Calmodulin assists during co-translational folding of the $K_v7.2$ channel calcium responsive domain

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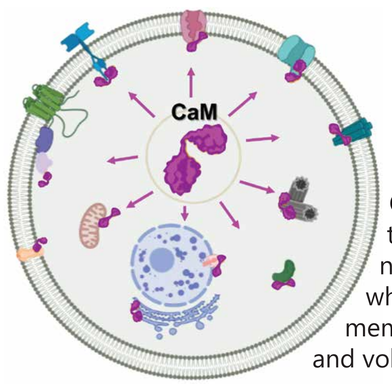
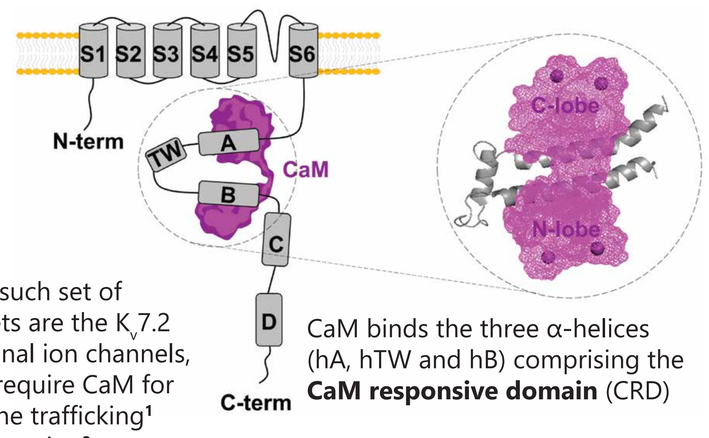
$K_v7.2$ neuronal ion channels and calmodulin
Introduction

Calmodulin (CaM) transmits Ca^{2+} signals to > 300 targets *in vivo* which cannot bind Ca^{2+} themselves.



One such set of targets are the $K_v7.2$ neuronal ion channels, which require CaM for membrane trafficking[1] and voltage gating[2].

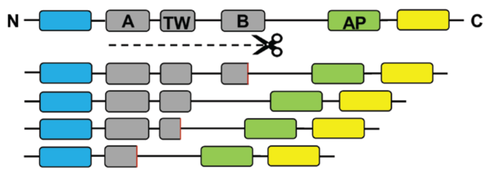


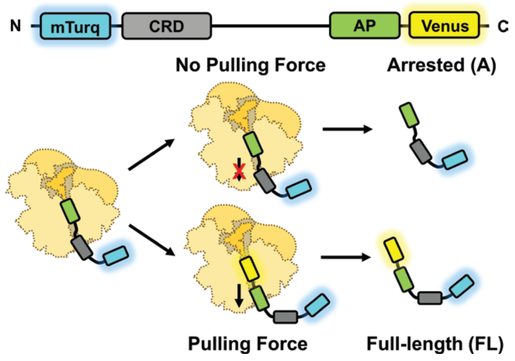
CaM binds the three α-helices (hA, hTW and hB) comprising the **CaM responsive domain** (CRD)

CaM is also involved in the assembly and folding of these channels[3], but the **underlying mechanisms are unknown**.

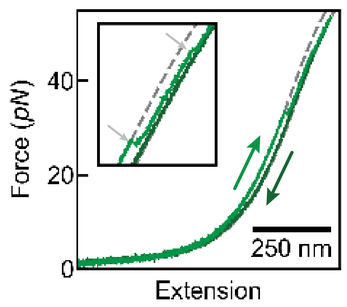
Co-translational folding of the CRD is CaM-dependent
Force Profile Analysis (FPA)

Co-translational folding investigated using a library of **folding biosensors**: the CRD sequence flanked by an arrest peptide (AP) and two fluorophores.

