DOI: 10.1002/anie.200705538

Artificial Life

Life as a Nanoscale Phenomenon**

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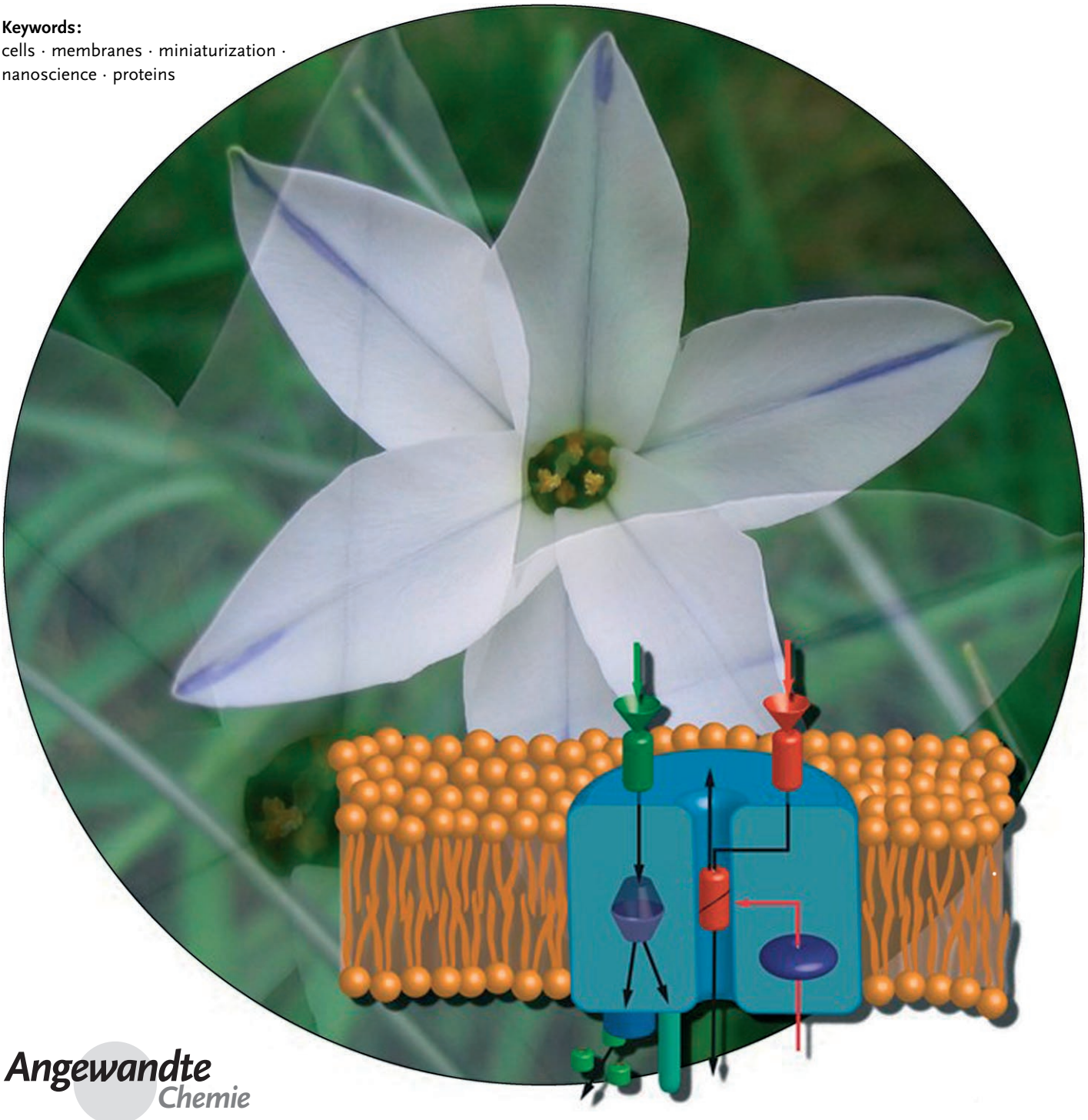
Keywords:
cells · membranes · miniaturization · nanoscience · proteins



The nanoscale is not just the middle ground between molecular and macroscopic but a dimension that is specifically geared to the gathering, processing, and transmission of chemical-based information. Herein we consider the living cell as an integrated self-regulating complex chemical system run principally by nanoscale miniaturization, and propose that this specific level of dimensional constraint is critical for the emergence and sustainability of cellular life in its minimal form. We address key aspects of the structure and function of the cell interface and internal metabolic processing that are coextensive with the up-scaling of molecular components to globular nanoobjects (integral membrane proteins, enzymes, and receptors, etc) and higher-order architectures such as microtubules, ribosomes, and molecular motors. Future developments in nanoscience could provide the basis for artificial life.

From the Contents

1. Introduction—The Significance of Nanoscience

Given that nanoscience has grown exponentially over the last decade, producing important contributions at the interfaces between chemistry, physics, biomedicine, and analytical science, it seems expedient to ask why this research area is so scientifically distinguished. It is now over twenty years since certain semiconductors[1,2] and iron oxides[3,4] were shown to exhibit markedly different electronic and magnetic properties, respectively, compared with corresponding bulk materials when confined to length scales of a few nanometers. This was followed some years later by innovations in scanning tunneling microscopy that enabled individual atoms to be manipulated and positioned into quantum structures,[5,6] and the discovery of nanotubular forms of carbon.[7] Together, these important advances justify the commonly held raison d'etre of nanoscience that confinement is of key importance in determining the intrinsic properties of structured matter. But size alone is not the defining parameter of many nanoscale-derived effects. More generally, these phenomena are determined by the surface area to volume ratio, with the consequence that particles with constant volume but variable shape can differ significantly in their confinement properties. Thus, much work continues to be undertaken on the synthesis of nanoparticles in the form of rods,[8] plates,[9] and multipodal structures,[10,11] which have high shape anisotropy. Similarly, structure as well as size, plays a key role in determining the metallic/semiconducting properties of carbon nanotubes,[12] and the depletion of crystal defects, moderation of surface roughness, and selected expression of crystal faces[13–16] are additional parameters that contribute significantly to the continuing surge of activity in nanoscience today.

Taking into account the above considerations, it remains nevertheless true that only a limited number of materials exhibit significant and useful changes in their intrinsic properties as a function of scale. Yet nanoscience remains wide ranging and eclectic. This is because natural progressions in the science and engineering of miniaturization, as well as structural amplification in supramolecular chemistry, are direct consequences of the development of nanoscience. Nanoscale miniaturization is of immediate relevance particularly to a large and active community concerned with storage density, functional displays and screening protocols, isolation of molecules and their conjugates, and device integration. Moreover, collective properties such as super-hydrophobicity,[17] plasmonic coupling,[18] and magnetic/electronic behavior of devices[19,20] can be modulated by miniaturization of structural components and fabrication of superlattice assemblies. And from a bottom-up perspective, the ability to synthesize complex nanoscale objects with hybrid structure and composition is seen as a major challenge and opportunity in the chemistry of organized matter.[21]

The very large scope of the above activities indicates how nanoscience has shifted in significance in recent years from an initial focus on the confinement-induced modification of intrinsic properties towards the exploration of extrinsic properties associated with miniaturization. This transition brings nanoscience close to biology, although the engineering challenges associated with reduction in length scale are very different from the problems involved with the amplification of molecular-based architectures. It is self-evident that nanoscale miniaturization is a principle attribute of biochemistry, and it follows that the evolution of life was contingent on the emergence and integration of multiple forms of nanostructured objects (Figure 1, Table 1). These remarkable architectures serve as miniaturized components of complex processing systems, and given the molecular basis of metabolism it is pertinent to ask why these structures are as large as they are; that is, what key properties are associated with nanoscale objects that are absence in small molecules, and which

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[**] We thank Dr. Erik Dujardin, Toulouse, for designing the frontispiece.

