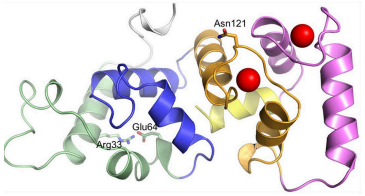
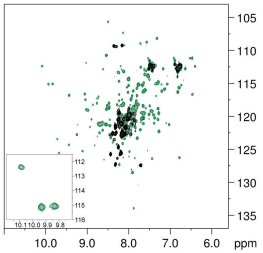
PRIN 2017

Kick-off Meeting

Bologna, September 5, 2019

Effects of point mutations on the structure and stability of calcium sensor proteins (UNIT 3)





Daniele Dell'Orco

UNIVERSITÀ di VERONA | Dipartimento di **NEUROSCIENZE, BIOMEDICINA E MOVIMENTO**

Mariapina D'Onofrio

UNIVERSITÀ di VERONA | Dipartimento di **BIOTECNOLOGIE**

The groups

1 - BMB@UniVR – Biochemistry and Molecular Biophysics

- Characterization of ***protein-protein*** and ***protein-ion*** interactions of biomedical relevance
- Structure-function properties of ***Neuronal Calcium Sensors (NCS)*** and target regulation in normal conditions and in ***genetic diseases***
- Multidisciplinary approach that integrates *in house* ***experimental*** and ***computational*** techniques to understand complex cell behaviours

Specific research topics:

- ***photoreceptor biochemistry & biophysics*** in health and disease;
- ***nanodevices as carriers*** of proteins (nanoparticles and liposomes)
- ***system-level description*** using a bottom-up startegy (from sub-protein level to the cell)

The groups

2 - Chimica delle biomacromolecole

- Structural characterization of proteins
- Characterization of ***protein-ligand*** *and* ***protein-nanoparticles*** interactions using biophysical techniques, mainly NMR
- Influence of post traslational modifications on protein aggregation propensities

Specific research topics:

- *Ubiquitination machinery and its influence in Alzheimer's* disease;
- *Modulation of aggregation properties of Intrinsically disordered proteins invloved in neurodegenerative disorders;*
- *Characterizazion of protein-nanoparticles interaction and modulation of protein function*

What shall we do in the project:

WP3: Generation of new experimental data: structural, functional and stability
WP4: Generation of new experimental data: binding affinity variations

- Express and purify selected proteins and their variants (disease-associated SAVs deriving from SNV) *("min 11 + 7 variants in CaM"+ variants from Unit 4)*

- Characterize protein structure/folding properties by CD spectroscopy (near & far UV), fluorescence spectroscopy and NMR (^{1}H and ^{1}H-^{15}N HSQC experiments)

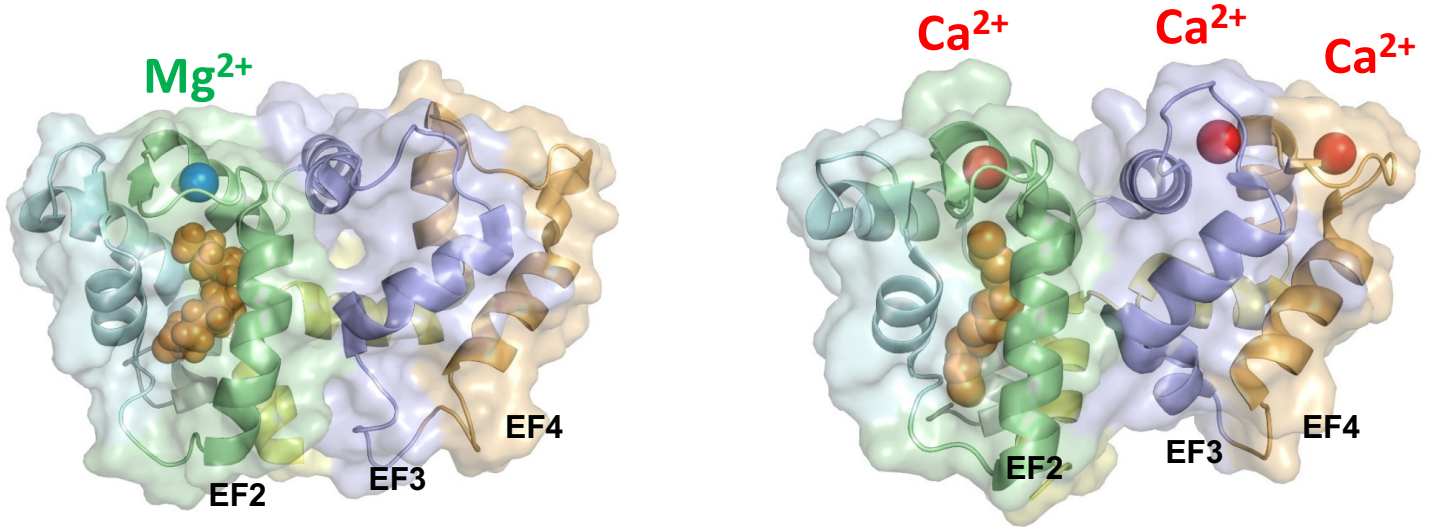
- Determine relative stabilities (**folding**) $\Delta\Delta G_f^\circ = \Delta G_f^{\circ mut} - \Delta G_f^{\circ wt}$ relative to the standard state for selected cases by thermal (CD, DSC) or chemical denaturation (CD, Flu)

- Determine relative affinities (**binding**) $\Delta\Delta G_b^\circ = \Delta G_b^{\circ mut} - \Delta G_b^{\circ wt}$ in binding experiments with selected targets by Surface plasmon resonance, ITC and NMR

Examples of previous work

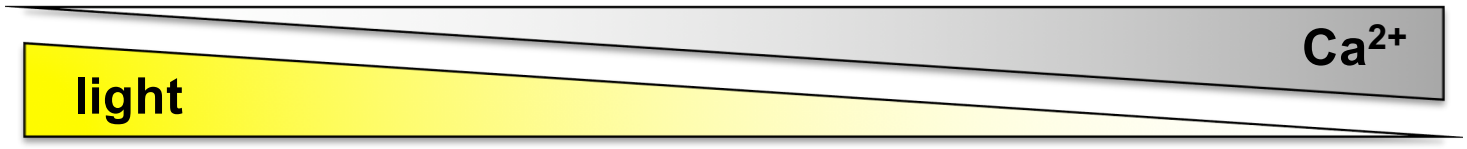
1 – Involvement of GCAP1 in autosomal dominant cone-rod dystrophies

