

ITC XIV – Amyloid and Prion Studies

Belosi B., Gaggelli E., Guerrini R., Kozlowski H., Luczkowski M., Mancini F. M., Remelli M., Valensin D., and Valensin G. (2004) Copper binding to the neurotoxic peptide PrP106-126: thermodynamic and structural studies. *ChemBiochem* **5**, 349-359.

Abstract: The human prion protein fragment PrP(106-126) is a highly fibrillogenic peptide, resistant to proteinases and toxic to neurons; it derives from the normal prion protein (PrP(C)), with which it can interact, thus inhibiting its superoxide dismutase-like activity. The same properties are also shown by the abnormal isoform of the prion protein (PrP(Sc)), and this similarity makes PrP(106-126) an interesting model for the neurotoxic action of PrP(Sc). A role for copper in PrP(106-126) aggregation and toxicity has recently been evidenced, and the interaction of terminal Lys, His and Met residues with the copper ion at neutral pH has been suggested. In order to shed more light on the complex-formation equilibria of PrP(106-126) with the copper ion, a thorough investigation has been carried out by means of several experimental techniques: potentiometry, solution calorimetry, VIS spectrophotometry, circular dichroism, EPR and NMR spectroscopy. A shorter and more soluble fragment-PrP(106-113), which lacks the hydrophobic C-terminal domain of PrP(106-126) but contains all the potential donor groups-has also been considered for the sake of comparison. The involvement of terminal amino, imidazolic and amido nitrogens in complex formation has been confirmed, while no evidence was found for the interaction of side chains of Met and Lys residues with the copper ion. Solution structures for the main complexes are suggested.

Brazier M. W., Davies P., Player E., Marken F., Viles J. H. and Brown D. R. (2008) Manganese binding to the prion protein. *J Biol Chem* **283**, 12831-12839.

Abstract: There is considerable evidence that the prion protein binds copper. However, there have also been suggestions that prion protein (PrP) binds manganese. We used isothermal titration calorimetry to identify the manganese binding sites in wild-type mouse PrP. The protein showed two manganese binding sites with affinities that would bind manganese at concentrations of 63 and 200 μM at pH 5.5. This indicates that PrP binds manganese with affinity similar to other known manganese-binding proteins. Further study indicated that the main manganese binding site is associated with His-95 in the so-called "fifth site" normally associated with copper binding. Additionally, it was shown that occupancy by copper does not prevent manganese binding. Under these conditions, manganese binding resulted in an altered conformation of PrP, displacement of copper, and altered redox chemistry of the metal-protein complex. Cyclic voltammetric measurements suggested a complex redox chemistry involving manganese bound to PrP, whereas copper-bound PrP was able to undergo fully reversible electron cycling. Additionally, manganese binding to PrP converted it to a form able to catalyze aggregation of metal-free PrP. These results further support the notion that manganese binding could cause a conformation change in PrP and trigger changes in the protein similar to those associated with prion disease

Brockhaus M., Ganz P., Huber W., Bohrmann B., Loetscher H. R. and Seelig J. (2007) Thermodynamic studies on the interaction of antibodies with beta-amyloid peptide. *J Phys. Chem B* **111**, 1238-1243.

Abstract: Antibodies against beta-amyloid peptides (Abetas) are considered an important therapeutic opportunity in Alzheimer's disease. Despite the vast interest in Abeta no thermodynamic data on the interaction of antibodies with Abeta are available as yet. In the present study we use isothermal titration calorimetry (ITC) and surface plasmon resonance to provide a quantitative thermodynamic analysis of the interaction between soluble monomeric Abeta(1-40) and mouse monoclonal antibodies (mAb). Using four different antibodies directed against the N-terminal, middle, and C-terminal Abeta epitopes, we measured the thermodynamic parameters for the binding to Abeta. Each antibody species was found to have two independent and equal binding sites for Abeta with binding constants in the range of $10(7)$ to $10(8)$ M^{-1} . The binding reaction was essentially enthalpy driven with a reaction enthalpy of $\Delta H(0)(\text{Abeta})$ approximately -19 to -8 kcal/mol, indicating the formation of tight complexes. The loss in conformational freedom was supported by negative values for the reaction entropy $\Delta S(0)(\text{Abeta})$. We also measured the heat capacity change of the $1\text{mAb}:2\text{Abeta}$ reaction. $\Delta C(0)(p, \text{abeta})$ was large and negative but could not be explained exclusively by the hydrophobic effect. The free energy of binding was found to be linearly correlated with the size of the epitope.

