Local Dynamics in Protease Recognition

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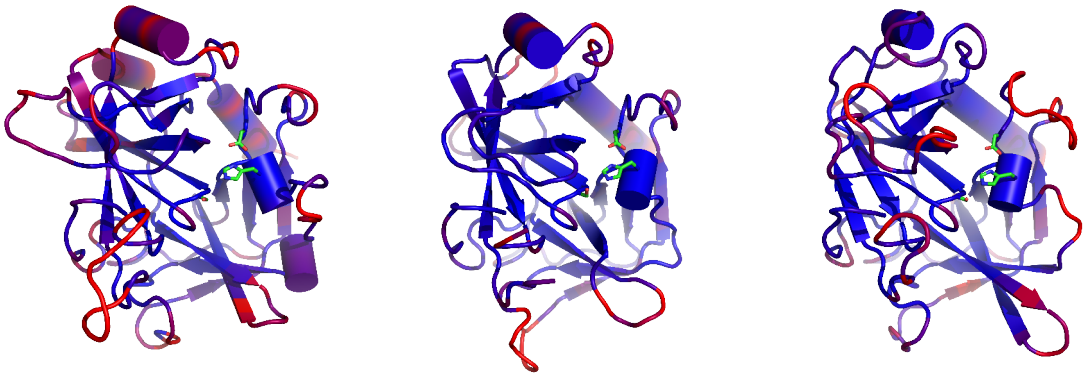
Proteases catalyze cleavage of peptide bonds and are vitally important in a wide range of fundamental cellular processes. Far more than 500 proteases have been identified in the human genome individually tied to a unique cleavage pattern [1]. These patterns reach from specificity for a single peptide in case of proteases involved in signaling cascades to broad spectra of cleaved peptides for digestive enzymes.

To analyze the impact of local dynamics on protease specificity, a series of homologous chymotrypsin-like serine proteases including highly specific as well as unspecific proteases was selected. Inspired by information theory, subpocket-wise substrate cleavage entropies are presented based on cleavage data from the MEROPS database [2]. Calculated entropy scores, ranging from 0 for a conserved substrate to 1 for a random distribution of substrates, appear to be qualitatively linked to local flexibility of the binding site region. Consequently, temperature factors from X-ray structures as well as all-atom 100ns molecular dynamics trajectories using the AMBER package [3] are compared in respect to subpocket specificity.



Analysis of specificity and flexibility patterns reveal a consistent correlation of binding site rigidity and specificity. As conformational plasticity is paralleled by a broader conformational space, a mechanism of conformational selection [4] in the binding process of proteases is proposed. According to this model, the whole conformational ensemble contributes to the substrate specificity of proteases rather than single interactions derived from a static point of view. This finding implies the need for refined rules for substrate cleavage considering binding site flexibility in accordance to earlier findings for snake venom metalloproteases [5].

[1] X. S. Puente, L. M. Sanchez, C. M. Overall, C. Lopez-Otin, *Net. Rev. Genet.*, **2003**, *4*, 544-558.
[2] N. D. Rawlings, A. J. Barrett, A. Bateman, *Nucleic Acids Res.*, **2010**, *38*, D227-D233.
[3] D. Case et al., AMBER10, University of California, San Francisco, **2010**.
[4] C. J. Tsai, R. Nussinov, *Protein Sci.*, **1997**, *6*, 24-42.
[5] H. G. Wallnoefer, T. Lingott, J. M. Gutierrez, I. Merfort, K. R. Liedl, *J. Am. Chem. Soc.*, **2010**, *132*, 10330-10337.