Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information for

The Role of Salt in the Mitochondria: Returning Cytochrome c to itsNative State after its Dissociation from Cardiolipin Containing Membranes**

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Materials and Methods.

Preparation of cytochrome c solutions. Equine cytochrome *c* was purchased from Sigma-Aldrich Co. (St Louis, MO) with no further purification and dissolved in 25 mM HEPES buffer. Potassium ferricyanide was added to the protein solution to oxidize any residual ferrous forms present in the sample. The protein solution was then titrated to pH 7.0 and passed through a Sephadex G-10 column (GE Healthcare) to remove any remaining oxidizing agents and impurities. The sample was then readjusted back to pH 7.0.

Preparation of liposomes. TOCL (1,1'2,2'-Tetraoleoyl Cardiolipin) and DOPC (1,2-dioleoyl-sn-glycero-3-phosphocholine) (Avanti Polar Lipids, Birmingham, AL) were dissolved in a 2:1 chloroform/methanol mixture. Solvent was removed by rotary evaporation at room temperature and the remaining suspension was left to sit in a vacuum desiccator overnight. The lipid film was rehydrated with 25 mM HEPES buffer (pH 7.4) to obtain a concentration of 5 µM. The mixture was then left to sonify in an ice bath at 100 W for one to two hours. The solution was centrifuged for 40 minutes at 12000 rpm to remove impurities. The supernatant was extracted and left to stabilize overnight. All liposome solutions were stored under nitrogen to prevent any oxidation.

Preparation of cytochrome c-liposome complex and supernatant binding experiments. Liposomes were first diluted to the required concentration using 25 mM HEPES buffer (pH 7.4). The concentration of cytochrome *c* in all experiments was 5 µM. In order to explore the influence of Na^+ and Cl^- ion on cytochrome c – liposome complexes, aliquots of NaCl were added to the cytochrome *c* – liposome mixtures until a final concentration of 100 mM was reached. Unbound cytochrome c was investigated by centrifuging cytochrome *c* - liposome mixture with a Beckman Optima TLX Ultracentrifuge (TLA-100.3 rotor, 50000 rpm) for 2 hours at

room temperature. The supernatant was immediately extracted to prevent any rebinding. In order to probe the direct influence of NaCl on unbound/dissociated cytochrome c aliquots of NaCl were added to the supernatant until a final salt concentration of 100 mM was reached.

Electronic Circular Dichroism Spectroscopy. Spectra were obtained using a Jasco J810 Spectrapolarimeter, which was purged and cooled with gaseous nitrogen. Aqueous cyt *c* - liposome solutions were measured with a 1 mm quartz cell purchased from International Crystal Laboratories (Gardfield, NJ). For the far-UV region, the spectra were measured in the range from 180-350 nm with a scanning speed of 500 nm/min, a data pitch of 0.05 nm, a bandwidth of 5 nm, and a response time of 0.5 seconds. Five spectra were accumulated per sample at 20˚ Celsius. Smoothing of spectra was performed using the Sigma Plot smoother function for the far-UV region. Care was taken to ensure that the general shape of the spectrum was not affected. For the Soret band region, the same parameters were used and the spectra were measured between 300 and 800 nm using a 1 cm quartz cell. The temperature was controlled with a Peltier solid-state heating and cooling module. All spectra were solvent corrected using Jasco spectral analysis program.

Dynamic Light Scattering. Particle radii measurements were obtained using a Horiba Lb-500 Dynamic Light Scattering Particle Size Analyzer (Edison, NJ). A 10 mm path-length quartz cuvette was used to collect data at room temperature.

CD Figures.

