the Pieces Together: Integrative Modeling Platform Software for Structure Determination of Macromolecular Assemblies. PLoS Biol 10(1): e1001244. doi:10.1371/journal.pbio.1001244

**Published:** January 17, 2012

**Copyright:** © 2012 Russel et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

**Funding:** The work was funded by NIH grants R01 GM083960, PN2 EY016525, and U54 RR022220. The research of Keren Lasker was supported by continuous mentorship from Prof. Haim J. Wolfson as well as a fellowship from the Clore Foundation PhD Scholars program. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Competing Interests:** The authors have declared that no competing interests exist.

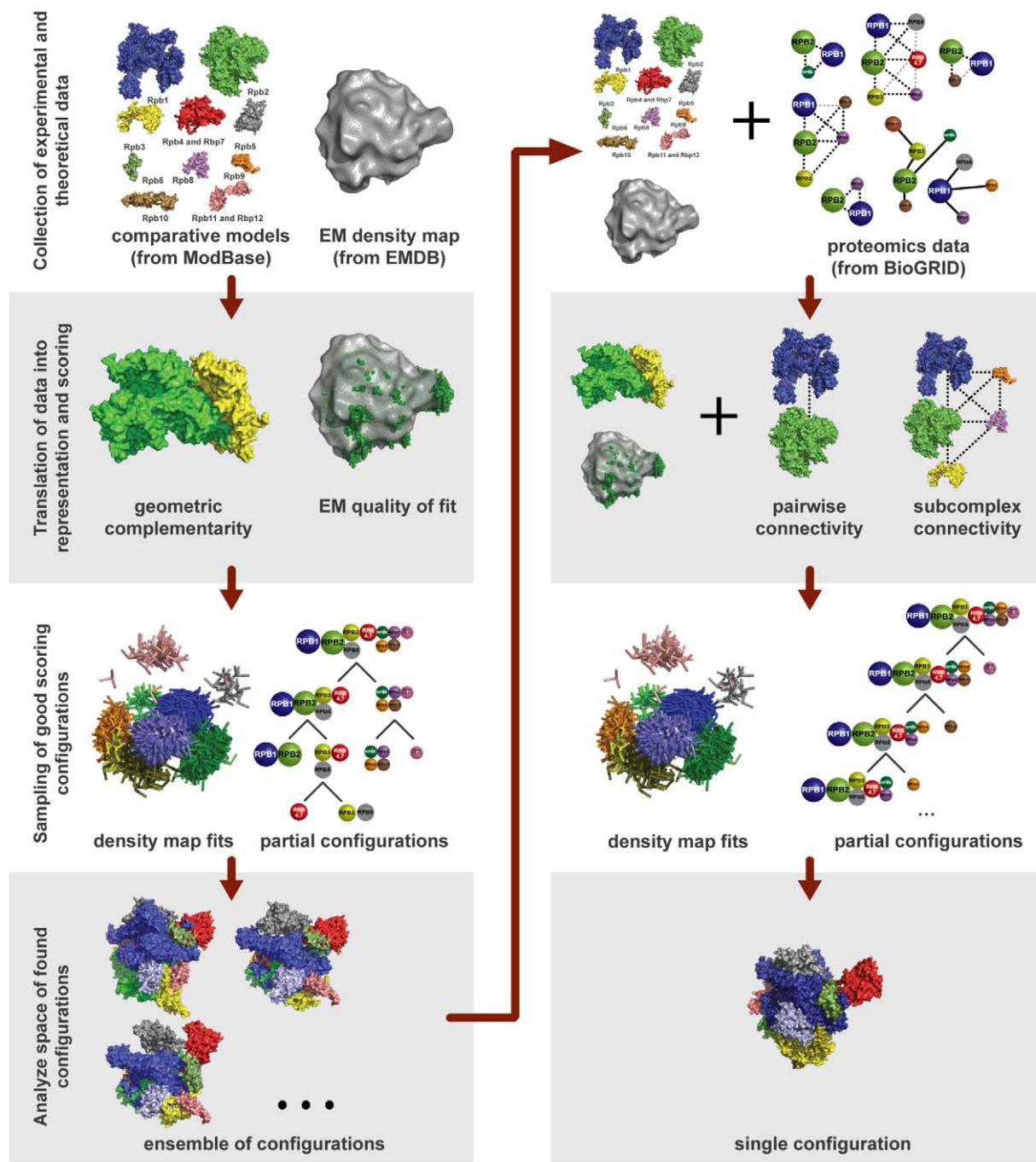
**Abbreviations:** EM, electron microscopy; IPM, Integrative Modeling Platform; NPC, nuclear pore complex; SAXS, small angle X-ray scattering

