Conformational change of protein cytochrome b-562 adsorbed on colloidal gold particles; absorption band shift

H. S. Zhou,*[a] S. Aoki,†[b] I. Honma,[c] M. Hirasawa,[b] T. Nagamune[b] and H. Komiyama[b]

[a] *Nanophotonics Laboratory, Frontier Research Program, The Institute of Physical and Chemical Research (RIKEN), Hirosawa 2-1, Wako-shi, Saitama 351-01, Japan*
[b] *Department of Chemical System Engineering, Faculty of Engineering, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo, Japan*
[c] *Electrotechnical Laboratory (ETL) 1-1-4, Umezono, Tsukuba, Ibaraki 305, Japan*

An enormous and continuous shift of a surface plasmon resonance of gold particles is observed when protein cytochrome b-562 is adsorbed on to the surface.

Organic–inorganic nanostructured composites provide a rich source of new functional materials for fundamental research and applications.[1–5] Examples include ordered mesoporous molecular sieves synthesized *via* a liquid-crystal surfactant template;[1,2] a low-cost, high-efficiency solar cell system of nanocrystallites covered by a chlorophyll derivative or related natural porphyrins;[3,4] and a polydiacetylene shell layer synthesized on a template of nanosized gold particles.[5] Although many interesting functional materials have been synthesized, it is still not clear how these organic functional molecules are deposited on, or affected by a solid boundary surface of nanoparticles; or what conformations and orientations are adopted. If the mechanism by which organic molecules are adsorbed on the solid surface of nanoparticles is clarified, it will open a wide opportunity in studying the basic physics and chemistry of nanostructure composite materials.

Here, we disclose our recent findings for optical coupling in an organic–inorganic nanostructure composite system of the protein cytochrome b-562 and colloidal gold particles. Although many studies, especially surface enhanced Raman scattering (SERS),[6–8] have been carried out for protein adsorption on solid surfaces, direct optical measurement at the protein's molecular level and conformational study of the protein on the surface of gold particles has scarcely been reported.

An aqueous gold colloidal solution was prepared by reduction of $HAuCl_4$ (5×10^{-3} mass%, solution 1) with citric acid (0.15 mass%, solution 2).[9] Solution 1 (750 ml) was heated at 100 °C for 12 min in an oil bath, and then solution 2 (37.5 ml) added. After *ca*. 2 min the boiling solution turned blue, and after 3 min became reddish purple. At this stage, the mean particle size was 31.0 nm with a narrow size distribution according to both transmission electron microscopy (TEM) and dynamic light scattering.[10,11] The dynamic light scattering method is a useful tool for measuring particle sizes in the range from 10 nm to several μm.

Cytochrome b-562 solutions of various concentrations were prepared (0.0003–0.16 g l^{-1}). The solution (0.2 ml) was added to the gold colloidal solution (20 ml) with vigorous stirring. Cytochrome b-562 is a small cylindrical[12] haem protein (height 5.0 nm, diameter 2.5 nm; Fig. 1) found in the periplasm of *Escherichia coli* with a chromophore (protohaem IX) about 3.5 nm from the base. A significant absorption occurs at 418 nm and smaller absorbances at 530 and 560 nm.

The surface plasma resonant absorption band of gold particles is located at *ca*. 530 nm.[13] The plasmon absorption band (at 530 nm) of the original 31.0 nm gold colloidal suspension was red-shifted and broadened upon adsorption of the protein. When only a small amount of the protein was added, much less than for full monolayer coverage, the interaction is weak and only a small shoulder emerged at *ca*. 650 nm. However, with increasing quantity of the protein, the interaction becomes stronger and the new peak is broadened and shifted to long wavelength (up to 750 nm).

Upon further addition of protein the absorption spectrum unexpectedly returned to that of the original colloidal solution and the absorption peak of isolated cytochrome b-562 appeared at *ca*. 418 nm arising from solution-phase protein. At this stage, composite particles and isolated protein co-exist and interaction between them becomes weak eventually non-existant at high protein concentration.

We introduce the concept of effective coverage to describe the status of adsorbed protein cytochrome b-562 on the surface of gold particles taking into account factors such as the shape of the protein and the protein electrostatic repulsive force to effect full coverage.

The effective coverage $\beta = 1.0$ and $\beta = 2.0$ refer to full coverage of cytochrome b-562 on the surface of gold particles in 'side-on' and 'tail-on' conformations respectively.

