# CORONAVIRUS – Part Eight: 5G, TONY PANTALLERESCO, TERAHERTZ, NANO,COVID-19 CODED



Update on India: I have got permission for other villages but not for the village where we have hostel I have applied for it as soon as I will get the permission I will let you know and sister. They have to get permission from their district Magistrate. Give my love to family there and to all Three Heart Congregation. With Christ love Pastor Dalbeer Singh and family **He has not been able to go to the hostel children at all since the lock down started.**
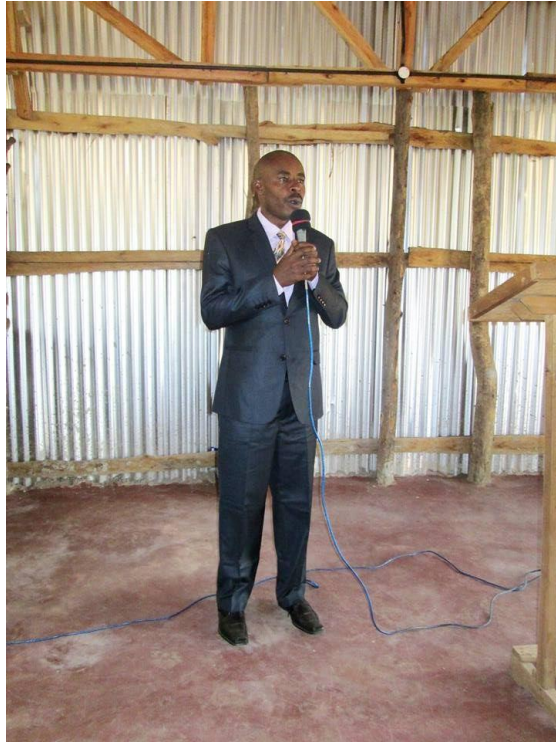
Update on Africa: Hello Pastor, praise our Lord and savior Jesus Christ.

God has continued to keep us safe. Kindly am requesting the team to pray with us, it's raining heavily here. It started raining last month, the low lands are now flooded and many families have been displaced, crops swept away. In some parts there is land slides burying some people and property. Most of our rivers are now overflowing, roads in a total mess now full of mud , potholes and water making them impassable especially here in the villages.

It's raining day & night. Our pit latrine is now filled with water now overflowing, same with our dug water well. Run-off water now flowing in our house through the mud walls. Not only us but many families are affected even some houses have collapsed.  I am thinking of moving my family to our village home on Monday which is a bit better than here. The weather people are telling us it will continue raining likely for another one month or more.

Despite all this we have faith in God that he will protect us.

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I did talk with Pastor and asked him if he was hearing a lot of loud and heavy booms.  He said yes, lots of them.  I told him I felt like it was probably the luciferians with their weather weapons.  They had just been hurrying to install 4g everywhere there at the beginning of the lockdown.  And Bill Gates has been saying that the developing nations like Africa and India were going to be hit very hard because of the coronavirus.  So if you are awake you can read between the lines there and know that they are trying to really take them out.  Plus, they can supposedly predict the weather for 1 month or even 2 in advance now?  They haven't been able to do that where we are.  But if you are the one causing the weather, then you would certainly know what your plans are.

Please keep our outreach ministries in prayer.



Millimeter waves are utilized by the U.S. Army in crowd dispersal guns called Active Denial Systems. Dr. Paul Ben-Ishai pointed to research that was commissioned by the U.S. Army to find out why people ran away when the beam touched them. "If you **are unlucky enough to be standing there when it hits you, you will feel like your body is on fire**." The U.S. Department of Defense explains how: "The sensation dissipates when the target moves out of the beam. The sensation is intense enough to cause a nearly instantaneous reflex action of the target to flee the beam."

It uses radio frequency millimeter waves in the 96GHz range to penetrate the top 1/64 of an inch layer of skin on the targeted individual, instantly producing an intolerable heating sensation that causes them to flee.

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5G operates on millimeter waves.   Remember Kay Griggs said the military runs the government.





Oxygen 65% and hydrogen 10% are predominantly found in water, which makes up about 60 percent of the body by weight.  It's practically impossible to imagine life without water.

Hemoglobin is the protein inside red blood cells.  It carries oxygen.

Red blood cell

Each red blood cell contains several hundred million hemoglobin molecules which transport oxygen

Hemoglobin molecule

Heme

Oxygen binds to heme on the hemoglobin molecule

✿A.D.A.M.
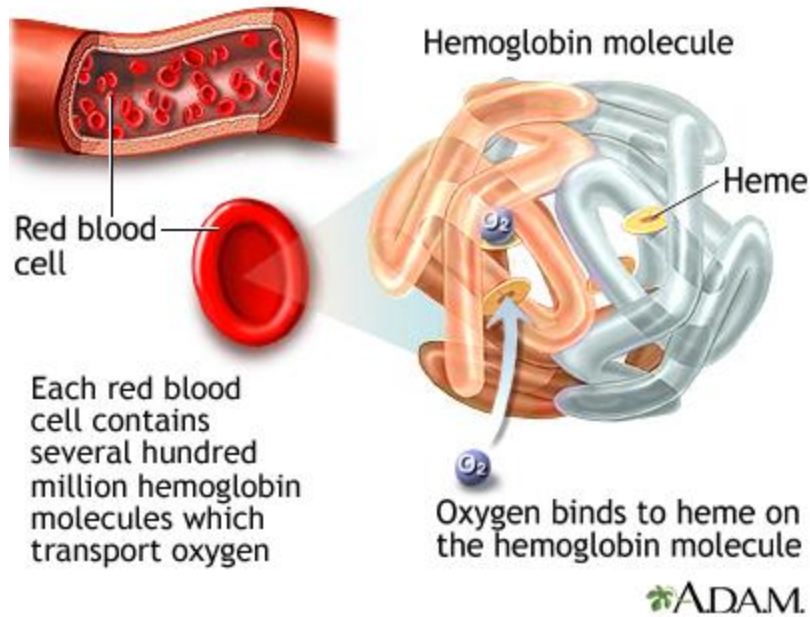
Dr. Thomas Cowan said that 5G at 60 GHz is a water destructuring device.
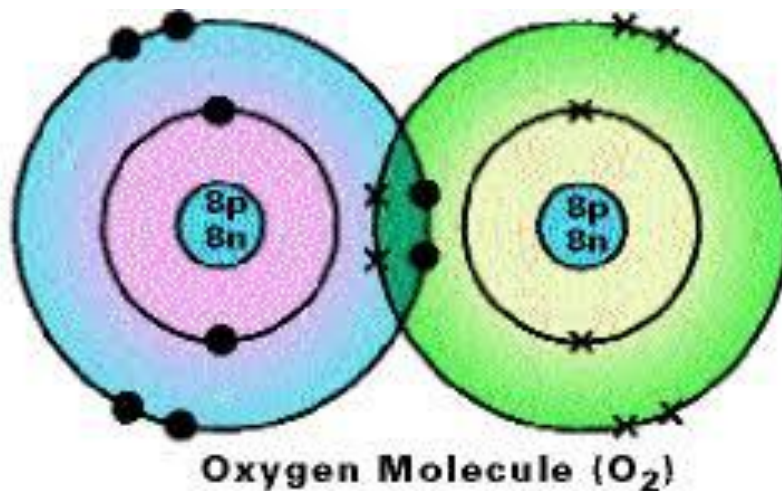


Oxygen Molecule ($O_2$)

Oxygen the atom is O. Oxygen the molecule is $0^2$. Two atoms together. These two atoms form the oxygen molecule and share some electrons. 60 GHz causes electrons surrounding oxygen molecules to spin, akin to how high-powered microwaves running on 2.4 impact molecules in food such as water. They're heating, in part, by impacting those molecules to rotate or oscillate with each wave. The movement energy from the rotation of these super tiny water molecules helps heat the rest of the food. In a similar way that 2.4 causes $H^20$ to

oscillate, 5G/60 GHz even at low power causes electrons on oxygen molecules to spin; changes to the spin frequencies on oxygen electrons impact human biology.

The *impact of oxygen molecules spinning the electrons is that it makes the hemoglobin unable to uptake the oxygen and get it to the rest of your body.*

The oxygen is contained in the water, and the hemoglobin inside the red blood cells carries the oxygen, now you can better understand how 5g at 60 GHz is damaging the very ability to breathe. At 60 GHz oxygen absorbs 98% of the energy – or frequency.

If you were in a city or a place that was covered in 5G, and they switched it on to 60 GHz, not only would it weaken your immune system, but can also produce virus like effects and the type of respiratory problems we've been hearing about with coronavirus.  One guy in my research was pointing out that at 60 ghz you can also have heart attacks, strokes, problems with muscles etc. because of lack of oxygen.  It doesn't travel far and that is why they are putting up so many transmitters.

At the molecular level, these frequencies affect the orbit of electrons, and that affects the ability of blood hemoglobin to bind with oxygen. If blood cannot hold oxygen, the result is death by suffocation. In short, 5G technology has the potential of being used as a weapon to quickly weaken, disable, or destroy individuals or populations.

The luciferians are successfully militarizing the air around us with their weaponized millimeter frequencies. The whole world is working to install 5g and beyond.

God breathed life…

**Gen.2:7-** And the LORD God formed man *of* the dust of the ground, and breathed into his nostrils the breath of life; and man became a living soul.

As I worked on this, this week, I had this thought about what I had learned about 5G and the 60 Ghz range: satan wants to take the very breath of life out of us by using these militarized frequencies starving us of oxygen.
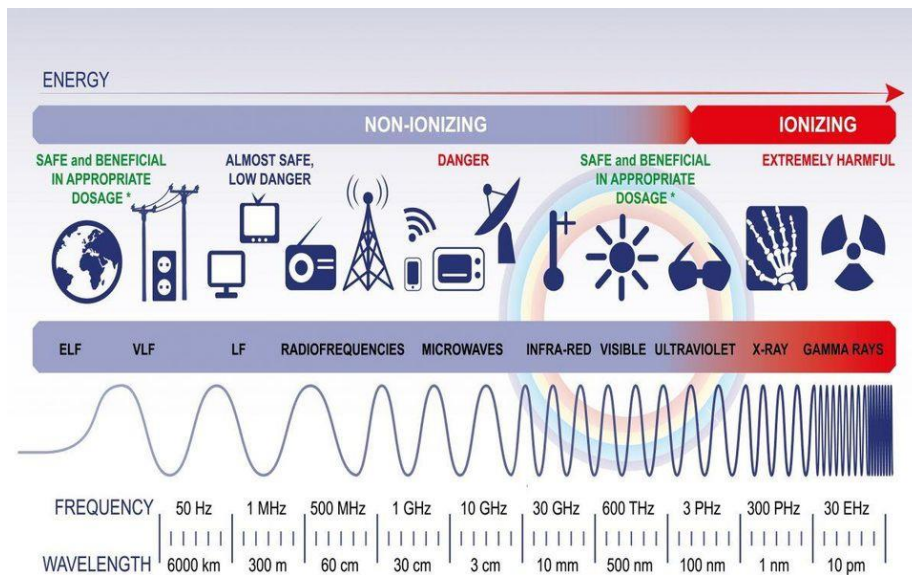


Just past the middle of this chart a little more to the right you will see where 30 GHz is marked.  So a little past that is where 60 GHz would be.  Just starting to get into the infra-red frequencies.  Note that the THz is even a little past the GHz range.

Remember what Barrie Trower said, "5G gives them all the information about you that they want.  It goes two ways, it can be used to harm you and it can be used to spy on you."

https://twitter.com/KeriHilson/status/1239355228291465216

**Keri Hilson** @KeriHilson

People have been trying to warn us about 5G for YEARS. Petitions, organizations, studies...what we're going thru is the affects of radiation.

5G launched in CHINA. Nov 1, 2019. People dropped dead. See attached & go to my IG stories for more. TURN OFF 5G by disabling LTE!!!
https://pbs.twimg.com/media/ETMS-4QXsAA0J5m.jpg

Twitter | Yesterday at 8:58 PM (121 kB) ▾

**Keri Hilson** ✔
@KeriHilson

Why do you think the virus is not happening in Africa like that? Not a 5G region. There may be a few bases there, but not as prevalent as other countries. It has nothing to do w/ melanin (for those theories)...

I did see a website that was basically trying to bash and discredit her for speaking out. At this stage in the game we know they are running their false flag on the whole world. They are reporting fake statistics and propaganda like crazy. I can tell you that at the start of all of this Pastor Erustus said there was a big push there in Kenya to get 4g installed. To me that is so they can hurry up and get them on 5g or higher. For those of you who have been watching truther videos, you know that they are just calling most deaths a covid-19 death right now to pad the numbers. That's a big part of why you now have numbers reported from everywhere. All countries are working together on this because they have one goal in mind and that is a Global Government or New World Order.

Some have speculated that during the lockdown they were installing 5G in the schools. One lady left her house and went to check on the nearby school. Let's see what she found.

*Please play this video – Lady drives out to the school while we are all on lockdown and discovers that they are installing 5G*(about 1.5 min's)
https://www.youtube.com/watch?v=zDe-RQmdef0&feature=youtu.be



Tony doesn't believe it is 5g. He thinks it has to do more with the THz. If you are not familiar with Tony Pantalleresco I suggest you listen to his podcasts, watch some of his youtube videos. He is trying to make people aware of the assault that the luciferians are doing to the entire world and help you to deactivate the nano and flush it out of your body. You may have to listen to his information a few times just to be able to process the information he is giving. It is deep and scientific and it is the world we are now living in.

All of his links are on my sermon notes and in the info. box on youtube. When you go to his podcasts, look at the research. He has put so much time in to trying to educate and share his research. I talked with Tony about all of this so that I could understand in layman's terms a simple explanation. He is living this stuff and can explain the nanotechnology and frequencies better than I can. So I will be sharing what we talked about as well. In addition to the research on his website and podcasts, he sent a ton more to me and that is here for you as well. I

am also sharing a link to AROY MAK's youtube channel, all of Tony's podcasts are housed there.

*Please play from 28:57 to 30:25 Came from snakes, then bats etc.  Tony says it's not even an epidemic, not even a virus, it's an infection created through synthetic biology activated by ThZ frequencies, that's where this is all coming from.*(about 1.5 min's)

https://independz.podbean.com/e/tonys-show-03072020/

*Please play from 26:49 to 30:48  and from 31:54 to 36:56   5G, 6G and 7G the perfect weapon, bunching terra hertz frequencies and releasing them causes a lot of damage on the inside.  You won't see it externally but  you will feel it internally.  This is the perfect killing machine.  If they hit you in the head you can get a terrible migraine out of nowhere.  Could be as a result of terahertz exposure.  Tony is stating about the 6G and the terahertz so that once everyone wakes up to this they will have a reference point to look back and see when it really began.  This allows them to more powerfully target whoever they are targeting.  This is the type of weapon that can simulate a virus outbreak.  For the person or people who were targeted, if the components of the viral outbreak have been injected or consumed then the frequency has the ability to zero in on whoever did what or ate what, cause or simulate the effect of what you would think would be happening if someone had the flu. We're talking about nanorobotics.  It may be setting up tiny little program nodules in the system to further expand and network.  Could be using things like quantum dots could be using things like dendrimers, origami, any of these things that can create a platform to create a bigger connection in the system (your body).  When you're looking at self replication of robots, robots that can just repair themselves and copy themselves and make more clones of themselves in order to follow it's directive – nanotechnology does this.  This is what they are spraying on us through the chemtrails. /Tony does  not recommend vaccines because they can cause genetic damage or augment a program into the child. Tony explains how when you are in a group of people sitting in a room somewhere and everyone has their cell phones, then frequencies are shooting all around that room and through each

other.  Everyone is getting hit with the frequencies. If you are in the car and people are using their cell phones, the magnitude of the frequencies is increasing and hitting everyone in clusters, if there is more than one cell phone in use.  And if they are operating on the terahertz band width, they are gonna be literally burning holes inside of you.  You won't see it on the outside of the skin. We should not be that addicted to these cell phones but we are addicted to them because of the biometrical programming that they have.  How they attach themselves to that person.  5G operates in a straight line.  It doesn't have the penetration power.  He says it could not do much to most people.  5G would not penetrate, not strong enough but 6G with the terra hertz can go right through a person just like an x-ray and leave no evidence except to drop its pay load into the person to activate the program.  The radiation is going to affect the genetic code. This is one of the biggest things you have to protect is the genetic code, the mitochondria, the ATP, you have to protect yourself because they want to break you down in this fashion (frequencies and nanotechnology). *(about 9 min's)

https://independz.podbean.com/e/tonys-show-03092020/

He is talking about the synthetic virus being released through something in their food in Wuhan or through a vaccine they were given laced with nano and metals. Then they hit it with a frequency and it activated and ran its program.


*Please play from 21:28 to 24:40  and from 27:57 to 30:35 Tony talks about if Canada gets approval for mandatory vaccines they will have chips or implants in the vaccines.  They will be injecting implants into everybody. Those implants will be subjected to any kind of frequency access to the body.   You can create a virus inside a body once the building block material has already been entered into the body.  And by adding a vaccine to our physiology will provide those proteins that are required for construction. The vaccine will provide the dna that is required for construction.  And because we have nano particles already inside of us, we are saturated with them, these nano particulates will then embed with the proteins and then start forming whatever the program is telling them to do. The protein, nano, and dna will work together to do whatever the "program" tells it to do. This

is how genetically modified seeds are being done as well.  They open up the seed, they embed a nanoparticle with a program, it then gets embedded into the protein and then starts creating an assemblage in the seed to create whatever dynamic or dysfunction they want to put into that seed.  So that as it grows and progresses it produces the component that is in it.  You are nothing more than a seed, a husk, loaded with protein and dna, enzymes and other things that cooperate in a certain coordination that now can assemble and build whatever is put into the body.  When our bodies break down nutrients and different things it starts to assemble and construct and build whatever is required or needed to sustain the body's health.  When you put in anything, good or bad, it is going to cause a chemical reaction.  With these proteins that we are consuming, and with all the nano loaded with carbon.  Once nano metals embed into the brain they start forming an assemblage of circuitry inside the brain and then can receive transmissions from any kind of frequency that gets shot at it.  Your phone is the conduit that is closest to you.  You can't put the phone down you can't shut it off.  You are always waiting for a phone call that doesn't happen.  Because the phone is talking to you, talking to your mental, to your subconscious, talking to your dna.  This is the hard reality. /Tony has been saying this whole thing is a hoax.  It is a fake pandemic just like all the other fake pandemics.  He said all the wars have been fake, not really about what we were led to believe they were about. It only took them 200 years to work their plan to dumb down the people to where they have no mind skills whatsoever.  Critical thinking is gone. The media can say anything they want and the people will believe it.  There is no right or left, no democrat no republican.  There is no vote, that is just an illusion.  There is absolutely no information whatsoever that shows that vaccines work.  He shares to watch about vaccines on youtube, a video from the 80's.*(about 5.5 min's)

https://independz.podbean.com/e/tonys-show-03132020/

*Please play from 5:37 to 6:22 and from 16:03 to 18:35 and from 21:24 to 22:16 If someone has the flu and you are staying in the same proximity as them, sooner or later you get it too.  Tony says the real coronavirus is nothing more than the common cold.  Says they have no test for the coronavirus because the one they are talking about doesn't really exist.  So what are they testing for? Their tests are

for tuberculosis and pneumonia./More people die from smoking cigarettes and from heart attack than from coronavirus. The chance of you getting this is slim to none because it is not even a real pathology, it is a synthetic one. Has only affected Asians in China. And it has killed them there because of the terra hertz band width that they were using. They are counting all deaths as a coronavirus caused death. If you had a hang nail, it is coronavirus. If you had a heart attack and died, it was the coronavirus. If you swing with monkeys and die, it was from coronavirus. If you were swimming in a lake with the serpents, you have coronavirus. They are not showing any dead bodies. They are making up diagnosis. / The media has fed you fear and now if someone comes along and says it is just a hoax you can't get your mind around it because fear has gripped your heart. And as long as you are in a state of fear you are going to respond in a fearful manner. Fear and faith are opposite sides of the same coin. Faith is a belief that can set you free and fear will keep you trapped in the quagmire that you are believing in. It is time to step up to the plate and have a little faith. You might hit a homerun. If you don't step up to the plate you don't hit nothin'.*(about 4 min's)

https://independz.podbean.com/e/tonys-show-03162020/

**Tony shared a video link in the research to this podcast that was about pathologies being activated by frequencies. Youtube has removed it.**

Tony mentioned a mandatory vaccine just ahead of the coronavirus outbreak. He said it was targeted to the Asians. Remember the freemasonic meeting held in 2010 that Bill Ryan shared about, where they said that "China would catch a cold."

*Please play 6:53 to 9:35 - Dr. Thomas Cowan on 5G talking about injecting metal into bodies through vaccines and having the perfect storm because the metals help to make us even more conductive to the frequencies.*(about 2.5 min's)

Our bodies are antenna's already and now they have been spraying metals on us, injecting them through vaccines, putting them in our medicines and food and even our clothing.

Youtube is censoring like crazy!!!

This video is unavailable.
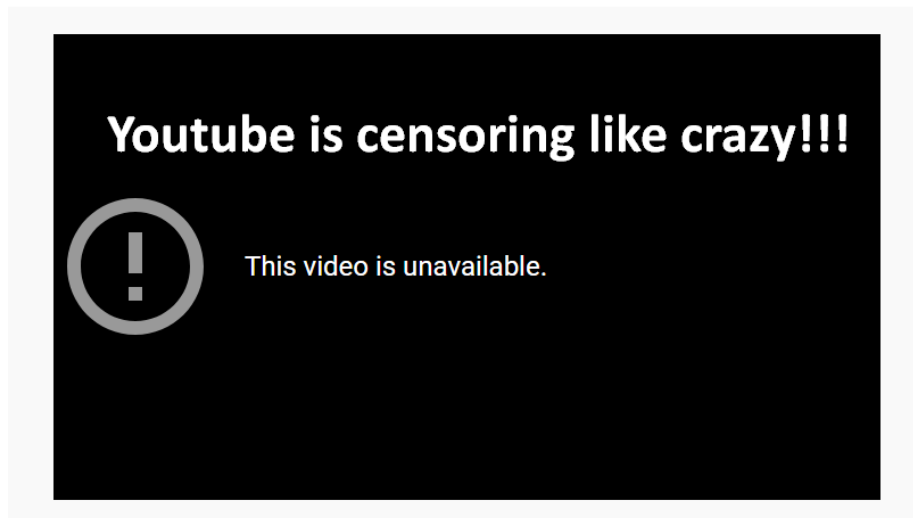
Tony shared the above video.  I did find it again, let's have a look:

*Please play from 4:07 to 4:25 and from 5:42 to 5:57 and from 9:22 to 9:33 and from 11:53 to 12:40  Shows many people in China just falling over and many are shaking like they are being electrocuted.*(about 1.5 min's)

https://www.youtube.com/watch?v=NbmCXiVUMHc

These people don't look to me like they were sick and not feeling good.  You don't get dressed up and go to a casino if you are that sick.  You don't go out in public and stand in the street.  These people were just up walking around and the next minute they fell straight over.  Some of them were vibrating or shaking like they were getting shocked or electrocuted.  This looks like weaponized frequencies. We will get a little deeper into this part next week.

