

# *Molecular dynamics analysis libraries with an example based on the dynamics in the physiopathology of gelsolin*



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[github.com/giorginolab/GSN-Tutorial-BCN-2022](https://github.com/giorginolab/GSN-Tutorial-BCN-2022)

Master projects available!

## **Part I. Motivation**

Finnish-type amyloidogenic gelsolin variant -  
an example of protein dynamics playing a role in  
proteotoxicity and drug design discovered by MD.

## **Part II. Practice**

MD analysis libraries: intro and reproduction of  
the analysis\* shown in the paper.

\* Marked with  
this symbol →



Giorgino T, Mattioni D, Hassan A, Milani M, Mastrangelo E, Barbiroli A, et al.  
Nanobody interaction unveils structure, dynamics and proteotoxicity of the Finnish-type amyloidogenic gelsolin variant. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*. 2019 Mar 1;1865(3):648–60.

[Journal link.](#)

[Preprint.](#)

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BBA - Molecular Basis of Disease

journal homepage: [www.elsevier.com/locate/bbadis](http://www.elsevier.com/locate/bbadis)



## Nanobody interaction unveils structure, dynamics and proteotoxicity of the Finnish-type amyloidogenic gelsolin variant



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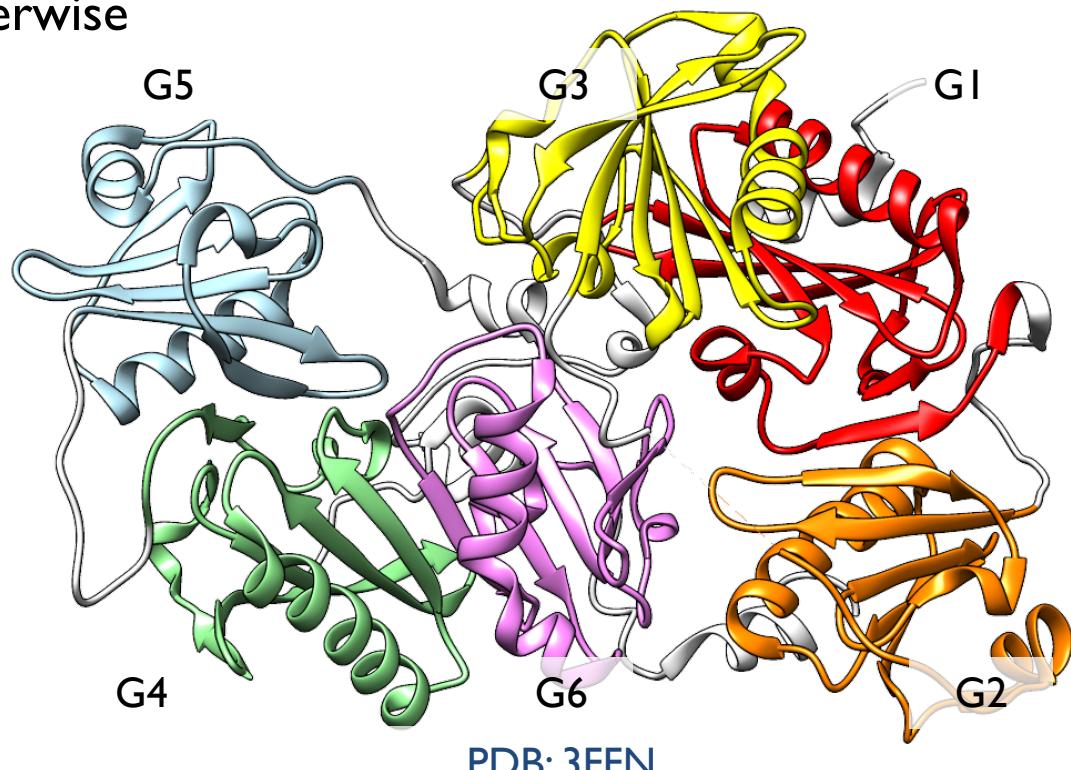
# **Part I.**

# **Gelsolin**

# Gelsolin

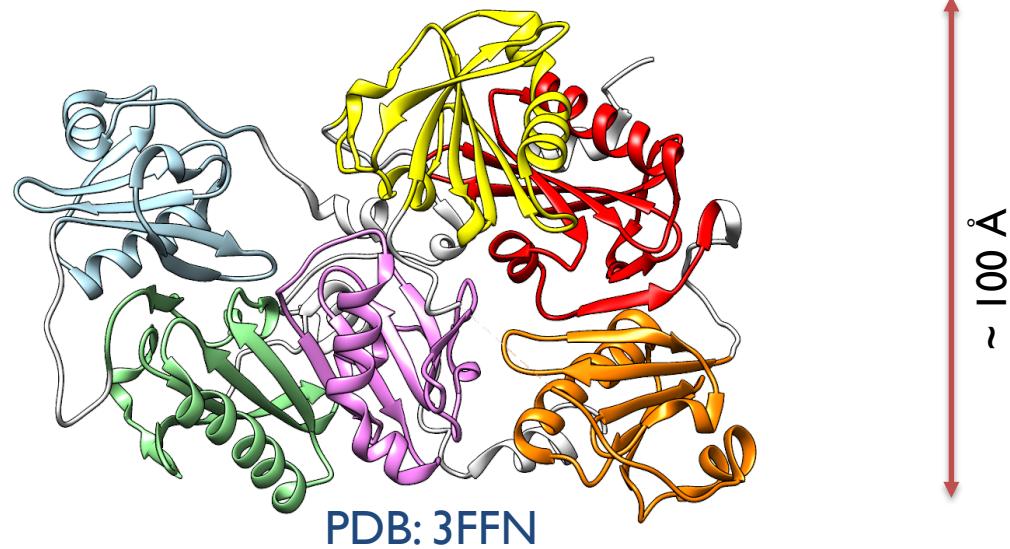
## A poster-child for multi-domain proteins

- Ubiquitous in cells *and* plasma (different isoforms)
- 6 repeats of the *Gelsolin* domain, G1-G6
- Binds and caps actin filaments *when activated*
- Activated/open by  $\text{Ca}^{++}$ , otherwise inactive/closed

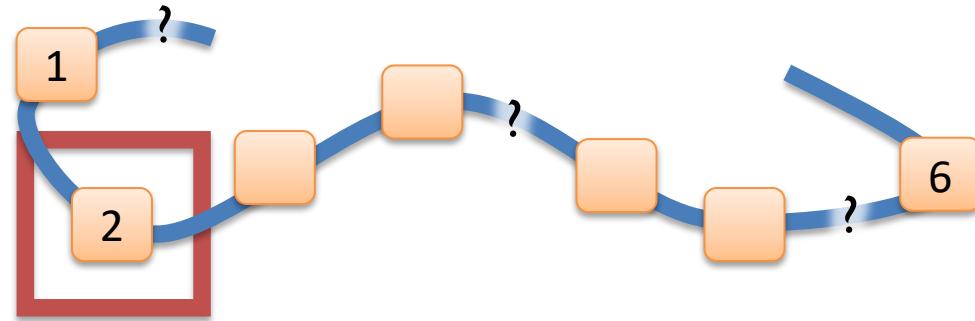




Inactive  
No  $\text{Ca}^{2+}$ .  
Closed form.  
Resolved (2008).



Active  
~ mM  $\text{Ca}^{2+}$ .  
Active form.  
Dynamic. Elusive.



However, crystallisation of  $\text{Ca}^{2+}$ -bound  
**isolated domains** has been successful.

# **Domain G2 and AGel Amyloidosys**

# AGel amyloidosis

Also known as...

FAMILIAL Am., FINNISH TYPE

Am., MERETOJA TYPE

Am. DUE TO MUTANT GELSOLIN

& permutations

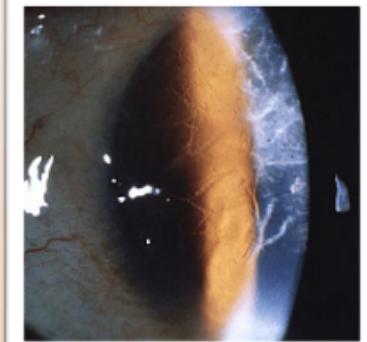
Am. V

Am. CRANIAL NEUROPATHY WITH LATTICE CORNEAL DYSTROPHY  
HEREDITARY GELSOLIN Am.

**"AGel amyloidosis** is a rare, usually systemic amyloidosis characterized by a triad of ophthalmologic, neurologic and dermatologic findings due to the deposition of **gelsolin amyloid fibrils** in these tissues".

