Understanding protein - ligand interactions for drug design: A structural perspective, Milton T. Stubbs[a], Daniel Rauh[a] and Gerhard Klebe[b], [a]*Institut für Biotechnologie, Martin-Luther Universität Halle-Wittenberg, Germany;* [b]*Institut für Pharmazeutische Chemie, Philipps-Universität Marburg, Germany.* E-mail: stubbs@biochemtech.uni-halle.de

Keywords: protein-ligand recognition; drug design; serine-proteinases

A high resolution crystallographic structure determination of a protein - ligand complex is generally accepted as the 'gold standard' for structure-based drug design. Yet how much does a crystal structure actually reveal about ligand affinity? In a combined crystallographic, kinetic and mutagenic approach, we have set out to analyse the multivariate determinants for ligand selectivity.

In so doing, we have observed pH-dependent changes in binding mode, as well as ligand-induced large scale reorganisation of secondary structure. The availability of multiple crystal forms allows us to factorise the relative contributions of competing processes to protein - ligand affinity. The implications for computational drug design will be discussed.

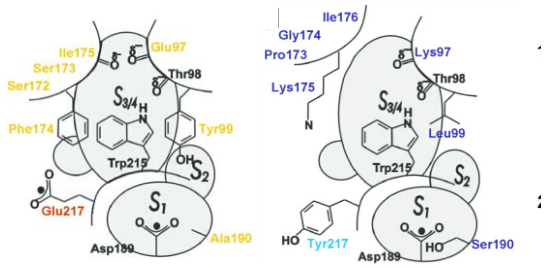

Fig.1 Schematic diagram showing the ligand binding sites of factor Xa (left) and trypsin (right). The aromatic pocket of factor Xa was introduced into that of trypsin through mutation of the residues shown.

[1] Renatus, M., Bode, W., Huber, R., Stürzebecher, J. and Stubbs, M.T. (1998) *Structural and functional analyses of benzamidine-based inhibitors in complex with trypsin: Implications for the inhibition of factor Xa, tPA and urokinase.* J. Med. Chem. 41, 5445-5456.

[2] Dullweber, F., Stubbs, M.T., Musil, D., Stürzebecher, J. and Klebe, G. (2001) *Factorising ligand affinity: A combined thermodynamic and crystallographic study of trypsin and thrombin inhibition.* J. Mol. Biol. 313, 593-614.

[3] Stubbs, M.T., Reyda, S., Dullweber, F., Möller, M., Klebe, G., Dorsch, D., Mederski, W.W.K.R. and Wurziger, H. (2002) *pH-dependent binding modes observed in trypsin crystals – Lessons for structure-based drug design.* Chem. Bio. Chem. 3, 246-249.

[4] Rauh, D., Reyda, S., Klebe, G. and Stubbs, M.T. (2002) *Trypsin mutants for structure based drug design: expression, refolding and crystallisation*. Biol. Chem. 383, 1309-1314.

[5] Reyda, S., Sohn, C., Klebe, G., Rall, K., Ullmann, D., Jakubke, H.-D. and Stubbs, M.T. (2003) *Reconstructing the binding site of factor Xa in trypsin reveals ligand-induced structural plasticity*. J. Mol. Biol. 325, 963-977.

[6] Rauh, D., Klebe, G., Stürzebecher and Stubbs, M.T. (2003) *ZZ made EZ: Influence of inhibitor configuration on enzyme selectivity*. J. Mol. Biol. 330, 761-770.

[7] Rauh, D., Klebe, G. and Stubbs, M.T. (2004) Understanding protein-ligand interactions: The price of flexibility. J. Mol. Biol. 335, 1325-1341.

www.biochemtech.uni-halle.de/xray