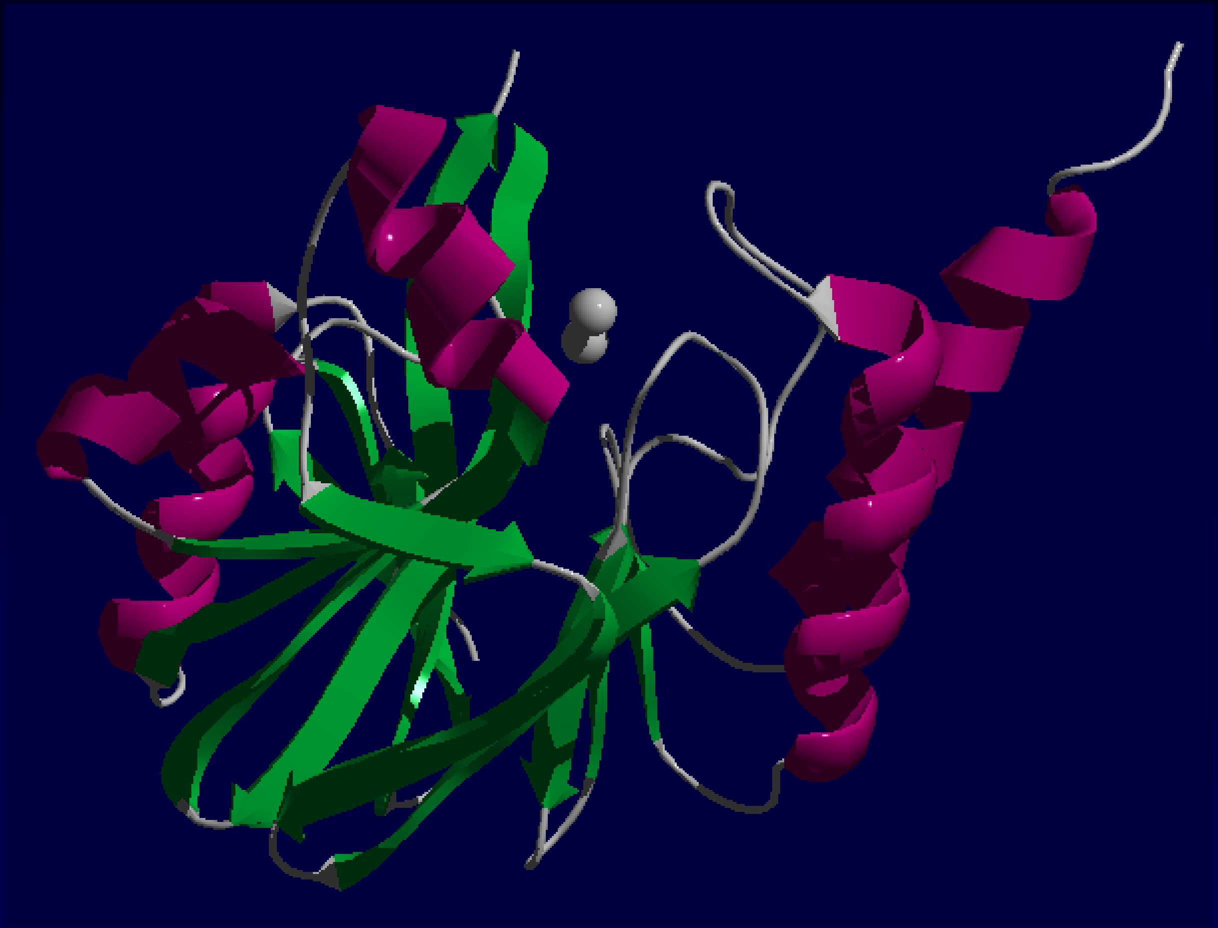
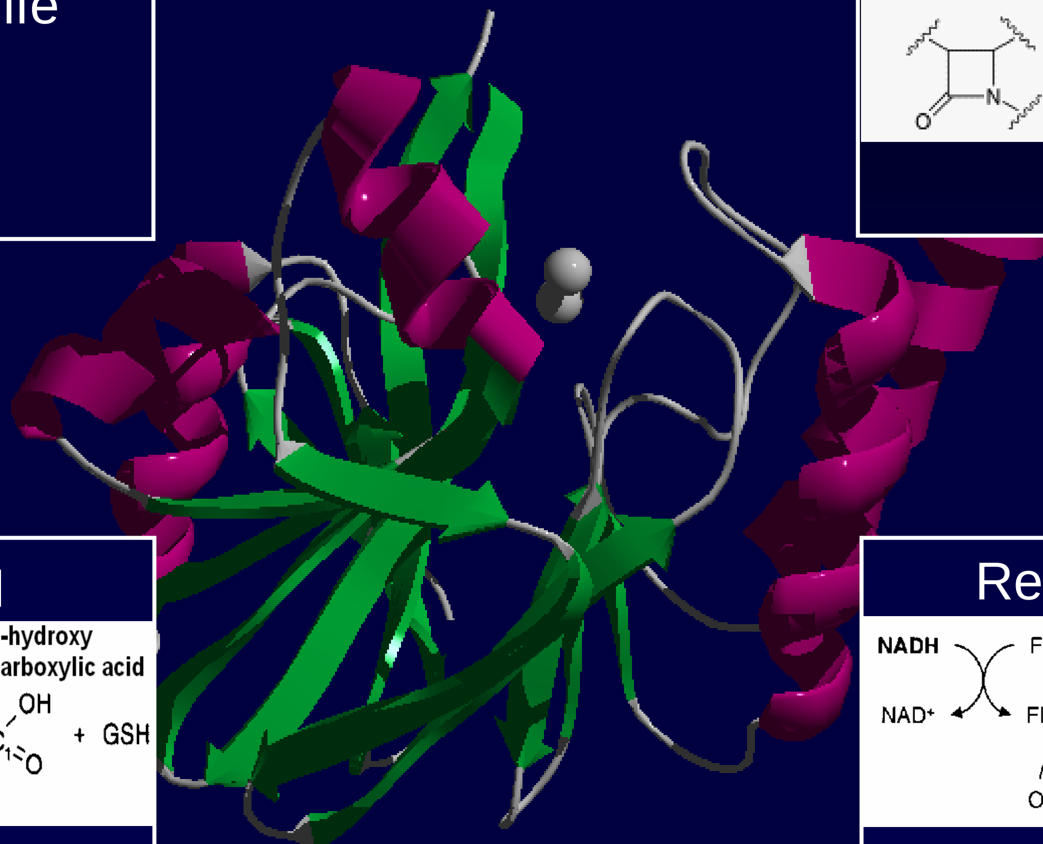
Functional diversity and metal selectivity of proteins sharing the metallo-β-lactamase fold