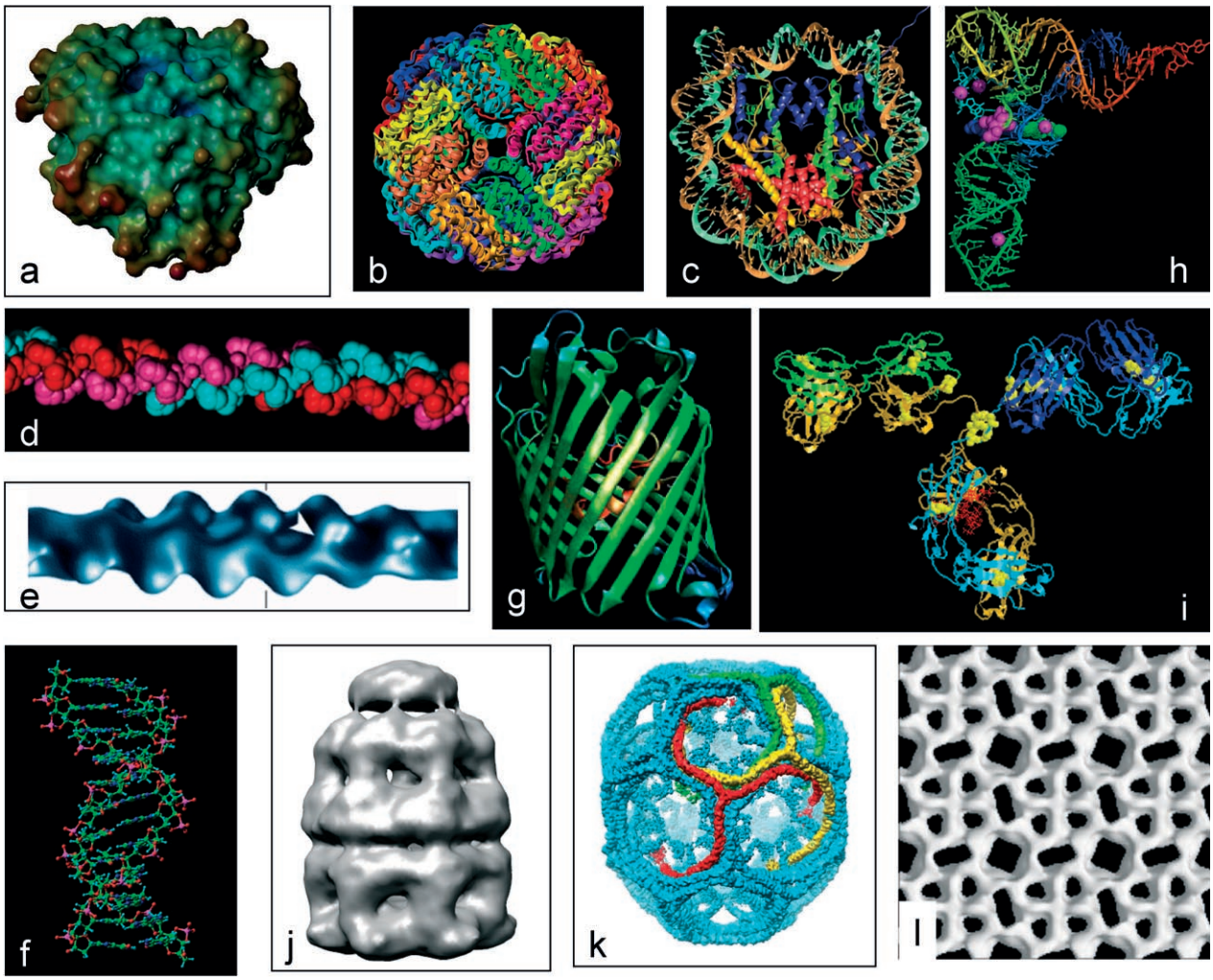

Figure 1. Examples of biological nanoobjects (see Table 1 for details and other examples): a–c) nanoparticles (a, globular (myoglobin); b, hollow/core–shell (ferritin); c, wrapped (nucleosome)); d–f) helical nanofilaments (d, triple coiled-coil (collagen); e, nanoparticle chain (F-actin); f, double-strand (DNA); g) nanotube (membrane protein (porin)); h,i) nanopods (h, L-shaped (tRNA)), i, Y-shaped (IgG)); j) nanobarrel (chaperonin groEL/ES complex); k) nanocage (clathrin); l) bacterial S-layer. Figures adapted from: c) ref. [28]; d) http:///www.med.unibs.it/~marchesi/pps97/course/section11/assembli.html; e) ref. [79]; f) http://www.nmr.cabm.rutgers.edu/photogallery/proteins/htm/page26; g) http://www.palaeos.com/Eukarya/Images/BetaBarrels.jpg; h) http://www.biochem.umd.edu/biochem/kahn/teach_res; i) http://www.sci.sdsu.edu/TFrey/Chem365/Proteins/AntibodyStructCh365.htm; j) http://www.rbvi.ucsf.edu/Outreach/Workshops/UCSF-Fall-2005/07-VolumeData/tutorial/chaperonin.html; k) ref. [73]; l) ref. [60].

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ultimately lead to sustainable chemical systems capable of self-renewal and replication?

In this Review, we propose that the correlation between biology and nanoscience is of deeper significance than generally acknowledged, and that the long-standing legacy of nanoscale phenomena lies in the emergence of sustainable systems of chemical complexity. We consider the living cell as an integrated self-regulating machine run by nanoscale miniaturization, and propose that this specific level of miniaturization is critical for the emergence and sustainability of the cell. We base this notion on the grounds that the regulation and optimization of systems based primarily on the input and reactivity of small molecules necessitate nanoscale operations and transformations to specifically facilitate the gathering, processing, and transmission of chemical information. Given the molecular nature of chemical systems, this

Table 1: Biological nanoobjects.

Type	Examples	Size [nm]
nanoparticles		
globular	(many proteins)	>2.5
hollow	apoferritins	12
	lumazine synthase	15
	cowpea chlorotic mottle virus	28
core–shell	lipoproteins (LDL)	20
	ferritins	8 (core) + 4 (shell)
wrapped	nucleosomes	11
nanofilaments (helical)		
double-strand	DNA	2 (width)
coiled coil	collagen	1.4×300
multiheaded	myosin	2×135
nanoparticle chains	F-actin	$[7\times36]_n$
	deoxyhemoglobin S	20 (width)
nanotubes	tobacco mosaic virus	18×300
	microtubules	25 (width)
	porins	1×5
	α-hemolysin	1.5/4.5×10
nanopods	clathrin triskelion	2×45 (per leg)
	IgG (Y-shaped)	4×5×8
	tRNA (L-shaped)	5.5×7
nanobarrels	chaperonins	14×15 (4.5 pore)
	proteasomes	15×11 (5×2 pore)
nanocages	clathrin	60
nanorotors	F_0F_1- ATPase	10×8 (F_1), 13×5 (F_o)
nanosheets	lipid bilayer	5 (thickness)
	S-layers	5–20 (thickness)

dependence is an inevitable consequence of the up-scaling required for processing, and a natural prerequisite for self-renewal mechanisms to emerge.

We begin with a discussion of the preconditions and constraints placed on the cell as a viable self-regulating chemical system (Section 2), and then discuss the fundamental importance and advantages of nanoscale boundaries for the emergence of a systems interface (Section 3). The nanoscale aspects associated with the internalization of extended molecular structures to produce functional globular objects and miniaturized transmitters are discussed in Section 4. Section 5 highlights the fundamental benefits accrued from the modular assembly of nanoscale globular objects to produce higher-order structures such as molecular motors. The potential influence of synthetic nanoscale structures on the realization of artificial cellular life is considered from a chemical perspective in Section 6. Finally, Section 7 summarizes the main conclusions of the review.

