

ITC XIII - Protein subunit association/dissociation, protein stability and aggregation studies

Alford J. R., Kwok S. C., Roberts J. N., Wuttke D. S., Kendrick B. S., Carpenter J. F. and Randolph T. W. (2008) High concentration formulations of recombinant human interleukin-1 receptor antagonist: I. Physical characterization. *J Pharm. Sci* **97**, 3035-3050.

Abstract: At relatively high protein concentrations (i.e., up to 100 mg/mL), recombinant human interleukin-1 receptor antagonist (rhIL-1ra) was found to exist in a monomer-dimer equilibrium controlled by solution ionic strength. Sedimentation equilibrium at 25 degrees C was used to measure the increase in the dimer dissociation constant ($K(d)$) as a function of ionic strength. $K(d)$ increased from 2.0 to 12.6 mM as the solution ionic strength was increased from 0.011 to 0.184 molal. These $K(d)$ values were used with both static light scattering and membrane osmometry data collected over a protein concentration range of 1-100 mg/mL to determine second osmotic virial coefficients. Expanding the second osmotic virial coefficient model to account for separate monomer-monomer (B(22)), monomer-dimer (B(23)), and dimer-dimer (B(33)) interactions reveals net monomer-dimer interactions are attractive, whereas the others are repulsive. Lastly, isothermal titration calorimetry dilution experiments showed that rhIL-1ra dimerization is enthalpically driven ($\Delta H(\text{dimerization}) \ll 0$), which is consistent with intermolecular cation- π interactions previously proposed as the monomer-monomer contact sites in dimers

Augsten, M., Pusch, R., Biskup, C., Rennert, K., Wittig, U., Beyer, K., Blume, A., Reinhard Wetzker, R., Friedrich, K., and Rubio, I. (2006) Live-cell imaging of endogenous Ras-GTP illustrates predominant Ras activation at the plasma membrane. *EBMO Reports* **7**, 46-51.

Abstract: Ras-GTP imaging studies using the Ras-binding domain (RBD) of the Ras effector c-Raf as a reporter for overexpressed Ras have produced discrepant results about the possible activation of Ras at the Golgi apparatus. We report that RBD oligomerization provides probes for visualization of endogenous Ras-GTP, obviating Ras overexpression and the side effects derived thereof. RBD oligomerization results in tenacious binding to Ras-GTP and interruption of Ras signalling. Trimeric RBD probes fused to green fluorescent protein report agonist-induced endogenous Ras activation at the plasma membrane (PM) of COS-7, PC12 and Jurkat cells, but do not accumulate at the Golgi. PM illumination is exacerbated by Ras overexpression and its sensitivity to dominant-negative RasS17N and pharmacological manipulations matches Ras-GTP formation assessed biochemically. Our data illustrate that endogenous Golgi-located Ras is not under the control of growth factors and argue for the PM as the predominant site of agonist-induced Ras activation.

Baranauskiene L., Matuliene J. and Matulis D. (2008) Determination of the thermodynamics of carbonic anhydrase acid-unfolding by titration calorimetry. *J Biochem Biophys Methods* **70**, 1043-1047.

Abstract: The enthalpy of unfolding (ΔH) of carbonic anhydrase II was determined by titrating the protein with acid and measuring the heat using isothermal titration calorimetry (ITC) in the temperature range of 5 to 59 degrees C. By combining the ITC results with our previous findings by differential scanning calorimetry (DSC) in the temperature range of 39 to 72 degrees C, the ΔH dependence over a wide temperature range was obtained. The temperature dependence of the enthalpy displays significant curvature indicating that the heat capacity of unfolding (ΔC_p) is dependent on temperature. The T-derivative of ΔC_p was equal to $100 \pm 30 \text{ J}/(\text{mol} \times \text{K}^2)$, with the result that the ΔC_p is equal to 15.8 kJ/(molK) at 5 degrees C, 19.0 kJ/(molK) at 37 degrees C and 21.8 kJ/(molK) at 64 degrees C. The enthalpy of unfolding is zero at 17 degrees C. At lower temperatures, the ΔH becomes exothermic. This method of determining protein unfolding thermodynamics using acid-ITC, significantly widens the accessible T-range, provides direct estimate of the thermodynamic parameters at physiological temperature, and gives further insight into the third T-derivative of the Gibbs free energy of unfolding

Barranco-Medina S., Kakorin S., Lazaro J. J. and Dietz K. J. (2008) Thermodynamics of the dimer-decamer transition of reduced human and plant 2-cys peroxiredoxin. *Biochemistry* **47**, 7196-7204.

Abstract: Isothermal titration calorimetry (ITC) is a powerful technique for investigating self-association processes of protein complexes and was expected to reveal quantitative data on peroxiredoxin oligomerization by directly measuring the thermodynamic parameters of dimer-dimer interaction. Recombinant classical 2-cysteine peroxoredoxins from Homo sapiens, Arabidopsis thaliana, and Pisum

sativum as well as a carboxy-terminally truncated variant were subjected to ITC analysis by stepwise injection into the reaction vessel under various redox conditions. The direct measurement of the decamer-dimer equilibrium of reduced peroxiredoxin revealed a critical concentration in the very low micromolar range. The data suggest a cooperative assembly above this critical transition concentration where a nucleus facilitates assembly. The rather abrupt transition indicates that assembly processes do not occur below the critical transition concentration while oligomerization is efficiently triggered above it. The magnitude of the measured enthalpy confirmed the endothermic nature of the peroxiredoxin oligomerization. Heterocomplexes between peroxiredoxin polypeptides from different species were not formed. We conclude that a functional constraint conserved the dimer-decimer transition with highly similar critical transition concentrations despite emerging sequence variation during evolution

Behbehani G. R., Saboury A. A. and Taleshi E. (2008) A direct calorimetric determination of denaturation enthalpy for lysozyme in sodium dodecyl sulfate. *Colloids Surf B Biointerfaces* **61**, 224-228.

Abstract: Thermodynamics of the interaction between sodium dodecyl sulfate (SDS) with lysozyme were investigated at pH 7.0 and 27 degrees C in phosphate buffer by isothermal titration calorimetry. A new method to follow protein denaturation, and the effect of surfactants on the stability of proteins was introduced. The new solvation model was used to reproduce the enthalpies of lysozyme-SDS interaction over the whole range of SDS concentrations. The solvation parameters recovered from the new equation, attributed to the structural change of lysozyme and its biological activity. At low concentrations of SDS, the binding is mainly electrostatic, with some simultaneous interaction of the hydrophobic tail with nearby hydrophobic patches on the lysozyme. These initial interactions presumably cause some protein unfolding and expose additional hydrophobic sites. The enthalpy of denaturation is 160.81 +/- 0.02 kJ mol⁻¹ for SDS

Behbehani G. R., Saboury A. A. and Taleshi E. (2008) Determination of partial unfolding enthalpy for lysozyme upon interaction with dodecyltrimethylammonium bromide using an extended solvation model. *J Mol Recognit* **21**, 132-135.

Abstract: The interactions of dodecyltrimethylammonium bromides (DTABs) with hen egg lysozyme have been investigated at pH = 7.0 and 27 degrees C in phosphate buffer by isothermal titration calorimetry. DTAB interacts endothermically and activate lysozyme. The endothermicity of the lysozyme-DTAB interaction is in marked contrast to the exothermic interactions between sodium dodecyl sulphate (SDS) and lysozyme which have been attributed to specific binding between the anionic sulphate head groups and cationic amino acid residues. The enthalpies of interaction between the cationic surfactant (DTAB) and lysozyme are dominated by the endothermic unfolding of the native structure followed by an exothermic solvation of the lysozyme-DTAB complex by the addition of extra DTAB. A new direct calorimetric method to follow protein denaturation, and the effect of surfactants on the stability of proteins was introduced. The extended solvation model was used to reproduce the enthalpies of lysozyme-DTAB interaction over the whole range of DTAB concentrations. The solvation parameters recovered from the new equation, attributed to the structural change of lysozyme and its biological activity. At low concentrations of DTAB, the binding is mainly electrostatic, with some simultaneous interaction of the hydrophobic tail with nearby hydrophobic patches on the lysozyme. These initial interactions presumably cause some protein unfolding and expose additional hydrophobic sites. The DTAB-induced denaturation enthalpy of lysozyme is 86.46 +/- 0.02 kJ mol⁻¹

Bello M., Perez-Hernandez G., Fernandez-Velasco D. A., Arreguin-Espinosa R. and Garcia-Hernandez E. (2008) Energetics of protein homodimerization: effects of water sequestering on the formation of beta-lactoglobulin dimer. *Proteins* **70**, 1475-1487.

Abstract: Transient protein-protein interactions are functionally relevant as a control mechanism in a variety of biological processes. Analysis of the 3D structure of protein-protein complexes indicates that water molecules trapped at the interface are very common; however, their role in the stability and specificity of protein homodimer interactions has been not addressed yet. To provide new insights into the energetic bases that govern the formation of highly hydrated interfaces, the dissociation process of bovine beta Ig variant A at a neutral pH was characterized here thermodynamically by conducting dilution experiments with an isothermal titration calorimeter. Association was enthalpically driven throughout the temperature range spanned. DeltaH and deltaC(p) were significantly more negative than estimates based on surface area changes, suggesting the occurrence of effects additional to the dehydration of the contact surfaces between subunits. Near-UV CD spectra proved to be independent of protein concentration,

indicating a rigid body-like association. Furthermore, the process proved not to be coupled to significant changes in the protonation state of ionizable groups or counterion exchange. In contrast, both osmotic stress experiments and a computational analysis of the dimer's 3D structure indicated that a large number of water molecules are incorporated into the interface upon association. Numerical estimates considering the contributions of interface area desolvation and water immobilization accounted satisfactorily for the experimental ΔC_p . Thus, our study highlights the importance of explicitly considering the effects of water sequestering to perform a proper quantitative analysis of the formation of homodimers with highly hydrated interfaces

Bordbar A. K., Taheri-Kafrani A., Mousavi S. H. and Haertle T. (2008) Energetics of the interactions of human serum albumin with cationic surfactant. *Arch Biochem Biophys* **470**, 103-110.

