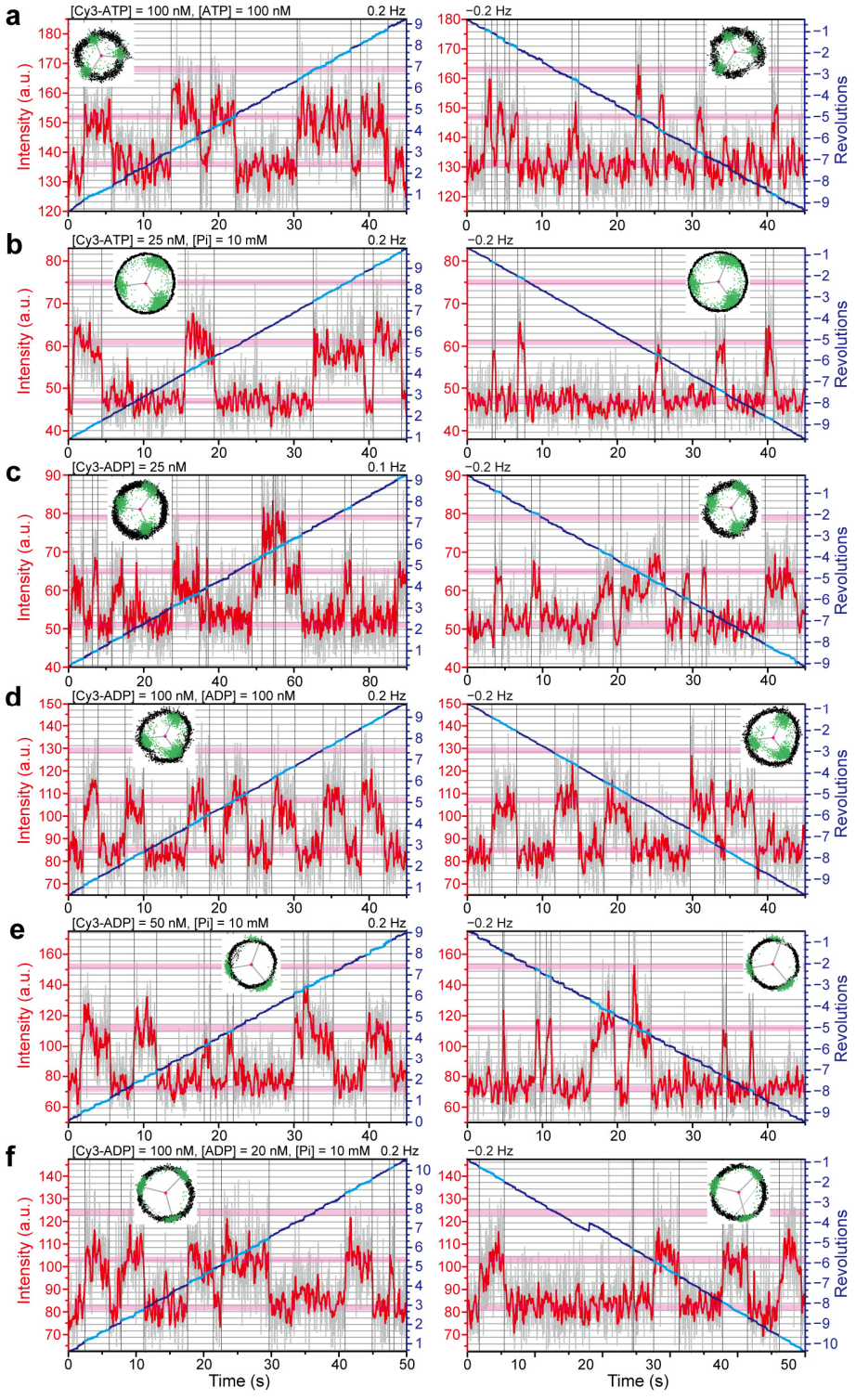
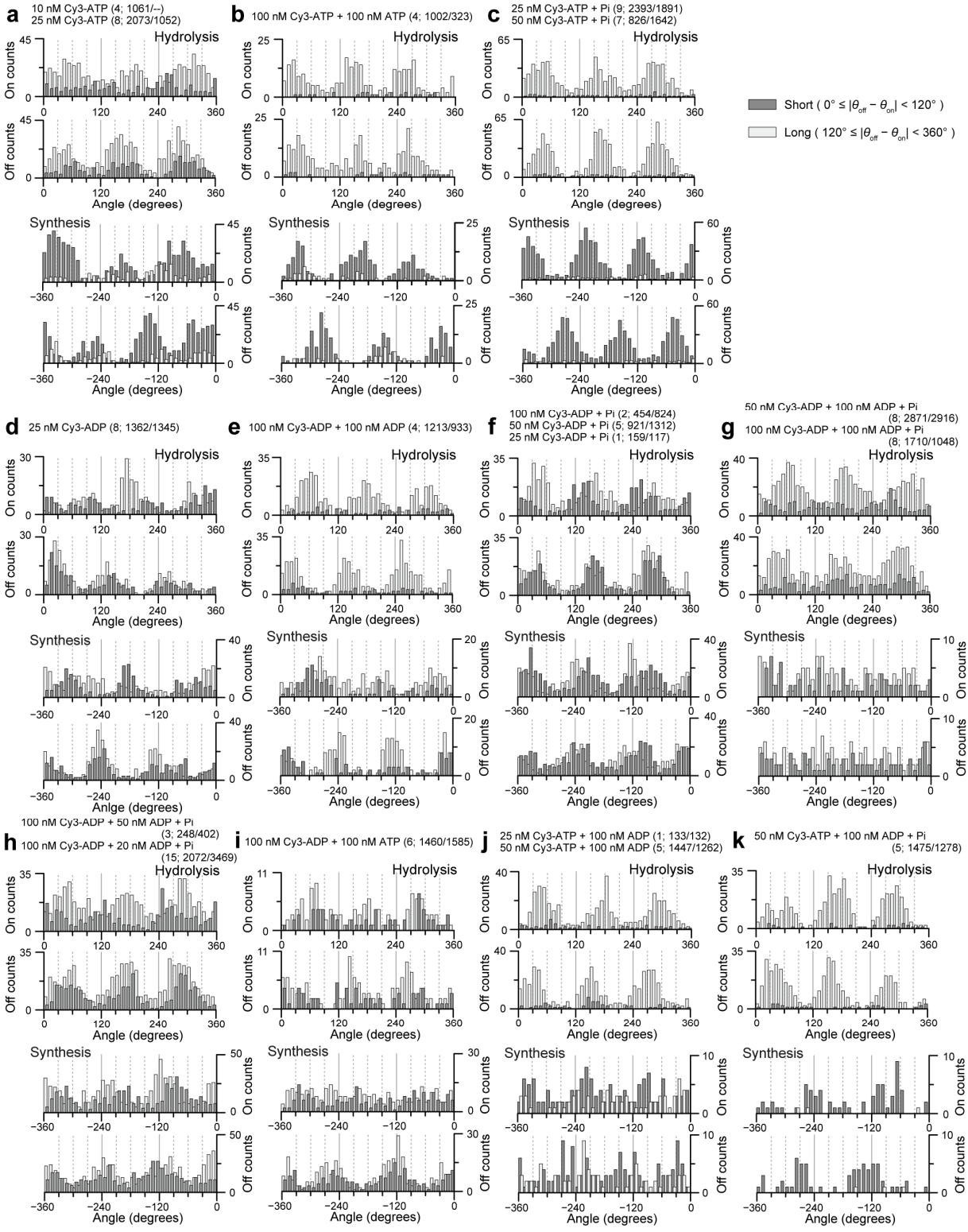


