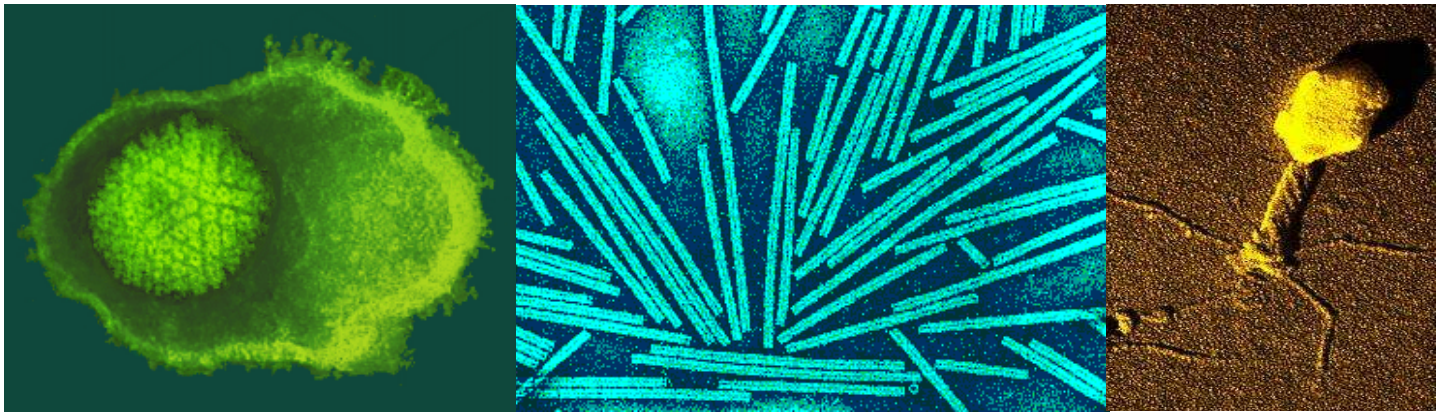
In vitro assembly of simple viruses: a soft matter approach

http://rhino.wisc.edu/virusworld/



Paul van der Schoot — TU/e

Roya Zandi — University of California Riverside

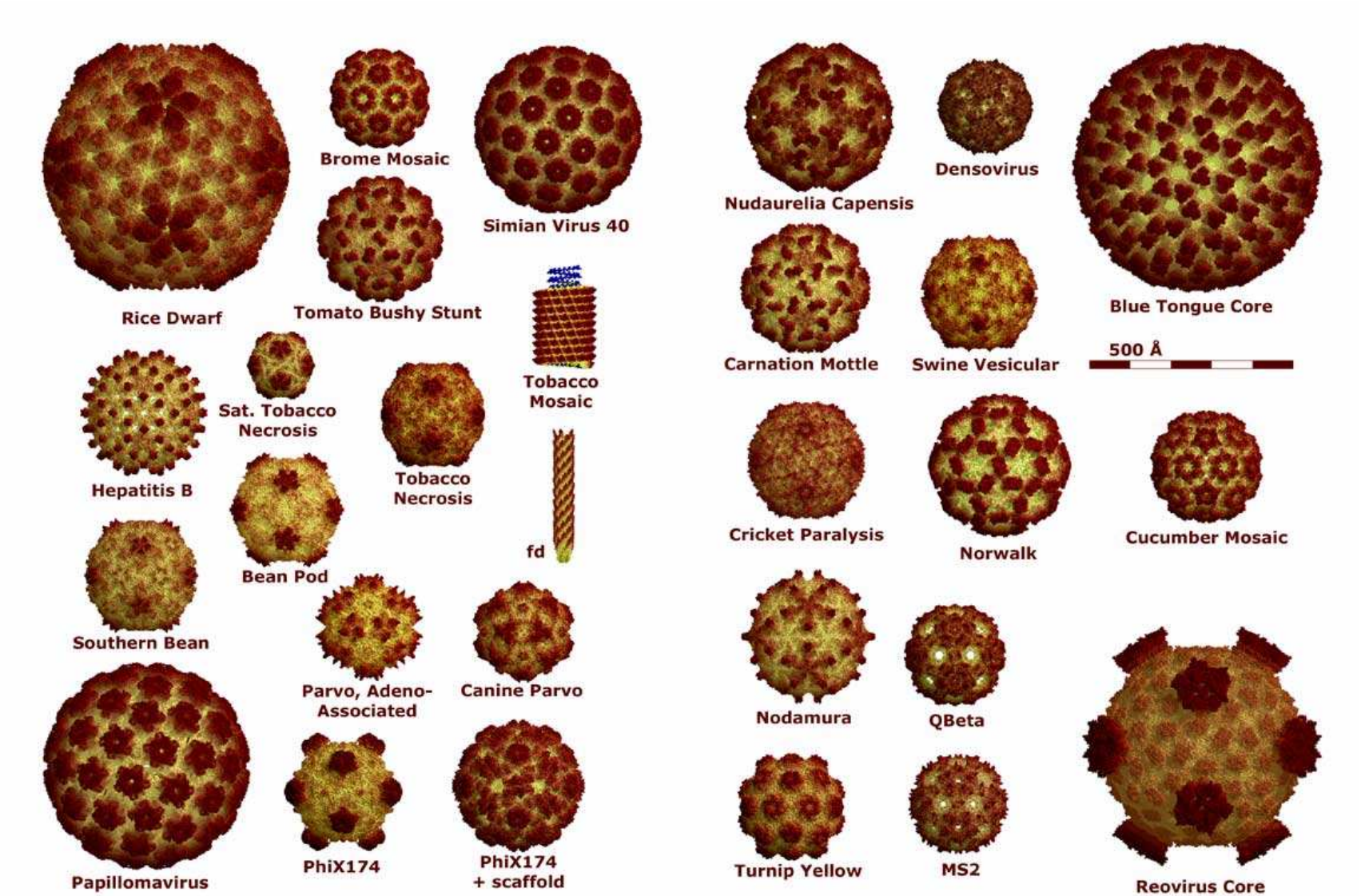
Willem Kegel — Universiteit Utrecht

David Reguera — Universitat de Barcelona

Robijn Bruinsma — UCLA

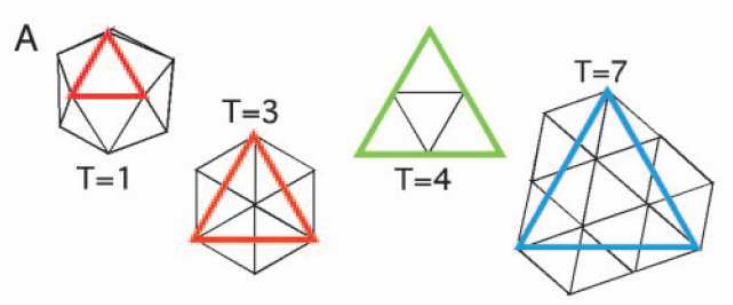
Howard Reiss — UCLA

Viruses large and small…



http://rhino.bocklabs.wisc.edu/virusworld/

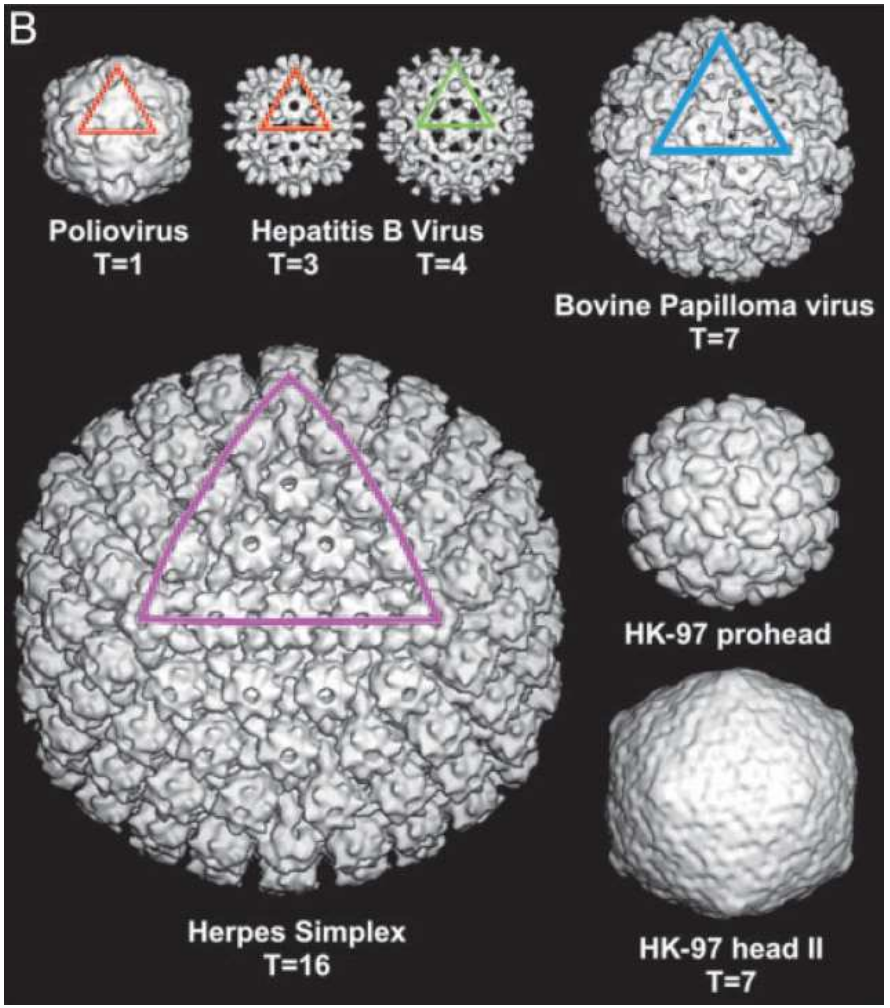
T numbers and all that…



capsid aggregation numbers

q = 60 x T = 60, 180, 240, 420, …

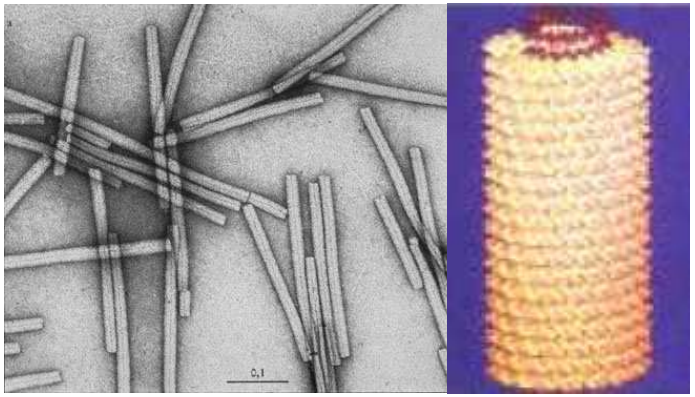
Quantised!



