Polydipyrrole- and polydicarbazole-nanorods as new nanosized supports for DNA hybridization†

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Novel functional polydipyrrole- and polydicarbazole nanorods have been AAO template-synthesized from COOH-dipyrrole/-dicarbazole monomers using Vapor Deposition and Liquid Phase Polymerizations (VDP and LPP). They were tested as insoluble supports for covalent DNA attachment and hybridization.

Amongst various classes of nanomaterials, conducting polymers (CPs)-based nanomaterials such as polymeric nanotubes/rods have recently piqued scientific interest due to their potential use in various biomedical applications.1–3 In general, CPs have been (electro)chemically template-synthesized from simple non-functional oxidizable monomers, such as pyrrole, aniline, and thiophene within nanoporous membranes, resulting in solid CPs-based nanorods and hollow nanotubules.1–3 If desired, membrane dissolution can release monodispersed CPs-based nanorods and/or nanotubules for further processing. Both anodized aluminum oxide (AAO) and track-etched polyester membranes have been used, since they contain a high density of size-defined, well-separated, discrete nanopores as shape-defining nanoreaction vessels.4–6 Astonishingly, the oxidative template-synthesis polymerization of more sophisticated, oxidizable monomers having chemical functionalities has never been reported. Depending on monomer structure, the resulting CPs-based nanotubes/rods should possess functional groups protruding from surfaces. These groups may be made accessible within polymeric matrices themselves for post-polymerization derivatizations.

In this work, we report the preparation of novel polymeric CPs-based polycarboxylated functional nanorods that were template-synthesized from dipyrrole (DPy) and dicarbazole (DCb) monomers 3 and 4–6,9 (Scheme 1). These new DPY- and DCb-precursors are bis-heterocyclic and mono-bis-carboxylated. DCb-monomers of type 3 that contained NH2-sensitive pentafluorophenyl esters exchangeable by surface NH2 groups of enzymes, have been electropolymerized onto Pt/Au microelectrodes.8 In addition, magnetically responsive nanocomposites of a magnetite-pDPy-pDCb core-shell morphology were readily prepared by chemical oxidation of a range of DPY-/DCb-monomers

of type 3–6 around nanosized magnetite particles.10 In all these examples, (electro)chemically stable polymer deposits resulted from a polymer reticulation caused by the innovative bis-heterocyclic chemical design of monomers. It should be noted that simple N-substituted mono carbazoles could not afford electrochemically stable polycarbazole films but rather shorter soluble tetrameric polycarbazole chains.11,12 Additionally, since carbazoles are more difficult to (electro)chemically oxidize than pyrroles, we identified ceric ammonium nitrate (CAN) as a unique oxidant able to efficiently polymerize bis-heterocyclic DCb-monomers around magnetite nanoparticles.10

The two COOH-containing DPy- and DCb-monomers 3 and 4 have been synthesized by condensing L-lysine 1 with 1,4-dimethoxy tetrahydrofuran 2 using a modified Clauson–Kaas reaction7,8 (Scheme 1). The two C2-symmetrical bis-COOH DCb-monomers 5 and 6 were readily obtained in a short three-step synthesis.9,10 A similar modified Clauson–Kaas reaction has been performed on the protected L-α-benzyl glutamate, followed by DCC-HOBt mediated amidation of the resulting benzylated pyrrole/carbazole-glutamates using linkers L1–3. Subsequent debenzylation (10% Pd/C, ¾ v/v cyclohexene/i-ProOH) of protected DPy-/DCb-intermediates cleanly afforded DPy- and DCb-monomers 5 and 6 (32–33% overall yields).

Monomers DPy-3 and DCb-4 have been chemically polymerized within nanosized pores of AAO membranes (Whatman International Ltd., Anodic 25; Ø = 21 mm, 3 membranes/experiment, 60 μm thickness, 100 nm average pore size, 109 pores cm–2 pore density) by vapor deposition (VDP,13,14 FeCl3 oxidant) and liquid phase (LPP,1,15 CAN oxidant) polymerization techniques. Detailed experimental procedures for

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‡ Electronic supplementary information (ESI) available: experimental description of fabrication of pDPy-pDCb-nanorods. See http://dx.doi.org/10.1039/b502483h
the preparation of pDPy-/pDCb-nanorods are described in the ESI.† The resulting polycarboxylated pDPy-/pDCb-nanorods are new nanosized insoluble polymeric supports compatible for DNA hybridizations. To our best of our knowledge, the preparation of such functional CPs-based nanorods from chemically more sophisticated COOH-containing DPy-/DCb-monomers, and their further use in DNA hybridizations, have never been reported.

The FT-IR spectrum (Bomem MB 100 FT-IR spectrometer, KBr pellet) of pDPy-nanorods, fabricated by the VDP method, showed the characteristic large peak of a polycationic doped \( \pi \)-conjugated polypyrrole system, e.g. the conjugated C–N stretching at 1384 \( \text{cm}^{-1} \).16 The C–H and O–H (COOH function) stretchings, respectively at \([2853, 2930, 2960]\) and 3443 \( \text{cm}^{-1} \), further supported the formation of polycarboxylated pDPy-nanorods.

