Catalyst Discovery Using Biomolecule Evolution

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Objectives

This project hypothesizes that large random sequence libraries of RNA, and RNA containing key chemical modifications, can be "directed" in vitro to evolve to form novel catalyst materials with enhanced activities. The benefit of the biomolecule in vitro evolution approach to materials synthesis is that it is highly combinatorial. Vast landscapes of biomolecule sequence and structure space are sampled quickly and efficiently (ca 10^{14} unique sequences) to identify even rare sequences capable of assembling new functional catalyst materials. Our current objectives are thus to develop the tools and methods necessary for exploiting RNA in vitro evolution in the search for photo- and electro- catalyst materials.

Technical Barriers

The remarkable properties found in naturally occurring biomaterials have inspired a growing research effort in which biomolecules are used to synthesize and assemble materials in the laboratory. In an attempt to mimic natural evolutionary processes, in vitro selection methods that employ large random sequence biomolecule libraries are now being used to discover biomolecules that mediate the formation of materials. In addition to potentially affording more environmentally friendly routes to inorganic materials, the sometimes highly selective recognition capabilities of biomolecules selected in vitro can facilitate the assembly of nanoscale materials into more complex functional assemblies and devices. Our work is helping to define the range of possibilities in the use of biomolecules for synthesizing and assembling inorganic nanoparticle photochemical and electrochemical catalysts.

Abstract

The remarkable properties found in naturally occurring biomaterials have inspired a growing research effort in which biomolecules are used to synthesize and assemble materials in the laboratory. In an attempt to mimic natural evolutionary processes, *in vitro* selection methods that employ large random sequence biomolecule libraries are now being used to discover biomolecules that mediate the formation of materials. In addition to potentially affording more environmentally friendly routes to inorganic materials, the sometimes highly selective recognition capabilities of biomolecules selected *in vitro* can facilitate the assembly of nanoscale materials into more complex functional assemblies and devices.

Prior results from our labs have shown that RNA has the remarkable ability to catalyze and/or mediate the formation of solid-state materials under mild conditions, and to control materials size, shape, and physical properties.[1-4] Our recent work has focused on explicating basic RNA structure-function relationships and developing new methods for screening large

biomolecule libraries for activity. The fundamental knowledge, tools and methods generated in this project will enable exploration of vast compositional landscapes to potentially identify new materials for photo- and electro-catalytic reactions of interest in the production and utilization of hydrogen fuels.

Progress Report

Our groups have been applying in vitro selection methods toward the isolation of RNA sequences capable of mediating the formation of inorganic nanoparticles. Our initial work showed that a subset of RNA families could be isolated from an initial random sequence RNA library (ca. 10^{14} unique sequences) that mediate the formation of inorganic materials. In one in vitro selection, RNA sequences were isolated that mediate the formation of large (Figure 1) hexagonal crystals from the precursor complex $Pd_2(DBA)_3$ (DBA is dibenzylidene acetone). In a second series of experiments, RNA sequences were selected using magnetic field partitioning that mediate the formation of magnet-responsive cobalt iron oxide nanoparticles. These magnetic nanoparticles formed at room temperature in pH 7 aqueous solution (not shown). Preparative iron oxide synthesis typically requires high pH and elevated temperatures (pH >10, T > 80 °C).

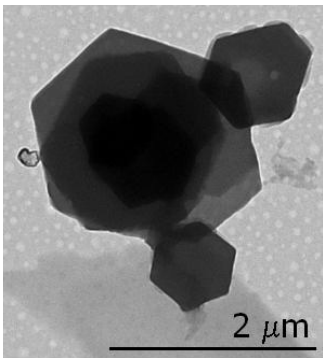

Figure 1. TEM image of crystals resulting from the incubation of $Pd_2(DBA)_3$ and an RNA sequence isolated using in vitro selection.

These important demonstrations served to illustrate the ability of RNA to nucleate and grow materials with novel morphologies and physical properties, under more environmentally friendly conditions than possible previously. However, the molecular-level details by which RNA promotes these solid-state reactions were completely unknown. One of the aims of our current work in this area is thus to determine the substrate specificity of these sequences. A central question is whether changing the metal and ligand set of the precursor complex would change the size and shape of the resulting nanoparticles grown in the presence of the selected RNA sequences.

The results showed that the RNA sequences selected to interact with $Pd_2(DBA)_3$ could in fact make particles—albeit significantly smaller ones—and retain some shape control when using related organometallic complexes such as $Pt_2(DBA)_3$. However, when substantially different organometallic zero-valent Pd or Pt precursors such as $Pd(PPh_3)_4$ or $Pt(PPh_3)_4$ were used particle formation was possible, but shape control was lost (PPh_3 is triphenylphosphine).

