Biomimicry: The Bottom-Up Approach

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What You Will Learn in This Chapter

1. Model systems used for analysis via synthesis.
2. Principles of engineering biomolecules.
3. Understanding of biomolecules; alchemy to urea again.
4. Membrane mimetics.
5. Membrane components.
6. Understanding of the concept of synthesis from first principles.
7. Understanding of why this is done.
8. Understanding of the various ways this principle is applied.
9. Awareness of examples.
10. The student should be able to explain how the bottom-up approach is applied to synthetic biology.
11. He or she should also be able to explain the uses of this approach.
12. The student should be able to cite and illustrate this use, with examples.

In the search for the minimal cell, it is expected that an entire cell might be difficult to model using current technology. We explain how the bottom-up approach is used to model specific parts, or functions, of cells instead. Such model structures include the cell membrane, proteins, and even the genetic material. Each system is constructed using basic materials, which are rationally combined to form functional structures. Some of these systems might even use materials that are different from their counterparts in the biosphere. Finally, we introduce the idea of synthetic organisms comprising or using noncanonical materials as a safeguard against their accidental release into the environment.

4.1 Synthetic Bioarchitecture: The Bottom-Up Approach

The bottom-up approach thrives on the idea of self-assembly. In the light of self-organizing structures, the dimension of life leans on self-organization. To adapts this concept is only a natural consequence in research applications, dealing with the dimension of molecules—otherwise, it is quite tedious (if not impossible) to manufacture molecular assemblies with mechanical tools. In the 1980s, the famous physicist Richard Feynman stated, "What I cannot create, I do not understand." This is still valid for physics and for biology; this motto is still far in the future for all "living" species. Even Craig Venter, the godfather of "synthetic life," who has "implanted" a functional genome in a living species, has not "created" life from the bottom up. Creating life has always been a dream of mankind, and synthetic bioarchitecture is a realistic view of how far and possibly impossible this goal is, as life might not be encoded in a structure; there may be something more to it.

Let us give a real-world, state-of-the-art example of a bottom-up approach in synthetic biology.

The concept of DNA origami has been developed over the past decade. Of course, DNA does not consist of just four monomers; it also provides unusual charge density and, as a consequence, an inherent and precise spatial control.

- Can we build functional (molecular) Hybrid structures employing the blueprints of Nature?
- Does Nature (living cells), understand' such hybrid architectures?

Fig. 4.1 Gustave Doré - The Monkey and the Dolphin (1867), a scene from the book "Fontaine's Fables". This example is intended to illustrate the idea of functional assembly - quasi 'incompatible species', monkey and dolphin, merge into a functional unit

With this famous example, let us start in the world of bottom-up approaches with a concept aiming for employment of functional building blocks and achievement of self-sustaining (or even autopoietic) systems. In the end, it is a protocell, which has been sketched by many but is still unattained, as confinement of the building blocks of life in small spherical objects is still hampered by lack of control of energy influx/efflux and, as a consequence, it is still unable to sustain itself in laboratory conditions. Interestingly, the "end of the game" is the status of equilibrium. At this point, the far end goal is understanding "far from equilibrium reactions with the intention to build in such concepts in bottom-up approaches". If we understand how to combine macromolecules in functional assemblies, we might achieve "sustaining" objects with even unconventional abilities, as Gustave Doré depicted in his graphics of an "impossible" hybrid creature, such as the dolphin in Fig. 4.1, carrying a dog's head and being guided by a monkey. This image depicts the gap to be closed in order to preserve function, while being composed of impossible matches from the evolutionary point of view. How this might go, along with unintended consequences, is discussed in this book under safety issues related to the field of synthetic bioarchitectures (see ▶ Chap. 6).

In this book, we focus on membranous interfaces as a relevant and eligible example of a highly organized, self-assembled, functional structure from nature. We use the concept of bottom-up approaches, as these structures inherently consist of lipid molecules, organized as a two-dimensional crystal structure in a liquid ordered state. We can approach a self-organized spherical object with our available methods and tools for characterizing embedded proteins and even mimetics of simple biochemical feedback cycles (Fig. 4.2).

