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Kinesin Walks Hand-Over-Hand

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Kinesin is a processive motor that takes 8.3 nm center-of-mass steps along microtubules for each ATP hydrolyzed. Whether kinesin moves by a "hand-over-hand" or an "inchworm" model has been controversial. We have labeled a single head of the kinesin dimer with a Cy3 fluorophore and localized the position of the dye within 2 nm before and after a step. We observe that single kinesin heads take 17.3 ± 3.3 nm steps. A kinetic analysis of the dwell times between steps shows that the 17 nm steps alternate with 0 nm steps. These results strongly support a hand-over-hand mechanism, and not an inchworm mechanism. In addition, our results suggest that kinesin is bound via both heads to the microtubule while waiting for ATP in between steps.

Conventional kinesin (referred to simply as kinesin) is a highly-processive, dimeric motor that takes 8.3 nm center-of-mass steps along microtubules for each ATP hydrolyzed (*1*–*3*). Kinesin transports a variety of cargo, including membranous organelles, mRNAs, intermediate filaments and signaling molecules (*4*). Mutations in a neuron-specific conventional kinesin have been linked to neurological diseases in humans (*5*).

Kinesin is a homodimer with identical catalytic cores (heads) that bind to microtubules and ATP (*6*). Each head is connected to a "neck-linker", a mechanical element that undergoes nucleotide-dependent conformational changes that enable motor stepping (*7*). The neck linker is in-turn connected to a coiled-coil that then leads to the cargo binding domain (*8*). In order to take many consecutive steps along the microtubule without dissociating, the two heads must operate in a coordinated manner, but the mechanism has been controversial. Two models have been postulated: the hand-over-hand "walking" model in which the two heads alternate in the lead (*7*), and an "inchworm" model in which one head always leads (*9* and references therein).

The hand-over-hand model predicts that, for each ATP hydrolyzed, the rear head moves twice the center of mass, while the front head does not translate. For a single dye on one head of kinesin, this leads to a prediction of alternating 16.6 nm and 0 nm translation of the dye (Fig. 1A). In contrast, the inchworm model predicts a uniform translation of 8.3 nm for all parts of the motor, which is equal to the center-of-mass translation (Fig. 1A). In addition, each model makes predictions about rotation of the stalk. The inchworm model predicts that the stalk does not rotate during a step. A symmetric version of the hand-over-hand, in which the kinesin-microtubule complex is structurally identical at the beginning of each ATP cycle, predicts that the stalk rotates 180 degrees, while an asymmetric hand-over-hand model does not require stalk rotation (*9*, *10*). Based on biophysical measurements that showed no rotation of the stalk, Hua et al. (*9*) concluded that an inchworm model was more likely for kinesin, although they could not rule out an asymmetric hand-over-hand mechanism.

Recently we have developed a technique (Fluorescence Imaging One Nanometer Accuracy, FIONA) that is capable of tracking the position of a single dye with nanometer accuracy and sub-second resolution (*11*) (and references therein). In FIONA, the position of a dye before and after a step is monitored by imaging the dye's fluorescence onto a charge-coupled detector through a total-internal-reflection fluorescence microscope. The image, or point-spread-function (PSF), is a diffraction limited spot with a width of ~280 nm, but the center of the image, which corresponds to the position of the dye (*13*), can be located with nanometer accuracy. We previously applied the technique to show that myosin V walks in a hand-over-hand manner, with each head moving alternating 74 nm and 0 nm (*11*).

Here we have performed analogous experiments with a "cys-light" kinesin (*7*) with a solvent-exposed cysteine inserted on each head for labeling with a Cy3 fluorophore (Fig. 1B) (*14*). The dye's position was monitored as the kinesin moved on microtubules that were immobilized on a coverslip (*14*). Three different constructs were used, a homodimer with E215C, a second homodimer with T324C, and a heterodimer with one head lacking solvent-exposed cysteines and the other head containing cysteines at S43C and T324C, which are 2 nm apart (Fig. 1B). Sub-stoichiometric labeling was used for the homodimers, and single quantal bleaching of fluorescence confirmed that only a single dye was present on each kinesin analyzed (fig. S1B). The heterodimer was labeled with an excess of dye and both single- and double-quantal bleaching was observed (*14*). In the absence of ATP, kinesins were stationary. In the presence of 340 nM ATP, discrete steps were observed for the three different kinesin constructs (Fig. 2). A total of 354 steps from 35 kinesins were observed. We typically collected 4000 photons per 0.33 sec image. Traces from relatively bright kinesins (>5000 photons/image) are shown in Fig. 2, a histogram of 143 steps from 26 molecules is shown in Fig. 3A. The precision of step size determination was 1.5 – 3 nm based upon measuring the distance between average positions of PSF centers before and after a step (*11*, *12*). The average step size derived from histogram is 17.3 ± 3.3 nm. 8.3 nm steps or odd multiples of 8.3 nm were not observed. This data is therefore strongly supportive of a hand-over-hand mechanism, and not an inchworm mechanism.

