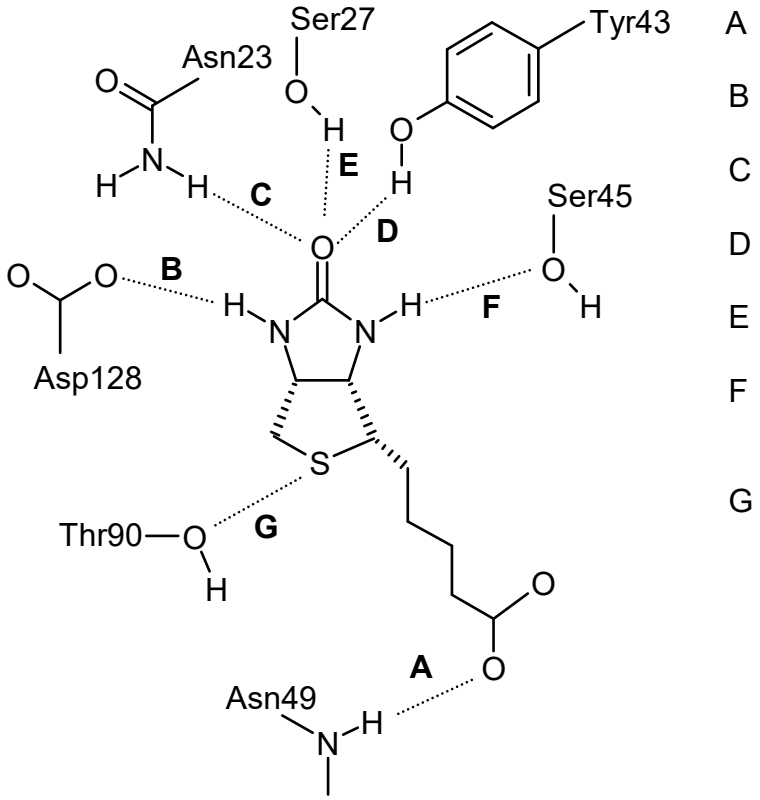
Exercises to the lecture „Modern Methods in Drug Discovery“ WS20/21

1. Biotin is a highly affine ligand of streptavidin (K_i = 2.5 ·10^{-13} M) that shows a total of 6 hydrogen bonds in the binding pocket (see scheme below).

a) Determine the lengths of these hydrogen bonds (A to F) from the X-ray structure with the pdb entry code 2RTF. This structure contains hydrogen atoms. Use chain D of streptavidin.
What is the distance G between the oxygen atom of Thr90 and the sulfur atom?
Would an alternative orientation of the OH-atom make sense, why?
(35 points)



H-bond	length [Å]
A	
B	
C	
D	
E	
F	
G	

b) Explain (briefly) the physical reason why X-ray structures (usually) do not show hydrogen atoms. (5 points)

c) Assume that the free energy of binding ΔG = –RT ln(K_i) at 298 K (R = 8.314 J K^{-1} mol^{-1}) stems from the hydrogen bonds A to F, only. Calculate the average contributing energy of one hydrogen bond. (10 points)

d) For the S45A mutant (Ser45 replaced by alanine) of streptavidin a ΔG value of 57266 J mol^{-1} was measured at 37° Celsius. Calculate the corresponding K_i value using the formula given in c). (10 points)

e) How many hydrogen bonds between biotin and streptavidin are left in this mutant? Compute the corresponding energy of binding using the average energy from one hydrogen bond in part c). Compare that to the experimental value given in part d). (5 points)

2. Compare the X-ray structures of tACE with the bound inhibitors lisinopril (1O86.pdb) and captopril (1UZF.pdb).
a) Which amino acids form interactions with the zinc ion? (list these with amino acid type and their residue number) (15 points)

b) What can be said about the difference in binding to zinc comparing captopril and lisinopril? (polar, ionic, coordinative,…) (5 points)

c) Which other, specific interactions (hydrogen bonds, salt bridges) are possible inside the binding pocket for other inhibitors? List three protein residues (with residue number), which are not among the list in part b) and the kind of interaction. (15 points)