

Basi molecolari delle amiloidosi

PV

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IT

NMR Udine (Esposito et al.)

AFM Genova (Relini et al)

Cristallografia Milano
(Bolognesi et al)

Proteomica ed espressione
Biochimica Biotecnologia
(Napoli Pucci/Piccoli)

EU

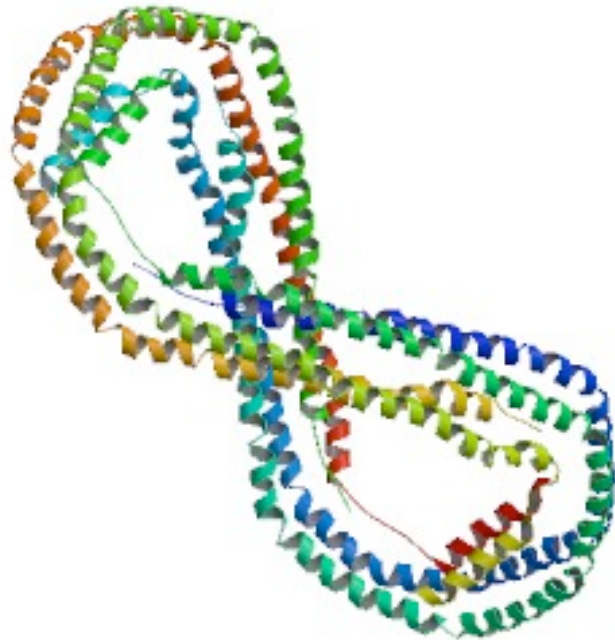
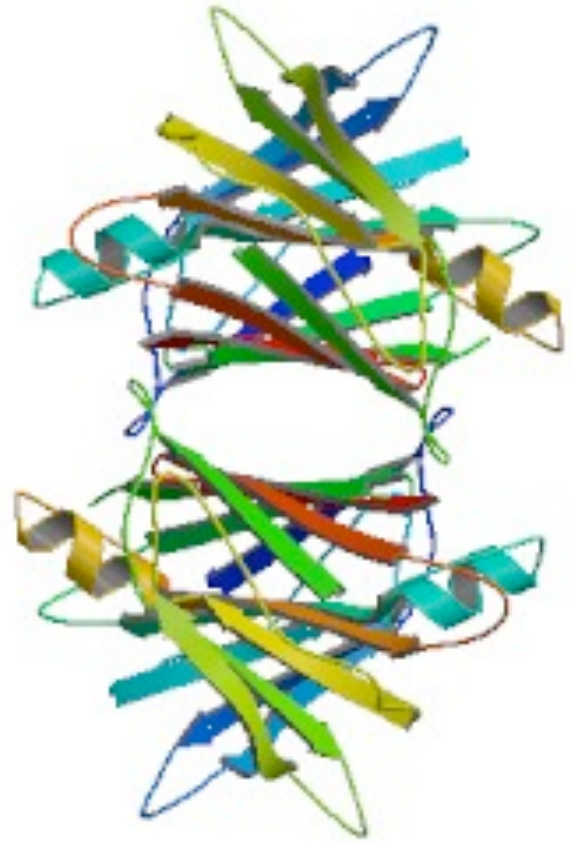
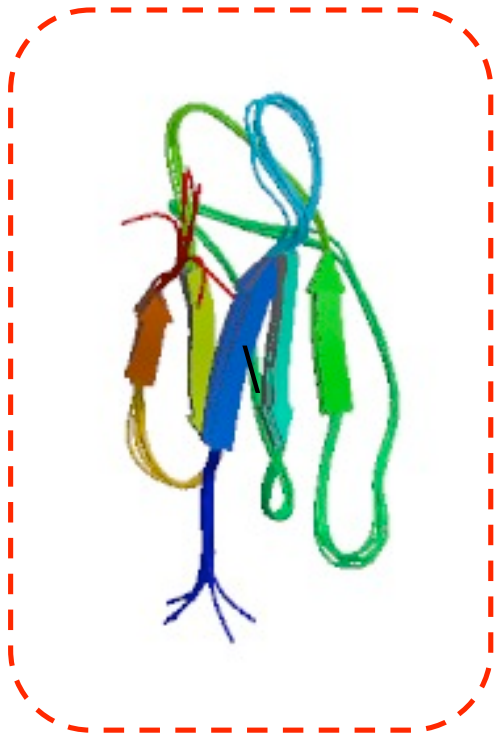
UK amyloid disease
Referral centre MB Pepys

VIB (camelide mAb)
Lode Wyns

Finanziamenti: MIUR-Ministero Sanità-Cariplo- EU. (*Regione Lombardia?!*)

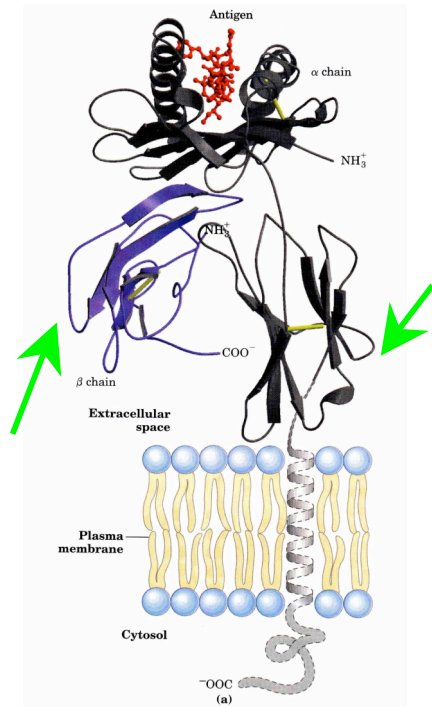
Table 1. Amyloid Proteins and Their Precursors.*

Amyloid Protein	Precursor	Distribution	Type	Syndrome or Involved Tissues
A β	A β protein precursor	Localized Localized	Acquired Hereditary	Sporadic Alzheimer's disease, aging Prototypical hereditary cerebral amyloid angiopathy, Dutch type
A PrP	Prion protein	Localized Localized	Acquired Hereditary	Sporadic (iatrogenic) CJD, new variant CJD (alimentary?) Familial CJD, GSSD, FFI
ABri	ABri protein precursor	Localized or systemic?	Hereditary	British familial dementia
ACys	Cystatin C	Systemic	Hereditary	Icelandic hereditary cerebral amyloid angiopathy
A β 2M	Beta ₂ -microglobulin	Systemic	Acquired	Chronic hemodialysis
AL	Immunoglobulin light chain	Systemic or localized	Acquired	Primary amyloidosis, myeloma-associated
AA	Serum amyloid A	Systemic	Acquired	Secondary amyloidosis, reactive to chronic infection or inflammation including hereditary periodic fever (FMF, TRAPS, HIDS, FCU, and MWS)
ATTR	Transthyretin	Systemic Systemic	Hereditary Acquired	Prototypical FAP Senile heart, vessels
AApoA1	Apolipoprotein A-I	Systemic	Hereditary	Liver, kidney, heart
AApoAII	Apolipoprotein A-II	Systemic	Hereditary	Kidney, heart
AGel	Gelsolin	Systemic	Hereditary	Finnish hereditary amyloidosis
ALys	Lysozyme	Systemic	Hereditary	Kidney, liver, spleen
AFib	Fibrinogen A α chain	Systemic	Hereditary	Kidney

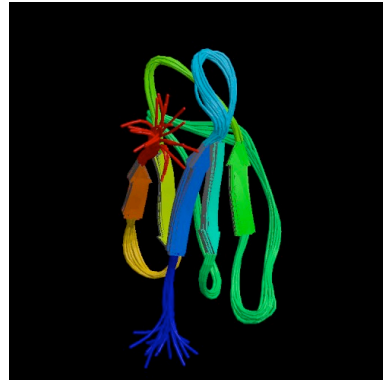


β 2-m and dialysis related amyloidosis

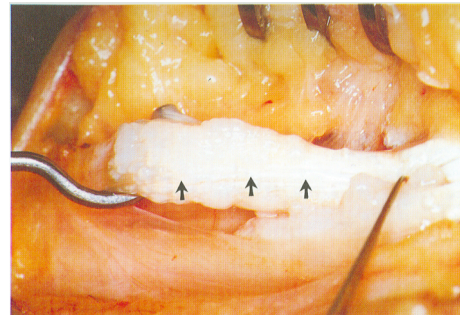
Cell membranes



Plasma



Amyloid deposits



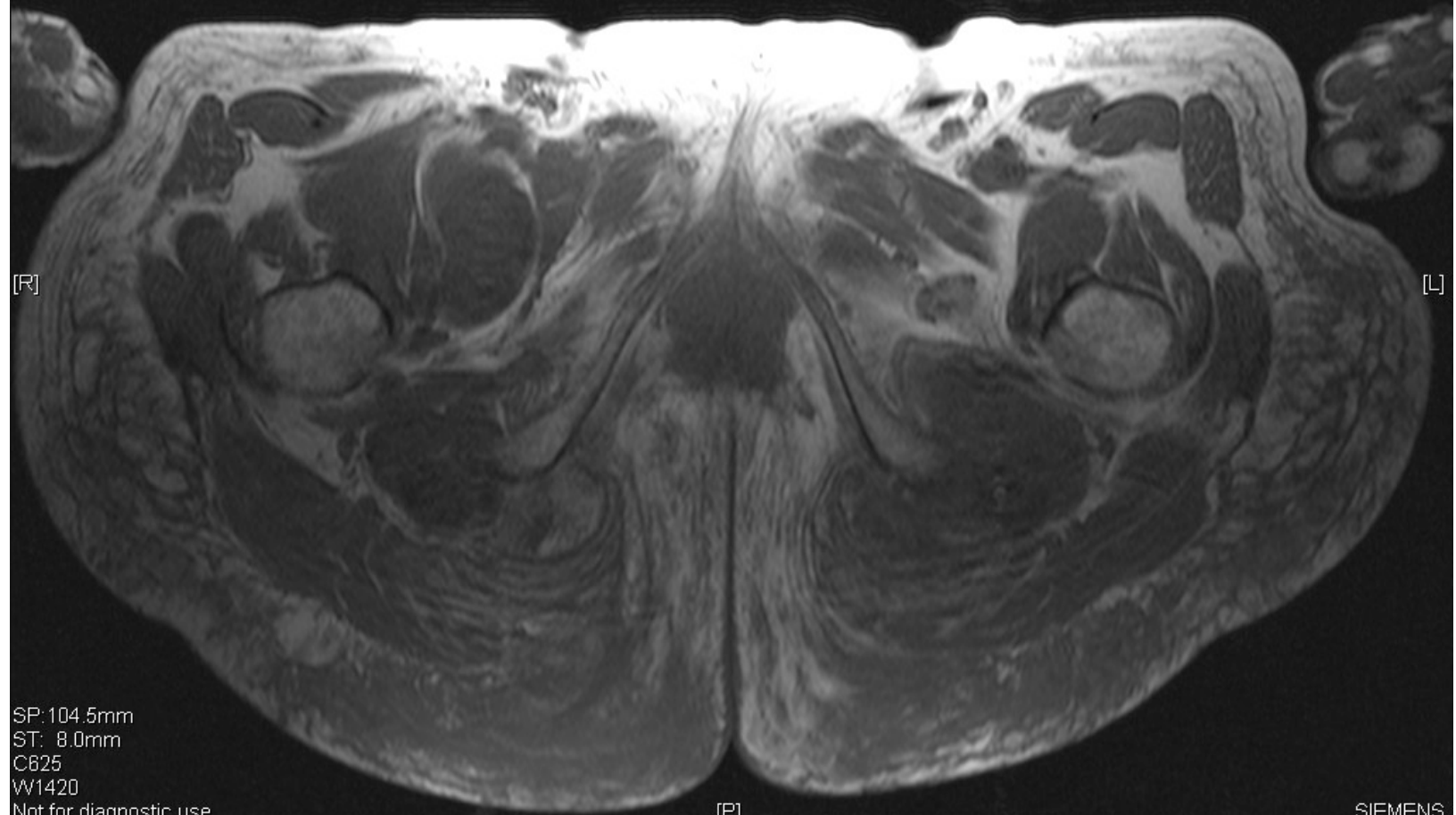
6B



FERRARA, FRANCESCA
10

[A]

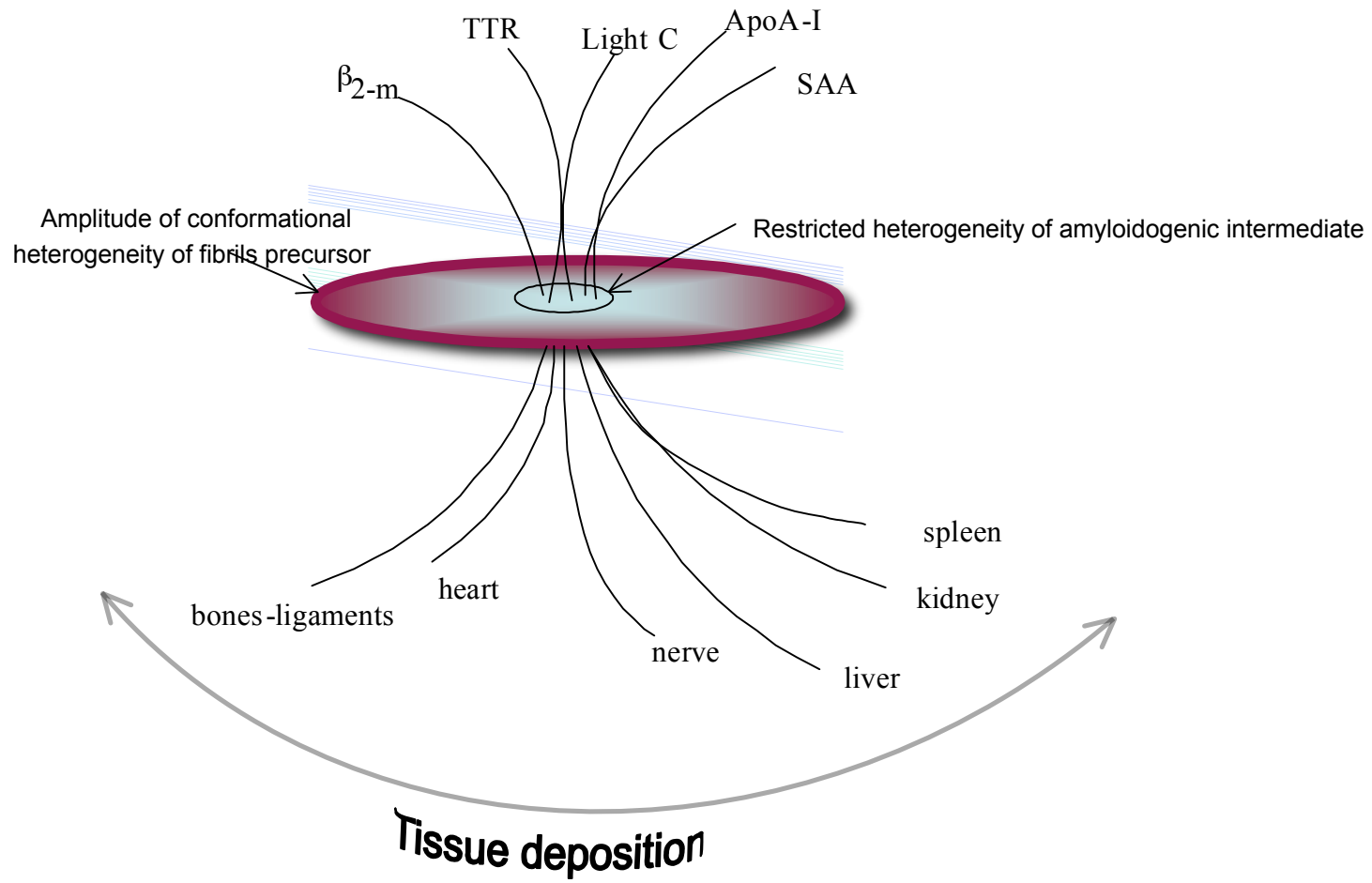
RM ANCHE^general
03/05/2007
10.38.54
2007014245



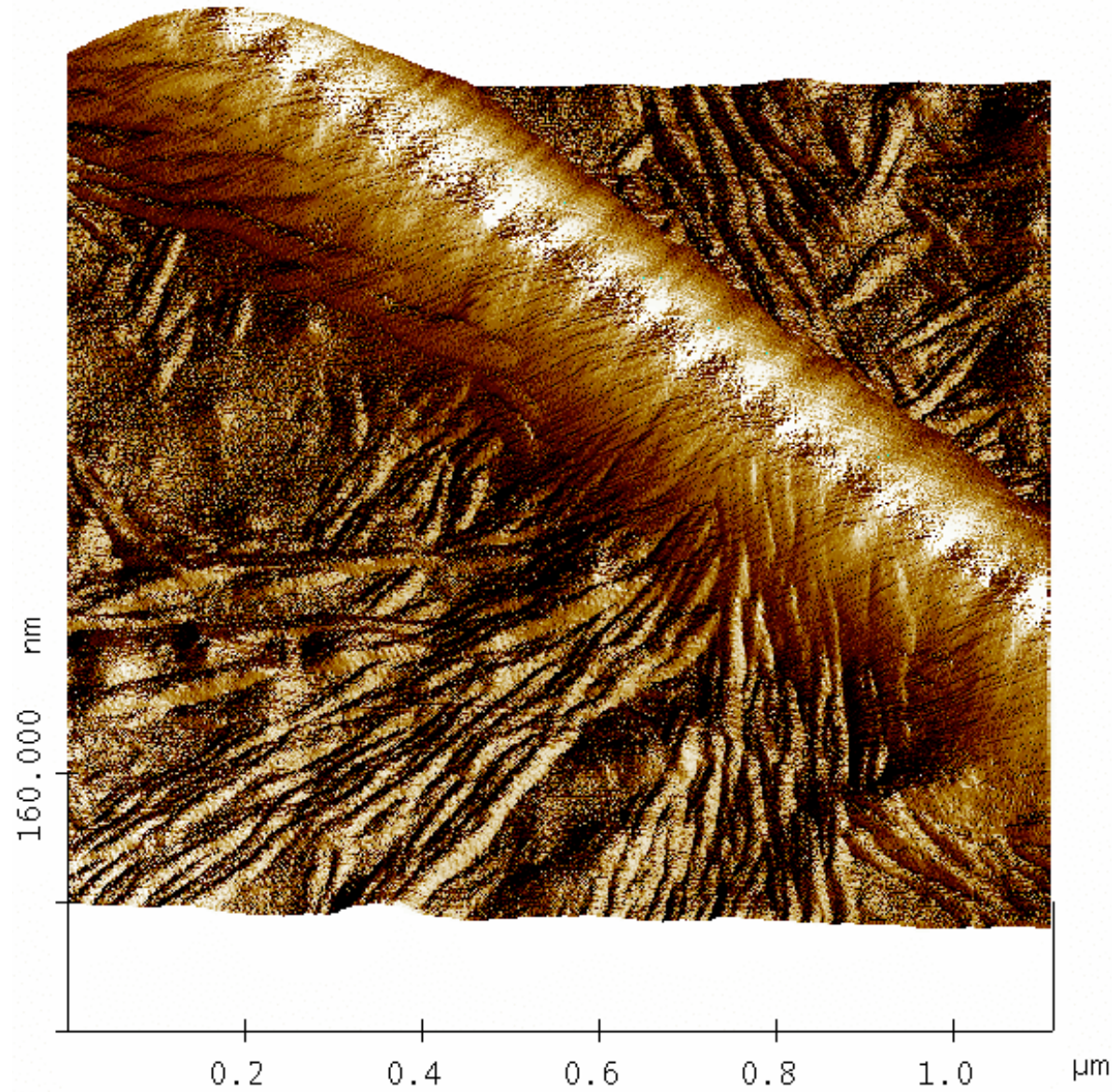
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ST: 8.0mm
C625
W1420
Not for diagnostic use

[P]

