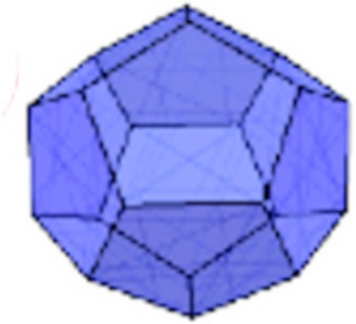
Genome Selection by RNA Viruses.

Inbal Mizrahi & Joseph Rudnick (UCLA)

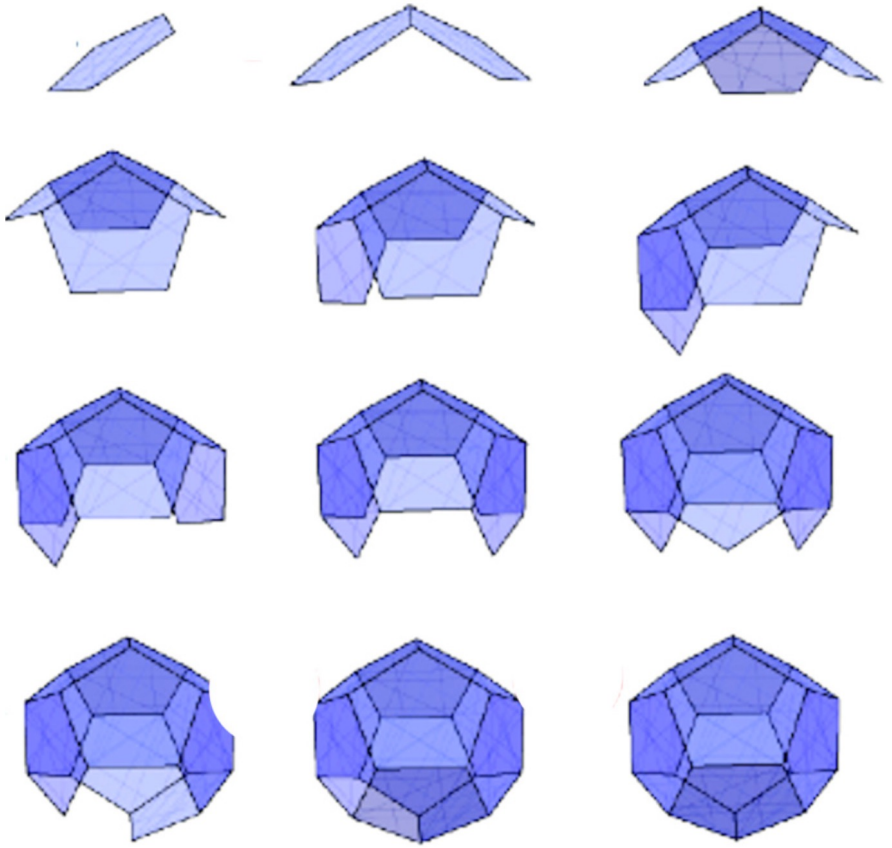
- Minimization of the Virion Free Energy (Roya Zandi).
- Nucleation and Growth (Rees Garmann).
- Statistical physics of viral RNA selection via nucleation and growth?
- *Enormous number of competing RNA configurations.* Simplified but soluble model.

Zlotnick Model for Capsid Assembly

A. Zlotnick. To Build a Virus Capsid - an Equilibrium-Model of the Self-Assembly of Polyhedral Protein Complexes. *J. Mol. Biol.*, 241(1):59–67, 1994.



- Dodecahedral capsid: 12 pentamers



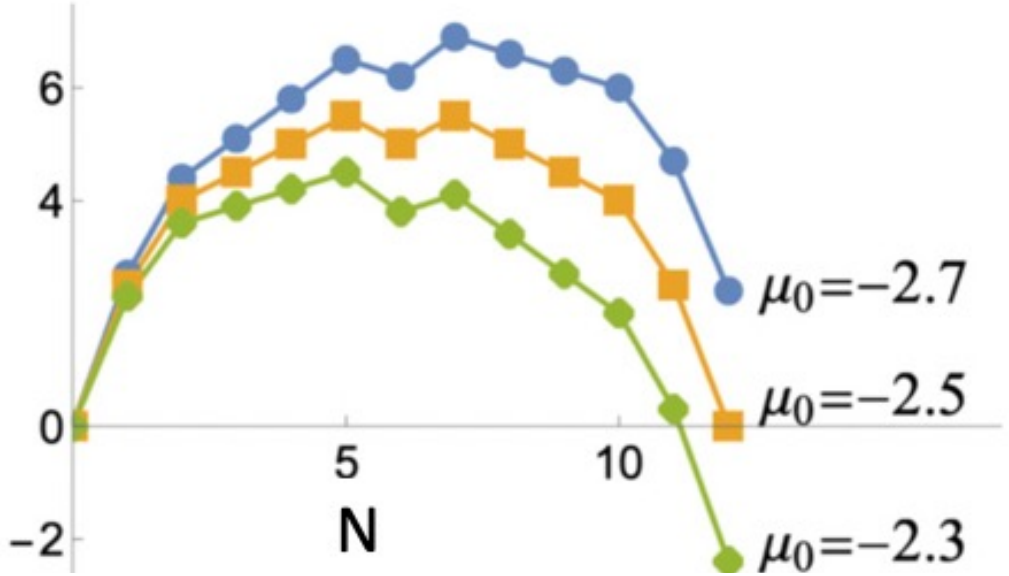
- Minimize assembly energy ΔE at every assembly step.
- $\Delta E = -\epsilon_1 \times n_1$ (n_1: # of shared pentamer-pentamer edges)
- About 10^5 *degenerate* minimum energy assembly pathways.
- State-Space: 12 states.

Assembly Free Energy

$$\Delta F = \Delta E(N) - \mu_0 N$$

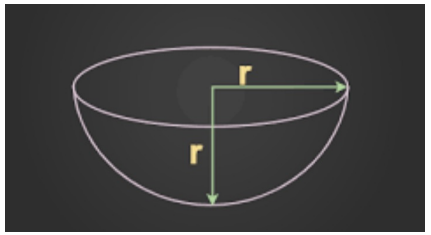
μ_0 = chemical potential of pentamers free in solution.