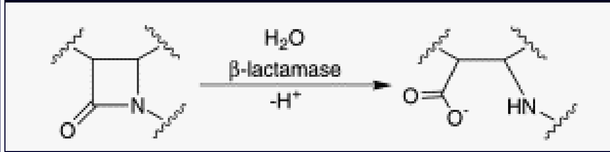
Oliver Schilling

Metallo-β-Lactamase Fold

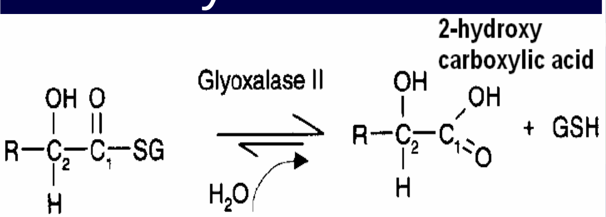


ElaC
- all 3 domains of life
- highly conserved
- uncharacterized (year 2000)

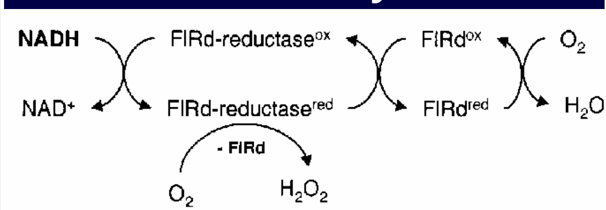
"real" lactamases



Glyoxalase II



Redox enzymes

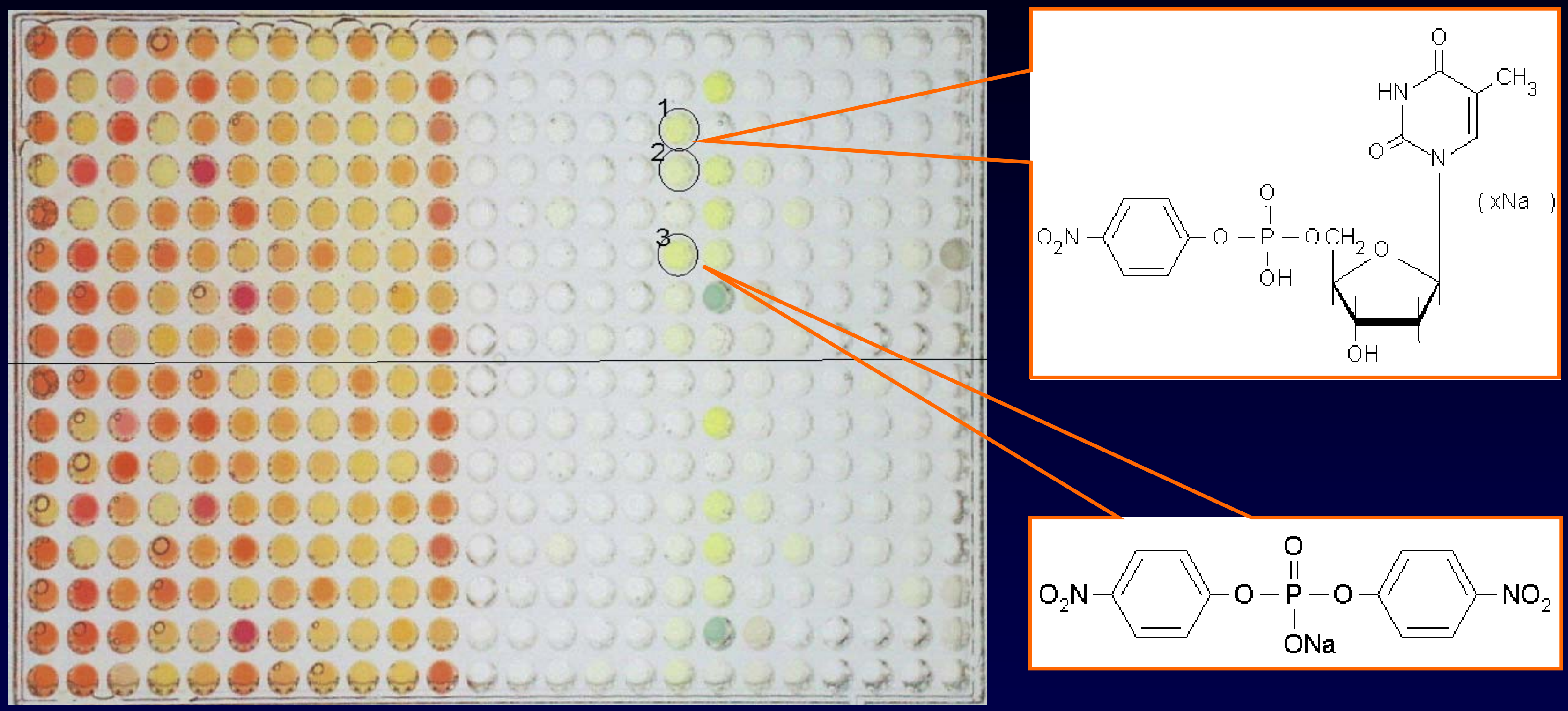


