Scalable Architecture for Nanotechnology

Introduction

This white paper outlines the development of a flexible and scalable platform for nanotechnology that utilizes a combination of top-down and bottom-up self-assembly methods. A key feature of the technology is the utilization of engineered proteins, polymers that are able to spontaneously self-assemble to organize thousands of atoms with atomic precision, as basic structural components. It is shown how a flexible set of architectural components can be engineered to allow assembly of an essentially infinite set of designed nanostructures with one, two, and three-dimensional organization. The proposed technology can provide a flexible platform for assembling a wide range of novel materials and devices. The approaches outlined are under active development at Imiplex, a company founded to create and develop protein-based nanotechnology.

Nanotechnology: What is It?

Nanotechnology generally refers the fabrication of materials that have structural features on the nanometer scale. A nanometer (nm) is $1x10^{-9}$ meters, the scale of atomic structures, and substantially less than the wavelength of visible light (~400 nM). The ultimate challenge in nanotechnology is the development of scalable manufacturing methods that can organize and control assembly processes from the nanoscale to the macroscale.

Why is Nanotechnology Potentially Useful?

Miniaturization: The efficiency of many electronic or other devices ultimately devolves on maximizing the density with which discrete components can be manufactured on an appropriate substrate. Silicon-based semiconductor fabrication technology has continuously progressed over the last 40 years, resulting in current generation integrated circuits with 40 nm features. Although there have been many (incorrect) predictions that the practical limits of device miniaturization had been reached, many believe that pushing device densities beyond the latest generation may be subject to fundamental physical limitations (International Technology Roadmap for Semiconductors (http://www.itrs.net/reports.html)).

Emergence of New Properties: There is considerable interest in exploiting the emergence of novel physical properties that arise in very small systems for the development of new kinds of functional devices. New properties can range from effects that stem from the statistical or quantum characteristics that emerge in very small systems, to novel physical characteristics that emerge through the interaction of light with structures having dimensions less than optical wavelengths.

Biological System Interfaces: One of the most exciting possibilities for nanotechnology involves the development of materials or devices that can directly interact with biological systems and/or directly incorporate biological macromolecules as intrinsic structural or functional components. Virtually all of the functionality and interaction specificity of biological systems is manifest in the chemical features of macromolecules structurally organized at the nanoscale, principally proteins and nucleic acids, which are macromolecules respectively specialized for structure-function and information storage and processing roles.

The Central Problem in Nanotechnology

A key impediment to the practical realization of useful nanodevices is manufacturing scalability. In the context of engineered nanostructures, two different aspects of scalability are important. The first aspect involves the ability to manufacture structures using a parallel manufacturing process. For example, early conceptions of nanostructure fabrication –

motivated by studies demonstrating the ability to manipulate single atoms using atomic force microscopy - envisioned that devices could be assembled essentially atom-by-atom using molecular scale atomic manipulators (Drexler, 1992). However, this approach is impractical for large scale production owing to the time required to perform the thousands, if not millions of serial assembly steps required for each nanostructure. Although the economic importance of nanotechnology has been clearly recognized in the US (http://www.nano.gov/), the "Precise and arbitrary manipulation and positioning of large numbers of nanostructures" remains the number one "Difficult Problem in Nanotechnology": (http://www.mitre.org/tech/nanotech/nanosystems/problems.html).

As outlined below, it seems technically feasible that the combination of a top-down approach, able to specify the position of few nucleation sites, coupled with a self-assembly process around a limited number of assembly nucleii, can produce highly engineered nanostructures in a parallel production mode.

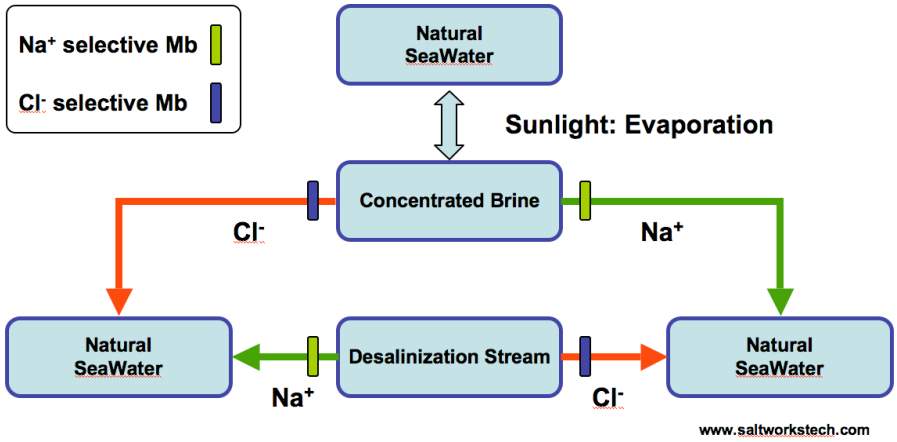

Figure 1. A desalinization "circuit" driven by sunlight (www.saltworkstech.com). The ultimate efficiency of the scheme depends on the density and selectivity of the ion-specific permeable membranes used to separate the charged ion flows. Several proteins have been identified with properties potentially useful in such applications.

A second aspect of scalability applies to the use of nanostructures for large-scale applications where material costs will be an important factor for successful commercialization. Examples include systems incorporating functional nano-membranes for water purification or desalinization (**Fig 1**). Such systems would be optimally developed from membrane structures organized on the nanoscale, but would have to allow production on a large scale to provide useful capacity. **Figure 2** illustrates a nanomembrane design that incorporates an anion-selective protein (pdb code 1e54, www.rcsb.org), just one of many proteins found in microorganisms that act as channels to unidirectionally conduct specific ions or molecules. Additional applications requiring large-scale surface assemblies could include energy storage devices or photovoltaic cells. Continuing advances in protein expression technology, particularly using renewable sources like green plants, may reduce engineered protein costs to a few dollars a pound, making such large-scale applications economically feasible.

Why Protein-Based Nanotechnology?

Proteins are polymers of amino acids (chiral polyamide derivatives) that spontaneously fold to form uniquely organized, tightly packed structures that self-organize thousands of atoms with atomic precision over ranges of 1 to 100 nm, and manifest a wide range of structural, catalytic, binding, and signal transduction properties. Proteins are readily modified through alteration of their encoding DNA sequence using modern tools of molecular biology. Recent worldwide initiatives to expand the knowledge of structural folds

formed by proteins has created a database of over 50,000 diverse structures (www.rcsb.org), many derived from extremophile organisms whose proteins function at temperatures approaching 100^{O}C or in other hostile environments. Thermostable proteins, whose 3D structures are known at atomic resolution through protein crystallography, form the basis for the engineered protein architecture under development at Imiplex.