**CLOSING**

Thank you Youtuber Sheri Leyva for sharing the following with us:

Covid 19.  C=3 in gematria. In Strong's Concordance the number 3 is Abaddon. Definition: Abaddon, the angel of the abyss. (that is satan)

Ovid is derived from the Latin name 'Ovidius,' itself derived from the Latin word 'ovis,' which means 'sheep.'

In Strong's Concordance the number 19 is ibchah.  Definition: Slaughter

Covid-19 literally mans: The angel of the abyss slaughter sheep.

You might think this person is reaching, but they play these little games.  Always using symbols, colors, numbers, nature, dates, events, coding etc.

**Romans 8:31-39 -** What shall we then say to these things? If God *be* for us, who *can be* against us? He that spared not his own Son, but delivered Him up for us all, how shall He not with Him also freely give us all things?  Who shall lay any thing to the charge of God's elect (who will bring any charge against those whom God has chosen)? *It is* God that justifieth. Who *is* he that condemneth (no one)? *It*

*is* Christ that died, yea rather, that is risen again, Who is even at the right hand of God, Who also maketh intercession for us. Who shall separate us from the love of Christ? *shall* tribulation (trouble), or distress (hardship / calamity), or persecution, or famine (or hungry), or nakedness (destitute), or peril (danger), or sword (or threatened with death)? As it is written, For Thy sake we are killed all the day long (we face death all day long / we are killed every day); we are accounted as sheep for the slaughter(considered sheep to be slaughtered). Nay (No), in all these things we are more than conquerors through Him that (Who) loved us. For I am persuaded (convinced), that neither death, nor life, nor angels (fallen angels), nor principalities (demons), nor powers (not even the powers of hell), nor things present (fears for today), nor things to come (nor our worries about tomorrow), Nor height (no power in the sky above), nor depth (nor in the earth below), nor any other creature (nor anything else in all of creation), shall be able to separate us from the love of God, which is in Christ Jesus our Lord.

I chose this passage because it plays into what was revealed by the youtuber. However, Jesus was the One Who was slain so we could be set free and forgiven of our sins. He is the spotless Lamb of God.  And through Him we are more than conquerors!!  Online, people refer to the ones who are asleep as sheeple, they call the ones that go along with whatever the luciferians dish out, sheeple.
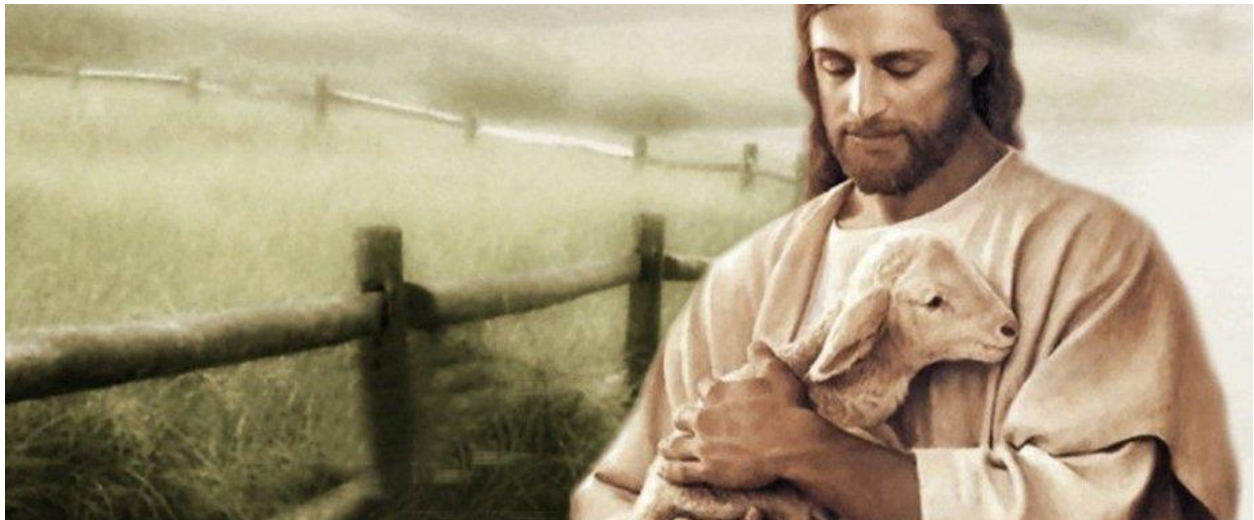


Well I am a sheep of the Lord Christ Jesus.  And He said do not fear for I am with you.

**PRAYER**

PROOF! 5G Being Installed in Many Schools
https://www.youtube.com/watch?v=zDe-RQmdef0&feature=youtu.be

ɥʇnɹʇ ǝɥʇ snɹᴉʌʌ ƃ u o ɹ o ɔ Part 2
https://www.youtube.com/watch?v=NbmCXiVUMHc

What happens with the 5G frequency of 60GHz to the Oxygen and to our biology!
https://www.youtube.com/watch?v=xLFggUbMWjc&feature=youtu.be

https://www.rfsafe.com/5g-network-uses-nearly-same-frequency-as-weaponized-crowd-control-systems/

Oxygen and hemoglobin research
https://needtoknow.news/2018/06/will-5g-technology-bring-human-extinction/

More on Oxygen and hemoglobin research
https://stop5g.cz/us/category/health-risks/


**LINKS FOR EVERY PART:**

Dr. Tent shares about Vaccines
http://www.threeheartschurch.org/index.php/sermons/sermon/243-stop-the-vaccinations-they-are-injecting-us-with-cancer-and-viruses

Vaccination MUST SEE! - The Hidden Truth - Australian Documentary (FULL)
https://www.youtube.com/watch?v=P9CGfYPXDKg&t=4228s

Weather and Chemical Warfare
http://www.threeheartschurch.org/index.php/sermons/sermon/256-weather-and-chemical-warfare-part-one

Promotes using vitamin C against coronavirus and as a preventative
http://doctoryourself.com/

Coronavirus AID SUPPORT Possible cure High doses of Vitamin C Covid 19
https://www.youtube.com/watch?v=g6HCrzGY2kM&feature=youtu.be

Tony's website

http://augmentinforce.50webs.com/

Tony's Youtube Channel

https://www.youtube.com/user/HerbsPlusBeadWorks

Tony's technical stuff

https://www.bitchute.com/channel/independz/

Tony's podcasts *Tony said to start with 3/7/2020 podcast and go to 3/17/2020*

http://independz.podbean.com/

https://independz.podbean.com/e/tonys-show-03072020/

https://independz.podbean.com/e/tonys-show-03092020/

https://independz.podbean.com/e/tonys-show-03132020/

https://independz.podbean.com/e/tonys-show-03162020/

https://independz.podbean.com/?s=Tonys+show+03172020

https://blog.iyannis.com/tony-pantalleresco-2020-03-18-immune-system-booster-and-more-on-viruses-and-namo/

Aroy Mak's youtube channel houses all of Tony's podcasts

https://www.youtube.com/channel/UCoGv8Zjgf4VXWIOOfb-uoAw

**RESEARCH TONY SHARED TO ME WHEN I BEGAN BUGGING HIM FOR A SIMPLIFIED WAY TO SHARE:**

## Bio-Nanorobotics — A Field Inspired by Nature

A nanorobot is essentially a controllable machine at the nanometer or molecular scale that is composed of nano-scale components and algorithmically responds to input forces and information. It explains the mimetics at two levels when nano scale is considered. One is the "machine nano mimetics" principle meaning the creation of nano-machine components inspired by the equivalent machine components at the macro-scale and the other is the "bio nano mimetics" principle where biological entities such as proteins and DNA are used to create the nano-machine components.
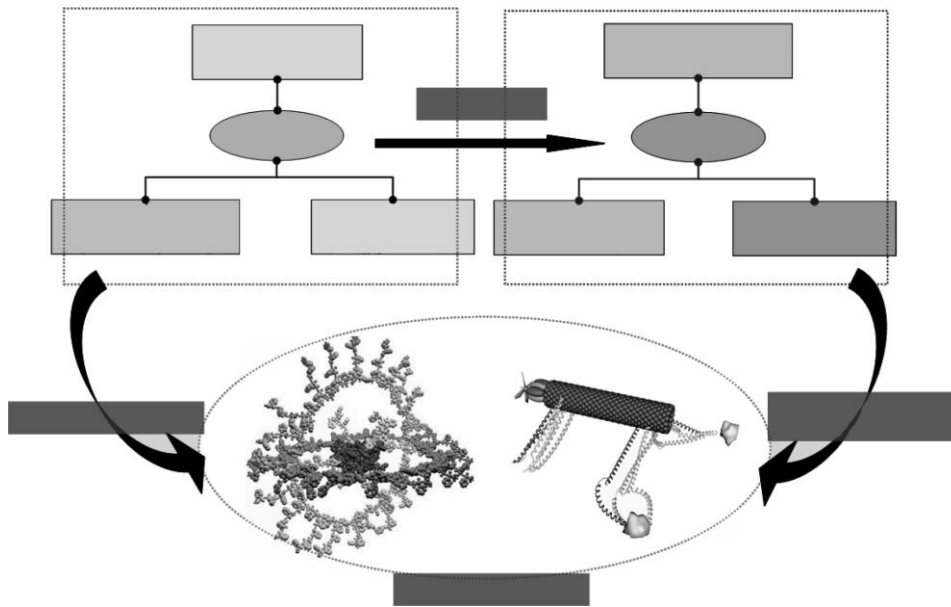


Figure 7.1 (See color insert following page xxx) Biomimetics — bio-nano robotics, inspired by nature and
machine Materials --*(proteins and DNA) De*sign and Mechanisms (revolute joint, actuators)
Characteristics
*(self-assembly, selfreplication,self-healing)*
Processes
*(sensing, actuation, energy production)*
Usability range
*(applicability in diverse enviroments)*

Characteristics
*(durability, rigidity)* Machine
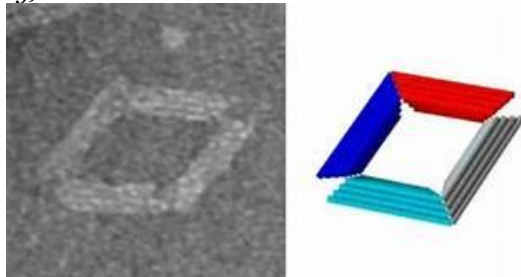


**This is a lattice the pre form for the origami** to overlay to build a circuit board for the assembly of the fullerene network to allow quantum dots bots and other carbon nano to move through in it's network

The field of nanorobotics studies the design, manufacturing, programming, and control of the nano- scale robots. This review chapter focuses on the state of the art in the emerging field of nanorobotics and its applications and discusses in brief some of the essential properties and dynamical laws which make this field more challenging and unique than its macro-scale counterpart. This chapter is only reviewing nano-scale robotic devices and does not include studies related to nano-precision tasks with macro-robotic devices that usually are also included in the field of nanorobotics (e.g., AQ1 ATMs and other forms of proximal probe microscopy).

**Nanorobots would constitute any active structure (nano-scale) capable of actuation, sensing, signaling, information processing, intelligence, and swarm behavior at nano-scale. These functionalities could be illustrated individually or in combinations by a nanorobot (swarm intelligence and cooperative behavior). So, there could be a whole genre of actuation and sensing or information processing nanorobots having ability to interact and influence matter at the nano-scale. Some of the characteristic abilities that are desirable for a nanorobot to function may** include:

**(i)**
**Swarm intelligence — decentralization and distributive intelligence;**
**(ii)**
**Self-assembly and replication — assemblage at nano-scale and "nano-maintenance";**
**(iii)**
**Nano-information processing and programmability — for programming and controlling nanorobots (autonomous nanorobots);**
**(iv)**
**Nano- to macro-world interface architecture — an architecture enabling instant access to the nanorobots and its control and maintenance;**

Comment [i2]: Complex nanodevices can be devised and designed through biomimetics. Bio-nanorobotics, namely biomolecular robots, represents a specific class of nanorobots where proteins and DNA could act as motors, mechanical joints, transmission elements, or sensors. If all of these different components were assembled together they can form bio-nanorobots with multi-degree-of-freedom, able to apply forces and manipulate objects in the nanoscale world

Comment [i3]: There are many differences between macro- and nano-scale robots. However, they occur mainly in the basic laws that govern their dynamics. Macro-scaled robots are essentially in the Newtonian mechanics domain whereas the laws governing nanorobots are in the molecular quantum mechanics domain. Furthermore, uncertainty plays a crucial role in nanorobotic systems. The fundamental barrier for dealing with uncertainty at the nano-scale is imposed by the quantum and the statistical mechanics and thermal excitations

Comment [i4]: The nanorobots are invisible to the naked eye, which makes them hard to manipulate and work with
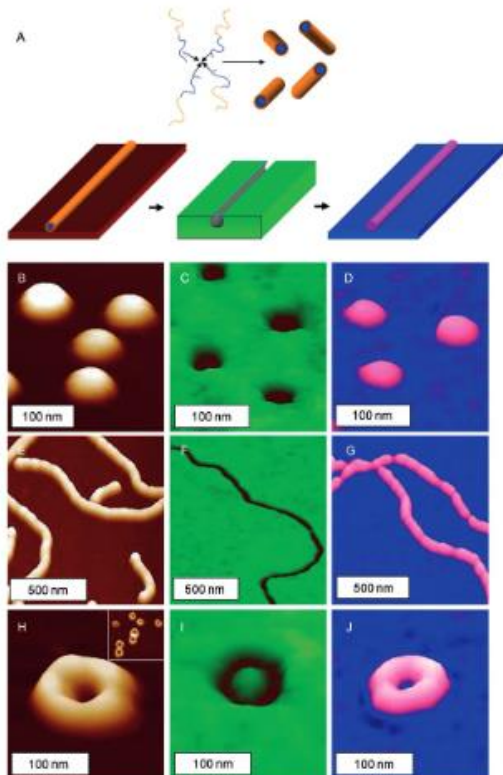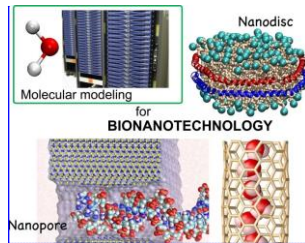
are 1. PS-b-PI micelle replication. Depending on the block-copolymer composition, self-assembly of b-PI in heptane results in micelles with well-defined shapes. A) Schematic image depicting self-embly of micelles and their deposition onto substrates (brown/white), molding (green/black) and lication (blue/magenta); B) spherical micelle master, prepared by self-assembly of a 39 kDa-b-kDa PS-b-PI copolymer and solution deposition onto mica; vertical scale = 100 nm. C) PFPE mold of a 1erical micelle master; vertical scale = 20 nm. D) Triacrylate replica of spherical micelles; vertical le = 130 nm. E) Cylindrical micelle master, prepared by self-assembly of a 40 kDa-b-10 kDa PS-b-PI 1olymer and solution deposition onto mica; vertical scale = 300 nm. F) PFPE mold of a cylindrical :elle master; vertical scale = 200 nm. G) Triacrylate replica of cylindrical micelles; vertical le = 300 nm. H) Toroidal micelle master, prepared by self-assembly and deposition of a 21 kDa-b-Da PS-b-PI copolymer and solution deposition onto mica; vertical scale = 45 nm. Inset: larger atomic :e microscopy (AFM) image showing a collection of toroidal micelle nano-objects; vertical sca-:150 nm. I) PFPE mold of a toroidal micelle; vertical scale = 25 nm. J) Triacrylate replica of a toroidal 1lle master; vertical scale = 60 nm.

Figure 1. PS-b-PI micelle replication. Depending on the block-copolymer composition, self-assembly of PS-b-PI in heptane results in micelles with well-defined shapes. A) Schematic image depicting selfassembly
of micelles and their deposition onto substrates (brown/white), molding (green/black) and replication (blue/magenta); B) spherical micelle master, prepared by self-assembly of a 39 kDa-b-94 kDa PS-b-PI copolymer and solution deposition onto mica; vertical scale=100 nm. C) PFPE mold of a
spherical micelle master; vertical scale=20 nm. D) Triacrylate replica of spherical micelles; vertical ACHTUNGTRENUNGscale=130 nm. E) Cylindrical micelle master, prepared by self-assembly of a 40 kDa-b-10 kDa PS-b-PI coACHTUNGTRENUNGpolymer and solution deposition onto mica; vertical scale=300 nm. F) PFPE mold of a cylindrical micelle master; vertical scale=200 nm. G) Triacrylate replica of cylindrical micelles; vertical scale=300 nm. H) Toroidal micelle master, prepared by self-assembly and deposition of a 21 kDa-b-4 kDa PS-b-PI coACHTUNGTRENUNG polymer and solution deposition onto mica; vertical scale=45 nm. Inset: larger atomic force microscopy (AFM) image showing a collection of toroidal micelle nano-objects; vertical scale= 150 nm. I) PFPE mold of a toroidal micelle; vertical scale=25 nm. J) Triacrylate replica of a toroidal micelle master; vertical scale=60 nm.

**Naturally occurring supramolecular objects, such as proteins,micelles, and viruses, exhibit sophisticated morphological shapes or surface motifs that conventional synthetic and fabrication techniques cannot replicate.** These structures owe their interesting shapes and shape-related properties spheres, cylinders, vesicles, and toroids[9, 10] **We have created master templates of these motifs by dispersing micellar solutions of PS-b-PI copolymers in heptane onto mica substrates. A variety of master structures including 50-nm spheres, largely to noncovalent chemical interactions that can produce unique, "evolutionarily designed" shapes with nanometer precision.** These "self-assembly"-driven approaches can be tremendously successful in controlling nanoscale shape in organic and inorganic materials, but the chemical structure of each component must be carefully designed and precisely synthesized to ensure that the desired morphology is obtained.
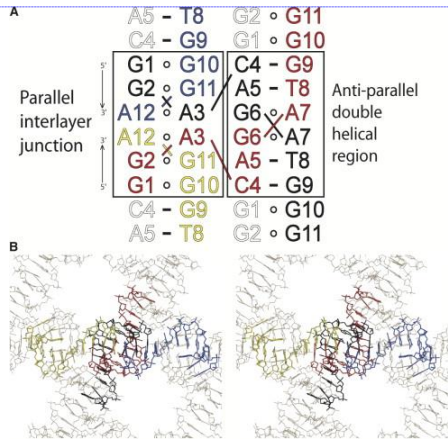
**How it can be Done**
photo-curable perfluoropolyether (PFPE) **elastomers to replicate naturally occurring objects** ("master templates").[2–4] The naturally occurring motif is replicated in the PFPE elastomer by pouring the curable PFPE resin over the master template **and photopolymerizing the resin into a flexible "mold" that transfers the details of the master morphology into a crosslinked fluoropolymer** (cf. Figure 1 A). Due to the extremely low surface energy (8–10 dyncm_1 depending on chemical structure) of the PFPE precursor resin**, it will spontaneously spread on materials with a critical surface tension greater than 8–10 dyncm_1, which includes almost all substrates found in nature, including organic materials**.[

Comment [i8]: Bio-Nanorobotics of nature's mechanism, while the DNA-based molecular machines use the basic properties of DNA to design various synthetic mechanisms (which might not be present in the nature). Nature deploys
proteins to perform various cellular tasks—from moving cargo to catalyzing reactions, while it has kept DNA as an information carrier. It is hence understandable that most of the natural machinery is built from proteins. With the powerful crystallographic techniques available in the modern world,
the protein structures are clearer than ever. The ever increasing computing power makes it possible to dynamically model protein folding processes and predict the conformations and structure of lesser known proteins (Rohl et al., 2004). All this helps unravel the mysteries associated with the molecular machinery and paves the way for the production and application of these miniature machines in various fields including medicine, space exploration, electronics, and military.

