EXPERT REVIEWS

Hunting for predictive computational drug-discovery models

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The Keystone Symposium on Computer-Aided Drug Design was held at Steamboat Springs (CO, USA), from March 29th to the 3rd of April, 2008. The organizers brought together approximately 180 participants, representing a cross-section of viewpoints from academia and the pharmaceutical industry. Since it is a young discipline, it was a privilege to have a keynote introduction from one of the original pioneers of the field, Irwin Kuntz. By avoiding pitfalls, and addressing active debates, the young field can become more reliably predictive. Accordingly, this report focuses on best practices. As reliability improves, drug-discovery programs will increasingly use models to determine which high-throughput screens to run.

Banking on protein data

Many computational approaches use the protein databank (PDB) as an essential resource for training of energy functions or sampling algorithms. Unfortunately, the PDB has significant limitations for these uses, limitations that have often been ignored. Examples presented include crystal structures deposited with implausible substrate or side-chain conformations. Many existing PDB models lack the source data needed to correct such errors properly. Fortunately, effective from February 2008, new PDB submissions must be accompanied by structural factors or nuclear magnetic resonance (NMR) restraints (a plea to structural biologists – now is an excellent time to unearth and deposit older source data). Structural models accompanied by source data will retain their full value as methodology advances.

In particular, there is mounting support for large-scale re-refinement of models in the PDB. An early example is the Electron Density Server [1], which provides electron density maps where possible. Other motivations include refining to ensembles (rather than single structures) or refining with new models for anisotropic thermal motion [2,3]. Another methodological advance, particularly important for drug design, is the application of more sophisticated energetic models during crystallographic refinement. In one case study, Kenneth Merz (University of Florida, FL, USA) described how the classic parameters (Engh and Huber) incorrectly favored a planar configuration for an aromatic diamidine ligand. By contrast, a mixed quantum–mechanical molecular–mechanical model, used as the chemistry portion of the refinement objective, accurately captured the preferred geometry. This capability will be included in the upcoming Assisted Model Building with Energy Refinement (AMBER) 10 release [4].

Merz also emphasized that the common global measures of crystallographic quality, R and R_{free}, are not sensitive indicators for the quality of local side-chain placement into an electron density map. This is particularly crucial for drug design where a portion of the structure is of higher intrinsic interest. An increased use of local quality metrics, such as local versions of the real-space correlation coefficient, may help address this shortfall. A related issue arose in the context of PDB model coordinate precision, discussed by Paul Hawkins (OpenEye software; NM, USA). In principle, metrics like the diffraction-component precision index (DPI) are better indicators of model precision than commonly used indicators, such as resolution or temperature factors [5].

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When to sample, when to cancel

Molecules are complicated and somewhat devious. Any mistake can adversely impact binding-free energy calculations. The question of intrinsic difficulty divides the community into those that favor cancelation (e.g., the relative free-energy calculations of William Jorgensen) and those that proceed with intensive sampling (e.g., the free-energy calculations of Benoit Roux).

Jorgensen argues that there are many potential sources of error when modeling ligand–protein energetics, and that the errors are large with respect to the precision needed to rank small-molecule inhibitors. Sources of error include modeling choices, such as atom typing, ionization state, solvation and counter-ion details, starting configuration and system truncation. For instance, the ligand penalty for assuming the bound form (conformer focusing) is left out of the most simple ligand ranking schemes. Unfortunately, if this significant (up to 15 kcal/mol) effect is included; Jorgensen estimates that current forcefields will generate cumulative uncertainties of 5–10 kcal/mol [6]. However, by studying series of related compounds and calculating relative binding-free energies, many potentially confounding effects cancel out [7]. Roux also argues that ignoring entropic differences with end-point scoring of predicted complexes is an oversimplification. The philosophical difference is that Roux more optimistically proceeds by extracting absolute binding-free energies from nanosecond timescale free-energy perturbation simulations [8].

Relying on cancelation of errors is not risk free. Gerhard Klebe (Philipps Universitat, Germany) presented elegant crystallography and isothermal calorimetry results for a series of related inhibitors, demonstrating significant differences in the enthalpic and entropic components of the binding energy, even for ligands that varied in quite subtle ways [9,10].