- Rare, but endemic in Finland

*“... was 1/1,040 among 182,000 inhabitants, whereas in 1860 in the parish Valkeala the prevalence was calculated at 1/155. Today the true prevalence probably lies between these figures.”* (Meretoja 1973)
- Inherited, autosomal dominant
- Caused by GSN gene mutations
- No cure, just symptomatic treatments



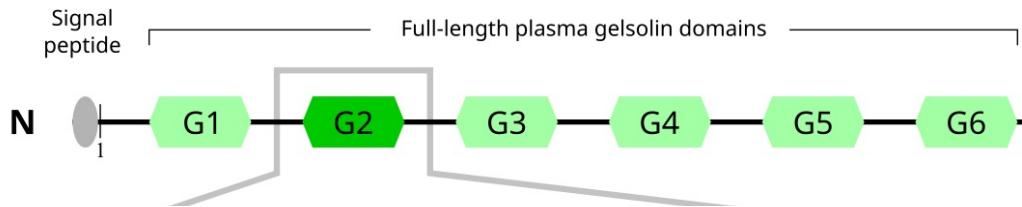
Corneal lattice dystrophy



Cutis laxa

## Mutations causing AGel

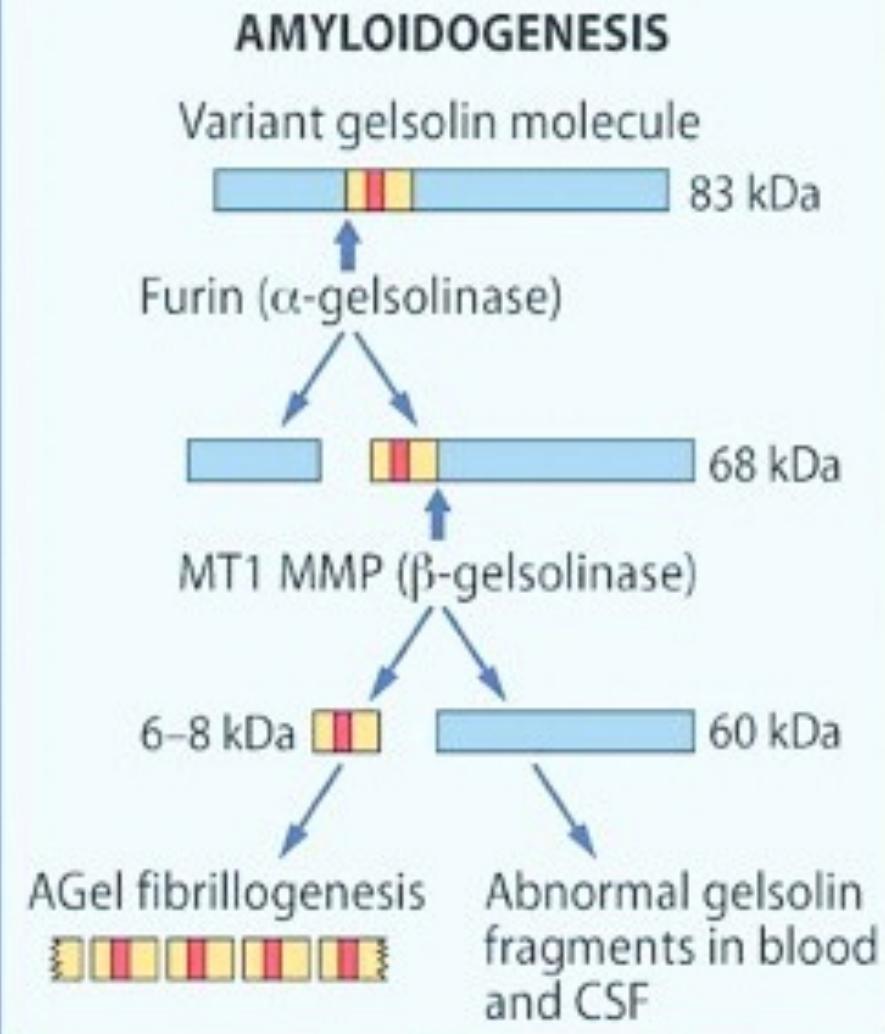
- D187N Systemic, Finnish
- D187Y Systemic, Danish
- G167R Renal
- N184K Renal



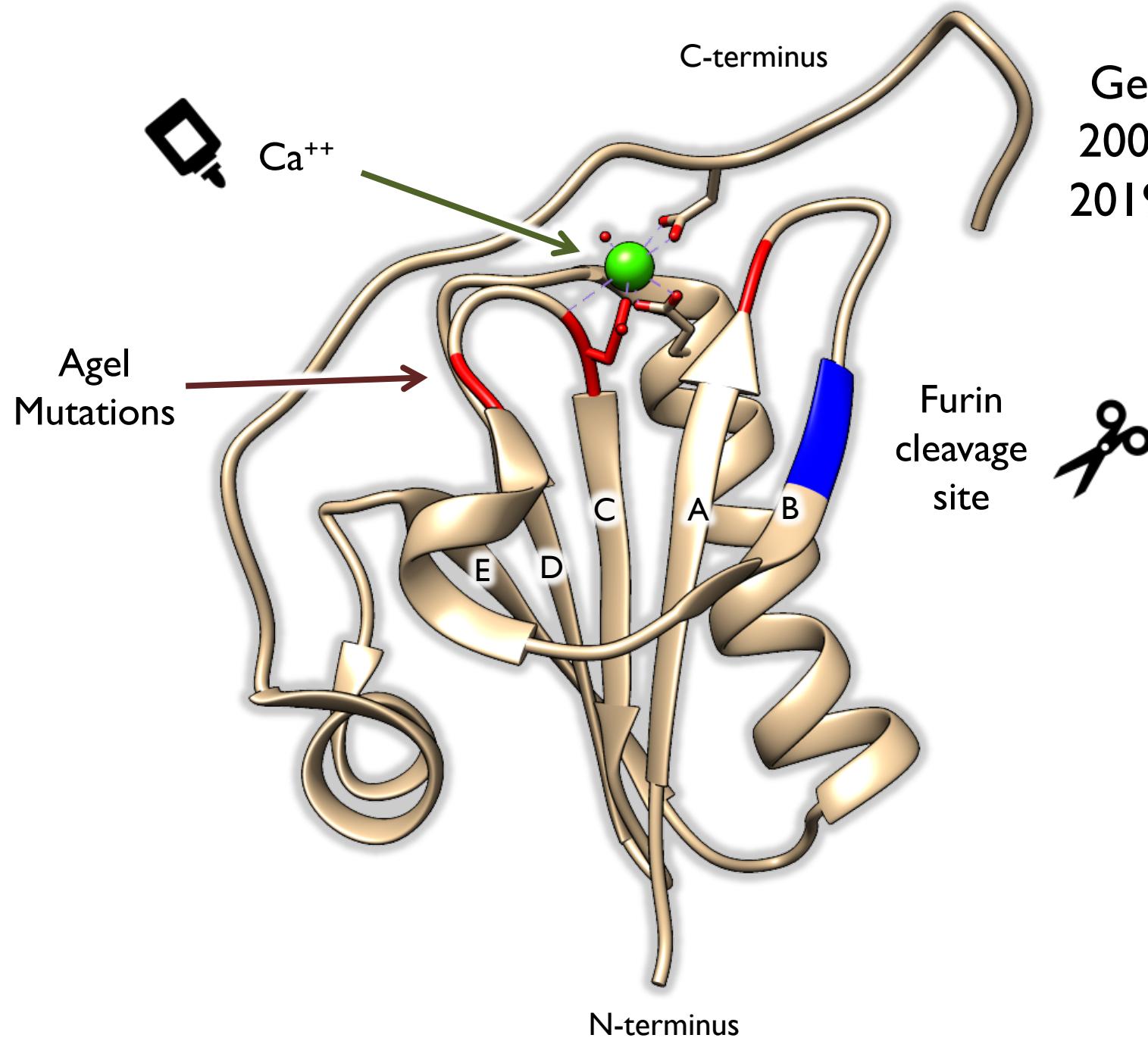
All in G2 domain



- WT plasma GSN encounters furin without consequence since WT is correctly folded.
- Mutant plasma GSN has a structural intermediate state exposing the cleavage site, and undergoes pathological furin processing.



Gelsolin G2 WT  
2002 Cd<sup>2+</sup> IKCQ  
2019 Ca<sup>2+</sup> **6QW3**



Mutant	Structure	Year	Disease form
WT	1KCQ	2002	Sporadic
N184K	5FAF	2015	Renal (rare)
G167R	5O2Z	2017	Renal (rare)
D187N	???		Systemic, Finnish
D187Y	???		Systemic, Danish

Observation: D187X mutants lose  $\text{Ca}^{2+}$  coordination

Hypothesis: Is this indication of an **order-disorder** shift?

