Supporting Information

Disentangling Electron Tunneling and Protein Dynamics of Cytochrome *c* Through a Rationally Designed Surface Mutation

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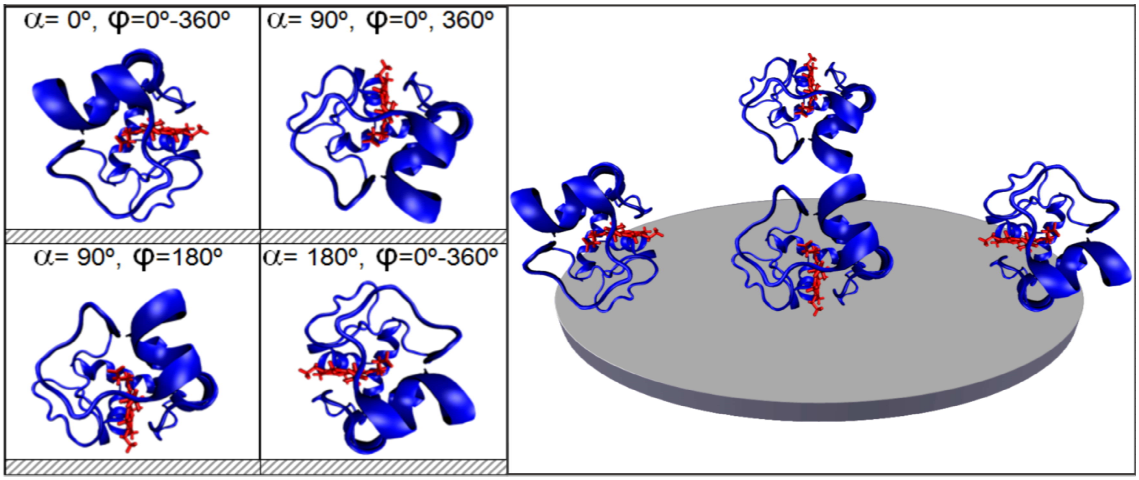

Scheme S1. Representation of the different orientations of the Cyt-SAM complexes for key α and φ values (left) and their corresponding positions in the α vs φ plots.

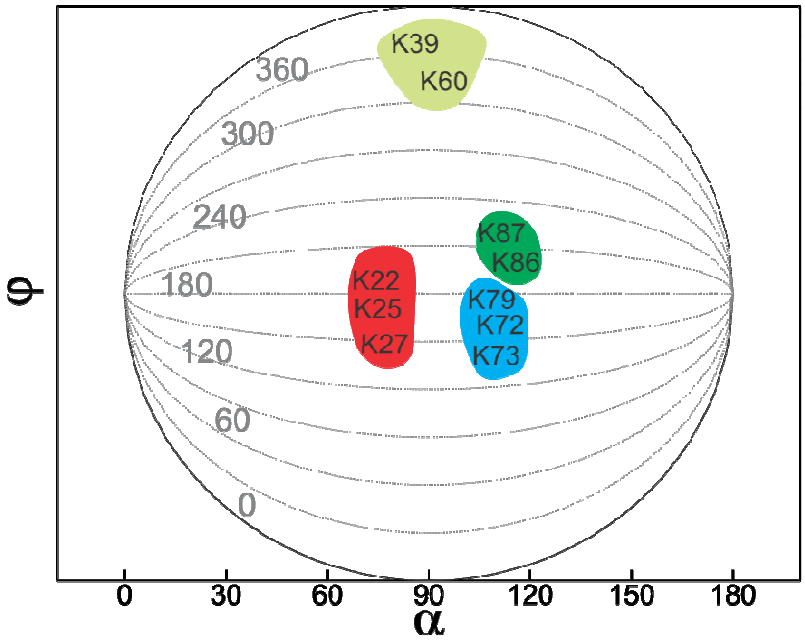

Figure S1. Representation of the key lysine (K) residues that establish Cyt-SAM contacts in the different orientations defined by the α and φ angles. Comparison with Figure 3 shows that K87 stabilizes Cyt in orientations that exhibit a low average electronic coupling. Further details regarding lysine residues and their role in the complex formation may be found elsewhere.[1,2]

