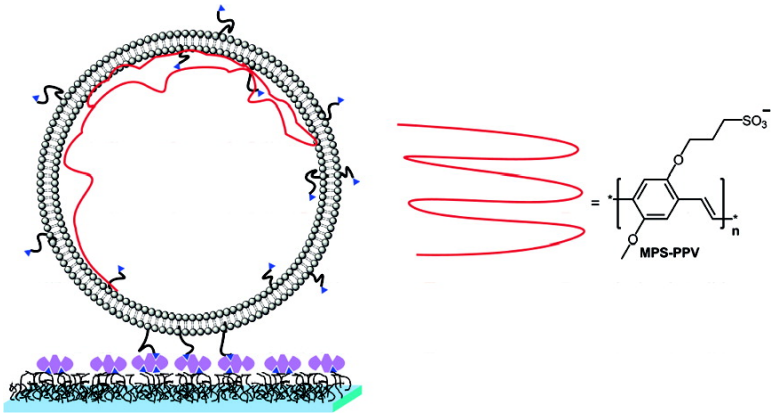
Communication

Liposome Encapsulation of Conjugated Polyelectrolytes: Toward a Liposome Beacon

An Thien Ngo, Pierre Karam, Elisa Fuller, Melanie Burger, and Gonzalo Cosa

J. Am. Chem. Soc., **2008**, 130 (2), 457-459 • DOI: 10.1021/ja076217b



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

J|A|C|S
COMMUNICATIONS

Published on Web 12/21/2007

Liposome Encapsulation of Conjugated Polyelectrolytes: Toward a Liposome Beacon

An Thien Ngo, Pierre Karam, Elisa Fuller, Melanie Burger, and Gonzalo Cosa*

Department of Chemistry, McGill University, 801 Sherbrooke Street West, Montreal, QC, H3A 2K6, Canada

Received August 17, 2007; E-mail: gonzalo.cosa@mcgill.ca

The use of conjugated polymers (CPs) in fluorescent sensing has received increasing attention since the original report by Swager et al. on CPs amplified fluorescence quenching.[1,2] In the past decade, the availability of conjugated polyelectrolytes (CPEs) has further allowed incorporating the amplified fluorescence quenching scheme into fluorescent sensors for small biomolecules,[3] DNA,[4] and proteins.[5]

We describe in this Communication the encapsulation of CPEs within 100 nm diameter liposomes, their subsequent specific immobilization onto polyethylene glycol (PEG)-coated glass coverslips and their observation via single molecule spectroscopy (SMS).[6] Our design exploits the efficient exciton migration[1,7] along CPE and a large CPE hydrodynamic radius. We believe that this novel architecture, a surface tethered liposome beacon, should prove instrumental in developing high-throughput liposome array[8] platforms designed to, for example, monitor membrane binding and permeation by pharmaceutical drugs capable of quenching the CPE emission.

Our results were obtained with a negatively charged CPE poly-[5-methoxy-2-(3-sulfopropoxy)-1,4-phenylenevinylene] (MPS−PPV) (Figure 1), whose hydrodynamic radius ranges between 10 and 40 nm as determined via dynamic light scattering (DLS). The lipid fraction consisted of dioleoylphosphatidyl-choline (DOPC) or up to 1/300 lipophilic dye/DOPC mole ratio mixtures, where the dye was incorporated for the purpose of liposome visualization. We chose a zwitterionic lipid to minimize electrostatic interactions with the negatively charged MPS−PPV; the low phase transition temperature of DOPC further ensures that under our experimental conditions the lipids are in the fluid phase. We selected the dye 1,1′-dioctadecyl-3,3,3′,3′-tetra-methylindodicarbocyanine perchlorate (DiD, Figure 1) given its high lipophilicity and its low emission yield unless membrane embedded.[9]

To encapsulate MPS−PPV, dry lipid films were prepared and later hydrated to a final lipid concentration of 20 mM, well above the DOPC critical micelle concentration (cmc), with buffer solutions containing MPS−PPV ca. 1.6 mM in monomer units. Following lipid film hydration, the samples were subjected to freeze−thaw sonication cycles and, unless otherwise specified, extruded through a 100 nm pore diameter polycarbonate membrane.[10] This yielded particles of ca. 120 nm diameter, measured by DLS.

