The Molecular Mechanisms of Crystal Growth

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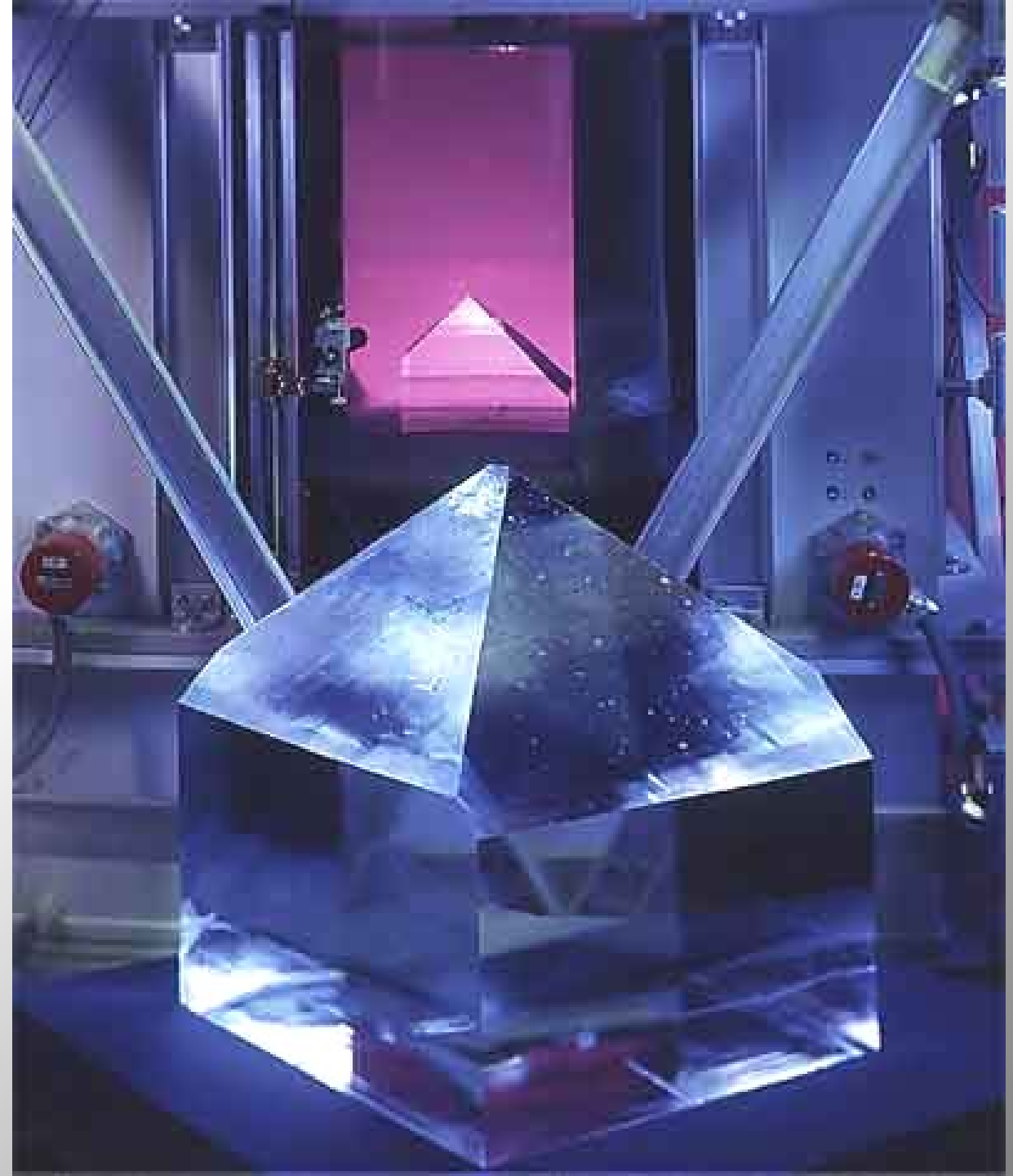
KDP

~ 20 cm crystal
grows in ~ 1 day



"FAST GROWTH Method
Sets Crystal Size Record,"
LASER FOCUS WORLD,
July 1999 Cover
Crystal from LLNL

Ferritin

~ 700 μm crystal
grows in ~ 1 month

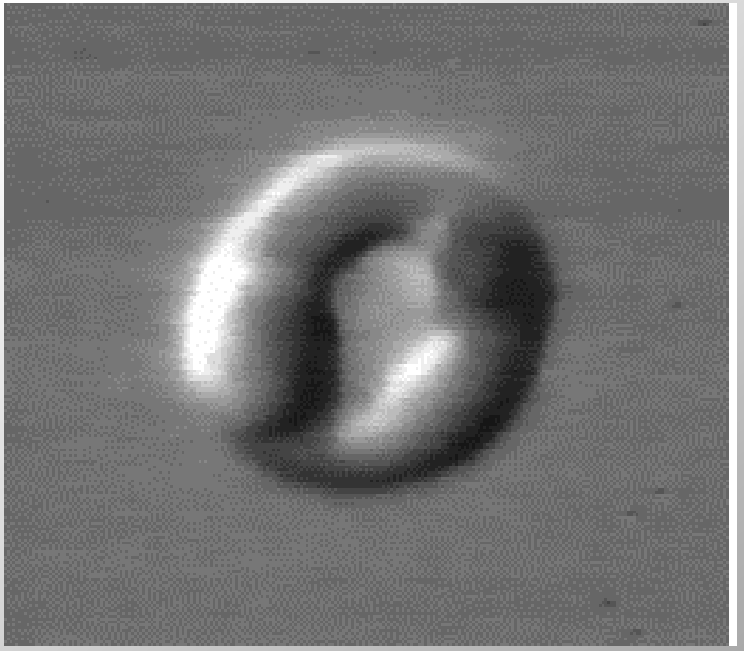
IPC, Sofia

Crystals of Hemoglobin C in Red Blood Cells

Erythrocytes from
HbC Transgenic Mice

- crystallization induced by 4 hour incubation in 3% NaCl, 37°C
- crystal dissolution induced by addition of 0.09 M NaCl solution

  5 s original = 0.1 s as played



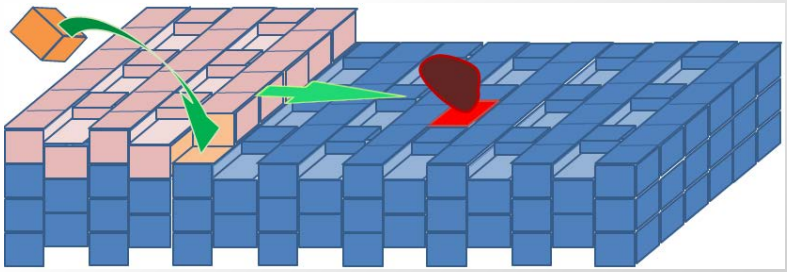
J. E. Canterino, *et al.*,
Biophys. J. ***95, 4025 (2008).***

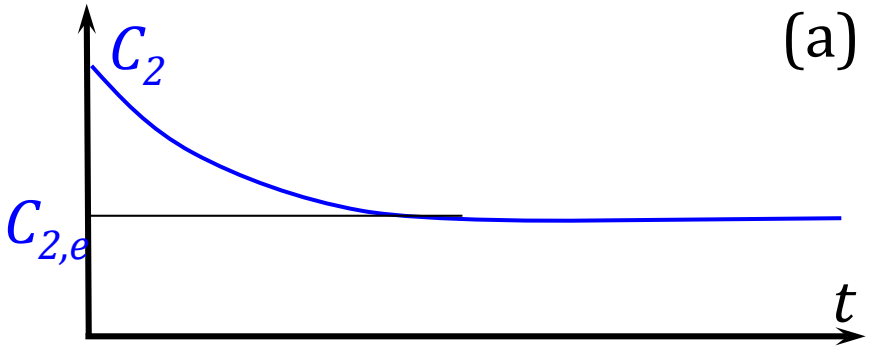
Macroscopic Methods of Solubility Determination

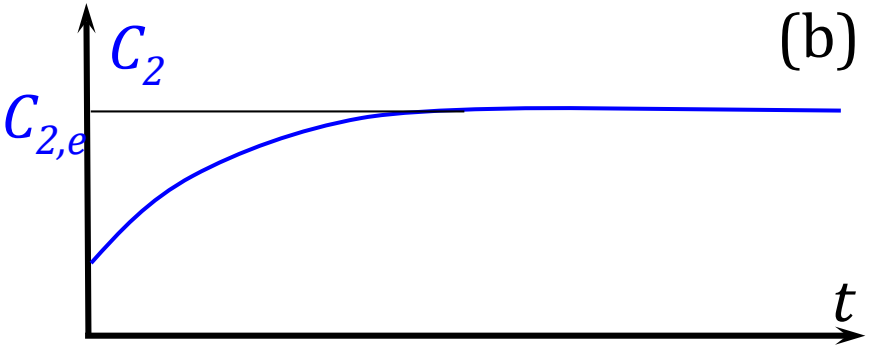
Crystal growth

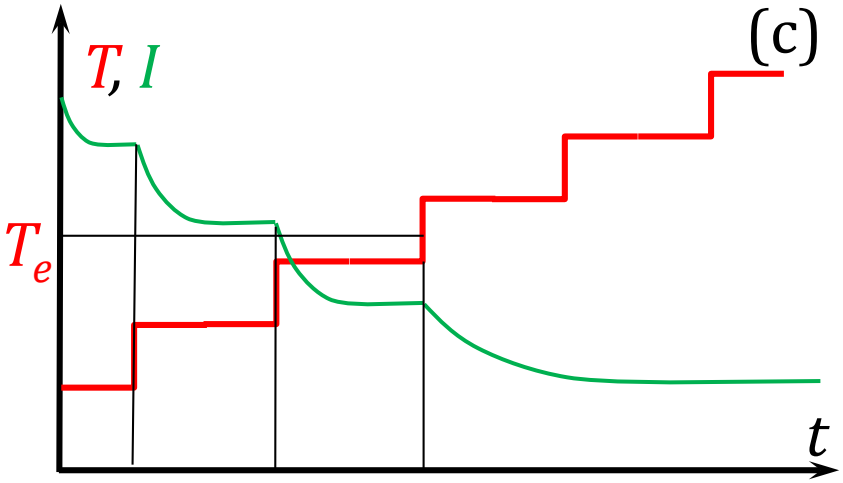
Crystal dissolution

Step wise dissolution

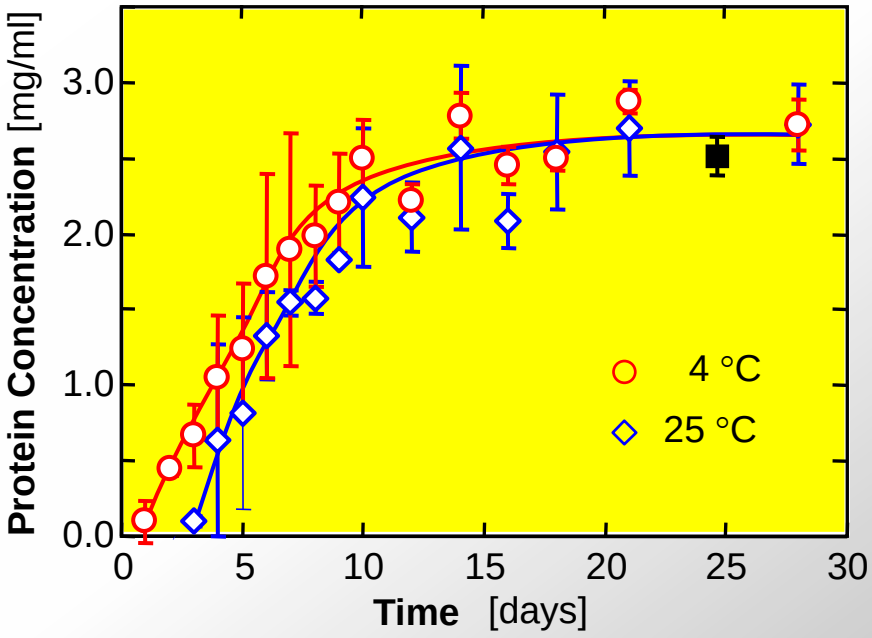


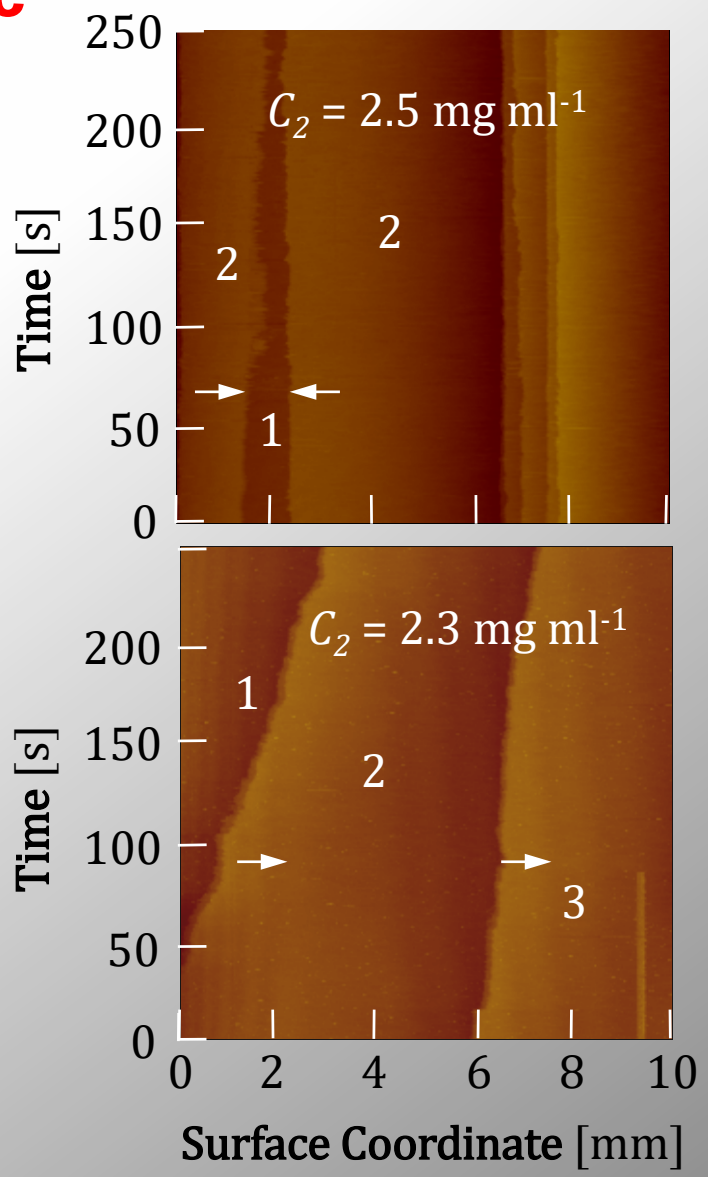




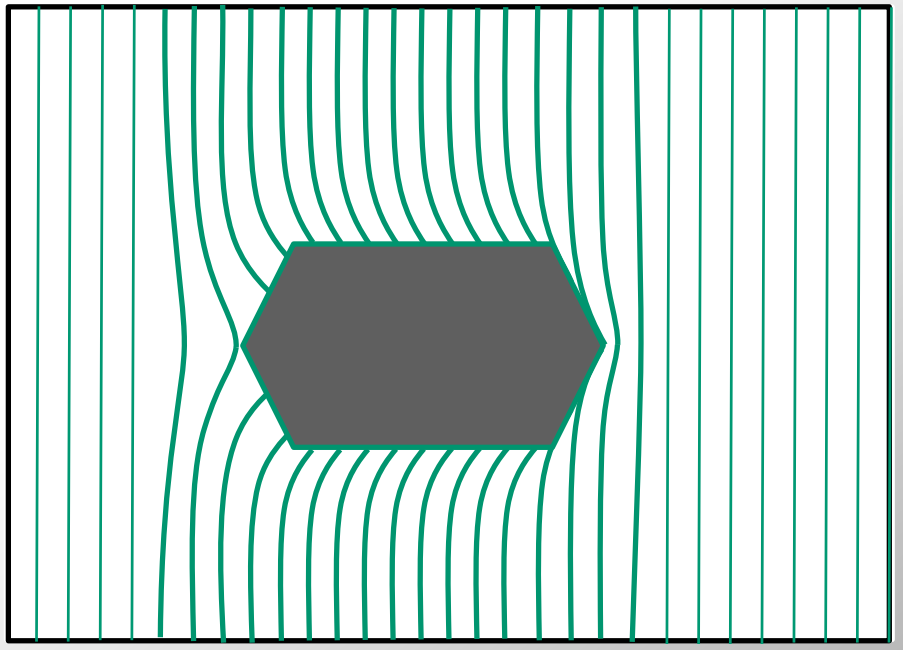


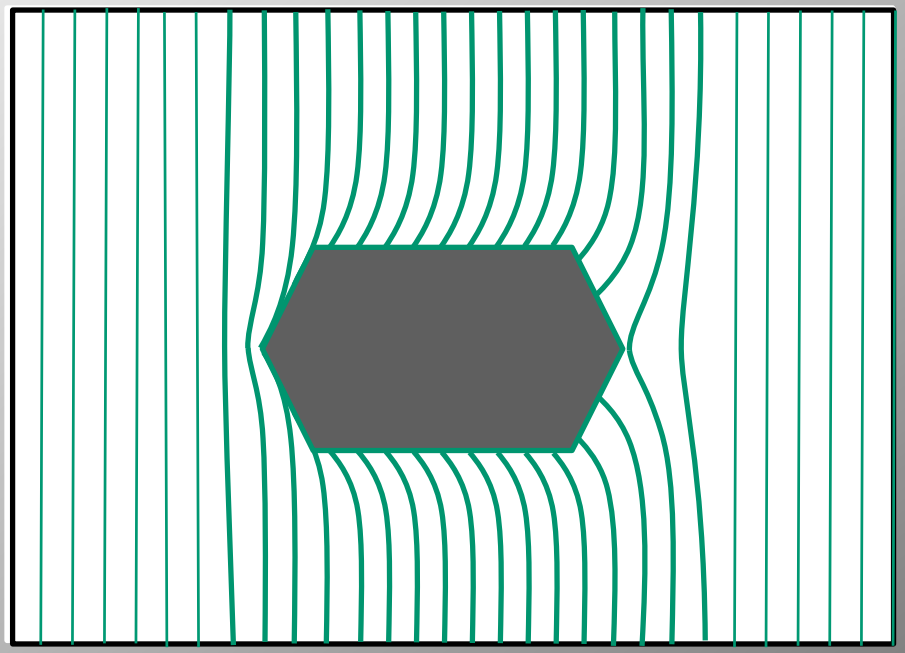
Macroscopic and Microscopic Methods of Solubility Determination