Chatani E. and Goto Y. (2005) Structural stability of amyloid fibrils of beta(2)-microglobulin in comparison with its native fold. *Biochim Biophys Acta* **1753**, 64-75.

Abstract: Among various amyloidogenic proteins, beta(2)-microglobulin (beta2-m) responsible for dialysis-related amyloidosis is a target of extensive study because of its clinical importance and suitable size for examining the formation of amyloid fibrils in comparison with protein folding to the native state. The structure and stability of amyloid fibrils have been studied with various physicochemical methods, including H/D exchange of amyloid fibrils combined with dissolution of fibrils by dimethylsulfoxide and NMR analysis, thermodynamic analysis of amyloid fibril formation by isothermal calorimetry, and analysis of the effects of pressure on the structure of amyloid fibrils. The results are consistent with the view that amyloid fibrils are a main-chain-dominated structure with larger numbers of hydrogen bonds and pressure-accessible cavities in the interior, in contrast to the side-chain-dominated native structure with the optimal packing of amino acid residues. We consider that a main-chain dominated structure provides the structural basis for various conformational states even with one protein. When this feature is combined with another unique feature, template-dependent growth, propagation and maturation of the amyloid conformation, which cannot be predicted with Anfinsen's dogma, take place.

Gasset-Rosa F., Mate M. J., Vila-Fajardo C., Bravo J. and Giraldo R. (2008) Binding of sulphonated indigo derivatives to RepA-WH1 inhibits DNA-induced protein amyloidogenesis. *Nucleic Acids Res* **36**, 2249-2256.

Abstract: The quest for inducers and inhibitors of protein amyloidogenesis is of utmost interest, since they are key tools to understand the molecular bases of proteinopathies such as Alzheimer, Parkinson, Huntington and Creutzfeldt-Jakob diseases. It is also expected that such molecules could lead to valid therapeutic agents. In common with the mammalian prion protein (PrP), the N-terminal Winged-Helix (WH1) domain of the pPS10 plasmid replication protein (RepA) assembles in vitro into a variety of amyloid nanostructures upon binding to different specific dsDNA sequences. Here we show that di- (S2) and tetra-sulphonated (S4) derivatives of indigo stain dock at the DNA recognition interface in the RepA-WH1 dimer. They compete binding of RepA to its natural target dsDNA repeats, found at the repA operator and at the origin of replication of the plasmid. Calorimetry points to the existence of a major site, with micromolar affinity, for S4-indigo in RepA-WH1 dimers. As revealed by electron microscopy, in the presence of inducer dsDNA, both S2/S4 stains inhibit the assembly of RepA-WH1 into fibres. These results validate the concept that DNA can promote protein assembly into amyloids and reveal that the binding sites of effector molecules can be targeted to inhibit amyloidogenesis

Groenning M., Norrman M., Flink J. M., van de W. M., Bukrinsky J. T., Schluckebier G. and Frokjaer S. (2007) Binding mode of Thioflavin T in insulin amyloid fibrils. *J Struct. Biol* **159**, 483-497.

Abstract: Amyloid fibrils share various common structural features and their presence can be detected by Thioflavin T (ThT). In this paper, the binding mode of ThT to insulin amyloid fibrils was examined. Scatchard analysis and isothermal titration calorimetry (ITC) showed at least two binding site populations. The binding site population with the strongest binding was responsible for the characteristic ThT fluorescence. This binding had a capacity of about 0.1 moles of ThT bound per mole of insulin in fibril form. The binding capacity was unaffected by pH, but the affinity was lowest at low pH. Notably, presence of a third binding process prior to the other processes was suggested by ITC. Binding of ThT resulted in only minor changes in the fibril structure according to the X-ray diffraction patterns, where a slightly more dominant equatorial reflection at 16Å relative to the intersheet distance of 11Å was observed. No change in the interstrand distance of 4.8Å was observed. On the basis of our results, we propose that ThT binds in cavities running parallel to the fibril axis, e.g., between the protofilaments forming the fibrils. Such cavities have been proposed previously in insulin fibrils and several other amyloid fibril models.