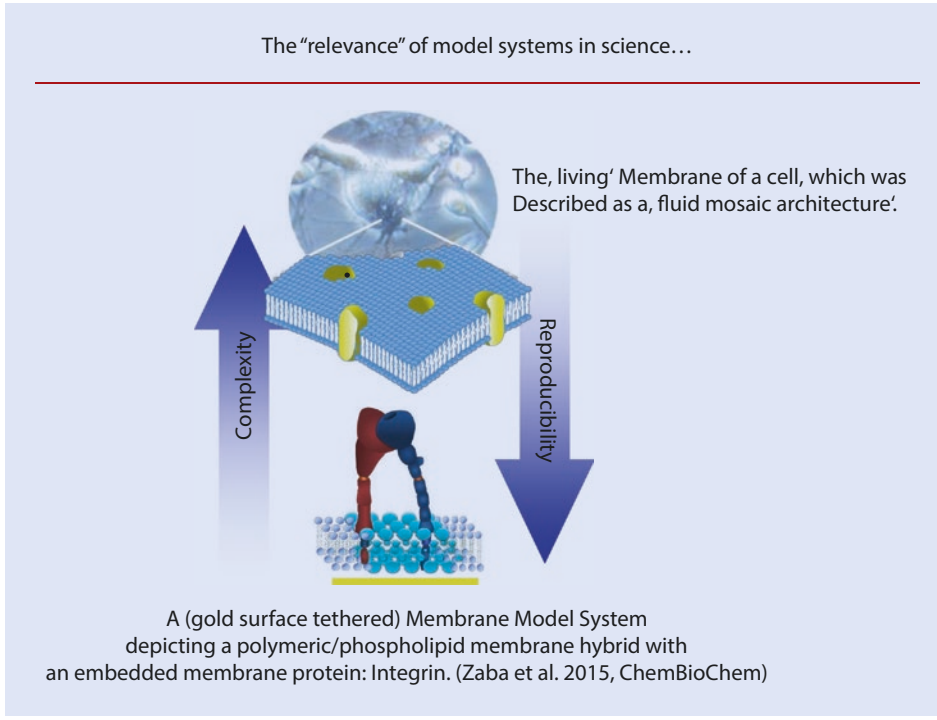


Fig. 4.2 We will always have to compromise with the "robustness, e.g., reproducibility" offered by a model system, reflecting only some properties of the naturally integrated system. Cells, as natural locations of interesting biomolecules, such as membrane proteins, are subtle units with complex, intercalating biochemical responses and the inherent feature of aging in the course of an experiment. On the other hand, we have to start somewhere with the attempt to "catch" a glimpse of biomaterials; as such, we "freeze" objects, such as membrane proteins, in a tethered, metal-anchored, planar lipid membrane architecture in order to understand, for example, ligand receptor interactions

4.2 The Minimal Cell Revisited

As explained in ▶ Chap. 2, synthetic biology employs two basic approaches in its work: the top-down approach and the bottom-up approach. Where these two techniques meet, at their extremes, is the minimal cell. The concept of the minimal cell posits that all cells are advanced variations of a much simpler cell.

An analogy would be a car that consists of just a frame, an engine, axles, and wheels. With this simple chassis, you can then modify it to make a luxury car, an ambulance, or even something novel. The idea of the minimal cell is similar. This method of stripping down to the minimum is the top-down approach.

Unlike the top-down approach, the bottom-up approach attempts to put together diverse building materials to construct complex structures. In the process of trying to recreate parts of (or whole) cells, we will hopefully learn how each part evolved and how each improvement solves a problem (Fig. 4.3). By observing what exists in nature, we hope to find solutions to engineering problems.

