Supporting Information

Native Top-Down ESI-MS Study of 158 kDa Protein Complex by High Resolution Fourier Transform Ion Cyclotron Resonance Mass Spectrometry

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EXPERIMENTAL METHODS

Materials. Aldolase from rabbit muscle and ammonium acetate were purchased from Sigma-Aldrich (St. Louis, MO). Acetonitrile and formic acid were obtained from Fisher Scientific (Pittsburgh, PA).

Sample Preparation. Aldolase was dissolved in MilliQ water to a concentration of 100 μM and then buffer exchanged three times with 200 mM ammonium acetate solution (300 μL each time) using Amicon centrifugal filters (Millipore Inc., Billerica, MA) with a molecular weight cut-off (MWCO) of 50 K. The buffer exchanged protein sample was then diluted with 200 mM ammonium acetate solution to a concentration of 20 μM for native nano-ESI-MS analysis.

FT-ICR MS Analysis. Protein solutions were loaded into metal-coated borosilicate capillaries (Au/Pd-coated, 1 μm I.D.; Thermo Fisher Scientific, West Palm Beach, FL) and sprayed at a flow rate of 10 - 40 nL/min through a nanospray ion source. High resolution measurements for the native protein solutions were acquired using a 12 T Bruker solariX^{TM} XR FT-ICR MS (Bruker Daltonics, Bremen, Germany) with the ParaCell.[16, 20] Other experiments were performed using a 15 T Bruker solariX FT-ICR MS with an infinity cell. The ESI capillary voltage was set to 0.9~1.2 kV. The temperature of the drying gas was 80 °C and the flow rate was 2.5 L/min. The RF amplitude of the ion-funnels was 300

V_{pp}, and the applied voltages were 200 V and 6 V for funnels 1 and 2, respectively. The voltage of skimmer 1 was varied up to 150 V to pre-heat ions but without inducing ion fragmentation and the skimmer 2 voltage was maintained at 20 V. The lowest values of RF frequencies were used in all ion-transmission regions: multipole 1 (2 MHz), quadrupole (1.4 MHz), and transfer hexpole (1MHz). Ions were accumulated for 500 ms in the hexapole collision cell before being transmitted to the ICR cell. The time-of-flight of 2.5 ms was used. Vacuum pressures for different regions of the 15 T system were ~2.3 mbar for the source region, ~2.4×10^{-6} mbar for the quadrupole region, and ~2.6×10^{-9} mbar for the UHV-chamber pressure. The UHV pressure for the 12T system with the new cell is ca. 6×10^{-10} mbar. ECD experiments were performed with an ECD pulse length of 0.01~0.02 s, ECD bias of 1.5 V, and ECD lens of 15 V. The ECD hollow-cathode current was 1.6 A. Up to 300 scans were averaged for each spectrum and all spectra were externally calibrated with cesium iodide. Data was processed in DataAnalysis and interpreted manually.

Table S-1. The isotopic mass-list table of the 26+ non-adducted tetrameric aldolase $[(C_{1733}H_{2773}N_{489}O_{525}S_{11})_4+26H]^{26+}$.

