We prepared the kinesin motor domain (K355) mutant that has a single cysteine at neck linker region and His-tag at C-terminal. Subsequently the kinesin mutant wasimerized with photochromic bifunctional cross-linker, azobenzene dimaleimide (ABDM). And the photo-reversible regulation of the ATPase and motor activities of the kinesin dimer cross-linked with ABDM was studied. We also tried to develop photo-responsive vesicle composed of photochromic molecules as a cargo for the photo-controlled kinesin. Diacetyl glycerol was coupled with carboxypyrrol-propioanpyron to be phospholipid analogue using carbonyldiimidazole condensation reagent. The spiroanpyon moiety performs photo-reversible isomerization between hydrophobic spiro form and merocyanine zwitterion form upon visible light and ultraviolet light, respectively. Therefore, it is expected that the merocyanine form of the mimick phospholipid results in formation liposome like vesicle. The photo-reversible formation of the vesicle was studied using water-soluble fluorescent probe.

680-Pos Board B460 Study of Phospho-Regulation of a Mitotic Kinesin using a Directed Evolution Approach Alina Goldstein1,2, Nurit Siegler1,2, Liam Holt1, Leah Gheber1,2, 1Department of Chemistry, Ben Gurion University of the Negev, Beer Sheva, Israel, 2Ilse Katz Institute for Nanoscale Science and Technology, Ben Gurion University of the Negev, Beer Sheva, Israel, 1Department of Molecular and Cell Biology, University of California, Berkeley, Berkeley, CA, USA.

The S. cerevisiae Cin8 belongs to the kinesin-5 sub-family of mitotic motor proteins. During mitosis, Cin8 orchestrates the mitotic spindle assembly and its elongation. Recent work from our laboratory indicated that phosphorylation of Cin8 by Cdkl1 governs its localization to the mitotic spindle during mitosis. Here we tested the rigidity of phosphorylation sites in Cin8, and examined whether phosphorylation at newly created Cdkl1 sites can mimic the known phospho-regulation or create new regulation. For this purpose, we generated phosphorylation-deficient mutant of Cin8 and introduced new Cdkl1 sites by single amino acid replacement. This resulted in thirty-one novel Cdkl1 phosphorylation sites. In part of the sites, partial and full Cdkl1 consensus sites were created. Next we analyzed Cin8 localization to the spindle during anaphase. We found that only novel Cdkl1 phosphorylation site at position 276 is able to restore the original phosphory-regulation of Cin8, and is located in high proximity to a native Cdkl1 phosphorylation site (S277). Although several sites were created nearby, only this site exhibits localization pattern which is similar to WT-Cin8. This result suggests that phospho-regulation of Cin8 by Cdkl1 at this region is rigid and highly dependent on the structural context. Several additional novel Cdkl1 mutants exhibited new phenotypes, suggesting that there are regions in Cin8 where phospho-regulation by Cdkl1 is more flexible. These results imply that phospho-regulation of Cin8 is more elusive than previously anticipated and further study of its mechanism is required.

681-Pos Board B461 Transport by a Kinesin in the Presence of Magnetic Nanoparticles Elsan Mirzakhalili1, Eleni Gourgou, Bogdan Epureanu1, Mechanical Engineering, University of Michigan, Ann Arbor, MI, USA.

Superparamagnetic nanoparticles are used to influence the medium in which a kinesin-5 motor protein acts. Using our recent work with Arabidopsis thaliana (AtKinesin-5, AtKinesin-7) in vitro, we observed that the kinesin protein shows a higher affinity for magnetic nanoparticles. Moreover, we noticed that the presence of magnetic nanoparticles affects the speed of kinesin transport. However, characterizing the motion of a kinesin in the presence of many magnetic nanoparticles requires stochastic simulations at a variety of conditions. The required computational time is prohibitive. Hence, a generalized model is developed to estimate the force on the cargo without solving the full-order system dynamics every time. Finally, the motion of cargo under varying magnetic fields is studied. These results can be used to detect possible deficiencies in kinesin - microtubule interactions.


