

within the L5 loop in allosteric communication and why there are differing efficacies in drug inhibition. Here we demonstrate an integrated approach to build, test, and refine a model of how the L5 loop alters the conformation of the motor domain in solution. Over 30 perturbations of the L5 loop, either by sequence variation or drug binding, were analyzed using kinetic data, vibrational spectroscopy, and multivariate analysis. Principal component analysis organized mutant kinesins into two populations of Eg5 conformers, distinguished by changes mainly in 3<sub>10</sub> helices and unordered regions. The presence of inhibitors also resulted in coincident, steady-state structural changes in this kinesin. We surmise that the above conformational changes are localized to the L5 loop. Unexpectedly, conformational changes were not restricted to the drug-binding pocket alone: we have directly measured long-distance changes to the beta-sheet core of the kinesin protein, a requirement for allostery that is quantifiable in this analysis. Such tools can ultimately permit prediction of pleiotropic changes in structure and consequently protein function and drug efficacy.

### 853-Pos

#### Structure-Function Studies of Loop L5 in the Mitotic Kinesin Eg5

Steven S. Rosenfeld, William M. Behnke-Parks.  
Columbia University, New York, NY, USA.

All processive kinesins contain an unusual structural motif consisting of an alpha helix ( $\alpha 2$ ) interrupted in the middle by a stem and loop motif known as L5. The role of L5 in the overall enzymatic function of kinesin motors remains unknown. However, its importance is highlighted by the finding that a variety of small molecule inhibitors of the mitotic kinesin Eg5, which contains the longest L5 in the kinesin superfamily, bind to this region with high affinity. These inhibitors induce a folding of L5, and trap the motor in an ADP bound, weak microtubule binding conformation. In order to gain greater insight into the function of L5, we have characterized three site-directed mutants in the loop (position 121), stem (position 131) or in the portion of  $\alpha 2$  amino-terminal to this stem and loop motif (position 113). These three mutations have profound effects on the kinetics of structural transitions that occur with nucleotide and microtubule binding, and our results indicate that this conserved structural motif plays an important role in tuning the kinetics of kinesin motors.

### 854-Pos

#### A Conserved Element in Kinesin-5 Motors Couples ADP Release to a Forward Step

Adam Larson<sup>1</sup>, Nariman Naber<sup>2</sup>, Roger Cooke<sup>2</sup>, Edward Pate<sup>3</sup>, Sarah Rice<sup>1</sup>.  
<sup>1</sup>Northwestern University, Chicago, IL, USA, <sup>2</sup>University of California-San Francisco, San Francisco, CA, USA, <sup>3</sup>Washington State University, Pullman, WA, USA.

Kinesin superfamily motor proteins contain a structurally conserved loop near the ATP binding site, termed L5. The function of L5 is unknown, although several drug inhibitors of the mitotic kinesin Eg5 bind to L5. Here, we performed electron paramagnetic resonance spectroscopy on Eg5 with spin labels site-specifically attached to ADP, to L5, and to the neck linker element that docks along the enzymatic head to drive forward motility on microtubules. Our results indicate that L5 undergoes a conformational change that enables Eg5 to bind to microtubules in a pre-powerstroke state, with its neck linker undocked. Deletion or inhibition of L5 blocks this pre-powerstroke state and abolishes the fast, coordinated stepping of the Eg5 dimer. This L5-dependent motile mechanism should prevent single Eg5 dimers from moving on two separate microtubules and forming aberrant microtubule cross-links in the mitotic spindle.

### 855-Pos

#### Single Molecule Analysis of the Mitotic Kinesin Eg5

Joshua S. Weinger, Tarun M. Kapoor.  
Rockefeller University, New York, NY, USA.

In vertebrates, proper formation of the mitotic spindle requires the activity of Eg5, a motor protein of the kinesin-5 family. Loss of Eg5 function leads to monopolar spindles and mitotic arrest. Eg5's homotetrameric configuration, in which two pairs of motor domains are connected by a central stalk, allows it to crosslink and slide microtubules. Previous single molecule fluorescence studies of Eg5 have shown that in physiological ionic strength its motion along single microtubules is predominantly diffusive and ATP-independent, but becomes predominantly directional upon crosslinking two microtubules. The structural and mechanistic basis of this allosteric regulation of Eg5's motor activity is poorly understood. One possibility is that Eg5's non-motor domains, including the central stalk and C-terminal tail, transmit signals of microtubule binding state across the Eg5 tetramer and regulate motor activity. To probe the roles of Eg5's non-motor domains and to establish structure-function relationships for these domains, we have generated a series of GFP-labeled Eg5 deletion constructs, including a dimeric version and tetrameric versions containing deletions of the motor domain and of the C-terminal tail domain. Each of the constructs, including one that lacks the kinesin motor domain, binds microtu-

bules with varying affinities. In a multiple motor microtubule gliding assay, deletion of the tail domain results in a 50% increase in gliding velocity, from 15 nm/s for full-length Eg5, to 23 nm/s for tail-less Eg5. This indicates that the tail domain is capable of attenuating Eg5 motility. To understand the contributions of the Eg5's domains to its directional and diffusive movement, we are using single molecule fluorescence microscopy to characterize the motility along microtubules for each construct. These studies suggest a model wherein the non-motor domains of Eg5 modulate its motor activity and contribute to its ability to associate with microtubules.

