

Surface Plasmon Fluorescence Investigation of Energy-Transfer-Controllable Organic Thin Films

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Thin functional organic films on a gold substrate were fabricated by adsorbing tetrakis(carboxyphenyl)porphyrin (TCPP) on a spacer layer, which was prepared by the layer-by-layer adsorption of a dendrimer and a linear polymer. The thickness and photoluminescence of the films were investigated by surface plasmon resonance and surface plasmon fluorescence techniques, respectively. TCPP adsorbed on the spacer layer in aqueous solutions of different ionic strengths resulted in a thick TCPP adlayer at high ionic strength and a shrunk spacer layer at low ionic strength. The fluorescence was quenched at high ionic strength but could be observed at low ionic strength. The effects are explained by the states of dye aggregation. This study shows the control of energy transfer from a metal surface to a dye layer by changing the dye adlayer. It can contribute to the development of molecular devices involving energy-transfer systems.

Introduction

Functional organic thin films have been the focus of the development of nanoscale devices, sensors, and so on. Energy-transfer phenomena are particularly important for the design of electronic and optical devices. Along these lines, energy transfer between metal substrates or metallic nanoparticles and dyes such as macrocycles (porphyrin and phthalocyanine) has been studied. For the characterization of such films, surface plasmon phenomenon on metals can be utilized. Because the surface plasmon enhances the electromagnetic field of the evanescent wave near the surfaces, the energy of the incident light is effectively transferred to the adsorbates on the metal surface. Hence, a surface plasmon resonance (SPR) apparatus based on this phenomenon can be used as a sensor in the efficient detection of small amounts of analytes. In addition to the SPR phenomenon, the enhanced evanescent wave can excite a fluorescent probe on a metal substrate, generating photoluminescence light (PL) observed as a surface plasmon fluorescence (SPF) spectrum.^{1,2} In that case, the probe on a thin metal film displays a significantly higher PL signal than in the absence of a metal film. Thus, SPF spectroscopy is a new and valid technique for the characterization of films involving energy transfer, and the PL signal is a barometer of the energy transfer where part of the incident energy transfers to dye through evanescent wave and the energy migrates to fluorescence from the dye.

However, it must be noted that the SPF signal from a fluorescent probe strongly depends on the distance between the probe and the metal surface because of the effect of energy transfer from the probe to the metal (i.e., quenching increases with decreasing the probe–metal distance^{3,4}). Thus, to obtain a sufficient PL

signal, it is necessary to keep an adequate distance between the probe molecules and the metal surface. In previous reports, a polymer,⁴ a layer of antibodies,⁵ surfactant bilayers,⁶ or a self-assembled thiol monolayer (SAM)⁷ has been applied as a spacer, and the fluorescent probe has been attached to the spacer via covalent bonds, hydrogen bonds, and so forth.

In this study, we have focused on layer-by-layer deposition for the formation of the spacer because the spacer thickness can be easily controlled by this method. One of the building blocks for the layers is an ionic dendrimer. Dendrimers possess many functional groups, and their spherical or spheroidal shapes are well defined in solution. Such functional groups and this uniform shape are advantageous because of the strong attachment on an oppositely charged surface and the retention of thickness as a spacer.⁸ The other building block is a linear polymer with an opposite charge to that of the dendrimer. The linear polymer binds electrostatically and extends onto the dendrimer surface. Finally, the organic thin film allowing for energy transfer was prepared on the multilayer spacer by adsorbing a water-soluble porphyrin as a PL probe, and the effect of ionic strength on the PL intensity was investigated by means of SPF.

Experimental Section

Materials. Fourth-generation (G4) amine-terminated poly(amido amine) (PAMAM) dendrimers, poly(vinylsulfonate) (PVS), and NaH₂PO₄/Na₂HPO₄ pellets including NaCl were purchased from Sigma-Aldrich. Tetrakis(carboxyphenyl)porphyrin (TCPP) was obtained from Porphyrin Systems, Germany. These reagents were used without further purification. Ultrapure water (18.2 Ω/m) was used throughout the experiments.

