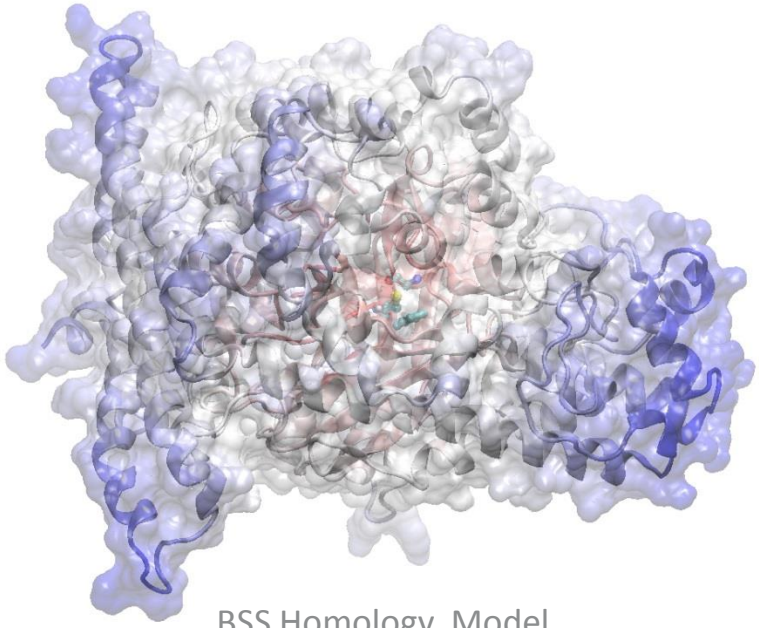
Role of Free Radical Kinetics in Biocorrosion