Guilloureau L., Damian L., Coppel Y., Mazarguil H., Winterhalter M., and Faller P. (2006) Structural and thermodynamical properties of CuII amyloid-beta16/28 complexes associated with Alzheimer's disease. *J Biol Inorg Chem* **11**, 1024-1038.

Abstract: The aggregation of the peptide amyloid-beta (Aβ) to form amyloid plaques is a key event in Alzheimer's disease. It has been shown that CuII can bind to soluble Aβ and influence its aggregation properties. Three histidines and the N-terminal amine have been proposed to be involved in its coordination. Here, for the first time, we show isothermal titration calorimetry (ITC) measurements of the CuII binding to Aβ16 and Aβ28, models of the soluble Aβ. Moreover, different spectroscopic methods were applied. The studies revealed new insights into these CuII-Aβ complexes: (1) ITC showed two CuII binding sites, with an apparent K_d of 10⁻⁷ and 10⁻⁵ M, respectively; (2) the high-affinity site

has a smaller enthalpic contribution but a larger entropic contribution than the low-affinity binding site; (3) azide did not bind to CuII in the higher-affinity binding site, suggesting the absence of a weak, labile ligand; (4) azide could bind to the CuII in the low-affinity binding site in Abeta28 but not in Abeta16; (5) 1H-NMR suggests that the carboxylate of aspartic acid in position 1 is involved in the ligation to CuII in the high-affinity binding site; (6) the pKa of 11.3 of tyrosine in position 10 was not influenced by the binding of 2 equivalents of CuII.

Hatcher L. Q., Hong L., Bush W. D., Carducci T. and Simon J. D. (2008) Quantification of the binding constant of copper(II) to the amyloid-beta peptide. *J Phys. Chem B* **112**, 8160-8164.

Abstract: The amyloid beta (A beta) peptide of Alzheimer's disease binds copper(II), and the peptide-bound metal may be a source of reactive oxygen species and neurotoxicity. To circumvent peptide aggregation and reduce redox activity, there is growing interest in using metal chelates as drug therapeutics for AD, whose design requires accurate data on the affinity of A beta peptides for copper(II). Reports on Cu²⁺ binding to A beta range from approximately 10⁽⁵⁾ to approximately 10⁽⁹⁾; these values' being obtained for different peptide lengths (1-16, 1-28, 1-40, 1-42) at varying pH. Herein, we report that Cu²⁺'s binding to A beta(1-40) at 37 degrees C occurs in a 1:1 stoichiometry with a pH-dependent binding constant: 1.1 (+/-0.2) x 10⁽⁹⁾ M⁽⁻¹⁾ and 2.4 (+/-0.2) x 10⁽⁹⁾ M⁽⁻¹⁾ at pH 7.2 and 7.4, respectively. Under identical conditions, A beta(1-16) reveals a comparable binding constant, confirming that this portion of the peptide is the binding region. Several previously reported values can be reconciled with the current measurement by careful consideration of thermodynamics associated with the presence of competing ligands used to solubilize copper

He X., Zhu G., Koelsch G., Rodgers K. K., Zhang X. C., and Tang J. (2003) Biochemical and structural characterization of the interaction of memapsin 2 (beta-secretase) cytosolic domain with the VHS domain of GGA proteins. *Biochemistry* **42**, 12174-12180.

Abstract: Memapsin 2 (beta-secretase) is a membrane-associated aspartic protease that initiates the hydrolysis of beta-amyloid precursor protein (APP) leading to the production of amyloid-beta and the onset of Alzheimer's disease (AD). Both memapsin 2 and APP are transported from the cell surface to endosomes where APP hydrolysis takes place. Thus, the intracellular transport mechanism of memapsin 2 is important for understanding the pathogenesis of AD. We have previously shown that the cytosolic domain of memapsin 2 contains an acid-cluster-dileucine (ACDL) motif that binds the VHS domain of GGA proteins (He et al. (2002) FEBS Lett. 524, 183-187). This mechanism is the presumed recognition step for the vesicular packaging of memapsin 2 for its transport to endosomes. The phosphorylation of a serine residue within the ACDL motif has been reported to regulate the recycling of memapsin 2 from early endosomes back to the cell surface. Here, we report a study on the memapsin 2/VHS domain interaction. Using isothermal titration calorimetry, the dissociation constant, K_d, values are 4.0 x 10⁻⁴, 4.1 x 10⁻⁴, and 3.1 x 10⁻⁴ M for VHS domains from GGA1, GGA2, and GGA3, respectively. With the serine residue replaced by phosphoserine, the K_d decreased about 10-, 4-, and 14-fold for the same three VHS domains. A crystal structure of the complex between memapsin 2 phosphoserine peptide and GGA1 VHS was solved at 2.6 Å resolution. The side chain of the phosphoserine group does not interact with the VHS domain but forms an ionic interaction with the side chain of the C-terminal lysine of the ligand peptide. Energy calculation of the binding of native and phosphorylated peptides to VHS domains suggests that this intrapeptide ionic bond in solution may reduce the change in binding entropy and thus increase binding affinity.

