Nucleo-copolymers: oligonucleotide-based amphiphilic diblock copolymers†

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For the first time, poly(butadiene) has been covalently linked to an oligonucleotide sequence and the resulting nucleo-copolymer exhibits amphiphilic properties in dilute aqueous solution, self-assembling into nanometer-sized vesicular structures.

Research on DNA has been constantly driving a great deal of interest. For instance, the shared expectations in gene therapy to cure genetically acquired diseases hold the attention of everyone and major efforts are put in the establishment of the human genomic map.[1]

Genetics recalls that Nature produces 'state-of-the-art' polymers, which organize into highly functional structures. Complementary DNA strands are sequences based on only four nucleotides, which assemble into the double helix that codes life: a single defect in the nucleotide sequences which compose the complementary DNA strands may lead to severe diseases.

Nature thus provides a quasi-infinite number of water-soluble polymers, merely by combining the four letters of this alphabet. Inherent to the composition, length and structure, a nucleotide sequence is a polyelectrolyte naturally sensitive to the pH and ionic strength of its aqueous surrounding. However, solely nucleotide-based polyelectrolytes are able to undergo non-covalent, yet highly specific interaction by base-pairing. Besides, they are recognized by cell surface receptors[2] and various enzymes.[3]

Nevertheless, a nucleotide sequence has never been used as the water-soluble polymer segment to build an amphiphilic diblock copolymer. Driven by the chemical incompatibility between the two covalently linked hydrophobic and hydrophilic polymers, diblock copolymers undergo self-assembly in aqueous solution. This process is currently the most versatile approach to control and drive the organization of polymers at the nanometer length-scale[4] and mimic the behavior of biological molecules.

We thus describe here, for the first time, the synthesis of a nucleotide-based amphiphilic diblock copolymer and its self-assembly into vesicular structures in dilute aqueous solution.

Hydroxyl-terminated poly(butadiene), PB (MW = 2000 Da), was purchased from PolySciences, Ind. and modified to present a terminal amine function. PB was selected as the non-polar segment because of its low glass transition temperature and the possibility to further perform a cross-linking reaction. This mechanism will enable, if necessary, the mechanical stabilization of the self-assembly.[5]

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The nucleotide sequence was synthesized using an EXPEDIT DNA Synthesizer 8909 and consists of 12 dCMP units, cytidine$_{12}$. This number of bases was chosen in order to provide stability to the formation of the double helix[6] upon hybridization of the nucleo-vesicles with the complementary nucleotide strands, which will be performed in forthcoming investigations.

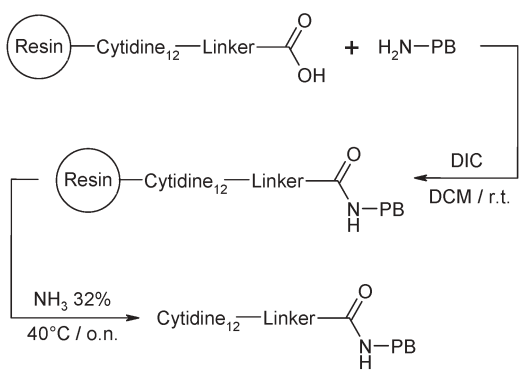
In order to prevent tedious chemistry and purification steps, the reaction between the amino-terminated PB and the carboxy-modified nucleotide sequence, which is synthesized by common solid-phase synthesis,[7] is carried out prior to the cleavage of the oligonucleotide from the phosphoramidite solid support (ESI†) (Scheme 1).

The product of this one-step copolymer synthesis was purified through size exclusion chromatography (SEC), in order to remove the non-reacted species, using phosphate buffer saline, PBS as eluent. FTIR (ESI†) was used to characterize the product (IR/cm^{-1}): 3528 (ν_{C-OH} cytidine$_{12}$); 3369 (ν_{NH_2} cytidine$_{12}$); 3179 ($\nu_{N-H,\ amide}$); 1650 ($\nu_{C=C}$ PB, $\nu_{C=O,\ amide}$); 1121–1104 (d, $\nu_{P=O}$ cytidine$_{12}$); 1055 ($\nu_{C-O-C,\ cycl}$ cytidine$_{12}$)).