Zlotnick, *PNAS* 101 (2005) 15549

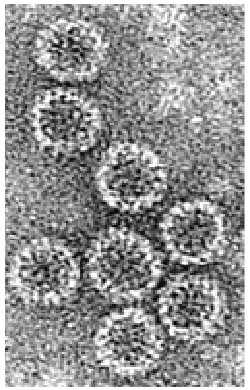
In vitro “assembly diagrams” of coat proteins

TMV

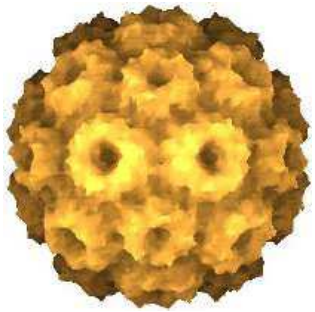


http://rhino.wisc.edu/virusworld/

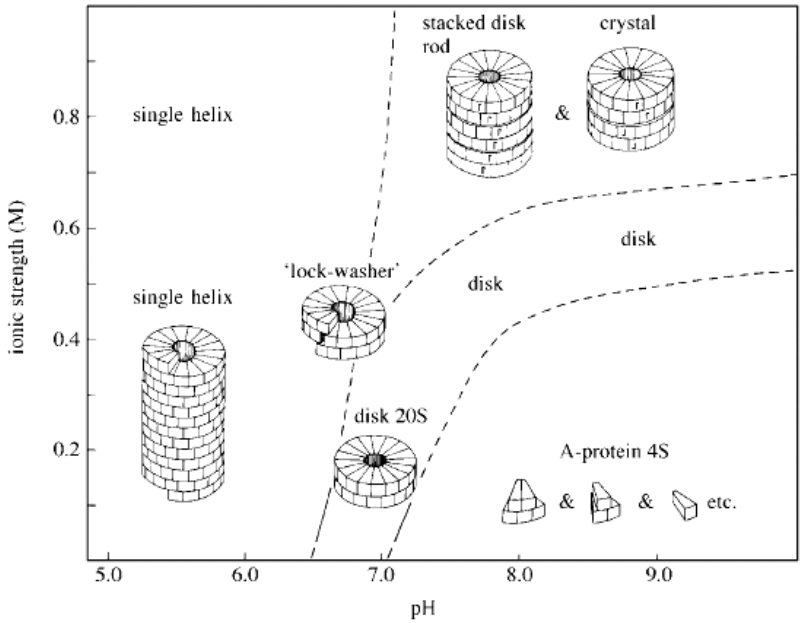
CCMV



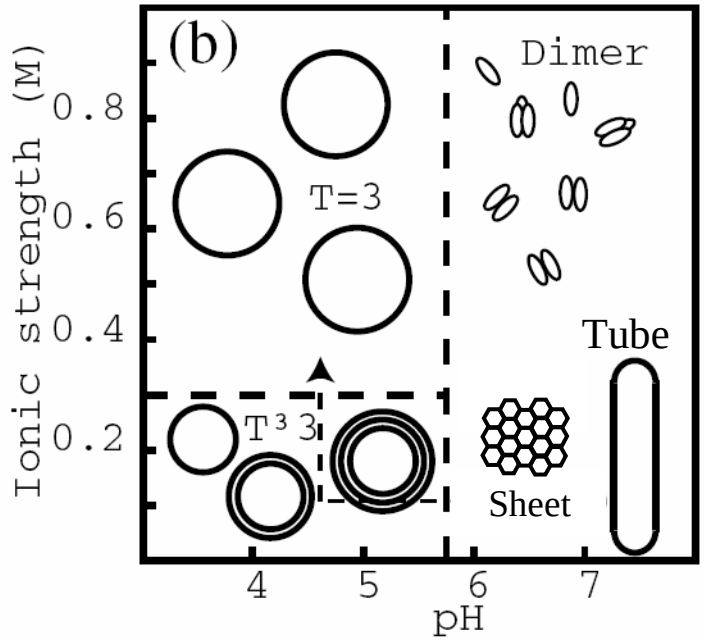
http://virus.chem.ucla.edu/



http://viperdb.scripps.edu



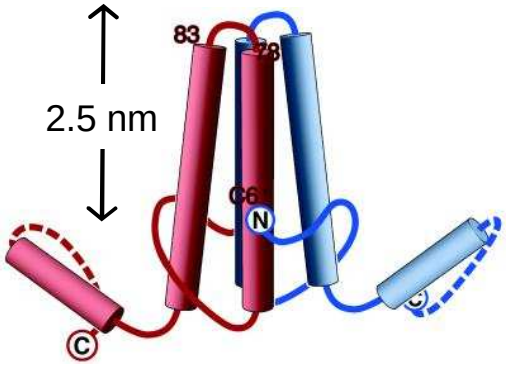
Klug *PTRS Lond* B 354 (1999) 531



Adolph and Butler *PTRS Lond* B 276 (1976) 113.

Self assembly of HBV capsids

Hepatitis B virus coat protein:



assembly domain

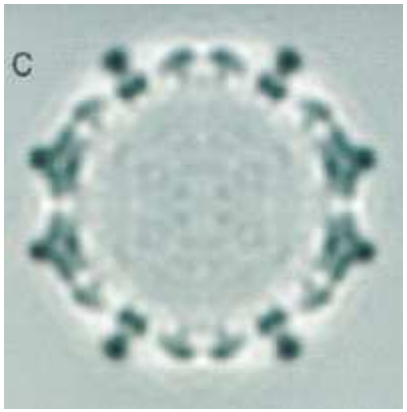
protamine domain

7 nm

remove protamine domain

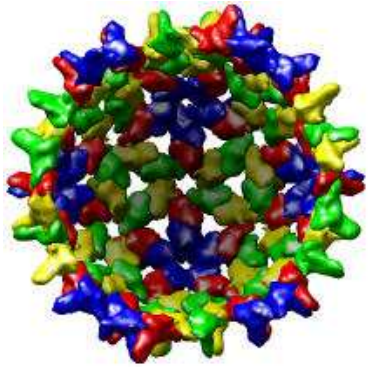
keep protamine domain

30 nm

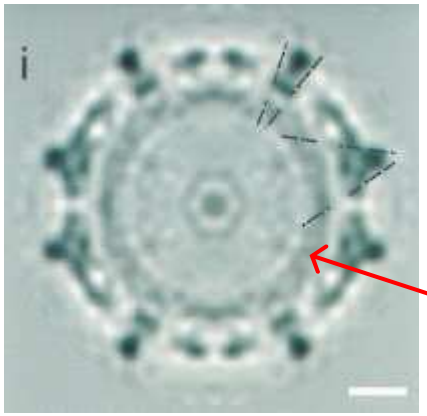


empty capsid

http://viperdb.scripps.edu



T = 4



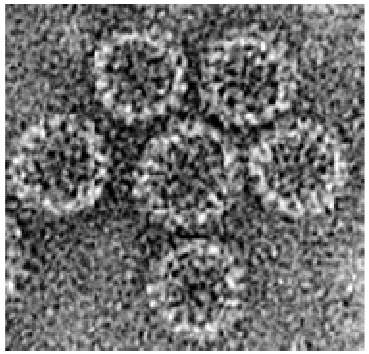
E. Coli RNA

filled capsid

Zlotnick et al *PNAS* 94 (1997) 9556

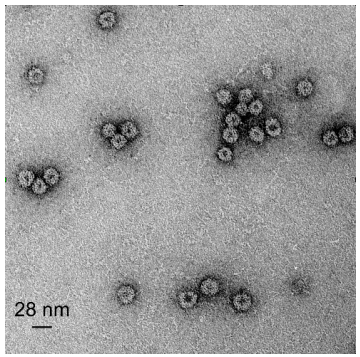
State selection of virus-like particles…

CCMV

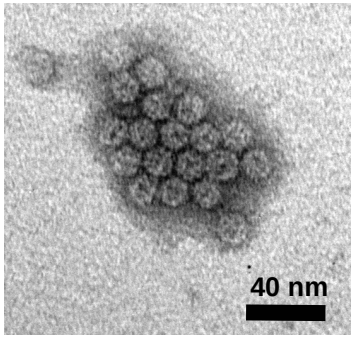


pH = 4

CCMV + PSS



http://virus.chem.ucla.edu/



pH = 7

The Virus Research Group UCLA

Yufang Hu
Chuck Knobler
Bill Gelbart

"T = 3 → 4"

Radboud Universiteit Nijmegen

Friso Sikkema
Jeroen Cornelissen
Roeland Nolte

"T = 3 → 1"

free protein ⇄ capsids ⇄ structure

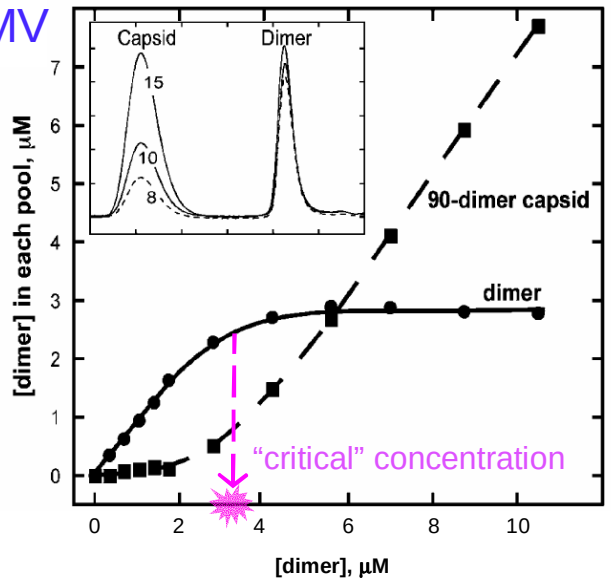
- protein structure
- salt
- pH
- concentration
- temperature
- kinetics
- RNA/PE
- stoichiometry

