

Effects of pleiotropic molecules on alpha-crystallin aggregation

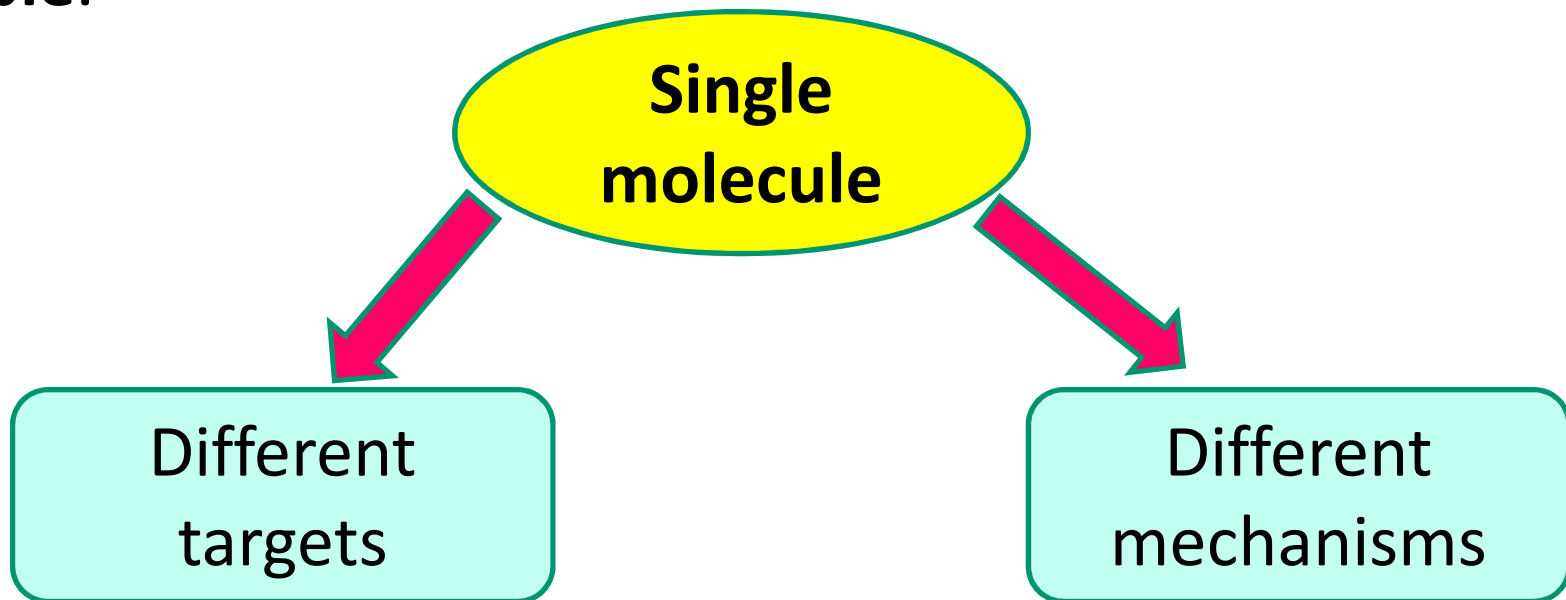
Vincenzo Giuseppe Nicoletti



*Università di Catania - Scuola "Facoltà di Medicina"
Dipartimento di Scienze Bio-mediche sez. Biochimica*

Pleiotropismo

- Termine coniato nel 1910 da Ludwig Plate.
- Fenomeno genetico (pleiotropia): un gene può produrre **svariati effetti sul fenotipo**.
- Viene usato anche per indicare **molecole con proprietà multiple**.

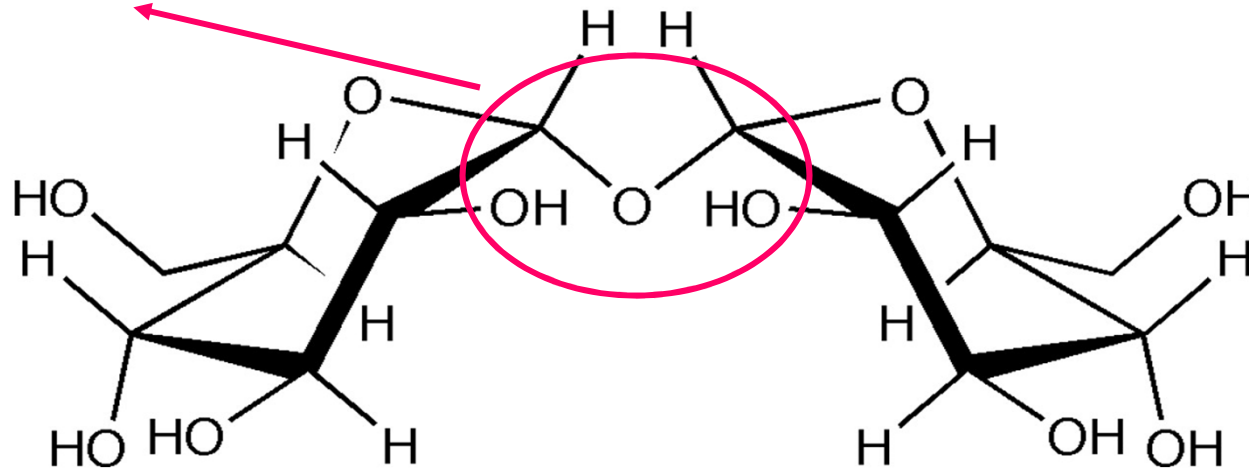


Il disaccaride **trealosio e il dipeptide **carnosina** sono tipiche molecole pleiotropiche:**

- **stabilizzazione del ripiegamento delle proteine;**
- **inibizione dell'aggregazione;**
- **elevata capacità protettiva contro vari tipi di stress.**

What is Trehalose?

α,α -1,1-glycosidic linkage



- Non-reducing disaccharide joint by two glucoses
- Protects cells against various **stress** conditions
- it may serve as a **source of energy** and carbon
- Confers resistance to desiccation (**anhydrobiosis**) (Crowe 1970)

Where is Trehalose Found?

- Until 1997, trehalose was thought to be present in a few **desiccation-tolerant plants**
- can be found in: bacteria, fungi, insects, plants and invertebrates.
- but **not in mammals**.



Selaginella lepidophylla
Resurrection Plants

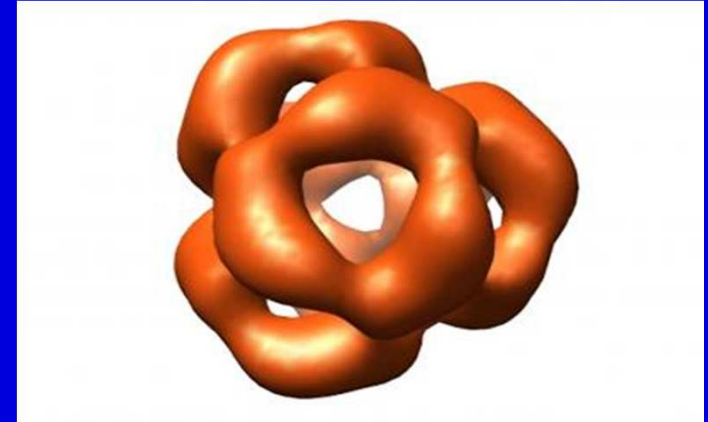
Tardigrades



Carnosine (β -alanyl-L-histidine)

- It is synthesized by carnosine synthase from **L-histidine** and **beta-alanine**
- It is degraded by **carnosinase**.
- attenuate muscle acidosis.
- content **declines with age**.

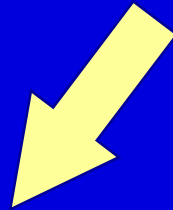
crystallins



- the family is composed of three classes: **α** , **β** and **γ** .
- **predominant proteins** within the eye lens.
- α -Crystallin belongs to the **heat shock proteins family**;
- prevents misfolding (aggregation and/or precipitation) of other proteins, itself included due to a **self-chaperone property**.

Nel suo stato nativo l' α -cristallina forma un grosso **aggregato eterogeneo** a basso peso molecolare (LMW) solubile in acqua

