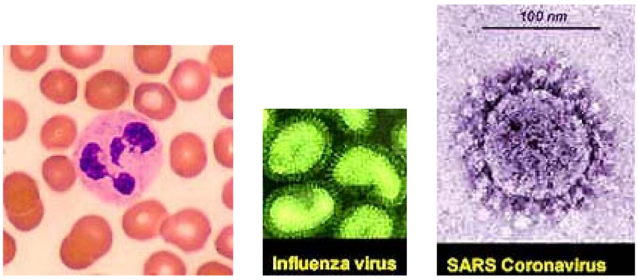
Bio-Nanotechnology Breakthrough: Building "Nanomotors" and "Nanostructures" with RNA



Left: Human red blood cells = 7 micrometers (microns). **Middle:** Influenza viruses = about 80-100 nanometers.
Right: SARS coronavirus = 100 nanometers.

1 micrometer (micron) = 1 / millionth of a meter (39.37 inches)
1 nanometer = 1 / billionth of a meter

August 21, 2004 West LaFayette, Indiana - When you enter the world of bio-nanotechnology, you are so small that if one nanometer were the size of a single pea, a regular meter of 39.37 inches would be the size of the Earth! In that nano world, virus sizes vary from 18 to 300 nanometers.

Earthfiles, news category.

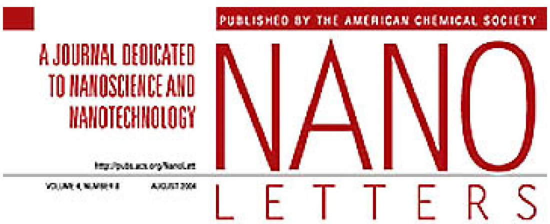
A team of scientists at Purdue University, led by Peixuan Guo, Ph.D. - a molecular virologist in Purdue's Cancer Research Center - have been studying what viruses do to invade and re-program cells in disease.

One particular virus that invades bacteria gets the bacteria to synthesize an unusual and powerful phi29 encoded RNA to build little motors so the virus can drive *its* DNA into protective protein shells that are then inserted into the bacteria and take over the bacteria cells' programming.

What's incredible is how the virus builds an organic motor to get its own DNA into the protein shell and into the bacteria. The virus gets six bacteria RNA molecules to come together in a ring. The scientists use the metaphor of six children linking hands. The virus is able to tell one RNA molecule to clasp its right hand to the left hand of another RNA molecule and to clasp its left hand to the right hand of another RNA molecule.

The little ring nano motor then surrounds the virus's DNA, apparently turning like a motor, which drives the virus's DNA into the protein shell that is inserted by the virus into the host bacteria.

Dr. Guo and his colleague, Dieter Moll, Ph.D., at Purdue's Cancer Research Center, have been able to copy the virus's creation of the tiny RNA motors and have been able to build the nanomotors in their laboratory to do what *they* want the RNA molecules to do. Recently, the research team has gone even further in breakthrough work that was recently published in *NANO Letters*, a journal dedicated to nanoscience and nanotechnology. Peixuan Guo's lab is supported by NIH (National Institutes of Health), NSF (National Science Foundation), DOD (Department of Defense) grants, and Dieter Moll is funded as an Erwin Schrödinger fellow of the Austrian Science Fund FWF.



Dr. Guo and Dr. Moll have been able to make RNA molecules build structures on a large scale that are several micrometers in size. That means the scientists have been able to modify what the virus does to make the nanomotors and go beyond to build with thousands of RNA molecules.

Before this RNA breakthrough, other scientists had been able to manipulate proteins and DNA, but not to make motors and delivery systems at this minute nano scale. All this means that humans working in the totally invisible microscopic world of viruses and RNA, and copying what the microbes do, might be able to deliver medicines directly into cells of diseased patients or to build molecular electronics that would be the first marriage of organic and inorganic, a marriage of the living with the non-living.

This week I talked with molecular biologist Dieter Moll, Ph.D., whose background was in protein bio-nanotechnology at the University of Agricultural Sciences in Vienna, Austria, before joining Dr. Guo's lab about two years ago.

