Supplementary Information for the Manuscript

Oxygen Binding and Activation by the Complexes of PY2- and TPA-appended Diphenylglycoluril Receptors with Copper and Other Metals

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Contents:

Supplementary Material available. Experimental describing the preparation of the complexes, and the catalytic, UV, and XAS experiments. Figures for the best simulations of the EXAFS, including one highlighting the Cu contribution, and complete Tables with the EXAFS simulation parameters and electrospray MS data for the Fe complex of **2**.

Experimental

Standard UV-vis experiment. The UV-vis setup was assembled as described earlier.[1] The glass UV-chamber, containing a stirrer bar and a fibre optics probe for measuring UV-vis data, was deoxygenated by at least three alternating cycles of argon and vacuum purges. All solvents used for either UV-vis or XAS experiments were distilled under nitrogen and transferred to the UV-vis chamber under either nitrogen or argon. For a standard UV-vis experiment, 20 mL of a solution of the complex to be measured was prepared in a Schlenk flask of 50 mL. This solution was subsequently introduced in the UV-vis chamber under Schlenk conditions. UV-vis scans were collected, using the fibre optics probe coupled to the UV-vis spectrometer, between 900 nm and 290 nm with a scan speed of 4800 nm per min. After recording an initial spectrum at room temperature, the solution of the copper complex of a receptor was cooled to –78 °C (over a time of 1.5 h) using a closed cycle cryostat. Then, dioxygen was introduced through a needle in a septum and bubbled slowly through the cooled solution of the sample. The dioxygen uptake by the complex to be measured was followed in time, using cycle mode UV-vis spectroscopy taking 30 scans, which started 1 minute apart. After the uptake of dioxygen was completed, as determined by no further changes in the spectra, the flow of dioxygen was stopped. The oxygenated sample solution was then allowed to slowly warm up to room temperature. During the step of warming up, another cycle of UV-vis spectra can be recorded to study the temperature dependence of the sample solution, while monitoring the internal temperature of the solution.

Preparation of XAS samples with the new type of XAS cell. The central part, viz. the sample chamber with windows, of a new type of XAS cell was assembled as described earlier.[1] The UV-vis setup was assembled and deoxygenated as described above. Subsequently, a sample solution was prepared and a room temperature scan of the deoxy complex was recorded as described above. Then, to prepare a deoxy XAS sample, the fibre optics probe was removed from the glass UV-vis chamber and in its place the central part of a XAS cell was introduced through its inlet, while the XAS cell was connected via one of its filling inlets to a Hamilton syringe of 100 μL by means of a Teflon connector. The plunger of the syringe was then slowly lifted, thereby creating a vacuum within the XAS cell, allowing the sample chamber to be filled through a second filling inlet. After the XAS cell had been completely filled, the filling inlets were closed using Teflon caps and the XAS cell was frozen in liquid nitrogen until the XAS measurements were performed. To prepare an oxy sample, the fibre optics probe was replaced in the glass UV-vis chamber, after which the solution was cooled to –78°C, while stirring. Then, dioxygen was introduced as described

above, while monitoring the oxygenation by UV-vis. After the flow of dioxygen was stopped, the fibre optics probe was removed again and a XAS cell was filled at -78 °C and frozen using the same procedure as described above. Subsequently, the thus prepared XAS cells were attached to the cell holders under liquid nitrogen, stored in a filled Dewar vessel with liquid nitrogen for transportation, and mounted on the cryostat of the spectrometer under liquid nitrogen.

Preparation of XAS samples with the old type of XAS cell. In line with earlier work[2] we initially used a very simple XAS cell, consisting of an aluminium body (29x24x1mm) with a rectangular aperture (15x10 mm), forming the sample chamber (150 μL). Two rectangular pieces (windows) of Kapton (polyimide film) (24x18 mm) were glued on both sides of the cell surrounding the aperture using cyanoacrylate glue (Permacol). Two radial perforations were provided through which the cell could subsequently be filled with a (cooled) solution of the sample. These perforation were then sealed by a small drop of glue (two-component UHU plus Schnellfest), after which the samples were rapidly frozen in liquid nitrogen to prevent leakages. They were stored in a filled Dewar vessel for transportation. Oxygenated samples were prepared by bubbling dioxygen through a dry ice cooled cell, filled as described above, for 30 seconds, after which it was closed with glue and frozen as mentioned above.