Abstract: The heat capacity changes for interaction of human serum albumin (HSA) and a cationic surfactant-cetylpyridinium chloride (CPC), were studied at conditions close to physiological (50mM HEPES or phosphate buffer, pH 7.4 and 160mM NaCl) carrying out isothermal calorimetric titrations (ITC) at various temperatures (20-40 degrees C). ITC measurements indicated that the small endothermic changes associated with CPC demicellization were temperature independent at these conditions. Surprisingly, important enthalpy changes associated with binding of CPC to HSA were exothermic and temperature independent at lower concentrations (below 0.022mM) of CPC and endothermic and temperature dependent at higher concentrations of CPC. The values of heat capacity changes were obtained for each studied concentration of CPC from the plot of enthalpy changes vs temperature. The obtained results demonstrate the temperature independence of heat capacity changes at entire range of studied CPC concentrations. Both enthalpograms and heat capacity curves indicate the two-step mechanism of HSA folding changes due to its interactions with CPC. The first step corresponds to transition from native state to partially unfolded state and the second to unfolding and to the loss of tertiary structure. The analysis of the results indicates that predominant cooperative unfolding occurs at CPC/HSA molar ratio region between 25 and 30. Such information could not be extracted from thermograms and describes the role of heat capacity as a major thermodynamic quantity giving insight on physical, mechanistic and even atomic-level into how HSA may unfold and interact with CPC. The effect of CPC binding on HSA intrinsic fluorescence and CD spectra were also examined. Hence, the analysis of spectral data confirms the ITC results about the biphasic mechanism of HSA folding changes induced by CPC. The CD measurement also represents the conservation of considerable secondary structure of HSA due to interaction with CPC.

Bosl B., Grimminger V., and Walter S. (2005) Substrate binding to the molecular chaperone Hsp104 and its regulation by nucleotides. *J Biol Chem.* **280**, 38170-6.

Abstract: The Hsp104 protein from *Saccharomyces cerevisiae* is a member of the Hsp100/Clp family of molecular chaperones. It mediates the solubilization of aggregated proteins in an ATP-dependent process assisted by the Hsp70/40 system. Although the principal function of Hsp104 is well established, the mechanistic details of this catalyzed disaggregation are poorly understood. In this work, we have investigated the interaction of Hsp104 with reduced, carboxymethylated alpha-lactalbumin (RCMLa), a permanently unfolded model substrate. Our results demonstrate that the affinity of Hsp104 toward polypeptides is regulated by nucleotides. In the presence of ATP or adenosine-5' -O-(3-thiotriphosphate), the chaperone formed complexes with RCMLa, whereas no binding was observed in the presence of ADP. In particular, the occupation of the N-terminally located nucleotide-binding domain with ATP seems to be crucial for substrate interaction. When ATP binding to this domain was impaired by mutation, Hsp104 lost its ability to interact with RCMLa. Our results also indicate that upon association with a polypeptide, a conformational change occurs within Hsp104 that strongly reduces the dynamics of nucleotide exchange and commits the bound polypeptide to ATP hydrolysis.

Burrows S. D., Doyle M. L., Murphy K. P., Franklin S. G., White J. R., Brooks I., McNulty D. E., Scott M. O., Knutson J. R., Porter D., and . (1994) Determination of the monomer-dimer equilibrium of interleukin-8 reveals it is a monomer at physiological concentrations. *Biochemistry* **33**, 12741-12745.

Abstract: Interleukin-8 has been shown by X-ray crystallography and NMR to be a homodimer, suggesting that this is the form which binds to its receptor. Here we measure, for the first time, the monomer-dimer equilibrium of interleukin-8 using analytical ultracentrifugation and titration microcalorimetry and find that it dissociates readily to monomers with an equilibrium dissociation constant of $18 \pm 6 \mu\text{M}$ at 37 degrees C. The present findings suggest that the monomer is the form which binds to the receptor. Comparison of

experimental and structure-based calculated thermodynamics of interleukin-8 dimerization argues for limited subunit conformational changes upon dissociation to monomer.

Cheema M. A., Taboada P., Barbosa S., Castro E., Siddiq M. and Mosquera V. (2007) Energetics and conformational changes upon complexation of a phenothiazine drug with human serum albumin. *Biomacromolecules*. **8**, 2576-2585.

Abstract: The interactions and complexation process of the amphiphilic phenothiazine fluphenazine hydrochloride with human serum albumin in aqueous buffered solutions of pH 3.0 and 7.4 have been examined by zeta-potential, isothermal titration calorimetry (ITC), UV-vis spectroscopy, and dynamic light scattering (DLS) techniques with the aim of analyzing the effect of hydrophobic and electrostatic forces on the complexation process and the alteration of protein conformation upon binding. Thus, the energetics and stoichiometry of the binding process were derived from ITC data. The enthalpies of binding obtained are small and exothermic, so the Gibbs energies of binding are dominated by large increases in entropy, consistent with hydrophobic interactions at an acidic pH. However, at physiological pH, binding to the first class of binding sites is dominated by an enthalpic contribution due to the existence of electrostatic interactions and probably some hydrogen bonding. Binding isotherms were obtained from microcalorimetric data by using a theoretical model based on the Langmuir isotherm. zeta-Potential data showed a reversal in the sign of the protein charge at pH 7.4, as a consequence of the binding of the drug to the protein. Gibbs energies of drug binding per mole of drug were also derived from zeta-potential data. On the other hand, binding of the phenothiazine that causes a conformational transition on the protein structure was followed as a function of drug concentration using UV-vis spectroscopy, and the data were analyzed to obtain the Gibbs energy of the transition in water ($\Delta G(\text{degree})_w$) and in a hydrophobic environment ($\Delta G(\text{degree})_{hc}$). Finally, the population distribution of the different species in solution and the size of the complexes were analyzed through dynamic light scattering. The existence of an aggregation process of drug/protein complexes, as a consequence of the expanded structure of the protein induced by the drug and subsequent further binding, is in agreement with ITC data. In addition, detection of drug aggregates at concentrations below the drug critical micelle concentration was also detected by this technique.

Chen S., Chen L., Tan J., Chen J., Du L., Sun T., Shen J., Chen K., Jiang H., and Shen X. (2005) Severe acute respiratory syndrome coronavirus 3C-like proteinase N terminus is indispensable for proteolytic activity but not for enzyme dimerization. Biochemical and thermodynamic investigation in conjunction with molecular dynamics simulations. *J Biol Chem* **280**, 164-173.

Abstract: Severe acute respiratory syndrome (SARS) coronavirus is a novel human coronavirus and is responsible for SARS infection. SARS coronavirus 3C-like proteinase (SARS 3CL(pro)) plays key roles in viral replication and transcription and is an attractive target for anti-SARS drug discovery. In this report, we quantitatively characterized the dimerization features of the full-length and N-terminal residues 1-7 deleted SARS 3CL(pro)s by using glutaraldehyde cross-linking SDS-PAGE, size-exclusion chromatography, and isothermal titration calorimeter techniques. Glutaraldehyde cross-linking SDS-PAGE and size-exclusion chromatography results show that, similar to the full-length SARS 3CL(pro), the N-terminal deleted SARS 3CL(pro) still remains a dimer/monomer mixture within a wide range of protein concentrations. Isothermal titration calorimeter determinations indicate that the equilibrium dissociation constant ($K(d)$) of the N-terminal deleted proteinase dimer (262 microm) is very similar to that of the full-length proteinase dimer (227 microm). Enzymatic activity assay using the fluorescence resonance energy transfer method reveals that N-terminal deletion results in almost complete loss of enzymatic activity for SARS 3CL(pro). Molecular dynamics and docking simulations demonstrate the N-terminal deleted proteinase dimer adopts a state different from that of the full-length proteinase dimer, which increases the angle between the two protomers and reduces the binding pocket that is not beneficial to the substrate binding. This conclusion is verified by the surface plasmon resonance biosensor determination, indicating that the model substrate cannot bind to the N-terminal deleted proteinase. These results suggest the N terminus is not indispensable for the proteinase dimerization but may fix the dimer at the active state and is therefore vital to enzymatic activity.

Chung K., Kim J., Cho B. K., Ko B. J., Hwang B. Y. and Kim B. G. (2007) How does dextran sulfate prevent heat induced aggregation of protein? The mechanism and its limitation as aggregation inhibitor. *Biochim Biophys Acta* **1774**, 249-257.

Abstract: The effect of dextran sulfate on protein aggregation was investigated to provide the clues of its biochemical mechanism. The interaction between dextran sulfate and BSA varied with the pH values of the solution, which led to the different extent of aggregation prevention by dextran sulfate. Light scattering data with thermal scan showed that dextran sulfate suppressed BSA aggregation at pH 5.1 and pH 6.2, while it had no effect at pH 7.5. Isothermal titration calorimetric analysis suggested that the pH dependency of the role of dextran sulfate on BSA aggregation would be related to the difference in the mode of BSA-dextran sulfate complex formation. Isothermal titration calorimetric analysis at pH 6.2 indicated that dextran sulfate did not bind to native BSA at this pH, but interacted with partially unfolded BSA. While stabilizing native form of protein by the complex formation has been suggested as the suitable mechanism of preventing aggregation, our observation of conformational changes by circular dichroism spectroscopy showed that strong electrostatic interaction between dextran sulfate and BSA rather facilitated the denaturation of BSA. Combining the data from isothermal titration calorimetry, circular dichroism, and dynamic light scattering, we found that the complex formation of the intermediate state of denatured BSA with dextran sulfate is a prerequisite to suppress the aggregation by preventing further oligomerization/aggregation process of denatured protein.

Contreras L. M., Gomez J., Prieto J., Clemente-Jimenez J. M., Las Heras-Vazquez F. J., Rodriguez-Vico F., Blanco F. J. and Neira J. L. (2008) The family 52 beta-xylosidase from *Geobacillus stearothermophilus* is a dimer: Structural and biophysical characterization of a glycoside hydrolase. *Biochim Biophys Acta* **1784**, 1924-1934.