SIEMENS

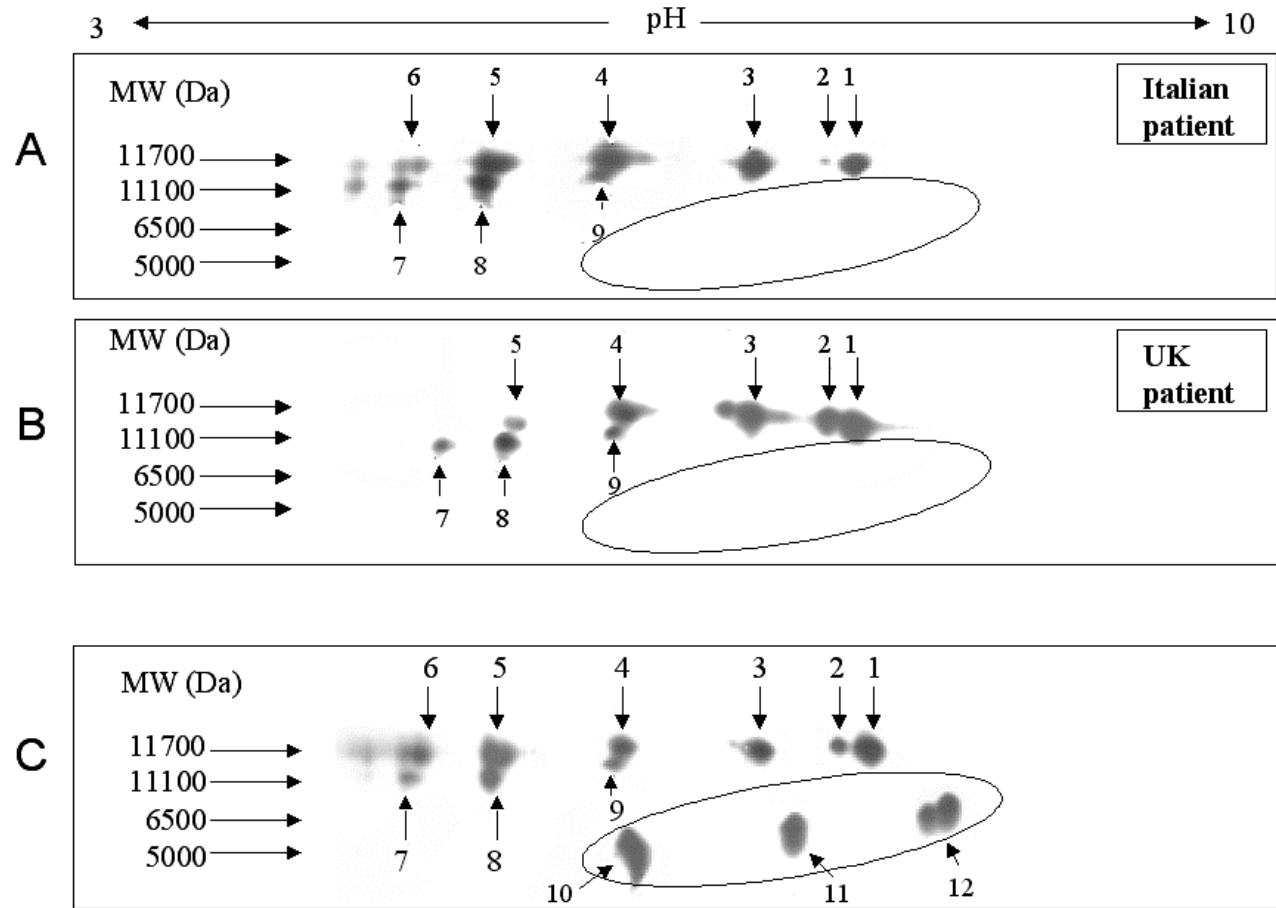
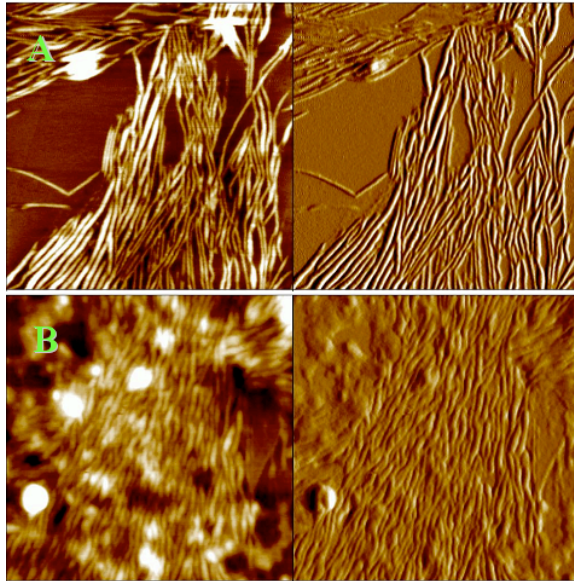


AFM of natural amyloid fibrils extracted from an amyloid deposit



Relini et al. JBC 2006

"Apparent interaction of collagen fibres and amyloid fibrils"



Spot number

1-6

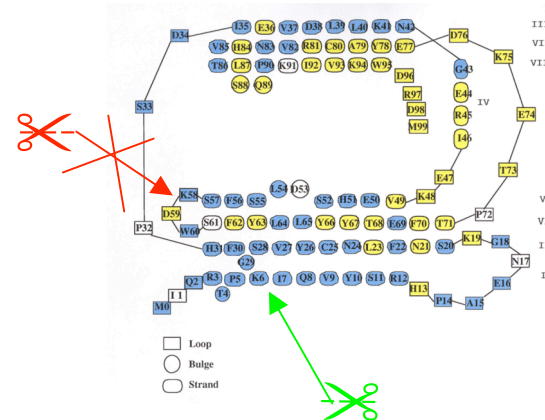
full length

7-9

$\Delta N6$ $\beta 2$ -m

10-12

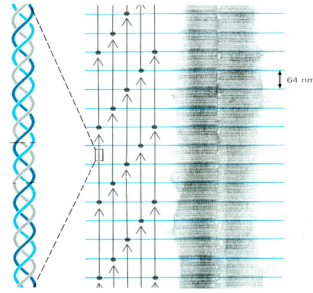
Lys 58 cleaved species
(Corlin et al Clin Chem 2005)



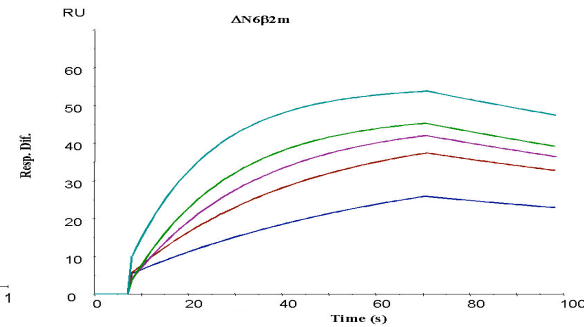
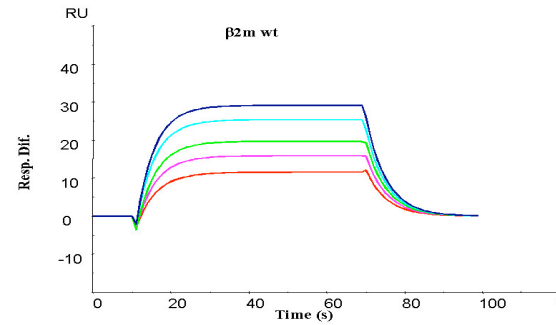
Giorgetti et al Protein Science 2007

1	Naiki, et al 1997. <i>Amyloid</i> 4: 223–232 *	Na Citrate 50mM pH 2.5-4	β 2-m 100 μ M + seeds	37 deg
2	McParland et al 2000. <i>Biochemistry</i> 39: 8735–8746 *	Na citrate 50 mM pH 2.5 100 mM NaCl	β 2-m 100 μ M No seeds	“
3	Esposito et al <i>Protein Science</i> 2000, °	Na Citrate Na citrate 50 mM pH 6.5	β 2-m N-terminal truncated 100 μ M +seeds	“
4	Chiti et al <i>J Biol Chem.</i> 2001 °	Na Citrate Na citrate 50 mM pH 7.3	Refolding intermediate 100 μ M + seeds	“
5	Yamamoto al, 2004, <i>J Am Soc Nephrol</i> , °	Na Phosphate 50 mM 100 mM NaCl pH 7.4 20%TFE	β 2-m 100 μ M +seeds heparin	“
6	Yamamoto al, <i>Biochemistry</i> 2004 43, 11075-11082 Kihara et al,2005, <i>JBC</i> ,280:120 2-8 °	Na Phosphate 50 mM 100 mM NaCl pH 7.4 0.5% SDS	β 2-m 25 μ M +seeds	“
7	Myers et al <i>Biochemistry</i> 2006 °	Na acetate-Phosphate pH 7 conditioned seed by Heparin 60 μ g/ mg apoE 14 μ g/mg	β 2-m 45 μ M / Δ N6 truncated + seeds (Collagen type II)	“
8	Jahn Thomas. <i>Nat Struct Biol</i> °	Buffer A pH 7	β 2-m refold. intermediate + seeds	“
9	Borysik AJ et al ° <i>Kidn. Int</i> 2007	PBS Buffer pH 7.4	Δ N6 beta 2m + GAGs	
10	Relini et al <i>JBC</i> 2006 °	Phosphate buffer pH 6.4	Collagen type I	37-40°C

Collagen β_2 -m interaction (Homma et al Nephron 1989)



Immobilisation of collagen triple helix on SPR system (BIAcore)

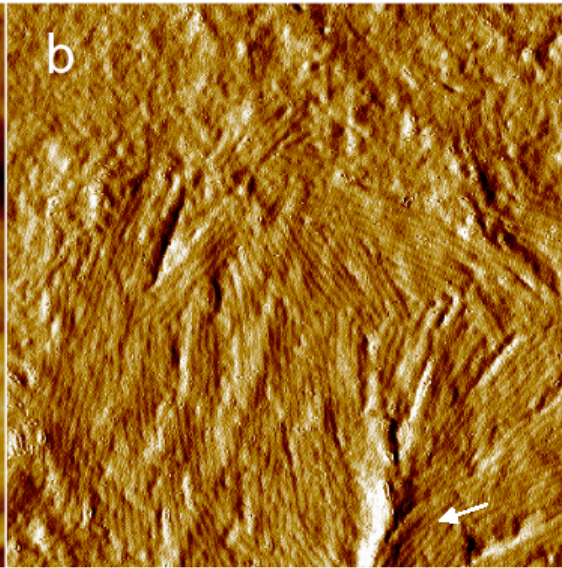
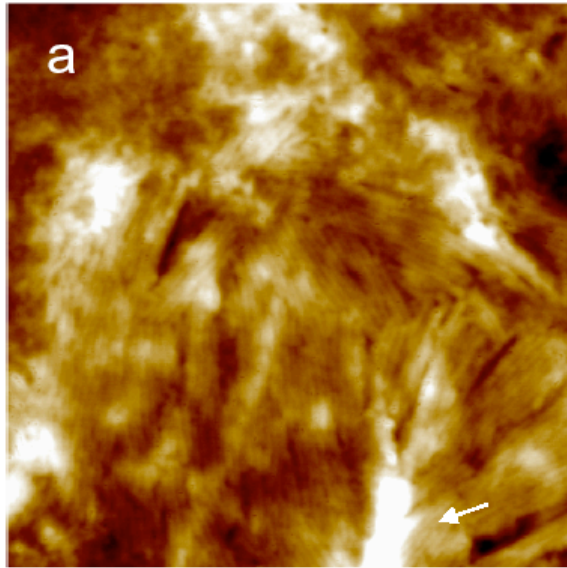


Kinetics and thermodynamics of β_2 -m isoform interaction with collagen type I determined by surface plasmon resonance measurements

Ligand	pH	k_{on} ($M^{-1} sec^{-1}$)	k_{off} (sec^{-1})	K_d (M)
β_2 -m	7.4	6.3×10^2	2.6×10^{-1}	4.1×10^{-4}
β_2 -m	6.4	4.1×10^2	9.0×10^{-2}	2.2×10^{-4}
β_2 -m I ₂ , T= 30"	7.4	3.4×10	2.6×10^{-1}	7.6×10^{-3}
β_2 -m I ₂ , T= 600"	7.4	2.8×10	2.3×10^{-1}	4.3×10^{-3}
β_2 -m I ₂ , T= 1200"	7.4	6.6×10^2	2.4×10^{-1}	4.4×10^{-4}
R3A β_2 m	7.4	2.1×10^2	1.4×10^{-1}	6.7×10^{-4}
H31Y β_2 m	7.4	3.0×10^2	1.4×10^{-1}	6.8×10^{-4}
Δ N6 β_2 m	7.4	1.4×10^3	4.7×10^{-2}	3.4×10^{-5}
Δ N6 β_2 m	6.4	1.0×10^3	5.0×10^{-3}	4.9×10^{-6}

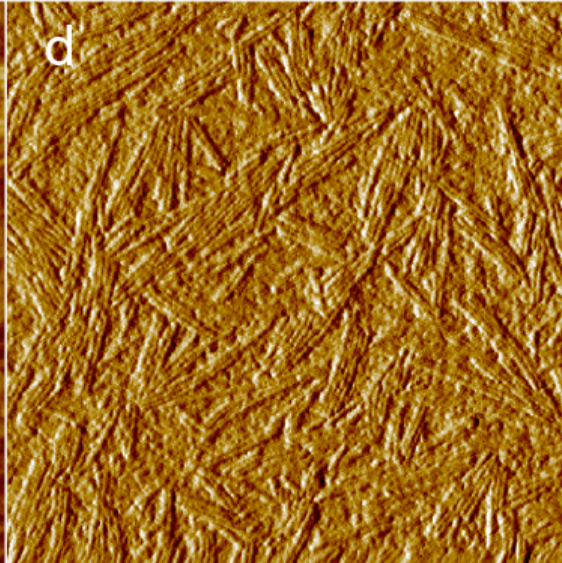
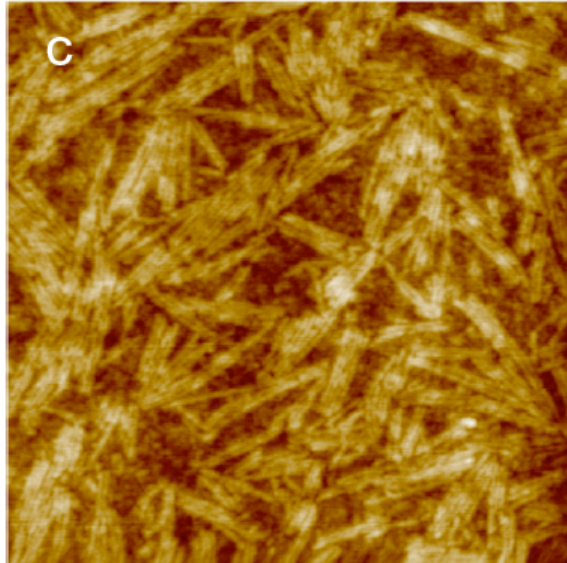
Incubation of 50 μ M β 2-m at 37-40°C pH 6.4 with fibrillar collagen type I

**Height data
4 days after
20nm filtration**



**Amplitude data
4 days after
20nm filtration**

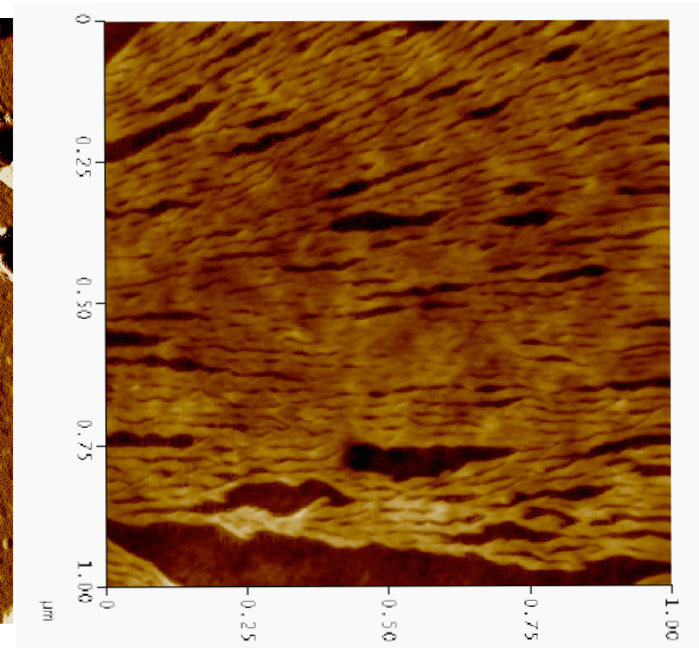
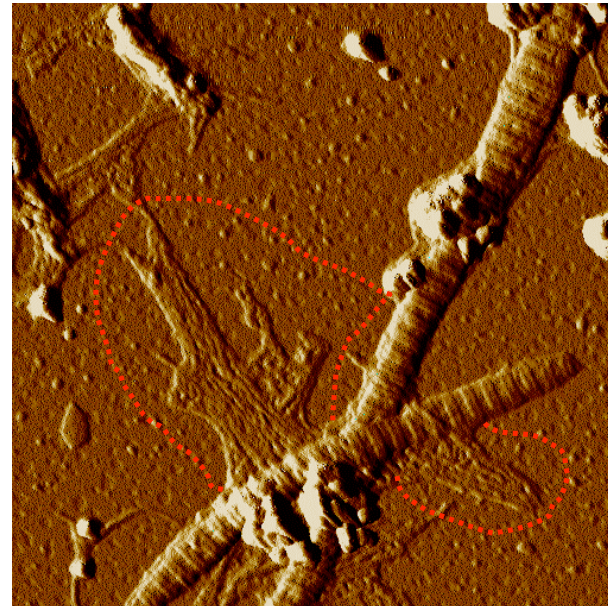
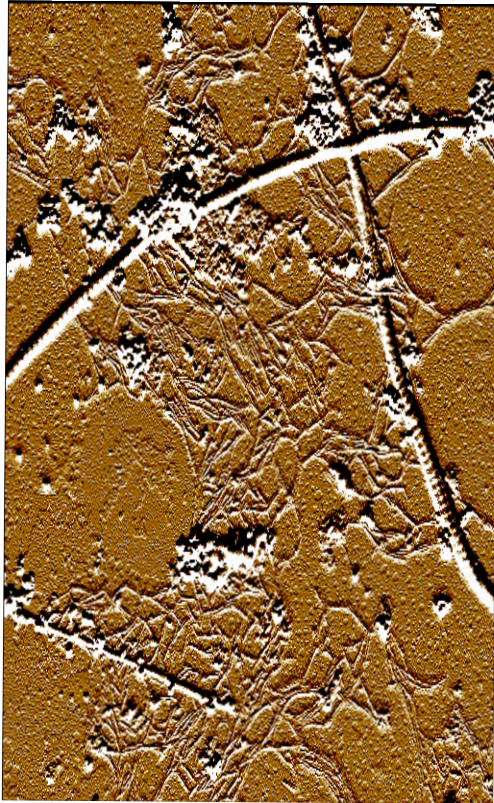
**Height data
2 days after
200nm filtration**



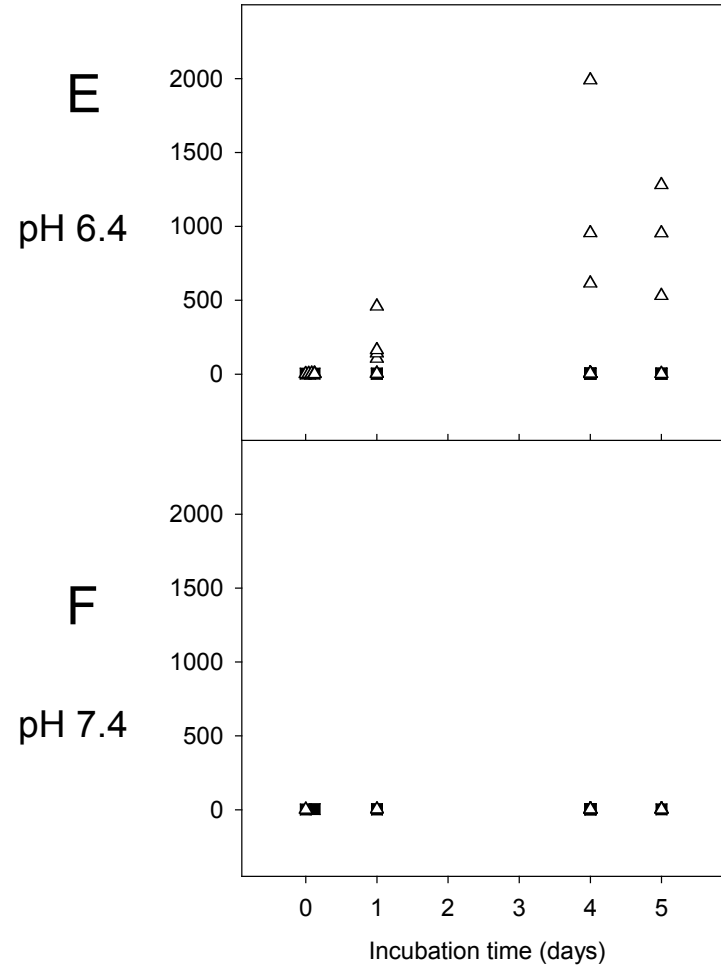
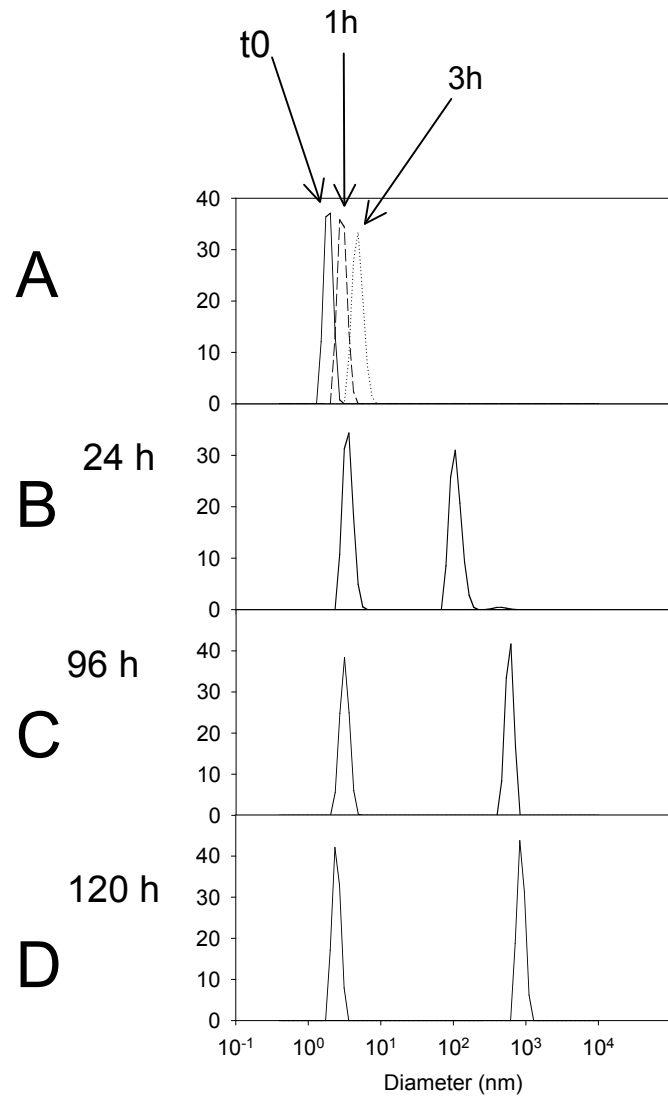
**Amplitude data
2 days after
200nm filtration**