Standard XAS experiments. All experiments reported here were carried out at the European Molecular Biology Laboratory (EMBL) Outstation in the Hamburg Synchrotron Laboratory (HASYLAB) of the *Deutsches Elektronen Synchrotron* (DESY) in Hamburg, Germany using the EXAFS beamline. For fluorescence-mode measurements the detector was placed at an angle of 90° in the horizontal plane with respect to the radiation beam. The cell must be placed under an angle of 45° to the beam to allow fluorescence radiation to be collected onto the detector. The EXAFS scans were acquired around the Cu K-edge (8980 eV) between 8700 and 9700 eV. Between 15 and 50 scans per sample were taken, depending on the copper-concentration in the samples. The samples were kept at 20 K in the He exchange gas atmosphere of a closed-cycle cryostat during the measurements. The DORIS storage ring was operating at 4.5 GeV with currents between 143 and 70 mA. The station was equipped with a Si(111) double crystal monochromator (set to 50% of peak intensity to suppress harmonics),[3] a focusing mirror, a CANBERRA 13 element solid-state fluorescence detector, and an energy calibration device.[4] It was necessary to decrease the opening of the lateral slit to reduce contamination of the signal with Cu-contribution, which most likely arose from contaminants in the stainless steel of the cell.

Data reduction and simulation of the EXAFS spectra. Data reduction was carried out at the EMBL Outstation using the EMBL data reduction package,[5] including the energy calibration programmes CALIB and ROTAX, the averaging programme AVERAGE, and the background subtraction programme REMOVE. Simulations were carried out on the UNIX computer BUTTHEAD at the EMBL Outstation, using the programme EXCURV98 developed at the CLRC Daresbury Laboratory.[6,7] Phase shifts were calculated with EXCURV98 in the default (Hedin-Lundqvist/von Barth) settings. Multiple scattering effects within the pyridine rings and the Cu_2O_2 unit were calculated in the default (small atom approach) settings.[8] In the refinement of the simulations, restraints[9] based on the geometry of the pyridine unit[12] were applied, and the Debye-Waller type factors of equivalent atoms in the multiple scattering units, such as C2 and C3, and C4 and C5 in the pyridine ring and the oxygens in the Cu_2O_2 unit, were kept at identical values. In the case of the Cu_2O_2 unit, a series of simulations were refined from starting points in which the geometry was varied from a flat diamond structure to a bent butterfly with a dihedral angle of 90°.[2]

Gas chromatography. GC analysis was carried out in several experiments and the reference number of the gas chromatograph used will be mentioned in each separate experiment:
GC 1) Varian 3800 instrument with a Supelco fused silica capillary column (15m length, 35 μm ED, d_f 1.0 μm) containing a FFAP stationary phase, data were analysed with Varian Star 5.2 software.
GC 2) HP 5890 Series II gas chromatograph with a HP-1 cross-linked methyl silicone gum (25 m length, 32 μm ED, d_f 0.17 μm) and a HP 3396 II integrator.
GC 3) HP 5890 Series II gas chromatograph with a CP-wax 52 series CB fused silica column and a HP 3396 II integrator.
GC 4) HP 6890 Series with a non-polar HP 5 - 5% phenyl-methylpolysiloxane column. Data were analysed using Chemstation software.

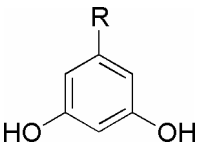
Catalytic experiments with Cu(I) complexes of receptors using three different methods.
Method 1: A pale yellow solution of 2 equiv. of $[Cu(I)(CH_3CN)_4](ClO_4)$ and 1 equiv. of receptor was prepared *in situ* and cooled to -80 °C. Dioxygen was bubbled through the solution for 10 min. and the resulting solution was dark yellow/brown. Subsequently, substrate was added.

