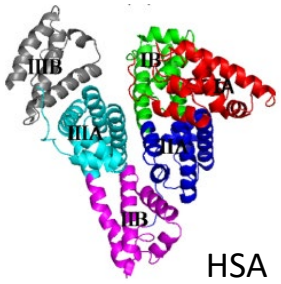
Understanding the contribution of allosteric binding to drug-drug interactions

Detlef Smilgies [1,2] and Katie Edwards [2,3]

[1] R. F. Smith School of Chemical and Biomolecular Engineering, Cornell University
[2] School of Pharmacy and Pharmaceutical Sciences, SUNY Binghamton
[3]Department of Microbiology, Cornell University

Human serum albumin (HSA) is a plasma protein present at high concentrations (~30-50 g/L) in blood serum. Among its functions, it acts as a carrier for endogenous and exogenous small molecules (e.g., pharmaceutical agents, dietary supplements, vitamins) throughout the body. Some drugs, such as warfarin (a blood thinner), are extensively bound (99%) to circulating HSA. The non-covalent interactions between drugs and HSA yield an equilibrium between protein-bound and free drugs, with only the free drug capable of exerting a therapeutic effect.



HSA

$$[HSA] + [drug] \leftrightarrow [HSA - drug\ complex]$$

The binding affinity of drug molecules, as represented by the dissociation constant (K_D), varies by a factor of about 100, from 10^{-6} M to 10^{-4} M.

$$K_D = \frac{[HSA][drug]}{[HSA - drug\ complex]}$$

HSA has two main binding pockets (Sudlow sites I and II) with varying structural preferences for drug molecules. Competition for these binding sites through ligand exchange reactions can occur when certain medications or nutrients are consumed concurrently. In addition, the association of a drug molecule with one of the binding sites may cause a conformational change of the protein, which can affect the binding of a second ligand to other binding sites. Both direct competition and allosteric effects can influence the free drug concentration in the bloodstream and impact clinical outcomes if not considered during drug dosing. Changes in the free drug concentration can be significant for drugs such as warfarin and phenytoin (an anti-epileptic) that have a narrow range between toxic and therapeutic effects.

Ligands of interest in our group include naproxen and ibuprofen, both of which are commonly used over-the-counter non-steroidal anti-inflammatory drugs and have moderate binding affinity to the HSA Sudlow II site. In this project, we will study how the association of these drugs with HSA is affected by drugs such as warfarin and phenytoin that bind to the Sudlow I site. Using these drugs alone and in combination, isothermal titration calorimetry will be used to determine the dissociation constant and the associated entropy change in HSA. We will use fluorescence spectroscopy to monitor changes in intrinsic protein fluorescence, which indicates the changes in the local environment experienced by specific amino acids induced by protein conformational changes. Students will gain hands-on experience with state-of-the-art equipment and techniques used in the pharmaceutical industry at the Binghamton University School of Pharmacy and Pharmaceutical Sciences and have an opportunity to contribute to the body of knowledge on drug-drug interactions through this project.

Suggested reading

F. Yang, Y. Zhang and H. Liang, Interactive Association of Drugs Binding to Human Serum Albumin, *Int. J. Mol. Sci.* 2014, 15, 3580-3595. (doi:10.3390/ijms15033580)

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Isothermal Titration Calorimetry

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E.S. Mozafaria, E. Tazikeh-Lemeskia, and A.A. Saboury, Isothermal Titration Calorimetry and Molecular Dynamics Simulation Studies on the Binding of Indomethacin with Human Serum Albumin, *Biomacromol. J.* 2016, 2, 34-43.

Fluorescence quenching method

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I. Lammers, V. Lhiaubet-Vallet, F. Ariese, M.A. Miranda, C. Gooijer, Binding of naproxen enantiomers to human serum albumin studied by fluorescence and room-temperature phosphorescence, *Spectrochim Acta A Mol Biomol Spectrosc* 105 (2013) 67–73.