

that we see every day during a morning or evening walk, the chemical contents present on the green leaves – Chlorophyll. Again, with red patches being dominant on Mars, the chlorophyll story had an add-on; red vegetation may be dominating or may represent lichens or algae. Whatever it may be, there is life on Mars; such a statement remained in the headlines, irrespective of how small the finding may be. Without doubt, the press releases were influencing scientific conclusion. Later, however, Earth-based observations at a different wavelength revealed that ‘it’s all wind-blown sand’. Quoting from the book, *No more trees; no more moss; no more lichens; no more algae. Just windblown sand.*

As the era of modern exploration on the surface of Mars from its orbit began, the stage was set for the next set of results. This time the news that triggered the debate was methane, another biomarker. By mid-20th century it became clear that macro-Martians could not be seen but the idea of micro-Martians, however, remained in the debate. The thought for plausible presence of micro-Martians was strengthened when bio-signature gases were released, while water and labelled chemicals carried from Earth were made to interact with the Martian soil. Abiotic and biotic processes can synthesize the same product(s) through different pathways. Therefore, extra care must be taken in the scientific analysis. However, the important moment in announcing the definitive confirmation that ‘there is life on Mars’ masked the unbiased analysis by many scientists. This was reflected when the Martian meteorite was shown to contain a structure, few tens of nanometres in size, that was straightaway considered to be fossil remain of a once-formed microbe on the Martian surface without any strong support that can be tested over time. Quoting from the book, *We do not yet have the extraordinary evidence... and The search goes on.*

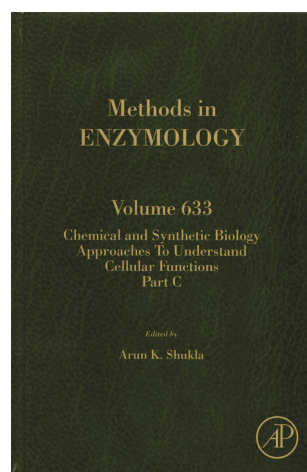
Methane on Mars was not easily forgotten as it reappeared over and over again. The orbiter and measurements using the sophisticated lander and rover, played their role in keeping the methane story alive, despite the very low abundances of the gas. This was in a way convincing because we were examining the biomarker on the Martian surface or atmosphere avoiding terrestrial molecules; so whatever was measured was devoid of any terrestrial contamination. The real surprise came when it was confirmed that gases from the Earth were carried along with the rover. While

eliminating the contamination was one part of the story – the sudden presence and absence of methane, based on sensitive and reliable measurements, is still unclear. What pumps it to the Martian atmosphere and where does it get lost? Only experiments, either *in situ* or laboratory-based can help answer this. Nevertheless, it must be a thorough and scientifically rigorous work rather than doing the same mistakes in understanding Mars over the centuries. Quoting from the book, *On the critical question of whether life exists on Mars, the jury is still out.*

One aspect is clear; scientists at any moment must stick to their science rather than rushing to make catchy headlines. This is most important while exploring unknowns. Quoting from the book, *Scientists don’t always discover what they are looking for when they design their experiments, but once an experiment is underway, they almost always discover things worth knowing.* This book is an interesting account of how our understanding about Mars constantly changed and advanced with better and new observations.

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Methods in Enzymology: Chemical and Synthetic Biology Approaches to Understand Cellular Functions – Part C, Vol. 633. Arun K. Shukla (ed.). Academic Press, an imprint of Elsevier, 50, Hampshire Street, 5th Floor, Cambridge MA 02139, USA. 2020. 308 pages. Price: US\$ 199.00.

The *Methods in Enzymology* series has served the signal function of presenting detailed protocols for a range of biochemical experiments and assays – not limited to the study of enzymes. This book is one among three that describe methods and tools of chemical and synthetic biology for the study of structure, function and dynamics of proteins and physiological processes. In a departure from the early norms, topical reviews of areas which have seen multiple approaches develop in the recent past are included. There are 15 chapters, all useful and informative, a few of them are discussed in this review.

