Self-assembly of virus capsids

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TU/e

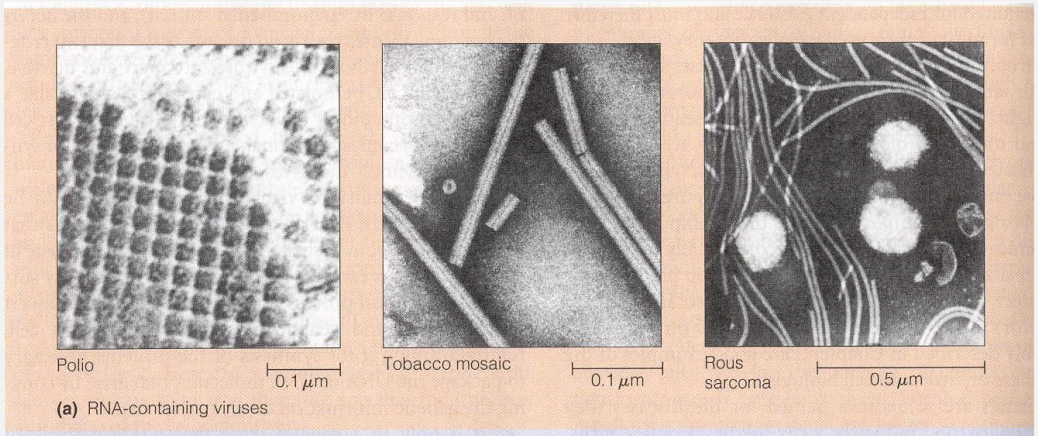
(1) Spherical virus capsids: HBV

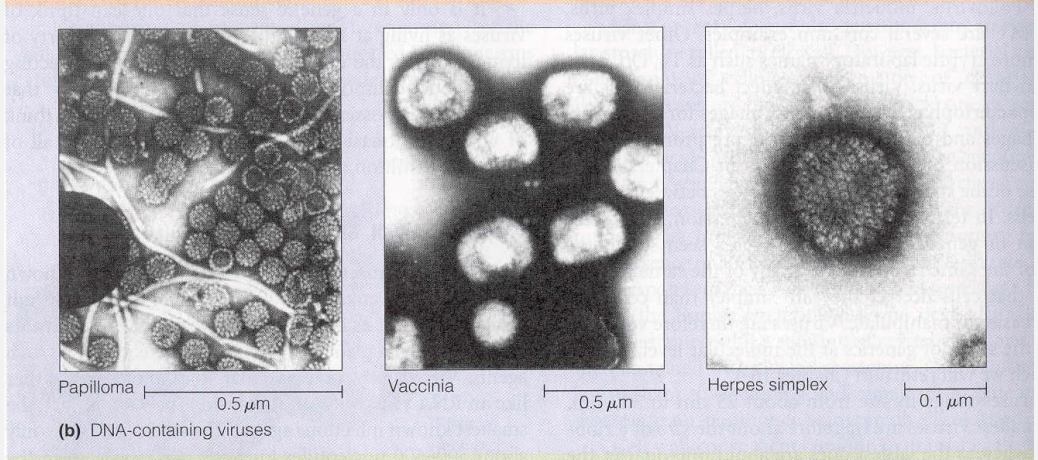
See also: ‘Physical Biology of the Cell’ Rob Phillips ea
T&F 2009; 2nd ed 2012.

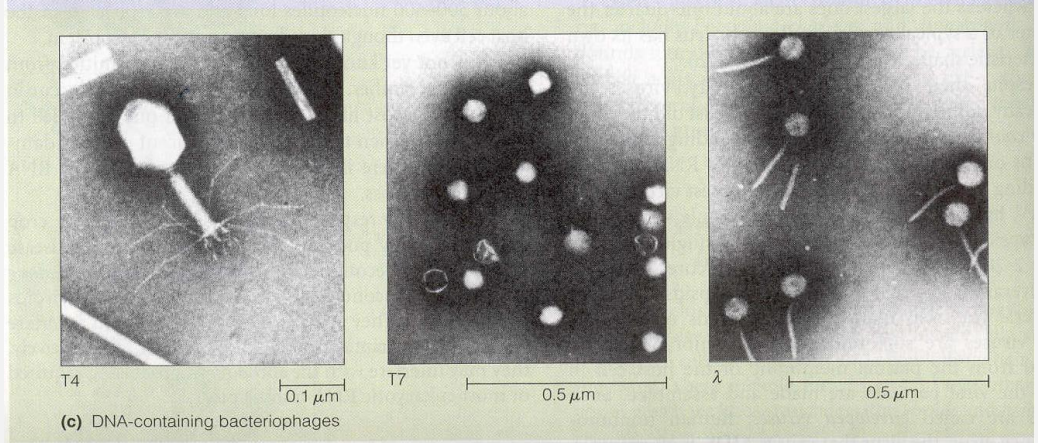
(2) (Briefly) cylindrical viruses: TMV disk-helix –
comparing the numbers

(3) A virus shell – like structure made of colloids

Viruses are (often) assemblies of protein subunits around RNA or DNA

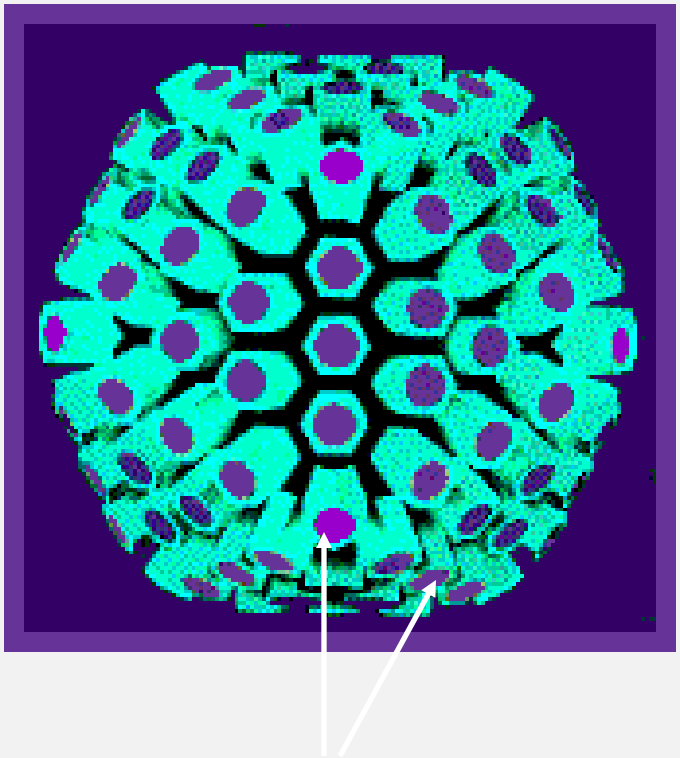






(Bad news wrapped in protein)

Spherical virus **capsid**

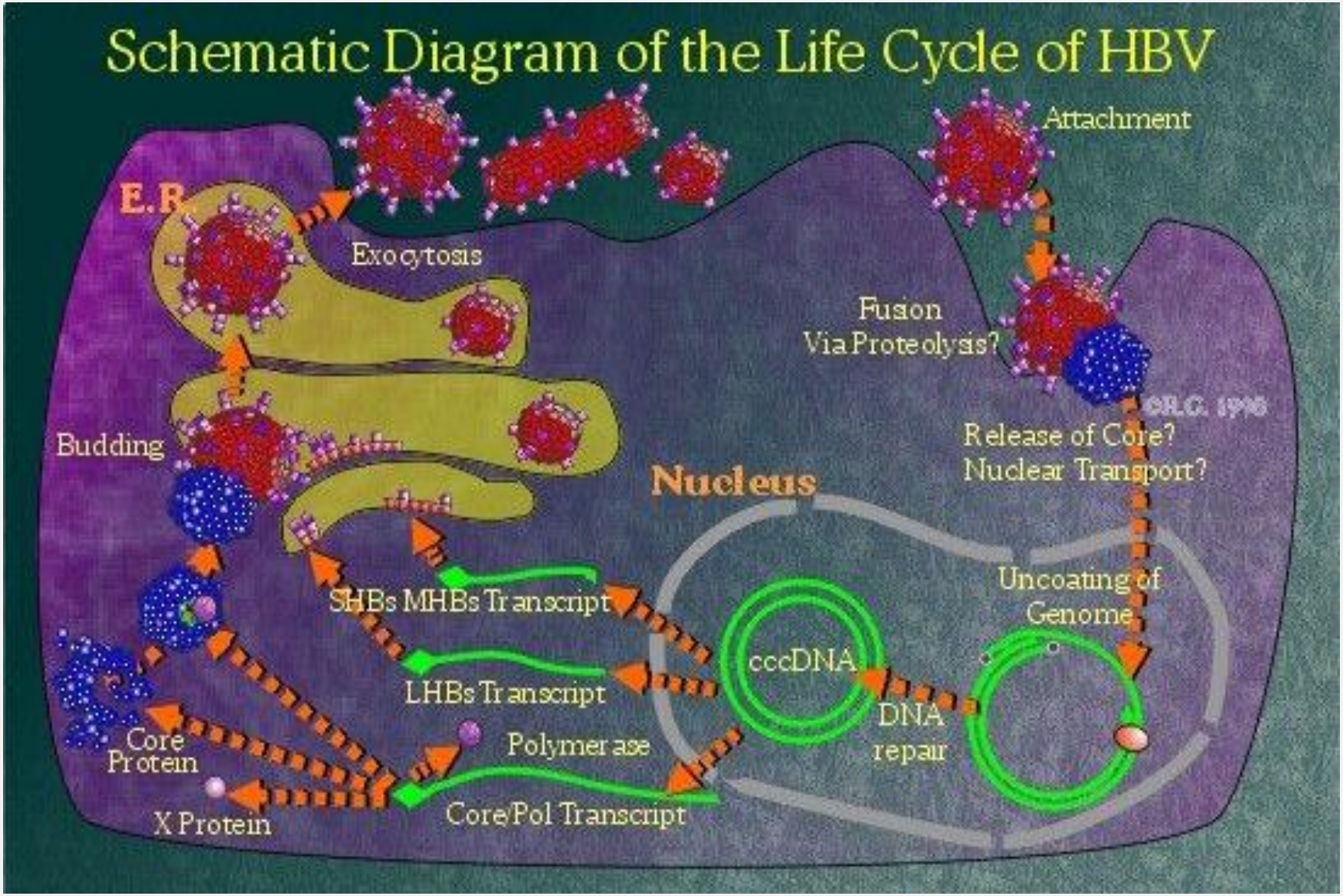


Coat protein subunits

capsomers

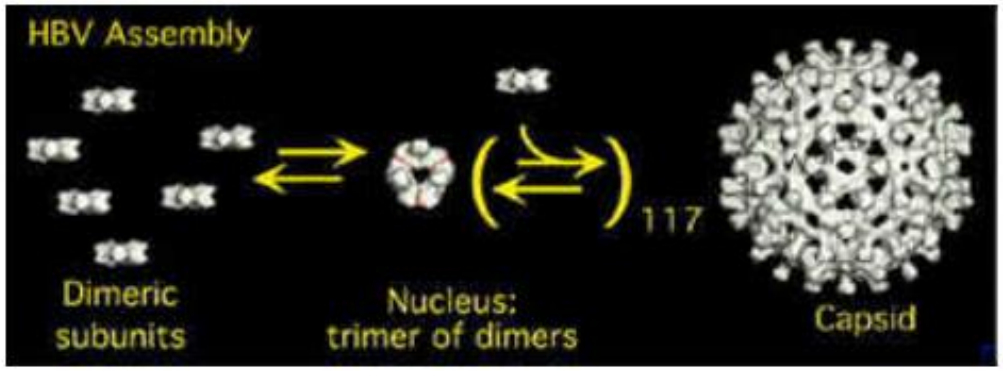
Quiz

- Why subunits?