Support for the hypothesis:

- Inability to crystallize
- GSN NMR data \*
- Known  $\text{Ca}^{2+}$ -regulated disorder/order transitions: sortase, a-cyc toxin, etc.
- Thermodynamic stabilities<sup>§</sup>: WT ≠ mutant

\* Kazmirski et al. Nat Struct Mol Biol. 2002 Feb;9(2):112

§ Thermal and chemical

**Lamas**  
**Nanobodies**  
**to the rescue**

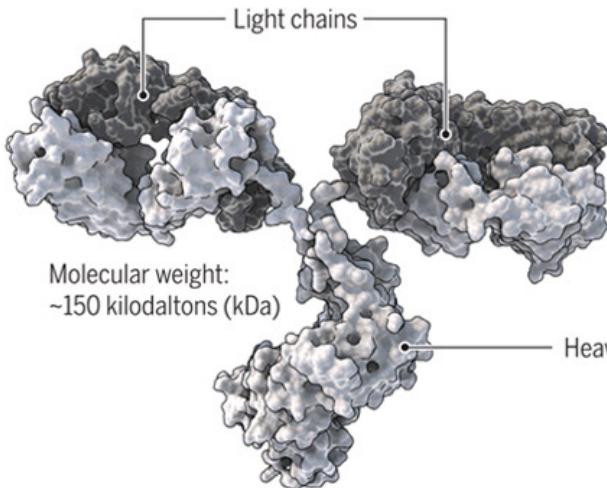


# Nanobodies

VHH, variable domain of hcAbs  $\sim$ 15 kDa

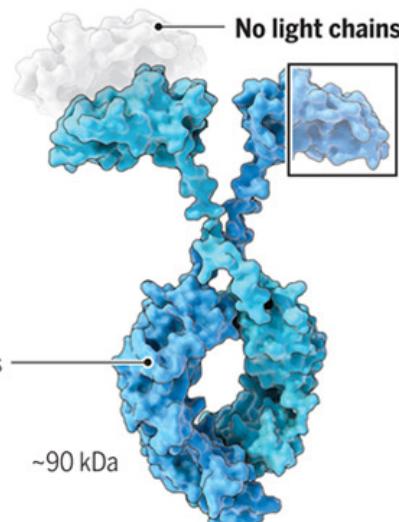
## Downsizing antibodies

Human blood teems with conventional antibodies—bulky, Y-shaped proteins that home in on bacteria and viruses. The small antibodies produced by sharks and the camel family differ from those immune molecules not only in size, but also in their structure and binding ability.



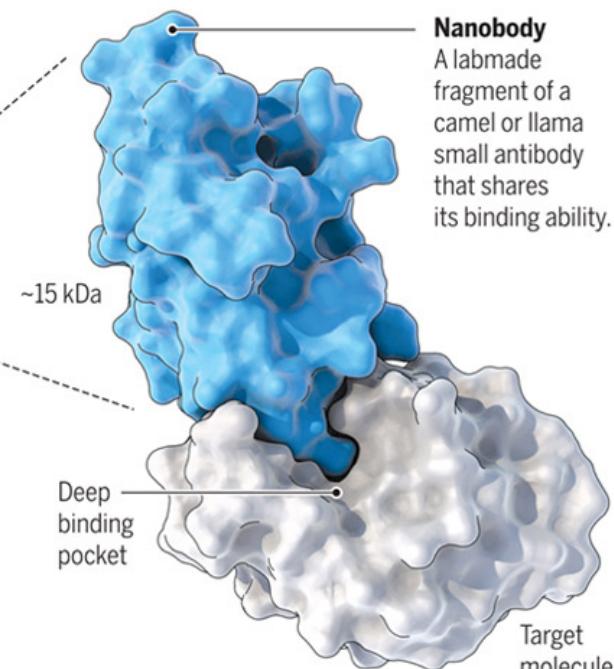
### Typical antibody

Two light and two heavy chains intertwine to make a protein that can identify and affix to bits of pathogens or other molecules.



### Small antibody

This slimmed-down variety lacks light chains but can still bind to its targets.



### Nanobody binding

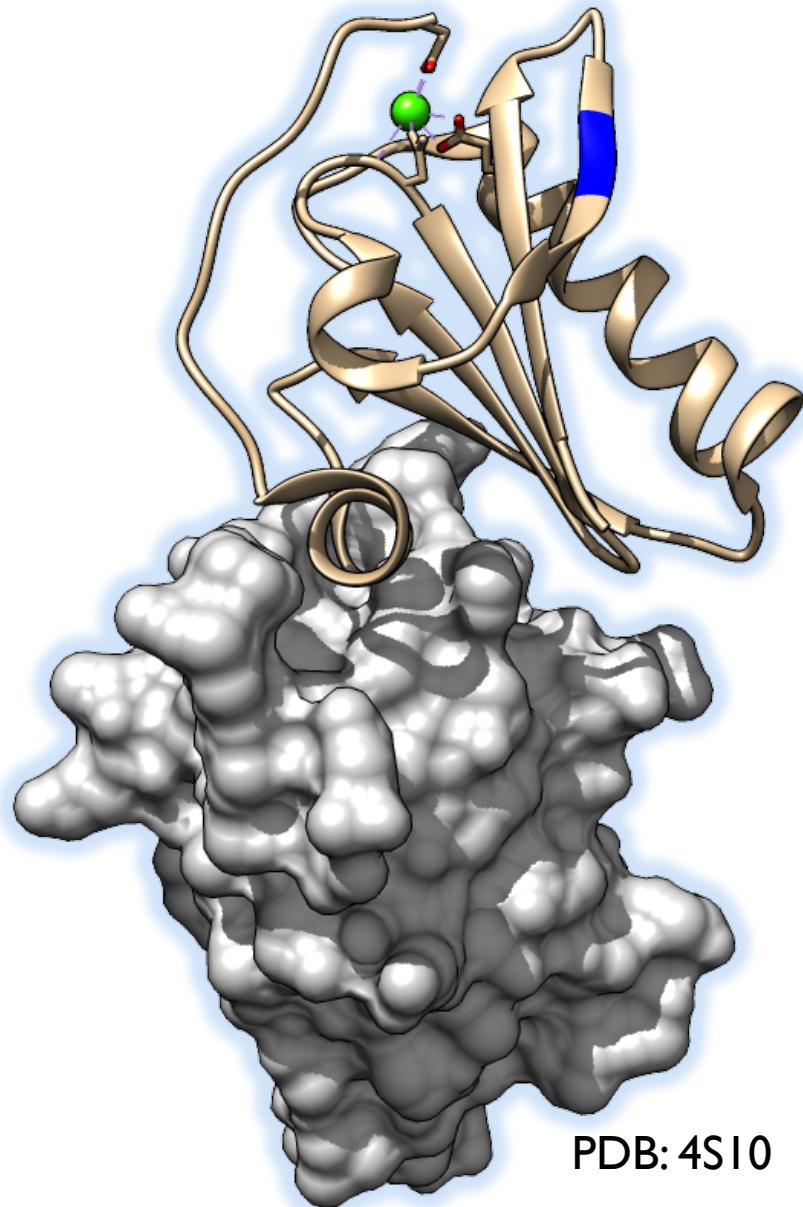
Because of its binding style, a nanobody can fit into crevices on molecules.

# 2015: Gettemans et al. – G2 Nanobodies

- Inoculate llamas with GSN **WT** G2
- Extract nanobodies (NbII)
- Sequence them
- Obtain WT:Nb structure

## Idea

- Can the WT-raised NbII re-stabilize D187N enough to allow crystallization?

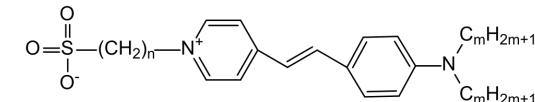
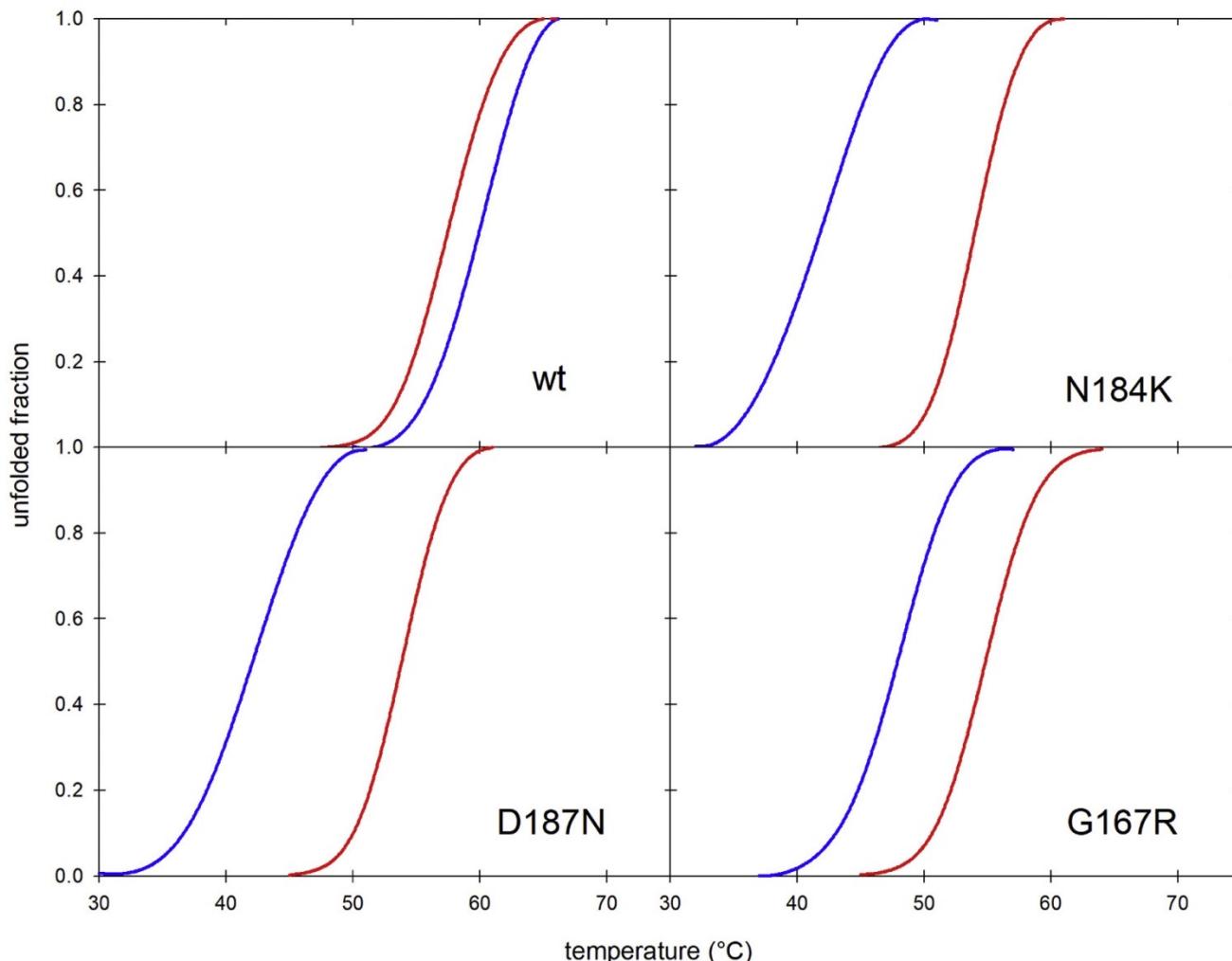


