Supporting Information:

Native ambient mass spectrometry enables analysis of intact endogenous protein assemblies up to 145 kDa directly from tissue.

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EXPERIMENTAL DETAILS

Nano-DESI

An XYZ-stage (Zaber Technologies Inc., Vancouver, Canada) was mounted at the mass spectrometer inlet. Solvent and sampling capillaries were flame-pulled from fused silica tubing (O.D. 275 μm, I.D. 75 μm prior to modification) and cut to a final outer diameter of approx. 100 μm. The sampling capillary was positioned approx. 0.5 mm within the mass spectrometer inlet to aspirate solvent with the inlet vacuum. The exit of the sampling capillary was not flame-pulled but had the coating removed. Solvent was delivered through the solvent capillary by a 10 mL gas-tight syringe (Hamilton, Reno, NV) at a flow rate of 1.9-2.0 μL/min and a liquid junction was formed between the two capillaries. High voltage was provided by a wire from the mass spectrometer power supply connected directly to the syringe needle and optimized for sensitivity and spray stability (typically between 0.8 and 1.5 kV). Electrospray optimization was performed on the protonated detergent ion signal (C8E4: m/z 307.24) to achieve an RSD% of <10% and a spray current between 0.1 and 0.5 μA. Each nano-DESI probe has slightly different characteristics and will optimize differently but this method provided consistent performance for native protein analysis with the solvent systems used.

Stage movement was controlled directly by Zaber Control (Zaber Technologies Inc.) or automated by custom software written in LabVIEW (NI, Austin, Texas). *In situ* intact protein profiling and top-down mass spectrometry analyses were performed by positioning thin tissue sections underneath the nano-DESI probe and scanning the probe across the surface at between 1 and 20 μm/s. A relay connected to an Arduino Uno v3 microcontroller triggered contact closure to signal the start of a new line scan in MSI experiments.

LESA microextraction and nanoelectrospray ionization

Proteins were extracted from tissue by LESA microextraction using a Triversa Nanomate robot (Advion Biosciences, Ithaca, NY) and extraction solvents comprising ammonium acetate (200 mM) containing detergent (specified with relevant data). A 96 well plate was cut in half and loaded into the well plate mount. The glass slide containing the thaw mounted tissue was secured in the remaining space of the well plate mount using adhesive putty. A method was defined in the advanced user interface (AUI) of the ChipSoft software whereby 5 μL of the sampling solvent was aspirated from one of the wells of the well plate into a pipette tip. The pipette was positioned over the tissue and the pipette was brought in to contact with the tissue surface ("contact" LESA)[1]. 2 μL solvent was dispensed and the tissue-liquid junction was maintained for 30 seconds including 10 dispense-respirate cycles before the solvent (2 μL) was re-aspirated into the pipette tip. The pipette was repositioned above a clean well

and the sample extract was deposited (5 µL). 4 µL of the solvent extract was transferred into a nanoESI emitter by use of a manual pipette fitted with a gel loading tip.

Haemotoxylin and eosin staining procedure

Haemotoxylin and eosin staining was performed as described in Table S1. Thaw mounted tissue sections were sequentially submerged in solvent baths containing the described solvent and for the time period listed. Where a repeat submersion was required, a fresh bath of solvent was used. Tissue was mounted in DPX and a coverslip was applied before optical imaging.

Table S1: Haemotoxylin and eosin staining procedure.

Solvent Bath	**Time (min)**	**Repeat**
Water	2	1
Haemotoxylin Harris	4	0
Water	2	1
Acid Alcoholc	0.5	0
Water	2	1
Scott's tap Water Substitute	0.5	0
Water	2	1
Eosin	1	0
Water	2	1
Industrial denatured alcohol	2	3
Xylene	2	2

Table S2: Theoretical and measured average mass of identified protein assemblies and subunits.

