Site-specifically wired engineered glucose dehydrogenase

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Technology

Prof. Alfonta developed improved glucose sensing enzyme wired to an electrode allowing more accurate and sensitive glucose measurements. A designed fusion enzyme in a combination with a biocatalytic function from a redox enzyme domain fused to a natural minimal electron transferring domain. The catalytic domain used is the α sub-unit of a Flavin adenine dinucleotide dependent glucose dehydrogenase (FAD-GDH) from *Burkholderia Cepacia*. It is a thermostable enzyme and known as non-sensitive to oxygen. The minimal electron transferring unit is the c-type cytochrome domain MCR-2, from a protein called MamP which originates from magneto-tactic bacteria. MCR-2 is one of the shortest natural c-type cytochromes known today (23 amino-acids) and thus can be used to achieve DET. Using genetic code expansion, non-canonical amino acids (ncAAs) was introduced into FGM. The recombinant protein was then coupled to an electrode by absorption or by site-specific wiring though the ncAA. Several sites specifically wired mutant enzymes were compared to each other and to a non-specifically wired enzyme, and the surface and activity analyses suggest that the site-specific wiring through different sites maintains the correct folding of the enzyme and have a positive effect on the apparent electrochemical electron transfer rate constant. The glucose sensing achieved was more accurate for glucose with high sensitivity at the tens of micro-molars regime which makes it suitable for wearable biosensing devices. Moreover, higher selectivity was observed towards glucose with low/no reaction to other sugars and commonly used medications that can be influence the measurement.

Application

The current enzymes and wiring to the electrode can replace the ones being used in invasive, minimally invasive, and non-invasive glucose sensors, including continuous glucose (CGM) sensors to gain more accurate and sensitive measurements.

Advantages

- High specificity, selectivity, and sensitivity.
- The use of unnatural amino acids (UAAs) with a designated residue that allows biorthogonal chemical modification resulting in a precise and controlled site-specific wiring of the enzyme to the electrode.
- The enzyme can be used to measure glucose from different body fluids such as blood, sweat, tears and urine.
- Can easily replace the current enzymes being used and integrated into existing monitoring devices.
- Resolves the problem of inaccurate Glucose measurement caused by different medications such as Paracetamol (acetaminophen), dopamine, ascorbic acid (vitamin C), and mannitol.

Patents

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