Interactively exploring supramolecular assembly: A molecular dynamics approach to virus construction

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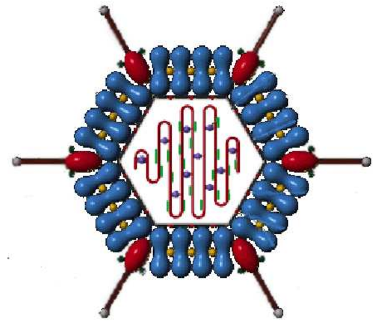
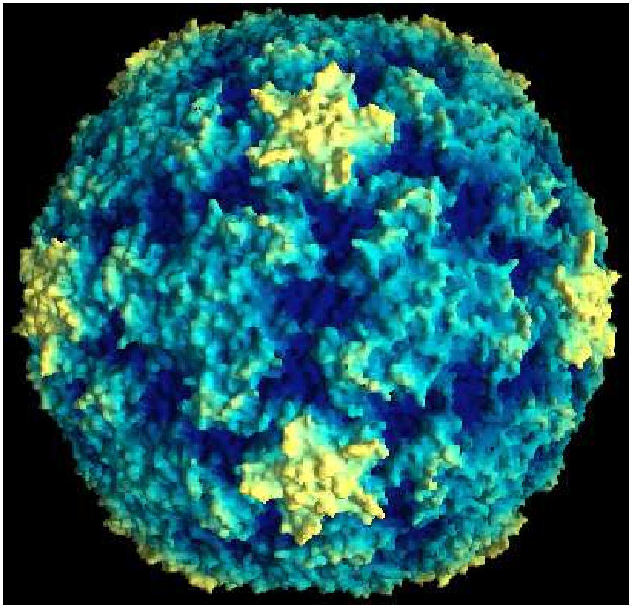
Viruses for pedestrians

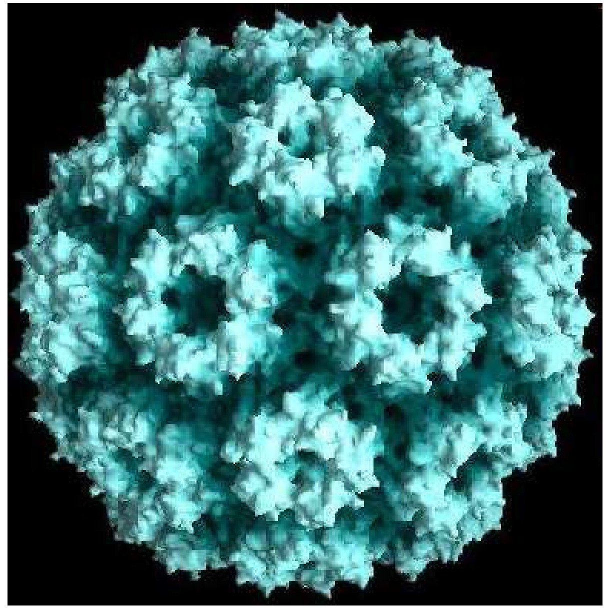
- Virus: genetic material (RNA/DNA) packaged inside protein shell (simplest kinds, additional features optional) —



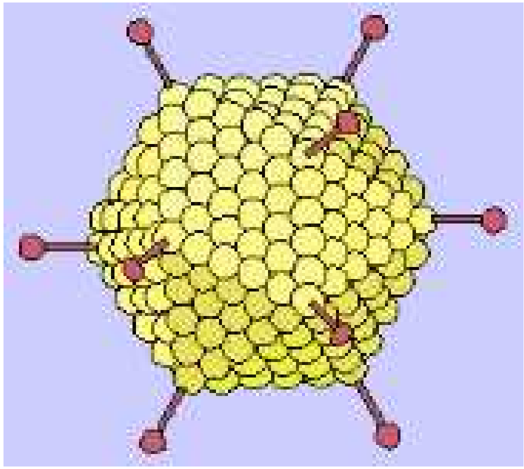
- Many possible geometrical forms for shell; nature tends to use two basic shapes – helical tubes and icosahedral shells; very similar structures occurring in animal/plant/bacterial viruses of different types hints at common design principles.
- Highly symmetric shapes minimize genetic ('engineering' design) information required for shell construction, since the same structural unit(s) can be used repeatedly.

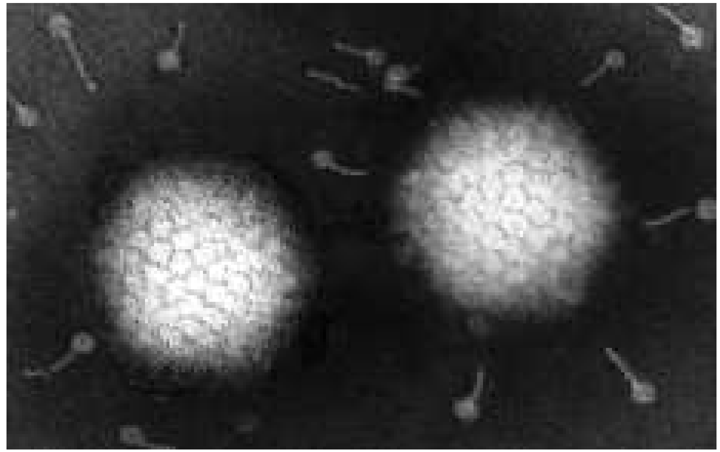
- Near-spherical shape provides maximal volume for given surface area.
- Examples: rhinovirus (T=1) with 60 units; cowpea virus (T=3) with 180 units; images by J. Sgro using x-ray crystallography, cryo-electron microscopy —





- Another example: adenovirus (T=25) with 252 units (240 × 6-valent, 12 × 5-valent), schematic and electron microscopy —





- Icosahedral symmetry (60-fold, excluding reflection) ubiquitous; each triangular face of the icosahedron consists of 3 smaller asymmetric protein subunits; this corresponds to tiling sphere with 60 identical triangles (cf. fullerenes).

Self-assembly

- Virus shell assembly provides example of the complex processes occurring in the very simplest of (biological) organisms.
- Process lies on the border of chemistry and biology.
- Similar to crystallization: both are governed by thermodynamics and driven by bond formation, but virus growth is self-limiting; goal (?) is minimum free-energy state (reminiscent of protein folding).
- Reversible shell formation is observed *in vitro*, even without genetic material; protein-protein interactions stabilize structure.
- Assembled shell structures extensively studied; little known about pathways and assembly mechanisms because partial intermediates are highly transient.

