Biomimetic carpentry of self-assembling functional protein nanostructures based on phage tail fibers. NSF Functional Nanostructures Grant 9834603 **Edward Goldberg, Tufts**

GOAL

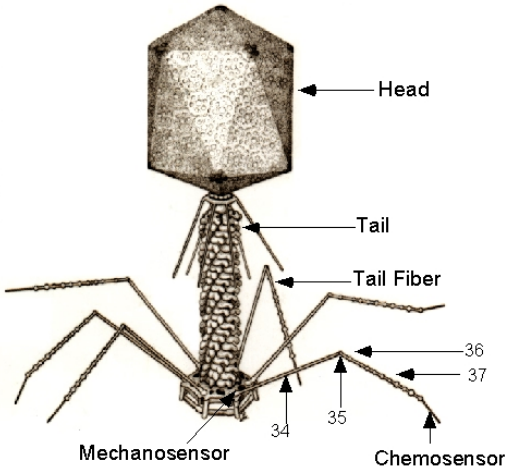
Our goal is to integrate structural design with 3-D patterning of function by controlled assembly of functionalized protein rod units into open nanostructural arrays.

APPROACH

To accomplish this goal, we will create what is essentially a molecular TinkerToy for practical manufacture of functional nanostructures. We will use a biological paradigm for sequential self-assembly of multiunit molecular arrays. There are three principal steps toward achieving our goal:

1. Engineer protein rod units (3nm diameter) with terminal binding domains for joint formation.
2. Place functional moieties both (organic and inorganic) at defined sites in the rodlike (central) portion of the units.
3. Build arrays in a massively parallel manner.

Figure 1. T4 phage

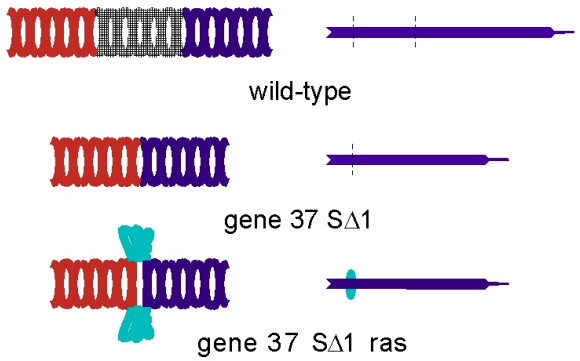


Characteristics of the Protein Rod Material

Cells are the ultimate factory for manufacture by self-assembly. Examples of such self-assembly (and major function) are: ribosomes (protein synthesis); proton pump (energy transduction); flagellum (movement); pilus (tethering); virus (nucleic acid delivery).

To take advantage of cellular manufacture, we engineer our units genetically from T4 phage tail fibers and produce them in bacterial cells. Figure 1 shows the structure of phage T4. The tail fibers are composed of long rigid rod segments made of extended β-sheets which assemble by forming rigid joints between complementary terminal binding domains. Thus the natural equivalent of step 1 occurs in vivo. We will modify the parts and process to make useful constructs in vitro.

Figure 2. A 15 aa H-ras epitope in the SΔ1 junction

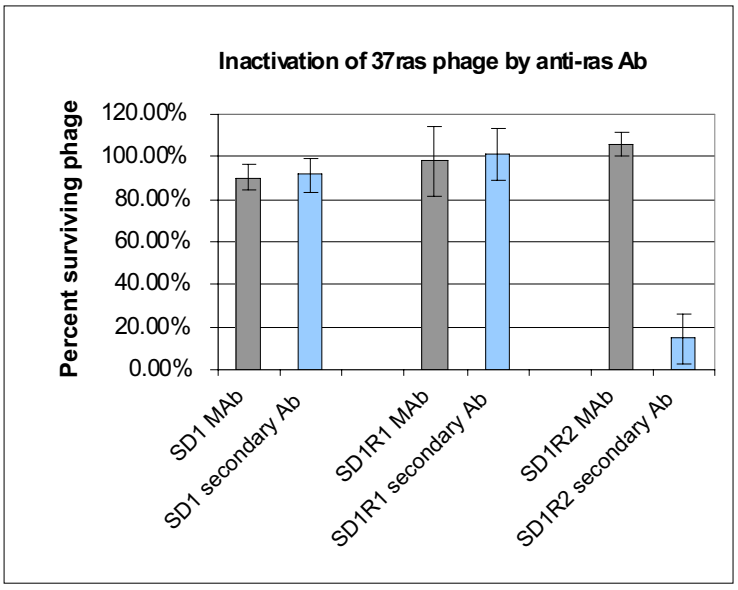


The State of Our Art

How do we place a functional moiety at a precise site in the rod-like region of the unit without disrupting the structural integrity of the unit? We insert the moiety into a loop in the β–sheet. We postulate that such an insertion will not affect the surrounding structure adversly. Furthermore we believe that such a loop can be found at the junction of a deletion, in a tail fiber gene, that permits normal infection.

Figure 2 shows a cartoon of such a deletion of 346 residues (SΔ1 - identified by Dr. P. Hyman, now of NanoFrames Inc.) in the gene 37 protein (of 1026 residues). When we transferred this

deletion into a phage it remained normal and fully infective. Thus the structural integrity of the rod-like tail fibers was not compromised by this large deletion.



To demonstrate the placement of a functional moiety into the tail fiber, we inserted a 15 residue H-ras epitope (flanked on each side by a tetraglycine) into the SΔ1 fusion site. The phage carrying this 23 residue insert remained infective and formed plaques.

