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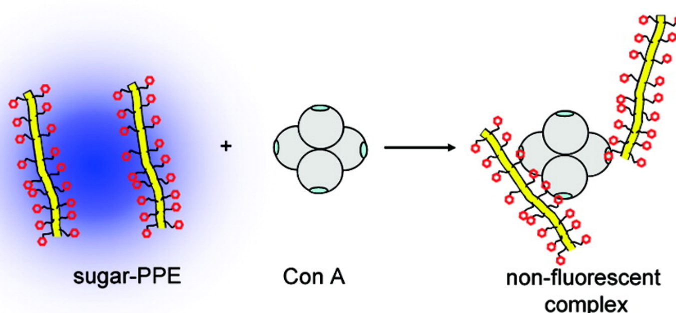

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Fluorescence Self-Quenching of a Mannosylated Poly(*p*-phenyleneethynylene) Induced by Concanavalin A

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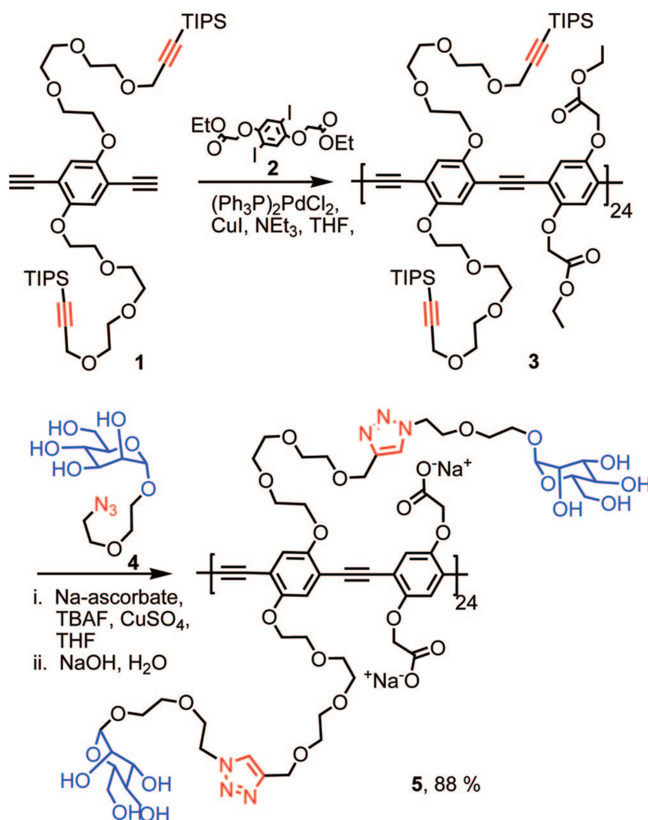
Conjugated polymers (CP) have attained considerable status in the detection and quantitation of biologically active molecules.[1] CPs have either been used in the solid state as sensory coatings for electrodes, or, transparently in solution as species that change their absorption spectra and more sensitively, their emission spectra upon exposure to a specific analyte. They are often superior to nonconjugated, nonfluorescent polymeric scaffolds,[2] as they elegantly combine recognition and transmission elements. We now probe a protein/CP interaction using fluorescence quenching of the mannose-substituted poly(*p*-phenyleneethynylene) (PPE) **5** by Concanavalin A (Con A), the tetrameric lectin of the jack bean,[3] and investigate its unusual mechanism.

The optical/fluorescence-based detection of biologically important species in solution profits from the endless synthetic variability of CPs. Different tailored backbones are fitted to the intended purpose by the attachment of suitable recognition elements. The sensing or probing of biomolecular targets by CPs rests on either (a) fluorescence resonance energy transfer, (b) ratiometric response or (c) quenching of the fluorescence after binding to a specific analyte. All three of these mechanisms are of significant interest and have demonstrated use in the probing of biomolecules. The properties that make CPs so much more powerful than small dyes are (a) their facility for acting as molecular antennae, in which one exciton can "patrol" up to one hundred repeat units, (b) their ability for supporting multivalent interactions,[4] and, (c) most complex and least well understood, their aggregation phenomena, which can lead to a phenomenally large signal amplification that allows detection of zeptomolar concentrations of DNA by CPs.[5]

