

# Dr. Frank M. Raushel

Professor of Chemistry,  
Texas A&M University

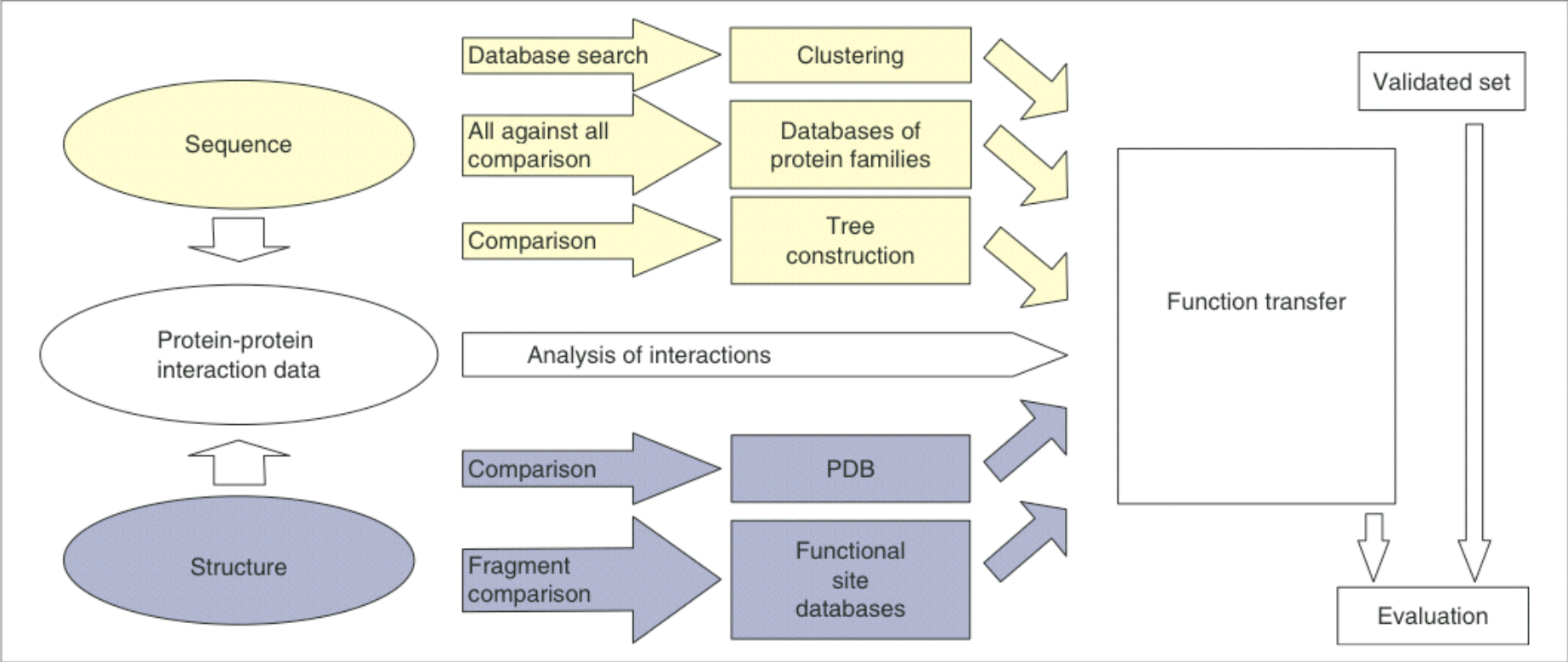


Deciphering Enzyme Specificity  
VICB Seminar April 8, 2009

# Dr. Raushel's Education and Experience

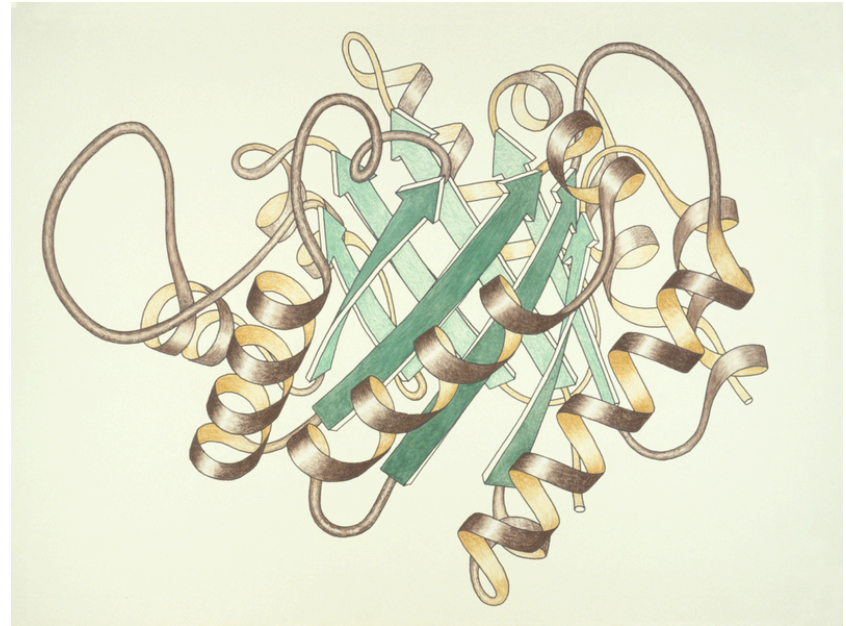
- B.A. College of St. Thomas, Chemistry (1972)
- Ph.D. University of Wisconsin-Madison, Biochemistry (1976)
- Post-Doctoral: Pennsylvania State University  
Advisor: J. J. Villafranca (1976-1980)
