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Recent Patents in Bionanotechnologies: Nanolithography, Bionanocomposites, Cell-Based Computing and Entropy Production

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Abstract: This article reviews recent disclosures of bio-inspired, bio-mimicked and bionanotechnologies. Among the patents discussed is a nanoscale porous structure for use in nanocomposites and nanoscale processing. Patents disclosing methods for printing biological materials using nanolithography techniques such as dip-pen technology are discussed, as are patents for optimizing drug design. The relevance of these technologies to disease prevention, disease treatment and disease resistance is discussed. The paper closes with a review of cell-based computing and a brief examination of how information technology has enabled the development of these technologies. Finally a forecast of the how these technologies are likely to accelerate global entropization is discussed as well as a new classification of machine types.

Keywords: Bionanotechnology, biomimickery, bio-inspired, dip-pen, nanotechnology, printing, information, entropy.

INTRODUCTION

In the upcoming years we are likely to see nanotechnology maintaining the momentum of the recent biology revolution and to enable what Freeman Dyson refers to as "Our Biotech Future" [1]. Much of what has occurred since the discovery of the structure of DNA has been enabled by molecular biology, computer technology, computer science, and advanced imaging methods. Emerging nanotechnological methods that not only image and measure, but that also synthesize and manufacture are likely to transform medical technologies and to improve human health. Preeminent among the fields of health care to be impacted will be drug discovery, tissue engineering, and human-machine interfaces. Automated gene sequencing technologies and DNA manufacturing technologies, coupled with NMR and protein crystallography results are beginning to elucidate structure-function relationships between genes and proteins, something that was only proposed fifty years ago [2], and which came to be known as the "Central Dogma" of biology [3]. As correlations and relationships between individual genomes and individual health patterns are established, the rate of discovery and prevention is likely to accelerate, especially through projects such as the Human Variome Project [4]. This paper discusses some of the recent patents of devices, methods and models that are likely to enable the engineering of devices that can interface directly with the human nervous system, alter human behavior by tailored drug delivery, or even DNA replacement. This will be enabled by machines that directly manipulate either single cells and molecules either rapidly in small batches or in a highly parallel manner, replacing traditional flask and stir-plate chemistry techniques. Already, novel technologies and techniques are enabling the communication and interrogation of individual cells and molecules. See for example, advances in single DNA manipulation and detection [5-7].

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SURFACE PATTERNING PATENTS

Nanolithography

Nanolithography technologies are likely to play a prominent role in affecting the rate of drug discovery by promoting other technologies such as cell-based sensing e.g. [8, 9] or cell-based computing e.g. [10-16]. The ability to pattern surfaces, especially electrically active surfaces is critical to the success of these technologies, especially as cells of various phenotypes are employed. The following is an abridgement of several patents that are likely to enable such technologies. A recent patent entitled "Method and apparatus for mesoscale deposition of biological materials and biomaterials," invented by Gregory Marquez, and Michael J. Renn [17], describes a method for deposition of an aerosolized biomaterial consisting of an aerosol stream using a carrier gas that deposits material onto a target surface in a digitized pattern. Aerodynamic focusing of the aerosol stream attached to a print head deposits patterns with feature sizes ranging from 5 – 200µm, onto flat or featured targets. This method has the advantage of avoiding the use of masks, but its limited feature size places it in a category of devices similar to the Nano eNabler™, available commercially from BioForce Nanosciences Inc. Potential deposition materials are numerous and are listed in the disclosure to include: conductive metal precursors, nanoparticle metal inks, dielectric and resistor pastes, biocompatible polymers, and a range of biomolecules including peptides, viruses, proteinaceous enzymes, extracellular matrix biomolecules, as well as whole bacterial, yeast, and mammalian-cell suspensions. This type of application is especially valuable for technologies such as biosensor rapid prototyping and microfabrication, lab-on-chip manufacturing, biocompatible electroactive polymer development, hybrid BioMEMS, bio-optics, and microfabrication of biomedical devices.

In this technology, fluid viscosity and inclusion size dictate the aerosolization mode. For example, biological solutions with viscosities of approximately 1-10 cP require ultrasonic transducers or pneumatic nebulizers. Ultrasonic

aerosolization is also frequently used for cell wall disruption and could thus be used for an application where this process is required. For solutions with viscosities of approximately 10-1000 cP, pneumatic aerosolization is used. Above 1000 cP, aerosolization cannot be performed without dilution with an appropriate solvent. Pneumatics and ultrasonic aerosolization allow for the generation of droplets or droplet/particles with sizes typically in the 1-5 μm size range. One drawback of this technology is that the pneumatic aerosolization process typically requires a carrier gas flow rate that exceeds the maximum allowable gas flow rate through the deposition head. To accommodate this need, a "virtual impactor" is frequently used such as in the M3D[®] process to reduce the flowrate of the carrier gas after aerosolization.

The patent also mentions, but does not claim right to use electromagnetic fields such as generated by a laser to heat, evaporate, cross link, sinter thermally decompose, melt or photochemically decompose features. This has particular relevance for biocompatible culturing targets and can be used to simultaneously deposit multiple materials through a single or multiple deposition heads. The deposition mechanism uses picoliter amounts of biomaterials, depositing them via arrays of microneedles tapered from 50 μm at the base to 5 μm at the tip, with a length of 200 μm . If this technology were to be coupled with even smaller microneedles from emerging nanofabrication techniques, e.g. [18], and open-loop or closed-loop 3D nanomanipulation strategies e.g. [19], printing resolution would be enhanced.

In another nanolithography application, a group of inventors from Grand Ledge, Michigan describe an apparatus and process for manufacturing changes of a substrate in a work region that is 100 \times 100 \times 100 μm or smaller [20]. The apparatus uses a plasma source adjacent to the work region to produce radiation or matter that changes the surface. The authors also suggest that an AFM or laser can additionally be used. One of the primary uses cited is MEMS production. For example, some of the processes claimed are adding and removing, dividing and assembling, or manufacturing materials and parts to enhance reliability and efficiency in manufacturing of micro or nano devices.

The authors claim that manual nanomanipulation is complex and time-consuming. For a recent example of an open-loop nanomanipulation task see [19]. They also cite the problems with thermal drift during AFM imaging. This is exemplified especially in imaging biological samples e.g. [21]. Instead they contend that automatic path planning is crucial for nanomanufacturing [22]. The authors intend to use microplasma sources with a nanoscale ion beam source having beam energies from 50 – 100 eV, a free radical capable of operating in several gases, and a UV light. The preferred minimum size for the ion, free radical and photon beams is 50 nm in diameter using a grid/aperture technology in a nanoscale processing system mounted adjacent to existing AFM technology allowing the operation of a plasma source and subsequent AFM imaging. The source is mounted with nanometer-resolution position control. Evaluation of both deposition and etching processes is done initially on flat surfaces and then the processing is applied to more complex three dimensional shapes.

