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INTERNATIONAL WORKSHOP ON PROTEOMICS: PROTEIN STRUCTURE, FUNCTION AND INTERACTIONS

(5 - 16 May 2003)

"Misfolding diseases and aggregation"

presented by:

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Structural hypotheses for understanding poly-glutamine diseases

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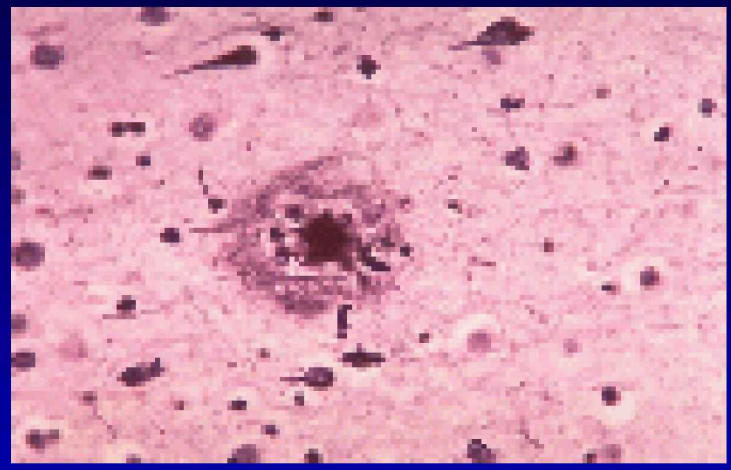
NIMR, London

A new family of diseases

-Alzheimer'disease
-Prion diseases
-Poly-glutamines
-Parkinson's disease
-Tauopathy
-Familial amyotropic lateral schlerosis

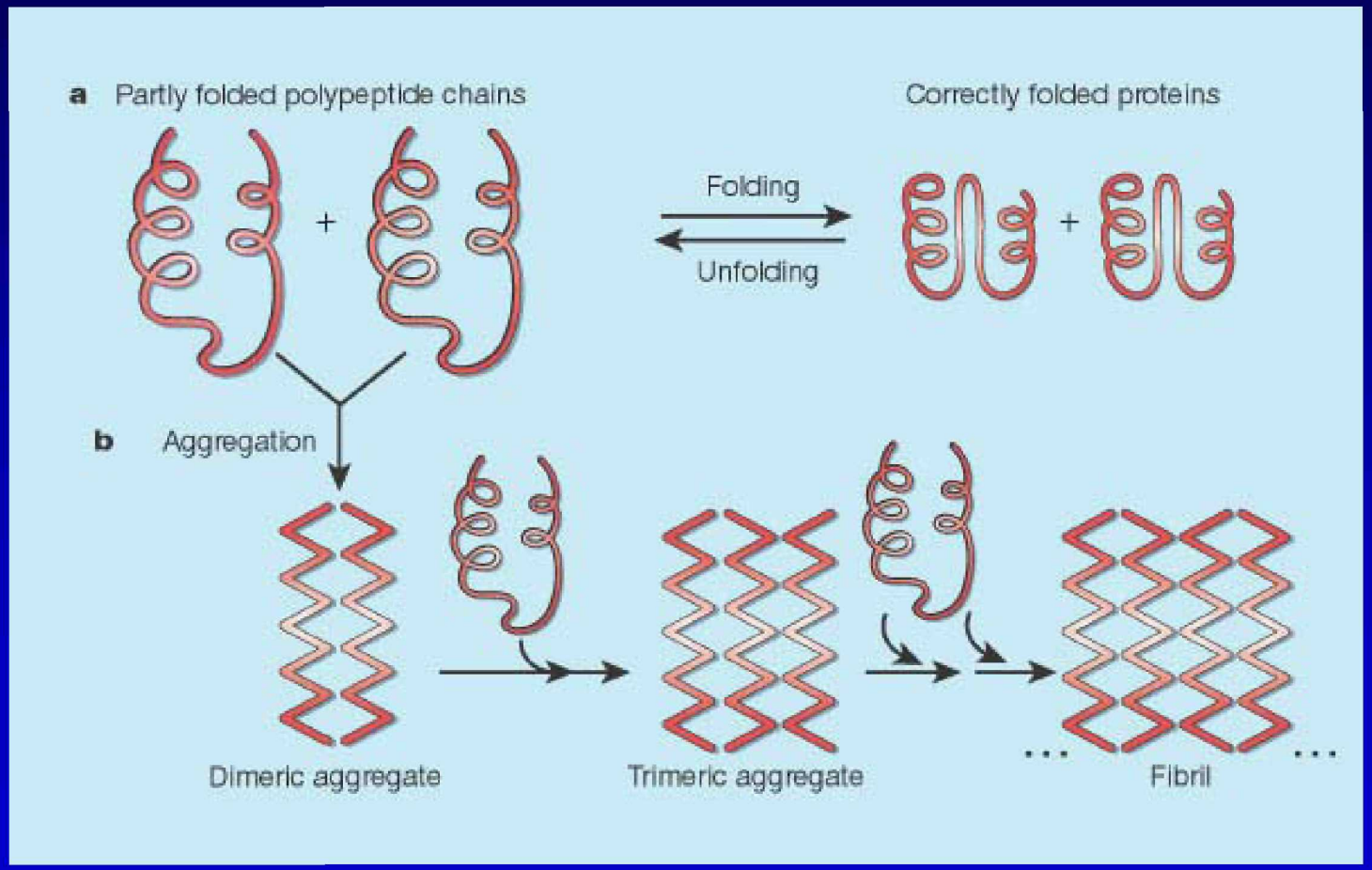
All associated with
toxic aggregation and protein misfolding

Aging and Alzheimer

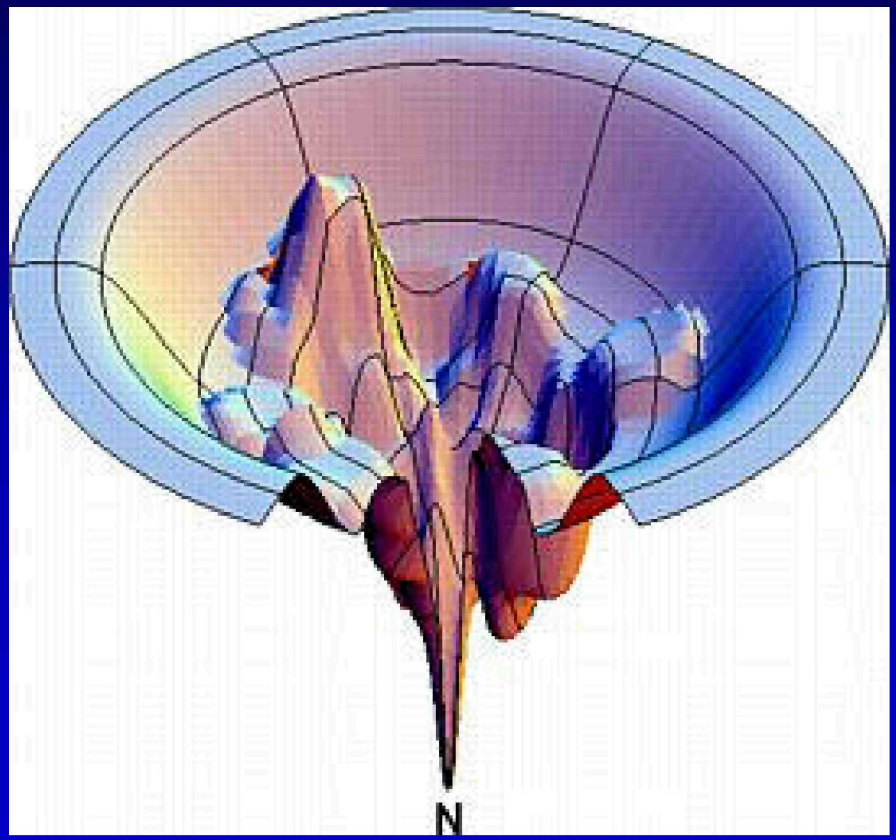


Alzheimer was of a German professor of Psychology in Breslau. Together with Franz Nissl they established the pathologic anatomy of mental illness. Alzheimer published several treatises on cerebro arteriosclerosis in 1904 and on Huntington's chorea early in 1911. In 1907 appeared the monumental work on Alzheimer's Disease for which he will always be remembered.