Hong L., Bush W. D., Hatcher L. Q. and Simon J. (2008) Determining thermodynamic parameters from isothermal calorimetric isotherms of the binding of macromolecules to metal cations originally chelated by a weak ligand. *J Phys. Chem B* **112**, 604-611.

Abstract: An accurate data analysis method for determining stoichiometry and thermodynamic parameters from isothermal titration calorimetry data for the binding of macromolecules to metal cations that are solubilized through an association with a weak ligand is presented. This approach is applied to determine the binding constant for the association of Cu(II) to the first 16 residues of the Alzheimer's amyloid beta peptide, Abeta(1-16) under conditions where Cu(II) is rendered soluble through weak binding to glycine. At pH 7.2 and 37 degrees C, a binding constant of 1.5 x 10⁽⁹⁾ M⁻¹ (K_d = 0.7 nM) is determined for the association of Cu(II) with Abeta(1-16)

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

Kardos J., Yamamoto K., Hasegawa K., Naiki H., and Goto Y. (2004) Direct measurement of the thermodynamic parameters of amyloid formation by isothermal titration calorimetry. *J Biol Chem.* **279**, 55308-55314.

Abstract: Amyloid fibril deposition is associated with over 20 degenerative diseases, including Alzheimer's, Parkinson's and prion disease. Although research over the last few years has revealed the morphology and structural features of the amyloid form, knowledge about the thermodynamics of amyloid formation is limited. Here, we report for the first time a direct thermodynamic study of amyloid formation using isothermal titration calorimetry. beta2-Microglobulin, a protein responsible for dialysis-related amyloidosis, was used for extending amyloid fibrils in a seed-controlled reaction in the cell of the calorimeter. We investigated the enthalpy and heat capacity changes of the reaction, where the monomeric, acid-denatured molecules adopt an ordered, cross-beta sheet structure in the rigid amyloid fibrils. Despite the dramatic difference in morphology, beta2-microglobulin exhibited a similar heat capacity change upon amyloid formation to that of the folding to the native globular state, while the enthalpy change of the reaction proved to be markedly lower. In comparison with the native state, the results outline the important structural features of the amyloid fibrils: a similar extent of surface burial even with the supramolecular architecture of amyloid fibrils, a lower level of internal packing, and the possible presence of unfavorable side-chain contributions.

Kim Y. S., Randolph T. W., Manning M. C., Stevens F. J., and Carpenter J. F. (2003) Congo red populates partially unfolded states of an amyloidogenic protein to enhance aggregation and amyloid fibril formation. *J Biol Chem* **278**, 10842-10850.

Abstract: Congo red (CR) has been reported to inhibit or enhance amyloid fibril formation by several proteins. To gain insight into the mechanism(s) for these apparently paradoxical effects, we studied as a model amyloidogenic protein, a dimeric immunoglobulin light chain variable domain. With a range of molar ratios of CR, i.e. $r = [\text{CR}]/[\text{protein dimer}]$, we investigated the aggregation kinetics, conformation, hydrogen-deuterium exchange, and thermal stability of the protein. In addition, we used isothermal titration calorimetry to characterize the thermodynamics of CR binding to the protein. During incubation at 37 degrees C or during thermal scanning, with CR at $r = 0.3, 1.3,$ and 4.8 , protein aggregation was greatly accelerated compared with that measured in the absence of the dye. In contrast, with CR at $r = 8.8$, protein unfolding was favored over aggregation. The aggregates formed with CR at $r = 0$ or 0.3 were typical amyloid fibrils, but mixtures of amyloid fibrils and amorphous aggregates were formed at $r = 1.3$ and 4.8 . CR decreased the apparent thermal unfolding temperature of the protein. Furthermore, CR perturbed the

tertiary structure of the protein without significantly altering its secondary structure. Consistent with this result, CR also increased the rate of hydrogen-deuterium exchange by the protein. Isothermal titration calorimetry showed that CR binding to the protein was enthalpically driven, indicating that binding was mainly the result of electrostatic interactions. Overall, these results demonstrate that at low concentrations, CR binding to the protein favors a structurally perturbed, aggregation-competent species, resulting in acceleration of fibril formation. At high CR concentration, protein unfolding is favored over aggregation, and fibril formation is inhibited. Because low concentrations of CR can promote amyloid fibril formation, the therapeutic utility of this compound or its analogs to inhibit amyloidoses is questionable.