For example, a 1-mm diameter discharge operating with 5% hydrogen in argon at pressures of 10's of Torr has a power density of several 100 W/cm^3 and the electron/ion densities are of the order of 10^{12} cm^{-3} at the low mTorr pressures and are above 10^{14} cm^{-3} in the higher operating pressure regime. Because of tip geometry, smaller diameter discharges are even more intense having power densities of around 1000 W/cm^3 . However, even while discharge power densities are high, because of the small volumes involved, the total absorbed power is low: typical absorbed power levels are a few watts for short discharges, a few tens of watts for centimeter-long discharges. These devices thus only require a 25-150 watt microwave power supply.

The authors also employ an "augmented reality system," the aim of which is to provide the operator with real-time visual dynamic AFM image of the operating environment. The image is updated locally based on real-time force information obtained by the AFM tip. Similar technologies exist for haptic feedback during AFM experiments [23]. In this scheme, the AFM tip acts as a robot end effector for manipulation, and a sensing device to obtain the interaction force between the tip and the environment. A manipulation of tens of nano particles can take several weeks to finish using scan-design-manipulation-scan scheme. But it only took about two hours to assemble a complex pattern consisting of an "MSU" logo which consists of several hundred nanoparticles.

ARRAYING PATENTS

Microtissue Arrays

Microtissue arrays are likely to become an increasingly relevant biotechnology and will be driven by the need for high-throughput drug screening. In a recent patent for analyzing microtissue arrays, Foran and Chen [24] incorporate several established image processing algorithms for performing quantitative immunohistochemistry for the purpose of identifying the presence and prevalence of a wide array of biomarkers relevant to a multitude of diseases and conditions. The majority of the patent outlines methods for storage, manipulation, retrieval and sharing of microarray data via the internet using web-based technologies. The inventors describe a process for aligning microarrays of tissue that have been stained with either one or two stains then for decomposing the color intensities of individual regions of the array so that protein expression levels may be quantified. The techniques have been applied to tissue microarray specimens stained with 3, 3' diaminobenzidine (DAB) chromogen and counter-stained with hematoxylin, resulting in various shades and combinations of the two dye colors. The patent includes a statement that provides for identifying regions of interest and normalization of the data to a standard image so that protein expression levels may be compared across samples. It covers an algorithm for using low magnification to initially align the array under a microscope where initial data acquisition is performed, followed by a series of high-magnification image captures so that a higher quality images can capture multi-resolution texture and morphometric measurements. Of major significance of the patent is its coverage of a protocol that allows clinicians at remote locations to simultaneously view and discuss images via web-based technology. The patent

does not cover RNA expression levels. The patent also covers systems for controlling the system over the internet. This technology holds promise to accelerate the rate of disease detection and to reduce the risk of false positives by allowing multiple clinicians simultaneous access to histology samples. However, in order to be effective, it must be coupled with other recent processes for initial detection such as contrast-enhanced MRI e.g. [25] and targeted delivery treatment methods e.g. [26].

Nanoscale array sensing combined with patterning is perhaps the next step in nanolithography evolution; representing a hybridization of nanolithography and imaging, or in some instances, nanomanipulation and imaging [27]. A recent patent, "Biomarker sensors and method for multi-color imaging and processing of single-molecule life signatures" [28], describes another nanoarraying device e.g. [29-32] for use in life sciences and diagnostics. This and related nanoarraying technologies e.g. [33-35] are likely to become increasingly relevant for disease screening, tissue engineering, and forensics research. The disclosure describes a device consisting of an array of active regions for use in reacting one or more chemical or molecular species in at least two of the active regions in a sequential process. The device has a transparent surface substrate covered by a silane material and an active region overlying the silane. Typical dimensions of the feature sizes and separations between the arrayer are less than 1 μ m. The device is capable of making multiple passes to specific regions of the substrate for the purpose of providing a means of performing chemical reactions in pulses of approximately 10 μ s.

The inventors claim that their invention "has a much broader range of applicability than typical dip-pen technologies since it can be applied to the formation of patterns using biological materials, chemical materials, metal materials, polymer materials, solid state materials, molecule structures of 100 atoms and less, dendrimers, DNA, proteins, semiconductors, insulators, organic thin films, inorganic thin films, any combination of these, and the like." Similar to existing nanolithography stations, the environment can maintain a specified humidity from about 22% to about 92%. The system also has a substrate temperature control strategy. The probe has a ~5 - 20 nm thick coating that is chemically inert, relative to a chemical and/or protein ink, and is a hydro-phobic material, such as Teflon™. Optionally, the ink solution includes a surfactant such as polysorbate 20. The active regions are spatially separated by 30 - 100 nm with one active region separated by an arbitrarily small distance from another active region using the dip pen process. The active regions have dimension of 100 - 300 nm. The system has also been designed to perform fluorescence monitoring with a two-component fluorophore. The inventors claim to use a combination of steps including an undisclosed way of forming a micro-array of deposited molecules or with a surfactant species to transfer single or multiple molecules from an AFM probe tip onto a selected region of the substrate. The authors also describe an elaborate plan for protein synthesis that combines the advantages of aqueous media chemistry with the spatial precision of atomic force microscopy. Since synthetic schemes rely on multiple sequential steps in aqueous or organic solvents, and nanoscopic patterning is usually carried out in air, an

electrochemical AFM-based nanolithographic technique is proposed. In the inventors' scheme, a conducting AFM probe tip serves as an amperometric electrode to generate extremely localized amounts of excess acid in buffered aqueous solution through the electrolysis of water limiting the diffusion field of the free acid to just a few nanometers of the probe. Rapid reactions of the electro-generated protons with the conjugate base of a weak acid result in a sharp pH gradient near the tip. The acid near the probe surface selectively removes acid-labile dimethoxytrityl (DMT) protecting groups from the 5' ends of oligonucleotides immobilized on a substrate and in close proximity to the probe. However, since the pH of the bulk solution is much higher, the acid-labile protecting groups on the functionalized substrate outside of the tip interaction region remain intact. Subsequent steps add a specific monomer to the deprotected oligonucleotides only at regions defined by the probe tip. This electrochemical synthetic scheme has been successfully applied and described in two publications. One involves the construction of fluorescent patterns on glass at 40 μ m pitch, using dimethylformamide (DMF) deprotection chemistry [36], and in the other demonstrates the production of nucleic acid and polypeptide micro-arrays (100 μ m diameter sites separated by 100 μ m) [37].

