XAS Applications

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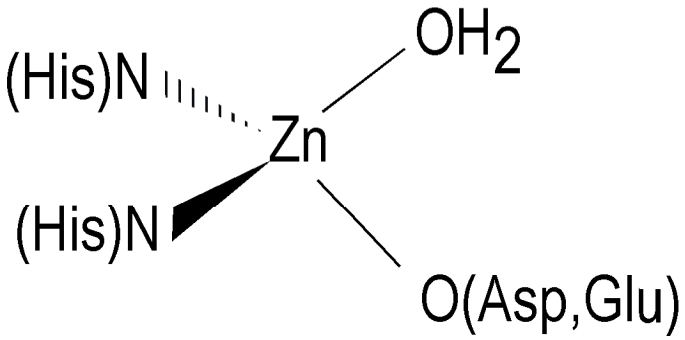
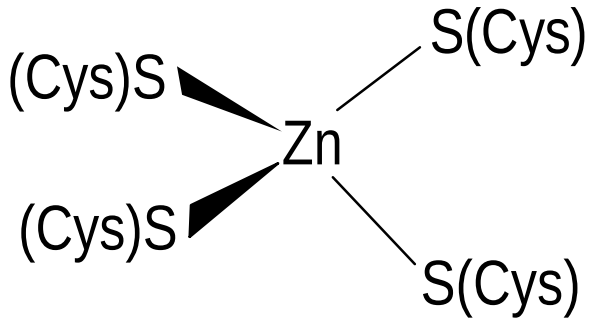
- Characterization of unknown protein
- Comparison with crystal structure
- Determination of solution structure
- When crystal structures are not enough
- Spatial localization

Biological Zn sites

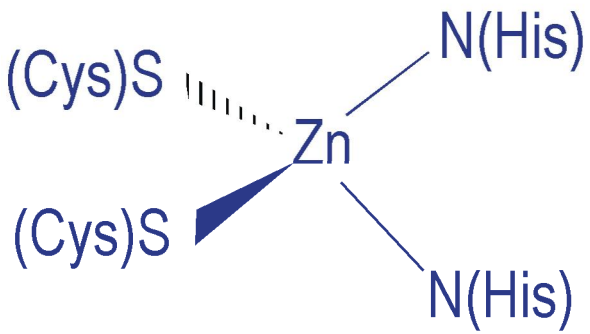
Catalytic



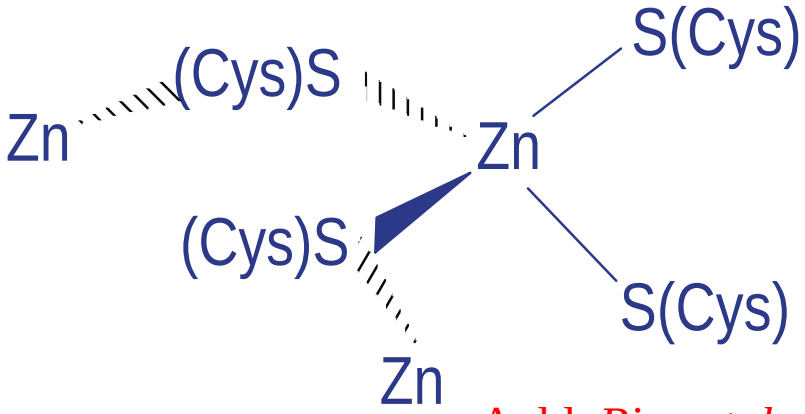
Structural



Regulatory



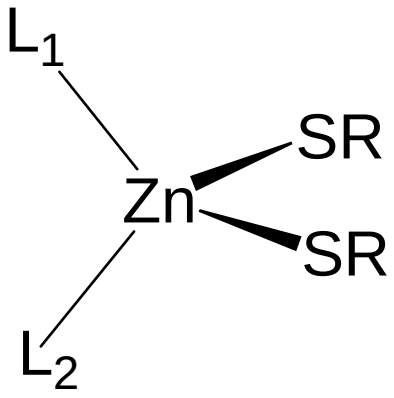
Storage



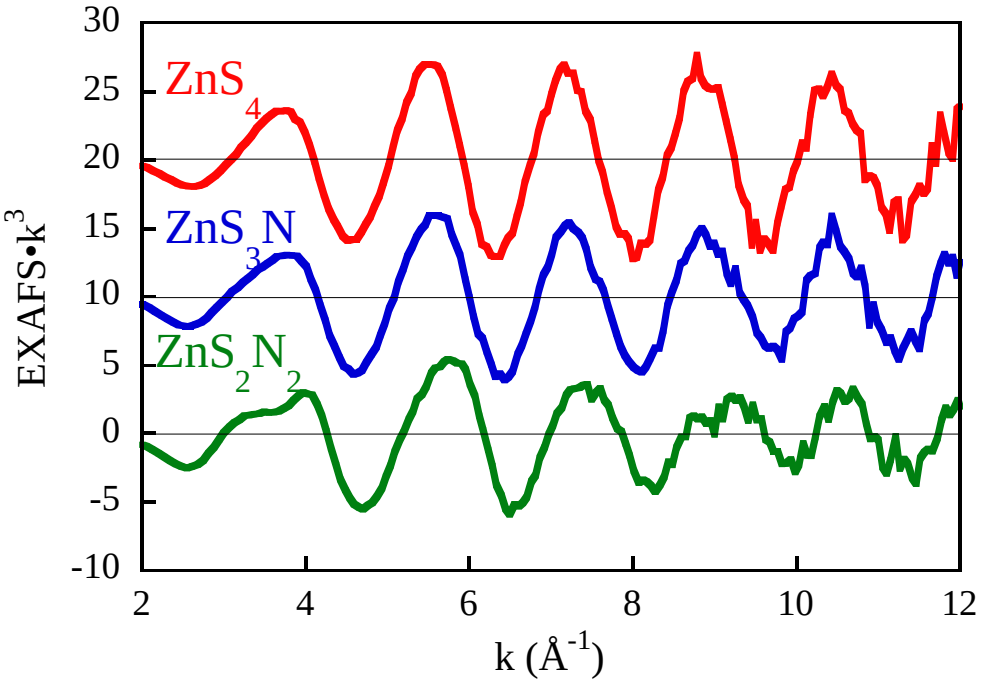
e.g., Auld, *Biometals*, 2001

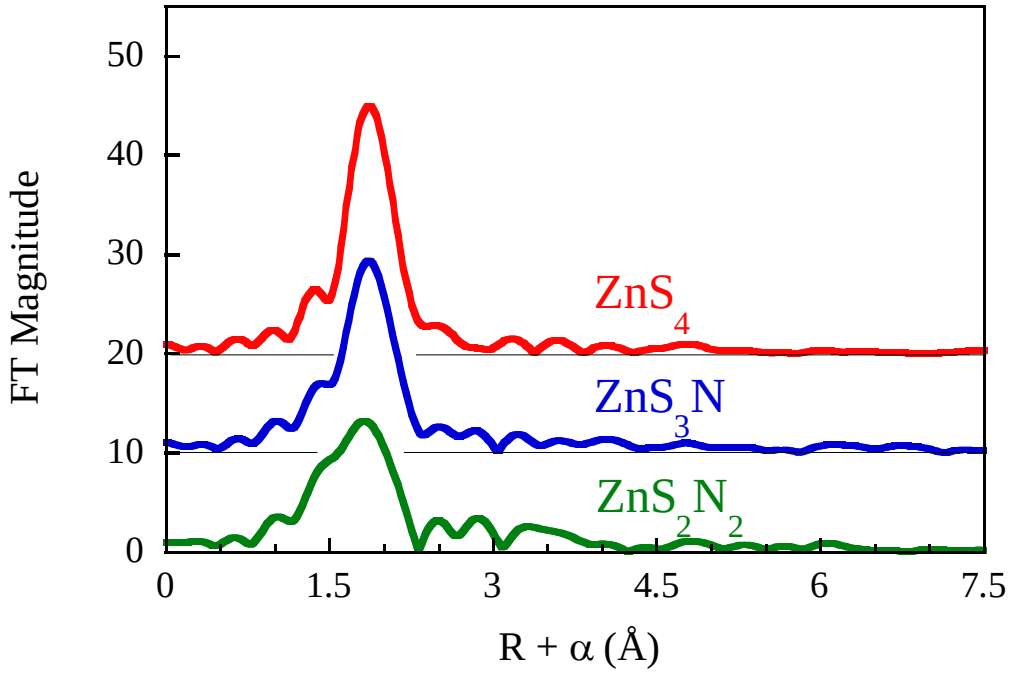
A case study in data under-determination

Clark-Baldwin, et al. "The limitations of X-ray absorption spectroscopy for determining the structure of zinc sites in proteins. When is a tetrathiolate not a tetrathiolate?" *J. Am. Chem. Soc.* **1998**, *120*, 8401-8409.



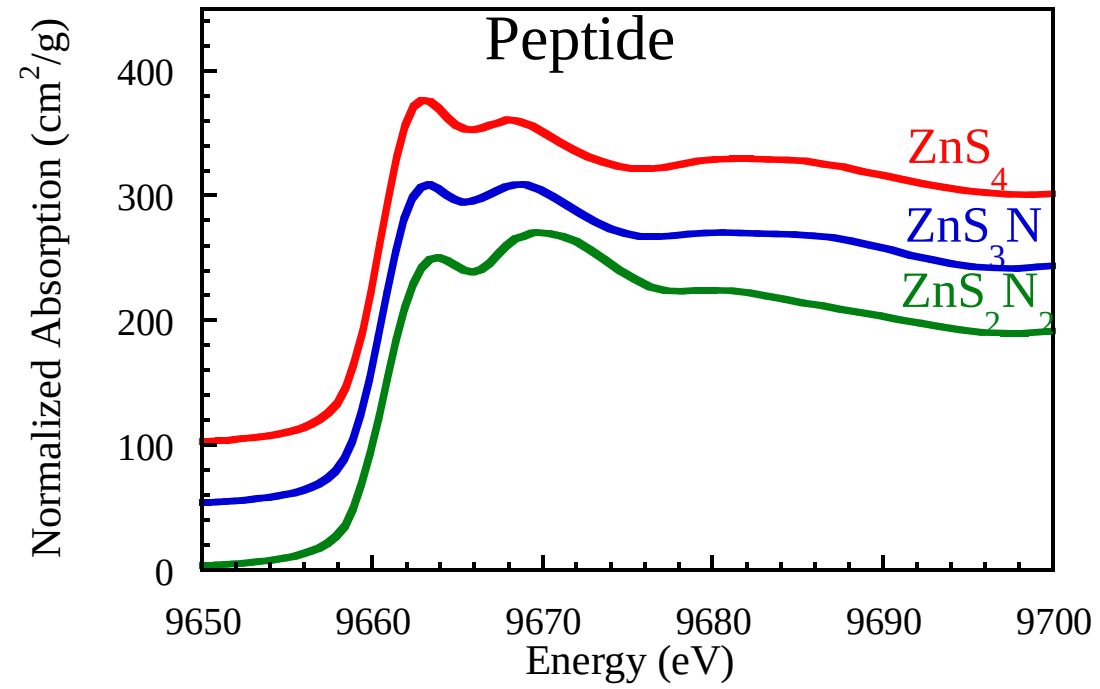
Zn EXAFS is remarkably insensitive to changes in ligation.

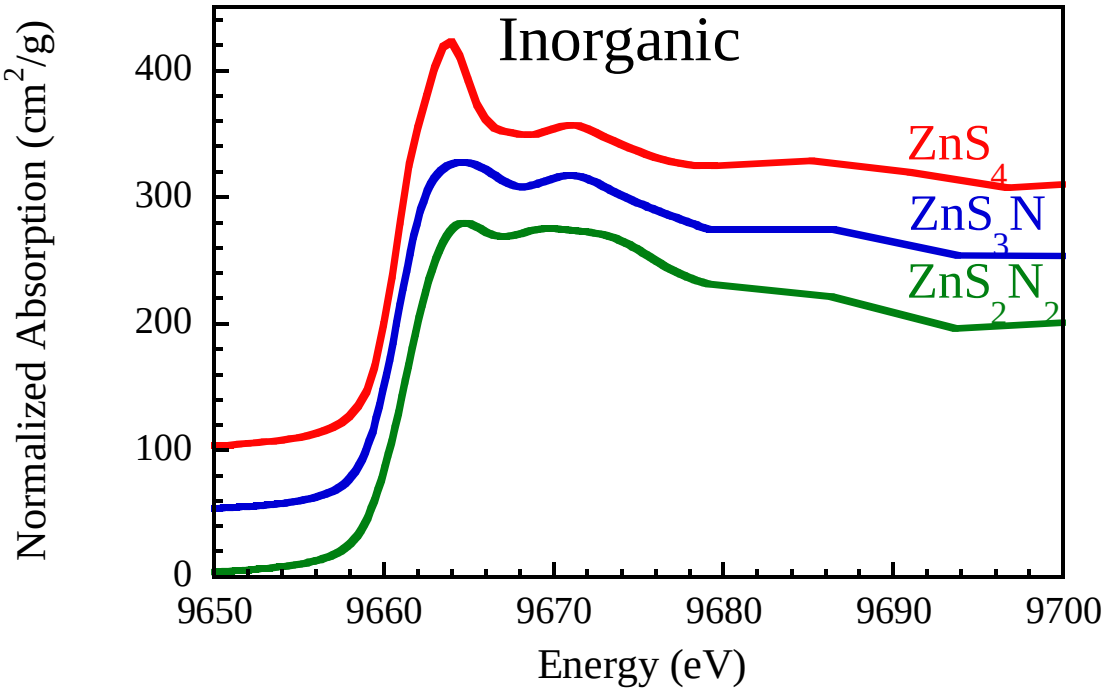




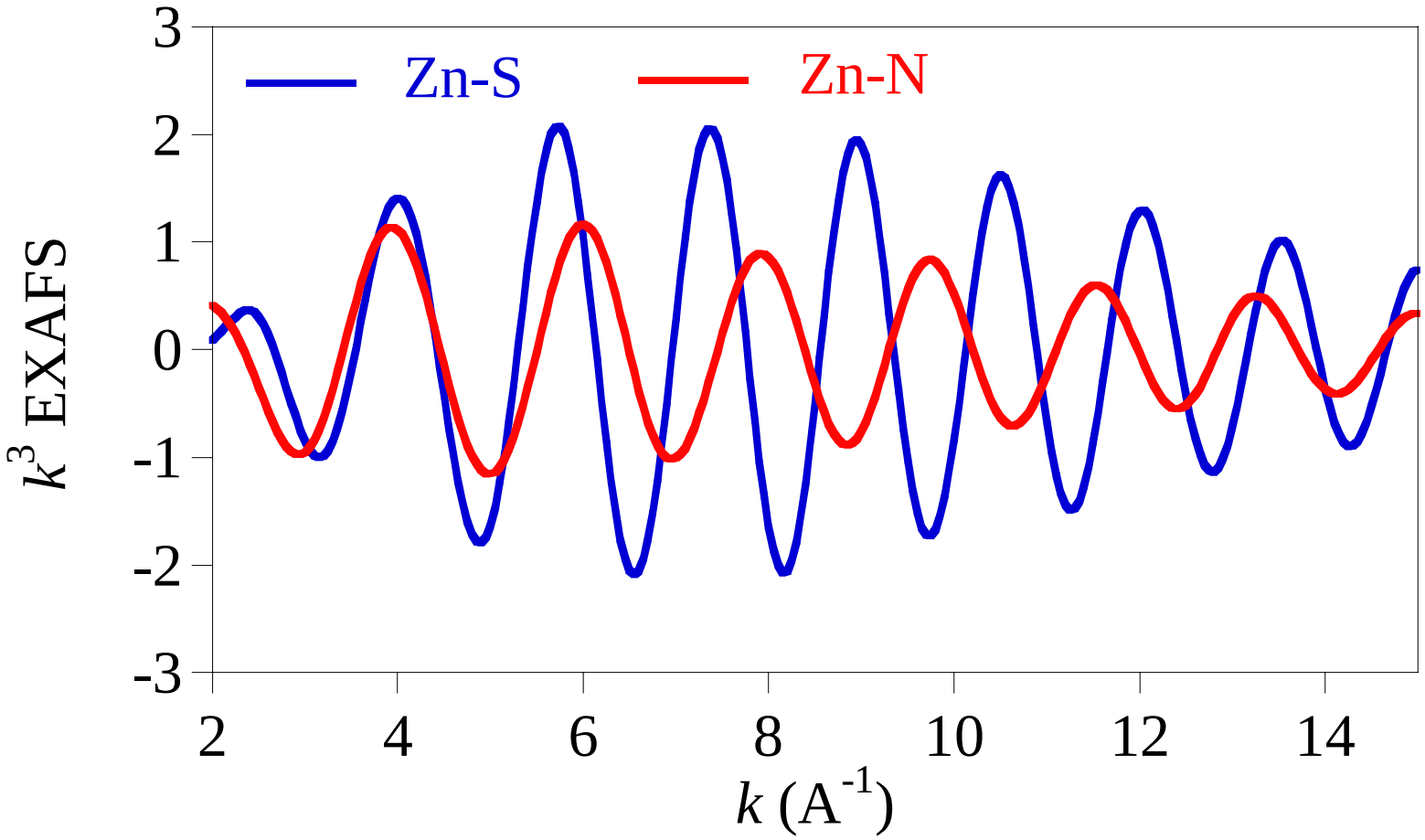
XANES spectra *are* sensitive to ligation

but show greater variation between different compounds than with changes in ligation.