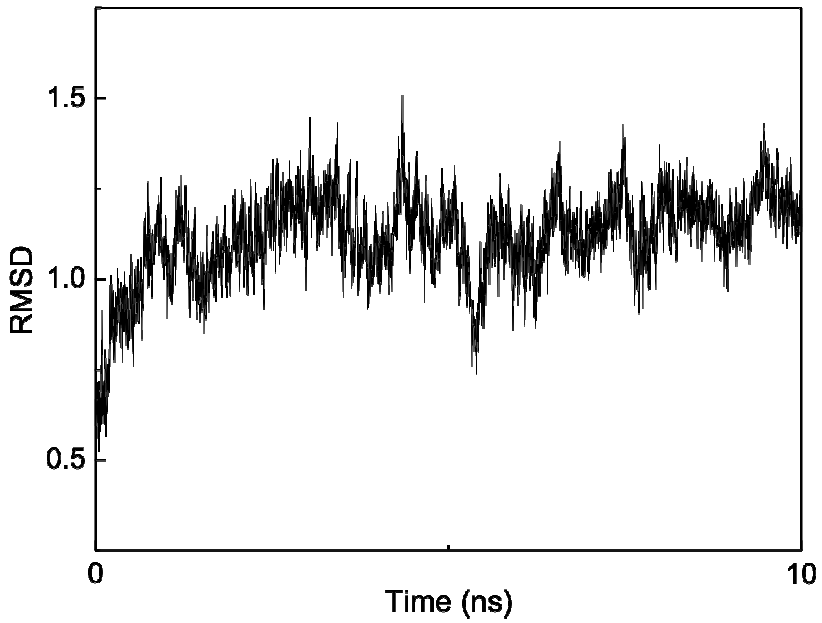

Figure S2. RMSD of the backbone structure of K87C for a 10 ns thermalization MD. The RMSD value is below 1.5 Å, indicative of a preserved protein folding.

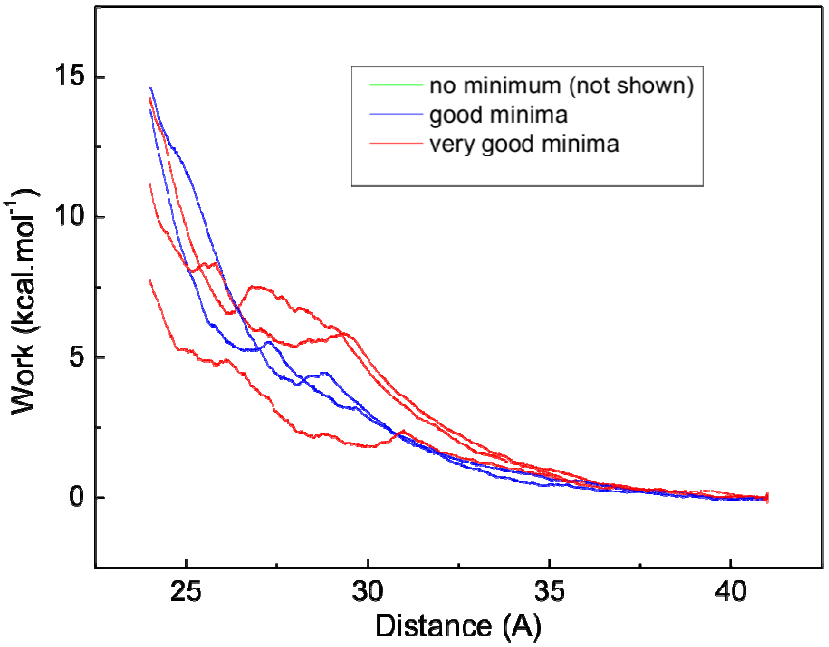

Figure S3. Work-vs-distance profiles from the SMD simulations of K87C. The trajectories that yielded an energy minimum are displayed. The structures corresponding to said minima were employed as starting point for the MD simulations in explicit solvent.