BIONANOMATERIALS PATENTS

Biological performs may begin to replace some classes of light-duty composite materials. Preeminent among all biomaterials are the naturally occurring proteins. Nearly three billion years of evolution has produced the versatile collagen [38, 39], capable of supporting tensile loads in tissues such as ligament [40], cornea [41], nerve [42-44] (Fig. 1) and skin [45] compressive loads in tissues such as cartilage e.g. [46] and bone e.g. [47] and is likely to remain critical to tissue engineering applications [48].

Biomimicry, the ability to exploit existing biologically derived materials with a nanoporous structure is likely to obviate the need for artificial nanocomposites in some applications. A patent by Darby and Darby [51], "Process for making biopreform from monocotyl caudex plant, biopreform" describes a method of making biopreform from the stems of monocotyledonous plant suitable for liquid infiltration and gaseous transportation of materials. The trees used include coconut, *Cocos nucifera*, palmyra palm, *Borassus flabellifer*, and date palm, *Phoenix dactylifera*. The plants are dried under pyrolytic conditions to create a material with microstructural features consistent with those of the parent plants. Similar processes for making silicon - silicon carbide ceramics using the same biopreform, but at temperatures of 1450° to 1600° C could potentially create materials with similar properties, but with higher humidity tolerance. Compared with zeolites, the process is performed at lower temperatures, has porosities of similar size and distribution, and may prove to have better mechanical properties than other emerging nanocomposite materials. Briefly, the process involves slowly heating a stem in the range of 350° to 1000° C, maintaining the stem piece at the peak temperature under self-generated ambient atmosphere, then cooling it to obtain the biopreform. The microstructure of the resulting material is nearly identical to the precursor plants. The inventors claim their biopreform to be useful in

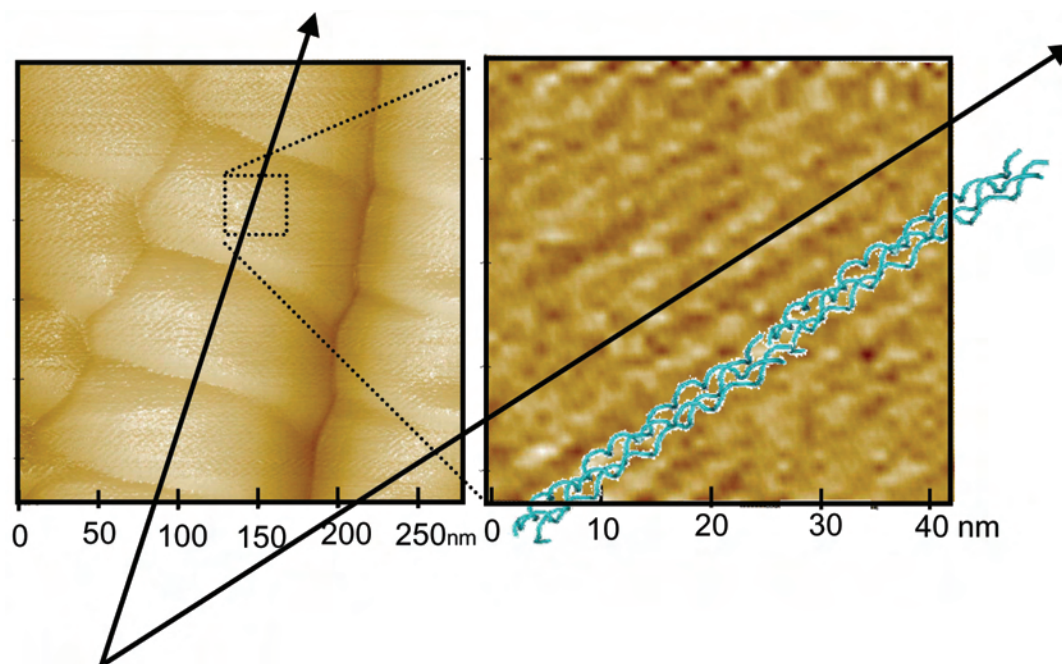


Fig. (1). At the nanoscale, a single collagen fibril looks much like a helical rope. The image on the left is an atomic force microscopy image of an individual collagen fibril with two neighboring fibrils [49]. The figure on the right is a 40 nm × 40 nm portion of the fibril superimposed with a backbone model of the collagen triple helix, the full length of which is approximately 300 nm. The two arrows represent the fibril axis (left) and the molecular axis (right) [50]. As nanotechnology and advanced microscopy techniques advance, greater resolution images of individual molecules are expected. However, the level of resolution seen in this image, taken in 2001 of a single fibril with minimal sample preparation, remains unsurpassed.

preparation of numerous composite materials: ceramics, carbon-epoxy, carbon-metal, carbon-silicon carbide, carbon-metal oxide, silicon-silicon carbide, metal-silicon carbide, transitional metal silicide-silicon carbide, aluminum oxide-aluminum nitride, and silica silicon nitride. The structures may also be used for gaseous transportation or liquid infiltration, reaction and substitution or suitable combination of such processes. These materials have particular application for industries such as aerospace, automotives, sports equipment and even replacement-limb prosthetics.

The temperatures used indicate that pyrolytic weight loss is practically completed by 600° C and that heating at a rate up to 5° C per minute up to 750 to 800° C indicates complete pyrolysis and typically no collapse, distortion, or graphitization of cellular structure, and that mineral residues are not converted into stable compounds, ensuring that the biological structural features of the parent plant are preserved. What remains to be determined is the material's susceptibility to water damage. As this bio-derived matrix has a structure that evolved to imbibe and transport water, it may be susceptible to soaking in water, leading to degradation. Other similar structures include naturally grown porous structures such as those found in ocean sponges e.g. [13]. Presumably if the material were to be used as an impregnated composite, or if it were to be treated with a shell of impenetrable material, this would be less likely. The materials used were grown in tropical climates such as those found in India, the southeastern United States and other countries where palm trees are prevalent. The biopreform characteristics vary depending in the specific species used

and preparation protocol, but they have been observed to contain pore sizes of a bimodal or trimodal distribution: 70 to 120 μm, 6 to 20 μm and 1 to 5 μm. The biopreforms have been characterized by X-ray diffraction and found to contain non-graphitic carbon. What the authors do not discuss, but something that future investigators might benefit from, would be the intentional formation of a graphitic phase such as that recently developed [52]. If successful, and if used in combination with a diffusion of atomic carbon gas, the technology of this patent could have the potential to make continuous carbon nanotubes with larger diameters than those currently achievable using conventional catalytic processes.

