Nanotechnology

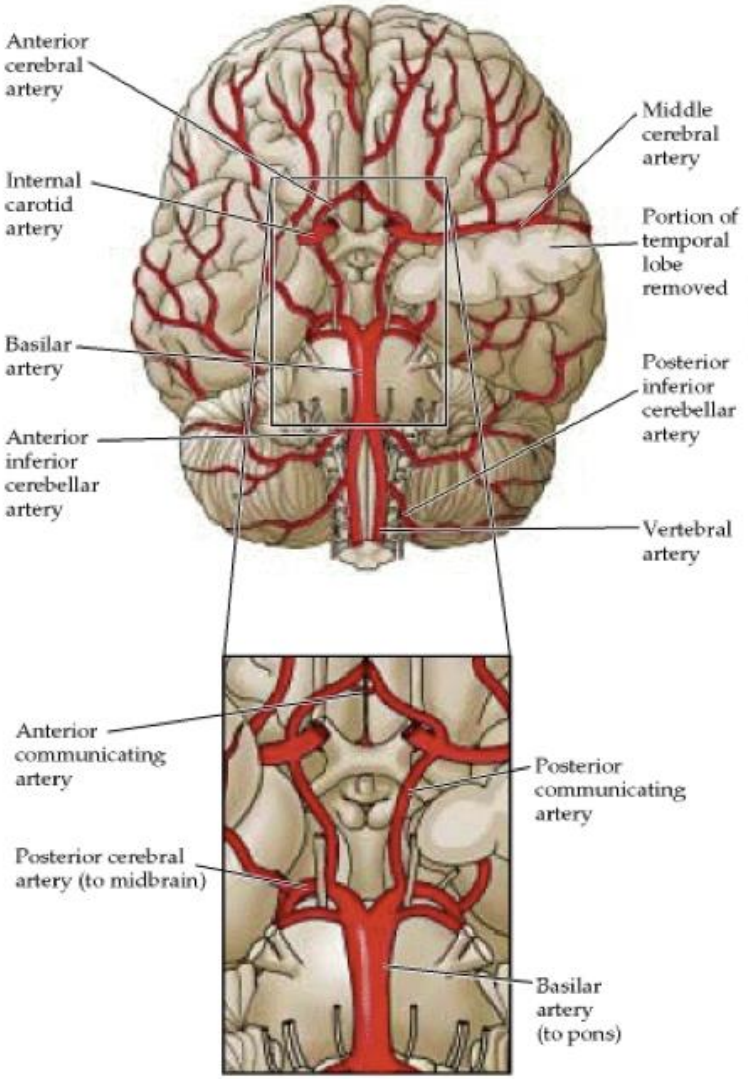
&

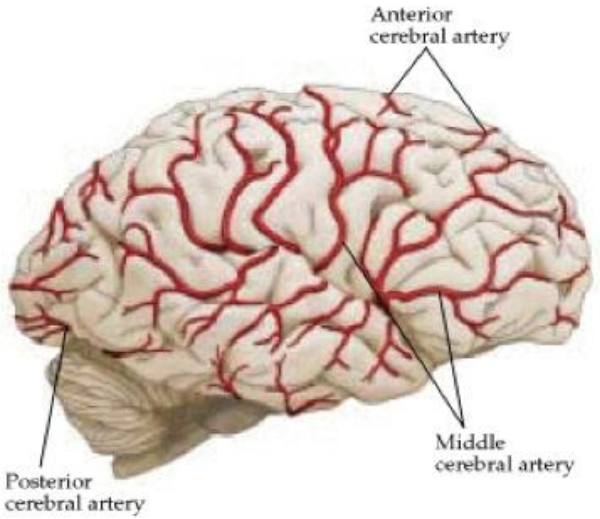
Medicine

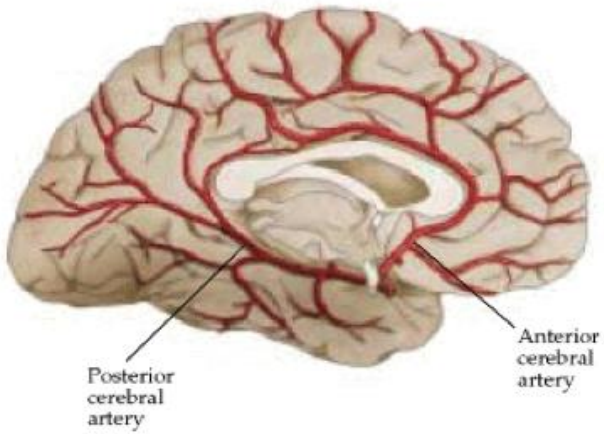
In the case of the nanopore gene sequencing technology, a sample is extracted from the target organism and the sequencing is performed externally. In the case of tracing the connectome using a rabies virus, the experiments are performed in vitro using cells grown in a culture. How might we operate directly on cells in vivo, that is to say in live animals without requiring surgery, inserting probes or sacrificing the animal to extract information for analysis.

One possible answer is to use recombinant DNA technology to re-program cells to perform new operations while not interfering with their normal function. Where in the case of employing a retrovirus to trace the connectome we used the existing inter-cellular transport mechanism, in the case of delivering a viral vector to a large population of target cells — neurons in our case — we might exploit the system of arteries and capillaries that deliver nutrients and oxygen to every cell in the human body. In the particular case of the brain, delivery is complicated by the intervention of the blood-brain barrier which consists of a membrane called the endothelium that surrounds each capillary and through which every molecule entering the brain must pass. Once inside the brain glial cells called astrocytes assist in the exchange of oxygen and glucose and the production of enzymes and neurotransmitters essential for neurons to perform their duties.

Brain's Blood Supply as Communication Network

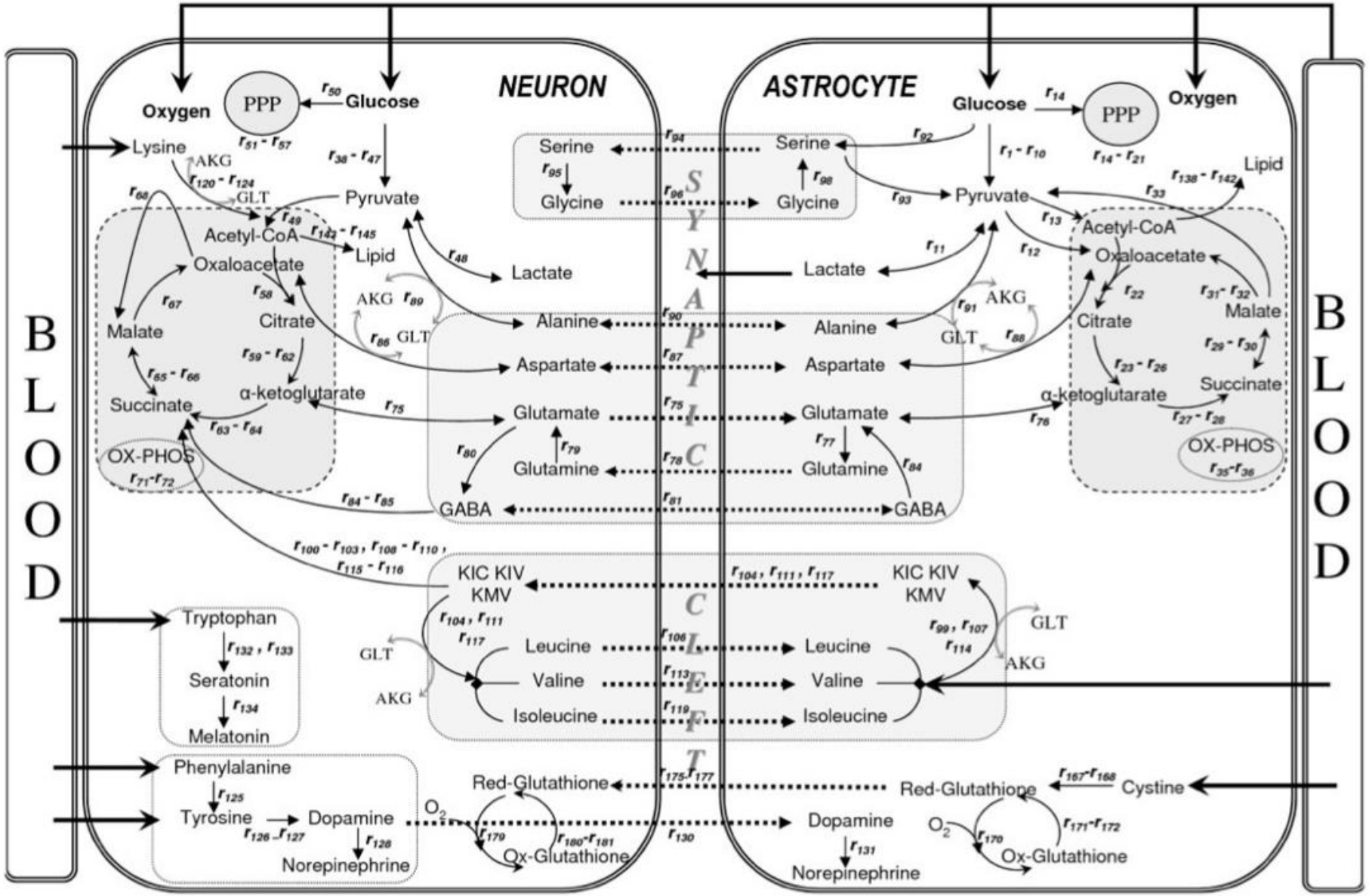






The blood brain barrier protects the brain from toxins and pathogens that might disrupt the neural machinery controlling vital processes throughout the body. Unfortunately for those affected by viral-borne brain diseases, nature has figured out how to bypass the barrier and the HIV and rabies retroviruses mentioned earlier are examples of such pathogens. The silver lining is that we are figuring out how to use these same viral vectors to repair cell damage and deliver drug payloads selectively to targets throughout the body and the brain in particular. We're also figuring out ways to foil natural viruses so they can't cross the blood-brain barrier.

