Reactivity of Chromium(III) Nutritional Supplements in Biological Media: a X-Ray Absorption Spectroscopic Study

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Supporting Information

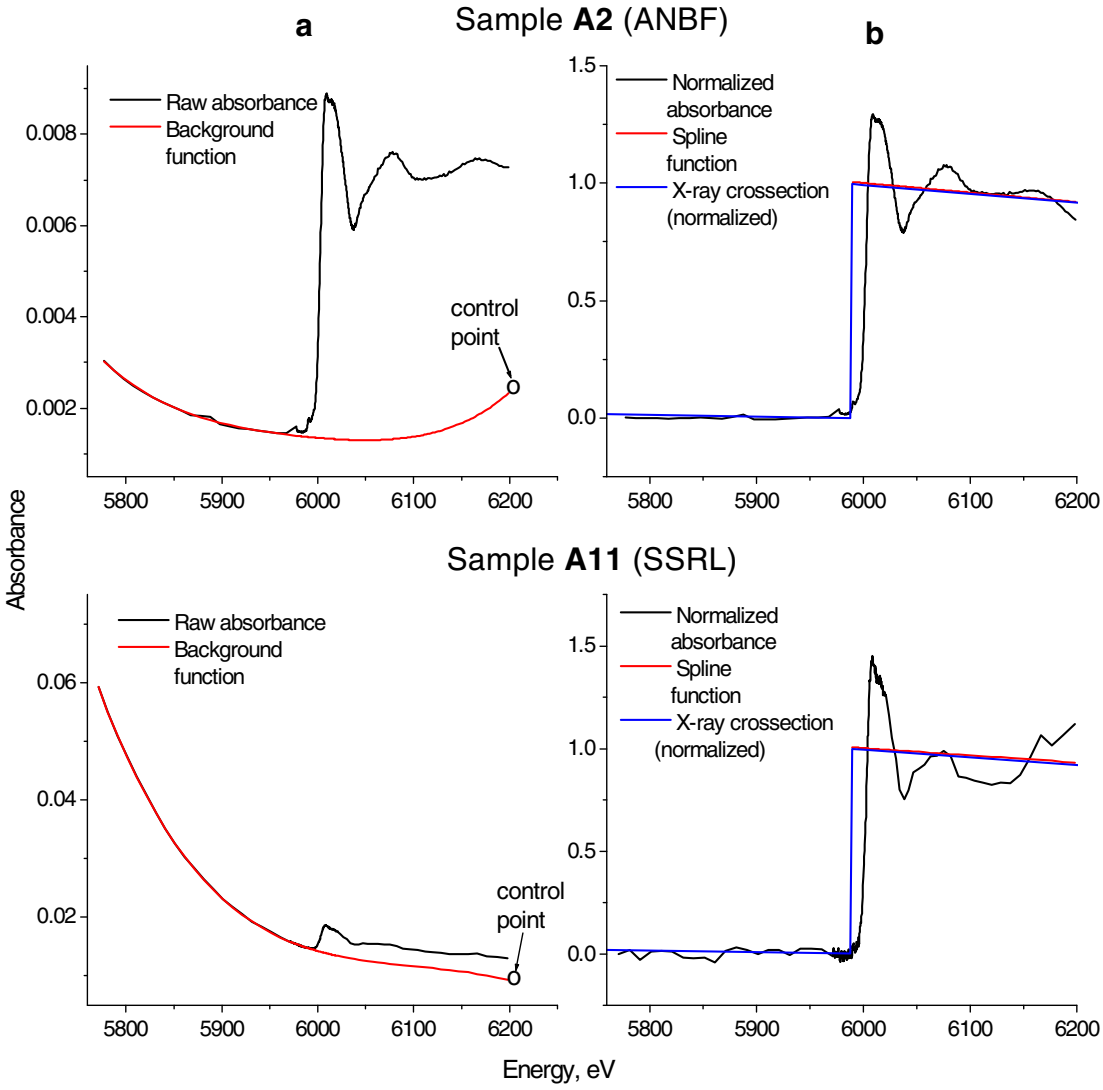


Figure S1. Typical examples of background subtraction and normalization of XANES spectra, including those with strong (e.g., **A2**) and weak (e.g., **A11**) edges, collected at different beamlines (ANBF or SSRL). Designations of the samples correspond to Tables 1-3 (main text). The upper energy limit for data collection was 200 eV above the Cr edge.[33] The normalization algorithm, performed in the Spline program within the XFit software package,[32] included the following steps: (i) the pre-edge background (red line in **a**) was fitted with a fourth-order polynomial; (ii) the spline function (red line in **b**) was set as a first-order polynomial; and (iii) the post-edge part of the background function was adjusted using a control point (**a**), so that the slope of the resultant spline function corresponded to that of the normalized X-ray crossection for Cr (red and blue lines in **b**).[33] The control points are the points with weight 100 (compared to 1 for the absorbance data points and the *k*-weighting for the normalised absorbance points) in the least-squares fitting procedure for the corresponding spline curve. They, therefore, act by strongly 'attracting' the spline curve toward themselves and can be used to influence or control the curve.[32]

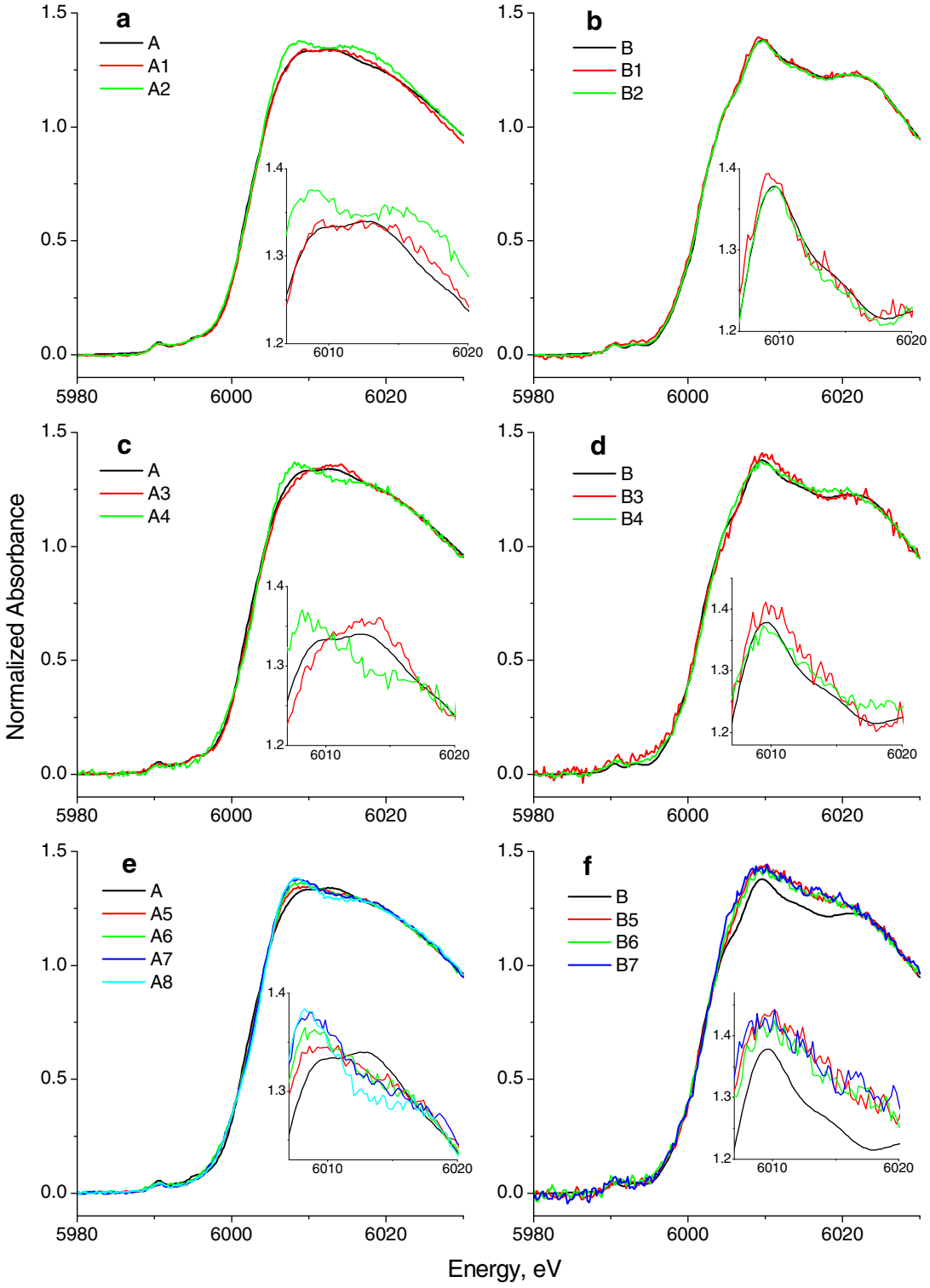


Figure S2. Comparison of XANES spectra (295 K) of Cr(III) complexes **A** and **B** before and after the reactions with artificial digestion systems and blood components. Designations of the samples correspond to Tables 1-3 (main text). The insets are expansions of the main peaks in the XANES.