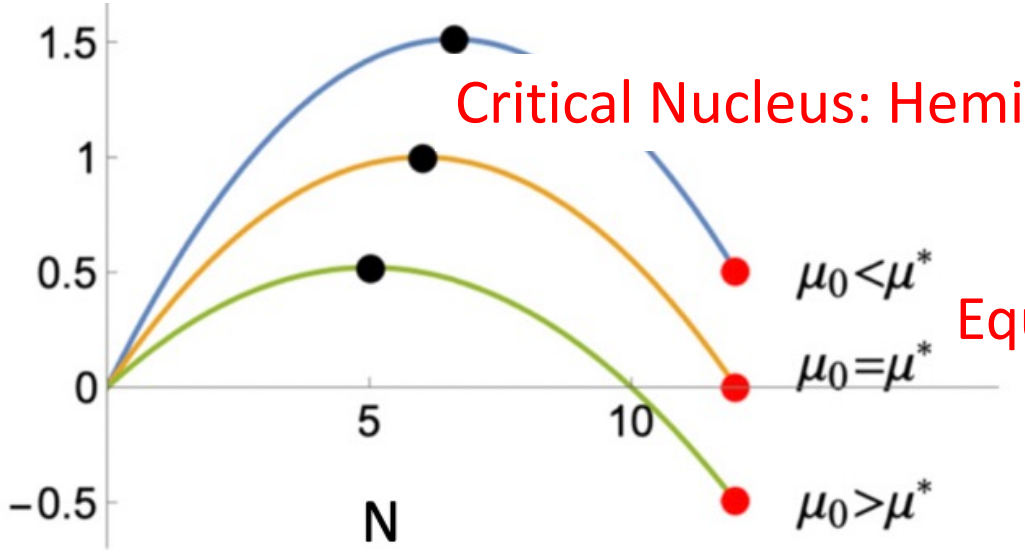
N = Number of Pentamers



Nucleation-and-Growth

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



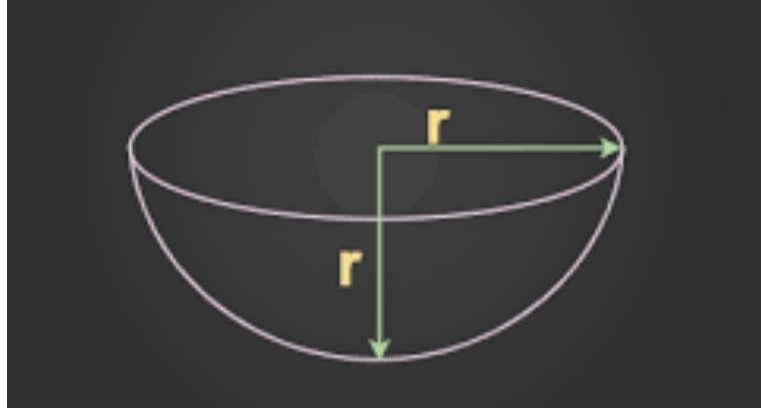


Critical Nucleus: Hemispherical Shell

Equilibrium Assembly
$\Delta F = 0$

Supersaturated Assembly: $\Delta F < 0$

Thermal Equilibration Time: T_{eq}



- Activation Energy Barrier:
  Perimeter of Hemispherical Shell x Bond energy/Unit length:

$$\Delta E \sim \sqrt{N_{max}} \times \epsilon \sim \sqrt{60} \times 3\, k_B T \sim 23.2\, k_B T \quad \text{(T=1)}$$

- Equilibration Time: $T_{eq} \sim T_{micro} \exp \frac{\Delta E}{k_B T} \sim$ (10^{-9} × exp 23) sec $\sim 10\ sec.$

- T=3: $N_{max} = 180.$ $\Delta E \sim 40.2\, k_B T$ **$T_{eq} \sim$ 10 years** (M. Cates)

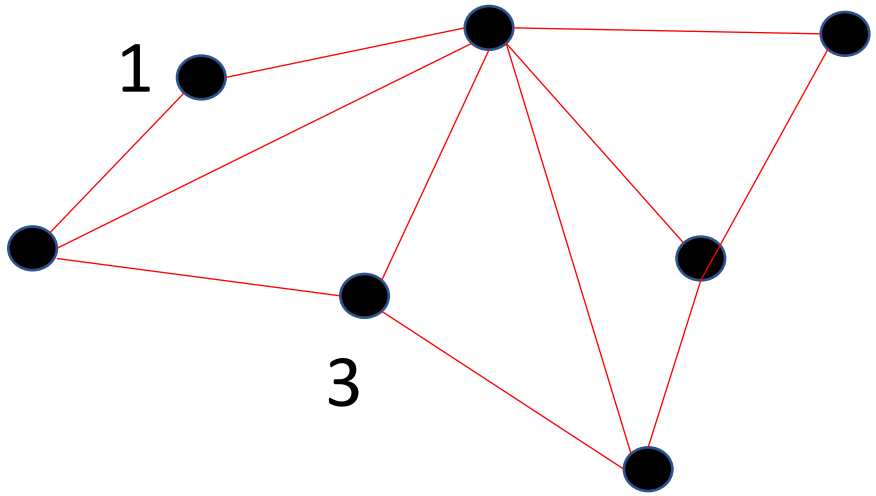
- Kinetics?

Master Equation

G.Uhlenbeck
Chapman-Kolmogorov

- Connectivity Matrix $A_{n,m}$

$$A_{1,2} = 1$$
$$A_{1,3} = 0$$



State-Space Graph

$P_n(t)$: Probability that state n is occupied at time t

- Master Equation.

Matrix of Transition Rates $W_{m,n}$

$$\frac{dP_n(t)}{dt} = \sum_m A_{m,n} W_{m,n} P_m(t) - P_n(t) \sum_m A_{n,m} W_{n,m}$$

- Detailed Balance: $\frac{W_{m,n}}{W_{n,m}} = exp - \Delta E_{m,n}/k_B T$

- $\Delta E_{m,n} = E_n - E_m$

  E_n : State-space energy levels

- Diffusion-Limited Assembly Kinetics / Smoluchowski Limit.

