SUPPLEMENTAL MATERIALS

Several equilibrium models potentially applicable to the polymerization of cell division protein FtsZ are formulated in simple free energy terms; this includes cooperative, adsorption, membrane attachment and cyclic models, as well as an extended monomer activation model.

Polymerization of a double-stranded flat filament is cooperative with a dimer nucleus (Figure 6). Given an axial monomer association free energy Ga (notation abbreviated from ΔGa^0_{app}), a lateral association free energy change Gl and a combined axial and lateral association free energy change Gd, the dimer nucleation and the elongation free energy changes for a staggered polymer are Gl and Gd respectively (Figure 6A). Due to the different number of contacts involved, Gl >> Gd, the formation of the dimer nucleus is less favorable than its elongation. The single-stranded elongation free energy change is less favorable than the double-stranded elongation, Ga >> Gd. The free energy change of lateral association of two protofilaments of length n to give a double-stranded polymer is G(n,single->2n,double) = Gl + nGd-(n-1)Ga. For a non-staggered double-stranded polymer G(n, single->2n, double) = Gl + (n-1)Gd (Figure 6B). Therefore the lateral association is progressively favored, leading to a majority of double-stranded polymers, even in the case of an indifferent lateral monomer association, Gl = 0. Note that typically Gd < (Ga + Gl), because the unfavorable mixing entropy change due to the formation of a bimolecular complex in the solution counts only once. In an ideal system, Gd = Ga + Gl – Gcratic, where Gcratic is the cratic free energy change Gcratic = ΔG^0_c = -TδΔSmix ≈ 2.4 Kcal mol^{-1} at room temperature (S1). In the non-staggered double filament (Figure 6B) the nucleus is really a trimer and its formation free energy change is Gl + Ga; the elongation free energy change averaged per monomer is (Gd + Ga)/2. Cooperative polymerization in this case, requires that Gd < (Ga + 2Gl). Finally, if the two protofilaments in the double filament are related by a translation, triple, quadruple, etc., filaments may also form by lateral repetition of identical interactions; if the two protofilaments are symmetric or antiparallel, their lateral interaction cannot be propagated.

Polymerization with adsorption to a surface is cooperative with an adsorbed monomer or oligomer nucleus (Figure 7). This model may be initially considered as formally related to double-stranded polymerization, where the surface substitutes for the second strand. Consider a single protofilament with an axial monomer association free energy change Ga, an adsorption free energy change Gb and a combined association plus adsorption free energy change Gs, the nucleation and elongation free energy changes are Gb and Gs respectively. The formation of the adsorbed monomer nucleus is less favorable than its elongation, Gb >> Gs. Single-stranded elongation in solution is less favorable than elongation of an adsorbed filament, Ga >> Gs. The free energy change of adsorption of a protofilament is G (n, solution-> adsorbed) = Gb + (n-1)(Gs-Ga); therefore, even for Gb=0, filament adsorption is favored over monomer adsorption and adsorption and the protein self-association are mutually enhanced. Protofilaments may also associate into two-dimensional polymers, in which case adsorption and lateral association favor each other. Models describing the opposing effects of excluded surface area and the formation of protein clusters on surface adsorption have been developed (S2, and references therein). Conversely, in a case of protofilament lateral association into staggered double filaments (Figure 6A) and competing adsorption of the lateral association interface on a surface (Figure 7), protofilament adsorption will prevail over double filament formation if Gb + (n-1)(Gs-Ga) < Gl + nGd - (n-1) Ga, which for an ideal system as above implies Gb < Gl and Gs < Gd. For non-staggered double filaments (Figure 6B), protofilament adsorption will prevail over double filament formation if Gb + (n-1)(Gs-Ga) < Gl + (n-1)Gd, which similarly implies Gb < Gl + Ga(n-1)/n.