A potent promoter of fibrillogenesis on collagen is also heparin

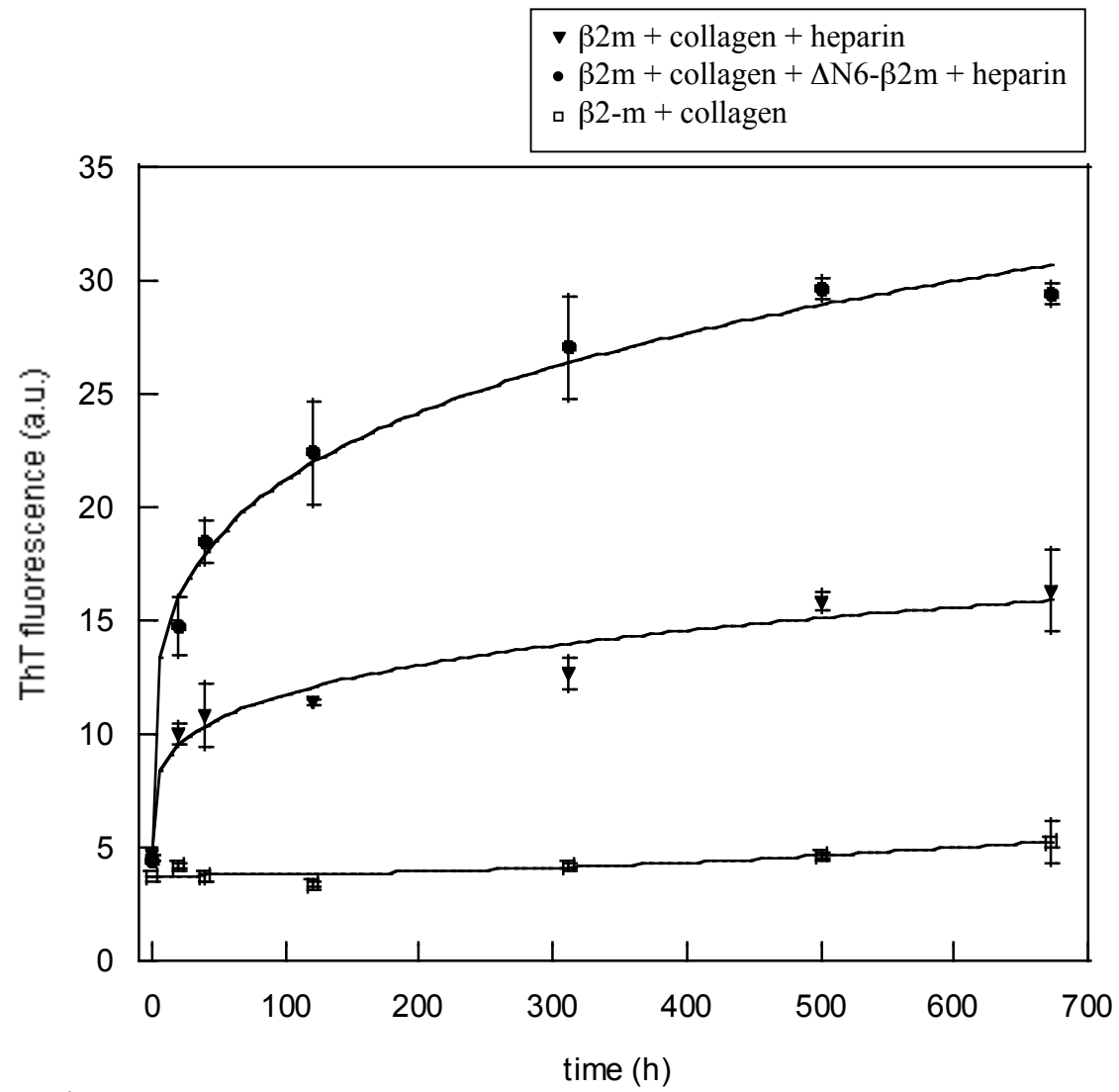
β_2 -m 0.1 mg/ml, heparin 3 μ g/ml, t of amyloid fibrils observation= 24 ore



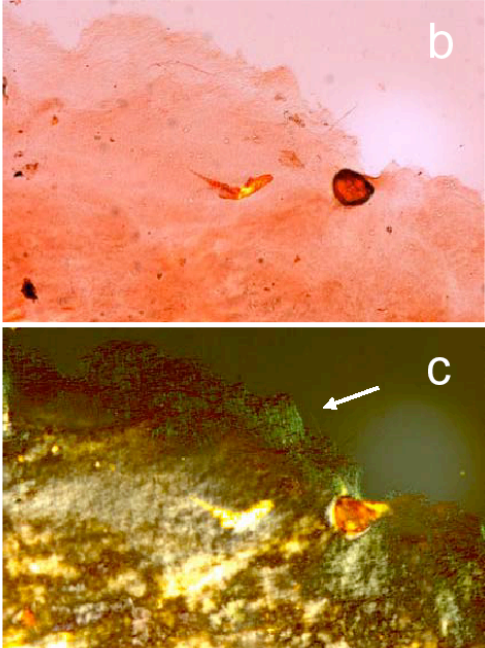
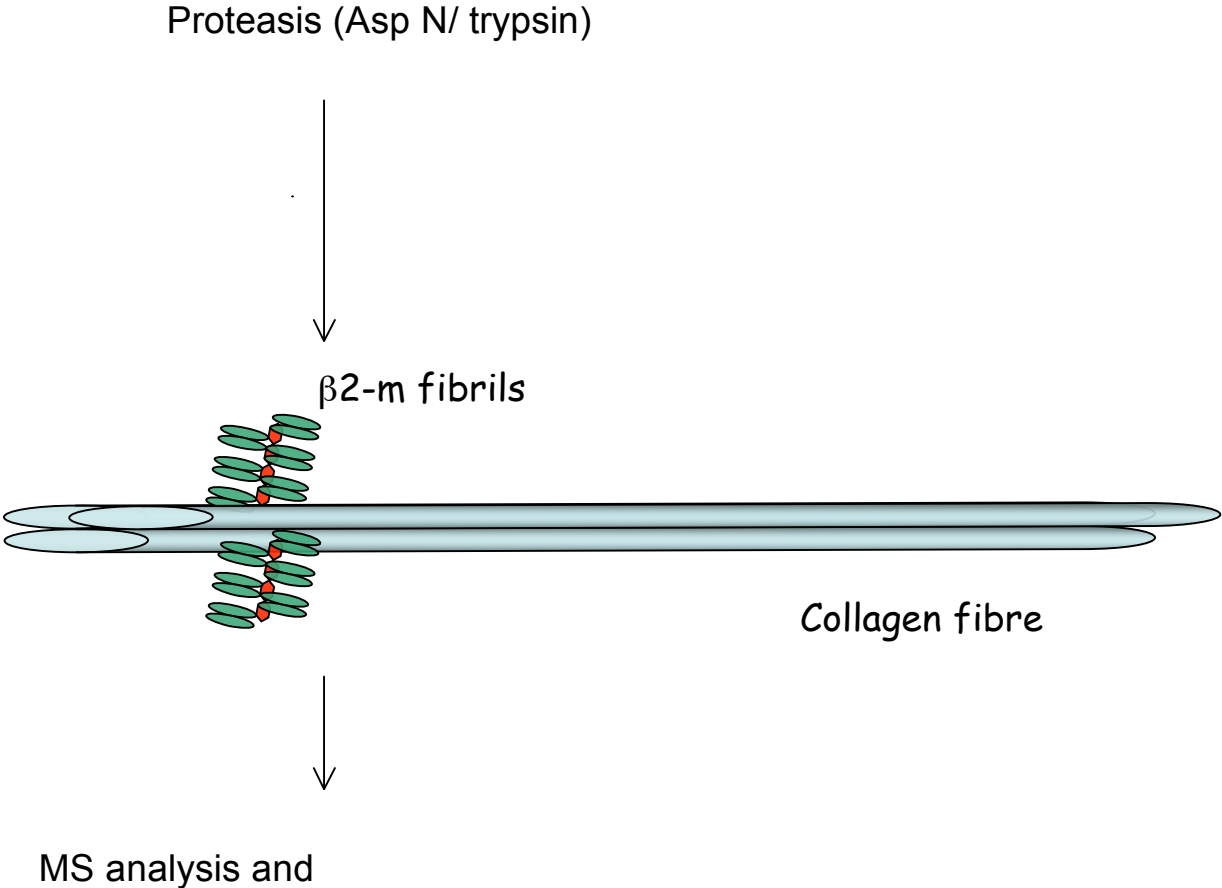
β 2-m in solution in the presence of heparin after filtration 20 nm



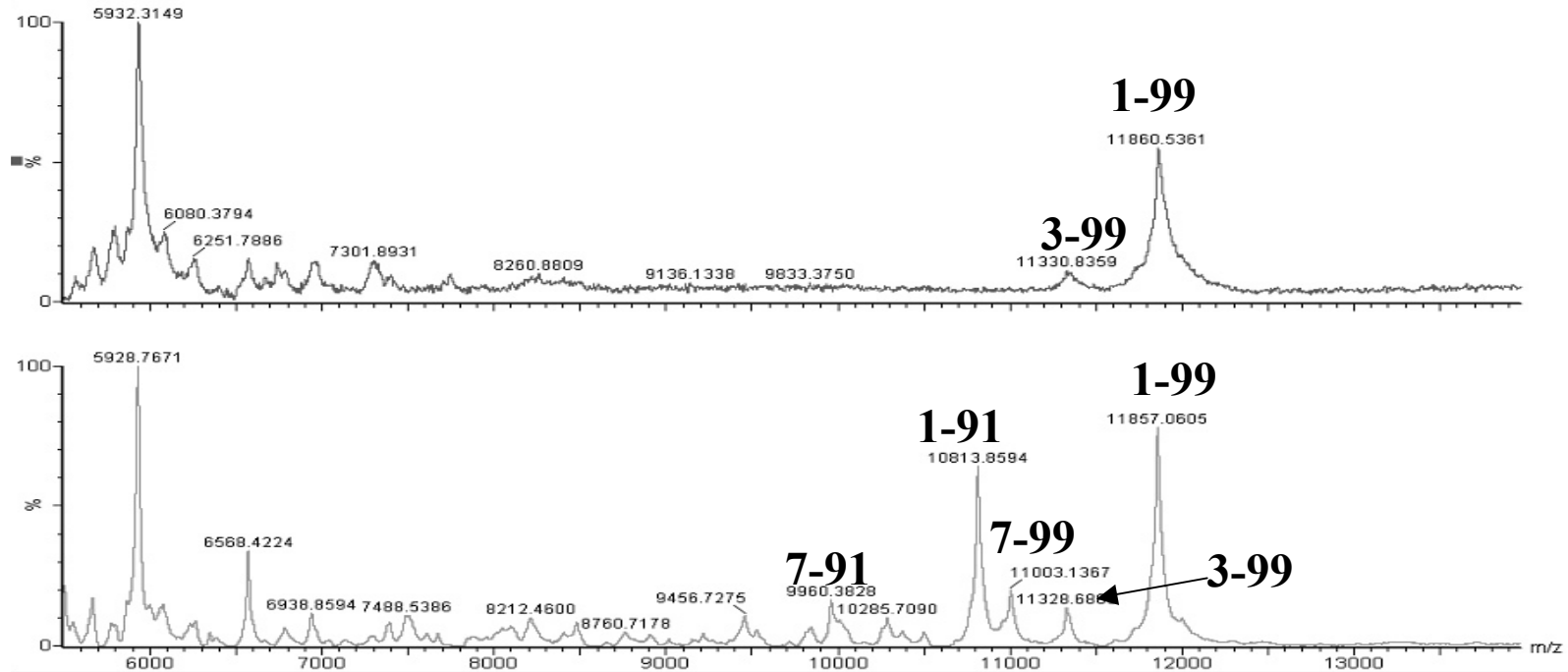
Thioflavin assay on bulk solution



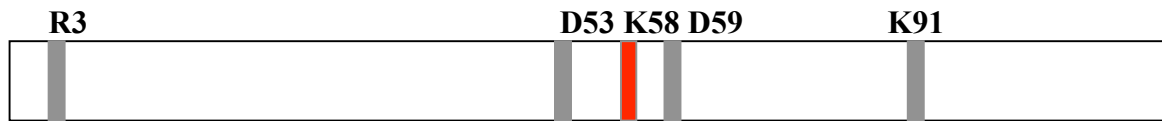
Limited proteolysis



Limited proteolysis

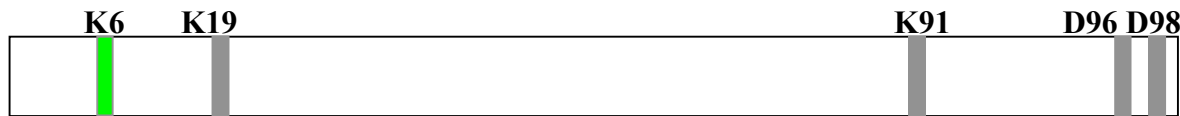


Monti et al
Protein Science
2002

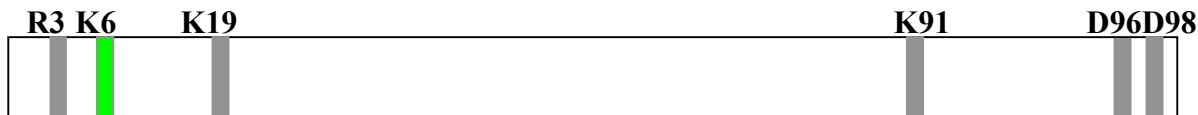


β 2-m globular

Monti et al
Protein Science
2002



β 2-m fibrillar
(TFE 20%)



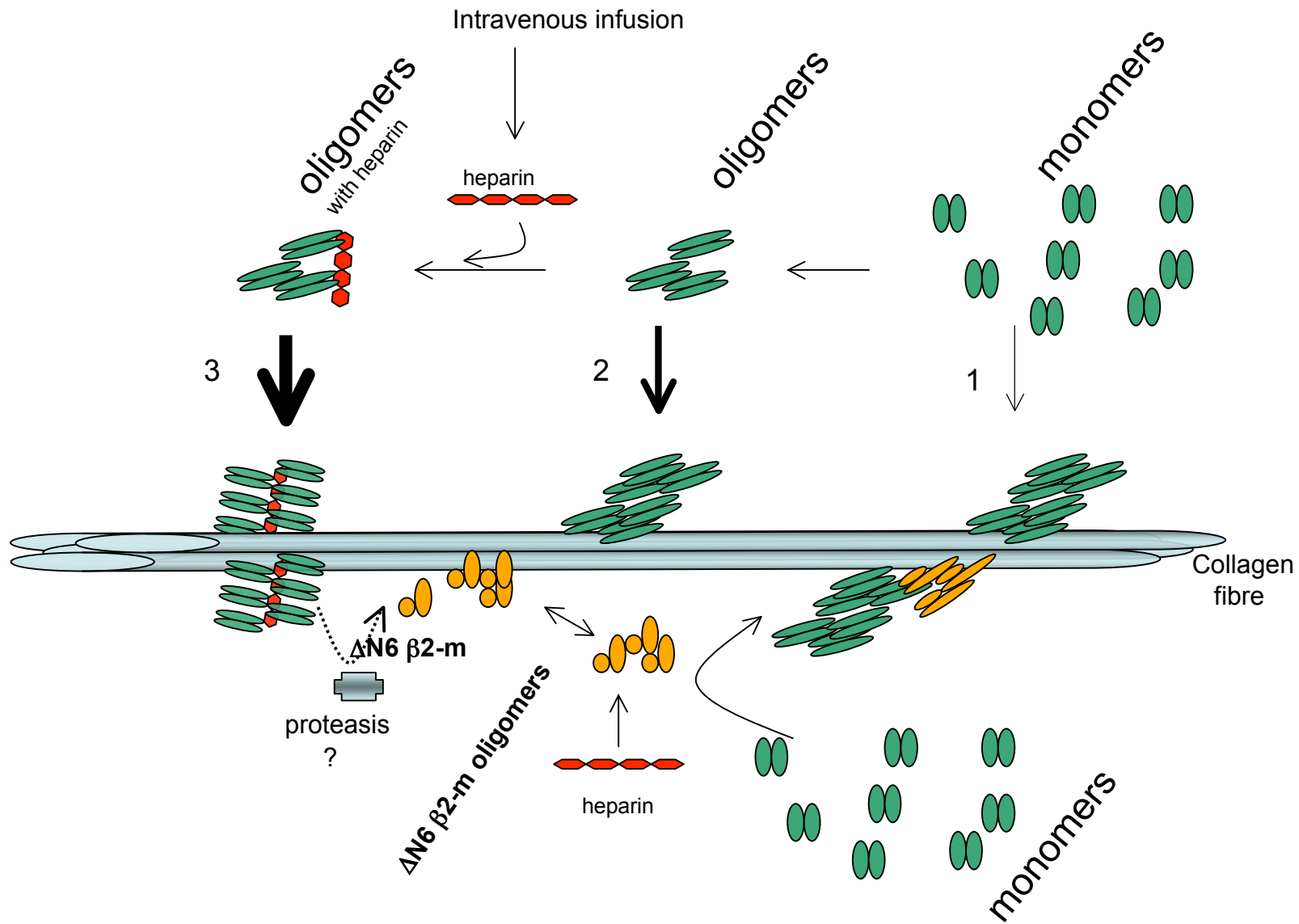
β 2-m fibrillar
(on collagen)

Esposito et al. Protein Sci. 2000 May;9(5):831-45

Myers et al Biochemistry. 2006 Feb 21;45(7):2311-21.

Borysik AJ et al ^o Kidney Int. 2007 Jul;72(2):174-81

An hypothetical model for the tissue specific localisation of β 2-m amyloid fibrils



interactors of the amyloidogenic proteins

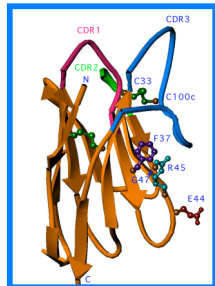


Lode Wyns



Mireille Dumoulin

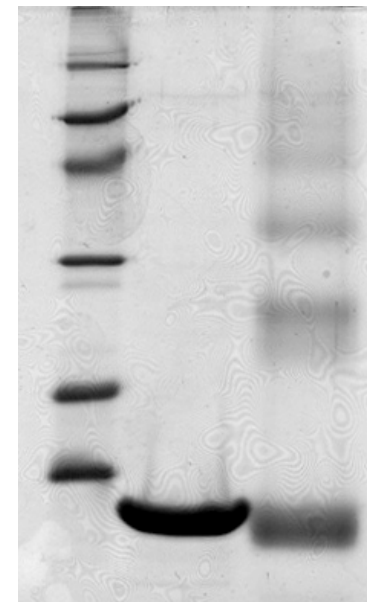
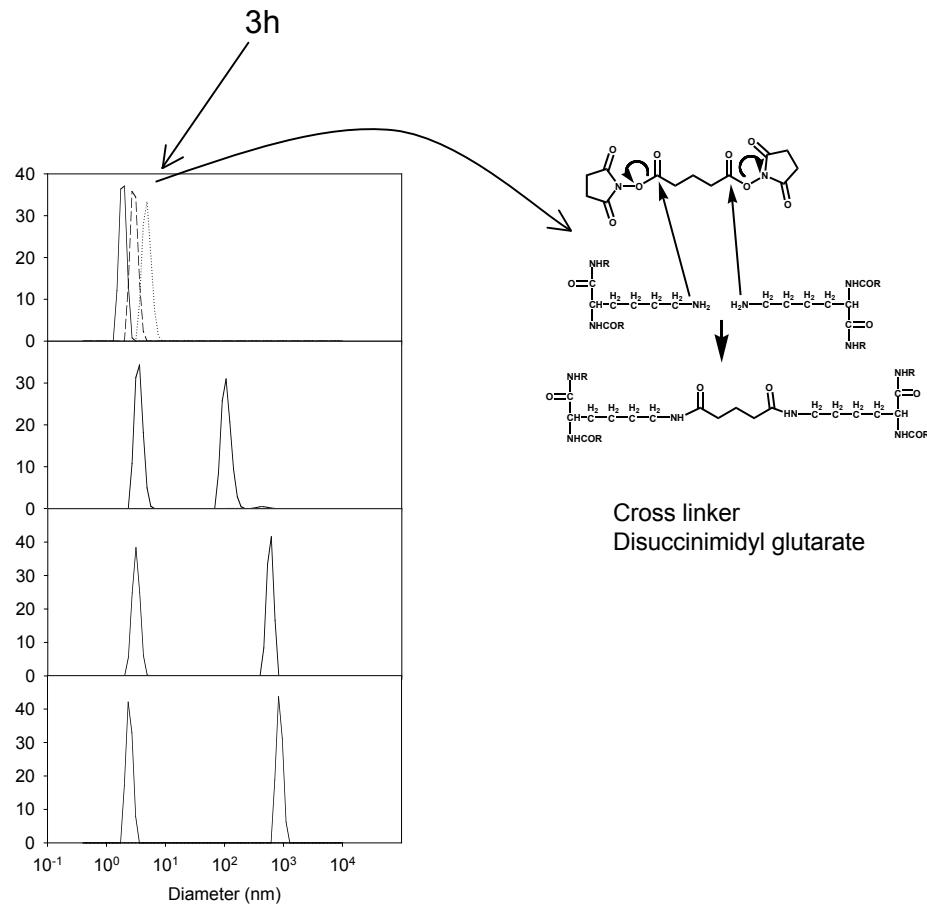
<http://www.vib.be/VIB/EN/>