Carfi, A., *et al.* (1995) EMBO J. 14, 4914-21
Concha, N. O. *et al.*, (1996) Structure 4, 823-36

ElaC is a Phosphodiesterase

ElaC

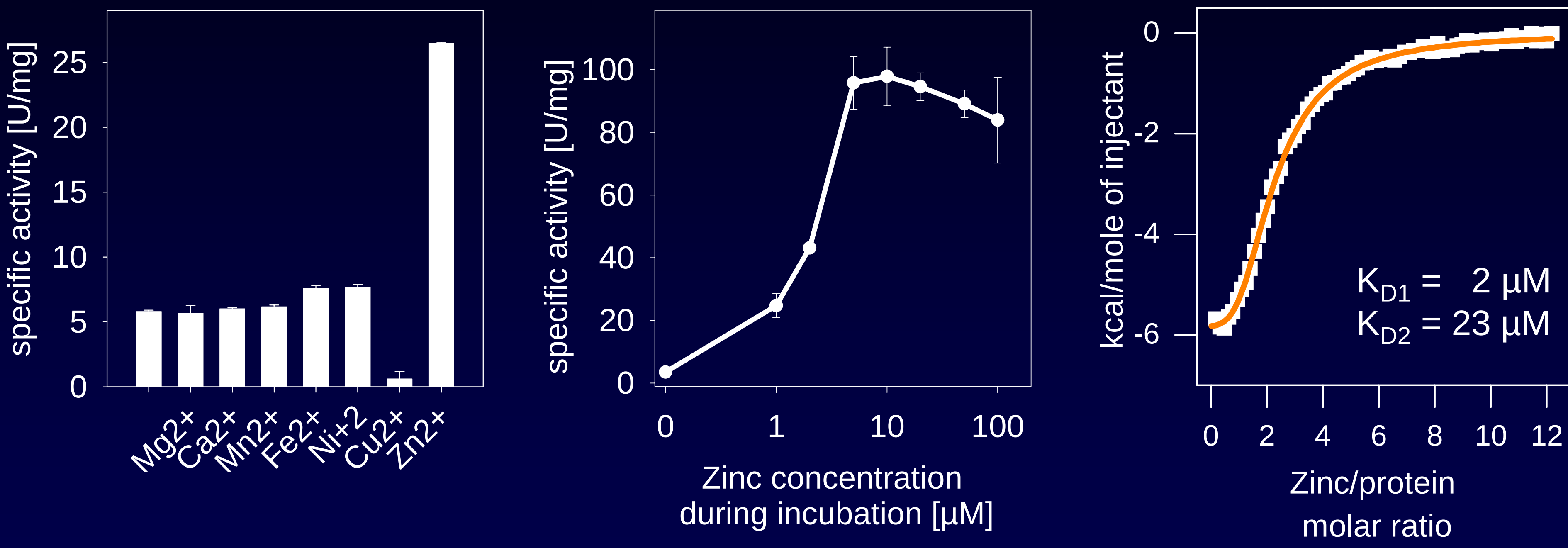
buffer



1 and 2: thymidine-5'-*p*-nitrophenylphosphate sodium and ammonium salt, respectively
3: bis (*p*-nitrophenyl)phosphate

Vogel, A., Schilling, O., *et al.* (2002)

