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Determining the Local Structure of Platinum Streptidine using X-Ray Absorption Spectroscopy

Michaëlle Mayalu

Office of Science, SULI Program

Massachusetts Institute of Technology

Stanford Linear Accelerator Center

Stanford, CA

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Participant: _____

Signature

Research Advisor: _____

Signature

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ABSTRACT

Determining the Local Structure of Platinum Streptidine using X-Ray Absorption Spectroscopy. MICHAËLLE MAYALU (Massachusetts Institute of Technology Cambridge, MA 0213-4307) SERENA DEBEER GEORGE (Stanford Linear Accelerator Center Stanford, CA 94025)

X-Ray absorption spectroscopy (XAS) is a technique that utilizes high energy X-rays commonly obtained from synchrotron radiation to determine the structure of known and unknown substances and materials. By examining the absorption vs. energy pattern, one can determine the local structure surrounding the absorbing atom. Analysis of a region of the absorption vs. energy graph called extended x-ray absorption fine structure (EXAFS) leads to information about the identity of the atoms surrounding the absorber, the number of atoms surrounding the absorber, and the distances between the absorber and neighboring atoms. Using XAS, structural descriptions of platinum streptidine, a newly synthesized platinum anti-cancer agent, have been obtained. The results show that the platinum is in fact coordinated to the streptidine, which was the main question that needed to be answered about the drug.

I. INTRODUCTION

The discovery of platinum anti-cancer agents has lead to extensive research into the mechanisms by which these complexes succeed in inhibiting cell division. It is believed that the platinum complex may bind to a certain site in the DNA thus preventing further division of the cell. Although the discovery of platinum anti-cancer drugs has made considerable advances in the treating certain cancers, many mysteries regarding these drugs still remain.

It is not known what the mechanisms are that lead to the drug being taken up by the cell membrane and integrated into the DNA, and why the drug is successful in treating some types of cancers but ineffectual in treating other types. Also, the platinum has been found to be very toxic causing nephrotoxity, neuropathy, ototoxicity, hematological toxicity, neuropathology and seizures [1]. A fair amount of research has been directed at developing agents that will reduce the toxicity of the platinum, as well as synthesizing new platinum complexes that may have less toxic side effects.

Cisplatin is one of the most successful platinum anti-cancer agents and is very effective in treating testicular and ovarian cancers [2]. Although the drug is quite efficient, it is still highly toxic and only is successful in certain types of cancers. Also, some cancers develop resistance to the drug after the first treatment[2]. Consequently, the search for improved platinum drugs that treat a wider range of cancers and display fewer toxic side effects continues.

Many other drugs are being synthesized including a Platinum-Streptidine complex [3]. Although several analytical methods have been performed on this drug, such as elemental analysis and nuclear magnetic resonance (see Figure i), much is still unknown

about the solid state structure, and even more importantly the solution state structure of the Platinum-Streptidine complex. Because the structure and coordination of the drug is essential to understanding how the drug interacts with molecules in the body, X-ray absorption spectroscopy (XAS) is a powerful tool for determining the structure of this newly developed drug.

X-Ray absorption spectroscopy is a technique that utilizes high energy X-rays commonly obtained from synchrotron radiation to determine the structure of known and unknown substances and materials. The X-rays are absorbed into the atom of a sample with detectors mounted at the front and back of the sample. Relative absorbance is measured by taking the log of the currents in each detector [4] .Another way of measuring the absorbance is to measure the fluorescence intensity. The graph of the fluorescence intensity vs. the energy is equivalent to the graph of the absorbance vs. energy[4]. By examining the absorption vs. energy pattern, one can determine the local structure surrounding the atom as well as in some cases the angle between the atom and the absorber.

At a certain energy, the absorption vs. energy graph shows an abrupt increase in absorption which signifies the release of an electron from the atoms core shells. This jump is referred to as the absorption edge. Beyond the edge, at higher energies, is a region called the extended x-ray absorption fine structure or EXAFS. The oscillatory behavior in this region is due to the interactions between the photoelectron wave generated by the absorbing atom and the backscattering of the wave from atoms nearby. This interaction causes constructive and destructive interference which leads to the sinusoidal curves that characterize the EXAFS[4] (see Figure ii). Analysis of the EXAFS

region leads to information about the identity of the atoms surrounding the absorber, the number of atoms surrounding the absorber, and the distances between the absorber and neighboring atoms.

Since obtaining useful data on dilute samples is only possible using synchrotron radiation, this type of radiation is essential to the Platinum Streptidine experiment because dilute Platinum Streptidine is a better representative of how the complex will operate *in vivo* [4]. The main questions to be answered by analysis of XAS data measured with XAS are: Is it the Pt coordinated to the Streptidine? And if this is the case, through which atoms? [3]

II. MATERIALS AND METHODS

XAS measurements and data analysis were conducted at the Stanford Synchrotron Radiation Laboratory (SSRL). Pt L_3-edge data were measured on beam-line 9-3 on solid and solution samples in transmission and fluorescence mode, respectively. The energy was calibrated using a platinum foil and assigning the first inflection point to 11563.0 eV. The data scan range was 11330-12690eV.The flux was ~ $2*10^{12}$ photons per second at 100 mA with a 1 (vertical) by 4 (horizontal) mm spot size. The data represent 3- and 11-scan averages (scan time, 30 min per scan) for the solid and solution, respectively. Samples were monitored for radiation damage throughout the procedure. It should be noted that the platinum streptidine was dissolved in dimethyl-sulfoxide. The final concentration was ~2mM in Pt.

Analysis of the data was conducted with the programs EXAFSPAK [5] and FEFF[6,7]. Also, the Cambridge Structural Database was used as a reference of known complexes [8].

The pre-edge region of the data was subtracted by fitting it with a first order polynomial and then subtracting the polynomial so it lied flat. The smooth background due to atomic interference was eliminated by fitting the region above the edge with a three region cubic spline that was then subtracted [5]. The EXAFS was then k^3-weighted to enhance the oscillations at high k.

Several theoretical models of the EXAFS were produced using FEFF for the calculation of the single scattering phase and amplitude parameters and EXAFSPAK for the fitting. The data were fitted with a k-range of 2-15$Å^{-1}$ and 2-12 $Å^{-1}$ for the solid and solution data respectively. Parameters such as the bond length(R), the mean square displacement (σ^2) and the experimental energy threshold (E_0) were allowed to float while the coordination number (N) was changed systematically with every fit. It should be noted that the energy threshold (E_0) was constrained to the same value for all paths. In working with the EXAFS and FEFF software it must be noted that although the approximations might make sense mathematically, they may not be of physical or chemical relevance. Therefore a balance must be reached between both mathematical and physical significance. The Cambridge Structural Database was therefore used as a reference to compare similar known structures with the fits generated in EXAFSPAK.

