Conformational Changes and Substrate Recognition in *Pseudomonas aeruginosa* D-Arginine Dehydrogenase

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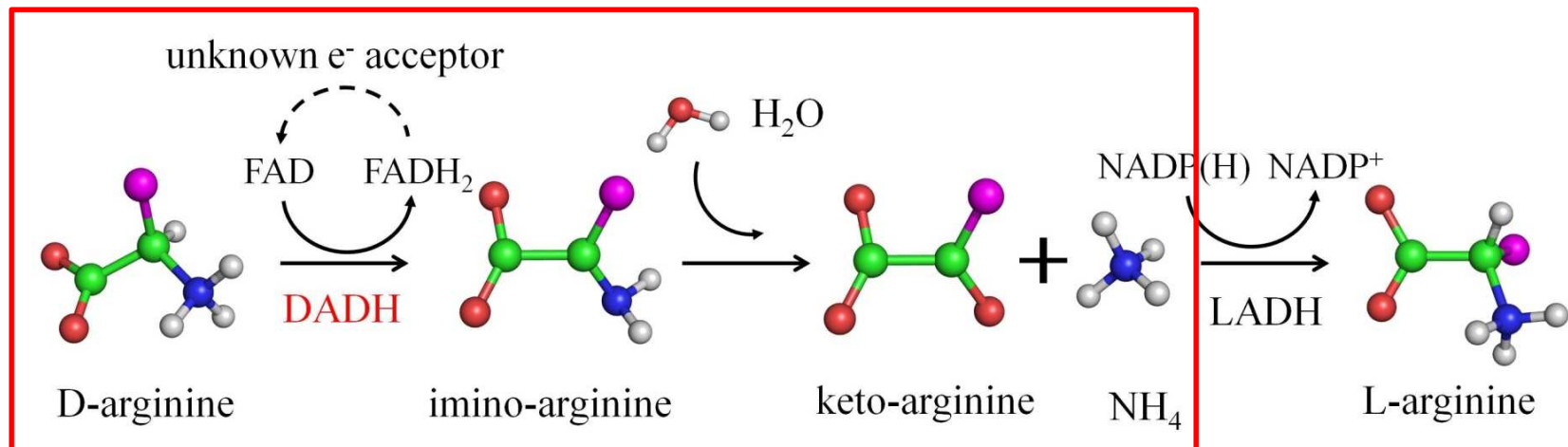# Biological function of D-amino acids

- ## L-amino acids
  - Building blocks of proteins.

- ## D-amino acids
  - Bacteria cell wall synthesis (D-Ala, D-Glu)

  - Trigger bacteria biofilm disassembly (D-Tyr, D-Met, D-Trp, D-Leu. Kolodkin-Gal et al., *Science*, 2010)

  - Related to brain development and neural signaling in mammals (D-Ser, D-Asp, Yoshimura et al., *J Biosci Bioeng*, 2003)

D-Alanine   L-Alanine

Mirror

COOH   C   CH₃   NH₂   H
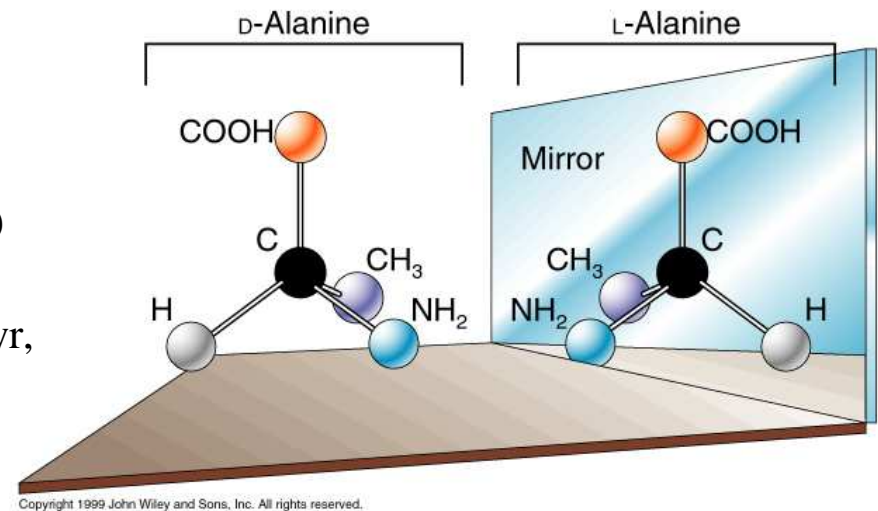
- ## L- and D- amino acids are inter-converted by enzymes like D-amino acid oxidase.

