# Functionalization of DNA by electrostatic bonding

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**Summary**

Functional nanostructures have stimulated the evolution of the fields of nanoscience and nanotechnology through fabricating unprecedented architectures from the molecular level to achieve materials with novel properties in the macroscopic world. In these areas, biomacromolecules, i.e. proteins, DNA and their assemblies, like viruses, due to their inherent 3D structures, have attracted enormous attention to transform their biological function into applicable materials in technological systems. Efforts have been undertaken to overcome their poor structural stability in absence of an aqueous environment and maintain them in high concentration during manufacturing processes. By mixing a double-stranded (ds) DNA-surfactant complex with a NLO-active dye and a second dye, Disperse Red 1, in organic phase, second-order nonlinear optical (NLO) materials [1] and electro-optic waveguide modulators [2] were successfully fabricated. The dsDNA-lipid film was casted from organic solution, exhibiting anisotropic electric conductivity due to the base-pair stacking. [3] The general approach is to complex these biological entities with oppositely charged molecules, like surfactants and polymers, to minimize the aggregation and preserve their native conformation. At the same time the formation of complexes enables easy processing of the biomacromolecules.

In **chapter 1**, two manners from the literature have been introduced to form biomacromolecule complexes through electrostatic interactions. One is through ion exchange with charged polyethylene glycol (PEG) moieties in aqueous solution. Another one is co-operative precipitation. Solvent-free nucleic acid-, protein- and virus-PEG complexes were obtained as liquids at room temperature with intact structures and exhibiting good solubility in common organic solvents, so that their biological functions were preserved in water-free state and the materials could be processed in organic phase. Co-operative precipitation enabled the complexation between biomacromolecules and cationic lipids in a stoichiometric fashion. The

properties of the obtained complexes, like phase transition temperature and compact ordering can be tuned by changing the structure and alkyl chain length of lipids. Although two approaches have been developed, each of them has some short comings. The ion exchange requires exceeding amount of ligands and time consuming dialysis to achieve a reasonable complexation degree. The co-operative precipitation is only applicable to certain cationic lipids with aliphatic tails exhibiting 8 to 16 carbons. To broaden the choices of lipids, we developed a two-step method in **chapter 2** to introduce functional lipids like π-systems onto DNA. Water soluble 4-(hexyloxy)anilinium (ANI) was synthesized as a key ingredient to precipitate DNA from the aqueous phase then solubilize it in the organic phase where ANI can subsequently be exchanged for a more hydrophobic amine-derived surfactant. We demonstrated that this method yields complete exchange of the surfactant and allows for the modification of DNA with hydrophobic primary, secondary and tertiary alkylamines. Even an amine derived from aromatic terthiophene was successfully complexed with DNA through this way adopting a right-handed helix as a result of being complexed with the DNA molecule. Starting from such aromatic molecule architectures, a DNA-based light harvesting system was fabricated through complexing with amine-derived pyrene units, which absorb light at 350 nm and then transfer the energy along the DNA to a chromophore at the terminus of the DNA strand to emit photons at 610 nm.

In **chapter 3**, we applied the two-step lipid exchange method to negatively charged sulfated cyclodextrin (CD). CD was complexed with ANI in aqueous solution then subjected to the exchange of tris[2-(2-methoxyethoxy)ethyl]amine in organic phase. Fully sulfated substituted α-cyclodextrin and β-cyclodextrin (α- and β-full-CD), as well as heptakis(6-*O*-sulfo)-β-cyclodextrin (β-half-CD) were manufactured into complexes exhibiting fluidic property at room temperature. The viscosity and transition temperatures are ranging from -5 to 22 °C of the complexes and increase in the order of β-full-CD < α-full-CD < β-half-CD. All of them are thermally stable up to

220 °C, behaving as room temperature CD ionic liquid (IL). The cavity of CD was maintained to incorporate guest molecules like pyrene. The successful incorporation was demonstrated by the fluorescence change of pyrene before and after mixing with CD-ILs. This finding indicated the incorporation of pyrene into the hydrophobic cavity of CD-ILs.

In **chapter 4**, we applied this lipid exchange method to complex amine-derived PEGs with DNA. As a standard procedure, ANI was firstly applied to precipitate DNA from the aqueous phase and was then subjected to the displacement of PEGs with Mw 350, 500, 750, 1000 and 1500 Dalton in organic phase. It was found that only the first four can be successfully introduced onto DNA electrostatically forming DNA-PEG complexes. The complexes were characterized by NMR, UV/Vis and CD spectroscopy. The Mw of DNA-PEG complexes were further studied through GPC coupled to static light scattering (SLS), which showed that the substitution degree of PEG can only reach full conversion when low molecular weight PEG with a mass of 350 Da is used. The substitution degree gradually decreased to30% as the Mw of the PEGs was increased to 1000 Da.

In **chapter 5**, a new generation of lipid exchange method was developed based on a similar working principle as the previous one. This allowed the introduction of quaternary ammonium compounds onto negatively charged DNA by electrostatic interactions. ANI was used to precipitate DNA from aqueous phase. Then it was replaced by quaternary ammonium molecules containing acetylacetonate as counterion. This exchange process can be realized because the conjugated base of acetylacetonate is very strong allowing to abstract a proton from ANI. As a result, ANI becomes neutral and acetylacetonate accepts the proton to form the neutral diketone. The positively charged quaternary ammonium then binds electrostatically with the negatively charged phosphate groups of DNA forming the new DNA complexes. (Polyethylene glycol) trimethylammonium (TMA-PEG) and tetra-alkyl

ammonium compounds were introduced onto DNA in this manner and were characterized thoroughly. In addition, the stability of DNA-TMA-PEG750 complex was compared with DNA-PEG complexes made in chapter 4 in different buffer solutions, which revealed that quaternary ammonium is more vulnerable to ion displacement than the primary aminePEGs due to the low binding constant of quaternary ammonium to phosphate groups.

In summary, two new exchange methods have been developed to introduce a wide variety of cationic moieties as connterions to the anionic DNA backbone. The scope of counterions ranges from amines carrying long non-water-soluble alkyl chains over amines with π-systems to quaternary ammonium groups lacking a proton at the nitrogen center. Moreover, small molecules with low molecular weight were complexed with DNA up to polymers with a molecular weight of more than 1.5 KDa. The latter type of molecules leads to DNA bottle brush structures. It is anticipated that the novel methods introduced in this thesis have an impact on DNA used in technical applications or the area of biomedicine. For the latter application field nucleic acids were frequently covalently functionalized with PEG to slow down their degradation by nucleases in biological media. The new electrostatically bonded DNA-PEG hybrids might lead to an increase of the stability of the nucleic acid part. Thereby, laborious chemical functionalization of the DNA could be avoided and at the same time the novel electrostatic functionalization strategy might help nucleic acid drugs to be more effective.

**References**


1. Wanapun, D., Hall, V. J., Begue, N. J., Grote, J. G., Simpson, G. J., *ChemPhysChem*, **2009**, *10*, 2674.
2. Heckman, E. M., Grote, J. G., Hopkins, F. K., Yaney, P. P., *Appl. Phys. Lett*. **2006**, *89*, 181116.
3. Okahata, Y., Kobayashi, T., Shimomura, M., *J. Am. Chem. Soc*. **1998**, *120*, 6165.