



UNIVERSITA' DEGLI STUDI DI PAVIA

DALLA SCIENZA DEI MATERIALI ALLA BIOMEDICINA MOLECOLARE
Percorsi scientifico-formativi per giovani ricercatori



Regione Lombardia

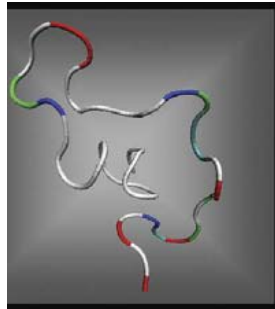
"Proteine amiloidogeniche e piccole molecole: studi di binding"

Raffaella Colombo PhD

Capogruppo: Ersilia De Lorenzi

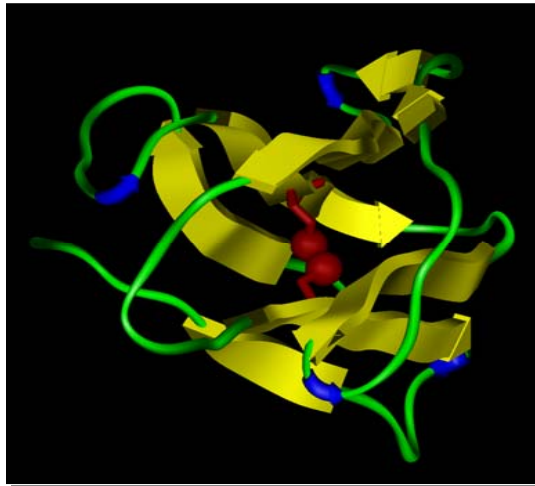
Pavia, 12 giugno 2009

$A\beta_{1-42}$ PM: 4514.15 Da



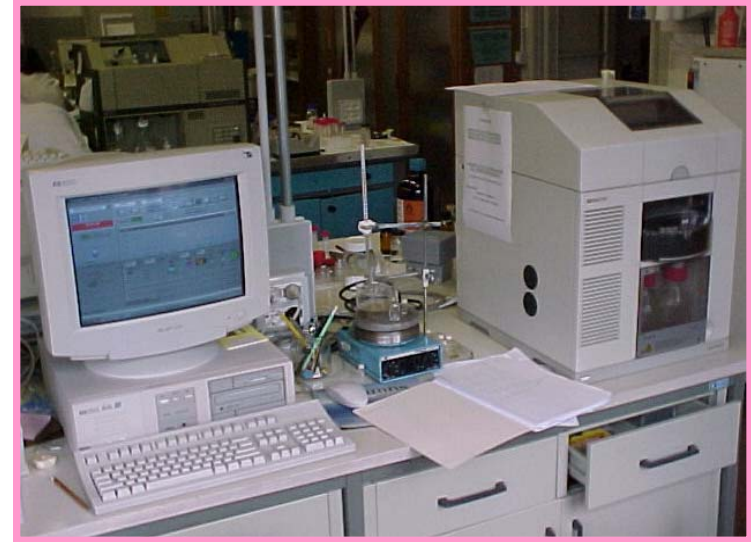
MALATTIA DI ALZHEIMER

β_2 -microglobulina PM: ~11800 Da



AMILOIDOSI
CORRELATA ALLA DIALISI

Elettroforesi capillare (CE)



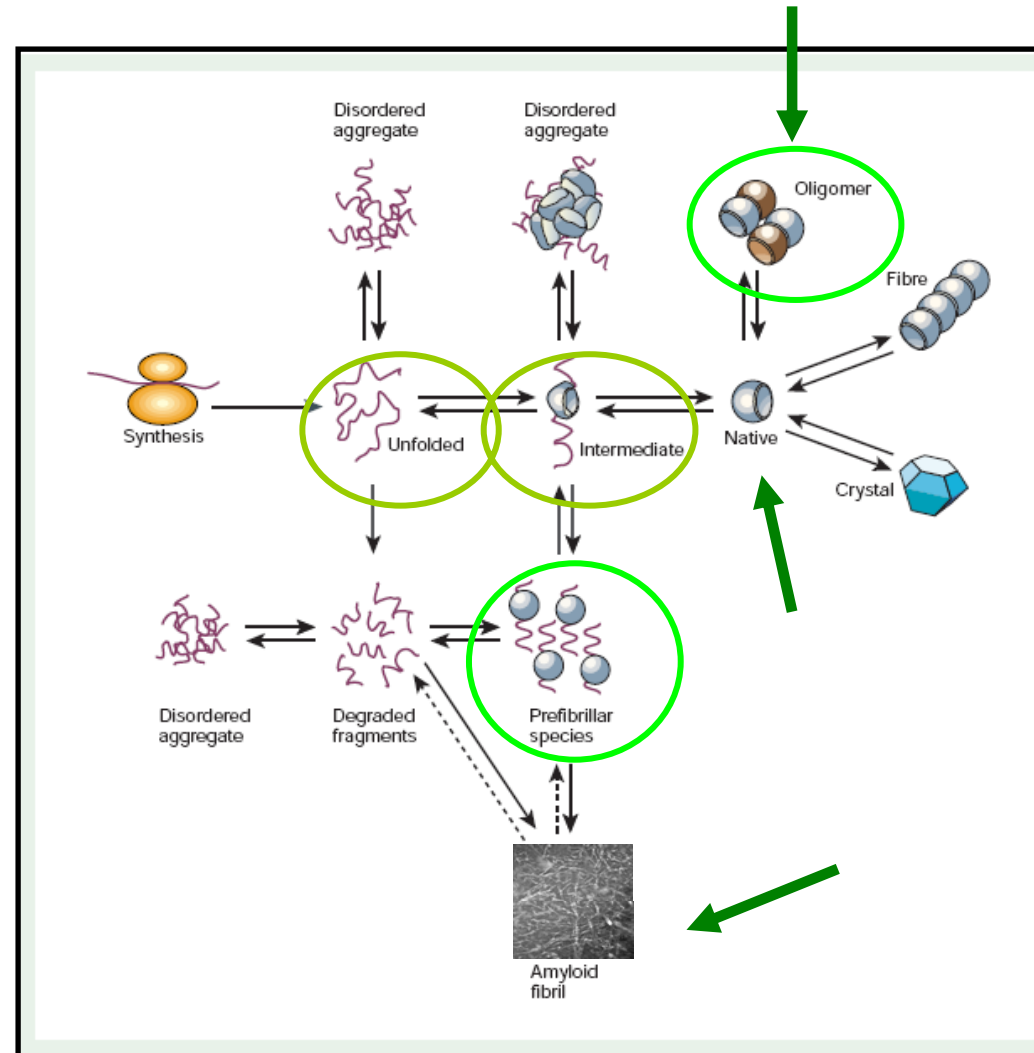
Flow Field-flow fractionation (FIFFF)

Spettrometria di massa a trappola elettrostatica (ORBITRAP)



Folding e misfolding

Studi di *binding*



Dobson, C.M., *Nature* 2003, 426, 884



Elettroforesi capillare (CE)

- Soluzione libera
- Alta efficienza e risoluzione
- Bassi consumi di campione

- Separazione di conformeri, intermedi e oligomeri
- Studi di equilibri
- Studi di cinetiche di *refolding*
- Studi di coincubazione e complessazione (ACE)