Biotinylated molecules are used extensively in bioanalysis. (Strep)avidins bind biotin with high affinity and have been used to detect, locate and quantify biotinylated molecules. However, the assays show variability for different derivatives, and also poor sensitivity and accuracy. The competitive binding assay described enhances the robustness of the assay as well as its sensitivity. Hytonen and his group have developed avidin variants with differing ligand specificity. The newly expanded range of ligands includes some that are currently detected using either more complex methods or more expensive reagents. As a case in point, a variant binding progesterone is shown to be a promising alternative to antibody-based detection and quantitation of the steroid hormone.

Much as (strep)avidins have been the biotechnologists’ tool for detecting biotinylated molecules, siderophores have been utilized by bacteria for complexing and accumulating Fe^{3+} , which is poorly soluble. They also serve as virulence factors. Rutscher and Bottcher present protocols for enzymatic engineering of siderophores with a range of ring sizes which can be tuned to inhibit the swarming of pathogenic bacteria, such as *Vibrio alginolyticus* by blocking their access to Fe^{3+} . Another approach to tackling pathogenic bacteria is to inhibit the enzymes required for the synthesis of metabolites used for quorum sensing, virulence and interspecies competition. Bottcher and Prothwa describe a competitive inhibitor profiling strategy to screen for inhibitors of an enzyme central to the synthesis of such metabolites in *Pseudomonas aeruginosa*.

A particularly intriguing chapter by Tzakos and his group is the NMR tube bioreactor. Saturation transfer difference NMR is used to identify potential substrates of an enzyme under conditions where the substrates can bind, but the reaction

cannot proceed. The technique not only identifies potential substrates, but also pinpoints portions of the substrate that interact with the enzyme. Once the reaction is initiated, products can be monitored in real time without fractionation. In this stage, the reaction conditions can be played with. Moreover, by adding several putative substrates, one can monitor the course(s) of all the reactions that proceed in real time. Finally, both starting materials and products can be screened for binding to protein targets.

The Crispr/Cas9 system is extensively used for genome editing. Utility of the technique would be enhanced if it could be regulated temporally and spatially. A number of groups are developing methods to induce Cas9 *in vivo* for genome editing on command. One such approach is presented by Fowler and his group. They have engineered a single-component Cas9, which is normally non-functional as it is auto-inhibited. However, it can be chemically induced by a small molecule activator A115, which is cell permeant. A115 rapidly activates the auto-inhibited Cas9 in cells at concentrations below 10 μM .

An emerging area is that of synthetic receptors. Manipulating receptors can provide insights into signalling processes within a cell. Moreover, they can be harnessed to rewire and study a range of cellular functions, and also to engineer sensors. Chang and Bonnet have reviewed the field and highlight three strategies.

- (i) Modifying the ligand-binding domain of an extant receptor to recognize a small molecule of interest. Here the starting receptor is functional, but it is not always clear whether the modified protein will still couple ligand binding to signal transduction.
- (ii) Mixing and matching binding and signalling domains to generate cellular outputs to a range of environmental or physiological ligands. Here both modules are taken from extant systems. An example presented is a modular GPCR system.
- (iii) Using synthetic sensing domains such as single-domain camelid antibodies and coupling them to extant signalling domains. One example discus-

sed is coupling to Notch signalling, where binding of the ligand results in cleavage of an intracellular actuator domain. Given the success reported for developing avidin variants with a wide range of ligand specificities, one may find them being introduced here as well. However, a platform for truly modular sensing and transduction is yet to be optimized.

In summary, this book delivers on its promise to both introduce the reader to a range of techniques in chemical and synthetic biology to study cellular processes, and also present detailed protocols in each case. It is a volume well worth browsing through for the casual reader and one that will be well thumbed in the laboratory of a practitioner.

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