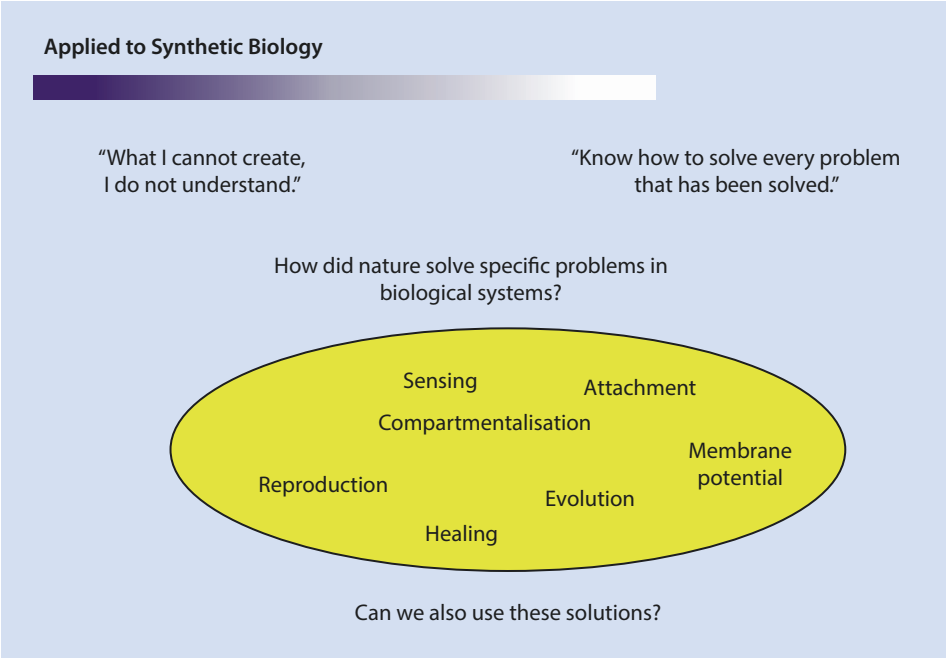


Fig. 4.3 Studying, reverse engineering and mimicking various structures and processes in living systems can teach us how to develop solutions to complex biological problems. The figure quotes from Richard P. Feynman

4.3 The Reductionist Approach

However, a cell is more than just a structure. We also have to recreate some of the processes that occur in it (Fig. 4.4). As such, attempting to construct whole cells from scratch is not trivial. A more feasible approach would be to start by making parts of a living cell, such as the cell membrane.

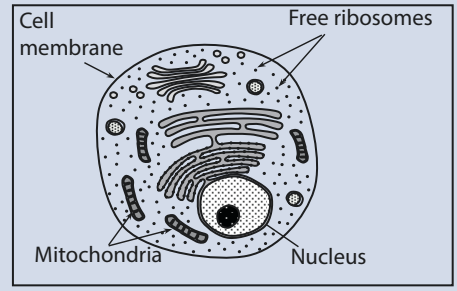
Cell membranes comprise (1) various lipids, which form the bilayer; (2) proteins, which are embedded in or attached to them; (3) carbohydrates, which modify both; and (4) cholesterol, which modulates the membrane fluidity (Fig. 4.5). Using combinations of lipids and cholesterol, one can produce artificial membranes of various forms.

However, such membranes would lack the critical membrane proteins. Various methods exist to synthesize proteins and incorporate them into artificial membranes. One powerful approach is to supplement cell-free protein synthesis reaction mixes with artificial membranes (Fig. 4.6). During protein production, membrane proteins would integrate into the artificial membranes.

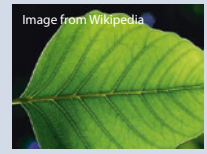
With artificial membrane vesicles, one could mimic the surface structure of bacteria. Most bacteria are coated with surface-layer (S-layer) proteins. They are found to form regular patterns on the surface membranes of both Gram-positive and Gram-negative bacteria, and serve various functions including surface attachment and virulence. One can mimic this surface by preparing pure S-layer proteins and, under the right conditions, allowing them to crystallize on the surface of artificial liposomes (Fig. 4.7).

