

In Focus **DNA Nanotechnology**

Excerpts from an interview on January 29, 2013

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Within the TUM-IAS Biophysics Focus Group, hosted by Prof. Andreas Bausch in the TUM Physics Department, one line of research is bending DNA toward new ends – that is, exploring how DNA can be used as a programmable building material for self-assembly of nanoscale structures and devices. Broader than the design approach popularly known as “DNA origami,” the field of molecular self-assembly with DNA has seen recent progress justifying a more industrial-sounding handle: “DNA nanotechnology.”



Prof. Hendrik Dietz, a Hans Fischer Tenure Track Fellow of the TUM-IAS, leads the Laboratory for Biomolecular Nanotechnology, which receives additional support from the Excellence Clusters CIPSM (Center for Integrated Protein Science Munich) and NIM (Nanosystems Initiative Munich), the collaborative research center Forces in Biomolecular Systems (DFG SFB 863), the European Research Council, and the Chemical Industry Fund. Dietz's lab works closely with partners at MIT, the MRC Laboratory of Molecular

Biology in Cambridge, England, and the TUM research groups of Prof. Matthias Rief, Prof. Friedrich Simmel, and Dr. Ulrich Rant, a TUM-IAS Fellow and group leader at the Walter Schottky Institute. Four members of the lab met with interviewer Patrick S. Regan (PSR) to look back over a remarkably productive year and consider prospects for the future: Prof. Hendrik Dietz (HD) and doctoral candidates Thomas Gerling (TG), Thomas Martin (TM), and Jean-Philippe Sobczak (JPS).



PSR: Hendrik, you've described the aim of your research as gaining access, through technology, to a domain where nature employs special kinds of mechanical structures to accomplish chemical and biological processes. Could you please elaborate on that?

HD: Every biological cell contains thousands of macromolecules that have well defined three-dimensional shapes, with absolute dimensions on scales from a few to a few tens of nanometers and with atomically precise features. Most commonly these objects are known as proteins. People typically don't appreciate how wonderful these objects are and what they can accomplish, but they are our inspiration.

Proteins are built according to genetic information, and they're made from amino acid chains. The sequence of these amino acid chains already fully encodes the three-dimensional shape that these objects will adopt in solution. Once this shape has been formed, through a self-assembly process, it can already be functional. And the functions range from making new molecules from smaller ones, or breaking down larger molecules, to transport on a nanometer scale, conversion of energy from light into chemical compounds, or even a kind of computation. And basically all of this makes cells live, and enables us to live.

In a way this is nanotechnology that has emerged through evolution. We can look at this in awe and say how wonderful these objects are, and we may want to learn how to actually build something with similar capabilities. But these objects are smaller and more structurally intricate than anything we can build with state-of-the-art top-down fabrication methods. On the other hand, they are far bigger and structurally more complex than anything we can make with state-of-the-art bottom-up chemical synthesis. So really these objects are in a gap, a technology gap, where we haven't yet learned to build with this sophistication.

Nature uses an interesting principle to build these objects, and that's exactly what we want to learn. Using another kind of macromolecule, DNA, we want to learn how to tailor the sequences of biomolecular building blocks so that they already encode a well



defined three-dimensional shape; and ideally the shapes that we build that way could be as complex and as sophisticated as the shapes of these natural macromolecular machines that we find in our cells. Ideally one day we will also be able to encode sophisticated functionality in these shapes. DNA nanotechnology gives us access to this regime between state-of-the-art top-down and bottom-up approaches.

PSR: Thinking about your own inspiration and motivation, do you have favorite examples of nature's biomolecular nanomachines?

HD: ATP synthase. It's a nanoscale rotary motor.

TG: The flagellum motor, that's my favorite machine.

TM: Ion channels are also good. The way they selectively let through some ions and block others, that's pretty amazing.

JPS: I wouldn't say I have a favorite one, but always, when my family asks what I do, what are these molecules good for, I explain that almost everything is made in some way from proteins. Everything in this room for example, like the wood for the desk, the plastic, the paper, the material of your pants, everything was in some part made by proteins. They always want some kind of specific example, but it's easier to point to things that aren't, because there's only a few.