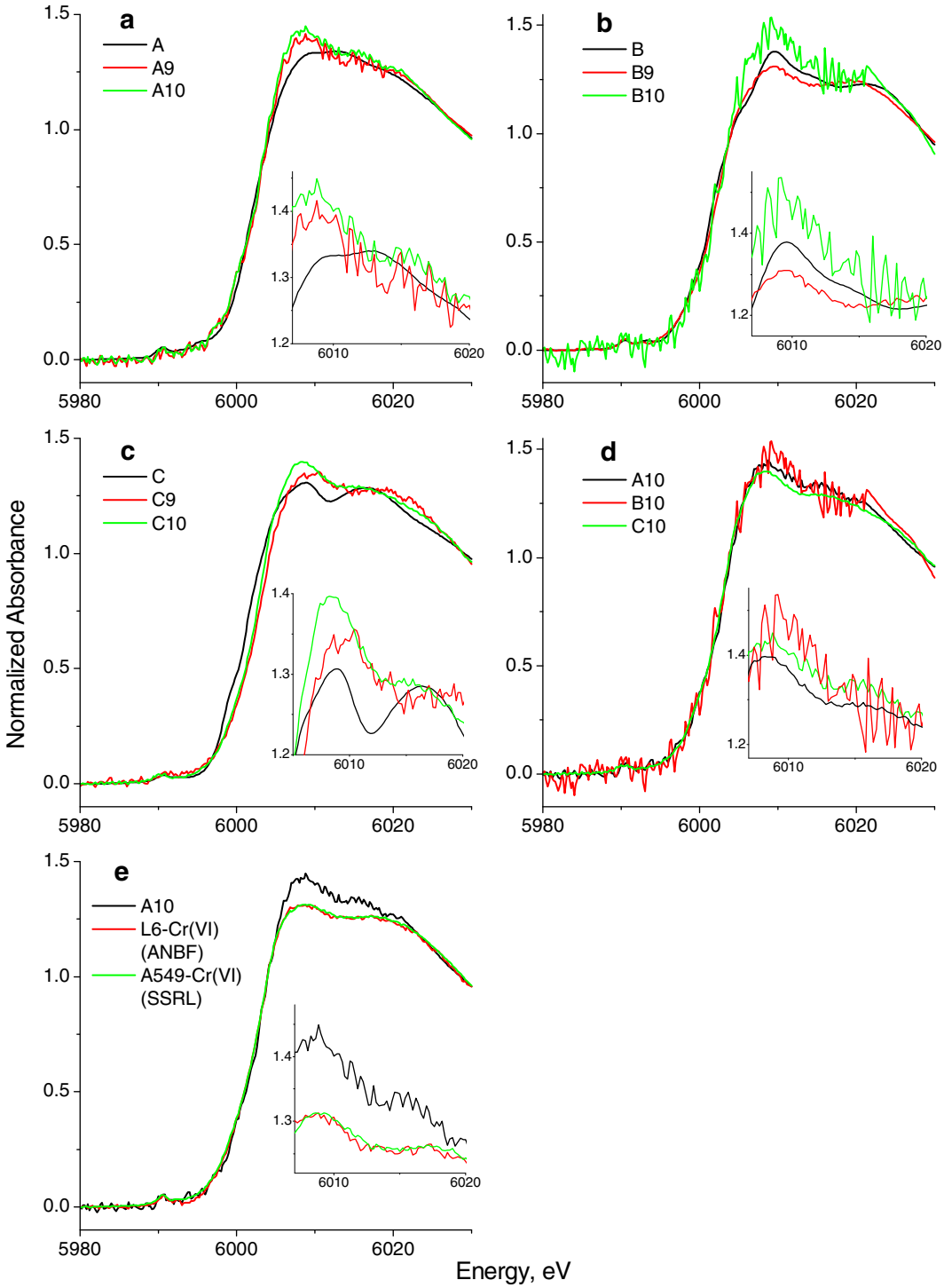


Figure S3. Comparison of XANES spectra (295 K) of Cr(III) complexes **A**-**C** before and after the reactions with cultured mammalian cells or cell culture media. Designations of the samples: L6-Cr(VI) are differentiated rat muscle cells (L6), treated with Cr(VI) (10 μM for 20 h at 310 K); A549-Cr(VI) are human lung carcinoma cells (A549), treated with Cr(VI) (100 μM for 4 h at 310 K; data from Ref. 12); other designations correspond to Tables 1-3 (main text).

