Bio-inspired Polymers for Nanoscience Research

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ABSTRACT

Peptoids are a novel class of non-natural biopolymer based on an N-substituted glycine backbone that are ideally suited for nanomaterials research [1]. This bio-inspired material has many unique properties that bridge the gap between proteins and bulk polymers [2]. Like proteins, they are a sequence-specific heteropolymer, capable of folding into specific shapes and exhibiting potent biological activities; and like bulk polymers they are chemically and biologically stable and relatively cheap to make. Peptoids are efficiently assembled via automated solid-phase synthesis from hundreds of chemically diverse building blocks, allowing the rapid generation of huge combinatorial libraries [3]. This provides a platform to discover nanostructured materials capable of protein-like molecular recognition and function.

Keywords: peptoids, biomimetic polymers, combinatorial chemistry, self-assembly, helix bundle.

1 BIOMIMETIC POLYMERS

One of the fundamental challenges in nanoscience is to develop methods that allow the synthesis of materials with precisely defined 3-dimensional structures. Such techniques would allow for the positioning of molecular moieties at defined distances and angles in space. This kind of control over materials synthesis would be extremely powerful, as chemically reactive groups, chromophores, metals, nanocrystals, biologically active molecules, etc could all be arranged in precise geometries relative to each other. This will undoubtedly lead to new generations of nanostructured materials with very sophisticated properties.

In order to build such nanostructured materials, we look to Nature for inspiration. Natural nanostructures exhibit a fantastic array of precisely defined shapes, and yet their underlying architecture is profoundly simple. Enzymes, receptors, antibodies, structural proteins, DNA and RNA are all biopolymers based upon the predominantly linear repetition of a relatively small number of monomer building blocks. The key is that the 20 amino acids or the 4 nucleotides are arranged into specific sequences that determine the information content, structure and function.

Our group and others have focused on techniques to chemically synthesize sequence-specific heteropolymers that can not only position a chemically diverse set of monomers in a particular order, but can fold into specific secondary and tertiary structures the way nucleic acids and proteins do [4]. By choosing oligomerization chemistry that is more robust than Nature's polypeptide or nucleic acid backbones, we should be able to construct folded synthetic nanostructures that are much more stable. As we continue to understand the chemical and physical mechanisms of biopolymer function, we should be able to import these structural features into completely synthetic, industrially useful materials.

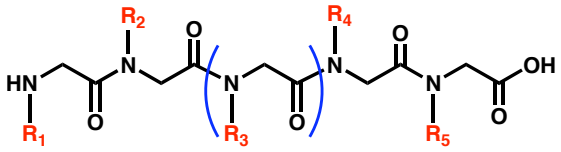

Figure 1. Peptoids are a novel class of bio-inspired materials.

Man-made bulk polymers can be highly robust, useful materials. However, the methods used to produce them lack the ability to precisely control the monomer sequence. Although techniques like atom transfer radical polymerization (ATRP) allow unprecedented control of polymer composition [5], they can only make linear or branched block copolymer-type structures. The self-organization in man-made polymers is currently limited mainly to simple forms of striation and layering -- lamellae, micelles, hexagonal and discotic phases, and bicontinuous structures.

In recent years chemists have made great strides in the synthesis of non-natural sequence-specific heteropolymers [2]. Like biopolymers, these materials can fold into defined structures, but have the advantage that they can incorporate a much wider range of chemical functionalities. Because each monomer is added one at a time, this class of materials must be made by solid-phase synthesis, where monomers are added iteratively. It is now possible to synthesize such materials in the length regime of small proteins [6] (~50 monomers). Thus, the challenge facing nanoscientists is now turning from synthesis to design.

2 PEPTOID OLIGOMERS

Peptoids are a class of non-natural biomimetic oligomer based on an N-substituted glycine backbone [1] (Fig. 1) that combine many of the advantageous properties of bulk polymers with those of proteins. Peptoid oligomers are of particular interest for building defined nanostructured materials because of their ease of synthesis [7] and their chemical and biological stability. Peptoid oligomers are

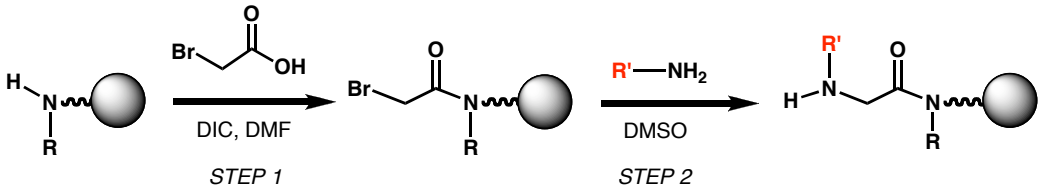

Figure 2. The submonomer method allows the rapid solid-phase synthesis of peptoids from cheap and readily available starting materials. Hundreds of diverse side chains can be introduced from the corresponding primary amine.

resistant to degradation by many common proteases [8], for example. Because the peptoid backbone has a similar side chain spacing and polarity to peptides, it is not surprising that peptoids have been shown by several laboratories to have a wide variety of potent biological activities [1].