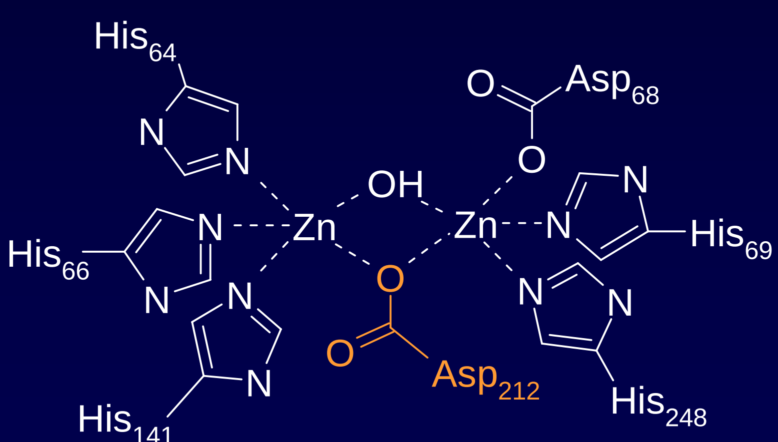
ElaC: Zinc Dependent Phosphodiesterase

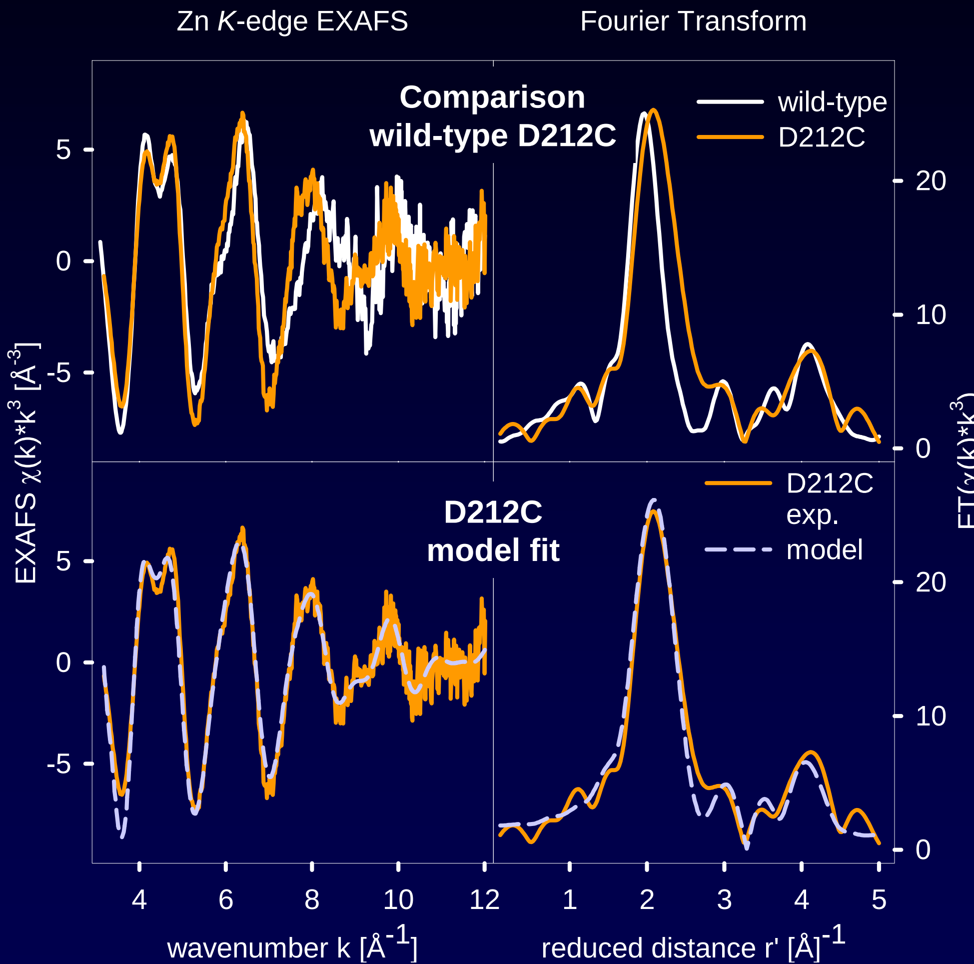


Vogel, A., Schilling, O., *et al.* (2002)
Schilling, O., Vogel, A., *et al.* (2005)

ElaC active site

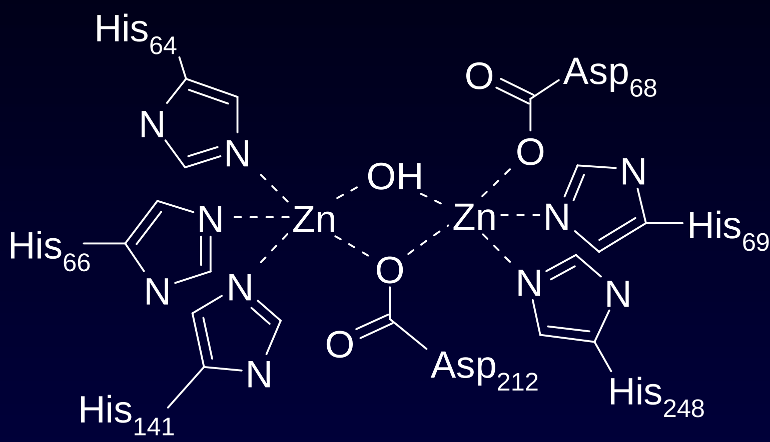
Sequence analysis
Site specific mutagenesis
Enzyme kinetics
X–ray absorption spectroscopy