\* E-mail: sali@salilab.org

---

The Community Page is a forum for organizations and societies to highlight their efforts to enhance the dissemination and value of scientific knowledge.





**Figure 1. Integrative structure modeling of the human RNA Polymerase II [10].** The first round of modeling was performed using only the 2nm EM density map of the assembly from EMDB [51] and subunit comparative models from ModBase [47], on the basis of the crystallographic structures of the yeast RNAPII proteins. The data were found to be insufficient to uniquely resolve the structure. To overcome this challenge, protein interaction networks extracted from BioGrid [48] were added. The addition of these data resulted in a single structure. The scripts are available as part of IMP. doi:10.1371/journal.pbio.1001244.g001

experimental lab, through direct adoption by an experimental lab, or by experimentalists modifying existing integrative modeling applications. To facilitate widespread adoption, we have developed the Integrative Modeling Platform (IMP) software package.

## A Platform for Integrative Modeling

The IMP software package facilitates the writing of integrative modeling applications; the development of new model representations, scoring functions, sam-

pling schemes, and analysis methods; and the distribution of integrative modeling applications.

In IMP, models are encoded as collections of particles, each representing a piece of the system. Depending on the data available, particles can be used to create

## **Box 1. The Four Stages of the Integrative Modeling Cycle.**

**Stage 1: Gathering Information.** Information is collected in the form of data from wet lab experiments, as well as statistical tendencies such as atomic statistical potentials, physical laws such as molecular mechanics force fields, and any other feature that can be converted into a score for use to assess features of a structural model.

**Stage 2: Choosing How To Represent And Evaluate Models.** The resolution of the representation depends on the quantity and resolution of the available information and should be commensurate with the resolution of the final models: different parts of a model may be represented at different resolutions, and one part of the model may be represented at several different resolutions simultaneously. The scoring function evaluates whether or not a given model is consistent with the input information, taking into account the uncertainty in the information.

**Stage 3: Finding Models That Score Well.** The search for models that score well is performed using any of a variety of sampling and optimization schemes (such as the Monte Carlo method). There may be many models that score well if the data are incomplete or none if the data are inconsistent due to errors or unconsidered states of the assembly.

**Stage 4: Analyzing Resulting Models and Information.** The ensemble of good-scoring models needs to be clustered and analyzed to ascertain their precision and accuracy, and to check for inconsistent information. Analysis can also suggest what are likely to be the most informative experiments to perform in the next iteration.

Integrative modeling iterates through these stages until a satisfactory model is built. Many iterations of the cycle may be required, given the need to gather more data as well as to resolve errors and inconsistent data.

atomic, coarse-grained, or hierarchical representations. It is straightforward to represent a protein at any resolution, from fully flexible atomic models (one particle per atom), to rigid bodies, to coarse-grained models consisting of only one or a few particles for the whole protein (see Figure 1 for a worked-through example, structural modeling of the human RNA polymerase II [10]). Different parts of the model can be represented differently, as dictated by the available information. Each particle has associated attributes, such as coordinates, radius, atom type, rigid body composition, residue information, and mass. If the attributes already in IMP are not sufficient, new attributes can be created and used similarly to the predefined ones. For example, for coarse-grained small angle X-ray scattering (SAXS) scoring, a scattering factor attribute could be associated with the particles representing amino acid residues.

