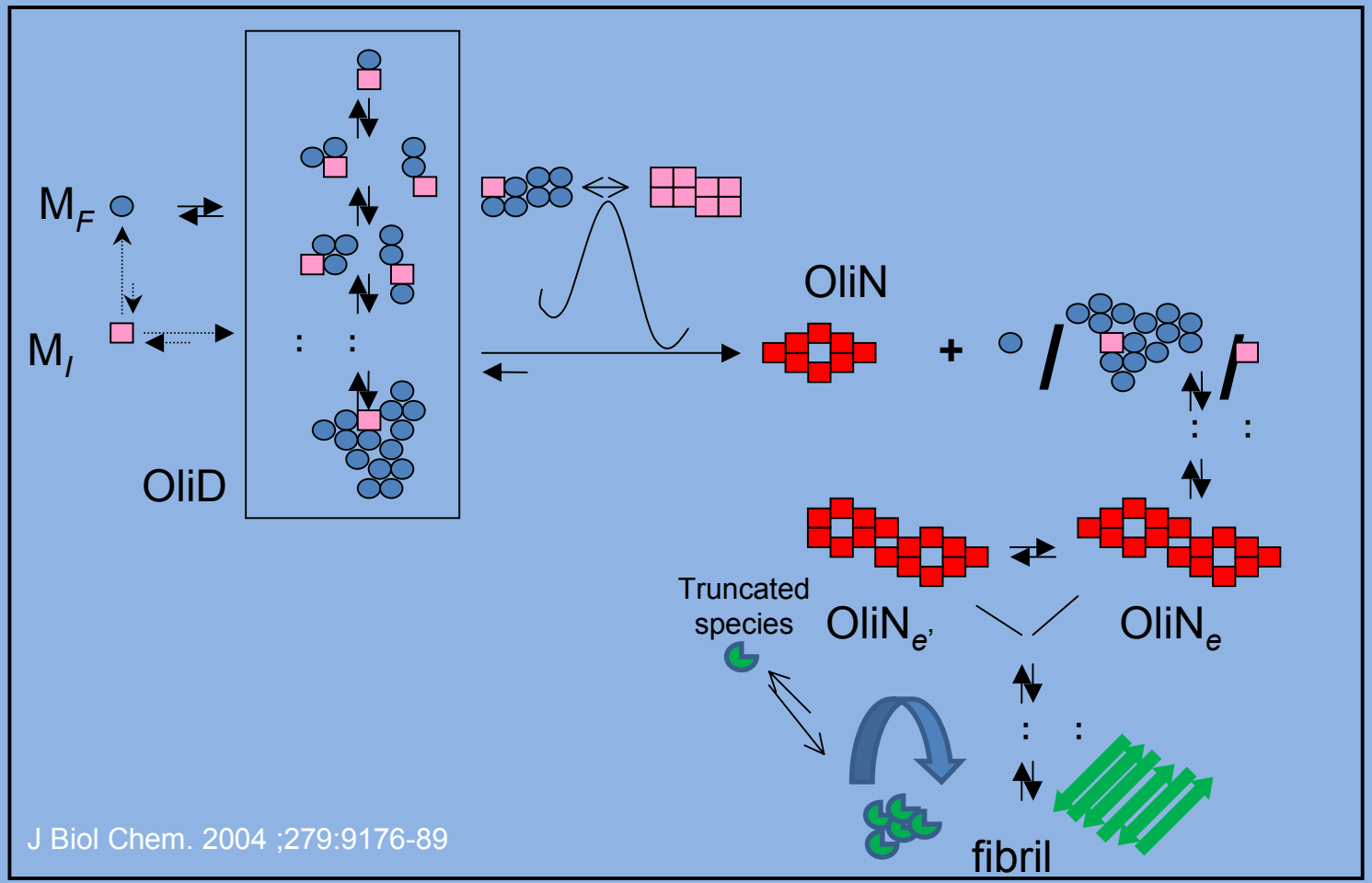
Misfolding of β2-m: new tools for inhibiting protein aggregation



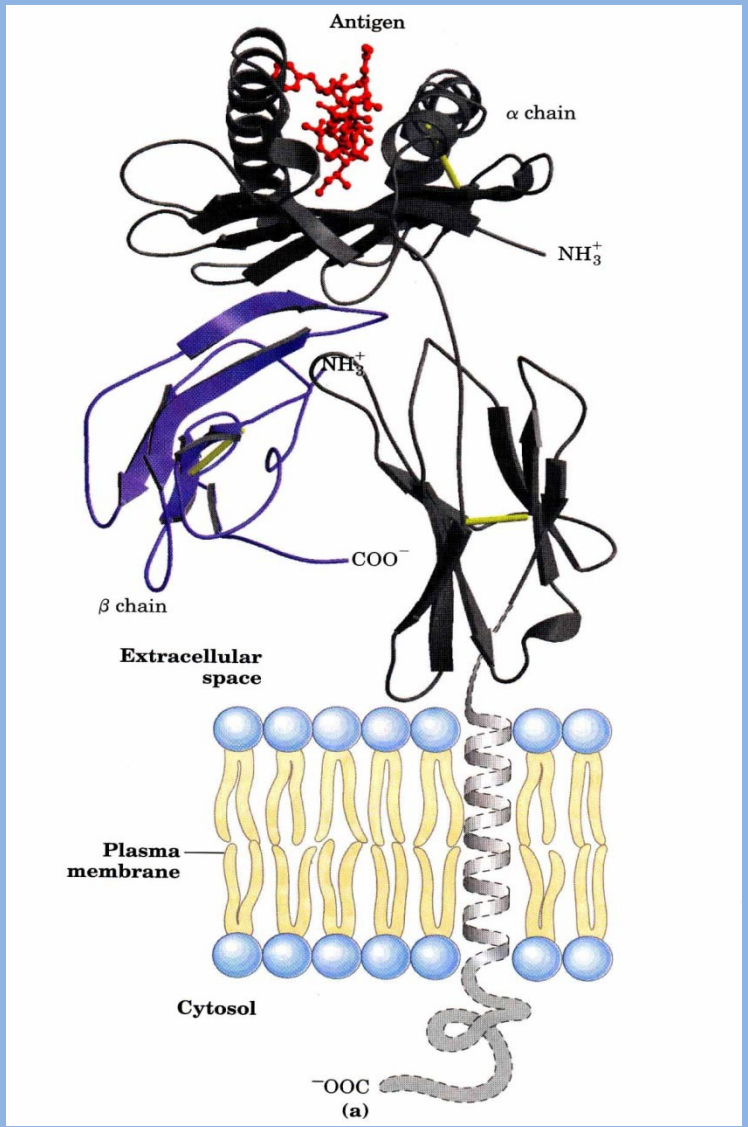
✓ Detection of two partially structured species in the folding process of the amyloidogenic protein beta 2-microglobulin.J Mol Biol. 2001 ;307:379-91.

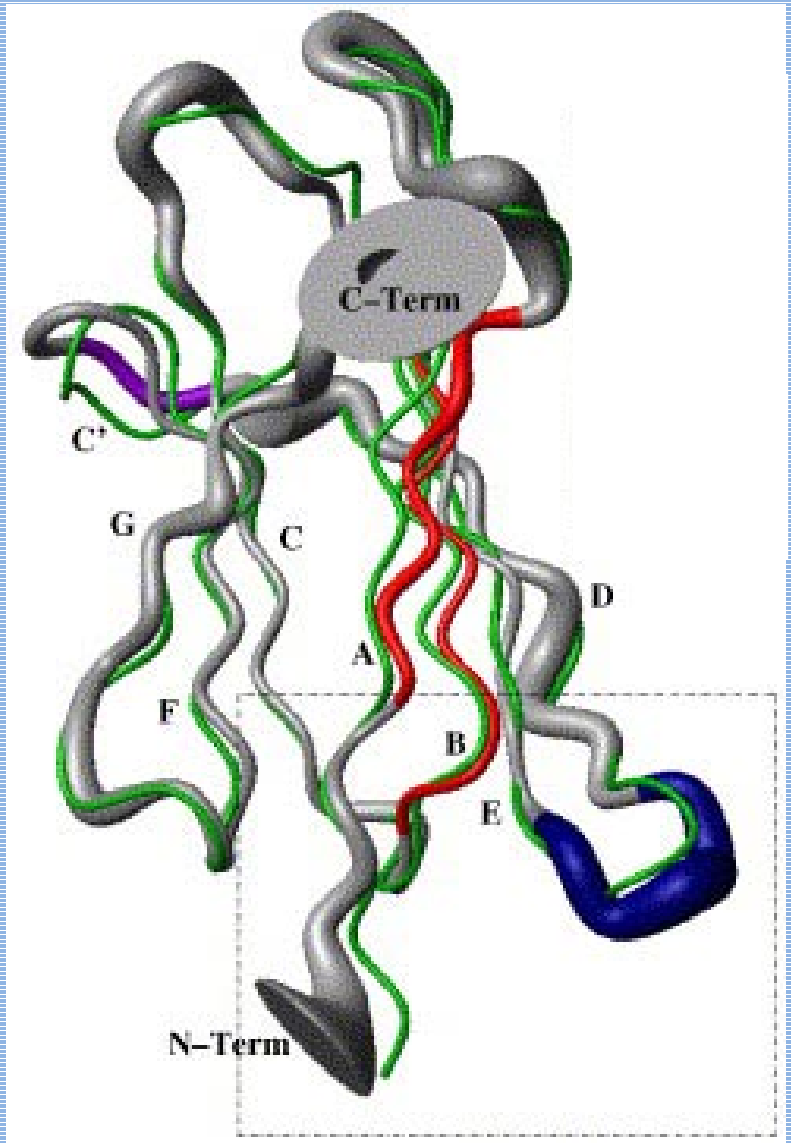
✓ A partially structured species of beta 2-microglobulin is significantly populated under physiological conditions and involved in fibrillogenesis. J Biol Chem. 2001

✓ The solution structure of human beta2-microglobulin reveals the prodromes of its amyloid transition. Protein Sci. 2002 ;11:487-99.