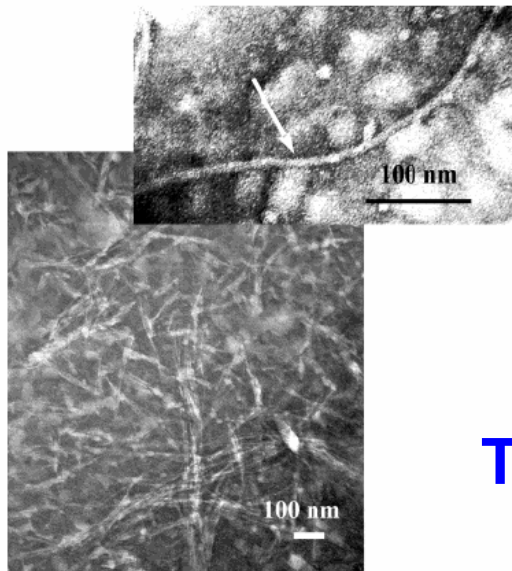
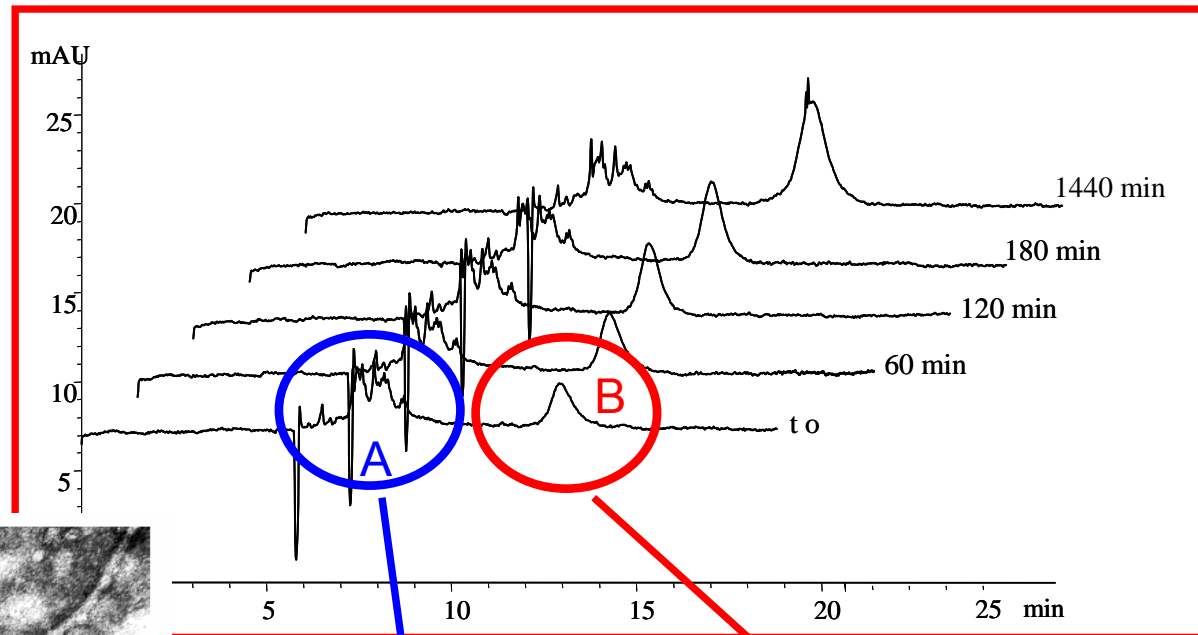


Stefania Sabella¹
Milena Quaglia^{1,2}
Cristina Lanni³
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Gabriele Caccialanza¹
Alberto Calligaro⁴
Vittorio Bellotti²
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Electrophoresis 2004, 25, 3186–3194

Capillary electrophoresis studies on the aggregation process of β -amyloid 1-42 and 1-40 peptides