Mg^{2+} / Ca^{2+} structural effects - GCAP1



GC-***activating*** state

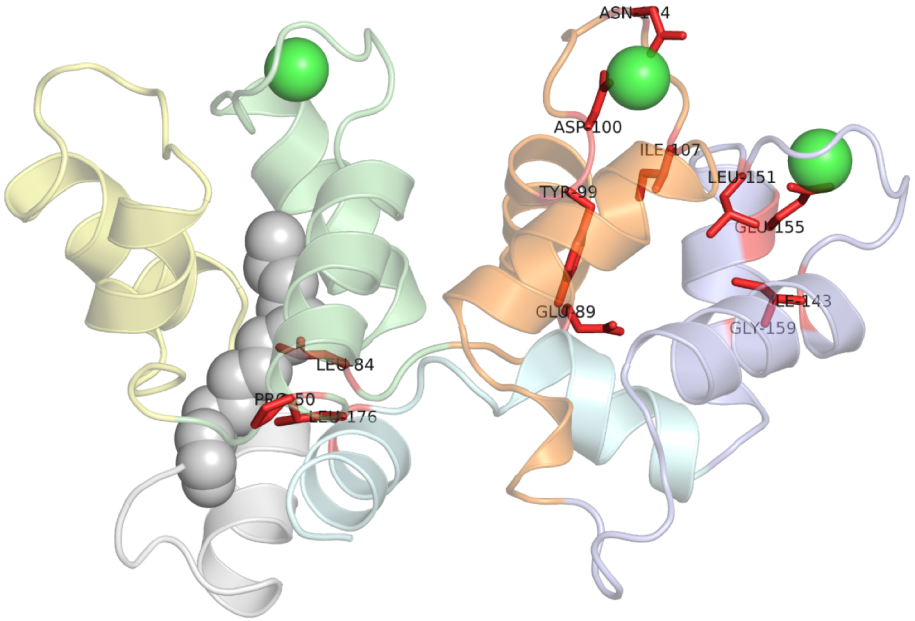
GC-***inhibiting*** state



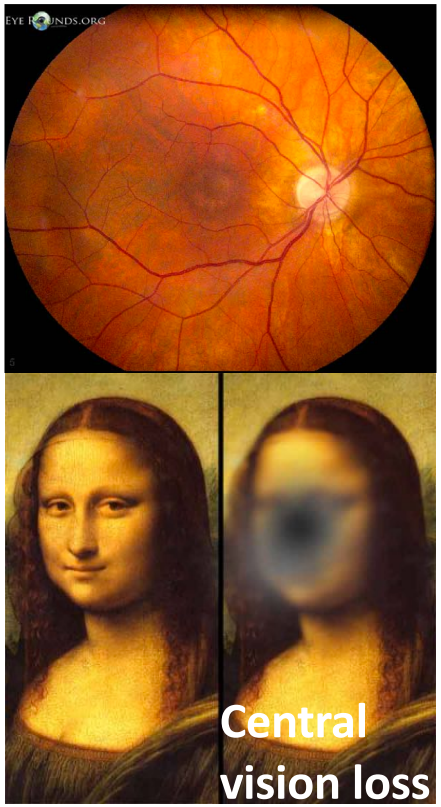
Biochim Biophys Acta. **2015**;1853(9):2055-65

Why GCAP1?

20 missense mutations in *GUCA1A* associated to retinal dystrophies

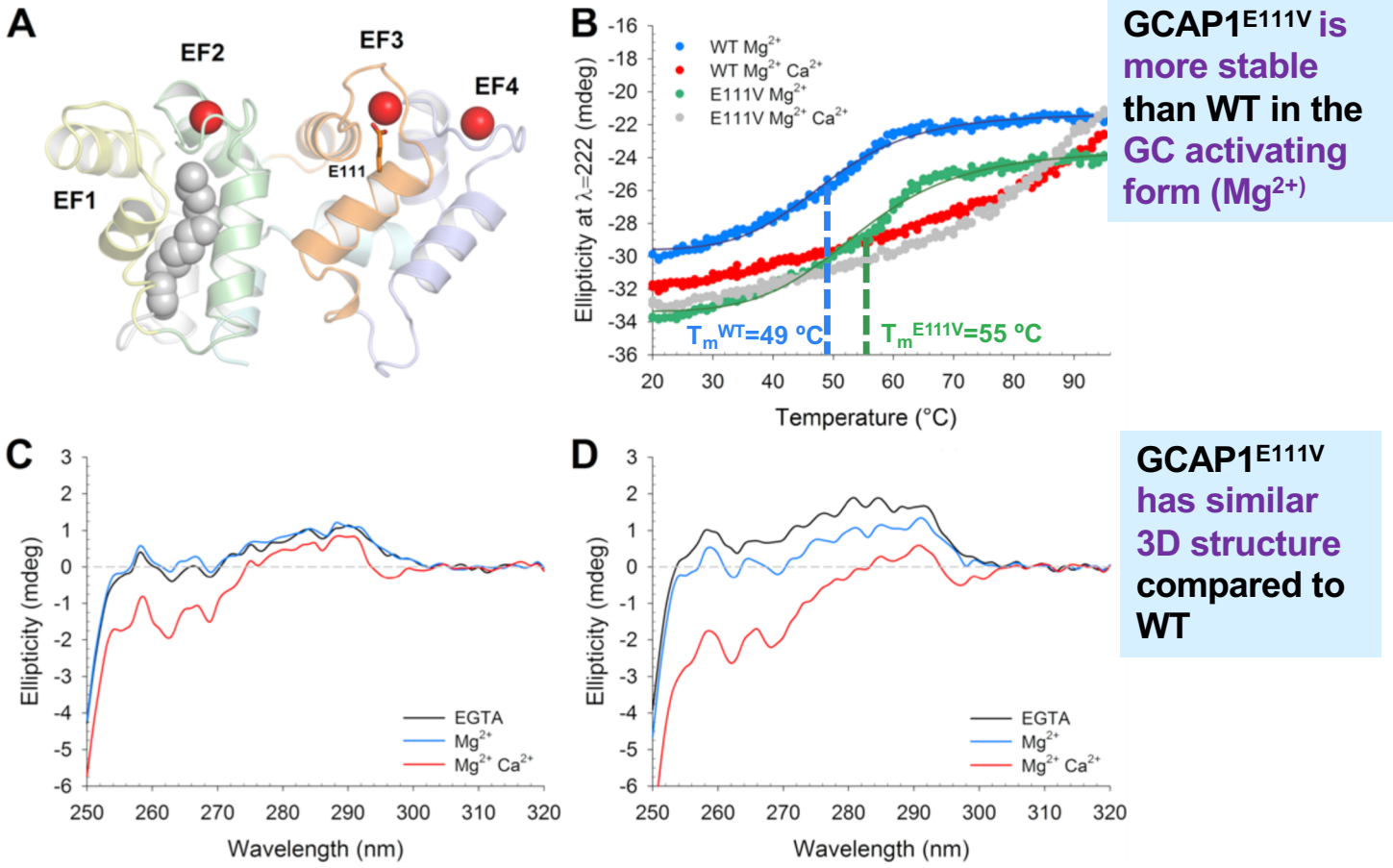


Hum Mol Genet. **26**(1):133-144. (2017)