✓Human beta-2 microglobulin W60V mutant structure: Implications for stability and amyloid aggregation. Biochem Biophys Res Commun. 2009 ;38:543-7

✓Detection of fragments of beta2-microglobulin in amyloid fibrils. Kidney Int. 2000;57:349-50


Antigen
α chain
NH_3^+
NH_3^+
COO^-
β chain
Extracellular space
Plasma membrane
Cytosol
^-OOC
(a)



— β2m wt structure in MHC-I crystal

— β2m wt structure in solution

- strands A and B
- interstrand loop D-E
- strand C'
- strands D and E

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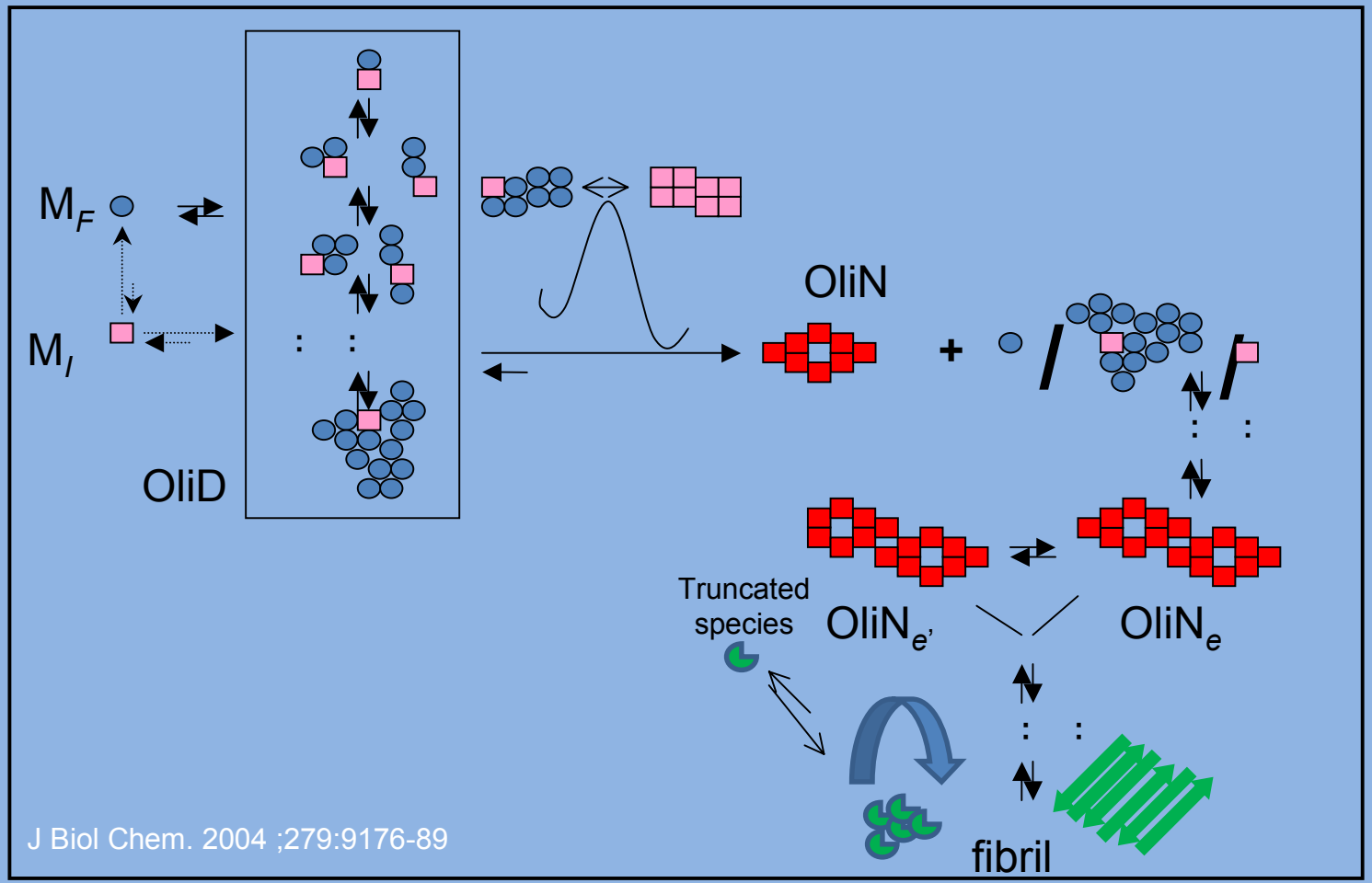

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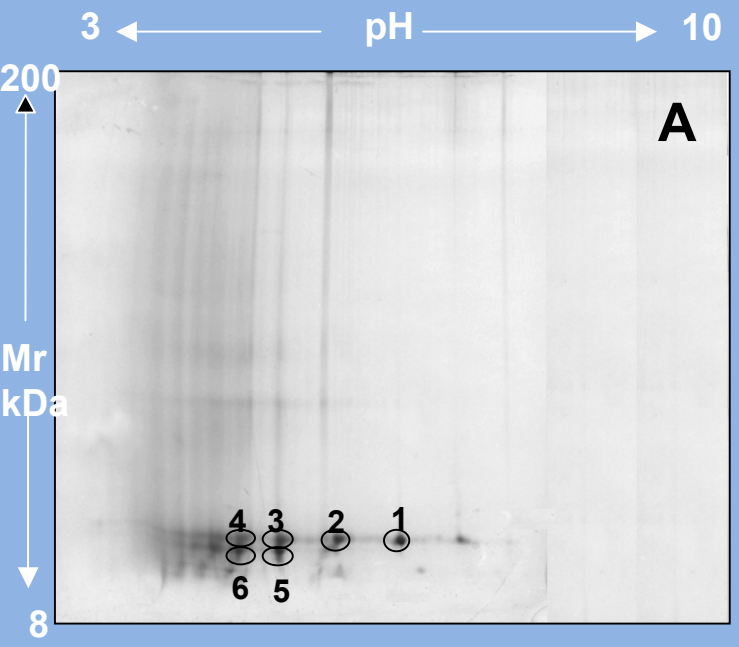
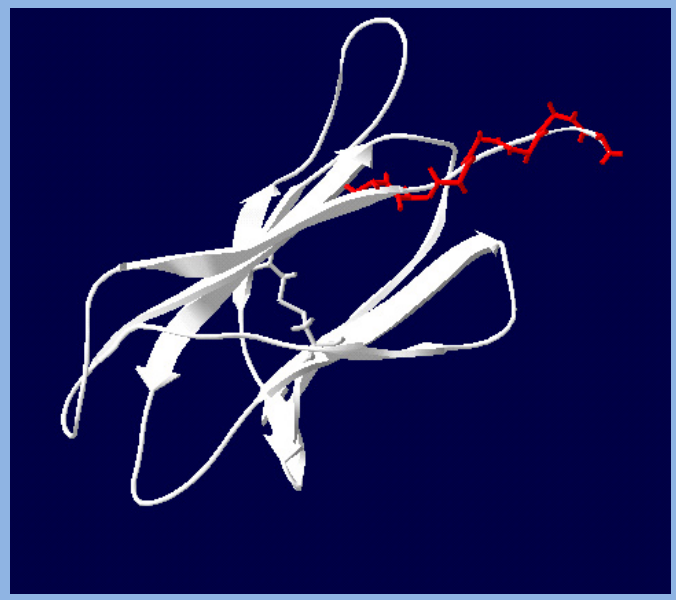




Stoppini M et al Biochim Biophys Acta. 2005;1753:23-33

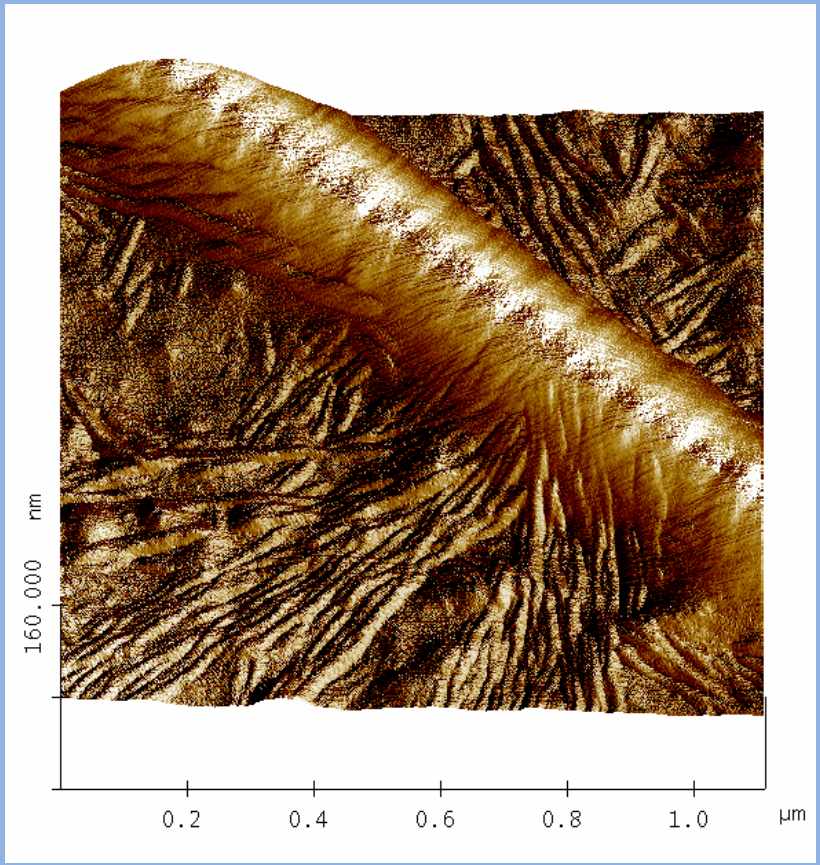
ΔN6β2-m

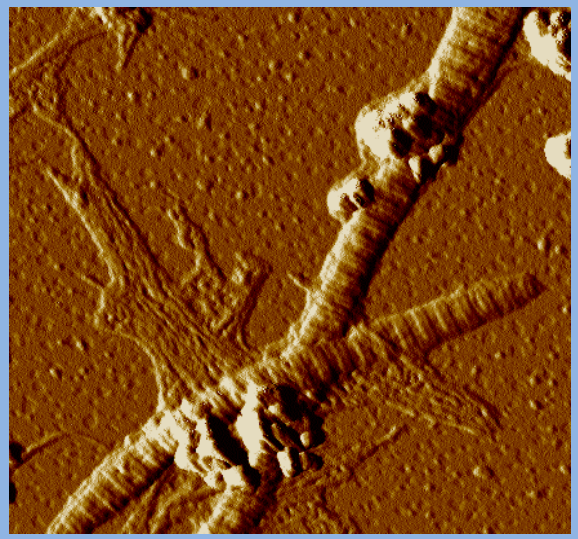
- rappresenta il 30% del contenuto proteico delle fibrille naturali (*Eur J Biochem*, 1998; **258**:61-7)

Possiede un'elevata instabilità termodinamica e una forte tendenza a d aggregare e formare fibrille anche in condizioni di pH fisiologiche (*Protein Sci,* 2000; **9**:831-845**)**

- Non raggiunge una conformazione completamente strutturata (*Eur J Biochem*, 1998; **258**:61-7)
- Presenta una conformazione simile a quella presente nelle fibrille (*Protein Sci,* 2002; **11**: 2362-2369)

