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Insights into the autoinhibition of a kinesin-4 family member

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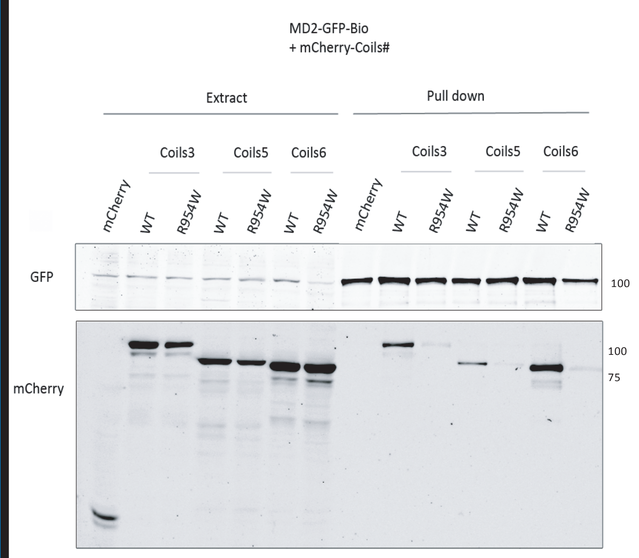
Abstract

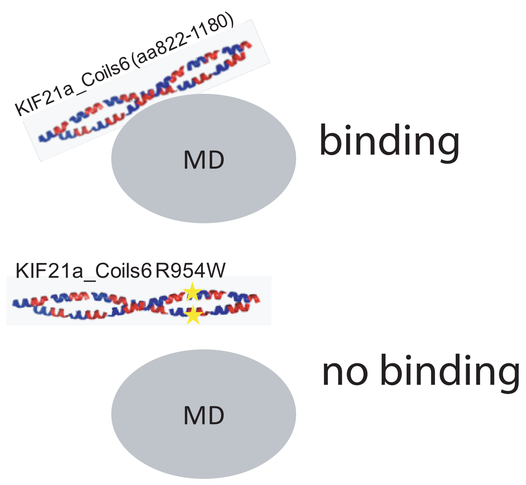
Intracellular cargo transport is a fundamental process to guarantee cell division, polarisation and morphology. This important task involves the kinesin family members of microtubule motor proteins (also called KIFs). Kinesins transport cellular organelles, vesicles and newly synthesized proteins along microtubules to their cellular destination, using adenosine tri-phospohate (ATP) hydrolysis as driving force. A conventional kinesin consists of an N-terminal motor domain (MD) involved in MT binding and ATP hydrolysis, followed by a α-helical stalk domain required for dimerisation and a tail domain necessary for cargo binding. KIF21a is a neuronal expressed kinesin-4 protein, which is vital for the development of the neuronal system. One of KIF21a interaction partners is KANK1, which is transported by the kinesin motor to the plasmamembrane where it negatively regulates actin stress fiber formation, as well as cell migration. It is crucial to tightly regulate the motor activity of kinesins because their malfunction is often related to severe neurological diseases and cancer. This is also true for KIF21a: several point mutations in the KIF21a gene are related to congenital fibrosis of the extraocular muscles type 1 (CFEOM1), which is characterized by the dysfunction of the oculomotor nerve resulting in inhibition of the muscles elevating the eye lid and globe. Interestingly, the majority of the point mutations lie in the coiled-coil stalk region of the motor protein with only one mutation located in the motor domain.

Here, we show that a part of the KIF21a stalk domain, which contains the residues involved in CFEOM1 (aa822-1180) binds to its own motor domain. This interaction is disrupted by introducing one of the CFEOM1 causing point mutations (R954W), suggesting that CFEOM1 patients probably have an overactive KIF21a protein that is not able to autoinhibit itself. Furthermore, we set out to biochemically and biophysically characterize the shortest KIF21a coiled-coil (cc) construct (aa938-1017) containing all the residues identified in CFEOM1 patients. Surprisingly, this peptide forms a monomeric antiparallel coiled-coil. We were able to produce crystals of this peptide for X-ray diffraction experiments and collected a native dataset at 2.3 Å and a Hg-derivative data set at 3.8 Å resolution. However, the datasets are not sufficient to allow for structure solution yet. Therefore, we plan to fuse the coiled-coil peptide to seryl tRNAsynthetase (SRS) from *Thermus thermophilus*.

