UNIVERSITÄT LEIPZIG

b-ACT matter
Forschungs- und Transferzentrum für bioaktive Materie

Lecture 6

Soft Matter Physics

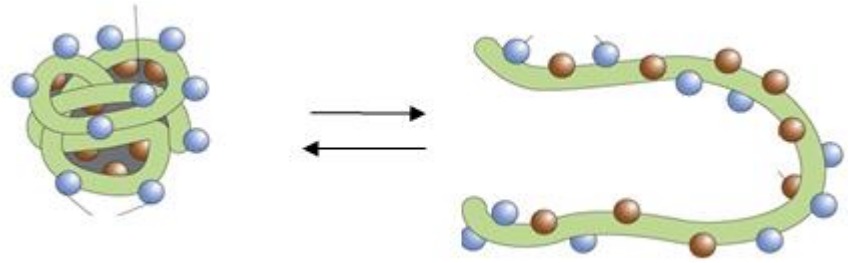
Thermal & phase transitions

- Thermal denaturation of proteins
- Cooperativity in thermal melting
- Phase transitions and classifications
- Unmixing of liquid phases

Thermal denaturation of proteins, DNA and lipids

Increasing temperature

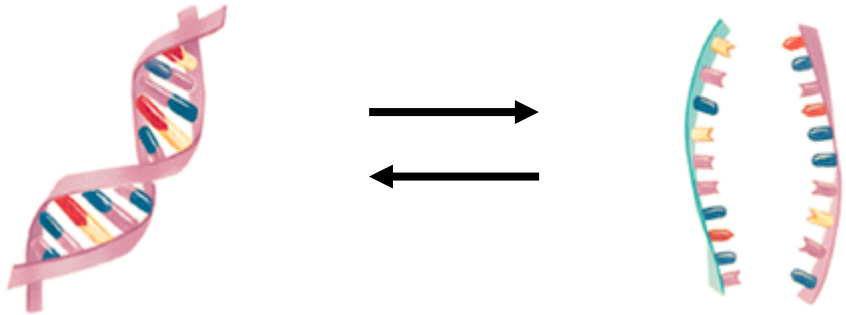
protein



(folded)

(unfolded, molten)

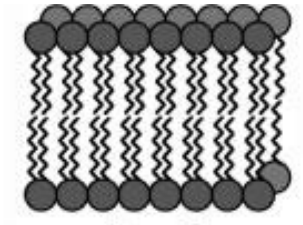
DNA



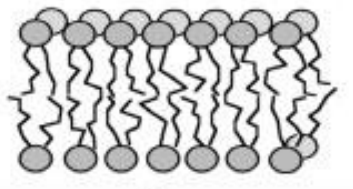
(double-stranded)

(single-stranded)

Lipids



(lamellar gel)



(lamellar fluid)

Increasing temperature

Recap: Chemical potential & Mass action law

μ_0 → Standard state chemical potential

(at standard conditions: p = 101.3 kPa, T = 298.15 K, c_0 = 1M)

For mixture(s): (homo/hetero)

$$\mu_i = \mu_{i,0} + k_B T \ln\left(\frac{c_i}{c_{i,0}}\right)$$

Relation chemical potential (μ) – Gibbs free energy (G):

$$\mu(T,p,N) = \left(\frac{\partial G}{\partial N}\right)_{T,p}$$

Chemical reaction:

$$-\nu_A\,A + -\nu_B\,B + \ldots \rightarrow \ldots + \nu_Y\,Y + \nu_Z\,Z$$

(stoichiometry factors ν_i)

Free energy change:

$$\Delta_r G = \underbrace{\sum_i \nu_i \mu_i}_{\Delta_r G^0} + k_B T \sum_i \ln\left(\frac{c_i}{c_{i0}}\right)^{\nu_i} = \Delta_r G^0 + k_B T \sum_i \ln\left(\frac{c_i}{c_{i,0}}\right)^{\nu_i}$$

Recap: Mass action law

Chemical reaction: $-\nu_A\,A + -\nu_B\,B + \ldots \rightarrow \ldots + \nu_Y\,Y + \nu_Z\,Z$

(ν_A and ν_B negative)

Free energy change:

$$\Delta_r G = \Delta_r G^0 + k_B T \sum_i \ln\left(\frac{c_i}{c_{i,0}}\right)^{\nu_i} = \Delta_r G^0 + k_B T \ln\left(\prod\left(\frac{c_i}{c_{i,0}}\right)^{\nu_i}\right)$$

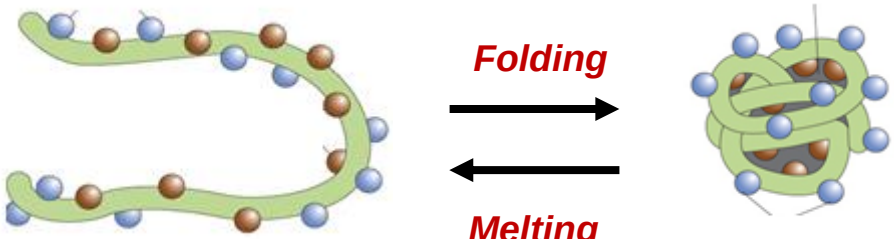
$$\Delta_r G = \Delta_r G^0 + k_B T \ln\left(\underbrace{\prod_i {c_i}^{\nu_i}}_{K_{eq}} \underbrace{\prod_i {c_{i,0}}^{-\nu_i}}_{c_0}\right) \longrightarrow \Delta_r G = \Delta_r G^0 + k_B T \ln\left(c_0 K_{eq}\right)$$

At equilibrium:

$$\Delta_r G = 0 \longrightarrow K_{eq} = c_0\, e^{-\Delta_r G^0/k_B T} = \frac{\ldots [Y][Z]}{[A][B]\ldots}$$

Thermal denaturation of a small 2-state system

Example: protein



(**U**nfolded, molten)

(**F**olded)

$$U \rightleftharpoons F$$

$$[P]_{total} = [U] + [F]$$

Free energy of folding:

$$\Delta_r G_{fold} = \Delta_r G^0_{fold}(p,T) + k_B T \ln\left(\frac{[F]}{[U]}\right)$$

"end-product"

"substrate"

What happens at melting temperature (T_m)?

50% fraction **F**olded + 50% fraction **U**nfolded

$$[U] = [F] = [P]_{total}/2$$

Thermal denaturation of a small 2-state system

At melting temperature (T_m):

$$[U] = [F] = [P]_{total}/2$$

Free energy of folding: $\Delta_r G_{fold} = \Delta_r G^0_{fold}(p,T) + \underbrace{k_B T \ln\left(\frac{[F]}{[U]}\right)}_{0}$

$$\Delta_r G_{fold} = \Delta_r G^0_{fold} = \Delta_r H^0_{fold} - T\Delta_r S^0_{fold}$$

During folding reaction: $\Delta_r H^0_{fold} < 0;$ (heat release; e.g. H-bond formation) $\Delta_r S^0_{fold} < 0$ (lower configurational entropy)

What happens when we change T?

T small (folding): $\Delta_r G_{fold} < 0$ … $\Delta_r H^0_{fold}$ dominates

T high (melting): $\Delta_r G_{fold} > 0$ … $\Delta_r S^0_{fold}$ dominates

T = T_m: $\Delta_r G_{fold} = 0$ … both states eq. populated

Thermal denaturation of a small 2-state system

When T = T_m:

("Equilibrium")

$$\Delta_r G_{fold} = \Delta_r H^0_{fold} - T_m \, \Delta_r S^0_{fold} = 0$$

$$\boldsymbol{T_m = \frac{\Delta_r H^0_{fold}}{\Delta_r S^0_{fold}}}$$ ⟵ "competition" between $\Delta_r H$ and $\Delta_r S$