		Magnitude Mode			**Absorption Mode**		
#	**Theo. *m/z***	**Exp. *m/z***	**Resolving Power**	**Mass Error (ppm)**	**Exp. *m/z***	**Resolving Power**	**Mass Error (ppm)**
1	6032.20209	6032.20018	422632	-0.32	6032.20224	1006237	0.02
2	6032.24065	6032.24049	342045	-0.03	6032.24202	592319	0.23
3	6032.27921	6032.27686	183779	-0.39	6032.28029	682948	0.18
4	6032.31776	6032.31357	255115	-0.69	6032.31759	557074	0
5	6032.35632	6032.35443	520656	-0.31	6032.35679	726866	0.08
6	6032.39488	6032.39531	604127	0.07	6032.39697	874429	0.35
7	6032.43344	6032.43548	276851	0.34	6032.43686	897715	0.57
8	6032.47199	6032.46537	344641	-1.1	6032.47378	452396	0.30
9	6032.51055	6032.50785	487330	-0.45	6032.51111	459409	0.09
10	6032.54911	6032.55164	518302	0.42	6032.55311	504063	0.66
11	6032.58766	6032.58611	172729	-0.26	6032.58785	285087	0.03
12	6032.62622	6032.62159	336353	-0.77	6032.62752	486055	0.22
13	6032.66478	6032.66618	441620	0.23	6032.66694	775977	0.36
14	6032.70333	6032.69814	547722	-0.86	6032.69991	353134	-0.60
15	6032.74189	6032.74321	648864	0.22	6032.74508	918383	0.53
16	6032.78045	6032.77891	367031	-0.26	6032.78130	733458	0.14
17	6032.81900	6032.81653	748585	-0.41	6032.82112	551656	0.37
18	6032.85756	6032.85824	275587	0.11	6032.85978	623611	0.37
19	6032.89611	6032.89265	582885	-0.57	6032.89550	466279	-0.10
20	6032.93467	6032.92919	441325	-0.91	6032.93180	709151	-0.50
21	6032.97322	6032.9663	212980	-1.15	6032.97647	433793	0.54
22	6033.01178	6033.01297	502877	0.20	6033.01396	988277	0.36
23	6033.05033	6033.0494	538993	-0.15	6033.05237	668649	0.34
24	6033.08889	6033.08944	847021	0.09	6033.09008	953087	0.20
25	6033.12744	6033.12821	524429	0.13	6033.12920	664118	0.29
26	6033.16600	6033.16738	617729	0.23	6033.16854	690630	0.42
27	6033.20455	6033.20564	435898	0.18	6033.20734	458836	0.46
28	6033.24311	6033.24647	565043	0.56	6033.24580	775095	0.45
29	6033.28166	6033.28416	611173	0.41	6033.28382	588346	0.36
30	6033.32022	6033.32246	693065	0.37	6033.32215	707593	0.32
31	6033.35877	6033.35991	1041911	0.19	6033.36062	740219	0.31
32	6033.39733	6033.39915	354978	0.30	6033.39243	915614	-0.80
33	6033.43588	6033.43859	471404	0.45	6033.43883	588854	0.49
34	6033.47443	6033.47787	383642	0.57	6033.47777	652330	0.55
35	6033.51299	6033.51406	795011	0.18	6033.51614	559541	0.52
36	6033.55154	6033.55282	819098	0.21	6033.55252	551361	0.16

37	6033.59009	6033.59124	515945	0.19	6033.59216	820060	0.34
38	6033.62865	6033.63077	545586	0.35	6033.63130	686454	0.44
39	6033.66720	6033.66829	515220	0.18	6033.66854	762847	0.22
40	6033.70575	6033.70772	515220	0.33	6033.70768	718291	0.32
41	6033.74431	6033.74520	764790	0.15	6033.74513	762127	0.14
42	6033.78286	6033.78509	764790	0.37	6033.78573	925006	0.48
43	6033.82141	6033.82300	568715	0.26	6033.82372	646045	0.38
44	6033.85997	6033.86075	530478	0.13	6033.86118	689966	0.20
45	6033.89852	6033.89344	547332	-0.84	6033.90097	472419	0.41
46	6033.93707	6033.93678	286519	-0.05	6033.93763	435541	0.21
47	6033.97562	6033.97601	669950	0.06	6033.97633	709382	0.12
48	6034.01418	6034.01656	511694	0.39	6034.01610	766613	0.32
49	6034.05273	6034.04895	502153	-0.63	6034.05265	571571	0
50	6034.09128	6034.09369	352219	0.40	6034.09300	526283	0.29
51	6034.12983	6034.13138	416532	0.26	6034.13041	695412	0.10
52	6034.16838	6034.16703	397551	-0.22	6034.16856	666724	0.03
53	6034.20694	6034.20399	398683	-0.49	6034.20580	535109	-0.20
54	6034.24549	6034.24539	960978	-0.02	6034.24597	1068154	0.08
55	6034.28404	6034.28443	593822	0.06	6034.28426	774756	0.04
56	6034.32259	6034.32403	822748	0.24	6034.32114	627279	-0.20
57	6034.36114	6034.36229	398006	0.19	6034.36157	730919	0.07
58	6034.39969	6034.40100	465158	0.22	6034.39851	749229	-0.20
59	6034.43825	6034.43647	522493	-0.29	6034.43738	711194	-0.10
60	6034.47680	6034.46353	760253	-2.20	6034.47628	756098	-0.10
61	6034.51535	6034.51440	745469	-0.16	6034.51488	895533	-0.10
62	6034.55390	6034.55324	589437	-0.11	6034.55330	901327	-0.10
63	6034.59245	6034.59110	389482	-0.22	6034.59132	832195	-0.20
64	6034.63100	6034.62731	597304	-0.61	6034.62858	532470	-0.40
65	6034.66955	6034.66891	432092	-0.11	6034.66912	767342	-0.10
66	6034.70810	6034.70437	500808	-0.62	6034.70626	588842	-0.30
67	6034.74665	6034.74364	477116	-0.50	6034.74480	660063	-0.30
68	6034.78520	6034.78351	462863	-0.28	6034.78409	665255	-0.20
69	6034.82375	6034.82443	420344	0.113	6034.82261	662213	-0.20
70	6034.86230	6034.86042	920482	-0.31	6034.86170	627844	-0.10
71	6034.90085	6034.90035	542899	-0.08	6034.90003	846868	-0.10
72	6034.9394	6034.93747	527620	-0.32	6034.93703	747905	-0.40
73	6034.97795	6034.97520	524641	-0.46	6034.97553	636822	-0.40
Average Resolving Power			525902			681709	
Average Absolute Deviation (ppm)				0.36		0.28	