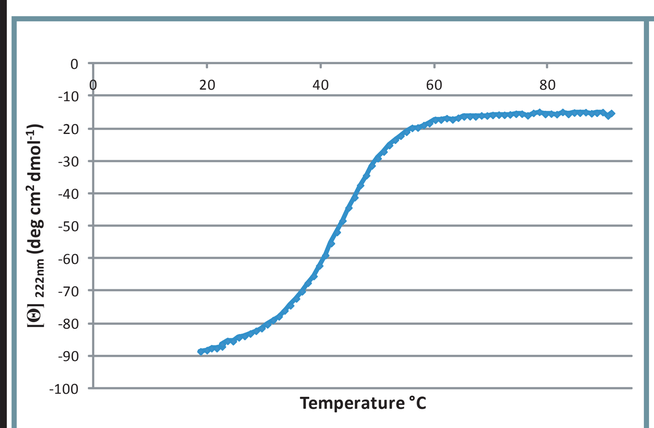
The structure of the KIF21a coiled-coil combined with structural analysis of the CFEOM1 mutants will lead to a better understanding of the disease. Furthermore, we aim to structurally, biochemically and biophysically characterize the complex of the KIF21a coiled-coil bound to its motor domain, which will provide insights into a new type of kinesin autoinhibition mechanism.

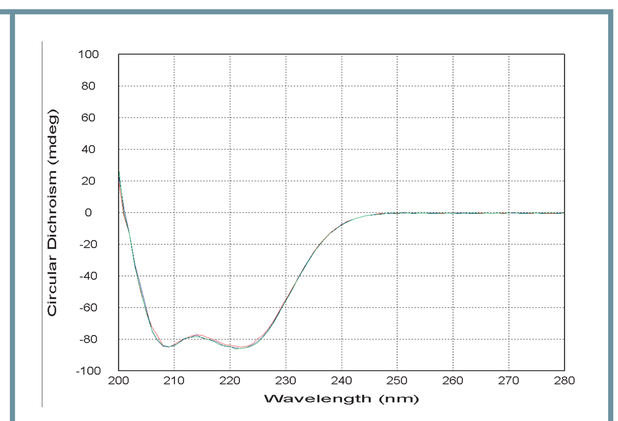
hsKIF21a stalk binds to its MDC- binding is disrupted by introducing a CFEOM1 pointmutation

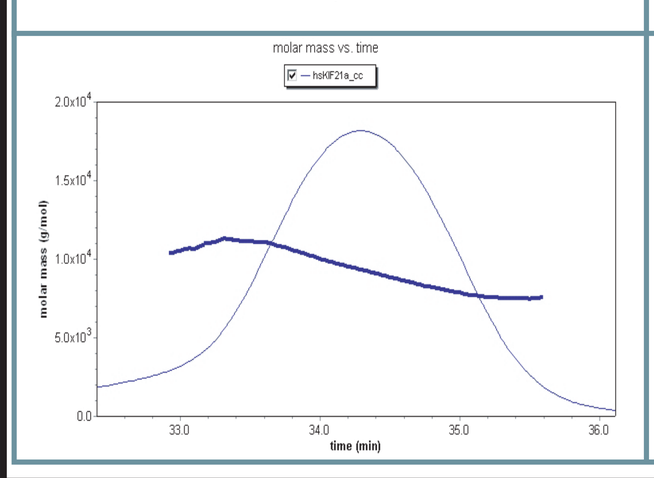


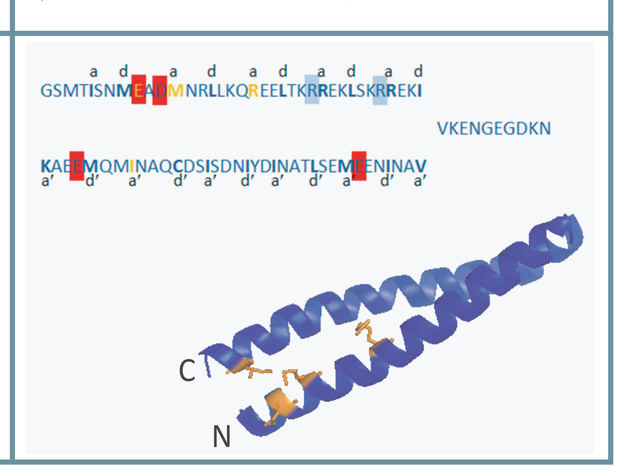


hsKIF21a_cc (aa938-1017) is monomeric and most likely an antiparallel coiled-coil

