

ITC XXIII – Polymer and nanoparticle studies, including protein-polymer interactions, and DNA-polymer interactions

Almeida N. L., Oliveira C. L., Torriani I. L., and Loh W. (2004) Calorimetric and structural investigation of the interaction of lysozyme and bovine serum albumin with poly(ethylene oxide) and its copolymers. *Colloids Surf B Biointerfaces* **38**, 67-76.

Abstract: This work reports investigations aiming at verifying the occurrence of specific interactions between lysozyme or bovine serum albumin (BSA) and poly(ethylene oxide) and its copolymers with poly(propylene oxide). Thermal stability of these proteins, followed by means of high sensitivity DSC, was found to be mostly unaffected by the presence of these polymers. Chromatographic experiments (reverse-phase HPLC and size exclusion chromatography) did not reveal any sign of specific interaction for these mixtures, either. Isothermal titration calorimetry revealed an increase in enthalpy for the mixtures, represented by a positive enthalpy of transfer for these proteins from buffer to polymer solutions. Moreover, SAXS analyses confirmed that at ambient temperatures these polymers do not affect lysozyme structure. In summary, no evidence is found to support earlier suggestions that some kind of complex could be formed between these proteins and poly(ethylene oxide) or its copolymers, but the present results suggest the occurrence of entropically driven hydrophobic effects.

Arnaud A. and Bouteiller L. (2004) Isothermal titration calorimetry of supramolecular polymers. *Langmuir* **20**, 6858-6863.

Abstract: A method to characterize the self-association of supramolecular polymers by isothermal titration calorimetry (ITC) has been designed. Association constants in the range 10^4 - 10^6 dm³ mol⁻¹ have been successfully determined from the heat exchange occurring when a supramolecular polymer solution is injected into a calorimetric cell containing pure solvent. Very good agreement with literature values has been obtained. Compared to other techniques (such as NMR or Fourier transform infrared spectroscopy), the use of ITC presents several advantages: (i) the enthalpy of association is obtained together with the association constant from the same experiment, (ii) the measurements can be performed in almost any solvent, and (iii) systems with higher association constants can be characterized.

Asadi A., Saboury A. A., Moosavi-Movahedi A. A., Divsalar A. and Sarbolouki M. N. (2008) Interaction of bovine serum albumin with some novel PEG-containing diblock copolymers. *Int J Biol Macromol.* **43**, 262-270.

Abstract: A comparative study on the interaction of different PEG-containing diblock copolymers including SA400, SA600, SA1500 and OA1500 (stearyl and oleyl esters of polyethylene glycol with 400, 600 and 1500 molecular weights, respectively) with bovine serum albumin (BSA) was carried out using isothermal titration calorimetry (ITC), attenuated total reflectance Fourier transform infrared (ATR-FTIR), circular dichroism (CD), and fluorescence spectroscopies. ITC data show that SA400, SA600, SA1500 and OA1500 bind to BSA, with association constants of (14.5, 3.16, 50.7 and 17.6) × 10³ M⁻¹, respectively. Results also show that the binding is enthalpically driven, disfavored by conformational entropy. Quantitative analysis of the FTIR absorbance spectra at amide I' (1600-1700 cm⁻¹) as well as far UV circular dichroism data show that these polymers do not disturb the BSA structure and only cause a slight increment in helicity along with a slight decrease in the beta-structure. Only stearyl esters SA400 and SA1500 slightly decreased the random structure content of the BSA. The diblock copolymers inhibit protein aggregation and bind to BSA better than their constituent PEGs causing an increment in its T_m; SA1500 is showing the strongest effect

Bellot M. and Bouteiller L. (2008) Thermodynamic Description of Bis-urea Self-Assembly: Competition between Two Supramolecular Polymers. *Langmuir (epublication)*.

Abstract: Supramolecular polymers are chains of small molecules held together through reversible noncovalent interactions. In general, a given monomer self-assembles into a single type of supramolecular polymer. However, in a few cases, two different self-assembled structures can coexist; this yields interesting responsive systems. To improve the understanding of these systems, we report an association model describing the self-assembly of a supramolecular polymer into two competing forms. The parameters controlling the system were measured by high sensitivity differential scanning calorimetry and isothermal titration calorimetry in the case of a hydrogen-bonded bis-urea supramolecular polymer solution

in toluene. The model enables us to compute the proportion and length of all components in the system at any temperature and concentration. The results of these calculations are in agreement with the experimental phase diagram and with independent viscosity measurements

Braia M., Porfiri M. C., Farruggia B., Pico G. and Romanini D. (2008) Complex formation between protein and poly vinyl sulfonate as a strategy of proteins isolation. *J Chromatogr. B Analyt. Technol. Biomed. Life Sci* **873**, 139-143.

Abstract: The complex formation between the basic protein trypsin and the strong anionic polyelectrolyte poly vinyl sulfonic acid was studied by using turbidimetric and isothermal calorimetric titrations. The trypsin-polymer complex was insoluble at pH lower than 5, with a stoichiometric ratio polymer mol per protein mol of 1:136. NaCl, 0.5M inhibited the complex precipitation in agreement with the proposed coulombic mechanism of complex formation. The protein structure and its thermodynamic stability were not significantly affected by the presence of the polyelectrolyte. The enzymatic activity of trypsin increases throughout time, even in the presence of the polymer

Cedervall T., Lynch I., Lindman S., Berggard T., Thulin E., Nilsson H., Dawson K. A. and Linse S. (2007) Understanding the nanoparticle-protein corona using methods to quantify exchange rates and affinities of proteins for nanoparticles. *Proc. Natl. Acad. Sci U. S. A* **104**, 2050-2055.

Abstract: Due to their small size, nanoparticles have distinct properties compared with the bulk form of the same materials. These properties are rapidly revolutionizing many areas of medicine and technology. Despite the remarkable speed of development of nanoscience, relatively little is known about the interaction of nanoscale objects with living systems. In a biological fluid, proteins associate with nanoparticles, and the amount and presentation of the proteins on the surface of the particles leads to an in vivo response. Proteins compete for the nanoparticle "surface," leading to a protein "corona" that largely defines the biological identity of the particle. Thus, knowledge of rates, affinities, and stoichiometries of protein association with, and dissociation from, nanoparticles is important for understanding the nature of the particle surface seen by the functional machinery of cells. Here we develop approaches to study these parameters and apply them to plasma and simple model systems, albumin and fibrinogen. A series of copolymer nanoparticles are used with variation of size and composition (hydrophobicity). We show that isothermal titration calorimetry is suitable for studying the affinity and stoichiometry of protein binding to nanoparticles. We determine the rates of protein association and dissociation using surface plasmon resonance technology with nanoparticles that are thiol-linked to gold, and through size exclusion chromatography of protein-nanoparticle mixtures. This method is less perturbing than centrifugation, and is developed into a systematic methodology to isolate nanoparticle-associated proteins. The kinetic and equilibrium binding properties depend on protein identity as well as particle surface characteristics and size.

Chen W. Y., Chen C. S., and Lin F. Y. (2001) Molecular recognition in imprinted polymers: thermodynamic investigation of analyte binding using microcalorimetry. *J Chromatogr A* **923**, 1-6.

