

# Structural Properties of the Kinesin "Coiled-Coil Break 1" Region

S. MADATHIL, K. FAHMY, A.H. CREVENNA<sup>1</sup>, D.N. COHEN<sup>1</sup>, J. HOWARD<sup>1</sup>

Submitted to *Biophys. J.*

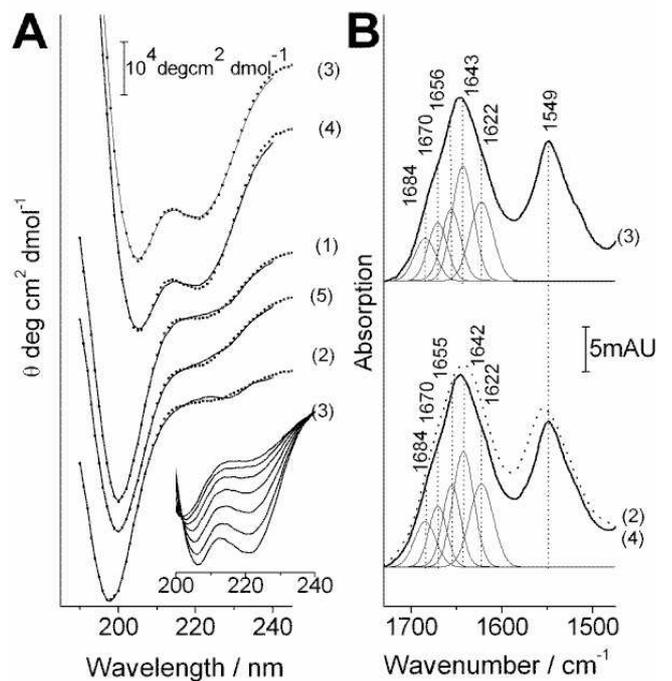
## Abstract

Kinesin-1 transports cargo along microtubules. In vivo, several kinesins move a single organelle and it is important that they operate in a coordinated fashion so that they do not interfere with each other. Coordination is thought to be facilitated by flexible domains. The tail of kinesin-1 is predicted to be a coiled-coil with two main interruptions, the swivel (380-442aa) and the hinge (560-624aa). In a gliding assay, [1] deletion of the swivel impaired motor cooperation at large kinesin densities. The torsion elasticity constant ( $k$ ) was measured by analyzing the rotational random motion of microtubules tethered to a glass surface by single kinesin molecules. Removal of the swivel has no effect on the stiffness of the motor ( $k = 7 \pm 1 k_b T / rad$ ) nor has the removal of the hinge and subsequent tail domains ( $k = 8 \pm 1 k_b T / rad$ ) when compared to the full length ( $9 \pm 3 k_b T / rad$ ). The structure and thermal stability of the swivel region was studied by CD, FTIR, and fluorescence spectroscopy of model peptides. The data indicate the existence of secondary structure within the  $\approx 50$  central residues in the Swivel flanked by random stretches. Dimer formation between these more stable helical domains of the Swivel in the two kinesin heavy chains may account for the unexpected large torsional rigidity of the Swivel observed here at the single molecule level. We propose that the localized helical propensity in the swivel provides a mechanism for a reversible structural transition that generates the required flexibility at high motor densities by strain-dependent helix unwinding.

## Results and Discussion

The functional studies carried out in this project convincingly show that the Coiled-Coil Break-1 behaves differently from what is expected for a disordered structure: (1) its torsion elasticity is higher than predicted by the WLC model, (2) its deletion doesn't cause an increase of stiffness. These results suggest that a certain degree of secondary and / or tertiary structure may be present in this region which was experimentally demonstrated by the structural studies on the five 30 aa long overlapping pieces of synthetic model peptides derived from this region. CD spectroscopy at 20°C revealed that the peptides 3 and 4 (corresponding to the central part of Coiled-Coil Break-1) show characteristic  $\alpha$ -helical far UV-CD spectra and display the greatest molar ellipticity corre-

sponding to around 70% total helical content. Peptide 1 and 5 have a low percentage of helicity and peptide 2 is the most unstructured one, supporting previous findings. The FTIR data of the most helical peptides 3 and 4 suggest that these helices form coiled-coils [2]. The energetics of the structural transitions of the model peptides, addressed by fluorescence-based thermal unfolding experiments, is in full agreement with the presence of defined secondary structure demonstrated by CD and FTIR spectroscopy. Based on the ensemble of our functional and structural studies we suggest that only at high motor densities the strain exerted on the swivel induces the transition of the structured regions from helical to disordered stretches, thereby providing the required flexibility in a function-dependent manner.



**Fig. 1** (A) CD spectra of the 5 model peptides at 20°C and the inset shows the CD spectra of peptide 3 from 2-60°C. (B) FTIR spectra of peptide 3 and 4 which form coiled coils and of peptide 2 which is unstructured.

- [1] J. Howard, A.J. Hudspeth, R.D. Vale, *Nature*. 342 (1989) 154-158
- [2] S. Krimm, J. Bandekar. *Adv. Prot. Chem.* 38 (1986) 181-236

<sup>1</sup> MPI-CBG Dresden