- Assistant (1980), Associate (1986), Professor of Chemistry (1989-present) Texas A&M University

# Strategies for Assigning Protein Function



# Amidohydrolase Superfamily

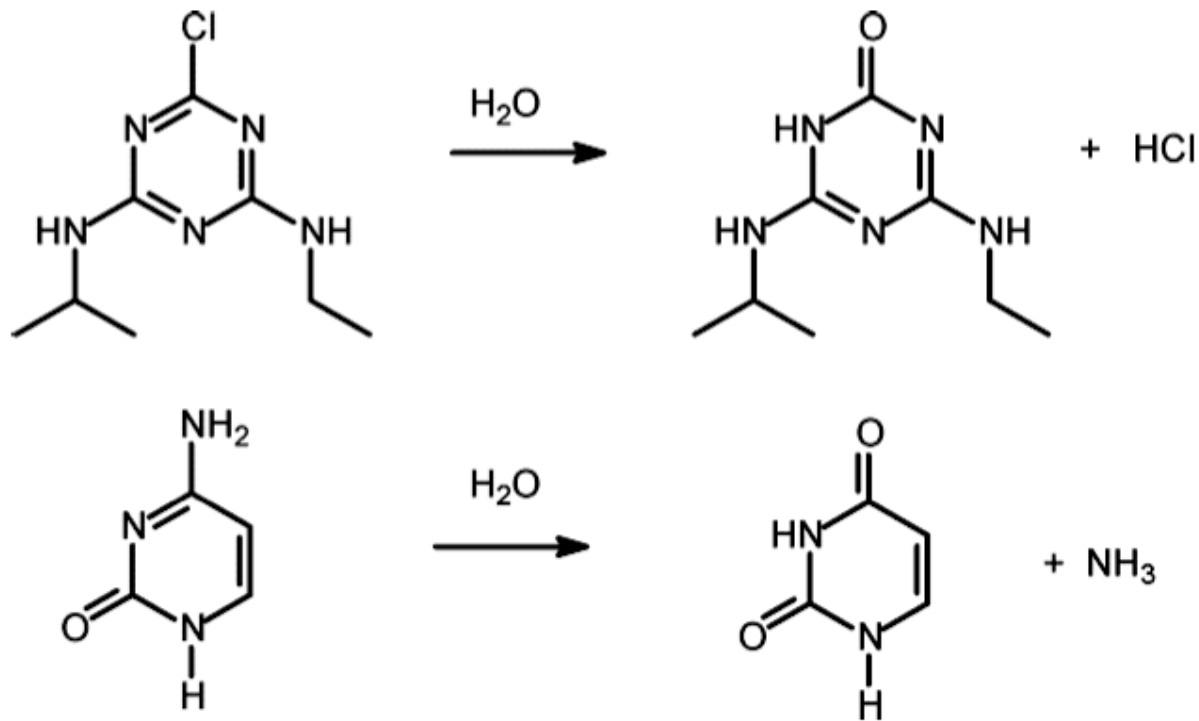
- ~ 6,000 catalogued members,  
> 30 known reactions
- Generally catalyze hydrolysis of amide and ester bonds in carboxylate and phosphate substrates
- Provide a subset of proteins ideal for exploring techniques of predicting substrate specificity for enzymes of unknown function