MOLECULAR DESIGN PATENTS

The ultimate nanotechnology is one in which an object is manufactured one atom or molecule at a time. This mode of assembly has been postulated for years by innovators such as Robert Freitas [53] and Eric Drexler [54]. However, other more practical methods for building materials from so-called self-assembly methods, especially in monolayer formations, have proved to be more efficacious, at least in the short-term e.g. [55-57]. Arguably, the most advanced molecular synthesis system is the pentad of protein synthesis molecules: ribosomes, tRNA, aminoacyl-tRNA synthetases, mRNA, and protein factors [58]. However, there have also been recent attempts to mimic the molecular manufacturing machinery developed by nature. For example, in "Algorithmic design of peptides for binding and/or modulation of the functions of receptors and/or other proteins," Mandell, Selz, and Shlesinger [59] cover with a comprehensive abstract, "Methods of

synthesizing a peptide or peptide-like molecule to a polypeptide or protein target based on mode-matching each member of a set of peptide constituents of the peptide or peptide-like molecule to peptide constituents of the target polypeptide or protein target." The primary purpose of the broadly defined invention is to enable greater specificity and targeted labeling of proteins either *in vivo* or *in vitro*. The authors state that "the ability to accurately identify and track protein expression is fundamental to the study of all diseases and to the study of all of bioscience." The method disclosed uses three interrelated techniques to statistically characterize a polypeptide's physicochemical properties for constructing peptides with a high probability of binding to a target protein, polypeptide or peptide. The first method is eigenvector-based. An eigenvector is determined from the autocovariance matrices of a sequentially tagged physicochemical property data series of the peptides, polypeptides and proteins for both the original data series and the target series. An eigenfunction is then constructed from the convolution of these two eigenvectors. The second technique uses a maximum entropy power spectral transformation to categorize, classify or otherwise describe a protein based on its entropy or information content. The third uses discrete and continuous wavelet transformations, one-dimensional wavelet packets and multiple convolved wavelets to confirm the dominant statistical wavelengths of the eigenfunctions and locate them as phase amplitudes or absolute valued moduli in the constituent sequences. The target sequence list is exhaustive, covering "any protein involved in protein-protein interaction" but explicitly names cell membrane receptors, nuclear membrane receptors, circulating receptors, enzymes, membrane and circulating transporters, membrane proteins involved in the translocation of viral and other infective agents into the cell, chaperonins and chaperonin-like proteins, monoclonal antibodies and antibody derivatives. The method is based on creating graphs of the leading eigenfunctions of the target protein's smoothed hydropathy sequence, to form an ordered set of hydrophobic free energy (ΔG_{hp}) eigenfunctions.

The primary impact of this invention will be in the fields of laboratory medicine as well as enabling new discoveries in molecular biology. For example, a sequence of a particular polypeptide that is associated with a disease condition is known, then peptides that will bind, modulate the function of, activate, or inhibit those polypeptides may be synthesized using the methods of this invention. When used to treat a tumor, the peptide could be conjugated to or incorporated with a cytotoxic agent, such as a radioisotope or a toxin, or could be attached to a nanoparticle sensitive to ultrasonic heating as recently demonstrated by [60]. When used for detection, the peptides could be conjugated to a molecule that can be visualized or otherwise detected, such as a radioisotope, a chromophore or a fluorophores such as a quantum dot. The peptides developed using the invention could be used to screen tissue or blood samples for the presence or absence of a particular polypeptide. Sample types listed by the authors include blood, plasma, blood products, urine samples, fecal samples, tissue biopsy samples, skin samples, semen samples, and epithelial cell samples. When used to screen for tumors or disease conditions, or when used as a therapeutic, the developed

peptides could be included as a component in a diagnostic or therapeutic kit, respectively. While the use of peptides for screening in such applications is well known, the primary utility of the invention is to reduce the need for undue experimentation.

Molecular design for drug delivery has seen an explosion of forms in recent years. In a patent entitled "Polymeric micelles for drug delivery" [61] the inventors describe a method for designing a drug-loaded micelle comprised of a multiblock copolymer consisting of several poly amino acid blocks. One intended purpose for the invention is the treatment of cancer. The primary intellectual property of this invention is that it employs three main components: a polymeric hydrophilic block, a crosslinked poly amino acid block, and a third poly-amino acid block. At the tertiary scale, the micelle has three main characteristics: an inner core, a crosslinked outer core, and a hydrophilic shell. The inventors cite doxorubicin and camptothecin as two examples of drugs that may be delivered through their invention, but the patent states that the general method may be used to deliver a multitude of nanovectors such as siRNAs or MRI contrast agents. The patent also covers micelles with reactive surface functionality for attachment of drugs, permeation enhancers, and targeting groups. The patent describes a method of sequential polymerization, wherein a first monomer such as N-carboxy anhydride (NCA), lactam, or imide is incorporated into the polymer, followed by a second monomer to form a second amino acid block. The multiblock copolymers covered consist of one synthetic polymer portion and two or more poly amino acid portions ($W-X'-X''$), where W is a synthetic polymer portion and X and X' are poly amino acid chains or "amino acid blocks". For example, the multiblock copolymers could consist of triblock copolymers if three polymer portions are employed or tetrablock copolymers if four regions are present. The hydrophilic polymers used for this particular application are the well-known polyethylene oxide (also referred to as polyethylene glycol or PEG), poly(N-vinyl-2-pyrrolidone), poly(N-isopropylacrylamide), poly(hydroxyethyl acrylate), poly(hydroxyethyl methacrylate), and polymers of N-(2-hydroxypropoyl)methacrylamide (HMPA). The patent claims to cover the use of covalently linked natural or synthetic poly amino acid or amino acid blocks of the L-configuration and include those with "protected" functional groups such as amino acid monomers with hydroxyl or amino moieties.

The inventors denote that a "living polymer chain-end" is the terminus resulting from a polymerization reaction that maintains the ability to react further with additional monomer or with a polymerization terminator. This "living end" is analogous to the end of the C-terminus of a protein where polymerization takes place on a ribosomal complex. The inventors also define "detectable moiety" or "label" as any moiety such as primary or secondary labels capable of being detected. For example, a primary label might be a radioisotope-containing moiety such as ^{32}P , ^{33}P , ^{35}S , or ^{14}C . Primary moieties could also be mass-tags such as gold nanospheres. They could also take the form of fluorescent labels, which generate a light signal when excited. Secondary labels as defined by the inventors include "moieties such as biotin, or protein antigens that require the

presence of a second compound to produce a detectable signal." For example, for biotin labels, the second compound might be a streptavidin-enzyme conjugate. In the case of an antigen label, the second compound could be an antibody-enzyme conjugate. Fluorescent groups are also frequently used as secondary labels and are possible candidates for secondary labels in this application as well. A popular emerging technology for protein detection is a process known by the acronym, FRET (fluorescent resonance energy transfer) whereby energy is transferred to another compound or group in a non-radiative manner, causing the second compound or group to generate a detectable radiative signal.

