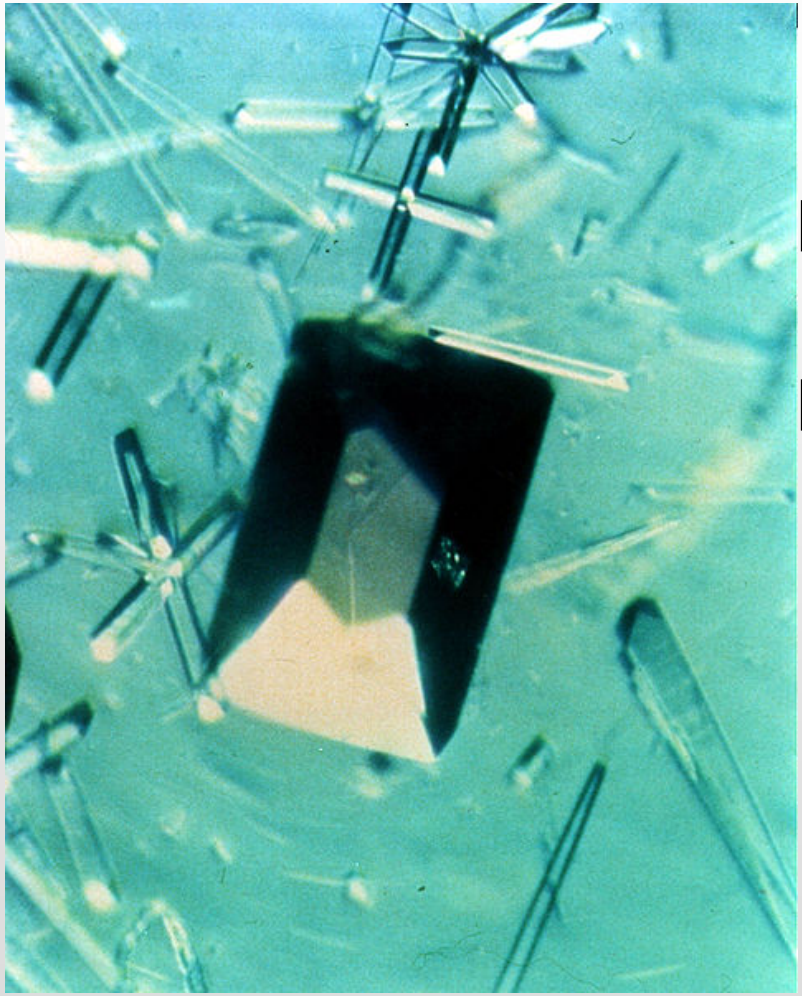
Joint use of SAXS and NMR

Annalisa Pastore
King's College London
Scuola Normale Superiore

The three brothers of Structural Biology

Lele Luzzati

The limits of X-ray…



Necessity of crystals

Little information on dynamics

The limits of cryo-EM

The protein size

It is expensive

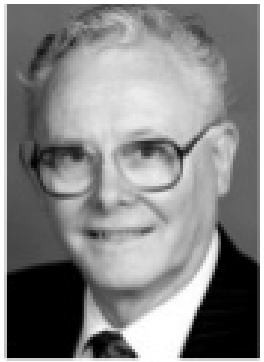
The samples get radiation damnaged

A bit of NMR history

2002 *Chemistry* Wüthrich (ETH)

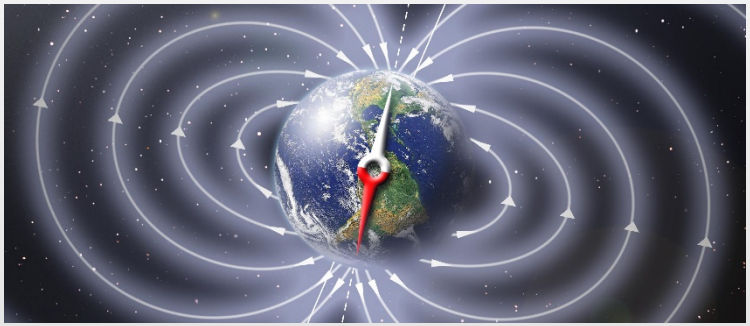
"for his development of nuclear magnetic resonance spectroscopy for determining the three-dimensional structure of biological macromolecules in solution"

2003 *Medicine* Lauterbur (University of Illinois in Urbana), Mansfield (University of Nottingham)



"for their discoveries concerning magnetic resonance imaging"

Nuclear Magnetic Resonance

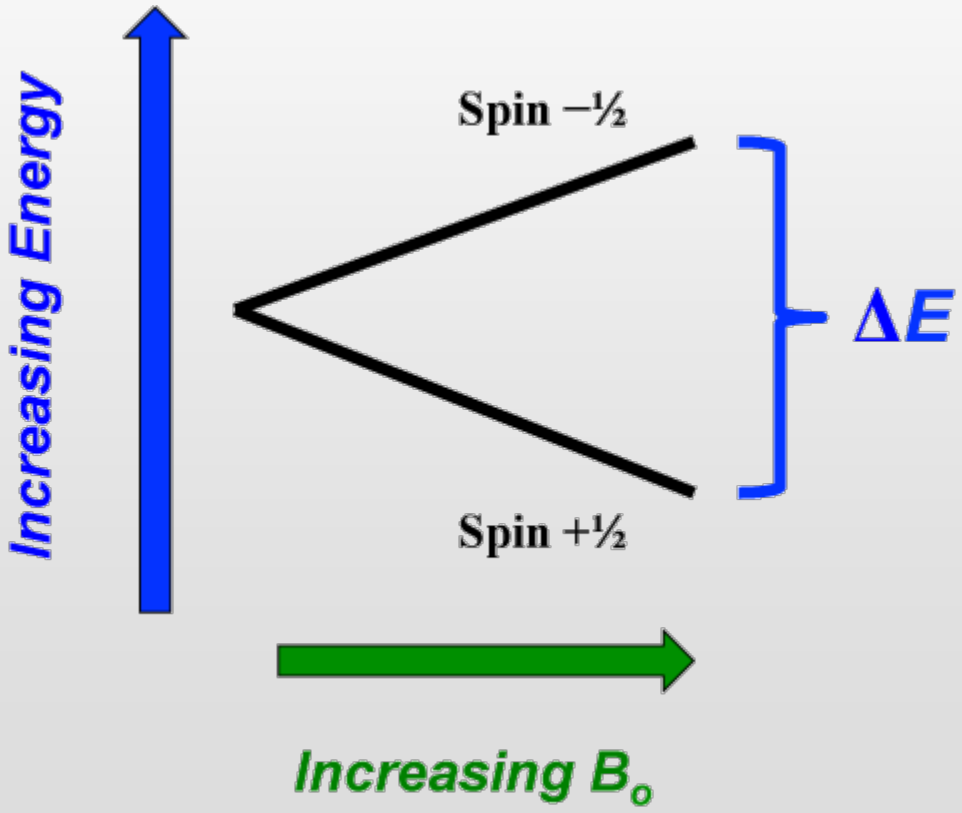


1 Gauss

23.5 Tesla

Circa 500.000 times >>

The magnetic field removes the degeneracy of the nuclear spin levels



NMR as a radio…

Tune the frequency to observe a certain… element….

The 1H spectrum of a protein

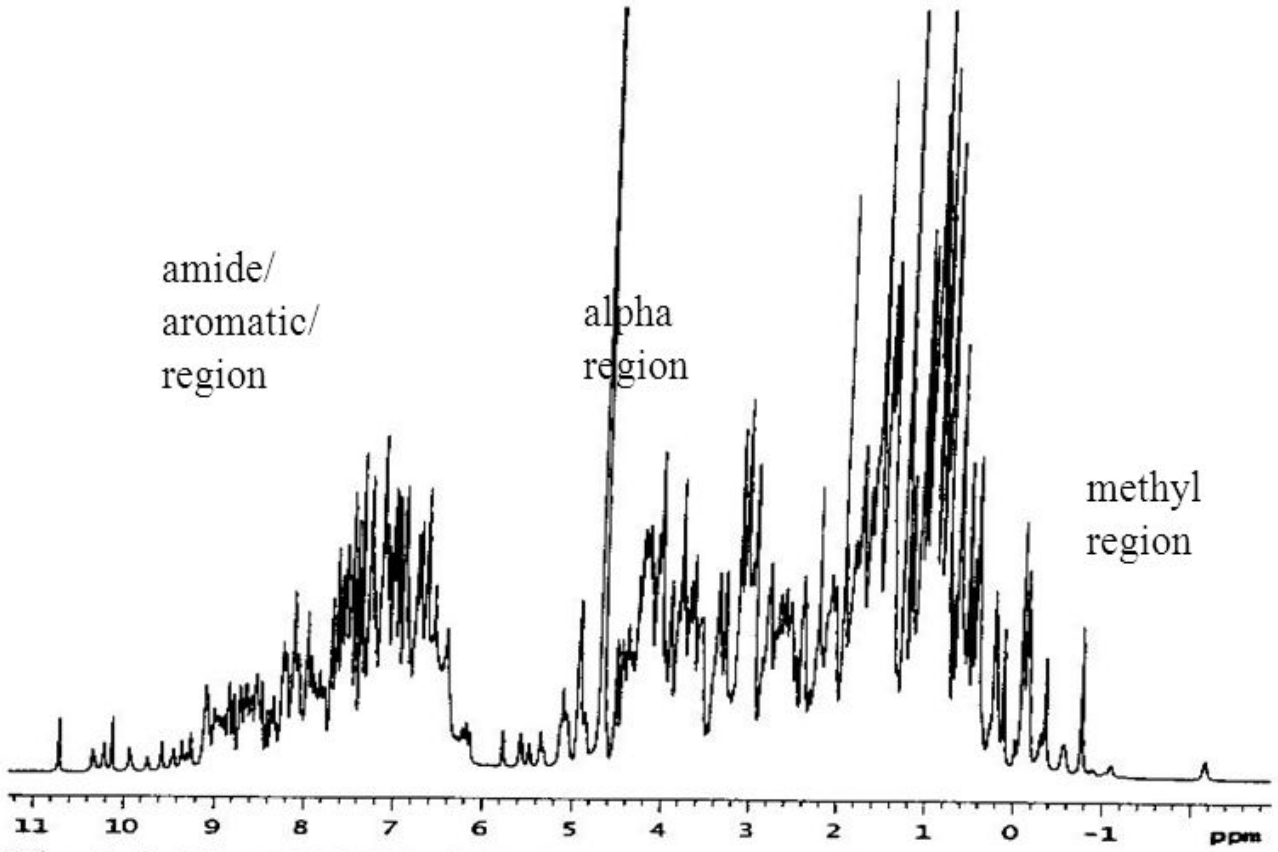
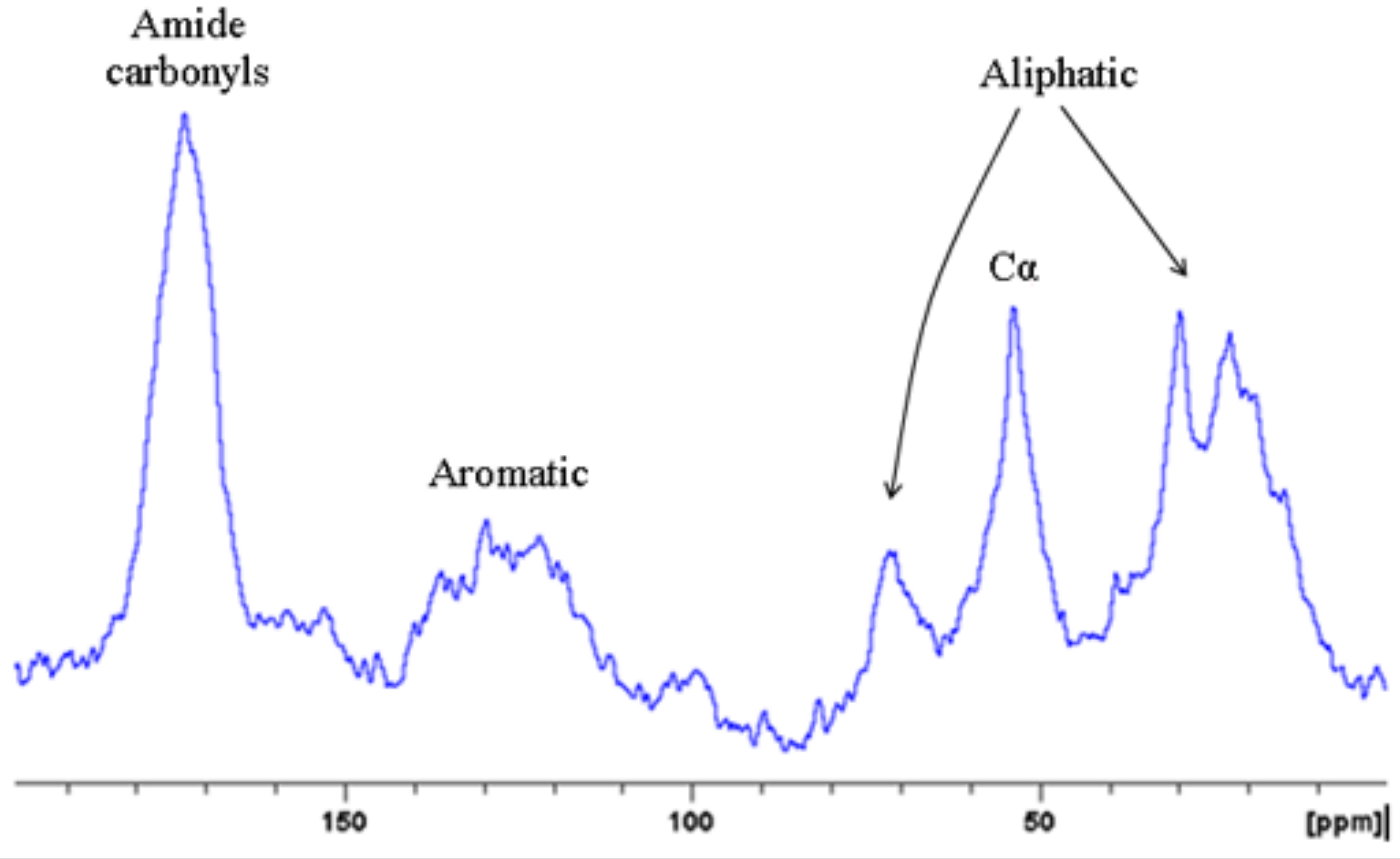


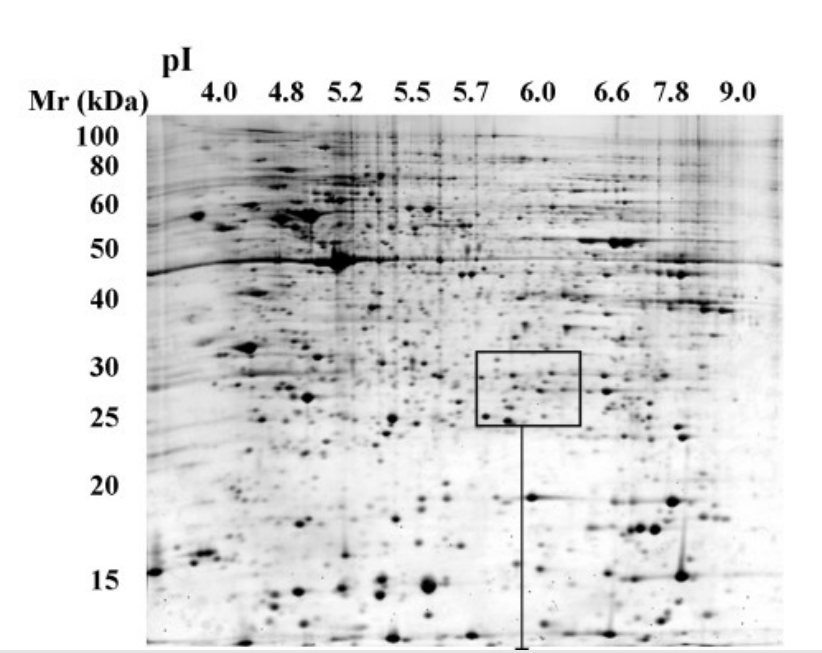
Fig. 1.2 The 750 MHz ^{1}H NMR spectrum of lysozyme.