Li H., Koshiba S., Hayashi F., Tochio N., Tomizawa T., Kasai T., Yabuki T., Motoda Y., Harada T., Watanabe S., Inoue M., Hayashizaki Y., Tanaka A., Kigawa T. and Yokoyama S. (2008) Structure of the C-terminal phosphotyrosine interaction domain of Fe65L1 complexed with the cytoplasmic tail of amyloid precursor protein reveals a novel peptide binding mode. *J Biol Chem* **283**, 27165-27178.

Abstract: Fe65L1, a member of the Fe65 family, is an adaptor protein that interacts with the cytoplasmic domain of Alzheimer amyloid precursor protein (APP) through its C-terminal phosphotyrosine interaction/phosphotyrosine binding (PID/PTB) domain. In the present study, the solution structures of the C-terminal PID domain of mouse Fe65L1, alone and in complex with a 32-mer peptide (DAAVTPEERHLSKMQQNGYENPTYKFFEQMQN) derived from the cytoplasmic domain of APP, were determined using NMR spectroscopy. The C-terminal PID domain of Fe65L1 alone exhibits a canonical PID/PTB fold, whereas the complex structure reveals a novel mode of peptide binding. In the complex structure, the NPTY motif forms a type-I beta-turn, and the residues immediately N-terminal to the NPTY motif form an antiparallel beta-sheet with the beta5 strand of the PID domain, the binding mode typically observed in the PID/PTB.peptide complex. On the other hand, the N-terminal region of the peptide forms a 2.5-turn alpha-helix and interacts extensively with the C-terminal alpha-helix and the peripheral regions of the PID domain, representing a novel mode of peptide binding that has not been reported previously for the PID/PTB.peptide complex. The indispensability of the N-terminal region of the peptide for the high affinity of the PID-peptide interaction is consistent with NMR titration and isothermal calorimetry data. The extensive binding features of the PID domain of Fe65L1 with the cytoplasmic domain of APP provide a framework for further understanding of the function, trafficking, and processing of APP modulated by adapter proteins

Li Y., Cao M., and Wang Y. (2006) Alzheimer amyloid beta(1-40) peptide: interactions with cationic gemini and single-chain surfactants. *J Phys Chem B Condens Matter Mater Surf Interfaces Biophys* **110**, 18040-18045.

Abstract: The aggregation of amyloid beta-peptide [Abeta(1-40)] into fibril is a key pathological process associated with Alzheimer's disease. The effect of cationic gemini surfactant hexamethylene-1,6-bis-(dodecyldimethylammonium bromide) [C(12)H(25)(CH(3))(2)N(CH(2))(6)N(CH(3))(2)C(12)H(25)]Br(2) (designated as C(12)C(6)C(12)Br(2)) and single-chain cationic surfactant dodecyltrimethylammonium bromide (DTAB) on the Alzheimer amyloid beta-peptide Abeta(1-40) aggregation behavior was studied by microcalorimetry, circular dichroism (CD), and atomic force microscopy (AFM) measurements at pH 7.4. Without addition of surfactant, 0.5 g/L Abeta(1-40) mainly exists in dimeric state. It is found that the addition of the monomers of C(12)C(6)C(12)Br(2) and DTAB may cause the rapid aggregation of Abeta(1-40) and the fibrillar structures are observed by CD spectra and the AFM images. Due to the repulsive interaction among the head groups of surfactants and the formation of a small hydrophobic cluster of surfactant molecules, the fibrillar structure is disrupted again as the surfactant monomer concentration is increased, whereas globular species are observed in the presence of micellar solution. Different from single-chain surfactant, C(12)C(6)C(12)Br(2) has a much stronger interaction with Abeta(1-40) to generate larger endothermic energy at much lower surfactant concentration and has much stronger ability to induce the aggregation of Abeta(1-40).