After conjugation of the PB to the cytidine$_{12}$, the nucleo-copolymer, cytidine$_{12}$-block-poly(butadiene), is no longer soluble in tetrahydrofuran, THF. The resulting nucleotide-based copolymer is an amphiphilic diblock copolymer, which dissolves in an aqueous solution. With water being a poor solvent for the hydrophobic PB polymer segments, spontaneous self-assembly of the nucleo-copolymer occurs upon dilution in an aqueous solution.

Dynamic light scattering measurements show a bimodal size distribution of the self-assembly (data not shown). Small, nanometer-sized and large, micrometer-sized structures coexist.

In order to resolve the morphology of these structures we first performed transmission electron microscopy, TEM. As shown in Fig. 1(a), structures with a size of 80 nm can be observed. Since the



Scheme 1 Synthesis of the oligonucleotide-based amphiphilic diblock copolymer, cytidine$_{12}$-block-poly(butadiene).

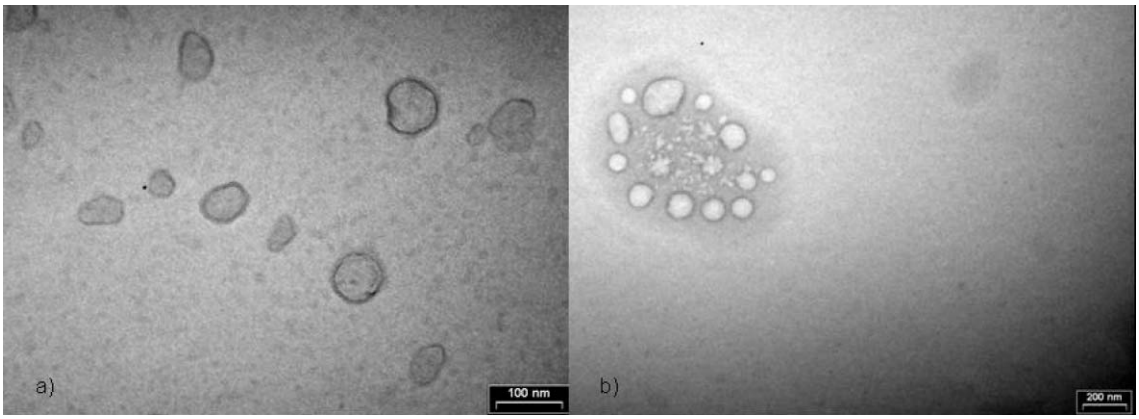


Fig. 1 Self-assembly of the oligonucleotide-based amphiphilic diblock copolymer cytidine$_{12}$-block-poly(butadiene) into: (a) 80 nm vesicular structures and (b) higher-order vesicle structures in dilute aqueous solution, observed by transmission electron microscopy, TEM.

nucleotide sequence contains atoms such as phosphorus, providing high contrast under electron irradiation, we clearly observe spherically closed dark shells.

The larger self-assembled structures result from a complex organization of the smaller vesicles (Fig. 1(b)). As described by Geng *et al.*,[8] charged diblock copolymers in water may assemble into tethered vesicles, encapsulated vesicles or large compound vesicles, depending on the ionic strength and pH of the solution.

These are vesicular structures adsorbed from the aqueous solution onto the carbon-coated grid and analyzed in vacuum, which explains the irregular shape of the structures.

The morphology of the nucleo-copolymer self-assembled into micrometer-sized structures was investigated by optical microscopy, which enables the characterization of the self-assembly in an aqueous environment.

In a first set of experiments, a 100 nM solution of Bodipy® 630/650-Xt (Invitrogen) was added to the nucleo-copolymer aqueous solution. This fluorescent hydrophobic dye is entrapped in the poly(butadiene) environment of the nucleo-copolymer self-assembly. As can be observed in Fig. 2(a), micrometer-sized fluorescent structures are detected by fluorescence microscopy.

In a subsequent experiment, we adapted a common 'life/dead' assay used in biology to stain the genomic DNA of bacteria. When their membrane is viable, a DNA chelating agent, Syto9®, is able to enter the nucleus, which then shines upon light excitation. This chelating agent was therefore used to image the nucleotide segments of the nucleo-copolymers present in the self-assembled particles (Fig. 2(b)).