# Does NbII increase stability?

→ Thermofluor (differential scanning fluorimetry) experiments: measure the temperature exposing the hydrophobic core.



Mutants are destabilized  
NbII-binding re-stabilizes them

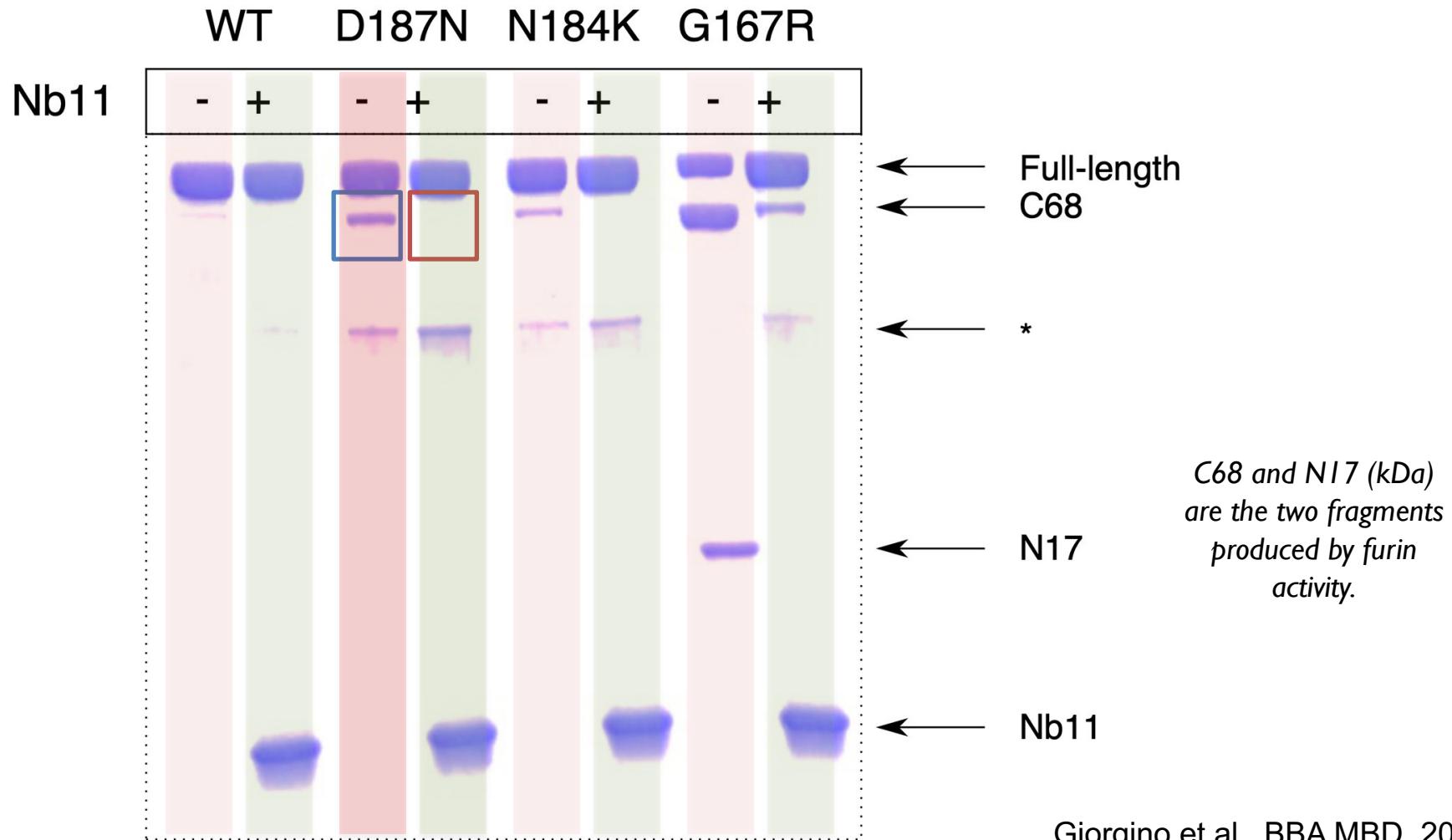


*SYPRO Orange binds nonspecifically to hydrophobic surfaces, and water strongly quenches its fluorescence. When the protein unfolds, the exposed hydrophobic surfaces bind the dye, resulting in an increase in fluorescence by excluding water.*

# Does NbII protect from proteolysis?

## → Furin proteolysis experiments

Mutants are cleaved  
NbI I-binding protects them



**G2 + Nb → structure**

# Does NbII enable crystallization of D187N?

Yes, it does.