Nanobodies

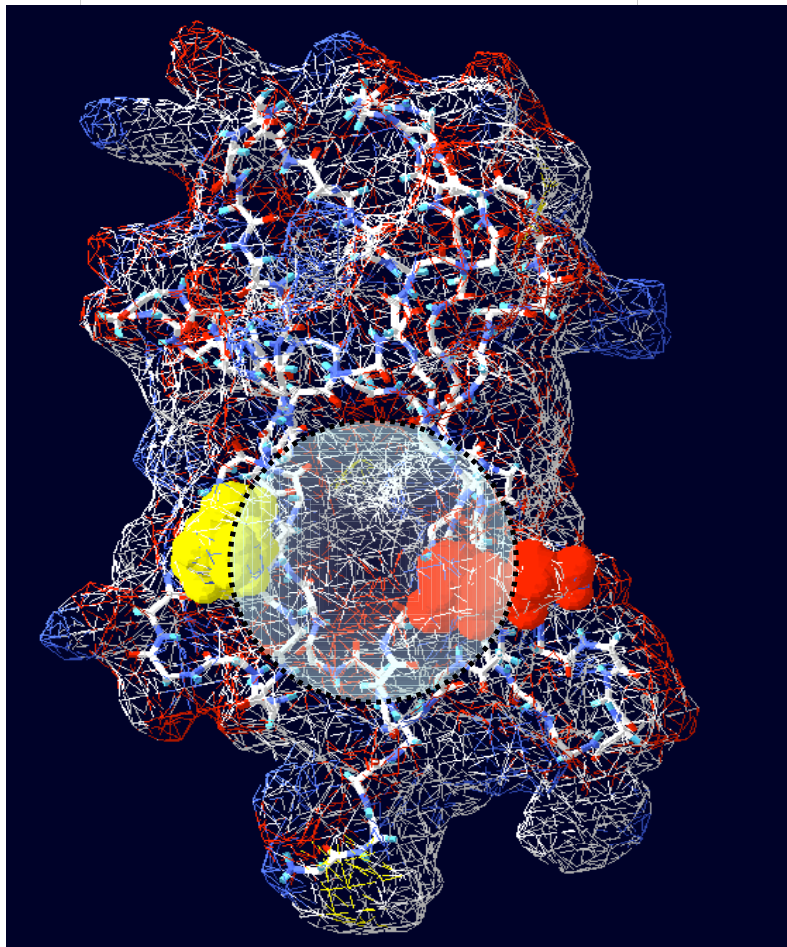
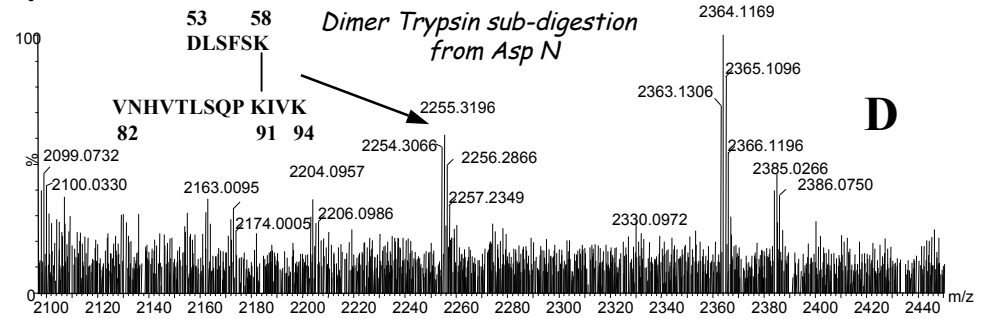
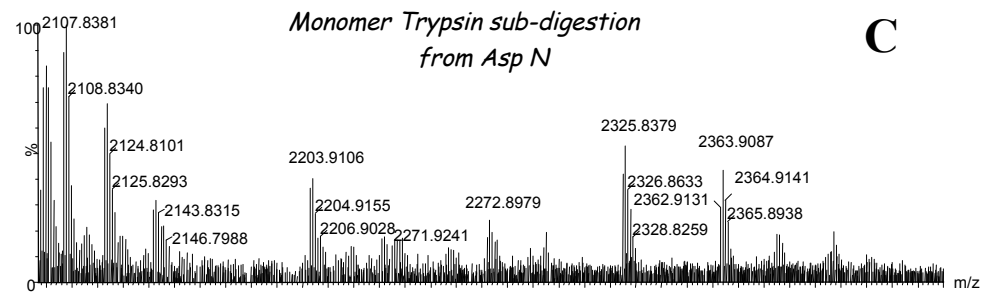
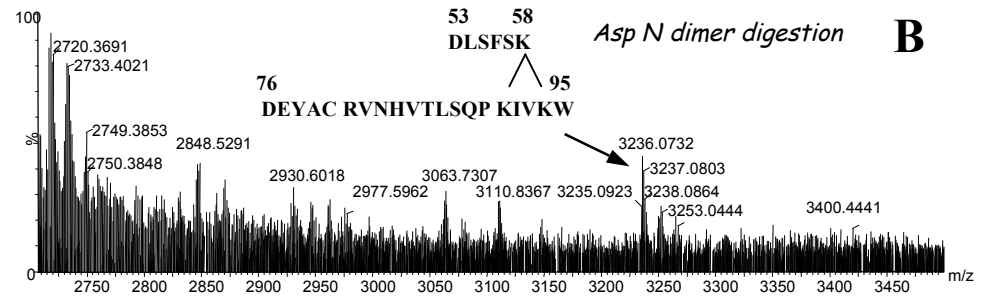
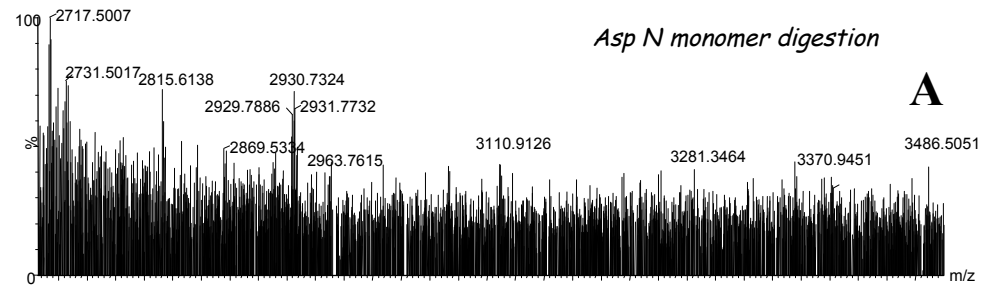
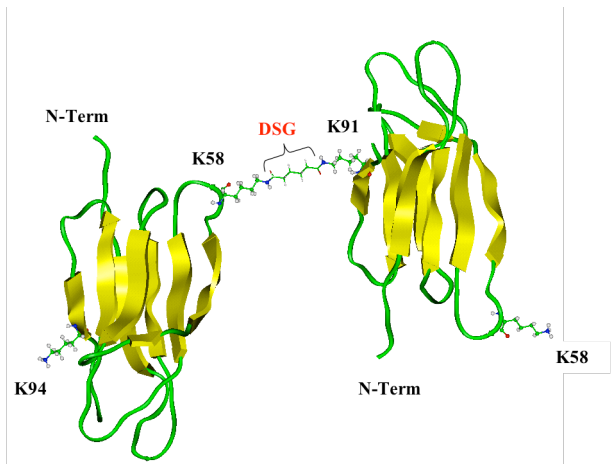
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Nb_20a (=Nb_b2m1a) EP502 1,333mg/ml VUB-ULTR 07/03/07	5ml	6,665mg
Nb_20b (=Nb_b2m1b) EP503 1,017mg/ml VUB-ULTR 07/03/07	5ml	5,085mg
Nb_21(=Nb_b2m4) EP539 0,752mg/ml VUB-ULTR 07/03/07	6,5ml	4,888mg
Nb_22a (=Nb_b2m2a) EP505 1,153mg/ml VUB-ULTR 07/03/07	4ml	4,612mg
Nb_24 (=Nb_b2m3) EP506 1,989mg/ml VUB-ULTR 07/03/07	5ml	9,945mg
Nb_25 (=Nb_b2m5) EP668 1,916mg/ml VUB-ULTR 07/03/07	4ml	7,664mg
Nb_29a (Δ b2m) CA94 0,441mg/ml VUB-ULTR 07/03/07	10ml	4,41mg
Nb_29c (Δ b2m) CA69 0,437mg/ml VUB-ULTR 07/03/07	13,5ml	5,8995mg
Nb_31 (Δ b2m) CA7069 0,417mg/ml VUB-ULTR 07/03/07	9,5ml	3,9615mg

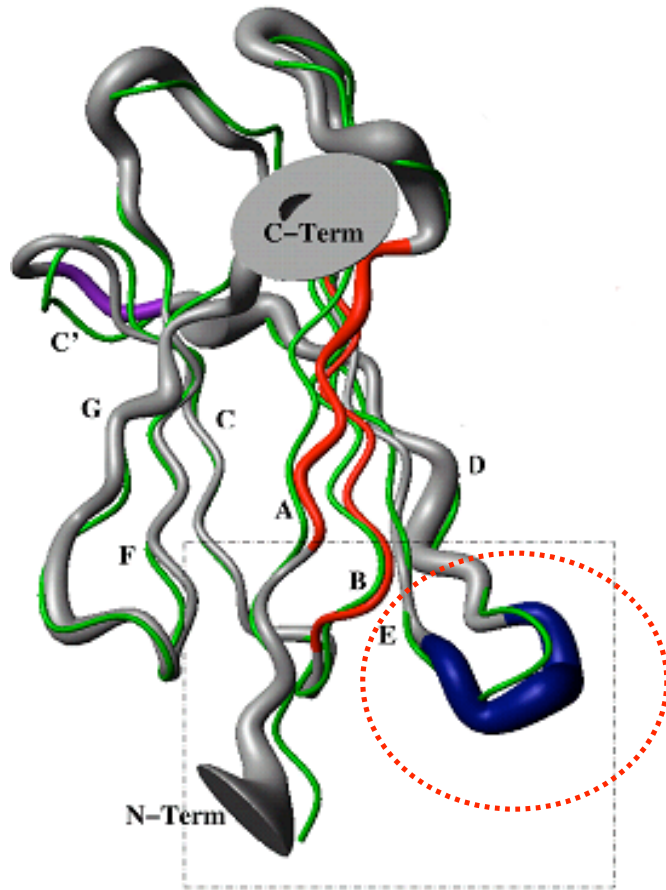
Use of chemical cross linkers



st b2mst b2merlink

oligomers

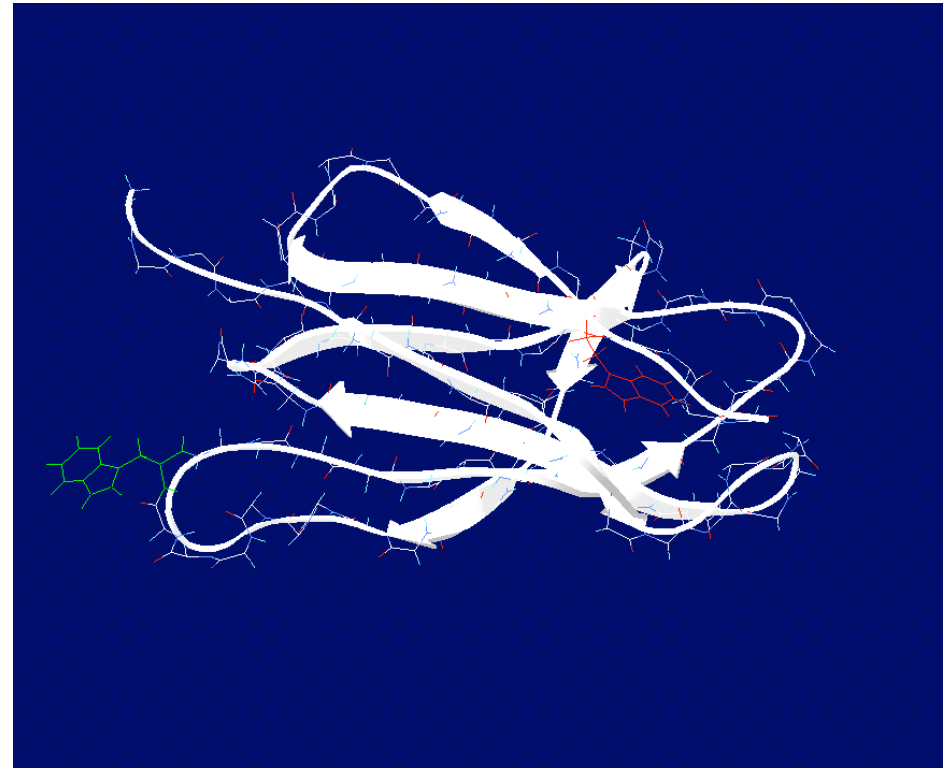




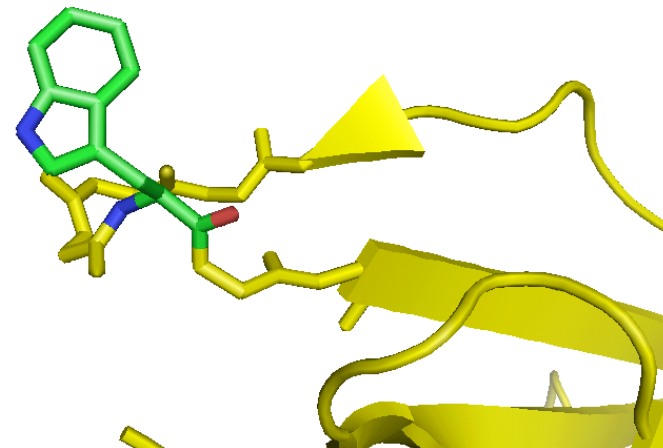
Corazza et al. JBC 2004

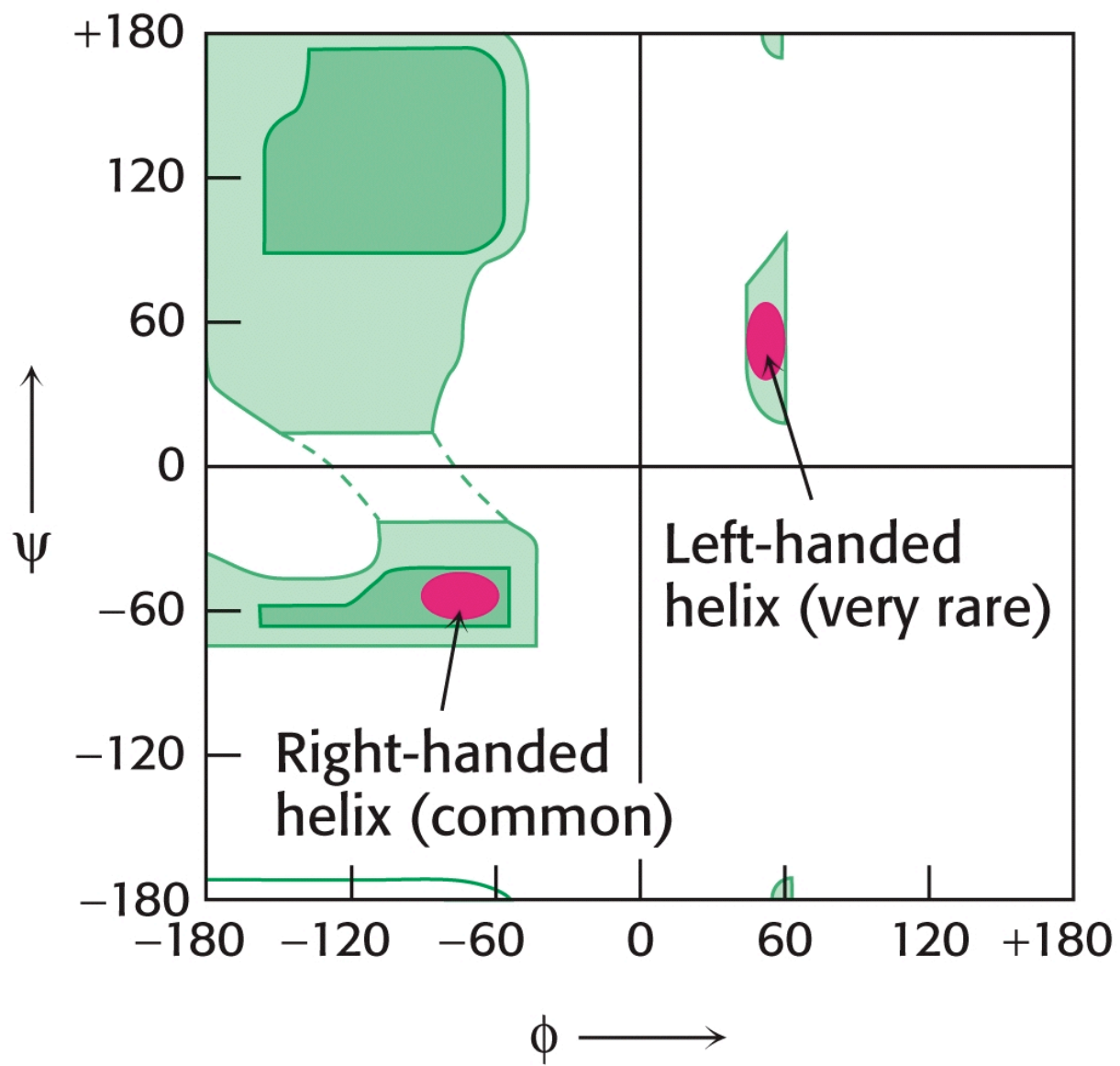
Fogolari et al Biophys J 2007

Kihara et al JBC 2006

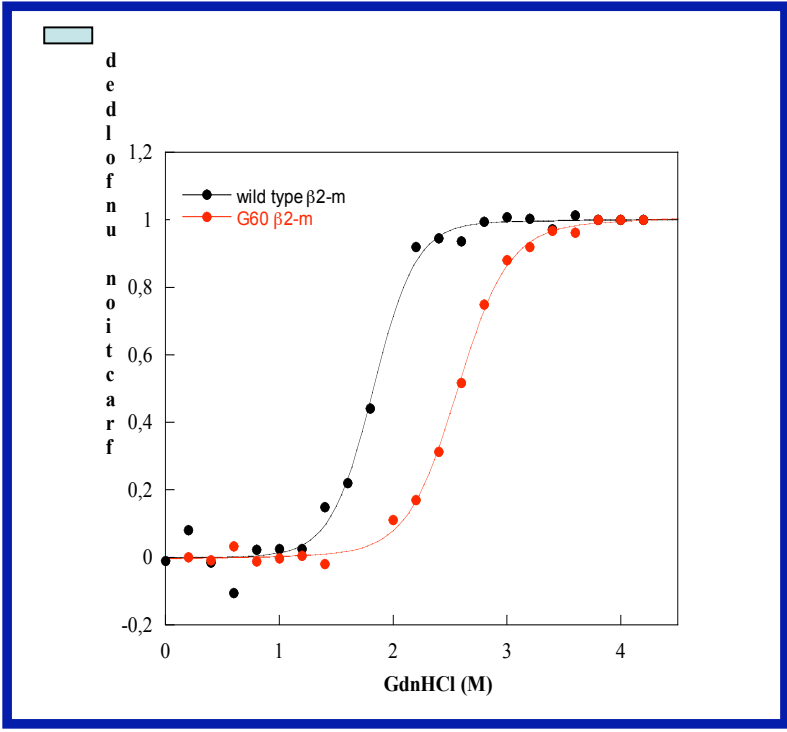
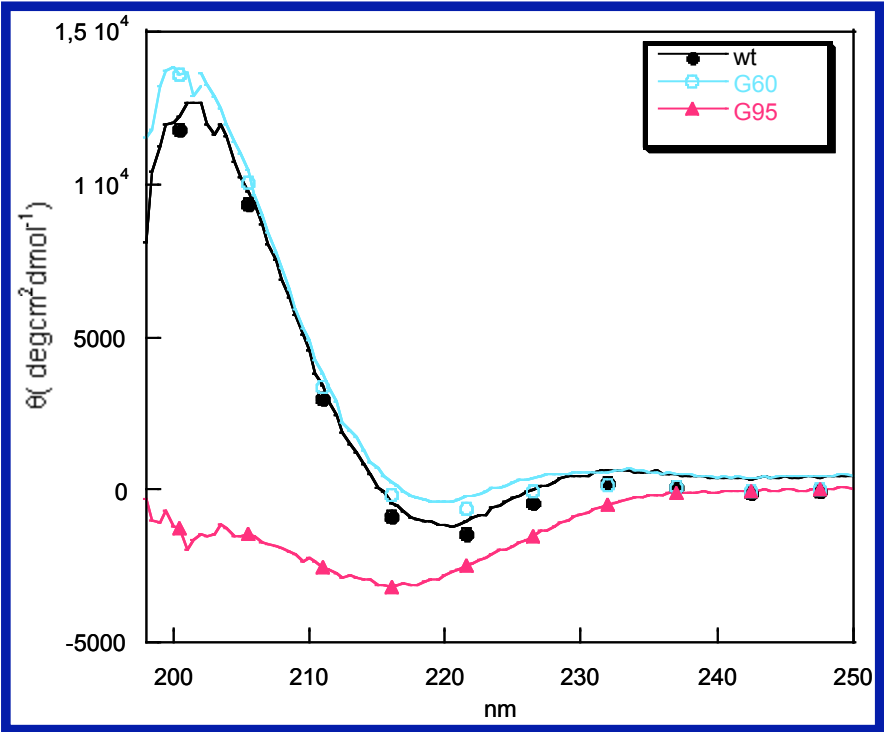