Polymerization with attachment to a membrane (Figure 8) is related to adsorption-polymerization. Attachment may be mediated by a membrane protein. In excess of this linker protein, all subunits can become attached and the model becomes formally identical to adsorption-polymerization. If there are fewer attachment protein molecules that monomers, only *m* of the *n* subunits (m < n) in a filament become attached (Figure 8). Let Ga be the axial association free energy change, Gb the monomer attachment free energy change and Gm the combined association and attachment free energy change. Similarly to the models described above, Ga >> Gm. The free energy change of formation of a solution n-mer is (n-1)Ga, whereas that of formation on a n-mer with m monomers attached is more favorable, (n-1)Ga + m(Gm-Ga). The free energy change of substoichiometric attachment on the n-mer is Gb + m(Gm-Ga), which favors filament attachment. Coupled lateral association and attachment favor each other. However, in a hypothetical case of competing protofilament association into double filaments and membrane attachment (excluding each other), attached protofilaments will predominate if Gb + m(Gm-Ga) < Gl + nGd - (n-1) Ga (Figure 1A) or Gb + m(Gm-Ga) < Gl + (n-1)Gd (Figure 1B), which for a low m/n would require that Gm << Gd, a substantially more favorable attachment-association than double-stranded elongation.

Polymerization of a single protofilament with a cyclic end-product (Figure 9) may appear cooperative. Let Ga be the protofilament elongation free energy change and Gnc the closure free energy change. Gnc can be decomposed as Gnc = Ga – Gcratic + Gr, where Gcratic comes from the entropic advantage of a unimolecular versus a bimolecular association (see above) and Gr includes all other contributions, such as the loss of configurational entropy from the open state and any monomer deformation required to close the polymer. If Gnc < 0, that is Ga-Gcratic < Gr, most n-mers will circularly close. In such a system, above a certain concentration, the cyclic products accumulate while the monomers and short open oligomers remain at nearly constant concentration; this type of system gives characteristically bimodal sedimentation velocity profiles, in which above a certain concentration the fast boundary appears and the size of the slow boundary remains nearly constant (S3). These properties may appear similar to a cooperative polymerization with nucleus size n, but the closed polymers increase in number instead of elongating.

Polymerization of a single protofilament coupled to monomer conformational change can behave cooperatively with a dimer nucleus. Figure 10 is an extended version of Figure 5 in the main text. Let the isolated monomer exist in two interconvertible structures with a free energy difference Gc1. The free energy change of the structural change of an associated subunit is Gc2; in general, Gc1 ± Gc2, the free energy of the structural change depends on the association state. The axial association free energy changes are Ga. The free energy change of dimerization is G2 = Gc1 + Ga2 (going from 1 to 2 in the scheme in Figure 19). The free energy change of elongation for any step above dimer is G(n>2) = Gc2 + Ga2 (going from 2 to 3 in the scheme). If the structural change and association favor each other, Gc2 < Gc1 and G(n>2) < G2, which is a polymerization with a dimer nucleus.

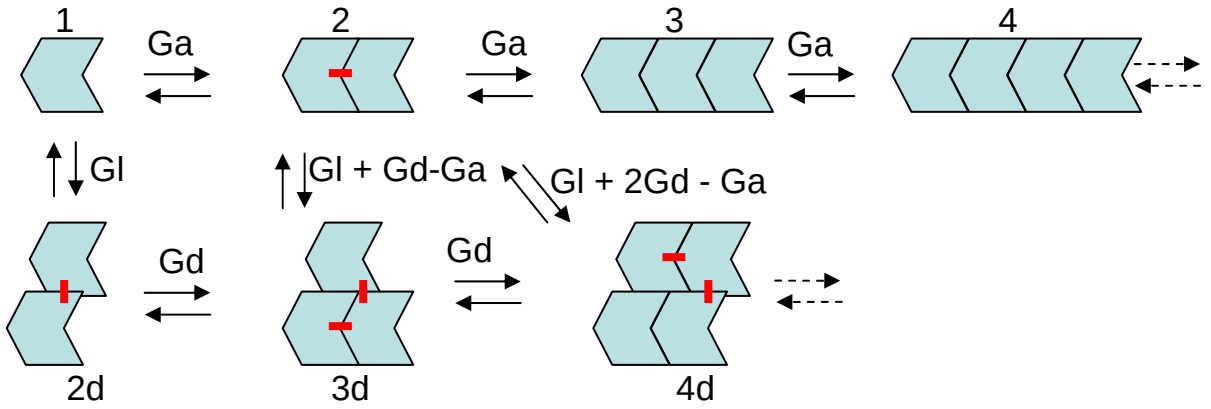
Suplemental REFERENCES

S1. Andreu, J.M., and S.N. Timasheff. 1982. Interaction of tubulin with single ring analogues of colchicine. Biochemistry 21:534-543.

