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A biosensing model system: selective interaction of biotinylated PPEs with streptavidin-coated polystyrene microspheres†

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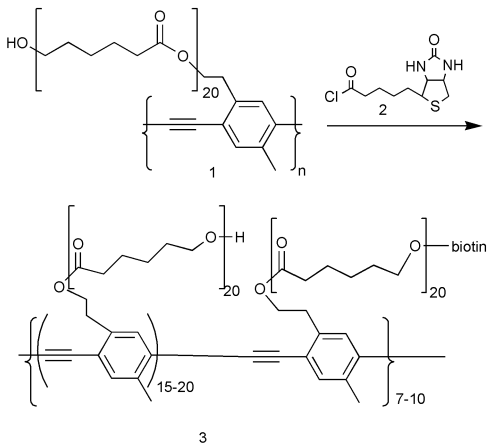
Formation of a highly fluorescent composite formed from the biotinylated PPE 3 and streptavidin covered polystyrene microspheres is reported.

Conjugated materials are valuable sensors[1] that just begin to penetrate the biological world.[2] Spectacular examples are the sensing of DNA strands by cleverly designed polythiophenes, water soluble poly*para*phenylenvinylene derivatives,[3–5] and the use of polydiacetylene vesicles for toxin detection.[6]

Efficient fluorescence[7] and chromic behavior[8] make poly(*para*phenyleneethynylene)s (PPE) attractive as candidates in sensory schemes[1] and water soluble PPE-derivatives are known.[9] However, PPEs substituted with biogenic moieties are largely uncharted waters,[5,10] and we were interested in a biotin-substituted PPE as a model compound to study interactions of suitably functionalized conjugated polymers with bacterium. In this study, a streptavidin coated polystyrene bead is a primitive model for a cell/bacterium and the biotin/streptavidin interaction mimics the recognition process between conjugated polymer and a "cell surface". To obtain a biotinylated PPE, the polymer **1**[11] was dissolved in dry THF and treated with the biotin-attached acid chloride **2**[12] at 0 °C (Scheme 1).† The acid chloride **2** was prepared according to literature procedures. After allowing the reaction mixture to reach ambient temperature stirring was continued for 4 h. The reaction mixture was precipitated into 250 mL of methanol under vigorous stirring. The polymer **3** was isolated by suction filtration, redissolved in 1 mL of THF and precipitated into water to remove all excess of biotin. The successful biotinylation was qualitatively evidenced by IR spectroscopy of the polymer **3**, while its approximate degree of biotinylation was determined by an agglutination assay utilizing free streptavidin.



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Scheme 1 Synthesis of a biotinylated PPE

† Electronic supplementary information (ESI) available: experimental, including details of preparation and spectroscopic characterization of all new compounds and biotinylation assay of **3** by streptavidin. See http://www.rsc.org/suppdata/cc/b3/b303700m/

Based upon this assay every 15th to 20th monomer unit in the PPE chain (P_n = 140, gel permeation chromatography) was biotinylated. As a consequence only 7–14 biotin units are attached to a single polymer chain. This low "loading" of the PPE made it impossible to evidence the presence of biotin by ^{1}H NMR spectroscopy. However, the agglutination studies showed convincingly the presence of biotinylated PPEs.

It was of interest to see if the biotinylated PPE **3** and its precursor **1** would behave differently when exposed to streptavidin-coated microspheres. In a first experiment polymer **1** was mixed with streptavidin-covered microspheres. Fig. 1 (right) shows that the polymer solution is unchanged and does not alter its emission color. If a solution of **3** was mixed with a suspension of streptavidin-coated microspheres (Fig. 1, left), the polymer precipitated out as a consequence of the tight binding of the polymer bound biotin to the immobilized streptavidin. The precipitate obtained by the reaction of **3** with streptavidin-coated beads was examined by fluorescence microscopy (Fig. 2a,b,d). The formation of dense "mats" of beads was observed. Surprisingly the beads appeared both blue and red fluorescent when viewed through a DAPI or Texas Red filter respectively (Fig. 2a,b). Preparations of polymer **1** exposed to streptavidin beads produced isolated islands of fluorescence upon co-evaporation under otherwise identical conditions. The isolated islands of PPE-fluorescence are only visible under a DAPI filter, while under a Texas Red filter the sample is non-fluorescent. PPE aggregated onto spheres therefore has a measurable fluorescence in the red, while the PPE itself in the solid state does not show this red-shifted feature.

To explain this behaviour we took emission spectra of **3** in solution, **1** with streptavidin in solution and the complex of **3** with streptavidin as a suspension. The change in fluorescence is significant (Fig. 3) and the aggregation causes a disappearance of the blue shoulder visible for (**1** + streptavidin) and for uncomplexed **3**.

