Imperial College London

Protein Engineering for Biosensor Design

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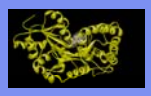
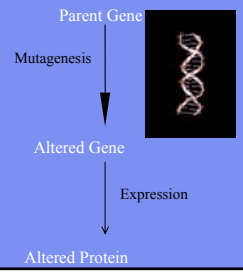
A Protein Engineering Perspective

- Wild type ('natural') proteins have evolved to fulfil a specific physiological function
- The needs of biosensors are very different.





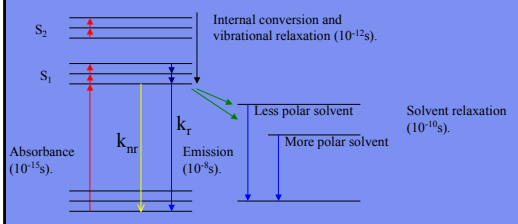
A Modular Approach

- *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:

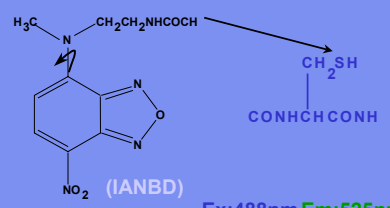


k_{nr} can be environment sensitive

Environment Sensitive Fluorophores

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

Introducing Environmental Sensitivity



(IANBD)

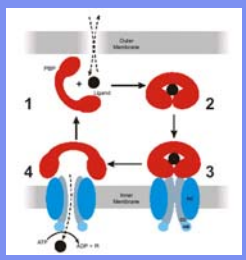
Natural or engineered cysteine residue

Ex:488nm Em:525nm

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

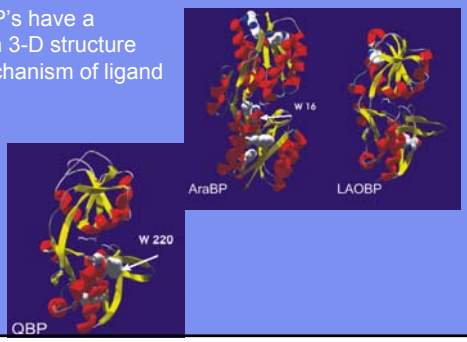
Periplasmic Binding Proteins-Ligand Diversity and a Common Scaffold

- *E.coli* PBP's are the main route to trace nutrient uptake

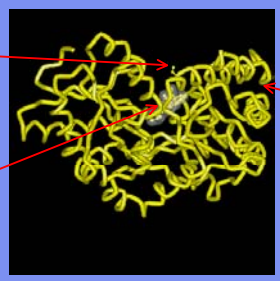


Diverse Sequences but a Common Structure

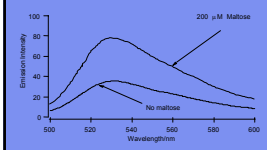
The PBP's have a common 3-D structure and mechanism of ligand binding

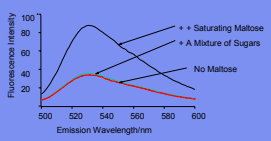


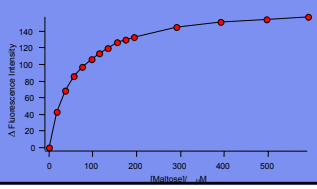
Signal Generation via Site Specific Labelling