We next performed fluorescence quenching studies to evaluate the extent of MPS−PPV encapsulation, as well as to assess potential electrostatic/hydrophobic interactions between DOPC molecules and MPS−PPV. As a quencher we chose methyl viologen (MV^{2+}),[3] a charged molecule unable to cross the lipid bilayer.[12] In addition to the encapsulated MPS−PPV, two control samples were prepared: free MPS−PPV in buffer and MPS−PPV solution to which preformed DOPC liposomes had been added. The Stern−Volmer

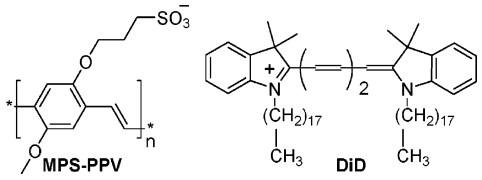


Figure 1. Structure of MPS−PPV and of DiD.

plot of encapsulated MPS−PPV (red circles) shows a plateau in the $[MV^{2+}]$-dependence of the quenched (I) versus unquenched (I_o) fluorescence intensity. The plateau is indicative of an unquenchable population. We estimated the quencher-inaccessible (encapsulated) fraction (ϕ) of MPS−PPV from the I/I_o value where the Stern−Volmer plot exhibits a plateau, that is, $\phi = I/I_o$.[13] For encapsulated MPS−PPV the inaccessible fraction is ca. 9% at $[MV^{2+}] \geq 20$ mM (Figure 2, red trace). The 9% value is in close agreement with expectations.[10,14]

No plateau is seen in the quenching of MPS−PPV to which preformed liposomes had been added (green open diamonds). The latter sample exhibits a linear trace with a 100-fold decrease in quenching sensitivity compared to free MPS−PPV (black filled squares), as expressed by the Stern−Volmer quenching constant (K_{sv}) obtained from the slope of the I_o/I vs $[MV^{2+}]$ plot. A K_{sv} of ca. 1.0×10^3 M^{-1} is obtained for MPS−PPV + preformed DOPC liposomes.[15] For MPS−PPV in buffer solution with no lipids, the K_{sv} is ca. 1.0×10^5 M^{-1}. The results from the quenching experiments point to nonspecific interactions between DOPC and MPS−PPV, a conclusion further supported by the blue-shift and

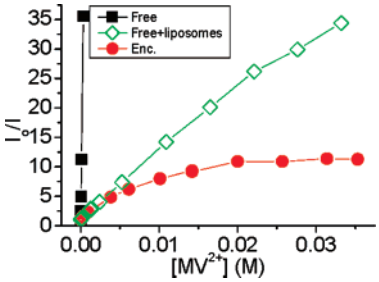


Figure 2. Fluorescence quenching upon adding increasing $[MV^{2+}]$ to (■) an MPS−PPV solution 1.6×10^{-5} M in monomer units; (◇) an MPS−PPV solution 1.6×10^{-5} M in monomer units to which a suspension of preformed DOPC liposomes (final $[DOPC] = 2 \times 10^{-4}$ M) is initially added; (●) a liposome encapsulated MPS−PPV solution (DOPC lipid films hydrated with MPS−PPV solutions to yield a final 20 mM DOPC and 1.6 mM MPS−PPV in monomer units solution which is further diluted 100-fold). All experiments are done in 150 mM NaCl and 10 mM HEPES buffer solutions. The lines connecting the experimental points are a visual aid.

