Minisemester on Evolution of Interfaces
Sapporo, Japan, July 26-30, 2010

Kink Generation by the Association of 2D Clusters

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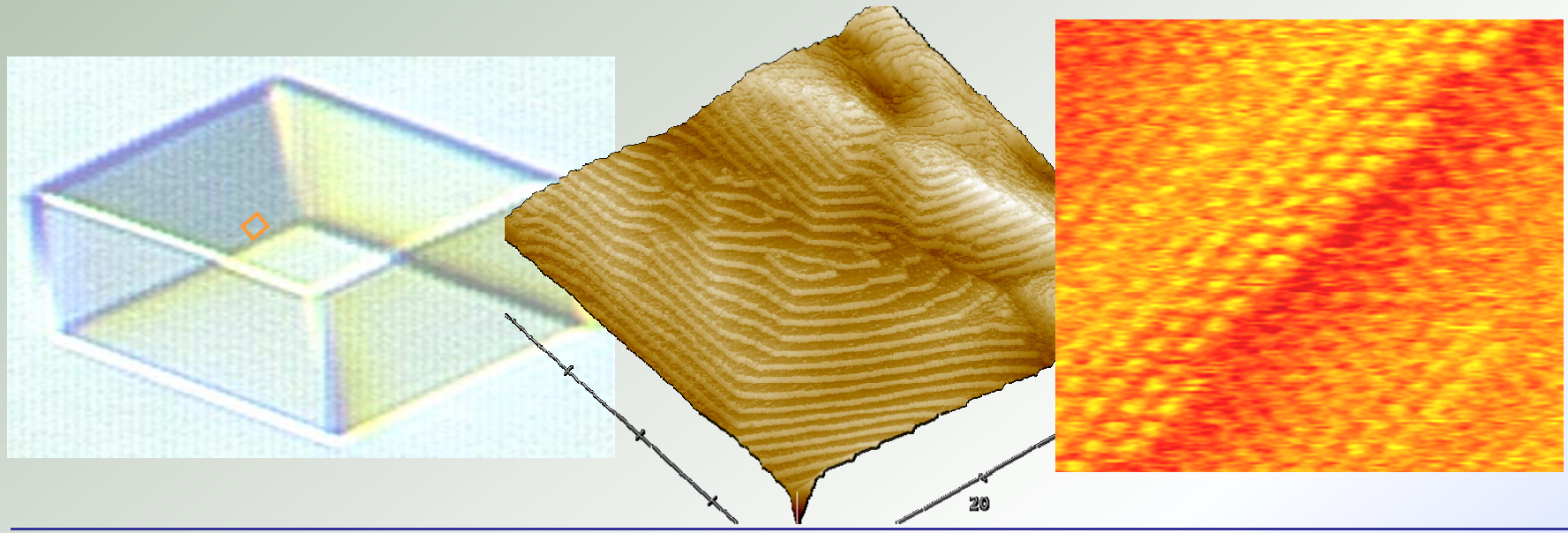
Departments of Chemical and Biomolecular Engineering and Chemistry
University of Houston



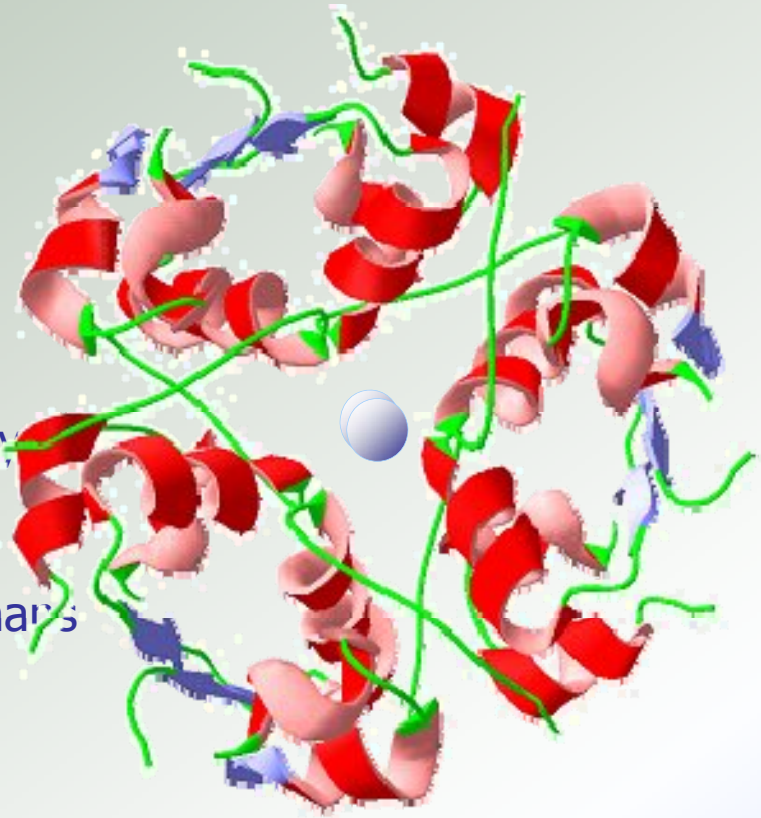
The University of Houston, The Welch Foundation of Texas

Insulin and Diabetes

Endocrine hormone regulating metabolism

Controls blood glucose levels

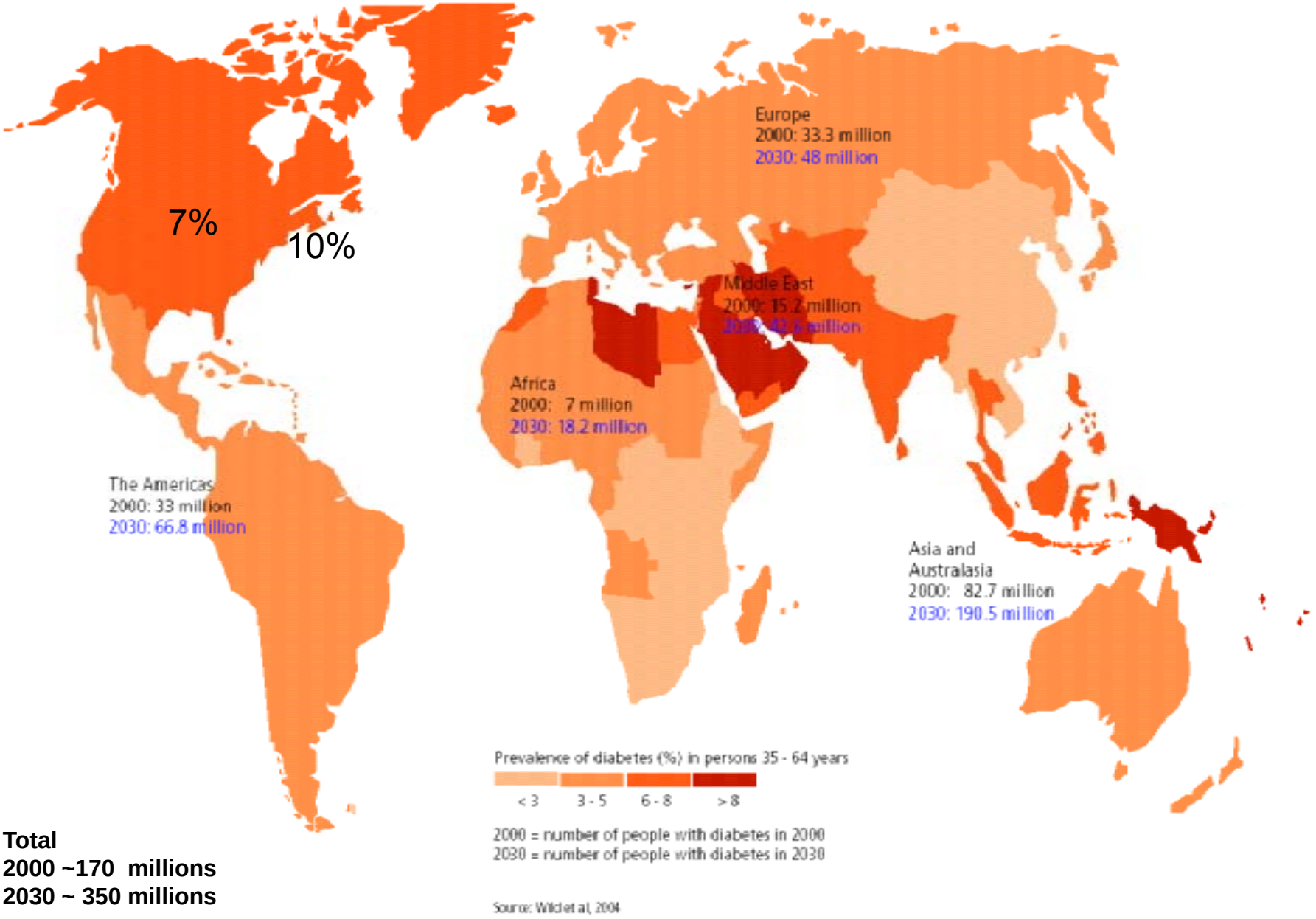
Diabetes type I: inability to produce insulin
Diabetes type II:
insulin resistance → deficiency
Diabetes type III:
dysfunction of neurons ← insulin deficiency



Insulin in pancreas:
- Secreted by the β-cells of the islets of Langerhans
- Forms hexamers with 2 Zn^{2+}
- Hexamers stored as **rhombohedral crystals**

Unique example of **benign** crystallization
- Protection of insulin
- Increase of degree of conversion from soluble proinsulin

