Engineering a biosensor for the herbicide glyphosate

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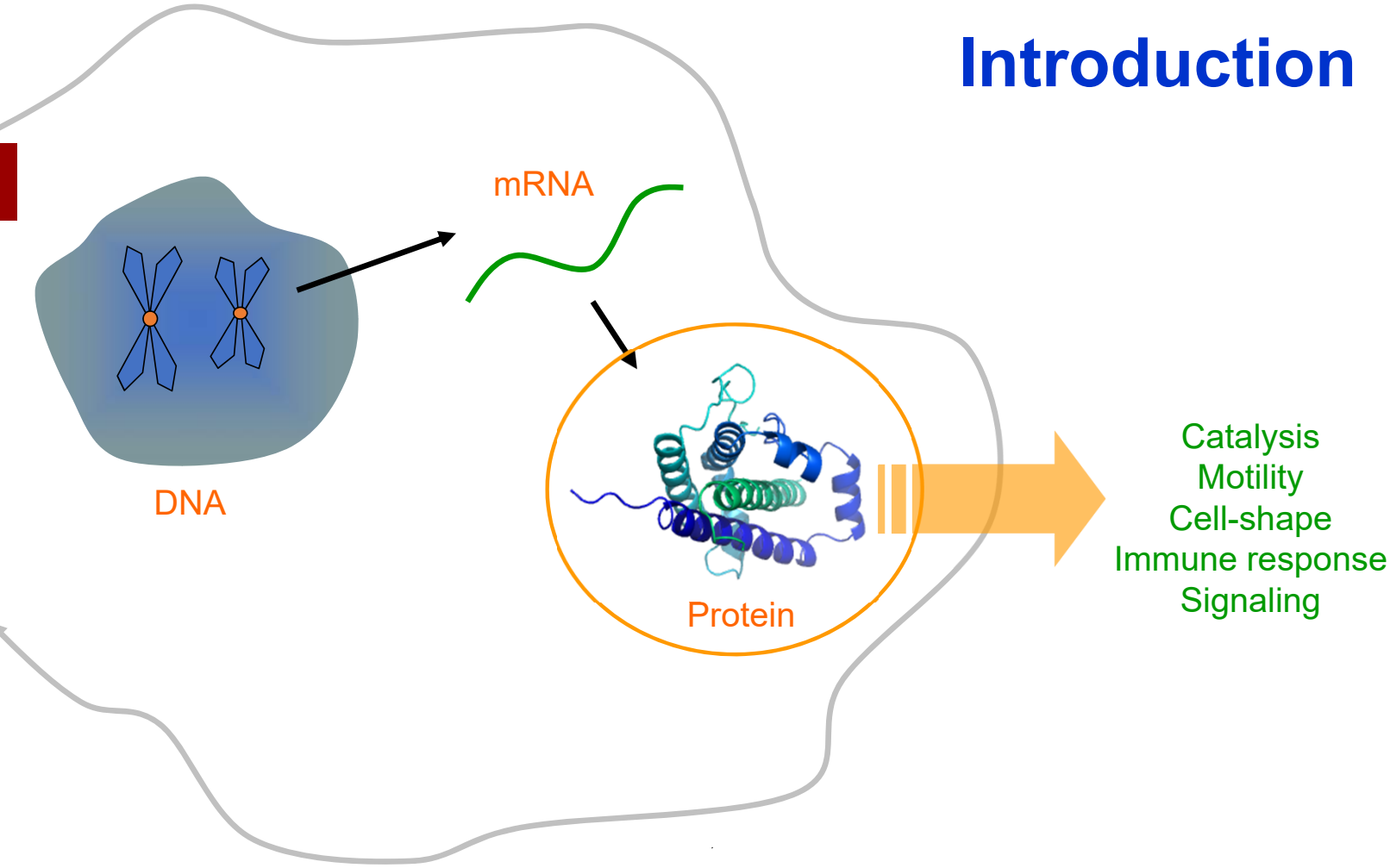
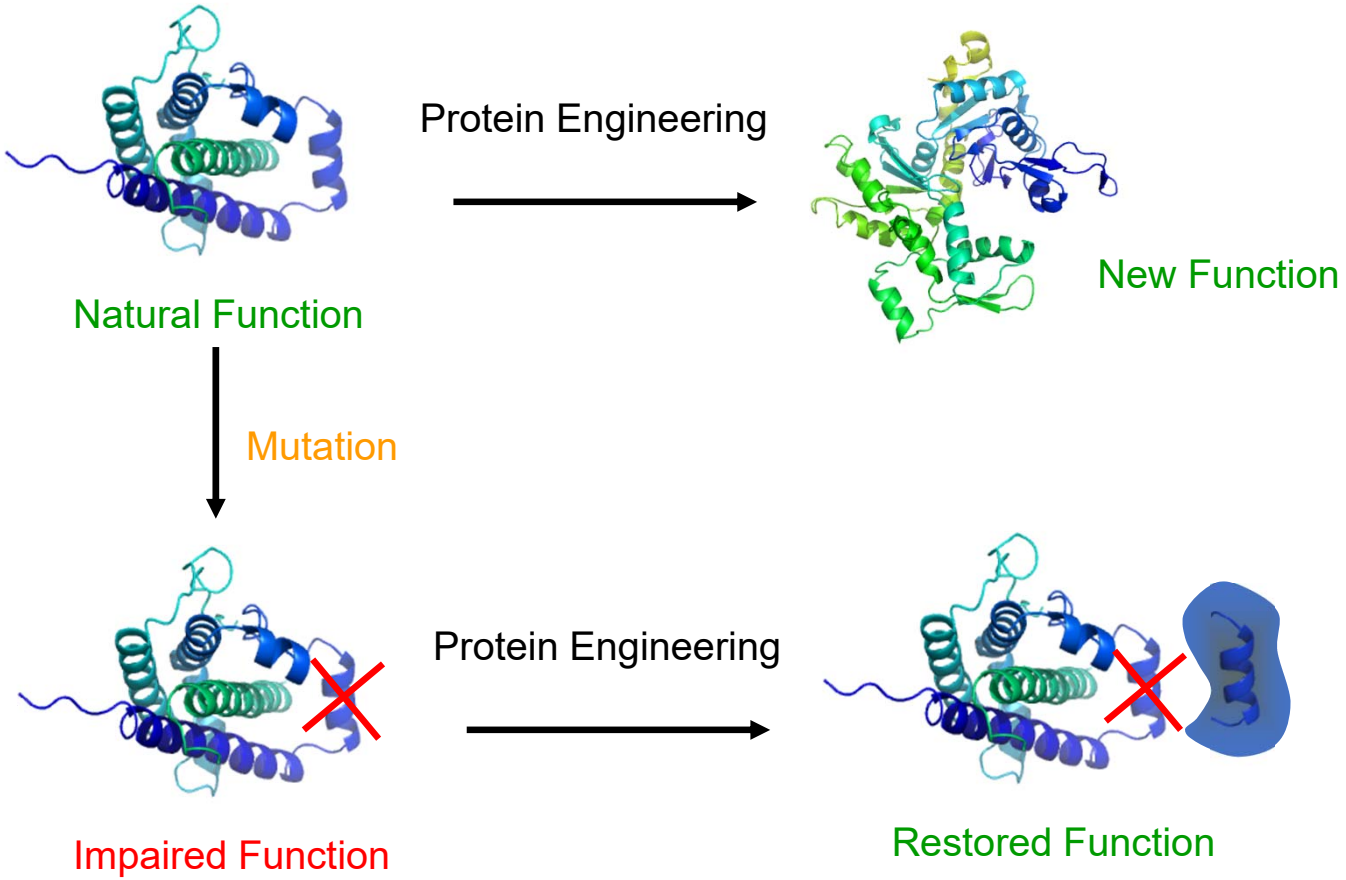
Introduction