Bancroft *Adv Virus Res* 16 (1970) 99

Virus capsids as supramolecular assemblies

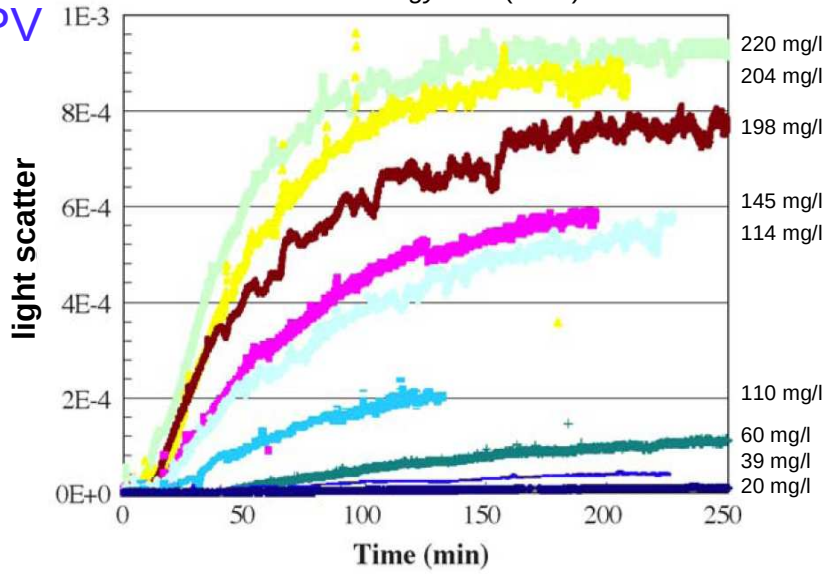
CCMV

Johnson et al. *Nanolett* 5 (2005) 765

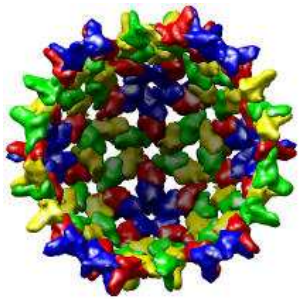


HPV

Casini et al. *Virology* 325 (2004) 390

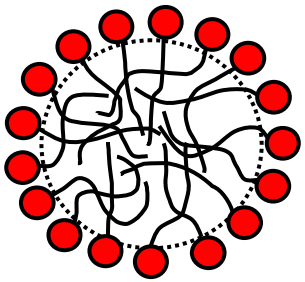


coat proteins as sticky amphiphiles



capsid

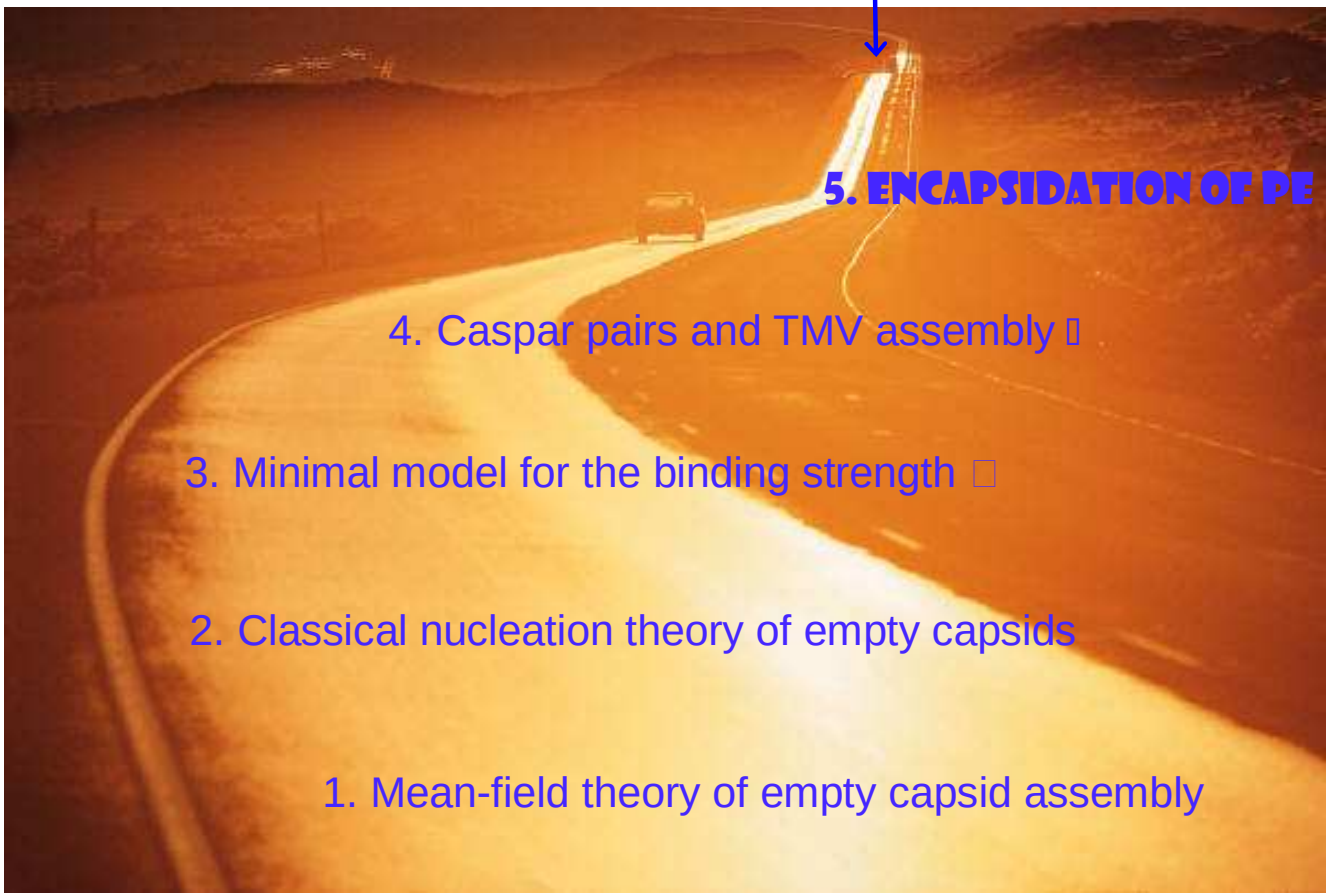
≈



micelle

McPherson *Bioassays* 26 (2005) 447

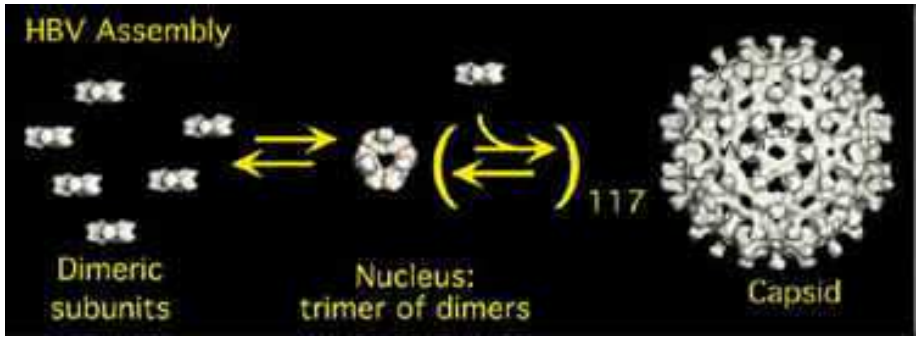
Road map to lunch…



Mean-field theory of equilibrium capsid assembly

multiple chemical equilibria:

- cylinders: Lauffer, Caspar, …
- spheres: Zlotnick, …



Zlotnick '96

total free energy

$$F = \sum_{N=1}^{\infty} \rho(N)\left[\ln \rho(N) - 1 + G(N)\right] + \cancel{F_{exc}(\phi)}$$

equilibrium

$$\frac{\delta F}{\delta \rho} = \mu N \quad \Rightarrow \quad \rho(N) = \exp\left[\mu N - G(N)\right]$$

size distribution

$$\sum_{N=1}^{\infty} N\rho(N) = \phi$$

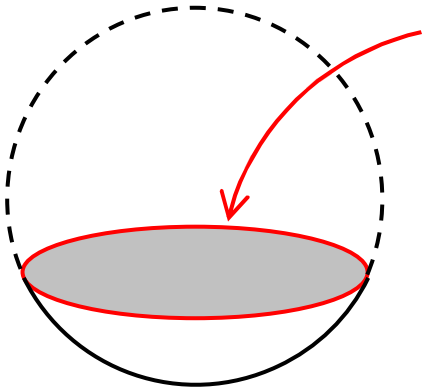
protein concentration

free energy of an assembly

$$G(N) = Ng + \Delta G(N)$$ ← "rim energy"

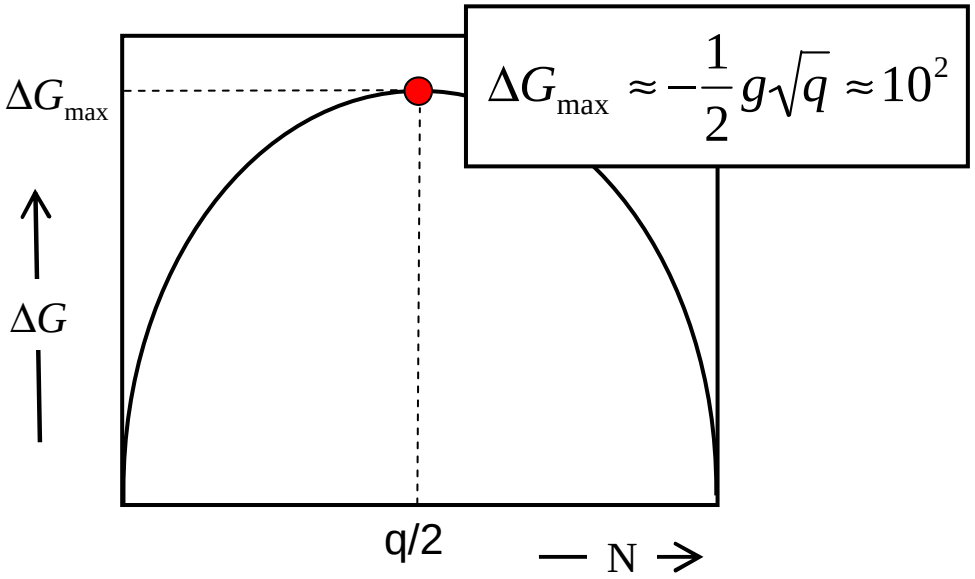
"binding strength" ≈ – *O*(10) $k_B T$

Spherical capsids: rim suppresses intermediates!

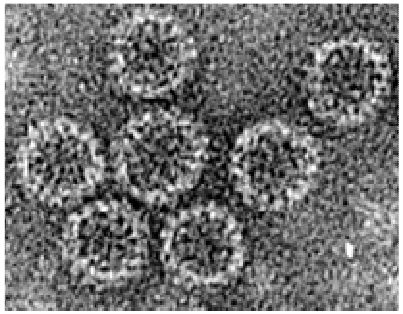


partially complete capsid
of fixed T number

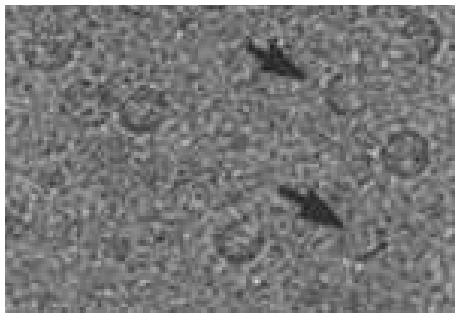
rim energy $\Delta G(N) \approx -g\sqrt{\frac{N(q-N)}{q-1}} \Rightarrow G(1) \equiv 0$



- *assembly thermodynamics:*
  - $\Rightarrow$ intermediates unimportant
  - $\Rightarrow$ 2-species model sufficient
- *assembly kinetics:*
  - $\Rightarrow$ intermediates important
  - $\Rightarrow$ lag time
  - $\Rightarrow$ hysteresis