Trp60 occurs in α_L backbone conformation in MHCI

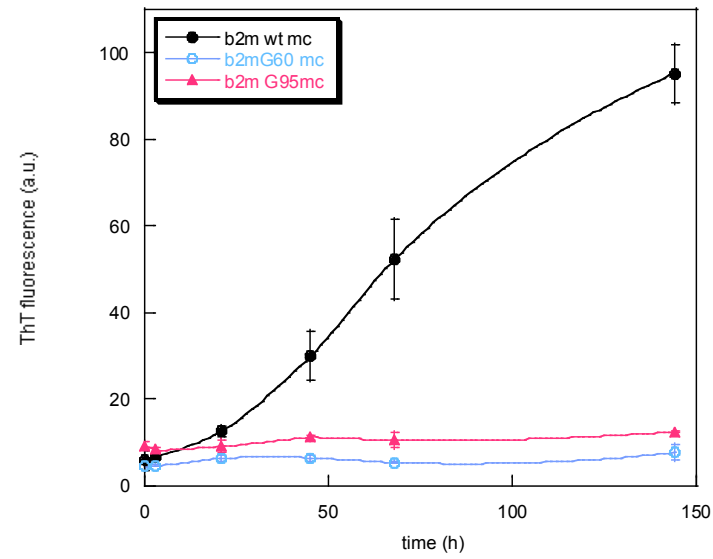




Effect of mutagenesis of Trp 60- to Gly



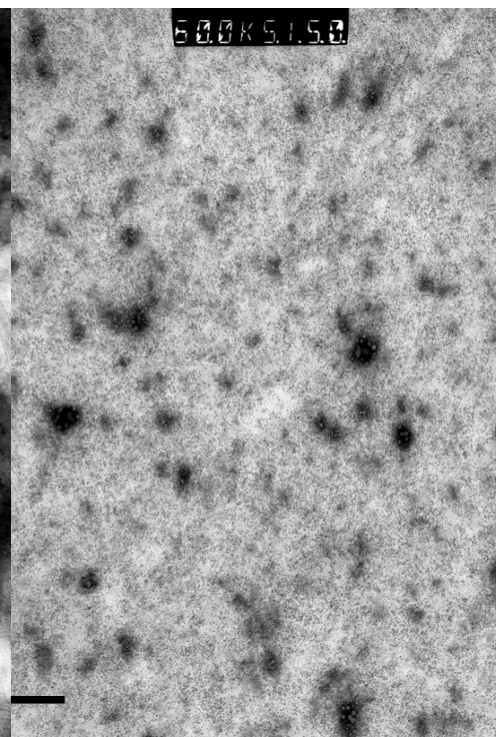
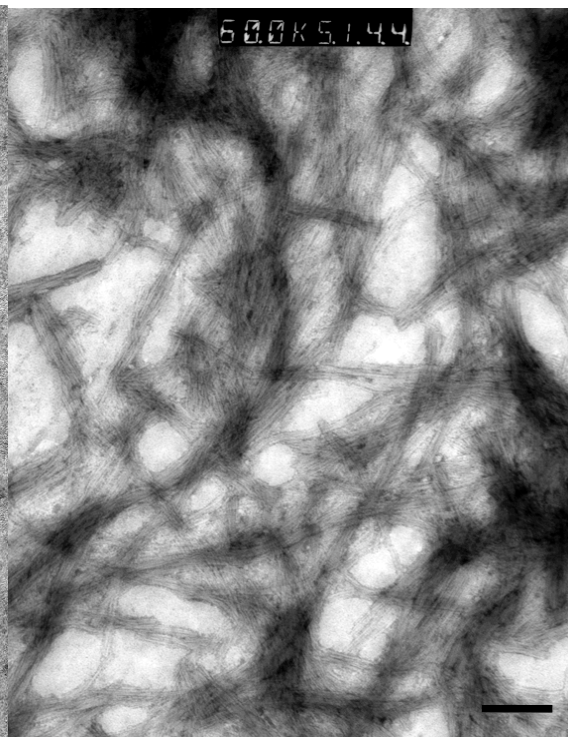
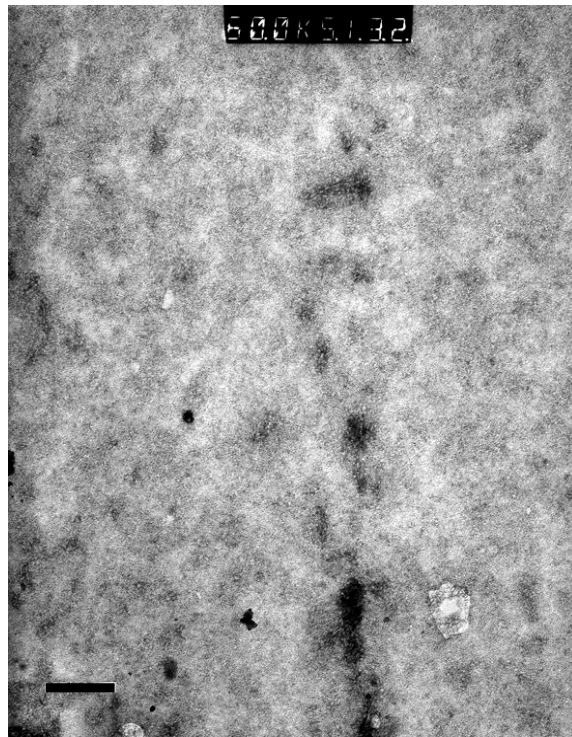
fibrillogenesi 10/04/2007



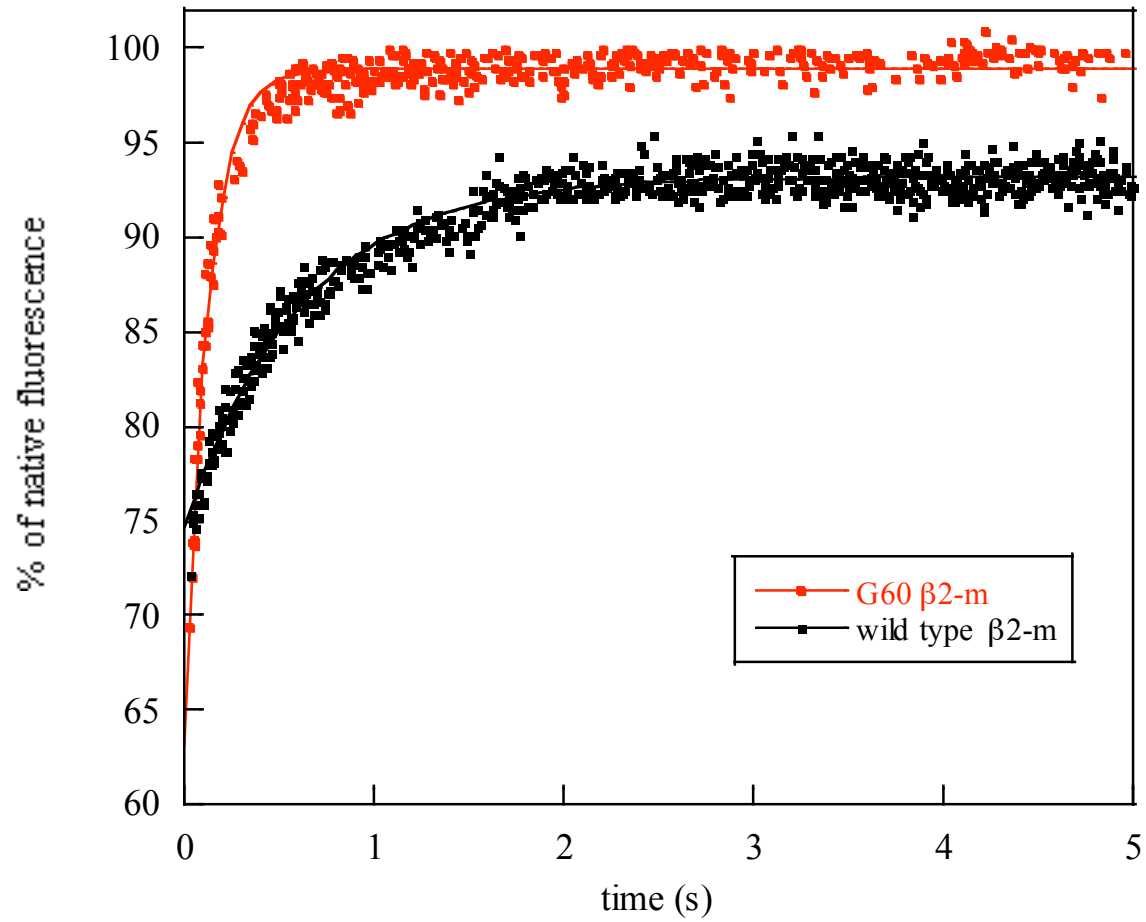
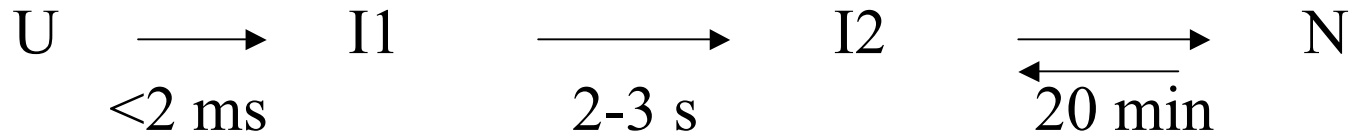
G60

WT

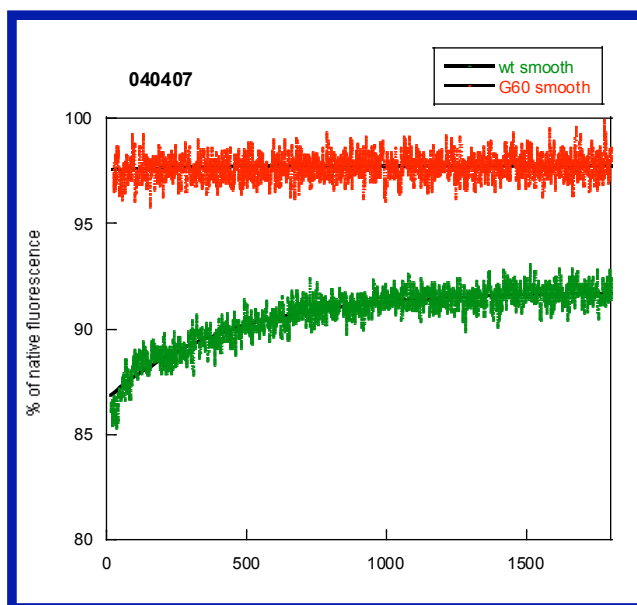
G95



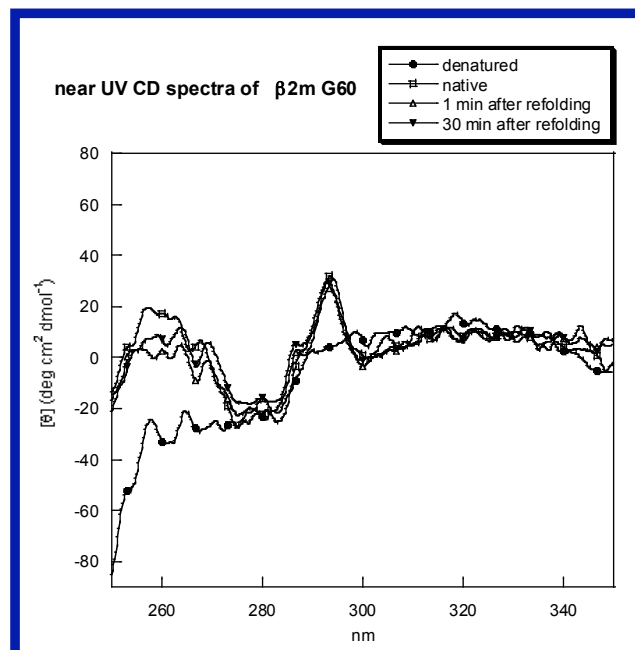
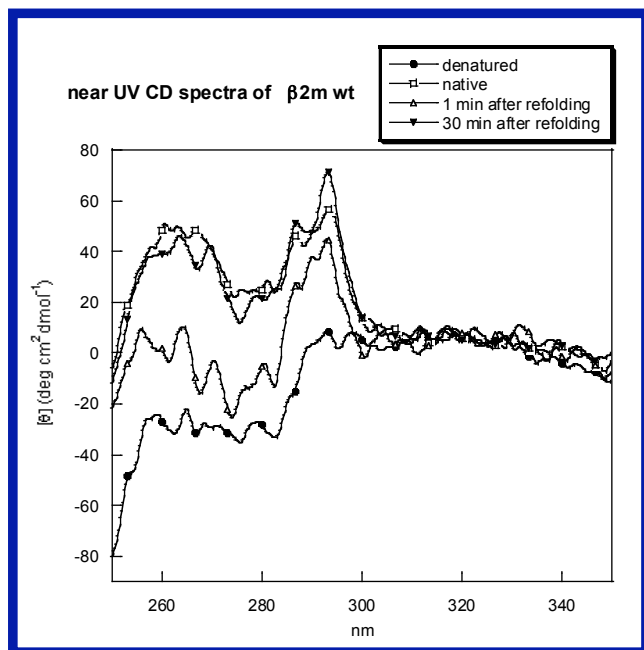
Effect of Trp replacement of β 2-m folding kinetics



Effect of Trp replacement of β 2-m folding kinetics

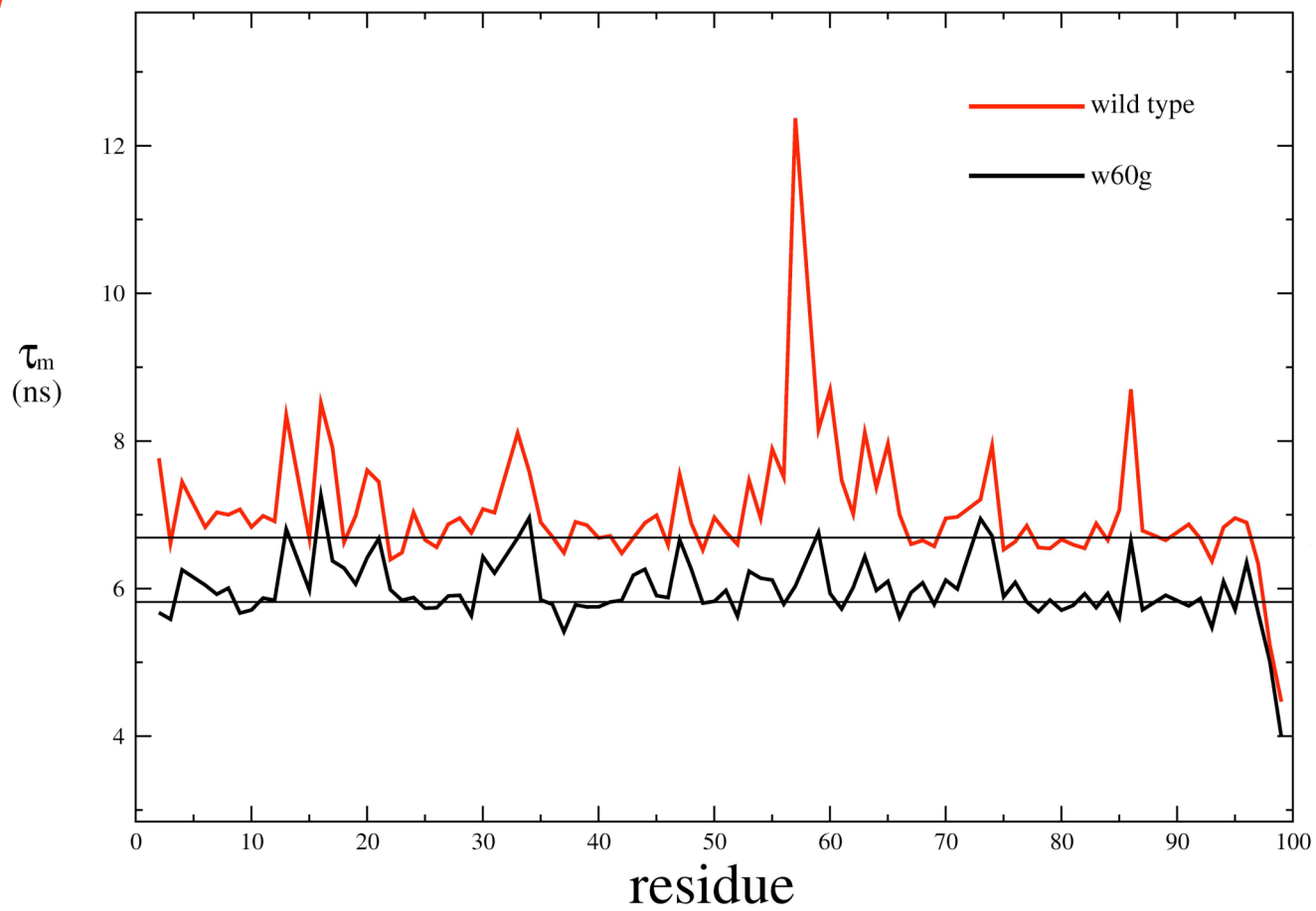


Intrinsic fluorescence

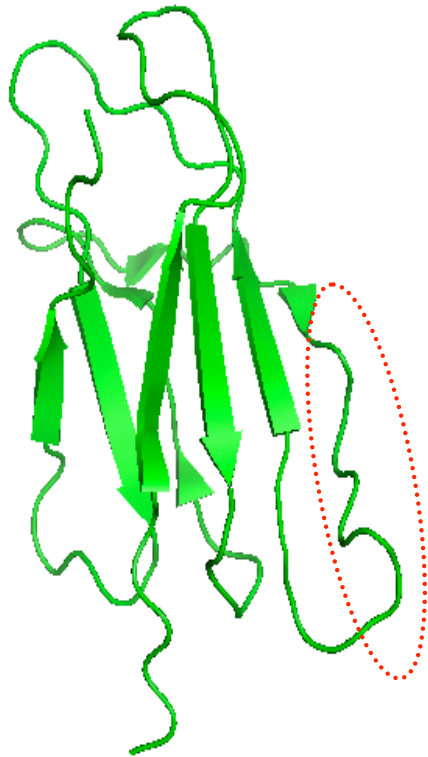


Near UV CD

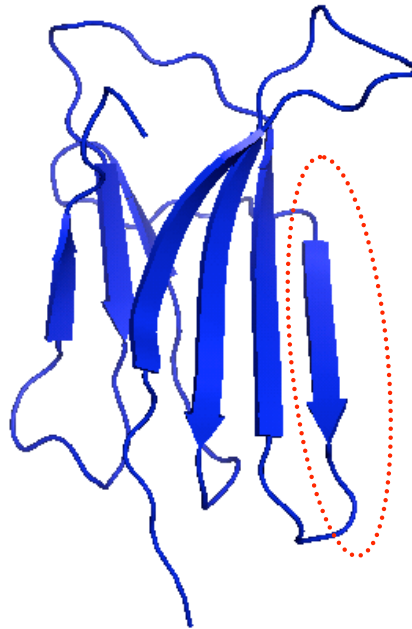
NMR: ^{15}N relaxation measurements



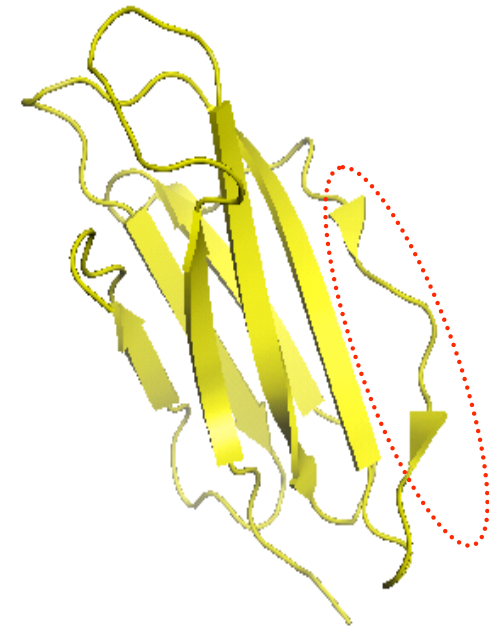
NMR: W60G



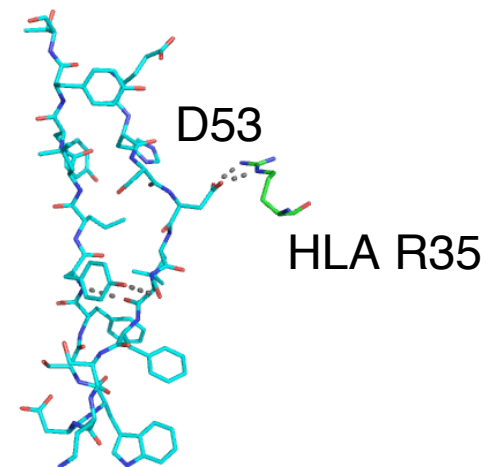
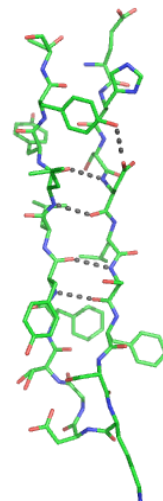
X-ray: W60G