Misfolding and aggregation

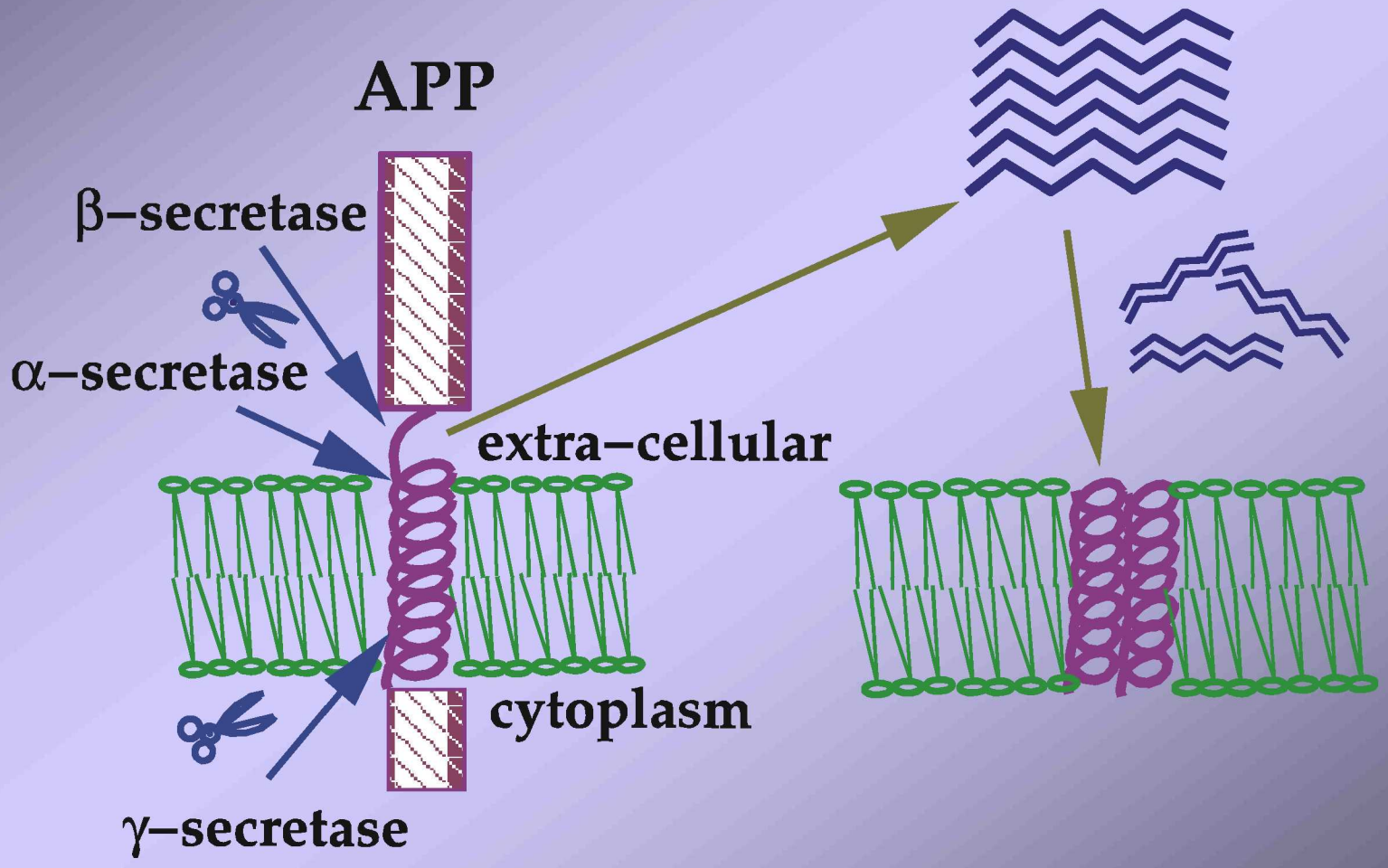


Energy funnels

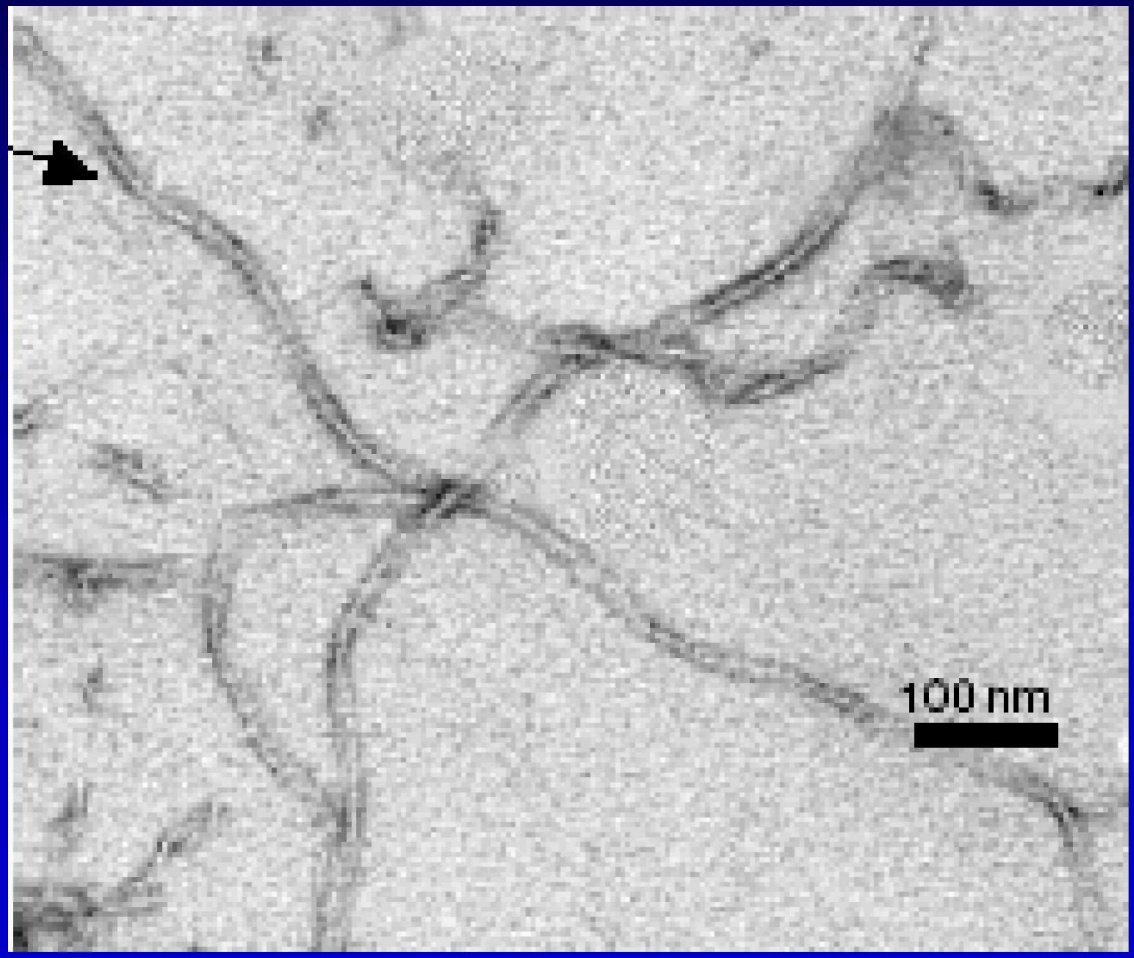


Protein misfolding and diseases

Misfolding diseases: Alzheimer



Amyloid fibers



How to study the insoluble?

X-ray
NMR of liquids

amorphous material
insoluble in water

Electron microscopy
Infrared spectroscopy

NMR of solids
Fiber diffraction

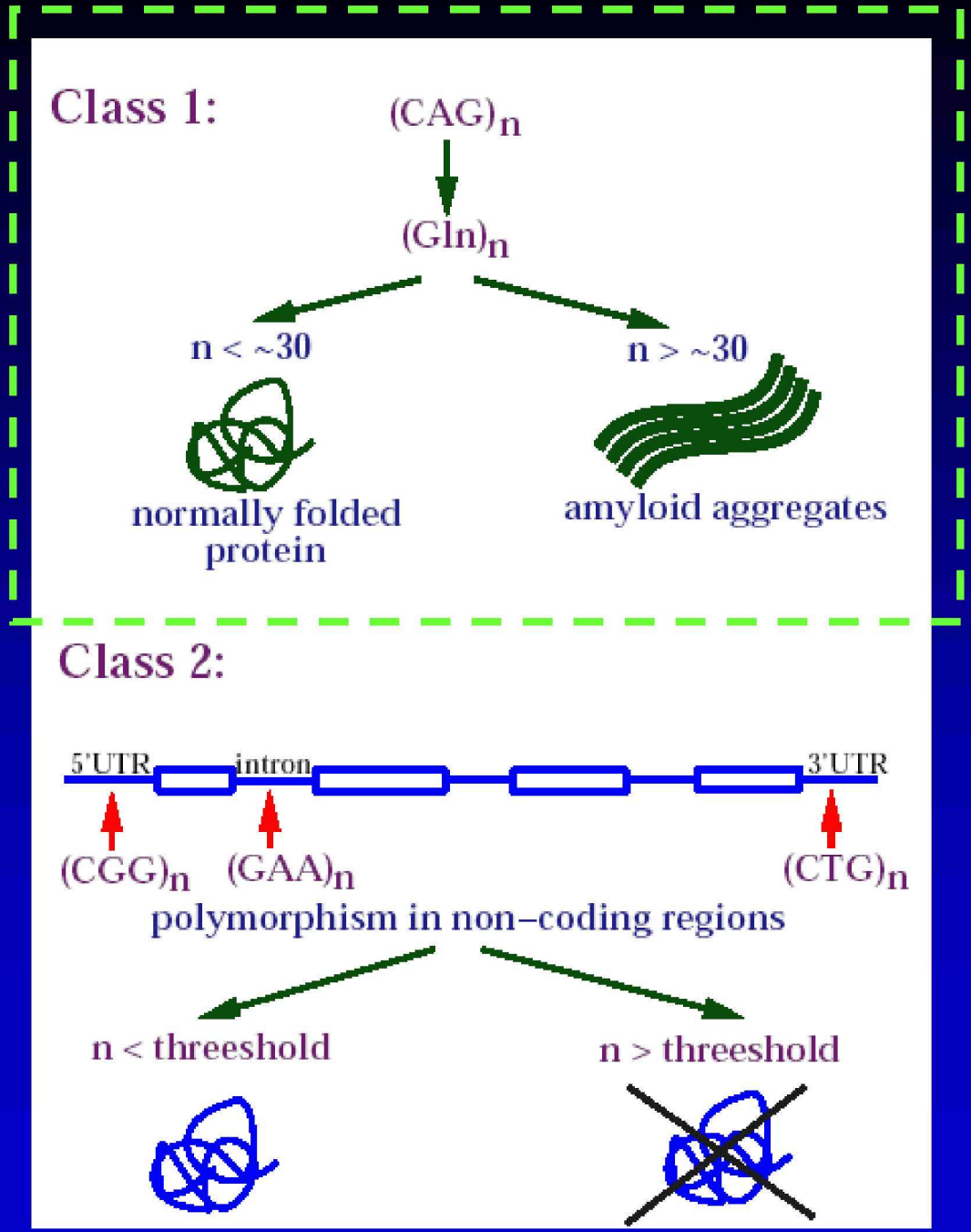
A different approach!

