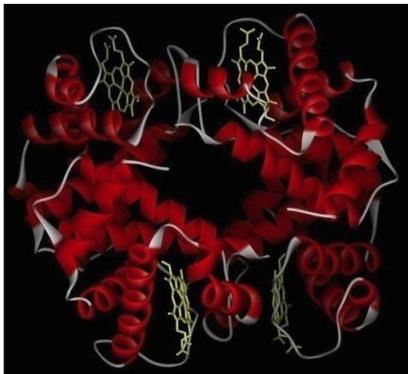
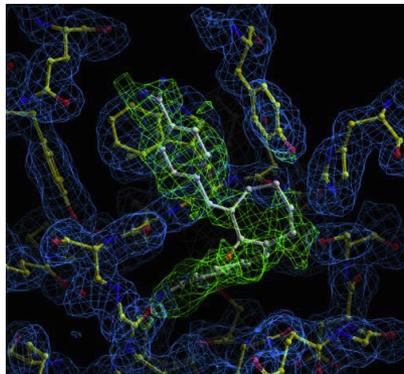
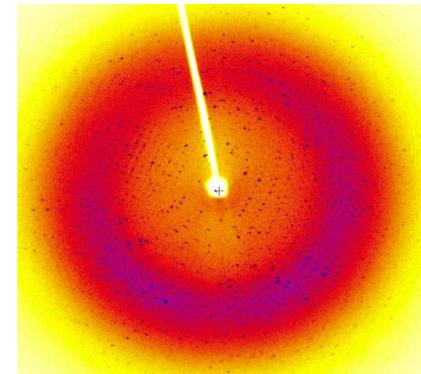
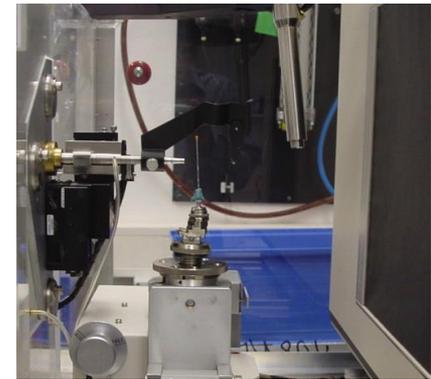
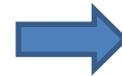
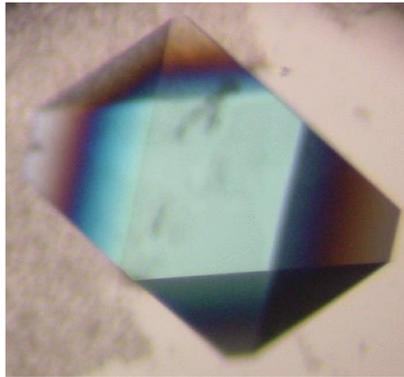


Proteine ricombinanti per studi
strutturali:
cristallizzazione

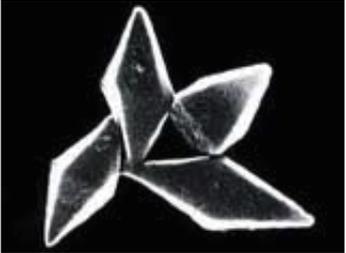
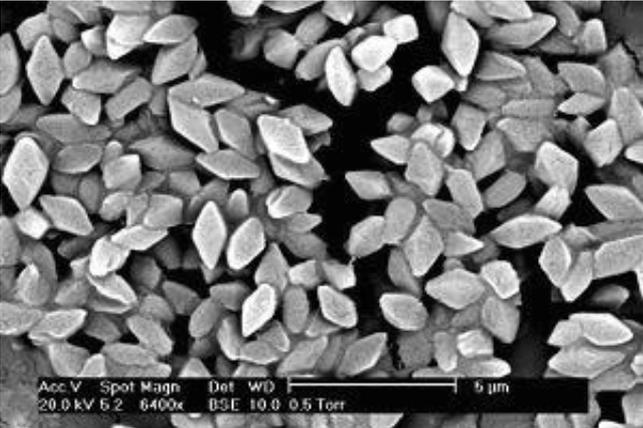
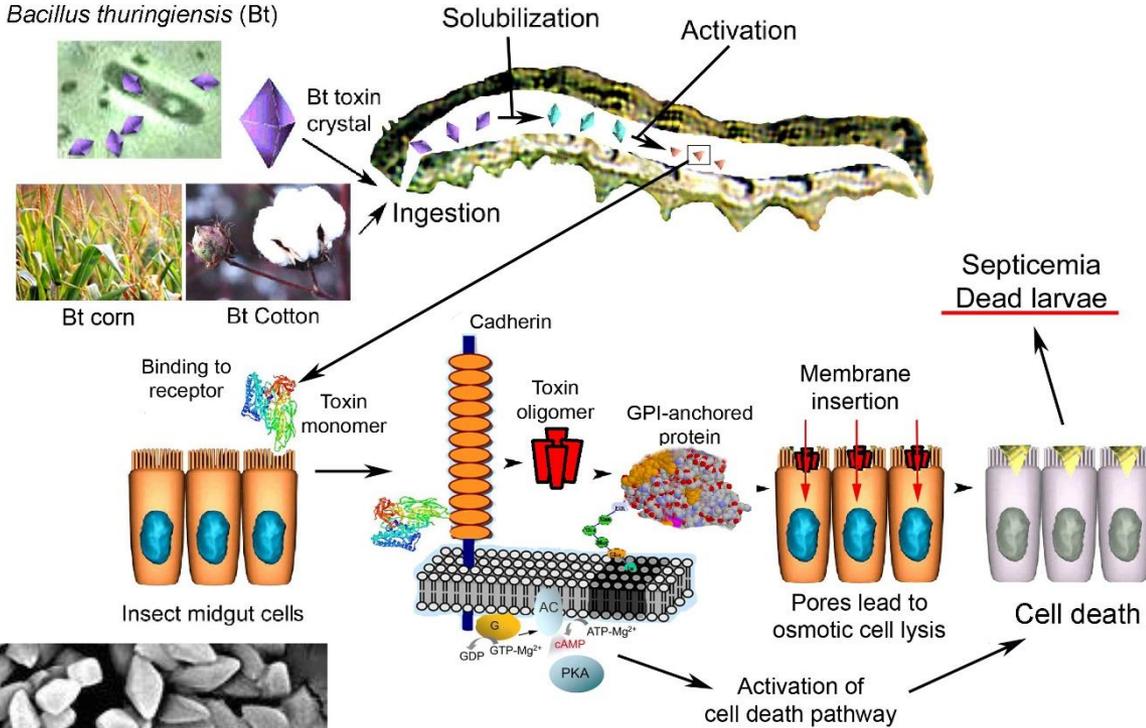
Cristallizzazione:

Fasi per risolvere una struttura cristallografica:



Cristallizzazione:

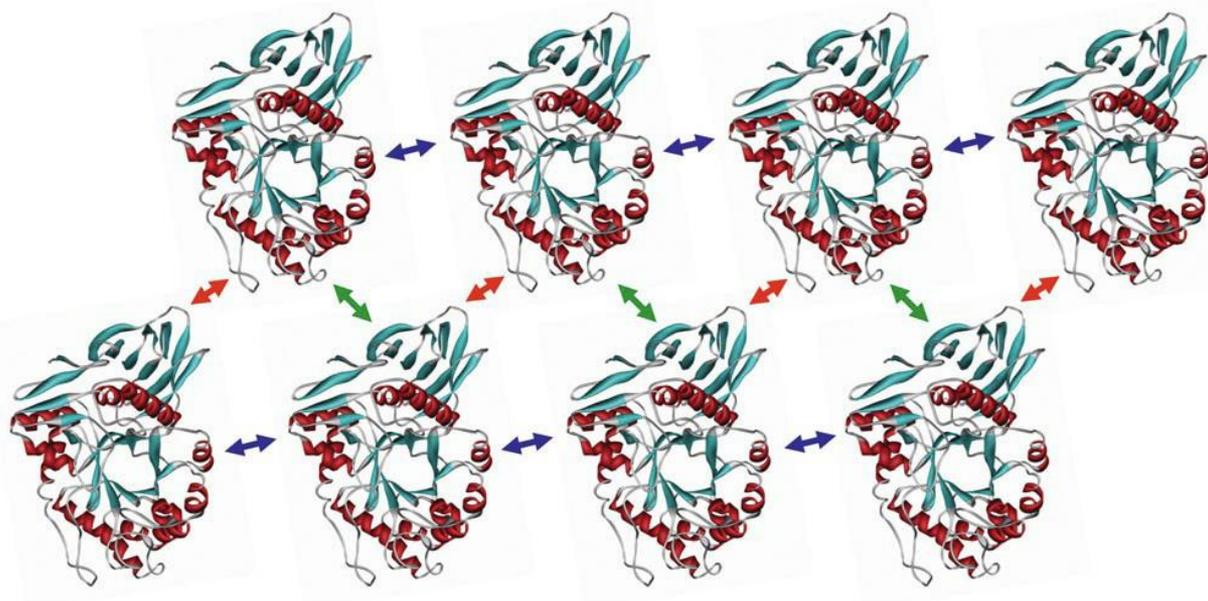
Cristalli in natura



Cristallizzazione:

Caratteristiche dei cristalli

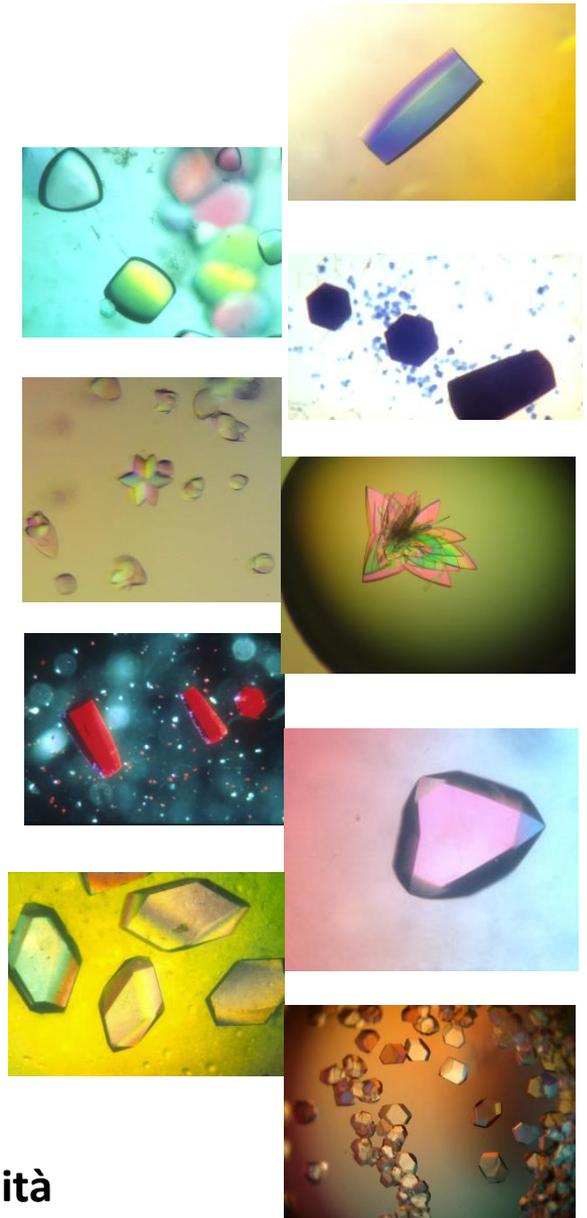
- in media composti da circa il 50% di solvente
- numero relativamente piccolo di legami intermolecolari deboli



© Garland Science 2010

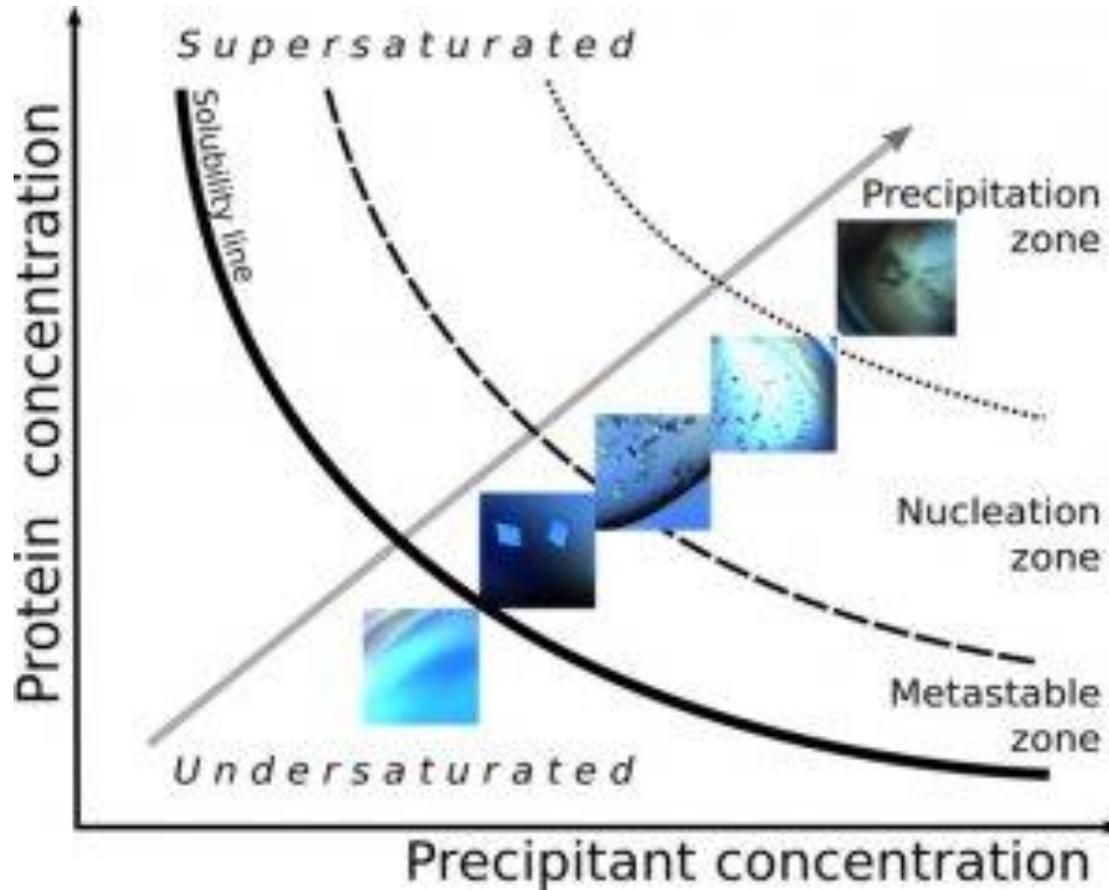
Parametri chimico-fisici

Parametri biologici/microeterogeneità



Cristallizzazione:

Diagramma di fase della Solubilità

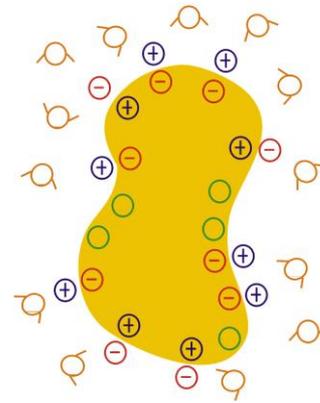


Cristallizzazione:

Solubilità delle proteine

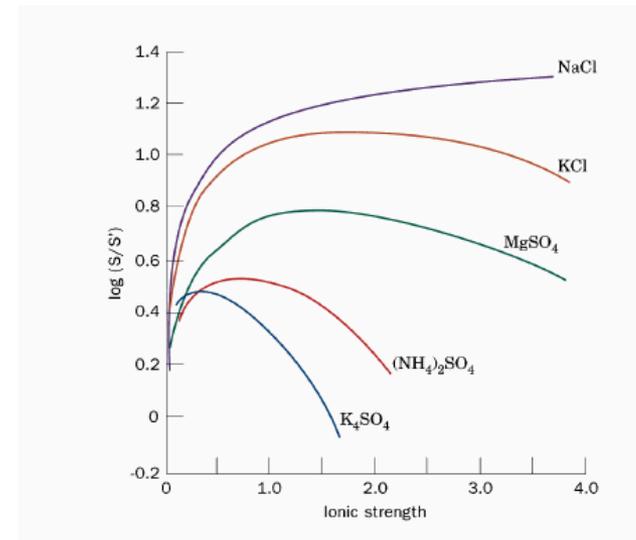
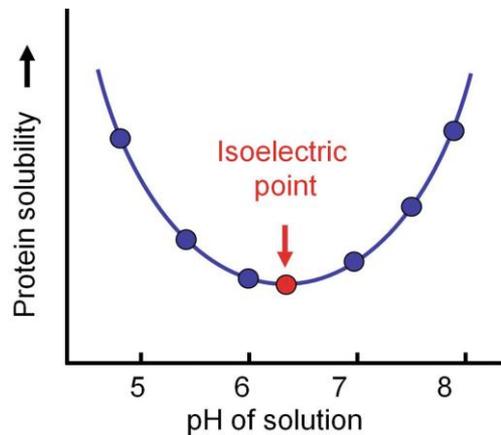
dipende da

- T
- Costante dielettrica del mezzo
- pH



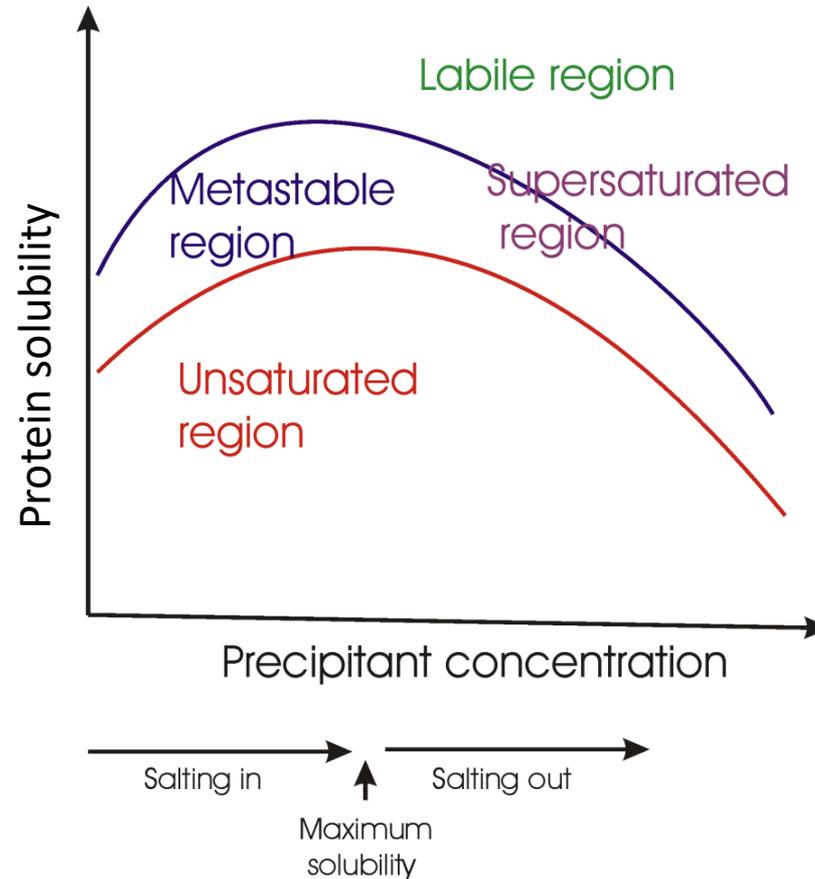
•

Forza ionica ($I = \frac{1}{2} \sum z_i^2 c_i$)



Cristallizzazione:

Diagramma di fase della Solubilità

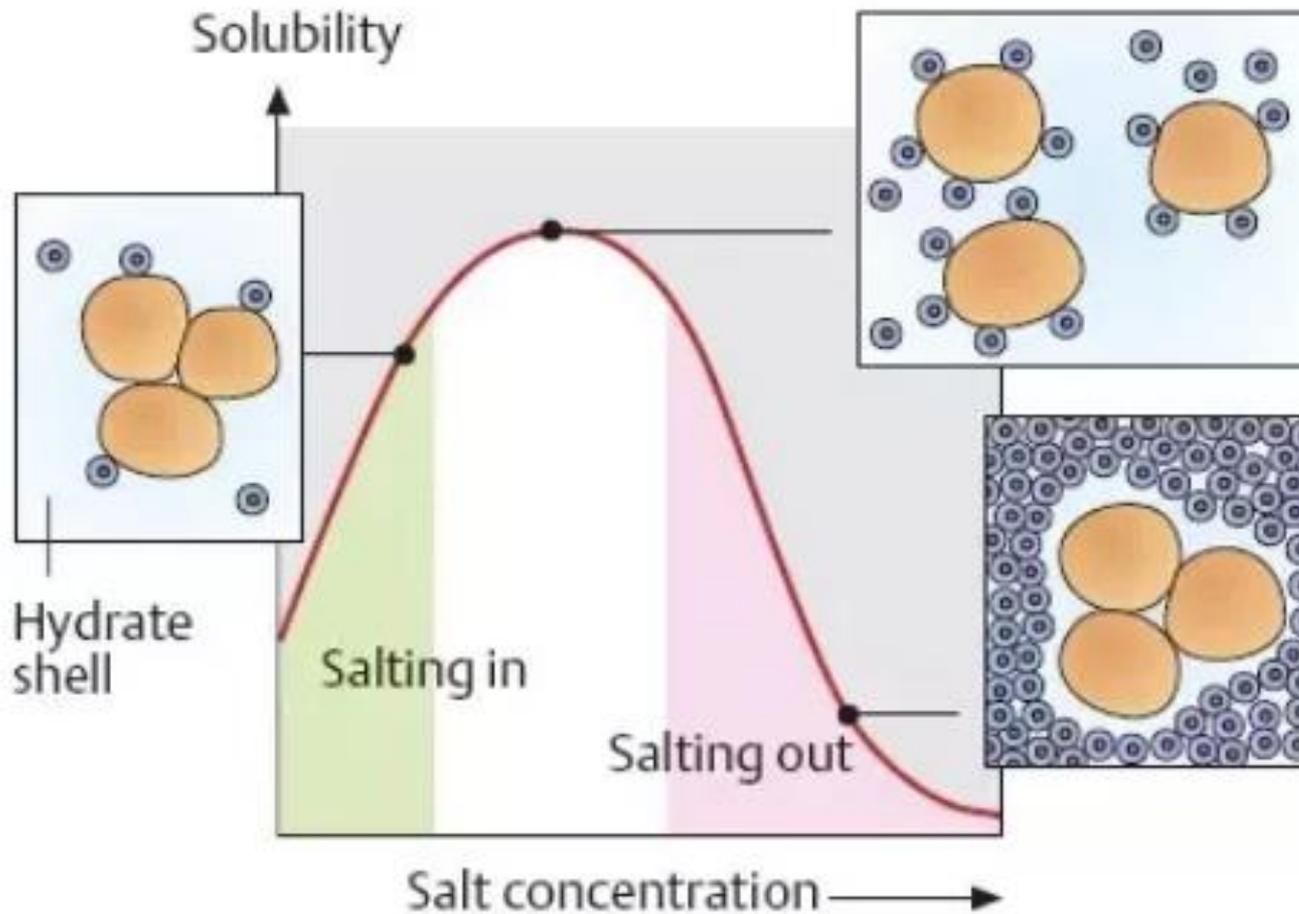


Cristallizzazione:

Solubilità e forza ionica

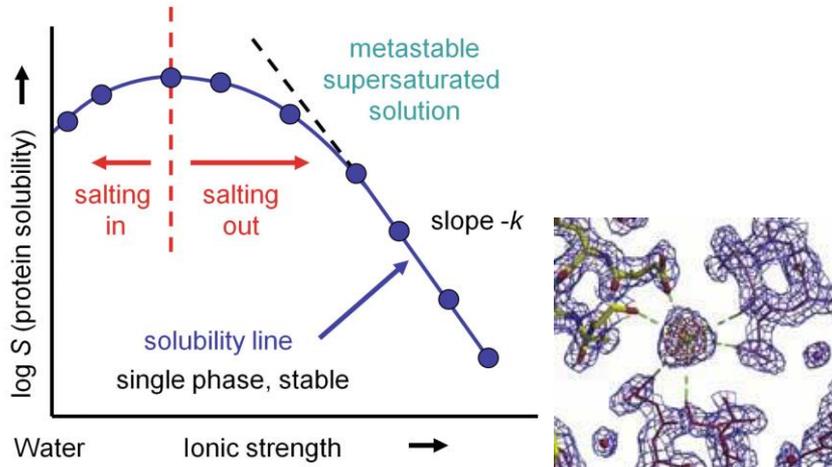
Salting in (bassa solubilità a bassa forza ionica)

Salting out (bassa solubilità ad alta forza ionica)

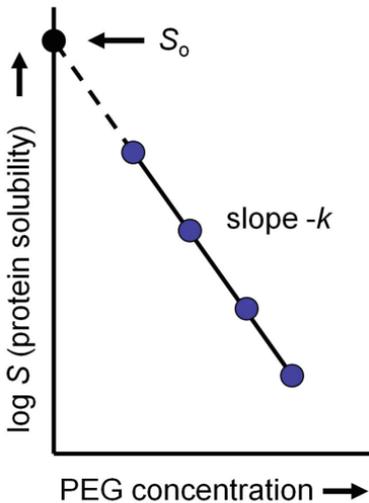


Cristallizzazione:

Retta di Solubilità



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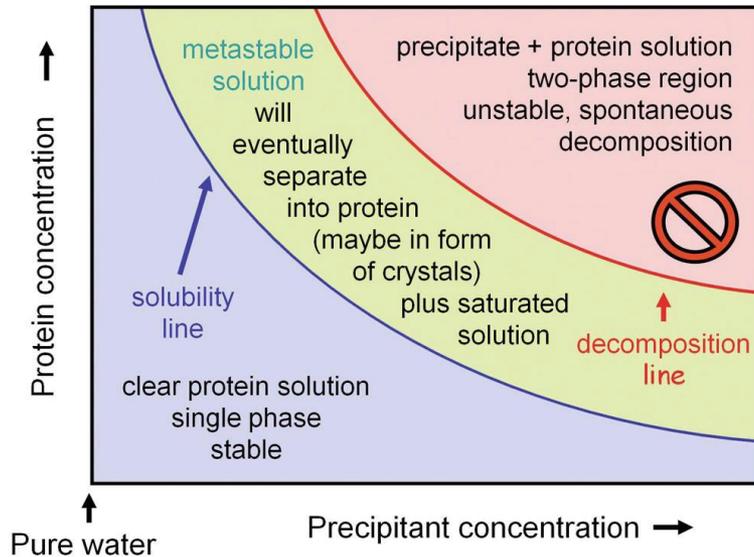
$$\log(S) = \log(S_0) + kI$$

k specifico per il sistema solvente-soluto
I forza ionica

$$\log(S) = \log(S_0) + k[\text{PEG}]$$

Cristallizzazione:

Diagramma di fase: zona metastabile



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limiti della zona metastabile definiti dalla condizione di stabilità:

$$(\delta^2 \bar{G} / \delta x^2) > 0$$

\bar{G} = energia molare media di Gibbs

x = frazione molare della proteina

curvatura di $\bar{G} < 0$

soluzione altamente sovrasatura instabile (decomposizione).

Metodi:

- aggiunta di un precipitante,
- scambio di solvente mediante dialisi,
- diffusione dell'interfaccia libera,
- variazione del pH

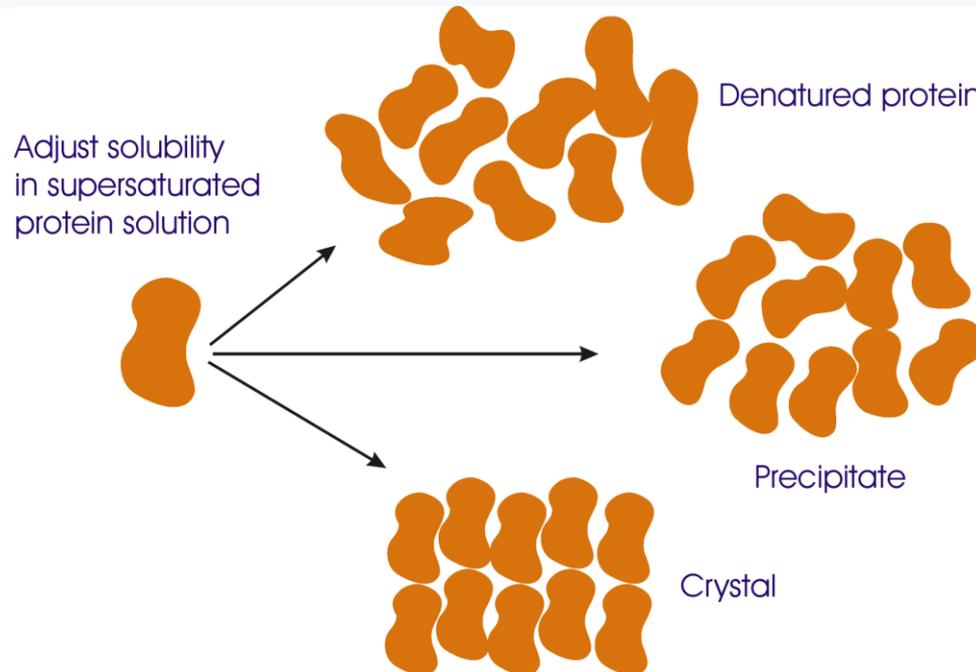
Cristallizzazione:

Driving forces

Una proteina è stabile in soluzione quando sono presenti interazioni nette con il solvente

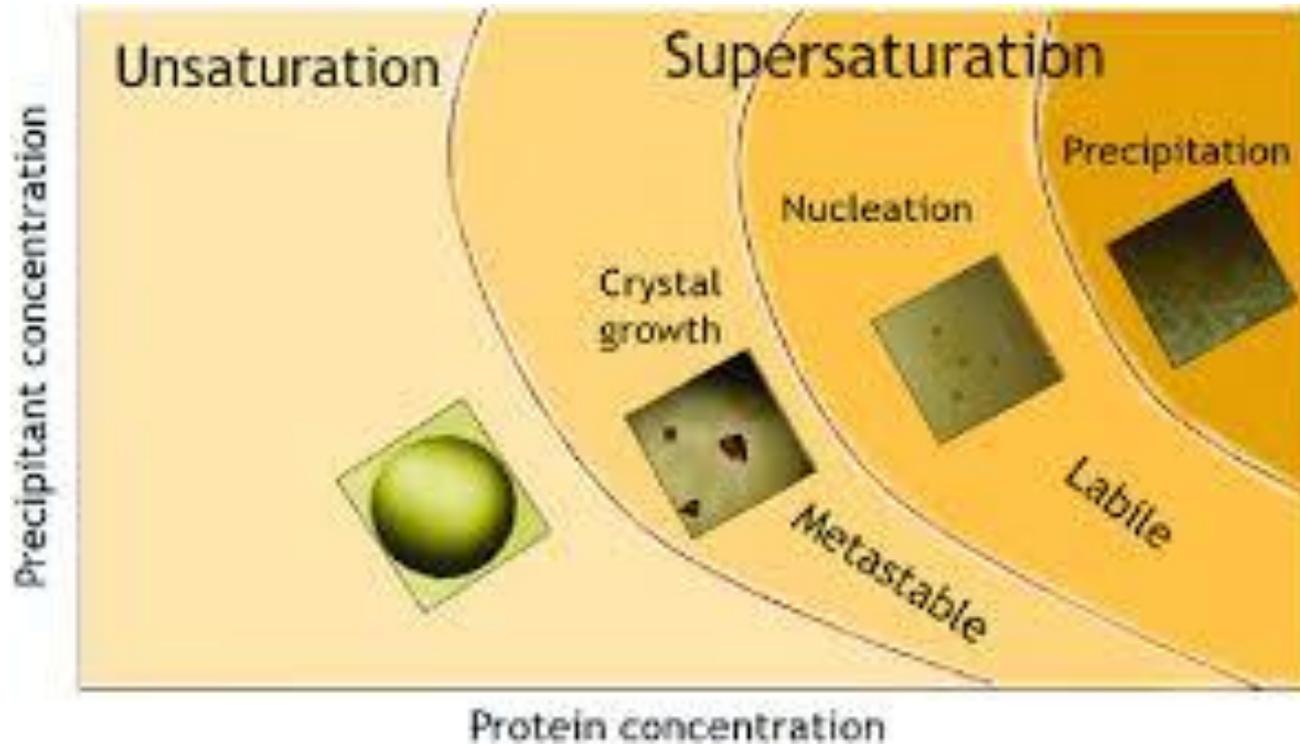
Energia libera di solvatazione $\Delta G_s = \Delta H_s - T\Delta S_s$

Energia libera di cristallizzazione $\Delta G_c = \Delta H_c - T(\Delta S_{\text{protein}} - \Delta S_{\text{solvent}})$



Cristallizzazione:

Fasi



Cristallizzazione:

Energia di Nucleazione

Dipende dal raggio critico di nucleazione r

equazione di Gibbs-Thompson:

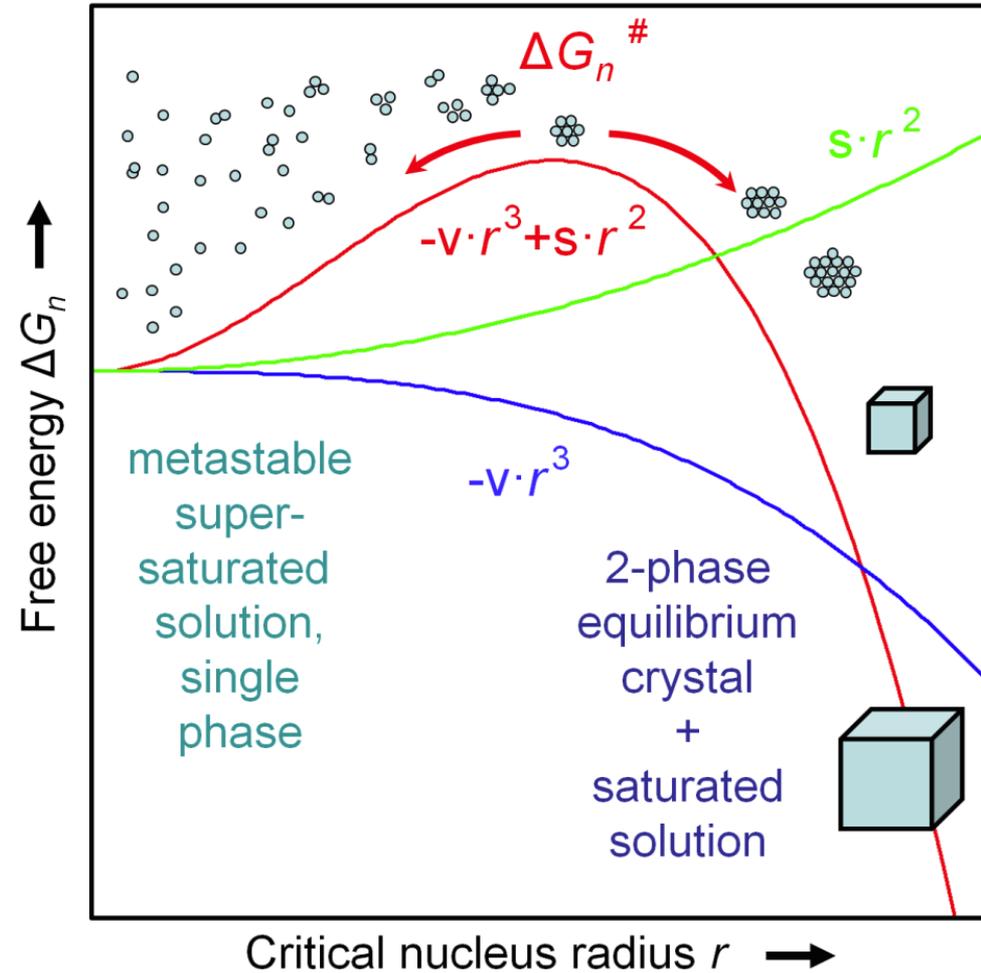
$$\Delta G_n^\# = -\frac{4}{3} \pi r^3 kT \ln \beta + 4\pi r^2 \gamma$$

$\beta = C / C_s$ è il coefficiente di supersaturazione

C_s concentrazione massima all'equilibrio

C concentrazione effettiva della soluzione
supersatura

γ aumento dell'energia dell'interfaccia libera



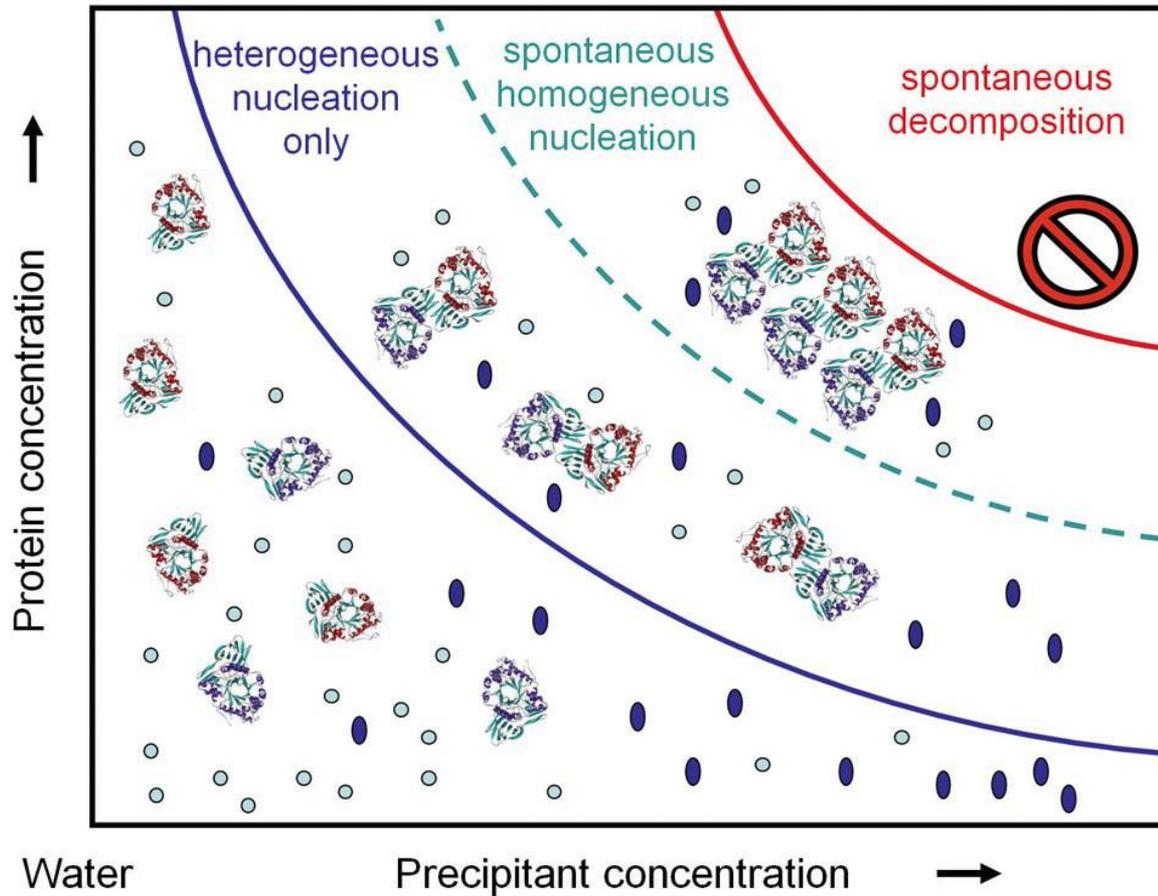
Per una data sovrasaturazione ed energia superficiale, raggio critico se $\delta G / \delta r = 0$ (picco curva rossa)

Cristallizzazione:

Nucleazione

zone di nucleazione in un diagramma di fase

stable → ← metastable ← ← unstable

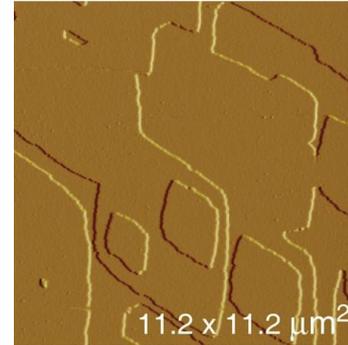


Cristallizzazione:

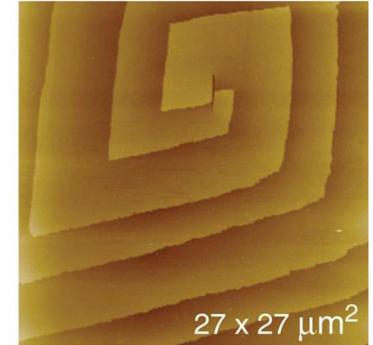
Crescita

Immagini AFM:

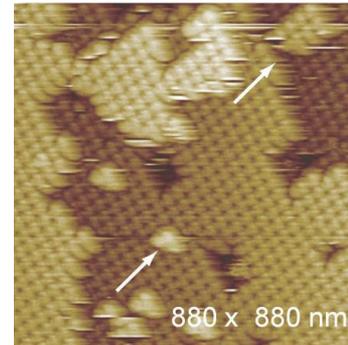
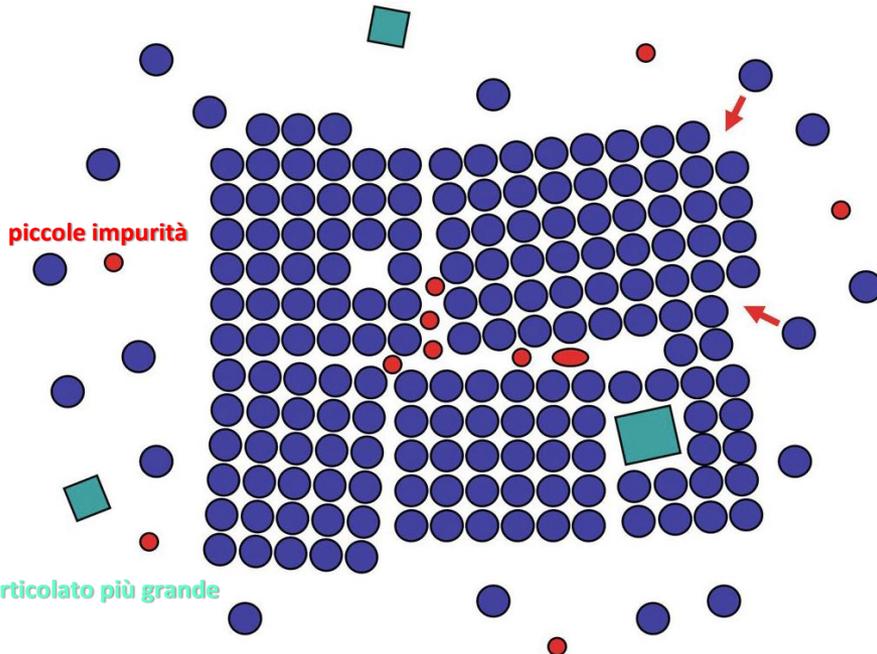
superficie di cristalli di glucosio isomerasi:
modelli di crescita a isola e a spirale (più comuni)



