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A Single-Molecule Probe Based on Intramolecular Electron Transfer

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Detecting structure, dynamics, chemical reactions, and physical processes at the *single-molecule level* represents the ultimate degree of sensitivity for sensing and imaging. Fluorescence-based single-molecule spectroscopy (SMS) has evolved as an important method for studying the behavior of single molecules under ambient conditions.[1−4] Molecules composed of a fluorescent chromophore (reporter), a binding site (receptor), and a mechanism of communication between the two have found use in chemosensory and biological applications.[5−10] While there have been limited SMS studies of commercially available dyes that respond to binding events,[11] there is a tremendous need to develop new molecular systems and methodology for single-molecule-based sensing. Molecular systems in which photoinduced *intramolecular electron transfer* (IET) from a high-energy nonbonding electron pair efficiently quenches the excited state of the chromophore form an important class of chemosensory materials.[10] Reactions of this electron pair, with protons, metal atoms, organic electrophiles, or surfaces, lower the energy of the electron pair below the highest occupied molecular orbital (HOMO) of the chromophore, turning *off* IET and turning *on* fluorescence (Figure 1). This class of molecules has yet to be investigated at the single-molecule level. Here we describe how these molecules can be used as extremely sensitive fluorescent *single-molecule probes* of local structure, reversible chemical reactions, and interfacial processes such as electron transfer.

Perylene 3,4,9,10-tetracarboxyl bisimides[12] are useful molecules for single-molecule fluorescence-based studies because of their high photochemical stability, high quantum yield of fluorescence (>99%), low quantum yield of intersystem crossing, and versatile reactivity. To make a perylene-based single-molecule sensor, we have synthesized *N*-(1-nonyldecyl)-*N*′-(*p*-aminophenyl) perylene 3,4,9,10-tetracarboxyl bisimide (NDAPP),[13] using standard methods.[12] The modifiable conjugated linker allows for efficient IET between the amine and the excited chromophore, resulting in nearly complete quenching of fluorescence in the unbound state. Similar free amine-based perylene molecules have previously been investigated for chemosensory applications.[14]

Figure 2 shows the absorption and emission spectra of NDAPP in dioxane for the free base and protonated forms, showing a dramatic increase in fluorescence upon protonation. Titration with solutions of $ZnCl_2$ in THF, $Pt(SEt_2)_2Cl_2$ in $CHCl_3$, TiO_2 nanoparticles in THF, and aldehydes in $CHCl_3$ all resulted in similar fluorescence increases. In all cases, at titration end point fluorescence was restored nearly to the level of normal, unquenched perylene bisimides. Fluorescence was also observed at the surfaces of glass, gold-coated glass, $ZnCl_2$ crystals in $CHCl_3$, and in polymer matrices of polyvinylbutyral (PVB).

The SMS of the NDAPP system can be used to locally probe nanoscale surface structure. Figure 3 shows scanning confocal fluorescence images of single isolated molecules dispersed on glass and quartz surfaces under argon atmosphere. Bright fluorescence is observed for NDAPP molecules on glass (Figure 3A). Metal and metal oxide impurities in the glass (TiO_2 7%, ZnO 10%, Al_2O_3 3%) are imaged by the NDAPP sensor, since binding of the free amine to these centers turns on the fluorescence. No significant fluorescence is observed from molecules dispersed on freshly cleaned quartz (99.999% SiO_2) since there are no such binding sites available (Figure 3B).

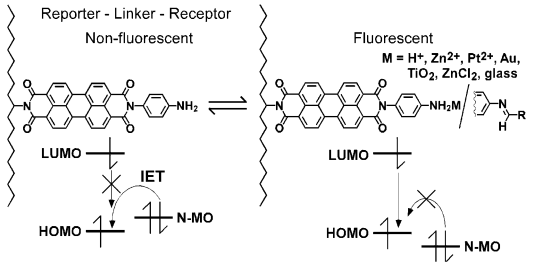

Figure 1. Structure and molecule orbital diagrams of single-molecule probe NDAPP in the unbound and bound states.

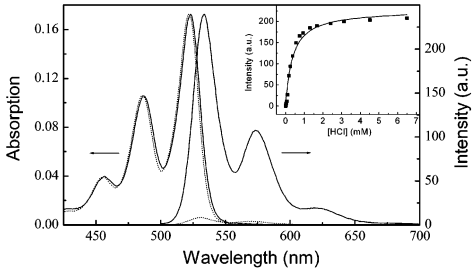

Figure 2. Absorption and fluorescence spectra of free base NDAPP in dioxane 2×10^{-6} M (dotted) and after addition of 6.5×10^{-3} M of HCl protonated (solid). (Inset) Fluorescence titration curve obtained by addition of HCl/dioxane solution, giving a pK_a of 3.45.