III.RESULTS AND ANALYSIS

i. Comparison: Solid vs. Solution (Figure 1, 2,3)

Figure 1 shows a comparison of the normalized Pt L_3-edge data of platinum tetrachloride (K_2PtCl_4) to solid platinum streptidine complex ($C_8H_{18}N_6O_7Cl_4Pt$). As can been seen from the graph, significant differences exist between the two edges. The platinum tetrachloride edge is to a lower energy indicating that it is more reduced than

the platinum streptidine. The difference in white-line intensities for the platinum tetrachloride and solid platinum streptidine should also be noted. Also the initial EXAFS oscillations of the two complexes are out of phase. These differences indicate that the solid platinum streptidine is not merely a simple platinum tetrachloride complex, as could have been inferred through other analysis methods (i.e. on the basis of elemental analysis). The results indicate that the streptidine must interact with the platinum in a significant enough way to alter the structure, yielding a complex distinct from $[PtCl_4]^{2-}$.

A comparison of the solid and solution Pt L_3 edges is shown in Figure 2. As can be seen, the rising edge positions of the solid and solution data are at different energies, the solution edge being at a slightly higher energy than the solid edge. This is consistent with the elements present in the solid structure being replaced with lighter atoms in the solution structure (see EXAFS analysis, vide infra).

The k^3-weighted EXAFS for the Pt-Std solid and solution data are shown in figure 3(a). As can be seen, the amplitude of the EXAFS decreases slightly on going from the solid to the solution, while the frequency is essentially the same. The decrease in amplitude may indicate a decrease in coordination number or may also be indicative of heavier atoms in the solid being replaced by lighter atoms in solution. This agrees with the conclusions drawn from the edge data. Because the frequencies of the Pt EXAFS are relatively the same in solid and solution, it may be that the same element or one of similar weights surrounds the absorber in both solid and solution.

The corresponding Fourier Transforms for the Pt-Std solid and solution EXAFS are shown in figure 3(b). As can be seen from the graph the peak intensity greatly decreases from solid to solution. Similar to the decrease in EXAFS amplitude, the

decrease in peak intensity indicates a decrease in coordination number and/or lighter atoms ligated to the Platinum. The relative distances of the peak from the absorber however remains constant and therefore supports the previous assumption that same element or one of similar weight surrounds the atom in both solid and solution.

ii. Solid Fit (Figure 4, Table 2)

The k^3-weighted data and fits for the solid form of Pt-Std with the corresponding Fourier Transforms (k=2-15 $Å^{-1}$) are shown in Figure 4. FEFF-based fits were preformed, and the data were best fit by 4 Pt-Cl at 2.30 Å and 1 Pt-N at 2.02 Å (Figure 4b). The fit for 4 Pt-Cl at 2.30 Å (Figure 4a) was also reasonable. But, as previously discussed, evidence suggests that the solid Pt-Std is not merely a platinum tetrachloride complex. Also as can be seen from Table 1 there was a decrease in the normalized error when a nitrogen atom was added to the platinum tetrachloride, which indicates that the 4 Pt-Cl with 1 Pt-N is the more likely structure of the solid Pt-Std.

iii. Solution Fit (Figure 5, Table 2)

The k^3-weighted data and fits with the corresponding Fourier transforms for the dilute form of Pt-Std are shown in Figure 5. However, it should be noted that the EXAFS were only usable out to k=12 $Å^{-1}$, which greatly reduced the resolution. Therefore, because sulfur and chlorine are so close in atomic number, the fits for Pt-Cl or Pt-S vectors were indistinguishable. The structure is most likely a mixture of Pt-Cl and Pt-S with Pt-N because, as previously noted, the intensity of the Fourier transform from solid to solution decreased indicating the replacement of chlorine for a lighter atom.

The best fit was obtained by inclusion of 4 Pt-S/Cl at 2.30 Å and 1 Pt-N at 2.03 Å (Figure 5c). A very similar fit resulted in the inclusion of 3 Pt-S/Cl at 2.31 Å and 2 Pt-N at 2.08 Å (Figure 5a). Although the error for 4 Pt-S/Cl with 1 Pt-N is slightly lower (as shown in Table 2) it is less likely that this is the correct structure of dilute Pt-Std since previous examinations of the solid and solution Pt-Std edges and EXAFS indicated lighter atoms present in the solution. Another possibility, however is that the nitrogen is only loosely associated with the Pt which could account for the dilute Pt-Std edge being at a slightly higher energy than the solid.

Another very similar fit was obtained by 2 Pt-Cl/S at 2.33 Å and 3 Pt-N at 2.12 Å (Figure 5b). It should be noted however that coordination to the streptidine ligand via 3 nitrogens would be sterically quite hindered, and thus it is possible that one of the light atom interaction may be attributed to an oxygen atom from H_2O. Also, it should be emphasized that there not enough evidence to distinguish between the three possible structures. The three fits are far too similar.

IV.CONCLUSION

The results of this study show that the solid Pt-Std ($C_8H_{18}N_6O_7Cl_4Pt$) is not merely a platinum tetrachloride . This is demonstrated by the comparison of Pt L_3-edge XAS results of the Pt-Std and $PtCl_4$ which shows a significant difference between the two complexes. EXAFS data on the solid and solution Pt-Std show that the Pt-Std undergoes a change from solid to solution. More specifically, there are lighter atoms present in the solution Pt-Std. This is supported by examining the corresponding Fourier Transforms which also indicate lighter atoms in solution.

Furthermore, it can be concluded that Platinum is in fact coordinated to the streptidine through a bond with nitrogen. In the solid, the streptidine is coordinated to the Pt by one nitrogen at 2.02 Å. In the solution the streptidine could be coordinated to 1-3 nitrogens. This is shown by analysis and fitting of the EXAFS data of the solid and solution Pt-Std.

Future work should include the determination of how many chlorine and sulfur atoms are ligated in the solution structure and also the determination of how many nitrogens are coordinated to the dilute Pt-Std. This can be done by further XAS experiments to obtain Pt L_3 edge data at higher k. Data obtained to higher k would give an increased resolution rendering a better distinction between the sulfur and chlorine atoms. Another alternative would be to examine the edge of another element in the complex, such as chlorine. This would give additional information about the structure of the complex.

V.ACKNOWLEDGMENTS

This research was conducted at the Stanford Synchrotron Radiation Laboratory at the Stanford Linear Accelerator Center. I thank the U. S. Department of Energy, Office of Science for giving me the opportunity to participate in the SULI program and giving me this wonderful learning experience. Special thanks go to my mentor Serena DeBeer George for her patience and guidance. I also thank Apruva Metha for his stimulating lectures. And last, but definitely not least, I would also thank Martin George, who fixed my laptop computer! ☺

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TABLES

Fits	Component	CN	R(A)	$\sigma^2(A^2)$	E_0(eV)	Normalized Error(χ^2) F/(No.pts)	Reduced Error(χ^2) F/(No.pts-No.Vars)
Fit 1	Pt-Cl	4	2.304907	0.002451	-5.996988	0.567001	0.574148
Fit 2	Pt-Cl Pt-N	4 1	2.303149 2.018205	0.002311 0.006925	-6.960680	0.547486	0.558014
Fit 3	Pt-Cl Pt-N	4 2	2.303241 2.020574	0.002332 0.013314	-7.083107	0.556331	0.567029
Fit 4	Pt-Cl	3	2.304137	0.001301	-6.346557	0.657848	0.670499
Fit 5	Pt-Cl Pt-N	3 1	2.314112 2.143173	0.001308 0.000541	-4.339458	0.657848	0.670499
Fit 6	Pt-Cl Pt-N	3 2	2.316230 2.157105	0.001471 0.002038	-3.440386	0.626655	0.638706