Lin M. S., Chiu H. M., Fan F. J., Tsai H. T., Wang S. S., Chang Y. and Chen W. Y. (2007) Kinetics and enthalpy measurements of interaction between beta-amyloid and liposomes by surface plasmon resonance and isothermal titration microcalorimetry. *Colloids Surf B Biointerfaces* **58**, 231-236.

Abstract: The objective of this research is to understand the interaction mechanism of beta-amyloid (Abeta) with cell and were basically divided into two parts. The first part focused on the time-dependent structural changes of Abeta (1-40) by circular dichroism (CD) spectroscopy, thioflavin T (ThT)

fluorescence assay, and atomic force microscopy (AFM). The second part emphasized the kinetics and enthalpy of interaction between Abeta (1-40) and liposome by surface plasmon resonance (SPR) and isothermal titration microcalorimetry (ITC). Results obtained from CD, ThT and AFM confirmed the formation of 1 microm fibril after single day incubation. The driving force of kinetic interaction between Abeta and liposomes was revealed by SPR to be electrostatics. Further studies indicated that fresh Abeta has high GM1 affinity. Besides, addition of cholesterol to the liposome could alter membrane fluidity and affect the interactions of fresh Abeta with liposomes especially in the amount of Abeta absorbed and preserving the structure of liposome after adsorbing. Hydrophobicity was found to be the driving force leading to the interaction between Abeta fibrils and liposomes. These reactions are endothermic as supported by ITC measurements. When the composition of liposomes is zwitterionic lipids, the interaction of Abeta with liposomes is predominantly hydrophobic force. In contrast, the driving force of interaction of charged lipids with Abeta is electrostatic.

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

Linse S., Cabaleiro-Lago C., Xue W. F., Lynch I., Lindman S., Thulin E., Radford S. E. and Dawson K. A. (2007) Nucleation of protein fibrillation by nanoparticles. *Proc. Natl. Acad. Sci U. S. A* **104**, 8691-8696.

Abstract: Nanoparticles present enormous surface areas and are found to enhance the rate of protein fibrillation by decreasing the lag time for nucleation. Protein fibrillation is involved in many human diseases, including Alzheimer's, Creutzfeld-Jacob disease, and dialysis-related amyloidosis. Fibril formation occurs by nucleation-dependent kinetics, wherein formation of a critical nucleus is the key rate-determining step, after which fibrillation proceeds rapidly. We show that nanoparticles (copolymer particles, cerium oxide particles, quantum dots, and carbon nanotubes) enhance the probability of appearance of a critical nucleus for nucleation of protein fibrils from human beta(2)-microglobulin. The observed shorter lag (nucleation) phase depends on the amount and nature of particle surface. There is an exchange of protein between solution and nanoparticle surface, and beta(2)-microglobulin forms multiple layers on the particle surface, providing a locally increased protein concentration promoting oligomer formation. This and the shortened lag phase suggest a mechanism involving surface-assisted nucleation that may increase the risk for toxic cluster and amyloid formation. It also opens the door to new routes for the controlled self-assembly of proteins and peptides into novel nanomaterials

Liu L. L. and Franz K. J. (2007) Phosphorylation-dependent metal binding by alpha-synuclein peptide fragments. *J Biol Inorg Chem* **12**, 234-247.

Abstract: Alpha-synuclein (alpha-syn) is the major protein component of the insoluble fibrils that make up Lewy bodies, the hallmark lesions of Parkinson's disease. Its C-terminal region contains motifs of charged amino acids that potentially bind metal ions, as well as several identified phosphorylation sites. We have investigated the metal-binding properties of synthetic model peptides and phosphopeptides that correspond