Not quite the minimal cell……

Mimicking just the parts?
- Cell surface membrane
- Nucleus
- Cell wall
- etc



Mimicking just the processes?
- Information transfer
- Mass transfer
- Photosensing
- etc



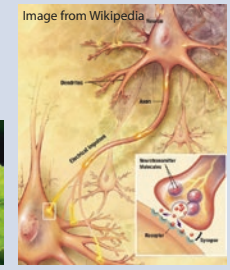
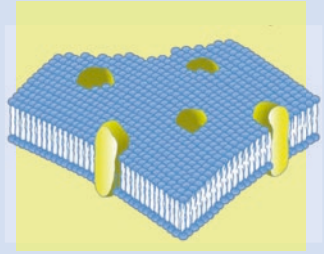


Fig. 4.4 Do we mimic biological structures or biological processes?

Mimicking structures

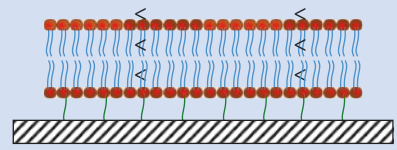
Cell membranes



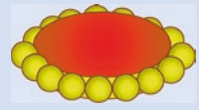
Lipids
- Phospholipids
- Sphingolipids
- Glycolipids

Cholesterol
Proteins
Carbohydrates

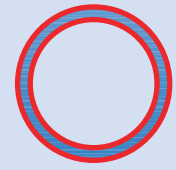
Artificial membranes



Tethered membranes



Nanodics



Liposomes

Fig. 4.5 Examples of artificial lipid membranes. Membranes can be constructed from some, or all, of the basic components found in natural cell membranes. Different membrane structures such as tethered planes and liposomes allow synthetic membranes to be used in diverse ways

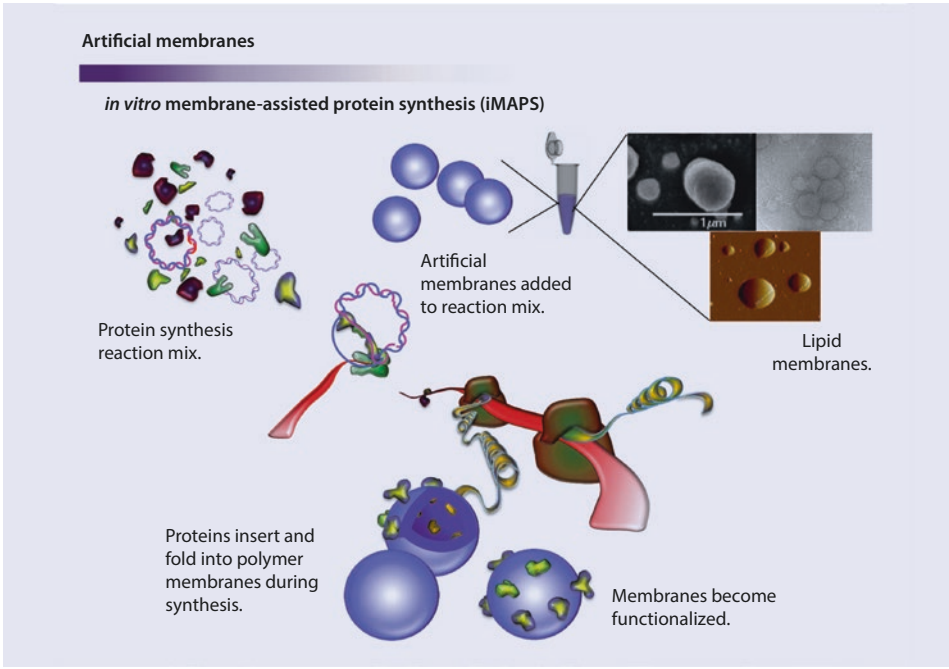


Fig. 4.6 The figure illustrates *in vitro* membrane-assisted protein synthesis. Artificial membranes are added to cell-free protein synthesis reaction mixes. As nascent proteins are produced, they are though to insert into, fold and orient themselves in, the membranes