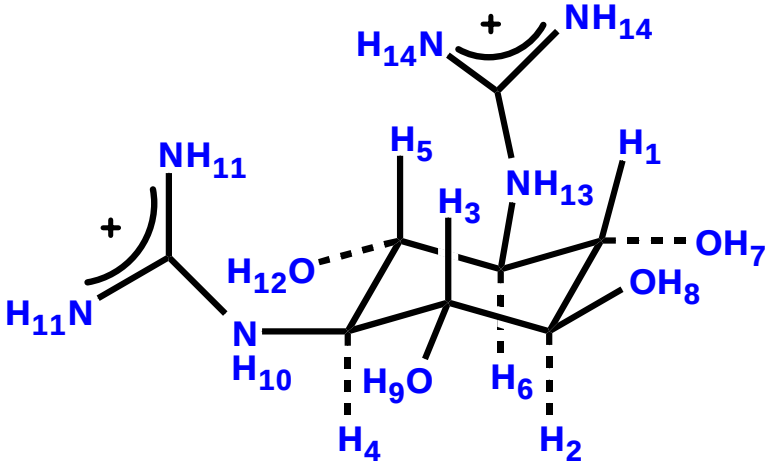
Table 1: Fit Results for the solid Pt L3 XAS Data

Fits	Component	CN	R(A)	$\sigma^2(A^2)$	E_0(eV)	Normalized Error(χ^2) F/(No.pts)	Reduced Error(χ^2) F/(No.pts-No.Vars)
Fit 1	Pt-S/Cl	4	2.297600	0.005870	-4.376000	0.420567	0.426844
Fit 2	Pt-S/Cl Pt-N	4 1	2.297591 2.026988	0.005589 0.005848	-5.306335	0.358606	0.367616
Fit 3	Pt-S/Cl Pt-N	3 1	2.308216 2.065793	0.003563 0.000716	-3.874737	0.388795	0.398564
Fit 4	Pt-S/Cl Pt-N	3 2	2.310000 2.082000	0.004120 0.005820	-3.467305	0.362555	0.371664
Fit 5	Pt-S/Cl Pt-N	3 3	2.307600 2.095000	0.004480 0.010780	-3.301708	0.371329	0.380658
Fit 6	Pt-S/Cl Pt-N	2 2	2.328183 2.103048	0.001509 0.000891	-1.346530	0.415886	0.426335
Fit 7	Pt-S/Cl Pt-N	2 3	2.327185 2.113151	0.002211 0.004120	-1.088723	0.388843	0.398613

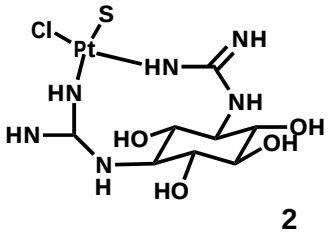
Table 2: Fit Results for the solution Pt L3 XAS Data

FIGURES

(a)



(b)



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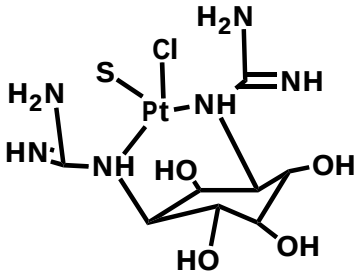


Figure i: (a) Diagram of Streptidine[1] (b), (c) Putative structures[1]

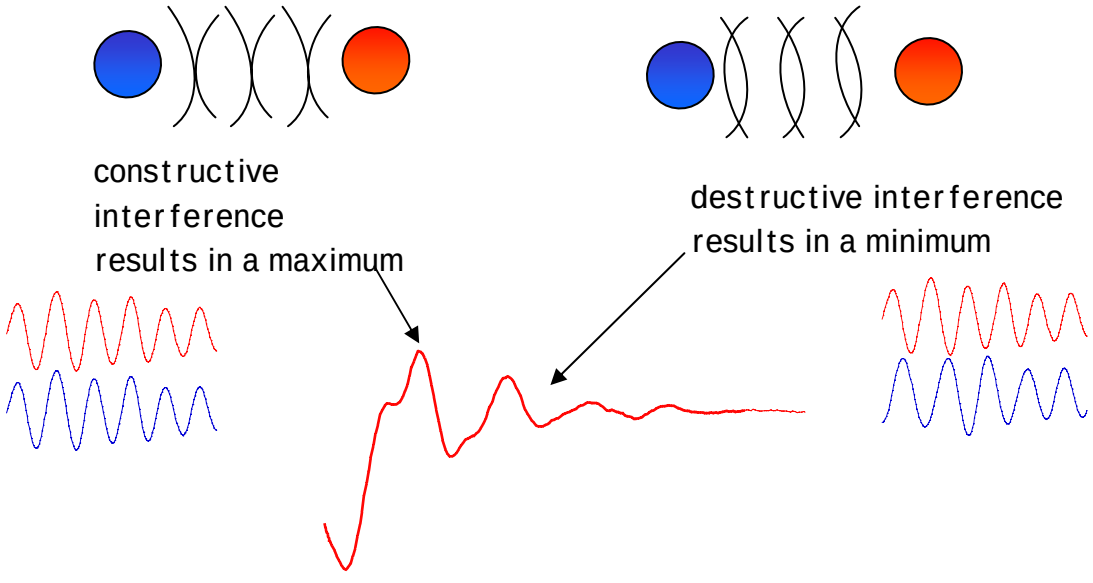


Figure ii: Diagram of EXAFS: constructive interference between the photoelectron wave and the backscatter wave results in a local maximum in the while destructive interference results in a local minim.