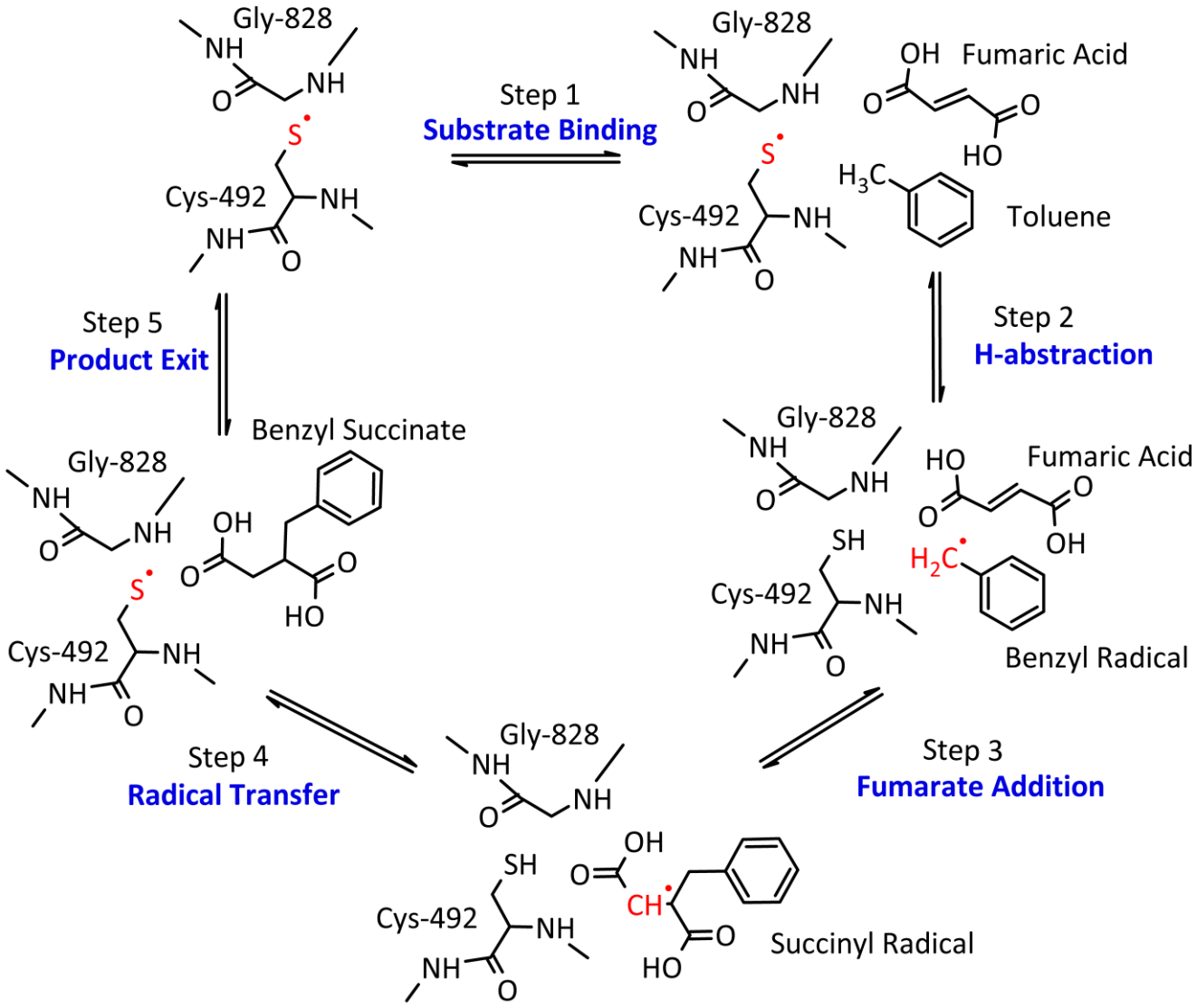
BSS Homology Model

C. Mark Maupin

22nd July 2013

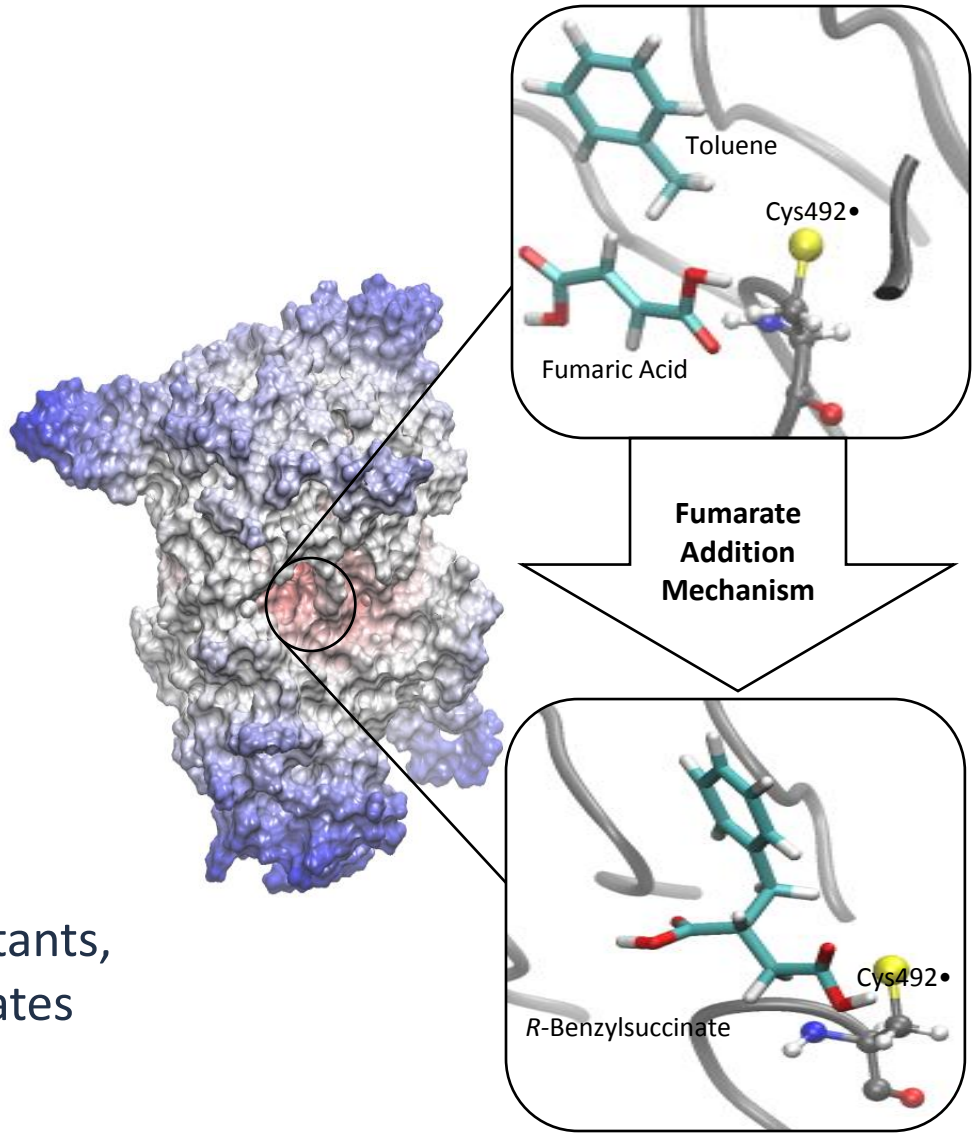
Workshop on Alternative Fuels and Materials: Biocorrosion

Fuel Bio-Degradation by Free Radical Chemistry



Importance of Accounting for Protein Structure

- Substrate (Fuel) selectivity
  - –Substrate binding
  - –Active site topology
- Reaction energetics
  - –Electrostatic
  - –Solvation effects
- Reaction entropies
  - –Steric effects
    - conformations of the reactants, products and transition states