Prevalence of Diabetes

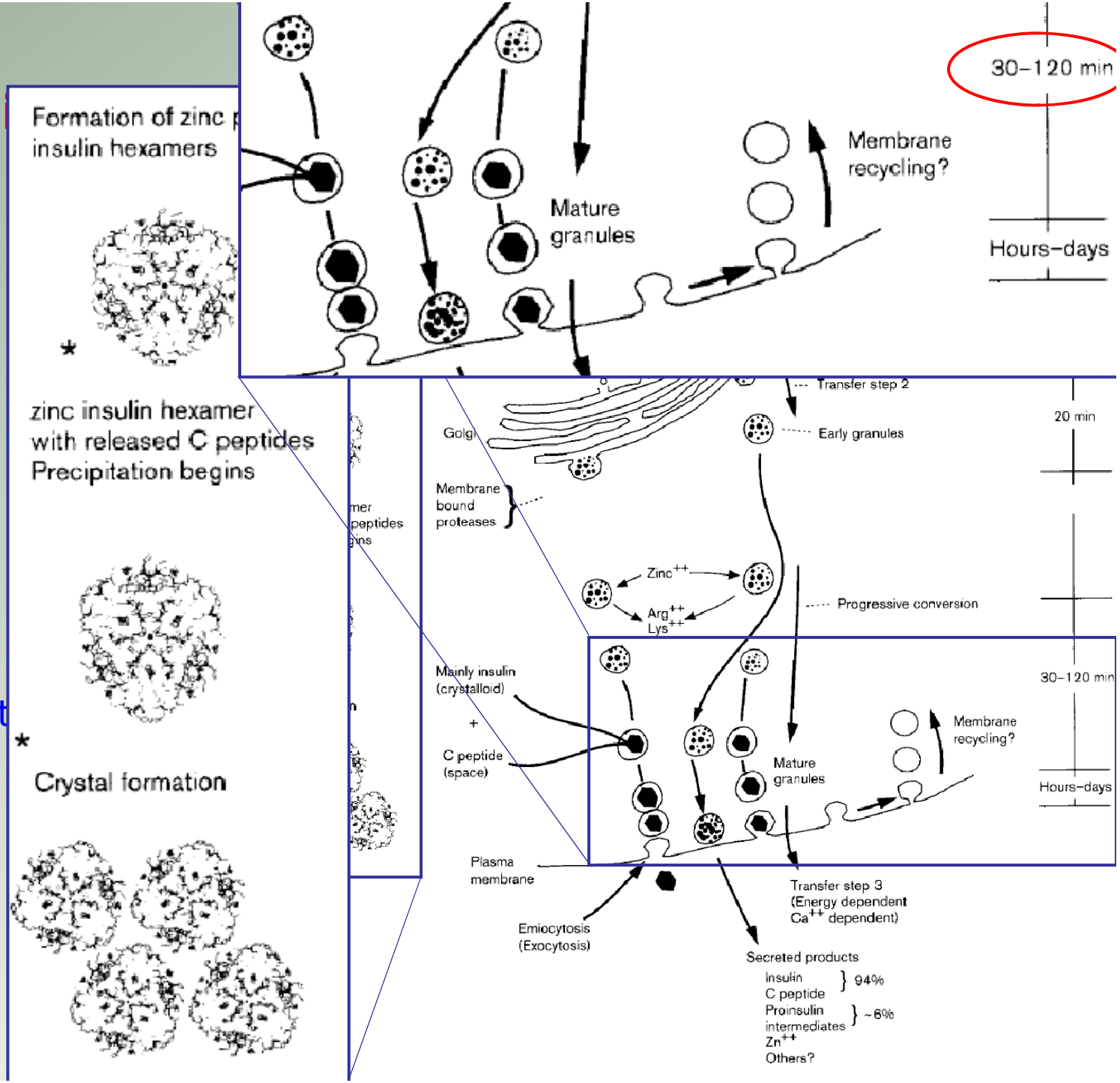


Total
2000 ~170 millions
2030 ~ 350 millions

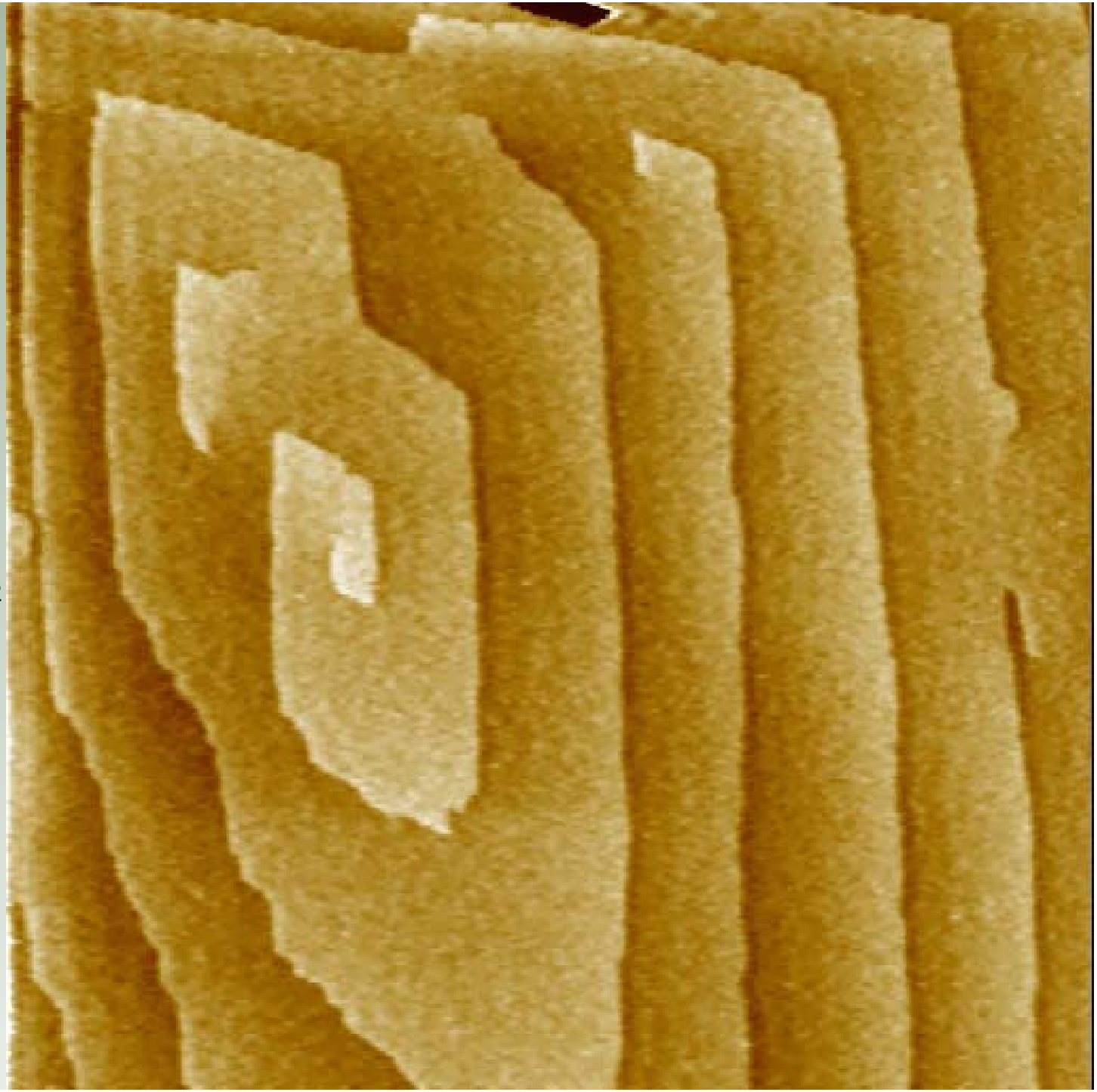
Insulin Biosynthesi

G. Dodson,
D. Steiner,
Curr. Op.Struct. Biol.
8 (1998) 189

- Fast crystal growth
- Ready response to fluctuations in conversion rate
- Crystals exclude proinsulin present in islet cells
- Slow dissolution at undersaturation
- Single crystal per vesicle



Growth of Insulin Crystals



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

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

Step Motion Characterized by Kinetic Coefficient β

C(0) -- concentration at interface
C_{eq} -- solubility

$$v = \beta \Omega C_e [C(0)/C_{eq} - 1]$$

Chernov
Sov. Phys. Uspekhi **4**, 116 (1961)

$$[C(0)/C_{eq} - 1] = \left[\exp\left(\mu_{\text{at interface}} / k_B T\right) - \exp\left(\mu_{\text{in crystal}} / k_B T\right)\right]$$

Ω -- volume of one molecule in crystal
ΩC_e = n_L/n_c -- molecular densities in solution and crystal

β -- not affected by depletion at interface

PROTEIN	Insulin	Insulin w/acetone	Apoferritin	Ferritin	Canavalin	Catalase	Lysozyme (101)	Lysozyme No bunching (110)	Thaumatin	Lumazine S.	HemoglobinC	STMV	KDP (100)	BaNO3	amyloid fibrils	DNA base pair format
size a [nm]	6.5	13		3.5	11.5	3		4	16	5.5	16	0.5	0.4	1	1	
β [μm/s]	90	420	6	6	5.2-26	0.32	2 - 3	22-45	2 - 3	2	3.6	0.2	4 - 8	500 - 1200	130			
k [s-1]	14,000	65,000	450		3,000	30	800	13,000	800	500	225	36	375	2,000,000	325,000	0.00002	1.E+07	

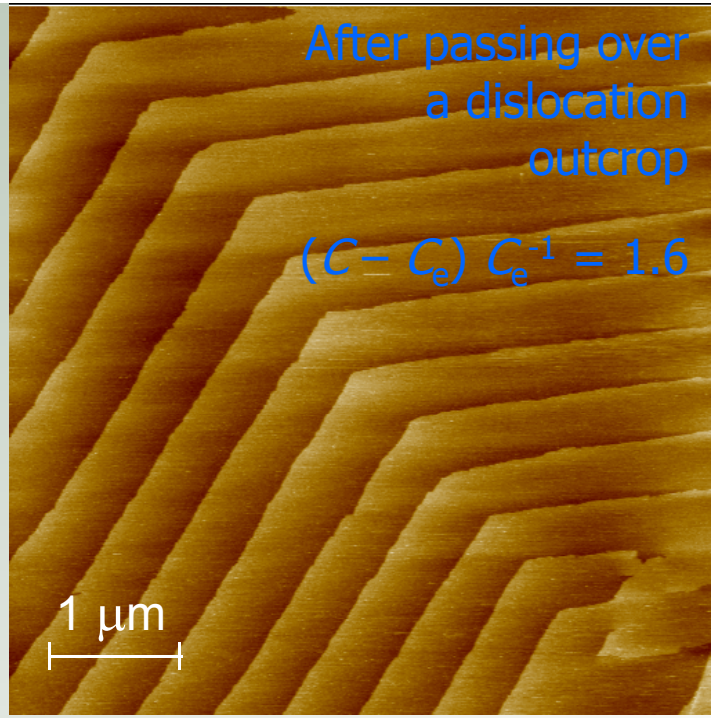
Stable Equidistant Step Trains

At all locations, in a wide supersaturation range equidistant step trains observed
Steps on insulin ***do not*** bunch

$$Pe_k = \beta_{st} p\delta/D = 3.5$$

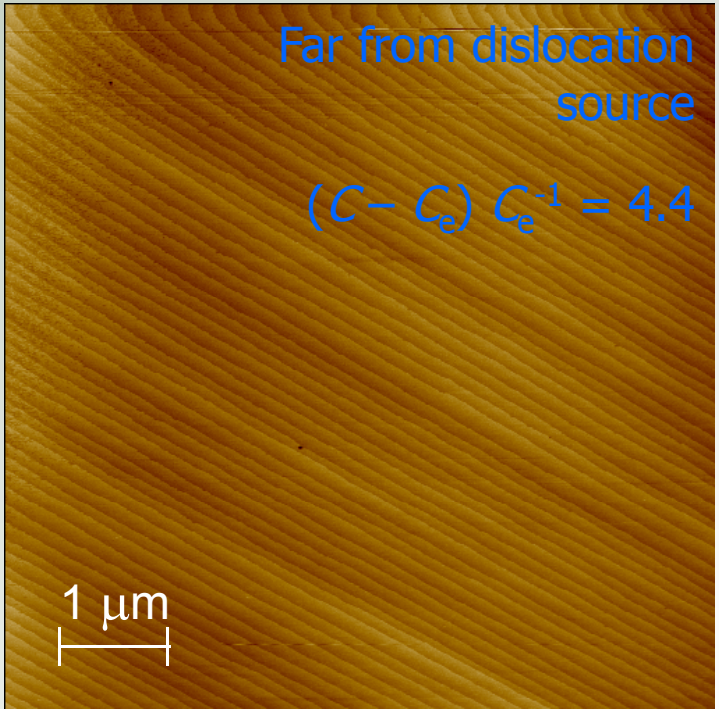
Strong transport control

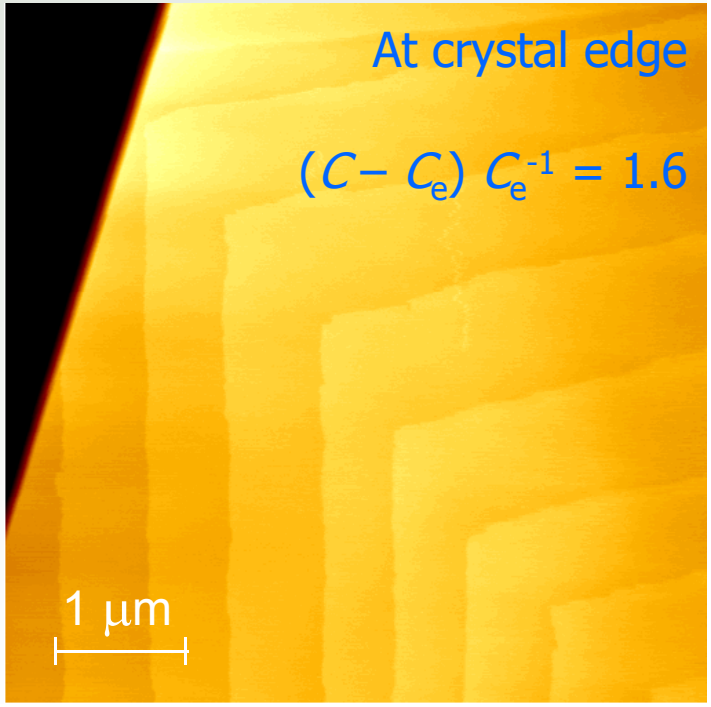
O. Gliko, *et al.*,
Phys. Rev. Lett **90**, 225503 (2003)