A β ₁₋₄₂

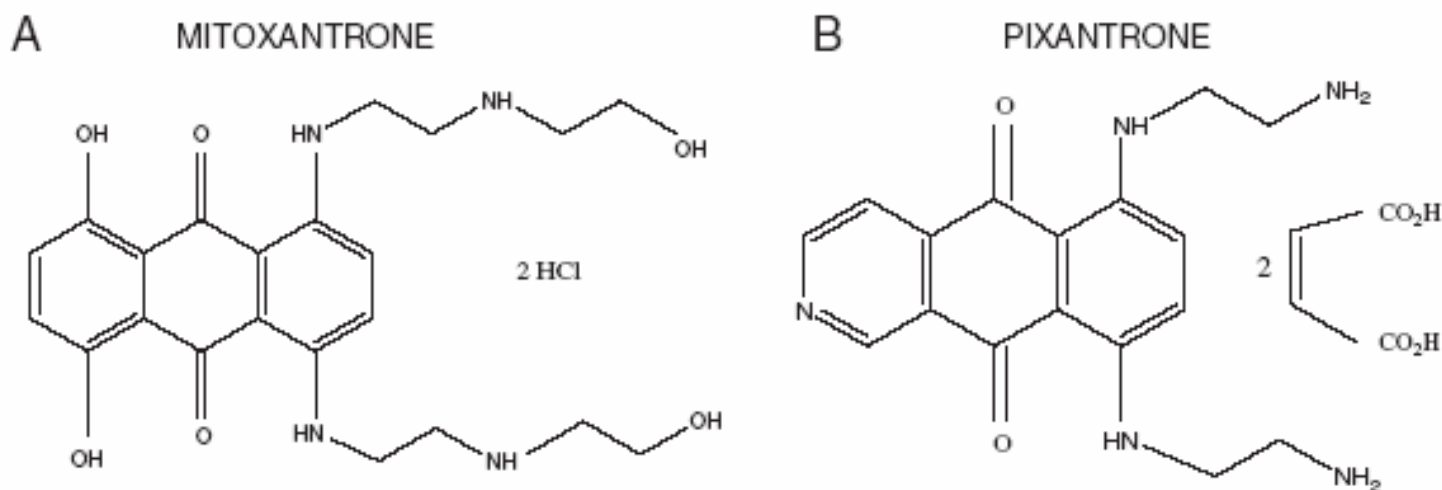


10kDa < A < 50kDa
TRIMERI-UNDECAMERI

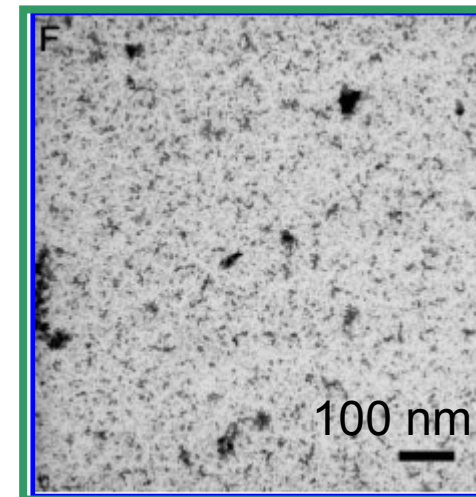
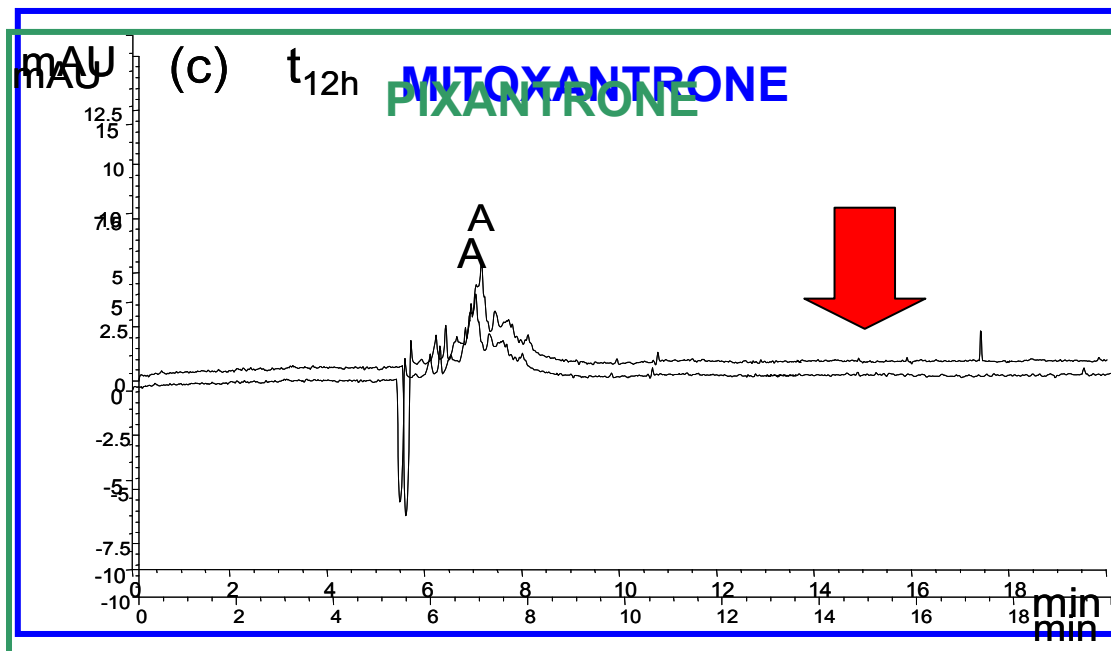
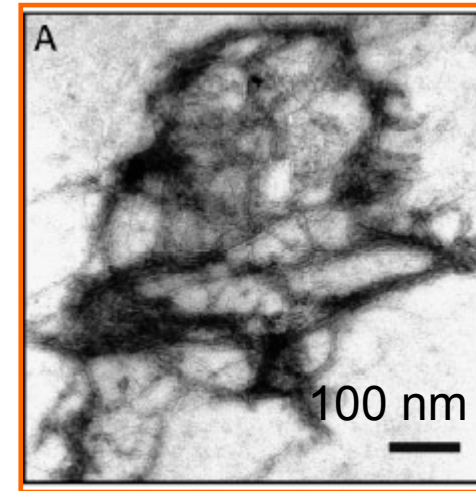
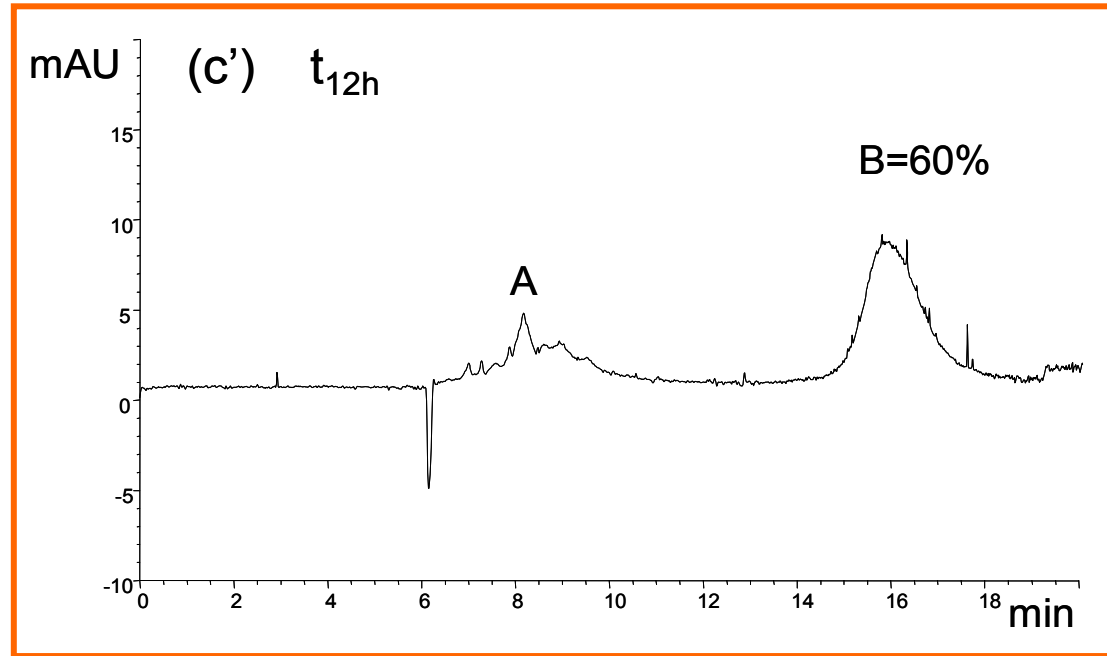
B > 100kDa
> 22-MERI

Research Article

CE can identify small molecules that selectively target soluble oligomers of amyloid β protein and display antifibrillogenic activity



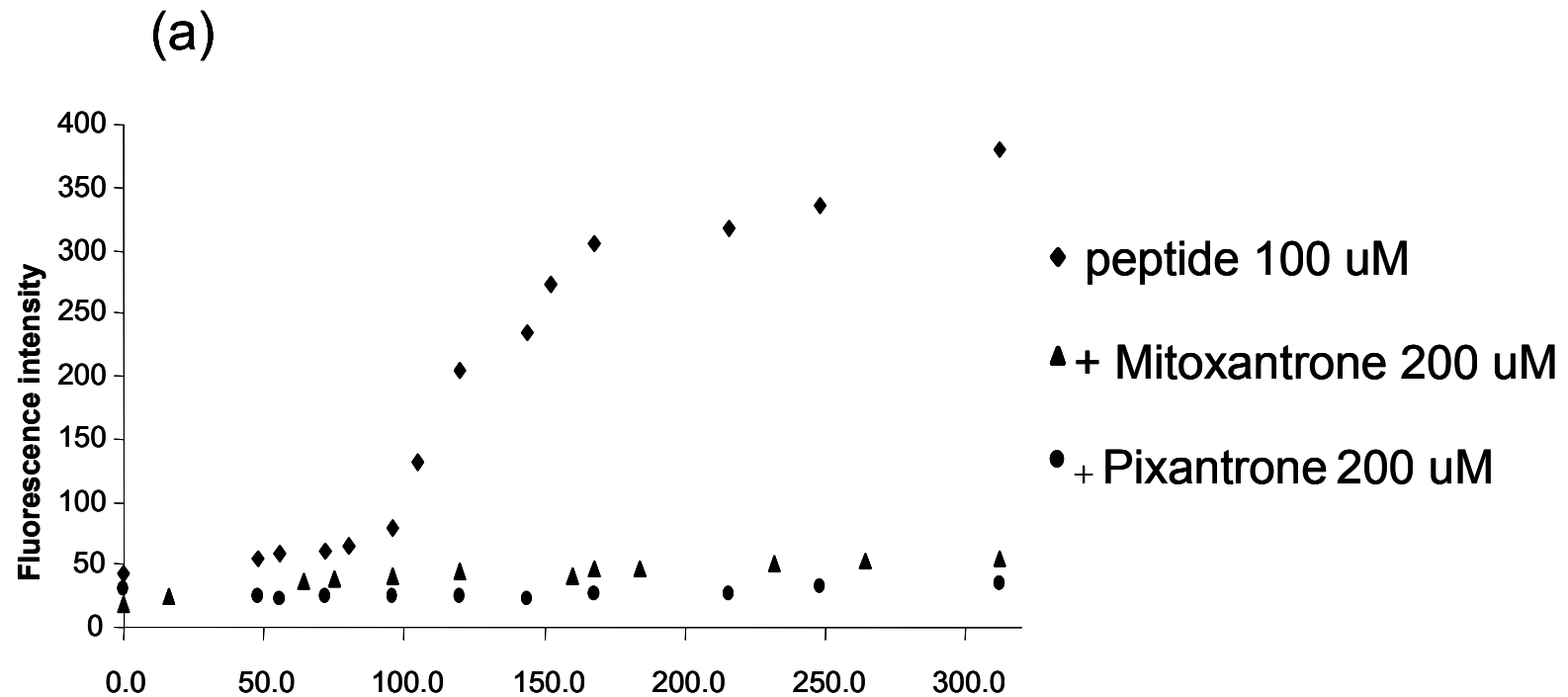
Colombo, R., et al. "Derivati antracenedionici e aza-antracenedionici come agenti capaci di inibire l'aggregazione di peptidi amiloidi" *Patent n. MI2008A366, 2008.*



Saggio ThT : studi di fibrillogenesi



Colombo R. et al., *Electrophoresis* 2009, 30, 1418



PIXANTRONE: RECUPERO VITALITÀ CELLULARE

Flow Field-flow fractionation (FIFFF)