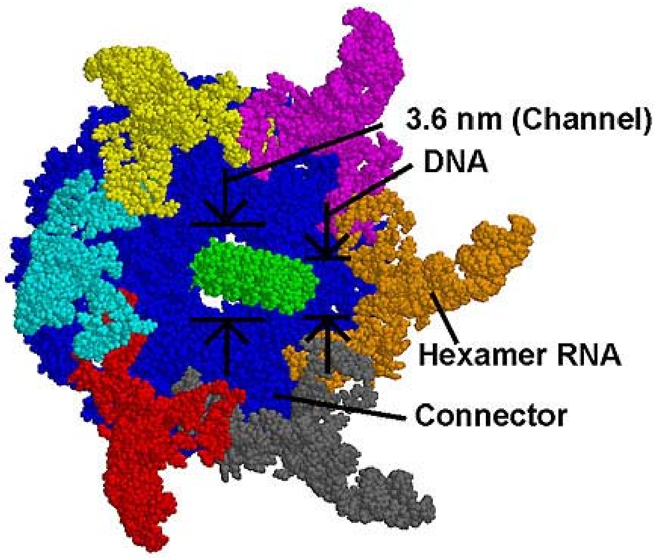
Interview:

Dieter Moll, Ph. D., Post Doctorate Research Associate since February 2003, Laboratory of Prof. Peixuan Guo (PAI-shun gwoe), Ph.D., who is Professor of Molecular Virology, Purdue Cancer Research Center, Purdue University, West Lafayette, Indiana: "Only recently, we've found out that if the virus can get such good use out of this molecule, then maybe we could get some good use out of it, too.

WHEN THE VIRUS PROVOKES THE RNA TO MAKE THIS MOTOR RING, HOW DOES THE VIRUS USE THIS 'MOTOR?'

This virus, when it infects a bacterial cell, it gets the cell to produce viral components, the next generation of viruses. These viral components have to come together and form a new, complete virus particle. Part of that involves an empty protein shell the virus has and it involves the DNA, a long molecule carrying the genetic information. this DNA molecule needs to go inside the protein head so it's protected there. This DNA molecule does not really want to go in there (empty protein shell). So, the virus needs a motor to be able to stuff the DNA molecule into the protein shell. This RNA molecule is an essential component of the molecular motor that does that job for the virus.

And what we think may be happening is that 6 RNA molecules will turn the connector and the connector will act like a hex nut driving the bolt, which is the DNA, into the protein shell.



"Nanomotor" that is naturally produced by a virus that infects bacteria. The virus wants to insert its DNA (green tube at center) into a protective protein shell the virus makes to invade a bacterium. The six colored "arms"
(yellow, magenta, orange, grey, red and turquoise) are six RNA molecules the virus manipulates to form "Hexamer RNA."
(The perspective is at a slight angle so the turquoise arm is curved up, not outward.) Each of the six RNA molecules
has a "right and left hand" which can link up with the other RNA molecules to forma ring, the Hexamer RNA. The dark
blue area around the green DNA is a protein plate called a "Connector"that connects the six RNA molecules, leaving a 3.6 nanometer (nm) "donut hole" called a "Channel."After the virus lines up its DNA with the 3.6nm hole, the Hexamer RNA is rotated around the DNA"like a hex nut would drive a bolt."

The "grains" throughout the 3-d computer model represent individual atoms of the biomolecules.
Graphic © 2004 by Peixuan Guo, Ph.D. and Dieter Moll, Ph.D.

WHERE THE 6 MOLECULES ARE RINGED TOGETHER, THE ASSUMPTION IS THAT IT IS TURNING. AND THAT WOULD BE THE 'MOTOR' DESIGNATION THAT WOULD BE MOVING THAT PROTRUDING DNA AT THE CENTER INTO THE VIRUS'S PROTEIN SHELL?

That's right. Another reason why this whole structure qualifies as a motor is because it uses energy. It takes ATP, which is molecular fuel of the cell the gasoline of this motor. It hydrolyzes ATP and converts the energy of ATP into mechanical motion. That's why we call it a motor.

Modifying Nature's RNA Motor Design for Human Bio-Nanotechnology

HOW DO YOU AND DR. PEIXUN GUO THINK THAT THESE NATURAL RNA MOTORS CAN BE USED IN HUMAN BIO-NANOTECHNOLOGY?

What we did was to modify these RNA molecules. On the virus, they form this motor structure by holding hands. We modified the hands of these RNA molecules. So, one particular RNA molecule cannot just hold hands with any other partner, but it can only hold hands with a particular partner. so, we can basically tell one RNA molecule, 'You take this other RNA molecule to your right and this other one to your left.' That way, we can tell each RNA molecule exactly where to go.

