Protein Engineering for Biosensor Design

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A Protein Engineering Perspective
- Wild type ('natural') proteins have evolved to fulfill a specific physiological function
- The needs of biosensors are very different.

A Modular Approach
- **Signalling Module**-indicates ligand occupancy
- **Attachment Module**-mediates protein-surface interactions
- **Binding Site Module**-determines specificity

Module 1: Signalling
Fluorescence: Environmental Sensitivity

Sensitive to Solvent Polarity and Local Environment:

- Internal conversion and vibrational relaxation (10^-12s).
- Less polar solvent
- More polar solvent
- Solvent relaxation (10^-10s).
- Absorbance (10^-15s).
- Emission (10^-8s).

S1
S2

k_{nr}

Emission (10^-8s).

k_{nr} can be environment sensitive

Environment Sensitive Fluorophores

- Nitrobenzoxadiazole (Mobility)
- Acrylodans (Dielectric constant)
- Aladan (Electrostatics)
- DyMPO (Dielectric constant)
- Coumarins (Polarity)

Donor-acceptor pairs and twisted intramolecular charge transfer (TICT) ensure excited state relaxation is sensitive to rotational freedom and/or solvation i.e. molecular binding.

Natural or engineered cysteine residue

Ex:488nm Em:525nm

Periplasmic Binding Proteins-Ligand Diversity and a Common Scaffold

- E.coli PBP’s are the main route to trace nutrient uptake
Diverse Sequences but a Common Structure

The PBP's have a common 3-D structure and mechanism of ligand binding.

Signal Generation via Site Specific Labelling

Analytical Performance of S337C MBP

Other PBP Fluorophore Combinations

<table>
<thead>
<tr>
<th>Protein</th>
<th>Acrylodan</th>
<th>IAANS</th>
<th>IAEDANS</th>
<th>IANBD</th>
<th>MDCC</th>
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<tbody>
<tr>
<td>WT (C64)</td>
<td>1.52</td>
<td>0.08</td>
<td>0.98</td>
<td>0.04</td>
<td>1.01</td>
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<tr>
<td>D67C</td>
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<tr>
<td>S176C</td>
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<tr>
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<tr>
<td>AraBP</td>
<td>L298C</td>
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<td>0.15</td>
<td>0.97</td>
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<tr>
<td>S10C</td>
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<td>0.11</td>
<td>0.99</td>
<td>0.11</td>
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<tr>
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<td>0.02</td>
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<tr>
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<tr>
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</table>
Electrochemical Signalling - An Engineered Enzyme-Mediator Composite

Module 2: Immobilization

Tagging to Immobilize Proteins

Why Tags?
- Fusion tags match surface chemistry
- Controlled orientation
- Site-specific
- Retention of activity
- Generic

Construction of a Hybrid Protein

OBP   Linker   E12

QBP-E12
Hydrophobic Immobilization

(QBP E12 Protein is Functional when Bound to polystyrene)

Engineered Glucose Oxidase

Monomer structure
Linker is (gly-ser)$_5$

Improved Performance

Electrochemical
Optical
Module 3: Binding Site

Can the Specificity be Altered?
- Physiologically the PBP’s are highly specific. For example, phosphate binding proteins show almost no binding to sulphate.
- Can this specificity be changed by mutagenesis to broaden the range of potential ligands?

Typical Dose Response Curves

Narrow Range

Broad Range

Phosphate Binding Protein
Phosphate Binding Protein Mutants

Changing the Affinity...

...and Specificity of Phosphate Binding Protein

Target residues in the protein that change oxyanion specificity

It works for protein analytes too

- Use the same approach as with PBP’s but with single chain antibodies

scFv against hen lysozyme.

- HEWL
- TEWL
- HEWL in serum

[Table and graphs related to phosphate binding and specificity]
A Different Paradigm: Olfactory Sensing

- Approximately 1000 genes code for olfactory receptors (G-protein coupled) in the human genome (3x more in mouse)
- Even an untrained nose can detect >10,000 odours
- There are no ‘primary’ odorants and corresponding receptors (like taste of colour)

Olfaction relies on cross reactive receptors (sensors) and neuronal processing

- A protein array as a mimic of the olfactory system.
- Needs
  - A cross reactive set of proteins capable of further diversification
  - A read out mechanism
  - Data processing models

Bovine OBP: Open Binding Sites in a barrel...

...lined with hydrophobic residues
Site Specific Labeling with NBD

The Label Sits at the Edge of the Barrel

An OBP Microarray

A Combinatorial Response: Nitro-Aromatics

- Nitrobenzene
- 1,2-dinitrobenzene
- 1,4-dinitrobenzene
- 2,3-dinitrotoluene
- 2,6-dinitrotoluene
- 3,4-dinitrotoluene

Mutants 1-5

Replicates

Spot Size 200µm
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