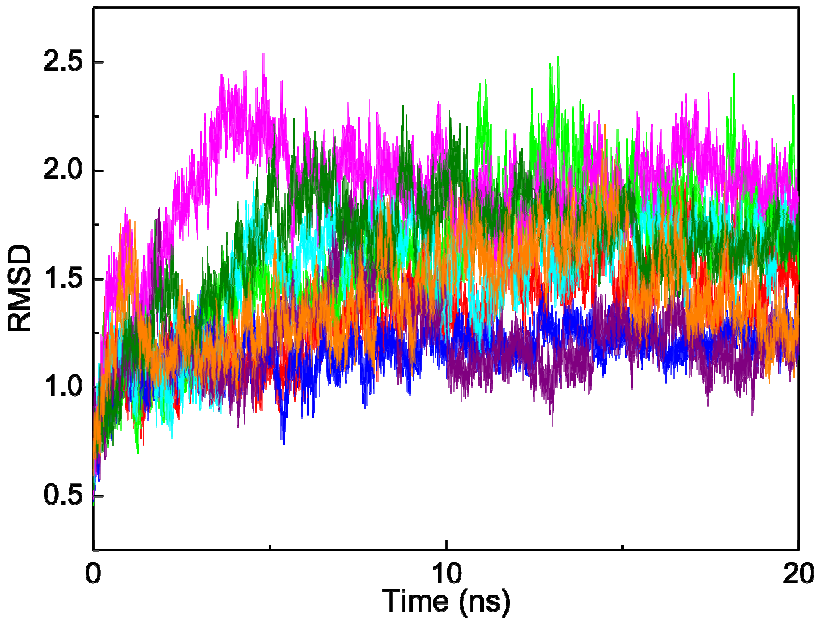

Figure S4. RMSD plots for the 20 ns MD simulations of K87C adsorbed on SAMs in explicit solvent. Each color represents a different trajectory. In every case the RMSD value is below 2.5 Å, indicative of a preserved protein folding.

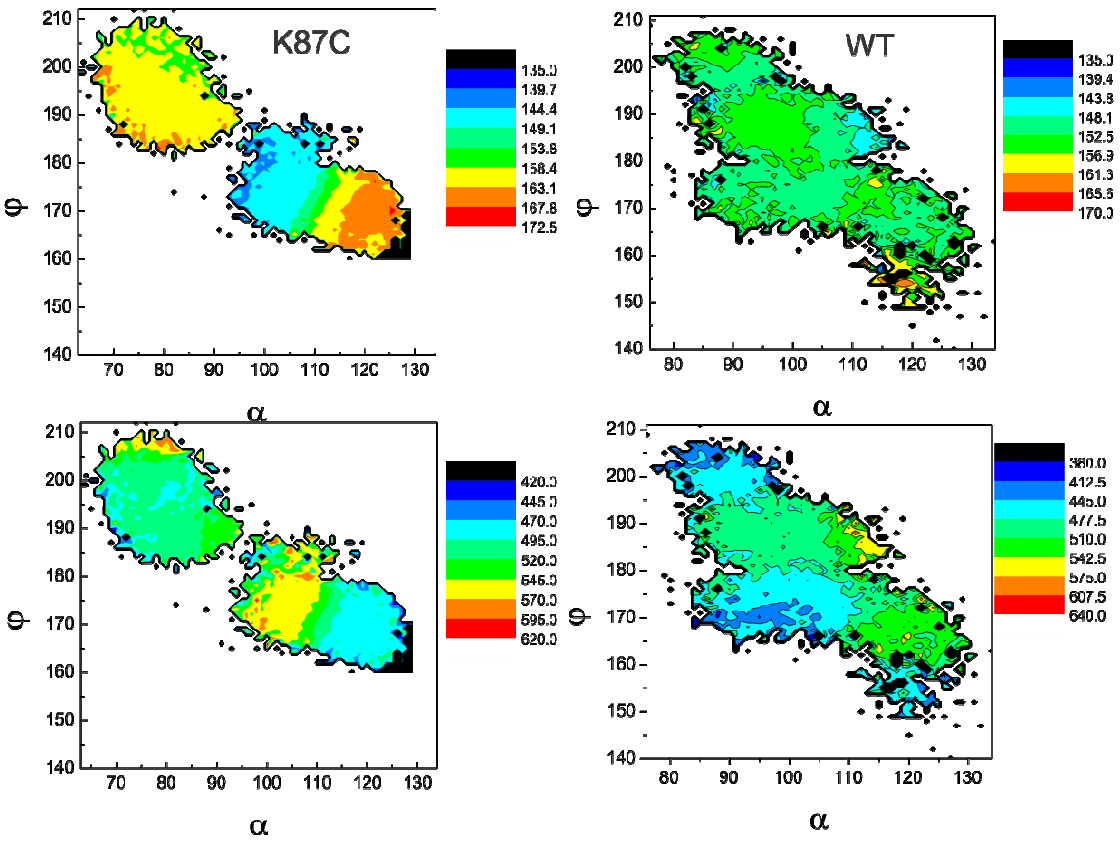

Figure S5. Dipole moment orientation vs *Z* axis (top) and modulus (bottom) for K87C (left) and WT Cyt variants (right) as a function of the orientational angles α and φ.