WHEN YOU CAN DO THAT, HOW DOES THAT HELP HUMANS AT THE NANOTECHNOLOGY LEVEL?

One of the big challenges in nanotechnology is to make defined architectures, defined structures that are at the nanometer scale. We can tell RNA molecules exactly where to go and in which position to arrange themselves and that is a way for us to make structures that have nanometer dimensions and properties.

GIVE ME AN EXAMPLE OF WHAT YOU THINK YOU COULD BUILD IF YOU COULD CONTROL THESE RNA NANOMOTORS?

First, let me explain what we have built already. We modified the hands of these RNA molecules so we can tell each RNA molecules which neighbor to choose. For this hand to hand interaction, the body and legs of the RNA molecules are not really required. So we can change them. It doesn't matter what they look like. So, what we did was to make one RNA molecule and give it a very long leg and tell it, 'You go to the right of another RNA molecule that has got its legs chopped off. And go to the left of another RNA molecule that has its legs fused together. So, that way we can create a variety of shapes and sizes of molecules, molecular assemblies, at the nanometer scale.

Beyond Building RNA Nanomotors to Building RNA Nanostructures

So far, it's not really useful because it's only long or short legs or something. But the next step we are working on right now is to make one RNA molecule that holds, for instance, a nano particle and another that holds a nano wire. Then if you can make RNA assemblies that will line up, for instance, a nano particle with a nano tube and a nano wire, then that gets very interesting and elegantly surreal nanotechnology.

I SEE, AND WHEN YOU USE THE WORD 'WIRE,' YOU'RE NOT TALKING ABOUT ORGANIC PROTEIN CHEMISTRY. YOU'RE TALKING ABOUT SOMETHING LIKE A VERY FINE COPPER WIRE THAT THE RNA NANO MOTOR COULD BE USED TO ASSEMBLE A COPPER WIRE WITH SOMETHING ELSE?

It wouldn't be a copper wire. Let me stress here that nanotechnology is always an inter-disciplinary approach. We need the input from nanotechnology from other fields such as material science that can make nano wires out of semi-conductors or who can handle nano tubes. Then we can link these components that other nanotechnologies make to our RNAs and use our RNAs as a scaffold to assemble these nano components in a defined way.

SO, HUMANS ARE COPYING THE VIRUS WORLD THAT USES RNA TO ASSEMBLE THINGS FOR VIRUSES.

Exactly. And the reason that molecules of life just work on a nanometer scale and work very well on a nanometer scale is because they have learned to do that over billions of years. For that reason, we are turning to the biological world for inspiration for nanotechnology. But also to take the actual building blocks. We are recruiting the building blocks that this particular virus has already developed and we are taking those building blocks and all we have to do is modify it. And get it to do what we want it to do, rather than what the virus wants it to do.

Organic Computers?

IS THE IMPLICATION THAT WE COULD END UP WITH COMPUTERS MADE OUT OF ORGANIC MATERIAL?

I think there are several different applications here. One is to use these nanostructures in molecular electronics, basically to try to make electronic devices now by different methods on a much smaller and more powerful scale.

Another issue is that these nanostructures are the same size of the molecules of life. So that is the reason they are also very good at interacting with living cells. So, another possible application is in the biomedical fields where we can use these nanostructures for example, as bio-sensors or eventually to connect our microscopic world and computerized digital world with the living world, with cells and establish a communication between cells and our microscopic world. It can be a two-way communication. We might be able to get information out of the cells and the cells will be able to tell us how they are. It might also work the other direction. We could get information into the cells and tell them what to do in a new sort of way.

RNA Delivery Structures Can Target Specific Cells for Medical Treatment

CAN YOU GIVE ME AN EXAMPLE OF SOMETHING YOU HAVE DONE ALREADY THAT WOULD SHOW PROMISE OF USING THIS KIND OF TECHNOLOGY TO TREAT SOMEBODY? OR HEAL SOMEBODY?

Yes, we targeted a gene that played a role in cancer and by knocking out this particular gene, we were able to kill cancer cells in the lab. But it's not working in a patient yet.

BUT THAT WAS STILL WORKING WITH RNA AND MAKING THE RNA MOLECULES DO WHAT YOU WANTED THEM TO DO?