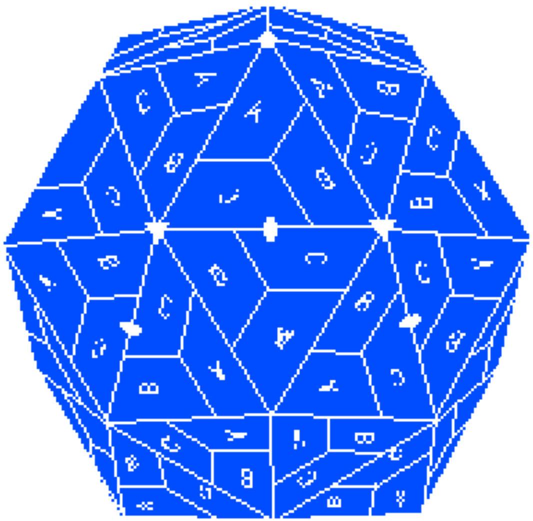
Plan

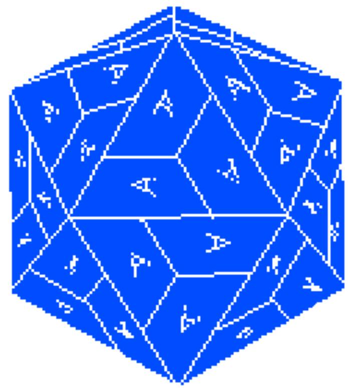
- Importance: Virus shells – knowledge of structure, assembly and interactions with cell essential for therapeutic reasons.
- Assumption: Given that the protein shells surrounding 'spherical' viruses tend to have certain common geometrical features, postulate general assembly mechanism for the 'capsid' (the protein coat surrounding the genetic material).
- Simplification: Use MD to study self-assembly of simple geometrical shapes (model 'capsomers') subject to specified interactions and association rules.
- Motivation for MD approach: Since virus assembly pathways difficult to access experimentally, important to have simulation testbed on which alternative construction scenarios can be examined.
- Extensions: Possibility of eventual quantitative estimates, but present work considers only simplified 'geometrical' models.

Quasi-equivalence

- Internal volume of minimal (60 subunit) shell holds very little DNA/RNA (simple volume calculation $\Rightarrow$ bound on amount of genetic material within).
- 'Quasi-equivalence' principle (explains many observed structures) with > 60 capsomers, the maximum that can have complete equivalent neighborhoods.
- Larger shells consist of $60 \times T$ units, with observed $T \leq 25$ (shell sizes typically in range 20-80 nm).
- Unique structure with 60-fold symmetry made from 12 pentagons and certain numbers of hexagons.
- Inspired by geodesic domes (Buckminster Fuller); such shells have minimal range of variation of bond lengths and angles.
- Cannot deduce assembly rules from final structure; although protein subunits are identical, differentiation ('autostery') occurs so they can occupy distinct quasi-equivalent positions (e.g., valence 5 and 6).

- Soccer ball: has 12 pentagons + 20 hexagons; triangulate faces ⇒ 180 facets ⇒ T=3.
- Can make paper figures from planar triangular nets using suitable cuts and folds.
- Schematic example: triangulation numbers T=3 and T=1.





Molecular dynamics simulation – some interactive examples

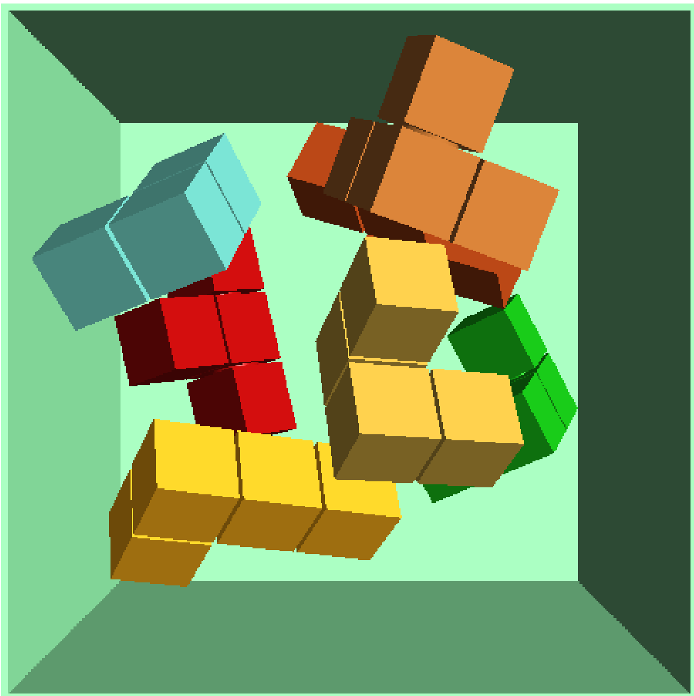
- Simple fluid . . .
  - MD: classical atomistic approach to studying bulk matter; Newton's second law governs motion.
  - Monatomic system – short-range Lennard-Jones interaction

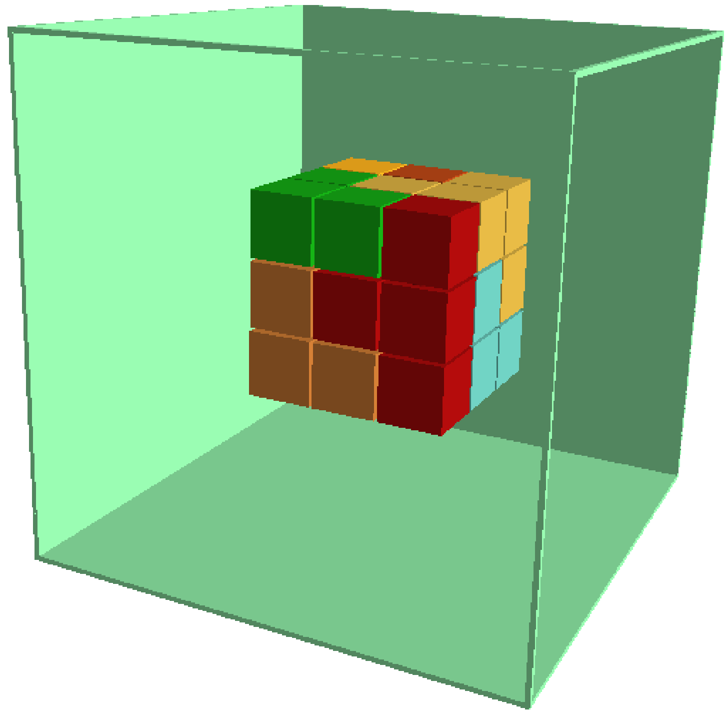
$$u(r_{ij}) = 4\epsilon \left[(\sigma/r_{ij})^{12} - (\sigma/r_{ij})^{6} + \tfrac{1}{4} \right], \quad r_{ij} \le r_c = 2^{1/6}\sigma$$

  - Efficient computation based on neighbor lists reduces $O(N^2)$ problem to $O(N)$.
  - Leapfrog integration of equations of motion.
  - Other details: boundary conditions, initial state, measurements, . . .
  - Study trajectories, temperature and density dependence, fluid-solid transition, etc.

- Rigid-body fluid . . .
  - Rotational dynamics described by Euler equation.
  - Linear and tetrahedral molecules.
- Flexible chains . . .
  - Link spherical atoms with rigid bonds, optional attractive forces; can examine helix-coil transition and folding.
- Micelle formation . . .
  - Simple LJ solvent and solute, short amphiphilic surfactant chains.
  - At low solute concentration observe micelle formation.
- Clusters of tapered rods . . .
  - Selective pair interactions to ensure alignment.
  - Typical cluster size determined by taper.

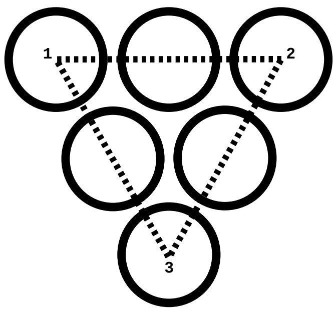
- Self-assembly of ‘Soma’ cube with selective interactions; does if happen? ...