S2. Minton, A.P. 2001. Effects of excluded surface area and adsorbate clustering on surface adsorption of proteins. II. Kinetic models. Biophys J 80:1641-1648.

S3. Frigon, R.P., and S.N. Timasheff. 1975. Magnesium-induced self-association of calf brain tubulin. I. Stoichiometry. Biochemistry 14:4559-4573.

A



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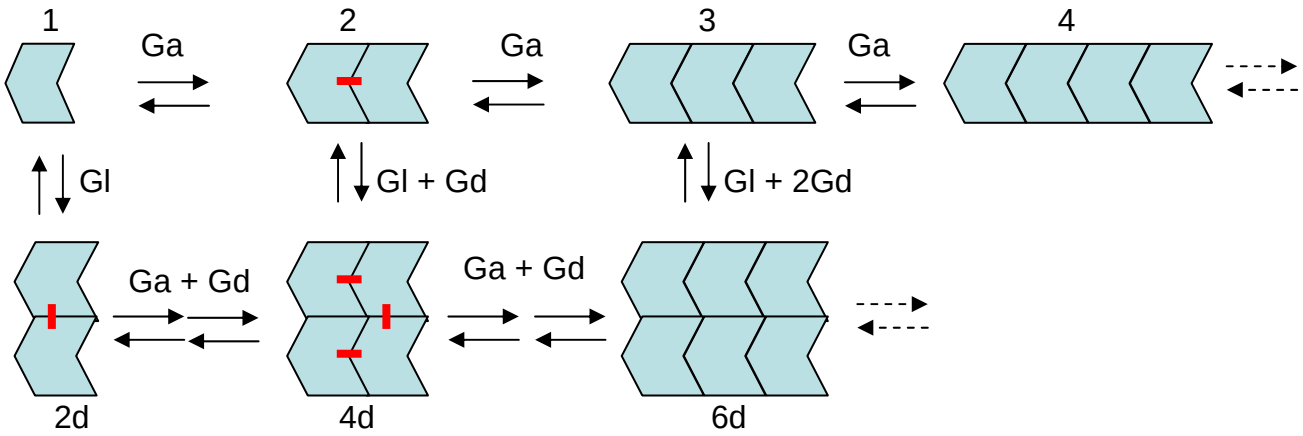

Figure 6. Polymerization of a double-stranded filament is cooperative with a dimer or trimer nucleus. In this scheme of single- and double-stranded polymerization, each protein monomer is represented by a chevron. Red marks indicate new contacts made. The free energy change of each association equilibrium represented is indicated in the scheme. Ga and Gl are the axial and lateral association free energy changes respectively. Gd is the combined axial and lateral association free energy change. Panel A, staggered protofilaments; for simplicity, Gd has been made to coincide in even and odd association steps. Panel B, non-staggered filaments; growth of this double filament implies odd and even association steps with free energies Ga and Gd respectively, which have been added for the purpose of comparison with Figure 7.

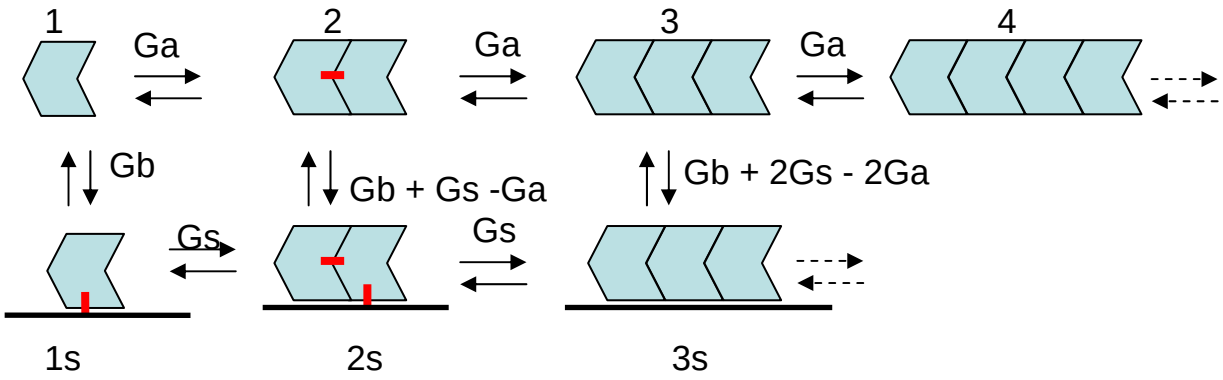

Figure 7. Adsorption-polymerization of a single protofilament is cooperative with an adsorbed monomer nucleus. Ga and Gb are the axial association and adsorption free energy changes respectively, Gs the combined axial and adsorption free energy change (see legend to Fig. 6).