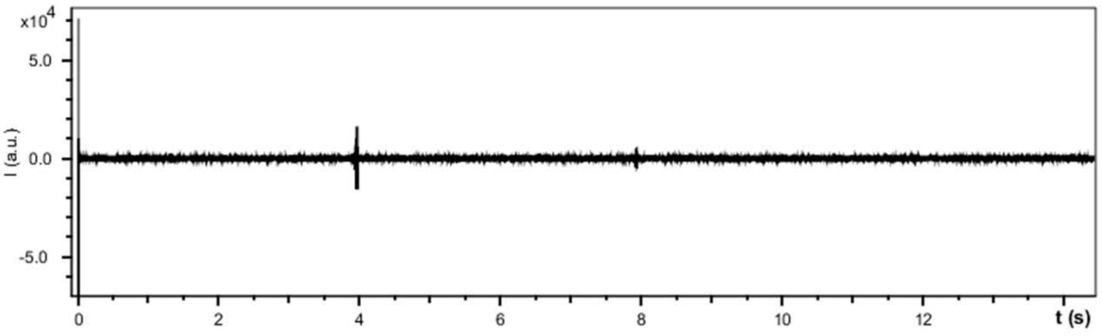

Figure S-1. Transient obtained using a 15 T FT-ICR MS with an infinity cell for the 27+ charged ions at *m/z* 5082±50 Da.