Left to right: Jean-Philippe Sobczak, Thomas Martin, Hendrik Dietz, Patrick Regan, Thomas Gerling

PSR: For building with DNA, what are the basic principles and methods?

HD: The building block is double-helical DNA domains, which we consider a secondary structural unit. Let me explain: In biology, when people talk about the structure of protein molecules, they differentiate between several levels of structure. The primary structure is just a sequence of amino acids, the building blocks that make up the amino acid chain. Secondary structure is for example beta sheets, alpha helices. And then there is tertiary structure, which refers to the 3-D arrangement of these secondary structural elements. And then there's the quaternary structure, which refers to the 3-D arrangement of multiple protein molecules. So here I think we can make the same distinction. The primary structure is the sequence of bases in each strand of a double-helical DNA domain, and we consider double-helical DNA domains as secondary structural elements, which you then connect in a user-defined 3-D topology to give rise to a tertiary structure. Our secondary structural elements, these double-helical DNA domains, can have user-defined length and helicity. A double-helical DNA domain is a right-handed helix that's formed from two DNA strands, and it has certain geometrical properties. And those can be tuned, so the helix can be overwound or underwound. Individual DNA double

helices can also be bent, but the bending is induced as a consequence of constraints that arise in the tertiary structure.

Since each double-helical DNA domain consists of two strands, we have four "outlets," two at each end. These outlets are phosphate backbone linkages, and they can just be transferred into the next secondary structural element. This means you don't need to think so much about the crossovers; we only think about the double-helical DNA domains and how we want to arrange them in 3-D space.

PSR: And the rest follows from that?

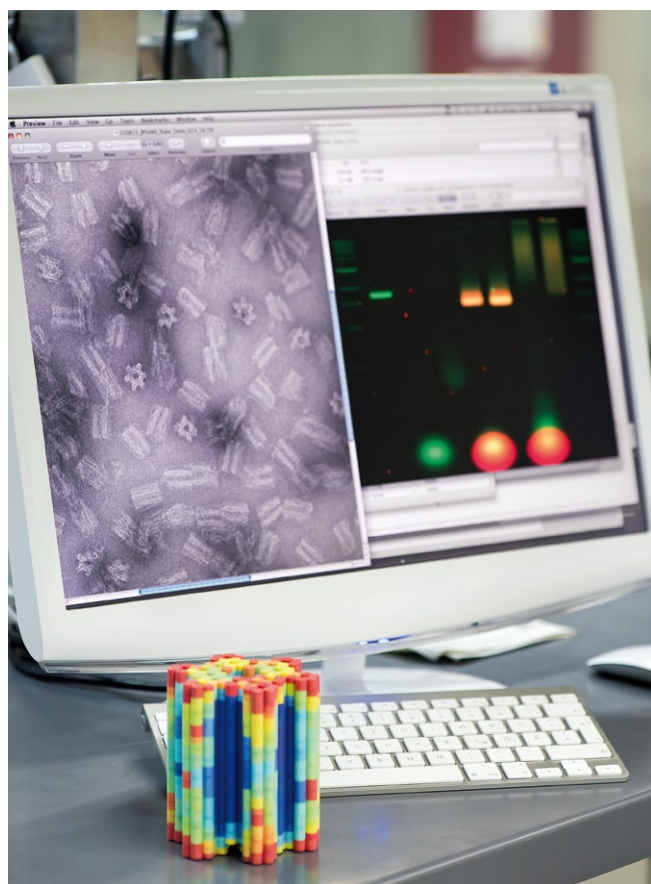
HD: That's right. And then you have to figure out the routing, where you route each strand through the 3-D arrangement of these double-helical DNA domains. That's a very simple design paradigm: You only think about double-helical DNA domains and how to connect them in 3-D. And by restricting yourself to B-form DNA and this paradigm, simple considerations can take you very far in terms of structural complexity.