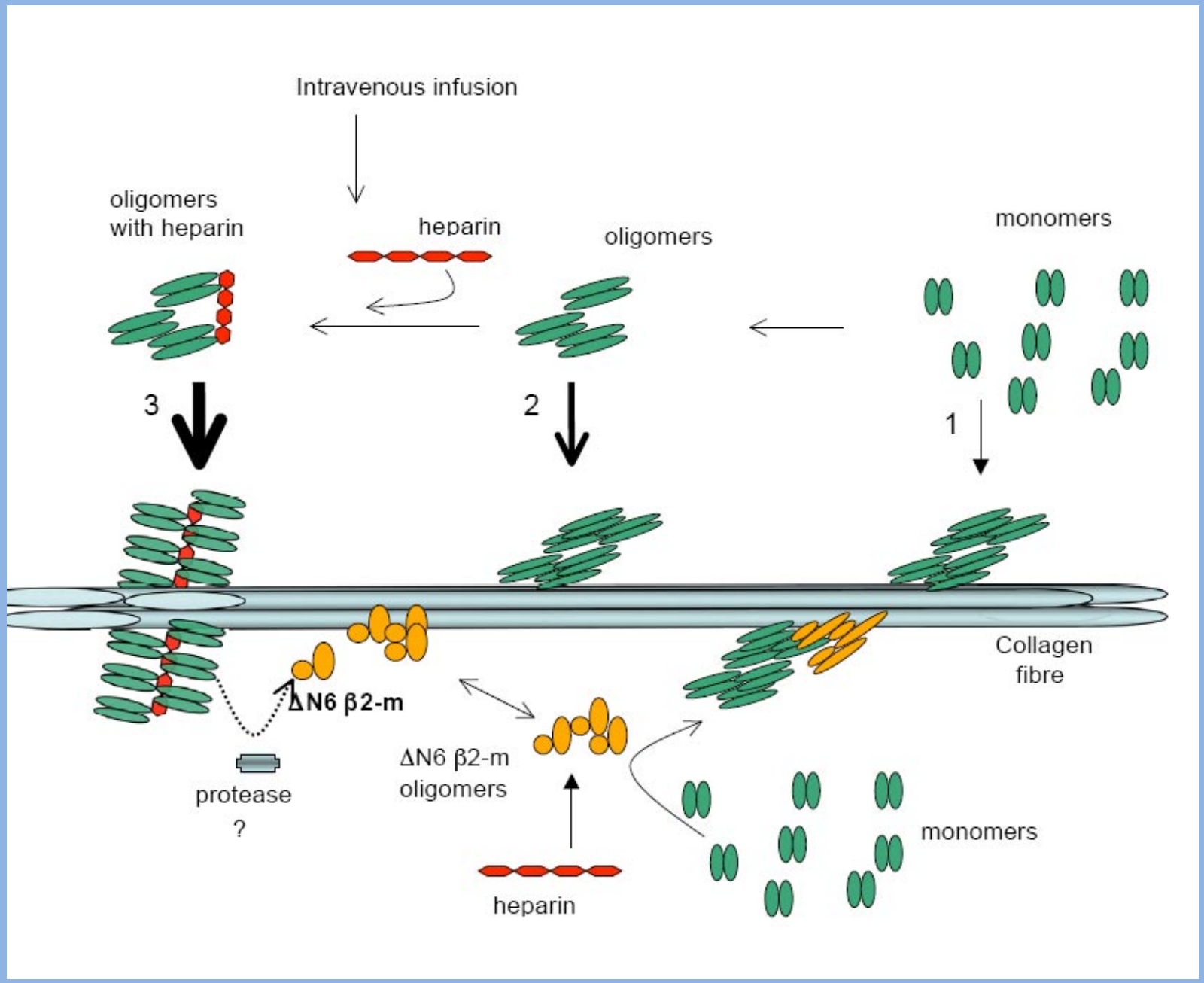
Naiki, et al 1997.*Amyloid* **4:** 223–232	Na Citrate 50mM pH 2.5 - 4	β2-m 100 uM + seeds
McParland et al 2000. *Biochemistry* **39:** 8735–8746	Na citrate 50 mM pH 2.5 100 mM NaCl	β2-m 100 uM No seeds
Esposito et al *Protein Science* 2000, *9*:831–845.	Na Citrate 50 mM pH 6.5	β2-m N-terminal truncated 100 uM +seeds
Chiti et al *J Biol Chem.2001* 14; 276(50): 4714-21	Na Citrate 50 mM pH 7.3	Refolding intermediate 100 uM + seeds
Yamamoto al, 2004, J *Am Soc Nephrol,* 15 :126-133	Na Phosphate 50 mM 100 mM NaCl pH 7.4 20%TFE	β2-m 100 uM +seeds
Yamamoto al, *Biochemistry* 2004 43, 11075-11082 Kihara et al,2005,JBC,280:120 2-8	Na Phosphate 50 mM 100 mM NaCl pH 7.4 0.5% SDS	β2-m 25 uM +seeds
Relini A et al. J Biol Chem. 2006 ; 1:16521-9. J Biol Chem. 2008;283:4912-20	Ammonium Acetate 50mM pH 6.4, 20 uM heparin, fibrillar collagen type	β2-m 30uM
Borysik AJ, et al *Kidney Int*. 2007 2:174-81	PBS pH 7,4, GAGs	β2-m N-terminal truncated 200 υM





Collagen Plays an Active Role in the Aggregation of β2-Microglobulin under Physiopathological Conditions of Dialysis-related Amyloidosis . J Biol Chem. 2006 ; 281:16521-9.

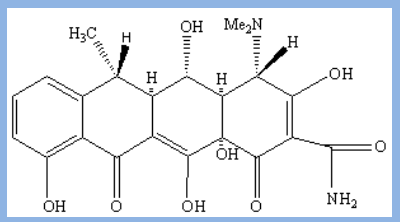
. Heparin strongly enhances the formation of β2-microglobulin amyloid fibrils in the presence of type *I* collagen J Biol Chem. 2008;283:4912-20.

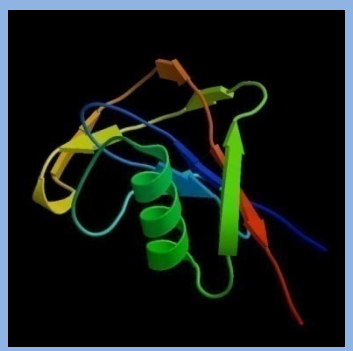

Intravenous infusion
oligomers
with heparin
heparin
oligomers
monomers
3
2
1
ΔN6 β2-m
protease
?
ΔN6 β2-m
oligomers
Collagen
fibre
monomers
heparin

Ricerca di piccole molecole e macromolecule in grado di inibire la fibrillogenesi:

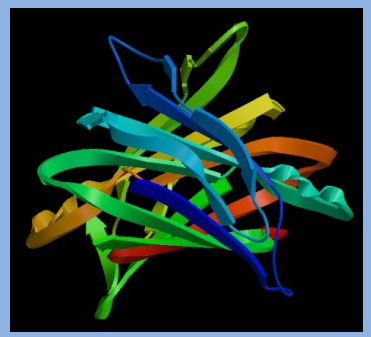
1- Tetracicline
2- Nanobody

1- Tetracicline

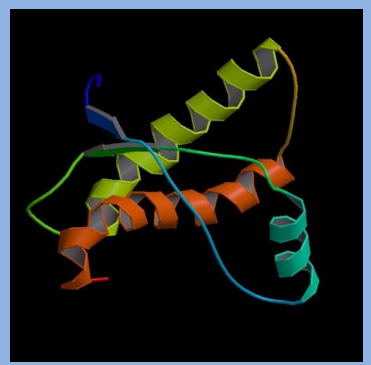




Howlett, D.R.; Biochem J. 1999 ;343 Pt 2:419-23



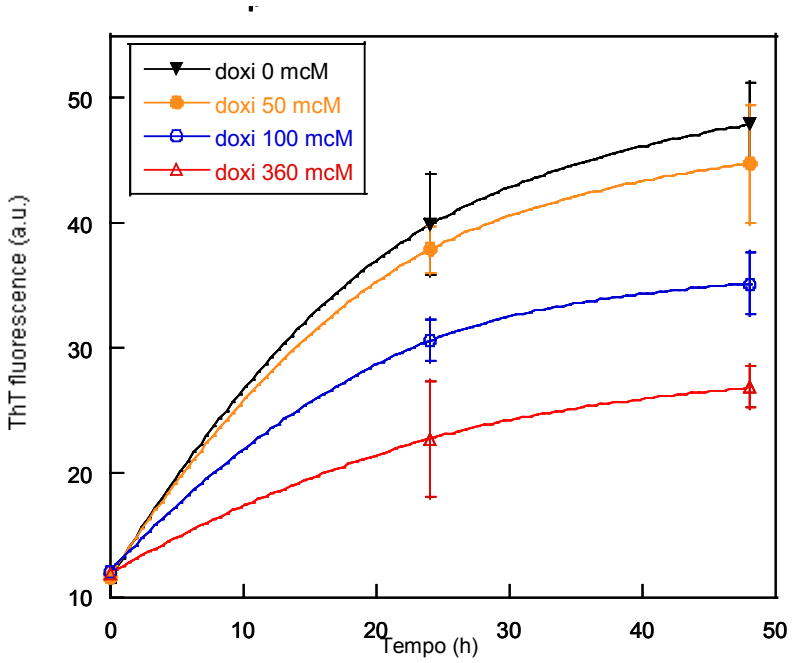
Cardoso I, Merlini G, Saraiva MJ. FASEB J. 2003;17:803-9.

