Uncovering the structural logic of Cnn autoinhibition: an integrative NMR, SAXS, and AlphaFold approach

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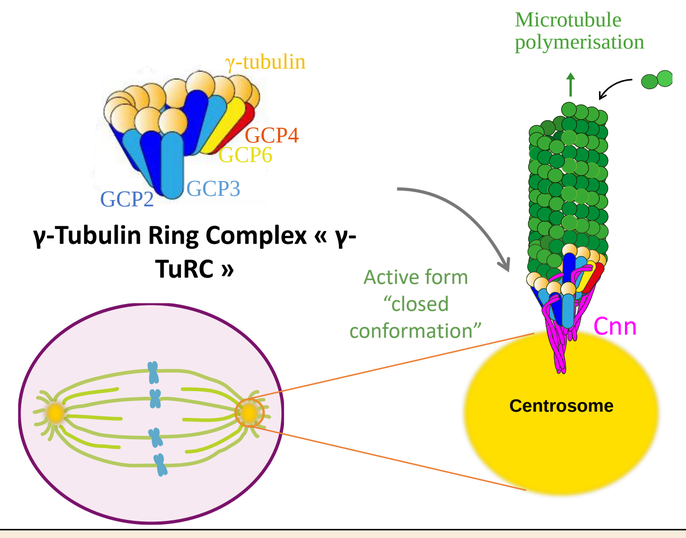
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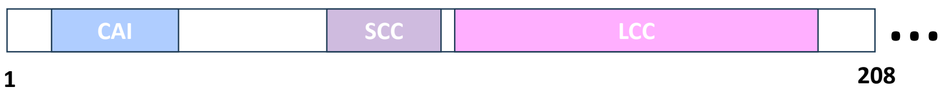
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Cnn: a modular centrosomal scaffold with unresolved structural regulation

Centrosomin (Cnn) is a key component of the *Drosophila melanogaster* centrosome, involved in microtubule nucleation. During cell division, Cnn is recruited to the centrosomes to form a scaffold essential for recruiting centrosomal factors. Among those factors, g-TuRC complex is recruited by Cnn N-ter region to template microtubules nucleation during mitosis.



Microtubule nucleation is tightly regulated, with Cnn maintained in an autoinhibited state in the cytosol to prevent ectopic γ-TuRC activation. The N-terminal region (residues 1–208) comprises three main sub-regions: an N-terminal autoinhibitory segment (CAI) present only in species close with drosophilae, a short α-helical motif (SCC), and a long coiled-coil domain (LCC).



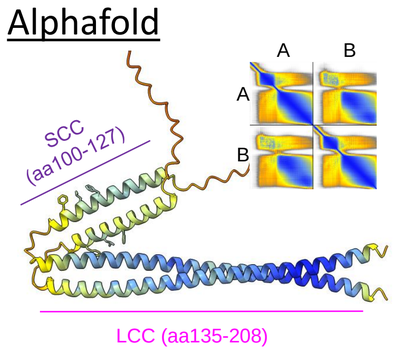
In *Drosophila*, autoinhibition is mediated exclusively by the CAI domain, whereas both the SCC and LCC are required for activation. In humans, by contrast, the SCC-equivalent alone is sufficient to activate γ-TuRC, while the LCC-equivalent negatively regulates SCC activity.

	Activation	inhibition
CDK5RAP2 (human)	Y-TuNA (equivalent of SCC)	γ-TuNA-In (equivalent of LCC)
Cnn (drosophilae)	**CM1 (SCC+LCC)**	**CAI**

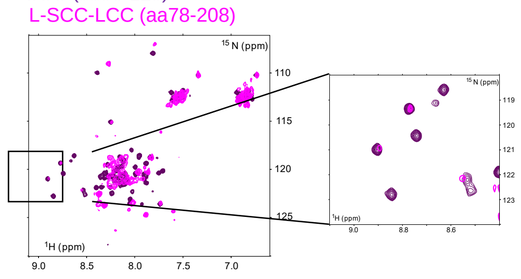
Key question : How does the CAI domain structurally inhibit the CM1 domain?