alpha crystallin



α -A

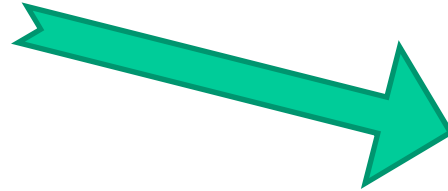
α -B

20 kDa 173 AA

20 kDa 175 AA

**LMW
crystallin
↓800 Kda**

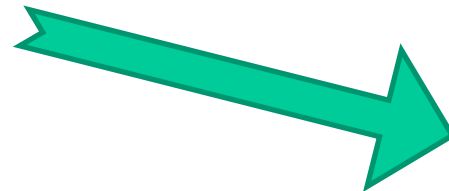
Aggregation



**HMW crystallin
↑1000 Kda**

**Aging
cataract**

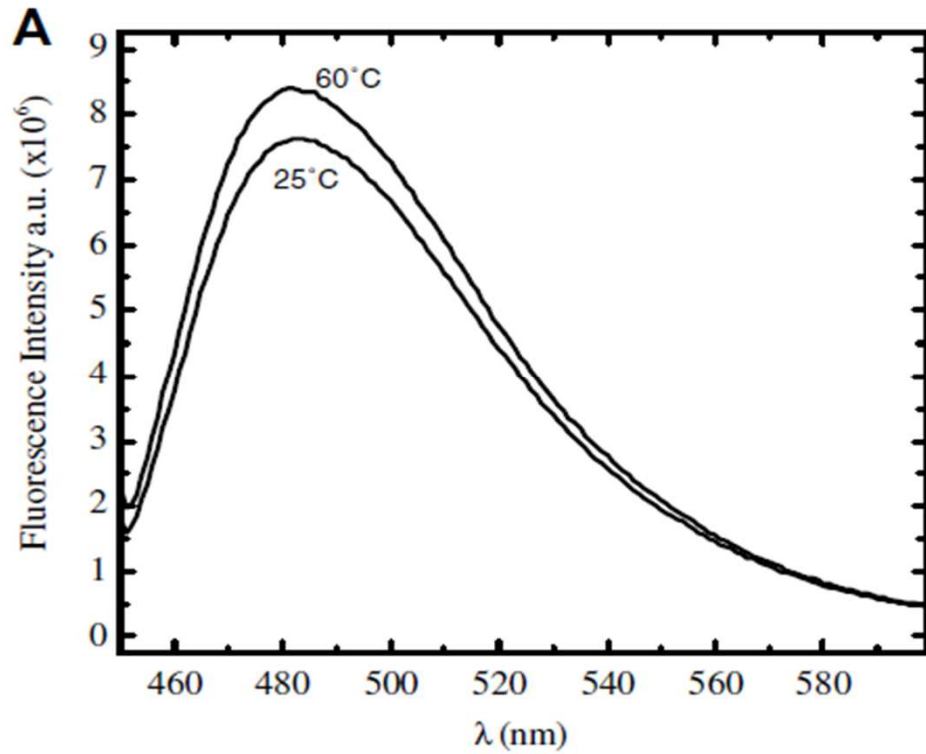
**Folded
crystallin**



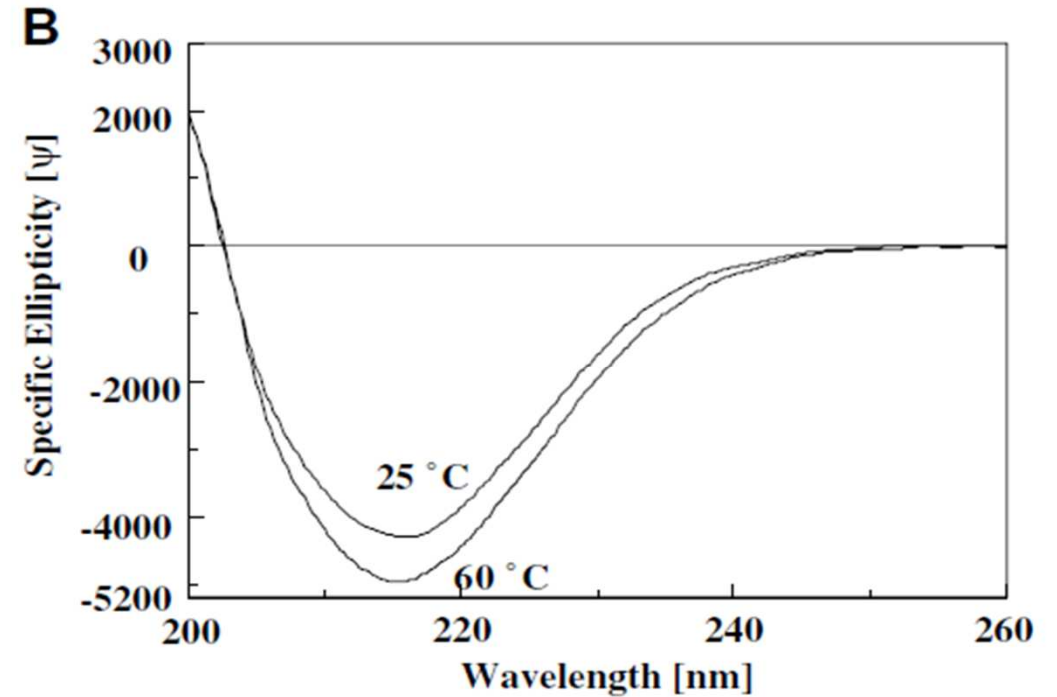
**misfolded
crystallin**

**Posttranslational
modification**

Effect of temperature on α -crystallin aggregates

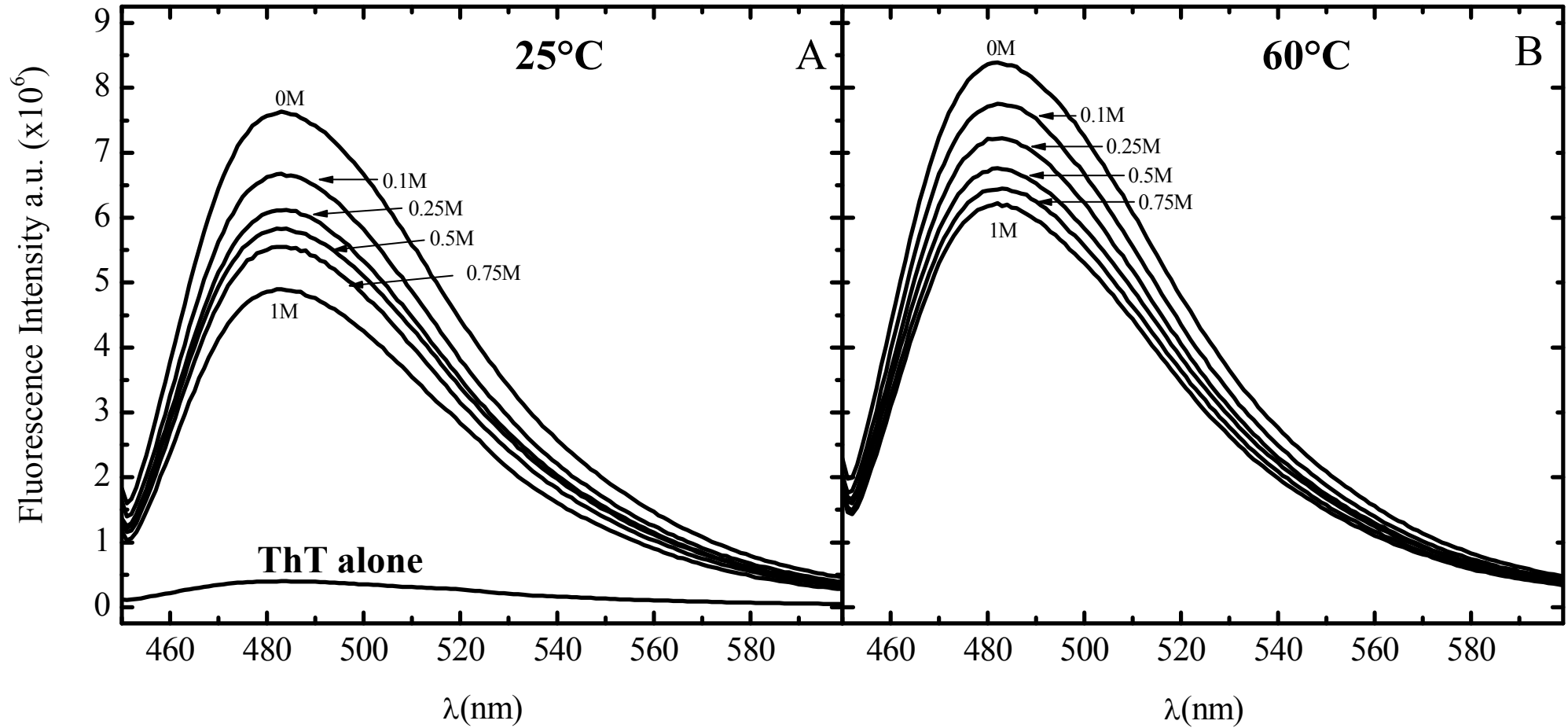


ThT (Thioflavin T)
fluorescence spectrum

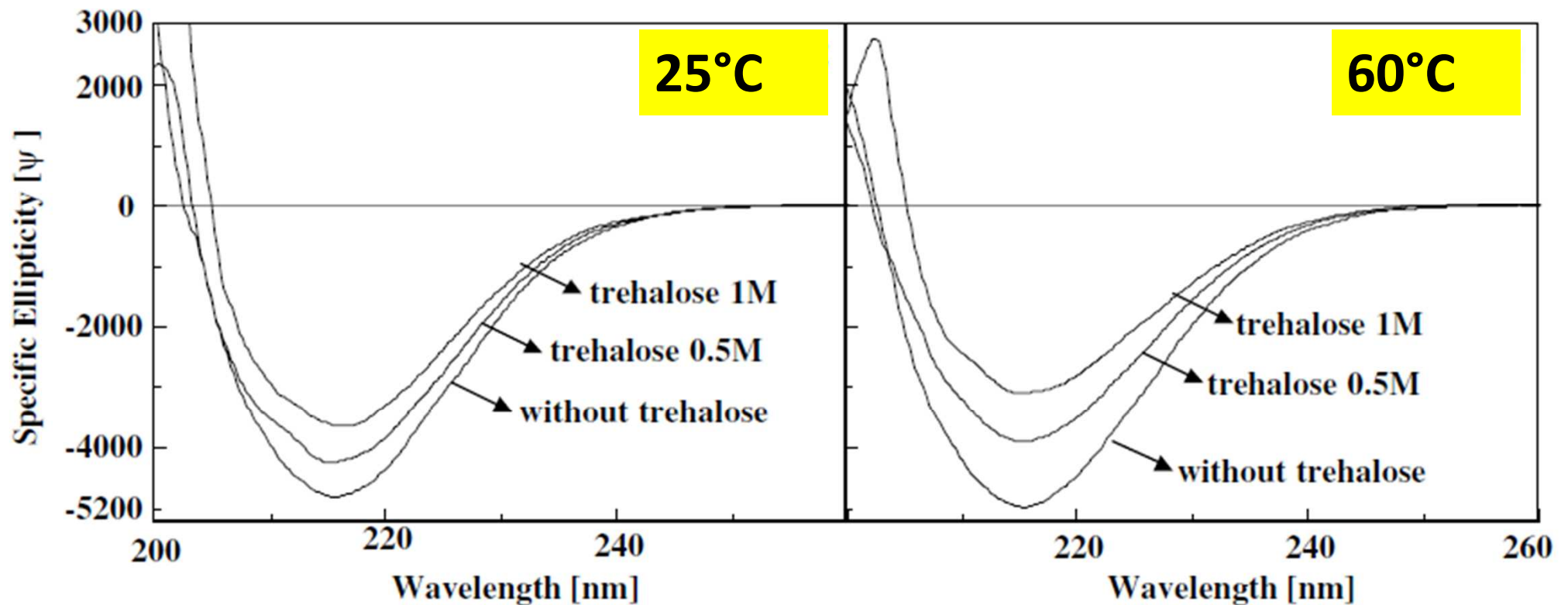


CD (circular dichroism) spectrum

Fluorescenza di ThT in soluzione con α -cristallina a diverse concentrazioni di trealosio (0M-1M)