The quenching of the fluorescence of PPEs by paraquat derivatives was first described by Swager and Zhou in a classic paper.[6] The PPE-analyte interactions could be correctly described by the Stern−Volmer formalism assuming static quenching, induced by excited-state electron transfer from the PPE to paraquat. The molecular antenna effect led to signal amplification by a factor of up to 100 when compared to similar monomeric fluorophores. Wudl and Whitten[7] later demonstrated superquenching when examining the interaction of paraquat with a sulfonated PPV and explained the observed thousand-fold gain in sensitivity by a combination of the antenna effect compounded by paraquat-induced aggregate formation. While quenching with paraquat involves excited-state electron transfer, Förster energy transfer can also be employed to quench the fluorescence of a water soluble conjugated polymer by the addition of the deeply colored cytochrome C as demonstrated by Heeger et al.[8] A combination of mechanisms is believed to be responsible for the superquenching ability of gold nanoparticles on the fluorescence of conjugated polymers.[9] However, the quenching of CP fluorescence can also occur if the analyte does not carry any obvious chromogenic or electron transfer center. This is the case in the interaction of PPEs with several proteins such as lysozyme, etc.,[10] or more importantly, Con A (tetrameric at pH 7.2 in phosphate buffer (PB)) as a model protein for biotoxins such as Ricin or *E. coli* toxin.[11,12]

Scheme 1. Synthesis of the PPE **5** via Pd-Catalyzed Coupling of **1** to **2** Followed by Dipolar Cycloaddition



The classic tool for investigating quenching is the Stern−Volmer equation:

$$F_o/F_{[Q]} = 1 + K_{SV}[Q] \tag{1}$$

in which $F_o/F_{[Q]}$ is the ratio of the initial fluorescence intensity F_o and the fluorescence intensity $F_{[Q]}$ in the presence of the quencher Q at a concentration [Q]. K_{SV} is the Stern−Volmer constant and may refer to static quenching if the complex between fluorophore and quencher is preformed and does not undergo diffusion or to dynamic quenching if diffusion occurs during the excited-state lifetime. In such a case, K_{SV} represents the equilibrium constant. In static quenching it is assumed that the quencher and the fluorophore form a nonfluorescent ground-state complex and the slope of $F_o/F_{[Q]}$ for different concentrations of Q provides the binding constant of fluorophore to quencher. In cases of static quenching the fluorescence lifetime of the fluorophore is independent of the concentration of added quencher Q. This is generally

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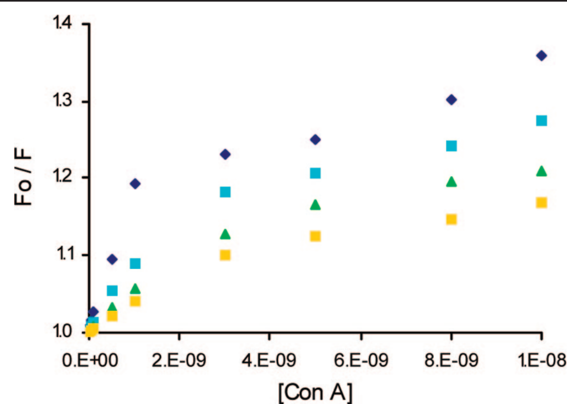

Figure 1. Stern−Volmer graph for different PPE concentrations: dark blue, [PPE] = 5×10^{-7} M; turquoise, [PPE] = 2.5×10^{-7} M; green, [PPE] = 1.25×10^{-7} M; gold, [PPE] = 6.25×10^{-8} M.

the case for PPEs, which have a lifetime of 0.3−0.4 ns.[13] The other necessary prerequisite for a straightforward application of the Stern−Volmer formalism is that a one-to-one complex forms between quencher and fluorophore. It is often argued that at low quencher/ fluorophore concentrations this assumption is valid. One should also note that the concentration of the fluorophore does not show up in this analytical expression of the Stern−Volmer equation.[14] However, for numerous examples of fluorescence quenching of CPs by small molecule quenchers published in the literature the polymer concentration *does* matter.[15] The observed Stern−Volmer "constants" are inversely dependent upon the concentration of the CP. As a consequence K_{SV} decreases with increasing concentration of CPs.