Current “paradigm”: monomers switch between 2 conformations: assembly-active and assembly-inactive state

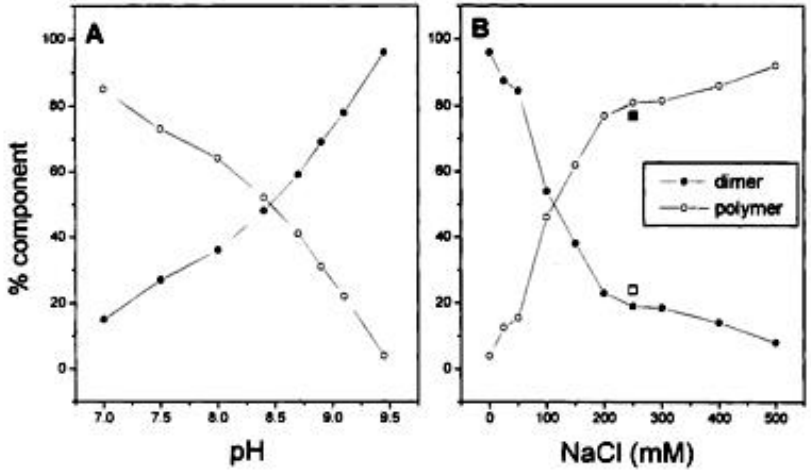
In vitro self-assembly of hepatitis B virus capsids



Association

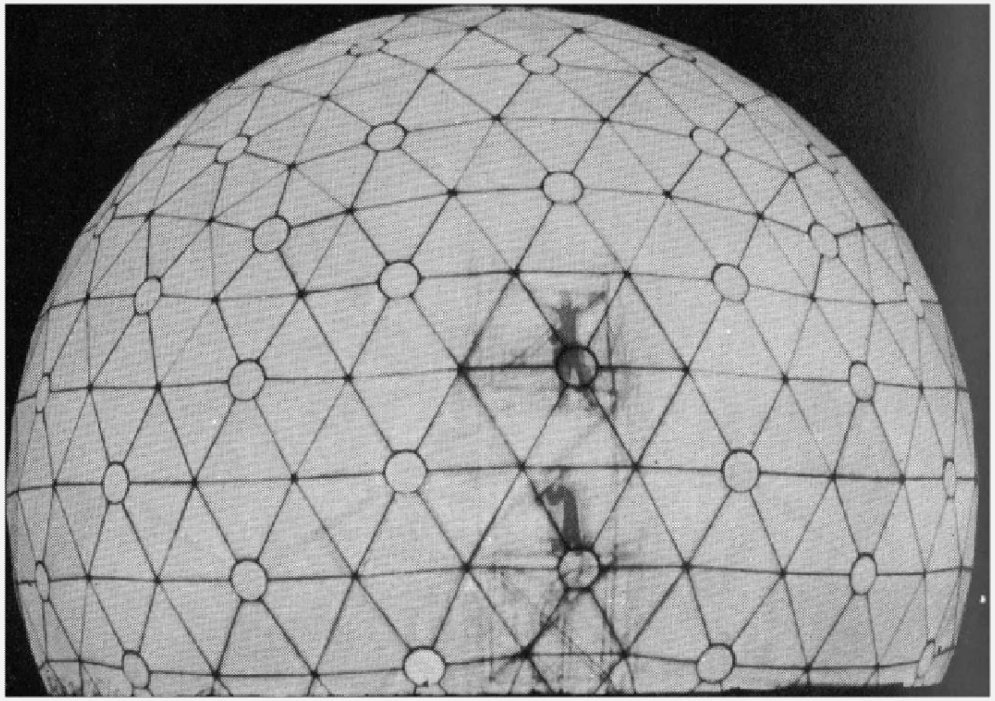
=

Reversible!



Wingfield et al. *Biochem* **34** (1995) 4919.

SHAPE of virus capsids [Caspar & Klug, 1962]:



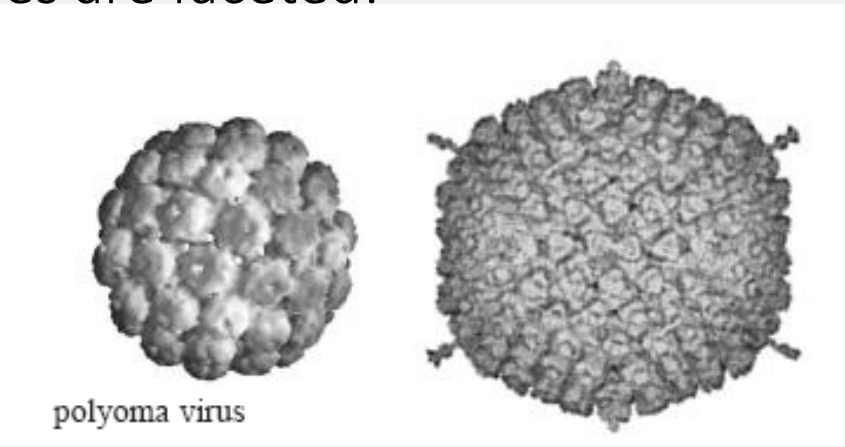
Spherical viruses are structured as miniature geodesic domes (quasi-equivalent triangular subunits arranged into groups of five and six)

"Magic number" $S=60T=60(h^2+hk+k^2)$ – dodecahedron T=1
- buckyball T=3

(triangle) vertices

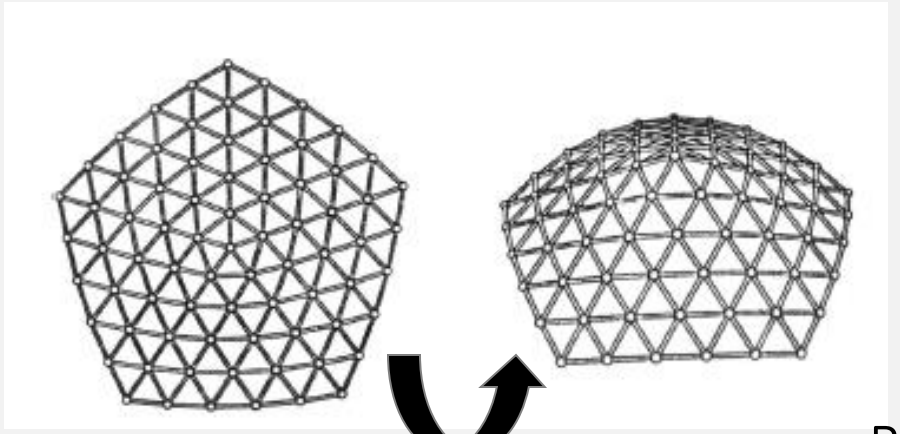
'triangulation number'

Large viruses are faceted:



(not to scale)

Caused by elastic energy ~ R^2 [Lidmar Mirny Nelson PRE 2003]



Buckling

Foppl-von Kármán number $YR^2/\kappa \geq 154$.

Young modulus

Bending elastic modulus

Pick your target

Conformational change necessary for assembly regulation?

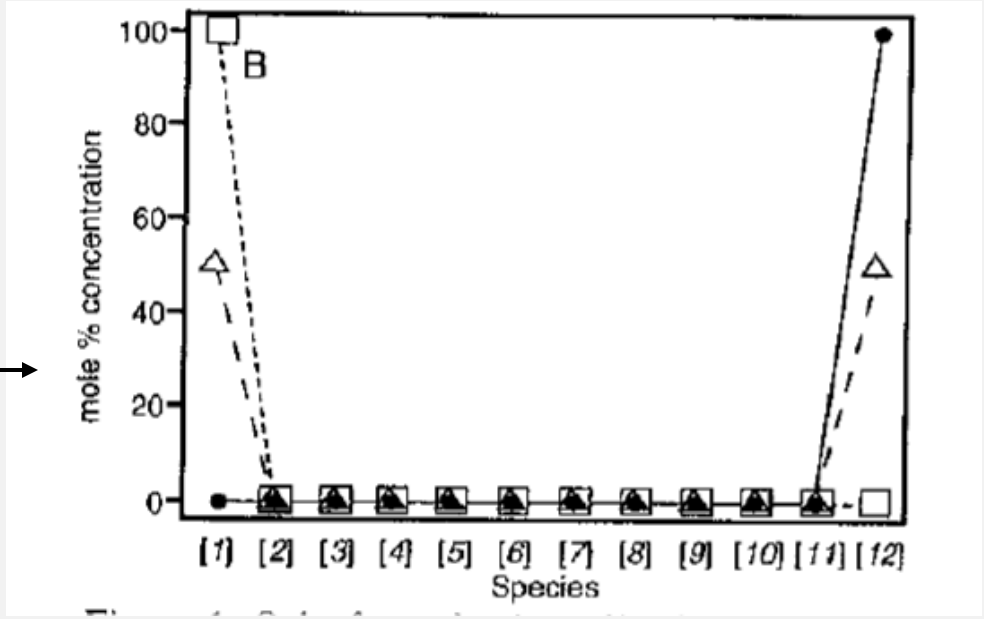
Can we get away with ‘simple’ physics / phys chem?

Choose level of description

Thermodynamic reason for dominant occurance of closed shells of coat proteins– example: dodecahedron (T=1)

q	# bonds/ monomer
1	0
2	1/2
3	3/3
4	5/4
5	7/5
6	10/6
7	12/7
8	15/8
9	18/9
10	21/10
11	25/11
12	30/12

Maximizing # bonds under geometrical constraints leads to either monomers OR 12-mers being present



[A. Zlotnick, J.Mol.Biol. (1994)]

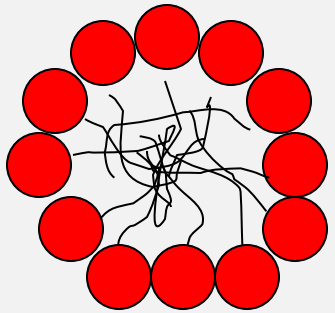
Boltzmann weight $\propto$ exp[- #bonds x bond energy]

Analog: micelles (archetypical self-assembly)

LIGHT SCATTERING IN SOAP SOLUTIONS

By P. Debye*
Baker Laboratory, Cornell University, Ithaca, New York

[Ann. NY Acad. Sci. **51**, 575, (1949)]



Consider the following idealized reaction between fatty ions A and micelles A_n, where n is the number of fatty ions per micelle:

$$nA \rightleftarrows A_n \tag{9}$$

If we let c_n be the concentration of micelles, c_1 the concentration of unaggregated paraffin chains, and c the total concentration of fatty ions the following relationships hold:

$$\frac{c_1^n}{c_n} = K \tag{10}$$

$$c = c_1 + nc_n \tag{11}$$

K is the equilibrium constant. It has been assumed that, for this simple treatment, activity coefficients are equal to unity.

The equilibrium constant K has the dimension of a concentration to the power $(n-1)$. We write $K = c_0^{n-1}$ and express our concentrations as multiples of c_0. For the relative concentrations

$$\gamma_1 = \frac{c_1}{c_0}, \qquad \gamma_n = \frac{cn}{c_0}, \qquad \gamma = \frac{c}{c_0}, \tag{12}$$

the relations

$$\gamma_1^n = \gamma_n, \qquad \gamma_1 + n\gamma_n = \gamma \tag{13}$$

hold.

For very large values of n, it turns out that the relative concentration γ_1 of the monomer is equal to γ for $\gamma < 1$. From $\gamma = 1$ on the concentration γ_1 remains constant. The relative concentration of the polymeric particle, on the other hand, is 0 from $\gamma = 0$ to $\gamma = 1$ and equal to $\gamma - 1$ from there on. It is seen that $\gamma = 1$ corresponds to a critical point and we shall have to identify c_0 with the critical concentration.

Modeling self-assembly of protein subunits into capsids.