The position of each peak on the spectrum is called chemical shift

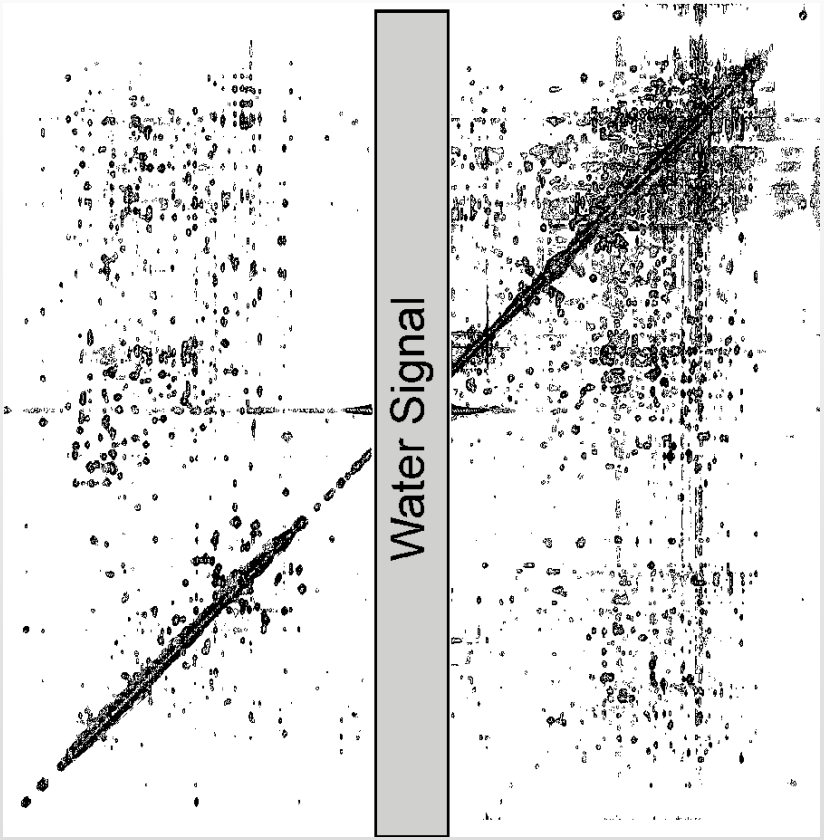
A 13C spectrum



You may gain resolution increasing the dimensions…



Like a 2D Gel



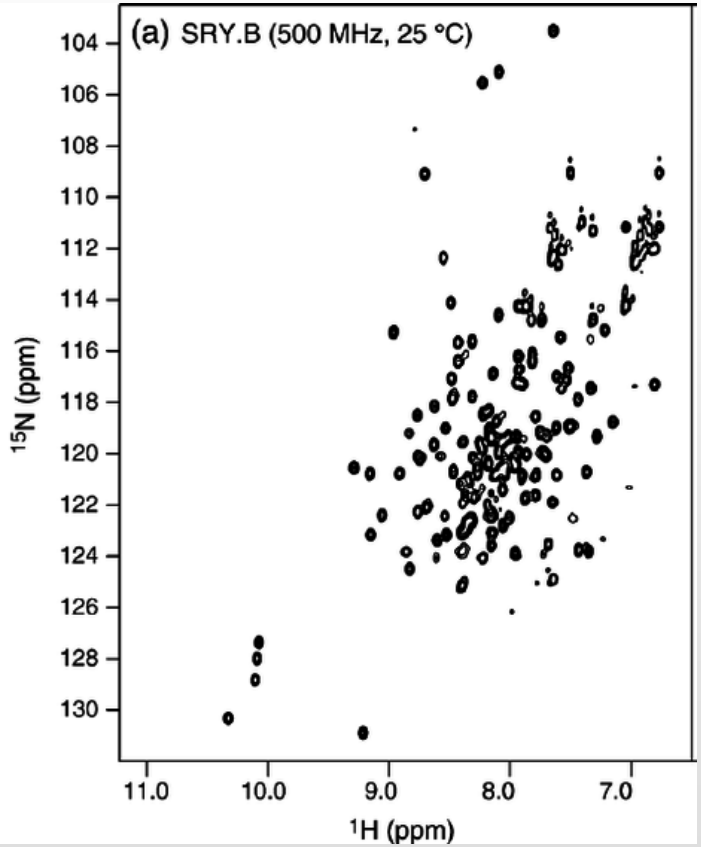
…or using more than one nucleus

Excite one nucleus,

transfer the magnetization to another,

and than back to the first

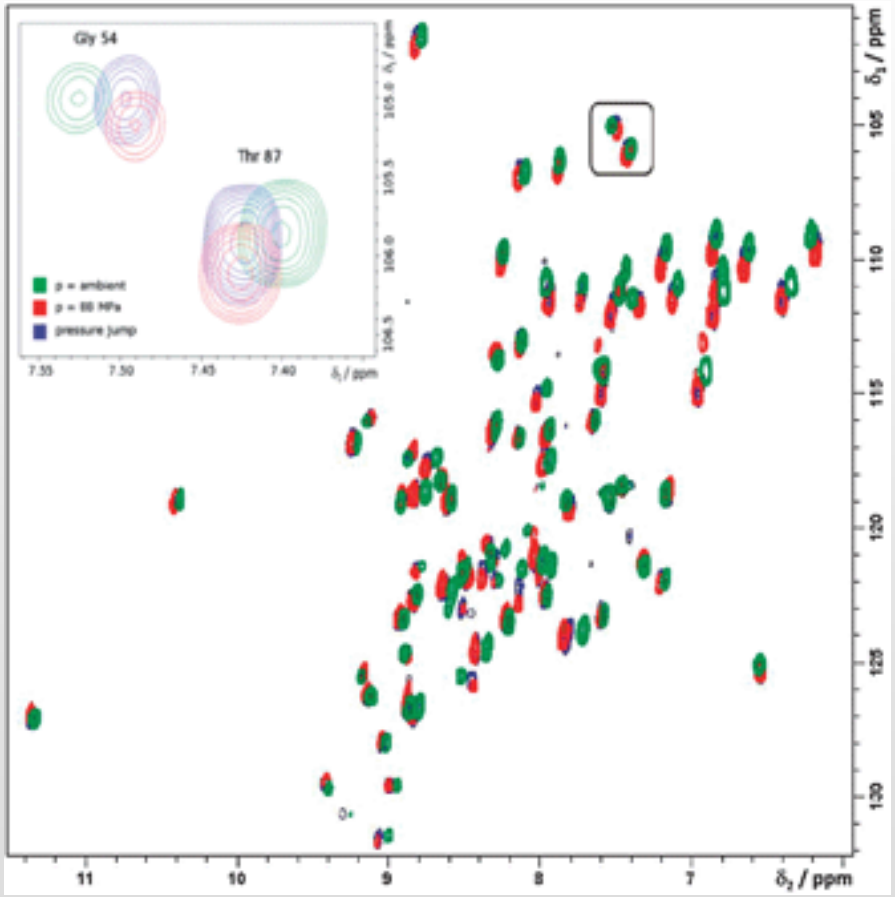
2D maps of 1H-15N correlations



As many resonances as many HN in the protein (i.e. the Nres – Npro)

The fingerprint

Chemical shift perturbation as a tool to map interactions…

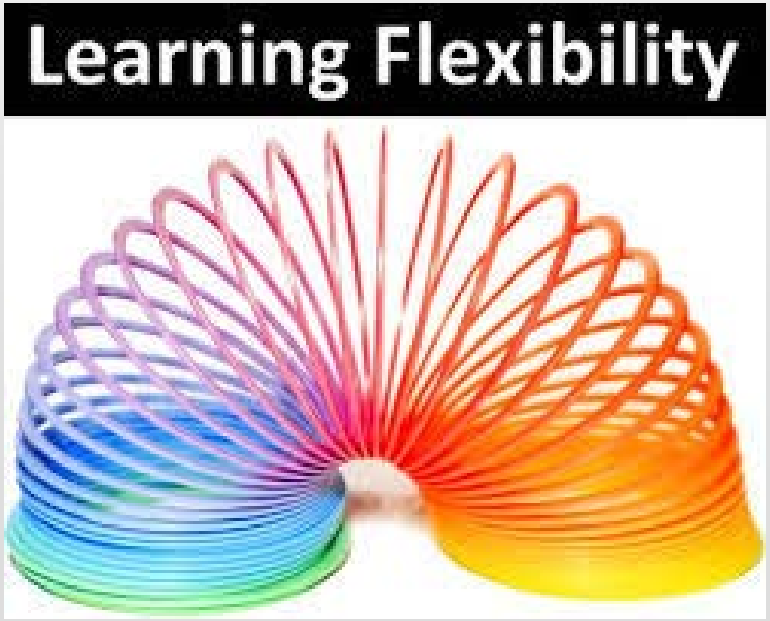


Sensitivity to the chemical environment

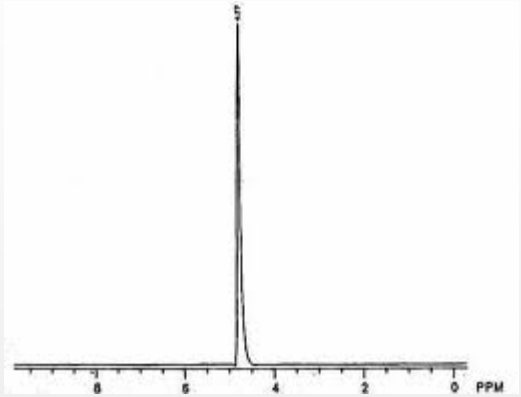
Distance restraints

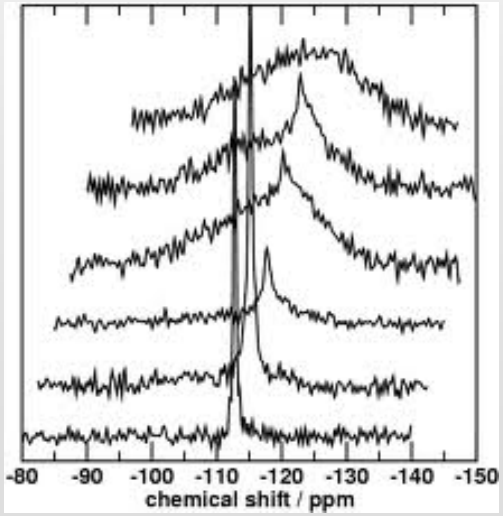
The advantanges:

- **Structure determination in solution**
- **Intermolecular interactions**
- **Dynamics**
- **Weak interactions**



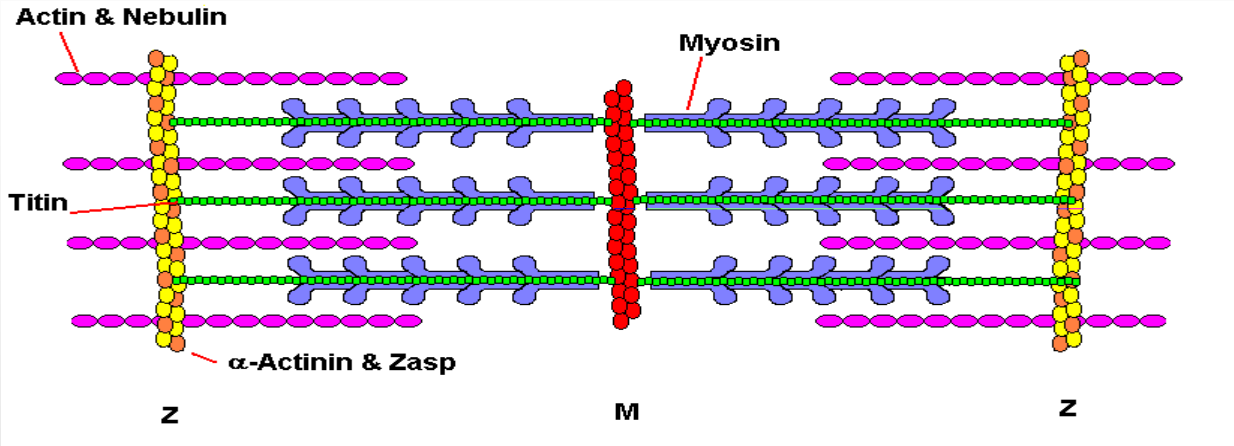
Disadvantages: The linewidth is proportional to the tumbling time