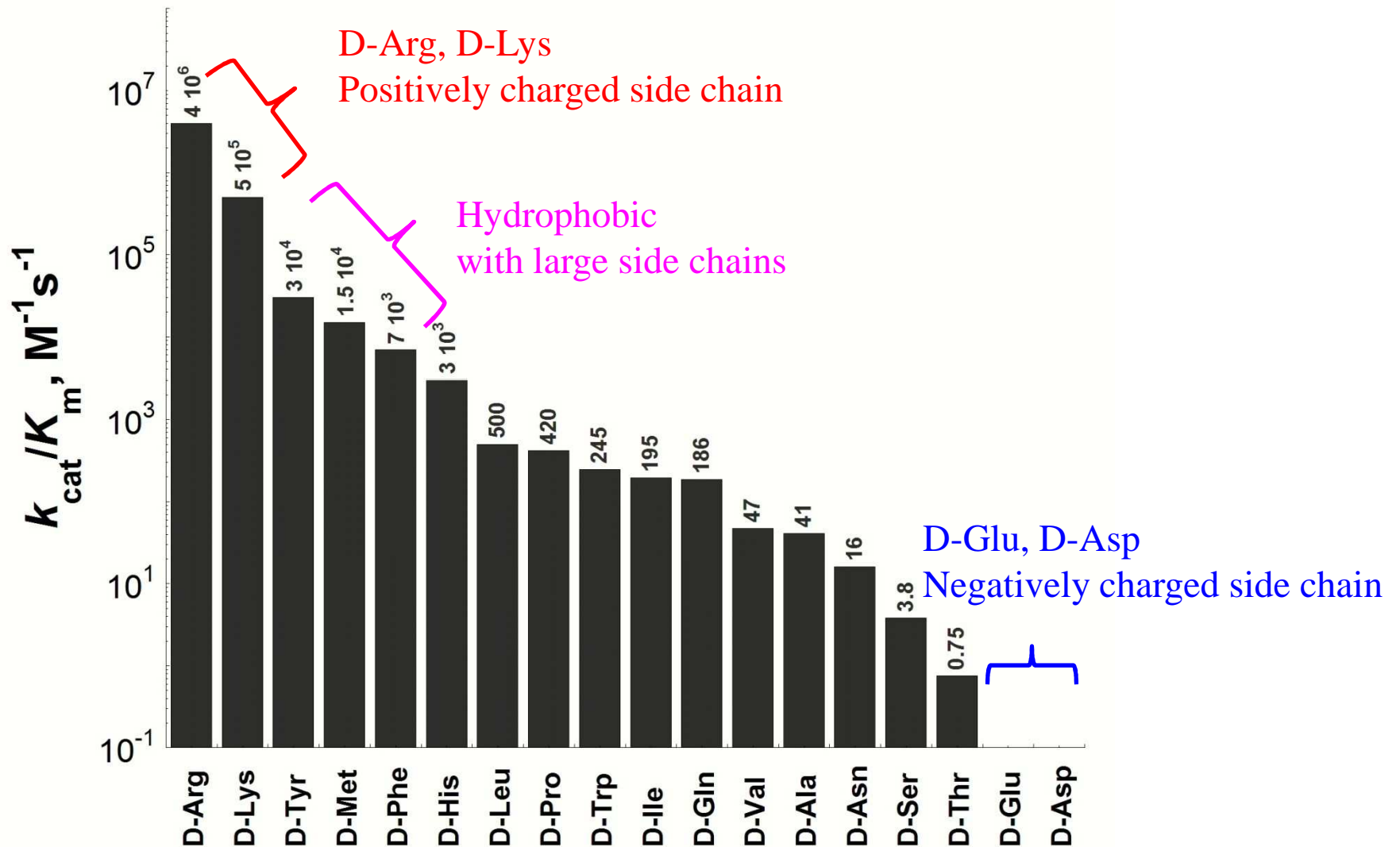# D-arginine dehydrogenase

- *dauBAR* operon in *P.aeruginosa* is highly induced by D-arginine.

- D-arginine dehydrogenase (DADH) and L-arginine dehydrogenase (LADH) perform D-to-L inversion of Arginine.
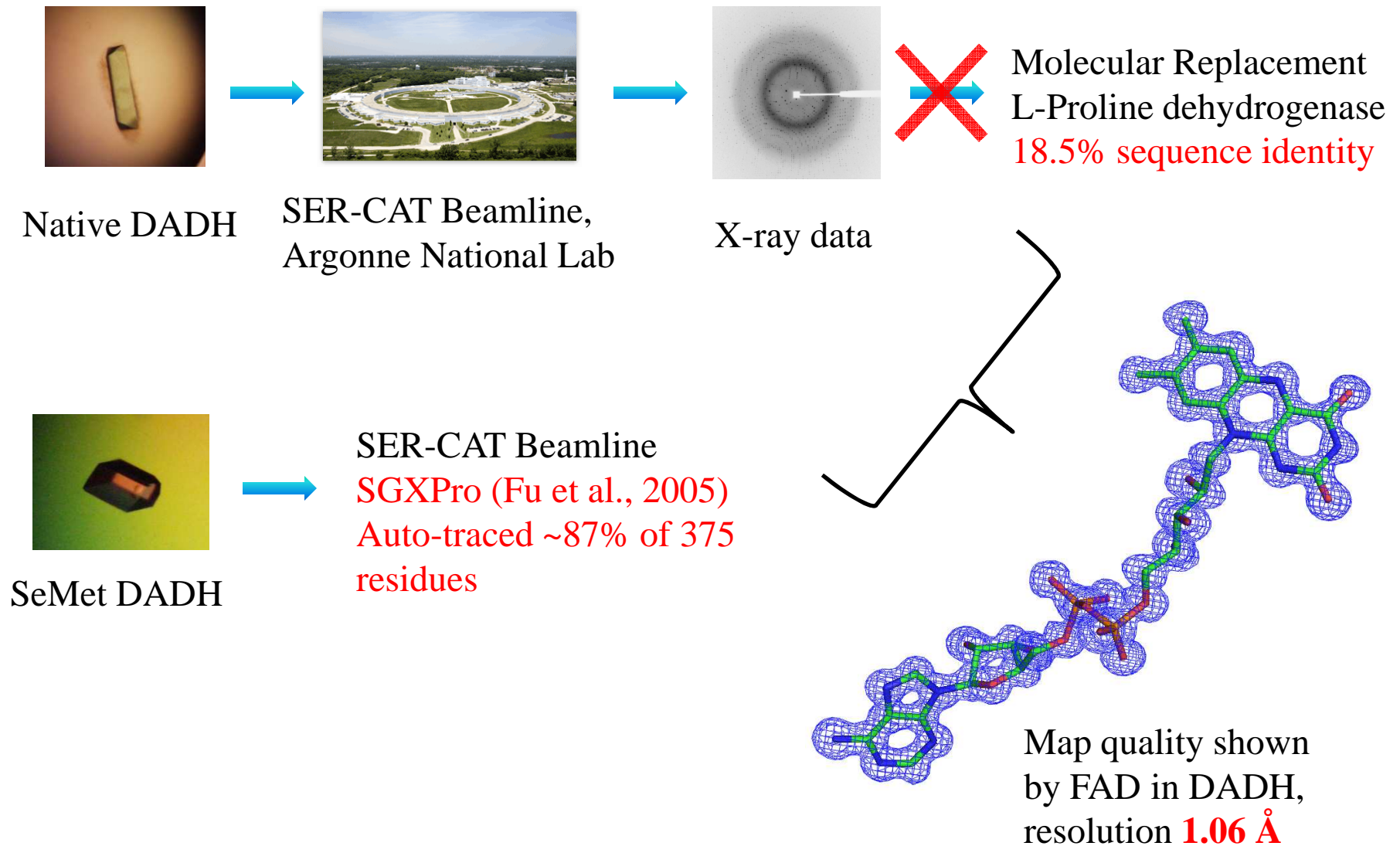