Abstract: This study aimed at elucidating the interaction mechanism between an imprinted polymer and its template in aqueous environment with thermodynamic aspects. The herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) was chosen as a model template to imprint a co-polymer of 4-vinylpyridine (4-VP) and ethyleneglycol dimethacrylate. Equilibrium binding isotherm analysis and isothermal titration microcalorimetry were used to quantify the contribution of enthalpy and entropy to the binding process, identify the nature of the interactions involved and confirm the existence of binding pockets with shape-complementarity to the template. For the binding process of 2,4-D to the imprinted polymer, we postulate three subprocesses: (1) dehydration of the binding pocket and of the 2,4-D, (2) adsorption of 2,4-D, and (3) rearrangement of the water molecules from the dehydration process. We found that binding in aqueous environment was due to the cumulative effect of pi-stacking and electrostatic interactions between the template and the functional monomers. At pH<6, entropy is the dominating driving force, while at pH>6 where the highest difference in binding between the imprinted and a non-imprinted reference polymer was observed, the enthalpy change accounts for most of the binding free energy. The developed microcalorimetric method sheds light on the binding mechanism of analyte molecules with imprinted polymers, in particular if the polymers are used in aqueous solvents.

Coles D. J., Yang S., Minchin R. F. and Toth I. (2008) The characterization of a novel dendritic system for gene delivery by isothermal titration calorimetry. *Biopolymers* **90**, 651-654.

Abstract: Understanding the nature of binding of polycationic dendrimers to DNA provides useful information on their role in gene delivery. In the present study, we have characterized the interaction of several peptide-based polycationic dendrimers with salmon sperm DNA using isothermal titration calorimetry. The dendrimers consisted of the cell penetrating peptide TAT, a nuclear localization signal peptide and dendritic polylysine. The binding affinity and thermodynamic parameters were found to increase as the number of positive charges on the dendrimer increased, indicating that ionic interactions were the major binding forces between the two molecules. The effect of acidic pH (3.2) compared to a more neutral pH (7.2) was also examined. The binding affinity was stronger at the lower pH but precipitation of the complex was more prominent at pH 7.2 which was shown by large enthalpies. The results indicate that our dendrimers are forming stable complexes with DNA

Dai X. H., Dong C. M. and Yan D. (2008) Supramolecular and biomimetic polypseudorotaxane/glycopolymer biohybrids: synthesis, glucose-surfaced nanoparticles, and recognition with lectin. *J Phys. Chem B* **112**, 3644-3652.

Abstract: A new class of supramolecular and biomimetic glycopolymer/poly(epsilon-caprolactone)-based polypseudorotaxane/glycopolymer triblock copolymers (poly(D-gluconamidoethyl methacrylate)-PPR-poly(D-gluconamidoethyl methacrylate), PGAMA-PPR-PGAMA), exhibiting controlled molecular weights and low polydispersities, was synthesized by the combination of ring-opening polymerization of epsilon-caprolactone, supramolecular inclusion reaction, and direct atom transfer radical polymerization (ATRP) of unprotected D-gluconamidoethyl methacrylate (GAMA) glycomonomer. The PPR macroinitiator for ATRP was prepared by the inclusion complexation of biodegradable poly(epsilon-caprolactone) (PCL) with alpha-cyclodextrin (alpha-CD), in which the crystalline PCL segments were included into the hydrophobic alpha-CD cavities and their crystallization was completely suppressed. Moreover, the self-assembled aggregates from these triblock copolymers have a hydrophilic glycopolymer shell and an oligosaccharide threaded polypseudorotaxane core, which changed from spherical micelles to vesicles with the decreasing weight fraction of glycopolymer segments. Furthermore, it was demonstrated that these triblock copolymers had specific biomolecular recognition with concanavalin A (Con A) in comparison with bovine serum albumin (BSA). To the best of our knowledge, this is the first report that describes the synthesis of supramolecular and biomimetic polypseudorotaxane/glycopolymer biohybrids and the fabrication of glucose-shelled and oligosaccharide-threaded polypseudorotaxane-cored aggregates. This hopefully provides a platform for targeted drug delivery and for studying the biomolecular recognition between sugar and lectin

De Stefano C., Gianguzza A., Piazzese D., and Sammartano S. (2005) Modelling of proton and metal exchange in the alginate biopolymer. *Anal Bioanal Chem* **383**, 587-596.

Abstract: Acid-base behaviour of a commercial sodium alginate extracted from brown seaweed (*Macrocystis pyrifera*) has been investigated at different ionic strengths ($0.1 \leq I/\text{mol l}^{-1} \leq 1.0$) and in different supporting electrolytes (Et₄N⁺I⁻, NaCl, KCl, LiCl, NaCl+MgCl₂), with the aim of examining the influence of ionic medium on the proton-binding capacity and of quantifying the strength of interaction with light metal ions in the perspective of speciation studies in natural aqueous systems. Potentiometric ([H⁺]-glass electrode) and titration calorimetric data were expressed as a function of the dissociation degree (alpha) using different models (Henderson-Hasselbalch modified, Hogfeldt three parameters and linear equations). The dependence on ionic strength of the protonation constants was taken into account by a modified specific interaction theory model. Differences among different media were explained in terms of the interaction between polyanion and metal cations of the supporting electrolytes. Quantitative information on the proton-binding capacity, together with the stabilities of different species formed, is reported. Protonation thermodynamic parameters, at alpha=0.5, are log K_H=3.686±0.005, DeltaG₀=-21.04±0.03 kJ mol⁻¹, DeltaH₀=4.8±0.2 kJ mol⁻¹ and TDeltaS₀=35.7±0.3 kJ mol⁻¹, at infinite dilution. Protonation enthalpies indicate that the main contribution to proton binding arises from the entropy term. A strict correlation between DeltaG and TDeltaS was found, TDeltaS=-9.5-1.73 DeltaG. Results are reported in light of building up a chemical complexation model of general validity to explain the binding ability of naturally occurring polycarboxylate polymers and biopolymers. Speciation profiles of alginate in the presence of sodium and magnesium ions, naturally occurring cations in natural waters, are also reported.

Ehtezazi T., Govender T., and Stolnik S. (2000) Hydrogen bonding and electrostatic interaction contributions to the interaction of a cationic drug with polyaspartic acid. *Pharm Res* **17**, 871-878.

Abstract: PURPOSE: To determine the mechanism and identify forces of interaction between polyaspartic acid and diminazene (a model drug). Such knowledge is essential for the design of polymeric drug delivery systems that are based on molecular self-assembly into complexes or micellar type systems. METHODS: Complex formation was studied by isothermal titration microcalorimetry and the McGhee von Hippel model was applied to obtain K_{obs} , ΔH_{obs} , and n_{obs} . The calorimetry data were compared with both an optical density study and the amount of free/complexed drug. RESULTS: The diminazene-polyaspartic acid interaction is enthalpically driven, whereby one diminazene molecule interacts with two monomers of polyaspartic acid. The dependence of K_{obs} on salt concentration reveals a contribution of electrostatic interactions. However, applying Manning's counter ion condensation theory shows that the major driving force for the complex formation is hydrogen bonding, with interfacial water molecules remaining buried within the complex. The modelling of the pH dependence of K_{obs} and ΔH_{obs} demonstrates that the ionization of carboxylic groups of polyaspartic acid is a prerequisite for the interaction. CONCLUSIONS: Complex formation between diminazene and polyaspartic acid is driven by both electrostatic interactions and hydrogen bonding, with the latter being the dominating force. Although electrostatic interactions are not the major driving force, ionization of the drug and polymer is essential for complex formation.

Gomy C. and Schmitzer A. R. (2006) Rational design of new polymerizable oxyanion receptors. *J Org Chem* **71**, 3121-3125.

