SUPPLEMENTAL TEXT

Relation between Trap Stiffness and Voltage Applied to Magnetic Tweezers

At low magnetic field as used in this study (less than 100 Gauss) magnetization of magnetic beads, M can be assumed to be proportional to the strength of external magnetic field, H (1).

$M \propto H$

As the strength of magnetic field is proportional to the applied voltage, the relation between trap stiffness k and the applied voltage is expected to be,

$k \propto V^2$ ($\propto H^2 \propto MH$)

To check this expectation, the trap stiffness was assessed experimentally. Among the magnetic beads attached to F_1(-ε) we carefully selected beads that exhibited free rotational diffusion. We trapped them with various magnitudes of voltage and angular fluctuations of beads were recorded (Fig. S1A). The angular distribution was fitted with Gaussian probability density function.

$P(\phi) = (2\pi\sigma^2)^{-1/2}\cdot\exp(-\phi^2/2\sigma^2)$

where ϕ is the bead angle in radian and σ is the variance.

The trap stiffness is given as (2),

$k = k_B T\sigma^{-2} \sim 4.1\sigma^{-2}$ (pN nm)

where k_B is the Boltzmann constant and T is the absolute temperature. Obtained k values were plotted against square of applied voltage (Fig. S1B). Data can be fitted well with a line from the origin, and the mean value of correlation coefficients is 0.94 (n = 8, 4 molecules). The result in Fig. S1 ensured that trap stiffness of the magnetic tweezers was really proportional to the square of voltage applied under the conditions we used.

Magnetic Torque Formulation

We assume that the magnetic bead has homogeneous magnetic susceptibility and that its shape can be approximated by a prolate ellipsoid. That is, $a > b = c$, where a, b, and c are the ellipsoidal semi-axes. The magnetic torque τ in the presence of external magnetic field can be written as (3),

$$\tau = \frac{1}{2}(\kappa'_a - \kappa'_c)\, vH^2 \sin 2\theta \quad [1]$$

where θ is the polar angle of the external magnetic field axis with respect to the long axis of the ellipsoid and v is the volume of the ellipsoid (v = $4\pi/3ac^2$); κ'_a and κ'_c are the apparent susceptibilities along the long and short axes of ellipsoid, respectively, with

$$\kappa'_i = \frac{\kappa}{1 + N_i\kappa} \quad (i = a, c)$$

where κ is the intrinsic magnetic susceptibility and N_a and N_c, the geometric demagnetizing factors along the long and short axes, respectively. They are given as (4),

$$\frac{N_a}{4\pi} = \frac{1}{m^2-1}\left[\frac{m}{2\,(m^2-1)^{\frac{1}{2}}}\ln\left(\frac{m+(m^2-1)^{\frac{1}{2}}}{m-(m^2-1)^{\frac{1}{2}}}\right)-1\right]$$

$$\frac{N_c}{4\pi} = \frac{m}{2(m^2-1)}\left[m-\frac{1}{2\,(m^2-1)^{\frac{1}{2}}}\ln\left(\frac{m+(m^2-1)^{\frac{1}{2}}}{m-(m^2-1)^{\frac{1}{2}}}\right)\right]$$

where $m = a/c$.