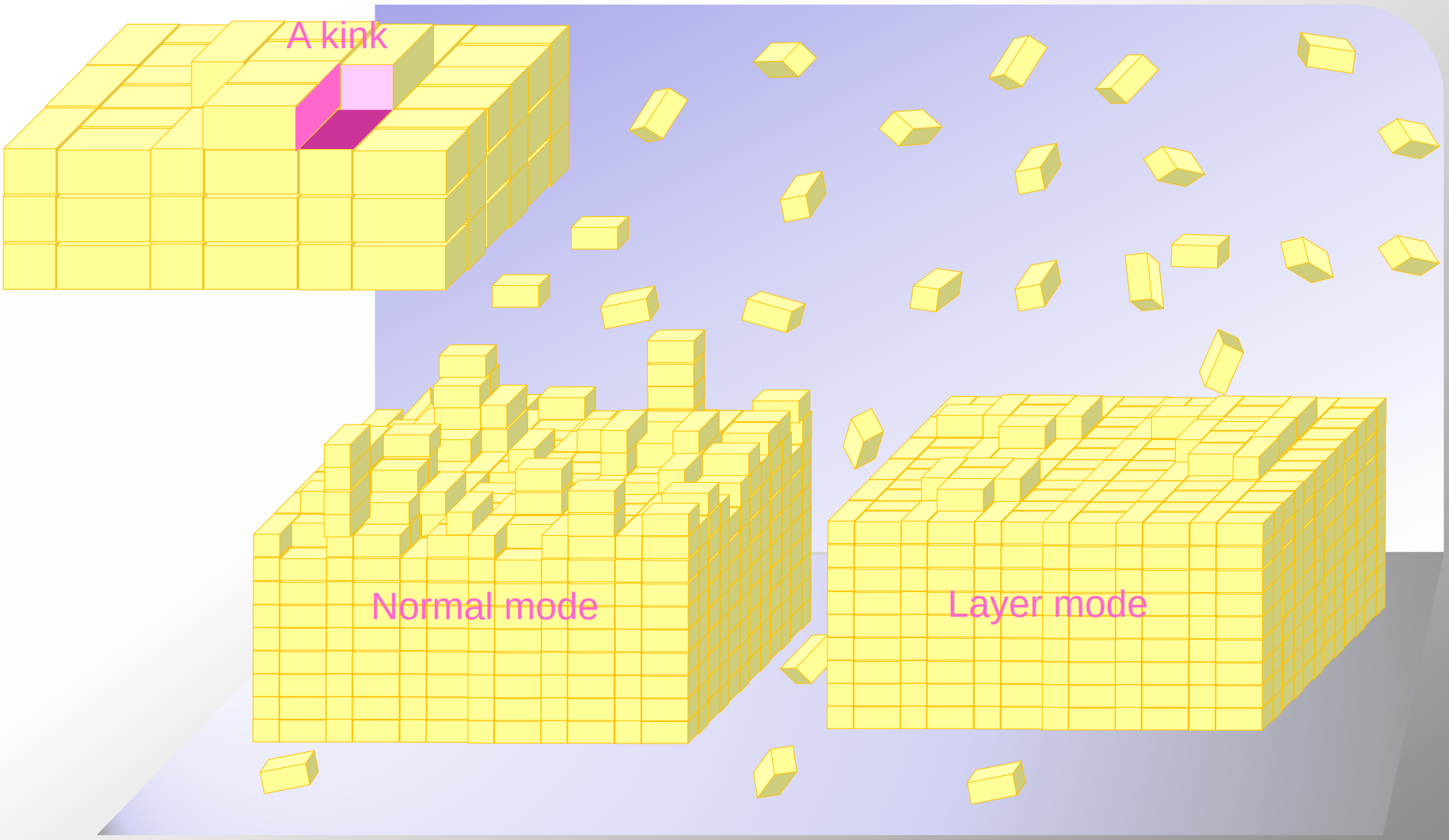


Microscopic Methods of Solubility Determination





Normal and Layer Growth



How Are Normal and Layer Modes Selected?

The free energy of a crystal surface Δg

$$\frac{\Delta g}{k_B T} = \alpha\theta(1-\theta) + \theta \ln \theta + (1-\theta)\ln(1-\theta)$$

$0 \leq \theta \leq 1$ surface coverage

$\theta = 0$ no molecules on surface

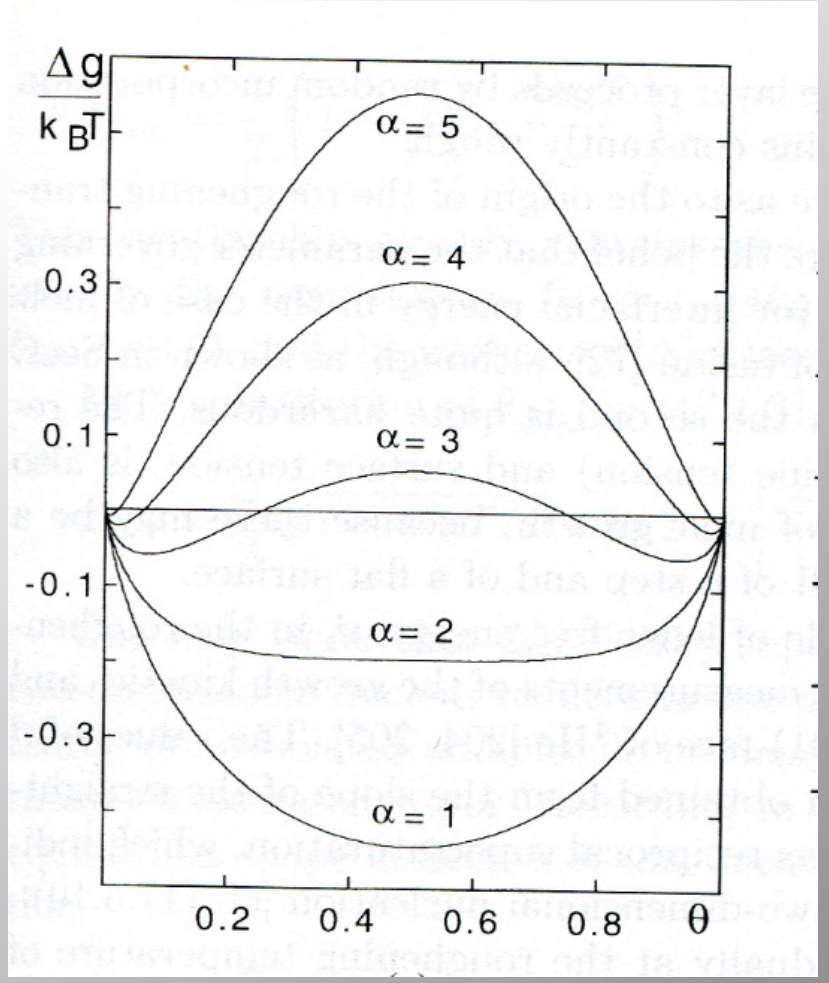
$\theta = 1$ full coverage

$$\alpha = \frac{\omega}{2k_B T} \qquad \gamma = \frac{\omega}{2a}$$

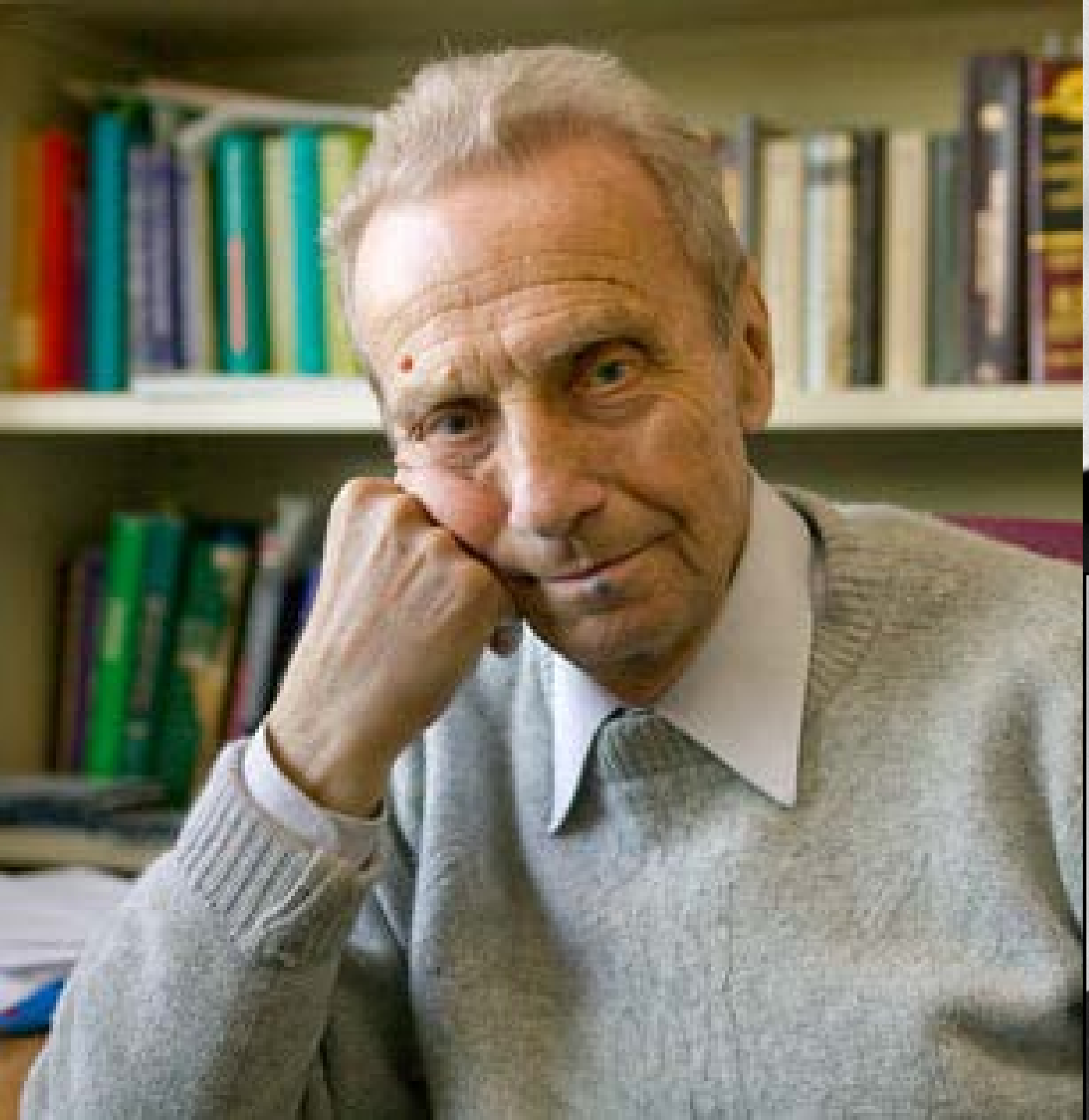
ω bond energy

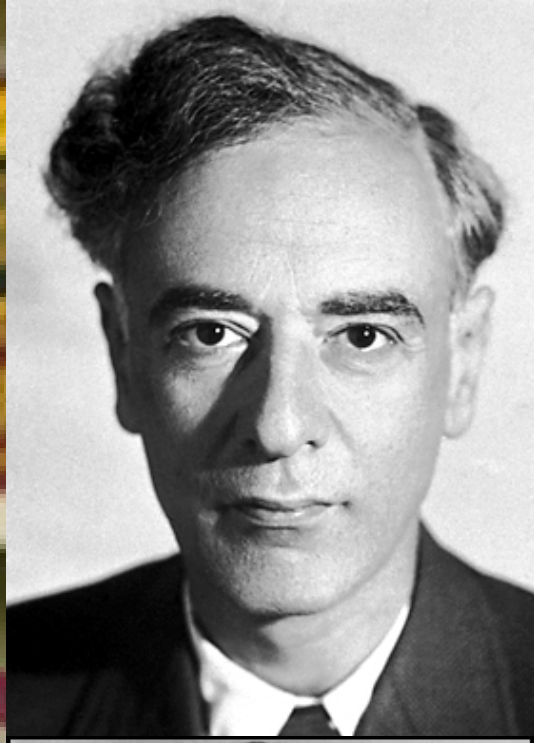
α proportional to surface free energy γ

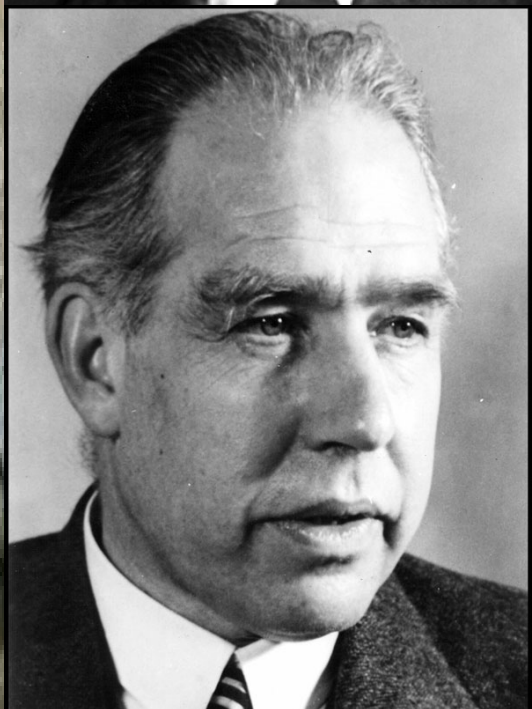
Solution grown crystal grow by
the generation and spreading of layers



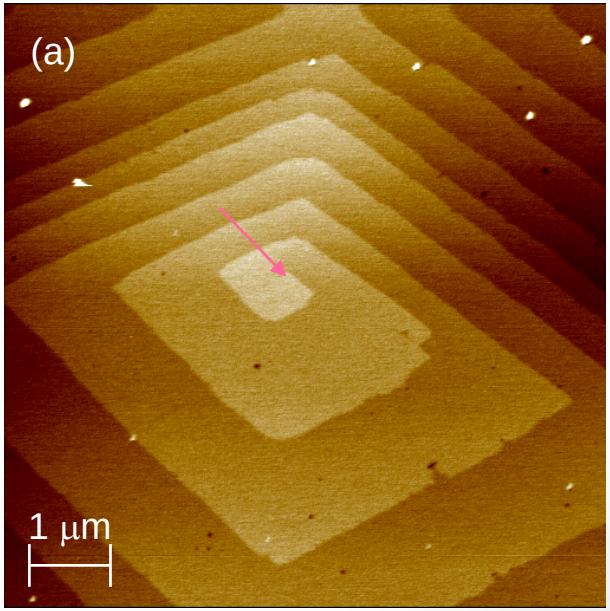
Jackson, K. A.
In *Growth and Perfection of Crystals*;
Doremus, R. H., Roberts, B. W., Turnbull, D., Eds.;
Chapman and Hill: London, 1958, p 319.