http://virus.chem.ucla.edu/

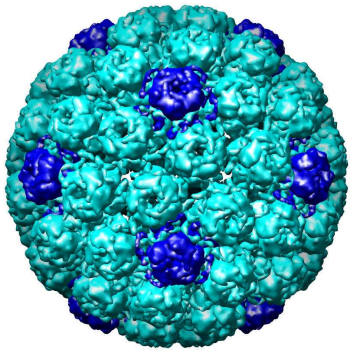


Berthet et al. EBJ 15 (1987) 159

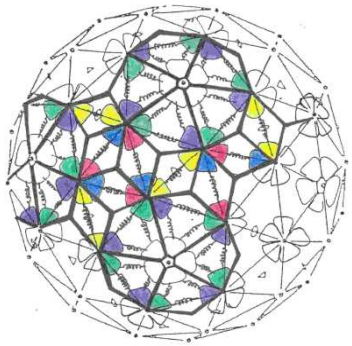
Zandi et al. *Biophys J* 19 (2006) 1939

Continuum vs discrete model…

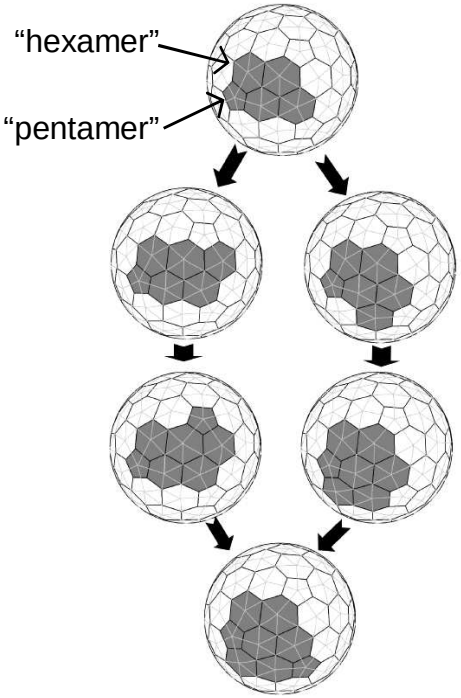
SV40
q = 72
“T” = 7

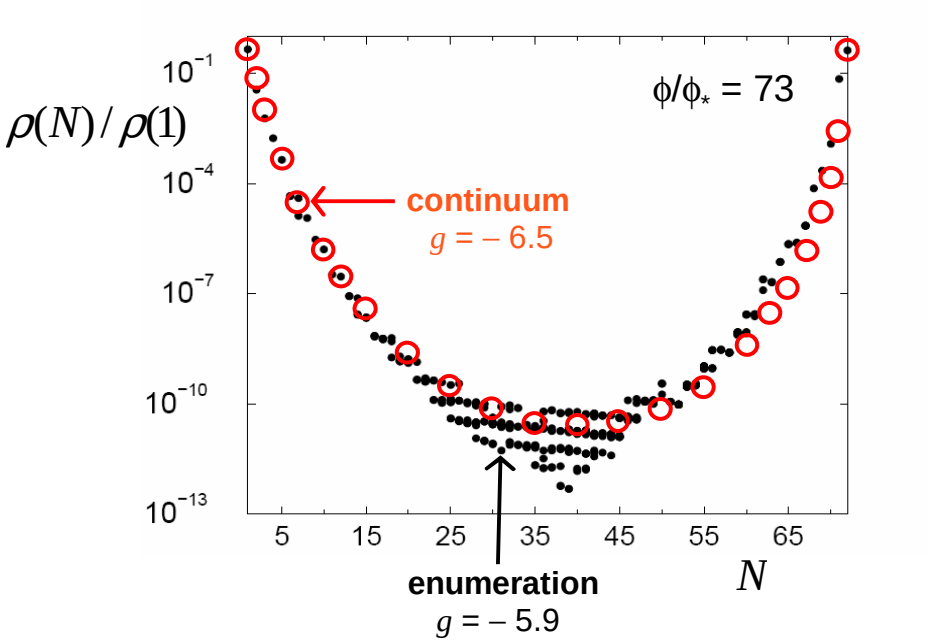


http://viperdb.scripps.edu



Keef et al. q-bio.bm/0508030





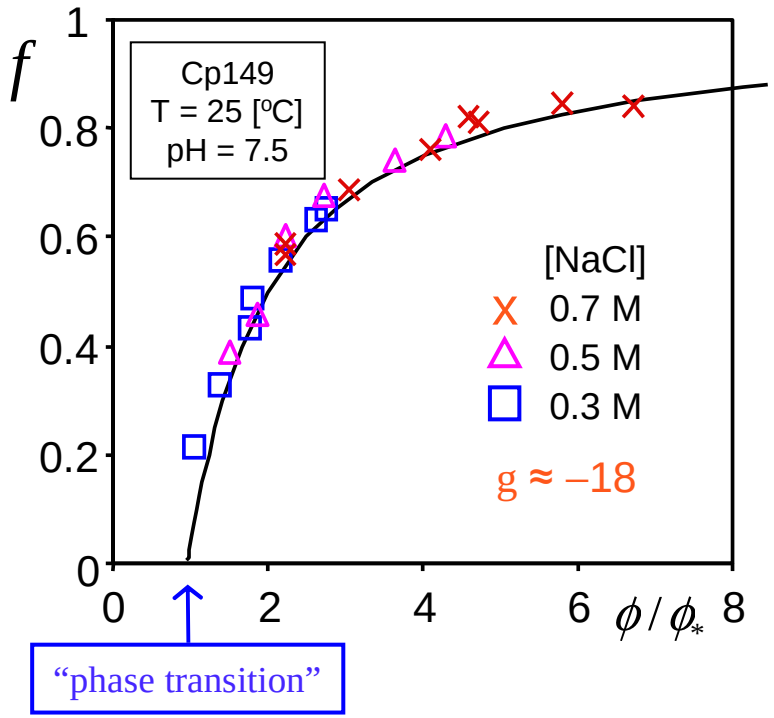
Two-species model of capsid assembly

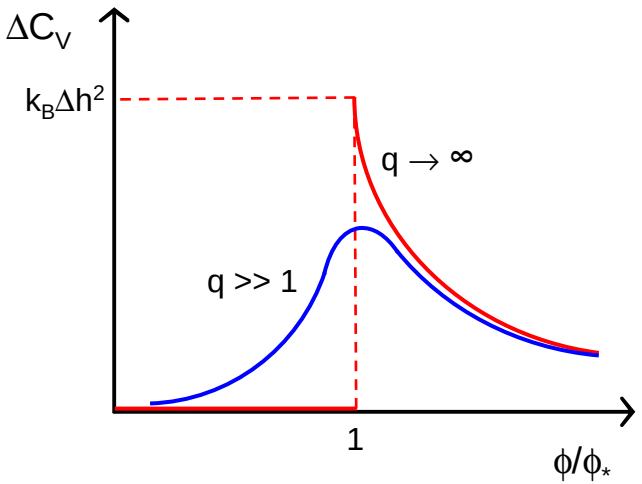
capsid fraction

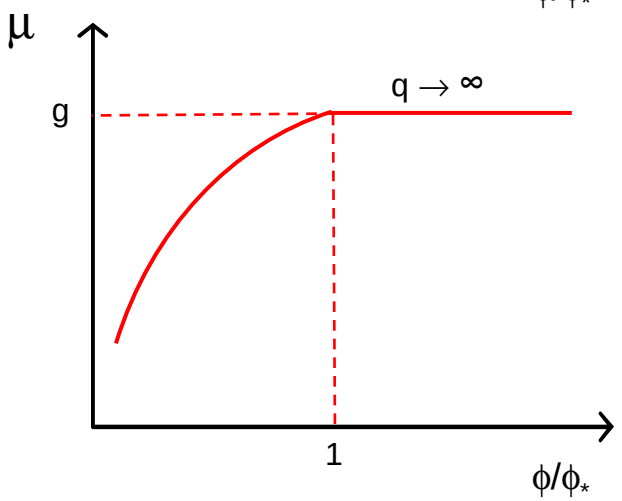
$$f = \frac{q\rho(q)}{\rho(1) + q\rho(q)} = 1 - f^{1/q}\left(\frac{\phi_*}{\phi}\right)^{1-1/q} \sim 1 - \frac{\phi_*}{\phi} \qquad (q >> 1)$$

critical protein concentration

$$\phi_* \sim \exp g$$



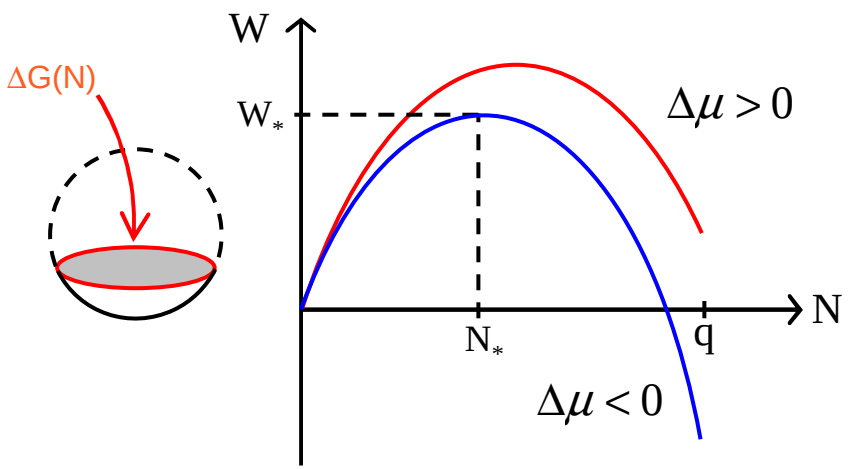




Debye *ANYAS* 59 (1949) 575
Ceres and Zlotnick *Biochem* 41 (2002) 11525
Kegel and vdS *Biophys J* 86 (2004) 3905

Classical nucleation theory of capsid assembly

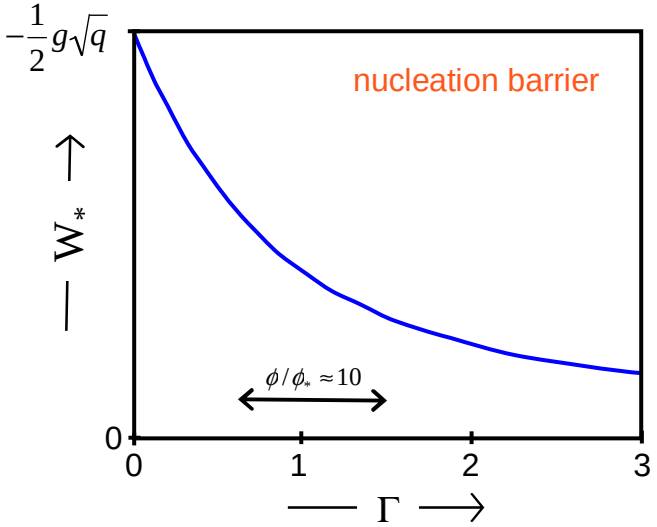
cluster free energy: $W(N) = N\Delta\mu + \Delta G(N)$

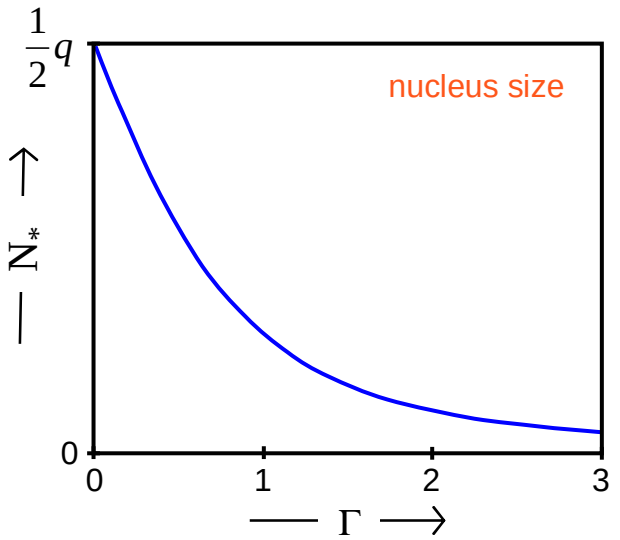


steady-state nucleation rate $J \propto \exp -W_*$

lag time $\tau \propto 1/|W_*''|$

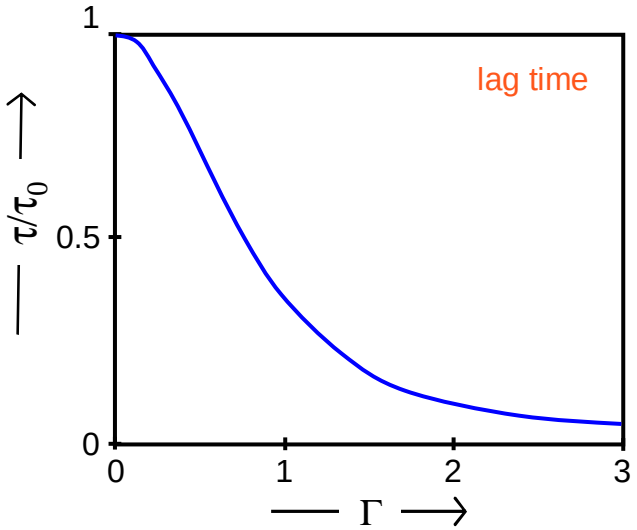
quench depth $\Gamma \propto \frac{\sqrt{q}}{|g|} \ln \frac{\phi}{\phi_*}$