Fig. 1A represents both SEM and TEM images of smooth and uniform meniscus-ended pDPy-nanorods (SEM and TEM: JEOL JSM-6700F Scanning Electron and JEOL EM-2000 EX II cast microscopy analyses. FT-IR spectroscopy revealed characteristic vibration peaks at 1640–1540, and 1473 \( \text{cm}^{-1} \) (indole ring), at 1384 \( \text{cm}^{-1} \) (conjugated C–N stretchings), at 1680 \( \text{cm}^{-1} \) (N–H stretchings), and at 3443 \( \text{cm}^{-1} \) (O–H stretchings). In the case of pDCb(6)-nanorods, additional characteristic peaks appeared for symmetric C–O (–OCH\(_2\)–) and C–H (ether group) stretchings at 1130 \( \text{cm}^{-1} \) and 2835–2955 \( \text{cm}^{-1} \) respectively. SEM and TEM images showed smooth surfaces of pDCb(5)- and pDCb(6)-nanorods (Figs. 1C and 1D respectively). Their calculated average lengths (5.0 and 7.0 \( \mu\text{m} \)) and diameters (190.0 and 130.0 \( \mu\text{m} \), 50 counted pDCb(56)-nanorods) resulted in high average aspect ratios ca. 26 and 53, respectively.

The four polycarboxylated pDPy(3-) and pDCb(4–6)-nanorods have been separately tested for DNA covalent immobilization and hybridization using an HRP-based enzymatic amplifying system (HRP: Horse Radish Peroxidase, Scheme 2 and ESI†). First an amine-modified 20-mer oligonucleotide NH\(_2\)-DNA H\(_2\)N-(CH\(_2\))\(_{12}\)-5\'GCACCTGGGAGCATTTAGGCT (14.1 nmol), that characterizes the 20210 mutation in the Human Factor II gene,17 has been covalently attached onto pDPy(3-) and pDCb(4–6)-nanorods (600.0 \( \mu\text{g} \)) following COOH activation by the water-soluble carbodiimide EDC (EDC: N\(^\prime\)-(3-dimethylaminopropyl)-N-ethyl-carbodiimide, 0.4 M MES buffer at pH 5.0, 2 h incubation at 20 °C).18,19

After hybridization with the fluoresceine-labeled antisense 20-mer oligonucleotide FITC-DNA (fluoresceine-\(^3\)AGCCTCAAT-GCTCCCCAGTG, 10 min, 60 °C, six parallel experiments, 50.0 \( \mu\text{g} \) of nanorods per Elisa plate well/experiment), an anti-FITC HRP-labeled mouse monoclonal antibody was added and incubated (10 min, 20 °C). After addition of the HRP substrate TMB (3,3\(^\prime\),5,5\(^\prime\)-tetramethyl-benzidine, Aldrich), both pDPy- and
hybridizations have been validated by an enzymatic HRP-based using AAO membrane templates. Nanorod-supported DNA polyCOOH-pDPy(3)-/pDCb(4–6)-nanorods. In a high efficiency factor EF (EF = 8.0) for these nanorods. On the contrary, more hydrophobic pDCb(4–6)-nanorods disclosed poor to moderate EFs in a 0.6-2.1 range due to much higher NSB signals. Further use of pDCb-nanorods for the detection of DNA hybridization will require additional passivation steps.

In conclusion, we have fabricated, for the first time, functional polyCOOH-pDPy(3)-/pDCb(4–6)-nanorods by template-synthesis using AAO membrane templates. Nanorod-supported DNA hybridizations have been validated by an enzymatic HRP-based amplifying system opening an interesting avenue toward DNA-based self-assembly processes of polymeric nanorods for supra-molecular biosensing assemblies.

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Notes and references
9 Full details on the preparation and the analytical/spectroscopic characterization of DCb-monomers 5 and 6 will soon be published in a forthcoming article.
14 This VDP experimental set-up has been previously described: J. Jang and B. Lim, Angew. Chem. Int. Ed., 2003, 42, 5600–5603.
18 A typical protocol for DNA attachment/hybridization onto polymeric nanorods has been reported in the ESL. It includes details about specific solutions and buffers used in these experiments.
19 The covalent attachment of the NH2-DNA capture probe onto pDPy-/pDCb-nanorods is quantitative in these conditions according to the following data. First, concentrations of COOH groups accessible on nanorods, these values have been found in a range of 750–805 nmol mg⁻¹ of nanorods, which much exceeded the quantity of reacting NH2-DNA (141 nmol). Additionally, reverse-phase HPLC checking of post-coupling washings did not allow detection of any unbound NH2-DNA probe (UV-diode array detection, Iₘₜₐₓ = 260 nm).