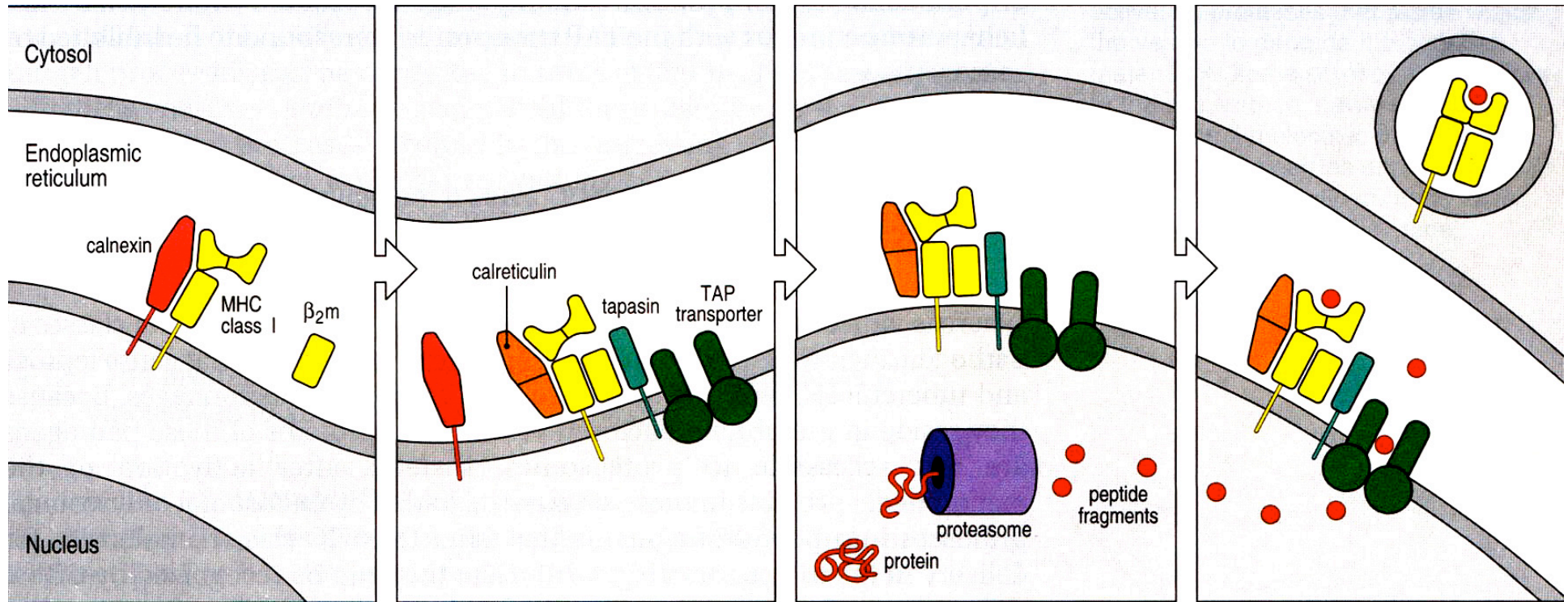
X-ray: wt+HLA
PDB 2B55

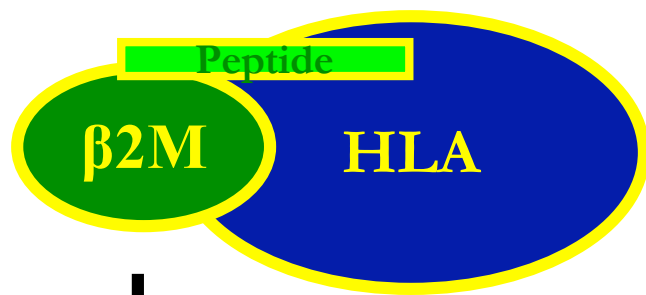


Trinh CH et al
Crystal structure of monomeric human
 β -2-microglobulin reveals clues to its
amyloidogenic properties
Proc Natl Acad Sci U S A. 2002
PDB 1LDS

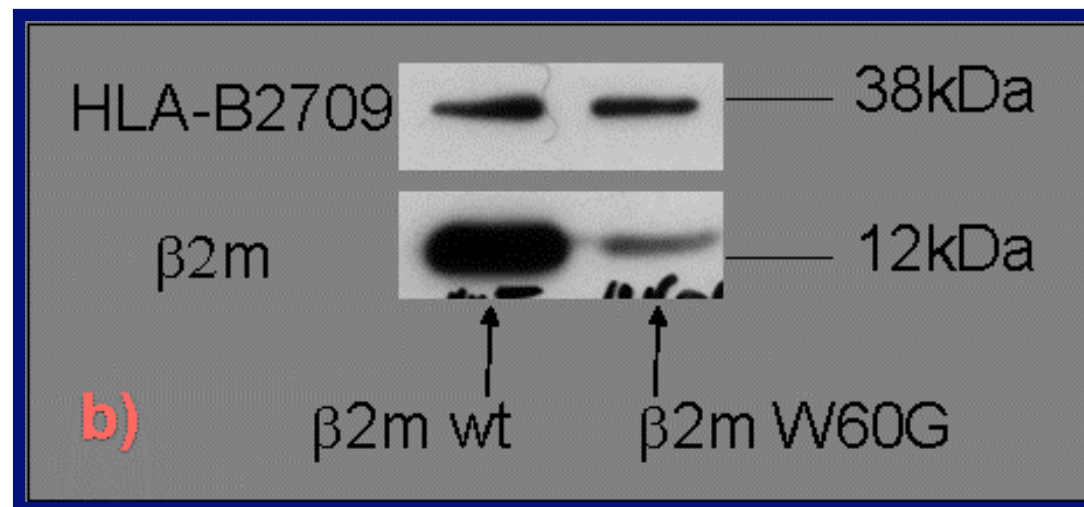
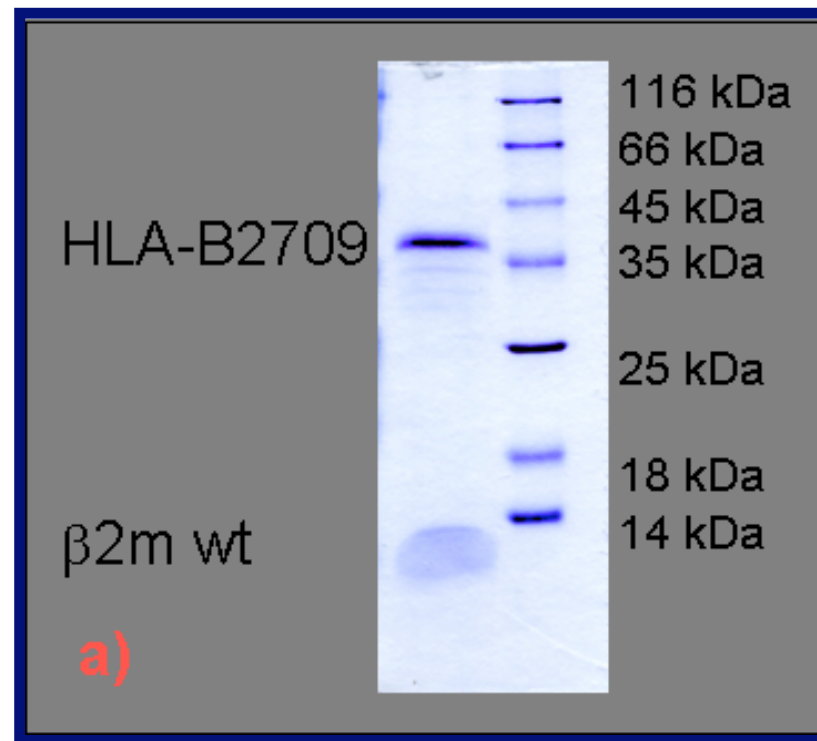
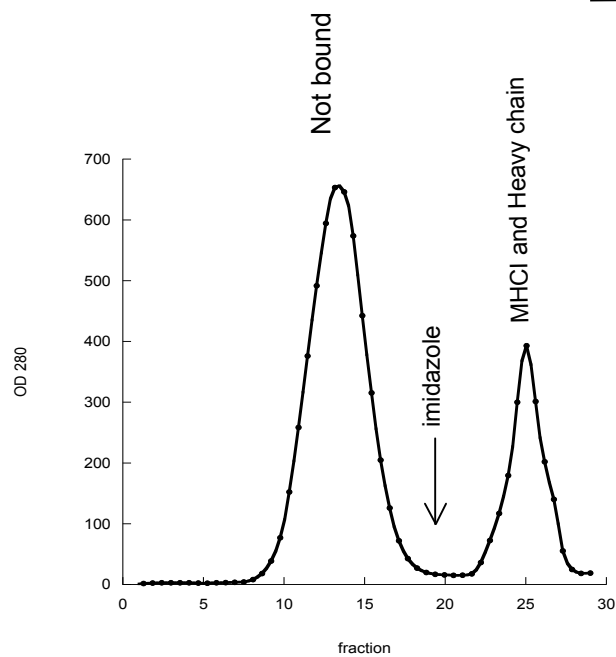


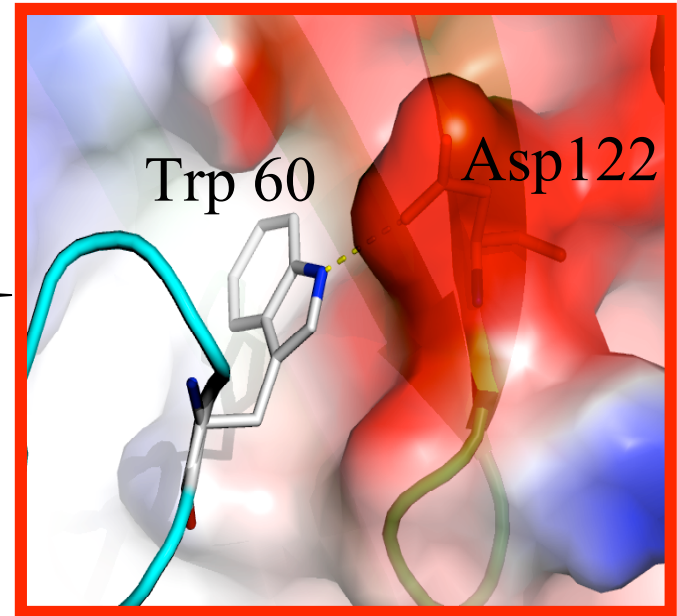
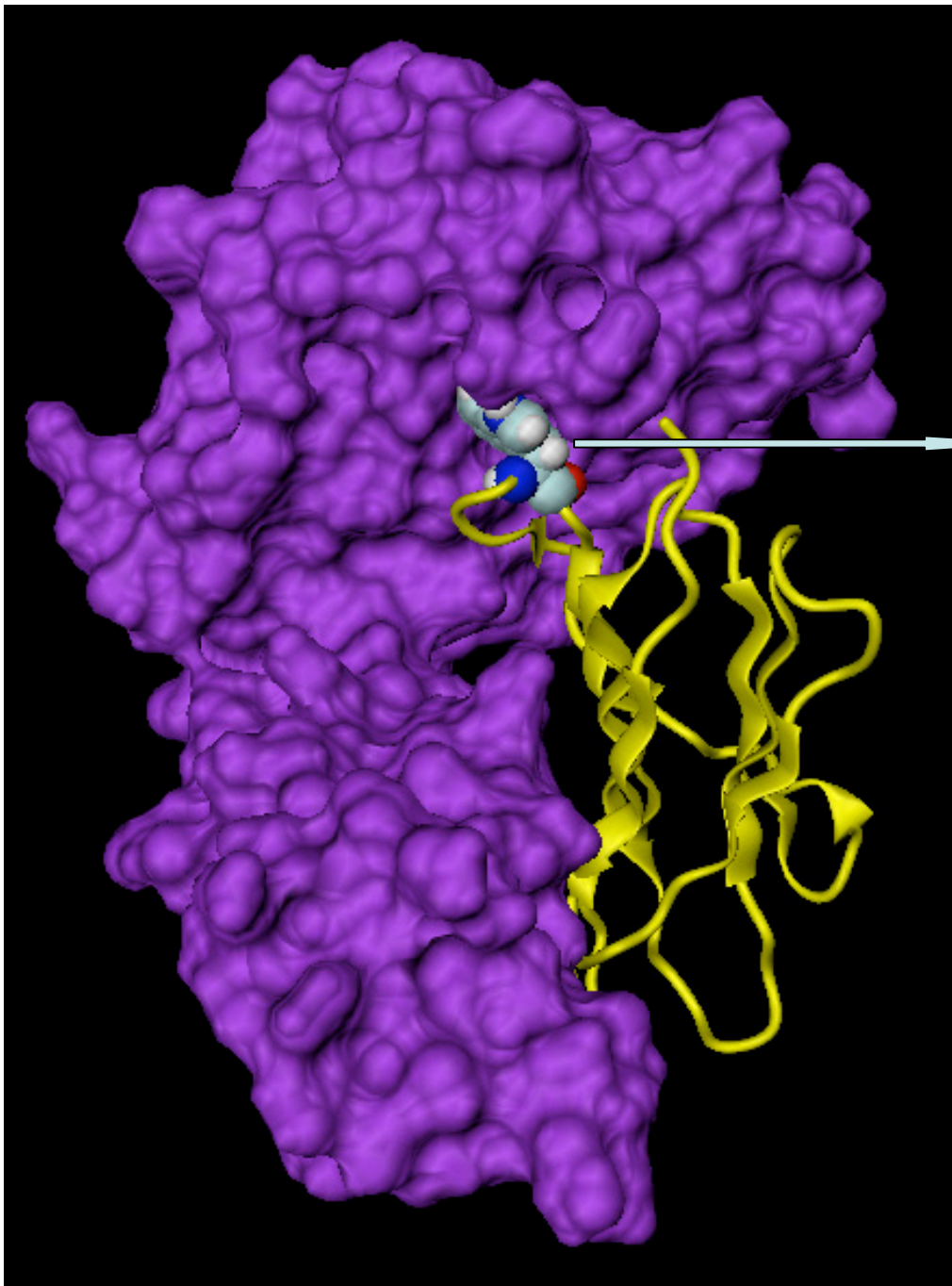
Intracellular assembly of the β 2-m in the MHCI



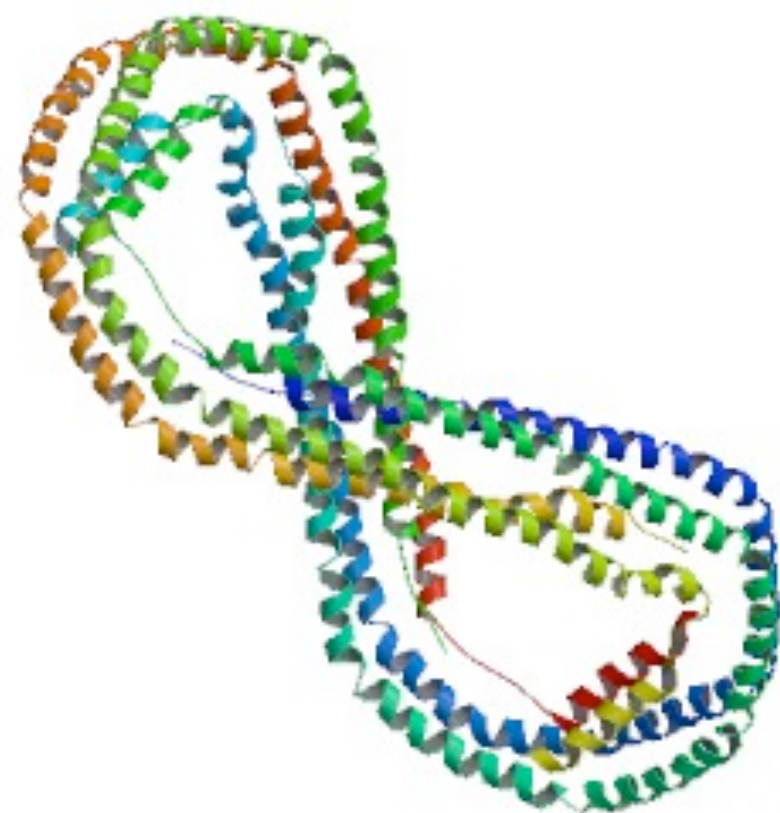


Affinity chromatography
(Nickel-His Tag)

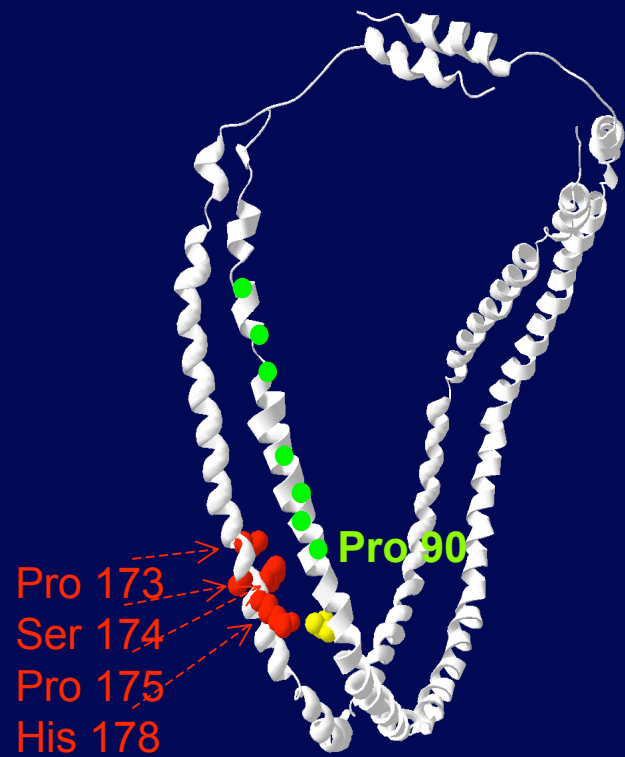




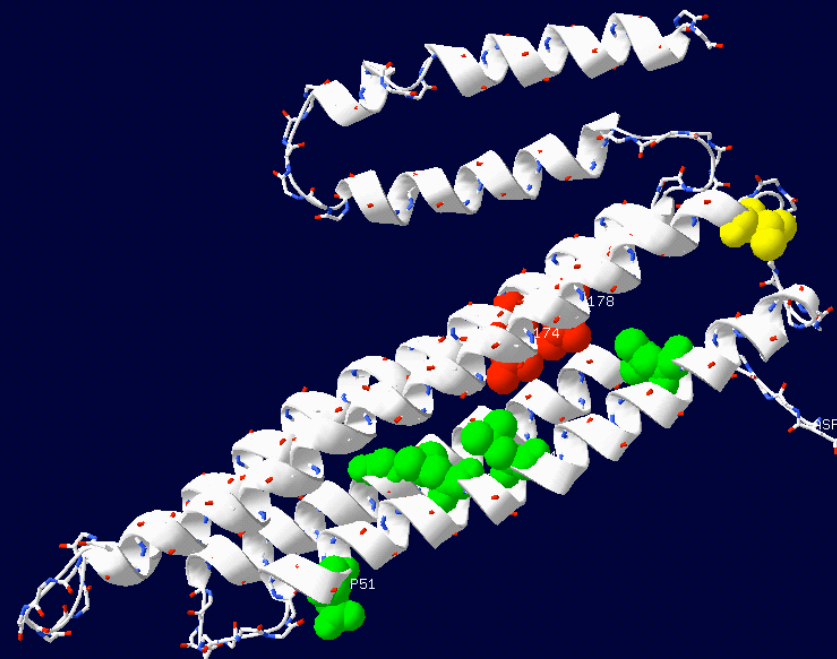
PDB 2BSS



<i>ApoA-I Variant</i>	<i>Fragments</i>	<i>ref</i>
Gly26Arg	1-83	BBA 156:762-768, 1998
Leu60Arg	1-88, 1-92, 1-93, 1-94	PNAS 89 :7389-7393, 1992
Trp50Arg	1-86, 1-92, 1-93	QJ Med 88 :695-702, 1995
Glu70Phe71Trp72 Deletion	ND	Kidney Int 53:276-281, 1998
Leu90Pro	1-88, 1-94	Am J Pathol 154:221-227
Arg173Pro	1-90 to 1-100	BBRC 257:584-588, 1999
60-71 Deletion/ ValThr Insertion	1-83,1-92	J Clin Invest 97:2714-2721, 1996
Leu174Ser	1-93	Am J Pathol 155: 695-702, 1999
Leu178His	NA	BBRC 242 :534-539, 1998
Leu75Pro	≈ 1-96	Amyloid 10:215-223, 2003 Gastroenterology. 126:1416-1422, 2004
Ala175Pro	ND	New Engl J Med 346:1786-91, 2002
Leu64Pro	≈ 1-96	Am J Kidney Dis. 44:1103-9, 2004



PDB 1AV1



2A01



1 DEPPQSPWDRVKDLATVYVDVLKDSGRDYVSQFEGSALGKQLNLKLLDNWDSVTSTFSKLRQLGPVTQEFWDNLEKETEGLRQEMSKDLEEV 93

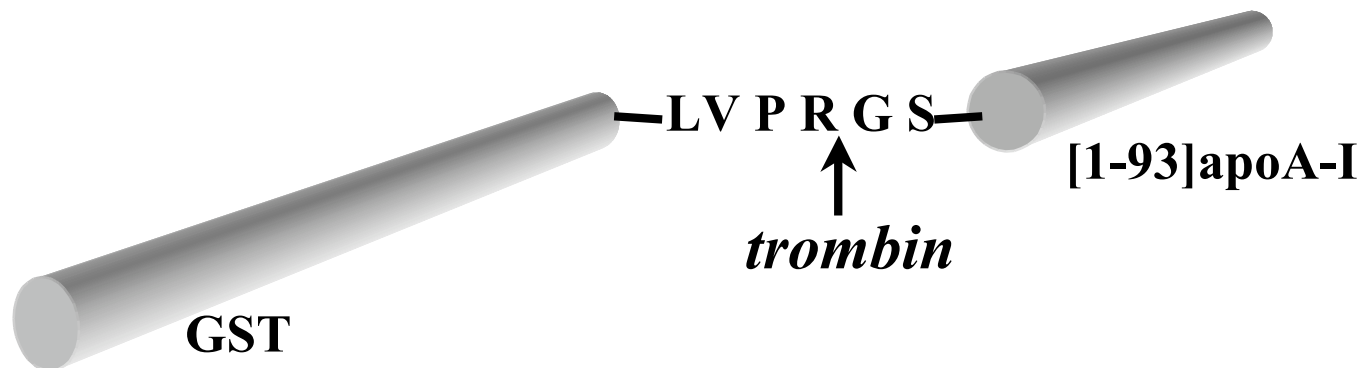
	length (a.a)	mass (Da)	pI	net charge	mean net charge (R)	mean hydrophobicity (H)
ApoA-I (1-93)	93	10720	4.3	-9	0.097	0.412
ApoA-I (full length)	243	28078	5.27	-9	0.037	0.409

