Electronic Supplementary Material (ESI) for Dalton Transactions.

Selective hydrolysis of oxidized insulin chain B by a Zr(IV)-substituted Wells-Dawson polyoxometalate

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Supporting Information

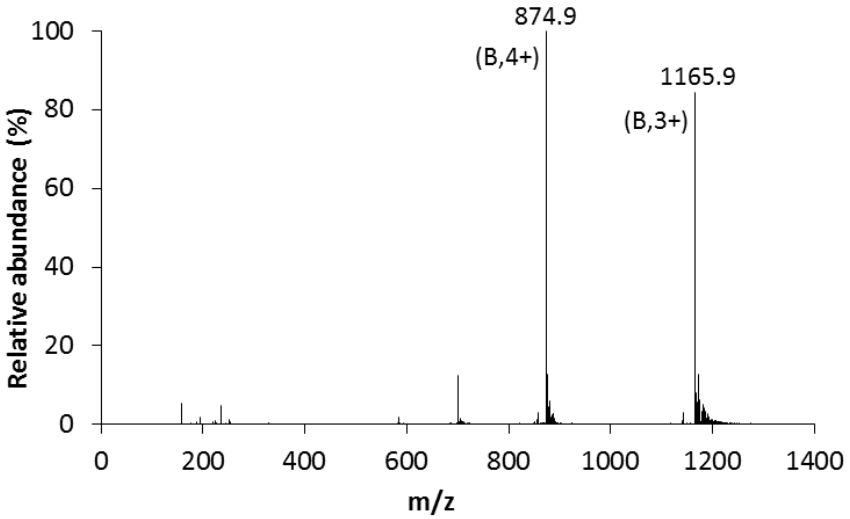

Figure S1: HPLC-ESI-MS spectrum of oxidized insulin chain B (full parent peptide B) with an HPLC elution time of 47.4 min. Both the triply (1165.9) and quarterly (874.9) charged mass of the parent peptide are shown.

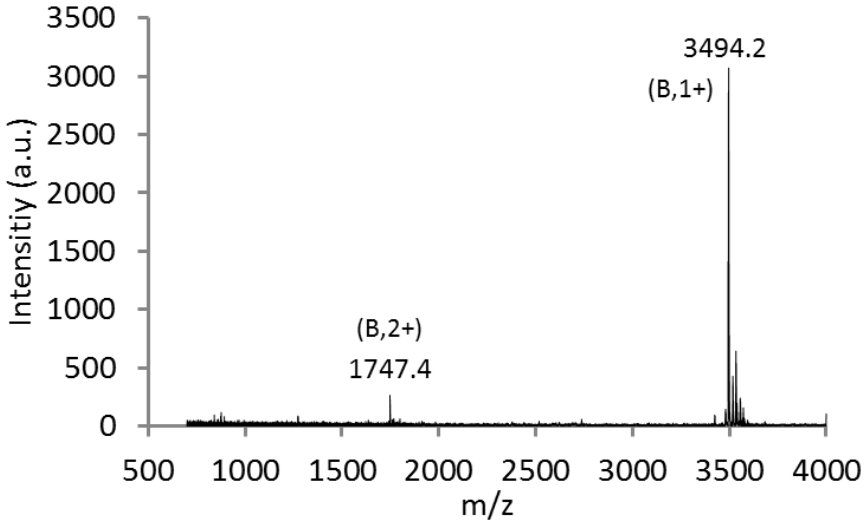

Figure S2: MALDI-TOF spectrum of oxidized insulin chain B (full parent peptide B) showing both the singly charged (3494.2) and doubly (1747.4) charged mass of the parent peptide.