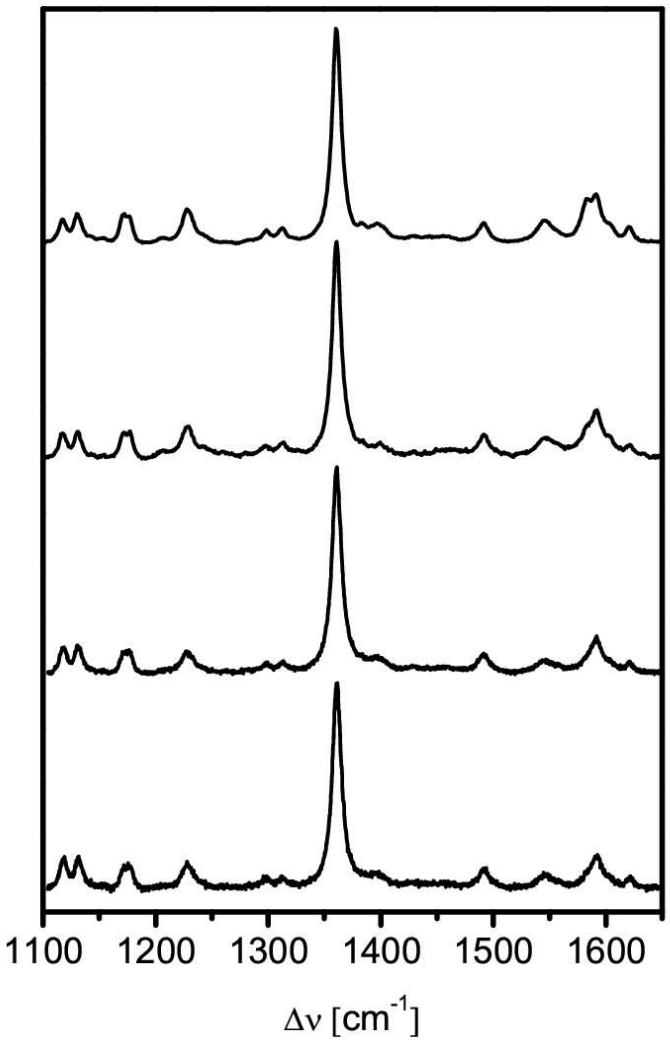

Figure S6. Comparison of SERR spectra of ferrous Cyt on a C10-SAM coated Ag electrode at -0.4 V with the RR spectra of ferrous WT Cyt and K87C. From top to bottom: RR spectrum of the WT Cyt, RR spectrum of K87C, SERR spectrum of the WT Cyt, SERR spectrum of K87C. Spectra were measured with 413 nm excitation. Reduction of the proteins in solution was achieved chemically by adding dithionite. Potentials refer to the Ag/AgCl (3M KCl) reference electrode.