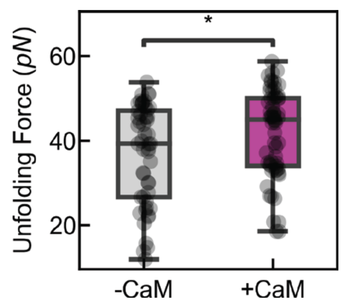




The AP causes translation to stall. Nascent chain folding induces a mechanical force which overcomes the stall, allowing translation to continue[4].



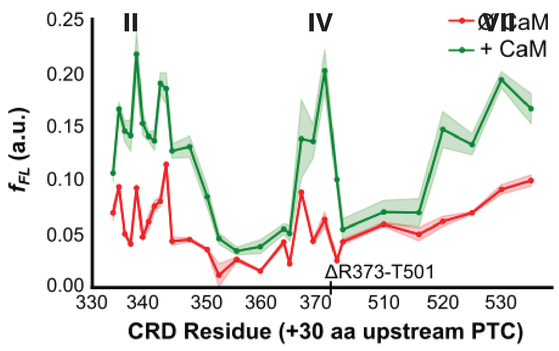
Real experimental data, showing a pull-retract cycle with two unfolding events



CaM binding stabilises the nascent CRD against mechanical unfolding.

Measuring the fraction of full-length protein synthesised (f_{FL}) at multiple points during translation allows us reconstruct a folding profile across the translation pathway[5]:

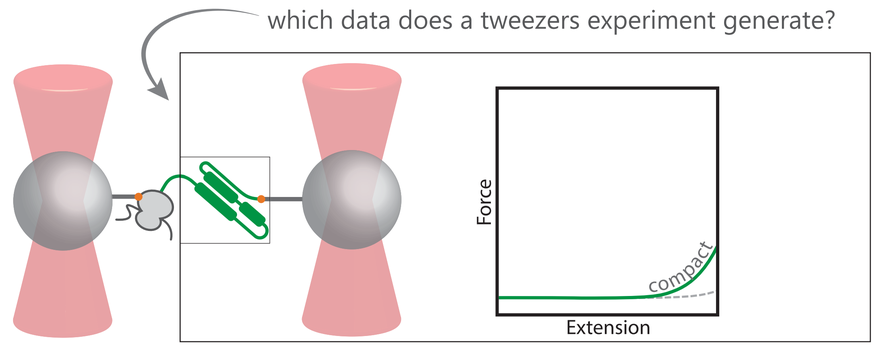
$$f_{FL} = \frac{I_{yellow}}{I_{yellow} + I_{blue}}$$



Three major peaks in f_{FL} (**II**, **IV** and **VII** respectively) result, corresponding to the exit of the three α-helices from the ribosomal exit tunnel:.

Co-translational folding is clearly promoted by CaM.

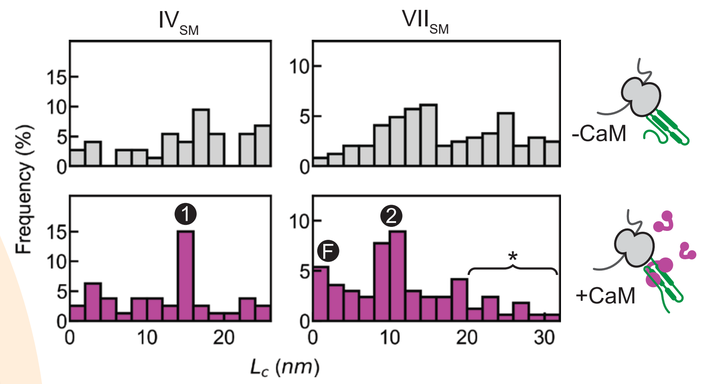
CaM induces co-translational hairpin formation
Single-Molecule Force Spectroscopy



Ribosomes expressing the CRD are tethered between two optically-trapped beads using DNA handles. By moving the beads we can apply a small force to unfold the nascent chain.

Repetitive pull-relax cycles allow us to probe possible folding states of the nascent chain.