Abstract: Xylans are the most abundant polysaccharides forming the plant cell wall hemicelluloses, and they are degraded, among other proteins, by beta-xylosidase enzymes. In this work, the structural and biophysical properties of the family 52 beta-xylosidase from *Geobacillus stearothermophilus*, XynB2, are described. Size exclusion chromatography, analytical centrifugation, ITC, CD, fluorescence (steady state and ANS-binding) and FTIR were used to obtain the structure, the oligomerization state and the conformational changes of XynB2, as pH, chemical denaturants or temperature were modified. This report describes the first extensive conformational characterization of a family 52 beta-xylosidase. The active protein was a highly hydrated dimer, whose active site was formed by the two protomers, and it probably involved aromatic residues. At low pH, the protein was not active and it populated a monomeric molten-globule-like species, which had a conformational transition with a pK(a) of ~4.0. Thermal and chemical-denaturations of the native protein showed hysteresis behaviour. The protein at physiological pH was formed by alpha-helix (30%) and beta-sheet (30%), as shown by CD and FTIR. Comparison with other xylosidases of the same family indicates that the percentages of secondary structure seem to be conserved among the members of the family

Czyptionka A., de los Panos O. R., Mateu M. G., Barrera F. N., Hurtado-Gomez E., Gomez J., Vidal M. and Neira J. L. (2007) The isolated C-terminal domain of Ring1B is a dimer made of stable, well-structured monomers. *Biochemistry* **46**, 12764-12776.

Abstract: The Ring1B is a core subunit protein of the PRC1 (polycomb repressive complex 1), which plays key roles in the regulation of the Homeobox gene expression, X-chromosome inactivation, stem cell self-renewal, and tumorigenesis. The C-terminal region of Ring1B interacts with RYBP, a transcriptional repressor in transiently transfected cells, and also with M33, another transcriptional repressor involved in mesoderm patterning. In this work, we show that the C-terminal domain of Ring1B, C-Ring1B, is a dimer in solution, with a dissociation constant of 200 microM, as shown by NMR, ITC, and analytical gel filtration. Each monomer is stable at physiological conditions in a wide pH range (approximately 5 kcal mol⁻¹ at 298 K), with a well-formed core and a spherical shape. The dimer has a high content of alpha-helix and beta-sheet, as indicated by FTIR spectra, and it is formed by the mutual docking of the preformed folded monomers. Since the C-terminal region is important for interaction with other proteins of the PRC1, the dimerization and the presence of those well-structured monomers might be a form of regulation.

Ekblad C. M., Friedler A., Veprintsev D., Weinberg R. L., and Itzhaki L. S. (2004) Comparison of BRCT domains of BRCA1 and 53BP1: a biophysical analysis. *Protein Sci* **13**, 617-625.

Abstract: 53BP1 interacts with the DNA-binding core domain of the tumor suppressor p53 and enhances p53-mediated transcriptional activation. The p53-binding region of 53BP1 maps to the C-terminal BRCT domains, which are homologous to those found in the breast cancer protein BRCA1 and in other proteins involved in DNA repair. Here we compare the thermodynamic behavior of the BRCT domains of 53BP1

and BRCA1 and examine their ability to interact with the p53 core domain. The free energies of unfolding are of similar magnitude, although slightly higher for 53BP1-BRCT, and both populate an aggregation-prone partly folded intermediate. Interaction studies performed in vitro by analytical size-exclusion chromatography, analytical ultracentrifugation, and isothermal titration calorimetry reveal that 53BP1-BRCT interacts with p53 with a K_d in the low micromolar range. Despite their homology with 53BP1-BRCT domains, the BRCT domains of BRCA1 did not bind p53 with any detectable affinity. In summary, although other studies have indicated that the BRCT domains of both BRCA1 and 53BP1 interact with p53 core domain, the quantitative biophysical measurements performed here indicate that only 53BP1 can bind. Although both proteins may be involved in the same DNA repair pathways, our study indicates that a direct role in p53 function is unique to 53BP1.

Fernando H., Chin C., Rosgen J., and Rajarathnam K. (2004) Dimer dissociation is essential for interleukin-8 (IL-8) binding to CXCR1 receptor. *J Biol Chem* **279**, 36175-36178.

Abstract: Chemokines play a fundamental role in trafficking of immune cells and in host defense against infection. The role of chemokines in the recruitment process is highly regulated spatially and temporally and involves interactions with G protein-coupled receptors and cell surface glycosaminoglycans. The dynamic equilibrium between chemokine monomers and dimers, both free in solution and in cell surface-bound forms, regulates different components of recruitment such as chemotaxis and receptor signaling. The binding and activity of the chemokine interleukin-8 (IL-8) for its receptors, previously studied using "trapped" non-associating monomers and non-dissociating dimers, show that the monomer has a native-like function but support conflicting roles for the dimer. We have measured the binding of native IL-8 to the CXCR1 N-domain, using isothermal titration calorimetry and sedimentation equilibrium techniques. The N-domain constitutes a critical binding site, and IL-8 binding affinity to the receptor N-domain is in the same concentration range as the IL-8 monomer-dimer equilibrium. We observed that only the IL-8 monomer, and not the dimer, is competent in binding the receptor N-domain. Based on our results, we propose that IL-8 dimerization functions as a negative regulator for the receptor function and as a positive regulator for binding to glycosaminoglycans and that both play a role in the neutrophil recruitment process.

Ferreon A. C. and Deniz A. A. (2007) Alpha-synuclein multistate folding thermodynamics: implications for protein misfolding and aggregation. *Biochemistry* **46**, 4499-4509.

Abstract: Alpha-synuclein aggregation has been tightly linked with the pathogenesis of Parkinson's disease and other neurodegenerative disorders. Despite the protein's putative function in presynaptic vesicle regulation, the roles of lipid binding in modulating alpha-synuclein conformations and the aggregation process remain to be fully understood. This study focuses on a detailed thermodynamic characterization of monomeric alpha-synuclein folding in the presence of SDS, a well-studied lipid mimetic. Far-UV CD spectroscopy was employed for detection of conformational transitions induced by SDS, temperature, and pH. The data we present here clearly demonstrate the multistate nature of alpha-synuclein folding, which involves two predominantly alpha-helical partially folded thermodynamic intermediates that we designate as F (most folded) and I (intermediately folded) states. Likely structures of these alpha-synuclein conformational states are also discussed. These partially folded forms can exist in the presence of either monomeric or micellar forms of SDS, which suggests that alpha-synuclein has an intrinsic propensity for adopting multiple alpha-helical structures even in the absence of micelle or membrane binding, a feature that may have implications for its biological activity and toxicity. Additionally, we discuss the relation between alpha-synuclein three-state folding and its aggregation, within the context of isothermal titration calorimetry and transmission electron microscopy measurements of SDS-initiated oligomer formation.

Fokkens M., Schrader T., and Klarner F. G. (2005) A molecular tweezer for lysine and arginine. *J Am Chem Soc* **127**, 14415-14421.

Abstract: Lysine and arginine play a key role in numerous biological recognition processes controlling, inter alia, gene regulation, glycoprotein targeting and vesicle transport. They are also found in signaling peptide sequences responsible, e.g. for bacterial cell wall biosynthesis, Alzheimer peptide aggregation and skin regeneration. Almost none of all artificial receptor structures reported to date are selective and efficient for lysine residues in peptides or proteins. An artificial molecular tweezer is introduced which displays an exceptionally high affinity for lysine (K_a approximately 5000 in neutral phosphate buffer). It features an electron-rich torus-shaped cavity adorned with two peripheral anionic phosphonate groups. Exquisite selectivity for arginine and lysine is achieved by threading the whole amino acid side chain

through the cavity and subsequent locking by formation of a phosphonate-ammonium/guanidinium salt bridge. This pseudorotaxane-like geometry is also formed in small basic signaling peptides, which can be bound with unprecedented affinity in buffered aqueous solution. NMR titrations, NOESY and VT experiments as well as ITC measurements and Monte Carlo simulations unanimously point to an enthalpy-driven process utilizing a combination of van der Waals interactions and substantial electrostatic contributions for a conformational lock. Since DMSO and acetonitrile compete with the amino acid guest inside the cavity, a simple change in the cosolvent composition renders the whole complexation process reversible.

Gaetani M., Mootien S., Harper S., Gallagher P. G. and Speicher D. W. (2008) Structural and functional effects of hereditary hemolytic anemia-associated point mutations in the alpha spectrin tetramer site. *Blood* **111**, 5712-5720.

Abstract: The most common hereditary elliptocytosis (HE) and hereditary pyropoikilocytosis (HPP) mutations are alpha-spectrin missense mutations in the dimer-tetramer self-association site. In this study, we systematically compared structural and functional properties of the 14 known HE/HPP mutations located in the alpha-spectrin tetramer binding site. All mutant alpha-spectrin recombinant peptides were well folded, stable structures, with only the R34W mutant exhibiting a slight structural destabilization. In contrast, binding affinities measured by isothermal titration calorimetry were greatly variable, ranging from no detectable binding observed for I24S, R28C, R28H, R28S, and R45S to approximately wild-type binding for R34W and K48R. Binding affinities for the other 7 mutants were reduced by approximately 10- to 100-fold relative to wild-type binding. Some sites, such as R28, were hot spots that were very sensitive to even relatively conservative substitutions, whereas other sites were only moderately perturbed by nonconservative substitutions. The R34W and K48R mutations were particularly intriguing mutations that apparently either destabilize tetramers through mechanisms not probed by the univalent tetramer binding assay or represent polymorphisms rather than the pathogenic mutations responsible for observed clinical symptoms. All alpha0 HE/HPP mutations studied here appear to exert their destabilizing effects through molecular recognition rather than structural mechanisms

Garzon M. T., Lidon-Moya M. C., Barrera F. N., Prieto A., Gomez J., Mateu M. G., and Neira J. L. (2004) The dimerization domain of the HIV-1 capsid protein binds a capsid protein-derived peptide: a biophysical characterization. *Protein Sci* **13**, 1512-1523.

Abstract: The type 1 HIV presents a conical capsid formed by approximately 1500 units of the capsid protein, CA. Homodimerization of CA via its C-terminal domain, CA-C, constitutes a key step in virion assembly. CA-C dimerization is largely mediated by reciprocal interactions between residues of its second alpha-helix. Here, we show that an N-terminal-acetylated and C-terminal-amidated peptide, CAC1, comprising the sequence of the CA-C dimerization helix plus three flanking residues at each side, is able to form a complex with the entire CA-C domain. Thermal denaturation measurements followed by circular dichroism (CD), NMR, and size-exclusion chromatography provided evidence of the interaction between CAC1 and CA-C. The apparent dissociation constant of the heterocomplex formed by CA-C and CAC1 was determined by several biophysical techniques, namely, fluorescence (using an anthraniloyl-labeled peptide), affinity chromatography, and isothermal titration calorimetry. The three techniques yielded similar values for the apparent dissociation constant, in the order of 50 μ M. This apparent dissociation constant was only five times higher than was the dissociation constant of both CA-C and the intact capsid protein homodimers (10 μ M).