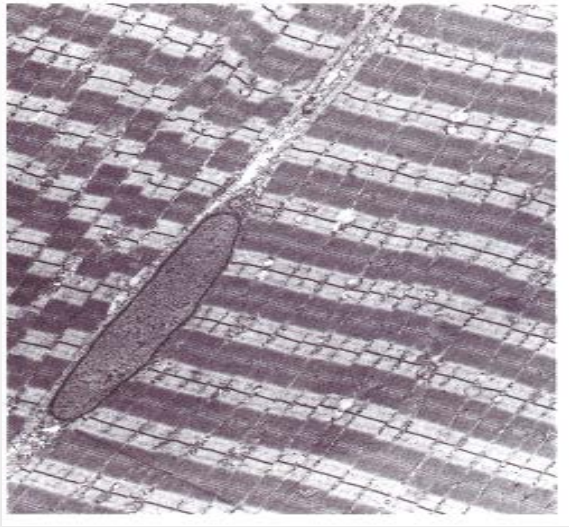


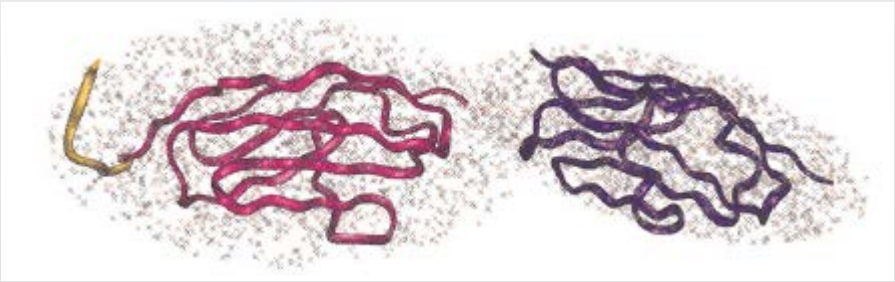


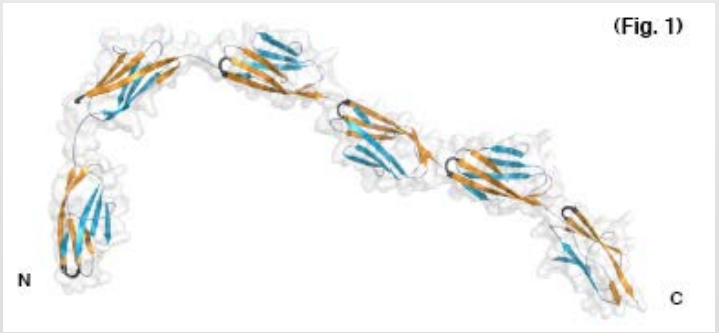
We are limited in the molecular size we can afford…

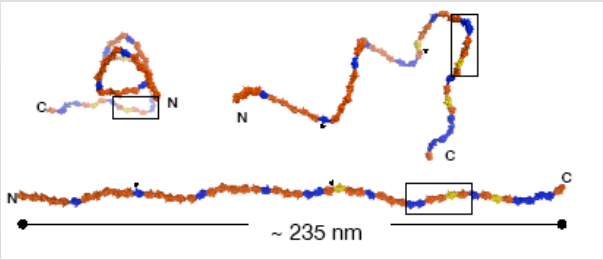
A way to solve the structure of giant proteins





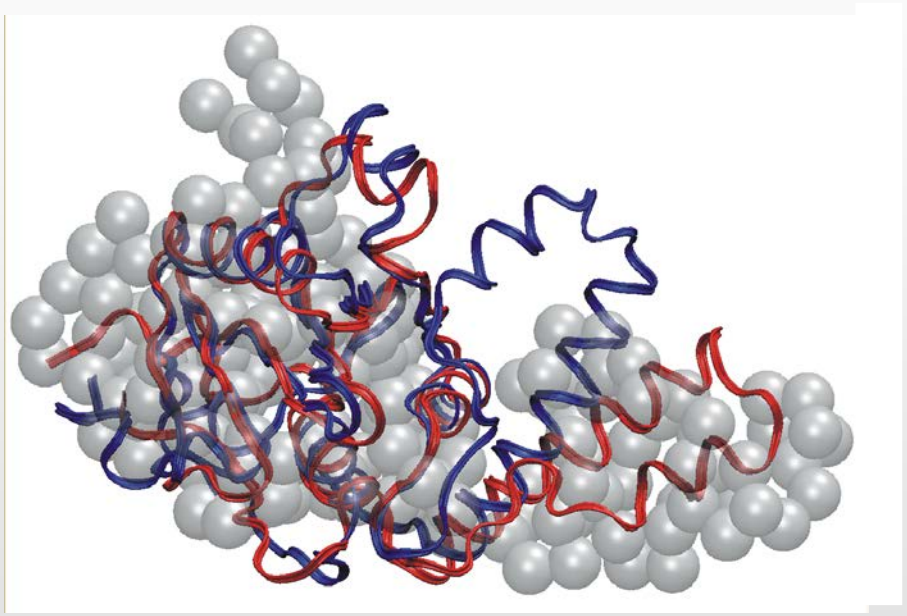


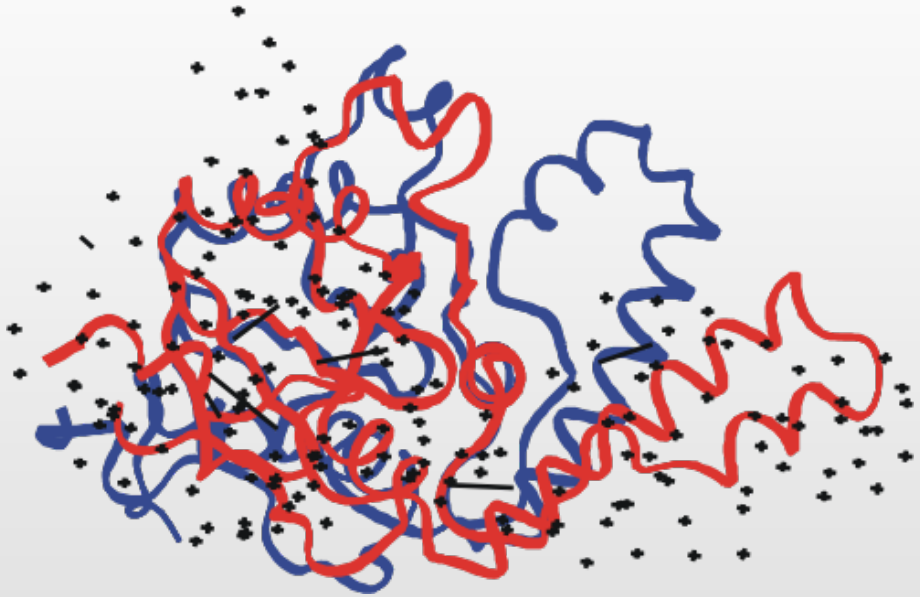




Improta et al. Structure 1997
Von Castelmur et al., PNAS 2007

A way to validate structures

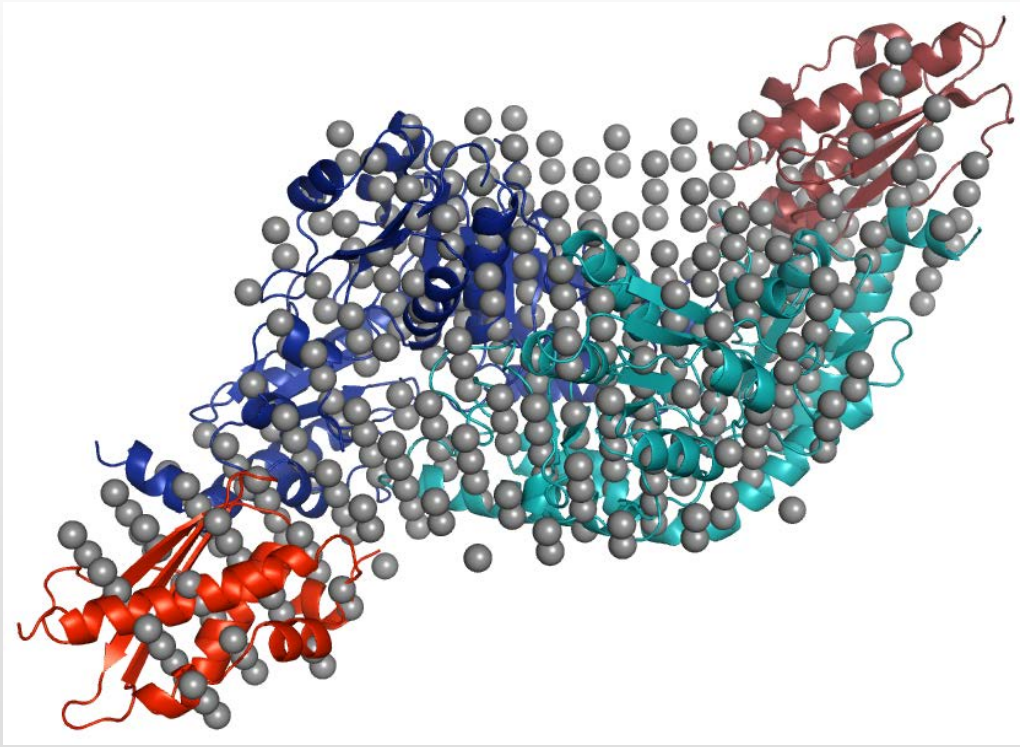




Only one structure fits the data: ours!!!

Nicastro et al., J. Biomol. NMR 2006

A way to solve the structure of molecular complexes

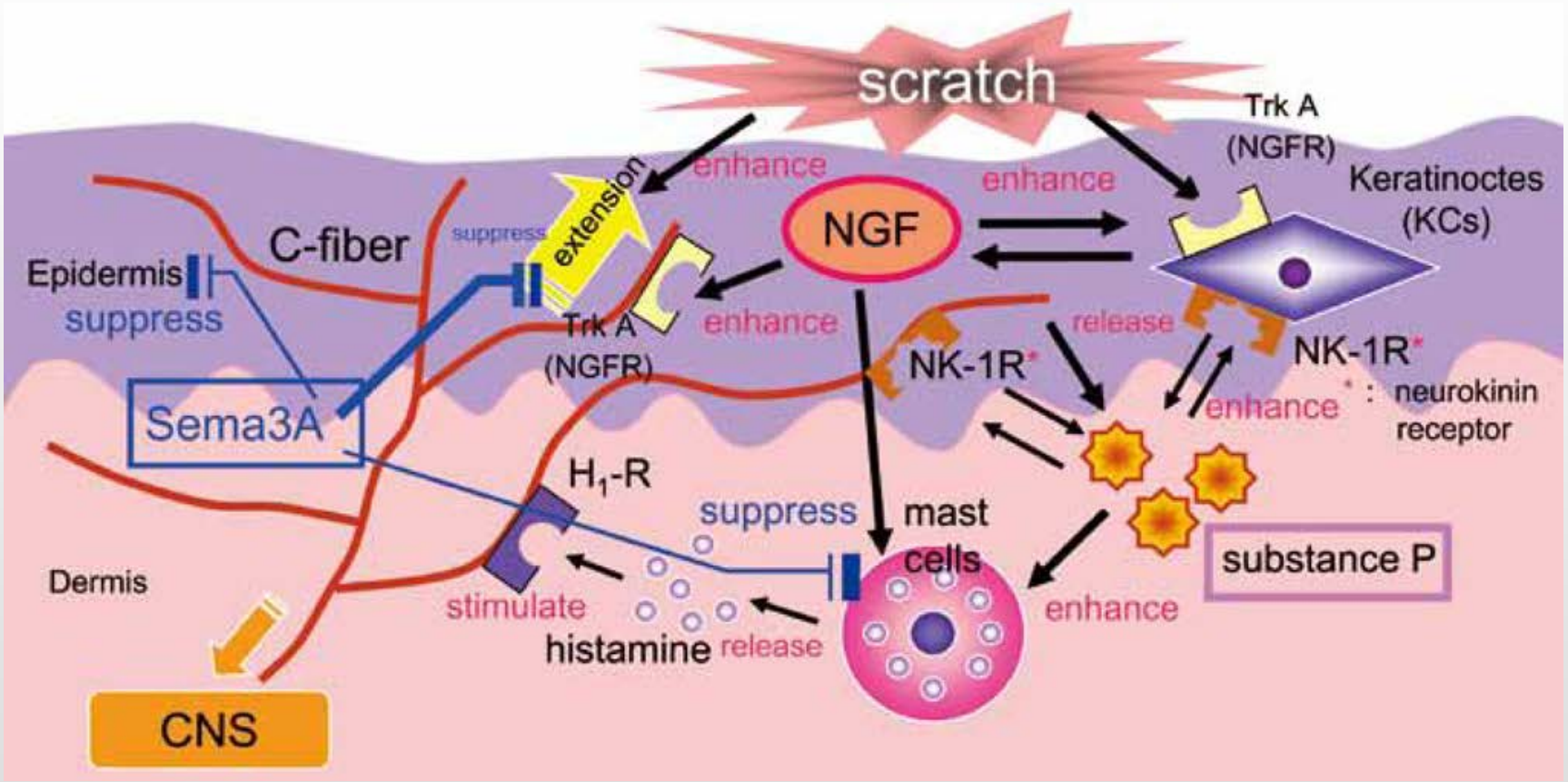


Prischi et al. NSMB 2010

The Nobel prize Rita Levi Montalcini

(1901 – 2012)

Nerve growth factor

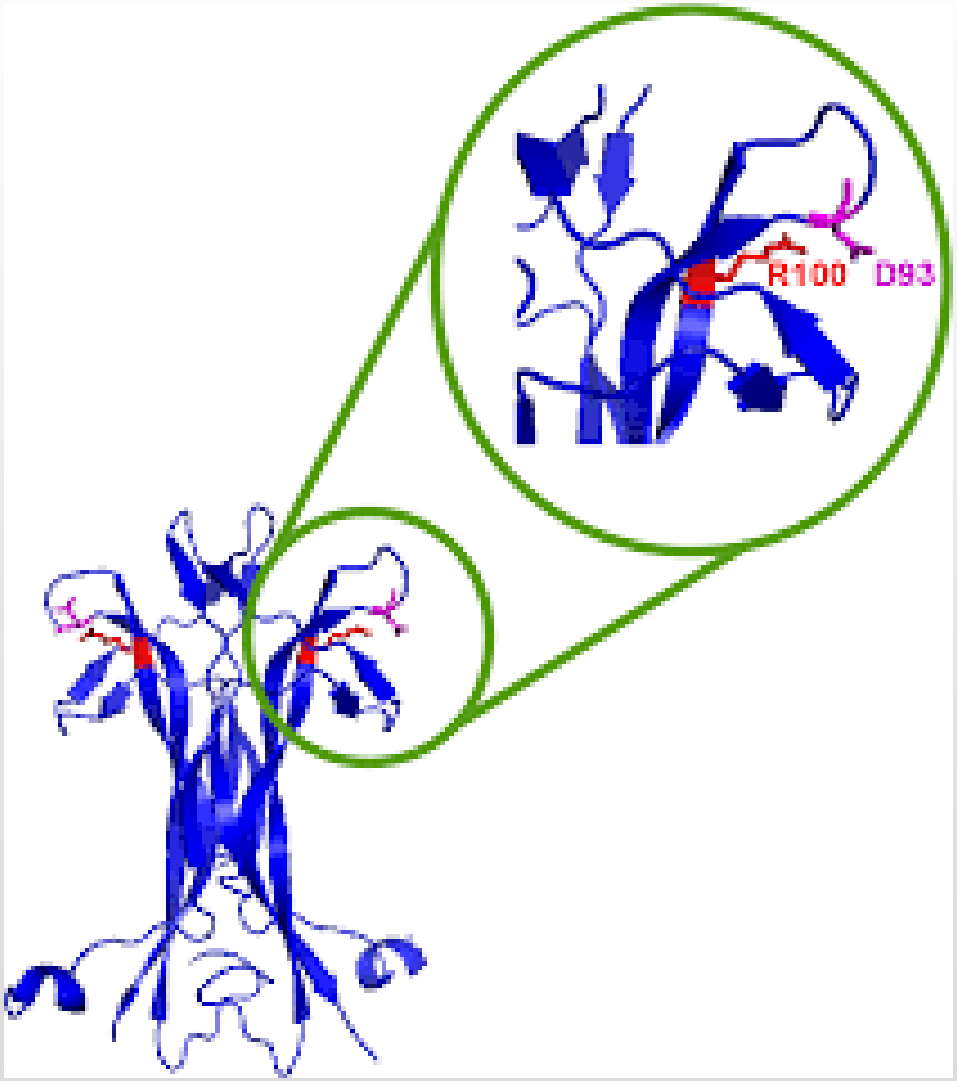


Nerve Growth Factor (NGF) plays an important role in cognitive function, depression, inflammation, autoimmunity, histamine intolerance, western disease, pain, cancer and more. It belongs to the neurotrophin group.