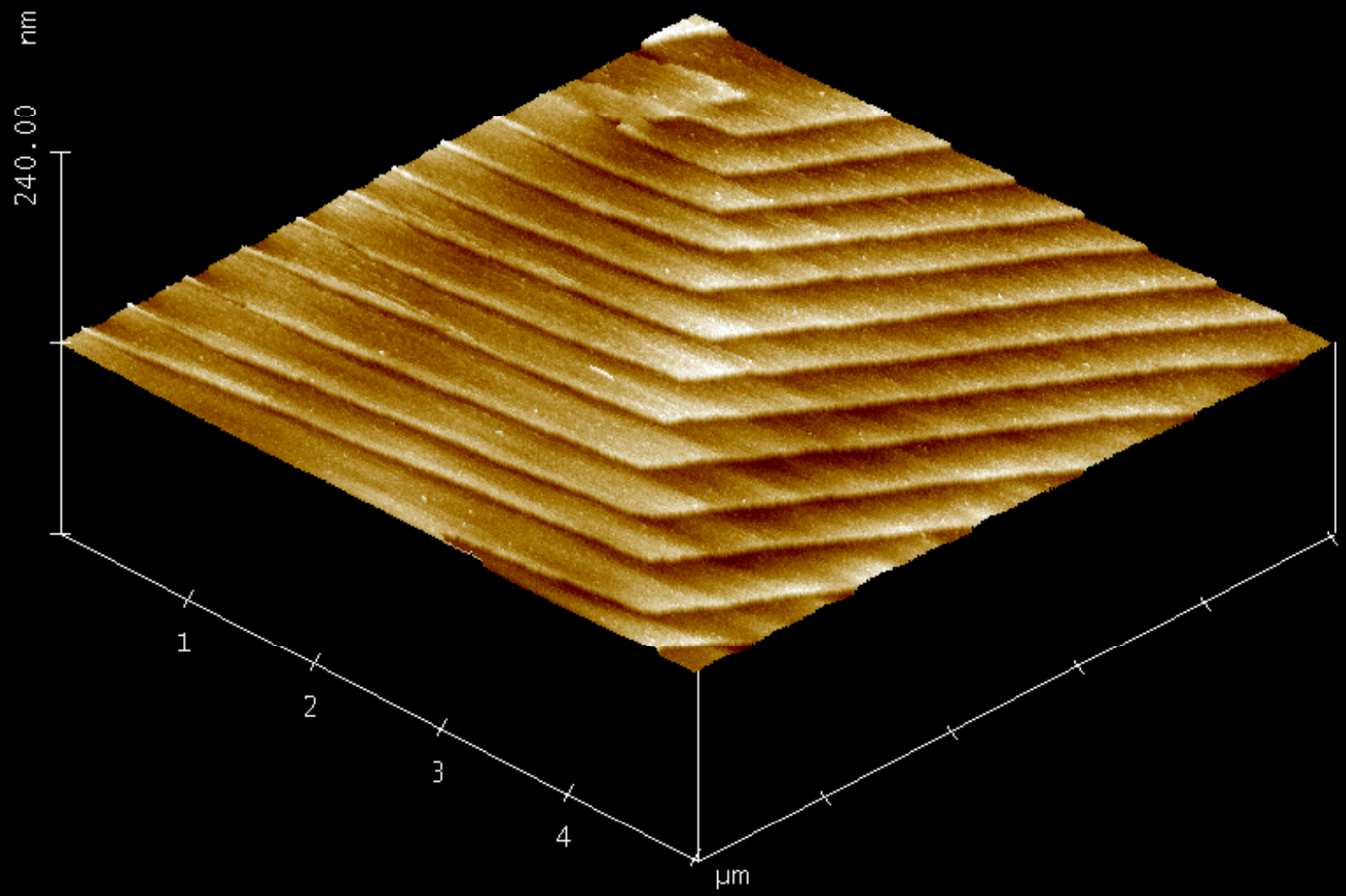
Lack of bunching ensures
~ 10× faster growth

P. G. Vekilov
J. I. D. Alexander
Chem. Rev. **100**, 2061 (2000)

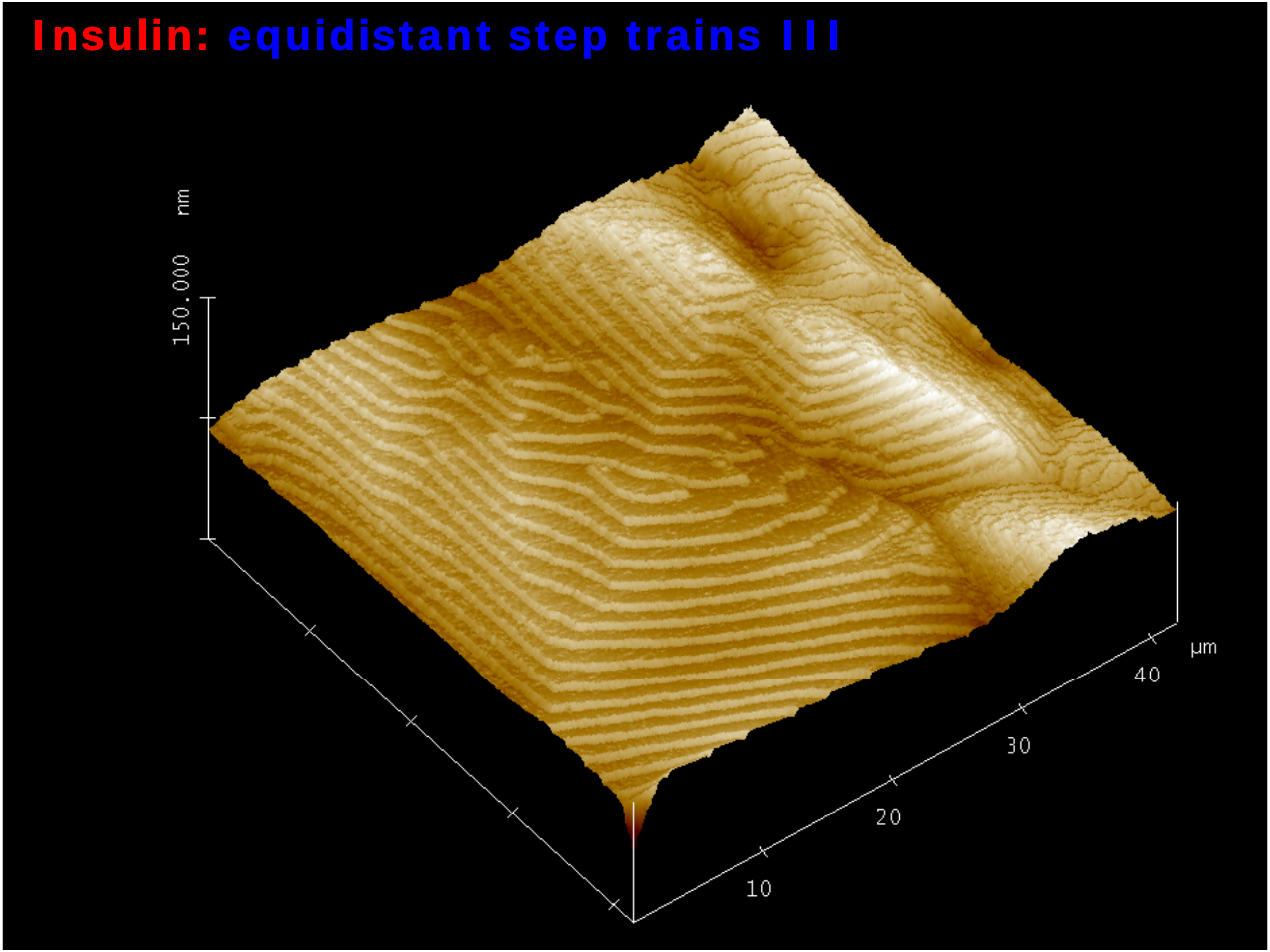




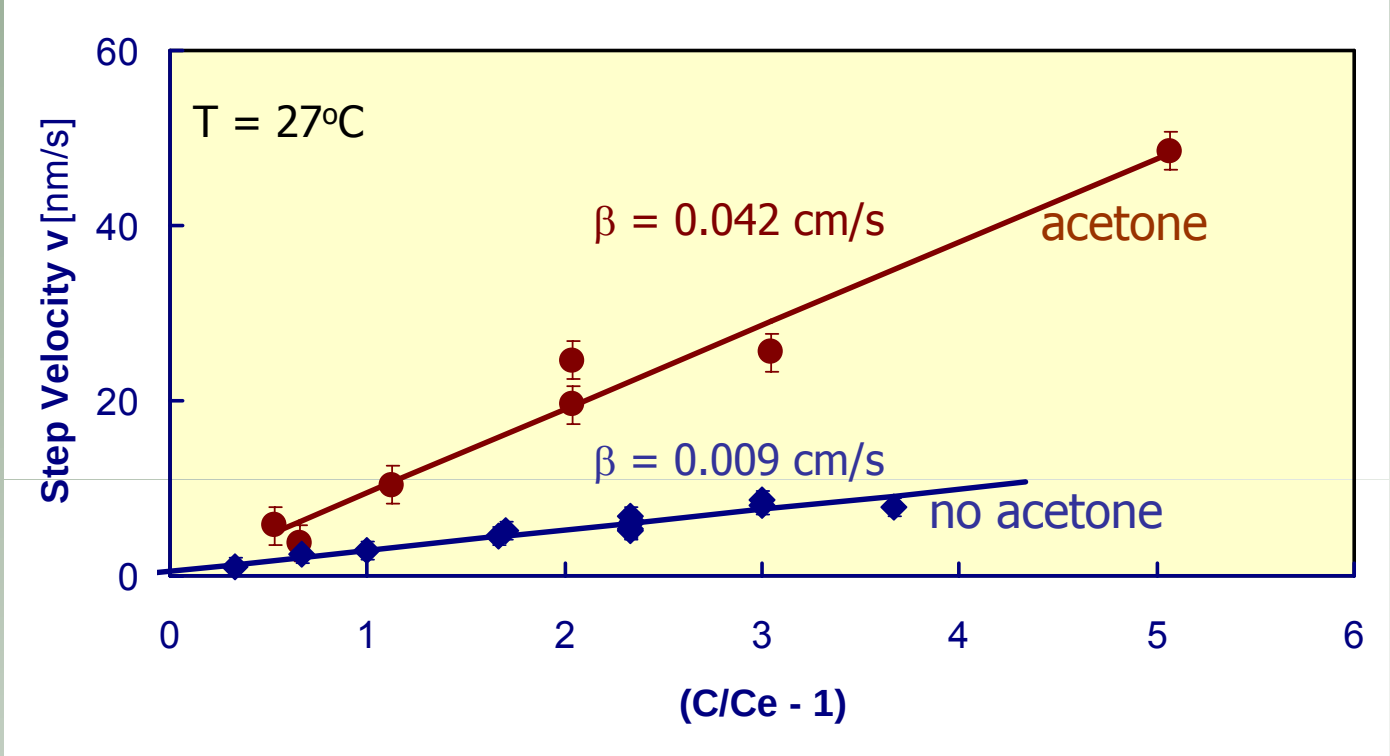
Insulin: equidistant step trains II



Insulin: equidistant step trains III



Is the Rate of Steps Biochemically Regulated?



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

- β in presence of acetone is higher than β in the absence of acetone
- Acetone destroys hydrophobic shells around insulin molecules

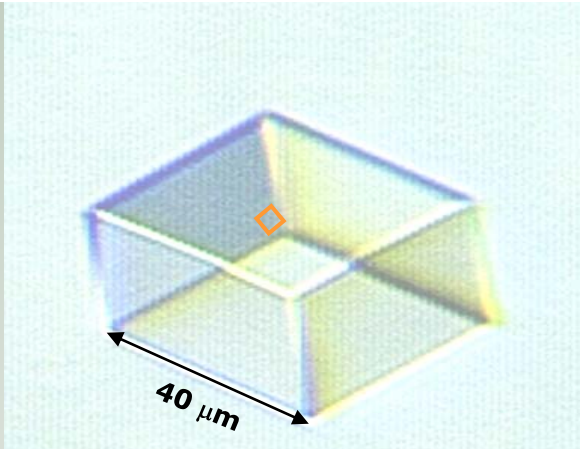
  from thermodynamic analyses in — L. Bergeron *et al.* *Biophys.J.* **85** (2003) 6

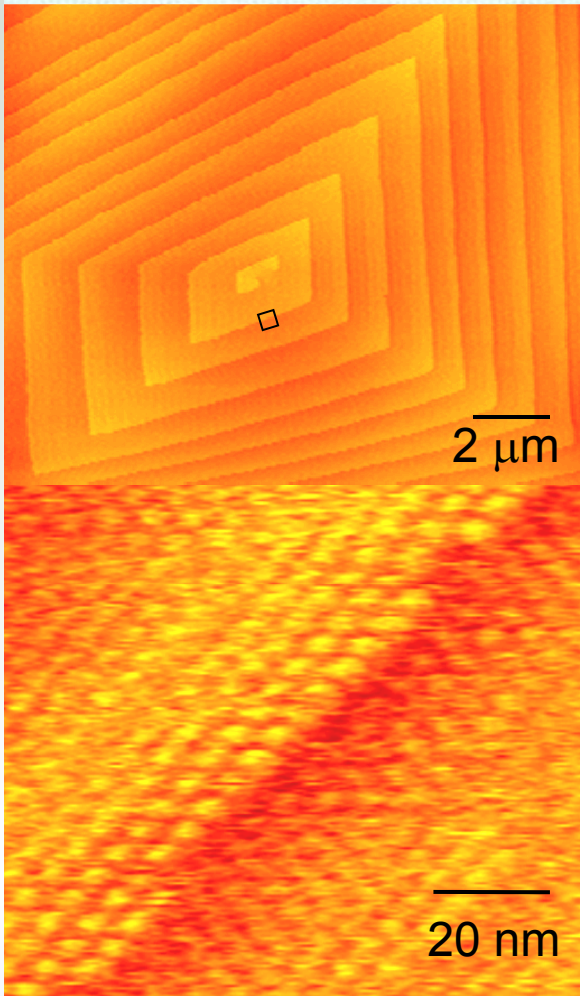
  supported by MD simulations — B.M. Pettitt *et al.* unpublished