Strategy:

1. "Guess" potential of mean force between coat protein subunits

2. Use 'all or nothing' approach (monomers or fully assembled virus capsids);

3. Predict behavior of capsid/ monomer ratio with T, ionic strength, pH, (…); compare with experiments.

Focus on Hepatitis B virus capsids

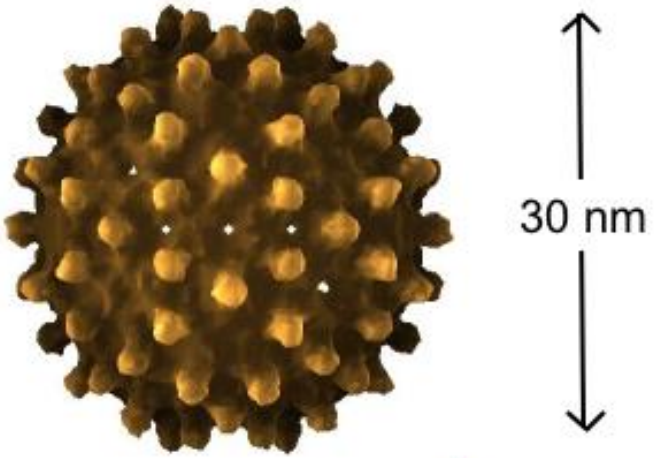
– results expected to be more general

See: [WKK & Paul van der Schoot, Bioph. J. 2004, 2006]

Potential applications e.g., artificial drug / gene delivery,

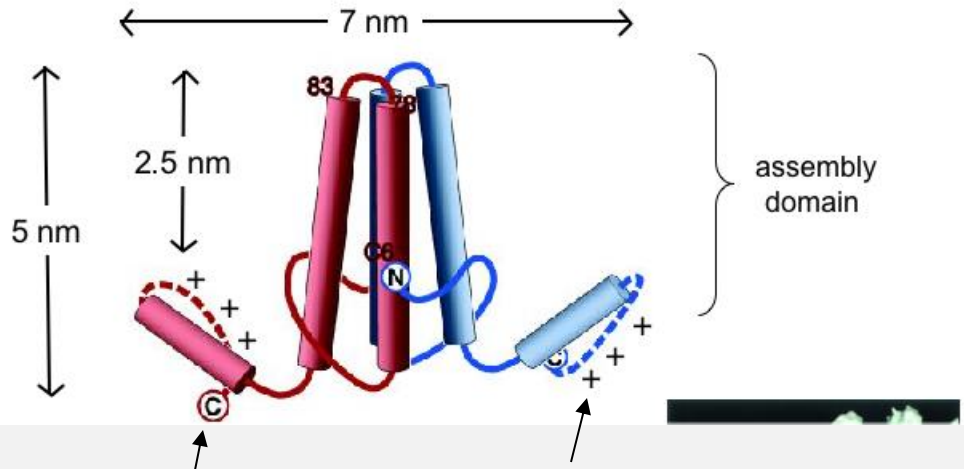
controlled release,…

Structure of the hepatitis B virus capsid



T=4

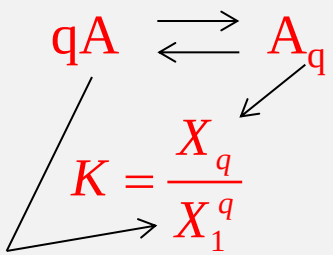
Hepatitis B virus 22 kDa core protein dimer



RNA-binding protamine domain
(absent in experiments shown here)

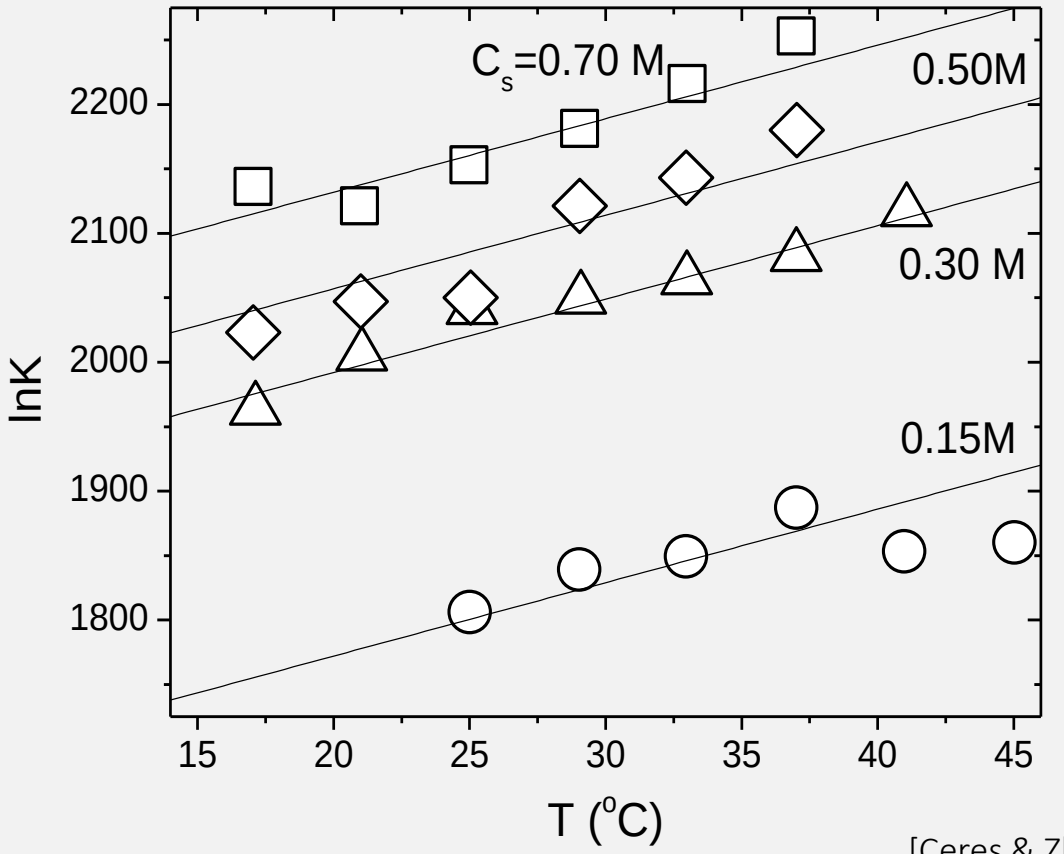
What's driving virus formation?

Virus in equilibrium with capsomers:



'in vitro'

Equilibrium constant

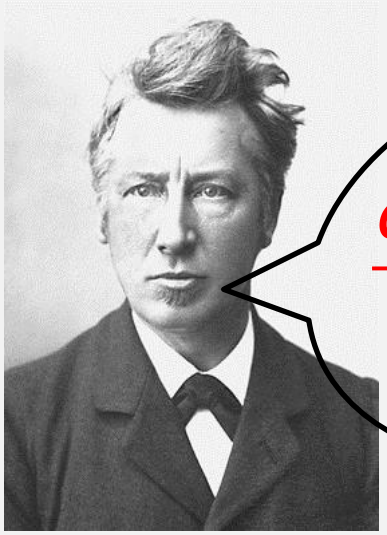


More virus:

- Higher T
- Higher ionic strength

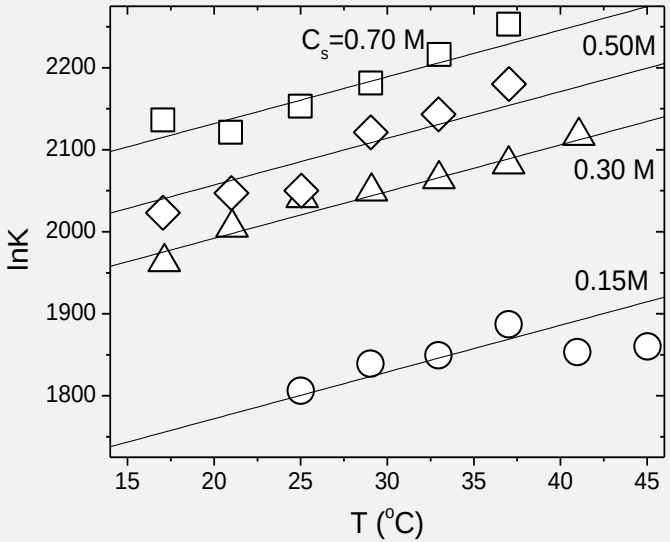
[Ceres & Zlotnick Bioch 2002]

Thermodynamics



$$\frac{d \ln K}{dT} = \frac{\Delta H^0}{kT^2}$$

Van ‘t Hoff law

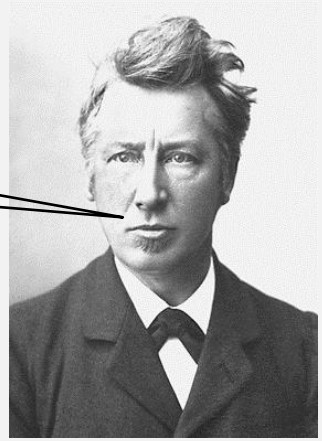


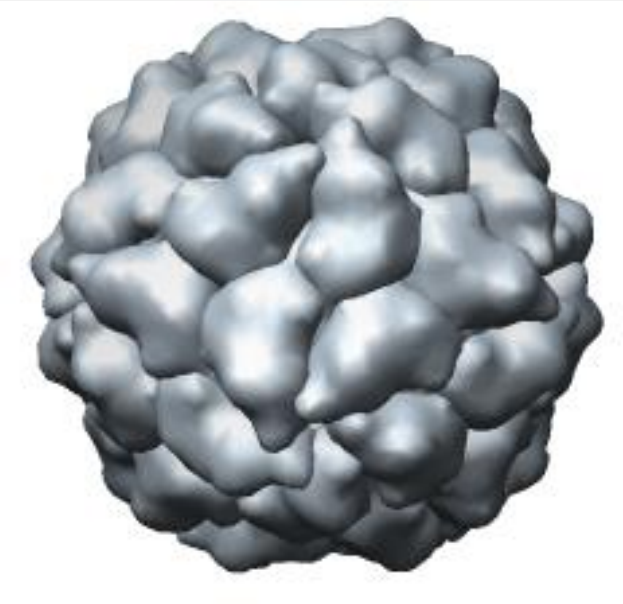
$$\Delta H^0 > 0$$ Virus formation is ENDOTHERMAL

$$\Delta G^0 = -kT \ln K = \Delta H^0 - T\Delta S^0$$

< 0 > 0 > 0

Entropy INCREASES upon virus formation!