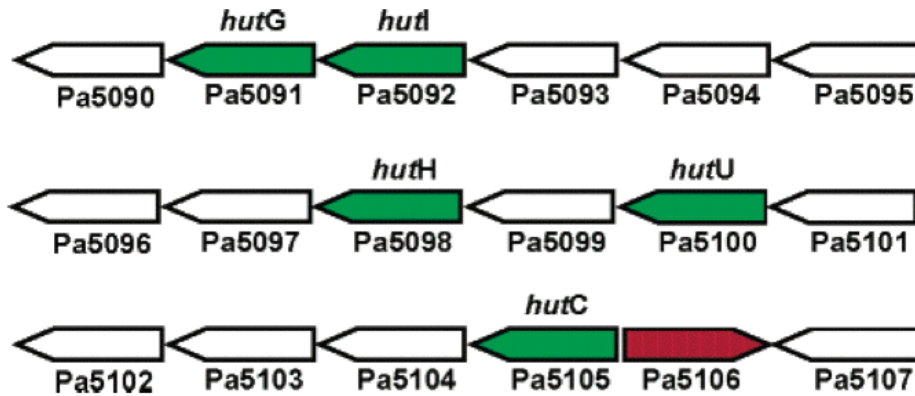
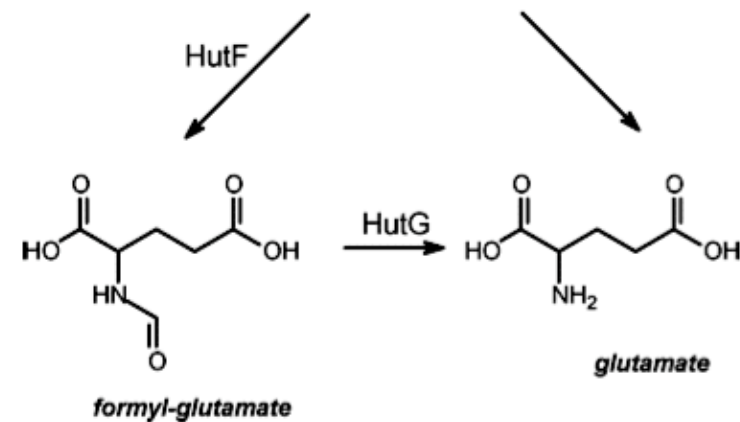
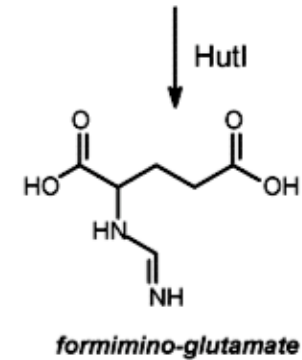
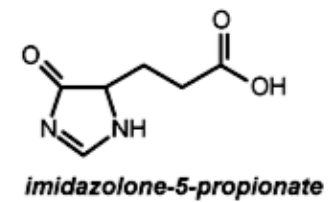
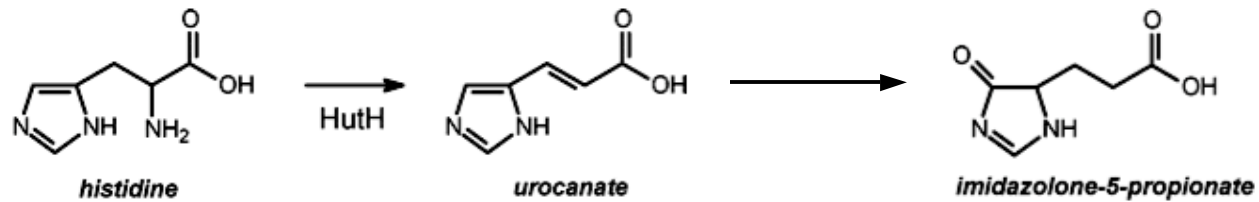
$(\beta/\alpha)_8$ -Barrel Fold

# Amidohydrolase Superfamily

Due to sequence homology, most AHS members of unknown function are annotated as *probable chlorohydrolases* or *probable cytosine deaminases*.