(Li and Lu, *PNAS*, 2009)

DADH has a broad substrate specificity

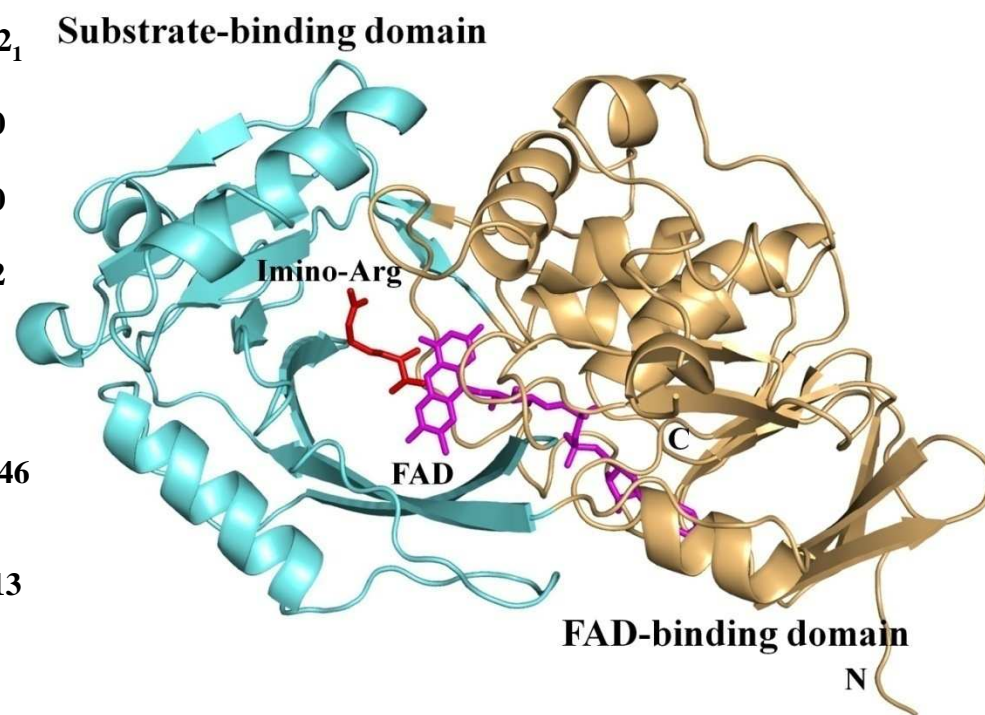Li et al., *Microbiology*, 2010; Fu et al., *Biochemistry*, 2010