- No interazioni
- No degradazione
- Informazioni dimensionali e PM

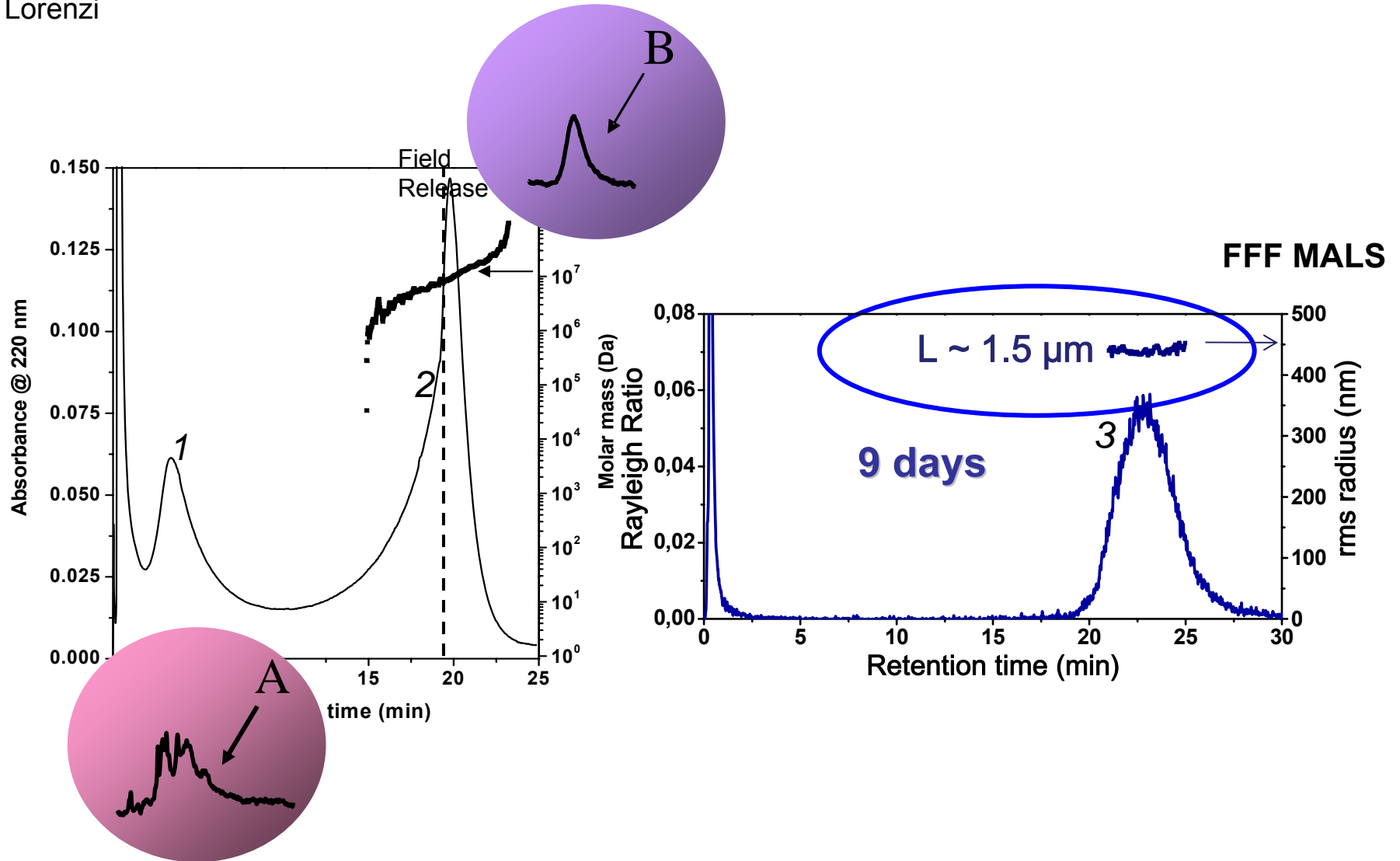
➤ **MACROMOLECOLE (10^{15} PM e μm)**

➤ **PROTEINE, COMPLESSI, CELLULE, AGGREGATI**

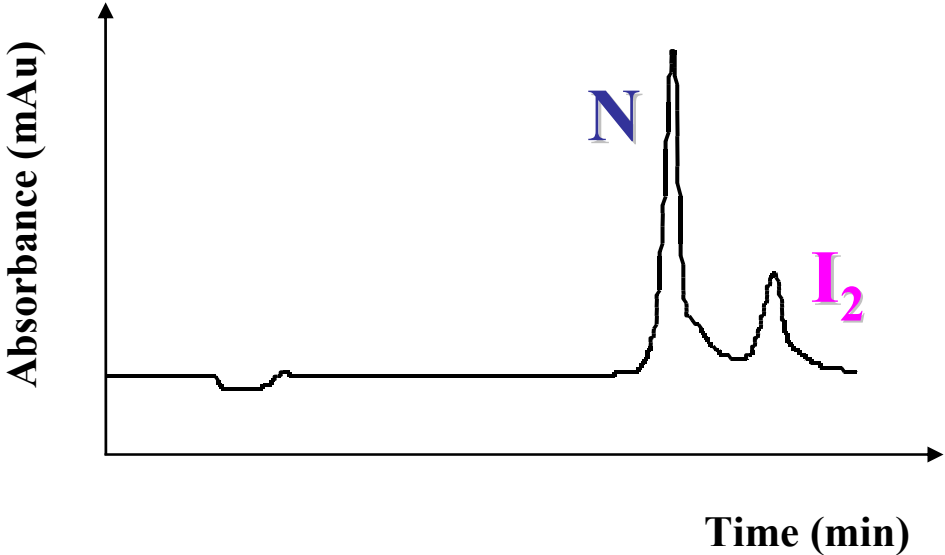
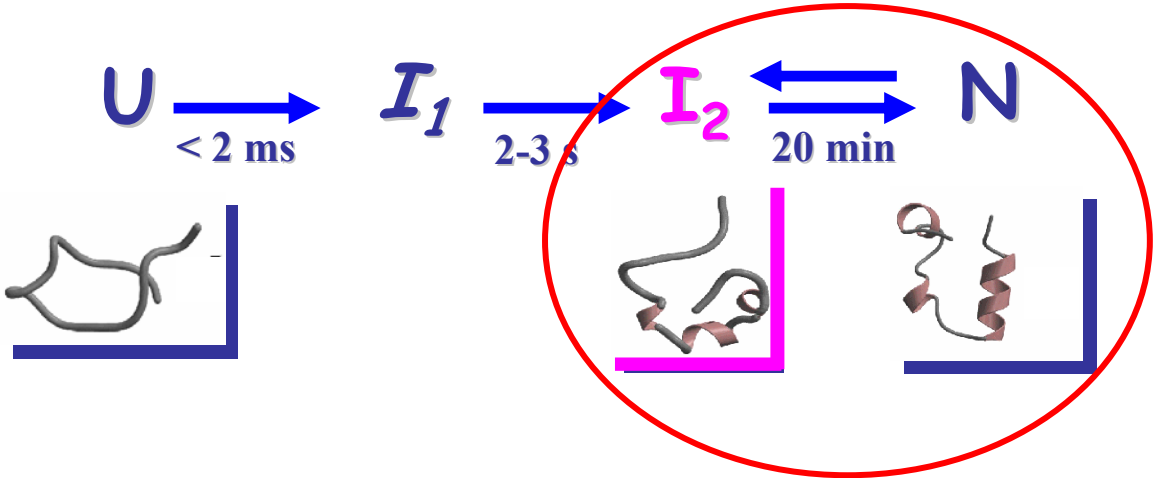