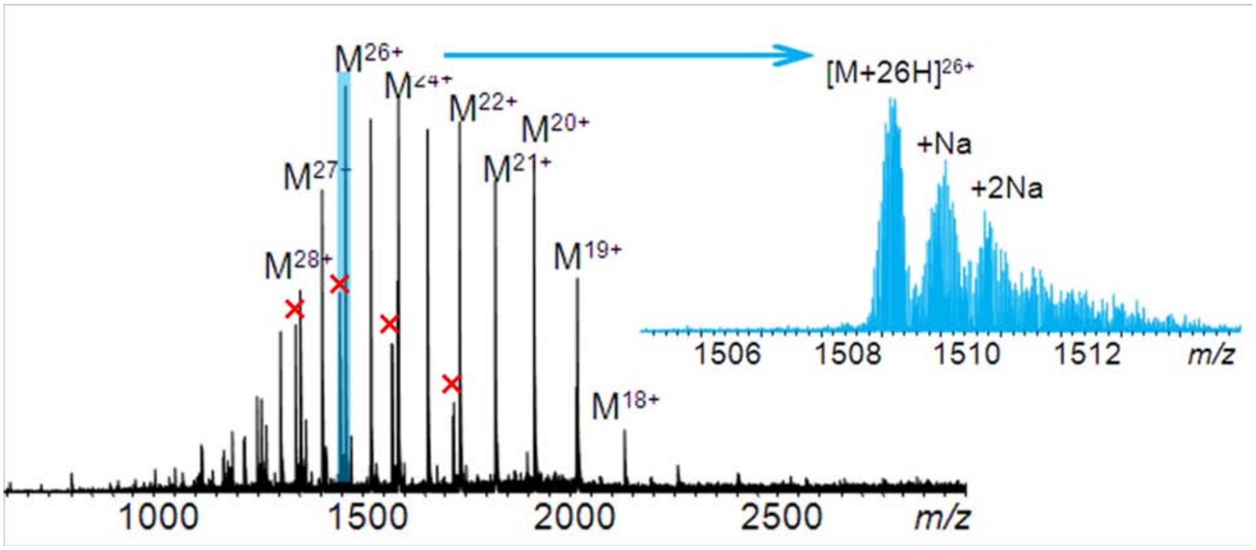

Figure S-2. Full mass spectrum of denatured aldolase monomer. The MS region of 26+ charge state is expanded and shown in the inset. Note: The second group of species with the label of "×" is the hydrolysis product of aldolase monomer.