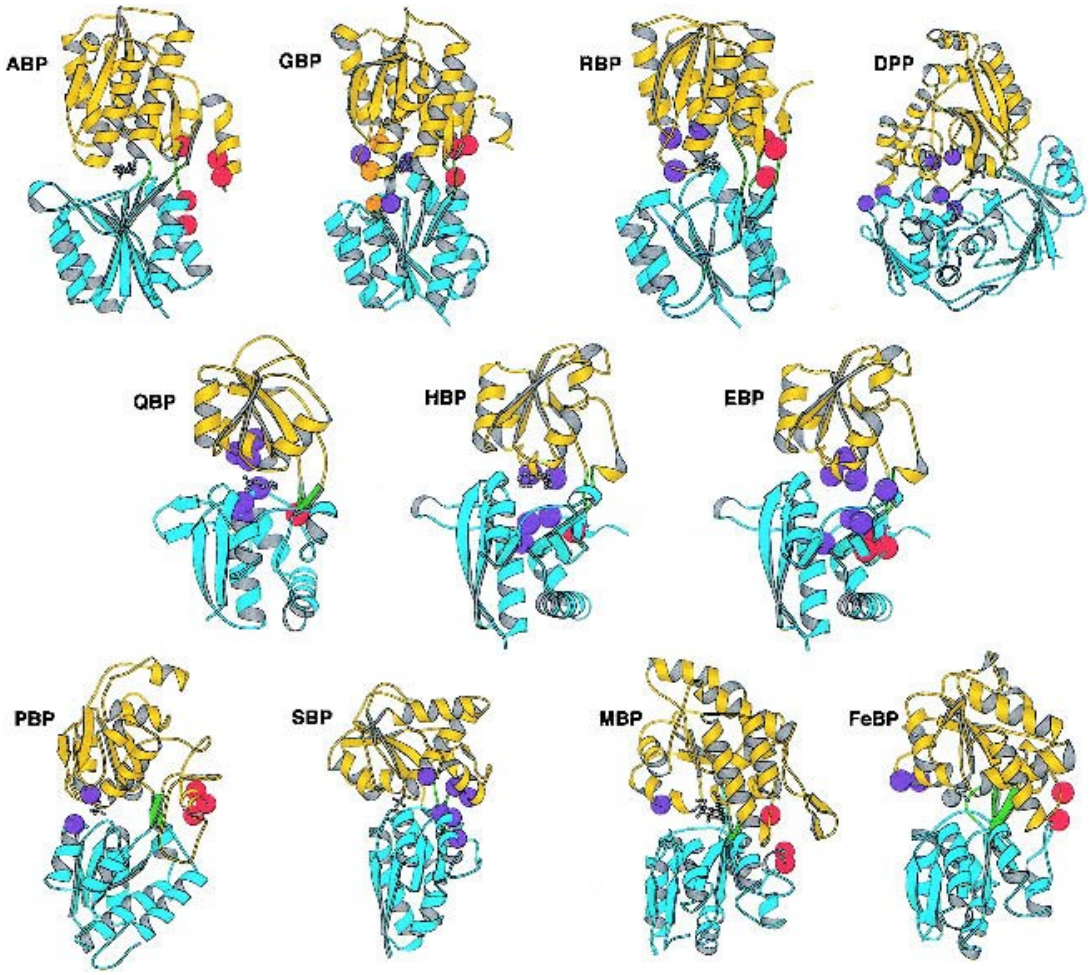
Catalysis
Motility
Cell-shape
Immune response
Signaling

Protein Engineering

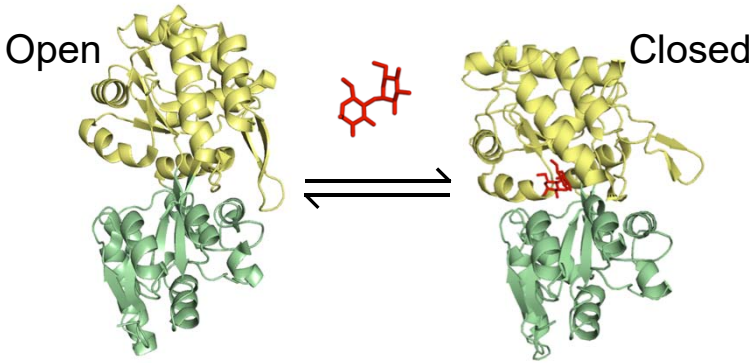


Periplasmic Binding Protein Superfamily

- Bacterial Ligand binding proteins
- Structure: Two domains
- Ligand binds between the domains
- Diversity in ligand type:
  - Sugars, amino acids, anions, metals, other small molecules