D.C. Rambaldi,
A. Zattoni,
P. Reschiglian,
R. Colombo,
E. De Lorenzi

In vitro Amyloid Ab1-42 Peptide Aggregation Monitoring by Asymmetrical Flow Field-Flow Fractionation with Multi-Angle Light Scattering Detection (Anal. Bioanal. Chem. *accepted*)



β_2 -microglobulina

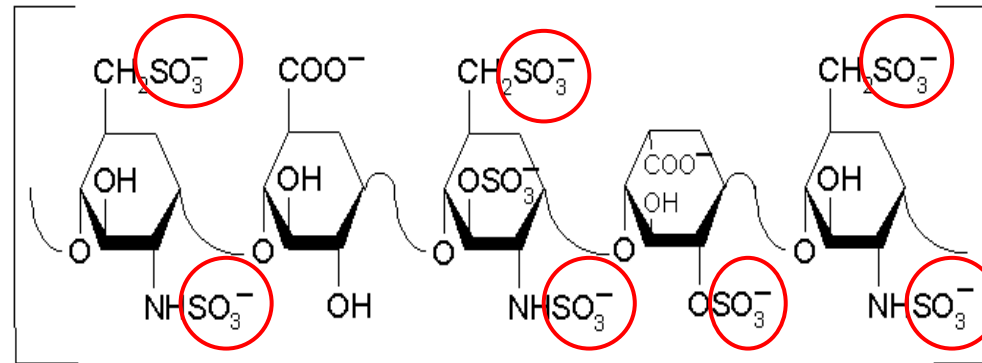


Chiti F. et al., *J. Biol. Chem.* 2001, 276, 46714

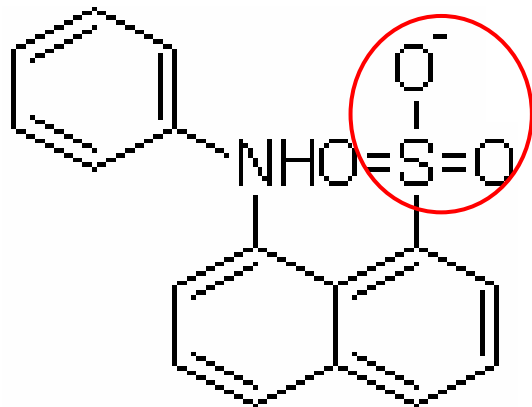
International Patent: De Lorenzi et al . 2003



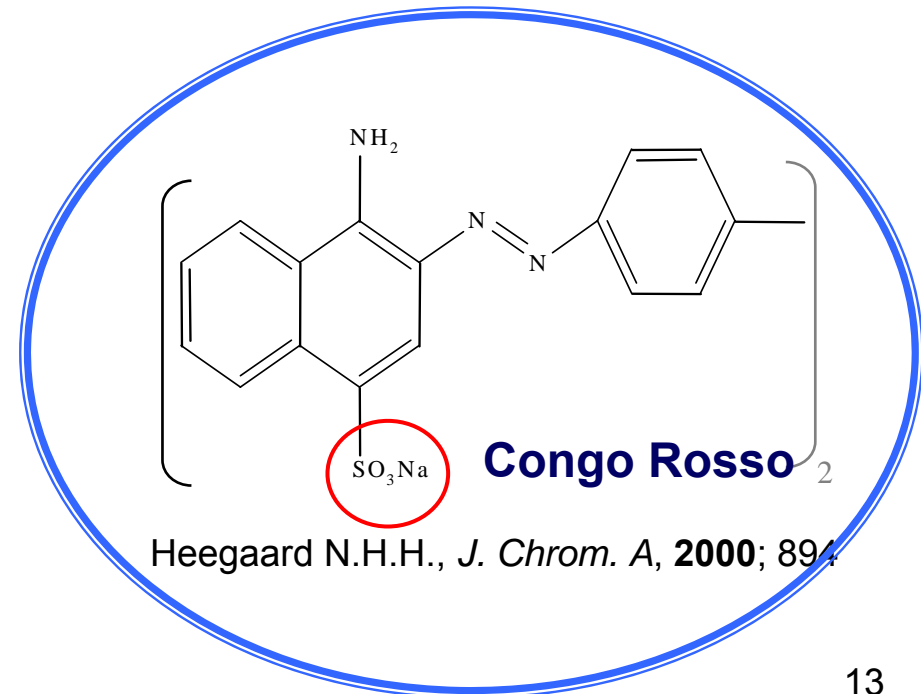
Eparina



Ohashi K., *Nephron.*, **2002**; 90



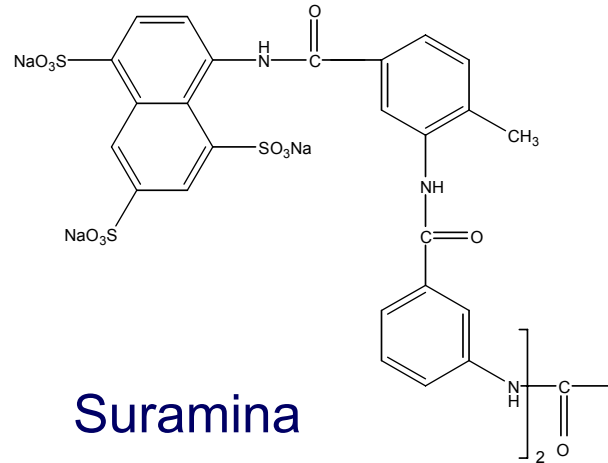
Acido 8-Anilino-1-naftalensolfonico



Heegaard N.H.H., *J. Chrom. A*, **2000**; 894

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 Ersilia De Lorenzi¹

Search of ligands for the amyloidogenic protein β_2 -microglobulin by capillary electrophoresis and other techniques