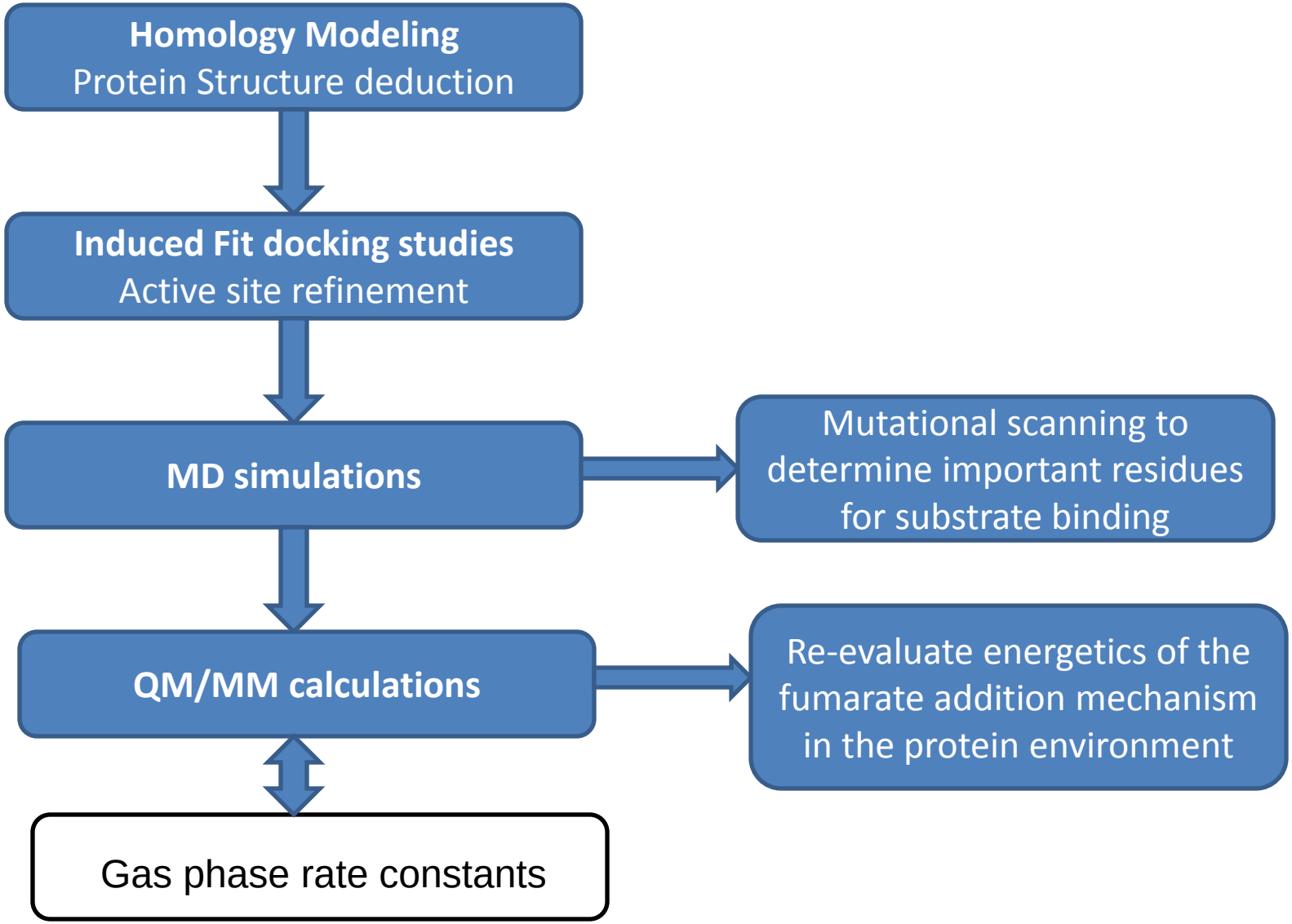
Enzymatic Hydrocarbon Biodegradation

- Glycyl Radical enzymes (GREs)
  - Utilize amino acid based radicals for catalysis
    - Glycine residue to harbor the radical
    - Cysteine residue for catalysis

- Fumarate addition reaction catalyzed by the GRE enzyme family
  - Benzylsuccinate synthase (BenzSS) – Aromatic fuels
  - Alkylsuccinate synthase (AlkSS) – Alkane fuels

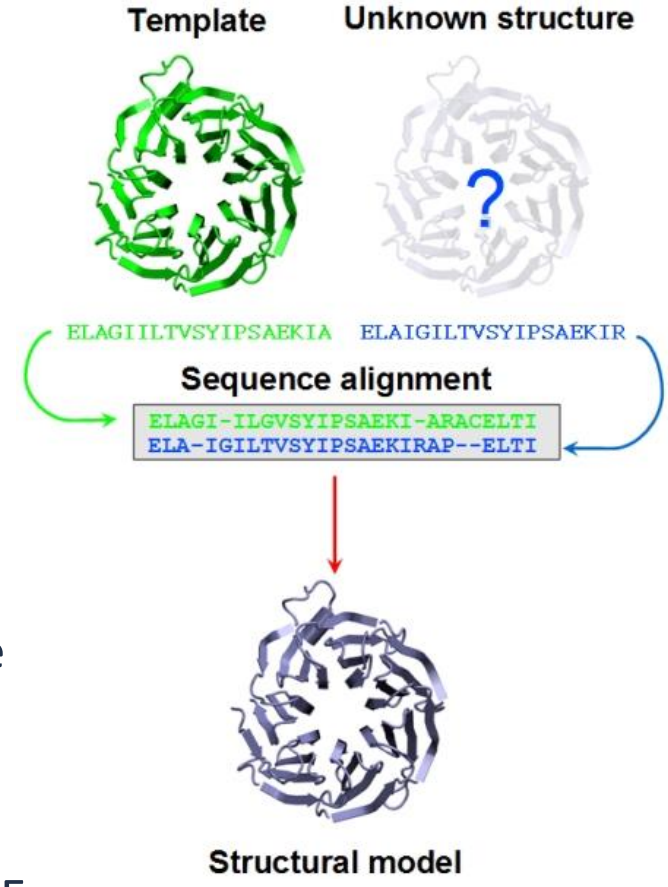
- X-ray crystal structures not available for BenzSS or AlkSS
  - Sensitivity to oxygen has precluded structural characterization