Preparation of Gold Substrates. High-index ($n = 1.70$) glass plates were cleaned twice with a detergent and twice with ethanol in an ultrasonic bath. After drying under a stream of nitrogen, chromium was deposited at up to 2 nm thickness on the glass plates in a vacuum chamber (5×10^{-6} Pa), followed by gold evaporation

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(1) (a) Naumann, R.; Jonczyk, A.; Kopp, R.; Esch, J.; Ringsdorf, H.; Knoll, W.; Gräber, P. *Angew. Chem.* **1995**, *34*, 2056–2058. (b) Kambhampati, D.; Nielsen, P. E.; Knoll, W. *Biosens. Bioelectron.* **2001**, *16*, 1109–1118.

(2) Krupka S.; Wiltzsch, B.; Reuning, U.; Hölscher, K.; Hara, M.; Sinner, E. *Biosens. Bioelectron.* **2006**, *22*, 260–267.

(3) Liebermann, T.; Knoll W. *Colloids Surf., A* **2000**, *171*, 115–130.

(4) Vasilev, K.; Knoll, W.; Kreiter, M. *J. Chem. Phys.* **2000**, *120*, 3439–3445.

(5) Lochner, N.; Lobmaier, C.; Wirth, M.; Leitner, A.; Pittner, F.; Gabor, F. *Eur. J. Pharm. Biopharm.* **2003**, *56*, 469–477.

(6) Sinner, E.; Reuning, U.; Kok, F. N.; Sacca, B.; Moroder, L.; Knoll, W.; Oesterhelta, D. *Anal. Biochem.* **2004**, *333*, 216–224.

(7) Liebermann, T.; Knoll, W.; Sluka, P.; Herrmann, R. *Colloids Surf., A* **2000**, *169*, 337–350.

(8) Li, C.; Mitamura, K.; Imae, T. *Macromolecules* **2003**, *36*, 9957–9965.

of a thickness of 50 nm. The prepared gold substrates were kept under an argon atmosphere until used.

Preparation of Spacer Films on Gold Substrates. A gold substrate was immersed for 1 h in the first step and for 15 min in the second and following steps in an aqueous solution (0.1 wt %) of the G4 PAMAM dendrimer at pH 7.5 and then rinsed with phosphate buffer (pH 7.5) including 0.15 M NaCl. Then the substrate was immersed for 15 min in a phosphate buffer solution (0.25 wt %) of PVS and rinsed with phosphate buffer (pH 7.5) including 0.15 M NaCl. These immersion procedures were repeated until 3.5 dendrimer/PVS bilayers topped by a dendrimer layer were formed.

Preparation of the Organic Thin Film on Spacer Films. The spacer-deposited substrates were immersed for 15 min in an aqueous solution (pH 7.5) of 0.05 mM TCPP at two different ionic strengths (i.e., (a) in phosphate buffer including a 0.15 M NaCl and (b) in pure water without buffer and salt). Finally, the TCPP-coated substrates were rinsed with phosphate buffer (pH 7.5) including 0.15 M NaCl.

Apparatus. The setup of an SPR sensor is described elsewhere.^{1,3} Briefly a p-polarized He/Ne laser (632 nm) was used as the incident light source. The cell part was set up by the Kratschmann configuration. An LaSFN9 prism was index matched to a metal-coated glass slide (LaSFN9), which was attached a Teflon cell of 1 cm³ volume with the front glass window. The cell body was equipped with liquid inlet and outlet for the fluid handling procedure. The prism was well washed with ethanol before use. Matching oil ($n = 1.70$) was used for optical contact between the sample glass slide and the prism. The rotation of the sample stage was computer-controlled to $\theta - 2\theta$ with the detector. The reflected light was measured by a detector equipped with a photodiode. For the SPF measurements, a photomultiplier was coupled to the SPR setup with an optical filter ($\lambda > 670$ nm).^{1,3} The SPR and SPF measurements were carried out at each step of the deposition of the 3.5 bilayers and the adsorption of TCPP. A kinetics profile of SPF during the TCPP adsorption was measured at a fixed angle, where the reflectivity drop is 30% less than at the surface plasmon resonance angle of the spacer layer alone because the PL intensity variation that must be detected is large.

Results

Fabrication of Dendrimer/PVS Multilayer Assemblies. SPR curves at each step in the fabrication of the layers are shown in Figure 1A. The SPR band was clearly shifted step by step to larger angles during the fabrication process. This indicates the increase in film thickness on the metal substrate originating from the adsorption of the molecules. At first, the NH₂-terminated PAMAM dendrimer was adsorbed directly onto the bare gold surface (Scheme 1). At pH 7.5, the dendrimer is positively charged because the pK_a values of the primary and tertiary amines of the dendrimer are ~ 9 and 6, respectively.⁹ Then, the negatively charged PVS polymer was adsorbed to the positively charged first dendrimer layer (Scheme 1). This electrostatic binding of dendrimers and PVS also happened during successive immersion steps.

