

**MATERIALS FOR THE PRODUCTION OF
NANOMETER STRUCTURES AND USE THEREOF**

FIELD OF THE INVENTION

5 The present invention pertains to nanostructures, i.e., nanometer sized structures useful in the construction of microscopic and macroscopic structures. In particular, the present invention pertains to nanostructures based on bacteriophage T4 tail fiber proteins and variants thereof.

10 **BACKGROUND TO THE INVENTION**

 While the strength of most metallic and ceramic based materials derives from the theoretical bonding strengths between their component molecules and crystallite surfaces, it is significantly limited by flaws in their
15 crystal or glass-like structures. These flaws are usually inherent in the raw materials themselves or developed during fabrication and are often expanded due to exposure to environmental stresses.

20 The emerging field of nanotechnology has made the limitations of traditional materials more critical. The ability to design and produce very small structures (i.e., of nanometer dimensions) that can serve complex functions depends upon the use of appropriate materials that can be
25 manipulated in predictable and reproducible ways, and that have the properties required for each novel application.

 Biological systems serve as a paradigm for sophisticated nanostructures. Living cells fabricate proteins and combine them into structures that are perfectly formed and can resist damage in their normal environment. In some
30 cases, intricate structures are created by a process of self-assembly, the instructions for which are built into the component polypeptides. Finally, proteins are subject to proofreading processes that insure a high degree of quality
35 control.

 Therefore, there is a need in the art for methods and compositions that exploit these unique features of

proteins to form constituents of synthetic nanostructures. The need is to design materials whose properties can be tailored to suit the particular requirements of nanometer-scale technology. Moreover, since the subunits of most macrostructural materials, ceramics, metals, fibers, etc., are based on the bonding of nanostructural subunits, the fabrication of appropriate subunits without flaws and of exact dimensions and uniformity should improve the strength and consistency of the macrostructures because the surfaces are more regular and can interact more closely over an extended area than larger, more heterogeneous material.

SUMMARY OF THE INVENTION

In one aspect, the present invention provides isolated protein building blocks for nanostructures, comprising modified tail fiber proteins of bacteriophage T4. The gp34, 36, and 37 proteins are modified in various ways to form novel rod structures with different properties. Specific internal peptide sequences may be deleted without affecting their ability to form dimers and associate with their natural tail fiber partners. Alternatively, they may be modified so that they: interact only with other modified, and not native, tail fiber partners; exhibit thermolabile interactions with their partners; or contain additional functional groups that enable them to interact with heterologous binding moieties.

The present invention also encompasses fusion proteins that contain sequences from two or more different tail fiber proteins. The gp35 protein, which forms an angle joint, is modified so as to form average angles different from the natural average angle of $137^\circ (\pm 7^\circ)$ or $156^\circ (\pm 12^\circ)$, and to exhibit thermolabile interactions with its partners.

In another aspect, the present invention provides nanostructures comprising native and modified tail fiber proteins of bacteriophage T4. The nanostructures may be one-dimensional rods, two-dimensional polygons or open or closed sheets, or three-dimensional open cages or closed solids.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A and 1B show a schematic representation of the T4 bacteriophage particle (Figure 1A), and a schematic representation of the T4 bacteriophage tail fiber (Figure 1B).

Figure 2 shows a schematic representation of a unit rod.

Figures 3A-3D show schematic representations of: a one-dimensional multi-unit rod joined along the x axis (Figure 3A); closed simple sheets (Figure 3B); closed brickwork sheets (Figure 3C); and open brickwork sheets (Figure 3D).

Figure 4 shows a schematic representation of two units used to construct porous and solid sheets (top and bottom), which, when alternatively layered, produce a multi-tiered set of cages as shown.

Figure 5 shows a schematic representation of an angled structure having an angle of 120°.

Figure 6 shows the DNA sequence (SEQ ID NO:1) of genes 34, 35, 36, and 37 of bacteriophage T4.

Figure 7 shows the amino acid sequences (shown in single-letter codes) of the gene products of genes 34 (SEQ ID NO:2, ORFX SEQ ID NO:3), 35 (SEQ ID NO:4), 36 (SEQ ID NO:5), and 37 (SEQ ID NO:6) of bacteriophage T4. The amino acid sequences (bottom line of each pair) are aligned with the nucleotide sequences (top line of each pair.) It is noted that the deduced protein sequence of gene 35 (from NCBI database) is not believed to be accurate.

Figures 8A-8B show a schematic representation of: the formation of a P37 dimer initiator from a molecule that self-assembles into a dimer (Figure 8A); and the formation of a P37 trimer initiator from a molecule that self-assembles into a trimer (Figure 8B).

Figure 9 shows a schematic representation of the formation of the polymer (P37-36)_n with an initiator that is a self-assembling dimer.

DETAILED DESCRIPTION OF THE INVENTION

All patents, patent applications and literature references cited in the specification are hereby incorporated by reference in their entirety. In the case of
5 inconsistencies, the present disclosure, including, definitions, will prevail.

Although the invention is described in terms of bacteriophage T4 tail fiber proteins, it will be understood that the invention is also applicable to tail fiber proteins
10 of other T-even-like phage, e.g., of the T4 family (e.g., T4, Tu1a, Tu1b), and T2 family (T2, T6, K3, Ox2, M1, etc.)

DEFINITIONS:

"Nanostructures" are defined herein as structures
15 of different sizes and shapes that are assembled from nanometer- sized protein components.

"Chimers" are defined herein as chimeric proteins in which at least the amino- and carboxy-terminal regions are derived from different original polypeptides, whether the
20 original polypeptides are naturally occurring or have been modified by mutagenesis.

"Homodimers" are defined herein as assemblies of two substantially identical protein subunits that form a defined three-dimensional structure.

25 The designation "gp" denotes a monomeric polypeptide, while the designation "P" denotes homooligomers. P34, P36, and P37 are presumably homodimers or homotrimers.

An isolated polypeptide that "consists essentially of" a specified amino acid sequence is defined herein as a
30 polypeptide having the specified sequence or a polypeptide that contains conservative substitutions within that sequence. Conservative substitutions, as those of ordinary skill in the art would understand, are ones in which an acidic residue is replaced by an acidic residue, a basic
35 residue by a basic residue, or a hydrophobic residue by a hydrophobic residue. Also encompassed is a polypeptide that lacks one or more amino acids at either the amino terminus or

carboxy terminus, up to a total of five at either terminus, when the absence of the particular residues has no discernable effect on the structure or the function of the polypeptide in practicing the present invention.

5 The present invention pertains to a new class of protein building blocks whose dimensions are measured in nanometers, which are useful in the construction of microscopic and macroscopic structures. Without wishing to be bound by theory, it is believed that the basic unit is a
10 homodimer composed of two identical protein subunits having a cross- β configuration, although a trimeric structure is also possible. Thus, as will be apparent, references to a "homodimer" or "dimerization" as used herein will in many instances be construed as also referring to a homotrimer or
15 trimerization. These long, stiff, and stable rod-shaped units can assemble with other rods using coupling devices that can be attached genetically or *in vitro*. The ends of one rod may attach to different ends of other rods or similar rods. Variations in the length of the rods, in the angles of
20 attachment, and in their flexibility characteristics permit differently-shaped structures to self-assemble *in situ*. In this manner the units can self-assemble into predetermined larger structures of one, two or three dimensions. The self-assembly can be staged to form structures of precise
25 dimensions and uniform strength due to the flawless biological manufacture of the components. The rods can also be modified by genetic and chemical modifications to form predetermined specific attachment sites for other chemical entities, allowing the formation of complex structures.

30 An important aspect of the present invention is that the protein units can be designed so that they comprise rods of different lengths, and can be further modified to include features that alter their surface properties in predetermined ways and/or influence their ability to join
35 with other identical or different units. Furthermore, the self-assembly capabilities can be expanded by producing chimeric proteins that combine the properties of two

different members of this class. This design feature is achieved by manipulating the structure of the genes encoding these proteins.

As detailed below, the compositions and methods of the present invention take advantage of the properties of the natural proteins, i.e., the resulting structures are stiff, strong, stable in aqueous media, heat resistant, protease resistant, and can be rendered biodegradable. A large quantity of units can be fabricated easily in microorganisms. Furthermore, for ease of automation, large quantities of parts and subassemblies can be stored and used as needed.

The sequences of the protein subunits are based on the components of the tail fiber of the T4 bacteriophage of *E. coli*. It will be understood that the principles and techniques can be applied to the tail fibers of other T-even phages, or other related bacteriophages that have similar tail and/or fiber structures.

The structure of the T4 bacteriophage tail fiber (illustrated in Figure 1) can be represented schematically as follows (N= amino terminus, C= carboxy terminus): N[P34]C - N[gp35]C - N[P36]C - N[P37]C. P34, P36, and P37 are all stiff, rod-shaped protein homodimers in which two identical β sheets, oriented in the same direction, are fused face-to-face by hydrophobic interactions between the sheets juxtaposed with a 180° rotational axis of symmetry through the long axis of the rod. (The structure will vary if P34, P36, and P37 are homotrimers.) gp35, by contrast, is a monomeric polypeptide that attaches specifically to the N-terminus of P36 and then to the C-terminus of P34 and forms an angle joint between two rods. During T4 infection of *E. coli*, two gp37 monomers dimerize to form a P37 homodimer; the process of dimerization is believed to initiate near the C-terminus of P37 and to require two *E. coli* chaperon proteins. (A variant gp37 with a temperature sensitive mutation near the C-terminus used in the present invention requires only one chaperon, gp57, for dimerization.) Once dimerized, the N-terminus of P37 initiates the dimerization

of two gp36 monomers to a P36 rod. The joint between the C-terminus of P36 and the N-terminus of P37 is tight and stiff but noncovalent. The N-terminus of P36 then attaches to a gp35 monomer; this interaction stabilizes P36 and forms
5 the elbow of the tail fiber. Finally, gp35 attaches to the C-terminus of P34 (which uses gp57 for dimerization). Thus, self assembly of the tail fiber is regulated by a predetermined order of interaction of specific subunits whereby structural maturation caused by formation of the
10 first subassembly permits interaction with new (previously disallowed) subunits. This results in the production of a structure of exact specifications from a random mixture of the components.

In accordance with the present invention, the genes
15 encoding these proteins may be modified so as to make rods of different lengths with different combinations of ends. The properties of the native proteins are particularly advantageous in this regard. First, the β -sheet is composed of antiparallel β -strands with β -bends at the left (L) and
20 right (R) edges. Second, the amino acid side chains alternate up and down out of the plane of the sheet. The first property allows bends to be extended to form symmetric and specific attachment sites between the L and R surfaces, as well as to form attachment sites for other structures. In
25 addition, the core sections of the β -sheet can be shortened or lengthened by genetic manipulations e.g., by splicing DNA regions encoding β -bends, on the same edge of the sheet, to form new bends that exclude intervening peptides, or by inserting segments of peptide in an analogous manner by
30 splicing at bend angles. The second property allows amino acid side chains extending above and below the surface of the β -sheet to be modified by genetic substitution or chemical coupling. Importantly, all of the above modifications are achieved without compromising the structural integrity of the
35 rod. It will be understood by one skilled in the art that these properties allow a great deal of flexibility in

designing units that can assemble into a broad variety of structures, some of which are detailed below.

STRUCTURAL UNITS

5 The rods of the present invention function like wooden 2 X 4 studs or steel beams for construction. In this case, the surfaces are exactly reproducible at the molecular level and thereby fitted for specific attachments to similar or different units rods at fixed joining sites. The surfaces
10 are also modified to be more or less hydrophilic, including positively or negatively charged groups, and have protrusions built in for specific binding to other units or to an intermediate joint with two receptor sites. The surfaces of the rod and a schematic of the unit rod are illustrated in
15 Figure 2. The three dimensions of the rod are defined as: x, for the back (B) to front (F) dimension; y, for the down (D) to up (U) dimension; and z, for the left (L) to right (R) dimension.