As outlined above, and illustrated in the 2D planar nanomembrane of **Figure 2**, many nanostructural assemblies based on engineered proteins directly incorporate the functional properties that have evolved in nature for the individual components. In addition to forming structural elements, naturally occurring proteins exemplify many practical binding, catalytic, and signal transduction functions, including chemomechanical, electromechanical, optomechanical, and optoelectronic mechanisms, and frequently form hierarchical assemblies of great structural and functional complexity that exemplify the potential for protein-based nanotechnology.

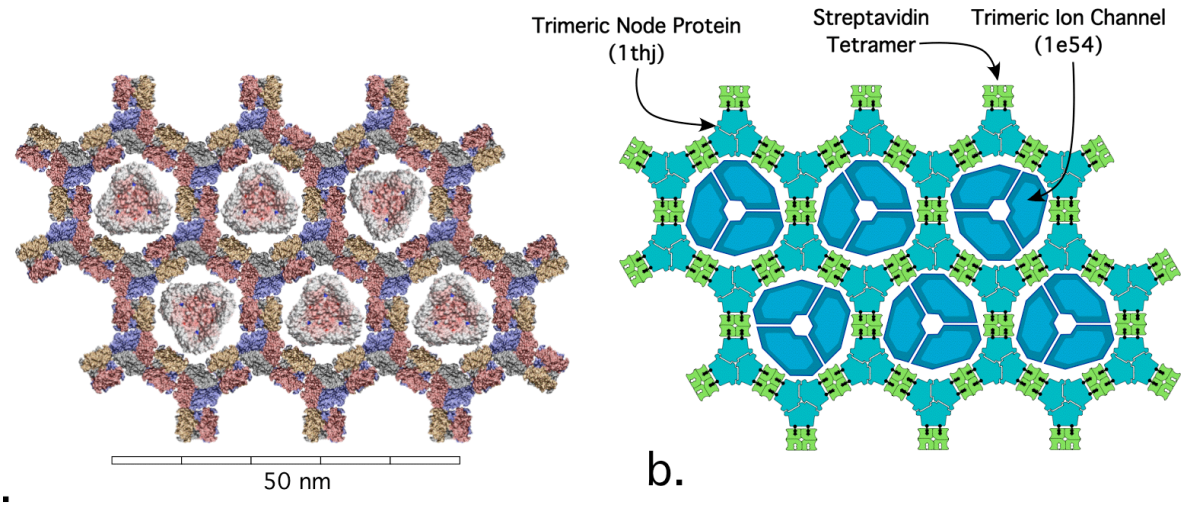

Figure 2. A hypothetical desalinization membrane. Part a. shows molecular images and part b. schematically shows construction features. The hexagonal grid support framework is assembled from C3-symmetric "node" trimers (pdb code: 1thj) chemically interlinked with streptavidin tetramers. The holes in the grid can accommodate numerous ion- and molecule-selective pore proteins such as the anion-selective channel pdb code: 1e54(www.rcsb.org).

Technology Development at Imiplex

Imiplex's objective is to develop a set of engineered protein components to broadly enable the fabrication of materials and devices with nanoscale structural features and organization.

As outlined in **Figure 3**, there are two limiting end-uses for nanostructures assembled with proteins. In one limiting application (Part C), the protein nanostructures are integral parts of functional biomaterials or devices. In a second limiting application (Part D), the protein nanostructures serve as a resist or template, enabling the patterning of other materials. It is also possible to envision hybrid structures that incorporate nanostructures using a combination of these methods (Part E).

The basic engineering strategy involves the development of a set of modular molecular building blocks generated by modification of protein molecules and complexes found in nature. Individual protein molecules are amino acid polymers that are encoded by DNA and synthesized by the protein synthesis machinery of living organisms. Proteins spontaneously fold from a linear polymer to form specific and compactly-packed three-dimensional structures that organize thousands of atoms with atomic precision.

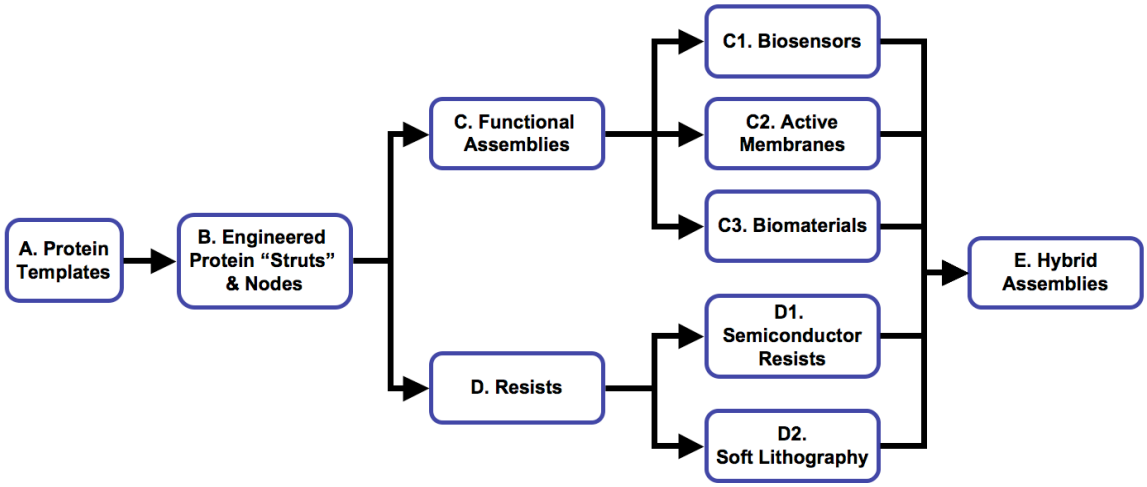

Figure 3. Scalable Architecture for Nanotechnology. The basic concept is to use known 3D structures of thermostable proteins (A) and methods of protein engineering (computer modeling, site specific modification, etc.) as the basis for creating a flexible set of nanoscale components (B) that can be assembled into 1, 2, and 3-dimensional structures (e.g. See Fig 6). Assembled nanostructures could have two limiting end use applications. One application set (C) includes functional assemblies, where the underlying protein architecture has been modified, e.g. through the attachment of immunoglobulins, binding molecules, etc. so that the protein architecture forms a functional biological interface. Potential applications include biosensors for pathogen and/or disease marker diagnosis, active membranes for water purification or filtration, substrates for biomaterials such as artificial skin, cartilage, and bone. The second set of applications (D) simply envisions using the protein-based nanoarchitecture as a resist or patterning agent that can be used to pattern semiconductor materials or coated with metal. The protein nanoarchitecture is used for initial patterning, but is subsequently removed from the assembly by etching or heating. The patterns formed using protein nanoarchitecture can provide a step in a semiconductor fabrication process or be used to generate stamps using soft lithography. Hybrid structures (E), are also possible, where e.g. substrate nanostructures are produced with soft lithography and then coated with functional protein nanoarchitectures.