The key to the technology is the use of amphiphilic multiblock copolymers. An amphiphilic molecule is one that has a hydrophobic and a hydrophilic end. Perhaps the most common example of an amphiphilic (or amphipathic) molecule is a phospholipid such as is found in nearly every living cell. It consists of one end that is typically comprised of a carbon atom bound with three hydrogen atoms. Its fourth bond holds it to the molecular backbone. The hydrophilic end of the molecule can vary in its chemical characteristics, but is likely to be charged, thus attracting polarized water molecules. The most common amphiphilic artificial substance is soap: polarized water sticks to one side of the water molecule and unpolarized skin oils bind the other. If these molecules form a monolayer that becomes spherical, a micelle forms. By contrast, a liposome is a bilayered sphere of amphiphilic molecules. In the present invention, the inventors have developed a method for self-assembling nano- and micron-sized structures in water. When present in solution above the critical micelle concentration (CMC), their amphiphilic multiblock copolymers assemble by "multi-molecular micellization." There are various theories as to how this occurs, but the inventors favor the mechanism whereby the hydrophobic poly(amino acid) portion or "block" of the copolymer collapses to form the micellar core, while the hydrophilic PEG block forms a peripheral corona and imparts water solubility.

The inventors also cite their work as falling under a larger realm of chemistry, known as "click chemistry," a term accredited to 2001 chemistry Nobel laureate, K. Barry Sharpless. Click chemistry is commonly defined as "selective, exothermic reactions which occur under mild conditions in water." The inventors state that certain R3 moieties, or molecules with three functional groups, of the present invention are suitable for click chemistry. The R3 moiety is useful for conjugating their micellar compounds to biological macromolecules such as proteins, viruses, and cells. Because most proteins contain a multitude of lysines and arginines, with their long, charged sidechains, micelle conjugation occurs uncontrollably at multiple sites on the protein. The authors contend that this is problematic when lysines or arginines are located around the active site of an enzyme or other biomolecule. They thus present another method for conjugating the R1 groups of a compound to a macromolecule via click chemistry.

They state that since click reactions tend to involve high-energy, "spring-loaded" reagents with well-defined reaction coordinates, they give rise to a wide scope of selective bond-

forming events. Examples include the nucleophilic-trapping or strained-ring electrophiles (epoxide, aziridines, aziridinium ions, episulfonium ions), certain forms of carbonyl reactivity (aldehydes and hydrazines or hydroxylamines, for example), and several types of cycloaddition reactions. The azide-alkyne 1,3-dipolar cycloaddition is named as one such reaction. In discussing the release mechanism, the authors contend that although the design of materials that respond to small pH variations is challenging, this mechanism, coupled with the enhanced permeability and retention (EPR) effect that their micelles may represent an effective method for limiting drug release to solid tumors. The EPR effect [62] has been observed in tumor treatment whereby the increased micro-vasculature at a tumor site makes it selective for small liposomes or macromolecular drugs. The advantage the authors state to their invention is that their amphiphilic block copolymers and cell-responsive polymer micelles are designed to combine the concepts of crosslinked polymer micelles and pH-sensitive drug targeting to construct "smart" nanovectors that are infinitely stable to dilution in the bloodstream but are "chemically programmed" to release their therapeutic payload in response to pH changes commonly found in solid tumors and cancer cells. They conclude their discussion on delivery by contending that by utilizing cancer-responsive nanovectors in conjunction with chemotherapeutic agents, clinical problems such as post-injection micelle stability and the targeted delivery of encapsulated therapeutics to cancer cells are mitigated. Unlike previous examples of micelle crosslinking strategies such as core and shell crosslinking, the multi-block approach of this group allows for the effective crosslinking of polymer segments located at the interface of the hydrophobic and hydrophilic polymer blocks as shown in Fig. 2. This approach is advantageous because stable micelles are prepared without sacrificing loading efficiency or altering the drug molecule during core crosslinking. The remainder of the patent lists several hundred drugs, either synthetic or biologically derived, that may be used in their targeting strategy.

BIOLOGICAL-BASED COMPUTING

The now well-known heuristic theory of computer technologist, Gordon Moore proposed in 1965 and now commonly referred to as Moore's Law has been applied to everything from the number of transistors on a chip [63, 64], to the number of cell phones in use [65], to the general rate of biological and technological development from the Big Bang to the present [66, 67]. In fact, Ray Kurzweil has used Moore's Law to predict that technological computation will surpass individual "human computation" within this century and indeed all of humanity's computational power shortly thereafter [65]. But what is biological computation? DNA has been used to solve the traveling salesman problem [68], but arguably, computation also occurs in the brain at a rate of approximately $10^{18} - 10^{18}$ computations per second, ($10^{12} - 10^{13}$ neurons with 10^3 connections each operating at 1 kHz). What computation occurs within a single cell?

In "Knowledge-Based Methods for Genetic Networks and Systems Based Thereon," Edwin Addison of Wilmington, NC, describes "A new computing architecture that mimics the behavior of biological cells, called a "Whole

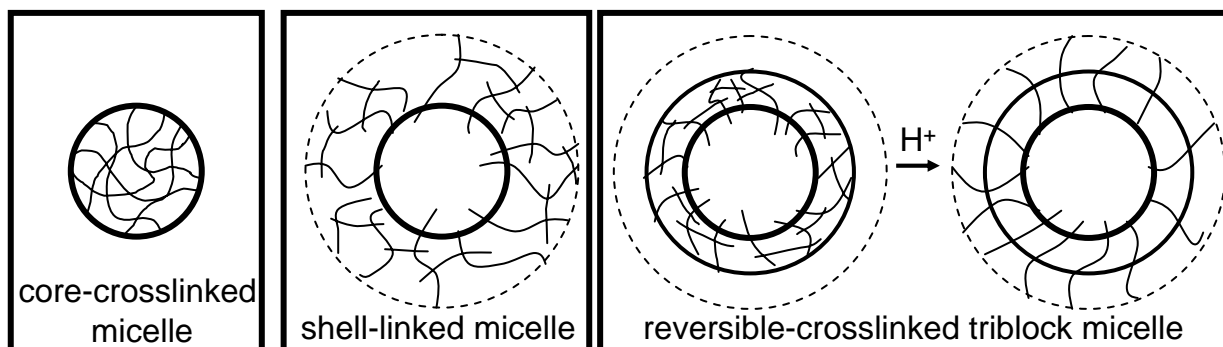


Fig. (2). Schematic comparing the triblock co-polymer method Breitenkamp *et al.* (2006) [61] to existing micelle drug-release methods. Image adapted from [61].