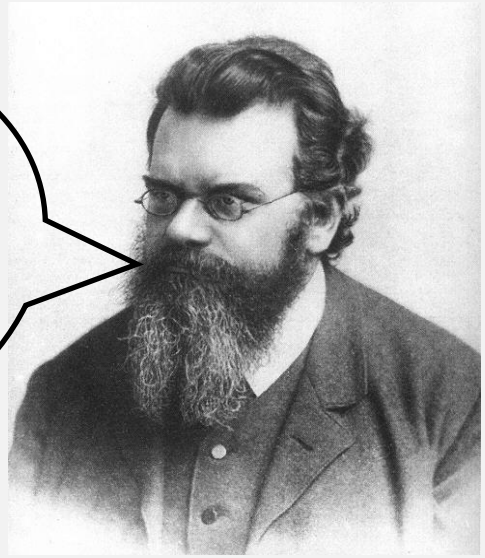




… has higher entropy than its ‘free’ subunits

More ‘ordered’ yet higher entropy

$S = k \ln W$



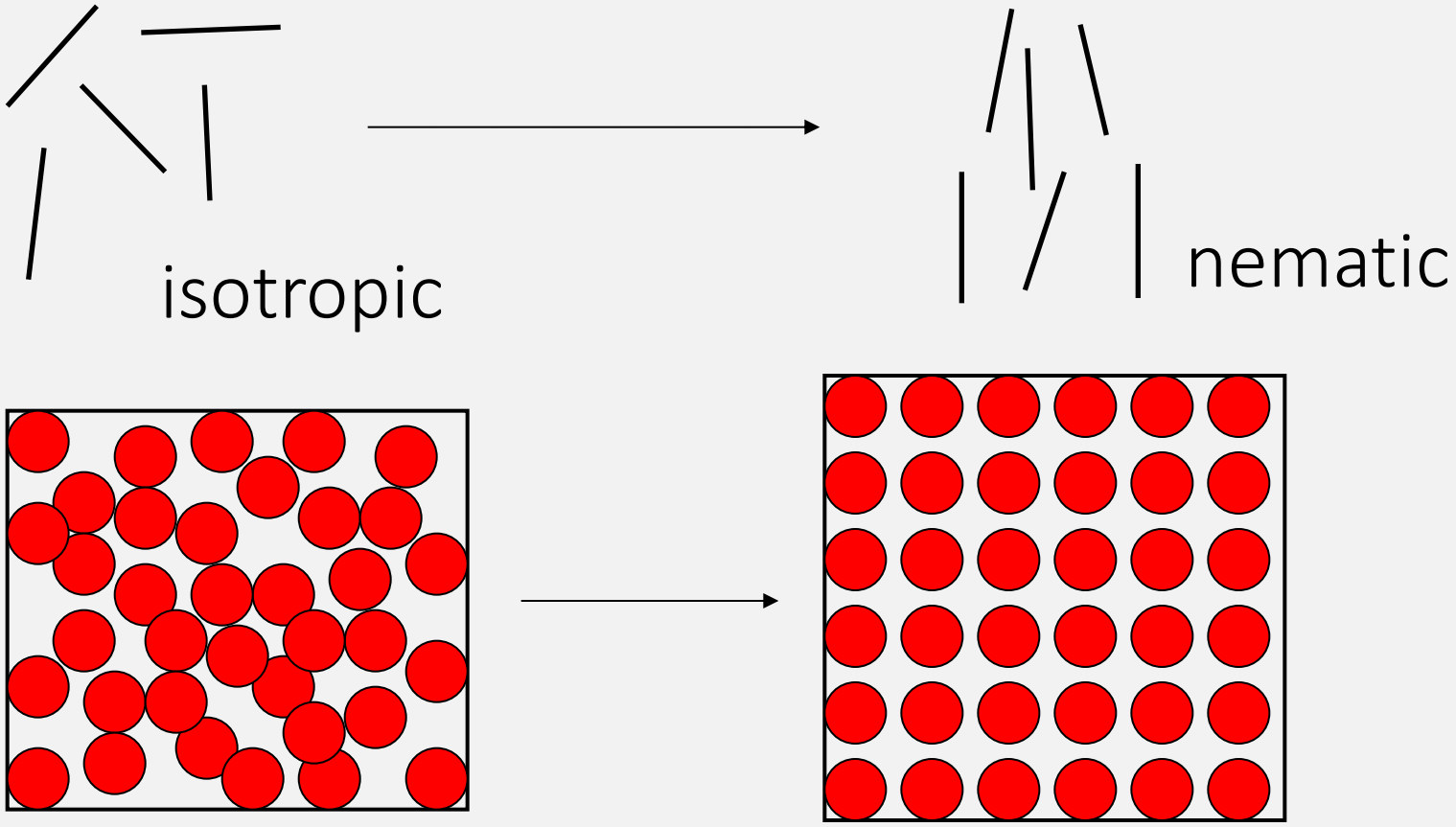
L. Boltzmann

Realizations / complexions
-Positions / momenta
-Distribution over energy levels

How does a virus get more entropy??

Entropy-increase without increase of ‘disorder’

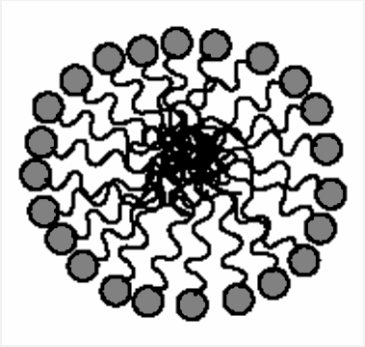
Onsager 1943

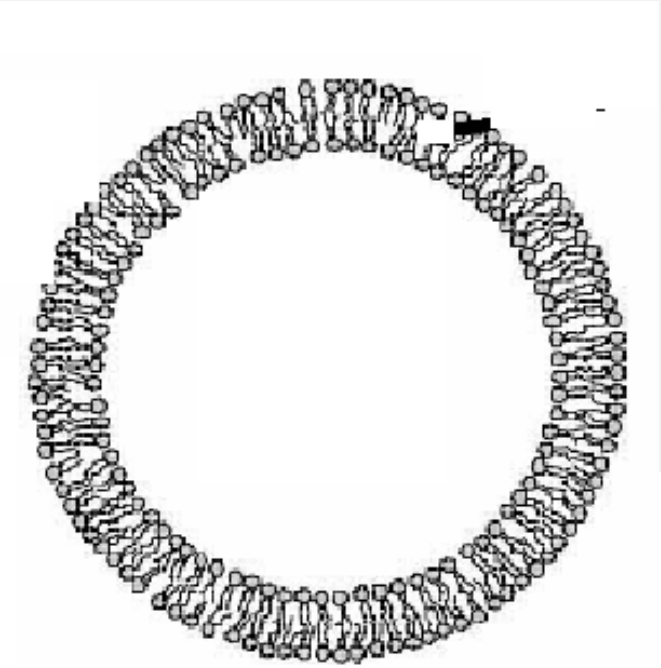


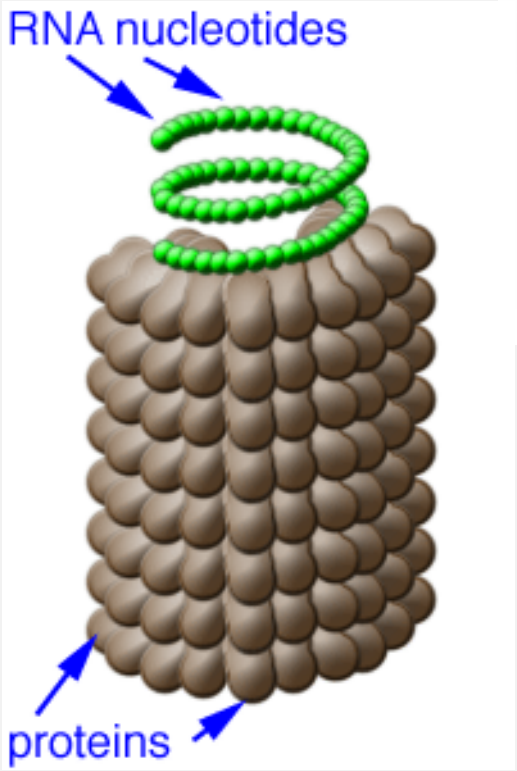
Configurational entropy high
Translational entropy low

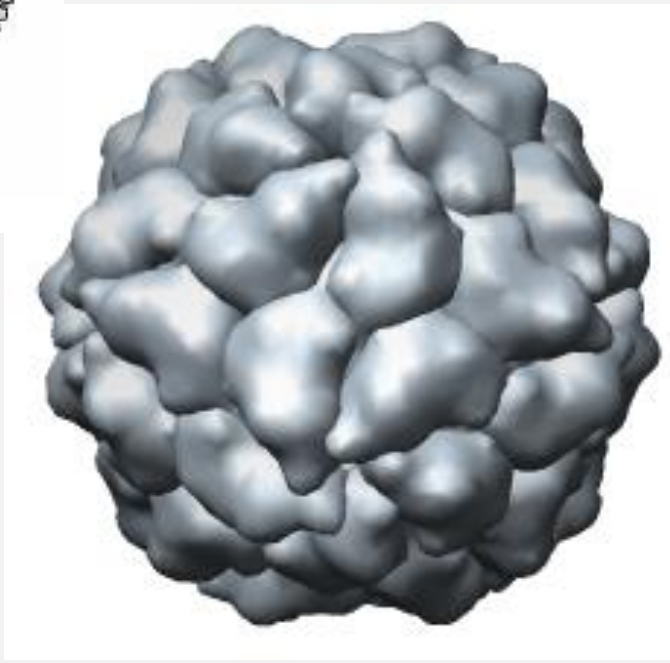
Configurational entropy low(er)
Translational entropy high(er)
~ Log(available volume)

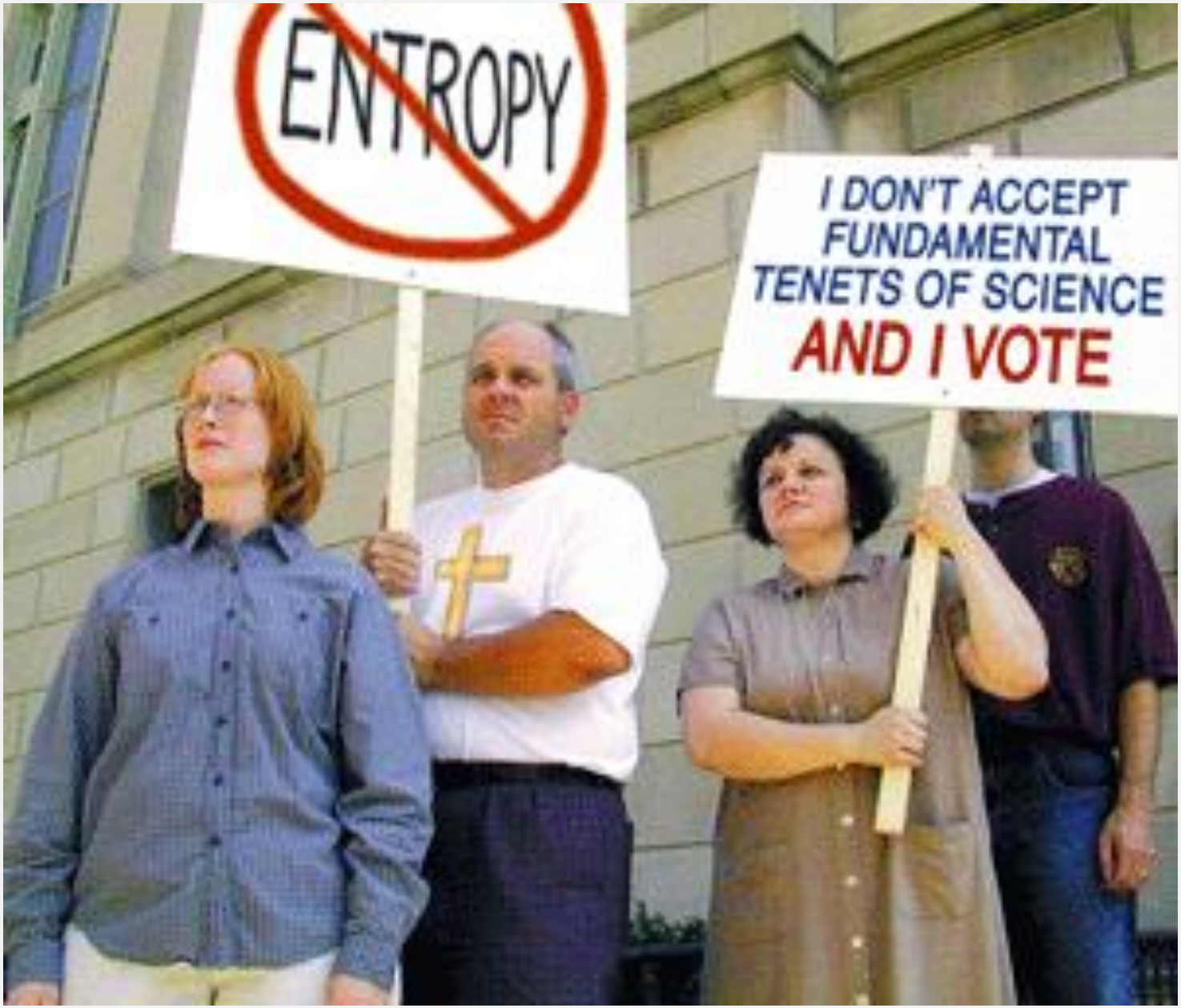
(dense) objects with larger entropy than their - dilute - building blocks










ENTROPY
I DON'T ACCEPT
FUNDAMENTAL
TENETS OF SCIENCE
AND I VOTE

‘The Onion’

1. Interactions between coat protein subunits: **increasing attraction with temperature, ionic strength**

Quiz: what interactions are expected to be important?