JPS: The thing is, it's difficult for people to imagine why you can build any shape. We have basically stiff pipes, the double-helical DNA, and we can link an arbitrary amount of them in whatever way we want to.

So we can build any kind of layer, like scaffolding on a building, for example, or like a wire mesh. It can have any kind of shape, but it's just straight lines connected at different points. That's our design freedom. We can make any shape, because that's the only restraint. We just have to connect at some point a lot of straight pipes. And they don't even need to be straight. We can bend them, so we have even more choices.

PSR: And once you have built a DNA-based nanostructure, how do you know what you've made?

TM: We have several tools. One is gel analysis. We know if the structure is correctly formed and compact, then it should run faster in a gel than an unstructured object. We just apply a voltage, and because our structures are charged, they run through the gel matrix; depending on the size they run at different speeds, and with that we see if they are well formed or not. And we also see in the gel how many dimers or unfolded structures we have, compared to different kinds of folding products. In the gel we have intercalating dyes, which fluoresce, and with that we see the defined bands.



Electron microscopy is the next step. We take the fastest band, the whole folded set, and we look at it, and with that we get a rough idea how the shape is. It's not as exact as you need for real 3-D reconstruction, but the approximate shape is already visible.

TG: That's the only way you know what you did. You have to look at them. And you have electron microscopy or atomic force microscopy methods. To see it with your eyes, this is the only way.

HD: You can still look directly at the folding products that you have in the solution. And then there are indirect methods that report on certain properties of the structure, for example global size, or radius of gyration, stuff like that. And these properties can be interrogated, for example, using chromatographic methods or electrophoretic methods.

PSR: During 2012, your group and collaborators demonstrated DNA-based structures for two potential applications – a "smart lid" for Uli Rant's solid-state nanopore sensors and, with Fritz Simmel's group, a synthetic membrane channel that mimics nature's way of tunneling through cell walls. These seem like big achievements, but are they just the beginning?

HD: Just demonstrating a self-assembled synthetic membrane channel makes it easier to imagine a lot of things: molecular sensors, nano-needles, artificial virus-like devices – for therapeutic purposes of course – even nanomachines powered by the ion flux.

TM: Nanopores, on their own, are pretty amazing, considering the way they allow single-molecule sensing without labeling. But they lack something because they are everywhere chemically the same. With DNA nanotechnology, you really have the possibility to modify a specific site, and this changes the nanopore into something that can be customized, tailored to different purposes.

Tools for determining quality and yield include gel analysis (right) and electron microscopy (left). 3-D prints (below) also make it easier to visualize DNA-based nanostructures.

104 **HD:** The thing is, this DNA-based molecular self-assembly is unique in the sense that it is the only fabrication technology right now that can give you structural complexity and a certain degree of positional control on this length scale of a few to a few tens of nanometers in solution. It can produce chemically registered objects, so that if you want for example to place a fluorescent molecule in a certain position in that structure, you can do it; and if you want to place another reactive group elsewhere, you can do that. And that may seem a mundane feat, not very impressive, but in a way that opens up a whole world of potential applications, because now you can build objects that are commensurate in size to natural macromolecular machines like protein molecules. That means that we can now start building little tools that we can use to manipulate these natural objects. Along these lines, we are pursuing a number of applications where we try to use this molecular self-assembly of DNA to build nanoscale tools and devices that can help us study protein molecules. One example is these nanoplates or “smart lids” for nanopore-based sensing applications. Another example is these synthetic membrane channels, which again may be used as single-molecule sensing devices.

We're also working on grippers that could enable us potentially to hold onto single protein molecules. This could play a role in studying the mechanical properties of the folded state of the protein molecule with the help of optical tweezers, like what Matthias Rief is doing. In this case our structures could increase the resolution of this technique, to measure the fluctuation dynamics of a protein molecule, and there isn't currently any other means to make such stiff grabbers on that scale.