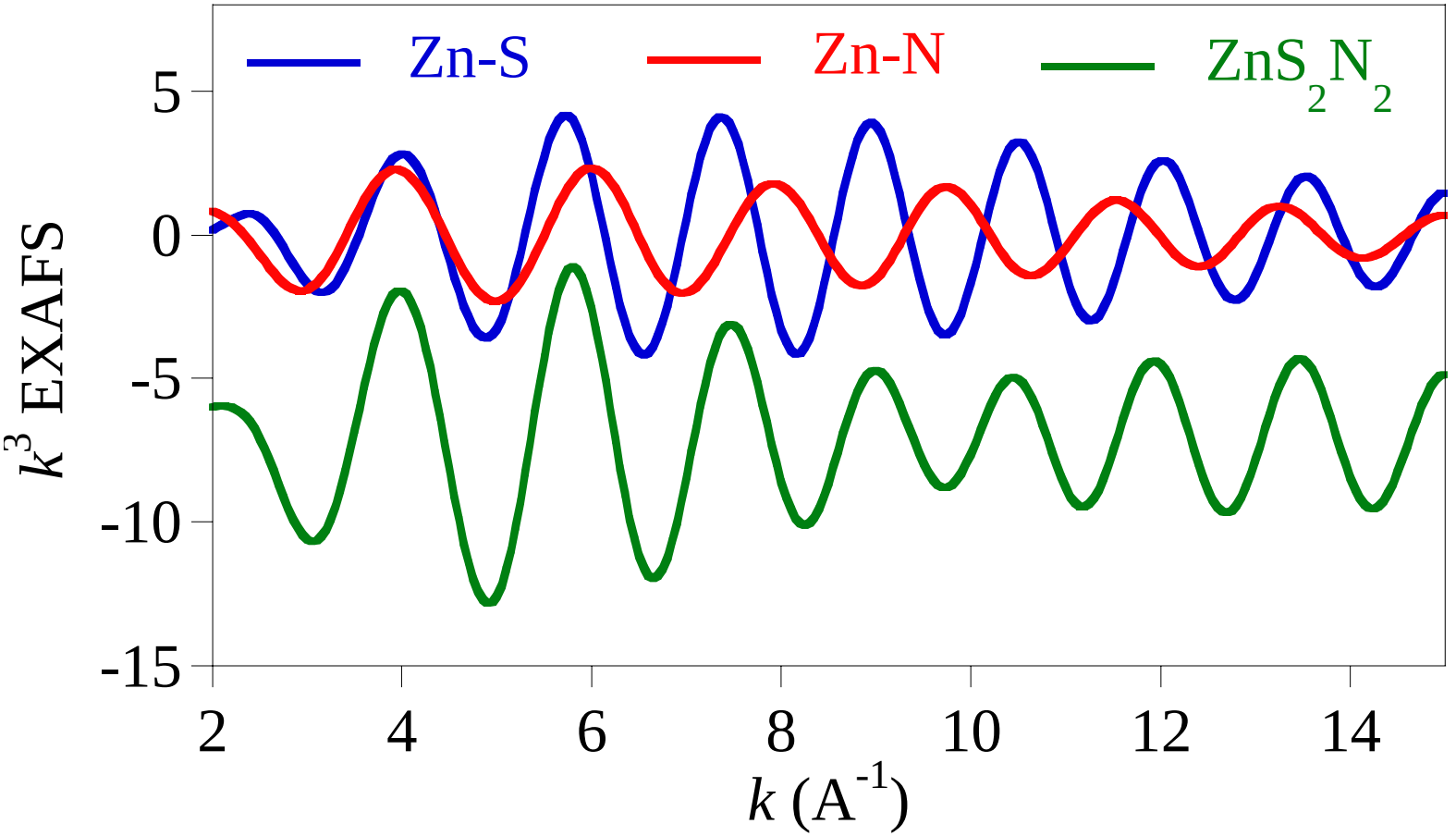




Zn-S and Zn-N EXAFS signals are approximately out of phase

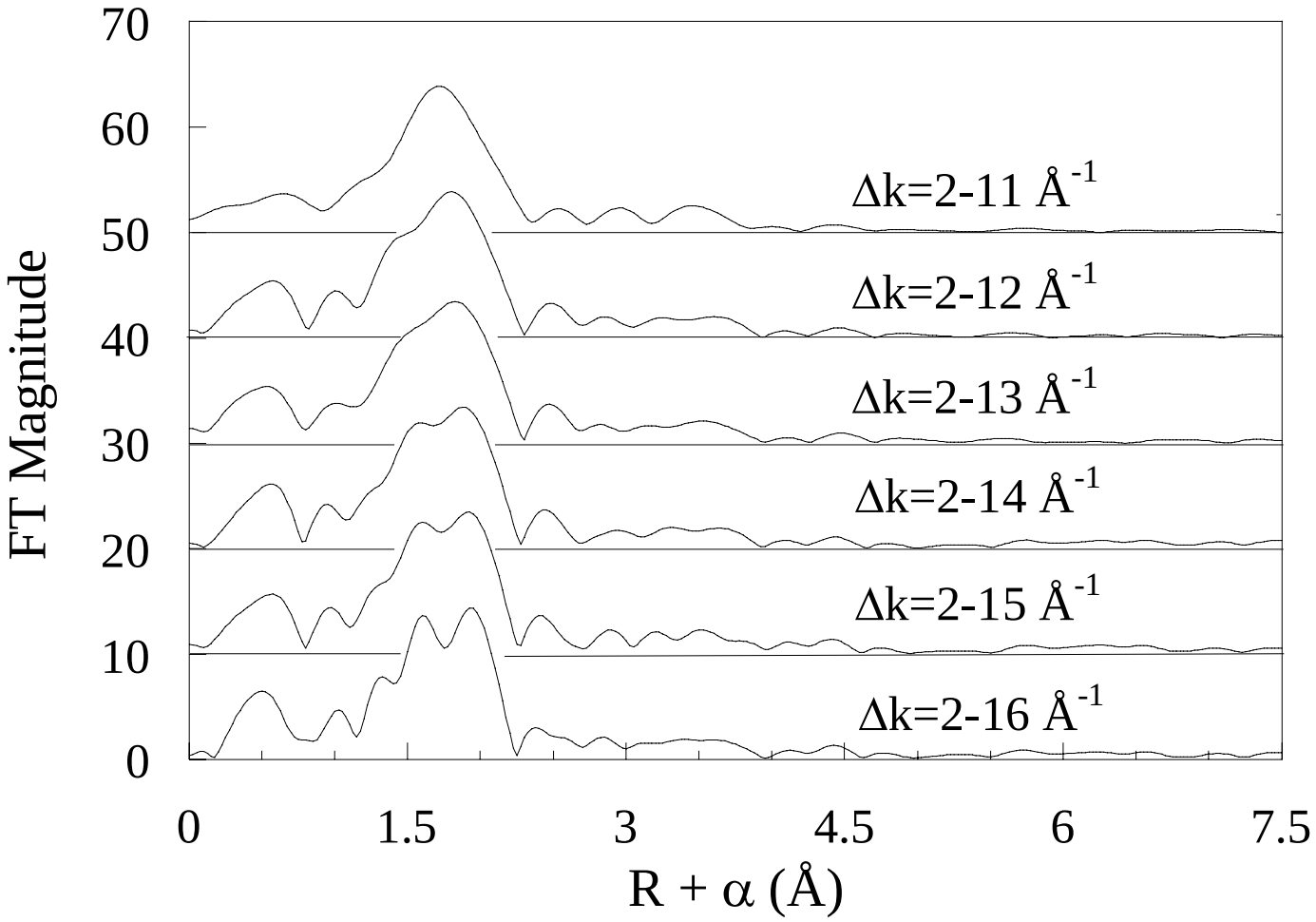


The observed EXAFS for mixed S/N sites is dominated by Zn-S scattering



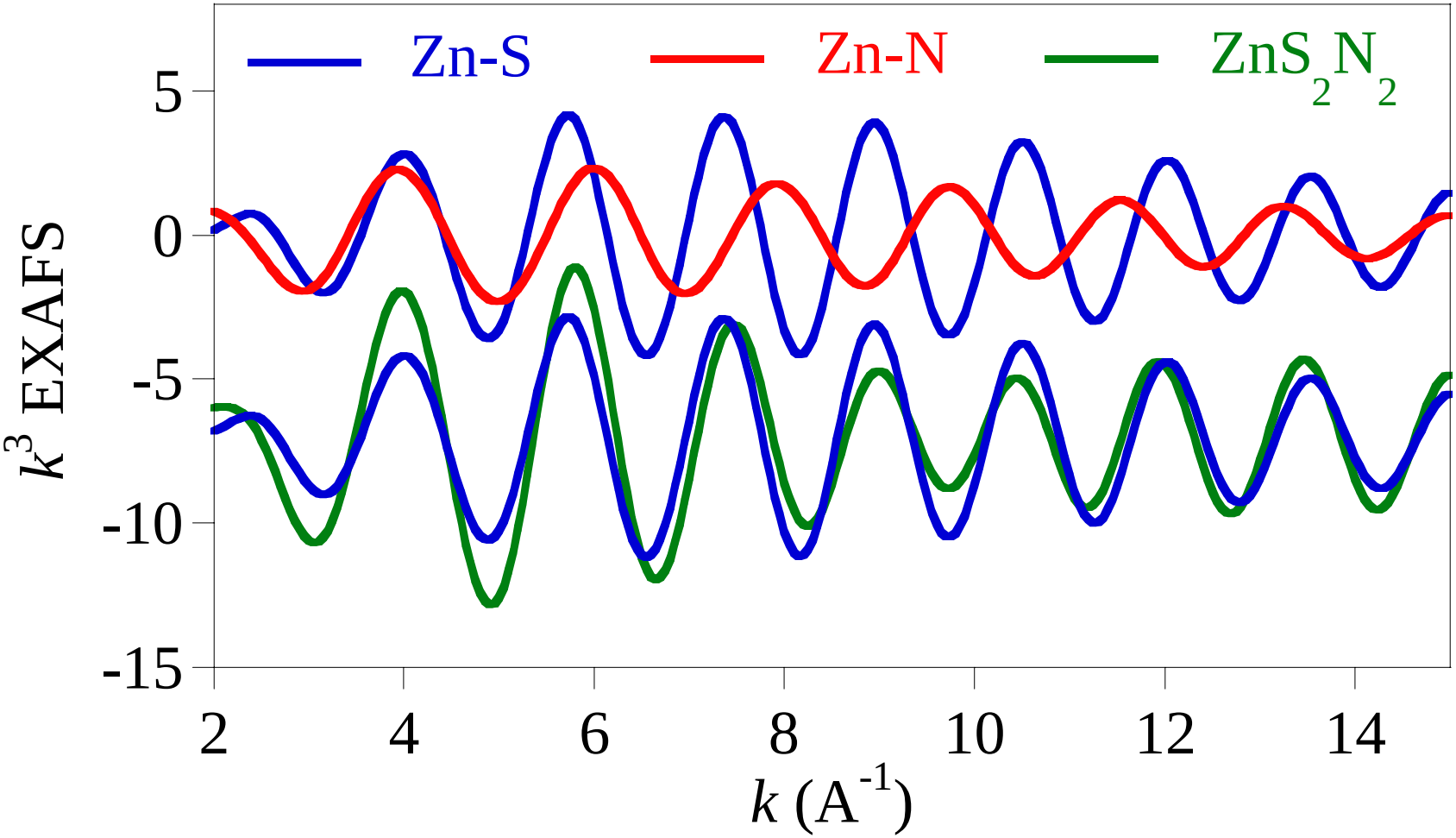
One solution is to measure data over wide *k* range

(ZnS_2N_2 inorganic)

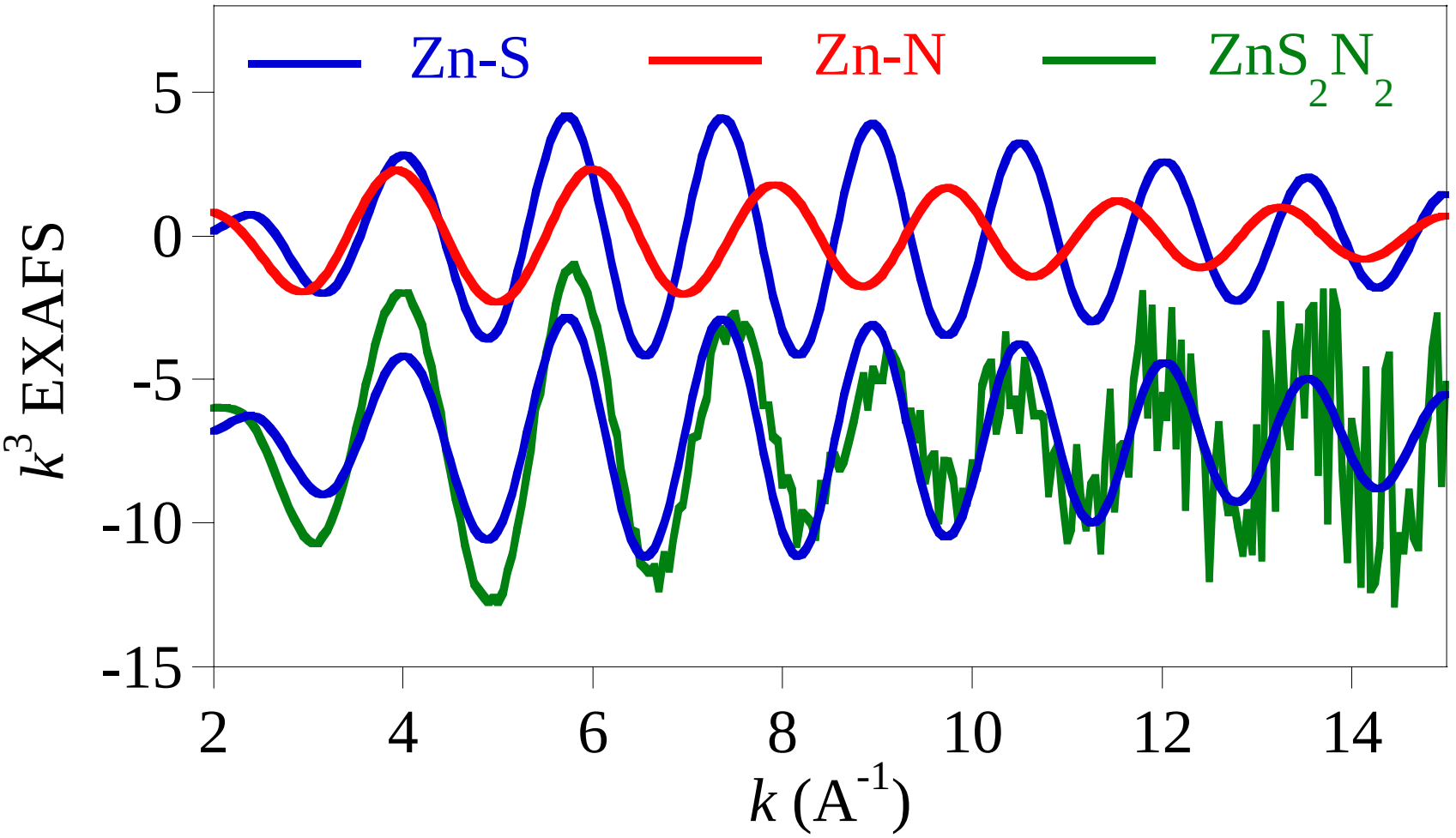


Note – $\Delta R \sim 0.25 \rightarrow \pi/2\Delta k = 0.25$ Å $\rightarrow \Delta k_{min} \sim 6.3$

High resolution EXAFS is required to reliably distinguish Zn-S from Zn-N …



…and even with high resolution data, extremely high signal/noise ratios are required to detect Zn-N in the presence of Zn-S

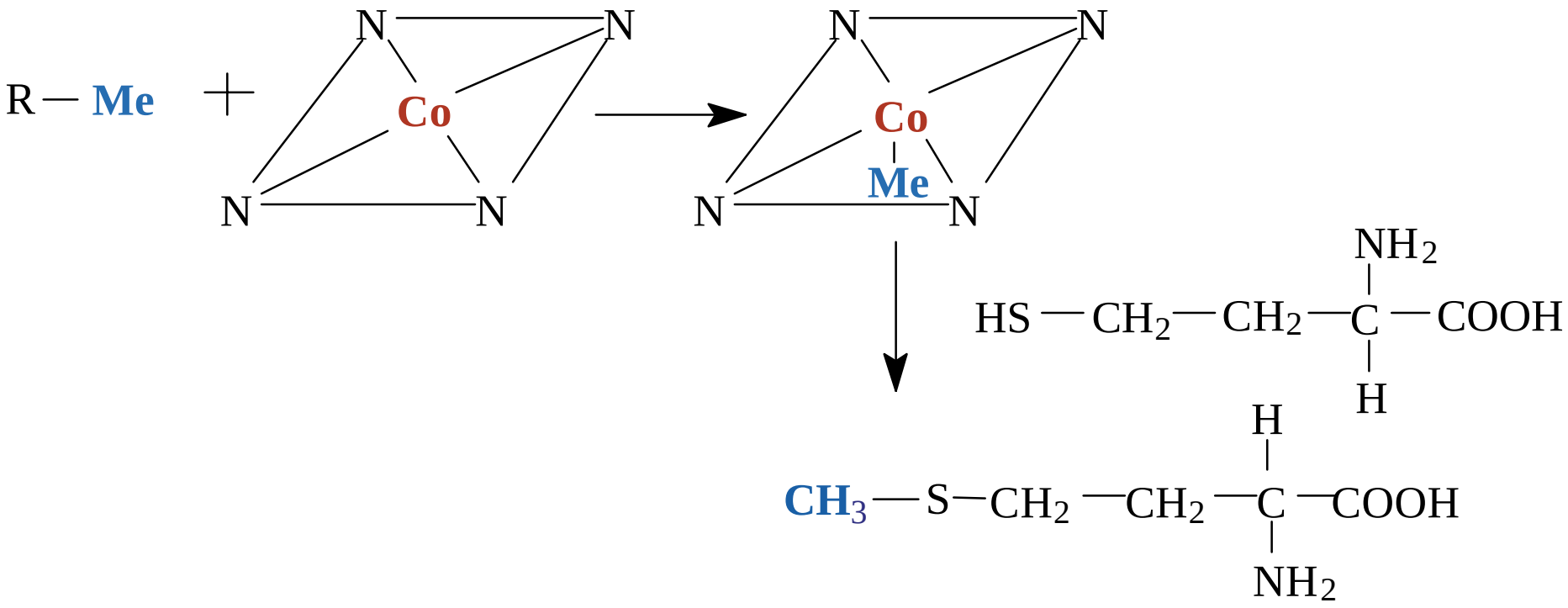


Homocysteine

Homocysteine + Me-X = Methionine + HX

E. coli has two methionine synthases

MetH – cobalamin dependent methionine synthase



MetE – cobalamin independent methionine synthase

MetE (cobalamin independent MetSyn) contains Zn

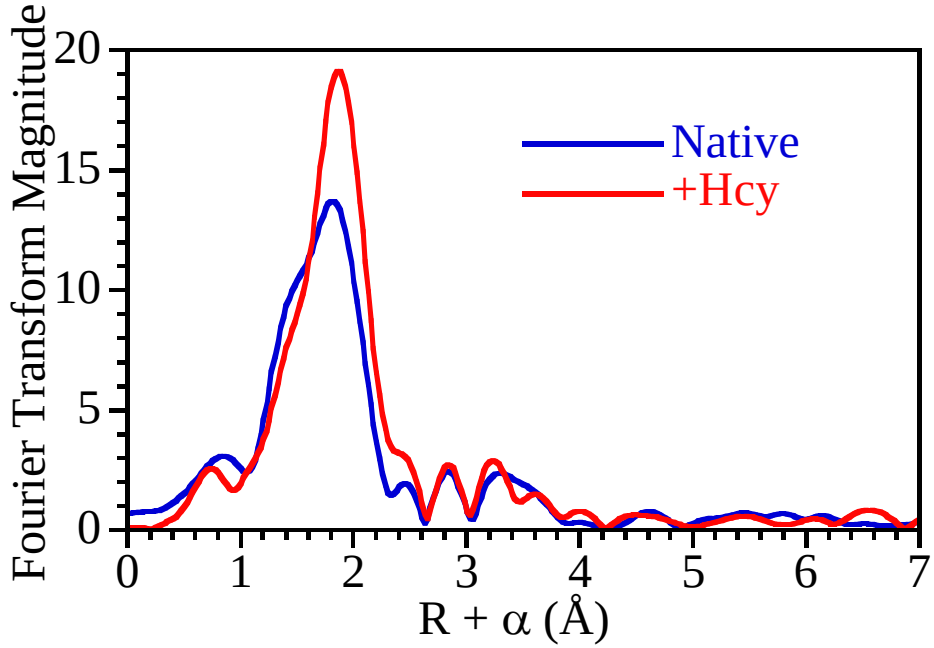
Zn is tightly bound

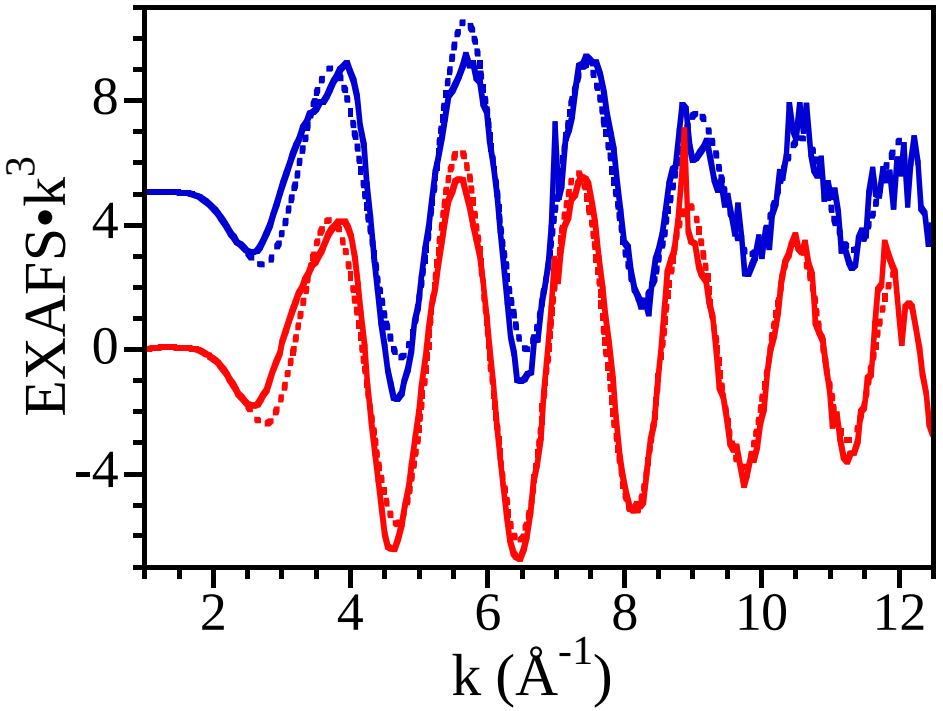
Zn is required for activity

Is Zn involved in reaction, or does it play a structural role?

The Zn site in MetE (cobalamin independent MetSyn) has $ZnS_2(O/N)_2$ ligation.