Candidate models are evaluated by a scoring function composed of terms called restraints, each of which measures how well a model agrees with the information from which the restraint was derived. The restraints encode both what is known about structures in general and what is known about this particular structure. Thus, a candidate model that scores well is consistent with all used information. The precision and accuracy of the resulting model increases with the amount and quality of information that is encoded in the restraints. IMP's ever-growing set of scoring function types includes ones for SAXS profiles [11], proteomics data [9], EM images and density maps [10,12], NMR spectroscopy [2], the CHARMM force-field [13], alignment with related structures [14], and a variety of statistical potentials [15]. IMP has been designed to make it easy for others to develop, use, and distribute new restraints. Other research groups are currently implementing restraints for various mass spectrometry measurements, SAXS, 5C data [3], and atomic structure prediction.

For experimental data, the scoring is generally implemented using a “forward model” [16], which simulates the measurements on the basis of the candidate model and then compares the simulated measurements to the actual measurements. For example, to evaluate the fit to an EM density map, a restraint uses the coordinates, radii, and masses of a set of particles representing the assembly to simulate its density map and then evaluates the cross-correlation with the experimental map.

## **Box 2. Advantages of the Integrative Structure Modeling Approach.**

**Using New Information.** Integrative modeling makes it easy to take advantage of new information and new types of information, resulting in a low barrier for using incremental information that is generally not applied to structure characterization. Even when a single data type is relatively uninformative, multiple types can give a surprisingly complete picture of an assembly [9,10].

**Maximizing Accuracy, Precision and Completeness.** Integrative models fit multiple types of information, and can thus be more accurate, precise, and complete than models based on the individual sources.

**Understanding and Assessing the Models.** By exhaustively sampling the space of models fitting the information, integrative modeling can find all models fitting the information, not only one. A full sampling of the models of a structure can improve the understanding of its function [49]. Because the data are encoded in scoring functions and the full set of models can be found, integrative modeling facilitates assessing the input information and output models in terms of precision and accuracy.

**Planning Experiments.** Integrative modeling provides feedback to guide future experiments, by computationally testing the impact of hypothetical datasets. As a result, experiments can be chosen to best improve our knowledge of the assembly.

**Understanding and Assessing Experimental Accuracy.** Data errors present a challenge for all methods of model building. Integrative modeling can detect inconsistent data as no models will exist that fit all the data. In addition, integrative modeling facilitates the application of more sophisticated methods for error estimation, such as Inferential Structure Determination [16].



### Box 3. Key Requirements for Integrative Modeling Infrastructure.

**Modular structure.** To allow a community of developers to easily add sources of information, sampling schemes or analysis methods, IMP is structured as a collection of self-contained modules that can be developed and distributed independently.

**Simple abstractions.** Having a set of simple common abstractions allows independently written modules to be used together. The restraint/scoring function abstraction provides one such, allowing arbitrary data to be combined. Representing models as collections of particles with associated data provides another, allowing easy mixing of coarse grained and atomic models.

**Easy sharing.** IMP provides a platform-independent high level scripting interface for writing integrative modeling applications from data to analysis; this reduces the burden of supporting the applications and so reduces the cost of sharing.

**Higher level entry points.** IMP provides a set of high level tools to facilitate application of established protocols to new systems. These include Multifit for assembling multiple subunits based on an EM density map, proteomics data and molecular docking [10], and FoXS for computing a SAXS profile of a given structure [50], both of which can be used via web interfaces, from Chimera [24] or from the command line.

As with most computational structure efforts, the main demand for computational time in integrative modeling comes from sampling models that satisfy the restraints (good-scoring models). IMP provides a wide variety of tools for building these sampling protocols, including optimization algorithms such as Monte Carlo [17] and conjugate gradients [18], the simplex optimizer from Gnu Scientific Library (GSL) [19], simulation schemes such as molecular dynamics and Brownian dynamics [20], and the Bullet rigid body dynamics engine (<http://www.bulletphysics.com>), as well as full sampling schemes such as the Gibbs sampler [16], replica exchange [21], and a divide-and-conquer sampler called DOMINO [22].

