Protonation effects of endothiapepsin-fragment complexes

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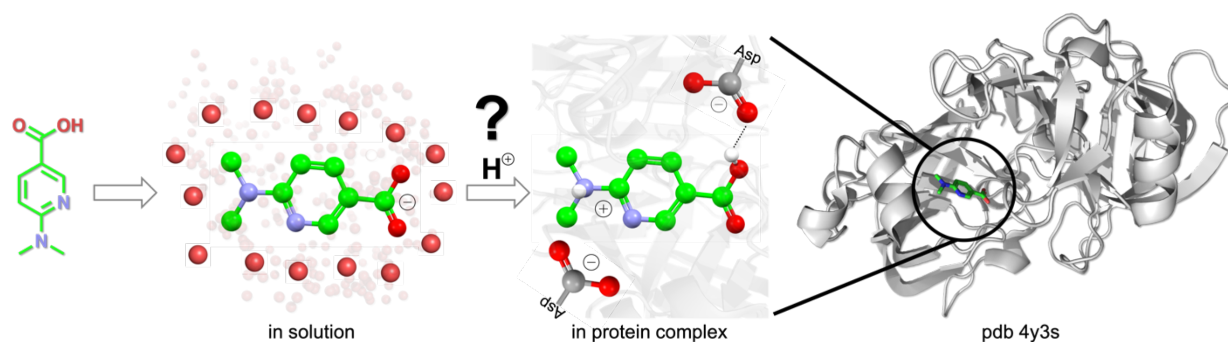
Endothiapepsin (EP) is an aspartic protease isolated from the ascomycete *Cryphonectria (Endothia) parasitica* and is often used as a milk clotting enzyme in cheesemaking processes. [1] Furthermore, EP often serves as a surrogate for renin (blood pressure) and β-secretase (Alzheimer's disease) in structure-based inhibitor design. [2] Due to a straightforward purification of EP with high yields, we use it as a model enzyme for the investigation of protonation changes upon protein-ligand complex formation (see figure below). Protonation changes upon binding of a ligand to a protein is often an overlooked phenomenon of molecular recognition. In fact, changes in protonation of titratable groups of either the ligand or the protein can have a serious impact on the binding geometry as well as the binding affinity. [3] In order to experimentally determine protonation changes, we perform Isothermal Titration Calorimetry (ITC) experiments with known EP-binding fragments in various buffers with differing heats of ionization. By supporting the ITC measurements with pK_a calculations (Poisson-Boltzmann calculations) we can predict the residue or ligand functional group responsible for the protonation change. [3] In a next step, we additionally want to determine the binding mode of the fragments via X-ray crystallography.



[1] N. Radeva, J. Schiebel, X. Wang, S. Krimmer, K. Fu, M. Stieler, F. Ehrmann, A.r Metz, T. Rickmeyer, M. Betz, J. Winquist, A. Park, F. Huschmann, M. Weiss, U. Mueller, A. Heine, G. Klebe , *J. Med. Chem.,* **2016**, *59(16)*, 7561–7575.
[2] M. Kuhnert, H. Köster, R. Bartholomäus, A. Park, A. Shahim, A. Heine, H. Steuber, G. Klebe, W. Diederich, *Angew. Chem. Int. Ed.,* **2015**, *54*, 2849–2853.
[3] M. Neeb, P. Czodrowski, A. Heine, L. Barandun, C. Hohn, F. Diederich, G. Klebe, *J. Med. Chem.,* **2014**, *57(13)*, 5554–5565