Figure 1B gives a plot of the SPR angle shift as a function of the number of immersion (binding) steps. From the angle shift, the thickness of the G4 PAMAM dendrimer (as the first layer) adsorbed on a gold substrate was evaluated on the basis of the Fresnel reflection theory.¹⁰ It was ~ 1.5 nm (assuming $n = 1.5$), consistent with a previous report.⁸ The thickness was lower than the diameter (~ 4.0 nm) of a spherical dendrimer in solution because the shape of the dendrimer is changed to oblate on the substrate.⁸ However, the angular shifts upon dendrimer deposition of the second and successive layers were almost

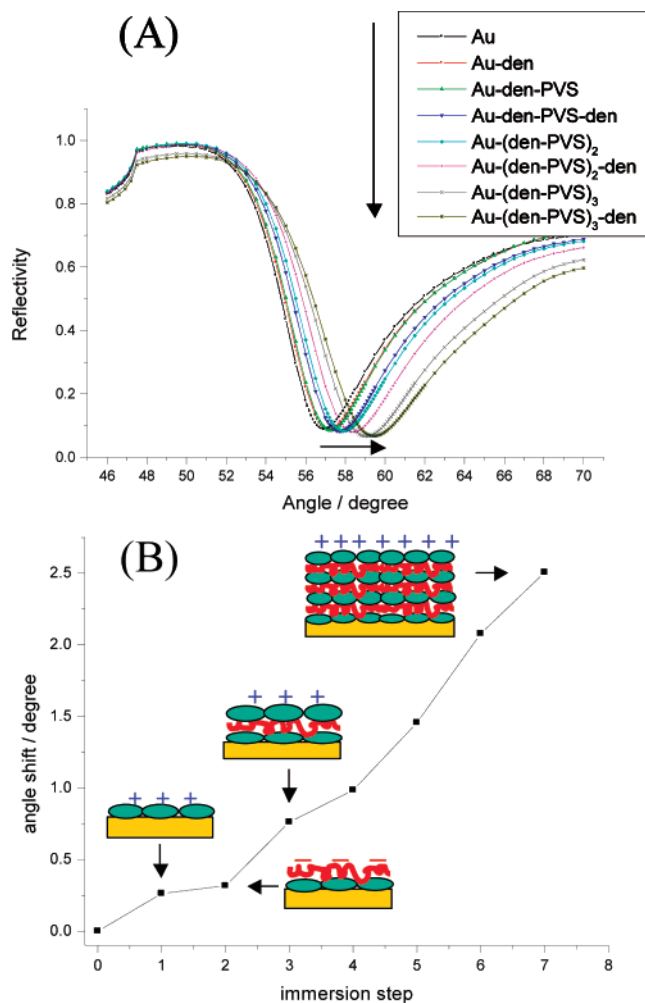


Figure 1. (A) SPR curves after each step of the layer preparation according to Scheme 1. (B) SPR angular shift vs number of immersion step. Zero angular shift indicates the SPR angle of the bare gold substrate.

constant ($\sim 0.45^\circ$). This indicates that, except for the first layer, the dendrimer layer maintains a uniform thickness (~ 2.7 nm) in the layer-by-layer deposition, although the dendrimers are still oblate. The observation that the earlier layers behave differently from later ones is often found in the layer-by-layer assembly process and has been explained by the reduced charge compensation in the earlier steps due to the low charge density of the substrate.¹¹

The SPR angle shifts upon binding the first PVS layer (i.e., the thickness of the first PVS layer was smaller than that of the dendrimer). Because the PVS is a linear, flexible polymer with a small cross-sectional diameter that is different from that of a spherical dendrimer, it is reasonable to assume that PVS molecules spread out on the dendrimer surface. A similar phenomenon was observed on successive layer-by-layer deposition of a dendrimer/polyaniline system.⁸ The angle shift induced by the PVS deposition increased with increasing number of immersion steps. The phenomenon is also explained by the low charge compensation for the small number of adsorption steps as described above.