Understanding Enzymatic Free Radical Kinetics



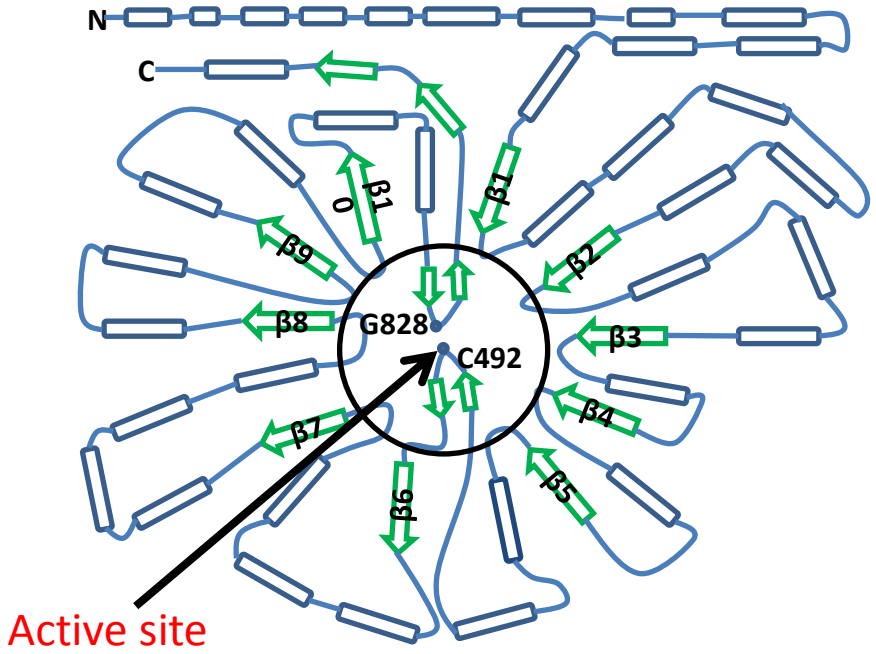
Homology Modeling: Protein Structure Determination

- Uses structural information from related proteins
  - –Template GREs for BenzSS's model
    - Glycerol Dehydratase (GDH)
    - GRE from *Archaeoglobus Fulgidus*
    - Pyruvate Formate Lyase (PFL)
- All these GREs have a conserved
  - –Glycine/Cystiene dyad
  - –Common 3-D structural motif
    - 10 stranded α-β barrel around the active site
- 3-D structure of BenzSS
  - –based on X-ray crystal structures from these GREs



Homology Modeling Results

	BSSa	GDH	GRE (Arch Fulg.)	PFL	HPA-D
BSSa	-	30%	27%	26%	24%
GDH	0.7 Å	-	36%	28%	29%
GRE (Arch. Fulg)	1.1 Å	1.1 Å	-	23%	27%
PFL	1.1 Å	1.1 Å	1.2 Å	-	22%
HPA-D	1.2 Å	1.2 Å	1.3 Å	1.3 Å	-



- Significant structural similarity in spite of relatively low sequence identity
- 10 stranded α-β barrel around the active site is also conserved

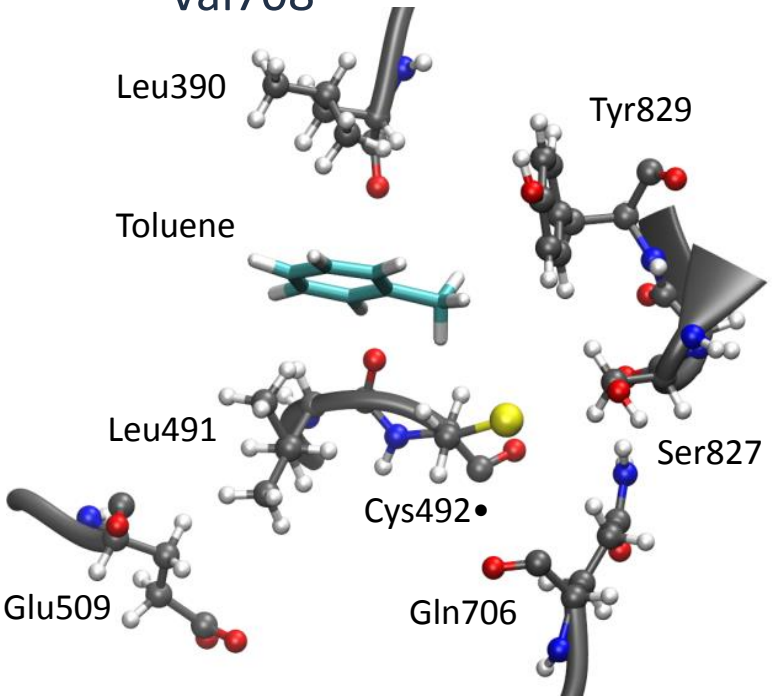
Vivek S. Bharadwaj, V. S., Dean, A. M., Maupin, C. M. (2013) JACS (accepted)