Maltose binding Protein (MBP)

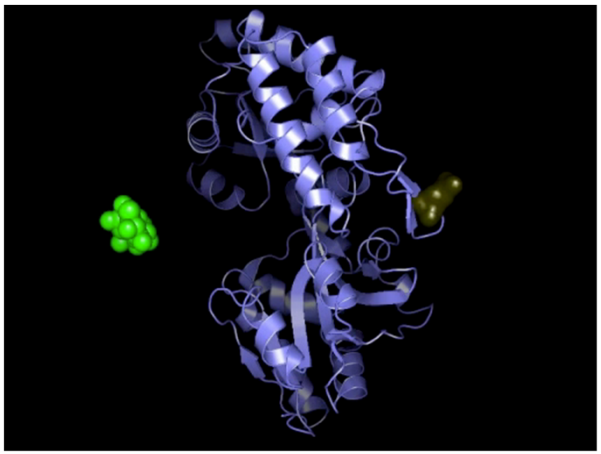


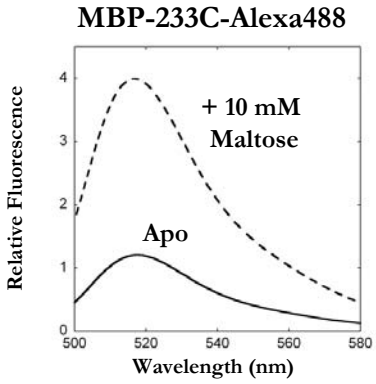
Maltose binding ⟶ conformational change

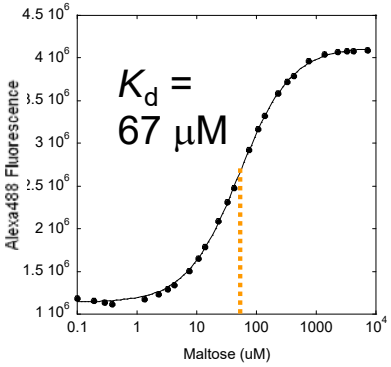
Dissociation constant (K_d):

$$K_d = \frac{[\text{Mal}]\,[\text{MBP}]}{[\text{Mal-MBP}]}$$

High affinity = low K_d





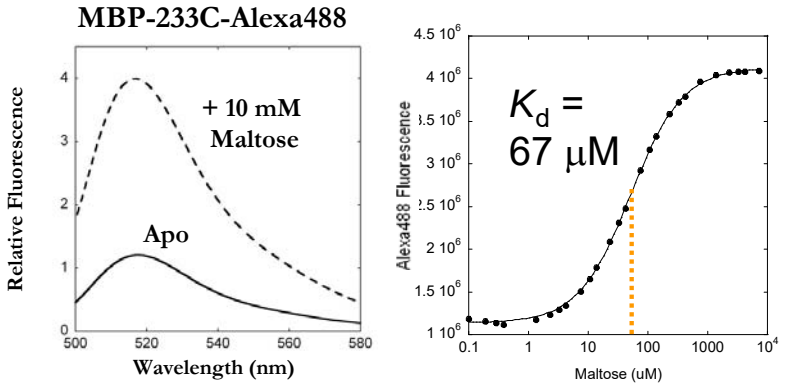


Periplasmic binding proteins as sensors

Level of fluorescence indicates the concentration of the ligand

Fluorescence sensor for the detection and measurement of the ligand

Limited to natural ligands



What about synthetic molecules?

No natural binding proteins for synthetic molecules

Can we reengineer periplasmic binding proteins to bind to synthetic molecules?

Strategy:

1. Understand binding mechanism

2. Make modification in protein to accommodate “new ligand”

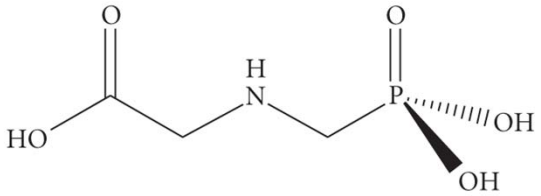
Generating a glyphosate sensor

Glyphosate: main ingredient in RoundUp

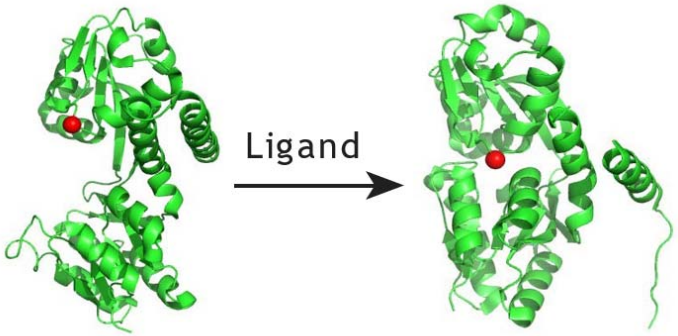
Most commonly used herbicide

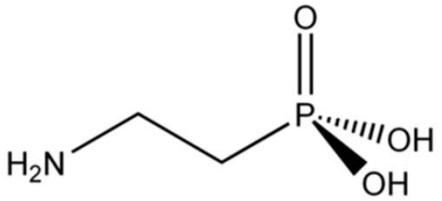
No naturally-occurring glyphosate binding protein

Closest is a “phosphonate binding protein” PhnD: binds to 2-AEP



Glyphosate

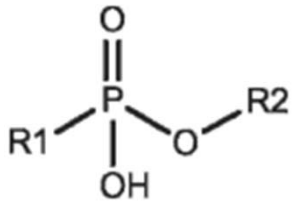


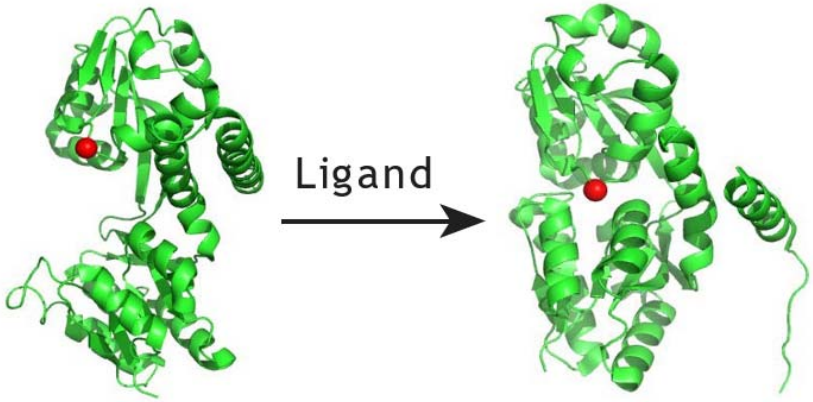


2-aminoethylphosphonate (2-AEP)