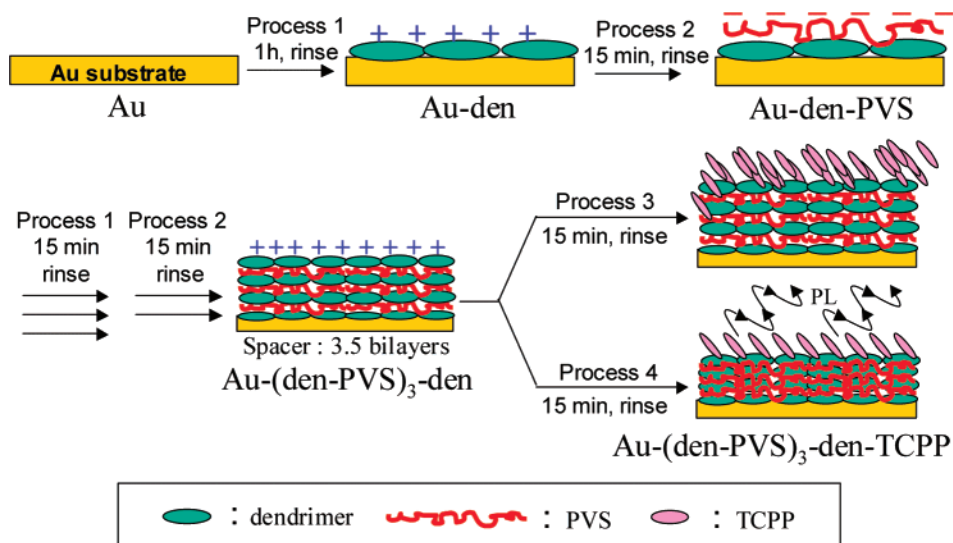
The attachment of the dendrimers onto a solid substrate was sometimes mediated by a binder layer such as a thiol (R-SH) SAM for gold substrates or organosilane (R-Si-(OCH₃)₃) layers for glass substrates.¹²⁻¹⁴ In the present work, no binder was

(9) (a) Kleinman, M. H.; Flory, J. H.; Tomalia, D. A.; Turro, N. J. *J. Phys. Chem. B* **2000**, *104*, 11472-11479. (b) Leisner D.; Imae, T. *J. Phys. Chem. B* **2004**, *108*, 1798-1804.

(10) Raether, H. *Surface Plasmons on Smooth and Rough Surface and on Gratings*; Springer-Verlag, Berlin, 1988.

(11) Santos, J. P.; Welsh, E. R.; Gaber, B. P.; Singh, A. *Langmuir* **2001**, *17*, 5361-5367.

Scheme 1. Schematic Illustration of the Fabrication of a Spacer Multilayer and an Organic Thin Film



required. The direct attachment of the amine-terminated dendrimers on a gold substrate simplifies the procedure of the layer-by-layer assembly.

The whole thickness of the resulting multilayer was larger than 10 nm. Because this thickness is large enough to reduce energy transfer from dye to substrate to a significant degree, as indicated in the previous report,⁷ a film of 3.5 bilayers was used as a spacer in the following the experiments.

Adsorption of TCPP to Space Multilayers from Aqueous Solutions at Different Ionic Strengths. The adsorption of TCPP onto a dendrimer/PVS multilayer film was carried out in an aqueous solution of high ionic strength (0.15 M NaCl) at pH 7.5, and the SPR and SPF curves before and after the adsorption process were compared, as seen in Figure 2. A significant SPR angle shift was observed, indicating the stable adsorption of TCPP onto the Au-(den-PVS)₃-den spacer even after rinsing. The value of the angular shift $\Delta\theta$ ($\sim 2.0^\circ$) corresponds to an ~ 12 nm thickness (assuming a refractive index of $n = 1.5$). Because this thickness is larger than that of one TCPP molecule (< 1 nm), the adlayer must be an aggregate of TCPP.

However, the PL intensity at high ionic strength increased upon the injection of the TCPP solution, reaching a maximum after 120 s, followed by a decrease to a constant plateau after ~ 500 s. However, upon rinsing, the PL intensity decreased back to the original background level before injection. These results indicate that, in spite of the adsorption of TCPP onto the spacer layer, no PL was emitted from the film after the rinse and from the spacer before the TCPP adsorption. Only during the injection is a time-dependent contribution of the TCPP molecules seen. It should be noticed that the absence of PL is independent of the resonance angle shift. As seen in Figure 3, the PL intensity at high ionic strength was almost constant at every angle between 45 and 70° , which covers the range of the SPR angle shift in Figure 2A and is different from the case at low ionic strength as described below.