Con A displays sensitive quenching of the fluorescence of mannosylated PPEs.[11] As Con A does not contain an easily identifiable electron or charge transfer center we decided to investigate the mechanism of this quenching. We chose the PPE **5** as a suitable object for our investigation, as it (a) carries a negative charge and (b) has the mannose residue attached to the PPE chain by a 20-atom tether, suggesting easy interaction of **5** with Con A. The synthesis of **5** is displayed in Scheme **1**; in the last step the pre-PPE **3** is desilylated and a copper catalyzed 1,3-dipolar cycloaddition to **4** is performed to give **5** in high yield.[16]

Figure 1 displays the dependence of the fluorescence quenching of **5** on the concentration of Con A, the classic Stern−Volmer formalism, for different concentrations of the polymer **5**. It is immediately noticeable that, at low concentrations of Con A, the *S−V* plot is linear. At higher concentrations of the quencher Q, the plot is also linear but with a different slope. Surprisingly, the extracted initial K_{SV} is proportional to the PPE concentration as well, which is contrary to the existence of a 1:1 complex, but rather commensurate with a 1:2 complex. If the concentration of PPE **5** is below 3×10^{-8} M, no quenching whatsoever is observed.

The lifetime of the PPE excited-state was measured as 450 ps and did not depend on [Con A], in agreement with a purely static quenching mechanism. The presence of a "two-phase" *S−V* plot, as opposed to a second order plot, suggests the intervention of two such quenching mechanisms. The general treatment of such kinetics is provided by eq 3, where f_1 and f_2 represent the relative fractions of quenching mechanisms ($f_1 + f_2 = 1.0$) and K_{SV1} and K_{SV2} are the respective Stern−Volmer constants.[17] Using the data in Figure 1 and eq 2, we determine by least-squares analysis the equilibrium constants shown in Table S2 and Figure 2. Figure 3 illustrates that K_{SV1} is linearly dependent on [PPE] and K_{SV2} is partially dependent on [PPE]. We also notice that the two rate constants differ by a factor of 10^4, but that, within experimental error, the contribution

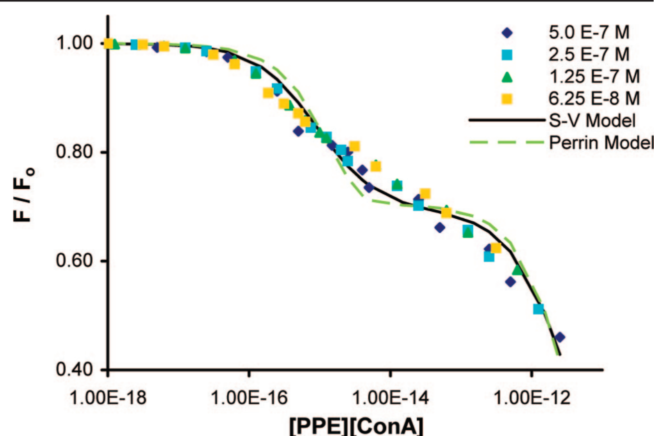

Figure 2. Master plot of all quenching data, including fit to eq 3 and eq 4.

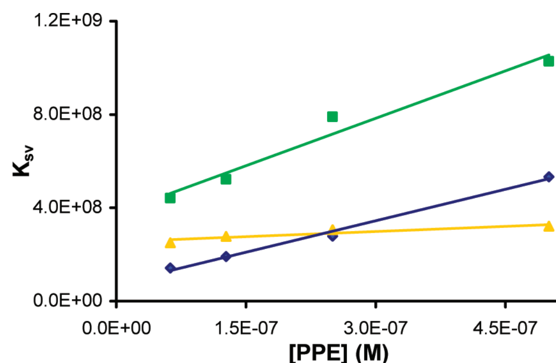

Figure 3. Plot of K_{sv1} and K_{sv2} and of f_1 [PPE]: dark blue = K_{sv1}; gold = $f_1 \times 10^9$; green = $K_{sv2} \times 10^4$.