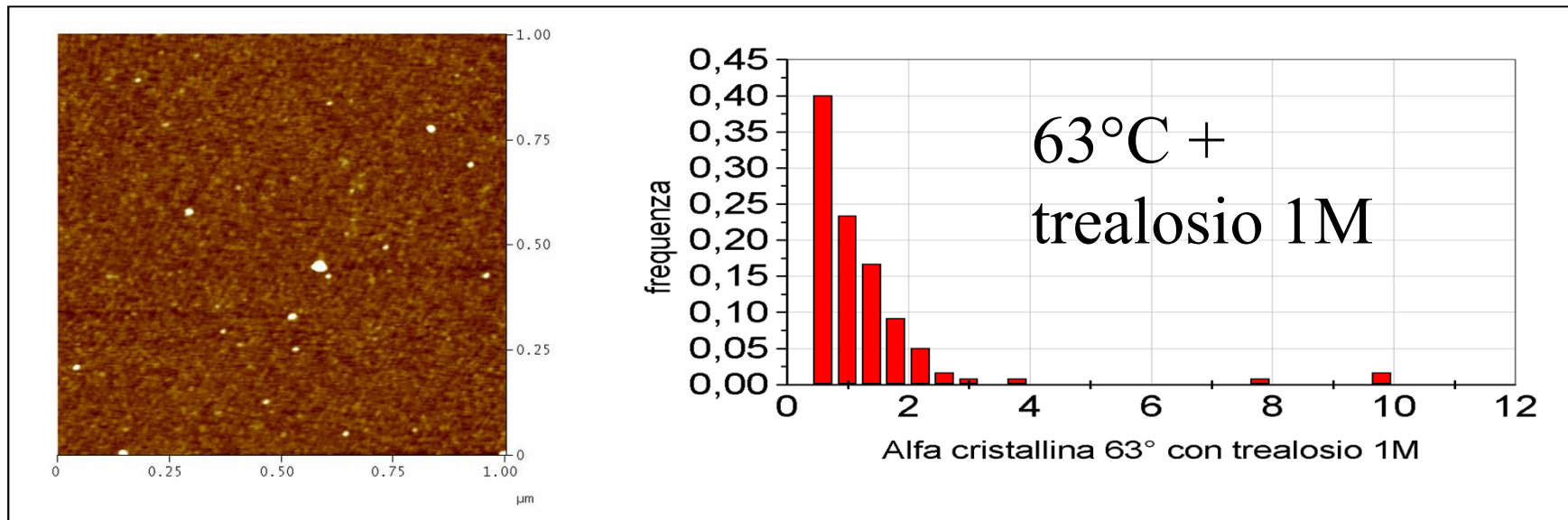
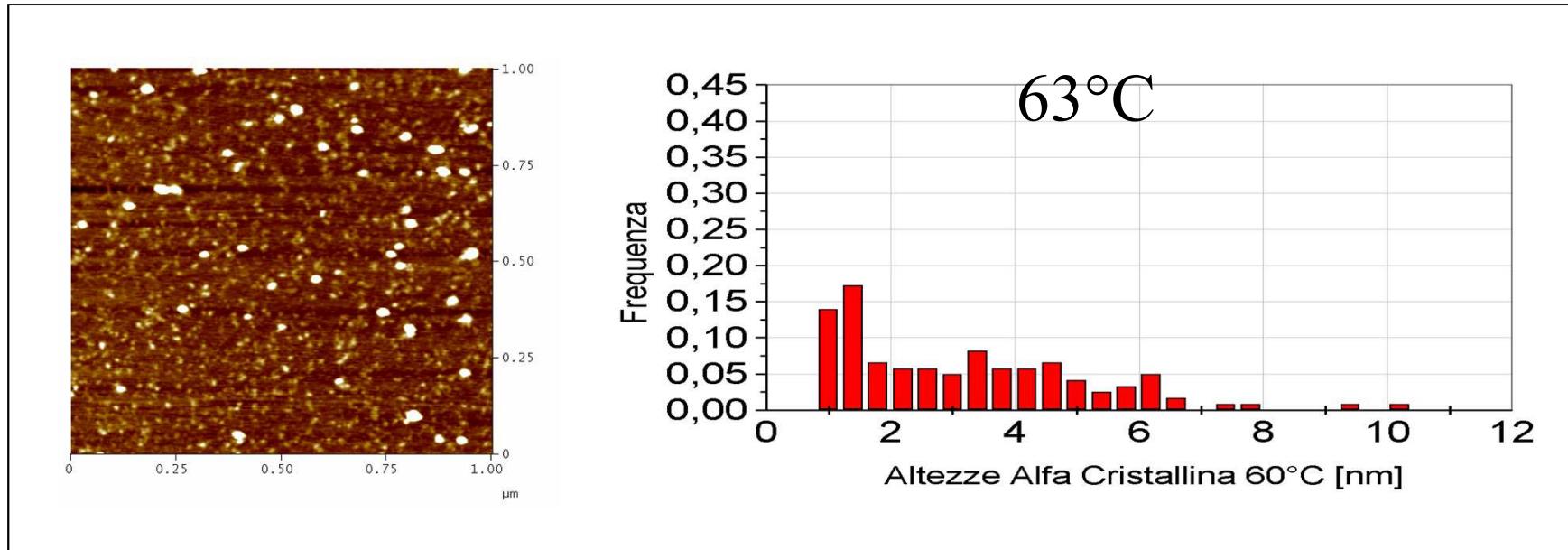


CD spectra of α -crystallin

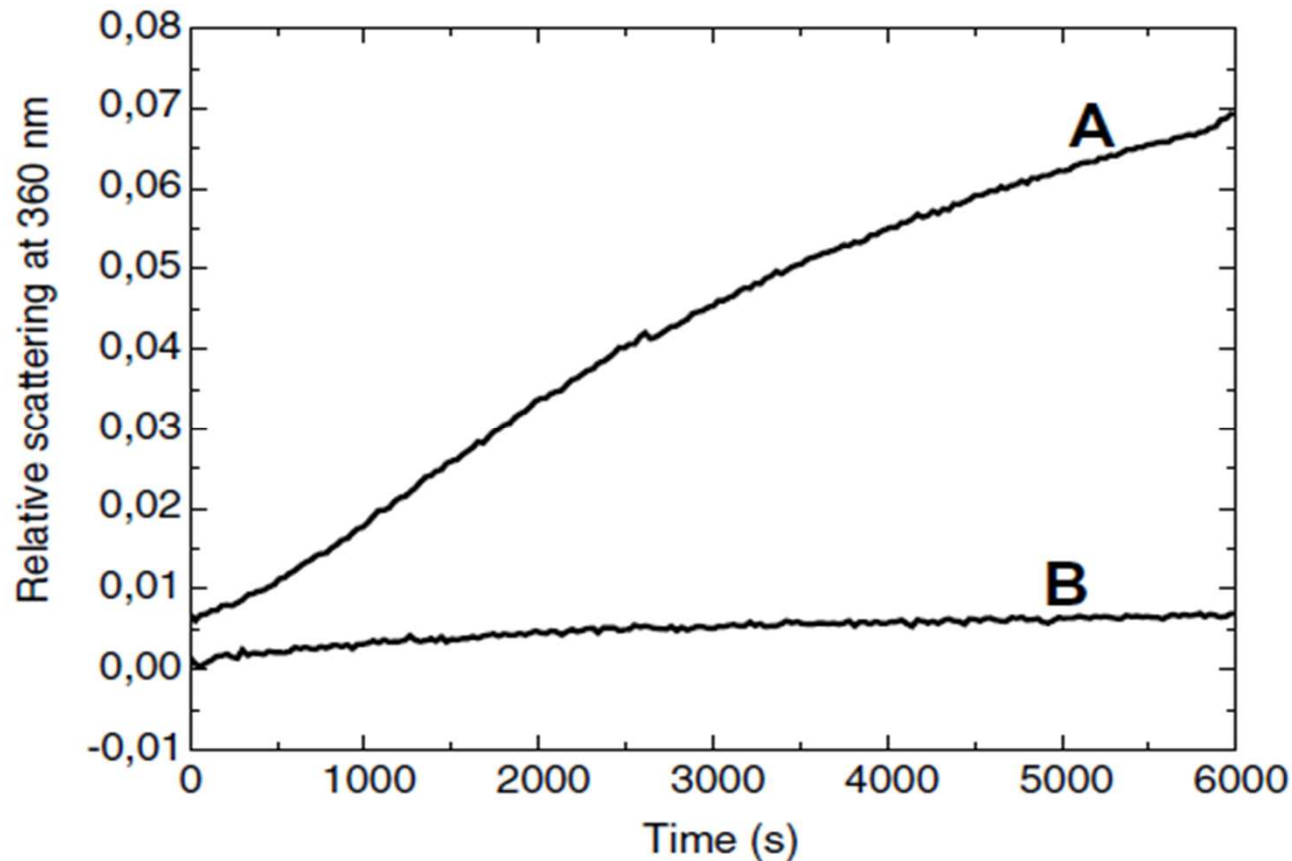


Dose dependent **decrease of the CD negative ellipticity** (formation of HMW aggregates) after incubation of α -crystallin solutions (0.5 mg/ml in 0.0025M phosphate buffer solution, pH 7) in the presence of different trehalose concentrations at 25°C or 60°C.

