Supplementary Information:
Incorporating H\(^+\) - and Na\(^+\) -specific probes into the luminal volumes of PLs and SMPs

i) 8-hydroxypyrene-1,3,6-trisulphonate (pyranine) and carboxy-SNARF-1 (Invitrogen Molecular Probes) are pH-dependent fluorescent probes; pyranine has been incorporated into PLs and used to study H\(^+\) -translocation by ATP synthase (1,2), and carboxy-SNARF-1 has been used to measure internal pH values in cell lines and organelles (3,4). To test whether they can be incorporated into the PL lumen, the probes were added to the incubation prior to the addition of the Biobeads (see Experimental Procedures), at concentrations of 0.1 – 10 mM pyranine or 50 μM carboxy-SNARF-1. However, pyranine adsorbed strongly to the Biobeads, and the high concentrations required to compensate for this led to slow but irreversible loss of complex I activity. Using α-cyclodextrin as an alternative to Biobeads (5) (40 – 80 mM (Sigma-Aldrich) was added in 10 mM steps over 1 – 12 hours) produced active pyranine-loaded PLs, but they were poorly-coupled and we were unable to separate them from the highly viscous cyclodextrin-detergent complex. Extrusion and freeze-thaw procedures that attempted to fracture and recombine PLs (6) in the presence of pyranine also led to losses in activity and/or coupling. Conversely, coupled PLs containing carboxy-SNARF-1 could be prepared, but we were unable to eliminate the probe from the external surface of the PLs; the same problem has been described previously (7).

ii) Sodium Green (8) is a fluorescent Na\(^+\) -sensitive dye that is based on a crown ether Na\(^+\) -binding functionality. It is a hydrophilic and relatively Na\(^+\) -specific example of a family of crown-ether based Na\(^+\) -sensitive dyes that include the SBFI (sodium-binding benzofuran isophthalate) dye that is widely used in cellular studies (9). Sodium Green could be successfully incorporated into complex I-containing PLs by adding it before the Biobeads (2 – 10 μM, from Invitrogen Molecular Probes, in a darkroom to prevent photobleaching). However, even though the cell-impermeable form was used, it leaked out of the vesicles within 30 – 60 s of them being diluted into an experimental solution; the same result was observed with protein-free liposomes. When the Sodium Green was pre-incubated with SMPs, in order to load them with the dye, it was similarly lost rapidly from the vesicular lumen when diluted into the experimental solution.

iii) TCHpH and Glu3 are large, pH-dependent, dendrimer-based probes that differ significantly from the fluorescent probes described above (10,11). TCHpH (2 – 5 mg mL\(^{-1}\)) (11) and Glu3 (1 – 3 mg mL\(^{-1}\)) (10) (kindly provided by Professor S. Vinogradov, University of Pennsylvania) could be trapped inside protein-free liposomes formed using Biobeads, but when complex I was present only PLs with very low activity could be formed, and the probes did not respond to complex I-mediated ΔpH formation. The probes do not inhibit complex I directly so they probably interfere with its reconstitution, and are not incorporated into enzyme-containing vesicles.

iv) Pyranine was included in the sonication step that is used to form SMPs (see Experimental Procedures) but it compromised the coupling and the rates of both NADH oxidation and ATP hydrolysis; its final concentration was very low, and it did not respond to expected changes to the intravesicular pH. Subsequently, ferricyanide was included during the sonication step, and then a highly sensitive iron assay (see Experimental Procedures) was used to evaluate the level of incorporation into the vesicular lumen; no iron could be detected. Together, these observations suggest that when SMPs form the intravesicular space does not mix with the external solution; the SMPs form by existing cristae structures closing off. As for PLs, extrusion and freeze-thaw procedures led only to the loss of complex I activity and/or coupling.

References