### 856-Pos

#### Tetrameric Chimera DK4mer is a Tool to Study Mechanisms Of Kinesin-5 Regulation. A Tetrameric Chimera of a Kinesin 1 and a Kinesin 5 is a Fast Microtubule Sliding Motor

Christina Thiede, Stefan Lakämper, Alok D. Weßel, Stefanie Reiter, Christoph F. Schmidt.

Georg-August-University, Goettingen, Germany.

The homo-tetrameric motor protein Eg5 from *X. laevis* drives relative sliding of anti-parallel microtubules by the processive action of its two opposing sets of dimeric motor. As shown by Kwok *et al.* (2006, Nat. Chem. Biol. 2:480) and Kaptein *et al.* (2008, J. Cell Biol. 182:421), tetrameric motors move slowly (~20nm/s), but processively on a single microtubule alternating between diffusional and directional episodes, while motors moving between two microtubules move in a highly directional and processive fashion.

In order to obtain a tetrameric model system with more clearly defined properties and motile phases, we have constructed a tetrameric chimera by replacing Eg5-motor domain and neck-linker by the homologous regions of D. melanogaster Kinesin 1 (DK4mer).

In surface-gliding assays, Dk4mer showed fast motility (553 ± 31nm/s), irrespective of a C-terminal his- or GFP-his-tag. Comparison to DmKHC shows a similar  $k_{0.5, ATP}$  of ~0.06mM, suggesting that the GFP-tagged version is suitable for single-molecule fluorescence studies. Single GFP-tagged DK4mer motors moved processively along the MT at speeds comparable to those seen in surface-gliding assays (499 ± 3nm/s). We observe clearly distinguished directional and diffusional episodes and an overall run length of ~9µm on average. We further performed relative sliding assays using DK4mer and observe the expected trimodal distribution of velocities at  $v=0$ ,  $v=v_1$  and  $v=2v_1$ ,  $v_1$  being 500nm/s, clearly showing that DK4mer is capable of sliding microtubules apart simultaneously using both pairs of motor domains.

The DK4mer is thus an excellent model system to study regulatory aspects of Kinesin-5 due to its high speed, its long processivity and its clear separation of diffusive and directional motility and its fast and efficient relative sliding of microtubules.

(\*authors contributed equally)

### 857-Pos

#### Analysis of Conformational Change of Conventional Kinesin Chimeric Protein Fused with GFP using Small Angle X-Ray Solution Scattering

Keiko Tanaka<sup>1</sup>, Yasunobu Sugimoto<sup>2</sup>, Katsuzo Wakabayashi<sup>3</sup>, Shinsaku Maruta<sup>1</sup>.

<sup>1</sup>Division of Bioinformatics, Graduate school of Engineering, Soka University, Tokyo, Japan, <sup>2</sup>Graduate school of Engineering Science, Osaka University, Osaka, Japan, <sup>3</sup>Graduate school of Engineering Science, Osaka University, Tokyo, Japan.

Recently, we have successfully dissolved the crystal structure of ADP bound K16 motor domain. The overall structure of the K16MD is similar to that of conventional kinesin motor domains, as expected from the high similarity of amino acid sequence. However, neck-linker region of K16 showed an ordered conformation in a position like that of Eg5. Previously, we have designed the K16 motor domain chimera protein fused with GFP at the neck-linker in order to monitor the conformational change of the neck-linker during ATP hydrolysis by small angle X-ray solution scattering. We determined the Radius gyration (Rg) values of K16-GFP in the presence or absence of nucleotides by X-ray solution scattering. The Rg of nucleotide-free K16-GFP was about 42 Å. In the presence of ADP and ATP, the Rg values were 38 Å and 39 Å, respectively. In this study, conventional kinesin fused with GFP (KIF5A-GFP) was prepared and analyzed by small-angle X-ray scattering in order to compare its neck-linker conformation with K16-GFP. The Rg value of ADP and AMPPNP states are 34.5 Å while that of nucleotide-free is 35.4 Å. For KIF5A-GFP, the Rg difference between nucleotide-free state and nucleotide-docked state is three times smaller than K16. These results suggest that the conformational change of K16 neck-linker is more significant than KIF5A. Moreover, Eg5 fused with GFP (Eg5-GFP) was also successfully expressed in *E. coli.*, which has different orientation of neck-linker in crystal structure. The conformation of the kinesin in the solution was also analyzed.