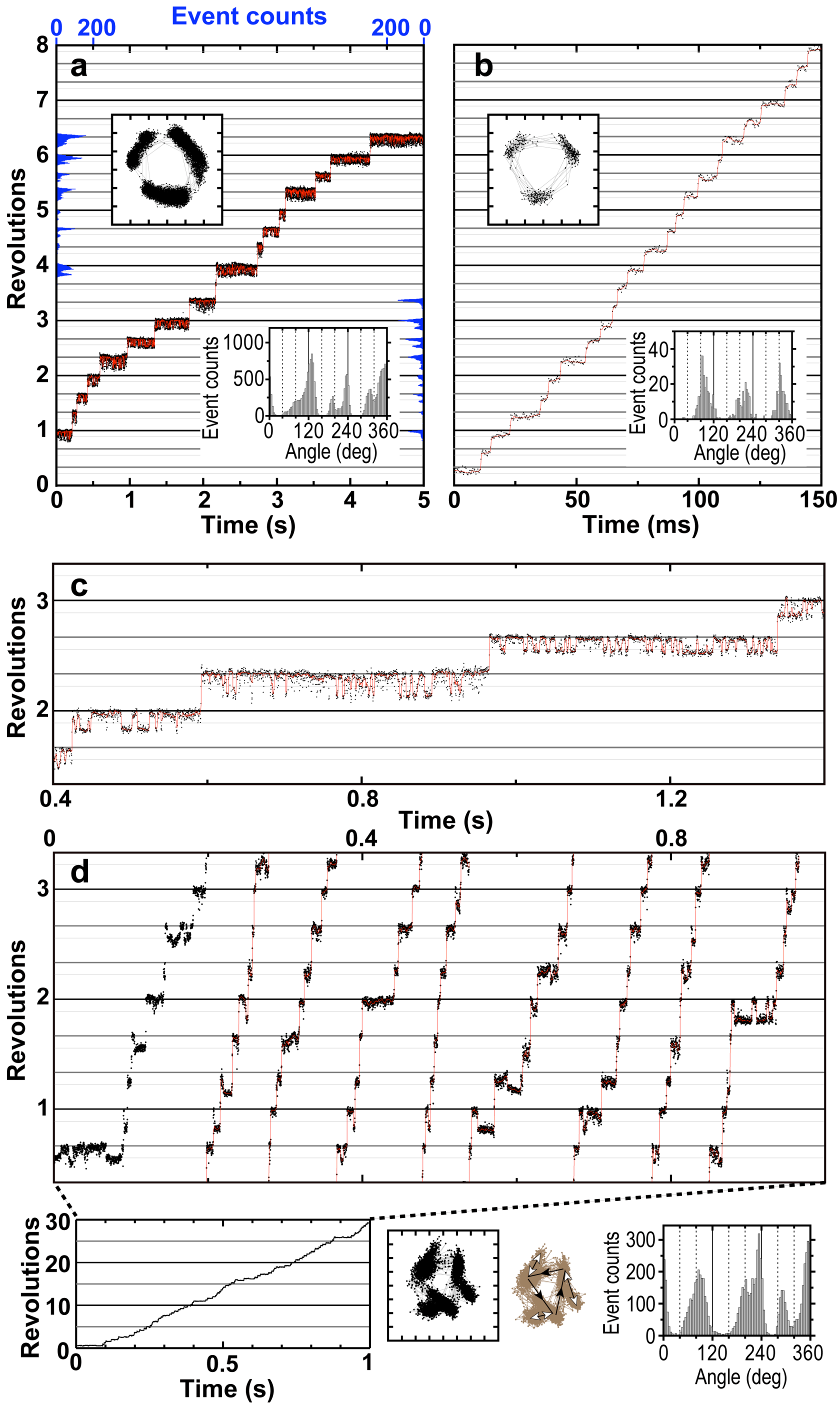
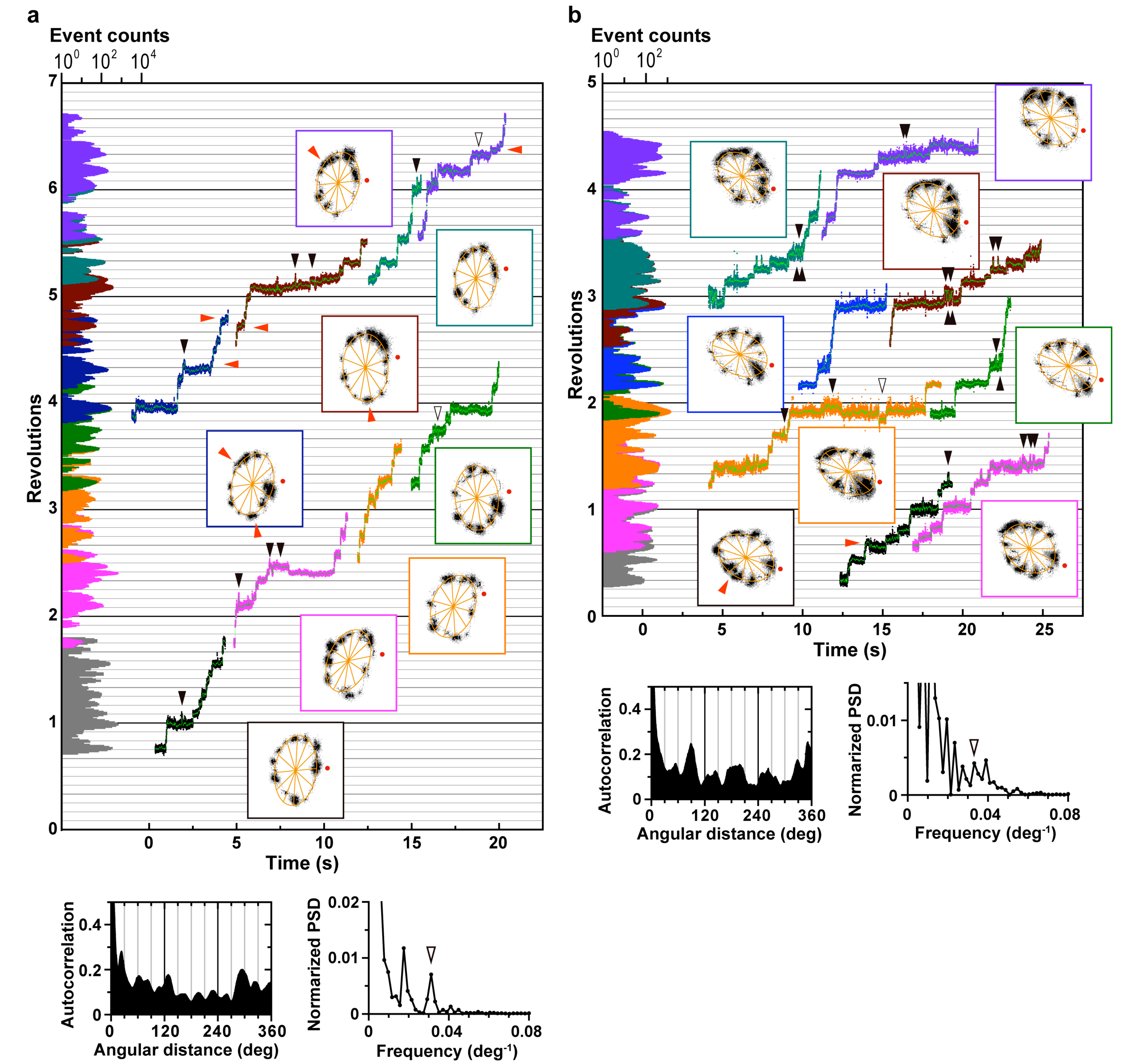