Gell D., Kong Y., Eaton S. A., Weiss M. J., and Mackay J. P. (2002) Biophysical characterization of the alpha-globin binding protein alpha-hemoglobin stabilizing protein. *J Biol Chem* **277**, 40602-40609.

Abstract: Alpha-hemoglobin stabilizing protein (AHSP) is a small (12 kDa) and abundant erythroid-specific protein that binds specifically to free alpha-(hemo)globin and prevents its precipitation. When present in excess over beta-globin, its normal binding partner, alpha-globin can have severe cytotoxic effects that contribute to important human diseases such as beta-thalassemia. Because AHSP might act as a chaperone to prevent the harmful aggregation of alpha-globin during normal erythroid cell development and in diseases of globin chain imbalance, it is important to characterize the biochemical properties of the AHSP.alpha-globin complex. Here we provide the first structural information about AHSP and its interaction with alpha-globin. We find that AHSP is a predominantly alpha-helical globular protein with a somewhat asymmetric shape. AHSP and alpha-globin are both monomeric in solution as determined by

analytical ultracentrifugation and bind each other to form a complex with 1:1 subunit stoichiometry, as judged by gel filtration and amino acid analysis. We have used isothermal titration calorimetry to show that the interaction is of moderate affinity with an association constant of $1 \times 10^7 \text{ m}^{-1}$ and is thus likely to be biologically significant given the concentration of AHSP (approximately 0.1 mM) and hemoglobin (approximately 4 mM) in the late pro-erythroblast.

Girard M., Turgeon S. L., and Gauthier S. F. (2003) Thermodynamic parameters of beta-lactoglobulin-pectin complexes assessed by isothermal titration calorimetry. *J Agric Food Chem* **51**, 4450-4455.

Abstract: Isothermal titration calorimetry (ITC) was used to determine the binding constant, stoichiometry, enthalpy, and entropy of beta-lactoglobulin/low- and high-methoxyl pectin (beta-lg-LM- and HM-pectin) complexes at 22 degrees C and at pH 4. The binding isotherms revealed the formation of soluble intrapolymer complexes (C1) further followed by their aggregation in interpolymer complexes (C2). The interaction between beta-lg and LM- or HM-pectin in C1 and C2 occurred spontaneously with a Gibbs free energy around -10 kcal/mol. The C1 were enthalpically driven, whereas enthalpic and entropic factors were involved in the C2 formation. Because ITC did not allow the dissociation of different enthalpic contributions, the values measured as pectin and beta-lg interacted could partially be attributed to conformational changes. The C1 had a binding stoichiometry of 8.3 and 6.1 beta-lg molecules complexed per LM- or HM-pectin molecule, respectively. The C2 had about 16.5 and 15.1 beta-lg molecules complexed per LM- and HM-pectin, respectively.

Harper S. L., Begg G. E., and Speicher D. W. (2001) Role of terminal nonhomologous domains in initiation of human red cell spectrin dimerization. *Biochemistry* **40**, 9935-9943.

Abstract: Human erythrocyte spectrin is an antiparallel heterodimer comprised of a 280 kDa alpha subunit and a 246 kDa beta subunit which further associates into tetramers in the red cell membrane cytoskeleton. Lateral association of the flexible rodlike monomers involves a multiple-step process that is initiated by a high affinity association near the actin-binding end of the molecule (dimer nucleation site). In this study, recombinant alpha and beta proteins comprising two or four "spectrin type" motifs with and without adjacent, terminal nonhomologous domains were evaluated for their relative contributions to dimer initiation, and the thermodynamic properties of these heterodimer complexes were measured. Sedimentation equilibrium studies showed that in the absence of the heterologous subunit, individual recombinant proteins formed weak homodimers ($K_d > 0.3 \text{ mM}$). When 2-motif (alpha20-21 and beta1-2) and 4-motif (alpha18-21 and beta1-4) recombinants lacking the terminal nonhomologous domains were paired with the complementary protein, high affinity heterodimers were formed in sedimentation equilibrium analysis. Both the alpha20-21/beta1-2 complex and the alpha20-21EF/betaABD1-2 complex showed stoichiometric binding with similar binding affinities (K_d approximately 10 nM) using isothermal titration calorimetry. The alpha20-21/beta1-2 complex showed an enthalpy of -10 kcal/mol, while the alpha20-21EF/betaABD1-2 complex showed an enthalpy of -13 kcal/mol. Pull-down assays using alpha spectrin GST fusion proteins showed strong associations between all heterodimer complexes in physiological buffer, but all heterodimer complexes were destabilized by the presence of Triton X-100 and other detergents. Complexes lacking the nonhomologous domains were destabilized to a greater extent than complexes that included the nonhomologous domains. The detergent effect appears to be responsible for the apparent essential role of the nonhomologous domains in prior reports. Taken together, our results indicate that the terminal nonhomologous domains do not contribute to dimer initiation nor are they required for formation of high affinity spectrin heterodimers in physiological buffers.

Hoyer W. and Hard T. (2008) Interaction of Alzheimer's A beta peptide with an engineered binding protein--thermodynamics and kinetics of coupled folding-binding. *J Mol Biol* **378**, 398-411.

Abstract: The oligomerization and aggregation of the amyloid-beta (A beta) peptide, a cleavage product of the amyloid precursor protein predominantly 40 or 42 amino acids in length, has been implicated in the pathogenesis of Alzheimer's disease. The identification of A beta-binding agents, e.g., antibodies or peptides, constitutes a promising therapeutic approach. However, the amount of structural and biophysical data on the underlying A beta interactions is currently very limited. We have earlier determined the structure of A beta (1-40) in complex with the affibody protein Z(A beta 3), a selected binding protein based on a three-helix bundle scaffold (Z domain). Z(A beta 3) is a dimer of affibody subunits linked via a disulfide bridge involving a selected cysteine mutation at position 28. Z(A beta 3) binds to the central and C-terminal part of A beta (residues 17-36), which adopts a beta-hairpin conformation in the complex. Here

we present a detailed biophysical analysis of the Z(A beta 3):A beta (1-40) interaction, employing NMR, circular dichroism spectroscopy, 8-anilino-1-naphthalenesulfonic acid and tyrosine fluorescence, size-exclusion chromatography, thermal denaturation profiles and isothermal titration calorimetry. We conclude that (i) free Z(A beta 3) is characterized by conformational exchange and the loss of helix 1 of the three-helix bundle scaffold; (ii) a high-energy barrier is associated with the conversion of an initial Z(A beta 3):A beta (1-40) recognition complex into the native complex structure, entailing slow binding kinetics; (iii) both A beta and Z(A beta 3) fold upon binding, which, e.g., becomes manifest in the binding thermodynamics that feature a large negative change in heat capacity; (iv) the C28-disulfide does not merely afford dimerization, but its impact on the binding interfaces of the affibody subunits and A beta is a prerequisite for tight binding. The extensive folding coupled to binding observed here likely constitutes an obligate feature of biomolecular interactions involving the central and C-terminal part of A beta. Options for improvement of Z(A beta) binding proteins are discussed

Huang S. L., Lin F. Y., and Yang C. P. (2005) Microcalorimetric studies of the effects on the interactions of human recombinant interferon-alpha2a. *Eur J Pharm Sci* **24**, 545-552.

Abstract: The applicability of the physical stability of protein solution monitored by isothermal titration calorimetry (ITC) was evaluated. The second virial coefficient, b_2 , derived from the dilution enthalpies of protein solution measured by ITC under various experimental conditions was studied. The protein applied in this work is human recombinant interferon-alpha2a (hrIFN-alpha2a), which is a commercial drug applied for the treatment of virus-infected diseases. The results obtained were used to predict the possibility of hrIFN-alpha2a aggregation, and the prediction can be further confirmed by size-exclusion chromatography (SEC). Various factors affecting the stability of protein solution were investigated, for example, temperature, salts, surfactants, and mechanical stress. Specifically, the results show that the dilution enthalpy of hrIFN-alpha2a increased with increasing temperature and NaCl concentration, while b_2 decreased, indicating that the attraction between hrIFN-alpha2a molecules was enhanced under these conditions. On studying the effect of mechanical stress, the data obtained reveals that the introduction of centrifugal or vortex force strengthened the attractive forces between hrIFN-alpha2a molecules. These implications were supported by SEC data, demonstrating that the amount of aggregated hrIFN-alpha2a was increased. As a consequence, the methodologies presented in this investigation offer a possibility of monitoring the physical stability of protein solution at various stages of recovery, purification as well as the development of appropriate drug storage formulations.

Jelinska C., Conroy M. J., Craven C. J., Hounslow A. M., Bullough P. A., Waltho J. P., Taylor G. L., and White M. F. (2005) Obligate heterodimerization of the archaeal Alba2 protein with Alba1 provides a mechanism for control of DNA packaging. *Structure (Camb)* **13**, 963-971.

Abstract: Organisms growing at elevated temperatures face a particular challenge to maintain the integrity of their genetic material. All thermophilic and hyperthermophilic archaea encode one or more copies of the Alba (Sac10b) gene. Alba is an abundant, dimeric, highly basic protein that binds cooperatively and at high density to DNA. *Sulfolobus solfataricus* encodes a second copy of the Alba gene, and the Alba2 protein is expressed at approximately 5% of the level of Alba1. We demonstrate by NMR, ITC, and crystallography that Alba2 exists exclusively as a heterodimer with Alba1 at physiological concentrations and that heterodimerization exerts a clear effect upon the DNA packaging, as observed by EM, potentially by changing the interface between adjacent Alba dimers in DNA complexes. A functional role for Alba2 in modulation of higher order chromatin structure and DNA condensation is suggested.

Jorgensen A. D., Nohr J., Kastrup J. S., Gajhede M., Sigurskjold B. W., Sauer J., Svergun D. I., Svensson B. and Vestergaard B. (2008) Small angle X-ray studies reveal that *Aspergillus niger* glucoamylase has a defined extended conformation and can form dimers in solution. *J Biol Chem* **283**, 14772-14780.