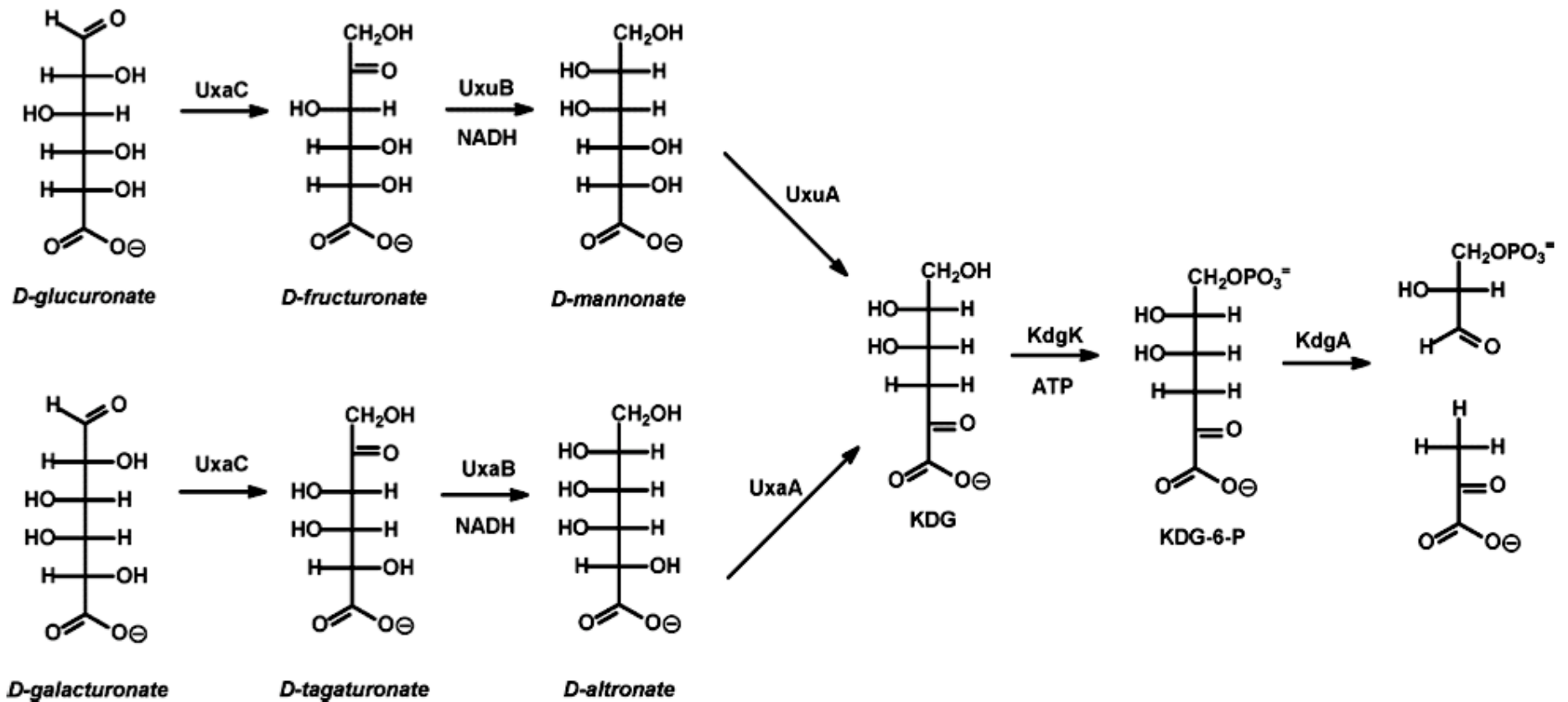
# Genomic and Operon Context



Pa5106 = HutF

# Genomic and Operon Context

URIs initiate uronic acid degradation; most bacteria have only one.