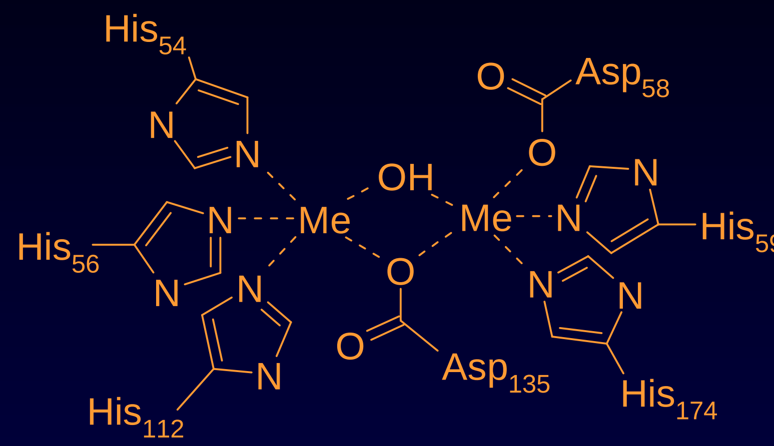


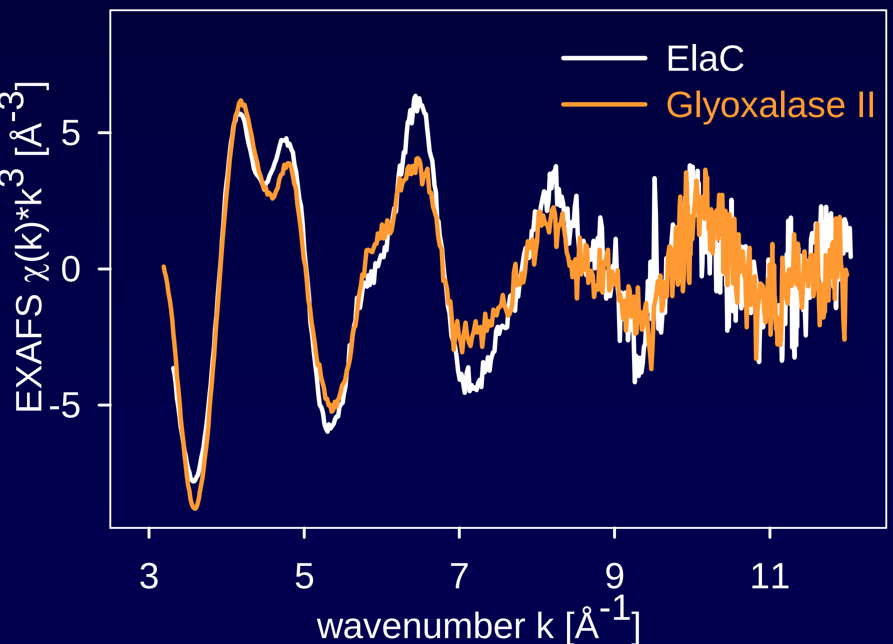


Vogel, A., Schilling, O., *et al.* (2004)

ElaC and Glyoxalase II: similar metal binding sites

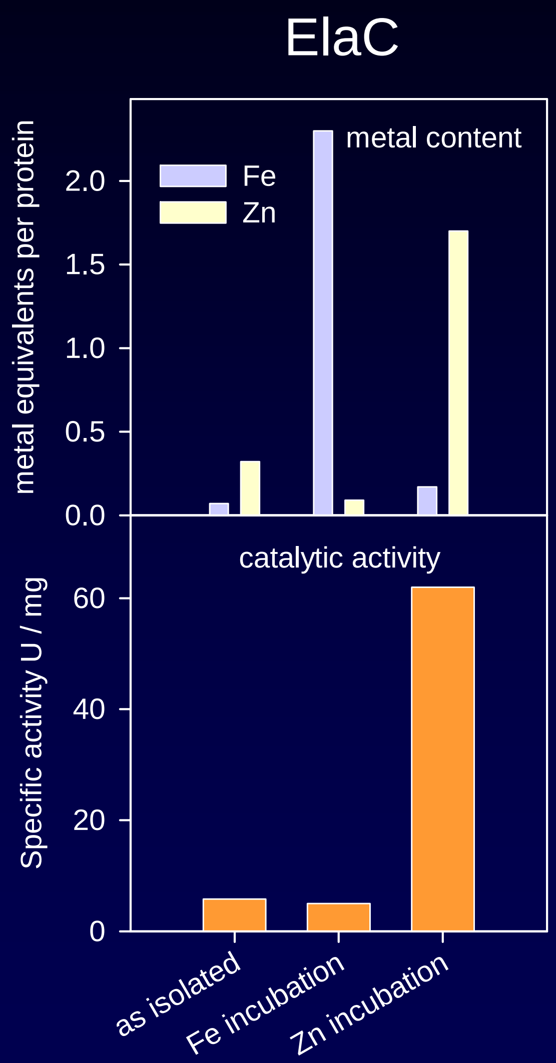


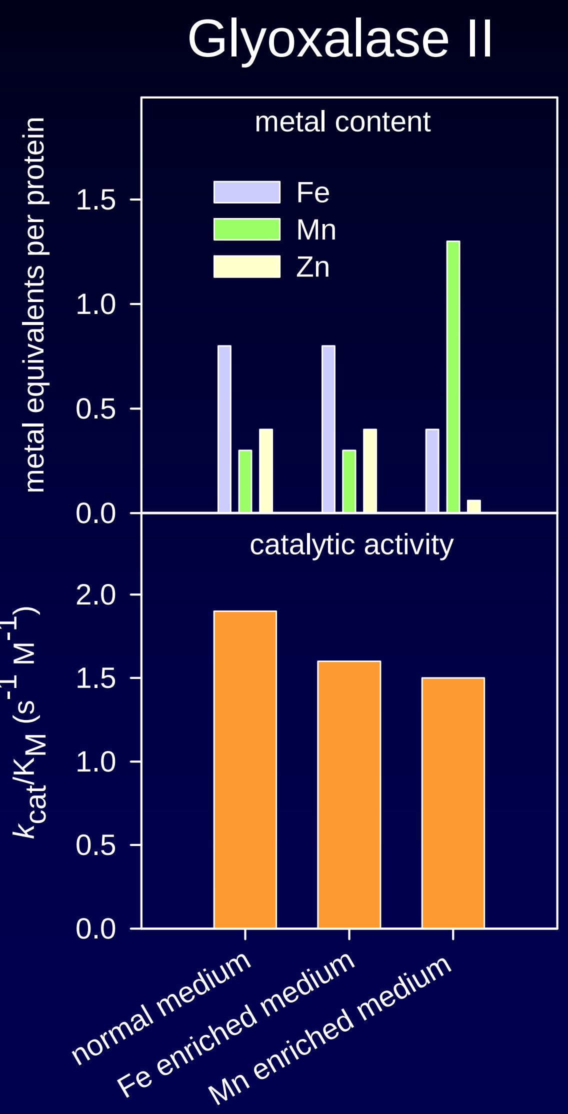




Vogel, A., Schilling, O., *et al.* (2004)