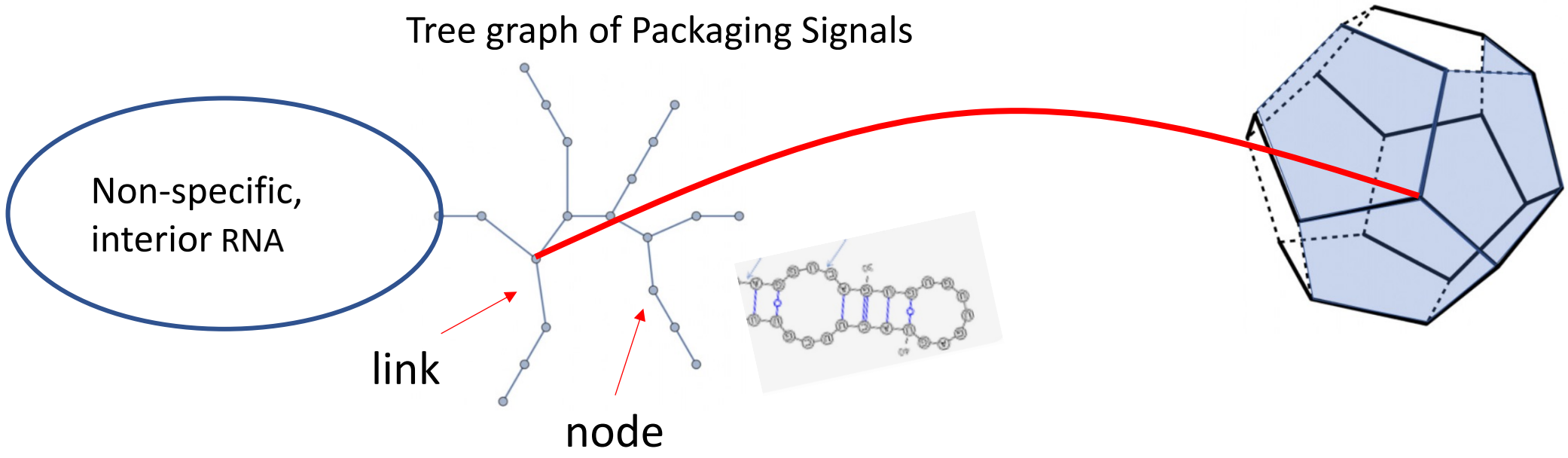
  $W_{n,m}$ is known once the energy spectrum and the state-space are known.

- Zlotnick Model:

A. Y. Morozov, R. F. Bruinsma, and J. Rudnick. Assembly of viruses and the pseudo-law of mass action. *J. Chem. Phys.*, 131(15):155101, 2009.

Include RNA?

- RNA



- RNA packaging signals: links of the **Spanning Tree Graphs** of the dodecahedron.
- Spanning Tree of a polyhedron: nodes cover all vertices just once.
- # of nodes = # vertices of the dodecahedron = 20.
- # of links = 19. Dodecahedron has 30 edges so 11 edges are not covered.
- About 10^5 distinct spanning trees.

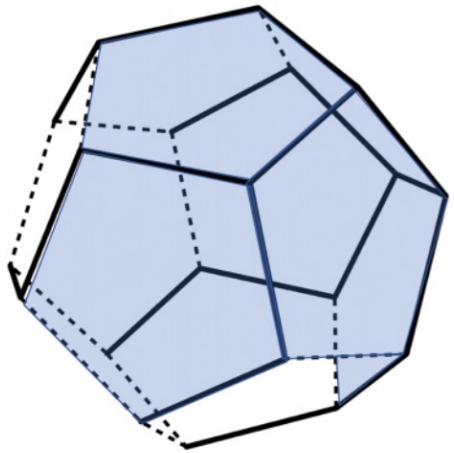
Lift degeneracy of the Zlotnic Model?

- $9 \leq$ **Maximum Ladder Distance** (MLD) ≤ 19

A. Yoffe et al.

PNAS | October 21, 2008 | vol. 105 | no. 42 | 16153–16158

Assembly Free Energy



Four different kinds of pentamer edges:

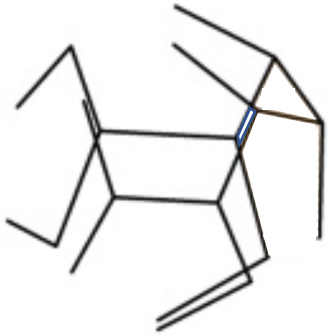
1) # of shared pentamer-pentamer edges without a link: n_1 Energy = - ε_1

2) # of shared pentamer-pentamer edges with a link: n_2 Energy = - ε_2

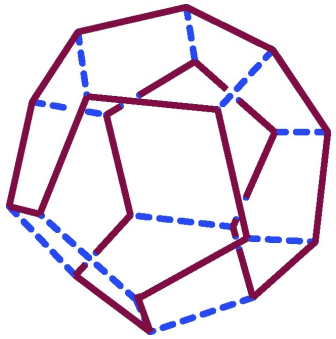
3) # of single pentamer edges without a link: n_3 Energy = - ε_3