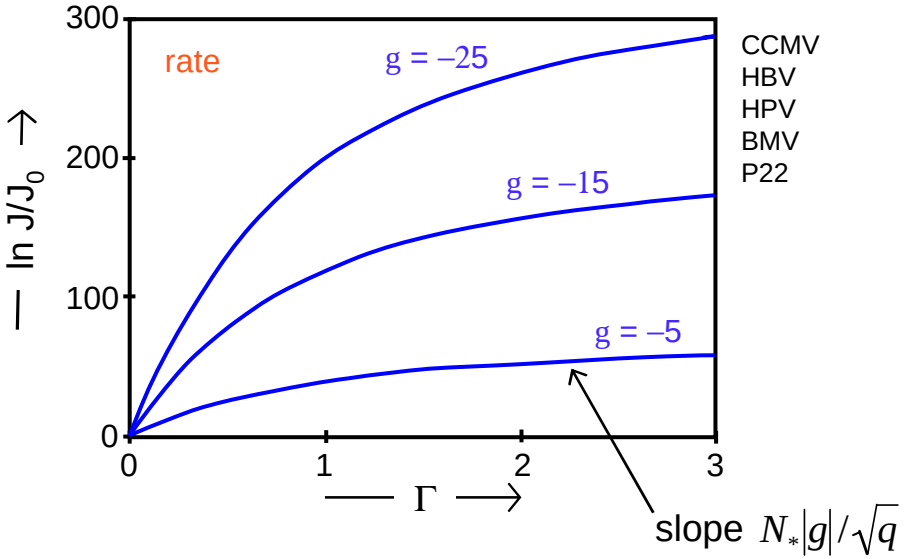




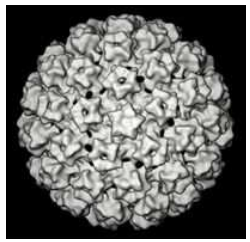
Zandi et al. *Biophys J* 19 (2006) 1939

Lag time and nucleation rate of capsid assembly



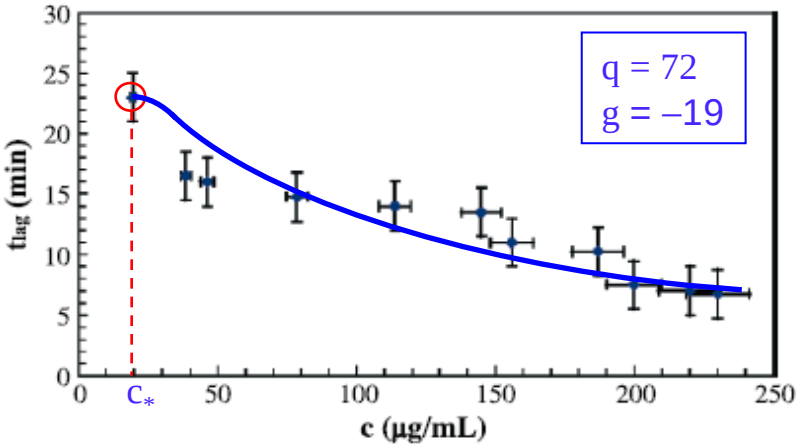


HPV



pH = 5.2
[NaCl] = 0.5M
T = 24ºC

Casini et al, *Virology* 325 (2004) 390

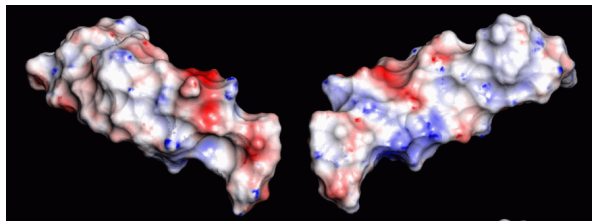


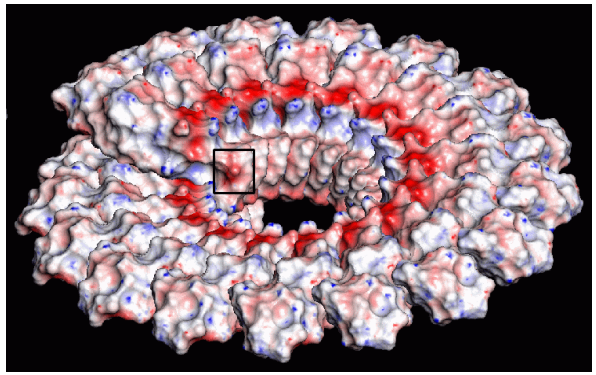
http://en.wikipedia.org/wiki/Papillomavirus

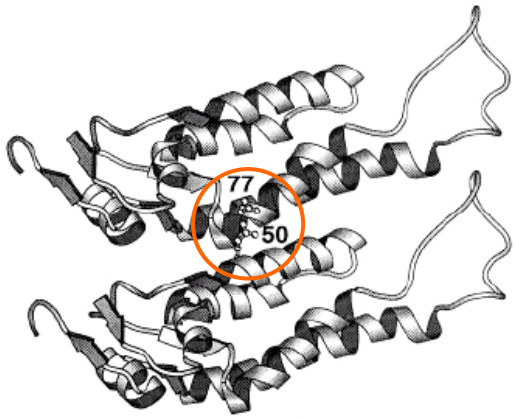
Zandi et al. *Biophys J* 19 (2006) 1939

Binding strength: competing interactions

TMV

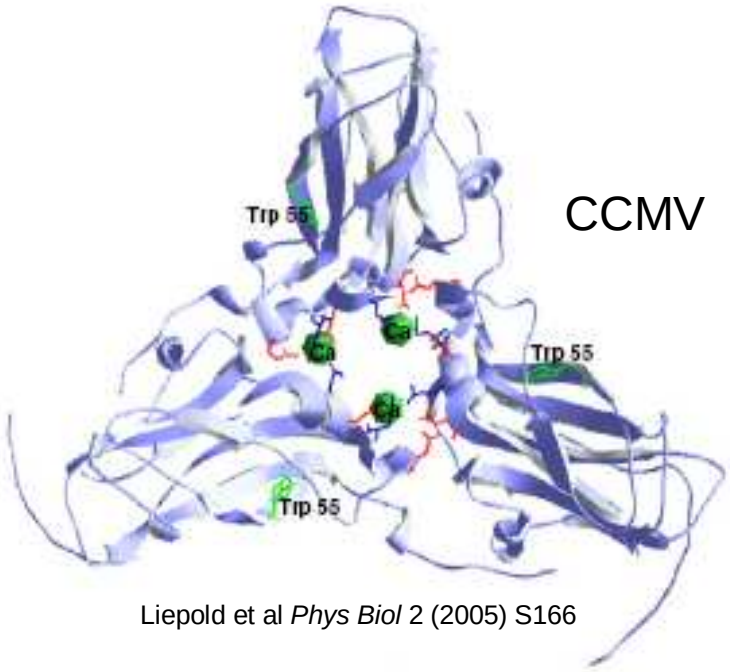






http://fibernet.vanderbilt.edu/

CCMV

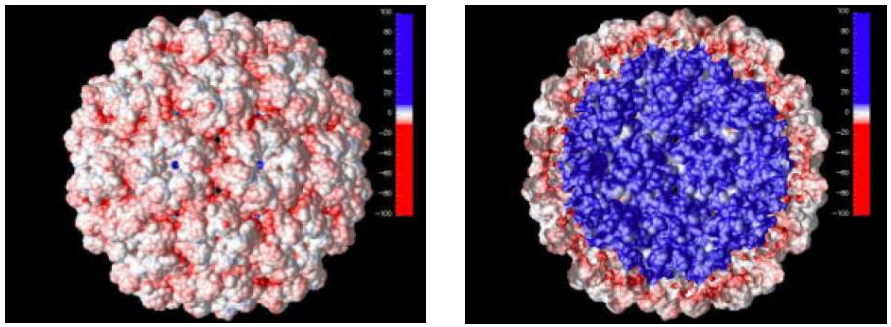


Liepold et al *Phys Biol* 2 (2005) S166

- Coulomb interactions
- "Caspar pairs"
- ionic bonds
- hydrogen bonds
- hydrophobic interactions ← Bancroft, Caspar, Lauffer, …
- steric interactions
- conformational switching
- van der Waals interactions
- chemical bonds

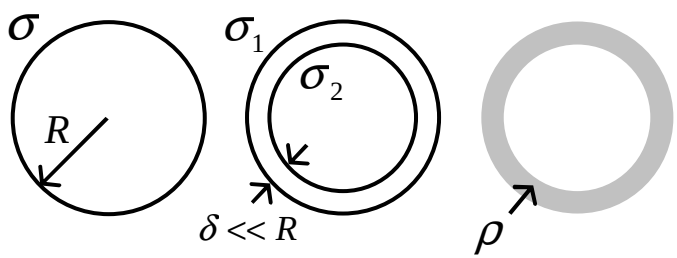
Electrostatics of virus capsids

CCMV



Konecny et al. *Biopolym* 82 (2006) 106

capsid models:



1) Debye-Hückel theory

$$\nabla^2\psi(r) = \lambda_D^{-2}\psi(r)$$

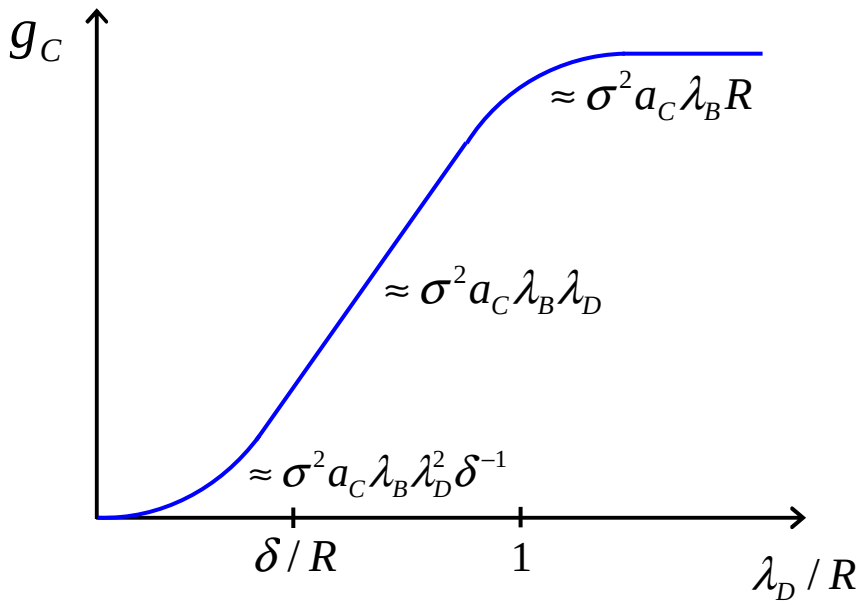
$$\lambda_D = 1/\sqrt{8\pi\lambda_B I}$$

$$\lambda_B = e^2/4\pi\varepsilon k_B T$$

$$I = \tfrac{1}{2} z_+^{\,2}\rho_+ + \tfrac{1}{2} z_-^{\,2}\rho_-$$

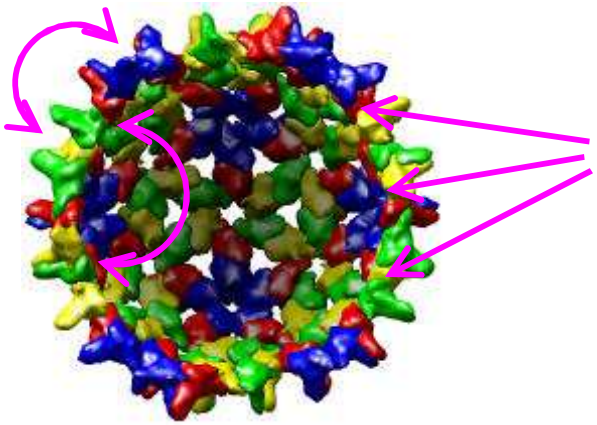
2) Debye charging process

3) subtract reference state



Kegel and vdS *Biophys J* 86 (2004) 3905

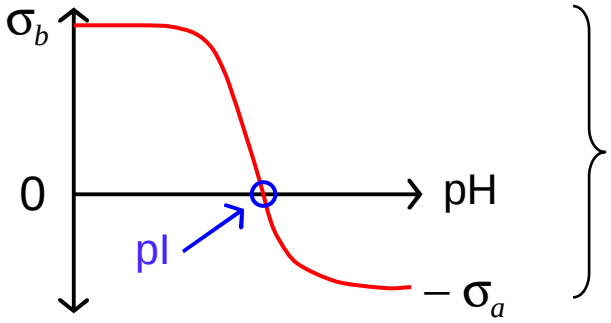
Minimal model for the binding strength



http://viperdb.scripps.edu

binding strength: $g = g(T, pH, I) \approx \underbrace{-\gamma_H a_H}_{\text{hydrophobic attraction}} + \underbrace{\lambda_B \lambda_D \sigma^2 a_C}_{\text{Coulomb repulsion}} + \cdots$

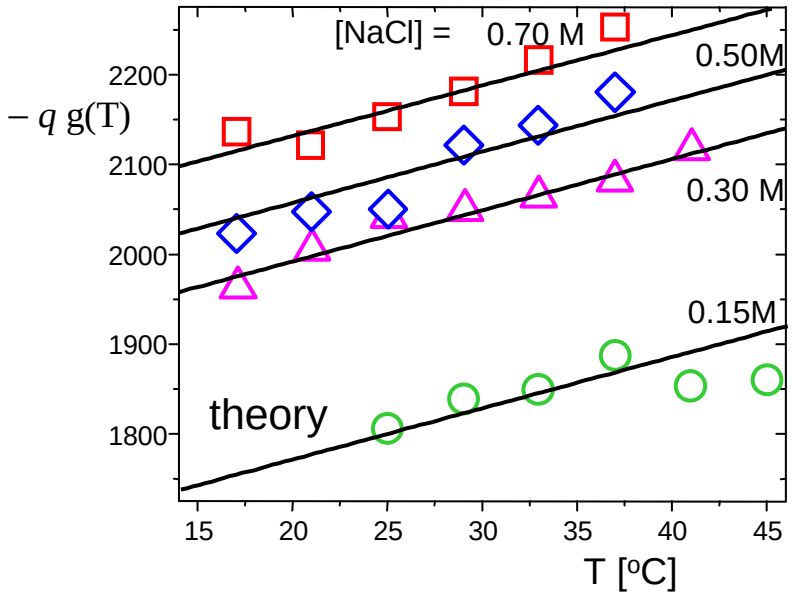
charge density:



$$\sigma \approx \frac{\sigma_b}{1+10^{pH-pK_b}} - \frac{\sigma_a}{1+10^{pK_a-pH}}$$

temperature: $g(T) = g(T_0) - h(T_0)(T/T_0 - 1) \approx g(T_0) + h_H(T_0)(T/T_0 - 1)$