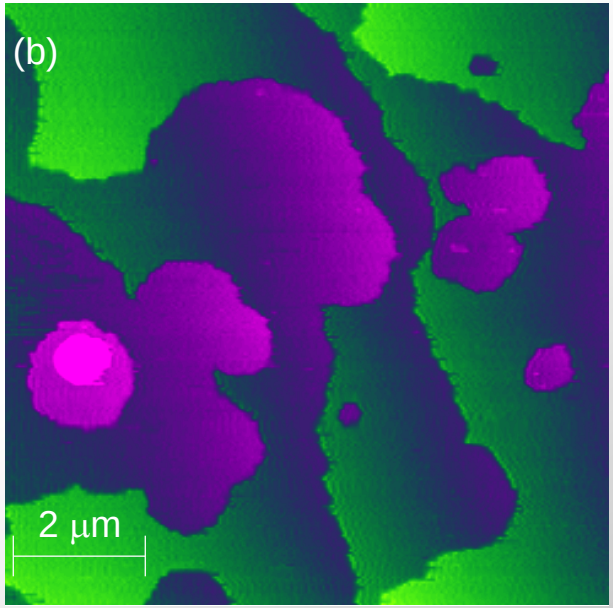


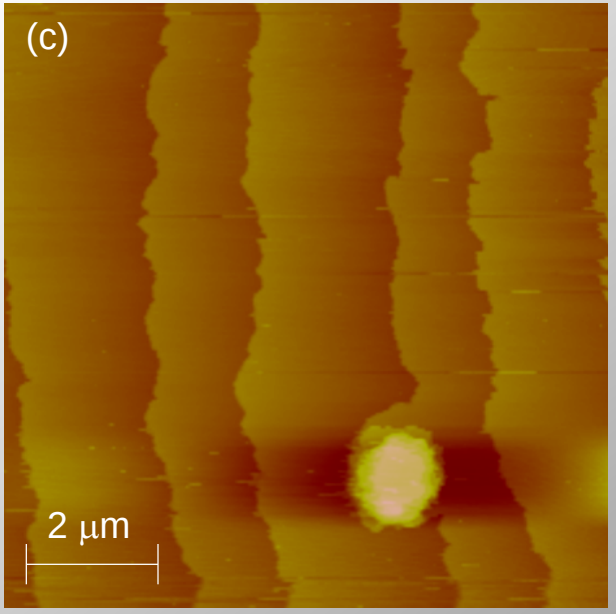




How Are Layers Generated?







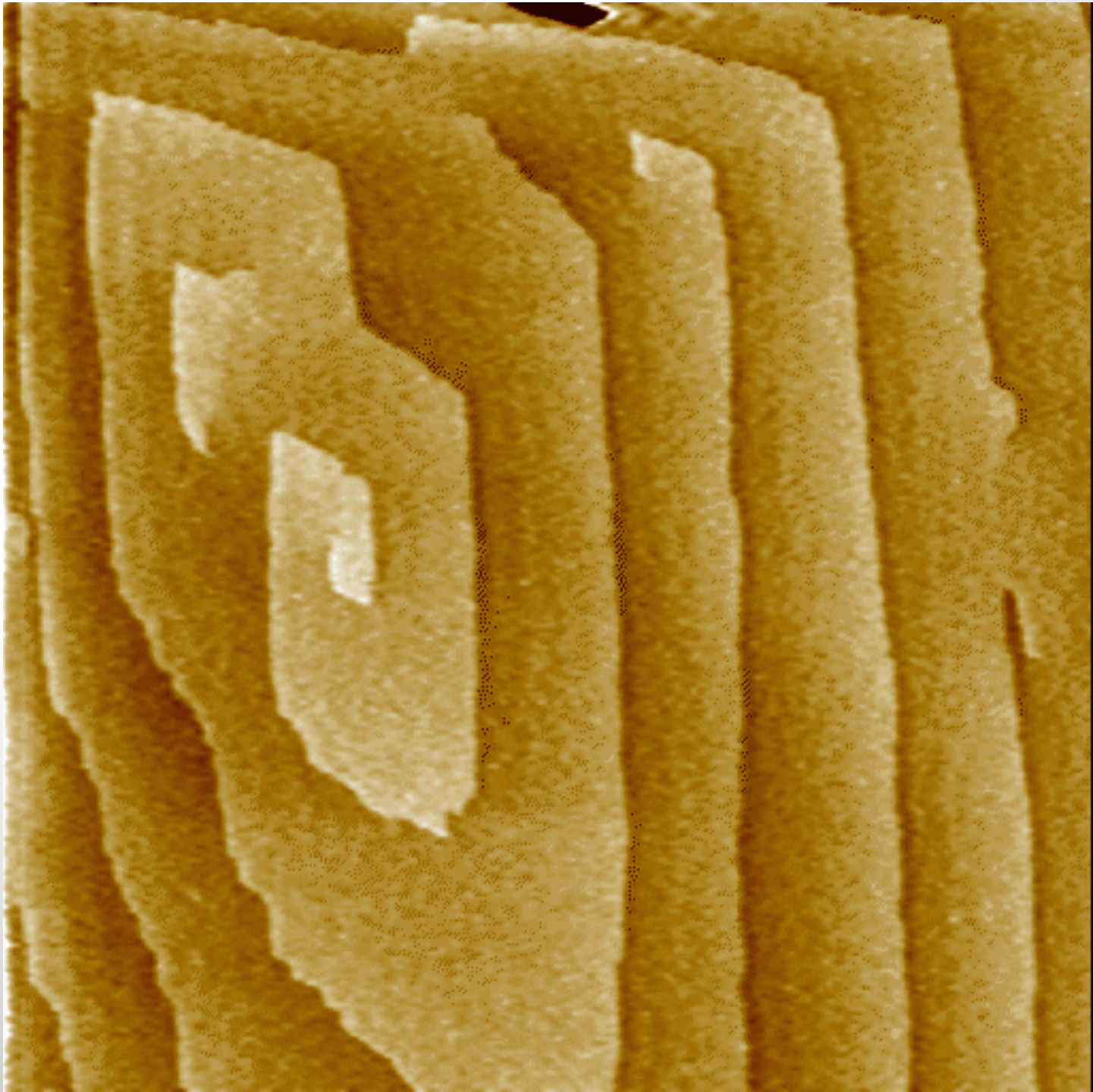
On dislocations

By 2D nucleation

By the landing of dense liquid clusters

JACS **127**, 3433 (2005)
Biophys. J. **92**, 267 (2007)
JPC **111**, 3106 (2007)

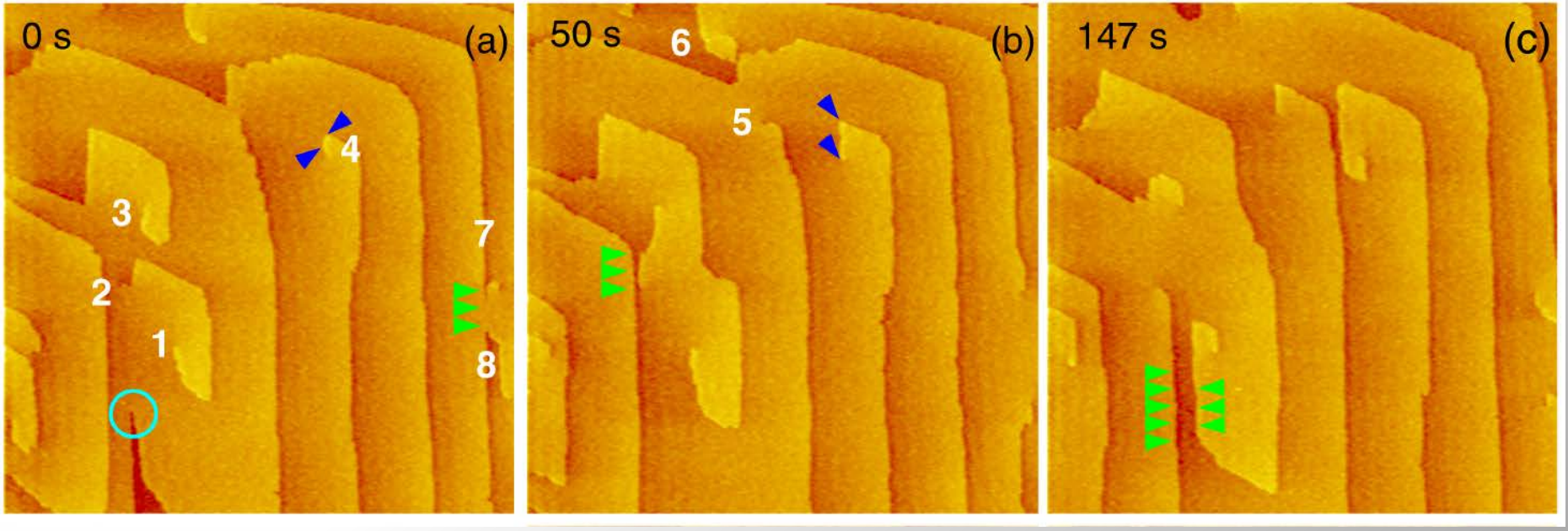
Growth of Insulin Crystals



94 frames
Size: 9.5 x 9.5 μm^2
50 s per frame
Real time: 95 min

I. Reviakine, *et al.*,
J. Am. Chem. Soc.
125, 11684 (2003)
O. Gliko, *et al.*,
Phys. Rev. Lett
90, 225503 (2003)

How Is the Step Density Determined?



$L_c \approx 320$ nm

$$L_c = \frac{2\gamma\Omega}{\Delta\mu}$$

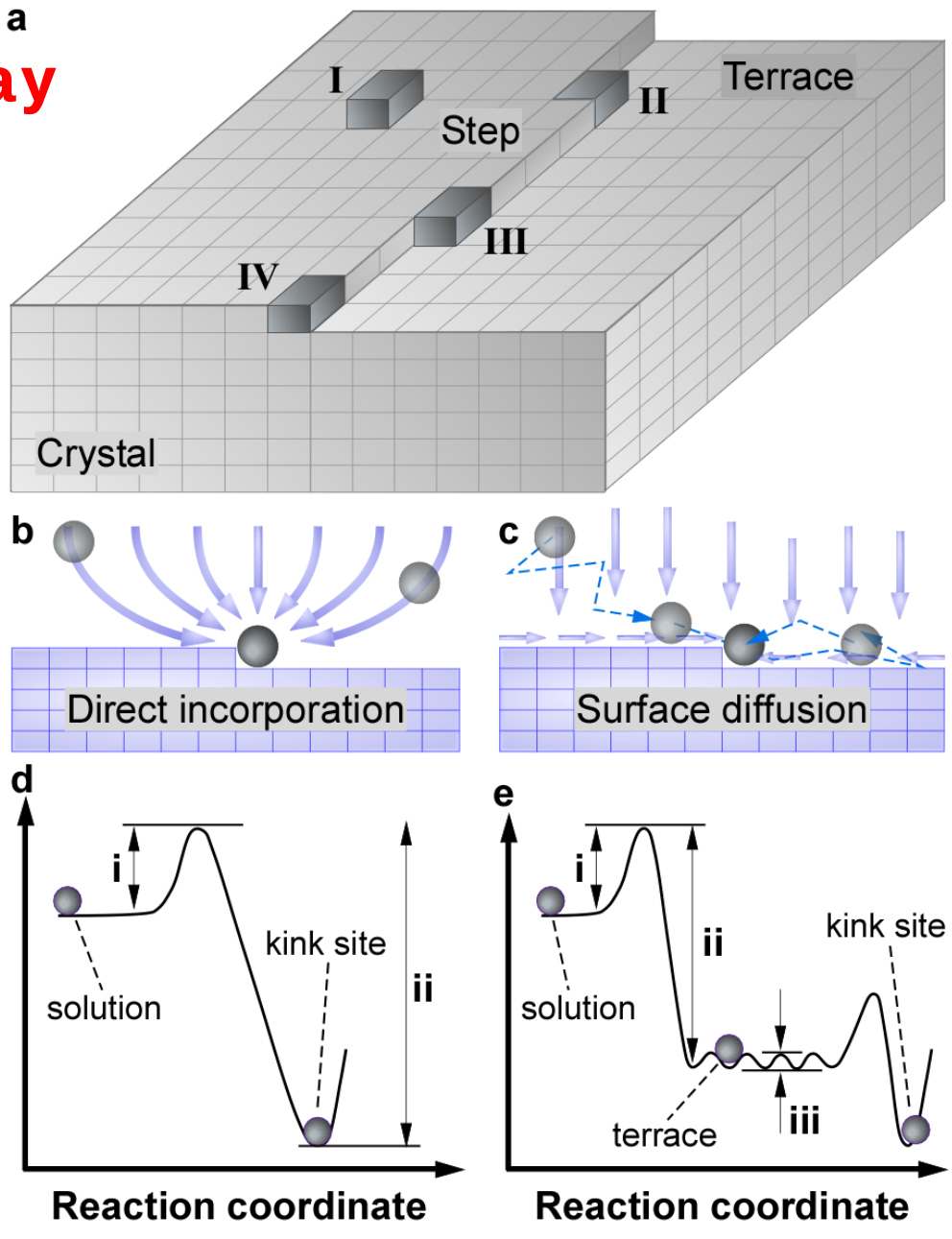
$\gamma \approx 14$ mJ/m^2

Reviakine, I., *et al.*, *J. Am. Chem. Soc.* **125**, 11684-11693, (2003)

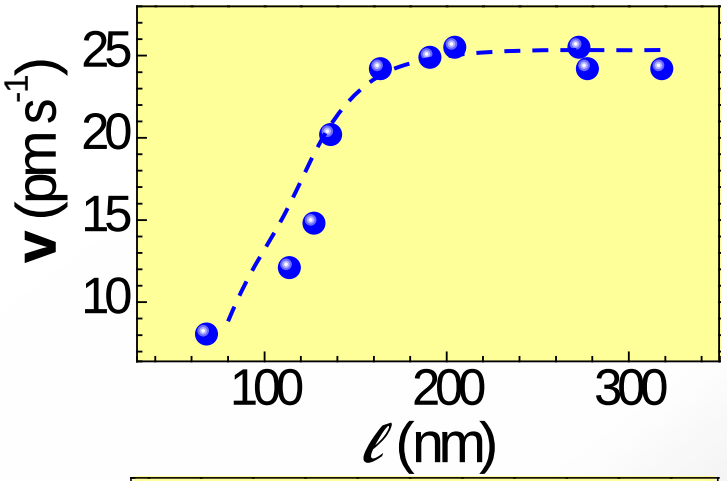
$l = 9.5\, L_c$ $\qquad$ $p = \frac{h}{l}$

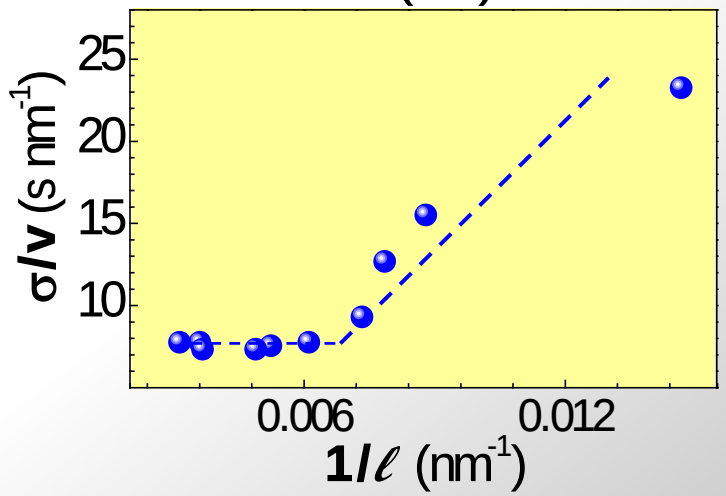
The Molecular Pathway to a Kink

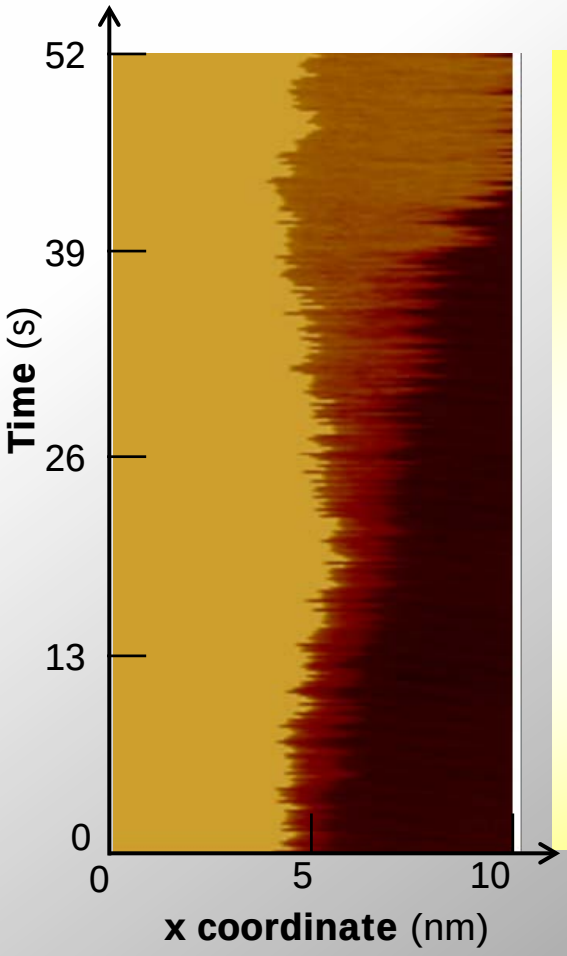
- The SD mechanism provides additional handles for control of step growth
- Can be detected from the strong competition for supply between the steps