The ability to easily manipulate the amino acid sequences of proteins and express them in non-native protein expression systems is the basis for the biotechnology revolution. Over 50,000 3D protein structures have been determined using X-ray crystallography and NMR, providing an extensive set of structural and functional molecular components as starting points for device design (www.rcsb.org). As a result of worldwide structural genomics programs, a substantial number of know protein structures have been derived from extremophiles, organisms that live in extreme environments of temperature, pH, or salinity, whose proteins are unusually stable, offering advantages both in protein production and ease with which they can be successfully engineered or modified chemically without adverse effects on protein 3D structural integrity. Extensive computational methods have been developed to model protein structures, while the tools of recombinant DNA technology enable the synthesis of virtually any polypeptide sequence or functional domain fusion, providing the basis for rapidly designing and optimizing novel assemblies from engineered biological macromolecules.

From an architectural standpoint, the basic engineering strategy involves the development of molecular components termed "struts" and "nodes". Struts and nodes are engineered protein molecules that provide the underlying architecture for the devices and materials ultimately manufactured using the technology. Struts are basically linear structural elements, while nodes are generally protein structures or assemblies with Cn rotational or 3-dimensional point group symmetry.

A practical approach to the development of a first-generation architecture is to use struts that incorporate streptavidin, a 60K MW protein tetramer (~4nm x 5nm x 6nm) with D2 symmetry that incorporates 4 high-affinity (Kd~10^{-14}) biotin binding sites oriented

approximately as the legs of an "H". Nodes are site-modified proteins with plane or point group symmetry (typically modified forms of protein multimers) that incorporate covalently bound biotin groups that are pairwise-complementary to the biotin binding sites on streptavidin, and are designed to allow the assembly of 1D, 2D, and ultimately 3D structures with defined geometrical organization.

Ringler and Schulz (2003) first demonstrated streptavdin-linked nanostructures produced by engineering a C4-symmetric protein tetramer to incorporate cysteine residues as sites of biotinylation for connection to streptavidin, potentially allowing formation of 2D square lattices. In addition, the protein tetramers incorporated chemical groups that anchored them to phospholipid molecules able to freely diffuse in a two-dimensional self-assembling membrane (SAM), creating a system allowing the spontaneous formation of streptavidin-linked 2D square lattices. A retrospective analysis of the Ringler and Schulz work showed that there were geometrical inaccuracies in the orientation of the site-specific modifications introduced into the C4 tetramer that would be expected to introduce twist into lattices otherwise constrained to lie on a 2D surface. Equally significant, the investigators reported that it was extremely difficult to control assembly conditions owing to the essential irreversibility of the streptavidin:biotin interaction. As a result, Ringler and Schulz could only assemble relatively small 2D lattices at their prototype stage of technology development.

Imiplex's development of the streptavidin-linked nanostructure concept as a *precision manufacturing technology* requires both improved node design, as well as development of new methods allowing sequential control of assembly steps.

Node Architecture

As noted above, Imiplex has focused on using thermostable proteins for its streptavidin-linked node:strut architectures owing to the generally improved stability of such proteins.

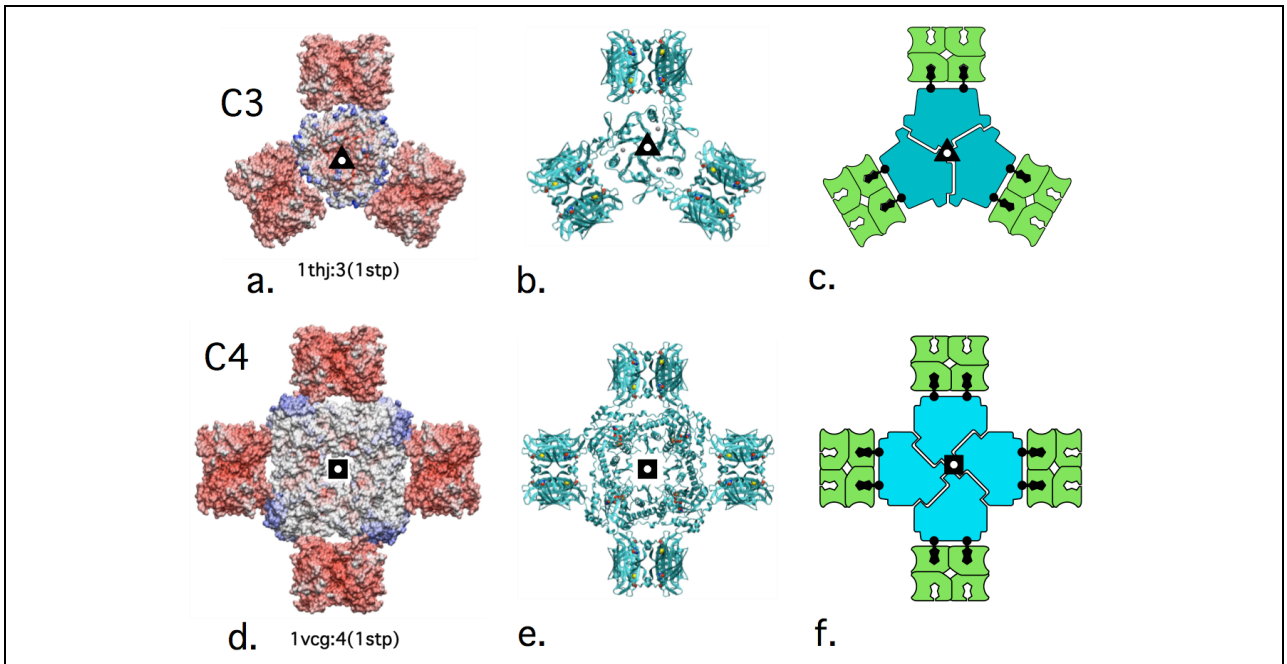

Figure 4. C3 and C4-Symmetric Nodes and Streptavidin Complexes. Top row shows C3-symmetric node:streptavidin complexes in space filling molecular representation (a.), polypeptide backbone ribbon representation (b.), and schematic representation (c.). The protein used as the template for the C3 node is the carbonic anhydrase from the thermophilic microorganism *Methanosarcina thermophila* (pdb code 1thj). Bottom row shows corresponding C4-symmetric node:streptavidin complexes (d.e.f). The C4 template node protein shown is the IPP isomerase from the thermophilic microorganism *Thermus thermophilus* (pdb code 1vcg). 1stp is the Protein Data Bank (pdb) code for streptavidin.

Figure 4 shows examples and schematics C3 and C4-symmetric node structures based on thermostable protein multimers complexed with streptavidin. These structures have been designed and expressed with two biotinylation sites per subunit and terminal polyhistidine tags that can be linked to Ni-chelated phospholipids, making them suitable for the assembly of infinite two-dimensional nano-lattices (e.g. See **Figure 6**, 2D lattices) on self assembling monolayers. Application of protein engineering methods to the Cn nodes to connect the individual chains into a single polypeptide chain, allowing the generation of nodes with reduced ligation number (**Figure 5**), greatly expands the variety of structures that can be generated (e.g. See **Figure 6**).