2. Limits to Cellular Life

The living cell can be considered as a spatially enclosed complex chemical system that is self-maintained and self-generated internally by metabolic processes acting under the flow of genetic information. Cellular components are produced, transformed, and arranged within the system, and this process—often referred to as *autopoiesis*[22]—is considered a necessary, and possibly sufficient, condition of life.[23] The cell is organized not only in the form of physically ordered structures undergoing time-dependent renewal and degradation, but also as fluctuating/cyclical patterns of flows of information, metabolites, materials, and energy that arise from the action of long-range constraints on local conditions.[24] Significantly, the internal structural and dynamical organization associated with autopoiesis must coexist throughout evolution with changing conditions in the local environment such that metabolic processes are fundamentally coupled in origin, operation, and adaptation to their milieu. Mechanistically, this takes place by transport and screening of molecules and materials at the cell boundary, which together maintain cell function, homeostasis, and viability by feedback between the inner organizational state and local environment. This relational feature, taken in its broadest context, is described as *cognition*,[25] and has been identified, along with autopoiesis, as a necessary condition of minimal cellular life.[26] Moreover, it has been argued that because interactions and active interventions with the environment are fundamental to the viability and adaptation of the internal organization of the cell, then autopoiesis is a precondition of cognition, and cognition is coextensive to life.[27]

Defining the edge of life as minimally cognitive, and hence autopoietic, it follows that two primary mechanistic features must emerge; 1) a systems interface with the environment and 2) a systems network for internalized self-processing (Figure 2). Together, these systems appear to be a

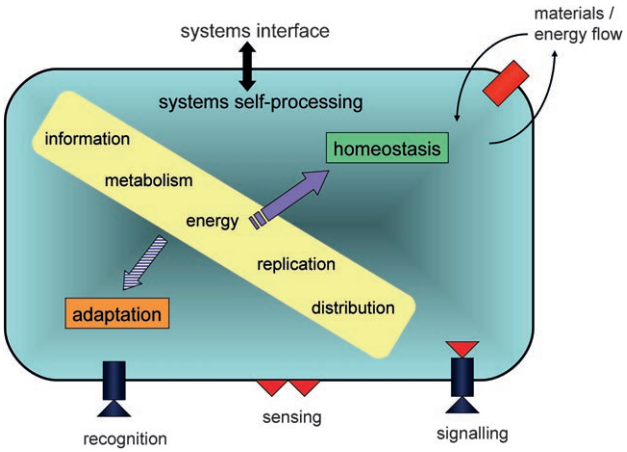

Figure 2. Systems of cellular life.

necessary and sufficient condition for biological life, as well as the realization of artificial cellular life.[27] They are indissolubly linked and their co-dependence is a consequence of

complex interfacial and internal organizational states based on dynamical patterns of flow of materials, energy, metabolites etc. As we will discuss in detail, both these systems operate and are integrated through nanoscale miniaturization.

The systems interface is delineated physically by the cell membrane in the form of a nanometer-thin phospholipid/sphingolipid/glycolipid bilayer with embedded (Figure 1g) or peripherally attached proteins that together serve as a boundary for the containment, transfer, and exchange of materials and energy, and as a sensorium for cell/cell and cell/molecule recognition and signalling (Table 2a). It seems irrefutable that emergence of the phospholipid/protein bilayer boundary as a fluid-like nanocomposite was a major event in the origin of cellular life. Moreover, the fact that so many fundamental processes are associated with the cell boundary—membrane proteins typically make up around a third of the proteome of a cell—supports the notion that the 5 nm thickness of the lipid bilayer sets a key boundary condition for the miniaturization and operation of the systems interface. In this regard, small variations in the nanoscale thickness of the lipid bilayer, along with associated properties such as membrane elasticity and fluidity, would have been important fitness parameters for the emergence and optimization of functional properties via integration of transmembrane proteins. In particular, a critical thickness of at least 3 nm is required for lipid bilayer stability, whilst lipid bilayers with thicknesses greater than 10 nm are less compatible with a functioning systems interface (see Section 3).

Internal processing networks are involved with the storage and generation of energy and information, metabolic activity, gene replication, and cellular distribution (protein sorting, trafficking, servicing, etc) (Table 2b). These processes constitute a dissipative nonequilibrium system that is maintained by continuous active exchange between the intracellular milieu and surrounding environment via the cell boundary. In prokaryotes, the cytoplasm is continuous and contains dispersed biopolymers, whereas in eukaryotes there are many different types of organelles (nucleus, mitochondria, chloroplasts etc) and compartments (lysosomes, vesicles) that function as discrete membrane-bounded subsystems. Significantly, self-processing is sustained against considerable chemical and redox gradients,[24] which enable elevated concentrations of energy-rich (reduced) carbon macromolecules and metabolites to be retained within the cell against a substantial osmotic pressure gradient. This is achieved by the outward pumping of sodium and chloride ions, and a similar process involving the efflux of calcium ions against a concentration gradient of approximately 10^4 prevents deleterious cross-linking of the intracellular biopolymers.

Underpinning the operation of metabolic processing networks is the emergence of macromolecular machinery that operates at a primary level on small organic molecules. These larger scale architectures are dependent on two interdependent factors–-extension of molecular structures to nanoscale dimensions in association with multiple domains of amphiphilicity. Together, these promote intramolecular internalization and the formation of three-dimensional globular nanoscale objects with folded tertiary structures (proteins, receptors, enzymes, certain RNAs (but not DNA); see Figure 1). Due to structural and energetic considerations, this necessitates a length scale of at least 2 nm for globular proteins (see Section 4). Moreover, the patterned exteriors and structural asymmetry of these objects often give rise to quaternary architectures with multiple domains and complex

Table 2: Examples of nanoscale miniaturization of biosystems operations.

Operation		Examples
a) Systems interface		
materials/energy flow		
	passive transport	porins, gap junctions
	active transfer	pumps (amino acids/Na^+, lactose/H^+)
	synport exchange	ATPases (Na^+/K^+, Ca^{2+}/H^+)
	capture	siderophores, endocytosis, clathrin pits
		photoreceptors (bacteriorhodopsin)
sensing		
	chemotaxis	chemoreceptors (methylation)
	hormone signalling	receptor/G-proteins
		receptor/phospholipase C
		EGF receptors (tyrosine kinases)
	signalling cascades	adenylate cyclase/$G_\alpha GTP$
	neurotransmission	acetylchoine/catecholamine receptors
		GABA receptors/Cl^-
	cell/matrix	integrins/RGD
	antigens	B-lymophocyte immunoglobins,
	MHC peptides	T-cell/CD4/CD8 receptors
b) Internal processing		
general metabolism		
	structure	F-actin, tubulin (microtubules), collagen
		spectrin, intermediate filaments
	enzymatic catalysis	globular proteins
	signalling	calmodulin/Ca^{2+}
	recognition	immunoglobins
	motors	microtubules/kinesin, actin/myosin
	protein synthesis	ribosomes
	protein folding/	chaperonins. endoplasmic reticu-
	transport	lum membrane
	energy	mitochondrial membrane
	storage	ferritins, metallothioneins
	detoxification	lysosome/P450, peroxisome/catalase
	destruction	ubiquitin/proteasome
		caspases (apoptosis)
information		
	storage	nucleosomes
	unwinding	helicase, gyrase
	replication	DNA polymerase, RNA primase
	error correction	DNA polymerases
	clipping	restriction endonucleases
	repair	ligase
	translation	tRNA/mRNA
	splicing	spliceosome proteins

functions. A key aspect of these biological nanostructures is that they facilitate directionality in the processing of small and large molecules through the spatial and chemical delineation of pathways that are able to exploit and sometimes oppose incessant random Brownian motion in solution. Such constraints are of special importance for the emergence of movement, internal trafficking, morphogenesis, and segregation of genetic material, all of which necessitate the controlled conversion of chemical energy into directed mechanical motion under isothermal conditions. While this occurs in macroscale machines through the confinement of heat, random thermal dissipation at the molecular level necessitates that Brownian motion be restrained selectively, and this is accomplished through the use of interacting nanoscale components (see Section 5).