The Molecular Pathway to a Kink





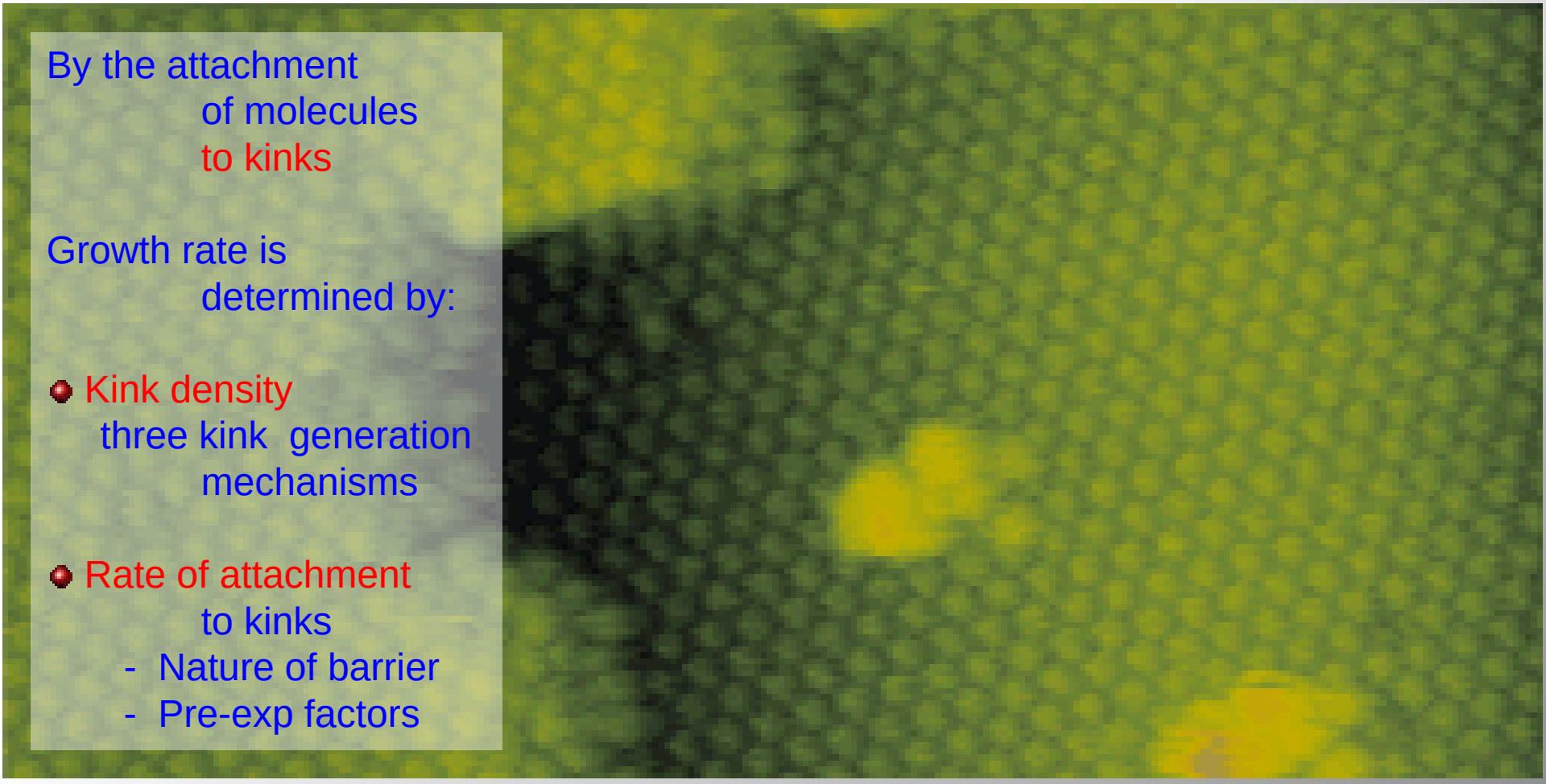


- Strong step slow down at ℓ < 150 nm indicates surface diffusion pathway
- Fast growth of underlying step indicates faster incorporation from lower terrace: Ehrlich-Schwoebel effect

- Controlling the structure of the solvent layer over crystal terraces is a potentially powerful way of crystal growth control

How Do Layers Spread?

Apoferritin



By the attachment
of molecules
to kinks

Growth rate is
determined by:

- Kink density
  three kink generation
  mechanisms

- Rate of attachment
  to kinks
  - Nature of barrier
  - Pre-exp factors

Kink Generation by Thermal Fluctuations

- Thermal fluctuations

"... several of the outermost layers of molecules on each side of the crystal are incomplete towards the edges. The boundaries of these imperfect layers probably fluctuate as molecules join them and depart from them." p.325

Gibbs, J. W. On the equilibrium of heterogeneous substances
Trans. Connect. Acad. Sci. **3**, 108-248 (1876)

- Equilibrium kink density—preserved during growth

Burton, W.K., Cabrera, N. &. Frank, F.C.
The growth of crystals and equilibrium structure of their surfaces.
Phil. Trans. Roy. Soc. London Ser. A **243,** 299- 360 (1951)

Kink Generation by Thermal Fluctuations

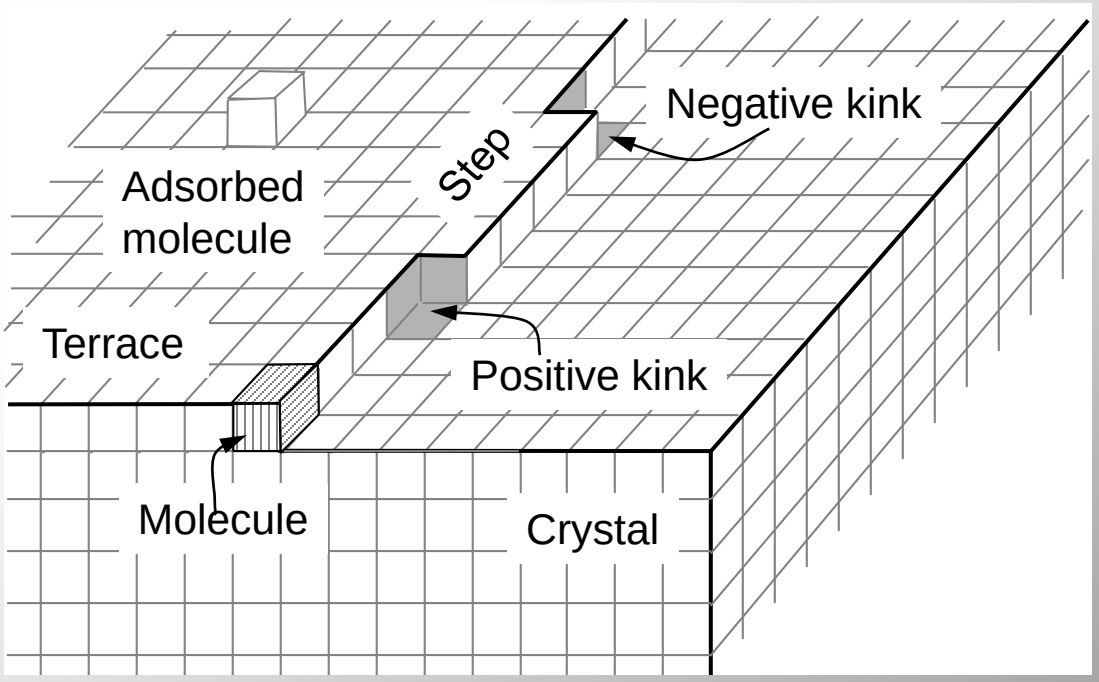
$\overline{n}_k$ number of molecules between kinks

ω free energy of kink

ϕ free energy of bond

$\overline{n}_k = ½ \exp(\omega/k_B T) + 1$

$= ½ \exp(\phi/2k_B T) + 1$



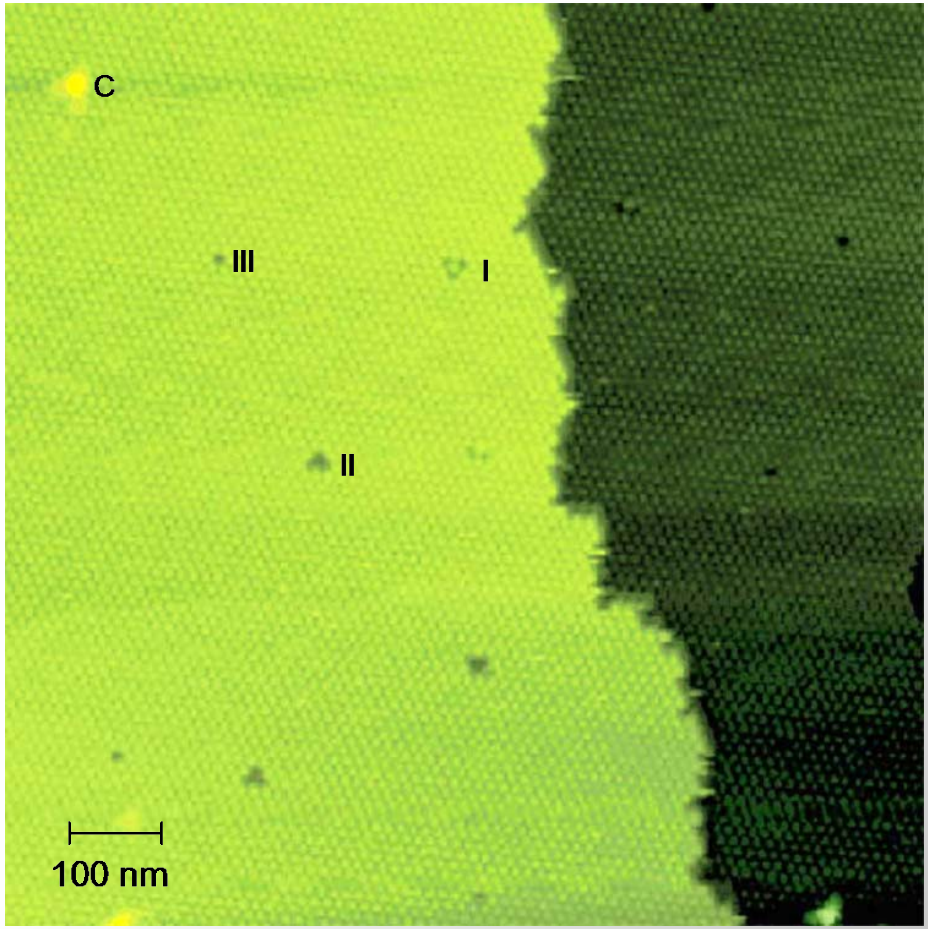
$\phi = 2(\Delta G^o - T\Delta S^o_{solute})/ZN_A$

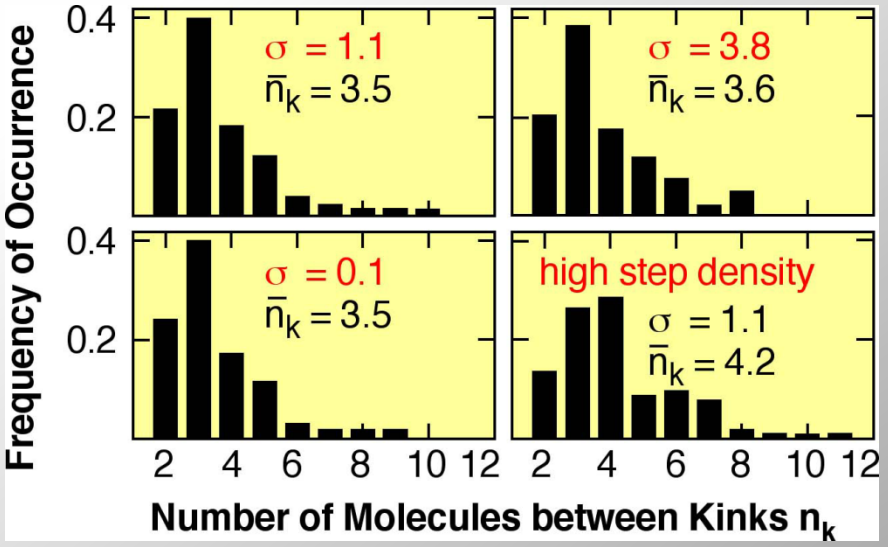
Kink density depends on bond strength

Kink Generation by Thermal Fluctuations

- Tests with the protein apoferritin

S.-T. Yau, *et al.*, *PRL* **85** (2000) 353





$\omega = 1.6\ k_B T$

$\phi = 3.2\ k_B T = 7.8$ kJ/mol

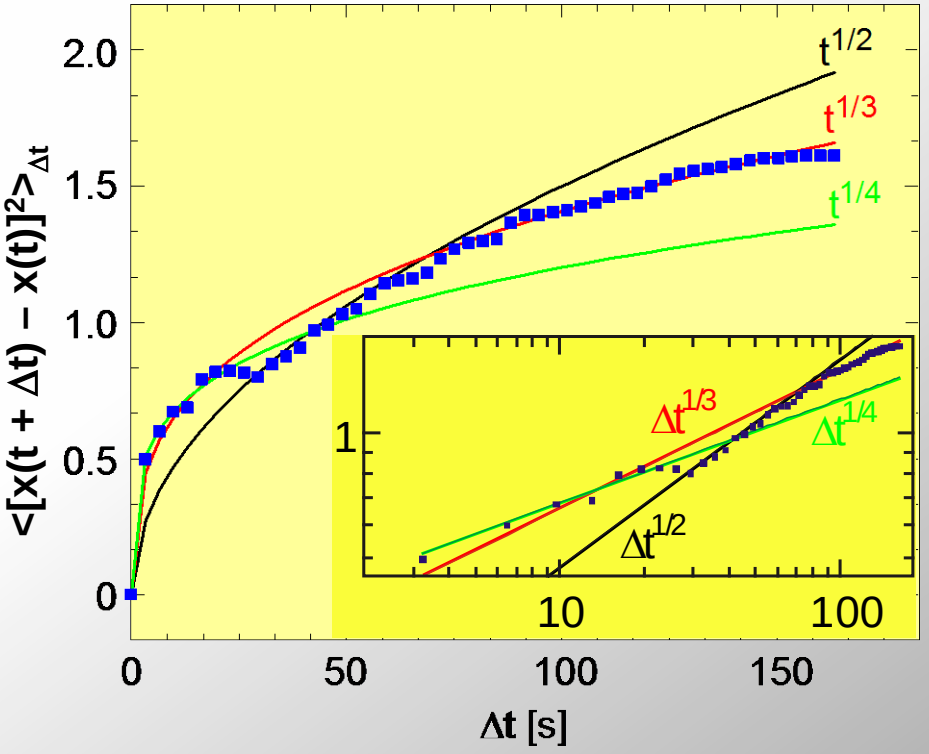
Agrees with macroscopic thermodynamic determinations

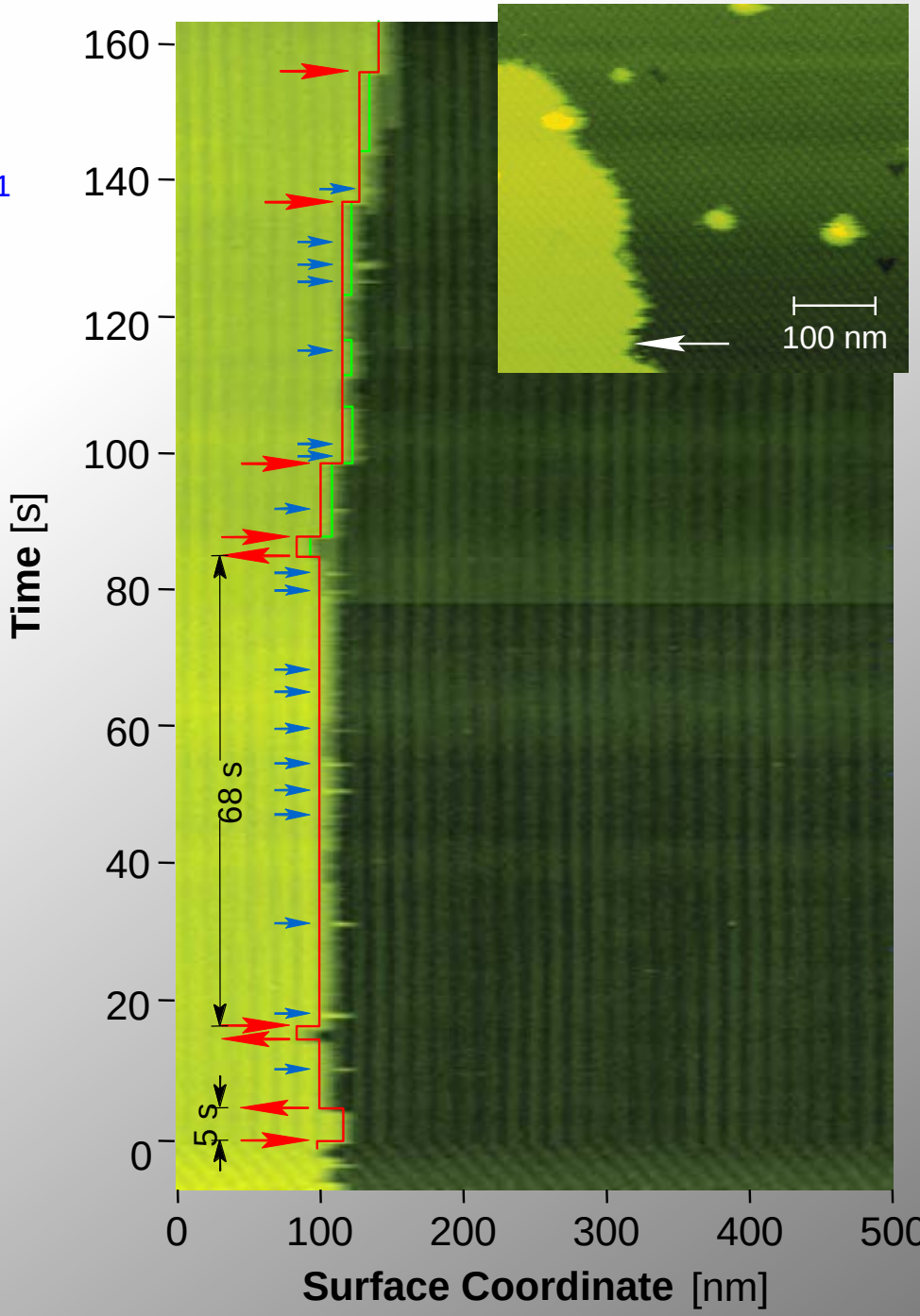
S.-T. Yau, *et al.*, *J. Mol. Biol.*, ***303,*** 667 **(2000)**