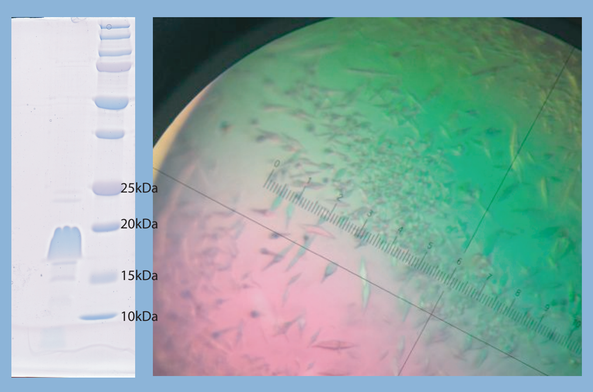


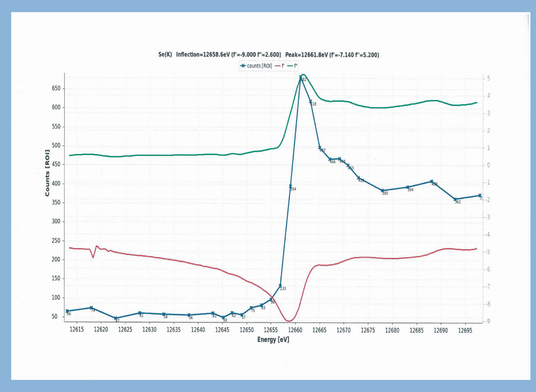




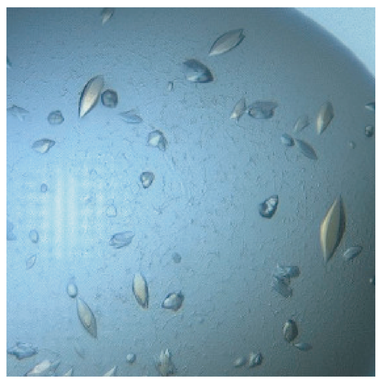


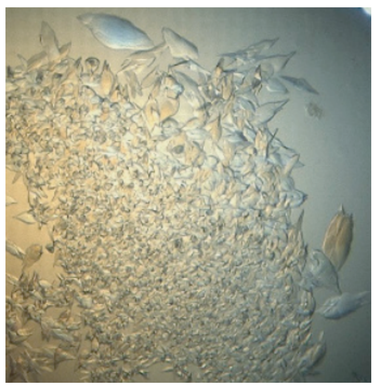
Se-Methionine labelling of hsKIF21a_cc





hsKIF21a_cc (aa938-1017) crystallisation





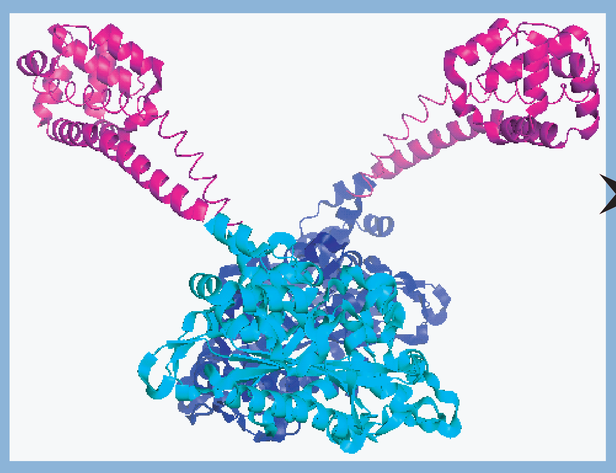
$P6_222$ or $P6_422$
native: 2.3 Å
S SAD: 2.6 Å

P312 or P321
native: 2.9Å
Hg-soaked: 3.8 Å

S SAD phasing--> no solution
Hg MAD phasing --> no solution
AMPLE --> no solution, but preliminary result is an antiparallel coiled-coil

➤ twinning problem!

Fusion protein of hsKIF21a_cc and SRS



➤ the wildtype antiparallel coiled-coil of SRS will be replaced by KIF21a_cc

PDB 3ERR, showing the structure of SRS (monomers cyan and blue) fused to dynein antiparallel coiled-coil and its globular MT binding domain (magenta), Gibbons *et al.* 2008 SCIENCE.

Summary

➤ KIF21a_cc is a monomeric antiparallel coiled-coil
➤ twinning problem with collected data sets

Outlook

- crystallizing SRS-KIF21a_cc fusion protein
- optimising Se-Methionine crystals--> structure!
- biophysical characterisation of coiled-coil CEOFM1 mutants
- crystallisation of KIF21a-MD and COILS6