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Artificial hemoprotein nanotubes

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Materials and apparatus.

All reagents were purchased from commercial sources as special grades and used without further purification. Recombinant HSA expressed in *Pichia pastoris* was purchased from Sigma-Aldrich. The synthetic heme (2-[8-{*N*-(2-methylimidazolyl)}octanoyloxy-methyl]-5,10,15,20-[tetrakis{α,α,α,α-*o*-(1-methylcyclohexanamido)}phenyl]porphinatoiron(II), FeP) and aqueous HSA-FeP solution (FeP/HSA = 4, mol/mol) were prepared according to our previously reported procedures.[9a] The water was deionized using a Millipore Elix and Simpli Lab-UV. The UV-vis absorption spectra were recorded using an Agilent 8453 UV-visible spectrophotometer with an Agilent 89090A temperature control unit.

Fabrication of HSA-FeP nanotubes.

The anodic porous alumina template membrane (Anodisc 25, 25 mmϕ, pore-size 0.2 μm, Whatman Corp.) was fixed into a stainless steel filter holder (Millipore). The HSA-FeP solution (2 mg/mL = 30 μM, 5 mL) at pH 3.8 (10 mM citric acid buffer) was slowly injected into the filter and passed through under a certain vacuum. After drying the template, the HSA-FeP solution (2 mg/mL = 30 μM, 5 mL) at pH 7.0 (10 mM sodium phosphate buffer) was passed through the porous template to produce a second layer under a certain vacuum. The alternative adsorption of at least 3 cycles led to the formation of the stable multilayer HSA-FeP films onto the pore walls of the anodic template membrane.

In order to liberate the nanotubes from the alumina template, the red colored membrane was fixed on the silicon wafer (Aldrich, Silicon wafer, (111), 2, 647101-5EA) or quartz plate (35 × 9 mm) with epoxy resin and then immersed in a 10% H_3PO_4 solution at room temperature. The salt was washed away by rinsing with pure water.

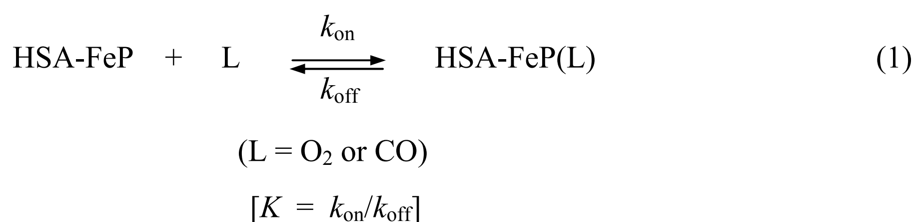
SEM and TEM observations.

For the SEM measurements, the samples on the silicon wafer were sputtered with Pd-Pt using a HITACHI E-1030 Ion Sputter. The SEM observations performed using a HITACHI S-4500S Scanning Electron Microscope.

For the TEM observations, the red-colored anodisc membrane with the HSA-FeP film was directly immersed into 10% H_3PO_4 and the solution was then filtered using a poly(carbonate) membrane (Millipore Isopore membrane, pore-size 50 nm). The obtained tubules were redispersed into pure water. Droplets of this solution were placed onto a carbon-coated copper grid (200 mesh), which was lightly hydrophilized by a JEOL DATUM HDT-400 hydrophilic treatment device. After 1 min, excess fluid on the grid surface was blotted off by filter paper. The remaining thin film of the sample was air-dried and stained with 0.25% uranyl acetate. After removal of the excess fluid, the grids were air-dried. The obtained specimens were observed in a JEOL JEM-1011 Electron Microscope at an accelerating voltage of 100 kV.

O_2 and CO binding equilibria and kinetics.

O_2 or CO binding to the HSA-FeP nanotubes was generally expressed by eq. 1 with the binding constant (K), association rate constant (k_{on}) and dissociation rate constant (k_{off}).



$$\text{HSA-FeP} + \text{L} \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} \text{HSA-FeP(L)} \qquad (1)$$

$$(\text{L} = O_2 \text{ or CO})$$

$$[K = k_{on}/k_{off}]$$

The quartz plate with HSA-FeP nanotubes was fixed in a 10 mm path length optical quartz cuvette tightly sealed with a rubber septum. The UV-vis absorption spectra were recorded within the range of 350–700 nm. The O_2 binding affinity (gaseous pressure at half O_2 binding, $P_{1/2} = K^{-}$

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^{1}) was determined by spectral changes at various partial pressures of O_2. The gas mixture with the desired partial pressure of O_2/N_2 prepared by the KOFLOC Gasbrender GB-2C was flowed into the sample cuvette for equilibration. The O_2 and CO binding kinetics were measured using a Unisoku TSP-1000WK time-resolved spectrophotometer with a Spectron Laser Systems SL803G-10 Q-switched Nd:YAG laser, which generated a second-harmonic (532 nm) pulse of 5-ns duration (10 Hz).[14a]