Comment [i9]: In this work, we report a nanofabrication method that is able to reproduce shapes normally associated with self-assembly using robust nanoscale replication methods, thereby combining the morphological sophistication of the
natural world with the scalable processing technologies associated with lithography

A

```
         A5 – T8     G2 ∘ G11
         C4 – G9     G1 ∘ G10
        G1 ∘ G10 | C4 – G9
5'      G2 ∘ G11 | A5 – T8      Anti-parallel
Parallel  A12 × A3 | G6 ∘ A7     double
interlayer  A12 × A3 | G6 ∘ A7   helical
junction  3'                      region
        G2 ∘ G11 | A5 – T8
5'      G1 ∘ G10 | C4 – G9
         C4 – G9     G1 ∘ G10
         A5 – T8     G2 ∘ G11
```

B
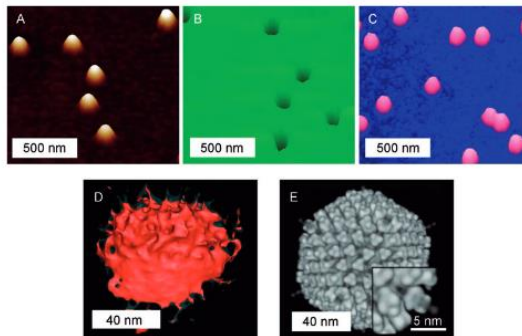




**Figure 2.** Transmission electron microtomography (TEMT) images depicting molding and replication of adenovirus particles. A) AFM image of an adenovirus master, prepared by depositing adenovirus particles onto a silicon surface; vertical scale = 100 nm. B) AFM image of a PFPE mold formed from an adenovirus master; vertical scale = 50 nm. C) AFM image of a triacrylate/bisphenol A dimethacryate adenovirus replica; vertical scale = 100 nm. D) TEMT reconstruction of a triacrylate/bisphenol A dimethacrylate adenovirus replica. E) Cryo-electron microscopy reconstruction of adenovirus (reprinted with permission from Ref. [23].)
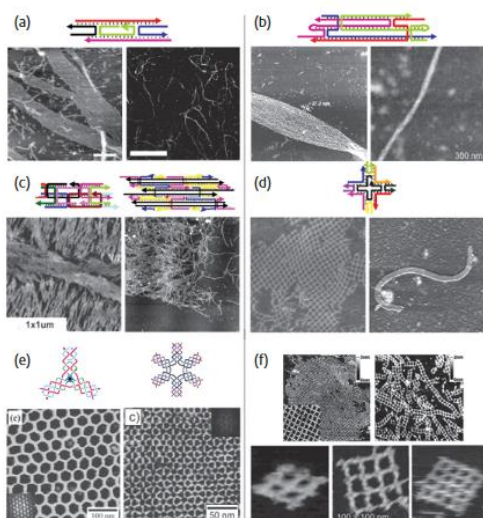
Fig. 1 Schematic diagrams of commonly used building blocks in DNA nanofabrication. (a) DNA double crossover complex and AFM images of resulting 2D arrays and tubes. (Adapted with permission from[12, 23]. © 1993, 2004 ACS.) (b) DNA triple crossover complex and resulting 2D arrays and tubes. (Adapted with permission from[13, 22]. © 2000 ACS, 2004 National Academy of Sciences.) (c) 3-helix-bundle and 6-helix bundle DNA tile complex and AFM images of resulting 2D lattices and nanotubes. (Adapted with permission from[16, 17]. © 2005, 2007 ACS.) (d) 4×4 cross-tile and resulting nanogrid and nanoribbon structures. (Adapted with permission from[26]. © 2003 AAAS.) (e) Schemes of three-point-star and six-point-star motifs and AFM images of 2D crystalline DNA arrays. (Adapted with permission from[29, 30]. © 2005, 2006 ACS.) (f) DNA nanoarrays with increased complexity and defined sizes based on the cross-tile system: nanogrid and nanotrack assembled from 2-tile system (top), molecular pegboard, 4x4 and symmetric 5x5 arrays (bottom from left to right). (Adapted with permission from[28, 32-34]. © 2005 ACS, 2006 Wiley-VCH.)
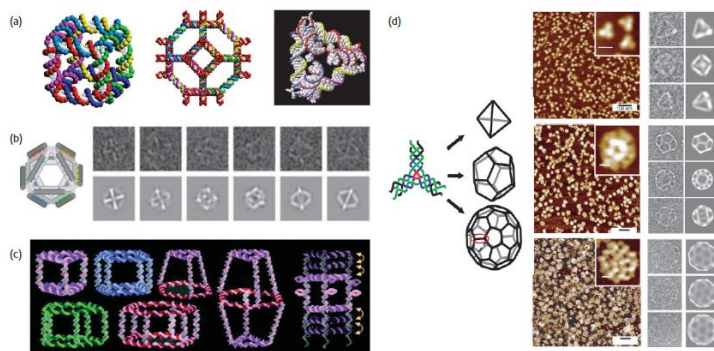


Fig. 2 Three-dimensional structures assembled via DNA self-assembly. (a) DNA molecules with the connectivity of a cube, an octahedron and a tetrahedron. (Adapted with permission from[37, 38, 40]. © 1991 Nature Publishing Group, 1994 American Chemical Society, 2004 Royal Society of Chemistry.) (b) Design of DNA octahedron from 1,669-nucleotide DNA and cryo-electron micrograph images of individual octahedron particles corresponding projections of their 3D map. (Adapted with permission from[39]. © 2004 Nature Publishing Group.) (c) 3D triangular prism, cube, pentameric and hexameric prisms, heteroprism and biprism generated from single-stranded and cyclic DNA triangles, squares, pentagons and hexagons with organic vertices. (Adapted with permission from[42]. © 2007 American Chemical Society.) (d) DNA tetrahedra, dodecahedra and buckyballs assembled from three-point-star motifs. (Adapted with permission from[44]. ©2008 Nature Publishing Group.)
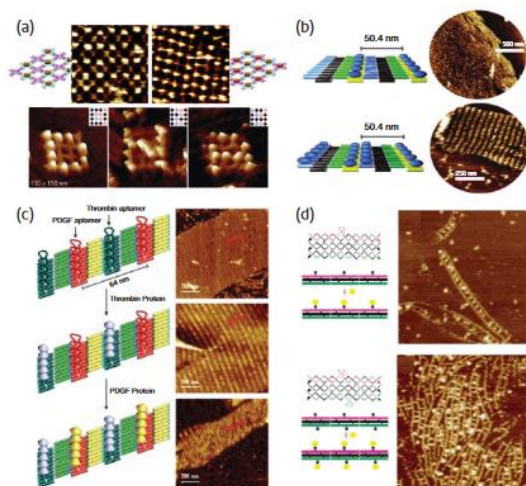
Fig. 4 DNA nanolattice directed self-assembly of protein molecules.
(a) Programmable assembly of streptavidin arrays on nanogrids and addressable 4x4 cross-tile nanogrids. (Adapted with permission from[27, 34]. © 2006 Wiley-VCH, 2005 American Chemical Society.) (b) Polyamide-biotin conjugates directed site-specific display of streptavidin along DX lattice scaffolds. (Adapted with permission from[62]. © 2008 American Chemical Society.) (c) Periodic 2D multiprotein nanoarrays directed by aptamer tags on DX nanolattices. (Adapted with permission from[68]. © 2007 American Chemical Society.) (d) TX tile lattice templated display of single chain antibodies. (Adapted with permission from[71]. © 2006 Royal Society of Chemistry.)

# 7

# Bio-Nanorobotics — A Field Inspired by Nature

**A. Ummat, A. Dubey, and C. Mavroidis**

**CONTENTS**

## 7.1   INTRODUCTION

The underlying principle of biomimetics deals with the understanding, conceptualization, and mimicking nature's way of handling various problems and situations. Nature has inspired mankind for ages and has been a key source from which we can learn and adapt. Natural processes are extremely efficient in terms of energy and material usage and provide us with many inspiring and thought provoking designs and principles. This chapter discusses biomimetics at the nano-scale, where we talk about nanorobotics and its design principles, which are inspired by nature's way of doing things at that scale.

Figure 7.1 describes the biomimetics principle and details the various aspects of mimetics. It explains the mimetics at two levels when nano-scale is considered. One is the "*machine nanomimetics*" principle meaning the creation of nanomachine components inspired by the equivalent machine components at the macro-scale and the other is the "*bionanomimetics*" principle where biological entities such as proteins and DNA are used to create the nanomachine components. The field of nanorobotics hence encapsulates these two mimetic principles and inherits their various characteristics, design logic, and advantages.

Nanotechnology can best be defined as a description of activities at the level of atoms and molecules that have applications in the real world. A nanometer is a billionth of a meter, that is, about 1/80,000 of the diameter of a human hair, or ten times the diameter of a hydrogen atom. The size-related challenge is the ability to measure, manipulate, and assemble matter with features on the scale of 1 to 100 nm. In order to achieve cost-effectiveness in nanotechnology, it will be necessary to automate molecular manufacturing. The engineering of molecular products needs to be carried out by robotic devices, which have been termed *nanorobots* (Freitas, 1999, 2003). A nanorobot is essentially a controllable machine at the nanometer or molecular scale that is composed of nano-scale components and algorithmically responds to input forces and information.
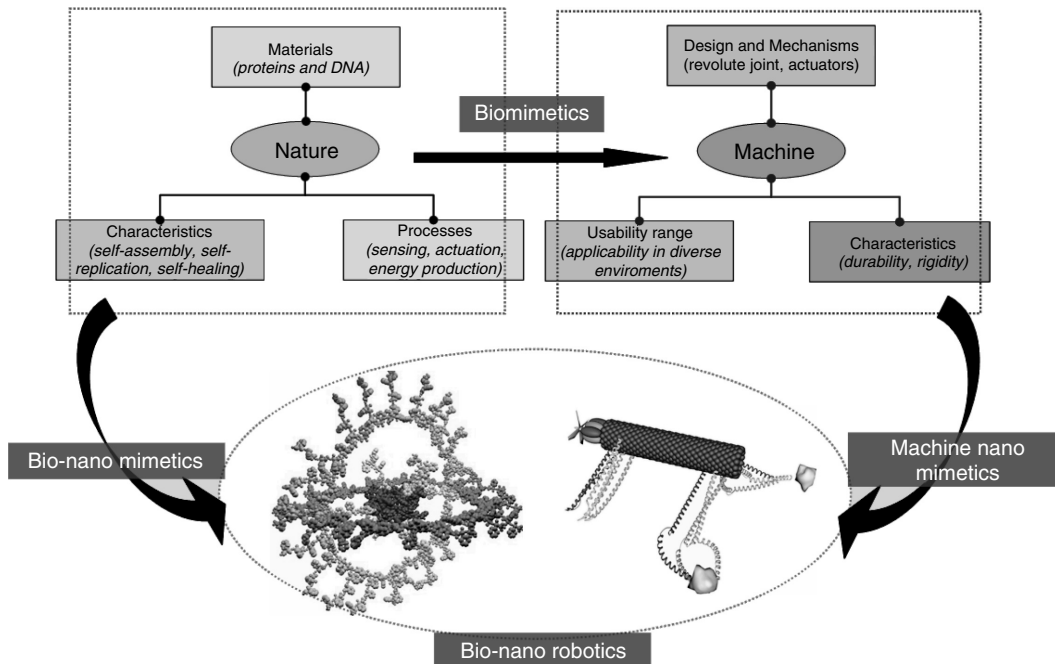


**Figure 7.1 (See color insert following page xxx)**   Biomimetics — bio-nano robotics, inspired by nature and machine.

The field of nanorobotics studies the design, manufacturing, programming, and control of the nano-scale robots.

This review chapter focuses on the state of the art in the emerging field of nanorobotics and its applications and discusses in brief some of the essential properties and dynamical laws which make this field more challenging and unique than its macro-scale counterpart. This chapter is only reviewing nano-scale robotic devices and does not include studies related to nano-precision tasks with macro-robotic devices that usually are also included in the field of nanorobotics (e.g., ATMs and other forms of proximal probe microscopy). AQ1

Nanorobots would constitute any active structure (nano-scale) capable of actuation, sensing, signaling, information processing, intelligence, and swarm behavior at nano-scale. These function-alities could be illustrated individually or in combinations by a nanorobot (swarm intelligence and cooperative behavior). So, there could be a whole genre of actuation and sensing or information processing nanorobots having ability to interact and influence matter at the nano-scale. Some of the characteristic abilities that are desirable for a nanorobot to function may include:

(i)    *Swarm intelligence* — decentralization and distributive intelligence;
(ii)   Self-assembly and replication — assemblage at nano-scale and "nano-maintenance";
(iii)  *Nano-information processing and programmability* — for programming and controlling nanor-obots (autonomous nanorobots);
(iv)   Nano- to macro-world *interface architecture* — an architecture enabling instant access to the nanorobots and its control and maintenance;

There are many differences between macro- and nano-scale robots. However, they occur mainly in the basic laws that govern their dynamics. Macro-scaled robots are essentially in the Newtonian mechanics domain whereas the laws governing nanorobots are in the molecular quantum mechanics domain. Furthermore, uncertainty plays a crucial role in nanorobotic systems. The fundamental barrier for dealing with uncertainty at the nano-scale is imposed by the quantum and the statistical mechanics and thermal excitations. For a certain nanosystem at some particular temperature, there are positional uncertainties that cannot be modified or further reduced (Drexler, 1992).

The nanorobots are invisible to the naked eye, which makes them hard to manipulate and work with. Techniques like scanning electron microscopy (SEM) and atomic force microscopy (AFM) are being employed to establish a visual and haptic interface to enable us to sense the molecular structure of these nano-scaled devices. Virtual reality (VR) techniques are currently being explored in nano-science and biotechnology research as a way to enhance the operator's perception (vision and haptics) by approaching more or less a state of "full immersion" or "telepresence." The development of nanorobots or nanomachine components presents difficult fabrication and control challenges. Such devices will operate in microenvironments whose physical properties differ from those encountered by conventional parts. Since these nano-scale devices have not yet been fabricated, evaluating possible designs and control algorithms requires using theoretical estimates and virtual interfaces or environments. Such interfaces or simulations can operate at various levels of detail to trade-off physical accuracy, computational cost, number of components, and the time over which the simulation follows the nano-object behaviors. They can enable nano-scientists to extend their eyes and hands into the nano-world, and they also enable new types of exploration and whole new classes of experiments in the biological and physical sciences. VR simulations can also be used to develop virtual assemblies of nano and bio-nano components into mobile linkages and to predict their performance.

Nanorobots with completely artificial components have not been realized yet. The active area of research in this field is focused more on molecular machines, which are thoroughly inspired by nature's way of doing things at nano-scale. Mother Nature has her own set of molecular machines that have been working for millions of years, and have been optimized for performance and design over the ages. As our knowledge and understanding of these numerous machines continues to

increase, we now see a possibility of using the natural machines or creating synthetic ones from scratch using nature's components. This chapter focuses more on molecular machines and explores various designs and research prevalent in this field. The main goal in the field of molecular machines is to use various biological elements — whose function at the cellular level creates motion, force, or a signal — as machine components. These components perform their preprogrammed biological function in response to the specific physiochemical stimuli but in an artificial setting. In this way proteins and DNA could act as motors, mechanical joints, transmission elements, or sensors. If all these different components were assembled together in the proper proportion and orientation, they would form nanodevices with multiple degrees of freedom, able to apply forces and manipulate objects in the nano-scale world. The advantage of using nature's machine components is that they are highly efficient and reliable.

Nanorobotics is a field which calls for collaborative efforts between physicists, chemists, biologists, computer scientists, engineers, and other specialists to work towards this common objective. Figure 7.2 details the various fields which come under the field of bio-nano robotics (this is just a representative figure and not exhaustive in nature). Currently this field is still developing, but several substantial steps have been taken by great researchers all over the world who are contributing to this ever challenging and exciting field.

The ability to manipulate matter at the nano-scale is one core application for which nanorobots could be the technological solution. A lot has been written in the literature about the significance and motivation behind constructing a nanorobot. The applications range from medical to environmental sensing to space and military applications. Molecular construction of complex devices could be possible by nanorobots of the future. From precise drug delivery to repairing cells and fighting tumor cells, nanorobots are expected to revolutionize the medical industry in the future. These applications come under the field of nanomedicine (Freitas, 1999, 2003), which is a very active area of research in nanotechnology. These molecular machines hence form the basic enablers of future applications.

In the next section, we shall try to understand the principles, theory, and utility of the known molecular machines and look into the design and control issues for their creation and modification. A majority of natural molecular machines are protein-based which involve using the exact replica
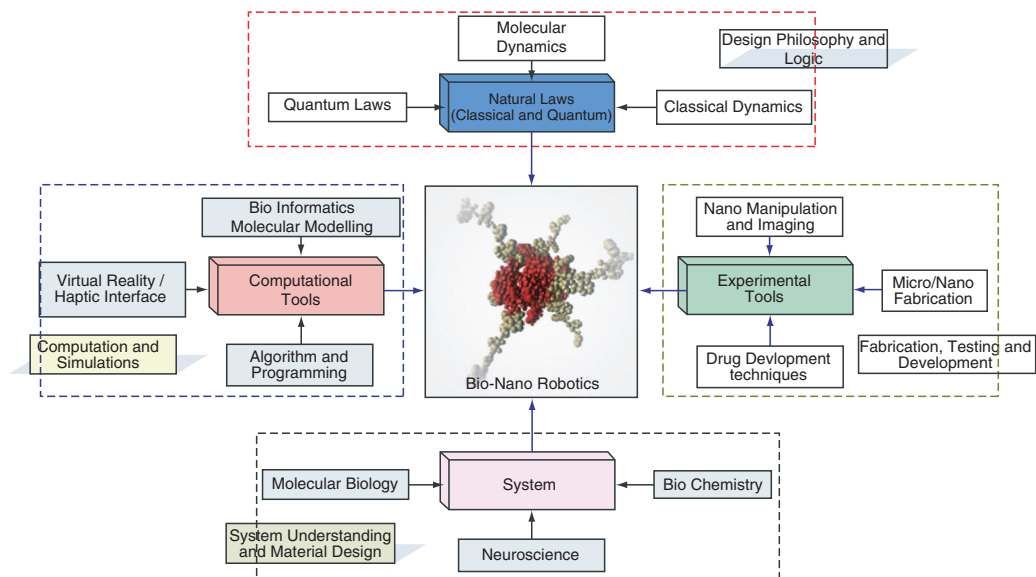


**Figure 7.2**   Bio-nano robotics — a truly multidisciplinary field.

of nature's mechanism, while the DNA-based molecular machines use the basic properties of DNA to design various synthetic mechanisms (which might not be present in the nature). Nature deploys proteins to perform various cellular tasks — from moving cargo to catalyzing reactions, while it has kept DNA as an information carrier. It is hence understandable that most of the natural machinery is built from proteins. With the powerful crystallographic techniques available in the modern world, the protein structures are clearer than ever. The ever increasing computing power makes it possible to dynamically model protein folding processes and predict the conformations and structure of lesser known proteins (Rohl et al., 2004). All this helps unravel the mysteries associated with the molecular machinery and paves the way for the production and application of these miniature machines in various fields including medicine, space exploration, electronics, and military.

## 7.2  BIOMOLECULAR MACHINES: BACKGROUND AND SIGNIFICANCE

### 7.2.1  Significance

The recent explosion of research in nanotechnology, combined with important discoveries in molecular biology have created a new interest in biomolecular machines and robots. The main goal in the field of biomolecular machines is to use various biological elements — whose function at the cellular level creates motion, force, or a signal, or stores information — as machine components. These components perform their preprogrammed biological function in response to the specific physiochemical stimuli but in an artificial setting. In this way proteins and DNA could act as motors, mechanical joints, transmission elements, or sensors. If all these different components were assembled together in the proper proportion and orientation, they would form nanodevices with multiple degrees of freedom, able to apply forces, and manipulate objects in the nanoscale world. The advantage of using nature's machine components is that they are highly efficient (Kinosita et al., 2000) and reliable. Just as conventional macro-machines are used to generate forces   AQ2
and motions to accomplish specific tasks, bionanomachines can be used to manipulate nano-objects to assemble and fabricate other machines or products and to perform maintenance, repair, and inspection operations.

Such bio-nano robotic devices will hopefully be part of the arsenal of future medical devices and instruments that will: (1) perform operations, inspections, and treatments of diseases inside the body, and (2) achieve ultra-high accuracy and localization in drug delivery, thus minimizing side effects. Figure 7.3 shows an idealized rendition of a biomolecular nanorobot repairing an infected cell in a blood vessel. The bio-nanorobot will be able to attach to the infected cell alone and deliver a therapeutic drug that can treat or destroy only the infected cell, sparing the surrounding healthy cells.

Development of robotic components composed of simple biological molecules is the first step in the development of future biomedical nanodevices. Since the planned complex systems and devices will be driven by these components, we must first develop a detailed understanding of their operation. From the simple elements such as structural links to more advanced concepts as motors, each part must be carefully studied and manipulated to understand its functions and limits.

Figure 7.4 lists the most important components of a typical robotic system or machine assembly and the equivalence between macro and potential bio-nano components. Beyond the initial component characterization is the assembly of the components into robotic systems. Figure 7.5 shows one such concept of a nano-organism, with its "feet" made of helical peptides and its body using carbon nanotubes while the power unit is a biomolecular motor. For this phase to be successful, a library of biological elements of every category must be available. At that point, conventional robotics can be used as a guide for fabrication of bio-nanorobots that function in the same manner. There will be systems that have mobile characteristics to transport themselves, as well as other objects, to desired locations.
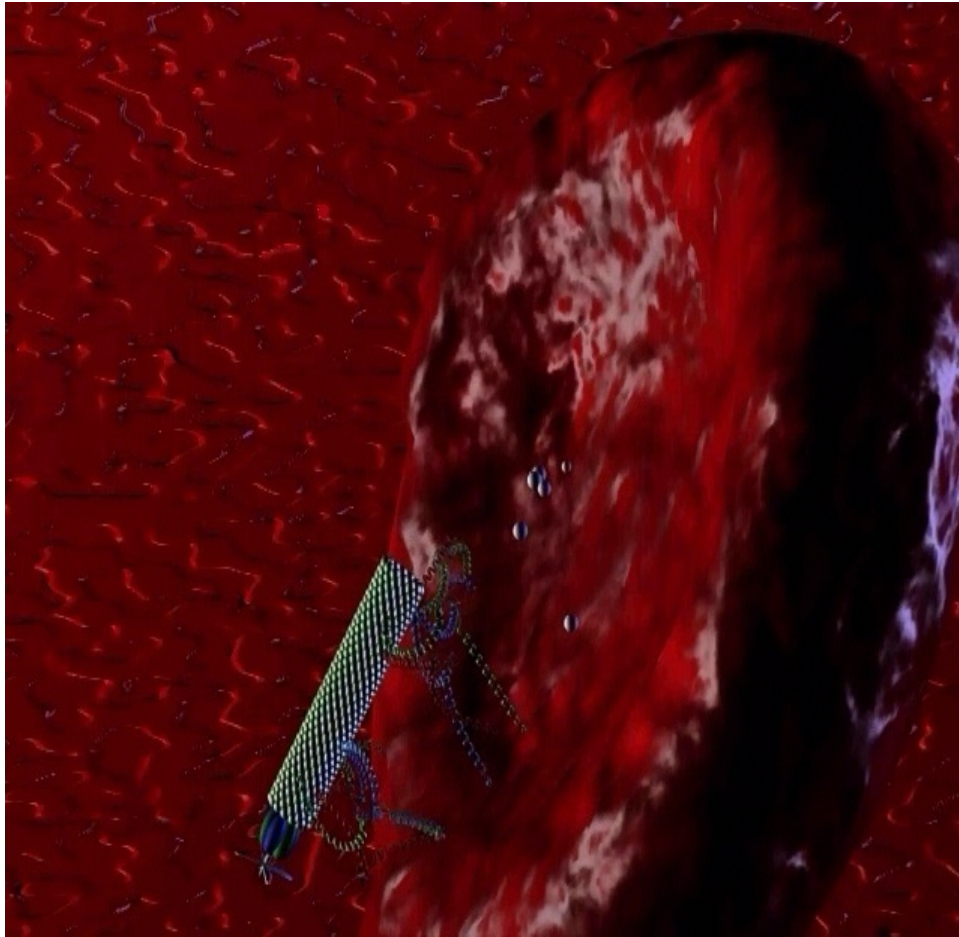
**Figure 7.3**   A 'nanorobot' flowing inside a blood vessel finds an infected cell. The nanorobot attaches to the cell and projects a drug to repair or destroy the infected cell.