10.1021/ja076217b CCC: $40.75

14-fold fluorescence increase that occurs upon adding DOPC liposomes to MPS−PPV solution (Supporting Information). The 100-fold decrease in the K_{sv} of MPS−PPV + preformed DOPC liposomes, compared to that of free MPS−PPV, suggests that DOPC may be hindering the association of MV^{2+} and extending the polymer backbone.[13] Similar results have been reported as evidence for interaction between MPS−PPV and cationic surfactants such as dodecyltrimethylammonium bromide (DTA) or 1,2-dioleoyl-3-trimethylammonium propane (DOTAP), where DOTAP associates with MPS−PPV monomers in a 0.6/1 mole ratio.[11,16] Arguably, DOPC/MPS−PPV interactions may be governed by hydrophobic and electrostatic effects.

We conclude at this point that (a) MPS−PPV interacts with the lipid molecules and that (b) ca. 9% of the total MPS−PPV is additionally encapsulated within liposomes. We may further consider that not all liposomes encapsulate MPS−PPV. Encapsulation being a probabilistic process, we expect that the fraction of liposomes encapsulating MPS−PPV should be related to the [MPS−PPV] in the hydrating solution.[10,17]

We next monitored individual surface-tethered liposomes via SMS to establish the feasibility of immobilizing liposomes encapsulating MPS−PPV and to further assess their sensing potential.[8,17,18] Liposomes were prepared as described above, starting from a 1/100 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine-*N*-(biotinyl) (biotin-DPPE)/DOPC mole ratio lipid film hydrated with MPS−PPV solution. Liposomes were immobilized on PEG-coated glass coverslips via biotin−streptavidin interactions.[18,19] To visualize all liposomes, that is, liposomes with no MPS−PPV and liposomes encapsulating MPS−PPV, we incorporated DiD in the lipid fraction at a mole ratio of 1/300 DiD/DOPC. A stage-scanning inverted fluorescence microscope was used for imaging purposes. We employed the 457.9 nm output (450 nW) from an Ar^+ laser to selectively excite MPS−PPV or the 633 nm output (320 nW) from a HeNe laser to selectively excite the membrane embedded DiD. Emission was separated into green and red channels by a 640 nm dichroic beam splitter and was directed to two avalanche photodiode (APD) detectors.

The total number of surface-tethered liposomes (N_{633}) was obtained by exciting at 633 nm and monitoring emission in the red channel (Figure 3C). The number of liposomes that encapsulate MPS−PPV (N_{457}) was obtained by exciting at 457.9 nm and monitoring emission in either channel (Figure 3A,B). Both excitation wavelengths yield about the same number of liposomes; (N_{633} = 104 and N_{457} = 99; total from two images), thus most liposomes contain at least one MPS−PPV molecule (Figure 3C).

The occurrence of Förster Resonance Energy Transfer (FRET) was evaluated by comparing the green channel intensity $\langle I_{green} \rangle$ and the average red/green intensity $\langle I_{red}/\ I_{green} \rangle$ for MPS−PPV encapsulated in surface-tethered liposomes that were prepared with and without DiD. Samples containing DiD (3/1/300 biotin-DPPE/DiD/DOPC mole ratio) exhibited comparable intensity in both channels (Figure 3A and 3B) upon 457.9 nm excitation. $\langle I_{green} \rangle$ was equal to 107 ± 72, while $\langle I_{red} \rangle$ was equal to 142 ± 106, yielding an average red/green intensity value ($\langle I_{red}/\ I_{green} \rangle$) of 1.41 ± 0.54.

Liposomes prepared without DiD exhibited higher green-channel intensity, with $\langle I_{green} \rangle$ = 196 ± 103. We further observed a lower $\langle I_{red}/\ I_{green} \rangle$ = 0.69 ± 0.18, where all red-channel intensity was consistent with MPS−PPV emission cross-talk. (N_{457} = 527, total from four images). A control sample, containing 1:300 DiD/DOPC without MPS−PPV exhibited no emission upon 457.9 nm excitation (data not shown). Therefore, the lower $\langle I_{green} \rangle$ and the higher $\langle I_{red}/\ I_{green} \rangle$ in liposomes with DiD can be attributed to FRET. Thus the single-molecule fluorescence experiments reveal