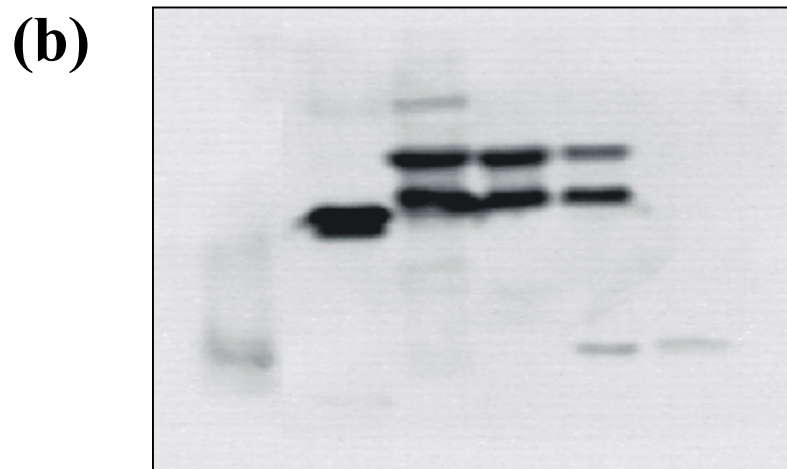
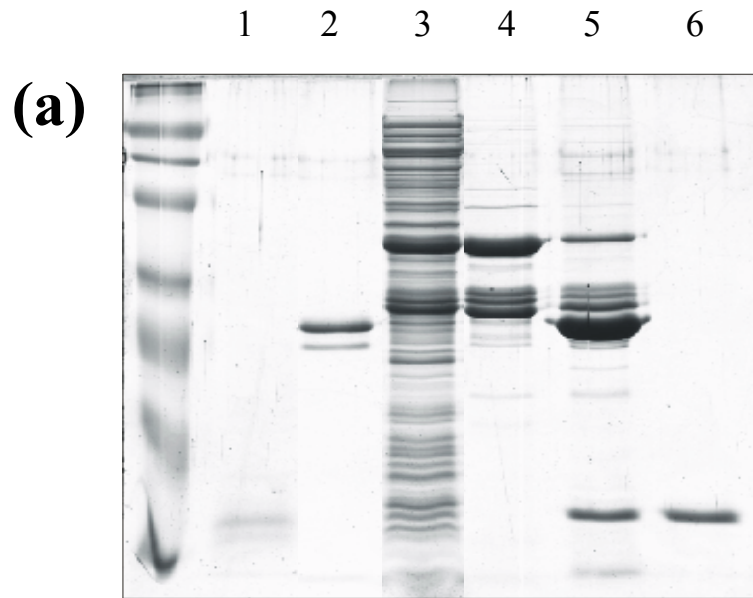
(a)

Apolipoprotein A-I (apoA-I)

¹DEPPQSPWDRVKDLATVYVDVLKDSGRDYVSQFEGSALGKQLNLKLLDNWD
SVTSTFSKLREQLGPVTQEFWDNLEKETEGLRQEMSKDLEEVKAKVQPYLDD
FQKKWQEEMELYRQKVEPLRAELQEGARQKLHELQEKLSPLGEEMRDRAR
AHVDALRTHLAPYSDELQRRLAARLEALKENGGARLAEYHAKATEHLSTLSE
KAKPALEDLRQGLLPVLESFKVSFLSALEEYTKKLNTQ²⁴³

(b)

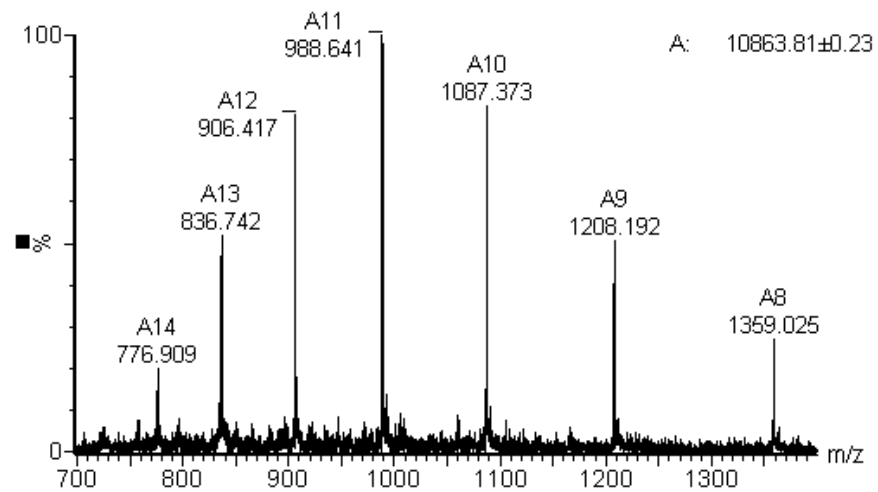
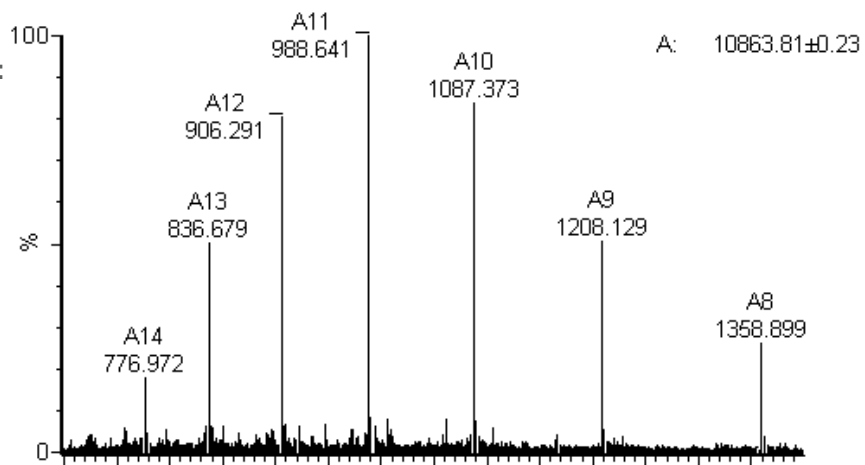
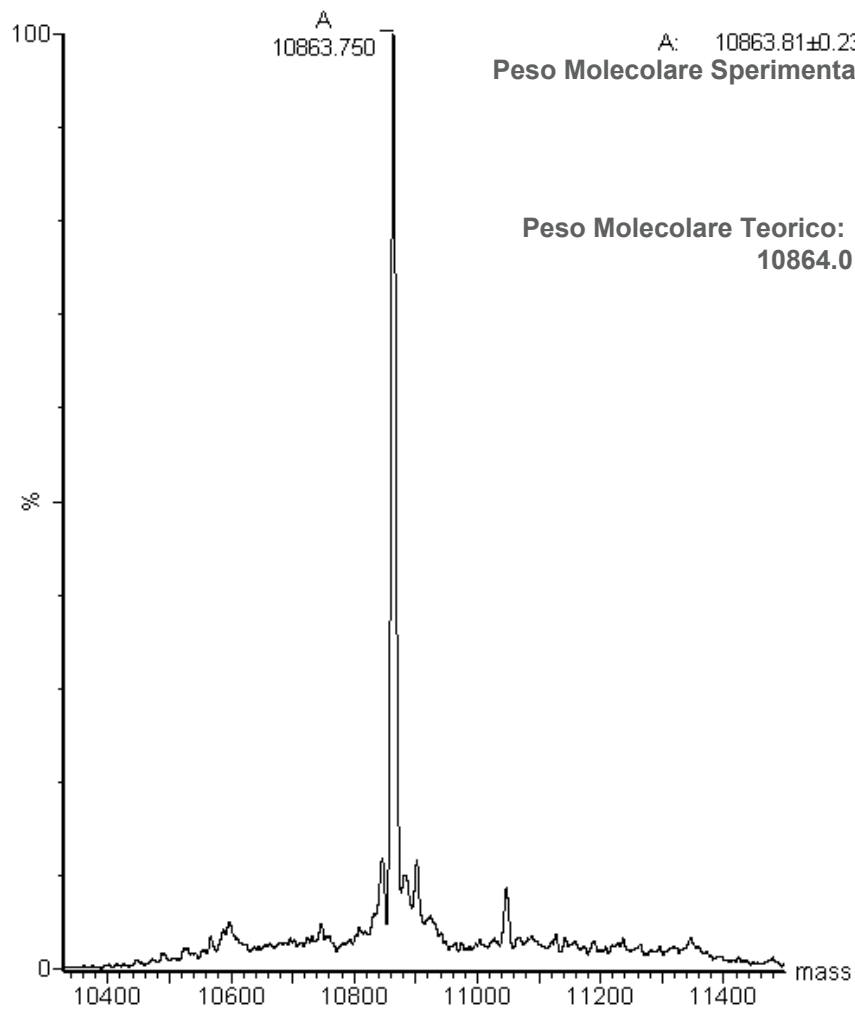




a: Coomassie staining

b: Western blotting

1. [1-93]apoA-I from *ex vivo* fibrils
2. wt apoA-I
3. soluble fraction of bacterial cells transformed with pGEX-4T-3/[1-93]apoA-I
4. GST- containing proteins selected by GSH- agarose affinity chromatography
5. products of trombin digestion
6. recombinant [1-93]apoA-I isolated by HPLC

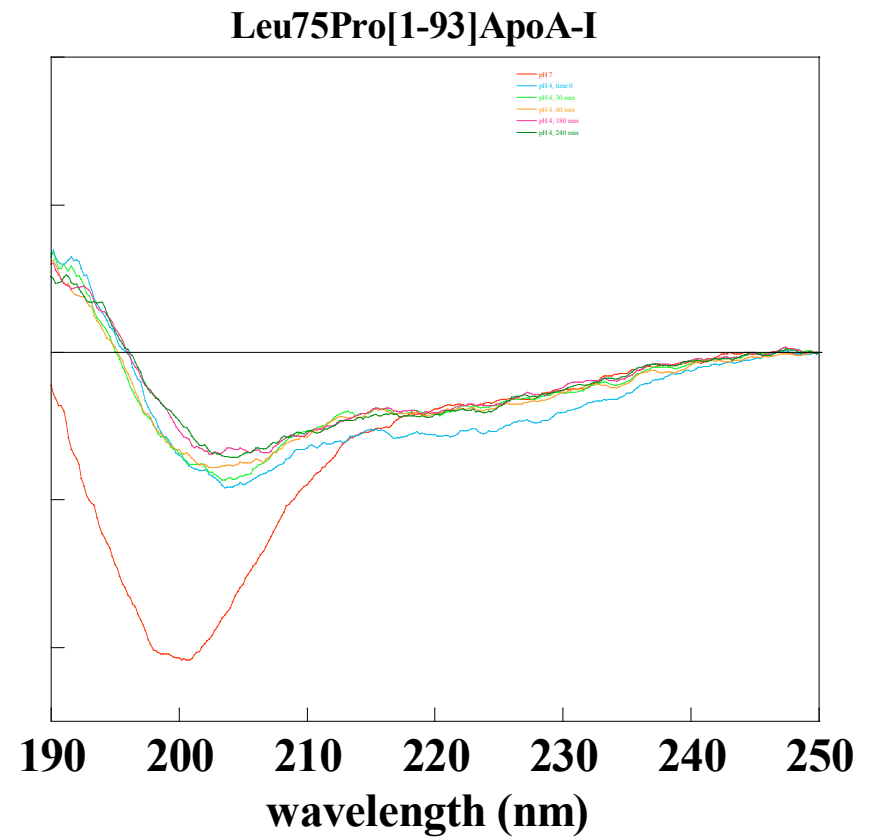
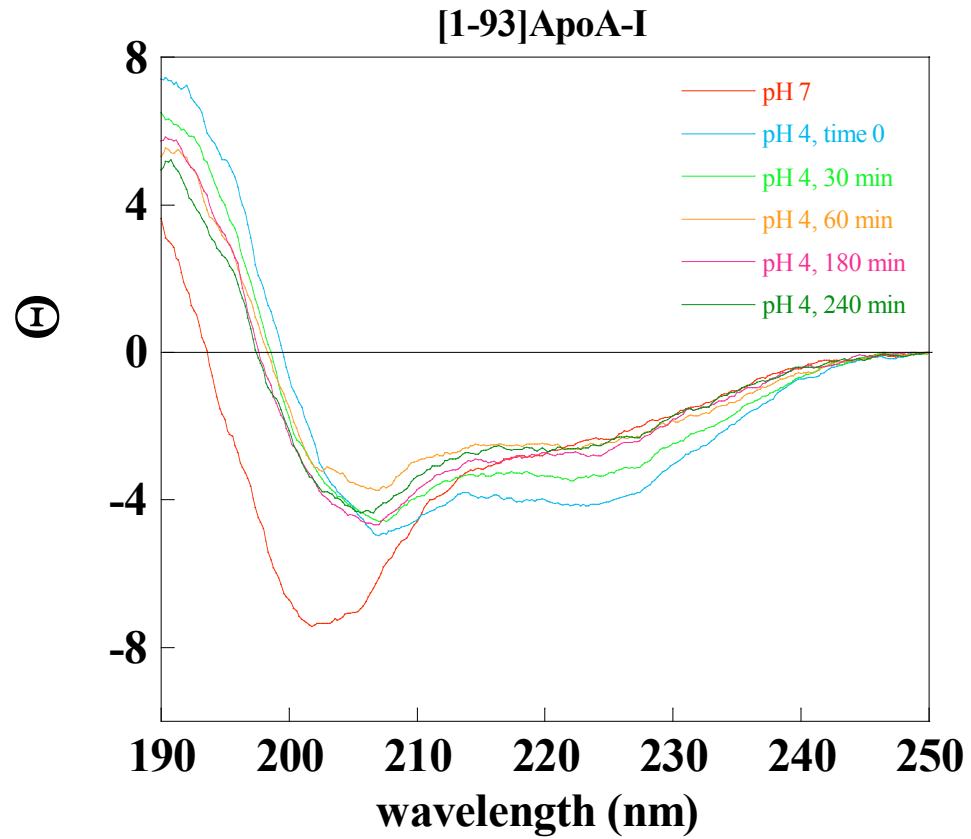


Z aggregation propensity

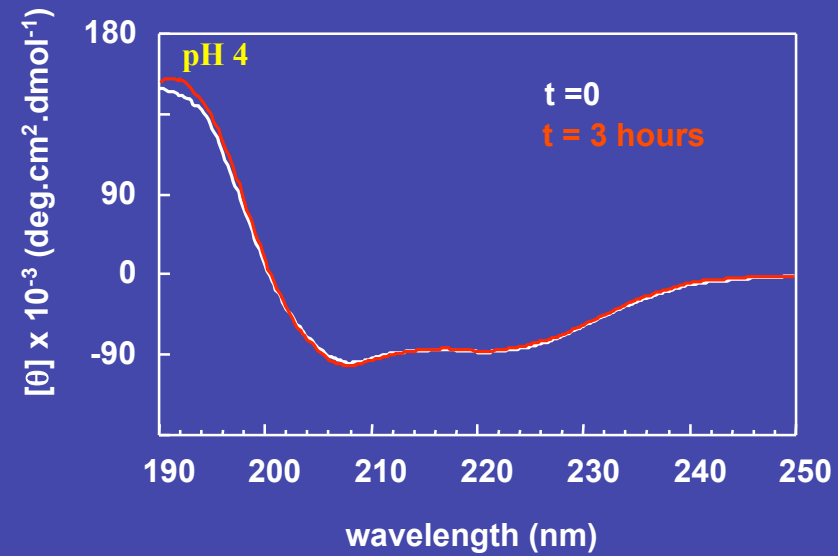
	pH 4	pH 7
WT	0,185358	-1,093692
Gly26Arg	0,016048	-1,10802
Trp50Arg	-0,063729	-1,19181
Leu60Arg	-0,027002	-1,153235
Leu64Pro	0,049564	-1,236317
Leu75Pro	0,049564	-1,236317

According to the algorithms for the prediction of aggregation propensity the amyloidogenic mutations in the 1-93 polypeptide do not favour the aggregation
Dobson and Pawlowski personal comm.

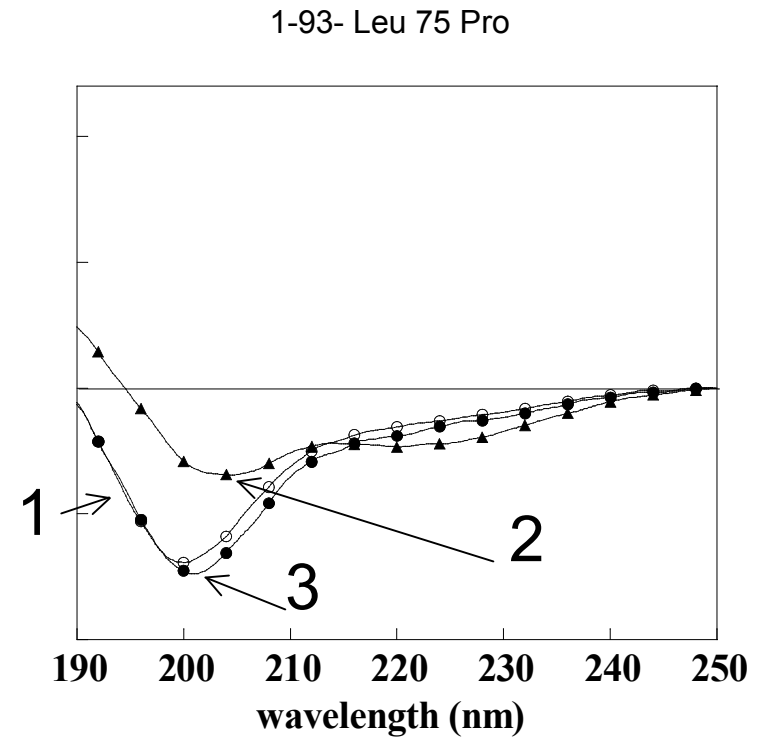
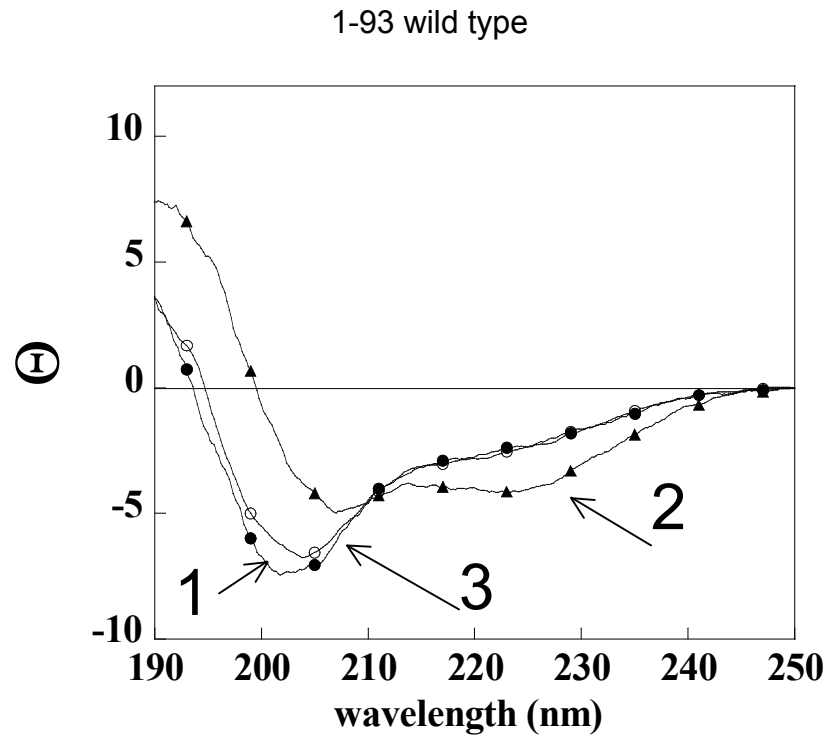
Secondary structure transition induced by a pH jump



Effect of TFE (2, 2, 2- trifluoroethanol) on the ApoA-I (1-93) secondary structure