Blood Brain Barrier as Highly Selective Interface



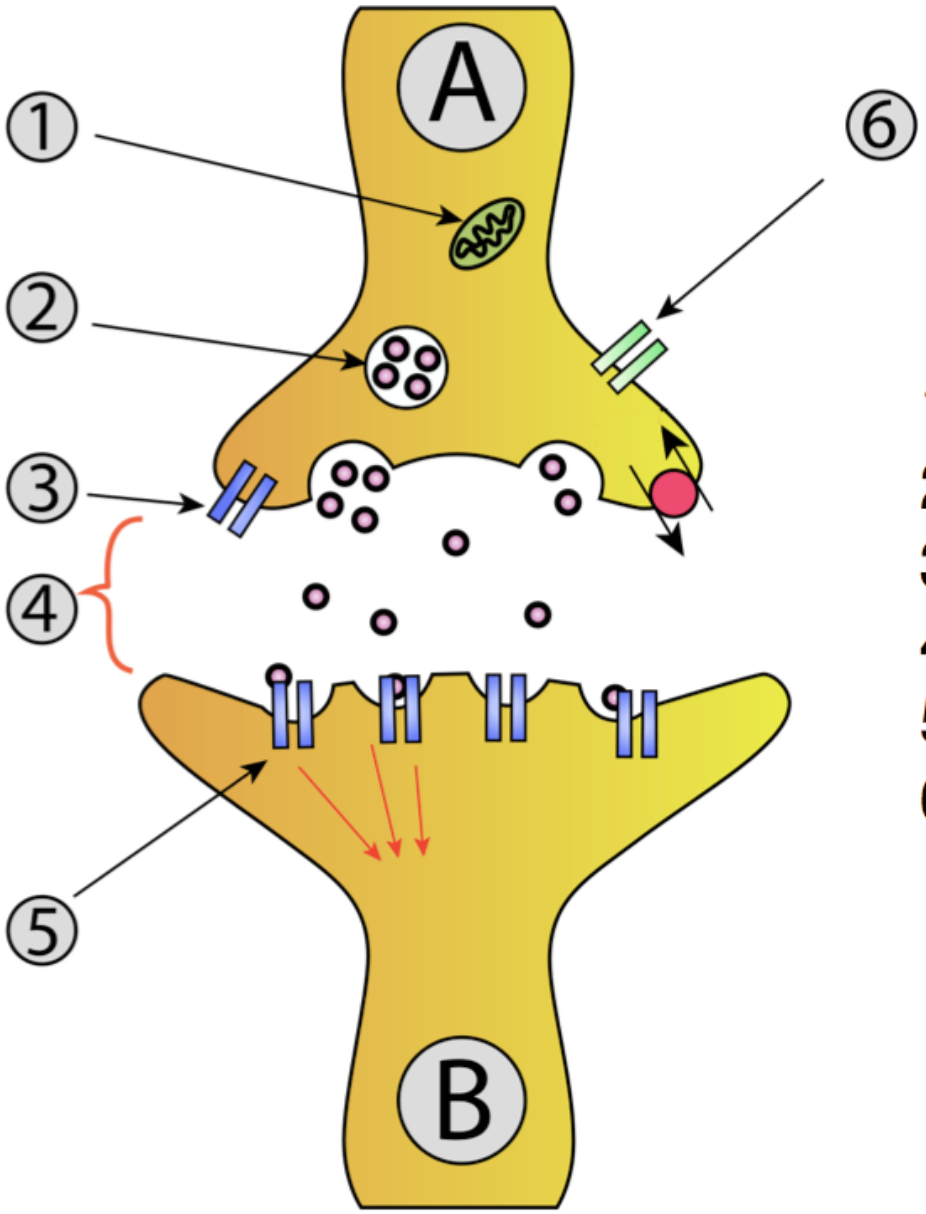
If we can use the arteries and capillaries to distribute and deliver molecular machines, then we might also use the lymph system and the vessels that return blood to the lungs and heart and waste products to the kidneys as a means of conveying information to locations external to the central nervous system where it might be more easily processed, say, using an artificial filtering process akin to dialysis7. This would provide an expedient for reading out neural-state information in lieu of more complicated nanotechnology solutions employing tiny radio transmitters that have been suggested in the literature.

Now we have a means of providing input and extracting output from the brain9. Granted that the input modality we've been exploring requires we infect each cell with virus and modify its DNA, and that would likely not serve as the input side of a real-time computer interface. On the output side however, we might have a shot at being able to observe a behaving brain at an unprecedented scale and level of detail. Assuming that our virally-delivered molecular machines don't interfere with the normal operation of the cells, we could in principle develop technology for reading off states of the brain that would not harm the host and could operate indefinitely10. What sort of information might we want to collect and how would we go about doing so?

Traditionally the focus has been on recording spike trains in the form of changes in the membrane potential of individual neurons. However, the signaling pathways in the brain are subtle and multitude; they include electrical pathways12 in the form of action potentials and voltage-gated ion channels, genetic pathways in the form of DNA translated into RNA and proteins expressed and transported within the cell, and chemical pathways in form of neurotransmitters which are emitted into the synaptic cleft separating an axon and a dendrite and serve to open ligand-gated ion channels on the dendrite.

Here we see a schematic synapse showing the neurotransmitters packaged in vesicles (2) in the pre-synaptic neuron A, ligand-gated ion channels (5) in the post-synaptic neuron B, and a mitochondrial organelle (1) that provides energy in the form of ATP. Other components include voltage-gated calcium13 channels (6) that are activated by action potentials and cause the vesicles to merge with the cell membrane and neurotranmitters to flood into the synaptic cleft and additional machinery responsible for scavenging neurotransmitters in a process called reuptake.

Molecular Machines: Synaptic Signal Transmission

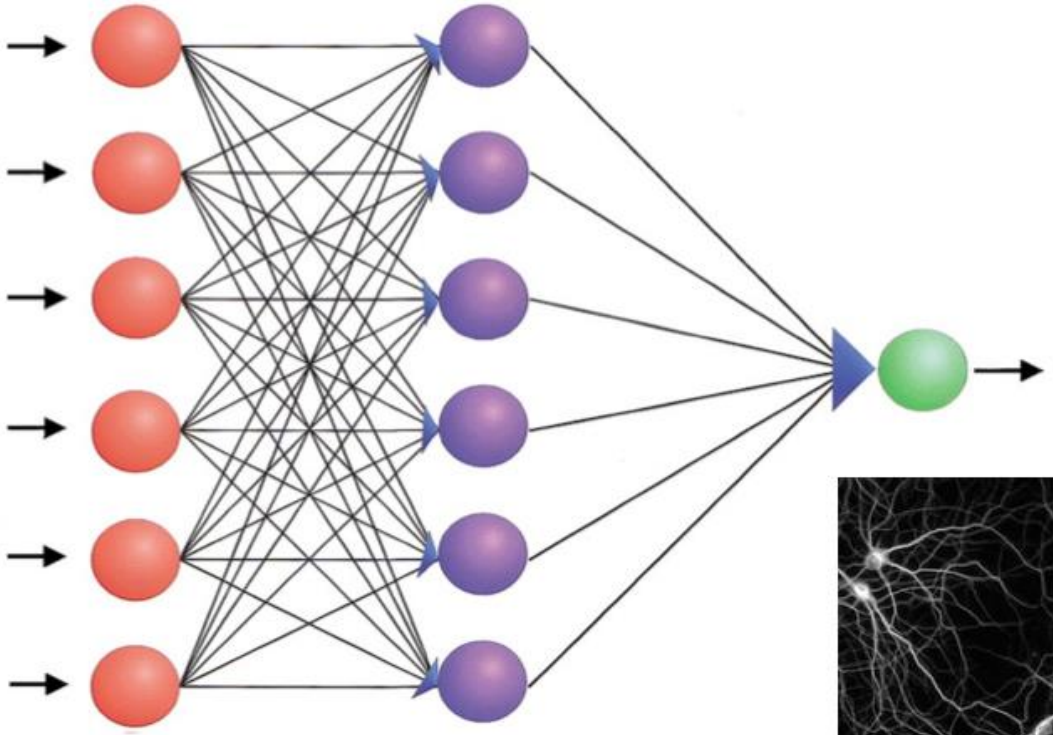


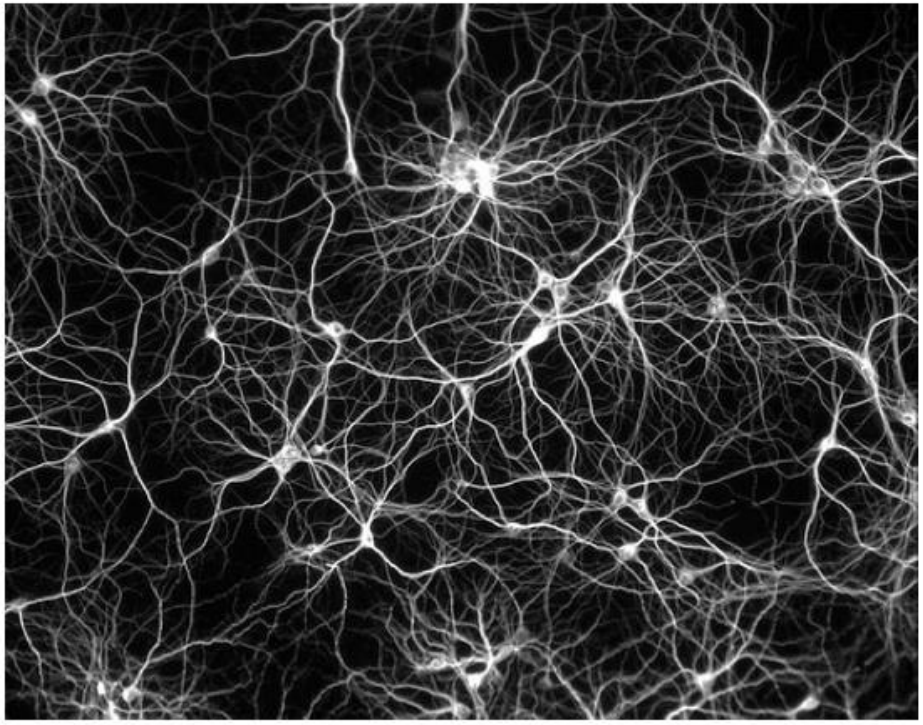
1. Mitochondria
2. Synaptic vesicle
3. Autoreceptor feedback
4. Synaptic Cleft
5. Postsynaptic receptor
6. Calcium ion channel

Must we record the state of all these components in order to obtain a complete picture? Perhaps, but it may be that the proteomic history — the record of specific proteins expressed and transported across synapses — is sufficient to infer most of what is going on informationally and computationally within the brain. In any case, we are going to assume so for the remainder of this discussion, and make the additional simplifying assumption that the production and transfer of neurotransmitters provide enough information.

Our grand goal is to collect enough data to infer not only the structure and circuitry of the brain — what we have been calling the connectome, but the function of smaller, anatomically-localized neural circuits, larger super-complexes of neurons that implement functional areas such as the visual cortex, and the recurrent pathways linking these functional areas to support high-level cognition. We hope to abstract the behavior of these diverse neural circuits and build models to test our understanding. Our progress so far suggests that we will have to record simultaneously from large collections of neurons to make additional progress on these challenging problems.