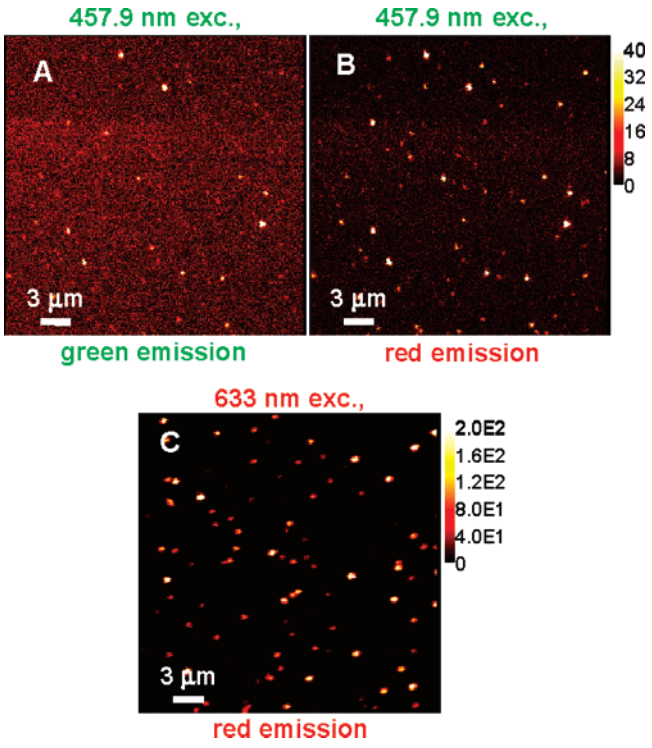


Figure 3. Fluorescence intensity images obtained for the green channel (**A**) and red channel (**B**) upon 457.9 nm excitation of MPS−PPV or for the red channel upon 633 nm excitation of DiD (**C**). The right bar illustrates the counts per millisecond per pixel. Liposomes were prepared following hydration of lipid films with a solution 1.60 mM MPS−PPV in monomer units, 150 mM in NaCl, and 10 mM in HEPES buffer pH 7.4.

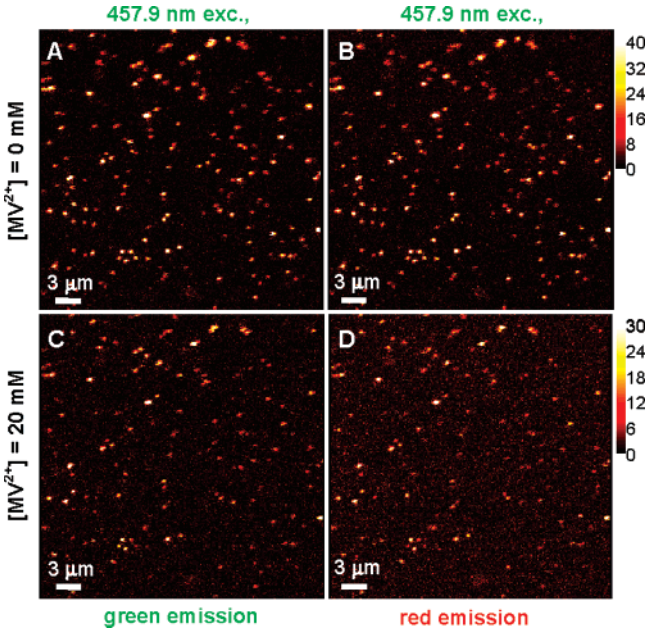


Figure 4. Fluorescence intensity images obtained for the green (**A** and **C**) and red (**B** and **D**) channels upon 457.9 nm excitation of surface tethered liposomes with MPS−PPV and no DiD before (**A** and **B**) and after (**C** and **D**) flowing 20 mM MV^{2+}. The right bar illustrates the counts per millisecond per pixel. Liposomes were prepared as described above.

