Interaction of Kif7 and Gli2-ZF Probed by Hydrogen Deuterium Exchange Mass Spectrometry

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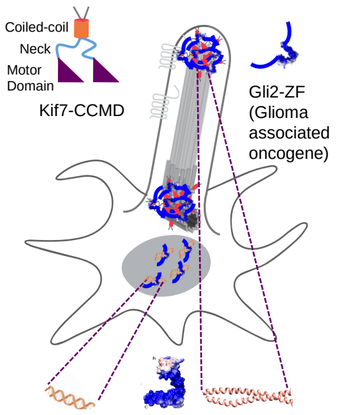
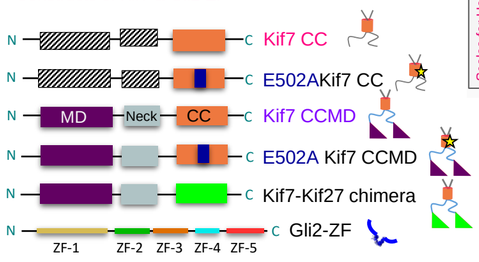
INTRODUCTION

- A conserved feature of Hedgehog signaling is localization of the main effector proteins, **Gli** (glioma associated protein) transcription factors to microtubules in the primary cilium.



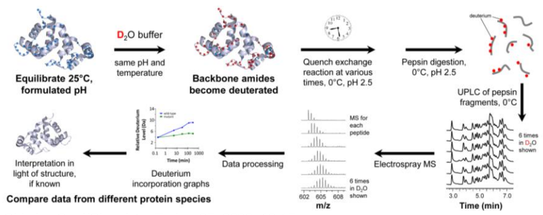
- The non-motile kinesin-4 protein **Kif7** mediates the recruitment of Gli to microtubules.
- DNA-binding zinc-finger domains of Gli2 (Gli2-ZF) interact with two unusual domains in Kif7, coiled-coil dimerization domain (Kif7-CC) and the motor domain (Kif7-MD).
- Furthermore, Gli binding increases the microtubule affinity of Kif7.

Constructs of Kif7 & Gli2-ZF



METHODS

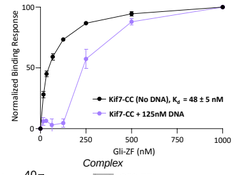
- Hydrogen Deuterium eXchange (HDX) was performed on each constructs. After labeling and quenching, proteins were digested online with Nepenthesin II for LC/MS.
- Mass spectrometry measurements were performed with a Waters Synapt G2Si HDMS^E. Mass spectra were analyzed with DynamX software.



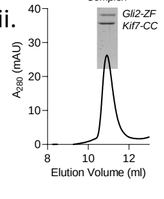
Chimica Oggi/Chemistry Today, **27**, 2009, 12-15.

Gli2-ZF and Kif7-CC binding assay

i.



ii.



i: Bio-layer Interferometry (BLI) assay to measure the binding affinity of Kif7-CC and Gli2-ZF with (purple) and without DNA (black)

ii: Size exclusion chromatography (Superdex 200 10/300 GL); Peak represents the complex formation between Kif CC and the Gli2-ZF

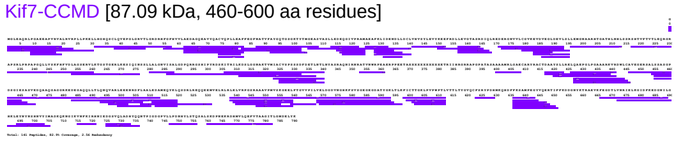
Nature Cell Biol, 24, 2022, 1088-1098

RESULTS AND DISCUSSION

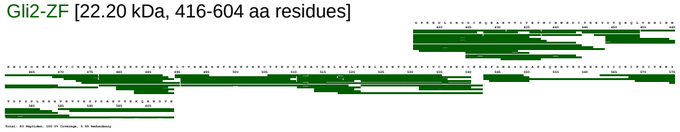
Sequence Coverage

Kif7-CC [15.67 kDa, 1-543 aa residues]