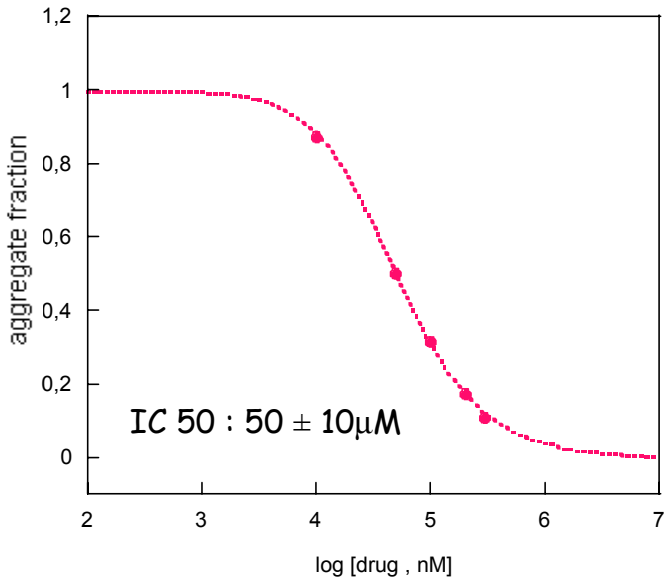


Forloni G, et al. Proc Natl Acad Sci U S A. 2002;99:10849-54

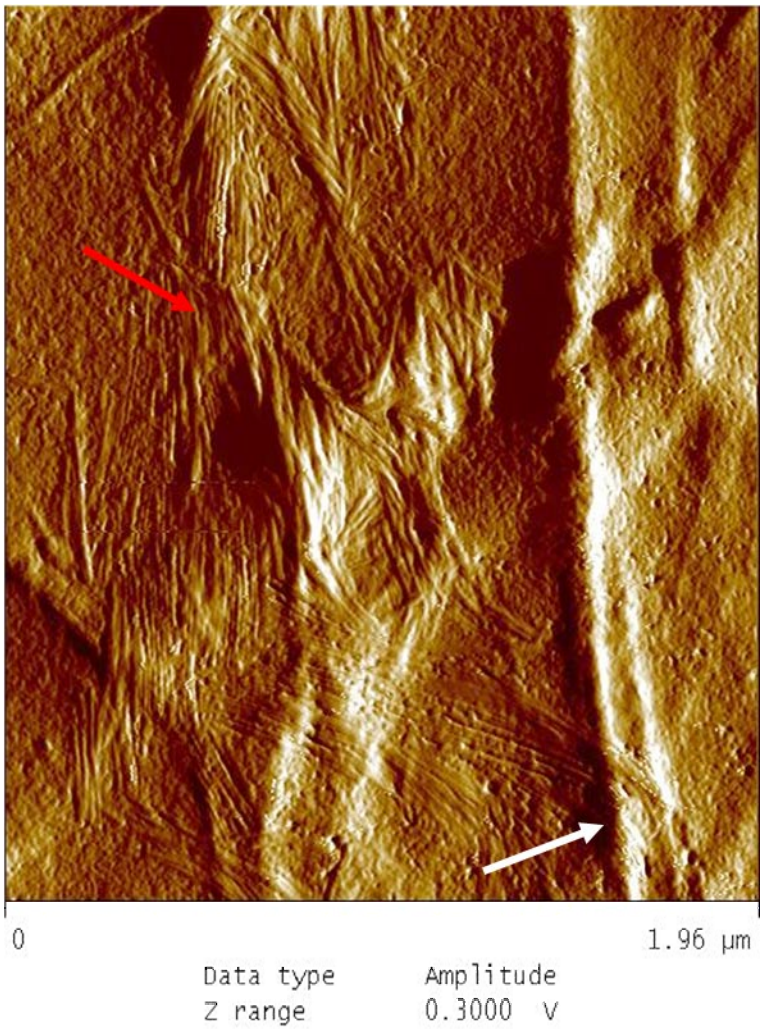
Cosentino U, et al J Mol Model. 14:987-94, 2008

β2-m fibrillogenesi in presenza di TFE

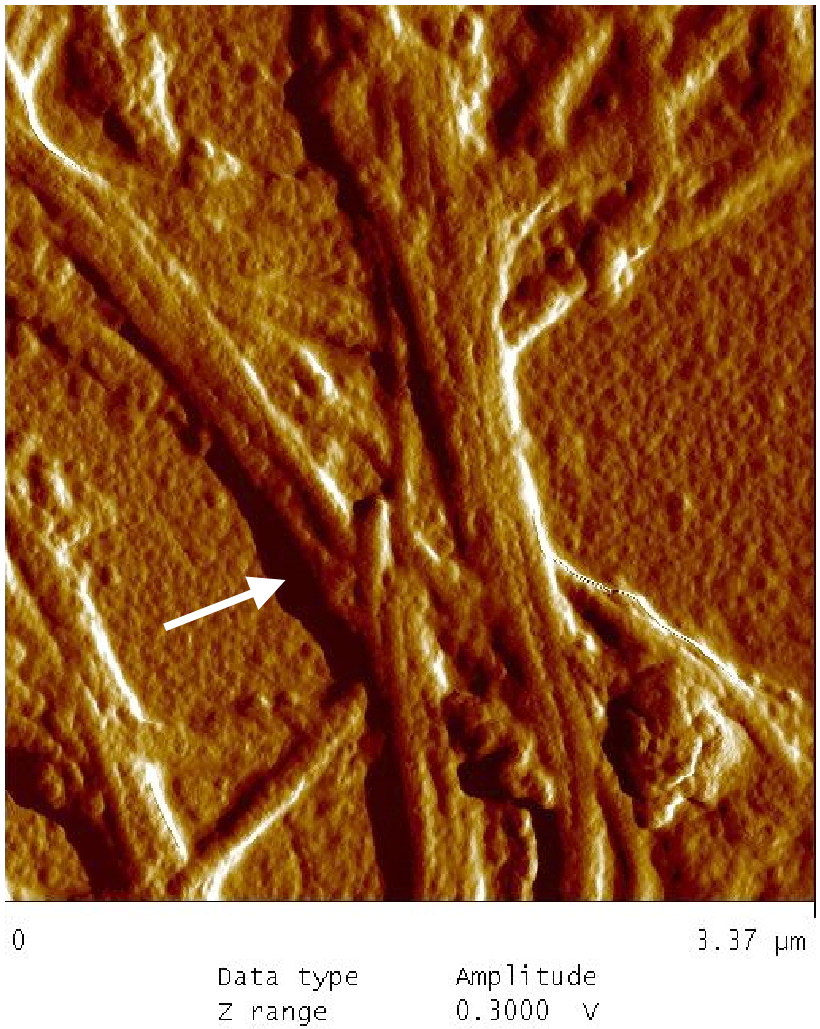




fibrillogenesi in presenza di collagene fibrillare

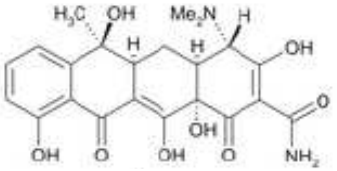


- doxicyclina

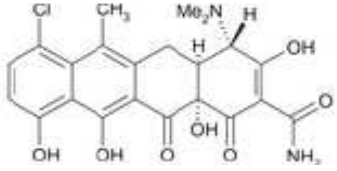


+ doxicyclina

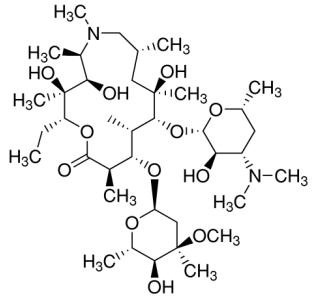
Relini A, et al., J Biol Chem. 2008; 283:4912-20