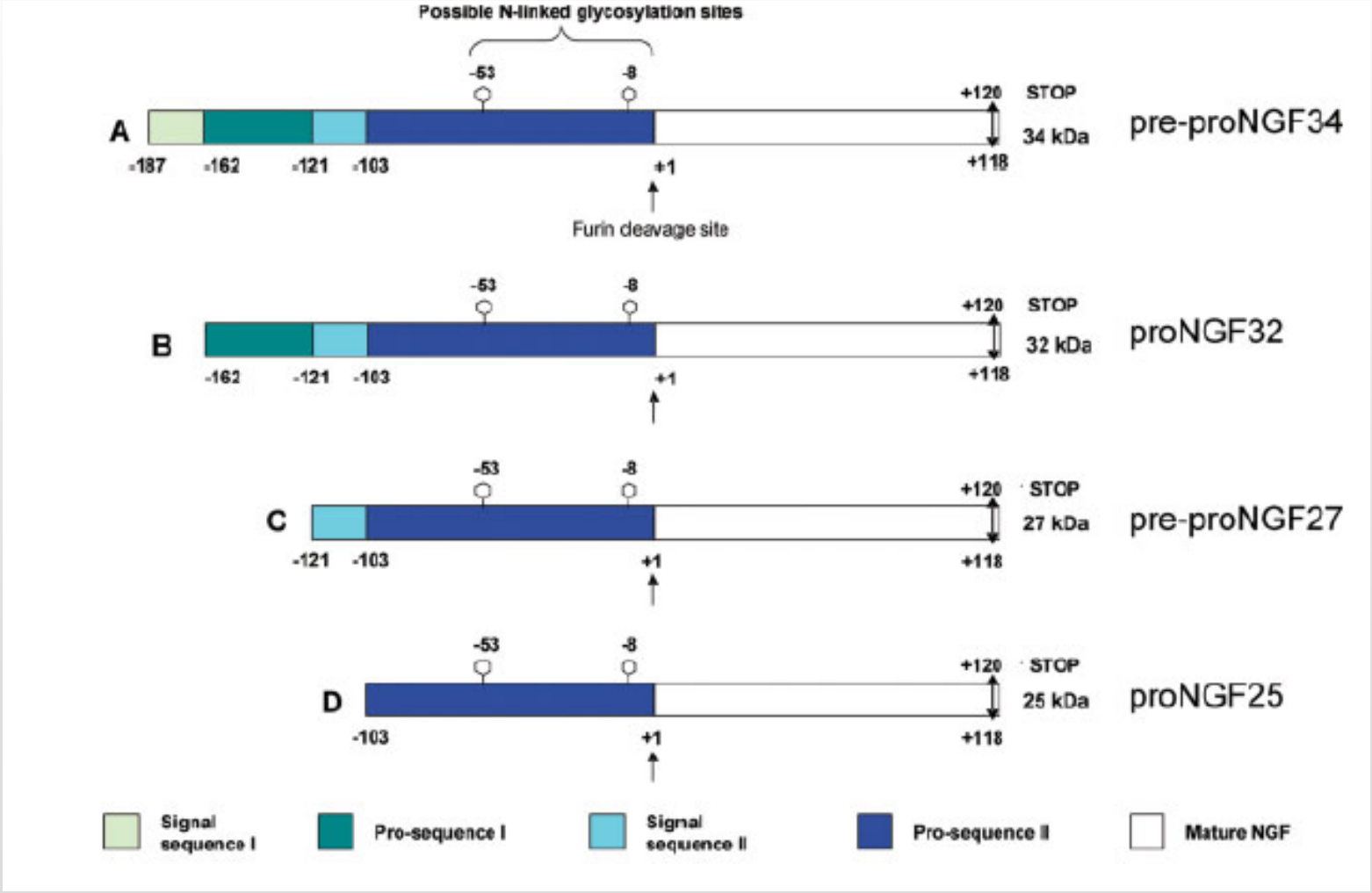
R100W mutants are painless…

Painless = fearless

The structure of NGF was solved in 1991 by Blundell and cow.



But NGF is expressed as a precursor

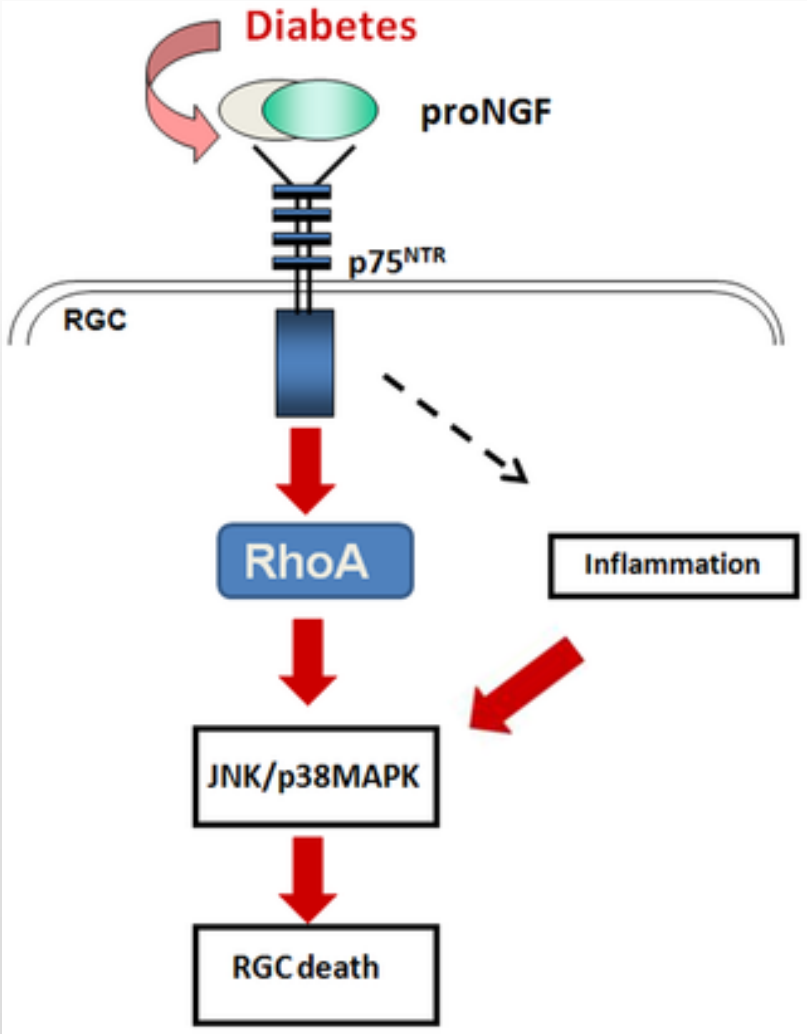


Evidence that proNGF has an independent life

ProNGF is the more abundant form in CNS tissues, mature NGF is barely detectable

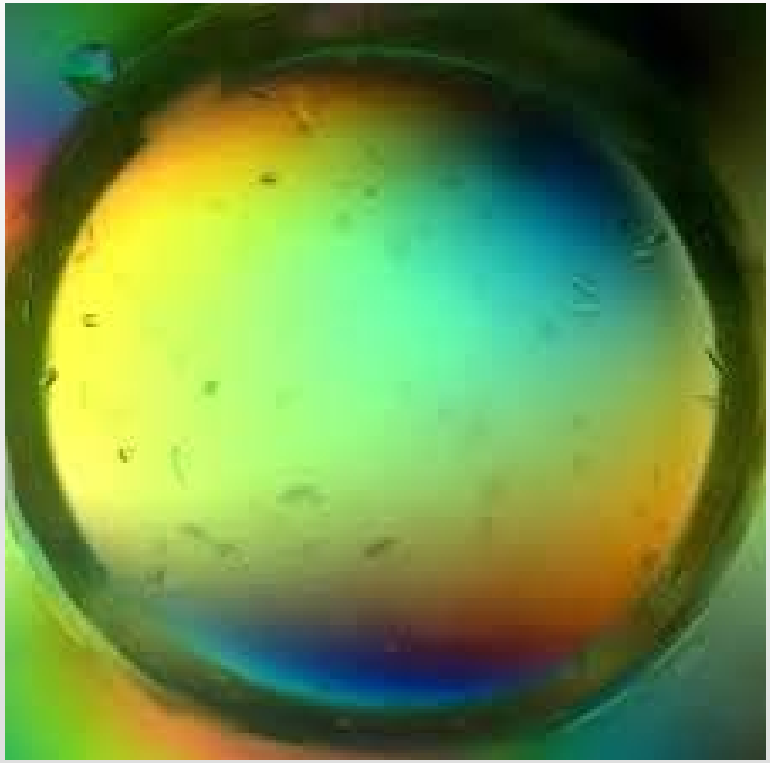
Cleaved pro-domain exists in vivo with uncleaved proNGF and mature NGF

In HEK TrkA stable cells, proNGF binds to TrkA at a site distinct from that of NGF



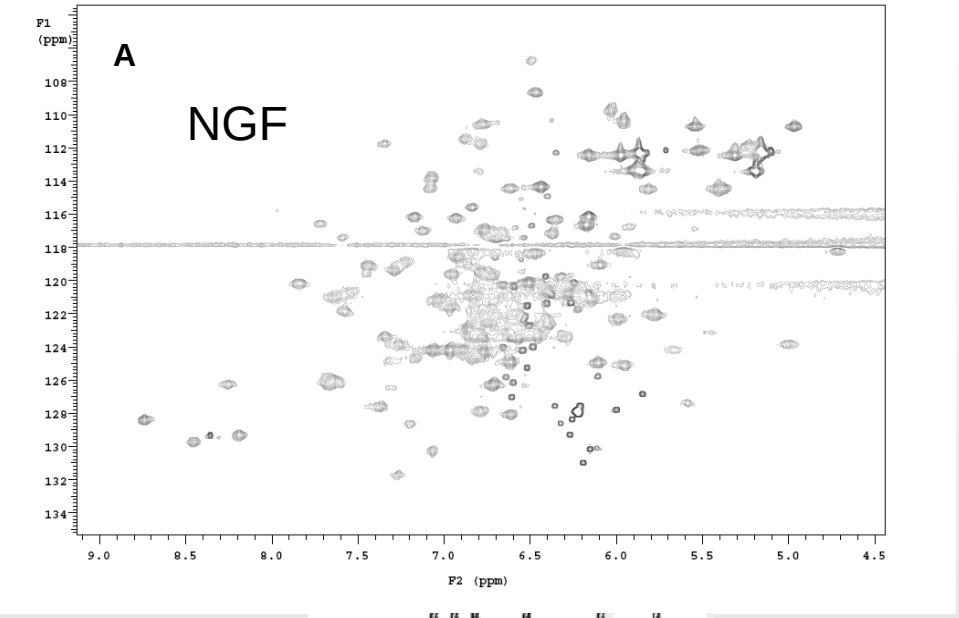
What is the structure of proNGF?

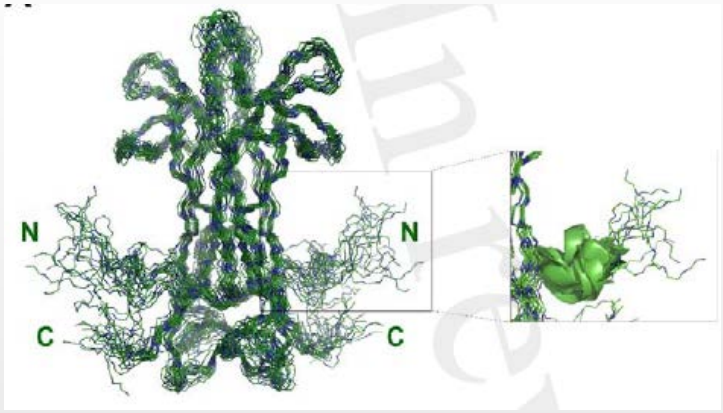
X-ray crystallography?

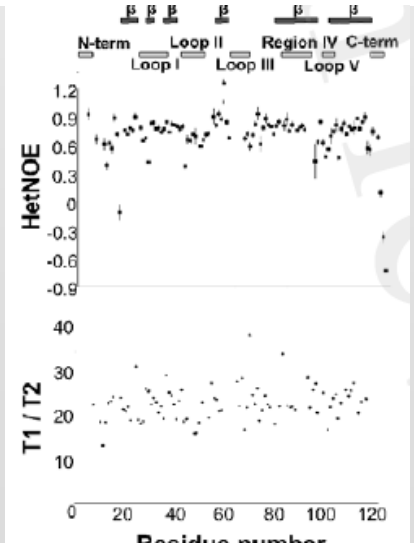


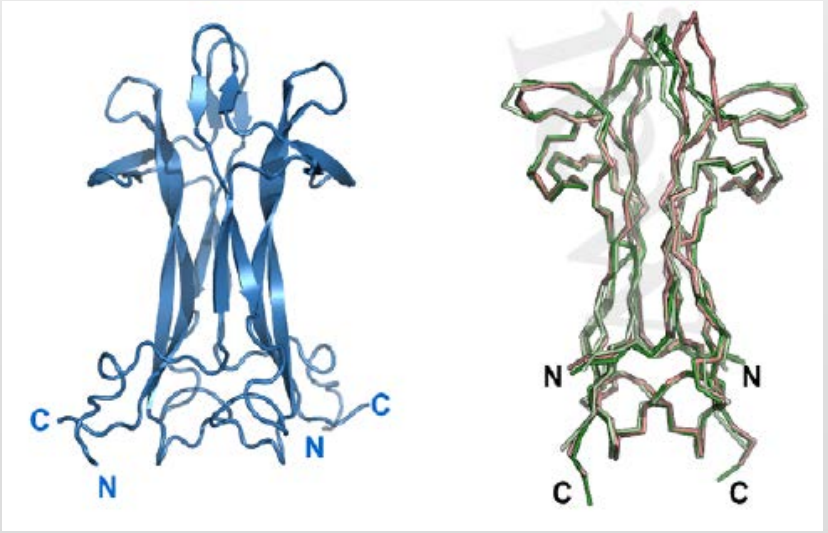
No crystals!!!