Molecular Machines: Neural Networks





Here's a very rough sketch for how we might record the proteomic history of a behaving brain. First off we need to be able to identify what neurotransmitter is being conveyed, which neuron is transmitting the information and which neuron is receiving it. Essentially we need a unique identifier for each class of neurotransmitter and each individual neuron. A recent paper in Nature described a scalable method for generating self-assembling barcodes using DNA origami as a substrate and fluorescent tags to encode the digital information [19]. Something like this method might suffice to encode the unique identifiers that we require.

Scalable Neuroscience: Big Data and Tiny Machines

Order of 10^{14} edges (synapses) in the cortex connection graph.

Order of 10^{11} nodes (neurons) in the cortex connection graph.

Encode any integer 0 to 18,446,744,073,709,551,615 in 64 bits.

Tag each neuron and each of its synapses with a unique integer.

Assign each neurotransmitter class a unique integer identifier.

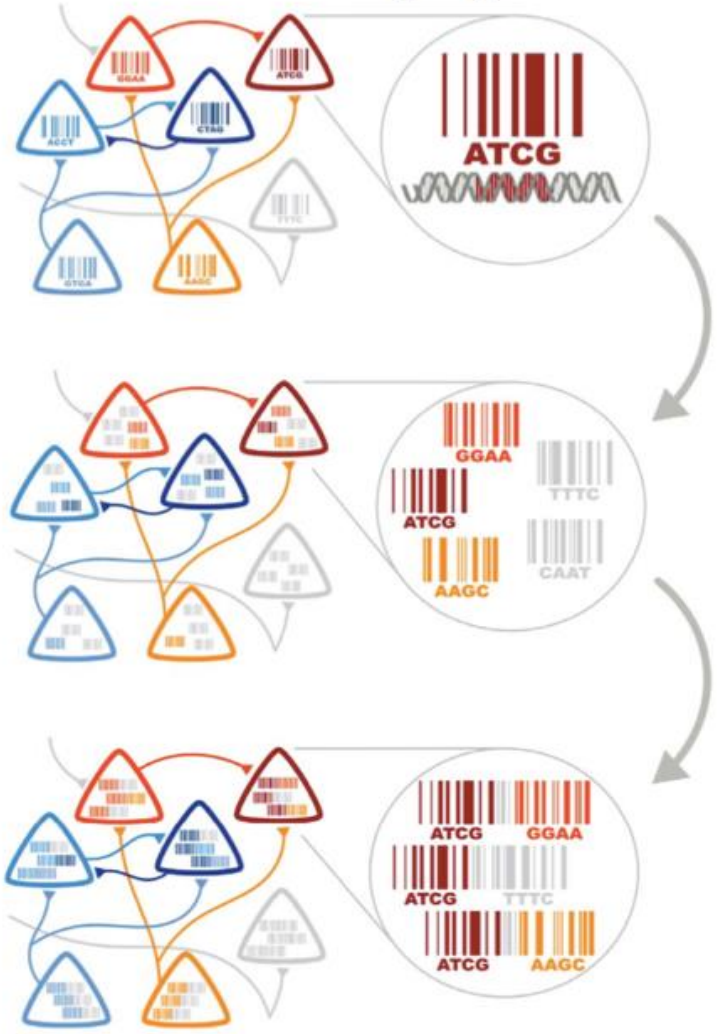
Could we record every event of the form: neurotransmitter *X* originating from neuron *A* was received at neuron *B* at time *T*?

Next we need to associate neurotransmitters with their identifying barcodes and convey these barcodes along with the neurotransmitters making sure that they find their way into the receiving neuron where they can be assembled into packets that describe each event as a triple of three barcodes encoding the transmitting neuron, the receiving neuron and the class of neurotransmitter conveyed. Once assembled these packets would be flushed into the cerebrospinal fluid to be subsequently eliminated from the brain via the lymph and blood circulation system.

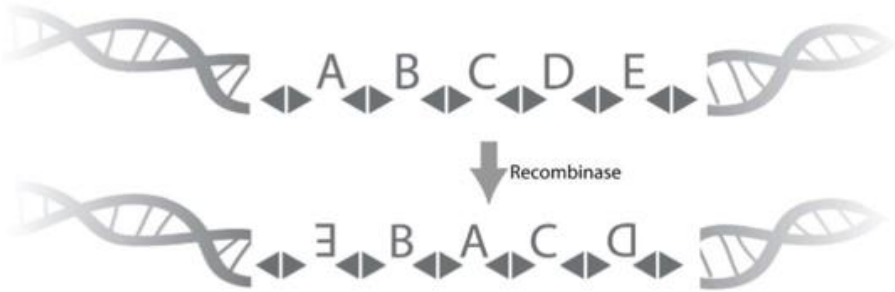
Fleshing this out would require a great deal of speculation, and so to reduce the hand waving to a tolerable level let's consider one more-or-less concrete proposal suggested in a paper [28] out of Tony Zador's group at Cold Spring Harbor Laboratory. The authors of this paper propose the idea of sequencing the connectome in their paper of the same title. They break down the problem into three components: (a) label each neuron with a unique DNA sequence or barcode, (b) propagate the barcodes from each source neuron to each synaptically-adjacent sink neuron — this results in each neuron collecting a "bag of barcodes", and (c) for each neuron combine its barcodes in source-sink pairs for subsequent high-throughput sequencing.

Sequencing the Connectome

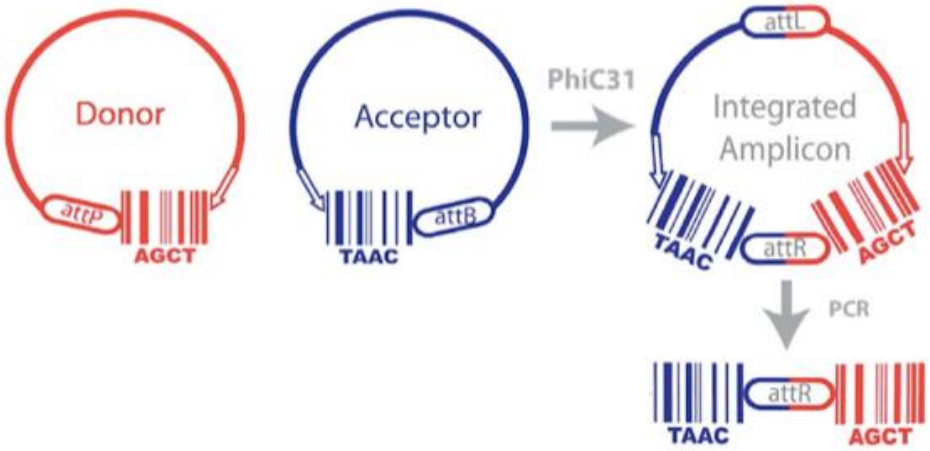
Barcode Propagation:



Barcode Generation:



Barcode Combination:



Source: Zador *et al*, 2012

Nanoscience

- At IBM in the US, a technique called electron beam lithography was used to create nanostructures and devices as small as 40 to 70 nm in the early 1970s

Nanotechnology in Medicine

Nanotechnology is a new field with many possible uses, medicine being one of them