Use mass action law to study changes in **state fraction as function of *T***

$$K_{eq} = K_{fold} = e^{-\Delta_r G^0_{fold}/k_B T} = \frac{[F]}{[U]} = \frac{[P]-[U]}{[U]}$$

$$[P]-[U] = [U]\, K_{fold} \Leftrightarrow [P] = [U]\, K_{fold} + [U]$$

$$[P] = [U]\left(K_{fold} + 1\right)$$

Fraction of unfolded protein

$$\rho_{unfold} = \frac{[U]}{[P]} = \frac{1}{K_{fold}+1}$$

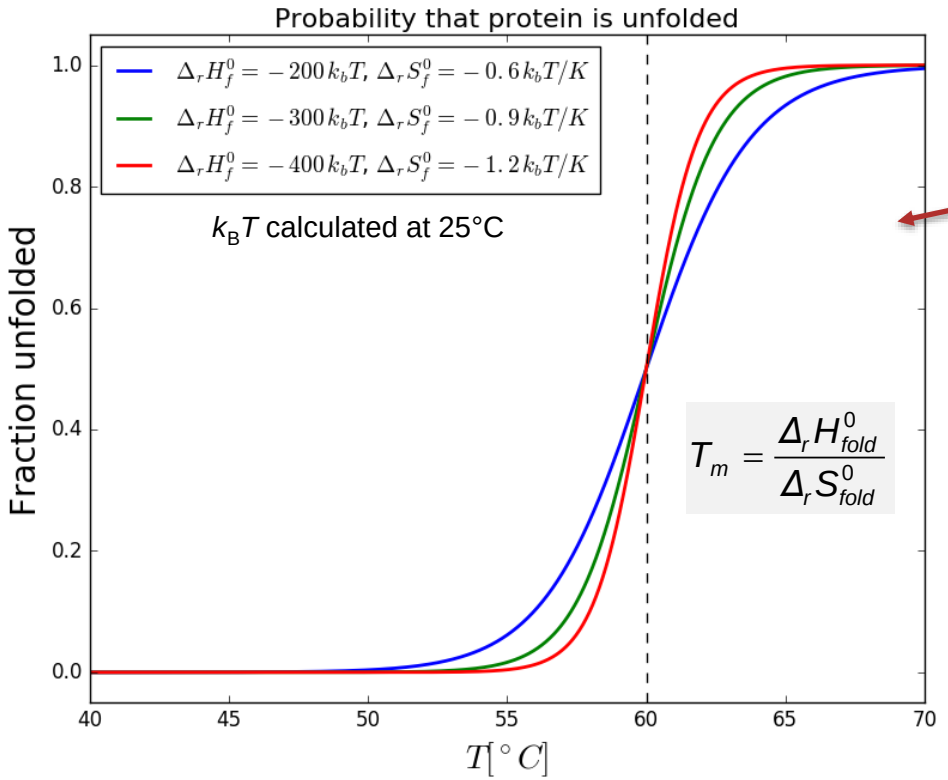
$$\rho_{unfold} = \frac{1}{e^{-\Delta_r G^0_{fold}/k_B T}+1}$$

$$\rho_{unfold} = \frac{1}{e^{-\Delta_r H^0_{fold}/k_B T}\, e^{-\Delta_r S^0_{fold}/k_B}+1}$$

Thermal denaturation of a small 2-state system

When T = T_m: $\Delta_r G_{fold} = \Delta_r H^0_{fold} - T_m \, \Delta_r S^0_{fold} = 0$

$$T_m = \frac{\Delta_r H^0_{fold}}{\Delta_r S^0_{fold}}$$ ← "competition" between $\Delta_r H$ and $\Delta_r S$



Fraction of unfolded protein

$$\rho_{unfold} = \frac{1}{e^{-\Delta_r H^0_{fold}/k_BT}\ e^{-\Delta_r S^0_{fold}/k_B} + 1}$$

Proteins with different standard enthalpy/entropy, but same T_m

With increased entropy change ($\Delta_r S^0_{fold} \ll 0$)

→ steeper curves

→ more sensitive to temperature

Heat capacity during denaturation of a 2-state system

Enthalpy changes $\Delta_r H^0_{fold}$ can be directly measured using calorimetry → Measure of heat taken up in the reaction

$$H_{total} = \rho_{fold} H_{fold} + \rho_{unfold} H_{unfold} = \rho_{fold} H_{fold} + \rho_{unfold} \left(H_{fold} - \boldsymbol{\Delta_r H^0_{fold}} \right)$$

$$\Delta_r H^0_{fold} = H_{fold} - H_{unfold}$$

$$H_{total} = \underbrace{\rho_{fold} H_{fold} + \rho_{unfold} H_{fold}}_{H_{fold}} - \rho_{unfold} \Delta_r H^0_{fold} = H_{fold} - \rho_{unfold} \Delta_r H^0_{fold}$$

$$H_{fold}(T) = constant$$

Heat capacity at constant pressure

$$c_p = \left(\frac{\partial H_{tot}}{\partial T} \right)_p$$

Heat capacity
as function of *T*

$$c_p(T) = -\Delta_r H^0_{fold} \left(\frac{\partial \boldsymbol{\rho_{unfold}}}{\partial T} \right)_p = \frac{\left(\Delta_r H^0_{fold} \right)^2}{k_B T^2} \frac{e^{-\Delta_r H^0_{fold}/k_B T} e^{\Delta_r S^0_{fold}/k_B}}{\left(e^{-\Delta_r H^0_{fold}/k_B T} e^{\Delta_r S^0_{fold}/k_B} + 1 \right)^2}$$

Heat capacity during denaturation of a 2-state system

Heat capacity as function of *T*

$$c_p(T) = -\Delta_r H^0_{fold} \left(\frac{\partial \rho_{unfold}}{\partial T} \right)_p$$

$$c_p(T) = \frac{\left(\Delta_r H^0_{fold}\right)^2}{k_B T^2} \frac{e^{-\Delta_r H^0_{fold}/k_B T} e^{\Delta_r S^0_{fold}/k_B}}{\left(e^{-\Delta_r H^0_{fold}/k_B T} e^{\Delta_r S^0_{fold}/k_B} + 1\right)^2}$$

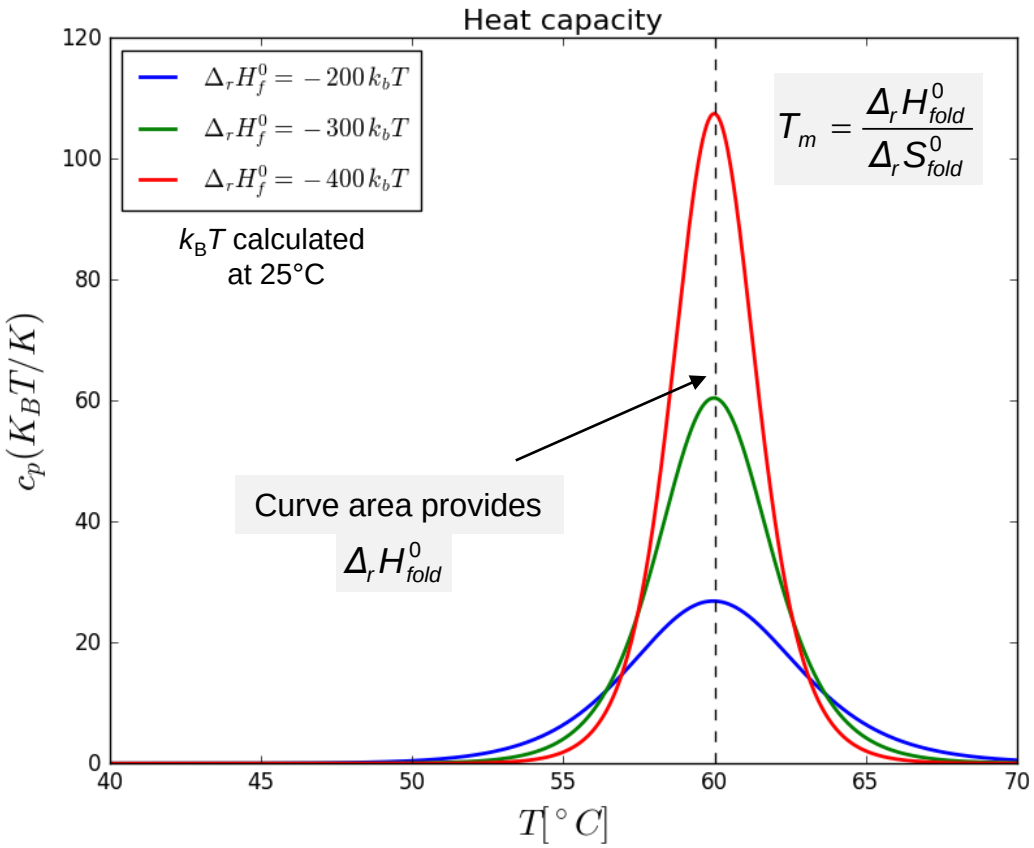
Characteristic bell-like curve near T_m

→ *T* range with strongest change in ρ_{unfold}

→ Curve width … indicates sharpness of transition

(extract $\Delta_r H^0_{fold}$ and $\Delta_r S^0_{fold}$)

→ $\int \rho_{unfold}\, dT = 1$, curve area provides $\Delta_r H^0_{fold}$



Proteins with different standard enthalpy/entropy, but same T_m

Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC)