PhnD

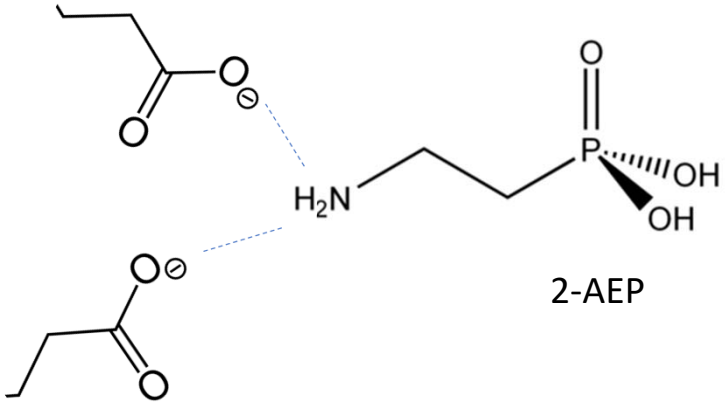
Binds to a variety of phosphonates and related molecules



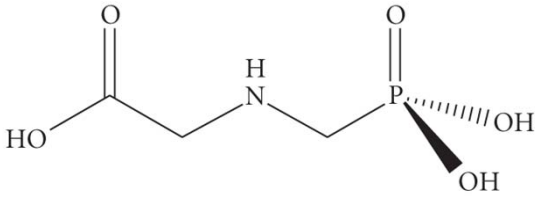


Ligand	R1[a]	R2[a]	K_d (μM)
2-AEP	$CH_2CH_2NH_2$	H	0.005
EP	CH_2CH_3	H	0.3
MP	CH_3	H	1
Phosphonoacetate	$CH_2C(=O)OH$	H	0.9
Phenylphosphonate		H	2.4
AMP	CH_2NH_2	H	5
Phosphate	OH	H	50
Glyphosate	$CH_2NHCH_2C(=O)OH$	H	650

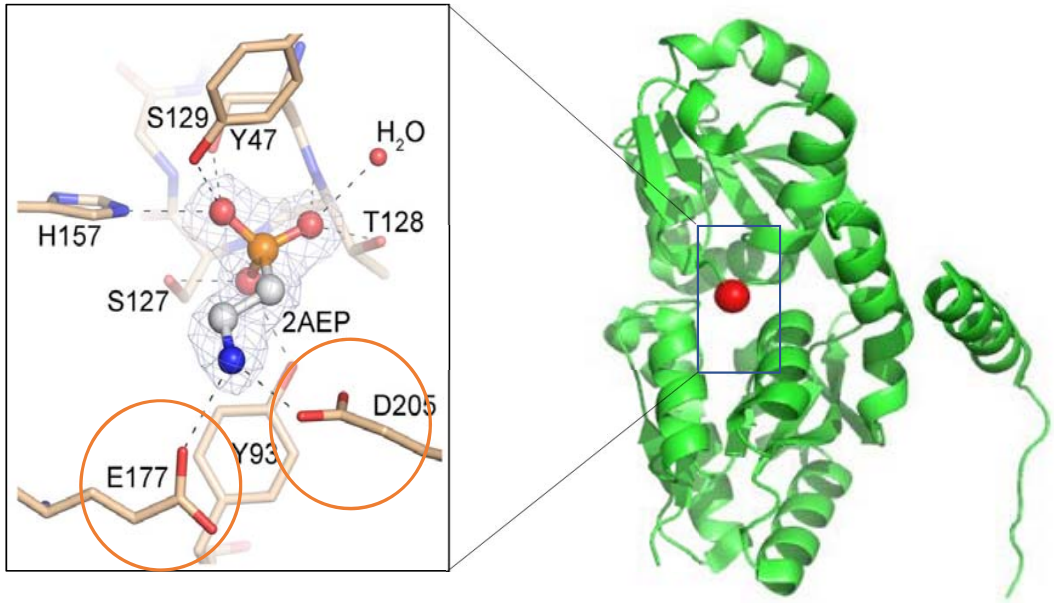
What accounts for the difference in affinity?



2-AEP



Glyphosate



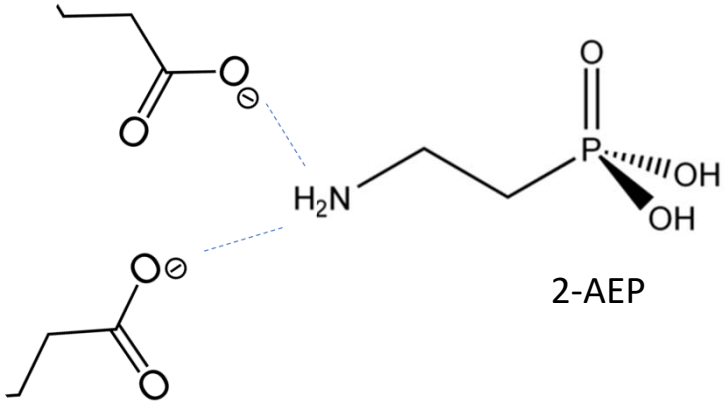
Glyphosate is longer than 2-AEP

Glyphosate has a negative charge

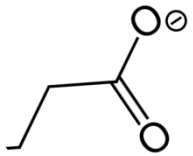
Changing PhnD into a glyphosate sensor

Change amino acids 177 and 205:

- Make them smaller to accommodate the larger glyphosate
- Remove the negative charge to accommodate the carboxylate

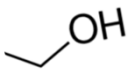


2-AEP

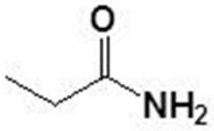


Glutamic acid (E)
or Aspartic acid (D)