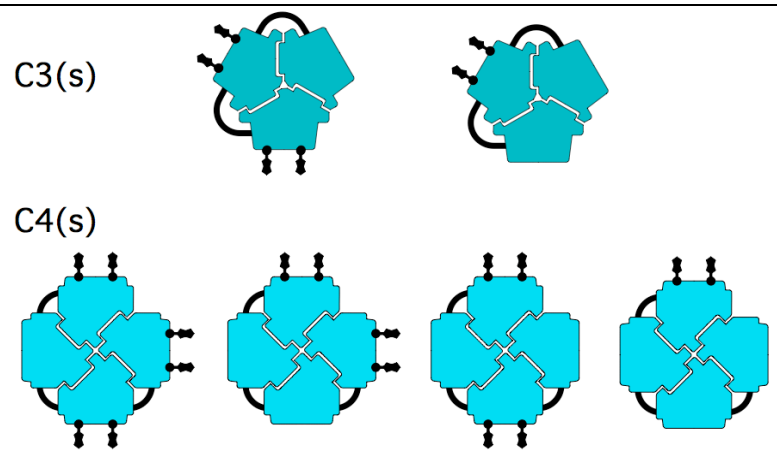

Figure 5. Single Chain C3 and C4-Symmetric Nodes with Reduced Ligation. In many cases it is possible, using protein engineering and recombinant DNA methods, to connect subunits of symmetric node structures into a single polypeptide chain. This allows a reduction in the number of biotinylation sites in a structure, resulting in an expanded set of node structures with defined geometry and ligation number for attached streptavidin struts. Top row shows C3 symmetric single-chain structures, including a 120 degree divalent node connector and a monovalent cap. Bottom row shows various possibilities for C4 single-chain ligation number and geometry. Imiplex is developing these single chain constructs from the template proteins outlined in Figure 4.

Owing to recent efforts in protein structural genomics funded by the NIH, there are numerous thermostable, symmetric, protein multimers that have been structurally characterized with 3,4,5, and 6-fold symmetry potentially suitable for use as node "templates" that can be modified by the introduction of biotin binding sites. These include multimers with Cn symmetry suitable for forming 2D structures, as well as multimers with Dn, tetrahedral, octahedral and higher symmetries suitable for building 3-dimensional assemblies. Several Cn-nodes have already been engineered and expressed at Imiplex, including single-chain nodes with sequences engineered to interconnect multimer subunits.

As outlined in **Figure 6**, a wide variety of structures can be envisioned with one, two, and three-dimensional geometry as determined by the geometry and symmetry of the nodes used to interconnect struts. In many of the structures shown, all of the nodes used in the nanostructure assembly are equivalent, leading to the formation of 1D, 2D, and 3D infinite lattices, closed planar polygons, and polyhedra. Generally, structures with nodes having Cn rotational symmetry (or corresponding single-chain constructs) are appropriate for structure formation 2D surfaces and are additionally engineered to incorporate a surface-binding amino acid sequence. For example, addition of a poly-histidine sequence at one of the protein chain termini can allow attachment to metal surfaces or to metal-chelated phospholipids imbedded in bilayers to allow 2D diffusional self-assembly on self assembling monolayers (SAMs).

The diversity of potentially accessible structures is enormously expanded when nodes of different symmetry and/or node attachment geometry can be combined within a specific nanostructure (e.g. the 2D and 3D "radial" structures in **Figure 6**.)

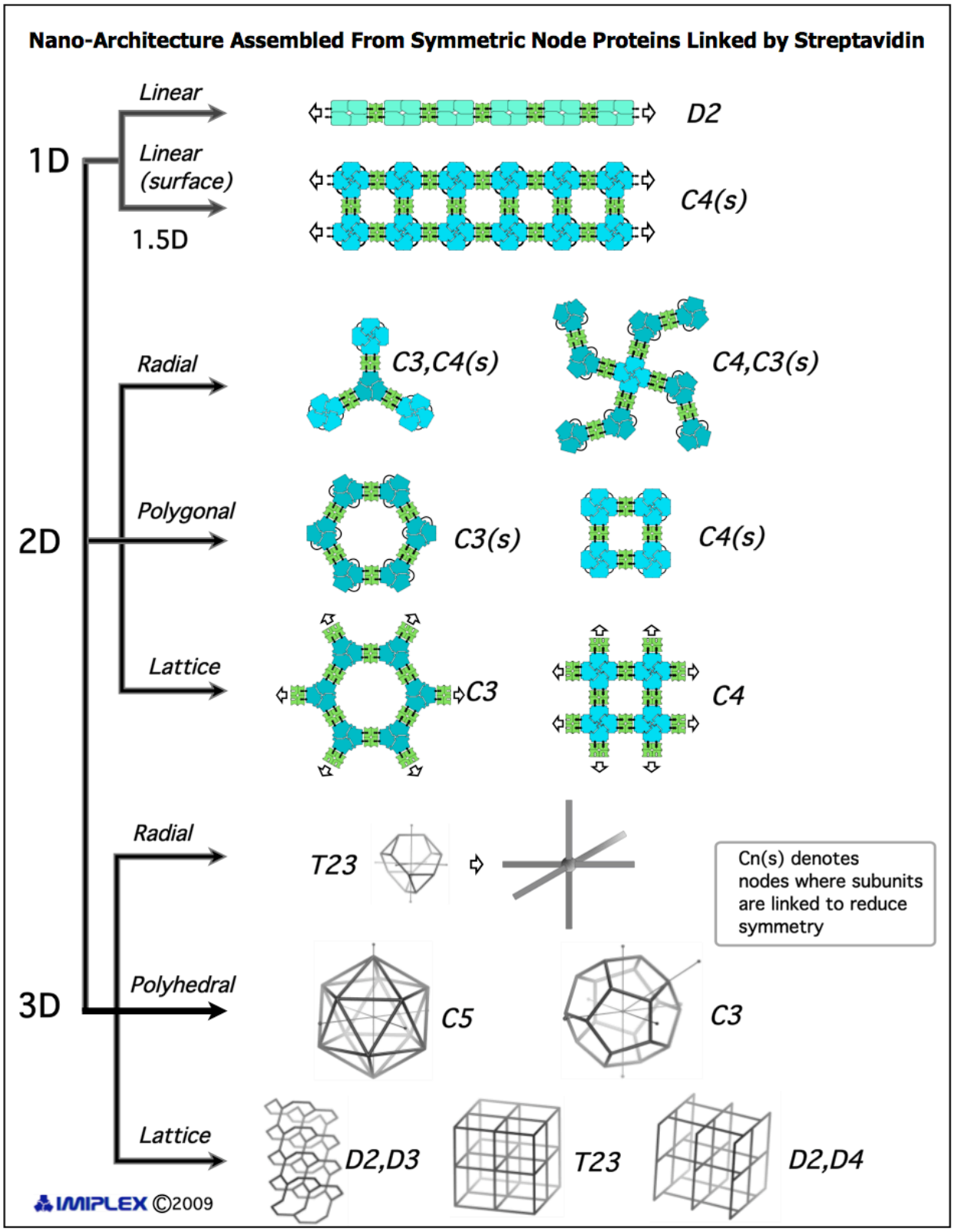

Figure 6. A Hierarchy of Protein-Based Nanostructure Architecture. Nature affords a large number of thermostable multimeric proteins potentially useful for 1D, 2D, and 3-dimensional nanostructure assemblies. These include both infinite (lattice) and finite structures.

