X-ray absorption spectroscopy of Zn sites in biology

When is a thiolate not a thiolate?

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Zn is one of the most wide-spread elements in biology

- Zn is typically coordinated to S (thiolate), N (Histidine) or O (water, carboxylate)
- Zn is usually tetrahedral
- Question of interest is determining the relative number of S vs. N/O ligands

EXAFS can readily distinguish S from N/O ligation

- EXAFS can distinguish ligands that differ by ca. 10 in atomic number

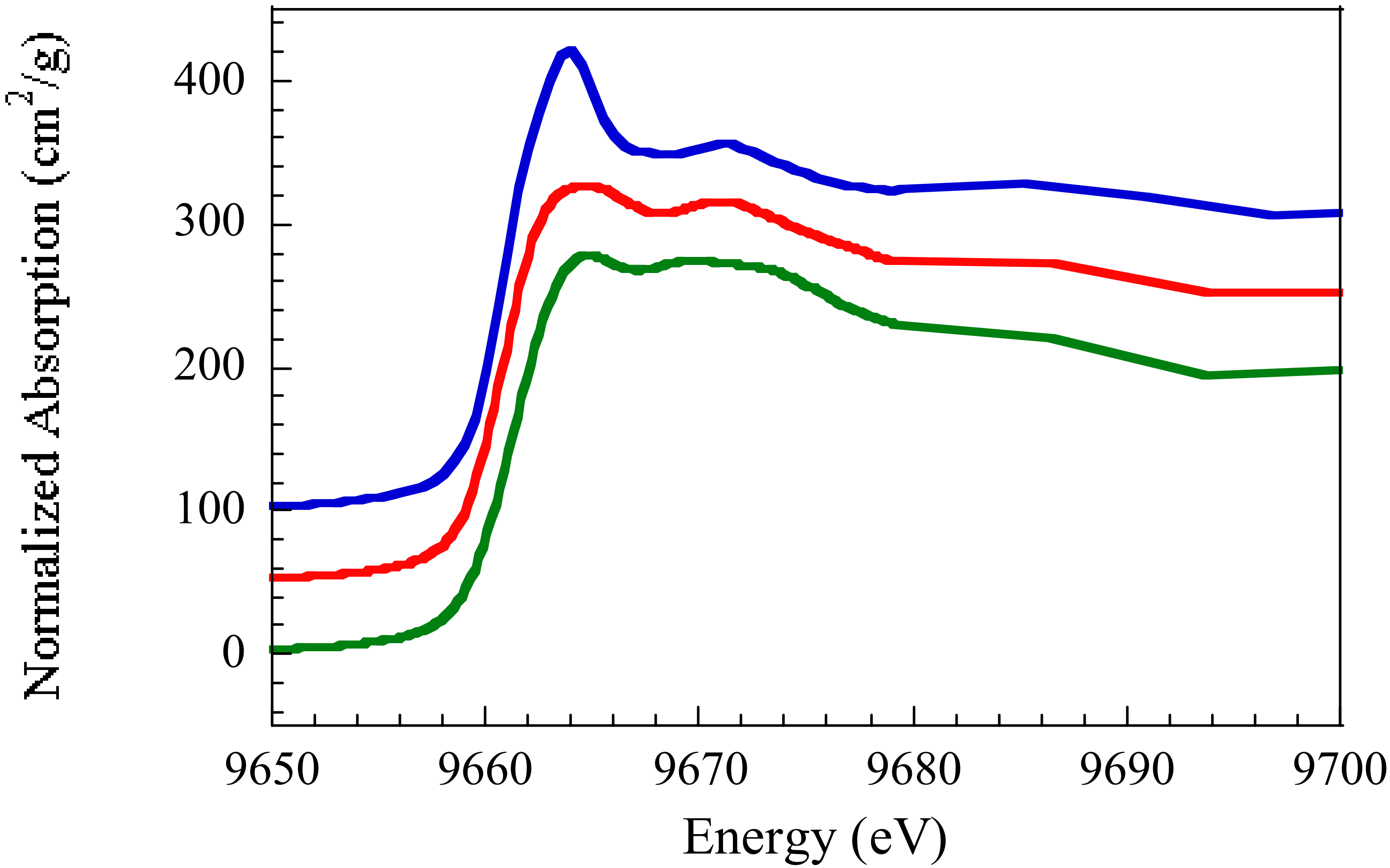
However

- There are several examples in which EXAFS has overestimated the number of sulfur ligands.
- And there are cases where EXAFS has underestimated the number of sulfur ligands.

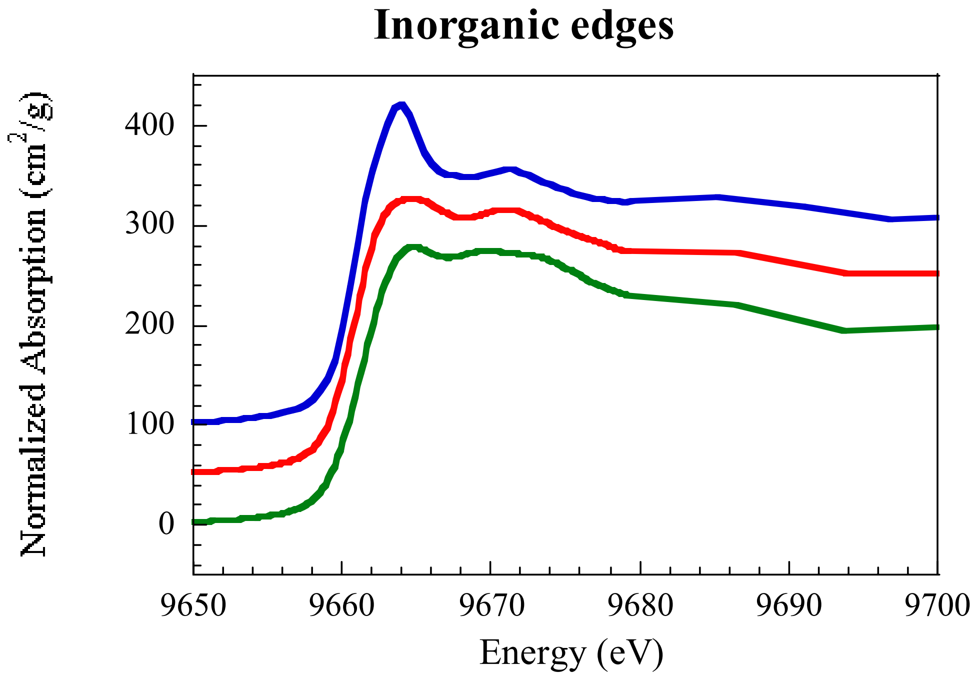
Goal: Use well-defined model compounds to understand limitations of ligation determination

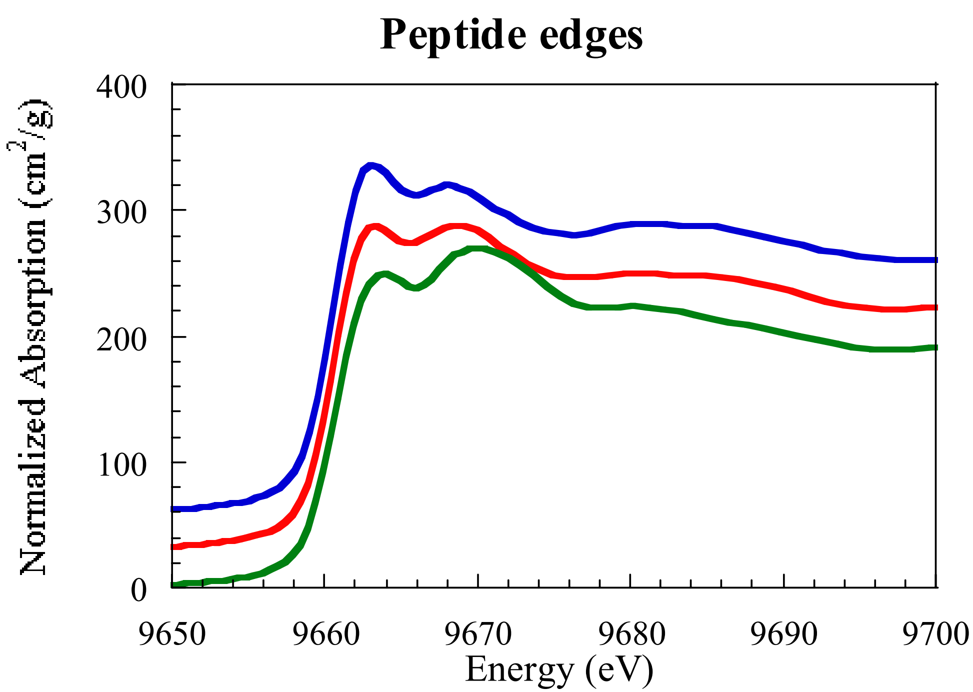
- "Inorganic" – thiolate/imidazole models (S. Koch).
- "Peptide" – cysteine/histidine peptide models (J. Berg)