The hand-over-hand mechanism predicts that these 17 nm steps alternate with 0 nm steps, which are not directly observable in a position versus time graph. However, if the observed 17 nm steps arise from the convolution of two sequential steps, i.e., 17 nm, 0 nm… then a dwell-time histogram of the number of steps versus step-time duration is the convolution of two exponential processes (*11*). This yields the dwell time probability, $P(t) = tk^2\exp(-kt)$, which is zero at t = 0, rises initially, and then falls. In contrast, if the 17 nm steps arise from a single process, then the dwell time histogram would be expected to yield an exponential decay (Poisson-distributed rate). The dwell time histogram of 347 steps for E215C and T324C (Fig. 3B) is well fit by the above convolution function (with $k = 1.14 \pm 0.03$ steps/sec), and not by the single-step decaying function. The rise near t = 0 is not due to instrument artifacts: an exponential process for myosin V stepping (with dyes located to show every step) at very similar rates yields the expected monotonic decay using the same instrument (*11*). We also have immobilized beads on a coverslip and moved them in 17 nm steps via a nanometric stage at the same average step rate and an exponentially-distributed dwell time, which yields the expected dwell-time histogram (fig. S2). The dwell-time histogram therefore provides strong support of the hand-over-hand model.

The average step size of each mutant also can be analyzed and compared. 318 steps from 30 kinesins singly labeled at E215C were observed, and of these, 124 steps from 22 E215C kinesins were chosen for their high-quality images. The average step size was 17.4 nm ± 3.2 nm (st. dev., σ), with σ_μ = 0.3 nm (Fig. 2 upper left and middle traces; movie M1). Three T324C kinesins displayed 12 steps with an average step size of 16.6 nm ± 4.4 nm (σ) and σ_μ = 1.3 nm. One molecule of S43C/T324C kinesin heterodimer was analyzed (Fig. 2, red colored), showing 7 steps with an average step size of 17.0 nm ± 3.4 nm and σ_μ = 1.3 nm. Consequently, all mutants have a 17 nm average step size that are experimentally indistinguishable and support a hand-over-hand model.

Our experiments also have implications for the number of kinesin heads bound while kinesin is waiting for ATP. Kinesin is a highly processive motor, implying that at least one head stays bound to the microtubule during multi-step motility. Both singly- and doubly-bound kinesin have been found in the presence of different nucleotides (*15*, *16*), and a two-headed bound species has been inferred to exist during the catalytic cycle based on a kinetic analysis (*17*) and by fluorescence polarization measurements at saturating ATP (*18*). However, whether or not kinesin is bound via one or two heads while waiting for ATP during motility has been unclear. If only one head is bound, then the step size would alternate between 16.6-*x* and *x*, where *x* is the distance along the direction of motion from where the dye would be if both heads were bound (fig. S3). We see no evidence for this modulation. For example, the average of every other step of E215C in the blue trace (upper left) shown in Fig. 2, is 16.4 ± 2.9 nm (σ_μ = 1.3 nm) for the even steps, and 16.9 ± 3.4 nm (σ_μ = 1.5 nm) for the odd steps. Similarly, for the green (middle) trace of E215C, the averages are 17.9 ± 3.2 nm (σ_μ = 1.2 nm) for the even steps, and 19.2 ± 2.9 nm (σ_μ = 1.2 nm) for the odd steps. Hence alternating steps are experimentally indistinguishable, indicating χ is less than 2 nm. Furthermore, in the one-foot dangling model, χ is expected to be different for each of the different mutants with different dye positions, which again, is not observed. Our measurements therefore strongly indicate that the two kinesin heads in the ATP-waiting state are either both bound, or if one head is detached, then it is sitting in a conformation such that it is within 2 nm from a tubulin binding site along the direction of motion.

