



Nucleotide-Induced Flexibility Change in Neck Linkers of Dimeric Kinesin as Detected by Distance Measurements Using Spin-Labeling EPR



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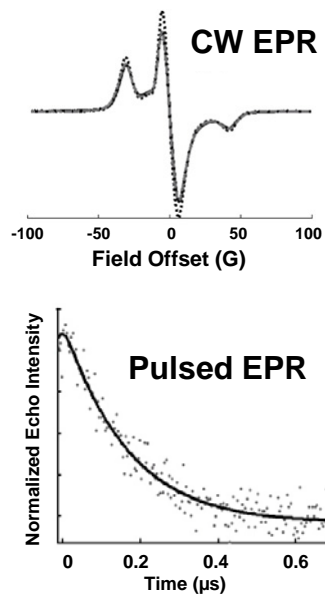


Figure 1. Continuous wave (CW) EPR and pulsed EPR DEER (Double Electron Electron Resonance) spectra of interacting spin pairs in microtubule-bound no-nucleotide kinesin.

Upper panel: kinesin dimer (black line), monomer control (dashed line). Lower panel: DEER spectra (points), fitted curve (continuous line).

Using dipolar continuous wave and pulsed electron paramagnetic resonance methods, we have determined the distribution of the distances between two spin labels placed on the middle of each of the neck linkers of dimeric kinesin. Some of the spin labels at Cys332 of the linkers in the microtubule-free or microtubule-bound no-nucleotide state are >5 nm apart and display a wide distribution, suggesting a high level of flexibility and extension of the linkers (loose state). In the presence of microtubules and nucleotides, the distance distribution decreases, implying a reduction in linker flexibility. However, the linkers are in equilibrium between the two populations: one is well-restricted at 1.6 nm (tight or docked state), and the other is widely distributed over 2–5 nm (loose or undocked state). We propose that large nucleotide-dependent flexibility changes in the linkers contribute to the directional bias of the kinesin molecule stepping 8 nm along the microtubule.

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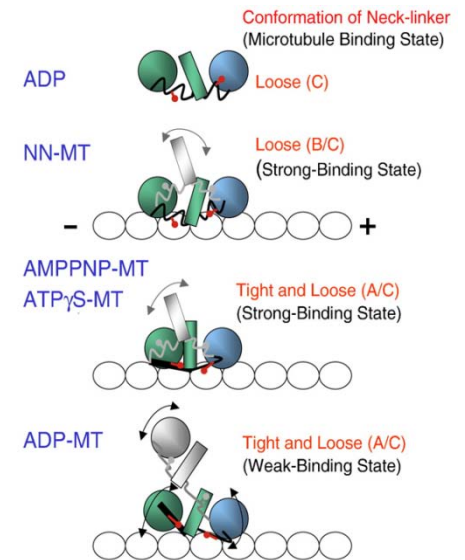


Figure 2. Proposed conformations of kinesin neck linkers. The neck linkers are in an equilibrium among three conformations (A-, B- and C-linkers), with different flexibility $A < B < C$. A-linkers: interspin distance of 1.6nm; B-linkers: 1.3-2.5 nm; C-linkers: 2-5nm. Microtubule (MT). No-nucleotide (NN).