Supplementary Figure S1. A typical example of ~40° jumps observed in some V_1 molecules. (**a** and **b**) Rotation of a same V_1 molecule was observed first at 4 μM ATP (**a**) and then after infusion of 1 mM ATP (**b**). Images were captured at 4000 frames s^{-1}(fps), and *red lines* on the time courses are median-filtered over 11 points (2.5 ms). Upper insets, trajectories of the bead centroid, axis divisions being 11.1 nm. Lower insets, histograms of angular positions for the indicated portion of the records. In **a**, histograms along the time courses (horizontal projection of the time courses onto the vertical axes) are also shown as *blue bars*. (**c**) An expanded view of **a** between 0.4 and 1.4 s. Such peculiar fluctuations, jumping to and fro between two angular positions separated by ~40°, were observed in less than 1/3 of rotating beads (see Supplementary Table S1). The intervals between the jumps were of the order of 10 ms, apparently independent of [ATP] and not synchronous with the 120° stepping (**c**). These relatively infrequent jumps were difficult to discern directly at mM ATP where the basic 120° steps occur at ~5 ms intervals, but their presence could be inferred from the random shift of dwelling positions by ~40° (**b**). (**d**) Another example at 400 μM ATP, showing a similar jump frequency but in off-circular directions. Images were captured at 8000 fps, and *red lines* on the time courses are median-filtered over 21 points (2.5 ms). Lower panels from left to right show: 1) the entire time course from which the expansion in the upper panel is made; 2) bead trajectory, axis divisions 11.1 nm; 3) movements in the trajectory where *black arrows* show normal anticlockwise stepping and *white arrows* off-circular jumps; 4) histogram of angular positions for the indicated portion of the records.