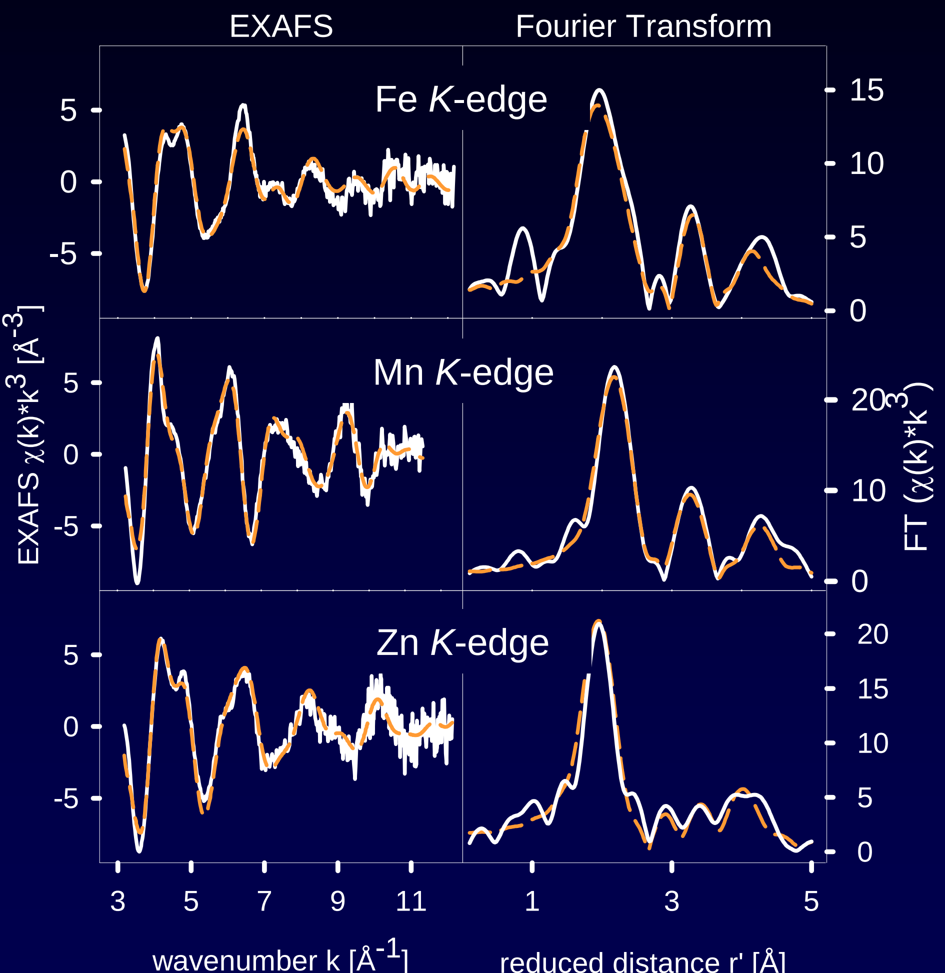
…, but different metal requirements





Schilling, O., *et al.* (2003)

Glyoxalase II has a flexible active site



Metal	Ligand	First shell distance (Å) characteristic	First shell distance (Å) glyoxalase II model
Fe(III)	oxygen (carboxylate)	2.01 (5)	1.96 (1)
	oxygen (water)	2.06 (6)	
	nitrogen (imidazole)	2.08 – 2.22	2.14 (1)
Mn(II)	oxygen (carboxylate)	2.15 (7)	2.11 (1)
	oxygen (water)	2.19 (4)	
	nitrogen (imidazole)	2.19 (8)	2.26 (1)
Zn(II)	oxygen (carboxylate)	2.00 (7)	1.98 (3)
	oxygen (water)	2.06 (9)	
	nitrogen (imidazole)	2.00 (2)	2.00 (2)

numbers in parentheses indicate error of last digit

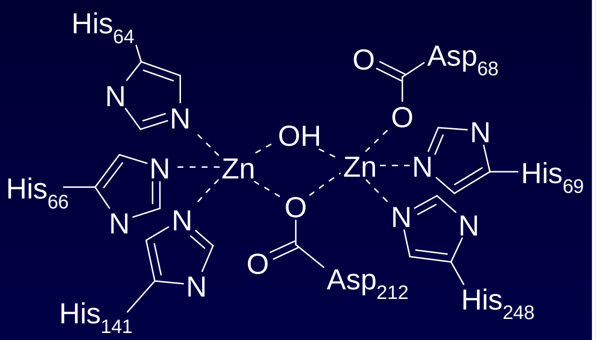
Harding, M. M. (1999) Acta Cryst. D 55, 1432-1443.
Harding, M. M. (2001) Acta Cryst. D 57, 401-411.

Schilling, O., *et al.* (2003)