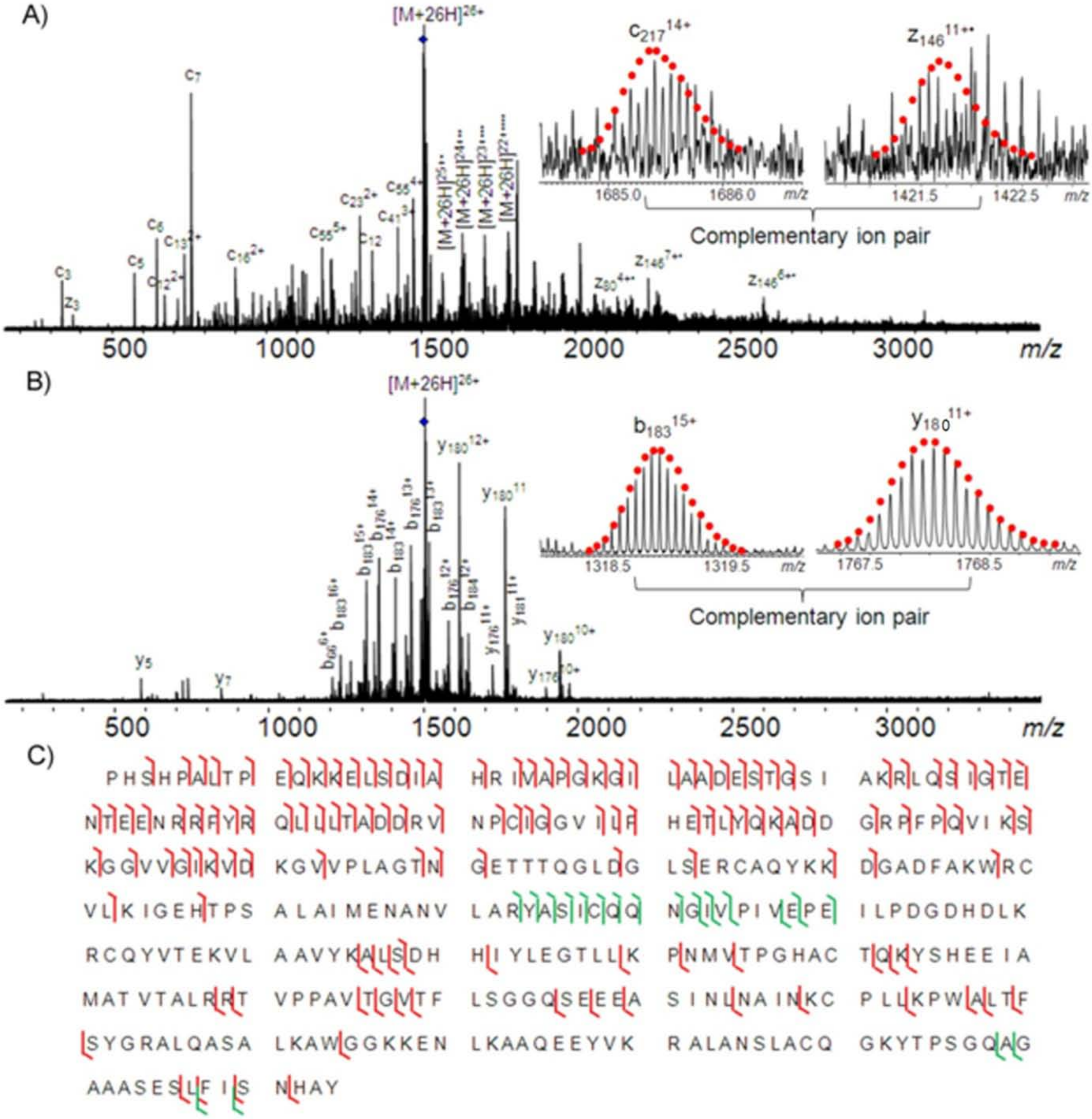

Figure S-3. A) Top-down ECD of denatured aldolase monomer (26+), (B) top-down CAD of the denatured aldolase monomer (26+), and C) backbone cleavage sites (b/y fragments are in green and c/z• fragments are in red).

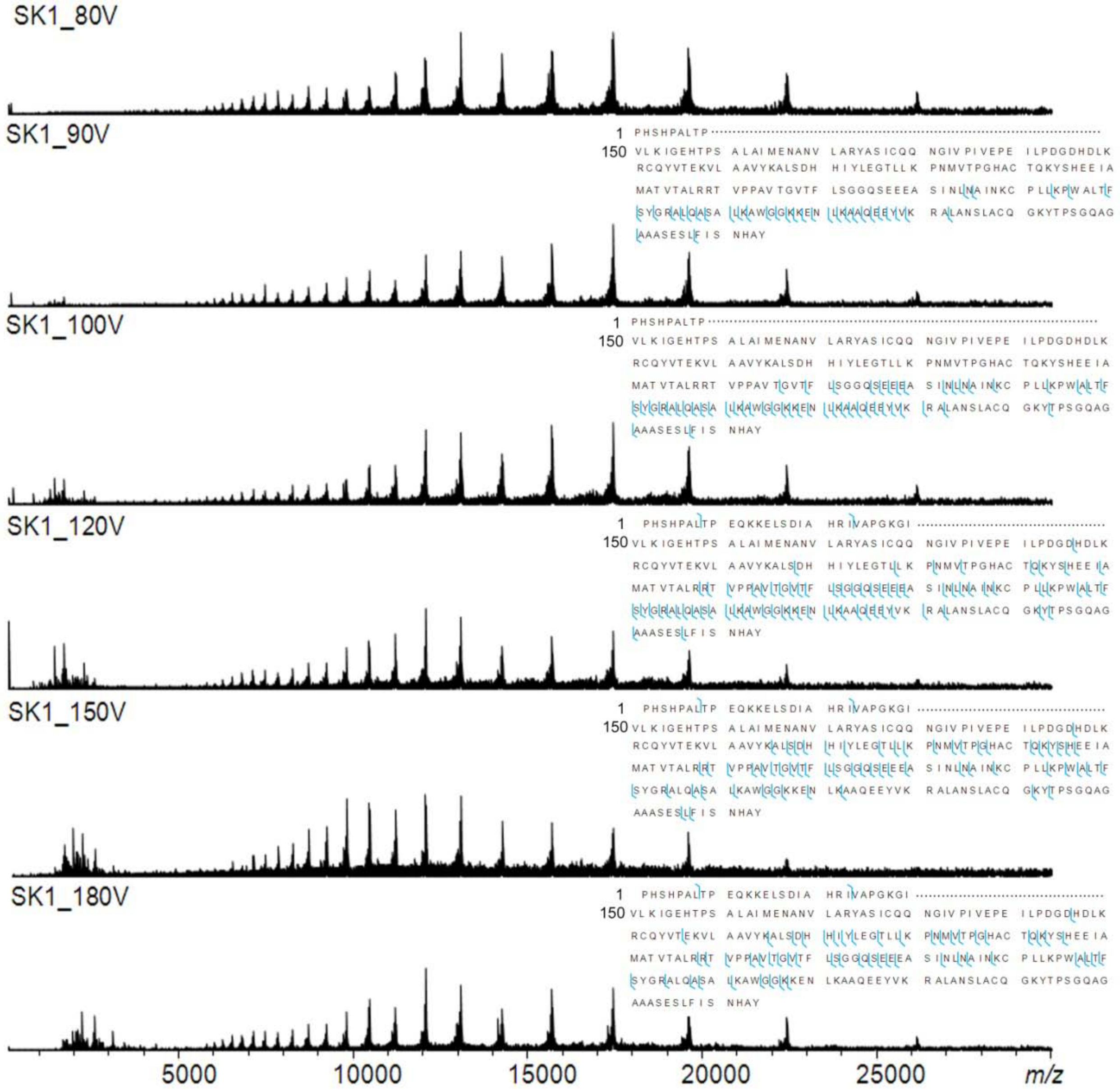

Figure S-4. Native top-down ECD of aldolase tetramer with various skimmer potentials for pre-activation.