A new family of diseases

-Alzheimer'disease
-Prion diseases
-Poly-glutamines
-Parkinson's disease
-Tauopathy
-Familial amyotropic lateral schlerosis

All associated with
toxic aggregation and protein misfolding

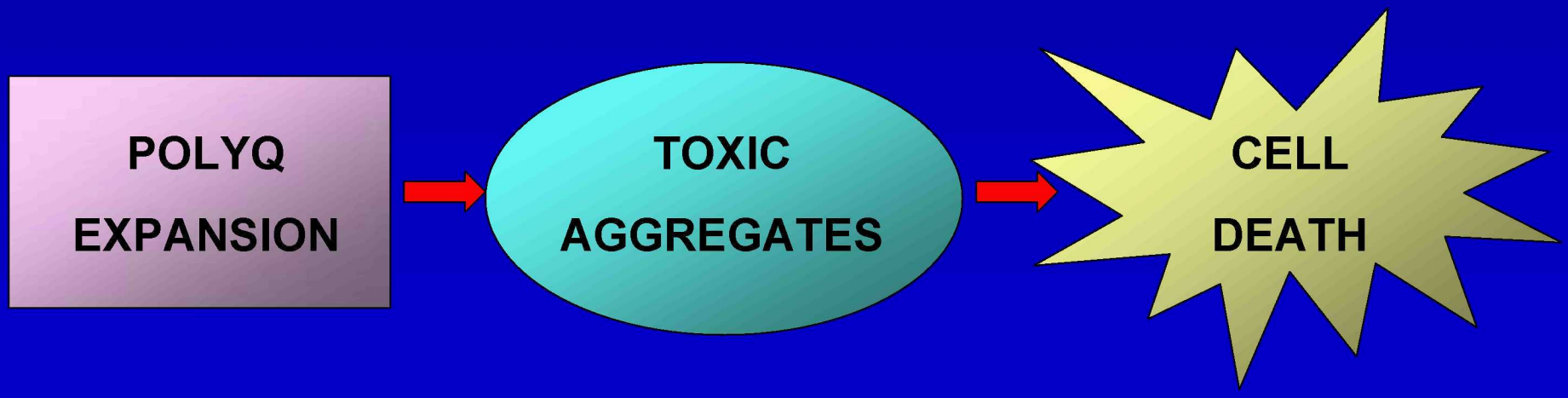
TRINUCLEOTIDE DISEASES



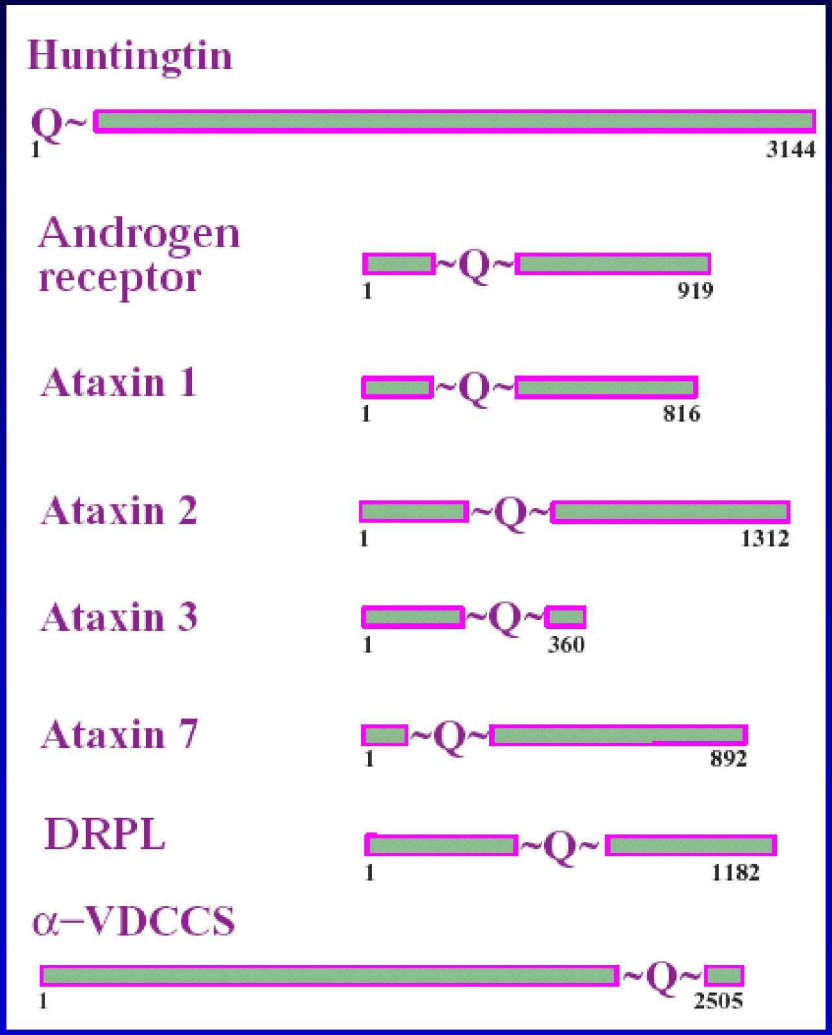
POLYGLUTAMINE DISEASES

- Progressive neuronal disfuctions
- Pathogenic THRESHOLD ~ 35 glutamines
- Nuclear Inclusions → fibres (amyloids)

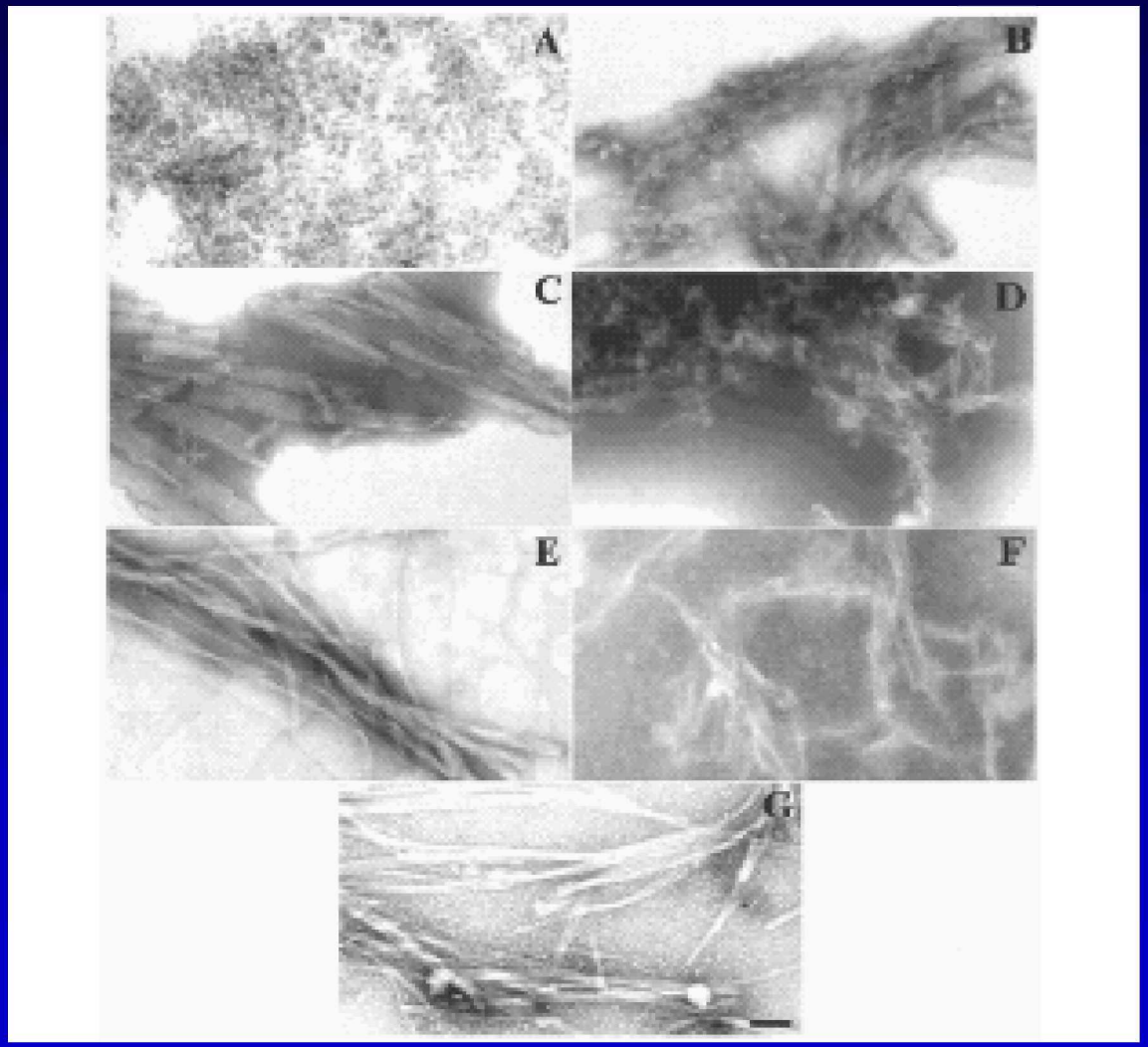
COMMON DISEASE MECHANISM?



An increasing number of polyQ proteins is associated to human diseases



Various types of fibers…



Polyglutamine proteins

- No sequence homology
- Different size
- Poly-Q stretches at different positions
- Different cellular localization
- Different functions

- Polyglutamine proteins are unrelated except for polyQ

A special feature of poly-Q diseases

The length of poly-Q correlates with the age of onset:

QQQQQQQQQQQQ → **Disease onset**

The longer… **… the younger**

POLYGLUTAMINE AGGREGATION

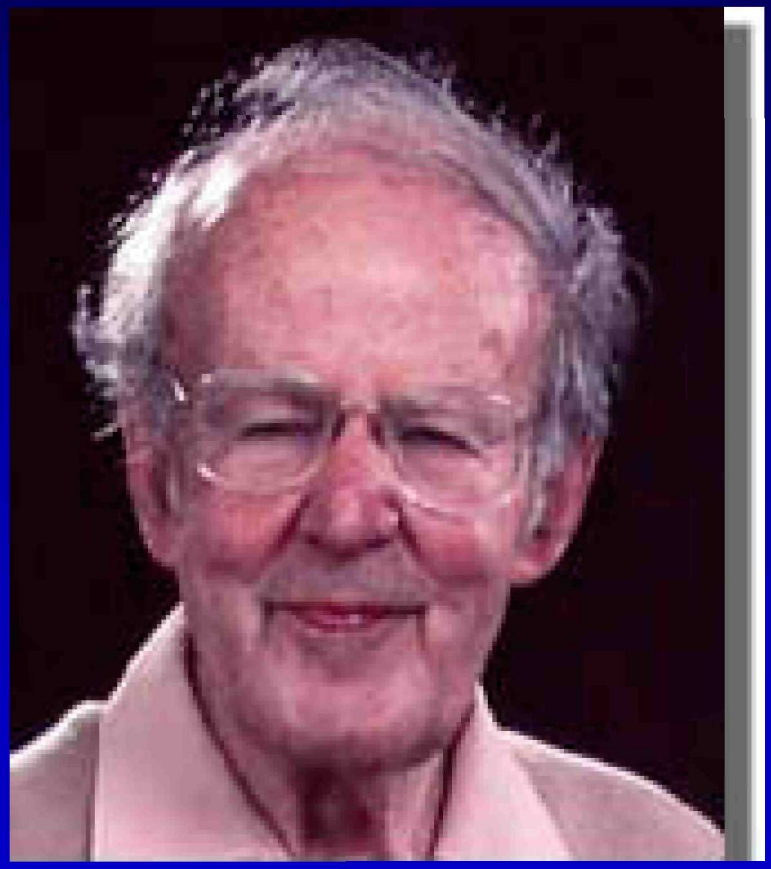
E. Wanker

Aggregation *in vitro*

- **is self-driven**
- **is independent on a specific protein**
- **depends on**
  - **repeat length**
  - **concentration**
  - **time**

POLYQ EXPANSION → **CONFORMATIONAL CHANGE** **?**

WHAT IS SPECIAL ABOUT POLY-Q?