Abstract: The industrially important glucoamylase 1 is an exo-acting glycosidase with substrate preference for alpha-1,4 and alpha-1,6 linkages at non-reducing ends of starch. It consists of a starch binding and a catalytic domain interspersed by a highly glycosylated polypeptide linker. The linker function is poorly understood and structurally undescribed, and data regarding domain organization and intramolecular functional cooperativity are conflicting or non-comprehensive. Here, we report a combined small angle x-ray scattering and calorimetry study of *Aspergillus niger* glucoamylase 1, glucoamylase 2, which lacks a starch binding domain, and an engineered low-glycosylated variant of glucoamylase 1 with a short linker. Low resolution solution structures show that the linker adopts a compact structure rendering a well defined

extended overall conformation to glucoamylase. We demonstrate that binding of a short heterobidentate inhibitor simultaneously directed toward the catalytic and starch binding domains causes dimerization of glucoamylase and not, as suggested previously, an intramolecular conformational rearrangement mediated by linker flexibility. Our results suggest that glucoamylase functions via transient dimer formation during hydrolysis of insoluble substrates and address the question of the cooperative effect of starch binding and hydrolysis

Jung J. M., Savin G., Pouzot M., Schmitt C. and Mezzenga R. (2008) Structure of heat-induced beta-lactoglobulin aggregates and their complexes with sodium-dodecyl sulfate. *Biomacromolecules*. **9**, 2477-2486.

Abstract: We report on the conformation of heat-induced bovine beta-lactoglobulin (betalg) aggregates prepared at different pH conditions, and their complexes with model anionic surfactants such as sodium dodecyl sulfate (SDS). The investigation was carried out by combining a wide range of techniques such as ultra small angle light scattering, static and dynamic light scattering, small angle neutron scattering, small-angle X-ray scattering, electrophoretic mobility, isothermal titration calorimetry (ITC) and transmission electron microscopy. Three types of aggregates were generated upon heating betalg aqueous dispersions at increasing pH from 2.0 to 5.8 to 7.0: rod-like aggregates, spherical aggregates, and worm-like primary aggregates, respectively. These aggregates were shown not only to differ for their sizes and morphologies, but also for their internal structures and fractal dimensions. The main differences between aggregates are discussed in terms of the ionic charge and conformational changes arising for betalg at different pHs. The formation of complexes between SDS and the various protein aggregates at pH 3.0 was shown to occur by two main mechanisms: at low concentration of SDS, the complex formation occurs essentially by ionic binding between the positive residues of the protein and the negative sulfate heads of the surfactant. At complete neutralization of charges, precipitation of the complexes is observed. Upon further increase in SDS concentration, complex formation of SDS and the protein aggregates occurs primarily by hydrophobic interactions, leading to (i) the formation of an SDS double layer around the protein aggregates, (ii) the inversion of the total ionic charge of each individual protein aggregate, and (iii) the complete redispersion of the protein aggregate-SDS complexes in water. Remarkably, the SDS double layer around the protein aggregates provides an efficient protective shield, preventing precipitation of the aggregates at any possible pH values, including those values corresponding to the isoelectric pH of the aggregates

Kodama T., Watson I. D., and Woledge R. C. (1977) Calorimetric studies of the ADP binding to myosin subfragment 1, heavy meromyosin, and to myosin filaments. *J Biol Chem* **252**, 8085-8087.

Abstract: A calorimetric titration method was used to study the ADP binding to the chymotryptic subfragments of myosin, heavy meromyosin (HMM) and myosin subfragment 1 (S-1), and to myosin aggregated into filaments at low ionic strength. The binding constant (K) and heat of reaction (ΔH , kiloJoules (moles of ADP bound)⁻¹) were determined. For HMM in 0.5 M KCl, 0.01 M MgCl₂, 0.02 M Tris (pH 7.8) at 12 degrees, $\log K = 5.92 \pm 0.13$ and $\Delta H = -70.9 \pm 3.6 \text{ kJ mol}^{-1}$. These results agree with our previous findings for myosin in 0.5 M KCl at 12 degrees. When the KCl concentration was reduced to 0.1 M, the binding constant did not change significantly ($\log K = 6.09 \pm 0.06$) but the binding was more exothermic ($\Delta H = -90.1 \pm 3.3 \text{ kJ mol}^{-1}$). Similar results were obtained for myosin filaments in 0.1 M KCl and also for both the isoenzymes of S-1 (S-1(A1) and S-1(A2)) in 0.1 M KCl. In 0.5 M KCl, the binding curves suggest that about one ADP is bound per active site, but as 0.1 M KCl, the apparent stoichiometry drops from 0.7 to 0.75. The most probable explanation is that there is some site heterogeneity which is more evident at lower ionic strength.

Lahiri S., Devi P. G., Majumder P., Das S. and Dasgupta D. (2008) Self-association of the anionic form of the DNA-binding anticancer drug mithramycin. *J Phys. Chem B* **112**, 3251-3258.

Abstract: The aqueous-phase self-association of mithramycin (MTR), an aureolic acid anticancer antibiotic, has been studied using different spectroscopic techniques such as absorption, fluorescence, circular dichroism, and ¹H nuclear magnetic resonance spectroscopy. Results from these studies indicate self-association of the anionic antibiotic at pH 8.0 over a concentration range from micromolar to millimolar. These results could be ascribed to the following steps of self-association: M + M left arrow over right arrow M₂, M₂ + M left arrow over right arrow M₃, and M₃ + M left arrow over right arrow M₄, where M, M₂, M₃, and M₄ represent the monomer, dimer, trimer, and tetramer of mithramycin, respectively. Dynamic light scattering and isothermal titration calorimetry studies also support aggregation.

In contrast, an insignificant extent of self-association is found for the neutral drug (at pH 3.5) and the [(MTR)₂Mg²⁺] complex (at pH 8.0). Analysis of 2D NMR spectra of 1 mM MTR suggests that the sugar moieties play a role in the self-association process. Self-association of the drug might occur either via hydrophobic interaction of the sugar residues among themselves or water-mediated hydrogen bond formation between sugar residue(s). On the other hand, absence of a significant upfield shift of the aromatic protons from 100 microM to 1 mM MTR suggests against the possibility of stacking interactions between the aromatic rings as a stabilizing force for the formation of the dimer and higher oligomers

Lakshminarayanan R., Vivekanandan S., Samy R. P., Banerjee Y., Chi-Jin E. O., Teo K. W., Jois S. D., Kini R. M. and Valiyaveetil S. (2008) Structure, self-assembly, and dual role of a beta-defensin-like peptide from the Chinese soft-shelled turtle eggshell matrix. *J Am. Chem Soc.* **130**, 4660-4668.

Abstract: Biomineral matrix formation and molecular recognition are two important processes associated with eggshell biomineralization. To understand these two processes, a major intracrystalline peptide, pelovaterin, was isolated from turtle (*Pelodiscus sinensis*) eggshell and its tertiary and quaternary structures were established. The global fold of pelovaterin is similar to that of human beta-defensins but has a large hydrophobic core and a short hydrophilic N-terminal segment, which is not preserved in defensins. Pelovaterin exhibits strong antimicrobial activity against two pathogenic gram-negative bacteria, *Pseudomonas aeruginosa* and *Proteus vulgaris*, and stabilizes a thin film of metastable vaterite. We show that pelovaterin self-aggregates in the form of micellar nanospheres and the aggregation in solution is entropy-driven. It is suggested that the micellar aggregation of pelovaterin is responsible for the induction and stabilization of the metastable phase by altering the interfacial energy. The results demonstrate the adaptability of an extracellular matrix protein to perform multiple tasks: polymorph discrimination and protection of the contents of the egg against bacterial invasion

Lendel C., Dincbas-Renqvist V., Flores A., Wahlberg E., Dogan J., Nygren P. A., and Hard T. (2004) Biophysical characterization of Z(SPA-1)--a phage-display selected binder to protein A. *Protein Sci* **13**, 2078-2088.

Abstract: Affibodies are a novel class of binding proteins selected from phagemid libraries of the Z domain from staphylococcal protein A. The Z(SPA-1) affibody was selected as a binder to protein A, and it binds the parental Z domain with micromolar affinity. In earlier work we determined the structure of the Z:Z(SPA-1) complex and noted that Z(SPA-1) in the free state exhibits several properties characteristic of a molten globule. Here we present a more detailed biophysical investigation of Z(SPA-1) and four Z(SPA-1) mutants with the objective to understand these properties. The characterization includes thermal and chemical denaturation profiles, ANS binding assays, size exclusion chromatography, isothermal titration calorimetry, and an investigation of structure and dynamics by NMR. The NMR characterization of Z(SPA-1) was facilitated by the finding that trimethylamine N-oxide (TMAO) stabilizes the molten globule conformation in favor of the fully unfolded state. All data taken together lead us to conclude the following: (1) The topology of the molten globule conformation of free Z(SPA-1) is similar to that of the fully folded structure in the Z-bound state; (2) the extensive mutations in helices 1 and 2 destabilize these without affecting the intrinsic stability of helix 3; (3) stabilization and reduced aggregation can be achieved by replacing mutated residues in Z(SPA-1) with the corresponding wild-type Z residues. This stabilization is better correlated to changes in helix propensity than to an expected increase in polar versus nonpolar surface area of the fully folded state.

Li D., Tang H. Y. and Speicher D. W. (2008) A structural model of the erythrocyte spectrin heterodimer initiation site determined using homology modeling and chemical cross-linking. *J Biol Chem* **283**, 1553-1562.

Abstract: Spectrin assembles into an anti-parallel heterodimeric flexible rod-like molecule through a multistep process initiated by a high affinity interaction between discrete complementary homologous motifs or "repeats" near the actin binding domain. Attempts to determine crystallographic structures of this critical dimer initiation complex have so far been unsuccessful. Therefore, in this study we determined the subunit-subunit docking interface and a plausible medium resolution structure of the heterodimer initiation site using homology modeling coupled with structural refinement based on experimentally determined distance constraints. Intramolecular and intermolecular cross-links formed by the "zero length" cross-linking reagent, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide were identified after trypsin digestion of cross-linked heterodimer complex using liquid chromatography-tandem mass spectrometry analysis. High

confidence assignment of cross-linked peptides was facilitated by determination of cross-linked peptide masses with an uncertainty of a few parts per million using a high sensitivity linear ion trap mass spectrometer equipped with a Fourier-transform ion cyclotron resonance detector. Six interchain cross-links distinguished between alternative docking models, and these distance constraints, as well as three intrachain cross-links, were used to further refine an initial homology-based structure. The final model is consistent with all available physical data, including protease protection experiments, isothermal titration calorimetry analyses, and location of a common polymorphism that destabilizes dimerization. This model supports the hypothesis that initial docking of the correct alpha and beta repeats from among many very similar repeats in both subunits is driven primarily by long range electrostatic interactions

Lin M. S., Chen L. Y., Tsai H. T., Wang S. S., Chang Y., Higuchi A. and Chen W. Y. (2008) Investigation of the mechanism of beta-amyloid fibril formation by kinetic and thermodynamic analyses. *Langmuir* **24**, 5802-5808.