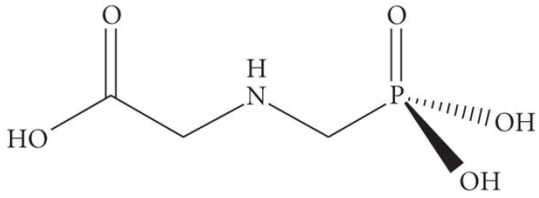
Mutations



Serine (S)

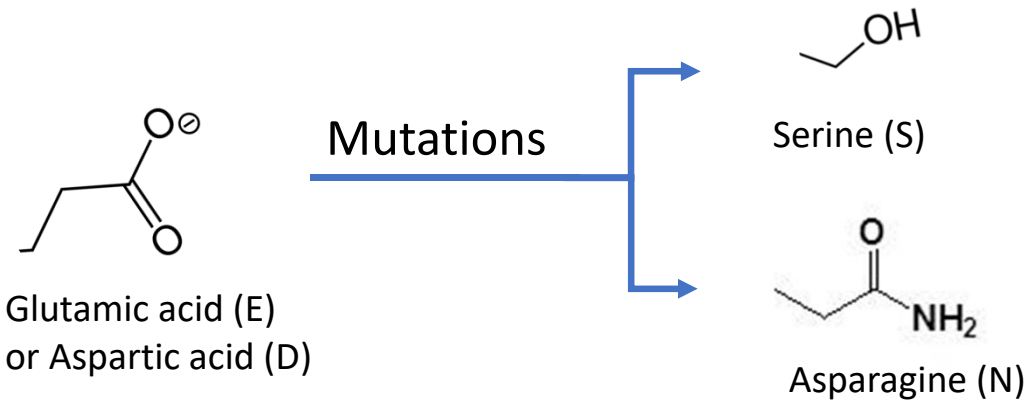


Asparagine (N)

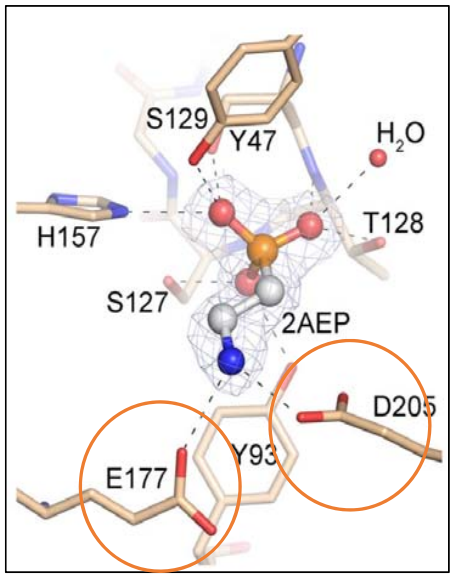


Glyphosate

Mutation results



Mutant	K_d for glyphosate
No mutation	650 μM
D205N	600 μM
E177N, D205S	98 μM
E177N	8.0 μM

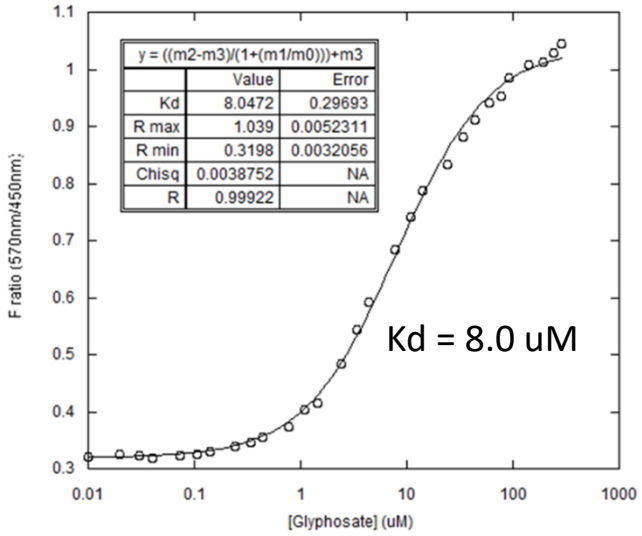


A single mutation from glutamic acid (E) to asparagine (N) at position 177 enhances the affinity by nearly 100 fold!

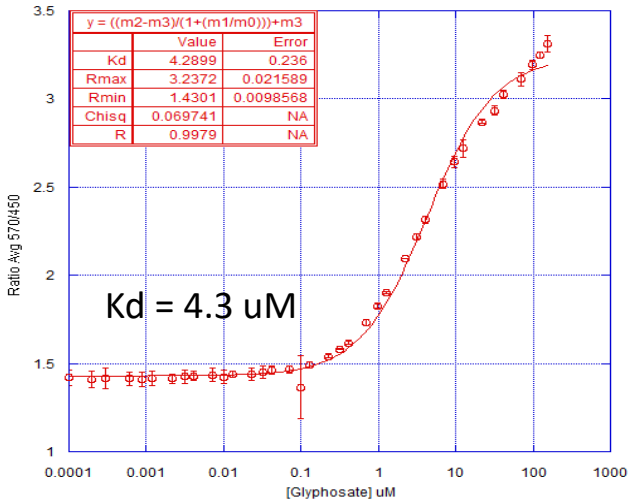
Improving the design

According to Alicia, et al, 2011, JMB, removing the last 6 amino acids converts the protein from a dimer to a monomer

PhnD177N (dimer)