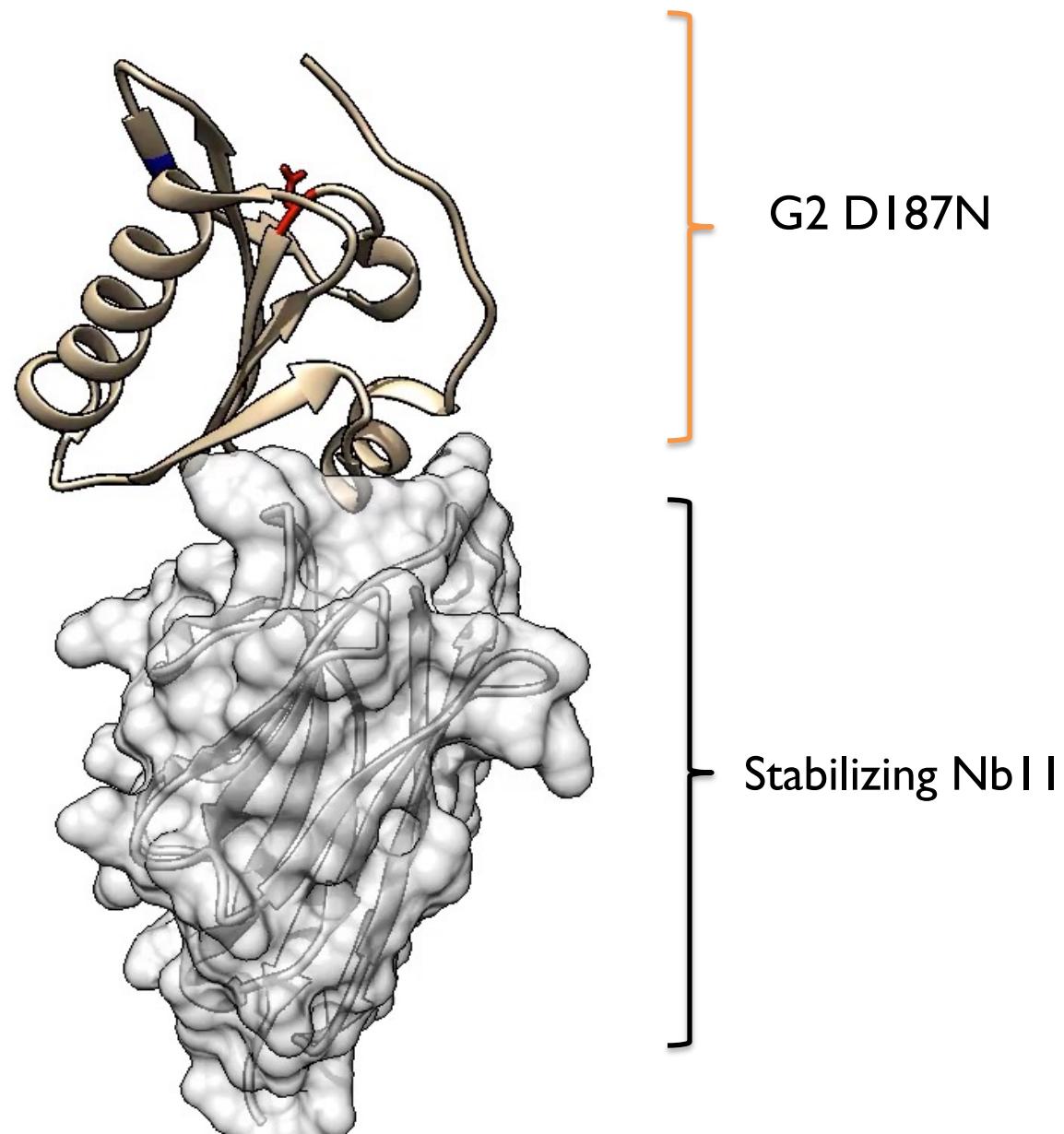
6HIF, 1.9 Å

Red: D187N mutation

Blue: furin cleavage site

Ca<sup>++</sup> is missing

Yet C-terminal tail in place.



Nanobody interaction unveils  
Finnish-type amyloidogenic ge

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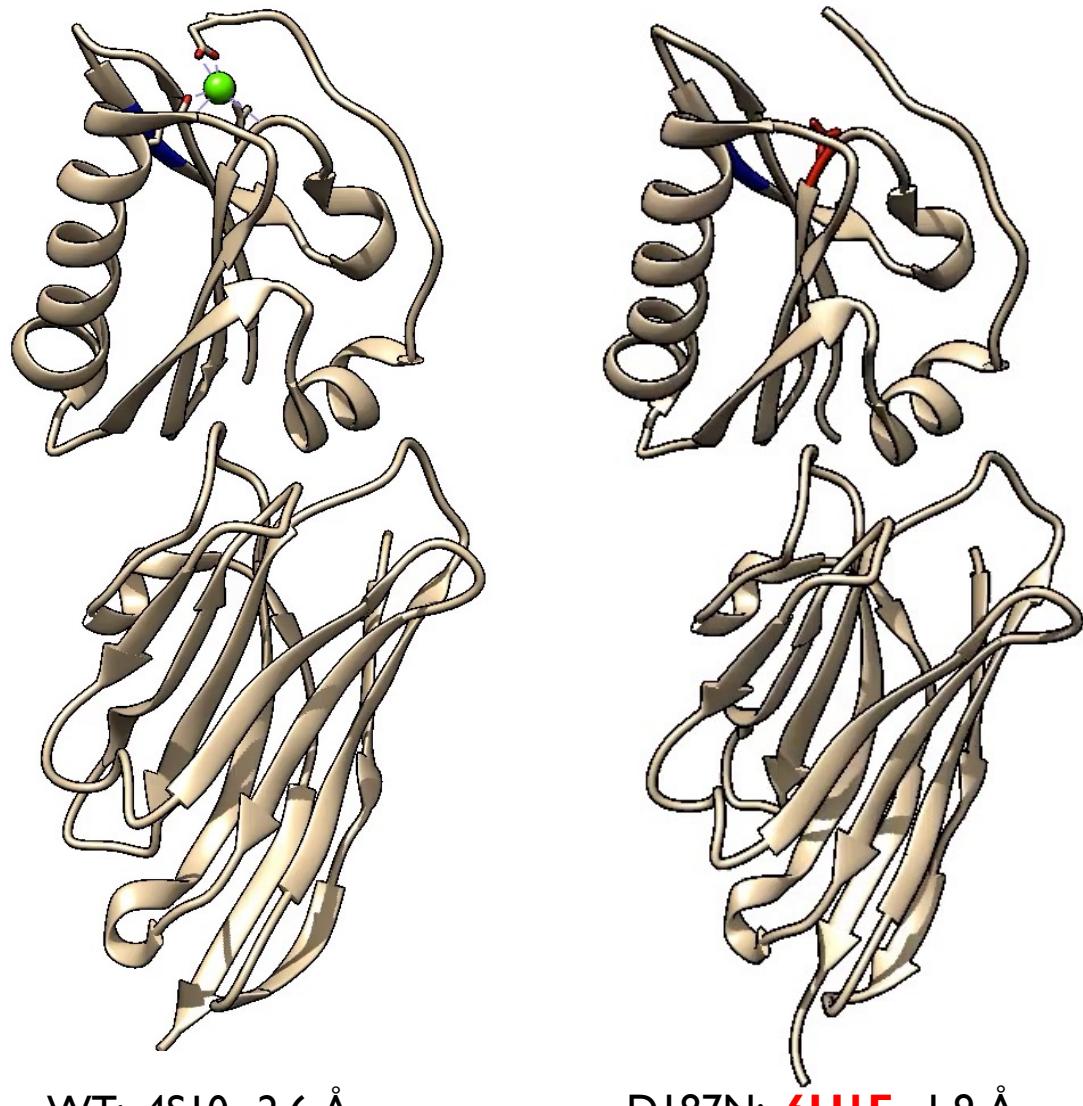
<sup>c</sup> Department of Molecular Biochemistry and Pharmacology, Istituto

<sup>d</sup> Dipartimento di Scienze sull'Alimentare, Unversità di Milano, Pa

# Three puzzles!

WT:NbII complex compared to D187N:NbII.

- I. WT and **D187N** are **virtually identical**\*: same structure, different function
2. NbII binds far from the furin cleavage site...
3. ...and far from the **Ca<sup>2+</sup>** ion



\* Except Ca<sup>2+</sup> binding

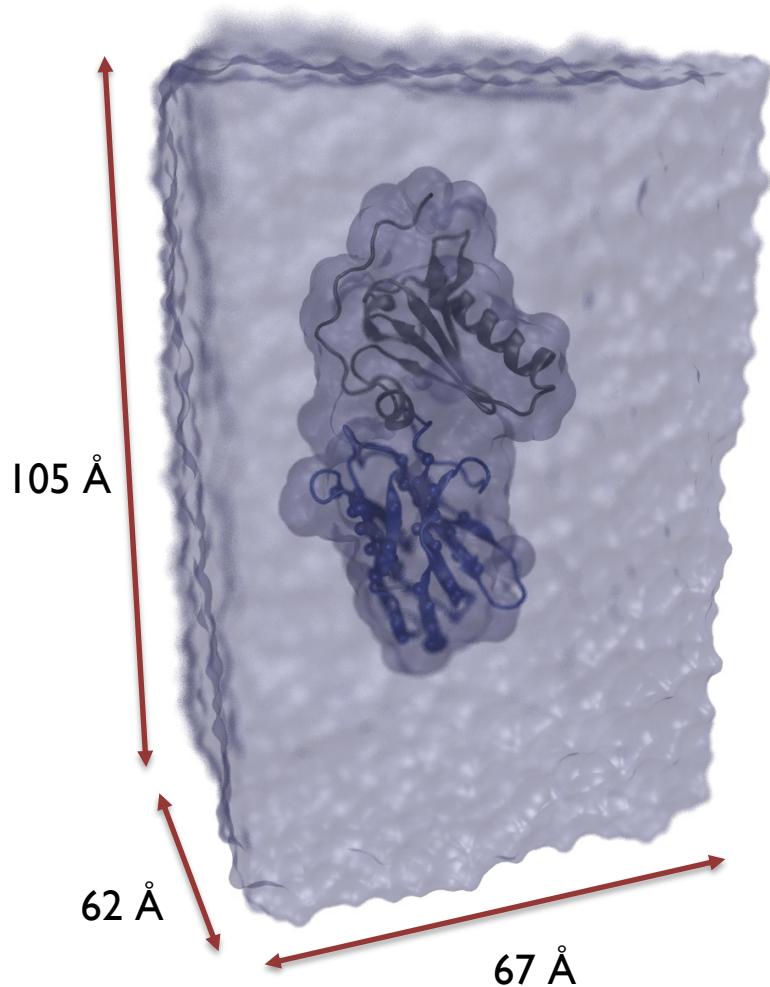
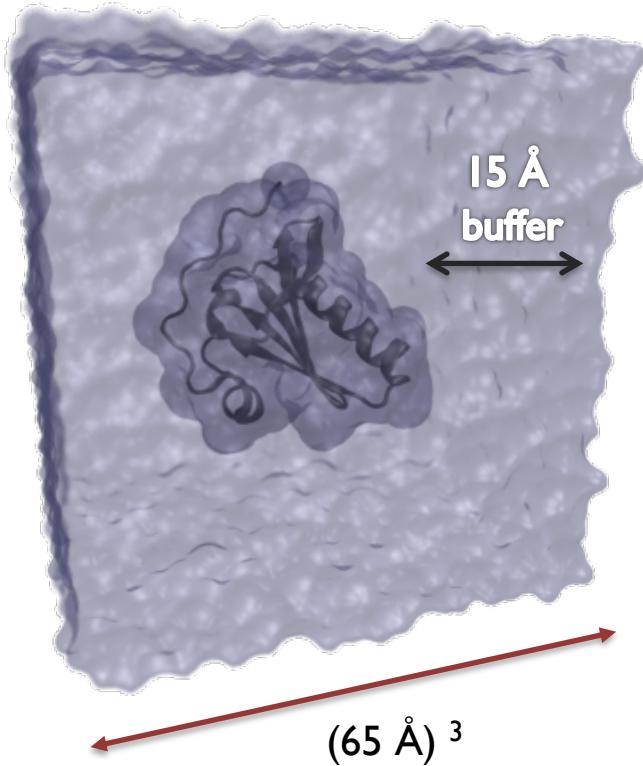
**G2 + Nb →  
modulation of the  
conformational  
ensemble**