Cell Computer” (WCC) [69]. The WCC computational architecture is based on the biochemical processing of cells. It represents both a specialization and an extension of “membrane computing.” The computing strategy is derived from the properties of biological cells and has extensive statistical redundancy built in. Presumably, cells can also be “programmed” using genetic programming techniques. The inventor describes the WCC as “a computing node that operates in a network of many other WCCs of similar or different types, wherein:

- a) the WCC operates by the principles of stochastic dataflow computing;
- b) the WCC is classified as a membrane computer;
- c) the WCC substantially obeys one or more basic principles selected from the group of:
 - i. the WCC has at least one membrane bound instruction within it;
 - ii. the WCC instructions convert one or more operands into one or more operand products and may optionally require the presence of additional operands that do not undergo change, and instructions execute upon the arrival of all necessary operands within a proximal distance to the instruction, which distance is determined by rule or instruction;
 - iii. a state vector of the WCC provides information about results or status of which are determined by the statistical aggregate expression level and state vector as a result of the random motion and aggregate execution of instructions;
 - iv. programs are stored as one or more strings of embedded operands in a latent state, and programs execute by arrival of activating or regulating operands;
 - v. the WCC contains a “cache” memory, which maintains a single copy of each individual instruction that is active in a given WCC;
 - vi. the WCC contains at least one of the following types of input/output instruction: 1) instructions that cause operands to cross membrane boundaries, and/or 2) instructions that respond to ligands;
 - vii. the WCC contains an operating system that provides the mechanism for copying instructions and operands from programs, delivering them to the proper location, and

providing the power for their execution upon the arrival of their operands;

viii. WCC program execution is massively parallel, distributed, multithreaded and otherwise contains no central processing unit;

ix. memory and computing results are stored via the state of WCC in a distributed manner, with short term memory of the WCC being the current state vector, and the long term memory of the WCC being the active pathways;

x. when there are differing types of WCCs in a network, there must be more than ten copies of each type;

xi. a WCC is robust to computing errors because there are many copies of any given instruction, operand or WCC in a network;

xii. a WCC may cease to function by its own decision if it becomes defective; and

d) the WCC may be programmed by manually fixing the instruction set and operands or through genetic programming.”

As described by the inventor, and as outlined in a 2000 NSF report, biological computing is a research area wherein the goal is to determine how biology performs information technology from the subcellular level to the systems and population level, and determines computing architectures to mimic these processing techniques [70]. Although controversial [71], the cell itself may be considered to be a computing machine. Observations supporting the notion that a cell is a computer are that a cell contains many enclosed organelles, each of which perform specific simultaneous operations. The operations likely have varying degrees of interrelatedness. For example, the cell nucleus, typically located near the geometric center of the cell, contains and protects its code in the form of DNA. The DNA is read by a complex of enzymes, is transcribed into RNA, and ultimately produces operands in the form of proteins, which then either diffuse, or are chemically directed spatiotemporally throughout the cell or indeed throughout the organism to perform signaling operations. This cluster of processes provides the computational kernel. Cellular computations are also mediated by external stimuli such as protein hormones, metabolism of food (proteins, fatty acids or sugars) or

metabolites such as electrolytes or oxygen. The primary operations of the cellular computer are constitutive; maintaining basal the power supply of the cell, while other processes conduct duties that may promote the importance of an individual cell such as exocytosis regulation and signal transduction. The substrates upon which most of these processes take place may be considered to occur in one of two places: the cytosol or the cell membrane. The cytosol, primarily consists of water and a multitude of proteins in various phases of construction and editing and the cytoskeleton. The cell membrane, nuclear membrane and membranes of the various organelles consist of a lipid bilayer and its associated integral proteins such as ion channels (Fig. 3), which may be considered as the I/O interface of the cellular computer.

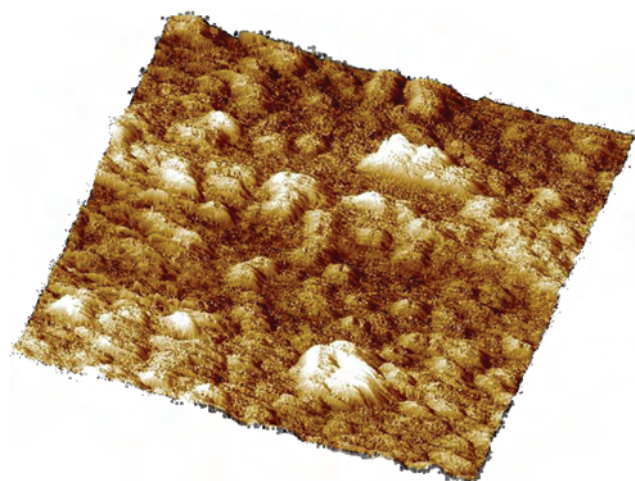


Fig. (3). Atomic force microscopy image of a nuclear membrane imaged on a gluteraldehyde-fixed intact nucleus extracted from an embryonic rat liver cell. Clearly visible are individual ion channels, which appear as small volcano-like structures. Image taken during a mitochondria isolation and imaging protocol [21]. Larger structures seen as large white clusters may be individual nuclear membrane pore complexes. Image size = $2\mu\text{m} \times 2\mu\text{m}$.

Since the cellular computing platform is highly parallel, highly redundant, and highly distributed, no single operation is ultimately critical to producing a final result. The results of computation are based on the concentration of operands, which are based on thousands or millions of chemical reactions. The inventors saliently state that

“Unlike traditional computing where every line of code could potentially crash a program, cells “compute” with the second law of thermodynamics.”

The uncertainties and inefficiencies often attributed to the second law also govern silicon-based digital computers, where voltages must “threshold” when changing states. The second law also dictates that purely reversible computing [72] is not possible, but approachable, just as perpetual motion is an idealization of a machine that is frictionless. Another intriguing property that the inventors ascribe to whole cell computing is “evolvability,” where programmability is based on genetic recombination. The notion that

entropy production, life and indeed information theory [73] are deeply intertwined has been explored in detail by others [74-77].

What remains to be determined, and something that is likely to become of deep importance to science, the environment, and indeed global technology policies is the rate of entropy production of a given technology or entity. For example, the well-known second law of thermodynamics states that when energy is transformed from one form to another, for example from thermal to mechanical, the entropy of the system increases. The same is true if the same system exchanges some of its mechanical energy for thermal energy. Thus, as repeated exchanges are made, entropy continues to rise until sufficient disorder exists such that further exchange becomes either impractical, uneconomical, or impossible.