Abstract: Extracellular beta-amyloid (A beta) deposit is considered as one of the primary factors that induce Alzheimer's disease (AD). The effects of various environmental factors, including temperature, ionic strength, and pH, on A beta (1-40) aggregation mechanisms were investigated in this study by spectrometry, isothermal titration calorimetry (ITC), and hydrophobic fluorescence assay. In the aggregation process, the secondary structure of A beta (1-40) transforms to the beta-sheet conformation, which could be described as a two-state model. As the temperature and ionic strength increase, the conformation of A beta converts to the beta-sheet structure with an increased rate. Results of circular dichroism monitoring demonstrate that the rate constant of nucleation is smaller than that of elongation, and the nucleation is the rate-determining step during the overall A beta aggregation. The beta-sheet structure was stabilized by hydrophobic forces, as revealed by the ITC measurements. The different structural aggregates and forming pathways could be identified and discriminated at high and low ionic strengths, resulting in distinctive fibril conformations. Furthermore, the thermodynamic analysis shows that hydrophobic interaction is the major driving force in the nucleation step. Our study provides an insight into the discriminative mechanisms of beta-amyloid aggregation via kinetics and thermodynamics, especially the first reported thermodynamics information obtained by ITC

Luke K., Apiyo D., and Wittung-Stafshede P. (2005) Role of the Unique Peptide Tail in Hyperthermostable Aquifex aeolicus Cochaperonin Protein 10. *Biochemistry* **44**, 14385-14395.

Abstract: All known cochaperonin protein 10 (cpn10) molecules are heptamers of seven identical subunits noncovalently linked by beta-strand interactions. Cpn10 from the deep-branching, hyperthermophilic bacterium Aquifex aeolicus (Aacpn10) shows high homology with mesophilic and other thermophilic cpn10 sequences, except for a 25-residue C-terminal extension not found in any other cpn10. Prior to atomic structure information, we here address the role of the tail by biophysical means. A tail-lacking variant (Aacpn10-del25) also adopts a heptameric structure in solution and exhibits natively-like substrate-refolding activity. Thermal and chemical perturbations of both Aacpn10 and Aacpn10-del25, probed by far-UV circular dichroism, demonstrate that both proteins have high thermodynamic stability. Heptamer-monomer dissociation midpoints were defined by isothermal titration calorimetry; at 25 degrees C, the values for Aacpn10 and Aacpn10-del25 are within 2-fold of each other and close to reported midpoints for mesophilic cpn10 proteins. In contrast, the monomer stabilities for the A. aeolicus proteins are significantly higher than those of mesophilic homologues at 30 degrees C; thus, heptamer thermophily is a result of more stable monomers. Electron microscopy data reveals that Aacpn10-del25 heptamers are prone to stack on top of each other forming chainlike molecules; the electrostatic surface pattern of a structural model can explain this behavior. Taken together, the unique tail in Aacpn10 is not required for heptamer structure, stability, or function; instead, it appears to be an ancient strategy to avoid cochaperonin aggregation at extreme temperatures.

Luke K., Apiyo D., and Wittung-Stafshede P. (2005) Dissecting homo-heptamer thermodynamics by isothermal titration calorimetry: entropy-driven assembly of co-chaperonin protein 10. *Biophys J* **89**, 3332-3336.

Abstract: Normally, isothermal titration calorimetry (ITC) is used to study binding reactions between two different biomolecules. Self-association processes leading to homo-oligomeric complexes have usually not been studied by ITC; instead, methods such as spectroscopy and analytical ultracentrifugation, which only provide affinity and Gibbs-free energy (i.e., $K(D)$ and ΔG), are employed. We here demonstrate that

complete thermodynamic descriptions (i.e., $K(D)$, ΔG , ΔH , and ΔS) for self-associating systems can be obtained by ITC-dilution experiments upon proper analysis. We use this approach to probe the dissociation (and thus association) equilibrium for the heptameric co-chaperonin proteins 10 (cpn10) from *Aquifex aeolicus* (Aacpn10-del25) and human mitochondria (hmcpn10). We find that the midpoints for the heptamer-monomer equilibrium occur at $0.51 \pm 0.03 \mu\text{M}$ and $3.5 \pm 0.1 \mu\text{M}$ total monomer concentration (25 degrees C), for Aacpn10-del25 and hmcpn10, respectively. For both proteins, association involves endothermic enthalpy and positive entropy changes; thus, the reactions are driven by the entropy increase. This is in accord with the release of ordered water molecules and, for the thermophilic variant, a relaxation of monomer-tertiary structure when the heptamers form.

McDonald C. B., Seldeen K. L., Deegan B. J., Lewis M. S. and Farooq A. (2008) Grb2 adaptor undergoes conformational change upon dimerization. *Arch Biochem Biophys* **475**, 25-35.

Abstract: Grb2 is an adaptor protein that couples activated receptor tyrosine kinases to downstream effector molecules such as Ras and Akt. Despite being a central player in mitogenic signaling and a target for therapeutic intervention, the role of Grb2 oligomerization in cellular signaling is not well understood. Here, using the techniques of size-exclusion chromatography, mass spectrometry, analytical ultracentrifugation and isothermal titration calorimetry, we demonstrate that Grb2 exists in monomer-dimer equilibrium in solution and that the dissociation of dimer into monomers is entropically-driven without an unfavorable enthalpic change at physiological temperatures. Our data indicate that enthalpy and entropy of dimer dissociation are highly temperature-dependent and largely compensate each other resulting in negligible effect of temperature on the overall free energy. From the plot of enthalpy change versus temperature, the magnitude of heat capacity change derived is much smaller than that expected from the rather large molecular surfaces becoming solvent-occluded upon Grb2 dimerization, implying that Grb2 monomers undergo conformational rearrangement upon dimerization. 3D structural models of Grb2 dimer and monomers suggest strongly that such conformational rearrangement upon dimerization may arise from domain swapping. Taken together, our study provides novel insights into the role of Grb2 as an adaptor in cellular signaling circuitry and how Grb2 dimerization may impart high fidelity in signal transduction as well as lead to rapid signal amplification upon receptor stimulation

Meier-Andrejszki L., Bjelic S., Naud J. F., Lavigne P. and Jelesarov I. (2007) Thermodynamics of b-HLH-LZ protein binding to DNA: the energetic importance of protein-DNA contacts in site-specific E-box recognition by the complete gene product of the Max p21 transcription factor. *Biochemistry* **46**, 12427-12440.

Abstract: The Myc/Mad/Max network of dimeric basic region-helix-loop-helix-leucine zipper (b-HLH-LZ) transcription factors bind to enhancer box sequences (E-box) in the promoters of a large set of genes that control cell metabolism, proliferation, and differentiation. Max (Myc-associated factor X) is the obligate heterodimerization partner of Myc and Mad proteins. On the other hand, Max is the only member of the family capable of forming a stable homodimer. As part of the transcriptional regulation mechanism, Myc/Max and Mad/Max heterodimers and Max homodimers are thought to compete for binding to the E-box target sequences. E-box recognition is structurally supported by the b-HLH-LZ structural motif, which also promotes dimerization. However, the actual dimerization and heterodimerization constants of the complete gene products and their affinities for E-box sequences are not known. Also, the detailed thermodynamic characterization of DNA binding by these transcription factors has not been done yet. Such knowledge is necessary for complete understanding of the transcriptional regulation carried out by the Myc/Mad/Max network. Here, we report the first in-depth thermodynamic characterization of the stability and specific DNA binding of a full length gene product of the Myc/Mad/Max family, namely, Max protein isoform p21 (Max p21). Using calorimetric methods (DSC and ITC) we have determined the dimerization constant of Max p21 in the low micromolar range, and the Max p21/E-box complex dissociation constant in the low nanomolar range at 37 degrees C. The association is driven by a large exothermic effect, which is partly compensated by entropic factors. The energetic contribution to binding affinity of seven highly conserved residues that contact the DNA was probed by X-to-Ala mutagenesis. The results demonstrate that high binding affinity critically relies on the side chain of Arg 26. Furthermore, the mutational analysis points to the important role of the persistent helical turn that comprises this residue at the junction of the basic region and helix H1. Altogether, the study supports the idea that Max p21 can bind E-box sequences in vivo and likely participates directly in the regulation of transcription as homodimer.

Nigen M., Croguennec T., Renard D. and Bouhallab S. (2007) Temperature affects the supramolecular structures resulting from alpha-lactalbumin-lysozyme interaction. *Biochemistry* **46**, 1248-1255.

Abstract: The interaction between alpha-lactalbumin and lysozyme, two globular proteins with highly homologous tertiary structures but opposite electric charges, was investigated. As assessed by isothermal titration calorimetry, lysozyme did not bind to the native form of alpha-lactalbumin but did interact with calcium-depleted alpha-lactalbumin (apo alpha-LA). This interaction leads to the formation of different supramolecular structures depending on the temperature. Heterogeneous, amorphous aggregates are formed at 5 degrees C, while droplets, coacervate-like structures, exist at 45 degrees C. The coacervates are formed by equimolar quantities of the two proteins, but their size and number depend on the initial protein molar ratio. These supramolecular structures are found to be stable when the temperature is decreased to 5 degrees C, while prolonged heating at 45 degrees C induces the formation of larger coacervates through a coalescence phenomenon. Surprisingly, interplay occurs between aggregates and coacervates when the temperature is increased from 5 to 45 degrees C. We discuss the results in terms of subtle heat-induced conformational changes in apo alpha-LA. In conclusion, our results show an association between globular proteins that leads to the formation of a variety of supramolecular structures in a temperature-dependent manner and confirm the primordial role of certain alpha-lactalbumin unfolding intermediates in protein-driven assembly.

Panse V. G., Swaminathan C. P., Surolia A., and Varadarajan R. (2000) Thermodynamics of substrate binding to the chaperone SecB. *Biochemistry* **39**, 2420-2427.