Attractive

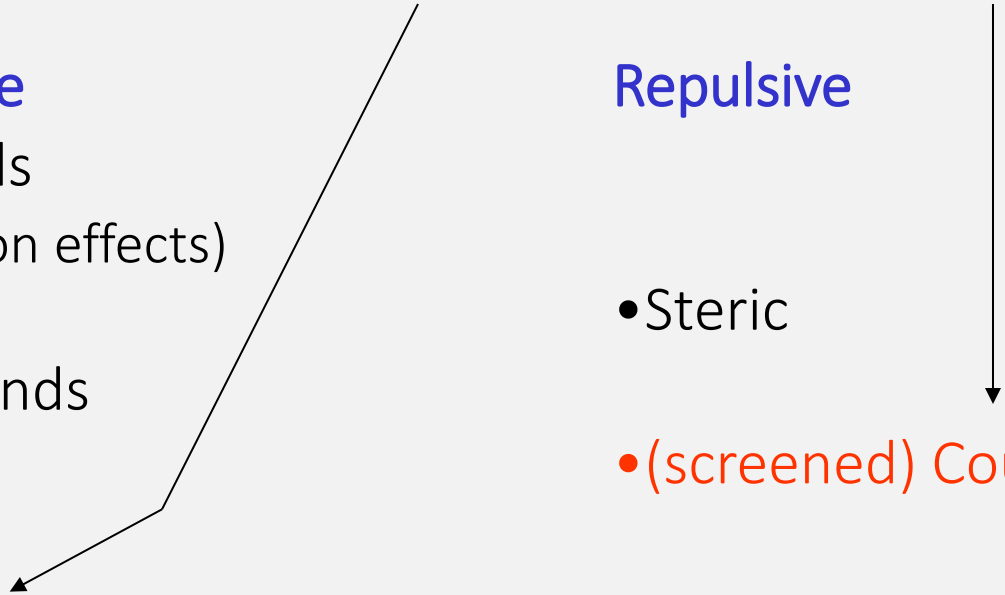
- Van der Waals (incl. specific ion effects)
- Hydrogen bonds
- Hydrophobic interactions
- Ion bonds

Repulsive

- Steric
- (screened) Coulomb

1. Interactions between coat protein subunits:

increasing attraction with temperature, ionic strength



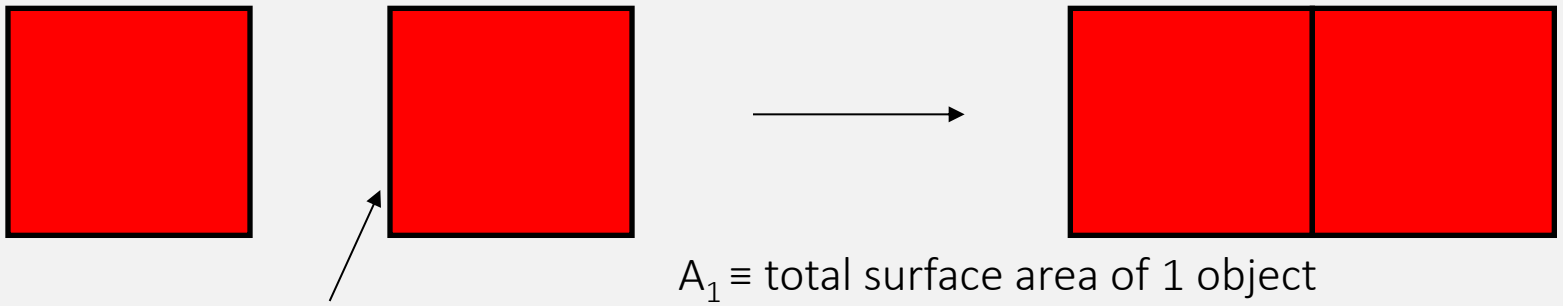
Attractive

- Van der Waals
  (incl. specific ion effects)
- Hydrogen bonds
- Hydrophobic interactions
- Ion bonds

Repulsive

- Steric
- (screened) Coulomb

Hydrophobic interactions (macroscopic view)



A

A_1 ≡ total surface area of 1 object
A ≡ overlap area per object

surface free energy

$F_1 = 2\gamma A_1$

$F_2 = 2\gamma(A_1 - A)$

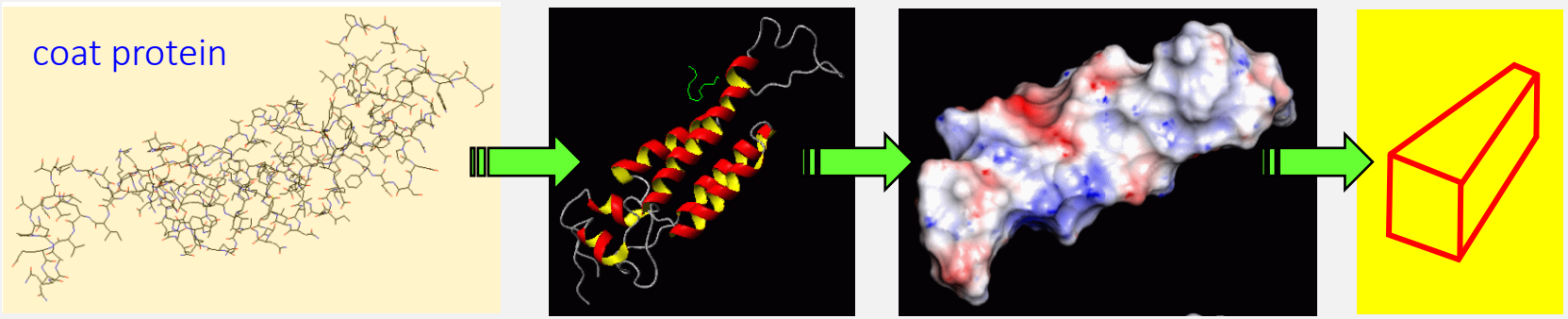
Gain upon sticking: $G_H = F_2 - F_1 = -2\gamma A$

Temperature dependence:

$$\gamma(T) = \gamma(T_0) + \left(\frac{\partial \gamma}{\partial T}\right)_{T=T_0} (T - T_0) + \ldots \approx \gamma(T_0) - s(T_0)(T - T_0)$$

s ≡ surface excess entropy (here <0 : stronger attraction with T!).

Coarse – graining: average out details



area A

interfacial tension γ

charge density σ

Potential of mean force between/per subunits: $G = G_H + G_C$

(objects in a "dressed vacuum")

Sticky part: hydrophobic interactions $G_H \approx -2\gamma A e^{-x/D}$

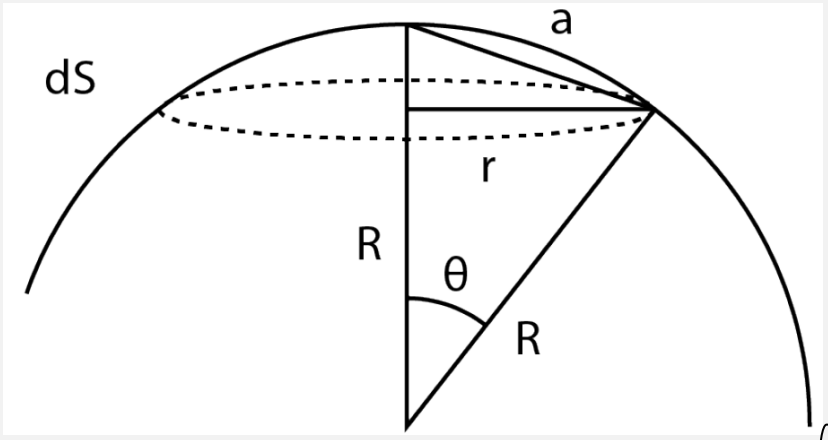
Charge density

Debye length

Soft part: screened Coulomb $G_C \approx k_B T A \sigma^2 \lambda_B \kappa^{-1}$

$\kappa^{-1} \approx 0.3/\sqrt{c_{salt}}$

Bjerrum length



1 charge with all other charges:

$$G_c = \frac{kTq}{2}\int_S \varrho \frac{z^2\lambda_B}{a} e^{-\kappa a} dS$$

Screened-Coulomb potential

$$\lambda_B = \frac{e^2}{4\pi\varepsilon\varepsilon_0 kT}$$

$$\rho = \frac{q}{4\pi R^2}$$

$$\kappa^{-1} \approx 0.3/\sqrt{c_{salt}}$$

(partially) integrate etc – see handout.

Result:

$$G_c \approx AkT\sigma^2\lambda_B\kappa^{-1}$$

2. Equilibrium between (q) monomers & fully assembled virus capsids

$qA \leftrightarrow A_q$ TD equilibrium: $q\mu_1 = \mu_q$

$$\mu_i = \mu_i^0 + kT \ln x_i$$

Equilibrium constant $$K = \frac{X_q}{X_1^q} = \exp[-(\mu_q^0 - q\mu_1^0)/kT] \approx \exp[-G(q)/kT]$$

Expand lnK (*via* G) to linear order in T; result:

$$\ln K \approx \ln K(T_0) - \frac{qA_H h}{k_B T_0^2} \quad (T - T_0)$$

$$h(T_0) = \gamma(T_0) + T_0 s(T_0)$$

Excess surface enthalpy (< 0)

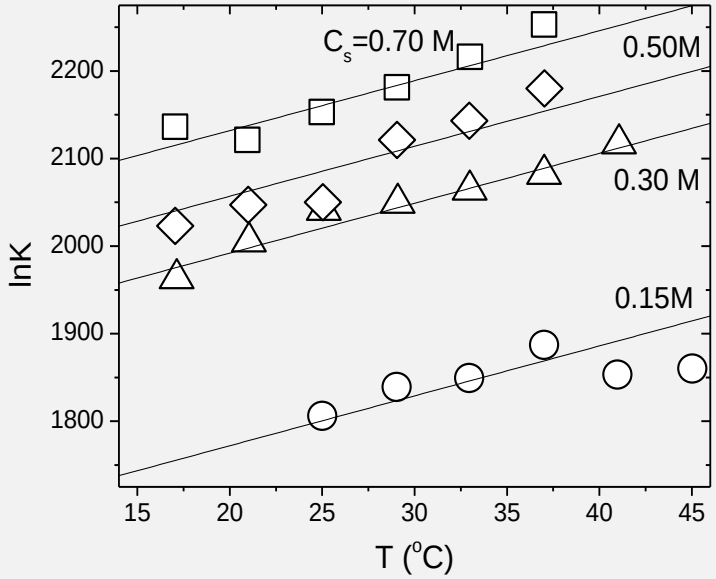
$$\ln K(T_0) = q\left(\frac{A_H \gamma}{k_B T_0} - A_C \sigma^2 \lambda_B \kappa^{-1}\right)$$

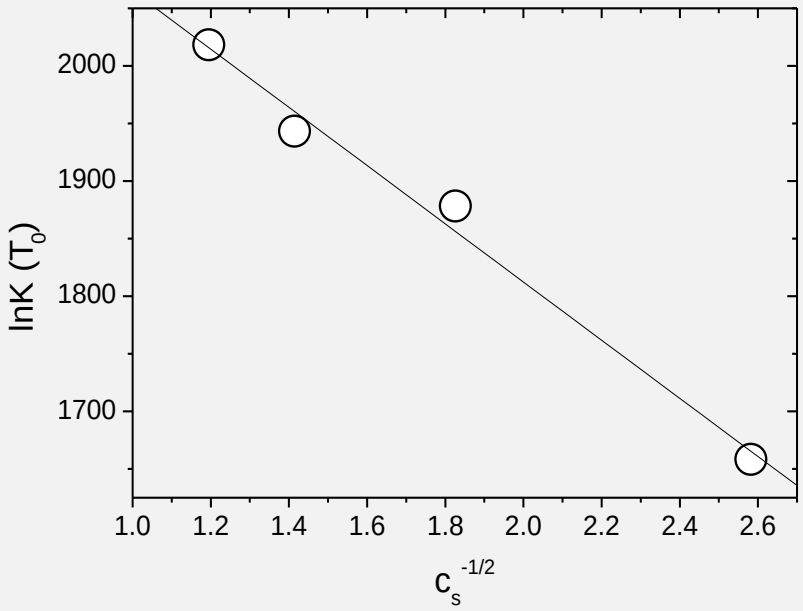
Calculate equilibrium constant

$$K = \frac{X_q}{X_1^q} \approx \exp[-G(q)/kT]$$

Predict: (1) lnK ∝ T

(2) lnK ∝ $c_s^{-1/2}$





Large K – mainly capsids: large γ

small σ – regulation by pH!