For comparison, the adsorption of TCPP was performed in an aqueous solution at low ionic strength, which was prepared only by adjustment to pH 7.5. The SPR and SPF results are shown in Figure 4. The SPF kinetic profile of the TCPP adsorption

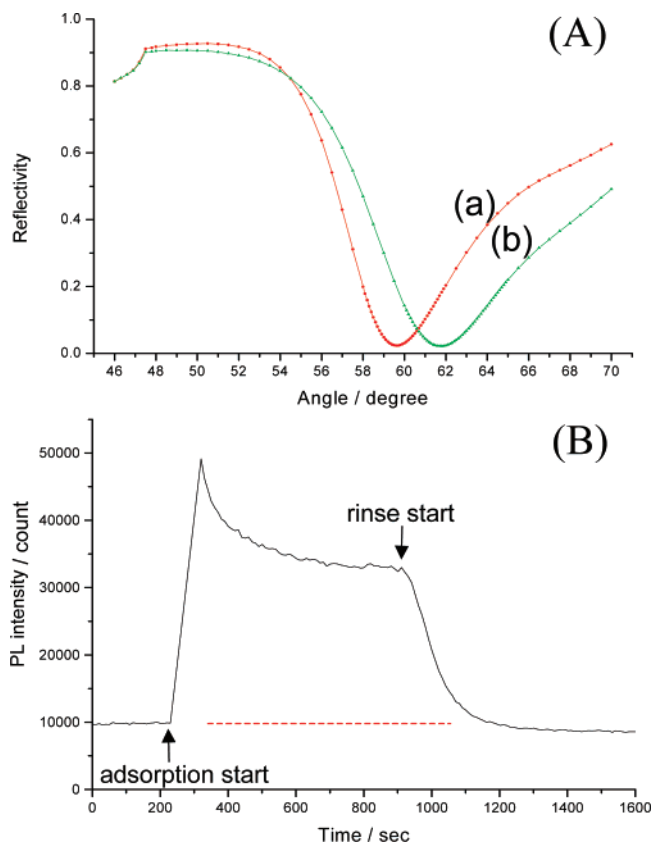


Figure 2. (A) SPR curves of (a) Au-(den-PVS)₃-den and (b) Au-(den-PVS)₃-den-TCPP at high ionic strength. (B) Kinetics profile of SPF during the TCPP adsorption at high ionic strength, followed by a rinsing step.

seems to be similar to that at high ionic strength (Figure 2B), although the emission during the injection at low ionic strength is 7 times stronger than that at high ionic strength, indicating the contribution from the TCPP solution. However, different from the case at high ionic strength, the PL intensity at low ionic strength decreased sharply ~ 10 s after the beginning of the rinsing step and slowed down after that. It did not reach the original level (before the injection) even after 1500 s. This fact suggests that TCPP molecules are adsorbed as a PL probe on the film and emit PL.

(12) Crespo-Biel, O.; Dordi, B.; Reinhoudt, D. N.; Huskens J. *J. Am. Chem. Soc.* **2005**, *127*, 7594–7600.

(13) Mark, S. S.; Sandhyarani, N.; Zhu, C.; Campagnolo C.; Batt, C. A. *Langmuir* **2004**, *20*, 6808–6817.

(14) Liu, Z.; Amiridis, M. D. *J. Phys. Chem. B* **2005**, *109*, 16866–16872.

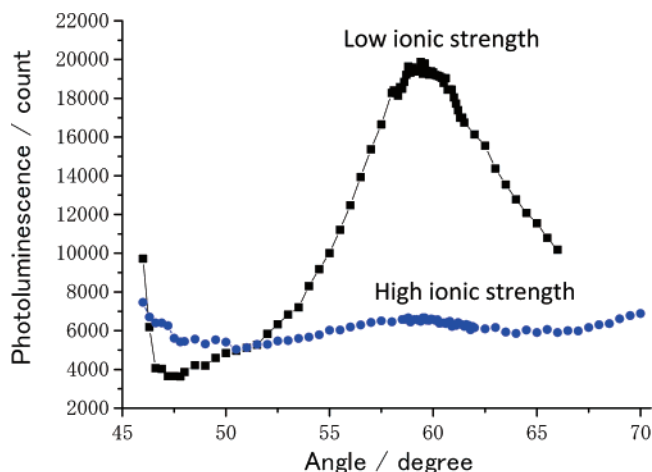


Figure 3. SPF curves vs angle of Au-(den-PVS)₃-den-TCPP at high and low ionic strengths, followed by a rinsing step.