We have developed a rapid and highly efficient method to synthesize peptoids [7]. The solid-phase submonomer method uses a two-step monomer addition cycle wherein each monomer is assembled from cheap and readily available starting materials (Fig. 2). The overall coupling efficiency for each cycle is typically in excess of 99%. This allows us to make materials in the size regime of small proteins and polymers (Fig. 3).

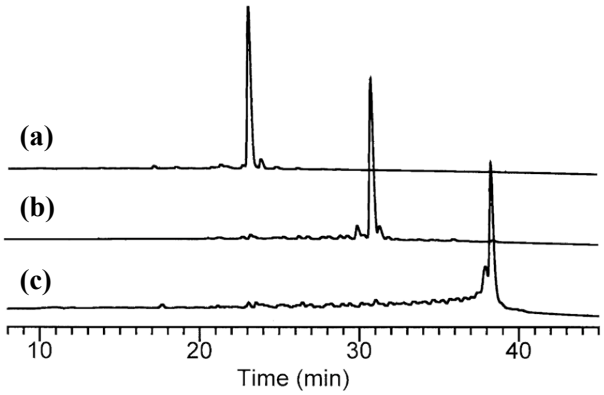

Figure 3. Length series of a peptoid synthesis as monitored by analytical HPLC during the synthesis of a peptoid 50 mer via the submonomer method. (a) 10mer, (b) 38mer, (c) 50mer.

A distinguishing feature of peptoids is that we can incorporate literally hundreds of different side chain moieties into a specific sequence of defined polymer length. Since the side chain functionality is introduced via a primary amine submonomer, we can use a tremendous number of commercially available amines directly as building blocks [3]. In addition, we have developed a variety of methods to incorporate many kinds of polar, reactive and heterocyclic functionalities into peptoids [9]. This allows rapid synthesis of biomimetic oligomers with far more chemical diversity than natural peptides, which vastly increases the probability of discovering novel oligomers with the desired activity. Methods to incorporate chemoselective ligation functionalities in high yield have also been developed [10] which allow peptoids to be readily incorporated into devices.

Despite the fact that the peptoid backbone is achiral, peptoid oligomers can be folded into helical secondary structures [11-16] (Fig.4). This is accomplished by incorporating bulky, chiral side-chains into the oligomer. These helical secondary structures are extremely stable to chemical denaturants and temperature [17]. The unusual stability of the helical structure may be a consequence of the steric hindrance of backbone ø angle by the bulky chiral side-chains [11, 13].

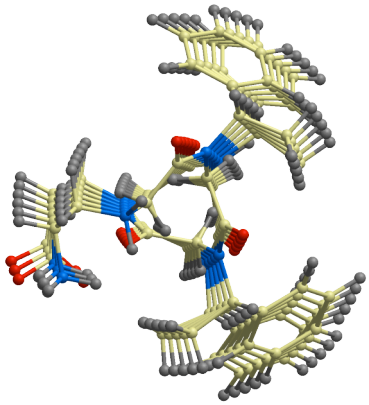

Figure 4. View down the axis of a model of a 15mer peptoid helix.

Amphiphilic peptoid helices can be packed together to form helical bundles, a significant step toward synthesizing a completely artificial protein [18]. Recently, a single-chain multi-helical compact protein-like nanostructure was synthesized by linking individual helical units together [6].

The ability to synthesize robust helices, displaying a wide variety of chemical functionalities that can self assemble into ordered structures offers us a unique platform to create novel functional nanostructured materials.

3 COMBINATORIAL DISCOVERY OF NANOMATERIALS

Ultimately, we aim to create stable nanostructures with protein-like functions from non-natural polymers. But many challenges remain before we can rationally design a sequence that will fold into a predictable defined tertiary structure. Despite decades of study, the rules that govern the kinetics and thermodynamics of folding polymer chains into stable tertiary structures are still not fully understood.