One dimensional multi-unit rods can be most readily
20 assembled from single unit rods joined along the x axis (Figure 3A) but regular joining of subunits in either of the other two dimensions will also form a long structure, but with different cross sections than in the x dimension.

Two dimensional constructs are sheets formed by
25 interaction of rods along any two axes. 1) Closed simple sheets are formed from surfaces which overlap exactly, along any two axes (Figure 3B). 2) Closed brickwork sheets are formed from interaction between units that have exactly overlapping surfaces in one dimension and a special type of
30 overlap in the other (Figure 3C). In this case there must be two different sets of complementary joints spaced with exactly 1/2 unit distance between them. If they are centered (i.e., each set 1/4 from the end) then each joint will be in the center of the units above and below. If they are offset,
35 then the joint will be offset as well. In this construction, the complementary interacting sites are schematized by * and **. If the interacting sites are each symmetric, the

alternating rows can interact with the rods in either direction. If they are not symmetric, and can only interact with interacting rows facing in the same or opposite direction, the sheet will be made of unidirectional rods or 5 layers of rods in alternating directions. 3) Open brickwork sheets (or nets) result when the units are separated by more than one-half unit (Figure 3D). The dimensions of the openings (or pores) depend upon the distance (dx) separating the interacting sites and the distance (dy) by which these 10 sites separate the surfaces.

Three dimensional constructs require sterically compatible interactions between all three surfaces to form solids. 1) Closed solids can assemble from units that overlap exactly in all three dimensions (e.g., the exact 15 overlapping of closed simple sheets). In an analogous manner, closed brickwork sheets can form closed solids by overlapping sheets exactly or displaced to bring the brickwork into the third dimension. This requires an appropriate set of joints on all three pairs of parallel 20 faces of the unit. 2) Porous solids are made by joining open brickwork sheets in various ways. For example, if the units overlap exactly in the third dimension, a solid is formed with the array of holes of exact dimensions running perpendicular to the plane of the paper. If instead, a 25 material is needed with closed spaces, with layers of width dz (i.e., in the U->D dimension), a simple closed sheet is layered on the open brickwork sheet to close the openings. If the overlap of the open brickwork sheet is e.g., 1/4 unit, then a rod of length 3/4 units is used to make the sheet. 30 Joints are then needed in the z dimension. The two units used to polymerize these alternate layers, and the layers themselves, are schematized in Figure 4.

All of the above structures are composed of simple linear rods. A second unit, the angle unit, expands the type 35 and dimensionality of possible structures. The angle unit connects two rods at angles different from 180°, akin to an angle iron. The average angle and its degree of rigidity are

built into this connector structure. For example, the structure shown in Figure 5 has an angle of 120° and different specific joining sites at a and at b. The following are examples of structures that are formed
5 utilizing angle joints:

1) Open brickwork sheets are expanded and strengthened in the direction normal to the rod direction by adding angles perpendicular to the sheet. In this case, a three dimensional network forms. Attachment of 90° angles to
10 the ends of the rods makes an angle almost in the plane of the sheet, allowing new rods added to those angles (which must have some play out of the plane of the original sheet to attach in the first place) to form a new sheet, almost parallel, with an orientation normal to its upper or lower
15 neighbor.

2) Hexagons are made from a mixture of rods and angle joints that form 120° angles. In this case, there are two exclusive sets of joints. Each set is made up of one of the two ends of the rod and one of the two complementary
20 sites on the angle. This is a linear structure in the sense that the hexagon has a direction (either clockwise or counterclockwise). It can be made into a two dimensional open net (i.e., a two dimensional honeycomb) by joining the sides of the hexagons. It can form hexagonal tubes by
25 joining the top of the hexagon below to the bottom face of the hexagon above. If the tubes also join by their sides, they will form an open three dimensional multiple hexagonal tube.

3) Helical hexagonal tubes are made analogously to
30 hexagons but the sixth unit is not joined to the first to close the hexagon. Instead, the end is displaced from the plane of the hexagon and the seventh and further units are added to form a hexagonal tube which can be a spring if there is little or no adhesive force between the units of the
35 helix, or a stiff rod if there is such a force to maintain the close proximity of apposing units.

It will be apparent to one skilled in the art that the compositions and methods of the present invention also encompass other polygonal structures such as octagons, as well as open solids such as tetrahedrons and icosahedrons 5 formed from triangles and boxes formed from squares and rectangles. The range of structures is limited only by the types of angle units and the substituents that can be engineered on the different axes of the rod units. For example, other naturally occurring angles are found in the 10 fibers of bacteriophage T7, which has a 90° angle (Steven et al., *J. Mol. Biol.* 200: 352-365, 1988).

DESIGN AND PRODUCTION OF THE ROD PROTEINS

The protein subunits that are used to construct the 15 nanostructures of the present invention are based on the four polypeptides that comprise the tail fibers of bacteriophage T4, i.e., gp34, gp35, gp36 and gp37. The genes encoding these proteins have been cloned, and their DNA and protein sequences have been determined (for gene 36 and 37 see Oliver 20 et al. *J. Mol. Biol.* 153: 545-568, 1981). The DNA and amino acid sequences of genes 34, 35, 36 and 37 are set forth in Figures 6 and 7 below.

Gp34, gp35, gp36, and gp37 are produced naturally following infection of *E. coli* cells by intact T4 phage 25 particles. Following synthesis in the cytoplasm of the bacterial cell, the gp34, 36, and 37 monomers form homodimers, which are competent for assembly into maturing phage particles. Thus, *E. coli* serves as an efficient and convenient factory for synthesis and dimerization of the 30 protein subunits described herein below.

In practicing the present invention, the genes encoding the proteins of interest (native, modified, or recombined) are incorporated into DNA expression vectors that are well known in the art. These circular plasmids typically 35 contain selectable marker genes (usually conferring antibiotic resistance to transformed bacteria), sequences that allow replication of the plasmid to high copy number in

E. coli, and a multiple cloning site immediately downstream of an inducible promoter and ribosome binding site. Examples of commercially available vectors suitable for use in the present invention include the pET system (Novagen, Inc.,
5 Madison, WI) and Superlinker vectors pSE280 and pSE380 (Invitrogen, San Diego, CA).

The strategy is to 1) construct the gene of interest and clone it into the multiple cloning site; 2) transform *E. coli* cells with the recombinant plasmid; 3)
10 induce the expression of the cloned gene; 4) test for synthesis of the protein product; and, finally, 5) test for the formation of functional homodimers. In some cases, additional genes are also cloned into the same plasmid, when their function is required for dimerization of the protein of
15 interest. For example, when wild-type or modified versions of gp37 are expressed, the bacterial chaperon gene 57 is also included; when wild-type or modified gp36 is expressed, the wild-type version or a modified version of the gp37 gene is included. The modified gp37 should have the capacity to
20 dimerize and contain an N-terminus that can chaperon the dimerization of gp36. This method allows the formation of monomeric gene products and, in some cases, maturation of monomers to homodimeric rods in the absence of other phage-induced proteins normally present in a T4-infected
25 cell.

Steps 1-4 of the above-defined strategy are achieved by methods that are well known in the art of recombinant DNA technology and protein expression in bacteria. For example, in step 1, restriction enzyme
30 cleavage at multiple sites, followed by ligation of fragments, is used to construct deletions in the internal rod segment of gp34, 36, and 37 (see Example 1 below). Alternatively, a single or multiple restriction enzyme cleavage, followed by exonuclease digestion (EXO-SIZE, New
35 England Biolabs, Beverly, MA), is used to delete DNA sequences in one or both directions from the initial cleavage site; when combined with a subsequent ligation step, this

procedure produces a nested set of deletions of increasing sizes. Similarly, standard methods are used to recombine DNA segments from two different tail fiber genes, to produce chimeric genes encoding fusion proteins (called "chimera" in this description). In general, this last method is used to provide alternate N- or C-termini and thus create novel combinations of ends that enable new patterns of joining of different rod segments. A representative of this type of chimera, the fusion of gp37-36, is described in Example 2.

10 The preferred hosts for production of these proteins (Step 2) is *E. coli* strain BL21(DE3) and BL21(DE3/pLyss) (available commercially from Novagen, Madison, WI), although other compatible *recA* strains, such as HMS174(DE3) and HMS174(DE3/pLyss) can be used. Transformation with the

15 recombinant plasmid (Step 2) is accomplished by standard methods (Sambrook, J., *Molecular Cloning*, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY; this is also the source for standard recombinant DNA methods used in this invention.) Transformed bacteria are selected by virtue of their

20 resistance to antibiotics e.g., ampicillin or kanamycin. The method by which expression of the cloned tail fiber genes is induced (Step 3) depends upon the particular promoter used. A preferred promoter is *plac* (with a *lacI^s* on the vector to reduce background expression), which can be regulated by the

25 addition of isopropylthiogalactoside (IPTG). A second preferred promoter is pT7 ϕ 10, which is specific to T7 RNA polymerase and is not recognized by *E. coli* RNA polymerase. T7 RNA polymerase, which is resistant to rifamycin, is encoded on the defective lambda DE lysogen in the *E. coli*

30 BL21 chromosome. T7 polymerase in BL21(DE3) is super-repressed by the *lacI^s* gene in the plasmid and is induced and regulated by IPTG.

Typically, a culture of transformed bacteria is incubated with the inducer for a period of hours, during

35 which the synthesis of the protein of interest is monitored. In the present instance, extracts of the bacterial cells are

prepared, and the T4 tail fiber proteins are detected, for example, by SDS-polyacrylamide gel electrophoresis.

Once the modified protein is detected in bacterial extracts, it is necessary to ascertain whether or not it
5 forms appropriate homodimers (Step 4). This is accomplished initially by testing whether the protein is recognized by an antiserum specific to the mature dimerized form of the protein.

Tail fiber-specific antisera are prepared as
10 described (Edgar, R.S. and Lielausis, I., *Genetics* 52: 1187, 1965; Ward et al, *J. Mol. Biol.* 54:15, 1970). Briefly, whole T4 phage are used as an immunogen; optionally, the resulting antiserum is then adsorbed with tail-less phage particles, thus removing all antibodies except those directed against
15 the tail fiber proteins. In a subsequent step, different aliquots of the antiserum are adsorbed individually with extracts that each lack a particular tail fiber protein. For example, if an extract containing only tail fiber components P34, gp35, and gp36 (derived from a cell infected with a
20 mutant T4 lacking a functional gp37 gene) is used for absorption, the resulting antiserum will recognize only mature P37 and dimerized P36-P37. A similar approach may be used to prepare individual antisera that recognize only mature (i.e., homodimerized) P34 and P36 by adsorbing with
25 extracts containing distal half tail fibers or P34, gp35 and P37, respectively. An alternative is to raise antibody against purified tail fiber halves, e.g., P34 and gp35-P36-P37. Anti gp35-P36-P37 can then be adsorbed with P36-P37 to produce anti-gp35, and anti-P36 can be produced by
30 adsorption with P37 and gp35. Anti-P37, anti-gp35, and anti-P34 can also be produced directly by using purified P37, gp35, and P34 as immunogens. Another approach is to raise specific monoclonal antibodies against the different tail fiber components or segments thereof.

