proteinspotlight
> ONE MONTH, ONE PROTEIN <

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yellow

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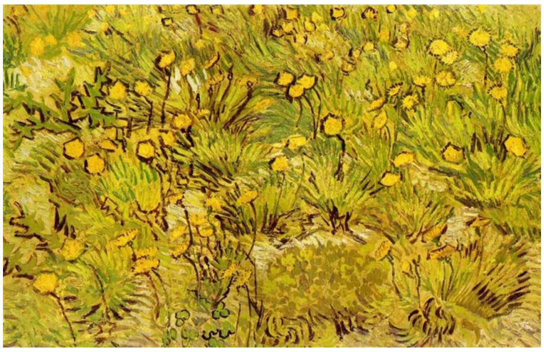
Chimpanzees use twigs to catch ants. Crows use roads to crack nuts. Humans too have always been good at diverting things for their own benefit – far more than any other species for that matter. We use water to make electricity, cows to provide us with milk and atoms to create heat. With the arrival of biotechnology, the habit has continued. A variety of molecules are now more known for what we do with them than for their original purpose. Green fluorescent protein (GFP) comes to mind – a protein that creates light in jellyfish and, for years now, has been used in research and medicine to label and track molecules and cells. Another is glucose oxidase, or GOx. This enzyme feeds on glucose and oxygen producing hydrogen peroxide in its wake. Since the 1950s, the enzymatic link between these three molecules has provided scientists with a limitless source of inspiration. GOx is currently used to preserve all sorts of consumable items while monitoring their sweetness and warding off microbes. It is also used in medicine to regulate glucose levels in fluids as it is used in the textile industry for bleaching and even in engineering to improve the viscosity of cements. A sort of success story for an enzyme that was discovered exactly 100 years ago.



A Field of Yellow Flowers

Vincent van Gogh (1853-1890)

In 1925, a young Danish botanist, Detlev Müller, was experimenting with the fungus *Aspergillus niger* when he noticed that bacteria were unable to flourish in its presence. He realised that when glucose was extracted from the preparation, the bacteria grew. This implied that *A.niger* probably uses glucose as a means to keep bacteria at bay. The botanist then noticed that when glucose is added to his preparation, oxygen is readily consumed. These observations finally led to his discovery of glucose oxidase, or GOx. In the presence of glucose, oxygen and water, GOx produces gluconic acid and hydrogen peroxide: glucose + O_2 + H_2O → gluconic acid + H_2O_2. It is the hydrogen peroxide that kills off bacteria.

So Detlev Müller was the first to discover glucose oxidase. He was also the first to discover flavoproteins though, surprisingly, he seems to have been unaware of this at the time. Flavoproteins are characteristically yellow. This is because they carry cofactors known as flavins which, when oxidised, become yellow – hence their name, from the Latin *flavus*. GOx is a flavoprotein but Müller never mentions the colour yellow in his writings. Consequently, for many years, the German physiologist Otto Warburg was recognised as the first scientist to have observed a flavoprotein in the 1930s – in his case a respiratory enzyme, glucose 6-phosphate dehydrogenase, in baker's yeast *Saccharomyces cerevisiae*. Researchers today wonder why Müller omitted to consider the importance of the yellow pigment. Some argue that, as a botanist, he was used to pigments and will have thought that the colour of his precipitate was not significant within the framework of his study. He was interested in how *A.niger* dealt with bacteria. What he failed to see was that the yellow pigment held what was at the heart of the enzymatic reaction.

Despite this, the flavoenzyme Müller unveiled became one of the first enzymes to be used in the field of biotechnology – and the most extensively. Today, GOx is added to items as diverse as toothpaste and bread dough. It is used to preserve foods and beverages as well as to enhance their colour and keep their flavour. It is used to improve the firmness of breadcrumbs and lower the content of alcohol in wines, as an anti-microbial agent in oral hygiene, as bleach in the textile industry and to add resistance to cement. In the medical field, GOx is used to fight off the growth of cancer cells and monitor the levels of sugar in diabetes. Like squeezing the last drops of juice from a lemon, we seem to be extruding from GOx everything we can.

It is not difficult to understand why. Each molecule GOx is intimately involved with – glucose, oxygen, water and hydrogen peroxide – is hugely popular in the framework of our planet's chemistry because they are crucial to myriads of biochemical and physicochemical processes. Pop GOx into wine and it will sup up glucose and regulate alcohol content. Add it to foods and it will rid them of the oxygen that makes them turn brown. Introduce it to textiles and the hydrogen peroxide will act as bleach. Insert it into toothpaste and the same peroxide will kill off oral bacteria. Present it to tumours and it will deprive them of energy (glucose) to grow. Couple it with biosensors and it will measure glucose levels in blood. The list seems endless.

This is why GOx is known almost inside out today. Extracellular, it belongs to the glucose-methanol-choline oxidoreductase superfamily, where each member depends on the same flavin cofactor: flavin adenine dinucleotide, or FAD. FAD is mainly bound to residues located at the N-terminal of GOx and is at the heart of the enzyme's redox reactions. GOx functions as a dimer. The FAD groups of each monomer meet without binding to one another and nestle down in the apex of a cone-shaped active site that forms at the dimer interface. It seems, too, that a water molecule lodges at the centre of the active site where it forms hydrogen bonds – perhaps conferring a certain stability to the site's overall structure. When glucose is present, it slips down into the cone-shaped active site, possibly displacing the molecule of water as it does. In the cone's apex, thanks to the presence of O_2 and to FAD, the glucose substrate is oxidised to produce gluconic acid and hydrogen peroxide.

Naturally, it was not for the benefit of humans and their activities that GOx first came to be. To date, this particular flavoenzyme has only been found in fungi and a few insects. As Müller first described, it is used to fend off bacteria by producing hydrogen peroxide (H_2O_2). Bees actually inject GOx into honey no doubt to check bacteria growth as the nectar ripens. This is why honey is sometimes used to treat chronic wounds and burns. GOx has a fast turnover and high stability which is why it has proved to be so useful in industry and medicine. Though the enzyme has been stripped down to its very bones and thoroughly examined from every angle, no one yet has been able to define the structure of the dimer with its glucose substrate. Understanding how things happen at the active site will undoubtedly widen up a horizon that is already astoundingly vast. Little did Detlev Müller know how far his initial observations would stretch. Nor did he seem to have realised that he was the first to discover flavoproteins. Such is the unexpectedness of research.

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Glucose oxidase, *Aspergillus niger :* P13006

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