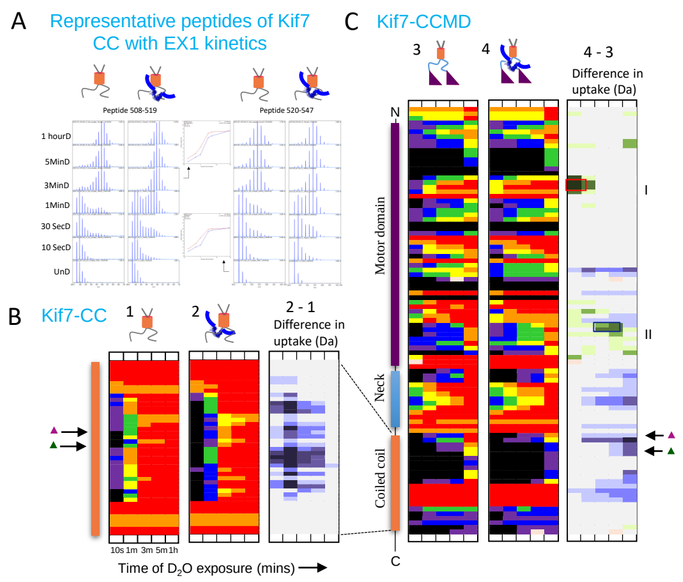
Kif7-CCMD [87.09 kDa, 460-600 aa residues]



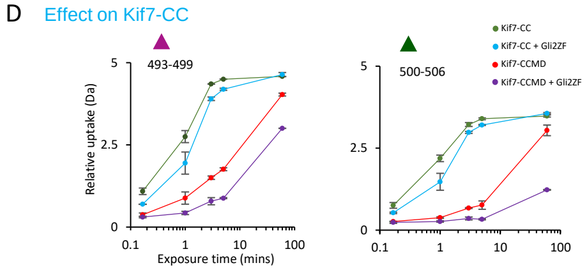
Gli2-ZF [22.20 kDa, 416-604 aa residues]



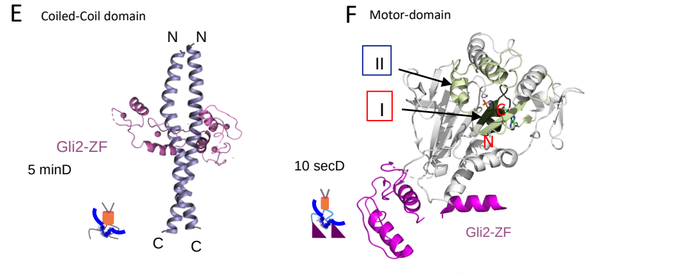
1. Effect of Gli2-ZF binding on Kif7



1. Stabilization of Coiled-coil domain of Kif7: **A**. Bimodal distribution of some peptides at the CC-domain, **B & C**. Differential deuterium exchange of Kif7-CC and Kif7-CCMD with and without Gli2-ZF bound,

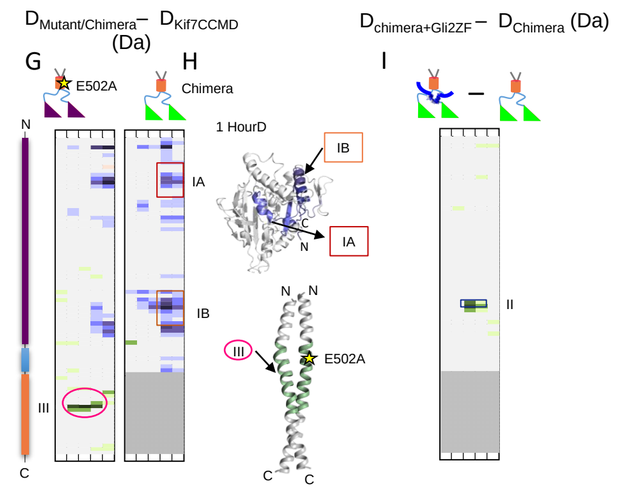


HDX data mapped on the crystal structure of Kif7-CC and Gli2ZF complex



D. Uptake plots of two peptides at the CC region showing difference in the deuterium uptake between the states (Kif7-CC and Kif7CCMD with and without Gli2ZF bound), **E.** data from difference in uptake (Da) for the kif7-CC:Gli2ZF complex at 5min mapped on the homology model of Kif7-CC domain, **F.** Data at 10 SecD for Kif7-CCMD:Gli2-ZF complex mapped on PRXO

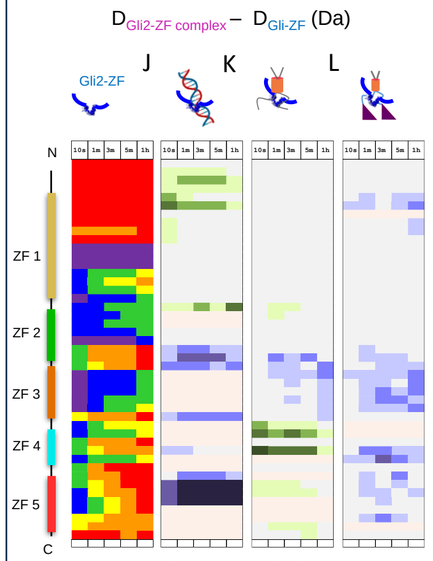
2. Effect of Mutation & truncation on Kif7 3. Ki7-Kif27chimera : Gli2ZF complex