https://webeye.ophth.uiowa.edu/eyeforum/atlas/pages/cone-rod-dystrophy.htm

Biochemical and biophysical investigations p.E111V vs. WT

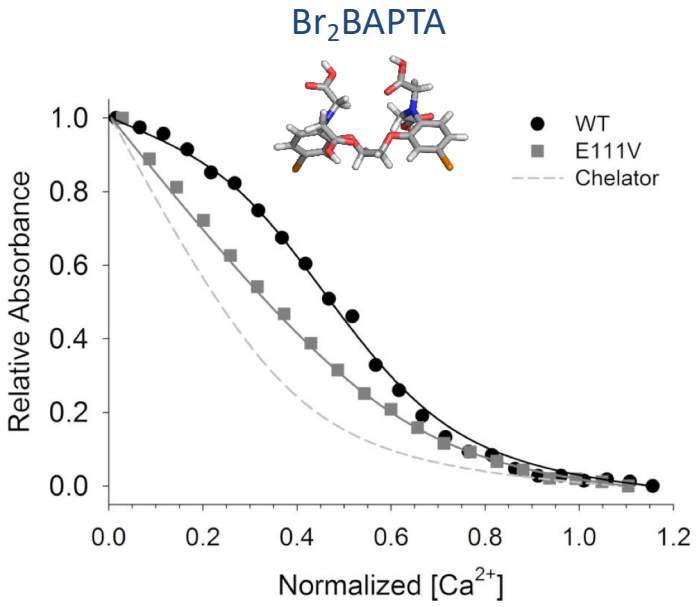


GCAP1^{E111V} is more stable than WT in the GC activating form (Mg^{2+})

GCAP1^{E111V} has similar 3D structure compared to WT

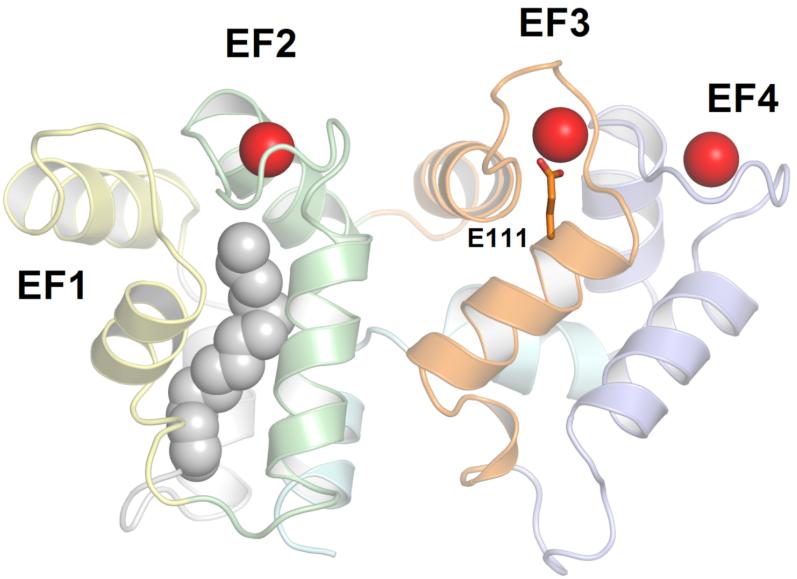
Hum Mol Genet. **2018**;27(24):4204-4217