To get a better idea of the microstructure of this composite, we performed scanning electron microscopy of the complex. In Fig. 4a. the egg crate structure of the composite is visible. The conjugated polymer covers the beads evenly giving testimony to

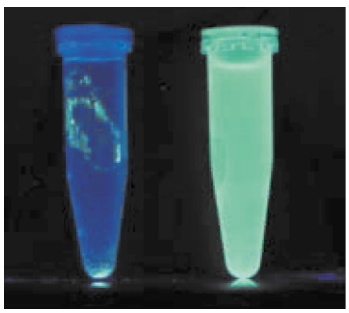

Fig. 1 Left: composite of polymer **3** and streptavidin-coated microspheres agglutinated at the bottom of the Eppendorf cap. The blueish fluorescence is innate to the Eppendorf cap. Right: control experiment in which polymer **2** and streptavidin coated microspheres are mixed. No agglutination is observed.

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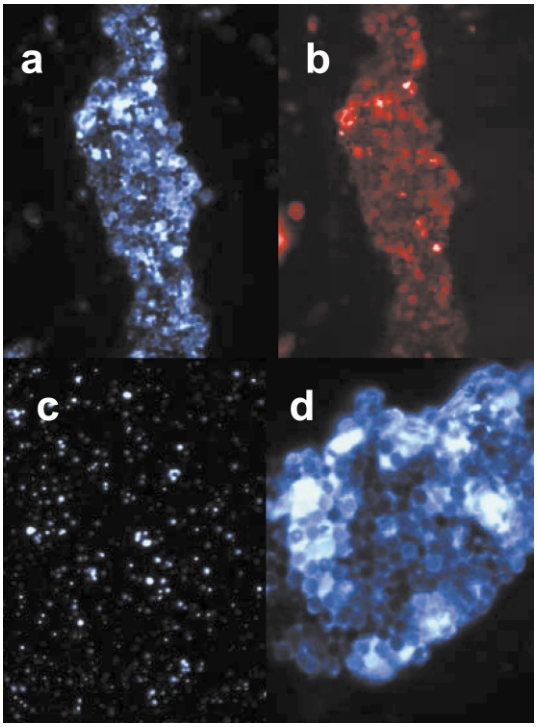

Fig. 2 Fluorescence micrographs of a) aggregates of polymer **3** with streptavidin-covered microspheres viewed under DAPI filter; b) same preparation but viewed under Texas Red filter; c) control experiment: non biotinylated polymer **2** co-preciptated with streptavidin-coated microspheres viewed under DAPI filter. Viewed under Texas Red filter the same preparation is non emissive. In a–c the base (width) of the picture is 250 μm; d) magnified picture of the cemented microspheres. The base in d) is 165 μm.

the binding between biotin and streptavidin. In Fig. 4b. the 3D arrangement of the polymer covered beads is apparent. The control experiment (**1** + streptavidin-coated beads) on the other hand (Fig. 4c) does not show *any* defined structure, only islands of polymer **1** are visible in the upper half, while three streptavidin-coated beads are isolated in the lower half of the picture.

In conclusion we have demonstrated that lightly biotin functionalized PPEs form nanocomposites with streptavidin-coated microspheres. This primitive system can be seen as a model for the interaction of cells (emulated by the beads) with functionalized conjugated polymers. This model could play an important role in the simple, colorimetric or fluorimetric detection of pathogens and toxins by PPE-types.

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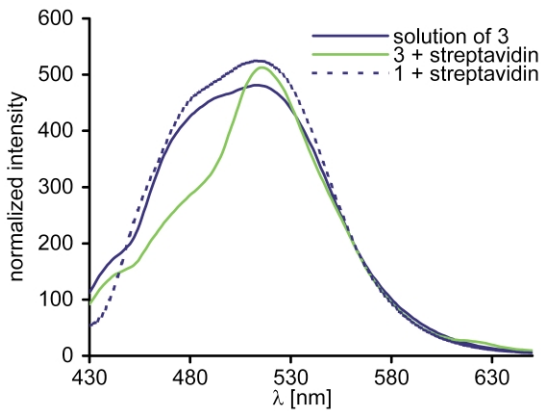

Fig. 3 Emission spectra of **3**, **1** with streptavidin and **3** with streptavidin (suspension).