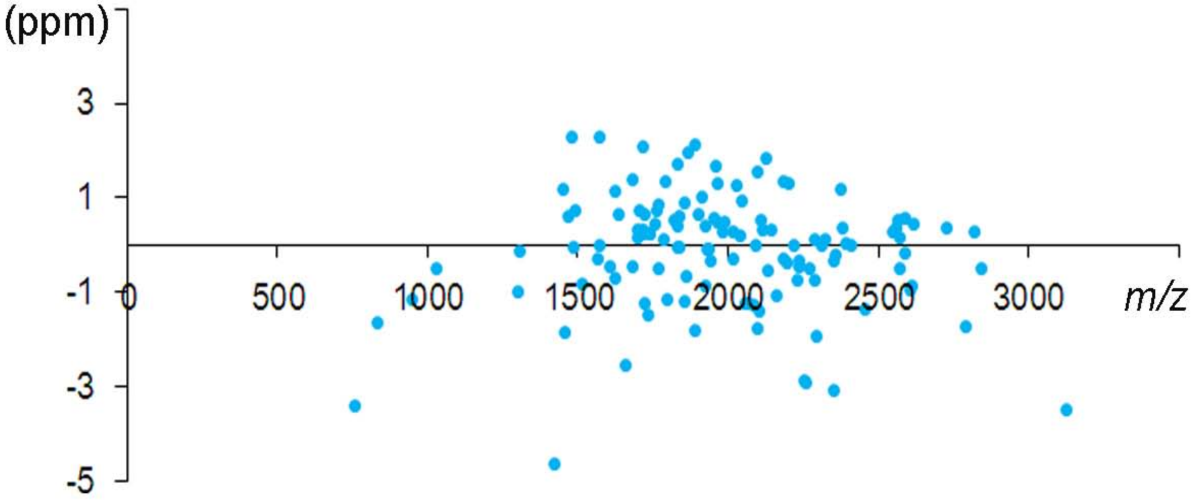

Figure S-5. Mass accuracy of product ions from native top-down ECD of aldolase tetramer.

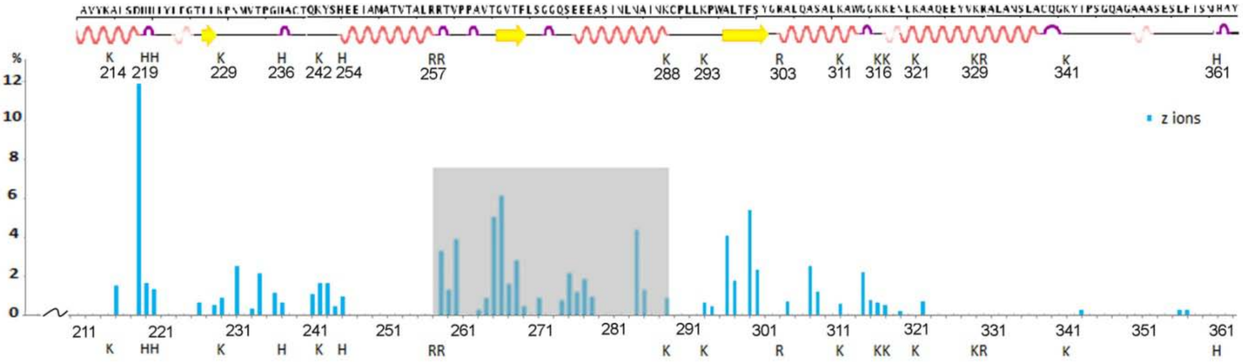

Figure S-4. Normalized abundance of $z^{\bullet}$-fragment ions from native top-down ECD of the aldolase tetramer. The top of the Figure shows the secondary structure and sequence of aldolase. The potential positive charge carrier sites (R, K, H) are indicated.