In conclusion, our results strongly support a hand-over-hand (walking) model for kinesin motility. Combined with the lack of a stalk rotation detected by Hua et al for kinesin (*9*), our data imply that kinesin moves by an asymmetric hand-over-hand mechanism. Myosin V also walks hand-over-hand (*11*, *19*), while likely not rotating the stalk (*20*), implying it too is likely asymmetric. Such a mechanism has rather stringent biophysical constraints (*9*), including implications for how the rear head passes by the front head. Hoenger et al., for example, (*10*) has postulated a model where the rear head passes the front head in such a manner that the neck-linker wraps and unwraps around the stalk with alternating steps to minimize the build-up of torsional strain in the stalk region. Interestingly, sideways drag slows the kinesin motor asymmetrically, which suggests left-right asymmetry to the forward stepping motion and is consistent with, although does not compel, an asymmetric hand-over-hand model (*21*). Direct detection of motion during the step, however, requires faster time-resolution than presented here.

References and Notes

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12. An uncertainty, or the standard error of the mean (sm), of the PSF centers for relatively bright spots (5000 photons/image) is <3 nm. Bright spots (>7000 photons/image) yields +– 2 nm localization of PSF centers (from 7 molecules; traces shown in Fig. 2). The step size was determined by calculating the distance between average positions of plateaus before and after a step. The standard error of the mean of two adjacent plateaus gives the precision of the step size (*11*).
13. For unpolarized dyes, the center of the PSF corresponds to the dye position. For dyes with fixed orientation emitting polarized fluorescence, the PSF can take unusual shapes, particularly when viewed with spherical aberrations, and the PSF center does not necessarily correspond to the dye position (*23*). In our case the PSF is well fit to a symmetric 2–D Gaussian, implying the center of the PSF corresponds to the dye position. Furthermore, the orientation of the dye in both the hand-over-hand and inchworm model is expected to be unchanged when on the front and rear head, and hence there should be no effect of PSF shape or dye orientation on step-size determination.
14. Materials and methods are available as supporting material on *Science* Online.
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Supporting Online Material
www.sciencemag.org/cgi/content/full/1093753/DC1
Materials and Methods
Figs. S1 to S3
References and Notes
Movie S1