Systems of autopoiesis and cognition are contingent on the storage and retrieval of large amounts of information, and in cells this is based on the processing of a linear code through molecular recognition using DNA and RNA macromolecules. The code must be maintained in pristine form, and this is achieved structurally in linear nanofilaments with a molecular backbone constructed from invariant 3′-5′phosphodiester covalent bridges. Polynucleotide nanofilaments are only 2 nm in width but macroscopic in length—nearly 1 m in certain human chromosomes—and access to the nanometer architecture must be preserved if the myriad protein–DNA interactions required, for example, during DNA replication (cell division), or transcription to mRNA for protein synthesis, are to take place. For this, DNA molecules must not only be packaged with retention of their native nanostructure—in eukaryotic cells this is achieved by association with histone octamers to produce chains of 11 nm-sized modular structures (nucleosomes,[28] Figure 1c)—but also remain susceptible to strand separation and negative supercoiling for processing of the coded information by an array of enzymes (Table 2b). In each case, the enzymes operate as miniaturized nanoscale machines of great complexity.

The strong feedback implicit in the coupling of complex systems of interfacial and internal processing is geared at the deepest level to cell viability, which in turn is honed by natural selection. At the level of the individual cell, the self-referential nature of life is manifest in the steady state of materials and energy fluxes (homeostasis), which necessitates that the hierarchical networks and loops of internal self-processing must be capable of passively or actively assimilating novel environmental inputs into the pre-existing autopoietic processes without undermining viability. This implicitly conservative nature is achieved by a high degree of systems vigilance and tolerance associated with degeneracy of the genetic code as well as mechanisms of error-correction and repair, molecular degradation, and vesicle-mediated exocytosis (Table 2b). This is the day-to-day business of life. In tension with this, is the enduring ability of the cell to adapt to novel disturbances by permanent transformations in the systems interface and internal processing networks to maintain and enhance viability. This is not realized at the level of the individual cell but by selection pressures on cell populations, that is at the super-system level of species.

3. Nanometer-Thin Boundaries

In this section we address three key questions that pertain to the emergence of a systems interface based on a nanometer-thin cellular boundary: 1) Is the nanoscale thickness of the lipid/protein bilayer an inevitable consequence of non-biological self-organization? 2) How was the lipid bilayer modulated by insertion of integral membrane proteins to produce a functional ultra-thin nanocomposite? 3) What fundamental biological advantages accrue from miniaturization of the interface on this length scale?

Phospholipids in bacteria and eukaryotes are structurally derived from D-glycerol with ester-linked saturated and unsaturated diacyl chains, whereas in archaea two isoprenoid chains are connected via ether linkages to a L-glycerol moiety. In both cases, the isolated phospholipid molecules can spontaneously self-assemble in water to produce vesicles and liposomes, suggesting that physicochemical rather than biological processes are sufficient to account for the ubiquity of the bilayer structural platform.[29] The self-assembly of phospholipid molecules is strongly dependent on both molecular size and shape, such that collective interactions between the hydrophobic domains increase with chain length, with the consequence that the interface changes from curved to planar as the tails become longer and the packing parameter P ($=V/al$)—which represents the ratio between the molecular volume (V) and effective volume (al) of a cylinder defined by the headgroup area a and molecular length l—rises above 0.75 to values of around 1.0.[30] Thus, diacylphosphatidylcholines with chain lengths of eight or less carbon atoms self-assemble into micelles, whereas higher chain derivatives spontaneously form bilayers.[31,32] As a consequence, the thickness of the bilayer must be greater than 3 nm, and this sets a lower limit on the length scale that a lipid platform could operate as a systems interface in cell biology.

In reality, the thickness of the lipid bilayer of the cell membrane is well above this cut-off value determined by structural and energetic considerations. Phospholipid biomolecules usually consist of acyl chains that are between 14 and 24 carbon atoms in length, with a typical mean value of 16 to 18, and variations in chain length, chain asymmetry, and degree of unsaturation, as well as cholesterol incorporation, have a significant influence on bilayer thickness.[33,34] Changes in length across this range do not significantly affect the packing parameter, which remains within the stability region of the fluid bilayer phase.[30] These molecular dimensions correspond to a lipid bilayer consisting of a hydrocarbon core of approximately 3 nm in thickness with an interfacial region of polar headgroups and bound water molecules of around 1.5 nm, giving a total thickness of 5 to 6 nm.[35] The dependence on this specific lipid length scale may have been inevitable because membranes comprising acyl carbon chains with lengths close to the stability limit (for example C_{10} or C_{12}) would be highly fluid and susceptible to micellization, which would compromise the structural integrity and dynamical properties of embedded proteins, particularly in bacterial cell membranes, which do not contain cholesterol. On the other hand, as the bending rigidity

increases progressively with the number of carbon atoms for saturated/mono-unsaturated phospholipids,[36] bilayers comprising extremely long chains ($>C_{32}$) would be significantly more rigid, which would compromise the membrane fluidity necessary for integration, segregation and lateral mobility of integral membrane proteins.

Lateral heterogeneity within the lipid bilayer plane is considered to be an important factor determining the function of biological membranes.[37] This arises in mixed component membranes due to fluid-like properties that promote raft formation associated with lateral molecular clustering, particularly in the presence of mixtures of sphingolipids and cholesterol.[38] The presence of large concentrations of integral membrane proteins—up to 30000 per μm^2 for rhodopsin in the rod outer segment[39]—will also significantly influence the in-plane nanoscale organization of the bilayer sheets (Figure 3a).[40] As a consequence, the ensuing protein/lipid nanocomposite membrane comprises a hierarchical arrangement of 1–10 nm-sized clusters of lipid molecules that are highly dynamic and preferentially associated within boundary layers of the integral membrane proteins.[40] These nonequilibrium structures play a key role in nanoscale miniaturization of the cell membrane composite and would have been of major significance in optimization of the systems interface.

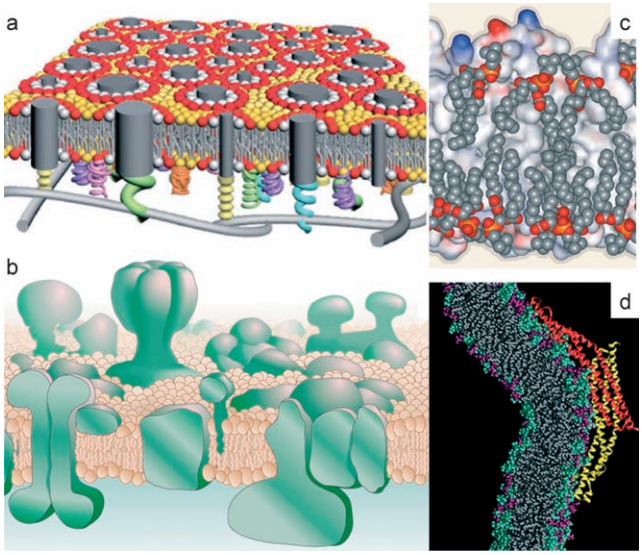

Figure 3. a) Schematic showing hierarchical arrangement in a lipid/protein nanocomposite membrane. The high concentration of membrane proteins induces local clustering on a length scale of 1–10 nm. Lipid molecules located in the first (white), second (red) boundary layers, as well as between the clustered shells (yellow) are shown. From ref. [40]. b) Modified model for the lipid/protein nanocomposite membrane. Variations in the thickness of the lipid bilayer are associated with patchiness arising from segregated regions of membrane proteins, some of which have extensive extracellular domains (e.g. F_0F_1-ATPase, as depicted in the center left of the drawing). From ref. [42]. c) Electron crystallographic structure showing a side view of one face of an aquaporin AQP0 tetramer stabilized by ordered packing of lipid molecules of the bilayer membrane. (AQP0 tetramer is shown as a surface plot (light background) with regions of negative charge (red), positive charge (blue), and hydrophobic domains (gray); lipid molecules are shown as a space-filled image (foreground) with polar headgroups (O, red; P, orange) and hydrophobic tails (gray)). Adapted from ref. [54,55]. d) Molecular dynamics simulation showing induced curvature in a lipid bilayer by binding of a protein N-BAR domain ($t=27$ ns). The bilayer bends to match the curvature of the surface of the N-BAR domain facing the membrane; charged phosphatidylserine (purple) and polar phosphatidylcholine (green) head groups are shown. From ref. [58].