Method 2: A pale yellow solution of 2 equiv. of $[Cu(I)(CH_3CN)_4](ClO_4)$ and 1 equiv. of receptor was prepared *in situ* and cooled to -80 °C. Dioxygen was bubbled through the solution for 10 min. and the resulting dark yellow/brown solution was subsequently treated with 3 nitrogen/vacuum cycles to remove excess of dioxygen from the solution followed by addition of substrate.
Method 3: A pale yellow solution of 2 equiv. of $[Cu(I)(CH_3CN)_4](ClO_4)$ and 1 equiv. of receptor was prepared *in situ* and cooled to -80 °C. Substrate was added to the solution and subsequently dioxygen was bubbled through the solution for 10 min..

Catalytic experiment with Cu(II) complexes.

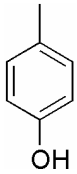
For the experiment with 50 equiv. of substrate a solution was prepared by dissolving 0.144 mg or 0.148 mg of receptors **1** and **2**, respectively, in 1 mL of chloroform and 0.8 mL of methanol. A stock solution of Cu(II) acetate monohydrate was prepared by dissolving 0.56 mg of Cu(II) acetate monohydrate in 1.4 mL of methanol and sonicating until it dissolved. An aliquot of 100 μL of the Cu(II) solution was added to the ligand solution. The absorption at 400 nm was recorded and subsequently 100 μL of a stock solution of 11 mg of DTBC in 1 mL of methanol was added. A total solution of 2.0 mL was obtained with a Cu(II) concentration of 1×10^{-4} M and a ligand concentration of 0.5×10^{-4} M. The formation of DTBQ was recorded by measuring the change in absorbance at 400 nm in time. The experiments with 1 equiv. of DTBC and the titration with DTBC were carried out in a similar fashion, only changing the amount of substrate added.

Dihydroxybenzene derivatives.



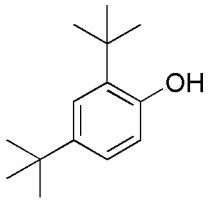
Silylation of dihydroxybenzenes was carried out by treating an aliquot of 50 μL of a solution of the substrate (1 mg/mL) with 25 μL MSTFA (N-methyl-N-(trimethylsilyl) trifluoroacetamide). After stirring for a few minutes the products were tested on GC 1 with the following temperature program: 80 (0)-5-230 (0) (total time of program: 30 min); the disilylated products were also tested by GC/MS to check the assignment. The retention times of the compounds were di-silylresorcinol (11.2 min); di-silylorcinol (12.7 min); tri-silyl-1,3,5-trihydroxybenzene (17.1 min); tri-silyldihydroxybenzylalcohol (19.0 min); and tri-silyldihydroxybenzoic acid (21.2 min). In a catalysis experiment, the MSTFA was added to an aliquot of the reaction mixture. This aliquot was passed over a short column of activated alumina (III) in order to remove the metal-complex; the resulting solution was injected on GC 1 to determine which products were formed.

p-Cresol.



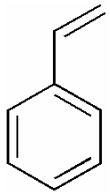
GC analysis of *p*-cresol was carried out on GC 1 with the following temperature program: 90(0)-5-190(60) (total time of program: 80 min). The retention times of the compounds were: *p*-cresol (16.1 min) and 4-hydroxybenzaldehyde (53.0 min). Copper was removed from the receptor by washing the dichloromethane reaction mixture with aqueous ammonia or by sonication of the evaporated reaction mixture with methanol.

2,4-di-tert-Butylphenol.



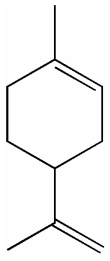
GC analysis of 2,4-di-tert-butylphenol and of the *ortho*-coupled dimer was carried out on GC 2 with the following temperature program: 100(0)-15-250(10) (total time of program: 20 min). The retention times of the compounds were: di-*tert*-butylphenol (4.5 min) and radical coupled dimer (10.5 min). An aliquot of the reaction mixture was passed over a small column of activated alumina (III), which was rinsed with 1 vol. of methanol. The resulting solution was injected on GC to determine which products were formed.

Styrene.



GC analysis of styrene and styrene oxide was carried out on GC 1 with the following temperature program: 70(0)-5-190(6) (total time of program: 30 min). The retention times of the compounds were: styrene (3.7 min) and styrene oxide (10.5 min). In order to measure a precise amount of styrene a solution in dichloromethane was prepared. To remove all dioxygen, this solution was deoxygenated by repeated freeze-pump-thaw cycles after which the styrene solution was added to the solution of metal complex. After reaction, an aliquot of the reaction mixture was passed over a small column of activated alumina (III), which was rinsed with 1 column vol. of acetone. The resulting solution was injected on GC to determine which products were formed.