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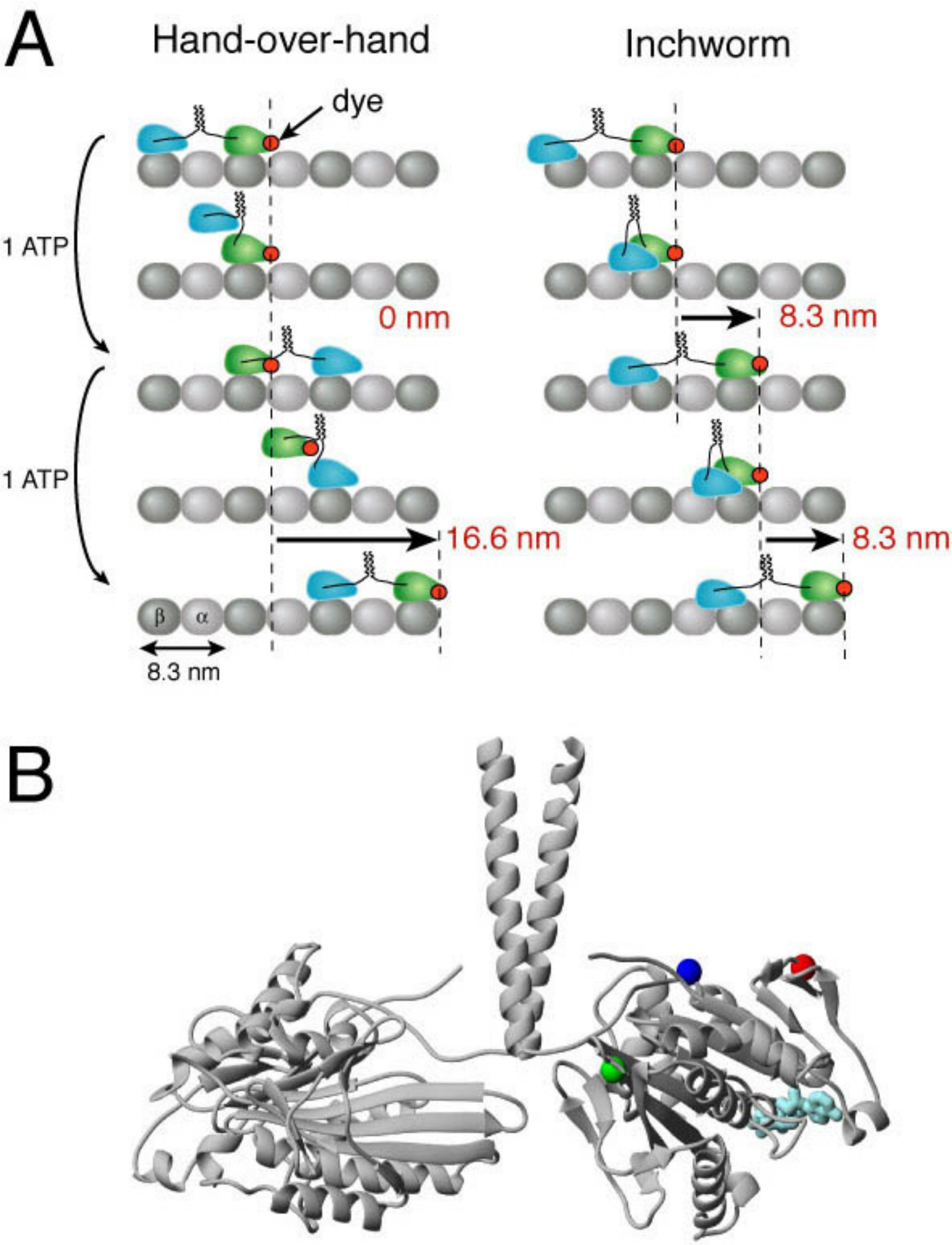
Fig. 1. Hand-over-hand versus inchworm model. (**A**) Examples of two alternative classes of mechanisms for processive movement by kinesin. Hand-over-hand model (left) predicts a dye on the head of kinesin moves alternatively 16.6 nm, 0 nm, 16.6 nm, while the inchworm mechanism (right) predicts uniform 8.3 nm steps. Inchworm model was adapted with slight modification from (*9*) (**B**) Position of S43 (red), E215 (green), and T324 (blue) shown on the rat kinesin crystal structure (from (*6*), PDB 2KIN). These residues, whose numbers above correspond to conventional human kinesin, were mutated to cysteines for fluorescent dye labeling as described in the text. The bound nucleotide (ADP) is shown as a space-filling model in cyan. This figure was made using MolMol (*22*).

Fig. 2. Position versus time for kinesin motility. The blue and green traces are from E215C homodimer kinesin, the red trace from the heterodimer S43C/T324C kinesin. The numbers correspond to the step size $\pm \sigma_\mu$. The uncertainties were calculated as described (*11*).