Different Ca^{2+} affinity: WT vs. E111V



Protein variant	K_d^{app} (μM)*
WT	0.49
E111V	40↑

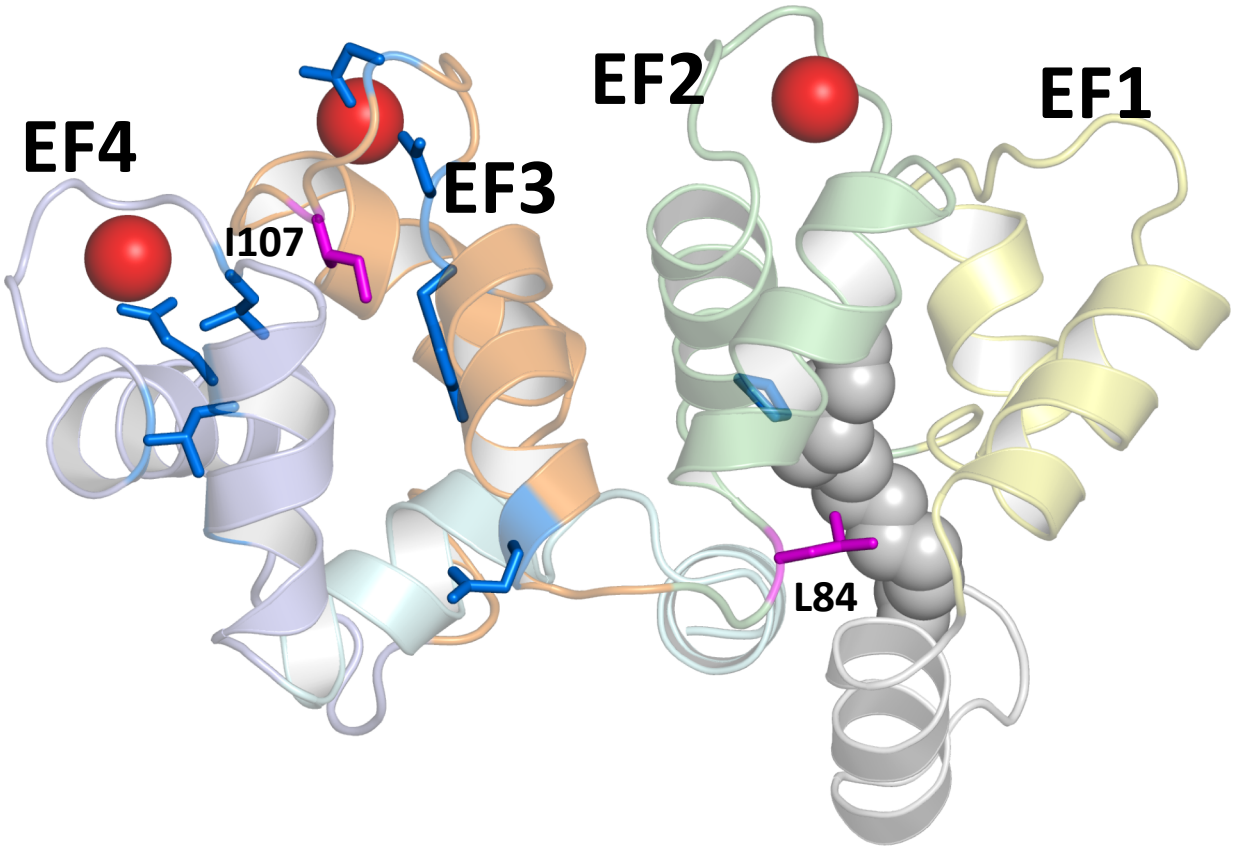
*** In the presence of 1 mM Mg^{2+}**



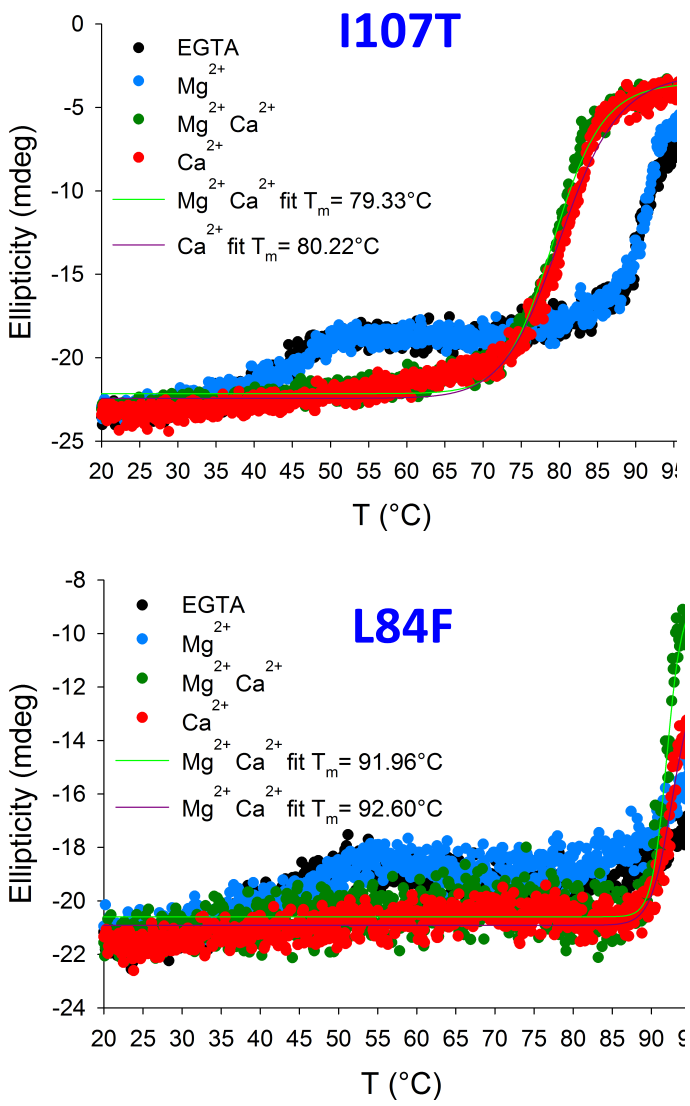
The bidentate Ca^{2+}-coordinator of EF3 is lost in $GCAP1^{E111V}$

Hum Mol Genet. **2018**;27(24):4204-4217

L84 and I107 are located in *remote* structural regions

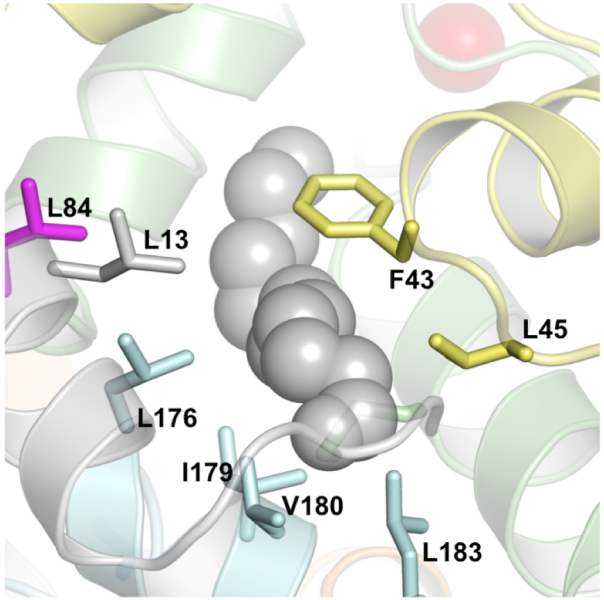


Thermal denaturation profiles following θ_{208} (T)



In the presence of Ca^{2+} L84F is extremely stable!

What is the origin of such stability?

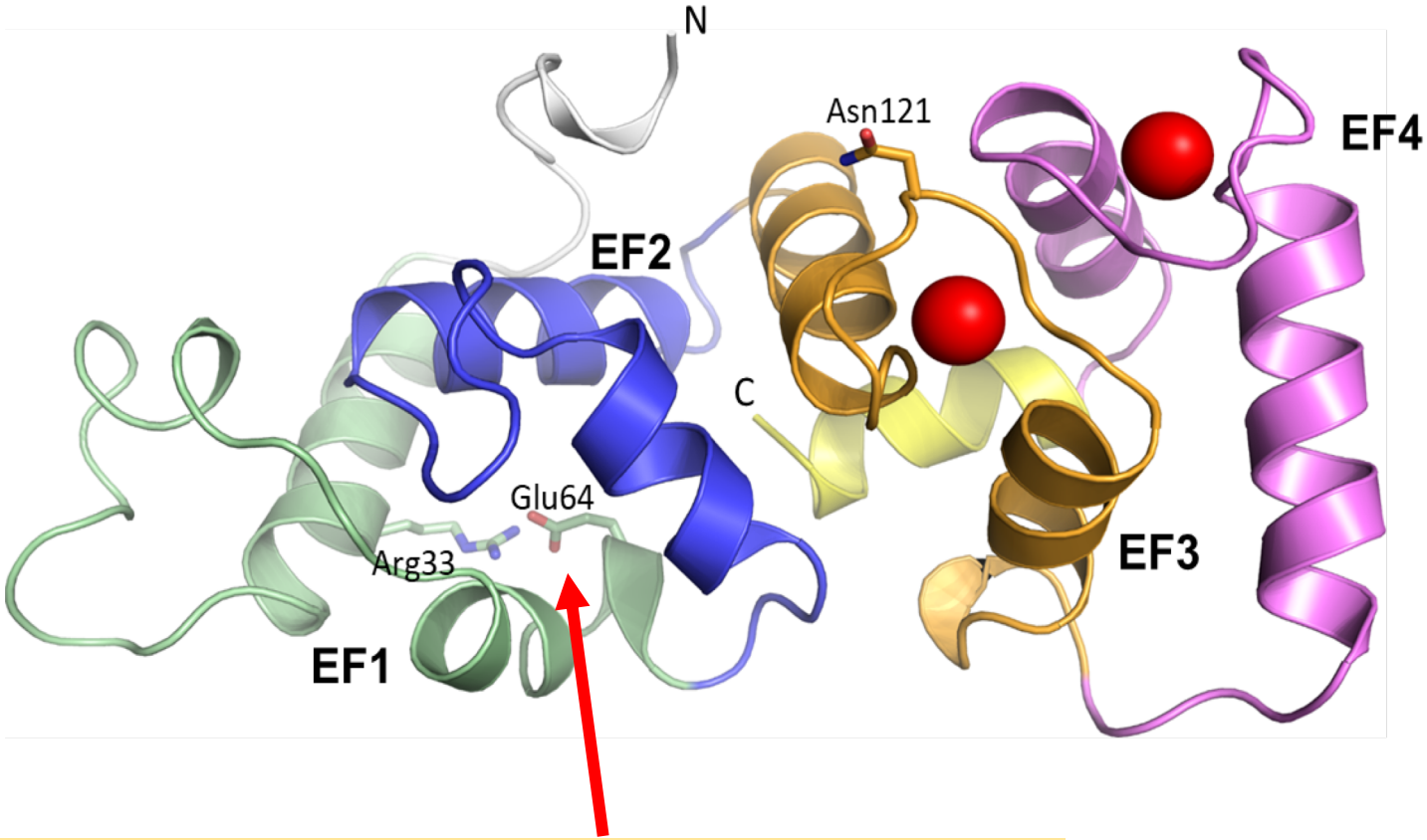


Hum Mol Genet. **2015**; 24(23):6653-66.