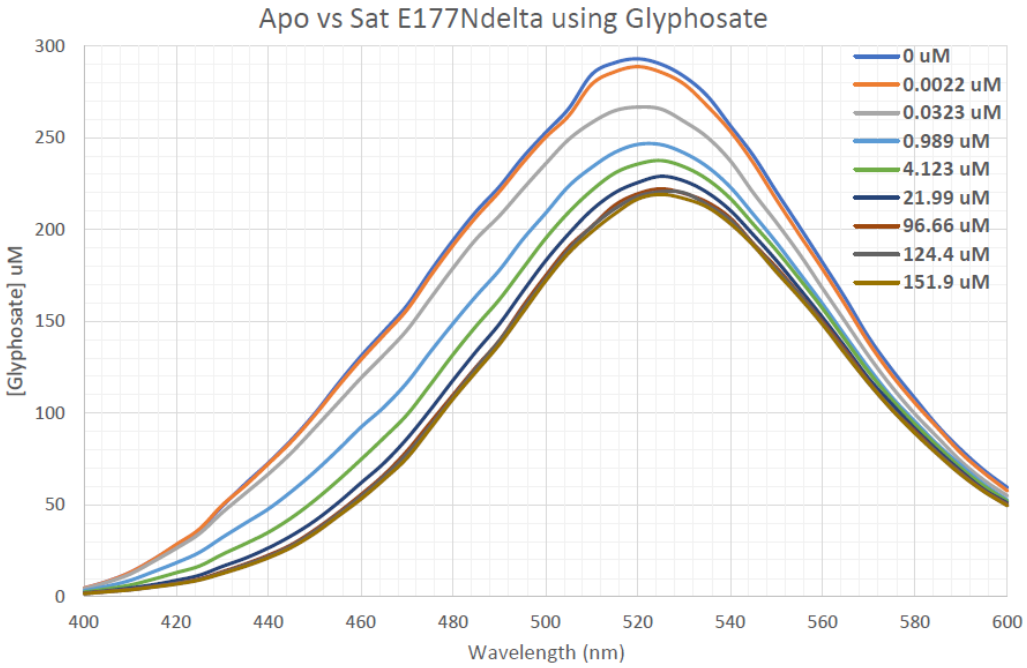


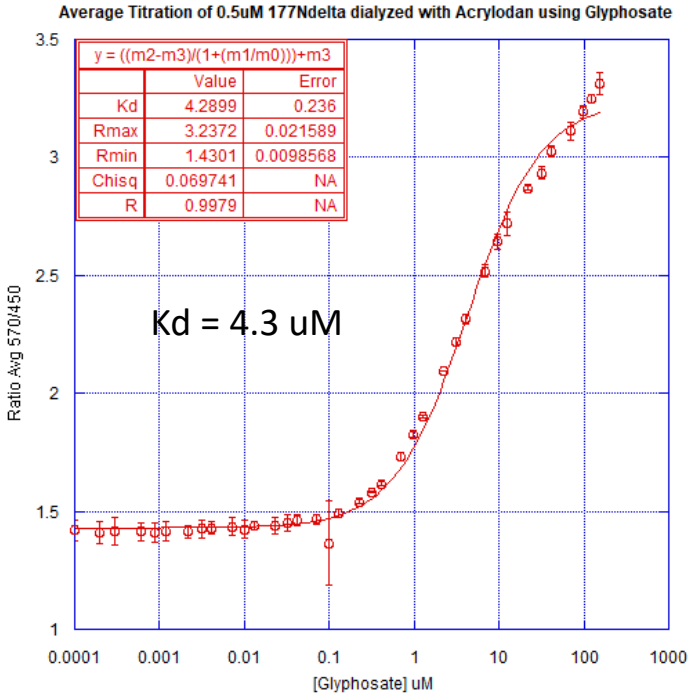
PhnD177NΔ (monomer)



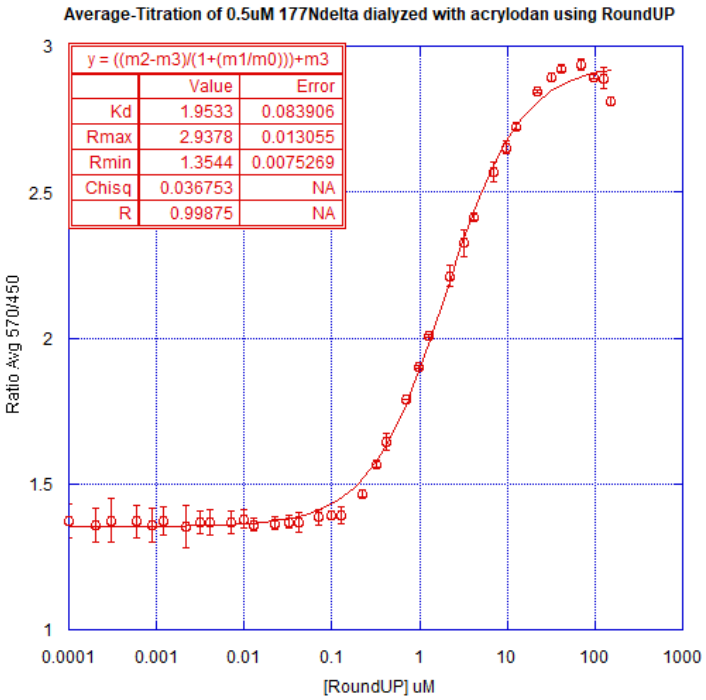
PhnD 177NΔ fluorescent sensor

Ratiometric fluorescence response



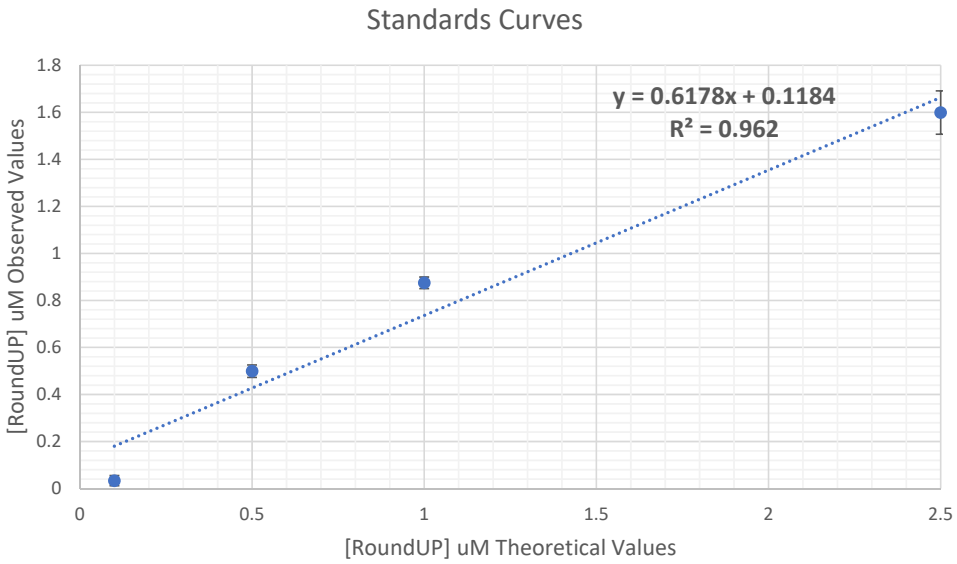


What about RoundUp?