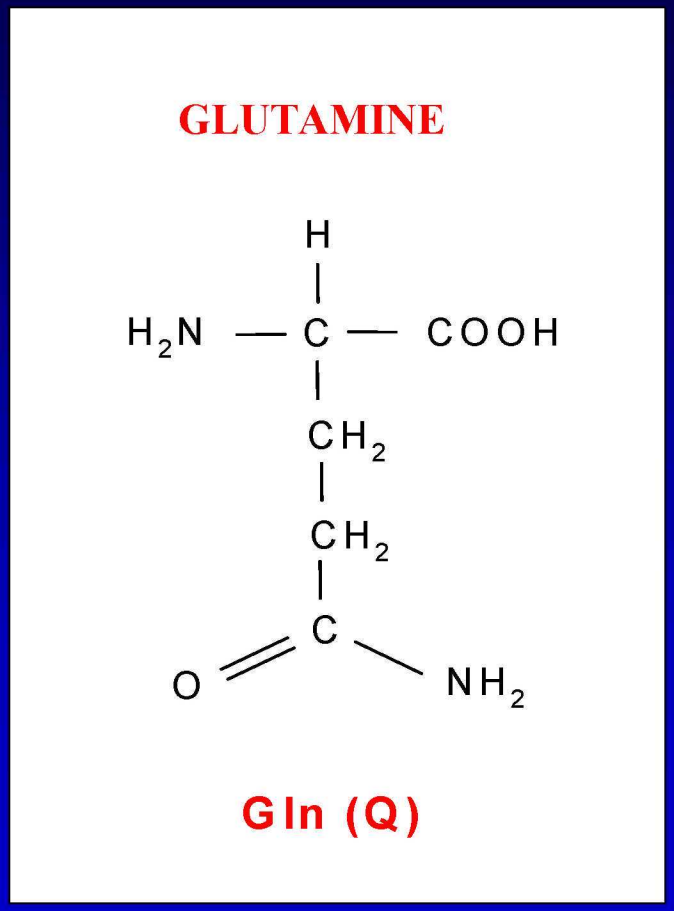
MAX PERUTZ

- Poly-Q stretches (>20Q) have been found in several proteins (>60)

- Many are transcription activators

- No known structure contains more than 10 tandem Q

PERUTZ's MODEL

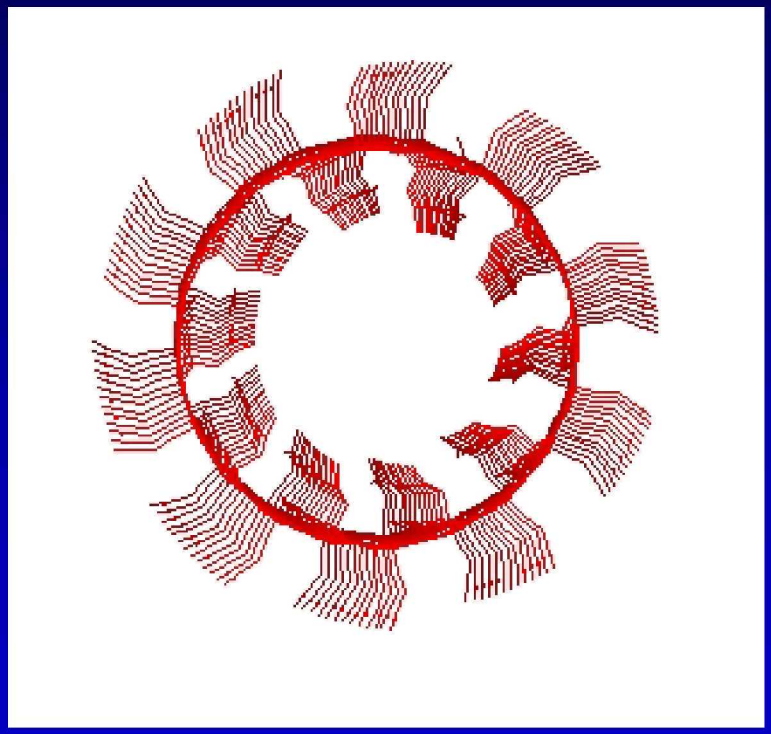


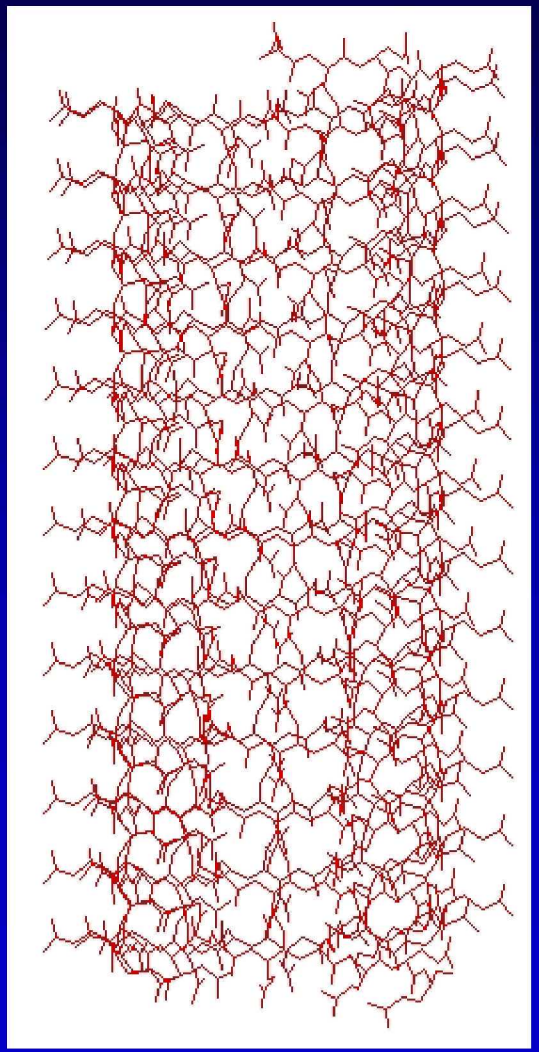
PolyQ form **POLAR ZIPPERS**

antiparallel **β-SHEETS**

held by **hydrogen bonds** between main-chain and side-chain amides

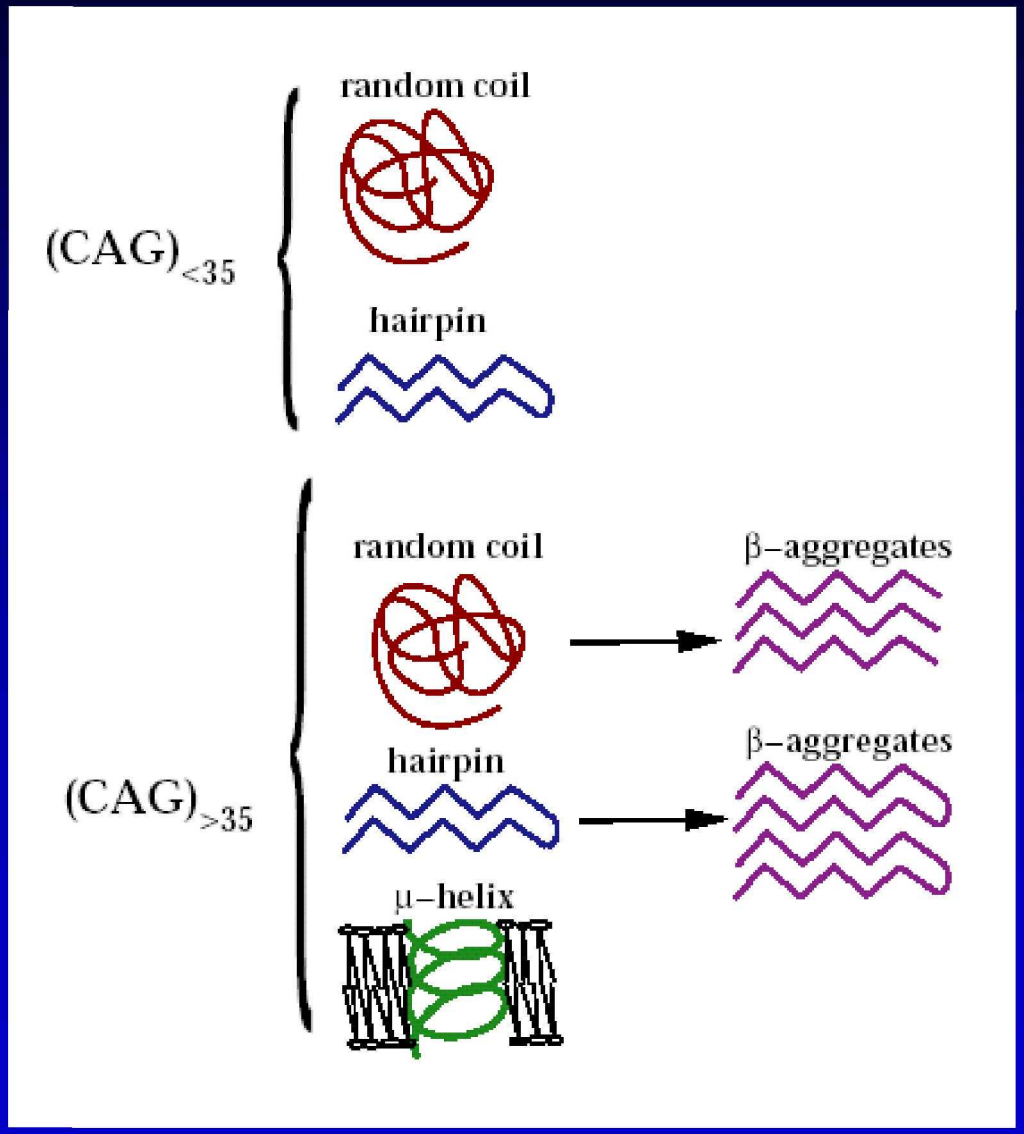
Model of water-filled nanotubes





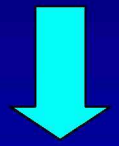
Perutz et al. (2002)