Abstract: The thermodynamics of binding of unfolded polypeptides to the chaperone SecB was investigated in vitro by isothermal titration calorimetry and fluorescence spectroscopy. The substrates were reduced and carboxamidomethylated forms of RNase A, BPTI, and alpha-lactalbumin. SecB binds both fully unfolded RNase A and BPTI as well as compact, partially folded disulfide intermediates of alpha-lactalbumin, which have 40-60% of native secondary structure. The heat capacity changes observed on binding the reduced and carboxamidomethylated forms of alpha-lactalbumin, BPTI, and RNase A were found to be -0.10, -0.29, and -0.41 kcal mol⁻¹ K⁻¹, respectively, and suggest that between 7 and 29 residues are buried upon substrate binding to SecB. In all cases, binding occurs with a stoichiometry of one polypeptide chain per monomer of SecB. There is no evidence for two separate types of binding sites for positively charged and hydrophobic ligands. Spectroscopic and proteolysis protection studies of the binding of SecB to poly-L-Lys show that binding of highly positively charged peptide ligands to negatively charged SecB leads to charge neutralization and subsequent aggregation of SecB. The data are consistent with a model where SecB binds substrate molecules at an exposed hydrophobic cleft. SecB aggregation in the absence of substrate is prevented by electrostatic repulsion between negatively charged SecB tetramers.

Panyukov Y. V., Nemykh M. A., Dobrov E. N. and Drachev V. A. (2008) Surfactant-Induced Amorphous Aggregation of Tobacco Mosaic Virus Coat Protein: A Physical Methods Approach. *Macromol. Biosci* **8**, 199-209.

Abstract: The interactions of non-ionic surfactant Triton X-100 and the coat protein of tobacco mosaic virus, which is an established model for both ordered and non-ordered protein aggregation, were studied using turbidimetry, differential scanning calorimetry, isothermal titration calorimetry, and dynamic light scattering. It was found that at the critical aggregation concentration (equal to critical micelle concentration) of 138×10^{-6} M, Triton X-100 induces partial denaturation of tobacco mosaic virus coat protein molecules followed by protein amorphous aggregation. Protein aggregation has profound ionic strength dependence and proceeds due to hydrophobic sticking of surfactant-protein complexes (start aggregates) with initial radii of 46 nm. It has been suggested that the anionic surfactant sodium dodecyl sulfate forms mixed micelles with Triton X-100 and therefore reverses protein amorphous aggregation with release of protein molecules from the amorphous aggregates. A stoichiometric ratio of 5 was found for Triton X-100-sodium dodecyl sulfate interactions.

Piszczek G., Rozycki J., Singh S. K., Ginsburg A., and Maurizi M. R. (2005) The molecular chaperone, ClpA, has a single high affinity peptide binding site per hexamer. *J Biol Chem* **280**, 12221-12230.

Abstract: Substrate recognition by Clp chaperones is dependent on interactions with motifs composed of specific peptide sequences. We studied the binding of short motif-bearing peptides to ClpA, the chaperone component of the ATP-dependent ClpAP protease of *Escherichia coli* in the presence of ATPgammaS and Mg²⁺ at pH 7.5. Binding was measured by isothermal titration calorimetry (ITC) using the peptide,

AANDENYALAA, which corresponds to the SsrA degradation motif found at the C terminus of abnormal nascent polypeptides in vivo. One SsrA peptide was bound per hexamer of ClpA with an association constant ($K(A)$) of $5 \times 10^6 \text{ m}^{-1}$. Binding was also assayed by changes in fluorescence of an N-terminal dansylated SsrA peptide, which bound with the same stoichiometry of one per ClpA hexamer ($K(A)$ approximately $1 \times 10^7 \text{ m}^{-1}$). Similar results were obtained when ATP was substituted for ATP γ S at 6 degrees C. Two additional peptides, derived from the phage P1 RepA protein and the E. coli HemA protein, which bear different substrate motifs, were competitive inhibitors of SsrA binding and bound to ClpA hexamers with $K(A) > 3 \times 10^7 \text{ m}^{-1}$. DNS-SsrA bound with only slightly reduced affinity to deletion mutants of ClpA missing either the N-terminal domain or the C-terminal nucleotide-binding domain, indicating that the binding site for SsrA lies within the N-terminal nucleotide-binding domain. Because only one protein at a time can be unfolded and translocated by ClpA hexamers, restricting the number of peptides initially bound should avoid nonproductive binding of substrates and aggregation of partially processed proteins.

Portnaya I., Ben-Shoshan E., Cogan U., Khalfin R., Fass D., Ramon O. and Danino D. (2008) Self-assembly of bovine beta-casein below the isoelectric pH. *J Agric. Food Chem* **56**, 2192-2198.

Abstract: Beta-casein is an intrinsically unstructured amphiphilic protein that self-assembles into micelles at neutral pH. This paper reports that beta-casein self-organizes into micelles also under acidic conditions. The protein association behavior and micelle characteristics at pH 2.6, well below the pI, are presented. The pH was found to strongly affect the micelle shape and dimensions. Cryogenic transmission electron microscopy (cryo-TEM) experiments revealed disk-like micelles of 20-25 nm in length and approximately 3.5 nm in height in acidic conditions. An aggregation number of 6 was determined by sedimentation equilibrium under these conditions. Isothermal titration calorimetry experiments verified the association below the pI and allowed determination of the micellization enthalpy, the critical micellar concentration, and the micellization relative cooperativity (MR). Small-angle X-ray scattering results at concentrations below the critical micellization concentration (CMC) suggest that the monomeric protein is likely in a premolten globule state at low pH. Calculations of the protein charge at acidic and neutral pH reveal a similar high net charge but considerable differences in the charge distribution along the protein backbone. Overall the results show that beta-casein is amphiphilic at low pH, but the distribution of charge along the protein chain creates packing constraints that affect the micelle organization, leading at concentrations above the CMC to the formation of disk micelles

Roy S., Katayama D., Dong A., Kerwin B. A., Randolph T. W., and Carpenter J. F. (2006) Temperature dependence of benzyl alcohol- and 8-anilinonaphthalene-1-sulfonate-induced aggregation of recombinant human interleukin-1 receptor antagonist. *Biochemistry* **45**, 3898-3911.

Abstract: The critical role played by temperature in ligand-induced protein aggregation was investigated. Recombinant human interleukin-1 receptor antagonist (rhIL-1ra) and the ligands benzyl alcohol and 8-anilinonaphthalene-1-sulfonate (ANS) were used. We investigated aggregation kinetics and the conformation and cysteine reactivity of rhIL-1ra in buffer alone or in the presence of 0.9% (w/v) benzyl alcohol or 4.2 or 21 mM ANS at 25 and 37 degrees C. In buffer, protein aggregation was not detected at 25 degrees C but occurred at 37 degrees C. At 25 degrees C, neither benzyl alcohol nor 4.2 mM ANS enhanced aggregation. However, at 37 degrees C, both compounds greatly accelerated protein aggregation. With 21 mM ANS, rhIL-1ra aggregation was accelerated at both temperatures, but the effect was more pronounced at 37 degrees C than at 25 degrees C. Increasing the temperature from 25 to 37 degrees C caused a minor perturbation in the tertiary structure of rhIL-1ra in buffer but no detectable alteration in secondary structure. Benzyl alcohol enhanced the tertiary structural perturbation at 37 degrees C, but the secondary structure was not affected by the ligand. The reactivity of buried free cysteines of rhIL-1ra was enhanced by benzyl alcohol at 37 degrees C but not at 25 degrees C, consistent with the structural results. Isothermal titration calorimetry documented that the interaction of benzyl alcohol with rhIL-1ra was hydrophobic and that the degree of hydrophobic interactions increased with temperature. At 25 degrees C, the interaction of ANS with rhIL-1ra was electrostatic, but at 37 degrees C, both electrostatic and hydrophobic interactions were important. Taken together, our results support the conclusion that benzyl alcohol and ANS interact hydrophobically with partially unfolded aggregation-prone protein molecules, resulting in temperature-dependent increases in their levels and acceleration of protein aggregation.

Sakurai K., Oobatake M., and Goto Y. (2001) Salt-dependent monomer-dimer equilibrium of bovine beta-lactoglobulin at pH 3. *Protein Sci* **10**, 2325-2335.

Abstract: Although bovine beta-lactoglobulin assumes a monomeric native structure at pH 3 in the absence of salt, the addition of salts stabilizes the dimer. Thermodynamics of the monomer-dimer equilibrium dependent on the salt concentration were studied by sedimentation equilibrium. The addition of NaCl, KCl, or guanidine hydrochloride below 1 M stabilized the dimer in a similar manner. On the other hand, NaClO(4) was more effective than other salts by about 20-fold, suggesting that anion binding is responsible for the salt-induced dimer formation, as observed for acid-unfolded proteins. The addition of guanidine hydrochloride at 5 M dissociated the dimer into monomers because of the denaturation of protein structure. In the presence of either NaCl or NaClO(4), the dimerization constant decreased with an increase in temperature, indicating that the enthalpy change ($\Delta H(D)$) of dimer formation is negative. The heat effect of the dimer formation was directly measured with an isothermal titration calorimeter by titrating the monomeric beta-lactoglobulin at pH 3.0 with NaClO(4). The net heat effects after subtraction of the heat of salt dilution, corresponding to $\Delta H(D)$, were negative, and were consistent with those obtained by the sedimentation equilibrium. From the dependence of dimerization constant on temperature measured by sedimentation equilibrium, we estimated the $\Delta H(D)$ value at 20 degrees C and the heat capacity change (ΔC_p) of dimer formation. In both NaCl and NaClO(4), the obtained ΔC_p value was negative, indicating the dominant role of burial of the hydrophobic surfaces upon dimer formation. The observed ΔC_p values were consistent with the calculated value from the X-ray dimeric structure using a method of accessible surface area. These results indicated that monomer-dimer equilibrium of beta-lactoglobulin at pH 3 is determined by a subtle balance of hydrophobic and electrostatic effects, which are modulated by the addition of salts or by changes in temperature.

Tellez L. A., Blancas-Mejia L. M., Carrillo-Nava E., Mendoza-Hernandez G., Cisneros D. A. and Fernandez-Velasco D. A. (2008) Thermal unfolding of triosephosphate isomerase from *Entamoeba histolytica*: dimer dissociation leads to extensive unfolding. *Biochemistry* **47**, 11665-11673.