Assembly

Nanostructures incorporating engineered proteins can be assembled using several different strategies. Although many of the structures composed of only two types of components (e.g. 2D infinite lattices composed of streptavidin and Cn nodes) can in principle be simply admixed and allowed to spontaneously polymerize, the irreversibility of the streptavidin:biotin interaction makes process control difficult and frustrates lattice "annealing" processes that can contribute to production of lattices with improved long-range order.

More complicated issues arise when structures that incorporate several node symmetries and/or geometry are to be assembled, or a preexisting structure is to be "functionalized" through the chemical attachment of e.g. an immunoglobulin binding domain. In these cases, it is necessary to provide a means of geometrically-controlled, sequential assembly. Imiplex is developing several different solutions enabling both lattice annealing and controlled sequential assembly. These include the development of new chemical linkng reagents that incorporate imino-biotin binding groups, and whose affinity for streptavidin is pH-dependent.

Figure 7 outlines the concept of a reversible streptavidin adaptor molecule under development that effectively functions as a reversible protecting group for two of the four biotin binding sites on streptavidin. The availability of this reagent will provide all of the classical utilities of chemical protecting groups developed for polymer chemistry, including sequential and geometrical control of polymerization sites and the ability to immobilize nano-structures while being assembled. The latter property is key to drive assembly steps to completion and also to facilitate purification of assembled nanostructures from unreacted reagents.

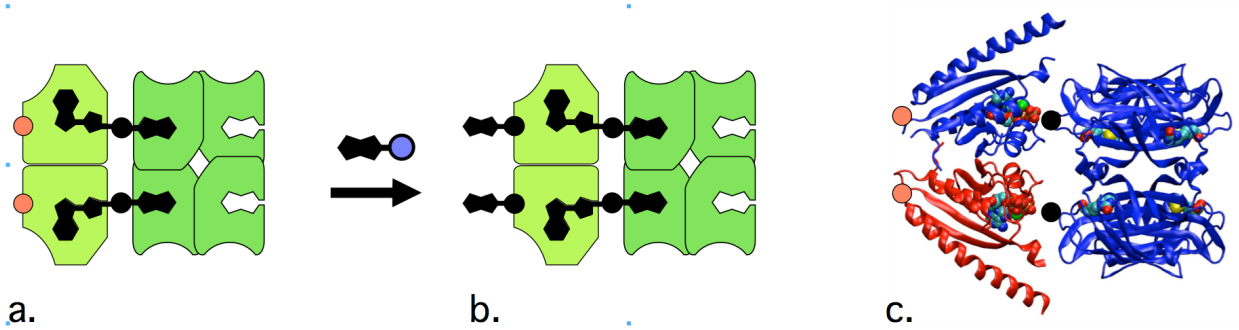

Figure 7. A Reversible Streptavidin Adaptor. Part a schematically shows a streptavidin tetramer that has two biotin binding sites free and two sites occupied by biotin groups attached to biotin-azido-ATP cross-links that link streptavidin to a dimeric ATP binding protein. Orange dots on the ATP binding protein indicate cysteine sites that are potential sites of biotinylation. Part b shows a schematic of the biotinylated complex, which "regenerates" streptavidin binding activity and gives the effect of a "reversible" protecting group for two of the four biotin binding sites on streptavidin. Incorporation of these adaptors into strut assemblies allows the controlled assembly of streptavidin-linked nanostructures. Part c. shows streptavidin docked with the thermostable, dimeric ATP binding protein MJ0577 (pdb code: 1MJH) that is being developed as a reversible protecting group for streptavidin for nanostructure assembly applications. Adaptor proteins can also be engineered to incorporate functional domains like immunoglobulin binding domains useful in biosensors (Fig 9).

Many potential applications of protein-based nanotechnology, such as the development of linear polymers as substrates for biomaterials, the separation membranes described in **Figure 2**, or the nanoassemblies for biosensors described as illustrative examples below (**Figure 9**), will have useful performance even if the structures incorporate some local defects in structural organization. Nevertheless, the ultimate objective is to develop a robust technology enabling the consistent and precision assembly of nanostructures with arbitrarily defined features, outlined above as the key "Difficult Problem in Nanotechnology" on the MITRE website.