35 Specific antibodies to subunits or tail parts are used in any of the following ways to detect appropriately homodimerized tail fiber proteins: 1) Bacterial colonies are

screened for those expressing mature tail fiber proteins by directly transferring the colonies, or, alternatively, samples of lysed or unlysed cultures, to nitrocellulose filters, lysing the bacterial cells on the filter if
5 necessary, and incubating with specific antibodies. Formation of immune complexes is then detected by methods widely used in the art (e.g., secondary antibody conjugated to a chromogenic enzyme or radiolabelled Staphylococcal Protein A.). This method is particularly useful to screen
10 large numbers of colonies e.g., those produced by EXO-SIZE deletion as described above. 2) Bacterial cells expressing the protein of interest are first metabolically labelled with ³⁵S-methionine, followed by preparation of extracts and incubation with the antiserum. The immune complexes are then
15 recovered by incubation with immobilized Protein A followed by centrifugation, after which they may be resolved by SDS-polyacrylamide gel electrophoresis.

An alternative competitive assay for testing whether internally deleted tail fiber proteins that do not
20 permit phage infection nonetheless retain the ability to dimerize and associate with their appropriate partners utilizes an *in vitro*, complementation system. 1) A bacterial extract containing the modified protein of interest, as described above, is mixed with a second extract prepared from
25 cells infected with a T4 phage that is mutant in the gene of interest. 2) After several hours of incubation, a third extract is added that contains the wild-type version of the protein being tested, and incubation is continued for several additional hours. 3) Finally, the extract is titered for
30 infectious phage particles by infecting *E. coli* and quantifying the phage plaques that result. A modified tail fiber protein that is correctly dimerized and able to join with its partners is incorporated into tail fibers in a non-functional manner in Step 1, thereby preventing the
35 incorporation of the wild-type version of the protein in Step 2; the result is a reduction in the titer of the resulting phage sample. By contrast, if the modified protein is unable

to dimerize and thus form proper N- and/or C-termini, it will not be incorporated into phage particles in Step 1, and thus will not compete with assembly of intact phage particles in Step 2; the phage titer should thus be equivalent to that observed when no modified protein is added in Step 1 (a negative control.)

Another way in which to test whether chimeras and internally deleted tail fiber proteins retain the ability to dimerize and associate with their appropriate partners is done *in vivo*. The assay detects the ability of such chimeras and deleted proteins to compete with normal phage parts for assembly, thus reducing the burst size of a wild-type phage infecting the same host cell in which the chimeras or deleted proteins are recombinantly expressed. Thus, expression from an expression vector encoding the chimera or deleted protein is induced inside a cell, which cell is then infected by a wild-type phage. Inhibition of wild-type phage production demonstrates the ability of the recombinant chimera or protein to associate with the appropriate tail fiber proteins of the phage.

The above-described methods are used, alone and in combination, in the design and production of different types of modified tail fiber proteins. For example, a preliminary screen of a large number of bacterial colonies for those expressing a properly dimerized protein will identify positive colonies, which can then be individually tested by *in vitro* complementation.

Non-limiting examples of novel proteins that are encompassed by the present invention include:

- 1) Internally deleted gp34, 36, and 37 polypeptides (See Example 1 below);
- 2) A C-terminally truncated gp36 fused to the N-terminus of N-terminally truncated gp37;
- 3) A fusion between gp36 and gp37 in which gp37 is N-terminal to gp36 (*i.e.*, in reverse of the natural order), termed herein "gp37-36 chimera" (See Example 2 below);

- 4) A fusion between gp34 and gp36 in which gp36 is N-terminal to gp34 (i.e., in reverse of the natural order), termed herein "gp36-34 chimer";
- 5) A variant of gp36 in which the C-terminus is mutated such that it lacks the capability to interact with (and dimerize in response to) the N-terminus of wild-type P37, termed herein "gp36*";
- 6) A variant of gp37 in which the N-terminus is mutated such that it forms a P37 that lacks the capability to interact with the C-terminus of wild-type gp36, termed herein "*P37";
- 7) Variants of gp36* and *P37 that can interact with each other, but not with gp36 or P37.
- 8) A variant "P37-36 chimer" in which the gp36 moiety is derived from the variant as in 5), i.e., "P37-36*". (For 5-8, See Example 3 below.)
- 9) A variant "P37-36 chimer" in which the gp37 moiety is derived from the variant as in 6) above, i.e., "*P37-36".
- 10) A variant P37-36 chimer, *P37-P36*, in which the gp36 and gp37 moieties are derived from the variants in 7).
- 11) A fusion between gp36 and gp34 in which gp36 sequences are placed N-terminal to gp34, the dimer of which is termed herein "P36-34 chimer";
- 12) Variants of gp35 that form average angles different from 137° or 158° (the native angle) e.g., less than about 125° or more than about 145° under conditions wherein the wild-type gp35 protein forms an angle of 137° when combined with the P34 and P36-P37 dimers, and/or exhibit more or less flexibility than the native polypeptide;
- 13) Variants of gp34, 35, 36 and 37 that exhibit thermolabile interactions or other variant specific interactions with their cognate partners; and
- 14) Variants of gp37 in which the C-terminal domain of the polypeptide is modified to include sequences that confer specific binding properties on the entire

molecule, e.g., sequences derived from avidin that recognize biotin, sequences derived from immunoglobulin heavy chain that recognize Staphylococcal A protein, sequences derived from the Fab portion of the heavy chain of monoclonal
5 antibodies to which their respective Fab, light chain counterparts could attach and form an antigen-binding site, immunoactive sequences that recognize specific antibodies, or sequences that bind specific metal ions. These ligands may be immobilized to facilitate purification and/or assembly.

10 In specific embodiments, the chimers of the invention comprise a portion consisting of at least the first 10 (N-terminal) amino acids of a first tail fiber protein fused via a peptide bond to a portion consisting of at least the last 10 (C-terminal) amino acids of a second tail fiber
15 protein. The first and second tail fiber proteins can be the same or different proteins. In another embodiment, the chimers comprise an amino acid portion in the range of the first 10-60 amino acids from a tail fiber protein fused to an amino acid portion in the range of the last 10-60 amino acids
20 from a second tail fiber protein. In another embodiment, each amino acid portion is at least 20 amino acids of the tail fiber protein. The chimers comprise portions, i.e., not full-length tail fiber proteins, fused to one another. In a preferred aspect, the first tail fiber protein portion of the
25 chimera is from gp37, and the second tail fiber protein portion is from gp36. Such a chimera (gp37-36 chimera), after oligomerization to form P37-36, can polymerize to other identical oligomers. A gp36-34 chimera, after oligomerization to form P36-34, can bind to gp35, and this unit can then
30 polymerize. In another embodiment, the first portion is from gp37, and the second portion is from gp34. In a preferred aspect, the chimers of the invention are made by insertions or deletions within a β turn of the β structure of the tail fiber proteins. Most preferably, insertions into a tail
35 fiber sequence, or fusing to another tail fiber protein sequence, (preferably via manipulation at the recombinant DNA level to produce the desired encoded protein) is done so that

sequences in β turns on the same edge of the β -sheet are joined.

In addition to the above-described chimers, nanostructures of the invention can also comprise tail fiber
5 protein deletion constructs that are truncated at one end, e.g., are lacking an amino- or carboxy- end (of at least 5 or 10 amino acids) of the molecule. Such molecules truncated at the amino-terminus, e.g., of truncated gp37, gp34, or gp36, can be used to "cap" a nanostructure, since, once
10 incorporated, they will terminate polymerization. Such molecules preferably comprise a fragment of a tail fiber protein lacking at least the first 10, 20, or 60 amino terminal amino acids.

In order to change the length of the rod component
15 proteins as desired, portions of the same or different tail fiber proteins can be inserted into a tail fiber chimer to lengthen the rod, or be deleted from a chimer, to shorten the rod.

20 ASSEMBLY OF INDIVIDUAL ROD COMPONENTS INTO NANOSTRUCTURES

Expression of the proteins of the present invention in *E. coli* as described above results in the synthesis of large quantities of protein, and allows the simultaneous expression and assembly of different components in the same
25 cells. The methods for scale-up of recombinant protein production are straightforward and widely known in the art, and many standard protocols can be used to recover native and modified tail fiber proteins from a bacterial culture.

In a preferred embodiment, native (nonrecombinant)
30 gp35 is isolated for use by growing up a bacteriophage T4 having an amber mutation in gene 36, in a su^0 bacterial strain (not an amber suppressor), and isolating gp35 from the resulting culture by standard methods.

P34, P36-P37, P37, and chimers derived from them
35 are purified from *E. coli* cultures as mature dimers. Gp35 and variants thereof are purified as monomers. Purification is achieved by the following procedures or combinations thereof,

using standard methods: 1) chromatography on molecular sieve, ion-exchange, and/or hydrophobic matrices; 2) preparative ultracentrifugation; and 3) affinity chromatography, using as the immobilized ligand specific antibodies or other specific binding moieties. For example, the C-terminal domain of P37 binds to the lipopolysaccharide of *E. coli* B. Other T4-like phages have P37 analogues that bind other cell surface components such as OmpF or TSX protein. Alternatively, if the proteins have been engineered to include heterologous domains that act as ligands or binding sites, the cognate partner is immobilized on a solid matrix and used in affinity purification. For example, such a heterologous domain can be biotin, which binds to a streptavidin-coated solid phase.

Alternatively, several components are co-expressed in the same bacterial cells, and sub-assemblies of larger nanostructures are purified subsequent to limited *in vivo* assembly, using the methods enumerated above.

The purified components are then combined *in vitro* under conditions where assembly of the desired nanostructure occurs at temperatures between about 4°C and about 37°C, and at pHs between about 5 and about 9. For a given nanostructure, optimal conditions for assembly (*i.e.*, type and concentration of salts and metal ions) are easily determined by routine experimentation, such as by changing each variable individually and monitoring formation of the appropriate products.

Alternatively, one or more crude bacterial extracts may be prepared, mixed, and assembly reactions allowed to proceed prior to purification.

In some cases, one or more purified components assemble spontaneously into the desired structure, without the necessity for initiators. In other cases, an initiator is required to nucleate the polymerization of rods or sheets. This offers the advantage of localizing the assembly process (*i.e.*, if the initiator is immobilized or otherwise localized) and of regulating the dimensions of the final

structure. For example, rod components that contain a functional P36 C-terminus require a functional P37 N-terminus to initiate rod formation stoichiometrically; thus, altering the relative amount of initiator and rod component will influence the average length of rod polymer. If the ratio is n , the average rod will be approximately $(P37-36)n$ N-terminus P37-P37 C-terminus.

In still other cases, the final nanostructure is composed of two or more components that cannot self-assemble individually but only in combination with each other. In this situation, alternating cycles of assembly can be staged to produce final products of precisely defined structure (see Example 6B below.)

When an immobilized initiator is used, it may be desirable to remove the polymerized unit from the matrix after staged assembly. For this purpose specialized initiators are engineered so that the interaction with the first rod component is rendered reversibly thermolabile (see Example 5 below). In this way, the polymer can be easily separated from the matrix-bound initiator, thereby permitting: 1) easy preparation of stock solutions of uniform parts or subassemblies, and 2) re-use of the matrix-bound initiator for multiple cycles of polymer initiation, growth, and release.

In an embodiment in which a nanostructure is assembled that is attached to a solid matrix via gp34 (or P34), one way in which to detach the nanostructure to bring it into solution is to use a mutant (thermolabile) gp34 that can be made to detach upon exposure to a higher temperature (e.g., 40°C). Such a mutant gp34, termed T4 tsB45, having a mutation at its C-terminal end such that P34 attaches to the distal tail fiber half at 30°C but can be separated from it *in vitro* by incubation at 40°C in the presence of 1% SDS (unlike wild-type T4 which are stable under these conditions), has been reported (Seed, 1980, Studies of the Bacteriophage T4 Proximal Half Tail Fiber, Ph.D. Thesis, California Institute of Technology), and can be used.