Effetto antiaggregante del trealosio osservato tramite microscopia a forza atomica



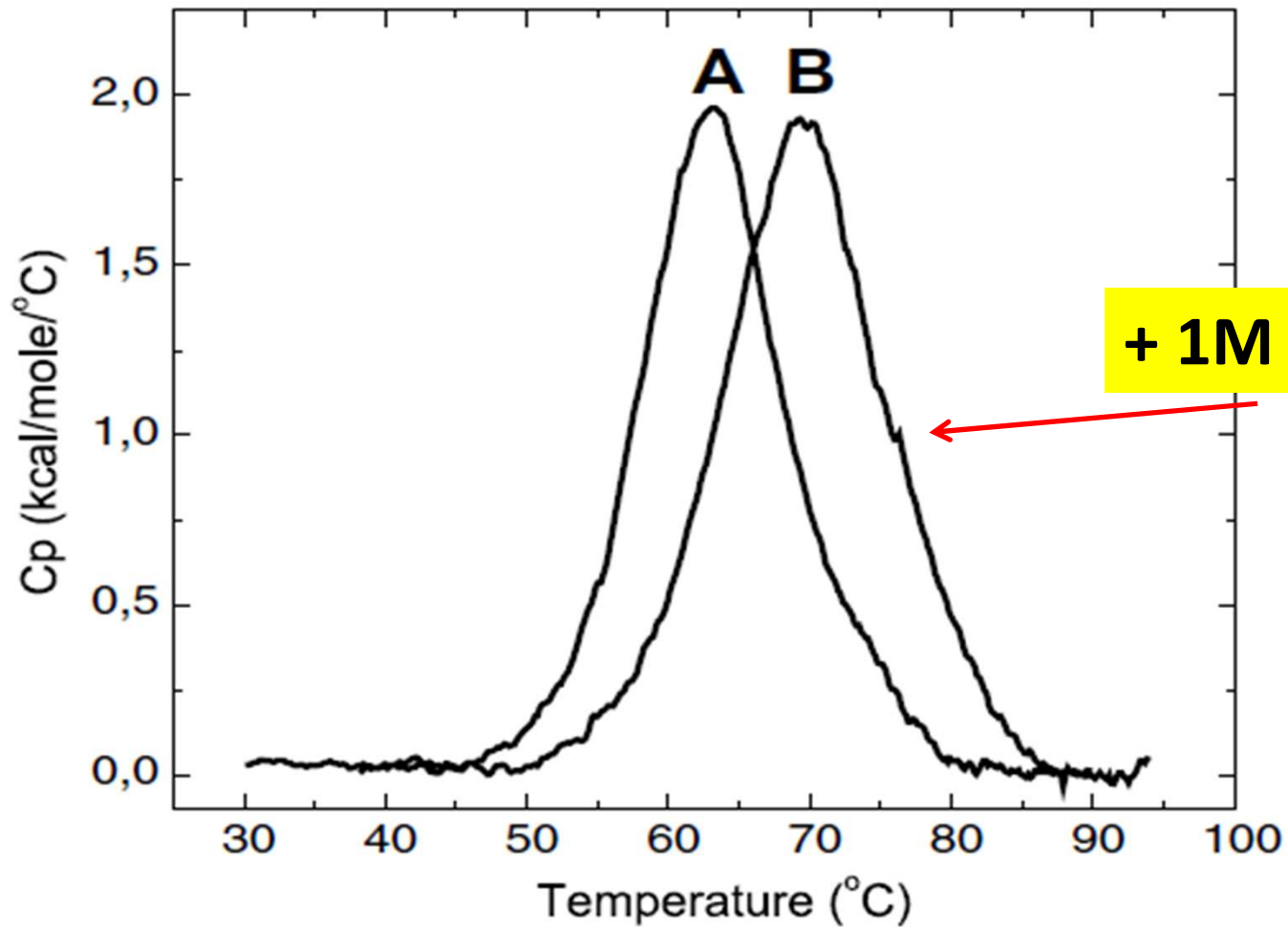
Trehalose cannot affect the chaperone activity of α -crystallin to protect the β L-crystallin from heat-induced aggregation.

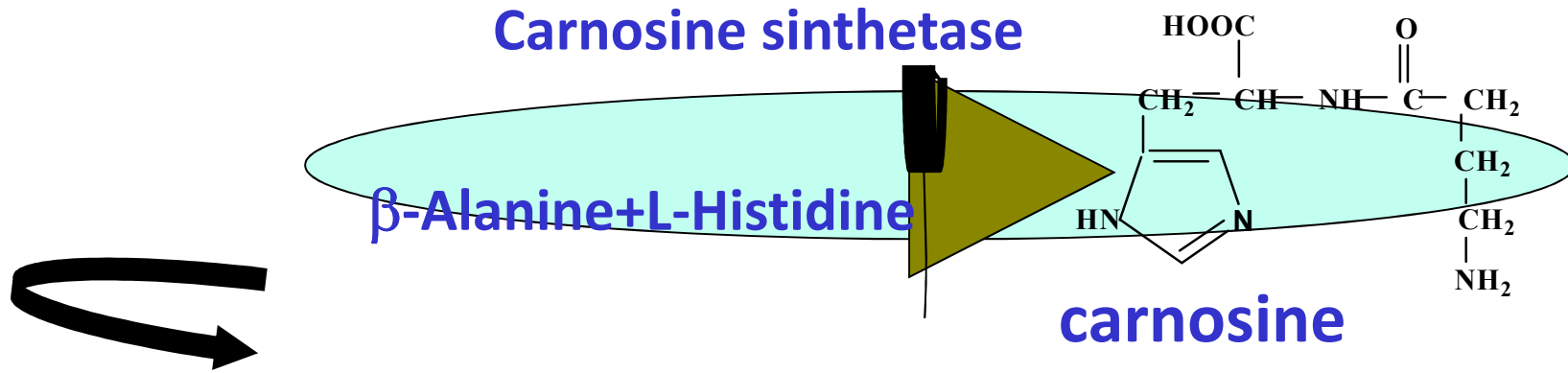


- 1 M trehalose
- 60°C
- (A) no α -crystallin
- (B) + α -crystallin

Aggregation of β L-crystallin occurred when the protein was heated at 60°C and was completely suppressed in the presence of α -crystallin (dissolved in 1 M trehalose) at a molar ratio of 1:2 with β L-crystallin (Fig. 4B).

Differential scanning calorimetry (DSC) profiles of α -crystallin to characterize temperature induced conformational changes





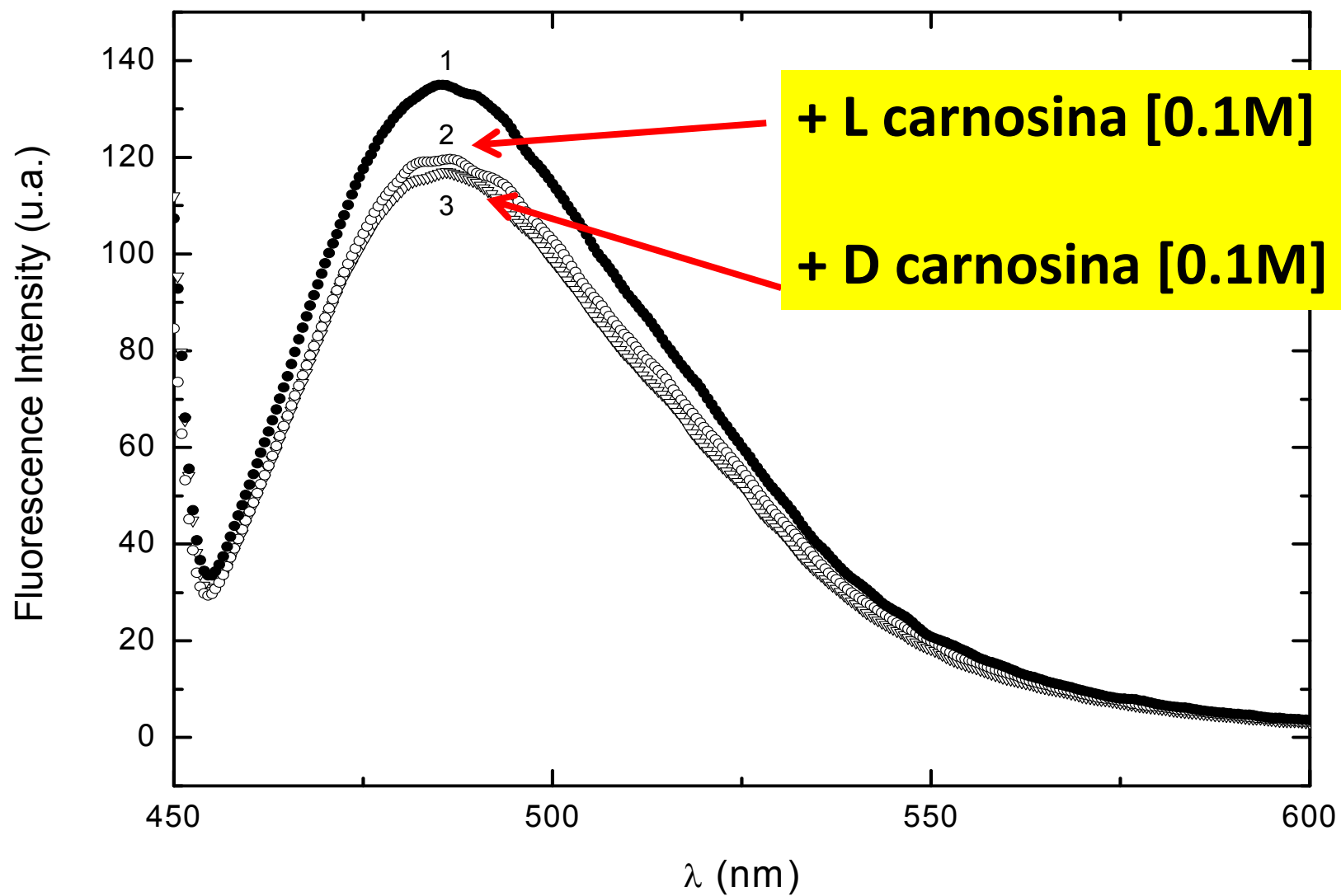
Distribution up to 20mM in:

- **Skeletal muscle**
- **brain**
- **heart**

Functions

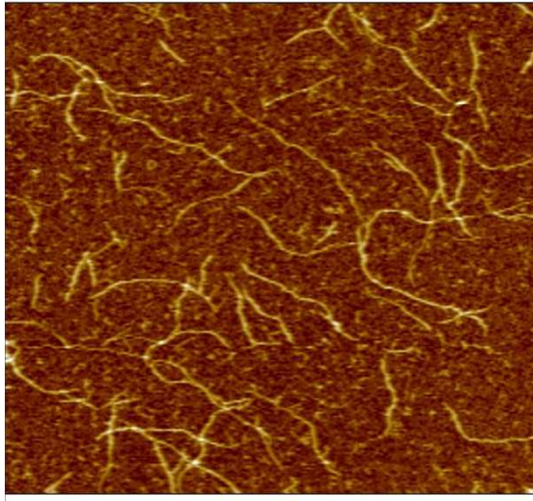
- Physiological intracellular buffer
- Metal ion chelator
- Antioxidant
- an antiglycant agent
- free radicals scavenger

Spettri di fluorescenza di ThT di campioni di α -cristallina in guanidina 1M (curva 1) e co-incubata con L o D carnosina

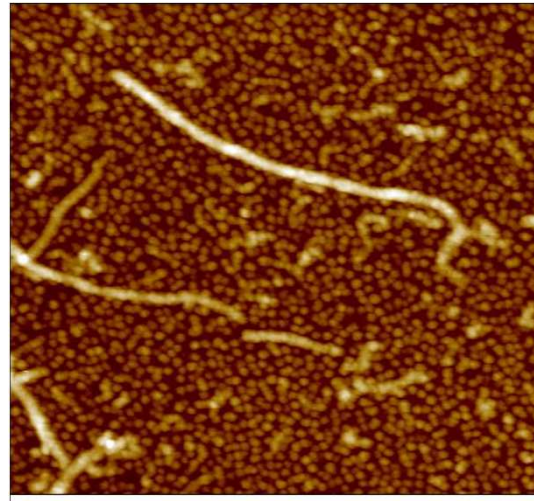


AFM: α -Cristallina (10 mg/ml)

co-incubata (60°C / 24h)
+ guanidina 1M

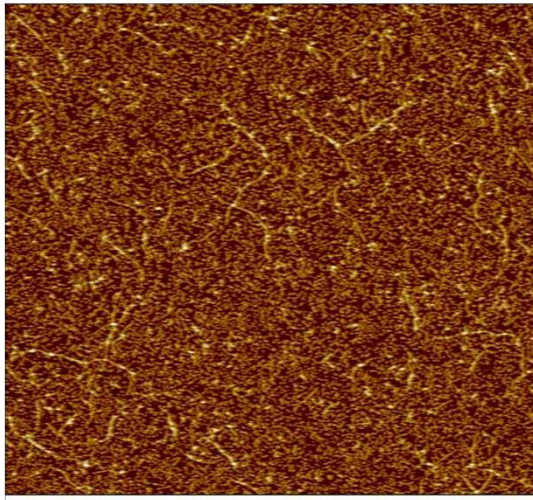


0 5.00 μ m
Data type Height
Z range 30.0 nm

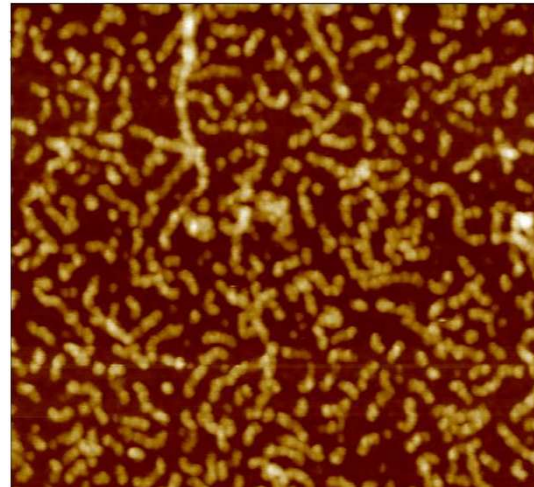


0 1.00 μ m
Data type Height
Z range 30.0 nm

+ guanidina 1M e
D-carnosina 100 mM



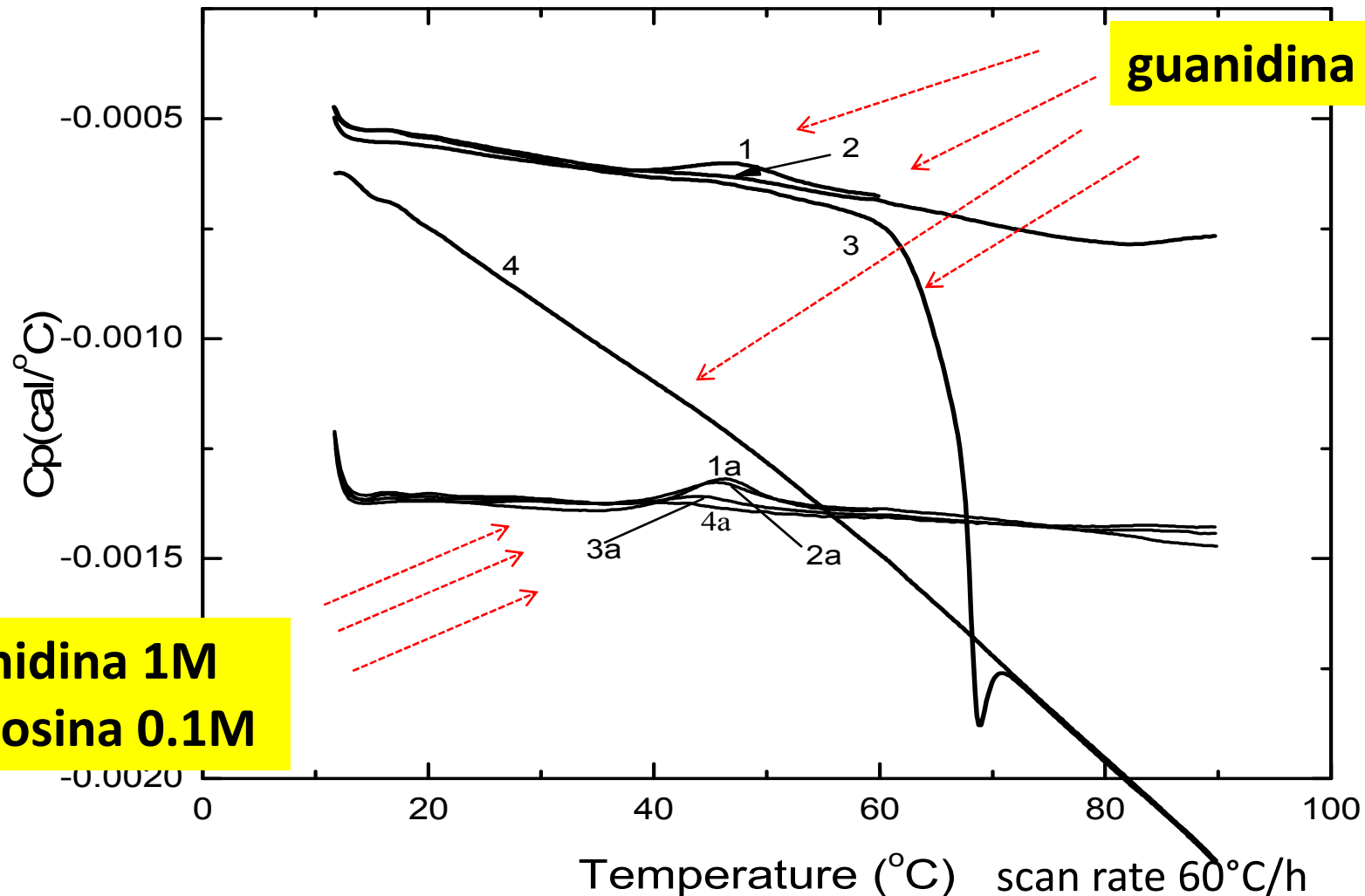
0 5.00 μ m
Data type Height
Z range 30.0 nm



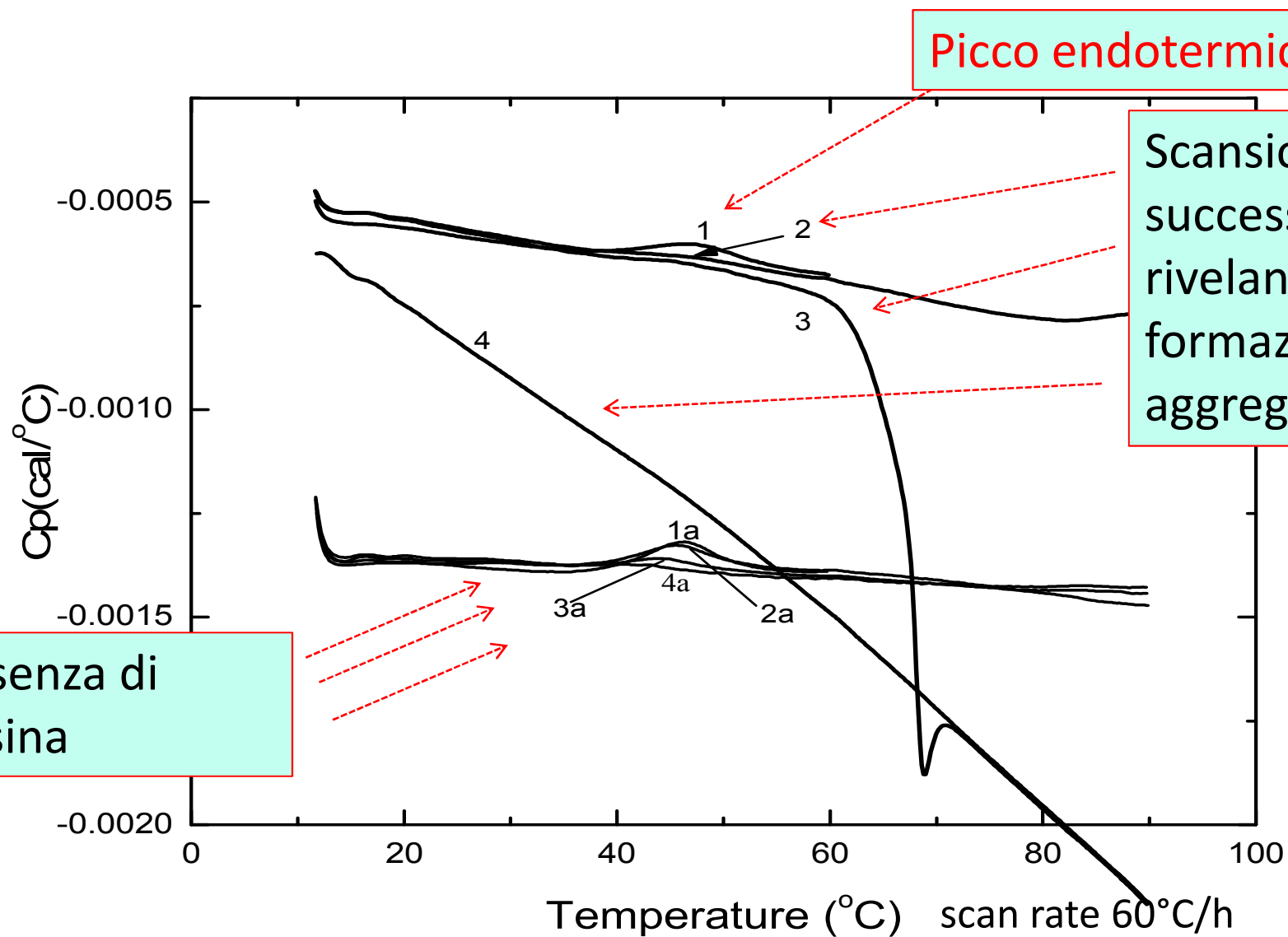
0 1.00 μ m
Data type Height
Z range 30.0 nm

