SUPPORTING INFORMATION FOR

Label-Free, All-Optical Detection, Imaging, and Tracking of a Single Protein

J. Ortega Arroyo,[†] J. Andrecka,[†] K. M. Spillane,[†] N. Billington,[‡] Y. Takagi,[‡] J. R. Sellers,[‡] and P. Kukura[*,†]

[†]Physical and Theoretical Chemistry Laboratory, South Parks Road, Oxford OX1 3QZ, United Kingdom

[‡]Laboratory of Molecular Physiology, National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, Maryland, 20892, United States

Description of the supporting movies S1-S4

Movie S1: iSCAT imaging of processive myosin 5a HMM molecules along actin filaments. Raw data was recorded at 1.7 kHz with 167× magnification (64 nm/pixel), background subtracted via temporal median filtering and then time averaged down to 10 Hz to produce the displayed movie. The movie frame rate is set to 25 Hz and the field of view corresponds to 6.6 μm × 6.6 μm.

Movie S2: Total internal reflection fluorescence imaging of single processive myosin 5a HMM molecules. Each myosin contains two GFP moieties that give rise to the fluorescence signal upon excitation at 473 nm. Frames were recorded with 100 ms exposure time under frame transfer mode at a frame rate of 10 Hz with 333× magnification (72 nm/pixel) by an iXon3 860 EM-CCD camera. The movie is played at 10 Hz and the field of view corresponds to 4.6 μm × 4.6 μm.

Movie S3: Consecutive imaging of the same myosin 5a HMM sample as in Movie S2 by interferometric scattering microscopy. Raw data was recorded at 1.0 kHz with 333× magnification (32 nm/pixel), background subtracted via temporal median filtering, spatially binned to 64 nm/pixel, and then time averaged to 10 Hz to produce the displayed movie. The movie is played at 10 Hz and the field of view corresponds to 5.08 μm ×5.08 μm.

Movie S4: Nanometric tracking of a single myosin 5a HMM molecule recorded by interferometric scattering microscopy. Raw data was recorded at 1.0 kHz with 333× magnification (32 nm/pixel), background subtracted via temporal median filtering, spatially binned to 64 nm/pixel, and then time averaged to 25 Hz to produce the displayed movie. Movie is played at 25 Hz and the field of view corresponds to 1.53 μm × 1.53 μm. The displayed movie has been up-scaled six-fold for ease of visualization with no further modification of the data.