Attachment Frequency

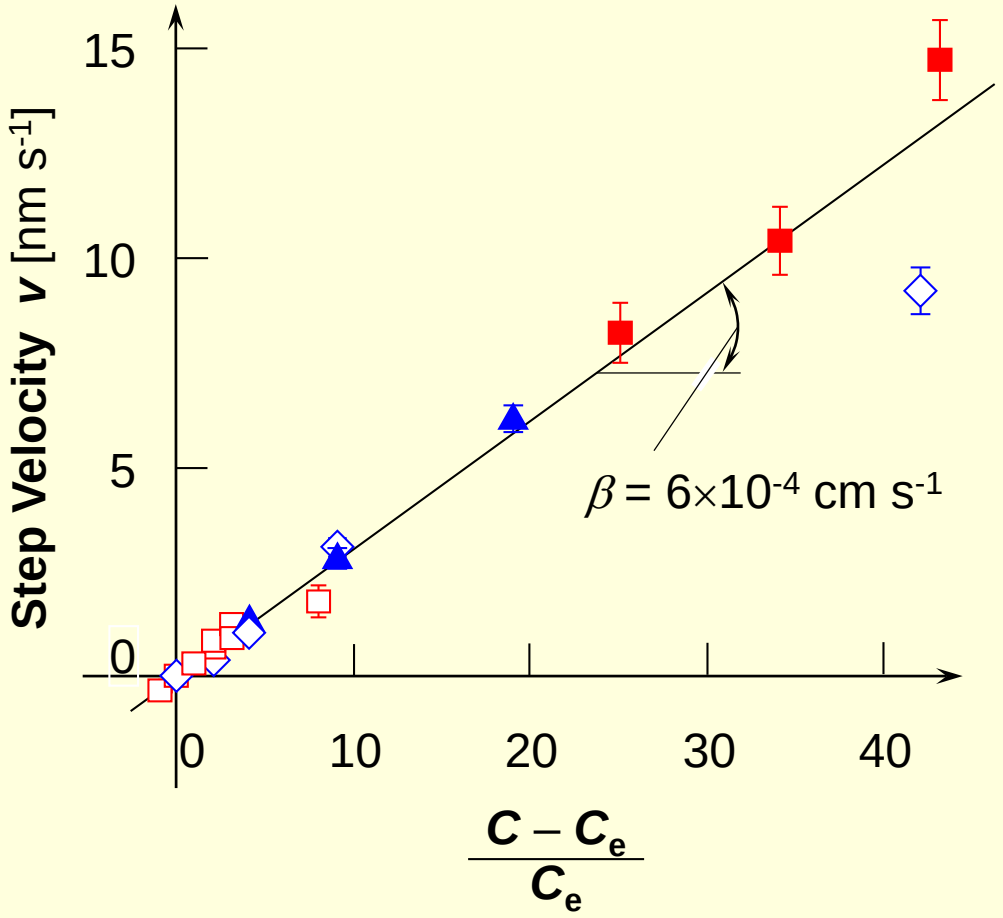
Net flux into kink ($j_+ - j_-$) = 0.065 s^{-1}

Test if
attachment-detachment events
are due to exchange with medium
rather than
to rearrangement of step





The Step Velocity



□	Ferritin	molecular level AFM
◇	Apoferritin	molecular level AFM
▲	Apoferritin	mesoscale AFM
■	Ferritin	interferometry

Petsev, D.N., *et al.*, *Proc. Natl. Acad. Sci. USA,* ***100, 792*** (2003)

Does Kink Density Scale Step Velocity?

$$v = (1/n_k) \cdot a \cdot (j_+ - j_-)$$

Ferritin at $(C - C_e)\, C_e^{-1} = 1$		Apoferritin at $(C - C_e)\, C_e^{-1} = 2$
$1/n_k = 1/3.5 = 0.28$	kink density	$1/n_k = 1/3.5 = 0.28$
$a = 13$ nm	molecular dimension	$a = 13$ nm
$(j_+ - j_-) = 0.054\ s^{-1}$	attachment frequency	$(j_+ - j_-) = 0.065\ s^{-1}$
$= 0.20$ nm/s	$(1/n_k) \cdot a \cdot (j_+ - j_-)$	$= 0.24$ nm/s
$v \approx 0.20$ nm/s		$v \approx 0.26$ nm/s

- Kinks generated by thermal fluctuations determine step velocity

Eyring, Kramers, or Smoluchowski Kinetics of

Eyr

Kra

Sm

-1/2

-1

Interaction potential

Separation

Diffusivity Does Not Depend on the Molecular Mass

Motion of molecules in solution follows *Langevin equation*

$$m\frac{dv}{dt} = -\zeta v + f(t)$$

ζ – effective friction coefficient, e.g, $6\pi\eta a$

$f(t)$ – random action of solvent molecules $\langle f(t)\rangle = 0$

Regrouping, averaging over t and solving for the mean squared displacement $\langle x^2\rangle$

$$\left\langle x^2\right\rangle = \frac{2k_BT}{\zeta}\left\{t - \frac{m}{\zeta}\left[1 - \exp\left(-\frac{\zeta t}{m}\right)\right]\right\}$$

Berry, P. S., Rice, S. A. & Ross, J. (2000) *Physical Chemistry*, Oxford, New York

For $t << m/\zeta$, $\langle x^2\rangle = (k_BT/m)t^2$, i.e., the molecule has a speed of $(k_BT/m)^{1/2}$

Mass dependent rate of diffusion

Leads to a mass dependent kinetic coefficient β

$\beta \propto m^{-1/2}$ only for events with characteristic times $t << m/\zeta$

For ferritin m =1.3×10^{-18} g, with h_{water} = 1 cP, $m/\zeta = 7\times10^{-13}$ s

We get $$\left\langle x^2\right\rangle = \frac{2k_BT}{\zeta}t = 2Dt \qquad D = \frac{k_BT}{\zeta} = \frac{k_BT}{6\pi\eta a}$$

Einstein law of Brownian diffusion
diffusivity independent of mass

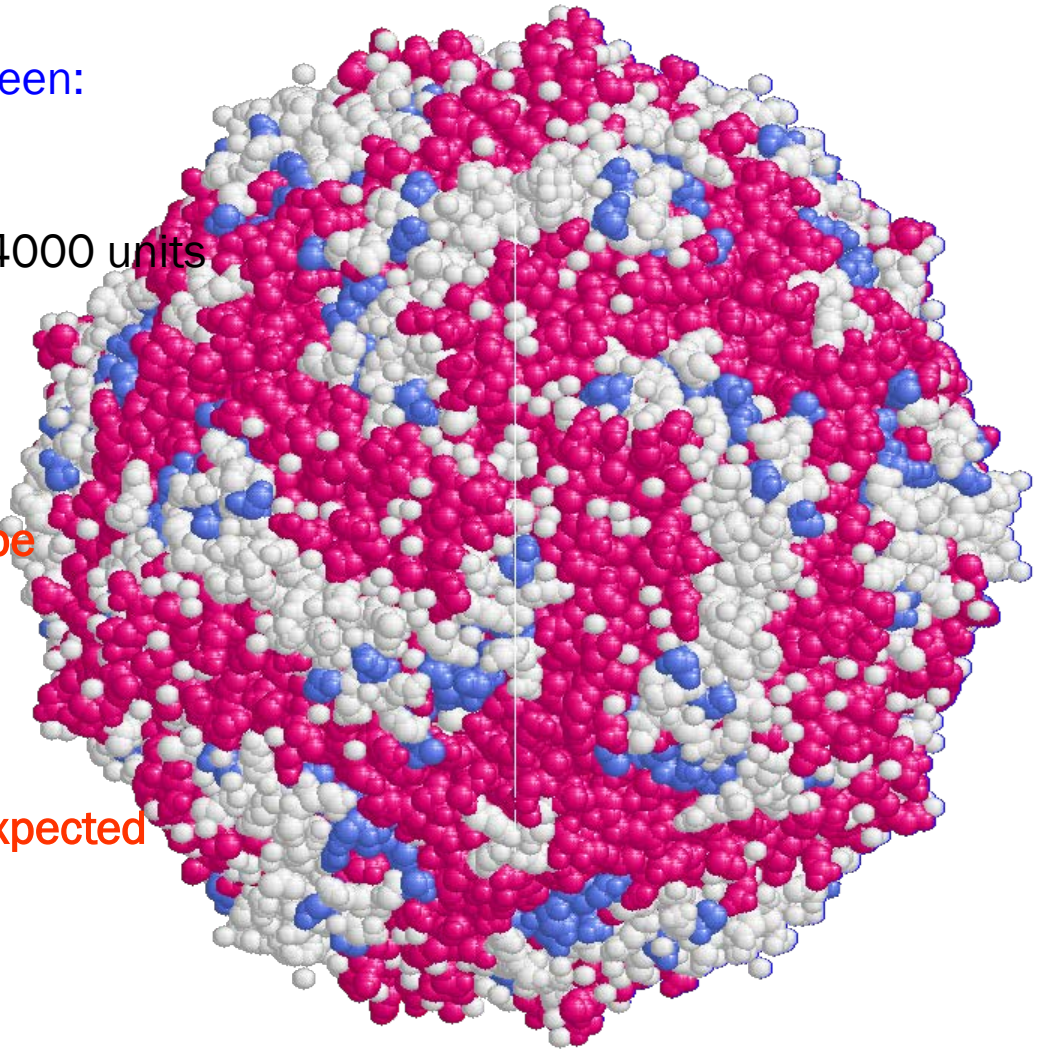
Why is this important:

Fundamental insight Control of instabilities Nanoassembly ...

Unique Pair of Model Proteins

Ferritin and Apoferritin from Horse Spleen:

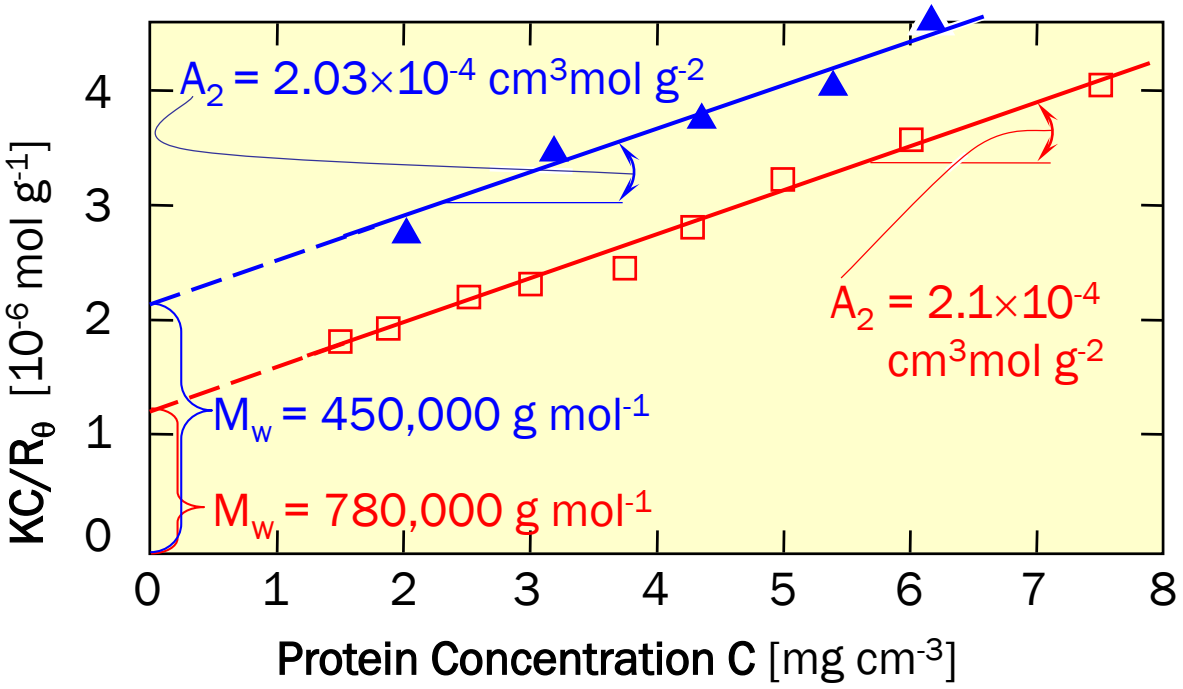
- Ferritin: iron storage protein
- core: FeOOH crystallite(s), 1000-4000 units
- Size of both proteins: 13 nm
- M_w = 450,000 for apoferritin
- M_w of ferritin varies
  650,000 – 900,000
- 24 subunits, quasi-spherical shape
- pI at pH = 5.5
- bonds in crystal—via
  two Cd^{2+} per contact
  - strong, chemical type bond
  - transition state limitations expected



The Molecular Mass of Ferritin

static light scattering with ferritin and apoferritin

$$\frac{KC}{\Delta R_\theta} = \frac{1}{M} + 2A_2C$$

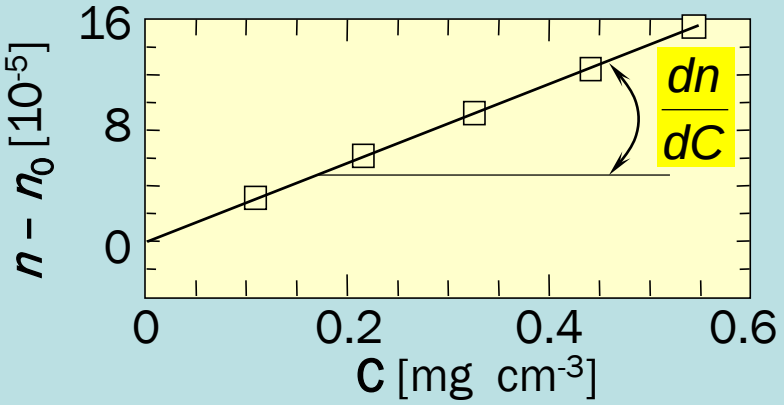


M agrees with biochemically determined

A_2 = A_2 – molecules identical shells

$$K = \frac{1}{N_A}\left(\frac{2\pi n_0}{\lambda^2}\right)^2\left(\frac{dn}{dC}\right)^2$$

n_0 – refractive index
dn/dC – *n*-increment
λ - wavelength



n_0 = 1.3320

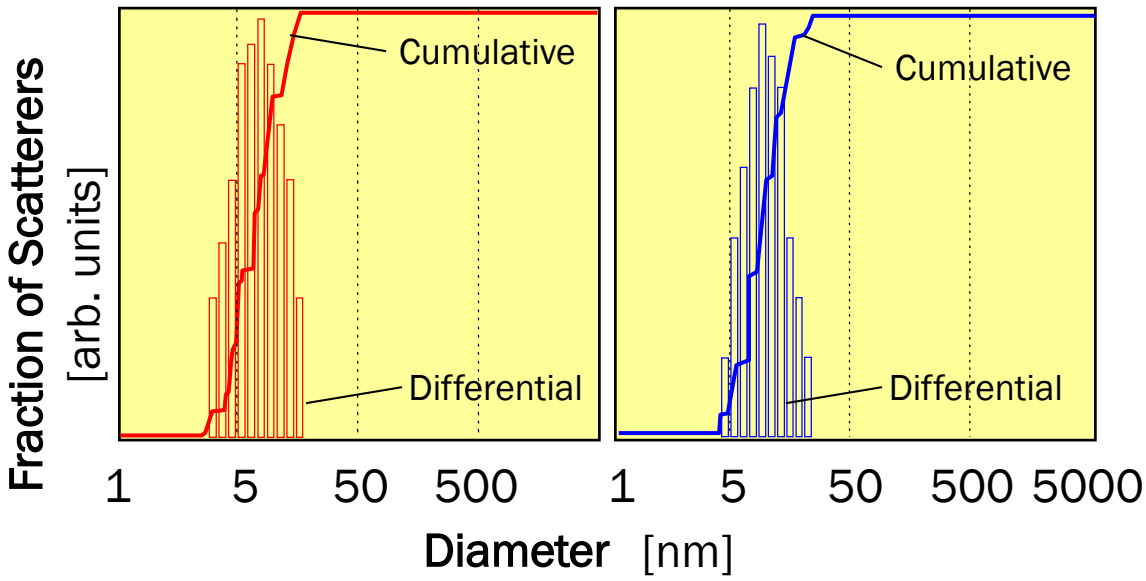
dn/dC = 0.290 cm^3g^{-1}

dn/dC = 0.159 cm^3g^{-1}

The Shell of Ferritin and Apoferritin

Dynamic light scattering characterization of chromatographically purified samples