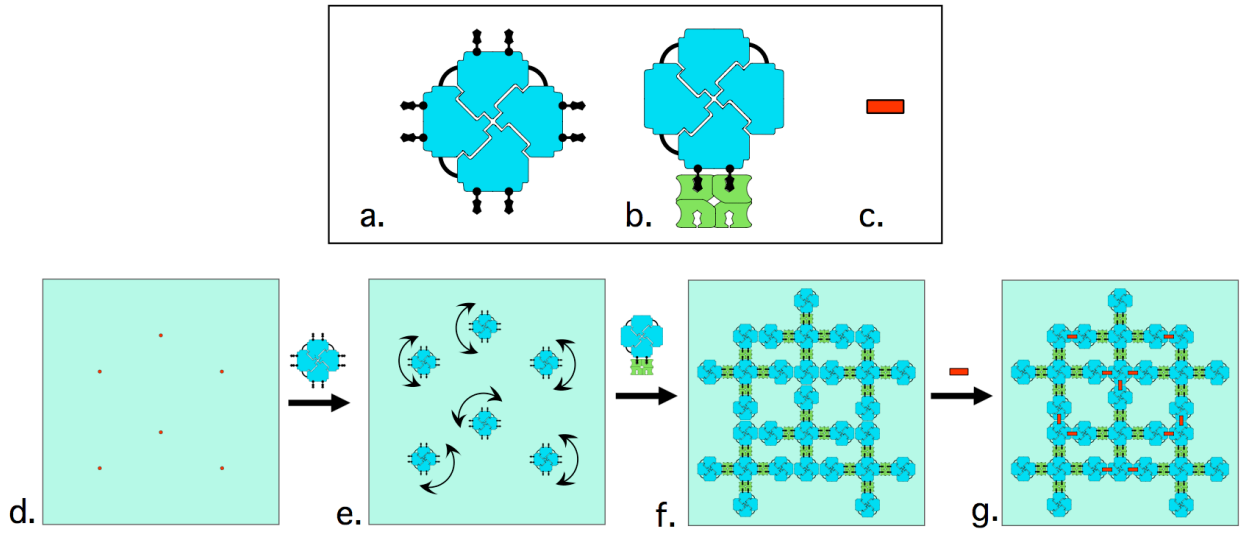

Figure 8. Nanostructures Assembly using a Combination of Top-Down and Bottom-Up Approaches. Part a. shows a C4-symmetric node with polypeptide termini enabling it to chemically bind to a substrate surface; e.g. through reaction of orienting cysteine thiol groups on the protein polypeptide chain with gold atoms on a substrate surface. Part b. shows a 1:1 complex of streptavidin with a C4-single-chain capping group. The single-chain capping group subunits can be engineered to incorporate chemically reactive or structural domains that will form geometrically defined interactions with subunits of other C4-single-chain capping groups (Interactions shows schematically in Part c.) Part d. shows a substrate material that has been precision patterned with a few chemically reactive sites (e.g. one or small clusters of gold atoms positioned using atomic force microscopy or other methods). Part e. shows these sites as points of immobilization of biotin-functionalized C4 nodes: e.g. through reaction of the gold atoms with terminal cysteine thiol groups on the protein polypeptide chain. Nodes immobilized at this stage generally will have substantial in-plane rotational flexibility in orientation. Part f shows the result of addition of a streptavidin:C4 single-chain capping node complex to form a finite patterned (tiled) structure. Part g. shows the structure "fixed" through interactions (red boxes) formed between the terminal single chain nodes when they are in proper relative orientation and proximity. According to the final pattern of static interactions that are desired in the structure, the terminal groups (e.g. the single-chain C4 node structures in b.) can be engineered to incorporate chemically reactive or structurally interacting polypeptide domains (red boxes in part g.) that will "lock" the components into their final orientation.

Figure 8 outlines an approach to nanostructure assembly that uses a combination of *directed* top-down and *spontaneous* bottom-up assembly strategies. The process is initiated with the formation of a few reactive chemical loci on a planar substrate, as could potentially be achieved using a variety of top-down nanopatterning methods or atomic force microscopy. These nucleation sites can in turn be reacted with protein molecules or larger complexes, which in themselves are precision, self-assembled nanostructures, to form larger assemblies. In principle, an arbitrarily large number of finite and semi-infinite structures can be created using this approach, where the initial immobilization of only a few atoms, or cluster of atoms, can serve to restrict the organizational degrees of freedom the structure can subsequently assume as it is progressively assembled.

The directed spontaneous assembly (DSA) approach can allow structures to be built up in a parallel, stepwise fashion to create structures of essentially arbitrary complexity. The DSA approach formally resembles the application of propagation rules for a cellular automata (CA), wherein the CA rules for occupancy of adjacent cells on a fixed lattice are represented by the geometrical and steric constraints of how complexes are bound to and subsequently interact when attached to nodes. A notable feature of CAs is the capability of generating very complicated patterns starting from a simple set of nuclei and iteratively applying a simple adjacent cell occupancy rule (**Figure 9**).

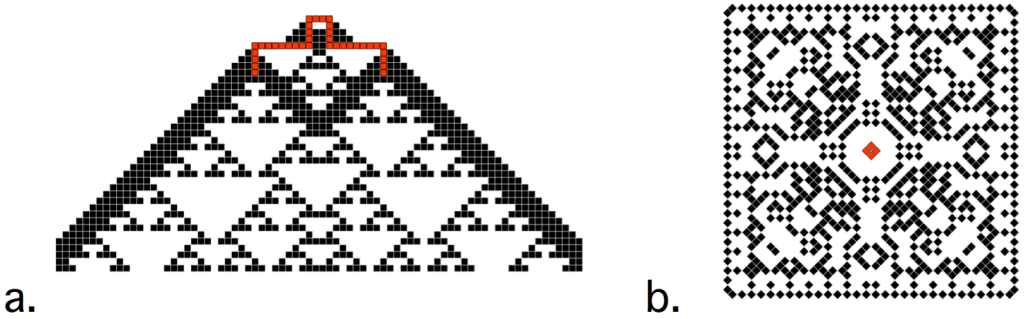

Figure 9. Part a. Cellular Automata pattern generated with the W22 CA rule from the red seed pattern after 29 generations. The W22 rule maintains every adjacent cell connection in successive generations. Part b. Cellular Automata pattern generated with the Persian Rug B234/S rule from the central 4 cells after 29 generations. The B234/S rule eliminates some connections to adjacent cells during pattern development (e.g. the red seed is not present in the final "rug").

An extensive set of molecular design tools stemming from computational modeling tools used in protein crystallography are readily adapted to protein-based nanotechnology applications. Nevertheless, topics such as the defining the relationship between the properties that can be physically incorporated into nodes (particularly those with reversible connection chemistry) and CAs that can be used to generate complex structures on surfaces, remain emerging research areas.

Equally important is the investigation and development of appropriate process technology for nanostructure fabrication, particularly as these relate to creating structures that incorporate biological functionality. Some of the important issues are addressed in the following section that describes initial applications of the technology.

Functional Structures

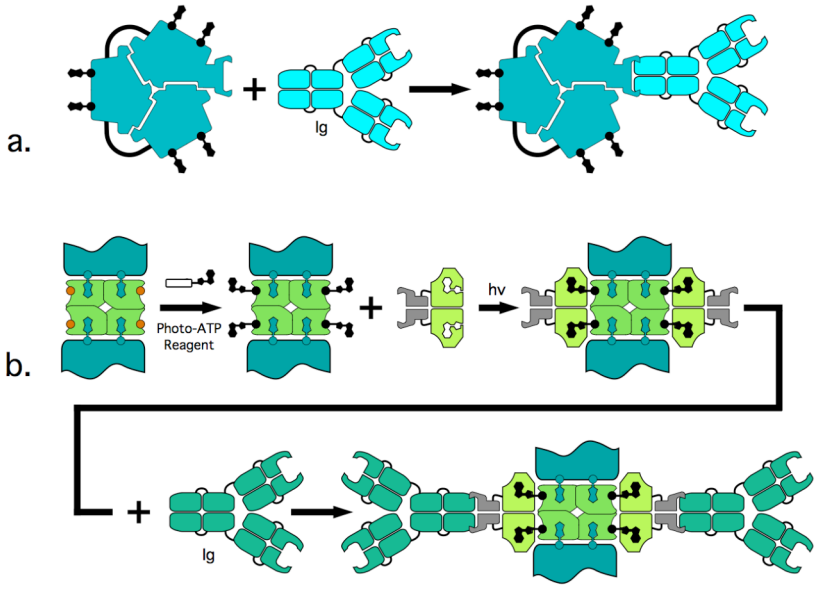

Figure 10. Nanostructures can be functionalized through the incorporation of additional proteins or functional domains. Part a. shows a C3 single-chain node incorporating an immunoglobulin (Ig) binding domain that has been incorporated as part of the node sequence. Part b shows a streptavidin variant functionalized with thiol groups allowing reaction with a photo-ATP reagent. The complex can bind a variant of the adaptor shown in Fig 7 that incorporates an Ig binding sequence within the adaptor protein sequence.

