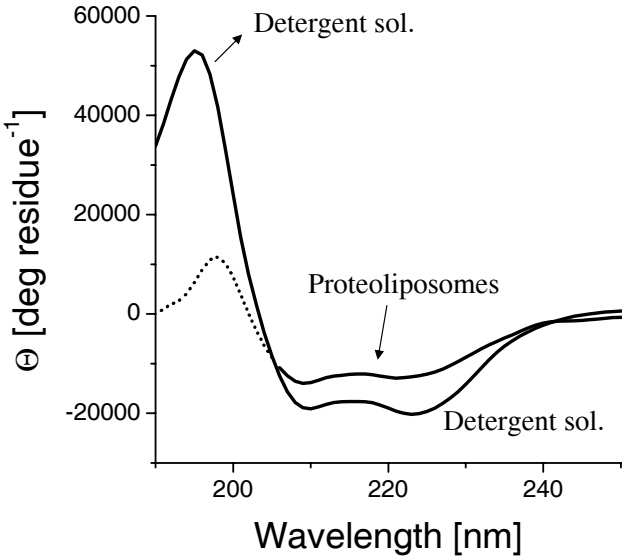

Figure S1: CD spectra of cbo_3 in detergent and after reconstitution (20° C, 5 mM phosphate buffer, pH 7.4).

Supporting information

Protein reconstitution and CD spectra

Circular dichroism (CD) spectroscopy has been used to confirm that cytochrome bo_3 (cbo_3) retains its integrity during reconstitution in *E. coli* 'polar' lipid vesicles. Figure S1 shows the CD spectra of the detergent solubilised protein (in 0.05% DDM) and cbo_3 after reconstitution (proteoliposomes). The protein concentration of the CD samples was determined using Schaffer-Weissman protein assay. For the proteoliposomes it was not possible to obtain good spectra at < 205 nm, due to light scattering of the vesicles, resulting in a depression of the signal at these wavelengths. Diluting the sample further resulted in a poor signal-to-noise ratio. Analysis of the CD was performed, but in line with literature,[1] the results of both samples did not correspond with the secondary structure obtained from the crystallographic data.[2] Still, both CD spectra - with bands at 220, 210 and 195 nm - are characteristic for α-helical proteins and confirm that cbo_3 is in its native from in the proteoliposomes.

Analysis of the electrochemical impedance spectroscopic (EIS) data

The equivalent circuit for a tBLM can be described as $R(R_mC_m)C_s$ in which the elements in brackets are parallel, R is the solution resistance ($\sim$50 Ω cm^2), R_m is the membrane resistance, C_m is the double layer capacitance of the membrane and C_s the double layer capacitance of the underlying surface. Data analysis indicated that this model was not able to accurately fit the data. Also when the capacitance elements were modeled using

Constant Phase Elements, often used to represent surface roughness, no adequate fit could be obtained (see Figure S2).

Instead, the data indicated that R_m and C_m exhibited a distribution of time-constants and best fits were obtained when modelling the dielectric relaxation of the membrane. This was done by equation (1) as described in ref.[3,4] which combines the empirical analytical expression of Havriliak and Negami (HN)[5] with a Constant Phase Element. The groups of Sackman and Wagner have shown that dielectric relaxation ecan be used to represent equivalent circuits containing multiple parallel RC elements and is useful when modeling supported lipid bilayer with lateral heterogeneities.[6]

$$Z = \frac{1}{(i\omega)^{\beta}\omega_0^{1-\beta}[\frac{\Delta C}{1+(i\omega\tau)^{\alpha}} + C_{\mathrm{inf}}]} \tag{1}$$

in which C_{inf} is the double capacitance of the membrane and ΔC the addition low-frequency capacitative element with the relaxation time constant τ. ω is the impedance frequency and α and β are variables that describe the relaxation and roughness of the surface (CPE element), respectively. As expected for the flat surfaces of the template stripped gold substrates, β is close to 1 (Table S1). Equation 1 does not only account for C_m, but also for its relaxation properties, thus replacing R_m. (Thus, the data is fitted with equation (1) in parallel with the solutions resistance only). The reason for this can be understood if one realises that $C_{inf} + \Delta C$ is the capacitance observed at the low-frequency limit as C_s would have done in the above circuit description [$R(R_mC_m)C_s$]. C_s could not always be determined using frequencies > 0.1 Hz and, for a similar reason, ΔC remains unresolved when fitting certain data.. Analysis indicated that the parameters τ and ΔC were highly correlated. When ΔC cannot be determined accurately by fitting the data, a value for τ can therefore not be given. Instead, Figure S2 shows C_m ['back' calculated using equation (1)] as a function of ω for the fits shown in Figure 2 of the article.

Figure S2 shows that at frequencies $> 10^3$ s^{-1} the bilayer capacitance is roughly equal to C_{inf}. At frequencies $< 10^3$ s^{-1}, C_m increases indicating that in this time domain ions can either pass the lipid bilayer or the dielectric of lipid bilayer relaxes. Finally, it can be seen that, in line with the Bode plots, C_m increases more steeply for tBLMs with cbo_3 than without, suggesting that the presence of cbo_3 slightly increases the permeability of the membranes. This can be either due to small defects or proton transfer via the cbo_3.