Some bio-nanorobots can be conceived as able to manufacture additional elements and various structures. There may also be robots that not only perform physical labor, but also sense the environment and react accordingly. There is no doubt that biomedical applications will be both a driving force and a beneficiary of these developments.

### 7.2.2   Brief Review of Biomolecular Machines

While the majority of the prior research in this field has largely focused on biomolecular motors, several other nano components such as sensors and even assemblies of components in the form of mechanisms have been studied. In the macroscopic world, what we understand by a 'motor' is a machine capable of imparting motion associated by the conversion of energy. Biomolecular motors have attracted a lot of attention recently because: (1) they operate at high efficiency, (2) some could be self-replicating and hence cheaper in mass usage, and (3) they are readily available in nature (Boyer, 1998). A number of enzymes function as nano-scale biological motors, such as kinesin (Block, 1998; Schnitzer and Block, 1997), RNA polymerase (Wang et al., 1998), myosin (Kitamura et al., 1999), and adenosine triphosphate (ATP) synthase, function as nano-scale biological motors (Montemagno and Bachand, 1999; Bachand and Montemagno, 2000; Soong et al., 2000; Noji et al., 1997; Yasuda et al., 1998; Walker, 1998).
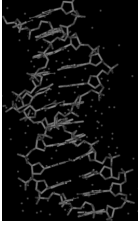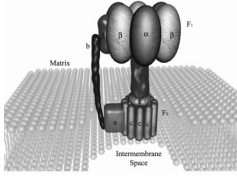
| Component | MacroRobots | Bio-Nano Robots |
|---|---|---|
| **Structural Elements**- Links | Metal, Plastic Polymer |  DNA Nanotubes [PDBfile:119D] |
| Joints | Metal, Plastic Polymer material *Revolute joints* *Prismatic joints* *Spherical joints* *Cylindrical joints* | *DNA* hinge *Molecular* bonds, Synthetic joints |
| Actuators | Electric motors, Pneumatic motors, Hydraulic motors, Smart material-based actuators |  ATPase protein flagella motors, DNA actuators, Viral protein motors etc. |
| **Transmission Elements** | Springs (Metal,Polyvinyl) Bearings Gears | β Sheets Molecular camshaft design Smith ss (2001). United States Patent No. 6,200,782 13 March 2001. |
| **Sensors** | Light sensors, force sensors, position sensors, temperature sensors |  Rhodopsin [PDB file-1JFP]    Heat Shock Factor [PDB file–3HSF] |

**Figure 7.4**   Macro- and bio-nano-equivalence of robot components.

### 7.2.2.1  The ATPase Motor

One of the most abundant rotary motors found in life forms is F0F1 ATP synthase, commonly known as the "ATPase motor." Oxidative phosphorylation was demonstrated over 50 years ago as an important process by which our bodies capture energy from the food we eat. The mechanism of this process was not known until 1997, when Boyer and Walker described the key role that ATP plays in the process (Boyer, 1998; Walker, 1998). Noji et al. published the structural and performance data of the ATPase motor in 1997 (Noji et al., 1997; Yasuda et al., 1998). According to this study, the $\gamma$?-subunit, which is about 1 nm in diameter, rotates inside the F1 subunit, which is about 5 nm in diameter, to produce approximately 40 pN-nm of rotary torque. Montemagno and his group were the first to indicate that the rotation of the $\gamma$?-subunit of the ATPase motor could be mechanically useful based on fabricated nanomechanical inorganic devices, which could be compatible with the force
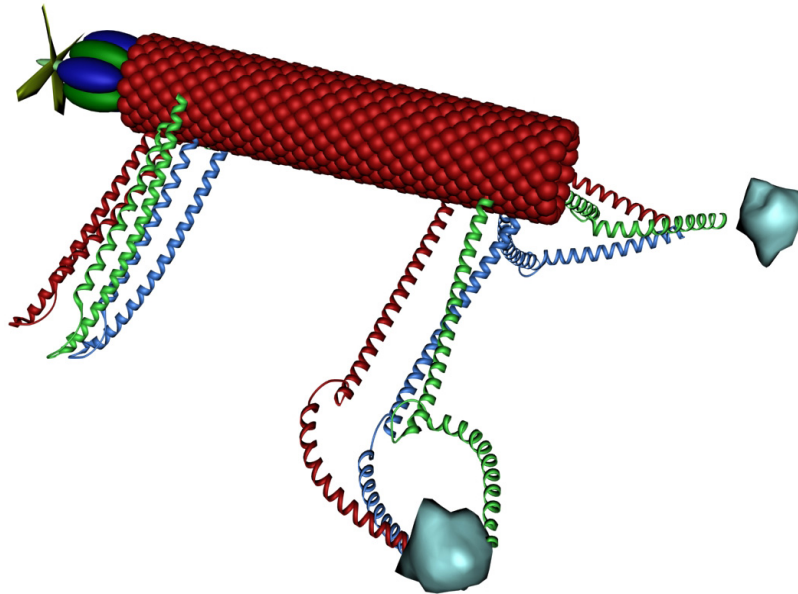
**Figure 7.5**   The biological elements will be used to fabricate robotic systems. A vision of a nano-organism: carbon nanotubes (CNT) form the main body; peptide limbs can be used for locomotion and object manipulation, a biomolecular motor located at the head can propel the device in various environments.

production and dimensions of the molecular motors (Montemagno and Bachand, 1999; Bachand and Montemagno, 2000; Soong et al., 2000). Frasch's group is currently studying the binding of metals to amino acids of the motor protein. These experiments are providing new insights into the means by which the energy obtained from the hydrolysis of ATP can be converted into the physical action of pumping a proton in a unilateral direction (Frasch, 2000).

### 7.2.2.2  Kinesin and Myosin

Motor proteins are tiny vehicles that transport molecular cargoes within cells. These minute cellular machines exist in three families: the kinesins, the myosins, and the dyneins (Farrell et al., 2002). Conventional kinesin was found to be a highly processive motor that could take several hundred steps on a microtubule without detaching (Block et al., 1990; Howard et al., 1989), whereas muscle myosin has been shown to execute a single "stroke" and then dissociate (Finer et al., 1994). A detailed analysis and modeling of these motors has been done (Vale and Milligan, 2000). Hackney's group has concentrated upon the usage of ATP energy by motors like kinesin, myosin, dynenin, and related motor families (Hackney, 1996). Unger's group is currently working towards developing a microtubule–kinesin system as a biological linear-motoric actuator. Their work is aimed at producing force multiplication by parallel action of numerous single driving units as well as a more efficient means for system control (Bohm et al., 1997). Other researchers have discovered a new member of the myosin-V family (Myo5c) and have implicated this myosin in the transport of a specific membrane compartment (Mehta et al., 1999). The role of ATP hydrolysis in kinesin motility has also been recently described (Farrell et al., 2002).

### 7.2.2.3  The Flagella Motors

*Escherichia coli* and similar organisms are equipped with a set of rotary motors only 45 nm in diameter. Each motor drives a long, thin, helical filament that extends several cell body lengths out

into the external medium. In addition to rotary engines and propellers, *E. coli*'s standard accessories include particle counters, rate meters, and gearboxes and thus has been described as a nanotechnologist's dream (Berg, 2000). Berg developed one of the earliest models for the rotary motor (Berg, 1974). Improved models came in 1992 (Ueno et al., 1992, 1994). Flagella motor analysis coupled to real-time computer assisted analysis of motion has also been performed (Khan et al., 1998). Researchers in Japan have applied crystallographic studies in order to understand the molecular structure of flagella motors as well as that of kinesin (Namba and Vonderveczt, 1997). Finally, Hess' group is attempting to build a nano-scale train system, complete with tracks, loading docks and a control system. Since motor proteins are a thousand times smaller than any man-made motor, they aim to utilize them in a synthetic environment as engines powering the nanotrains (Hess and Vogel, 2001).

### 7.2.2.4  Other Motors and Mechanisms

In addition to work on naturally existing motors, considerable effort is also being applied to develop synthetic molecular motors. The structure of the ATP synthase, a rod rotating inside a static wheel, suggests the use of rotaxanes as potential artificial models for natural motors (Harada, 2001). Rotaxanes are organic compounds consisting of a dumbbell-shaped component that incorporates one or more recognition sites in its rod section and is terminated by bulky "stoppers", encircled by one or more ring components. The possibility of manufacturing specific forms of rotaxane and creating molecular motors capable of guided rotary motion and the possibility of fueling such a motor by light, electrons, and chemical energy has been proposed (Schalley et al., 2001).

Schemes for using pseudorotaxanes, rotaxanes, and catenanes as molecular switches to perform chemical, electrochemical, and photochemical switching and controllable molecular shuttles have also been proposed recently (Balzani et al., 1998). Molecular shuttles have been reported using α-cyclodextrin — a parent of rotaxanes and catenanes (Harada, 2001). A light-driven monodirectional rotor made of helical alkene, with rotation around a central carbon–carbon covalent bond due to chirality has been reported (Koumura et al., 1999). Another simple way to convert chemical energy into mechanical motion in a controlled fashion is by using a metal ion which can be translocated reversibly between two organic compartments with the change of its ionization state, controllable by redox reaction or pH change (Amendola et al., 2001). Motility of unicellular organisms like vortecellids reminds us of energy storage and release by mechanical springs on a macromolecular scale. Spring-like action has been observed in sperm cells of certain marine invertebrates during fertilization. Springs and supramolecular ratchets by actin polymerization have yet to be built *in vitro,* but they theoretically can be generalized, as recently demonstrated (Mahadevan and Matsudaira, 2000).

### 7.2.2.5  DNA-Based Molecular Nanomachines, Joints, and Actuators

Several researchers are exploring the use of DNA in nano-scale mechanisms. DNA is small, relatively simple, and homogeneous and its structure and function is well understood. The predictable self-assembling nature of the double helix makes it an attractive candidate for engineered nanostructures. This property has been exploited to build several complex geometric structures, including knots, cubes, and various polyhedra (Seeman, 1998). Mathematical analyses of the elastic structure of DNA using energy minimization methods have been performed to examine its molecular stability, wherein short DNA strands were treated as an elastic rod (Tobias et al., 2000). Initial experiments on DNA visualization and manipulation using mechanical, electrical, and chemical means have been underway for a decade (Yuqiu et al., 1992; Hu et al., 2002). A dynamic device providing atomic displacements of 2–6 nm was proposed in Mao et al. (1999), wherein the chemically induced transition between the B and Z DNA morphologies acts as a

moving nano-scale device. A method for localized element-specific motion control was seen in the reversible transition between four stranded topoisomeric DNA motifs (PX and JX2) thereby producing rotary motion (Yan et al., 2002). A very important, though simple DNA machine that resembles a pair of tweezers has been successfully created, whose actuation (opening and closing) is also fueled by adding additional DNA fuel strands (Yurke et al., 2000).

### 7.2.3 Nanosensors

The technology of nanosensing is also under development. For example, silicon probes with single walled carbon nanotube (CNT) tips are being developed (MIT media laboratory Nanoscale    AQ3
Sensing, http://www.media.mit.edu/nanoscale/). For sensing certain analytes, genetically engi-    AQ4
neered versions of pore-forming proteins like *Staphylococcus aureus* alpha-hemolysin are    AQ5
also being studied (Kasianowicz and Bayley, 2003). Efforts to detect biological warfare agents like cholera toxins by utilizing their ability to bind to a bilayer membrane in the presence of gangliosides is another example (Plant and Silin, 2003). Light sensors could be made using certain photoreceptive polypeptides containing azobenzene or spyropyran units as they respond to light or dark environmental conditions by undergoing conformational change, for example, transition from random coil to a α-helix (Pieroni et al., 2001). An optical DNA biosensor platform has been reported using etched optical fiber bundles filled with oligonucleotide-functionalized microsphere probes (Ferguson et al., 1996). Finally, work is in progress to develop sensors for brain implantation, which would foretell the development of a stroke and be useful for perioperative online monitoring during coronary by-pass surgery (Manning and McNeil, 2001).

In addition to many of the examples mentioned above which generally correspond to one degree of freedom (DOF) rotary actuators, there are many other machine elements, the functional capabilities of which have not yet been represented by biomolecular elements. In addition, the assembly of different molecules in a multi-degree of freedom machine or the formation of hybrid systems composed of biomolecules and synthetic nonorganic elements has not yet been explored. In this context, our long term goal is to identify novel biomolecules that can be used as different types of machine components and to assemble them into controlled multi-degree of freedom systems using organic and synthetic nonorganic parts.

## 7.3  DESIGN AND CONTROL PHILOSOPHIES FOR NANOROBOTIC SYSTEMS

The design of nanorobotic systems requires the use of information from a vast variety of sciences ranging from quantum molecular dynamics to kinematic analysis. In this chapter we assume that the components of a nanorobot are made of biological components, such as proteins and DNA strings. So far, no particular guideline or a prescribed manner that details the methodology of designing a bio-nanorobot exists. There are many complexities that are associated with using biocomponents (such as protein folding and presence of aqueous medium), but the advantages of using these are also quite considerable. These biocomponents offer immense variety and functionality at a scale where creating a man-made material with such capabilities would be extremely difficult. These biocomponents have been perfected by nature through millions of years of evolution and hence these are very accurate and efficient. As noted in the review section on Molecular Machines, $F_1$-ATPase is known to work at efficiencies which are close to 100%. Such efficiencies, variety, and form are not existent in any other form of material found today. Another significant advantage in protein-based bio-nano components is the development and refinement over the last 30 years of tools and techniques enabling researchers to mutate proteins in almost anyway imaginable. These mutations can consist of anything from simple amino acid side-chain swapping, amino acid insertions or deletions, incorporation of nonnatural amino acids, and even

the combination of unrelated peptide domains into whole new structures. An excellent example of this approach is the use of zinc to control $F_1$-ATPase, which is able to rotate a nanopropeller in the presence of ATP. A computational algorithm (Hellinga and Richards, 1991) was used to determine the mutations necessary to engineer an allosteric zinc-binding site into the $F_1$-ATPase using site-directed mutagenesis. The mutant $F_1$-ATPase would rotate an actin filament in the presence of ATP with average torque of 34 pN nm. This rotation could be stopped with the addition of zinc, and restored with the addition of a chelator to remove the zinc from the allosteric binding site (Liu et al., 2002). This type of approach can be used for the improvement of other protein-based nano components.

These biocomponents seem to be a very logical choice for designing nanorobots. In addition, since some of the core applications of nanorobots are in the medical field, using biocomponents for these applications seems to be a good choice as they both offer efficiency and variety of functionality. This idea is clearly inspired by nature's construction of complex organisms such as bacteria and viruses which are capable of movement, sensing, and organized control. Hence our scope would be limited to the usage of these biocomponents in the construction of bio-nano robotics. A roadmap is proposed which details the main steps towards the design and development of bio-nanorobots.

### 7.3.1 The Roadmap

The roadmap for the development of bio-nano robotic systems for future applications (medical, space, and military) is shown in Figure 7.6. The roadmap progresses through the following main steps:

Step 1: Bio-Nano Components

Development of bio-nano components from biological systems is the first step towards the design and development of an advanced bio-nanorobot, which could be used for future applications (see
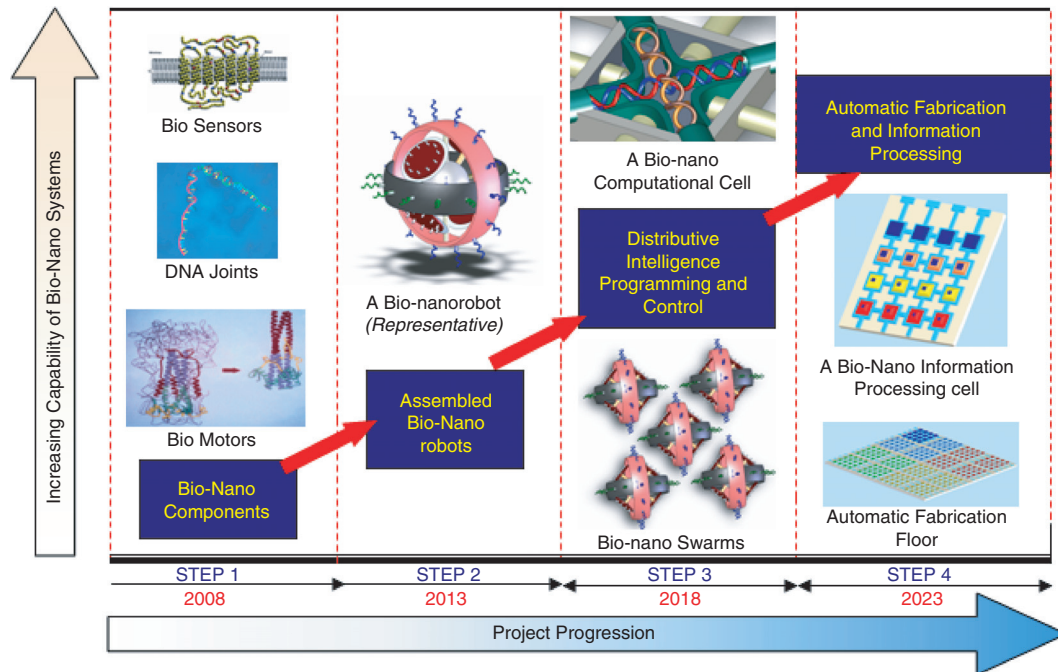


**Figure 7.6 (See color insert)** The roadmap illustrating the system capability targeted as the project progresses.

                    (a)                  (b)                                    (c)
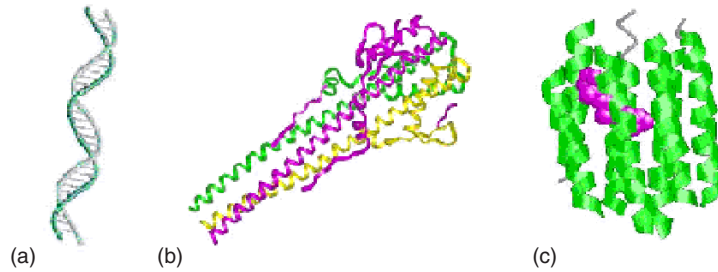
**Figure 7.7 (Step 1)**   Understanding of basic biological components and controlling their functions as robotic components. Examples are: (a) DNA which may be used in a variety of ways such as a structural element and a power source; (b) Hemagglutinin virus may be used as a motor; (c) Bacteriorhodopsin could be used as a sensor or a power source.

Figure 7.7). Since the planned systems and devices will be composed of these components, we must have a sound understanding of how these behave and how they could be controlled. From the simple elements such as structural links to more advanced concepts such as motors, each component must be carefully studied and possibly manipulated to understand the functional limits of each one of them. DNA and carbon nanotubes are being fabricated into various shapes, enabling possibilities of constructing newer and complex devices. These nanostructures are potential candidates for integrating and housing the bio-nano components within them. Proteins such as *rhodopsin* and *bacteriorhodopsin* are a few examples of such bio-nano components. Both of these proteins are naturally found in biological systems as light sensors. They can essentially be used as solar collectors to gather abundant energy from the sun. This energy could either be harvested (in terms of proton motive force) for later use or could be consumed immediately by other components, such as the ATP synthase nano rotary motor. The initial work is intended to be on the biosensors, such as heat shock factor. These sensors will form an integral part of the proposed bionano assemblies, where these will be integrated within a nanostructure and will get activated as programmed, for gathering the required information at the nano-scale. Tools and techniques from *molecular modeling* and *protein engineering* will be used to design these modular components.

Step 2: Assembled Bio-Nanorobots

The next step involves the assembly of functionally stable bio-nano components into complex assemblies. Some examples of such complex assemblies are shown in Figure 7.8.  Figure 7.8A shows a bio-nanorobot with its "*feet*" made of helical peptides and its body of CNT, while the power unit is a biomolecular motor. Figure 7.8B shows a conceptual representation of *modular organization* of a bio-nanorobot. The modular organization defines the hierarchy rules and spatial arrangements of various modules of the bio-nanorobots, such as the inner core (the brain or energy source for the robot), the actuation unit, the sensory unit, and the signaling and information processing unit. By the beginning of this phase, a "*library of bio-nano components*" will be developed, which will include various categories, such as actuation, energy source, sensory, signaling, etc. Thereafter, one will be able to design and develop such bionanosystems that will have enhanced mobile characteristics and will be able to transport themselves as well as other objects to desired locations at nano-scale. Furthermore, some bio-nanorobots need to assemble various biocomponents and nanostructures, including *in situ* fabrication sites and storage areas; others will manipulate existing structures and maintain them. There will also be robots that not only perform physical labor, but also sense the environment and react accordingly. There will be systems that will sense an oxygen deprivation and stimulate other components to generate oxygen creating an environment with stable homoeostasis.
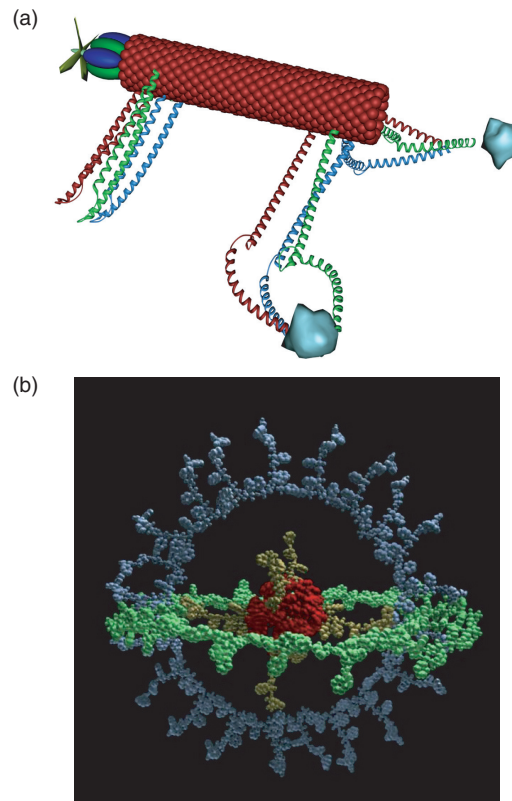
**Figure 7.8 (Step 2)**    (a) The bio-nano components will be used to fabricate complex bio-robotic systems. A vision of a nanorobot: carbon nanotubes (CNT) form the main body; peptide limbs can be used for locomotion and object manipulation and the biomolecular motor located at the head can propel the device in various environments. (b) Modular organization concept for the bio-nanorobots. Spatial arrangements of the various modules of the robots are shown. A single bio-nanorobot will have actuation, sensory, and information processing capabilities.