What is the molecular basis for recognition of D-amino acids by DADH?
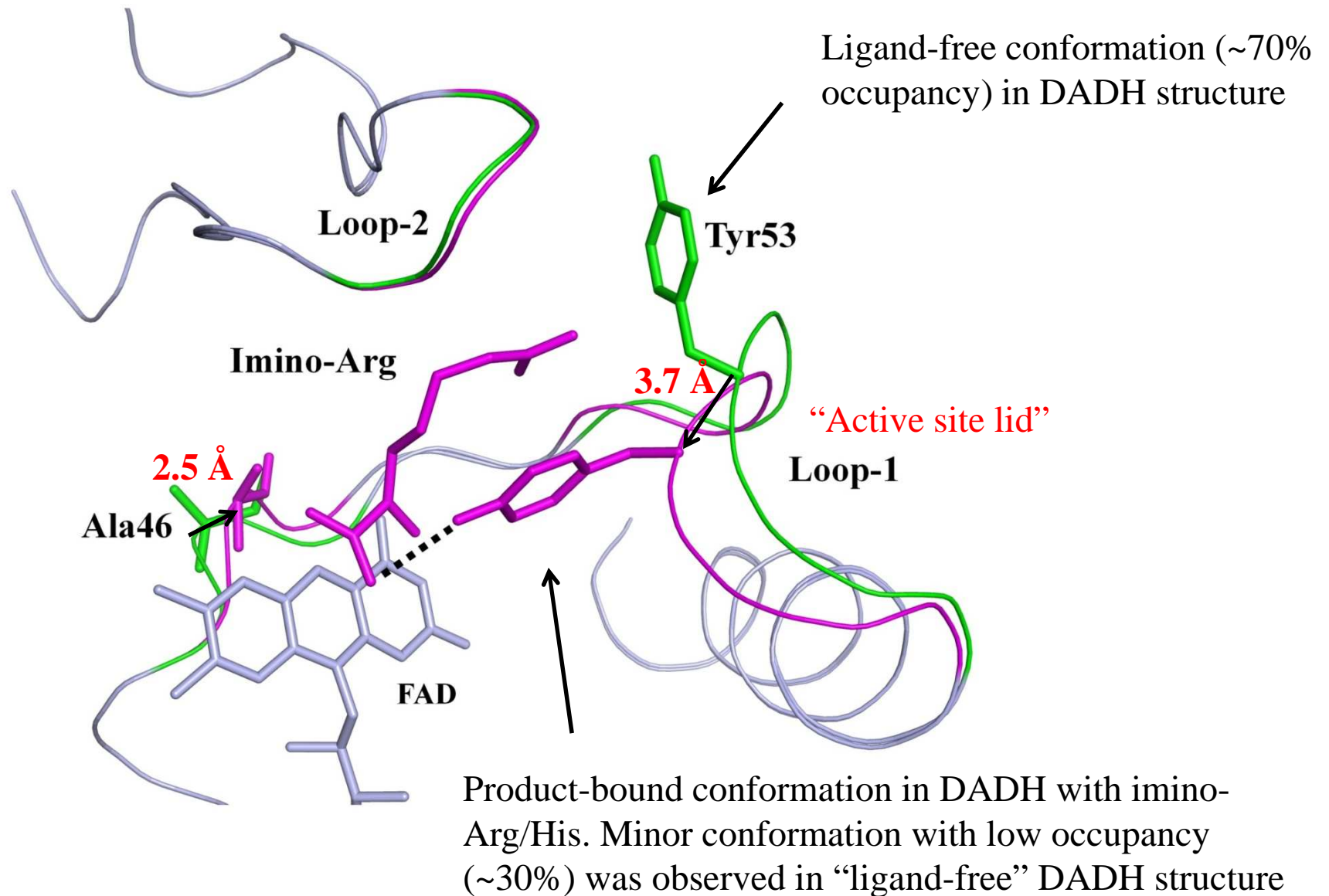
Crystallographic Method

Native DADH

SER-CAT Beamline,
Argonne National Lab

X-ray data

Molecular Replacement
L-Proline dehydrogenase
18.5% sequence identity

SeMet DADH

SER-CAT Beamline
SGXPro (Fu et al., 2005)
Auto-traced ~87% of 375
residues

Map quality shown
by FAD in DADH,
resolution 1.06 Å

Crystal Structure of DADH/imino acids

	DADH	DADH/ imino-Arg	DADH/ imino-His	DADH/ imino-Phe
Space group	$P\,2_12_12_1$	$P\,2_12_12_1$	$P\,2_12_12_1$	$P\,2_12_12_1$
a (Å)	62.18	62.17	62.10	62.20
b (Å)	78.08	78.43	78.15	78.40
c (Å)	89.72	89.95	89.59	89.92
Resolution (Å)	1.06	1.30	1.30	1.05
Total observation	1258,212	600,949	627,618	1296,946
Unique reflections	190,545	98,021	97,110	203,913
Completeness (%)	96.2	90.6	90.4	99.9
R-work (%)	13.2	13.4	12.8	14.6
R-free (%)	15.7	16.6	16.3	17.1

DADH/imino-Arg, resolution 1.30 Å

Fu et al., *Biochemistry*, 2010

Conformational changes upon substrate binding

Loop-2

Imino-Arg

2.5 Å

Ala46

Tyr53

3.7 Å

"Active site lid"