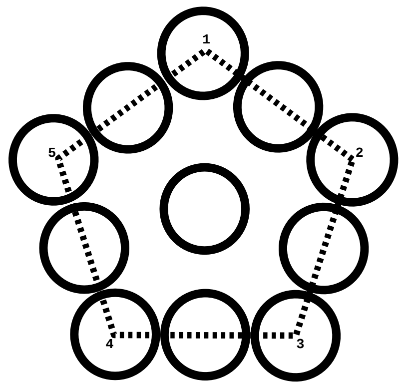




MD methodology for capsids

- Robustness of self-assembly suggests MD simulations using low resolution models ('shape'-based caricatures) might capture essence of process.
- First study of this kind (natural extension of old mechanical models for studying structure); provides simulated *in vitro* environment.
- Initial effort focused on assembly of pentagonal subunits into dodecahedra (also tetrahedra and octahedra – start small).

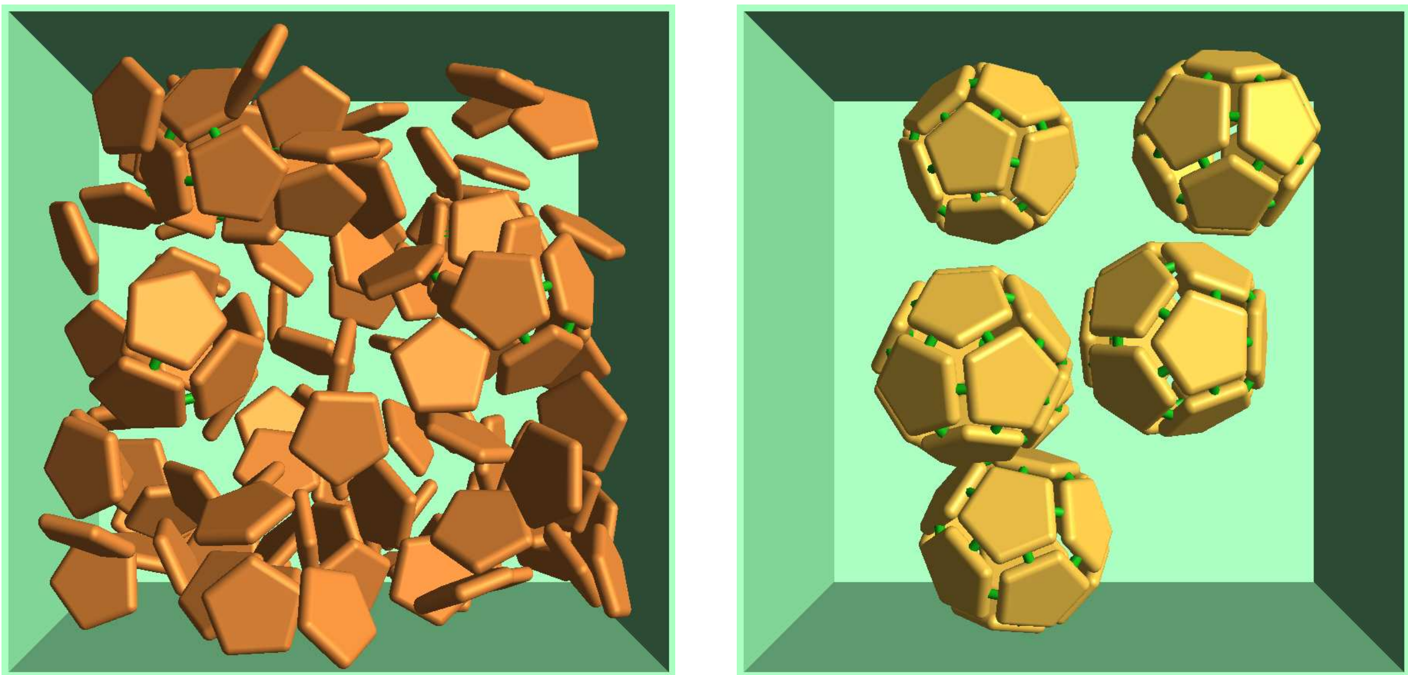




- Subunits consist of rigid planar assembly of closely-spaced spheres; vertices are ‘bonding sites’ that interact via LJ potential.
- Usually only repulsion (excluded-volume), but attraction allowed between prospective bonding sites – depending on assembly ‘rules’ and subunit status (other bonds, etc.).
- Bond formation: when attracting spheres first approach to within bonding range interaction is switched to steep and narrow potential well (unbreakable bond).
- Rules determine which spheres can attract at any instant; if all vertex spheres attract $\Rightarrow$ amorphous globule, but if subunit can bond with just one partner along each edge $\Rightarrow$ multiply-connected network; rules ensure correctly built structures.
- Use of rules eventually becomes unwieldy as shells get larger; increasing computer power allows larger monomers, with multiple interaction sites per face, and so help resolve problems that rules were designed to overcome.

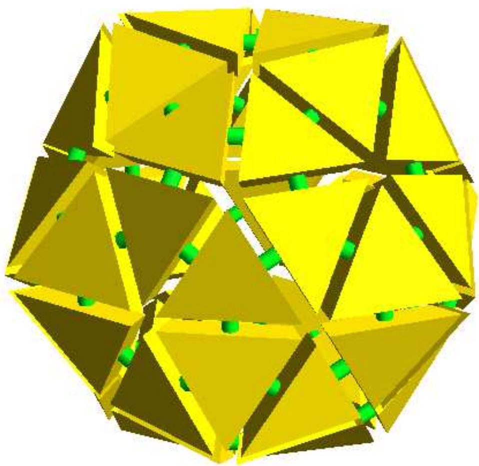
- Techniques (standard MD):
  - Neighbor lists for efficient force computation.
  - Rigid-body dynamics.
  - Leapfrog numerical integration.
  - Since visualization important – both for development and for monitoring behavior – initially used hard (elastically reflecting) wall boundary conditions (periodic boundaries visually confusing).
  - Gradual heating due to exothermal bond formation is avoided by adding a small velocity-dependent damping force to each bond.
- Omissions:
  - No solvent (heavy computational cost).
  - Poor biochemistry: missing ions, genetic material, ‘scaffold’ proteins.
  - No constraints to enforce structural rigidity, or angle-dependent forces to help alignment.
  - Rely on thermal motion to ensure that subunits eventually align, but ‘floppiness’ limits structure size and reduces assembly rate.

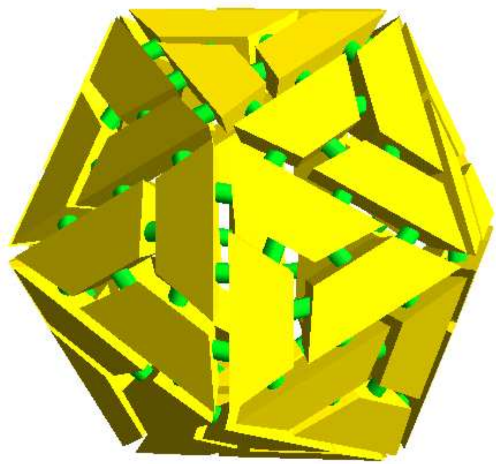
- System of 125 pentagons: early random state, and final state with 6 dodecahedra (everything else omitted).



Improved model

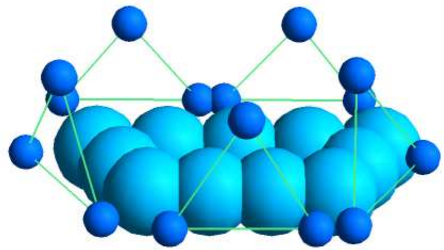
- Would like to construct larger polyhedra (e.g., pentakisdodecahedron with 60 triangular faces, and T=1 shell with 60 trapezoidal units):





- Previous model lacks rigidity; also complex *ad hoc* assembly rules (not biologically implausible but preferably avoided).