Estimation of Torque at the Maximum Angular Deviation

The unit of torque in Fig. 3C is thought to be comparable to the torque generated by F_1 during ATP-driven rotation at 1 mM ATP. The torque exerted by the magnetic tweezer depends on the angle between the direction of magnetic field and the long axis of magnetic bead (equation [1]). Therefore, for estimation of the unit torque, the angular deviation of the bead from the magnetic field ($\Delta\theta$') was analyzed in the following two cases: (i) when the beads deviated maximally ($\Delta\theta$) from the inhibitory angle during stiffness measurement of the inhibited F_1 (Fig. S2, $\Delta\theta'_{inhib}$), (ii) when ATP-driven F_1 rotation was clamped at MCV (Fig. S3, $\Delta\theta'_{ATP}$). The magnetic torque at $\Delta\theta'_{ATP}$ was used for the estimation of MCV (Fig. 3A), on the other hand, magnetic trap in actually exerted torque corresponding value of $\Delta\theta'_{inhib}$ deviated position (Fig. 3B).
Hence, the unit can be corrected as,
$\sin(2\cdot\Delta\theta'_{inhib})/\sin(2\cdot\Delta\theta'_{ATP})\times$($F_1$ torque).
Fig. S2 shows the histograms of $\Delta\theta'_{inhib}$ during the stiffness measurements (Fig. 3) of ε-inhibition and ADP-inhibition. The centers of $\Delta\theta'_{inhib}$ was 36° (Fig. S2C). Small difference from the expected value (45°) might be reflection of premature release of beads from the magnetic trap due to fluctuation of beads and of the trapping system. However, the torque at $\Delta\theta'_{inhib}$ was very close to the expected maximum magnetic torque (~ 0.95 fold (= sin(2·36°)/ sin(2·45°)).
Fig. S3B shows the angular distribution of beads when ATP-driven rotation of F_1 was clamped at a certain angle by various magnitudes of trap force. Torque of the magnetic trap is thought to be equal to that of the ATP-driven rotation at the center angle of Gaussian fit of each histogram. Here, as F_1 torque can be assumed to be constant at any angle, equation [1] can be rewritten as,
$1/H^2 = 1/(2\tau_{F1})(\kappa'_a - \kappa'_c)v\sin 2\theta$ [2]
Where, τ_{F1} is the constant torque of ATP-driven rotation. Equation [2] indicates that V^{-2} is proportional to $\sin 2\theta$. MCV for each molecule was also determined. The angular deviation values were plotted against inverse of squared voltage which was normalized by MCV (Fig. S3C). The directions of magnetic field were determined for each molecule by extrapolation of the fitted lines, *const*·$\sin 2\theta$. Data for each molecule were superimposed by setting the obtained direction of magnetic field as 0° and fitted again with the same function. Thus, $\Delta\theta'_{ATP}$ was obtained as 22°. According to these results, the unit of torque in Fig. 3C could be estimated as ~1.4 fold (= sin(2·36°)/ sin(2·22°)) of F_1 torque.

Reference for Supplemental Text

1. Romano, G., Sacconi, L., Capitanio, M., and Pavone, F. S. (2003) *Opt Comm* **215**, 323-331
2. Sielaff, H., Rennekamp, H., Wachter, A., Xie, H., Hilbers, F., Feldbauer, K., Dunn, S. D., Engelbrecht, S., and Junge, W. (2008) *Proc Natl Acad Sci U S A* **105**(46), 17760-17765
3. de Oliveira, J. F., Wajnberg, E., Esquivel, D. M., Weinkauf, S., Winklhofer, M., and Hanzlik, M. (2010) *J R Soc Interface* **7**(42), 143-152
4. Osborn, J. A. (1945) *Phys Rev* **67**, 351-357

Figure Legends for Supplemental Figures

Fig. S1. Relation of trap stiffness and voltage applied to magnetic tweezers.
(**A**) Angular distribution of magnetic beads trapped by magnetic tweezers. Magnetic beads were attached to F_1(-ε). The beads that showed free rotational diffusion were selected and trapped by magnetic tweezers. The angular motion caused by thermal energy was recorded. In this example, a bead was clamped at 1.5, 2.5, 4, 4.5 and 5V. Each angular distribution was fitted with Gaussian probability density function: $P(\phi) = (2\pi\sigma^2)^{-1/2}\cdot\exp(-\phi^2/2\sigma^2)$
(**B**) Relation between trap stiffness and applied voltage. Values of trap stiffness of magnetic tweezers, $k = k_BT\cdot\sigma^{-2} \sim 4.1\sigma^{-2}$ pN nm, were plotted against square of applied voltage. Data were fitted with straight lines from the origin. In this example, the correlation coefficients between data points and fitted line, R, was 0.99. The mean of R was 0.94 ± 0.05 (n = 8, 4 molecules).