A big can of interesting worms

Brian Shoichet (University of California, San Francisco, CA, USA) discussed collaborations with the US NIH Chemical Genomics Center [11,12]. The idea was simple: perform a large high-throughput screen (HTS) and a large virtual HTS in parallel on the same large set of unbiased molecules. One motivation was to learn not just from false positives but also from false negatives (not available from typical virtual HTS validation experiments). Shoichet described several pitfalls. First, a significant number of purchased compounds were not the expected chemical species. A more typical challenge is to ensure that the modeled ligand structure reflects the conformational and stereochemical diversity, and protonation and tautomer states found in nature. Another take-home lesson of the HTS was that 85–95% of the hits were not desirable inhibitors but, instead, formed colloidal aggregates that sequestered the target protein. Most of these false-positive aggregators could be detected by a counter screen with detergent or by screening against a second target protein. Unfortunately, 85–100% of the remaining hits were also false leads. The Shoichet results illustrated the precarious nature of an unbiased HTS; there is a significant probability that no genuine hits will be present. This provides motivation for fragment-based drug-discovery strategies.

Small is beautiful

In principle, by commencing lead optimization with smaller ligands (fragments), one is more likely to end up with an inhibitor that is higher in affinity while not being too large. By contrast, typical hits from a HTS are already fairly large, with less room for optimization. Industry talks demonstrated that fragment screening and the idea of ligand efficiency (affinity per ligand heavy atom) have become popular strategies for drug design. For example, Astex Therapeutics (Cambridge, UK) begins drug design by crystallizing up to approximately 100 small molecules bound to the target. Discussing research conducted at Wyeth, Deborah Loughney (Bristol–Myers Squibb, NY, USA) described a 'small is beautiful' strategy that included high-throughput NMR-saturation transfer difference experiments for rapidly detecting binding of highly soluble, low-affinity fragments.

However, Chuck Reynolds (Johnson & Johnson, USA) wondered if some of the euphoria for fragment ligand efficiency is overstated. Reynolds presented a statistical analysis of ligand efficiency data extracted from a database of binding data [101] that echoed a classic Kuntz paper [13,14]. The biophysical question is the degree to which binding free energy is additive with ligand size. Reynolds found that larger ligands have lower ligand efficiency. Reynolds suggested that the additional constraints of a larger ligand may produce a less optimal fit or that inherent nonlinearity in ligand surface area could play a role. It is difficult to establish causation; under-representation of picomolar inhibitors could also reflect the sufficiency of nanomolar inhibitors. Regardless, Reynolds recommends scaling ligand efficiency by compound size to calculate a size-independent 'fit quality' [14].

When questioned regarding additive inhibitor size effects observed by Hajduk, Reynolds suggested that the Hajduk examples start from fragments with low ligand efficiency, rather than fragments with peak ligand efficiency [15]. A related result from the Shoichet group demonstrated that several fragments derived from a β-lactamase inhibitor bound to noncognate sites [16,17]. To paraphrase, the Shoichet observation indicates that fragments with low-enough affinity need not bind in the expected orientation for the cognate substructure and the Reynolds analysis suggests that it might be unrealistic to expect additive ligand efficiency when starting from a fragment with high ligand efficiency. Therefore, these studies concern the proper interpretation of fragment-binding observations at either end of the affinity scale but leave an open window for fragment strategies. Certainly, these studies do not diminish cases where fragment design has been successfully applied [18].

Conclusion

Computational drug discovery is an extremely complex endeavor. Frequently, successful projects are not distinguished by an excess of insight but by the absence of errors. Besides spreading awareness of potential pitfalls, it is also important to consider the relative merits of retrospective (recapitulating known data) and prospective (predicting unknown data) analysis. Despite best intentions, retrospective analysis can easily turn into a form of multiple hypothesis testing. When a simulation disagrees with the expected result, the researcher tries to identify the error. When the simulation agrees, the researcher is consequently able to publish. Prospective analysis circumvents this trap and was a pervasive theme of the conference.

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Website

101 The Binding Database www.bindingdb.org/bind/index.jsp

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