# Genomic and Operon Context

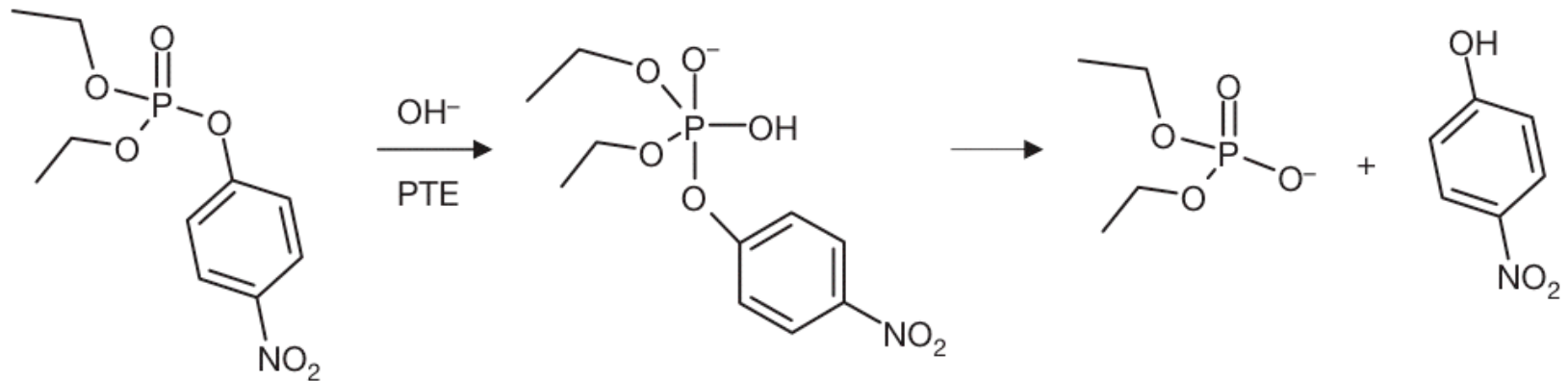
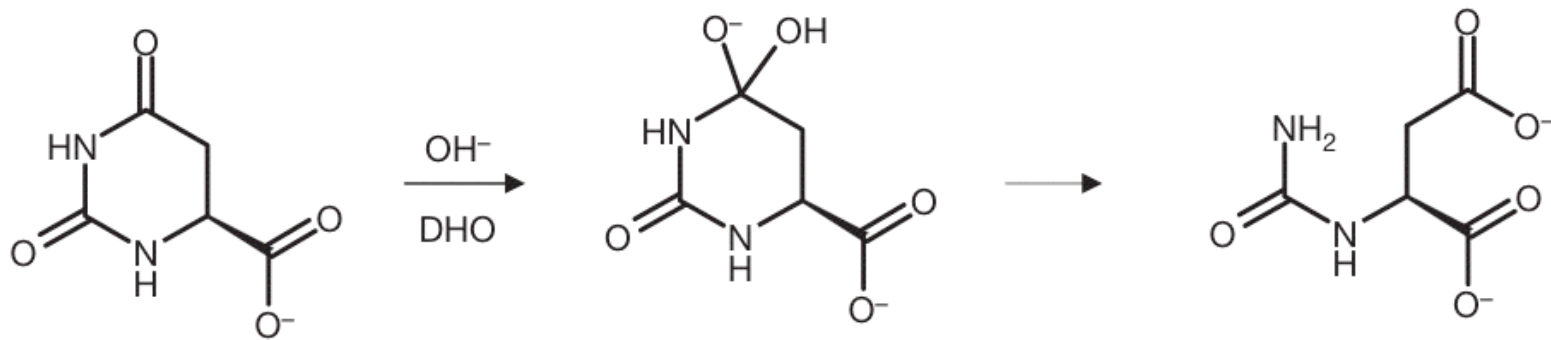
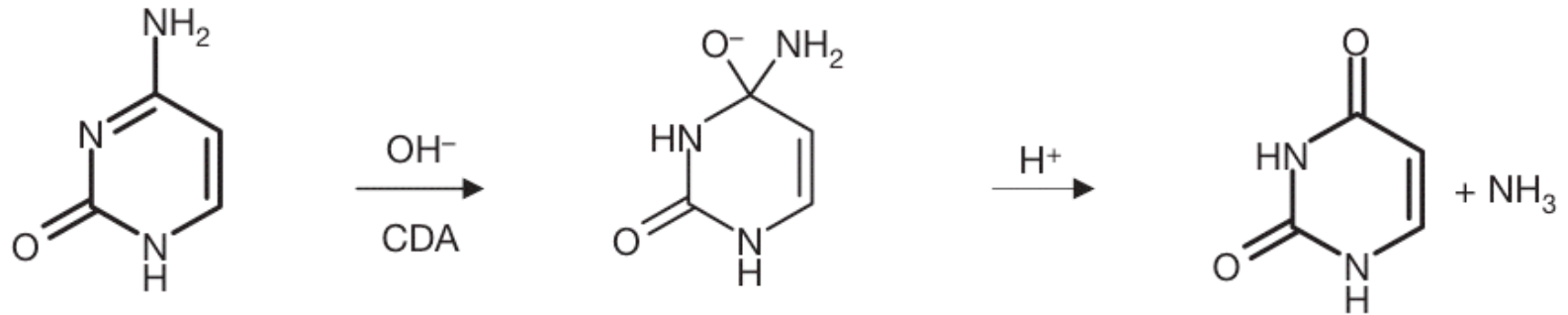
## Bh0493