Fig. S2. Angular deviation of beads from the direction of magnetic field during stiffness measurement of the inhibited F_1.
(**A**) Schematic illustration of measurement of angular deviation of beads from the direction of magnetic field. During stiffness measurement of the inhibited F_1 shown in Fig. 3, angular deviation of beads from the trap center at the moment when beads deviated maximally from the inhibitory position was measured ($\Delta\theta'_{inhib}$).
(**B**) Example of measurement of $\Delta\theta'_{inhib}$. ADP-inhibited F_1 was forcibly rotated in ATP synthesis direction. Direction of magnetic field position was assumed from manipulation speed (0.05 Hz).
(**C**) Histogram of $\Delta\theta'_{inhib}$. The center of $\Delta\theta'_{inhib}$ is 36 ± 15°.

Fig. S3. Angular deviation of beads from the direction of magnetic field when ATP-driven rotation was clamped at MCV.
(**A**) Schematic illustration of the measurement of angular deviation of the beads from the direction of magnetic field (Δθ'). To the actively rotating F_1(-ε) in 1 mM ATP (a), various magnitudes of magnetic field were applied (b-d). The angle of the tweezers was arbitrarily chosen because the torque of F_1 can be assumed to be constant at any rotation angle. The position of the clamped beads varied depending on the strength of the magnetic trap. When the voltage was equal to MCV, the Δθ' was equal to $\Delta\theta'_{ATP}$.
(**B**) Histograms of angular fluctuation of the beads at the position clamped by various voltages. x-axis (Δθ'), difference of the angles between the direction of magnetic field and the clamped position. The center of the histogram corresponded to the point at which the torque exerted to the bead by magnetic field became equal to the torque generated by ATP-driven rotation of F_1. Angle differences between the direction of magnetic field of magnetic tweezers and center values of each histogram were determined.
(**C**) The inverse of the squares of normalized voltage (applied voltage/MCV) was plotted to Δθ' (n = 34, 5 molecules). The direction of magnetic field of each experiment was determined by fitting data points by lines, $const\cdot\sin2(\theta\text{-}\theta c)$. Here, θc is the direction of magnetic field. Data from different molecules were superimposed by setting the obtained direction of magnetic field as 0°. According to the fitted line, $\Delta\theta'_{ATP}$ was estimated to be 22°.

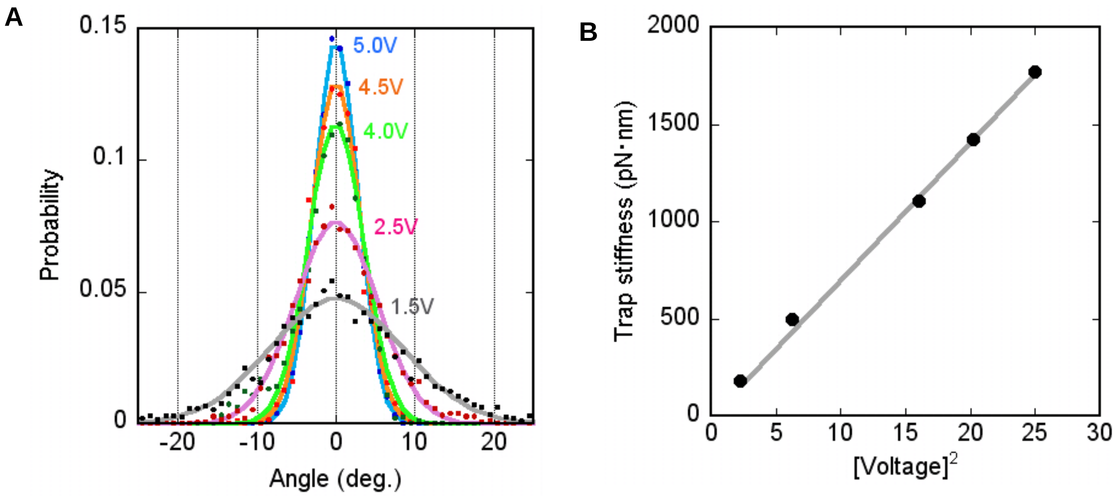

A
0.15
0.1
0.05
0
Probability
-20
-10
0
10
20
Angle (deg.)
5.0V
4.5V
4.0V
2.5V
1.5V
B
2000
1500
1000
500
0
Trap stiffness (pN·nm)
0
5
10
15
20
25
30
[Voltage]2