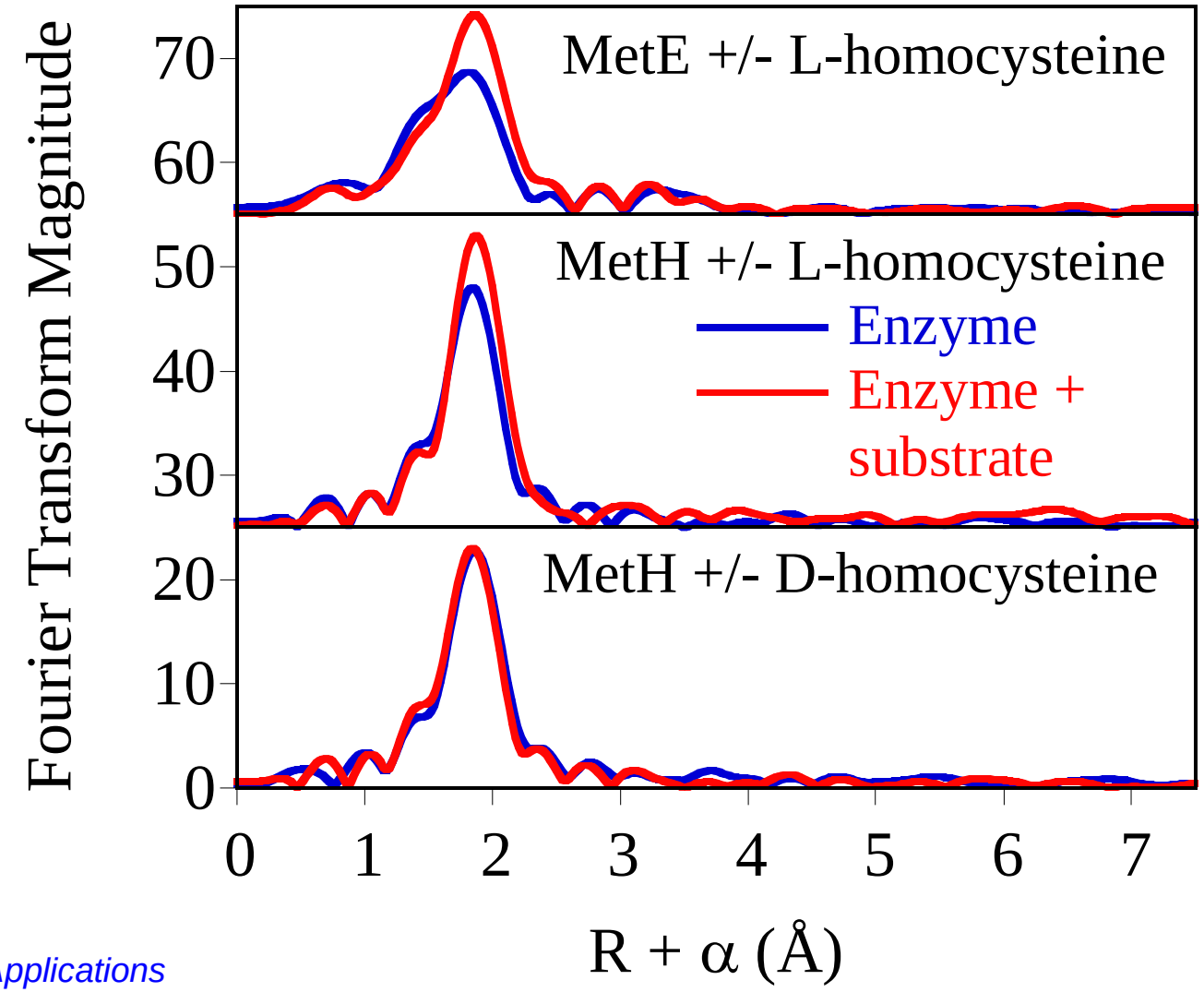
Addition of homocysteine changes ligation to $ZnS_3(O/N)$.





MetE Zn site changes on substrate binding

MetH (cobalamin dependent) also contains Zn



$ZnS_2(O/N)_2$

↓

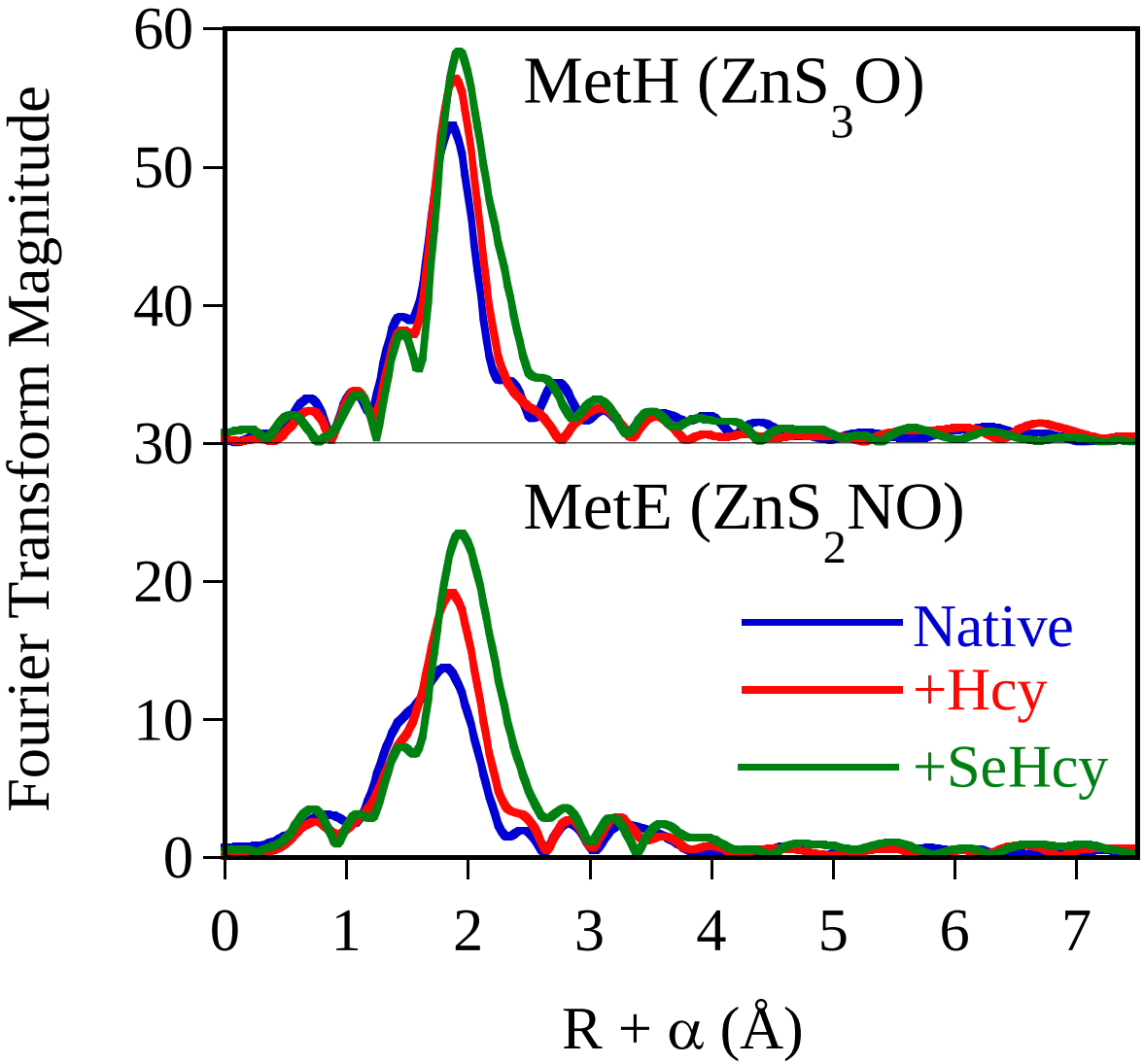
$ZnS_3(O/N)$

$ZnS_3(O/N)$

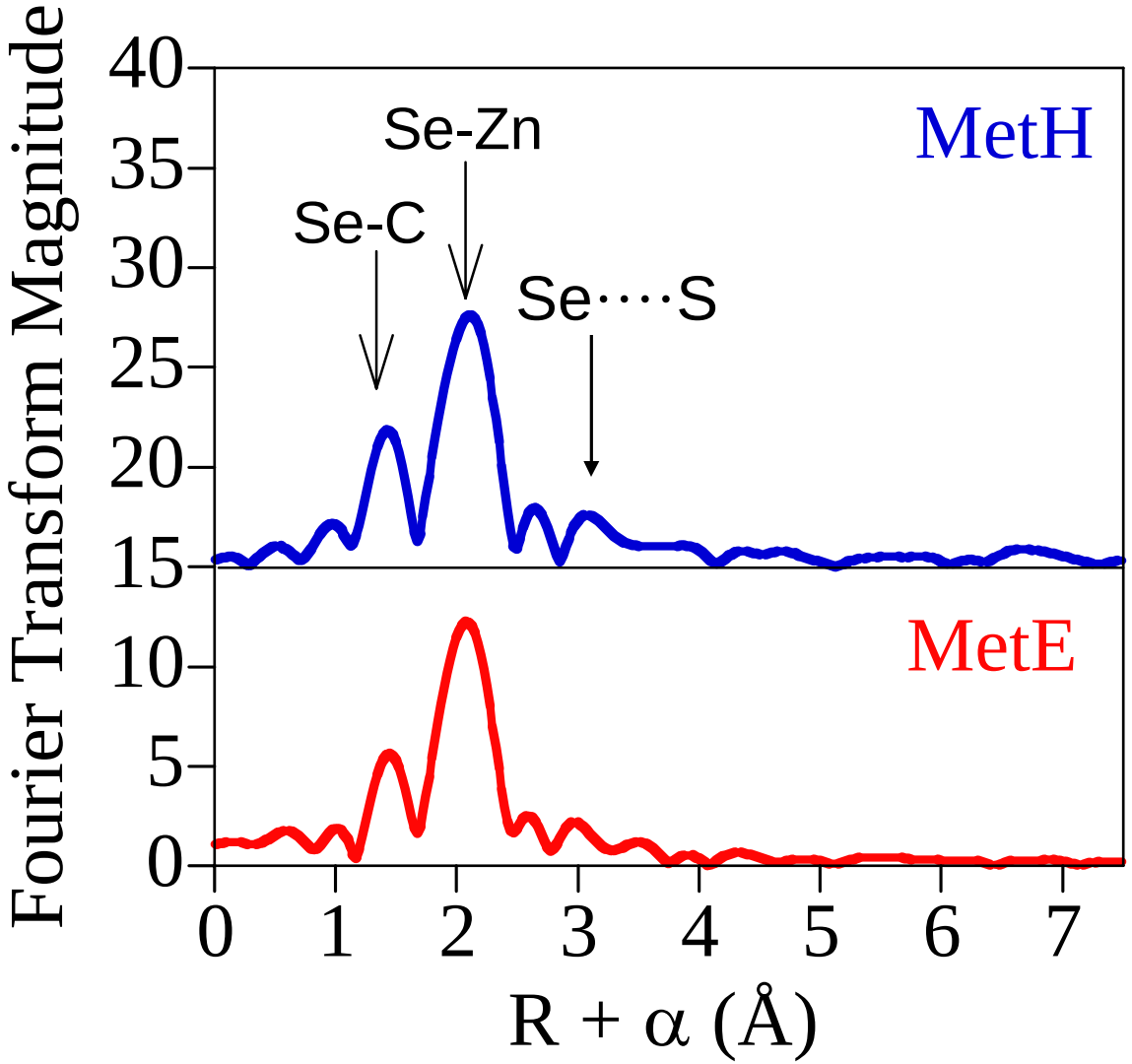
↓

ZnS_4

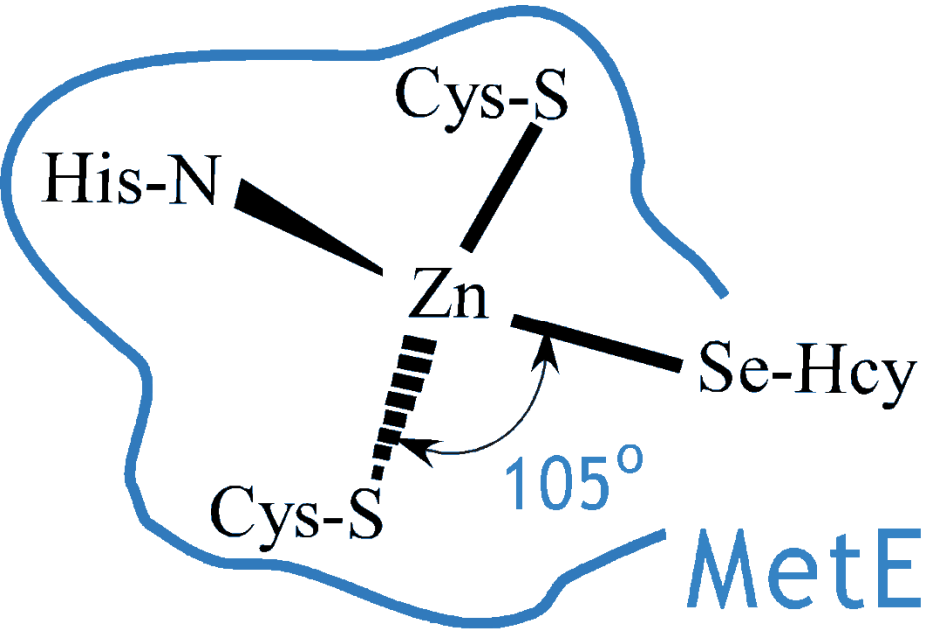
Changes in ligation are due to homocysteine binding to Zn

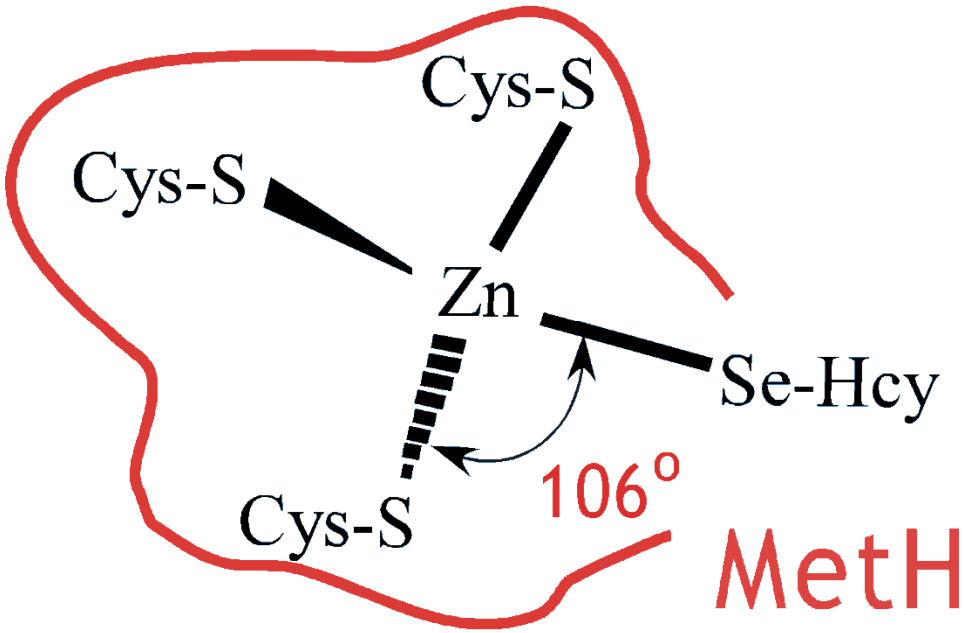


Se EXAFS confirms structural picture of MetE and MetH sites

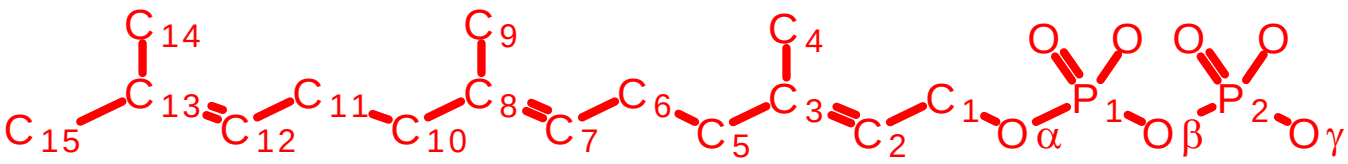


Combination of Zn + Se EXAFS consistent with only a small distortion from tetrahedral geometry in substrate-bound enzyme