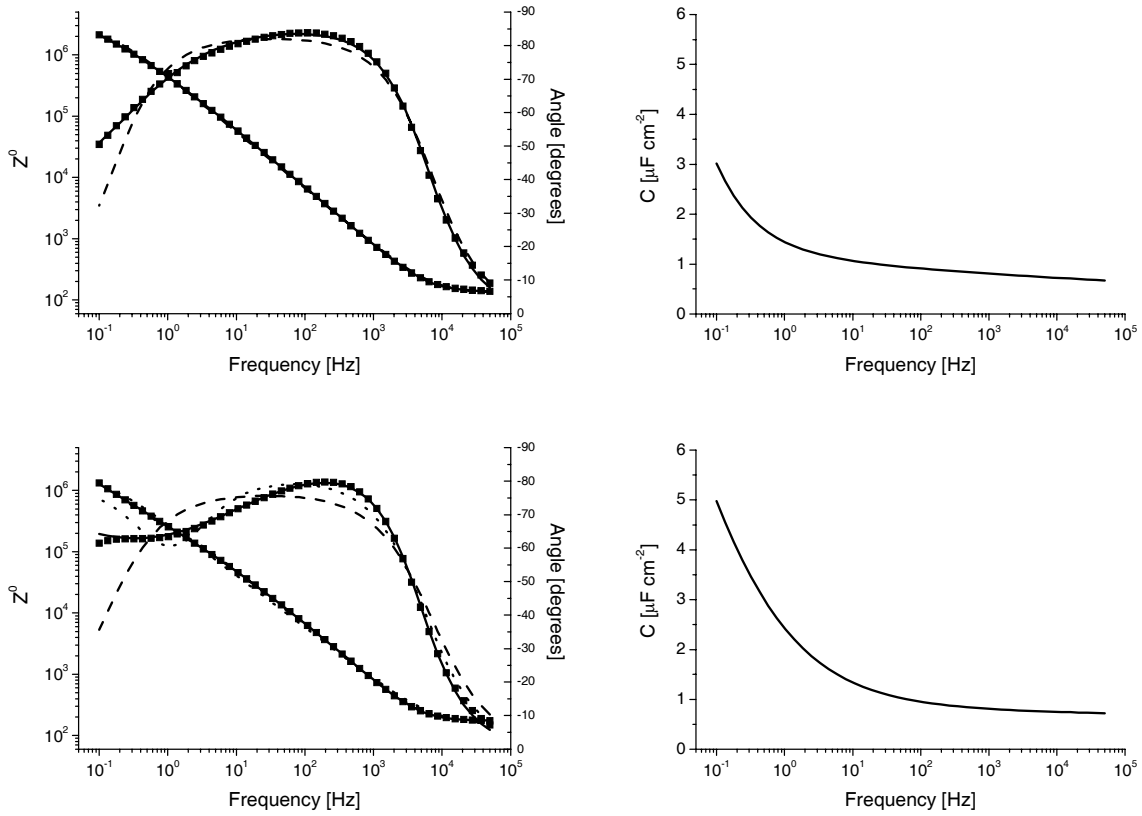

Figure S2: (Left) Bode Plots of the same data shown in Figure 2 of the article. The lines represent the best fits using the following equivalent circuits. (solid line) RC_m, in which C_m is modelled with eq. 1; (dashed line) $R(R_mC_m)$, in which C_m is modelled with a CPE; (dotted line) $R(R_mC_m)C_s$, in which C_m and C_s are modelled using CPEs. For the fits of the solid lines the fitted C_m as a function of frequency is given in the graphs on the right. (Top) Control vesicles (without cbo_3); (Bottom) Proteoliposomes.

Table S1: Parameters obtained when fitting the data shown in Figure S2.

Data	Equivalent Circuit	Element	Parameter	Value
Without cbo_3	$R(R_mC_m)$	R_m	R	0.7 $M\Omega cm^2$
		C_m (CPE[a])	Q^0	1.6 $\mu F cm^{-2}$
			α	0.92
	RC_m	C_m (Eq. 1)	ΔC	n.d.
			C_{inf}	1.2 $\mu F cm^{-2}$
			τ	n.d.
			α	0.60
			β	0.95
With cbo_3	$R(R_mC_m)$	R_m	R	0.5 $M\Omega cm^2$
		C_m (CPE)	Q^0	2.9 $\mu F cm^{-2}$
			α	0.85
	$R(R_mC_m)C_s$	R_m	R	0.03 $M\Omega cm^2$
		C_m (CPE)	Q^0	3.3 $\mu F cm^{-2}$
			α	0.91
		C_s (CPE)	Q^0	4.5 $\mu F cm^{-2}$
			α	0.89
	RC_m	C_m (Eq. 1)	ΔC	14 $\mu F cm^{-2}$
			C_{inf}	0.88 $\mu F cm^{-2}$
			τ	9 s^{-1}
			α	0.46
			β	0.98

[a] Constant Phase Element using $Z = 1/((i\omega)^{\alpha}Q^0)$

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