XANES spectra depend on Zn ligation

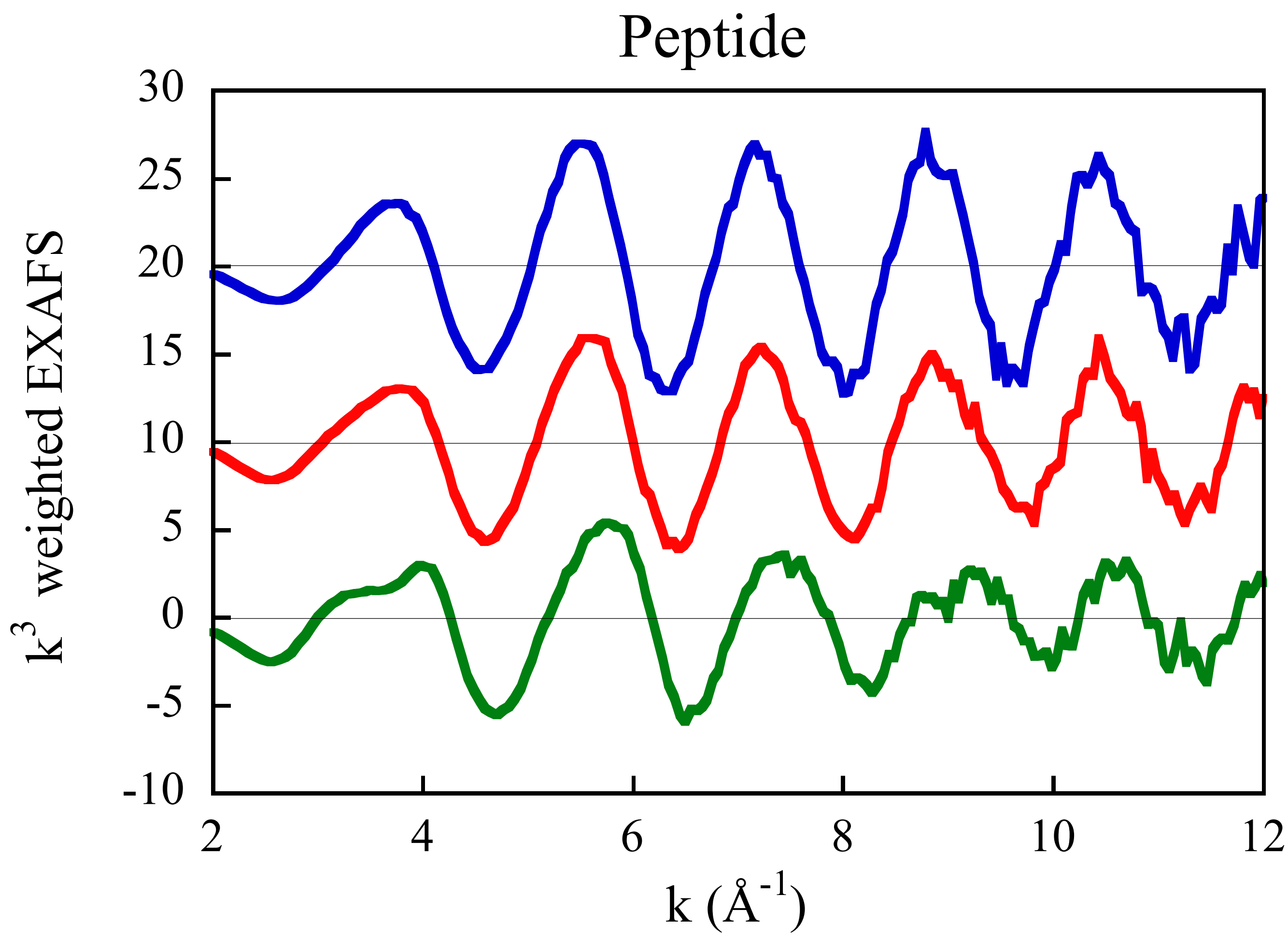


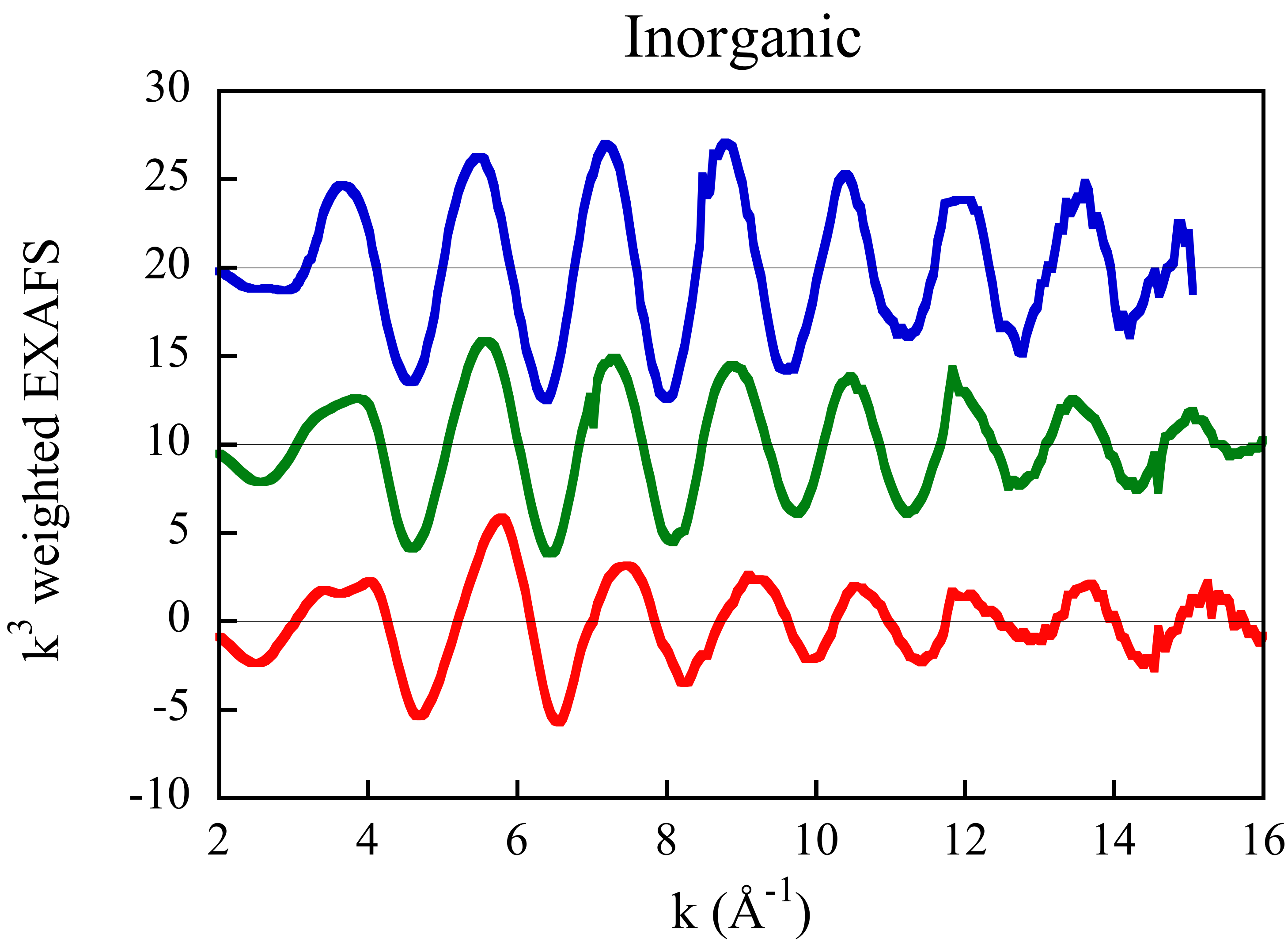
However, variation from sample to sample is larger than variation from ligation to ligation. (Despite the fact that 9660 eV feature is sometimes identified as “characteristic of ZnS4 ligation.





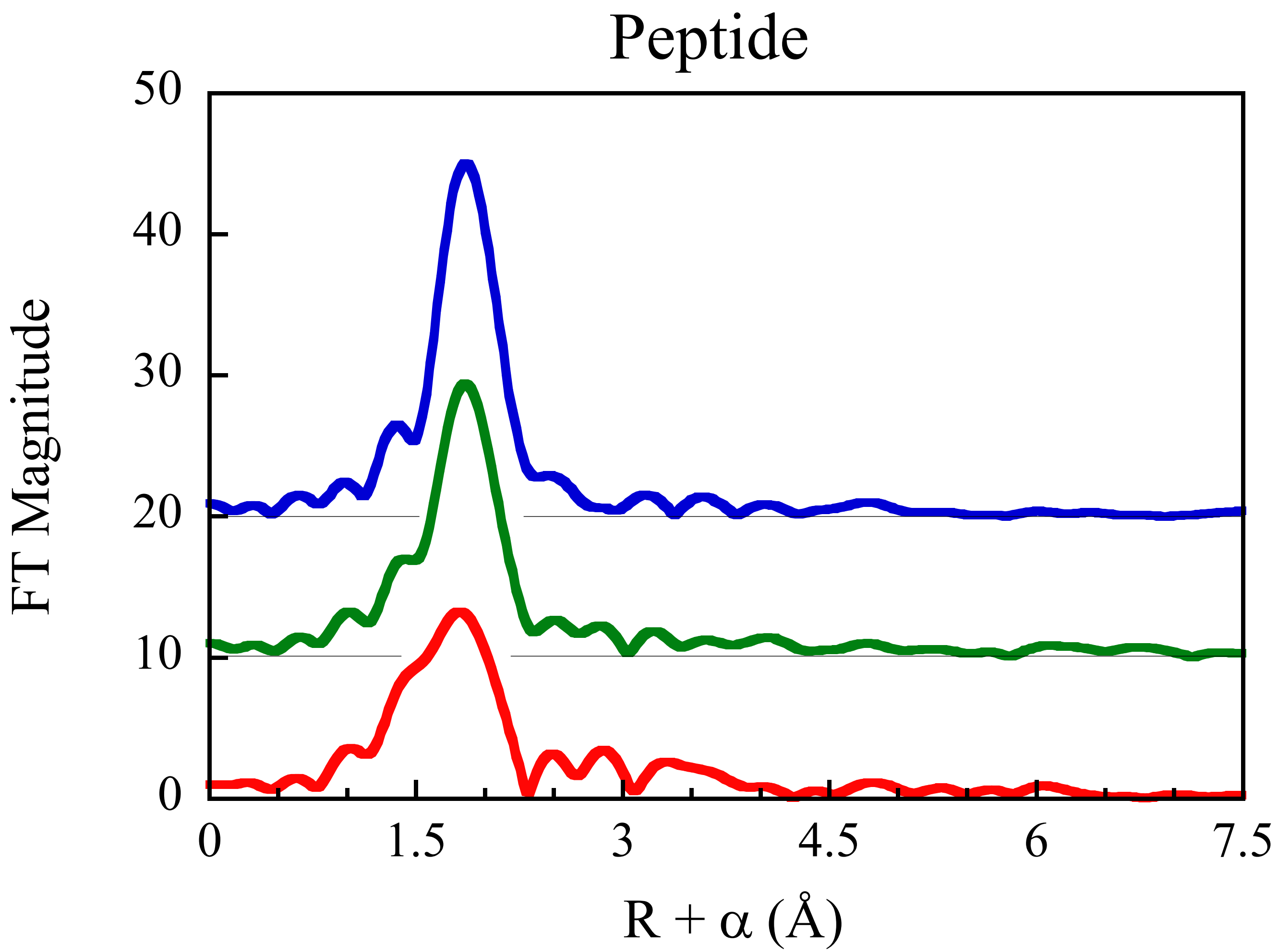
EXAFS shows only small amplitude changes

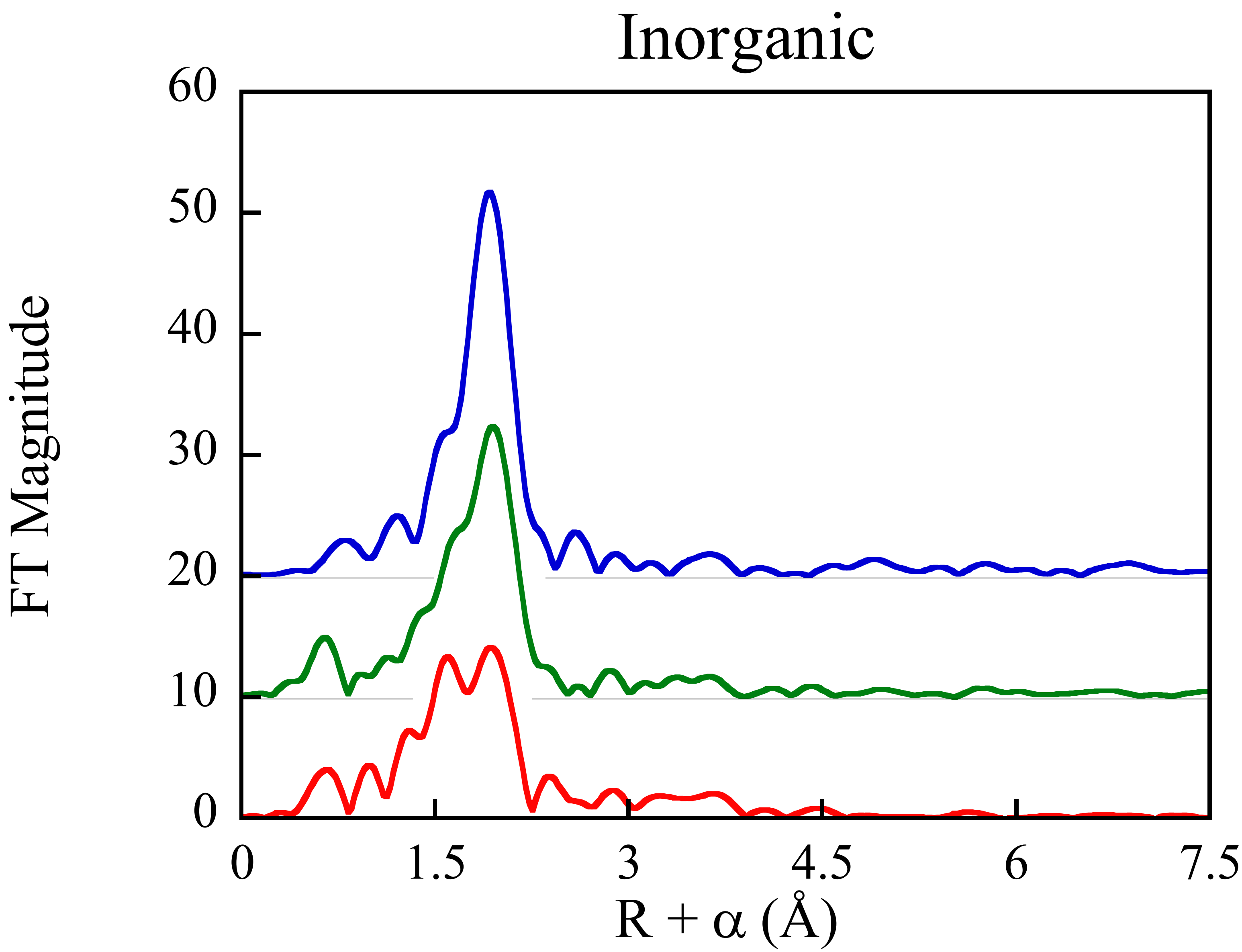




Inorganic
k3 weighted EXAFS
k (Å-1)
30
25
20
15
10
5
0
-5
-10
2
4
6
8
10
12
14
16

FTs do not show 2 obvious shells





Inorganic
FT Magnitude
R + α (Å)
60
50
40
30
20
10
0
0
1.5
3
4.5
6
7.5

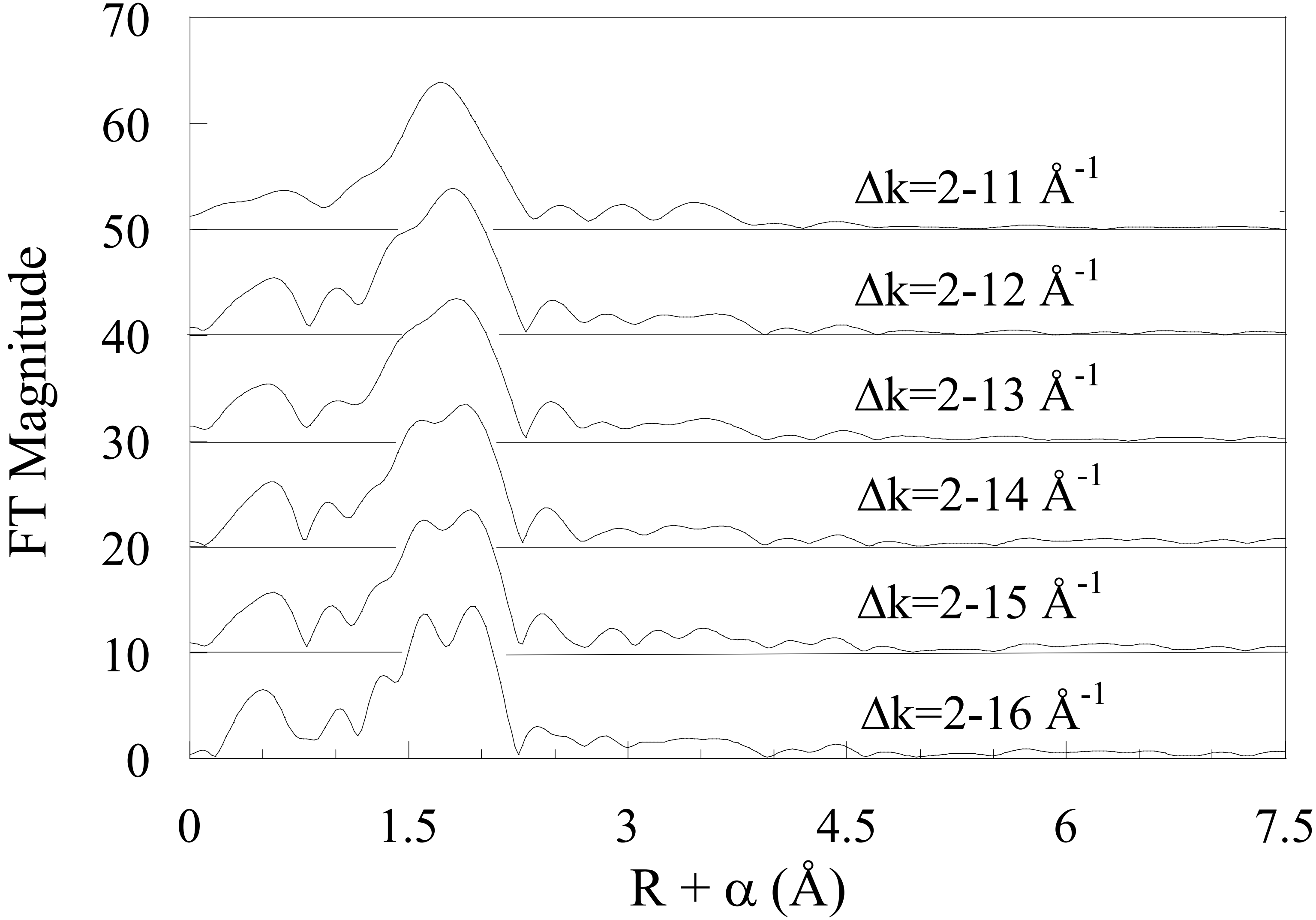
The lack of resolvable peaks is a consequence of:

- Unfortunate distances (Zn-N=2.05; Zn-S=2.3)
- Destructive interference -- $\varphi_N \approx \varphi_S + \pi$

This has the result that S and N oscillations are nearly out of phase for much of the accessible *k* range

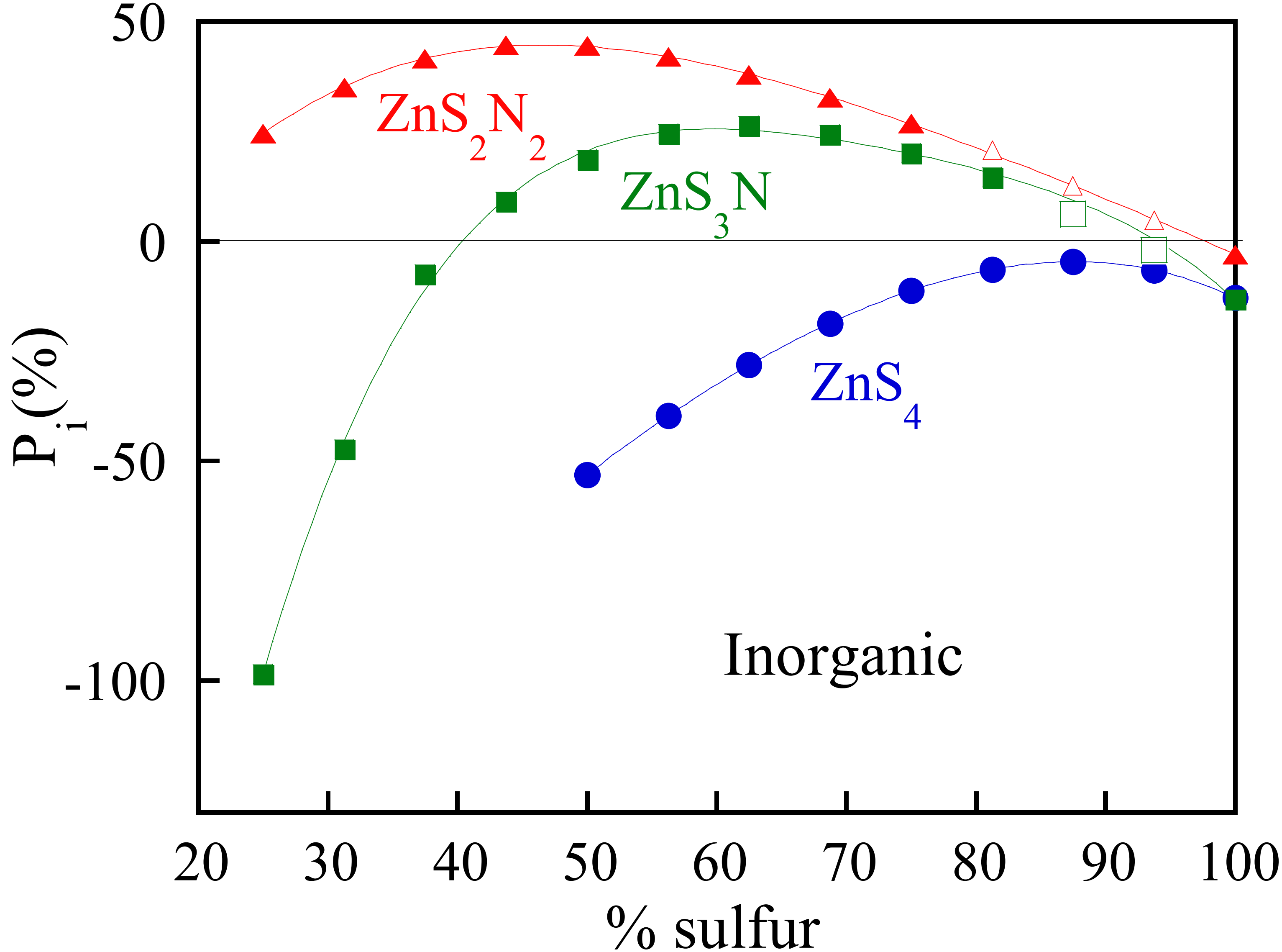
One solution is to measure data over wide *k* range

(ZnS_2N_2 inorganic)

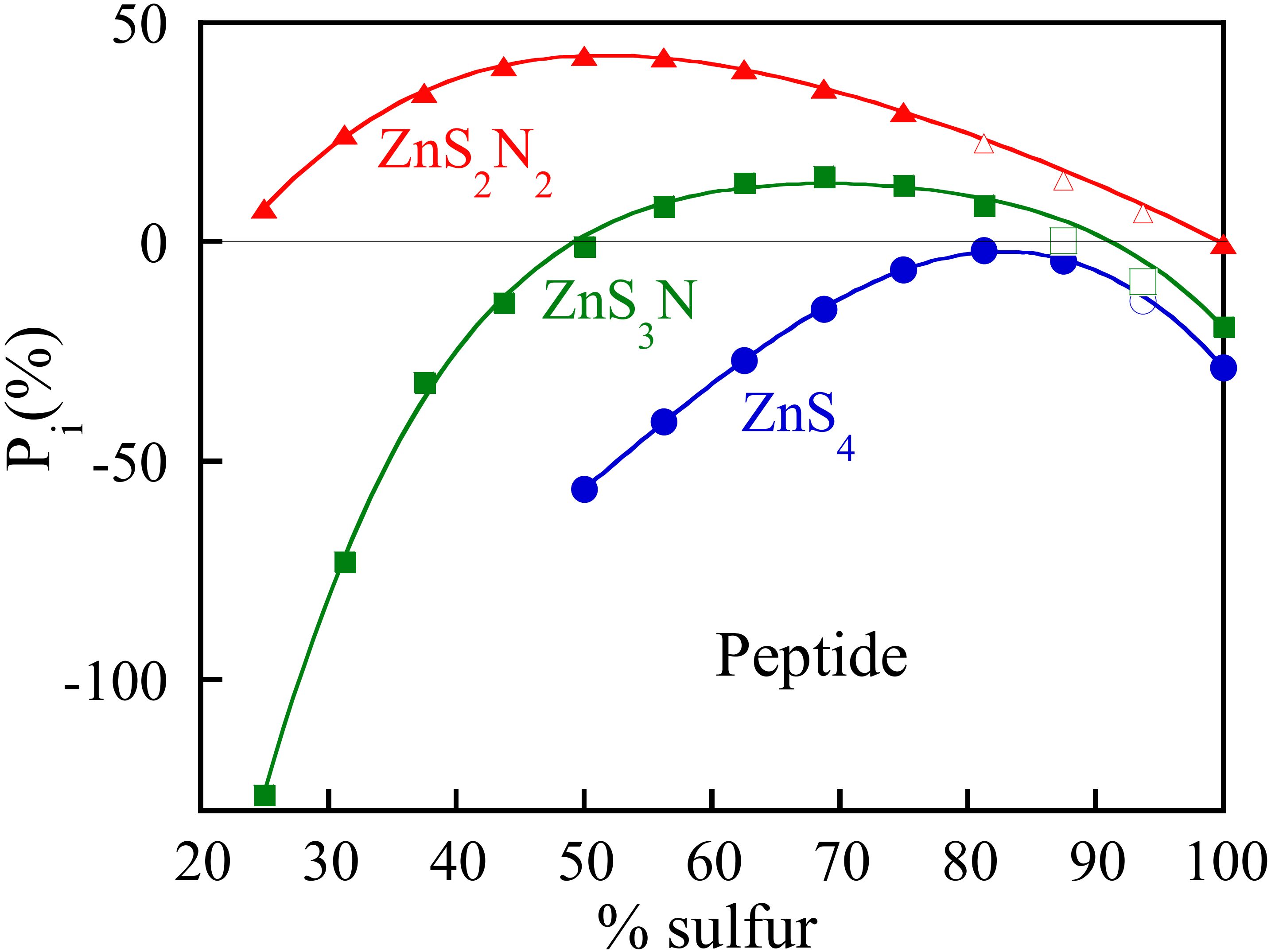


Treat %S as a continuous variable

- Define P_i as percent improvement in fit.
- To avoid changes in degrees of freedom, P_i defined with respect to a fictitious ZnS_2S_2 fit.
- Dependence of P_i on %S is highly characteristic of ligation – P_i=0 means that mixed ligation does not give any improvement in the fit.



ZnS2N2
ZnS3N
ZnS4
Inorganic
Pi(%)
% sulfur
50
0
-50
-100
20
30
40
50
60
70
80
90
100

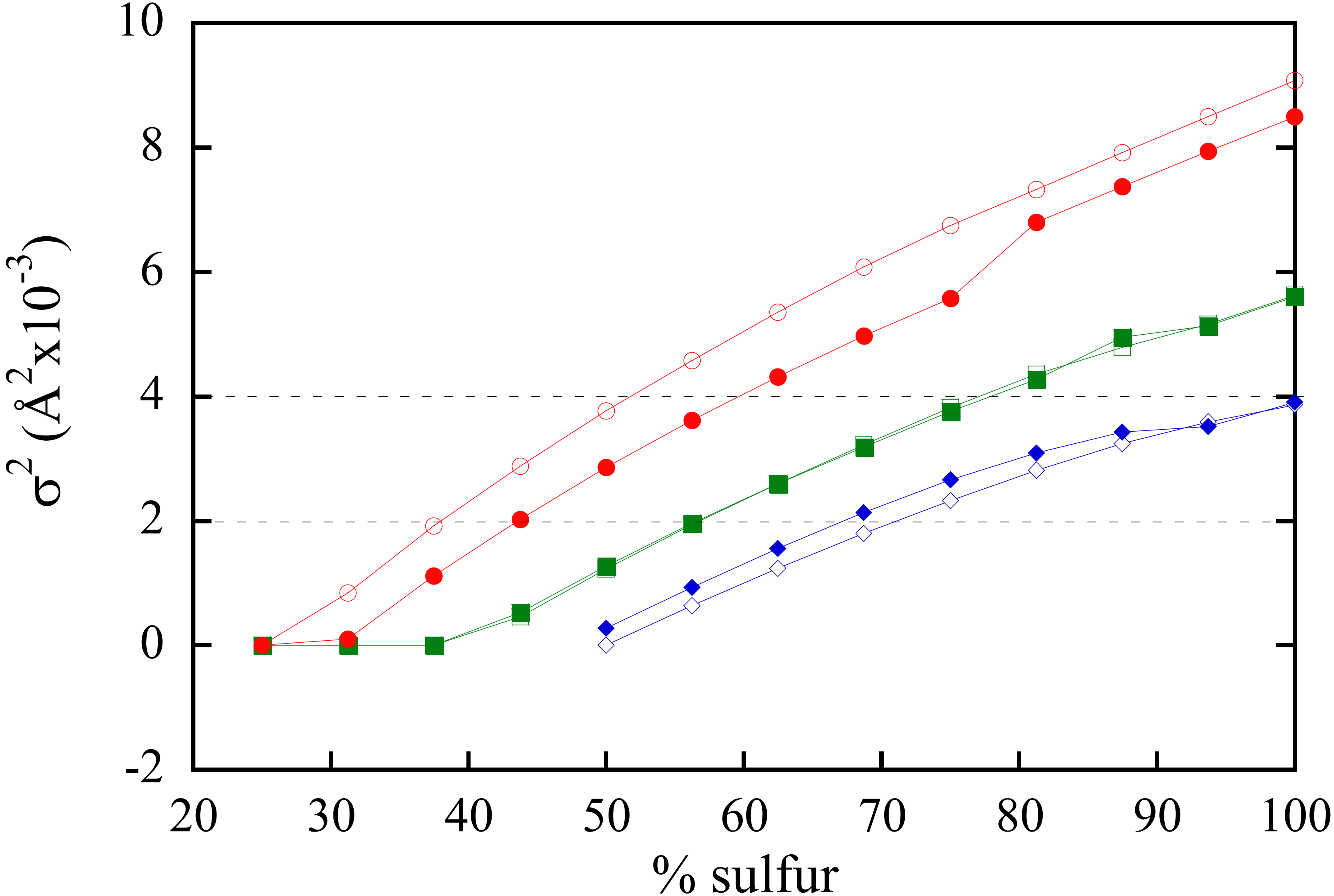


50
0
-50
-100
P_i(%)
20
30
40
50
60
70
80
90
100
% sulfur
ZnS_2N_2
ZnS_3N
ZnS_4
Peptide