Finally, IMP provides a variety of tools for comparing, clustering, and analyzing models. These tools can be used to check for quality-of-fit, the existence of multiple states of the system [3], and inconsistent information. Models can be clustered on the basis of root-mean-square deviation (RMSD), placement score [11], and various other metrics. Supported clustering algorithms include k-means, centrality

betweenness clustering [23], and simple binning. The resulting clusters and the constituent models as well as restraints can be exported to Chimera [24] and Pymol [25] for visual inspection and further analysis.

IMP has been used to produce a number of models; for example, a eukaryotic ribosome [26], a mammalian ribosome [27], a ryanodine receptor channel [28], the 26S proteasome [1], the Hsp90 chaperonin [29], the TRiC/CCT chaperonin [30], the actin-scrum complex [31], chromatin [3], and the NPC [4]. More information about IMP can be found at <http://integrativemodeling.org/>. The website provides a technical introduction, a tutorial, as well as a variety of examples to help users get started. In addition, it contains nightly tests, user and developer email lists, a wiki, and a bug tracker.

### Towards Open Structure Modeling

Publication of macromolecular structures has evolved from printed words and

pictures to include deposition of coordinates in the Protein Data Bank [32], and more recently deposition of raw input data such as X-ray scattering factors [32], NMR restraints [33], and EM particle images [34]. However, the conversion of the raw data to the final structures is often only briefly described and all too rarely available in a directly usable form [35–37], making reproduction and use of the published results laborious or even impossible.

If published papers included integrative modeling applications, a wide variety of researchers would benefit. In particular, experimental labs, which are unlikely otherwise to go through the effort of modeling systems themselves, would be able to use the state-of-the-art model to plan experiments by simulating potential benefits gained from new data. It would also be easy to see how much each new measurement contributes to and fits with the current model. Other computational groups could more easily experiment with new scoring, sampling, and analysis methods, without having to reimplement the existing methods from scratch. The common abstraction would make it easier to mix and match parts of other modeling packages [13,14,16,38–46] to improve the applications of integrative modeling. Finally, the authors themselves would maximize the impact of their work, increasing the odds that their results are incorporated into future modeling.

### Acknowledgments

We thank Frank Alber and Friedrich Förster for their contributions to the development of the integrative modeling paradigm; Ben Schwarz and Yannick Spill for contributing to the IMP code and design; Riccardo Pellarin, Massimiliano Bonomi, and Ben Schwarz for insightful comments on early iterations of the paper; and Jeremy Phillips, Frank Alber, Friedrich Förster, Marc Marti-Renom, and Davide Baù for being early users of the IMP library.

### References

1. Lasker K, Förster F, Bohn S, Walzhoeni T, Villa E, et al. (2012) Molecular architecture of the 26S proteasome holocomplex determined by an integrative approach. *Proc Natl Acad Sci U S A*: In press.
2. Simon B, Madl T, Mackereth CD, Nilges M, Sattler M (2010) An efficient protocol for NMR-spectroscopy-based structure determination of protein complexes in solution. *Angewandte Chemie (International ed in English)* 49: 1967–1970.
3. Baù D, Sanyal A, Lajoie BR, Capriotti E, Byron M, et al. (2010) The three-dimensional folding of the  $\alpha$ -globin gene domain reveals formation of chromatin globules. *Nature Struct Biol* 18: 107–114.
4. Alber F, Dokudovskaya S, Veenhoff LM, Zhang W, Kipper J, et al. (2007) The molecular architecture of the nuclear pore complex. *Nature* 450: 695–701.
5. Alber F, Dokudovskaya S, Veenhoff LM, Zhang W, Kipper J, et al. (2007) Determining the architectures of macromolecular assemblies. *Nature* 450: 683–694.
6. Wente SR, Rout MP (2010) The nuclear pore complex and nuclear transport. *Cold Spring Harb Perspect Biol* 2: a000562.
7. Devos D, Dokudovskaya S, Williams R, Alber F, Eswar N, et al. (2006) Simple fold composition and modular architecture of the nuclear pore complex. *Proc Natl Acad Sci U S A* 103: 2172–2177.