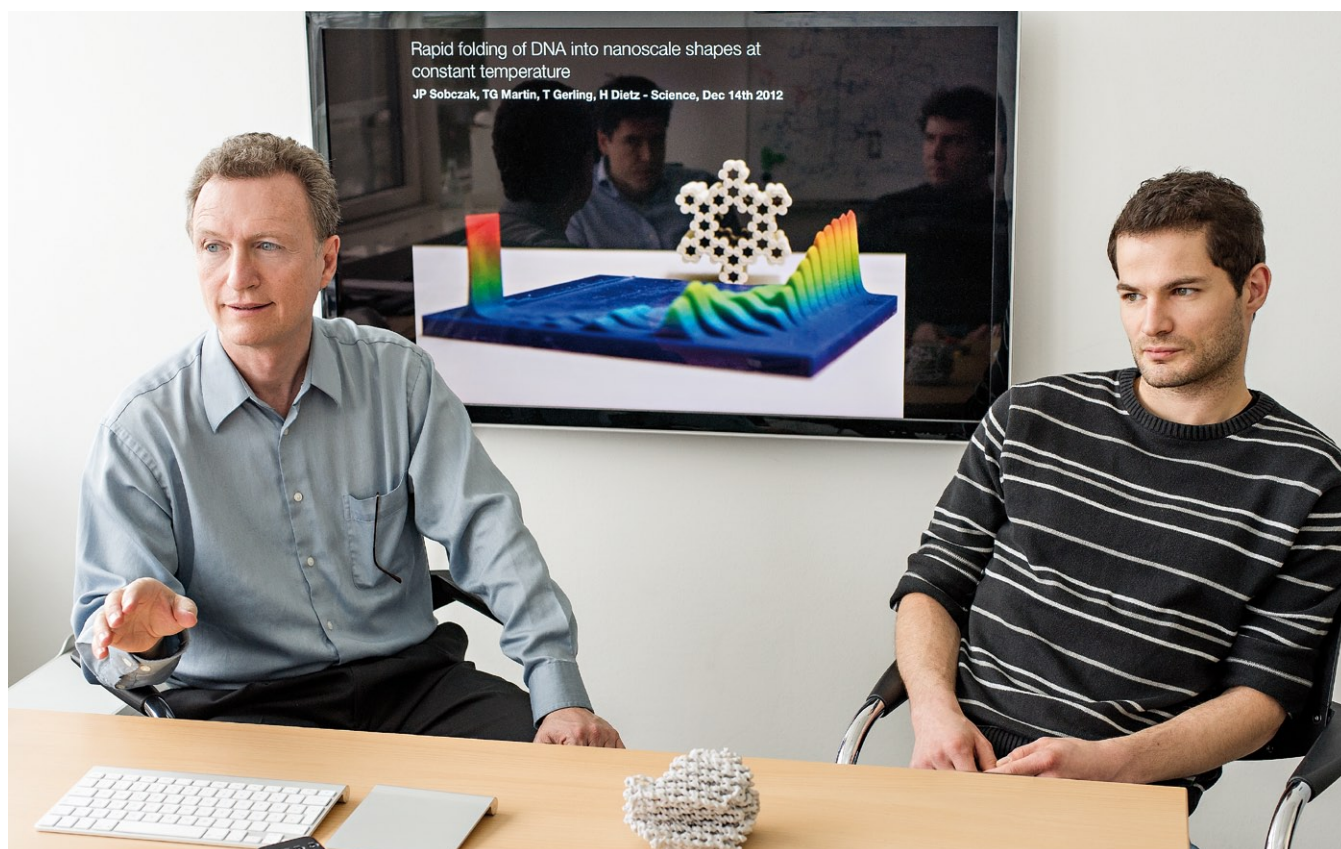
So you could see this as a series of relatively mundane applications where we take advantage of the fact that we can make an object that has a certain length with a certain stiffness, or we can place chemical groups in certain locations. On the other hand, if we only pursue applications that take advantage of the capabilities we have today, then we won't advance the technology.