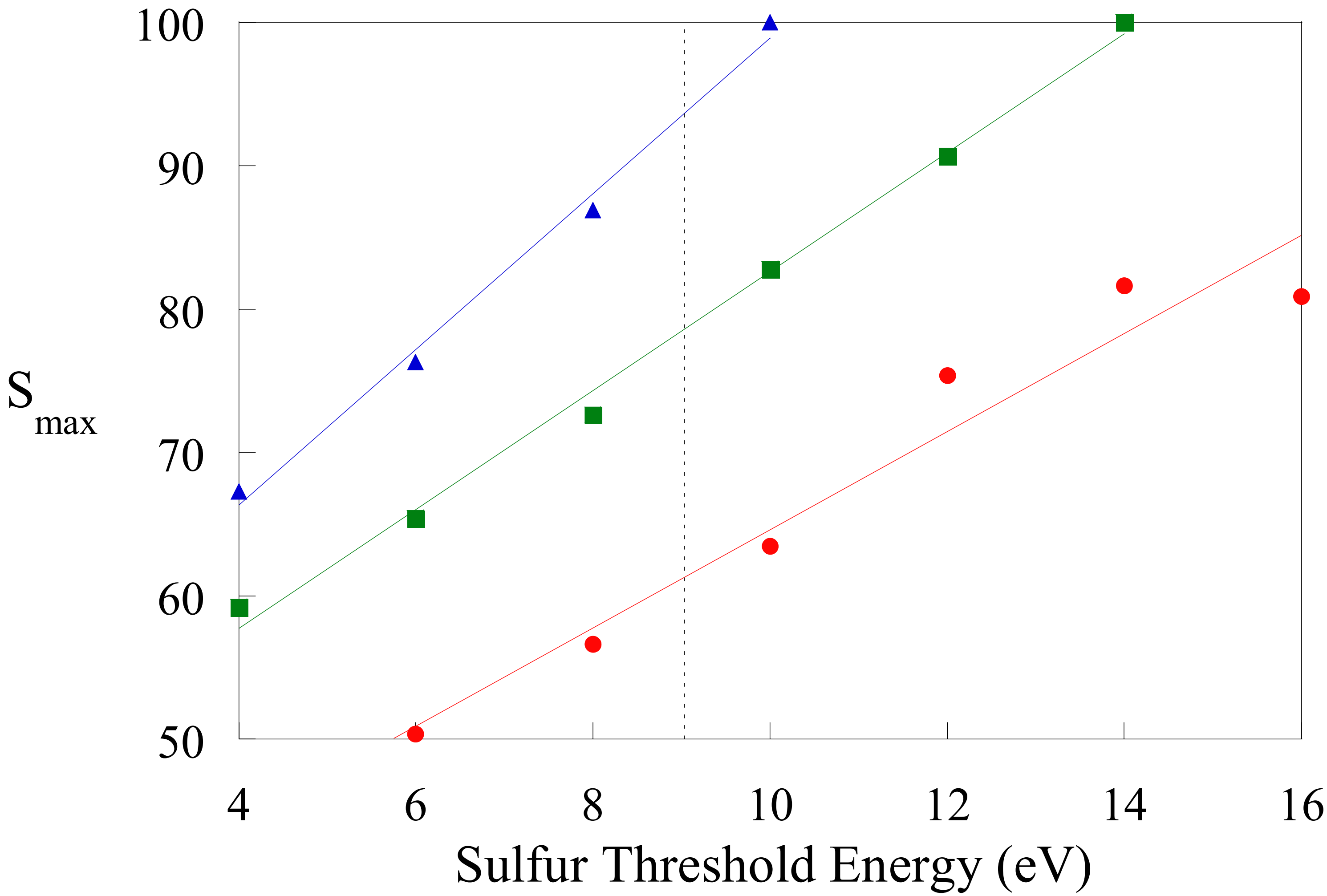
Dependence of P_i on %S

- Maximum in P_i approximately matches expected %S.
- The height of the maximum in P_i increases for samples that have authentic mixed ligation.
- P_i always increases when a nitrogen component is added.

In addition to P_i, σ^2 depends on ligation



S_{max} (% S giving optimum P_i) depends on E_0



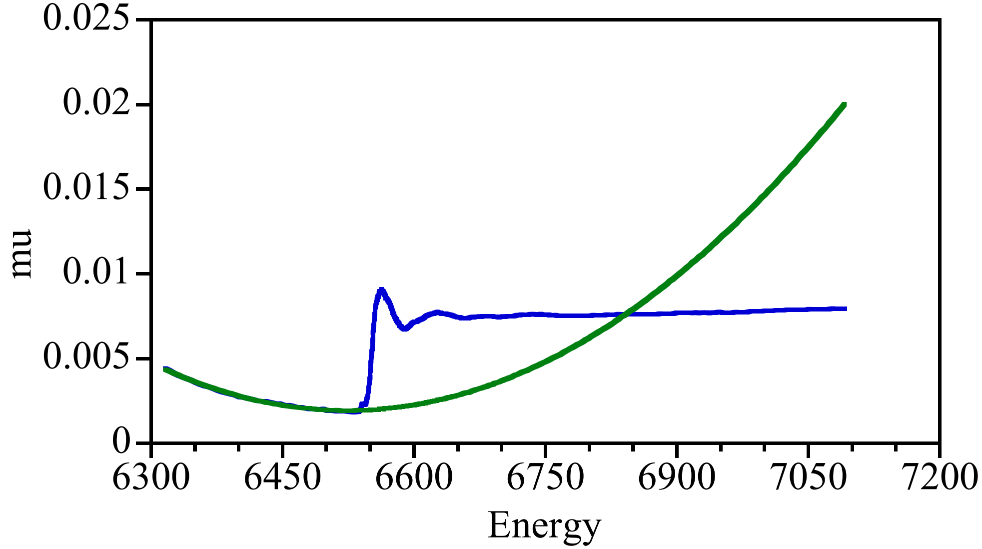
E_0 can have pronounced effect on apparent ligation

- Sensitivity to E_0 is a consequence of the fact that the difference between S and N is largely encoded in their phase difference.
- E_0 is often been treated as a freely variable parameter.
- Range of "chemically reasonable" variation has been given as ± 20 eV.
- Variation by of E_0 by more 3-4 eV from calibrated value (9 eV) changes the apparent ligation.

XANES spectra contain useful information regarding structure

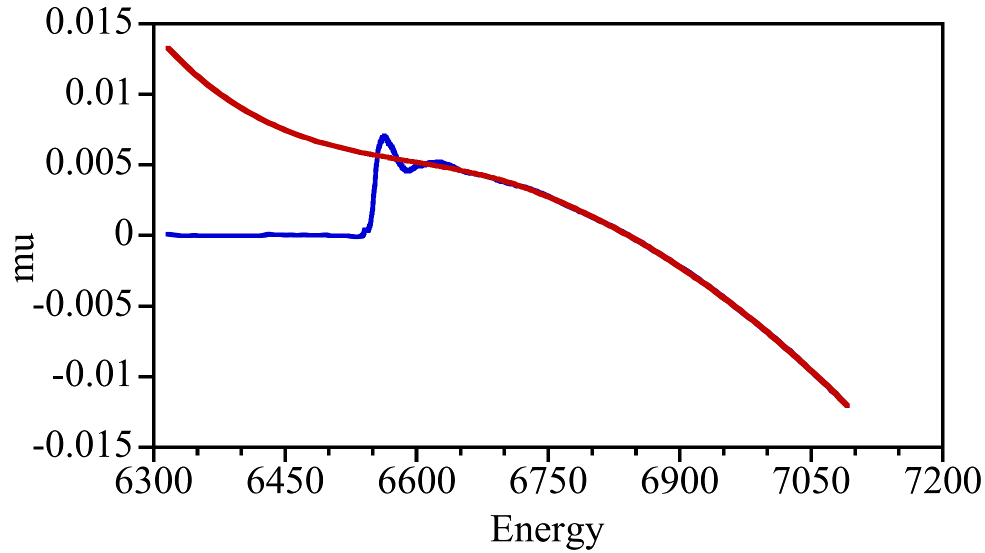
- Quantiative comparisions (e.g., titration) requires accurate normalization.
- Correction for various artifacts (self-absorption) requires accurate normalization.
- Common normalization procedures were developed for extracting EXAFS and do not necessarily work well for XANES.

Conventional normalization

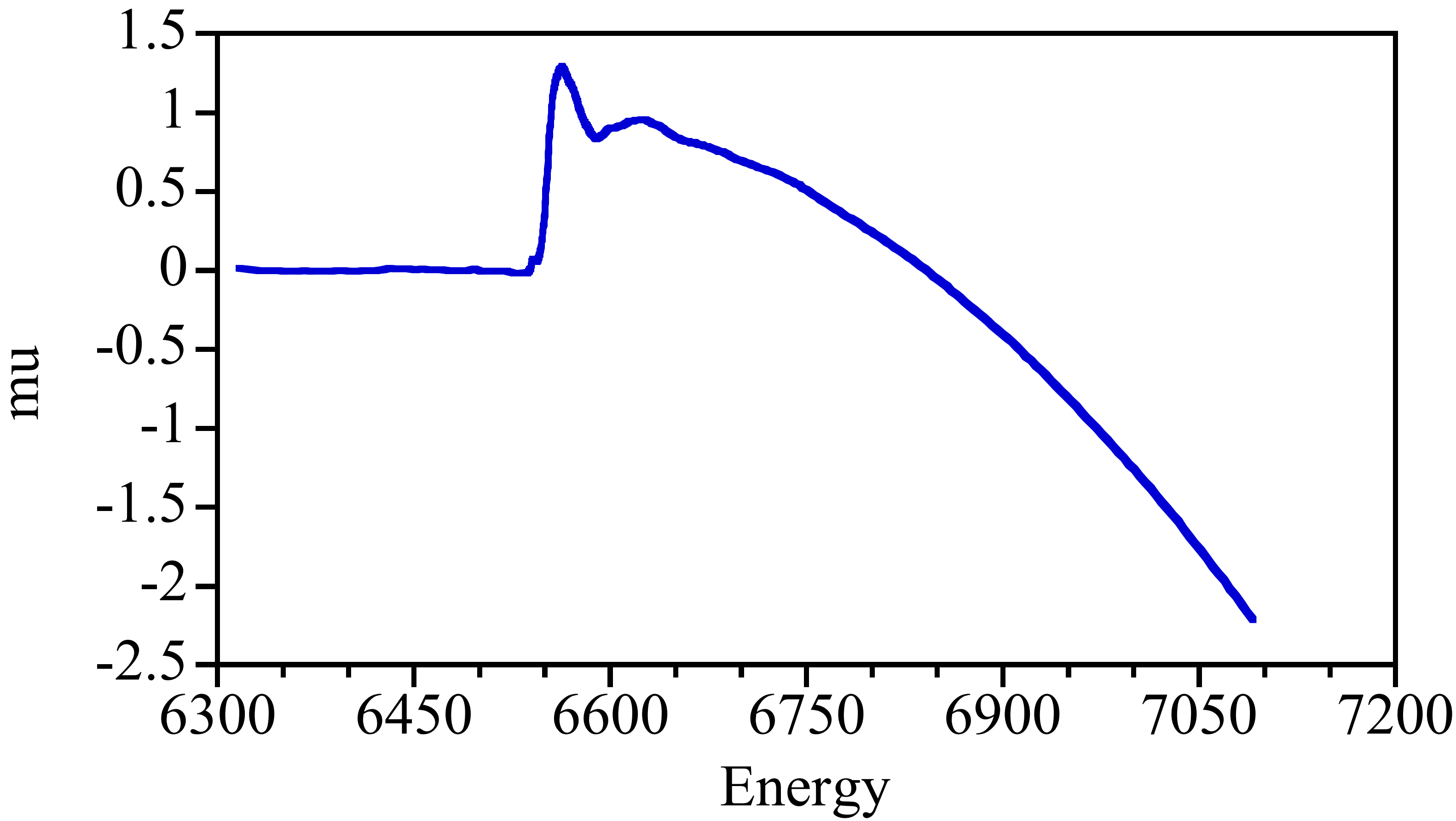


Pre-edge subtraction followed by extrapolation

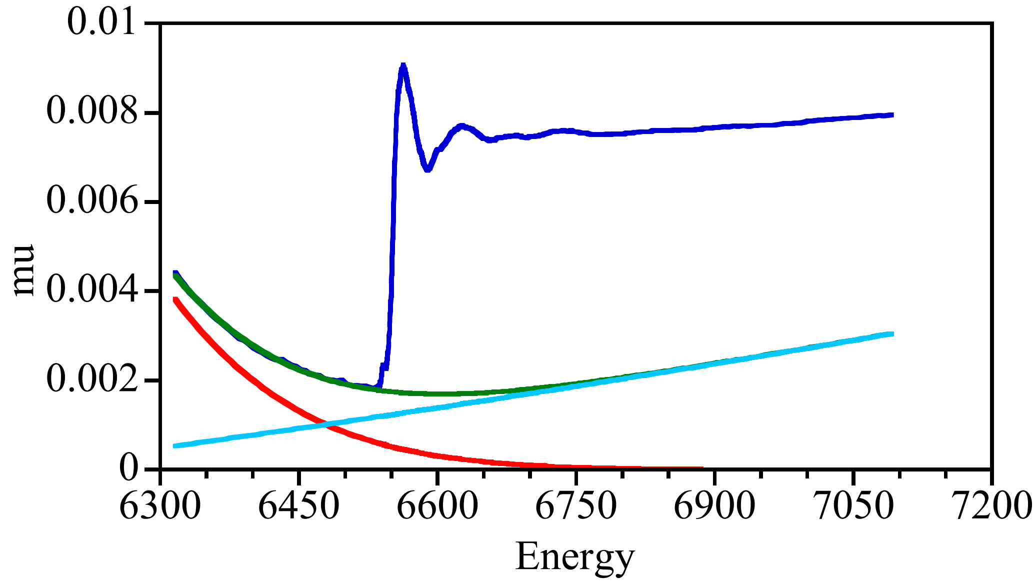
Post-edge (spline) subtraction followed by extrapolation