to residues 119-132 of the C-terminal, polyacidic stretch of human alpha-syn, with the sequence Ac-Asp-Pro-Asp-Asn-Glu-Ala-Tyr-Glu-Met-Pro-Ser-Glu-Glu-Gly (alpha-syn119-132). The peptide pY125 replaces tyrosine with phosphotyrosine, whereas pS129 replaces serine with phosphoserine. By using Tb(3+) as a luminescent probe of metal binding, we find a marked selectivity of pY125 for Tb(3+) compared with pS129 and alpha-syn119-132, a result confirmed by isothermal titration calorimetry. Truncated or alanine-substituted peptides show that the phosphoester group on tyrosine provides a metal-binding anchor that is supplemented by carboxylic acid groups at positions 119, 121, and 126 to establish a multidentate ligand, while two glutamic acid residues at positions 130 and 131 contribute to binding additional Tb(3+) ions. The interaction of other metal ions was investigated by electrospray ionization mass spectrometry, which confirmed that pY125 is selective for trivalent metal ions over divalent metal ions, and revealed that Fe(3+) and Al(3+) induce peptide dimerization through metal ion cross-links. Circular dichroism showed that Fe(3+) can induce a partially folded structure for pY125, whereas no change was observed for pS129 or the unphosphorylated analog. The results of this study show that the type and location of a phosphorylated amino acid influence a peptide's metal-binding specificity and affinity as well as its overall conformation.

Olofsson A., Borowik T., Grobner G. and Sauer-Eriksson A. E. (2007) Negatively charged phospholipid membranes induce amyloid formation of medin via an alpha-helical intermediate. *J Mol Biol* **374**, 186-194.

Abstract: Medin, a recently discovered 5.5 kDa peptide, is associated with amyloid deposits in the medial layer of human arteries and the prevalence is nearly 100% within individuals above 50 years. Presently, not much is known about its biochemical and biophysical properties or its pathway from soluble peptide to insoluble amyloid. Here we have characterized the behavior of medin in the presence of lipid membranes, using circular dichroism, isothermal titration calorimetry, differential scanning calorimetry, size exclusion chromatography, and atomic force microscopy (AFM). Medin was shown to exist as a monomer in solution with a predominantly random-coil structure. It binds lipid vesicles that have either a neutral or a negative surface potential. Upon association to membranes containing acidic lipids, it undergoes an electrostatically driven conformational change towards a mainly alpha-helical state. Prolonged incubation converts medin from an alpha-helical structure into an amyloid beta-sheet fibrillar state as confirmed by AFM. Based on these findings, we propose a mechanism of medin-amyloid formation where medin electrostatically associates in its monomeric form to biological interfaces displaying a negative potential. This process both increases the local peptide concentration and induces an aggregation-prone alpha-helical fold

Talmard C., Bouzan A. and Faller P. (2007) Zinc Binding to Amyloid-beta: Isothermal Titration Calorimetry and Zn Competition Experiments with Zn Sensors. *Biochemistry* **46**, 13658-13666.

Abstract: Aggregation of the peptide amyloid-beta (Abeta) to amyloid plaques is a key event in Alzheimer's disease. According to the amyloid cascade hypothesis, Abeta aggregates are toxic to neurons via the production of reactive oxygen species and are hence directly involved in the cause of the disease. Zinc ions play an important role, because they are able to bind to Abeta and influence the aggregation properties. In the present work isothermal titration calorimetry and Zn sensors (zincon, Newport Green, and zinquin) were used to investigate the interaction of Zn with the full-length Abeta1-40 and Abeta1-42, as well as the truncated Abeta1-16 and Abeta1-28. The results suggest that Zn binding to Abeta induces a release of approximately 0.9 proton by the peptide. This correspond to the expected value upon Zn binding to the three histidines and indicates that further ligands are not deprotonated upon Zn binding. Such behavior is expected for carboxylates, but not the N-terminus. Moreover, the apparent dissociation constant ($K_{d,app}$) of Zn binding to all forms of Abeta is in the low micromolar range (1-20 μ M) and rather independent of the aggregation state including soluble Abeta, Abeta fibrils, or Zn-induced Abeta aggregates. Finally, Zn in the soluble or aggregated Zn-Abeta form is well accessible for Zn chelators. The potential repercussions on metal chelation therapy are discussed.

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.

Thompsett A. R., Abdelraheim S. R., Daniels M., and Brown D. R. (2005) High affinity binding between copper and full length prion protein identified by two different techniques. *J Biol Chem* **280**, 42750-42758.