To some people it seems crazy to think of one day using genomic material, DNA, to build catalytically active objects. I think all you need to do that is sufficiently precise positional control and the ability to create complex objects. So we are trying to push that. Thomas Gerling is working on trying to build designed structures that can be actuated through the addition of chemicals. Jean-Philippe is trying to better understand the self-assembly, when and how these structures put themselves together, which in itself is interesting because basically everything in nature is formed through self-assembly. Maybe we can learn something about protein folding processes by studying the assembly process of these DNA nanostructures. And then Thomas Martin has been working on these channel applications, and another application for structural biology to try to enhance single-particle electron microscopy, basically mixing applications research with efforts to push the boundaries of the field.

PSR: Along those lines, you've also recently published results that suggest you're on the path toward making applications practical, even someday “industrial.” Let's start with the discovery that DNA-based nanostructures can be synthesized rapidly at constant temperatures. What are the implications for the field?

JPS: What people knew was that if you mixed everything and you annealed it for a very long time, you would at the end get the thing that you planned. And it made sense because you know, from base pairing, which is very simple, there's no reason you couldn't plan things in the first place and end up with a special shape. But you didn't know anything about what was going on along the way. It was just like a black box.

So we added a dye that allowed us to visualize the progress of folding actually in real time. We all did this together. And from this we learned a few things that led us to this constant-temperature folding.



People had different theories about how things would progress, but now we could actually see directly from the data: Here something happens, and in the other parts of our folding process nothing happens. And we could then focus on the temperature range where folding actually takes place.

PSR: This was a surprise in the beginning, wasn't it?

TM: Yes, it was a surprise. Before that we always had these seven-day runs, and we had no reason to expect that we could actually shorten it to a few hours.

HD: Previous protocols involved this chemical and thermal annealing. You had to wait a week and then you got maybe a little bit of your designed product. But these synthesis protocols suffered from low yields. You had a lot of by-products, and you lost a lot of good material on the way through the lengthy exposure to the high temperatures. So while these assembly protocols were good for proof-of-concept studies where we could show what we could make using DNA, you couldn't imagine doing robust manufacturing this way. Then we started looking into how the assembly proceeds and were

surprised to find that the procedure can be shortened, and that it actually can be shortened a lot, and then for some structures we got really satisfactory yields. That makes me believe this really is something we can convert into a robustly working manufacturing method that may have an industrial future, although there are still a couple of challenges that we need to solve.

PSR: As I understand it, to get these high yields in short time periods, you need to find out what's the magic temperature for the particular object you're making. Do you have rules yet, or is it still hit-and-miss?

TG: It's pretty easy to find out with an assay.

JPS: You can measure it, but right now you can't predict it yet, so that's another thing we're going to work on – being able to look at the design and tell what its temperature will be, so you don't have to do the measurement every time. That will be another important step in optimizing procedures, making things even faster. Now we have to run it once at least, to scan it. If you could predict it right away, you wouldn't even have to do that.

106 **TM:** Just from experience, you know whether some structures will fold at high temperatures or they will fold at low temperatures, but it's not so easy to actually understand why. In the broad range of plus or minus five degrees, you can probably estimate it, but to know exactly what you need for this constant-temperature folding, we're not there yet.

HD: I just want to emphasize what Jean-Philippe said. At least now there is a method by which you can rationalize the process of optimizing the assembly conditions. So you can monitor the assembly as a function of temperature and then pick the right temperature range in which the structure should fold. And that's an opportunity we have now, to get the high yields.

JPS: Another good thing is that this might be helpful to people who are trying to build proteins by design. That's a complicated thing because you can't tell right away how something will assemble, there are so many possible directions, and there are no easy rules. But for DNA-based self-assembly, where you have very straightforward interactions, it's reasonable to think you might be able to develop some kind of model.

PSR: Has it already improved your work life that you don't have to wait a week between batches?

TM: It's less excuses.

HD: The throughput has increased dramatically.

TM: Before, you had to work several projects at the same time, because you always had this step where you had to wait for a week.

TG: For most of the experiments, it's important to have a high yield of correctly folded structures, so I'm using this all the time.

PSR: The capability for subnanometer positional control – which you demonstrated for the first time using a specially designed test object and low-temperature electron microscopy – that's a separate issue but with a similar impact, right?