Yes, exactly. That is based on assembling RNA superstructures in a defined way that we can control by defining this hand-in-hand interaction we have been talking about. By targeting these RNA nano complexes to cells and letting them to do a particular job in the cells.

YOU'RE SEEING THAT NANOTECHNOLOGY RNA MOTORS COULD BE USED TO DELIVER MEDICINE OR PERHAPS EVEN TO CORRECT A DNA PROBLEM ON A LOCALIZED LEVEL THAT WOULD BE STILL TREATMENT, AS OPPOSED TO A UNIVERSAL STEM CELL CORRECTION OF ALL DNA IN ALL CELLS?

Yeah, that is right. There is a new technology that is under hot debate. It works very well in the lab. It is based on RNA molecules that can regulate gene expression and knock out a particular gene. This works very well in the lab and now people are trying to use it for therapeutic applications and in therapy, the big issue is how are we going to get these RNA molecules that are going to do the healing job into the cells? How are we going to target cells? And how are we going to deliver the functioning RNA sequence? We believe with our RNA nanostructures, we have a targeting and delivery vehicle because we can hook up a functional unit that can target a particular cell for instance, a cancer cell and connect this targeting RNA molecule with other RNA molecules that can hold the cure against the problem that the cell has.

YOU COULD TARGET AN ENTIRE TUMOR IN THE BRAIN OR LIVER OR PANCREAS THAT ARE VERY DIFFICULT AND USE THIS TARGETING MECHANISM WITH THESE NANOMOTORS TO DESTROY JUST THE CANCER CELLS?

We are hoping in the future that may be possible. In the moment, we are just working with cell structures in the lab on a small scale. We are making the initial steps to see what we can do with this with these nano complexes. But the first results are very promising.

Large RNA Nanostructures for Molecular Electronics: Mixing Living with Non-Living

WHAT WOULD YOU PERSONALLY LIKE TO SEE BE ONE OF THE RESULTS IN ALL OF YOUR WORK?

I would like to see that eventually that we can use our RNA nanostructures as scaffolds to make functioning nano devices. That could be nano devices that work in molecular electronics. Or they could be nano devices that work to help cure diseases.

IF YOU ARE WORKING IN ELECTRONICS, DOES THAT MEAN THAT IN THE FUTURE THERE WOULD BE A MARRIAGE BETWEEN THE ORGANIC RNA WORLD AND THE INORGANIC MINERAL WORLD?

That is what we are aiming at, yes. We have already got preliminary results where we have managed to link inorganic nano particles to RNA so that is the first step in linking the living world with the non-living world. In the long run, we are hoping to establish a communication between the living and the non-living world.

ONCE YOU DO THAT, HOW WOULD YOU SUSTAIN THE ORGANIC RNA STRUCTURES MEANING THAT'S LIFE THAT HAS TO HAVE SOME KIND OF FOOD AND ENERGY WITH THE INORGANIC WHICH DOES NOT?

There are two different scenarios. One is that they would use these structures just for micro-electronic applications. In that case, the job of the RNA would be to act as a scaffold to arrange nano components and after that, the job of the RNA is done and it can be replaced with something that is much more durable and sturdy.

The other scenario is that we use the nanostructures in cells, in cellular backgrounds, and then the cells would be doing the job of maintaining the organic molecules.

IT'S REALLY INCREDIBLE ISN'T IT?

Yeah, it's a hot area and a lot of things can be done. The most recent progress was that we got these RNA nanostructures to assemble on a large scale to form structures that were several micrometers in size. That is important because it matches the size range that we can achieve in conventional micro structuring technology. The way we did that was to fuse individual RNA building blocks together so that we form a twin that has two sets of arms. So, with that architecture we are not limited to building a finite ring of RNA molecules. But we can get the RNA molecules to assemble infinitely and form very large structures. And we got structures that are composed of several thousands of molecules and they are several micrometers in size.

THE RNA WAS ABLE TO ASSEMBLE EXACTLY THE WAY YOU WANTED IT TO ASSEMBLE?

That's right. By designing the RNA molecules in a particular way, we were able to tell the RNA molecules how to assemble and form the structures that we wanted to form. But we cannot completely bend the arm of Nature. We just have to redesign the biological building blocks so that they assemble in the way we want. There are not unlimited possibilities. We have to take into account what Nature wanted these molecules to do."

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http://pubs.acs.org/journals/nalefd/

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