Abstract: [structure: see text] We report the synthesis of a library of new polymerizable functional monomers designed for complexing with the oxyanionic moiety of the chemotherapeutic drug methotrexate. The ¹H NMR and ITC binding studies allowed for the selection of receptors possessing the best association parameters. Subsequently, the design of a broad library of polymerizable moiety-specific binding monomers for the imprinting of dicarboxylate containing drugs was accomplished. Di(ureidoethylenemethacrylate)stilbene possesses the highest association properties and shows potential to act as a monomer in the molecularly imprinting technique to obtain photoswitchable cavities.

Govender T., Ehtezazi T., Stolnik S., Illum L., and Davis S. S. (1999) Complex formation between the anionic polymer (PAA) and a cationic drug (procaine HCl): characterization by microcalorimetric studies. *Pharm Res* **16**, 1125-1131.

Abstract: PURPOSE: Due to the importance of drug-polymer interactions in, inter alia, drug loading/release, supramolecular assemblies and DNA delivery for gene therapy, the aim of this study was therefore to establish the mechanism of interaction between a model polymer (Polyacrylic acid, PAA) and a model drug (procaine HCl). METHODS: This was performed by studying the effect of salt (KCl) concentration on their heat released values using Isothermal Titration Microcalorimetry (ITM). The integrated released heat data were computer fitted to a one class binding model and the thermodynamic parameters (K_{obs} , ΔH , and N) were determined. RESULTS: As the KCl concentration was increased, K_{obs} decreased thus establishing the salt dependence of the interaction. The linear variation of ΔG_{obs} with ΔS_{obs} indicated that their interaction was entropically driven. The stoichiometry of the interaction was calculated to be one procaine molecule per monomer of PAA. Dissection of the total observed free energy at each KCl concentration indicated that the contribution of the non-electrostatic attractions to the interaction of PAA with procaine HCl was greater than those of the electrostatic attractions. CONCLUSIONS: We have shown that the interaction between PAA and procaine HCl is dependent upon the presence of counterions (monovalent ions) and is mainly entropically driven. The calculated stoichiometry indicated that one procaine HCl molecule neutralised one carboxylic acid group on PAA. Although electrostatic interactions were necessary for initiating complex formation, the non-electrostatic forces were dominant in stabilising the PAA-procaine HCl complex.

Greish Y. E., Bender J. D., Lakshmi S., Brown P. W., Allcock H. R., and Laurencin C. T. (2005) Low temperature formation of hydroxyapatite-poly(alkyl oxybenzoate)phosphazene composites for biomedical applications. *Biomaterials* **26**, 1-9.

Abstract: The formation of biodegradable composites which may be suitable as bone analogs is described. Polyphosphazene-hydroxyapatite (HAp) composites were produced via an acid-base reaction of tetracalcium phosphate and anhydrous dicalcium phosphate in the presence of polyphosphazenes bearing alkyl ester containing side-groups. The polyphosphazenes used were poly(ethyl oxybenzoate)phosphazene

(PN-EOB) and poly(propyl oxybenzoate) phosphazene (PN-POB). The effects of temperature and the proportions of polymers, PN-EOB and PN-POB on the kinetics, reaction chemistry and phase evolution during the formation of stoichiometric HAp were studied. Kinetics, phase evolution and microstructural development were evaluated using isothermal calorimetry, X-ray diffraction and scanning electron microscopy, respectively. Analysis of solution chemistry revealed that the increases in the pH during the formation of SHAp, resulted in partial hydrolysis of the polymer surfaces, which led in turn to the formation of a calcium cross-linked polymer surface. The calcium cross-linked polymer surface appeared to facilitate the nucleation and growth of apatite deposits on the polymer. The current study illustrates the in situ formation of HAp in the presence of polyphosphazenes, where HAp is chemically bonded to the polymer.

Hsu C. Y., Lin H. Y., Thomas J. L., Wu B. T., and Chou T. C. (2006) Incorporation of styrene enhances recognition of ribonuclease A by molecularly imprinted polymers. *Biosens Bioelectron* **22**, 355-363.
Abstract: Ribonuclease A (RNase A) is an RNA-cleaving enzyme characterized by its high conformational stability and strong catalytic activity. This enzyme is ubiquitous in living organisms and is difficult to inactivate. In polymerase chain reaction (PCR) RNase activity is removed by adding inhibitors. Molecularly imprinted polymers (MIPs) with high selectivity, high stability, low cost and facile synthesis could prove useful in extraction of target molecules, such as RNase A, from reaction mixtures. In this investigation, MIPs were synthesized from the monomers styrene and polyethyleneglycol 400 dimethacrylate (PEG400DMA) in several different ratios. Styrene as a functional monomer gave MIPs with a higher affinity for RNase A than other functional monomers tested, according to both enzyme-linked immunosorbent assay (ELISA) and isothermal titration calorimetry (ITC). The optimum volume ratio of styrene/PEG400DMA was 20/100 at 25 degrees C, and this ratio maximized the rebinding efficiency of RNase A to MIPs. Isothermal titration calorimetry was also used, and could be useful to design the composition of molecularly imprinted polymers for various target molecules.

Ikonen M., Murtomaki L. and Kontturi K. (2008) Controlled complexation of plasmid DNA with cationic polymers: effect of surfactant on the complexation and stability of the complexes. *Colloids Surf B Biointerfaces* **66**, 77-83.
Abstract: The aggregation of the cationic polymer-plasmid DNA complexes of two commonly used polymers, polyethyleneimine (PEI) and poly-L-lysine (PLL) were systematically compared. The complexation was studied in 5% glucose solution at 25 degrees C using dynamic light scattering and isothermal titration calorimetry. The aggregation of the complexes was controlled by addition of the surfactant polyoxyethylene stearate (POES). The stability of the complexes was evaluated using dextran sulphate (DS) as relaxing agent. The relaxation of the complexes in the presence of DS was studied using agarose gel electrophoresis. This study elucidates the role of surfactant in controlling the size of the PEI/pDNA complex and reveals the differences of the two polymers as complexing agents

Kimhi O. and Bianco-Peled H. (2007) Study of the interactions between protein-imprinted hydrogels and their templates. *Langmuir* **23**, 6329-6335.
Abstract: The interactions between lysozyme-imprinted hydrogel and their template protein were studied using adsorption measurements, competitive adsorption experiments, and isothermal titration calorimetry (ITC). The results were compared to the interactions between the imprinted polymer and a reference protein, cytochrome c. Experimental adsorption isotherms and competitive adsorption studies detected better affinity and higher capacity of the imprinted polymer toward the template protein. Moreover, analysis of ITC data identified major differences in the binding enthalpy of lysozyme when the imprinted and the non-imprinted polymers were compared. On the other hand, cytochrome C did not exhibit any major changes in the adsorption enthalpy when comparing the imprinted and the non-imprinted polymers. This is the first thermodynamic evidence for the creation of new binding sites in the process of protein imprinting.