De Lorenzi E., *Electroph.*, 2002; 23

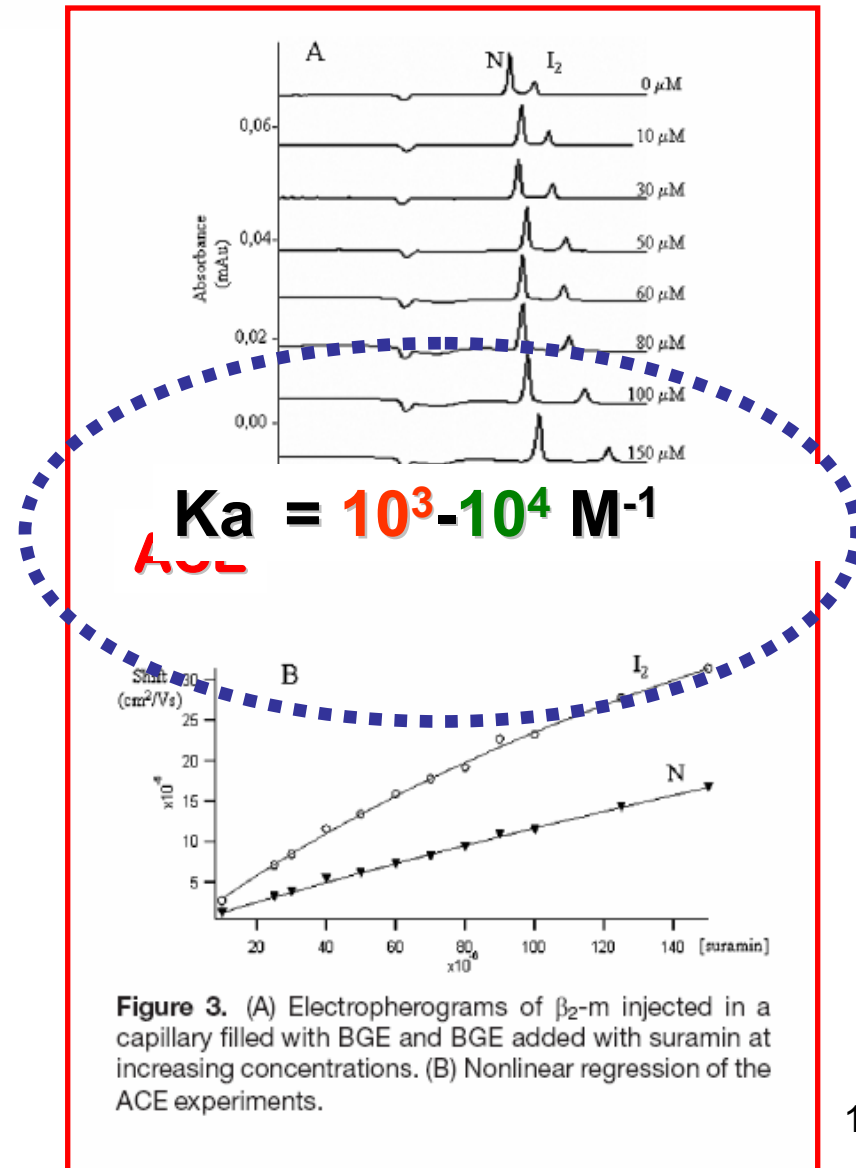
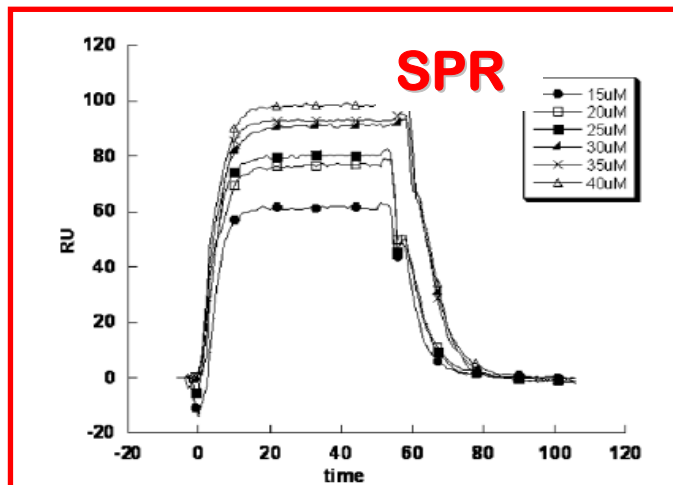
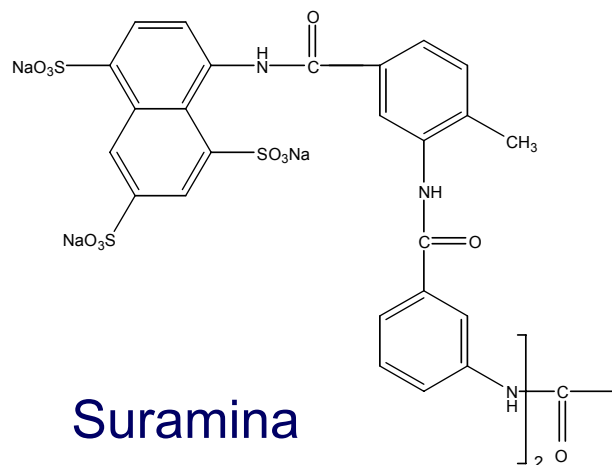
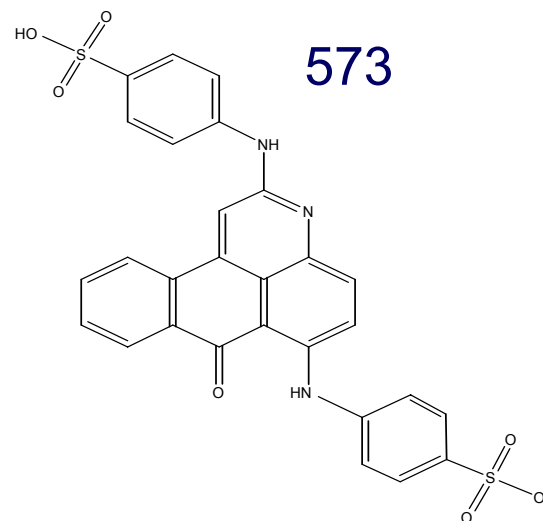


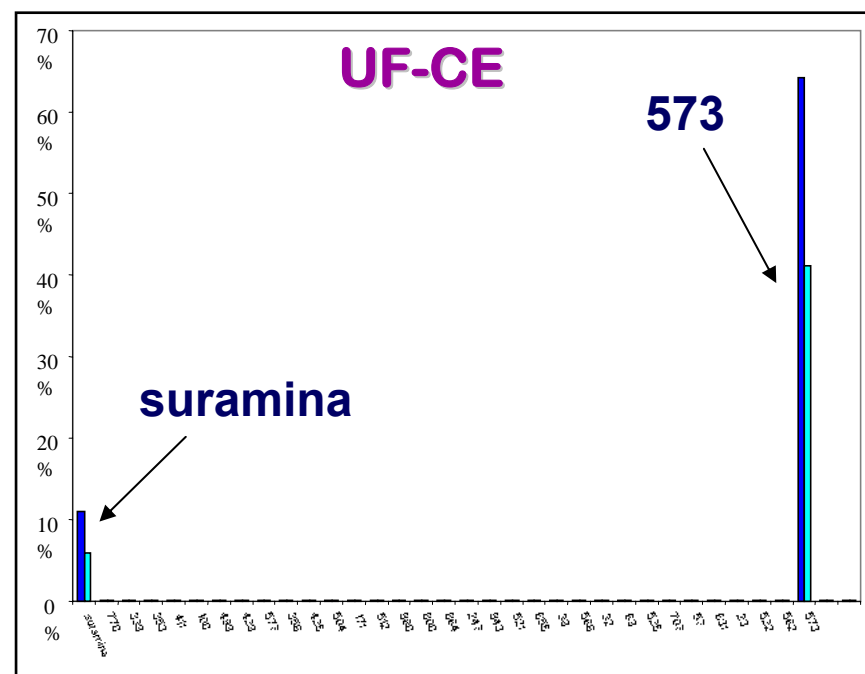
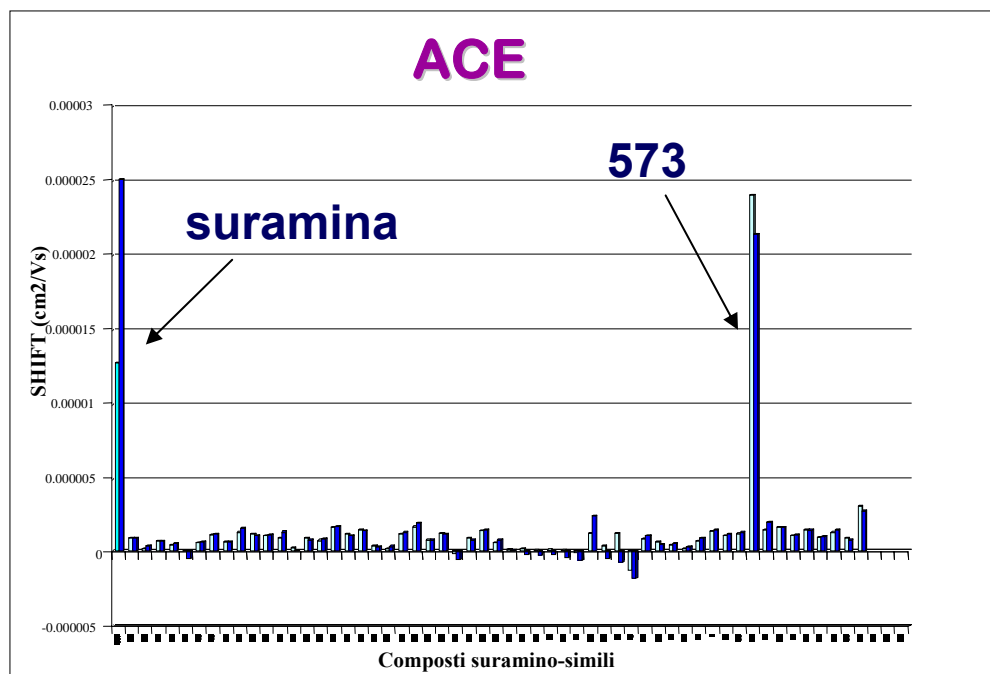
Figure 3. (A) Electropherograms of β_2 -m injected in a capillary filled with BGE and BGE added with suramin at increasing concentrations. (B) Nonlinear regression of the ACE experiments.