The integration, partitioning, and segregation of integral membrane proteins provide the primary functionality of the cell membrane, and these processes must have arisen under evolutionary pressures to transform the structural platform of the lipid bilayer into a dynamical interface capable of regulating the flow of energy and materials. Transmembrane proteins often have a helical bundle architecture with hydrophobic stretches that are approximately 3 nm (20 residues) long, which span the hydrocarbon core of the lipid bilayer.[41] They are typically inserted into the bilayer as they emerge from the ribosome through a protein-conducting translocon complex,[35] and it follows that matching of the hydrophobic protein domains with the lipid hydrophobic thickness would have been a primary fitness parameter. Recent structural studies indicate significant differences in the hydrophobic width of transmembrane proteins,[42] and these in turn induce variations in the thickness and curvature of the cell membrane that are tolerated by the intrinsic fluidity of the lipid matrix.[43,44] As a consequence, the classical fluid mosaic model of the cell membrane has been recently modified to incorporate architectural features such as variable patchiness, segregated regions of structure and function, variable thickness and area occupancy, and restricted lateral mobility (Figure 3b).[42]

Significantly, there is substantial evidence that modifications in membrane thickness play a significant role in fine-tuning interactions between the lipid matrix and integral membrane proteins.[45–48] For example, the activity of the leucine transport system of *Lactococcus lactis* in liposomes comprising phosphatidylcholine and phosphatidylethanolamine molecules with different acyl chain lengths decreased in the order, $C_{18} \approx C_{16} \gg C_{24} > C_{22} > C_{14}$.[49] Various factors such as membrane fluidity, elasticity, curvature, and asymmetry, as well as conformational matching/mismatching between the hydrophobic lipid chains and nonpolar domains of the integral membrane proteins, can influence the interplay between lipids and proteins of the cell membrane. The folding pathway of bacteriorhodopsin, for example, is modified by changes in lipid composition due to modifications in the lateral packing pressure.[50] Studies have also indicated that mismatches between the hydrophobic domains of the lipid chains and protein constituents can be tolerated by distortions in the lipid matrix to match the protein dimensions.[51] In particular, high-resolution structures of membrane protein/lipid interactions have shown that the specific packing of lipid fatty acyl chains against the rough hydrophobic interface of transmembrane proteins, such as cytochrome bc_1,[52] bacteriorhodopsin,[53] or aquaporin[54,55] (Figure 3c), facilitates the structural stabilization and functional integrity of the embedded macromolecules.

The above considerations highlight the important point that the fluidity and elasticity associated with nanometer-

thick lipid/protein composite films enable functional modulations in membrane thickness, patchiness, and curvature. The latter can also be influenced by the binding of peripheral proteins with N-BAR domains[56] that induce curvature in the bilayer membrane by binding of the N-terminal amphipathic helices and highly positive charged concave surface of the crescent-shaped dimer to patches of negatively charged lipids.[57] Atomistic simulations[58] indicate that bending is generated by the synergistic action of the embedding of the N-helices at the interface between the lipid tails and headgroups, in association with strong electrostatic interactions, which together force the membrane to locally adopt the intrinsic shape of the BAR domain (Figure 3d).

To summarize, in this section we have discussed from the perspective of miniaturization how the nanoscale thickness of the cell membrane provides physical properties that are compatible with the emergence of a systems interface. Cellular cognition is fundamentally dependent therefore on the structural evolution of membrane-bounded proteins under the nanoscale gauge specified by the thickness of the lipid bilayer. This necessitates a length scale of at least 3 nm, and an optimal thickness of around 5 to 6 nm. Composite 2D nanostructures, such as the lipid/protein bilayer, have properties that are fundamentally dependent on length scale because, unlike macroscopic films, organization of the internal nanostructure is strongly constrained by edge effects associated with the large surface area to volume ratio. Such structures are therefore responsive to biological adaptation via synergistic interactions between the lipid matrix and embedded proteins, with the consequence that complex systems of materials and energy flow can be generated across the cell membrane. In contrast, membranes with much greater thickness—such as the extensive cell wall thickening produced during cyst formation in protozoa[59]—would be significantly less sensitive to length scale modulation, and hence less susceptible to fine-tuning of their signalling and transport properties with respect to transmembrane functions. Whilst useful against adverse environmental conditions or during dormant periods in the life cycle, thickening of the cell wall under normal conditions is incompatible with a functioning systems interface. Interestingly, where the cell membrane is embellished—for example, the extracellular side of the cytoplasmic membrane of prokaryotes comprises additional structural layers, such as archaeal S-layer proteins[60] (Figure 1l), peptidoglycans (Gram-positive bacteria), or mixtures of peptidoglycans, lipopolysaccharides and lipoproteins (Gram-negative bacteria)—the disadvantages associated with the extended boundary are circumvented by adding the auxiliary layers as lamellar arrangements of nanometer-thin sheets and nanosized compartments. In this way, these multilayer superstructures increase the structural integrity, chemical resistance, and trafficking potential of the bacterial cell boundary, and provide confined periplasmic spaces for extracellular enzymatic processing.

In the following section we examine the role of internalization in extended molecular structures and the importance of this for the nanoscale miniaturization of globular objects (proteins, enzymes etc) and emergence of autopoiesis.

4. Globular Nanoobjects

Given the predominance of protein folding throughout structural biology,[61] the emergence of globular nanoobjects with complex and structurally persistent internalized architectures represents a key event in the evolution of life. We note too that many RNAs, unlike DNA, comprise single strands with complex folded architectures (Figure 1h). In proteins, these architectures are defined by linear chain sequences of extended molecular mass and multiple domains of amphiphilicity, and comprise delineated interior spaces and structures, chemically patterned exterior surfaces, and dynamical conformational states. Together, these mediate a wide range of functions such as small-molecule recognition, transport and reactivity, membrane signalling and transduction, and macromolecular activation, regulation and processing. The complexity of these activities and the increased informational content required for their realization are contingent on the up-scaling of molecular-based interactions, with the consequence that these functions are fundamentally dependent on nanoscale miniaturization.

What constraints are placed on this up-scaling? The domain size of globular proteins generally falls within the range of 100 to 200 amino acid residues.[62] In this regard, recent theoretical models based on size-dependent changes in the surface area to volume ratio of randomly packed (packing ratio = 0.64) residues predict an optimal domain size of 4.5 nm (156 amino acids) for globular proteins with spheroidal shape and approximately equivalent numbers of hydrophilic and hydrophobic side chains.[63] Larger globular structures are stabilized by an increase in the fraction of internalized hydrophobic residues, whereas small proteins such as ubiquitin, which consists of 76 amino acids,[64] adopt compact structures of β-sheets and α-helices by increasing the relative proportion of hydrophilic residues. Significantly, there is a marked transition at a chain length of around 20 to 25 residues, below which a polypeptide cannot fold into a globular domain due to insufficient hydrophobic residues.[63] This situation correlates with a minimum size for a folded globular domain of about 2.5 nm, and represents a critical boundary condition on the lower limit necessary for the evolution of functional 3D protein architectures.

Most enzymes are significantly larger than small proteins such as ubiquitin because high levels of intramolecular organization are required for recognition, capture, placement, activation, and regulation of low-molecular-weight organic compounds. The internalized microenvironments exhibit size, shape, and chemical specificity for the binding of substrates and cofactors, and the active sites are stereospecific, configured for transition state activation, and sensitive to local/global conformational rearrangements. These higher-order functions lead to chemo-, regio-, and stereoselective reactions that are catalyzed along specific trajectories and regulated through conformational rearrangements associated, for example, with the allosteric binding of substrates, proteolytic cleavage of proenzymes, cAMP-induced dissociation of protein kinases, inhibitory and stimulatory proteins, and reversible covalent modifications such as phosphorylation.