NMR of NGF?

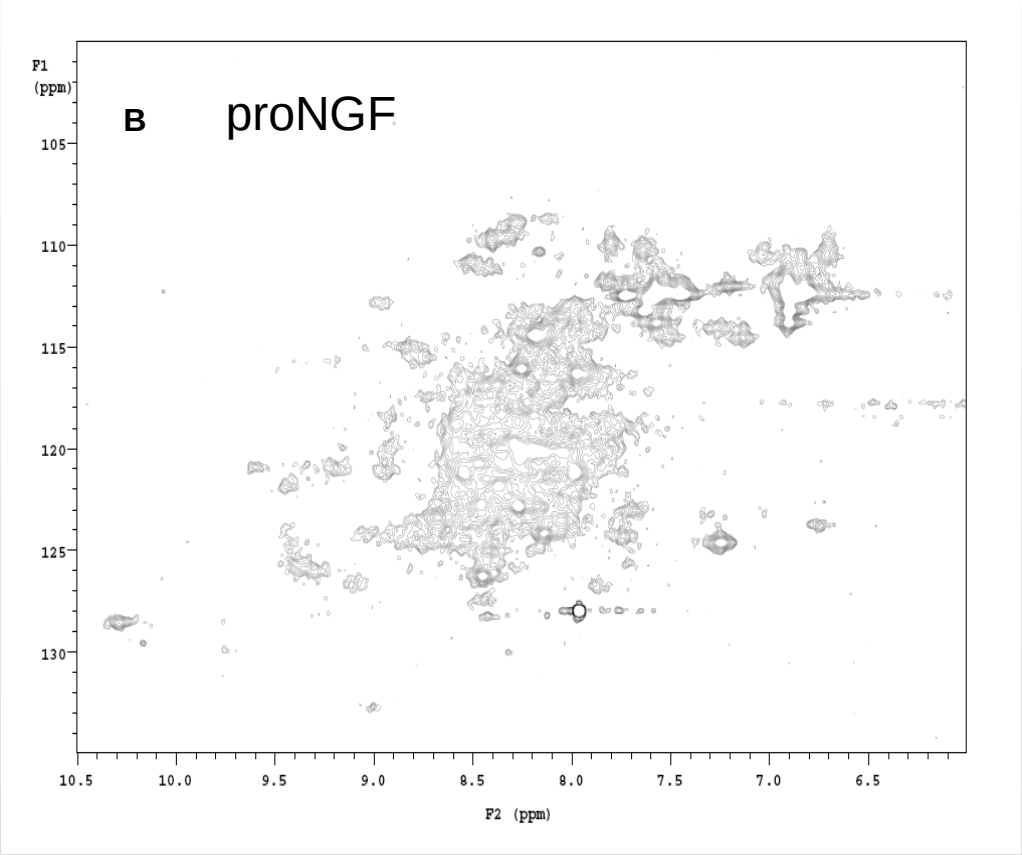

A
NGF
F1 (ppm)
F2 (ppm)


N
N
C
C

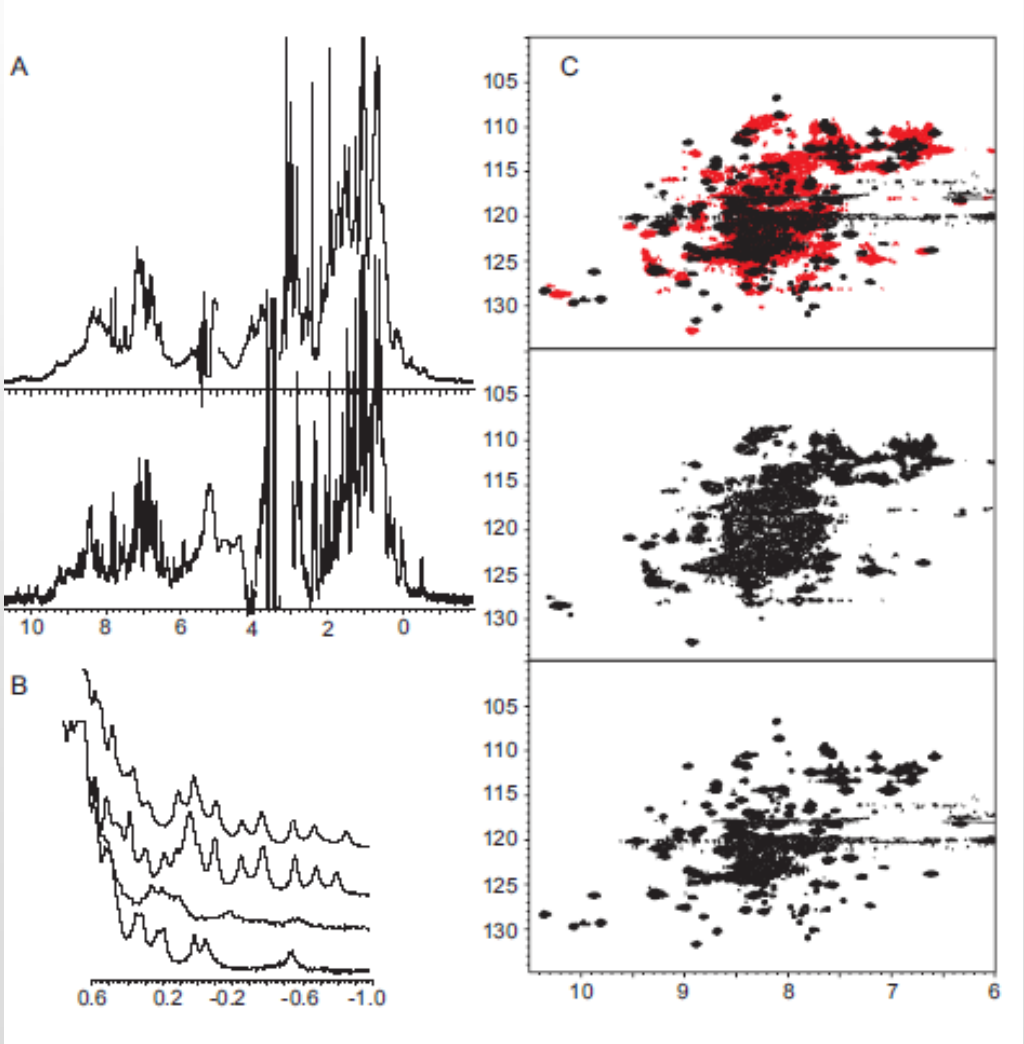

N-term
Loop I
Loop II
Loop III
Region IV
Loop V
C-term
HetNOE
T1 / T2
Residue number


C
N
N
C
N
N
C
C

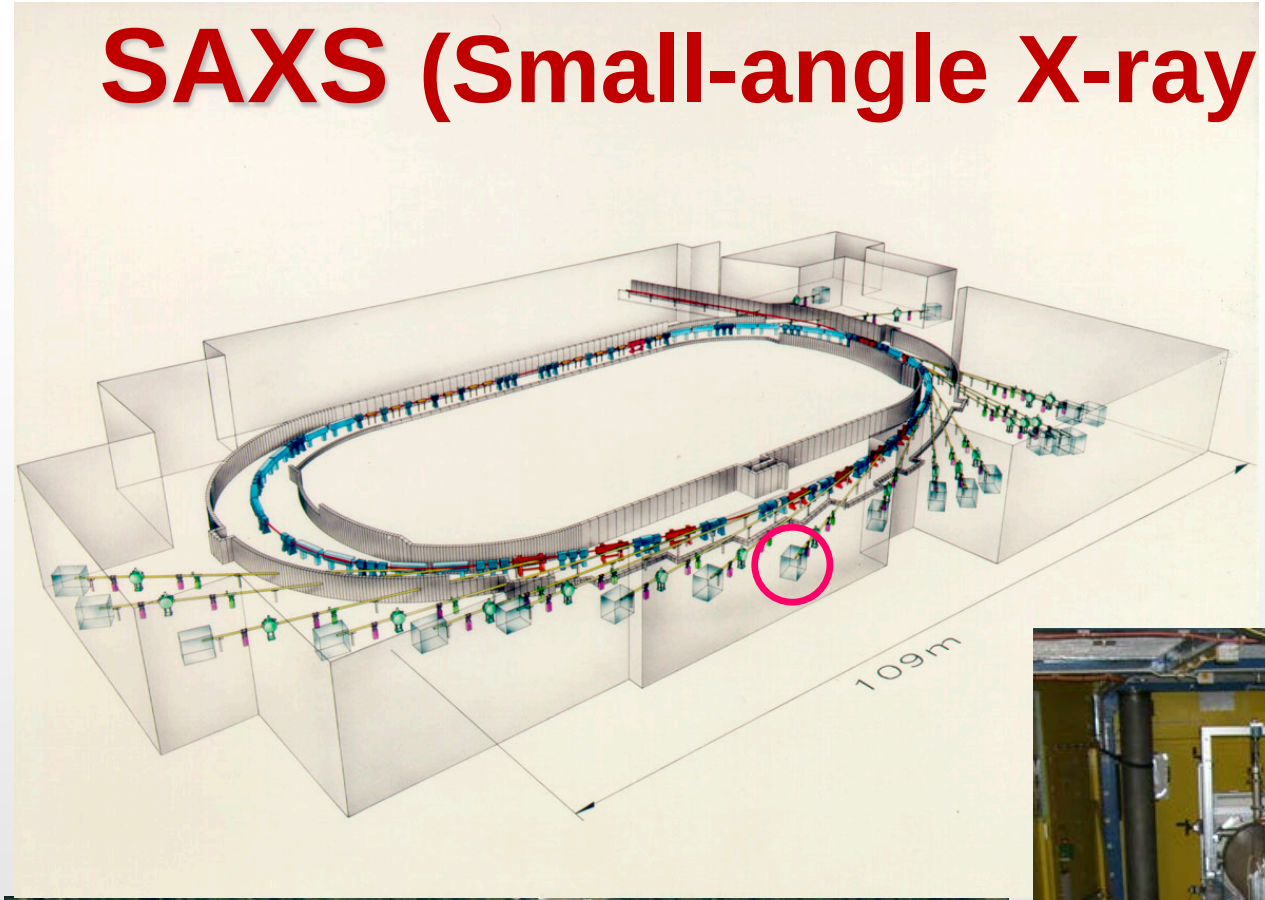
NMR of proNGF?