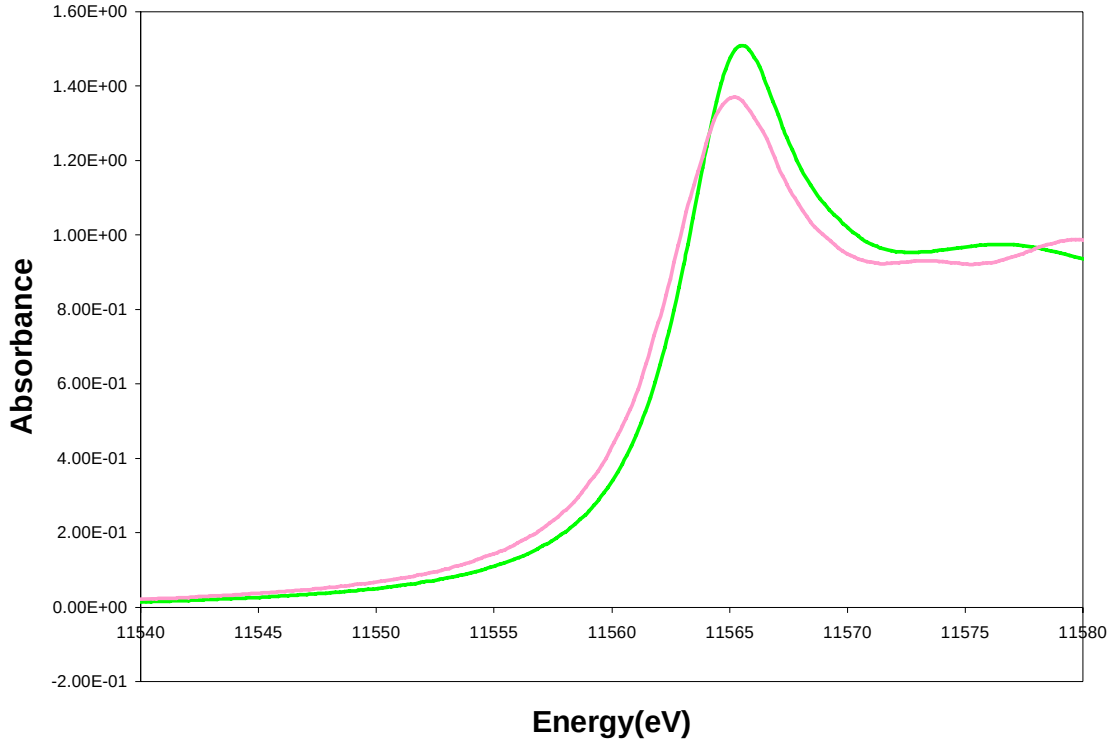


Figure 1: Comparison of the Pt L3-edge spectra of Solid Pt-Std ($C_8H_{18}N_6O_7Cl_4Pt$) (—) and platinum tetrachloride (K_2PtCl_4) (—).

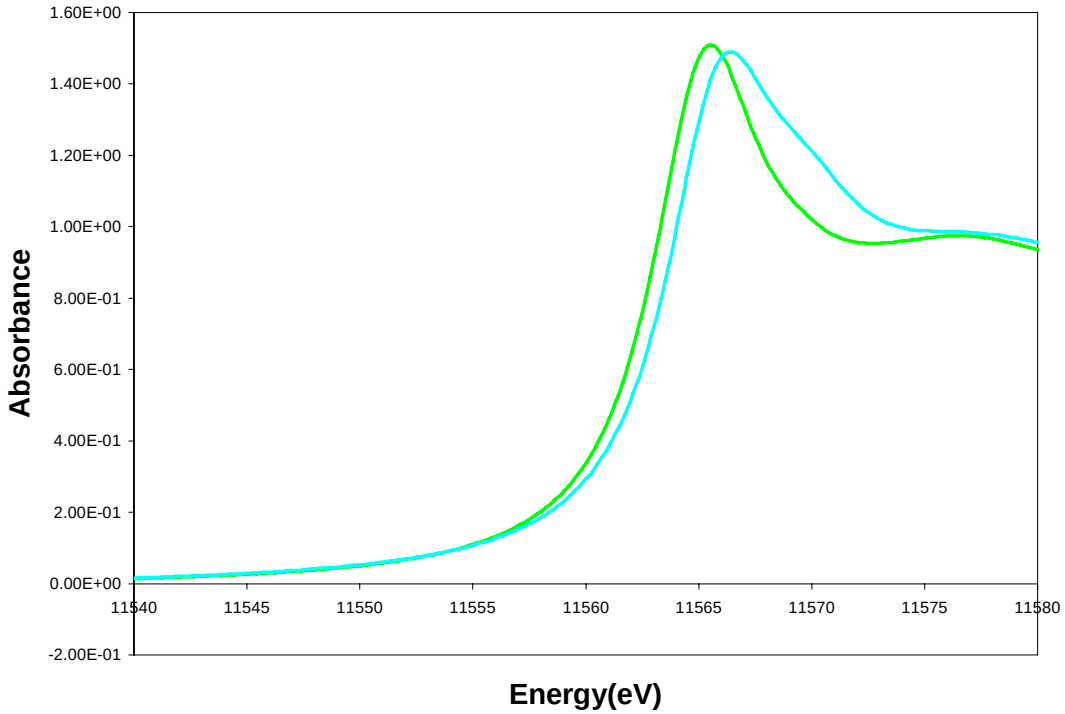
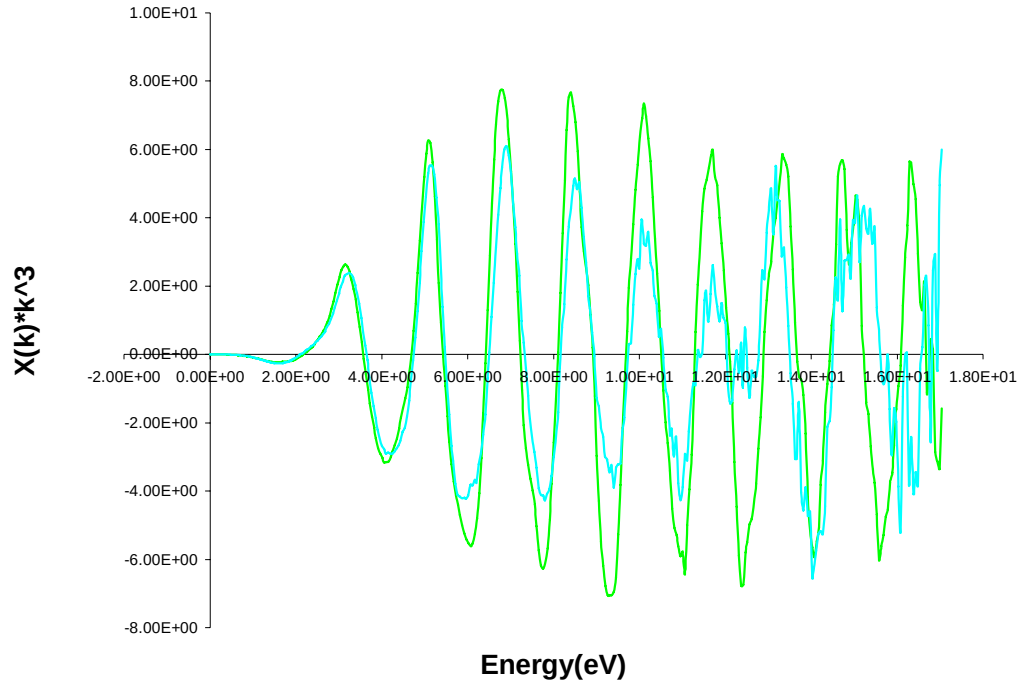


Figure 2: Comparison of the Pt L3-edge spectra of Solid (—) and Solution (—) Pt-Std