Self-assembly of empty HBV Cp149 capsids

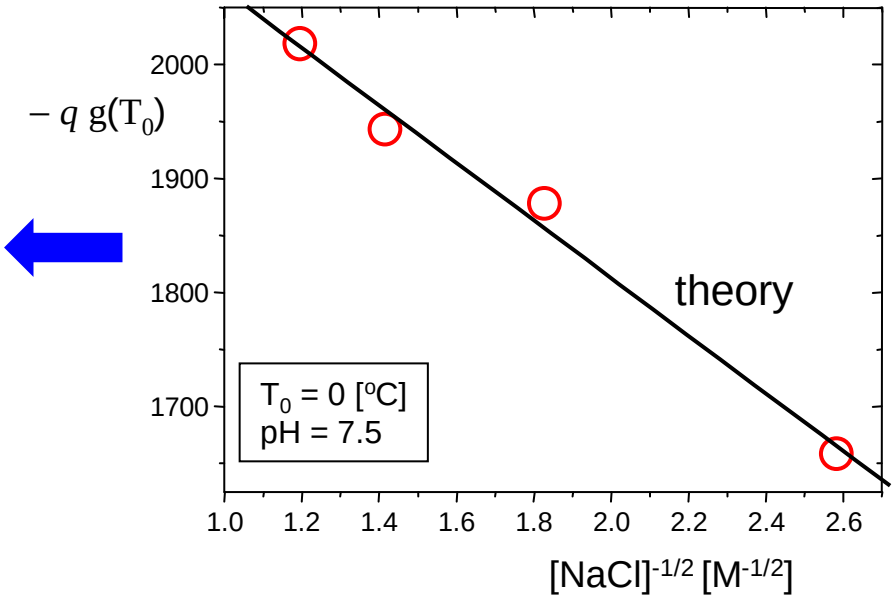


a_H	13 nm^2
γ_H	
h_H	–3.8 mN m^{-1}
s_H	

+19 k_BT_0

–13 k_BT_0

–32 k_BT_0

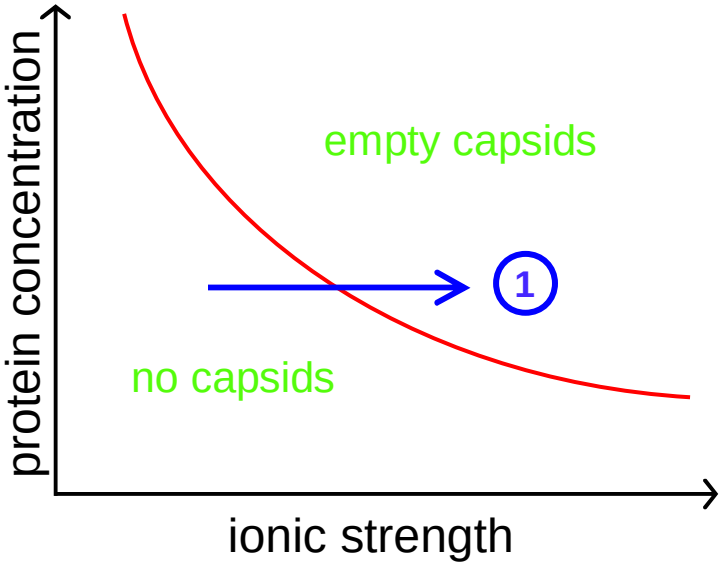


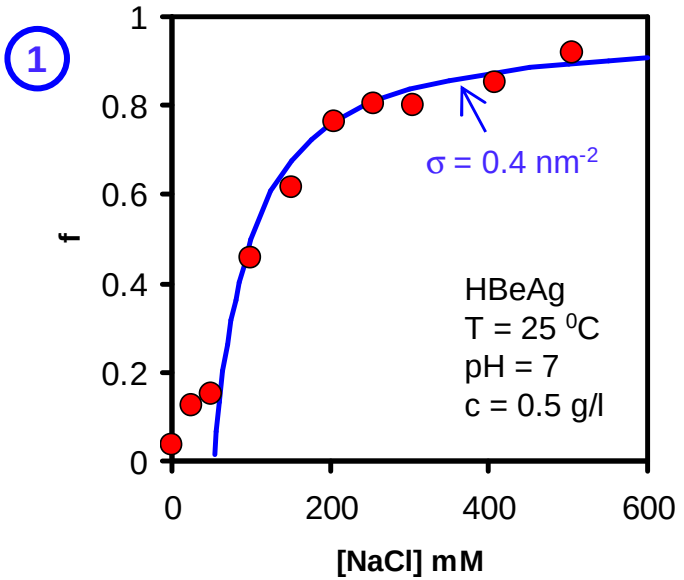
a_C	23 nm^2
σ	0.7 nm^{-2}

7 charges per protein

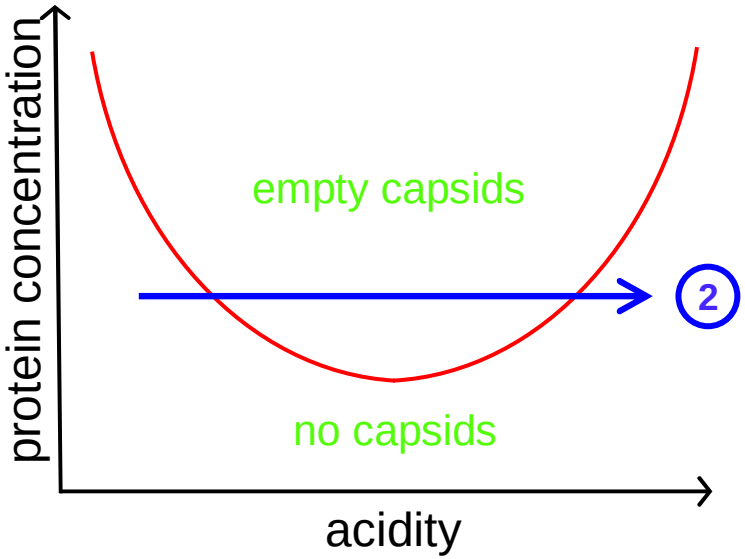
Ceres and Zlotnick *Biochem* 41 (2002) 11525
Kegel and vdS *Biophys J* 86 (2004) 3905

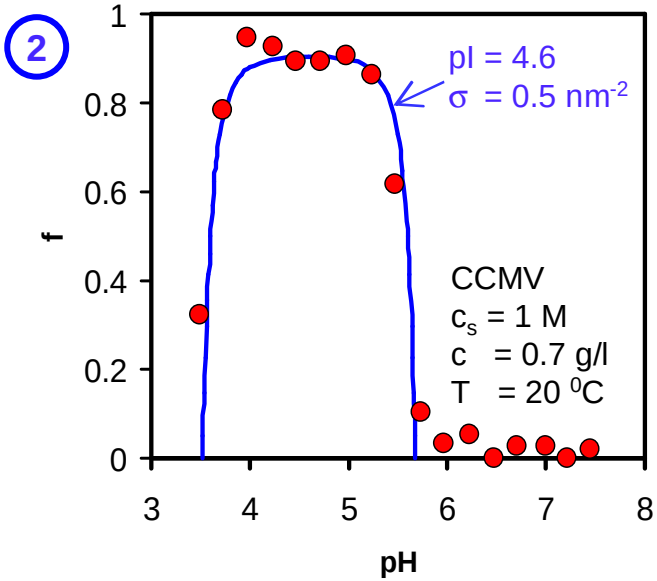
Theoretical assembly diagrams of empty capsids





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



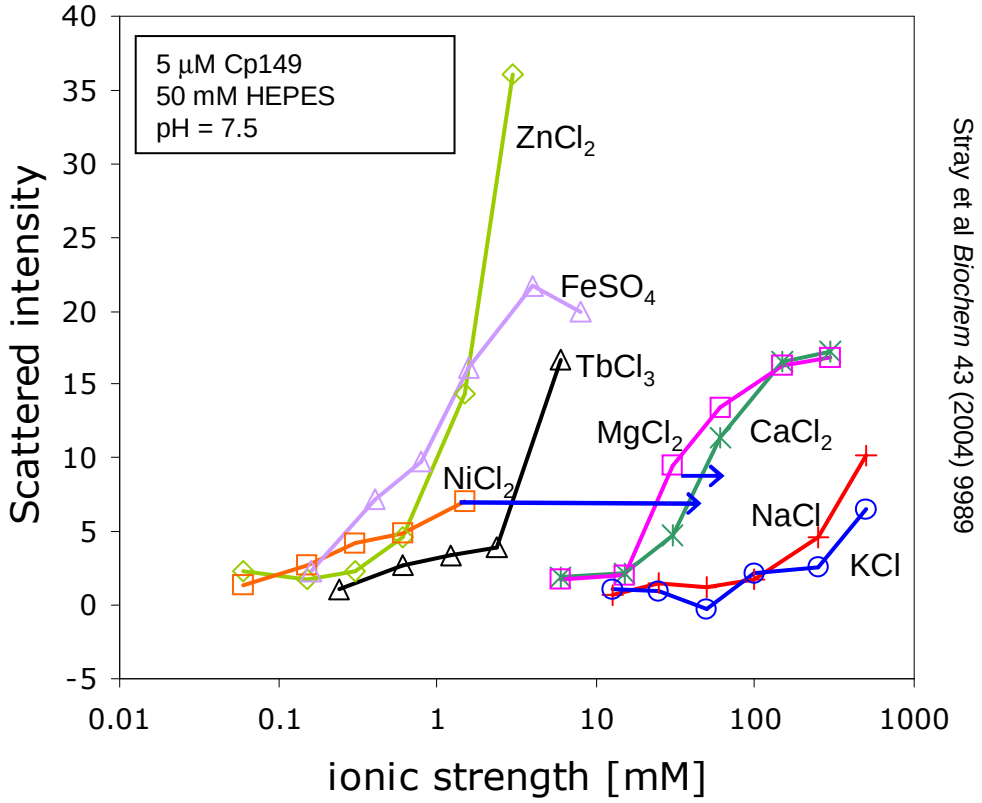


Adolph et al *J. Mol. Biol.* 88 (1974) 327

“Hofmeister” series of HBV capsid assembly

minimal capsid assembly model

$$
\begin{cases}
f \approx 1 - \phi_* / \phi \geq 0 \\
\ln \phi_* \approx -\dfrac{\gamma a_H}{k_B T} + \dfrac{\lambda_B \sigma^2 a_C}{\sqrt{8\pi\lambda_B I}} \\
\sigma \approx \dfrac{\sigma_b}{1+10^{pH-pK_b}} - \dfrac{\sigma_a}{1+10^{pK_a-pH}} \\
I = \frac{1}{2} z_+^{\,2} \rho_+ + \frac{1}{2} z_-^{\,2} \rho_-
\end{cases}
$$



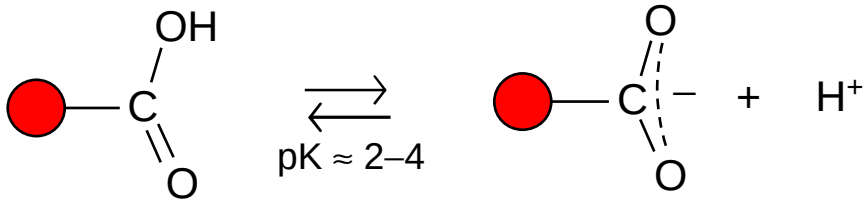
Stray et al *Biochem* 43 (2004) 9989

What’s wrong?