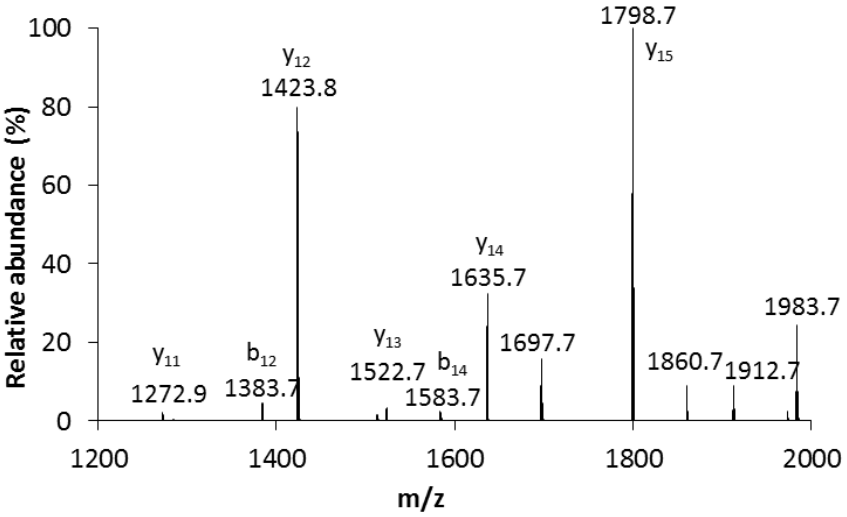

Figure S3: LC-MS/MS spectrum of oxidized insulin chain B. Both *N*-terminal b-ions and *C*-terminal y-ions are shown.

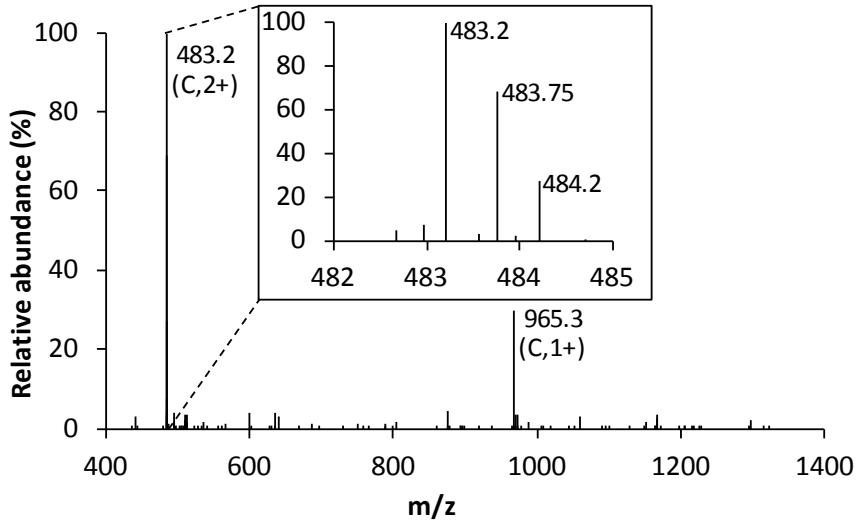

Figure S4: HPLC-ESI-MS spectrum of peptide fragment C, *Phe1 to Gly8*, with an HPLC elution time of 19.2 min. Both the doubly charged (483.2) and singly charged (965.3) mass corresponding to this hydrolysis fragment are shown. The corresponding isotopic pattern of the doubly charged fragment is shown as an inset.

