

## Supporting Information

### Mass spectrometry analysis of isolated bovine F-ATP synthase

Mass spectra of isolated bovine F-ATP synthase obtained as described in the text are shown in Supporting Figure 1.

### Assay system for F-ATPase activity

The present system contained pyruvate kinase for phosphate transfer from and lactate dehydrogenase for oxidation of NADH coupled with reduction of pyruvate to lactate (Pullman et al, 1960). The reaction mixture used in the present work contained NADH (200  $\mu$ M), ATP (2.5 mM), phosphoenolpyruvate (2.5 mM), lactate dehydrogenase (10  $\mu$ g/mL), and pyruvate kinase (10  $\mu$ g/mL) at 20 °C. The reaction was initiated by addition of an appropriate amount of the purified F-ATPase in 10  $\mu$ L to 2 mL of the reaction mixture. The rate of NADH oxidation was monitored at 340 nm. Under the present conditions, a clear lag-phase was detectable in the initial 20 sec. Thus, from the absorbance decrease from 20 sec to 80 sec (after the initial lag), the enzyme activity was determined. Although the absorbance decrease is not completely linear with respect to the elapsed time between 20 sec and 60 sec after the initiation of the enzyme reaction, the absorbance decrease (obtainable within 1.6% accuracy) was proportional to the amount of enzyme in the reaction mixture. This confirms the reliability of the measurement for assessment of the enzyme activity of each enzyme sample. For activity measurements of the two-dimensional crystals, the crystalline suspension (10  $\mu$ L/mL) was added to the reaction mixture containing all of the constituents as described above except for ATP. The reaction mixture was stirred with a magnetic stirrer to give a constant apparent absorption at

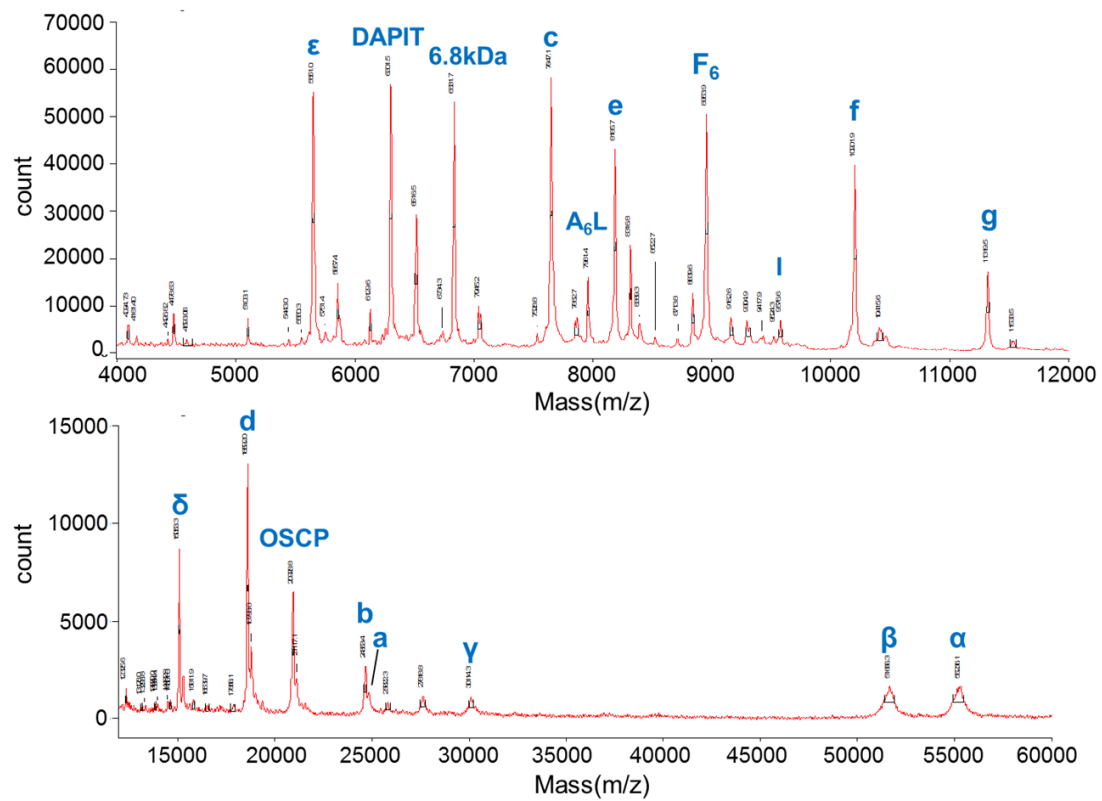
340 nm. The enzyme reaction was started by addition of ATP. Under these conditions, a significant initial lag-phase was not detected in the rate of absorbance decrease at 340 nm. However, the activity was determined by the absorbance decrease at 340 nm from the initial 20 sec to 80 sec for comparison of the activity of the crystalline sample with the solubilized sample. The amount of the enzyme in the two-dimensional crystalline sample was estimated by the amount of the enzyme sample applied to each dialysis button, assuming that all enzyme molecules are incorporated into the two-dimensional lattice.

### **The enzyme activity in the crystalline state**

The negatively-stained EM images of the two-dimensional crystalline sample prepared as described above showed that essentially all of the enzyme molecules were incorporated into the two-dimensional crystal lattice. This is consistent with the observation of much lower (about 10%) specific enzyme activity of the two-dimensional crystal suspension compared with that of the homogeneously solubilized enzyme. In general, any enzyme in crystalline state is expected to have much lower specific activity than that of the enzyme in solution. This effect depends upon the size of the crystal, since the effective enzyme concentration is decreased by crystal formation. Furthermore, protein-protein contacts in the crystal lattice are likely to restrict the conformational changes and to decrease the apparent enzyme activity.

After addition of dodecylmaltoside to a concentration of one CMC (critical micelle concentration) (0.01% w/w), the enzyme activity of the two-dimensional crystal suspension increased up to a level similar to the activity level observed before crystallization (90 – 100 %). Furthermore, the enzyme activity of the

detergent-treated two-dimensional crystals showed an oligomycin sensitivity of 75-85% which is also similar to the sensitivity observed before the two-dimensional crystallization (90%). Dodecylmaltoside is unlikely to cause deterioration of the enzyme because the enzyme preparation used for the present two-dimensional crystallization is stabilized with decylmaltoside, a detergent which is quite similar to dodecylmaltoside. Thus, it is quite unlikely that a trace amount of enzyme molecules which are not included in the two-dimensional crystal lattice are stimulated upon addition of the detergent to produce high enzyme activity and oligomycin sensitivity. Therefore, the high specific activity and oligomycin sensitivity strongly suggest that the two-dimensional crystal is composed of intact and non-impaired F-ATP synthase complexes.



**Supporting Figure 1.** Mass spectroscopic analysis of isolated bovine F-ATP synthase.