Proteins which catalyze the formation of correct (lowest energy) stable secondary (2°) structure of proteins are called chaperone proteins. (Often, especially in globular proteins, this stabilization is aided by tertiary structure, e.g., stabilization of β -sheets by their interaction in β -barrels or by interaction with α -helices). Normally chaperonins prevent intrachain or interchain interactions which would produce untoward metastable folding intermediates and prevent or delay proper folding. There are two known accessory proteins, gp57 and gp38, in the morphogenesis of T4 phage tail fibers which are sometimes called chaperonins because they are essential for proper maturation of the protein oligomers but are not present in the final structures.

The usual chaperonin system (e.g., groEL/ES) interact with certain oligopeptide moieties of the gene product to prevent unwanted interactions with oligopeptide moieties elsewhere on the same polypeptide or another peptide. These would form metastable folding intermediates which retard or prevent proper folding of the polypeptide to its native (lower energy) state.

Gp57, probably in conjunction with some membrane protein(s), has the role of juxtaposing (and aligning) and/or initiating the folding of 2 or 3 identical gp37 molecules. The aligned peptides then zip up (while mutually stabilizing their nascent β -structures) to form a beam, without further interaction with gp57. Gp57 acts in T4 assembly not only for oligomerization of gp37 but also for gp34 and gp12.

30 STRUCTURAL COMPONENTS FOR SELF ASSEMBLY OF BEAMS IN VITRO

Alternatively to starting the polymerization of chimers with the use of a preformed chimeric or natural oligomeric unit called an initiator produced *in vivo*, molecules (preferably peptides) that can self-assemble can be produced as fusion proteins, fused to the N- or C-terminus of tail fiber variants of the invention (chimers, deletion/insertion constructs) to align their ends and thus

to facilitate their subsequent unaided folding into oligomeric, stable β -folded rod-like (beam) units *in vitro*, in the absence of the normally required chaperonin proteins (e.g., gp57) and host cell membrane proteins.

5 As an illustration, consider the P37 unit as an initiator of gp37-36 oligomerization and polymerization. Normally, proper folding of gp37 to a P37 initiator requires a phage infected cell membrane, and two chaperone proteins, gp38 and gp57. In a preferred embodiment, the need for gp38
10 can be obviated by use of a mutation, ts3813 (a duplication of 7 residues just downstream of the transition zone of gp37) which suppresses gene 38 (Wood, W.B., F.A. Eiserling and R.A. Crowther, 1994, "Long Tail Fibers: Genes, Proteins, Structure, and Assembly," in Molecular Biology of
15 Bacteriophage T4, (Jim D. Karam, Editor) American Society for Microbiology, Washington, D.C., pp 282-290). If a moiety that self-assembles into a dimer or trimer or other oligomer ("self-assembling moiety") is fused to a C-terminal deletion of gp37 downstream or upstream of the transition region [the
20 transition region is a conserved 17 amino acid residue region in T4-like tail fiber proteins where the structure of the protein narrows to a thin fiber; see Henning et al., 1994, "Receptor recognition by T-even-type coliphages," in Molecular Biology of Bacteriophage T4, Karam (ed.), American
25 Society for Microbiology, Washington, D.C., pp. 291-298; Wood et al., 1994, "Long tail fibers: Genes, proteins, structure, and assembly," in Molecular Biology of Bacteriophage T4, Karam (ed.), American Society for Microbiology, Washington, D.C., pp. 282-290], when it is expressed, the self-assembling
30 moiety will oligomerize in parallel and thus align the fused gp37 peptides, permitting them to fold *in vitro*, in the absence of other chaperonin proteins.

 If P37 is a dimer (Figure 8A), the self-assembling moiety can be a self dimerizing peptide such as the leucine
35 zipper, made from residues 250-281 from the yeast transcription factor, GCN4 (E.K. O'Shea, R.Rutkowski and P.S. Kim, Science 243:538, 1989) or the self dimerizing mutant

leucine zipper peptide, pIL in which the a positions are substituted with isoleucine and the d positions with leucine (Harbury P.B., T. Zhang, P.S. Kim and T. Alper. 1993. A Switch Between Two-, Three-, and Four-Stranded Coiled Coils in GCN4 Leucine Zipper Mutants. *Science*, 262:1401-1407). If P37 is a trimer (Figure 8B), the self-assembling moiety can be a self trimerizing mutant leucine zipper peptide, pII in which both the a and d positions are substituted with isoleucine (Harbury P.B., et al. *ibid*). Alternatively, a collagen peptide can be used as the self-assembling moiety, such as that described by Bella et al. (J. Bella, M. Eaton, B. Brodsky and H.M. Berman. 1994. Crystal and Molecular Structure of a Collagen-Like Peptide at 1.9Å Resolution. *Science*, 226:75-81), which self aligns by an inserted specific non repeating alanine residue near the center.

Self-assembling moieties can be used to make initiators for polymerizations in the absence of the normal initiators. For example, to create an initiator for oligomerization and polymerization of the chimeric monomer, gp37-36, gp37-36-C₂ can be used as illustrated in Figure 9. (C₂ means that a dimer forming peptide is fused to the C-terminus of the gp36 moiety. This is used if the beam is a dimeric structure. Otherwise C₃ -- a trimer forming peptide fused to the C-terminus -- would be used.) Furthermore, use of the *E. coli* lac repressor N-terminus, e.g., which associates as a tetramer, with two coils facing in each direction could join two dimers (or polymers of dimers) end to end, either at their N- or C-termini depending upon which end the self-assembling peptides were placed. They could also join N- to C- termini. In any case, alone, they could only form a dimer, each end of which would be extensible by adding an appropriate chimer monomer (as shown for the simpler case in Figure 9).

In an alternative embodiment, the self-assembling moiety can be fused to the N-termini of the chimera. In a specific embodiment, the self-assembling moiety is fused to

at least a 10 amino acid portion of a T-even-like tail fiber protein.

A self assembling moiety that assembles into a heteroligomer can also be used. For example, if
5 polymerization between beams is directed by the surface of a dimeric cross- β surface, addition of a heterodimeric unit with one surface which does not promote further polymerization would be very useful to cap the penultimate unit and thus terminate polymerization. If the two types of
10 coiled regions of the self-assembling moiety are much more attractive to each other than to themselves, then all of the dimers will be heterodimers. Such is the case for the N-terminal Jun and Fos leucine zipper regions.

A further advantage to such heterodimeric units is
15 the ability to stage polymerization and thus build one unit (or one surface in a 2D array) at a time. For example, suppose surface A attaches to B but neither attaches to itself ($[A \leftrightarrow B]$ is used to symbolize this type of interaction). Mix A/A and B/B₀ (B₀ is attached to a matrix
20 for easy purification). This will form B₀/B-A/A. Now wash out A/A and add B/B. The construct is now B₀/B-A/A-B/B. Now add A/A₀. The construct is now B₀/B-A/A-B/B-A/A₀, and no more beams can be added. There are of course many other possibilities.

25

APPLICATIONS

The uses of the nanostructures of the present invention are manifold and include applications that require highly regular, well-defined arrays of fibers, cages, or
30 solids, which may include specific attachment sites that allow them to associate with other materials.

In one embodiment, a three-dimensional hexagonal array of tubes is used as a molecular sieve or filter, providing regular vertical pores of precise diameter for
35 selective separation of particles by size. Such filters can be used for sterilization of solutions (i.e., to remove microorganisms or viruses), or as a series of

molecular-weight cut-off filters. In this case, the protein components of the pores may be modified so as to provide specific surface properties (i.e., hydrophilicity or hydrophobicity, ability to bind specific ligands, etc.).

5 Among the advantages of this type of filtration device is the uniformity and linearity of pores and the high pore to matrix ratio.

In another embodiment, long one-dimensional fibers are incorporated, for example, into paper or cement or
10 plastic during manufacture to provide added wet and dry tensile strength.

In still another embodiment, different nanostructure arrays are impregnated into paper and fabric as anti-counterfeiting markers. In this case, a simple
15 color-linked antibody reaction (such as those commercially available in kits) is used to verify the origin of the material. Alternatively, such nanostructure arrays could bind dyes or other substances, either before or after incorporation to color the paper or fabrics or modify their
20 appearance or properties in other ways.

KITS

The invention also provides kits for making nanostructures, comprising in one or more containers the
25 chimeras and deletion constructs of the invention. For example, one such kit comprises in one or more containers purified gp35 and purified gp36-34 chimera. Another such kit comprises purified gp37-36 chimera.

The following examples are intended to illustrate
30 the present invention without limiting its scope.

In the examples below, all restriction enzymes, nucleases, ligases, etc. are commercially available from numerous commercial sources, such as New England Biolabs (NEB), Beverly, MA; Life Technologies (GIBCO-BRL),
35 Gaithersburg, MD; and Boehringer Mannheim Corp. (BMC), Indianapolis, IN.

EXAMPLE 1**DESIGN, CONSTRUCTION AND EXPRESSION OF INTERNALLY DELETED P37**

The gene encoding gp37 contains two sites for the restriction enzyme Bgl II, the first cleavage occurring after 5 nucleotide 293 and the second after nucleotide 1486 (the nucleotides are numbered from the initiator methionine codon ATG.) Thus, digestion of a DNA fragment encoding gp37 with BglII, excision of the intervening fragment (nucleotides 294- 1485) and re-ligation of the 5' and 3' fragments results 10 in the formation of an internally deleted gp37, designated Δ P37, in which arginine-98 is joined with serine-497.

The restriction digestion reaction mix contains:

	gp37 plasmid DNA (1 μ g/ μ l)	2 μ l
15	NEB buffer #2 (10X)	1 μ l
	H ₂ O	6 μ l
	Bgl II (10 U/ μ l)	1 μ l

The gp37 plasmid signifies a pT7-5 plasmid into which gene 37 20 has been inserted in the multiple cloning site, downstream of a good ribosome binding site and of gene 57 to chaperon the dimerization. The reaction is incubated for 1h at 37°C.

Then, 89 μ l of T4 DNA ligase buffer and 1 μ l of T4 DNA ligase are added, and the reaction is continued at 16°C for 4 hours.

25 2 μ l of the Stu I restriction enzyme are then added, and incubation continued at 37°C for 1h. (The Stu I restriction enzyme digests residual plasmids that were not cut by Bgl II in the first step, reducing their transformability by about 100-fold.)

30 The reaction mixture is then transformed into *E. coli* strain BL21, obtained from Novagen, using standard procedures. The transformation mixture is plated onto nutrient agar containing 100 μ g/ml ampicillin, and the plates are incubated overnight at 37°C.

35 Colonies that appear after overnight incubation are picked, and plasmid DNA is extracted and digested with Bgl II as above. The restriction digests are resolved on 1% agarose

gels. A successful deletion is evidenced by the appearance after gel electrophoresis of a new DNA fragment of 4.2 kbp, representing the undeleted part of gene 37 which is still attached to the plasmid and which re-formed a BglII site by ligation. The 1.2 kbp DNA fragment bounded by BglII sites in the original gene is no longer in the plasmid and so is missing from the gel.

Plasmids selected for the predicted deletion as above are transformed into *E. coli* strain BL21(DE3).

- 10 Transformants are grown at 30°C until the density (A_{600}) of the culture reaches 0.6. IPTG is then added to a final concentration of 0.4 mM and incubation is continued at 30°C for 2h, after which the cultures are chilled on ice. 20 μ l of the culture is then removed and added to 20 μ l of a
- 15 two-fold concentrated "cracking buffer" containing 1% sodium dodecyl sulfate, glycerol, and tracking dye. 15 μ l of this solution are loaded onto a 10% polyacrylamide gel; a second aliquot of 15 μ l is first incubated in a boiling water bath for 3 min and then loaded on the same gel. After
- 20 electrophoresis, the gel is fixed and stained. Expression of the deleted gp37 is evidenced by the appearance of a protein species migrating at an apparent molecular mass of 65-70,000 daltons in the boiled sample. The extent of dimerization is suggested by the intensity of higher-molecular mass species
- 25 in the unboiled sample and/or by the disappearance of the 65-70,000 dalton protein band.