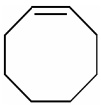
Limonene.



GC analysis of limonene and limonene oxide was carried out on GC 1 with the following temperature program: 90(0)-5-190(60) (total time of program: 80 min). The retention time of the compounds were: *S*-limonene (2.78 min), *R*-limonene (3.05 min), *cis*-oxide (6.9 min), and *trans*-oxide (7.1 min). The solvent used was acetone. After warm-up the reaction mixture was passed over a small column of activated alumina (III), which was rinsed with several column vol. of acetone. The

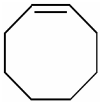
resulting solution was injected on GC to determine which products were formed.

Epoxidation experiments using Fe complexes.



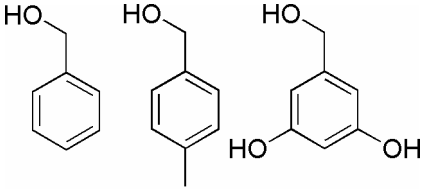
These experiments were carried out according to a literature procedure.[10] A Fe(II) complex was prepared *in situ* by preparing a solution of receptor **2** (0.44 mM) and $Fe(ClO4)_2$ (0.88 mM) in 2 mL of acetone. To this were added 1000 equiv. of cyclooctene and 500 equiv. of bromobenzene as an internal standard. After stirring under nitrogen in an ice bath for a few minutes, 50 equiv. of hydrogen peroxide were added. After four hours, the reaction was stopped by passing the reaction mixture over a short (1 cm) silica column, which was rinsed with 4 x 1.5 mL of diethyl ether/EtOH (9:1). The resulting solution was concentrated to 2 mL by a nitrogen flow and the products were analysed on GC 3 with a temperature program of 100(5)-10-200(0)-50-250(4) (total time of program: 20 min). The above experiment was repeated with dichloromethane as the solvent, however the iron complex is not soluble in this solvent and hence a suspension is obtained. This suspension was tested in the same way as the solution described above, except that an urea adduct of H_2O_2 was used instead of aqueous H_2O_2. No oxidation products were however, observed.

Epoxidation experiments using Mn complexes.



These experiments were carried out according to a literature procedure.[11] A Mn(III) complex was prepared *in situ* by mixing 1 or 2 equiv. of $Mn(OAc)_3 \cdot 2H_2O$ with 1 equiv. of receptor **1** in 2 mL of acetone. To this were added 1000 equiv. of cyclooctene and 500 equiv. of bromobenzene as an internal standard. After stirring under nitrogen in an ice bath for a few minutes, 8000 equiv. of hydrogen peroxide were added. After four hours, the reaction was stopped by passing the reaction mixture over a short (1 cm) silica column, which was rinsed with 4 x 1.5 mL of diethyl ether/MeOH (9:1). The resulting solution was concentrated to 2 mL by a nitrogen flow and the products were analysed on GC 3 with a temperature program of 100(5)-10-200(0)-50-250(4) (total time of program: 20 min).

Alcohol oxidation.



The experiments were carried out according to a literature procedure, in both acetone and dichloromethane.[12] A 1 mM solution of a dinuclear or mononuclear Mn(II) complex of receptor **1** was prepared *in situ* by mixing 1.44 mg (0.5 mmol) or 2.88 mg (1.0 mmol) of **1**, respectively, with 0.54 mg (1 mmol) of

Mn(III) acetate dihydrate in 2 mL of solvent and sonicating for 10 minutes. To this solution was added internal standard (500 equiv.) and substrate (1000 equiv.) (as a solution or as a solid). The resulting solution/suspension was cooled in ice and 5000 equiv. of hydrogen peroxide (470 mg of H_2O_2-urea adduct) were added. After stirring for 2 hrs an aliquot (of the reaction mixture) was taken and in the case of benzyl alcohol and *p*-methylbenzyl alcohol this was passed over a small (1 cm) silica column. In the case of 3,5-dihydroxybenzyl alcohol, 1 mL of diethyl ether and circa 300 μL of MSTFA were added to the aliquot, and after standing for 5 minutes the reaction mixture was passed over a small (1 cm) silica column. All columns were rinsed with 4 x 1.5 mL diethyl ether/MeOH (9:1 v/v). The resulting solutions were concentrated under a nitrogen flow to 2 mL and analysed by GC 4. An additional 5000 equiv. of hydrogen peroxide (urea adduct) were added to the reaction mixture and the reaction was stopped after 4 hrs. The work up and analysis were repeated.