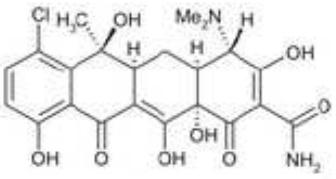
13- Tetracycline



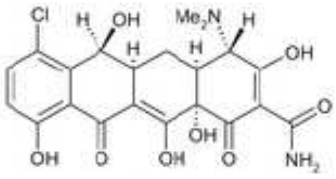
1-Anhydrochlortetracycline



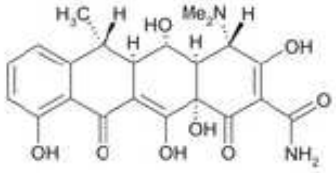
14-Azithromycin



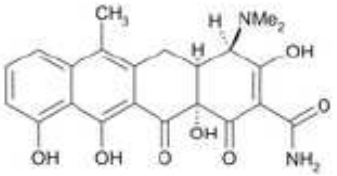
2- Chlortetracycline



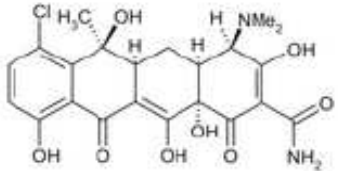
3- Demeclocycline



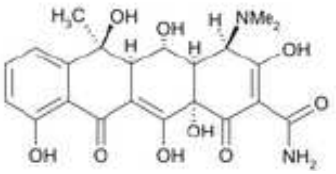
4-Doxycycline



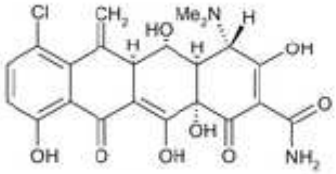
5- 4- Epianhydrotetracycli



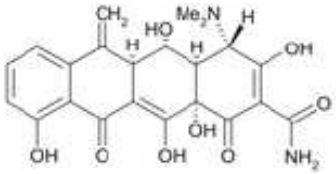
6- 4- Epichlortetracycline



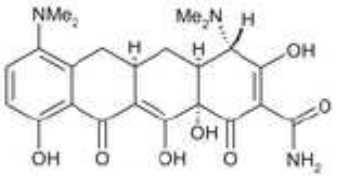
7- 4- Epioxytetracycline



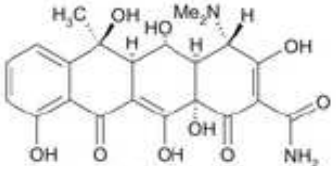
8- Meclocycline



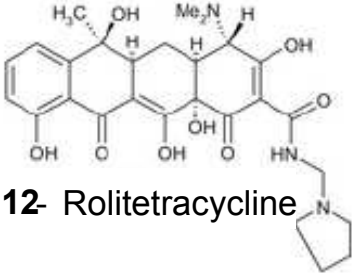
9- Methacycline



10-Minocycline

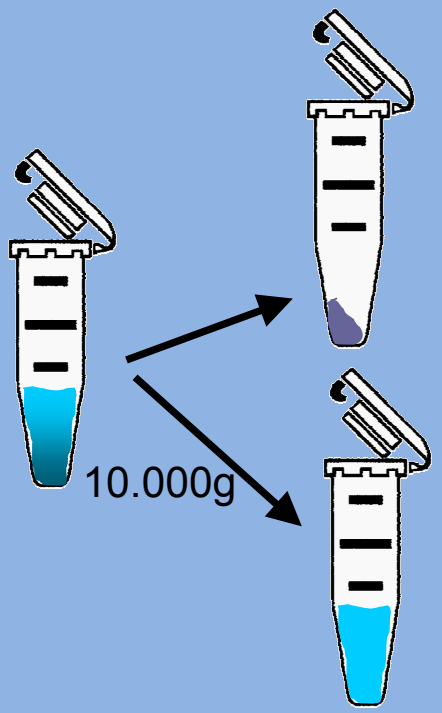


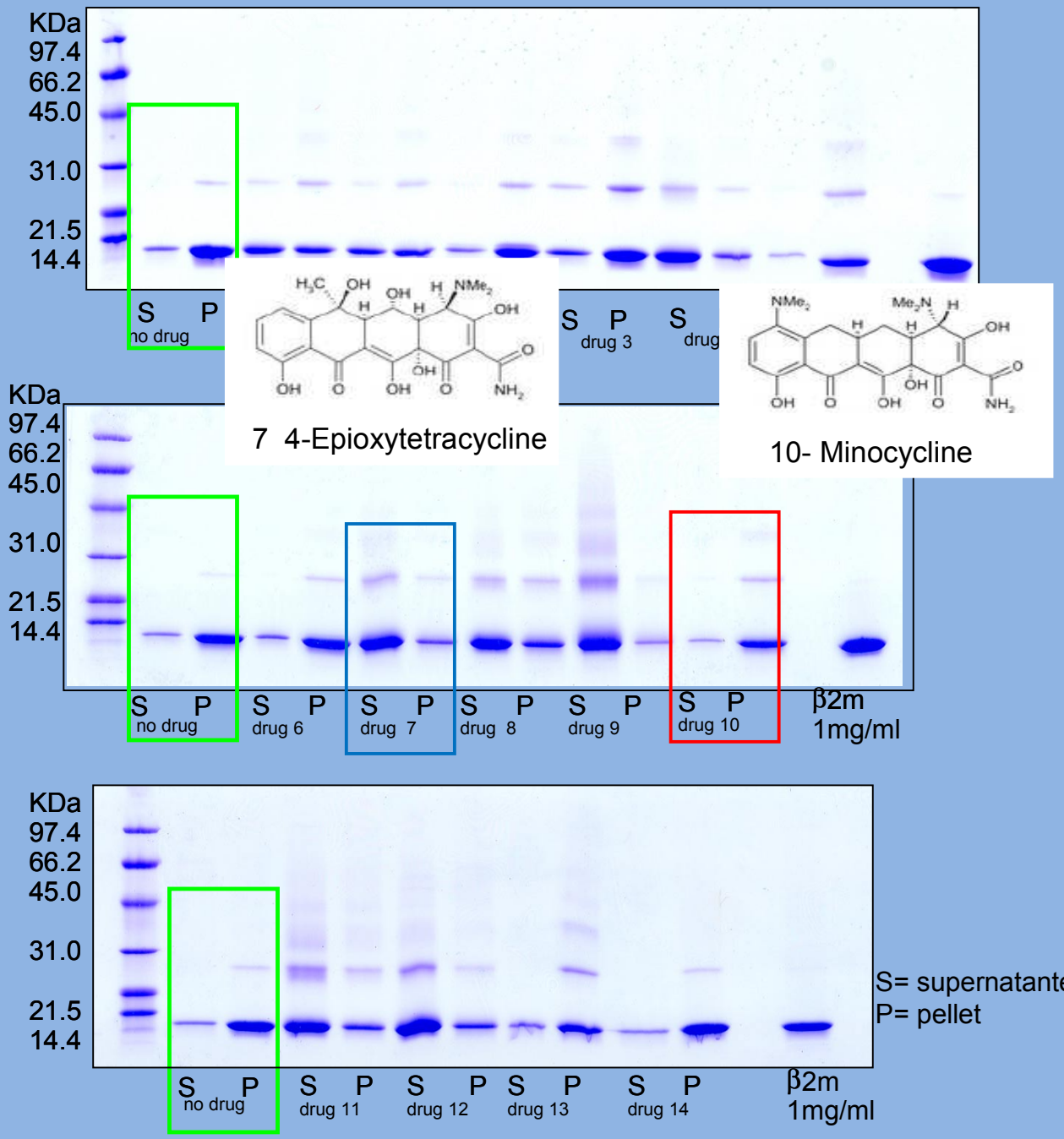
11- Oxytetracycline



12- Rolitetracycline

Analisi della frazione solubile

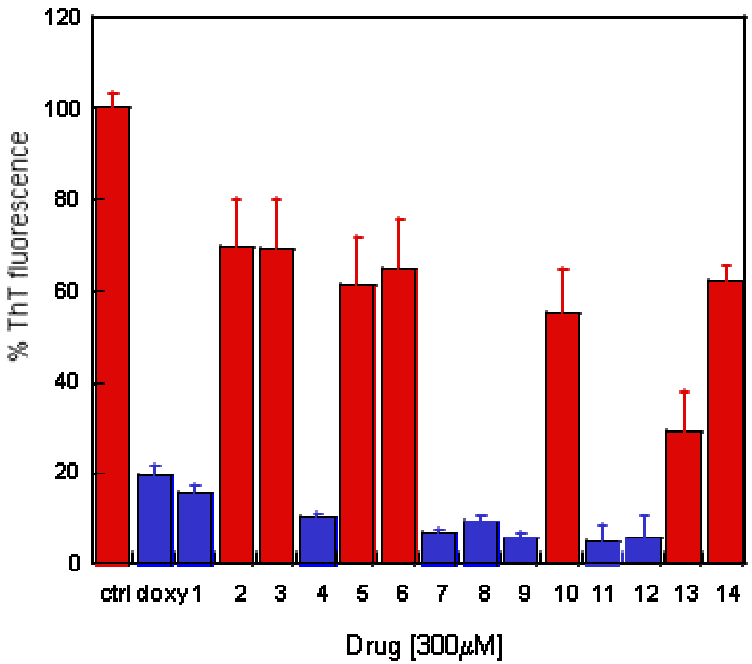


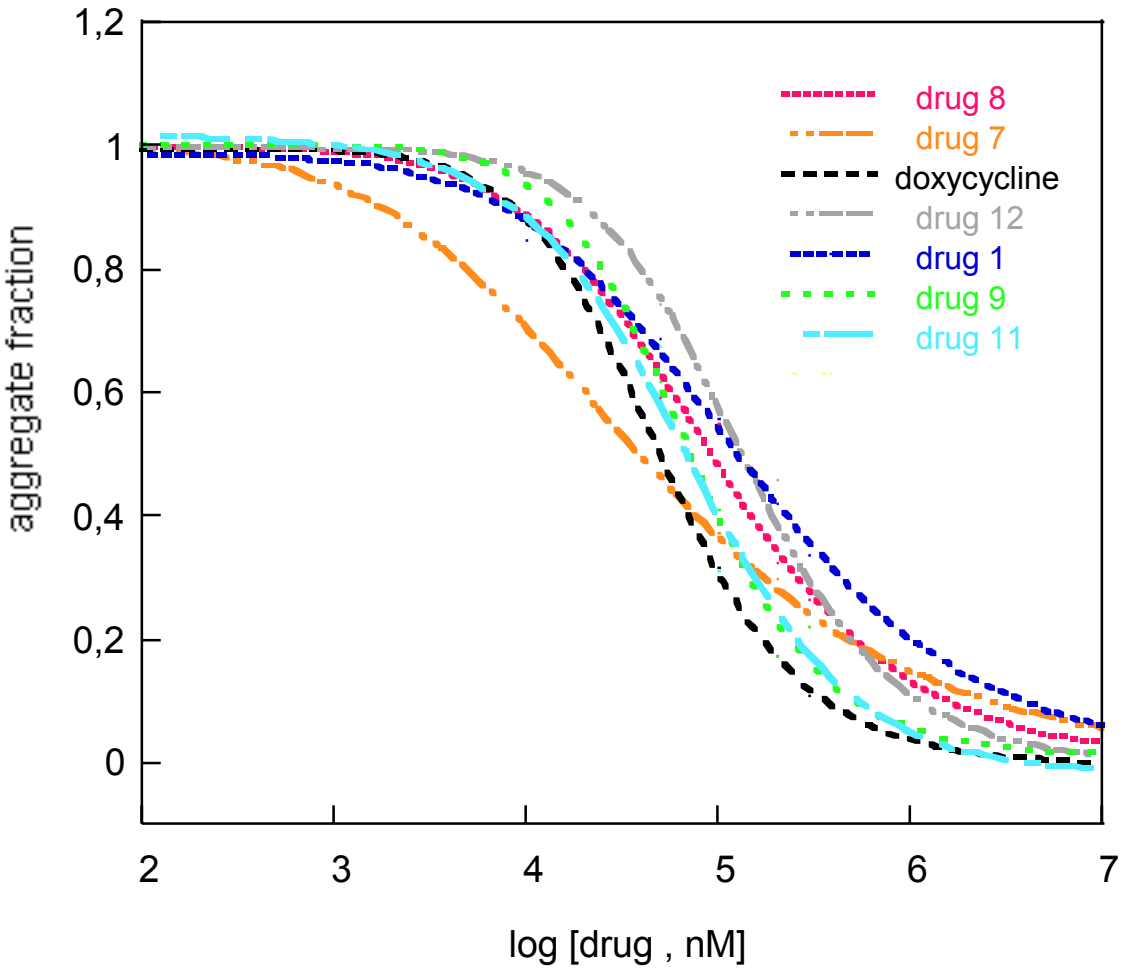


Analisi al microscopio a luce polarizzata dopo colorazione con rosso congo

sample	*Fibrils presence*
β2m	+ + +
β2m + Doxycycline	-
β2m + drug1	+ / -
β2m + drug 2	+ +
β2m + drug 3	+ + +
β2m + drug 4	-
β2m + drug 5	+ + +
β2m +drug 6	+ + +
β2m + drug 7	-
β2m + drug 8	+ / -
β2m + drug 9	-
β2m + drug 10	+ +
β2m + drug 11	-
β2m + drug 12	-
β2m + drug 13	+ +
β2m + drug 14	+ +

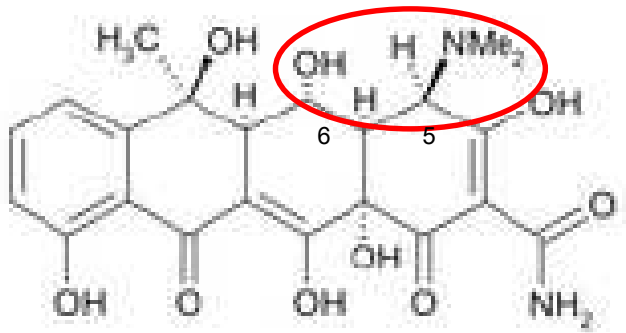
Saggio della ThT dopo 48h di incubazione





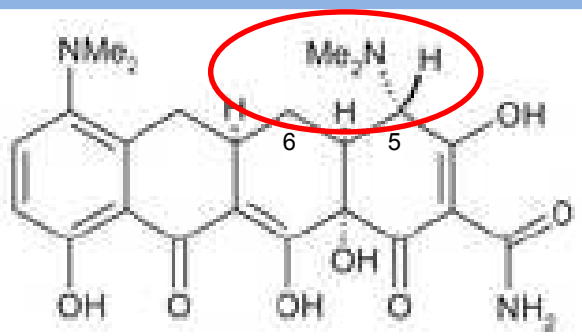
Drug	IC $_{50}$ (μM)
1	135±9
4	78 ±8
9	71 ±9
11	69 ±5
doxycycline	50 ±10
7	40 ±9
8	94 ±12
12	135 ±10