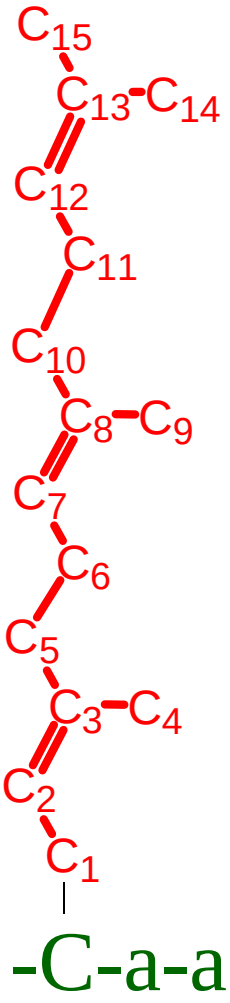
Protein Farnesyl transferase



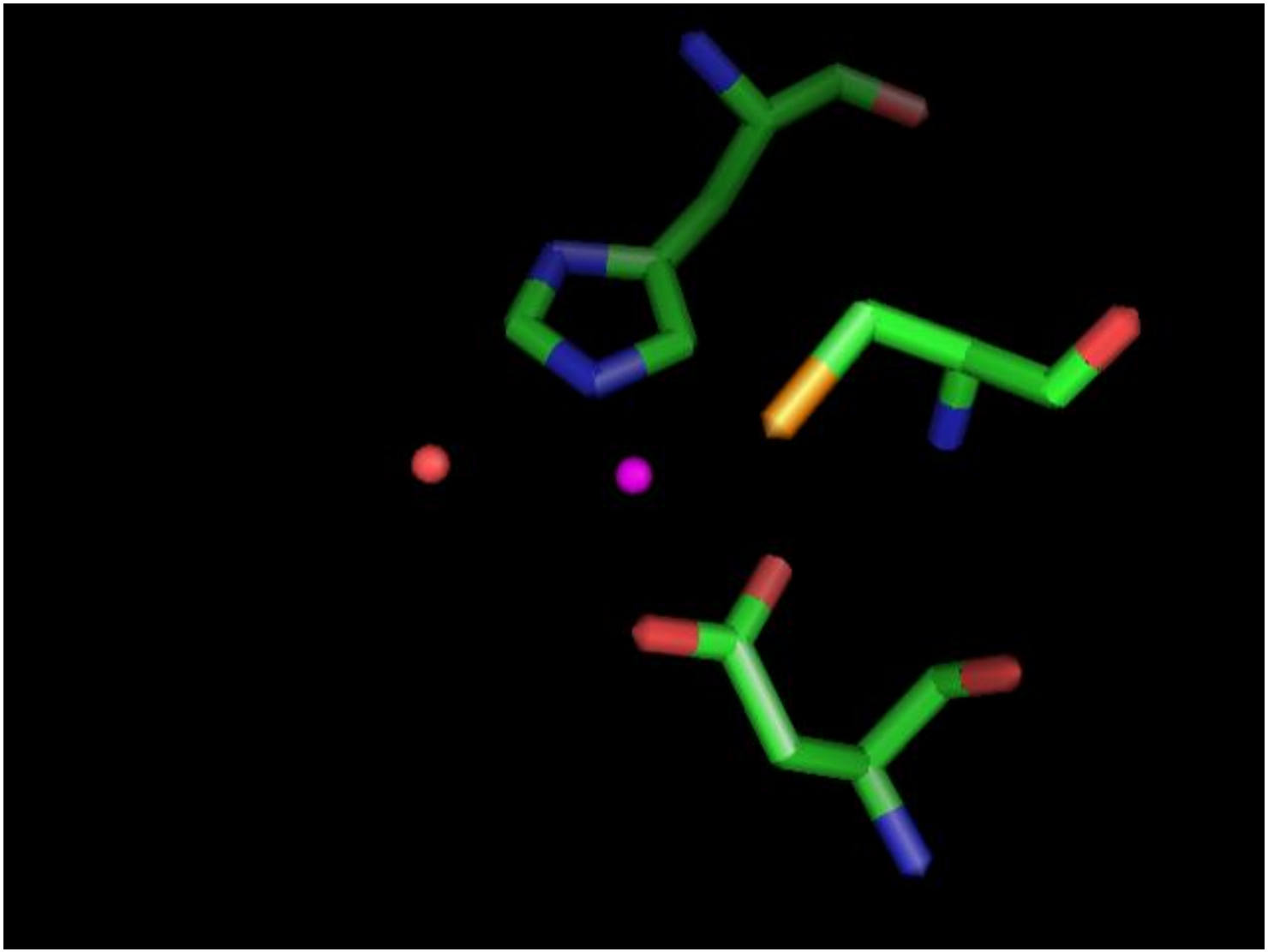
FPP

CaaX, X=S,M,Q,C,A

Peptide

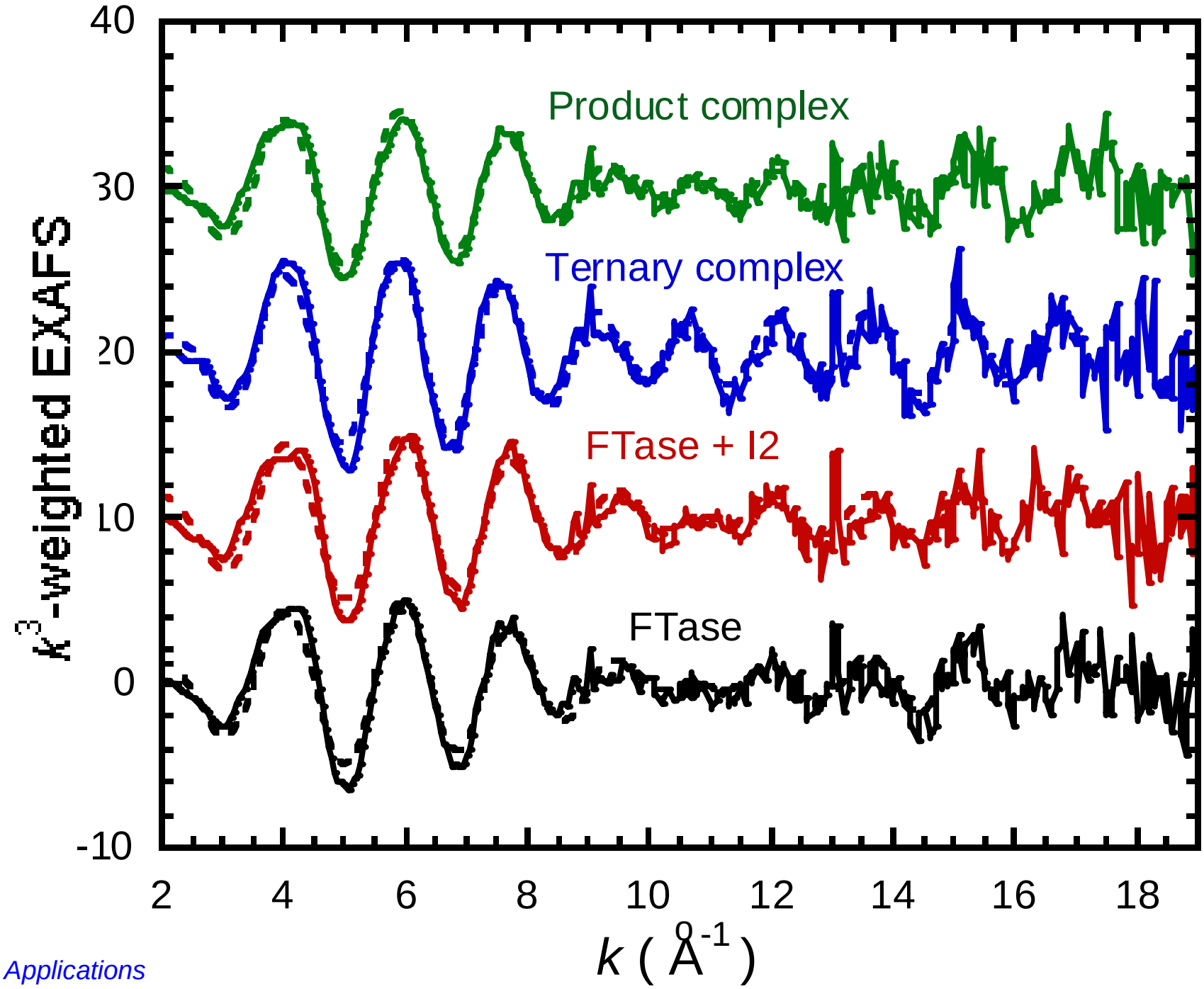


Protein Farnesyl transferase

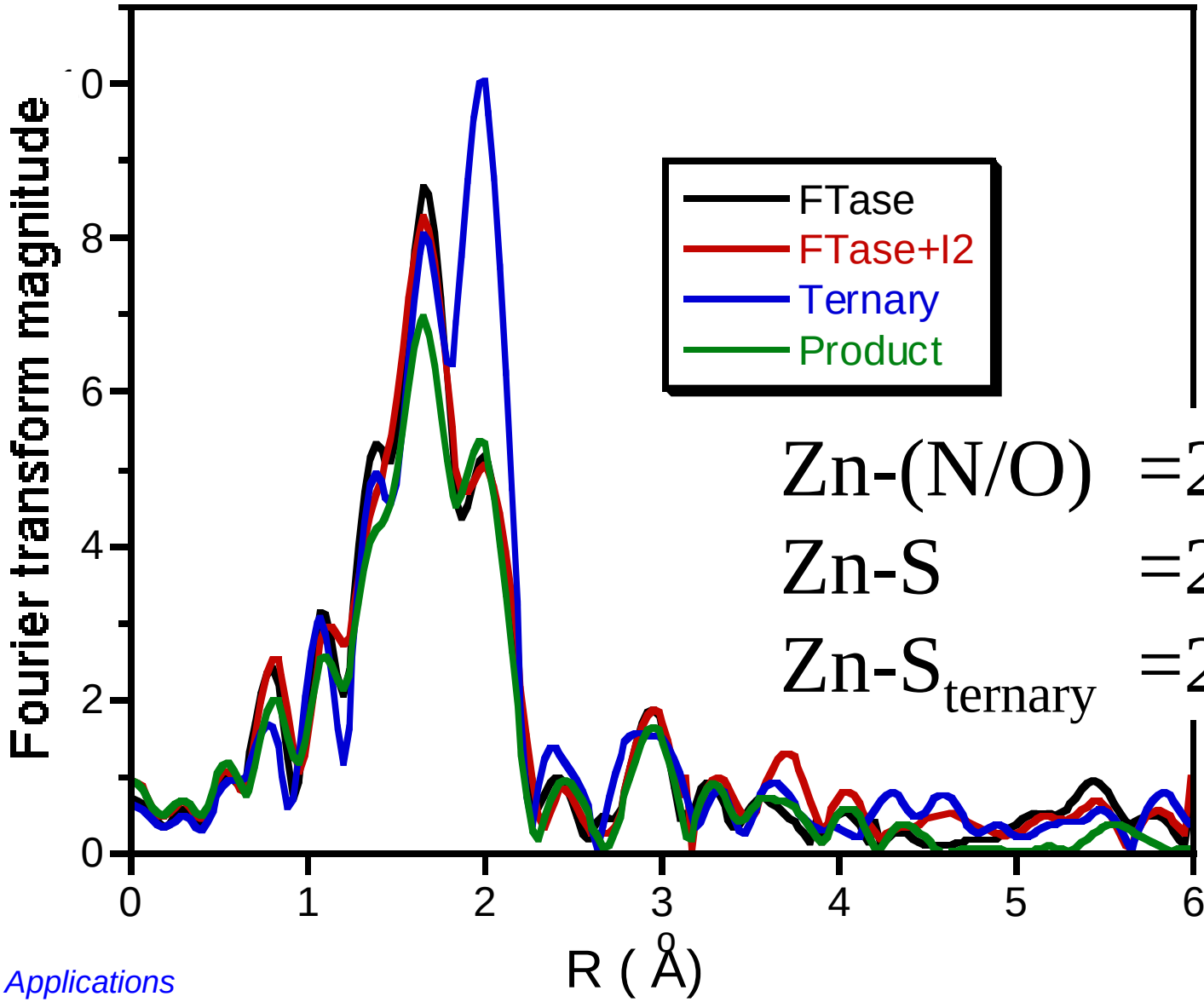


Ten FTase crystal structures are known

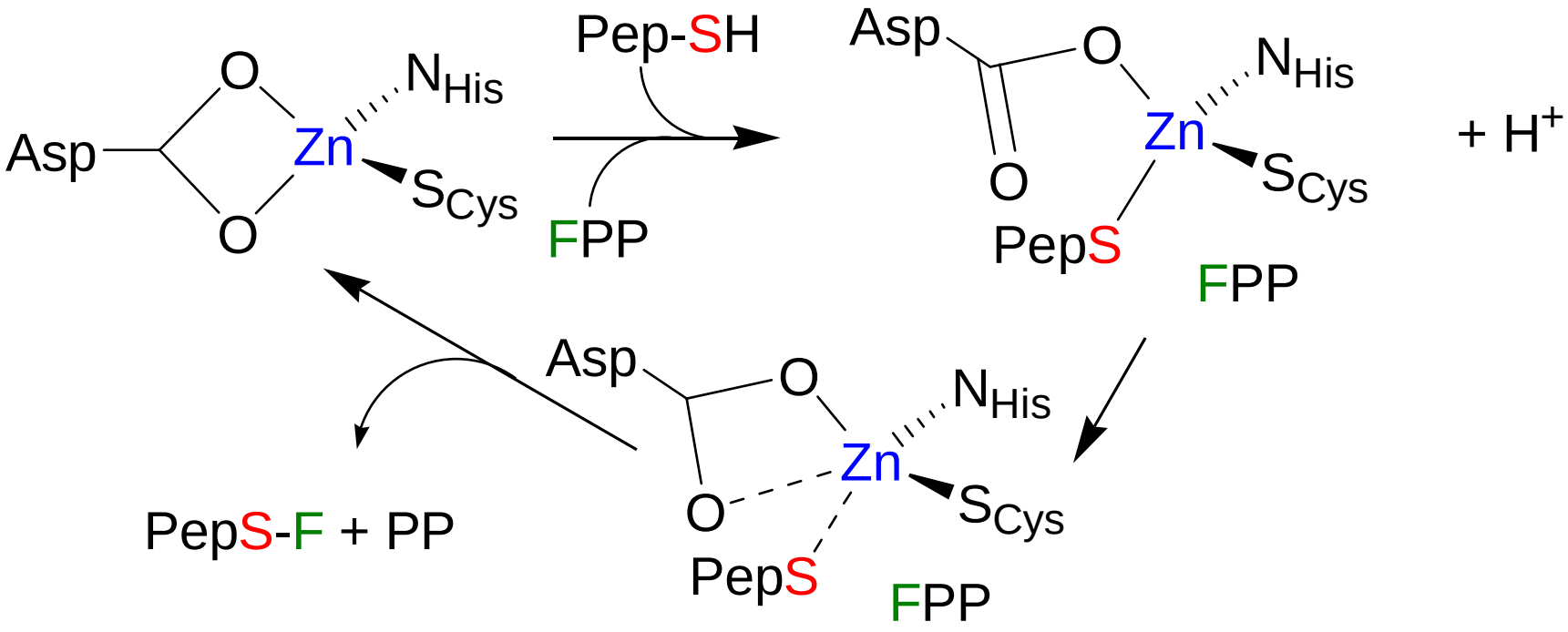
	Zn-S Pep	Zn-S C_{299}	Zn-O H_2O	Zn-O D_{297}	Zn-O D_{297}	Zn-N H_{362}
FTase	--	2.22	2.74	2.00	2.56	2.48
+FPP	--	2.27	3.22	1.99	2.03	2.10
	--	2.42	NA	2.38	3.05	2.64
Ternary	2.48	2.21	--	1.90	2.45	2.24
	2.40	2.26	--	1.99	2.61	2.18
	2.35	2.21	--	2.08	2.55	2.17
	2.41	2.33	--	1.97	2.53	2.21
	2.75	2.29	--	2.22	2.67	2.34
Product	2.66	2.27	--	2.06	2.42	2.18
Product+ FPP	--	2.30	--	2.13	2.42	2.25


Product complex
Ternary complex
FTase + I2
FTase
k^3-weighted EXAFS
k (Å^{-1})
40
30
20
10
0
-10
2
4
6
8
10
12
14
16
18

Zn is 4-coordinate; peptide sulfur binds only in ternary complex

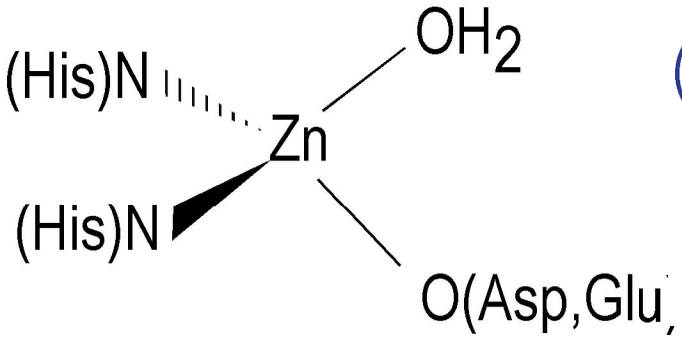


Carboxylate shift may play an important role in activating peptide thiolate

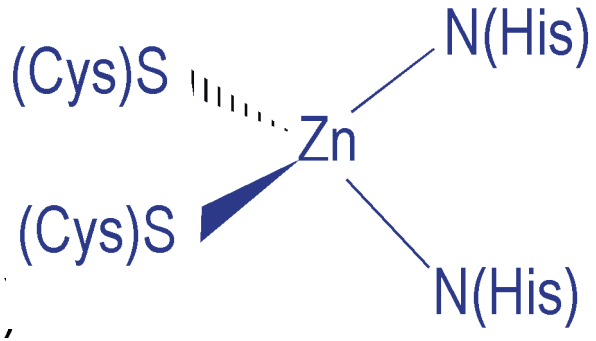


Biological Zn sites

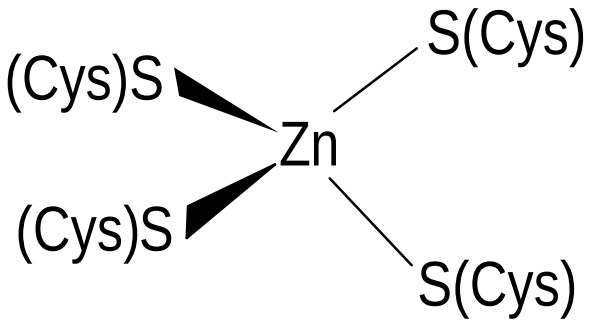
Catalytic



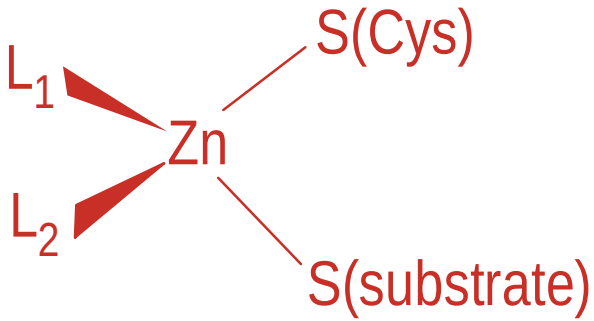
Regulatory



Structural



Alkyl-transfer



J. Am. Chem. Soc., **112** (10) 1990

p. 4031-4032

"Higher Order" Cyanocuprates $R_2Cu(CN)Li_2$: Discrete Reagents or "Lower Order" LiCN-Modified Gilman Cuprates?

Bruce H. Lipshutz,* Sunaina Sharma, and
Edmund L. Ellsworth†

as R_2CuLi·LiCN. We now describe, using spectroscopic studies, prima facie evidence in support of HO cyanocuprates.

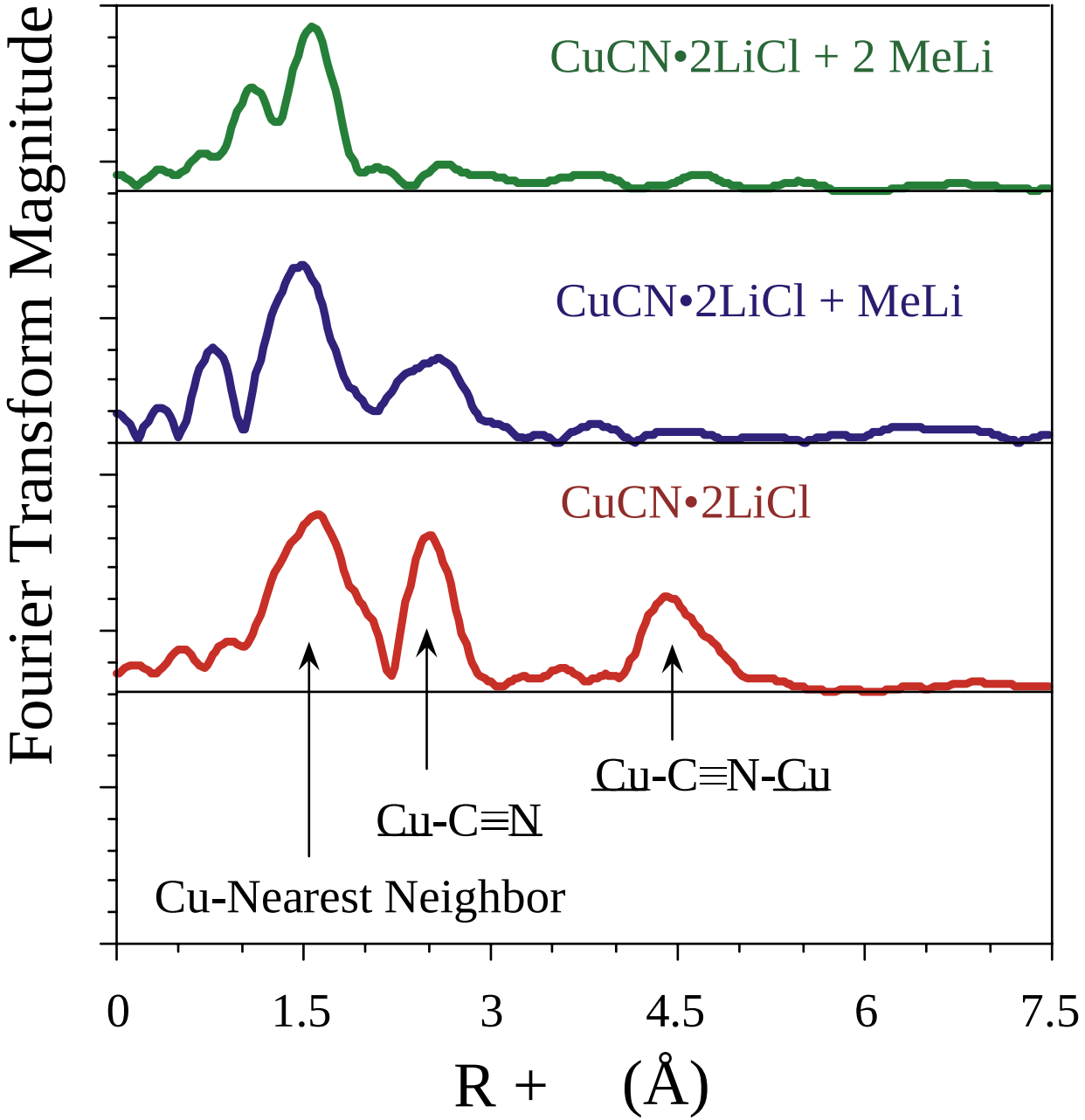
p. 4032-4034

"Higher-Order" Cyanocuprates: Are They Real?[1]

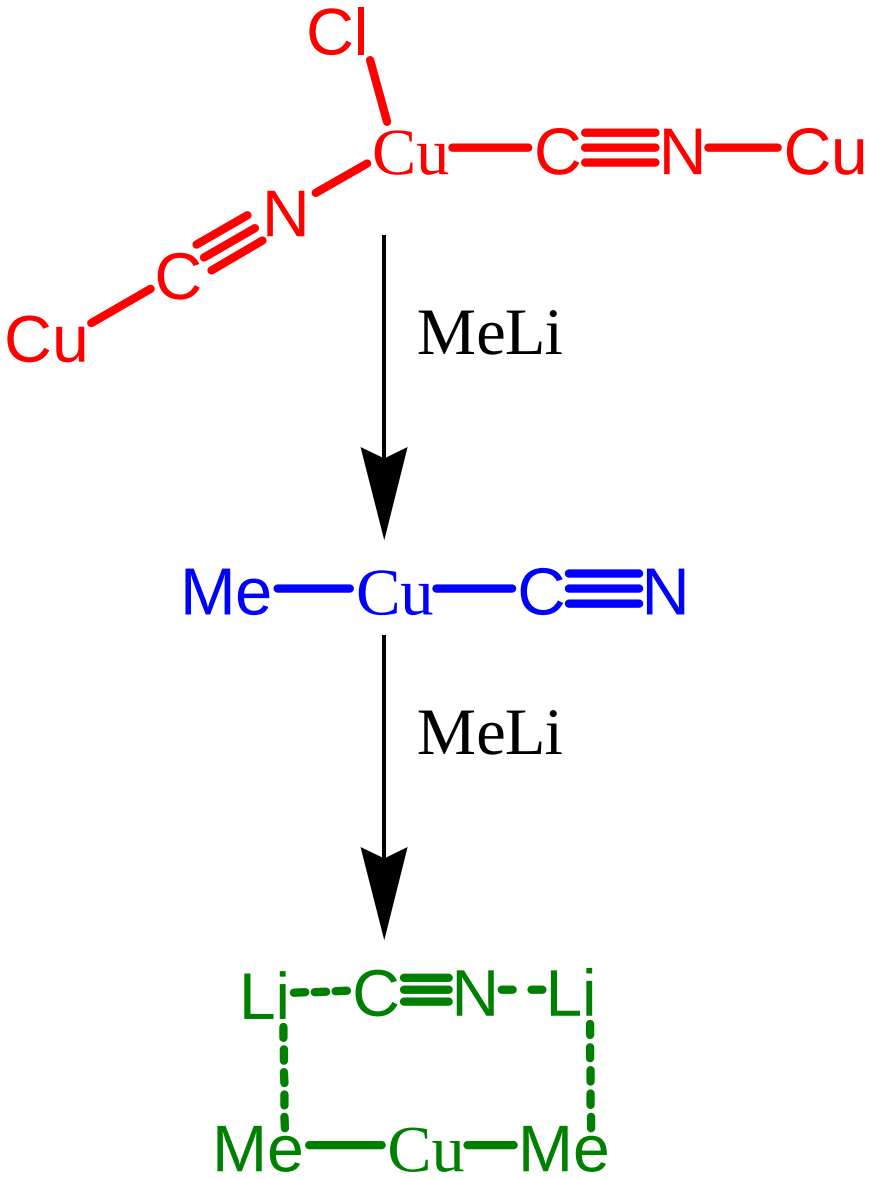
Steven H. Bertz

It can now be reported that the reagents prepared from 2 equiv of RLi (R = alkyl or aryl) and 1 equiv of CuCN may not be truly higher order *ate* complexes of Cu. ^{13}C NMR spectral evidence