Other proposed models…



The difficulty of validating the models

- Poly-Q peptides are insoluble in water
- Difficult expression of polyQ proteins



peptide models

- Artificial short tails (e.g. Asp2GlnnLys2)
- Studies in extreme pH and/or solvent conditions

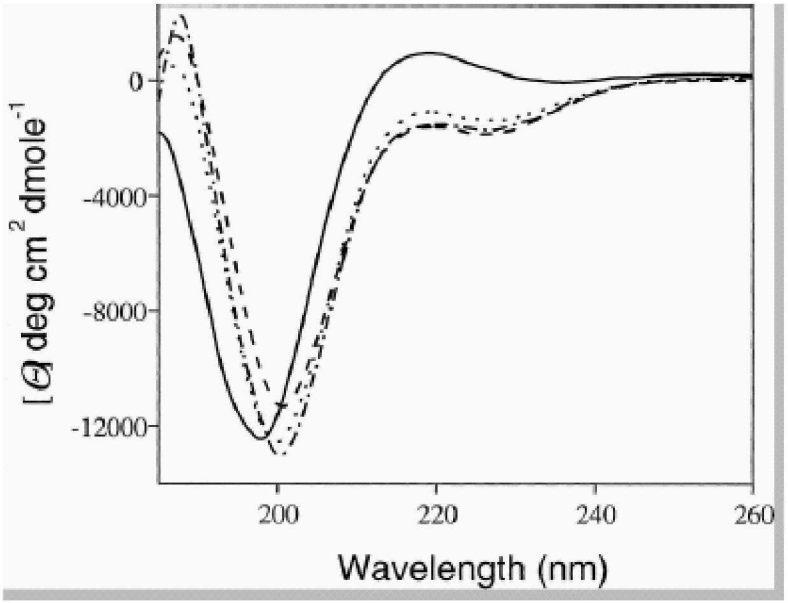
Random coil or β structures?

Mostly by CD

Some FTIR

Almost no NMR

Poly–Q peptides have been shown to be both in random coil and in β structures



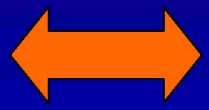
Perutz (1994) Pnas 91, 5355–5358.

Altschuler et al. (1997) J.Pept.Res. 50, 73–75

Sharma et al. (1999) Febs Lett. 456, 181–185

Chen & Wetzel (2000) Prot. Sci. 10, 887–891.

Necessity of model systems

Animal models  Biophysical models

Our approach:

```
MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL
EFPNLPYYID GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL
DIRYGVSRIA YSKDFETLKV DFLSKLPEML KMFEDRLCHK TYLNGDHVTH
PDFMLYDALD VVLYMDPMCL DAFPKLVCFK KRIEAIPQID KYLKSSKYIA
WPLQGWQATF GGGDHPPKDH PPKSDLVPRG SXEFPGRLER PHRD
```

GST — X=P

GST ~Q22 — X=MSLKP(Q)$_{22}$PPPA

GST ~Q41 — X=MSLKP(Q)$_{41}$PPPA

To use a well characterised protein to solubilise poly-Q

GST

Aim of the work

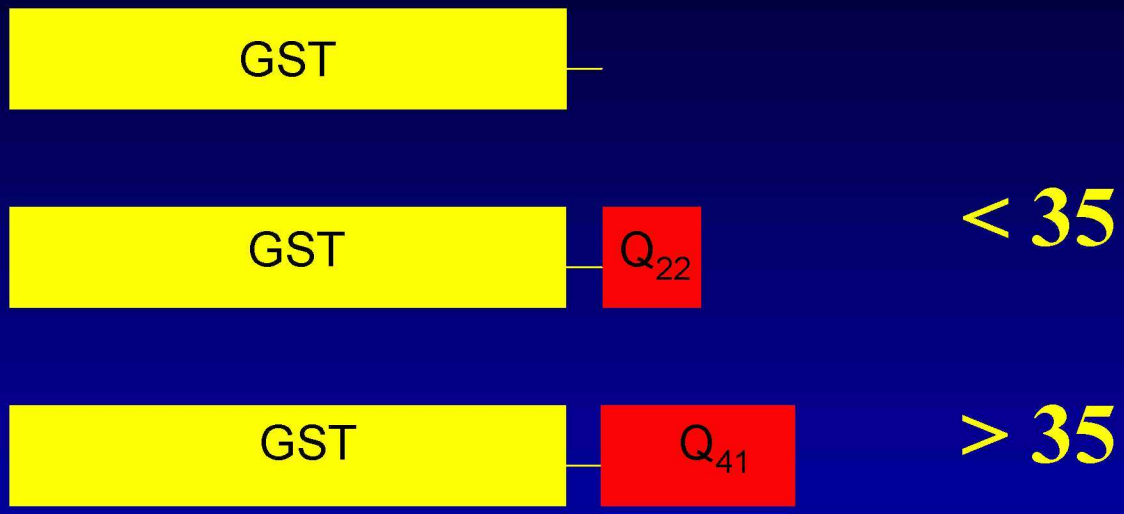
- PolyQ **structure** { α, β, random coil ? / flexible? / exposed to solvent? }
- **Differences** between Q22 and Q41 ?
- **Protein** context
- **Aggregation** properties

GST is a mostly α-protein

The structure of GST
(1gne.pdb)

helices turns
sheet insert

Our model system



Proteins **expressed in E. Coli**

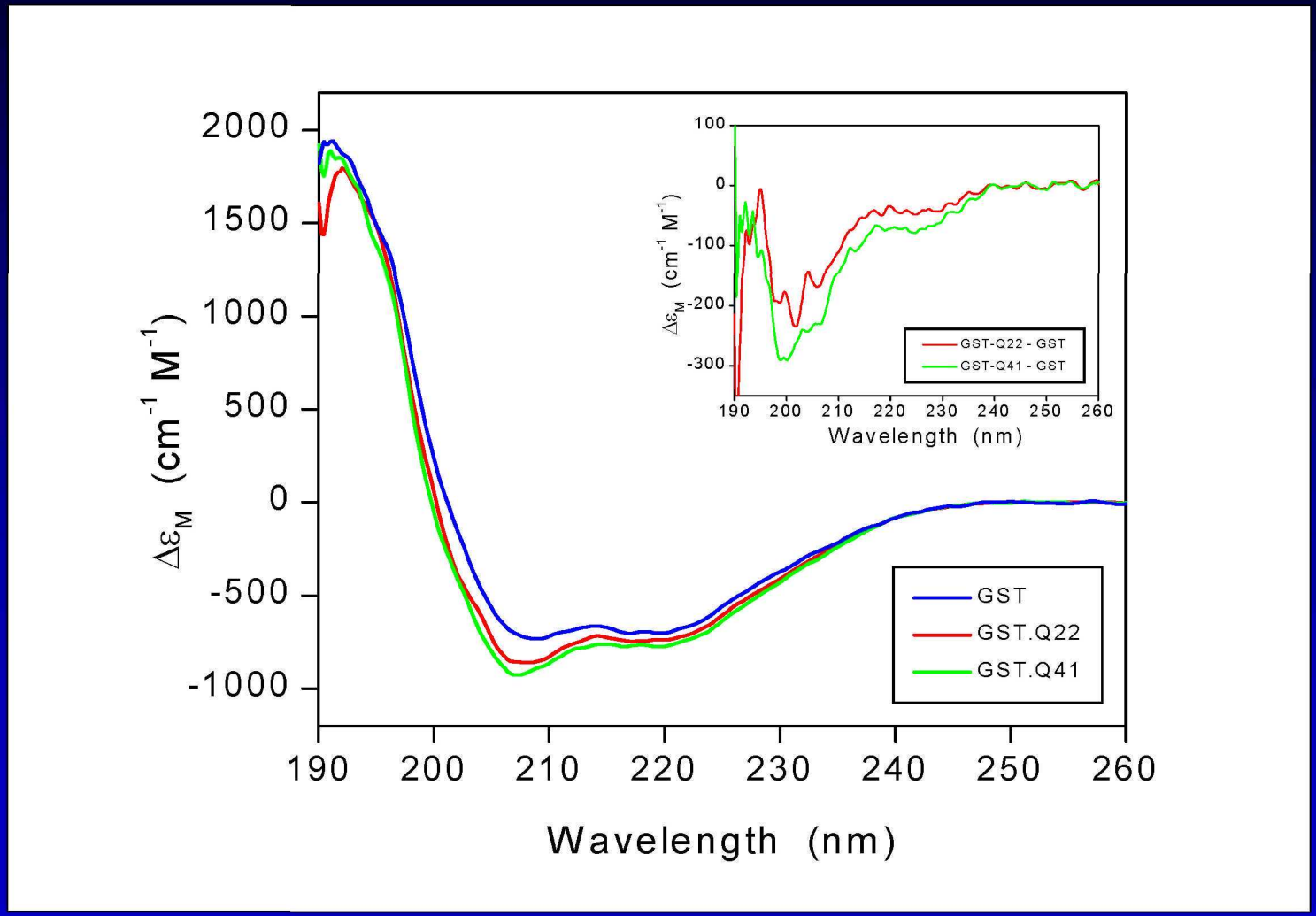
high yields

fully soluble

Behaviour of polyQ within PROTEIN CONTEXT

FAR-UV CD SPECTRA

poly-Q secondary structure ?