Analytical Performance of S337C MBP



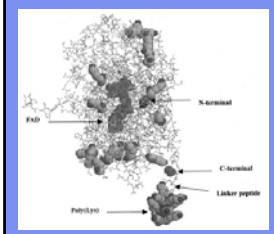




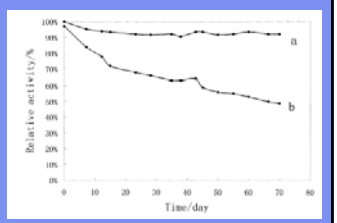
Other PBP Fluorophore Combinations

		Fluorophore									
		Acrylodan		IAANS		IAEDANS		IANBD		MDCC	
Protein			S.D.		S.D.		S.D.		S.D.		S.D.
AraBP	WT* (C64)	1.52	0.08	0.98	0.04	1.18	0.02	1.01	0.03	1.08	0.04
	D67C	1.02	0.08	0.98	0.08	0.96	0.05	1.00	0.06	1.02	0.06
	S176C	1.61	0.02	0.95	0.03	0.97	0.05	1.09	0.34	1.78	0.07
	S207C	1.00	0.06	0.96	0.05	1.00	0.04	1.00	0.03	1.00	0.04
	L298C	0.97	0.15	0.97	0.05	0.97	0.03	0.99	0.02	1.04	0.02
DEBP	S10C	0.85	0.11	0.99	0.11	0.98	0.03	0.86	0.05	0.92	0.04
	S11C	0.99	0.05	0.99	0.05	0.98	0.07	0.95	0.01	1.02	0.06
	S56C	0.99	0.02	0.97	0.03	0.99	0.04	0.98	0.01	1.04	0.07
	S121C	1.05	0.12	0.96	0.03	1.01	0.13	1.32	0.11	1.00	0.09
LAO-BP	S92C	0.97	0.07	1.01	0.02	0.97	0.07	1.38	0.03	1.51	0.02
	S120C	1.51	0.05	0.98	0.02	0.97	0.07	0.97	0.05	1.24	0.05

Electrochemical Signalling- An Engineered Enzyme-Mediator Composite



Engineered Structure
Poly(lysine) modified
With FMCA



Storage Stability

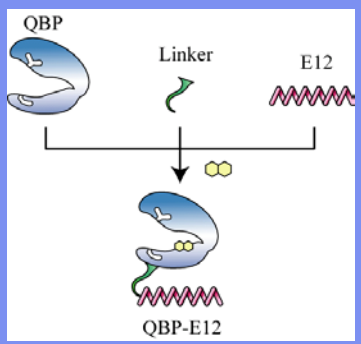
Module 2: Immobilization

Tagging to Immobilize Proteins

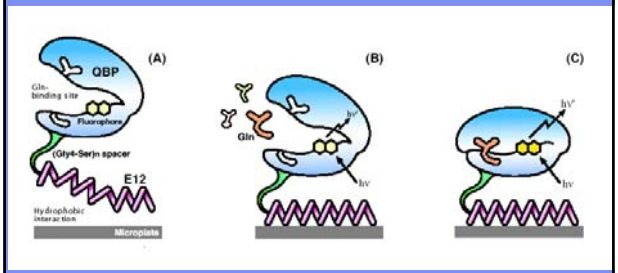
Why Tags?

- **Fusion tags match surface chemistry**
- **Controlled orientation**
- **Site-specific**
- **Retention of activity**
- **Generic**

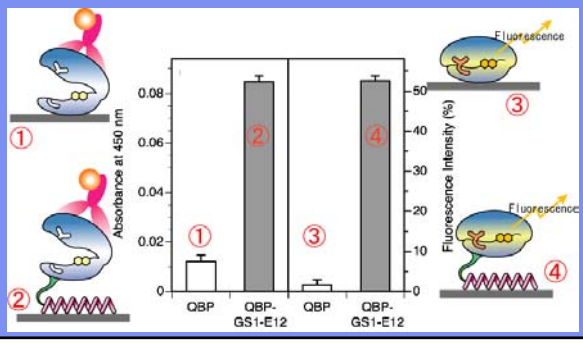
Construction of a Hybrid Protein



Hydrophobic Immobilization



QBP E12 Protein is Functional when Bound to polystyrene



Engineered Glucose Oxidase

QuickTime™ and a TIFF (LZW) decompressor are needed to see this picture.

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

QuickTime™ and a TIFF (LZW) decompressor are needed to see this picture.

Engineered Wild Type Bare Gold

Improved Performance

QuickTime™ and a TIFF (LZW) decompressor are needed to see this picture.

Electrochemical

QuickTime™ and a TIFF (LZW) decompressor are needed to see this picture.

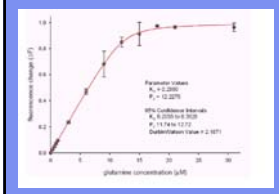
Optical

Module 3: Binding Site

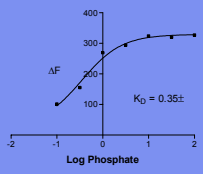
Can the Specificity be Altered?