The structural evolution of membrane-bounded globular proteins under nanoscale constraints imposed by the thickness of the lipid bilayer is a critical parameter in the operation of the systems interface of the cell (Section 3). These proteins are of fundamental importance as miniaturized transmitters of receptor-mediated and transport-mediated information flows across the cell boundary (Figure 4). In each case, fine-tuning of chemical activation and mechanical transmission is fundamentally dependent on structural internalization arising through folding pathways coded in the amino acid sequence. The principal structural motif consists of intersubunit or intramolecular hydrophilic pores and channels of specific size, shape, and chemical complementarity that reside within a complex hydrophobic globular nanoobject. The proteins can be activated by secondary interactions arising from coupling and transmission of spatially separated processes held within a single or locally conjugated unit. This is often accomplished through helically bundled/looped arrangements that are persistent but conformationally sensitive to ion binding, photo-excitation, electrochemical gradients across the membrane, and chemical functionalization at peripheral sites. For example, light-induced proton transport through the seven-helix motif of the transmembrane protein, bacteriorhodopsin, is dependent on precise structural placement of the auxiliary chromophore (all *trans* retinal) and its photoisomer (13-*cis*), as well as delineation of a pathway for proton migration.[65] In contrast, active transport through the Na^+/K^+-ATPase antiport involves two or more distinct conformational rearrangements that are triggered either by intracellular ATP binding and phosphorylation of the α-subunits (Na^+ efflux) or dephosphorylation (K^+ influx).[66]

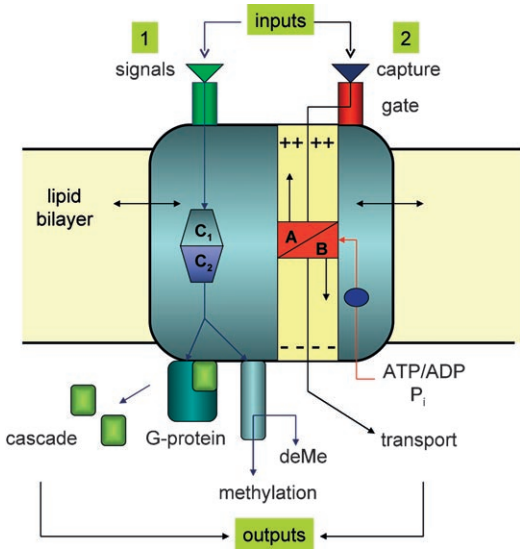

Figure 4. General scheme depicting the operation of integral membrane proteins in cellular cognition. These proteins are involved in diverse processes such as passive and active ion/molecule transport, proton gradients for ATP production, generation of action potentials via voltage-dependent charge migration, light harvesting and transduction, receptor-mediated signal transduction cascades, chemotaxis, and transduction of molecular motion. 1) Receptor-mediated information flow (signal transduction cascades). Extracellular binding of hormones and chemosensors produces conformational rearrangements (C_1, C_2) in the receptor that result in activation of intracellular processes. For example, chemosensors affect signal transmission to the flagella by influencing the rate of glutamate methylation/demethylation (deMe) on the cytoplasmic side of the receptor, whilst hormones activate the intracellular binding of peripheral G-proteins and the subsequent release of αGTP-bound subunits. The latter initiate cascades by activating other membrane-bounded proteins (adenylate cyclase) to produce second messengers (cAMP) that stimulate protein kinases for phosphorylation-mediated modulation of target proteins involved, for example, in glycogen degradation. 2) Transport-mediated information flow. Selected ions and molecules are bound close to the extracellular side depending on charge, size, and polarity, and transmission is activated by gated responses determined by proton gradients, electrochemical potentials, auxiliary ligand binding, or photoinduced conformational changes. Chemical activation via ATP binding and hydrolysis is common in many antiport systems in which the binding affinities associated with the two conformations required to transport the species (A and B) are switched either on or off by phosphorylation (P_i). Pathways (1) and (2) can also be modulated by interactions of the transmembrane proteins with the surrounding lipid bilayer (Section 3).

By using reversible conformation changes that are voltage-, ligand- or receptor-gated, steady state or cyclical patterns of materials flow can be established across and along the cell membrane. These information streams are a key feature of nanoscale miniaturization of the systems interface. They are particularly prevalent when the integral membrane proteins are coupled with peripheral proteins to generate intracellular signal transduction cascades. For example, interaction of adrenalin in the extracellular medium with a transmembrane seven-helix β-adrenergic receptor[67] initiates the adenylate cyclase cascade.[68] Thus, by using nanoscale components, the membrane is functionally traversed and the hormone-receptor complex is able to transduce an extracellular signal into intracellular activity. Moreover, many molecules of G_α-GTP and cyclic AMP are generated by a single hormone binding event, and fundamental to this cascade is the ability of both the hormone-receptor complex and adenylate cyclase to sustain multiple processing, which is achieved by the structural persistence of the protein nanostructures. In addition, the multifunctionality of these nanostructures make them sensitive to changes in hormone levels rather than absolute concentrations. They become desensitized, for example, under conditions of prolonged receptor binding by competitive processes such as serine phosphorylation of the hormone-receptor complex or latent GTPase activity of the α-subunit, which inhibit GTP/GDP exchange on the bound G-protein or deactivate adenylate cyclase, respectively.

Similarly, chemotaxis in bacteria involves the binding of soluble chemosensors (aspartate, galactose-binding proteins etc) to receptors present in the periplasmic space, which in turn influences the degree of reversible γ-methylation of glutamate residues present in the cytosolic segment of the transmembrane protein.[69] Fluctuations in concentration of the chemosensors are then read out temporally as changes in methylation. These induce changes in conformation within the triggering domain, which impact on the protein-mediated signal transmission to the flagella. Using nanoscale components to operate this system has the distinct advantage that

the information generated from twenty or so different chemoreceptors can be integrated and spatially processed prior to transmission via the Che proteins to the switch in the chemical motor.[70] Integration of these signals then determines the direction of rotation of the flagella, and hence the stop and start rates of tumbling motion of the cell.

In this section we have highlighted fundamental correlations between the operation of individual globular proteins and their structural persistence across nanoscale dimensions. Given the critical interdependence between metabolism and globular architectures, it seems reasonable to propose that the cell's ability to act as an autopoietic processing system was coextensive with the emergence of phenomena associated explicitly with the nanometer length scale. We have focused predominantly on the processing of small molecules by globular structures to illustrate the generic properties associated with internalized nanoscale architectures. Clearly, globular proteins (along with polynucleotides and polysaccharides) are themselves subject to higher-order processing (refolding, degradation, sorting etc), and this requires appropriate up-scaling in the miniaturization used for this purpose. Significantly, increases in processing complexity are associated predominantly with the modular assembly of multiple subunit architectures rather than the synthesis of folded single chains of extreme length. It appears that beyond a length scale of approximately 10 nm there are structural and functional constraints imposed on such structures. The latter would require extensive information codes and error correction mechanisms during translation, elaborate folding and refolding pathways, and increased demands on cellular trafficking, sorting and degradation. In contrast, modular assemblies of globular proteins have key properties that are central to the autopoetic operation of the cell. These are addressed specifically in the next section.

5. Modular Assembly and Nanomotors

The modular assembly of higher-order structures using nanoscale globular building blocks is a fundamental aspect of cell biology. This process is exploited for the structuration of complex biological objects, such as ribosomes,[71] chaperonins[72] (Figure 1j), clathrin cages[73] (Figure 1k), and proteasomes[74] that often span length scales of 15–25 nm, and for the reversible propagation of highly anisotropic cytoskeletal nanostructures, such as actin microfilaments and tubulin microtubules, that may extend to micrometers in length. There appears to be significant advantages associated with the self-assembly of complex structures using nanoscale objects as building blocks (nanotectonics) rather than by the spontaneous self-organization of molecules of small or intermediate size (supramolecular assembly). Both processes are reversible and involve collective intermolecular interactions, but it seems clear that embedded and integrated operations are more readily attained within miniaturized assemblages through nanotectonics because the intrinsic 3D nanostructure of their building blocks offers significantly greater functional capacity.