Growth of Insulin Crystals

- Insulin crystals grow by spreading of layers
- Layers—generated by screw dislocations
- No step bunching during insulin crystal growth
- New molecules attach to kinks located at the steps

- Kink density determines the rate at which steps advance

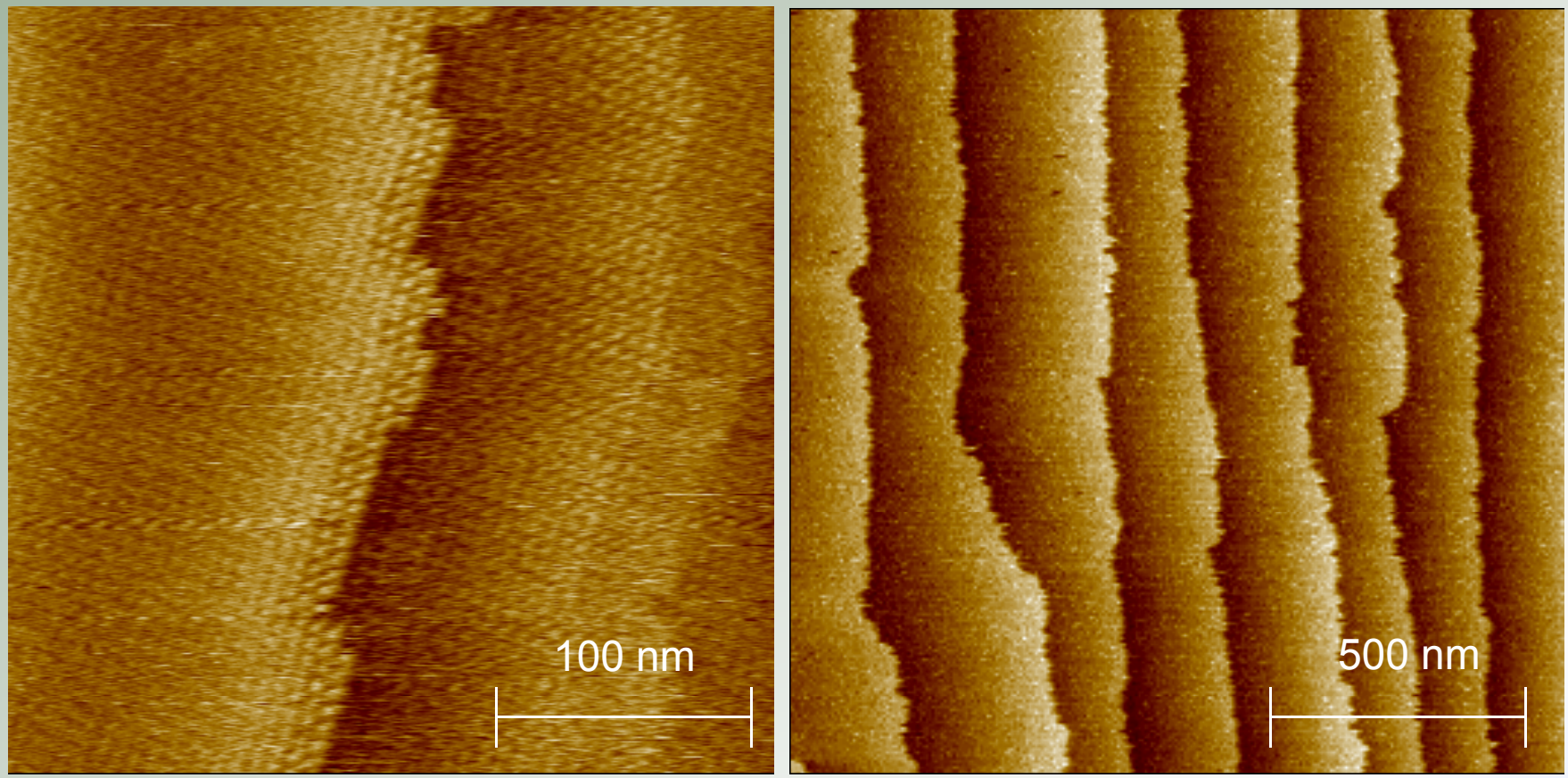




Structure of Step Edge

Low supersaturation—few kinks

High supersaturation--multiple kinks



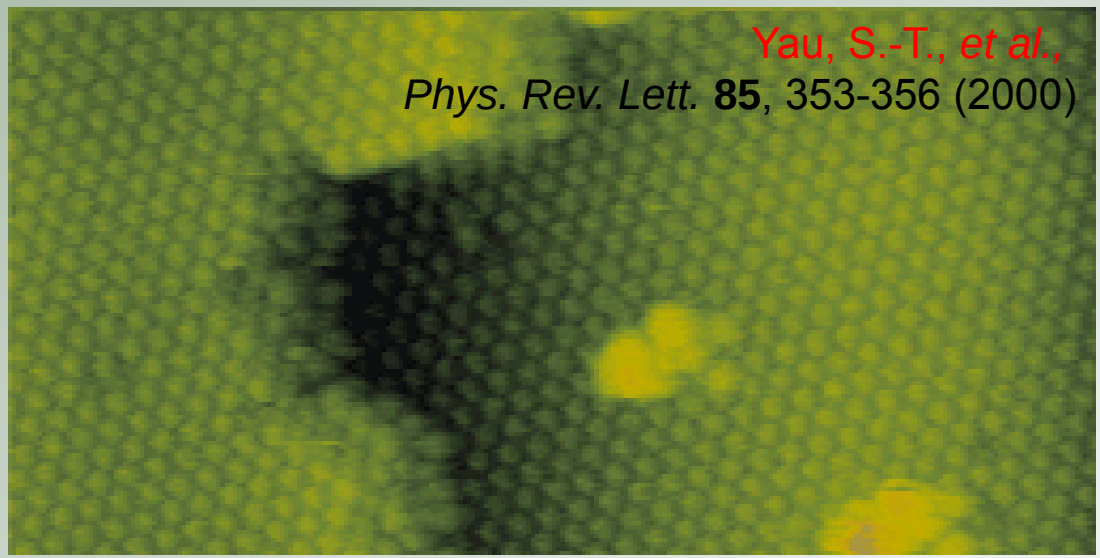
Where do the high-supersaturation kinks come from???

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

Mechanisms of Kink Generation

- Thermal fluctuations

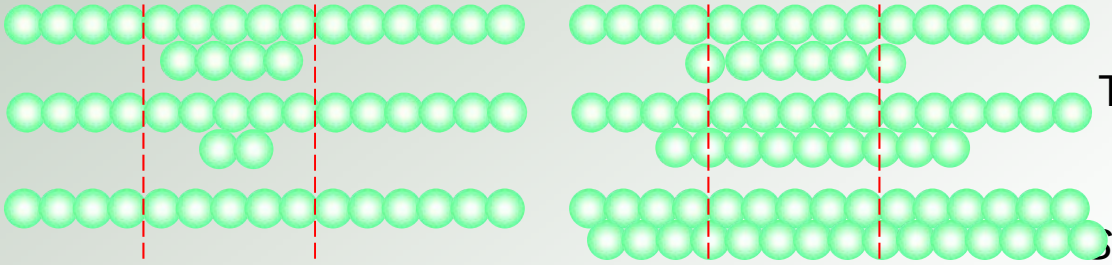
"… the edges of these [the crystal's] layers probably fluctuate as molecules join them and depart from them." p.237



Yau, S.-T., *et al.*,
Phys. Rev. Lett. **85**, 353-356 (2000)

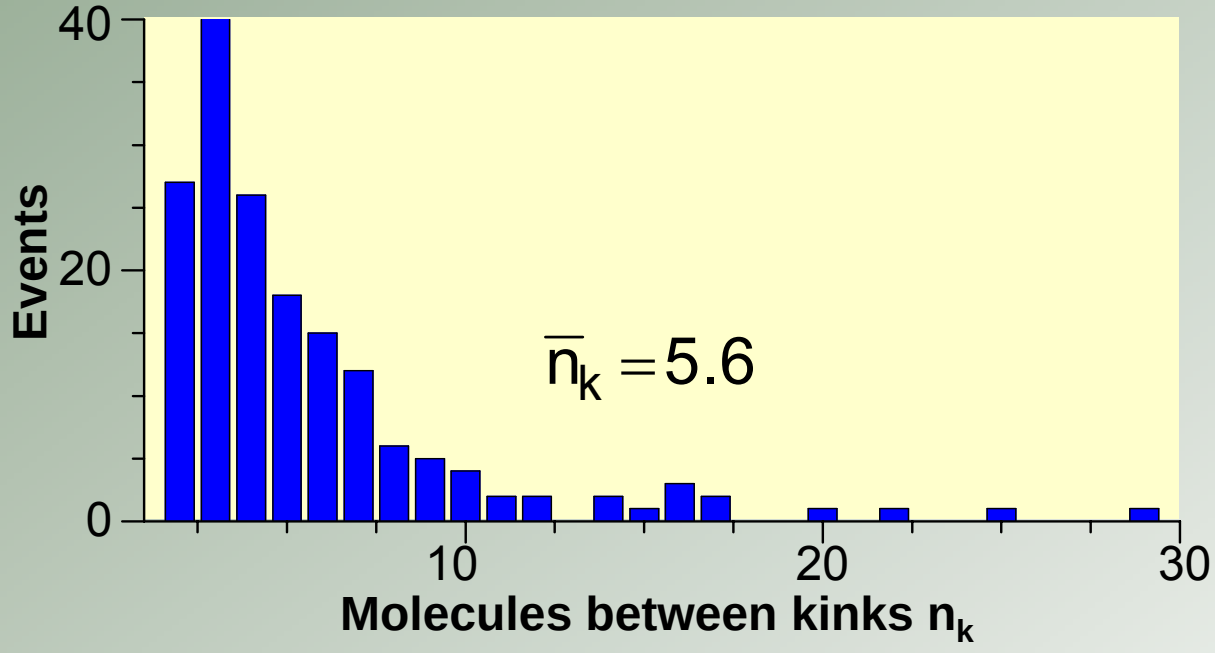
Gibbs, J. W.
On the equilibrium of heterogeneous substances
Trans. Connect. Acad. Sci. 3, 108-248 (1876)

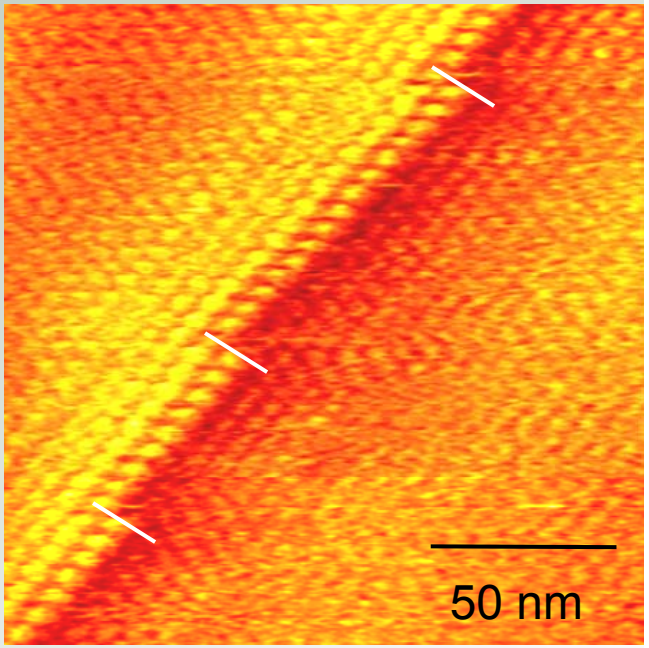
- 1D nucleation



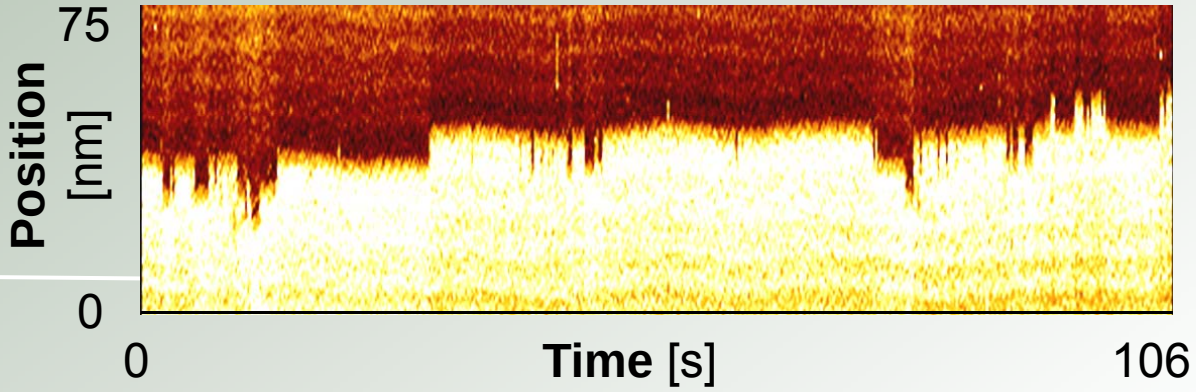
Voronkov, V. V.
The movement of an elementary step by means of the formation of one-dimensional nuclei
Sov. Phys.-Crystallogr. **15**, 8-13 (1970)