Molecular Dynamics Simulations

- Ensure stability of the homology model in a solvated environment
  - 185 ns MD simulations indicated a stable active site
- Evaluate critical enzyme-substrate interactions
  - Hydrogen bonding networks
  - Hydrophobic interactions
- Calculate binding energetics: Molecular Mechanics/Generalised Born Solvent Accessibility (MM/GBSA) calculations (40 ns)
  - Energy decomposition
  - Alanine mutational scanning
    - Evaluate contribution of amino acids to binding

Toluene Binding Pocket

- Hydrophobic residues aid toluene binding
  - –Leu390
  - –Tyr829
  - –Phe384
  - –Leu491
  - –Val708

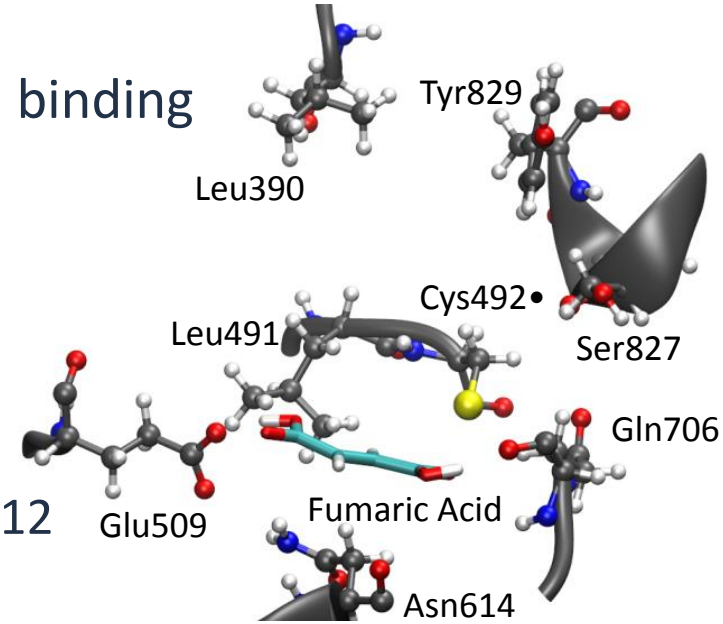


Residue	% Occupancy	
	Toluene	Toluene + Fumaric acid
Leu491	22.5%	3.6%
Leu390	14.3%	---
Val708	---	81.9%

- MD indicates feasible H-transfer distances
  - –~3.2Å between methyl group of toluene and Cys492•

Fumaric Acid Binding Pocket: Substrate Interactions

- Hydrogen bonding crucial for fumaric acid binding
- Toluene alters fumaric acid binding
- Critical amino acids
  - –Ser827, Glu509, Gln706 and Asn614, Gly512

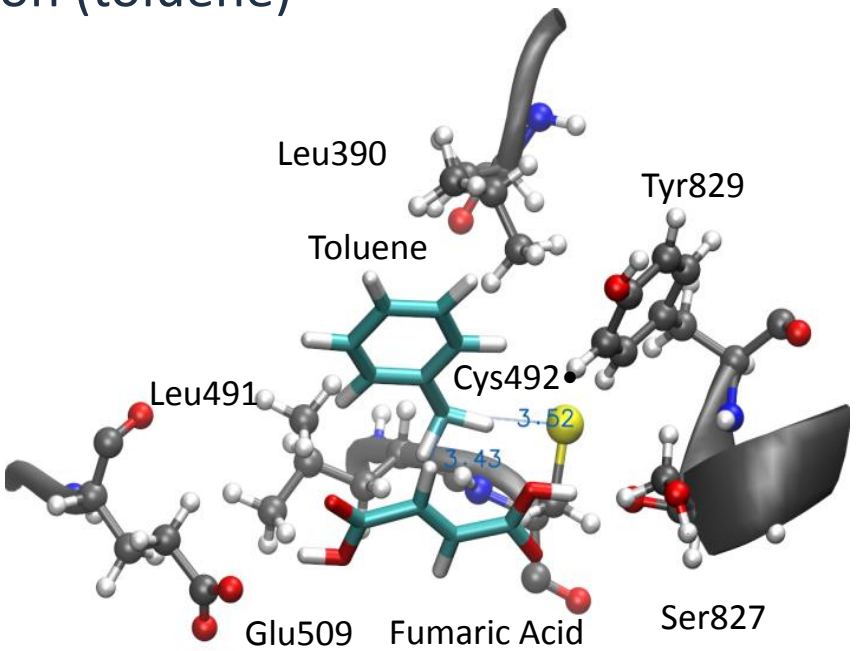


Residue	% Occupancy	
	Fumaric Acid	Fumaric acid + Toluene
Glu509	61.9%	81.9%
Ser827	---	55.4%
Gln706	81.9%	41.5%
Gln512	23.9%	---
Asn614	15.8%	---

