Association morphologies of amphiphilic polyelectrolyte diblock copolymers

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CHAPTER 6

Conclusions

In this thesis the structure of polyelectrolyte diblock copolymer micelles and vesicles at various length scales and under different conditions was considered in detail.

With increasing packing fraction, polyelectrolyte diblock copolymer micelles shrink irrespective corona charge and ionic strength of the supporting medium. The modest decrease in size with increasing concentration is due to the interaction among micelles, increased counterion adsorption, and/or Donnan salt partitioning between the coronal layer and the supporting medium (the functionality is fixed due to the glassy core). The corona chain statistics is rather insensitive to inter-micelle interaction. At sufficiently high corona charge under salt-free conditions, the chains remain almost fully stretched. For low charge, the corona chains are coiled and the structure factors comply with annealing of the charge towards the outer region due to the recombination/dissociation balance of the weak polyacid. In the presence of excess salt and through the whole range of concentrations, the radial decay of the monomer density is similar to the one observed in neutral star-branched polymers. Irrespectively the packing fraction, the counterions are strongly correlated with the corona forming segments with the same radial density profile. Our experiments do not allow an assessment of the extent to which the counterions are 2D localized around the stretched arms or 3D condensed inside in the coronal layer. However, almost all counterions are confined in either way, which results in a relatively small net micelle charge.

Due to the adsorption of the counterions in the coronal layer, inter-micelle correlation is rather insensitive to the corona charge fraction and can be satisfactorily

described by a hard sphere model. From a comparison of the outer micelle and hard sphere diameters, as obtained from the form and solution factor analysis, respectively, it was concluded that the coronas of sufficiently charged, salt-free micelles interdigitate once the concentration exceeds a certain critical value. Based on the fitted hard sphere diameter, this concentration corresponds with an effective micelle volume fraction 0.53 ± 0.02. At higher packing fractions, this value is effectively preserved by interpenetration of the coronal layers. For lower corona charge, interpenetration was observed at the higher weight concentrations. Interpenetration of the polyelectrolyte brushes also controls fluid rheology. As an example, the viscosity of a salt-free, 50% corona charge sample increases in value by 3 orders of magnitude, when the concentration is increased so that coronal layers interpenetrate. In addition, the parallel frequency scaling behavior of the dynamic moduli indicates the formation of an interconnected, physical gel. In the presence of excess salt, the coronal layers are less extended and they did not interpenetrate. Accordingly, the viscosity of the latter samples was in the range of the viscosity of the solvent.

Polyelectrolyte diblock copolymers can also be used to assemble vesicular structures. Here, the membrane is stabilized by complexation of the cationic copolymer with oppositely charged polyelectrolyte and a solvent quality induced collapse of the hydrophobic block in a single emulsion preparation procedure.

We have demonstrated that we can achieve efficient encapsulation and at least a 10-fold compaction of short fragment DNA (150 base pairs) with a single emulsion technique. With this technique, there are no restrictions to the molecular weight of the DNA and there is no need for precipitation onto template particles, since the material is inserted in the emulsion droplets before the membrane has been formed. Another advantage is that the size of the vesicles can be controlled by the emulsification procedure. Inside the vesicles, the DNA is tightly packed and organized in a liquid crystalline fashion; they exhibit the characteristic birefringent textures when they are observed with a polarized light microscope. As derived from the concentration, the interracial spacing between the molecules is around 3.3 nm, which is similar to that in DNA condensates and phage heads. With fluorescence staining experiments, we have shown that the copolymer membrane is highly permeable for small molecules such as fluorescence dyes. This is also supported by the permeability of the membrane for small ions, which results in release of the DNA from the vesicles in very high salt concentrations.

As a biotechnological example, we have shown that our model carrier system can be used for delivery of cloning vector DNA into in vitro cultured HeLa cancer cells in

a reverse transfection experiment. In a reverse transfection experiment, the cells are growing on top of the expression vector on a glass slide rather than the conventional method in which the DNA is added to the adherent cells. Potential advantages of the DNA/vesicle system are higher DNA coverage, control of release, protection of the DNA against nucleases produced by the cells, and possibilities for specific targeting by grafting of functional groups to the exterior of the vesicles. It is our contention that the easy and efficient deposition of the vesicles on a glass substrate and the control of the functionality of the carrier system have potential in the further development of transfected cell arraying techniques for functional genomics studies.

Our encapsulation procedure is not restricted to DNA; it can also be used to encapsulate other charged (bio)polymers. As an illustrative example, we have prepared the inverse system: capsules of cationic poly(ethylene imine) encapsulated by the anionic diblock poly(styrene-*b*-acrylic acid) copolymer. Since the copolymer is also the building block of the membrane, its chemical composition and molecular weight control the stability and functionality of the vesicle. In particular, the stability of the self-assembled structure in various environments needs to be optimized to suit the particular application. A promising option is chemical cross-linking (polymerizing) of the collapsed polymer layer. Other promising features are the possibilities to control biodegradability and tissue-specific adaptation by the specific choice of copolymer.