Suramina



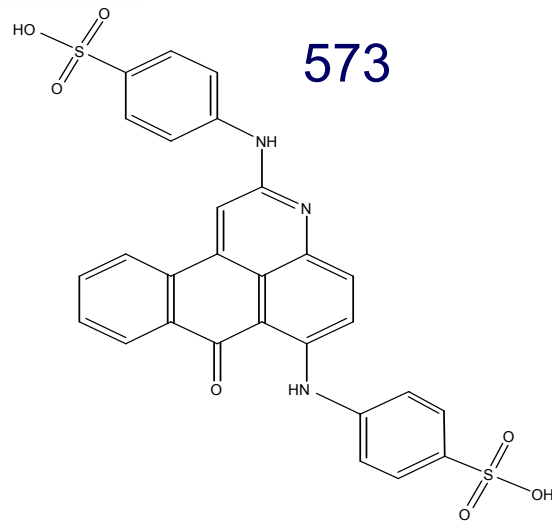
573



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 Vittorio Bellotti^{2,3}
 Ersilia De Lorenzi¹

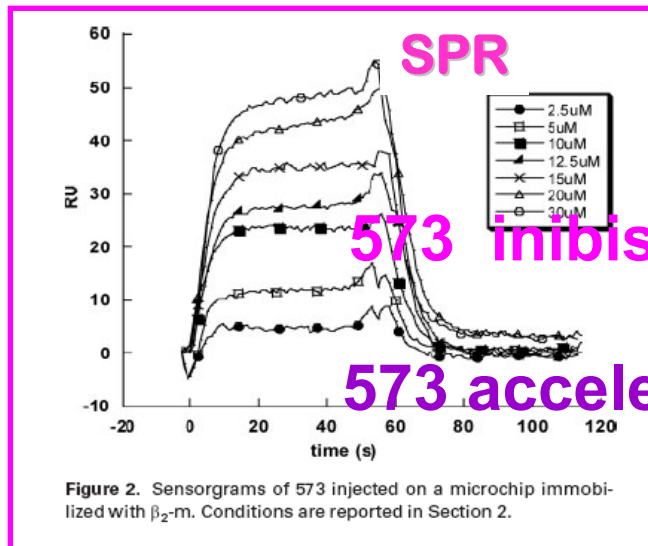
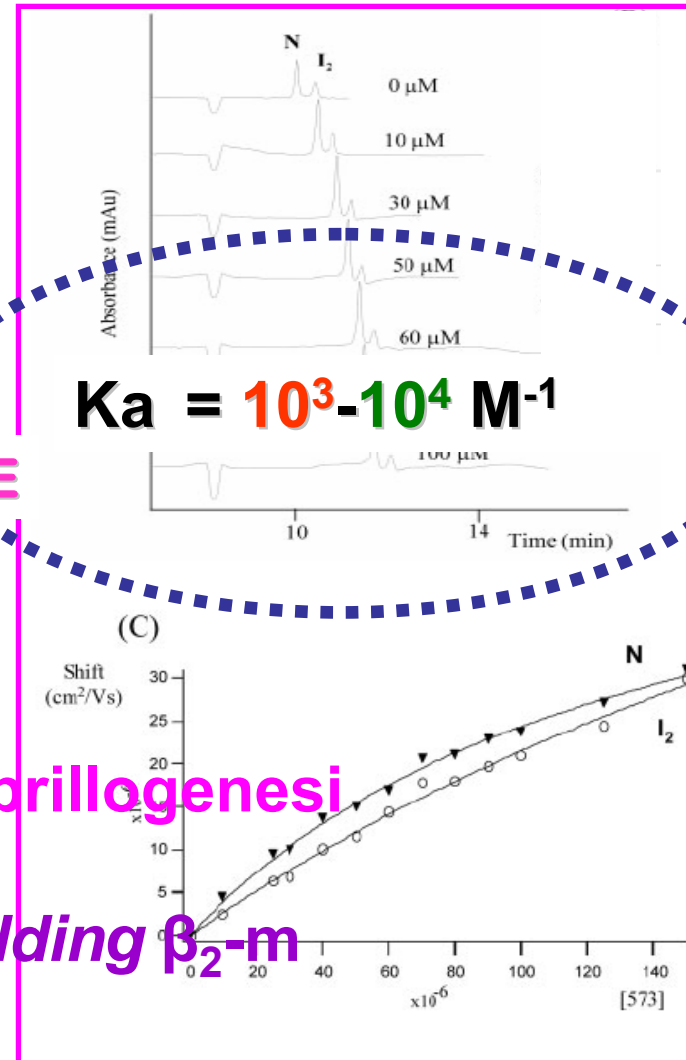
Research Article

Sulfonated molecules that bind a partially structured species of β_2 -microglobulin also influence refolding and fibrillogenesis



ACE

$K_a = 10^3 - 10^4 \text{ M}^{-1}$



Spettrometria di massa a trappola elettrostatica (ORBITRAP)