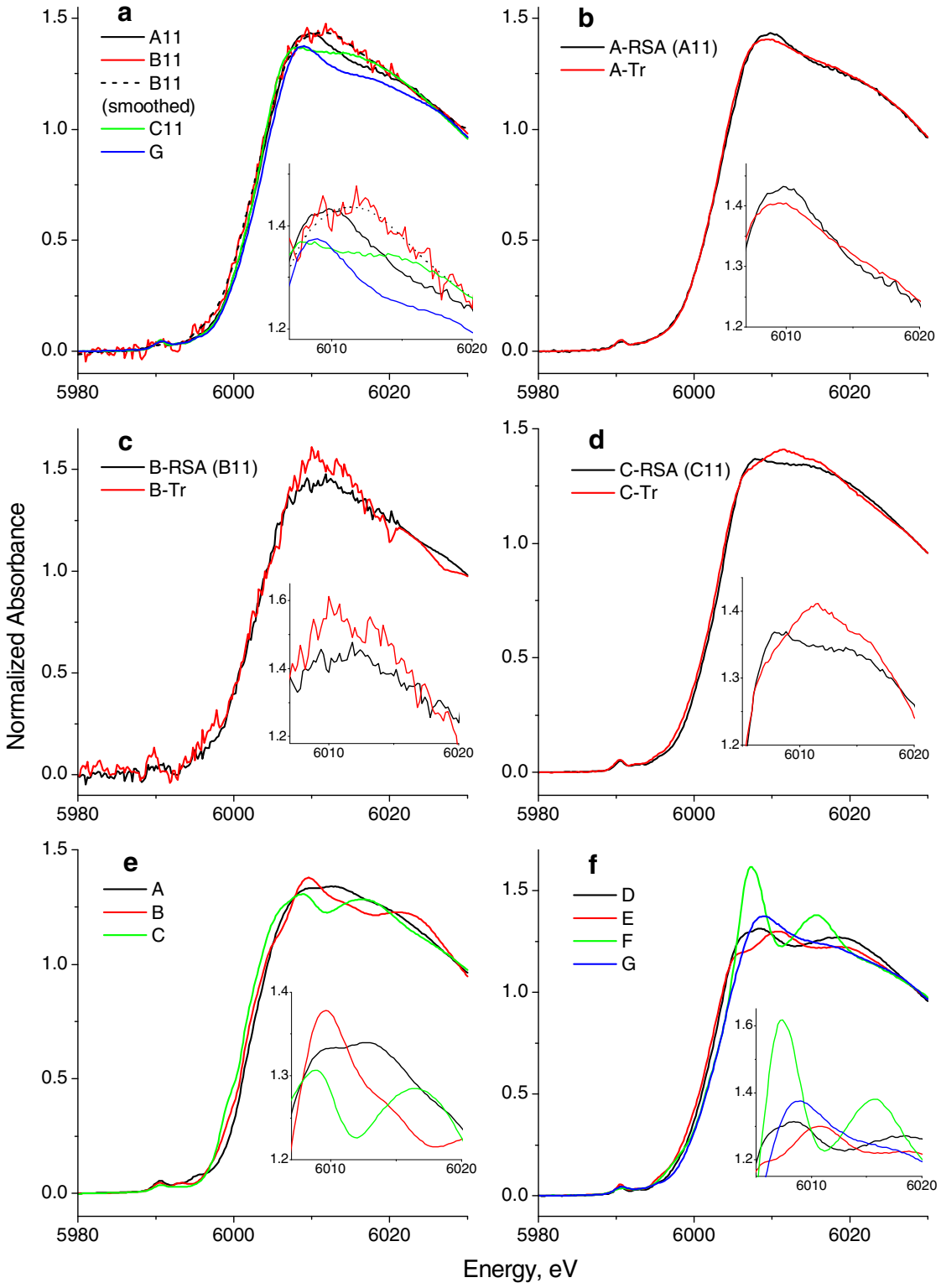


Figure S4. Comparison of XANES spectra (295 K) of Cr(III)-protein adducts (**a**-**d**; RSA is rat serum albumin and Tr is bovine transferrin; see Experimental Section for sample preparation) and of model Cr(III) complexes (**e** and **f**) . Designations of the samples correspond to those given inTables 1-3 (main text).

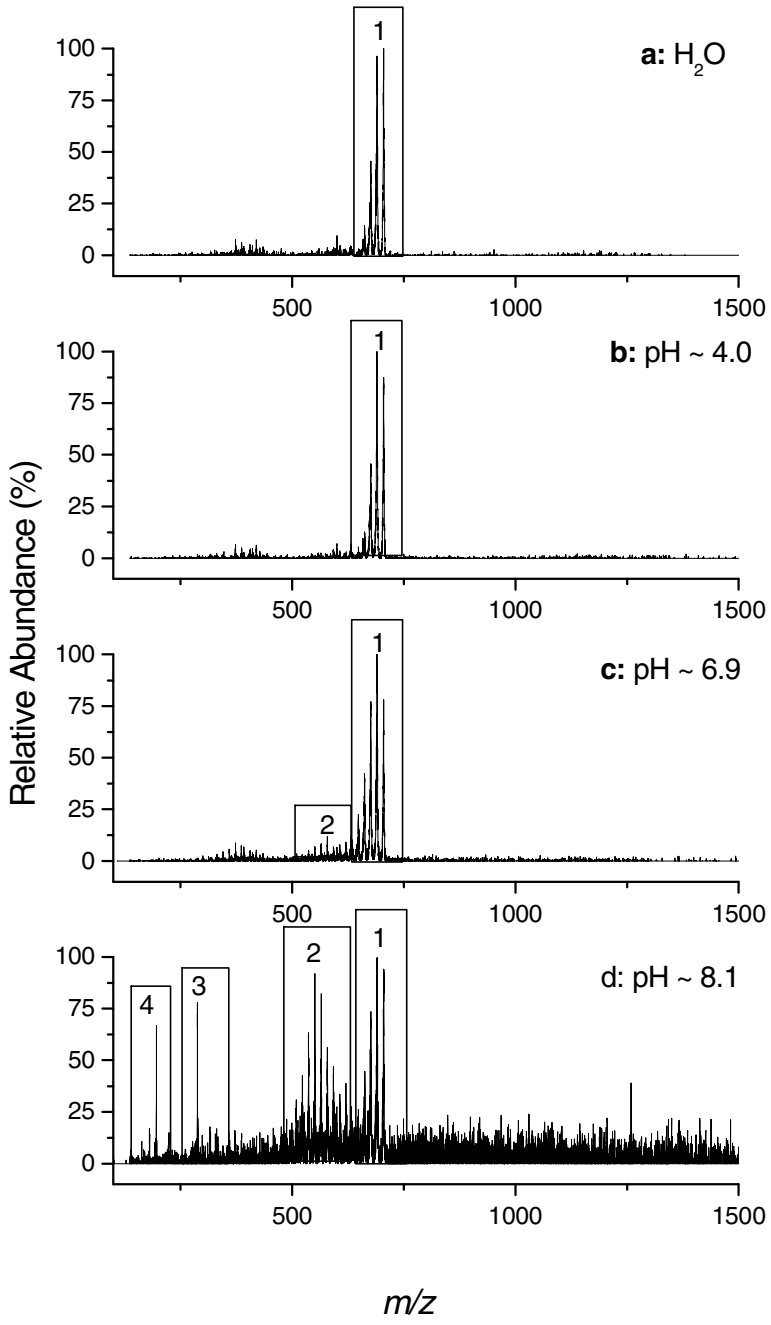


Figure S5. Typical ESMS signals (positive-ion mode) for a freshly prepared aqueous solution of **A** (1.0 mM; **a**) and for the reactions of **A** (1.0 mM) with NH_3/CH_3COOH buffer (10 mM) at various pH values for 32 h at 298 K (**b**-**d**). Assignments of the main signals (1-4) are given in Table S1.

Table S1. Assignment of Major ESMS Signals for the Reactions of **A** with Aqueous Buffer Solutions[a]

Signal	+*m*/*z*	Assignment [b]
1	663	$[Cr_3O(O_2CEt)_6(OH_2)_3]^+$
	677	$[Cr_3O(O_2CEt)_6(OH_2)_2(MeOH)]^+$
	691	$[Cr_3O(O_2CEt)_6(OH_2)(MeOH)_2]^+$
	704	$[Cr_3O(O_2CEt)_6(MeOH)_3]^+$
2	509	$[Cr_3O(O_2CEt)_3(OH)_3(OH_2)_3]^+$
	523	$[Cr_3O(O_2CEt)_3(OH)_3(OH_2)_2(MeOH)]^+$
	537	$[Cr_3O(O_2CEt)_3(OH)_3(OH_2)(MeOH)_2]^+$
	551	$[Cr_3O(O_2CEt)_4(OH)_2(OH_2)_3]^+$
	565	$[Cr_3O(O_2CEt)_4(OH)_2(OH_2)_2(MeOH)]^+$
	578	$[Cr_3O(O_2CEt)_4(OH)_2(OH_2)(MeOH)_2]^+$
	592	$[Cr_3O(O_2CEt)_4(OH)_2(MeOH)_3]^+$
3	288	$[Cr(O_2CEt)_3]NH_4^+$
4	196	$[Cr_2(OH)_2(O_2CEt)_2(OH_2)_6]^{2+}$

[a] Reactions of **A** (1.0 mM) with NH_3/CH_3COOH buffers (10 mM) at pH = 4.0, 6.9 or 8.1 (see Figure S5) for 32 h at 298 K. Designations of the signals (1-4) correspond to Figure S5. [b] MeOH ligands originate from the flushing solution used for ESMS (H_2O:MeOH = 1:1).