- The ability of the deleted polypeptide to dimerize appropriately is directly evaluated by testing its ability to be recognized by an anti-P37 antiserum that reacts only with
- 30 mature P37 dimers, using a standard protein immunoblotting procedure.

- An alternative assay for functional dimerization of the deleted P37 polypeptide (also referred to as Δ P37) is its ability to complement *in vivo* a T4 37⁻ phage, by first
- 35 inducing expression of the Δ P37 and then infecting with the T4 mutant, and detecting progeny phage.

A Δ P37 was prepared as described above, and found capable of complementing a T4 37⁻ phage *in vivo*.

EXAMPLE 2

5 DESIGN, CONSTRUCTION AND EXPRESSION OF A gp37-36 CHIMER

The starting plasmid for this construction is one in which the gene encoding gp37 is cloned immediately upstream (*i.e.*, 5') of the gene encoding gp36. The plasmid is digested with Hae III, which deletes the entire 3' region
10 of gp37 DNA downstream of nucleotide 724 to the 3' terminus, and also removes the 5' end of gp36 DNA from the 5' terminus to nucleotide 349. The reaction mixture is identical to that described in Example 1, except that a different plasmid DNA is used, and the enzyme is HaeIII. Ligation using T4 DNA
15 ligase, bacterial transformation, and restriction analysis are also performed as in Example 1. In this case, excision of the central portion of the gene 37-36 insert and religation reveals a novel insert of 346 in-frame codons, which is cut only once by HaeIII (after nucleotide 725). The
20 resulting construct is then expressed in *E. coli* BL21(DE3) as described in Example 1.

Successful expression of the gp37-36 chimera is evidenced by the appearance of a protein product of about 35,000 daltons. This protein will have the first 242
25 N-terminal amino acids of gp37 fused to the final 104 C-terminal amino acids of gp36 (numbered 118-221.) The utility of this chimera depends upon its ability to dimerize and attach end-to-end. That is, carboxy termini of said polypeptide will have the capability of interacting with the
30 amino terminus of the P37 protein dimer of bacteriophage T4 and to form an attached dimer, and the amino terminus of the dimer of said polypeptide will have the capability of interacting with other said chimera polypeptides. This property can be tested by assaying whether introduction of
35 Δ P37 initiates dimerization and polymerization. Alternatively, polyclonal antibodies specific to P36 dimer

may be used to detect P36 subsequent to initiation of dimerization by Δ P37.

A gp37-36 chimera was prepared similarly to the procedures described above, except that the restriction enzyme *TaqI* was used instead of *HaeIII*. Briefly, the 5' fragment resulting from *TaqI* digestion of gene 37 was ligated to the 3' fragment resulting from *TaqI* digestion of gene 36. This produced a construct encoding a gp37-36 chimera in which amino acids 1-48 of gp37 were fused to amino acids 100-221 of gp36. This construct was expressed in *E. coli* BL21(DE3), and the chimera was detected as an 18 kD protein. This gp37-36 chimera was found to inhibit the growth of wild type T4 when expression of the gp37-36 chimera was induced prior to infection (in an *in vitro* phage inhibition assay).

15

EXAMPLE 3

MUTATION OF THE GP37-36 CHIMER TO PRODUCE COMPLEMENTARY SUPPRESSORS

The goal of this construction is to produce two variants of a dimerizable P37-36 chimera: One in which the N-terminus of the polypeptide is mutated (A, designated *P37-36) and one in which the C-terminus of the polypeptide is mutated (B, designated P37-36*). The requirement is that the mutated *P37 N-terminus cannot form a joint with the wild-type P36 C-terminus, but only with the mutated *P36 N-terminus. The rationale is that A and B each cannot polymerize independently (as the parent P37-36 protein can), but can only associate with each other sequentially (i.e., $P37-36^* + *P37-36 \rightarrow P37-36^*---*P37-36$).

A second construct, *p37-P36*, is formed by recombining *P37-36 and P37-36* *in vitro*. When the monomers *gp37-36* and gp37-36 are mixed in the presence of P37 initiator, gp37-36 would dimerize and polymerize to (P37-36)_n; similarly, *P37 would only catalyze the polymerization of *gp37-36* to (*P37-36*)_n. In this case, the two chimeras could be of different size and different primary sequence with different potential side-group

interactions, and could initiate attachment at different surfaces depending on the attachment specificity of P37.

The starting bacterial strain is a *su^o* strain of *E. coli* (which lacks the ability to suppress amber mutations).

5 When this strain is infected with a mutant T4 bacteriophage containing amber mutations in genes 35, 36, and 37, phage replication is incomplete, since the tail fiber proteins cannot be synthesized. When this strain is first transformed with a plasmid that directs the expression of the wild type
10 gp35, gp36 and gp37 genes and induced with IPTG, and subsequently infected with mutant phage, infectious phage particles are produced; this is evidenced by the appearance of "nibbled" colonies. Nibbled colonies do not appear round, with smooth edges, but rather have sectors missing. This is
15 caused by attack of a microcolony by a single phage, which replicates and prevents the growth of the bacteria in the missing sector.

For the purposes of this construction, the 3'-terminal region of gene 36 (corresponding to the
20 C-terminal region of gp36) is mutagenized with randomly doped oligonucleotides. Randomly doped oligonucleotides are prepared during chemical synthesis of oligonucleotides, by adding a trace amount (up to a few percent) of the other three nucleotides at a given position, so that the resulting
25 oligonucleotide mix has a small percentage of incorrect nucleotides at that position. Incorporation of such oligonucleotides into the plasmid will result in random mutations (Hutchison et al., Methods.Enzymol. 202:356, 1991).

The mutagenized population of plasmids (containing,
30 however, unmodified genes 36 and 37), is then transformed into the *su^o* bacteria, followed by infection with the mutant T4 phage as above. In this case, the appearance of non-"nibbled" colonies indicates that the mutated gp36 C-termini can no longer interact with wild type P37 to form
35 functional tail fibers. The putative gp36* phenotypes found in such non-nibbled colonies are checked for lack of dimeric N-termini by appropriate immunospecificity as outlined above,

and positive colonies are used as source of plasmid for the next step.

Several of these mutated plasmids are recovered and subjected to a second round of mutagenesis, this time using 5 doped oligonucleotides that introduce random mutations into the N-terminal region of gp37 present on the same plasmid. Again, the (now doubly) mutagenized plasmids are transformed into the supo strain of *E. coli* and transformants are infected with the mutant T4 phage. At this stage, bacterial 10 plates are screened for the re-appearance of "nibbled" colonies. A nibbled colony at this stage indicates that the phage has replicated by virtue of suppression of the non-functional gp36* mutation(s) by the *P37 mutation. In other words, such colonies must contain novel *P37 15 polypeptides that have now acquired the ability to interact with the P36* proteins encoded on the same plasmid.

The *P37-36 and P37-36* paired suppressor chimeras (A and B as above) are then constructed in the same manner as described in Example 2. In this case, however, *P37 is used 20 in place of wild type P37 and P36* is used in place of wild type P36. A *P37-36* chimera can now be made by restriction of *P37-36 and P37-36* and religation in the recombined order. The *P37-36* can be mixed with the P37-36 chimera, and the polymerization of each can be accomplished independently 25 in the presence of the other. This is useful when the rod-like central portion of these chimeras have been modified in different ways.

EXAMPLE 4

30 DESIGN, CONSTRUCTION AND EXPRESSION OF A gp36-34 CHIMER

The starting plasmid for this construction is one in which the vector containing gene 57 and the gene encoding gp36 is cloned immediately upstream (i.e., 5') of the gene encoding gp34. The plasmid is digested with NdeI, which cuts 35 after bp 219 of gene 36 and after bp 2594 of gene 34, thereby deleting the final 148 C-terminal codons from the pg36 moiety and the first 865 N-terminal codons from the gp34 moiety.

The reaction mixture is identical to that described in Example 1, except that a different plasmid DNA is used, and the enzyme used is NdeI (NEB). Ligation using T4 DNA ligase, bacterial transformation, and restriction analysis are also performed as in Example 1. This results in a new hybrid gene encoding a protein of 497 amino acids (73 N-terminal amino acids of gp36 and 424 C-terminal amino acids of gp34, numbered 866-1289.)

As an alternative, the starting plasmid is cut with SphI at bp 648 in gene 34, and the Exo-Size Deletion Kit (NEB) is used to create deletions as described above.

The resulting construct is then expressed in *E. coli* BL21(DE3) as described in Example 1. Successful expression of the gp36-34 chimera is evidenced by the appearance of a protein product of about 55,000 daltons. Preferably, the amino termini of the polypeptide homodimer have the capability of interacting with the gp35 protein, and then the carboxy termini have the capability of interacting with other attached gp35 molecules. Successful formation of the dimer can be detected by reaction with anti-P36 antibodies or by attachment of gp35 or by the *in vitro* phage inhibition assay described in Example 2.

EXAMPLE 5

ISOLATION OF THERMOLABILE PROTEINS FOR SELF-ASSEMBLY

Thermolabile structures can be utilized in nanostructures for: a) initiation of chimera polymerization (e.g., gp37-36) at low temperature and subsequent inactivation of and separation from the initiator at high temperature; b) initiation of angle formation between P36 and gp35 (e.g., variants of gp35 that have thermolabile attachment sites for P36 N-termini or P34 C-termini, a variant P36 that forms a thermolabile attachment to gp35, and a variant P34 with a thermolabile C-terminal attachment site.) Thermolability may be reversible, permitting reattachment of the appropriate termini when the lower temperature is restored, or it may be irreversible.

To create a variant gp37 that permits heat induced separation of the P36 -- P37 junction, the 5' end of gp37 DNA is randomly mutagenized using doped oligonucleotides as described above. The mutagenized DNA fragment is then
5 recombined into T4 phage by infection of the cell containing the mutagenized DNA by a T4 phage containing two amber mutations flanking the mutagenized region. Following a low-multiplicity infection, non-amber phage are selected at low temperature on *E. coli* Su^o at 30°C. The progeny of these
10 plaques are resuspended in buffered and challenged by heating at 60°C. At this temperature, wild-type tail fibers remain intact and functional, whereas the thermolabile versions release the terminal P37 units and thus render those phage non-infectious.

15 At this stage, wild type phage are removed by: 1) adsorbing the wild type phage to sensitive bacteria and sedimenting (or filtering out) the bacteria with the adsorbed wild type phage; or 2) reacting the lysate with anti-P37 antibody, followed by immobilized Protein A and removal of
20 adsorbed wild type phage. Either method leaves the noninfectious mutant phage particles in the supernatant fluid or filtrate, from which they can be recovered. The non-infectious phage lacking terminal P37 moieties (and probably the rest of the tail fibers as well) are then urea
25 treated with 6M urea, and mixed with bacterial spheroplasts to permit infection at low multiplicity whereupon they replicate at low temperature and release progeny. Alternatively, infectious phage are reconstituted by *in vitro* incubation of the mutant phage with wild type P37 at 30°C;
30 this is followed by infection of intact bacterial cells using the standard protocol. The latter method of infection specifically selects mutant phage in which the thermolability of the P36-P37 junction is reversible.

Using either method, the phage populations are
35 subjected to multiple rounds of selection as above, after which individual phage particles are isolated by plaque purification at 30°C. Finally, the putative mutants are

evaluated individually for the following characteristics:

- 1) loss of infectivity after incubation at high temperatures (40-60°C), as measured by a decrease in titer; 2) loss of P37 after incubation at high temperature, as measured by decrease
- 5 in binding of P37-specific antibody to phage particles; and
- 3) morphological changes in the tail fibers after incubation at high temperatures, as assessed by electron microscopy.