- Most divergent AHS member in terms of sequence
- Located in proximity to D-galacturonate specific genes
- Recognizes both D-glucuronate and D-galacturonate equally

## Bh0705

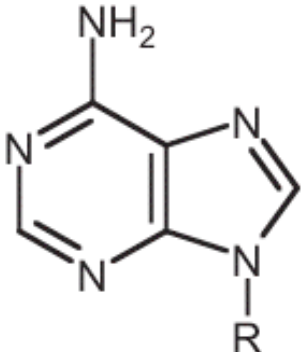
- More closely related to canonical URIs
- Located in proximity to D-glucuronate specific genes
- Relatively specific for D-glucuronate

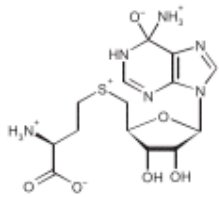
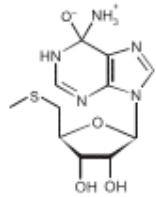
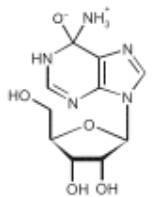
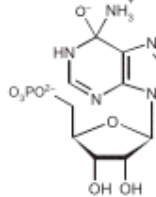
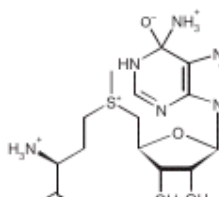
# Computational Docking



# Computational Docking

- Retrospective docking experiments
- Prospective prediction of substrate specificity for AHS member of unknown function (Tm0936)
- Seems to prefer adenine and adenosine analogues

Analogues in docking hit list	Top 10 ranked hits	Top 20 ranked hits	Top 100 ranked hits	Top 300 ranked hits
Adenine analogues				
	9	17	32	44

Substrate tested	Docked high-energy intermediate form	Dock rank	Relative docking scores (kcal mol <sup>-1</sup> )*	K <sub>m</sub> (μM)	k <sub>cat</sub> (s <sup>-1</sup> )	k <sub>cat</sub> /K <sub>m</sub> (M <sup>-1</sup> s <sup>-1</sup> )
S-adenosyl-L-homocysteine		5	0	210 ± 40	12.2 ± 0.8	5.8 × 10 <sup>4</sup>
5-Methyl-thioadenosine		6	4.4	44 ± 4	7.2 ± 0.2	1.4 × 10 <sup>5</sup>
Adenosine		14	9.5	250 ± 40	2.3 ± 0.2	9.2 × 10 <sup>3</sup>
Adenosine-5-monophosphate		80	20.2	ND	<10 <sup>-3</sup>	ND
S-adenosyl-L-methionine		511	35.2	ND	<10 <sup>-3</sup>	ND

# Computational Docking

- Some caveats:
  - Process is simplified by restriction to AHS and associated metabolites.
  - Tm0936 shows little conformational change along reaction coordinate.
- However:
  - Most other enzymes of unknown function also can be assigned to a broad class, allowing restriction to a subset of metabolite candidates.
  - Retrospective studies suggest that the challenge of conformational change is not insurmountable.

# Computational Docking

- Dr0930 identified as AHS member by 30% identity to *P. diminuta* phosphotriesterase
- Crystal structure determined; docking identified  $\gamma$ - and  $\delta$ -lactones as substrates
- Also showed weak PTE activity
- Two active-site mutations in Dr0930 conferred increased PTE activity
- Organophosphate triesters are not natural; introduced into environment mid-20th century
- Modern PTEs may have evolved from lactone-hydrolyzing AHS members

# References

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