

Supporting online material

Figure S1:

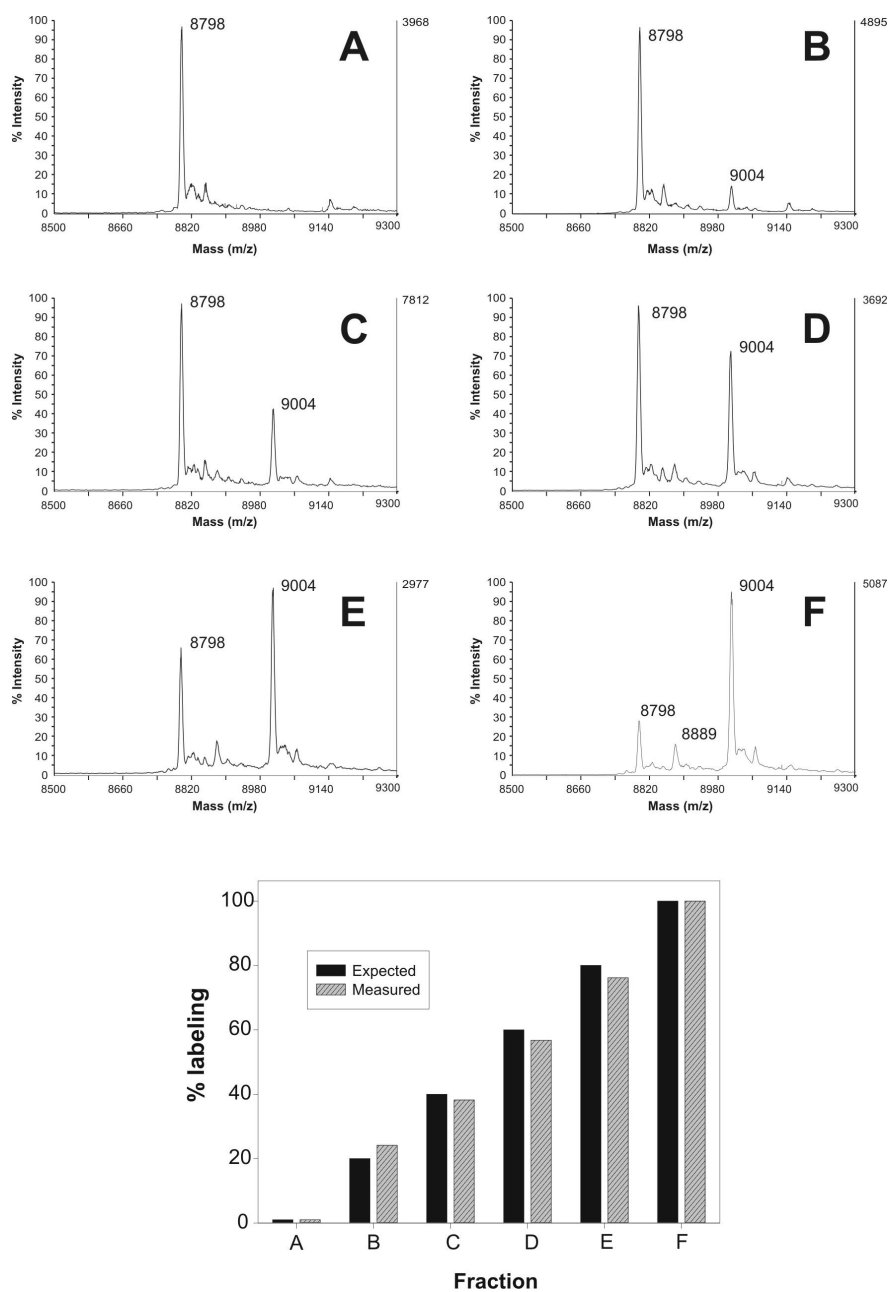


Figure S2:

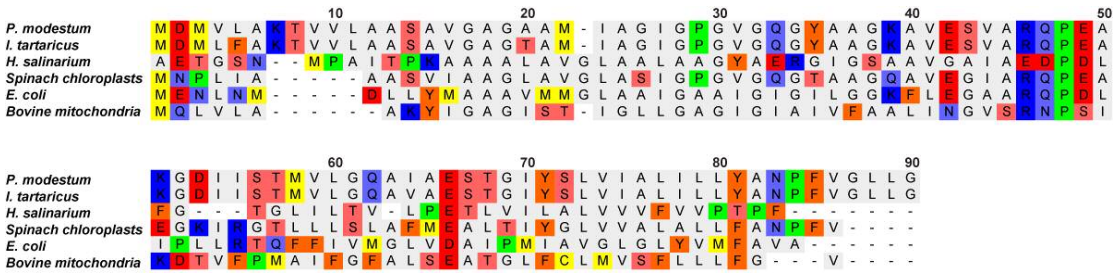
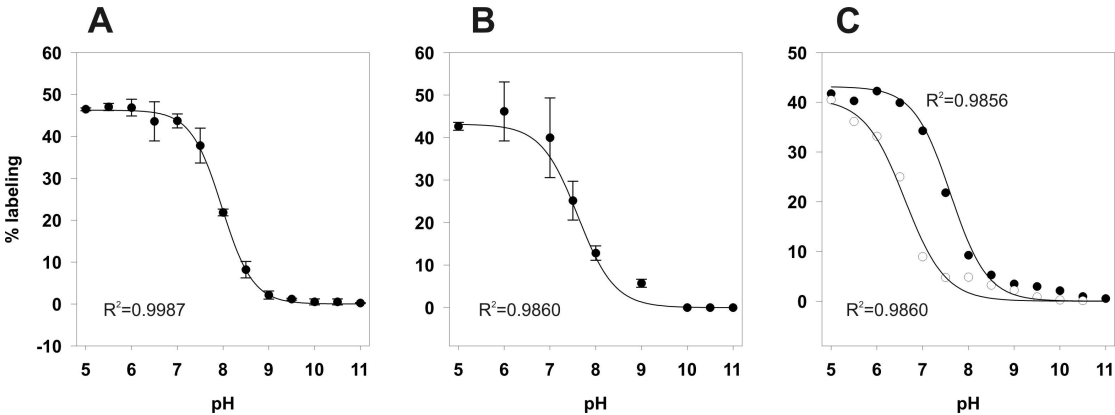


Figure S3:



Legends:

Figure S1

Top: MALDI TOF spectra of mixtures between DCCD-modified and unmodified c subunits. The c subunits were modified by incubation of the isolated c_{11} ring of the *I. tartaricus* enzyme (1) and the modified c subunits (M) were extracted with organic solvent. The unmodified c subunits (U) were prepared the same way from c_{11} which was not treated with DCCD. The two samples were mixed in the ratios (U:M) indicated below and subjected to MALDI mass spectroscopy (2). A, (100:0); B, (80:20); C, (60:40); D, (40:60); E, (20:80); F, (0:100). Bottom: The intensities of the peaks from these

measurements (A-F) were used to calculate the relative amount of c subunit modification. These values are compared with the expected ratios.

Figure S2:

Amino acid sequence alignment of the c subunits investigated in this study. The inner helix (residues 1-46) is separated by a conserved hydrophilic loop (residues 47-50) from the outer helix, harbouring the conserved acidic residue (66). Amino acids in the proximity of the ion binding site enclose residues 29-35 on the inner helix and residues 65-72 on the outer helix.

Figure S3:

Mathematical fitting of the sigmoidal pH dependencies.

Sigmoidal fitting was performed on the pH profiles of the ATP synthases of *Halobacterium salinarium* (A), *Ilyobacter tartaricus* (B), and the recombinantly expressed enzyme of *I. tartaricus* (C) in the absence (filled circles) and the presence of 15 mM Na⁺ (open circles). Depicted are the plots of a 3-parameter sigmoidal fitting and the corresponding R²-values. The program Sigma-Plot was used for presentation and mathematical calculation of the curves.

References:

- (1) Meier, T., Matthey, U., von Ballmoos, C., Vonck, J., Krug von Nidda, T., Kühlbrandt, W., and Dimroth, P. (2003) Evidence for structural integrity in the undecameric c-rings isolated from sodium ATP synthases. *J. Mol. Biol.* 325, 389-397.
- (2) von Ballmoos, C., Appoldt, Y., Brunner, J., Granier, T., Vasella, A., and Dimroth, P. (2002) Membrane topography of the coupling ion binding site in Na⁺-translocating F₁F₀ ATP synthase. *J. Biol. Chem.* 277, 3504-3510.