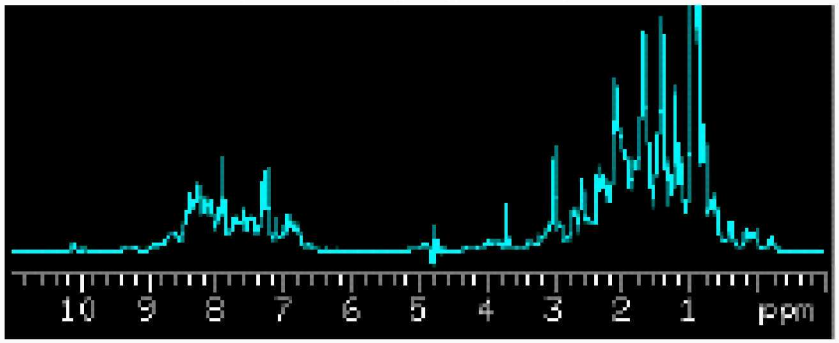
POLY-Q are in RANDOM COIL

NMR is an ideal tool to study protein fold

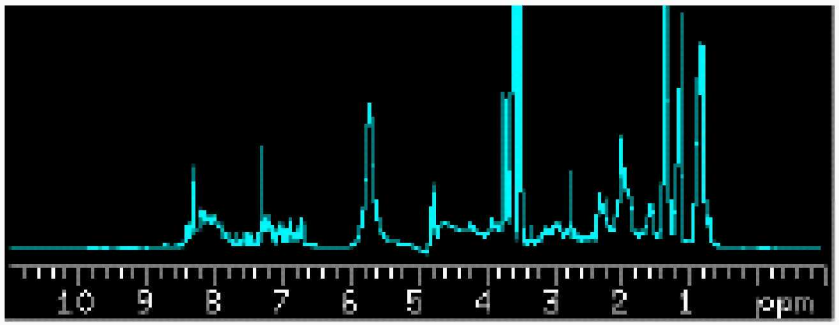
– probing the fold

NMR can give us the degree of folding (folded, partially folded, unfolded)

^1H spectrum of a folded protein



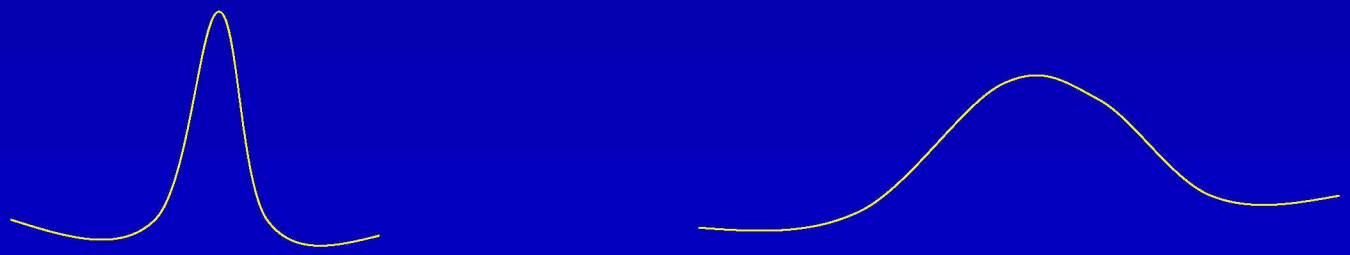
^1H spectrum of an unfolded protein



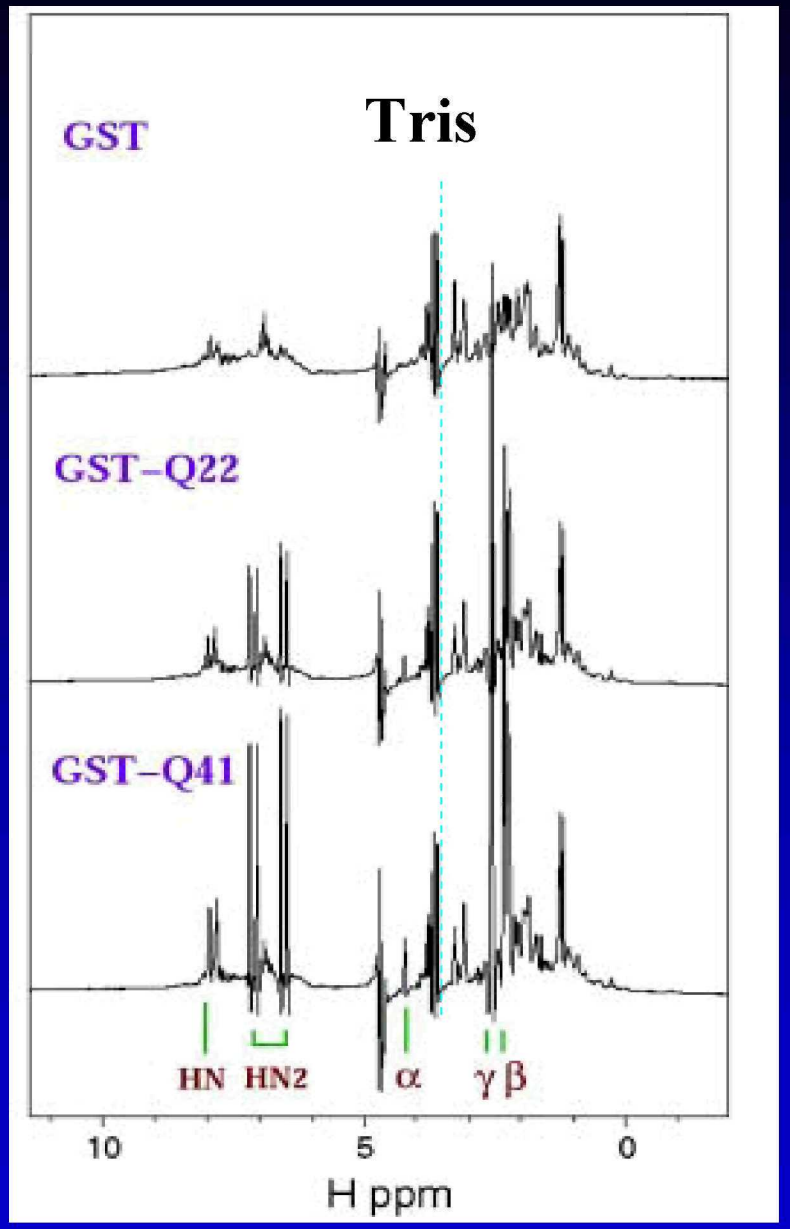
NMR studies of GST-Qs

NMR is tricky on GST: GST is a large protein (ca. 20 Kda x 2)

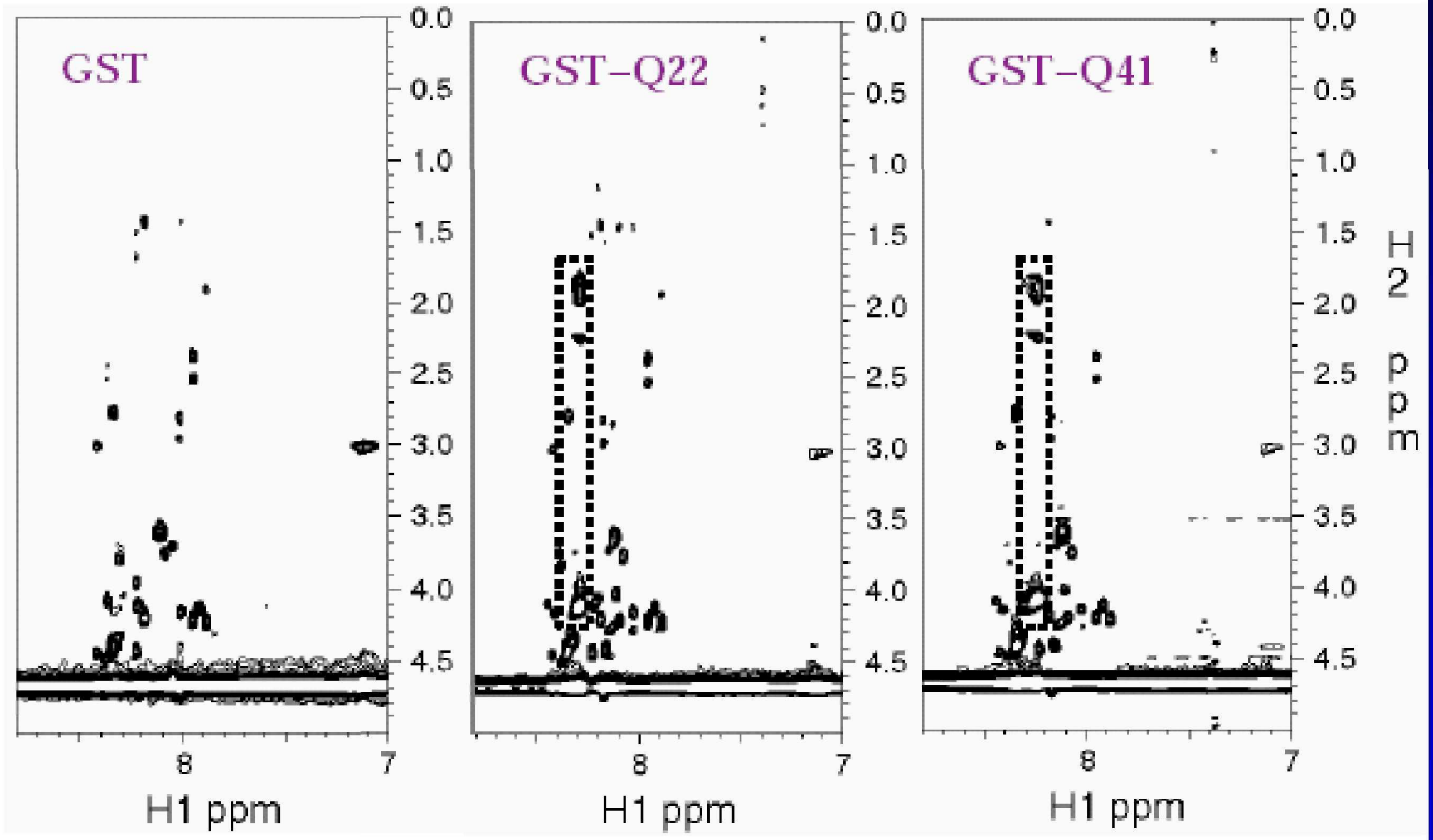
The NMR linewidth is proportional to the size