FRET between lipid-encapsulated MPS−PPV and lipid-embedded DiD, evident from the twofold drop in MPS−PPV emission intensity and the enhancement in red-channel emission when DiD is incorporated into the bilayer. Similar MPS−PPV intensity ratios are observed in ensemble experiments (Supporting Information), underscoring the sensing potential of the liposome beacon architecture.

We furthermore explored whether MPS−PPV would adsorb onto the liposome walls given the lipid-MPS−PPV interactions observed in ensemble experiments. Consistent with MPS−PPV adsorption, we see the co-localization of MPS−PPV and surface tethered liposomes when free MPS−PPV (3.2 mM MPS−PPV in monomer units) is combined in a 1:1 v:v ratio with preformed liposomes (3/1/300 biotin-DPPE/DiD/DOPC mole ratio, 40 mM DOPC), diluted, and flowed over the substrate. Single particle fluorescence quenching studies performed upon flowing 20 mM MV^{2+} completely suppress the MPS−PPV emission (Supporting Information). Prolonged buffer flowing does not result in intensity decrease; therefore the emission suppression involves the interaction of MPS−PPV and MV^{2+} rather than the removal of MPS−PPV.

For encapsulated MPS−PPV, we observe at most 30% quenching emission by comparing images before and after flowing MV^{2+} 20 mM. Without MV^{2+} $\langle I_{green} \rangle = 156 \pm 114$; with MV^{2+} $\langle I_{green} \rangle = 104 \pm 75$ (compare Figure 4 parts A and B with C and D, respectively). This emission quenching arguably arises from the interaction of MV^{2+} with MPS−PPV adsorbed on the external liposome walls. The MV^{2+} quenching experiments further underscore the sensing capability of the liposome beacon reported herein. The vectorial incorporation of MPS−PPV leads to external bilayer sensitivity, whereas the encapsulation leads to an architecture where the sensing occurs at the liposome core.

In conclusion, liposomes containing MPS−PPV have been prepared. The novel architecture described herein provides a new tool for addressing liposome arrays;[8] it will further facilitate SMS studies on the spectroscopic properties of CPEs, work currently in progress in our laboratory.

Acknowledgment. We are grateful to McGill University, NSERC, CFI New Opportunities Fund, and FQRNT Nouveaux Chercheur programs for financial assistance. A.T.N. and P.K. thank the McGill Chemical Biology Fellowship Program (CIHR) and A.T.N. also thanks FQRNT for postgraduate scholarships. M.B. thanks the U. of Calgary Teaching and Learning Funding Committee, and a Grant-in-Aid of Research from Sigma Xi. We want to thank Dr. H.-W. Liu (U. T. Austin) for assistance with the experimental setup; Prof. P. F. Barbara (U. T. Austin) and Prof. D. S. English (U. Maryland) for sharing their software; and Prof. K. S. Schanze (U. Florida) for helpful discussions.