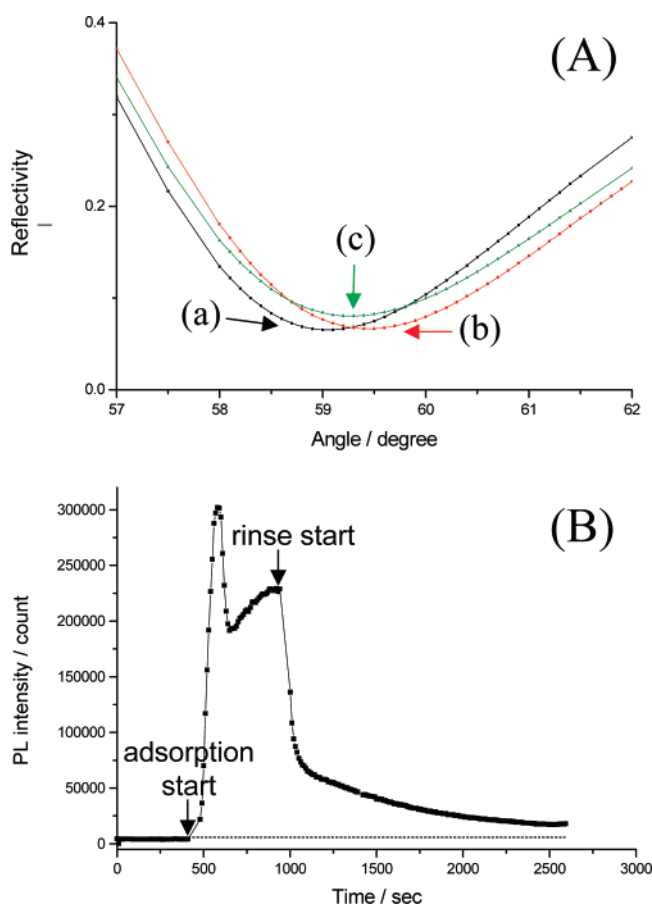


Figure 4. (A) SPR curves of (a) Au-(den-PVS)₃, (b) Au-(den-PVS)₃-den, and (c) Au-(den-PVS)₃-den-TCPP at low ionic strength. (B) Kinetics profile of SPF during the TCPP adsorption at low ionic strength, followed by a rinsing step.

This significant difference was also seen in the SPR behavior at low ionic strength. The SPR curve of Au-(den-PVS)₃-den-TCPP was located between the signals obtained before and after the formation of the outmost dendrimer layer (Figure 4A). This behavior indicates that the total film thickness decreases after the TCPP adsorption, although the thickness was larger than that of the Au-(den-PVS)₃ multilayer. Because the adsorption of TCPP is confirmed by the SPF result, a decrease in spacer thickness must be considered to be the dominant origin of the overall shrinkage. Two reasons are possible. One is the partial desorption

of spacer components, and the other is the shrinkage of the spacer by changing from high (during the preparation of the spacer) to low ionic strength (during the adsorption of TCPP).

If the ionic strength decreases, then the electric double layer increases as a result of the weakening of the electric shielding by the coexisting salt ions. Then polyelectrolytes with opposite charges interpenetrate between them. This results in a decrease in spacer thickness rather than the desorption of spacer components. At the same time, the adsorption of TCPP also may be influenced by the varied electrostatic effect of the spacer owing to the weakening effect of the electric shielding by salts. Because the surface charge on the spacer decreased as a result of the interpenetration of ions between the polyelectrolytes, the adsorption of TCPP molecules could be suppressed on the less-charged spacer surface. The SPR behavior in Figure 4A could reflect both the shrinkage of the polyelectrolytes in the spacer and the reduced adsorption of TCPP on the shrunken spacer.

