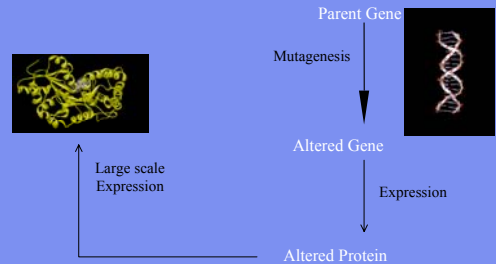


## Protein Engineering for Biosensor Design

Tony Cass  
Dept. of Biological Sciences

### A Protein Engineering Perspective

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



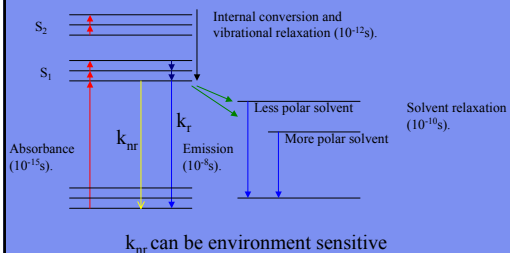
### A Modular Approach

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

### Module 1: Signalling

## Fluorescence: Environmental Sensitivity

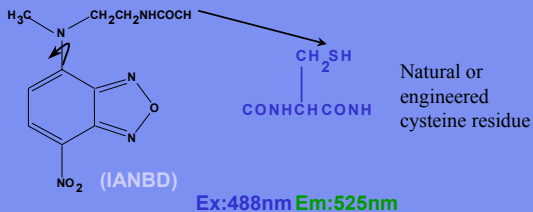
Sensitive to Solvent Polarity and Local Environment:



## Environment Sensitive Fluorophores

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

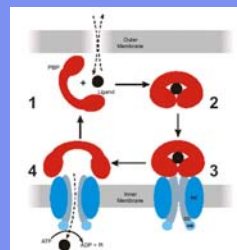
## Introducing Environmental Sensitivity



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

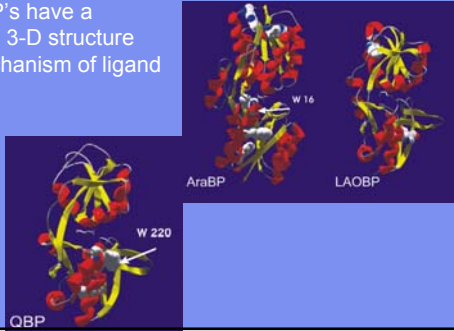
## Periplasmic Binding Proteins-Ligand Diversity and a Common Scaffold

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



## Diverse Sequences but a Common Structure

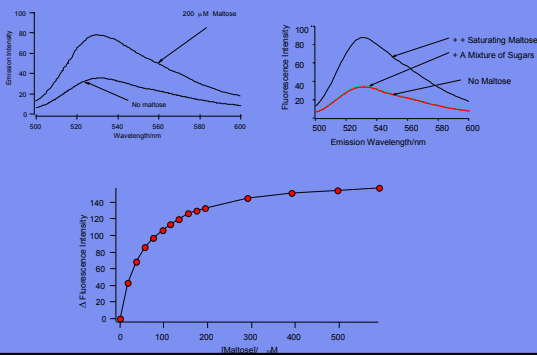
The PBP's have a common 3-D structure and mechanism of ligand binding



## Signal Generation via Site Specific Labelling



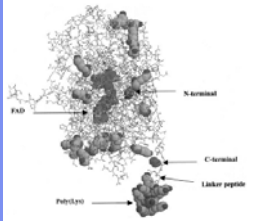
## Analytical Performance of S337C MBP



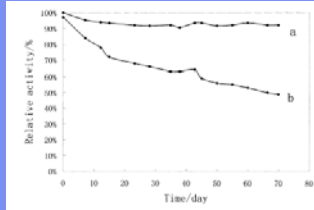
## Other PBP Fluorophore Combinations

Protein	WT*	Fluorophore									
		Acrylodan	S.D.	IAANS	S.D.	IAEDANS	S.D.	IANBD	S.D.	MDCC	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	1.09	0.34	1.02	0.06
	S176C	1.81	0.02	0.95	0.03	0.97	0.05	1.00	0.03	1.00	0.04
	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	0.82	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

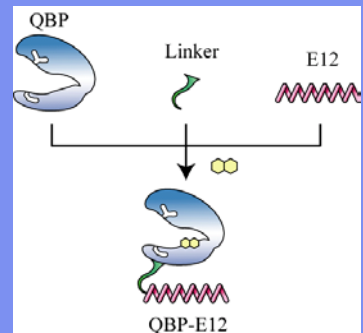


Surface

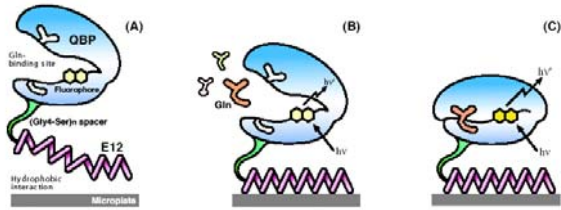
#### Why Tags?

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

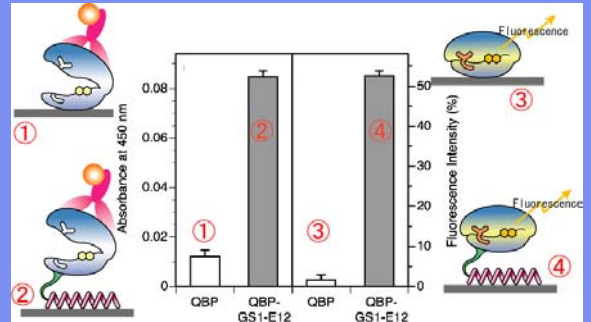
### Construction of a Hybrid Protein



## 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)<sub>5</sub>

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.