1. SCC+LCC: a necessary hairpin structure

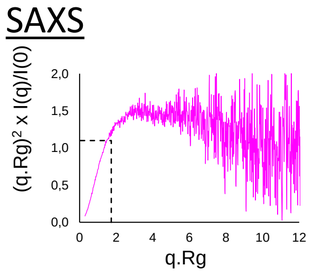
Alphafold



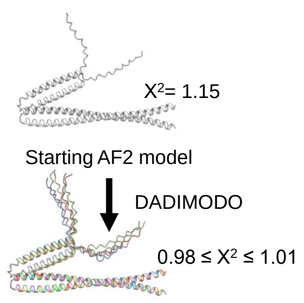
NMR



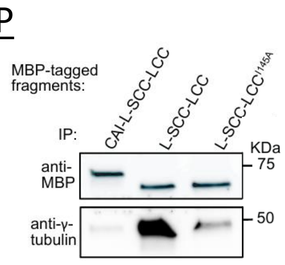
SAXS



Kratky plot shows an extended structure and some disordered region



Co-IP

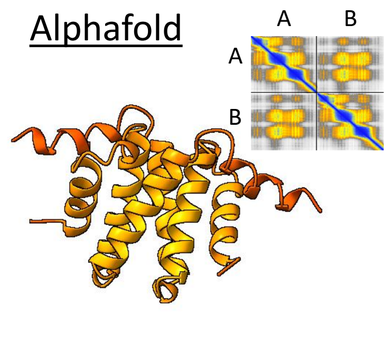


I145 is a key in the hairpin structure model

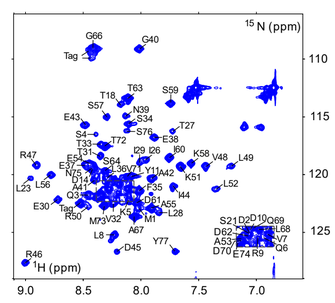
Validation of the hairpin structure

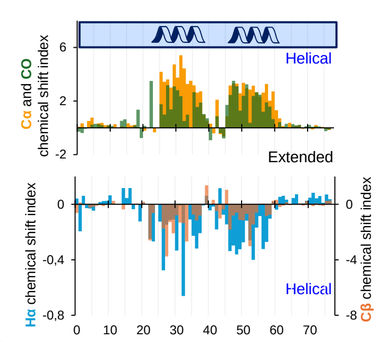
2. CAI: a domain hard to predict

Alphafold

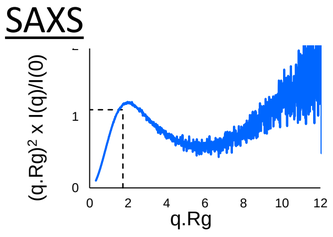


NMR

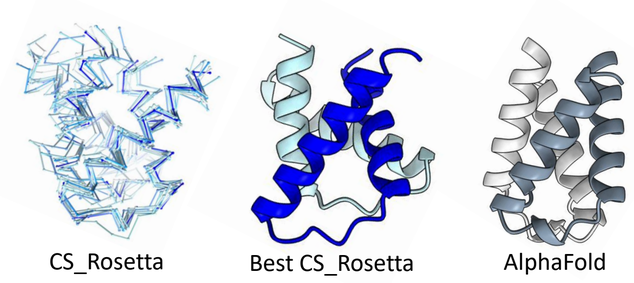




SAXS



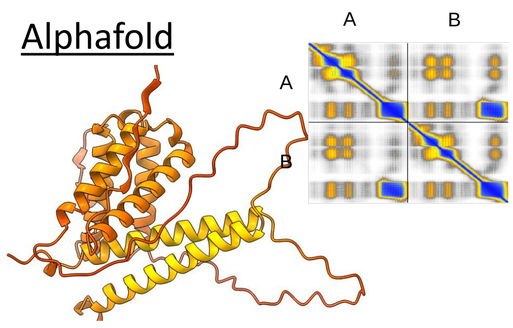
Kratky plot shows a globular structure with some disordered region



CAI adopts a double helix conformation

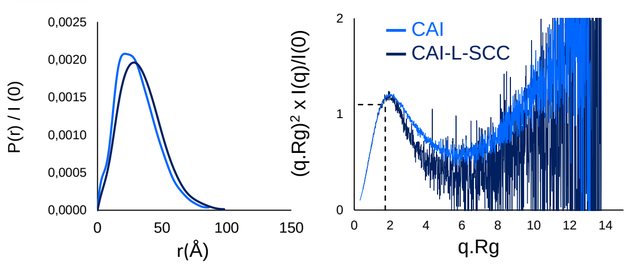
3. CAI in interaction with SCC

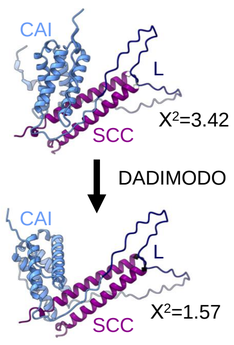
Alphafold



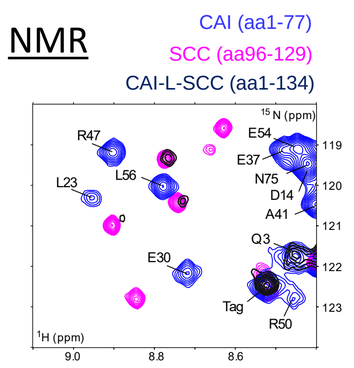
AF2 converges with a low confidence score on the interaction