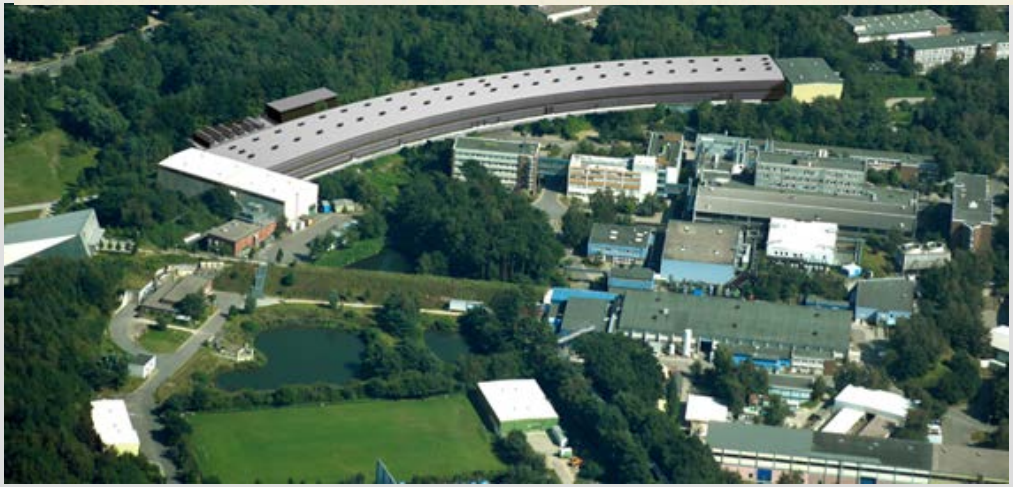


Comparison NGF vs proNGF

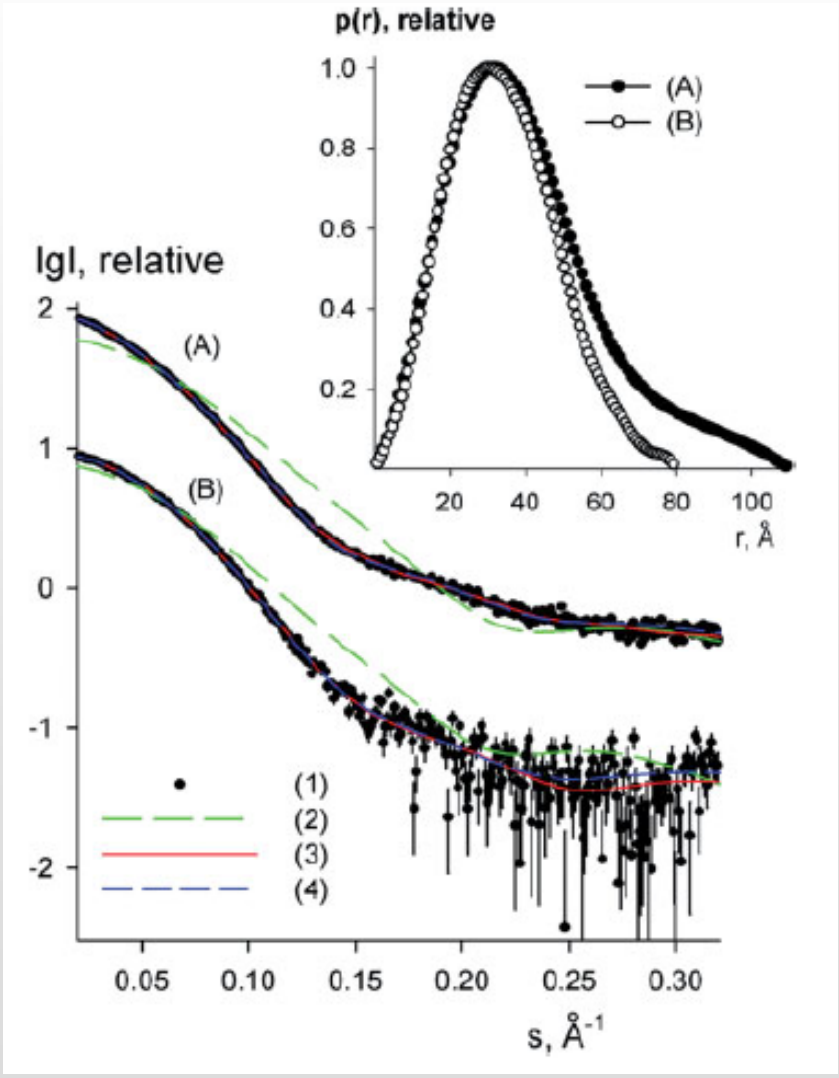


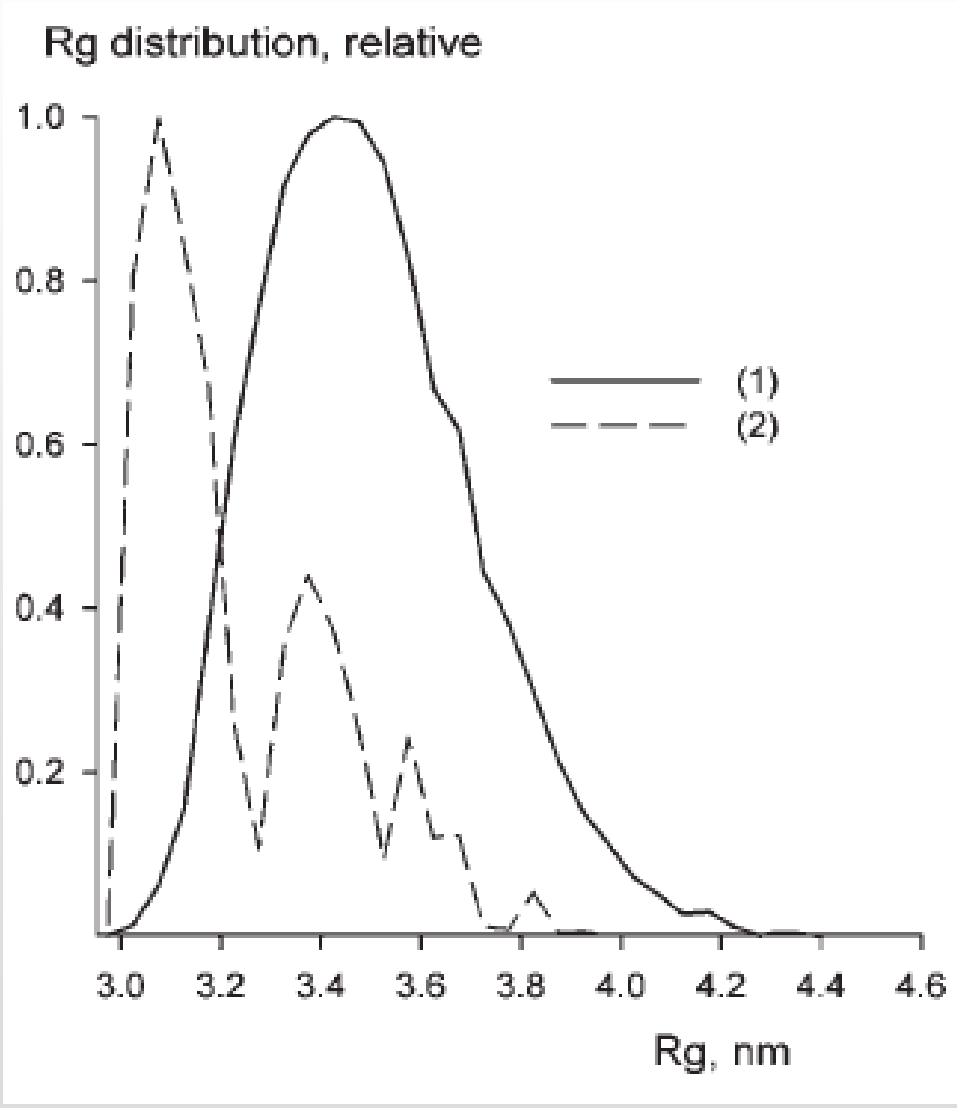
SAXS (Small-angle X-ray Scattering)



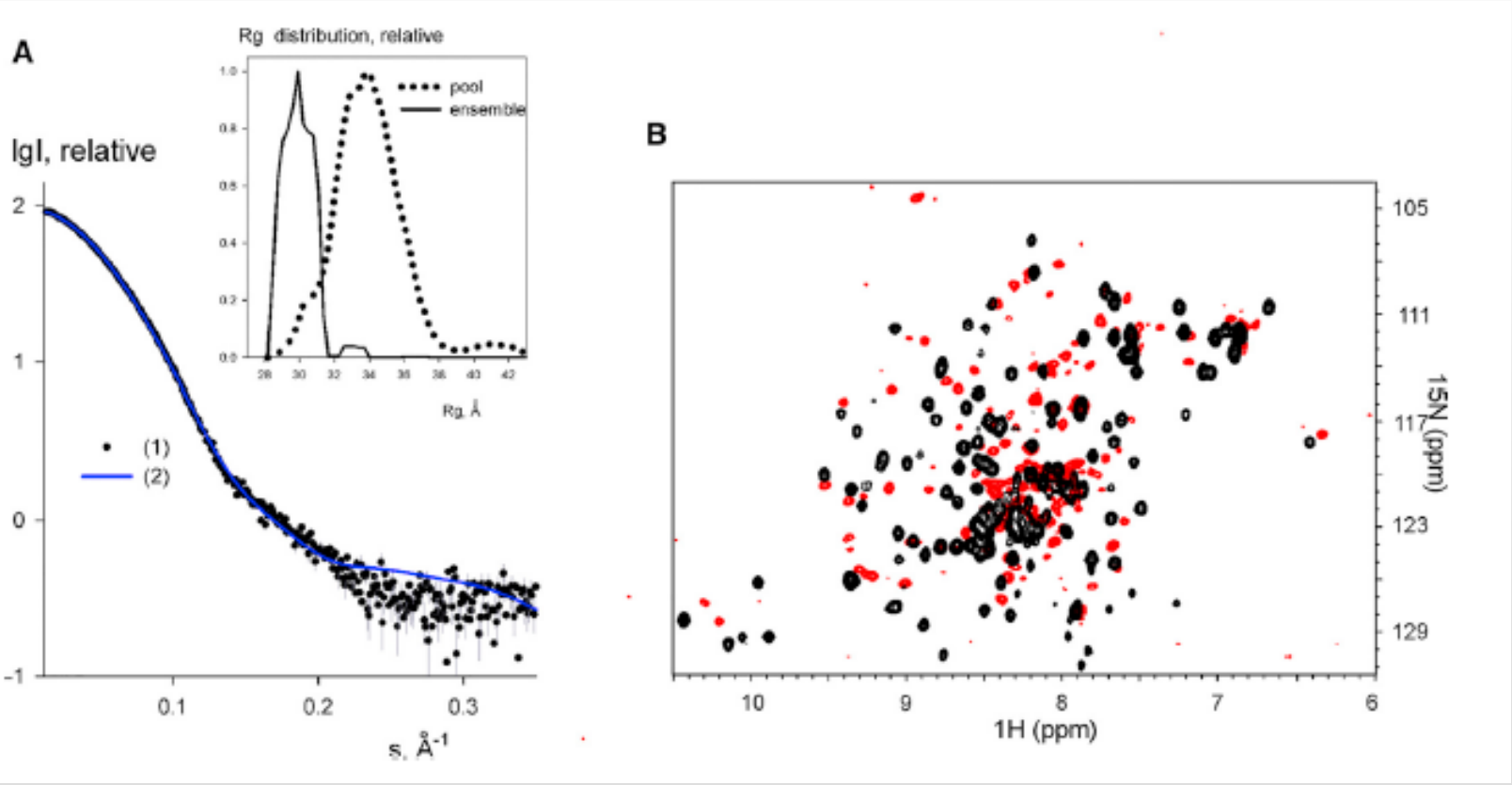


Validating the structures by SAXS data





SAXS and NMR validation



A collapsed intrinsically unfolded region

PROTEINS
STRUCTURE ■ FUNCTION ■ BIOINFORMATICS

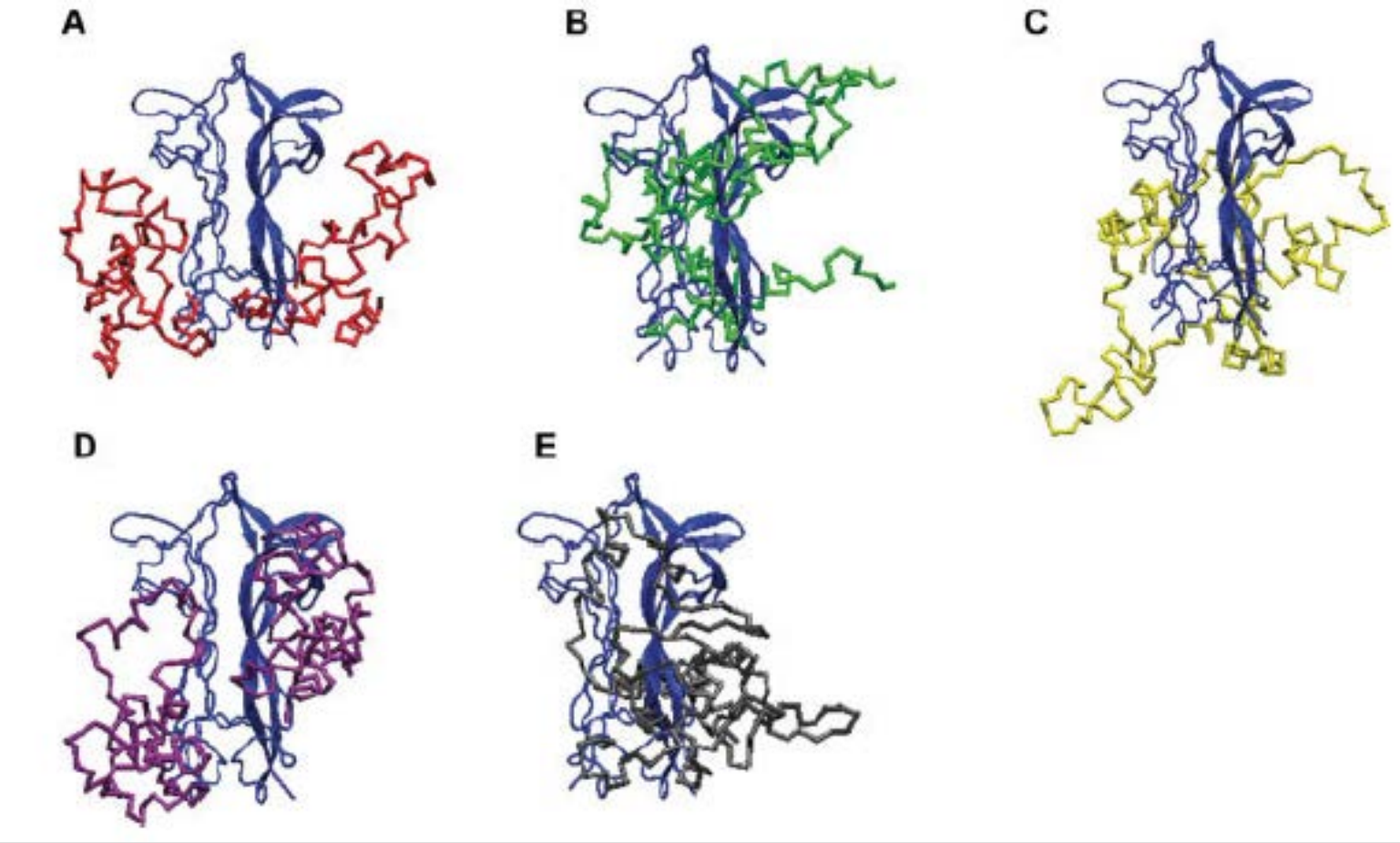
Intrinsic structural disorder of mouse proNGF

Francesca Paoletti,[1,2]* Sonia Covaceuszach,[3] Peter V. Konarev,[4,5] Stefania Gonfloni,[1,6] Francesca Malerba,[2] Elisabeth Schwarz,[7] Dmitri I. Svergun,[4,5] Antonino Cattaneo,[1,2] and Doriano Lamba[8]*

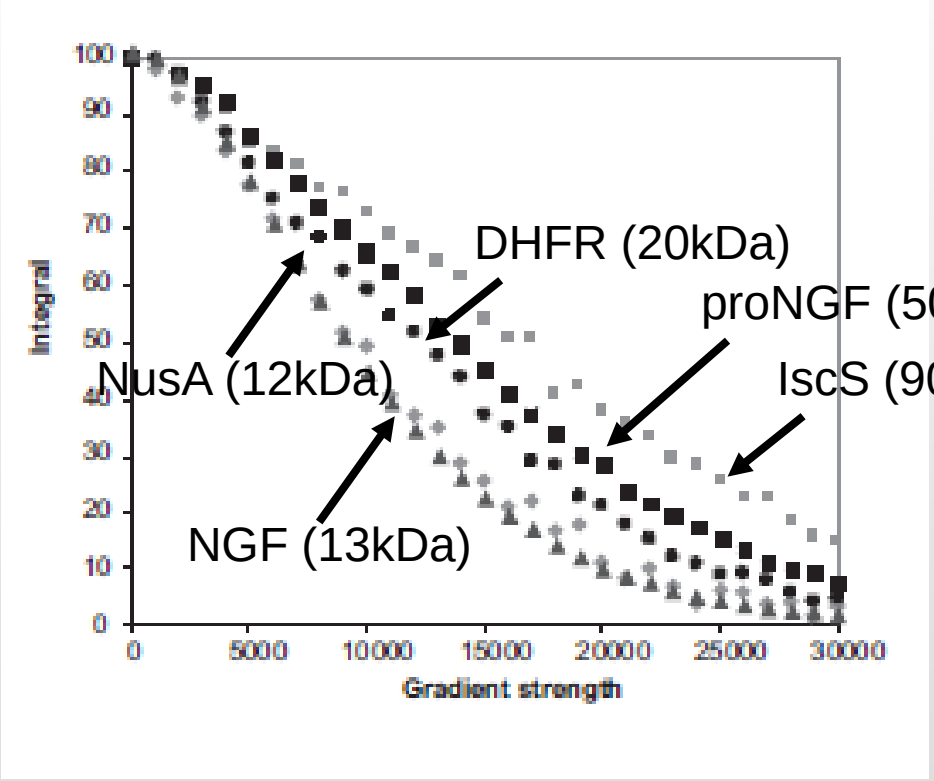
SAXS measurements revealed the proNGF is dimeric and anisometric, with the propeptide domain being intrinsically unstructured

