

a peculiar architecture

Vivienne Baillie Gerritsen

The space humans evolve in is divided into parts. It makes life easier. Each part is dedicated to a certain activity. We have homes to live in, pools to swim in, restaurants to socialise in, trains to travel on, roads to drive along and offices to work in. Cells, too, are divided into parts, and these are known as organelles or compartments. Mitochondria and chloroplasts produce energy, the nucleus transcribes DNA, the endoplasmic reticulum is the seat of protein trafficking, and vacuoles are destined for breaking down cellular waste. Like all cells, bacteria also have their compartments. Carboxysomes are a particularly intriguing example. Why? Because not only is their architecture reminiscent of crystals – sporting layers, straight lines, tips and angles – but their shells are built with proteins. One of the major shell proteins, dubbed CcmK2, assembles into cyclic hexamers which link to one another to form a twenty-faceted polyhedron.



Circle Limit III by M.S. Escher, 1959

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Carboxysomes are just one example of bacterial compartments whose shell is made out of protein – as opposed to lipid bilayers in organelles like mitochondria for example. The point of microcompartments is to isolate what is needed to perform certain metabolic pathways. Carboxysomes are where inorganic carbon – CO₂ – is fixed to ultimately produce sugar, and they were first observed in the cyanobacterium *Phormidium uncinatum* in the late 1950s. It took a further twenty years, however, to purify them and understand them in more molecular detail. Inside, as may have been

expected, researchers discovered the presence of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), the enzyme that fixes CO₂ to produce an intermediate molecule (3-phosphoglyceric acid, or 3-PGA) which is subsequently converted to energy (ATP).

Why sequester this particular step of energy production within a microcompartment? Some suggest carboxysomes may have appeared as the level of O₂ rose in the earth's atmosphere. It is a fact that RuBisCO quite happily fixes O₂ – an unfortunate circumstance since this actually costs the bacterium energy by way of a pathway known as photorespiration. If CO₂ and RuBisCO are sequestered somewhere together, then RuBisCO can concentrate on CO₂ and thus provide energy (in the intermediate form of 3-PGA) instead of consuming it. This may well be what actually occurred because most – if not all – of the cyanobacterial RuBisCOs are found in carboxysomes, and very few in the cytosol.

The overall architecture of carboxysomes seems almost out of place in the world of cells. When imagining things biological, the first thought that springs to mind is flow, smoothness and supple contours. The last structure you would think of is a polyhedron, i.e. a three-dimensional form made out of polygonal faces, straight edges and sharp corners. Carboxysomes are in fact icosahedrons, that is to say polyhedrons with no less than twenty faces. When compared to a cell membrane, the shell itself is relatively thin and the inside is packed with

concentric layers of RuBisCO. In between are found other proteins; the most important being carbonic anhydrase that processes bicarbonate to produce CO₂ – RuBisCO's substrate.

As an illustration, the main building block of the carboxysome shell in *Synechococcus elongatus* strain PCC 7942 is the structural protein CcmK2. CcmK2 is 102 amino acids long, adopts an alpha/beta fold and assembles into hexamers where one side is concave, and the other convex. The hexamers then bind to one another along their sides, gradually crafting each of the icosahedron's twenty faces. The tips of each face, or triangle, join to form what is known as an icosahedral vertex, and are usually capped by a pentameric shell protein. All in all, about 4,800 proteins are needed to build the carboxysome shell which is roughly about 10,000 times smaller than a pinhead. Besides being involved in constructing the shell, CcmK2 may also play a part in carboxysome intracellular location and distribution during cell division.

On the whole, a tightly-arranged enzymatic core of RuBisCO and, to a lesser degree, carbonic anhydrase forms the inside of carboxysomes. Taken singly, Rubisco happens to be one of the most slothful enzymes in town. However, if thousands of RuBisCOs are gathered in one small space to do exactly the same job – as in an assembly line – then the rate of total production will be heightened. This is exactly what occurs. Furthermore, not only does carbonic anhydrase provide RuBisCO with CO₂ but it also happens to be one of the fastest enzymes! Carboxysomes are therefore like high-speed production lines, able to produce vast amounts of energy not only in record time but in environments that are poor in CO₂. The fact that RuBisCO is encased in carboxysomes also prevents it from fixing O₂, and thus wasting cell energy.

Besides producing energy, molecules of various nature and size have to be able to travel in and out of carboxysomes. Their shells are thought to be sufficiently porous to let small molecules diffuse. But how do larger metabolites such as bicarbonate and 3-PGA enter and exit the shell, respectively? Undoubtedly through pores. Each hexamer forms a pore at its centre. In *Halothiobacillus neapolitanus* for instance, studies have shown that dimers of trimers may further stack at this point, creating central chambers which could let larger metabolites – namely bicarbonate – through, via an 'airlock' type mechanism. The pentamers that cap the icosahedral vertices also form pores. Pores, too, are located at regular intervals along the edges of the icosahedral faces. Could it be, then, that the sheer variety of carboxysome proteins are able to tune shell permeability?

The role of carboxysomes was understood quite early on, but no one realised that an astounding 10-25% of CO₂ is fixed globally by them! This is why scientists are keen to find out whether carboxysomes can be genetically engineered to fix CO₂ within various biotechnological applications. This could prove to be helpful, for example, in the fight against the surplus of CO₂ that human industry continues to fling into the atmosphere. Carboxysomes could also be introduced into plant chloroplasts as part of a CO₂ concentrating mechanism which could help to improve crop yield. Guttated carboxysomes could perhaps be designed, too, to ship cargo other than RuBisCO and co. Carboxysomes may, one day, prove to be a genetic engineer's dream. However, a lot remains to be understood. Not the least the fact that, much in the way choreographers direct their dancers, to build a carboxysome you need to know how to orchestrate the ratio and flow of a large number of self-assembling proteins.

Cross-references to UniProt

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