- New model
  - Several attraction sites located on lateral 'faces' of particle (capsomer).
  - Faces are beveled to give correct dihedral angles for closed shell.
  - Example (component balls and effective shape) —



- Torsional forces act during bonding to accelerate process; damping forces remove excess bond vibration energy.

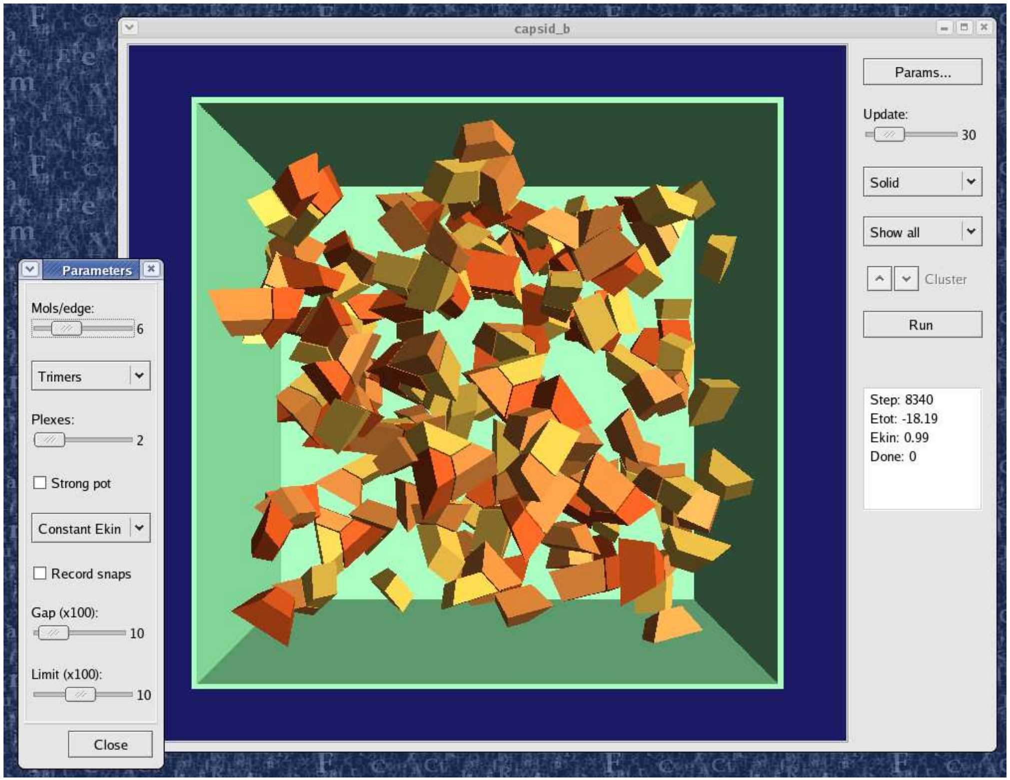
- Attractive forces (simple and effective) —

$$u(r) = \begin{cases} e(1/r_a^2 + r^2/r_h^4 - 2/r_h^2) & r < r_h \\ e(1/r_a^2 - 1/r^2) & r_h \le r < r_a \end{cases}$$

with (typically) $e = 0.1$, $r_h = 0.3$, and cutoff $r_a = 2$; only harmonic part applies for permanent bond.

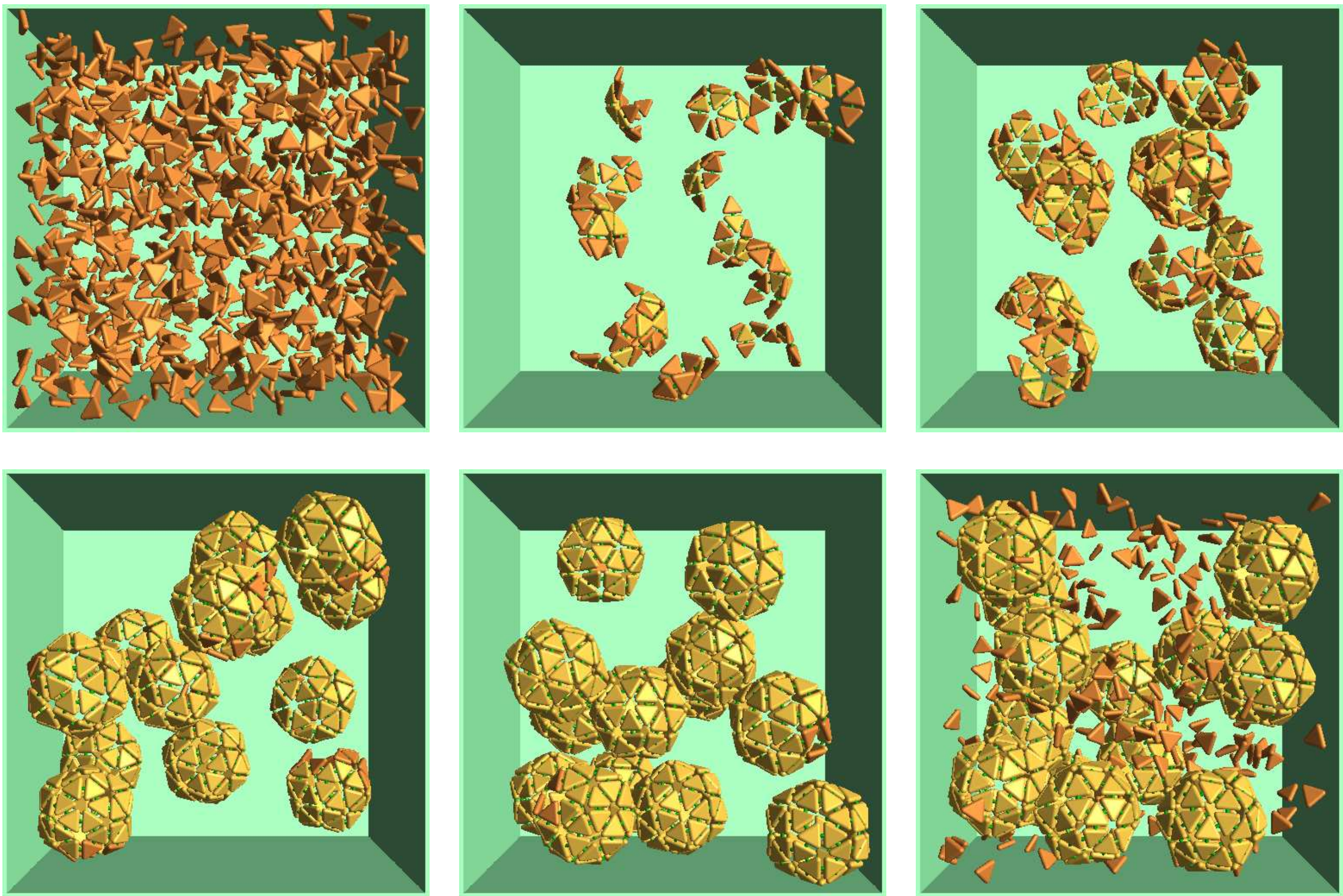
- Rigidity
  - When faces fully bonded, angular fluctuation between neighbors is $\approx 1^\circ$.
  - Fluctuations in complete assembly are even smaller.
- Assembly pathway
  - Assumption: growth of subassembly occurs by adding elements (monomers, or previously formed dimers or trimers); no combining of subassemblies.
  - Reduce incorrect assembly by only allowing one element at a time to bond to a growing subassembly, but no imposed assembly sequence.
  - Restrict allowed number of subassemblies that can nucleate to ensure adequate number of particles for growth.