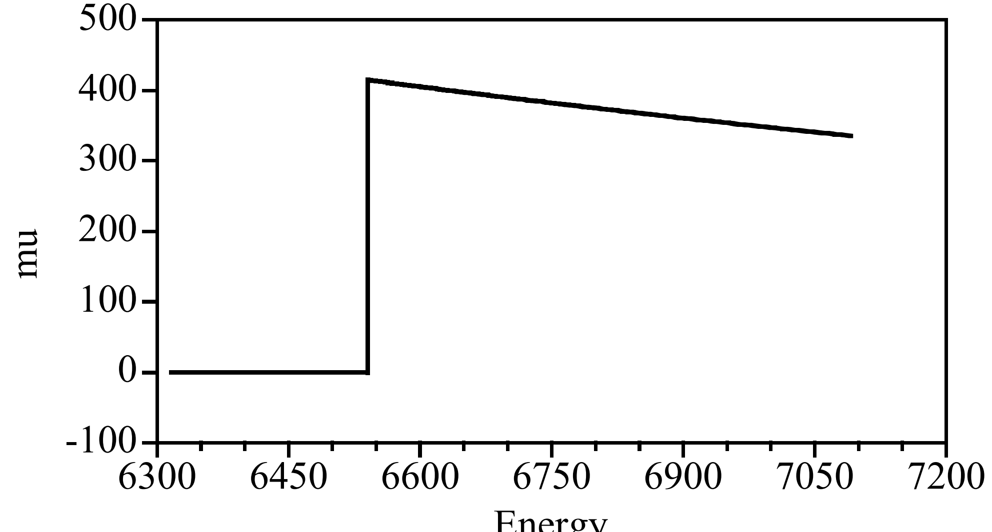
Conventionally normalized data



Alternative is to use a single background and tabulated cross-sections

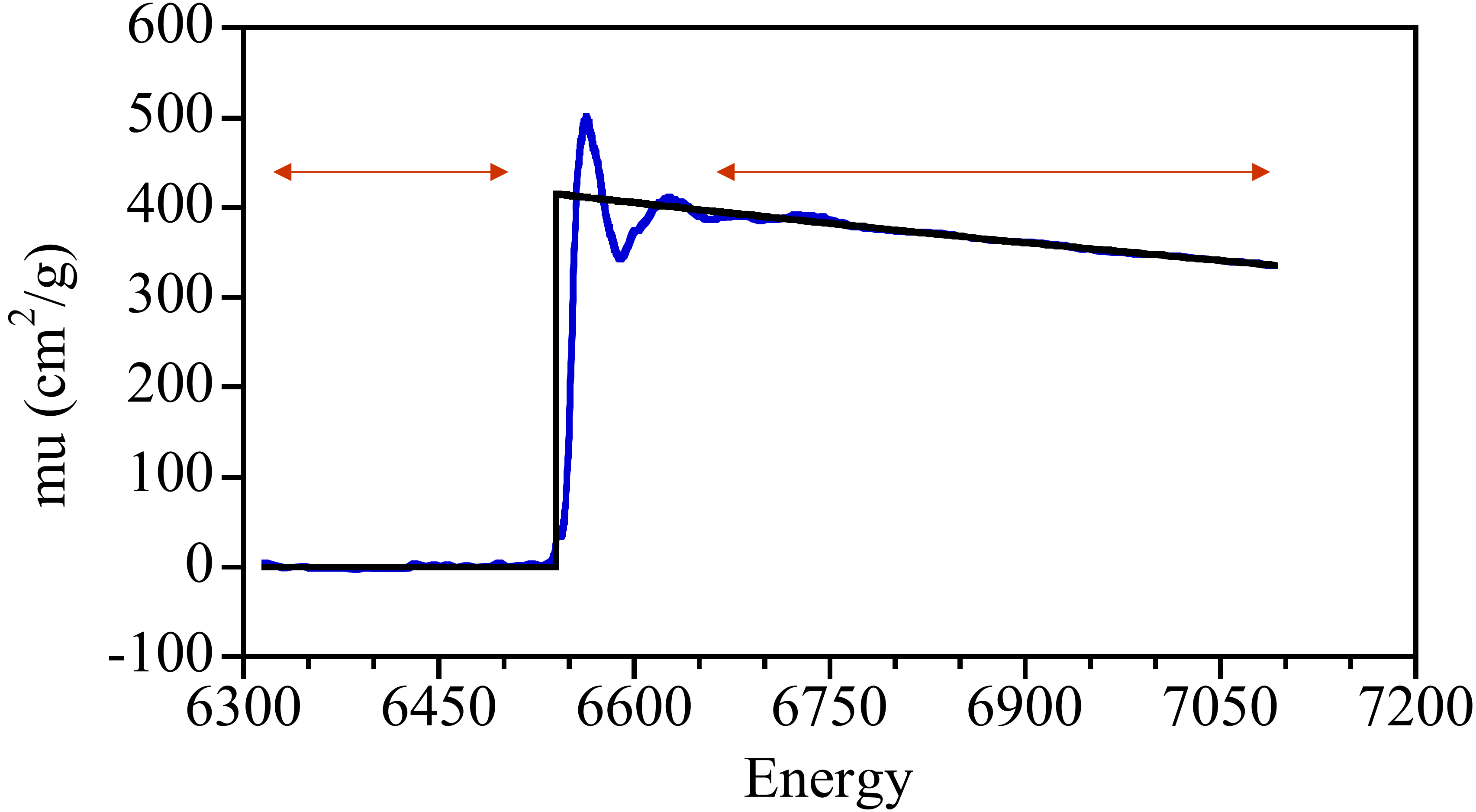


Polynomial + complementary error function background fit to all data

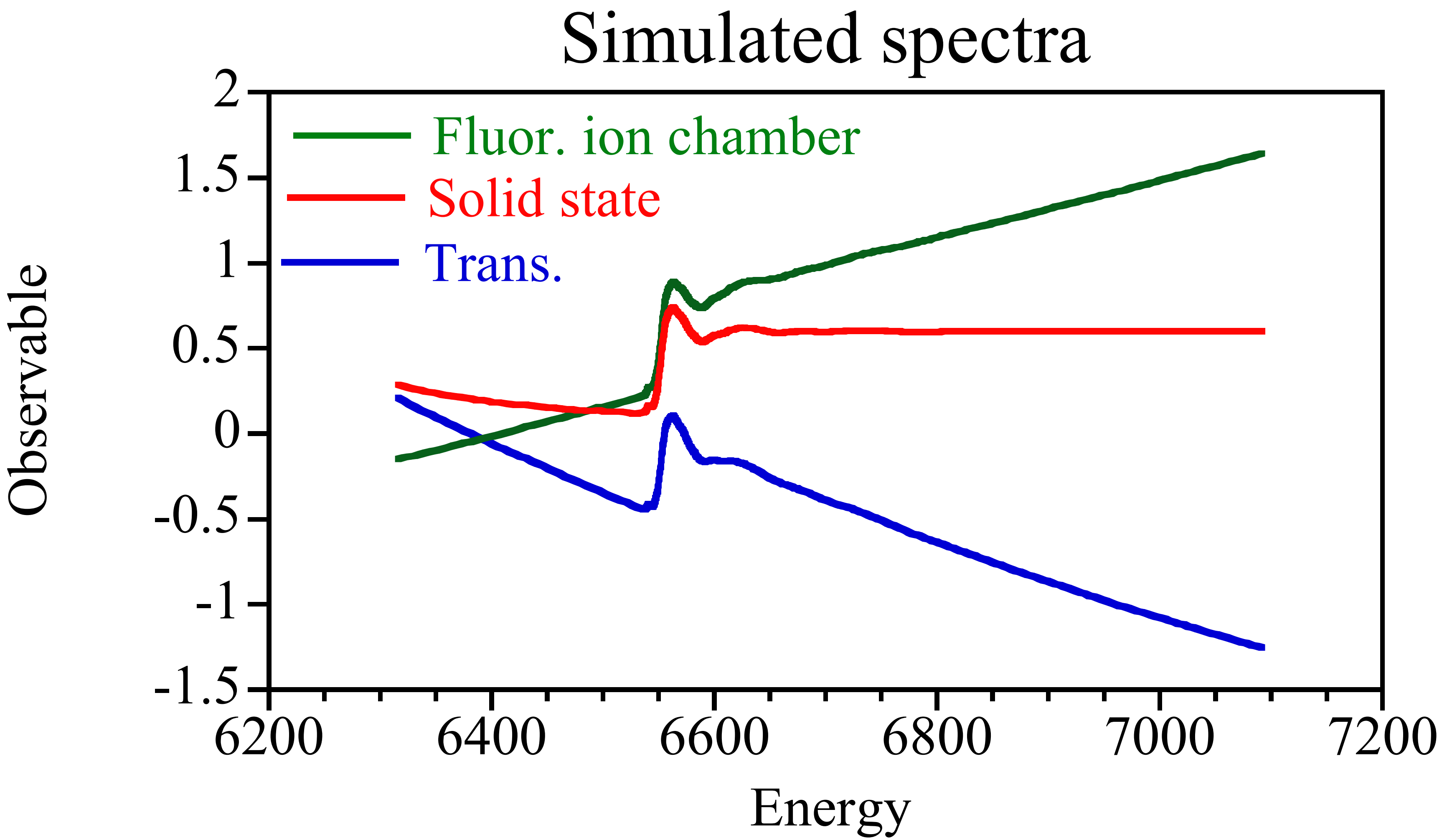


Fit to tabulated McMaster values

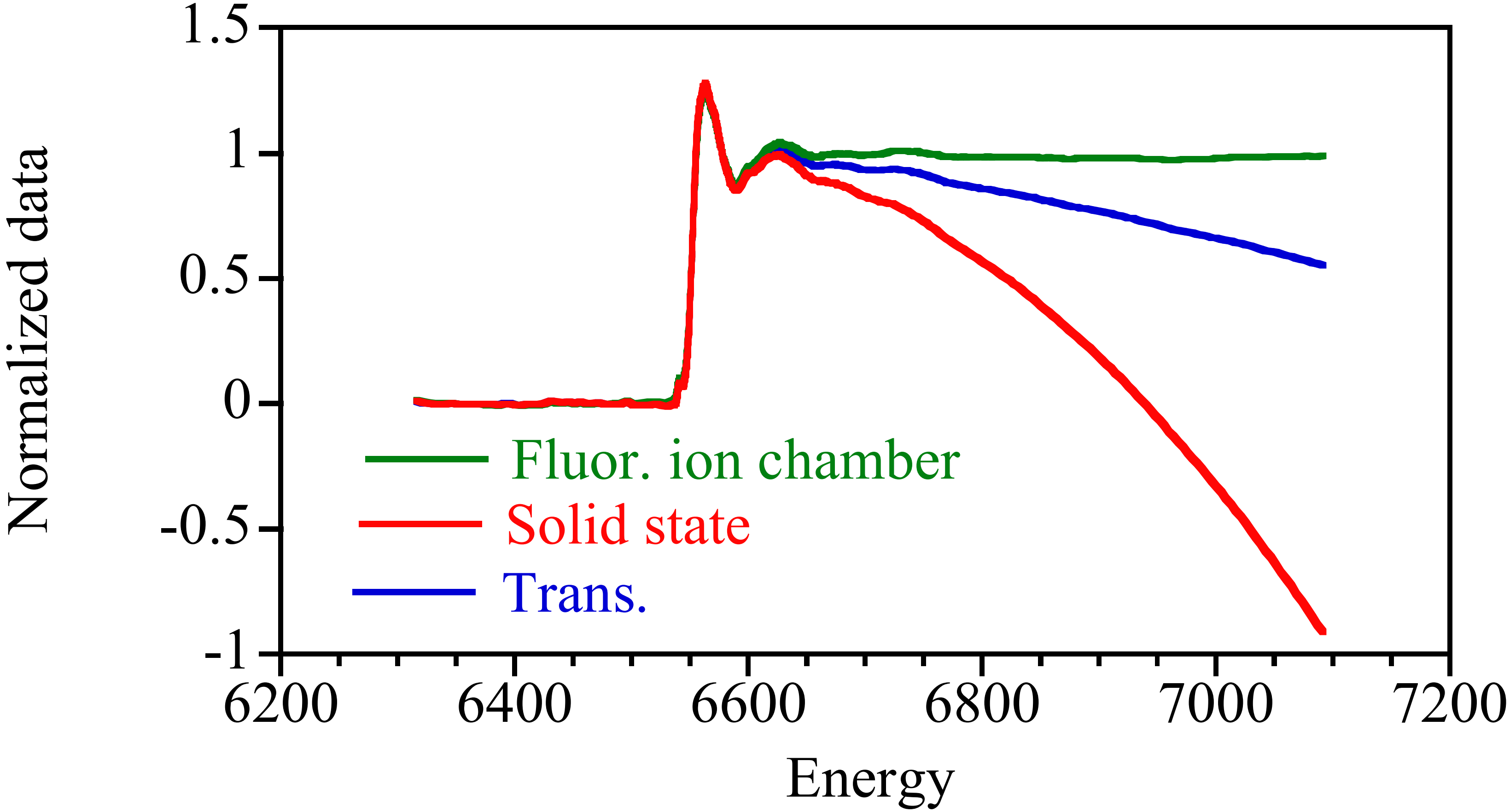
To avoid sensitivity to XANES features, only fit data below and above edge