Figure captions

Figure S1. Fourier filtered experimental (solid) k^3-weighted EXAFS (left) and phase-corrected Fourier transforms (right) with refined multiple scattering simulations (dashed, parameters in Table S1) of Cu(I) complexes. A, **1a** in acetone (Table S1, entry 1); B, **1a** in THF/acetonitrile (70/30) (Table S1, entry 2); C, **2a** in acetone (Table S1, entry 3).

Figure S2. Fourier filtered experimental (solid) k^3-weighted EXAFS (left) and phase-corrected Fourier transforms (right) with refined multiple scattering simulations (dashed, parameters in Table S2) of oxygenated Cu(I) complexes. A, 1mM **1b** in acetone (Table S2, entry 1); B, dilute **1b** in acetone (Table S2, entry 2); C, **1b** in THF/acetonitrile (70/30) (Table S2, entry 3); D, **2b** in acetone (Table S2, entry 4).

Figure S3. Fourier filtered experimental (solid) k^3-weighted EXAFS (top) and phase-corrected Fourier transform (bottom) with refined multiple scattering simulation (dots, parameters in Table S2, entry 1; dashed, Cu-Cu contribution to simulation) of oxygenated **1b** in acetone.

Tables

Table S1. Parameters for refined multiple scattering simulations of the bis-Cu(I) complexes **1a** and **2a**.[a]

	Entry 1	Entry 2	Entry 3
Sample	**1a** acetone	**1a** THF/ACN	**2a** acetone
Figure S1	A	B	C
Range (eV)	3.0-550.0	3.0-575.0	3.0-675.0
ΔEF (eV)	-2.2338	-5.126	-3.763
pyr-N	2.1 @ 1.954 (0.009)	2.1 @ 1.984 (0.010)	1.0 @ 1.958 (0.007)
Pyr-C1,C2[c]	2.948/2.947 (0.016)	2.964/2.967 (0.015)	2.872/2.876 (0.019)
Py3-C3,C4[c]	4.326/4.326 (0.028)	4.347/4.353 (0.030)	4.255/4.255 (0.039)
Pyr-C5[c]	4.758 (0.020)	4.803 (0.009)	4.749 (0.017)
Amine-N	0.9 @ 2.098 (0.001)	0.8 @ 2.103 (0.003)	1.1 @ 2.048 (0.009)
Cu-Cu	no sign. contribution	no sign. contribution	no sign. contribution
Fit index[b]	0.1624	0.2011	0.3859

(a) Distances in Å; Debye-Waller-type factors as $2\sigma^2$ in parentheses in $Å^2$; (b) Fit index on FF-data k^3-weighting; (c) the remote shells in the pyridine unit are refined with the same occupancy as the coordinating nitrogen.

Table S2. Parameters for simulations of the Cu(II) complexes of 1 and 2.[a]

	Entry 1	Entry 2	Entry 3	Entry 4
Complex	**1b** acetone, conc.	**1b** acetone, dil.	**1b** THF/ACN	2**b** acetone
Figure S2	A	B	C	D
Range (eV)	3.0 – 460.0	3.0 – 460.0	3.0 – 675.0	3.0 – 675.0
ΔEF (eV)	-5.844	-4.605	-3.026	-6.910
Pyr-N	2.5 @ 2.027 (0.018)	1.7 @ 2.030 (0.012)	2.3@2.014 (0.013)	1.3 @ 2.066 (0.002)
Pyr-C1,C2[d]	2.953/2.955 (0.010)	2.952/2.954 (0.004)	2.927/2.929 (0.012)	2.971/2.987 (0.025)
Pyr-C3,C4[d]	4.242/4.243 (0.031)	4.258/4.260 (0.021)	4.311/4.313 (0.018)	4.278/4.288 (0.027)
Pyr-C5[d]	4.803 (0.042)	4.841 (0.015)	4.787 (0.027)	4.829 (0.011)
Amine-N	No signif. contrib.	No signif. contrib.	No signif. contrib.	0.6 @ 2.241 (0.001)
O[e]	0.8 @ 1.887/1.964 (0.002)[c]	0.9 @ 1.866/1.984 (0.001)	0.8 @ 1.947 (0.012)	2.0 @ 1.952 (0.011)
Cu[e]	0.8 @ 3.593 (0.020)	0.9 @ 3.486 (0.022)	No signif. contrib.	1.7 @ 2.909 (0.030)
Fit index[b]	0.0767	0.0886	0.1369	0.0749