After mutants are isolated and their phenotypes confirmed, the P37 gene is sequenced. If the mutations

10 localize to particular regions or residues, those sequences are targeted for site-directed mutagenesis to optimize the desired characteristics.

Finally, the mutant gene 37 is cloned into expression plasmids and expressed individually in *E. coli* as

15 in Example 1. The mutant P37 dimers are then purified from bacterial extracts and used in *in vitro* assembly reactions.

In a similar fashion, mutant gp35 polypeptides can be isolated that exhibit a thermolabile interaction with the N-terminus of P36 or the C-terminus of P34. For thermolabile

20 interaction with P34, phage are incubated at high temperature, resulting in the loss of the entire distal half of the tail fiber (i.e., gp35-P36-P37). The only difference in the experimental protocol is that, in this case, 1) random mutagenesis is performed over the entire gp35 gene; 2) wild-

25 type phage (and distal half-fibers from thermolabile mutants) are separated from thermolabile mutant phage that have been inactivated at high temperature (but still have proximal half tail fibers attached) by precipitating both the distal half-fibers and the phage particles containing intact tail fibers

30 with any of the anti-distal half tail-fiber antibodies followed by Staphylococcal A-protein beads; 3) the mutant phage remaining in the supernatant are reactivated by incubation at low temperature with bacterial extracts containing wild type intact distal half fibers; and 4) stocks

35 of thermolabile gene, 35 mutants grown at 30°C can be tested for reversible thermolability by inactivation at 60°C and reincubation at 30°C. Inactivation is performed on a

concentrated suspension of phage, and reincubation at 30°C is performed either before or after dilution. If phage are successfully reactivated before, but not after, dilution, this indicates that their gp35 is reversibly thermolabile.

5 To create a gene 36 mutation with a thermolabile gp35--P36 linkage, the C-terminus of gene 36 is mutagenized as described above, and the mutant selected for reversibility. An alternative is to mutagenize gp35 to create a gene 35 mutant in which the gp35-P36 linkage will
10 dissociate at 60°C. In this case, incubation with anti-gp35 antibodies can be used to precipitate the phage without P36-P37 and thus to separate them from the wild-type phage and distal half-tail fibers (P36-P37), since the variant gp35 will remain attached to P34.

15

EXAMPLE 6

ASSEMBLY OF ONE-DIMENSIONAL RODS

A. Simple Assembly: The P37-36 chimera described in Example 2 is capable of self-assembly, but requires a P37
20 initiator to bind the first unit of the rod. Therefore, a P37 or a ΔP37 dimer is either attached to a solid matrix or is free in solution to serve as an initiator. If the initiator is, attached to a solid matrix, a thermolabile P37 dimer is preferably used. Addition of an extract containing
25 gp37-36, or the purified gp37-36 chimera, results in the assembly of linear multimers of increasing length. In the matrix-bound case, the final rods are released by a brief incubation at high temperature (40-60°C, depending on the characteristics of the particular thermolabile P37 variant.)

30 The ratio of initiator to gp37-36 can be varied, and the size distribution of the rods is measured by any of the following methods: 1) Size exclusion chromatography; 2) Increase in the viscosity of the solution; and 3) Direct measurement by electron microscopy.

35 B. Staged assembly: The P37-36 variants *P37-36 and P37-36* described in Example 3 cannot self-polymerize.

This allows the staged assembly of rods of defined length, according to the following protocol:

1. Attach initiator P37 (preferably thermolabile) to a matrix.
- 5 2. Add excess *gp37-36 to attach and oligomerize as P37-36 homooligomers to the N-terminus of P37.
3. Wash out unreacted *gp37-36 and flood with gp37-36*.
4. Wash out unreacted gp37-36* and flood with
10 excess *gp37-36.
5. Repeat steps 2-4, n-1 times.
6. Release assembly from matrix by brief incubation at high temperature as above.

The linear dimensions of the protein rods in the
15 batch will depend upon the lengths of the unit heterochimers and the number of cycles (n) of addition. This method has the advantage of insuring absolute reproducibility of rod length and a homogenous, monodisperse size distribution from one preparation to another.

20

EXAMPLE 7

STAGED ASSEMBLY OF POLYGONS

The following assembly strategy utilizes gp35 as an angle joint to allow the formation of polygons. For the
25 purpose of this example, the angle formed by gp35 is assumed to be 137°. The rod unit comprises the P36-34 chimera described in Example 4, which is incapable of self-polymerization. The P36-34 homodimer is made from a bacterial clone in which both gp36-34 and gp57 are expressed.
30 The gp57 can chaperone the homodimerization of gp36-34 to P36-34.

1. Initiator: The incomplete distal half fiber P36-37 is attached to a solid matrix by the P37 C-terminus. Thermolabile gp35 as described in Example 5 is then added to
35 form the intact initiator.

2. Excess P36-34 chimera is added to attach a single P36-34. Following binding to the matrix via gp35, the unbound chimera is washed out.

3. Wild-type (i.e., non-thermolabile) gp35 is then added in excess. After incubation, the unbound material is washed out.

4. Steps 2 and 3 are repeated 7-8 times.

5. The assembly is released from the matrix by brief incubation at high temperature.

10 The released polymeric rod, 8 units long, will form a regular 8-sided polygon, whose sides comprise the P36-34 dimer and whose joints comprise the wild-type gp35 monomer. However, there will be some multimers of these 8 units bound as helices. When a unit does not close, but
15 instead adds another to its terminus, the unit cannot close further and the helix can build in either direction. The direction of the first overlap also determines the handedness of the helix. Ten (or seven)-unit rods may form helices more frequently than polygons since their natural angles are 144°
20 (or 128.6°). The likelihood of closure of a regular polygon depends not only on the average angle of gp35 but also on its flexibility, which can be further manipulated by genetic or environmental modification.

The type of polygon that is formed using this
25 protocol depends upon the length of rod units and the angle formed by the angle joint. For example, alternating rod units of different sizes can be used in step 2. In addition, variant gp35 polypeptides that form angles different than the natural angle of 137° can be used, allowing the formation of
30 different regular polygons. Furthermore, for a given polygon with an even number of sides and equal angles, the sides in either half can be of any size provided the two halves are symmetric.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Goldberg, Edward B.
- (ii) TITLE OF INVENTION: MATERIALS FOR THE PRODUCTION OF NANOMETER STRUCTURES AND USE THEREOF
- (iii) NUMBER OF SEQUENCES: 6
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: Pennie and Edmonds
 - (B) STREET: 1155 Avenue of the Americas
 - (C) CITY: New York
 - (D) STATE: New York
 - (E) COUNTRY: US
 - (F) ZIP: 10036
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER: To be Assigned
 - (B) FILING DATE: 13-OCT-1995
 - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Misrock, S. Leslie
 - (B) REGISTRATION NUMBER: 18,872
 - (C) REFERENCE/DOCKET NUMBER: 8471-0005-999
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: (212) 790-9090
 - (B) TELEFAX: 212-869-8864
 - (C) TELEX: 66441 PENNIE

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 8855 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Bacteriophage T4
- (vii) IMMEDIATE SOURCE:
 - (B) CLONE: TAIL FIBER GENES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1289 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Bacteriophage T4

(vii) IMMEDIATE SOURCE:

- (B) CLONE: p34 amino acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Ala Phe Asn Ser Gly Arg Trp Arg Ala Leu Arg Thr Asp Ala Asn Trp
85          90          95
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100         105         110
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 420 425 430
 Asp Ser Leu Asn Asp Ser Thr Arg Ala Arg Leu Gly Val Ile Ala Leu
 435 440 445
 Ala Thr Gln Ala Gln Ala Asn Val Asp Leu Glu Asn Ser Pro Gln Lys
 450 455 460
 Glu Leu Ala Ile Thr Pro Glu Thr Leu Ala Asn Arg Thr Ala Thr Glu
 465 470 475 480
 Thr Arg Arg Gly Ile Ala Arg Ile Ala Thr Thr Ala Gln Val Asn Gln
 485 490 495
 Asn Thr Thr Phe Ser Phe Ala Asp Asp Ile Ile Ile Thr Pro Lys Lys

-46-

Pro Tyr Glu Leu Asn Arg Val Leu Ala Asn Tyr Leu Pro Leu Lys Ala
 865 870 875 880
 Lys Ala Ala Asp Thr Asn Leu Leu Asp Gly Leu Asp Ser Ser Gln Phe
 885 890 895
 Ile Arg Arg Asp Ile Ala Gln Thr Val Asn Gly Ser Leu Thr Leu Thr
 900 905 910
 Gln Gln Thr Asn Leu Ser Ala Pro Leu Val Ser Ser Ser Thr Gly Glu
 915 920 925
 Phe Gly Gly Ser Leu Ala Ala Asn Arg Thr Phe Thr Ile Arg Asn Thr
 930 935 940
 Gly Ala Pro Thr Ser Ile Val Phe Glu Lys Gly Pro Ala Ser Gly Ala
 945 950 955 960
 Asn Pro Ala Gln Ser Met Ser Ile Arg Val Trp Gly Asn Gln Phe Gly
 965 970 975
 Gly Gly Ser Asp Thr Thr Arg Ser Thr Val Phe Glu Val Gly Asp Asp
 980 985 990
 Thr Ser His His Phe Tyr Ser Gln Arg Asn Lys Asp Gly Asn Ile Ala
 995 1000 1005
 Phe Asn Ile Asn Gly Thr Val Met Pro Ile Asn Ile Asn Ala Ser Gly
 1010 1015 1020
 Leu Met Asn Val Asn Gly Thr Ala Thr Phe Gly Arg Ser Val Thr Ala
 1025 1030 1035 1040
 Asn Gly Glu Phe Ile Ser Lys Ser Ala Asn Ala Phe Arg Ala Ile Asn
 1045 1050 1055
 Gly Asp Tyr Gly Phe Phe Ile Arg Asn Asp Ala Ser Asn Thr Tyr Phe
 1060 1065 1070
 Leu Leu Thr Ala Ala Gly Asp Gln Thr Gly Gly Phe Asn Gly Leu Arg
 1075 1080 1085
 Pro Leu Leu Ile Asn Asn Gln Ser Gly Gln Ile Thr Ile Gly Glu Gly
 1090 1095 1100
 Leu Ile Ile Ala Lys Gly Val Thr Ile Asn Ser Gly Gly Leu Thr Val
 1105 1110 1115 1120
 Asn Ser Arg Ile Arg Ser Gln Gly Thr Lys Thr Ser Asp Leu Tyr Thr
 1125 1130 1135
 Arg Ala Pro Thr Ser Asp Thr Val Gly Phe Trp Ser Ile Asp Ile Asn
 1140 1145 1150
 Asp Ser Ala Thr Tyr Asn Gln Phe Pro Gly Tyr Phe Lys Met Val Glu
 1155 1160 1165
 Lys Thr Asn Glu Val Thr Gly Leu Pro Tyr Leu Glu Arg Gly Glu Glu
 1170 1175 1180
 Val Lys Ser Pro Gly Thr Leu Thr Gln Phe Gly Asn Thr Leu Asp Ser
 1185 1190 1195 1200
 Leu Tyr Gln Asp Trp Ile Thr Tyr Pro Thr Thr Pro Glu Ala Arg Thr
 1205 1210 1215
 Thr Arg Trp Thr Arg Thr Trp Gln Lys Thr Lys Asn Ser Trp Ser Ser

1220	1225	1230
Phe Val Gln Val Phe Asp Gly Gly Asn Pro Pro Gln Pro Ser Asp Ile		
1235	1240	1245
Gly Ala Leu Pro Ser Asp Asn Ala Thr Met Gly Asn Leu Thr Ile Arg		
1250	1255	1260
Asp Phe Leu Arg Ile Gly Asn Val Arg Ile Val Pro Asp Pro Val Asn		
1265	1270	1275
1280		
Lys Thr Val Lys Phe Glu Trp Val Glu		
1285		