One area where protein-based nanoassemblies can have a near-term economic impact is in the development of improved biosensors. The worldwide in vitro diagnostics (IVD) market is currently around $51 billion (about 7% of the world pharma market) and is expected to grow at an annual rate of about 8% to $76 billion in 2012. The "biomarker" component is one of the fastest growing components of IVD and is estimated to grow in real terms at a compound annual growth rate (CAGR) of 17.6%, from $5.5 billion in 2007 to $12.4 billion in 2012 (http://www.biophoenix.com/tocs/biomarkers.htm). Functionally there are two different market segments: nucleic acid biomarkers (e.g. single nucleotide polymorphisms-SNPs, genetic mutations) and protein biomarkers (cell surface proteins, cytokines, amyloid peptides, pathogen proteins, toxins, etc.). The key objectives of human DNA diagnostics generally involve determination of an individual's specific genetic background as an indicator of human disease proclivity. The objectives of human protein marker detection generally involve determination of the status of disease progression in an individual. In the protein biomarker space, most detection schemes utilize antibodies as the basic molecular mechanism for recognition of a protein marker or antigen. A number of studies have emphasized the observed or potential improvements in detector affinity and specificity that are associated with organized, high density immobilization of in IgGs on sensor array surfaces (e.g. Soukka et al. 2001).

The basic concept behind the development functional nanostructures for biosensors is to use the framework of the strut:node architecture as a scaffold to specifically attach protein motifs that can specifically bind IgGs with high affinity. Two representative approaches are outlined in **Figure 10.** One approach (**Fig 10a**) involves integrating the polypeptide sequence comprising a specific binding domain (e.g. a *Streptococcal* Protein G IgG-binding domain) into the polypeptide chain of the node protein itself (**Figure 11**).

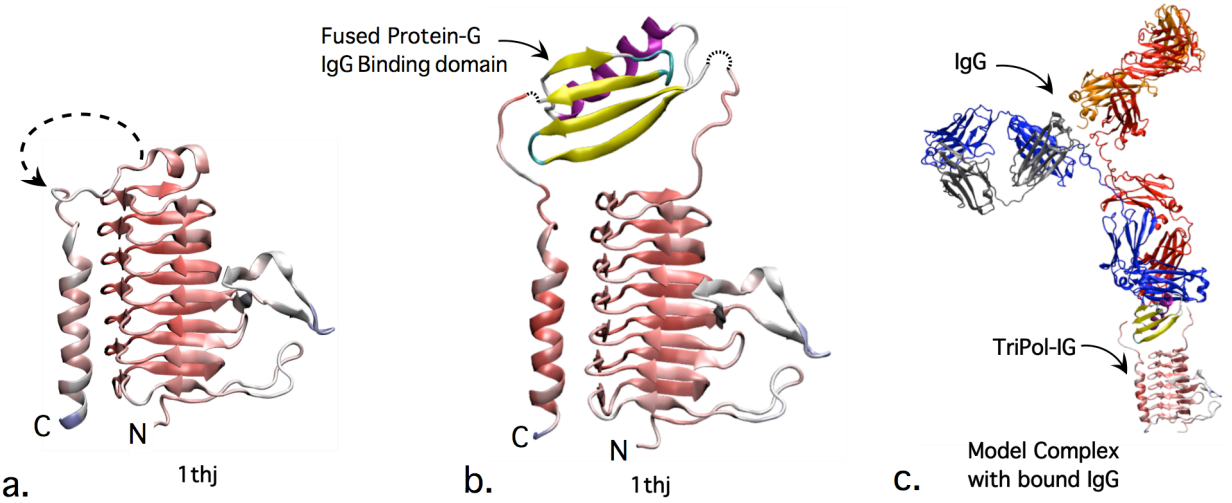

Figure 11 Node proteins with fused Ig binding domains. Part a shows a single chain of the trimeric node protein 1thj (Fig 4a). Part b shows an Protein-G Ig binding domain engineered into a surface loop of the protein. Part c shows a model of the structure with a bound IgG.

As outlined in **Figure 11**, one of the C3 nodes selected for development by Imiplex is well-suited for this application and additionally has the property that the N and C polypeptide chain termini are adjacent in the C3 trimer, allow easy engineering to create a single-chain variant as outlined in **Figure 5.**

Figure 12 shows a scheme for immobilizing a IgG-functionalized 2D hexagonal array on a coated glass surface. Binding of antigen to the array causes a change in the effective refractive index in the vicinity of the gold surface that can be detected using surface plasmon resonance methods (Smith & Corn 2003).