Fig. 3. Step sizes of an individual head of a kinesin dimer and dwell time analysis support a hand-over-hand mechanism. (**A**) Kinesin step size histogram from 124 steps of 22 molecules of E215C, 12 steps of 3 molecules of T324C and 7 steps of one S43C/T324C heterodimer. The average step size is 17.3 ±3.3 nm (N = 143, $\sigma_\mu = 0.27$ nm. The black solid line is a Gaussian fit. (**B**) Dwell-time histogram of 347 steps from 33 kinesin molecules including 317 steps from 29 molecules of E215C and 30 steps from 4 molecules of T324C at 340 nM ATP. The black line is a best-fit curve to the convolution function, $tk^2\exp(-kt)$ with $k = 0.38 \pm 0.01$ sec^{-1}, $r^2 = 0.984$.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Hand-over-hand
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1 ATP
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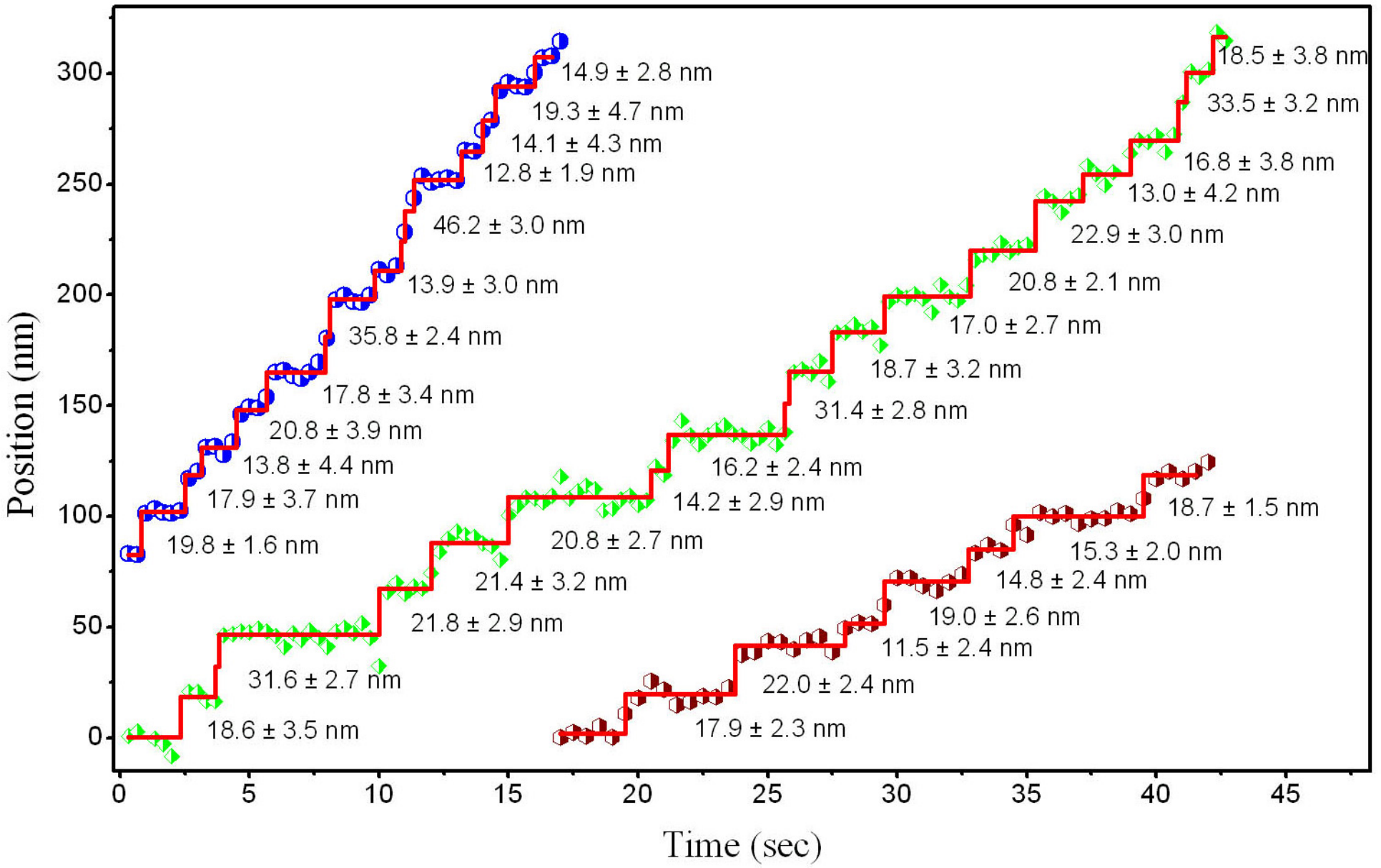
8.3 nm
16.6 nm
8.3 nm
β
α
8.3 nm
B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Time (sec)
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250
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45
19.8 ± 1.6 nm
17.9 ± 3.7 nm
13.8 ± 4.4 nm
20.8 ± 3.9 nm
17.8 ± 3.4 nm
35.8 ± 2.4 nm
13.9 ± 3.0 nm
46.2 ± 3.0 nm
12.8 ± 1.9 nm
14.1 ± 4.3 nm
19.3 ± 4.7 nm
14.9 ± 2.8 nm
18.6 ± 3.5 nm
31.6 ± 2.7 nm
21.8 ± 2.9 nm
21.4 ± 3.2 nm
20.8 ± 2.7 nm
14.2 ± 2.9 nm
16.2 ± 2.4 nm
31.4 ± 2.8 nm
18.7 ± 3.2 nm
17.0 ± 2.7 nm
20.8 ± 2.1 nm
22.9 ± 3.0 nm
13.0 ± 4.2 nm
16.8 ± 3.8 nm
33.5 ± 3.2 nm
18.5 ± 3.8 nm
17.9 ± 2.3 nm
22.0 ± 2.4 nm
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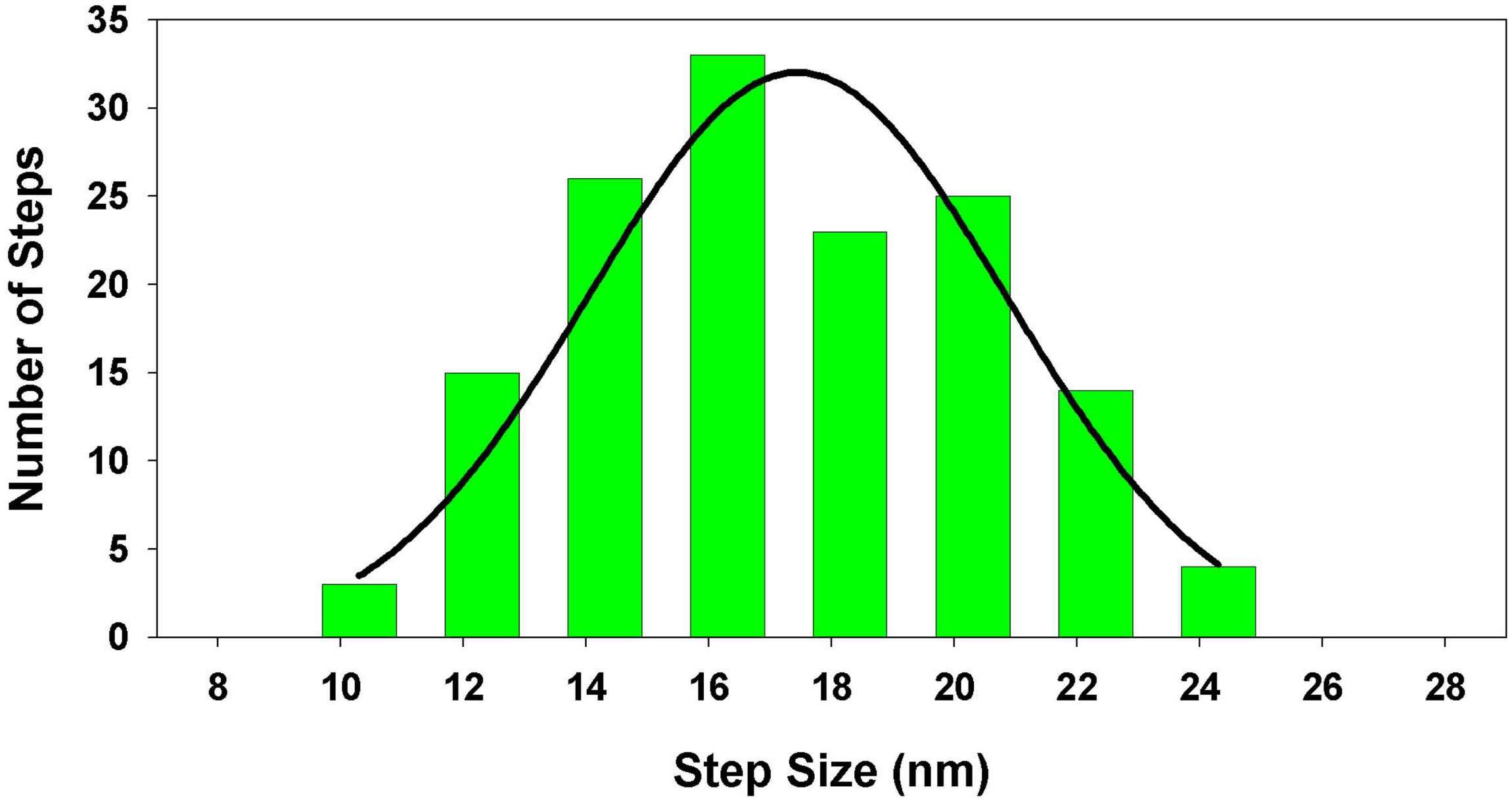
19.0 ± 2.6 nm
14.8 ± 2.4 nm
15.3 ± 2.0 nm
18.7 ± 1.5 nm