Kinks at Low Supersaturations $\Delta\mu/k_BT < 0.05$





Step dynamics—from AFM pseudo-image



One molecule shifts—due to attachment or detachment of molecules

Step velocity
v≈ 0.05-0.15 nm/s

Mechanism of kink formation

Kink free energy

$\omega = (3\phi_2 + \phi_3 - \phi_2 - \phi_3)/4$

$\omega = \phi_2/2$

Kink density

$\bar{n}_k = 1/2\exp(\omega/k_BT) + 1$

Assumes kinks generated by thermal fluctuations

With $\bar{n}_k = 5.6$, $\omega = 5.5$ kJ/mol

$\phi_2 = 11.1$ kJ/mol

Reduced crystallization free energy $\Delta G'$

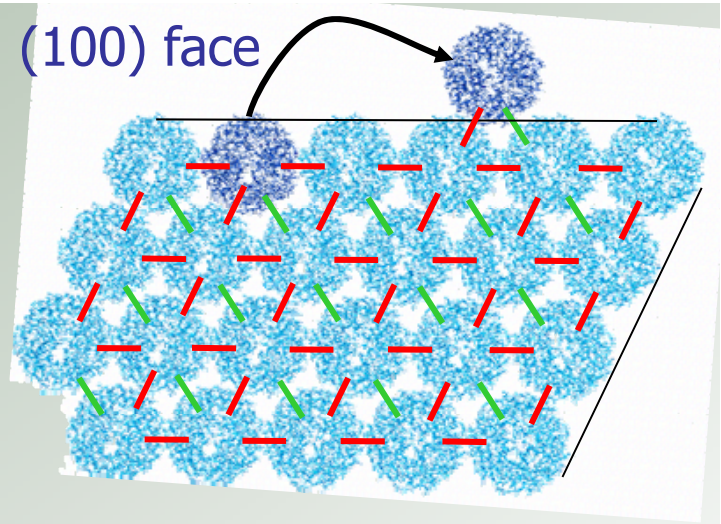
$\Delta G' = -1/2\ (2\phi_1 + 6\phi_2 + 6\phi_3)$,

$\Delta G' = -61.3$ kJ/mol

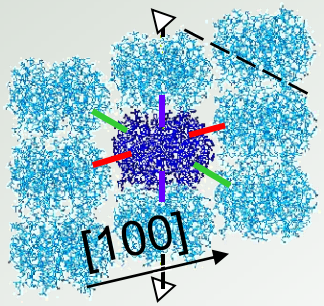
Bergeron, *et al., Biophys. J.* (2003)
Finkelstein, *et al., Prot. Eng.* (1989)

$\Delta G^\circ_{cryst} = -30$ kJ/mol

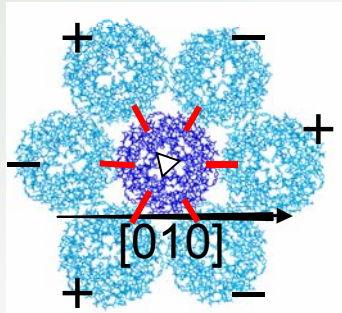
$\Delta S^\circ_{protein} = (\Delta G' - \Delta G^\circ_{cryst})/T \approx -105$ J/molK



(0-11) plane



(111) plane



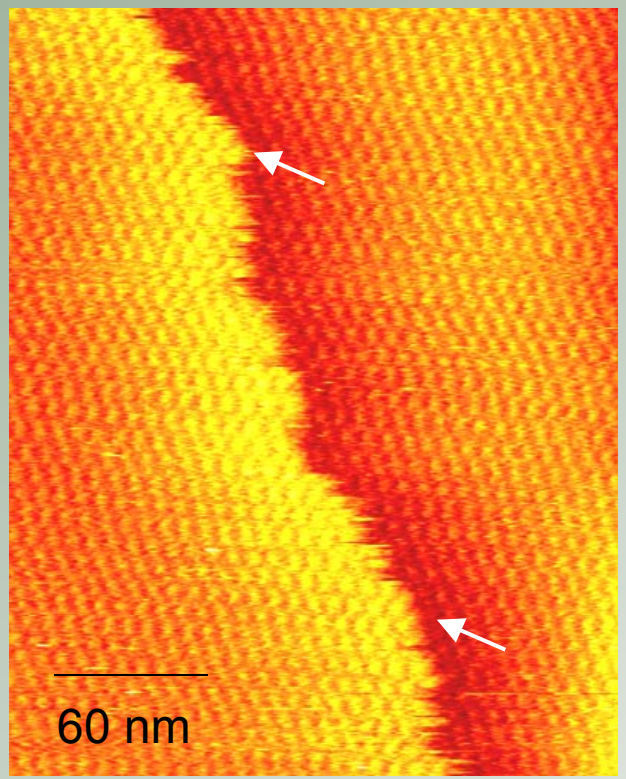
Bonds	Strength	Direction	Energy
Two	strong	[111]	$\phi_1 \approx \phi_2$
Six	strong	<010>	ϕ_2
Six	week	<01-1>	$\phi_3 \approx 1/2\phi_2$

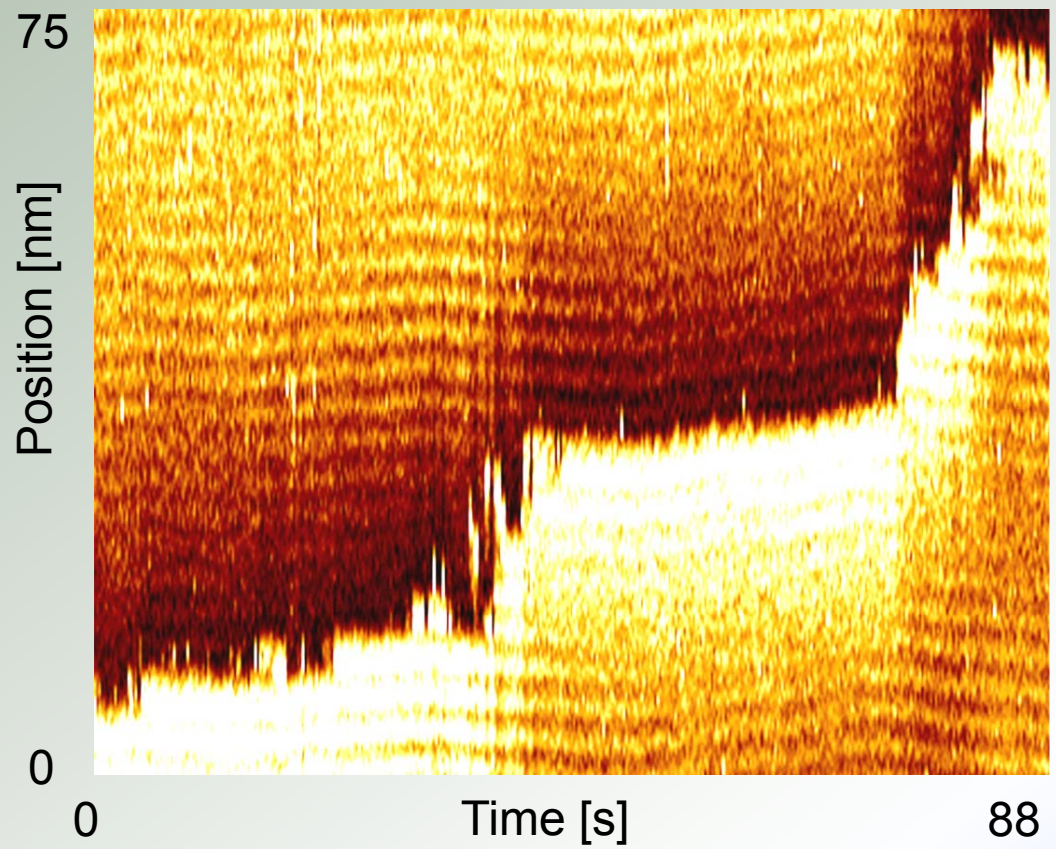
$\Delta S^\circ_{protein}$ determined from kink density—similar to published values