attivo

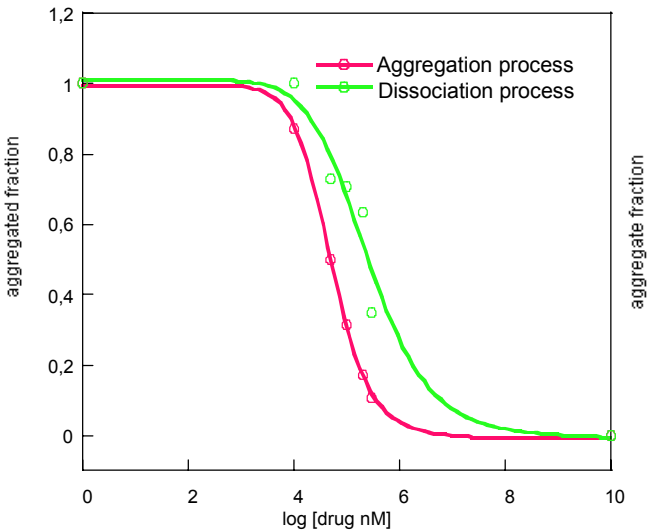


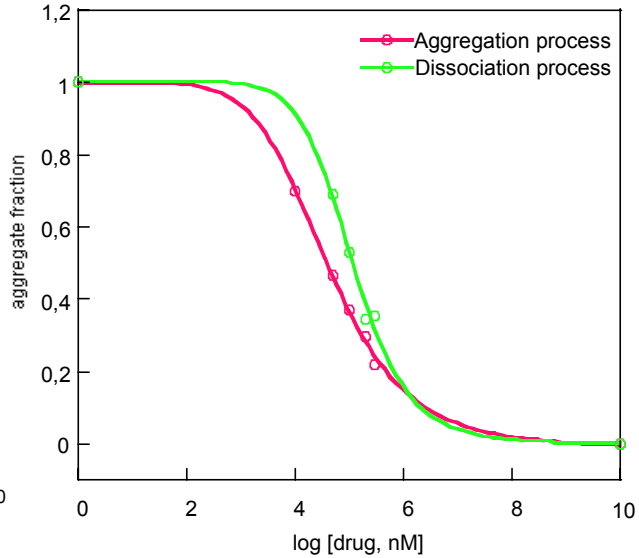
7 4-Epioxytetracycline

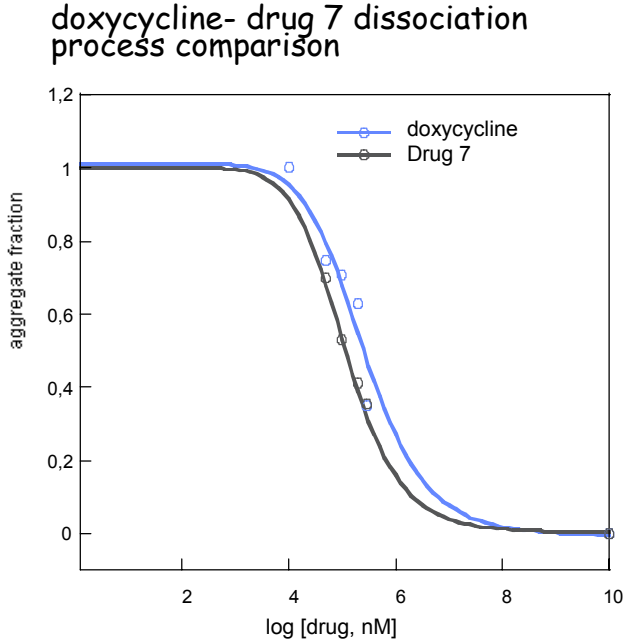
Non attivo



10- Minocycline

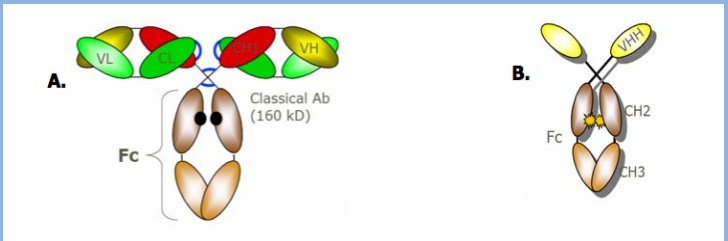


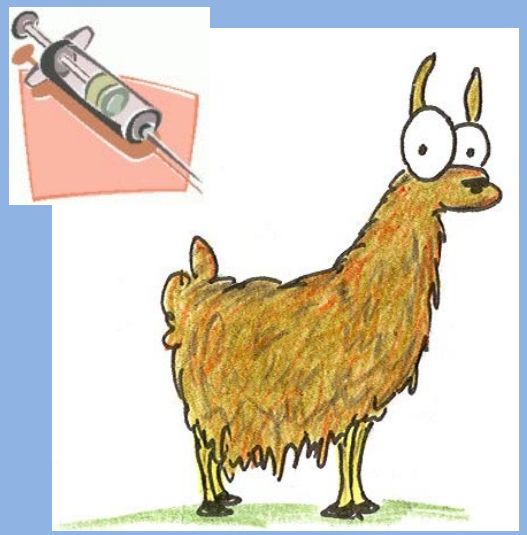




drug	IC50 aggregation (µM)	IC50 fibril dissociation (µM)
doxycycline	50 ±10	245 ±35
drug 7	40 ±9	117 ±26

2- Nanobody

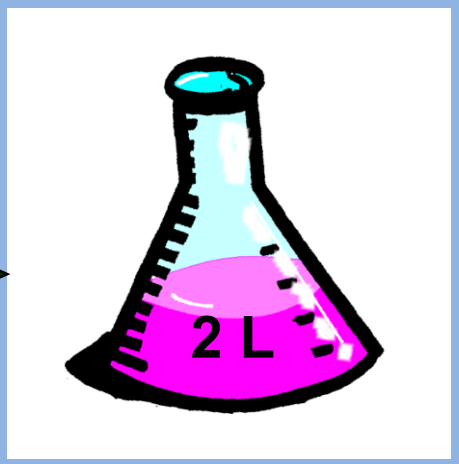




panning

phage display

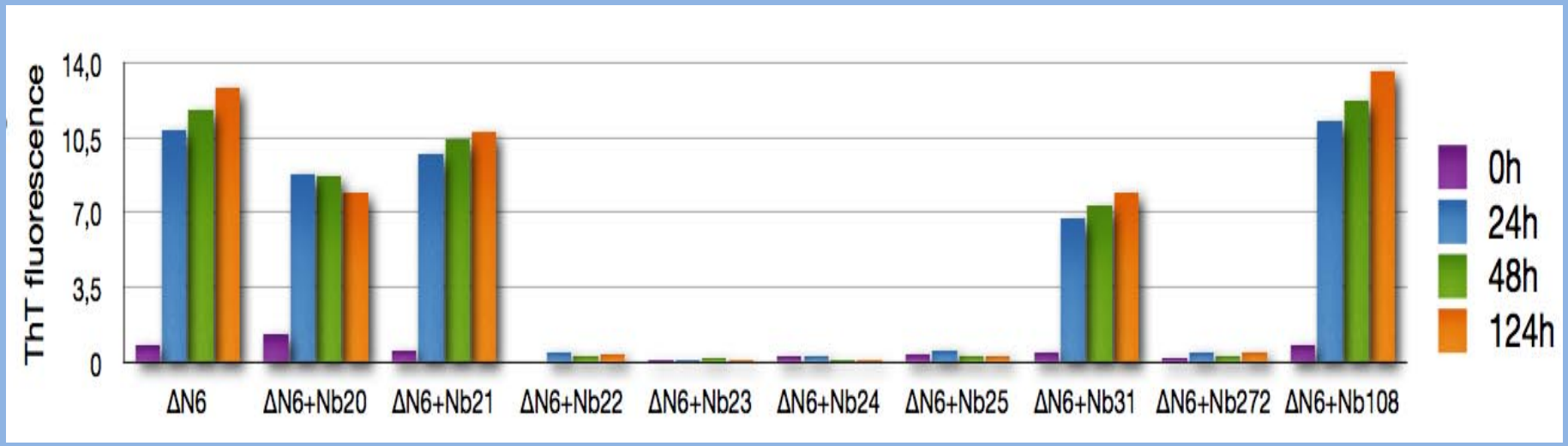
cloning…



Prof. Lode Wyns
Vrije Universiteit Brussel
Department of Ultrastructure
Institute of Molecular Biology & Biotechnology
Brussels

Dissociation constants in nM:

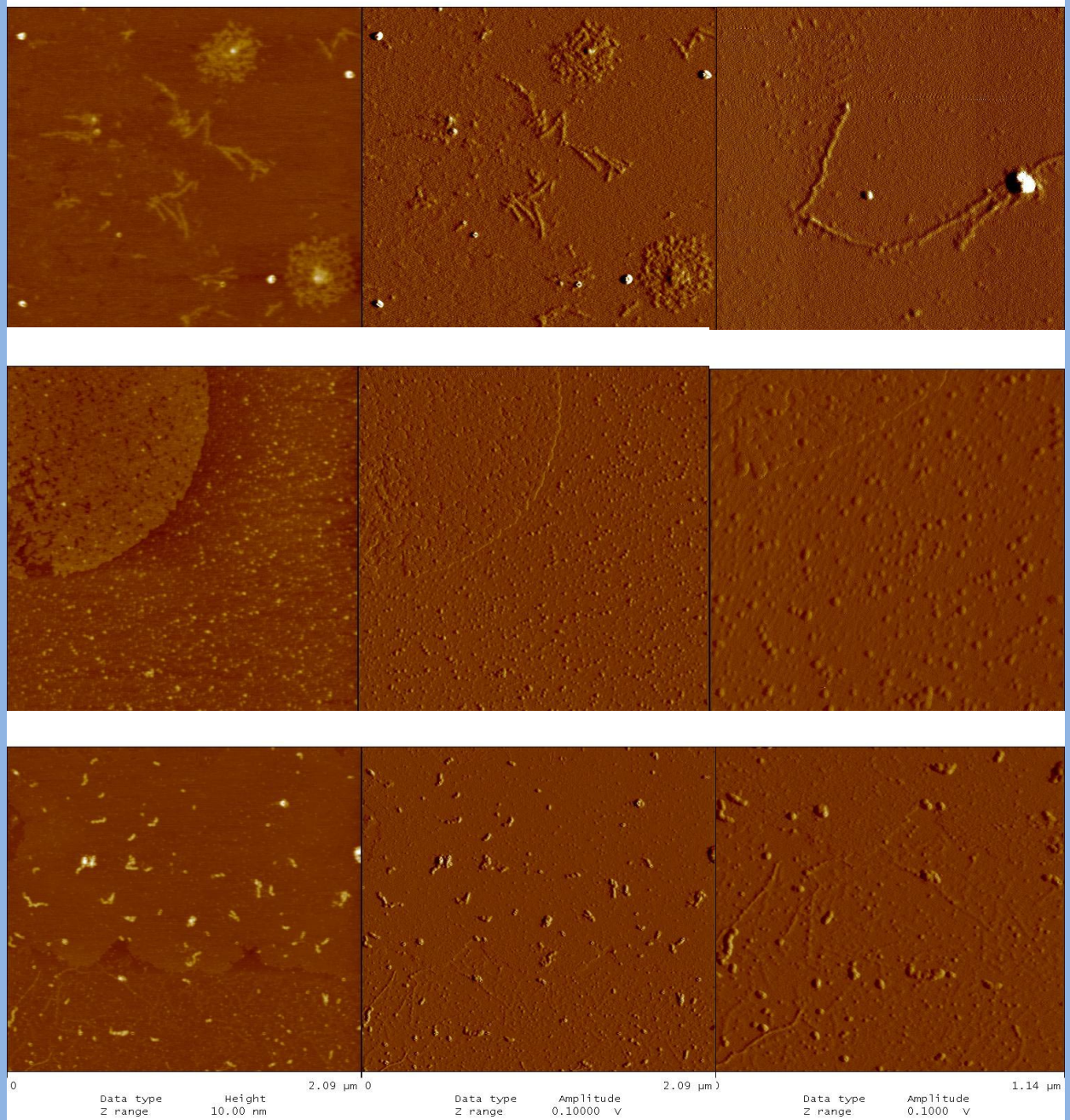
Nb	**20a**	**22a**	**23a**	**24**	**30a**	**30b**	**31**	**272b**	**273**
K_D [nM] b2m	24	269	50	58	2,6	1,6	6,8	129	52
K_D [nM] dN6 b2m	35	330	54	44	11,0	6,7	8,4	72	50