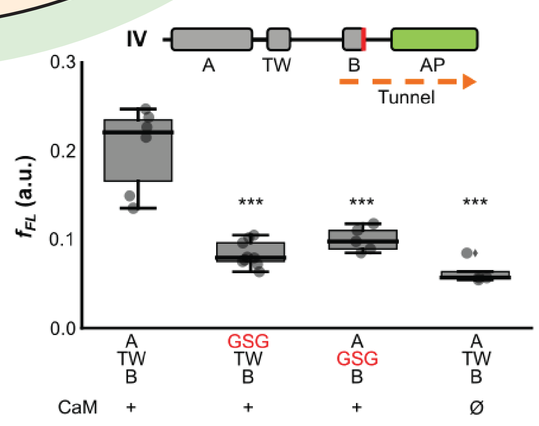
We can stall translation to investigate folding at different stages, and test the effect of CaM binding.



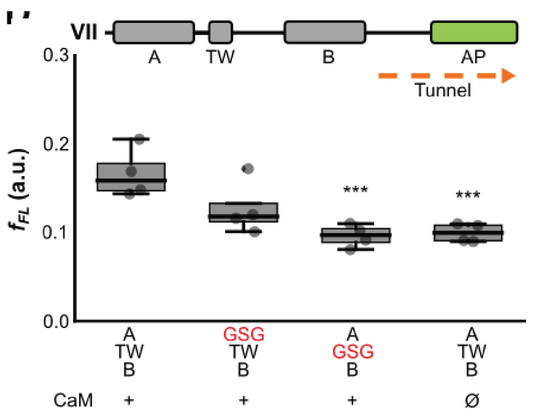
Once hTW has left the tunnel (IV, left), one clear peak emerges, corresponding to a hairpin formed between hA and hTW (**1**).

For the full CRD (VII, right), hTW is replaced with hB, forming the native conformation (**2**). The fully-folded conformation (**F**) is promoted, and the more unfolded states (*) are destabilised.

α-helices must fold sequentially
FPA + FRET

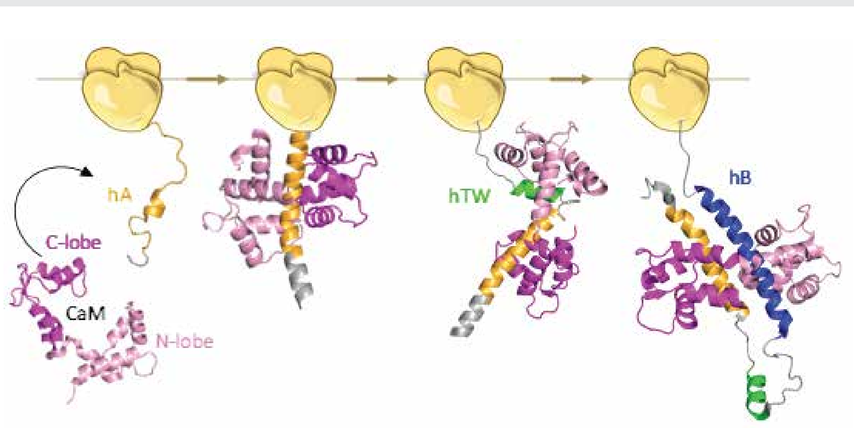


At IV, folding of hTW is dependent on hA: introducing a folding-incompetent hA mutant reduces f_{FL} to -CaM levels.



Similarly, folding of hB at stage VII is dependent on hTW (and hA, to a lesser extent).

Co-translational folding mechanism
Conclusions & Outlook



1. CaM recognises a pre-folded 'capping' conformation of hA;
2. CaM binding promotes folding of hA;
3. CaM forms a transient hairpin between hA and hTW once the latter exits the tunnel;
4. hB replaces hTW after exiting the tunnel, to obtain the native CRD conformation.

For the first time, we demonstrate the mechanism of CaM acting as a co-translational chaperone. But...
- how does CaM regulate both co-translational folding and ER membrane trafficking?
- is co-translational chaperoning by CaM in turn regulated by Ca^{2+} signalling in the cell?
- what is the precise role of hTW?

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