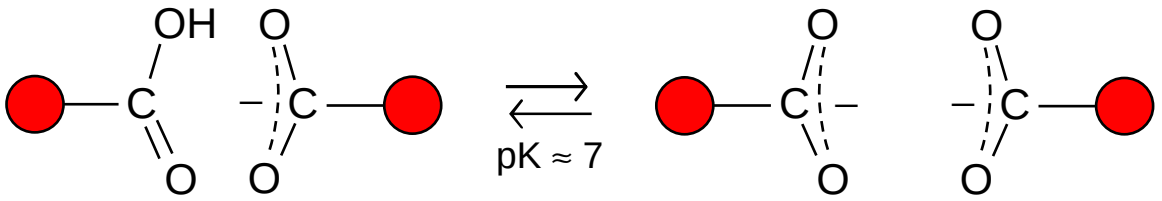
- buffer
- breakdown of DH theory
- conformational switching
- specific binding
- coarse graining
- …

"CASPAR" PAIRS AND CATION BINDING...

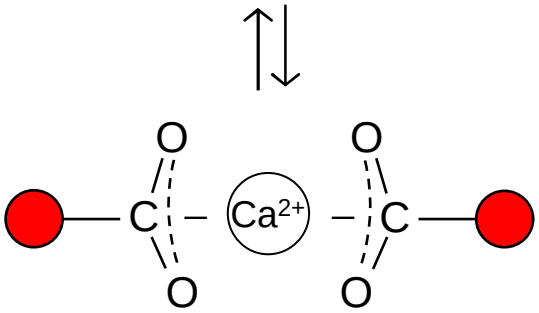
Glu/Asp:



"Caspar" pairs:

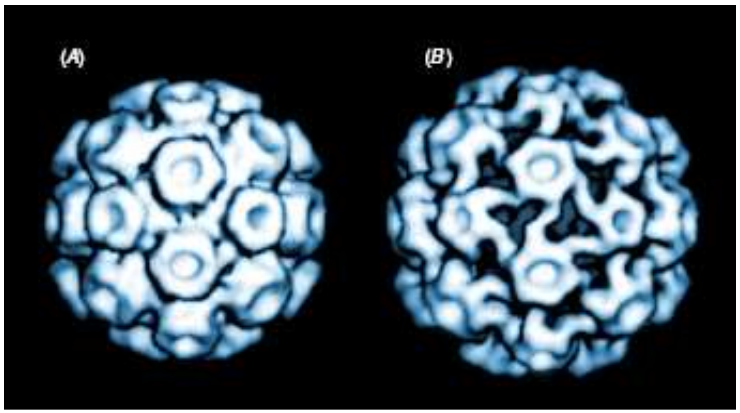


hydrogen bonding



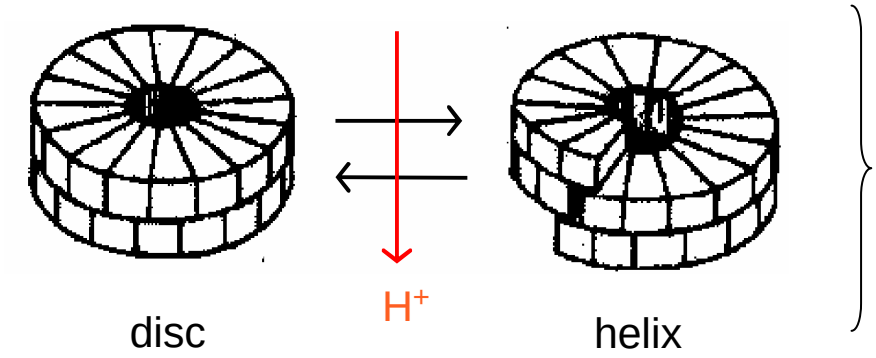
ionic bonding

CCMV



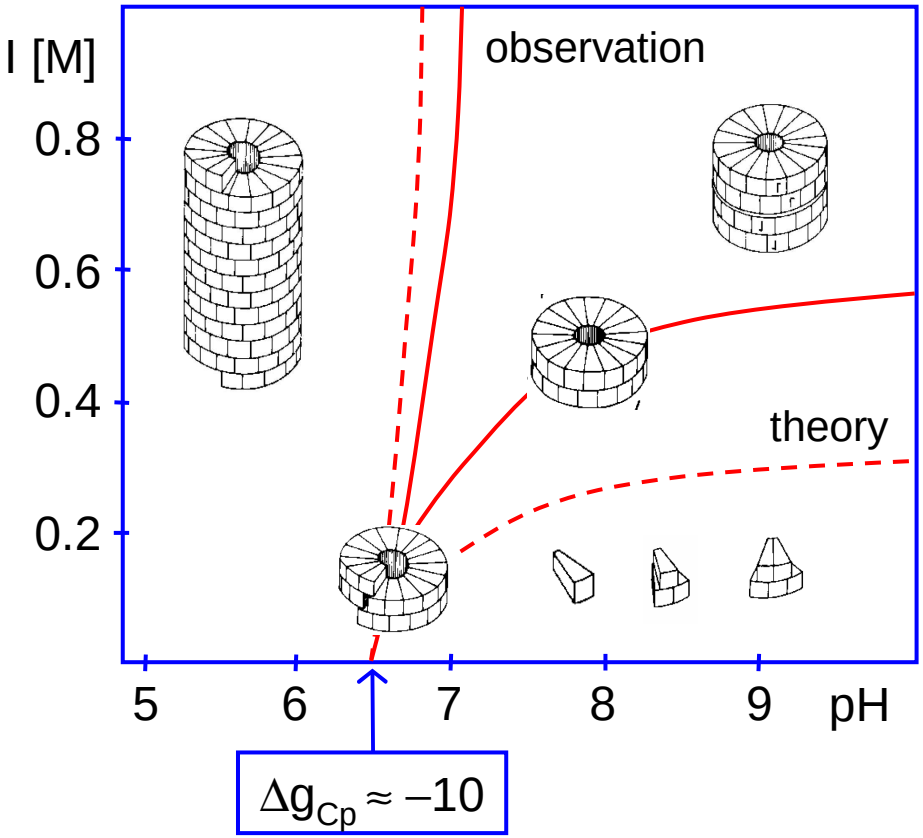
Liepold et al *Phys Biol* 2 (2005) S166

CASPAR PAIRS AND THE STABILITY OF TMV



Ansatz:
- different σ, a_C, a_H
- Caspar pairs

curve fitting:
- titration
- calorimetry
- "anomalous" pK

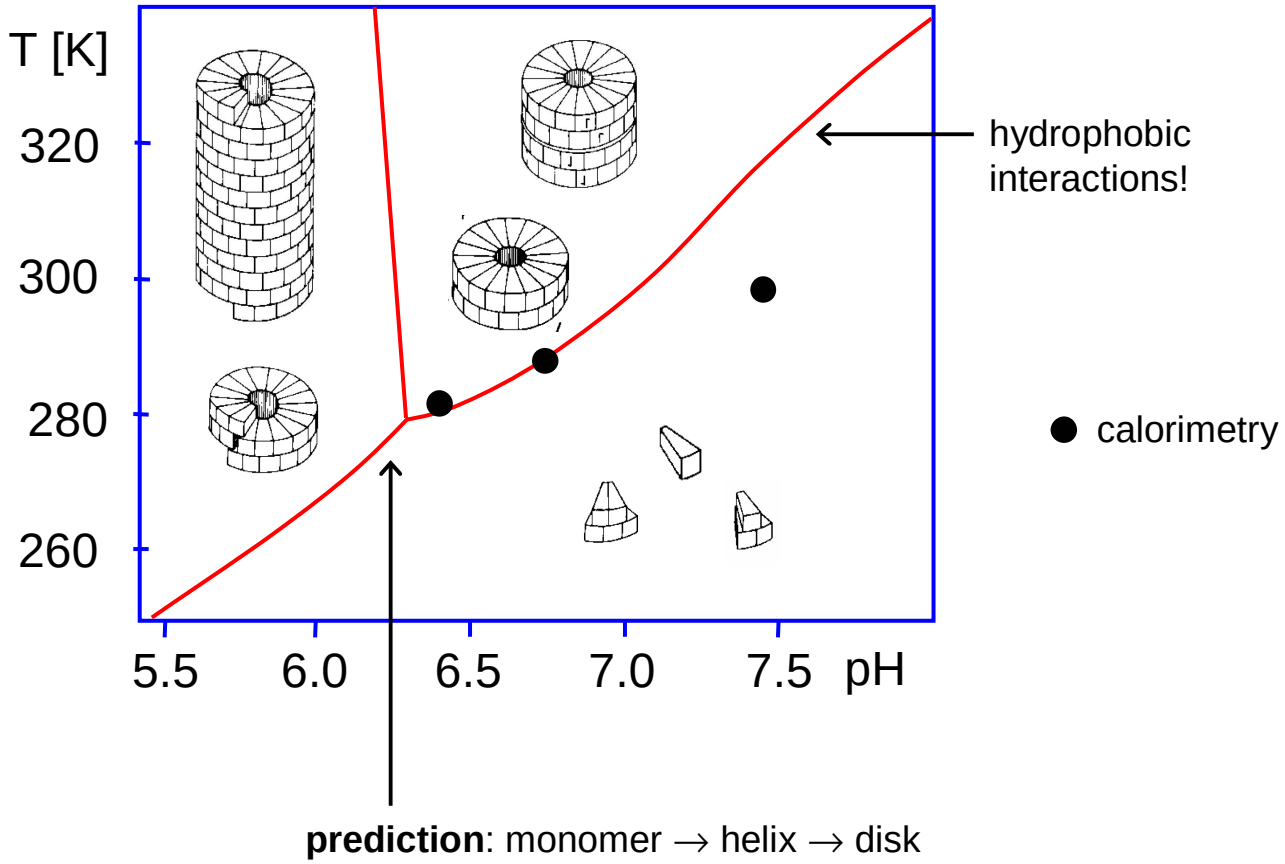


patch stickiness

	$\gamma_H a_H$	$h_H a_H$	$Ts_H a_H$
TMV disc	17	– 19	–36
TMV helix	16	– 18	–34
HBV	19	– 13	–32

Klug *PTRS Lond* B 354 (1999) 531
Kegel and vdS *Biophys J* (accepted)

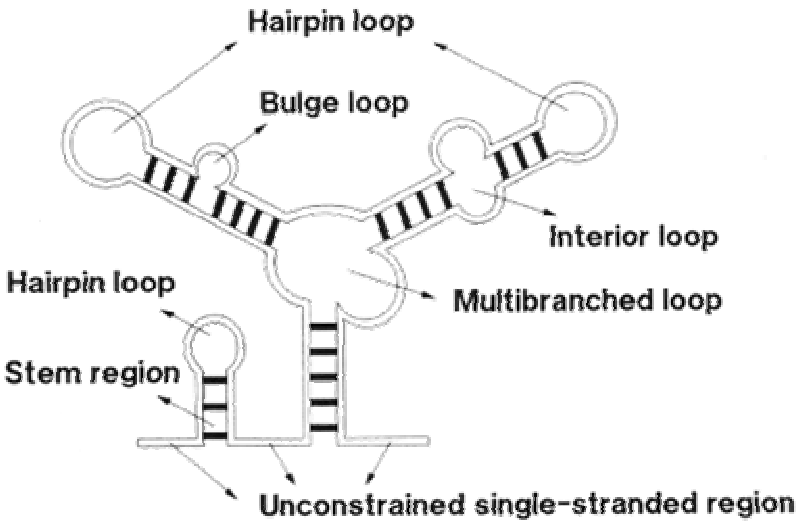
THEORETICAL PHASE DIAGRAM OF TMV



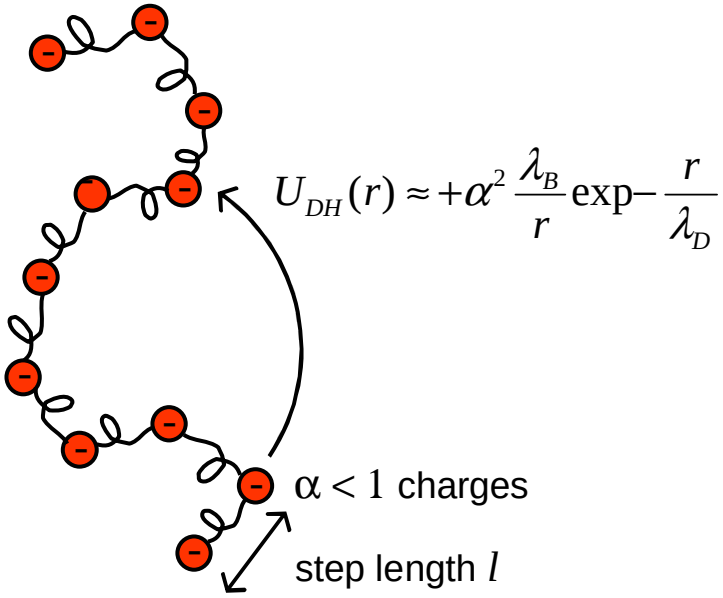
Kegel and vdS *Biophys J* (accepted)