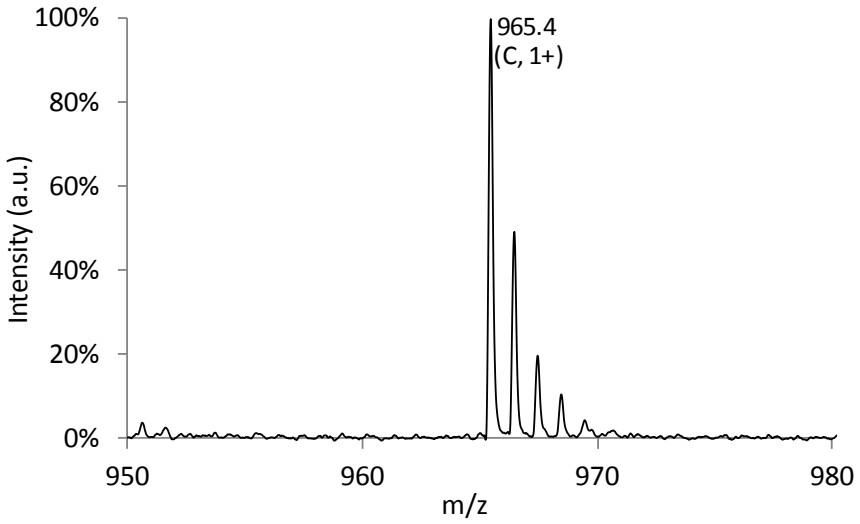

Figure S5: MALDI-TOF spectrum of singly charged peptide fragment C, *Phe1 to Gly8* (965.4) with its corresponding isotopic pattern.

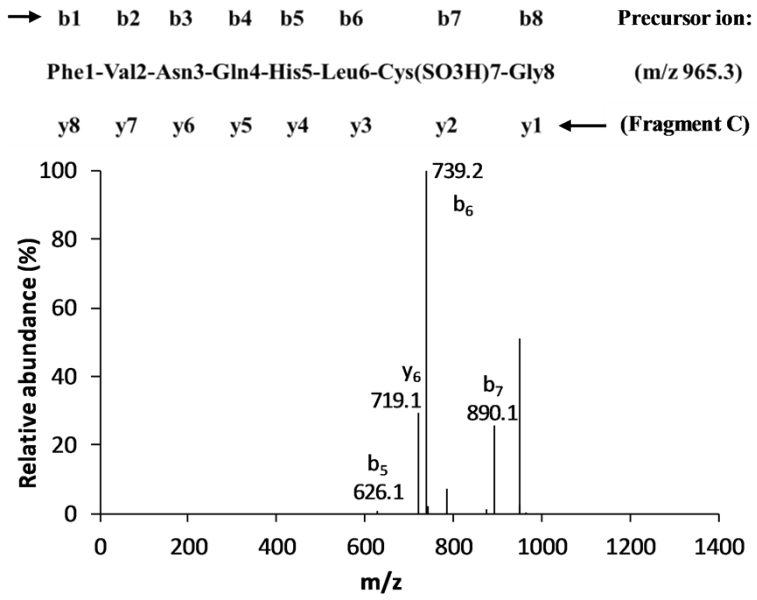

Figure S6: LC-MS/MS spectrum generated from the peptide fragment *Phe1 to Gly8*. Both *N*-terminal b-ions and *C*-terminal y-ions are shown.

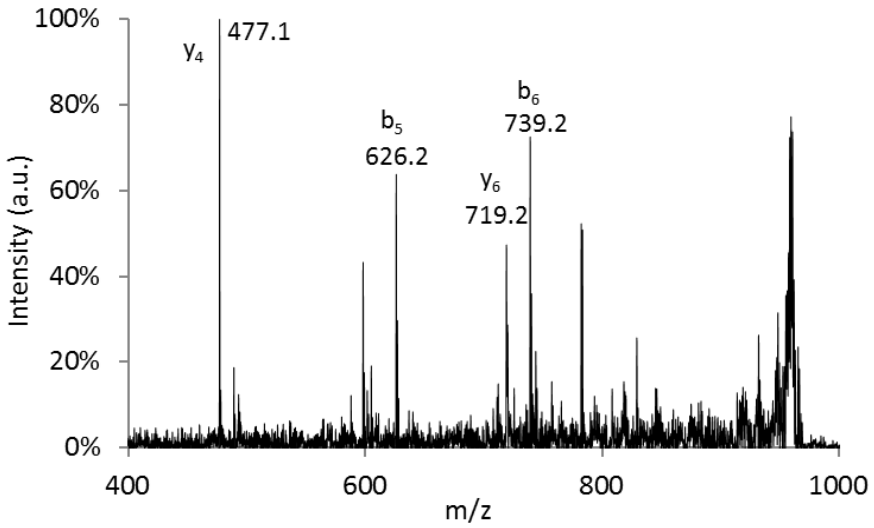

Figure S7: MALDI-TOF MS/MS generated from the peptide fragment *Phe1 to Gly8*. Both *N*-terminal b-ions and *C*-terminal y-ions are shown.