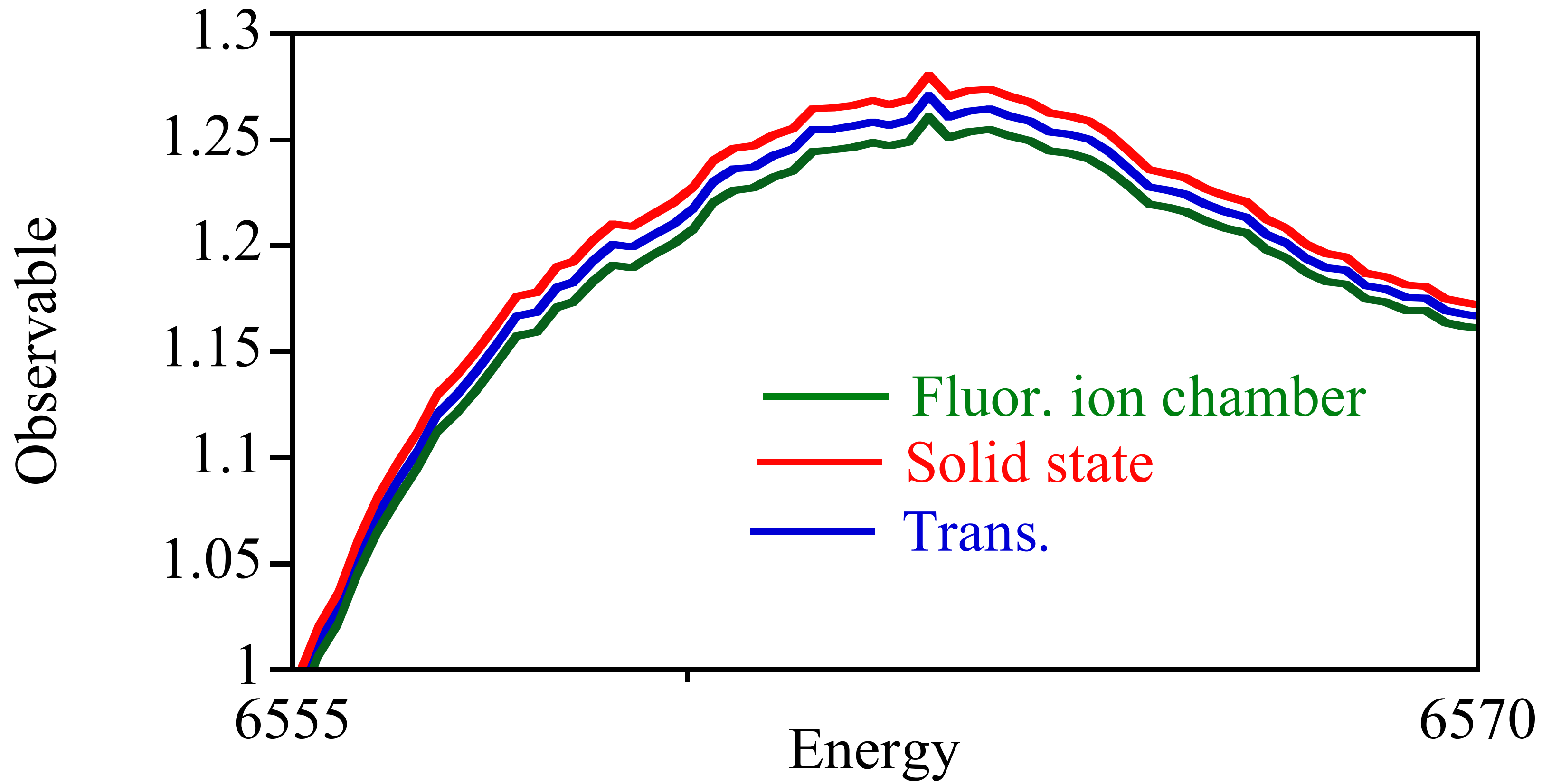
Ability to recover data using different backgrounds



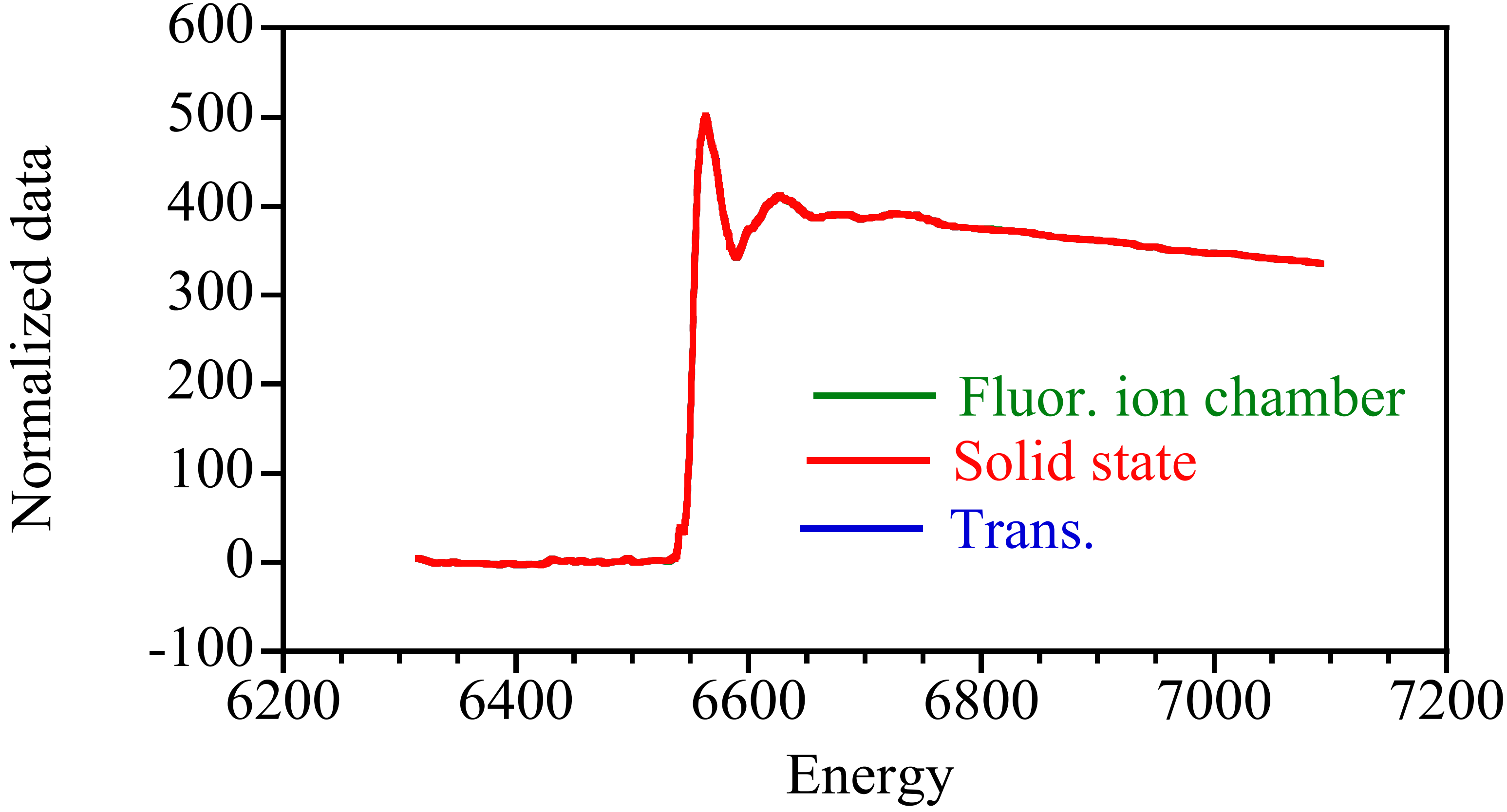
Conventional normalization is sensitive to background