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 65 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Bacteriophage T4
- (vii) IMMEDIATE SOURCE:
 - (B) CLONE: ORF X amino acid
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Met	Glu	Lys	Phe	Met	Ala	Glu	Ile	Trp	Thr	Arg	Ile	Cys	Pro	Asn	Ala
1				5					10					15	
Ile	Leu	Ser	Glu	Ser	Asn	Ser	Val	Arg	Tyr	Lys	Ile	Ser	Ile	Ala	Gly
		20						25					30		
Ser	Cys	Pro	Leu	Ser	Thr	Ala	Gly	Pro	Ser	Tyr	Val	Lys	Phe	Gln	Asp
		35					40					45			
Asn	Pro	Val	Gly	Ser	Gln	Thr	Phe	Arg	Arg	Arg	Pro	Ser	Phe	Lys	Ser
		50				55					60				
Phe															
65															

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 295 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Bacteriophage T4
- (vii) IMMEDIATE SOURCE:
 - (B) CLONE: p35 amino acid
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met	Leu	Phe	Arg	Leu	Gln	Met	Ile	Leu	His	Gln	Leu	Leu	Leu	Leu	Val
1				5				10						15	

Phe Met Asn Ser Leu Thr Asn Asn Arg Ile Val Ala Ile Leu Thr Ser
 20 25 30
 Gly Lys Val Asn Phe Pro Pro Glu Val Val Ser Trp Leu Arg Thr Ala
 35 40 45
 Gly Thr Ser Ala Phe Pro Ser Asp Ser Ile Leu Ser Arg Phe Asp Val
 50 55 60
 Ser Tyr Ala Ala Phe Tyr Thr Ser Ser Lys Arg Ala Ile Ala Leu Glu
 65 70 75 80
 His Val Lys Leu Ser Asn Arg Lys Ser Thr Asp Asp Tyr Gln Thr Ile
 85 90 95
 Leu Asp Val Val Phe Asp Ser Leu Glu Asp Val Gly Ala Thr Gly Phe
 100 105 110
 Pro Arg Arg Thr Tyr Glu Ser Val Glu Gln Phe Met Ser Ala Val Gly
 115 120 125
 Gly Thr Asn Asn Glu Ile Ala Arg Leu Pro Thr Ser Ala Ala Ile Ser
 130 135 140
 Lys Leu Ser Asp Tyr Asn Leu Ile Pro Gly Asp Val Leu Tyr Leu Lys
 145 150 155 160
 Ala Gln Leu Tyr Ala Asp Ala Asp Leu Leu Ala Leu Gly Thr Thr Asn
 165 170 175
 Ile Ser Ile Arg Phe Tyr Asn Ala Ser Asn Gly Tyr Ile Ser Ser Thr
 180 185 190
 Gln Ala Glu Phe Thr Gly Gln Ala Gly Ser Trp Glu Leu Lys Glu Asp
 195 200 205
 Tyr Val Val Val Pro Glu Asn Ala Val Gly Phe Thr Ile Tyr Ala Gln
 210 215 220
 Arg Thr Ala Gln Ala Gly Gln Gly Gly Met Arg Asn Leu Ser Phe Ser
 225 230 235 240
 Glu Val Ser Arg Asn Gly Gly Ile Ser Lys Pro Ala Glu Phe Gly Val
 245 250 255
 Asn Gly Ile Arg Val Asn Tyr Ile Cys Glu Ser Ala Ser Pro Pro Asp
 260 265 270
 Ile Met Val Leu Pro Thr Gln Ala Ser Ser Lys Thr Gly Lys Val Phe
 275 280 285
 Gly Gln Glu Phe Arg Glu Val
 290 295

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 221 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Bacteriophage T4

(vii) IMMEDIATE SOURCE:

(B) CLONE: p36 amino acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Met Ala Asp Leu Lys Val Gly Ser Thr Thr Gly Gly Ser Val Ile Trp
 1 5 10 15
 His Gln Gly Asn Phe Pro Leu Asn Pro Ala Gly Asp Asp Val Leu Tyr
 20 25 30
 Lys Ser Phe Lys Ile Tyr Ser Glu Tyr Asn Lys Pro Gln Ala Ala Asp
 35 40 45
 Asn Asp Phe Val Ser Lys Ala Asn Gly Gly Thr Tyr Ala Ser Lys Val
 50 55 60
 Thr Phe Asn Ala Gly Ile Gln Val Pro Tyr Ala Pro Asn Ile Met Ser
 65 70 75 80
 Pro Cys Gly Ile Tyr Gly Gly Asn Gly Asp Gly Ala Thr Phe Asp Lys
 85 90 95
 Ala Asn Ile Asp Ile Val Ser Trp Tyr Gly Val Gly Phe Lys Ser Ser
 100 105 110
 Phe Gly Ser Thr Gly Arg Thr Val Val Ile Asn Thr Arg Asn Gly Asp
 115 120 125
 Ile Asn Thr Lys Gly Val Val Ser Ala Ala Gly Gln Val Arg Ser Gly
 130 135 140
 Ala Ala Ala Pro Ile Ala Ala Asn Asp Leu Thr Arg Lys Asp Tyr Val
 145 150 155 160
 Asp Gly Ala Ile Asn Thr Val Thr Ala Asn Ala Asn Ser Arg Val Leu
 165 170 175
 Arg Ser Gly Asp Thr Met Thr Gly Asn Leu Thr Ala Pro Asn Phe Phe
 180 185 190
 Ser Gln Asn Pro Ala Ser Gln Pro Ser His Val Pro Arg Phe Asp Gln
 195 200 205
 Ile Val Ile Lys Asp Ser Val Gln Asp Phe Gly Tyr Tyr
 210 215 220

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1026 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Bacteriophage T4

(vii) IMMEDIATE SOURCE:

- (B) CLONE: p37 amino acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Ala Thr Leu Lys Gln Ile Gln Phe Lys Arg Ser Lys Ile Ala Gly
 1 5 10 15

Thr Arg Pro Ala Ala Ser Val Leu Ala Glu Gly Glu Leu Ala Ile Asn
 20 25 30
 Leu Lys Asp Arg Thr Ile Phe Thr Lys Asp Asp Ser Gly Asn Ile Ile
 35 40 45
 Asp Leu Gly Phe Ala Lys Gly Gly Gln Val Asp Gly Asn Val Thr Ile
 50 55 60
 Asn Gly Leu Leu Arg Leu Asn Gly Asp Tyr Val Gln Thr Gly Gly Met
 65 70 75 80
 Thr Val Asn Gly Pro Ile Gly Ser Thr Asp Gly Val Thr Gly Lys Ile
 85 90 95
 Phe Arg Ser Thr Gln Gly Ser Phe Tyr Ala Arg Ala Thr Asn Asp Thr
 100 105 110
 Ser Asn Ala His Leu Trp Phe Glu Asn Ala Asp Gly Thr Glu Arg Gly
 115 120 125
 Val Ile Tyr Ala Arg Pro Gln Thr Thr Thr Asp Gly Glu Ile Arg Leu
 130 135 140
 Arg Val Arg Gln Gly Thr Gly Ser Thr Ala Asn Ser Glu Phe Tyr Phe
 145 150 155 160
 Arg Ser Ile Asn Gly Gly Glu Phe Gln Ala Asn Arg Ile Leu Ala Ser
 165 170 175
 Asp Ser Leu Val Thr Lys Arg Ile Ala Val Asp Thr Val Ile His Asp
 180 185 190
 Ala Lys Ala Phe Gly Gln Tyr Asp Ser His Ser Leu Val Asn Tyr Val
 195 200 205
 Tyr Pro Gly Thr Gly Glu Thr Asn Gly Val Asn Tyr Leu Arg Lys Val
 210 215 220
 Arg Ala Lys Ser Gly Gly Thr Ile Tyr His Glu Ile Val Thr Ala Gln
 225 230 235 240
 Thr Gly Leu Ala Asp Glu Val Ser Trp Trp Ser Gly Asp Thr Pro Val
 245 250 255
 Phe Lys Leu Tyr Gly Ile Arg Asp Asp Gly Arg Met Ile Ile Arg Asn
 260 265 270
 Ser Leu Ala Leu Gly Thr Phe Thr Thr Asn Phe Pro Ser Ser Asp Tyr
 275 280 285
 Gly Asn Val Gly Val Met Gly Asp Lys Tyr Leu Val Leu Gly Asp Thr
 290 295 300
 Val Thr Gly Leu Ser Tyr Lys Lys Thr Gly Val Phe Asp Leu Val Gly
 305 310 315 320
 Gly Gly Tyr Ser Val Ala Ser Ile Thr Pro Asp Ser Phe Arg Ser Thr
 325 330 335
 Arg Lys Gly Ile Phe Gly Arg Ser Glu Asp Gln Gly Ala Thr Trp Ile
 340 345 350
 Met Pro Gly Thr Asn Ala Ala Leu Leu Ser Val Gln Thr Gln Ala Asp
 355 360 365
 Asn Asn Asn Ala Gly Asp Gly Gln Thr His Ile Gly Tyr Asn Ala Gly

370	375	380
Gly Lys Met Asn His Tyr Phe Arg Gly Thr Gly Gln Met Asn Ile Asn 385 390 395 400		
Thr Gln Gln Gly Met Glu Ile Asn Pro Gly Ile Leu Lys Leu Val Thr 405 410 415		
Gly Ser Asn Asn Val Gln Phe Tyr Ala Asp Gly Thr Ile Ser Ser Ile 420 425 430		
Gln Pro Ile Lys Leu Asp Asn Glu Ile Phe Leu Thr Lys Ser Asn Asn 435 440 445		
Thr Ala Gly Leu Lys Phe Gly Ala Pro Ser Gln Val Asp Gly Thr Arg 450 455 460		
Thr Ile Gln Trp Asn Gly Gly Thr Arg Glu Gly Gln Asn Lys Asn Tyr 465 470 475 480		
Val Ile Ile Lys Ala Trp Gly Asn Ser Phe Asn Ala Thr Gly Asp Arg 485 490 495		
Ser Arg Glu Thr Val Phe Gln Val Ser Asp Ser Gln Gly Tyr Tyr Phe 500 505 510		
Tyr Ala His Arg Lys Ala Pro Thr Gly Asp Glu Thr Ile Gly Arg Ile 515 520 525		
Glu Ala Gln Phe Ala Gly Asp Val Tyr Ala Lys Gly Ile Ile Ala Asn 530 535 540		
Gly Asn Phe Arg Val Val Gly Ser Ser Ala Leu Ala Gly Asn Val Thr 545 550 555 560		
Met Ser Asn Gly Leu Phe Val Gln Gly Gly Ser Ser Ile Thr Gly Gln 565 570 575		
Val Lys Ile Gly Gly Thr Ala Asn Ala Leu Arg Ile Trp Asn Ala Glu 580 585 590		
Tyr Gly Ala Ile Phe Arg Arg Ser Glu Ser Asn Phe Tyr Ile Ile Pro 595 600 605		
Thr Asn Gln Asn Glu Gly Glu Ser Gly Asp Ile His Ser Ser Leu Arg 610 615 620		
Pro Val Arg Ile Gly Leu Asn Asp Gly Met Val Gly Leu Gly Arg Asp 625 630 635 640		
Ser Phe Ile Val Asp Gln Asn Asn Ala Leu Thr Thr Ile Asn Ser Asn 645 650 655		
Ser Arg Ile Asn Ala Asn Phe Arg Met Gln Leu Gly Gln Ser Ala Tyr 660 665 670		
Ile Asp Ala Glu Cys Thr Asp Ala Val Arg Pro Ala Gly Ala Gly Ser 675 680 685		
Phe Ala Ser Gln Asn Asn Glu Asp Val Arg Ala Pro Phe Tyr Met Asn 690 695 700		
Ile Asp Arg Thr Asp Ala Ser Ala Tyr Val Pro Ile Leu Lys Gln Arg 705 710 715 720		
Tyr Val Gln Gly Asn Gly Cys Tyr Ser Leu Gly Thr Leu Ile Asn Asn 725 730 735		