Lin H. Y., Hsu C. Y., Thomas J. L., Wang S. E., Chen H. C., and Chou T. C. (2006) The microcontact imprinting of proteins: the effect of cross-linking monomers for lysozyme, ribonuclease A and myoglobin. *Biosens Bioelectron* **22**, 534-543.
Abstract: The performance of molecularly imprinted polymers (MIPs) is of interest to researchers in the field of analytical chemistry, and in the pharmaceutical and food industries. Because the choice of the

functional monomer(s) plays a key role in the selectivity of a MIP, the synthesis of an effective, tight-binding MIP can be difficult and time-consuming, involving the evaluation of the binding performance of MIPs of many different compositions. In this study, we report an express method combining molecular imprinting and microcontact printing techniques to prepare a polymer thin film as an artificial antibody. In addition to the microcontact printing technique, isothermal titration of monomers to proteins stamps was investigated to screen the functional monomer for MIPs. Finally, the importance of the choice of cross-linking monomers in MIPs was studied, and these studies suggest that monomers containing an optimal length PEG spacer give higher imprinting effectiveness. Several model antigens (lysozyme, ribonuclease A and myoglobin) were adsorbed on a cover glasses that were pretreated with hexamethyldisilazane (HMDS). These protein stamps were then contacted with different monomer solutions (cross-linking monomers) on a glass slide substrate. Photopolymerization yielded the molecularly imprinted polymer. This technique, analogous to microcontact printing, allows for the rapid, parallel synthesis of MIPs of different compositions, and requires very small volumes of monomers (ca. 4 μL). The technique also avoids potential solubility problems with the molecular targets. Of several cross-linking monomers screened, tetraethyleneglycol dimethacrylate (TEGDMA) gave the most selective lysozyme binding, while polyethyleneglycol 400 dimethacrylate (PEG400DMA) were most selective for ribonuclease A and myoglobin.

Lindman S., Lynch I., Thulin E., Nilsson H., Dawson K. A. and Linse S. (2007) Systematic investigation of the thermodynamics of HSA adsorption to N-iso-propylacrylamide/N-tert-butylacrylamide copolymer nanoparticles. Effects of particle size and hydrophobicity. *Nano. Lett* **7**, 914-920.

Abstract: Nanoparticles in biological fluids almost invariably become coated with proteins that may confer nanomedical and nanotoxicological effects. Understanding these effects requires quantitative measurements using simple systems. Adsorption of HSA to copolymer nanoparticles of varying hydrophobicity and curvature was studied using ITC, yielding stoichiometry, affinity, and enthalpy changes upon binding. The hydrophobicity was controlled via the co-monomer ratio, N-iso-propylacrylamide/N-tert-butylacrylamide. The most hydrophobic particles become fully covered with a single layer of protein, except at high curvature.

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, Creutzfeldt-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.

Maeda T., Yamamoto K., and Aoyagi T. (2006) Importance of bound water in hydration-dehydration behavior of hydroxylated poly(N-isopropylacrylamide). *J Colloid Interface Sci* **302**, 467-474.

Abstract: In this study, a differential scanning calorimetric analysis was performed to investigate the role of water existing around the polymer chains on their thermoresponsive behaviors using the novel functional temperature-sensitive copolymer, poly(N-isopropylacrylamide-co-2-hydroxyisopropylacrylamide) (poly(NIPAAm-co-HIPAAm)). The HIPAAm comonomers were incorporated into the polymeric chains as hydrophilic parameters, and then the hydration states of poly(NIPAAm-co-HIPAAm) with various HIPAAm compositions were examined. Bound water, which is affected by the polymeric chains to some extent, was produced by adding the copolymers to the water, and the temperature due to the melting of the bound water decreased as the HIPAAm content increased. On the basis of this result, we considered that the interaction between the bound water and the polymeric chains is reinforced by the increasing HIPAAm

composition. In addition, the cloud points of the copolymers shifted to a higher temperature, and the endothermic enthalpy for the phase transition decreased with increasing the HIPAAm content, suggesting that the number of water molecules disassociated from the polymeric chains decreased. Based on these results, we postulate that the changes in the interaction between the thermosensitive polymer and bound water exert a strong influence on its phase transition and/or separation, such as the cloud point or dehydration behavior.

Monteiro O. A., Jr. and Airoidi C. (2005) The influence of chitosans with defined degrees of acetylation on the thermodynamic data for copper coordination. *J Colloid Interface Sci* **282**, 32-37.

Abstract: The interaction of copper with three different chitosans having degrees of deacetylation of 77.5, 81.5, and 86.1%, named C, A, and F, respectively, was followed by the batch method at 298 \pm 1K and the values obtained were fitted to a modified Langmuir equation. These interactions were also obtained by calorimetric titration. Experimentally, 50.0 mg of each chitosan was suspended in doubly distilled water at 298.15 \pm 0.02K under mechanical turbine stirring. The titration was performed by adding increments of 10 μ mol of a 0.10 mol dm⁻³ Cu(NO₃)₂ aqueous solution and the calorimetric isotherms obtained were adjusted to a modified Langmuir equation. From the net thermal effects K and Δ H values were calculated, also permitting the acquisition of other thermodynamic data for the chitosan-copper interaction at the solid/liquid interface. The exothermic enthalpic values of -45.65 \pm 1.97, -49.91 \pm 1.57, and -48.64 \pm 0.82kJ mol⁻¹, for chitosans C, A, and F, respectively, reflect the degree of deacetylation. The spontaneity of the systems is shown by the negative Δ G values, -36.1 \pm 0.2, 36.8 \pm 0.1, and -38.1 \pm 0.3kJ mol⁻¹ for the same sequence of chitosans. The negative entropic values, -34, -44, and -35 J mol⁻¹ K⁻¹, are in agreement with an ordering of solvent as the complexation occurred. The intensity of the thermal effects and the thermodynamic data obtained from the copper/chitosan interactions can be associated with the ability of these biopolymers to extract copper from aqueous solutions.

Monteiro O. A., Jr. and Airoidi C. (1999) Some Thermodynamic Data on Copper-Chitin and Copper-Chitosan Biopolymer Interactions. *J Colloid Interface Sci* **212**, 212-219.

Abstract: Chitin and chitosan are good removers of cations from aqueous solution and wastewater. The interactive effect of cation with both biopolymers in aqueous medium was studied by the batch method at 298 \pm 1 K. The results were fitted to the modified Langmuir equation. The same adsorption was followed by calorimetric titration. In this process, 50.0 mg of each polymer was suspended in 19.0 cm³ of bidistilled water at 298.15 \pm 0.02 K, maintained under mechanical turbine stirring. The titration was performed by adding increments of 10 μ L of 0.10 mol dm⁻³ Cu(NO₃)₂ aqueous solution to the system. The resulting isotherm was also adjusted to a modified Langmuir equation. From the thermal effects K and Δ H values were determined, enabling the calculation of Δ G and Δ S for the interaction of copper cations with chitin and chitosan, giving the enthalpic values of -19.85 \pm 0.34 and -41.27 \pm 1.57 kJ mol⁻¹, respectively. The spontaneity of this interaction is shown from Δ G values of -35.9 \pm 0.1 and -36.8 \pm 0.1 kJ mol⁻¹, which are followed by Δ S values of +54 and of -15 J mol⁻¹K⁻¹, respectively. The complexation is probably associated with the lack of order of the chitin polymeric chain or with the freedom of water molecules initially bonded to cations. The copper ion is coordinated to the pendant groups of the polymeric chain to form stable complexes. Copyright 1999 Academic Press.

Paradossi G., Chiessi E., and Malovikova A. (1999) Study of the interactions of D- and L-polylysine enantiomers with pectate in aqueous solutions. *Biopolymers* **50**, 201-209.

Abstract: The interaction between D- and L-enantiomers of polylysine and potassium pectate was studied by means of CD, microcalorimetry, and osmometry. Upon binding with pectate, only poly(L-lysine) undergoes a coil to alpha-helix transition, while poly(D-lysine) remains in the disordered state. This suggests that the energetics of the interaction is influenced by stereochemical constraints besides electrostatic forces. Experimental findings from microcalorimetry suggest that a contribution to the overall enthalpy of binding comes from the polysaccharidic moiety. Stoichiometry of the macromolecular complexes studied by osmometry gives a polylysine:pectate ratio of 3:1, in agreement with the respective degree of polymerization of the two polyelectrolytes.