of each process to quenching remains nearly constant, that is, ca. 0.3 for K_{SV1} and 0.7 for K_{SV2}.

$$F_{[Q]}/F_o = f_1/(1 + K_{SV1}[Q]) + f_2/(1 + K_{SV2}[Q]) \quad (2)$$

$$F_{[Q]}/F_o = f_1/(1 + K'_{SV1}[Q][PPE]) + f_2/(1 + K'_{SV2}[Q][PPE]) \quad (3)$$

The near linear dependence of the K_{SV} values suggests that the quenching involves a complex of two PPE molecules. Indeed, if we assume that $K_{SV}(obs) = K'_{SV}[PPE]$, we can fit all the data in Figure 1 to a master equation, eq 3, as shown in Figure 2. The rms error for this least-squares treatment is a factor of 2 over that for the individual fits. Using this treatment,[18] we obtain a $K'_{SV1} = 1.1 \times 10^{15}$ L^2 mol^{-2} (31%) and $K'_{SV2} = 2.5 \times 10^{11}$ L^2 mol^{-2} (69%).

An alternate fit when static quenching is involved is to use the concept of quenching volume, based upon the Perrin model, in which fluorophores within the quenching volume V_q quench with 100% efficiency and those without quench with 0% efficiency.[19] Again, with the two quenching complexes, this model reduces to[17]

$$F_{[Q]}/F_o = f_1/(\exp(V_{q1}[Q][PPE])) + f_2/(\exp(V_{q2}[Q][PPE])) \quad (4)$$

Use of a nonlinear least-squares fit to this equation provided the fit shown in Figure 2 by the dashed line. We note the total sum of squares for this fit is 4 times that for equation 3. However, since to a first approximation $\exp(x) = 1 + x$, we note that V_q and K_{SV} are almost indistinguishable. The fit to equation 4 yields $V_{q1} = 7.6 \times 10^{14}$ and $V_{q2} = 2.1 \times 10^{11}$, nearly identical to the K_{SV} values.

To get a better understanding for the association processes that occur, we performed isothermal titration calorimetry (ITC) by adding the polymer solution to a solution of Con A in 0.01 M phosphate buffer at a pH of 7.2. We obtained a binding ratio of *N*

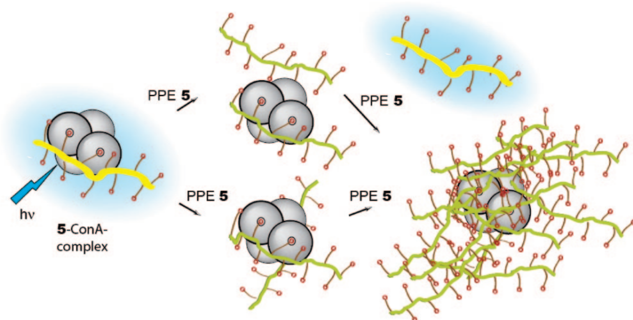

Figure 4. Stern−Volmer experiment: In the ITC experiment the PPE is added to tetrameric Con A at a pH of 7.2 in phosphate buffer, and the formation constant of the fluorescent, that is, spectroscopically nonobservable complex **5**−Con A is recorded. Upon addition of PPE nonfluorescent complexes ($\mathbf{5}$)$_2$−Con A form. Upon addition of more **5** the ($\mathbf{5}$)$_2$−Con A complex leads to a FRET-like transfer from the surrounding PPE molecules to the 2:1 complex, followed by efficient quenching mediated by the complex(es).

= 3.01 PPE/Con A; $\Delta G = -7.1$ kcal/mol·K, $\Delta H = -6.4$ kcal/mol·K, $\Delta S = 2.4$ cal/mol·K, and a resulting $K_a = 1.6 \times 10^5$ M^{-1}.