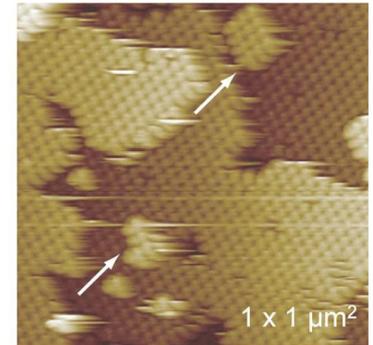
© Garland Science 2010



nuclei 2D sulla superficie di cristalli di citomegalovirus:
crescita di un strato



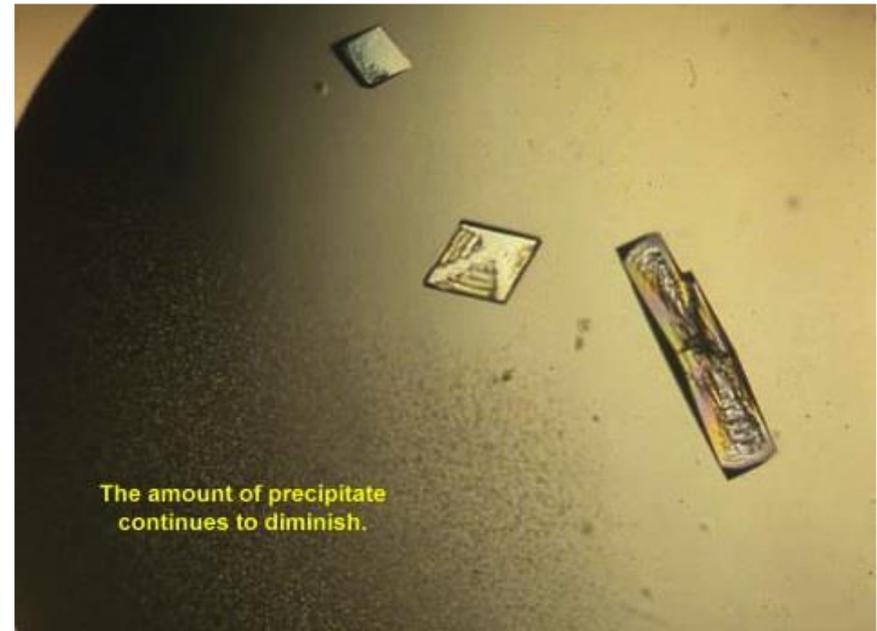
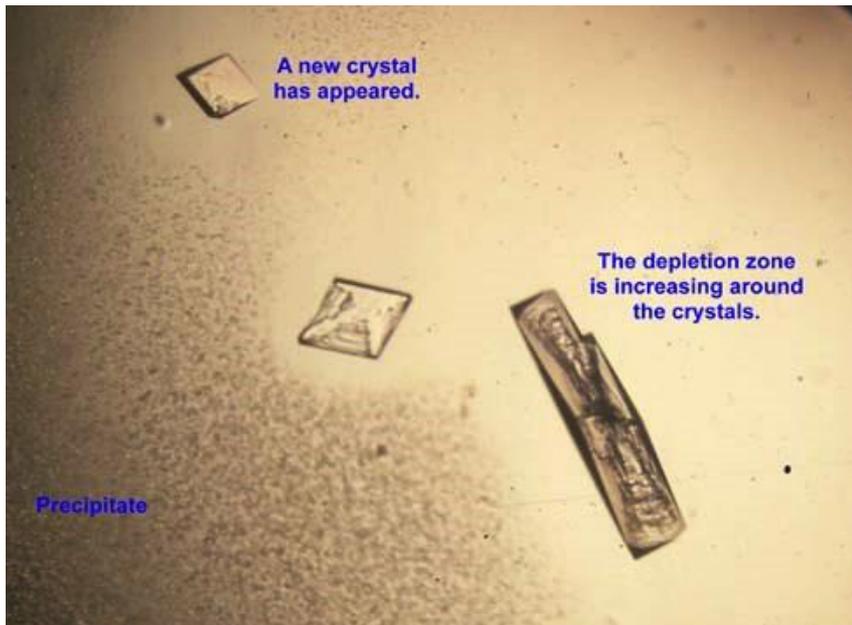
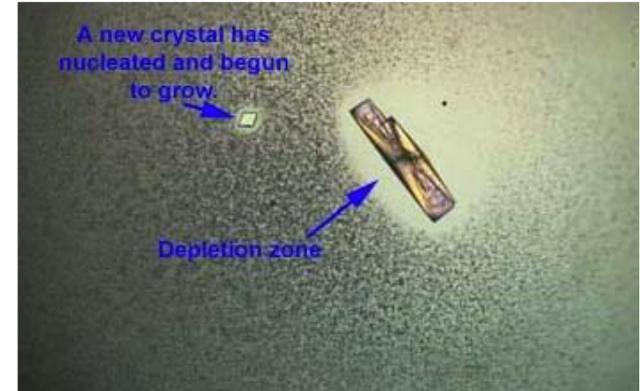
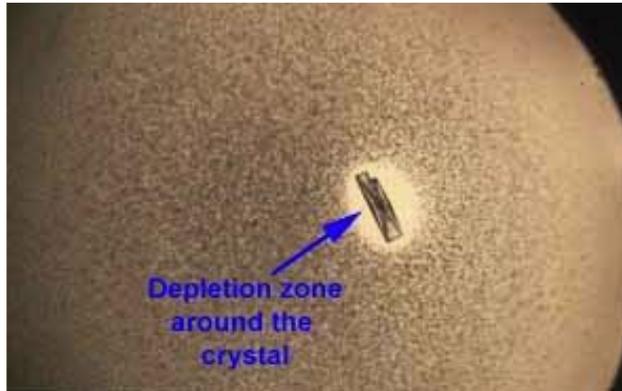
© Garland Science 2010



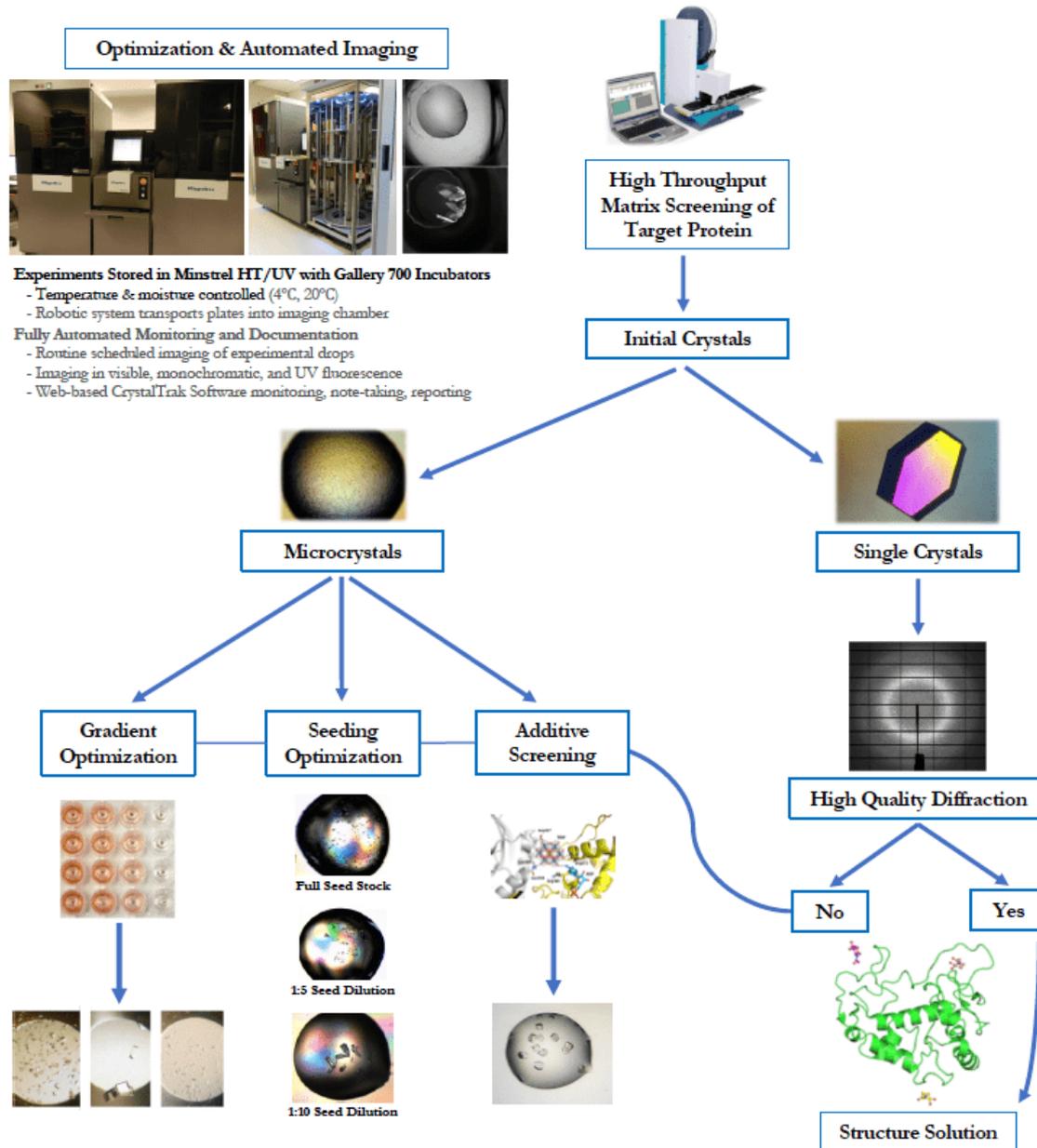
Un cristallo così altamente mosaico è inutile per esperimenti di diffrazione

Cristallizzazione: Maturazione di Ostwald

Processo spontaneo → i cristalli più grandi energeticamente favoriti

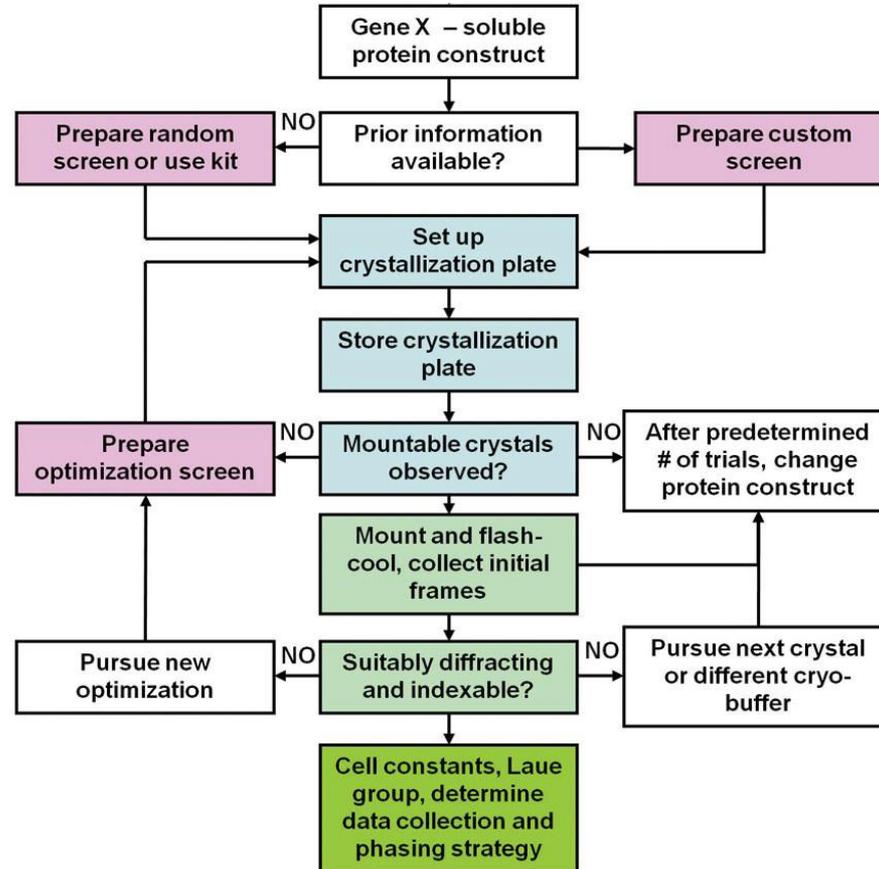


Cristallizzazione:



Cristallizzazione:

Workflow



Cristallizzazione:

problema di campionamento / ottimizzazione multivariata

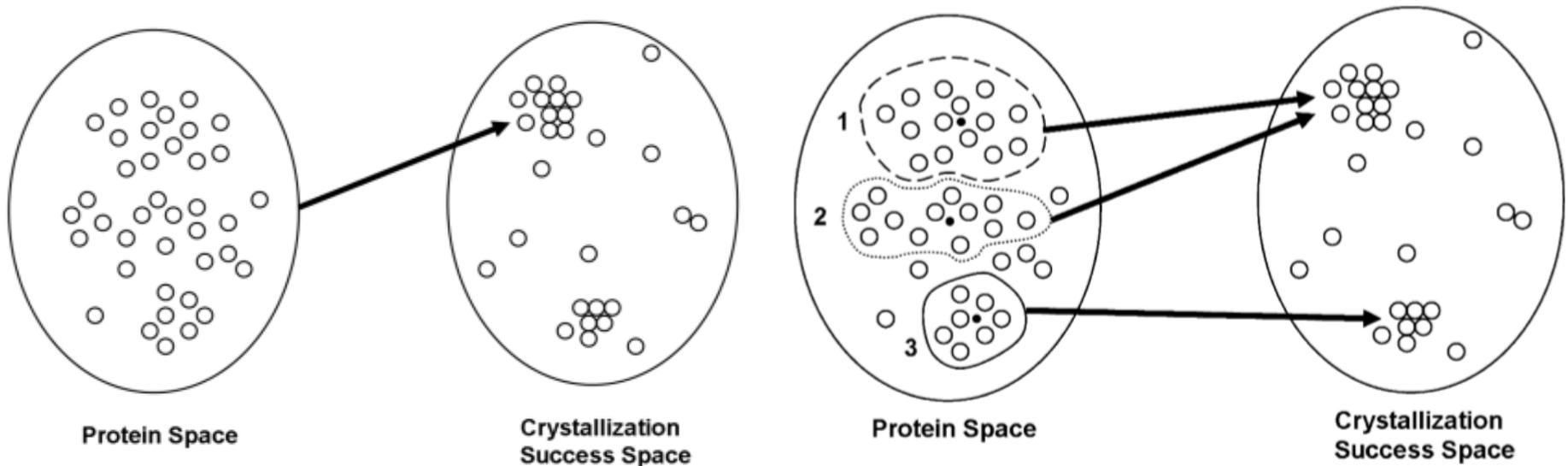
Infinite combinazioni di pH e tipo di tampone/ agente precipitante/Sali e additivi vs limitata quantità di campione:

↓ dimensionalità

↓ spazio

Approccio bayesiano

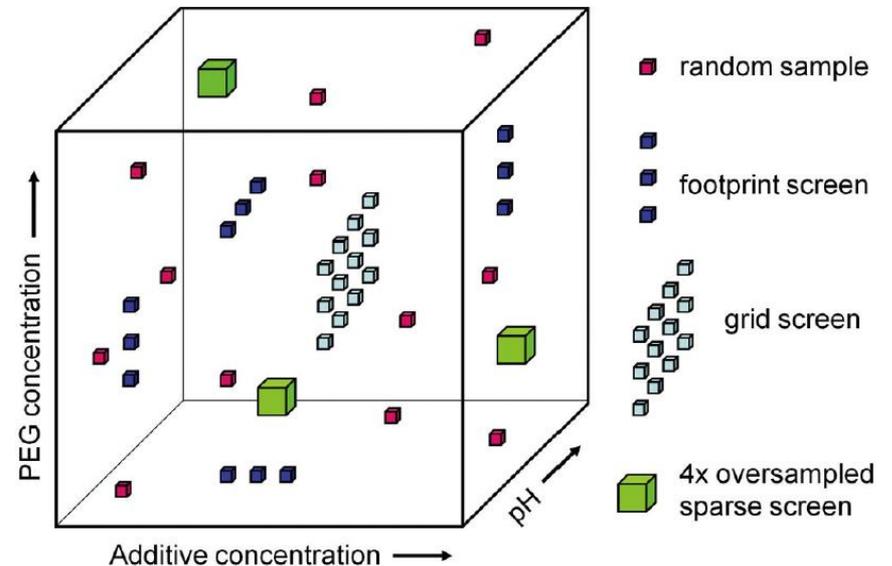
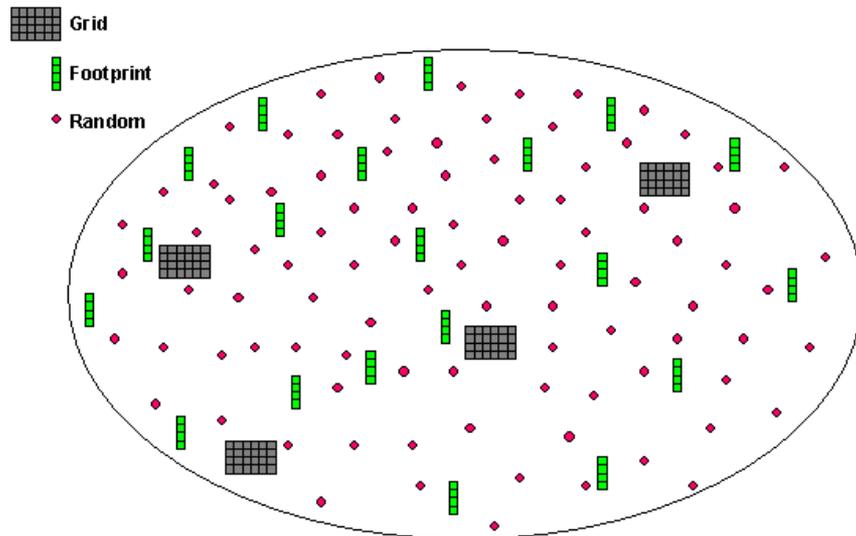
spazio di campionamento multivariato (multidimensionale) scarsamente popolato