Kd = 2.0 uM

RoundUp detection in buffered samples



Can detect as little as 0.2 uM RoundUP

The permitted limit for drinking water in the United States is 4 uM (Noori, et al, 2018, Sensors)

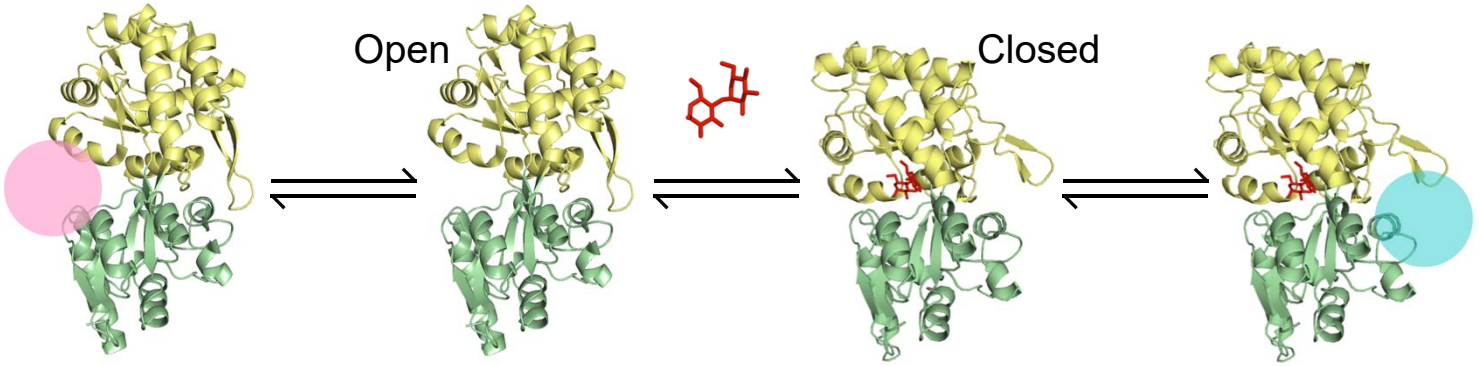
Specificity

What about interference from similar molecules?

Ligand	WT K_d	PND177NΔ K_d
Glyphosate	650 µM	4.29 µM
RoundUp	ND	1.95 µM
2-Aminoethyl Phosphonate (2-AEP)	0.005 µM	5.61 µM
Phosphate	260 µM	26.1 µM
Arsenate	230 µM	10.7 µM

Lower threshold of detection

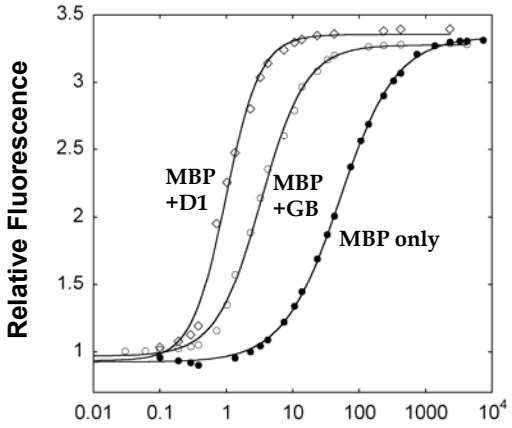
Can we increase the affinity even more?



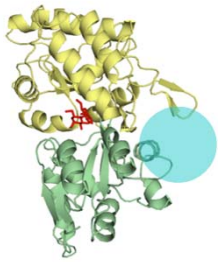
"*If a chemical system at equilibrium experiences a change in concentration … then the equilibrium shifts to counteract the imposed change*"

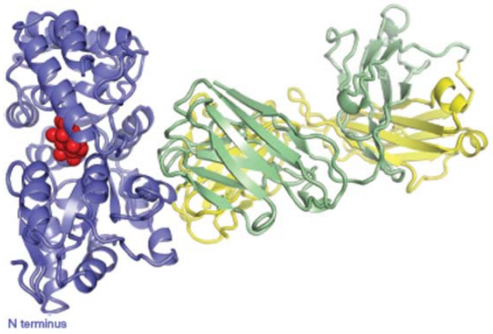
Le Chatelier's principal

Engineered Antibody Fragments (Fabs)



	K_d (μM)	ΔΔG (Kcal/mol)
MBP†	67	N/A
MBP† + D1	1.9	-2.1
MBP† + D2	9.6	-1.1
MBP† + GA	3.6	-1.7
MBP† + GB	2.1	-2.0