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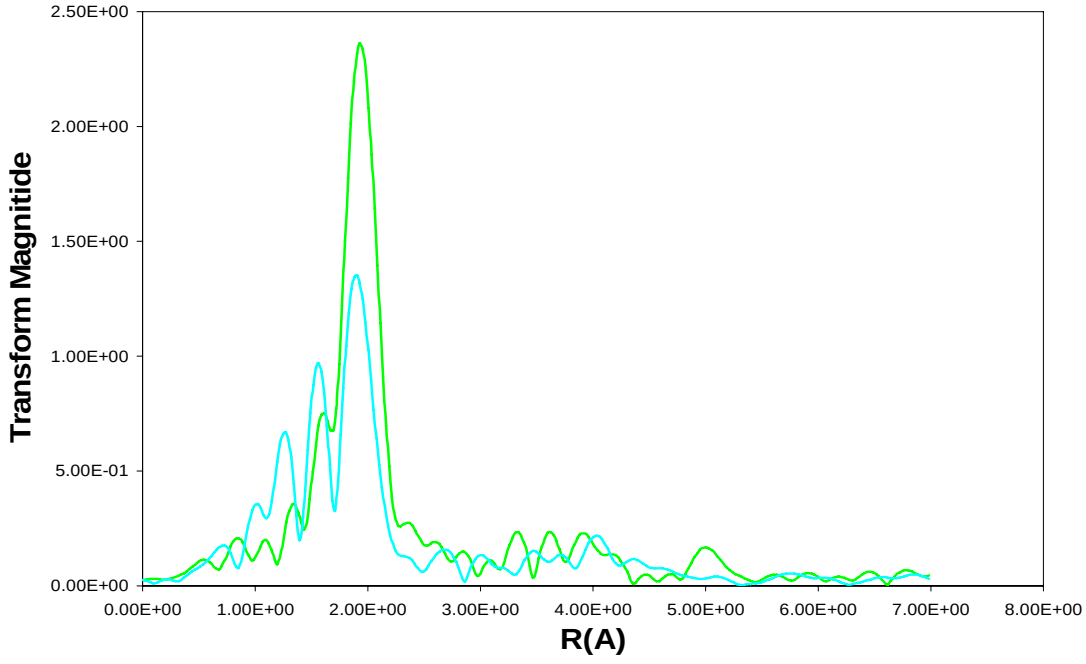
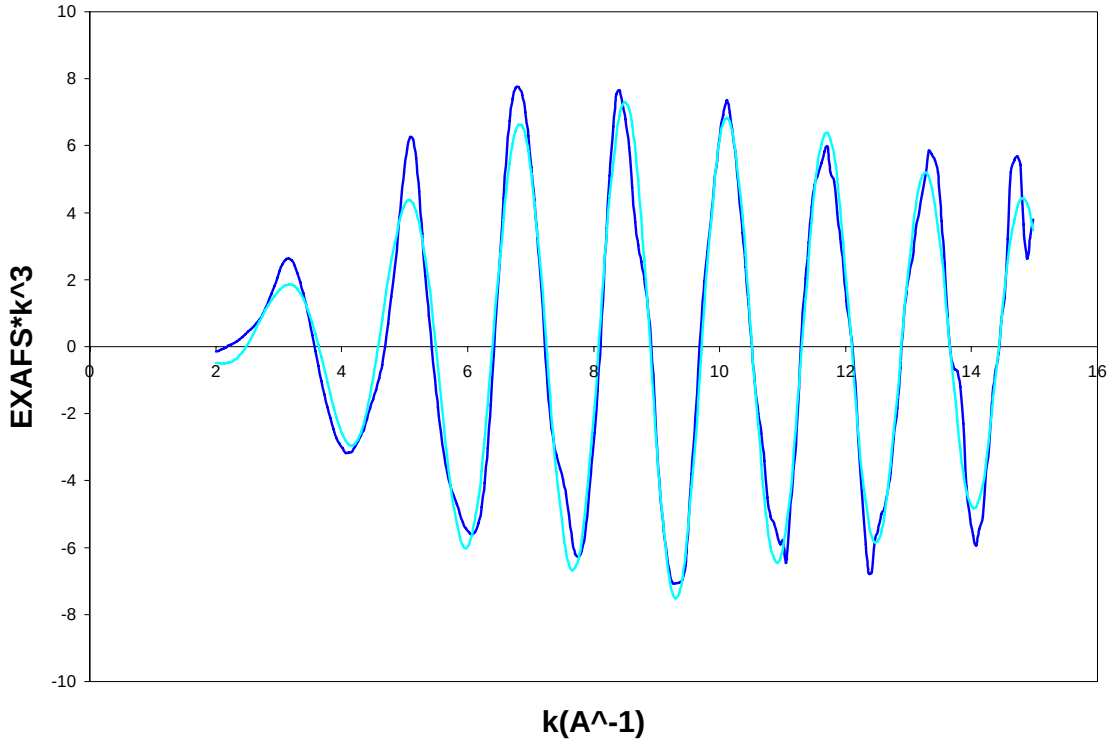


(b)

Figure 3: (a) Experimental EXASFS data for solid (—) and solution (—) Pt-Std (b) non-phase-shift corrected Fourier Transforms


EXAFS*k^3
k(A^-1)
10
8
6
4
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-2
-4
-6
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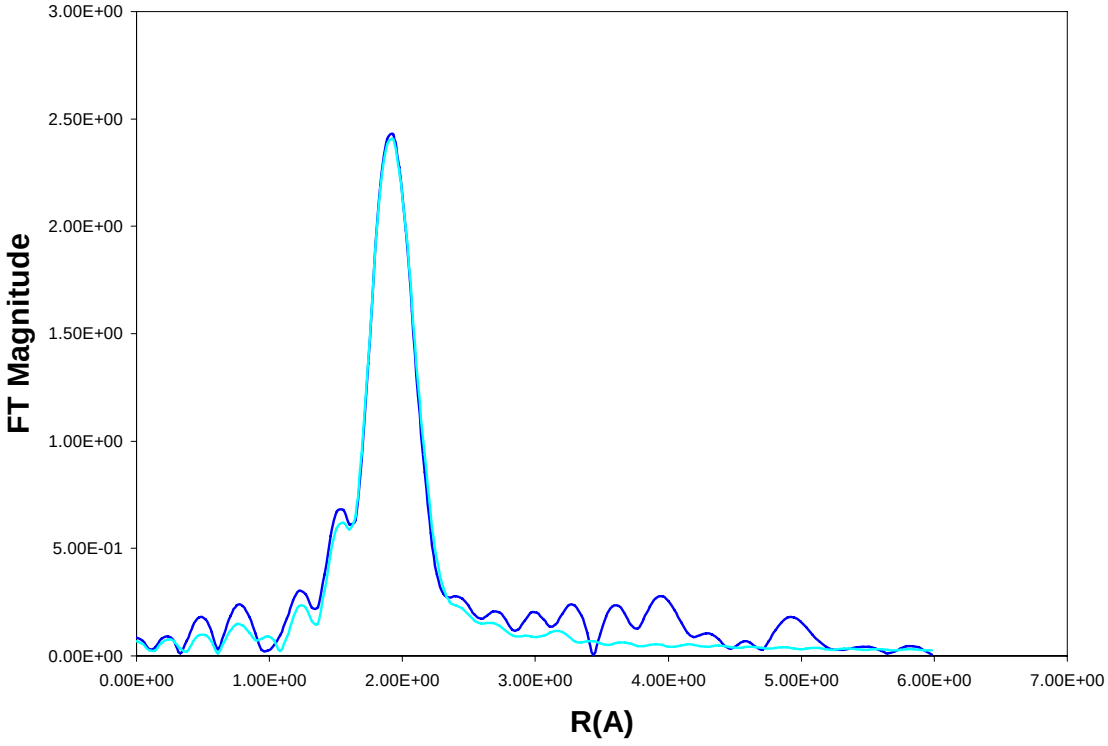
2
4
6
8
10
12
14
16