EXAFS shows that CN^- does not remain bound



Structures of cyanocuprates in THF



Solution speciation of CuI+PhLi

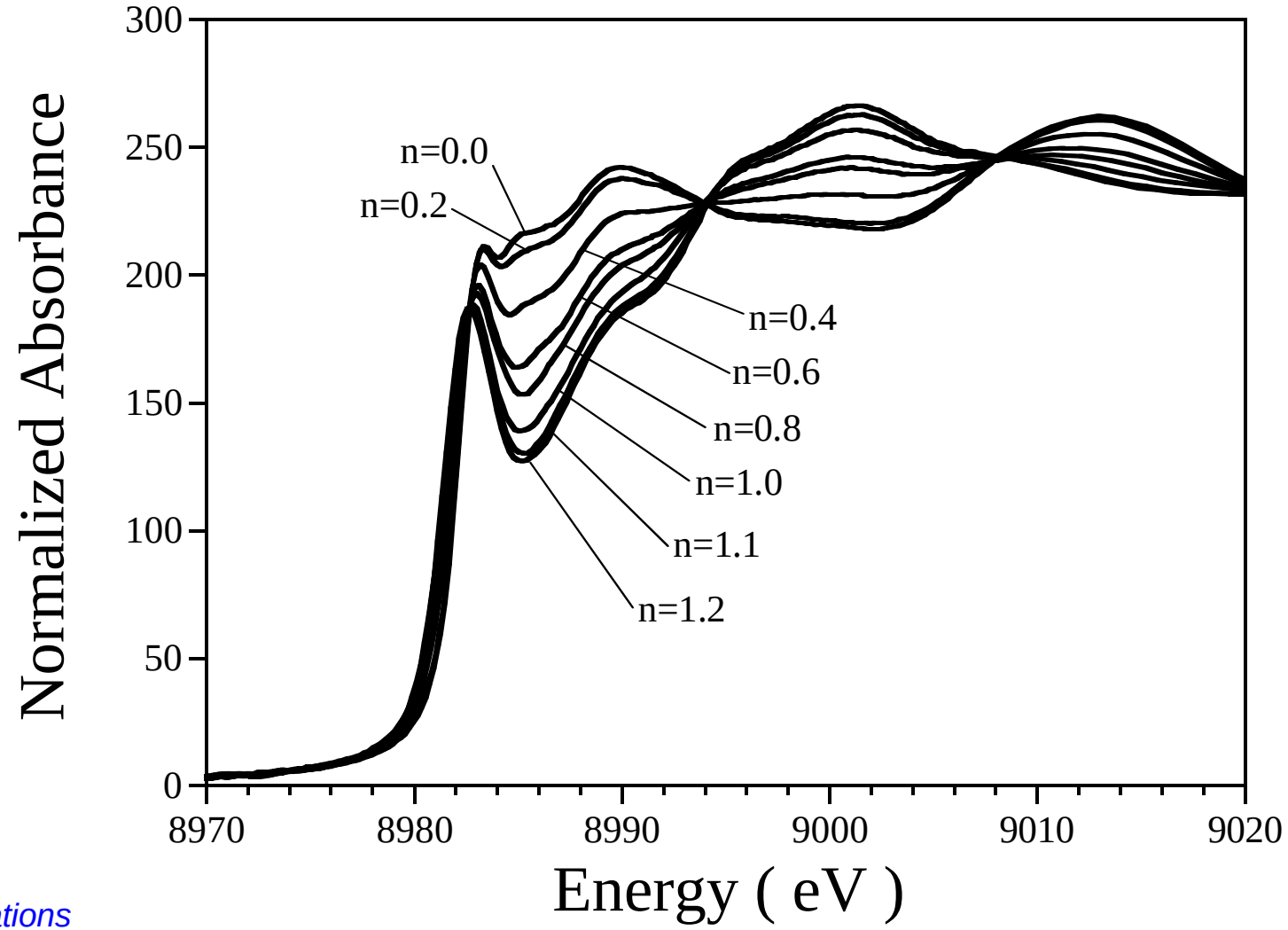
PhLi + CuI → "phenylcopper"

2PhLi+ CuI → "diphenylcuprate"

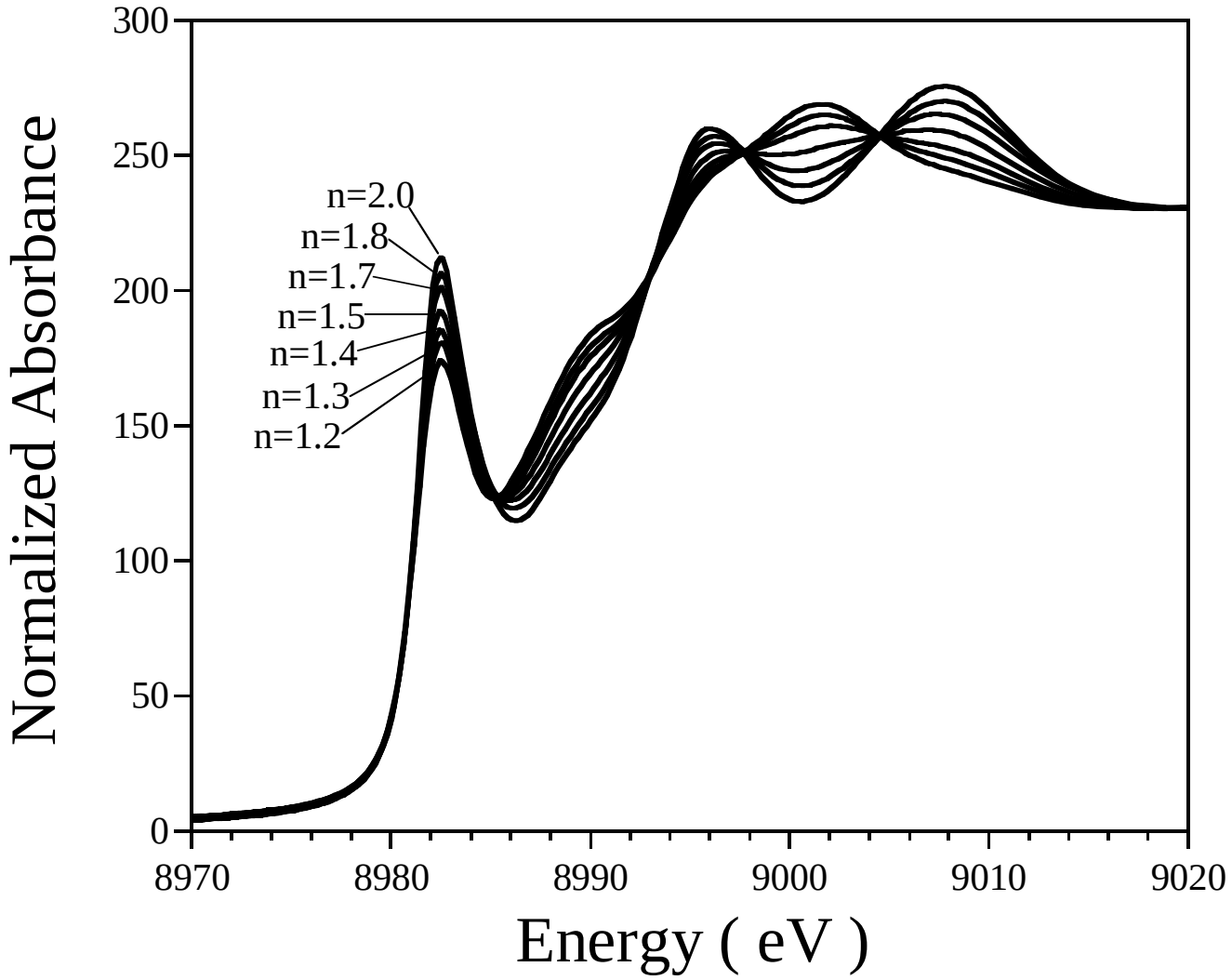
Crystalline phenyl:copper species

1:1	$Cu_4Ph_4(Me_2S)_2$	2:1	$[CuPh_2]^-$
	Cu_5Mes_5		$[CuPh_2Li]_2$
1.2:1	$[Cu_5Ph_6]^-$		$[Cu_3Li_2Ph_6]^-$
1.5:1	$[Cu_4LiPh_6]^-$		
	$[Cu_4MgPh_6]$		

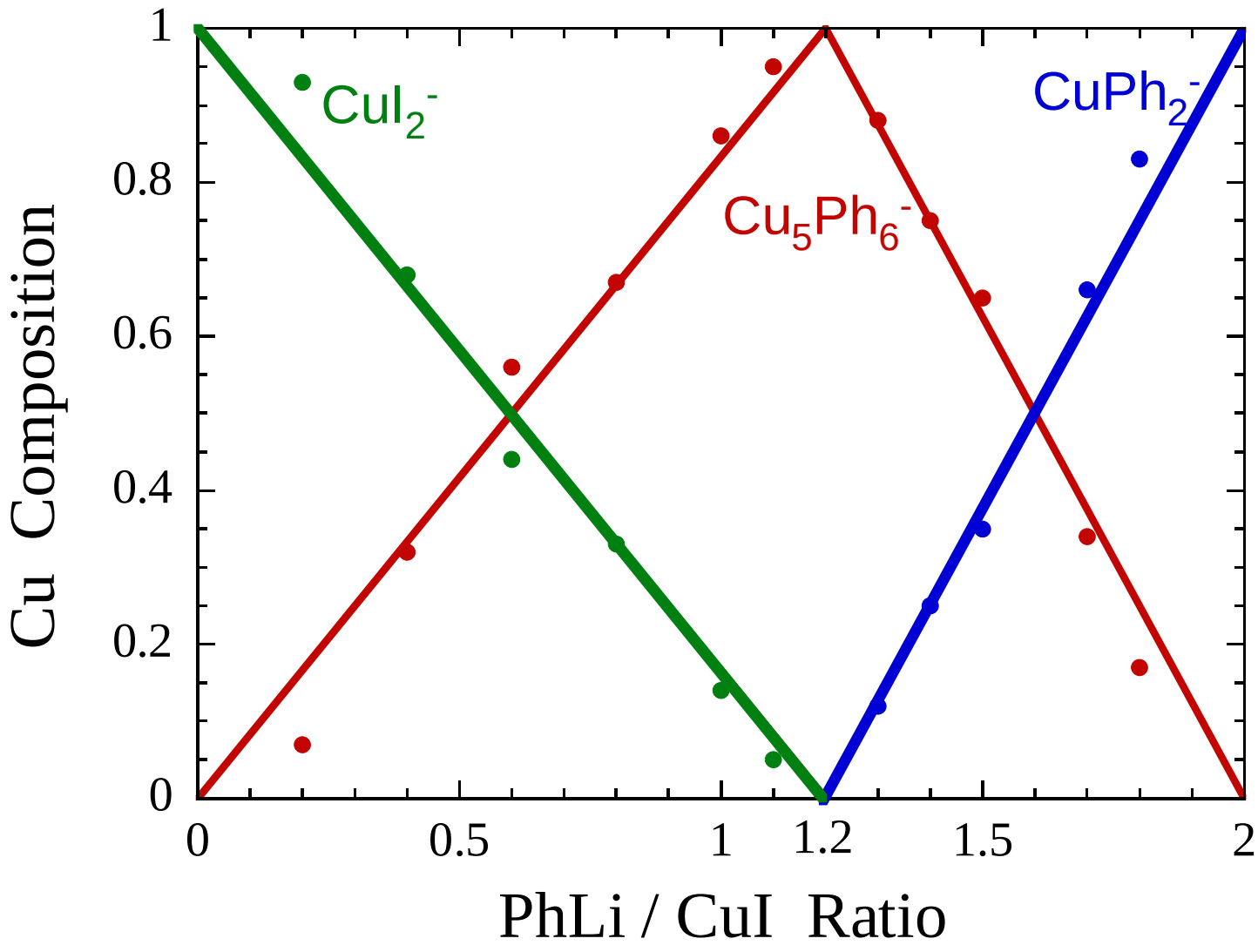
Titration of CuI+ *n* PhLi shows isosbestic behavior up to 1.2 equivalents



Titration of CuI+ *n* PhLi shows isosbestic behavior from 1.2-2.0 equivalents



EXAFS data support XANES speciation



$[CuI_2]^-$

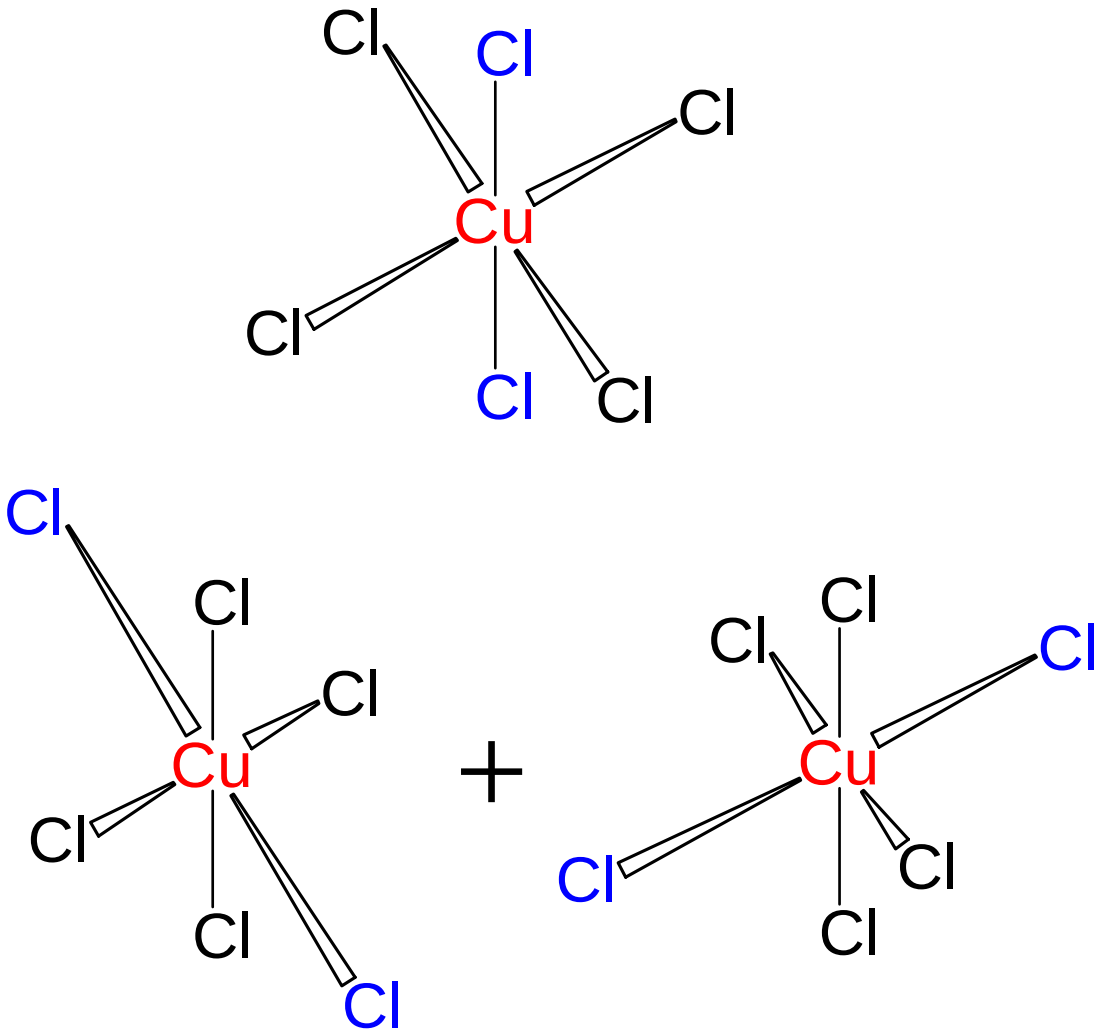
$[Cu_5Ph_6]^-$

$[CuPh_2]^-$

Inorg. Chem. **1994,** *33,* 1249–1250

EXAFS Evidence That the $CuCl_6^{4-}$ Ion in (3-Chloroanilinium)$_8$($CuCl_6$)Cl_4 Has an Elongated Rather Than Compressed Tetragonal Geometry

Paul J. Ellis,[†] Hans C. Freeman,[*,†] Michael A. Hitchman,[*,‡] Dirk Reinen,[§] and Burghard Wagner[§]



How to screw up you're the analysis of XAS data

or

some common errors, how to identify them, and how to (maybe) avoid them

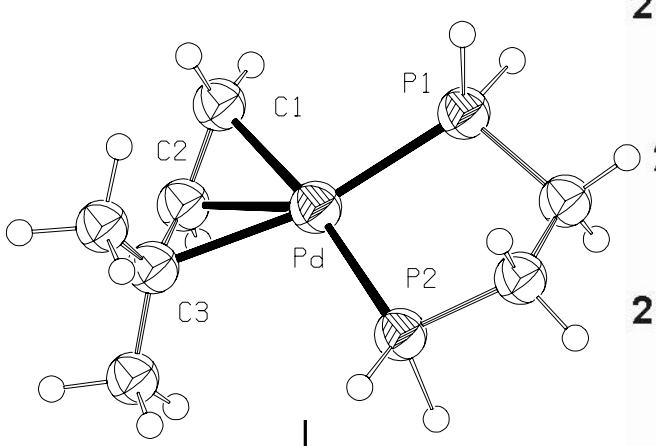
- The job of a least-squares fitting program is to give you the best (smallest deviation) solution, *not* to give you the right solution.

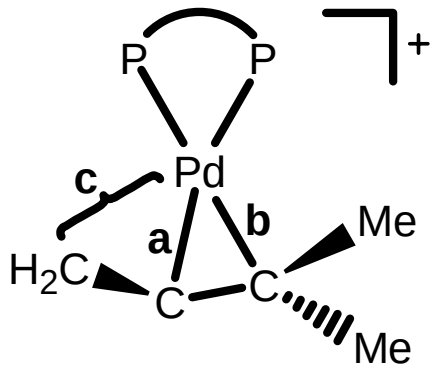
- If you see something, it tells you something; if you see nothing, it tells you nothing.