Petsev, D. N., *et al.* (2000) *Biophysical J.* **78**, 2060



Diffusivity $D = D = 3.2\times10^{-7}$ cm^{2}s^{-1}

Stokes law

With $\eta = 0.0095$ g cm^{-1}s^{-1}

$$D = \frac{k_B T}{3\pi\eta a}$$

Fredericks, W. J et al., *J. Crystal Growth* **141**, 183

Particle diameter $a = a = 13$ nm

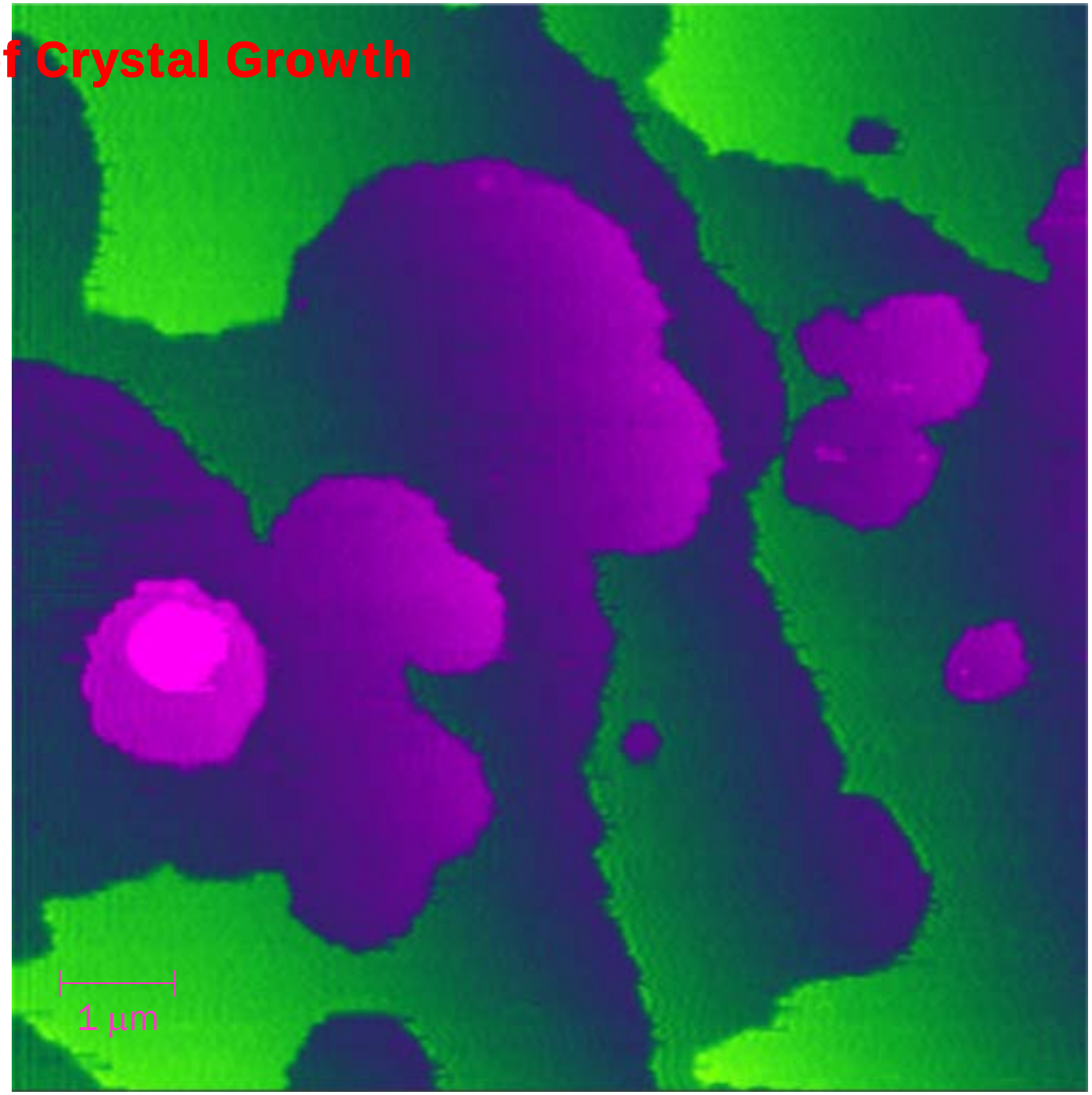
- Agrees with crystalographically determined
- Agrees with AFM results

Stokes diffusion of both proteins

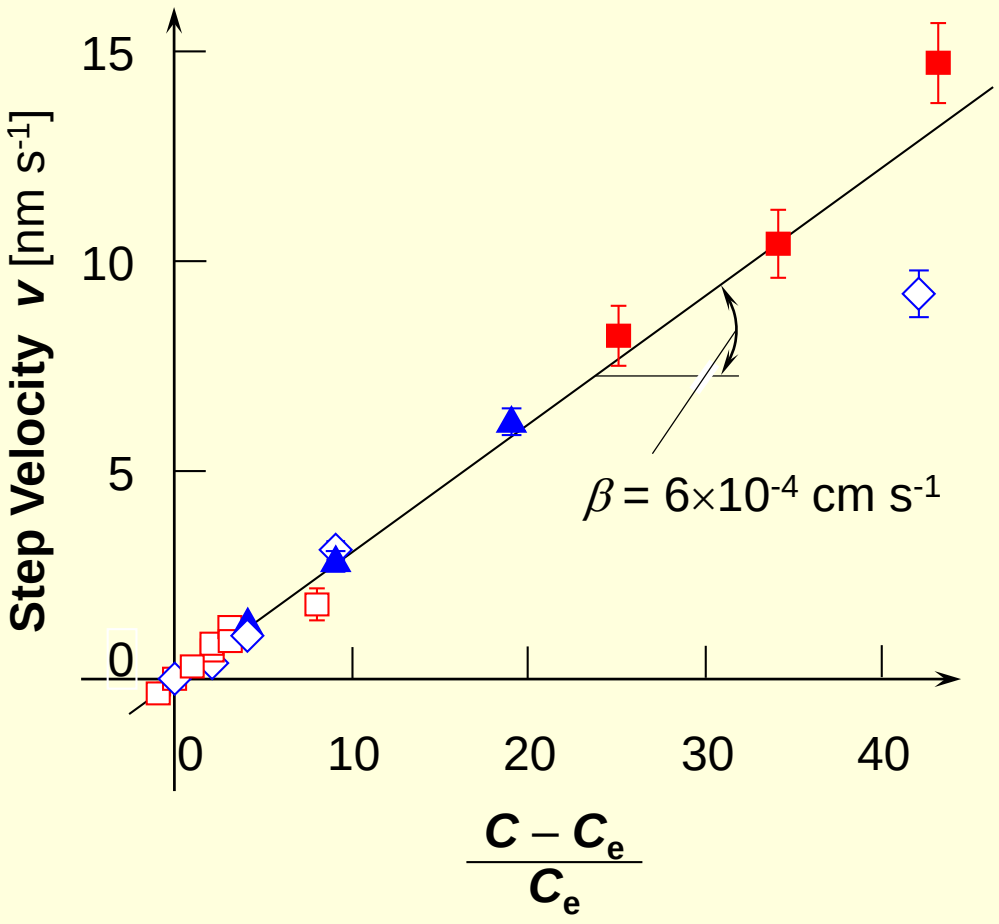
Mechanism of Crystal Growth

Spreading of layers generated by 2D nucleation

Molecules – incorporated into the crystal at kinks along steps



The Step Velocity: Ferritin and Apoferritin



Ferritin: M_w = 780,000 g mol^{-1}

Apoferritin: M_w = 450,000 g mol^{-1}

Kinetic coefficient of the steps for ferritin is equal to that of apoferritin

$\beta = 6\times10^{-4}$ cm s^{-1}

Mass-independent step velocity indicates Kramers-type (diffusion-limited) kinetics of attachment

$$v = a\bar{n}_k^{-1}(j_+ - j_-)$$

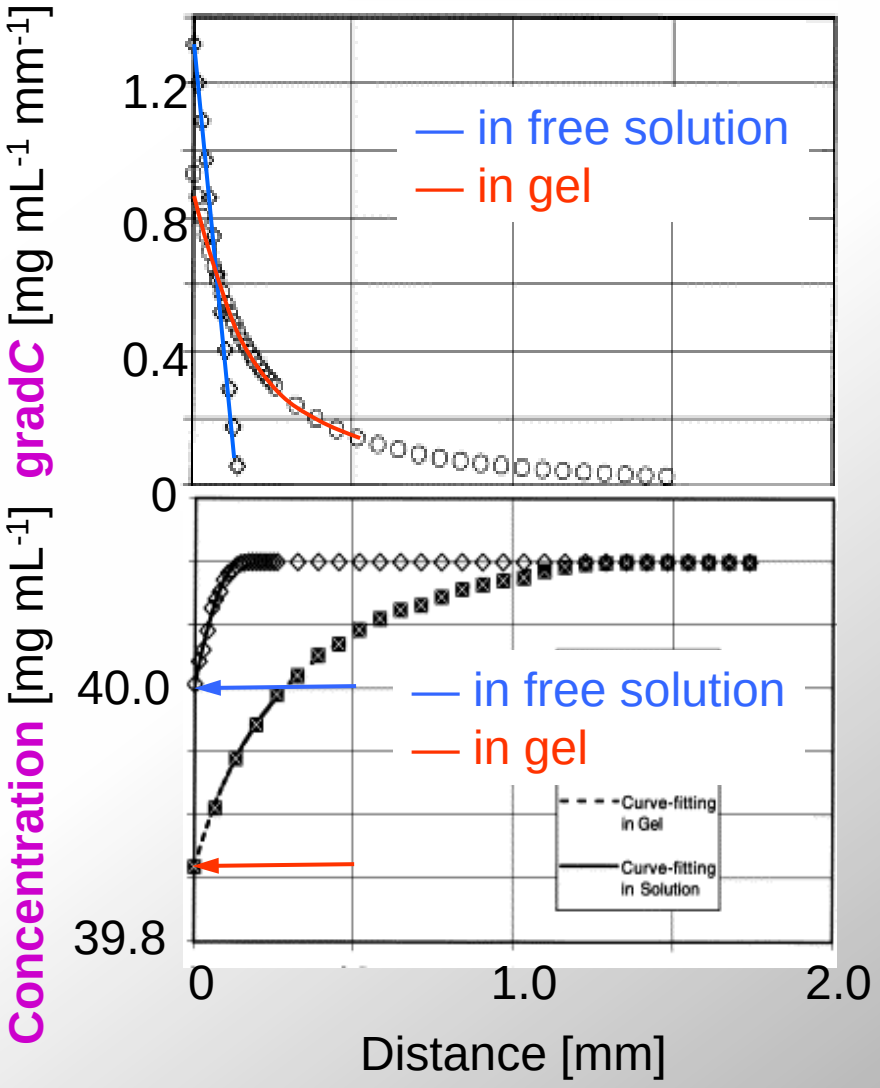
$$= \frac{a^3}{\bar{n}_k}\frac{D}{\Lambda}\exp\left(-\frac{U_{max}}{k_BT}\right)(n - n_e)$$

$$\beta = \frac{1}{\bar{n}_k}\frac{D}{\Lambda}\exp\left(-\frac{U_{max}}{k_BT}\right)$$

Petsev, D.N., *et al.*, *Proc. Natl. Acad. Sci. USA*, ***100, 792*** (2003)

Dependence of Kinetic Coefficient on Diffusivity

Concentration profiles at interface in gel in in free solution



Interfacial gradient — lower in gel

Interfacial concentration—unchanged

$\Omega D \, \mathrm{grad}C = R = \beta \cdot \mathrm{const} \cdot (C - C_e)$

- In gels:
  lower gradC → lower R → lower β (with preserved C)

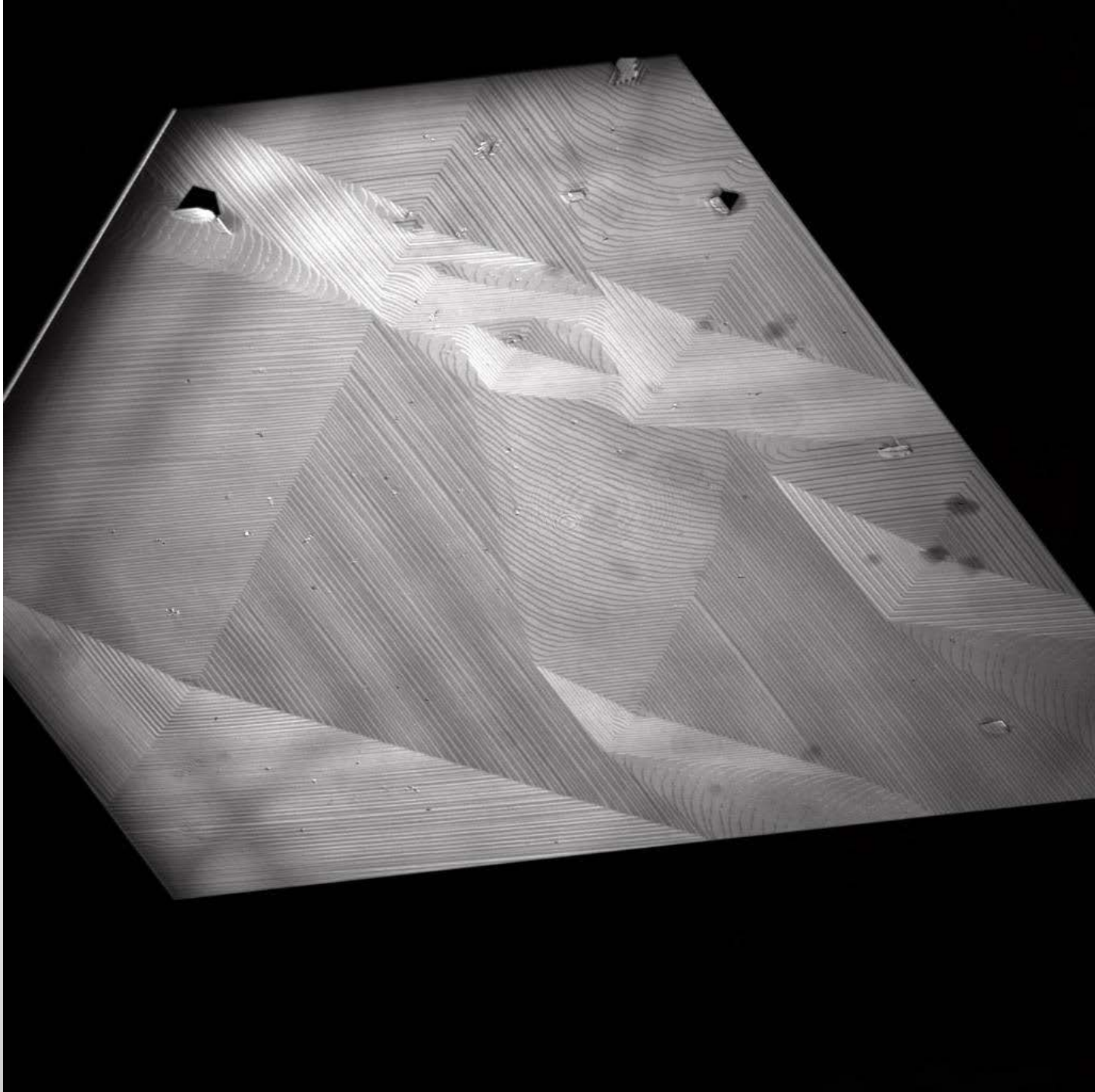
Further evidence:
growth of lysozyme and thaumatin initial interfacial gradient lower in gels than in free solutions

J.M. Garcia-Ruiz, A. Moreno
J. Crystal Growth 178 (1997) 393

D lower in gels
→ correlation between *D* and β

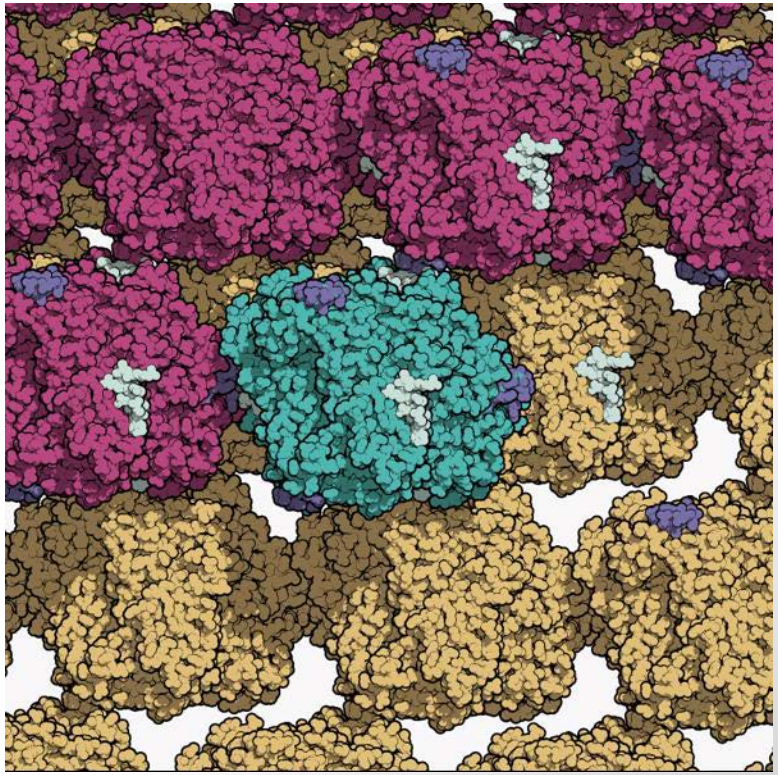
W.B. Hou, *et al.*,
J. Crystal Growth 232 (2001) 265.