With regard to biological computing, the inventor cites a lineage of leaders in neural networks [78], genetic programming [79, 80], artificial intelligence e.g. [81], DNA computing [68], molecular computing e.g. [82], and bacterial computing [83]. Returning to the Hickman NSF report [70], there is a “huge untapped potential” in cellular computing and currently research in the area is “scattered, not well focused, and often very application-oriented.” One cause perhaps is that computer scientists have thought biology to be uninteresting because of their slow metabolic speed. The realization that biological systems may be exploited for their parallelization is changing this notion. While this may be true for a single cell, when a cell is seen as a computing element and the focus is on what is inside of a cell as in the invention of Addison, computational steps can be very fast at the molecular level. Addison goes on to state that one goal of the research within the patent is to simulate a portion of a human immune system. He cites this as an example of using a network of WCCs cooperatively to do intelligent pattern recognition. He states that:

“The immune system is a highly distributed, complex and asynchronously parallel system of cells, antibodies, antigens and signaling molecules. To simulate lymphocyte response requires differently defined WCCs for each element of the system. For example, a different WCC architecture (instructions, compartments, concentrations) is needed for a B-lymphocyte, an antigen and an antibody, among others. Specific actions are simulated by defined instructions in each WCC type. For example, a common action in the immune system is when an antigen is presented to a lymphocyte causing the release of cytokines [84]. This leads to the specification of instructions and “cell organization” by modeling the specific action. The biochemical pathway causing the release of cytokines may be identified by referencing the KEGG database[85]. This pathway then represents the model of a WCC instruction. In the overall simulation, when an antigen comes in contact with the lymphocyte, that instruction sequence will be activated, causing an increase in the concentration of “cytokines”, an object which can trigger other WCC instructions. Then, the overall dynamics of a “system” or network of WCCs determines the overall outcome. In this case, the overall outcome may be a mapping of the number of active lymphocytes as a function of time, where an “active”

lymphocyte is one whose concentration level of a specific operand is above a certain level, the choice of which may be determined by genetic programming.”

Some further limitations of the current state of WCC listed by the inventor are that the proposed WCC architectures do not yet fully take advantage of high volume of cellular transactions and the second law of thermodynamics. He views a cell as a computing element or processor as an interrupt-driven dataflow machine that processes many instructions in parallel and uses many subordinated processors, and states that ultimately, the cell's role is to send and receive various communications (electrical, chemical) with other cells at various points in time. He states that a cell is a dataflow machine: instructions in the form of chemical reactions execute when their operands (i.e. reactants and enzymes) arrive. He then states that there is no sequential program of instructions as in the von Neumann machine. While this may be true for a single cell or a group of cells, there are a multitude of homeostasis enzymes that work serially checking for errors in DNA, maintaining ion gradients, and maintaining cell temperature. Thus while a whole cell certainly is not a von Neumann-type serial processor, it does contain several processes that are indeed serial.

A deeper question that lies at the heart of cellular computing is whether the human operator (scientist) can understand the processes of whole cells or groups of cells to a degree sufficient to compete with silicon transistor-based computers. For example, if we are to view machines as evolving through a process known as “mechanoevolution,” [86] then machines are selected for based on a fitness criteria. In the case of computation, the fitness criteria is based upon two metrics: the ability to perform calculations quickly and the ability of a significant number of human operators to program a given computational device to render it effective. For example, to be selected for, whole cell computing must either provide enough advantage to a limited few who are capable of conducting cell based computing, or it must be simple enough to allow a larger number of people to use it such that it becomes ubiquitous.

Addison states in his patent the often quoted adage that “it is easier to learn to fly by building airplanes than by dissecting birds”. Aircraft efficiency and bird efficiency may both be defined as the amount of energy used to overcome drag divided by the amount of chemical energy stored in the fuel. Computational efficiency must be defined in a different way. Since the purpose of the computer is not to move quickly and efficiently, but rather to organize data or information, its efficiency must be defined by the number of computations per second divided by the power used or computations per joule. A typical desktop computer performs a few hundred million computations per second, consuming approximately 100 joules per second. By comparison, the 10 to 100 trillion cells in the human body share approximately 5000 Calories (20 megajoules) of energy daily, thus each cell consumes on average, energy at a rate of 1 to 10 picowatts. Using the figures above, the desktop computer executes approximately one million computations per joule. A cellular computer with a mass of 10 kg consisting of one trillion cells would need to have each

of its cells performing 1,000,000 to 10,000,000 computations per second to compete with silicon-based computers.

An ideal Turing machine is zero-dimensional, or at most one dimensional. The computational framework of the present patent is perhaps two dimensional (if a membrane is being used as the input/output) or three dimensional if the whole cell is considered. The one-, two-, or three-dimensional state space of a computer may be defined, however by a single zero +1 space where all of the information contained in the one binary square of the Turing machine is given sufficient time to pass through it.

CURRENT & FUTURE DEVELOPMENTS

One underappreciated attribute of biologically manufactured materials is that they are all constructed molecule-by-molecule under enzymatic control and are always manufactured at ambient temperatures, whereas nearly all industrial processes involve heating either a pure material or a solution or combination of compounds to produce a desired shape. In the author's opinion, a true challenge for nanotechnology is to harness the “information” contained by the enzymatic processes of nature. Herein lies the secret to bottom-up manufacturing.

To the author's knowledge, no formal global efficiency analysis has been performed for manufacturing technologies. Typically, manufacturers try to keep their costs low by using a minimum amount of energy to make a part. In general, anything that is manufactured spends a period of time at an elevated temperature as it is being formed. It then cools and takes its final shape or configuration. This is true for manufacturing processes ranging from glass blowing to magnetic disk drive fabrication. Arguably, once a part has been manufactured, it has greater information content than the material had prior to the manufacturing and it is this information content that makes the manufactured item more valuable. This is true for anything from Michelangelo's David, which has a greater value than the stone from which it was carved to the kitchen stove, which has greater value than the sheet metal from which it was made. The specific spatial orientation of the atoms in these artifacts determine their value. What is frequently ignored however, is the rate of entropy production during the manufacturing process and the relative rate of entropy production among manufacturing processes. Since at the macroscale, no process is truly irreversible, when energy is exchanged from one form to another (i.e. thermal to mechanical), entropy is produced. An intriguing study would be to quantify the entropy production rate of a given nanofabrication technique and to compare it to a traditional fabrication technique. For example in a carbon vapor deposition process, a catalytic process is used and temperatures of 600°, up to 2000° C are reached at various stages in the process, to produce typically a few micrograms of material. By contrast, all of the bone, dentin and collagenous tissue in all organisms are produced at less than 40° C. Likewise, a nanomanipulation or microfabrication process typically involves movement of a robotic arm many million times as massive as the device being assembled, whereas enzymes such as DNA polymerase, which are true nanoassemblers, have masses equal to or less than the molecules they produce. Thus if a low-temperature enzymatic molecule were to be invented that “spun” carbon

nanotubes or other nanofibers, this would be a true step forward for making nanotechnology smarter, more efficient, and environmentally friendlier since less combustion products would be emitted during their manufacture.

