Gold Nanoparticle-mediated Electron Transfer of Cytochrome c on a Self-Assembled Surface

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Supporting Information
S1. Formation of gold nanoparticles

Fig. S1. UV-Vis spectra of the aqueous 1 mM HAuCl₄ solution (black line), and AuNP-PAH suspension (red line). Electronic spectra were recorded on Jasco V-670 spectrophotometer.
S2. Determination of average diameter of nanoparticles

![Transmission Electron Microscopy image of AuNPs-PAH and histogram of size distribution](image)

Figure S2. a) Transmission Electron Microscopy image of AuNPs-PAH; b) histogram of size distribution obtained by counting 100 particles. Images were recorded on FEI TECNAI G² F20 HRTEM.

S3. Biocatalytic properties of Cyt c in the presence of nanoparticles

The retention of electrocatalytic properties of Cyt c after immobilization was verified by analyzing the electrocatalytic activity of Cyt c towards electroreduction of hydrogen peroxide. Upon addition of H₂O₂, an increase in reduction current was observed with an onset at 0.20 V for Au/Cys/AuNP-PAH/Cys/Cytc (Figure S3a, red line). However, no electrocatalytic current was observed at the bare Au electrode within identical scanning potential range (Figure S3a, blue line), indicating that reduction of H₂O₂ was catalyzed by the Cyt c that was self-assembled on the modified electrode.
Figure S3. a) Cyclic voltammograms for Au/Cys/AuNP-PAH/Cys/Cytc (black line), and the bare Au electrode (green line) at a scanning rates of 50 mV s\(^{-1}\) in phosphate buffer without \(\text{H}_2\text{O}_2\) and with 0.37 mM \(\text{H}_2\text{O}_2\) (red and blue lines, respectively); b) Polarization curves for bioelectrodes Au/Cys/PAH/Cys/Cytc (black squares), and Au/Cys/AuNP-PAH/Cys/Cytc (red circles) in the presence of 0.37 mM \(\text{H}_2\text{O}_2\); c) Amperometric responses of Au/Cys/AuNP-PAH/Cys/Cytc (red line), and Au/Cys/PAH/Cys/Cytc (black line) at 0.0 V upon successive additions of 50 \(\mu\)L 30 mM \(\text{H}_2\text{O}_2\) solution to 25 mL phosphate buffer. A plot of current versus \(\text{H}_2\text{O}_2\) concentrations is shown in the inset.

Higher currents were observed for Au/Cys/AuNP-PAH/Cys/Cytc when the bioelectrodes with and without AuNPs were compared (Figure S3b). The red line indicates the typical current-
time amperometric curves for Au/Cys/AuNP-PAH/Cys/Cytc recorded under conditions of continuous stirring and successive addition of 50 µL 30 mM H₂O₂ into phosphate buffer. Based on the optimization experiments, 0.0 V was selected as the applied potential for H₂O₂ reduction. The reduction current increased abruptly and reached a stable value after the addition of each aliquot. About 6 s were needed to reach the maximum current, indicating a fast response process. A chronoamperometric curve for Au/Cys/PAH/Cys/Cytc (Figure S3c, black line) was recorded under identical conditions, where a similar behavior albeit with a discrete increase in the catalytic currents was observed. Both bioelectrodes exhibited increasing amperometric responses to H₂O₂ with good linear ranges from 6.0 x 10⁻⁵ M to 5.3 x 10⁻⁴ M (inset Figure S3c, r = 0.998). This behavior indicated that H₂O₂ could be easily reduced at low concentrations by Au/Cys/AuNP-PAH/Cys/Cytc and Au/Cys/PAH/Cys/Cytc bioelectrodes, further implying retention in Cyt c activity after its immobilization on the modified electrode.