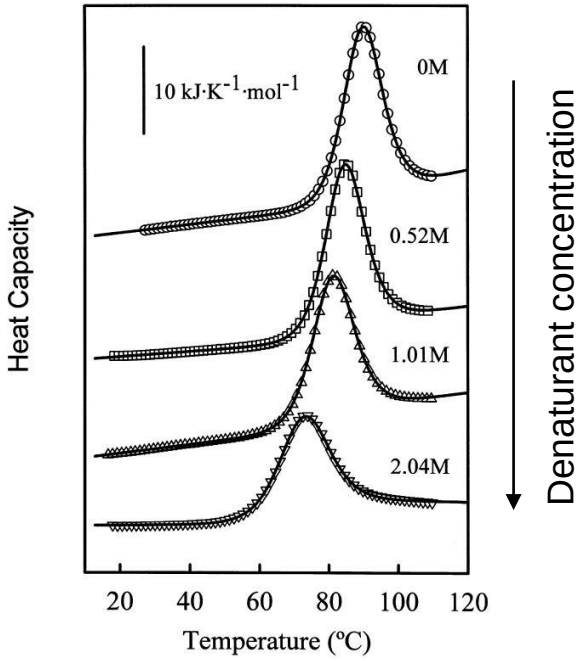
Calorimeter scans heat flux for given ΔT
with respect to reference volume

Heat capacity
as function of *T*

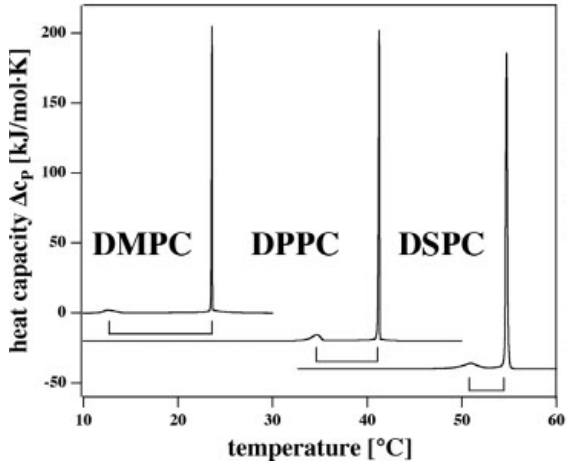
$$c_p = \left(\frac{\partial H_{tot}}{\partial T}\right)_p$$

DSC is widely applied folding/melting and phase transitions

(e.g., biomolecules, polymers, liquid crystals, …)

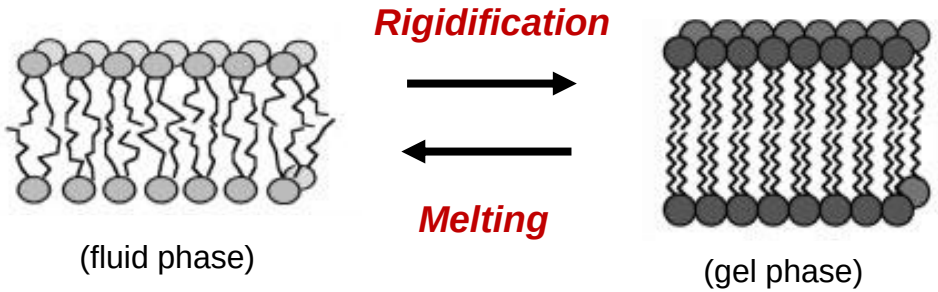


(Protein in presence of denaturant agent)



(Lipid bilayer membranes
undergoing
phase transition)

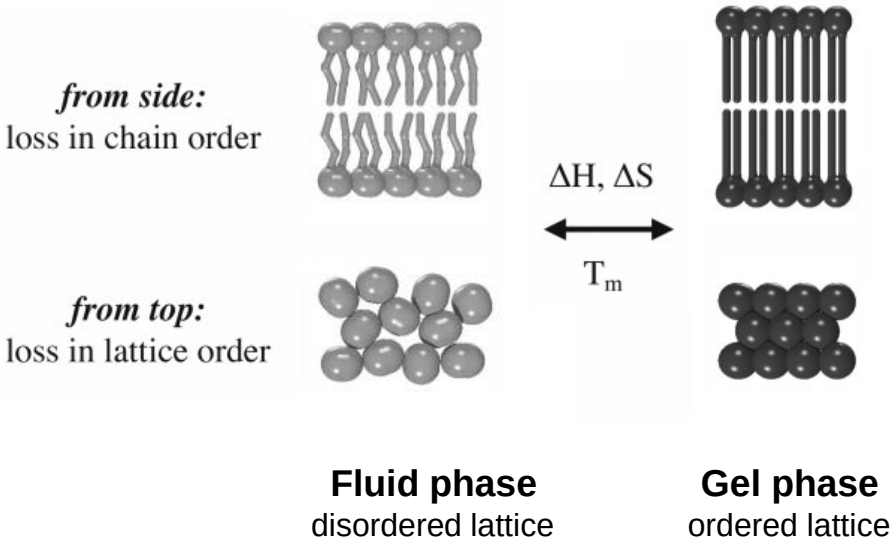
Cooperativity during thermal melting



Lipid bilayer membrane

$$Fluid \rightleftharpoons Gel$$

$$[L]_{total} = [F] + [G]$$



Heat capacity as function of *T*

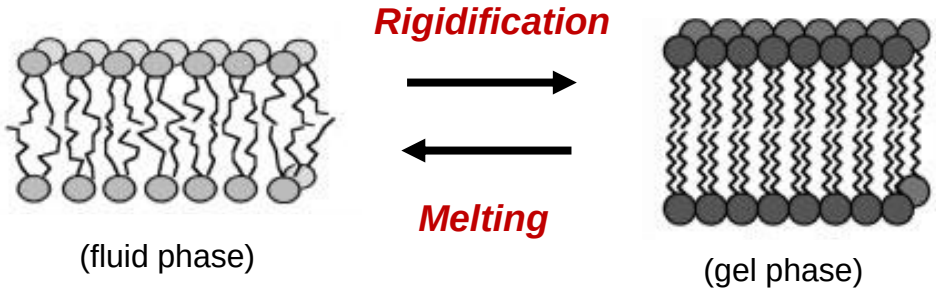
$$\rho_{melted} = \frac{[fluid\ lipid]}{[total\ lipid]} = \frac{1}{K_{eq} + 1}$$

$$c_p(T) = -\Delta_r H^0_{rig} \left(\frac{\partial \rho_{melted}}{\partial T} \right)_p$$

$$c_p(T) = \frac{\left(\Delta_r H^0_{rig}\right)^2}{k_B T^2} \frac{K_{eq}}{\left(K_{eq} + 1\right)^2}$$

These terms don't include cooperativity yet!

Cooperativity during thermal melting



Cooperativity factor n (like Hill's coefficient)

$$n\,L \rightleftharpoons L_n$$

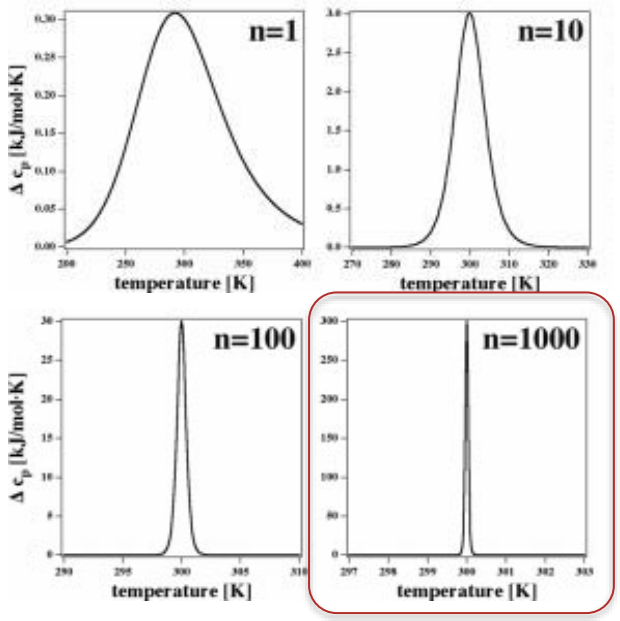
Lipid cluster

$$\Delta_r H^0_{rig} \rightarrow n\,\Delta_r H^0_{rig}$$
$$\Delta_r S^0_{rig} \rightarrow n\,\Delta_r S^0_{rig}$$
$$\Delta_r G^0_{rig} \rightarrow n\,\Delta_r G^0_{rig}$$

Heat capacity as function of *T*

$$c_p(T) = \frac{\left(n\Delta_r H^0_{rig}\right)^2}{k_B T^2} \frac{e^{-n\Delta_r H^0_{rig}/k_B T} e^{n\Delta_r S^0_{rig}/k_B}}{\left(e^{-n\Delta_r H^0_{rig}/k_B T} e^{n\Delta_r S^0_{rig}/k_B} + 1\right)^2}$$

Melting not independent → in cluster of n lipids



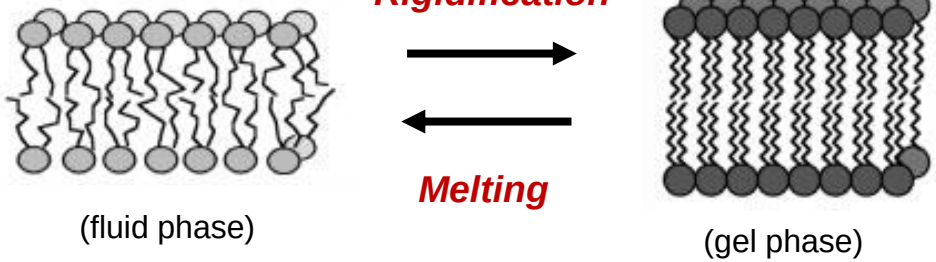
Cooperativity:

Larger n

→ Sharper peak

→ Narrow transition

Cooperativity during thermal melting



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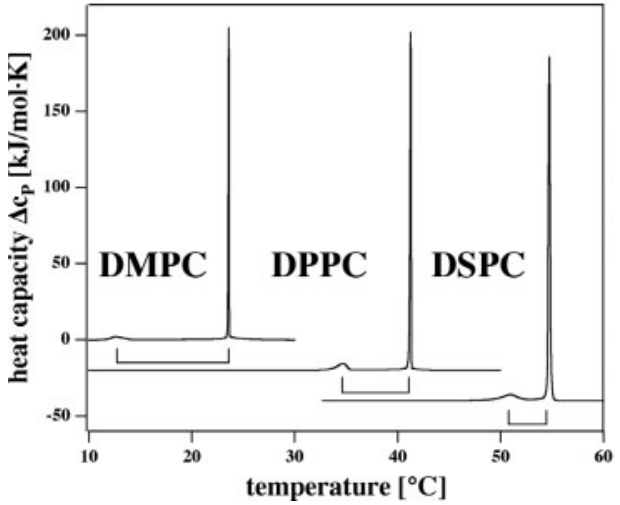
$$\Delta_r S^0_{rig} \rightarrow n\,\Delta_r S^0_{rig}$$
$$\Delta_r G^0_{rig} \rightarrow n\,\Delta_r G^0_{rig}$$

Heat capacity as function of *T*

$$c_p(T) = \frac{\left(n\Delta_r H^0_{rig}\right)^2}{k_B T^2} \frac{e^{-n\Delta_r H^0_{rig}/k_B T} e^{n\Delta_r S^0_{rig}/k_B}}{\left(e^{-n\Delta_r H^0_{rig}/k_B T} e^{n\Delta_r S^0_{rig}/k_B} + 1\right)^2}$$



Cooperativity:

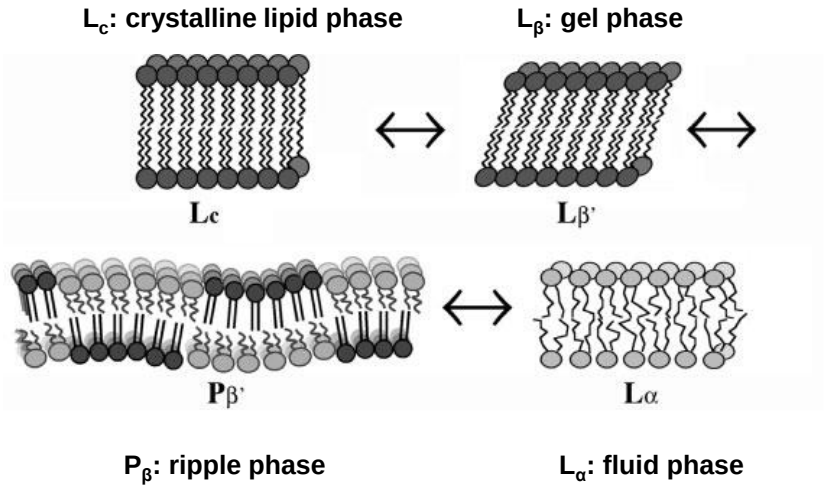
Larger n

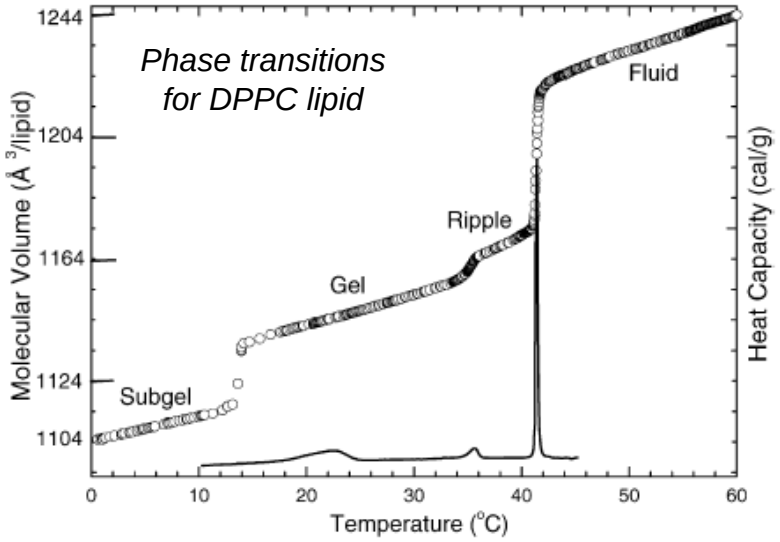
→ Sharper peak

→ Narrow transition

Melting not independent → in cluster of n lipids

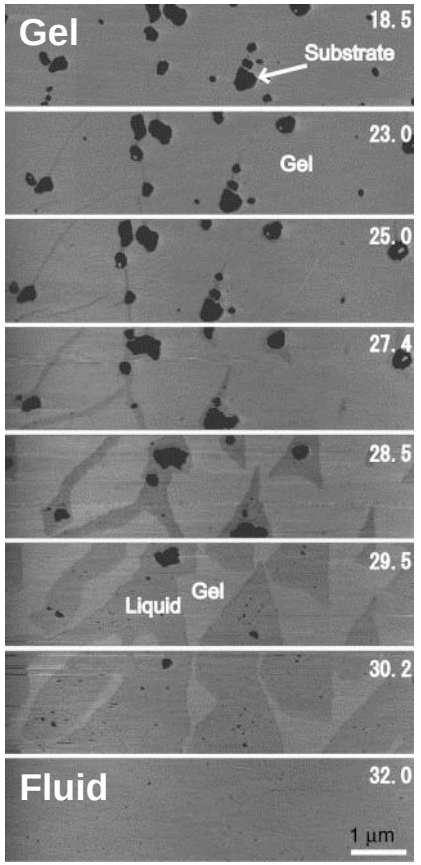
From thermal melting to phase transitions (e.g., in lipids)





AFM images/movies of DMPC supported lipid bilayers

Main transition

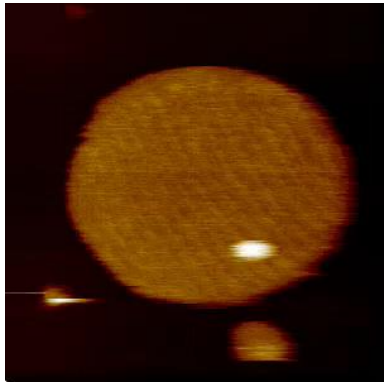


Heat

https://onlinelibrary.wiley.com/action/downloadSupplement?doi=10.1002%2Fsmll.201601549&file=smll201601549-sup-0006-S6.mov

