**Searching for Principles of Protein-Ligand Recognition by Varying Protein or Compound Structure**

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Pharmaceutical drug design depends on the success to build small a molecule that specifically and tightly binds to a target protein participating in disease progression. However, the principles of protein – ligand recognition are poorly understood and most new drugs are discovered by screening rather than by rational design. In the design of high-affinity and selective inhibitors, we applied an approach of augmenting the chemical structure of compounds. The increased size of the substituents determined the spatial limitations of the active sites of the 12 catalytically active human carbonic anhydrase (CA) isoforms until no binding observed because of the inability of the compounds to fit in the active site. This approach led to the discovery of high-affinity and -selectivity compounds for the anticancer target CA IX. The X-ray crystallographic structures of compounds bound to CA IX showed the positions of the bound compounds, whereas computational modeling confirmed that steric clashes prevent the binding of these compounds to other isozymes.

We also used a reverse engineering approach where mutation of the key six amino acids in the active site of human CAXII into the CAII isozyme performed to provide a protein chimera (chCA XII). The compounds that bound CA XII more strongly than CAII, switched their preference and bound more strongly to the engineered chimera, chCA XII, based on CA II, but containing the 6 key amino acids from CA XII. The structures of the compounds in the chimeric active site resembled those determined for complexes with CAXII. Both the compound augmentation and protein engineering provided information on the recognition mechanism based on the Lock-and-Key principle and validated both approaches in the development of new enzyme-specific drug candidate compounds.

**References**

1. Linkuvienė, V., A. Zubrienė, E. Manakova, V. Petrauskas, L. Baranauskienė, A. Zakšauskas, A. Smirnov, S. Gražulis, J.E. Ladbury, and D. Matulis. 2018. Thermodynamic, kinetic, and structural parameterization of human carbonic anhydrase interactions toward enhanced inhibitor design. Q. Rev. Biophys. 51:1–48.
2. Matulis (Ed.), D. 2019. Carbonic anhydrase as drug target: thermodynamics and structure of inhibitor binding. SPRINGER NATURE.
3. Dudutienė, V., A. Zubrienė, V. Kairys, A. Smirnov, J. Smirnovienė, J. Leitans, A. Kazaks, K. Tars, L. Manakova, S. Gražulis, and D. Matulis. 2020. Isoform-Selective Enzyme Inhibitors by Exploring Pocket Size According to the Lock-and-Key Principle. Biophys. J. 119:1513–1524.
4. Smirnovienė, J., A. Smirnov, A. Zakšauskas, A. Zubrienė, V. Petrauskas, A. Mickevičiūtė, V. Michailovienė, E. Čapkauskaitė, E. Manakova, S. Gražulis, L. Baranauskienė, W.-Y. Chen, J.E. Ladbury, and D. Matulis. 2021. Switching the Inhibitor-Enzyme Recognition Profile via Chimeric Carbonic Anhydrase XII. ChemistryOpen. 10:567–580.