TM: In the past, with normal negative stain electron microscopy, you could always see that these DNA-based

objects had a defined shape, but you never knew how exact it was, or if you had small differences between the structures. And with this cryo-EM study, we could show that you actually can get a very defined shape, and it doesn't vary much from one individual object to the other. We were able to get a very high-resolution cryo-EM 3-D structure out of it, and we could actually know where a specific base is in three-dimensional space.

PSR: Where would you place these results on the continuum of progress in the field?

HD: The broader field of molecular self-assembly has been around for three decades. Thirty years ago Ned Seeman, a crystallographer, started this whole field. He always cracks the same joke when he talks at conferences: no crystals, no crystallography, no crystallographer. So he was thinking about how to facilitate the preparation of crystals. He had the idea of using DNA as a template for 3-D crystals, which would serve as a host for guest proteins and thus would help in the structural analysis of those proteins. That's how this started back in the 1980s, stitching together DNA molecules to form bigger structures. In the 1990s he first demonstrated, for example, a little cube made of double-helical DNA domains, and then the field was slowly growing toward more complex structures, which were never really validated in detail.

Then in 2006 there was a breakthrough, a new approach to design that came to be known as DNA origami. Paul Rothemund from Caltech pioneered this. He wrote one beautiful single-author cover story in *Nature*, with a 95-page supplement, and he showed the tremendous potential of this new assembly method. He was the first who really made fairly complex objects. But still, the structural validation of these objects was rather coarse.

But all the people in this field were operating on the assumption that DNA-based self-assembly should give you subnanometer-precise positional control. And skeptics were criticizing the field for the lack of high-resolution structures, arguing that this indicated an inability to produce something that is structurally well ordered. So counter to the assumptions, the skeptics argued that what people were making was just pudding, or heterogeneous, or floppy, and therefore not useful for anything.

From this perspective, our high-resolution structure is very useful because it validates this assumption: that we can make structurally defined objects that have a high degree of order, comparable to what you find in protein molecules. This in turn suggests that you really may use these structures as high-resolution scaffolds to position reactive groups in space with subnanometer accuracy, and thus obtain more complex functionalities. And I think everybody in the future will be happy that we now have this structure. One should say though that the quality of synthesis, which is what enabled us to make such a large and complex test object in the first place, definitely owed a lot to the experience we had gained over the last three years or so.

PSR: With this kind of validation, is this the best time yet to be doing research in DNA nanotechnology?

TG: We are all physicists, and I think this area of biophysics is one of the only fields where a physicist can do something really new. For me, that's the most important thing.

TM: We all started before this breakthrough, where we could know for sure that we can build things with high accuracy. But the really interesting thing is that you never know what to expect. You may get some results

that you never thought of before, and that's possible largely because it's so new.

JPS: When I started, I was looking around to see which lab I wanted to work in. Then I came to Hendrik, and he said: nanomachines. I thought of nanomachines as something you would see in a science fiction movie. But OK, you're already doing it? I guess I'm going to stay here. Sounds good.

HD: One of the many interesting aspects of this field is that we're not at a point where we have 99 percent of everything figured out, and the next goal is to figure out 99.5 percent. Here we are at one percent or so of the actual potential of the field, and so everything we're doing is a huge step. Of course it's hard work, and there's a lot of suffering involved, because there's so little known and it's easy to make lots of little mistakes. You don't always know what's going on. So you try to establish a machine that has whatever conformation and dynamics you want, and then you find it hard to prove that it works that way, because you have to first figure out the assays to analyze it properly. And then you encounter artifacts that have to do with the measuring techniques and things like that, so it's still a hard business. But the risk-benefit ratio is right: high-risk, high-reward.



108 **PSR:** What are some of the most interesting problems facing you now?

TG: For me the next step is definitely to build dynamic DNA structures. So far all of these close-packed, 3-D DNA nanostructures are static in nature. Therefore the next step would be to build dynamic devices out of DNA.