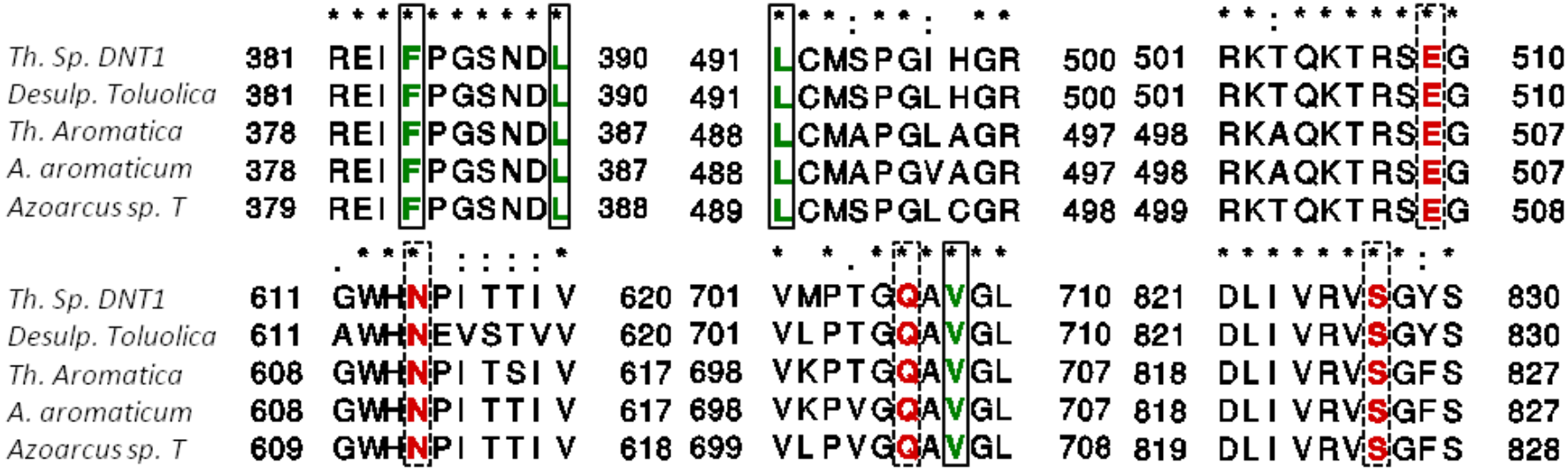
MM/GBSA & Alanine Scanning

- Binding energy calculations on mutants
- Evaluate specific residue contributions to binding energy
- Strong hydrogen bonding interactions (fumaric acid)
- Several weaker hydrophobic interaction (toluene)
- Proposed experimental mutations

Mutation	$\Delta\Delta G_{binding} = \Delta G_{mutant} - \Delta G_{wildtype}$
Effect on Toluene binding	
F384A	1.9 ± 0.9 kcal/mol
L491A	1.2 ± 0.7 kcal/mol
L390A	2.0 ± 0.8 kcal/mol
V708A	1.1 ± 0.6 kcal/mol
Y829A	1.8 ± 0.7 kcal/mol
Effect on Fumaric acid binding	
E509A	5.4 ± 1.9 kcal/mol
Q706A	3.9 ± 1.9 kcal/mol
S827A	5.7 ± 1.8 kcal/mol