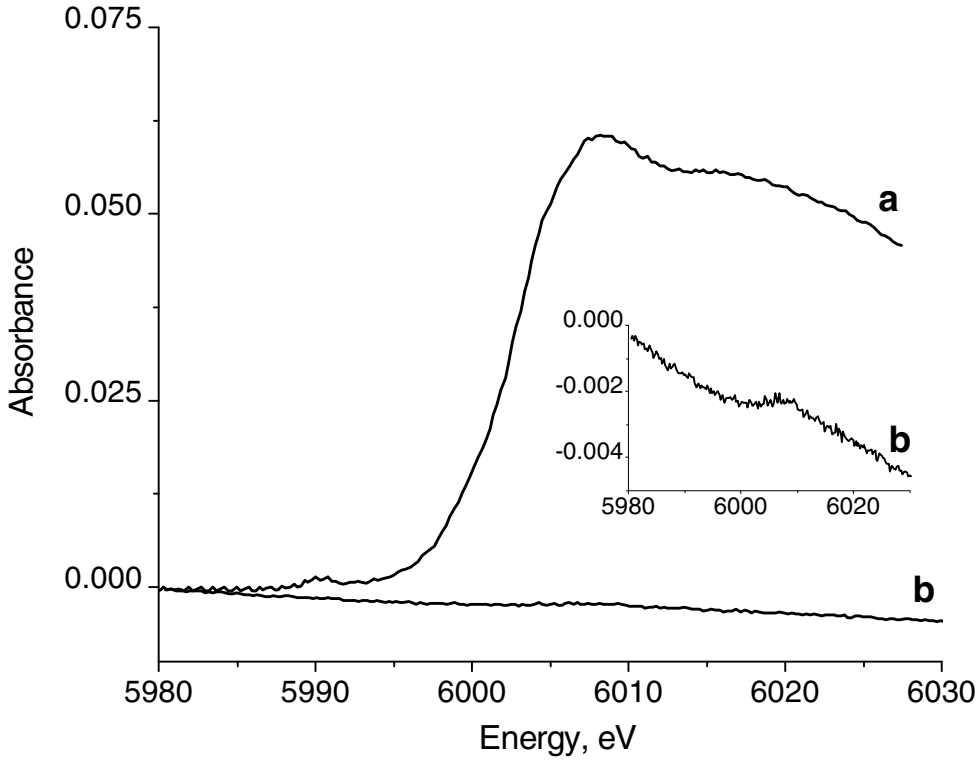


Figure S6. Cr K-edge XANES spectra (raw absorbance data, freeze-dried solids, 295 K) for cell pellets collected after the treatments of L6 cells with **C** (0.10 mM for 20 h at 310 K). Complex **C** was added to the cell culture medium either immediately (**a**) or 24 h prior to (**b**) the treatment of the cells. Conditions of sample preparation were identical for the both samples. Spectrum (**b**) was multiplied by a factor of 10, to compensate for the difference in ion chamber gain.

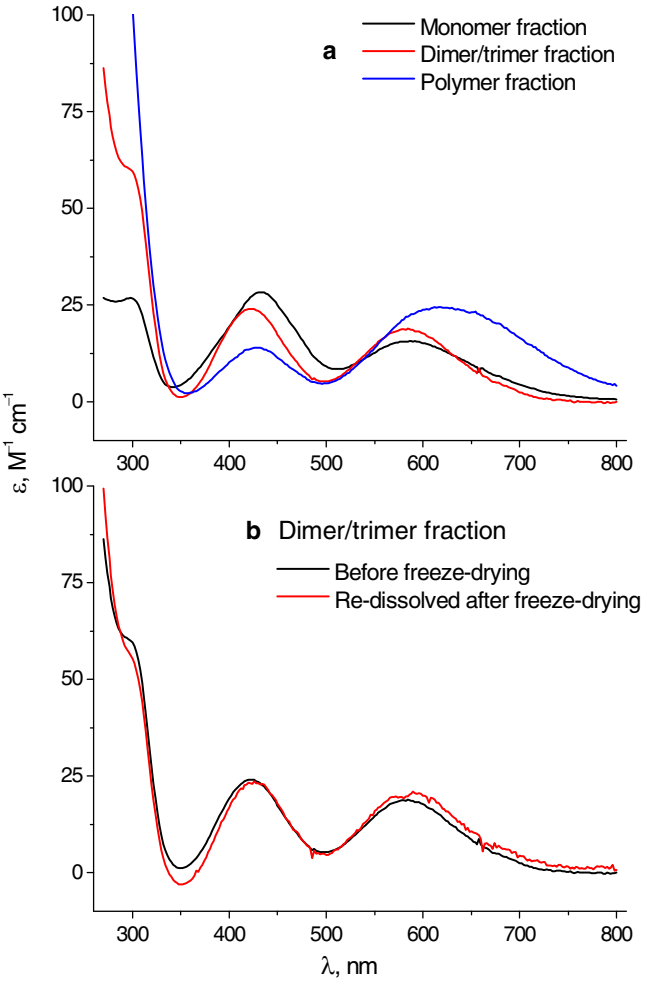


Figure S7. Electronic absorption spectra of Cr(III) aqua-hydroxo complexes:[23] (**a**) chromatographic fractions after the reaction of $[Cr(OH_2)_6]^{3+}$ (0.10 M) with NaOH (0.10 M) for 24 h at 298 K, followed by separation on Sephadex G25 gel filtration column (eluted with H_2O); and (**b**) comparison of spectra of the main chromatographic fraction immediately after the separation (black line) and after freeze-drying and re-dissolving in H_2O (red line). The extinction coefficients (ε, M^{-1} cm^{-1}) were calculated based on Cr concentrations in solutions, determined by AAS.

Table S2. Characteristic Features in the Electronic Absorption Spectra of Cr(III)-OH-OH_2 Complexes

Sample	**Max** [a]	**LW** [b]	**Min** [a]	**Max** [a]	**LW** [b]	**Min** [a]
Monomer (deprot.) [c]	586 (16)	159	511 (8.5)	432 (28)	97	342 (3.7)
Monomer (deprot.) [d]	590 (16)	ND[e]	ND[e]	430 (28)	ND[e]	ND[e]
Dimer/trimer [c]	584 (19)	118	498 (5.3)	422 (24)	79	350 (1.3)
Dimer/trimer [c,f]	590 (20)	125	498 (4.9)	424 (23)	78	350 (0)
Dimer [d]	582 (17)	147	490 (5.2)	417 (20)	78	345 (1.5)
Trimer [d]	584 (19)	121	499 (5.5)	425 (30)	80	346 (2.1)
Tetramer [d]	580 (16)	115	500 (5.6)	426 (30)	80	347 (1.9)
Hexamer [d]	585 (19)	129	503 (6.1)	426 (29)	82	353 (2.4)
Polymers [c]	619 (24)	182	495 (4.8)	428 (14)	87	355 (2.3)

[a] Wavelength in nm; extinction coefficients (M^{-1} cm^{-1}, per Cr ion) are given in parentheses. [b] Line width at half-height in nm. [c] Data from this work; samples obtained from the reaction of $[Cr(OH_2)]_6]^{3+}$ (0.10 M) with NaOH (0.10 M), followed by separation of the products on a Sephadex G-25 column (see Figure S5 and the Experimental Section in the main text). [d] Data from Ref. 23. [e] Data not available. [f] Sample re-dissolved after freeze-drying.