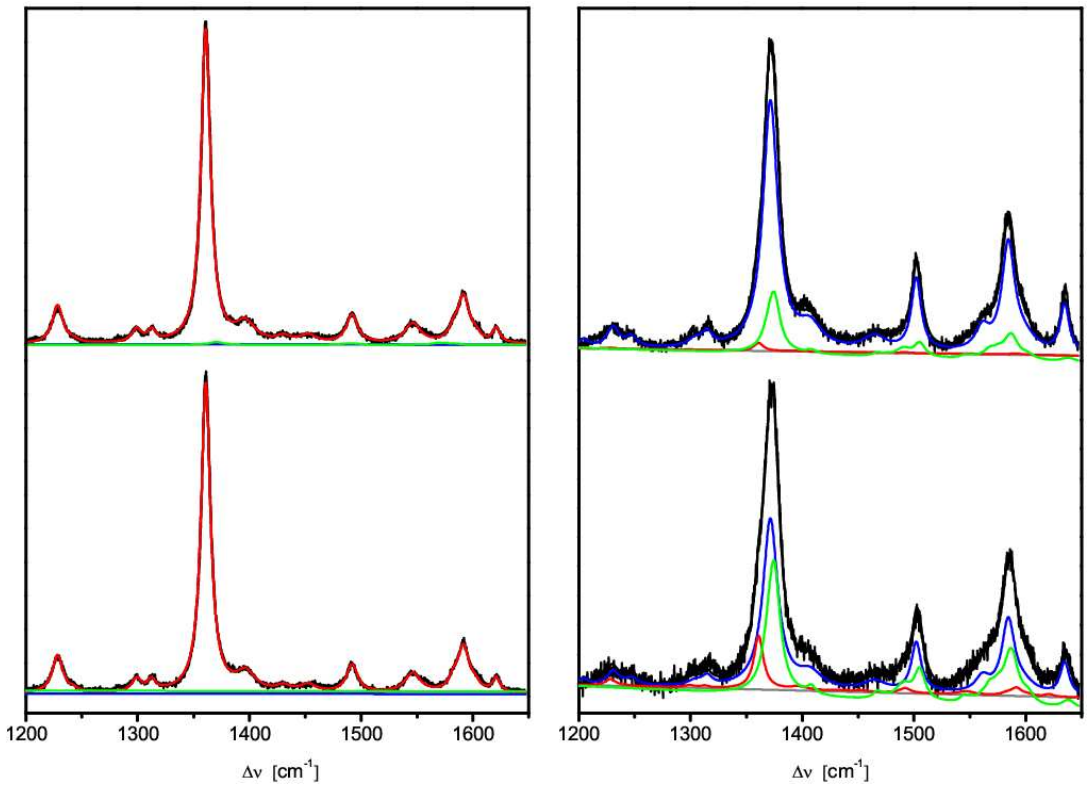

Figure S7A. SERR experimental spectra of WT Cyt (top) and K87C (bottom) on a C5-SAM coated electrode measured at -400 mV (left) and +150 mV (right) with 413 nm excitation. The component spectra of ferric B1, ferrous B1, and ferric B2 (HS and LS) are represented by the blue, red, and green lines. The baseline is displayed in grey and the overall fit (sum of the component spectra) is shown on black.