Pre-transition

Ripple-phase <-> Fluid phase



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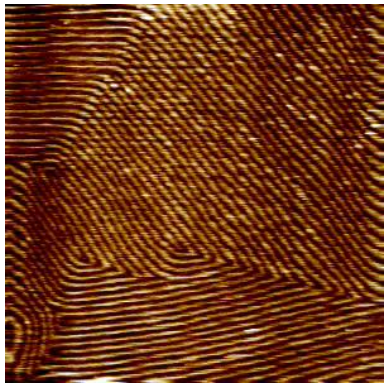
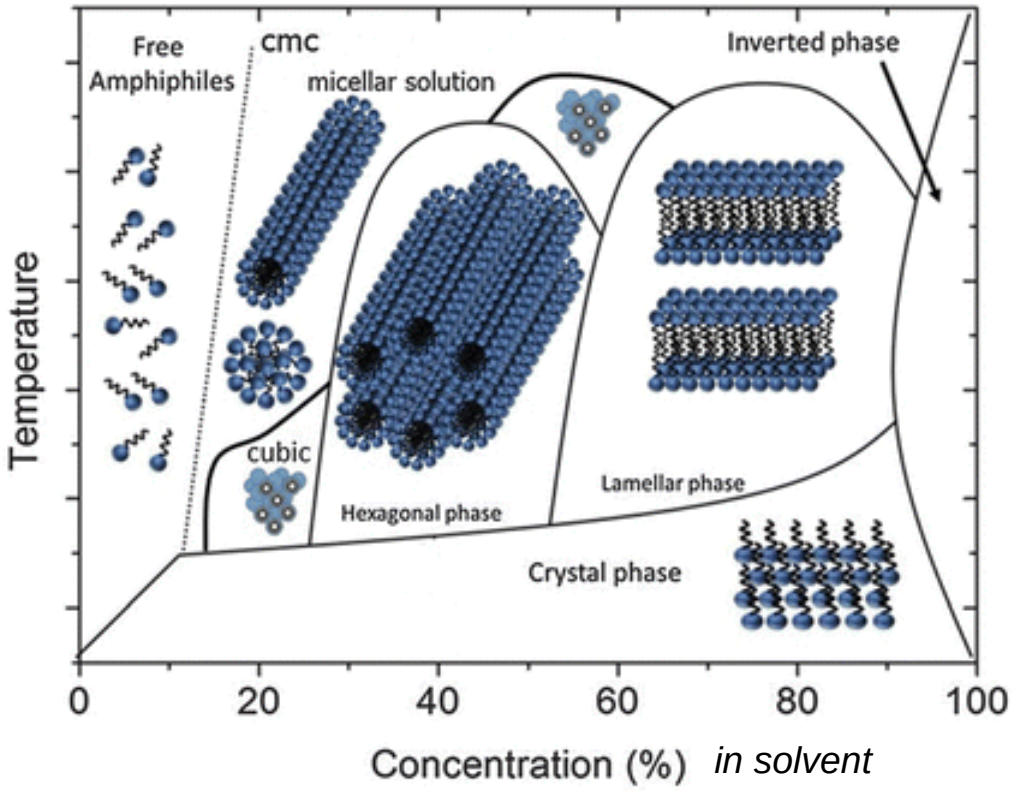
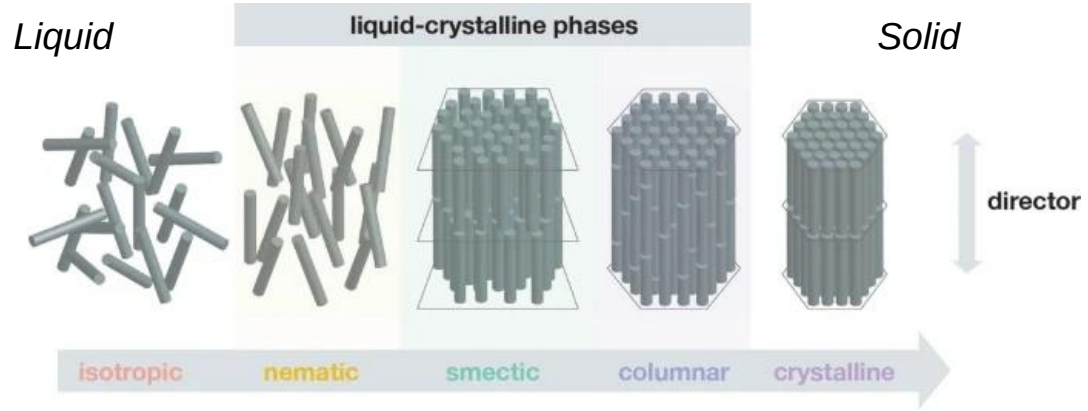


Phase transitions can be complex (e.g., in liquid crystals)

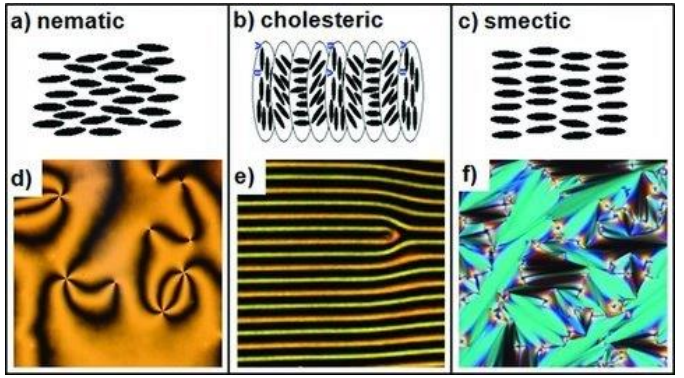
Amphiphiles, such as lipids, are **lyotropic** liquid crystals (depend on *T* and C in solvent)



Thermotropic liquid crystals → mesophase depends on *T*

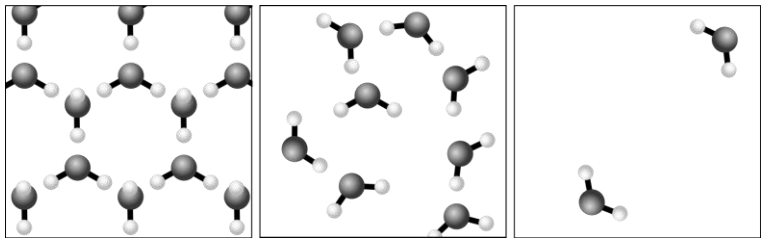


Increasing *T*



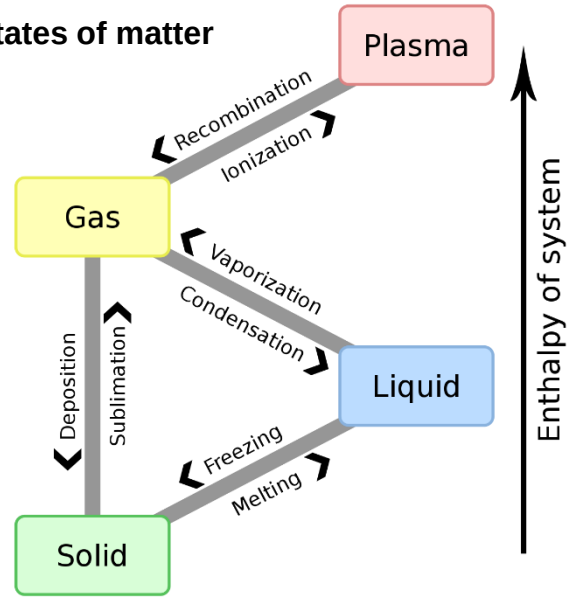
Phase transitions of a substance

Example: Water



Solid phase | Liquid phase | Gas phase

Classical states of matter

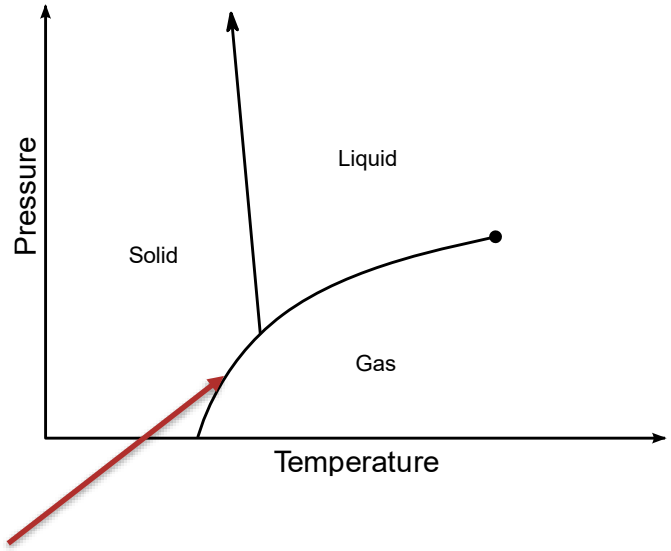


Phase transition of substance: spontaneous/abrupt transition between different states of qualitative uniform properties

Phase transitions occur at specific transition temperatures (e.g. T_m) **at given pressure**

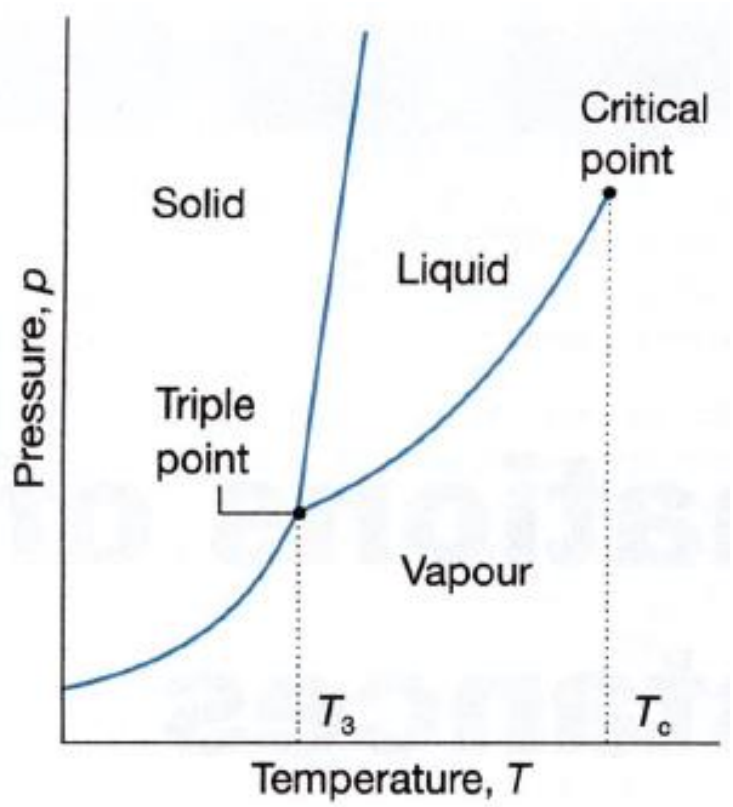
Phase diagrams show T, p regions at which phases are thermodynamically stable