Active sites with different metal requirements

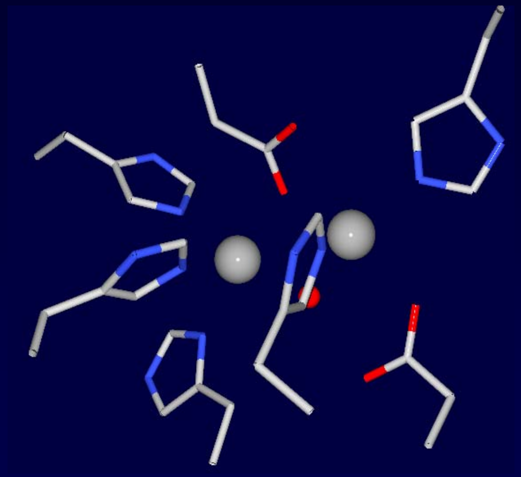
ElaC

Zn



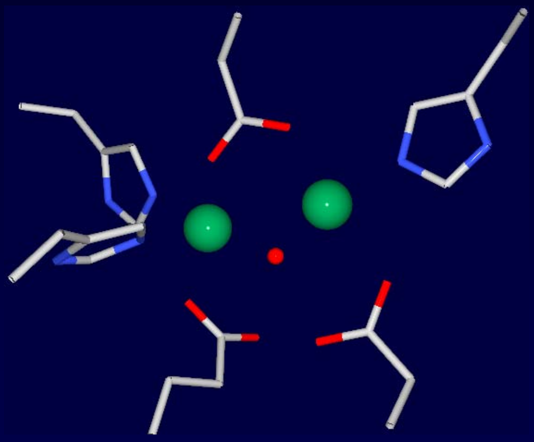
Glyoxalase II

Fe, Mn, Zn



Redox enzymes

Fe



FOR THE RECORD

Functional control of the binuclear metal site in the metallo-β-lactamase-like fold by subtle amino acid replacements

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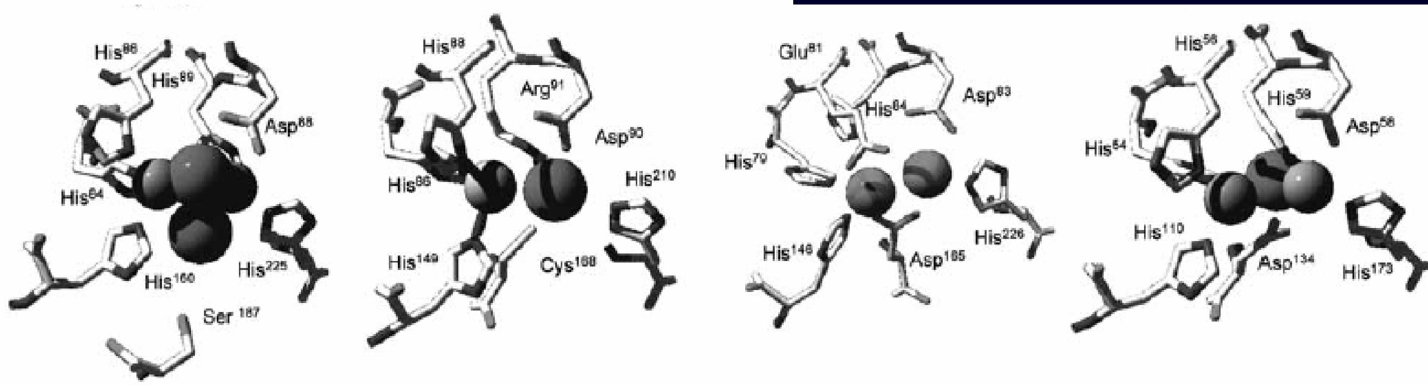
[3]Department of Biochemistry and M

(RECEIVED August 2, 2001; FINAL R

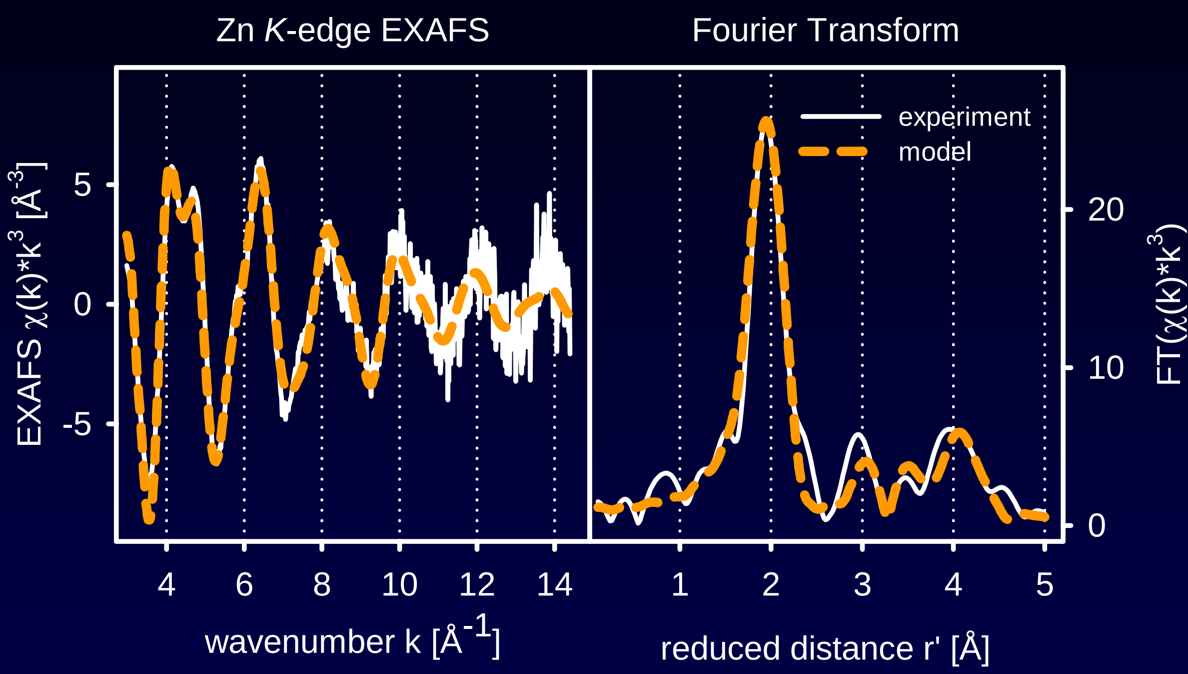


S. maltophilia **β-lactamase** | ***B. cereus*** **β-lactamase** | ***D. gigas*** **ROO** | ***H. sapiens*** **Glyoxalase II**