4) # of single pentamer edges with a link: n_4 Energy = - ε_4

$$\Delta F = \sum_{i=1}^{4} \varepsilon_i n_i - N\mu_0$$

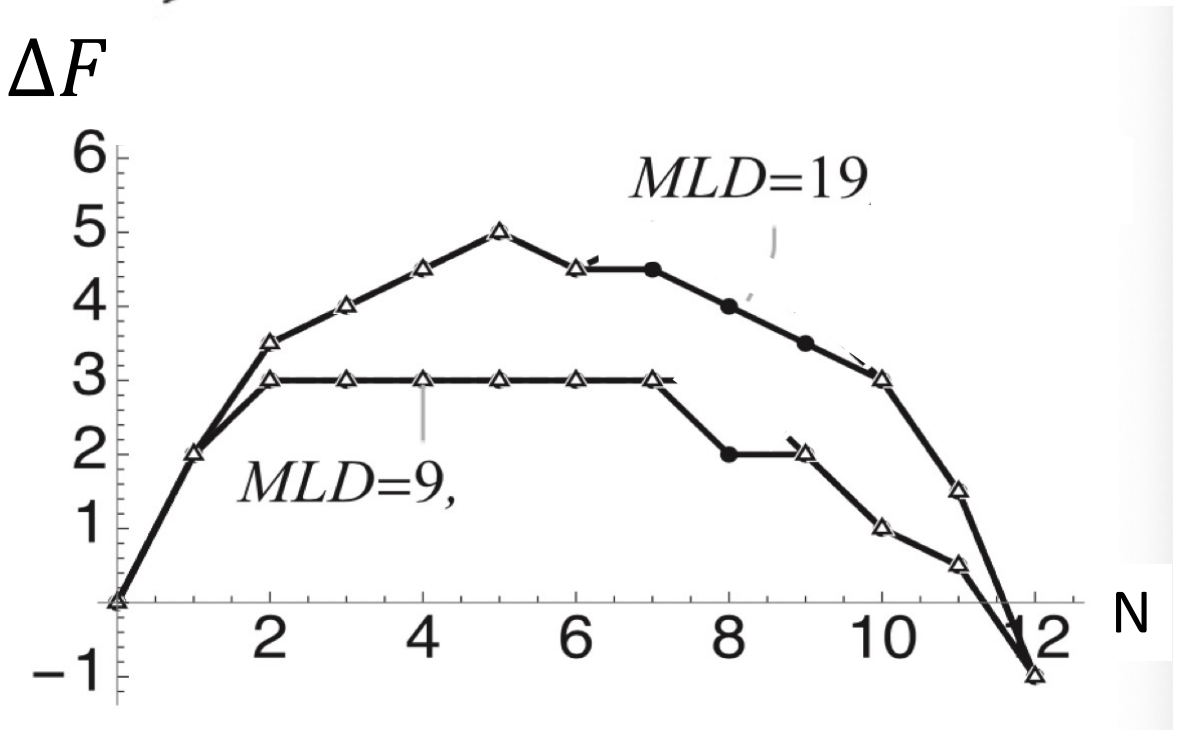


MLD = 9



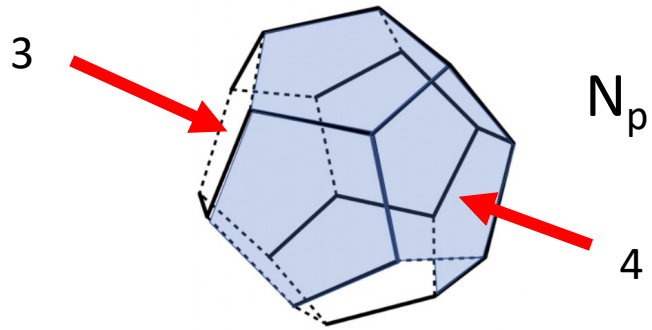
MLD = 19

"Hamiltonian Path" (Jure Dobnikar)

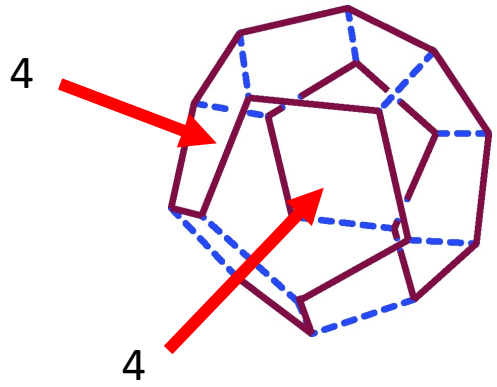


- Degeneracy of the assembly energy profile is lifted !

- **Wrapping Number.** $N_p \equiv$ Number of faces of the dodecahedron bordered by 4 tree links.



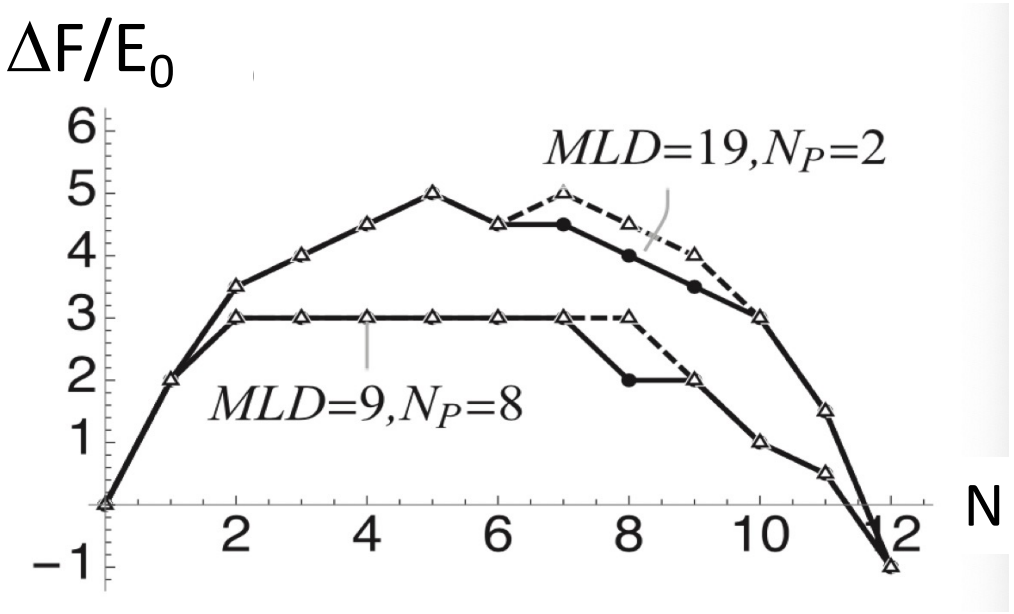
$N_p = 8$



$N_p = 2$

$2 \leq N_p \leq 8$

- Assembly energy profiles are *nearly* completely classified by the N_p and MLD numbers.