Profili DSC di α -cristallina (1mg/ml)

Reversibility of the unfolding transition estimated by scanning the samples four times after cooling.

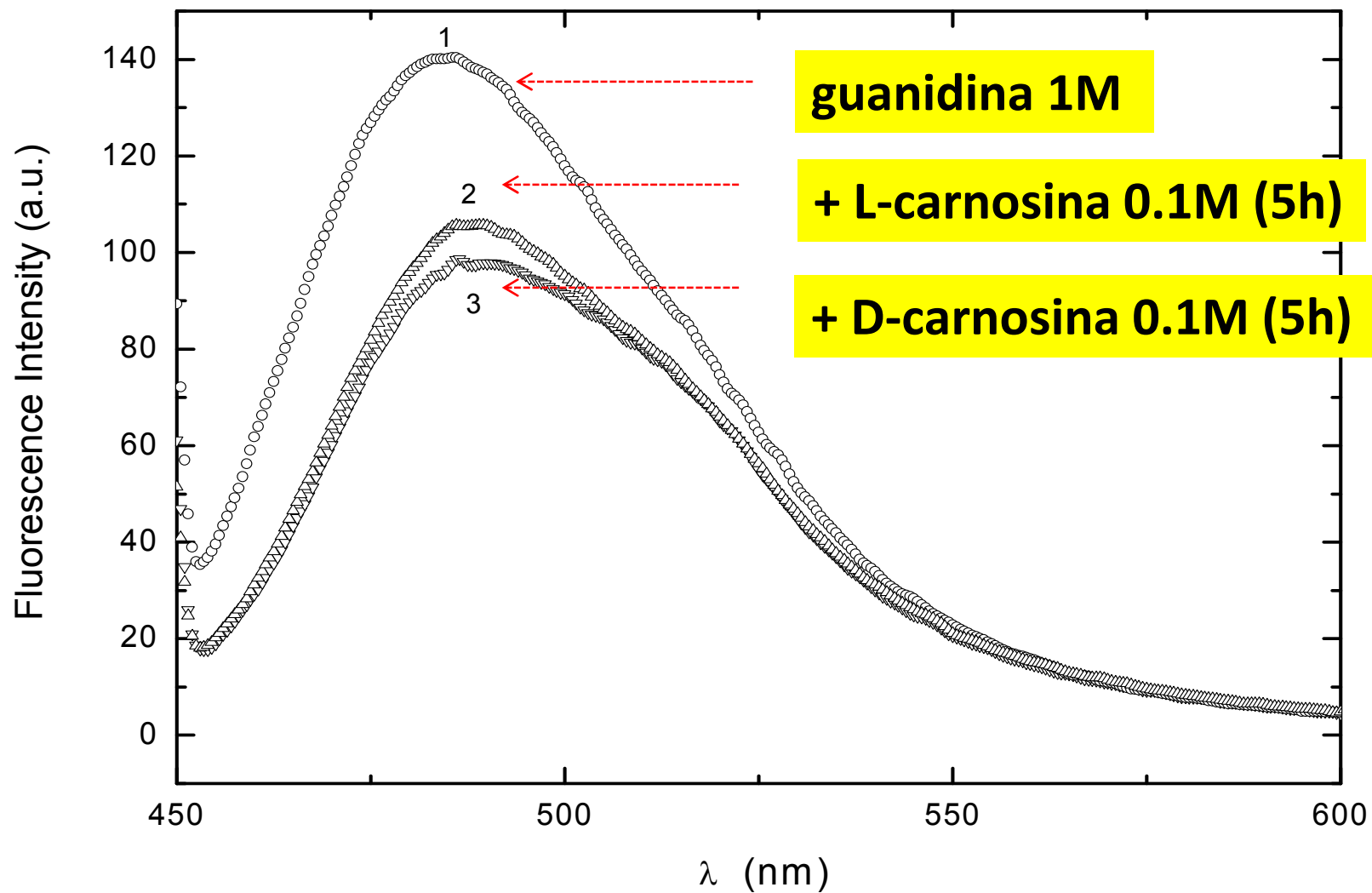


Profili DSC di α -cristallina (1mg/ml)



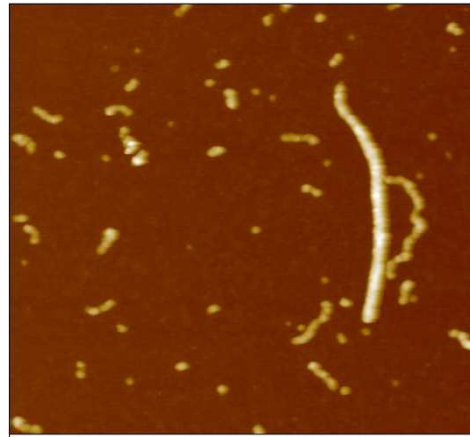
Spettri di fluorescenza di ThT di soluzioni di α -cristallina

Reversibility of the unfolding transition estimated by ThT spectra.



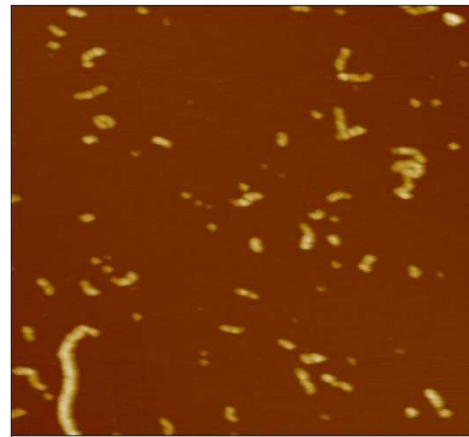
α -Cristallina (10 mg/ml) incubata con guanidina 1M (60°C/24h) e successivamente trattata a 25°C con D-carnosina ed L-carnosina 0,1M

L



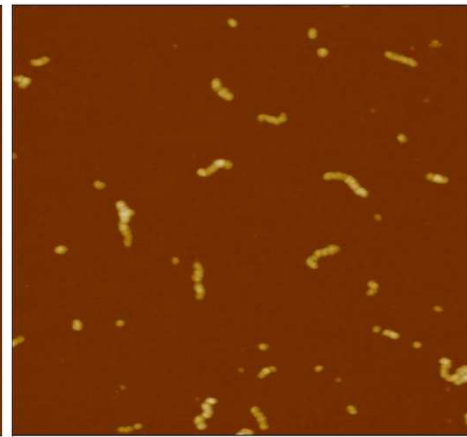
0 1.00 μ m
Data type: Z range
Height: 30.0 nm

240108_11.116
a-crystalline 10mg/ml Gua1M 60C/24h+L-carn25C/1h



0 1.00 μ m
Data type: Z range
Height: 30.0 nm

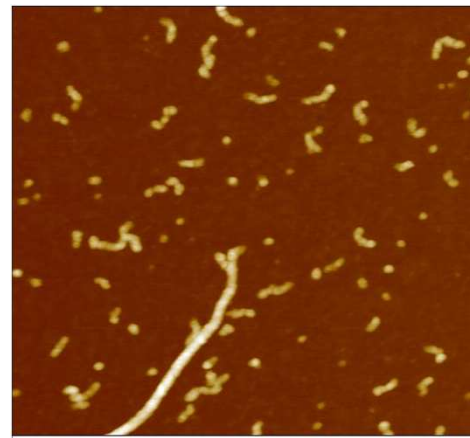
250108_13.101
a-cryst 10mg/ml Gua1M 60C/24h+L-carn 100mM 25C/3h