Figure S1

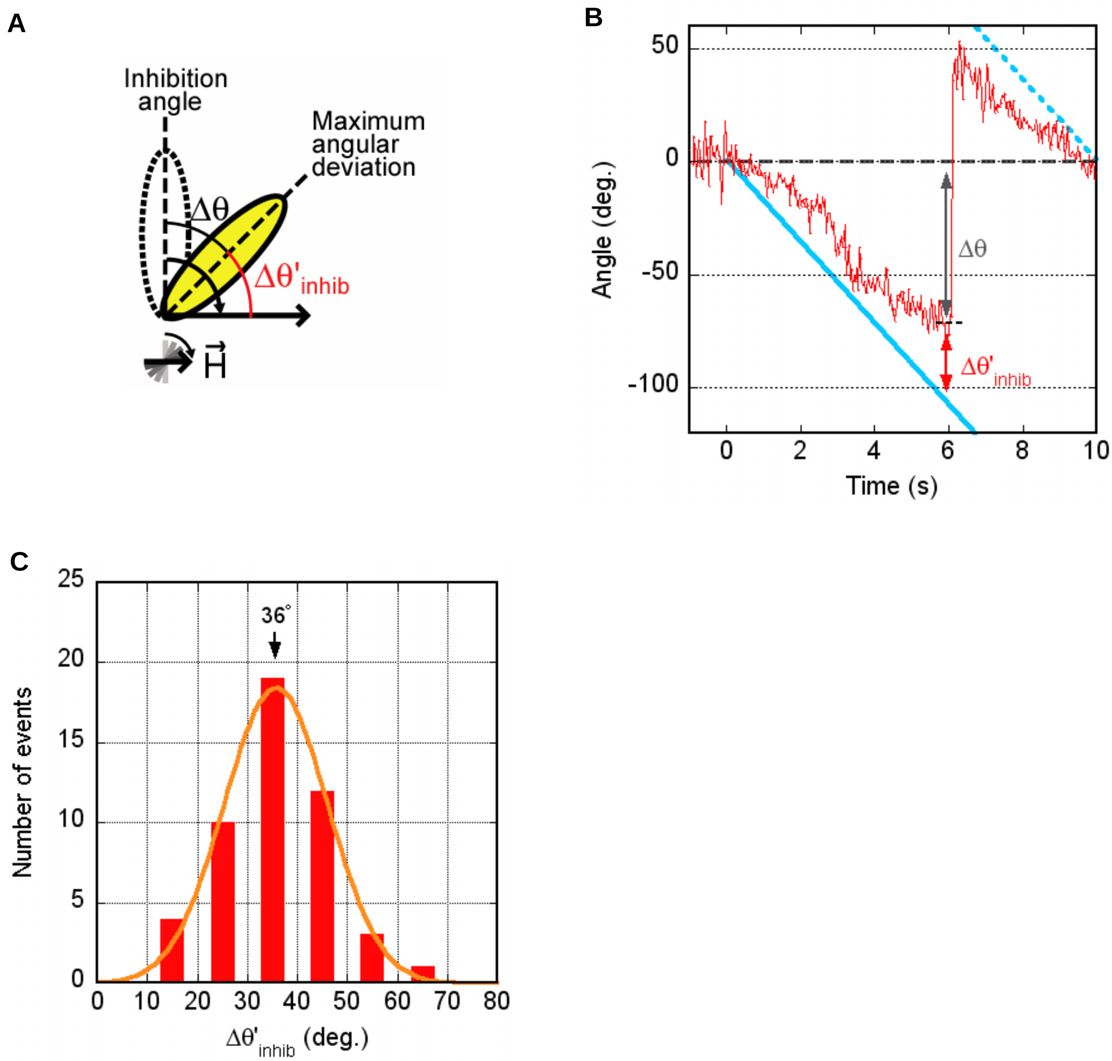
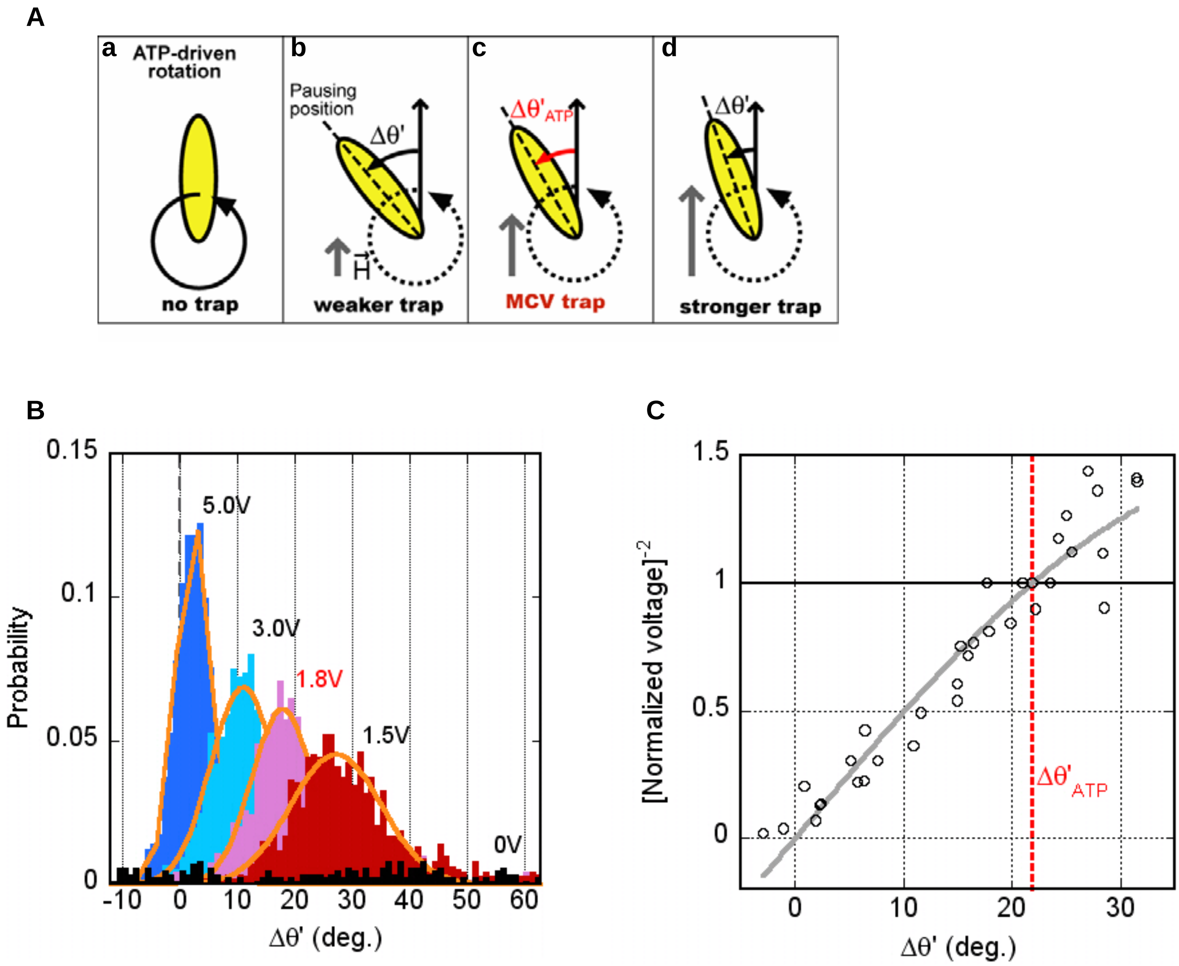

A
Inhibition angle
Maximum angular deviation
Δθ
Δθ'inhib
H
B
50
0
-50
-100
Angle (deg.)
0
2
4
6
8
10
Time (s)
Δθ
Δθ'inhib
C
25
20
15
10
5
0
Number of events
36°
0
10
20
30
40
50
60
70
80
Δθ'inhib (deg.)

Figure S2



A
a
ATP-driven rotation
no trap
b
Pausing position
Δθ'
H
weaker trap
c
Δθ'ATP
MCV trap
d
Δθ'
stronger trap
B
Probability
5.0V
3.0V
1.8V
1.5V
0V
Δθ' (deg.)
C
[Normalized voltage]-2
Δθ'ATP
Δθ' (deg.)

Figure S3