Zn | Zn | Fe | Fe Mn Zn

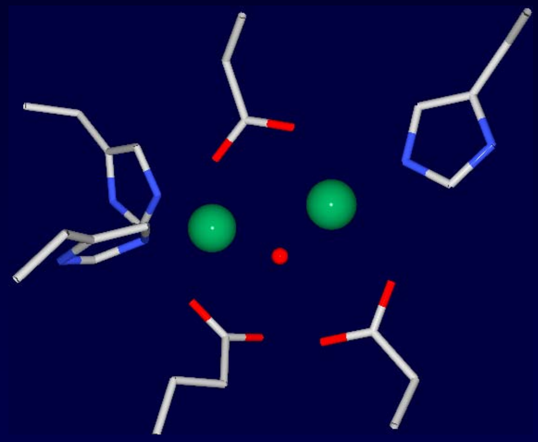
Gomes, C.M., Frazao, C., Xavier, A.V., Legall, J. and Teixeira, M. (2002) *Protein Sci.*, **11**, 707-712.

However, the atypical glutamate does not prevent zinc binding



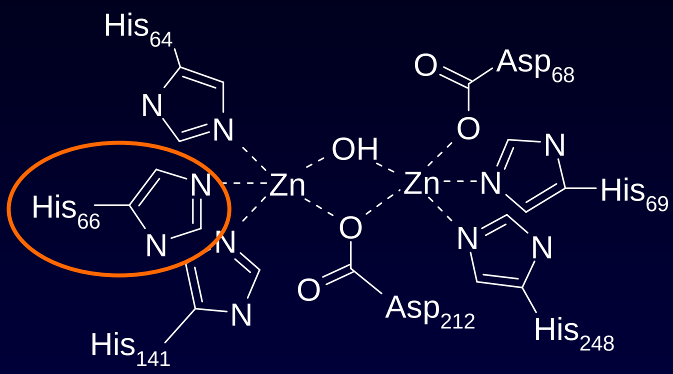
Redox enzymes

Fe

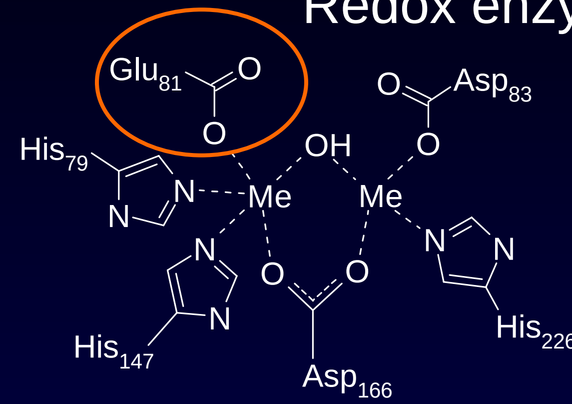


Role of the atypical glutamate

ElaC



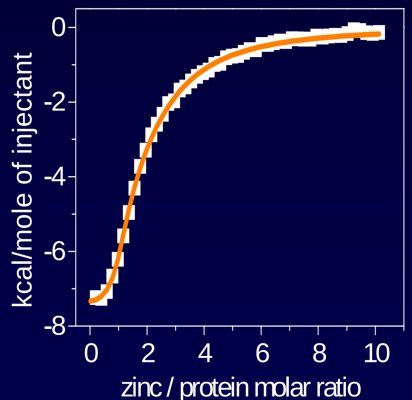
Redox enzyme



$His_{66} \rightarrow$ Glu: catalytically inactive

$His_{66} \rightarrow$ Ala: residual activity

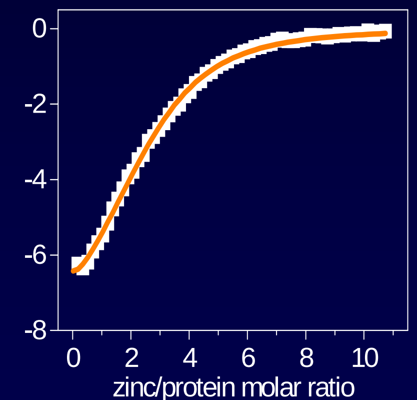
further metal ligands $\rightarrow$ Ala: residual activity



wild-type

$K_D^1 = 1\ \mu M$

$K_D^2 = 27\ \mu M$



$Glu_{81} \rightarrow$ His

$K_D^1 = 30\ \mu M$

$K_D^2 = 27\ \mu M$

Schilling, O., *et al.* (2004) Vogel, A., Schilling, O., *et al.* (2005)

Summary

- ✓ Metallo-β-lactamase fold: pool for diverse enzymatic reactions
- ✓ ElaC: zinc dependent phosphodiesterase
- ✓ ElaC and glyoxalase II:
  similar metal binding sites
  different metal requirements
- ✓ Iron dependent metallo-β-lactamase proteins:
  atypical glutamate does not control metal specificity,
  prevents side-reactions
- ✓ Not shown: Bacterial *elaC* deletion strain:
  functional diversity of ElaC proteins from different organisms
- ✓ Not shown: structural basis for physiological substrate
  selectivity of ElaC

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W. Meyer–Klaucke, P. Tucker, C. Müller, and W. Stöcker

Glyoxalase II

M. Crowder and C. Makaroff, Miami University, Oxford, Ohio, USA

Verein der Freunde und Förderer des DESY