8. DeGrasse JA, DuBois KN, Devos D, Siegel TN, Sali A, et al. (2009) Evidence for a shared nuclear pore complex architecture that is conserved from the last common eukaryotic ancestor. *Mol Cell Prot* 8: 2119–2130.
9. Alber F, Förster F, Korkin D, Topf M, Sali A (2008) Integrating diverse data for structure determination of macromolecular assemblies. *Annu Rev Biochem* 77: 443–477.
10. Lasker K, Phillips JL, Russel D, Velazquez-Muriel J, Schneidman-Duhovny D, et al. (2010) Integrative structure modeling of macromolecular assemblies from proteomics data. *Mol Cell Prot* 9: 1689–1702.
11. Schneidman-Duhovny D, Hammel M, Sali A (2011) Macromolecular docking restrained by a small angle X-ray scattering profile. *J Struct Biol* 173: 461–471.
12. Lasker K, Sali A, Wolfson HJ (2010) Determining macromolecular assembly structures by molecular docking and fitting into an electron density map. *Proteins* 78: 3205–3211.
13. Brooks BR, Brooks CL, Mackerell AD, Nilsson L, Petrella RJ, et al. (2009) CHARMM: the biomolecular simulation program. *J Comput Chem* 30: 1545–1614.
14. Sali A, Blundell TL (1993) Comparative protein modelling by satisfaction of spatial restraints. *J Mol Biol* 234: 779–815.
15. Shen M-Y, Sali A (2006) Statistical potential for assessment and prediction of protein structures. *Protein Sci* 15: 2507–2524.
16. Rieping W, Nilges M, Habeck M (2008) ISD: a software package for Bayesian NMR structure calculation. *Bioinformatics* (Oxford, England) 24: 1104–1105.
17. Metropolis N, Rosenbluth AW, Rosenbluth MN, Teller AH, Teller E (1953) Equation of state calculation by fast computing machines. *J Chem Phys* 21: 1087–1092.
18. Press WH, Flannery BP, Teukolsky SA, Vetterling WT (1997) Numerical recipes in C. Cambridge: Cambridge University Press.
19. Gough B, ed. (2006) GNU Scientific library reference manual Network Theory Ltd.
20. Schlick T (2002) Molecular modeling and simulation. New York: Springer.
21. Zhou R (2007) Replica exchange molecular dynamics method for protein folding simulation. *Methods Mol Biol* 350: 205–223.
22. Lasker K, Topf M, Sali A, Wolfson HJ (2009) Inferential optimization for simultaneous fitting of multiple components into a CryoEM map of their assembly. *J Mol Biol* 388: 180–194.
23. Freeman L (1977) A set of measures of centrality based on betweenness. *Sociometry* 40: 35–41.
24. Yang Z, Lasker K, Schneidman-Duhovny D, Webb B, Huang C, et al. (2012) UCSF Chimera, MODELLER, and IMP: an Integrated Modeling System. *J Struct Biol*. In press.
25. DeLano W (2002) The PyMOL molecular graphics system. Available: <http://pymol.org>.
26. Taylor DJ, Devkota B, Huang AD, Topf M, Narayanan E, et al. (2009) Comprehensive molecular structure of the eukaryotic ribosome. *Structure* 17: 1591–1604.
27. Chandramouli P, Topf M, Ménétret J-F, Eswar N, Cannone JJ, et al. (2008) Structure of the mammalian 80S ribosome at 8.7 Å resolution. *Structure* 16: 535–548.
28. Serysheva II, Ludtke SJ, Baker ML, Cong Y, Topf M, et al. (2008) Subnanometer-resolution electron cryomicroscopy-based domain models for the cytoplasmic region of skeletal muscle RyR channel. *Proc Natl Acad Sci U S A* 105: 9610–9615.
29. Krukenberg KA, Förster F, Rice LM, Sali A, Agard DA (2008) Multiple conformations of *E. coli* Hsp90 in solution: insights into the conformational dynamics of Hsp90. *Structure* 16: 755–765.