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

Typical Dose Response Curves

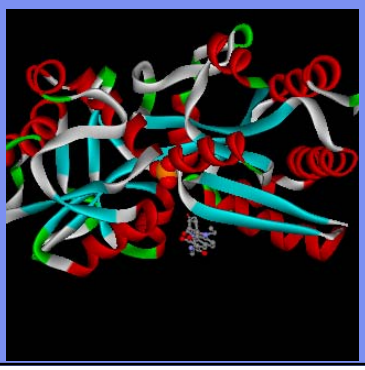


Narrow Range



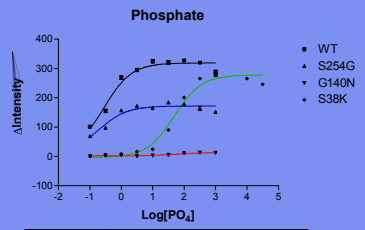
Broad Range

Phosphate Binding Protein



Phosphate Binding Protein Mutants

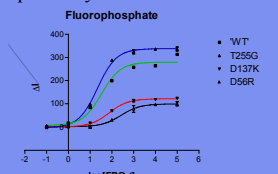
Changing the Affinity…



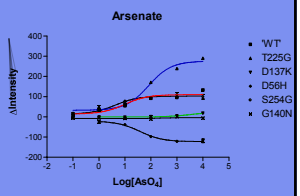
	WT	S254G	G140N	S38K
Best-fit values				
BOTTOM	17.79	15.65	2.364	-3.855
TOP	319.0	171.8	13.85	278.1
LOGEC50	-0.5373	-0.6847	1.833	1.682
EC50	0.2902	0.2067	68.04	48.11

…and Specificity of Phosphate Binding Protein

Target residues in the protein that change oxyanion specificity



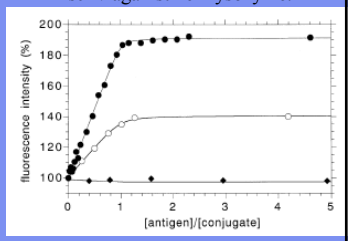
	'WT'	T255G	D137K	D56R
Best-fit values				
BOTTOM	6.855	-3.181	0.7345	1.326
TOP	279.7	337.8	121.8	99.70
LOGEC50	1.529	1.339	1.877	2.423
EC50	33.81	21.83	75.40	264.6



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

•[Renard et al. J. Mol. Biol. 318 429 (2002)]

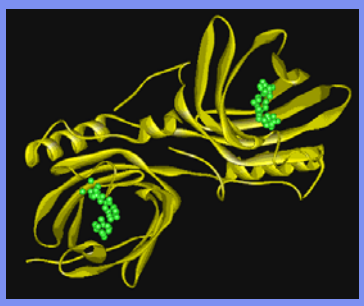
A Different Paradigm: Olfactory Sensing

- 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)

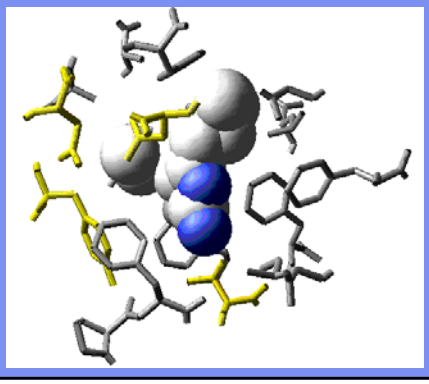
Olfaction relies on cross reactive receptors (sensors) and neuronal processing

- 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

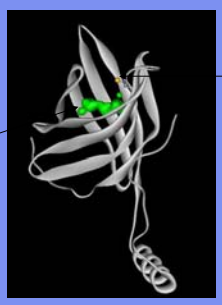
Bovine OBP: Open Binding Sites in a barrel…



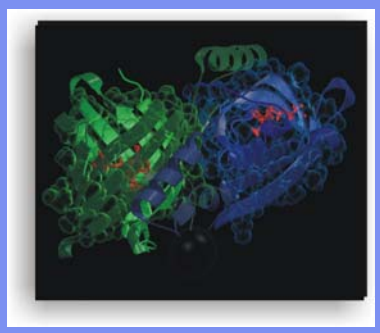
…lined with hydrophobic residues



Site Specific Labeling with NBD

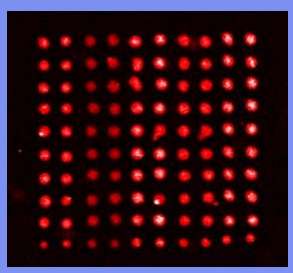


The Label Sits at the Edge of the Barrel



An OBP Microarray

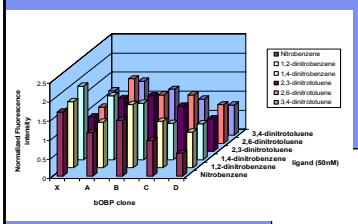
Mutants 1-5

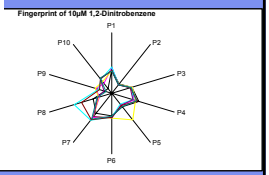


Replicates

Spot Size 200µm

A Combinatorial Response:
Nitro-Aromatics





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