Abstract: The cellular prion protein is known to be a copper binding protein. Despite the wide range of studies on the copper binding of PrP, there have been no studies to determine the affinity of the protein on both full length prion protein and under physiological conditions. We have used two techniques, isothermal titration calorimetry and competitive metal capture analysis to determine the affinity of copper for wild-type mouse PrP and a series of mutants. High affinity copper binding by wild type PrP has been confirmed by the independent techniques indicating the presence of specific tight copper binding sites up to femtomolar affinity. Altogether four high affinity binding sites of between femto and nanomolar affinities are located within the octameric repeat region of the protein at physiological pH. A fifth copper binding site of lower affinity than those of the octameric repeat region has been detected in full-length protein. Binding to this site is modulated by the histidine at residue 111. Removal of the octameric repeats leads to the enhancement of affinity of this fifth site and a second binding site outside of the repeat region undetected in the wild type protein. High affinity copper binding allows PrP to compete effectively for copper in the extracellular milieu. The copper binding affinities of PrP have been compared to those of proteins of known function and are of magnitudes compatible with an extracellular copper buffer or an enzymatic function such as superoxide dismutase like activity.

White J. T. and Kelly J. W. (2001) Support for the multigenic hypothesis of amyloidosis: the binding stoichiometry of retinol-binding protein, vitamin A, and thyroid hormone influences transthyretin amyloidogenicity in vitro. *Proc Natl Acad Sci U S A* **98**, 13019-13024.

Abstract: The amyloidoses are a large group of protein misfolding diseases. Genetic and biochemical evidence support the hypothesis that amyloid formation from wild-type or 1 of 80 sequence variants of transthyretin causes the human amyloid diseases senile systemic amyloidosis or familial amyloid polyneuropathy, respectively. The late onset and variable penetrance of these diseases has led to their

designation as multigenic--implying that the expression levels and alleles of multiple gene products influence the course of pathology. Here we show that the binding stoichiometry of three interacting molecules, retinol-binding protein, vitamin A, and L-thyroxine, notably influenced transthyretin amyloidogenicity in vitro. At least 70 genes control retinol-binding protein, vitamin A, and L-thyroxine levels in plasma and have the potential to modulate the course of senile systemic amyloidosis or familial amyloid polyneuropathy.

Zerovnik E., Skerget K., Tusek-Znidaric M., Loeschner C., Brazier M. W., and Brown D. R. (2006) High affinity copper binding by stefin B (cystatin B) and its role in the inhibition of amyloid fibrillation. *FEBS J* **273**, 4250-4263.

Abstract: We show that human stefin B, a protease inhibitor from the family of cystatins, is a copper binding protein, unlike stefin A. We have used isothermal titration calorimetry to directly monitor the binding event at pH 7 and pH 5. At pH 7 stefin B shows a picomolar affinity for copper but at pH 5 the affinity is in the nanomolar range. There is no difference in the affinity of copper between the wildtype stefin B (E31 isoform) and a variant (Y31 isoform), whereas the mutant (P79S), which is tetrameric, does not bind copper. The conformation of stefin B remains unaltered by copper binding. It is known that below pH 5 stefin B undergoes a conformational change and amyloid fibril formation. We show that copper binding inhibits the amyloid fibril formation and, to a lesser degree, the initial aggregation. Similarities to and differences from other copper binding amyloidogenic proteins are discussed.

Zhou B. R., Zhou Z., Hu Q. L., Chen J. and Liang Y. (2008) Mixed macromolecular crowding inhibits amyloid formation of hen egg white lysozyme. *Biochim Biophys Acta* **1784**, 472-480.

Abstract: The effects of two single macromolecular crowding agents, Ficoll 70 and bovine serum albumin (BSA), and one mixed macromolecular crowding agent containing both BSA and Ficoll 70, on amyloid formation of hen egg white lysozyme have been examined by thioflavin T binding, Congo red binding, transmission electron microscopy, and activity assay, as a function of crowder concentration and composition. Both the mixed crowding agent and the protein crowding agent BSA at 100 g/l almost completely inhibit amyloid formation of lysozyme and stabilize lysozyme activity on the investigated time scale, but Ficoll 70 at the same concentration neither impedes amyloid formation of lysozyme effectively nor stabilizes lysozyme activity. Further kinetic and isothermal titration calorimetry analyses indicate that a mixture of 5 g/l BSA and 95 g/l Ficoll 70 inhibits amyloid formation of lysozyme and maintains lysozyme activity via mixed macromolecular crowding as well as weak, nonspecific interactions between BSA and nonnative lysozyme. Our data demonstrate that BSA and Ficoll 70 cooperatively contribute to both the inhibitory effect and the stabilization effect of the mixed crowding agent, suggesting that mixed macromolecular crowding inside the cell may play a role in posttranslational quality control mechanism