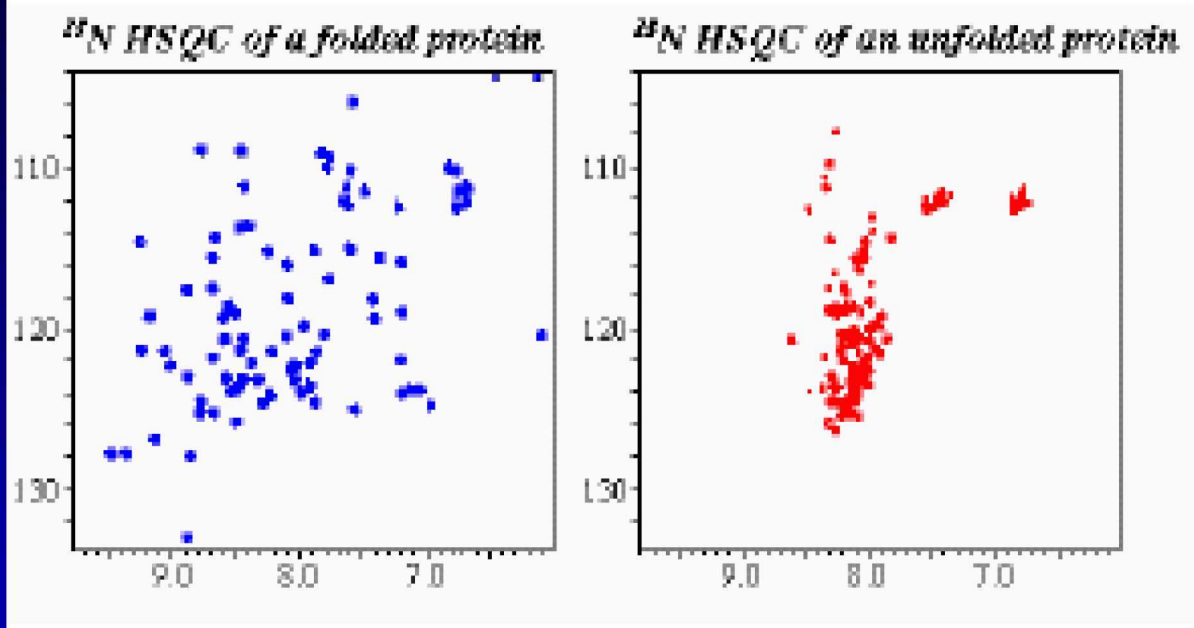
1D NMR SPECTRA



Homo-nuclear Tocsy experiments



– probing the fold

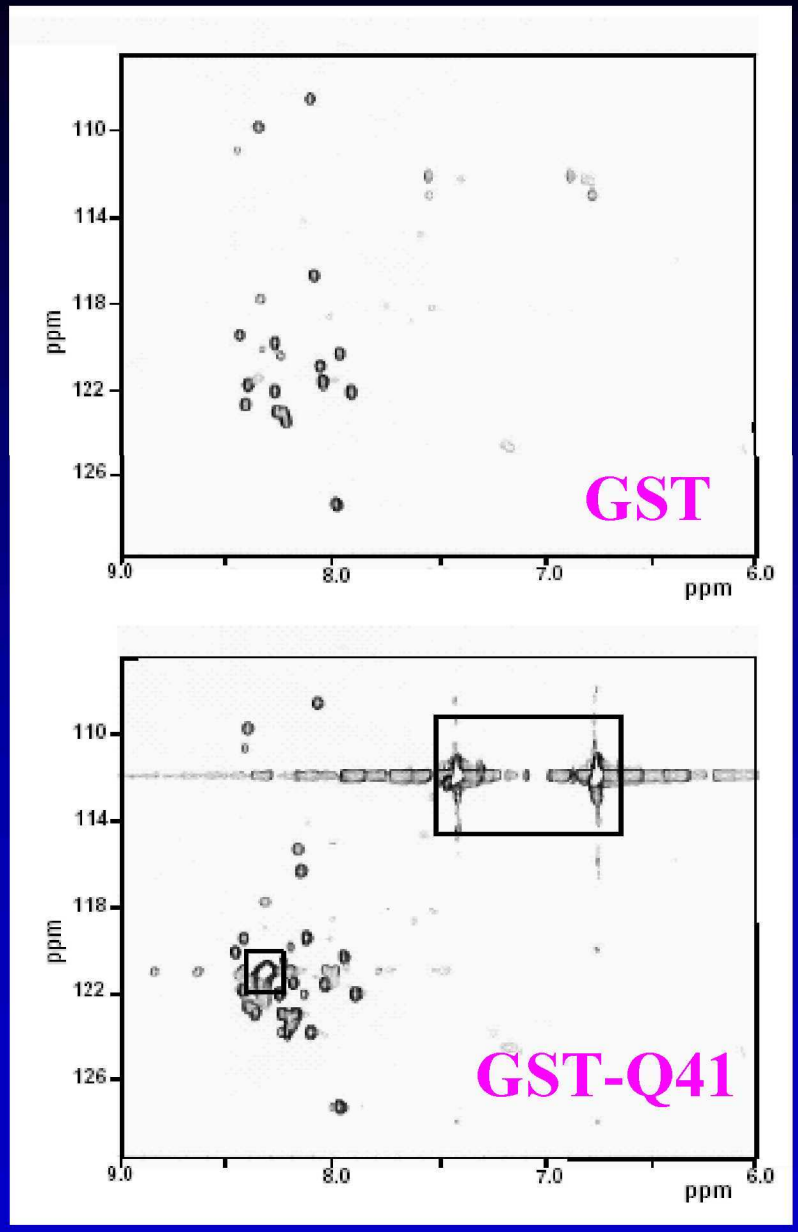


The spectra of unfolded proteins are characterised by a massive collapse of the resonances

Heteronuclear 15N spectra

T2 filtered

All glutamines are equivalent



NMR experiments on poly-Qs

- 1 and 2D homonuclear experiments
- 15N and 13C HSQC
- HNCO
- water saturation experiments
- diffusion experiments

Glutamine chemical shifts

	GST-Q22	GST-Q41	R.C.
Hα	4.25(0.02)	4.23(0.02)	4.37(0.2)
C’	176.3(0.1)	176.7(0.1)	176.3(1)
Cα	56.3(0.1)	56.7(0.1)	56.2(1.4)
Cβ	29.2(0.1)	29.2(0.1)	30.1(1.4)

Wishart et al. (1992) Biochemistry

NMR RESULTS

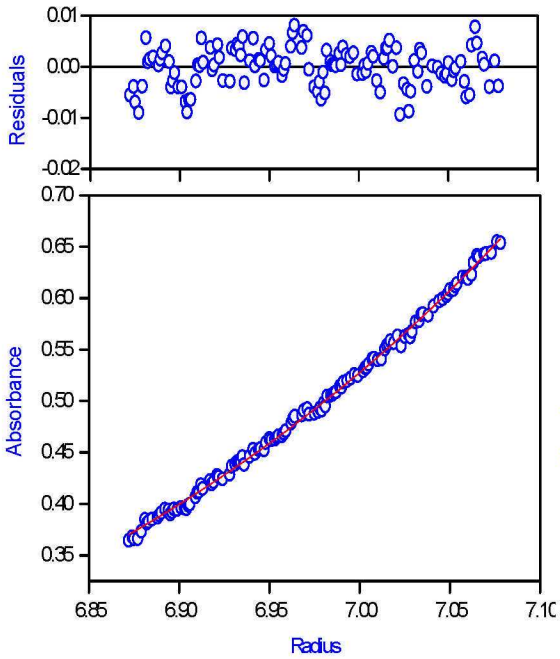
- All glutamines experience a similar chemical environment
- The Poly-Q region is highly flexible
- The glutamines are highly exposed to solvent
- No differences are observed between GST-Q22 and GST-Q41

The structure of soluble poly-Q is a random coil

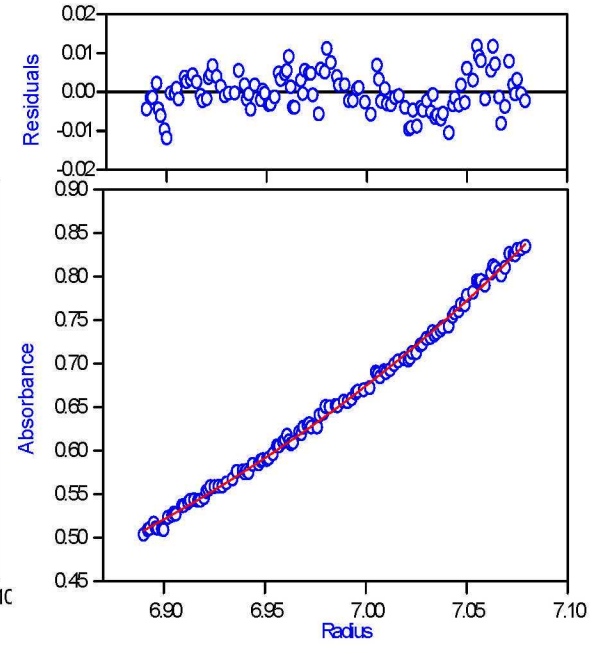
What about when they aggregate?