Perry T. D., Klepac-Ceraj V., Zhang X. V., McNamara C. J., Polz M. F., Martin S. T., Berke N., and Mitchell R. (2005) Binding of harvested bacterial exopolymers to the surface of calcite. *Environ Sci Technol* **39**, 8770-8775.

Abstract: Biologically produced exopolysaccharides (EPS) affect calcite dissolution and precipitation. In this study, natural alkaliphilic microbial isolates were collected from biofilms on historic limestone. The isolates were screened for their ability to produce significant quantities of EPS in cultures. The most productive isolates were identified by 16S rRNA sequence analysis as a close relative of *Bacillus cereus*. EPS with different chemical structures were harvested from the isolates. Isothermal titration calorimetry (ITC) was used to quantify the thermodynamics of binding by the harvested EPS to calcite. The binding was described by a Langmuir adsorption isotherm. Characterization of the EPS showed that binding strength to calcite depended on the chemical nature of the polymer.

Pico G., Bassani G., Farruggia B. and Nerli B. (2007) Calorimetric investigation of the protein-flexible chain polymer interactions and its relationship with protein partition in aqueous two-phase systems. *Int J Biol Macromol.* **40**, 268-275.

Abstract: The binding of polyethyleneglycol of molecular mass 1000, 3300 and 6000 and polyethylene-propylene oxide (molecular mass 8400) to lysozyme and ovoalbumin was measured by isothermal calorimetric titration. A binding process was found to be associated with a saturation effect, which suggests a protein-polymer interaction. The proteins showed an affinity for the polymers in the order of $10(2)M(-1)$ and it decreased with the increase in the polymer molecular mass. The number of polymer molecules bound per protein molecule varied from 0.01 to 0.2 for polyethyleneglycol 1000, 3300 and polyethylene-polypropylene oxide 8400, while for polyethyleneglycol 6000 such number got closer to the unity. The enthalpic change associated with the binding was positive in the order of 1 kcal/mol for lysozyme, while ovoalbumin showed values around 2-3 kcal/mol. Entropic changes were also positive with values around 17-20 e.u. for ovoalbumin and 1-7 e.u. for lysozyme. The heat associated with the protein transfer from a buffer to a medium containing the polymer or the salt (a process similar to protein partitioning in aqueous two-phase systems) was obtained. These results allow the direct calculation of the enthalpic change associated with a protein partition process in aqueous two-phase systems without applying the van'tHoff equation. In this way, it is possible to calculate the associated true heat when the protein is transferred from the bottom to the top phase.

Pollitt M. J., Buckton G., Brocchini S., and Alpar H. O. (2005) Calorimetric study of bovine serum albumin dilution and adsorption onto polystyrene particles. *Int J Pharm* **298**, 333-338.

Abstract: Titration calorimetry was used to investigate the interaction between a model antigen, bovine serum albumin (BSA), and a model particulate carrier, polystyrene (PS). The binding enthalpy was much higher than reported in the literature for a similar system and did not display a sigmoidal binding curve. These experiments may have accessed low coverage surface sites due to the irreversible nature of protein binding and stepwise titration. An important correction is the heat of dilution of the protein solution. Two regimes were observed: at low concentrations of BSA (below ca. 0.3% (w/v)) an exothermic dilution enthalpy of ca. $-100mJmg(-1)$ was determined, whereas at higher concentrations of BSA values of ca. $-20mJmg(-1)$ were obtained. Solution rheological data also showed a change at 0.3% (w/v) BSA, so we hypothesise that the fraction of the BSA as monomers, dimers and polymers in solution changes at approximately 0.3% (w/v).

Pourhosseini P. S., Saboury A. A., Najafi F. and Sarbolouki M. N. (2007) Interaction of insulin with a triblock copolymer of PEG-(fumaric-sebacic acids)-PEG: thermodynamic and spectroscopic studies. *Biochim Biophys Acta* **1774**, 1274-1280.

Abstract: A comparative study on the interaction of (PEG-co-P(FA/SC)-co-PEG) triblock copolymer with bovine and human insulins was carried out using isothermal titration calorimetry (ITC), circular dichroism (CD), and fluorescence spectroscopy. ITC data show that the copolymer has a low affinity for both proteins, with an association constant of about $7-9 \times 10(3) M(-1)$. Results also show that binding is enthalpically driven, and disfavored by conformational entropy. CD spectroscopy studies reveal a small increase in the helical content and a decrease in beta-structure as well as random coil in both proteins. Acrylamide quenching experiments display reduced accessibility of tyrosines, while intrinsic fluorescence spectra show lower tyrosine emission. Furthermore, thermal unfolding experiments, studied by far-UV CD at 222 and 217 nm, demonstrate that upon interaction with the copolymer helix structure becomes less stable while the stability of beta-structure remains unchanged. Altogether, these observations indicate that (PEG-co-P(FA/SC)-co-PEG) triblock copolymer has similar effect(s) on both proteins

Prevette L. E., Kodger T. E., Reineke T. M. and Lynch M. L. (2007) Deciphering the role of hydrogen bonding in enhancing pDNA-polycation interactions. *Langmuir* **23**, 9773-9784.

Abstract: There is considerable interest in the binding and condensation of DNA with polycations to form polyplexes because of their possible application to cellular nucleic acid delivery. This work focuses on studying the binding of plasmid DNA (pDNA) with a series of poly(glycoamidoamine)s (PGAAs) that have previously been shown to deliver pDNA in vitro in an efficient and nontoxic manner. Herein, we examine the PGAA-pDNA binding energetics, binding-linked protonation, and electrostatic contribution to the free energy with isothermal titration calorimetry (ITC). The size and charge of the polyplexes at various ITC injection points were then investigated by light scattering and zeta-potential measurements to provide comprehensive insight into the formation of these polyplexes. An analysis of the calorimetric data revealed a three-step process consisting of two different endothermic contributions followed by the condensation/aggregation of polyplexes. The strength of binding and the point of charge neutralization were found to be dependent upon the hydroxyl stereochemistry of the carbohydrate moiety within each polymer repeat unit. Circular dichroism spectra reveal that the PGAAs induce pDNA secondary structure changes upon binding, which suggest a direct interaction between the polymers and the DNA base pairs. Infrared spectroscopy experiments confirmed both base pair and phosphate group interactions and, more specifically, showed that the stronger-binding PGAAs had more pronounced interactions at both sites. Thus, we conclude that the mechanism of poly(glycoamidoamine)-pDNA binding is most likely a combination of electrostatics and hydrogen bonding in which long-range Coulombic forces initiate the attraction and hydroxyl groups in the carbohydrate comonomer, depending on their stereochemistry, further enhance the association through hydrogen bonding to the DNA base pairs.

Prevette L. E., Lynch M. L., Kizjakina K. and Reineke T. M. (2008) Correlation of amine number and pDNA binding mechanism for trehalose-based polycations. *Langmuir* **24**, 8090-8101.