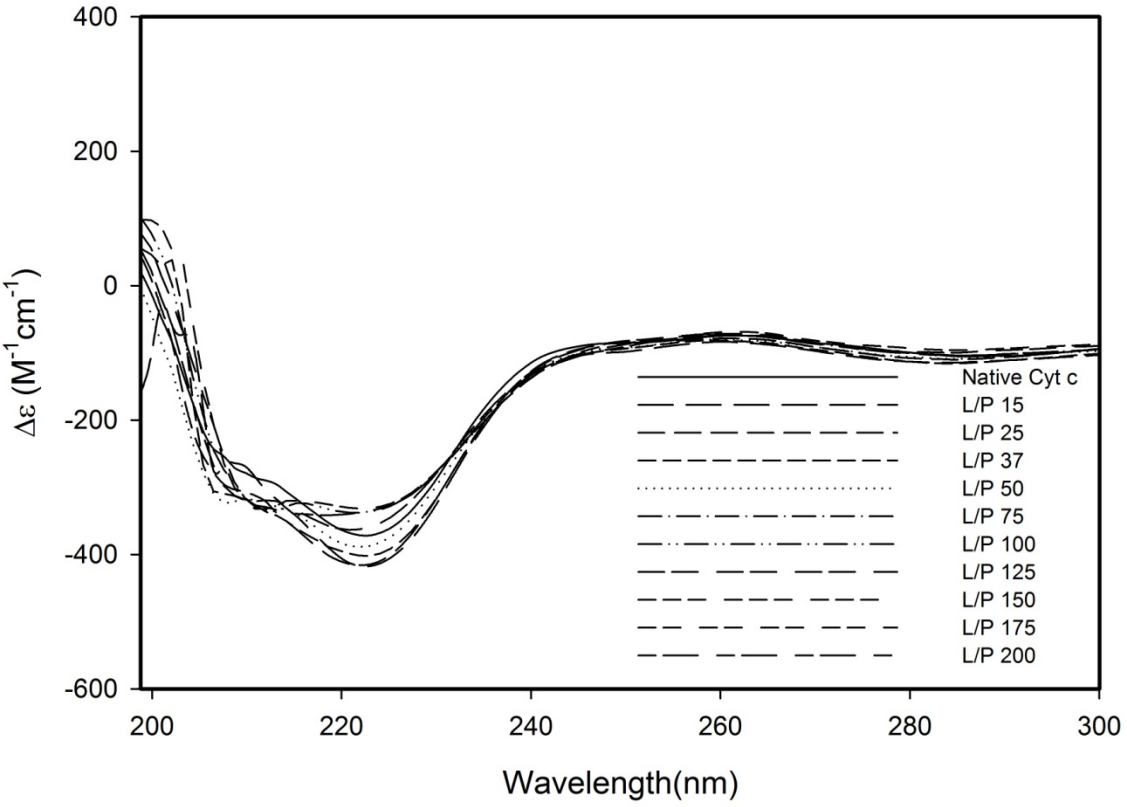

Figure S1: Far UVCD of cytochrome-liposome mixtures measured at the indicated lipid/protein ratios. Spectroscopic and solution parameters used to obtain these spectra are described in the Materials and Methods section.