Conserved Binding Site Residues

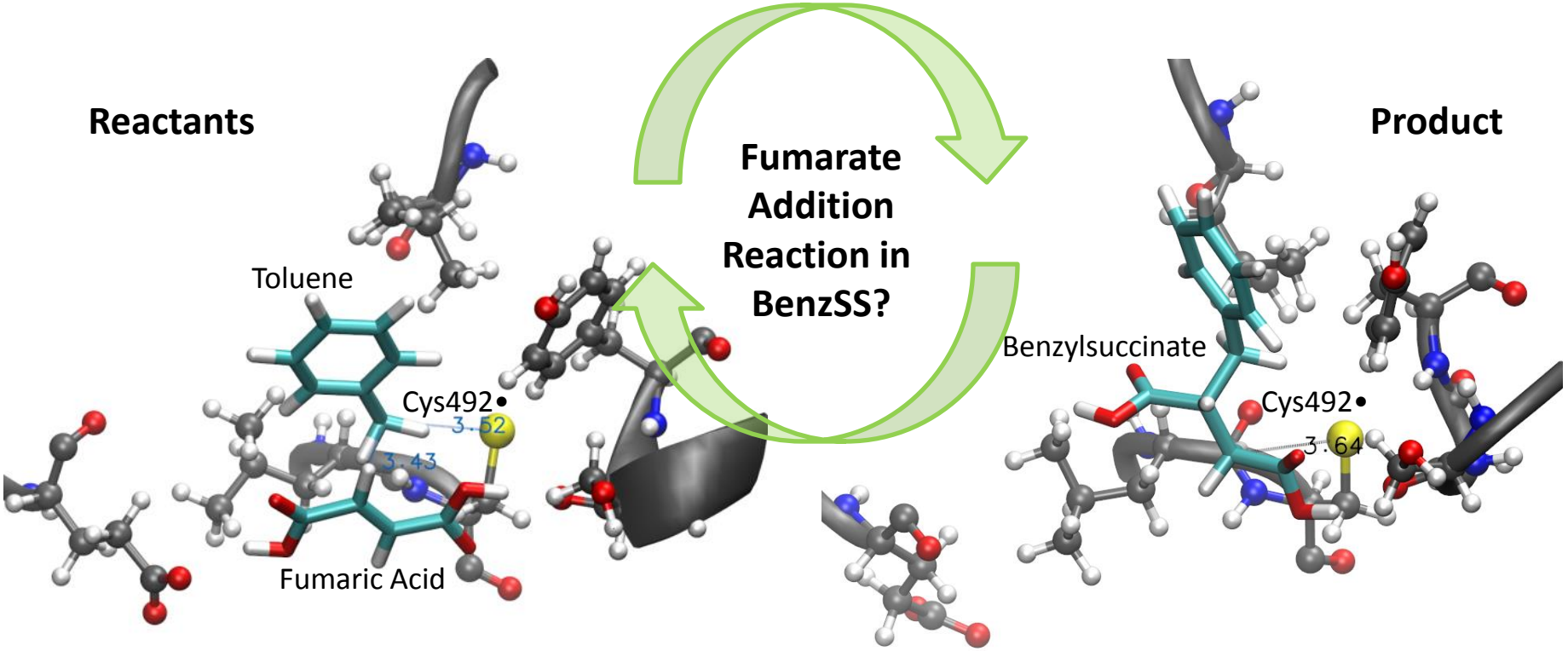


Hydrophobic residues aiding toluene binding (Phe 384, Leu390, Leu491 and Val 708) all are conserved. Tyr829 semi-conserved.

Hydrogen-bonding residues aiding fumaric acid binding (Ser827,Glu509 Asn614 and Gln706) are also conserved

Is the Structure Suitable for Catalysis?

- Hydrogen transfer distances in enzymes found to vary from 3.5 – 4.1 Å



- Radical transfer distances similar to QM calculations

MD Simulations Indicate Feasible H-transfer Distances

- Productive radical transfer distances consistently observed in MD

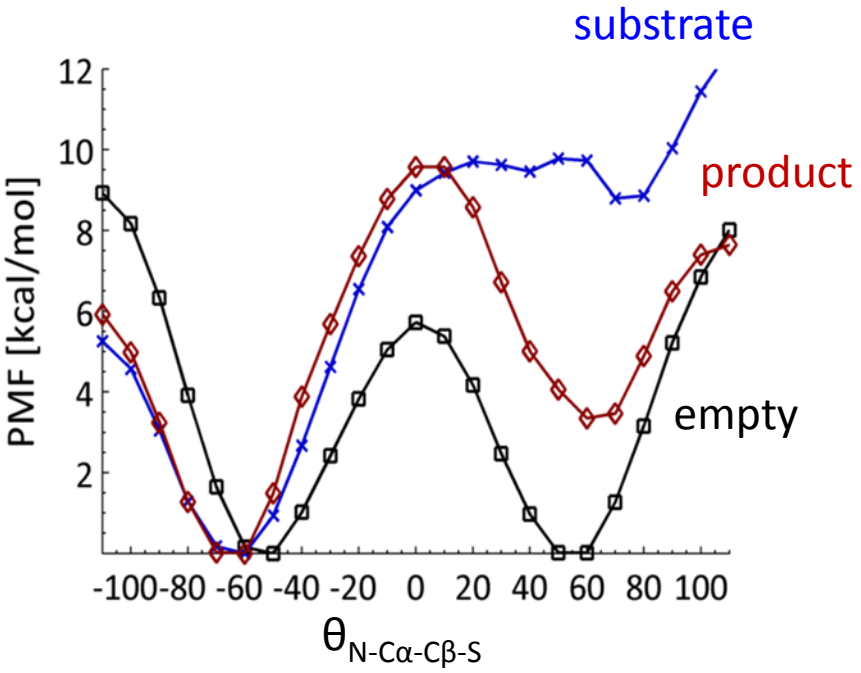
System	Cys492•S – Gly828Cα		Cys492•S – Toluene	
	Average (Å)	Productive*	Average (Å)	Productive*
BenzSS + Fumaric Acid	6.7 ± 1.2	10.5%	-	-
BenzSS + Toluene	5.6 ± 1.1	15.3%	6.7 ± 1.5	10.8%
BenzSS + Toluene + Fumaric Acid	4.7 ±1.1	51.4%	5.3 ± 0.7	5.8%

