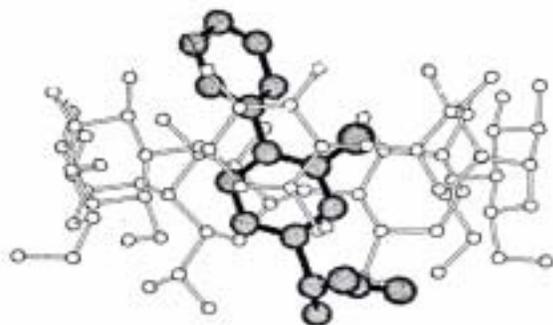


Drug-Binding Database: A Tool for Developing and Evaluating Algorithms Used to Select New Drug Candidates

NIST has developed a model system for studying the “fit” of a drug for its therapeutic target. Data from this model are used to populate a database for use by drug companies to validate the software algorithms they use to screen for new drug candidates. The new NIST database is expected to enable more cost-effective innovation of new pharmaceuticals.

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Drugs are designed to target and inhibit the function of proteins involved in pathways that advance the level of disease in cells. Drug development in the pharmaceutical industry involves high throughput screening of large libraries of diverse molecular structures to determine if they inhibit the protein target. A typical success rate for the initial screening of possible drug candidates is 3-6 hit series out of 750,000 compounds. If the structure of the protein target is known, either through crystallography or homology modeling, then the screening process and success rate can be significantly enhanced through computationally high affinity “docking” of the molecular structure into the active site on the target. Accordingly, pharmaceutical companies have made a significant investment in computational screening, with the docking-and-scoring software alone costing \$200k to \$1M per year per company. Although docking algorithms have been developed, it was found that there is very little correlation between the “fit” of the drug in the binding site and its binding affinity or potency. It is not clear as to whether newly developed docking algorithms are sufficient or as to whether the experimental data employed to validate the docking algorithm are accurate.



Crystal structure of FLP in the γ CD cavity

To address the accuracy of the binding data, the binding of drugs to cyclodextrins was identified as a binding system sufficient for use in the validation of newly developed docking algorithms.

The new NIST drug-fit database provides pharmaceutical companies with a valuable resource for better evaluation of the computational methods they use to screen the thousands of small molecule drug candidates for the 3-6 “hits” that move on for further development.

Thermodynamic binding parameters, of the drugs flurbiprofen (FLP), nabumetone (NAB), and naproxen (NPX) binding in the cavity of β -cyclodextrin (β CD) and in the larger cavity of γ -cyclodextrin (γ CD) in sodium phosphate buffer were determined from isothermal titration calorimetry (ITC) measurements over the temperature range from 293.15 K to 313.15 K. A fluorescence binding assay was developed for FLP and NAB, based on the enhancement of the drug fluorescence from an aqueous environment to the hydrophobic environment of the cyclodextrin cavity. The drug binding affinities range from 367 M^{-1} for NPX binding to γ CD at 313.15 K to 9520 M^{-1} for FLP binding to β CD at 293.15 K, over a factor of almost 30 and, thus, a viable range of values for validating binding affinities calculated from the docking algorithms. Since docking algorithms can also exhibit a dependence of binding affinity on pH and salt concentration, the binding affinities were also determined at 298.15 K as a function of pH from 6 to 8 and sodium chloride concentration up to 0.3 M. From comparisons of the results of the fluorescence assays to the fluorescence enhancement of the drug in a hydrophobic solvent such as isopropanol, it was found that water reorganization plays an important role in the thermodynamics of high affinity drug binding in the CD cavity. The role of water reorganization in the binding reaction was also confirmed by the heat capacity changes of the binding reactions.

Future Plans: A data base on drug binding to two isoforms of the p38 α MAP kinases, a popular protein drug target in the treatment of inflammation, is being developed for use in the development and validation of drug docking algorithms.

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Publications:

Todorova, N.A. and Schwarz, F.P., “**The role of water in the thermodynamics of drug binding to cyclodextrin**”, Journal of Chemical Thermodynamics in press.

Quote from reviewers: *“their careful control for pH, temperature, and salt concentration is very thorough and the data are of high quality. The basis for the experiments is well established in the paper, with the role of water release/retention a key concept in the interpretation of binding thermodynamics and drug design.”*