### Step 3: Distributive Intelligence, Programming and Control

With the individual bio-nanorobots capable of basic functions, we would now need to develop concepts that would enable them to collaborate with one another to develop "colonies" of similar nanorobots. This design step could lay the foundation towards the concept of *bionanoswarms* (distributive bio-nanorobots) (see Figure 7.9A). Here work has to be done towards the control and programming of such swarms. This will evolve concepts like distributive intelligence in the context of bio-nanorobots. Designing swarms of bio-nanorobots capable of carrying out complex tasks and capable of computing and collaborating amongst them will be the focus of this step. Therefore, the basic computational architectures needs to be developed and rules need to be evolved for the bio-nanorobots to make intended decisions at the nano-scale.

To establish an interface with the macro-world, the computers and electronic hardware have to be designed as well. Figure 7.10 shows the overall electronic communication architecture. Humans should be able to control and monitor the behavior and action of these swarms. This means that basic computational capabilities of the swarms will need to be developed. A representative computational bionanocell, which will be deployed within a bio-nanorobot, is shown in Figure 7.9B. This basic computational cell will initially be designed for data retrieval and storage at the nano-scale. This capability will enable us to program (within certain degrees of freedom) the swarm behavior in the bio-nanorobots. We will further be able to get their sensory data (from nano-world)

(a)



(b)
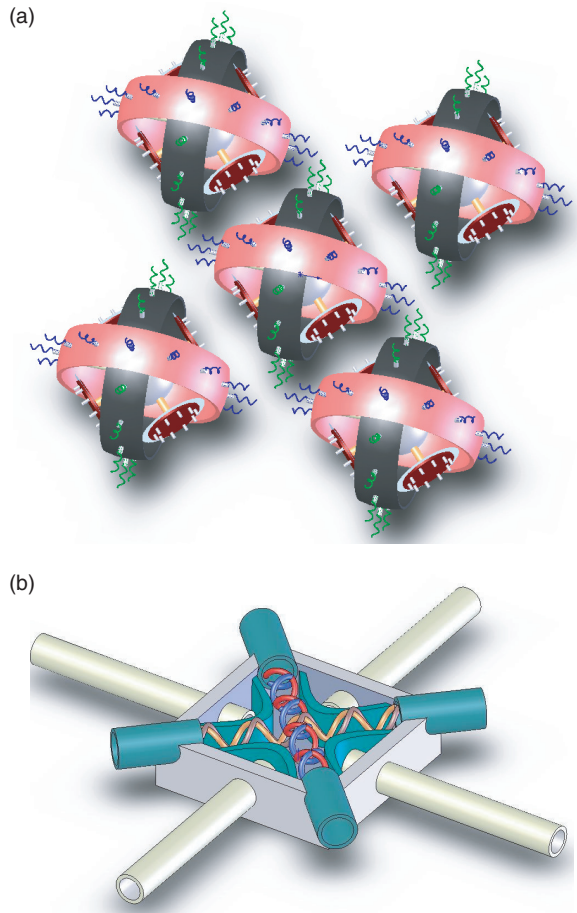


**Figure 7.9 (Step 3)**    (a) Basic bio-nanorobot forming a small swarm of five robots. The spatial arrangement of the individual bio-nanorobot will define the arrangement of the swarm. These swarms could be re-programmed to form bindings with various other types of robots. The number of robots making a swarm will be determined by the mission. Such swarms will attach additional bio-nanorobots at run time and replace any non functional ones. (b) A basic bio-nano computational cell. This will be based on one of the properties of the biomolecules, which is 'reversibility.'

back to the macro-world through these storage devices. This programming capability would control the bio-nano robotics system and hence is very important.

Step 4: Automatic Fabrication and Information Processing Machines

Specialized bio-nano robotic swarms would need to be designed to carry out complex missions, such as sensing, signaling, and data storage. The next step in nanorobotic designing would see the emergence of automatic fabrication methodologies (see Figure 7.11, *which only shows the floor concept of assembling bio-nanorobots*) of such bio-nanorobots *in vivo* and *in vitro*. Capability of information processing will be a key consideration of this step. This would enable bio-swarms to have capability of *adjusting* based on their interacting environment they will be subjected to. These swarms could be programmed for more than one energy source and hence would have an ability to perform in an alternate environment. Energy management, self-repairing, and evolving will be some of the characteristics of these swarms.
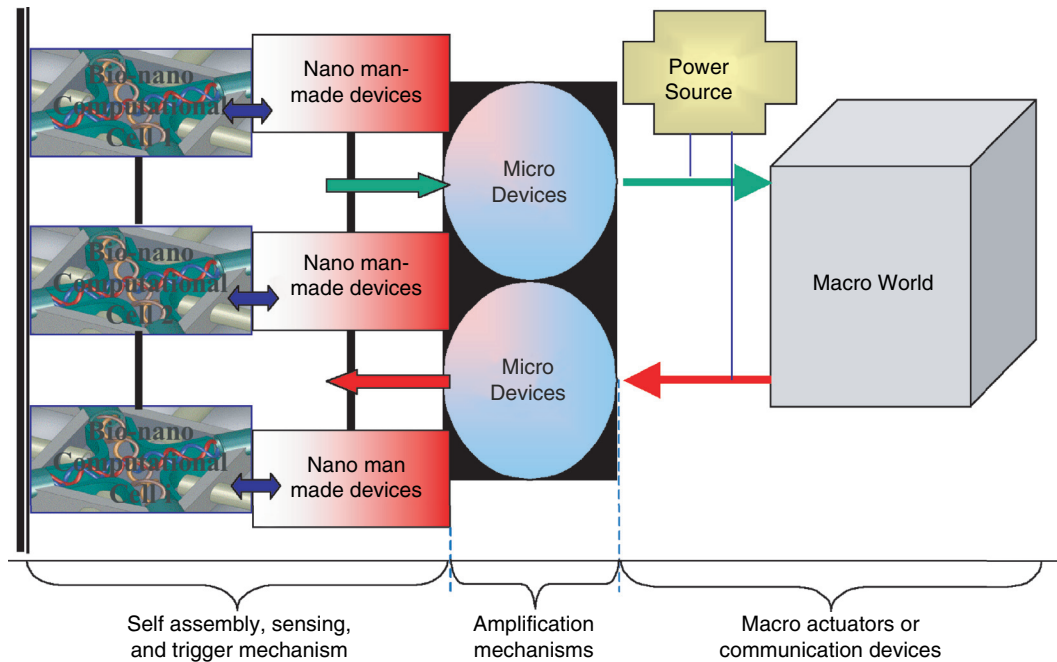
**Figure 7.10 (See color insert)**   Feedback path from nano- to macro-world route.
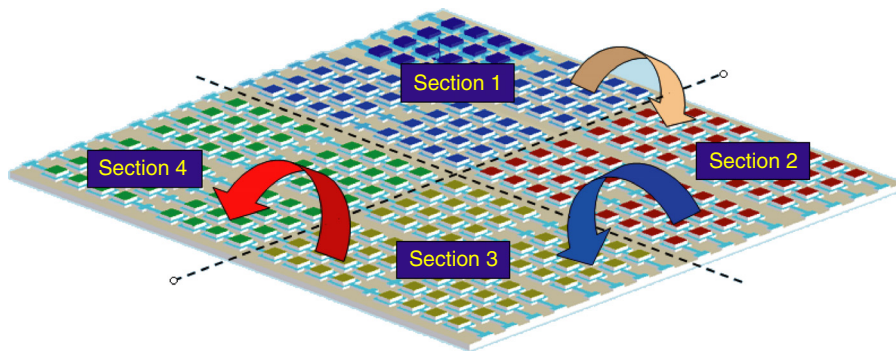


**Figure 7.11 (Step 4)**   An automatic fabrication floor layout. Different colors (Balaji: Is this a color insert as well?) represent different functions in automatic fabrication mechanisms. The arrows indicate the flow of components on the floor layout. **Section 1**→Basic stimuli storage — control expression; **Section 2**→Biomolecular component manufacturing (actuator or sensor); **Section 3**→Linking of bio-nano components; **Section 4**→Fabrication of bio-nanorobots (assemblage of linked bio-nano components).

## 7.3.2   Design Architecture for the Bio-Nano Robotic Systems

*(a) Modular Organization:* Modular organization defines the fundamental rule and hierarchy for constructing a bio-nano robotic system. Such construction is performed through *stable integration* (energetically in the most stable state) of the individual '*bio-modules or components*', which constitute the bio-nanorobot. For example, if the entity **ABCD**, defines a bio-nanorobot having some *functional specificity* (as per the Capability Matrix defined in Table 7.1) then A, B, C, and D are said to be the basic bio-modules defining it. The basic construction will be based on the techniques of molecular modeling with emphasis on principles such as *energy minimization* on

**Table 7.1    Defining the Capability Matrix for the Bio-Modules**

| Functionality | BioNanoCode | Capabilities Targeted | General Applications |
|---|---|---|---|
| Energy storage and carrier | E | Ability to store energy from various sources such as solar and chemical for future use and for its own working | Supplies the energy required for the working of all the bio-chemical mechanisms of the proposed bio-nano robotic systems |
| Mechanical | M | Ability to precisely move and orient other molecules or modules at nano-scale — includes the ability to mechanically bind to various target objects and carry them to desired locations | 1. Carry moities and deliver them to the precise locations in correct orientations<br>2. Move micro-world objects with nano-precision |
| Input Sensing | S | Sensing capabilities in various domains such as chemical, mechanical, visual, auditory, electrical, and magnetic | Evaluation and discovery of target locations based on either chemical properties, temperature, or other characteristics |
| Signaling | G | Ability to amplify the sensory data and communicate with bio-systems or with the micro controllers and ability to identify their locations through various trigger mechanisms such as fluorescence | Imaging for medical applications or for imaging changes in nanostructures |
| Information storage | F | Ability to store information collected by the sensory element — behave similar to a read–write mechanism in computer field | 1. Store the sensory data for future signaling or usage<br>2. Read the stored data to carry out programmed functions<br>3. Back bone for the sensory bio-module<br>4. Store nano-world phenomenon currently not observed with ease |
| Swarm behavior | W | Exhibit binding capabilities with 'similar' bio-nanorobots so as to perform distributive sensing, intelligence, and action (energy storage) functions | All the tasks to be performed by the bio-nanorobots will be planned and programmed keeping in mind the swarm behavior and capabilities |
| Information processing | I | Capability of following algorithms (Turing equivalent) | Programmable |
| Replication | R | Replicate themselves depending on the situation and requirement | Replicate by assembling raw components into nanorobots, and programming newly-made robot to form swarms that form automated fabricators consistent with the Foresight Guidelines for safe replicator development (Foresight Institute, 2000) |

the hypersurfaces of the bio-modules, *hybrid quantum-mechanical and molecular mechanical* methods, *empirical force field* methods, and *maximum entropy production* in least time.

   Modular organization also enables the bio-nanorobots with capabilities, such as organizing into *swarms,* a feature, which is extremely desirable for various applications. Figure 7.12A and Figure 7.12B show the conceptual representation of modular organization. Figure 7.12C shows a more realistic scenario in which all the modules are defined in some particular spatial arrangements based on their functionality and structure. A particular module could consist of other group of modules, just like a fractal structure (defined as *fractal modularity*). The concept of *bionanocode*
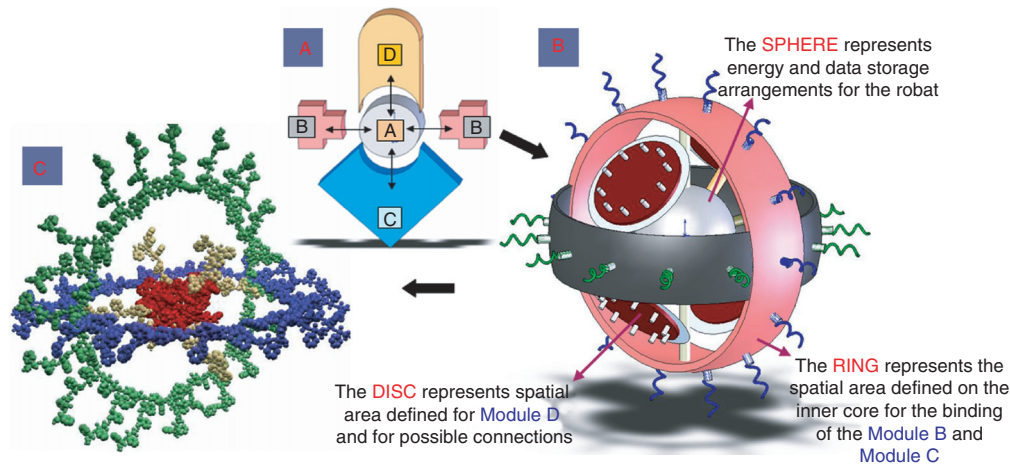
**Figure 7.12 (See color insert)**    (A) A Bio-nano robotic entity 'ABCD', where A, B, C and D are the various bio-modules constituting the bio-nanorobot. In our case these bio-modules will be set of stable configurations of various proteins and DNAs. (B) A bio-nanorobot (representative), as a result of the concept of modular organization. All the modules will be integrated in such a way so as to preserve the basic behavior (of self-assembly and self-organization) of the biocomponents at all the hierarchies. The number of modules employed is not limited to four or any number. It is a function of the various capabilities required for a particular mission. (C) A molecular representation of the figure in part B. It shows the red core and green and blue sensory and actuation bio-modules.

has been devised, which basically describes the unique functionality of a bio-nano component in terms of alphabetic codes. Each bionanocode represents a particular module defining the structure of the bio-nanorobot. For instance, a code like **E-M-S** will describe a bio-nanorobot having capabilities of energy storage, mechanical actuation, and signaling at the nano-scale. Such representations will help in general classifications and representative mathematics of bio-nanorobots and their swarms. Table 7.1 summarizes the proposed capabilities of the bio-modules along with their targeted general applications. The bio-nano code **EIWR ‖ M ‖ S ‖ FG** representing the bio-nano system shown in Figure 7.12B which could be decoded as shown in Figure 7.13.

*(b) The Universal Template* — BioNano STEM System: The modular construction concept involves designing a universal template for bionanosystems, which could be 'programmed and grown' into any possible bionanocoded system. This concept mimics the embryonic stem cells found in the human beings, that are a kind of primitive human cells which give rise to all other specialized tissues found in a human foetus and ultimately to all the three trillion cells in an adult human body. Our BioNano STEM system will act in a similar way. This universal growth template will be constituted of some basic bionanocodes, which will define the BioNano STEM system. This STEM system will be designed in a manner that could enable it to be programmed at runtime to any other required bio-module. Figure 7.14  shows one such variant of the BioNano STEM system, having the bionanocode: EIWR ‖ M ‖ S ‖ FG and having enhanced sensory abilities.

## 7.4  SELF-REPLICATION — MIMETICS: A NOVEL PROPERTY OF LIVING SYSTEMS

### 7.4.1  Significance

Mimetics of self-replication, as exhibited by nature, would influence any nano-level application. We would need an army of nanorobots (or living systems), mass-produced via techniques of self-replication (or life), to carry out many meaningful tasks at the nano-scale. These applications
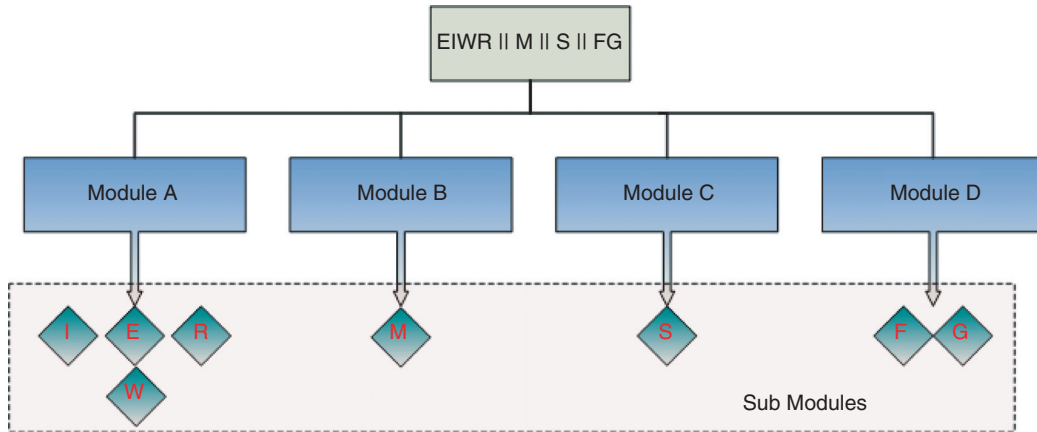
**Figure 7.13**    Showing the bio-nano code and the fractal modularity principle. The letter symbols have the values specified in Table 7.1. The '‖' symbol integrates the various bio-modules and collectively represents a higher order module or a bio-nanorobot.
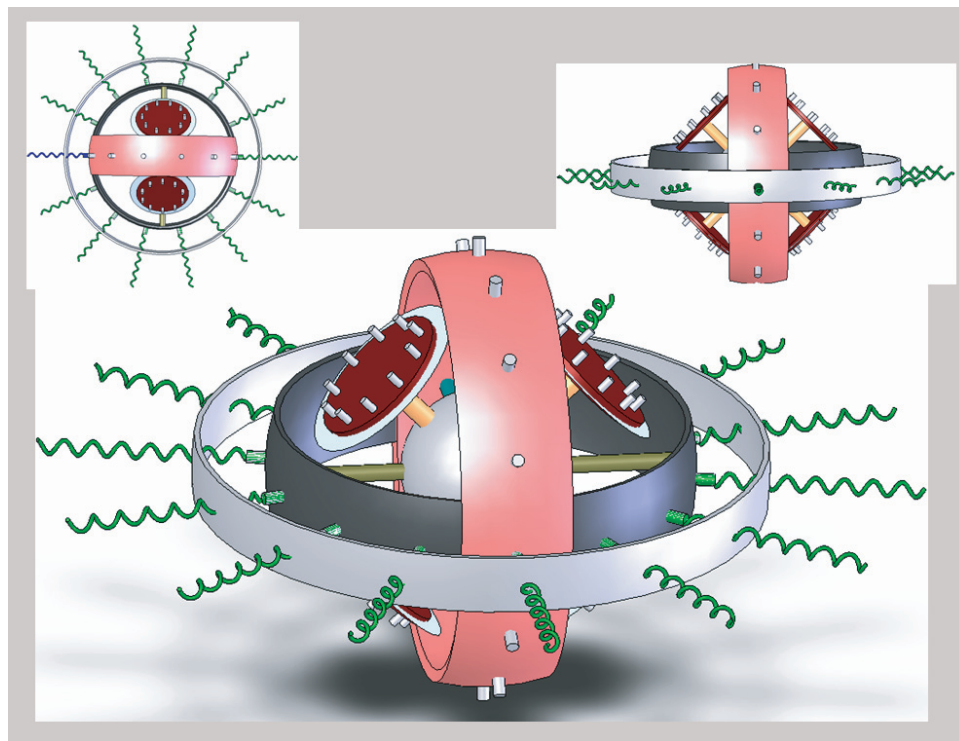


**Figure 7.14**    Shows a variant of the initial Bio-Nano STEM system (Figure 21B), fabricated with enhanced bio-nano code S, which defines it as a bio-nanorobot having enhanced sensory capabilities. The other features could be either suppressed or enhanced depending upon the requirement at hand. The main advantage of using Bio-Nano STEM system is that we could at runtime decide which particular type of bio-nanorobots we require for a given situation. The suppression ability of the Bio-Nano STEM systems is due to the property of **'Reversibility'** of the biocomponents found in living systems.

involving nanorobots demand that these machines are manufactured in millions or billions and in a timeframe reasonable for a particular application. One of nature's noblest properties is that of life. It is how nature progresses through its environment ever adapting and evolving. Although philosophically what life means and what constitutes it is not very clear, what is clear is how nature propagates itself with time and survives, every day and every moment! This is one attribute of nature which is of prime importance to us as researchers of science and engineering, and which, if understood, would bring a unique revolution that in a way would change the course of our lives.

The concept of self-replicating mechanisms (SRM) or mimetics of life is not new (Freitas and Merkle, 2004). We are perfect examples of these kinds of systems. We are wounded and our internal mechanisms heal it with some differences in some cases. Taking the example of the wound and its healing process, we move ahead and try to analyze how we can achieve such behaviors in the mechanisms that we design. At the core of the concept of self-replication lies the basic material (DNA/RNA) which undergoes such activity. Though we hardly know why these materials behave in this fashion, what we do know is how they behave and this provides the stepping stone for us to move ahead.

Before looking at some of the possible designs, a brief discussion on the application of such mechanisms is necessary. Why do at all we need living systems or self-replications? Where would they be best suited?

### 7.4.2  Applications

(a) Consider that our application depends upon a particular part —mechanical or electrical, or any other physical, biological, or chemical element — which fails or starts developing problems. We need self-rectifying mechanisms within our application to detect the problem and rectify it. It is similar to our example of the wound. We can think of many applications where we would desire such behavior. Given some initial material feedstock, it would be desired that the self-replicating mechanisms would rectify the problems. Having said that, we can classify the self-replicating mechanisms in the order we classify our main mechanisms or machines.