* <4 Å distance is considered productive

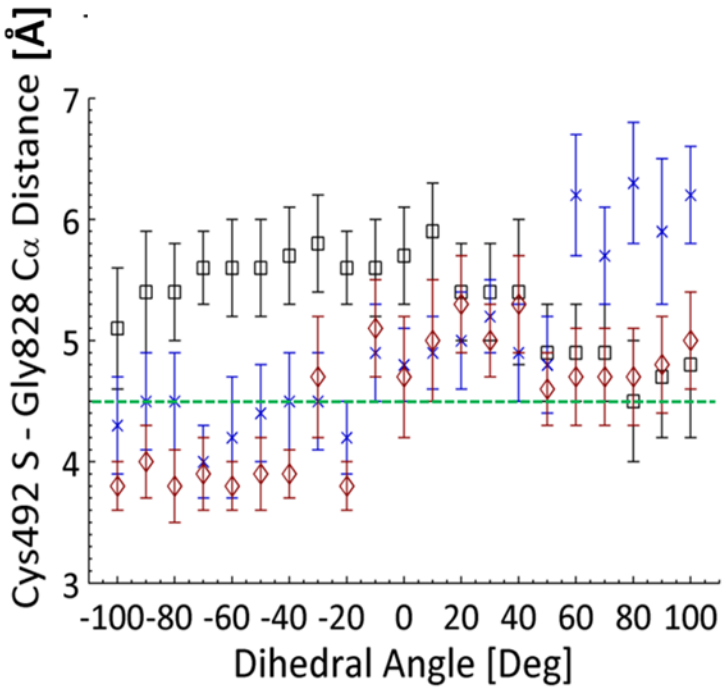
- Presence of both substrates leads to compact active site

- **Sets the stage for QM/MM studies to re-evaluate the energetics in the protein environment**

Dihedrals, Distances, and Feasible H-transfer



- Different free energy profiles
- Two distribution (empty)
- One distribution (substrate)



- Substrate & Product favor smaller dist.

Conclusions: Homology Modeling, Docking, and MD

- Consistent enzyme structural basis established via
  - Homology modeling, docking studies, MD simulations and MM/GBSA binding energetics
- Important amino acids for substrate binding identified
  - Toluene binding
    - Leu491, Leu390, Phe384, Val708, Tyr829
  - Fumaric acid binding
    - Ser827, Glu509, Gln706
- Binding sites are conserved
- Binding of substrate shifts preferred dihedral to favor smaller H-transfer distances

Questions?

Acknowledgements

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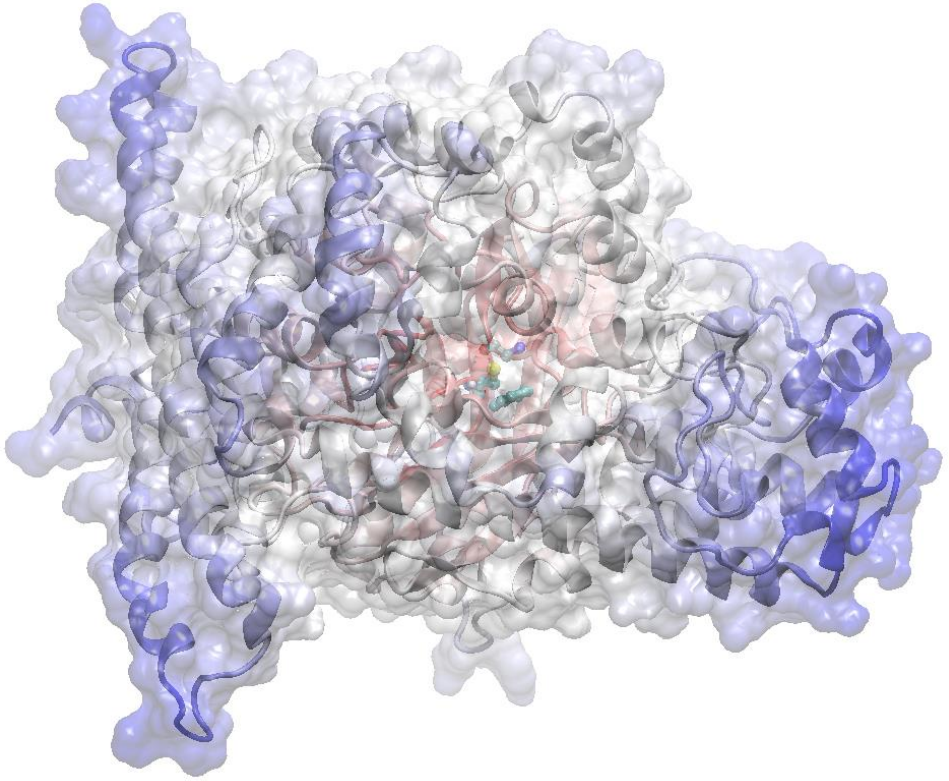

BSS Homology Model