Supporting Information Available: Absorption and fluorescence spectra, DLS, SMS, and general procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Thomas, S. W., III; Joly, G. D.; Swager, T. M. *Chem. Rev.* **2007**, *107*, 1339−1386.
(2) Zhou, Q.; Swager, T. M. *J. Am. Chem. Soc.* **1995**, *117*, 7017−7018.
(3) Chen, L.; McBranch, D. W.; Wang, H.-L.; Helgeson, R.; Wudl, F.; Whitten, D. G. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 12287−12292.
(4) (a) Ho, H.-A.; Boissinot, M.; Bergeron, M. G.; Corbeil, G.; Dore, K.; Boudreau, D.; Leclerc, M. *Angew. Chem., Int. Ed. Engl.* **2002**, *41*, 1548−1551. (b) Gaylord, B. S.; Heeger, A. J.; Bazan, G. C. *J. Am. Chem. Soc.* **2003**, *125*, 896−900.
(5) (a) Rininsland, F.; Xia, W.; Wittenburg, S.; Shi, X.; Stankewicz, C.; Achyuthan, K.; McBranch, D.; Whitten, D. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 15295−300. (b) Wang, D.; Gong, X.; Heeger, P. S.; Rininsland, F.; Bazan, G. C.; Heeger, A. J. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 49−53. (c) Dwight, S. J.; Gaylord, B. S.; Hong, J. W.; Bazan, G. C. *J. Am. Chem. Soc.* **2004**, *126*, 16850−16859.
(6) (a) Moerner, W. E.; Orrit, M. *Science* **1999**, *283*, 1670−1676. (b) Nie, S.; Zare, R. *Annu. Rev. Biophys. Biomol. Struct.* **1997**, *26*, 567−596. (c) Xie, X. S.; Trautman, J. K. *Annu. Rev. Phys. Chem.* **1998**, *49*, 441−480.
(7) (a) Tan, C.; Atas, E.; Muller, J. G.; Pinto, M. R.; Kleiman, V. D.; Schanze, K. S. *J. Am. Chem. Soc.* **2004**, *126*, 13685−13694. (b) Vanden Bout, D. A.; Yip, W.-T.; Hu, D.; Fu, D.-K.; Swager, T. M.; Barbara, P. F. *Science* **1997**, *277*, 1074−1077.
(8) Christensen, S. M.; Stamou, D. *Soft Matter* **2007**, *3*, 828−836.
(9) Buschmann, V.; Weston, K. D.; Sauer, M. *Bioconjugate Chem.* **2003**, *14*, 195−204.
(10) Torchilin, V. P.; Weissig, V. *Liposomes*, 2nd ed.; Oxford University Press Inc.: New York, 2003; Vol. 264.
(11) Dalvi-Malhotra, J.; Chen, L. *J. Phys. Chem. B* **2005**, *109*, 3873−3878.
(12) Lee, L. Y. C.; Hurst, J. K.; Politi, M.; Kurihara, K.; Fendler, J. H. *J. Am. Chem. Soc.* **1983**, *105*, 370−373.
(13) Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*, 3rd ed.; Springer: New York, 2006.
(14) On the basis of the surface area of DOPC (0.82 nm^2) and the liposome area (31.5×10^3 nm^2 per leaflet for a 100 nm diameter liposome) we estimate that each 100 nm diameter DOPC liposome has ca. 76600 lipid molecules. It follows that the liposome concentration is 260 nM when the liposomes are prepared starting from a 20 mM lipid suspension in water, that is, 20×10^{-3} M/76600 $\approx 260 \times 10^{-9}$ M. Considering that a single 100 nm diameter liposome encapsulate a volume of ca. 5×10^{-19} L, a 260 nM liposome solution will encapsulate a fraction of the total volume ≈ 0.08 as determined by the product of the volume per liposome times the total number of liposome particles in solution.
(15) Note that both the green and red traces have the same initial slope, that is, the samples in contact with DOPC have the same initial Stern−Volmer fluorescence quenching constant.
(16) Chen, L.; Xu, S.; McBranch, D.; Whitten, D. *J. Am. Chem. Soc.* **2000**, *122*, 9302−9303.
(17) (a) Boukobza, E.; Sonnenfeld, A.; Haran, G. *J. Phys. Chem. B* **2001**, *105*, 12165−12170. (b) Stamou, D.; Duschl, C.; Delamarche, E.; Vogel, H. *Angew. Chem. Int. Ed. Engl.* **2003**, *42*, 5580−5583.
(18) Cisse, I.; Okumus, B.; Joo, C.; Ha, T. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 12646−12650.
(19) Briefly, immobilization involved (a) the functionalization of glass coverslips with Vectabond reagent, (b) the covalent tethering of a 100/1 PEG (MW 5000)/biotin-PEG (MW 5000) mixture to the coverslip surface, (c) the assembly of a flow chamber, (d) a 10 min incubation of a streptavidin solution and (e) a 3−4 min incubation of the 5 pM liposome solution under study followed by a steady flow of buffer solution to remove all non-immobilized liposomes and non-encapsulated MPS−PPV.

JA076217B