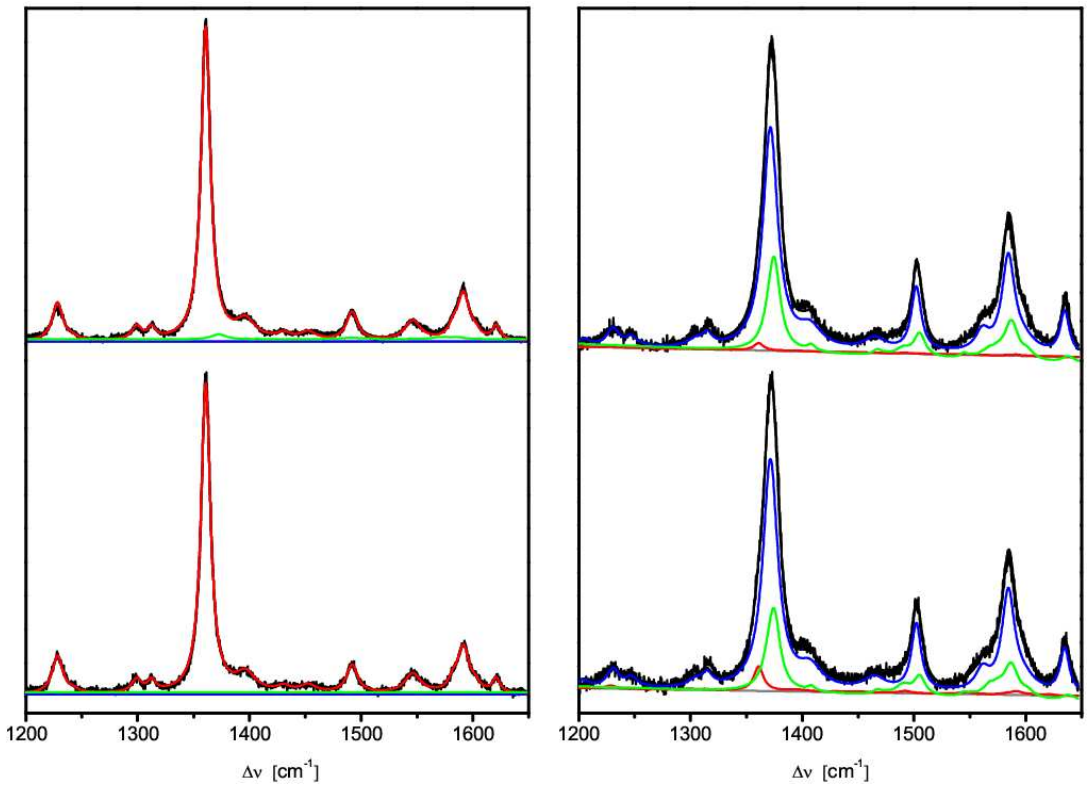

Figure S7B. SERR experimental spectra of WT Cyt (top) and K87C (bottom) on a C10-SAM coated electrode measured at -400 mV (left) and +150 mV (right) with 413 nm excitation. The component spectra of ferric B1, ferrous B1, and ferric B2 (HS and LS) are represented by the blue, red, and green lines. The baseline is displayed in grey and the overall fit (sum of the component spectra) is shown on black.

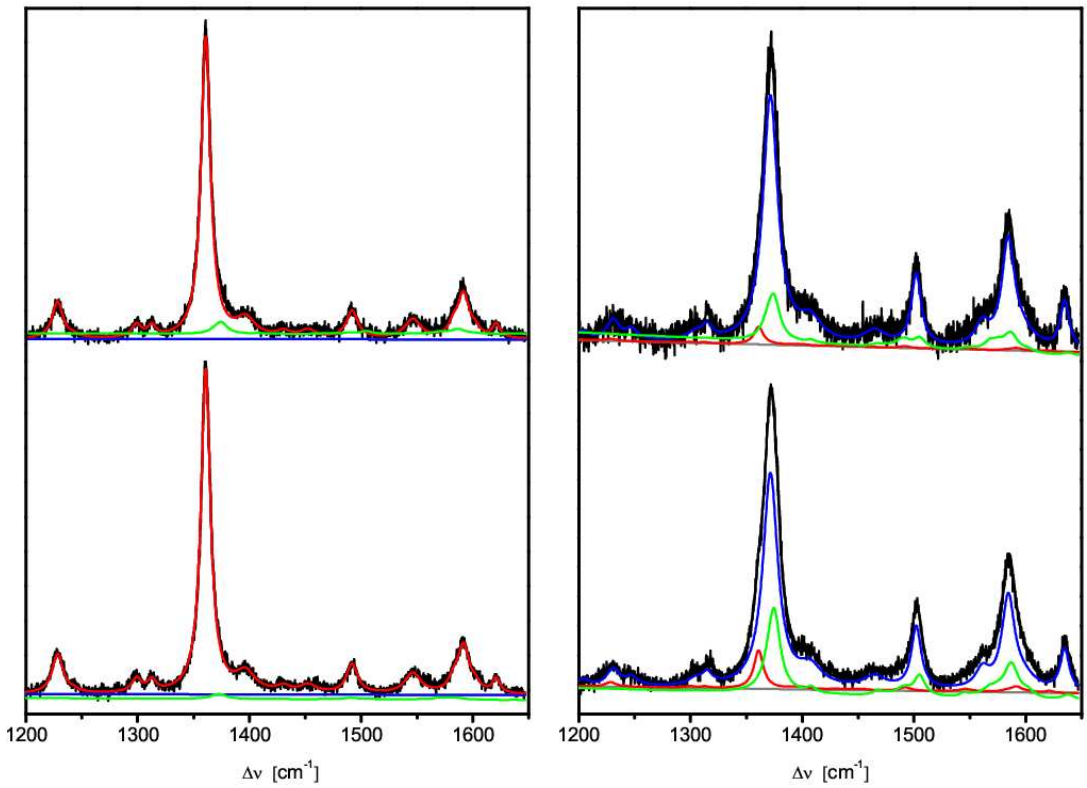

Figure S7C. SERR experimental spectra of WT Cyt (top) and K87C (bottom) on a C15-SAM coated electrode measured at -400 mV (left) and +150 mV (right) with 413 nm excitation. The component spectra of ferric B1, ferrous B1, and ferric B2 (HS and LS) are represented by the blue, red, and green lines. The baseline is displayed in grey and the overall fit (sum of the component spectra) is shown on black.