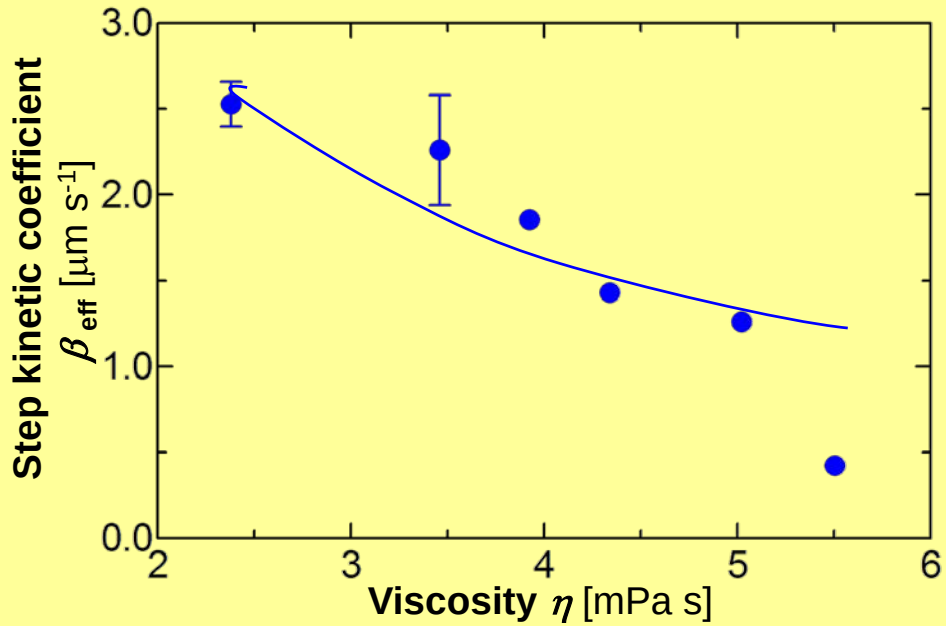
Glucose Isomerase

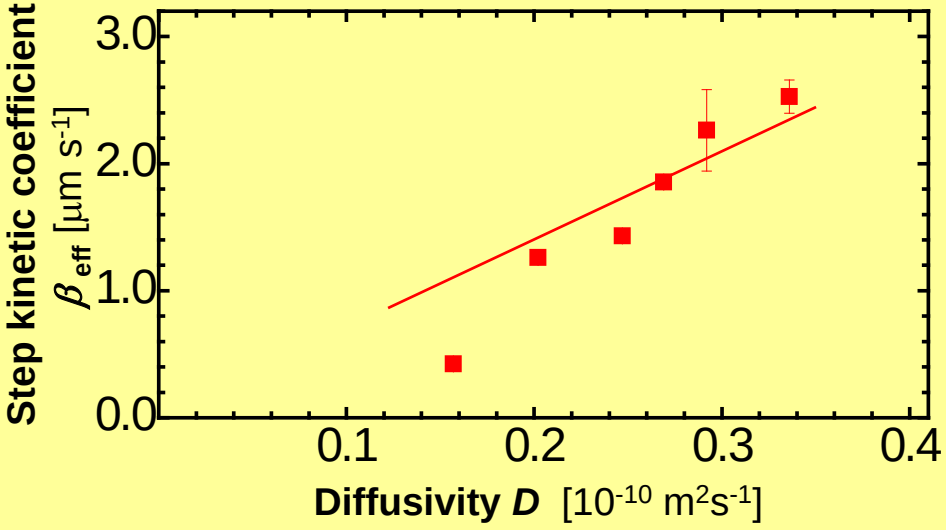


M. Sleutel *et al.*,
JPC Lett.
2012, 1258 (2011)

Glucose Isomerase







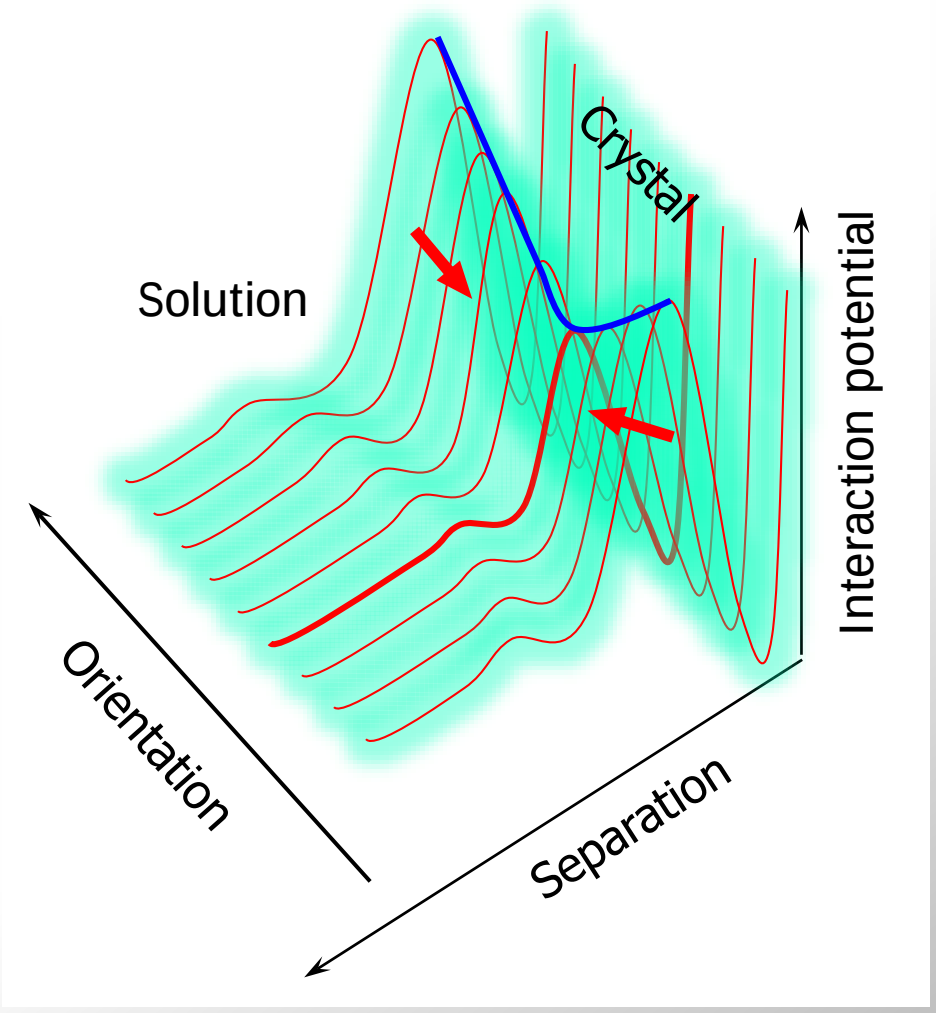
M. Sleutel *et al.*,
JPC Lett. **2012**, 1258 (2011)

Role of Symmetry

System	β, μm s^{-1}	D, 10^{-6} cm^2 s^{-1}	a nm	G	Z	source
Insulin	90	0.79	6.5	$\bar{3}$m	6	Reviakine, *et al.*
Insulin /acetone	420					
Apoferritin	6	0.32	13	432	24	Yau, *et al.*
Ferritin	6					Chen & Vekilov
Canavalin	5.8 – 26	0.4	3.5-8	3	3	Land, *et al.*
Catalase	0.32	n.a.	11.5	222	4	Malkin, *et al.*
Lysozyme [101]	2-3	0.73	3	1	1	Vekilov, *et al.*
No bunching	22 - 45					
Lysozyme [110]	2-3					
Thaumatin	2	0.6	4.0	1	1	Kuznetsov, *et al.*
Lumazine S.	3.6	0.2	16	m5	60	Gliko, *et al.*
Hemoglobin C	0.2	0.5	5.5	2	2	Feeling-Taylor, *et al.*
STMV	4 – 8	0.2	16	m5	60	Malkin, *et al.*
Inorganic salts	100-2000	10	0.5	1, 2	1	many works

Symmetry does not affect kinetic coefficient—supports Kramers-type kinetics

Role of Symmetry

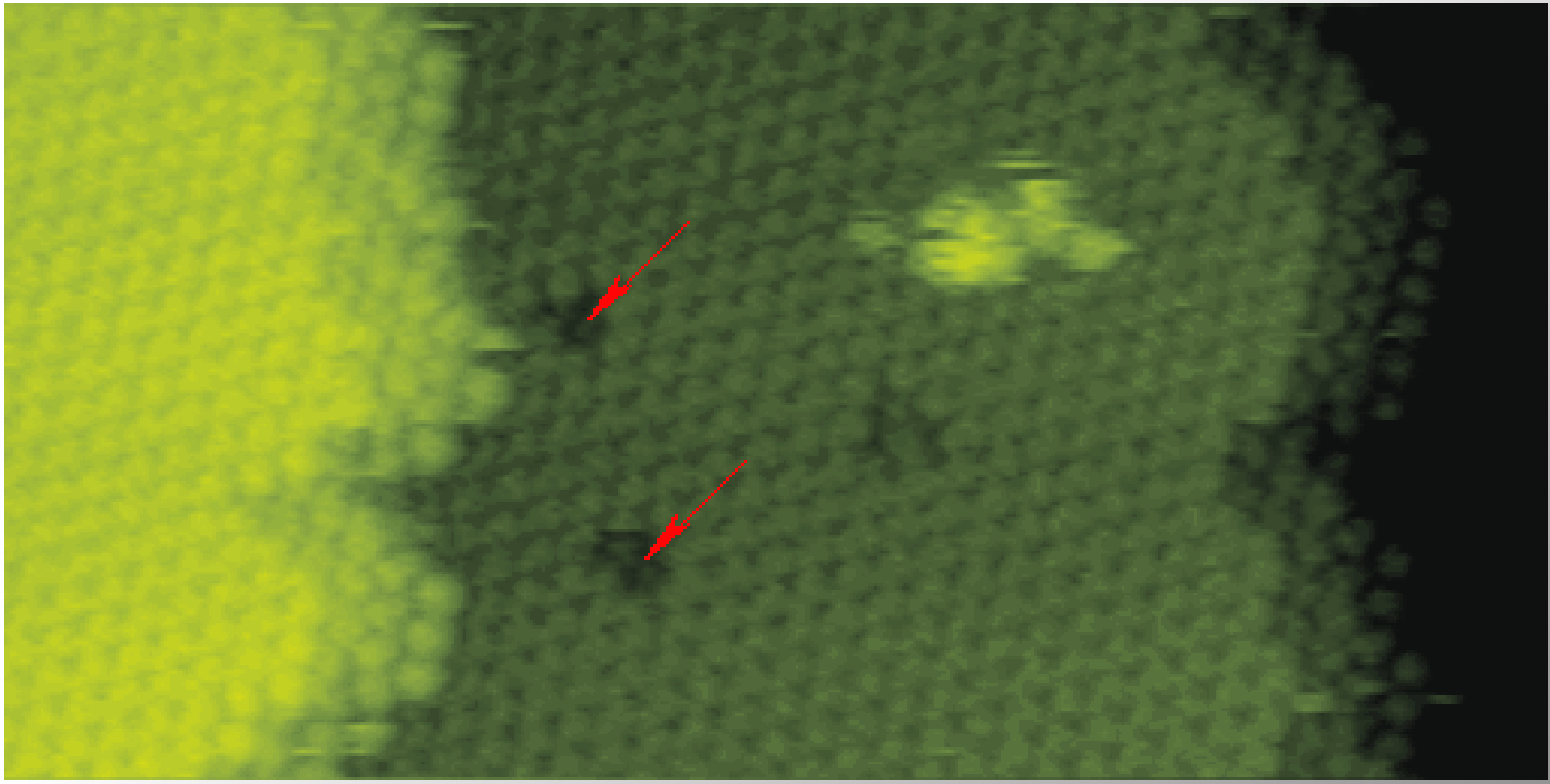


Molecules with “proper” orientation encounter lowest incorporation barriers

Misoriented molecules are driven to saddle point or proper orientation and incorporate

Only possible for diffusion-limited processes

Propagation of Steps Around Surface Vacancies



Viewfield width = 450 nm
Time between frames = 21 s
Sequence lasts 941 s

The Thermodynamics of Solution Crystallization: HbC

$\Delta G^o = \Delta H^o - T\,\Delta S^o_{protein} - T\,\Delta S^o_{solvent}$

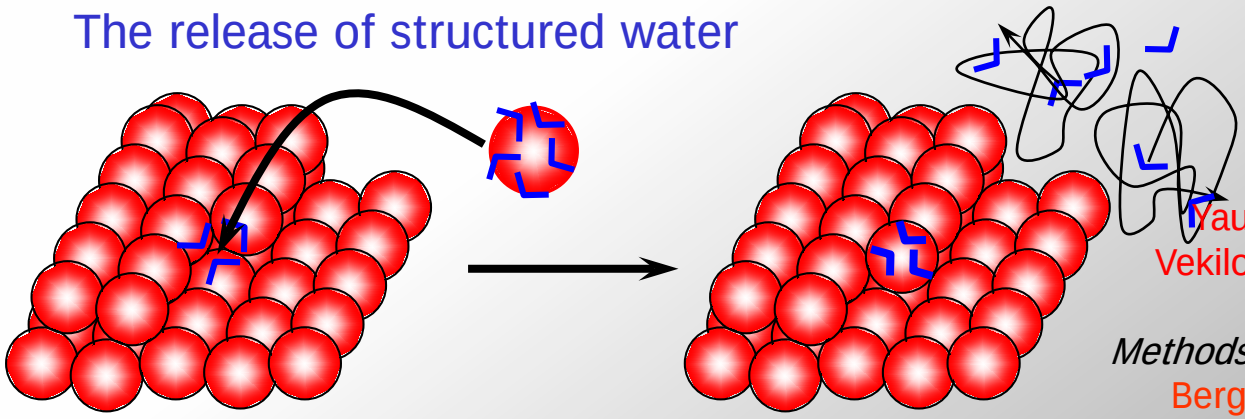
$\Delta H^o = 155$ kJ mol^{-1}

$T\,\Delta S_{protein} = -4$ kJ mol^{-1}, $\Delta S_{protein} \approx -13$ J mol^{-1}K^{-1},

With $\Delta G^o = -25$ kJ mol^{-1} $T\,\Delta S_{solvent} \approx$ **185** kJ mol^{-1}, $\Delta S^o_{solvent} \approx 620$ J mol^{-1}K^{-1}

$\Delta S_{solvent}$ – dominant contributor to the crystallization driving force

The release of structured water

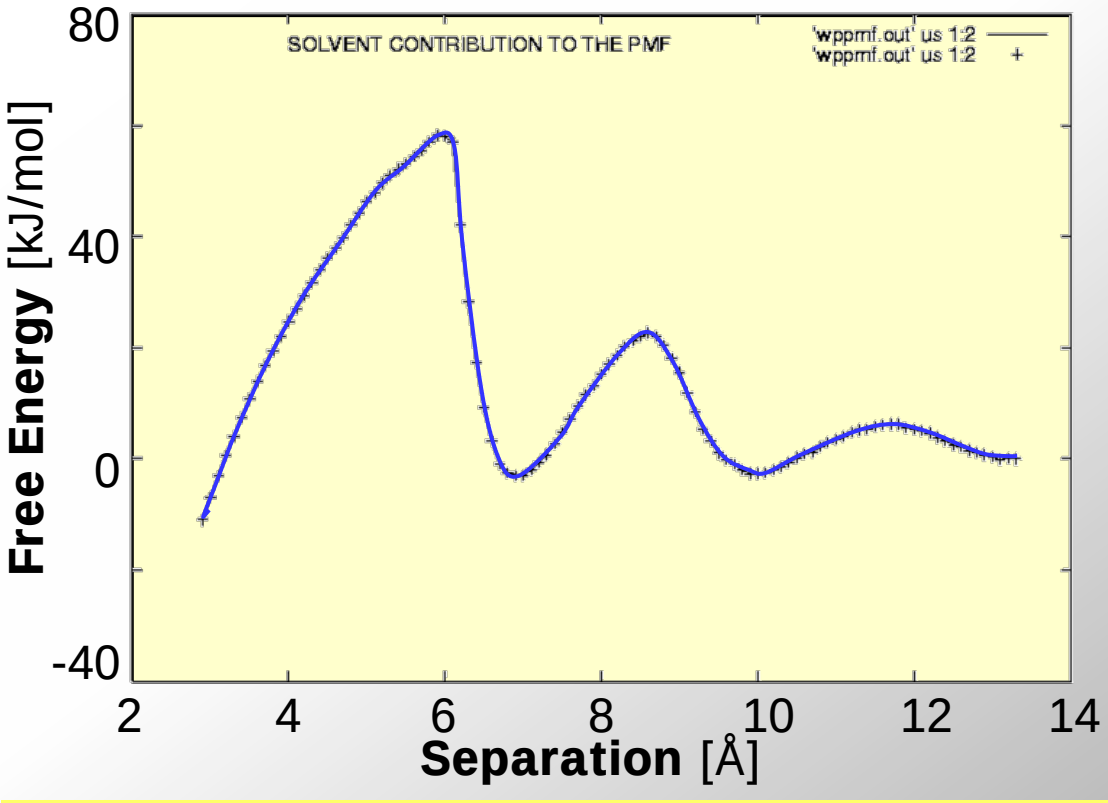


Yau, *et al.*, *J. Mol. Biol.* **303**, 667 (2000)
Vekilov, *et al.*, *Biophys. J.* **83**, 1147 (2002)
Acta Crystallogr. D **58**, 1611 (2002)
Methods in Enzymology vol. 368 (2003) p. 84
Bergeron, *et al.*, *Biophys. J.* **85,** 6 (2003)
Derewenda, Z.S. & Vekilov, P.G. *Acta Cryst. D,* ***62,*** 116 (2006)