ThT fluorescence
14,0
10,5
7,0
3,5
0
ΔN6
ΔN6+Nb20
ΔN6+Nb21
ΔN6+Nb22
ΔN6+Nb23
ΔN6+Nb24
ΔN6+Nb25
ΔN6+Nb31
ΔN6+Nb272
ΔN6+Nb108
0h
24h
48h
124h

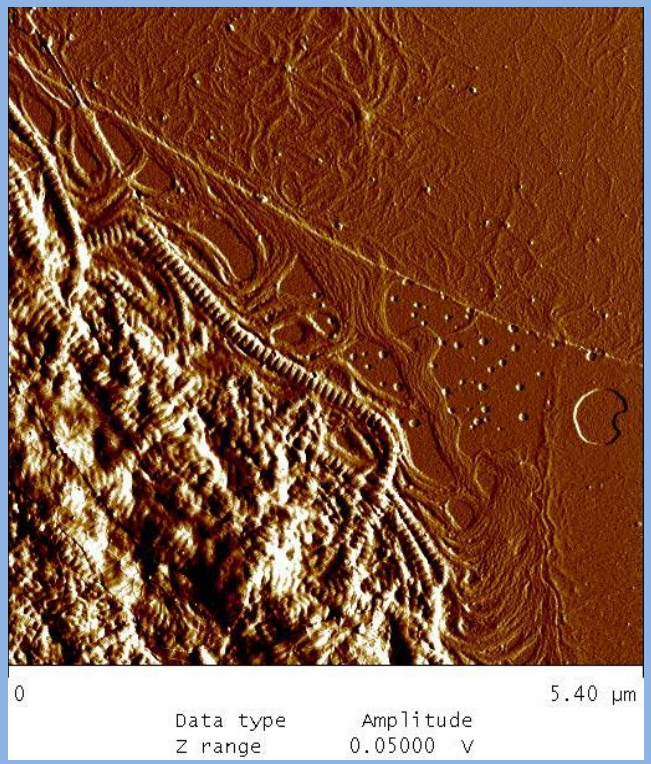
ΔN6b2m

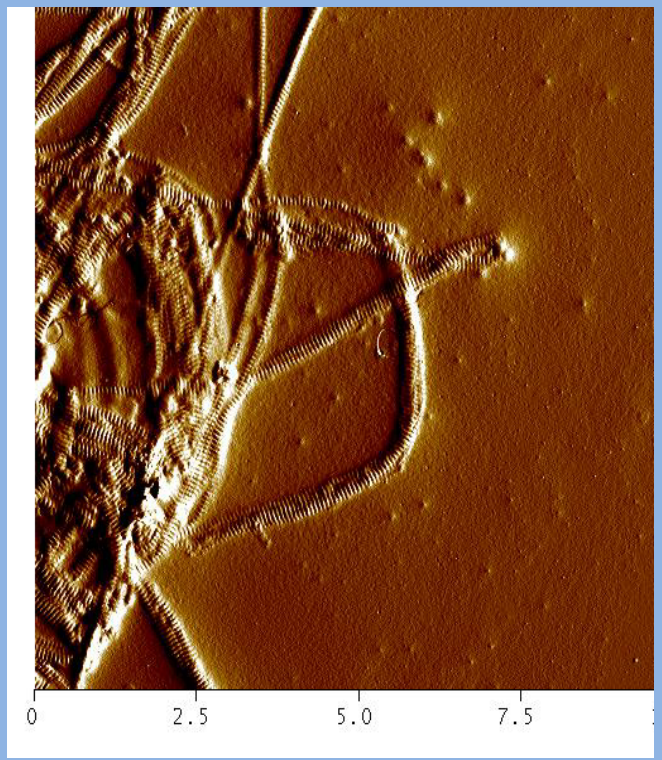
ΔN6b2m+Nb23a

ΔN6b2m+Nb20a



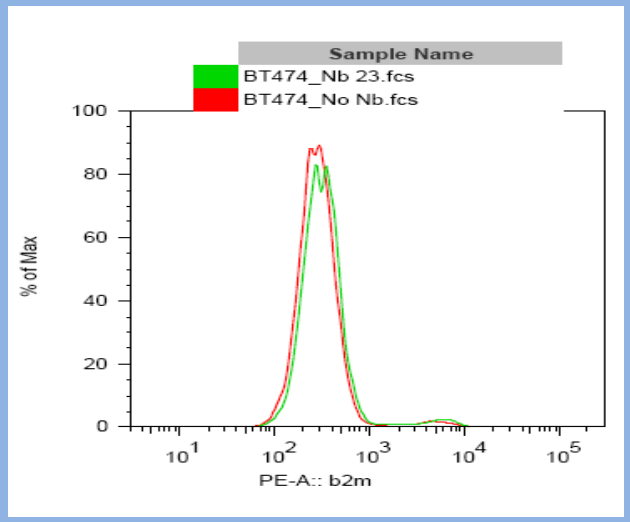
fibrillogenesi in presenza di collagene fibrillare

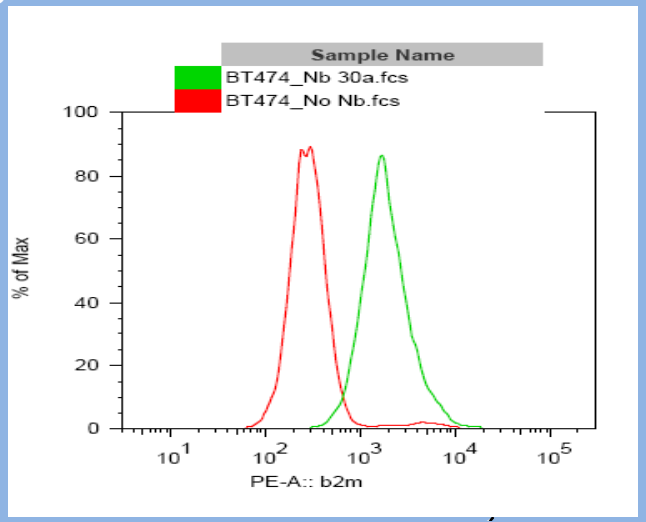


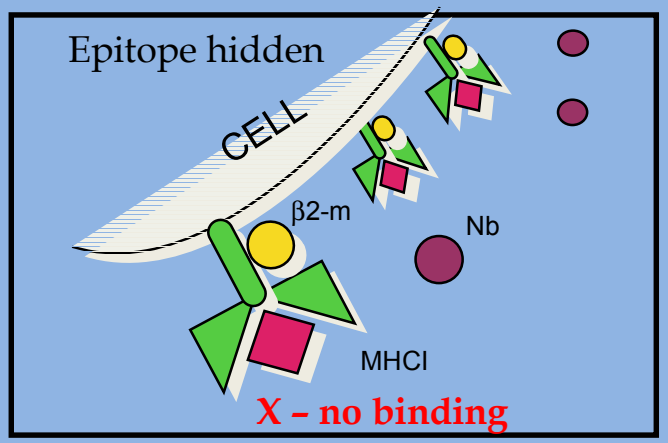


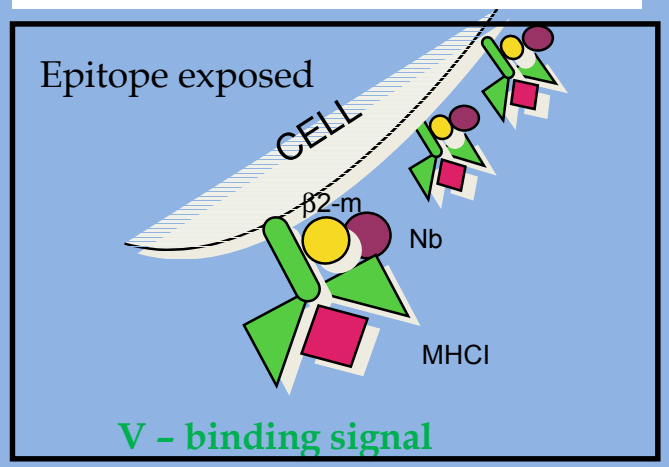
Legame con MHCI

FACS
Fluorescent-activated cell sorting





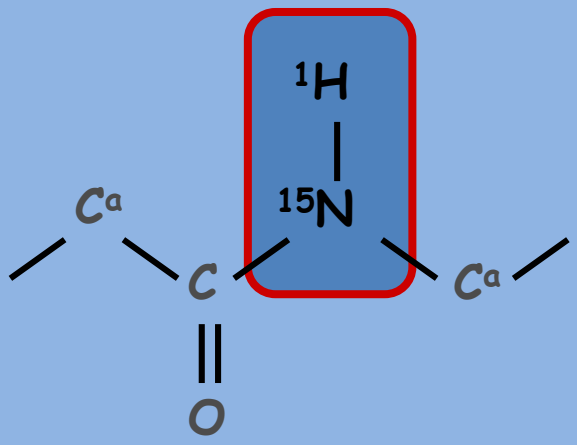




	Cell line	Nb20	Nb22	Nb23	Nb24	Nb30a	Nb30b	Nb31	Nb272	Nb273
1	BT 474	x	x	x	x	V	V	x	x	V
2	MDA-MB 435D	x	x	x	x	x	x	x	x	x
3	SKBR3	x	x	x	x	V	V	x	x	V