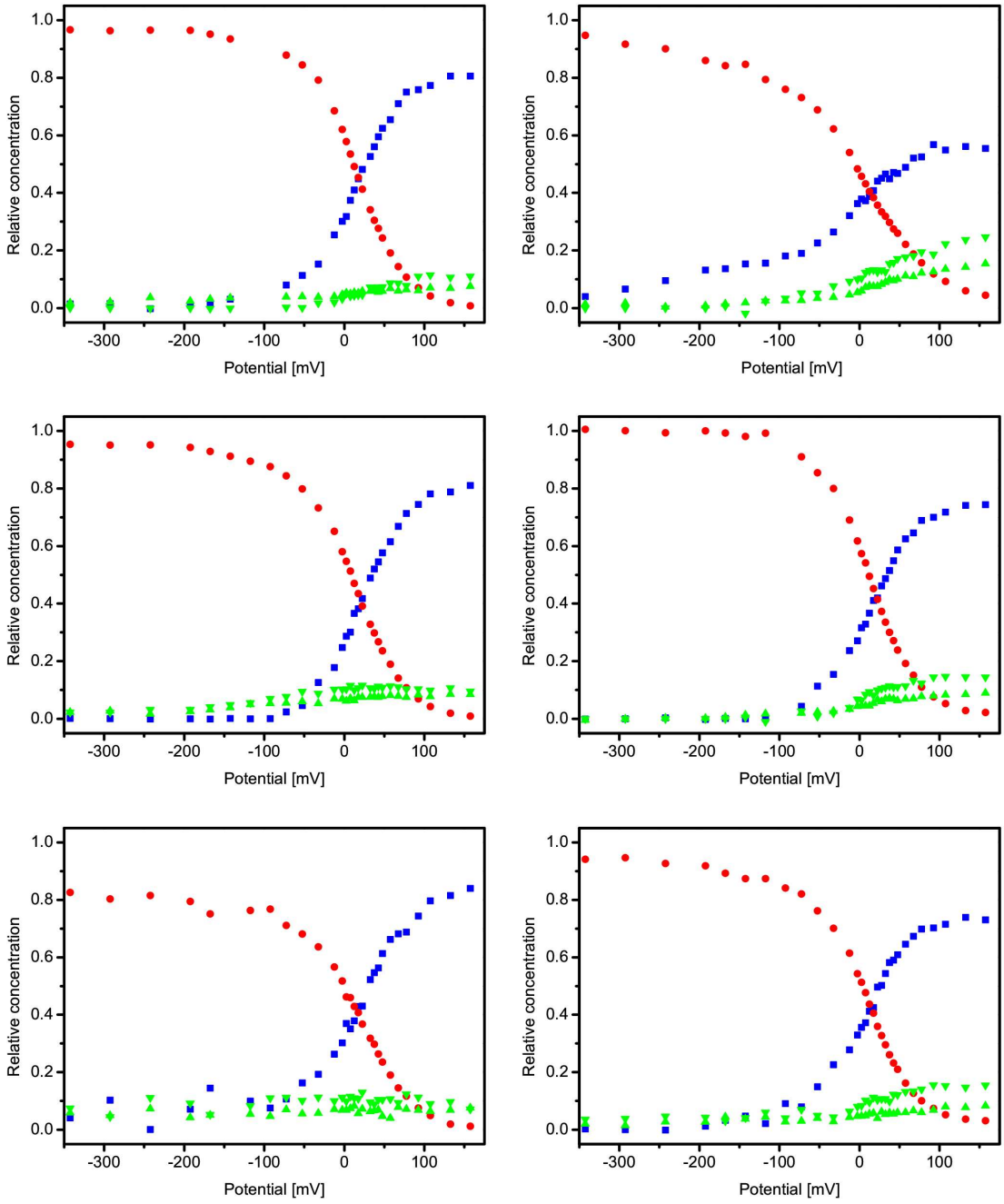

Figure S8. Potential dependence of the relative concentrations of the various Cyt species as derived from the component analysis of the experimental SERR spectra of WT Cyt (left) and K87C (right) on C5 (top), C10 (middle) and C15 (bottom) SAMs. The red circles and blue squares refer to the reduced and oxidized B1 species, respectively. The non-native oxidized B2 species are presented by green triangles (high spin – upright; low spin – reversed).