small κ^{-1} ≡ high ionic strength

Viruses regulate assembly – disassembly by physical conditions

From fits (& estimate of A_H, A_C):

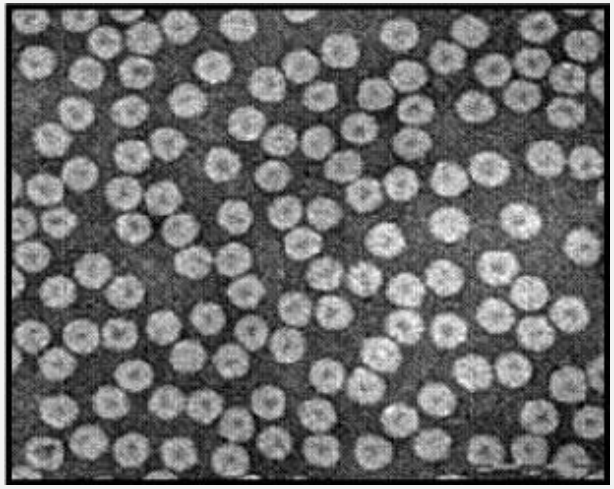
$\gamma(T_0) \approx 5.5$ mN/m (comparable to water-hexanol- [Villers & Platten JPC 1988])

$s(T_0) \approx -0.03$ mN/mK (consistent with data on hydrophobic surfaces – [Claesson ea J.Chem.Soc.Faraday Trans (1986)])

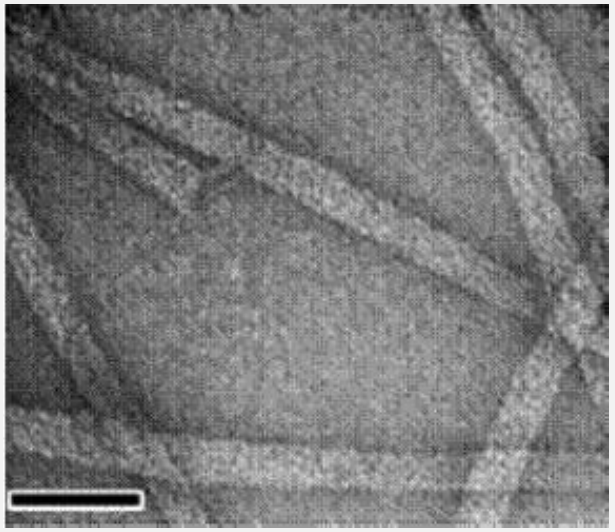
$\sigma \approx 0.7e/nm^2 \equiv 7\text{-}8$ charges/ subunit (From titrations, estimate ≈ 10 charges)

$h(T_0) \approx -3.8$mN/m ≡-13kT/ subunit (comparable to figure for surfactant micelles)

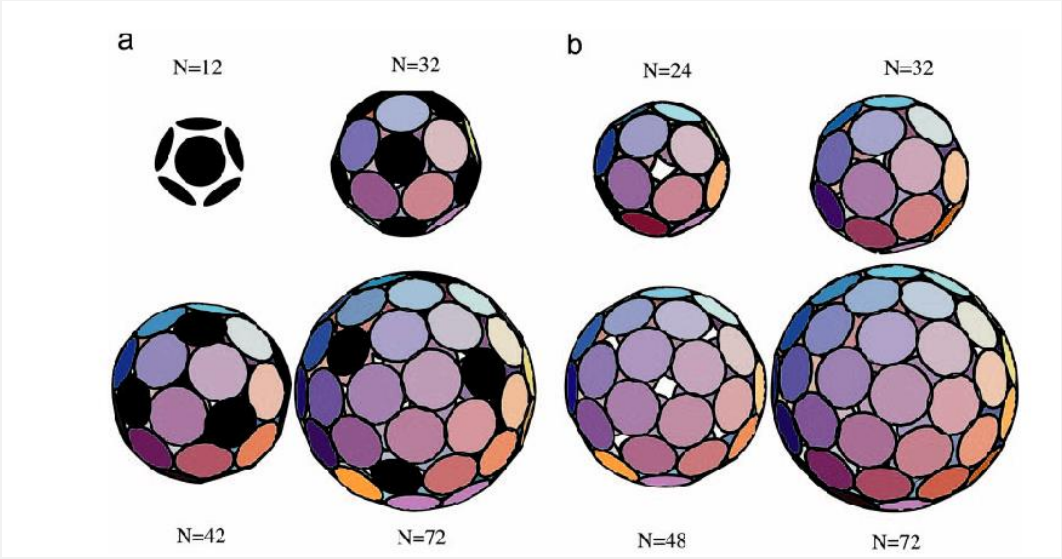
Challenge: predict ‘cargo’ (ccmv) influence, …



different template



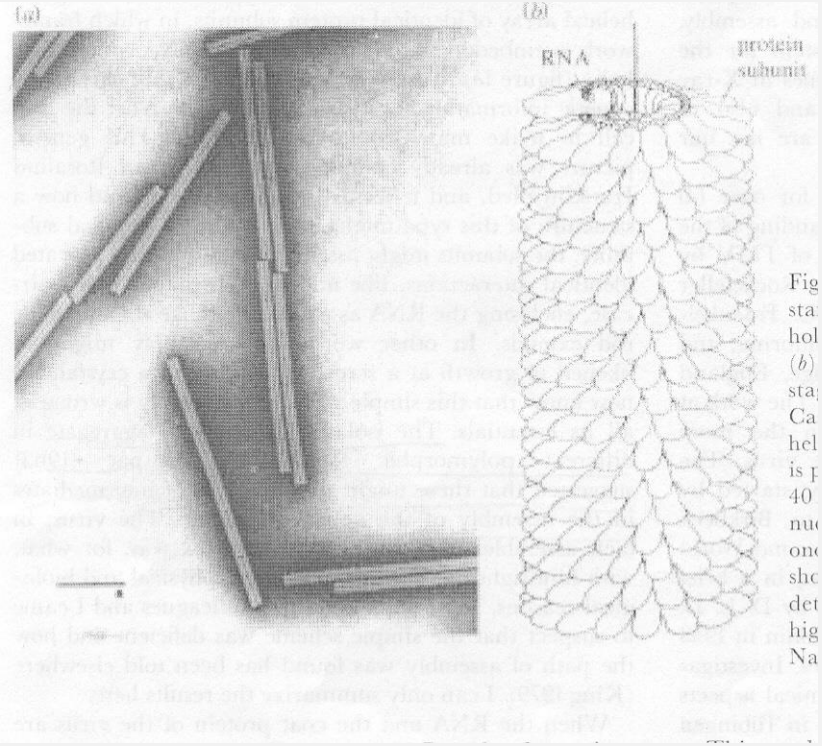
…Relative stability of T-number

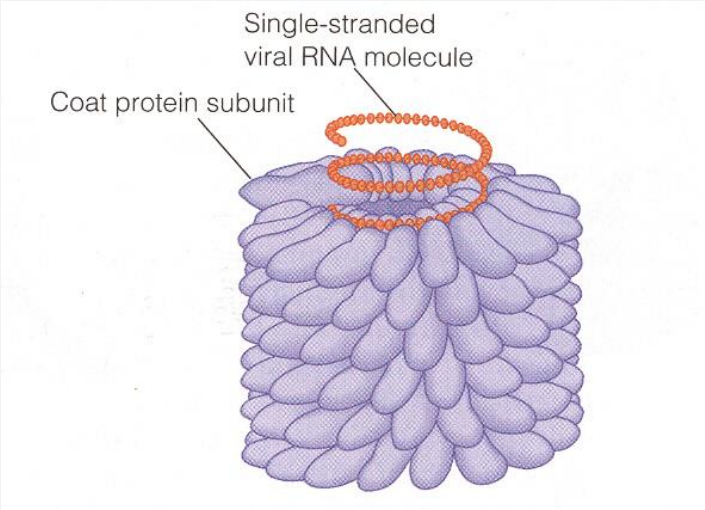


→ Requires fundamental insight in building block **geometry**

Something completely different: Tobacco Mosaic Virus (TMV)

Helix length ~ 300 nm
Diameter ~ 18 nm
# of subunits = 2130



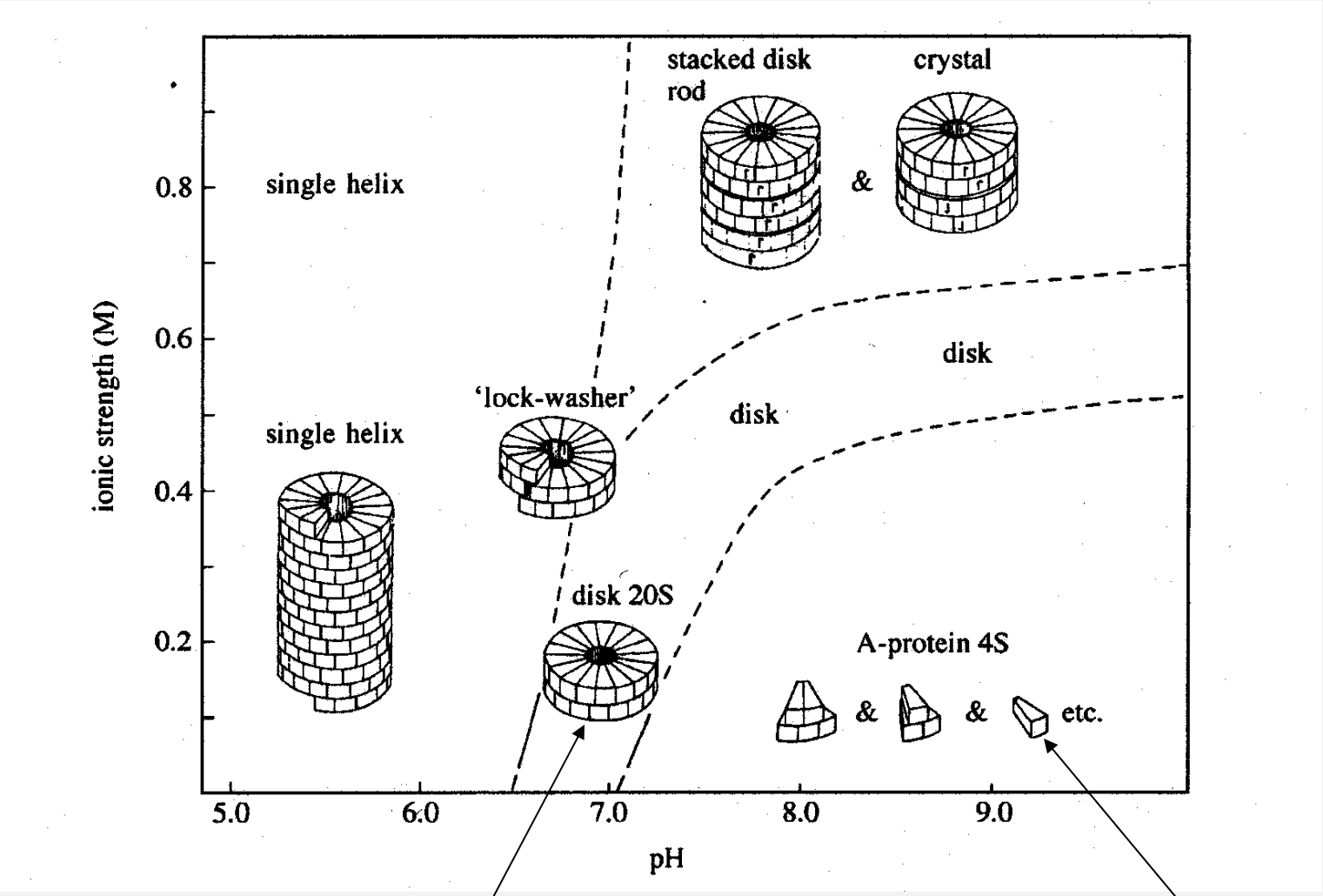


First observation of reversible virus formation: Fraenkel-Conrat & Williams, PNAS **41**, 690, (1955);