0 1.00 μ m
Data type: Z range
Height: 30.0 nm

250108_15.107
a-cryst 10mg/ml Gua1M 60C/24h+L-carn 100mM 25C/5h

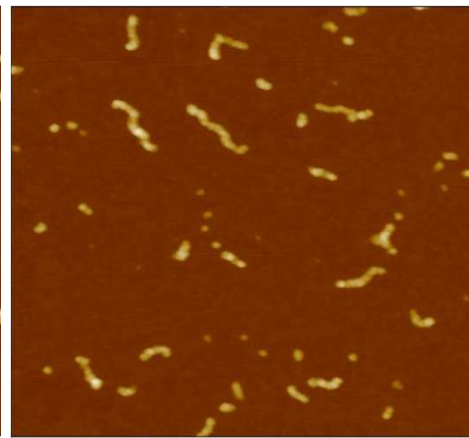
D



0 1.00 μ m
Data type: Z range
Height: 30.0 nm

240108_d1.112
a-crystalline 10mg/ml Gua1M 60C/24h D-carn25C/1h

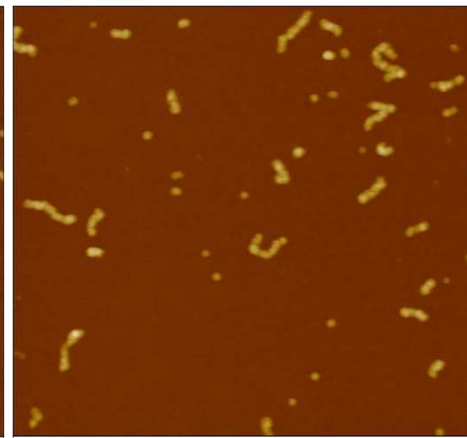
1h



0 1.00 μ m
Data type: Z range
Height: 30.0 nm

240108_d3.119
a-crystalline 10mg/ml Gua1M 60C/24h+D-carn25C/3h

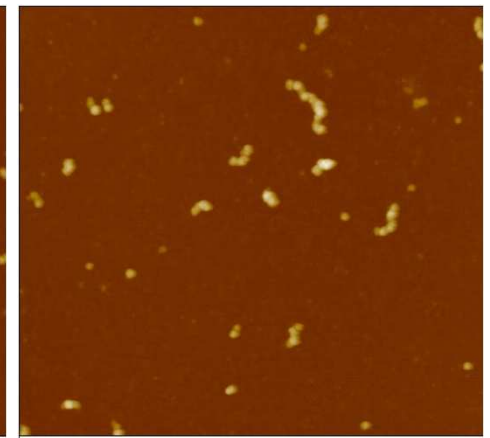
3h



0 1.00 μ m
Data type: Z range
Height: 30.0 nm

250108_d5.104
a-cryst 10mg/ml Gua1M 60C/24h+D-carn 100mM 25C/5h

5h

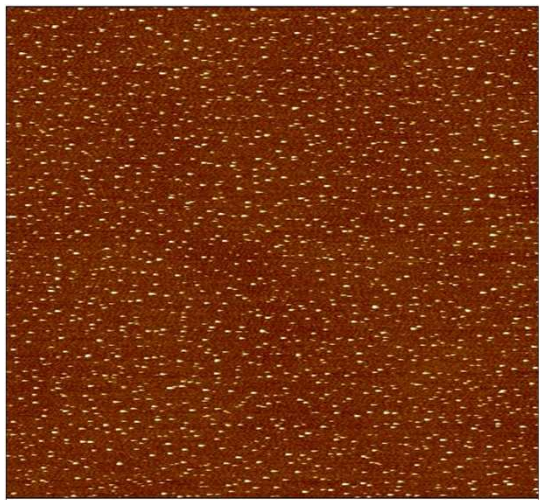


0 1.00 μ m
Data type: Z range
Height: 30.0 nm

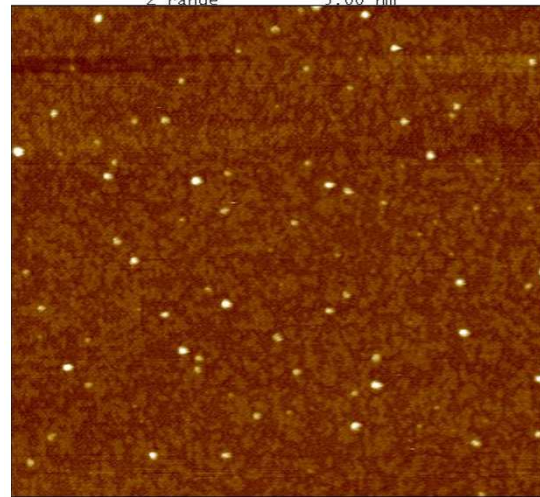
250108_d27.110
a-cryst 10mg/ml Gua1M 60C/24h+D-carn 100mM 25C/27h

27h

α -Cristallina (10 mg/ml) incubata con guanidina 1M (60°C/24h) e successivamente trattata con carnosina 0,1M a T°amb per 7gg .



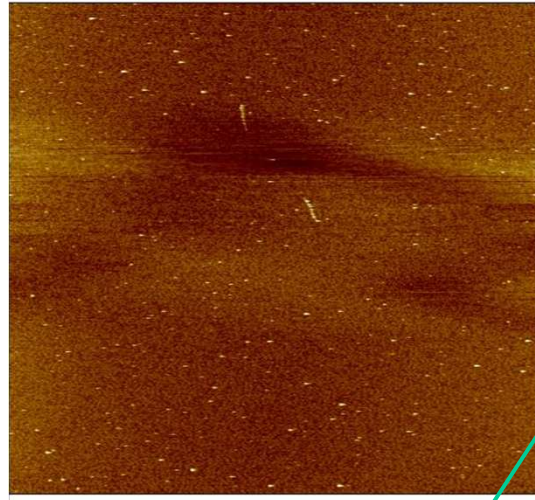
0 5.00 μ m
Data type Height
Z range 5.00 nm



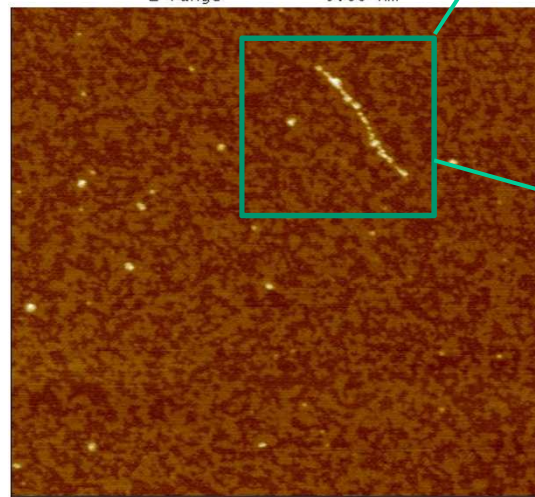
0 1.00 μ m
Data type Height
Z range 5.00 nm

180108.103
alpha-crystalline 1mg/ml+ L-carnosine 1week incub.

L-carnosina 1wk



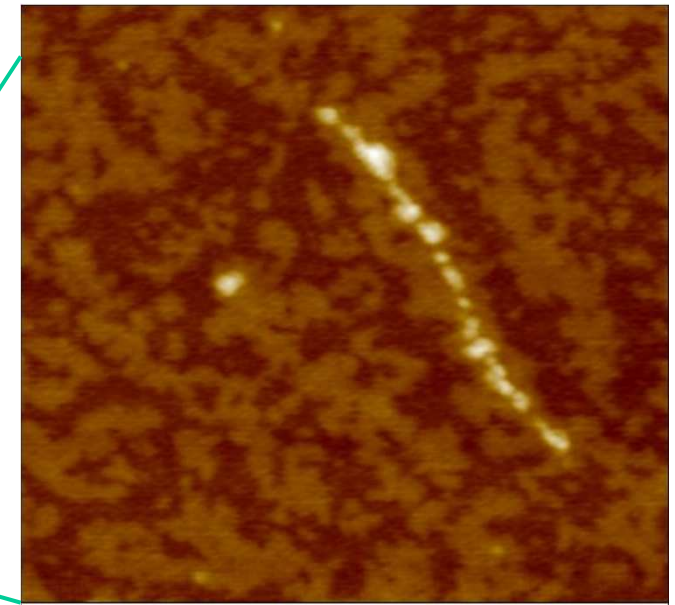
0 5.00 μ m
Data type Height
Z range 5.00 nm



0 1.00 μ m
Data type Height
Z range 5.00 nm

180108.106
alpha-crystalline 1mg/ml+ D-carnosine 1week incub.