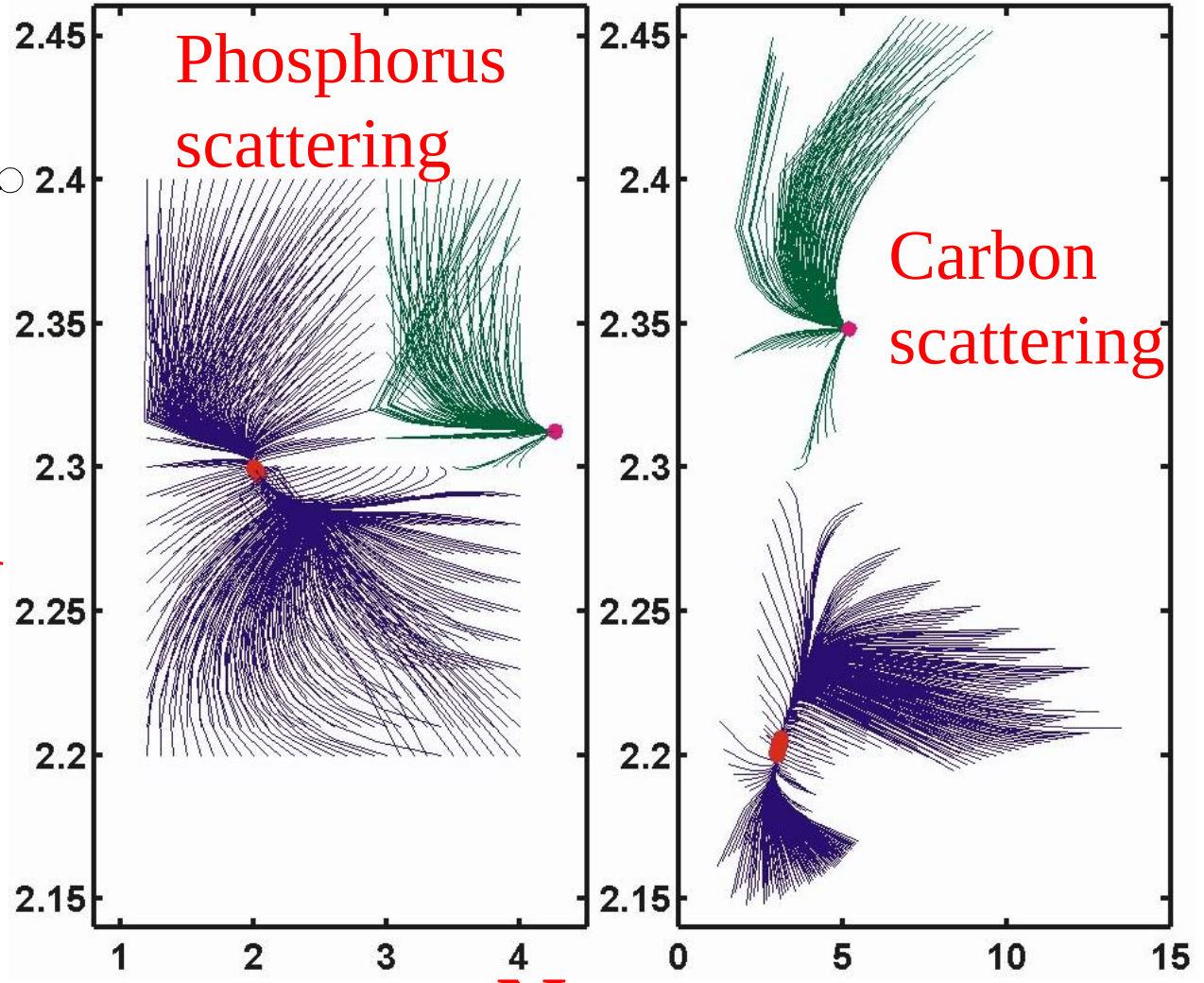
Common errors in EXAFS analysis

- Least-squares minimization
- Fourier Filtering
- Resolution

Iterative refinements are especially susceptible to multiple minima

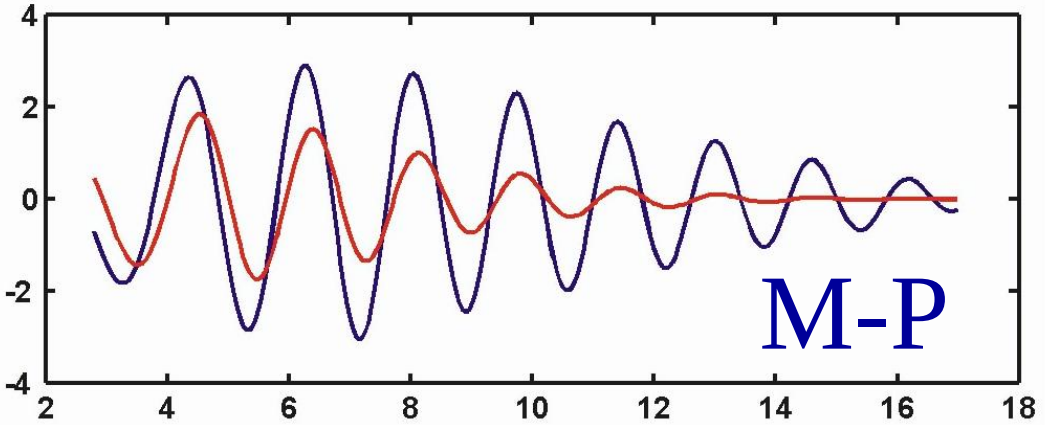


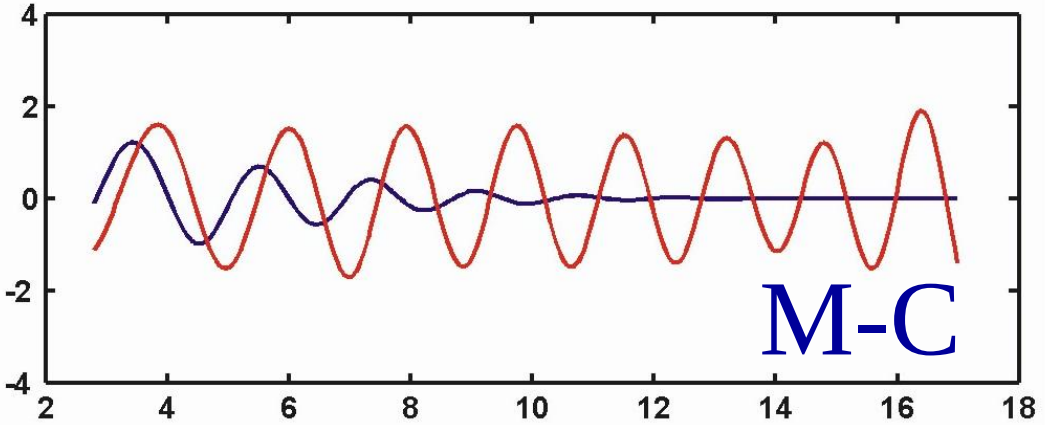


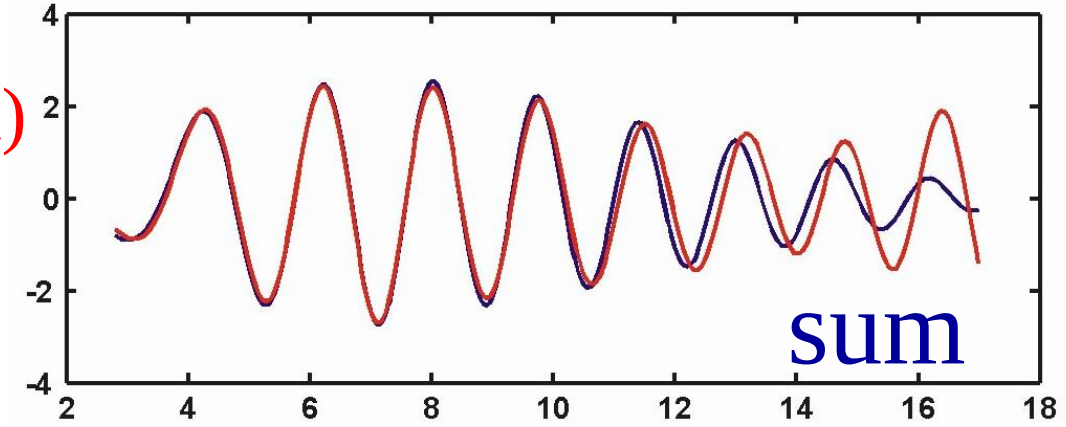


Clot, et al., *J. Am. Chem. Soc* **2004**, *126*, 9079-9084

Minima give very different structures but nearly identical EXAFS







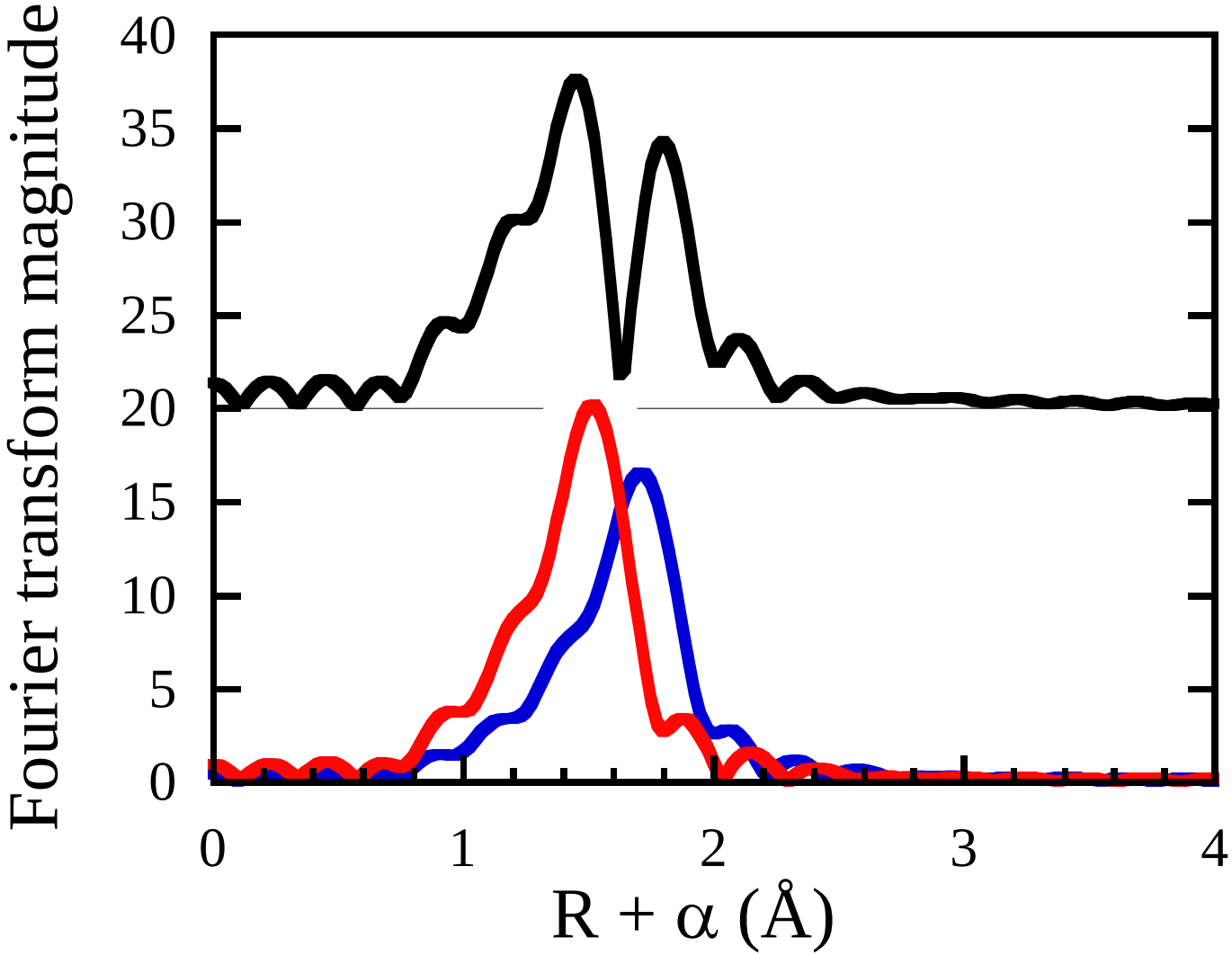
N_{free} vs. N_{obs}

$N_{obs} \sim 300$

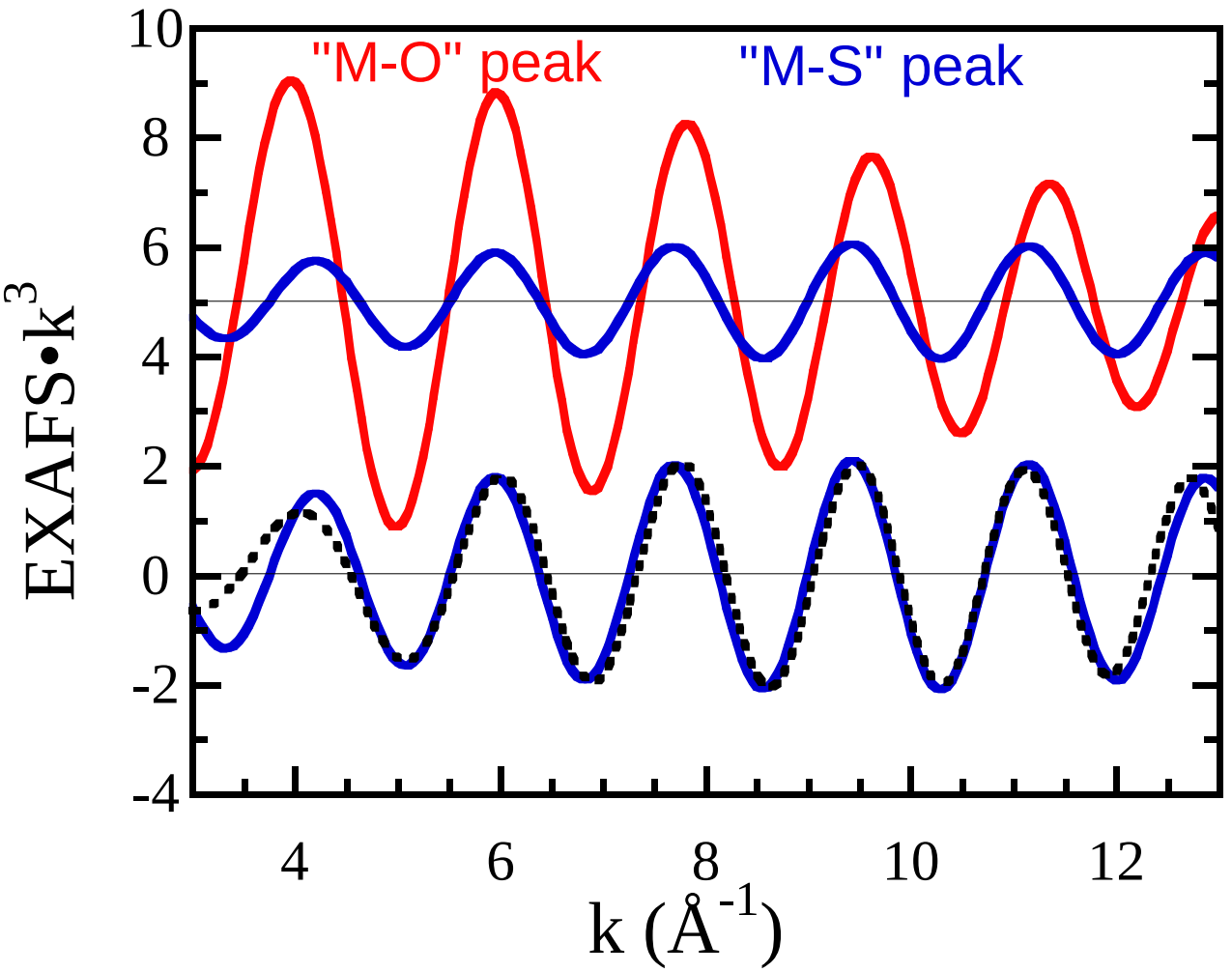
($\Delta k = 0.05$ Å; $k_{max} = 15$ Å)

$N_{free} \sim (2\ \Delta k\ \Delta R\ ) / \pi$

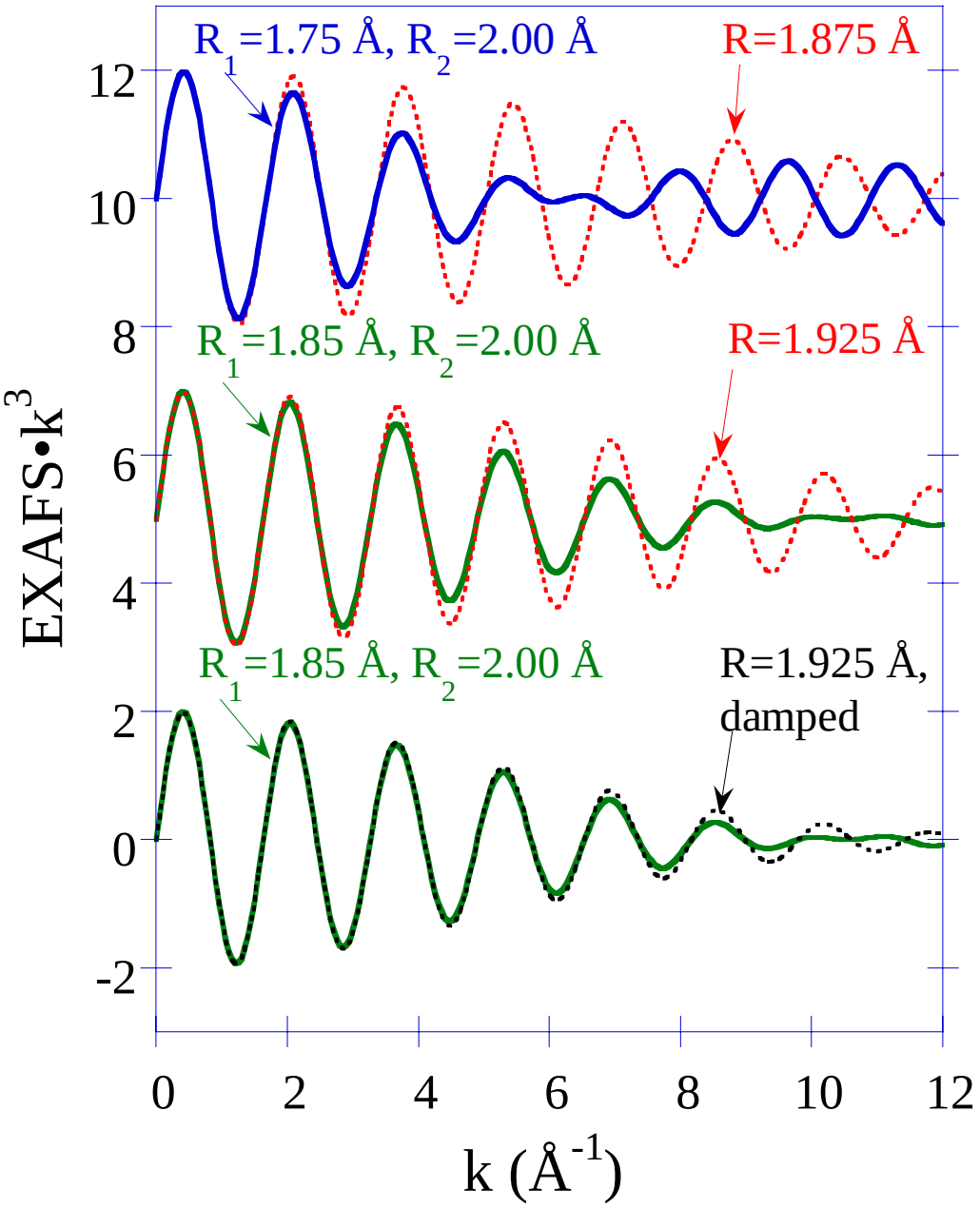
M-O at 2.0 and 2.2 Å give two apparently well resolved peaks in FT



Fitting each filtered peak gives the appearance of M-O and M-S EXAFS



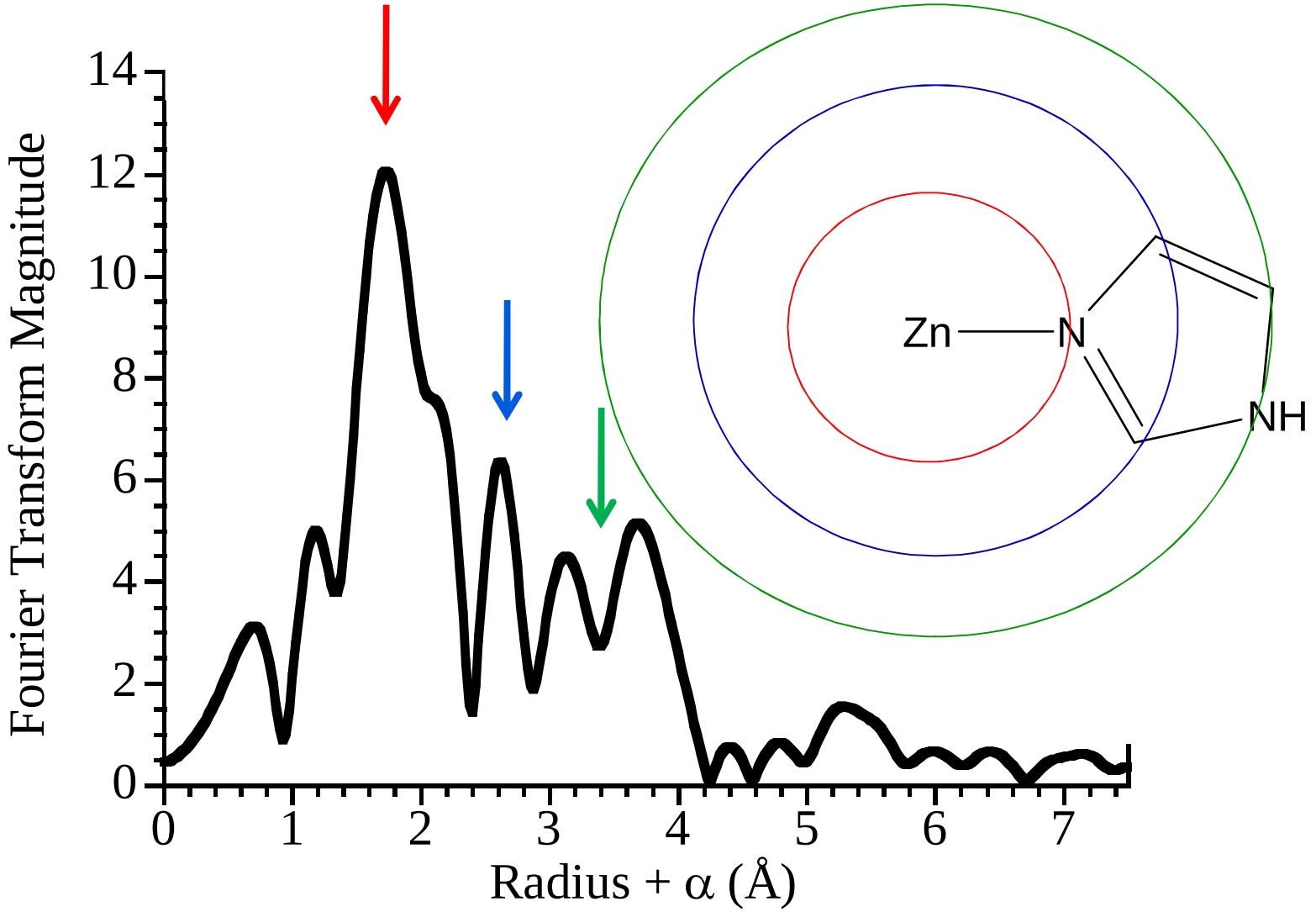
EXAFS resolution is $\sim \pi/2\Delta k$ = 0.13 Å