# GSN $\pm$ NbI MD simulations

- Unbiased sampling @300 °K
- 100 mM NaCl
- Harmonic restraints:  
SS NbI @ 0.03 kcal/mol/Å<sup>2</sup>

CHARMM36

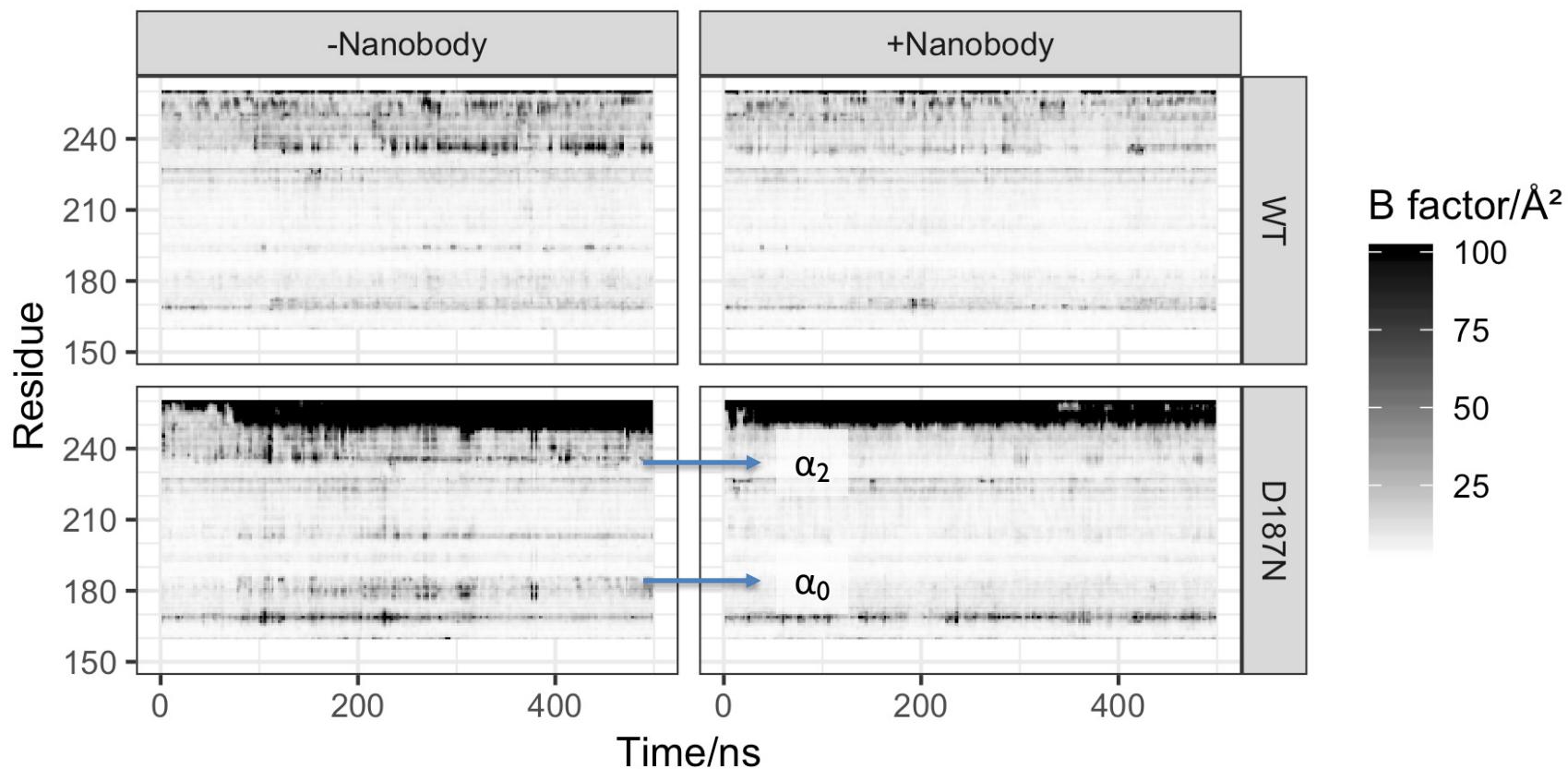
~3 μs tot. ~25k/43k atoms



# MD results



Sample	Nb11	Ca <sup>2+</sup>	Simulated time (ns)	C-terminal disorder onset
WT <sub>G2</sub>	-	+	800	Not observed
WT <sub>G2</sub>	+	+	750	Not observed
D187N <sub>G2</sub>	-	-	748	After 83 ns
D187N <sub>G2</sub>	+	-	512	After 40 ns