Abstract: Glycopolymers with repeat units comprised of the disaccharide trehalose and an oligoamine of increasing amine have been previously synthesized by our group and shown to efficiently deliver pDNA (plasmid DNA) to HeLa cells while remaining relatively nontoxic. Complexes formed between the most amine-dense of these polycations and pDNA were also found to be relatively stable in serum and have low aggregation, which is desirable for in vivo gene delivery. To lend insight into these interesting results, this study was aimed at investigating the binding strength and mechanism of interaction between these macromolecules, via isothermal titration calorimetry (ITC) and ethidium bromide exclusion assays. The size of these pDNA-polymer complexes, or polyplexes, at various states of formation was determined through light scattering and zeta-potential measurements. Varying degrees of pDNA secondary structure change occurred upon interaction with the polymers, as evidenced by circular dichroism spectra through increasing molar ratios of polymer amine to DNA phosphate, and Fourier transform infrared (FT-IR) results demonstrated stronger electrostatic binding with the phosphate backbone with the least amine-dense of the series. It was concluded that, depending on the number of secondary amines in the repeat unit, these polymers interact with pDNA via different mechanisms with varying extents of electrostatic interaction and hydrogen bonding. These differing mechanisms may affect the ability of trehalose to serve as a deterrent against aggregation in serum conditions and lend insight into the roles of polymer-pDNA binding during the complex transfection process

Quadir M. A., Radowski M. R., Kratz F., Licha K., Hauff P. and Haag R. (2008) Dendritic multishell architectures for drug and dye transport. *J Control Release (epublication)*.

Abstract: Here we present the efficiency and versatility of newly developed core-multishell nanoparticles (CMS NPs), to encapsulate and transport the antitumor drugs doxorubicin hydrochloride (Dox), methotrexate (Mtx) and sodium ibandronate (Ibn) as well as dye molecules, i.e., a tetrasulfonated indotricarbocyanine (ITCC) and Nile red. Structurally, the CMS NPs are composed of hyperbranched poly(ethylene imine) core functionalized by alkyl diacids connected to monomethyl poly(ethylene glycol). In order to evaluate their transport in aqueous media in vitro, we have used and compared SEC, UV, ITC, and NMR techniques. We observed that the CMS NPs were able to spontaneously encapsulate and transport Dox, Mtx and Nile red in both organic and aqueous media as determined by SEC and UV-VIS spectroscopy. For the VIS transparent Ibn Isothermal Titration Calorimetric (ITC) experiments show an exothermic interaction with the CMS NPs. The enthalpic stabilization (ΔH) upon encapsulation was in the order of approximately 7 kcal/mol which indicates stable interaction between Ibn and nanoparticles. A T(1) inversion recovery NMR experiment was carried out for (31)P and (1)H nuclei of Ibn and an

increment of spin-lattice relaxation time for respective nuclei was observed upon encapsulation. CMS NPs were also found to encapsulate ITCC dye with stoichiometry of 6-8 molecules/nanocarrier. For in vivo imaging studies the dye loaded CMS NPs were injected to F9 teratocarcinoma bearing mice and a strong contrast was observed in the tumor tissues compared to free dye after 6 h of administration

Rieger J., Stoffelbach F., Cui D., Imberty A., Lameignere E., Putaux J. L., Jerome R., Jerome C. and uzely-Velty R. (2007) Mannosylated poly(ethylene oxide)-b-poly(epsilon-caprolactone) diblock copolymers: synthesis, characterization, and interaction with a bacterial lectin. *Biomacromolecules*. **8**, 2717-2725.

Abstract: A novel bioeliminable amphiphilic poly(ethylene oxide)-b-poly(epsilon-caprolactone) (PEO-b-PCL) diblock copolymer end-capped by a mannose residue was synthesized by sequential controlled polymerization of ethylene oxide and epsilon-caprolactone, followed by the coupling of a reactive mannose derivative to the PEO chain end. The anionic polymerization of ethylene oxide was first initiated by potassium 2-dimethylaminoethanolate. The ring-opening polymerization of epsilon-caprolactone was then initiated by the omega-hydroxy end-group of PEO previously converted into an Al alkoxide. Finally, the saccharidic end-group was attached by quaternization of the tertiary amine alpha-end-group of the PEO-b-PCL with a brominated mannose derivative. The copolymer was fully characterized in terms of chemical composition and purity by high-resolution NMR spectroscopy and size exclusion chromatography. Furthermore, measurements with a pendant drop tensiometer showed that both the mannosylated copolymer and the non-mannosylated counterpart significantly decreased the dichloromethane/water interfacial tension. Moreover, these amphiphilic copolymers formed monodisperse spherical micelles in water with an average diameter of approximately 11 nm as measured by dynamic light scattering and cryo-transmission electron microscopy. The availability of mannose as a specific recognition site at the surface of the micelles was proved by isothermal titration microcalorimetry (ITC), using the BclA lectin (from *Burkholderia cenocepacia*), which interacts selectively with alpha-D-mannopyranoside derivatives. The thermodynamic parameters of the lectin/mannose interaction were extracted from the ITC data. These colloidal systems have great potential for drug targeting and vaccine delivery systems.

Simon M., Wittmar M., Bakowsky U., and Kissel T. (2004) Self-assembling nanocomplexes from insulin and water-soluble branched polyesters, poly[(vinyl-3-(diethylamino)-propylcarbamate-co-(vinyl acetate)-co-(vinyl alcohol)]-graft- poly(L-lactic acid): a novel carrier for transmucosal delivery of peptides. *Bioconjug Chem* **15**, 841-849.

Abstract: The design of carriers for protein delivery that provide protection against enzymatic degradation and facilitate protein transport across epithelial surfaces, thus avoiding parenteral administration, remains a challenge. Self-assembling nanoscale protein/polymer complexes might present a promising approach. We synthesized water-soluble, amphiphilic polyesters, poly[(vinyl-3-(diethylamino)-propylcarbamate-co-(vinyl acetate)-co-(vinyl alcohol)]-graft-poly(L-lactic acid), containing a positively charged backbone, and studied the spontaneous formation of nanocomplexes (NC) with insulin. NC were characterized using dynamic light scattering, zeta-potential measurements, and atomic force microscopy (AFM). Insulin loading was determined with HPLC, and the binding constants were obtained by isothermal titration calorimetry (ITC). The NC formation was followed using nephelometric and light scattering techniques. Water-soluble, positively charged, branched polyesters with amphiphilic properties were obtained in a three-step polymer-analogous reaction. The degree of amine substitution, DS, in the PVAL backbone was varied between 0.04 and 0.5, and grafting this backbone with L-lactide increased the molecular weight from 18 kDa to 81 kDa. The polymer composition was optimized to facilitate NC formation with insulin resulting in a DS of 0.09 and a poly(L-lactide) side chain substitution of 0.5 with an average chain length of two lactic acids. Depending on polymer composition, stable NC of 200-500 nm diameter were formed with insulin, and the binding constants ranged from 4.7×10^5 to $9.5 \times 10^6 \text{ M}^{-1}$. Positively charged surface charges ranging from +5 to +35 mV and an insulin loading up to 98% of 33 IU/mL were obtained. The NC visualized by AFM revealed spheroidal particles with an entangled internal structure. It was demonstrated that this class of multifunctional polymers is capable of self-assembly with a peptidic substrate. The resulting nanosized complexes offer the potential for mucosal insulin/protein delivery and merit further investigations under in vivo conditions.

Sou K., Endo T., Takeoka S., and Tsuchida E. (2000) Poly(ethylene glycol)-modification of the phospholipid vesicles by using the spontaneous incorporation of poly(ethylene glycol)-lipid into the vesicles. *Bioconjug Chem* **11**, 372-379.

