

Die Vorträge finden jeweils um 16.15 Uhr im Hörsaal H3, Egerlandstr. 3 statt.
Alle Interessenten sind herzlich eingeladen.

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Unfolding of proteins upon adsorption on chromatography surfaces

Hydrophobic interaction chromatography, hydroxyl apatite chromatography are orthogonal methods to ion exchange chromatography for separation of recombinant proteins, plasmids, virus and virus like particles. Partial unfolding of proteins upon adsorption on these surfaces is a common problem and often limits application. It has been hypothesised that the surface acts as catalyst for partial unfolding, which is also known as spreading. The fraction of partial unfolded protein is increasing with length of the alkyl chain and thus with the hydrophobicity of the surface in HIC. It also increases with the concentration of the kosmotropic salt but decreases with loading of the protein. Unfolding upon adsorption on hydrophobic interaction chromatography surfaces was confirmed by in-situ ATR FTIR measurements. With increasing residence time influences the unfolding progresses. This can be used to determine the activation energy assuming a certain ration rate and temperature dependence of the rate of adsorption. For set of references proteins the activation energy of the unfolding process has been determined in the range of 50-100 kJ/mol by isothermal titration calorimetry and. From temperature dependence of adsorption we calculated the thermodynamic quantities. We postulate a simple three state model describing this process. Mechanisms of protein adsorption and pDNA will be discussed. Interestingly the adsorption on hydroxyl apatite is strongly enthalpy driven whereas on charged surfaces a strong entropic part is observed.