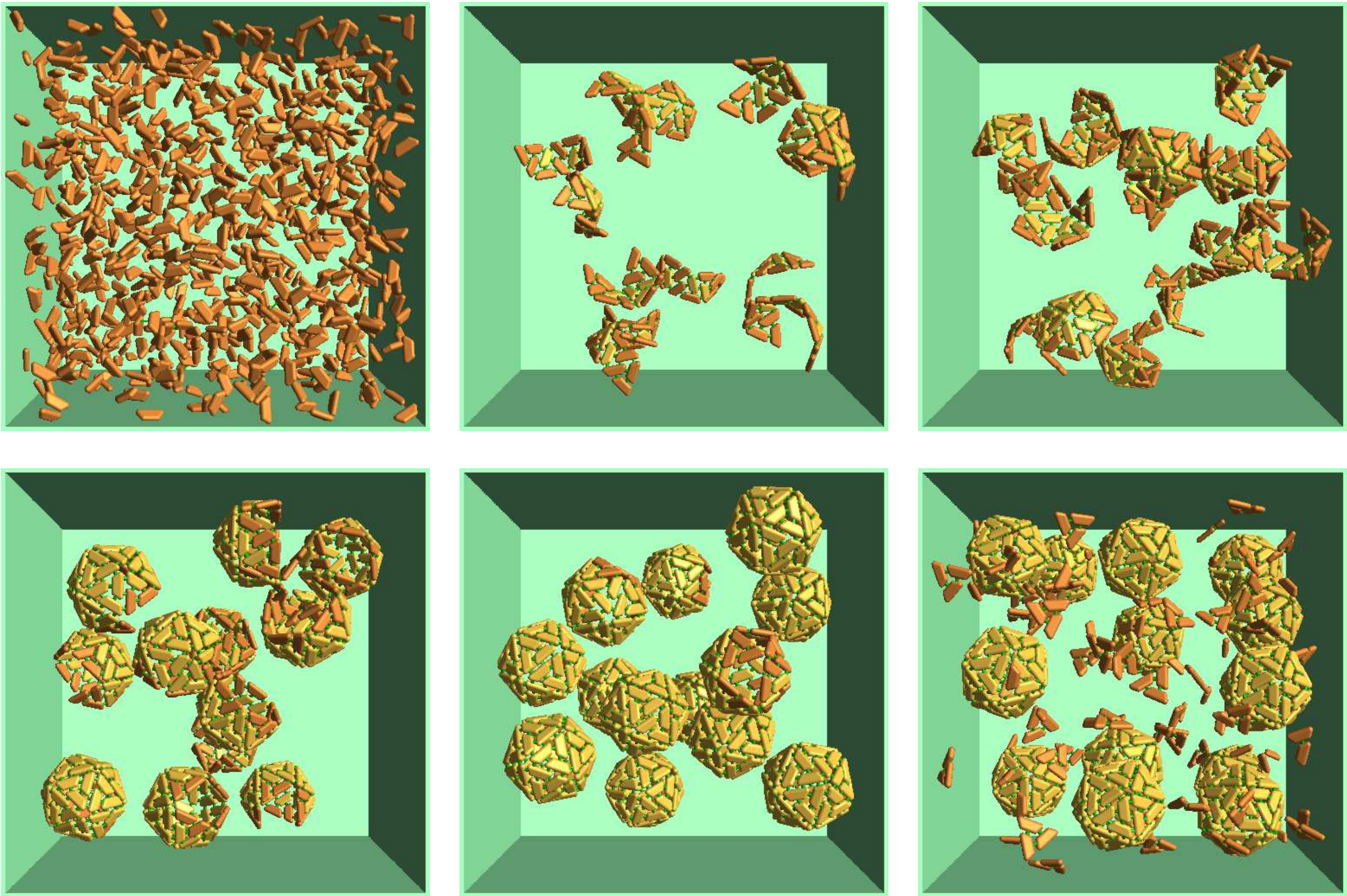
- Visualization: interactive computer graphics essential – color and various display options for greater information content . . .



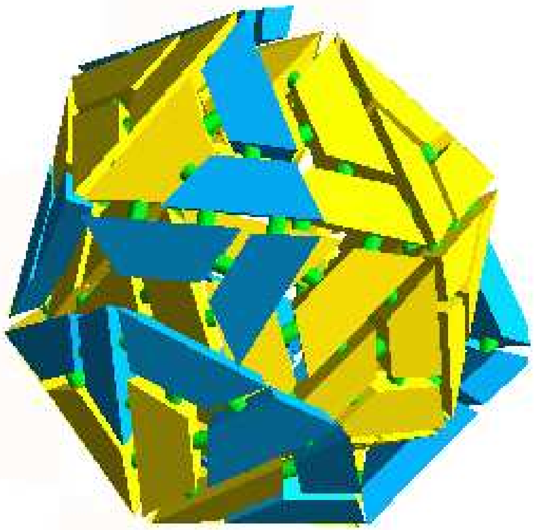
Exploratory results

- Examine variety of different models, parameters and assembly details, until behavior appears promising.
- System size: e.g., 1000 monomers; allow formation of up to 13 polyhedra; use either triangular or trapezoidal ‘capsomers’.
- Complete assembly of all the 60-hedra (in $\approx 4 \times 10^5$ timesteps).
- ‘Mutant’ structures rarely form since bond tolerance is tight.
- Color images show development of system (overleaf).
- Can make ‘movies’ to show dynamical history (show recordings); use for quantitative and visual analysis . . .
- Qualitative observation (tentative): partial assemblies tend to be compact rather than ramified (experimental relevance?).
- Growth of shells with 60 triangular and trapezoidal monomers —





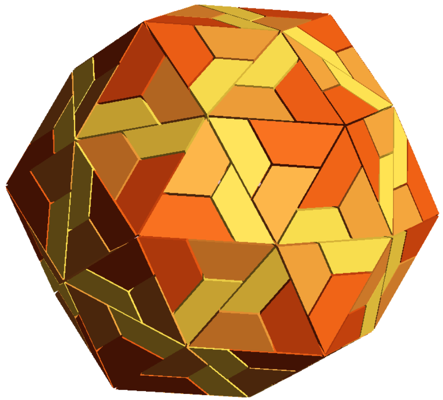
- Mutants rarely occur; example shows growth of 2nd shell (101 subunits, but could continue forever) —



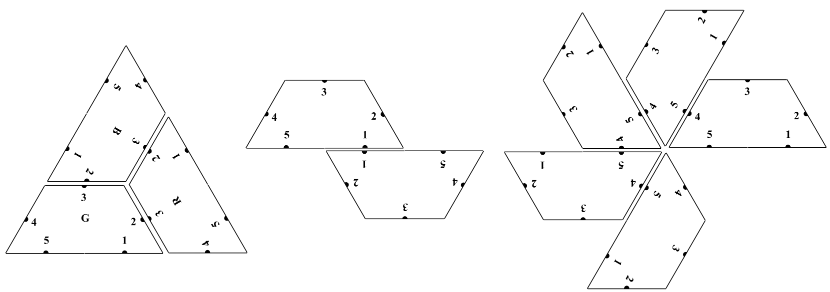
- Ability to encourage minor structural changes has potential medical value.

