A Cellfree S-layer Based Bisphenol A Biosensor

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Abstract

The development of sensitive and selective biosensors is an important topic in synthetic biology. Biosensors can be applied in a wide range - from the detection of environmental toxics up to clinical diagnostics. Because cells have to sense their surroundings, there are a lot of natural systems that are similar to a biosensor. Prejudicial cellular biosensors often show negative side effects that complicate any practical application. Common problems are the limited use outside a gene laboratory due to the use of genetically engineered cells, the low durability because of the usage of living cells and the appearance of undesired signals induced by endogenous metabolic pathways.

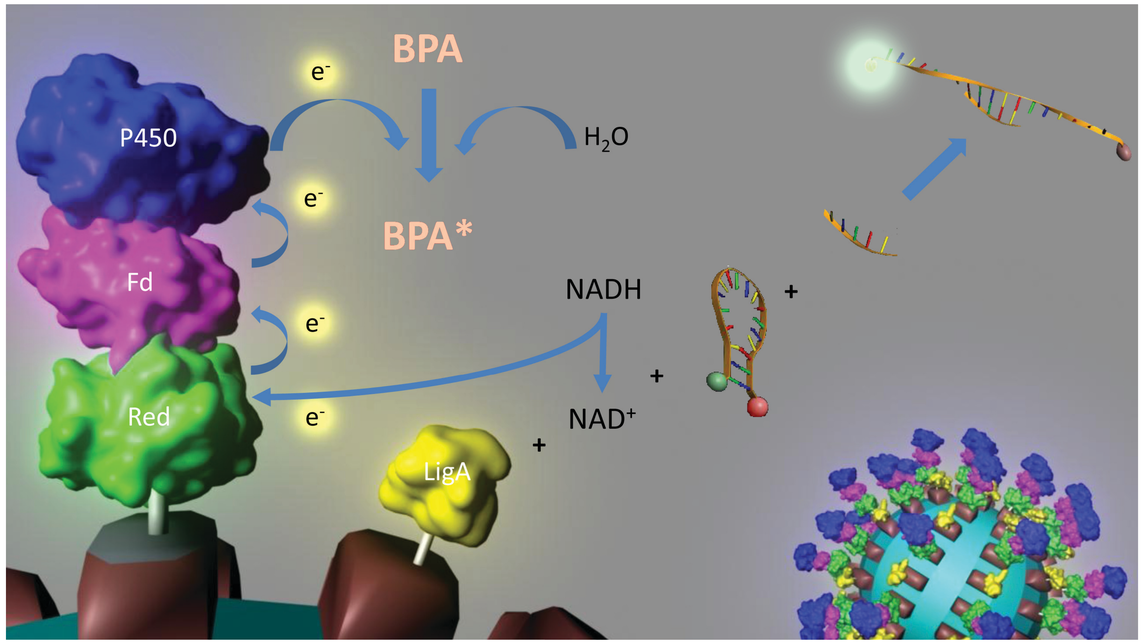
To solve these problems, the iGEM-Team Bielefeld 2011 aims at developing a cell-free Bisphenol A (BPA) biosensor based on a coupled enzyme reaction fused to S-layer proteins for everyday use. Bisphenol A is a supposedly harmful substance which is used in the production of polycarbonate. To detect BPA it is degraded by a fusion protein under formation of NAD^+ which is detected by an NAD^+ dependent enzymatic reaction with a molecular beacon. Both enzymes are fused to S-layer proteins which build up well-defined nanosurfaces and are attached to the surface of beads. By providing these nanobiotechnological building blocks the system is expandable to other applications.

Bisphenol A degradation

In 2005, Sasaki *et al.* isolated a soil bacterium from the *Sphingomonas* genus which is able to degrade the environmental poison Bisphenol A (BPA) with a unique rate and efficiency compared to other BPA degrading organisms. This strain was called *Sphingomonas bisphenolicum* AO1 and is able to completely decompose 120 mg BPA L^{-1} in about 6 hours. Three genes which are responsible for the first step of this effective BPA degradation by *S. bisphenolicum* AO1 were identified: a cytochrom P450 (*bisdB*), a ferredoxin (*bisdA*) and a ferredoxin-NAD^+ oxidoreductase (*Red*).