Single sensor molecules can also be used to probe reversible chemical reactions at the amine, such as protonation or imine formation. Exposure of the surface shown in Figure 3B to a dioxane/HCl vapor in a sealed jar for ∼30 min results in the appearance of fluorescent species due to the protonation of NDAPP molecules (Figure 3C). The protonation process is completely reversible—molecules remain protonated while a thin layer of solvent is present at the surface, but fluorescence is turned off when HCl is lost to the atmosphere once this layer eventually evaporates. Molecules of NDAPP dispersed in a thin film of PVB (∼30 nm, 3 mg/mL of PVB in $CHCl_3$, spin-coated at 2000 rpm) also fluoresce, since NDAPP reacts with the butyl aldehyde acetal on the polymer backbone to form the fluorescent imine. Fluorescence from NDAPP molecules on quartz is also observed after the slides remain in air for 4−5 days, even without exposure to HCl (Figure 3D). This fluorescence seems to arise from binding of the amine following molecular diffusion to protonated sites on the quartz surface,

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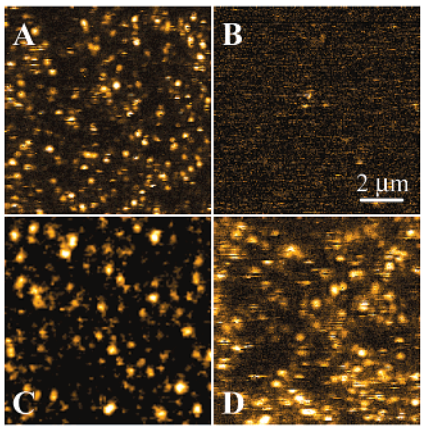

Figure 3. Single-molecule fluorescence scanning confocal images of NDAPP spin-coated (4000 rpm) onto glass (Corning) and quartz (Quartz Scientific) cover slips. All substrates were cleaned and determined to be free of fluorescent impurities before spin coating. Imaging was performed under an argon atmosphere, $\lambda_{exc} = 488$ nm, power ~50 W/cm^2, and 20 ms bin time. (A) 1×10^{-9} M NDAPP in $CHCl_3$ spin-coated on glass (0 to 316 counts/20 ms). (B) 2×10^{-8} M spin-coated on quartz (0 to 104 counts/20 ms). (C) Same sample as B, after exposure to dioxane/HCl vapor (0 to 203 counts/20 ms). (D) Sample prepared as B, but left in air for 4−5 days (0 to 114 counts/20 ms).

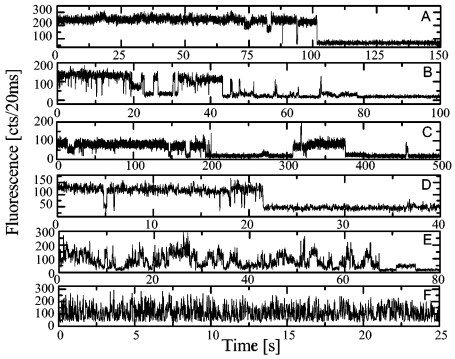

Figure 4. Characteristic single-molecule fluorescence time trajectories for the NDAPP system (A) in a PVB 30-nm thin film, (B) and (C) on glass, (D) on quartz following exposure to dioxane/HCl vapor, (E) and (F) on quartz after several days (no initial fluorescence observed).

generated during the acid cleaning of the slides: no fluorescence is observed, even after up to three weeks, if the slide is immersed in 1 M NaOH and rinsed before the NDAPP is spin-coated on the surface.

Fluorescence time trajectories of single molecules can reveal detailed information regarding binding and local environment of each chromophore. Single-molecule fluorescence traces of NDAPP under constant excitation are shown in Figure 4. Fluorescence traces of NDAPP in PVB (Figure 4A) display constant fluorescence levels for up to hundreds of seconds, with infrequent millisecond losses in fluorescence, before eventual irreversible photobleaching. This behavior is similar to what has been observed for other fluorescent perylene derivatives by SMS,[15] where short off-times have been attributed to triplet-state formation. Typical traces observed for molecules bound to glass substrates (Figure 4B and C) show longer off-periods, in some cases lasting longer than 100 seconds, as well as some brief millisecond off-times. Triplet lifetimes longer than milliseconds are unlikely, and the extended off-times are likely the result of electron transfer to the transition-metal sites in the glass, leading to long-lived, nonfluorescent charge-separated states. Such chromophoric radical cation/anion states have been proposed in other systems.[15,16] NDAPP bound to Ti(IV) oxide species on quartz also gives very similar fluorescence trajectories (not shown; see Supporting Information), supporting the suggestion that binding of NDAPP to such species is the source of fluorescence on glass.

HCl protonated NDAPP molecules on quartz show behavior similar to molecules in PVB, with infrequent millisecond off-times followed by irreversible loss of fluorescence (Figure 4D). The duration of fluorescence is shortened relative to that of NDAPP in the PVB matrix, since HCl may be lost to the atmosphere, resulting in the prompt loss of fluorescence.

The fluorescence that appears on quartz after several days displays a distinctly different appearance, with the intensity varying on the second time scale and displaying millisecond on- and off-times (Figure 4E and F), indicating that this fluorescence is due to binding to a different type of site than those present on glass. The erratic variations in fluorescence appear to result from molecular dynamics which change the absorption coefficient by changing the orientation of the chromophore relative to the polarized excitation light. We have observed similar fluorescent behavior for other perylenes when they are not tethered firmly to a surface or immobilized in a polymer matrix (see Supporting Information).

In conclusion, we present here for the first time the SMS of a new molecular probe which uses an intramolecular electron-transfer mechanism to detect binding. Information about the interaction of these molecules with their environment was obtained from analysis of the intensity, duration, and time-dependent behavior of the single-molecule fluorescence. Future work will be directed toward chemically modifying these molecules to alter their selectivity and sensitivity, to design specific single-molecule sensors for specific applications, and to incorporate other methods of monitoring single molecules, such as with polarization spectroscopy to follow molecular dynamics.

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Supporting Information Available: Cleaning procedures; fluorescence trajectories of NDAPP bound to Ti(IV) species on quartz and of unbound and immobilized perylene species (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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