We can interpret this data (Figure 4) by assuming that in the first step we form a fluorescent 1:1 complex of Con A and **5** (Scheme 1), with a K_a from ITC data of 1.6×10^5 M^{-1}. In the presence of an excess of **5**, a second molecule of **5** binds to Con A to form two fluorescence-quenched complexes. We cannot tell from these experiments the exact mechanism of binding, although it may be that one binding mechanism involves two mannose units on one PPE and one on another, and the other two and two. Again, taking V_{q1}(obs) = V_{q1}[PPE], we can estimate the quenching volume of the strongest 1:1 complex as $(7.6 \times 10^{14})(5.0 \times 10^{-7}) = 3.8 \times 10^8$ L mol^{-1}, or 6.3×10^8 nm^3 molecule^{-1}, a quenching distance of 430 nm! This extraordinary quenching distance is the direct result, we believe, of the fact that two molecules of PPE are involved, which results in the exciton-coupled amplification even greater than that produced with single molecule quenching[5] and may indicate the severe assumptions from using the Perrin model. The large quenching volume may also result from transfer of excitation energy from other molecules of **5**, leading to significant signal amplification.

In conclusion, we have demonstrated that the quenching of the fluorescence of the PPE **5** is induced by the addition of Con A through the formation of nonfluorescent aggregates. This system is very unusual as it displays increased quenching with increasing fluorophore concentration, approaching the apparent binding constants of streptavidin/biotin. Such aggregation-induced enhancement, while not unprecedented, has been seen by Leclerc[20] in the detection of zeptomolar quantities of DNA. Here as well an energy transfer from an ensemble of conjugated polymer chains to a quencher or a FRET dye gives a sensitivity that is incommensurate with any binding constant.

The exquisite sensitivity, the ease of data collection and data evaluation make the quenching of polymeric fluorophores alluring and−despite the large discrepancies with the ITC derived association constants−uniquely useful and promising for further sensory and probe-type applications.

