Supporting Information

Surface engineering of gold nanorods for cytochrome c bioconjugation: an effective strategy to preserve protein structure

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**Figure S1:** Zeta potential distribution recorded for Au NR, AuNRs@PCC and AuHCc.

**Figure S2:** Size distribution by intensity recorded for Au NR, AuNRs@PCC and AuHCc.
Table S1: Assignments of the intense bands of the IR spectra.\(^1,2\)

| A  | B    | C    | D    | Assignment                  |
|----|------|------|------|-----------------------------|
| 3415 | 3390 | 3340 |      | v (O-H)                     |
| /   | /    | 3288 | /    | v (N-H)                     |
|     |      |      |      | Amide                       |
| /   | 2921 | /    | 2921 | v\(_s\) (C-H)               |
| /   | 2852 | /    | 2852 | v\(_as\) (C-H)              |
| 1702 | /    | /    | /    | v (C=O)dimer                |
| /   | /    | 1652 | 1652 | v (C=O)                     |
|     |      |      |      | Amide I                     |
| /   | /    | 1545 | 1541 | v (C=O)                     |
|     |      |      |      | Amide II                    |
| 1558 | 1558 | /    | 1558 | v (C-O)                     |
|     |      |      |      | asymmetrical                |
| 1450 | 1455 | 1455 | 1456 | v (COO\(^-\))               |
| 1408 | 1410 | 1396 | 1417 | v (C-O)                     |
|     |      |      |      | symmetrical                 |
| 1248 | /    | /    | /    | v (C-O)                     |
|     |      |      |      | dimer                       |
| 814 | /    | /    | /    | \(\delta\) (O-H)            |
|     |      |      |      | dimer                       |
Figure S3: Resonance Raman spectra of AuHCC (b) compared with the spectra of pure (Fe$^{3+}$) (a) and (Fe$^{2+}$) (c) Cyt c solutions at the same concentration calculated from the electronic absorption spectra of AuHCC (1 μM, see Figure 1, green spectrum). Experimental conditions: 514.5 nm excitation wavelength, laser power at the sample 40 mW, 10 min integration time. The spectra have been shifted along the ordinate axis to allow better visualization.

Figure S4: Resonance Raman spectra of AuHCC compared with the spectra of pure (Fe$^{3+}$) and (Fe$^{2+}$) Cyt c solutions. Experimental conditions: 514.5 nm excitation wavelength; (Fe$^{3+}$) laser power at the sample 80 mW, average of 8 spectra with 40 min integration time; (AuHCC) laser power at the sample 70 mW, average of 15 spectra with 2 h 30 min integration time; (Fe$^{2+}$) laser power at the sample 35 mW, average of 2 spectra with 10 min integration time. The spectra have been shifted along the ordinate axis to allow better visualization.
Table S2: Resonance Raman frequencies and vibrational assignments of Cyt c and AuHCC$^a$.

| Mode  | Symmetry | $\nu_{exc} = 406.7$ nm Cyt c | $\nu_{exc} = 514.5$ nm Cyt c | $\nu_{exc} = 406.7$ nm AuHCc | $\nu_{exc} = 514.5$ nm AuHCc |
|-------|----------|------------------------------|------------------------------|------------------------------|------------------------------|
|       |          | Cyt c Fe$^{2+}$ | Cyt c Fe$^{3+}$ | AuHCc Fe$^{2+}$ | AuHCc Fe$^{3+}$ |
| v8    | $A_{1g}$ | 346             | 348             | 348             | 348             |
| v20   | $E_u$    | 357             | 360             | 359             | 359             |
| $\delta$(CpC6Ca) | | 372,380         | 372,382         | 374,382         | 374,382         |
| $\delta$(CpC6aS) | | 391,400         | 391,400         | 398             | 398             |
| $\delta$(CpC6bC) | | 413,420         | 413,420         | 413,418         | 413,418         |
| $\gamma_{22}$ | $E_g$    | 445             | 446             | 446             | 446             |
| $\nu_{33}$ | $B_{2g}$ | 478             | 480             | 480             | 480             |
| $\gamma_{12}$ | $B_{1u}$ | 520             | 522             | 522             | 522             |
| $\gamma_{21}$ | $E_g$    | 551,568         | 554,568         | 554,568         | 554,568         |
| $\nu$(C-S) | | 683,691         | 693             | 683,692         | 683,692         |
| v7    | $A_{1g}$ | 700             | 702             | 702             | 702             |
| v15   | $B_{1g}$ | 750             | 750             | 750             | 750             |
| $\nu_{22}$ | $A_{2g}$ | 1130            | 1125            | 1130            | 1130            |
| $\nu_{30}$ | $B_{2g}$ | 1173            | 1169            | 1173            | 1173            |
| $\nu_{13}$ | $B_{1g}$ | 1230            | 1234            | 1230            | 1230            |
| $\nu_{42}$ | $E_u$    | 1242            | 1247            | 1247            | 1247            |
| $\nu_{21}$ | $A_{2g}$ | 1313            | 1316            | 1313            | 1313            |
| $\nu_{4}$ | $A_{1g}$ | 1361            | 1375            | 1372            | 1363            |
| $\nu_{20}$ | $A_{2g}$ | 1400            | 1409            | 1402            | 1402            |
| $\nu_{3}$ | $A_{1g}$ | 1492            | 1504            | 1492,1502       | 1492,1502       |
| $\nu_{11}$ | $B_{1g}$ | 1546            | 1564            | 1566            | 1547,1561       |
| $\nu_{19}$ | $A_{2g}$ | 1584            | 1585            | 1583,1583       | 1585            |
| $\nu_{2}$ | $A_{1g}$ | 1592            | 1584            | 1585            | 1585            |
| $\nu_{37}$ | $E_u$    | 1604            | 1596            | 1604            | 1604            |
| $\nu_{10}$ | $B_{1g}$ | 1622            | 1635            | 1622,1635       | 1621,1635       |

$^a$The band assignments have been made on the basis of Hu 1993. The bands assigned to Fe$^{2+}$ and Fe$^{3+}$ HCC are indicated in green and black, respectively.
**Figure S5**: AuNRs@PAA + HCc before washing (blue), after (pink) washing by centrifugation and after $K_3[Fe(CN)_6]$ addition (green).

**Figure S6**: Resonance Raman spectra of AuHCc (b) compared with the spectrum of pure ($Fe^{3+}$) (a) HCc solution at the same concentration calculated from the electronic absorption spectra of AuHCc (1 μM, see Figure 1, green spectrum). Experimental conditions: 406.7 nm excitation wavelength, laser power at the sample 10 mW, 5 min integration time.
Figure S7: Resonance Raman spectra of AuHCc obtained with 406.7 (panel A) and 514.5 (panel B) nm excitation wavelengths. The difference spectra (a, a’) were obtained by subtraction of the blank spectrum (b, b’) from that containing Cyt c (c, c’). Experimental conditions: (b, c) laser power at the sample 10 mW, (b) average of 9 spectra with 3 h integration time, (c) average of 48 spectra with 4 h integration time; (b’, c’) laser power at the sample 70 mW, (b’) average of 30 spectra with 5 h integration time (c’) average of 45 spectra with 7 h 30 min integration time. The spectra have been shifted along the ordinate axis to allow better visualization.
References

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