PSR: So this is getting to your flagellum machine.

TG: Exactly. The first step would be to build a switch-like structure in which you can change the conformation upon some stimulus, chemical or temperature or pH or something else external, and thereby change its structure.

PSR: Like an actuator or a relay.

TG: Yes. This would be the very first step if you want to build something like the flagellum motor or ATP synthase or the ribosome or all these other fantastic nanomachines.

HD: With the natural macromolecular machines, there are certain analogies – to macroscopic motors, for example. They have movable parts, and these movable parts can be shifted from different structural arrangements through stimuli, mostly chemical. In the macroscopic world, if there's a motor with pistons and cylinders, you need a crank to run it through the different configurations. On the nanoscale, the different

conformations are attained thermally, through fluctuations, but they can be biased depending on the binding or unbinding of small molecules. And that's a quality we still need to implement into these nanostructures so they can be made active.

JPS: We have this step, from design to shape, that works pretty well I think. And now – the actuator is one example – we're facing the step from shape to function. I think everybody is working on this in some way right now. And that's probably where the most interesting things will happen.

HD: Another challenge will be scaling up the fabrication. There's typically 500 milligrams of salicylic acid in an aspirin tablet. Suppose you had a DNA nanodevice that could act as a drug delivery vehicle, and you wanted to make a tablet with 500 milligrams of that substance. With current material costs, that would amount to around 150,000 euros for one tablet, and that's a conservative estimate. And using current equipment and procedures, it would take you about two months. I don't think reducing the cost of synthesis and scaling up the throughput would be particularly difficult. But it remains to be done, and it's another aspect holding us back from broader applicability in areas such as health and chemistry.

PSR: Thinking ahead a few years, or even a few decades, what are your hopes and expectations for progress in biomolecular nanotechnology?



TG: What I would like to see is a synthetic DNA machine that has the functionality of biomolecules, like these machines we have talked about.

TM: I expect that there will be medical applications for DNA nanotechnology. It could also play an important role through fast prototyping. That is, when we understand the whole process better, it should be a simple matter to place chemical groups precisely where you want them.

JPS: You could say that people are using nanomachines now to produce therapeutic substances, but we can't build them ourselves yet, you have to use bacteria. But bacteria are not made for that. It's not optimal. Once you can build your own nanomachine, then you might be able to catalyze reactions and produce whatever you want with higher efficiency because it's designed only for that.

TM: You might be faster in reacting for specific things. Medicine always takes a lot of time to make something new, but with this technology you might have a very fast way to design something very specific.

HD: Well, you know, I have a wild imagination, so I can think of all kinds of crazy stuff. Imagine you could build anything bottom-up, with atomically precise control. Everything. So you could build some sort of synthetic wood for example. In a way, even wood is atomically precise. It is cells made out of certain components



arranged in 3-D structure, and it's all self-assembly. Imagine you understood the whole thing. So you could build molecular structures that interact with other molecular structures, and you could program their assembly on a "tape," as in nature, like a genome. And they form, they interact with each other, they build something like an analogon of a cell, but it could be arranged a different way. It grows, it combusts raw materials, it makes duplicates of itself, and you make a tree for example that grows into the shape of a house. It builds itself, basically, based on energy input from light and raw materials it finds in the soil.

Maybe more realistic – I think it's certainly conceivable that in 30 years we can build something like artificial viruses. The word has a negative connotation because it's usually associated with disease. But imagine you could build a synthetic particle that has the functionality of a virus and is capable, for example, of killing bacteria that cause disease in your body. You build some sort of nanodevice that selectively seeks out cancer cells and gets rid of them. Something that is useful but as sophisticated as we find in nature. Or think about vats filled with membranes in which you have little rotating nanomachines powered by ion current, and the rotary movement is used to control mechanically induced chemical reactions. So then you have these nanomachines that take in raw material, like smaller molecules, and stitch them together into long polymers: It could revolutionize chemistry.

And maybe thirty years from today we'll have a number of applications where this plays a role, but not in a very noticeable way – where it's invisible because it has become common technology.