1- Caratterizzazione degli epitopi riconosciuti: attraverso studi di NMR

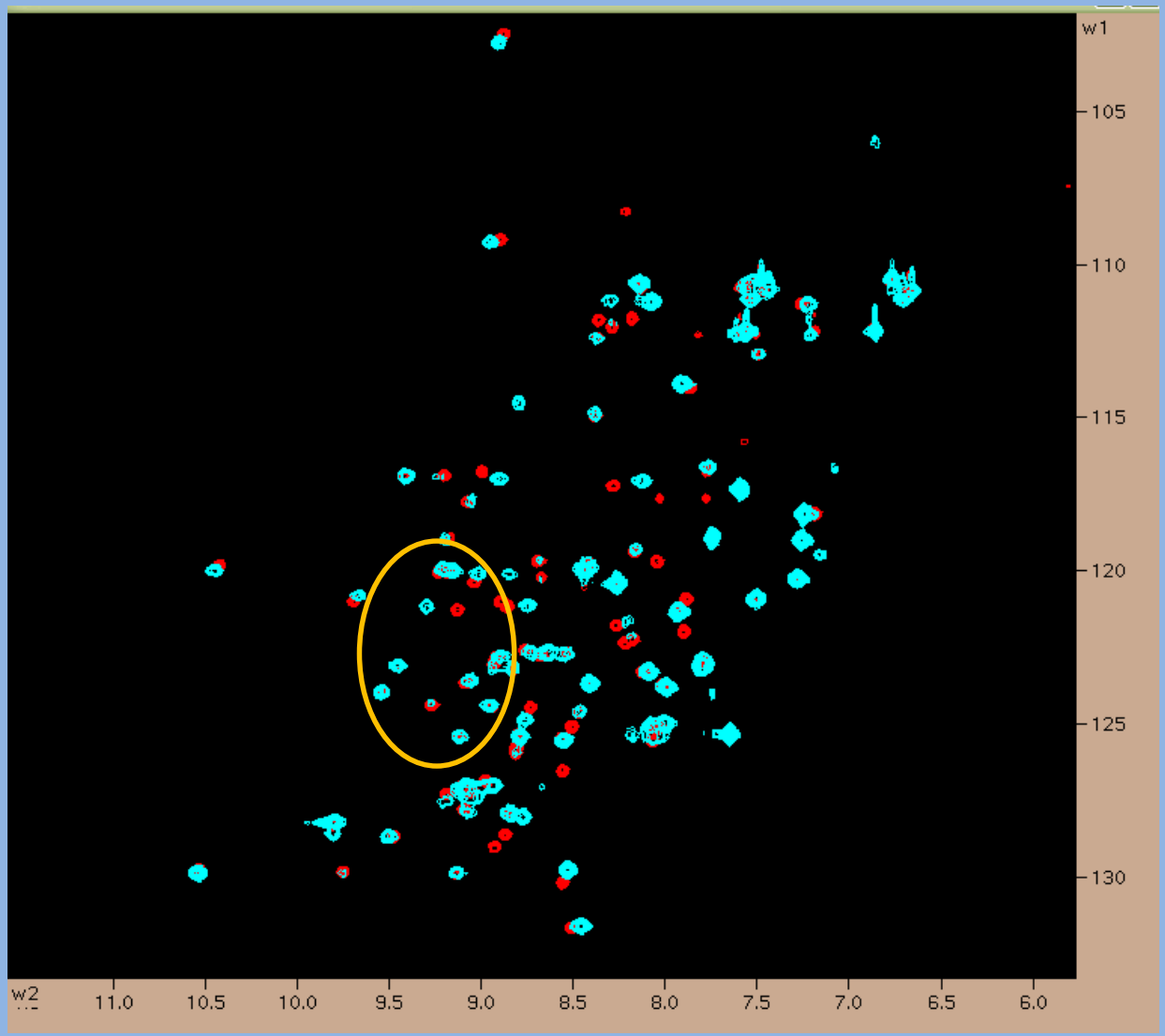
^{15}N-HSQC: titolazione del nb-23a non marcato in presenza di β2m marcata

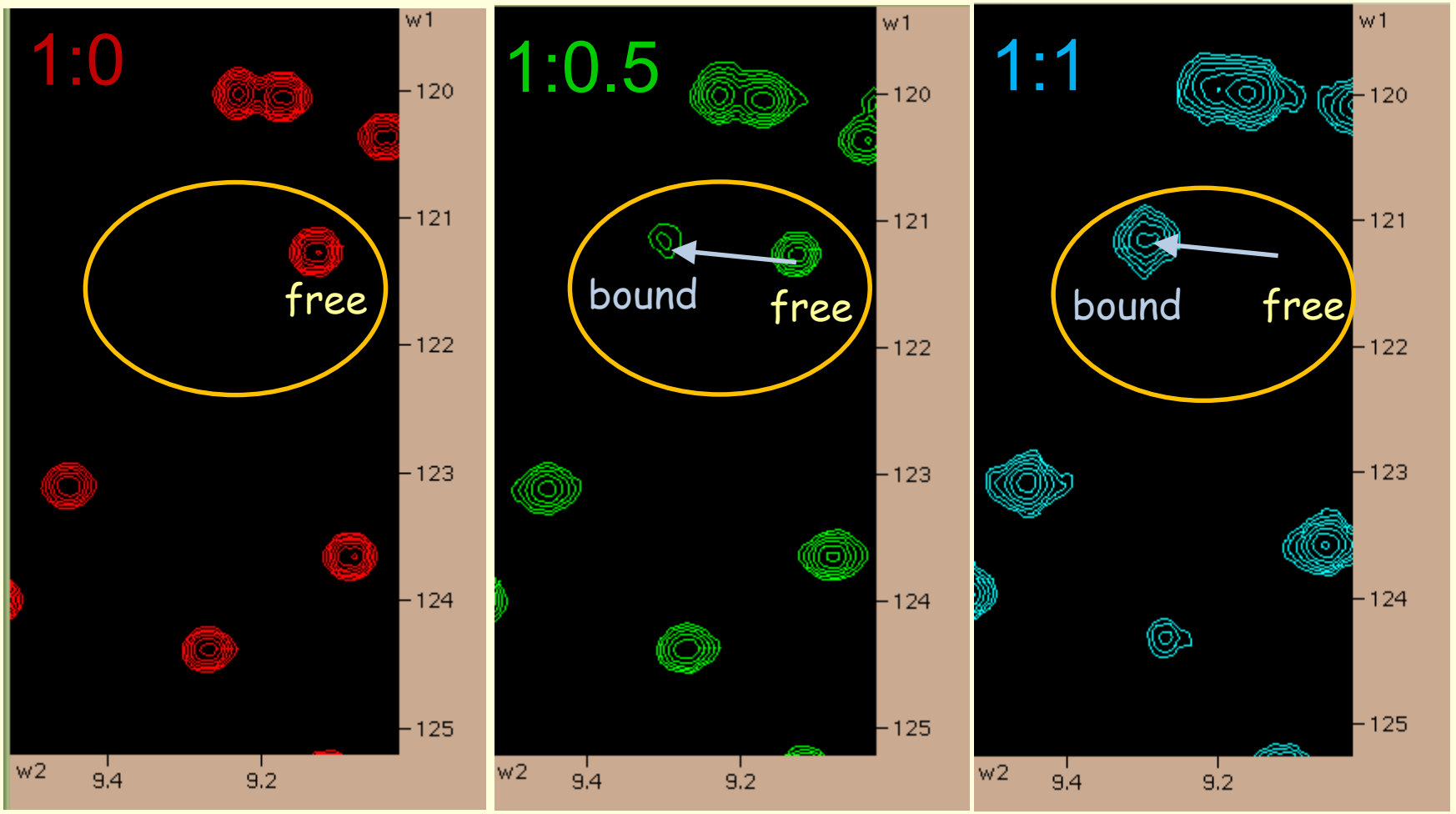


β2m:nb

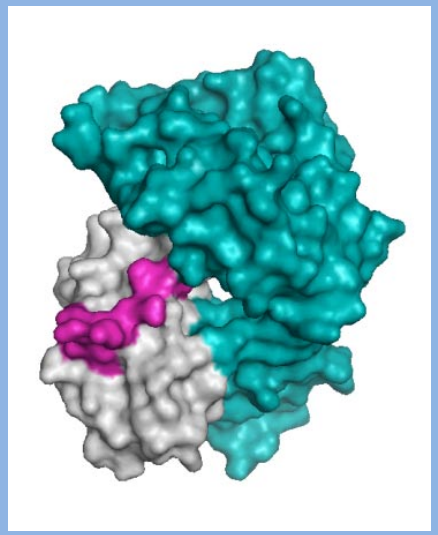
1:0

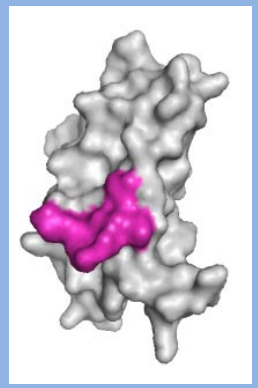
1:1





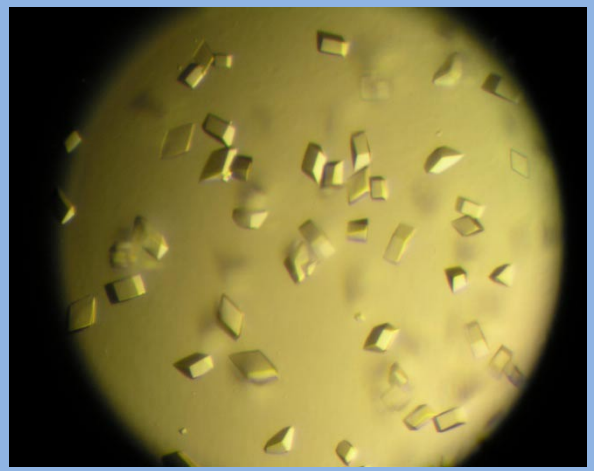
Scambio lento

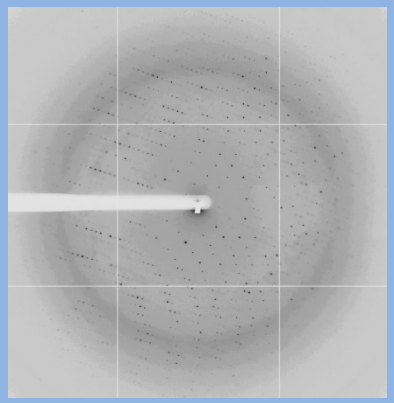


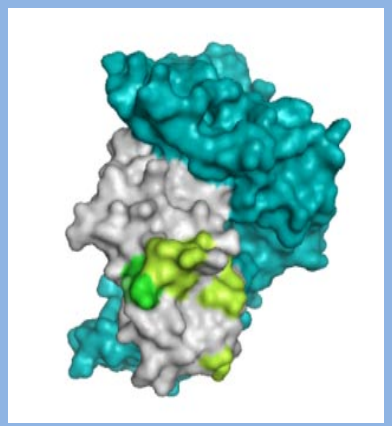


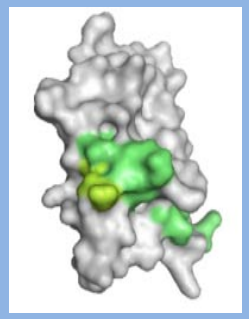
Nb 23 a

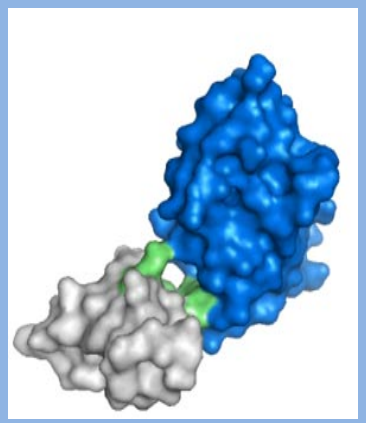
2- Caratterizzazione degli epitopi riconosciuti: attraverso studi di cristallografia ai raggi X











Gruppi coinvolti

PAVIA

Vittorio Bellotti

Monica Stoppini

Patrizia Mangione

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Loredana Marchese

Angelo Gallanti

Irene Zorzoli

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