Supplementary Figure S1 | Time courses of Cy3-nucleotide binding and manipulated rotation. (**a-f**) Left and right panels show time courses during hydrolysis and synthesis rotation, respectively. Nucleotide conditions and the rotary speed of magnetic field are indicated at the top. Red curves show

fluorescence intensity (grey curves) median-filtered over 8 frames. Pink horizontal lines, intensity levels for the number of bound Cy3-nucleotide of 0, 1, and 2. Vertical lines mark the on or the off events. Blue curves show bead rotation, cyan parts indicating the periods while a Cy3-nucleotide(s) was bound. Grey horizontal lines are ATP-waiting angles separated by 120°. Insets, trace of the centroid of the bead image; green, during free stepping in 60 nM unlabelled ATP; black, during forced rotation.


a
10 nM Cy3-ATP (4; 1061/--)
25 nM Cy3-ATP (8; 2073/1052)
b
100 nM Cy3-ATP + 100 nM ATP (4; 1002/323)
c
25 nM Cy3-ATP + Pi (9; 2393/1891)
50 nM Cy3-ATP + Pi (7; 826/1642)
Short (0° ≤ |θoff − θon| < 120°)
Long (120° ≤ |θoff − θon| < 360°)
d
25 nM Cy3-ADP (8; 1362/1345)
e
100 nM Cy3-ADP + 100 nM ADP (4; 1213/933)
f
100 nM Cy3-ADP + Pi (2; 454/824)
50 nM Cy3-ADP + Pi (5; 921/1312)
25 nM Cy3-ADP + Pi (1; 159/117)
g
50 nM Cy3-ADP + 100 nM ADP + Pi
(8; 2871/2916)
100 nM Cy3-ADP + 100 nM ADP + Pi
(8; 1710/1048)
h
100 nM Cy3-ADP + 50 nM ADP + Pi
(3; 248/402)
100 nM Cy3-ADP + 20 nM ADP + Pi
(15; 2072/3469)
i
100 nM Cy3-ADP + 100 nM ATP (6; 1460/1585)
j
25 nM Cy3-ATP + 100 nM ADP (1; 133/132)
50 nM Cy3-ATP + 100 nM ADP (5; 1447/1262)
k
50 nM Cy3-ATP + 100 nM ADP + Pi
(5; 1475/1278)
Hydrolysis
Synthesis
On counts
Off counts
Angle (degrees)
Anlge (degrees)

Supplementary Figure S2 | Histograms of binding (θ_{on}) and release (θ_{off}) angles for simple events. (**a**-**k**) Nucleotide conditions and the numbers of molecules and rotations in parentheses are indicated at the top. The histograms were compiled from the simple events (0→1→0) of **Fig. 3**, without taking into account the site assignment. Short ($0° \leq |\theta_{off} - \theta_{on}| < 120°$) and long ($120° \leq |\theta_{off} - \theta_{on}| < 360°$) bindings are distinguished. The histograms indicate that the distributions of the off angles for short and long bindings agree well, but the on angles do not match. Relative abundance of short and long binding events may be best judged in these histograms.