A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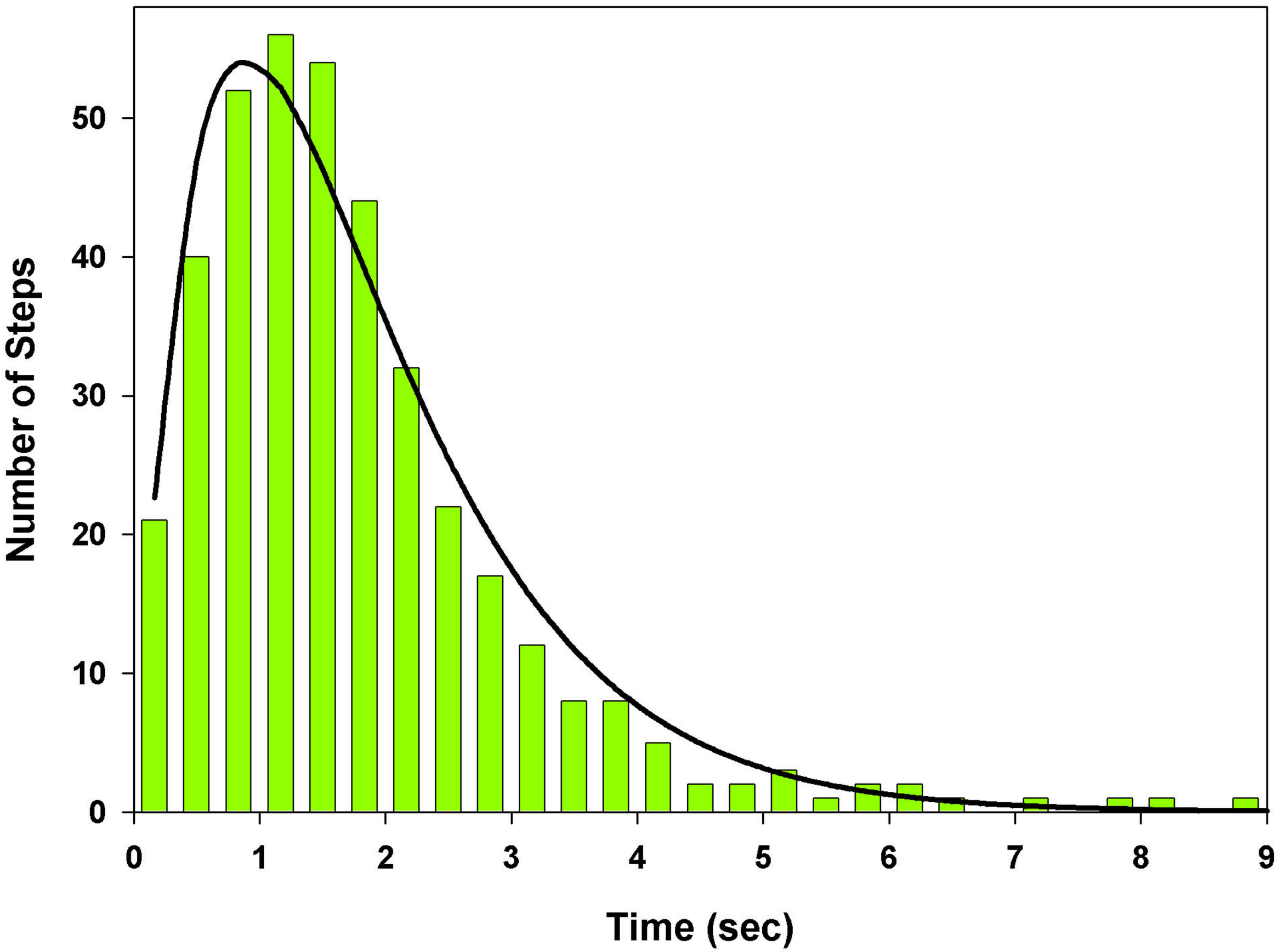
B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