Nucleation of Crystals in Solution

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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
Mass Crystallization

- **Insulin as medication**
  - In 2003, the worldwide insulin market
    - $4.3 billion
    - projected to grow to $7.1 billion by 2010
  - Other crystalline pharmaceuticals
    - acetaminophen, ..., interferon, ...
  - **Insulin** crystallization in pancreas
    - 7% of all humans with diabetes in 2030

- **Products and intermediates in chemical industry**
  - adipic acid for nylon produced in Texas
  - 5,500,000 tons / year worldwide
  - shipped to new jersey for nylon production
Insulin Biosynthesis


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

The Goal: Crystals with “Just-right”…

- Number
- Polymorph
- Morphology
- Habit
- Size
- Size distribution

Requires data on:
- Solution PChem
  - Phase diagrams
  - Metastable states
- Nucleation mechanisms
- Growth mechanisms
- Agglomeration
- …
Only God and Gibbs never erred
... and this has been strictly shown for Gibbs only

E.D. Shchukin
Crystallization and Nucleation

Crystallization

Nucleation

...
Classical Nucleation Theory: Thermodynamics

Solution—supersaturated: $\mu_{\text{soln}} > \mu_{\text{crystal}}$, $\Delta \mu = \mu_{\text{soln}} - \mu_{\text{crystal}} > 0$

Free energy gain  $= -n\Delta \mu$

Free energy loss  $= 6\gamma n^{2/3}$

Creation of new surface

$\Delta G(n) = -n\Delta \mu + 6\gamma n^{2/3}$

$\Delta G^* = \frac{32\gamma^3}{\Delta \mu^2} = \frac{1}{2} n^* \Delta \mu$

$6\gamma n^{2/3}$

$\Delta G^*$

$\Delta G(n)$

$n^*$

$n$

$-n\Delta \mu$
Classical Nucleation Theory: Kinetics

\[ J = A C \exp \left( -\frac{\Delta G^*}{k_B T} \right) \]

Volmer M (1939)
Kinetik der Phasenbildung (Steinkopff, Dresden)

Assumes that clusters are perfect crystals

Predicts steep \( I(C, \Delta \mu(C)) \)
The Nucleation Rate

Galkin, O. 
Vekilov, P. G. 
*PNAS* 97, 6277 (2000)

**Maximum in J(T)**

- Exponential increase at intermediate $\Delta T$'s; by weak decrease at higher $\Delta T$'s
- $T$ of maximum shifts with concentration
Crystallization as Sequential Transition along Two Order Parameters

- Classical viewpoint: direct nucleation along a “diagonal line” envisioned;

- Two-step mechanism: suggested by t W & F, T & O for critical point for L-L phase separation for proteins

- Everywhere else in phase diagram—classical crystal nucleation predicted

The Two-step Mechanism

- It operates in all areas of the phase diagram
- It may apply to all crystals (and other ordered solids) forming in solution


Two steps: Which One is Rate Determining

Rate of cluster formation
\[ J_1 \sim J_{01} \exp(-\Delta G_1^*/k_B T) \]

Rate of nucleation within clusters
\[ J_2 \sim J_{02} \exp(-\Delta G_2^*/k_B T) \]

- Is \( \Delta G_1^* > \Delta G_2^* \) ?
- Is \( J_{01} \) more important than \( \Delta G_1^* \) ?
- Is \( J_{02} \) more important than \( \Delta G_2^* \) ?
Nucleation of Dense Liquid Droplets

$T - T_{LL} = 0.7 \, ^{\circ}C$

3.53 s | 6.09 s | 10.58 s

$T - T_{LL} = 1.3 \, ^{\circ}C$

0.96 s | 1.92 s | 7.37 s

- Number of droplets increases with time
- Faster nucleation at higher $\Delta T$'s

→ Characteristics of nucleation regime of droplet generation
Nucleation Rate of Dense Phase Droplets

- Number of droplets increases in time—nucleation regime
- Nucleation rate ~10^9 cm^{-3}s^{-1} significantly higher than rates of crystal nucleation ~ 0.1 – 1 cm^{-3}s^{-1}

Structuring of dense liquid quasi-droplet is the rate determining stage

Equilibrium between solution and clusters: \( \mu_{\text{solution}} = \mu_{\text{clusters}} \)

\[ \Delta \mu(\text{solution,crystal}) = \Delta \mu(\text{clusters,crystal}) \]
Why is the maximum in $J(T)$ sharp?