Loop-1

FAD

Ligand-free conformation (~70% occupancy) in DADH structure

Product-bound conformation in DADH with imino-Arg/His. Minor conformation with low occupancy (~30%) was observed in "ligand-free" DADH structure
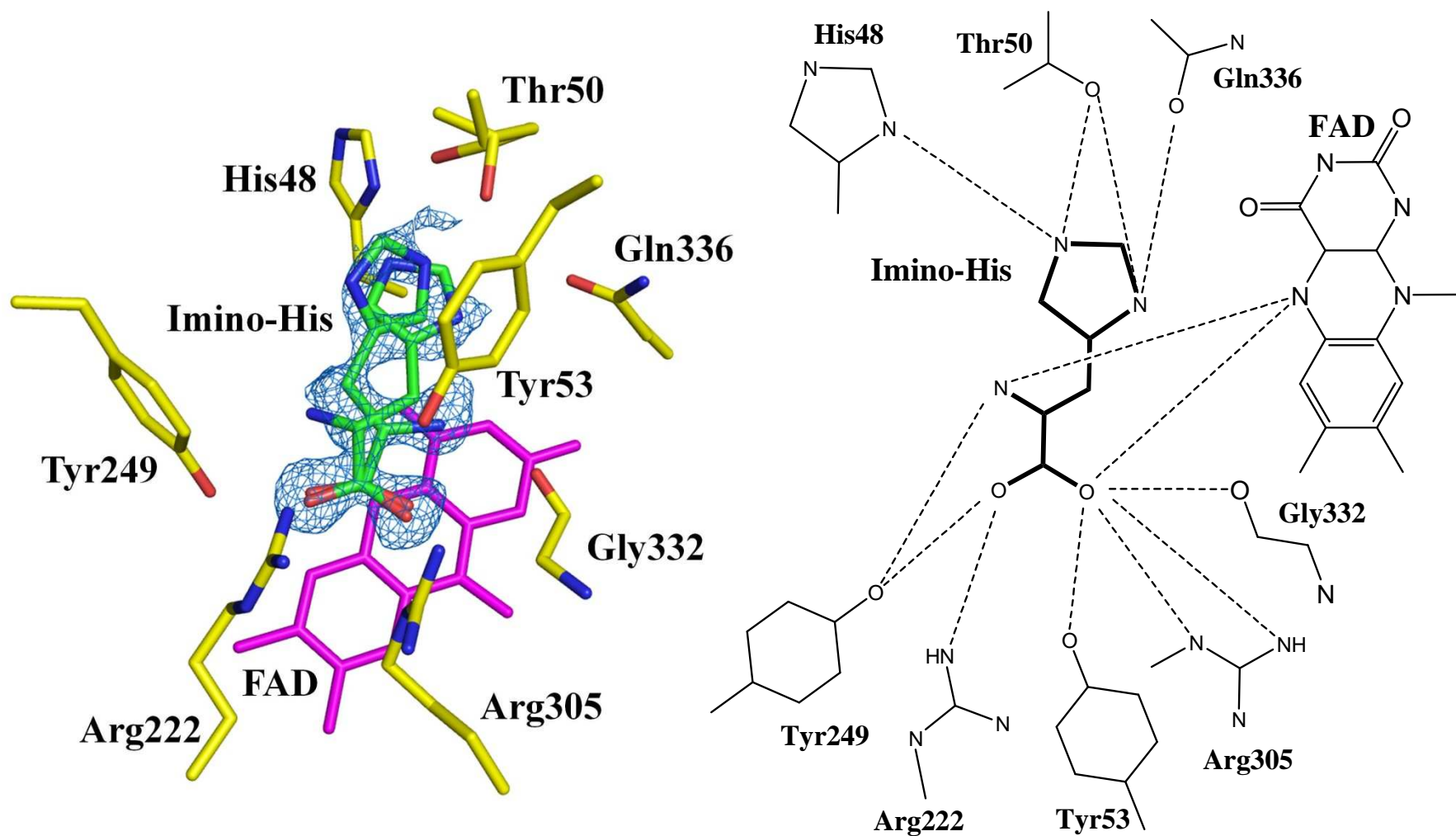