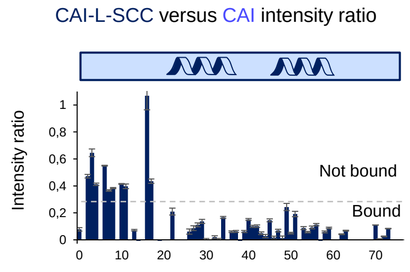
SAXS





NMR

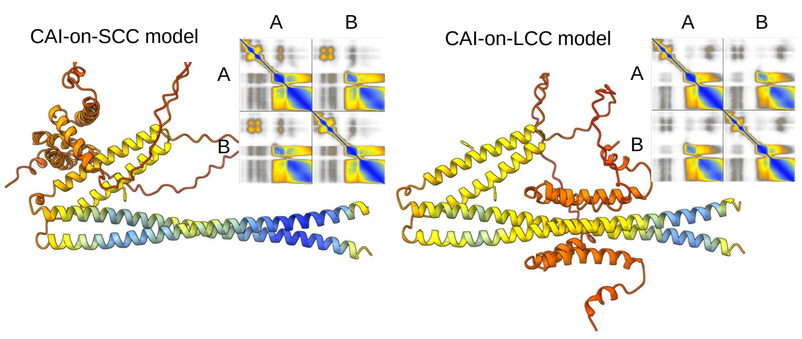




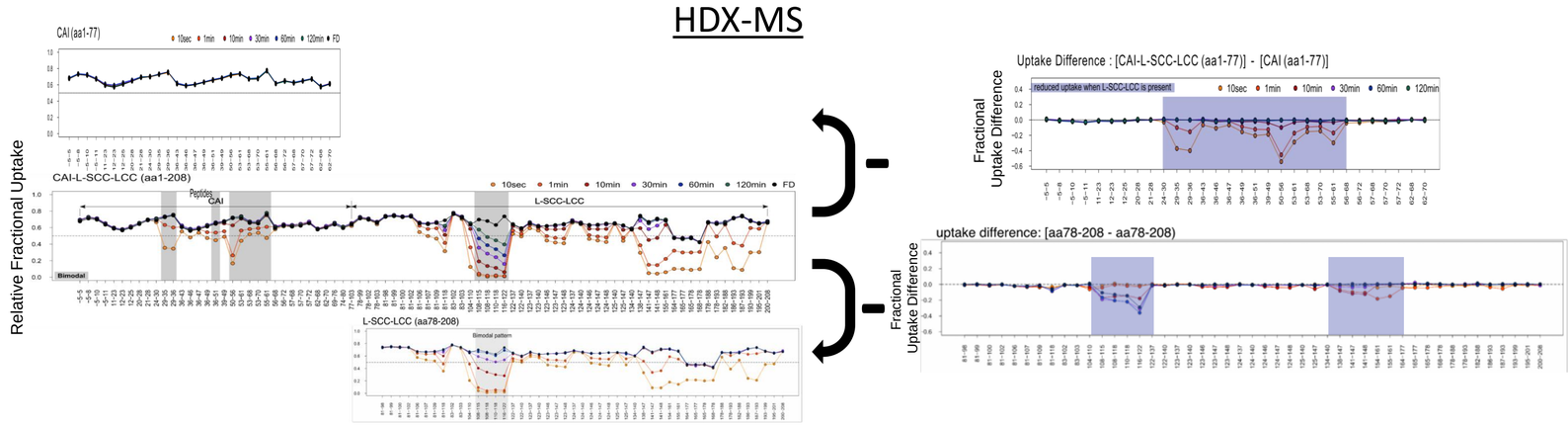
CAI interacts with SCC

4. CAI in interaction with SCC or LCC?

Alphafold



HDX-MS



CAI interacts with SCC even in the full construct

Conclusion & Perspectives

By combining HDX-MS, AlphaFold, SAXS, and NMR, we show that the CAI domain adopts a structured conformation and folds back onto the SCC motif, masking the γ-TuRC-binding interface and providing a structural basis for Cnn autoinhibition in *Drosophila*.

Future studies will focus on capturing the transition from the autoinhibited to the active state and elucidating how centrosomal cues, including phosphorylation, trigger SCC/LCC exposure and γ-TuRC activation.