(a) Distances in Å; Debye-Waller-type factors as $2\sigma^2$ in parentheses in $Å^2$; (b) Fit index of Fourier filtered data, with k^3-weighting; (c) distance for each of 2 O; (d) the remote shells in the pyridine unit are refined with the same occupancy as the coordinating nitrogen; (e) the Cu_2O_2 moiety has been refined as a unit with all occupancies refined at the same value.

Table S3.

Results of electro spray mass spectrometry of the bis-Fe(II) complex of receptor **2**.

Observed (MW)	Charge	# ligand (1481.7)	#Fe ions (55.8)	Extra components	Calculated (tot. MW/valence)
564.0	+3	1 (1481.7)	2 (111.6)	ClO_4^- (99.5)	564.3
412.0	+4	1 (1481.7)	2 (111.6)	Na(23) + MeOH (32)	412.1
398.3	+4	1 (1481.7)	2 (111.6)	-	398.3

Figures

Figure S1.

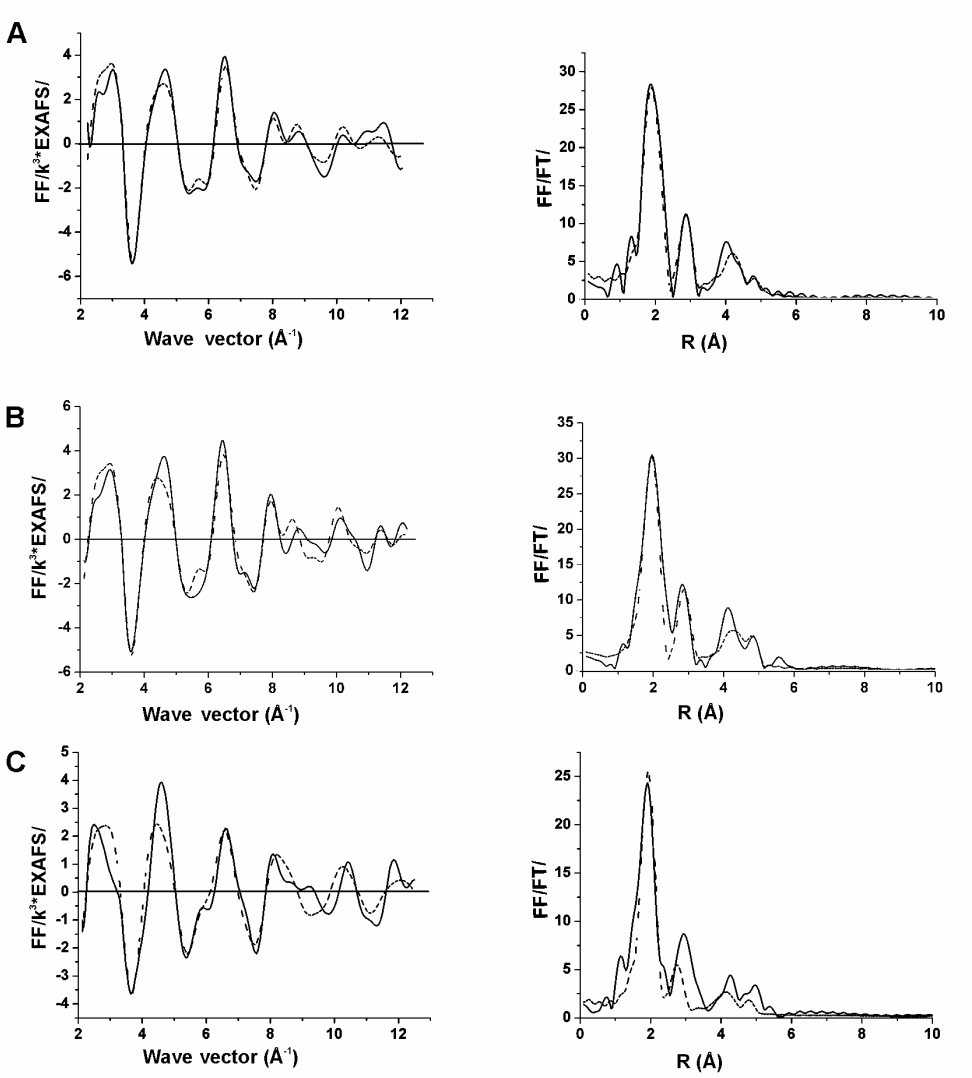


Figure S2.