Discussion

Upon the adsorption of TCPP molecules from a solution at high ionic strength, no PL was observed in spite of the substantial amount of adsorbed TCPP on the spacer film after rinsing. For the interpretation of this finding, various possibilities should be considered: (1) The spacing between the dye and the substrate may not be large enough to emit PL (i.e., the PL is quenched by energy transfer from TCPP to the metal substrate). However, because PL was observed on a thinner spacer at lower ionic strength, the spacer thickness does not explain the observation. (2) The PL could be quenched by charge transfer between acceptor and donor molecules. However, there is no report in the literature that PAMAM dendrimers form a charge-transfer complex with porphyrin in which they act as acceptor and donor molecules. Although an aged dendrimer solution displays PL at $\lambda = 450$ nm (excitation: $\lambda = 250$ and 390 nm),¹⁵ it is unlikely that dendrimer coupled electrostatically with TCPP (excitation: $\lambda = 630$ nm; emission: $\lambda = 645$ nm). (3) The NaCl concentration could act as a quencher for the PL intensity. However, because the emission of PL was observed during the injection of the TCPP solution, it can be concluded that NaCl barely quenches the PL of TCPP, although the salt concentration may affect the formation of aggregates.

(4) The lack of PL emission may be due to the orientation of TCPP. The dipole moment of a porphyrin ring is within the plane of the molecule. Thus, if the porphyrin ring is flat on the film, then it may not be excited by the p-polarized laser because the evanescent electric field is normal to the substrate. In this case, energy (light) is not supplied to the TCPP molecule, and no PL is emitted from the film. (5) The SPR result (Figure 2A) indicated the presence of highly aggregated TCPP molecules on the top of the spacer layer at high ionic strength. The aggregation of TCPP molecules might lead to the quenching of PL. The energy is dissipated in the aggregates of TCPP molecules via π - π stacking. Consequently, PL will not be observed. In a thick film of TCPP aggregates, it is unlikely that the orientations of all TCPP rings are parallel to the plane of the substrate. Therefore, possibility 5 is the most plausible.

The decrease in ionic strength caused the shrinkage of the polymers in the spacer but led TCPP on the spacer to emit PL. At low ionic strength, the aggregation of TCPP on the spacer is reduced because the electric shielding effect in the solution is weak, in addition to the reduced charge of the spacer layer surface. Thus, the PL from TCPP in this case is less suppressed by self-quenching.

The behavior of dyes in solution is different from that on a spacer film as far as the state of aggregation and orientation is concerned. As seen in the kinetic profiles (Figures 2 and 4), the PL intensity is not quenched completely in solution. In this case, although the absorbed incident light excites dye molecules, part of the excitation energy in the dyes is assumed to be dissipated without the emission of PL because energy transfer or dissipation occurs among dye molecules. This relaxation of excitation energy should depend on the size of the aggregate. In solution, aggregates are large at high ionic strength, and PL is strongly quenched. Thus, although the PL is weaker at high ionic strength than at low ionic strength, it was not quenched completely even in a solution at high ionic strength, as seen in Figures 2B and 4B.

Conclusions

Organic thin films of TCPP deposited onto a spacer layer were fabricated and investigated by SPR and SPF spectroscopy, providing information on the adsorption–desorption behavior and energy-transfer behavior, respectively. In this study, a spacer layer was indispensable because otherwise all excited dye molecules near the metal substrate (less than 10 nm in distance) could largely be quenched. A multilayer assembly of PAMAM dendrimers and PVS was prepared via layer-by-layer deposition. Because the PAMAM dendrimer has a large surface charge density and is a relatively large object, it is suitable as a component

in a layer-by-layer architecture. The assembly of 3.5 bilayers was evaluated to generate more than 10 nm in distance between the metal substrate and the dye.

The dye molecule (TCPP) was adsorbed onto this spacer in two different salt solutions. At high ionic strength, PL was totally quenched, although the TCPP did adsorb to the film. However, at lower ionic strength, the PL was not quenched completely. The high ionic strength promoted the aggregation of dye molecules by an electric shielding effect. However, for less electric shielding at low ionic strength, cationic dendrimers and anionic PVS interpenetrated more, and the spacer layer surface became less charged, giving rise to a reduction in spacer thickness and reduced TCPP adsorption. Thus, self-quenching was promoted by energy dissipation between neighboring dyes in aggregates, which was more dominant at high ionic strength than at lower ionic strength.

This study shows that energy transfer from a metal surface to a dye layer is controlled by changing the dye adlayer, contributing to the development of molecular devices involving energy-transfer systems.

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