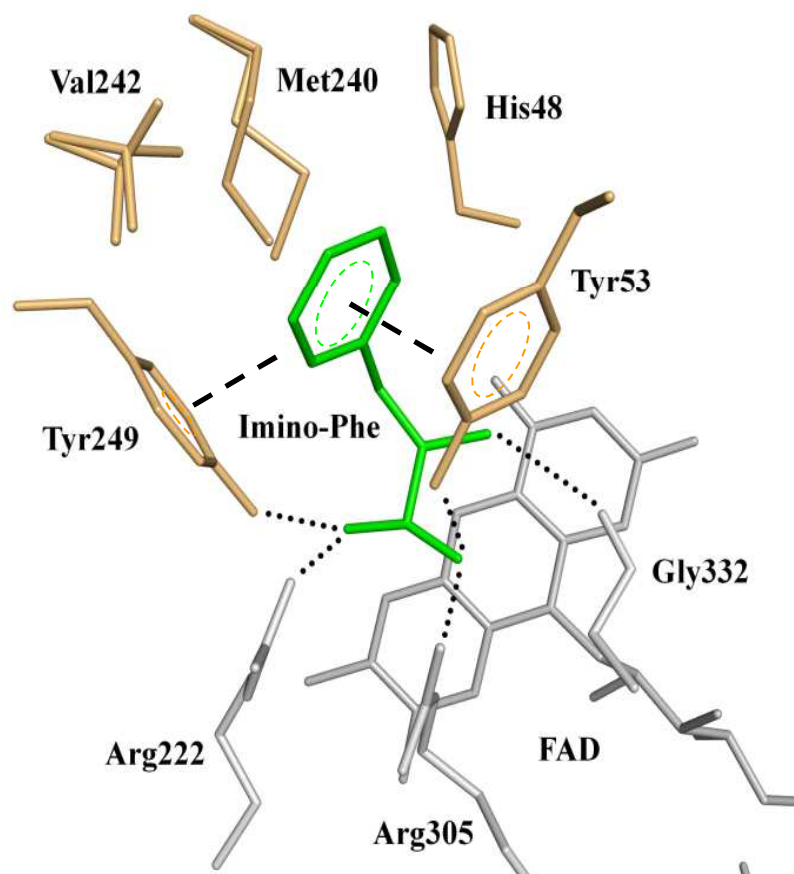# DADH interactions with imino-Arg
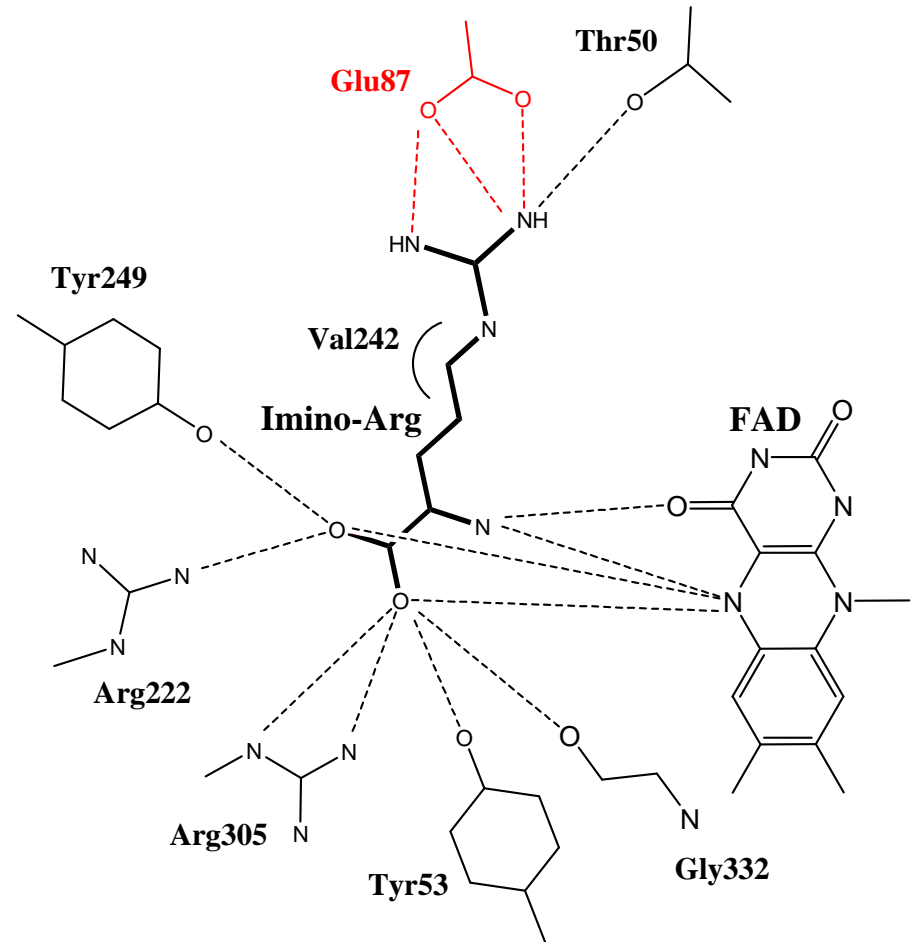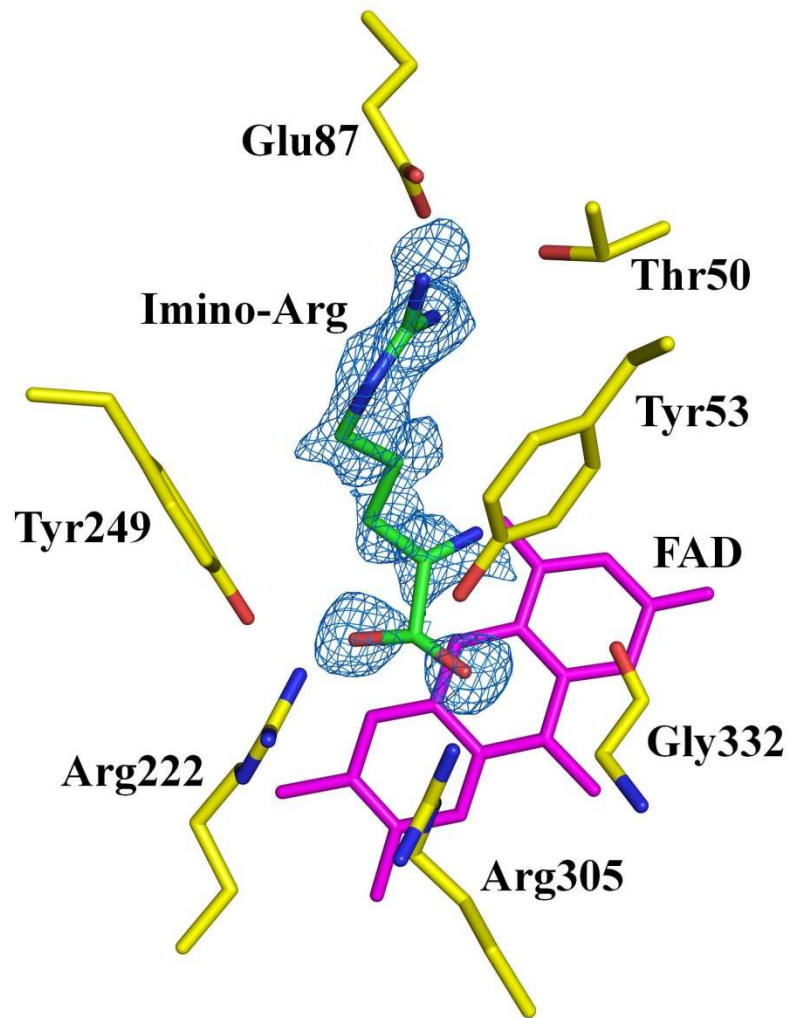
DADH interactions with imino-His
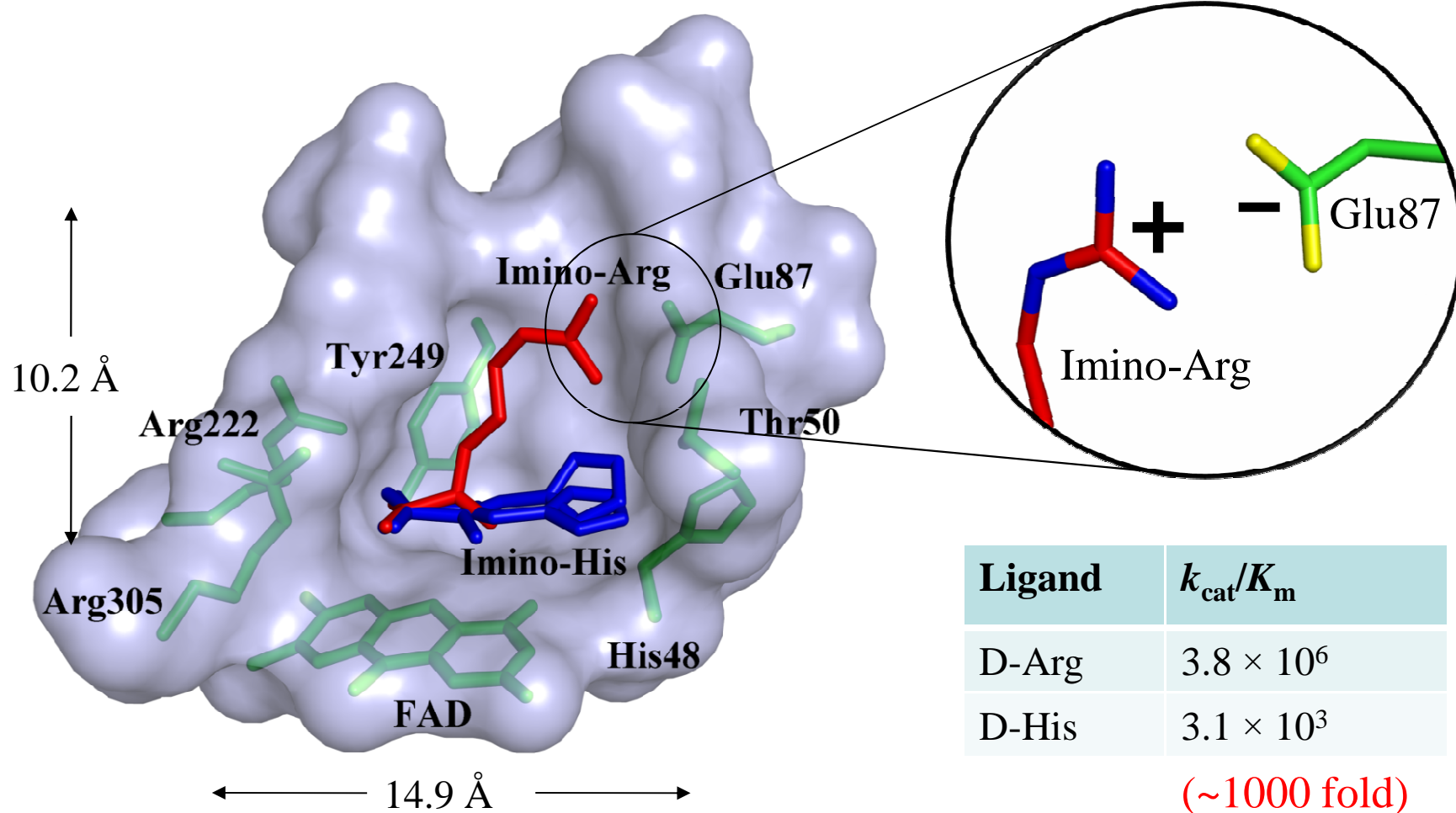
DADH interactions with imino-Phe