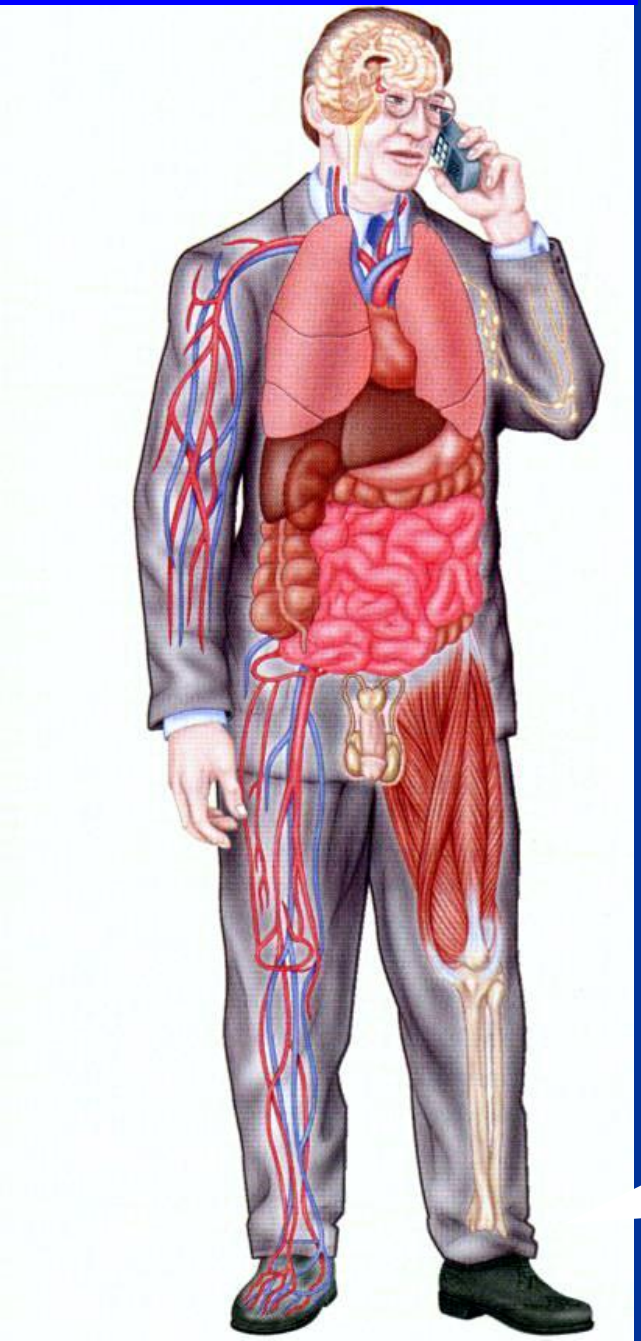
Nanotechnology

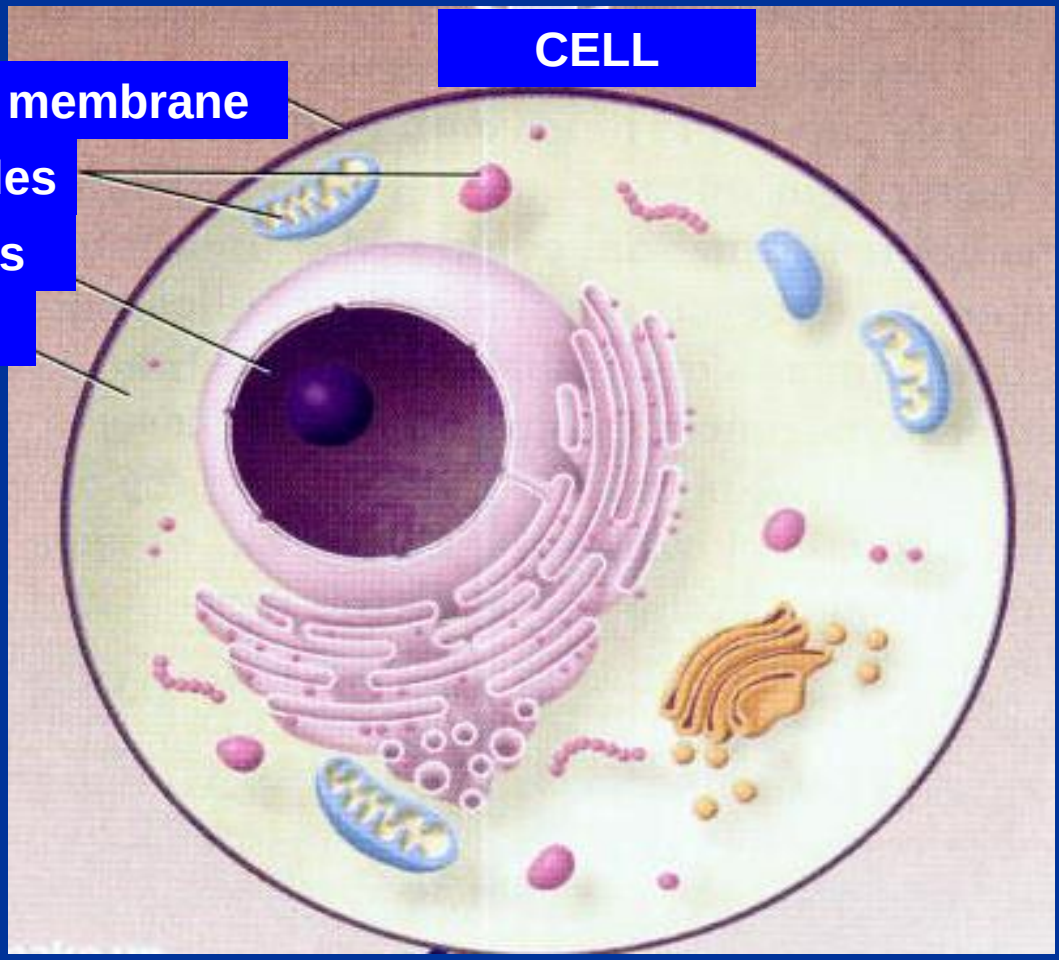
- “The manufacturing technology of the 21st century"
- The study and manufacture of devices of molecular dimensions, in the range of nanometers or one-billionth of a meter
- Most of industrial manufacturing processes are based on top-down technologies -- i.e., they take larger objects and make them smaller yielding products of fairly high precision and complexity

Nanotechnology

- Most products of living organisms are constructed by tiny molecular machines, such as cells and organelles, working from the bottom up.
- By organizing individual atoms and molecules into particular configurations, these molecular machines are able to create works of astonishing complexity and size, such as the human being

BODY SYSTEMS





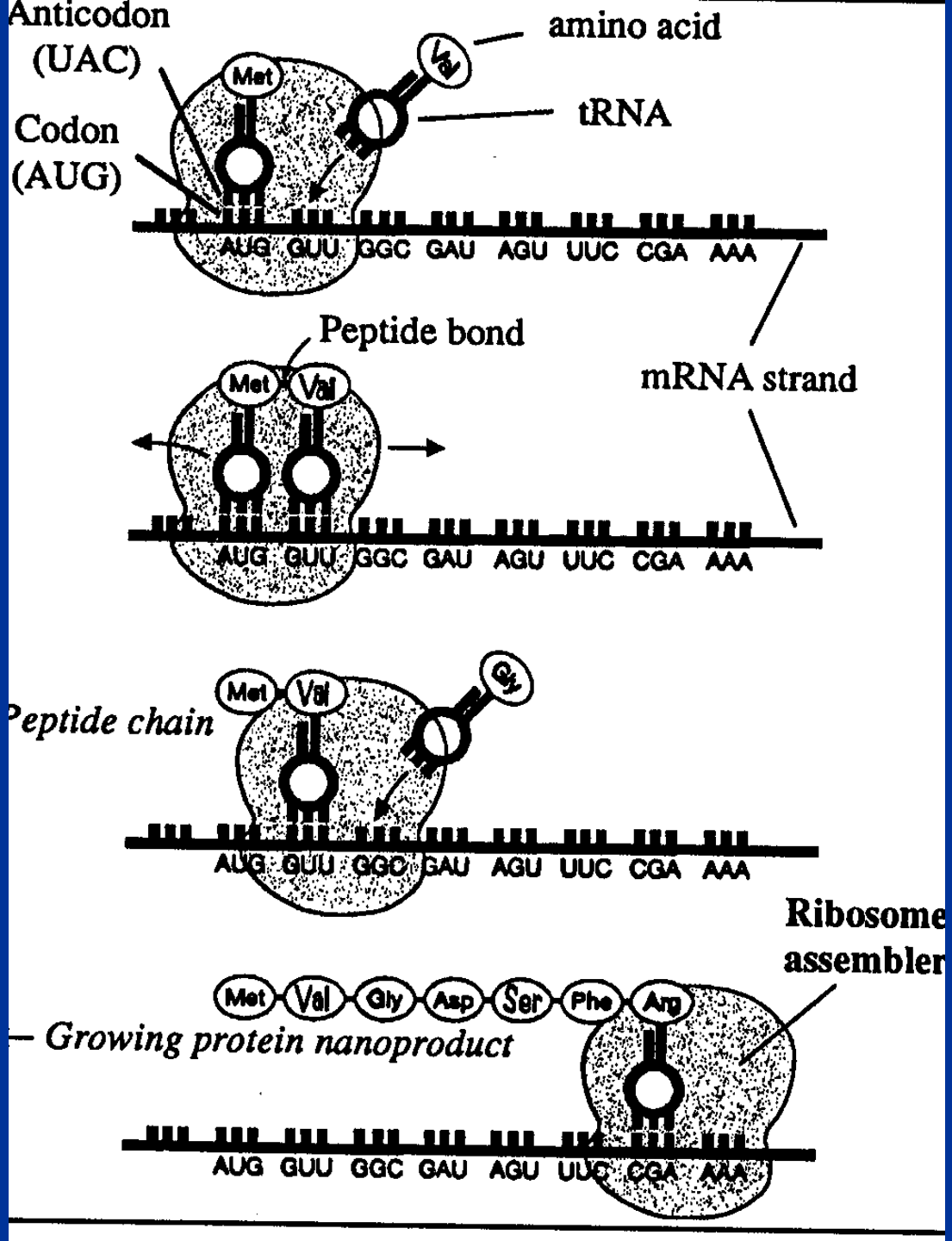
75 TRILLION CELLS

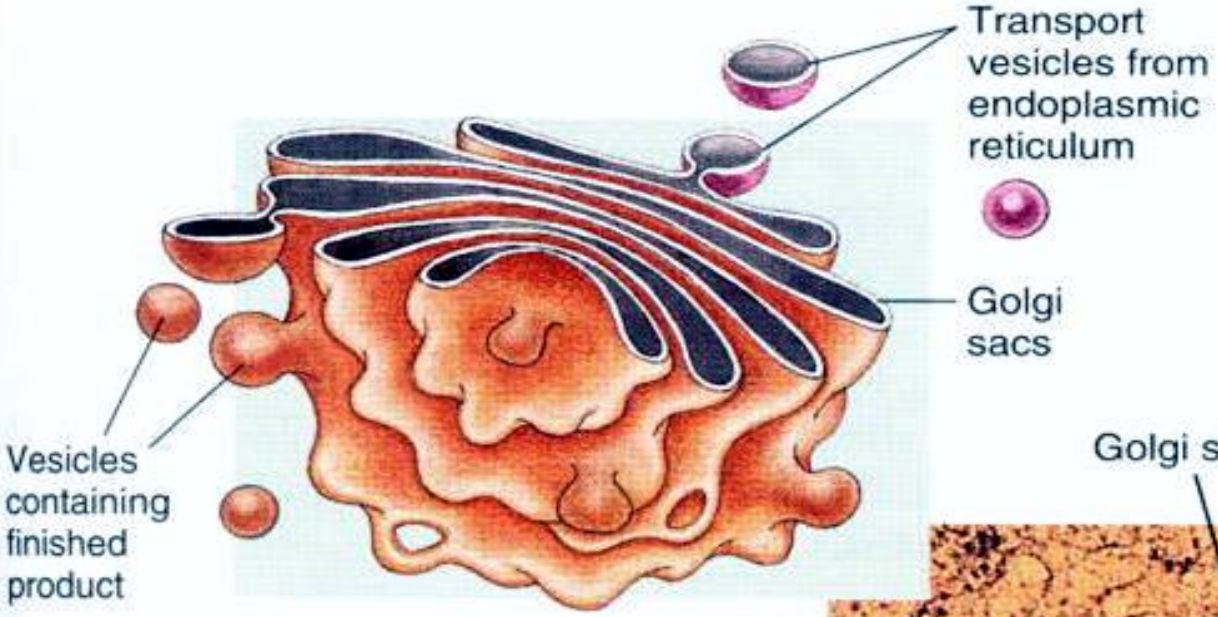
Nanotechnology

- Nature (Allah) shows that molecules can serve as machines because living things work by means of such machinery
- Enzymes are molecular machines that make, break, and rearrange the bonds holding other molecules together
- Muscles are driven by molecular machines that haul fibers past one another