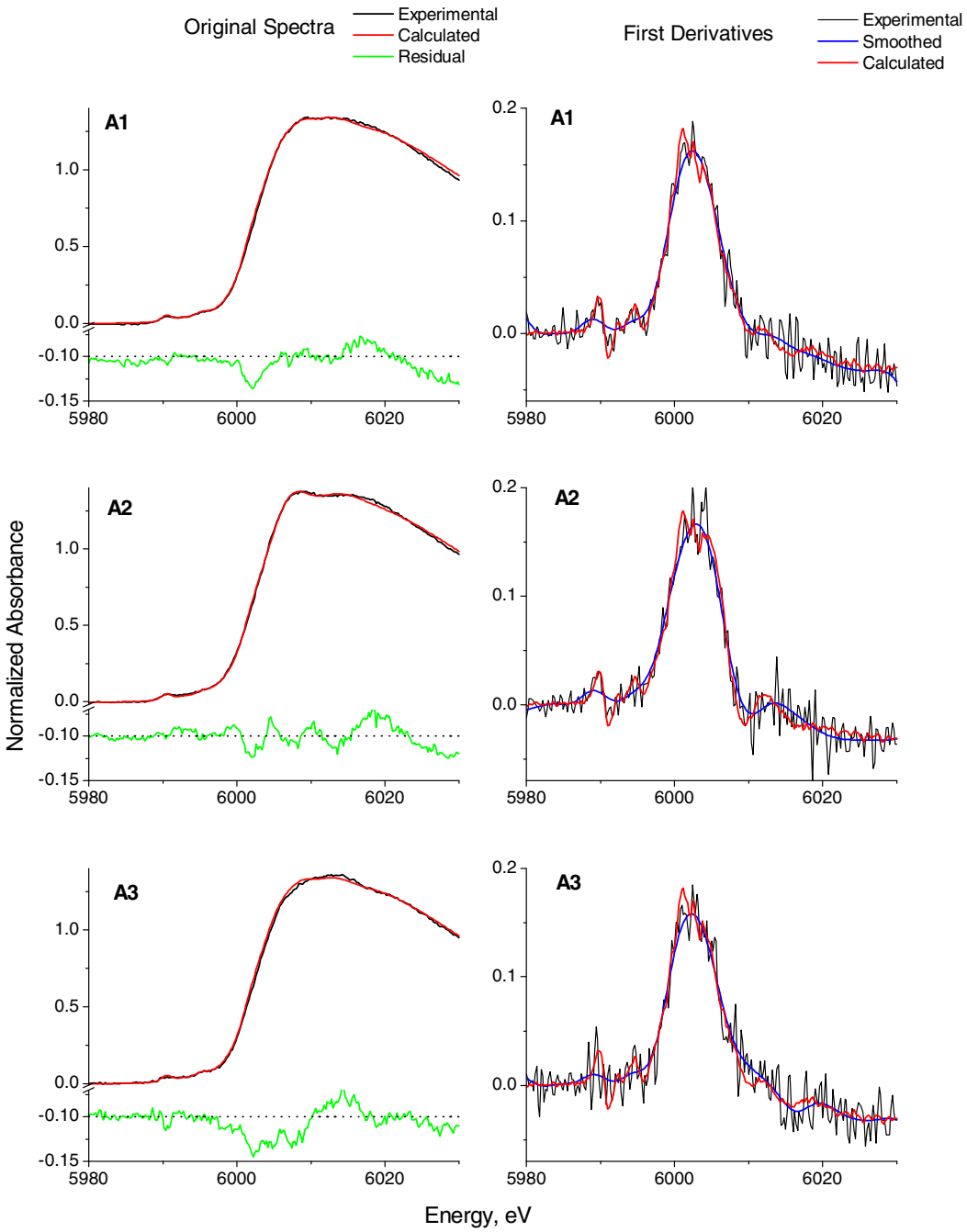


Figure S8. Comparison of experimental and calculated (based on the models listed in Table 3, main text) XANES spectra of biotransformation products of Cr(III) complexes **A**-**C**. Designations of the samples correspond to Tables 1-3, main text. Smoothed first-derivative spectra (blue lines) were obtained by the FFT procedure with 20-point window[35] (see the Experimental Section). The figure is continued on the next six pages. The data for the sample **A10** are shown in Figure 2, main text.

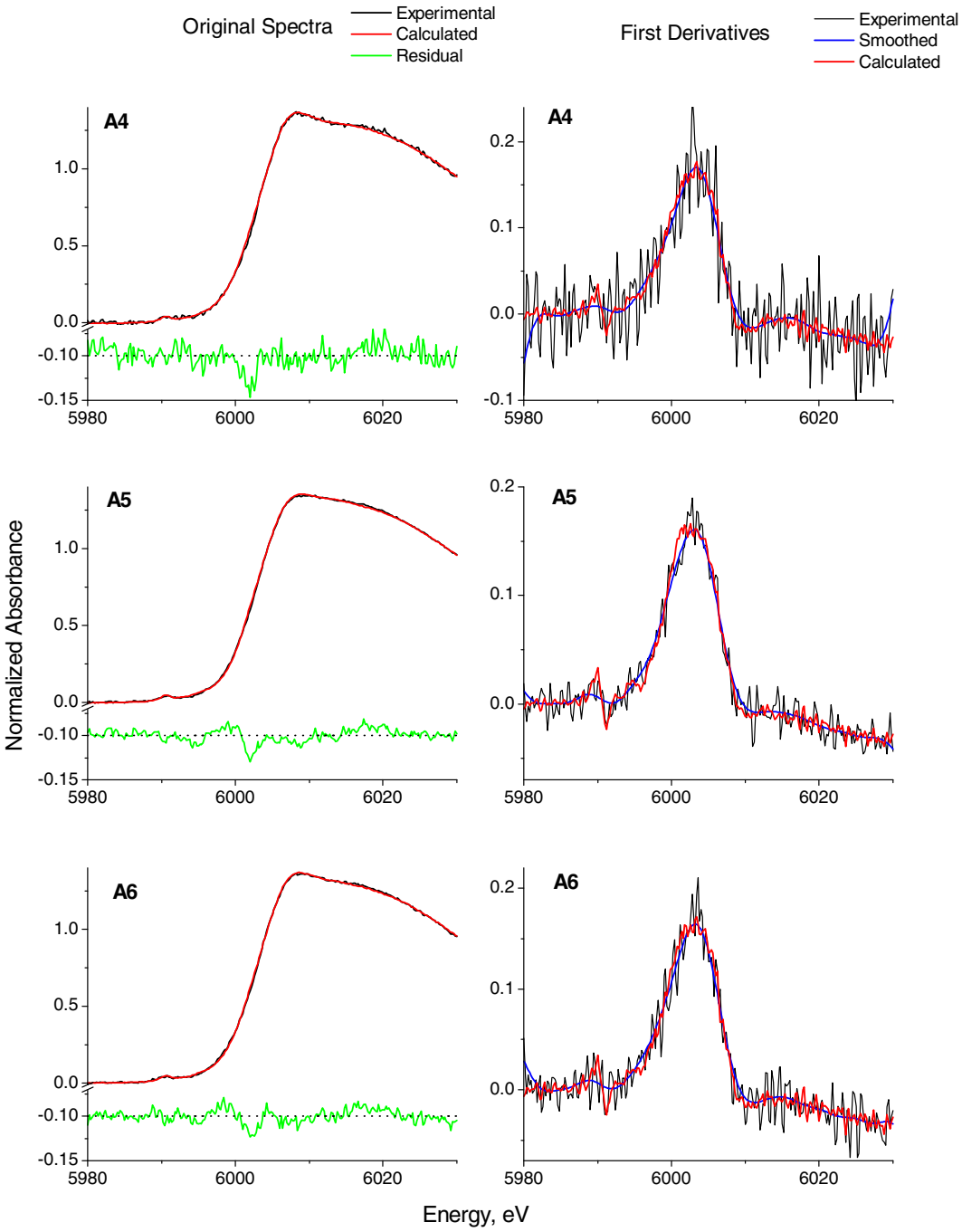
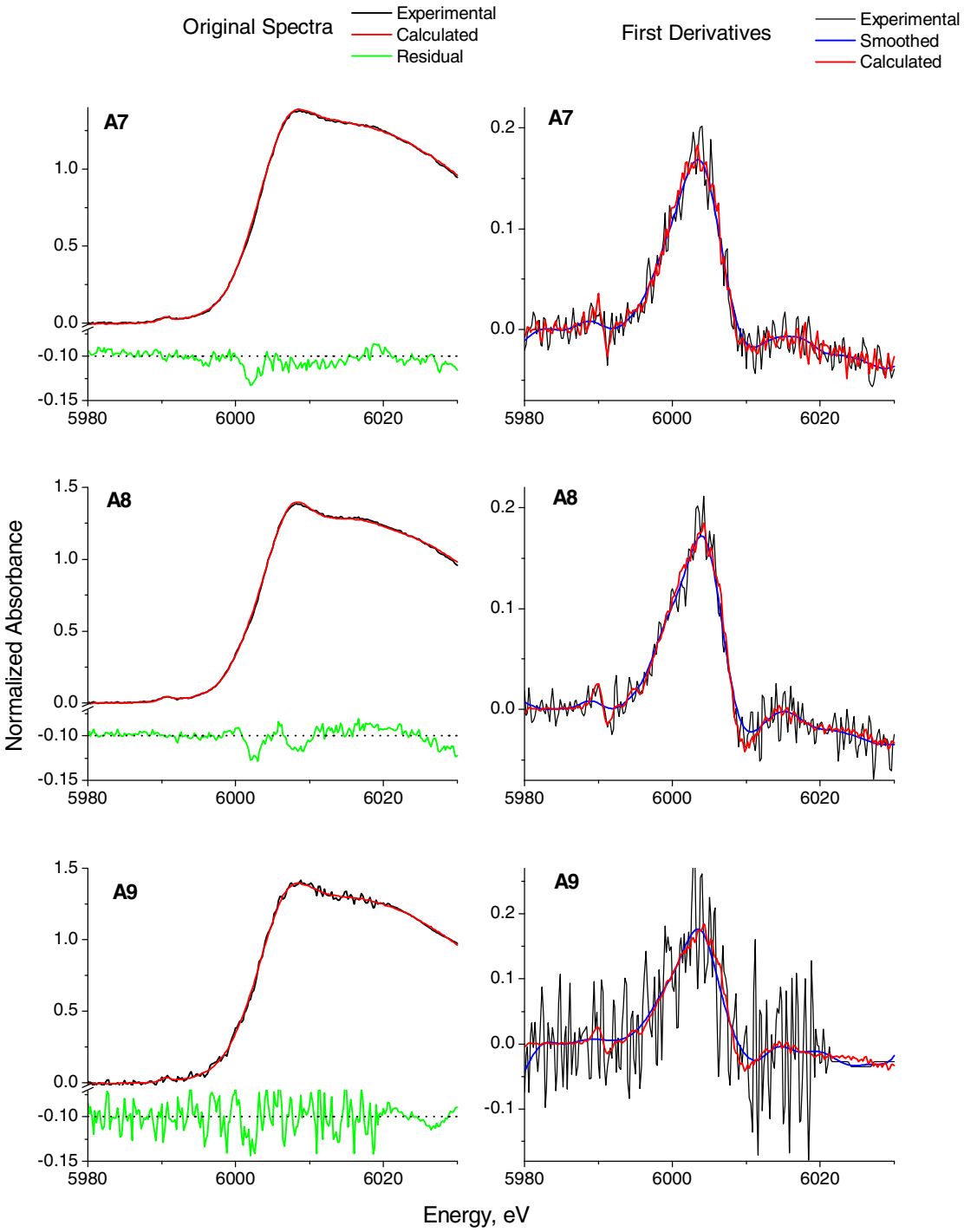