Examples of previous work

2 – Calcium and Integrin Binding Protein 2 (CIB2)

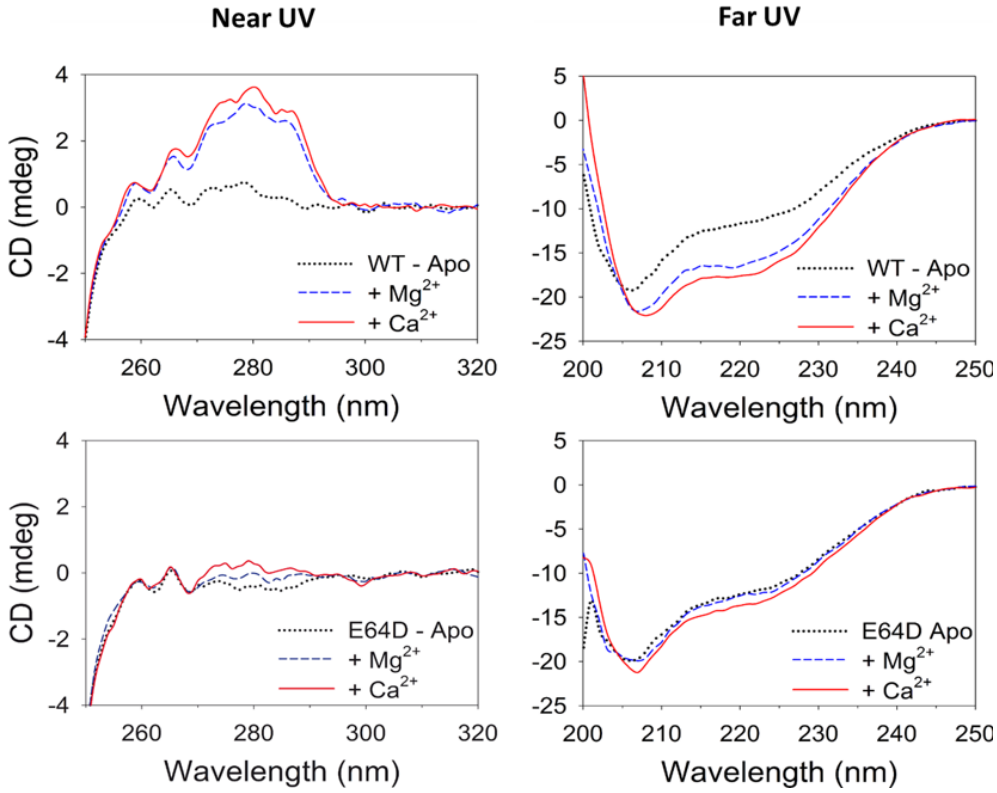
CIB2: an EF-hand protein involved in hearing physiology & disease



The conservative mutation **E64D** is associated with Usher Syndrome 1J

Front Mol Neurosci. **2018**;11:274

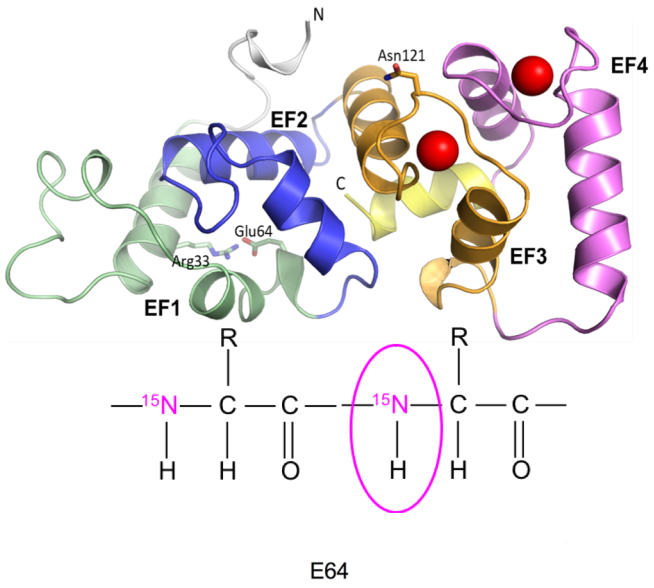
CIB2 folding properties (CD spectroscopy)



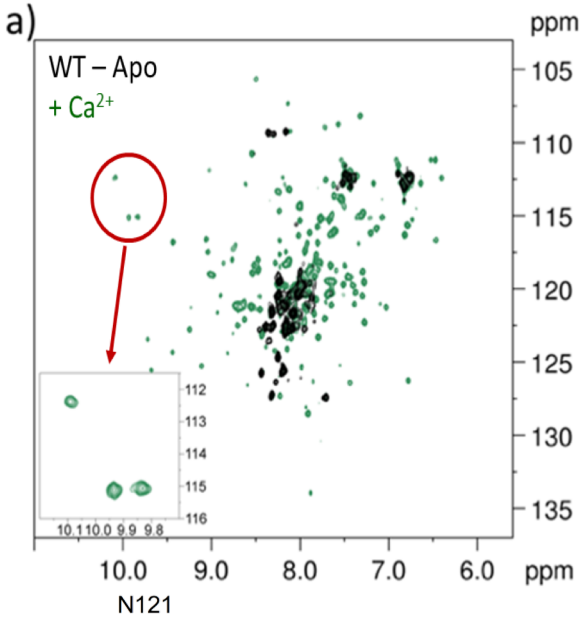
Front Mol Neurosci. **2018**;11:274

- **Apo- WT CIB2:** flexible molten-globule state.
- **Mg^{2+}** and **Ca^{2+} -bound WT CIB2**: high helical content and rigid tertiary structure.
- **p.E64D variant**: flexible molten-globule state.

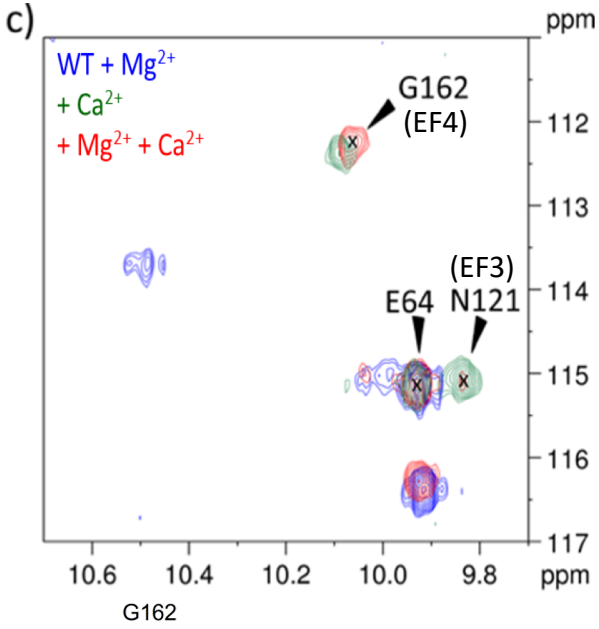
CIB2 conformational changes (NMR spectroscopy)