(a)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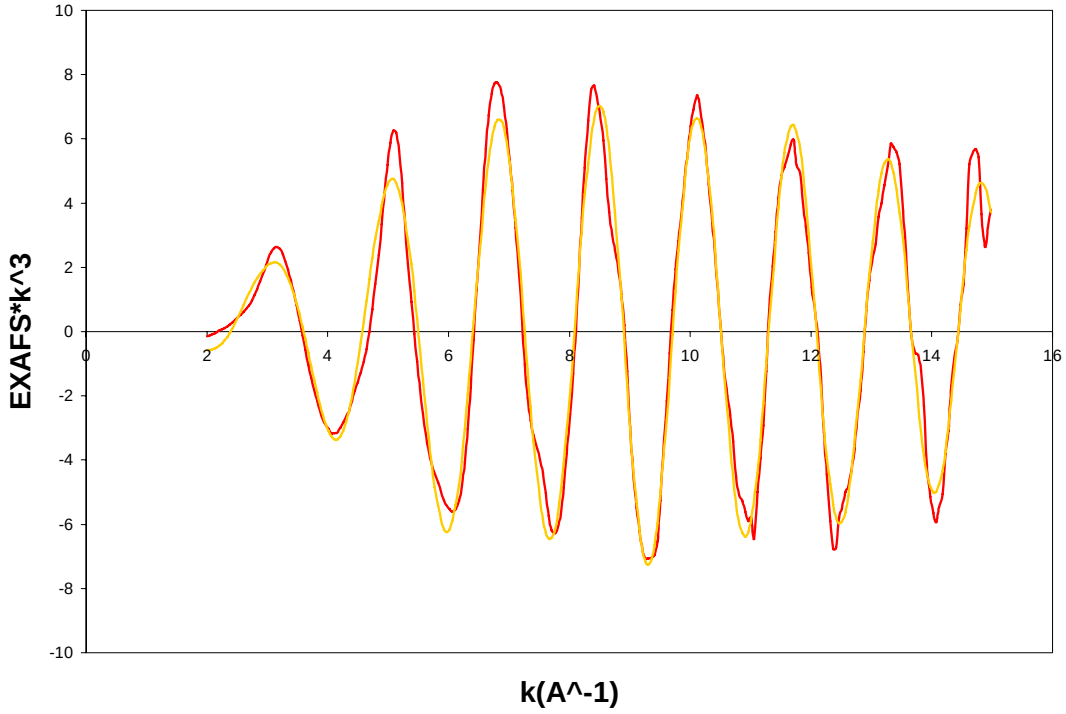

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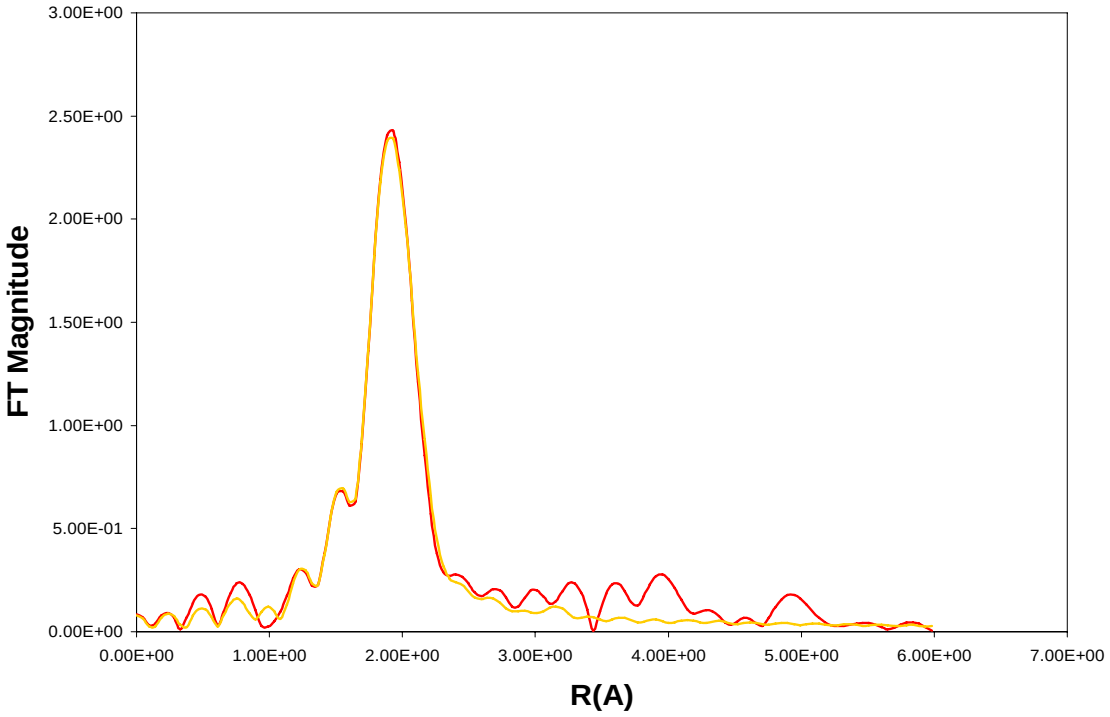
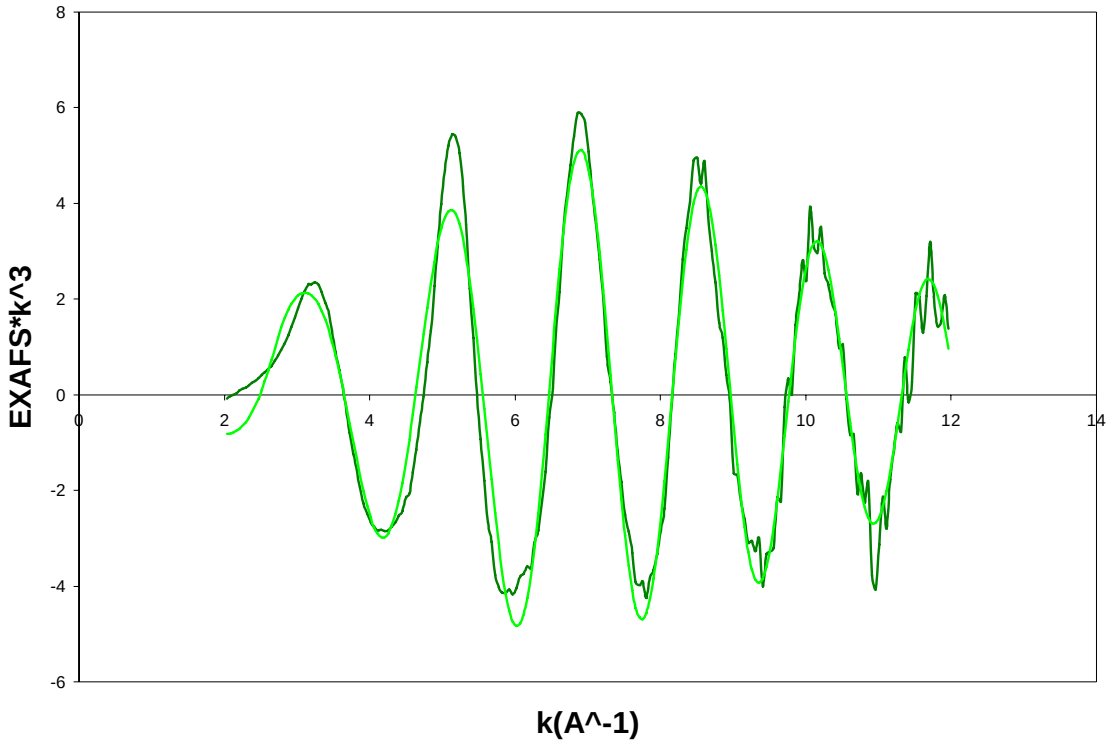