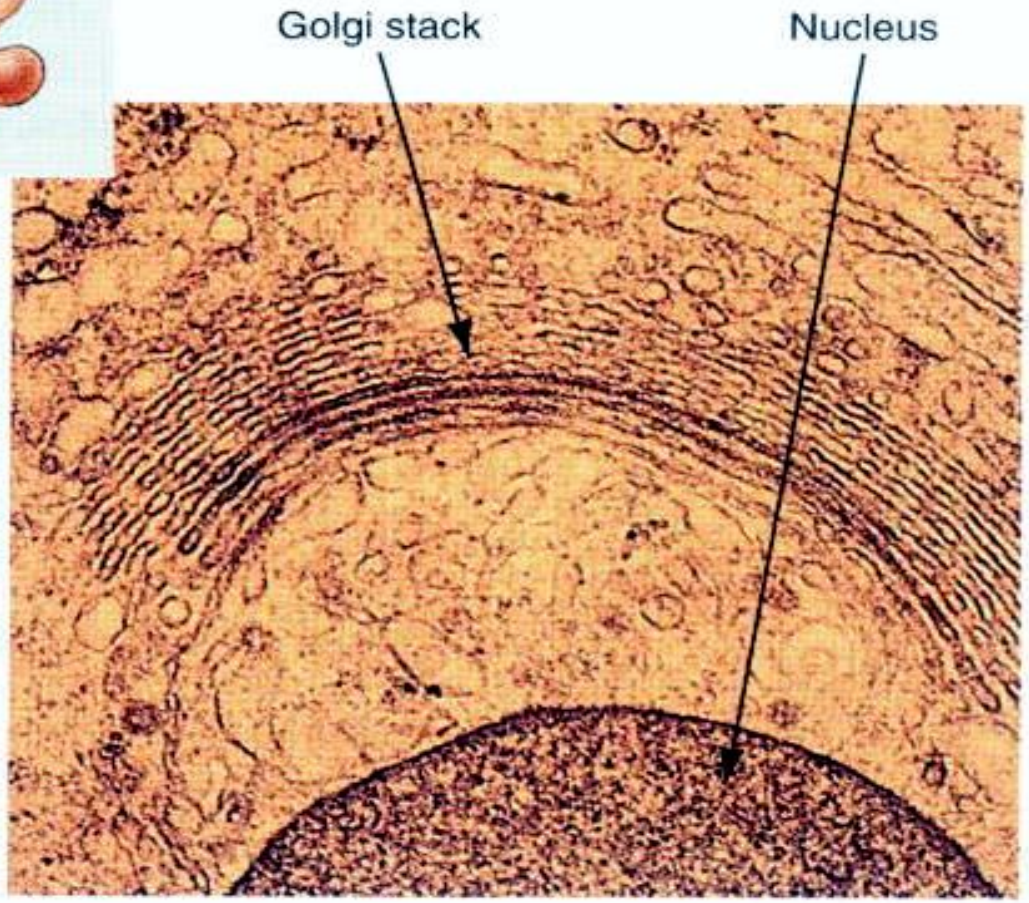
Nanotechnology

DNA serves as a data-storage system, transmitting digital instructions to molecular machines e.g., the ribosomes, that manufacture protein molecules.


Anticodon
(UAC)
Codon
(AUG)
amino acid
tRNA
Met
Val
AUG GUU GGC GAU AGU UUC CGA AAA
Peptide bond
mRNA strand
Met
Val
AUG GUU GGC GAU AGU UUC CGA AAA
Peptide chain
Met
Val
Gly
AUG GUU GGC GAU AGU UUC CGA AAA
Ribosome
assembler
Met
Val
Gly
Asp
Ser
Phe
Arg
Growing protein nanoproduct
AUG GUU GGC GAU AGU UUC CGA AAA

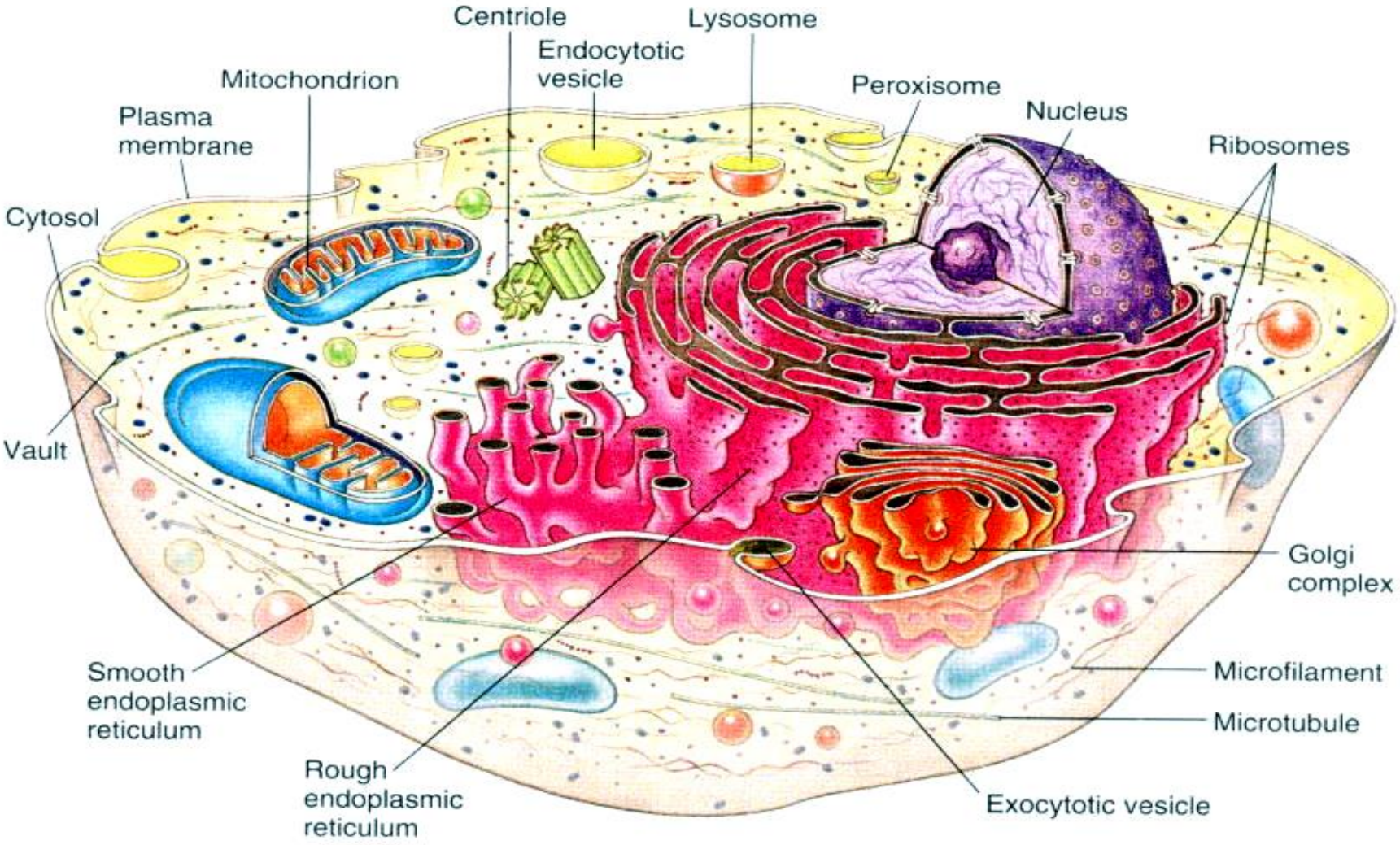


Illustrated Figure



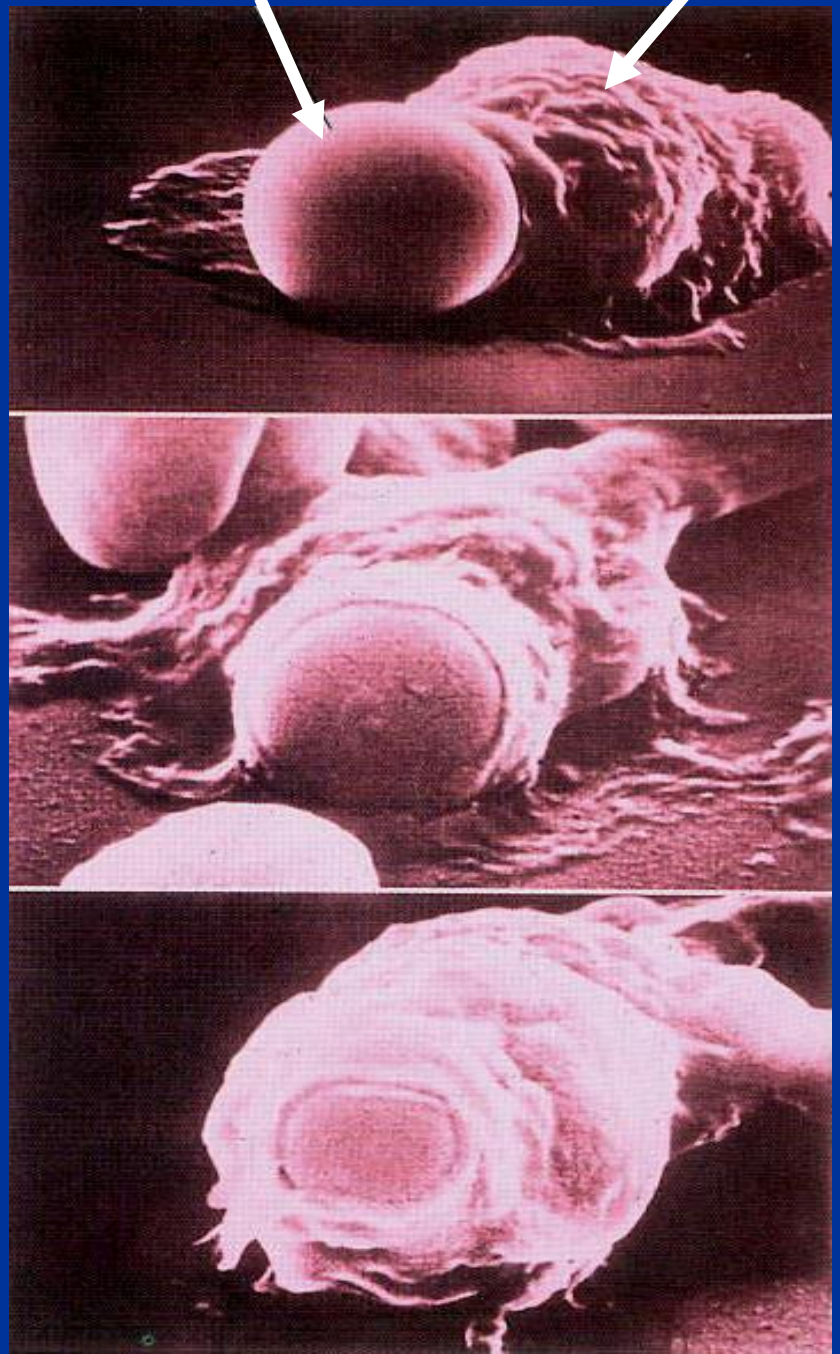
Electron Micrograph

GOLGI COMPLEX



THREE DIMENSIONAL ILLUSTRATION OF CELL STRUCTURES VISIBLE UNDER AN ELECTRON MICROSCOPE

ANATOMICAL CHARACTERISTICS OF ERYTHROCYTES (RBCS)