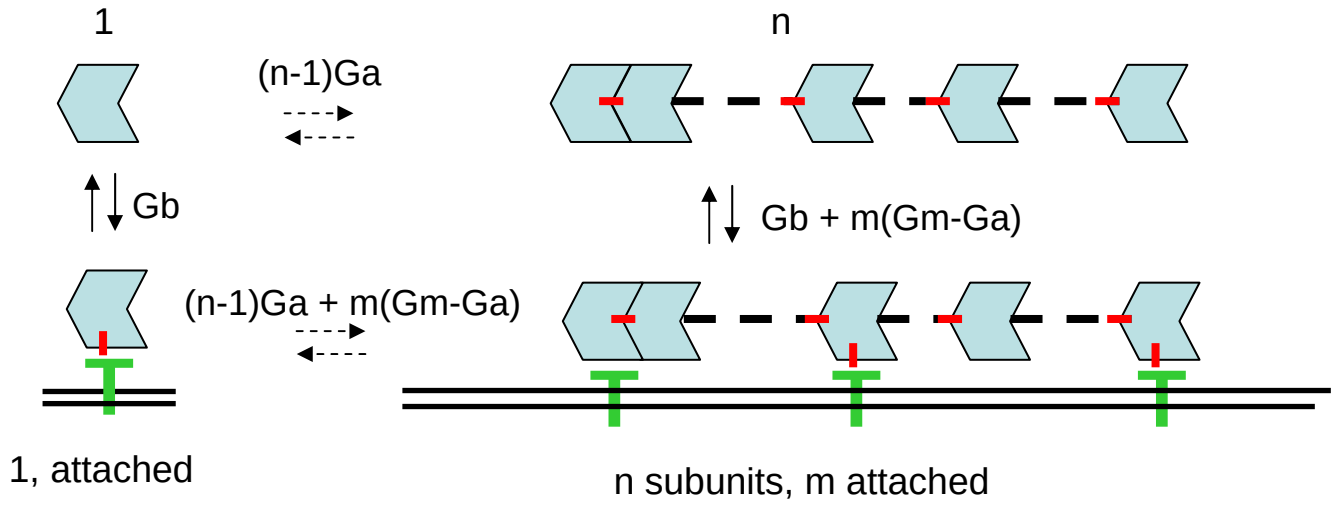

Figure 8. Polymerization of a single protofilament with substoichiometric binding to a membrane protein. Ga and Gb are the association and attachment free energy changes respectively and Gm is the combined association and attachment free energy change. The dashed arrows indicate multiple association steps to give a polymer of *n* subunits, *m* attached.