Gly Asn Phe Arg Val His Tyr His Gly Gly Gly Asp Asn Gly Ser Thr
 740 745 750
 Gly Pro Gln Thr Ala Asp Phe Gly Trp Glu Phe Ile Lys Asn Gly Asp
 755 760 765
 Phe Ile Ser Pro Arg Asp Leu Ile Ala Gly Lys Val Arg Phe Asp Arg
 770 775 780
 Thr Gly Asn Ile Thr Gly Gly Ser Gly Asn Phe Ala Asn Leu Asn Ser
 785 790 795 800
 Thr Ile Glu Ser Leu Lys Thr Asp Ile Met Ser Ser Tyr Pro Ile Gly
 805 810 815
 Ala Pro Ile Pro Trp Pro Ser Asp Ser Val Pro Ala Gly Phe Ala Leu
 820 825 830
 Met Glu Gly Gln Thr Phe Asp Lys Ser Ala Tyr Pro Lys Leu Ala Val
 835 840 845
 Ala Tyr Pro Ser Gly Val Ile Pro Asp Met Arg Gly Gln Thr Ile Lys
 850 855 860
 Gly Lys Pro Ser Gly Arg Ala Val Leu Ser Ala Glu Ala Asp Gly Val
 865 870 875 880
 Lys Ala His Ser His Ser Ala Ser Ala Ser Ser Thr Asp Leu Gly Thr
 885 890 895
 Lys Thr Thr Ser Ser Phe Asp Tyr Gly Thr Lys Gly Thr Asn Ser Thr
 900 905 910
 Gly Gly His Thr His Ser Gly Ser Gly Ser Thr Ser Thr Asn Gly Glu
 915 920 925
 His Ser His Tyr Ile Glu Ala Trp Asn Gly Thr Gly Val Gly Gly Asn
 930 935 940
 Lys Met Ser Ser Tyr Ala Ile Ser Tyr Arg Ala Gly Gly Ser Asn Thr
 945 950 955 960
 Asn Ala Ala Gly Asn His Ser His Thr Phe Ser Phe Gly Thr Ser Ser
 965 970 975
 Ala Gly Asp His Ser His Ser Val Gly Ile Gly Ala His Thr His Thr
 980 985 990
 Val Ala Ile Gly Ser His Gly His Thr Ile Thr Val Asn Ser Thr Gly
 995 1000 1005
 Asn Thr Glu Asn Thr Val Lys Asn Ile Ala Phe Asn Tyr Ile Val Arg
 1010 1015 1020
 Leu Ala
 1025

What is claimed is:

1. An isolated polypeptide consisting essentially of the gp37 tail fiber protein of bacteriophage T4 lacking
5 amino acids 99-496 (SEQ ID NO:6) when numbered from the amino terminus, wherein said polypeptide has the capability to form dimers and interact with the P36 protein oligomer of bacteriophage T4.

10 2. An isolated polypeptide consisting essentially of a fusion protein between the gp36 and gp37 proteins of bacteriophage T4, wherein amino acid residues 1-242 of gp37 (SEQ ID NO:6) are fused in proper reading frame to amino acid residues 118-221 of gp36 (SEQ ID NO:5).

15 3. The polypeptide of claim 2 wherein a plurality of carboxy termini of said polypeptide have the capability of interacting with the amino terminus of the P37 protein oligomer of bacteriophage T4 and to form an attached oligomer
20 and the amino termini of the oligomer of said polypeptide have the capability of interacting with the carboxy termini of gp36 polypeptides of bacteriophage T4.

4. An isolated polypeptide oligomer consisting
25 essentially of two gp37 polypeptides of bacteriophage T4, wherein the amino termini of said oligomer lack the capability of interacting with the carboxy termini of gp36 polypeptides of bacteriophage T4.

30 5. An isolated polypeptide oligomer consisting essentially of the P37 protein of bacteriophage T4, wherein the amino termini of said oligomer lack the capability of interacting with the carboxy termini of gp36 polypeptides of bacteriophage T4.

35 6. An isolated polypeptide consisting essentially of a variant of the gp36 protein of bacteriophage T4, wherein



said polypeptide lacks the capability of interacting with the amino terminus of the P37 protein oligomer of bacteriophage T4.

5 7. An isolated polypeptide consisting essentially of a fusion protein between the gp36 and gp34 proteins of bacteriophage T4, wherein amino acid residues 1-73 of gp36 (SEQ ID NO:5) are fused in proper reading frame amino-terminal to amino acid residues 866-1289 of gp34 (SEQ
10 ID NO:2).

8. An oligomer of the polypeptide of claim 7, wherein the amino termini of said dimer have the capability of interacting with the gp35 protein of bacteriophage T4.

15

9. An isolated polypeptide consisting essentially of a variant of the gp35 protein of bacteriophage T4, wherein said polypeptide forms an angle of less than about 125° when combined with the P34 and P36-P37 protein oligomers of
20 bacteriophage T4, under conditions wherein the wild-type gp35 protein forms an angle of 137° when combined with said oligomers.

10. An isolated polypeptide consisting essentially
25 of a variant of the gp35 protein of bacteriophage T4, wherein said polypeptide forms an angle of more than about 145° when combined with the P34 and P36-P37 protein oligomers of bacteriophage T4, under conditions wherein the wild-type gp35 protein forms an angle of 137° when combined with said
30 oligomers.

11. An isolated polypeptide consisting essentially of a variant of the gp35 protein of bacteriophage T4, wherein the interaction of said polypeptide with the P34 protein
35 oligomer of bacteriophage T4 is unstable at temperatures between about 40°C and about 60°C.



12. An isolated polypeptide oligomer consisting essentially of a variant of the P37 protein of bacteriophage T4, wherein the interaction of said oligomer with the P36 protein oligomer of bacteriophage T4 is unstable at
5 temperatures between about 40°C and about 60°C.

13. An isolated polypeptide oligomer consisting essentially of a variant of the P37 protein of bacteriophage T4, wherein the carboxy-terminal domain of said oligomer is
10 modified so as to confer the ability of the entire polypeptide to bind specifically to an immobilized ligand.

14. The polypeptide of claim 13, wherein said ligand is selected from the group consisting of biotin,
15 immunoglobulin, or divalent metal ions.

15. A nanostructure comprising a plurality of fusion proteins, said fusion proteins comprising a first portion consisting of at least the first 10 N-terminal amino
20 acids of a tail fiber protein fused via a peptide bond to a second portion consisting of at least the last 10 C-terminal amino acids of a second tail fiber protein, wherein the tail fiber proteins are selected from the group consisting of gp34, gp35, gp36, and gp37 proteins of a T-even-like
25 bacteriophage, wherein the first and second tail fiber proteins are the same or different.

16. The nanostructure of claim 15, wherein the first and second tail fiber proteins are different.
30

17. The nanostructure of claim 15, which further comprises a molecule that can self-assemble into a dimer or trimer, fused to at least a 10 amino acid portion of a T-even-like tail fiber protein.
35

18. The nanostructure of claim 17, wherein the molecule has the structure of a leucine zipper.



19. The nanostructure of claim 15, wherein said nanostructure comprises a linear one-dimensional rod.

20. The nanostructure of claim 15, wherein said nanostructure comprises a polygon.

21. The nanostructure of claim 15, wherein said nanostructure comprises a three-dimensional cage or solid.

22. The nanostructure of claim 15, wherein said nanostructure comprises a two-dimensional open or closed sheet.

23. An isolated fusion protein consisting essentially of a portion of a gp37 protein of a T-even-like bacteriophage consisting of at least the first 10-60 N-terminal amino acids of the gp37 protein fused to a second portion of a gp36 protein of a T-even-like bacteriophage consisting of at least the last 10-60 C-terminal amino acids of the gp36 protein.

24. An isolated fusion protein consisting essentially of a portion of a gp37 protein of a T-even-like bacteriophage consisting of at least the first 10 N-terminal amino acids of the gp37 protein fused to a second portion of a gp36 protein of a T-even-like bacteriophage consisting of at least the last 10 C-terminal amino acids of the gp36 protein.

25. An isolated fusion protein consisting essentially of a portion of a gp37 protein of a T-even-like bacteriophage consisting of at least the first 20 N-terminal amino acids of the gp37 protein fused to a second portion of a gp36 protein of a T-even-like bacteriophage consisting of at least the last 20 C-terminal amino acids of the gp36 protein.



26. An isolated fusion protein consisting essentially of a portion of a gp36 protein of a T-even-like bacteriophage consisting of at least the first 10-60 N-terminal amino acids of the gp36 protein fused to a second
5 portion of a gp34 protein of a T-even-like bacteriophage consisting of at least the last 10-60 C-terminal amino acids of the gp34 protein.

27. An isolated protein comprising at least 20
10 contiguous amino acids of the gp37, gp36, or gp34 protein of a T-even-like bacteriophage, and lacking at least 5 amino acids of the amino- or carboxy-terminus of the protein.

28. An isolated DNA encoding the polypeptide of
15 claim 1.

29. An isolated DNA encoding the polypeptide of
claim 2.

20 30. An isolated DNA encoding the polypeptide of
claim 4.

31. An isolated DNA encoding the polypeptide of
claim 5.
25

32. An isolated DNA encoding the polypeptide of
claim 6.

33. An isolated DNA encoding the polypeptide of
30 claim 7.

34. An isolated DNA encoding the polypeptide of
claim 9.

35 35. An isolated DNA encoding the polypeptide of
claim 10.



36. An isolated DNA encoding the polypeptide of claim 11.
37. An isolated DNA encoding the polypeptide of claim 12.
38. An isolated DNA encoding the polypeptide of claim 13.
39. An isolated DNA encoding the protein of claim 23.
40. An isolated DNA encoding the protein of claim 25.
41. An isolated DNA encoding the protein of claim 26.
42. An isolated DNA encoding the protein of claim 27.
43. A method for making a polygonal nanostructure comprising contacting the protein of claim 26 with purified gp35 proteins of a T-even-like bacteriophage.
44. A method for making a nanostructure comprising contacting a plurality of the proteins of claim 23 with each other.
45. A kit comprising in one or more containers the fusion protein of claim 23.
46. A kit comprising in one or more containers the fusion protein of claim 25.
47. A kit comprising in one or more containers the fusion protein of claim 26.



48. A kit comprising in one or more containers the fusion protein of claim 26, and an isolated gp35 protein of a T-even-like bacteriophage.

5 49. The protein of claim 23 wherein the T-even-like bacteriophage is T4.

50. The protein of claim 26 wherein the T-even-like bacteriophage is T4.

10

51. An isolated polypeptide consisting essentially of a variant of the gp36 protein of bacteriophage T4, wherein the interaction of said polypeptide with the P37 protein oligomer of bacteriophage T4 is unstable at temperatures
15 between about 40°C and about 60°C.

52. An isolated polypeptide consisting essentially of a variant of the gp36 protein of bacteriophage T4, wherein the interaction of said polypeptide with the gp35 protein of
20 bacteriophage T4 is unstable at temperatures between about 40°C and about 60°C.

53. An isolated polypeptide consisting essentially of a variant of the gp34 protein of bacteriophage T4, wherein
25 the interaction of said polypeptide with the gp35 protein of bacteriophage T4 is unstable at temperatures between about 40°C and about 60°C.

30

35