Kink generation is due to thermal fluctuations

Steps and Kinks at Intermediate Supersaturations

$0.05 < \Delta\mu/k_BT < 0.1$





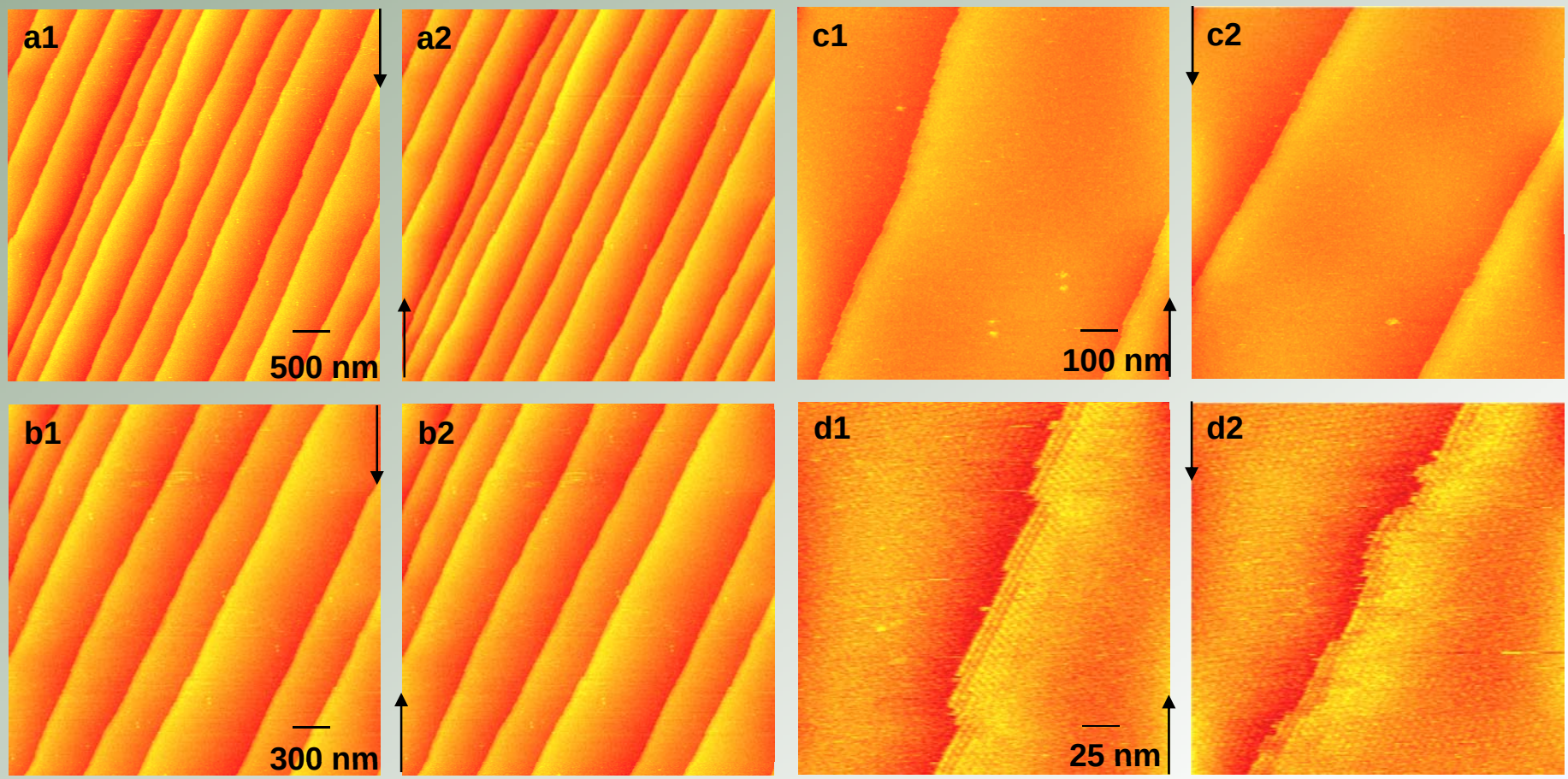
High kink density

Jumps $\leq$ 11 molecular diameters seen due to propagation of multiple kinks

Step velocity $v = a/\overline{\Delta t} \approx 0.6$ nm/s

D.K. Georgiou, P.G. Vekilov, *Proc. Natl. Acad. Sci. USA* **103** (2006) 1681

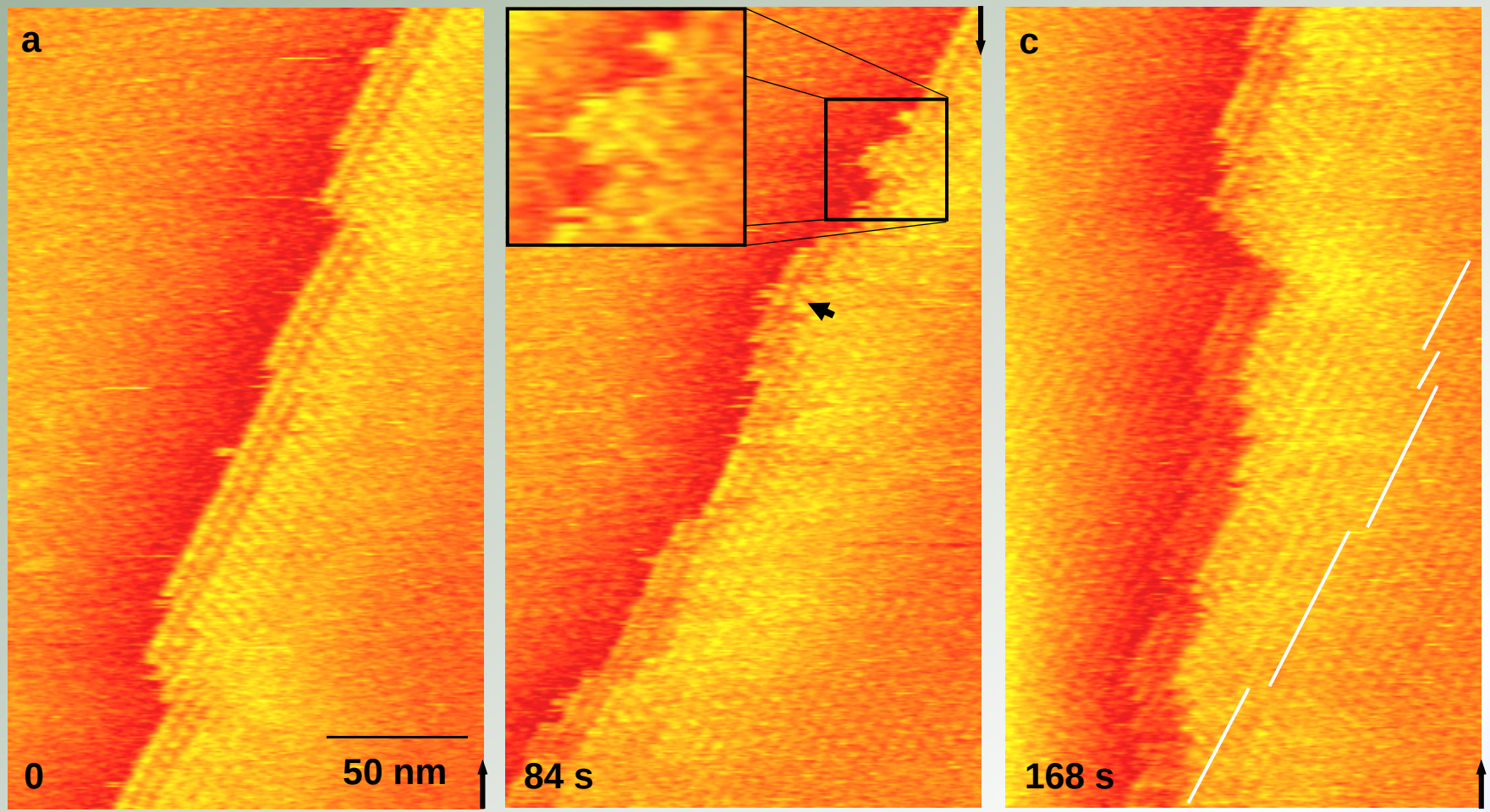
Imaging of Edges of Straight Steps



Zoom levels increased from **a**→**b**→**c**→**d**; molecular structure of step edge seen in **d**

D.K. Georgiou, P.G. Vekilov, *Proc. Natl. Acad. Sci. USA* **103** (2006) 1681

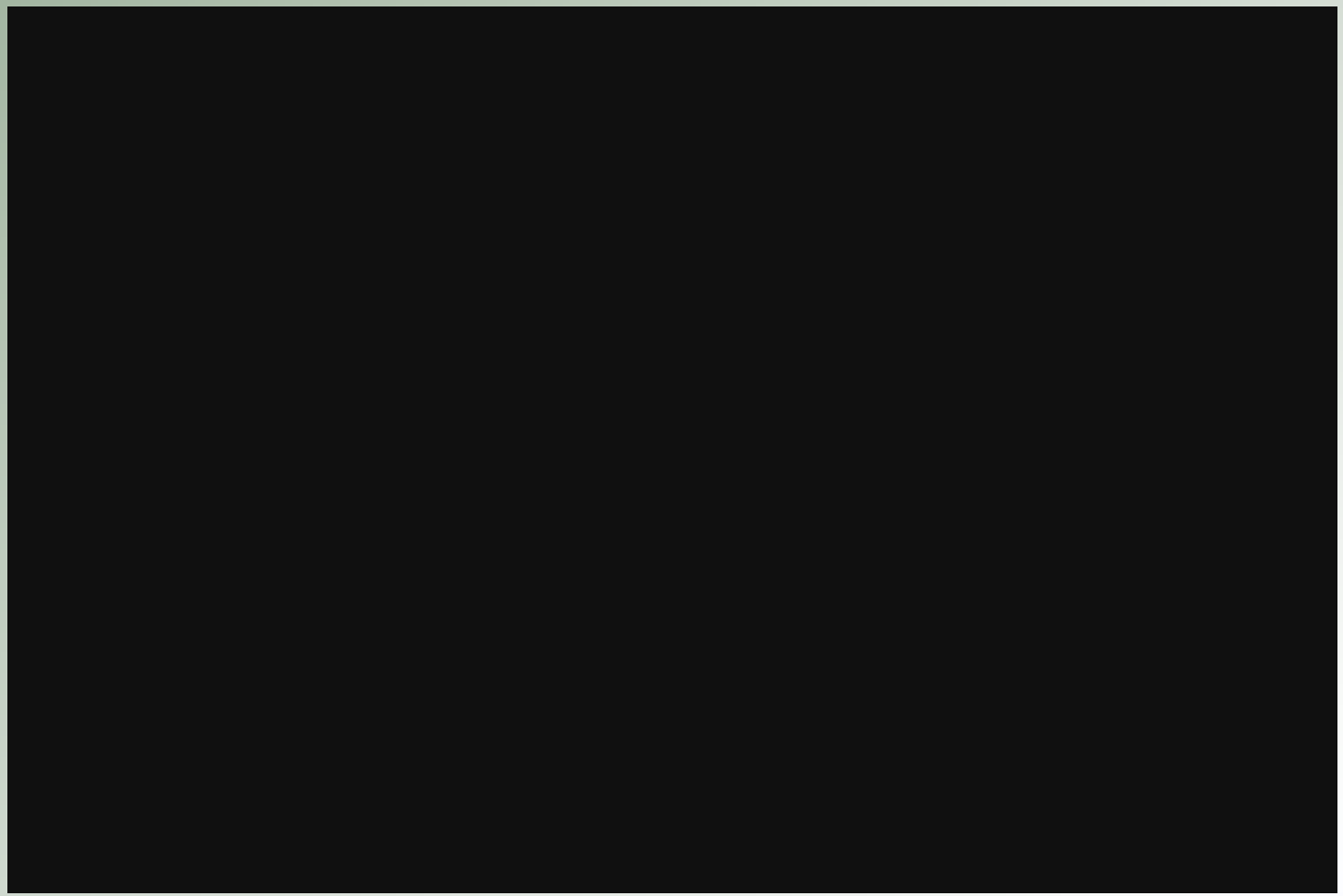
Mounds at Intermediate Δμ's



- Mounds appear at step edge and spread parallel to step
- Mounds generate multiple kinks
- Multiple kinks from mounds outside of viewfield enter viewfield

D.K. Georgiou, P.G. Vekilov, *Proc. Natl. Acad. Sci. USA* **103** (2006) 1681

Mounds: the Movie



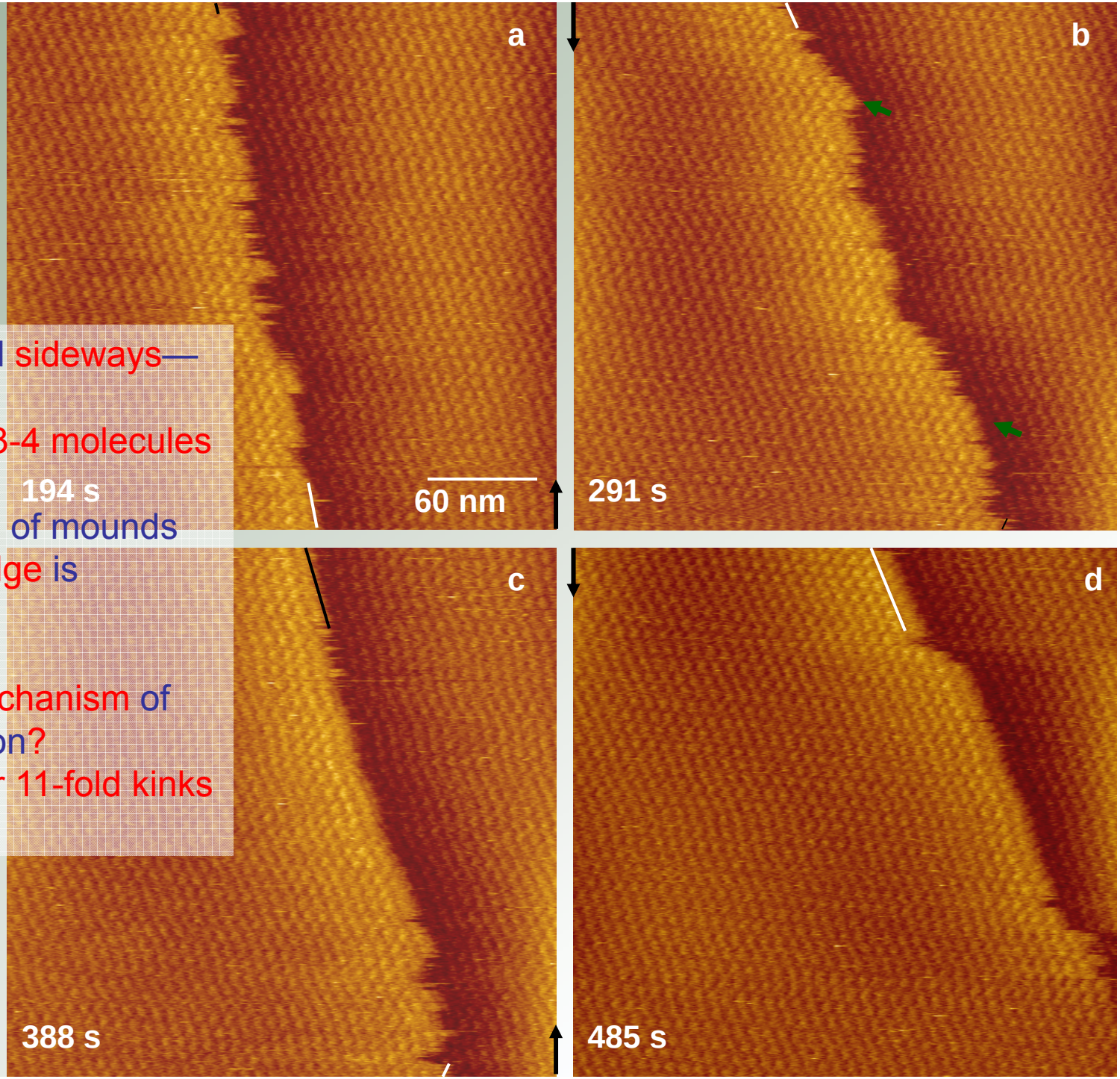
- Mounds appear at step and spread parallel to step
- After mounds spread out, straight step edge is restored
  - Mounds are NOT a form of instability

Further Instances of Mounds

- Mounds spread sideways—parallel to step
- Mounds are 2-3-4 molecules deep
- After spreading of mounds straight step edge is restored

➢ What is the mechanism of mound formation?