Figure S8 (continued)**.**



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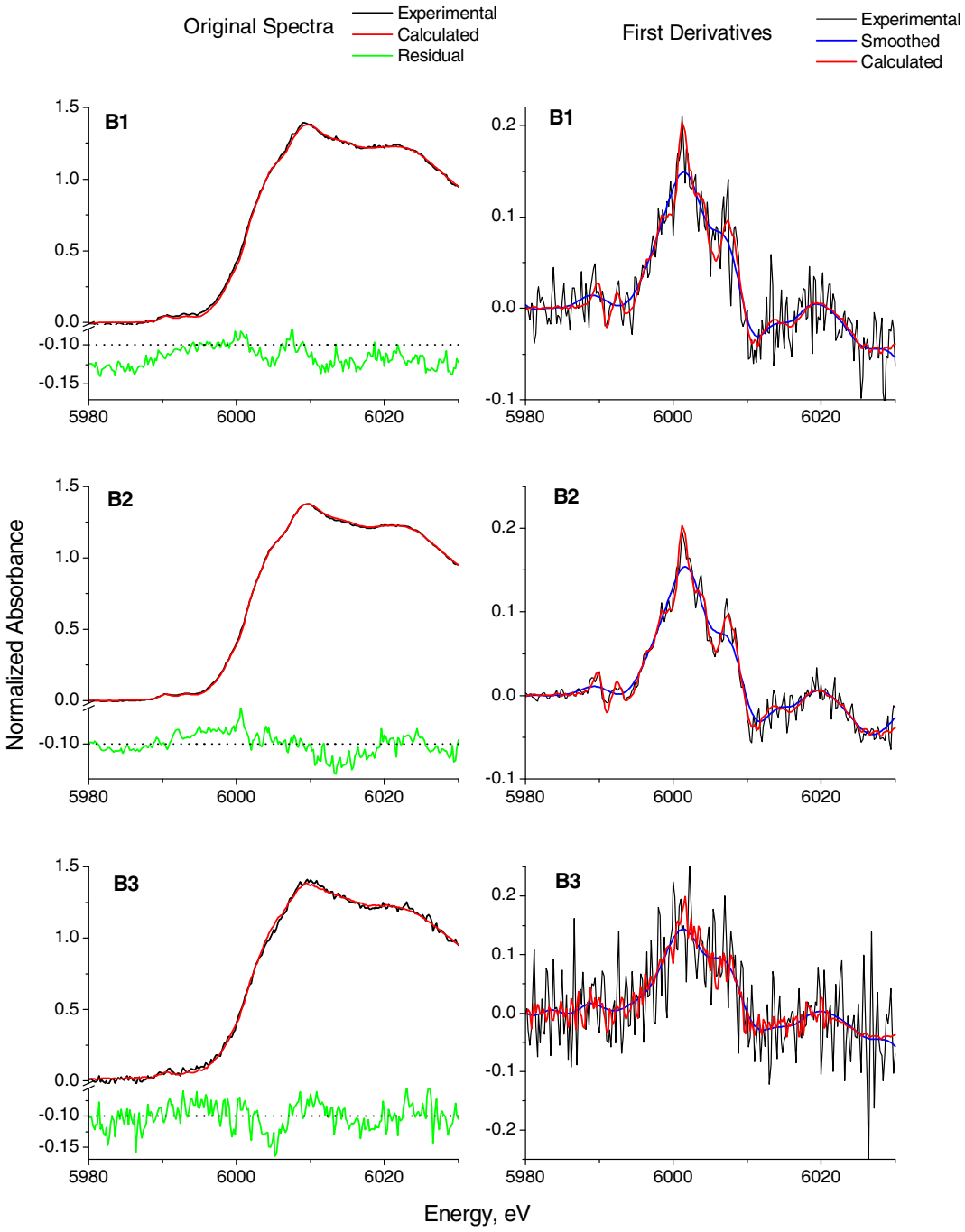