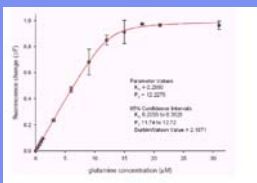
Optical

## Module 3: Binding Site

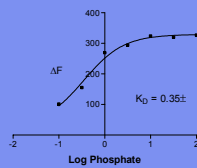
## Can the Specificity be Altered?

- 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

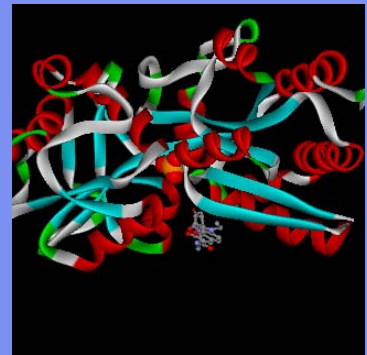


Narrow Range



Broad Range

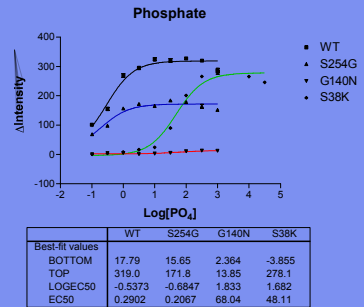
## Phosphate Binding Protein



## Phosphate Binding Protein Mutants

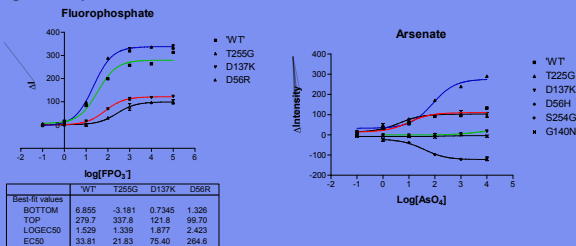


## Changing the Affinity...



## ...and Specificity of Phosphate Binding Protein

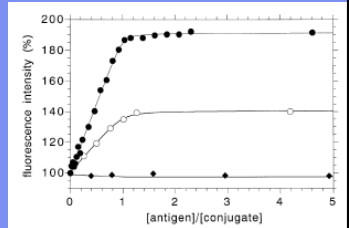
Target residues in the protein that change oxyanion specificity



## 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

\*[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)

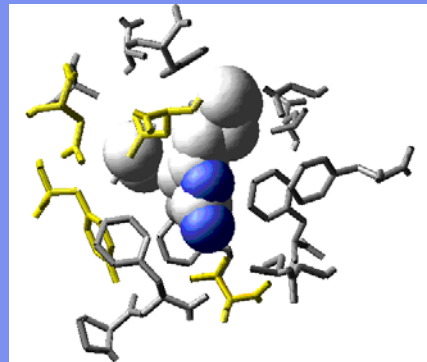
Olfaction relies on cross reactive receptors (sensors) and neuronal processing

- 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

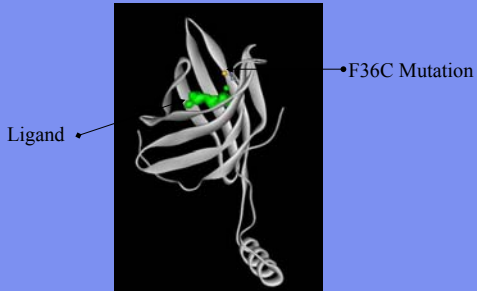
### Bovine OBP: Open Binding Sites in a barrel...



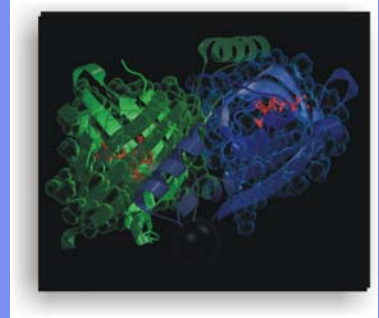
### ...lined with hydrophobic residues



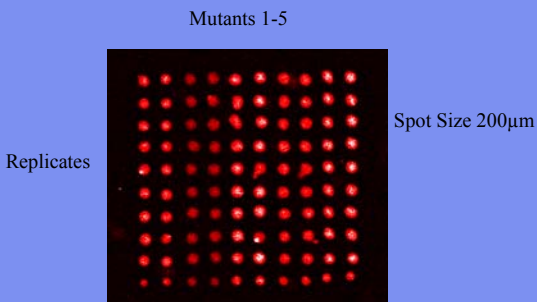
## Site Specific Labeling with NBD



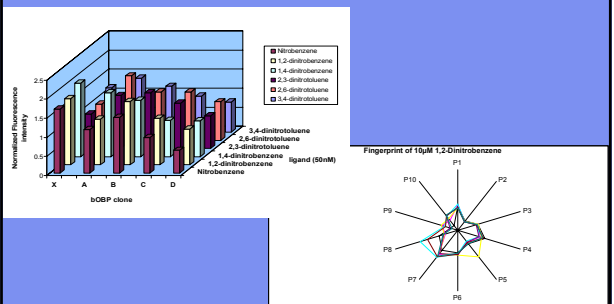
## The Label Sits at the Edge of the Barrel



## An OBP Microarray



## A Combinatorial Response: *Nitro-Aromatics*



## Acknowledgements

- Nathan Hobson
- Mel Fennah
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- DSTL
- Royal Society
- JSPS
- The Wellcome Trust