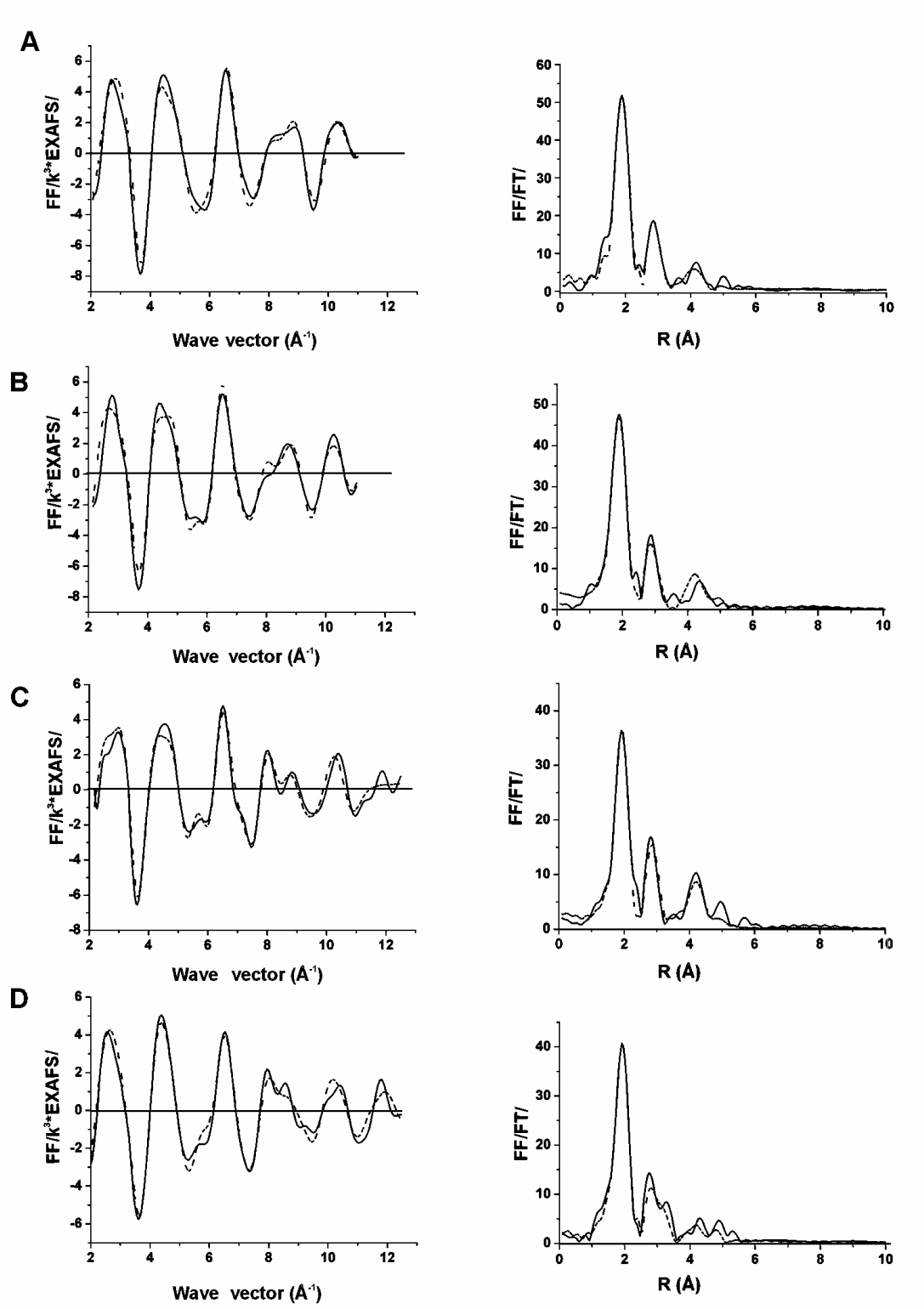
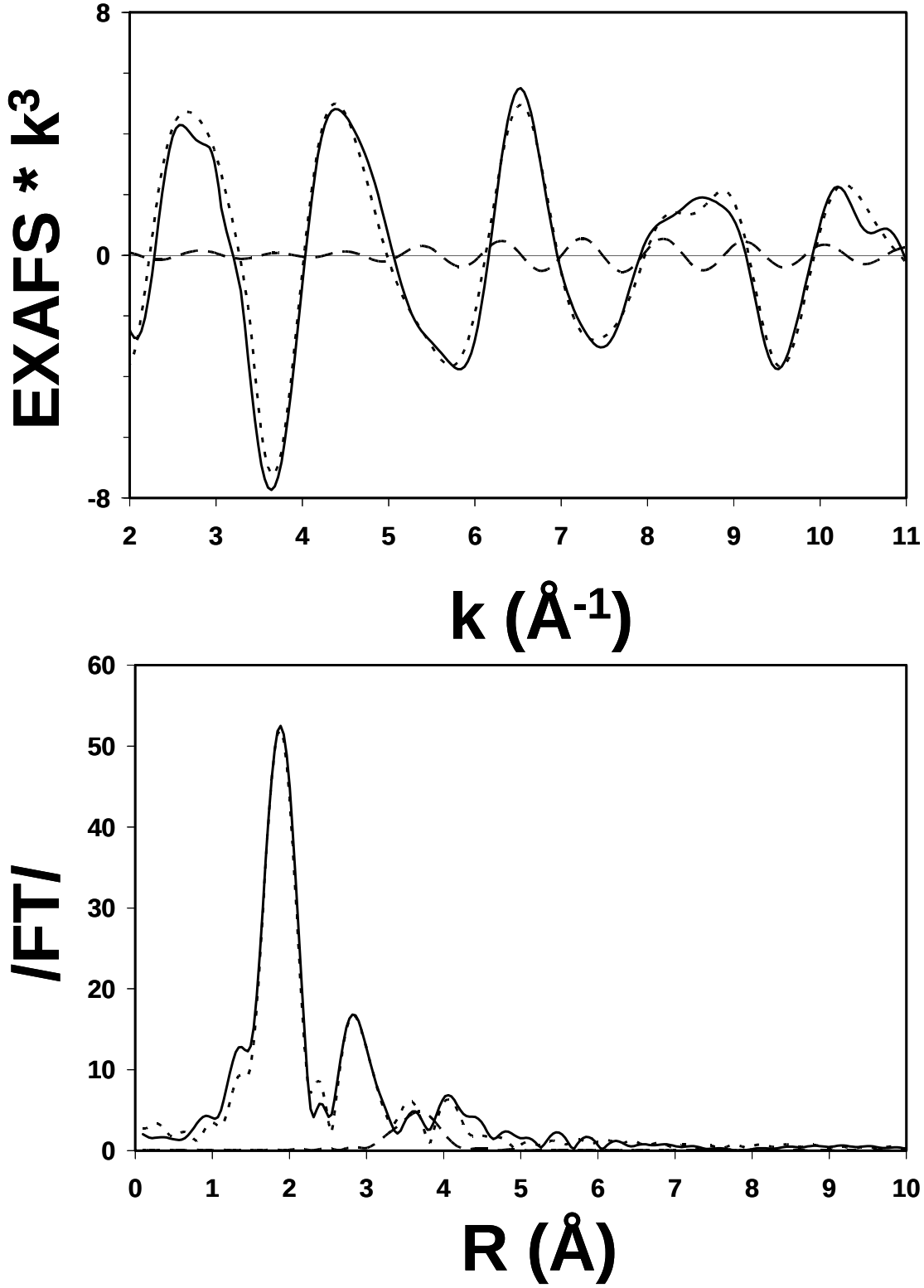


Figure S3.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