This is exemplified by the operation of the 20 nm-sized protein/RNA complex of the ribosome, which coordinates translation of mRNA codes to protein sequences by tRNA molecules (Figure 1h) that mediated anticodon recognition and linkage of activated amino acids at the aminoacyl (A) site. Orchestration of this complex process is fundamentally dependent on the modular assembly and organization of nanoscale subunits and associated proteins, as diverse functions must be coordinated in time and space. The prokaryotic ribosome has a molecular mass of over 2.5 million Dalton and consists of two rRNA subunits—the 30S subunit that mediates interactions between mRNA codons and tRNA anticodons, and a larger 50S subunit that catalyzes peptide bond formation and facilitates binding of G-protein initiation, elongation, and termination factors.[75] In addition, there are more than fifty associated proteins, approximately thirty of which are structurally ordered across the exterior of the larger 50S subunit of *Haloarcula marismortui*[71] (Figure 5a). Translocation of the chain-extended polypeptide from the A to peptidyl (P) site is fundamental to the operation of the ribosomal nanomachine, as it allows the polypeptide to grow in length rather than being released as a small molecule product. Thus, the sites must be spatially separated but interconnected, internalized within a nonpolar environment (to minimize peptide hydrolysis), and sensitive to conformational rearrangements and signals originating from regulatory proteins such as initiation and elongation factors. Moreover, rRNA-catalyzed peptide bond formation across the P/A sites occurs with extremely high fidelity to prevent sequence

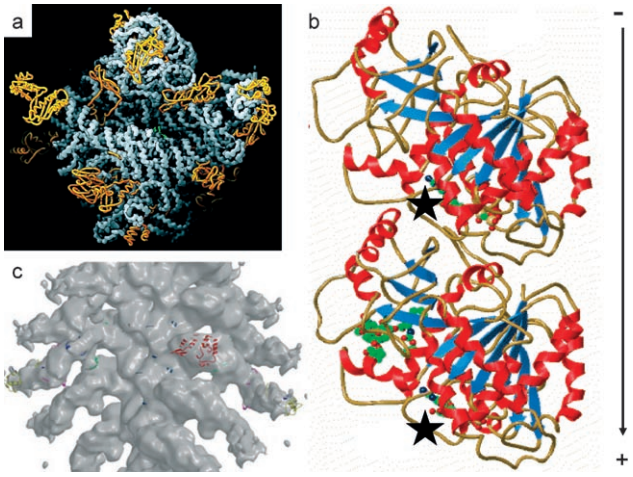

Figure 5. Modular assembly using globular nanoobjects. a) High-resolution structure of the 50S ribosome subunit of *H. marismortui*. The interface with the smaller 30S subunit is in the foreground. RNA (space-filled, gray) and ordered proteins (backbone, gold) are shown. The assembled nanostructure is about 25 nm across. From ref. [71]. b) Ribbon diagram showing orientation of α- (top) and β- (bottom) tubulin subunits in a microtubule protofilament (arrow indicates microtubule axis). The GTP/GDP binding sites are indicated (asterisk). From ref. [81]. c) Surface representation of actin filament in association with protruding myosin S1 cross-bridges (upper 50 kDa head domain). The closing of the actin-binding cleft is structurally coupled to the opening of the nucleotide-binding pocket. From ref. [82].

errors,[76] and this is accomplished by the relatively slow rate of GTPase-mediated release of a protein elongation factor (Tu) that is conjugated to the aminoacyl-tRNA present on the A site. This distorts the conjugate sufficiently to prevent peptide bond formation until the elongation factor is released.[77]

Modular assembly has the distinct advantage that the construction process can be highly directional, reversible, and regulated by conformational triggers. As a consequence, dynamic and adaptive miniaturized structures can be used for a wide range of cellular functions. Moreover, by employing nanoscale building blocks that are asymmetric, complex architectures with high anisotropy and structural polarity can be constructed. For example, F-actin filaments are 7 nm-wide (Figure 1e), and consist of a helical chain of globular subunits that are coaligned with respect to the orientation of their ATP binding sites.[78,79] Similarly, microtubules are relatively rigid, hollow 25 nm-diameter cylinders that are constructed from a helical array of alternating αβ-tubulin dimers[80,81] (Figure 5b). In both cases, reversible assembly is dependent on subunit activation/deactivation by ATP/ADP (actin) or GTP/GDP (tubulin), and the high degree of structural polarity gives rise to different rates of association/dissociation at opposite ends of the F-actin nanofilaments or at the microtubule growth tip. As a consequence, extended superstructures with highly organized architectures can be readily assembled from and dismantled into functional nanoscale units. Such structures are therefore transient and highly dissipative due to competing flows of assembly and disassembly.

The intrinsic structural polarity of F-actin and tubulin nanofilaments is utilized for directional force generation in biological motors. The transduction of chemical energy into directed mechanical motion under isothermal conditions is accomplished against the random thermal buffeting of Brownian motion by molecular motors operating through interacting nanoscale components. The absence of a local temperature gradient necessitates that the conversion of chemical energy associated with conformational rearrangements must be collectively harnessed and not lost by spontaneous transfer to the aqueous environment. This is achieved, for example, by coupling the random motion between the polar tack and motor head domain of an activator filament such as myosin (F-actin) or kinesin/dynein (microtubules) to produce stepwise net movements along a preferred direction.[82–84] In essence, this is a ratchet-like mechanism that provides both structural directionality (polarity) and stepwise (modular) increments for progressively linear (muscle contraction, vesicle transport), or rotary (F_0F_1-ATP synthase) motion. This is further regulated by auxiliary conformational triggers such as nerve-induced Ca^{2+} release and binding to troponin/tropomyosin proteins located on the actin nanofilaments.[85]

Directional movement along the polar track is critically dependent on the location and assembly of the functional subunits within these nanoscale machines (Figure 5c). For example, the binding sites for ATP hydrolysis and the track are both located in the globular catalytic head regions of the motor domains of mysosin and kinesin, such that these sites are coupled with asymmetric conformational rearrangements (hinged regions) that accompany the power stroke cycle during release of ADP and P_i.[86] Similar processes between dynein stalk regions and tubulin subunits of microtubules drive the force cycle in cilia.[87] In this case, however, dynein-induced sliding of adjacent microtubules around the axoneme is resisted by radial spokes and nexin linkages to produce synchronized bending rather than linear movement (kinesin) or contraction (mysosin).

A significant advantage associated with the operation of motors across nanometer length scales is that the directional movements are relatively fast and energy efficient. Biological motors move protein cargoes at typical speeds of 1 to 2 $\mu m\,s^{-1}$, which are significantly faster than passive diffusion of molecules through the cytoplasm.[88] In terms of energy conversion, the hydrolysis of a single ATP molecule is equivalent to an available energy budget of 100 pN nm (10^{-19} J), and this is converted by kinesin into a step movement of 8 nm against a load of 6 pN, which is equivalent to a conversion of around 50% efficiency.[88,89] Higher efficiencies of above 80% have been reported for the rotary motor F_1F_0-ATPase, which utilizes proton gradients to generate ATP.[90]

6. Nanoscience and Artificial Life

Given the critical dependence of life on nanoscale miniaturization, it seems reasonable to propose that the realization of artificial cellular systems will also be contingent on synthetic structures that operate on this length scale. Clearly, the complexities in this realm are overwhelming, but by delineating minimal cellular life as a constellation of chemical cognition and autopoiesis we can at least distinguish the basic archetypes necessary for the production of synthetic cells. Whilst the more realistic scenario at the present time is synthetic biology[91]—in which novel versions of life are made by established machinery—it is interesting to speculate on how minimal cells and their semi-artificial or synthetic counterparts could be realized through on-going advances, particularly in nanoscience.