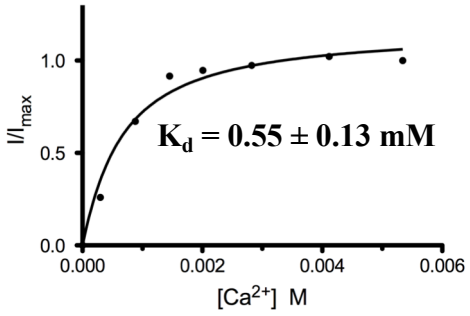
E64

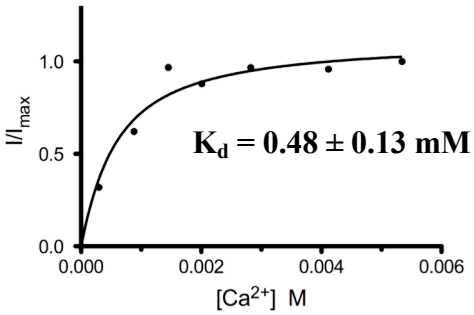


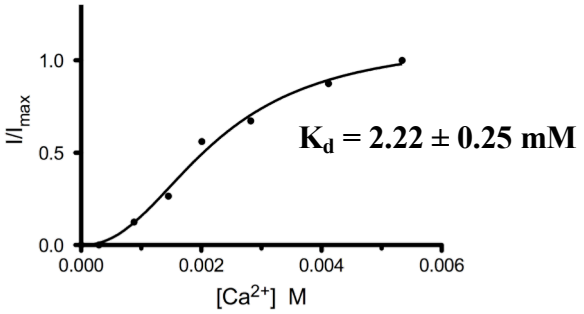
N121



G162







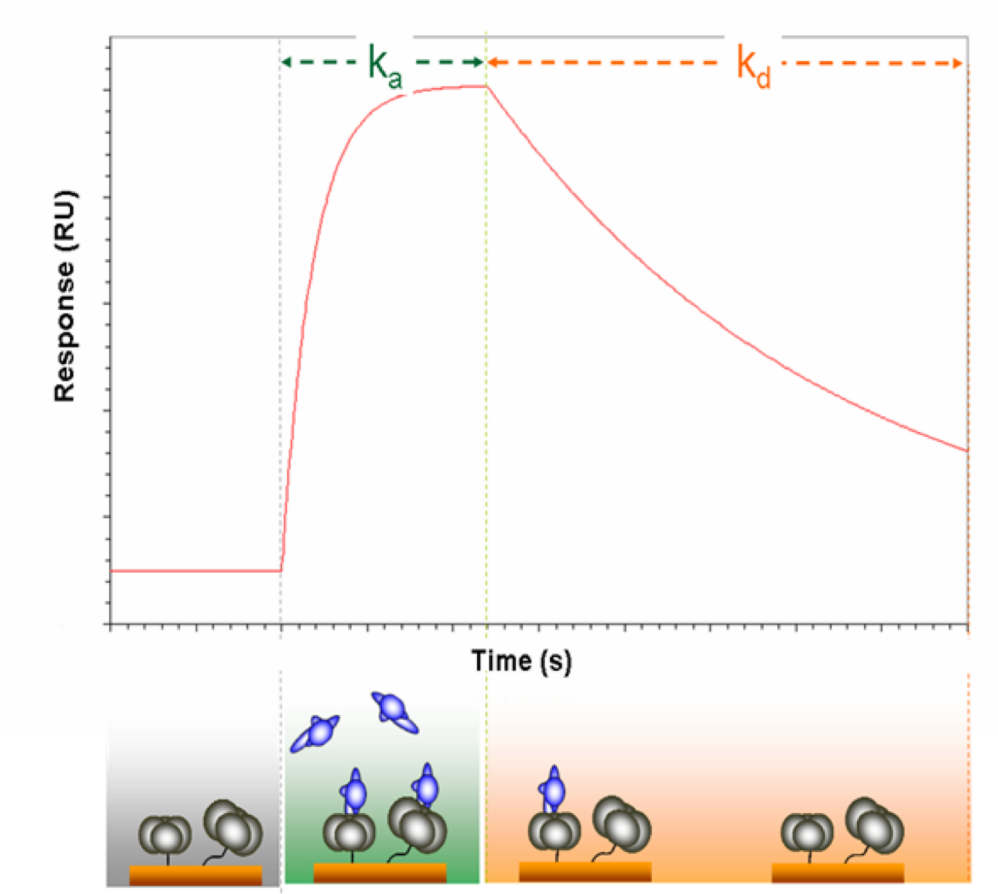
Binding of Mg^{2+} to EF3 motif creates a *long range allosteric communication* between EF3 and the residue E64