N_P MLD

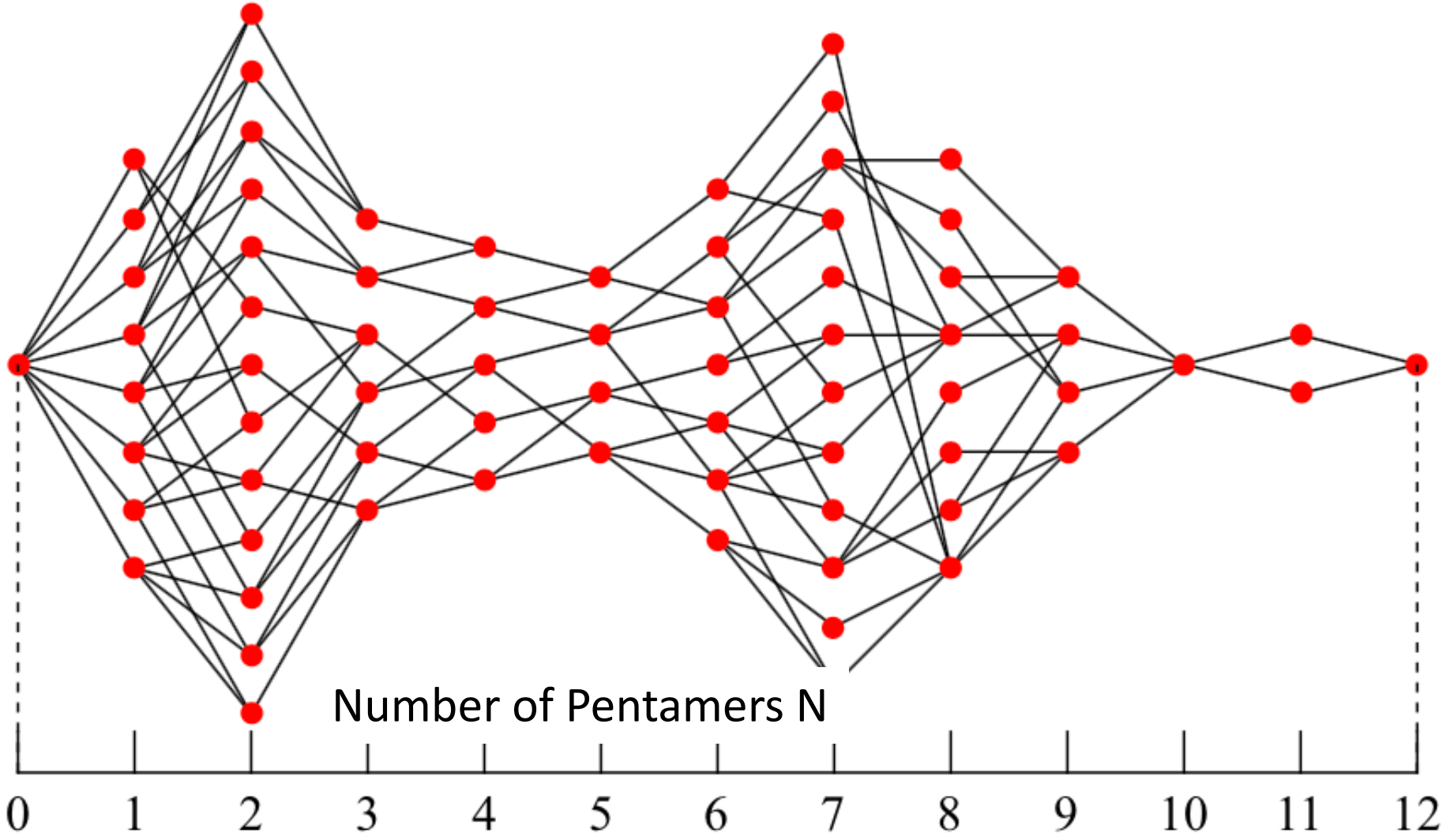
7 x 11 = 77 Degeneracy classes.

Class-war: packaging competition between different spanning tree classes.

State-Space for N_p = 8, MLD = 9

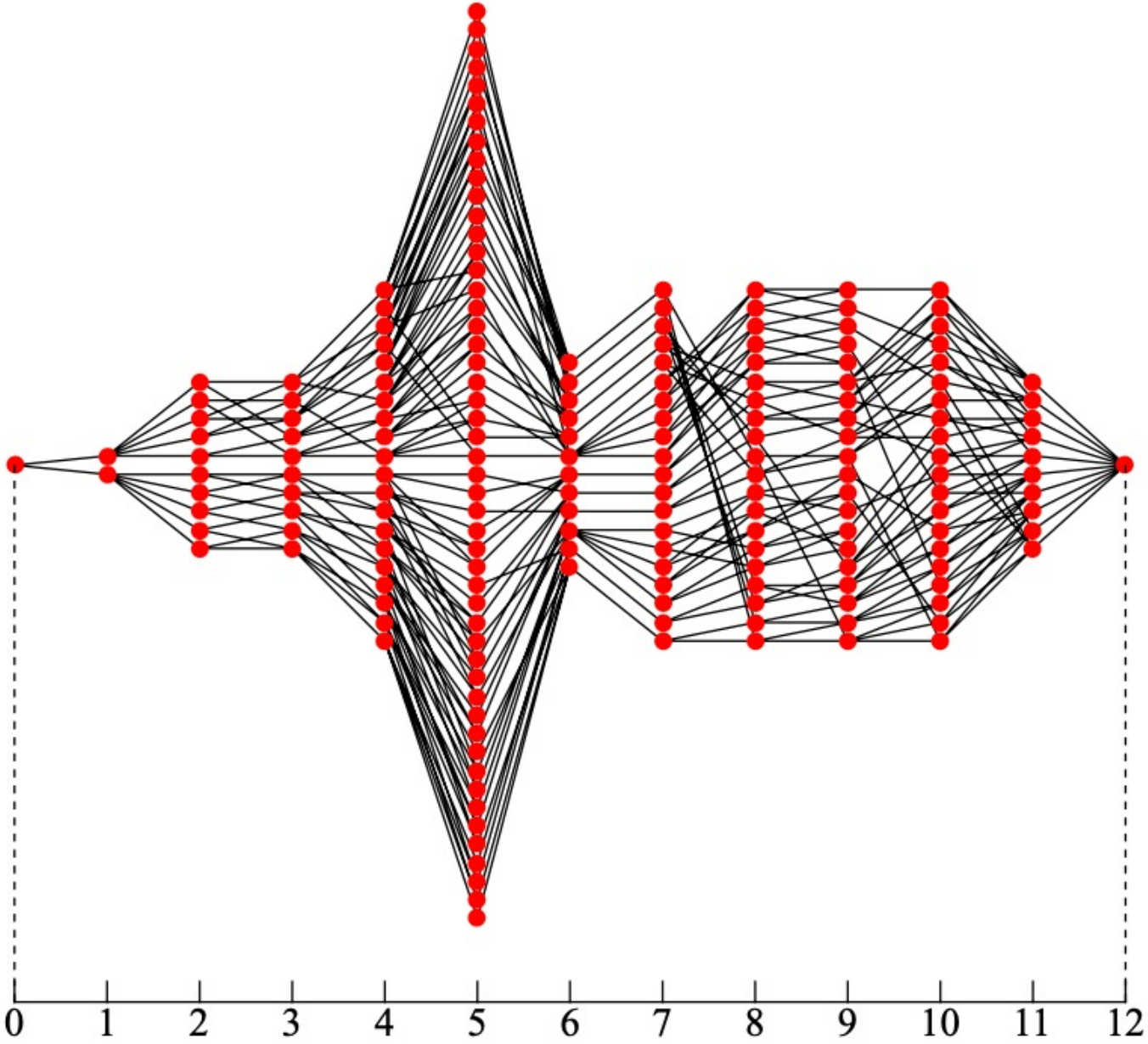
State Index i of degenerate states.