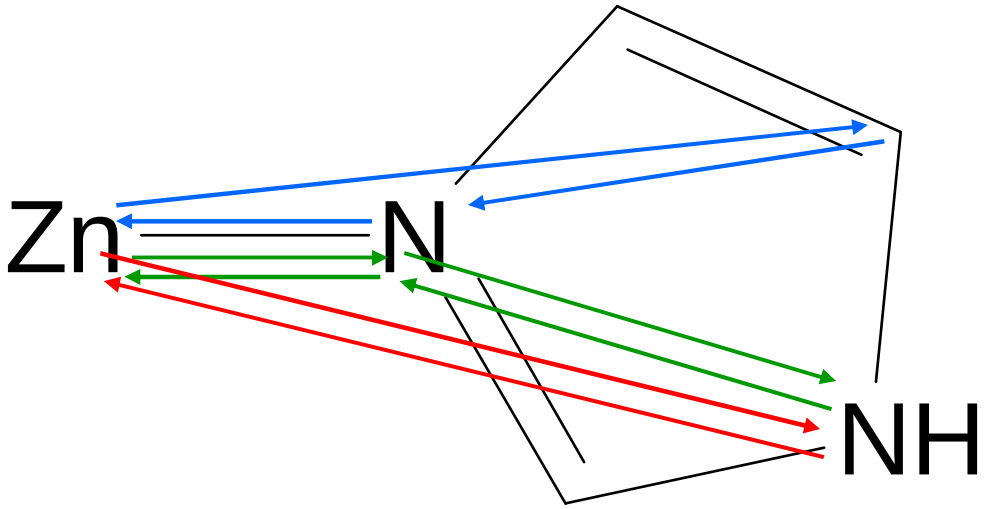
Summary

- The more variables you control, the more likely you are to obtain a unique solution
- Multiple data sets (elements, temperature, concentration, time, etc.) almost always help
- Conclusions are only as good as your model

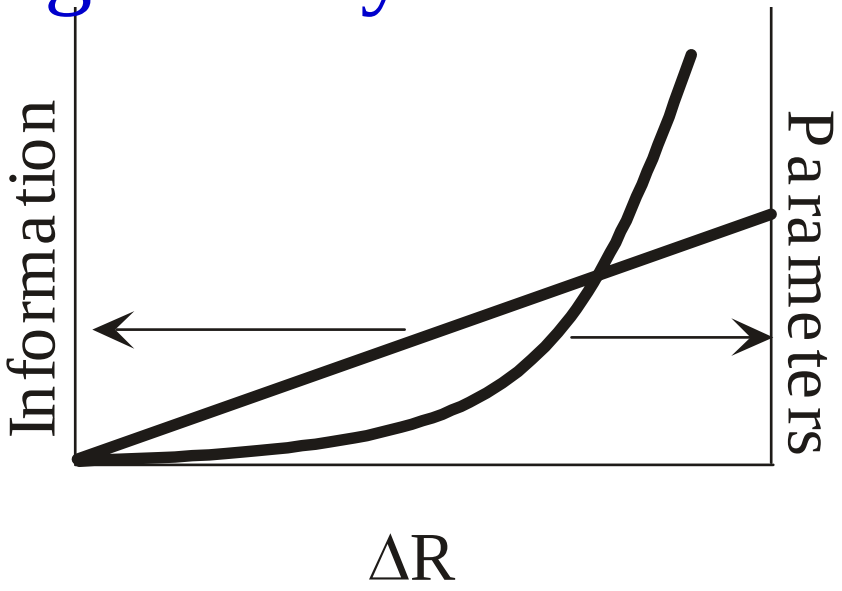
Outer shell scattering can provide ligand identification and geometric information

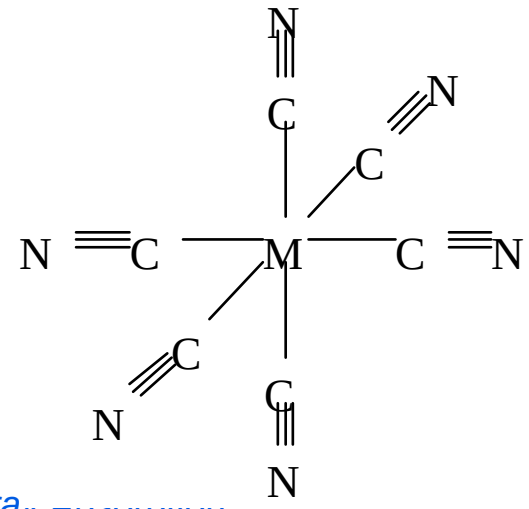


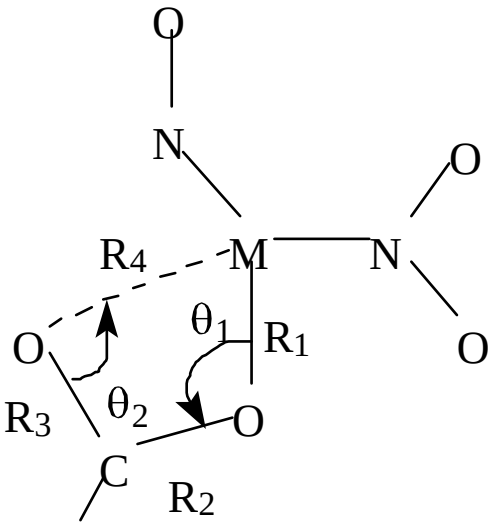
Multiple scattering makes EXAFS sensitive to angular arrangement of ligands



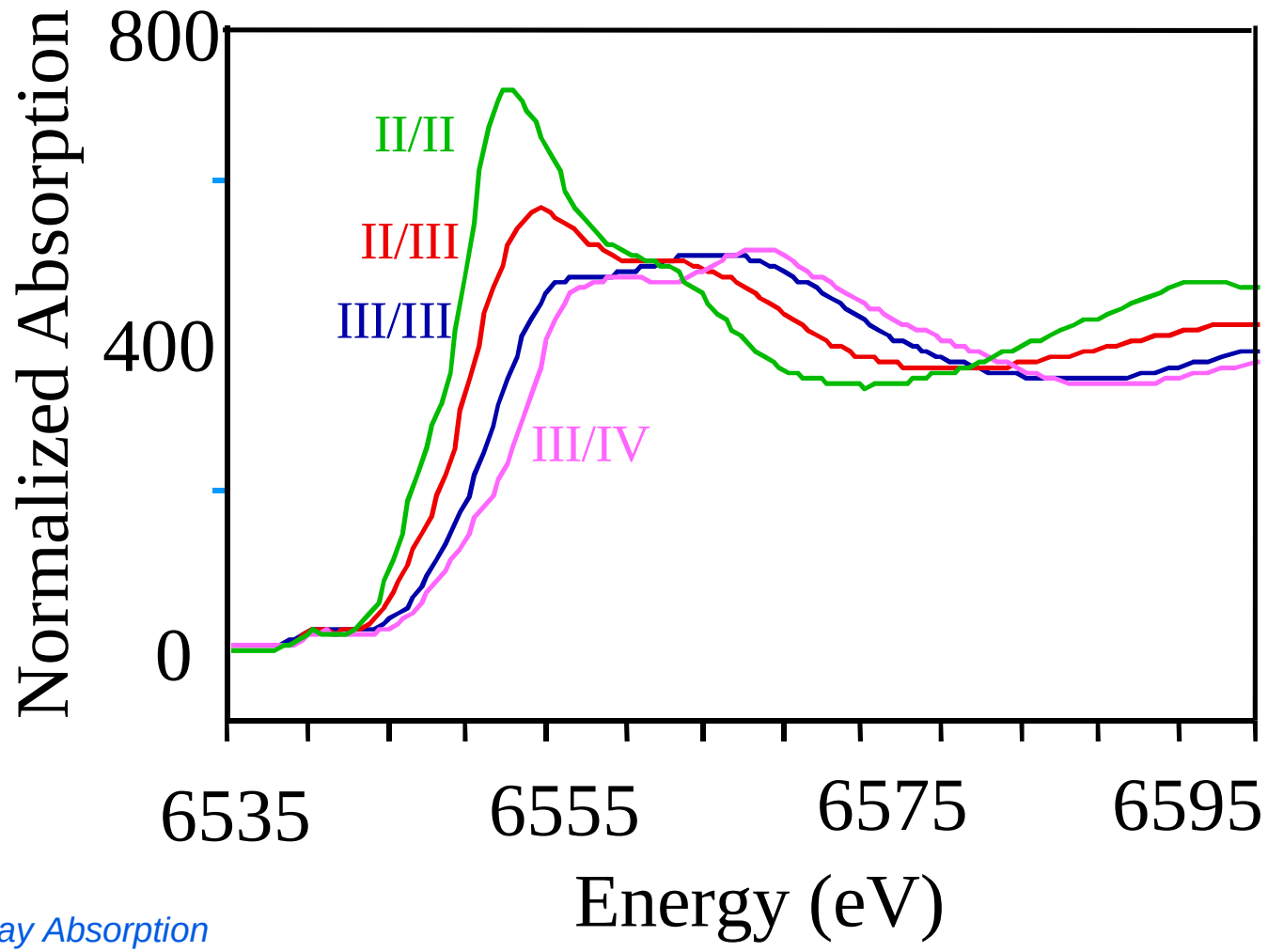
However, ability to reliably determine geometry is limited

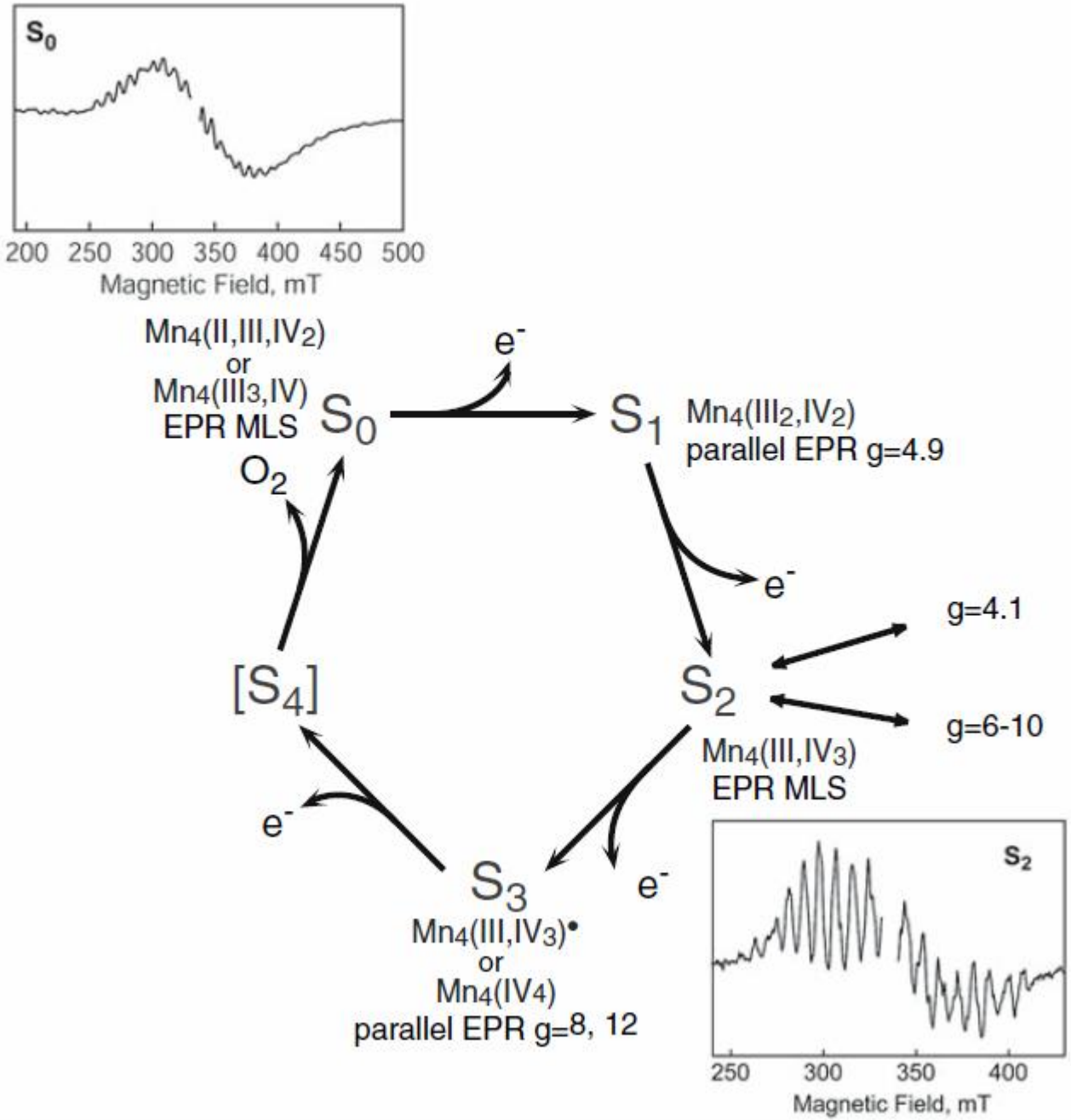




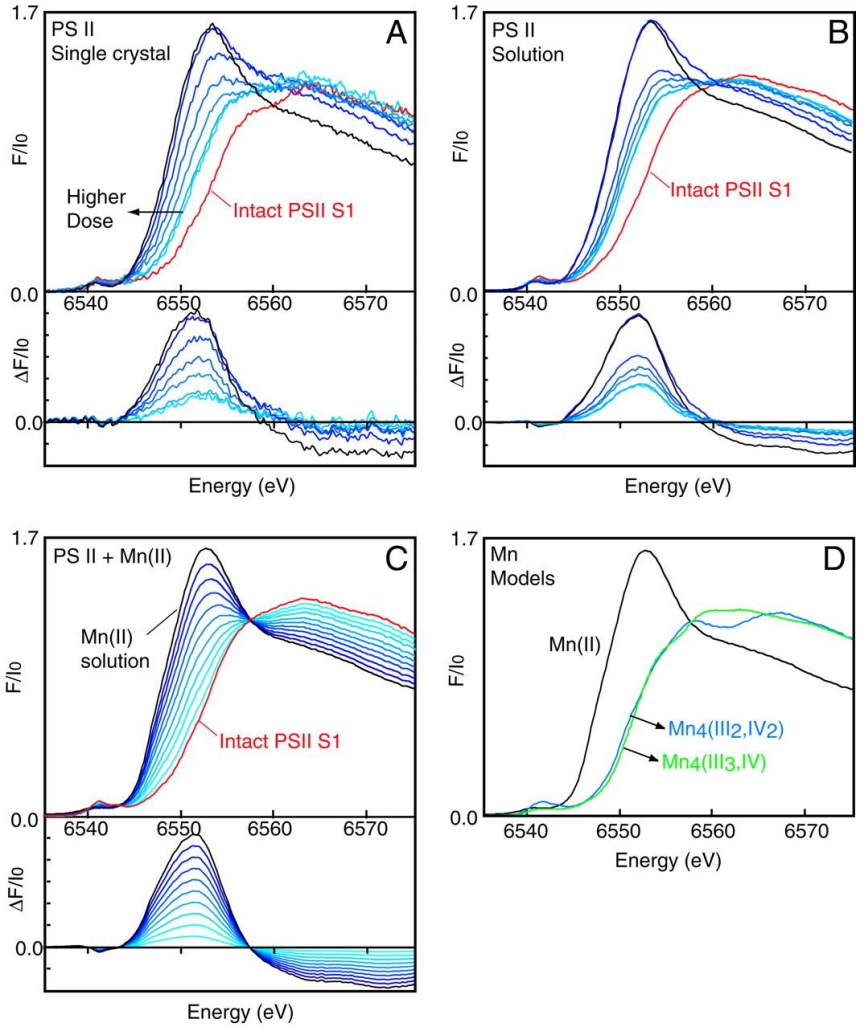


Dependence of XANES on Oxidation State




S0
200 250 300 350 400 450 500
Magnetic Field, mT
Mn4(II,III,IV2)
or
Mn4(III3,IV)
EPR MLS
S0
e-
S1
Mn4(III2,IV2)
parallel EPR g=4.9
O2
e-
g=4.1
[S4]
S2
g=6-10
Mn4(III,IV3)
EPR MLS
e-
e-
S3
Mn4(III,IV3)•
or
Mn4(IV4)
parallel EPR g=8, 12
S2
250 300 350 400
Magnetic Field, mT

Mn XANES of PS II versus x-ray dose and XANES of inorganic model compounds.



Yano J et al. PNAS 2005;102:12047-12052

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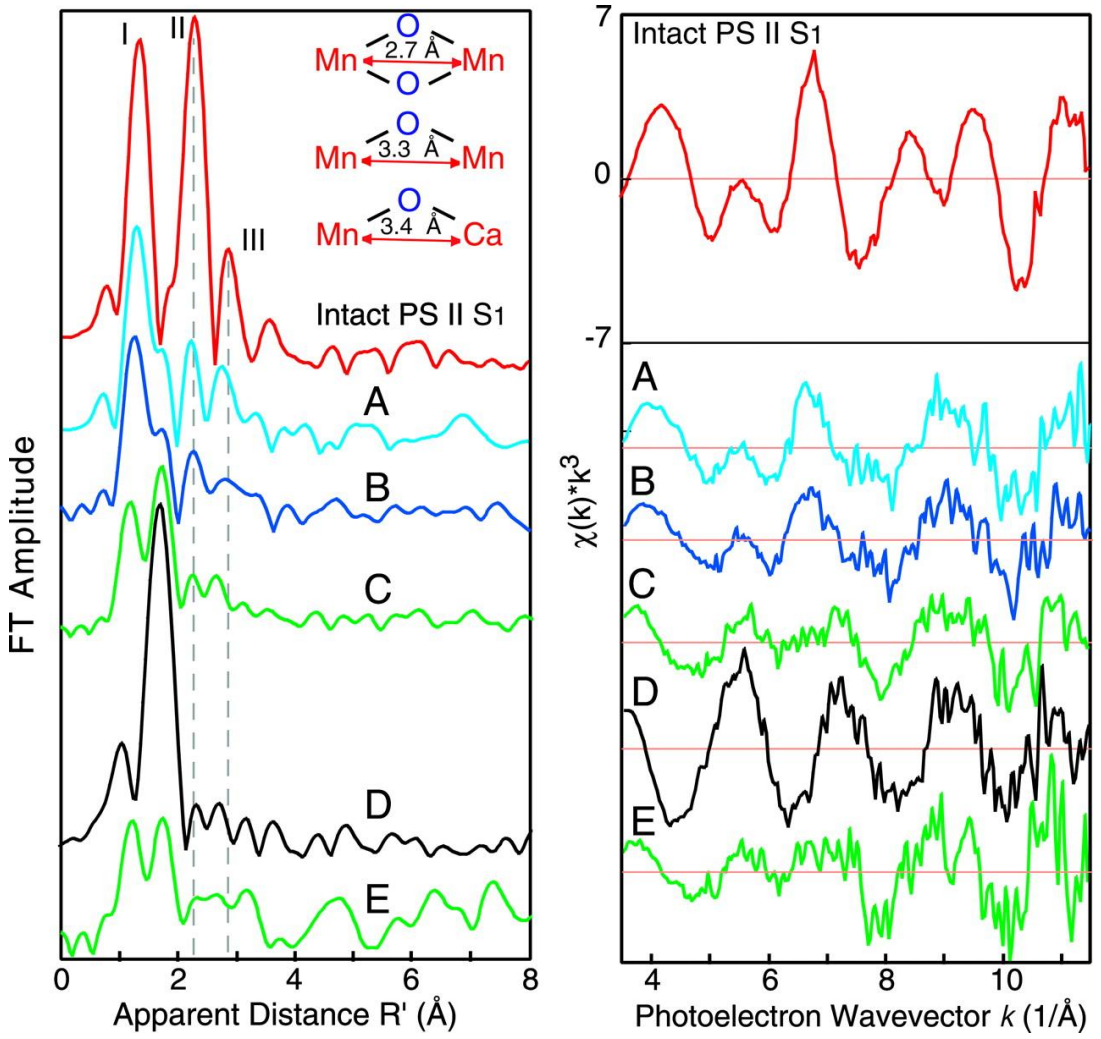
Spectral changes of PS II Mn EXAFS due to radiation damage with FTs (Left) and the k3-space EXAFS (Right).