Front Mol Neurosci. **2018**;11:274

Examples of previous work

3 – CIB2-target interaction probed by surface plasmon resonance

Surface Plasmon Resonance:
The typical experiment and response



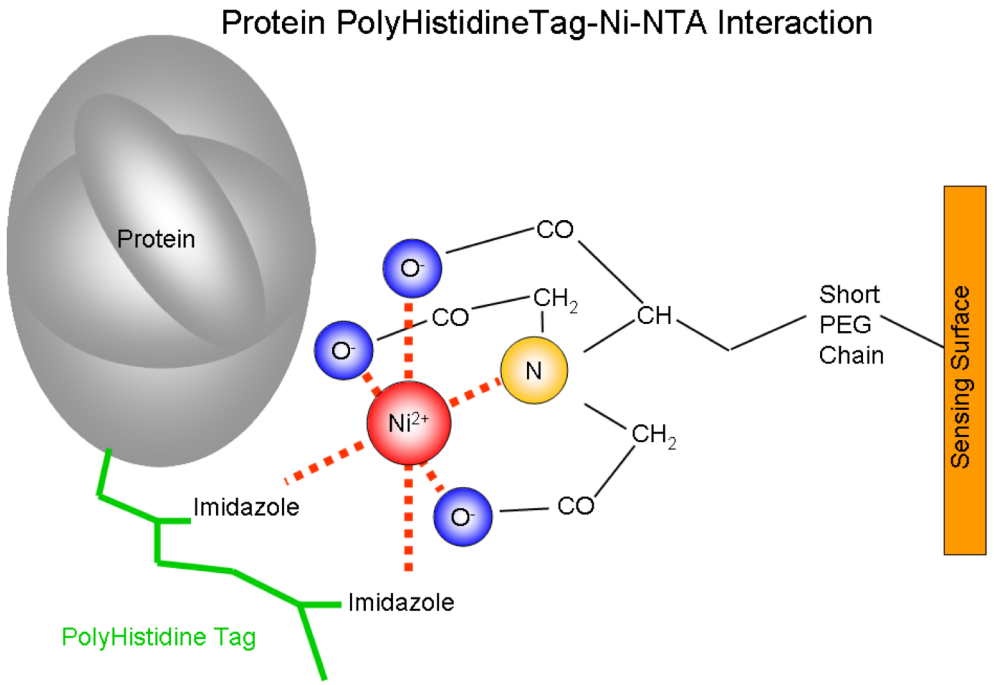
Complex (*AB*) Formation

$$A + B \underset{k_d}{\overset{k_a}{\Leftrightarrow}} AB$$

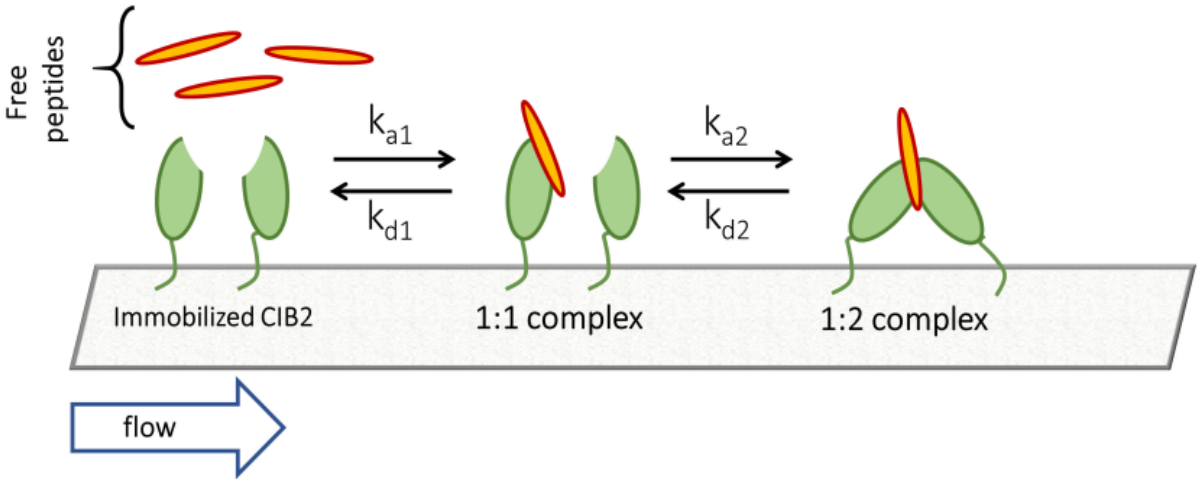
Where the forward (on) and reverse (off) rates are k_a and k_d, respectively.

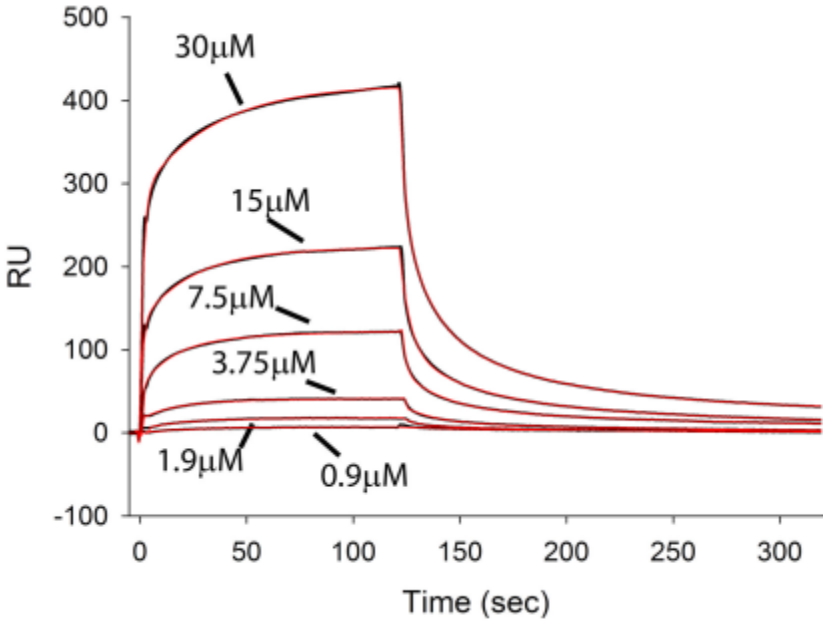
AFFINITY CAPTURE SURFACE (His-tagged proteins)

- His imidazole group coordinates with surface-attached nitrilotriacetic acid (NTA) - nickel complexes
- biosensor activation: injecting nickel chloride: the nickel ions coordinate with the surface NTA residues
- Biosensor regeneration: inject imidazole, SDS, or EDTA and reuse the chip



CIB2- α7M integrin interaction



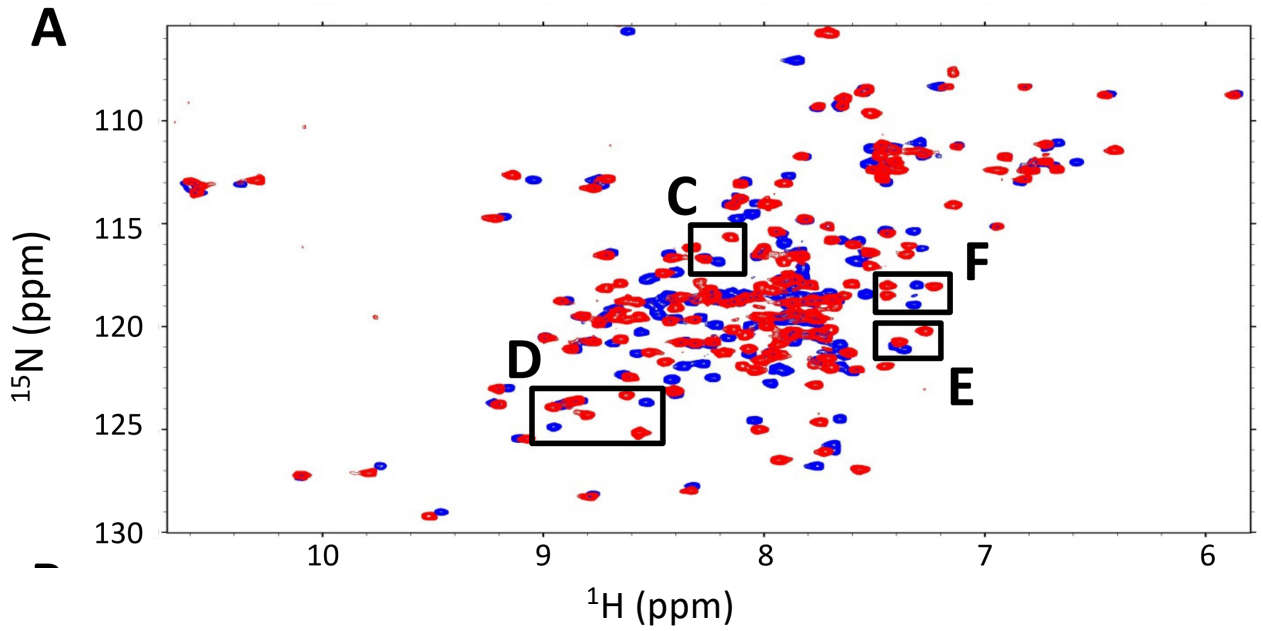


$$A + L \xrightarrow{k_{a1}} AL$$
$$AL \xrightarrow{k_{d1}} A + L$$
$$AL + L \xrightarrow{k_{a2}} AL_2$$
$$AL_2 \xrightarrow{k_{d2}} AL + L$$