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Supporting Information Available: Synthetic details and NMR spectra for **5**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Thomas, S. W.; Joly, G. D.; Swager, T. M. *Chem. Rev.* **2007**, *107*, 1339–1386. (b) McQuade, D. T.; Pullen, A. E.; Swager, T. M. *Chem. Rev.* **2000**, *100*, 2537–2574.
(2) (a) Kiessling, L. L.; Pohl, N. L. *Chem. Biol.* **1996**, *3*, 71–77. (b) Kanai, M.; Mortell, K. H.; Kiessling, L. L. *J. Am. Chem. Soc.* **1997**, *119*, 9931–9932.
(3) Lis, H.; Sharon, N. *Chem. Rev.* **1998**, *98*, 637–674.
(4) (a) Kim, I.-B.; Erdogan, Belma; Wilson, J. N.; Bunz, U. H. F. *Chem. Eur. J.* **2004**, *10*, 6247–6254. (b) Mammen, M.; Choi, S. K.; Whitesides, G. M. *Angew. Chem.* **1998**, *37*, 2755–2794.
(5) Dore, K.; Leclerc, M.; Boudreau, D. *J. Fluoresc.* **2006**, *16*, 259–265.
(6) Zhou, Q.; Swager, T. M. *J. Am. Chem. Soc.* **1995**, *117*, 12593–12602.
(7) Chen, L.; McBranch, D. W.; Wang, H.-L.; Helgeson, R.; Wudl, F.; Whitten, D. G. *Proc. Nat. Acad. Sci. U.S.A.* **1999**, *96*, 12287–12292.
(8) Fan, C.; Plaxco, K. W.; Heeger, A. J. *J. Am. Chem. Soc.* **2002**, *124*, 5642–5643.
(9) (a) Fan, C.; Wang, S.; Hong, J. W.; Bazan, G. C.; Plaxco, K. W.; Heeger, A. J. *Proc. Nat. Acad. Sci. U.S.A.* **2003**, *100*, 6297–6301. (b) You, C. C.; Miranda, O. R.; Gider, B.; Ghosh, P. S.; Kim, I. B.; Erdogan, B.; Krovi, S. A.; Bunz, U. H. F.; Rotello, V. M. *Nat. Nanotechnol.* **2007**, *2*, 318–323.
(10) (a) Kim, I. B.; Dunkhorst, A.; Bunz, U. H. F. *Langmuir* **2005**, *21*, 7985–7989. (b) Miranda, O. R.; You, C.-C.; Phillips, R.; Kim, I.-B.; Ghosh, P. S.; Bunz, U. H. F.; Rotello, V. M. *J. Am. Chem. Soc.* **2007**, *129*, 9856–9857. (c) Dwight, S. J.; Gaylord, B. S.; Hong, J. W.; Bazan, G. C. *J. Am. Chem. Soc.* **2004**, *126*, 16850–16859.
(11) (a) Kim, I. B.; Wilson, J. N.; Bunz, U. H. F. *Chem. Commun.* **2005**, 1273–1275. (b) Disney, M. D.; Zheng, J.; Swager, T. M.; Seeberger, P. H. *J. Am. Chem. Soc.* **2004**, *126*, 13343–13346.
(12) (a) Xue, C.; Jog, S. P.; Murthy, P.; Liu, H. Y. *Biomacromolecules* **2006**, *7*, 2470–2474. (b) Kaur, P.; Wu, M. Y.; Anzaldi, L.; Waldeck, D. H.; Xue, C. H.; Liu, H. Y. *Langmuir* **2007**, *23*, 13203–13208. (c) Xue, C. H.; Donuru, V. R. R.; Liu, H. Y. *Macromolecules* **2006**, *39*, 5747–5752.
(13) (a) Bunz, U. H. F. *Chem. Rev.* **2000**, *100*, 1605–1644. (b) Bourdin, E.; Davey, A.; Blau, W.; Delysse, S.; Nunzi, J. M. *Chem. Phys. Lett.* **1997**, *275*, 103–105. (c) Bunz, U. H. F.; Imhof, J. M.; Bly, R. K.; Bangcuyo, C. G.; Rozanski, L.; vandenBout, D. A. *Macromolecules* **2005**, *38*, 5892–5896.
(14) Stern, O.; Volmer, M. *Phys. Z.* **1919**, *20*, 183–188.
(15) Wang, J.; Wang, D. L.; Miller, E. K.; Moses, D.; Bazan, G. C.; Heeger, A. J. *Macromolecules* **2000**, *33*, 5153–5158.
(16) (a) Englert, B. C.; Bakbak, S.; Bunz, U. H. F. *Macromolecules* **2005**, *38*, 5868–5877. (b) Huisgen, R.; Szeimies, G.; Möbius, L. *Chem. Ber.* **1967**, *100*, 2494–2499. (c) Lewis, W. G.; Green, L. G.; Grynszpan, F.; Radic, Z.; Carlier, P. R.; Taylor, P.; Finn, M. G.; Sharpless, B. K. *Angew. Chem.* **2002**, *41*, 1053–1057. (d) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, B. K. *Angew. Chem.* **2002**, *41*, 2596–2599. (e) Helms, B.; Mynar, J. L.; Hawker, C. J.; Frechet, J. M. J. *J. Am. Chem. Soc.* **2004**, *126*, 15020–15021.
(17) Eftink, M. R. *Top. Fluoresc. Spectrosc.* **1991**, *2*, 53–126.
(18) In this context, [PPE] refers to repeat unit concentration. The degree of polymerization (DP) is 24, which can be used to correct to polymer concentration.
(19) (a) Murphy, C. B.; Zhang, Y.; Truxler, T.; Ferry, V.; Martin, J. J.; Jones, W. E. *J. Phys. Chem. B.* **2004**, *108*, 1537–1543. (b) Andre, J. C.; Niclause, M.; Ware, W. R. *Chem. Phys.* **1978**, *28*, 371–377. (c) Lakatos-Lindenberg, K.; Hemenger, R. P.; Pearlstein, R. M. *J. Chem. Phys.* **1972**, *56*, 4852–4867.
(20) (a) Ho, H. A.; Dore, K.; Boissinot, M.; Bergeron, M. G.; Tanguay, R. M.; Boudreau, D.; Leclerc, M. *J. Am. Chem. Soc.* **2005**, *127*, 12673–12676. (b) Dore, K.; Dubus, S.; Ho, H. A.; Levesque, I.; Brunette, M.; Corbeil, G.; Boissinot, M.; Boivin, G.; Bergeron, M. G.; Boudreau, D.; Leclerc, M. *J. Am. Chem. Soc.* **2004**, *126*, 4240–4244.

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