Example: for N=1 there are 8 states since N_p = 8



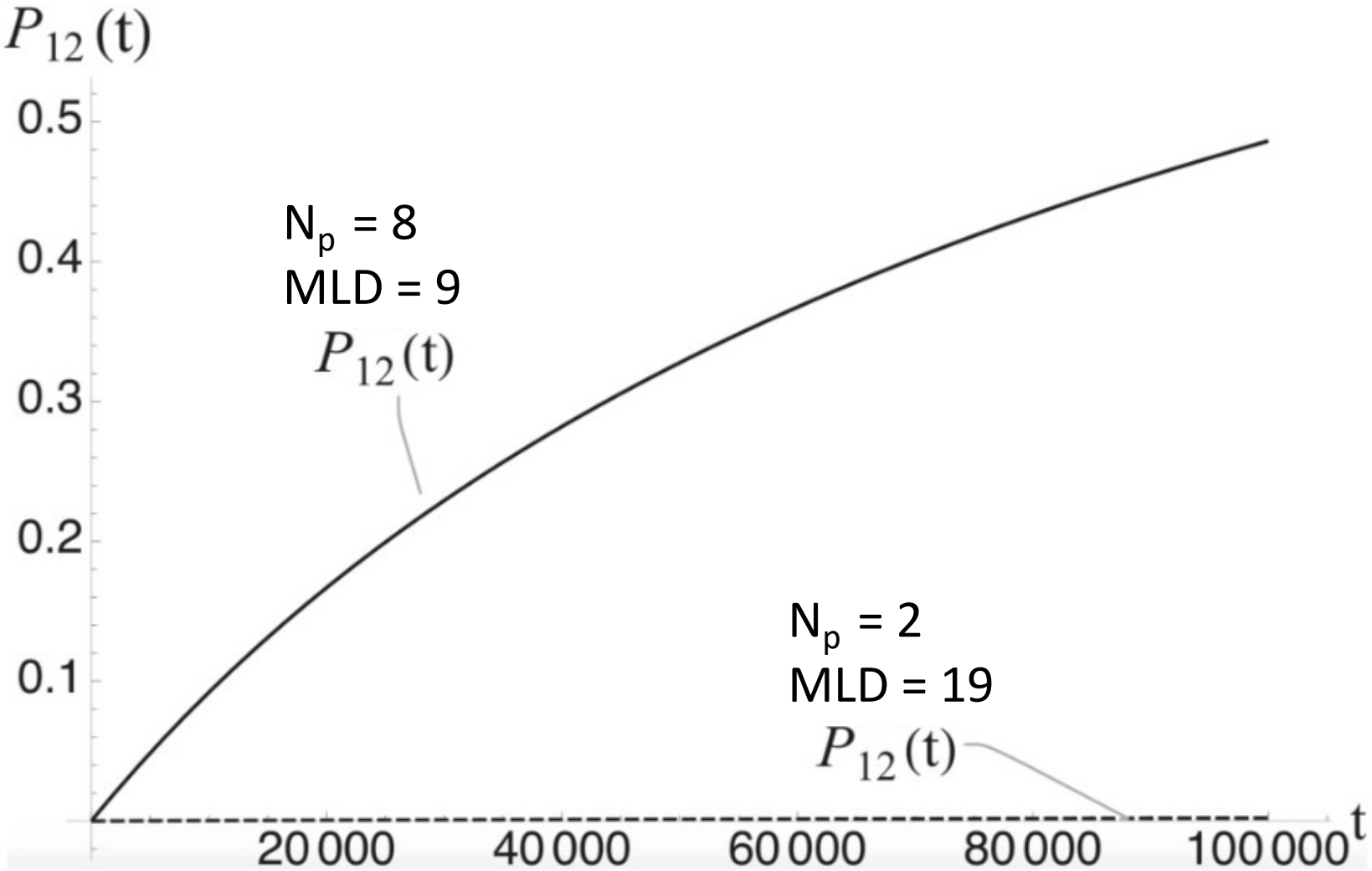
MLD = 19

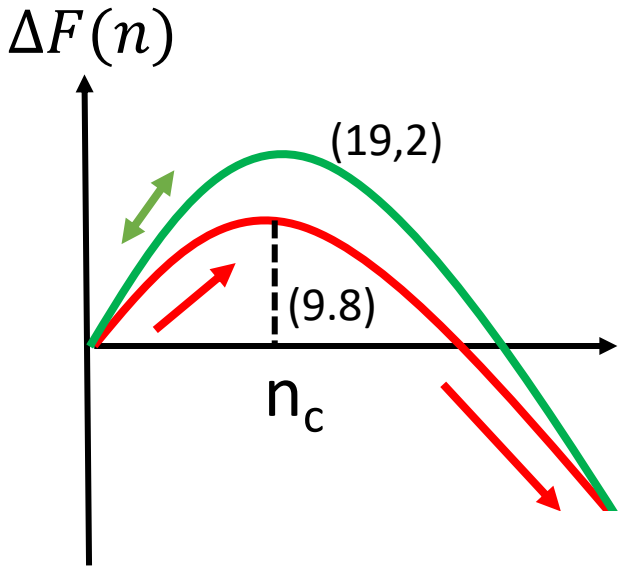
N_p=2



- Particle Assembly: Random walks through state space linking N = 0 to N = 12
- Master Equation method.