A globular ‘‘crab-like’’ and elongated shapes equally fit the scattering data

A crab-like structure?



proNGF mostly behaves as a collapsed structure



A different approach

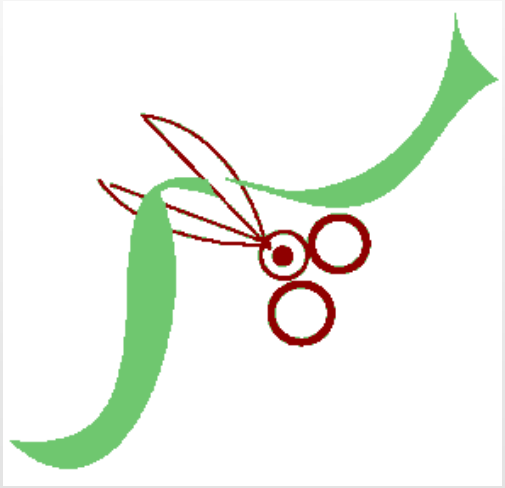
~~Plan A~~

~~Plan B~~

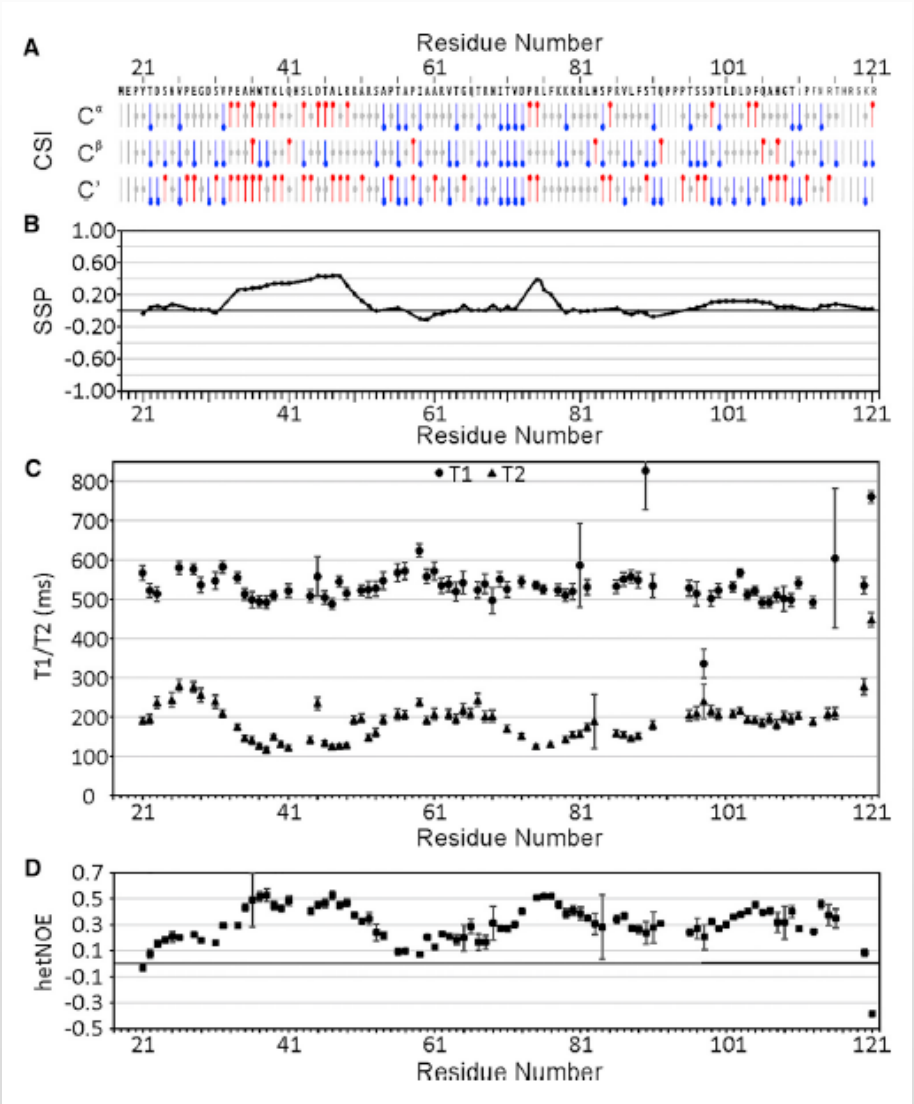
Plan C

We cut the pro-peptide (NGFpd)

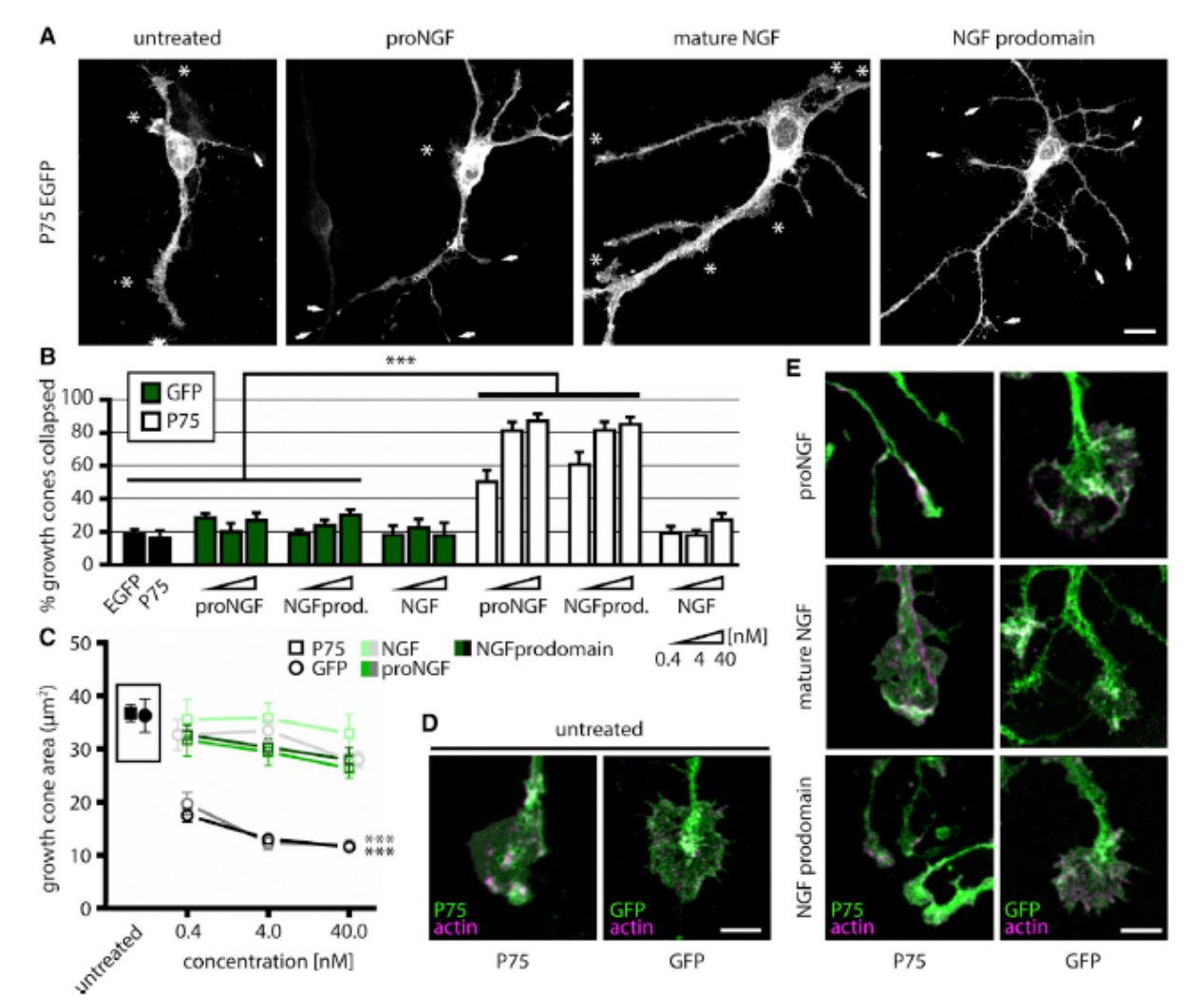
```
-121  -112        -102         -92         -82         -72
1        10          20          30          40          50
MSMLFYTLIT AFLIGVQAEP YTDSNVPEGD SVPEAHWTKL QHSLDTALRR
Signal peptide

        -62         -52         -42         -32         -22
         60          70          80          90         100
ARSAPTAPIA ARVTGQTRNI TVDPRLFKKR RLHSPRVLFS TQPPPTSSDT

        -12          -2   1        9          19          29
        110         120  122    130         140         150
LDLDFQAHGT IPFNRTHRSK RSSTHPVFHM GEFSVCDSVS VWVGDKTTAT
                          N-term                  Loop I

         39          49          59          69          79
        160         170         180         190         200
DIKGKEVTVL AEVNINNSVF RQYFFETKCR ASNPVESGCR GIDSKHWNSY
          Loop II               Loop III

         89          99         109         119
        210         220         230         240
CTTTHTFVKA LTTDEKQAAW RFIRIDTACV CVLSRKATRR G
Region IV     Loop V               C-term
```