The *bisdAB* genes from *S. bisphenolicum* AO1 were isolated, transformed into and expressed in *E. coli* and enabled this bacterium to degrade BPA, too. In addition, the BisdAB proteins from *S. bisphenolicum* AO1 were able to degrade BPA in a cell free system in which spinach reductase was added. So we assume that the BisdAB proteins also work in a cell free system together with the ferredoxin-$NAD(P)^+$ oxidoreductase from *E. coli*. The suggested reaction mechanism of the first BPA degradation step is shown in the central image.

In 2008, the iGEM team from the University of Alberta submitted the codon usage optimized *bisdAB* genes from *S. bisphenolicum* AO1 to the registry of standard biological parts in the so called Freiburg BioBrick assembly standard. Via this assembly standard it is very easy to build fusion proteins. These already existing protein domains will be fused together with the $NAD(P)^+$ oxidoreductase gene from *E. coli* to the fusion protein Red:Fd_{bisd}:$P450_{bisd}$ which subsequently will be fused to an S-layer gene. We have already shown that the Fd_{bisd}:$P450_{bisd}$ fusion protein is degrading BPA more effective in *E. coli* than the polycistronic *bisdAB* gene (data not shown).



NAD^+ detection

Our selected NAD^+ detection method displays a molecular beacon based approach. These have been initially described in 1996 as nucleic acid probes that fluoresce upon hybridization. For this effect the ends of a single-stranded DNA molecule are labeled with a fluorophore as well as with an appropriate quencher. Both are in close proximity to each other due to a formed stem-loop, so that the detection of any fluorescence signal is prevented.

The molecular beacon's closed state can be applied to a bioassay detecting NAD^+ even in very low concentrations (LOD 0,3 nM). Using two complementary targets hybridizing side-by-side with the hairpin enables NAD^+-dependent DNA ligation by *E. coli* DNA ligase. Only after closing the gap between both hybridized targets the stem melts and the secondary structure gets broken down to a linearized probe-target hybrid. The immediate consequence is a disruption of the close proximity of the fluorophore and the quencher, so that an excitation with light is converted into a visible fluorescence signal. Hence, NAD^+ concentration determines DNA ligase activity, which is responsible for the formation of the molecular beacon's open state and therefore directly correlates with the emerging fluorescence signal. Additionally, the highly selective bioassay has a low limit of detection compared to other methods.

Because of the signal's stability and the suitability for daily use it can be coupled to NADH-dependent BPA degradation in the context of biosensing.

S-layer

S-layers (crystalline bacterial surface layer) are crystal-like layers consisting of multiple protein monomers and can be found in various (archae-)bacteria. They constitute the outermost part of the cell wall. Especially their ability for self-assembly into distinct geometries is of scientific interest. At phase boundaries, in solutions and on a variety of surfaces they form different lattice structures. The geometry and arrangement is determined by the C-terminal self assembly-domain, which is specific for each S-layer protein. The most common lattice geometries are oblique, square and hexagonal. By modifying the characteristics of the S-layer through combination with functional groups and protein domains as well as their defined position and orientation to each other (determined by the S layer geometry) it is possible to realize various practical applications. The usability of such well defined nano-lattice structures is far-reaching from ultrafiltration membranes to the development of immobilized biosensors.

Especially for the production of cell-free biosensors functional fusion proteins are of great importance. Sleytr *et al.* fused fluorescent proteins with an S-layer glycoprotein from *Geobacillus stearothermophilus*. They demonstrated that the properties of the fusion protein were similar to the native fluorescent protein. The intensity of the fluorescence, the lifetime and the adsorption spectra showed comparable behavior at different pH-values. Enzymes fused to immobilized S-layers showed a significantly longer durability.

The iGEM-Team Bielefeld aims at the assembly, production and immobilization of S-layer fusion proteins for the detection of BPA by a coupled enzymatic reaction. S-layers from five different organisms are employed. The provision of various S-layers with different geometries offers the possibility for the scientific community to create functional nanobiotechnological surfaces with simple and standardized methods, quasi do it your self nanobiotechnology. First, different fusion proteins with fluorescent proteins and a luciferase are created. The functionality and efficiency of the immobilization to various materials such as silicon dioxide or cellulose is then characterized by measuring the fluorescence and luminescence, respectively.

References

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