Abstract: The critical micelle concentrations of 1, 2-dipalmitoyl-sn-glycero-3-phosphoethanolamine-N-[monomethoxy poly(ethylene glycol) (5000)] (PEG-DPPE) and its distearoyl analogue (PEG-DSPE) were 70 and 9 μM , respectively, in buffer solutions ([Tris] = 20 mM, [NaCl] = 140 mM, pH 7.4) at 37 degrees C. When these PEG-lipid micelle dispersions were mixed with the dispersions of phospholipid vesicles comprised of a C16 membrane, of which the carbon number is 16, or a C18 membrane, the PEG-lipid micelles were dissociated into monomers and then spontaneously incorporated into the surface of the preformed vesicles. The incorporation rates and the enthalpy changes during incorporation were measured with an isothermal titration microcalorimeter. The incorporation rate of PEG-DPPE was faster than that of PEG-DSPE, because the dissociation rate of the PEG-DPPE micelles was faster than that of PEG-DSPE micelles. The incorporation equilibrium constant of PEG-DSPE was larger than that of PEG-DPPE due to its slow dissociation rate from the membrane, caused by the stronger hydrophobic interaction. The combination of PEG-DSPE and the C18 membrane was the most thermodynamically stabilized pair. Furthermore, the dispersion stability of the surface-modified vesicles prepared by this spontaneous incorporation was analyzed by using the critical molecular weight of the polymer for the aggregation of vesicles. The aggregation of the vesicles was successfully suppressed with an increase in the molecular weight of the PEG in the PEG-lipid and its incorporation ratio.

Singh S. K. and Caram-Lelham N. (1998) Thermodynamics of kappa-Carrageenan-Amphiphilic Drug Interaction as Influenced by Specific Counterions and Temperature: A Microcalorimetric and Viscometric Study. *J Colloid Interface Sci* **203**, 430-446.

Abstract: The adsorption of amphiphilic drug molecules to a polyelectrolyte, kappa-carrageenan, has been shown to be related to hydrophobicity of drug and the conformation of the polyanion which in turn can be regulated by choice of counterion. The binding is of a strongly cooperative nature and the degree of cooperativity has been found to be related to the self-aggregation tendency of the drug molecules. This system has been examined by titration microcalorimetry and capillary viscometry to determine the thermodynamics of the binding phenomenon. The titration calorimetry data confirms the trends and conclusions drawn regarding the factors that control the binding. Viscometry shows that although there is a change in size of the polymeric chains when the drug molecules are adsorbed, the effect is primarily due to charge neutralization and not a conformation change. This allows the microcalorimetry data to be analyzed to recover the enthalpy of binding of the drug molecules to the polymer. Earlier published equilibrium binding data has been analyzed to determine the binding constants and free energy changes in the process (-25 to -90 kJ/mol). A phenomenological model has been derived for the cooperative binding process for this purpose. The binding process is primarily enthalpy driven with the major part of enthalpy change (-10 to -40 kJ/mol) arising from the aggregation of bound drug molecules, i.e., from hydrophobic interactions; the process is also entropically favorable. The size of these aggregates in polymer-bound state is of the order of 2-5 molecules of drug, similar to the pre-micellar aggregates of the drugs in solution. Copyright 1998 Academic Press.

South C. R., Higley M. N., Leung K. C., Lanari D., Nelson A., Grubbs R. H., Stoddart J. F., and Weck M. (2006) Self-assembly with block copolymers through metal coordination of SCS-Pd(II) pincer complexes and pseudorotaxane formation. *Chemistry* **12**, 3789-3797.

Abstract: Poly(norbornene)-based block copolymers containing side chains of palladated pincer complexes/dibenzo[24]crown-8 or palladated pincer complexes/dibenzylammonium salts were synthesized. Noncovalent functionalization was accomplished with their corresponding recognition units through simple 1:1 addition with association constants (K_a) greater than 10^5 m^{-1} . The self-assembly processes were monitored by using both ^1H NMR spectroscopy and isothermal titration calorimetry. In all cases, we found that the self-assembly of the recognition units along each polymer block does not preclude the self-assembly processes along the other block.

Srinivasachari S., Liu Y., Pevette L. E. and Reineke T. M. (2007) Effects of trehalose click polymer length on pDNA complex stability and delivery efficacy. *Biomaterials* **28**, 2885-2898.

Abstract: Cationic polymers are currently being studied as non-viral vectors to deliver therapeutic DNA into cells. In this study, a series of trehalose-based glycopolymers containing four secondary amines in the repeat unit were synthesized via the 'click reaction' [degrees of polymerization ($n(w)$)=35, 53, 75, or 100] to elucidate how the polymer length affects the bioactivity. The four structures bound and charge-neutralized pDNA with similar affinity that was independent of the length, as determined through gel electrophoresis,

heparin competitive displacement, and isothermal titration calorimetric assays. Dynamic light scattering measurements revealed that the polyplexes formed with the longer polymers ($n(w)=53, 75, \text{ or } 100$) inhibited flocculation in media containing serum, whereas the polyplexes formed with the shorter polymer ($n(w)=35$) aggregated rapidly. Similar results were observed via transmission electron microscopy studies, where the nanoparticles formed with the polymers having longer degrees of polymerization showed discrete particles in media containing 10% serum. Transfection experiments revealed that the polymers exhibited low cytotoxicity at low N/P ratios and could facilitate high cellular uptake and gene expression in HeLa and H9c2(2-1) cells, and the results were dependent on the degrees of polymerization (longer polymers yielded higher transfection and toxicity).

Stolnik S., Heald C. R., Garnett M. G., Illum L., and Davis S. S. (2005) Differences in the adsorption behaviour of poly(ethylene oxide) copolymers onto model polystyrene nanoparticles assessed by isothermal titration microcalorimetry correspond to the biological differences. *J Drug Target* **13**, 449-458.

Abstract: The adsorption behaviour of a tetrafunctional copolymer of poly (ethylene oxide)-poly (propylene oxide) ethylene diamine (commercially available as Poloxamine 908) and a diblock copolymer of poly (lactic acid)-poly (ethylene oxide) (PLA/PEG 2:5) onto a model colloidal drug carrier (156 nm sized polystyrene latex) is described. The adsorption isotherm, hydrodynamic thickness of the adsorbed layers and enthalpy of the adsorption were assessed. The close similarity in the conformation of the poly (ethylene oxide) (PEO) chains (molecular weight 5000 Da) in the adsorbed layers of these two copolymers was demonstrated by combining the adsorption data with the adsorbed layer thickness data. In contrast, the results from isothermal titration microcalorimetry indicated a distinct difference in the interaction of the copolymers with the polystyrene colloid surface. Poloxamine 908 adsorption to polystyrene nanoparticles is dominated by an endothermic heat effect, whereas, PLA/PEG 2:5 adsorption is entirely an exothermic process. This difference in adsorption behaviour could provide an explanation for differences in the biodistribution of Poloxamine 908 and PLA/PEG 2:5 coated polystyrene nanoparticles observed in previous studies. A comparison with the interaction enthalpy for several other PEO-containing copolymers onto the same polystyrene colloid was made. The results demonstrate the importance of the nature of the anchoring moiety on the interaction of the adsorbing copolymer with the colloid surface. An endothermic contribution is found when an adsorbing molecule contains a poly (propylene oxide) (PPO) moiety (e.g. Poloxamine 908), whilst the adsorption is exothermic (i.e. enthalpy driven) for PEO copolymers with polylactide (PLA/PEG 2:5) or alkyl moieties.