- Mechanical self-replicating mechanisms;
- Electrical self-replicating mechanisms;
- Chemical or biological self-replicating mechanisms.

There could be other classifications as well and numerous other examples following the lines of our wound example, can be thought of. It just depends on our imagination. For example, suppose that we build some SRM which mimics the living system. Its function is to detect the crucial defect in a mechanical element and then mend that defect. If we are able to devise such an application, it could significantly enhance the life and performance of the system. The system in this example would be designed and constructed to work at nano-scale, and therefore it would have an ability to detect the slightest of defects and start working towards rectifying it.

(b) Remote Applications would also benefit from SRM systems. Maintaining remote applications requires constant human interaction. If these systems mimic the coded logic and goals of the living system, then it would be able to performance optimally with minimal human interventions. For example, deep space explorations would require circuits, machines, and equipments to adjust and adapt with time and as per the conditions they would be subjected to.

(c) Applications at the nano-scale. This category of applications would be most influenced by our bio mimetic systems because they could lay the foundations of nanodevices that have the capability of manipulating molecular matter.

In the following section we try to define some of the guidelines and working philosophies for designing and fabricating such replicating systems. The details are the thoughts and ideas of the authors and are not verified or supported by experimental facts.

### 7.4.3  The Design of Life Mimetic Systems

The design of life-mimetic systems requires new innovative materials to be designed that behave in the same fashion as that designed by nature. These new materials are termed "intrinsic materials" from here on.

#### 7.4.3.1  Intrinsic Material

The unique arrangement of the constituent atoms of intrinsic materials would give rise to:

- unique potential field surface around them;
- unique charge distribution;
- unique internal energy gradients.

It is through these internal energy gradients that two particular intrinsic materials would interact with each other. Hence the behavior of the intrinsic material would be a direct function of its internal energy gradients.

#### 7.4.3.2  Interaction Laws

The final objective of any interacting intrinsic materials would be to achieve the intrinsic balance of the resulting system (termed *self-balancing*). This would translate to achieving minimum energy gradients in all directions for all interacting materials. The final system would then be defined by its new achieved internal energy gradients. These intrinsic energy gradients would also be influenced by the external fields.

#### 7.4.3.3  Self-Balancing

It implies that the materials considered would tend to align with its intrinsic energy gradients and would try to minimize the formed unbalance. The classical instance self-replication via energy-minimized self-assembly was first demonstrated in the late 1950s. The canonical example of this approach is called the Penrose Blocks (Penrose and Penrose, 1957; Penrose, 1958). The unbalance and the property of self-balancing are similar in essence to what is postulated by the law of entropy. This concept of self-balancing is motivated from the law of maximum entropy production according to which a system follows a path which minimizes the potential or maximizes entropy at the maximum rate (Archives of Science, 2001).

#### 7.4.3.4  Growth and the Reproductive Limit

An intrinsic material would have a property of growth (an important variable for replication). This property of growth only occurs when the system is provided with some energy maybe in the form of additional intrinsic material or external gradients. Growth cannot happen in isolation. This implies that in the process of self-balancing, it is possible that the particular configuration of the intrinsic material is stable up to a particular level. This level would be governed by the strength of the potential gradients for that intrinsic material and the extrinsic gradients. Therefore, the growth implies that the intrinsic material can achieve higher state by not disturbing its self-balance or increasing it further. But this growth can only be achieved to a particular extent; beyond it, it tends to disintegrate by following the paths defined by the laws of maximum entropy production. And this particular limit of growth is termed Reproductive Limit.

### 7.4.3.5  Self-Filtering and Self-Healing

The concept of replication further demands that the materials thus designed should exhibit the property of self-filtering and self-healing. Self-filtering implies that the material involved in the systems exhibiting self-replication will not allow any kind of growth pattern but only a particular one. This particular growth pattern (which inherently depends on the interactions of the components) will determine which material it binds to and to which it doesn't. Hence such systems will only interact with certain intrinsic materials.

In the process of this growth, a realignment of the intrinsic material occurs. As the addition of further intrinsic material takes place, the whole system tries to realign itself towards the most stable state and in the fastest possible time. This realignment goes on with the self-filtering process. If at any incremental stage system doesn't find the kind and type of material it is looking for, it doesn't realign and rejects that particular material and doesn't grow. By rejection, it might imply that either the system doesn't align with the material or marks it as an unstable configuration and seeks for the opportunity to replace it immediately. Hence it acts as a self-healer.

### 7.4.4  Self-Replication — A Thought Experiment

Let us try to replicate a system, based on the above properties. Consider a system A, consisting of some intrinsic materials (Figure 7.15). The system A is completely defined by the way these intrinsic materials are associated and aligned with in it. According to the above properties of self-replicating mechanisms, it is observed that, the intrinsic materials of the system could be broken down into further fundamental intrinsic materials.

Any stable configuration of the individual intrinsic material is in sync with the property of self-balancing. Now when a particular intrinsic material (say 1) gets in an interactive distance of another intrinsic material (say 2), then these two intrinsic materials try to form another subsystem A1 within the super system A, following the property of self-balancing. These two intrinsic materials combined will have some other function of intrinsic energy gradient and could be the sum of the individual intrinsic energy gradients of the intrinsic materials and the applied external fields.

Now this argument could be extended to the situation when the third intrinsic material (say 3) comes into the picture. This intrinsic material 3 would not only interact with intrinsic material 1 but also with intrinsic material 2. Finally, a system A comes into generation, because of self-balancing acts of these three intrinsic materials. The configuration they achieve becomes highly stable for that
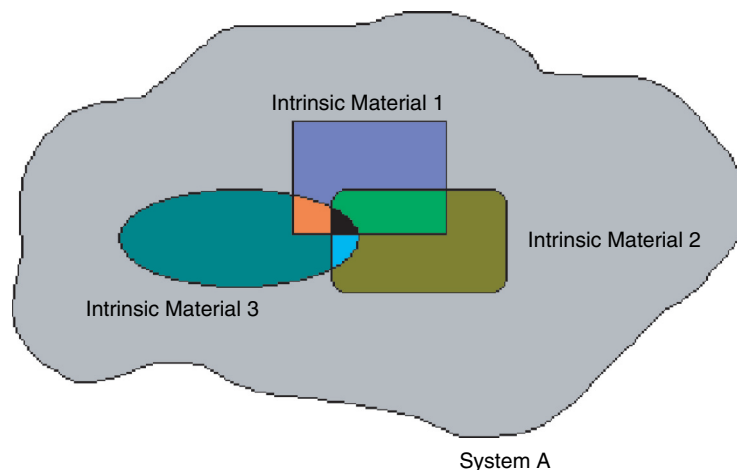


**Figure 7.15**  System of intrinsic materials — a self-replicative system A.
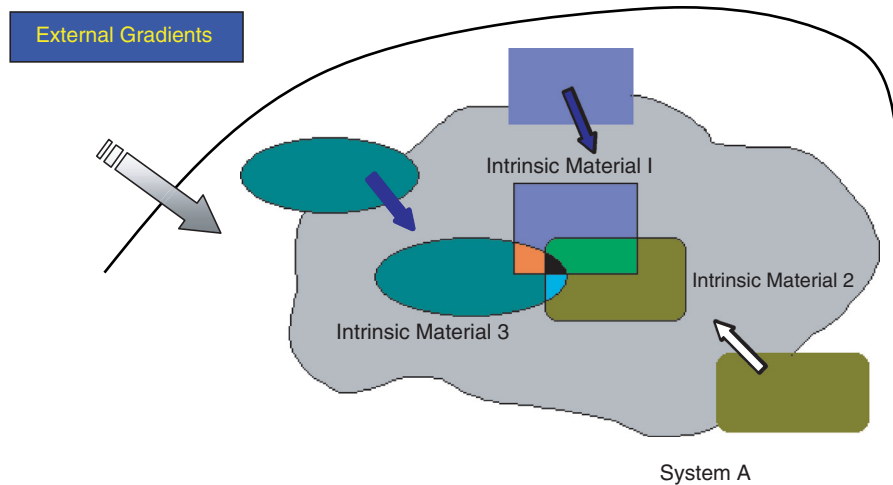
**Figure 7.16**    Energy being added to the self-replicative system A in the form of newer intrinsic material (1, 2 and 3) and external gradient (this external gradient is applied either to aid the interaction between the intrinsic materials or to impart a particular dynamics to the system for favorable environment for the interaction).
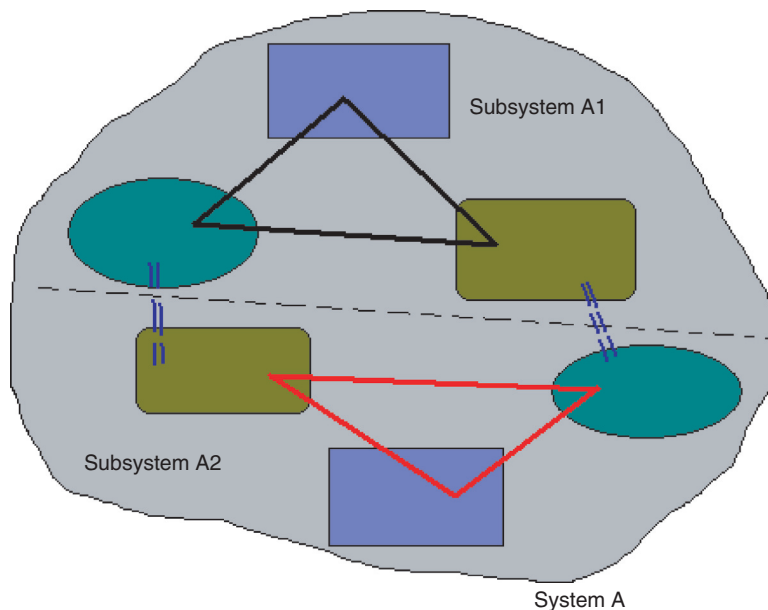


**Figure 7.17**    Creation of stable subsystems within the original system A (which as a whole is marginally unstable under the external gradients and two independently stable subsystems). This step is the most crucial in the process of attaining a self-replicative super system. This demands a unique selection of such replicative intrinsic materials in the initial place, namely, 1, 2 and 3.

particular situation. Now let's introduce more *energy* to the system A. It would be in the form of introducing intrinsic materials or applying external gradients to the system A or both. Figure 7.16 explains the concept.

Here because of the process of self-filtering, copies of intrinsic material 1, 2 and 3 are introduced. The property of self-balancing comes into dominance and the systems tries to adjust itself into the most stable state. As defined earlier, the initial state is the most stable state; following is what happens to the system A. Two subsystems within the main systems are made as shown in Figure 7.17. The alignment of *subsystems A1* and *A2* is similar to the one of the initial system, that is, *A*. Please note that
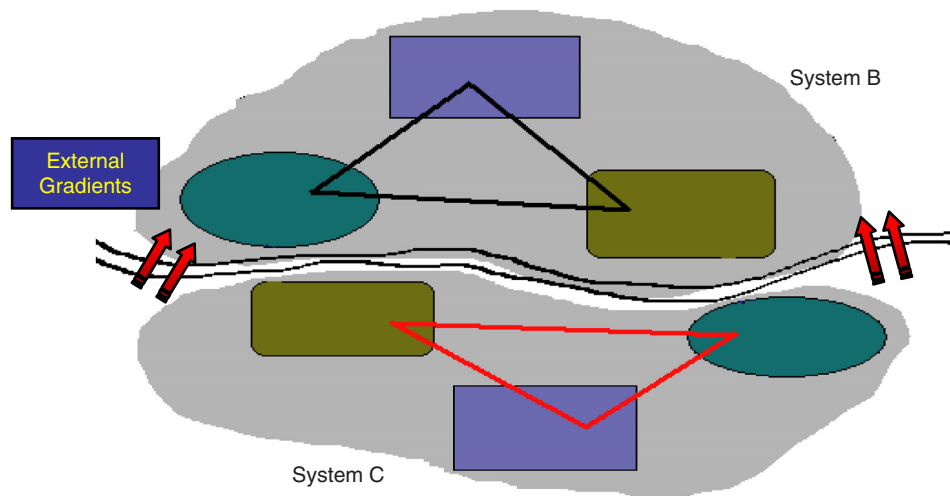
**Figure 7.18**   Replicating stage of the system A into system B and system C. Systems B and C which could be called the child systems are similar to system A in function and its configurations.

such system is possible, because we can control the external parameters, namely, extrinsic gradients and the intrinsic material introduced. The triangles drawn in the figure above shows the configuration of the intrinsic materials of *subsystems A1* and *A2*. The dotted lines, depict the interaction between the old intrinsic materials and the new ones and the possible configuration that could be achieved. Now because the external gradients are still applicable a unique instability in the system occurs. The system tries to self-balance and in the process leads to its most stable configurations, which was its initial one (the initial configuration, system A). Figure 7.18 explains the concept.

In the end, the original *system A* replicates into *systems B* and *C*. Both these new systems, have the same functionalities as defined by the original system (A), because the have received the same configuration and the same intrinsic materials.

## 7.4.5   Design Parameters for Self-Replicating Systems

Following are the various design parameters that need to be considered while designing a self-replicating system.

### 7.4.5.1   Selection of Intrinsic Materials

This is, of course, the most important parameter in designing the desired system. The obvious choices would be biomaterials and chemicals found in the human body, which have exhibited self-replication. Their choice is mainly because of their availability and the fact that they themselves are the materials resulting from a replicative process. This does not limit the selection of other replicative materials. Also a lot of data on nature's biomaterials is available from the experiments performed in the field of biology and genetic engineering. The field of nanotechnology is the biggest area where the concept of self-replication system would be a success and the biomaterials could be managed at that scale.

### 7.4.5.2   Defining the External Gradient Parameters

It is extremely important to define the external gradient parameters within which the system needs to perform. Our choice of the intrinsic materials would be greatly impacted by their behavior. Also their sensitivities to these external gradients need to be calculated so as to fine-tune the system.

### 7.4.5.3  *Generating Stable Alignment and Internal Gradients*

Selection of the appropriate intrinsic materials for our system implies that we need to also select the appropriate internal gradient functions and the alignment generated by these intrinsic materials. We need to calculate the most stable configuration for our system at no external gradient level and then fine tune our alignment as it is applied. Application of these external gradients could generate a situation where no stable configuration is possible within our operating conditions. This calls for adding some further intrinsic materials to the system, which would help us to get to the stable configuration (closure engineering) (Robert et al., 2004). This variation of the intrinsic gradient in this manner is termed as *intrinsic variational gradients* to distinguish it from the inherent intrinsic gradients generated due to the intrinsic materials. | AQ6 |

   The parameters mentioned above create the foundation for the development of mathematics for this field. To create any system with self-replicating mechanism we need to first find out its most stable state, then we need to calculate its behavior in the extrinsic gradients and then we need to excite it with energy and supply of intrinsic materials so that it replicates. Though these methodologies are not verified, further research in this area is being carried on by the authors and their collaborators.

## 7.5   CONCLUSIONS

Biomimetics and its principles would greatly influence the field of nanorobotics and nanotechnology. The way nature is designed and the way natures solves its problems is of great interest to us because they allow us to understand basic principles that would pave the way to practical nanotechnology.

   The recent explosion of research in nanotechnology, combined with important discoveries in molecular biology have created a new interest in bio-nano robotic systems. The preliminary goal in this field is to use various biological elements — whose function at the cellular level results in a motion, force or signal — as nanorobotic components that perform the same function in response to the same stimuli — but in an artificial setting. This way proteins and DNA could act as motors, mechanical joints, transmission elements, or sensors. Assembled together, these components would form nanorobots with multiple degrees of freedom, with the ability to apply forces and manipulate objects at the nano-scale, and transfer information from the nano- to the macro-scale world.

   The first research area is in determining the structure, behavior and properties of basic bio-nano components such as proteins. Specific problems include the precise mechanisms involved in molecular motors like ATP Synthase, and of protein folding. The next step is combining these components into complex assemblies. Next concepts in control and communication in swarms need to be worked out. Again, we plan to follow nature's path, mimicking the various colonies of insects and animals, and transforming principles learned to our domain. Since it would require specialized colonies of nanorobots to accomplish particular tasks, the concepts of cooperative behavior and distributed intelligence need to be developed, possibly by using knew hierarchical and other techniques. | AQ7 |

   Principles like self-replication are the ones of greatest importance for the field of nanorobotics. It is this life mimetics which will enable us to design and fabricate the future nanorobots having immense capabilities and potential. These would require innovative materials (intrinsic materials) and fabrication methodologies, with due regard to well-known manufacturing- and applications-related safety concerns. The safety issue is of paramount importance in this field for researchers and scientists. The proposed bio-nanorobots would be completely controlled molecular devices and are far from being dangerous to society. Though these devices would have many unique capabilities, which are not seen currently, they are harmful as projected in science fiction movies and books. There is an increasing need for educating the community about the exact nature of this research and its essential differences with the projections of the science fiction community.
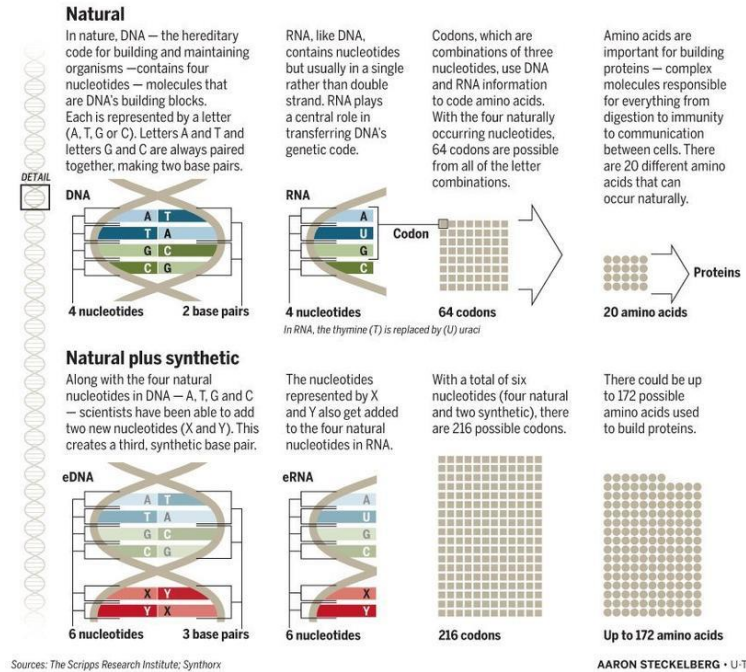
## REFERENCES

Archives of Science. (2001). All about entropy, the laws of thermodynamics, and order from disorder. *http://www.entropylaw.com,* (c) Copyright 2001.

Amendola V, Fabbrizzi L, Mangano C, Pallavicini P. (2001). Molecular machines based on metal ion translocation. *Acc. Chem. Res.* 34: 488–93.

Bachand GD, Montemagno CD. (2000). Constructing organic/inorganic NEMS devices powered by biomolecular motors. *Biomed. Microdev.* 2: 179–84.

Balzani V, Lopez MG, Stoddart JF. (1998). Molecular machines. *Acc. Chem. Res.* 31: 405–14.

Berg HC. (2000). Motile behavior of bacteria. *Phys. Today* 53: 24–9.

Berg HC. (1974). Dynamic properties of bacterial flagellar motors. *Nature* 249: 77–9.

Block SM. (1998). Kinesin: what gives? *Cell* 93: 5–8.

Block SM, Goldstein LS, Schnapp BJ. (1990). Bead movement by single kinesin molecules studied with optical tweezers. *Nature* 348: 348–52.

Bohm KJ, Steinmetzer P, Daniel A, Baum M, Vater W, Unger E. (1997) Kinesin-driven microtubule motility in the presence of alkaline-earth metal ions: indication for a calcium ion-dependent motility. *Cell Motil. Cytoskeleton* 37: 226–31.

Boyer PD. Energy, life and ATP, (Nobel Lecture). (1998). *Angewandte Chemie International Edition* 37: 2296–307.

Drexler EK (1992). *Nanosystems: Molecular Machinery, Manufacturing and Computation*. John Wiley and Sons.

Farrell CM, Mackey AT, Klumpp LM, Gilbert SP. (2002) The role of ATP hydrolysis for kinesin processivity. *J. Biol. Chem.* 277: 17079–87.

Ferguson JA, Boles TC, Adams CP, Walt DR. (1996). A fiber-optic DNA biosensor microarray for the analysis of gene expression. *Nat. Biotechnol.* 14: 1681–4.

Finer JT, Simmons RM, Spudich JA. (1994). Single myosin molecule mechanics: piconewton forces and nanometre steps. *Nature* 368: 113–9.

Foresight Institute. (2000). Molecular Nanotechnology Guidelines: Draft Version 3.7, 4 June 2000.

Frasch WD. (2000). Vanadyl as a probe of the function of the F1-ATPase-$Mg^{2+}$ cofactor. *J. Bioenergetics Biomembr.* 32: 2000.

Freitas Jr., RA. (1999). *Nanomedicine, Volume I: Basic Capabilities*, Landes Bioscience, Georgetown, Texas, 1999.

Freitas Jr., RA. (2003). *Nanomedicine, Volume IIA: Biocompatibility*, Landes Bioscience, Georgetown, Texas, 2003.

Freitas Jr., RA, Merkle. RC. (2004). *Kinematic Self-Replicating Machines*, Landes Bioscience, Georgetown, Texas, 2004; *http://www. MolecularAssembler.com/KSRM.htm*

Hackney DD. (1996). The kinetic cycles of myosin, kinesin, and dynein. *Annu. Rev. Physiol.* 58: 731–50.

Harada A. (2001). Cyclodextrin-based molecular machines. *Acc. Chem. Res.* 34: 456–64, 2001.16.

Hellinga HW, Richards FM. (1991). Construction of new ligand binding sites in proteins of known structure. I. Computer-aided modeling of sites with pre-defined geometry. *J Mol. Biol.* 222: 763–85.

Hess H, Vogel V. (2001). Molecular shuttles based on motor proteins: active transport in synthetic environments. *J. Biotechnol.* 82: 67–85.

Howard J, Hudspeth AJ, Vale RD. (1989). Movement of microtubules by single kinesin molecules. *Nature* 342: 154–8.

Hu J, Zhang Y, Gao H, Li M, Hartman U. (2002). Artificial DNA patterns by mechanical nanomanipulation. *Nanoletters* 2: 55–7.