Yano J et al. PNAS 2005;102:12047-12052

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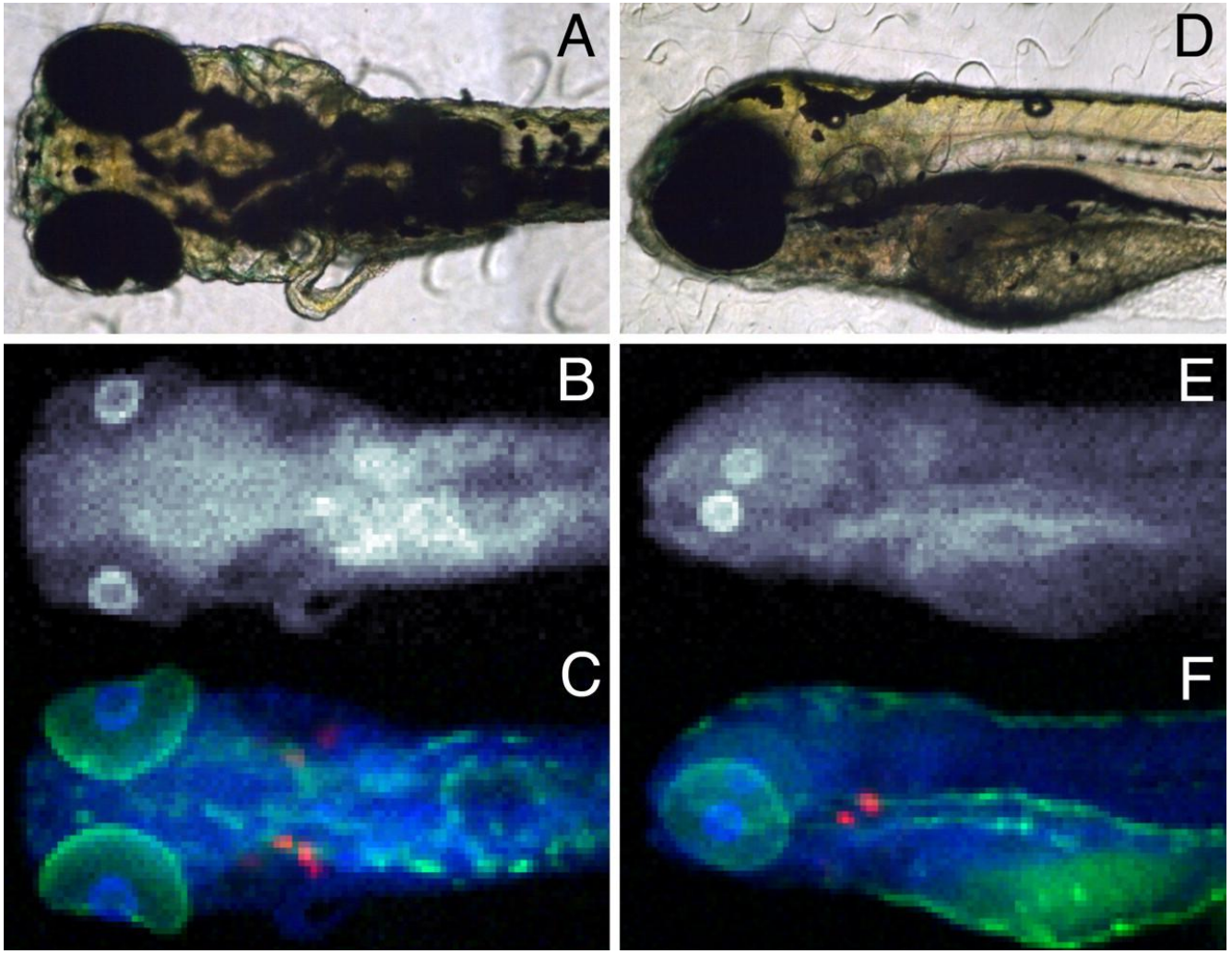
X-ray fluorescence imaging



Korbas M et al. PNAS 2008;105:12108-12112

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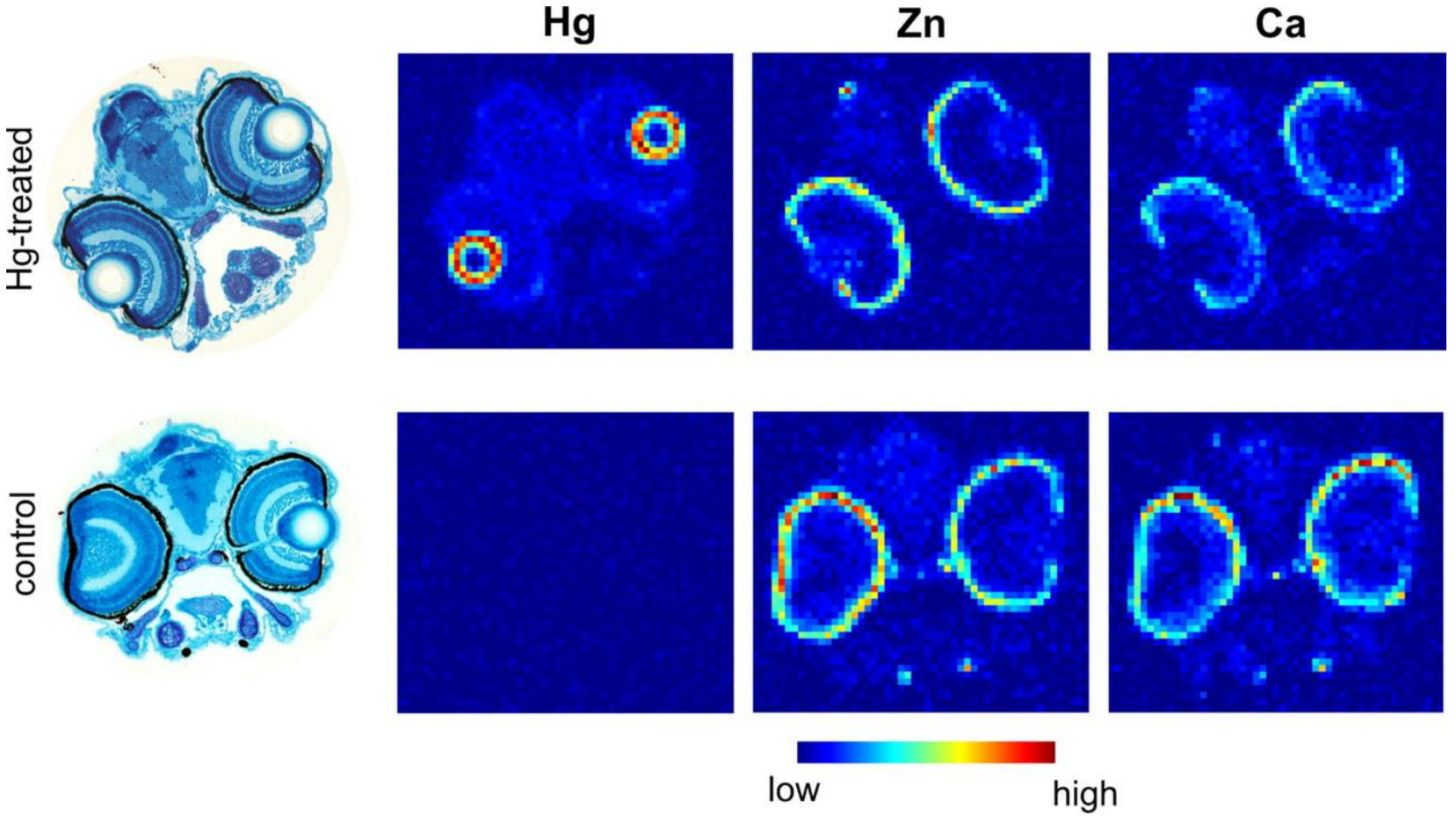
Elemental distributions in MeHg exposed and unexposed zebrafish.



Korbas M et al. PNAS 2008;105:12108-12112

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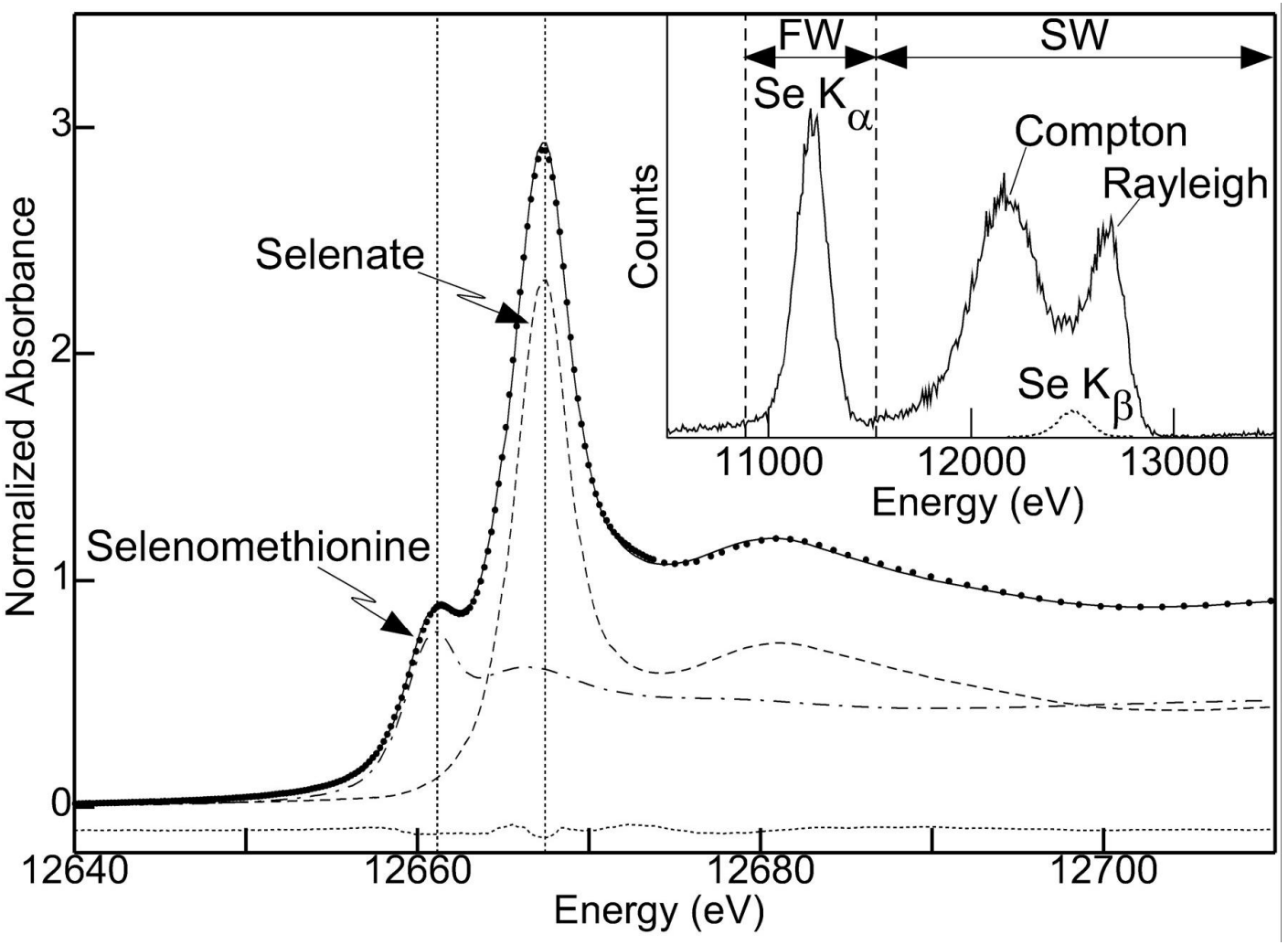
Se K x-ray absorption near-edge spectrum of A.



Pickering I J et al. PNAS 2000;97:10717-10722

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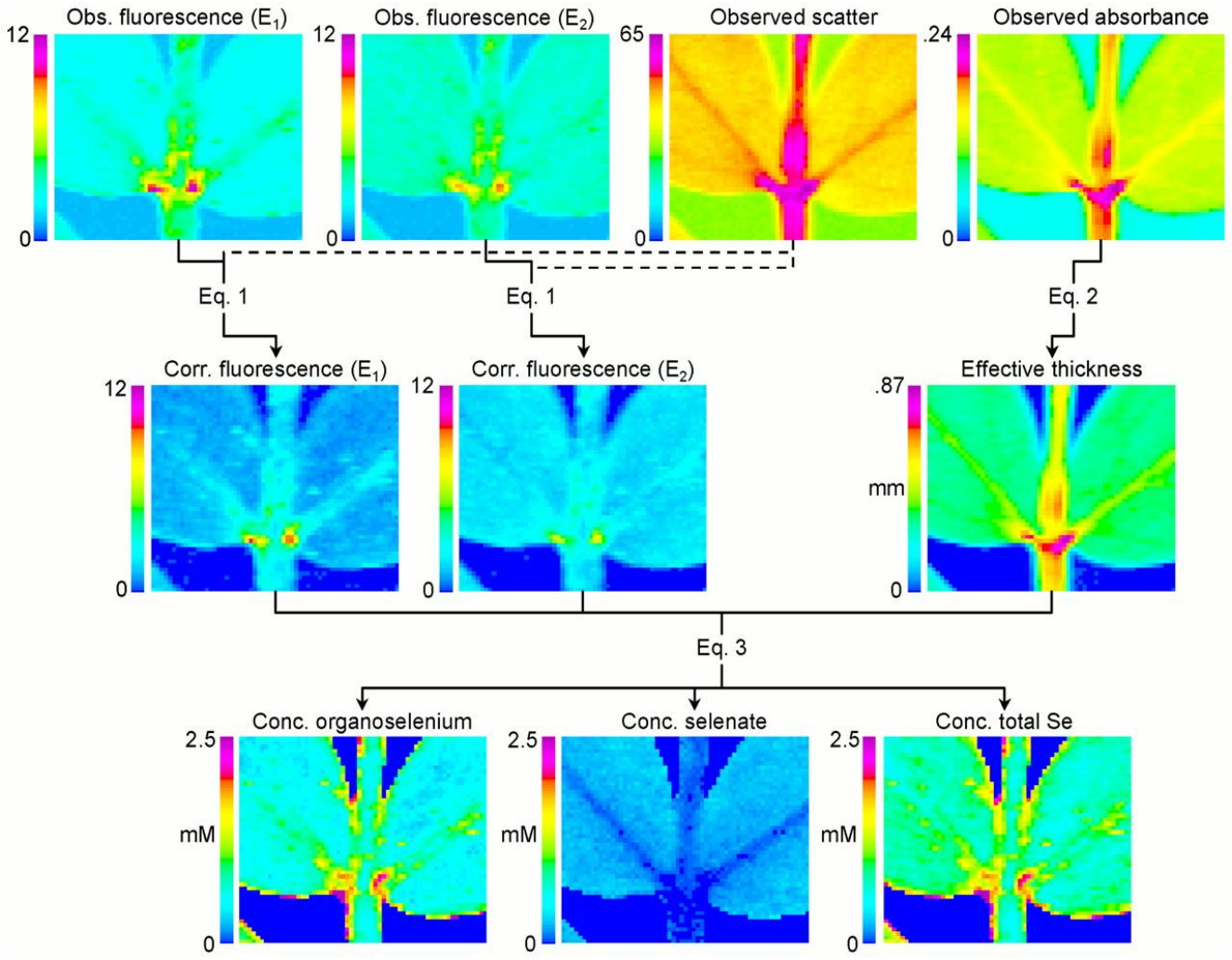
Data reduction scheme for the method of chemically specific imaging.



Pickering I J et al. PNAS 2000;97:10717-10722

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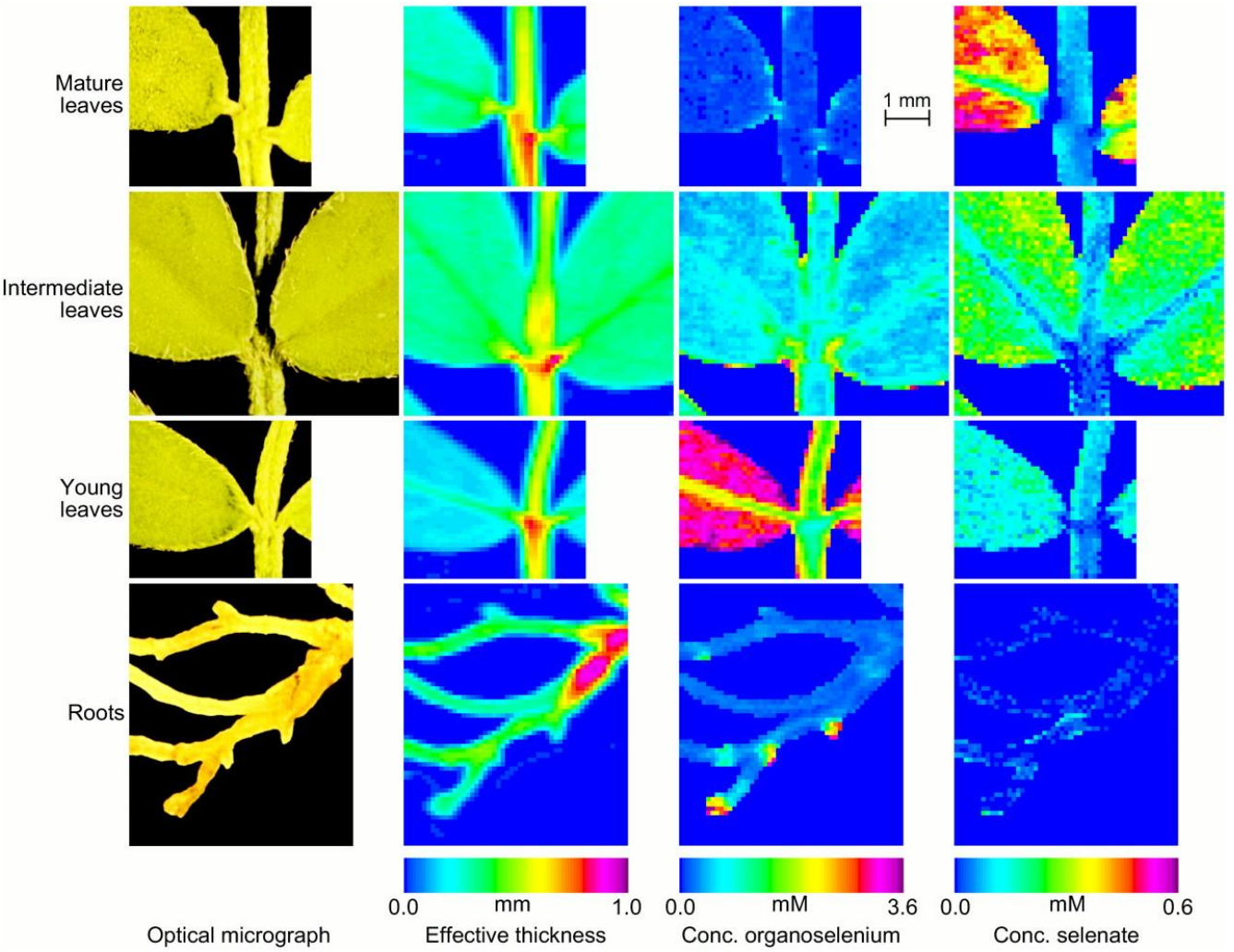
Chemically specific concentration images of different parts of A. bisulcatus.



Pickering I J et al. PNAS 2000;97:10717-10722

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