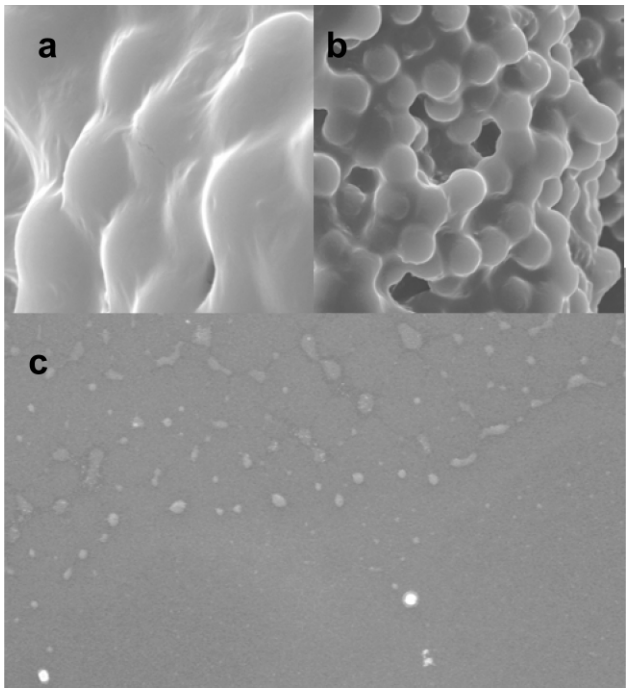

Fig. 4 Scanning electron micrographs of a) complex of **3** and streptavidin-coated microspheres (18 μm × 18 μm), b) same as in a) but with lower magnification (53 μm × 53 μm), c) control experiment in which non-biotinylated polymer **2** is co-precipitated with streptavidin-coated microspheres. There are no apparent interactions between polymer (islands on top half) and microspheres (bottom white spots, size 452 μm × 452 μm.). The size of the microspheres is in all cases 5 μm.

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Notes and references

1 M. Schlupp, T. Weil, A. J. Berresheim, U. M. Wiesler, J. Bargon and K. Müllen, *Angew. Chem.*, 2001, **40**, 4011; T. M. Swager, *Acc. Chem. Res*, 1998, **31**, 201; D. T. McQuade, A. E. Pullen and T. M. Swager, *Chem. Rev.*, 2000, **100**, 2537; Q. Zhou and T. M. Swager, *J. Am. Chem. Soc.*, 1995, **117**, 12593.
2 L. A. Samuelson, D. L. Kaplan, J. O. Lim, M. Kamath, K. A. Marx and S. K. Tripathy, *Thin Solid Films*, 1994, **242**, 50; K. Faid and M. Leclerc, *Chem. Commun.*, 1996, 2761; M. Hiller, C. Kranz, J. Huber, P. Bäuerle and W. Schuhmann, *Adv. Mater.*, 1996, **8**, 219.
3 S. Bernier, S. Garreau, M. Bera-Aberem, C. Gravel and M. Leclerc, *J. Am. Chem. Soc.*, 2002, **124**, 12463; H. A. Ho, M. Boissinot, M. G. Bergeron, G. Corbeil, K. Dore, D. Boudreau and M. Leclerc, *Angew. Chem.*, 2002, **41**, 1548.
4 B. S. Gaylord, A. J. Heeger and G. C. Bazan, *J. Am. Chem. Soc.*, 2003, **125**, 896; B. S. Gaylord, A. J. Heeger and G. C. Bazan, *Proc. Nat. Acad. Sci.*, 2002, **99**, 10954; D. L Wang, X. Gong, P. S. Heeger, F. Rininsland, G. C. Bazan and A. J. Heeger, *Proc. Nat. Acad. Sci.*, 2002, **99**, 49.
5 S. A. Kushon, K. D. Ley, K. Bradford, R. M. Jones, D. McBranch and D. Whitten, *Langmuir*, 2002, **18**, 7245.
6 S. Okada, S. Peng, W. Spevak and D. Charych, *Acc. Chem. Res.*, 1998, **31**, 229; J. J. Pan and D. Charych, *Langmuir*, 1997, **13**, 1365.
7 U. H. F. Bunz, *Chem. Rev.*, 2000, **100**, 1605.
8 C. E. Halkyard, M. E. Rampey, L. Kloppenburg, S. L. Studer-Martinez and U. H. F. Bunz, *Macromolecules*, 1998, **31**, 8655; T. Miteva, L. Palmer, L. Kloppenburg, D. Neher and U. H. F. Bunz, *Macromolecules*, 2000, **33**, 652.
9 N. DiCesare, M. R. Pinto, K. S. Schanze and J. R. Lakowicz, *Langmuir*, 2002, **18**, 7785; C. Y. Tan, M. R. Pinto and K. S. Schanze, *Chem. Commun.*, 2002, 446; M. R. Pinto and K. S. Schanze, *Synthesis*, 2002, 1293.
10 B. Erdogan, J. N. Wilson and U. H. F. Bunz, *Macromolecules*, 2002, **35**, 7863; F. Babudri, D. Colangiuli, P. A. Di Lorenzo, G. M. Farinola, O. H. Omar and F. Naso, *Chem. Commun.*, 2003, 130.
11 Y. Wang, B. Erdogan, J. N. Wilson and U. H. F. Bunz, see attached ms.
12 S. Crapatureanu, R. Serbanescu, S. B. Brevitt and R. Kluger, *Bioconjugate Chem.*, 1999, **10**, 105.