Kasianowicz JJ, Bayley H, *http://www.cstl.nist.gov/biotech/biomat/Projects/metal.html*

Khan S, Zhao R, Reese TS. (1998). Architectural features of the Salmonella typhimurium flagellar motor switch revealed by disrupted C-rings. *J. Struct. Biol.* 122: 311–9.

Kinosita K. Jr, Yasuda R, Noji H, and Adachi K (2000). A rotary molecular motor that can work at near 100% efficiency. *Phil. Trans. R. Soc. Lond. B* 355: 473–489.

Kitamura K, Tokunaga M, Iwane AH, Yanagida T. (1999). A single myosin head moves along an actin filament with regular steps of 5.3 nanometres. *Nature* 397: 129–34.

Koumura N, Zijlstra RW, van Delden RA, Harada N, Feringa BL. (1999). Light-driven monodirectional molecular rotor. *Nature* 401: 152–5.

Liu H, Schmidt JJ, Bachand GD, Rizk SS, Looger LL, Hellinga HW, Montemagno CD. (2002). Control of a biomolecular motor-powered nanodevice with an engineered chemical switch. *Nat. Mater*. 1: 173–7.

Mahadevan L, Matsudaira P. (2000). Motility powered by supramolecular springs and ratchets. *Science* 288: 95–100.

Manning P, McNeil C. (2001) Microfabricated Multi-Analyte Amperometric Sensors. *http://nanocentre. ncl.ac.uk/*

Mao C, Sun W, Shen Z, Seeman NC. (1999). A nanomechanical device based on the B–Z transition of DNA. *Nature* 397: 144–6.

Mehta AD, Rock RS, Rief M, Spudich JA, Mooseker MS, Cheney RE. (1999). Myosin-V is a processive actin-based motor. *Nature* 400: 590–3.

MIT media laboratory Nanoscale Sensing, *http://www.media.mit.edu/nanoscale/*

Montemagno CD, Bachand GD. (1999). Constructing nanomechanical devices powered by biomolecular motors. *Nanotechnology* 10: 225–331.

Namba K, Vonderveczt F. (1997). Molecular structure of bacterial flagellum. *Quarterly Reviews of Biophysics 30: 1*. 1–65.                                                                                    AQ8

Noji H, Yasuda R, Yoshida M, Kinosita K, Jr. (1997). Direct observation of the rotation of F1-ATPase. *Nature* 386: 299–302.

PDB: 1JFP, Yeagle PL, Choi G, Albert AD. (2001). Studies on the structure of the G-protein-coupled receptor rhodopsin including the putative G-protein binding site in unactivated and activated forms. *Biochemistry* 40: 11932.

PDB: 119D, Leonard GA, Hunter WN. (1993). Crystal and molecular structure of d(CGTAGATCTACG) at 2.25 A resolution. *J Mol Biol* 234: 198.

PDB: 3HSF, Damberger FF, Pelton JG, Liu C, Cho H, Harrison CJ, Nelson HCM, Wemmer DE. (1995). Refined solution structure and dynamics of the DNA-binding domain of the heat shock factor from *Kluyveromyces lactis*. J. *Mol. Biol.* 254: 704.

Penrose LS, Penrose R. (1957). A self-reproducing analogue. *Nature* 179: 1183.                          AQ9

Penrose LS. (1958). Mechanics of self-reproduction. *Ann. Hum. Genet.* 23: 59–72.

Pieroni O, Fissi A, Angelini N, Lenci F. (2001). Photoresponsive polypeptides. *Acc. Chem. Res.* 34: 9–17.

Plant AL, Silin V. *http://www.cstl.nist.gov/biotech/biomat/Projects/warfare.html*

Rohl CA, Strauss CE, Misura KM, Baker D. (2004)). Protein structure prediction using Rosetta. *Methods Enzymol.* 383: 66–93.

Schalley CA, Beizai K, Vogtle F. (2001). On the way to rotaxane-based molecular motors: studies in molecular mobility and topological chirality. *Acc. Chem. Res.* 34: 465–76.

Schnitzer MJ, Block SM. (1997). Kinesin hydrolyses one ATP per 8-nm step. *Nature* 388: 386–90.

Seeman NC. (1998). DNA nanotechnology: novel DNA constructions. *Annu. Rev. Biophys. Biomol. Struct.* 27: 225–48.

Soong RK, Bachand GD, Neves HP, Olkhovets AG, Craighead HG, Montemagno CD. (2000). Powering an inorganic nanodevice with a biomolecular motor. *Science* 290: 1555–8.

Smith SS. (2001). United States Patent No. 6,200,782, 13 March 2001.

Tobias I, Swigon D, Coleman BD. (2000). Elastic stability of DNA configurations. I. General theory. *Phys. Rev. E Stat. Phys. Plasmas Fluids Relat. Interdiscip. Topics* 61: 747–58.

Ueno T, Oosawa K, Aizawa S. (1992). M ring, S ring and proximal rod of the flagellar basal body of *Salmonella typhimurium* are composed of subunits of a single protein, FliF. *J. Mol. Biol.* 227: 672–7.

Ueno T, Oosawa K, Aizawa S. (1994). Domain structures of the MS ring component protein (FliF) of the flagellar basal body of *Salmonella typhimurium*. J. *Mol. Biol.* 236: 546–55.

Vale RD, Milligan RA. (2000). The way things move: looking under the hood of molecular motor proteins. *Science* 288: 88–95.

Wang MD, Schnitzer MJ, Yin H, Landick R, Gelles J, Block SM. (1998). Force and velocity measured for single molecules of RNA polymerase. *Science* 282: 902–7.

Walker JE. (1998). ATP Synthesis by Rotary Catalysis (Nobel Lecture). *Angewandte Chemie Intternational Edition* 37: 2308–19.

Yan H, Zhang X, Shen Z, Seeman NC. (2002). A robust DNA mechanical device controlled by hybridization topology. *Nature* 415: 62–5.

Yasuda R, Noji H, Kinosita K, Jr., Yoshida M. (1998). F1-ATPase is a highly efficient molecular motor that rotates with discrete 120 degree steps. *Cell* 93: 1117–24.

## SYNTHETIC BIOLOGY (SYNBIO)

**Expanding the genetic code**

A team at The Scripps Research Institute has created a way to add two letters to DNA, creating a third base pair that dramatically expands the genetic code. The new synthetic base pair could help in numerous avenues of research, such as developing new drugs, diagnostics and vaccines.

**Natural**

In nature, DNA — the hereditary code for building and maintaining organisms —contains four nucleotides — molecules that are DNA's building blocks. Each is represented by a letter (A, T, G or C). Letters A and T and letters G and C are always paired together, making two base pairs.

RNA, like DNA, contains nucleotides but usually in a single rather than double strand. RNA plays a central role in transferring DNA's genetic code.

Codons, which are combinations of three nucleotides, use DNA and RNA information to code amino acids. With the four naturally occurring nucleotides, 64 codons are possible from all of the letter combinations.

Amino acids are important for building proteins — complex molecules responsible for everything from digestion to immunity to communication between cells. There are 20 different amino acids that can occur naturally.

DETAIL

DNA — 4 nucleotides — 2 base pairs

A T
T A
G C
C G

RNA — 4 nucleotides — Codon — 64 codons

A
U
G
C

*In RNA, the thymine (T) is replaced by (U) uraci*

20 amino acids — Proteins

**Natural plus synthetic**

Along with the four natural nucleotides in DNA — A, T, G and C — scientists have been able to add two new nucleotides (X and Y). This creates a third, synthetic base pair.

The nucleotides represented by X and Y also get added to the four natural nucleotides in RNA.

With a total of six nucleotides (four natural and two synthetic), there are 216 possible codons.

There could be up to 172 possible amino acids used to build proteins.

eDNA — 6 nucleotides — 3 base pairs

A T
T A
G C
C G
X Y
Y X

eRNA — 6 nucleotides

A
U
G
C
X
Y

216 codons

Up to 172 amino acids

Sources: The Scripps Research Institute; Synthorx

AARON STECKELBERG · U-T

May 7, 2017 by [Dr Rajiv Desai](#)

**Synthetic Biology** (synbio):

Prologue:

The advent of digital technology is considered one of the biggest revolutions of all time for how quickly and deeply it changed the world forever. Even more powerful revolution is coming, which goes under the name of synthetic biology. The potential of this discipline is very well understood in the biological scientific world. It is time I make general public aware of the impact it will have on our lives. Synthetic biology brings together a bewildering number of disciplines: biotechnology, molecular biology, systems biology, biophysics, computer engineering, genetic engineering, and more. ==Synthetic biology will allow scientists and engineers to create biological systems that do not occur naturally as well as to re-engineer existing biological systems to perform novel and beneficial tasks==. The term "synthetic biology" was introduced in 1978 by molecular

biologist and geneticist Wacław Szybalski. **Synthetic biology is considered as an amalgamation of principles of engineering and biology**. At its core, it's all about the **selective assembly of genetic information**. This is where the connection with computer science comes into play. Synthetic biologists aren't just copying and pasting existing DNA from one place to another—they're looking to figure out **how specific sequences work and then putting them together into new configurations**. The idea is that you can figure out what given segments of DNA do and then patch them together, **much as you would with lines of computer code, effectively programming cells to behave in new ways.** The scientists involved treat DNA a bit like a computer programme. **The aim is to rewrite life's internal operating system: design a new programme, print it out and run it in a cell which has been cleaned of its own genetic material to perform functions that may or may not be found in nature or create genetic machines from scratch**. **Now we can read and write the genetic code, put it in digital form and translate it back into synthesized life**. Ten years ago, genetic engineering was limited to cutting and pasting DNA from existing organisms. **Today's biologists can write down gene sequences that have never existed anywhere, place an order over the Internet, and receive the desired DNA by return mail.** As the cost of sequencing and DNA synthesis continues to drop, ambitious ideas for synthetic biology are becoming more affordable and achievable. In Boston, scientists and students conduct so called "synbio" projects developing odorless E. coli cells meant to synthesize wonder protein containing essential amino acids. Synthetic biology can create synthetic food, chemicals, biofuels, diagnostics, antibiotics and even building materials. It's easy to imagine nightmarish synbio-powered science fiction scenarios such as terrorists designing custom viruses designed to target specific populations. Alternately, seemingly benign lab-grown organisms might behave in unexpected ways when they interact with ecosystems, threatening the natural balance. The danger is not just bio-terror, but bio-error. And, at a more structural level, some fear that these technologies could suddenly disrupt industries such as farming, potentially driving billions deeper into poverty. But that's all very far away. In many ways, it's the very definition of an emerging field. Its promises are enormous, but progress remains incremental.

> **Comment [i1]:** This is alarming to no end that something like this can be enroute and without any dire reasoning or cosequence-with the implications here--this is by far the most insidious means of weaponization to date

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**"A Scientist discovers that which exists; an Engineer creates that which never was."**

– Theodore von Karmen

Note: Please read my articles 'Genetically Modified' and 'Gene Therapy' published earlier in this website. **A genetically modified organism (GMO) is an organism (plant/ animal/ microorganism etc.) whose genetic material (DNA) has been altered using genetic engineering techniques by either adding a gene from a different species or over-expressing/ silencing a pre-existing native gene**. Genetic material in an organism can be altered without genetic engineering techniques which include mutation breeding where an organism is exposed to radiation or chemicals to create a non-specific but stable change, selective breeding (plant breeding and animal breeding), hybridizing and somaclonal variation. However, these organisms are not labelled as GMO. Gene

therapy can broadly be considered any treatment that changes gene function to alleviate a disease state. Gene therapy means man-made transfer/alteration/ expression/suppression of DNA/RNA in human/animal cells for the purpose of prophylaxis and/or treatment of a disease state. Replacing a defective gene by normal gene is one of the types of gene therapy. Other types include gene editing, gene silencing, insertion of novel genes, gene reprogramming, DNA vaccine etc. Gene therapy is one of the methods of genetic engineering and in the puritan medical terminology; any individual who has received gene therapy necessarily becomes genetically modified organism (GMO). Synthetic biology is an extreme version of genetic engineering and organism created by synthetic biology is labelled as synthetically modified organism (SMO).

———

**Abbreviations and synonyms:**

XNA = xeno-nucleic acids

SB = synthetic biology = synbio

AL = artificial life

AI = artificial intelligence

bp = base pairs

Mbp = million bp

GMO = genetically modified organisms

GE = genetic engineering

SMO = synthetically modified organisms

JCVI = J. Craig Venter Institute in Rockville, Maryland.

CRISPR = Clustered Regularly Interspaced Palindromic Repeats

PCR = polymerase chain reaction

SBPs = Synthetic biology projects

———

**Terminology**:

Here's a list of some important terms that will help you understand what we're talking about when discussing synbio technology.

**Amino acid:** The building block of proteins, which are essential to all life forms. There are twenty-two different amino acids, twenty of which are encoded by human genes and two are produced by other biological processes. These amino acids, also called residues, can be strung together in different combinations, each combination resulting in a unique protein.

**Protein:** A biological polymer made of amino acids strung together in a specific sequence.

**Base:** One of five chemical structures that can make up part of a nucleoside. The names of the bases are adenine (A), cytosine (C), guanine (G), thymine (T) and uracil (U). Adenine, cytosine, and guanine are used in both DNA and RNA whereas thymine is only used in DNA and uracil is its RNA counterpart.

**Base pair:** A specific pairing of bases that enable the helical structure of both DNA and RNA. Cytosine always pairs with guanine using three hydrogen bonds, and adenine with thymine (in DNA) or uracil (in RNA) with two hydrogen bonds

**Nucleoside:** Nucleosides consist of a **ribose** (or deoxyribose) sugar linked to a base. They are strung together to form DNA and RNA, with **a phosphate** group linking each nucleoside to the next.

_

**Nucleotide:** Nucleotides are the building blocks of nucleic acids; they are composed of three subunit molecules: a nitrogenous base, a five-carbon sugar (ribose or deoxyribose), and phosphate group. Nucleotide is nucleoside with phosphate.

**Codon:** A sequence of three nucleotides that codes for a specific amino acid.

**Oligonucleotide (oligo):** A short DNA or RNA polymer that is usually less than 100 nucleotides in length. It is often man-made (synthetic DNA or RNA).

**Recombinant DNA**: DNA that has been formed artificially by combining constituents from different organisms. Recombinant DNA (rDNA) molecules are DNA molecules formed by laboratory methods of genetic recombination (such as molecular cloning) to bring together genetic material from multiple sources, creating sequences that would not otherwise be found in the genome.

**Synthetic DNA**: DNA generated using synthetic biology tools rather than extracted from a living organism. The resulting synthetic DNA can be created in exactly the same sequence as the DNA from a living organism. Synthetic DNA cannot create new organisms (even microscopic ones) from scratch, but can be used to add beneficial

Comment [i3]: with nanobio you have the ligands ( protein polymers utilized by the hosts protein and attracts other nano to it for assembly forming a fullerene complex to create networks or to transport other building blocks of synthetic dna or other nano materials

Comment [i4]: Or Detrimental -- bio/nano warfare -targetting sequence looking for specific DNA to attach to to cause whatever conditions that is in the programming of the other dna

**character traits** to a plant (self-fertilizing, drought tolerant) or engineer new drugs or vaccines like artemisinin for malaria.

**Part (Biological part):** is a sequence of **DNA that encodes for a biological function**, for example promoters or protein coding sequences. At its simplest, a basic part is a single functional unit that cannot be divided further into smaller functional units. Basic parts can be assembled together to make longer, more complex composite parts, which in turn can be assembled together to make devices that will operate in living cells.

**BioBricks:** **Standard synthetic DNA sequences of known structure and function that can be used as "Lego-like" building blocks**. These can be combined in different ways to generate a specific form and function. **When inserted into living organisms, BioBricks create new, or replicate existing, biological systems**. Over 20,000 parts are currently available in the Registry of Standard Biological Parts.

> **Comment [i5]:** NANO equivalent lattices--these are basically what is put in to allow origami to assemble to allow other nano( bio) to attach assemble into a platform and integrate into cells tissue and DNA to fully be incorporated

**DNA-based circuits**: The rational **design of DNA sequences to create biological circuits with predictable, discrete functions, which can be combined in various cell hosts**.

**Chassis:** The cell or organism **into which BioBricks are inserted**, **producing a new biological system.**

**Minimal genome:** It is generally defined as the smallest set of genes that allows for replication of the organism in a particular environment. It is the minimal number of parts (genes) needed for life, to serve as a chassis for engineering minimal cell factories for new functions.

**Cloning:** Molecular cloning is the process of **inserting foreign DNA into a cell in order to create many copies of it and/or translate it into protein**.

**CRISPR/Cas9:** A naturally-occurring system that has recently been used as a synthetic biology tool **to edit genes**. **This system allows for the precise inactivation or recoding of any gene through the insertion, deletion or substitution of nucleotide sequences**.

> **Comment [i6]:** when you add NANOBIOLOGY ( synthetic bio) this is what happens with the integration and re programming of dna

**Data storage:** A term that refers to any method used **to store and archive digital data**. **Synthetic DNA can be used to store digital data**, which researchers predict would be error-free when recovered after up to 1 million years.

**DNA synthesis**: A process for producing DNA from individual nucleotides in the laboratory. **These DNA fragments can be used as gene parts or building blocks to assemble whole genes or libraries**. They are also used ubiquitously throughout academia and industry to study biology and develop medical products, such as vaccines and diagnostic tests

> **Comment [i7]:** the concept of whole gene or libraries indicates a over writing what maybe there and a new operation or systems are now being changed

**Engineering Biology:** another term used to refer to **synthetic biology (synbio)**.

**Expression:** The process by which genetic information stored in DNA is transformed into a cellular function. Expression is a-2 step process, first transcription of DNA into RNA, and second translation into protein. Example of expression is production of insulin to process sugar.

**Gene:** The basic unit of heredity; a specific DNA sequence that codes for a protein or RNA required for the organism to develop and function.

**Genome**: The complete set of genetic information of an organism, stored in genes made of DNA (most often) or RNA (rare).

**Gene editing:** The ability to insert a beneficial sequence, replace a mutated sequence or remove a diseased sequence of DNA using systems such as CRISP/Cas9.

**Gene regulation:** The control of gene expression. This can be effected by turning the expression of genes on and off at specific times through modulation of both direct and indirect factors such as promoters and enhancers.

**Genetic engineering:** Direct human manipulation of an organism's genetic material, to add new traits not already found in that organism, or to delete an unwanted disease gene.

**iGEM (international Genetically Engineered Machine competition**): A foundation dedicated to advancement of synthetic biology through education and competition. In its annual worldwide competition, college and high school level participants use standard parts and their own design to build new biological systems.

**Mutation:** Change in a gene that occurs randomly or in response to radiation or chemical mutagens. Alternatively, using the synthesis of DNA, specific mutations can be designed on purpose by researchers for precise and controlled experimentation.

**Nanobiology**: A branch of biology that deals with biological interactions at a very small (nano) scale, often involving structures that are only millionths of an inch in size.

**Pathway:** In biology, a pathway describes a series of actions between molecules that can lead to a change in a cell and/or a product such as sugar, protein, fat or organic molecules. A pathway is usually encoded as a string of genes in a long fragment of DNA.

**PCR (Polymerase Chain Reaction):** It is a technique used in molecular biology to amplify a single copy or a few copies of a segment of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence through the action of polymerases.

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**Comment [i8]:** Basically the operating system that that accesses the program to causse the body to function or other operations in the body to work

**Comment [i9]:** the operating system of the body

**Comment [i10]:** the ability to replace insert or program dna is not always beneficial -when you look at vaccines you can see what happens when this operations is being done and the mutations that occur and the disarray of function occurs as a result of insertion of genetic or nano paterials that now overwrite and initiate a new program wiping out the old one as a esult of the over write -similar to a cassette tape when you record and the re record over what you had

**Comment [i11]:** Self assemblying and Self replication

**Sequence:** The specific order in which amino acids, deoxynucleotides, and ribonucleotides are strung together to form a specific protein, DNA or RNA, respectively.

**Sequencing:** Process by which scientists determine the specific order of base pairs within DNA, RNA, or protein.

**Living modified organism:** Any living organism that possesses a novel combination of genetic material obtained through the use of modern biotechnology.

> **Comment [i12]:** This can apply to all life even of mankind or humanity

**Living organism:** Any biological entity capable of transferring or replicating genetic material, including sterile organisms, viruses and viroids.

> **Comment [i13]:** This can apply to all life even of mankind or humanity

**Protocell (artificial cell):** Protocells are living cells constructed from scratch capable of reproduction, self-maintenance, metabolism and evolution. Bottom-up synthetic biology approach produces protocells cells and compartments.

**Xenobiology:** The study and development of life forms based on biochemistry not found in nature. This includes xeno-nucleic acids XNA (synthetic alternatives to the natural nucleic acids DNA and RNA) and amino acids that are not found in the natural genetic code of organisms. Xenobiology could provide a biosafety tool by preventing interactions between synthetic organisms and the natural world (xeno-nucleic acids can prevent genetic exchange with wild organisms, as they cannot hybridise with natural genetic material).

**Digital logic gate:** An idealized or physical device that implements Boolean logic (such as AND, OR, NOT) on one or more inputs to produce a single output

**Orthogonal biosystems:** Engineering cells/organisms to include systems or parts not found in nature to impart new capacities or chemistry. Orthogonal systems operate independently of the cell's natural machinery.

**Bionanoscience:** Utilising and exploiting synthetic molecular (nano) machines based on cellular systems

> **Comment [i14]:** Another term can be as well utilized is a Mimetic ---these synthetic molecules can create what appears to be something else in biology --it Mimics there attributes

**Biofuels:** Sources of energy derived from biomass such as plants, algae and animal waste products. The use of biofuels could substantially reduce greenhouse gas emissions by recycling carbon dioxide from the air and replacing fossil fuels such as oil. Synthetic biology tools are often used to in the development of biofuels.