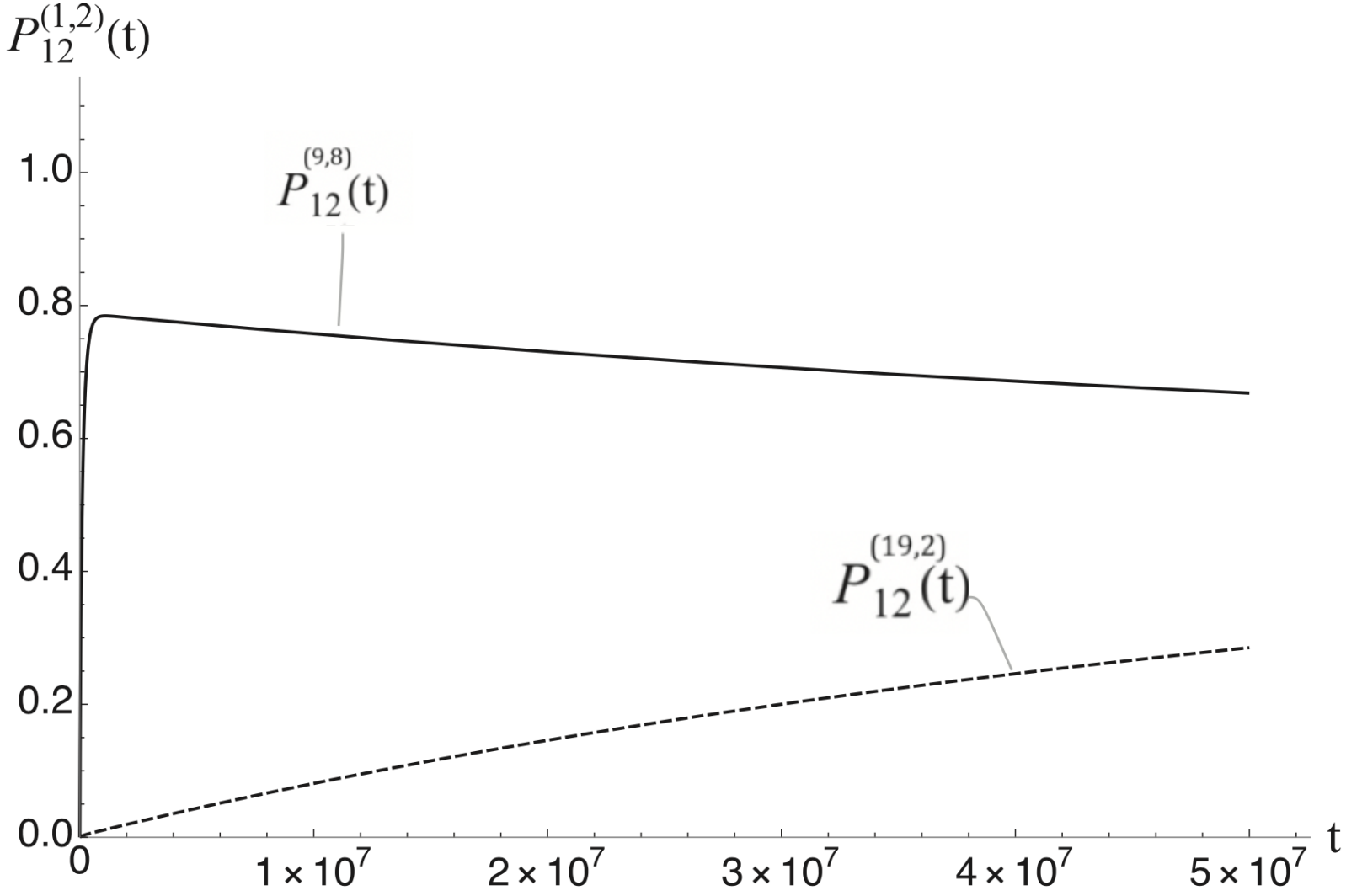
$P_{12}(t)$ = Probability that a complete dodecahedral particle has formed at time t.