Table S1: MS/MS data from LC-MS/MS and MALDI-TOF MS/MS of the peptide fragment, *Phe1 to Gly8*, showing both the *N*-terminal b-ions and *C*-terminal y-ions.

Experimental m/z value	Theoretical m/z value	Peptide fragment	Charge	Type of ion
477.1	477.2	His5 to Gly8	1+	y_4 ion
626.2	626.3	Phe1 to His5	1+	b_5 ion
719.2	719.3	Asn3 to Gly8	1+	y_6 ion
739.2	739.4	Phe1 to Leu6	1+	b_6 ion
890.1	890.4	Phe1 to Cys(SO_3H)7	1+	b_7 ion
947.1	947.4	Phe1 to Gly8	1+	b_8 ion

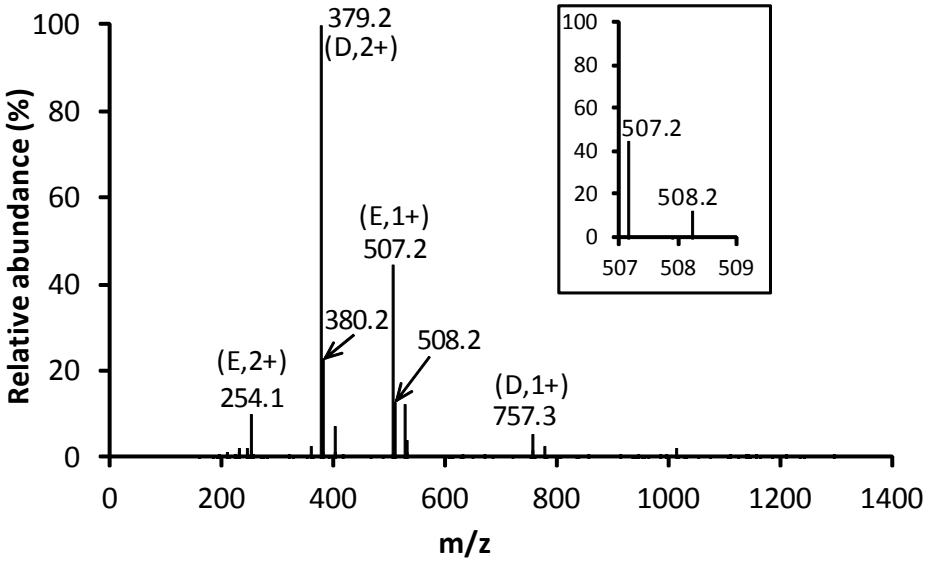

Figure S8: HPLC-ESI-MS spectrum of peptide fragments D and E, *Phe1 to Leu6* and *Phe1 to Gln4 respectively*, with an HPLC elution time of 13.4 min. Both the doubly charged (379.2) and singly charged (757.3) mass corresponding to hydrolysis fragment *Phe1 to Leu6* are shown. Both the singly charged (507.2) and doubly charged (254.1) mass corresponding to hydrolysis fragment *Phe1 to Gln4* are shown.