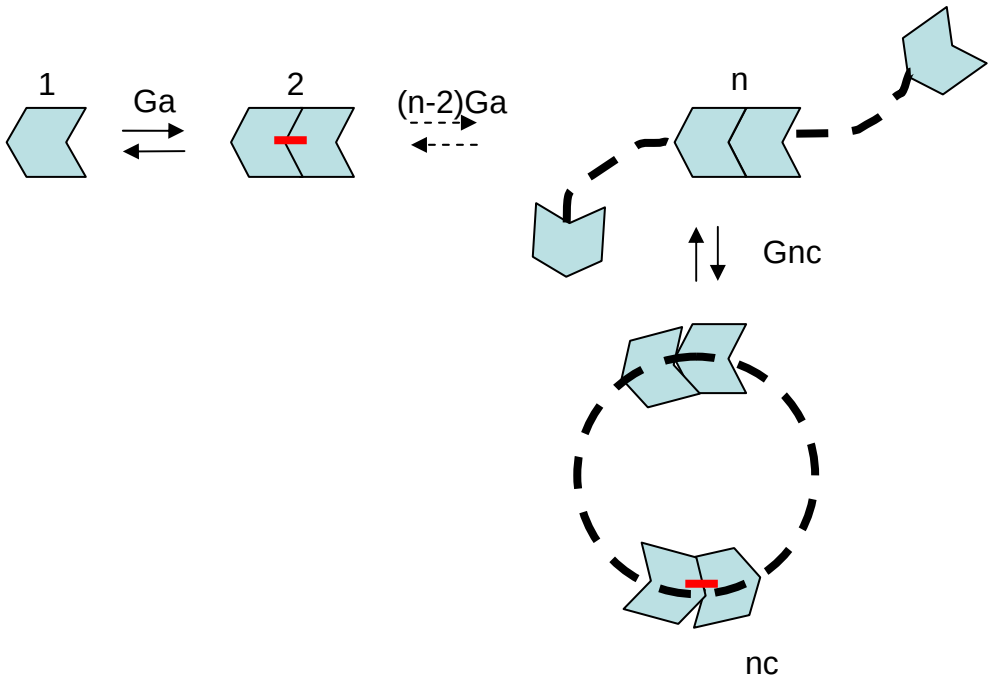

Figure 9. Polymerization of a single protofilament with a cyclic end product. Ga and Gnc are the association and cyclization free energy changes respectively. Red marks indicate the bonds made in dimerization and cyclization. Non cyclic n-mers are arbitrarily flexible in this scheme. All bonds are identical in the cyclic end product.

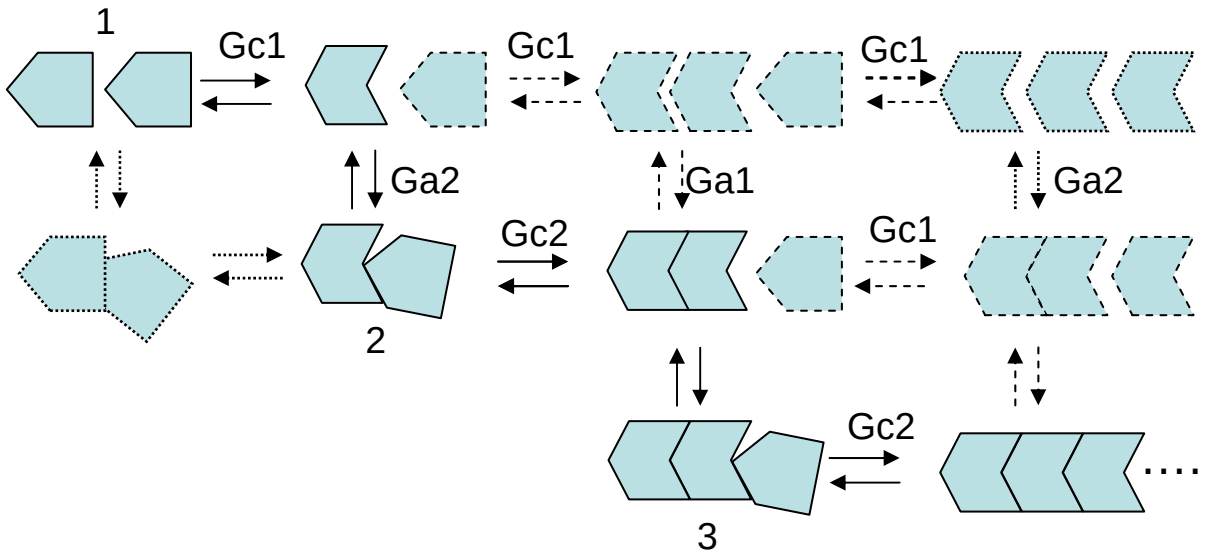

Figure 10. Polymerization of a single protofilament with a monomer conformational change can behave cooperatively with a dimer nucleus. In this scheme the monomers exist in two states, an actively self-associating structure (represented by a chevron) and a poorly self-associating structure (represented by an unfitting pentangle). Note that both association interfaces of the polar monomer change between conformations; all internal bonds are identical in the linear polymers. Ga´s are association free energy changes and Gc´s are conformational free energy changes; there are several possible pathways. Consider the thermodynamic box going from two different monomers (one chevron, one pentangle) to a dimer (two chevrons) in the middle of the scheme. The total free energy change is Ga1 + Gc1 = Gc2 + Ga2. Note that if Ga1 ≠ Ga2, Gc1 ≠Gc2, the association and the structural change are linked. The linkage free energy is Glink = Gc2 – Gc1 = Ga1 – Ga2. When Glink < 0 each process favors the other, when Glink > 0 both processes are unfavorably linked.