Phase diagram



Phase boundaries show T, p regions at which sets of two phases coexist in equilibrium

Phase transitions of a substance

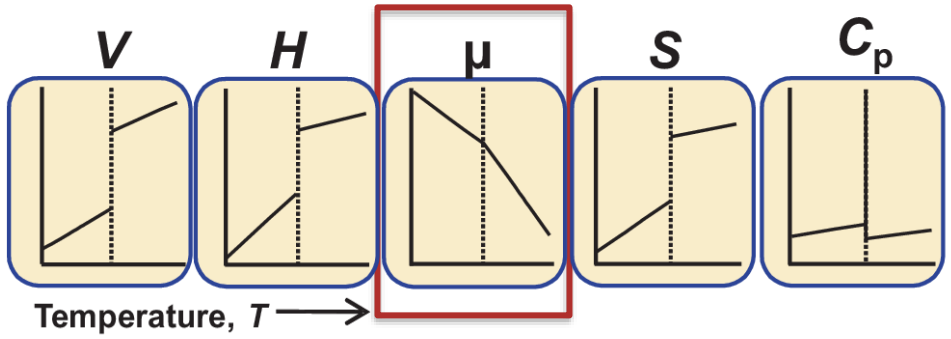


Triple point: *T, p* point with *s-l-g* coexistence

Critical point: *T, p* so high → super-critical fluid

Ehrenfest classification of phase transitions

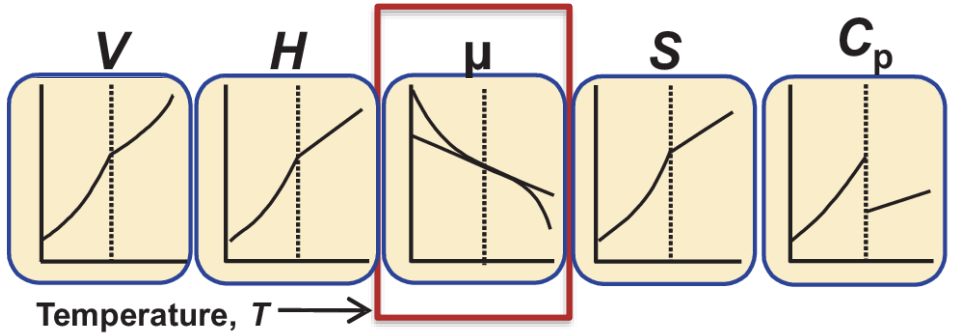
1st order



e.g., melting/freezing, evaporation/condensation

First derivate of μ discontinuous

2nd order



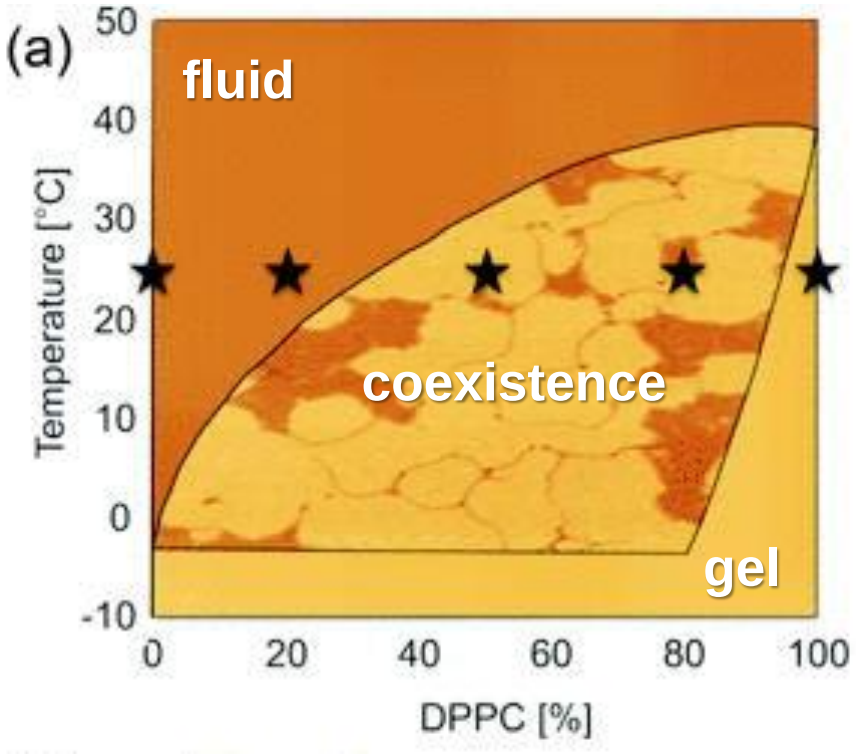
e.g., (super-)conducting, certain solid-solid transitions

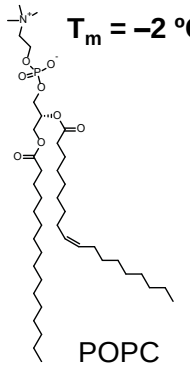
Second derivate of μ discontinuous

Phase transitions of a mixture of substances

Phase transitions for mixture of substances (e.g., *n* (im-)miscible compounds)

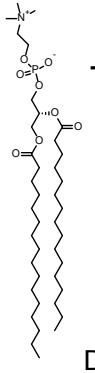
Phase separation with binary mixtures of lipids





T_m = –2 °C

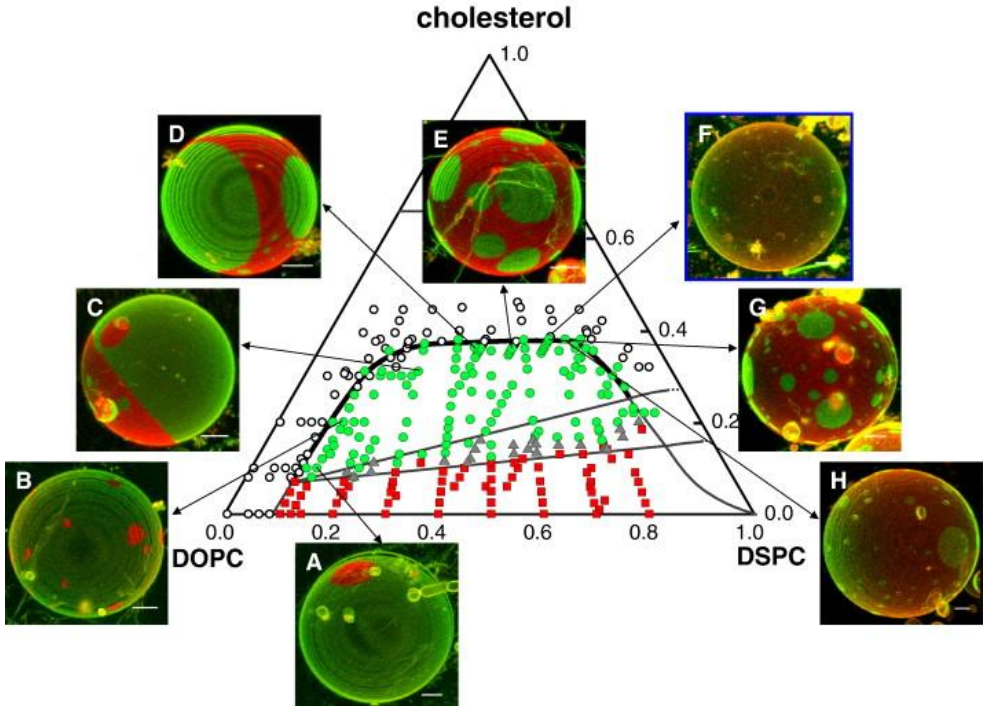
POPC



T_m = 41°C

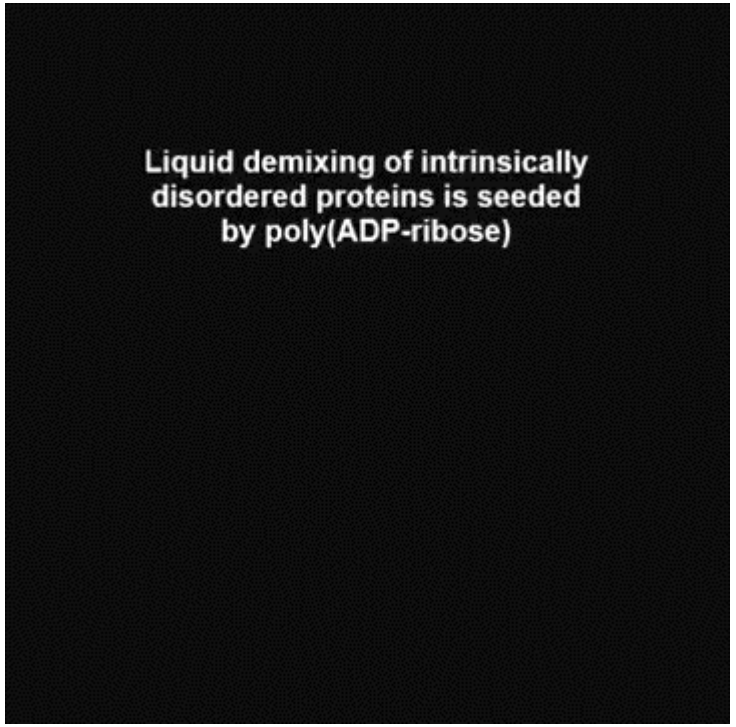
DPPC

Phase separation with ternary mixtures of lipids



Phase transitions of a mixture of substances

Liquid phase-sep. compartments in cells:



https://www.youtube.com/watch?v=QQFWPRMXzI0

Ouzo effect: *Ouzo (Anethole+EtOH) + H_2O*

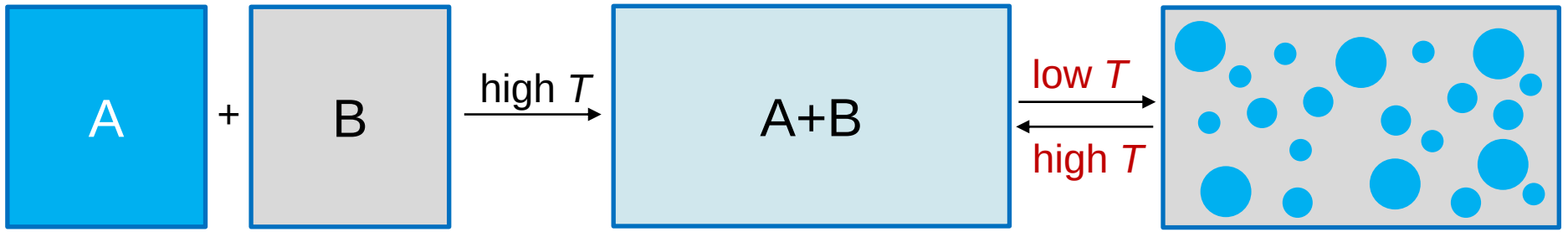
https://www.youtube.com/watch?v=t5jbxh0C0UU

All examples shown here describe the **unmixing of two liquid phases** (either in 2D or 3D)

Liquid-liquid unmixing

Regular solution model:

Volume fractions: $\Phi_A + \Phi_B = 1$



Two liquids at *T*, *V* constant

↓

Goal to calculate ΔF_{mix}

$$\Delta F_{mix} = \underbrace{F_{A+B}}_{mix} - \underbrace{(F_A + F_B)}_{unmix}$$

Using Boltzmann formula $S = -k_B \sum_i p_i \ln p_i$ (*Shannon Entropy*), calculate $S_{mix} = -k_B(\Phi_A \ln \Phi_A + \Phi_B \ln \Phi_B)$

Assumptions:

1) Molecules arranged into lattice with z nearest neighbors
2) Interaction energy terms are pairwise additive ($\varepsilon_{AA}, \varepsilon_{BB}, \varepsilon_{AB}$)
3) Given site (A & B molecule) has $z\Phi_A$ A neighbors and $z\Phi_B$ B neighbors

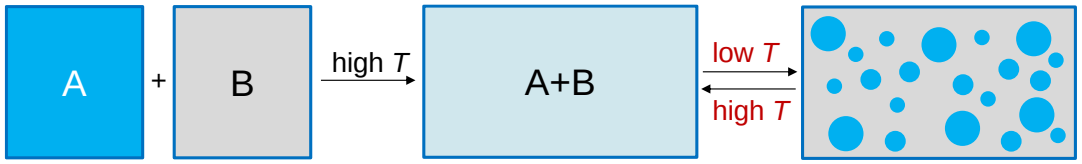
Internal energy for mixed vs. unmixed states:

$$U_{mix} = \frac{1}{2}\left(\underbrace{z\Phi_A^2\varepsilon_{AA} + z\Phi_B\Phi_A\varepsilon_{AB}}_{\text{A on site}} + \underbrace{z\Phi_A\Phi_B\varepsilon_{AB} + z\Phi_B^2\varepsilon_{BB}}_{\text{B on site}}\right)$$

$$U_{unmix} = \frac{1}{2}\left(\underbrace{z\Phi_A\varepsilon_{AA}}_{\text{site A}} + \underbrace{z\Phi_B\varepsilon_{BB}}_{\text{site B}}\right)$$

Liquid-liquid unmixing

$$\Phi_A + \Phi_B = 1$$



$$U_{mix} = \frac{1}{2}\left(\underbrace{z\Phi_A^2\varepsilon_{AA} + z\Phi_B\Phi_A\varepsilon_{AB}}_{\text{A on site}} + \underbrace{z\Phi_A\Phi_B\varepsilon_{AB} + z\Phi_B^2\varepsilon_{BB}}_{\text{B on site}}\right)$$

$$U_{unmix} = \frac{1}{2}\left(\underbrace{z\Phi_A\varepsilon_{AA}}_{\text{site A}} + \underbrace{z\Phi_B\varepsilon_{BB}}_{\text{site B}}\right)$$

$$\Delta U_{mix} = U_{mix} - U_{unmix} = \frac{z}{2}(\Phi_A^2\varepsilon_{AA} + \Phi_B^2\varepsilon_{BB} + 2\Phi_B\Phi_A\varepsilon_{AB} - \Phi_A\varepsilon_{AA} - \Phi_B\varepsilon_{BB})$$

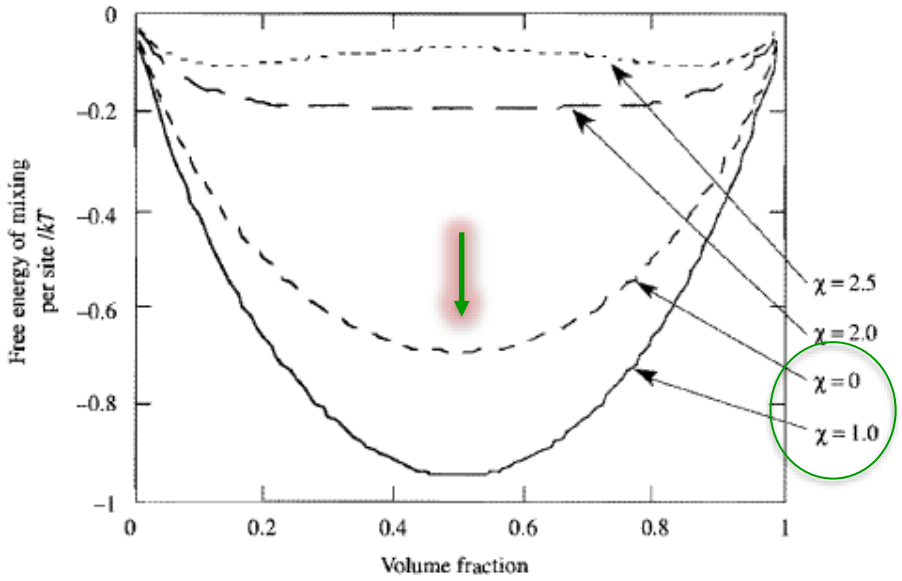
$$\Delta U_{mix} = \frac{z}{2}\left(\underbrace{(\Phi_A^2 - \Phi_A)}_{-\Phi_A\Phi_B}\varepsilon_{AA} + \underbrace{(\Phi_B^2 - \Phi_B)}_{-\Phi_A\Phi_B}\varepsilon_{BB} + 2\Phi_B\Phi_A\varepsilon_{AB}\right)$$

$$\chi = \frac{\Delta U_{mix}}{k_B T\Phi_A\Phi_B} = \frac{z}{2}(2\varepsilon_{AB} - \varepsilon_{AA} - \varepsilon_{AA})$$

Defined dimensionless parameter

$$\frac{\Delta F_{mix}}{k_B T} = \frac{\Delta U_{mix}}{k_B T} - \frac{S_{mix}}{k_B} = \chi\Phi_A\Phi_B + \Phi_A \ln \Phi_A + \Phi_B \ln \Phi_B$$

