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**Supporting Material**

**The conserved L5 loop establishes the pre-powerstroke conformation of the kinesin-5 motor, Eg5**

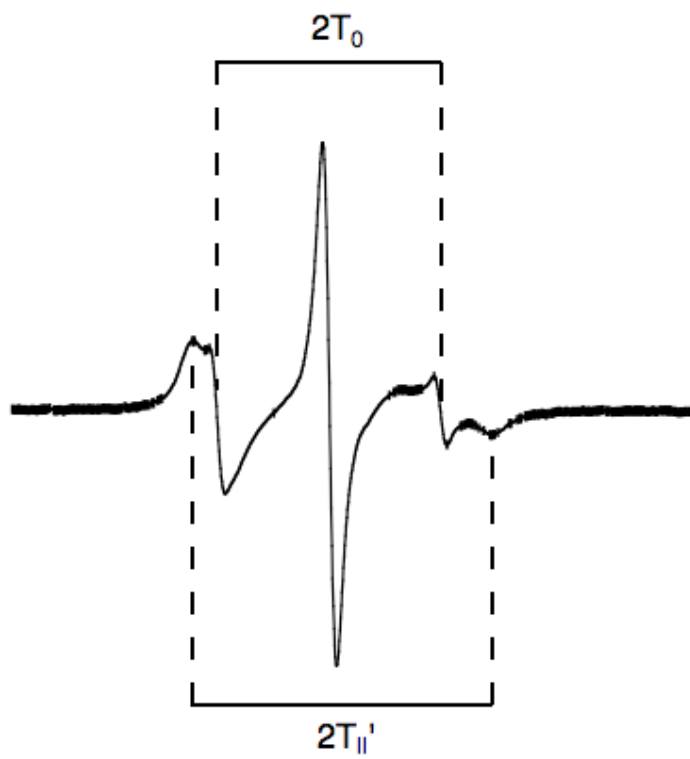
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## Supplemental figure legends

**Figure S1: Spectral parameters for cone angle determination.**  $2T_{||}$  is the low-field to high-field splitting for the observed spectrum.  $2T_0$  is the hyperfine splitting for probe tumbling rapidly in solution. In practice, the two parameters are determined from separate spectra, but are shown here superimposed on a single multi-component spectrum for brevity.

**Figure S2: Basis spectra and sample deconvolutions for E124C-MSL, L360C-MSL, and V365C-MSL.** Left, basis spectra used for probes at each location. For both E124C-MSL and L-360C-MSL, the spectrum of E124C-MSL bound to MTs in the presence of ADP•AlF<sub>4</sub> at 25°C was used as the mobile basis spectrum (red) and the spectrum of APO V365C-MSL bound to MTs at 2°C was used as the immobilized basis spectrum (blue). For V365C-MSL, the spectrum of E124C-MSL bound to ADP in solution was used as the mobile basis spectrum and the same immobilized basis spectrum was used as for E124C-MSL and L360C-MSL. Basis spectra shown are normalized to contain the same number of spins. Right, spectral deconvolutions. Right, sample deconvolutions and  $\chi^2$  values for probes at each location. Each deconvolution shown is for ADP-bound Eg5 bound to MTs. The experimental data is shown in blue, the linear combination of mobile and immobilized basis spectra that best fit the experimental data is shown in red, and the residual error is shown in black.  $\chi^2$  values for all data reported were  $\leq 2\%$ .

Figure S1



**Figure S2**

Deconvolution of spectral components