2. *Mutation and truncation induced structural alteration on Kif7MD:* **G & H**:Differences in the deuterium uptake w.r.t Wild type Kif7-CCMD for the mutant E502A and the chimera Kif7. The difference in the deuterium uptake data at 1 h is mapped on the PDB; 7RXO for the motor domain and on the homology model of the Kif7-CC domain for the coiled-coil domain.

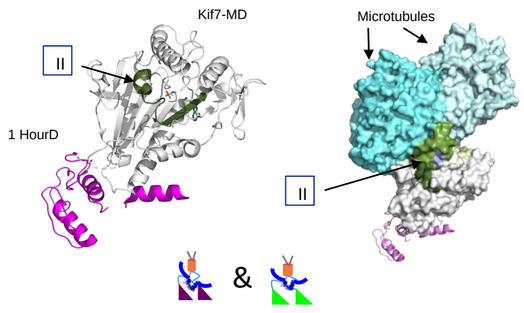
3. *Effect of Gli2-ZF binding on the motor domain of Kif7*: **I**. The difference of deuterium uptake of the chimera and Gli2-ZF complex w.r.t chimera

4. Binding effect of different ligands on Gli2-ZF



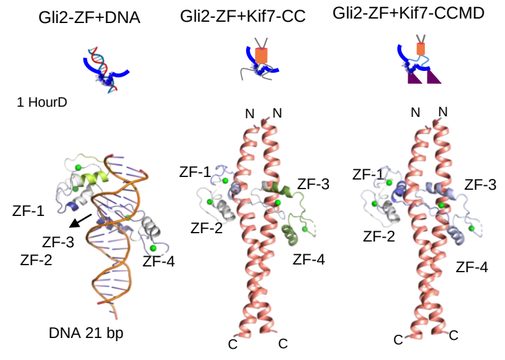
4. *Involvement of zinc fingers of Gli2 on binding to different ligands*: **J**, **K**, & **L**. Differences in the deuterium uptake of bound Gli2-ZF with DNA (21 bp), Kif7-CC and Kif7-CCMD respectively w.r.t un-bound Gli2-ZF

Effect on Kif7-Motor domain on interaction with GLi2-ZF in Ki7CCMD:Gli2ZF and Kif7-Kif27 Chimera:Gli2ZF complex



The difference in the deuterium uptake for the Kif7-CCMD:Gli2ZF complex w.r.t Kif7-CCMD alone at 1 Hour D_2O exposure is mapped on the structure, PRXO. 1 Hour D for the Kif7-chimera:Gli2ZF complex w.r.t Chimera also showed same difference (decreased protection) at peptide II (outlined with blue box). The surface representation shows the Kif7 MD:Gli2ZF complex bound to microtubule

Involvement of different zinc fingers of Gli2 in bound state



The difference in the deuterium uptake at 3 min time point for all three complexes is mapped on the structure. PDB:2Gli

CONCLUSIONS

1. Coiled coil domain of Kif7 gets stabilized upon interaction with Gli2-ZF. The CC domain from the Kif7-CC construct is more dynamic than the CC domain of Kif7-CCMD suggesting intradomain crosstalk

2. There were no measurable differences in HDX between CC- constructs of WT-Kif7 and E502A-Kif7. Both proteins behaved similarly and were more dynamic at the coiled-coil region. However, the comparison of HDX of the CCMD between WT and E502A showed the central region of the coiled-coil of the E502A mutant to be more deprotected in (residues 480-542). We hypothesize that the neck and the motor domains of Kif7 communicate with the coiled-coil region, either through direct quaternary contacts or through allosteric interactions

3. Although Kif7-CC mimics the DNA in overall structure the involvement of the different zinc fingers of Gli2 upon binding to Kif7-CC is different from binding to DNA. Additionally, Gli2-ZF binding to DNA showed overlapping in the ZF2-3 region (in agreement with structure) and distinct in flanking zinc fingers

ACKNOWLEDGEMENT

Prof. Thomas Wales for his input. Financial support from NIH R01-CA233978 for Prof. John R. Engen.
NIH & ACS for Radhika Subramanian. MGH ECOR-FMD fellowship for Farah Haque