Kinesin is an ATP-driven motor protein that plays important physiological roles in intracellular transport, mitosis and meiosis, control of microtubule dynamics, and signal transduction. Kinesin species derived from various organisms have been well characterized. In contrast, plant specific kinesins have yet to be adequately characterized. We have previously demonstrated that some kinesins derived from rice plant have unique biochemical characteristic properties and structures. In this study, we characterized rice plant specific kinesin E11 that belongs to the plant specific A4 subfamily in kinesin-7 family. E11 motor domain was expressed by E. coli expression system and purified with Co-chelate column in order to characterize biochemical and ATPase kinetic properties. The fluorescent ATP analogues, Mant-ATP and ADP-Mant were employed for the kinetic characterization. We have successfully observed significant FRET between Mant-ATP and intrinsic tryptophan (Trp23) residue in E11. The kinetic parameters of initial binding of Mant-ATP to E11 and release of Mant-ADP from E11 were analyzed by monitoring the FRET stopped flow apparatus and compared with other rice kinesins and conventional kinesins. The results revealed that the initial binding of ATP to E11 and release of ADP are slower than those of other rice plant specific kinesins.

683-Pos Board B463 Photo-Control of Mitotic Kinesin Eg5 using Thiol Group Reactive Fulgimide Derivative Yuki Tamura1, Dong Gyu Cho2, Tae Joon Jeon3, Shinsaku Maruta1,2, 1Div.Bioinfo.,Grad.Sch.Eng., Soka University, Tokyo, Japan, 2Chemistry, Inha University, Incheon, Korea, Republic of, 3Biological Engineering, Inha University, Incheon, Korea, Republic of, Dept.Bioinfo.,Fac.Eng., Soka University, Tokyo, Japan.

It is believed that the loop L5 of kinesin is important region for motor function. Interestingly mitotic kinesin Eg5 has a several times longer L5 in comparison with other kinesins. It has been demonstrated that the L5 of Eg5 performed as a stabilizer for the Eg5-specific inhibitors (STLC, melatoco) complexes. Aim of our study is to control the function of Eg5 photo-reversibly using photochromic molecules incorporated into L5. Previously, we have prepared Eg5 mutants (E116C, E118C, T125C, W127C, D130C) which have a single cysteine residue in L5 in order to incorporate photochromic molecules. We also synthesized thiol reactive photochromic molecules 4-phenylazomaleicinil(PAM) and lodoacetil-spiropyrain (IASP). PAM and IASP were incorporated into the mutants stoichiometrically. Some of the Eg5 mutants modified with PAM and IASP showed reversible alteration of ATPase activity upon ultraviolet (UV) and visible (VIS) light irradiations. In this study, we synthesized a novel thiol reactive photochromic molecules monoiodoacetoxyflugide(IFAG). Fulgimide performs photo-reversible isomerization between non-polar opened-ring form and polar closed-ring form upon visible light and ultraviolet light. IFAG was incorporated into Eg5 mutant W127C stoichiometrically. Although the modified Eg 5 mutant W127C-IFAG showed slightly decreased ATPase activity, the ATPase activity showed photo-reversible alteration upon UV and visible light irradiations. Alteration in the ATPase activity of W127C-IFAG in the presence of STLC upon UV and VIS light irradiations was also examined.

684-Pos Board B464 Photo-Regulation of Kinesin Intramolecularly Crosslinked by Bifunctional Azobenzen Derivative at the Coiled-Coil Stalk Region Haruka Fujio1, Kazunori Kondo2, Shinsaku Maruta1,2, 1Bioinformatics, Soka University, Tokyo, Japan, 2Bioinfo.,fac.eng, Soka University, Tokyo, Japan.

Kinesin is an ATP driven dimeric motor protein carries cellular cargoes along microtubules. The stalk region of kinesin is responsible for dimerization with coiled-coil interaction. Formation of dimer is essential for kinesin to perform processive movement along the microtubules. Aim of this study is to control dimerization of kinesin by the reversible conformational change at the cold-coil stalk region using photochromic molecule resulting in photo-reversible regulation of motility. Azobenzen-dimaleimide (ABDM) is a bifunctional SH reactive photochromic crosslinker and its crosslinking span is altered by cis-trans photo-isomerization of azobenzene moiety upon ultraviolet and visible light irradiations. We have previously demonstrated that the two reactive cysteine residues SH1(707) and SH2(697) in α-helix of myosin which region is believed to have a energy transducing role, were cross-linked by...