In order to demonstrate the specific binding function of this epitope, we added rat monoclonal anti-H-ras antibody (specific to the R2 epitope) to the phage (SΔ1R2) in solution. Figure 3 shows that no phage inactivation when anti H-ras antibody alone was added (grey bars). This surprised us. After scratching our heads, we decided to add polyclonal anti-rat IgG antiserum. The same figure (blue bars) show that only the phage carrying the specific epitope was inactivated. The controls carrying no epitope (SΔ1), or carrying a different 15 residue peptide from the H-ras protein (SΔ1R1), were not significantly inactivated. Inactivation of phage is most likely due to crosslinking of tail fibers, by anti-rat antibodies which bind to anti-H-ras antibodies bound to the epitope inserted into the tail fibers.

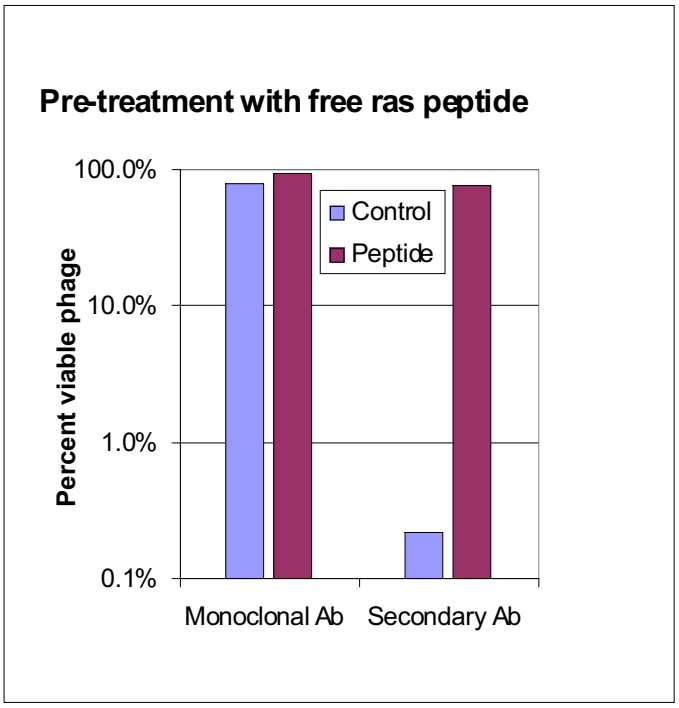
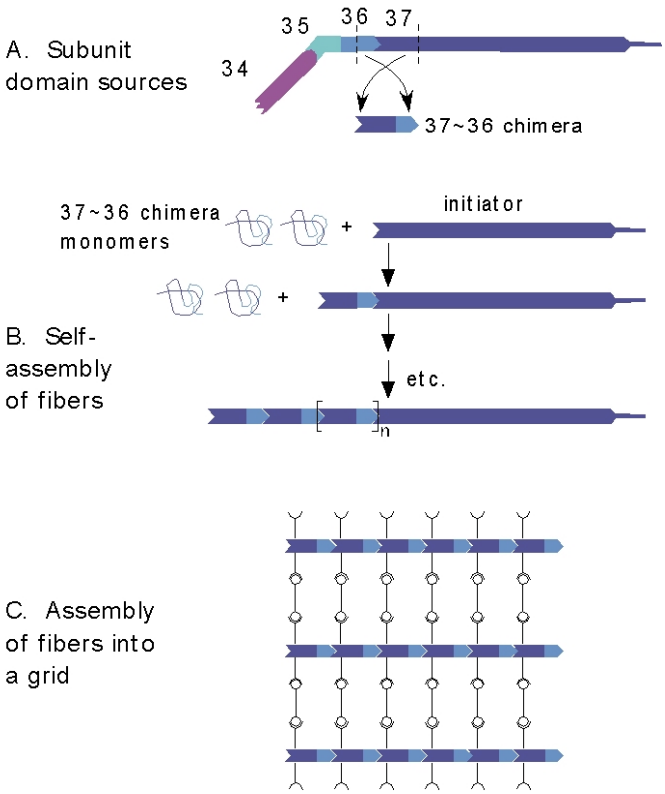


Figure 4 demonstrates that pretreatment of anti-H-ras with the free 15 residue H-ras peptide epitope inhibits phage inactivation. This proves that the H-ras epitope is the specific target leading to inactivation.

Thus, we have demonstrated the ability to alter the length of a unit rod and to functionalize this rod by engineering specific genetic alterations in a tail fiber gene.

Figure 5. Engineering a chimeric unit for assembly of a functionalized nanoarray.



Assembling functional arrays

Figure 5a illustrates one way to engineer a self-assembling chimeric unit to extend the length of an initator unit to a rigid rod (Figure 5b). If the chimeric extender unit carries a functional moiety (e.g. a semicircle, Figure 5c), a bifunctional bridging agent can join the extended rods into a 2-dimensional, structural array. Other functional moieties can be included in this array. This type of process can be extended into 3-dimensional open and closed structural arrays with functional moieties.

Impact of Technology

Our goal is to assemble nanostructural units into predefined arrays of 1-, 2- and 3-dimensions to support patterns of functional units at the mesoscopic level. These arrays will have applications in many industries including reinforcement, catalysis, processing, electron transport, optical and quantum applications. Our major challege to achieve this goal will be to implement controlled assembly in vitro.