Fig. 2 shows the shifting of the long-wavelength absorption peak position as a function of the protein's effective coverage β. In the present experiment, as all of the protein is adsorbed on the particle surfaces, the surface coverage is linearly proportional to the amount of protein added to the solution. As the protein

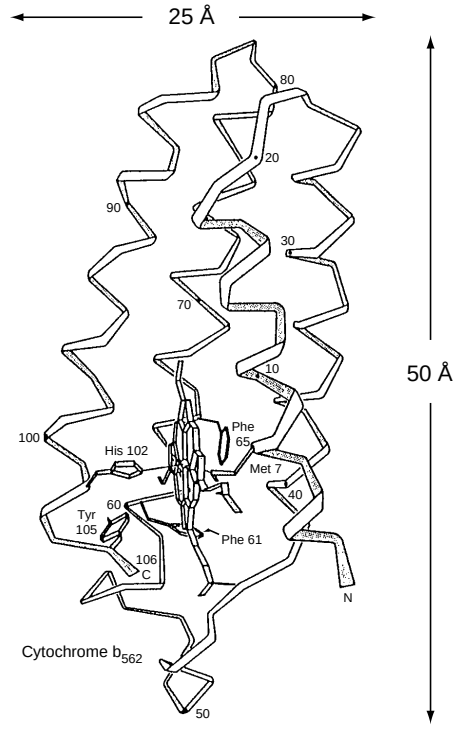

Fig. 1 Schematic structure of cytochrome b-562

coverage increases, the peak position moves to longer wavelength until it reaches 750 nm and the effective coverage β at this point is 1.0, where the particle surface is covered by proteins in a 'side-on' conformation. The expected size of such a structure is verified by dynamic light scattering measurements. Further addition of the protein moves the peak back to its original position at 530 nm, although the composite particle size increases monotonously with the protein coverage. This indicates that the phenomenon of λ_{max} moving back to its original value is not a consequence of separation of the protein and gold particles. We propose that the protein's adsorption conformation on the surface changes at a certain concentration. A model is proposed which considers two adsorption conformations of cytochrome b-562 on the gold surface. Namely, the 'side-on' conformation predominates at low concentrations ($\beta < 1.0$) while the 'tail-on' conformation predominates at high concentrations ($1 < \beta < 2$).

Different conformations of the protein lead to differences in the interaction distance between the gold surface and the interior chromophore of cytochrome b-562. In other words, the interaction between the gold particles and cytochrome b-562 depends strongly on the distance between the surface and the chromophore. The red-shift to 750 nm and spectral broadening occurred only for the 'side-on' conformation because the short separation in this case.

The model of the conformation change of the protein was supported by particle size measurement as determined by dynamic light scattering. Initially the diameter of the gold particles is 31.0 nm and it increases with protein coverage (Fig. 2). The composite particle size was 36 nm at the maximum spectral shift of the plasmon absorption band. At this stage, the protein b-562 is in a 'side-on' conformation on the gold surface. Since in such a conformation the width of the protein is 2.5 nm the value of 36 nm ($= 31 + 2 \times 2.5$ nm) is as expected for monolayer coverage. As the concentration is raised further the absorbed protein molecules start shifting conformation and eventually at a particle size of 40 nm, the interaction was so weak that the optical absorption spectrum was almost the same as the intrinsic absorption spectrum of the gold particles. The effective coverage β at this stage is 2.0 corresponding to effective full coverage of cytochrome b-562 on the surface of the gold particles in the 'tail-on' conformation. A size of 40 nm is also in accord with such a conformation. Further addition of protein leads to no change and no second layer coverage is observed with the additional protein isolated in solution as reflected by the absorption band od 418 nm.

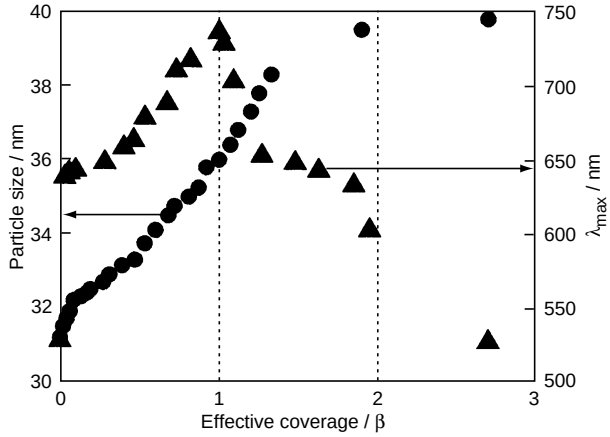

Fig. 2 Changes of particle size and peak position of the longer wavelength plasmon band with surface protein effective coverage β

To summarise, changes in conformation of adsorbed protein have been optically detected, for the first time, by the shift of the resonant plasmon absorption arising from the distance-sensitive dielectric interaction between the protohaem within the protein and the gold surface.

Footnote

† *Present address*: Electric Materials and Equipment Section 3, Sumitomo Corporation, 1-2-2, Hitotsubashi, Chiyoda-ku, Tokyo, 100 Japan.

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