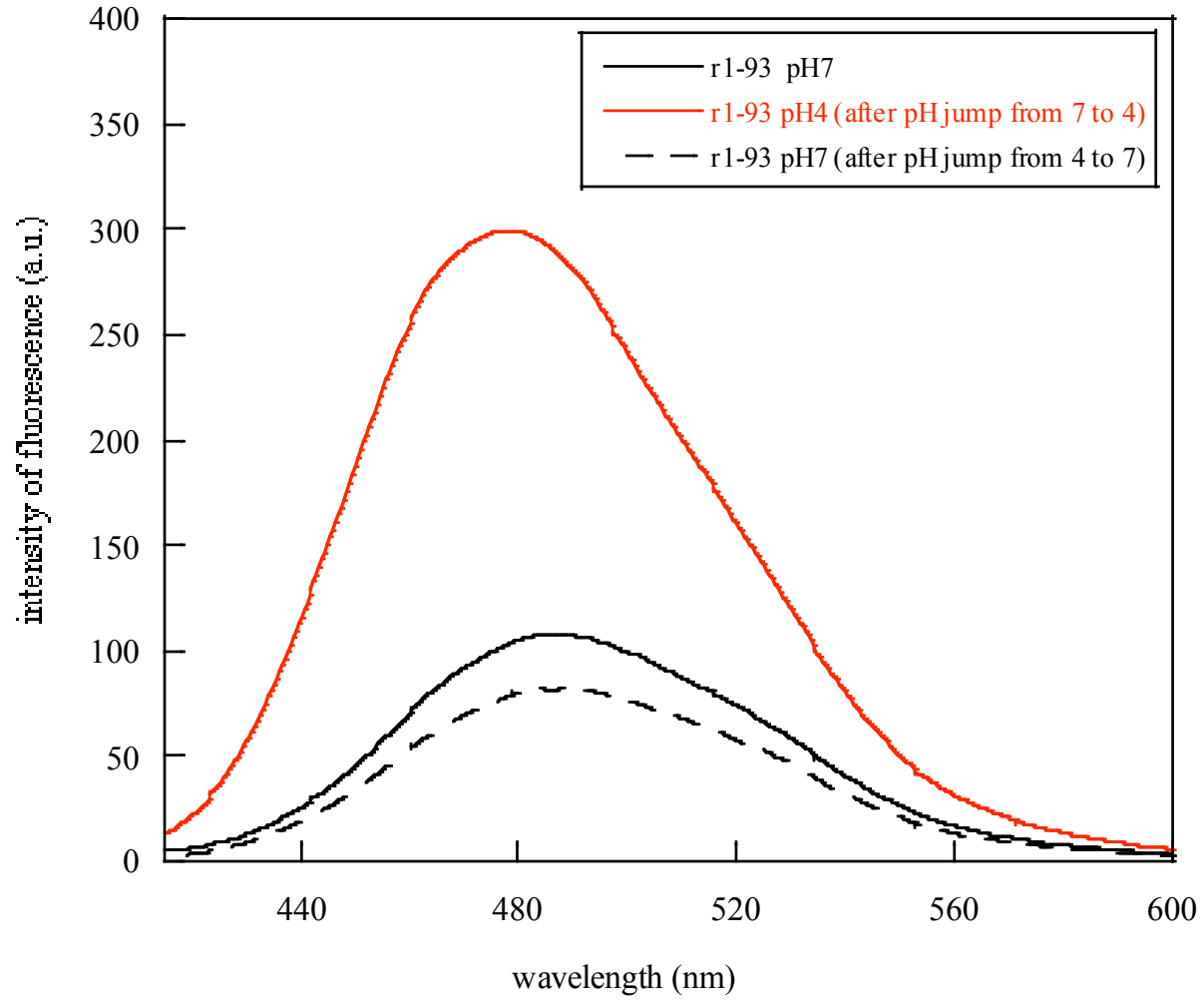


- 1 pH7
- 2 acidification
- 3 neutralisation

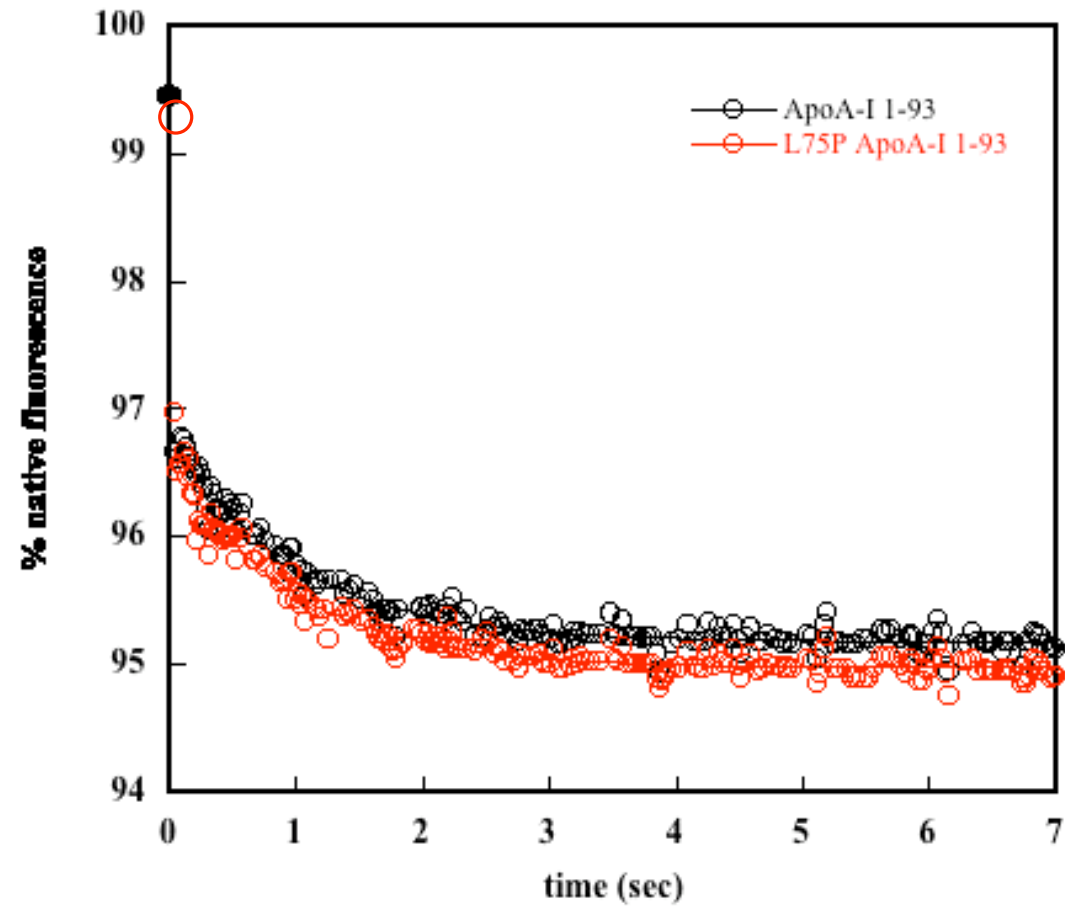


The transition is reversible in the early phase

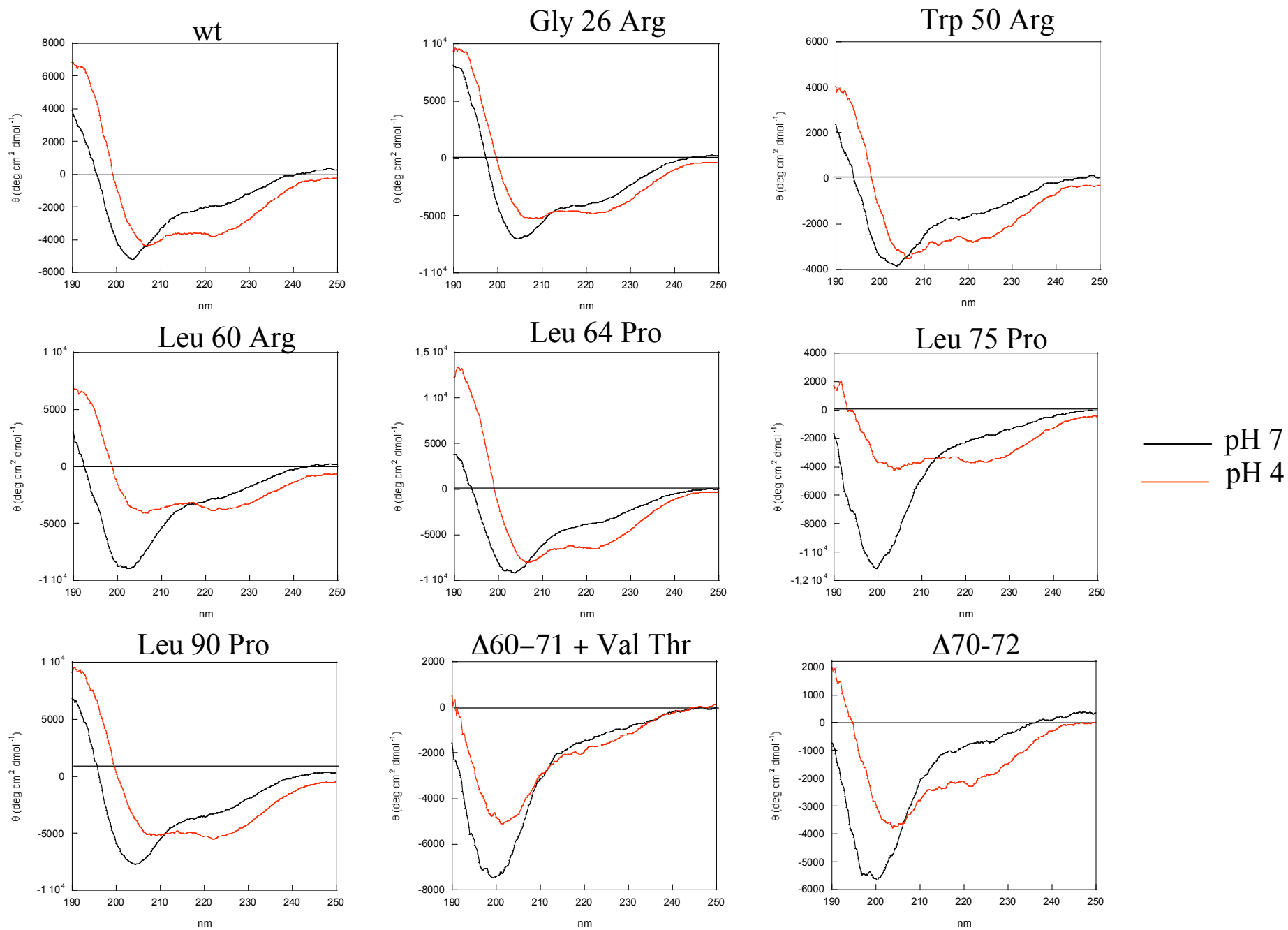
Binding to ANS



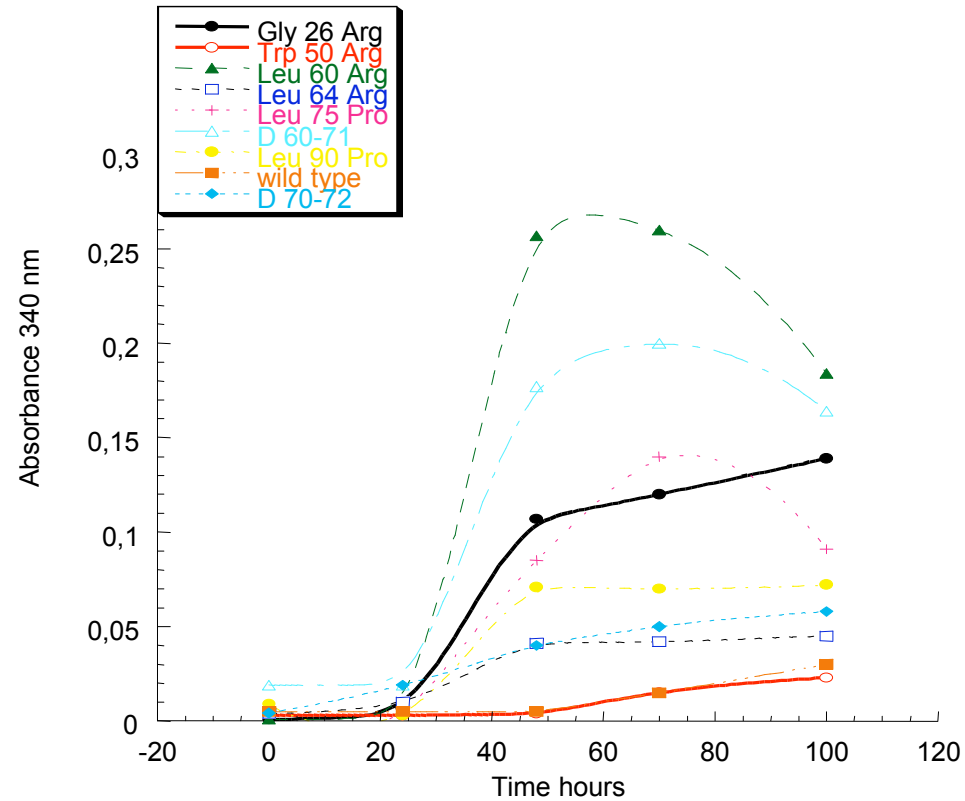
Stopped fluorescence for monitoring the first phase of the pH mediated structure transition



Far-UV CD of 1-93 Apolipoprotein AI variants

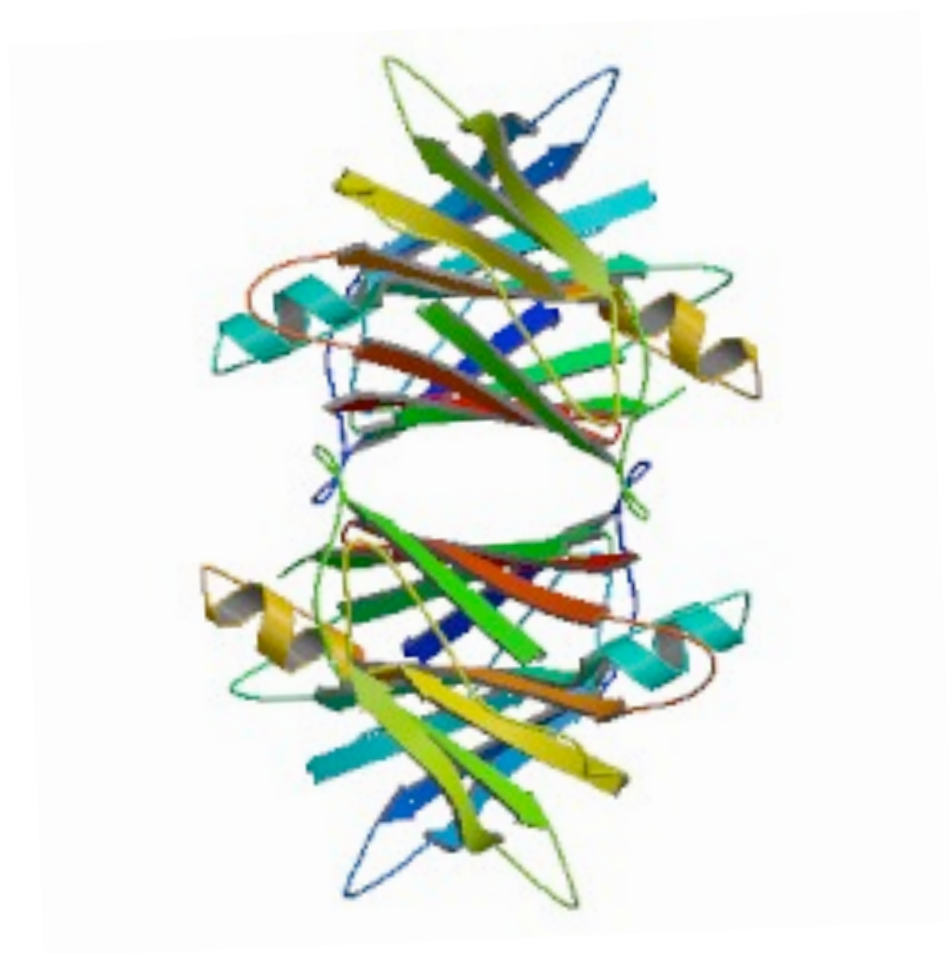


pH induced Aggregation of 1-93 Apolipoprotein AI variants



No massive fibrillar conversion in this time scale

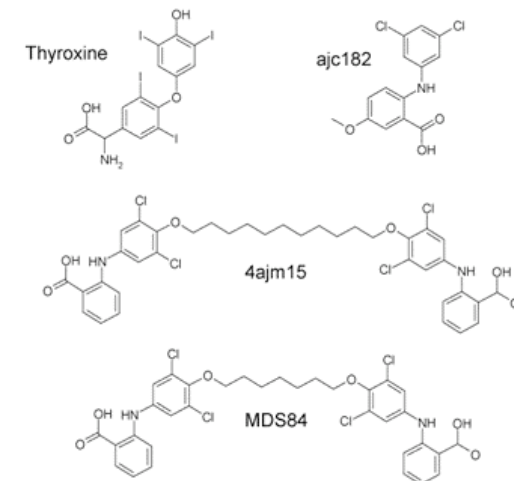
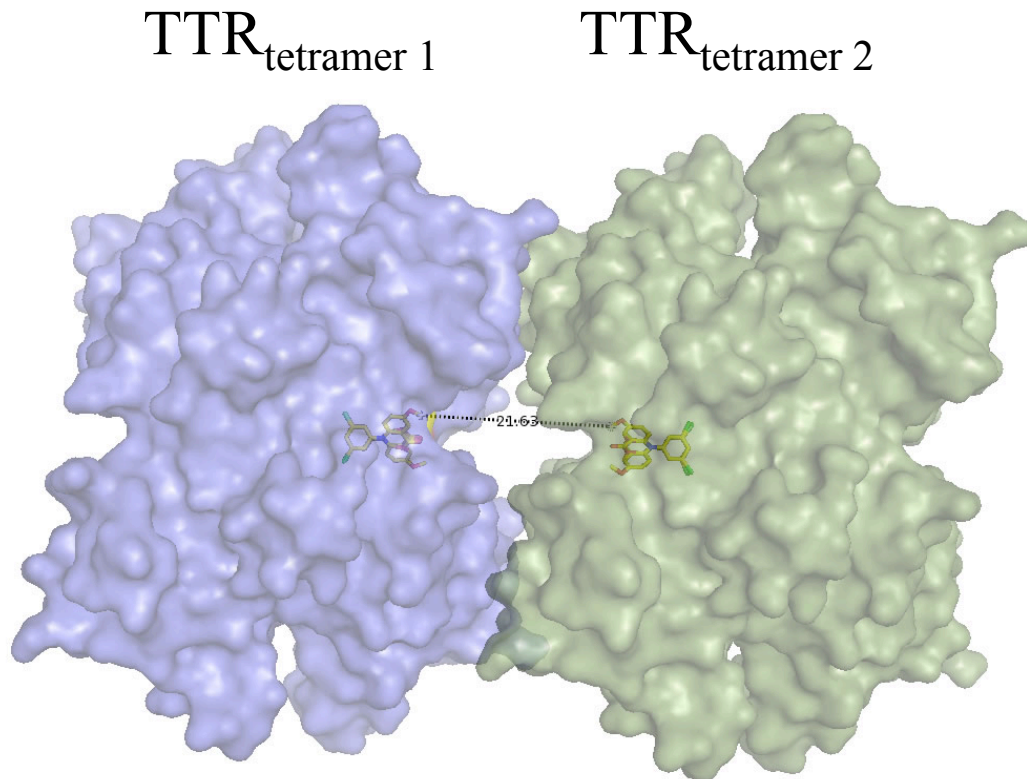
1. Identificazione della proteasi....
2. Descrizione delle condizioni compatibili con l'attività della proteasi....
3. Correlazione algoritmi-esperimenti di fibrillogenesi.....
4. QC di apo mutate.....



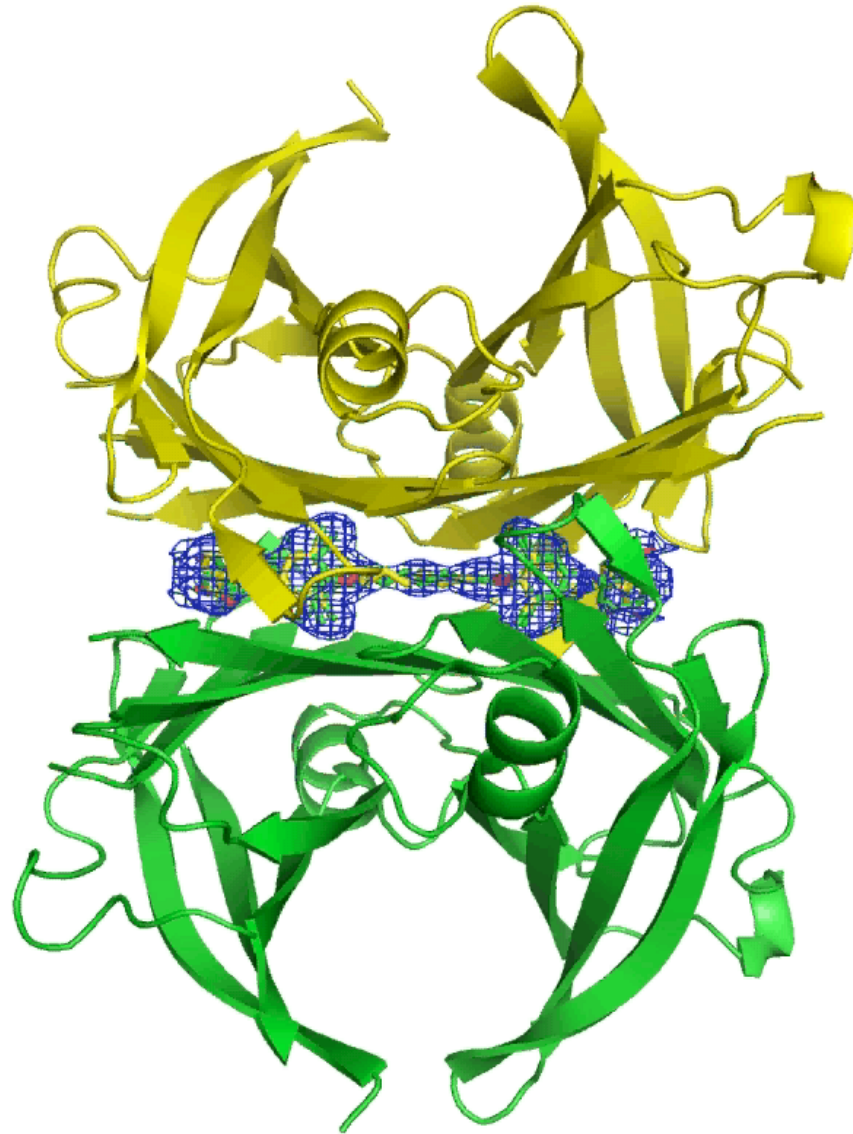
Transizione da struttura globulare a fibrillare

The discovery that amyloidogenic mutations destabilises the globular proteins has generated the medical approach of “ligand mediated stabilisation” (Maria Saraiva & Jeff Kelly)

Pavia is involved in designing new molecules through the collaboration with Mark Pepys lab.in London



*Transizione da struttura
globulare a fibrillare*



4ajm15 is the strongest stabiliser of the TTR tetramer so far described (Carol Robinson personal communication)- Olo-TTR does not make fibrils or oligomers

UCL Business spin-out Pentraxin Therapeutics awarded
grant from the Wellcome Trust Seeding Drug Discovery Initiative

Fine