It was then possible to determine if shape control could be restored using other organometallic Pd and Pt metal precursors if a series of completely new in vitro selections were performed using those precursors (e.g., beginning with a random sequence RNA library but using these new Pd and Pt precursors). The metal precursors employed included: $Pt_2(DBA)_3$, $Pd(PPh_3)_4$ and $Pt(PPh_3)_4$. Two fundamental questions were of interest. First, will related sequence motifs be selected when using $Pt_2(DBA)_3$ as a substrate that were found from a previous in vitro selection using $Pd_2(DBA)_3$? This is a subtle structural change in substrate that turned out to be telling with regard to the molecular recognition and mediation capabilities of RNA. Second, can sequences be evolved that function to give either improved yields or new shapes when using either $Pd(PPh_3)_4$ or $Pt(PPh_3)_4$, which are well known to be significantly less reactive than those complexes with DBA ligands? Some very encouraging results were obtained from these studies.

We now know that very similar, but not identical, sequence motifs were selected using $Pd_2(DBA)_3$ compared to $Pt_2(DBA)_3$. Perhaps more importantly, these newly selected sequences showed excellent shape control for formation of exclusively hexagonal particles Table 1. Note that Pd017, could not achieve this exquisite shape
control using $Pt_2(DBA)_3$ in place of $Pd_2(DBA)_3$, supporting the concept that sequences can be fine tuned via in vitro selection to work with specific substrates to yield specific particle shapes (the designation Pd017 corresponds to a particular sequence from the original selection). It was also of interest to determine if RNA sequence could only determine shape control for substrates with high reactivity toward ligand substitution like $Pt_2(DBA)_3$ and $Pd_2(DBA)_3$. This required us to perform more extensive sequencing and investigation of the nanoparticle mediating properties of sequences selected to make particles from either $Pd(PPh_3)_4$ or $Pt(PPh_3)_4$.

We have found that, similar to $Pd_2(DBA)_3$, sequences can be isolated that make non-spherical particle shapes using the less reactive zero-valent complex $Pt(PPh_3)_4$. We now know that organometallic complexes with relatively stable ligands such as phosphines can be suitable substrates for RNA-mediatiated metal nanoparticle formation. Further exploration is needed to better understand the limitations of RNA-mediated nanoparticle formation, but it is at least now clear that more highly reactive organometallic substrates such as $Pd_2(DBA)_3$ and $Pt_2(DBA)_3$ are not required.

Table 1 Shape and size distribution of Pd or Pt particles formed by individual RNA sequences using their specific organometallic precursor.

RNA Isolate	Particle Shape	Size (μm)	% Population
Pd017	hexagon	1.3 ± 0.6	100
Pd034	cubic	0.82± 0.4	100
Pt02	hexagonal	0.14 ± 0.05	100
Pt012	hexagonal	0.16 ± 0.08	100
Pt018	spherical	0.023 ± 0.008	73
	hexagonal	0.22 ± 0.14	17
	cubic	0.07 ± 0.02	10
Pt021	hexagonal	0.13 ± 0.04	100
Pt032	hexagonal	0.18 ± 0.09	100
Pt049	spherical	0.04 ± 0.02	100
Pt069	spherical	0.06 ± 0.03	100

Future Directions

Our studies of the molecular-level details of how RNA recognizes organometallic precursor compounds to build transition metal nanoparticles were derived from our interest in using such compounds to generate novel catalytic materials. These examples, however, all pertain to the synthesis of previously known materials. Moreover, with the exception of our magnetic field partitioning method to isolate RNA sequences capable of mediating the formation of magnetic nanoparticles, our prior work has utilized *size-based* rather than property based selection methods. That is, any solid material assembled in the presence of a biomolecule was isolated. For biomolecule evolution methods to truly have an impact in the field of materials discovery will require new tools for screening biomolecule libraries for their ability to assemble materials with a desired *property* (e.g., catalytic activity). We are thus currently seeking to build specialized microarray technology to fully enable biomolecule *in vitro* selection as a tool for materials discovery.