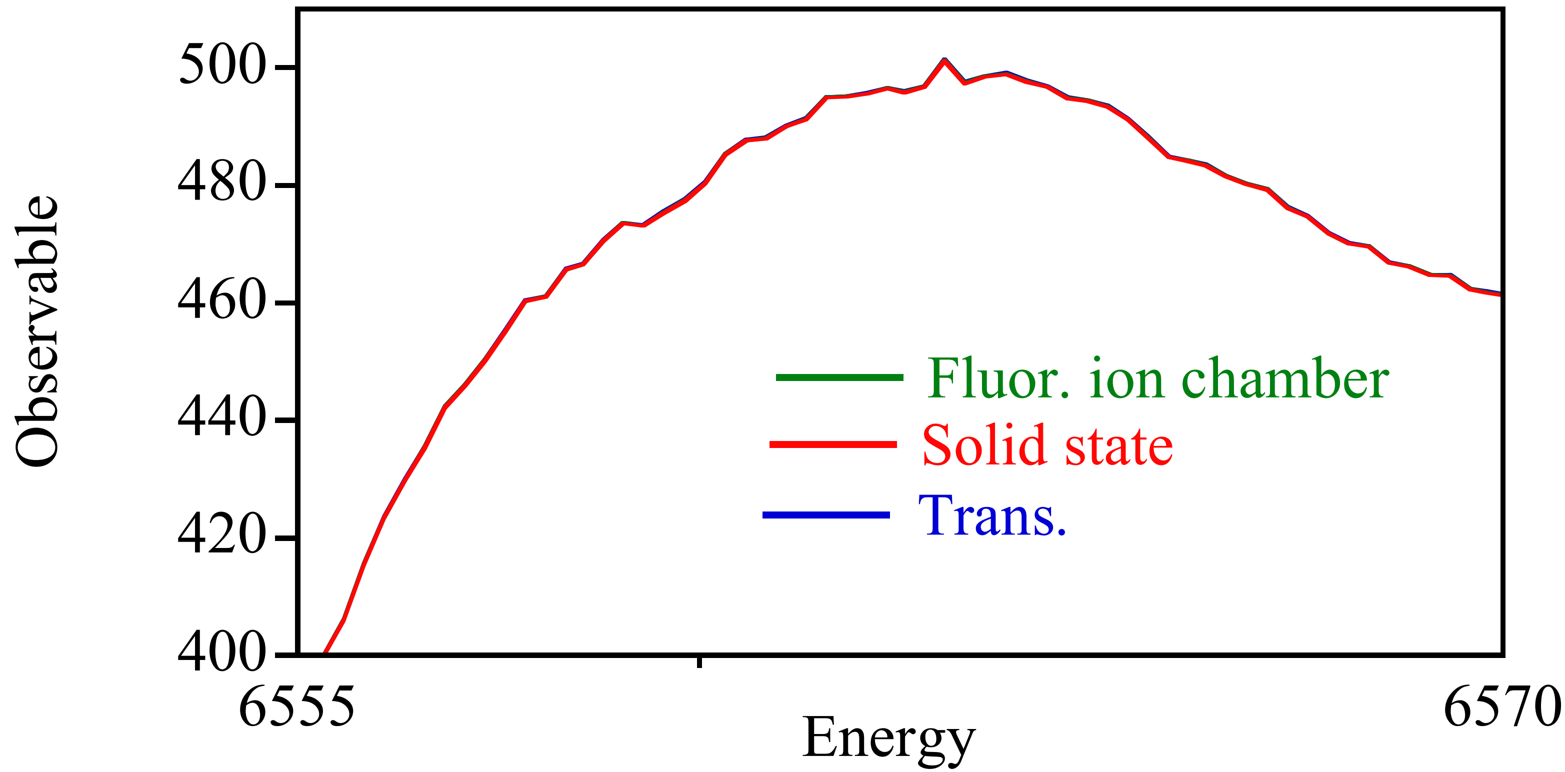


Errors in conventional normalization affect data even near edge



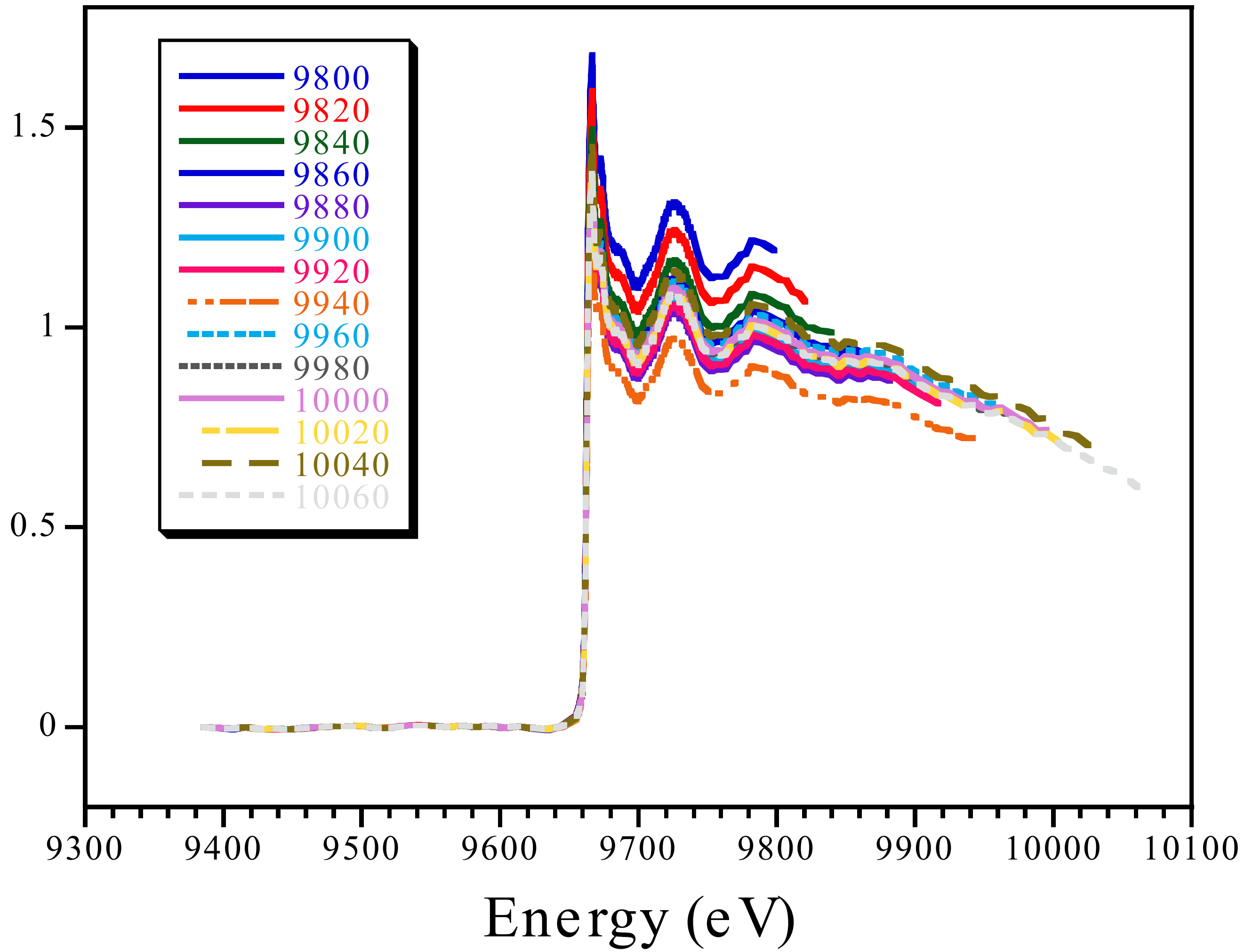
Proposed normalization is insensitive to background



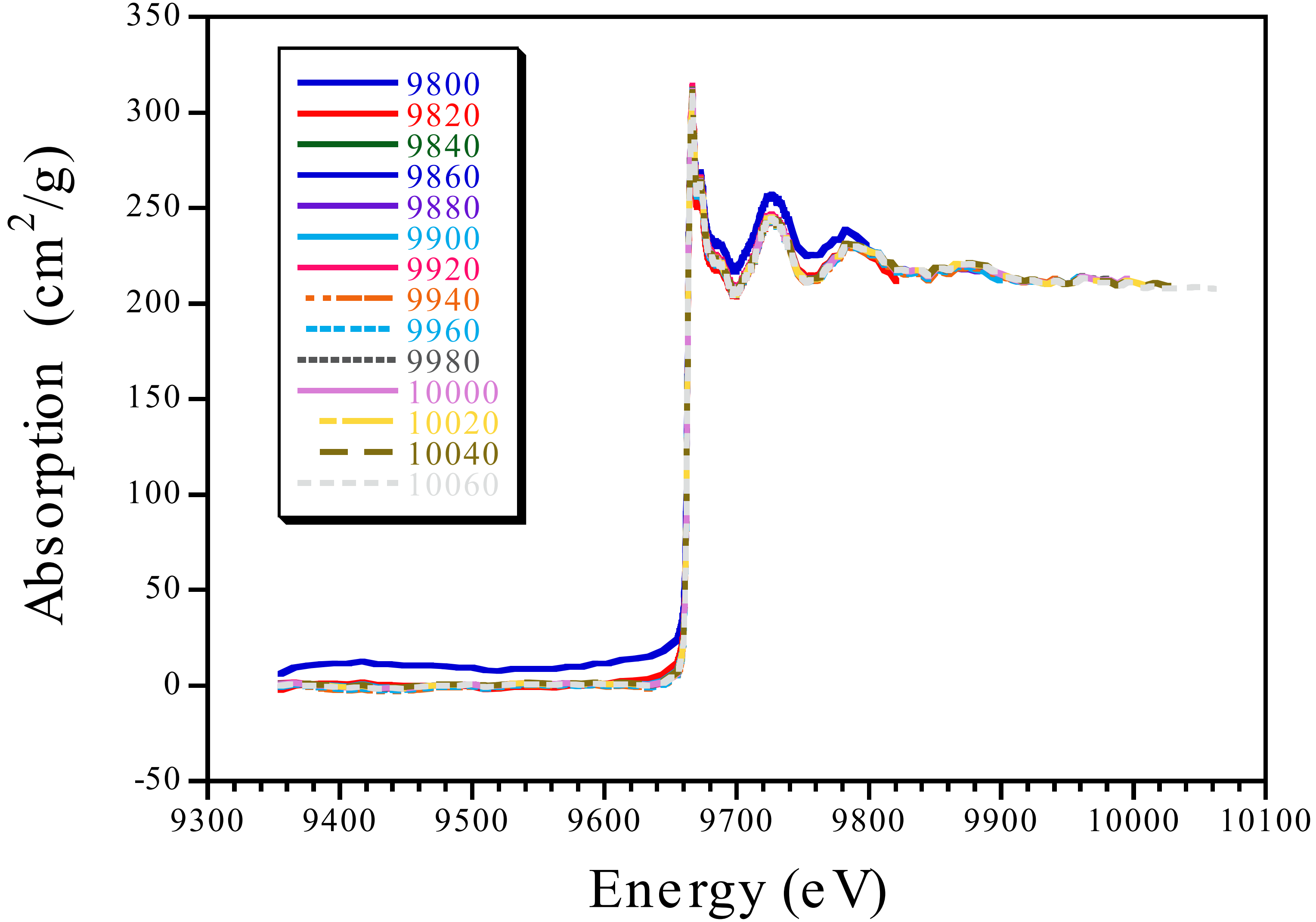


Observable
500
480
460
440
420
400
6555
6570
Energy
Fluor. ion chamber
Solid state
Trans.

Conventional normalization is sensitive to range of data



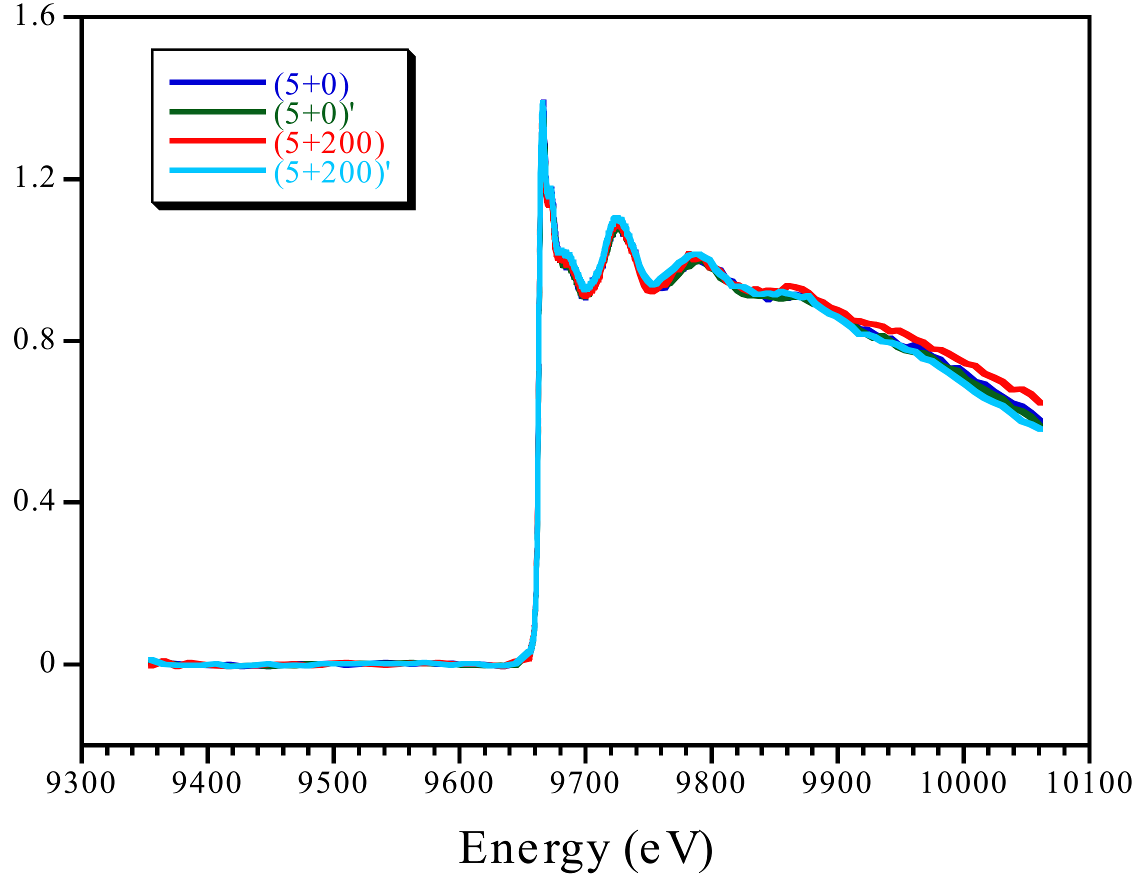
New method shows only slight sensitivity for E_{max}≥~150 eV above edge



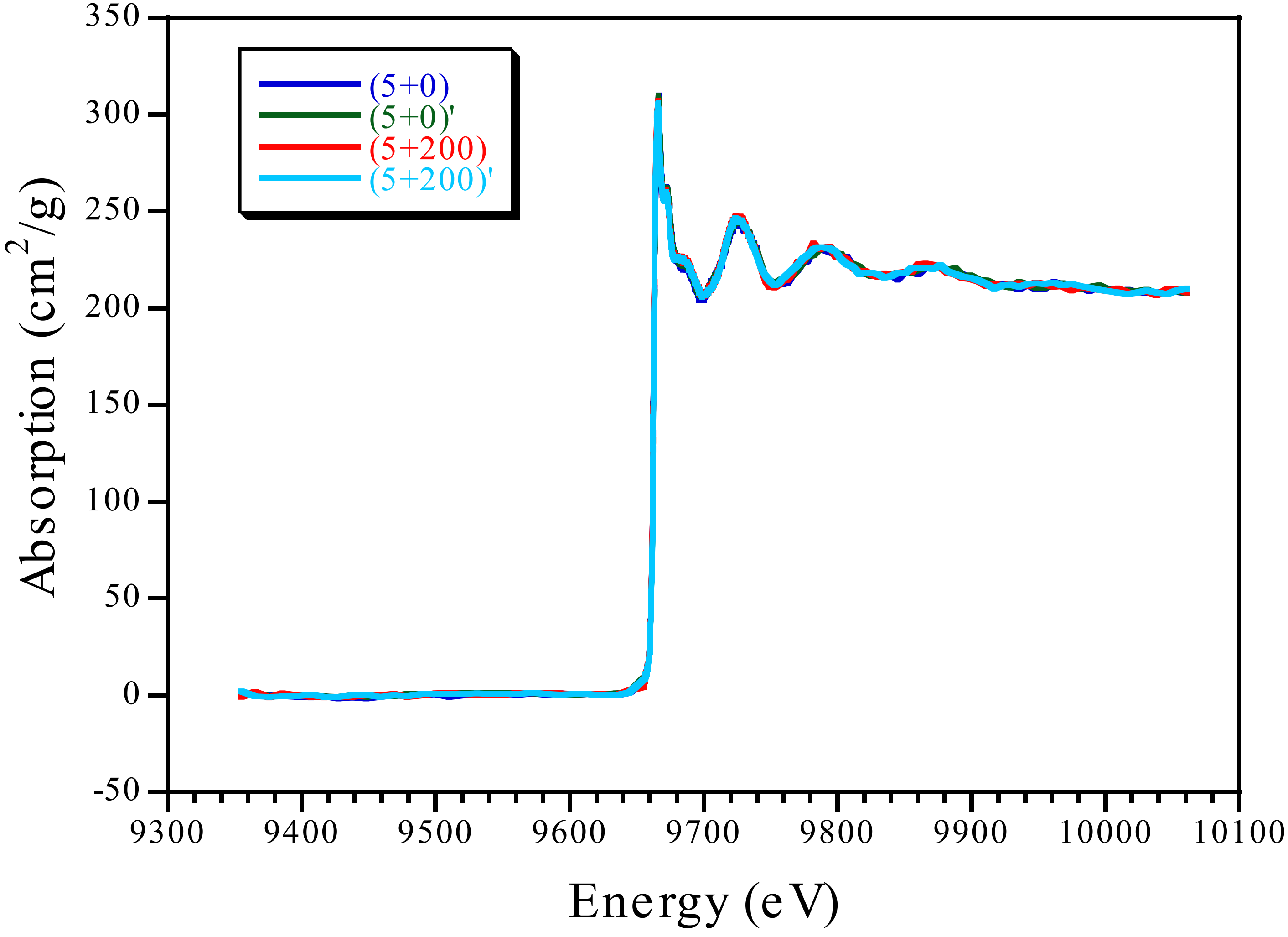
Although errors in conventional normalization are small, they affect conclusions