As a closing remark, I would like to introduce a classification of machines. These may be generally defined as Type I, Type II, Type III, or Type IV machines. A Type I machine is one that requires no power input and which is not itself responsible for entropy generation without human input. An example of a Type I machine is a hammer. A Type II machine is one that requires a non-human power source and thus performs energy conversion such as from electrical to mechanical, and is thus capable of entropy generation both during operation as well as maintenance. An example is an automobile, which converts chemical energy into thermal and mechanical energy during operation, but which also requires the use of several other Type I machines such as wrenches, pliers, etc., for its maintenance. A Type III machine on the other hand, is nearly capable or fully capable of self-maintenance and thus generates entropy whether or not a human is immediately present to operate it. Examples include advanced robots or some advanced assembly plants. Societies that are able to develop, maintain and utilize machines of increasing levels of complexity i.e. Type III versus Type I typically enjoy a greater standard of living. However, what has not been fully analyzed is the effect of the accelerating of “entropization,” that humans have on the environment. We know that the second law states that $dS > dE/T$, where S is entropy, E is energy, and T is temperature. Since we assume that energy is always exchanging states and that an increasing amount of this state change is driven by human innovation, the second law is sometimes stated as

$dS/dt > 0$, where t is time. However, what has not been considered, is whether this rate of entropy is accelerating, i.e., $d^2S/dt^2 > 0$, is constant, $d^2S/dt^2 = 0$, or is diminishing, $d^2S/dt^2 < 0$. It is my contention that since living systems are always working towards deceleration of their own entropy production rates and that of their technologies, that the net result is an acceleration in the global entropy level (Fig. 4). A definition of Type IV machine, is perhaps less clear, but may be generally defined as one that only serves a purpose when fully integrated with human anatomy. A common example is a pacemaker or insulin pump. Ultimately these machines will diminish further in size, perhaps reaching the dimensions outlined in the inaugural issue of this journal [87]. Once this scale has been reached, and indeed once nanorobots have become integral portions of our own germ cells, we will evolve into Type IV machines as predicted by Kurzweil [88].

For example, the majority of the technologies reviewed in this article operate on vanishingly small volumes of material. In some cases the volume is as small as a few picoliters (10^{-15} m^3 , or $10 \times 10 \times 10 \text{ }\mu\text{m}$) or femtoliters (10^{-18} m^3 , or $1 \times 1 \times 1 \text{ }\mu\text{m}$) of space. However, the equipment that must be maintained to perform these manipulations still requires up to a kilowatt of power. For example, to manually assemble a nanopipette, using a nanomanipulator requires that two or three computers be running as well as a high-voltage electron beam, not to mention one or two microscope operators [19]. By contrast, at the macroscale, to build a stone wall or to frame a house, considerably more mass is being moved per unit time, with only two hundred watts from a human. Perhaps the most extreme example of this inverse relationship between the amount of energy required

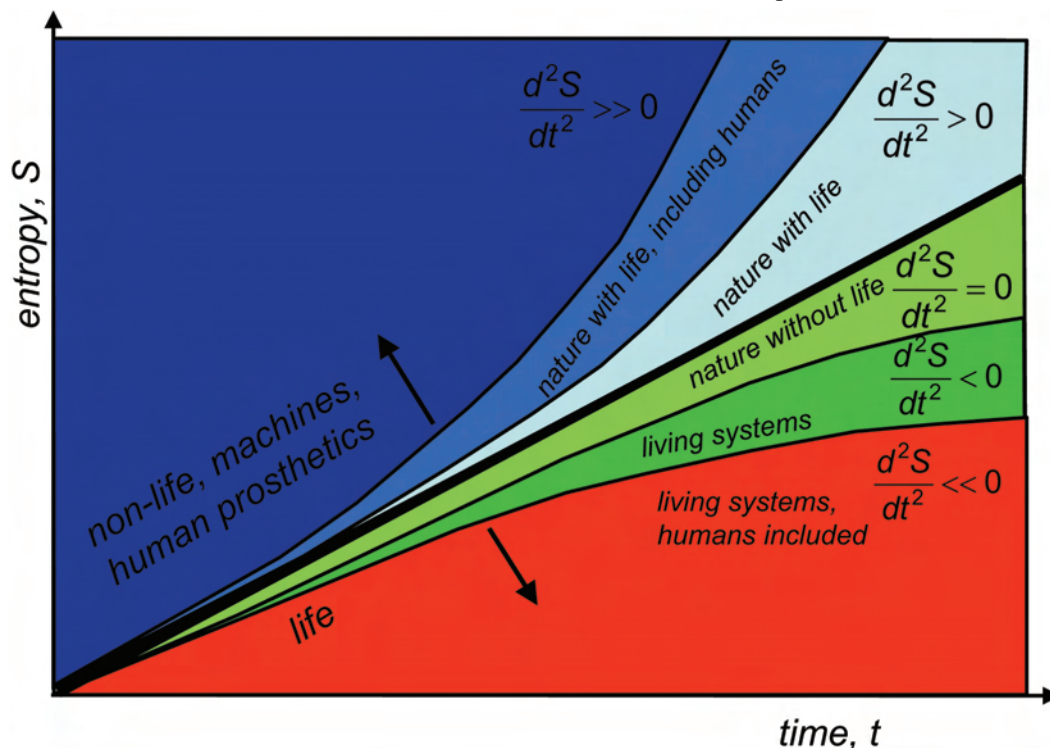


Fig. (4). Quantitative model of entropy production in a universe with and without life, which strives to reduce entropy locally. For example, it is well known from the second law that dS/dt is positive, but as advanced technologies are developed to reduce entropy within individual human organisms, will global entropy production accelerate?

to manipulate matter and the length scale upon which it is being manipulated is manifested in particle accelerators which are now reaching dimensions of several tens of kilometers in circumference, and where the critical operating volume is that of a single atomic nucleus 10^{-30} to 10^{-45} m³. The energy required to operate such a system can approach that of an average sized city. In other words, the devices used to probe matter at the subatomic scale must be tens of kilometers miles in diameter and consume tremendous energy to monitor events taking place in nanoseconds. Once true "picotechnology" comes on line, its cost per unit volume is likely to somewhere between the extreme seen in particle accelerators and that of current nanotechnology. The true question, however, is what the payoff will be for science and technology investors.

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