Thus, alternative methods are needed to circumvent this problem. The way Nature solves the problem is by a

process of sequence evolution: biopolymers sequences are varied by mutation and the fittest mutant survives on to the next generation. This iterative process is repeated over and over, yielding sequences with optimal function. Because peptoid synthesis itself is not a major limitation, we can also apply this iterative biomimetic discovery process by using combinatorial library discovery methods. We make very large combinatorial libraries of structured peptoid oligomers and screen them directly for function.

Combinatorial discovery techniques generate large numbers of compounds in parallel. Much in the way that the immune system is a library of billions of antibodies that can recognize foreign molecules, combinatorial libraries can yield compounds with high binding affinities to targets and/or potent biological activities [19].

We have used combinatorial chemistry techniques to make large libraries of peptoid helices, and have screened them for their ability to self-assemble into compact helical bundles [18]. We have linked these helices together to form a single-chain helical bundle structure [6]. We expect that this folded structure can serve as the basis to build functional folded protein-like nanostructures. By randomizing certain portions of this and related folded structures, we aim to select individual sequences capable of specific functions.

3.1 Automated Tools for Synthesis and Screening

In order to screen large libraries of peptoids for new functional structures, we have designed and custom-built several key combinatorial synthesis technologies. The high-throughput synthesis and evaluation of thousands of individual peptoids is made possible by robotic parallel synthesis, "mix & split" synthesis and single-bead array technology.

Since the submonomer method [7] requires the iterative addition of monomers, a very large number of reagent addition and resin washing steps are required. Automation of this process is essential to produce peptoid oligomers in a timely and reproducible fashion [20]. We have designed and built our own custom robotic synthesizers. Each instrument consists of 30 - 40 fritted reaction vessels, and allows the parallel synthesis of peptoid oligomers (Fig. 5). The automated two-step monomer addition cycle takes < 30 minutes, so that the synthesis of a series of 20mers can be synthesized overnight.

In addition to automation of the chemistry, the robotic synthesizer is also capable of performing resin mixing and resin splitting operations required by the "mix & split" combinatorial synthesis method [20]. This allows a very large number of different sequences to be synthesized simultaneously in such a fashion that each resin bead in the combinatorial mixture contains a single compound [21]. This means that we can generate tens of thousands of peptoid oligomers in a single robot run.

Figure 5. Custom robotic combinatorial peptoid synthesizer capable of fully automated mix & split library synthesis.

In order to study each individual oligomer as a separate compound, it can be advantageous to array out individual resin beads (each containing a unique compound) from the synthesis mixture into a multi-well plate. We have developed a bead-arraying instrument that can array out 1000 beads per day into multi-well plates. Peptoids can then be tested for activity as individual compounds. Assays can be performed while the compound is attached to the bead, or the compounds can be cleaved and screened in solution.

One key property of peptoids that is essential to the success of our library approach is that peptoid oligomers can be sequenced by tandem electrospray mass spectrometry. Thus, once a peptoid-bead is identified from a library that has the desired activity, the peptoid's identity can be unambiguously determined by single-bead sequencing. Since the peptoid backbone fragments along the amide bond in the mass spectrometer, a ladder of ions is obtained from which the monomer sequence can be determined (Fig. 6). We have developed synthesis linkers

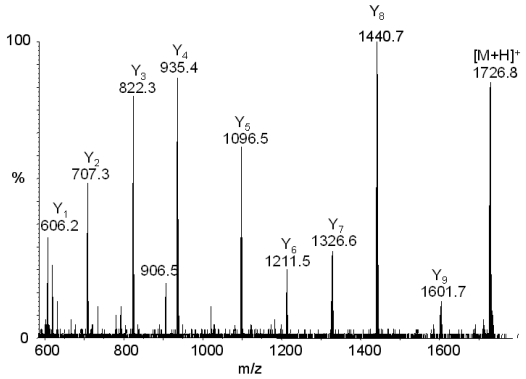

Figure 6. Peptoids can be rapidly sequenced by tandem electrospray mass spectrometry. Fragmentation at each backbone amide yields a ladder of ions that can be easily interpreted.

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that dramatically increase the speed of sequence determination [22]. The method is very rapid and requires very little (pmol) material.

Having these powerful tools in hand, we are poised to discover a variety of novel functional nanostructured materials. These tools are available to Users of the Molecular Foundry to solve problems in nanoscience and nanotechnology.

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