Cristallizzazione:

problema di campionamento / ottimizzazione multivariata

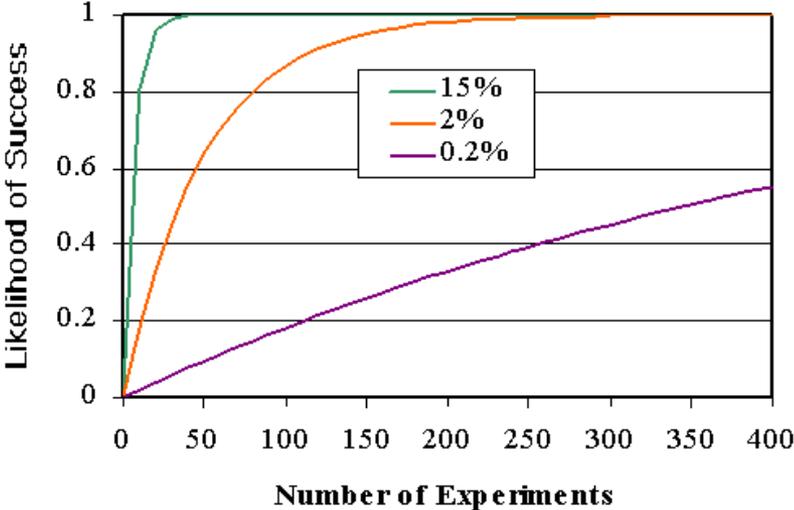
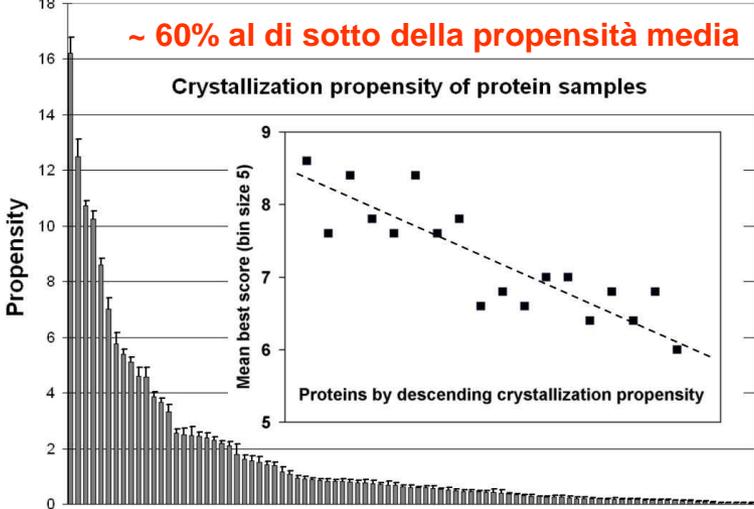
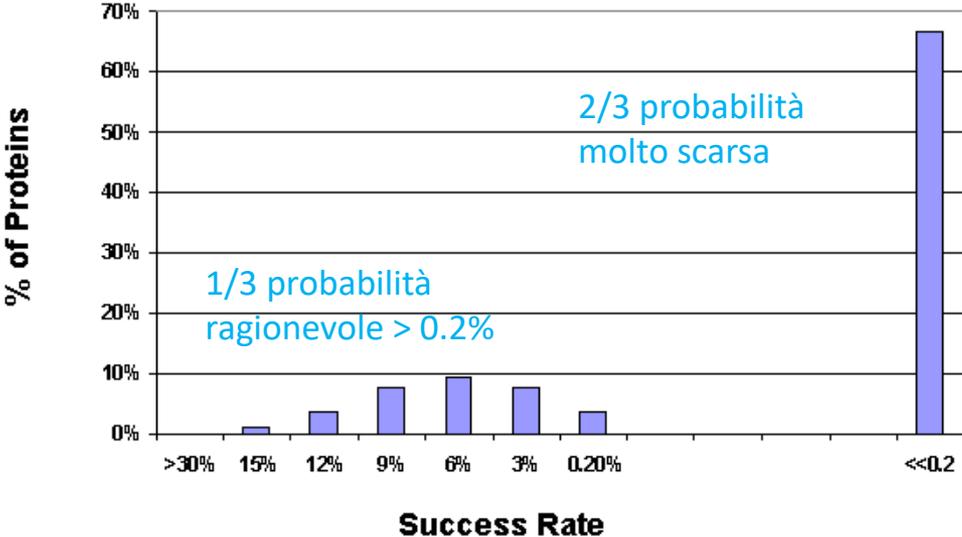
- Sparse matrix sampling (combinazioni già usate con successo)
- Random sampling
- Grid screens
- Footprint screen



senza conoscenza preliminare: Random sampling approccio più efficiente (specialmente per eventi rari)

Cristallizzazione:

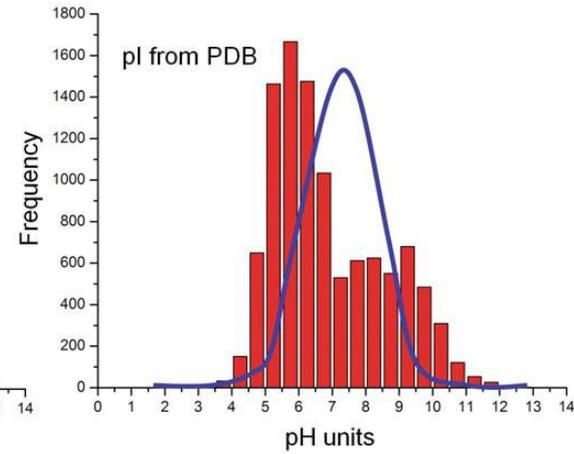
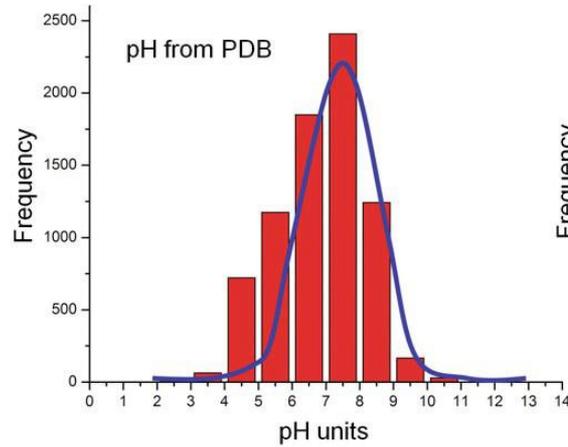
problema di campionamento / ottimizzazione multivariata



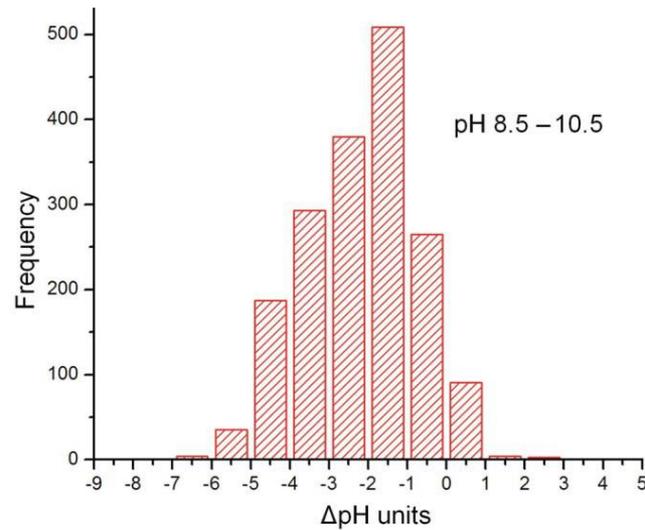
Anche per casi con probabilità molto bassa (1/500) nel 50% dei casi si ottiene un positivo dopo 288 esperimenti

Cristallizzazione:

Dipendenza dal pH



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pH ottimale \approx pI - 1.5

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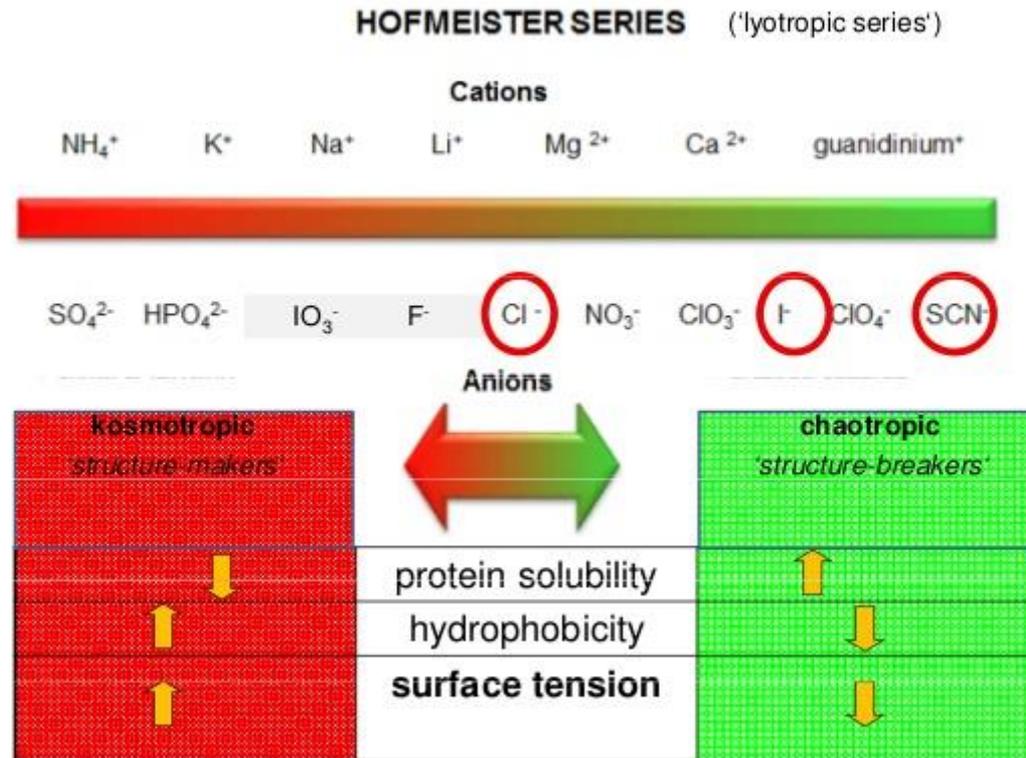
Cristallizzazione:

Agenti precipitanti

Salts	Volatile organic solvents	Polymers	Nonvolatile organic solvents
Ammonium sulfate	Ethanol	Polyethylene glycol 1000, 3350, 6000, 8000, 20000	2-Methyl-2,4-pentanediol (MPD)
Ammonium phosphate	Propanol	Jeffamine T, Jeffamine M	2,5-Hexanediol
Lithium sulfate	Isopropanol	Polyethylene glycol monomethyl ester	1,3-Propanediol
Lithium chloride	Dioxane	Polyethylene glycol monostearate	Polyethylene glycol 400
Sodium citrate	Acetone	Polyeneamine	Jeffamine 400
Ammonium citrate	Isobutanol		
Sodium phosphate	<i>n</i> -Butanol		
Sodium chloride	<i>tert</i> -Butanol		
Potassium chloride	Acetonitrile		
Sodium acetate	Dimethyl sulfoxide		
Ammonium acetate	1,3-Butyrolactone		
Magnesium sulfate			
Magnesium chloride			
Calcium chloride			
Sodium formate			
Sodium tartrate			

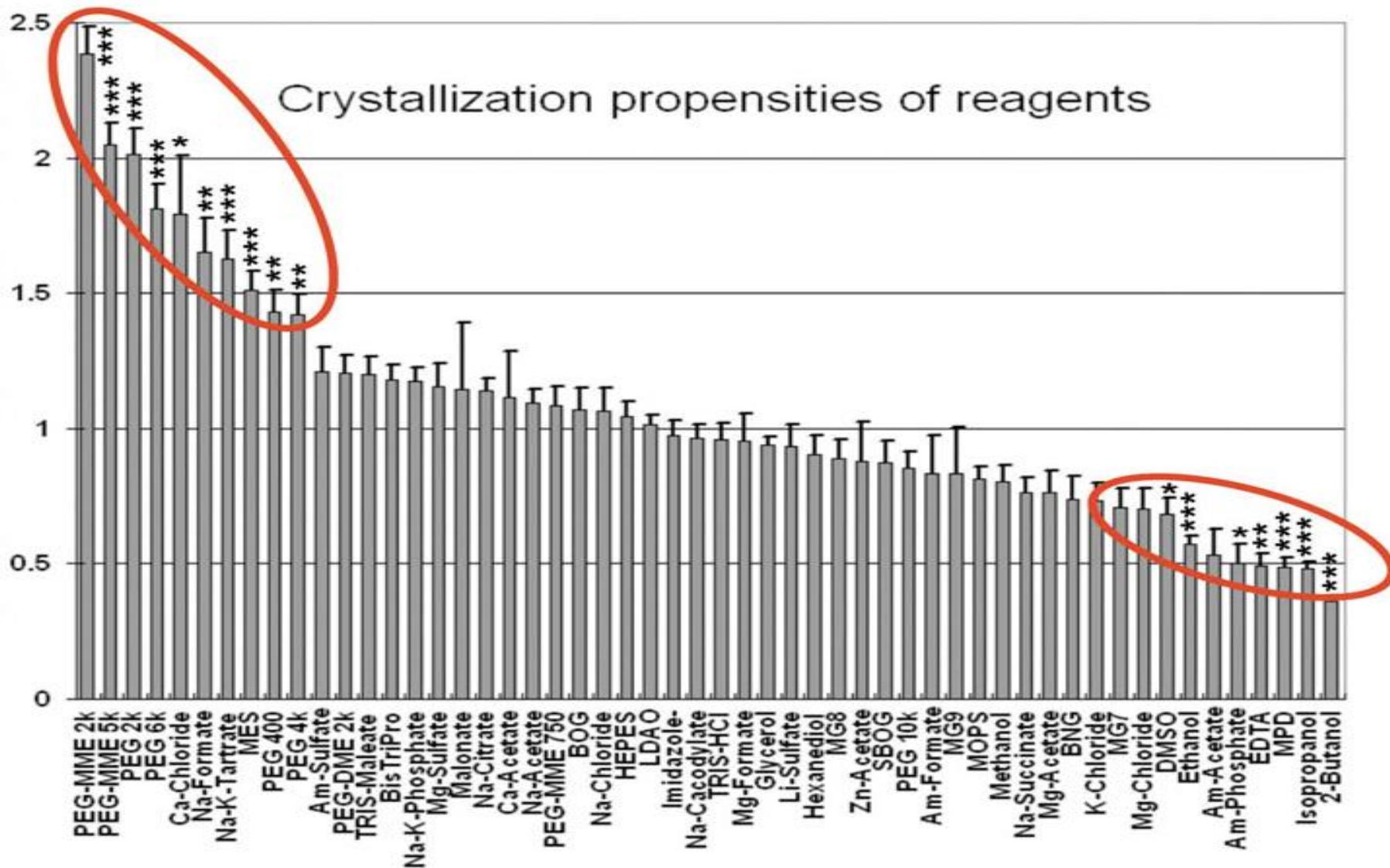
Cristallizzazione:

Agenti precipitanti: sali



Cristallizzazione:

Dipendenza dagli agenti precipitanti



Cristallizzazione:

Requisiti

Attrezzatura fondamentale:

- stanze a temperatura controllata (4 ° C e 20 ° C),
- Microscopio con polarizzatore (birifrangenti)
- piastre
- loops

Elevate quantità di proteine molto pure (~ 10mg / ml)

Reagenti di elevata purezza



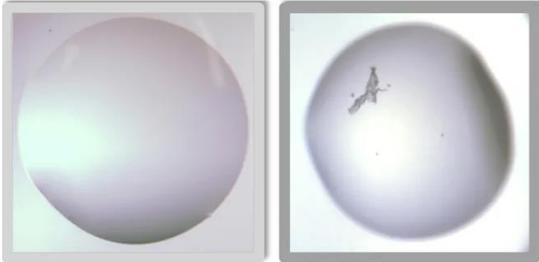
Cristallizzazione:

HT set up



Cristallizzazione:

Possibili risultati



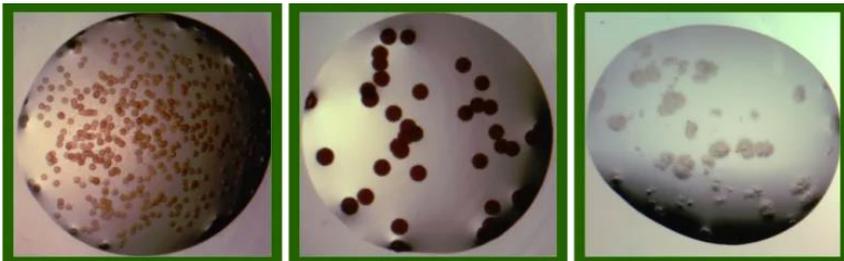
Gocce limpide



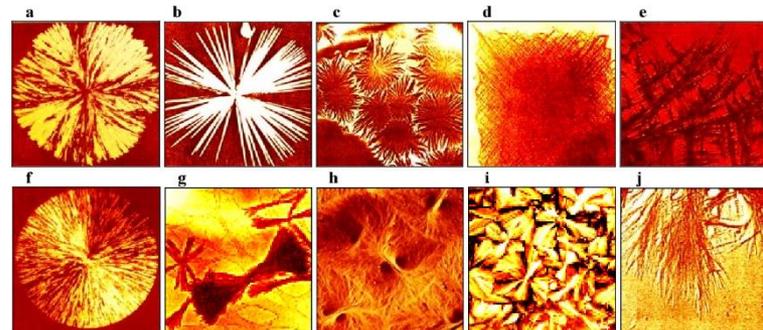
Precipitati



Separazione di fase



Sferuliti

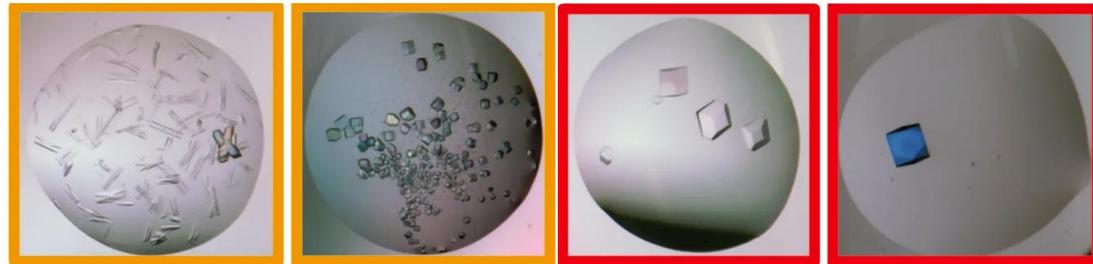


Cristallizzazione:

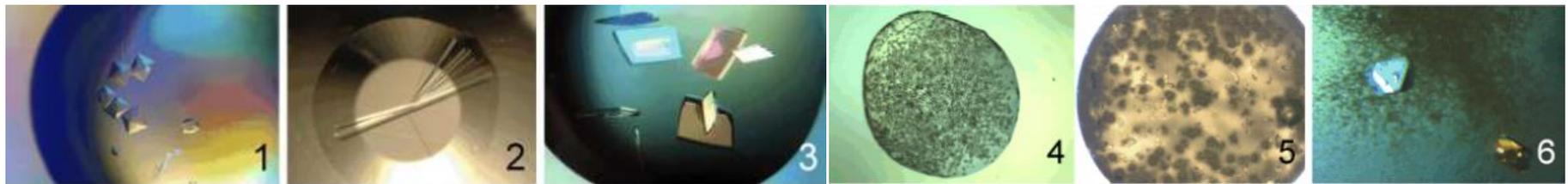
Possibili risultati



Materiale cristallino e micro-cristallino



Cristalli



1 non diffrangono

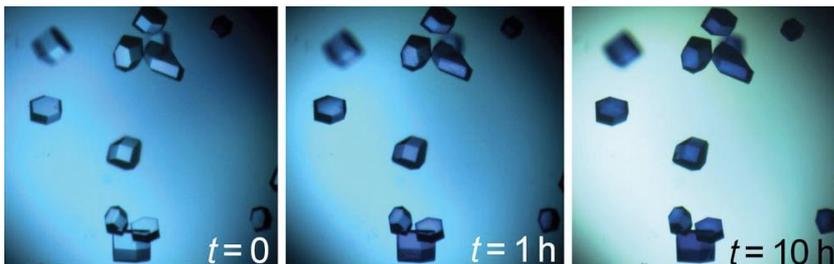
2 non di proteina

3 anisotropi

4 troppo piccoli

5 cristalli e ricci

6 maturazione Ostwald



ITZIT test (soaking con blu di metilene): discrimina da sali

Cristallizzazione:

Screen evaluation

Crystallization sheet for bovine trypsin Date:

Protein concentration: Drops: 5 μ l protein + 5 μ l buffer

Buffer system used: 0.1M MES/Tris Temp:

0.2 M ammonium sulphate			0.3 M ammonium sulphate			% PEG 8000
20	25	30	20	25	30	

A	B	C
D	E	F
G	H	I

Dates:

6.0						
7.0						
8.0						
9.0						

A:

B:

C:

D:

E:

F:

G:

H:

I:

- | | | |
|----------------------|----------------------|----------------------|
| 1: light precipitate | 2: precipitate | 3: heavy precipitate |
| 4: small spherolytes | 5: large spherolytes | |
| 6: small crystals? | 7: needles | 8: crystals |

Other notes:

Cristallizzazione:

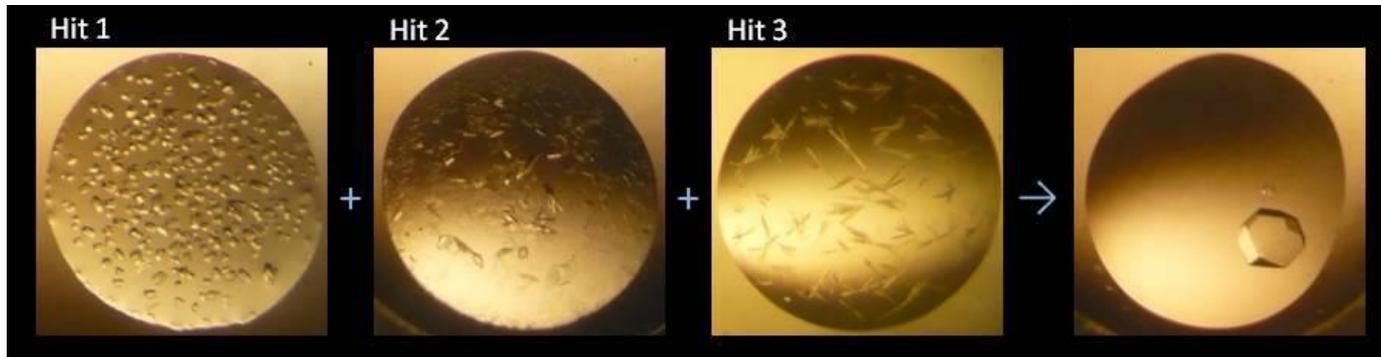
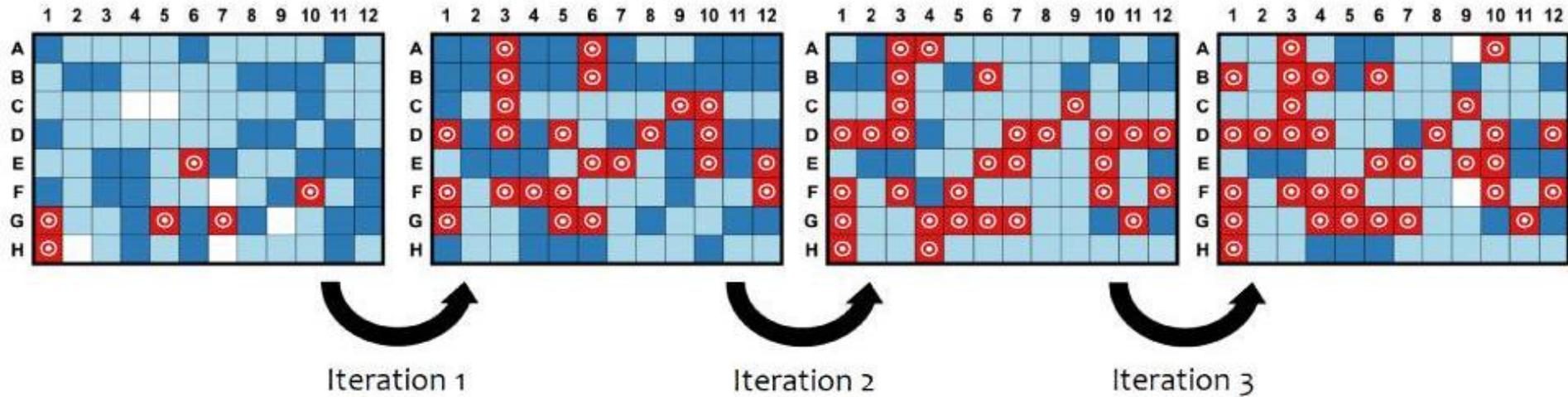
Ottimizzazione

Initial Screen

Optimized Screen 1

Optimized Screen 2

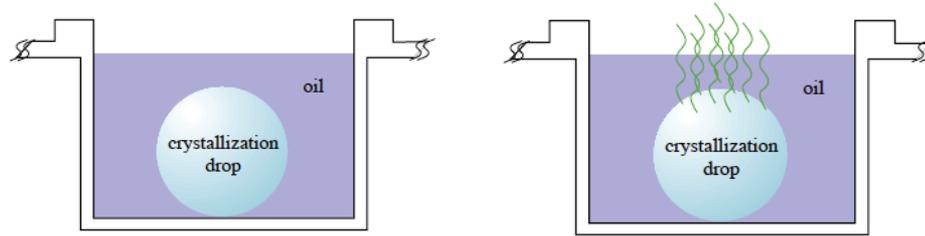
Optimized Screen 3



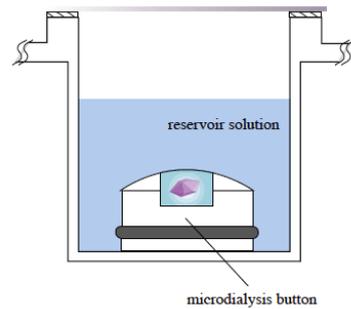
Cristallizzazione:

Metodi

1. batch method

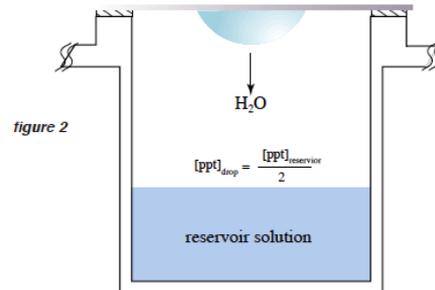


2. dialysis

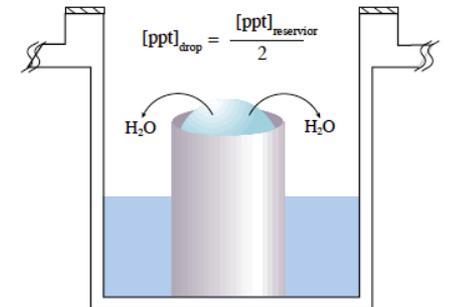


3. vapour diffusion:

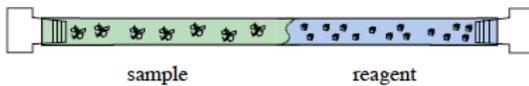
hanging-drop



sitting-drop



4. Free interface diffusion

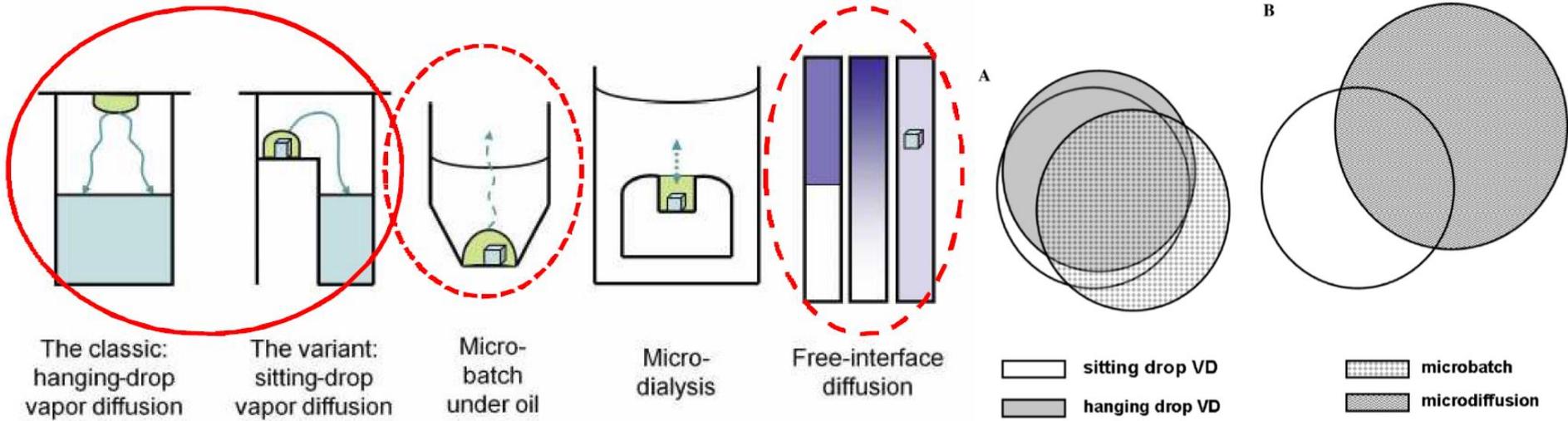


Cristallizzazione:

Metodi

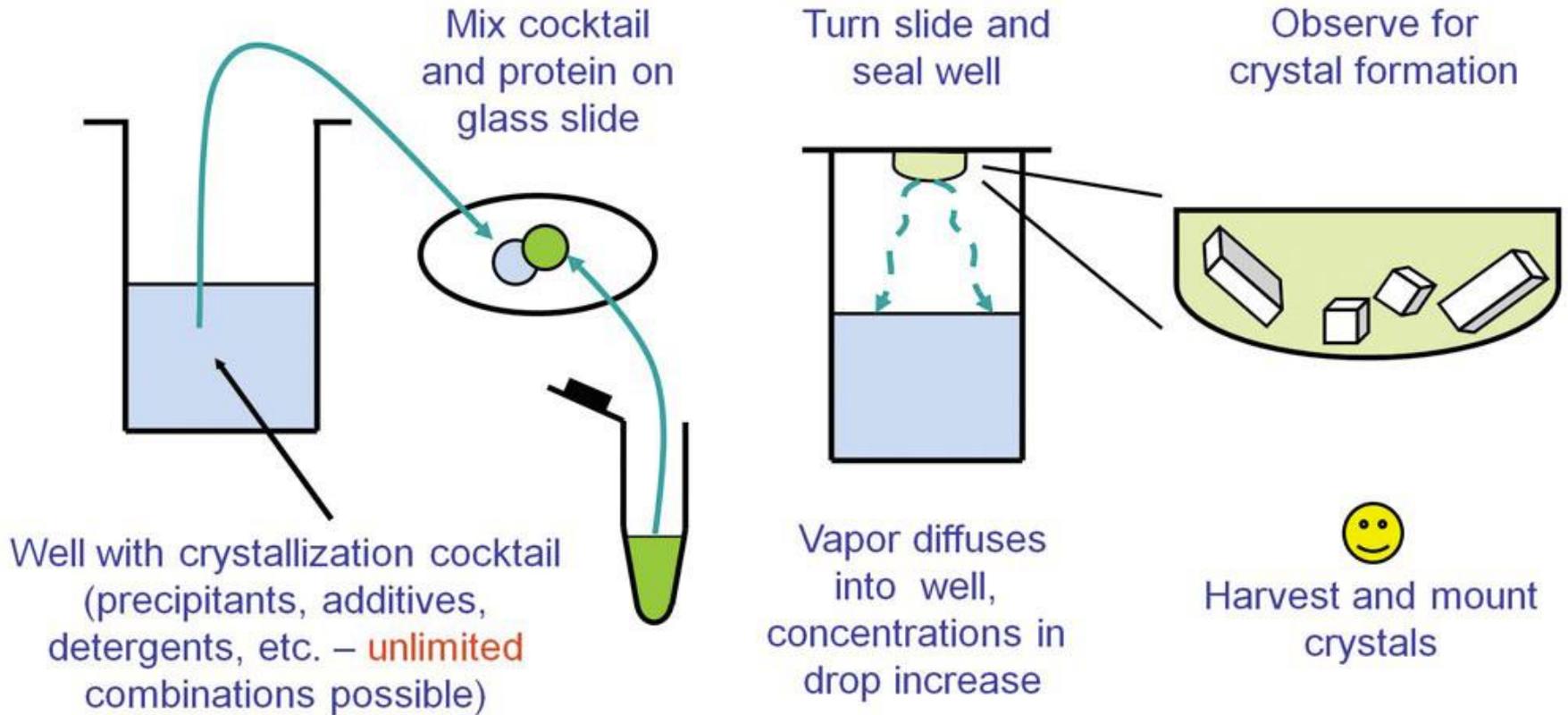
percorsi diversi nello spazio delle fasi cristallizzazione

Risultati molto diversi



Cristallizzazione:

Vapour diffusion:

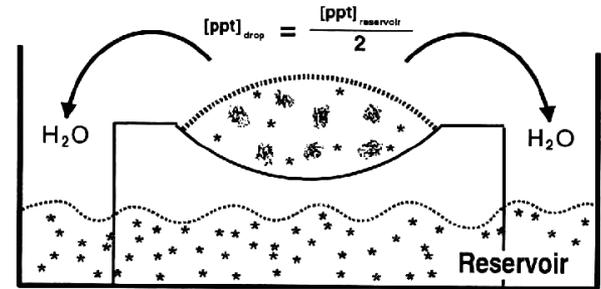


Cristallizzazione:

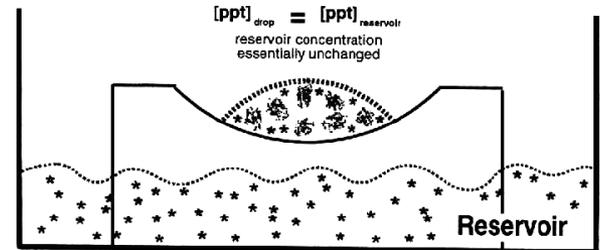
Sitting drop



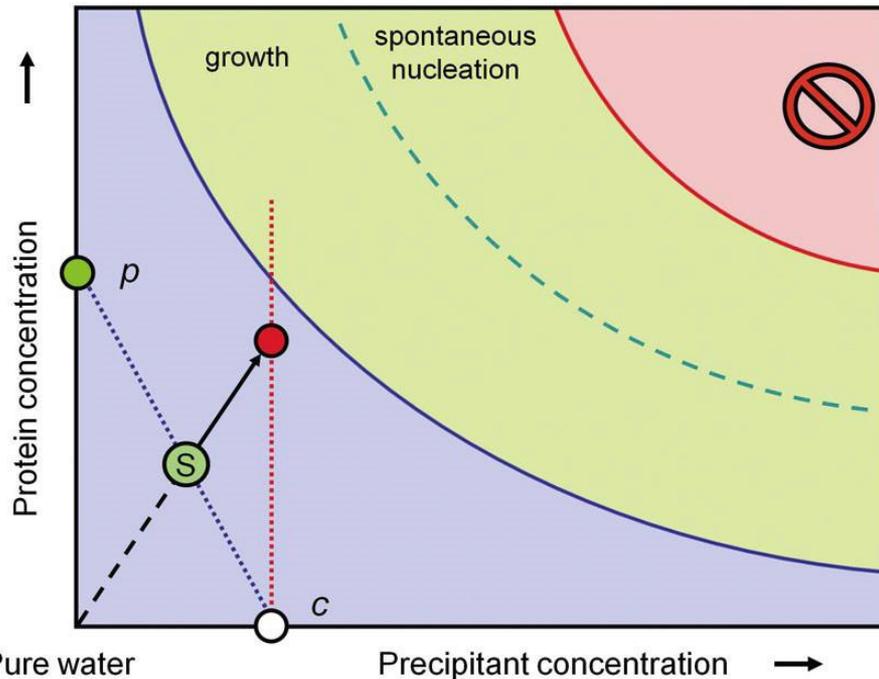
 Macromolecule * Precipitant ions or Molecules (ppt)



(A) Equilibration proceeds through vapor phase



(B) Drop volume decreases, increasing concentration of both precipitant and protein



Cristallizzazione:

Hanging drop

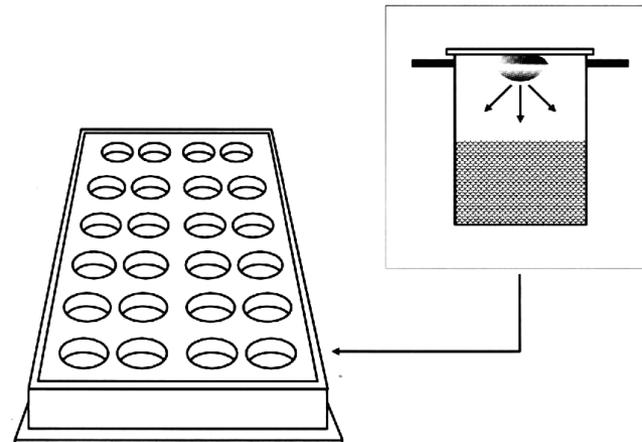
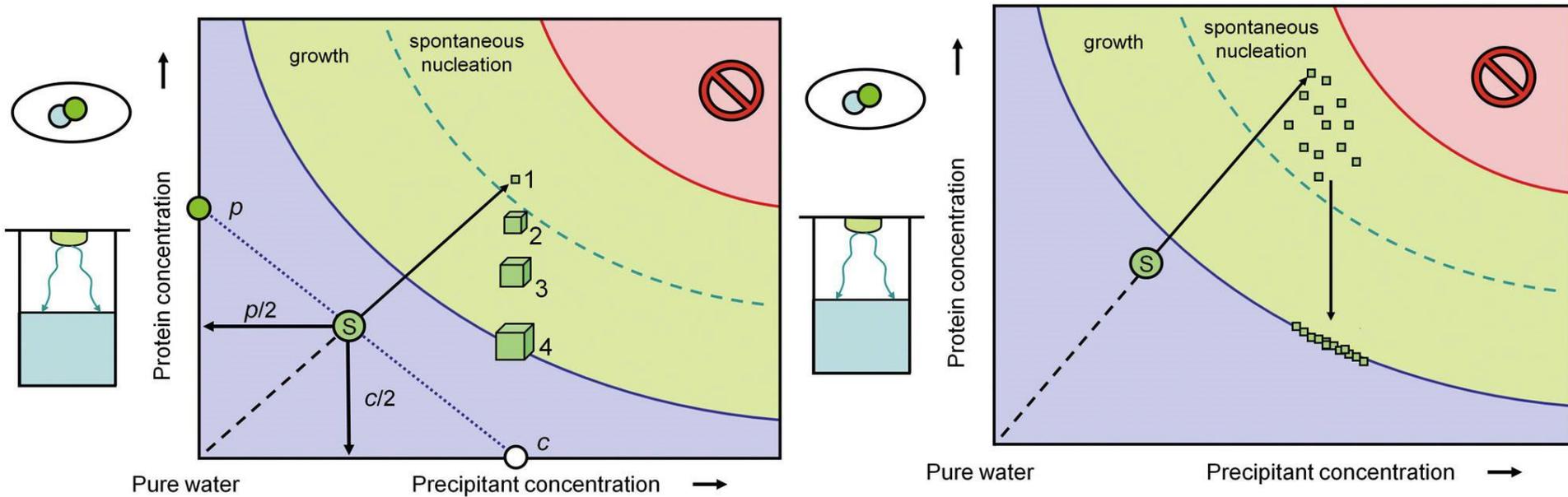


Figure 5.24. A standard configuration for hanging drop protein crystallization

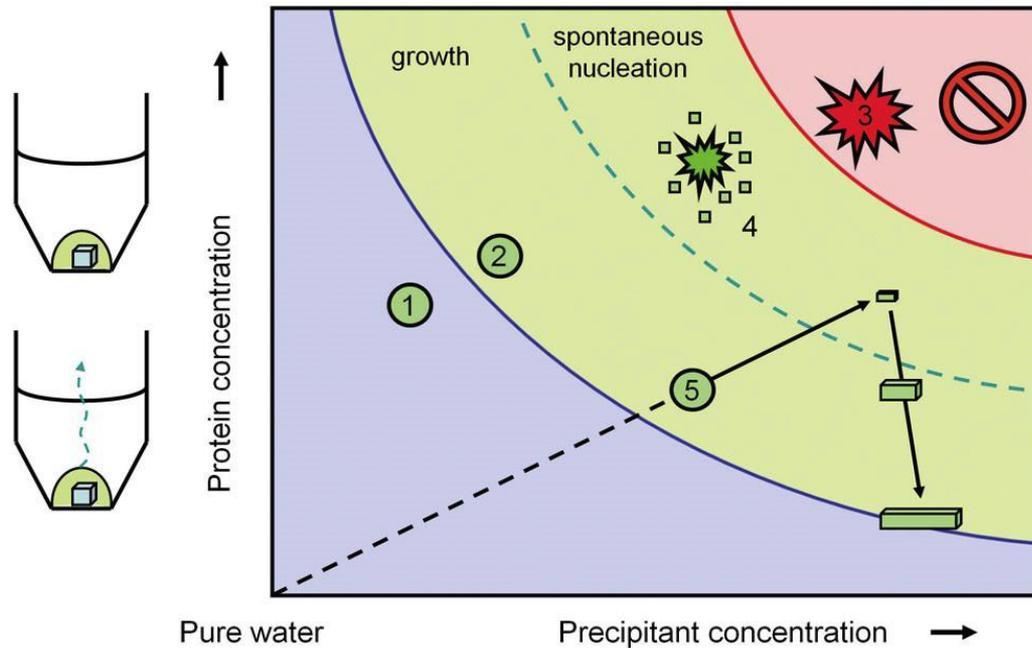
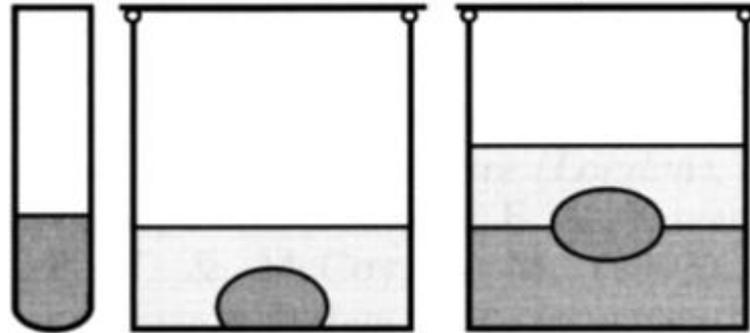


Cristallizzazione:

Batch method

Drop
under oil

Floating
drop in oil

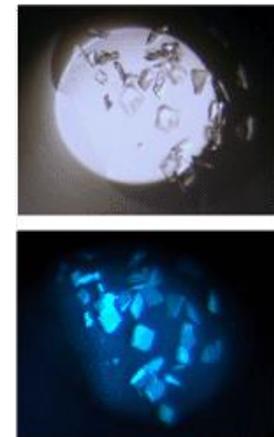
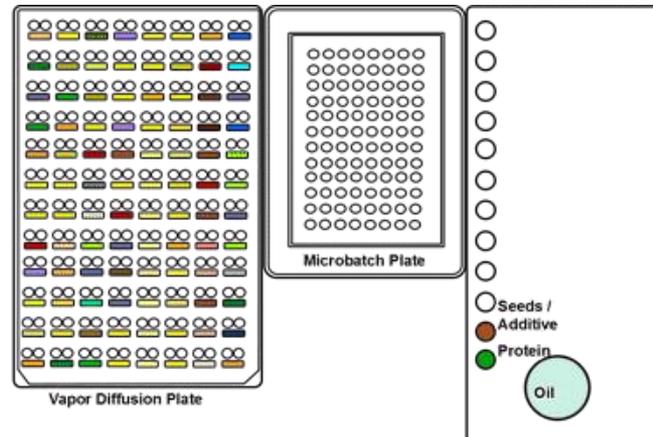
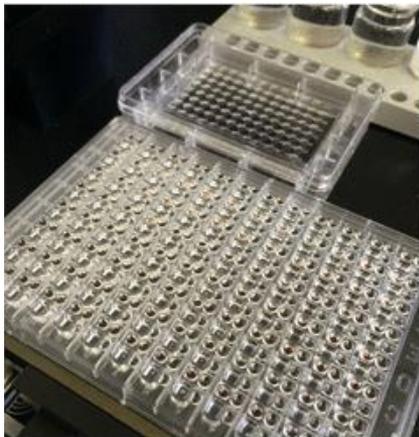
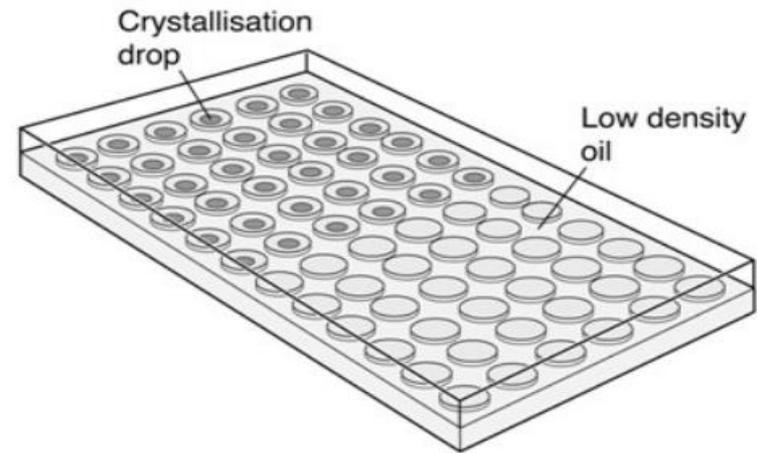
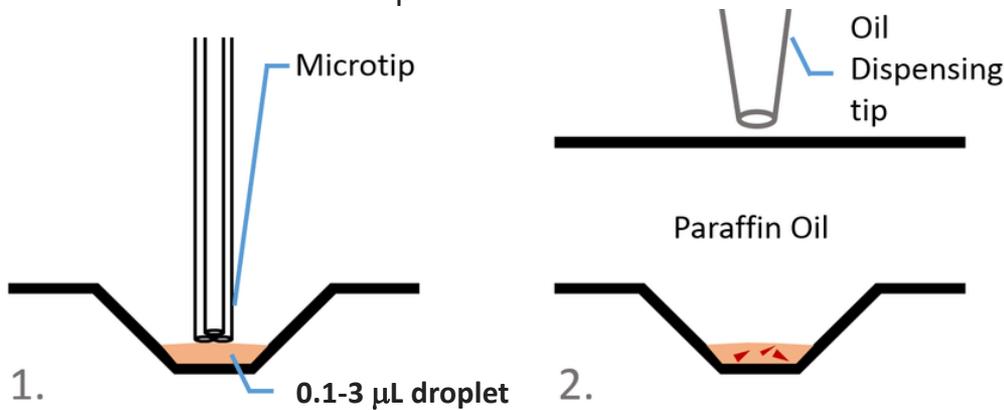


Cristallizzazione:

MicroBatch method

nucleazione controllata a seconda di tipo/spessore dell'olio

- Screening: miscela 50:50 di paraffina/silicone
- Ottimizzazione: olio di paraffina

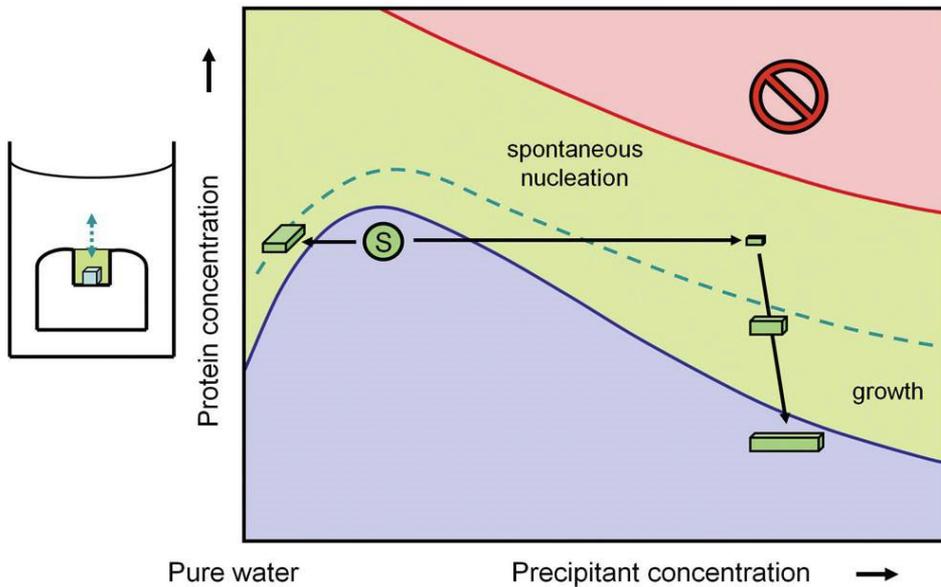


Cristallizzazione:

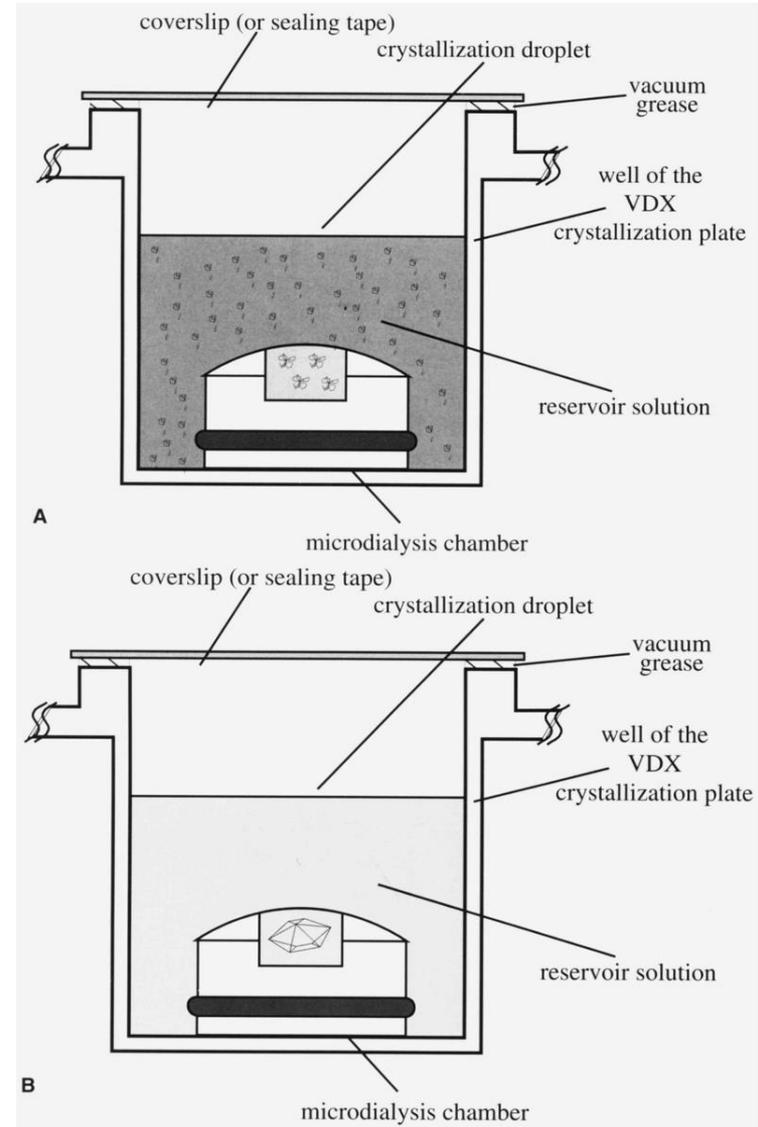
Dialisi

Due modalità:

acqua distillata: inversione del salting-in
precipitante concentrato: salting-out



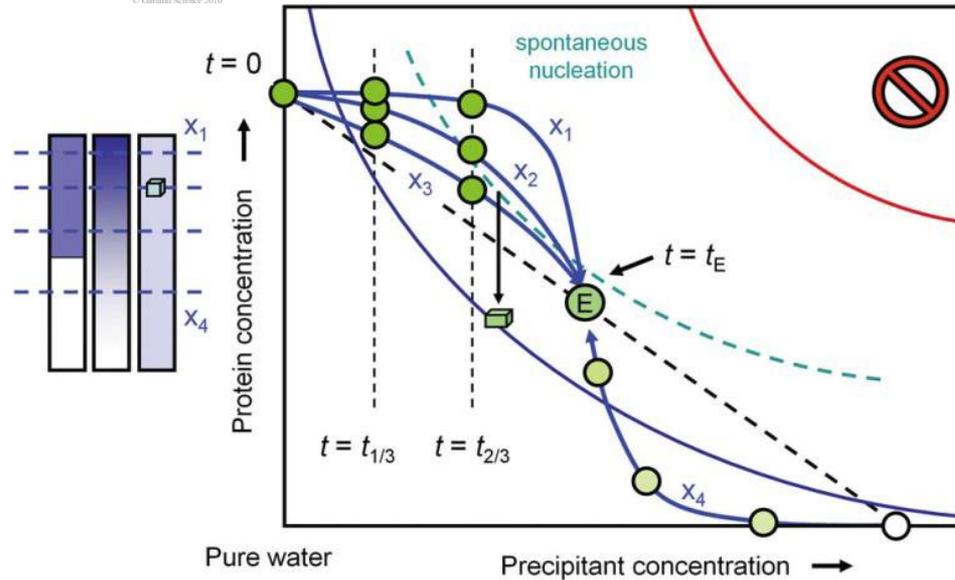
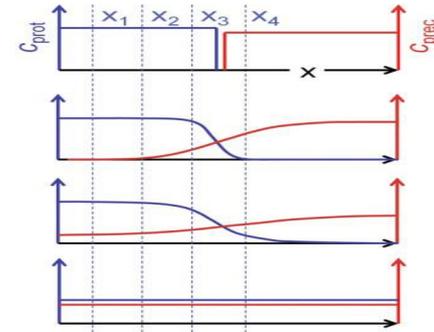
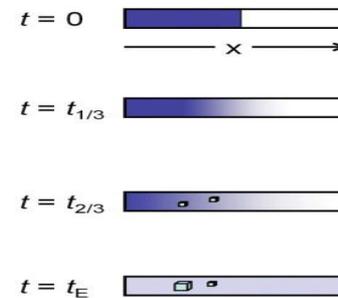
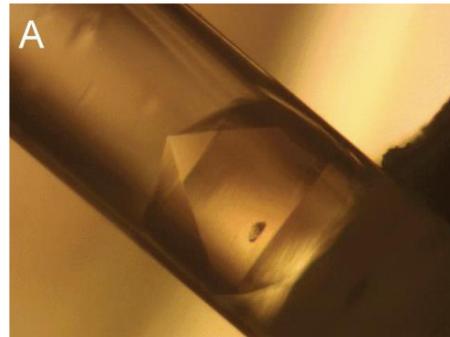
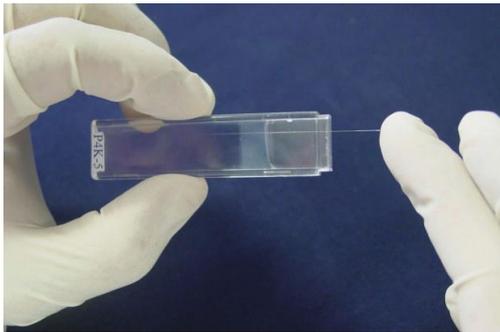
© Garland Science 2010



Cristallizzazione:

Free interface diffusion

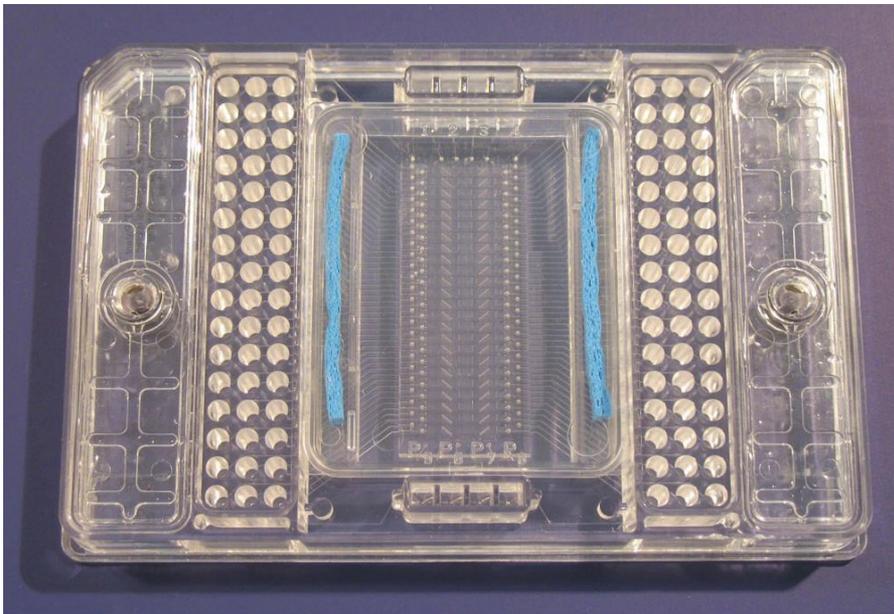
Capillare inserito in un gel saturo di soluzione precipitante: cristalli di una particella virale (diametro 640 Å).



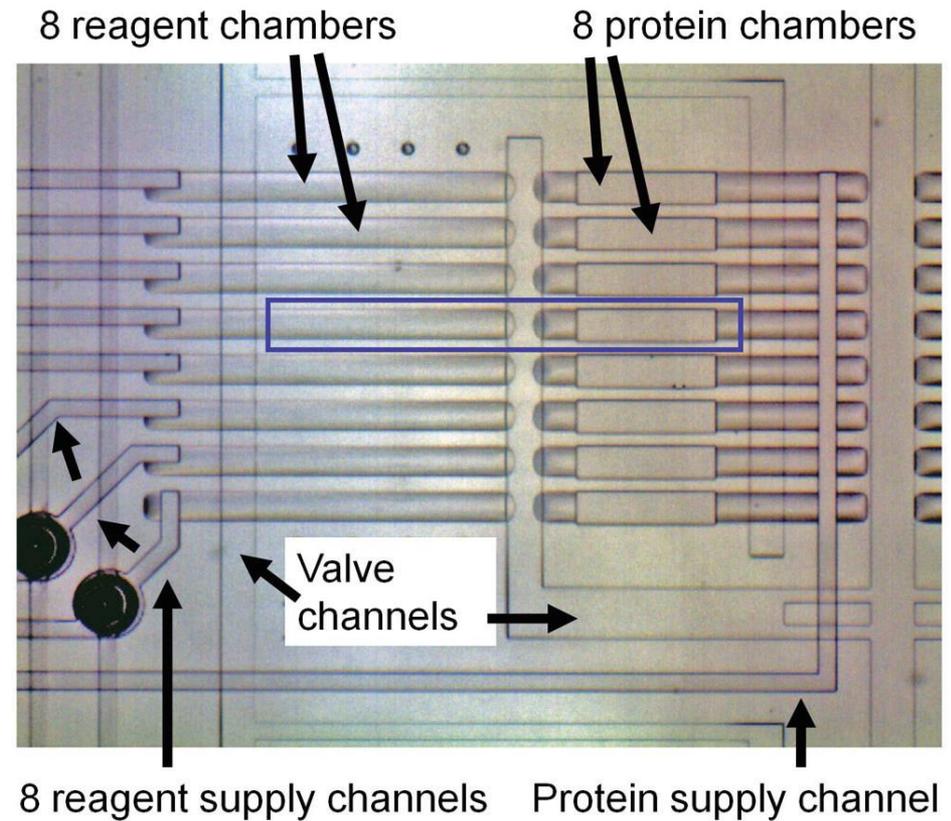
Cristallizzazione:

Free interface diffusion

Chip microfluidico per screening



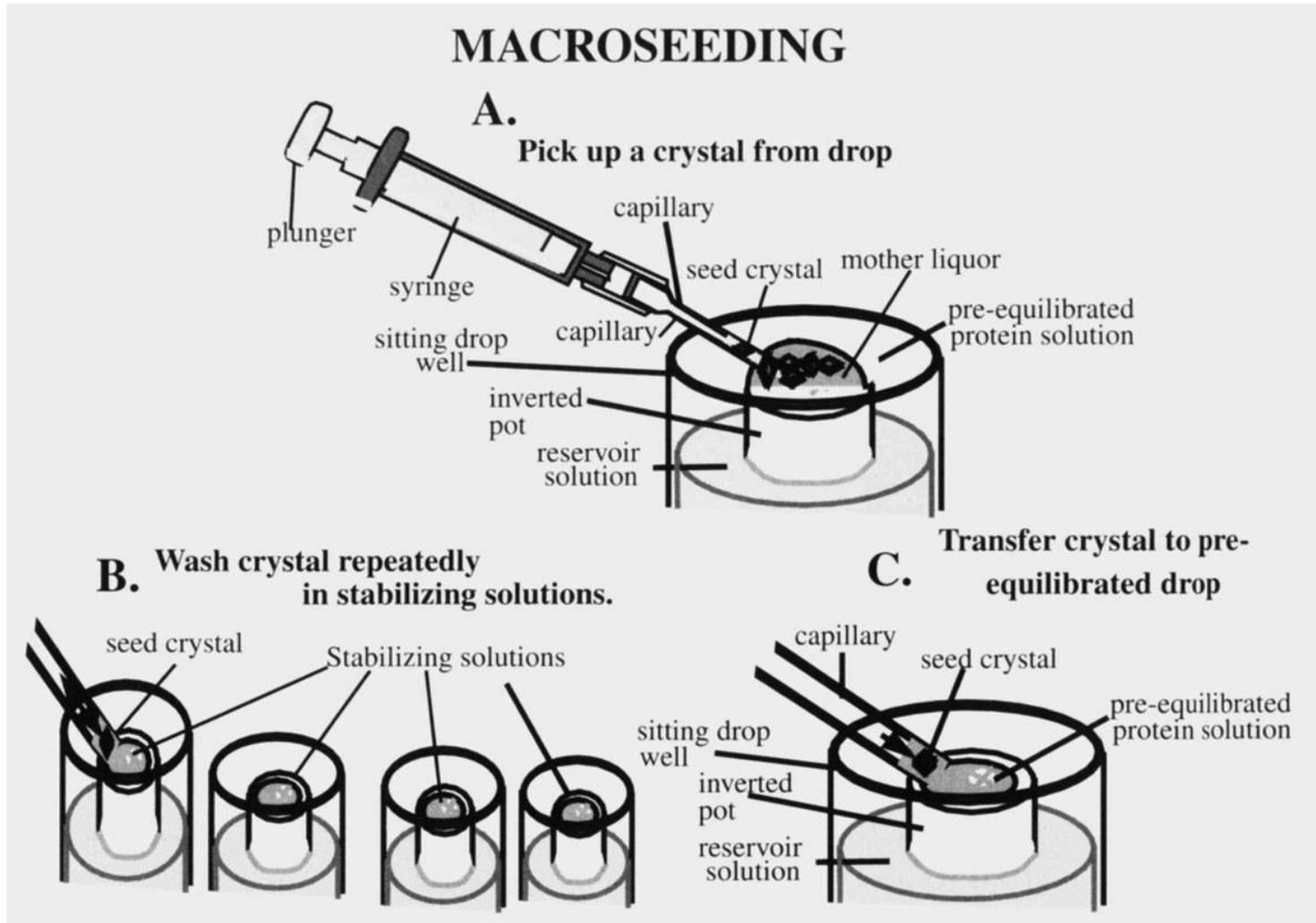
© Garland Science 2010



© Garland Science 2010

Cristallizzazione:

Seeding macroseeding:

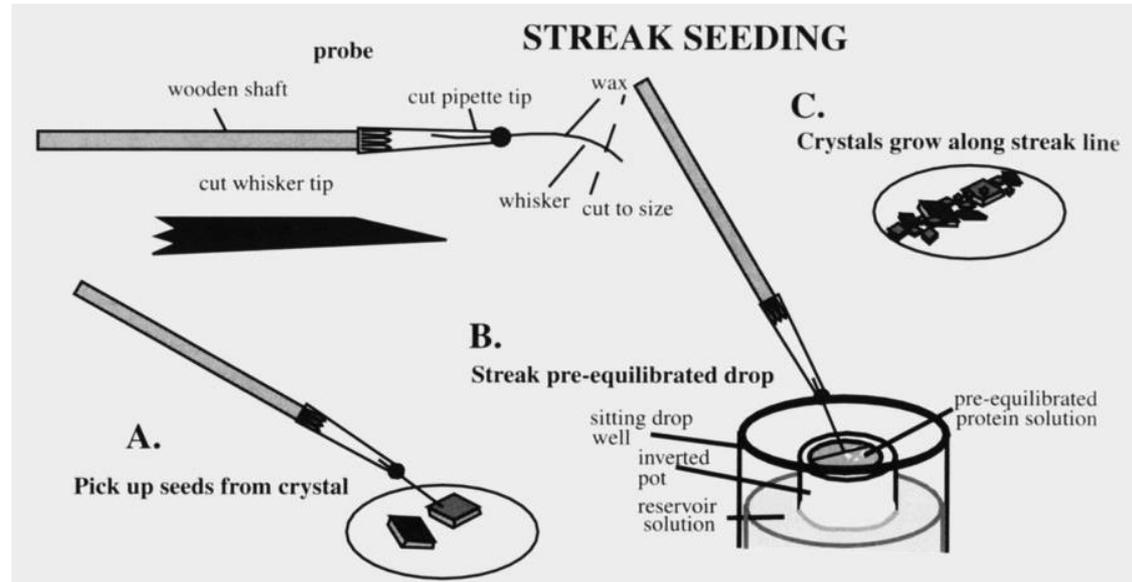
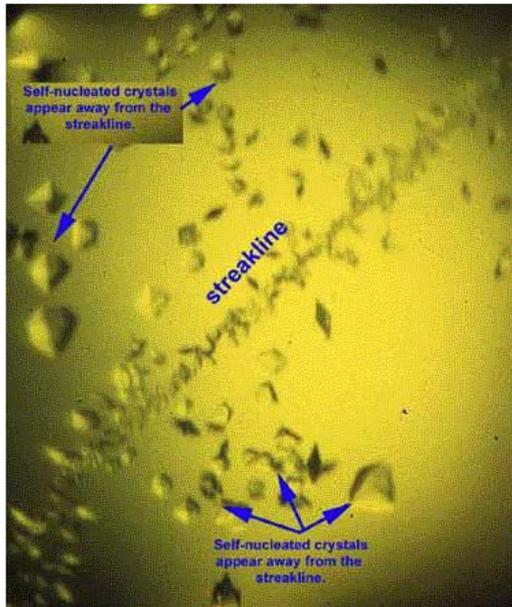
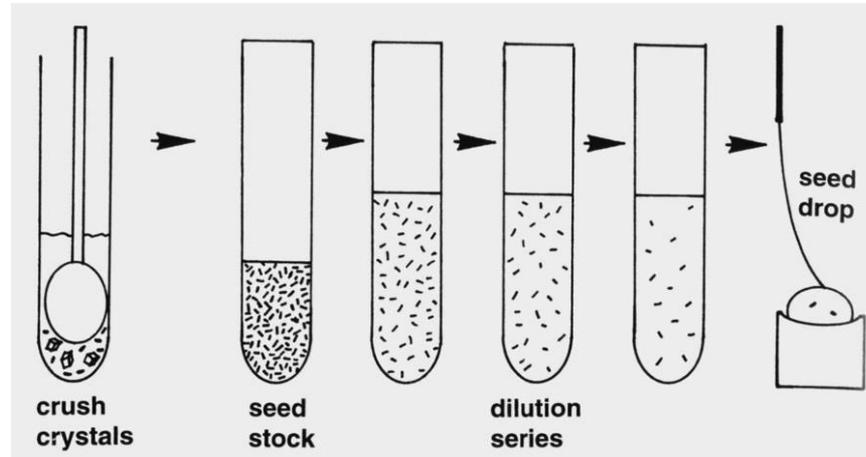


Cristallizzazione:

Seeding

microseeding

streak seeding

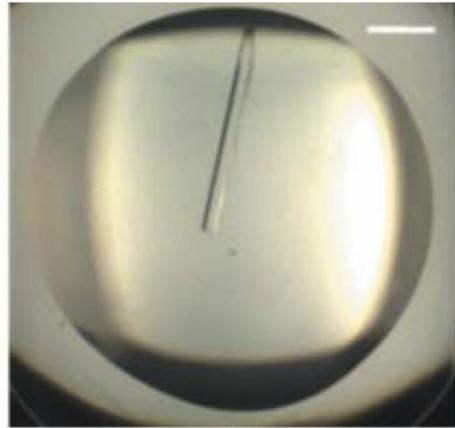


Cristallizzazione:

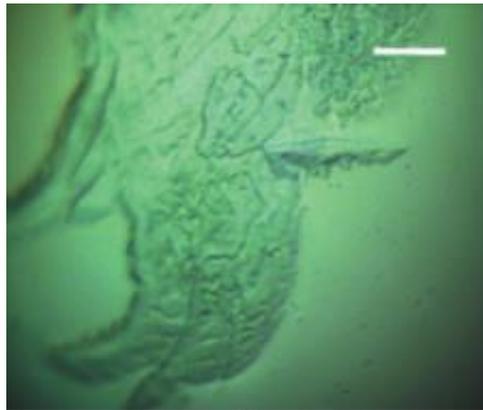
Microseeding & screening



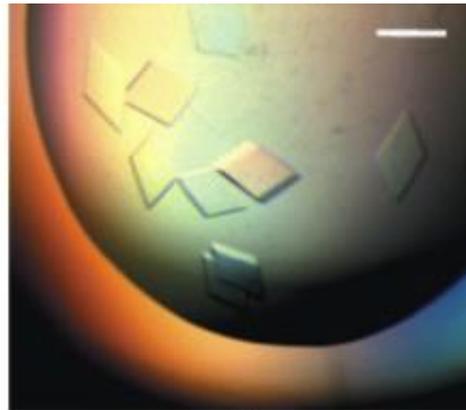
(a)



(b)

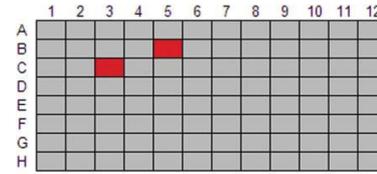


(a)

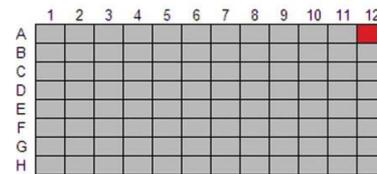


(b)

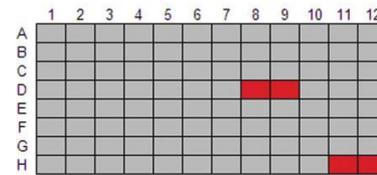
senza seeding



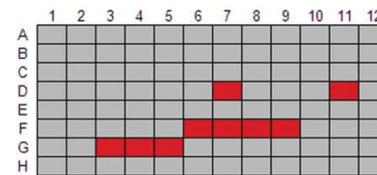
(a)



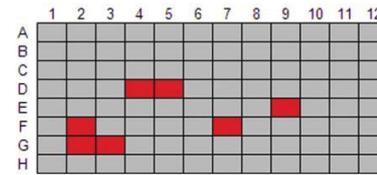
(c)



(e)

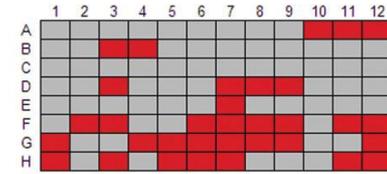


(g)

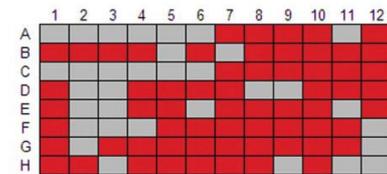


(i)

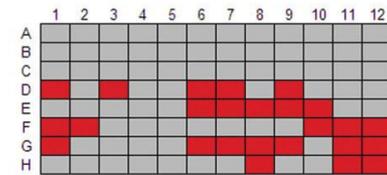
con seeding



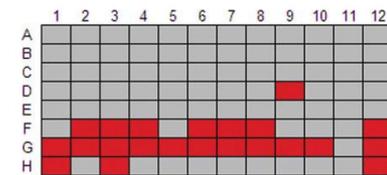
(b)



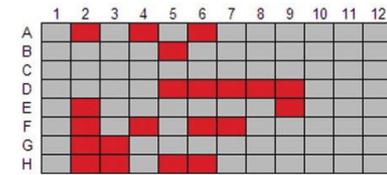
(d)



(f)



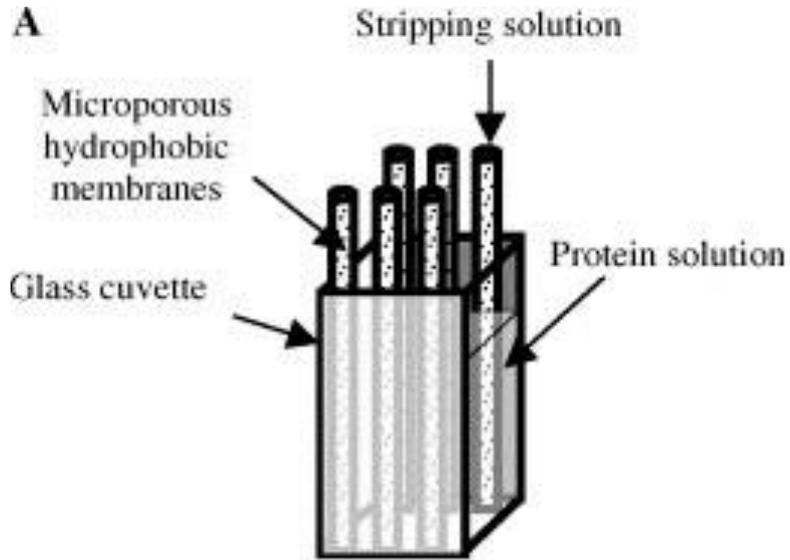
(h)



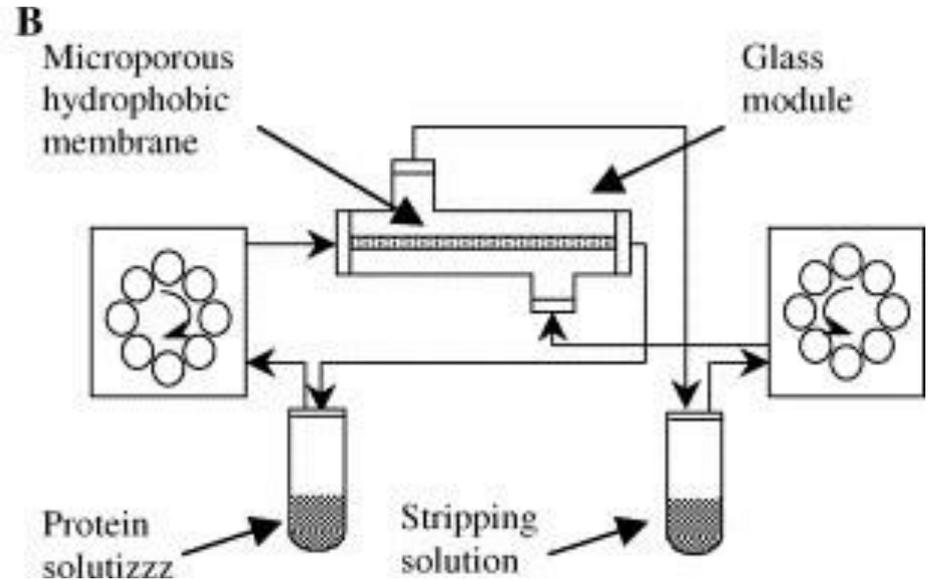
(j)

Cristallizzazione:

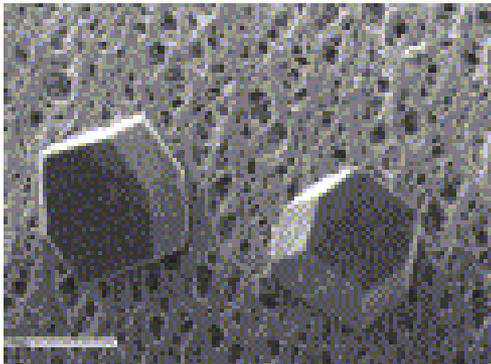
Membrane system



Configurazione statica (quiescente)



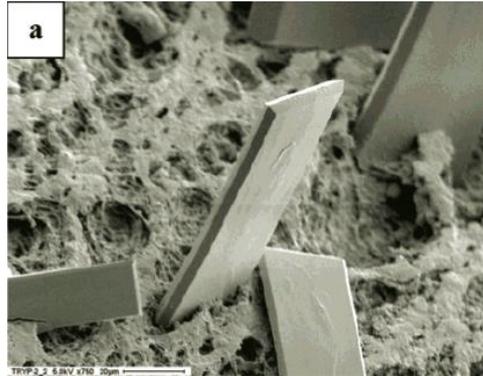
Configurazione a flusso forzato della soluzione



Cristallizzazione:

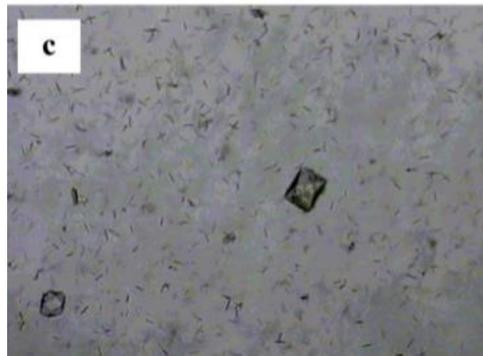
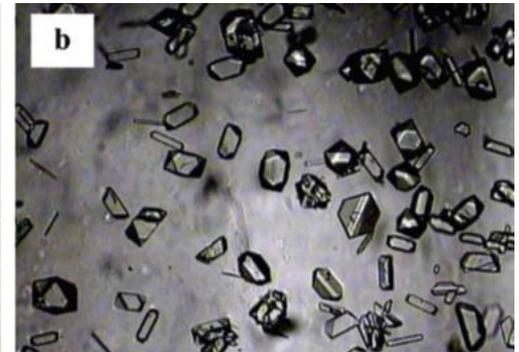
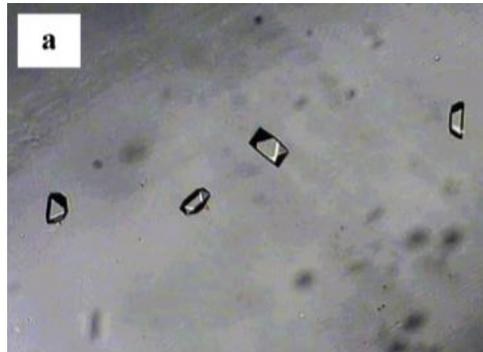
Membrane system

Configurazione statica
Cristalli BPT cresciuti sulla superficie di una
membrana polimerica



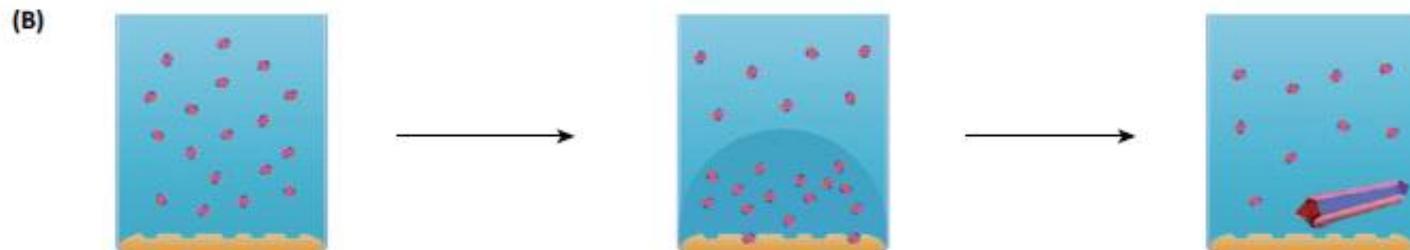
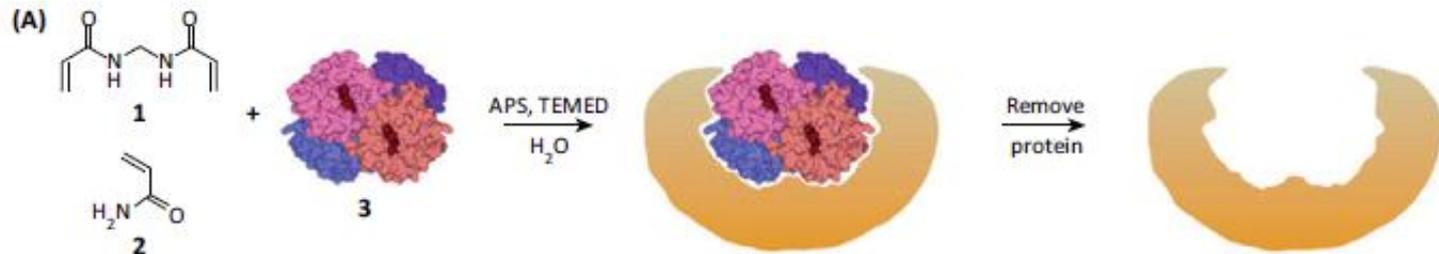
Cristalli BPT ottenuti in configurazione
a flusso forzato di soluzione con solfato di
ammonio come precipitante
velocità di ricircolo della soluzione

- (a) 423 $\mu\text{m} / \text{s}$;
- (b) 821 $\mu\text{m} / \text{s}$;
- (c) 1072 $\mu\text{m} / \text{s}$;
- (d) 1139 $\mu\text{m} / \text{s}$



Cristallizzazione:

Molecularly imprinted polymers



Review

Cell
PRESS

Imprinted polymers assisting protein crystallization

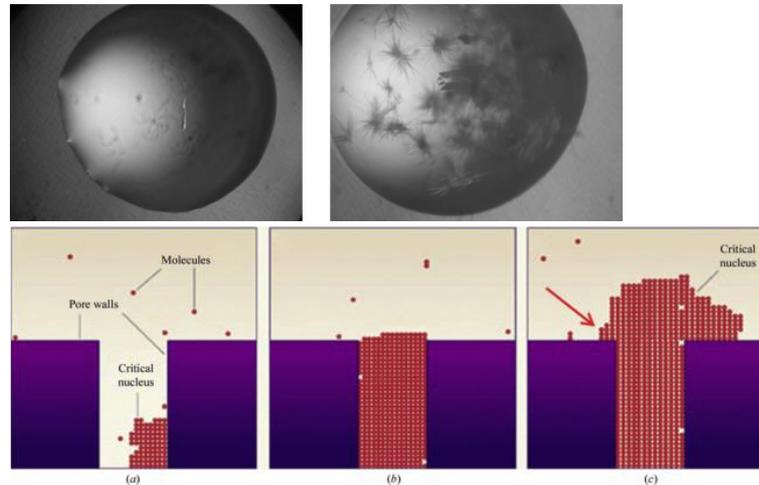
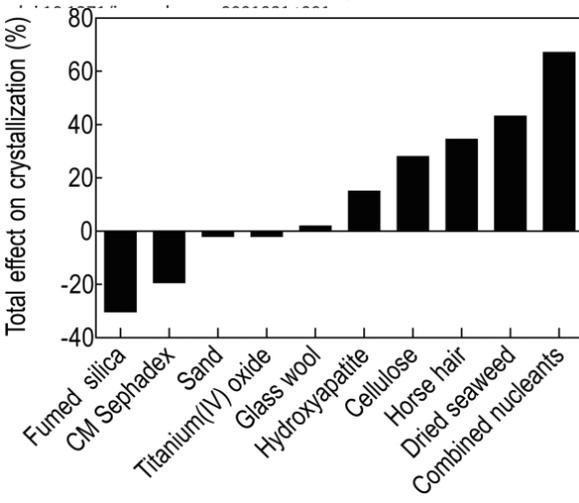
Emmanuel Saridakis¹ and Naomi E. Chayen²

Cristallizzazione:

Nucleazione eterogenea

Protein	Control (no nucleant)	Fumed silica		CM Sephadex		Sand		Titanium(IV) oxide		Glass wool		Hydroxyapatite		Cellulose		Horse hair		Dried seaweed		Combination of 9 nucleants		Combination of 4 nucleants	
	Total	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-
Lysozyme	5	1	3	0	1	0	0	1	1	0	0	1	0	2	0	1	0	4	0	7	3	7	0
Pepsin	3	0	1	1	2	0	0	1	0	0	0	2	1	0	0	2	0	2	0	4	1	ND	
Trypsin	5	1	2	0	2	0	0	0	1	1	0	2	1	3	0	2	0	3	0	5	1	ND	
Glucose Isomerase	4	0	3	0	1	0	0	0	0	0	0	1	2	2	1	1	0	2	0	3	1	ND	
Ribonuclease A	6	0	4	1	2	0	0	1	1	0	0	2	2	2	0	3	1	2	1	3	2	ND	
Myoglobin	4	0	3	0	1	0	1	0	1	0	0	1	1	2	0	1	0	2	0	4	0	ND	
α -lactalbumin	5	1	1	1	0	0	0	0	1	0	0	1	1	1	0	2	1	3	0	0	0	ND	
Catalase	5	2	2	0	1	0	0	0	0	0	0	2	1	1	1	3	1	1	0	6	2	ND	
Xylanase	5	1	1	0	1	0	0	1	1	0	0	3	1	0	0	3	1	2	1	6	1	ND	
Thaumatin	4	2	2	0	1	0	0	1	0	0	0	2	0	3	1	2	0	2	1	5	1	ND	
Sum over all proteins	46	8	22	3	12	0	1	5	6	1	0	17	10	16	3	20	4	23	3	43	12	ND	
Total effect (crystallization hits)		-14		-9		-1		-1		1		7		13		16		20		31		ND	
Total effect (%)		-30		-20		-2		-2		2		15		28		35		43		67		ND	

For each protein/heterogeneous nucleating agent pair and the sum over all proteins, the number of new and missing crystallization conditions (“+” and “-”, respectively) is shown, relative to the no-nucleant control. For the no-nucleant control, the total number of crystallization hits is shown. The total effect of the nucleating agent, relative to the no-nucleant control, is shown in the last two rows. “ND”, not determined.