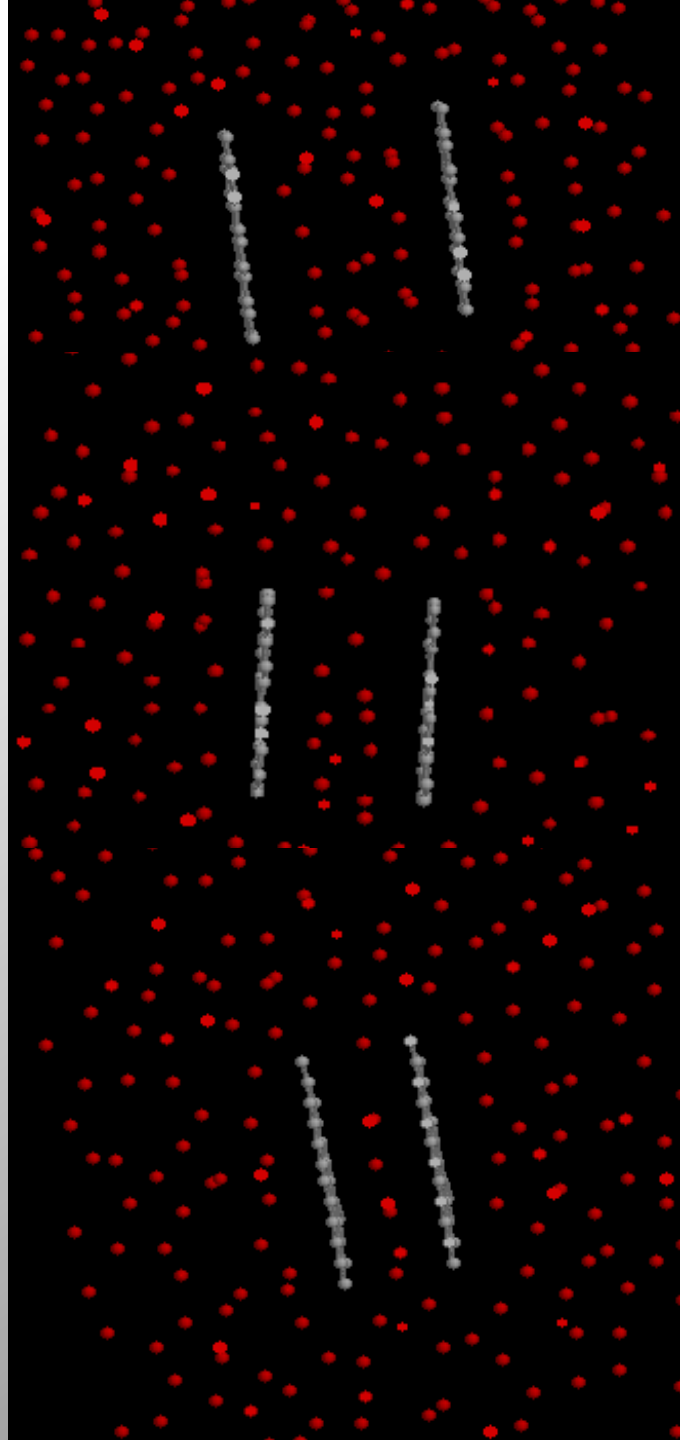
Hypothesis:
Release of water molecules at hydrophobic and hydrophilic patches determinant of slow protein crystallization and aggregation kinetics

Evidence from Molecular Dynamics Simulations

Calculation of potential of mean force as two hydrophobic surfaces approach in water



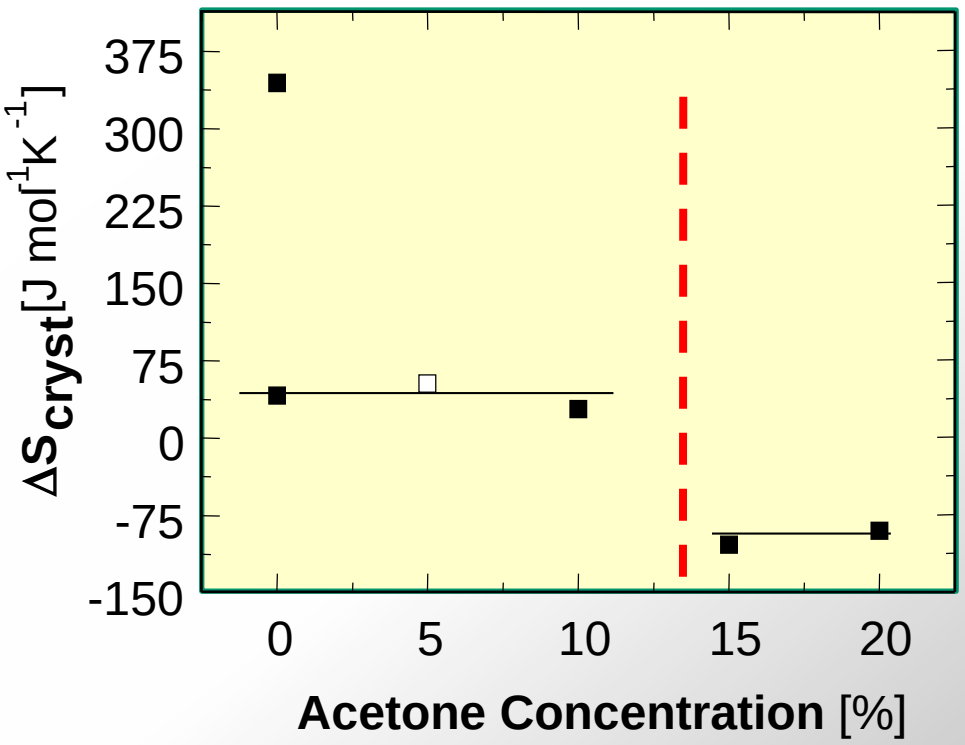
Expulsion of structured water leads to a significant barrier for approach



N. Choudhury & B.M. Pettit, *J. Phys. Chem. B*, **110,** 8459 (2007)

How Can One Destroy the Shell of Structured Water?

Thermodynamics of insulin crystallization in the presence of acetone



Upon addition of acetone
crystallization entropy drops from
+40 J/mol K
to – 115 J/mol K

ΔS^o of binding of 1 insulin molecule
– 105 J/mol K

Tidor, B. & Karplus, M. (1994)
J. Mol. Biol., **238,** 405

Negative value with acetone =
loss of entropy of insulin molecule

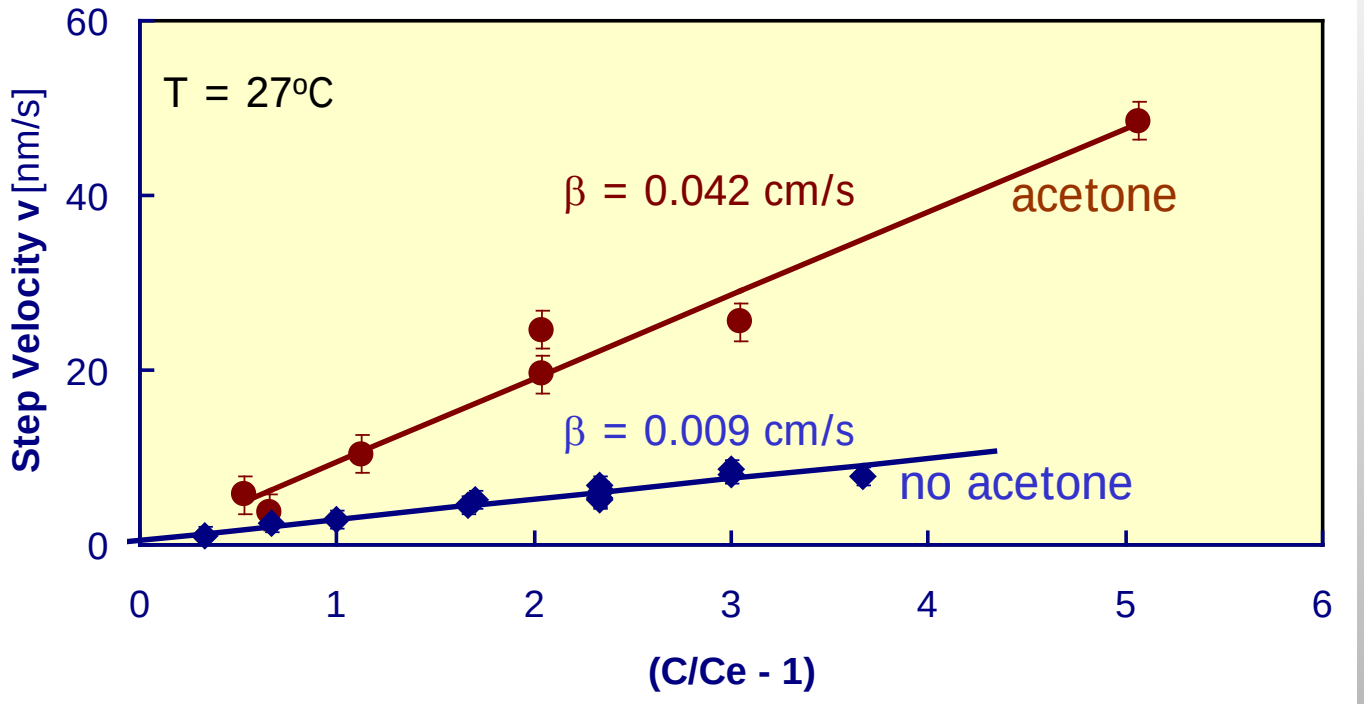
- shows lack of structured water

Acetone destroys water shell

L. Bergeron, *et al.*, *Biophys. J.* **85,** 6 (2003)

Is the Barrier due to Structured Water?

Insulin step velocity in the presence of acetone



Reviakine, *et al.*, *J. Am. Chem. Soc.* **125**, 11684 (2003)

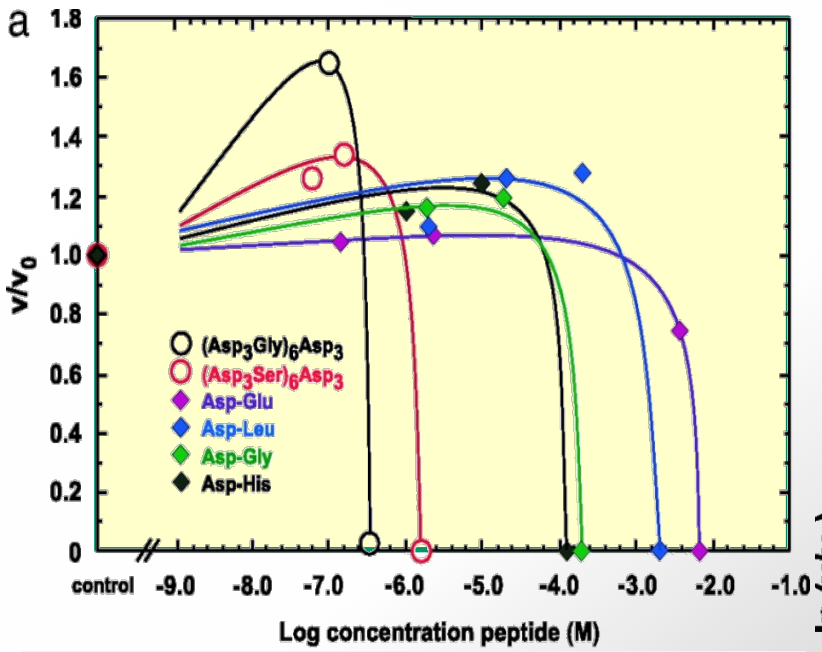
β in presence of acetone is higher than β in the absence of acetone

- Barrier to incorporation is due to structured water

Is the Barrier due to Structured Water?

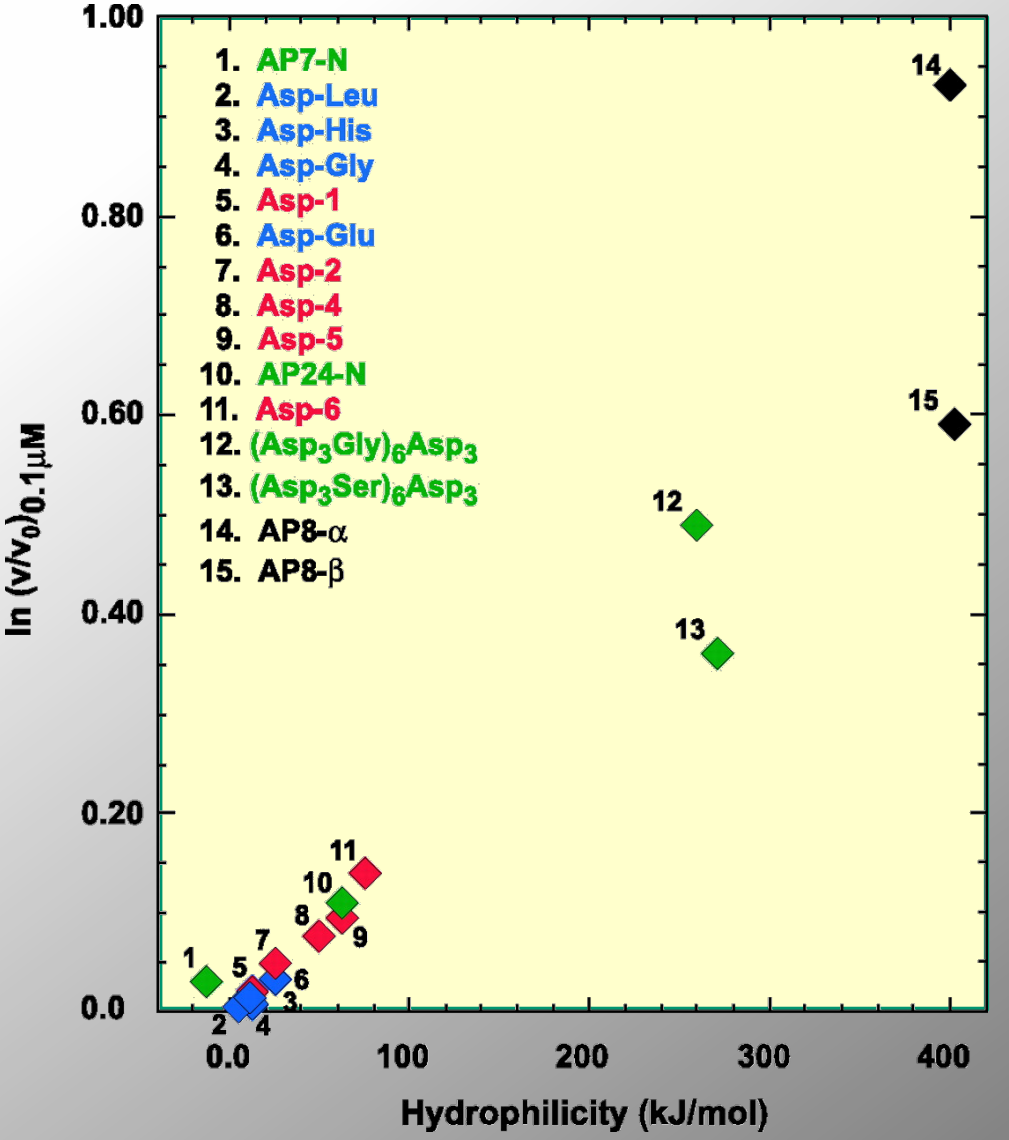
Calcite step velocity in the presence of additives

Elhadj, S.,*et al.*, *PNAS*, **103,** 19237 (2006)



Acceleration of step velocity by nanomolar amounts of additives correlates with their hydrophilicity

- Barrier to incorporation is due to structured water



Conclusions

Solution-grown crystals have important
physiological, pharmaceutical, industrial, etc.,
applications, and large market

The rate of growth of crystals is determined by:
the rate of layer generation
the kink density
the rate of incorporation into kinks

Kinks are generated by three mechanisms:
by thermal fluctuations
by “1D nucleation” of molecular rows
by association of 2Dclusters

Incorporation into kinks follows Kramers-type
(diffusion limited) kinetics

The incorporation barrier is caused by the water
structuring on the surface of solute and crystals