Tan J. F., Too H. P., Hatton T. A., and Tam K. C. (2006) Aggregation behavior and thermodynamics of binding between poly(ethylene oxide)-block-poly(2-(diethylamino)ethyl methacrylate) and plasmid DNA. *Langmuir* **22**, 3744-3750.

Abstract: The aggregation behavior and the thermodynamics of binding between poly(ethylene oxide)-block-poly(2-(diethylamino)ethyl methacrylate) (PEO-b-PDEAEMA) block copolymers and plasmid DNA were examined. Binding between the polymer and DNA were confirmed by gel electrophoresis. The high affinity between the polymer and DNA was demonstrated through the ethidium bromide (EtBr) displacement assay, and the binding was found to be related to the stoichiometric balance between the amine group of the polymer and the DNA nucleotide molar ratio (N/P molar ratio). The light scattering and TEM results showed that, at low polymer concentration, the hydrodynamic radii ($R(h)$) of the polymer/DNA complexes was around 90 nm; however, at sufficiently high polymer concentration, the complexes condensed to around 35 nm induced by a structural rearrangement of the amphiphilic nature of the block copolymer. The isothermal titration calorimetric results showed that the binding between the polymer and DNA is driven by a large favorable enthalpy.

Tian Y., Ravi P., Bromberg L., Hatton T. A. and Tam K. C. (2007) Synthesis and aggregation behavior of pluronic F87/poly(acrylic acid) block copolymer in the presence of doxorubicin. *Langmuir* **23**, 2638-2646.

Abstract: Poly(acrylic acid) (PAA) was polymerized on both termini of Pluronic F87 copolymer using the atom transfer radical polymerization technique to produce a novel block copolymer, PAA-b-F87-b-PAA (F87PAA). The loading of a cationic anticancer drug, doxorubicin (DOX), to F87PAA at different pH values was investigated using isothermal titration calorimetry (ITC), laser light scattering techniques, and UV-vis spectroscopy. At pH of 4.3-7.1, the ITC profile exhibited a significant exothermic peak, which indicated that the drug loading is an enthalpically driven process. At a pH of 4.3, the enthalpy maximum was significantly reduced in the presence of 2 M urea, indicating the existence of hydrogen bonds between

the DOX and F87PAA copolymer. At a pH of 7.1, the fraction of bound DOX was close to the stoichiometric proportion of 1:1 to the molar concentration of carboxyl groups in the copolymer, where the drug loading is governed by electrostatic and stacking interactions. The TEM image of the complex indicated the formation of large compound micelles induced by the binding of DOX to the PAA segments.

Wintgens V., oud-Mahammed S., Gref R., Bouteiller L. and Amiel C. (2008) Aqueous polysaccharide associations mediated by beta-cyclodextrin polymers. *Biomacromolecules*. **9**, 1434-1442.

Abstract: Macromolecular assemblies were elaborated by mixing in water hydrophobically modified dextrans (MDC(n)) and beta-cyclodextrin polymers (pbetaCD) interacting by inclusion complexation between the hydrophobic moieties of MDCn and the beta-cyclodextrin cavities of pbetaCD. Dextrans have been modified by grafting alkyl groups (C(n)) of varying chain lengths (n = 8-16) and grafting ratio (3-6 mol%). Different pbetaCD polymers were synthesized by polycondensation of beta-cyclodextrin and epichlorohydrin. The polymer-polymer interactions have been studied by fluorimetry, isothermal titration microcalorimetry, phase diagrams, and viscosimetry. The viscoelastic properties of the temporary networks (in the semidilute range) have been studied by rheology. The interaction mechanisms between the MDCn and pbetaCD can be understood taking into account the strength of the interaction between the alkyl group and the beta-cyclodextrin cavity (mainly controlled by the alkyl chain length), the density of junctions between the chains (depending on the alkyl grafting density and the pbetaCD molecular weight), and additional cooperative effect (arising for high alkyl grafting density)

Wollner K., Vollprecht M., Leopold N., Kasper M., Busche S. and Gauglitz G. (2007) Interaction behaviour of a PDMS-calixarene system and polar analytes characterised by microcalorimetry and spectroscopic methods. *Anal Bioanal. Chem* **389**, 1879-1887.

Abstract: Spectroscopic techniques and microcalorimetry were applied to investigate a polymer-(polydimethylsiloxane; PDMS) calixarene system during interaction with propylamine and n-propanol as analyte molecules. This was done to understand the sensitivity and selectivity of this system. By these means the interesting binding site of the calixarene selector was identified and dependencies on specific properties of the polymer and the functional groups were determined. Reflectometric interference spectroscopy (RIFS) was used to characterize the kinetics whereas isothermal titration calorimetry (ITC) yielded thermodynamic data. Infrared (IR) and ¹H NMR spectroscopy allowed identification of the sensing process as an interaction between the selective group of the PDMS-calixarene system and the amino group of propylamine, and measurement of the effects on hydrogen bonds. The combination of the different spectroscopic methods and the microcalorimetric measurements broadened the understanding of this system, regarded as a model system. Thus, future tailoring of functional groups designed for improved and more selective analyte detection is possible.

You C. C., Agasti S. S. and Rotello V. M. (2008) Isomeric control of protein recognition with amino acid- and dipeptide-functionalized gold nanoparticles. *Chemistry* **14**, 143-150.

Abstract: Amino acid and dipeptide-functionalized gold nanoparticles (NPs) possessing L/D-leucine and/or L/D-phenylalanine residues have been constructed in order to target the surfaces of alpha-chymotrypsin (ChT) and cytochrome c (CytC). Isothermal titration calorimetry (ITC) was conducted to evaluate the binding thermodynamics and selectivity of these NP-protein interactions. The chirality of the NP end-groups substantially affects the resultant complex stability, with up to 20-fold differences seen between particles of identical hydrophobicity, demonstrating that structural information from the ligands can be used to control protein recognition

Ziegler A. and Seelig J. (2008) Binding and clustering of glycosaminoglycans: a common property of mono- and multivalent cell-penetrating compounds. *Biophys J* **94**, 2142-2149.

Abstract: Recent observations in cell culture provide evidence that negatively charged glycosaminoglycans (GAGs) at the surface of biological cells bind cationic cell-penetrating compounds (CPCs) and cluster during CPC binding, thereby contributing to their endocytotic uptake. The GAG binding and clustering occur in the low-micromolar concentration range and suggest a tight interaction between GAGs and CPCs, although the relation between binding affinity and specificity of this interaction remains to be investigated. We therefore measured the GAG binding and clustering of various mono- and multivalent CPCs such as DNA transfection vectors (polyethylenimine; 1,2-dioleoyl-3-trimethylammonium-propane), amino acid homopolymers (oligoarginine; oligolysine), and cell-penetrating

peptides (Penetratin; HIV-1 Tat) by means of isothermal titration calorimetry and dynamic light scattering. We find that these structurally diverse CPCs share the property of GAG binding and clustering. The binding is very tight (microscopic dissociation constants between 0.34 and 1.34 μM) and thus biologically relevant. The hydrodynamic radius of the resulting aggregates ranges from 78 nm to 586 nm, suggesting that they consist of numerous GAG chains cross-linked by CPCs. Likewise, the membrane-permeant monovalent cation acridine orange leads to GAG binding and clustering, in contrast to its membrane-impermeant structural analogs propidium iodide and ethidium bromide. Because the binding and clustering of GAGs were found to be a common denominator of all CPCs tested, these properties might be helpful to identify further CPCs