

Supplementary information

(A) Statistics of photon bursts.

Photon burst were selected automatically from fluorescence time traces as described in Material and Methods. Table II summarizes the number of all photon bursts (donor only bursts are removed) and the number of bursts with one FRET level, that with two FRET levels and that with three and more FRET levels. The direction of a FRET transition is obtained only from bursts which show at least two FRET levels, i.e. with at least one transition. A FRET level was recognized as such, when the average FRET efficiency calculated in 1 ms intervals was constant within the standard deviation of 0.15 for at least 5 ms.

Table II: Statistics of photon bursts under different experimental conditions.

Conditions	Total number of photon bursts	Bursts with one FRET level	Bursts with 2 FRET levels	Bursts with 3 and more FRET levels	Transitions between FRET level with ATP hydrolysis sequence H→M→L→H	Transitions between FRET level with ATP synthesis sequence H→L→M→H
AMPPNP binding	965	888 (92.0%)	77 (8%)	0	48 (62.3%)	29 (37.7%)
ATP hydrolysis	2491	2037 (81.8%)	209 (8.4%)	245 (9.8%)	366 (80.6%)	88 (19.4%)
ATP synthesis	1689	1321 (78.2%)	181 (10.7%)	187 (11.1%)	60 (16.3%)	308 (83.7%)
Proton transport	1038	898 (86.5%)	112 (10.8%)	28 (2.7%)	48 (34.3%)	92 (65.7%)
Buffer pH 8	810	761 (94.0%)	49 (6%)	0	27 (55.1%)	22 (44.9%)
1 mM ADP buffer pH 8	1480	1428 (96.5%)	52 (3.5%)	0	33 (63.5%)	19 (36.5%)
3 mM Pi buffer pH 8	889	869 (97.8%)	20 (2.2%)	0	12 (60.0%)	8 (40.0%)
1mM ADP + 3mM Pi buffer pH 8	986	960 (97.4%)	26 (2.6%)	0	15 (57.7%)	11 (42.3%)
1mM ADP + 3mM Pi buffer pH 4.7	1023	985 (96.3%)	38 (3.7%)	0	23 (60.5%)	15 (39.5%)
After ATP synthesis	805	755 (93.8%)	50 (6.2%)	0	31 (62.0%)	19 (38.0%)
After dissipation of Δ pH	238	230 (96.6%)	8 (3.4%)	0	not significant	not significant

(B) Statistics of FRET level histograms

The FRET level histograms were constructed as follows: For catalytic conditions (Fig. 4B,C,D) photon bursts with 2 or more FRET levels with the corresponding FRET level sequence were selected and the mean of each FRET level was calculated. For ATP hydrolysis this resulted in 366 bursts with 1024 FRET levels, i.e. 2.80 levels per burst, for ATP synthesis 308 bursts with 812 FRET levels, i.e. 2.64 levels per burst, and for proton transport 92 bursts (ATP synthesis direction) with 210 levels, i.e. 2.28 level per burst. For non-catalytic conditions (Fig. 4A and Fig. 6) photon bursts with one FRET level were selected. The histograms were fitted with a Gaussian distribution and the fractions of enzymes in L-, M- and H-state were calculated from the normalized area of Gaussian fits. The mean FRET values are the maxima of the fits and the error limits are the standard deviation. All data are summarized in Table III.

Table III: Statistics of FRET level histograms under different experimental conditions.

Conditions	Number of all FRET levels	L-state [%]	M-state [%]	H-state [%]	Mean FRET value of L-state	Mean FRET value of M-state	Mean FRET value of H-state
AMPPNP binding	888	28.3	10.0	61.7	0.23±0.09	0.52±0.07	0.87±0.06
ATP hydrolysis	1024	36.1	25.6	38.3	0.24±0.06	0.52±0.06	0.89±0.07
ATP synthesis	812	31.8	34.9	33.3	0.22±0.09	0.53±0.09	0.87±0.07
Proton transport	210	26.3	38.7	35.0	0.22±0.06	0.51±0.11	0.89±0.09
pH 8	761	51.5	14.5	34.0	0.19±0.07	0.52±0.06	0.94±0.03
1 mM ADP pH 8	1428	52.4	13.2	34.4	0.17±0.07	0.51±0.07	0.94±0.03
3 mM Pi pH 8	869	49.7	15.7	34.6	0.17±0.06	0.52±0.07	0.95±0.03
1mM ADP + 3mM Pi pH 8	960	48.2	18.5	33.3	0.17±0.06	0.51±0.08	0.94±0.03
1mM ADP + 3mM Pi pH 4.7	985	49.1	16.6	34.3	0.17±0.06	0.50±0.08	0.94±0.03
After ATP synthesis	755	25.9	18.0	56.1	0.17±0.05	0.51±0.08	0.95±0.03
After dissipation of ΔpH	230	37.9	13.6	48.5	0.18±0.06	0.52±0.07	0.95±0.03