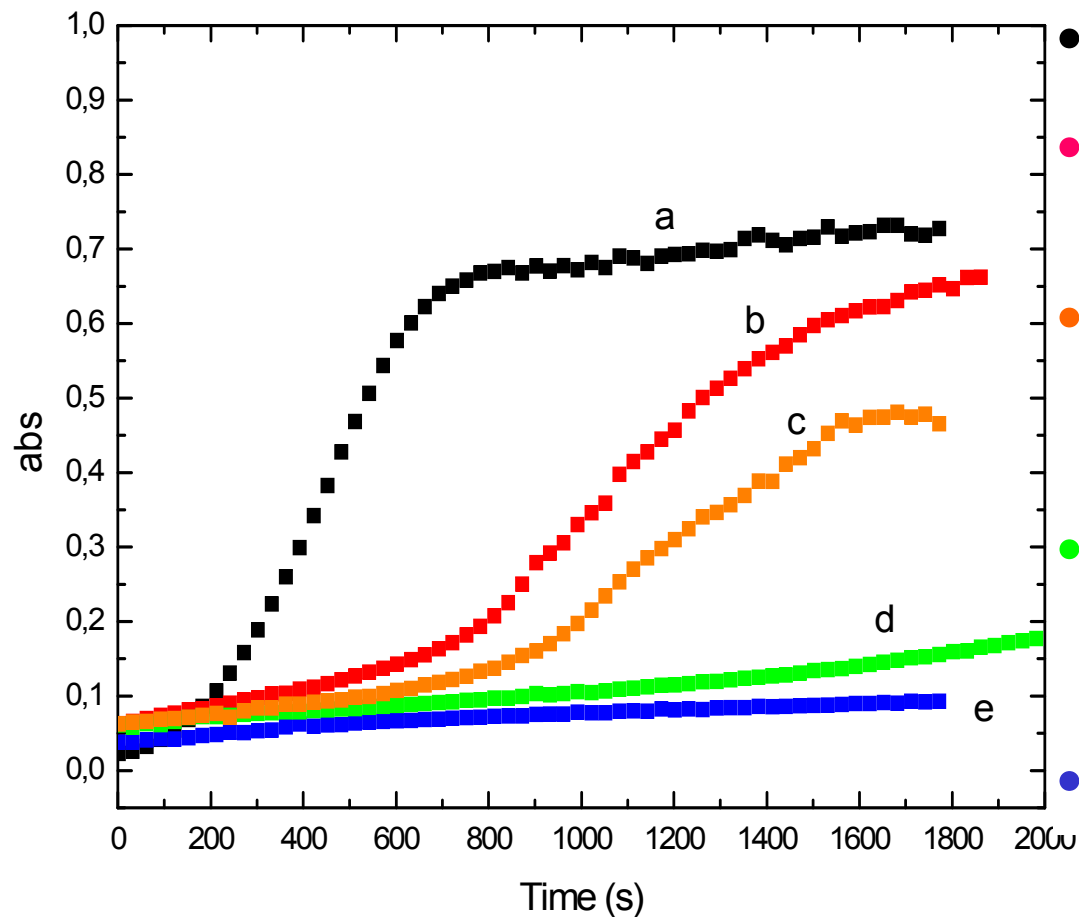
D-carnosina 1wk



0 400 nm
Data type Height
Z range 5.00 nm

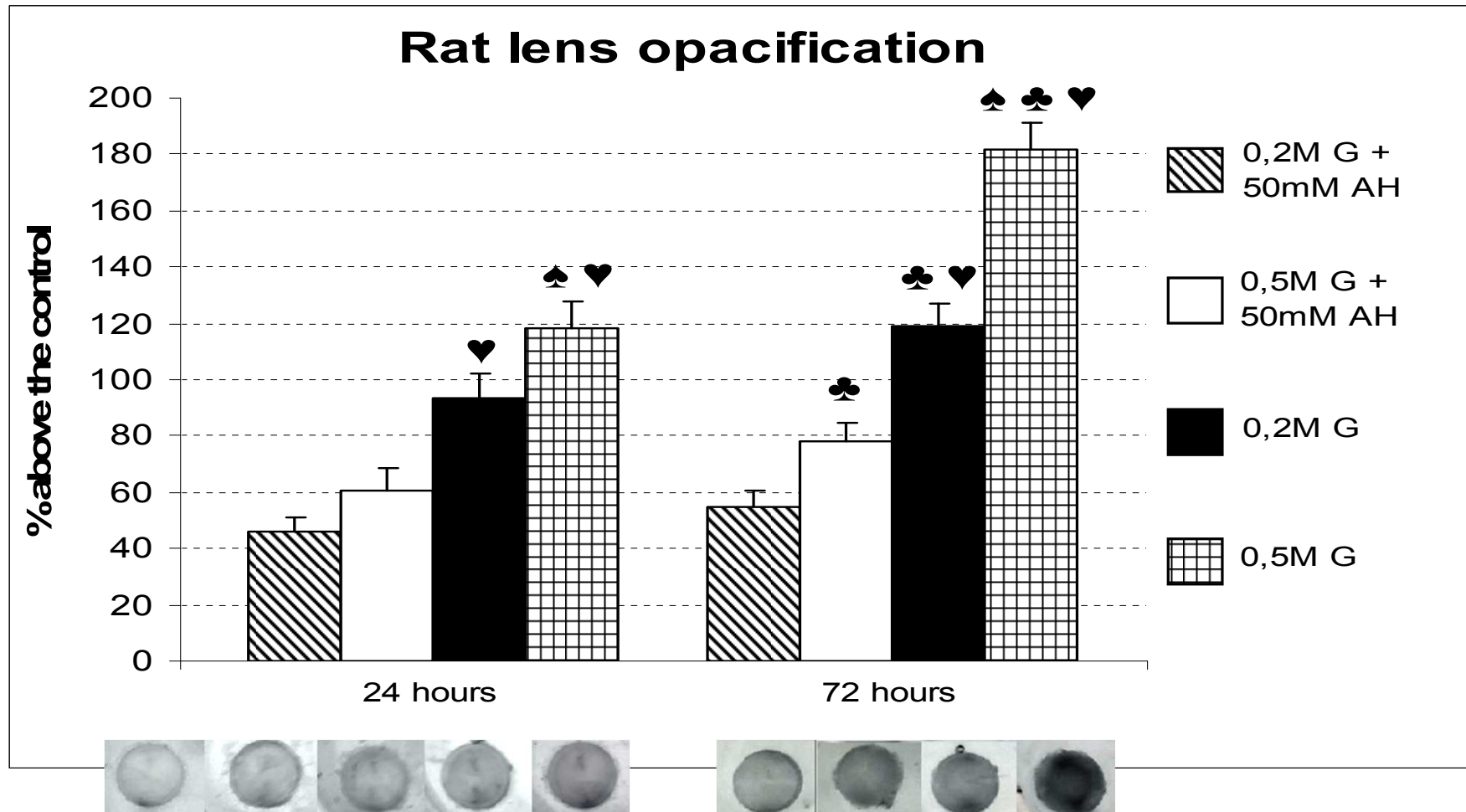
D-carnosina x 1 sett.
*Particolare della
dissoluzione di una
singola fibrilla*

Variazione di assorbanza relativa all'aggregazione di β -cristallina e attività chaperon dell' α -cristallina



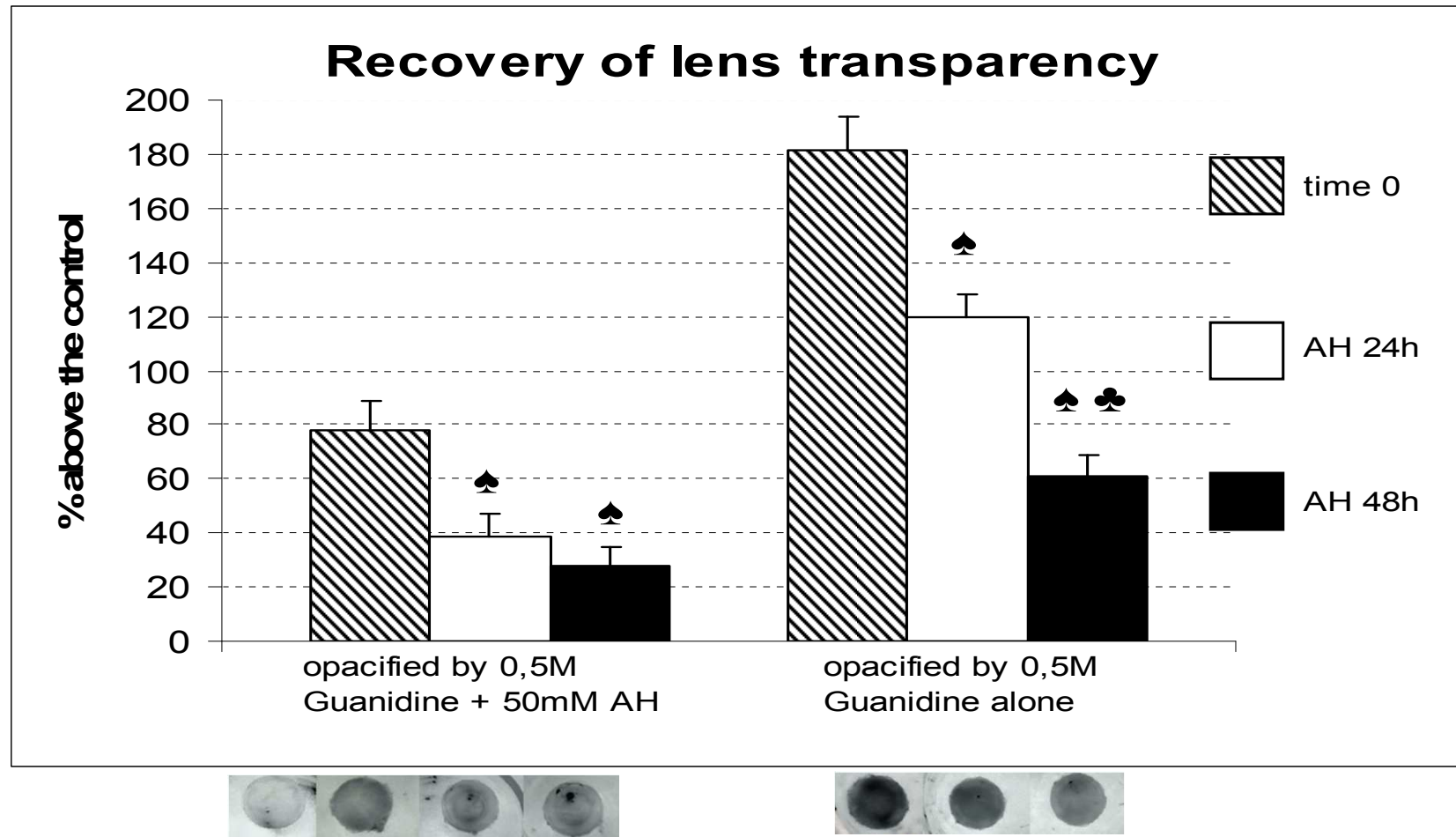
- **Incubazione a 55°C (a)**
- **+ α -cristallina incubata in condizioni destabilizzanti (b)**
- **+ α -cristallina incubata in condizioni destabilizzanti seguite da carnosina 1M (c)**
- **+ α -cristallina co-incubata con carnosina in condizioni destabilizzanti (d)**
- **+ α -cristallina nativa (e).**

Cristallini di ratto in coltura organotipica trattati con guanidina-HCl da sola e in presenza di carnosina.



♠ significativo vs. guanidina 0.2M; ♣ significativo vs. 24 ore; ♥ significativo vs. trattamento con carnosina.

Reversibilità del danno da guanidina tramite trattamento con carnosina.



Particolarmente evidente nei cristallini precedentemente opacizzati con una elevata concentrazione di guanidina.

♠ significativo vs. tempo 0; ♣ significativo vs. 24 ore.

Enrico Rizzarelli,

Salvatore Fisichella,

Dipartimento di Scienze Chimiche, Università di Catania.

Francesco Attanasio:

Istituto di Biostrutture e Bioimmagini (IBB), CNR, Catania.

Bruno Pignataro,

Sebastiano Cataldo:

Dipartimento di Chimica Fisica, Università di Palermo.

Silvia Nicoletti,

Anna Savarino: Dottorato di ricerca in Scienze Chimiche, Catania.