➢ Where do 10 or 11-fold kinks come from?



What is the Mounds' Mechanism?

Due to step intersections?

No, the observations of mounds—from straight step segments

"Kink Erlich-Schowoebel effect", other step-shape instability?

No, the mounds are well-defined, spread out and leave straight step segments

Nucleated on step edges?

No, such nucleation would require an energy barrier

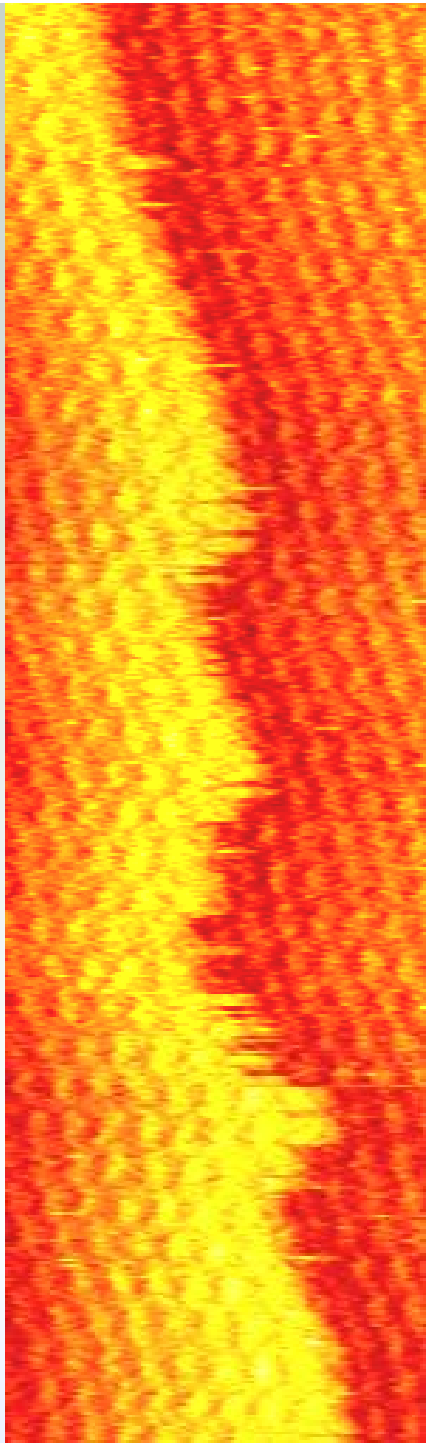
Possible sources of such barrier: Impurities
Lattice point defects
Lattice strain

No impurities were detected

A few point defects heal rapidly; not related to mounds

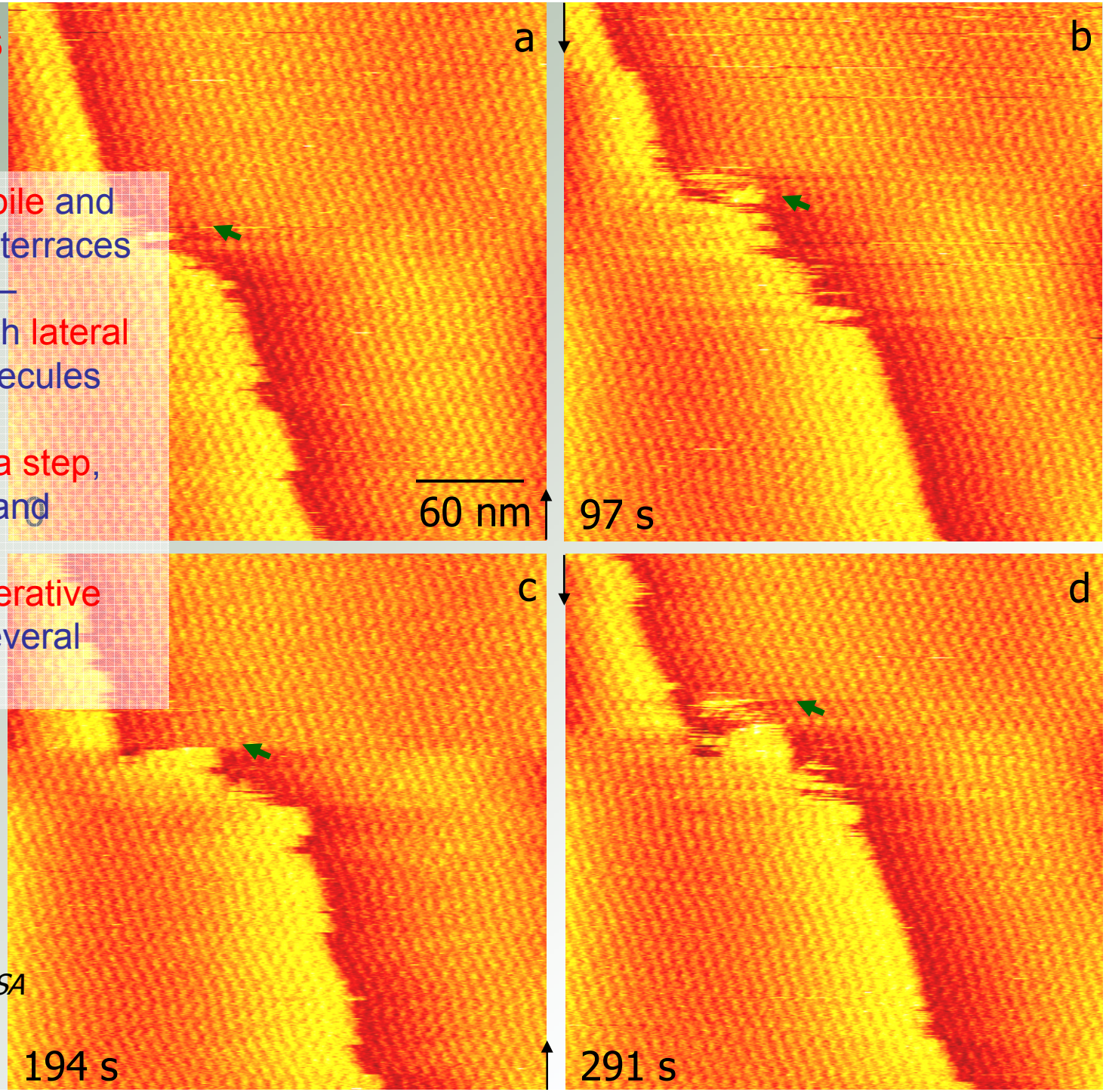
No lattice strain detected (cross-correlation analysis in GRIP)

Clusters pre–formed on the terraces and **associating** to steps?



2D Clusters

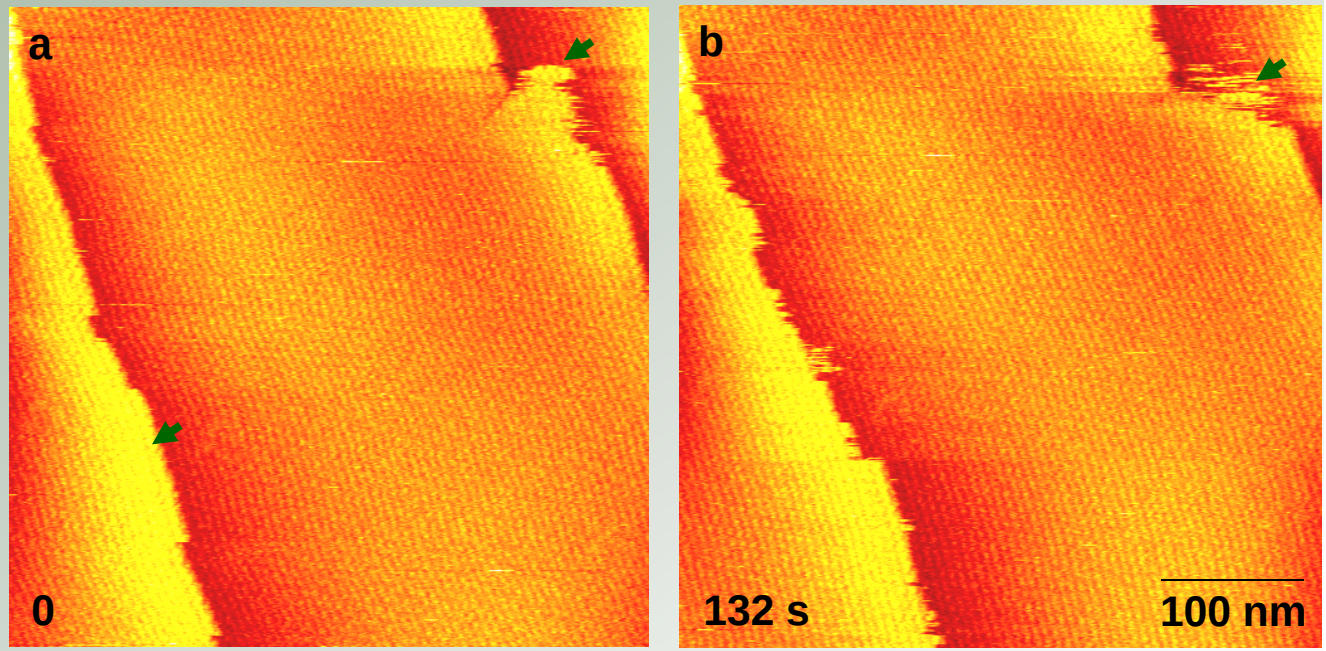
- Clusters are mobile and undetectable on terraces
- Cluster mobility—explained through lateral vibrations of molecules within them
- On approach to a step, clusters slowed and detected
- Sequential cooperative association of several clusters seen

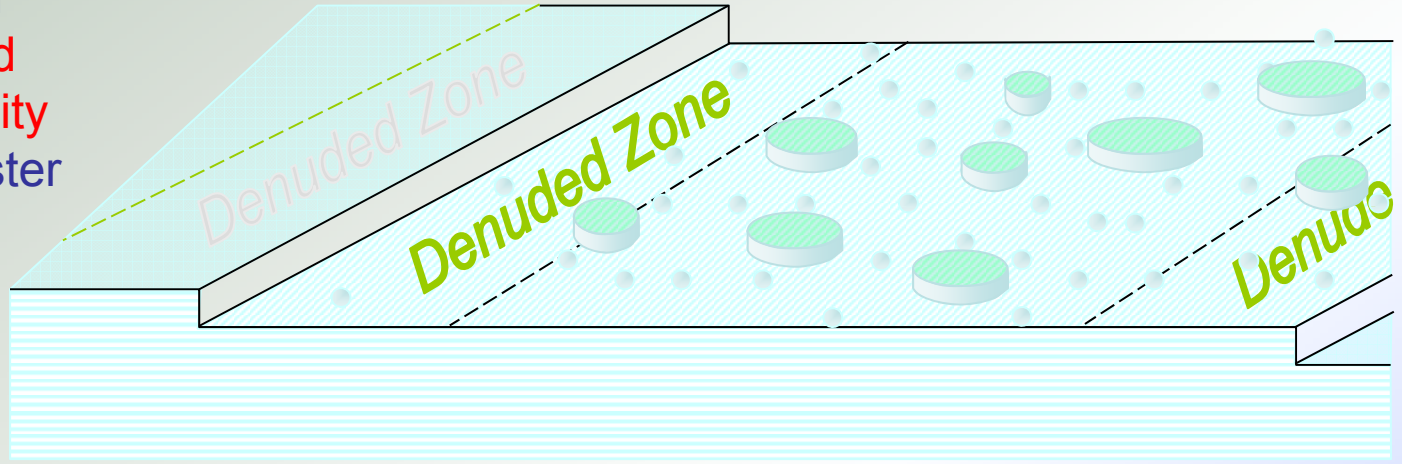


D.K. Georgiou,
P.G. Vekilov,
Proc. Natl. Acad. Sci. USA
103 (2006) 1681

Cooperative Association of Clusters

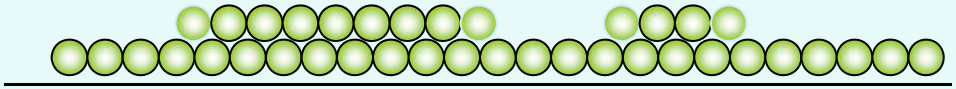
- Vicinity of step—denuded of adsorbed molecules and 2D clusters
- Randomly, clusters penetrate denuded zone and associate to steps
- Step promoted into a higher concentration zone
- Association of other clusters is facilitated
- Evolution of instability stopped by low cluster concentration