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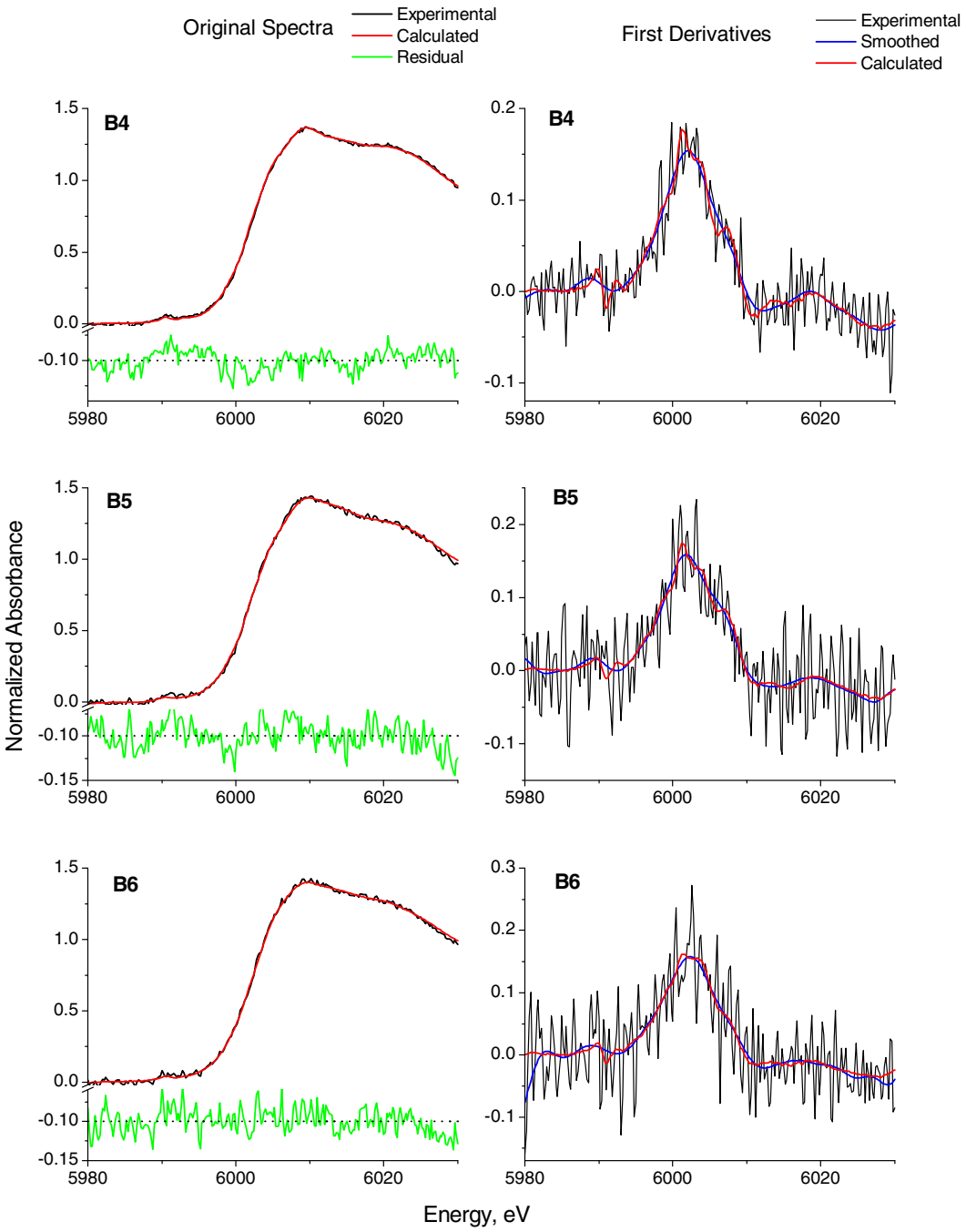

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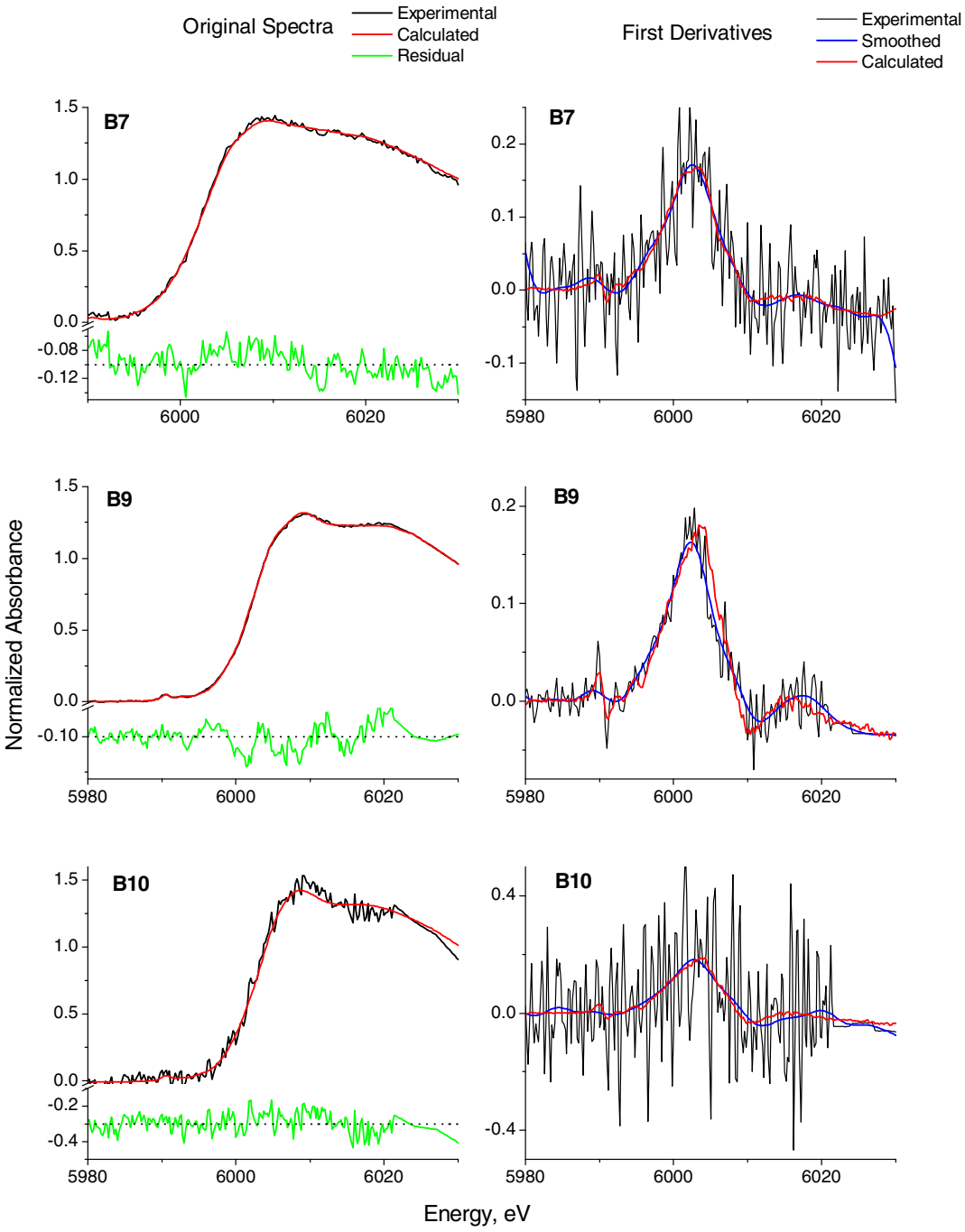

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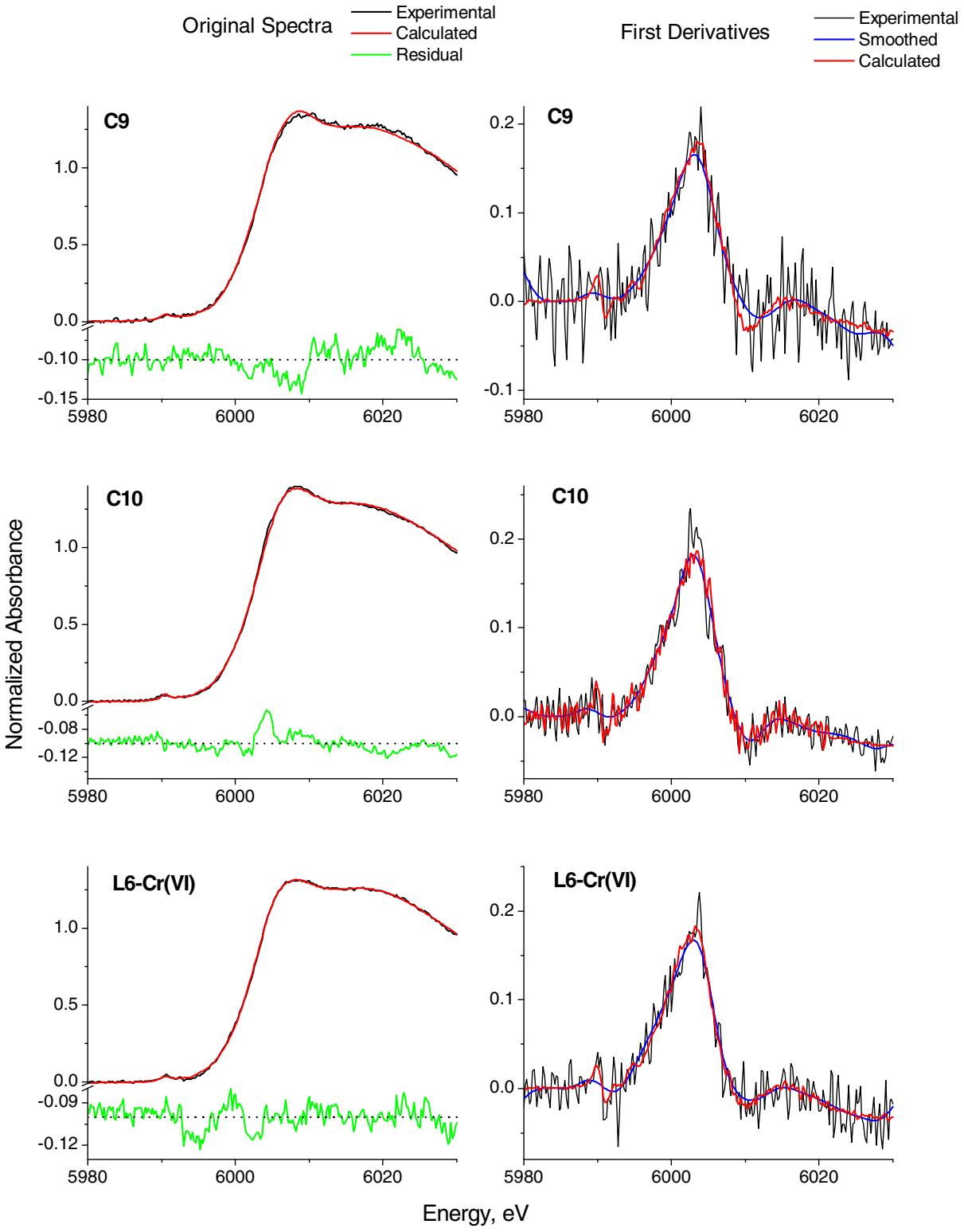