Abstract: In mesophiles, triosephosphate isomerase (TIM) is an obligate homodimer. We have previously shown that monomeric folding intermediates are common in the chemical unfolding of TIM, where dissociation provides 75% of the overall conformational stability of the dimer. However, analysis of the crystallographic structure shows that, during unfolding, intermonomeric contacts contribute to only 5% of the overall increase in accessible surface area. In this work several methodologies were used to characterize the thermal dissociation and unfolding of the TIM from *Entamoeba histolytica* (EhTIM) and a monomeric variant obtained by chemical derivatization (mEhTIM). During EhTIM unfolding, sequential transitions corresponding to dimer dissociation into a compact monomeric intermediate followed by unfolding and further aggregation of the intermediate occurred. In the case of mEhTIM, a single transition, analogous to the second transition of EhTIM, was observed. Calorimetric, spectroscopic, hydrodynamic, and functional evidence shows that dimer dissociation is not restricted to localized interface reorganization. Dissociation represents 55% ($\Delta H(Diss) = 146.8 \text{ kcal mol}^{-1}$) of the total enthalpy change ($\Delta H(Tot) = 266 \text{ kcal mol}^{-1}$), indicating that this process is linked to substantial unfolding. We propose that, rather than a rigid body process, subunit assembly is best represented by a fly-casting mechanism. In TIM, catalysis is restricted to the dimer; therefore, the interface can be viewed as the final nucleation motif that directs assembly, folding, and function

Terzi E., Holzemann G., and Seelig J. (1994) Reversible random coil-beta-sheet transition of the Alzheimer beta-amyloid fragment (25-35). *Biochemistry* **33**, 1345-1350.

Abstract: The beta-amyloid protein (39-43 amino acid residues) is the major constituent of the amyloid deposits found in brain of patients with Alzheimer's disease. Using circular dichroism spectroscopy, we have studied the secondary structure and the aggregation of fragment 25-35 of the beta-amyloid protein (beta AP(25-35)OH) under a variety of conditions. beta AP(25-35)OH in solution at pH 4.0 or 5.5 exhibits a concentration-dependent random coil \leftrightarrow beta-sheet transition. The equilibrium is characterized spectroscopically by an isodichroic point and can be described quantitatively by a simple association model with association constants between $1.8 \times 10^4 \text{ M}^{-1}$ (non-cooperative model, nucleation parameter $\sigma = 1$) and $2.9 \times 10^4 \text{ M}^{-1}$ (cooperative model, $\sigma = 0.2$). The enthalpy of association is ΔH approximately -3 kcal/mol as determined by titration calorimetry. The equilibrium is shifted completely toward beta-structured fibrils at pH 7.4 where the Met-35 carboxyl group is fully charged. In contrast, removal of the charged carboxy terminus by amidation locks the equilibrium in the random coil conformation. Model

calculations suggest an antiparallel beta-sheet structure involving residues 28-35 which is stabilized at both ends of the beta-sheet by ion pairs formed between Lys-28 and Met-35. Removal of fibrils via millipore filtration leads to solutions with random coil monomers only. Seeding these solutions with a few fibrils establishes a new random coil \leftrightarrow beta-sheet equilibrium.

Terzi E., Holzemann G., and Seelig J. (1995) Self-association of beta-amyloid peptide (1-40) in solution and binding to lipid membranes. *J Mol Biol* **252**, 633-642.

Abstract: The beta-amyloid peptide (beta AP), a 39 to 43 residue peptide, is the major component of Alzheimer plaques. Using circular dichroism spectroscopy, titration calorimetry, and analytical ultracentrifugation we have analyzed the self-association of beta AP(1-40) in aqueous solution and the binding of beta AP(1-40) to negatively charged lipid vesicles. The CD spectra of both aggregation and membrane binding are characterized by an isodichroic point at 212 nm, indicating a simple two-state equilibrium for both cases. In aqueous solution beta AP(1-40) exhibits a reversible, concentration-dependent random coil \leftrightarrow beta-structure transition which can be described by a cooperative aggregation model with an association constant of $s = 1.05 \times 10^4 \text{ M}^{-1}$ and a nucleation parameter of $\sigma = 0.012$. A similar conformational change is observed upon addition of lipid. At a given peptide concentration, the addition of negatively charged, small unilamellar vesicles also induces a conformational change from a random coil conformation to a conformation with 40 to 60% beta-structure. The binding isotherm can be measured with high sensitivity titration calorimetry. It is approximately linear in the initial binding phase and exhibits an apparent saturation behaviour. The apparent binding constant decreases with concentration from K_{app} approximately 2100 M^{-1} at low concentration to 700 M^{-1} at the highest concentration measured. Peptide penetration into the lipid membrane and peptide aggregation at the membrane surface are proposed as possible mechanisms to explain the lipid-induced random coil \leftrightarrow beta-structure transition.

Thoppil A. A. and Kishore N. (2007) Equimolar mixture of 2,2,2-trifluoroethanol and 4-chloro-1-butanol is a stronger inducer of molten globule state: isothermal titration calorimetric and spectroscopic studies. *Protein J* **26**, 507-516.

Abstract: A mixture of 4-chloro-1-butanol and 2,2,2-Trifluoroethanol (TFE) has been used to generate Molten globule (MG) state of structurally homologous but functionally different proteins bovine alpha-lactalbumin and hen egg-white lysozyme. The thermal denaturation was done using UV-Visible spectroscopy. From UV-Visible profile, thermal transition was not observed beyond a particular concentration. There was an indication of molten globule state in case of alpha-lactalbumin from circular dichroism experiments. By intrinsic tryptophan fluorescence, acrylamide and potassium iodide quenching, 8-anilino-naphthalene sulfonic acid (ANS) binding and energy transfer studies the presence of molten globule state was confirmed. Quantitative characterization of MG state and determining the binding thermodynamics of ANS to the MG state was done using Isothermal Titration Calorimetry (ITC). Results show that alpha-lactalbumin exists in MG state at a particular concentration but lysozyme does not show features of MG state.

Velazquez-Campoy A., Leavitt S. A., and Freire E. (2004) Characterization of protein-protein interactions by isothermal titration calorimetry. *Methods Mol Biol* **261**, 35-54.

Abstract: Isothermal titration calorimetry (ITC) is a powerful technique to study both protein-ligand and protein-protein interactions. This methods chapter is devoted to describing protein-protein interactions, in particular, the association between two different proteins and the self-association of a protein into homodimers. ITC is the only technique that determines directly the thermodynamic parameters of a given reaction: ΔG , ΔH , ΔS , and ΔC_P . Isothermal titration calorimeters have evolved over the years and one of the latest models is the VP-ITC produced by Microcal, Inc. In this chapter we will be describing the general procedure for performing an ITC experiment as well as for the specific cases of porcine pancreatic trypsin binding to soybean trypsin inhibitor and the dissociation of bovine pancreatic alpha-chymotrypsin.

White E. W., Tanious F., Ismail M. A., Reszka A. P., Neidle S., Boykin D. W. and Wilson W. D. (2007) Structure-specific recognition of quadruplex DNA by organic cations: influence of shape, substituents and charge. *Biophys Chem* **126**, 140-153.

Abstract: Combining structure-specific recognition of nucleic acids with limited sequence reading is a promising method to reduce the size of the recognition unit required to achieve the necessary selectivity and binding affinity to control function. It has been demonstrated recently that G-quadruplex DNA

structures can be targeted by organic cations in a structure-specific manner. Structural targets of quadruplexes include the planar end surfaces of the G-tetrad stacked columns and four grooves. These provide different geometries and functional groups relative to duplex DNA. We have used surface plasmon resonance and isothermal titration calorimetry to show that binding affinity and selectivity of a series of quadruplex end-stacking molecules to human telomeric DNA are sensitive to compound shape as well as substituent type and position. ITC results indicate that binding is largely enthalpy driven. Circular dichroism was also used to identify a group of structurally related compounds that selectively target quadruplex grooves.

Yang F., Zhou B. R., Zhang P., Zhao Y. F., Chen J. and Liang Y. (2007) Binding of ferulic acid to cytochrome c enhances stability of the protein at physiological pH and inhibits cytochrome c-induced apoptosis. *Chem Biol Interact.* **170**, 231-243.

Abstract: Ferulic acid (FA) is one of the most effective components of a traditional Chinese medicine, angelica, and cytochrome c plays a vital role in apoptosis. Here we report the application of fluorescence spectroscopy, isothermal titration calorimetry (ITC), differential scanning calorimetry (DSC), and circular dichroism (CD) to investigate the mechanism for the interaction of bovine heart cytochrome c with FA and the effect of the binding on native state stability of the protein at physiological pH. Fluorescence spectroscopic studies together with ITC measurements indicate that FA binds to cytochrome c with moderate affinity and quenches the intrinsic fluorescence of the protein in a static way. ITC experiments show that the interaction of cytochrome c with FA is driven by a moderately favorable entropy increase in combination with a less favorable enthalpy decrease for the first binding site of the protein. The melting temperature of cytochrome c in the presence of FA measured by DSC and CD increases 4.0 and 5.0 degrees C, respectively, compared with that in the absence of FA. Taken together, these results indicate that FA binds to and stabilizes cytochrome c at physiological pH. Furthermore, binding of FA to cytochrome c inhibits cytochrome c-induced apoptosis of human hepatoma cell line SMMC-7721. Our data provide insight into the mechanism of drug-protein interactions, and will be helpful to the understanding of the mechanism for FA-inhibited and cytochrome c-induced apoptosis.

Zeng H., Yang X., Flowers R. A., and Gong B. (2002) A noncovalent approach to antiparallel beta-sheet formation. *J Am Chem Soc* **124**, 2903-2910.

Abstract: Four tripeptide chains, when attached to the same end of a hydrogen-bonded duplex (1.2) with the unsymmetrical, complementary sequences of ADAA/DADD, have been brought into proximity, leading to the formation of four hybrid duplexes, 1a.2a, 1a.2b, 1b.2a, and 1b.2b, each of which contains a two-stranded beta-sheet segment. The extended conformations of the peptide chains were confirmed by 1D and 2D NMR. The peptide strands stay registered through hydrogen bonding and the beta-sheets are stabilized by side chain interactions. Two-dimensional NMR data also indicate that the duplex template prevents further aggregation in the peptide segment. When the peptide chains are attached to the two different termini of the duplex template, NMR studies show the presence of a mixture with no clearly defined conformations. In the absence of the duplex template, the tripeptides are found to associate randomly. Finally, isothermal titration calorimetry studies revealed that the hybrid duplex 1a.2a was more stable than either the duplex template or the peptides alone.