Nucleazione in pori/cavità

Cristallizzazione:

Nucleazione eterogenea

Agenti nucleanti realizzati in bio-vetro ($\text{CaO-P}_2\text{O}_5\text{-SiO}_2$)



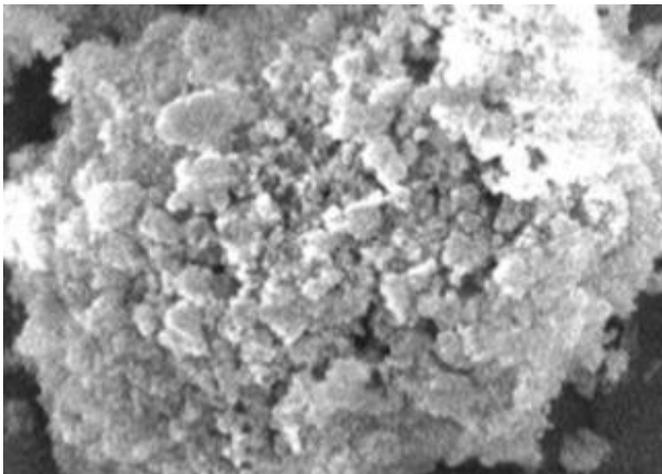
Crustacianina



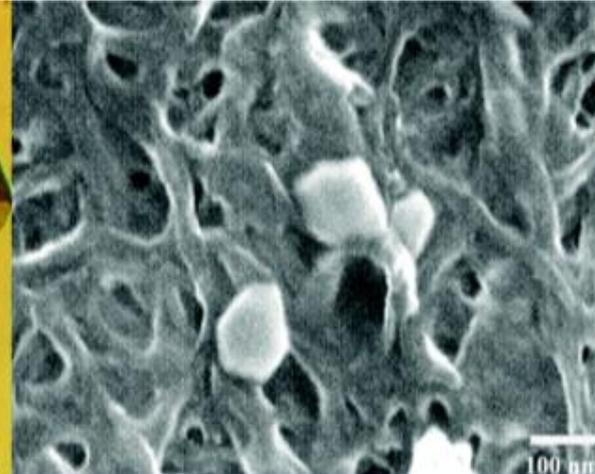
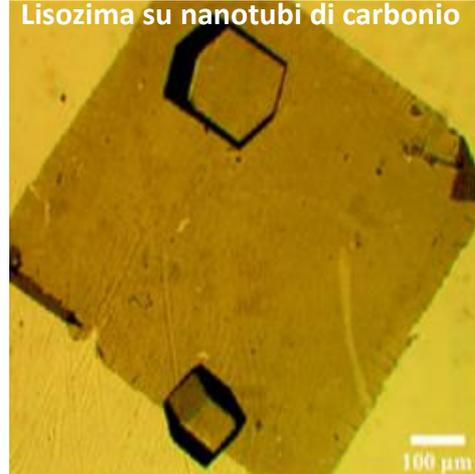
Lattamasi



Inibitore di serin proteasi



Lisozima su nanotubi di carbonio



Cristallizzazione:

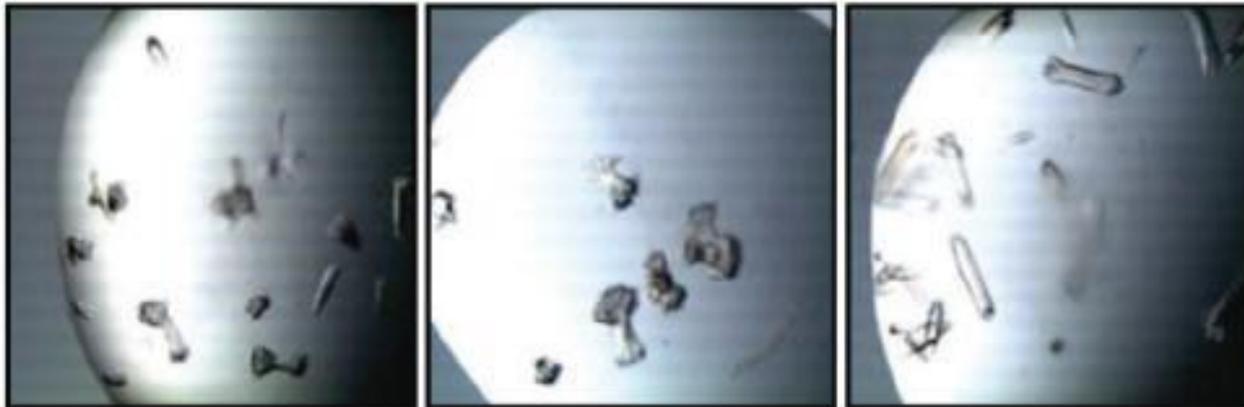
In acqua deuterata

effetti sulla struttura, stabilità e dinamica delle proteine

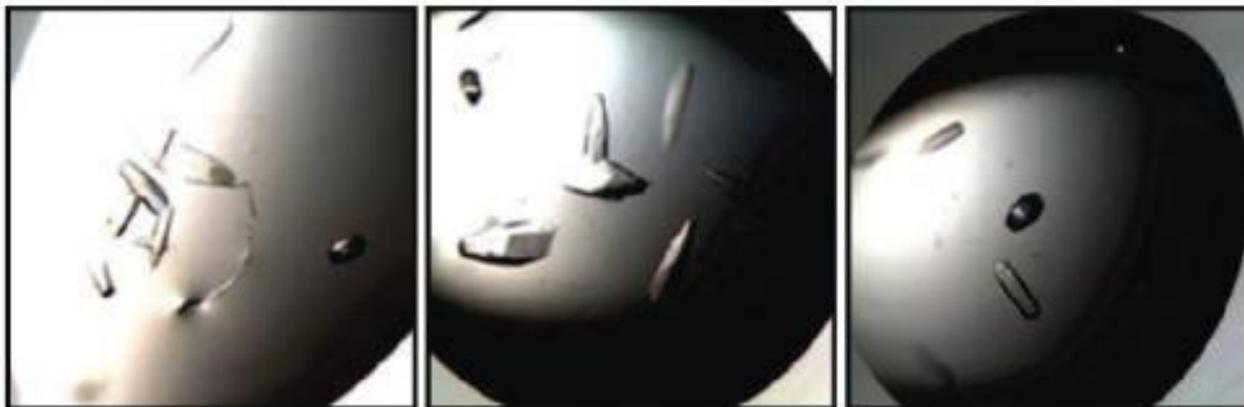
→ importanza dell'idratazione

→ influenza sulle cinetiche di assembly

50% D₂O



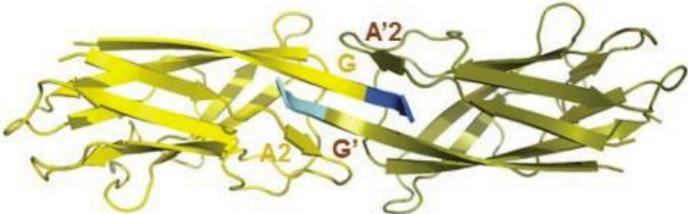
100% D₂O



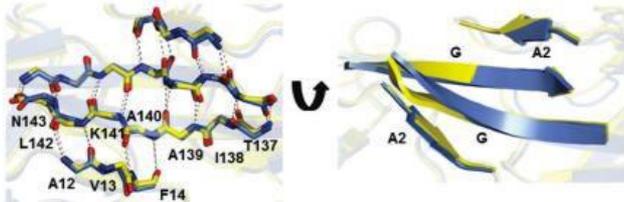
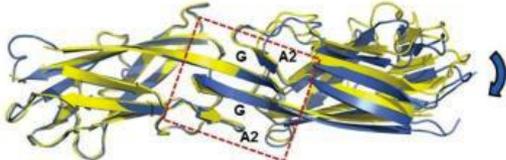
Salmonella enteritidis fimbriae 14 pilin SefD: Domain-swapped dimer

Cristallizzazione:

In acqua deuterata

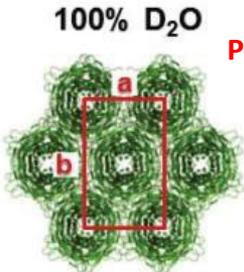
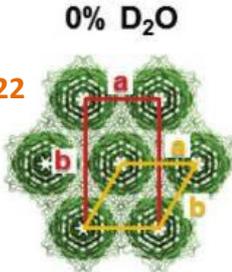


backbone H bonding

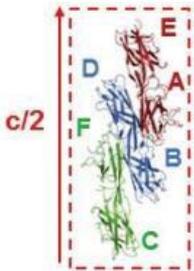
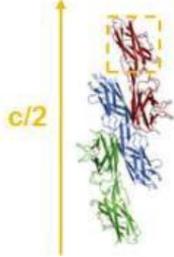


A)

$C222_1$
subgroup of P_6522

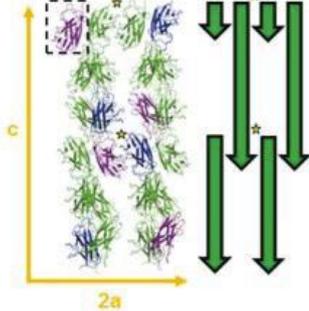


$P2_12_12_1$

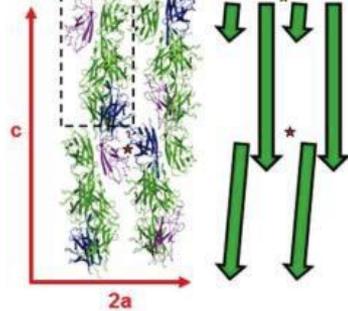


B)

0% D₂O
2 'face-to-face' dimers (*)



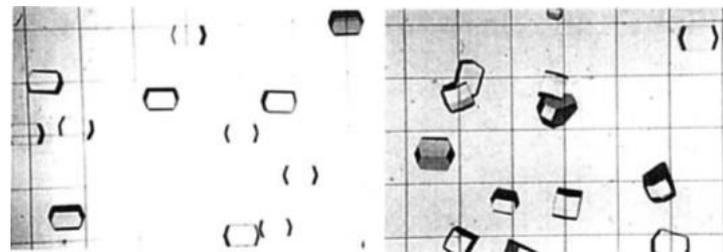
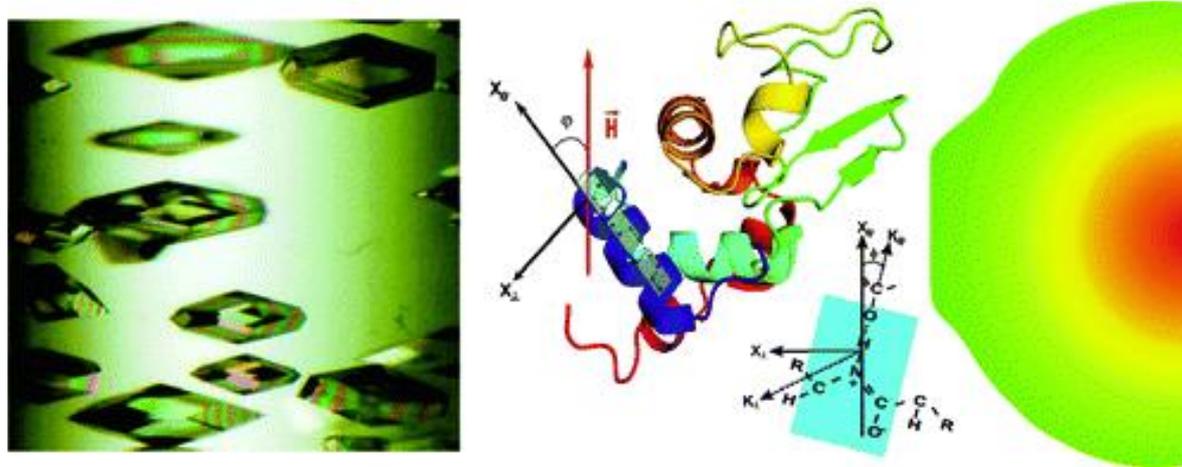
100% D₂O
only one of these dimers



B)

Cristallizzazione:

In campo magnetico



1 mm

(a) $H=0.64T$

(b) $H=0T$

→ H

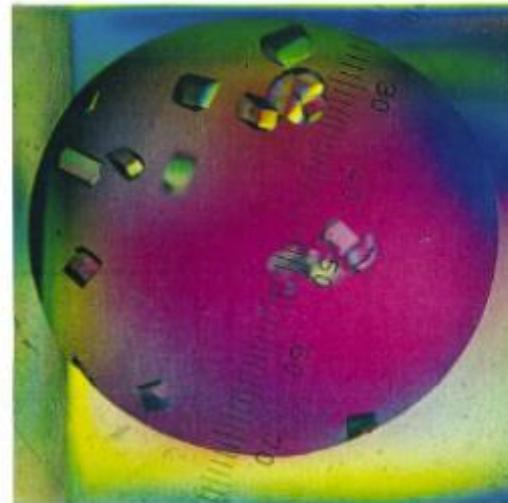
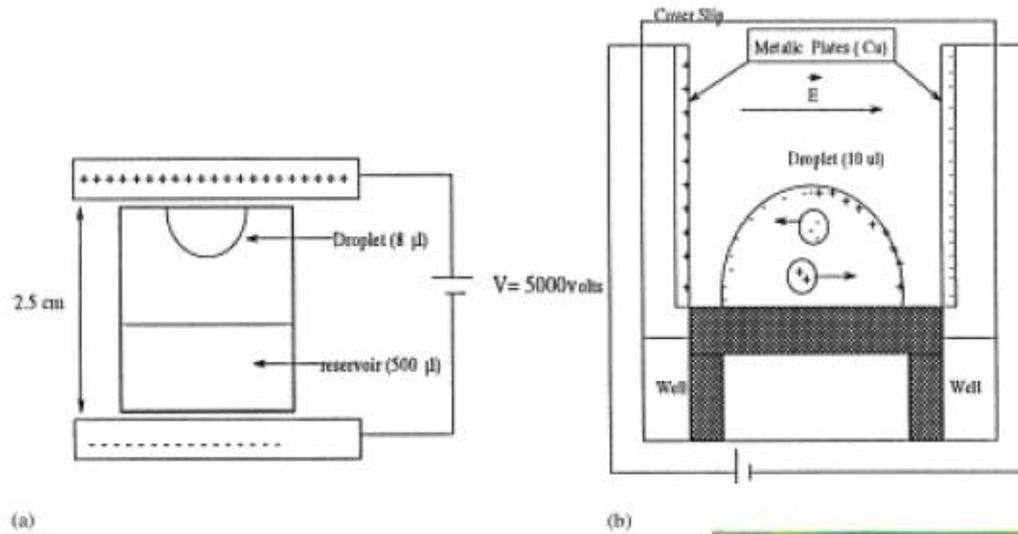
Effects of a Magnetic Field on Lysozyme Crystal Nucleation and Growth in a Diffusive Environment

Jose A. Gavira* and Juan Ma. García-Ruiz

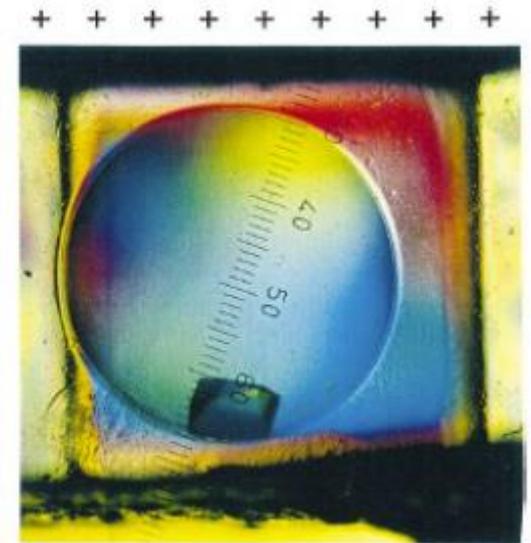
[View Author Information](#) ▾

Cristallizzazione:

In campo elettrico



(a)



(b)

New approaches on crystallization under electric fields.
(PMID:20025897)

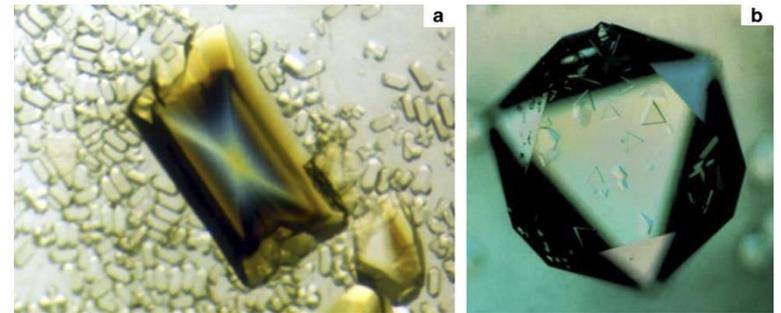
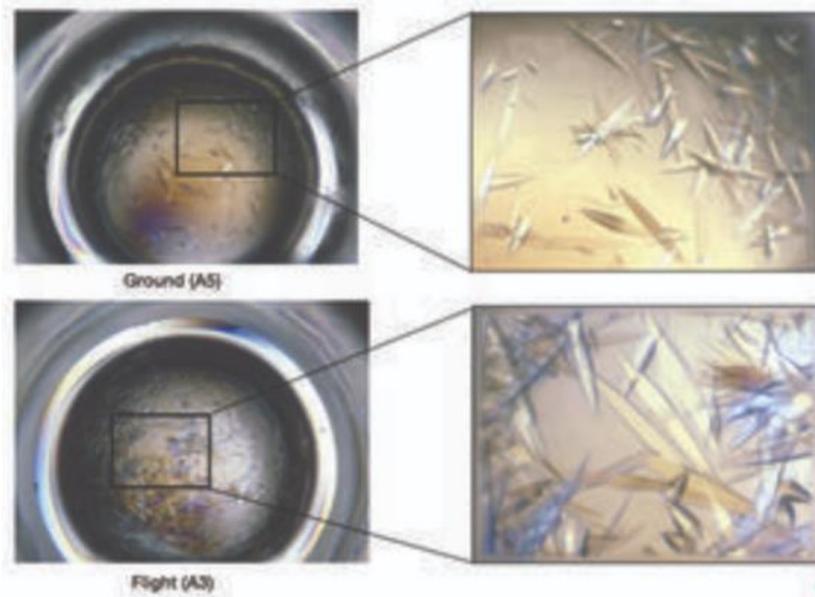
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[Hammadi Z, Veessler S](#)

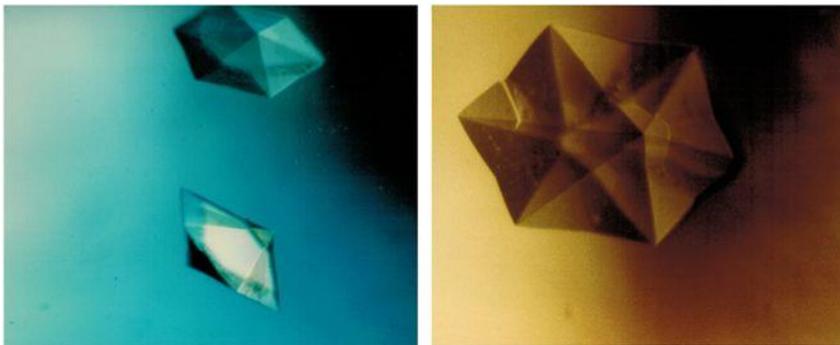
[Progress in Biophysics and Molecular Biology](#) [01 Nov 2009, 101(1-3):38-44]

Cristallizzazione:

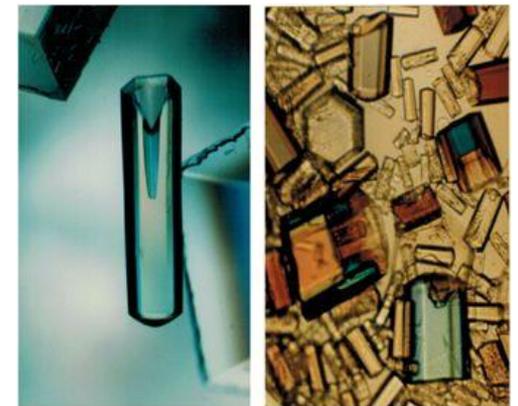
In microgravità



satellite tobacco mosaic virus (STMV): 30X



virus del mosaico giallo della rapa (TYMV)



canavalina