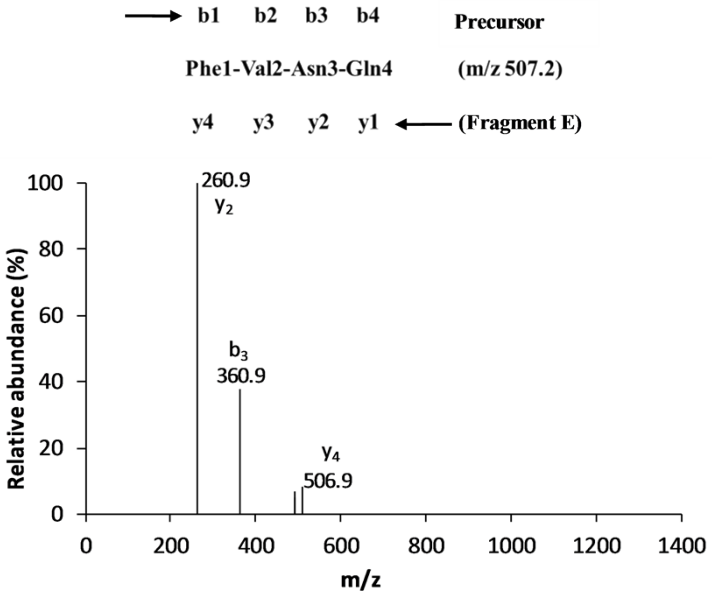

Figure S9: LC-MS/MS spectrum generated from the peptide fragment *Phe1 to Gln4*. Both *N*-terminal b-ions and *C*-terminal y-ions are shown.

Table S2: MS/MS data of the peptide fragment, *Phe1 to Gln4*, showing both the *N*-terminal b-ions and *C*-terminal y-ions.

Experimental m/z value	Theoretical m/z value	Peptide fragment	Charge	Type of ion
260.9	261.1	Asn3 to Gln4	1+	y_2 ion
360.9	361.2	Phe1 to Asn3	1+	b_3 ion
506.9	507.2	Phe1 to Gln4	1+	y_4 ion

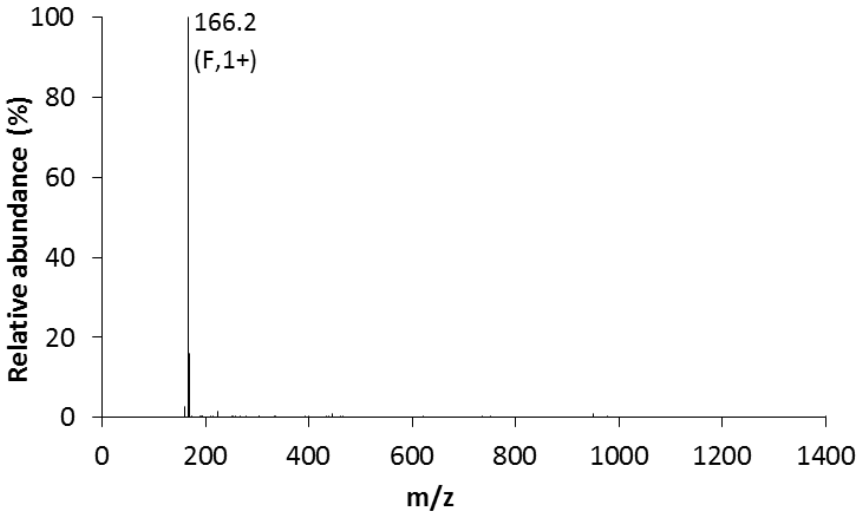

Figure S10: HPLC-ESI-MS spectrum of the terminal amino acid F, *Phe1*, with an elution time of 11.5 min. The singly charged (166.2) mass corresponding to *Phe1* is shown.

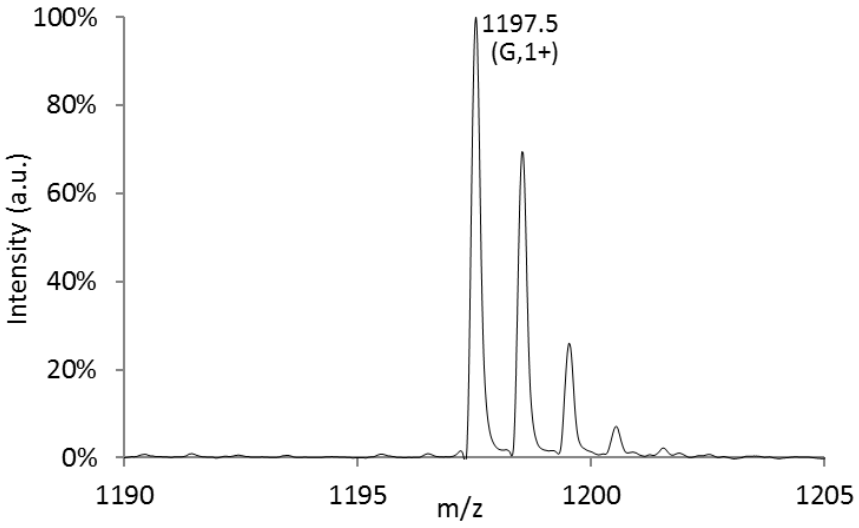

Figure S11: MALDI-TOF spectrum of singly charged peptide fragment G, *PyroGlu21 to Ala30* (1197.5) with its corresponding isotopic pattern.