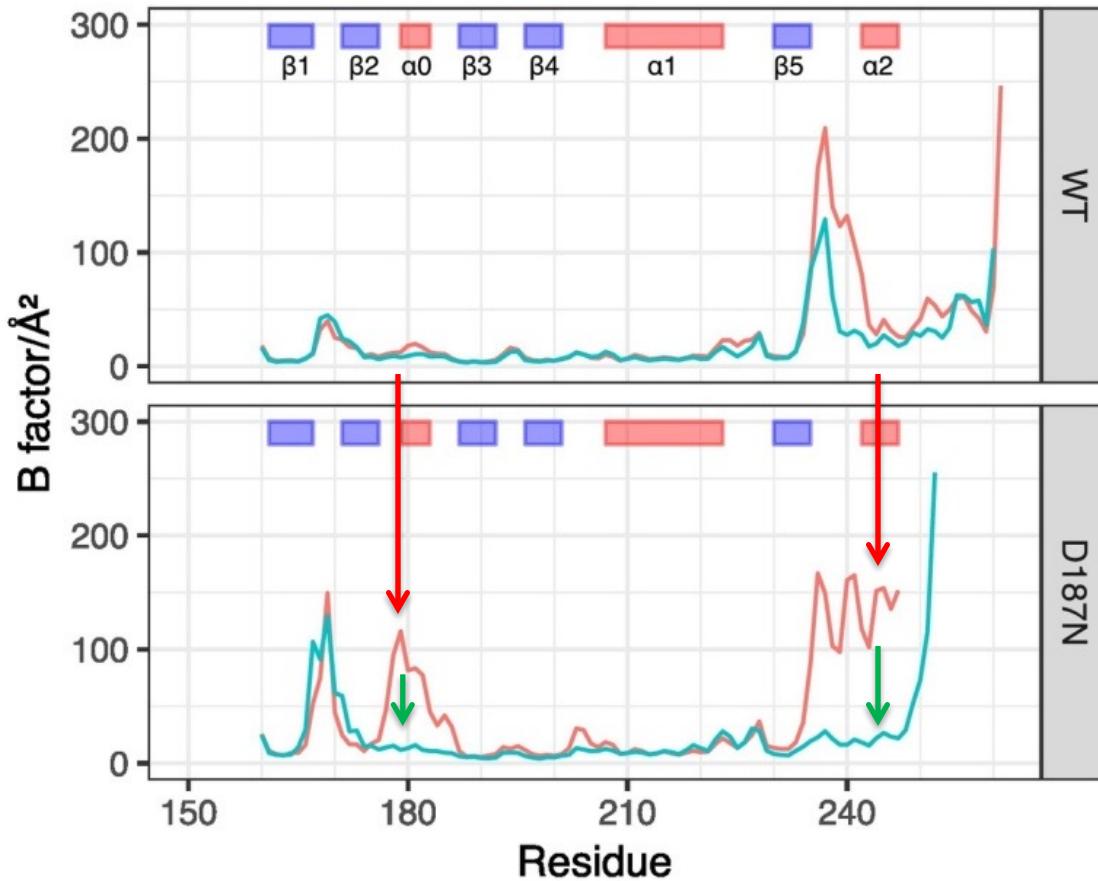


# A matter of dynamics?

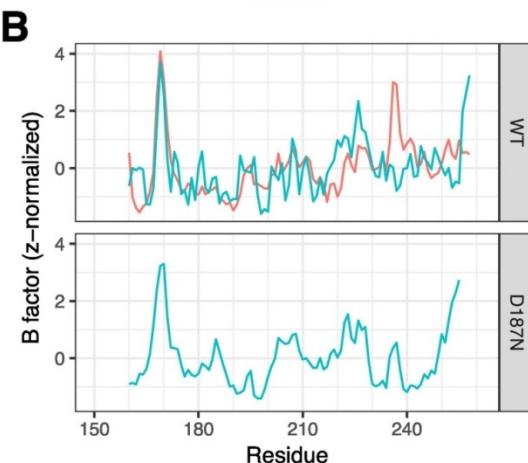
$$B = (8\pi^2/3) \text{ RMSF}^2$$



— -Nanobody  
— +Nanobody



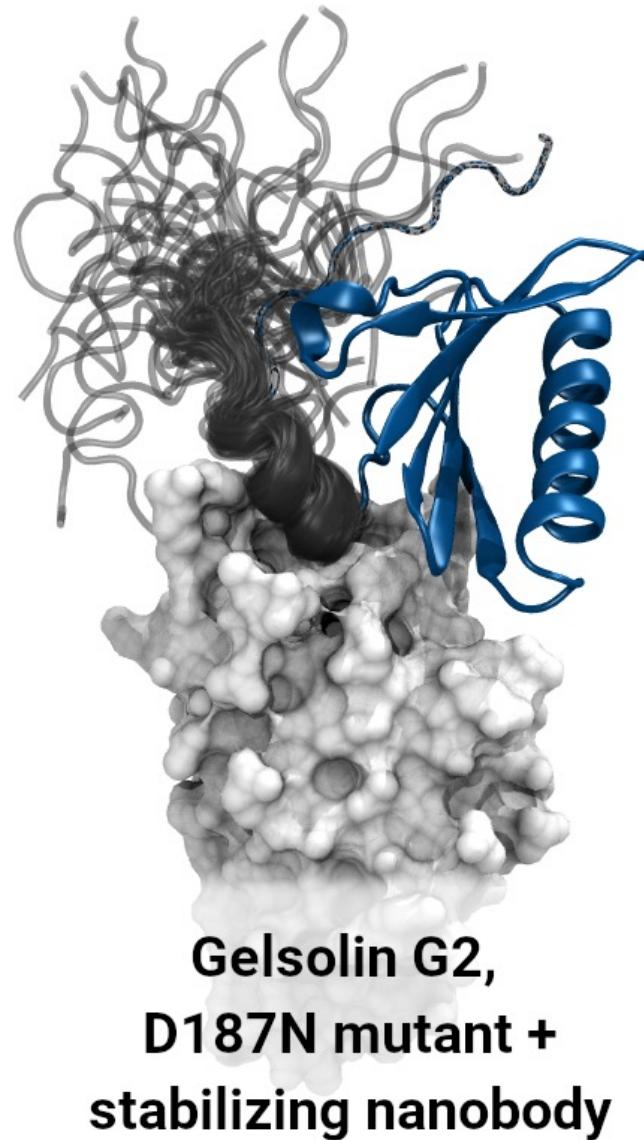
MD vs exp. B-factors →



# Reduction of disorder at C-terminus



**Gelsolin G2,  
D187N mutant**



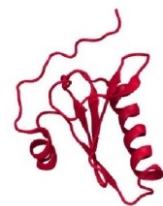
**Gelsolin G2,  
D187N mutant +  
stabilizing nanobody**

**C**

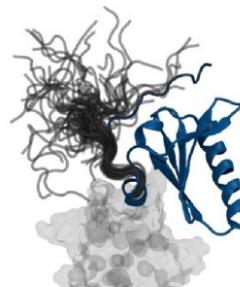
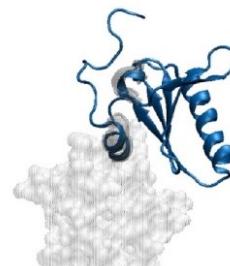
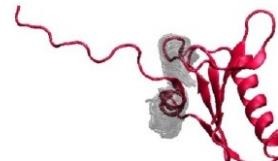
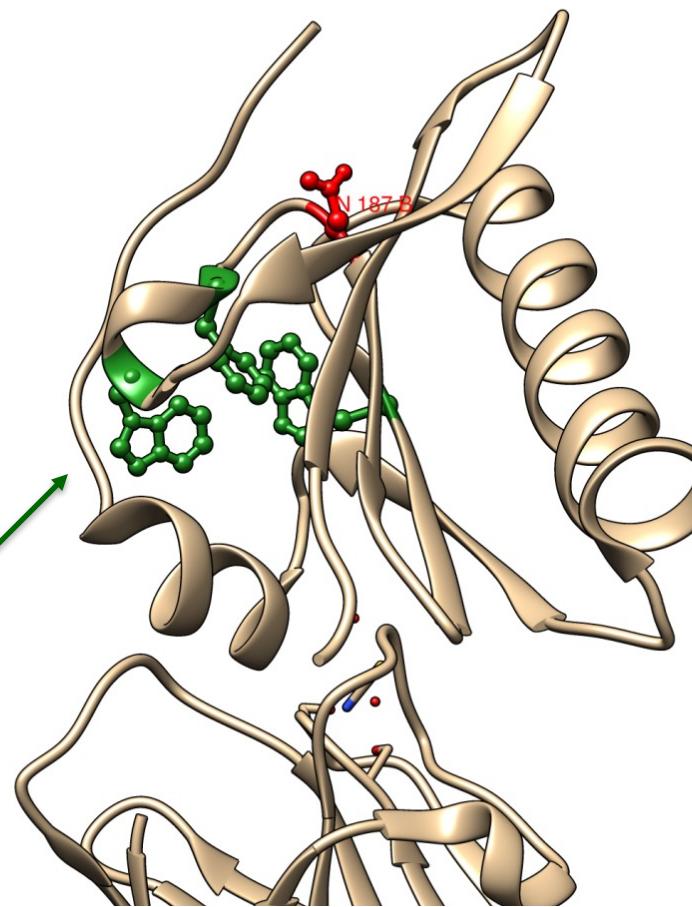
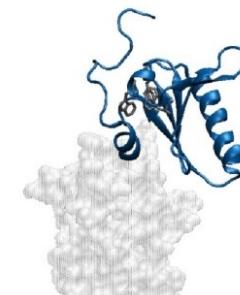
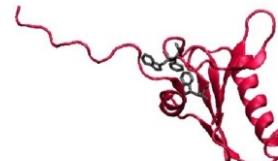
D187N