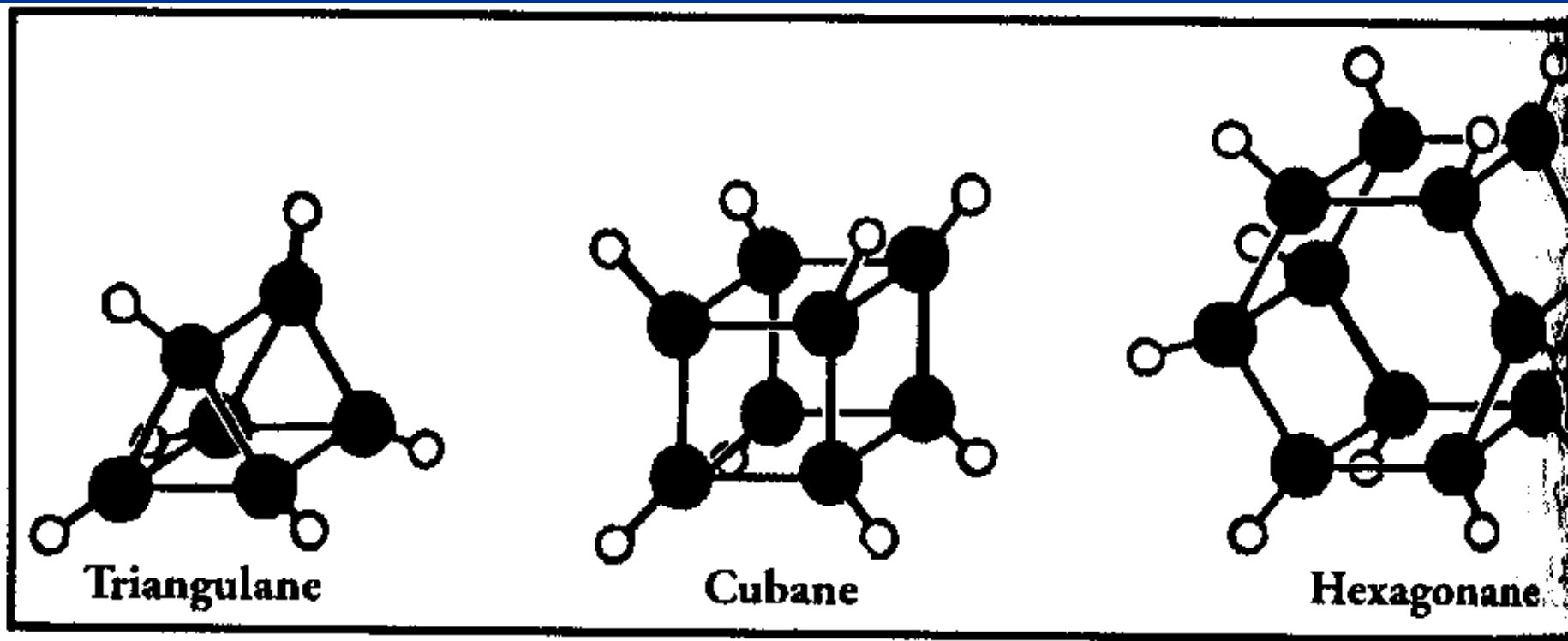


PHAGOCYTOSIS

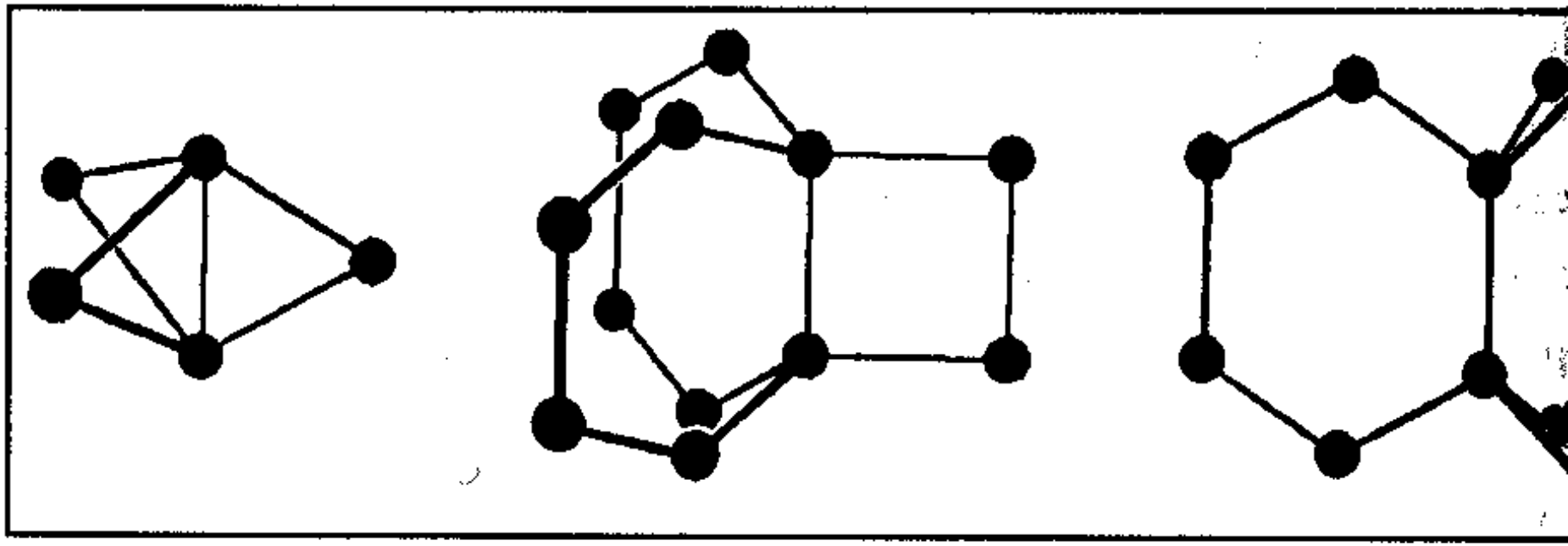
AN OLD RBC BEING ENGULFED BY A WBC

Nanotechnology

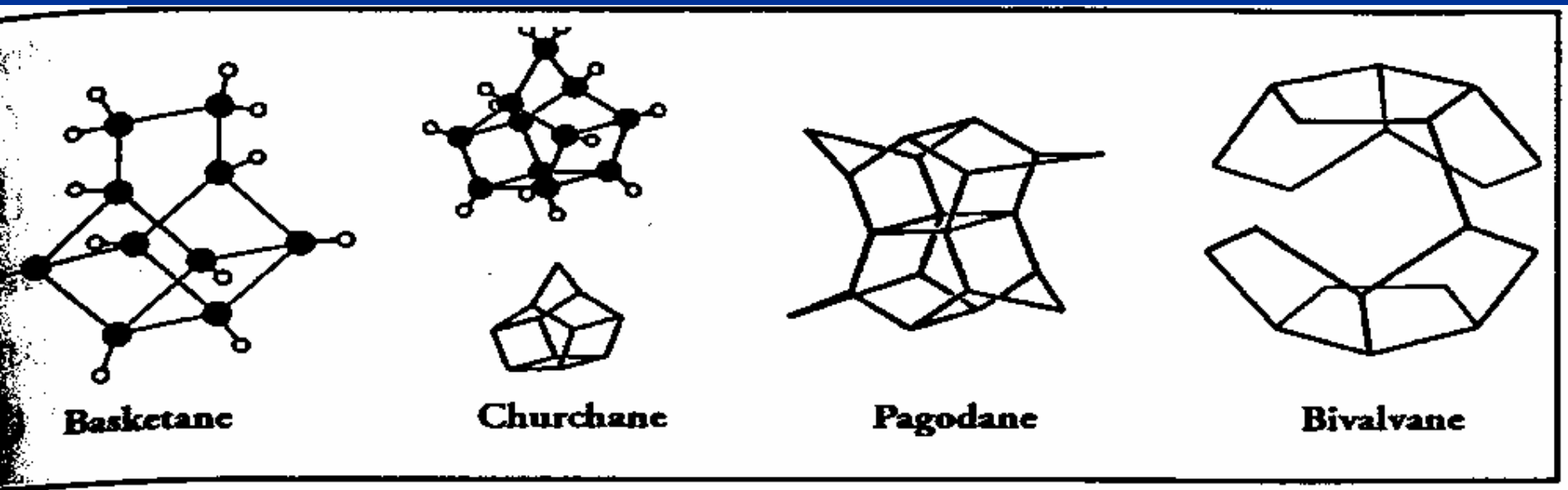
- Using special bacterium-sized "assembler" devices, nanotechnology would permit on a programmable basis exact control of molecular structures that are not readily manipulated by natural molecular machines and molecular techniques presently available.
- With nanotechnology, atoms will be specifically placed and connected, all at very rapid rates, in a fashion similar to processes found in living organisms



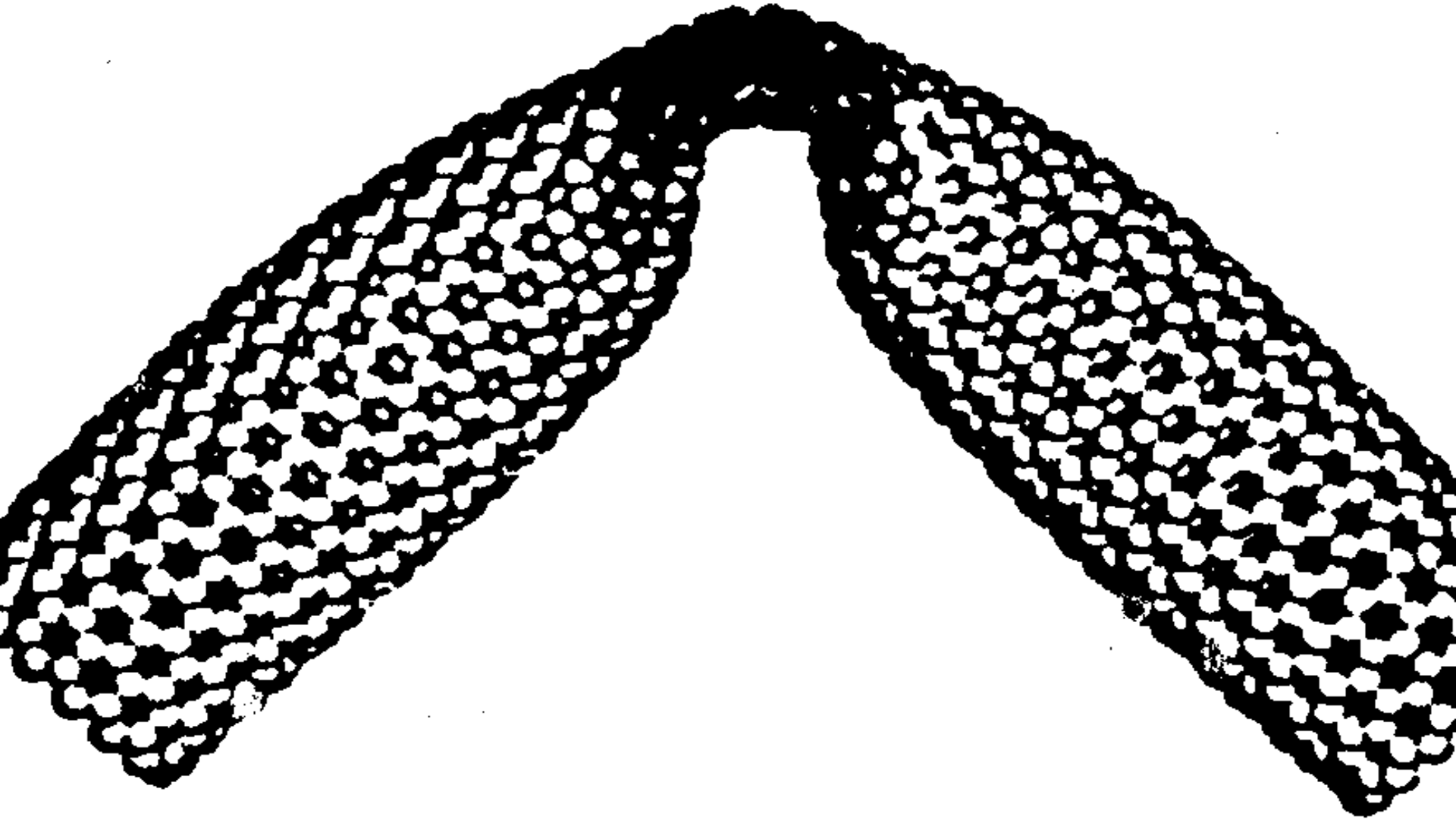
The prismanes.[382]