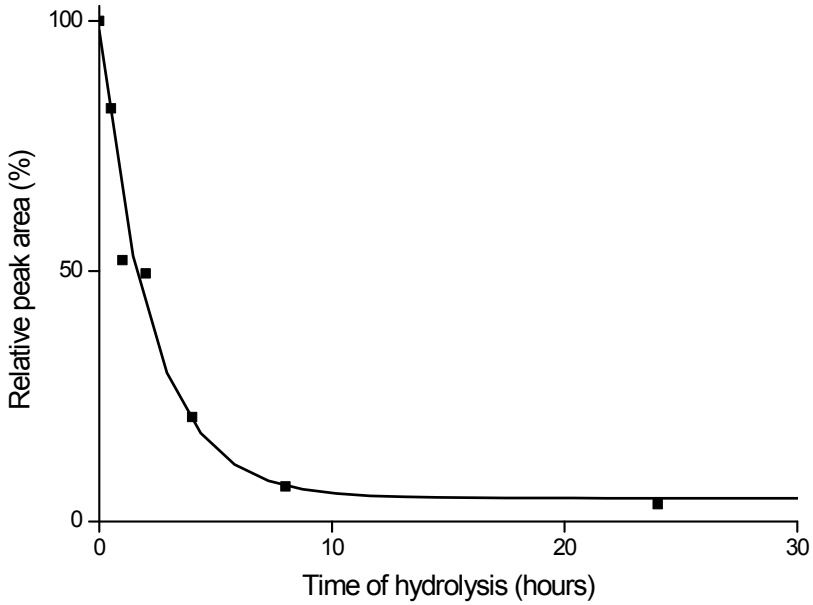

Figure S12: Kinetic plot showing the rate of disappearance of the parent peptide after incubation of oxidized insulin chain B with a Zr(IV) Wells-Dawson POM in a 2:1 molar ratio (Zr(IV) Wells-Dawson POM: oxidized insulin chain B) at 60 °C and pH 7.0.

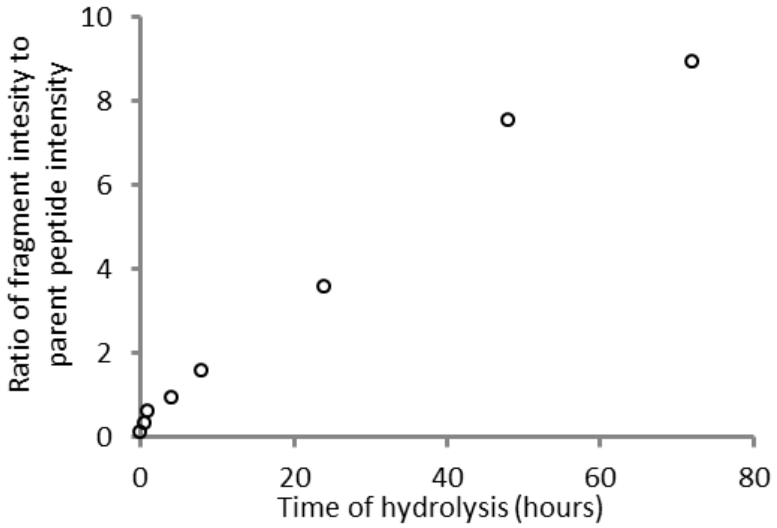

Figure S13: Ratio of intensity of peptide fragment, *Phe1----Gly8,* to intensity of oxidized insulin chain B over time at 37 °C and pH 7.0.