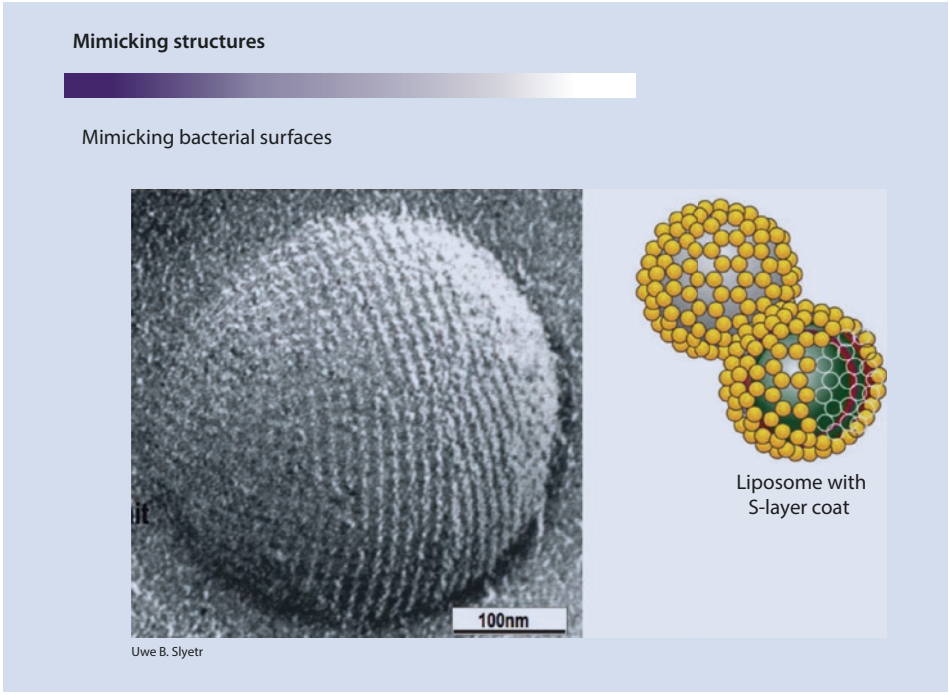


Fig. 4.7 An example of how vesicles might be modified with a coat of Surface-layer (S-layer) proteins, in order to mimic bacterial cell surfaces. (Sleytr)