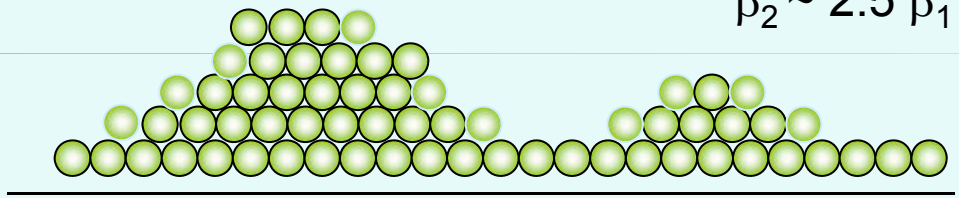
Non-linear Acceleration of Steps Due to Cluster Association

$0 < \sigma \leq 0.05$, no mounds, 4 kinks, β_1



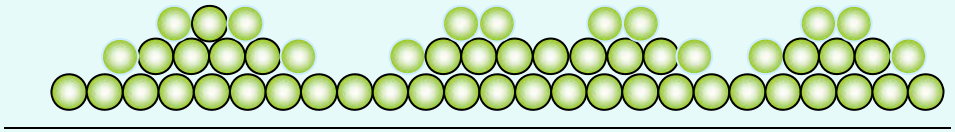
+

$0.05 \leq \sigma \leq 0.1$, 2 mounds, 10 kinks, $\beta_2 \sim 2.5\ \beta_1$



×

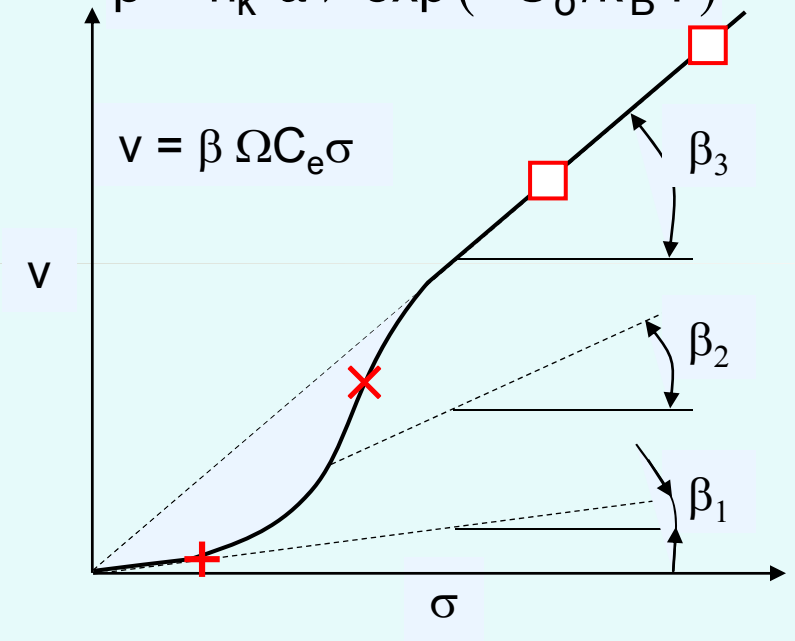
$0.1 < \sigma$, 4 mounds, 13 kinks, $\beta_3 \sim 3.2\ \beta_1$



□

$$\beta = \bar{n}_k^{-1} a \nu \exp\left(-U_o/k_B T\right)$$

$$v = \beta\, \Omega C_e \sigma$$



D.K. Georgiou, P.G. Vekilov, *Proc. Natl. Acad. Sci. USA* **103** (2006) 1681

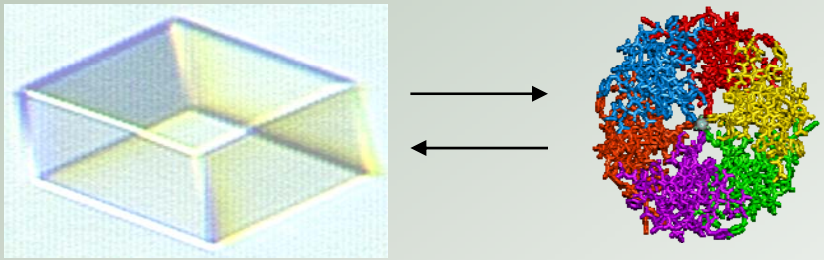
Ensures biological function of crystallization

Step and Face Kinetic Coefficients

Step kinetic coefficient: $v = \beta \Omega n_e \sigma$

Face kinetic coefficient: $R = \beta_f \Omega n_e \sigma$

v, nm s^{-1}	σ	β, cm s^{-1}	β_f, cm s^{-1}
0.1	< 0.05	2.2×10^{-3}	2.2×10^{-5}
0.6	≥ 0.1	6.3×10^{-3}	6.3×10^{-5}
>1	>0.2	6.3×10^{-3}	6.3×10^{-5}



v: step velocity **β**: kinetic coefficient
Ω: volume that one hexamer occupies in crystal
$\mathbf{n_e}$:number density of hexamers in a solution at equilibrium with crystals
σ : supersaturation, $\Delta\mu/kT$, $\mathbf{C_e}$: solubility
R : crystal growth rate
$\beta_f = \beta\ p$

What should σ *in vivo* be so that a 200 nm crystal can grow within 30 min? (R ≈ 0.05 nm·s^{-1})

If $\beta_f = 6.3 \times 10^{-5}$ cm s^{-1} $\sigma \approx 1$ is required
If $\beta_f = 2.2 \times 10^{-5}$ cm s^{-1} $\sigma \approx 3$ is required

Coupled Reactions of Proinsulin Conversion and Insulin Crystallization

What is the rate-determining step?

Is proinsulin conversion the slow step?
If yes, then C≈Ce and $\sigma \leq 0.1\text{-}0.2$

Is insulin crystallization the slow step?
If yes, it would result in accumulation of dissolved insulin and high σ values

but
For a 100nm crystal to grow within 30 min: supersaturation $\sigma \approx 1$
proinsulin conversion and insulin crystallization are kinetically coupled

Insulin Crystallization in the Presence Proinsulin

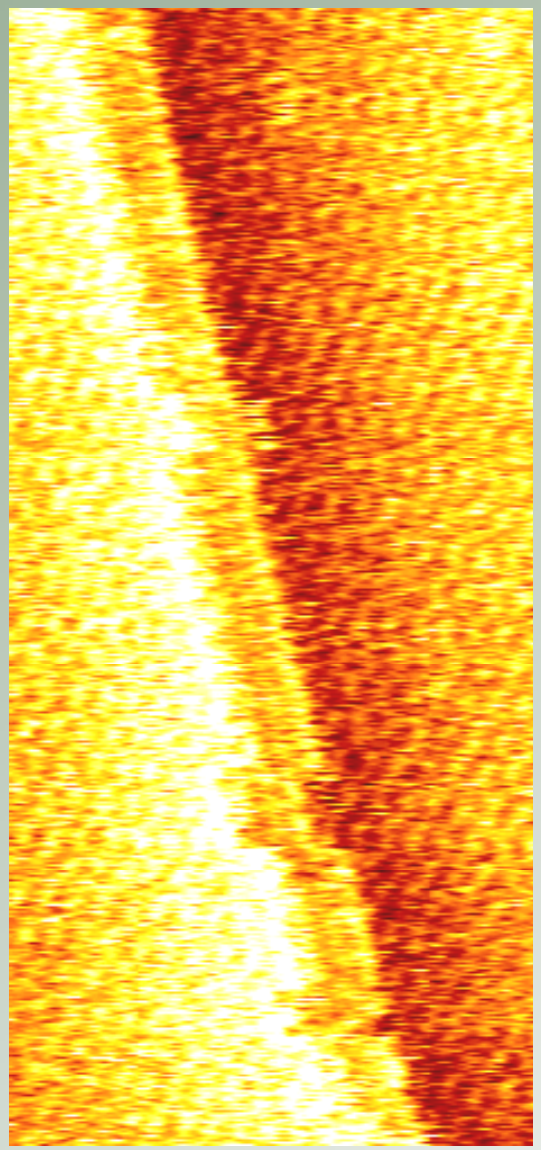
What happens at high proinsulin concentrations?

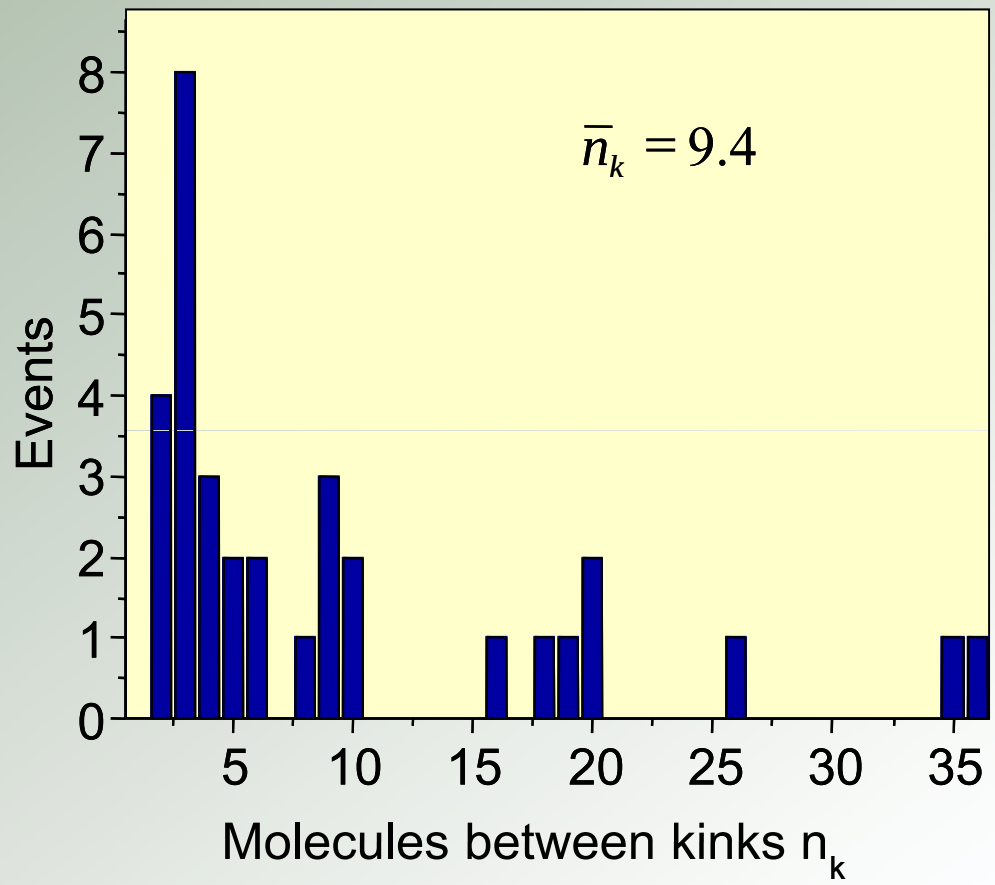
Proinsulin incorporates in the insulin crystals
Crystals become highly imperfect
Growth is delayed by hours and even days

Decrease the efficiency of insulin biosynthesis

Crystallization via mound-generated kinks mechanism provides a low supersaturation environment
Crystallization occurs in a nearly perfect manner and proinsulin is excluded

Kinks at Slightly Undersaturated Solutions





- Dissolution regulation mechanism: lower kink density ensures that crystal dissolution is slowed

Conclusions

New mechanism of step advancement through association of pre-formed clusters

The attached clusters result in higher
- kink density
- kinetic coefficient
- step velocity

Provides fast response to fluctuations of the rate of insulin production from pro-insulin

Chemical regulation of rate of crystal growth by destruction of water shells

Lack of step bunching ensures faster kinetics

Deletion of kinks in undersaturated solutions prevents dissolution

These mechanisms may be parts of **glucose-regulation pathways** in sick and healthy organisms