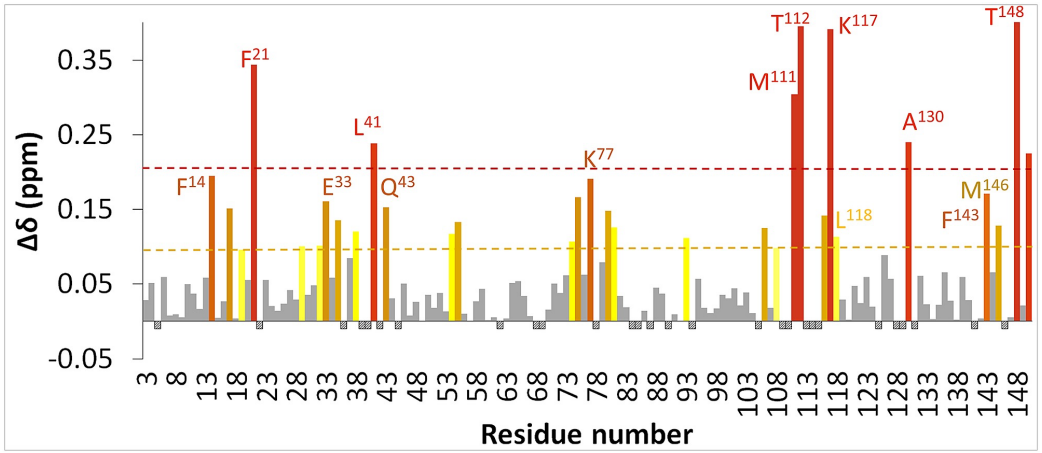
$$\frac{d[L]}{dt} = -(ka_1 \cdot [A] \cdot [L] - kd_1 \cdot [AL]) - (ka_2 \cdot [AL] \cdot [L] - kd_2 \cdot [AL_2])$$
$$\frac{d[AL]}{dt} = (ka_1 \cdot [A] \cdot [L] - kd_1 \cdot [AL]) - (ka_2 \cdot [AL] \cdot [L] - kd_2 \cdot [AL_2])$$
$$\frac{d[AL_2]}{dt} = ka_2 \cdot [AL] \cdot [L] - kd_2 \cdot [AL_2]$$
$$L(0) = R_{max}; AL(0) = AL_2(0) = 0$$

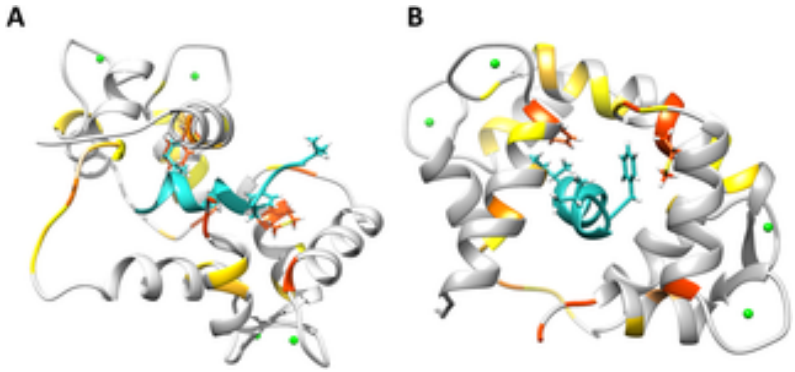
Submitted for publication

4 – Calmodulin-target interaction probed by NMR



Sovrapposizione di spettri HSQC della proteina calmodulina in assenza e presenza di peptide



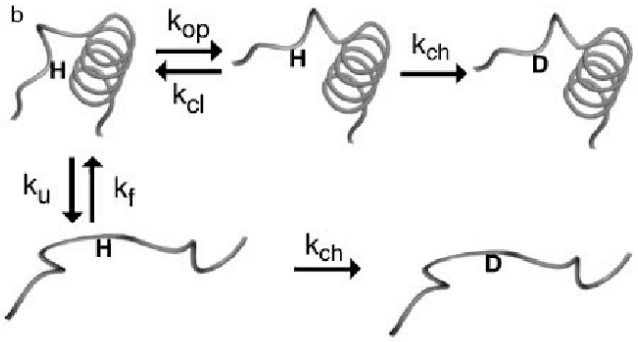


H/D exchange by NMR

a

$$A{-}H + OD^- \xrightarrow{k_{int}[OD^-]} A^- + H{-}OD$$
$$A^- + D_3O^+ \longrightarrow D_2O + A{-}D$$

b



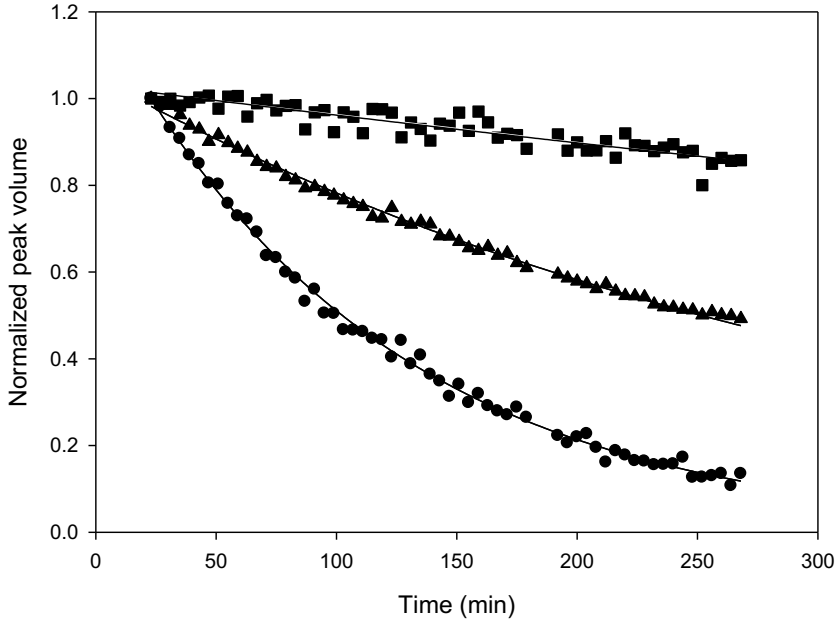
c

$$k_{obs} = \frac{k_{op} \cdot k_{ch}}{k_{cl} + k_{ch} + k_{op}} \approx \frac{k_{op} \cdot k_{ch}}{k_{cl} + k_{ch}}$$

(EX1) $k_{cl} << k_{ch}$; $k_{obs} = k_{op}$

(EX2) $k_{cl} >> k_{ch}$; $k_{obs} = \frac{k_{op}}{k_{cl}} \cdot k_{ch}$

Per meccanismo EX2 $K_{op}=k_{op}/K_{cl}$ $\Delta G_{op} = -RT\ln(K_{op})$



$$I(t) = I(0)\exp(-k_{obs}\, t)$$