(d)

Figure 4: (a) Experimental EXASFS data(—) and fits(—) for the solid Pt-Std, Fit 1: 4 Pt-Cl at 2.30 Å (b) corresponding Fourier Transforms and fit, Fit 1 (c) EXASFS data(—) and fits(—) for the solid Pt-Std, Fit 2: 4 Pt-Cl at 2.30 Å and 1 Pt-N at 2.02 Å (d) corresponding Fourier Transforms and fit, Fit 2


EXAFS*k^3
k(A^-1)
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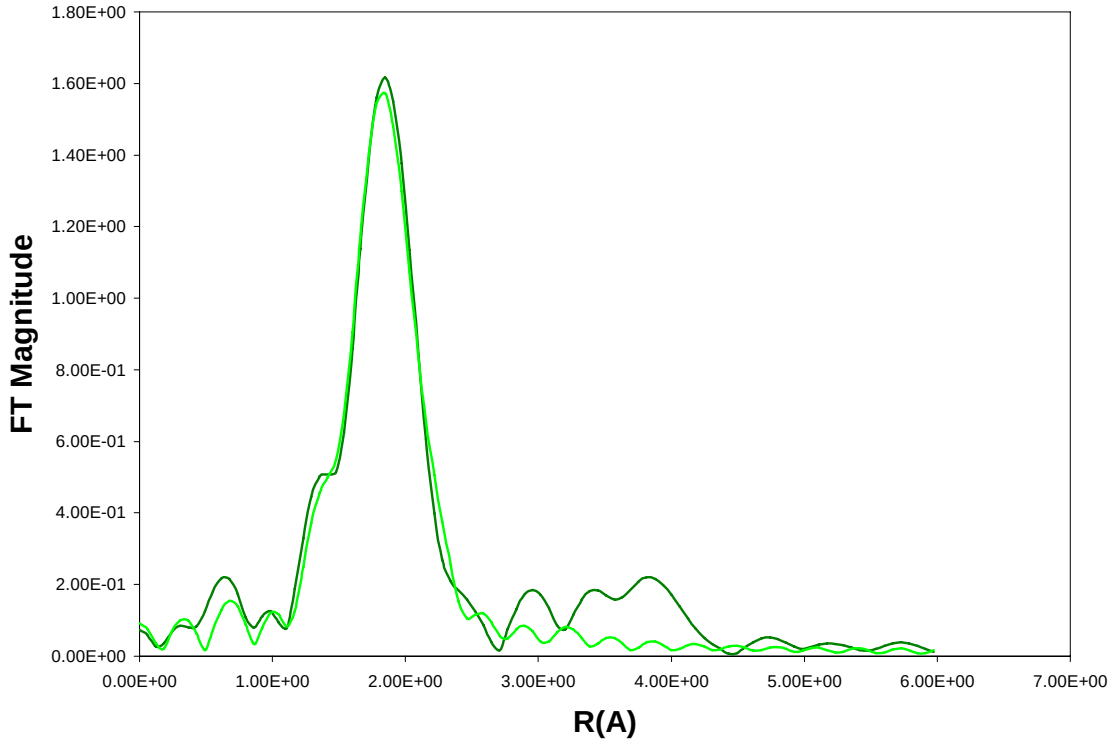
10
12
14

(a)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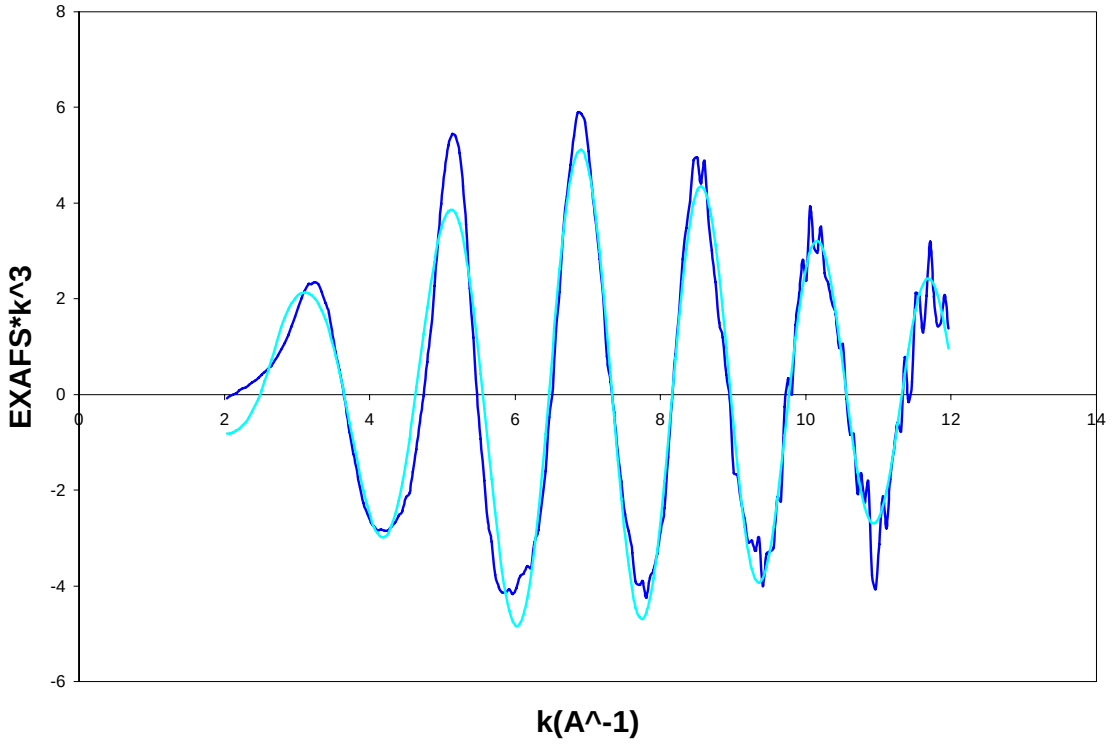
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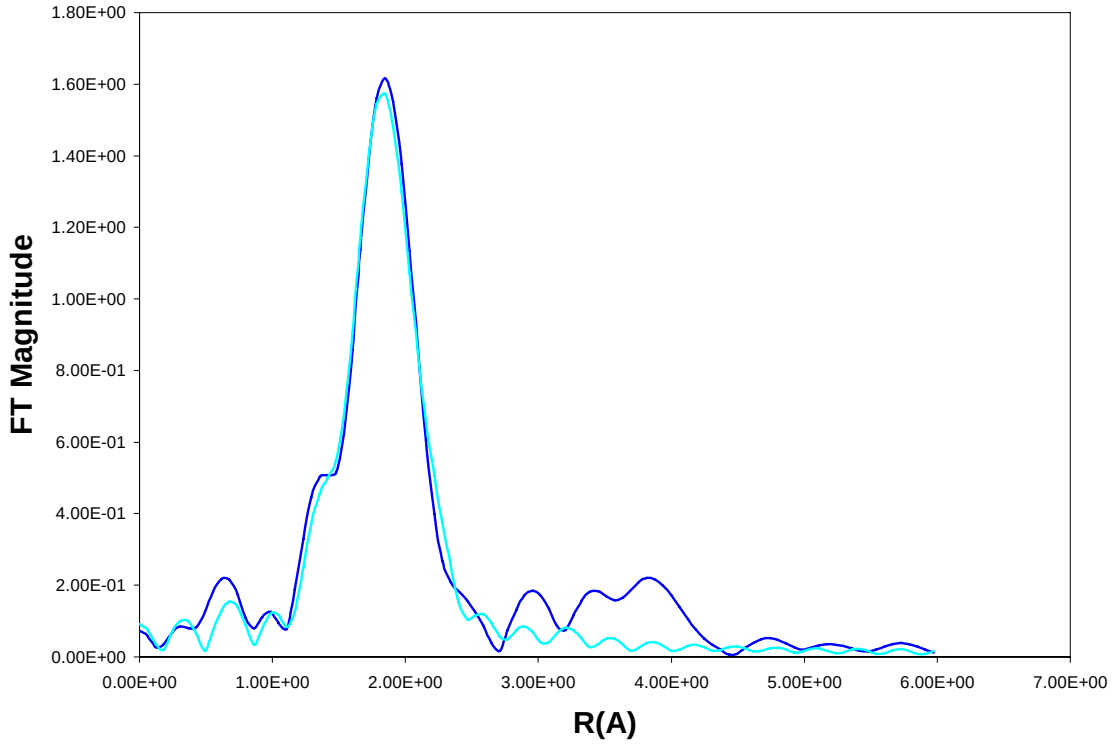
14