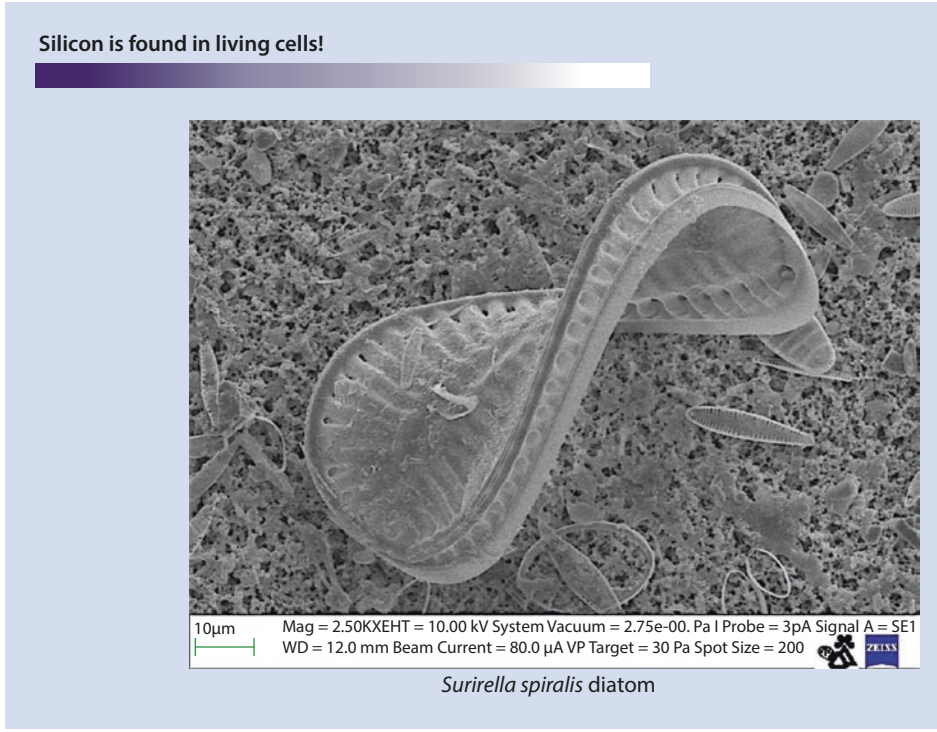


Fig. 4.8 An example of how, despite being associated with all things artificial in modern culture, silicon is often incorporated into the structure of living systems. (Angeli, 2016)

4.4 Considering Materials

In the attempt to mimic natural systems in terms of structure and function, researchers are free to use synthetic materials. This provides the advantage of a wider choice of materials, as well as versatility. As such, one can consider the use of atypical materials, such as silicon. Although it is associated with synthetic products, silicon can actually be found in living systems.

Silicon belongs to the same group of elements as carbon, and hence can form many molecules similar to carbon dioxide and methane. Like carbon, silicon is capable of concatenation. As such, some have posited that life based on silicon might also be possible. An early pioneer of this idea was the nineteenth-century German astrophysicist Julius Scheiner.

Many terrestrial lifeforms, such as diatoms, bacillariophytes, and sponges, use the silicon derivative silaffin as a building block (Fig. 4.8). In diatoms, they form exoskeletons with distinct shapes and properties. Instead of S-layer proteins, one can think of coating artificial liposomes with such material.

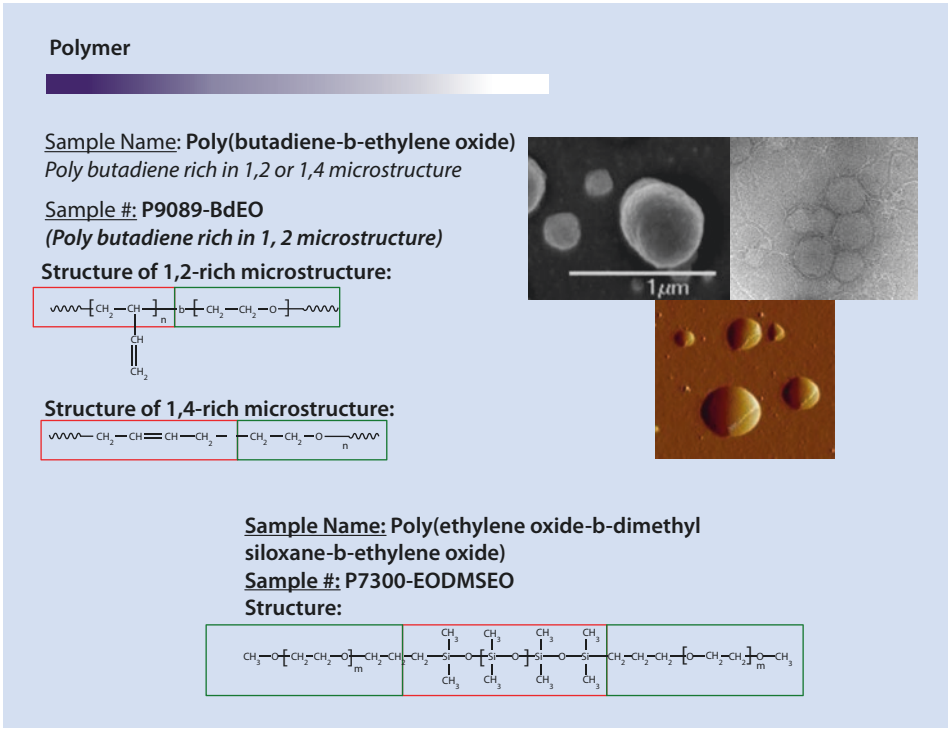


Fig. 4.9 Examples of amphiphilic polymers. Just like amphiphilic lipids, polymers with hydrophilic (*red box*) and hydrophobic (*green box*) domains may also self-assemble into membrane bilayers. Tri-block copolymers comprising hydrophilic domains flanking a hydrophobic domain may even form monolayers which would still yield the hydrophilic-hydrophobic-hydrophilic internal character typical of biological membranes