- $Zn(SR)_4^{2-}$ dissociates in solution
- Complex can be forced to 100% $Zn(SR)_4^{2-}$ by addition of excess RS^-
- Measure duplicate data for 5 mM $Zn(SR)_4^{2-}$ with and without added RS^-

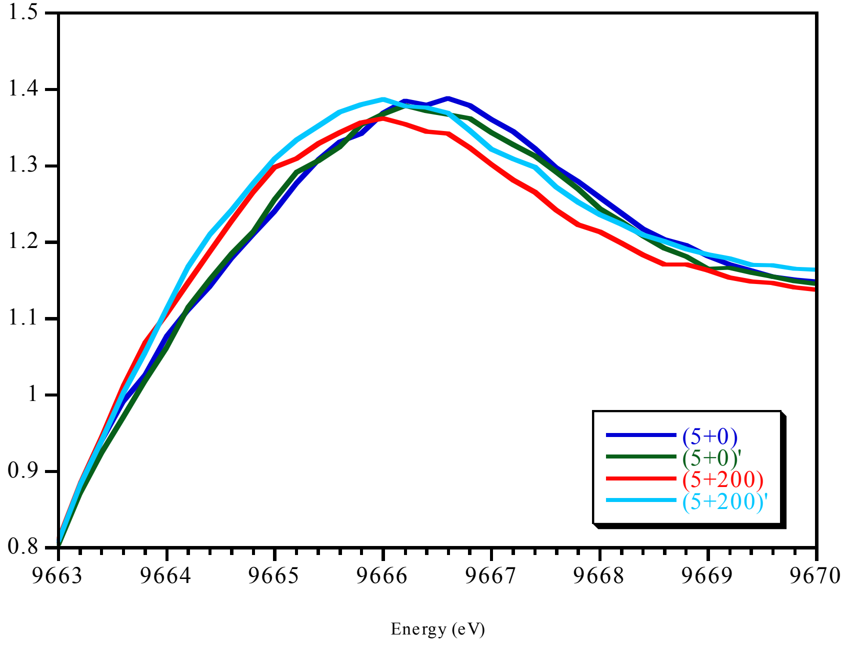
Conventional normalization

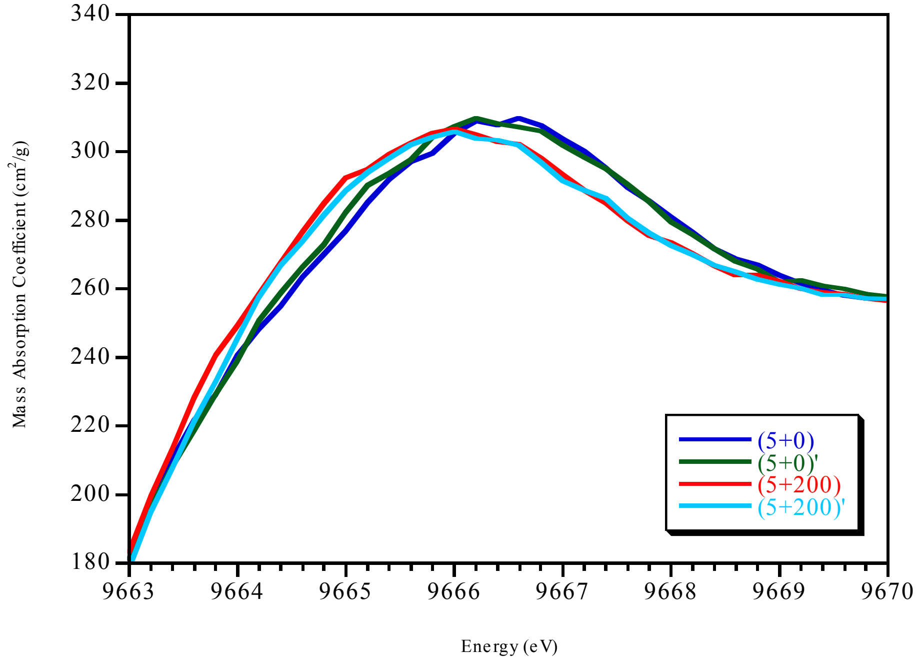


Proposed normalization method

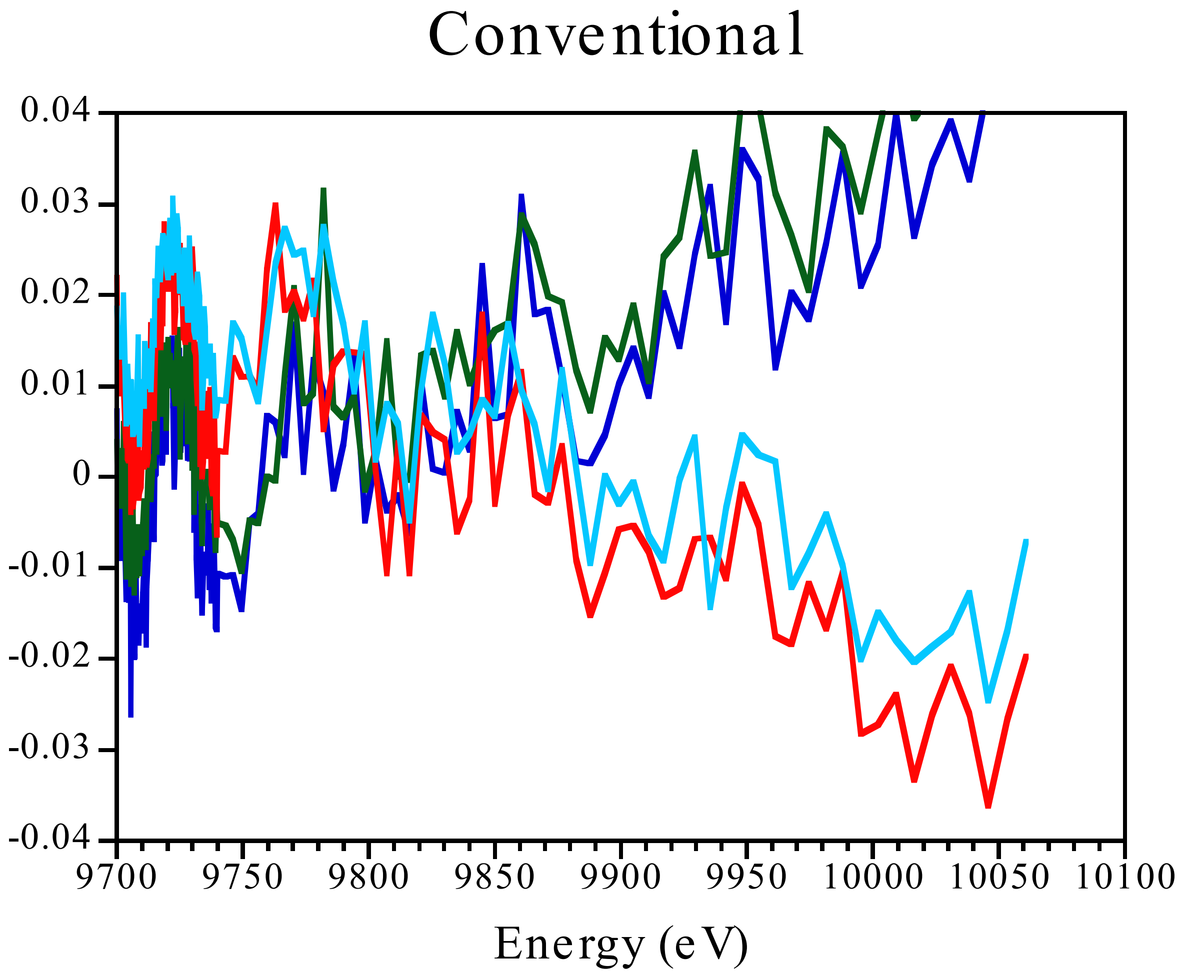


Variation in normalization obscures chemical effects

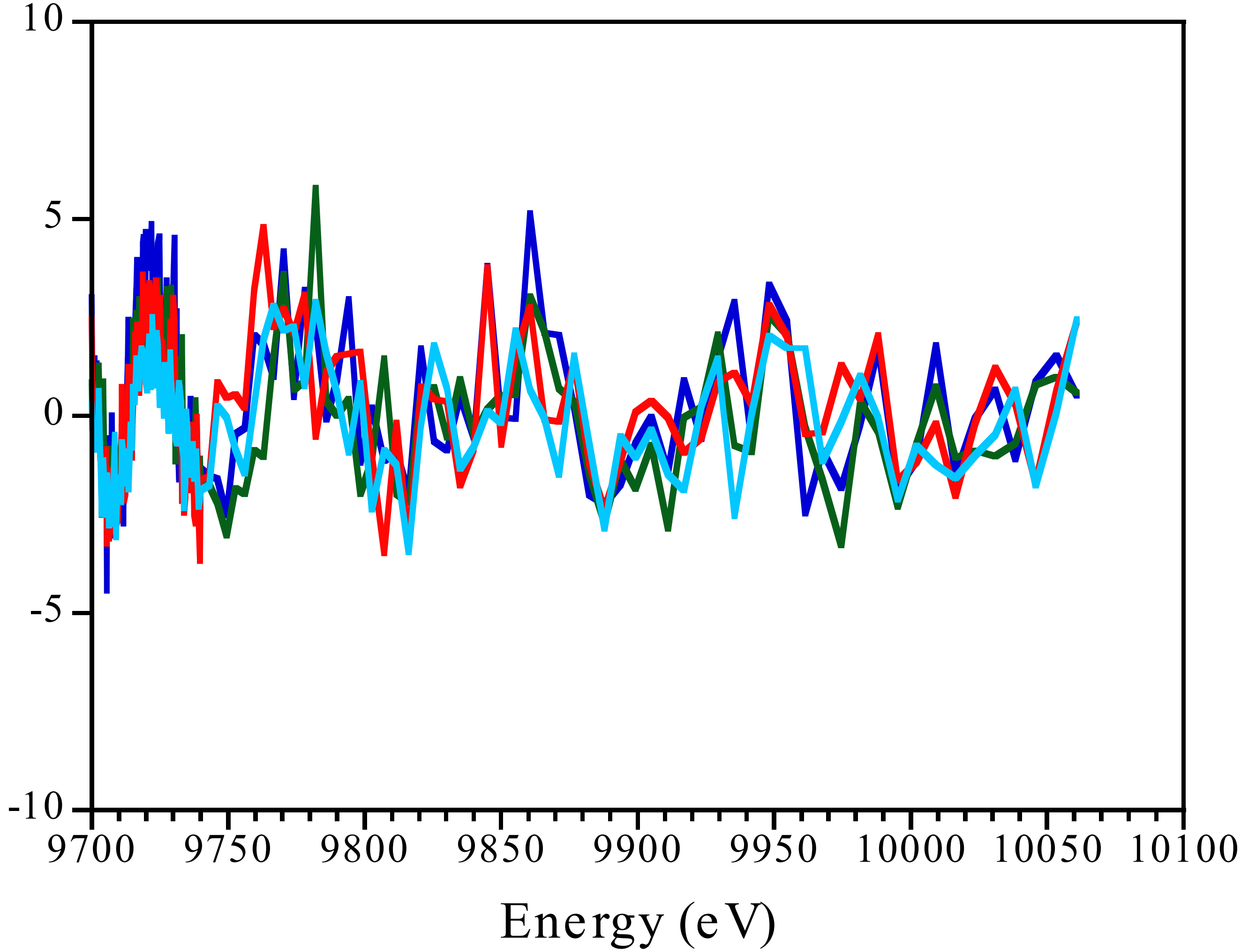




4 possible difference specta – should all be the same



With new normalization, difference signal is detectable



Acknowledgements

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International XAFS Society

Report on error analysis. See http://ixs.csrri.iit.edu/

Public comment period – please review and, if desired, comment.