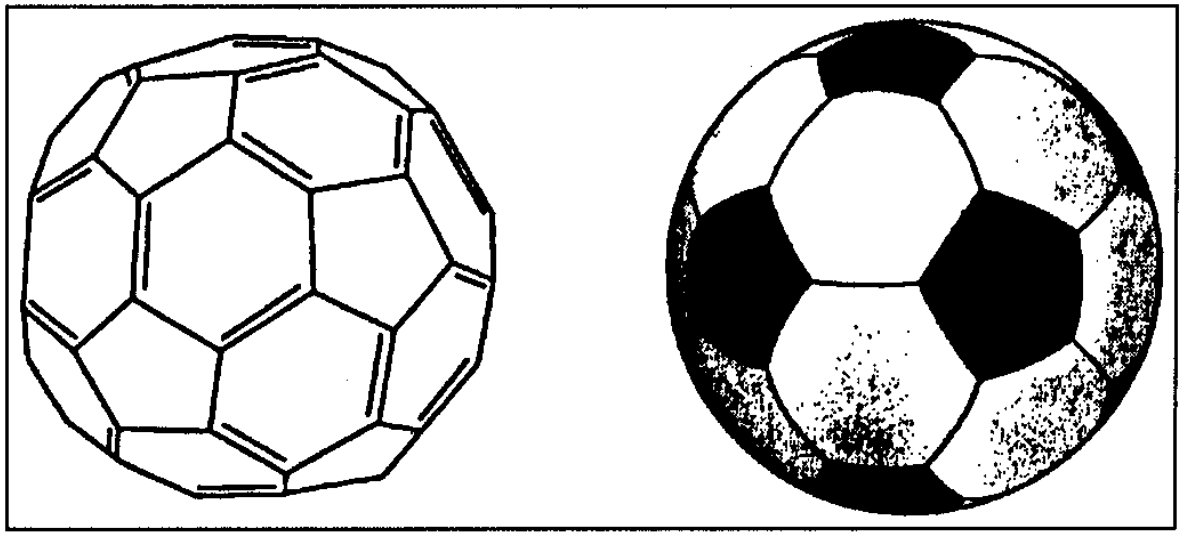
Propellanes (H atoms not shown).[382]



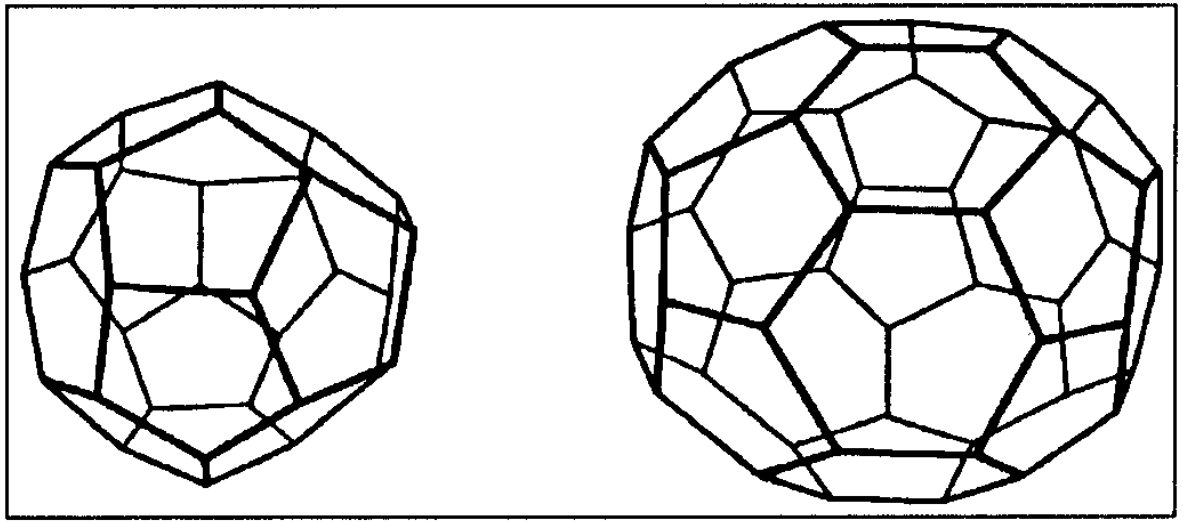
Other unusual molecular "parts".[382]



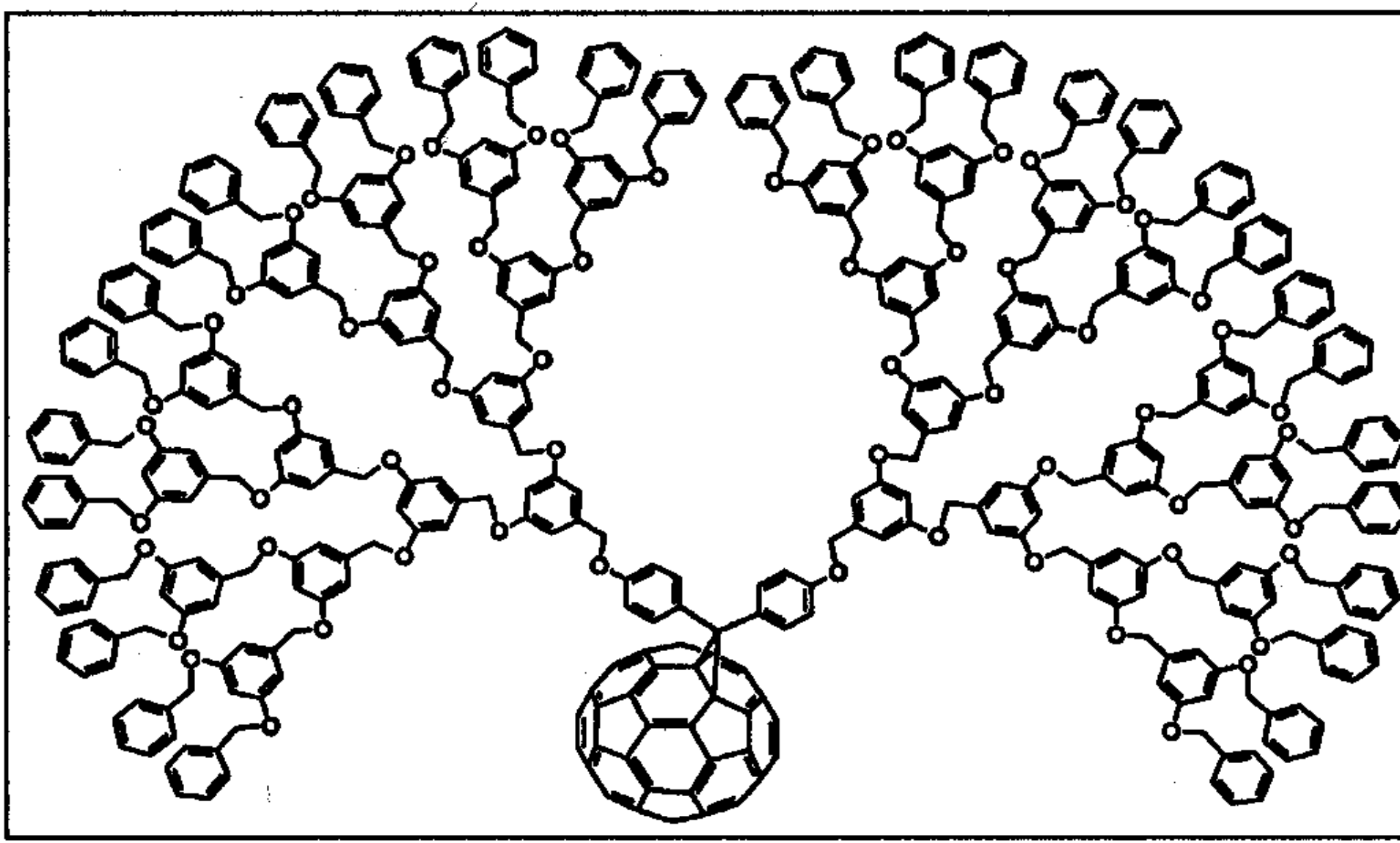
Carbon nanotubes kink when bent.[2661]