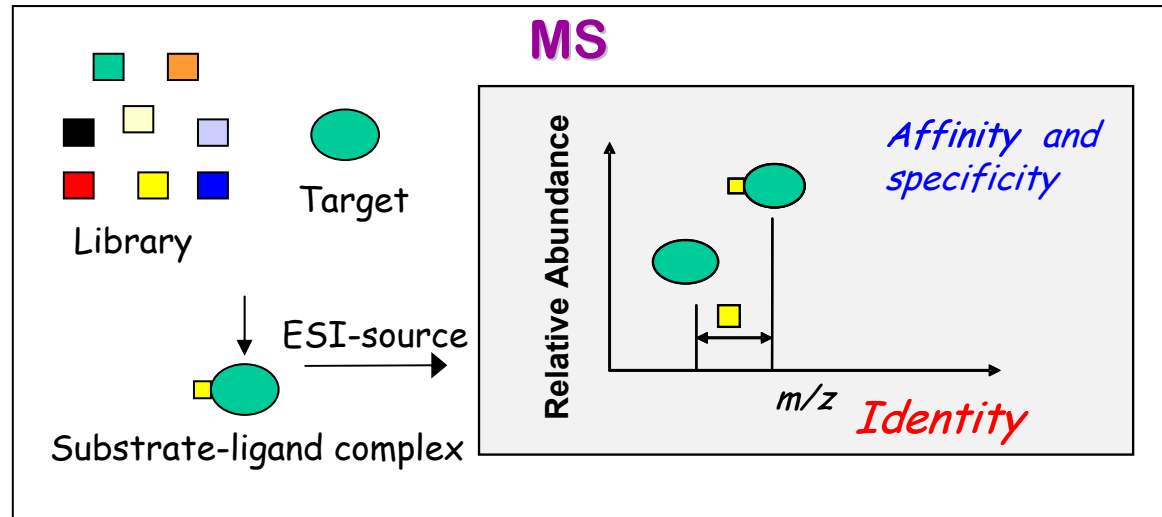


- Elevata sensibilità e risoluzione
- Alto *throughput*
- Bassi consumi

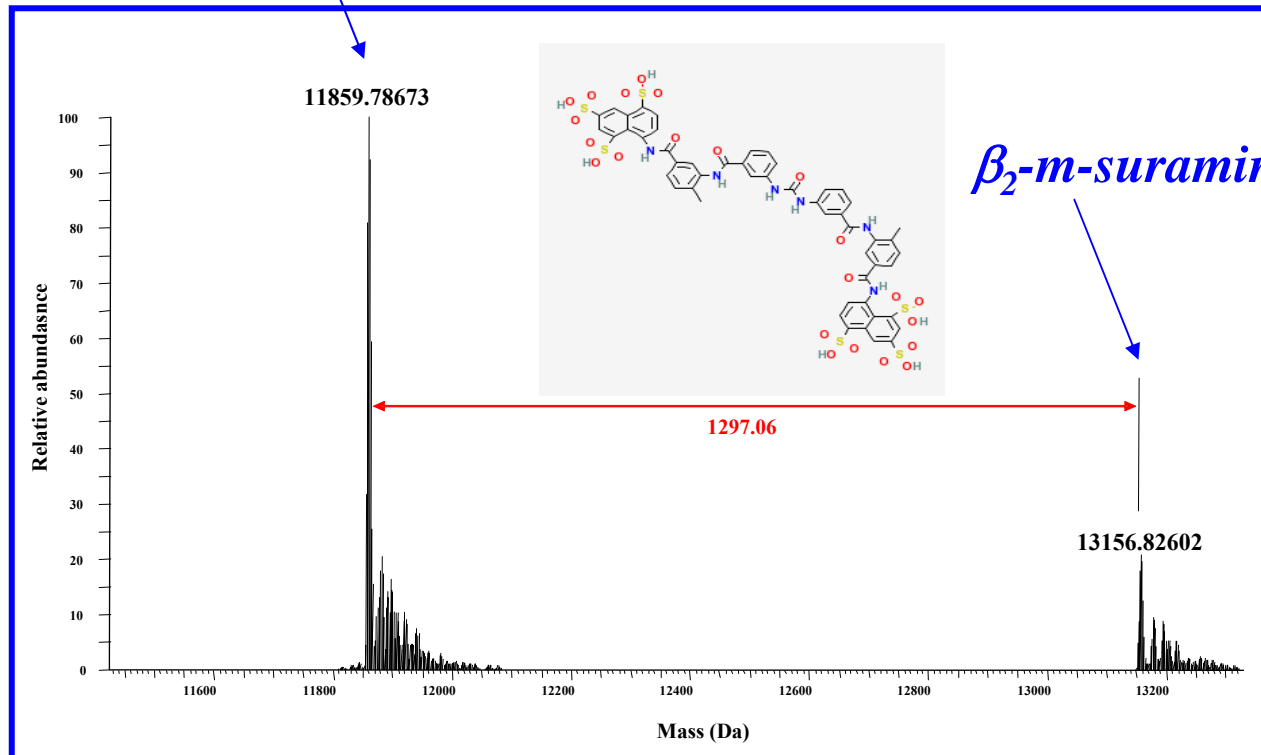
➤ AFFINITÀ

➤ STECHIOMETRIA DI LEGAME





β_2 -m

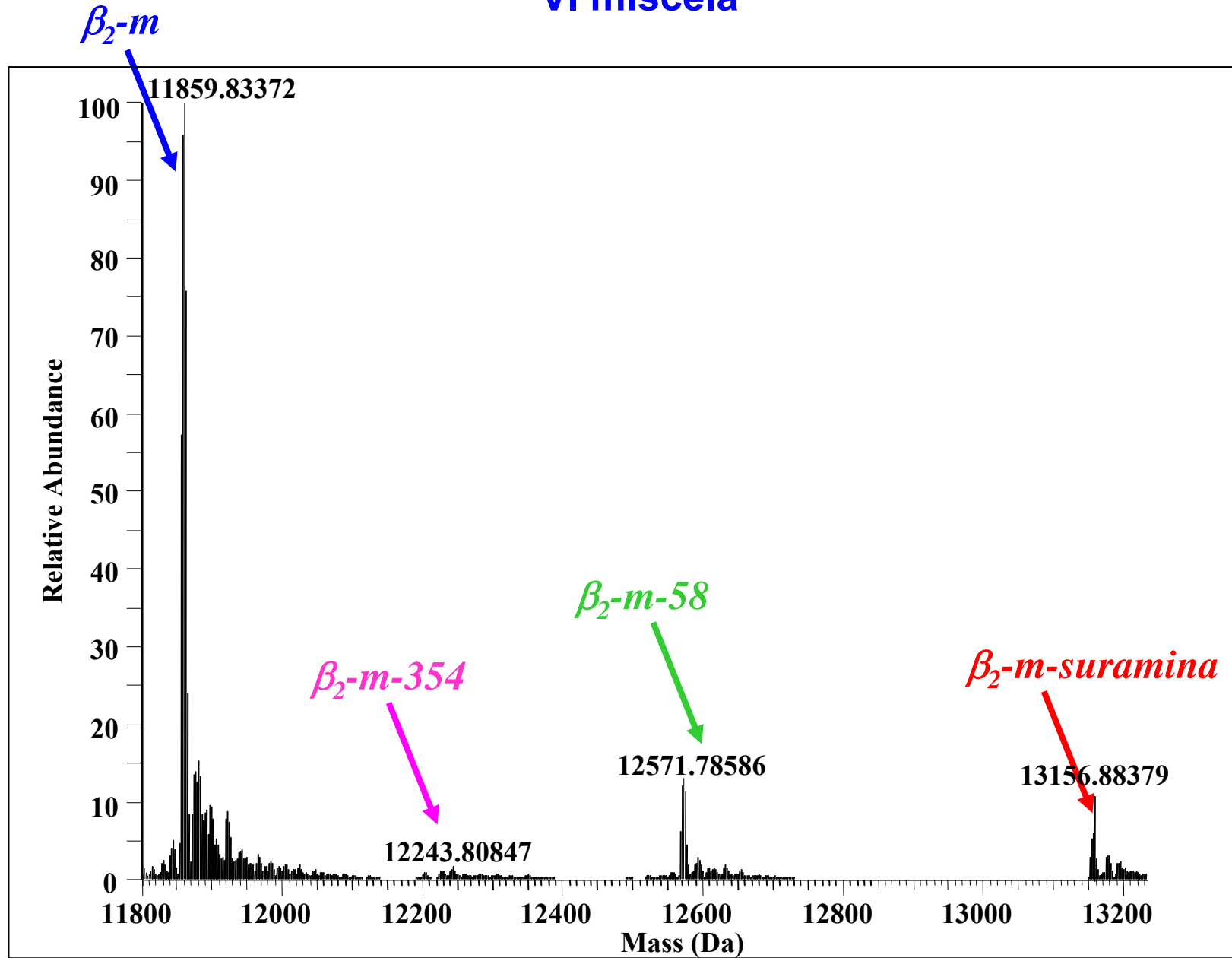


- voltaggio
 - temperatura
 - tampone
 - tempi di incubazione
-
- numero ligandi in miscela
 - rapporto proteina: ligandi

Screening di 100 molecole solfonate

n° miscela	composti SPECS
I	516, 876, 454, 457, 875, 349, 220, 215, 735, 980 + suramina
II	37, 42, 30, 33, 34, 661, 31, 358, 658, 885 + suramina
III	210, 800, 864, 247, 943, 521, 655, 38, 566, 32 + suramina
IV	152, 56, 391, 382, 53, 872, 35, 380, 362 + 573 + suramina
V	23, 522, 562, 707, 320, 572, 103, 359, 376, 567 + suramina
VI	22, 61, 486, 832, 54, 954, 354, 44, 55, 58 + suramina
VII	814, 819, 59, 62, 47, 813, 49, 28, 48, 46 + suramina
VIII	386, 246, 21, 825, 70, 561, 69, 525, 409, 473 + suramina
IX	190, 91, 92, 770, 339, 353, 411, 100, 499, 429 + suramina
X	577, 356, 425, 504, 171, 512, 524, 605, 659, 12 + suramina

VI miscela



Classe 1	Classe 2	Classe 3 ≈ SURAMINA	Classe 4 > SURAMINA
54	37	8 # 573	1 #: 58



"Proteine amiloidogeniche e piccole molecole: studi di binding"

Raffaella Colombo PhD

Capogruppo: Ersilia De Lorenzi

Pavia, 12 giugno 2009