

Supporting Information

Polydipyrrole- and Polydicarbazole-Nanorods as New Nanosized Supports for DNA Hybridization

Jean-Paul Lellouche,^{*a} Senthil Govindaraji,^a Augustine Joseph,^a Jyongsik Jang,^{*b} and Kyung Jin Lee^b

^a Bar-Ilan University, Department of Chemistry, Ramat-Gan 52900, Israel. Fax: 972-3-535 12 50; Tel: 972-3-531 83 24; E-mail: lellouj@mail.biu.ac.il

^b Hyperstructured Organic Materials Research Center and School of Chemical Engineering, Seoul National University, Shinlimdong 56-1, Seoul 151-742, Korea. Fax: (+82)2-888-1604; Tel: (+82)2-880-7069; E-mail: jsjang@plaza.snu.ac.kr

Preparation of pDPy(3)- and pDCb(5/6)-nanorods Using the VDP Methodology

Typically, AAO membranes (Whatman International Ltd., Anodisc 25; $\text{Ø} = 21$ mm, *three* membranes/experiment, 60 μm thickness, 100 nm average pore size, 10^9 pores/ cm^2 pore density) were first extensively wetted with a 10% w/v aqueous FeCl_3 solution (30 mL, 10 min contacting). Excess oxidant was rubbed off (sand paper) from both top and bottom membrane surfaces. Dried FeCl_3 -loaded AAO template membranes were then moved into a reaction vessel (100 mL) equipped with a monomer-loading reservoir (10 mL). The reaction chamber was evacuated (10^{-2} torr at 20 °C) and neat DPy-monomer **3** (50.0 mg, 0.20 mmol) was introduced into the reservoir. After insertion of the charged reactor into a pre-heated oven (90 °C), the DPy-monomer **3** vaporized under these conditions and polymerized inside FeCl_3 -loaded nanopores of AAO membranes during 6 h. Brown polymeric pDPy(**3**)-nanorod containing AAO membranes were then dissolved in aqueous 2M NaOH (10 mL), and diluted with water (50 mL). Precipitated pDPy(**3**)-nanorods were isolated by ultracentrifugation (4000 rpm, 20 min at 4 °C), and dried (10^{-2} torr vacuum, 12 h). This precipitation-washing-drying sequential process was repeated three times to afford high-purity brown pDPy(**3**)-nanorods (yield: 5.0 mg of nanorods/AAO template membrane).

pDCb(**5/6**)-nanorods were similarly prepared but using cerium ammonium nitrate (CAN)-charged AAO membranes (by dipping into a 50% w/v aqueous CAN solution followed by drying in a vacuum oven at 50 °C, 10^{-2} torr, 1 h). After removing the CAN oxidant in excess from top and bottom template

membrane surfaces, CAN-loaded membranes were moved into the reaction vessel that contained oxidizable DCb-monomers **5/6** (30.0 mg, 46.4 and 38.6 μmol respectively). The reaction vessel was evacuated until its internal pressure reached about 10^{-2} torr. The templated polymerization proceeded at 120 °C for 12 hours. After polymerization, insoluble greenish pDCb(**5/6**)-nanorods were precipitated and purified as described above for the former DPy-related procedure (yield: 6.0-7.0 mg of nanorods/AAO template membrane).

Preparation of pDCb(**4**)-nanorods Using the LPP Methodology

Similar AAO template membranes were charged with the CAN oxidant specific for the efficient polymerization of DCb-containing monomers (*five* CAN-loaded AAO template membranes/experiment, 10 min dipping in a 50% w/v aqueous solution of CAN). After drying (40 °C, 10^{-2} torr, 1 h), they were dipped into a CH_2Cl_2 solution of DCb-monomer **4** (0.5% w/v CH_2Cl_2 , 10 min contacting) for templated polymerization. Polymerization progress inside AAO membrane pores could be monitored by color changes of contacted membranes from light yellow (starting CAN color) to green (color of the doped pDCb(**4**)-based polymer). After 1 h polymerization, pDCb(**4**)-loaded template membranes were cleaned from any unreacted DCb-monomer **4** (CH_2Cl_2 washing, 30 mL), and digested (aqueous 2M NaOH solution, 20 mL) to afford light brown pDCb(**4**)-nanorods that were purified similarly to former pDPy(**3**)-ones (yield: 4.0-5.0 mg of pDCb(**4**)-nanorods/membrane).