- **After 3 months at 25 °C pH 6.5, NO significant aggregation was observed!!!**

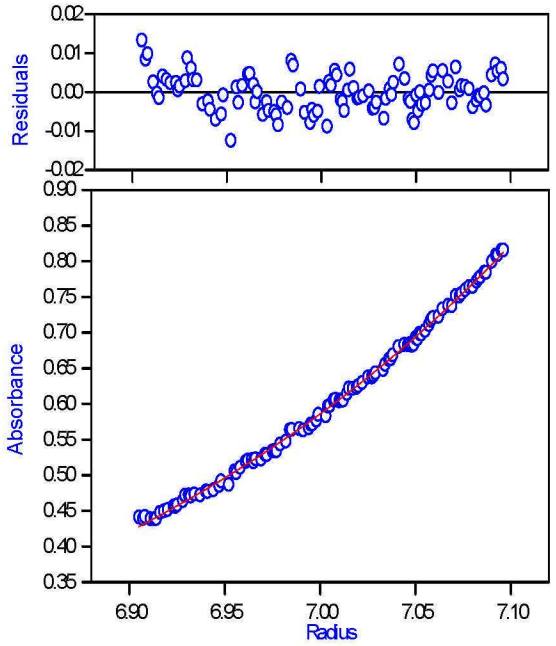
ANALYTICAL ULTRACENTRIFUGE



GST



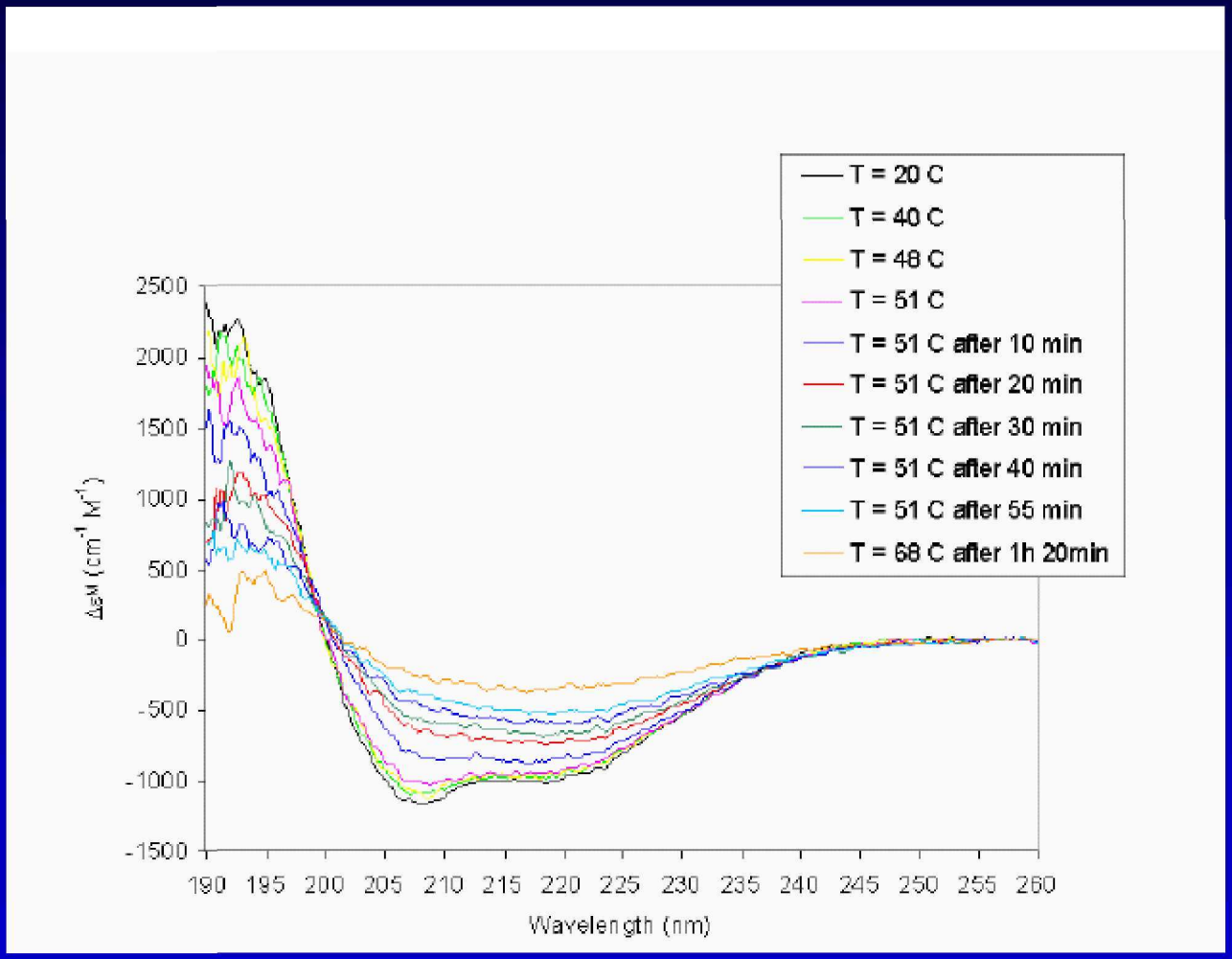
GST-Q22



GST-Q41

Only one species in solution → No Aggregation

Thermal denaturation of GST-Q41

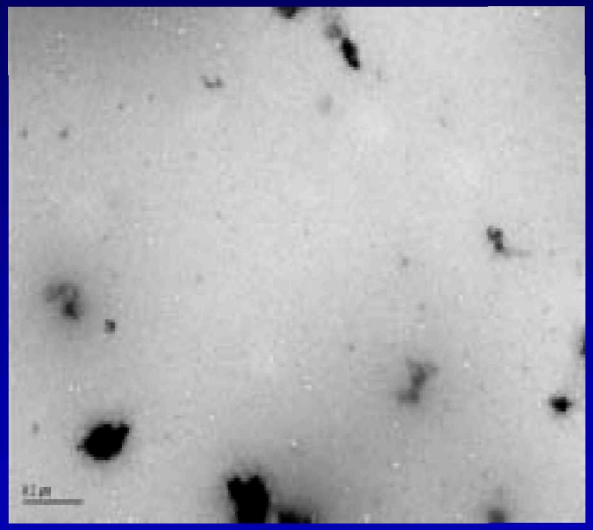


Results from thermal unfolding

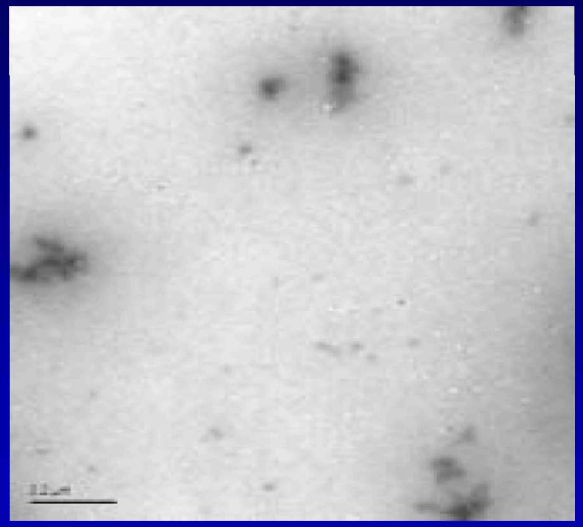
- All three samples are stable up to 52 C
- Above 52 C the samples start to precipitate
- The precipitation of GST and Q22 starts immediately while Q41 precipitates only after an incubation time

Analysis of the aggregates by EM

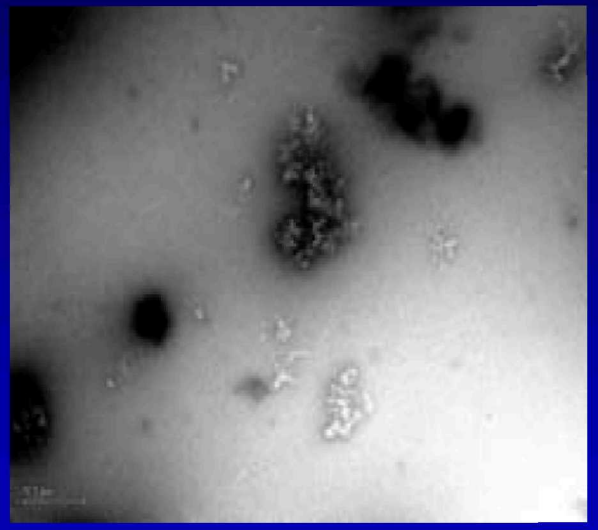
Samples incubated at T > 50 °C



GST



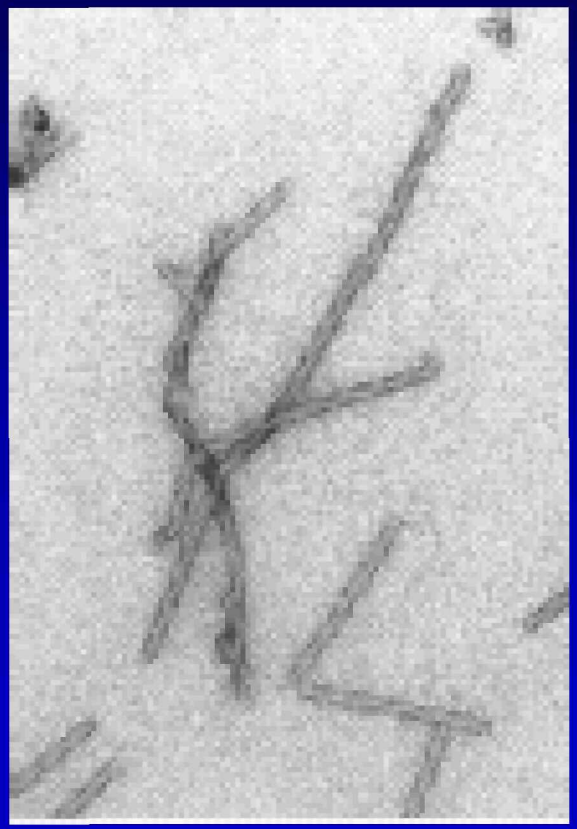
GST-Q22

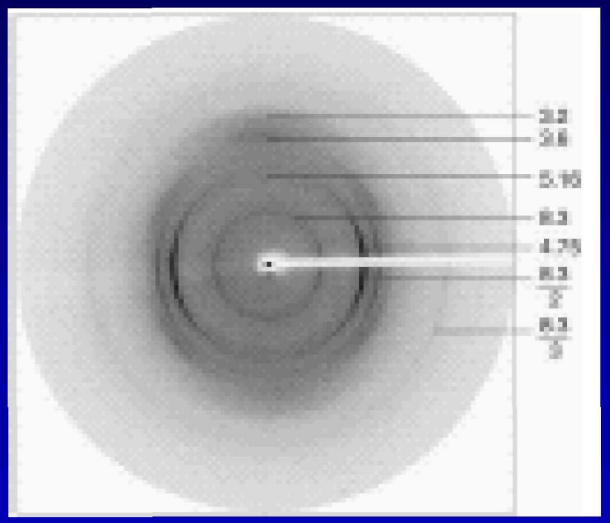


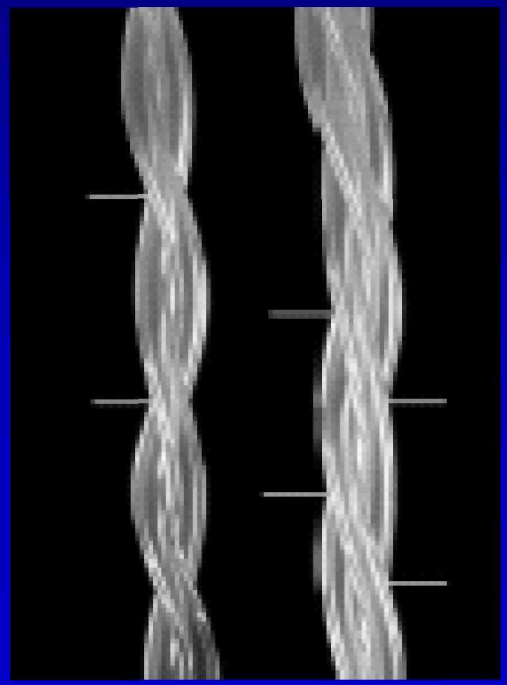
GST-Q41

GST-Q41 has a greater tendency to aggregate

Models of fiber structures







CONCLUSIONS

- NMR → direct and selective observation of the conformation of polyQ within a protein context
- When unaggregated POLYQ = RANDOM COIL
- This is consistent with a transition random coil → β-sheet upon aggregation
- The protein context strongly influences the solubility of polyQ regions
- Under destabilising conditions, the length of polyQ determines the tendency to aggregate

Future perspectives

- Studies of ‘real’ poly-Q proteins (ataxin 3)
- Characterization of conditions that promote amyloid formation
- Studies of the kinetics of aggregation

Acknowledgements

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