NGFpd is unfolded with helical tendency

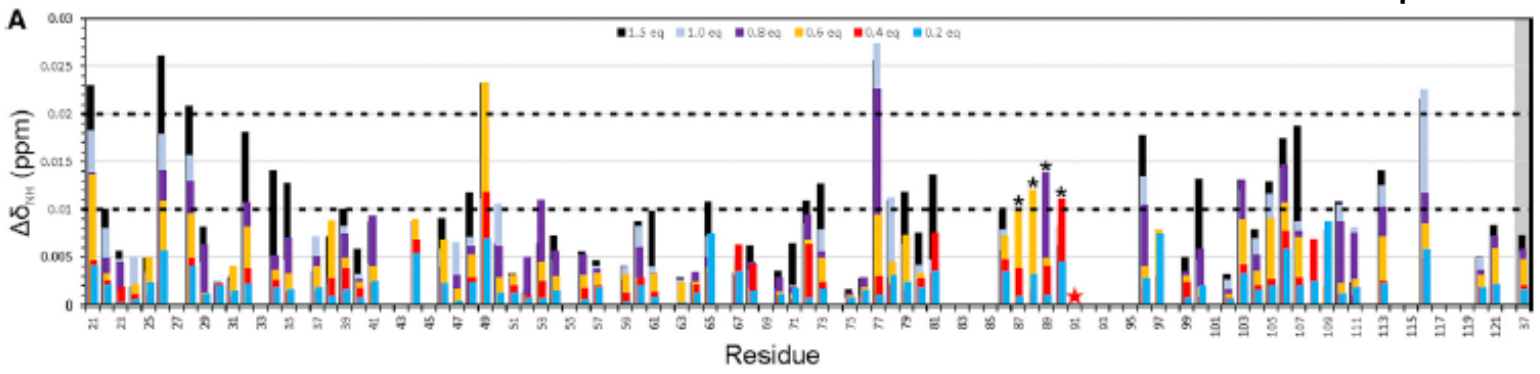


NGFpd is responsible for cone collapse

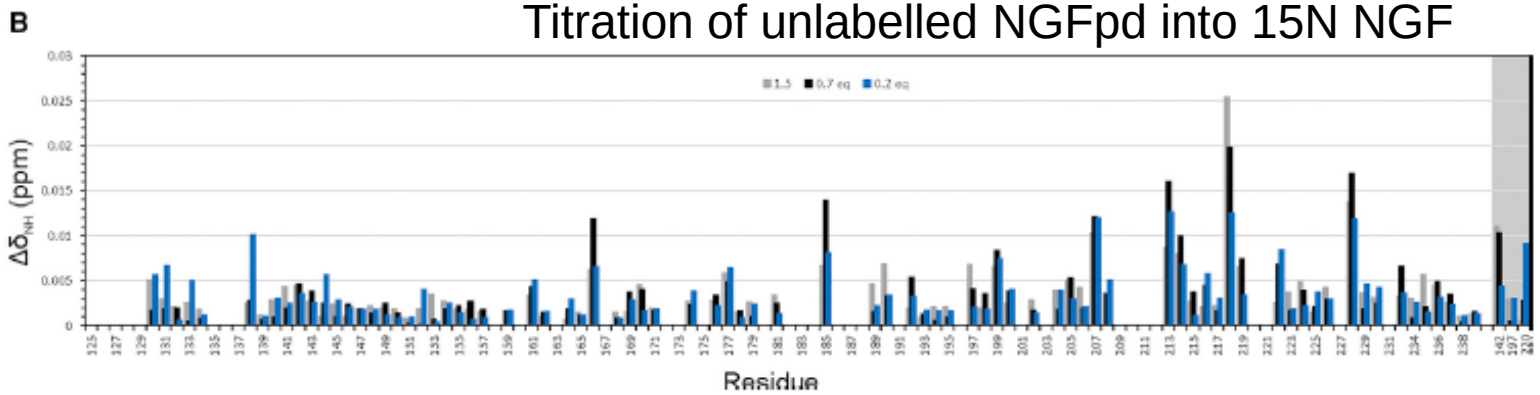


Mapping the effects of NGF on NGFpd and viceversa

Titration of unlabelled NGF into 15N NGFpd

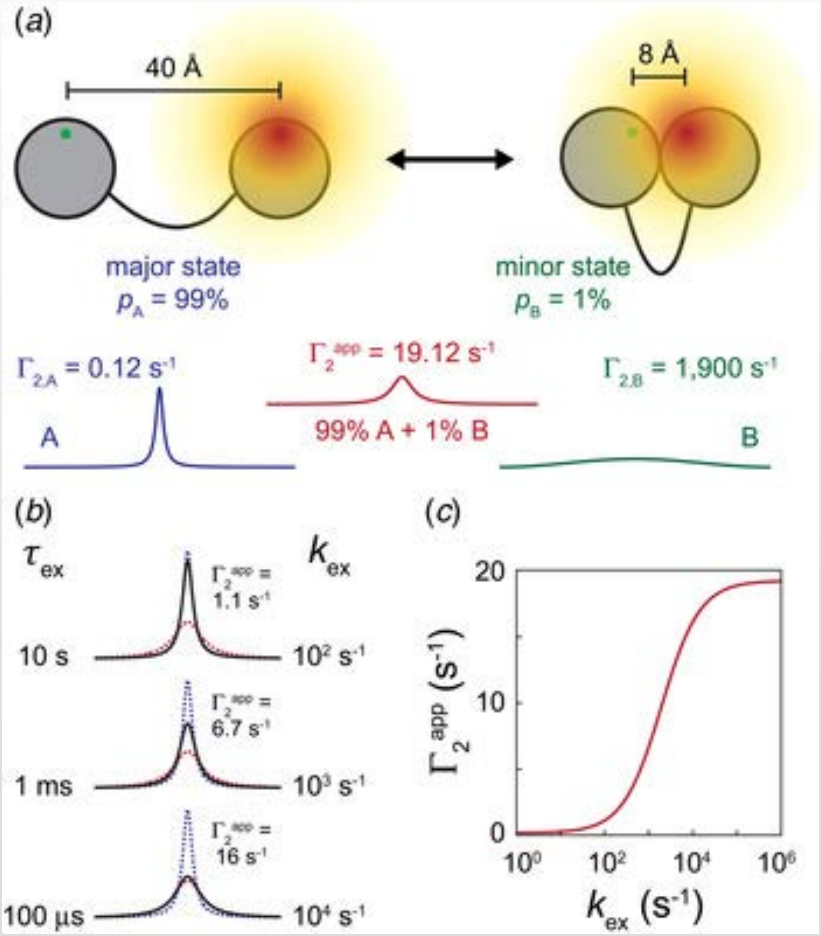


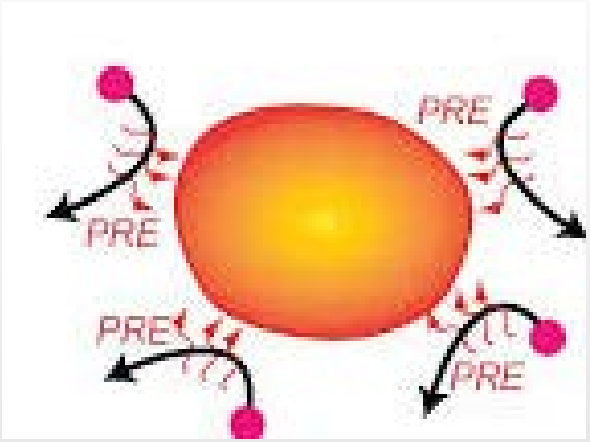
Titration of unlabelled NGFpd into 15N NGF



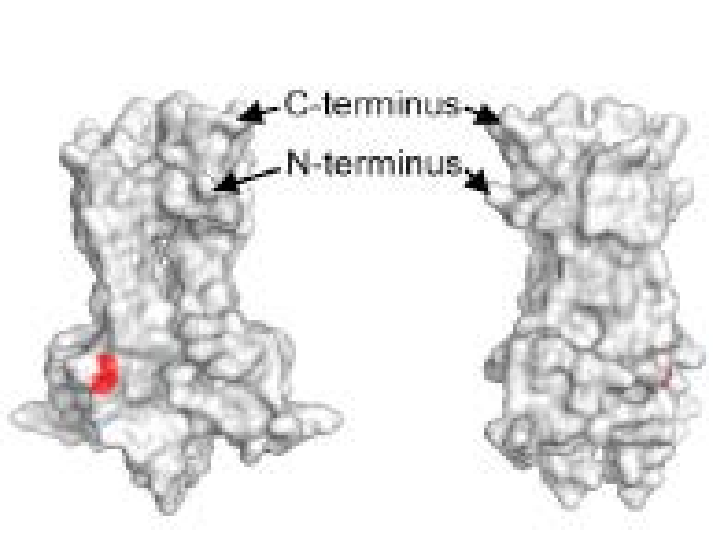
The individual two halves interact even though weakly

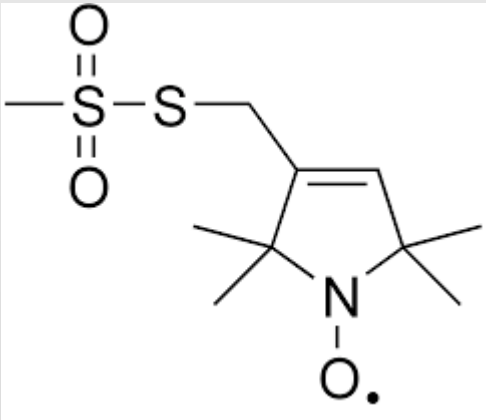
Paramagnetic relaxation enhancement

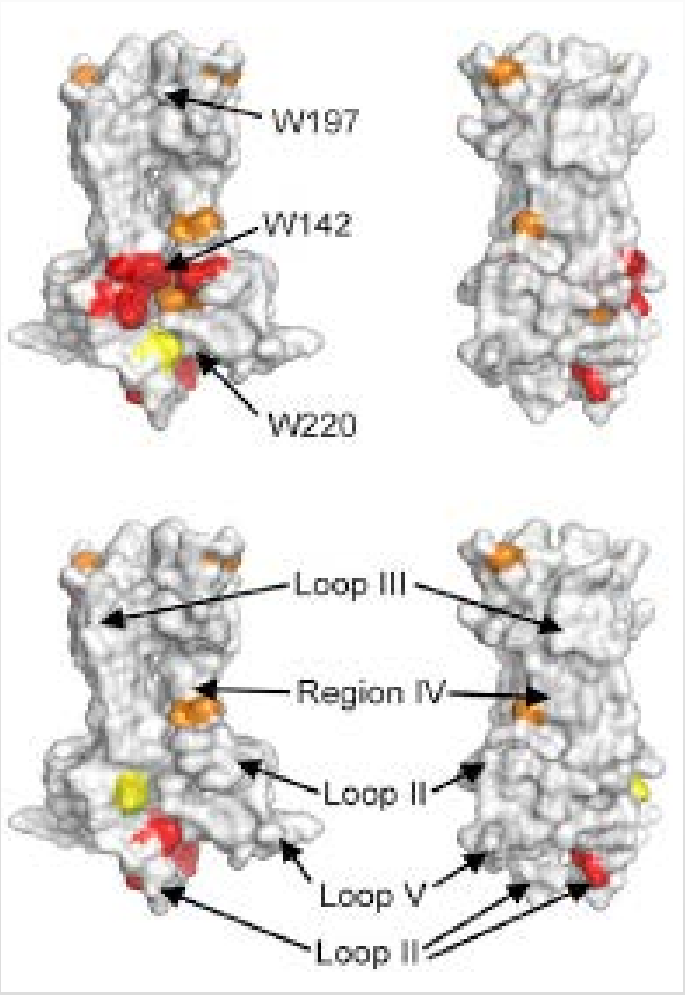




Mapping the surface of interaction

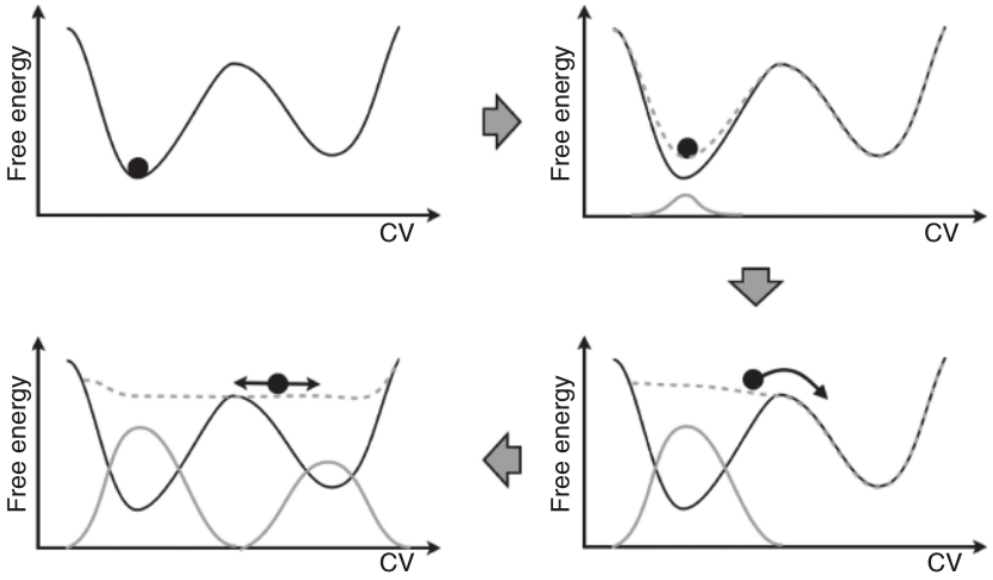


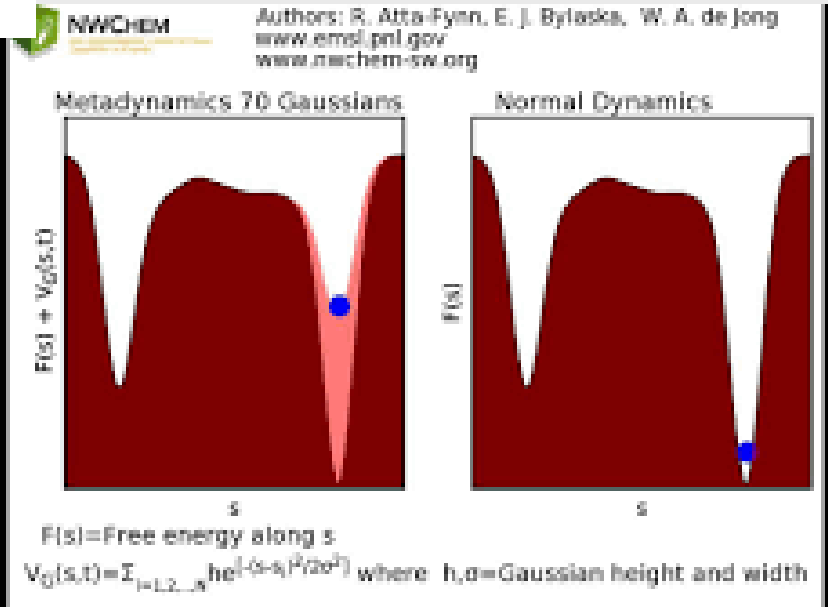




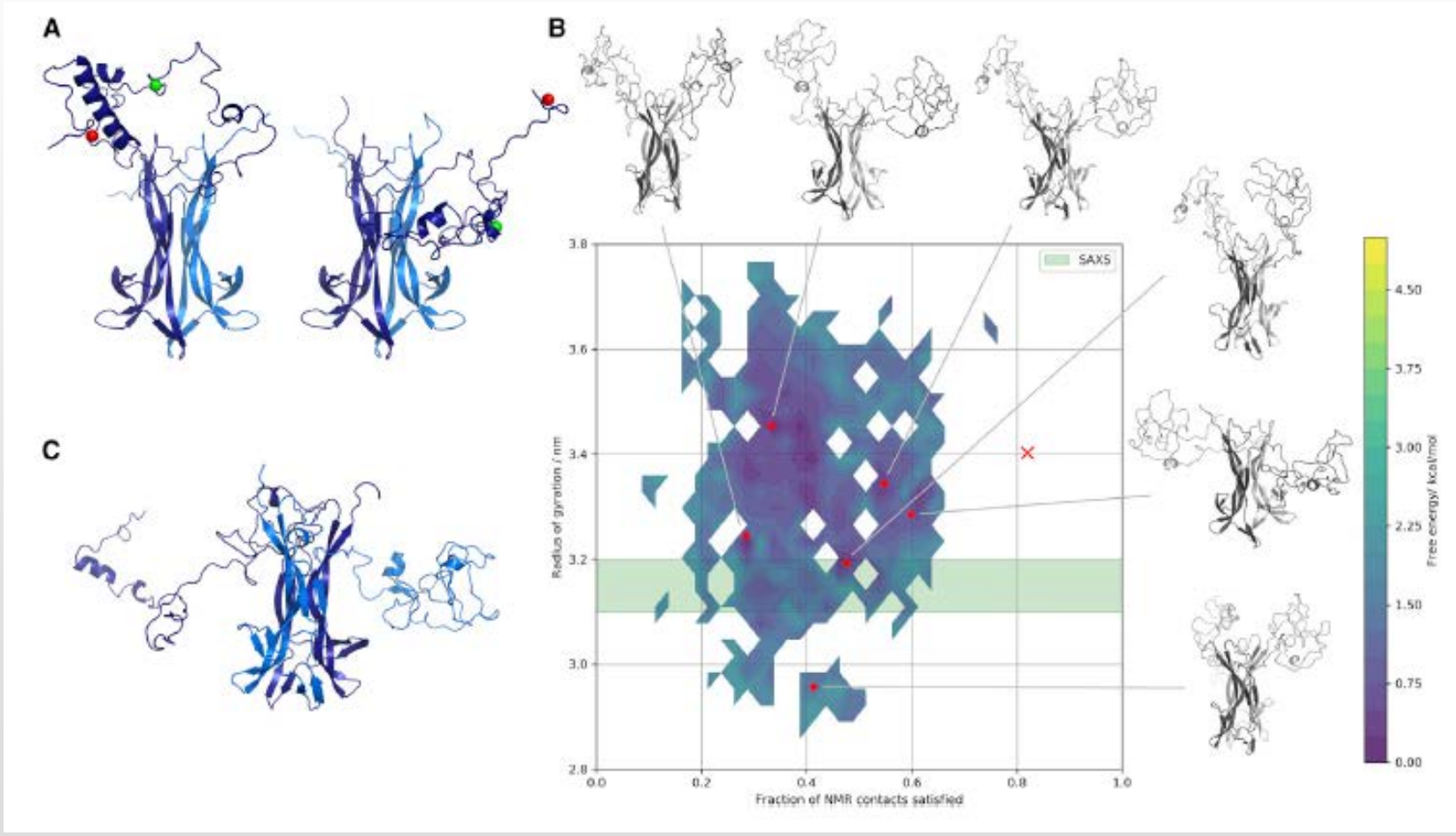
MTSL
(*S*-(1-oxyl-2,2,5,5-tetramethyl-2,5-dihydro-1H-pyrrol-3-yl)methyl methanesulfonothioate)

Restrained metadynamics





Using SAXS to restrict the MD results



Conclusions

ProNGF has distinct properties from NGF

The pro-domain has function of its own

The pro-domain is unstructured but Collapses on NGF to produce a flexible semi compact structure

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A pact of friendship...

NMR

SAXS