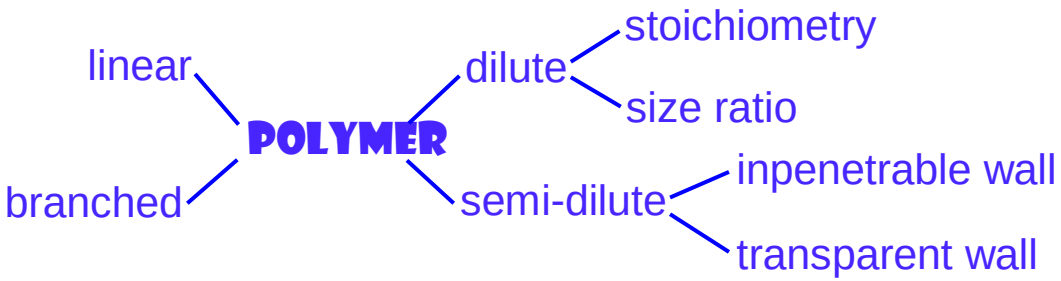
ENCAPSIDATION OF SS RNA/PE

ss viral RNA

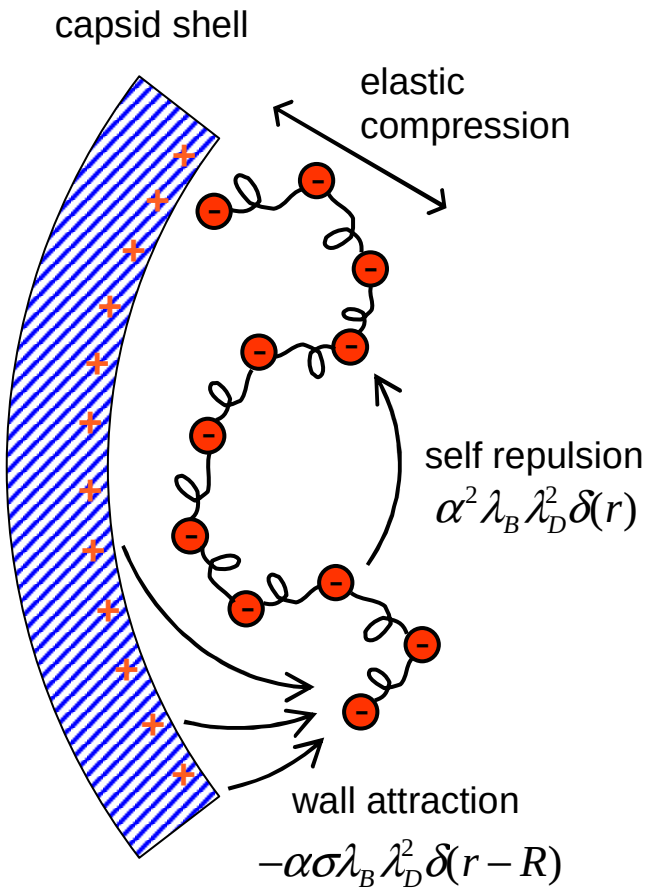


model RNA/PE





Mean-field theory of polyanion adsorption



full equilibrium in semi-dilute solution

$$\Delta G_P = \int d^3\vec{r}\left\{\frac{1}{6}l^2\left(\nabla\phi^{1/2}\right)^2 + \frac{1}{2}\nu(\phi^2-\phi_B^2) - \mu_B(\phi-\phi_B)\right\}$$

$$-4\pi R^2\alpha\sigma\lambda_B\lambda_D^2\left(\phi(R)-\phi_B\right)$$

Edwards equation

$$\frac{1}{6}l^2\Delta\phi^{1/2} = \phi^{1/2}(\nu\phi-\mu_B)$$

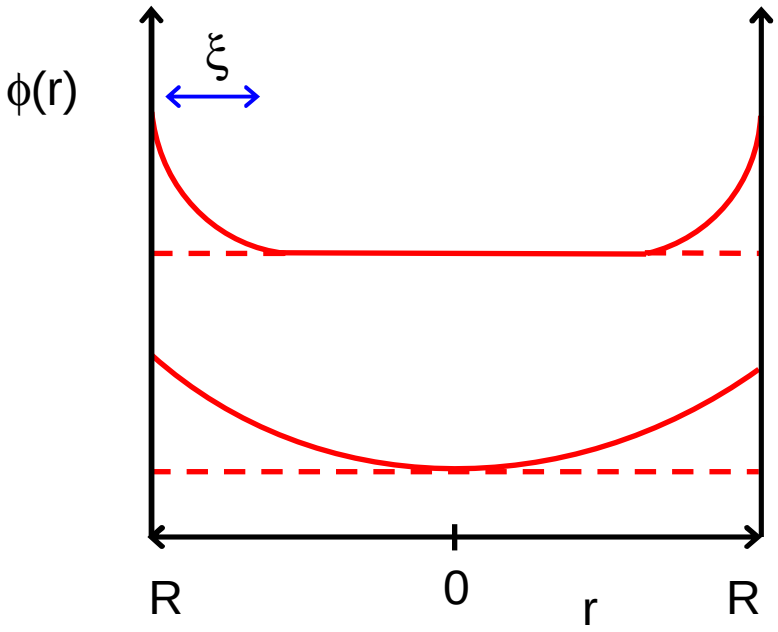
$$\frac{1}{\phi^{1/2}}\frac{d}{dr}\phi^{1/2}\bigg|_{r=R} = \frac{\alpha\sigma\lambda_B\lambda_D^2}{l^2}$$

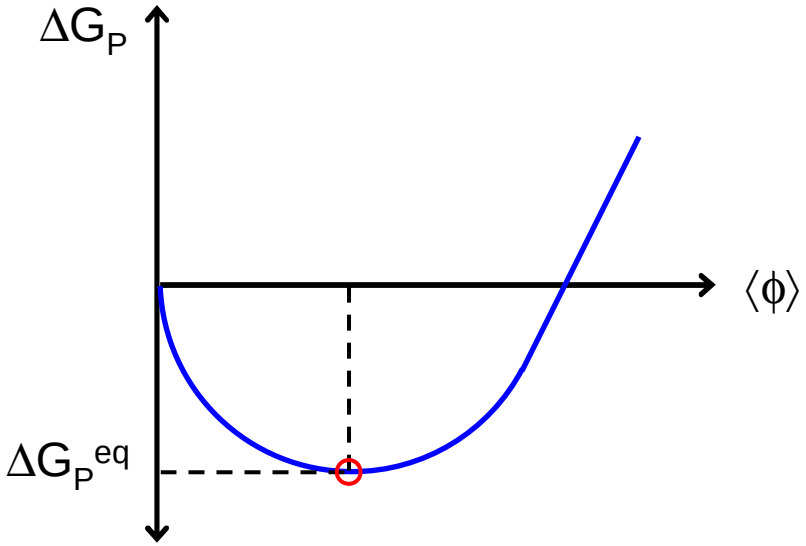
critical protein concentration

$$\ln\phi_* = g + \Delta G_P^{eq}/q$$

Ji and Hone *Macromolecules* 21 (1988) 2600
Joanny *EPJB* 9 (1999)117

PROFILE AND FREE ENERGY OF CAPTURED POLYELECTI





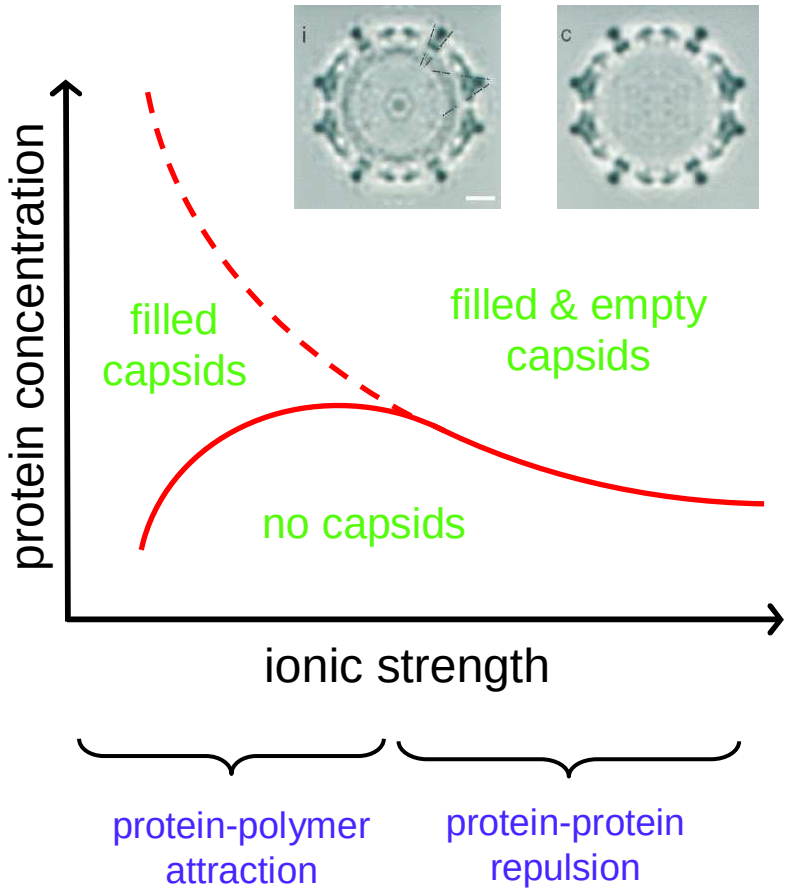
full equilibrium

$$\begin{cases} \langle\phi\rangle \Rightarrow \text{charge reversal …} \\ \ln\phi_* \approx -\gamma_H a_H + \sigma^2\lambda_B\lambda_D a_C - \alpha\sigma^3\lambda_B^2\lambda_D^4 l^{-2} a_C \end{cases}$$

attractive!

Bruinsma and vdS *Phys Rev E* 71 (2005) 061928

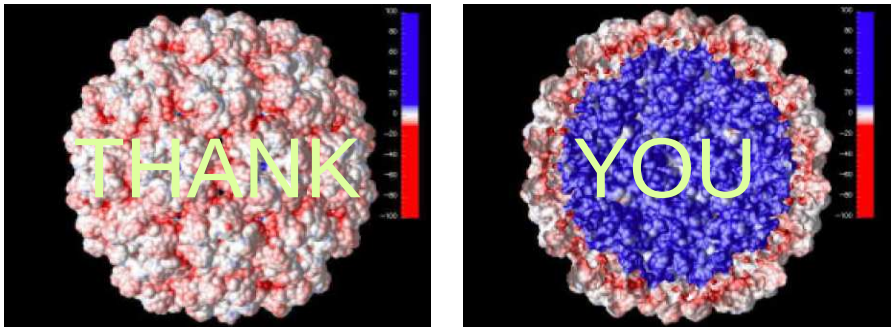
THEORETICAL ASSEMBLY DIAGRAM: FULL EQUILIBRIUM



Zlotnick et al. *PNAS* 94 (1997) 9556
Bruinsma and vdS *Phys Rev E* 71 (2005) 061928

Conclusions

- capsid assembly obeys the law of mass action
- the rim tension suppresses incomplete capsids
- the rim tension could cause nucleated assembly
- the coat proteins repel each other electrostatically
- encapsidation helps to overcome the self-repulsion



Konecny et al. *Biopolymers* 82 (2006) 106