Poster Presentation

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Enthalpic cost of water removal from a glucose binding cavity on HK620 TSP

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Bacteriophage HK620 recognizes and cleaves the O-antigen polysaccharide of Escherichia coli serogroup O18A1 with its tailspike protein (TSP). HK620TSP binds hexasaccharide fragments with low affinity, but single and double amino acid exchanges generated a set of high-affinity mutants with submicromolar dissociation constants. Isothermal titration calorimetry showed that only small amounts of heat were released upon complex formation via a large number of direct and solvent-mediated hydrogen bonds between carbohydrate and protein. At room temperature, association was both enthalpy- and entropy-driven, emphasizing major solvent rearrangements upon complex formation. Crystal structure analysis of a complete set of combinations of wildtype protein and point mutations with and without polysaccharide ligands was carried out. It could be shown that the extended sugar binding site can be dissected into two regions: first, a hydrophobic pocket at the reducing end with minor affinity contributions. Second, a region where the specific exchange of amino acids creates a site for additional water molecules. Sidechain rearrangements upon sugar binding lead to desolvation and additional hydrogen bonding which define this region of the binding site as the high-affinity scaffold.

[1] *Broeker, N. K., Gohlke, U., Müller, J. J., Uetrecht, C., Heinemann, U., Seckler, R. & Barbirz, S. (2013). Glycobiology 23, 59-68.*

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