The features of the new technology being developed are generally: (i) a planar substrate or microwell array onto/into which a random-sequence biomolecule library is loaded, (ii) a light source and potentiostat to input energy (e.g., to provide a driving force for electrocatalytic or photocatalytic reactions of interest), (iii) a Zeiss inverted laser scanning confocal fluorescence microscope capable of imaging large surface area chips (20 mm x 20 mm) with near single molecule resolution, and (iv) an injection system that can deliver or extract as little as femtoliter volumes of reagents located in any spot or well on the chip within 3 microns on demand. The chips or microwells may be loaded with ssRNA, ssDNA, or peptides (via phage). It is hypothesized that a subset of these sequences, when incubated with certain metal ions, will form nanoscale materials with unprecedented properties (e.g., catalytic activities). The microscope

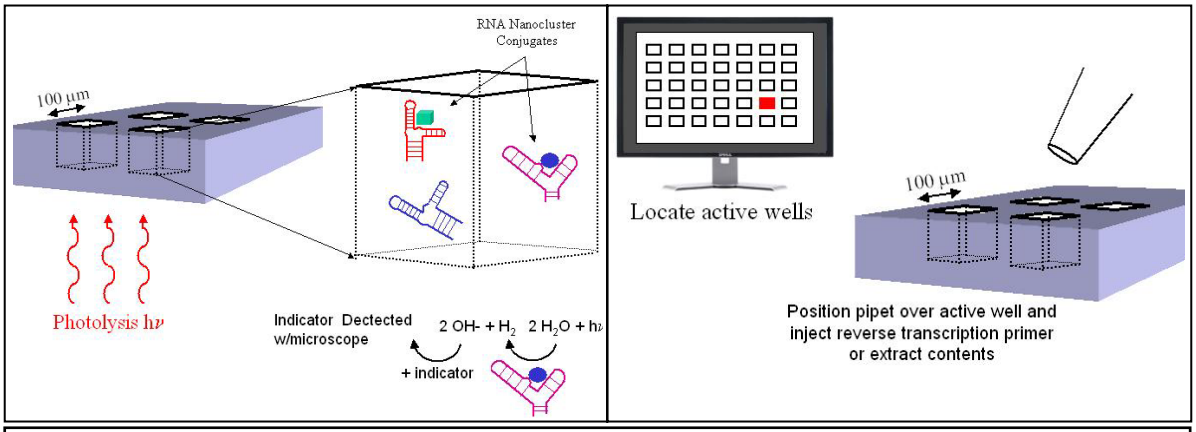

Figure 2. Schematic illustration of the use of the proposed instrument in the search for water-splitting photocatalyst materials. A random-sequence library of biomolecules (ca. 10^{14} unique RNA sequences) is incubated with metal precursors and distributed into the wells of a microwell array (Left Panel). The array is then mounted into the inverted microscope and irradiated with visible light to promote the photocatalytic conversion of H_2O to H_2 and OH^-. Any well that contains an active catalyst will become increasingly basic, turning on a fluorescent indicator. The entire chip is imaged with the fluorescent microscope, and the active wells are positioned under the femtoliter injection system (Right Panel). The entire contents of the well may be extracted or reverse transcription primer may be injected into the well for RT/PCR.

was designed to scan the entire array to identify the most active materials for catalyzing a desired reaction, while the injection system is responsible for isolating the sequences that assembled those materials. Figure 2 depicts schematically the operational principles of the instrument as they apply to the search for catalysts for photoinduced water splitting. Note that as long as one of the products of the desired reaction can be coupled to a fluorescent output, the microscope will be able to identify wells containing active catalysts. In the example, water splitting produces hydroxide ions that are coupled to a pH indicator.

Summary

Biological organisms have evolved over millions of years in response to countless selection pressures to synthesize remarkable nanostructured materials. Nanoparticles are synthesized in nature and then organized in well-defined ways to form teeth, shells, bones, and other mechanically durable composites. Iron is converted into magnetic oxide nanoparticles, which are then assembled into 1-dimensional bar magnets for navigation. In certain places, life exists arguably only because of mechanisms that have evolved to convert toxic ions to their more tolerable solid-state counterparts, as in the case of bacteria that sequester silver or cadmium ions in the form of silver and cadmium sulfide nanoparticles. At the heart of many of nature's

catalysts are metal oxide complexes and small nanoclusters. Photosystem II of oxygenating plants consists of a tetranuclear manganese oxide cluster, for example.

Thanks to recent work by our group and others, we now know that RNA and peptides can be coaxed to evolve materials in a laboratory setting. The benefits of these methods are that vast landscapes of compositions, structures, and sizes can be surveyed simultaneously, in a process that is highly dynamic because of natural evolutionary mechanisms, and is driven by materials selection pressures that are designed *a priori*. Just as biological organisms found in nature have solved incredible and myriad materials problems, biomolecule *in vitro* evolutionary materials synthesis could in principle be applied to the search for nearly any new material.

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