Figure S8 (end).

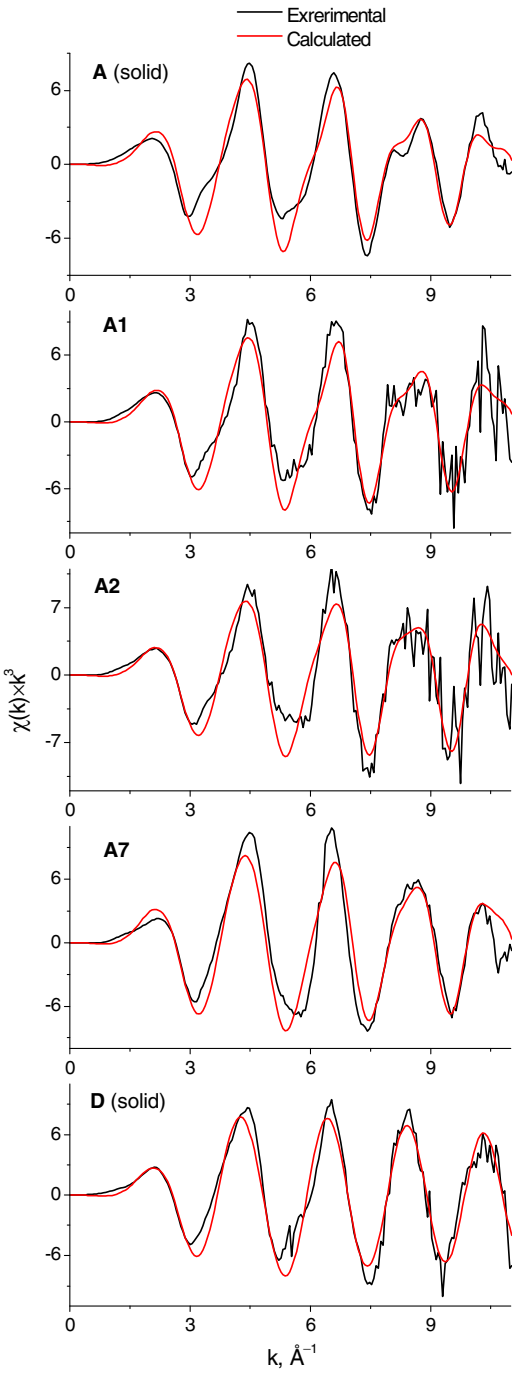

Figure S9. Experimental and fitted XAFS spectra (295 K) of **A** and **D** (solid mixtures with BN) and reaction products of **A** in biological media (**A1**, **A2** and **A7**, freeze-dried solids). Designations of the samples correspond to Tables 1-3 (main text). Details of the fits are given in Table S3.

Table S3. Summary of SS XAFS Fitting Results[a]

Parameter [b]	**A** (solid)	**A1**	**A2**	**A7**	**D**
k range, Å^{-1}			1-11		
FT range, Å			0.5-5.0		
N_i/p			4.5		
R, %	30.0	29.1	31.6	30.7	25.2
$-\Delta E_0$, eV	3.6(3)	3.6(3)	3.6(3)	3.6(3)	3.6(3)
S_0^2 [c]	0.80(1)	0.81(7)	0.81(7)	0.84(7)	0.81(7)
			Shell 1: Cr–O/N		
N [d]	6	6	6	6	6
X, Å	1.96(1)	1.96(1)	1.96(1)	1.96(1)	1.98(1)
σ^2, [e] Å^2	0.0050(1)	0.0036(4)	0.0020(5)	0.0022(9)	0.0015(2)
			Shell 2: Cr–Cr		
N	1.9 ± 0.3	2.3 ± 1.0	1.5 ± 0.7	0.7 ± 0.3	--
X, Å	3.24(2)	3.24(2)	3.15(2)	3.10(2)	--
σ^2, [e] Å^2	0.0048(6)	0.0043(5)	0.0055(4)	0.0060(6)	--

[a] Designations of the samples correspond to Tables 1-3 (main text). Errors in the last significant figures (arising from the noise in the data, as calculated by Monte-Carlo analysis) are shown in parentheses. [b] Designations of the parameters: N_i/p is the determinancy factor (were N_i is the number of independent observations and p is the number of varied parameters);[32] R is the goodness-of-fit parameter;[32] $\Delta E_0 = E_0 - 6005$ (eV) is the threshold energy; S_0^2 is the scale factor; N are the numbers of donor atoms in each shell; X (Å) are the average absorber-scatterer distances; and σ^2 (Å^2) are the Debye-Waller factors. [c] Restrained to be within the 0.80-1.0 range.[32] [d] The values were not varied during the optimization. [e] Restrained to be within the 0.0005-0.02 Å^2 range.

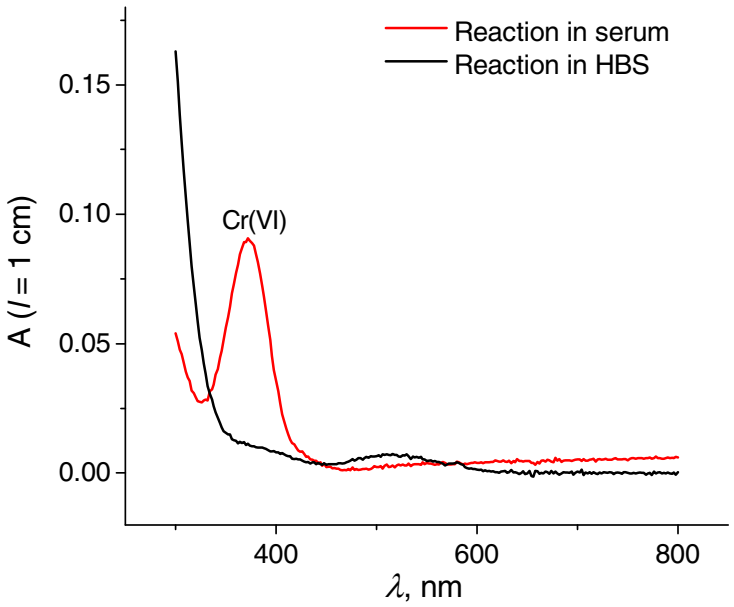

Figure S10. Typical electronic absorption spectra of the reaction mixtures containing **B** (1.0 mM) and H_2O_2 (5.0 mM) in undiluted rat serum or HEPES-buffered saline (HBS, pH = 7.4) after 1 h of reaction at 310 K. The reaction mixtures were diluted 10-fold with H_2O prior to the spectral measurement.