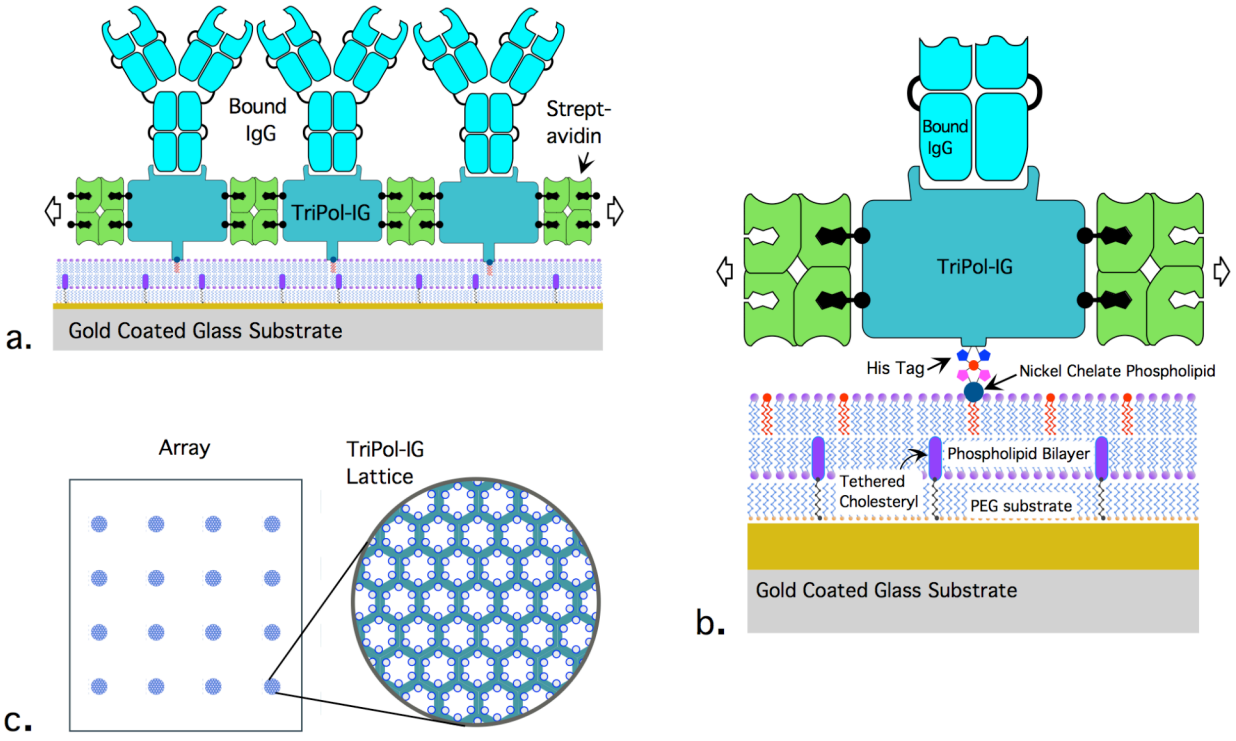

Figure 12 Biosensor Surface Assembly. Part a shows a TriPol-Ig structure assembled on an air-stable lipid bilayer that is formed on a PEG-substituted glass substrate incorporating a tethered cholesterol analog that can insert into and stabilize the assembly of the phospholipid bilayer (Deng et al. 2008). Part b details the Ni-chelate phospholipids inserted into the bilayer that serve as mobile anchoring points allowing the 2D diffusion of TriPol-Ig nodes to assemble 2D lattices. Part c illustrates an application where the TriPol-Ig components are spotted on an extended substrate as an array that can be subsequently functionalized with different antibodies at each spot. The gold coating on the glass surface allows detection of antibody binding using e.g. surface plasmon resonance (SPR).

An aspect that is unique to the constructs and devices potentially developed using protein-based nanoassemblies, is the ability to precisely position the relative orientation of two (or more) IgG molecules on a substrate surface, creating a detector that can attach to different epitopes of an antigen simultaneously. **Figure 10b** outlines an alternative method of functionalizing nanostructures using a streptavidin variant that has been engineered to incorporate cysteine thiol groups allowing specific modification with a photo-ATP reagent. The complex can subsequently bind a variant of the adaptor shown in **Figure 7** that incorporates an Ig binding domain as part of the adaptor protein sequence.

Figure 13 illustrates how the components outlined above might be assembled into architectures that immobilize two different antibodies in close proximity. The resulting, highly specific, capture agent can provide the basis for a biosensor, since the binding of an antigen would "freeze" the relative orientations of the bound IgGs (whose interdomain connections are otherwise quite flexible), an effect that could be potentially detected using a variety of biophysical methods including surface plasmon resonance (SPR, Smith & Corn 2003) or fluorescence resonant energy transfer (FRET, Lackowitz 1999) methods.

The near-term market driver for diagnostics based on protein biomarkers is the increasing recognition of the potential of "personalized medicine" where treatments are optimized based on genetic background or disease stage. Although it is clear that there are many correlations between single gene or protein markers and disease proclivity or state of disease progression, for most diseases the situation is much more complex and involves multiple markers. Nevertheless, patients, drug developers, physicians, and insurers are all demanding better diagnostics as the trend to personalized medicine accelerates.

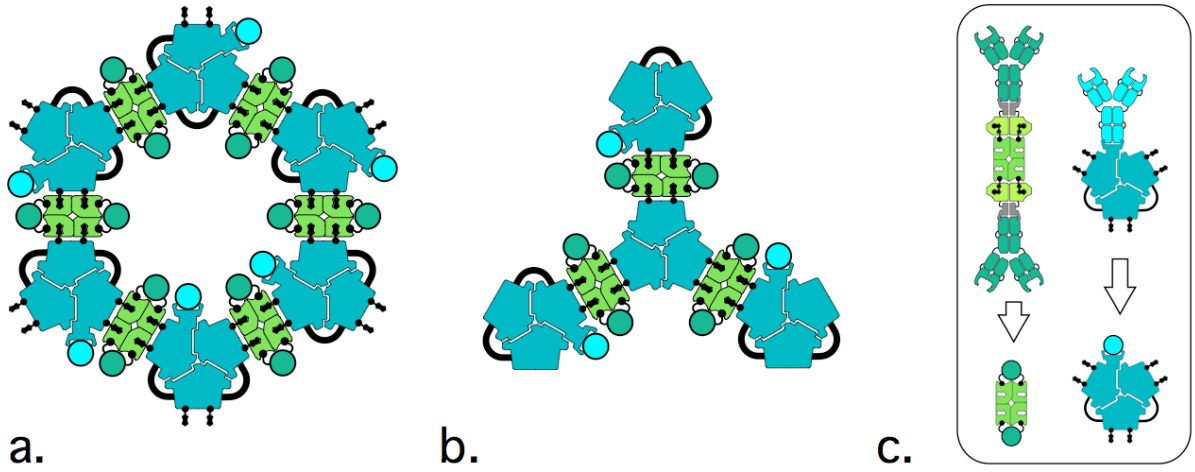

Figure 13 Examples of Biosensor Architecture. Protein-based nanostructures can be functionalized by incorporation or attachment of prosthetic groups, dyes, or other proteins with binding, sensing, or catalytic function. Structures a. and b, for example, are designed to allow simultaneous attachment of two different antibodies (circles of different colors) able to simultaneously bind two different epitopes on an antigen. Simultaneous interaction with different binding sites on an antigen is expected to increase affinity and specificity for antigen binding, potentially by several orders of magnitude. In the unbound state, the surface bound IgG molecules are still relatively flexible, but become immobilized on antigen binding. Immobilization of the antigen can be detected using a number of different biophysical methods including surface plasmon resonance (SPR, Smith & Corn 2003) or fluorescence resonant energy transfer (FRET, Lackowitz 1999), potentially providing a useful biosensor readout for antigen detection. Part c. shows a schematic inset that correlates this Figure and Figure 10.

This white paper has outlined the development of a scalable architecture for nanotechnology based on engineered protein structures. The technology capitalizes on the structural and functional diversity manifest in the thousands of known 3D protein structures, together with the power of molecular biology to readily engineer these proteins to create new biomaterials and biosensors. Although it will undoubtedly take years to realize the full potential of protein-based nanotechnology, we expect to develop a series useful devices and applications as the technology advances, much in the way that the technology required to build a transistor radio ultimately evolved into today's multiprocessor semiconductor fabrication technology.

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