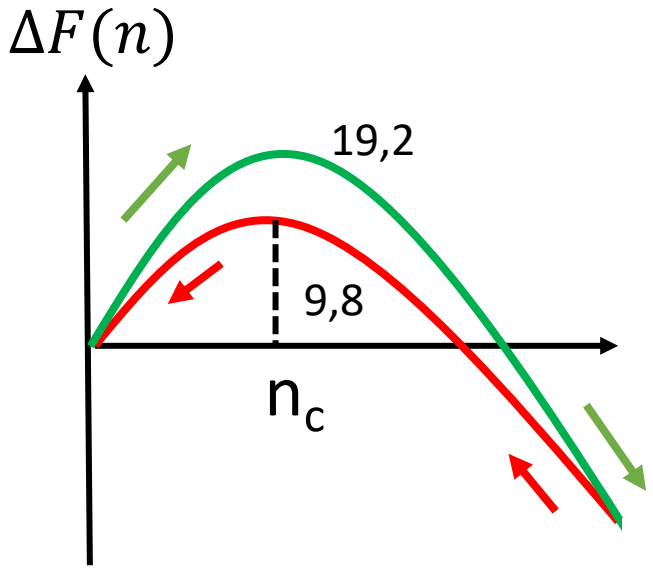




$t << T_{eq}$

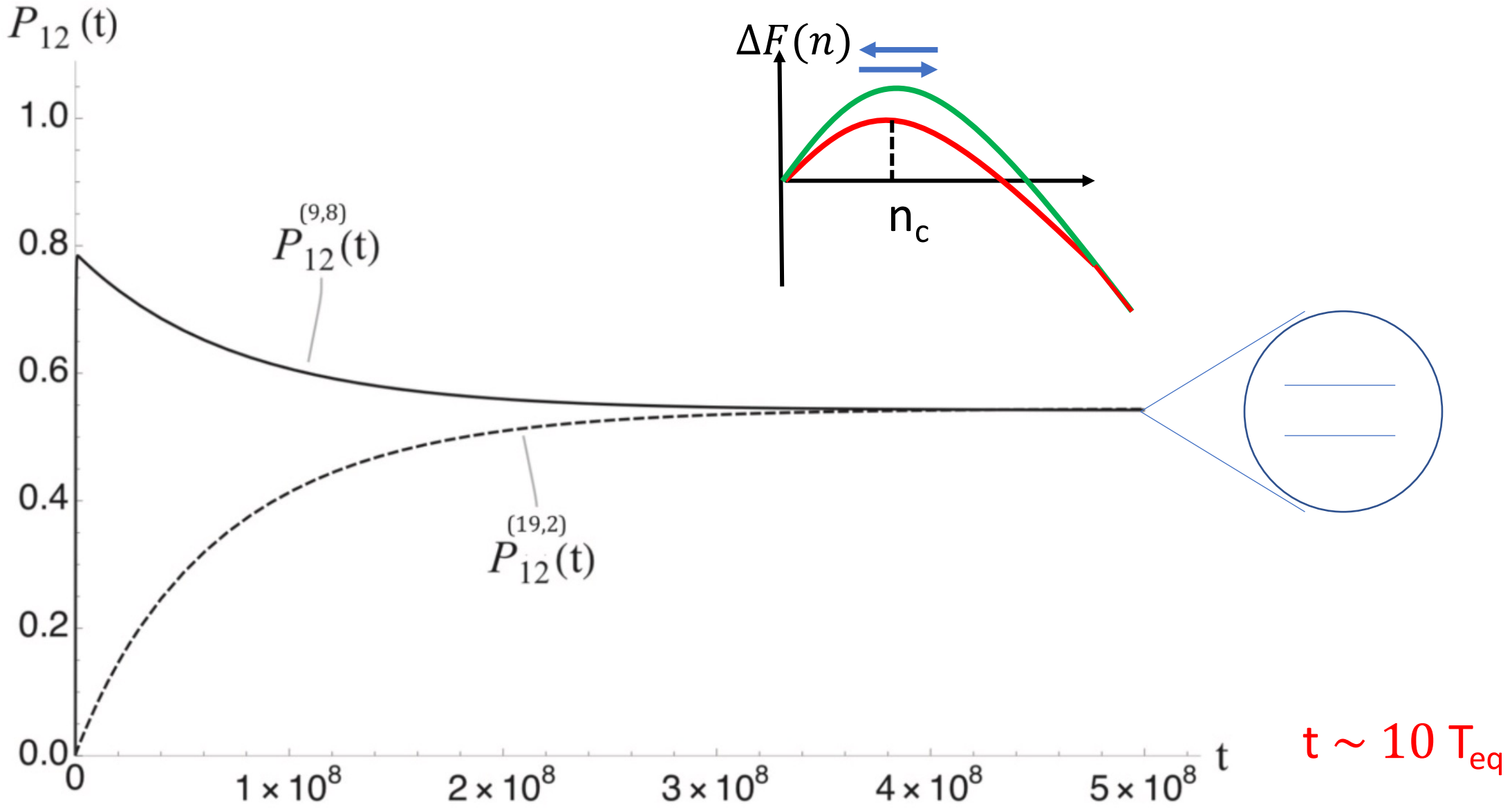
- Larger N_p and smaller MLD trees have higher growth rates.





$t \sim 0.5\ T_{eq}$

- Reduced Selectivity.
- Some MLD=9, N_p =8 particles disassemble due to thermal fluctuations!



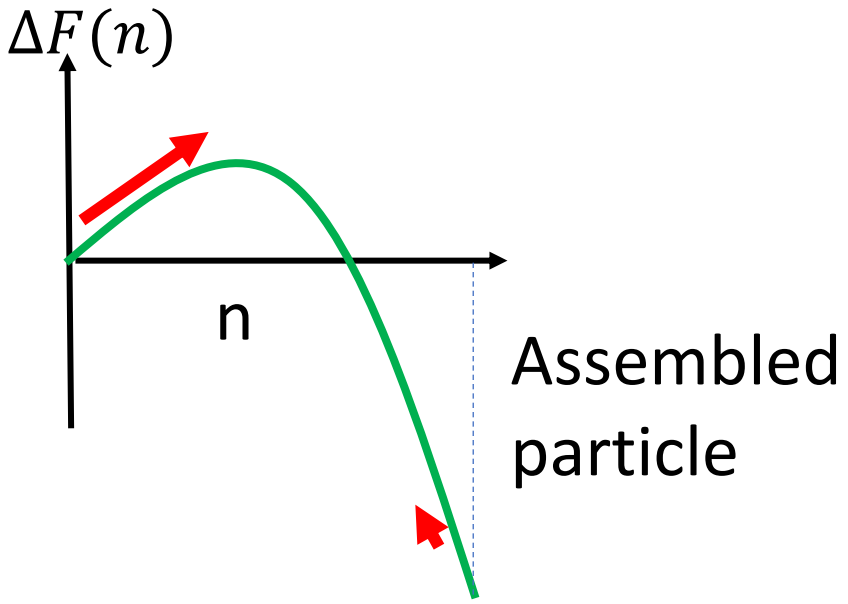
$t \sim 10\ T_{eq}$

- Thermal equilibrium
- Little selectivity left.

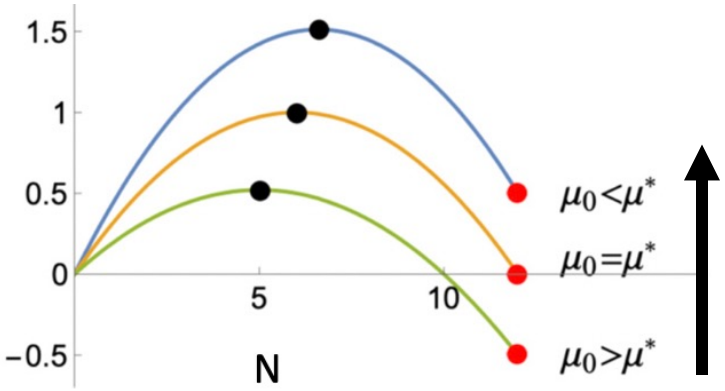
Conclusions

- *Selective nucleation*? Yes, but only on time scales small compared to the thermal equilibration time T_{eq}.

- Selective nucleation depends on packaging signal RNA topology (MLD) and geometry (Wrapping Number). Increases with moderate supersaturation!



- If assembled particles are placed in a solution with few capsid proteins, they will be *metastable*. (Wilson Poon)



- Thermal equilibrium: Steady-state assembly/disassembly of assembled viral particles. Loss of RNA selectivity.

- Defects: model disregards the role of RNA entropy and the role of the non-specific RNA.

Master Equation

$P_{i,n}(t) \equiv$ Occupation probability of node i,n on the assembly state space graph.

- Twelve Coupled Master Equations n = 1, ... , 12

Graph Connectivity Matrix

$$\frac{dP_{i,n}(t)}{dt} = \sum_j \left\{ A^{j,i}_{n-1} W_{n-1,n} P_{j,n-1}(t) + A^{i,j}_{n} W_{n+1,n} P_{j,n+1}(t) \right\}$$

$$- P_{i,n}(t) \sum_j \left\{ A^{j,i}_{n-1} W_{n,n-1} + A^{i,j}_{n} W_{n,n+1} \right\}.$$

Transition rates. Obey Detailed Balance.
"Dynamical Monte-Carlo"