A Typical Protocol for DNA Attachment/Hybridization on Polymeric Nanorods

Specific Reagents, Buffers and solutions and reagents: the neutral PBS buffer (*pH* 7.0) was prepared from Dubelcco's Phosphate Buffered saline (Sigma); 0.4M MES (*pH* 5.0): prepared using 99% anhydrous 2-morpholine ethane sulfonic acid, adjusted to *pH* 5.0 by adding 10M NaOH (storage at 4 °C); TNET buffer (*pH* 7.5): prepared from a mixture of 10mM Tris-HCl, 0.5M NaCl, 1mM EDTA and 0.02% Tween-20; Washing Solution: prepared from a mixture of 3M NaCl and 2M Tris-HCl (*pH* 7.5); Assay Solution: prepared from a mixture of 154mM NaCl, 50mM Tris-HCl (*pH* 7.8), 0.5% BSA, and

0.1% Tween-20. The 20-mer amine-modified oligonucleotide NH₂-DNA NH₂-(CH₂)₁₂-^{5'}GCACTGGGAGCATTGAGGCT (Danyiel Biotech Ltd, Israel, chemical purity ≥ 98%) has been prepared in a 0.4M MES buffer (pH 5.0, 1.68 × 10⁻⁵M; 70 μL, 14.11 nmol, 1.18 nmol/Eppendorf tube). The FITC-labeled *anti*-sense oligonucleotide FITC-DNA FITC-^{5'}AGCCTCAATGCTCCCAGTGC has been previously dissolved in a pH 7.5 TNET buffer (10⁻⁵M; Danyiel Biotech Ltd, Israel, chemical purity ≥ 98%; 100 μL, 10.0 nmol/Eppendorf tube). The buffered aqueous EDC solution has been freshly prepared using a cold 0.4M MES buffer (pH 5.0, 100 mg (0.64 mmol) EDC/mL of 0.4M MES). The commercially available reporter *anti*-FITC HRP-labeled mouse monoclonal antibody (Savyon Diagnostics part No. 9064, lot DO7952A0) has been diluted before use in the Assay Solution (final antibody concentration: 0.1 μg antibody/mL of Assay Solution).

An aliquot (1.2 mL, 600.0 μg) of a well-shaken pDPy(3)-/pDCb(4-6)-nanorod aqueous suspension (50.0 μg/100 μL) was centrifuged (13,000 rpm, 3 min) and the supernatant phase discarded. Precipitated polymeric polyCOOH nanorods were first washed with a neutral PBS buffer (3 × 1.2 mL), re-suspended in a freshly prepared 0.4M MES buffer solution of NH₂-DNA (840 μL, 14.11 nmol, 1.2 mL), and gently agitated for 30 min at room temperature (smooth vortex agitation) for homogenization. A solution of EDC in 0.4M MES (pH 5.0, 360 μL, 100.0 mg EDC/mL (0.64 μmol/μL), 230.4 μmol) was added and incubated with nanorods for COOH activation/DNA covalent attachment (2 h, 20 °C, smooth vortex agitation). After precipitation (centrifugation at 13,000 rpm during 3 min), DNA-decorated nanorods were washed with both neutral PBS (3 × 1.2 mL) and TNET (pH 7.5, 2 × 1.2 mL) buffers, and were distributed to *twelve* Eppendorf plastic tubes as 100 μL portions (TNET buffer, last washing, ~ 50.0 μg nanorods/Eppendorf tube). Each Eppendorf tube was added with the FITC-labeled *anti*-sense oligonucleotide FITC-DNA FITC-^{5'}AGCCTCAATGCTCCCAGTGC dissolved in a pH 7.5 TNET buffer (Danyiel Biotech Ltd, Israel, chemical purity ≥ 98%, 10⁻⁵M, 150 μL, 1.5 nmol/Eppendorf tube),

and incubated at 60 °C for 10 min. Then, DNA-hybridized nanorods were successfully washed with the pH 7.5 TNET buffer (3 × 100 μL) and with the Assay Solution (1 × 100 μL) before incubation with the reporter *anti*-FITC HRP-labeled mouse monoclonal antibody (100 μL, 0.1 μg antibody/mL of Assay Solution, 20 °C, 10 min). Following incubation, polymeric nanorods were washed with the Washing Solution (2 × 150 μL), and added with the TMB substrate (0.05% w/w TMB solution in de-ionized water, 100 μL, 20 °C) in each Eppendorf tube. Resulting nanorod suspensions were left undisturbed for blue color development for 5 min (20 °C) and centrifuged (13,000 rpm during 3 min). Following separation, 50 μL of separate supernatant fractions were transferred to wells of a thermowell polycarbonate non-sterile 96-well microtiter plate for optical reading at 620 nm using an Elisa plate reader Anthos ht II (TB data). For each pDPy-/pDCb-nanorods, *six* similar experiments were also conducted in parallel that omitted the FITC-DNA complementary sequence. Resulting optical data afforded NSB data that allowed the determination of EF factors characterizing each polymeric nanorod (Figure 2).

Aminated Dansylated Probe for the Determination of the Concentration of Accessible COOH Groups in Polymeric pDPy(3)-/pDCb(4-6)-Nanorods

The novel dansyl-containing fluorescent 1-amino-probe **DNSA** has been synthesized by mono-sulfamide condensation of the commercially available dansyl chloride with the linear diamine 1,4-diamino-butane (see below).