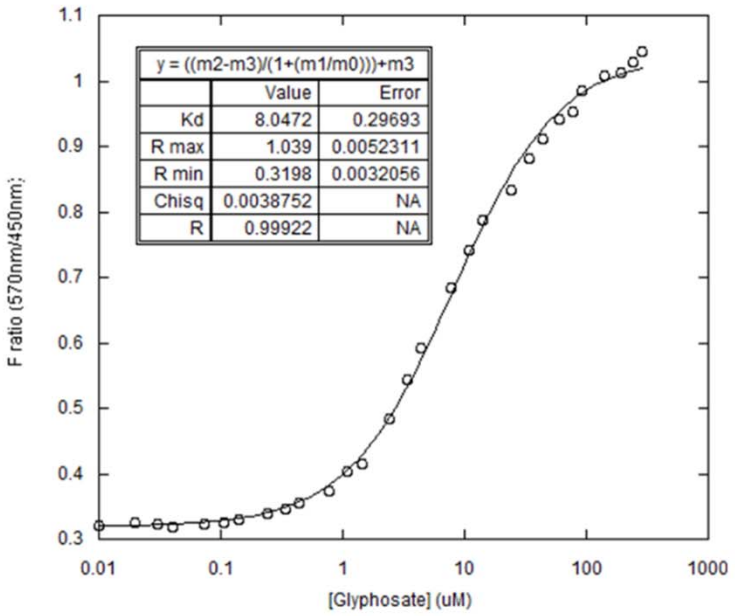




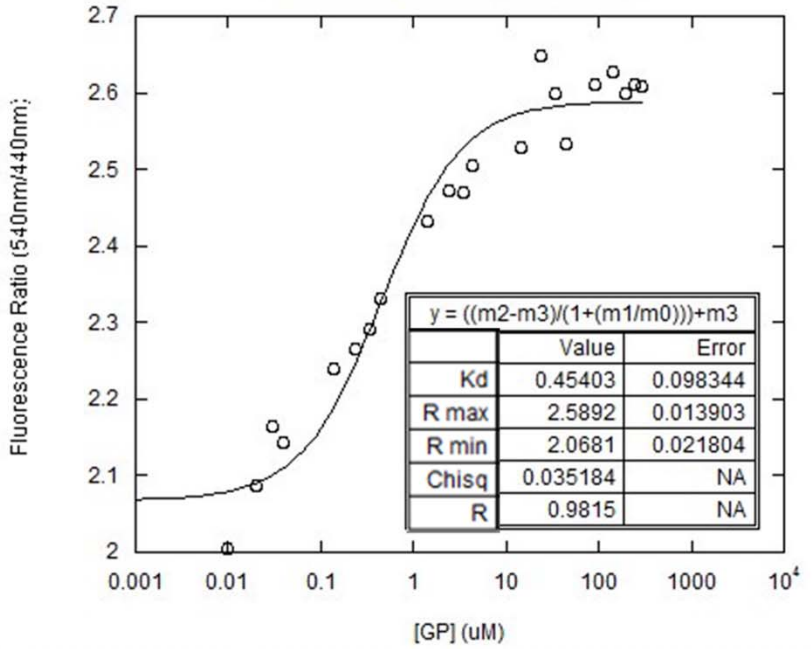
Maltose binding protein & closed-specific Fab

PhnD closed-specific Fabs

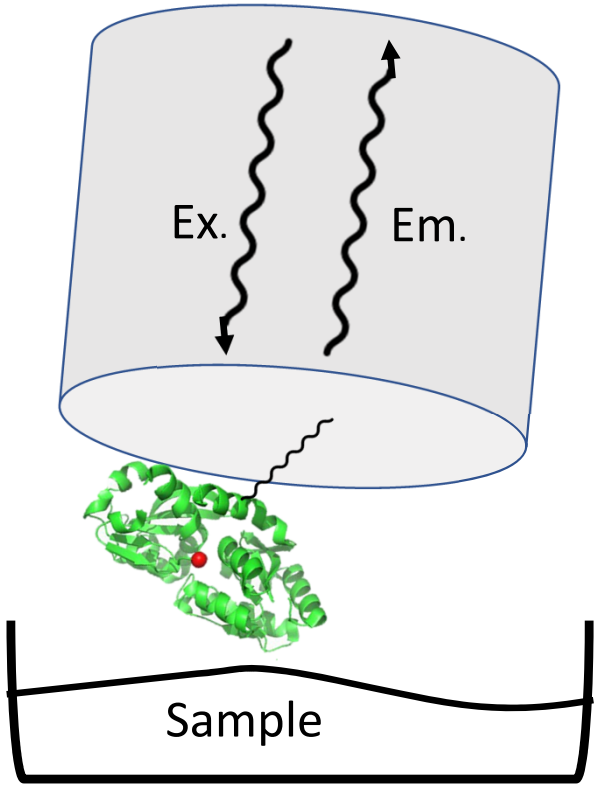
No Fab Kd = 8 uM



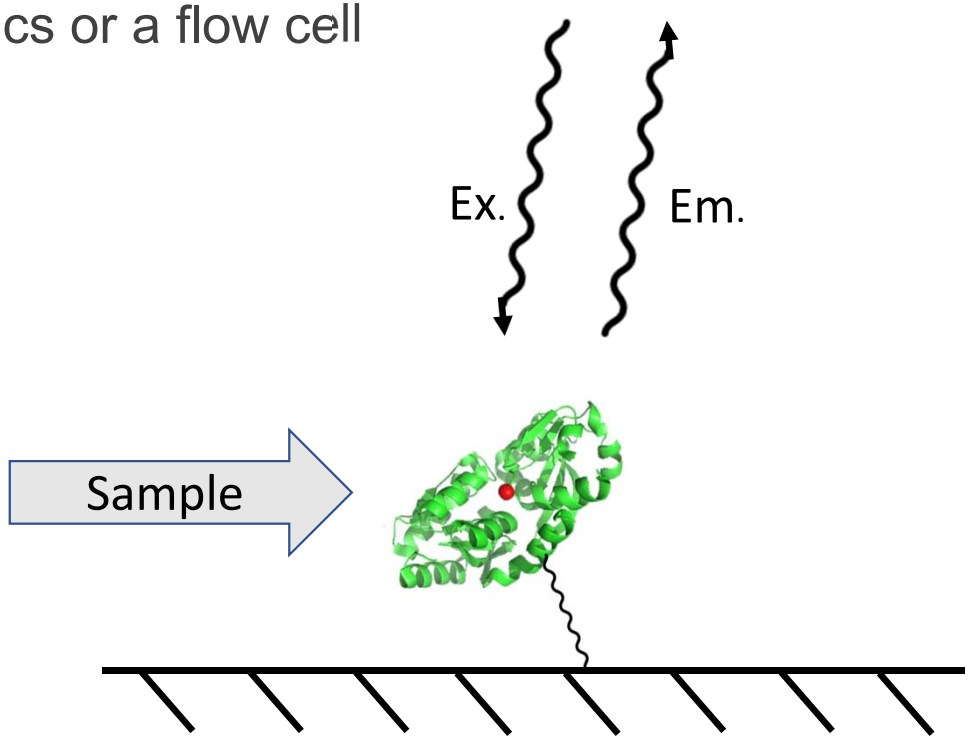
With Fab Kd = 0.4 uM



The future: a device



Fiber optics or a flow cell



Summary

- Redesign PhnD protein into a glyphosate biosensor
  - Fluorescence change in response to conformation change
  - Detection in the submicromolar range
- Future:
  - Incorporate into a device
  - Sensors for other phosphonates (pesticides, pollutants, nerve agents)

Thank you!

NEMC organizers

IUSB Chemistry and Biochemistry

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Pierre N'Guetta & Maggie Fink

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