Two-colour confocal laser scanning microscopy, CLSM, allows the observation of both the hydrophobic and hydrophilic parts of the structures. The homogeneous co-localization of the two fluorescence signals, Fig. 2(c), indicates that the larger particles consist of a complex organization of vesicular structures, with a size range of 1–2 μm. The shiny nucleotide shell surrounding the blurry core of the structures indicate that higher-order vesicles, HOV, consist of multi-vesicles encapsulated in one large vesicle.[8] Both TEM and CLSM measurements are in agreement.

Considering a nucleotide sequence as a water-soluble polyelectrolyte, we synthesized for the first time, an amphiphilic diblock copolymer which consists of a hydrophilic nucleotide-based polyelectrolyte covalently linked to a hydrophobic poly(butadiene) segment. As demonstrated by the microscopy studies, these nucleo-copolymers self-assembled into vesicular (Scheme 2) and higher-order vesicle structures in dilute aqueous solution, with the nucleotide strands pointing towards both the inner aqueous pool

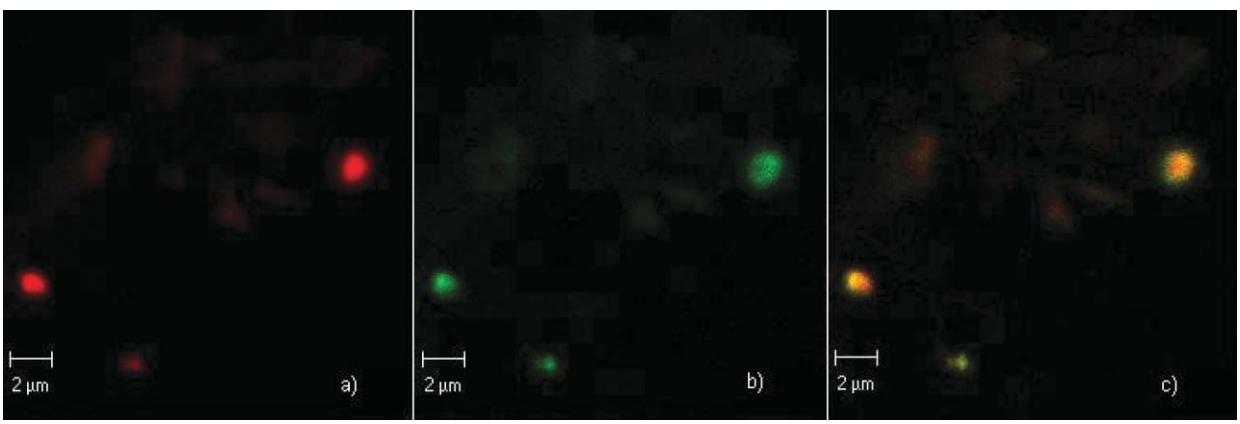
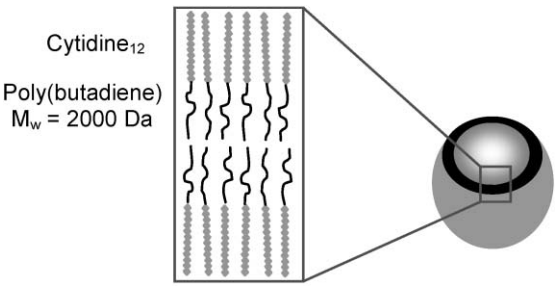


Fig. 2 Self-assembly of the oligonucleotide-based amphiphilic diblock copolymer cytidine$_{12}$-block-poly(butadiene) into micrometer-sized higher-order vesicle structures in dilute aqueous solution, observed by two-colour confocal laser scanning microscopy: (a) Bodipy staining of the hydrophobic environment; (b) Syto9® staining of the nucleotide segments, and (c) co-localization of the signals from both Bodipy and Syto9®.



Scheme 2 Schematic representation of the self-assembly of the oligonucleotide-based amphiphilic diblock copolymer cytidine$_{12}$-block-poly(butadiene) into vesicular structures in dilute aqueous solution.

and the surrounding of the spherically closed nucleotide-based polymer shell.

The influence of both the pH and the ionic strength on the configuration of the nucleotide within the self-assembled vesicles is being investigated. Driven by the unique ability of complementary nucleotide-based polyelectrolytes to assemble *via* base-pairing, those nucleo-vesicles are naturally recognized by microorganisms and studies on cell signaling or internalization and bacterial adhesion of the nucleotide-based shell are currently in progress. Advances in gene or drug delivery and genomics can be, hence, foreseen.

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