(c)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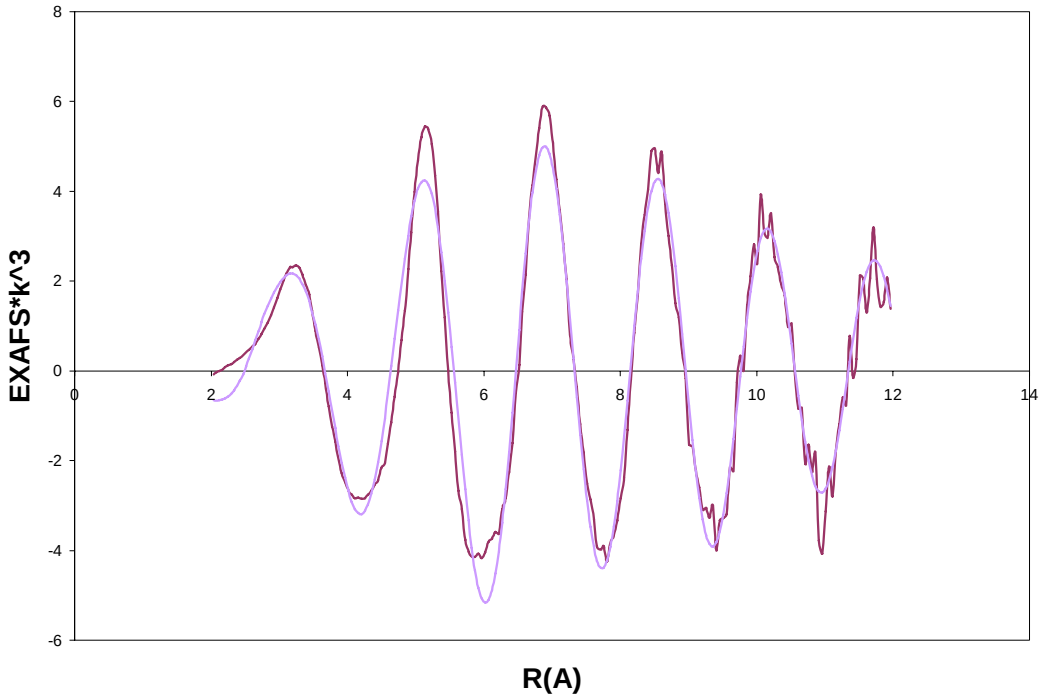

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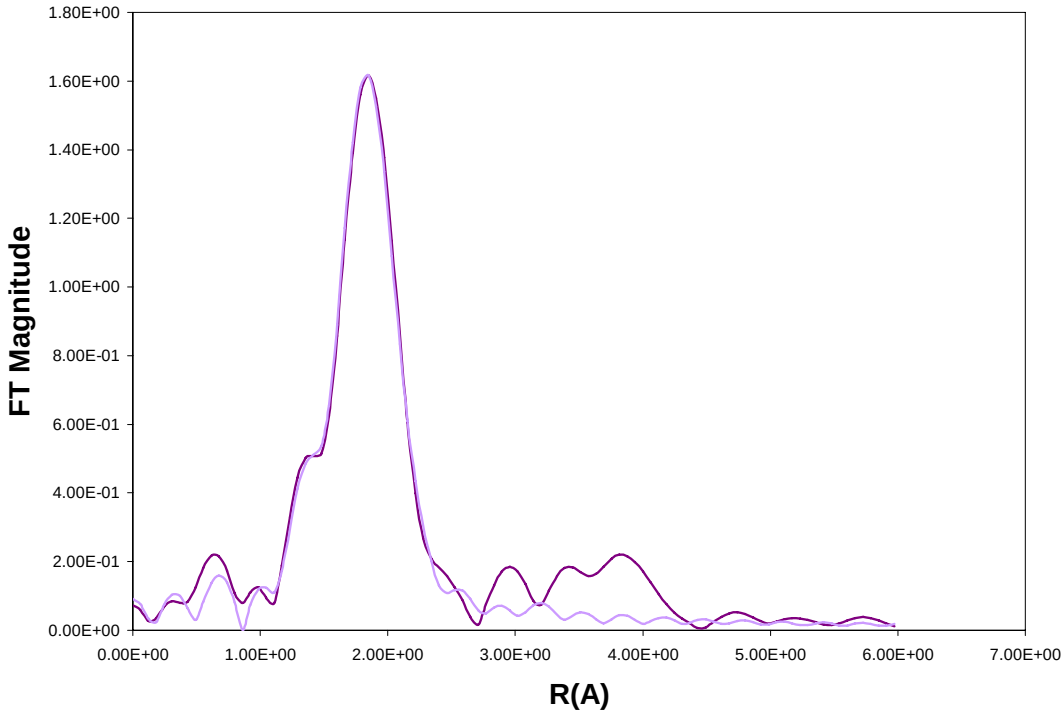

(f)

Figure 5: (a) Experimental EXASFS data (—) and fits (—) for the solution Pt-Std, Fit 4: 3 Pt-S/Cl at 2.31 Å and 2 Pt-N at 2.08 Å. (b) corresponding Fourier Transforms and fit, Fit 4 (c) EXASFS data (—) and fits (—)for the solid Pt-Std, Fit 7: 2 Pt-Cl/S at 2.33 Å and 3 Pt-N at 2.12 Å (d) corresponding Fourier Transforms and fit, Fit 7 (e) Experimental EXASFS data(—) and fits(—) for the solution Pt-Std, Fit 2: 4 Pt-S/Cl at 2.30 Å and 1 Pt-N at 2.03 Å (f) corresponding Fourier Transforms and fit, Fit 2