Bigger shells

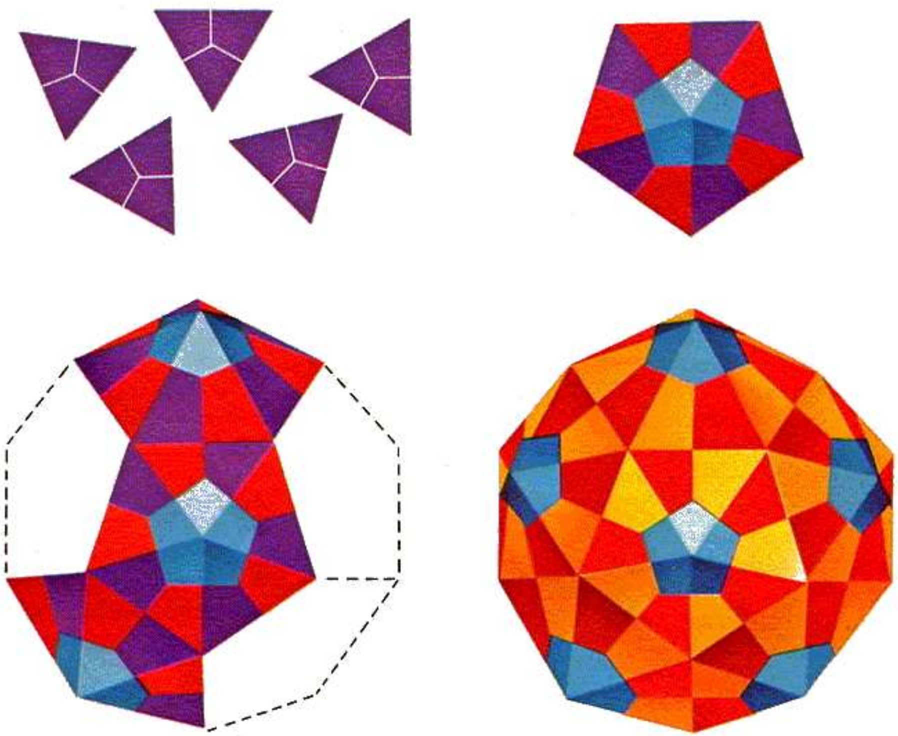
- Shell of size 180 (T=3) with 3 slightly different monomer types, to mimic effect of quasi-equivalence (without ‘autostery’) —



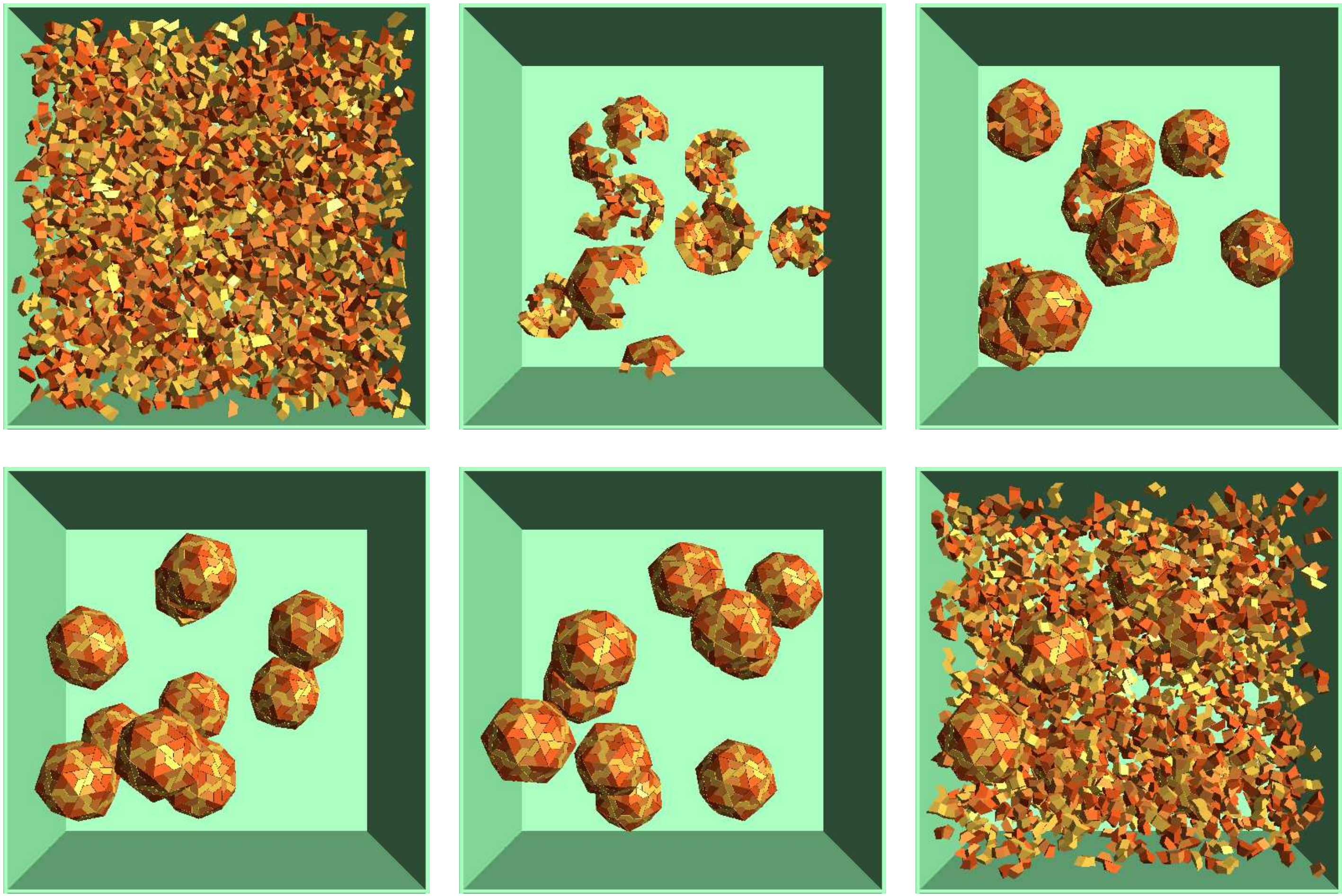
- Need to be more specific as to which faces can bond —



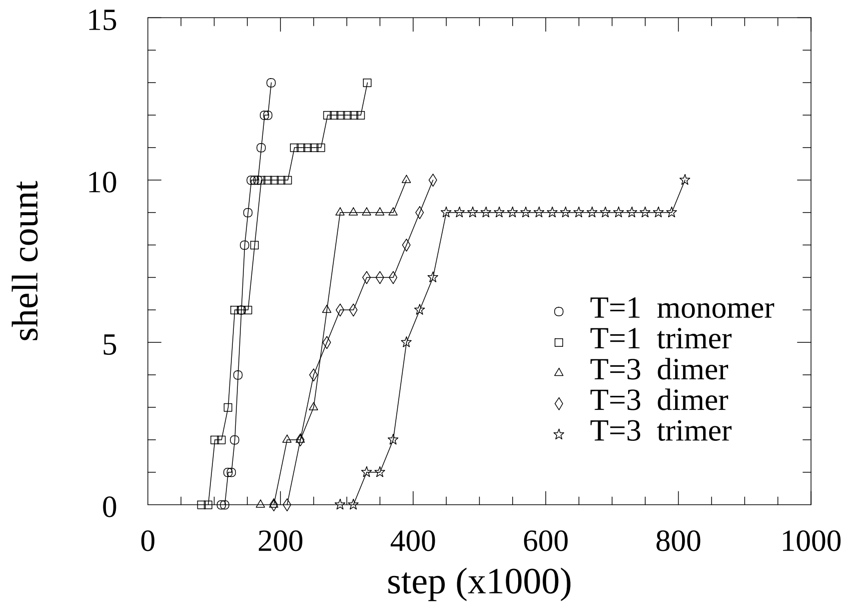
- Growth pathway can involve small intermediate structural elements, e.g., dimers or trimers —



- System of 4096 monomers ⇒ 10 T=3 shells via dimer pathway . . .