30. Booth CR, Meyer AS, Cong Y, Topf M, Sali A, et al. (2008) Mechanism of lid closure in the eukaryotic chaperonin TRiC/CCT. *Nature Struct Mol Biol* 15: 746–753.
31. Cong Y, Topf M, Sali A, Matsudaira P, Dougherty M, et al. (2008) Crystallographic conformers of actin in a biologically active bundle of filaments. *J Mol Biol* 375: 331–336.
32. Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, et al. (2000) The Protein Data Bank. *Nucleic Acids Res* 28: 235–242.
33. Ulrich EL, Akutsu H, Doreleijers JF, Harano Y, Ioannidis YE, et al. (2008) BioMagResBank. *Nucleic Acids Res* 36: D402–D408.
34. Lawson CL, Baker ML, Best C, Bi C, Dougherty M, et al. (2011) EMDataBank.org: unified data resource for CryoEM. *Nucleic Acids Res* 39: D456–464.
35. Mesirov JP (2010) Computer science. Accessible reproducible research. *Science* 327: 415–416.
36. Barnes N (2010) Publish your computer code: it is good enough. *Nature* 467: 753.
37. Merali Z (2010) Computational science: ...Error. *Nature* 467: 775–777.
38. Leaver-Fay A, Tyka M, Lewis SM, Lange OF, Thompson J, et al. (2011) Rosetta3 an object-oriented software suite for the simulation and design of macromolecules. *Methods Enzymol* 487: 545–574.
39. Ludtke SJ (2010) 3-D structures of macromolecules using single-particle analysis in EMAN. *Methods Mol Biol* 673: 157–173.
40. Shaikh TR, Gao H, Baxter WT, Asturias FJ, Boisset N, et al. (2008) SPIDER image processing for single-particle reconstruction of biological macromolecules from electron micrographs. *Nat Protoc* 3: 1941–1974.
41. Schwieters CD, Kuszewski JJ, Tjandra N, Clore GM (2003) The Xplor-NIH NMR molecular structure determination package. *J Magn Reson* 160: 65–73.
42. Adams PD, Afonine PV, Bunkoczi G, Chen VB, Davis IW, et al. (2010) PHENIX: a comprehensive Python-based system for macromolecular structure solution. *Acta Crystallogr D* 66: 213–221.
43. Case DA, Cheatham TE, 3rd, Darden T, Gohlke H, Luo R, et al. (2005) The Amber biomolecular simulation programs. *J Comput Chem* 26: 1668–1688.
44. Sharma S, Ding F, Nie H, Watson D, Unnithan A, et al. (2006) iFold: a platform for interactive folding simulations of proteins. *Bioinformatics* 22: 2693–2694.
45. Hess B, Kutzner C, van der Spoel D, Lindahl E (2008) GROMACS 4: algorithms for highly efficient, load-balanced, and scalable molecular simulation. *J Chem Theory and Computation* 4: 435–447.
46. Peter E, Vijay P (2010) OpenMM: a hardware-independent framework for molecular simulations. *Comput Sci Eng* 12: 34–39.
47. Pieper U, Webb BM, Barkan DT, Schneidman-Duhovny D, Schlessinger A, et al. (2011) ModBase, a database of annotated comparative protein structure models, and associated resources. *Nucleic Acids Res* 39: D465–D474.
48. Stark C, Breitkreuz B-J, Chatr-Aryamontri A, Boucher L, Oughtred R, et al. (2011) The BioGRID Interaction Database: 2011 update. *Nucleic Acids Res* 39: D698–D704.
49. Ma W, Trusina A, El-Samad H, Lim WA, Tang C (2009) Defining network topologies that can achieve biochemical adaptation. *Cell* 138: 760–773.
50. Schneidman-Duhovny D, Hammel M, Sali A (2010) FoXS: a web server for rapid computation and fitting of SAXS profiles. *Nucleic Acids Res* 38: W540–W544.
51. Kostek SA, Grob P, De Carlo S, Lipscomb JS, Garczarek F, Nogales E (2006) Molecular architecture and conformational flexibility of human RNA polymerase II. *Structure* 14: 1691–700.