**DNA and RNA:**

Deoxyribonucleic acid (DNA) is a molecule that carries the genetic instructions used in the growth, development, functioning and reproduction of all known living organisms and many viruses. DNA and RNA are nucleic acids; alongside proteins, lipids and complex carbohydrates (polysaccharides), they are one of the four major

types of macromolecules that are essential for all known forms of life. **Most DNA molecules consist of two biopolymer strands coiled around each other to form a double helix.** The two DNA strands are termed polynucleotides since they are composed of simpler monomer units called nucleotides. Each nucleotide is composed of one of four nitrogen-containing nucleobases—either cytosine (C), guanine (G), adenine (A), or thymine (T)—and a sugar called deoxyribose and a phosphate group. The nucleotides are joined to one another in a chain by covalent bonds between the sugar of one nucleotide and the phosphate of the next, resulting in an alternating sugar-phosphate backbone. The nitrogenous bases of the two separate polynucleotide strands are bound together according to base pairing rules (A with T, and C with G) with hydrogen bonds to make double-stranded DNA. The total amount of related DNA base pairs on Earth is estimated at 5.0 x 10^37 and weighs 50 billion tonnes.

**The structure of part of a DNA double helix:**

In living organisms, DNA does not usually exist as a single molecule, but instead as a pair of molecules that are held tightly together. These two long strands entwine like vines, in the shape of a double helix. The nucleotide contains both a segment of the backbone of the molecule (which holds the chain together) and a nucleobase (which interacts with the other DNA strand in the helix). A nucleobase linked to a sugar is called a nucleoside and a base linked to a sugar and one or more phosphate groups is called a nucleotide. A polymer comprising multiple linked nucleotides (as in DNA) is called a polynucleotide.

**Atoms in DNA:**

Figure below shows atoms in the DNA molecule colour-coded by element and the detailed structure of two base pairs are shown in the bottom right.

DNA stores biological information. DNA is a molecule that contains the instructions an organism needs to develop, live and reproduce. These instructions are found inside every cell, and are passed down from parents to their children. The DNA backbone is resistant to cleavage, and both strands of the double-stranded structure store the same biological information. This information is replicated as and when the two strands separate. A large part of DNA (more than 98% for humans) is non-coding, meaning that these sections do not serve as patterns for protein sequences. The two strands of DNA run in opposite directions to each other and are thus antiparallel. Attached to each sugar is one of four types of nucleobases (informally, bases). It is the sequence of these four nucleobases along the backbone that encodes biological information. The order of these bases is what determines DNA's instructions, or genetic code. Similar to the way the order of letters in the alphabet can be used to form a word, the order of nitrogen bases in a DNA sequence forms genes, which in the language of the cell, tells cells how to make proteins. The entire human genome contains about 3 billion bases and about 20,000 genes. RNA strands are created using DNA strands as a template in a process called transcription. Under the genetic code, these RNA strands are translated to specify the sequence of amino acids within

**proteins in a process called translation. The regular structure and data redundancy provided by the DNA double helix make DNA well suited to the storage of genetic information, while base-pairing between DNA and incoming nucleotides provides the mechanism through which DNA polymerase replicates DNA, and RNA polymerase transcribes DNA into RNA.**

**DNA is a long polymer made from repeating units called nucleotides.** The structure of DNA is dynamic along its length**, being capable of coiling into tight loops, and other shapes. In all species** it is composed of two helical chains, bound to each other by hydrogen bonds. Both chains are coiled round the same axis, and have the same pitch of **34 ångströms (3.4 nanometres). The pair of chains has a radius of 10 ångströms (1.0 nanometre). According to another study, when measured in a different solution, the DNA chain measured 22 to 26 ångströms wide (2.2 to 2.6 nanometres), and one nucleotide unit measured 3.3 Å (0.33 nm**) long. **Although each individual nucleotide repeating unit is very small, DNA polymers can be very large molecules containing millions to hundreds of millions of nucleotides**. For instance, the DNA in the largest human chromosome, chromosome number 1, consists of approximately 220 million base pairs and would be 85 mm long if straightened.

**A  gene is a sequence of DNA that contains genetic information and can influence the phenotype of an organism.** Within a gene, the sequence of bases along a DNA strand defines a messenger RNA sequence, which then defines one or more protein sequences. **The relationship between the nucleotide sequences of genes and the amino-acid sequences of proteins is determined by the rules of translation, known collectively as the genetic code. The genetic code consists of three-letter 'words' called codons formed from a sequence of three nucleotides (e.g. ACT, CAG, TTT**). **In transcription, the codons of a gene are copied into messenger RNA by RNA polymerase**

. **This RNA copy is then decoded by a ribosome that reads the RNA sequence by base-pairing the messenger RNA to transfer RNA, which carries amino acids**. Since there are **4 bases in 3-letter combinations, there are 64 possible codons (64 possible permutations, or combinations of three-letter nucleotide sequences that can be made from the four nucleotides). Of the 64 codons, 61 represent 20 natural amino acids, and three are stop signals. For example, the codon CAG represents the amino acid glutamine, and TAA is a stop codon**. **The genetic code is described as degenerate, or redundant, because a single amino acid may be coded for by more than one codon**. When codons are read from the nucleotide sequence, **they are read in succession and do not overlap with one another. Within eukaryotic cells, DNA is organized into long structures called chromosomes. During cell division these chromosomes are duplicated in the process of DNA replication, providing each cell its own complete set of chromosomes. Eukaryotic organisms (animals, plants, fungi, and protists) store most of their DNA inside the cell nucleus and some of their DNA in organelles, such as mitochondria or chloroplasts. In contrast, prokaryotes (bacteria and archaea) store their DNA only in the cytoplasm.** Within the eukaryotic chromosomes, chromatin proteins such as histones compact and organize DNA. **These compact**

> **Comment [i15]: Like a boot drive in a operating system**

structures guide the interactions between DNA and other proteins, helping control which parts of the DNA are transcribed.

**RNA:**

Like DNA, RNA (ribonucleic acid) is assembled as a chain of nucleotides, but unlike DNA it is more often found in nature as a single-strand folded onto itself, rather than a paired double-strand. In RNA, adenine and uracil (not thymine) link together, while cytosine still links to guanine. Cellular organisms use messenger RNA (mRNA) to convey genetic information (using the letters G, U, A, and C to denote the nitrogenous bases guanine, uracil, adenine, and cytosine) that directs synthesis of specific proteins. In protein synthesis RNA molecules direct the assembly of proteins on ribosomes. This process uses transfer RNA (tRNA) molecules to deliver amino acids to the ribosome, where ribosomal RNA (rRNA) then links amino acids together to form proteins. Many viruses encode their genetic information using an RNA genome. Some RNA molecules play an active role within cells by catalyzing biological reactions, controlling gene expression, or sensing and communicating responses to cellular signals.

**Genome size:**

Genome size is the total amount of DNA contained within one copy of a single genome. It is typically measured in terms of mass in picograms [trillionths of a gram, abbreviated pg] or less frequently in Daltons or as the total number of nucleotide base pairs typically in megabases (millions of base pairs, abbreviated Mb or Mbp). One picogram equals 978 megabases.

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bp = base pair (e.g. A with T)

1 kb = 1000 bp (e.g. viral genome)

1Mb = 1000,000 bp (e.g. bacterial genome)

I Gb = 1000,000,000 bp (e.g. mammal genome)

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**Comment [i16]:** XNA = xeno-nucleic acids
SB = synthetic biology = synbio
AL = artificial life
AI = artificial intelligence
bp = base pairs
Mbp = million bp
GMO = genetically modified organisms
GE = genetic engineering
SMO = synthetically modified organisms
JCVI = J. Craig Venter Institute in Rockville, Maryland.
CRISPR = Clustered Regularly Interspaced Palindromic Repeats
PCR = polymerase chain reaction
SBPs = Synthetic biology projects
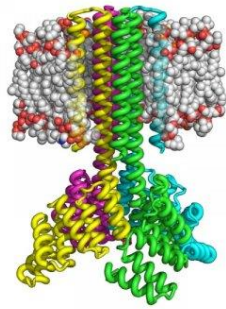
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**Scientists create complex transmembrane proteins from scratch**

**Advance paves the way for the design of transmembrane proteins with useful, new functions**



==This illustration shows how four copies of computer-designed transmembrane protein assembled to form a rocket-shaped tetramer with a wide cytoplasmic base that funnels into eight transmembrane helices and which orients correctly in membrane==.
*Credit: University of Washington Institute for Protein Design*

==It is now possible to create complex, custom-designed transmembrane proteins from scratch==, scientists report this week. The advance, led by molecular engineers at the University of Washington Institute for Protein Design, will ==enable researchers to create transmembrane proteins not found in nature to== perform specific tasks.

==In the living world, transmembrane proteins are found embedded in the membrane of all cells and cellular organelles==. ==They are essential for them to function normally==. For example, many ==naturally occurring transmembrane proteins act as gateways for the movement of specific substances across a biological membrane. Some== transmembrane proteins ==receive or transmit cell signals==. Because of such roles, many drugs are designed to target transmembrane proteins and alter their function. "Our results pave the way for the design of multispan membrane proteins that could mimic proteins found in nature or have entirely novel structure, function and uses," said David Baker, a University of Washington School of Medicine professor biochemistry and director of the UW Institute of Protein Design who led the project. The research is reported in the March 1 issue of the journal *Science*. Peilong Lu, a senior fellow in the Baker lab, is the paper's lead author. But understanding how transmembrane proteins are put together and how they work has proved challenging. Because they act while embedded within the cellular membrane, transmembrane proteins have proven to be more difficult to study than proteins that operate in the watery solution that make up the cells' cytoplasm or in the extracellular fluid. In the new study, Lu and his coworkers used a

**Comment [i1]:** A protein that spans the entire biological membrane.-- A transmembrane protein is a protein which spans the entire length of the cell membrane. It is embedded between the phospholipids, providing a channel through which molecules and ions can pass into the cell. Transmembrane proteins also facilitate communication between cells by interacting with chemical messengers. Many biological processes, such as the metabolism of glucose and the production of fatty acids, are triggered after a particular transmembrane protein is activated.

computer program, developed in the Baker lab and called Rosetta, that can predict the structure a protein will fold into after it has been synthesized. The architecture of a protein is crucial **because a protein's structure determines its function**. **A protein's shape forms from complex interactions between the amino acids that make up the protein chain and between the amino acids and the surrounding environment**. **Ultimately, the protein assumes the shape that best balances out all these factors** so that the protein achieves the lowest possible energy state. The Rosetta program used by Lu and his colleagues can predict the structure of a protein by taking into account these interactions and calculating the lowest overall energy state. It is not unusual for the program to create tens of thousands of model structures for an amino acid sequence and then identify the ones with lowest energy state. **The resulting models have been shown to accurately represent the structure the sequence will likely assume in nature**. Determining the structure of transmembrane proteins is difficult because portions of transmembrane proteins **must pass though the membrane's interior, which is made of oily fats called lipids.** In aqueous fluids, amino acid residues that have polar sidechains -- components that can have a charge under certain physiological conditions or that participate in hydrogen bonding -- tend to be located on the surface of the protein where they can interact with water, which has negatively and positively side charges to its molecule. As a result, polar residues on proteins are called hydrophilic, or "water-loving." Non-polar residues, on the other hand, tend to be found packed within the protein core away from the polar aqueous fluid. Such residues are called hydrophobic or "water-fearing**." As a result, the interaction between the water-loving and water-fearing residues of the protein and the surrounding watery fluids helps drive protein folding and stabilizes the protein's final structure**. In membranes, however, protein folding is more complicated **because the lipid interior of the membrane is non-polar, that is, it has no separation of electrical charges**. This means to be stable the protein must place nonpolar, water-fearing residues on its surface, and pack its polar, water-loving residues inside. Then it must find a way to stabilize its structure by creating bonds between the hydrophilic residues within its core. The key to solving the problem, says Lu, was to apply a method developed by Baker lab to design **proteins so that the polar, hydrophilic residues fit in such a way that enough would form polar-polar interactions that can tie the protein together from within**. "Putting together these 'buried hydrogen bond networks' was like putting together a jig-saw puzzle," Baker said. With this approach, Lu and his colleagues **were able to manufacture the designed transmembrane proteins inside bacteria and mammalian cells by using as many as 215 amino acids**. The resulting proteins proved to be highly thermally **stable and able to correctly orient themselves on the membrane**. Like naturally occurring transmembrane proteins, **the proteins are multipass, meaning they traverse the membrane several times, and assemble into stable multi-protein complexes, such as dimers, trimers and tetramers.** "We have shown

that it is now possible to accurately design complex, multipass transmembrane proteins that can be expressed in cells. This will make it possible for researchers to design transmembrane proteins with entirely novel structures and functions," said Lu. **Story Source-m**aterials provided by **[University of Washington Health Sciences/UW Medicine](#)**. **Journal Reference**-Peilong Lu, Duyoung Min, Frank DiMaio, Kathy Y. Wei, Michael D. Vahey, Scott E. Boyken, Zibo Chen, Jorge A. Fallas, George Ueda, William Sheffler, Vikram Khipple Mulligan, Wenqing Xu, James U. Bowie, David Baker. **Accurate computational design of multipass transmembrane proteins**. *Science*, 2018; 359 (6379): 1042 DOI: [10.1126/science.aaq1739](#) University of Washington Health Sciences/UW Medicine. "Scientists create complex transmembrane proteins from scratch: Advance paves the way for the design of transmembrane proteins with useful, new functions." ScienceDaily. ScienceDaily, 1 March 2018.
<www.sciencedaily.com/releases/2018/03/180301144148.htm>.

## Towards XNA nanotechnology- new materials from synthetic genetic polymers.

Pinheiro VB[1], Holliger P[2].

**Author information**

## Abstract

Nucleic acids display remarkable properties beyond information storage and propagation. **The well-understood base pairing rules have enabled nucleic acids to be assembled into nanostructures of ever increasing complexity. Although nanostructures can be constructed using other building blocks, including peptides and lipids, it is the capacity to evolve that sets nucleic acids apart from all other nanoscale building materials**. Nonetheless, the poor chemical and biological stability of DNA and RNA constrain their applications. Recent advances in nucleic acid chemistry and **polymerase engineering enable the synthesis, replication, and evolution of a range of synthetic genetic polymers (XNAs) with improved chemical and biological stability**. We discuss the impact of this technology on the generation of XNA ligands, enzymes, and nanostructures with tailor-made chemistry.

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## Synthetic genetic polymers capable of heredity and evolution.

Pinheiro VB[1], Taylor AI, Cozens C, Abramov M, Renders M, Zhang S, Chaput JC, Wengel J, Peak-Chew SY, McLaughlin SH, Herdewijn P, Holliger P.

**Author information**

## Abstract

Genetic information storage and processing rely on just two polymers, DNA and RNA, yet whether their role reflects evolutionary history or fundamental functional constraints is currently unknown. **With the use of polymerase evolution and design, we show that genetic information can be stored in and recovered from six alternative genetic polymers based on simple nucleic acid architectures not found in nature [xeno-nucleic acids (XNAs)].** We also select XNA aptamers, which bind their targets with high affinity and specificity, demonstrating that beyond heredity, specific XNAs have the capacity for Darwinian evolution and folding into defined structures. Thus, heredity and evolution, two hallmarks of life, are not limited to DNA and RNA but are likely to be emergent properties of polymers capable of information storage.

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## Artificial Cells –Self Repair Self Assembly and Self Production-An EU integrated project in IT- FP6-IST-FET-002035
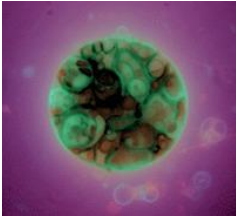


The European Commission has supported the Integrated Project PACE in its Future Emerging Technologies program from **2004-2008**. This is the final public report of results and experience in PACE.---**PACE has created the foundation for a new generation of embedded IT using programmable chemical systems that approach artificial cells in their properties of self-repair, self-assembly, self-reproduction and evolvability**.



Future projects will build on the technology and experience developed in PACE to **build the first artificial chemical cells and apply them to revolutionize complex construction in and outside IT**. PACE has established a new hybrid IT technology for programming complex chemical systems. PACE has explored the IT potential of future synthetic chemical cells: addressing both the novel embedded IT required to produce and program them and their technical opportunities, both within IT and to other fields. **In contrast with biological approaches to minimize existing cells, applications of these artificial chemical cells will exploit their chemical distinctiveness from biological cells, in particular their ability to function without proteins and below the complexity barrier posed by biological translation machinery.-**-A consortium of some 13 partners and 2 cooperating groups from 8 European countries, including Switzerland and Lithuania, and several leading USA organizations are pioneering this new approach under the IST-FET section of the EU 6th Framework Program (FP6).

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## XNA-Synthetic DNA That Can Evolve

**By swapping sugars in the DNA helix, scientists have created a new kind of genetic code that can function and evolve like regular DNA.**
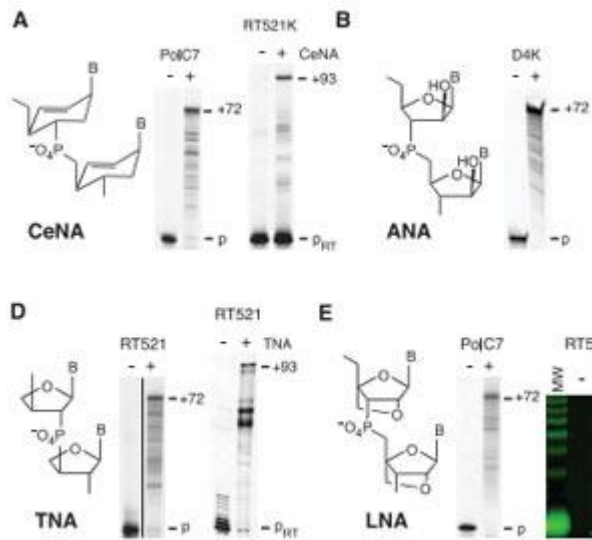


**Every living thing on Earth** uses DNA or RNA to carry its genetic instructions for life. These two nucleic acids have different names because they're built from different sugars: DNA uses deoxyribose sugars for a backbone of its double helix, while RNA uses ribose. But what if other sugars could be used too?

Now scientists have shown that at least six other types of sugars can form nucleic acid backbones—and they can be used to store and retrieve genetic information. The researchers built DNA molecules from scratch, but replaced the deoxyribose with six other kinds of sugar, including hexitol, threose, and arabinose. The six types of synthetic genetic chains are called XNAs, or xeno-nucleic acids ("xeno" is Greek for "foreign"). And because XNA shows the possibility of heredity—passing down their genetic information—the researchers say these molecules not only could address fascinating questions about the origin of life, but also could open up the possibility of another kind of life based not on DNA and RNA.

Jack Szostak, a geneticist and Nobel laureate at Harvard University, tells PM in an email that the work "is very interesting with respect to the origin of life—in principle, many different polymers could serve the roles of RNA and DNA in living organisms. Why then does modern biology use only RNA and DNA?"

**How to Make Synthetic DNA**

This isn't the first time that geneticists have cooked up synthetic nucleic acids in a lab. Some scientists had previously created DNA with [new kinds of base pairs](#) beyond the A-T and C-G connections in DNA, and others had already created [XNAs that incorporate foreign sugars](#). John Chaput, a molecular biologist at Arizona State University and an author on [the new study in *Science*](#), says this work asks a new question: "How can you perform Darwinian evolution on something other than DNA or RNA? Lots of DNA and RNA molecules have been evolved in the laboratory, but going the next step and doing it on other molecules has been very challenging. This is one of the first examples of that."

To prove that XNAs could evolve, the researchers first had to create a new kind of enzyme to build the XNAs. Although it's possible to manufacture XNAs by machine, the resulting nucleic acids are short chains that have limited functionality and evolvability. So instead of using the machinated approach, the researchers took thousands of DNA-building enzymes and evolved them into XNA-building enzymes.

That required taking thousands of enzymes and mixing them together with XNA building blocks, as well as DNA strands that served as templates for the scaffolding on which to build XNA molecules. If an enzyme turned out to be good at building XNA strands, it was captured using a filtering process and amplified it for the next round of testing; enzymes that were bad at making XNA were washed away. Over many rounds of filtering, the enzyme population evolved to become more adroit at building XNAs—in fact, they could produce polymers XNA chains that lasted were five times longer than machine-made XNAs.

"They took enzymes that already existed, and evolved mutants of them that are better at making XNAs," says Floyd Romesberg, a chemist at the Scripps Research Institute, who called the technique "impressive."

Next, the researchers tried to evolve the XNAs themselves. To do so, they used a similar filtering technique. In this case, the scientists selected for XNAs that could bind to a specific protein; XNAs that did not bind to the proteins were washed away. Those that did bind were transcribed back into DNA so that they could be replicated. After replication, the team transcribed the copies back into XNA. In this way, the XNAs that had evolved to bind the protein were able to pass on that talent to a new generation of XNAs.

**Synthetic DNA, Synthetic Life?**

Because the XNAs are able to pass genetic information from one generation to the next and can adapt to the constraints of test tube evolution, Chaput says, XNAs could serve as the building blocks for completely new genetic systems. "Could you create synthetic life with it? That's possible, but it's much further down the road."

Szostak agrees, saying that "in the longer run, a very interesting implication is that it may be possible to design and build new forms of life that are based on one or more of these non-natural genetic polymers." But Romesberg emphasized that creating completely new life forms using XNA would be a long and difficult mission. The foremost challenge: Researchers must find an efficient way to reproduce XNAs directly into more XNAs without having to convert them to DNA and back again.

Some folks are nervous that releasing XNA into the biosphere could allow it to intermingle with DNA and RNA with unpredictable results. But scientists such as Steven Benner and Markus Schmidt retort that XNAs are foreign enough to be invisible to natural organisms (read more about this issue here).

Regardless of whether XNAs can or will be used to create new life forms, the researchers have shown that it is possible to expand evolution into systems based not on DNA or RNA. And the XNAs and their enzymes may also lead to some answers about why life as we know it is based on those to molecules only. Are DNA and RNA the best molecules to store genetic information and catalyze biological reactions, or did they become the building blocks of life by sheer happenstance? Now researchers will have the tools to test the true efficiency against lab-created competitors.

Medicine, too, could benefit from XNAs, Romesberg says. Doctors already prescribe biological products such as enzymes and antibodies to treat certain diseases, but these drugs break down quickly in the stomach and the blood stream. Because XNAs are somewhat foreign, they're not broken down as quickly in the body, as it has not evolved enzymes to digest them.

The experiment also has implications for looking for life on other planets. "Maybe if you look hard enough out in space, you might find a life form based on XNA," Chaput says.