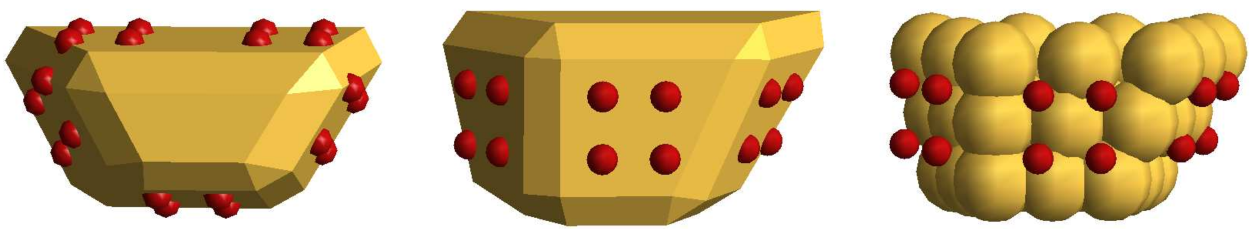


- Quantitative aspects: measure number of complete shells vs time (permanent bonding); observe slowdown near completion ('starvation') —

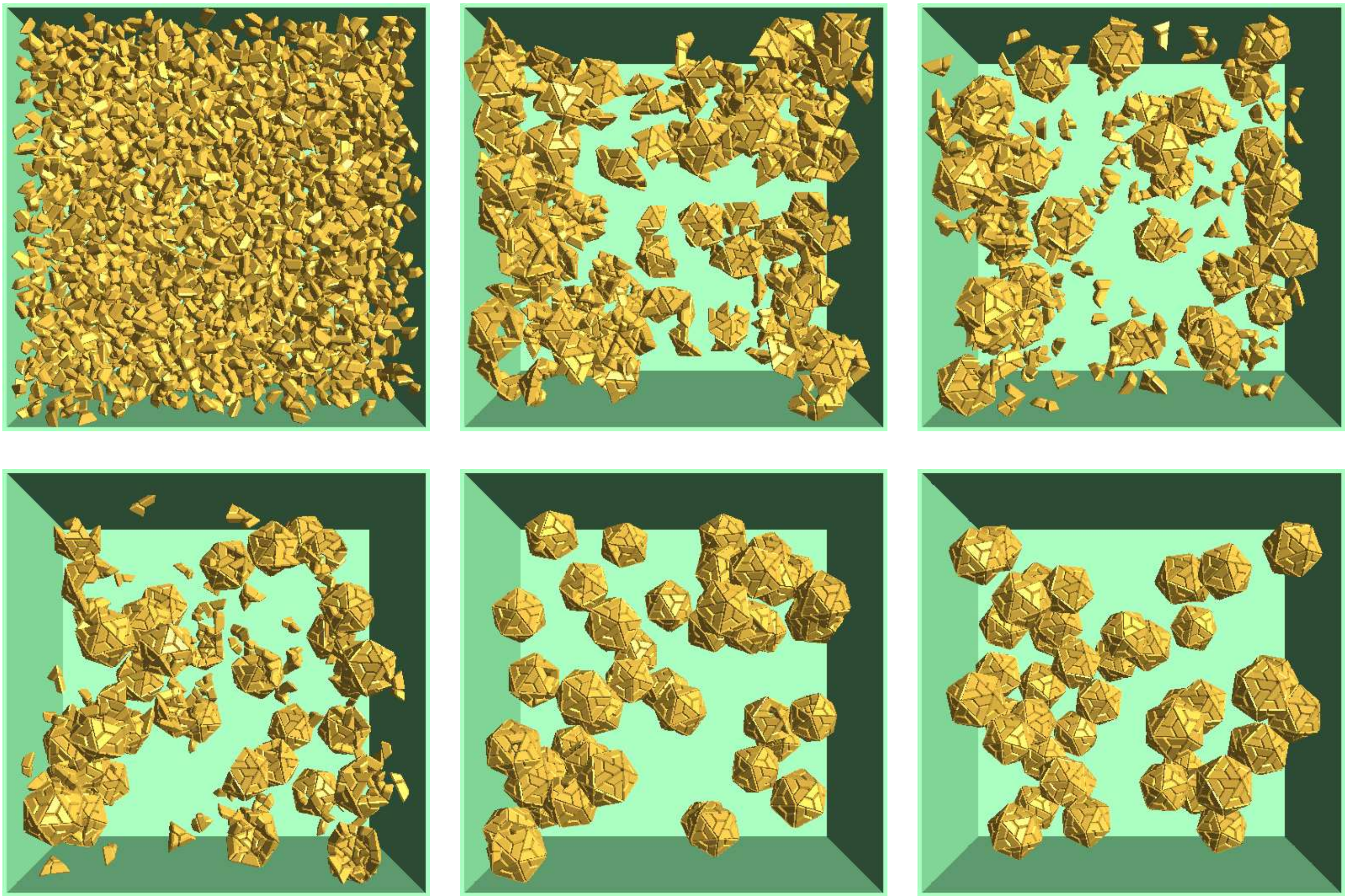


Reversible growth

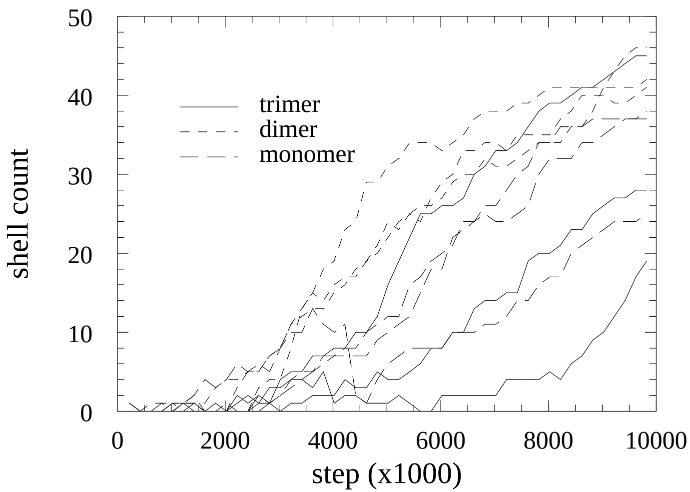
- Discard irreversible bonding; allow bonds to form and break reversibly without restriction (incorrect, energetically-unfavorable partial structures self-disassemble).
- Larger systems and longer runs needed for adequate yield.
- Destroy all shells below a certain size at regular intervals to provide monomers so larger shells can continue to grow.
- Monomer - details (note 'thickness' of unit) —