It might be asked whether it is allowed to calculate mean FRET values from photon bursts by averaging bursts with two, three or more FRET levels. Therefore, we compared in Fig. 8 histograms using either all photon bursts (A), photon bursts with two levels (B), photon burst with 3 and 4 levels (C) and photon burst with more than 4 levels (D).

For ATP hydrolysis (blue) and for ATP synthesis (red) the maxima of the distributions are found at the same mean FRET value, i.e. the data can be combined into one histogram as shown in Fig 4.

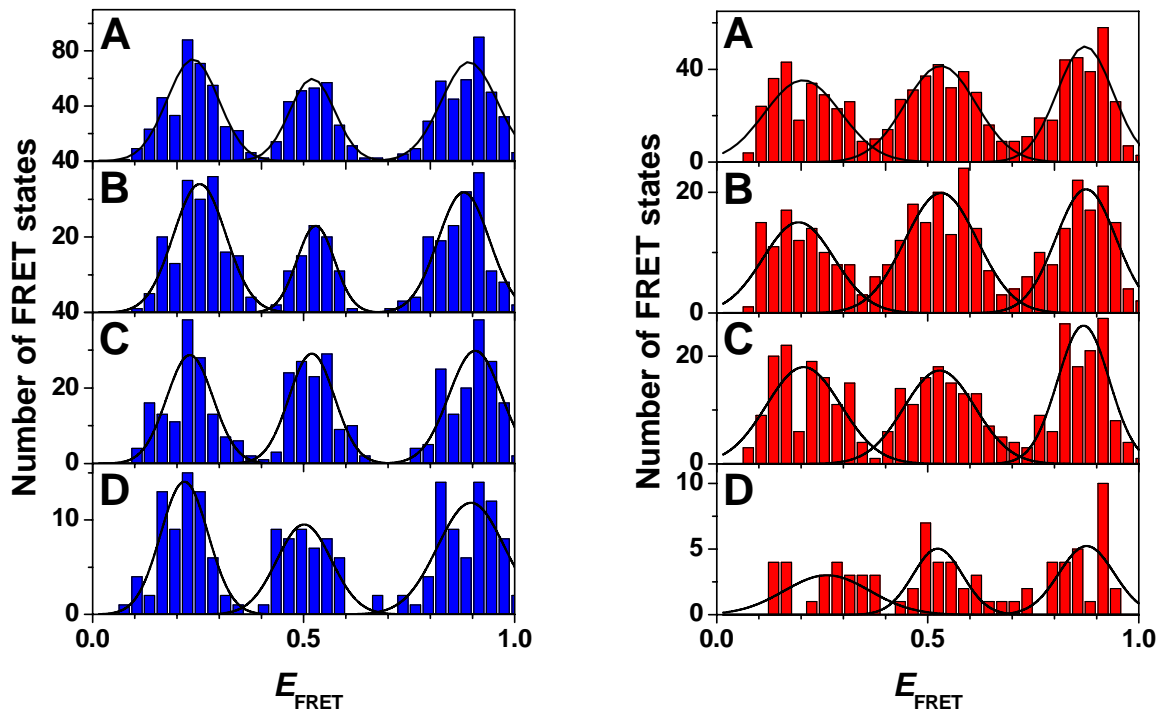


Fig. 8: FRET level histograms from photon bursts with different numbers of FRET levels.

Left: ATP hydrolysis. A, Average of all analyzed photon burst with FRET level sequence $H \rightarrow M \rightarrow L \rightarrow H$ (366, see Fig. 4B); B, photon bursts with 2 levels (209); C, photon bursts with 3 (90) and 4 levels (37), D; photon bursts with more than 4 levels (30).

Right: ATP synthesis. A, Average of all analyzed photon bursts with FRET level sequence $H \rightarrow L \rightarrow M \rightarrow H$ (308, see Fig. 4C); B, photon bursts with 2 levels (181); C, photon bursts with 3 (89) and 4 levels (26), D; photon bursts with more than 4 levels (12).