The cause of these jumps is yet unknown. If these are coupled with catalysis, candidates would be hydrolysis/resynthesis of ATP or release/rebinding of ADP within a catalytic pocket, because the jumps did not cease at [ATP] as low as 1 μM. A more likely explanation, in view of the [ATP] independence, asynchrony with 120° stepping, and deviation from circular trajectory as in the case of **d**, is that the bead can adopt two orientations relative to the D subunit (or the D subunit or its part relative to the rest of V_1) and thermally jumps between the two positions.



Supplementary Figure S2. Additional examples of ~30° substep rotation in upside down V_oV_1. Rotation was observed at 2000 fps at 40 μM ATP in the presence of 0.1 % (w/v) Triton X-100. See Fig. 4 for details. Drifts in these data were larger than in Fig. 4, and thus the coloured portions are chosen with mutual overlaps. Boxes enclosing trajectories measure 66 × 66 nm^2. Histogram bin size is 1° in **a**, and 2° in **b**. Lower left panels show the autocorrelation of its angular histogram; the continuous time courses over 1750° were 21-point median filtered and then binned at 0.25° intervals without the correction for rotational drift. Lower right panels show the power spectra, the *arrowheads* indicating a peak at $(32°)^{-1}$ in **a**, and $(30°)^{-1}$ in **b,** respectively.

Supplementary Table S1. Statics of V_1 and V_oV_1 rotations in Fig. 1.

V_1 rotations.

[ATP]	*n*	rotation rate (rps)		total # of revolutions		back&forth jumps per 10 dwells[*1]			
		mean ± SD	range	mean ± SD	range	<1	1-5	>5	N.D.
4 mM	12	58 ± 9	44-72	291 ± 166	85-600	2			
4 mM (+DDM)	6	51 ± 4	44-54	357 ± 194	170-650				
2 mM	20	58 ± 8	43-74	297 ± 155	44-678	2			
1 mM	8	53 ± 9	41-61	345 ± 326	28-750	1			
400 μM	12	40 ± 9	26-51	367 ± 172	134-660	2	2		1
200 μM	8	31 ± 6	23-38	264 ± 132	50-434	2	1		
100 μM	27	19 ± 7	11-40	125 ± 74.6	32-364	5	2		1
40 μM	15	7.9 ± 2.5	4.7-13	126 ± 61.7	20-211	5	1		1
40 μM (+DDM)	4	10 ± 2	7.2-12	279 ± 260	22-584		1		
10 μM	17	1.9 ± 0.4	1.1-2.6	56.9 ± 41.5	15-160	4	2		
4 μM	18	1.3 ± 0.8	0.74-2.9	64.3 ± 55.0	21-220	3	1	3	1
2 μM	10	0.80 ± 0.22	0.48-1.2	77.1 ± 68.6	10-196	3	1	1	
1 μM	12	0.24 ± 0.11	0.11-0.47	35.5 ± 26.0	10-105	3	3	2	1
total # (ratio)	**169**	-	-	-	-	**32** (19%)	**14** (8%)	**6** (4%)	**5** (3%)

V_oV_1 rotations

[ATP]	*n*	rotation rate (rps)		total # of revolutions	
		mean ± SD	range	mean ± SD	range
4 mM (Small steps)	12	2.9 ± 4.7	0.46-16	102 ± 195	20-720
4 mM (Clean 3-steps)	3	23 ± 14	6.3-33	232 ± 319	26-600
400 μM	6	0.57 ± 0.27	0.11-0.90	25.5 ± 9.2	16-40
40 μM	6	0.83 ± 0.65	0.23-1.7	36.5 ± 27.4	16-90
4 μM	8	0.20 ± 0.07	0.071-0.31	17.0 ± 4.3	9-22
0.4 μM	4	0.038 ± 0.11	0.030-0.047	8.3 ± 1.7	6-10

[*1]Occurrence of ~40° back and forth fluctuations. Dwells containing one or more return jumps were counted. <1, marginal cases where less than one dwell in 10 showed jumps. N.D., not determined because the dwells were too noisy. In Fig. S1, **a** is judged as >5, **b** as <1, and **d** as 1-5.