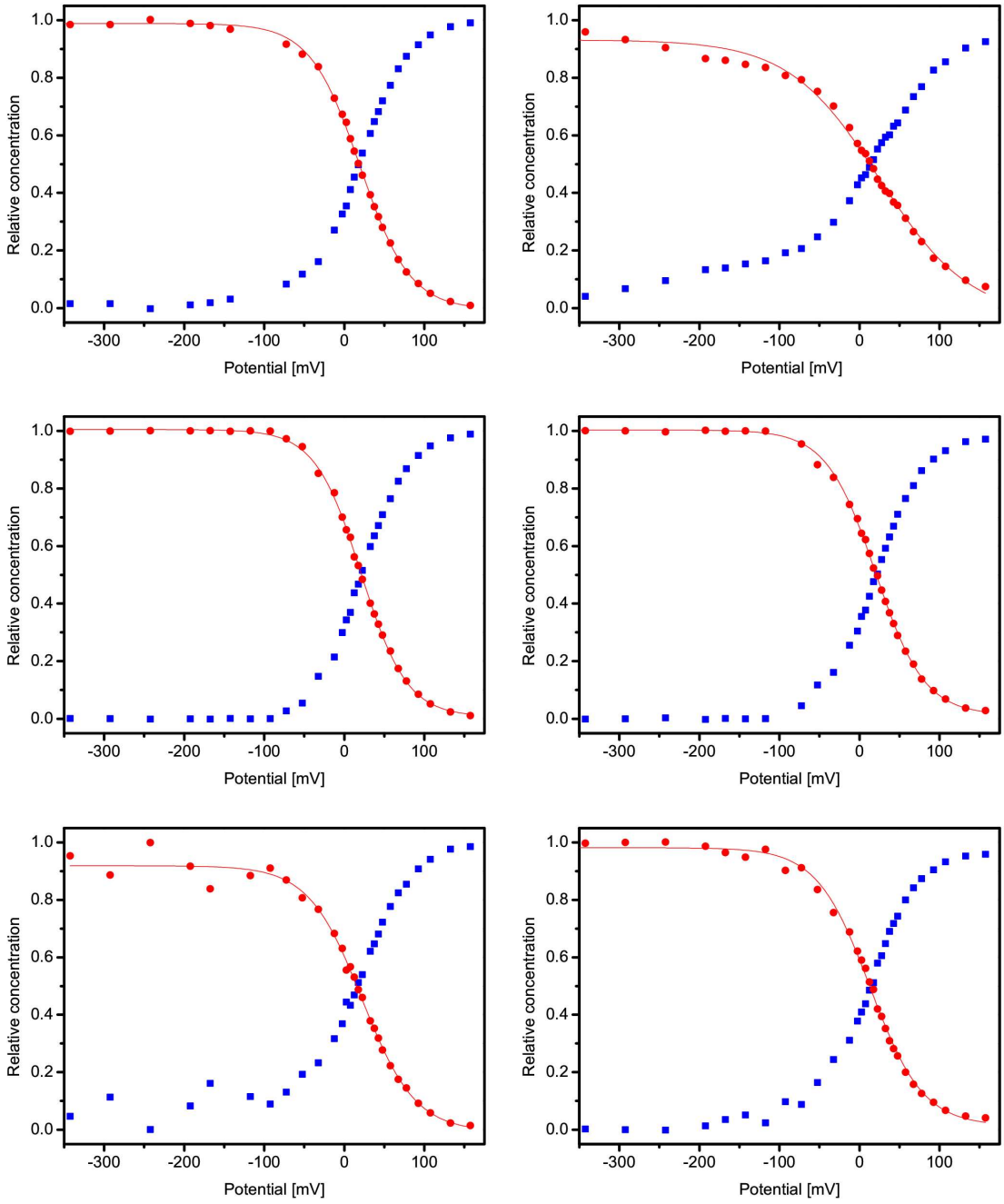

Figure S9. Nernst plots derived from the component analysis of the stationary experimental SERR spectra of the WT Cyt (left) and K87C (right) on C5 (top), C10 (middle) and C15 (bottom) SAMs. The red circles and blue squares refer to the reduced and oxidized B1 species, respectively.

References

(1) Alvarez Paggi, D.; Martin, D. F.; De Biase, P. M.; Hildebrandt, P.; Marti, M. A.; Murgida, D. H. Molecular Basis of Coupled Protein and Electron Transfer Dynamics of Cytochrome *c* in Biomimetic Complexes. *J. Am. Chem. Soc.* **2010**, *132* (16), 5769-5778 .
(2) Paggi, D. A.; Martin, D. F.; Kranich, A.; Hildebrandt, P.; Marti, M. A.; Murgida, D. H. Computer Simulation and SERR Detection of Cytochrome *c* Dynamics at SAM-coated Electrodes. *Electrochim. Acta* **2009,** *54* (22), 4963-4970.