The concept of a minimal genome size capable of sustaining self-maintenance, replication, and evolvability is now well established and thought to constitute around 200–300 genes in extant organsms,[92] although a lower number may have been sufficient for protocell viability at the origin of life.[93] From a bottom-up perspective, good progress has been made in reconstructing semi-artificial cells based on synthetic vesicles and liposomes in association with encapsulated enzymes and genes,[94] or integral membrane proteins, such as α-hemolysin, bacteriorhodopsdin and F_0F_1-ATPase, which facilitate ion transport and energy transduction.[95–97] In addition, PCR amplification of DNA,[98] ribosome synthesis of poly(Phe),[99] and DNA transcription[100] have been successfully achieved in vesicles by encapsulation of multiple components, although in each case polymerization was severely limited by rapid depletion of the encapsulated monomers. This problem can be partially circumvented by co-incorporation of two genes that translate for a protein marker (green fluorescent protein (GFP)) or a membrane

porin (α-hemolysin), such that protein synthesis is prolonged by influx of nutrients (ATP, amino acids) through the newly formed channels in the vesicle membrane.[101] However, accumulation of the porin will result ultimately in the collapse of membrane gradients unless the vesicles can self-replicate by growth and division. In this respect, self-replication of the vesicle shell can be achieved in association with RNA replication[102] or polynucleotide synthesis,[103] although the "core" and shell replications were not synchronous and the internal reactions were depleted within a few generations by dilution effects.

Clearly, the above studies are still far removed from the autopoietic coupling of metabolism and information flow that occurs within the cognitive boundary of extant cells capable of self-reproduction and evolution. The complexity of these model systems can be increased, for example, by expressing a gene for RNA polymerase in liposomes and the resulting enzyme used to promote mRNA for protein (GFP) synthesis.[104] Such a transcription activation cascade demonstrates that coupling of gene expression and protein synthesis in lipid vesicles is certainly feasible. However, a critical advance not yet met in synthetic protocell biology involves the coupling of carriers and catalysts of genetic information such that self-replication is maintained within the vesicles. In particular, encapsulation and integration of RNA molecules that function both as a template and enzyme (ribozyme) for self-replication would represent a major breakthrough and provide convincing evidence that the emergence of DNA and proteins was preceded by a RNA origin of life.[105]

The increasingly innovative pathways to synthetic biology and protocell modeling currently being developed are providing new groundbreaking opportunities, which will not only facilitate the elucidation of the origins of cellular life,[94] but also provide novel life and materials systems capable of transforming many aspects of medicine and technology.[106] The dependence on biological machinery is fundamental to these advances, and it seems almost unimaginable that a comparable system based solely on non-biologically derived components could ever be realized, even though such materials would dramatically increase the range of conditions under which artificial cells remain viable. As a first step, it seems feasible that artificial cells with systems interfaces based on non-lipid nanoscale boundaries will be developed in the future. For example, synthetic vesicles can be prepared in aqueous solutions from amphiphilic block copolymers,[107] or by layer-by-layer deposition of polyelectrolytes on sacrificial colloidal particles,[108] soluble proteins and membrane-bounded porins incorporated into polymersomes,[109,110] and polyelectrolyte shells used as artificial cells with high membrane permeability.[111] Significantly, fusion and division of the polymer vesicles can be achieved by swelling and fluidization associated with intercalation of certain cosolvents,[112] suggesting that polymersome self-replication might be possible if such compounds could be generated in situ within the vesicles.

The *de novo* construction of artificial living cells based on nonbiological components necessitates the development of complex processing systems comprising nanoscale components with collective and integrated properties. This is a challenge of enormous magnitude that is hardly conceivable at the current time, but one that could well be the long standing legacy of nanoscience as it develops through the twenty first century. There are promising indicators that the scope for nanoscale miniaturization of complex functional structures is certainly well within the reaches of synthetic chemistry. For example, nanopores and channels have been fabricated by using stacked arrays of cyclic peptides[113] or barrels of octiphenyl/peptide staves,[114] cytoskeletal-like filaments prepared from worm-like micelles of poly(ethylene oxide)-based diblock copolymers,[115] and linear or rotary molecular motors synthesized.[116,117] The latter can be activated photochemically[118,119] and in some cases perform work and move objects.[120–122] In a few cases the directionality in these systems can be increased against Brownian motion by immobilization on gold surfaces.[123] Moreover, studies on the catalytic activity of inorganic nanoparticles continue to reveal novel aspects of reactivity that could perhaps be integrated into cyclical pathways involving reactant/product feedback loops established by nanoparticles positioned in the interior and within the membrane of an artificial cell construct. For example, gold nanoparticles are highly effective catalysts for the relatively low-temperature oxidation of CO, H_2, alkenes, and alcohols in the presence of molecular oxygen,[124] suggesting that these nanoparticles might act as miniaturized catalysts in artificial cells. The formation of fatty acids from long-chain alcohols, for example, could provide a route to membrane growth and division, whilst simultaneously transforming glycerol to glycerate or an alkene to an epoxide to produce reaction intermediates for a primitive metabolic cycle. Functionalized gold nanoparticles have also been used in optical, redox, and analyte sensing,[125] gas monitoring,[126] template-directed nanocircuitry,[127] motor-driven transportation,[128] and nanoplasmonics,[18] and similar advances have been demonstrated for many other types of inorganic nanoparticles such as CdS, TiO_2, and CeO_2. Thus, it seems a realistic prospect that in the future the multifunctionality of inorganic nanoparticles in controlling transformations such as catalysis, photoactivation, energy capture, and transmission will be adapted and integrated for the advancement of synthetic protocells and sustainable systems with life-like properties.

7. Conclusions

In this Review we have considered the living cell as a self-regulated, self-maintained complex chemical system that operates principally through nanoscale miniaturization via a systems interface (cognition) and internal self-processing networks (autopoiesis). We postulated that the evolution of an integrated and functional cell membrane, as well as the emergence of metabolic processing networks based on globular macromolecules, were dependent on up-scaling of molecular interactions to length scales beyond 3 and 2.5 nm, respectively. These boundary conditions were imposed by structural and energy instabilities associated respectively with planar bilayers comprising short chain phospholipids, and polypeptide chains of insufficient length and amphiphilicity.

Factors such as membrane fluidity, bending rigidity, and conformational matching/mismatching between lipid chains and integral membrane proteins, appear to be optimized for a membrane thickness of around 5 to 6 nm, and as a consequence this length scale is coextensive with the emergence of molecules of nanometer size and the concomitant integration of globular objects such as pumps, light/energy converters, receptors, transmitters, and chemosensors into the lipid bilayer. Similarly, constraints on the folding of polypeptide secondary structures give rise to an optimum domain size for globular proteins of approximately 4.5 nm. This is commensurate with the emergence of complex and structurally persistent internalized architectures with delineated interior spaces and integrated conformational dynamics, which together are necessary for the up-scaling of small-molecule processing in activities such as enzyme catalysis, activated transport, signal transduction, and chemotaxis. Moreover, the use of globular nanoobjects in the modular assembly of ribosomes, cytoskeletal elements, and molecular motors, for example, is fundamental to the construction of higher-order assemblages with extended organizational complexity and multifunctionality.

Finally, we have highlighted the potential importance of nanoscale components for the development of artificial systems of cellular life. There are promising indicators that developments in synthetic and materials chemistry will be capable of producing a wide range of nonbiologically derived self-assembled nanostructures, such as polymer membranes and filaments, linear or rotary molecular motors, and membrane channels, as well as expanding libraries of nanoparticle-based catalytic reactions. These components offer promising prospects for the fabrication of miniaturized artificial systems with minimal life-like functions such as self-maintenance. It seems realistic to predict that such systems will be demonstrated as functioning ensembles in the near future. In contrast, the widespread difficulty in envisaging synthetic (non-DNA/RNA) processes of informational replication, transcription, and translation remains a fundamental obstacle to the realization of artificial cells that can be described as autopoietic and cognitive. In fact, it is debatable whether alternative self-replication systems of inorganic[129] or organic[130] origin can ever emulate the scope and fidelity of the DNA software and protein hardware of extant organisms. Indeed, the apparent singularity of life as a coextension of nucleotide-based logic raises profound scientific and philosophical questions that will remain open for many years to come.

Received: December 4, 2007
Published online: June 2, 2008

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