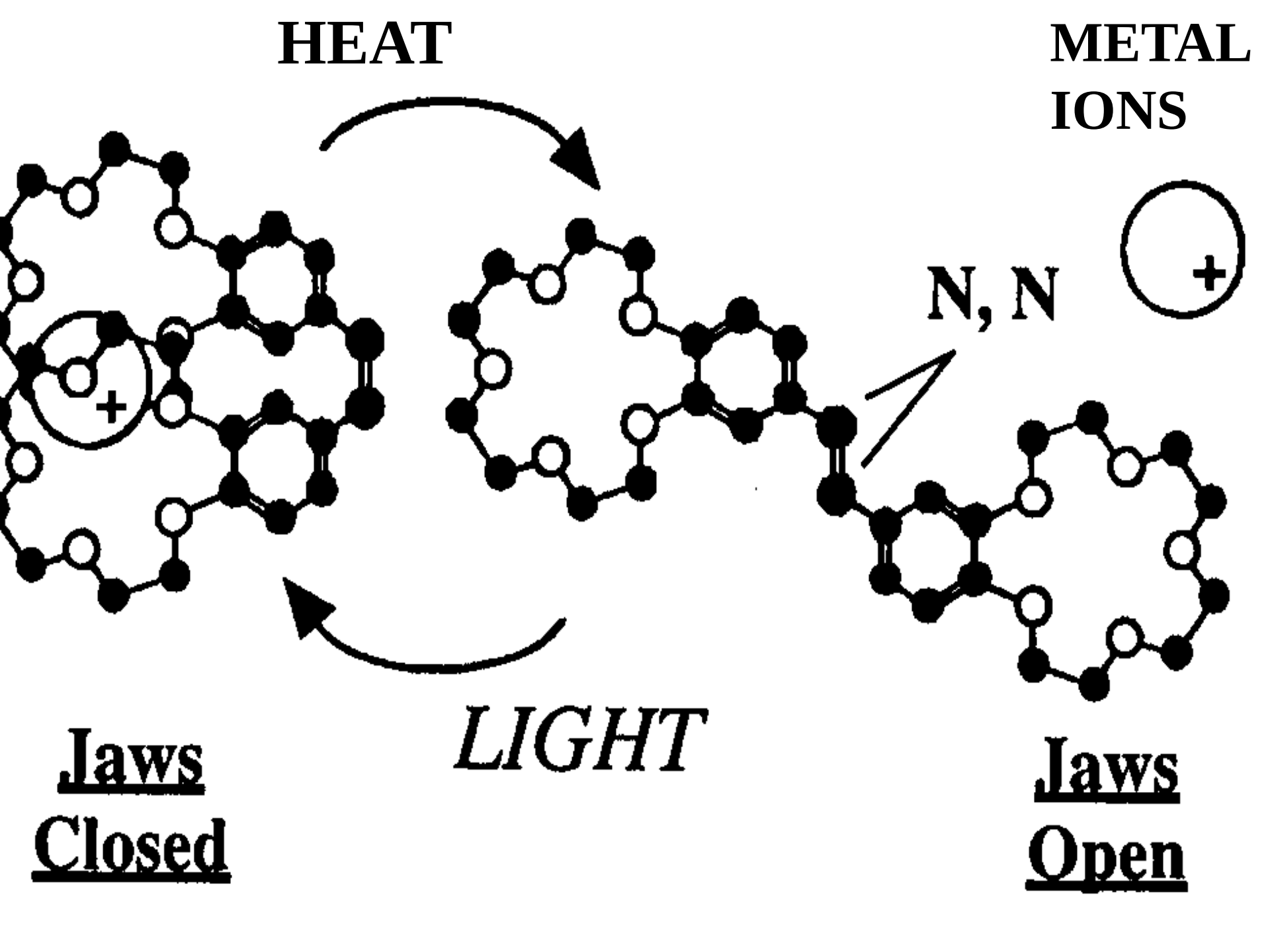
C_{60} fullerene "buckyball" and a soccer ball.[382]

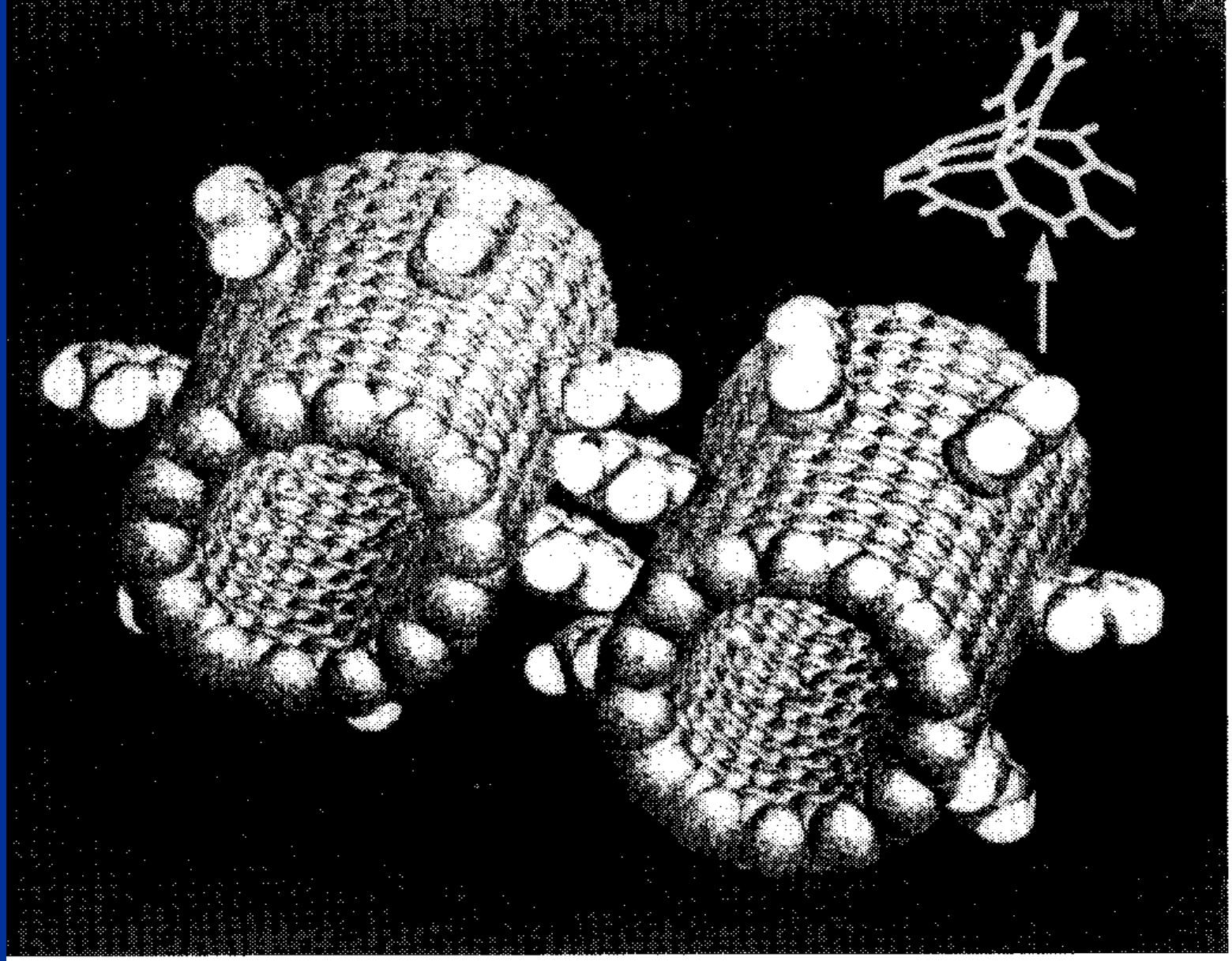


C_{32} and C_{50} fullerenes.[382]

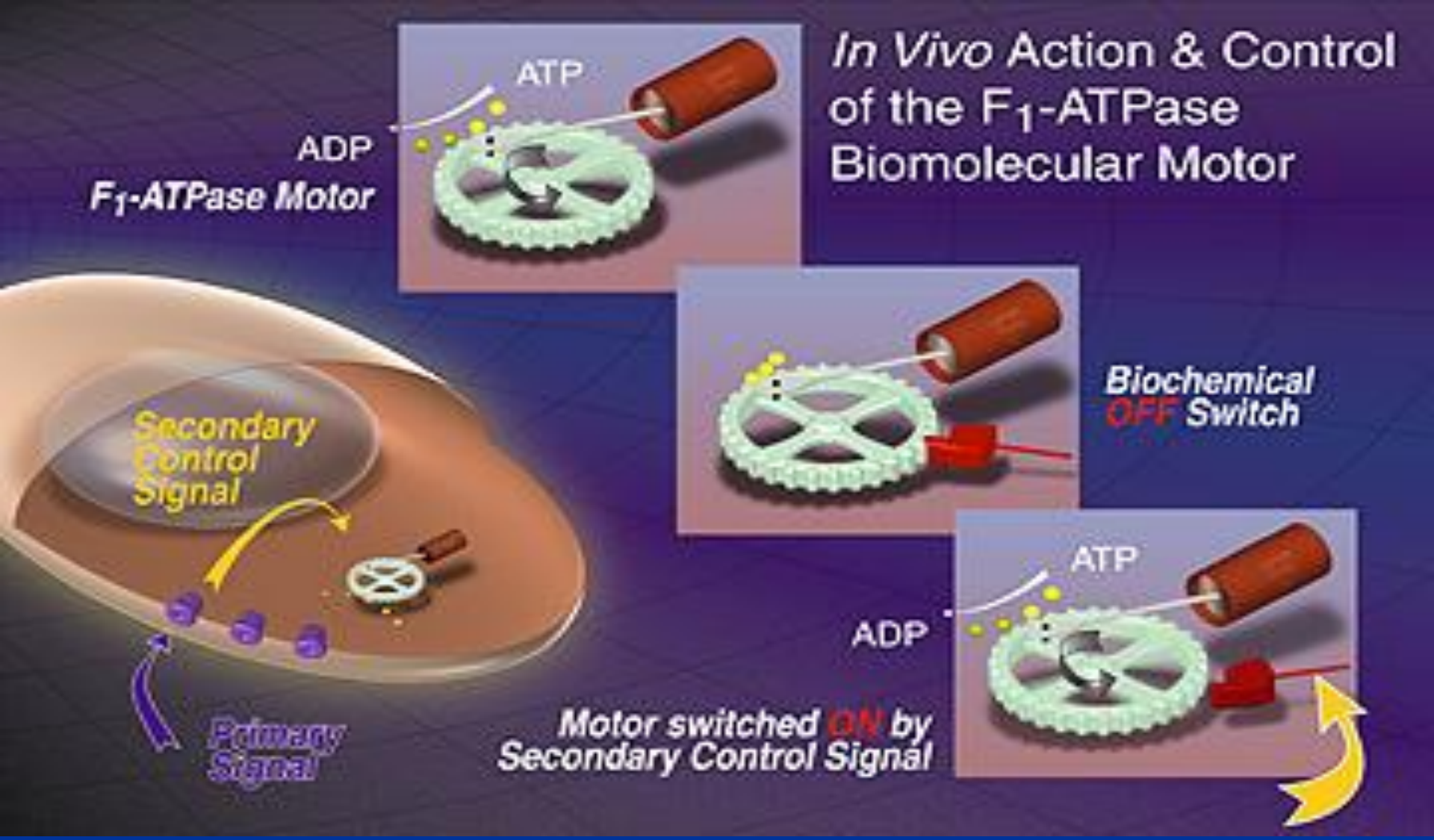


Fullerene dendrimer.[2620]


HEAT
METAL IONS
+
N, N
+
LIGHT
Jaws Closed
Jaws Open



Computer simulation of fullerene nanogears of the same size (courtesy of Al Globus, NASA/Ames).[2648,2667]



A future application of Cornell's molecular motor research: With the integration of biomolecular motor devices and cell-signalling systems -- by engineering a secondary binding site tailored to a cell's signalling cascade -- researchers plan to use the cell's sensory system to control nanodevices implanted in living cells. *Nanoscale Biological Engineering and Transport Group/Cornell University.*