$\chi(T)$ measure of the strength of A-B interactions



for $\chi < 2$

↓

Favorable A-B interactions

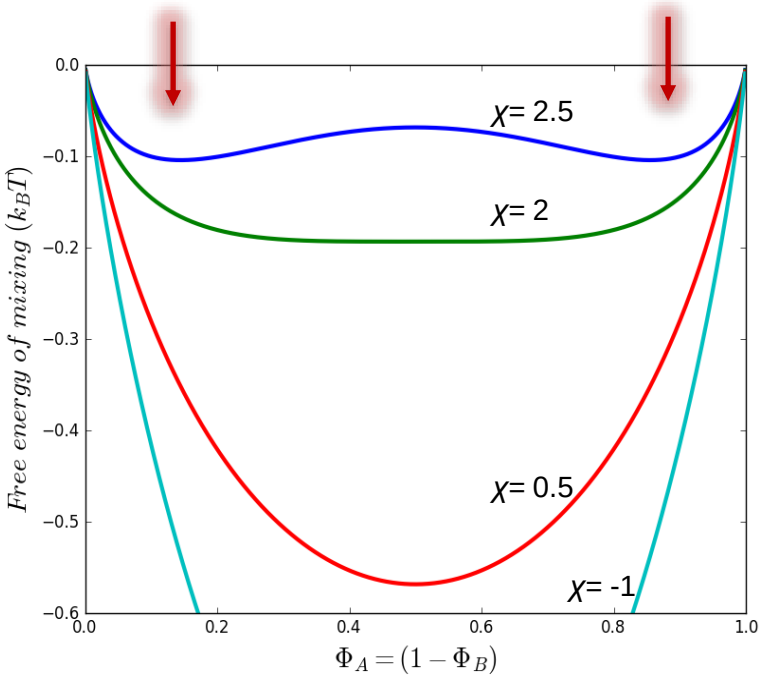
↓

Local miminum at Φ= 0.5

Free energy of mixing two substances

Unfavorable A-B interactions **for $\chi < 2$**

Two local mimina at $\Phi \neq 0.5$



*These mimima define the **binodal** separating unstable-stable states*

Hypothetical generation of two compartments from mixed liquid with Φ_0:

Since $\Phi_A + \Phi_B = 1$, shorten to $\Phi_A = \Phi$ and $\Phi_B = 1 - \Phi$

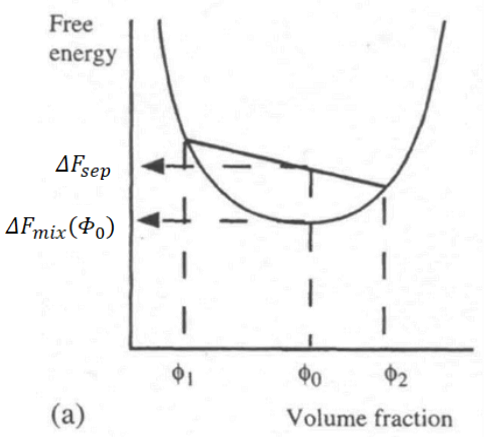
For a desired Φ_0, we can then mix:

$$\Phi_1 \leq \Phi_0 \leq \Phi_2$$

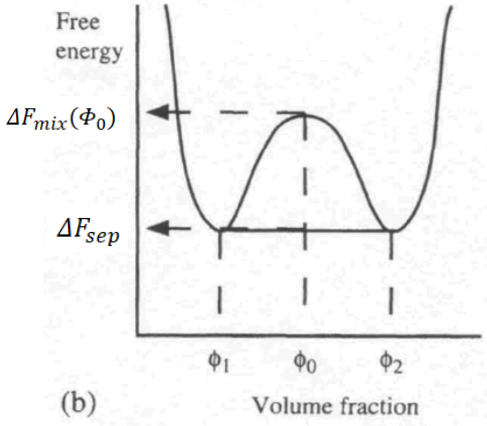
$$\boldsymbol{\alpha_1 \Phi_1 + \alpha_2 \Phi_2 = \Phi_0}$$

Partial separated system

mix is stable



mix is unstable



Free energy change of partially separated system:

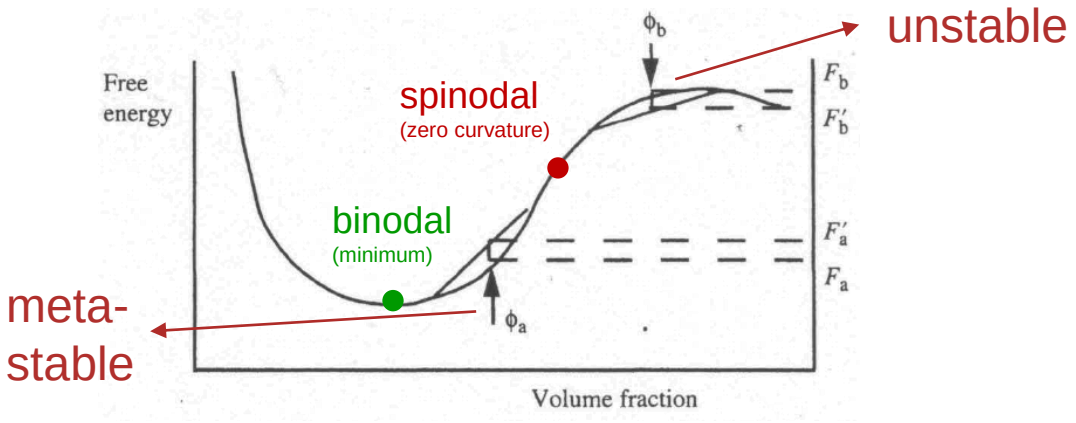
$$\Delta F_{sep} = \Delta F_{mix}(\Phi_1) + \Delta F_{mix}(\Phi_2)$$

Unstable if

$$\boldsymbol{\Delta F_{sep} < \Delta F_{mix}(\Phi_0)}$$

Stability during unmixing

Stable vs. metastable vs. unstable



Sign of the curvature of the free energy curve:

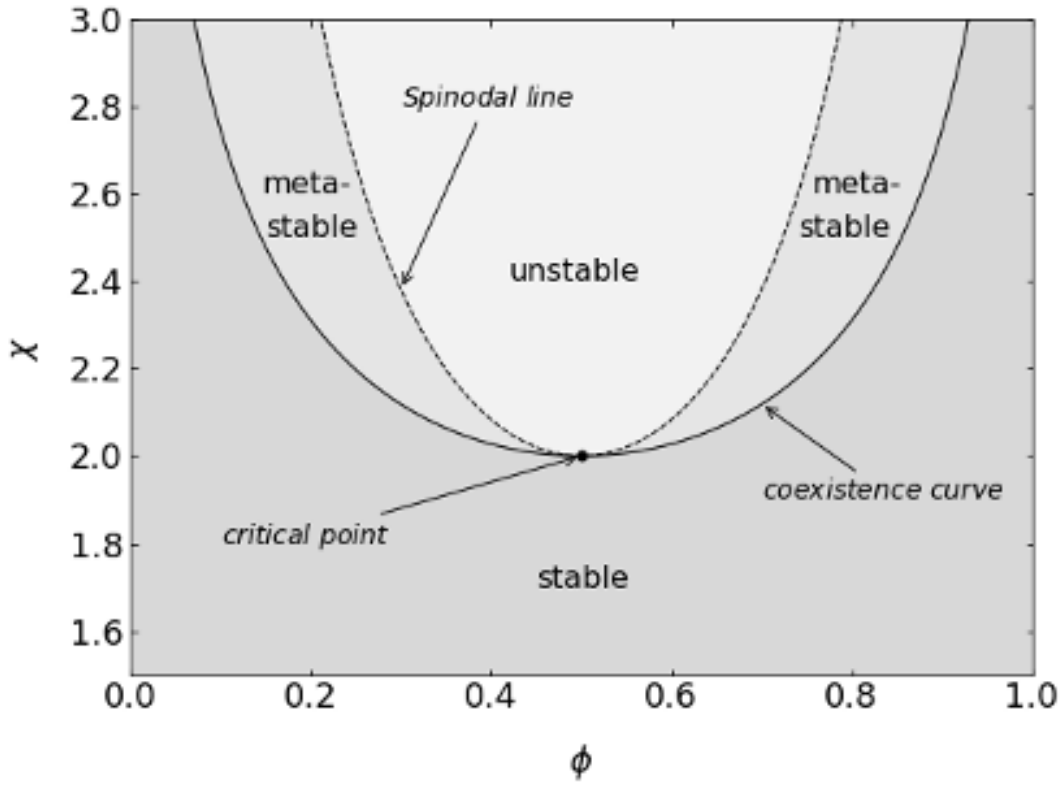
$$\frac{d^2F_{mix}}{d\phi^2} > 0 \ldots \text{stable}$$

$$\frac{d^2F_{mix}}{d\phi^2} < 0 \ldots \text{unstable}$$

$$\frac{d^2F_{mix}}{d\phi^2} = 0 \ldots \text{spinodal line}$$

$$\frac{d^3F_{mix}}{d\phi^3} = 0 \ldots \text{critical point}$$

Phase diagram



χ is proportional to $1/T$

Additional literature for material covered in lectures 5 & 6