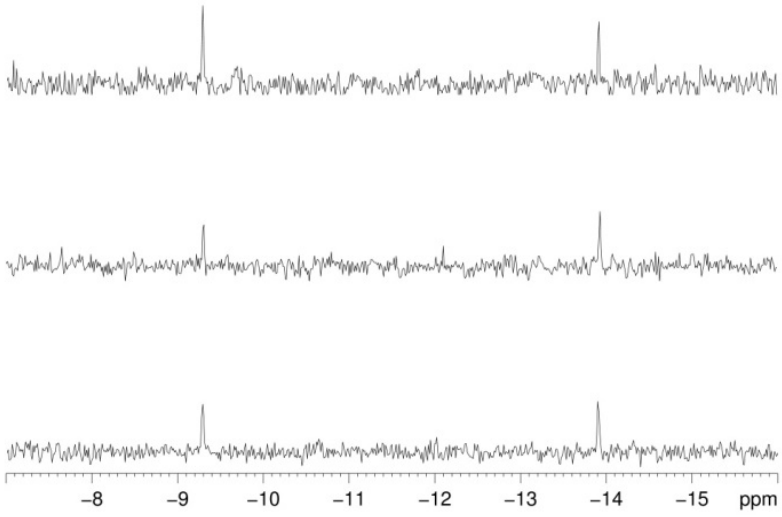

Figure S14: ^{31}P NMR spectra of Zr(IV) Wells-Dawson POM (0.5 mM, RT, pD 7.0) in the absence (bottom) and presence (middle) of oxidized insulin chain B (0.25 mM, RT, pD 7.0) and after heating the mixture (0.5 mM Zr(IV) Wells-Dawson POM + 0.25 mM oxidized insulin chain B, pD 7.0) at 60 ^{o}C for 6 days (top) in D_2O.

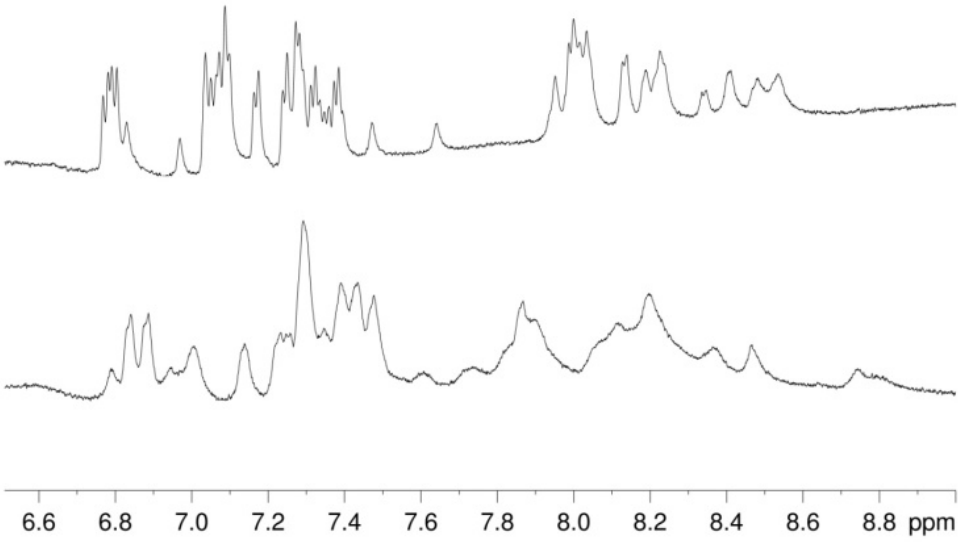

Figure S15: Aromatic and N-H region of ^{1}H NMR spectrum of oxidized insulin chain B in the absence (top) and presence (bottom) of Zr(IV) Wells-Dawson POM (pH 7.0, RT).