4.5 Unconventional Materials

One might even replace the lipids of liposomes with synthetic amphiphilic polymers, such as poly(butadiene)-poly(ethylene oxide) (Fig. 4.9). In aqueous environments, these are also able to self-assemble into membrane bilayers. Such membranes would be more stable against oxidation and mechanical force than liposomes.

Even fundamental cellular functions, such as gene expression, are amenable to mimicry using synthetic material. An example is xDNA, which is DNA comprising bases extended with an additional benzene ring, in addition to the natural four (Fig. 4.10). These extended bases are fluorescent and can base pair with normal nucleotides. Their use is expected to teach us more about the behavior of natural DNA.

Another example is the expanded genetic code. Here, stop or nonsense codons in bacteria are used to encode nonstandard amino acids (Fig. 4.11). To do this, a mutant

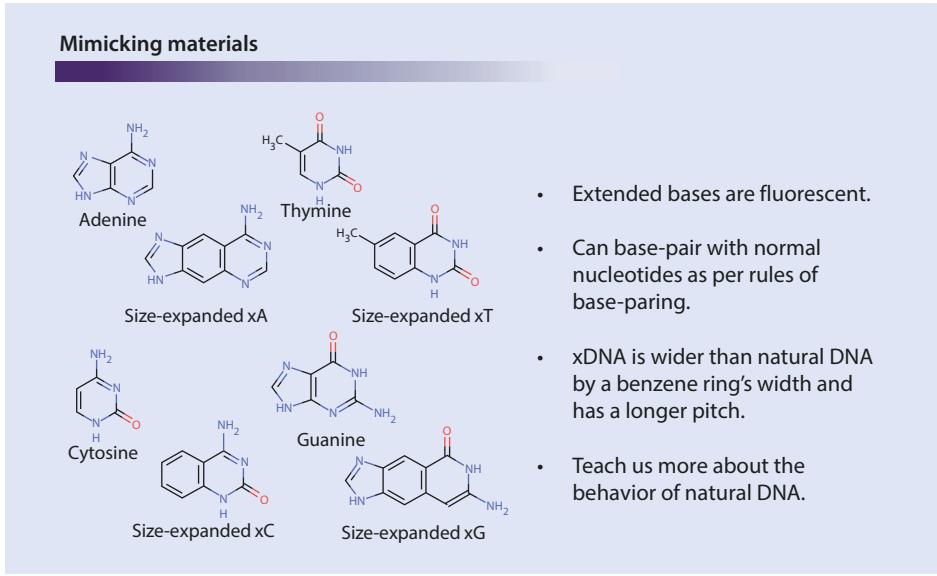


Fig. 4.10 Unlike their natural counterparts, size-expanded nucleic acids are fluorescent. Despite the addition of a benzene moiety, each is able to base-pair in the same way as their natural counterparts. (Lynch et al., 2006)