DADH/imino-Phe (1.05 Å)

Active site hydrophobic wall: Tyr53, Met240, Val242, and Tyr249

Side chain of imino-Phe forms:
parallel-displaced π-stacking with Tyr53
T-shaped π-stacking with Tyr249

Ligand	k_{cat}/K_m
D-Arg	3.8×10^6
D-Phe	6.9×10^3

(~550 fold)

D-Arg and D-His bind to DADH in distinct patterns

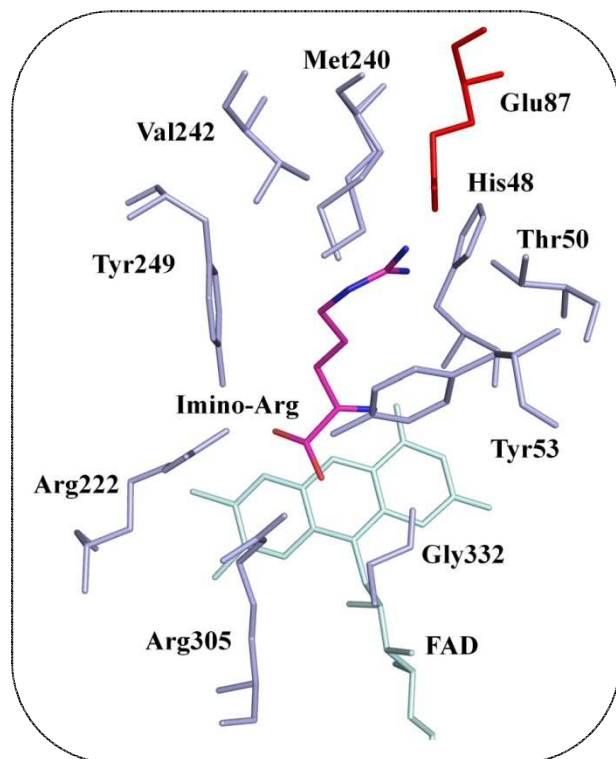| Ligand	k_{cat}/K_m
D-Arg	3.8×10^6
D-His	3.1×10^3

(~1000 fold)