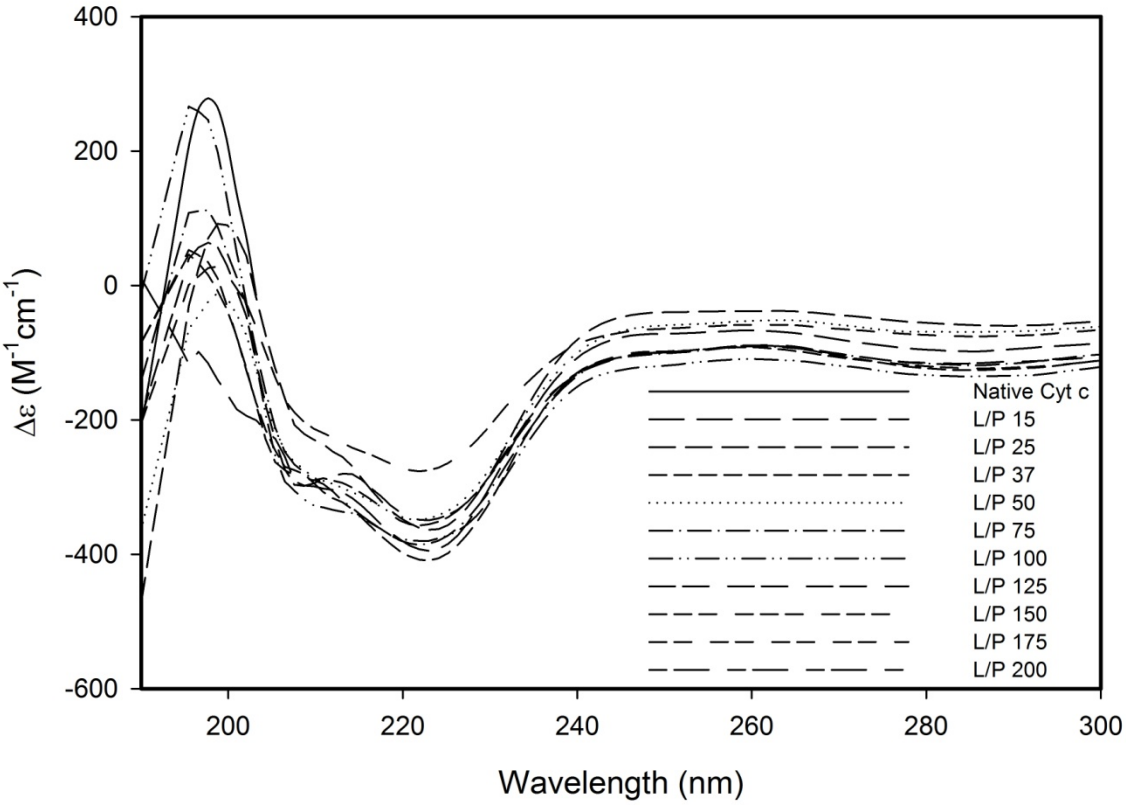

Figure S2: : Far UVCD of protein-liposome mixtures containing 100 mM NaCl measured at the indicated lipid/protein ratios. Spectroscopic and solution parameters used to obtain these spectra are described in the Materials and Methods section.

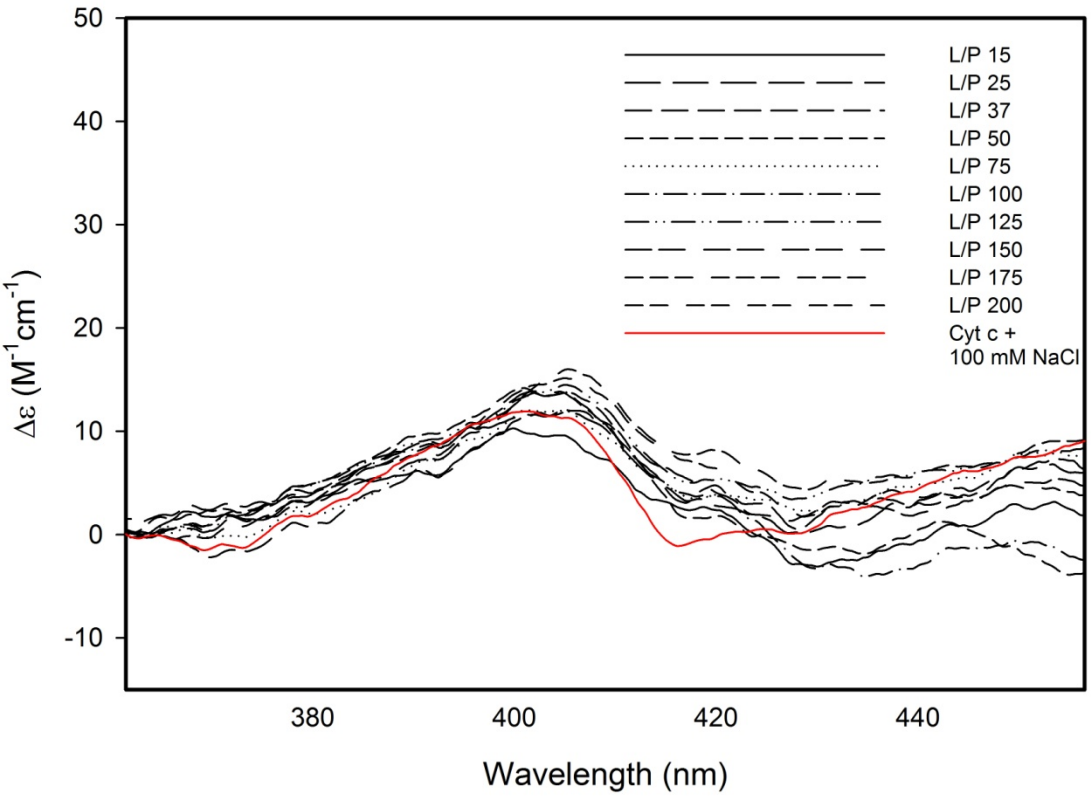

Figure S3: Soret band CD of ferricytochrome c in the supernatant obtained from a centrifugation of the indicated liposome/protein mixtures. NaCl was added to the supernatant prior to each measurement until a concentration of 100 mM was reached. For comparison the CD spectrum of native cytochrome c measured at physiological pH in the presence of 100 mM NaCl was added in red.