NO RNA

TMV stability diagram [Fraenkel-Conrat & Williams, PNAS **41**, 690, (1955); Klug, Phil. Trans. R. Soc. B**354**, 531, (1999)]

Total conc. subunit=$3*10^{-4}$M



Double disk of (total) 34 subunits

Taper shaped monomer;
largest dim.~8nm;
smallest dim. ~2.5 nm

Approach:

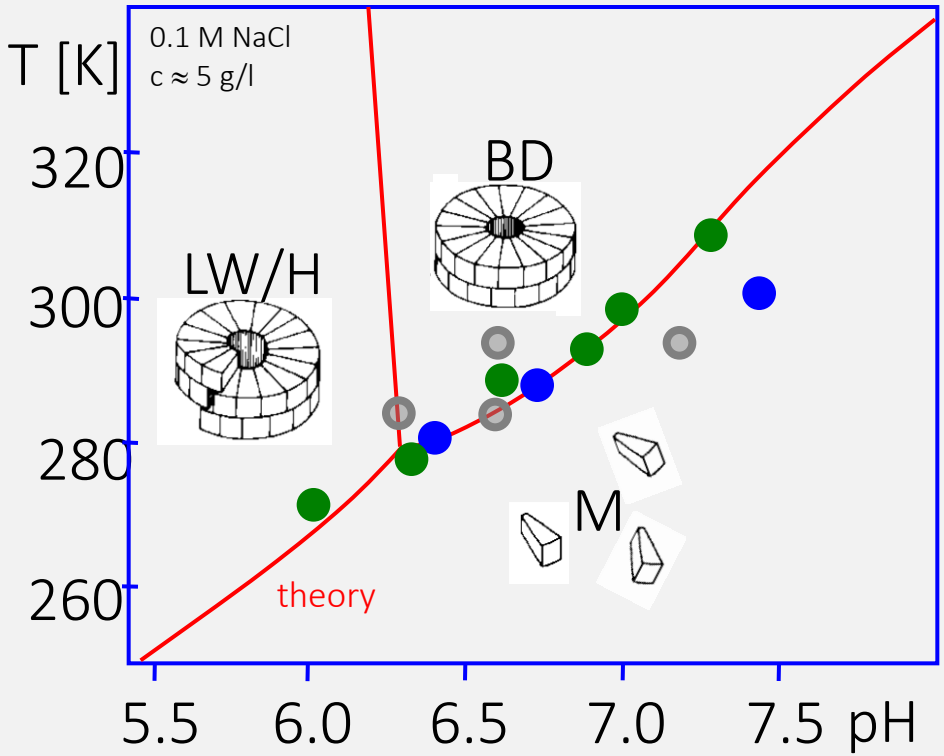
Same as HBV – assume potential of mean force:

$G = G_H + G_C$ (bound)

$G = 0$ (unbound)

Additional ingredient: ‘Caspar pairs’

Temperature and pH dependence



DSC
titration
sedimentation

Butler *Biochem* 20 (1981) 3792
Durham *J Mol Biol* 67 (1972) 289
Sturtevant et al *Biochem* 20 (1981) 3792

Kegel & vdSchoot *BPJ* 91 (2006) 1501

Comparing HBV & TMV

	Attr. Energy γA/kT	Excess entropy As/(kT/K)
TMV Disks	-17.3	-0.121
TMV Helices	-16.3	-0.115
HBV	-19.3	-0.107

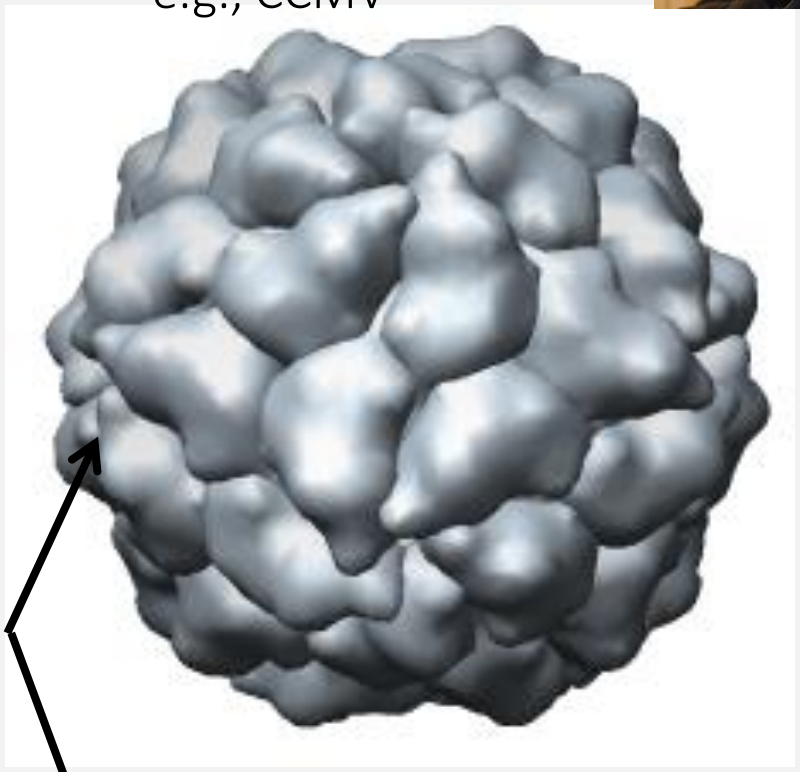
Different virus, (almost) equal numbers….

Colloids as model systems for virus capsids

with Chris Evers

e.g., CCMV

Sorry this story is under review so no pic – it looks like the structure on the right)



5-fold

a capsomer

~ 30 nm

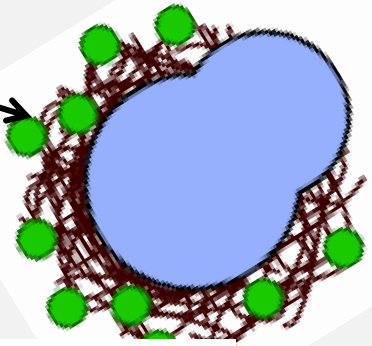
Evers et al., http://arxiv.org/pdf/1503.00552v1.pdf (2015)

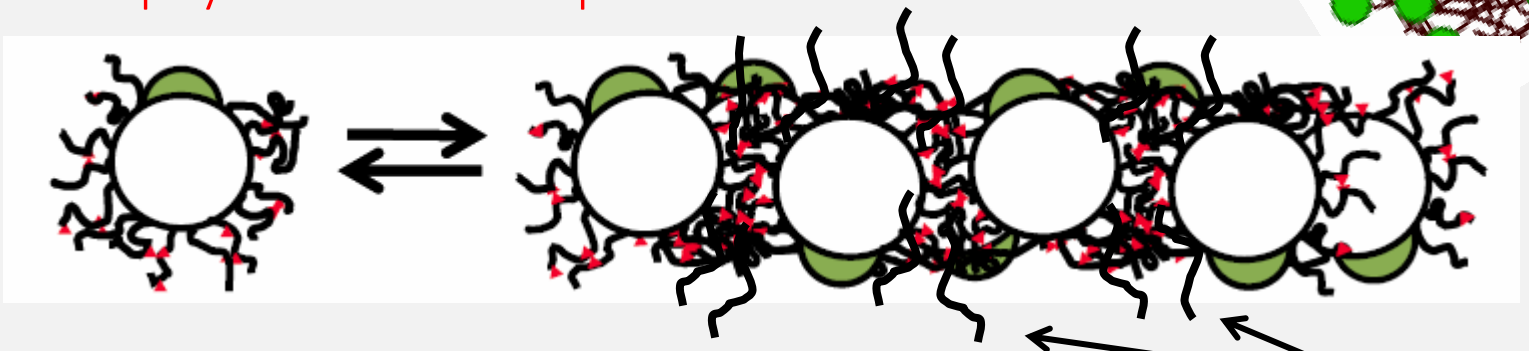
Capsid proteins are soft, asymmetric, deformable objects

-> asymmetry

-> charge + mild ‘hydrophobicity’ required

-> charged (PAA) flobby brush with hydrophobic bits..... physicist view of a protein!



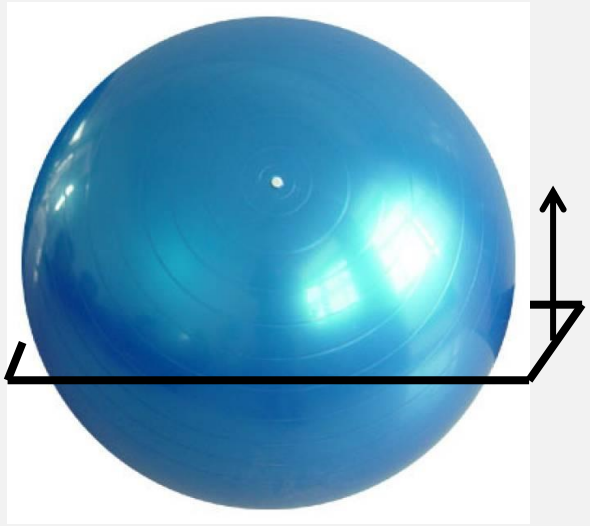


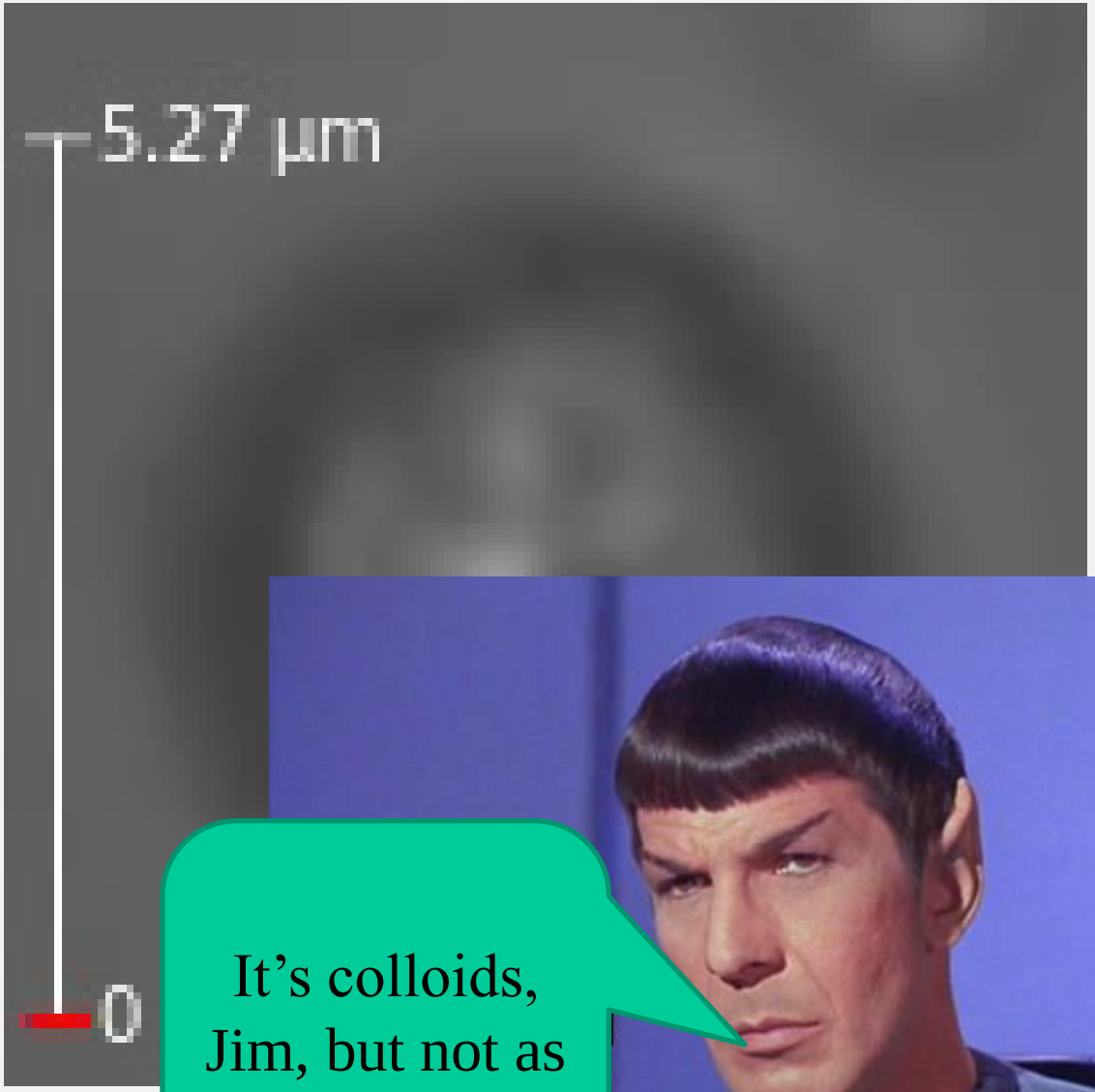
Redistribution of hydrophobic & hydrophilic parts upon approach:

(repulsive) PAA chains stick out (of plane)

(as a consequence of)

Attractive bits condense in plane

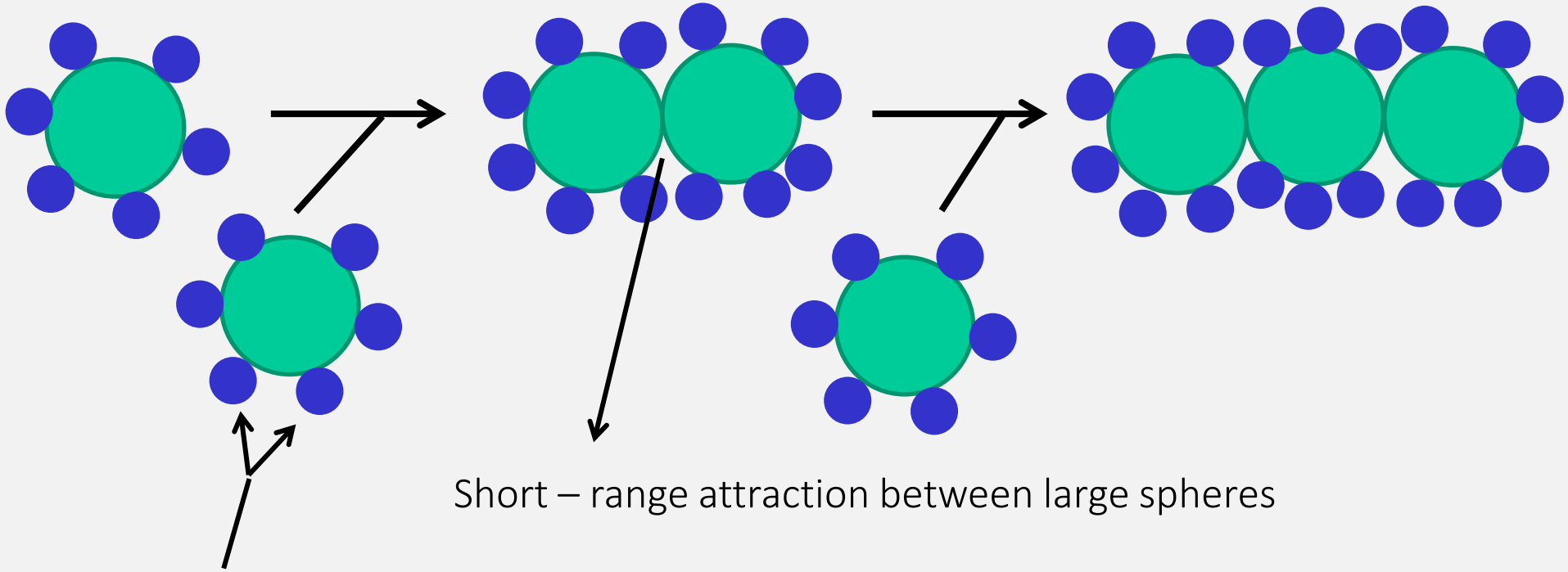




RIP, Leonard Nimoy

Toy model for ‘floppyness’ *---in computro---*

– with Jurriaan Luiken & Peter Bolhuis (UvA)



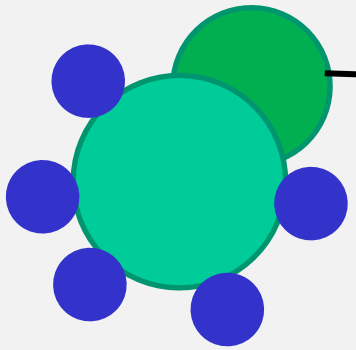
Short – range attraction between large spheres

Fixed number of small ‘satellite’ spheres that move over surface of large sphere;

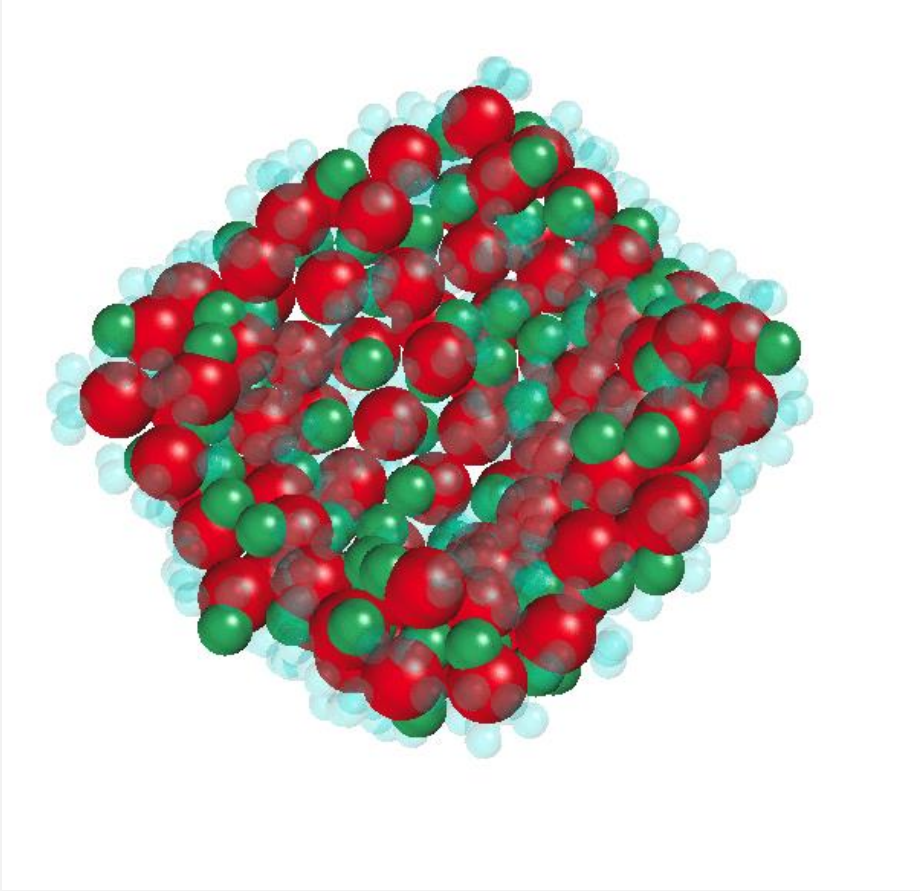
- Repulsion (excluded volume) between, small – large sphere.
- Small sphere interactions: (1) overlap (AO) ; (2) excluded volume

-> under appropriate conditions leads to stable FLAT sheets

Break symmetry:



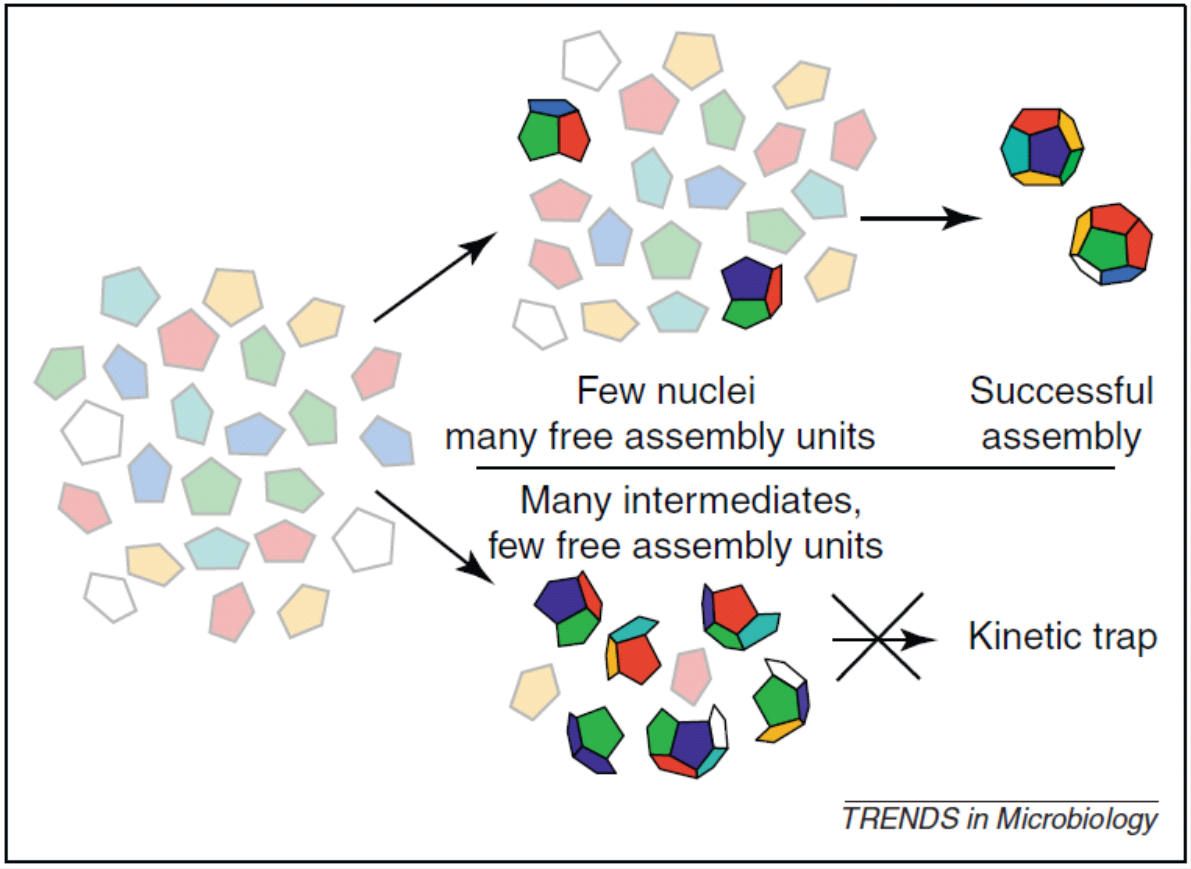
- Immobile (on particle surface)
- Excluded volume interactions (with all other objects)



-> curved sheet – globally as in colloidal shells

In progress:

Under what conditions are long-lived intermediate states expected?



[A. Zlotnick 2012]

- Take capsomer escape time as a measure for equilibration time
- Typical formation times of complete capsids ~ 10 s – several minutes
- -> Non-ergodic crossover time ~ seconds

Summary

- Hydrophobic attraction & Coulomb repulsion compete in virus capsid assembly: virus capsids are assemblies of soft, sticky objects –

  NO need to invoke conformational changes;

- Ionic strength, pH dependence by

  screened Coulomb interaction

- Typical interaction energy G ≈ - (10 – 20) kT / monomer

  (~ - (25 – 50) kJ/ mole)

- Virus –like shells form using 'colloidal bond hybridization' – new theory required.