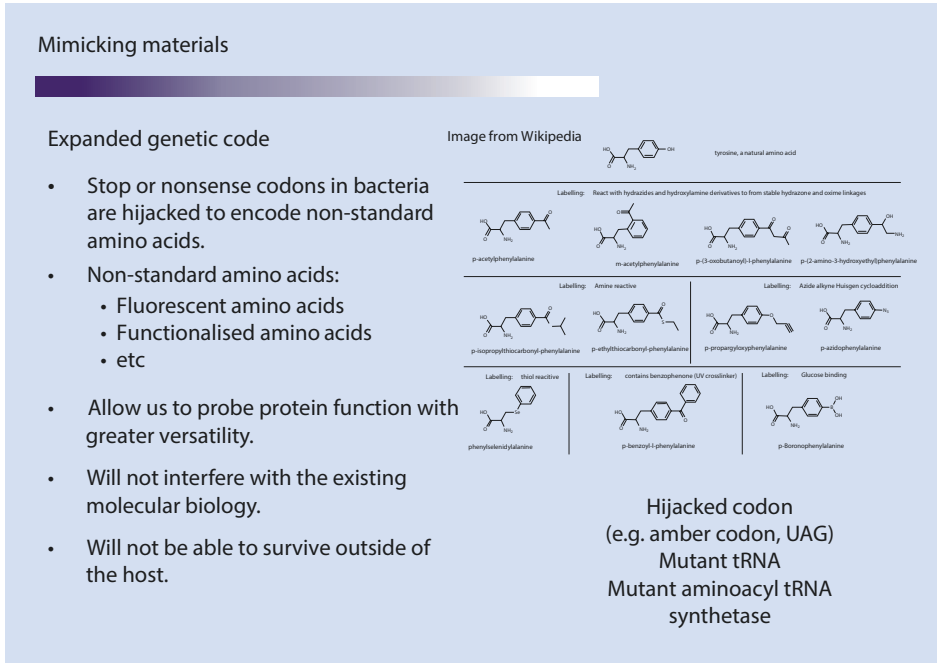


Fig. 4.11 Transfer RNA (tRNA) that complement stop or redundant codons can be harnessed to encode amino acids not among the 20 universal protein-forming ones. For this to work, a mutant amino acyl-tRNA synthetase must also be found which is capable of activating the chosen tRNA with the non-standard amino acid

transfer RNA (tRNA), which will only base pair with a stop or nonsense codon, is activated with a fluorescent or chemically reactive amino acid. Using them for gene expression will allow us to probe protein function with greater versatility.

Each of these endeavors will teach us basic principles of engineering, derived from observing how natural systems have evolved to adjust to obstacles. Eventually, the mimicry of biological systems might employ purely synthetic materials and perhaps give rise to novel biological functions. Finally, in the last chapter, we will see how this strategy might be exploited as a safety measure against the accidental release of synthetic organisms into the natural environment.

Take-Home Messages

1. The bottom-up approach attempts to use basic materials to construct complex structures mimicking either the structure or the function of living systems.
2. As whole cells are too complex to mimic entirely, some researchers attempt to mimic only specific structures, such as the plasma membranes of prokaryotic and eukaryotic cells.
3. These attempts might also include the use of unconventional materials for building such structures.
4. These materials include functioning substitutes for amino acids, nucleic acids, and phospholipids, among others.
5. Such synthetic characters might serve as a safeguard against the accidental release of synthetic organisms.

Further Reading

Krueger AT, Peterson LW, Chelliserry J, Kleinbaum DJ, Kool ET (2011) Encoding phenotype in bacteria with an alternative genetic set. J Am Chem Soc 133:18447–18451

Lynch SR, Liu H, Gao J, Kool ET (2006) Toward a designed, functioning genetic system with expanded-size base pairs: solution structure of the eight-base xDNA double helix. J Am Chem Soc 128:14704–14711

Nallani M, Andreasson-Ochsner M, Tan CW, Sinner EK, Wisantoso Y, Geifman-Shochat S, Hunziker W (2011) Proteopolymersomes: in vitro production of a membrane protein in polymersome membranes. Biointerphases 6:153–157

Pamirsky IE, Golokhvast KS (2013) Silaffins of diatoms: from applied biotechnology to biomedicine. Mar Drugs 11:3155–3167

Sumper MK, N. (2004) Silica formation in diatoms: the function of long-chain polyamines and silaffins. J Mat Chem 14:2059–2065

Tan DC, Wijaya IP, Andreasson-Ochsner M, Vasina EN, Nallani M, Hunziker W, Sinner EK (2012) A novel microfluidics-based method for probing weak protein-protein interactions. Lab Chip 12:2726–2735

Xie J, Schultz PG (2005) Adding amino acids to the genetic repertoire. Curr Opin Chem Biol 9:548–554