Tyr53, Gly332 and Gln336 are removed to view the internal active site

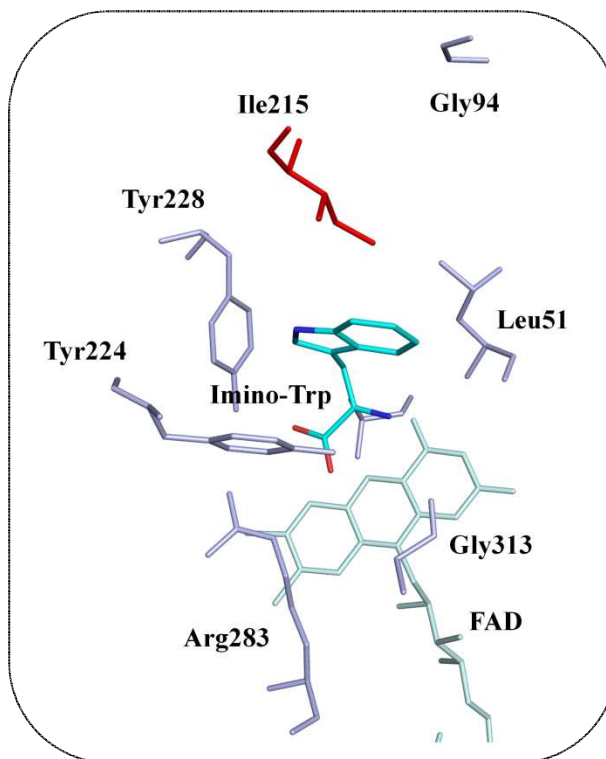Fu et al., *Biochemistry*, 2010
Yuan et al., *Biochemistry*, 2010

Comparison with related enzymes
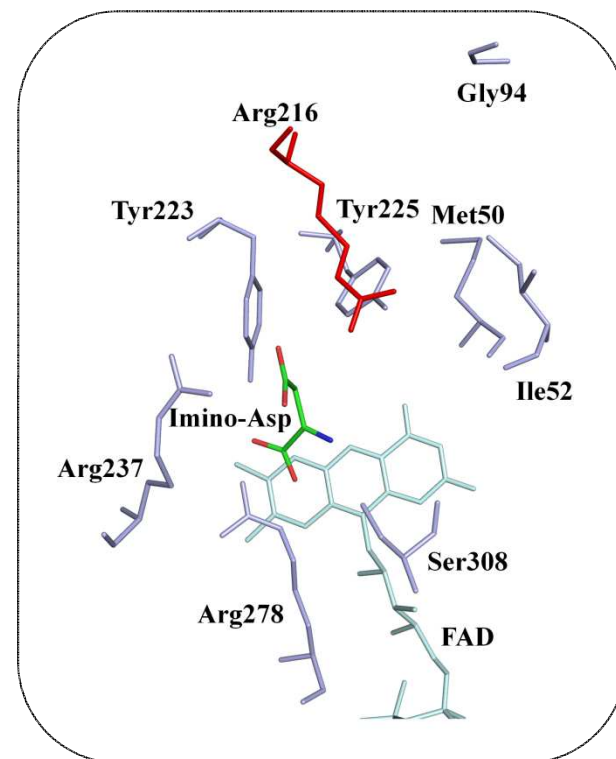

DADH/imino-Arg (1.30 Å)

D-amino acid oxidase/
imino-Trp (3.10 Å)

D-aspartate oxidase/
imino-Asp (Model)

Positive charge

(Fu et al., 2010)

Hydrophobic

(Todone et al., 1997, Setoyama
et al., 2006)

Negative charge

(Katane et al., 2011)

Conclusions

- The active site of DADH allows it to accommodate different D-amino acids.

- Glu87 is critical for DADH preference for positively charged residues (D-Arg, D-Lys).

- The conformational change of "active site lid" is involved in substrate binding and product release.

- Key structural features are important to the distinct substrate specificity of DADH, D-amino acid oxidase and D-aspartate oxidase
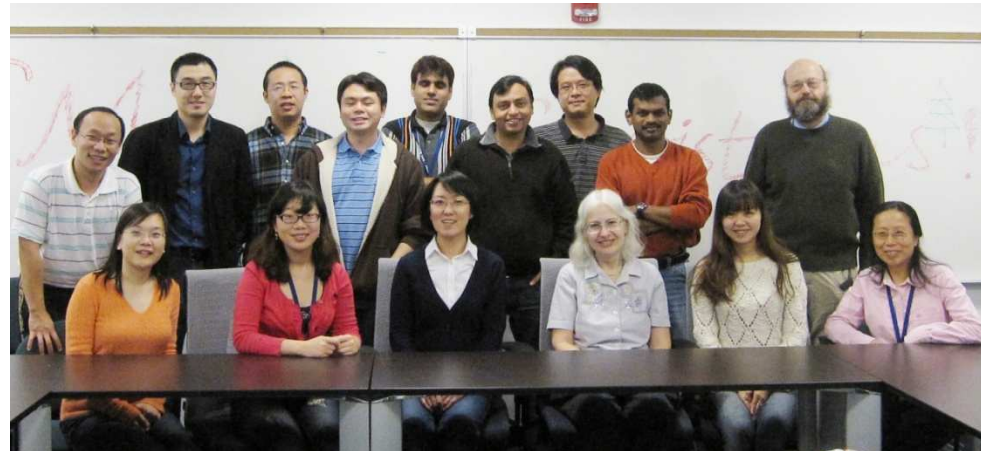
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