D187N  
+Nb11

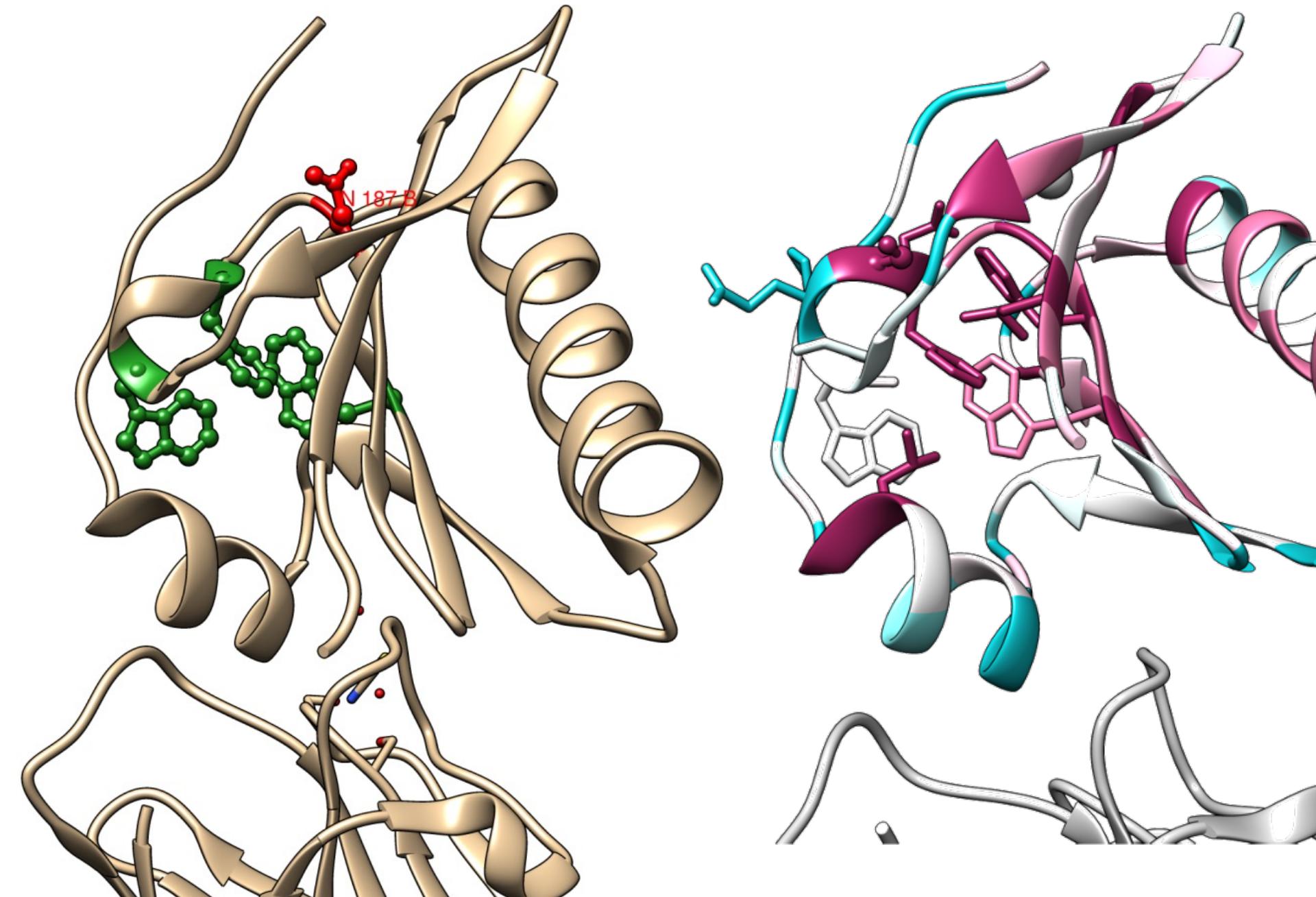
Initial configuration



Reduction of C-terminus entropy

Stabilization of  $\alpha_0$  and  $\alpha_2$ Preservation of the  $W_{180}F_{183}W_{200}$  core

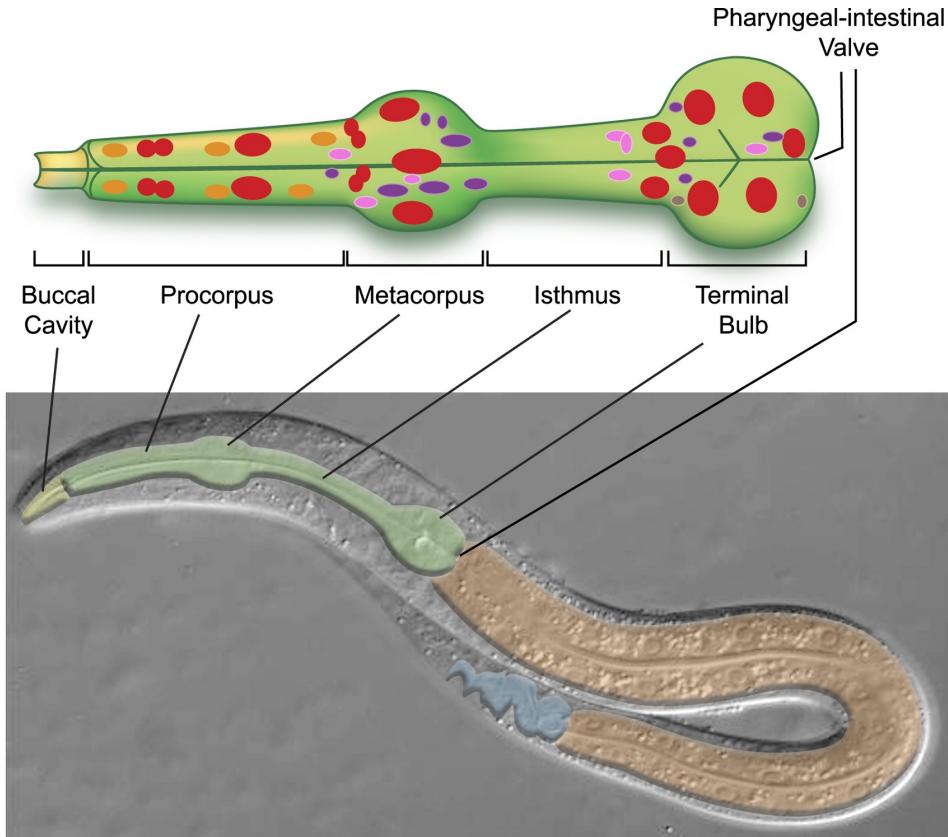
W180, F183, W200 form a **conserved** hydrophobic core centered ~ at F183 ( $\alpha 0$ )



**Any chance to make  
a drug out of it?**

# *Caenorhabditis elegans*

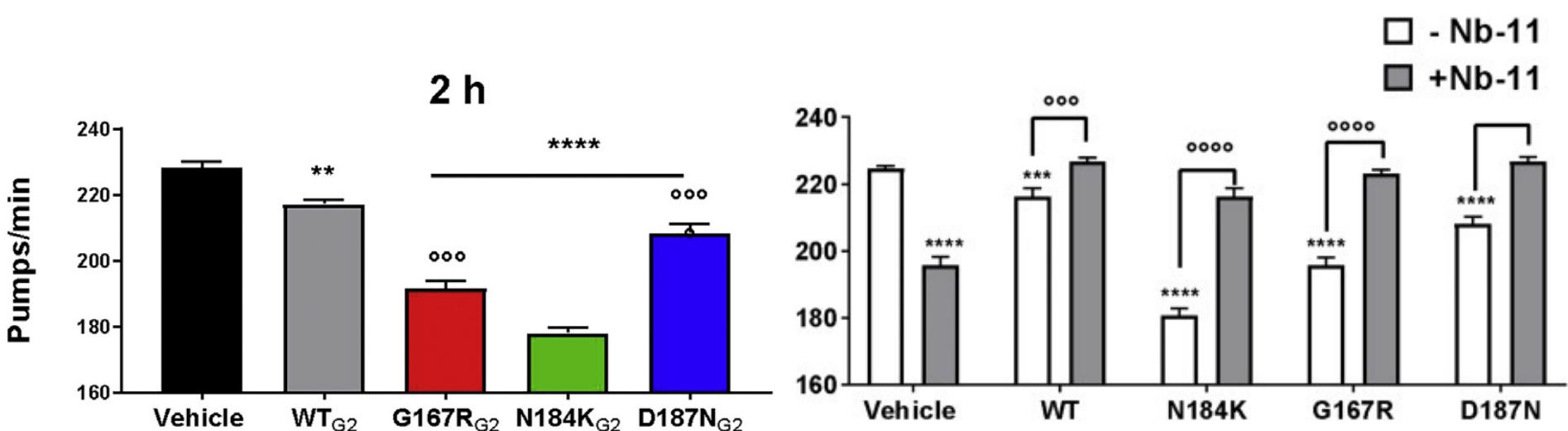
- A well-behaved model organism
- Transparent, relatively easy to handle
- 959 cells, 302 neurons
- Pharynx pumps continuously, orthologous to vertebrate heart
- **Pharynx pumping used for toxicity assays\***



\* LC<sub>50</sub> ranking in *C. elegans* was as predictive of acute toxicity in mammals, other than rat and mouse, as LD<sub>50</sub> ranking in rat or mouse. (Hunt et al. 2017, doi:10.1002/jat.3357)

# *Caenorhabditis elegans*

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G2 mutants are more toxic than WT and control

Nb11 reduces toxicity to WT levels

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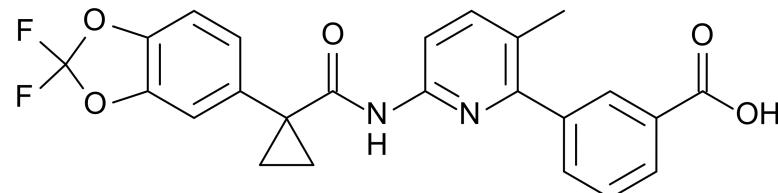
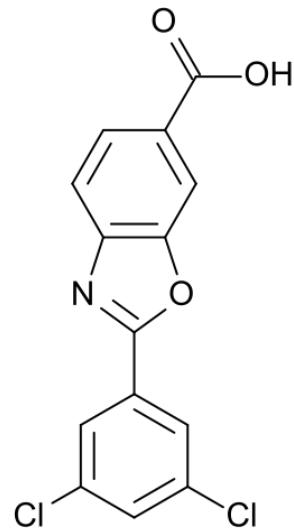
# Pharmacoperones

= pharmacological chaperones

Pharmacoperones stabilize the native conformation of misfolded or destabilized proteins, to prevent their degradation and promote correct trafficking to their functional site of action.

Not many examples:

- Tafamidis (Transthyretin amyloidosis): kinetically stabilizes the (mutated) tetramer, preventing monomer unfolding
- Lumacaftor (Cystic fibrosis): ΔF508 point mutant in CFTR does not reach membrane and is degraded in the ER.



# Conclusions

- D187N impairs G2 stability by loss of  $\text{Ca}^{2+}$ , shifting balance towards disordered tail conformations
- NbI1 re-stabilizes the domain fold locally protecting a conserved hydrophobic core
- Mechanism obtained via combined crystallography + **molecular dynamics simulations**