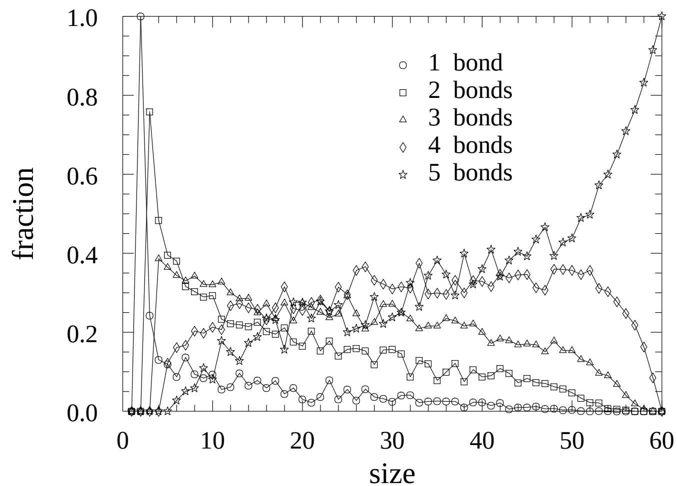


- For T=1 shells: 4096 monomers ⇒ 45 complete shells over 10^7 timesteps; interactions tailored to encourage initial trimer formation . . .

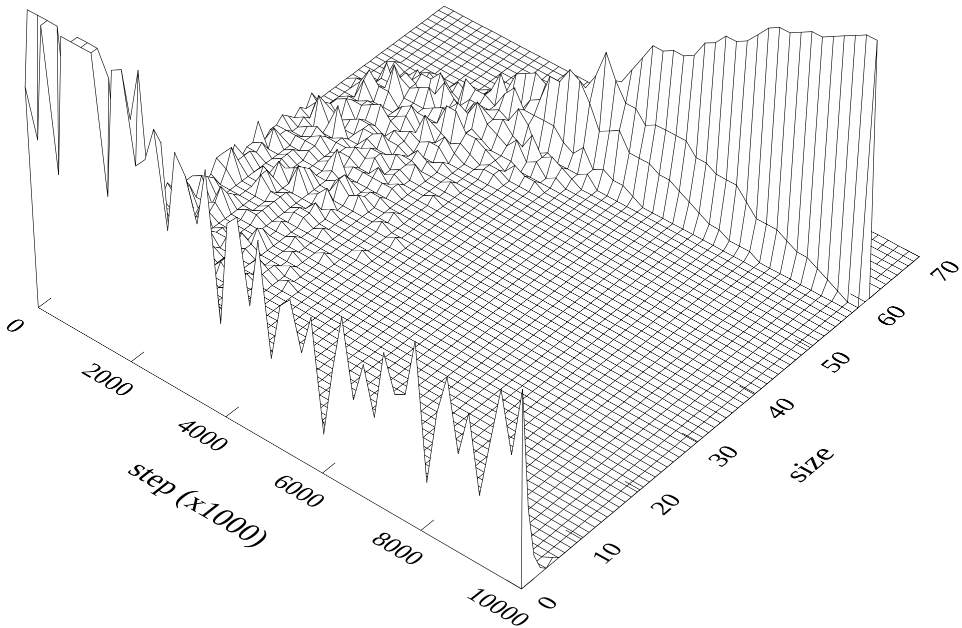


- Can use periodic boundaries to avoid wall bunching . . .
- Measure number of complete T=1 shells vs time (reversible bonding) and capsomer fraction with different bond counts (≤ 5) vs size —



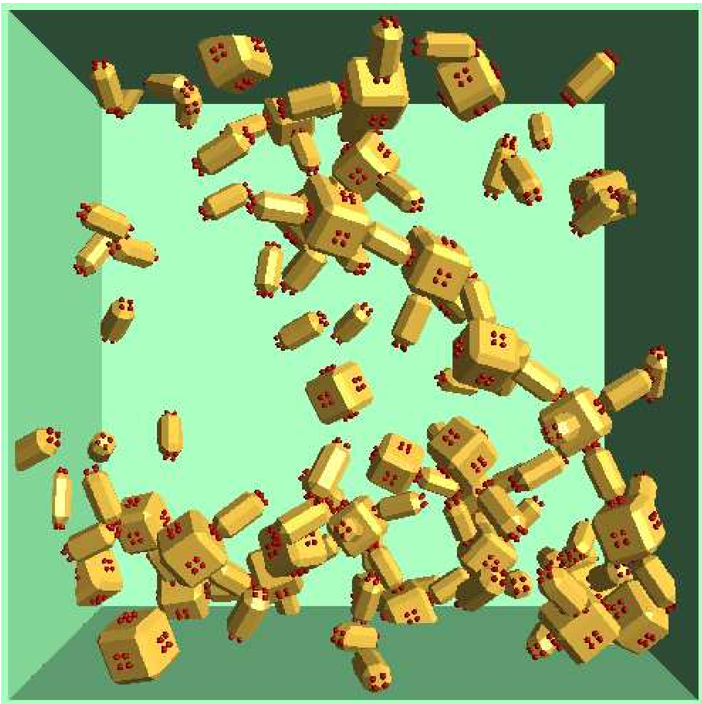


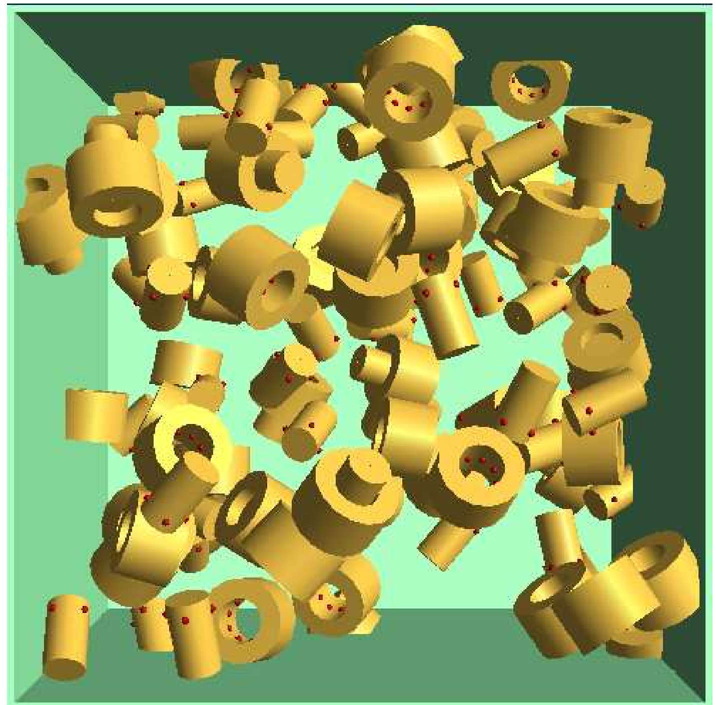
- Measure cluster-size distribution vs time (trimer-weighted growth) —



Other kinds of self-assembly

- Simple lattice elements and cylindrical objects ...





Future work

- Study populations and lifetimes of intermediate states; perhaps relate to scattering data.
- Sensitivity to particular subunit representation and choice of interactions?
- Coexistence of multiple pathways, with competition between different routes?
- Use of 'continuous feed' approach, with removal of completed products.
- Use of experimental / computed bonding energies to emulate real virus capsomers.
- Mechanism for quasi-equivalence to appear 'naturally'.
- References —

  - DCR, Johnson & Skolnick, Comp. Phys. Comm. **121** (1999) 231
  - DCR, Phys. Rev. E **70** (2004) 051905