- Spinodal – boundary between metastable and unstable two-phase areas

Other proteins:
- Ferritin crystals grown at $\sigma = 4.2$, where $n^* \approx 1$
- Are protein crystals always grown in spinodal regimes?

Spinodal can be defined from $n^* \to 1$

Pre-exponential Factors and Barriers for Structuring

Liquidus or solubility of crystals
Gelation line
Solution-crystal spinodal
L-L coexistence
L-L spinodal

Why is the maximum in $J(T)$ sharp?

- $J(T)$ reaches sharp max at solution-crystal spinodal.

![Graph showing temperature vs. concentration with various coexistence lines and points.](image)
Heterogeneous Nucleation

Contradiction between
molecular level-viewpoint — templating
mesososcopic level viewpoint — wetting “angle”, decrease of free energy barrier

\[ \Delta G^*_{\text{hetero}} = \Delta G^*_{\text{homo}} \times F(\alpha) \]

- Rate of heterogeneous nucleation
  - linear function of $\Delta \mu$
  - no peculiarity at $\Delta G^*_2$ jumps with $n^*$
- $N_{\text{hetero}}$ – not a function of $\Delta G^*_2$
  - $\Delta G^*_2$ is insignificant
- Heterogeneous centers enhance growth of structured clusters

Polymorph Selection and Substrates

- Barriers for structuring—insignificant
- Substrates accelerate growth rate of structured clusters
- Different substrates—enhance formation of different polymorphs
  — structural similarity; — enhanced intermediate; — catalyzed bonds

The Two-step Mechanism for Other Crystals

- **Glycine, urea**

- **Charged colloid crystals**

- **NaClO₃**

- **NaCl nucleation from solution (MD simulation)**

- **Calcite nucleation**
Theoretical Justification of Generality of 2step Mechanism


For protein molecules

\[ \Delta G/kT = \begin{cases} a & k_B T = 0.30e \\ b & k_B T = 0.40e \\ c & k_B T = 0.50e \end{cases} \]

For small molecules

\[ \Delta G/kT = \begin{cases} d & k_B T = 0.50e \\ e & k_B T = 0.60e \\ f & k_B T = 0.70e \end{cases} \]

Two-step barrier always lower than direct barrier
Clusters and HbS Polymer Nucleation

- $\theta(T)$ much stronger than $R(T)$
contradicts 1-step nucleation and agrees with 2-step
D. Kashchiev, et al.,

- Polymers are perpendicular to plane of polarization of polarized light

- Dependencies of $r$, $V_l$ and $N_l$ of mesoscopic metastable clusters
on C and T
follow those of nucleated polymers

⇒ Clusters are precursors for polymer nuclei

Aggregation Precedes Ordering in Biological Self-assembly

- Hemoglobin assembly—from 2 α-chains, 2 β-chains and 4 heme-moieties after translocation
  α- and β-chains associate prior to folding

  Hemes attach to α₂β₂ complex and then enter assigned slots

- Nucleation of prion-protein fibers—via a disordered toxic fluid-like cluster

Summary and Conclusions

- A spinodal for the solution-to-crystal phase transition exists
  - The nucleation barrier in the vicinity of the spinodal is negligible
  - The nucleation rate reaches saturation or a maximum at the spinodal

- Assembly of ordered arrays
  - crystals, oligomers, fibers, etc.
  - is preceded by association into disordered clusters

- The precursor is a metastable mesoscopic liquid cluster
- Rate of crystal nucleation is determined by structuring of dense quasi-droplet
- Polymorph selection is determined by kinetics factors rather than by high barriers

- The low volume fraction of the nucleation precursors delays nucleation by $\sim 10^{10}$

- Understanding and control of nucleation in solution requires insights into the solution physicochemical mechanism nano- and mesostuctures
So What?

- Clusters are needed for nucleation of crystals.
  To enhance clusters:
  - moderate intermolecular attraction or repulsion
  - proper water structure around the protein molecules
- Crystal nucleation occurs in a spinodal regime
  - $\gamma$ is not important
- Simpler picture of nucleation and role of additives
- Heterogeneous particles may affect polymorph selection via structural similarity