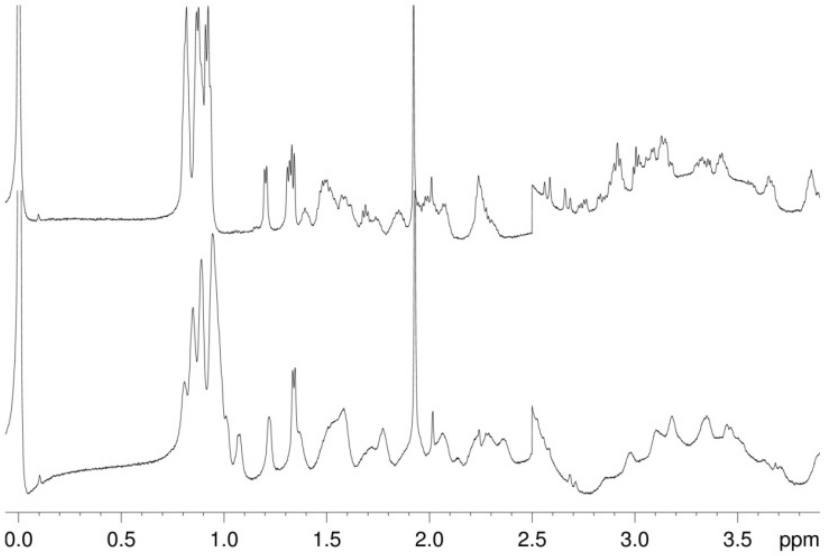

Figure S16: Aliphatic region of ^{1}H NMR spectrum of oxidized insulin chain B in the absence (top) and presence (bottom) of Zr(IV) Wells-Dawson POM (pH 7.0, RT).

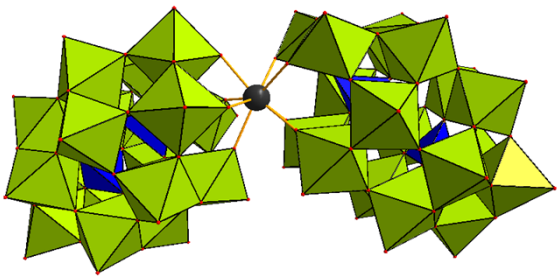

Figure S17: Combined polyhedral/ball-and-stick representation of $K_{13}(H_2O)[Eu(H_2O)_{3/4}(\alpha_2\text{-}P_2W_{17}O_{61})_2] \cdot 2\ KCl \cdot n\ H_2O$ **(2).** A 1:2 species is displayed in which one Eu (III) ion coordinates to two Wells-Dawson POM units. The WO_6 octahedrons are displayed in green, the PO_4 tetrahedrons in blue, and the Eu(III) ion center in dark grey.

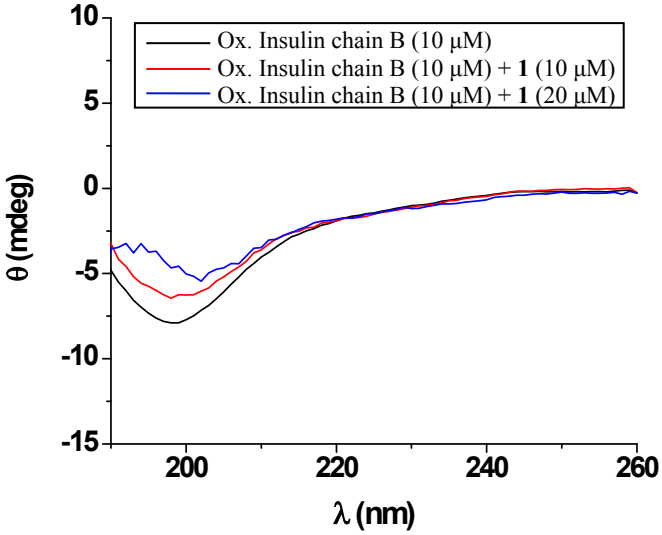

Figure S18: CD spectra of pure oxidized insulin chain B (10 µM) and oxidized insulin chain B (10 µM) in the presence of 1 (10 µM and 20 µM) in 100% H_2O (pH 7.0). A minimum at 200 nm is observed both in the absence and presence of Zr(IV) Wells-Dawson POM, representing a complete random coil conformation of oxidized insulin chain B.