	Protein	Uniprot entry	Assembly Stoichiometry	PTMs	Mass (assembly) (Da)	Theoretical mass (assembly) (Da)	Mass difference (ppm)	Measured mass (subunit) (Da)	Theoretical mass (subunit) (Da)	Mass difference (ppm)
Brain	MIF	P30904	Homotrimer	N/A	37038.5	37038.5	0.9	12346.1	12346.2	-9.0
	PGAM1	P25113	Homodimer	N-term acetylation, Y-phosphorylation	57649.6	57646.1	61.3	28822.6	28823.1	-14.3
	MDH2	P04636	Homodimer	N/A	66366.2	66365.4	13.0	33182.3	33182.7	-12.3
Kidney	α-enolase (apo)	P04764	Monomer	N-term acetylation	N/A	N/A	N/A	47039.2	47038.7	11.1
	α-enolase (holo)	P04764	Homodimer + 4x Mg^{2+}	N-term acetylation	94176.9	94175.7	12.8	47086.1	47087.9	-36.8
	MDH1	O88989	Homodimer	N-term acetylation	72789.6	72787.9	23.2	36393.7	36394.0	-7.8
	MDH2	P04636	Homodimer	N/A	66366.0	66365.4	10.0	33183.4	33182.7	21.6
	Ω-amidase	O88989	Homodimer	N-term acetylation	61224.7	61224.2	8.7	30610.3	30612.1	-57.4
Liver	OTC	P00481	Homotrimer	N/A	108831.7	108529.9	2773.5	36177.0	36176.6	10.2
	LDHA	P04642	Homotetramer	N-term acetylation	145449.0	145445.3	25.6	36360.2	36361.3	-30.6

Rat brain

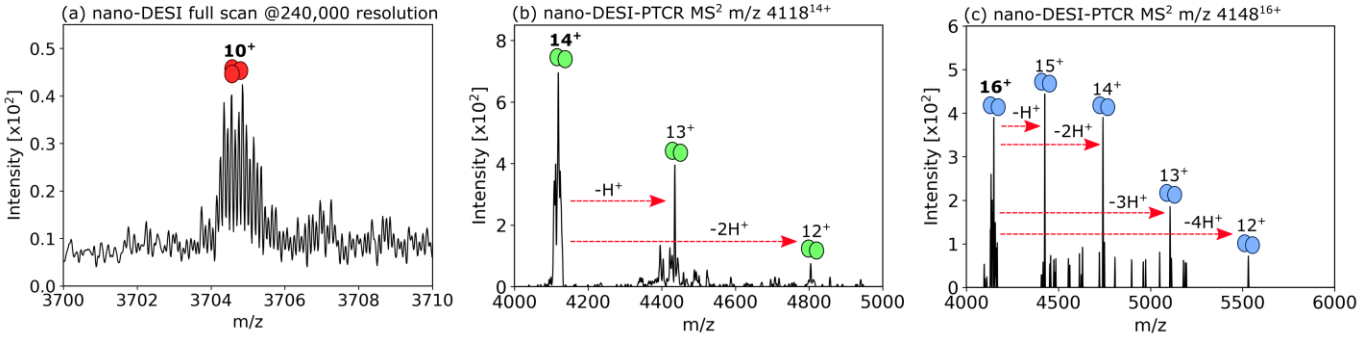


Figure S1: Determination of the intact mass of protein assemblies in rat brain. (a) nano-DESI full scan MS spectrum at a nominal resolution of 240,000 (m/z 200) showing isotopically resolved signals for MIF in the 10+ charge state (approx. 37.0 kDa). ((b) nano-DESI-PTCR MS2 spectrum for m/z 4118 indicating precursor ions in the 14+ charge state (approx. 57.6 kDa). (c) nano-DESI-PTCR MS2 spectrum for m/z 4148 indicating precursor ions in the 16+ charge state (approx. 66.3 kDa). PTCR spectra were acquired at orbitrap resolution = 7500 at m/z 200 using a 200 mM ammonium acetate + 0.5x CMC C8E4 solvent system.

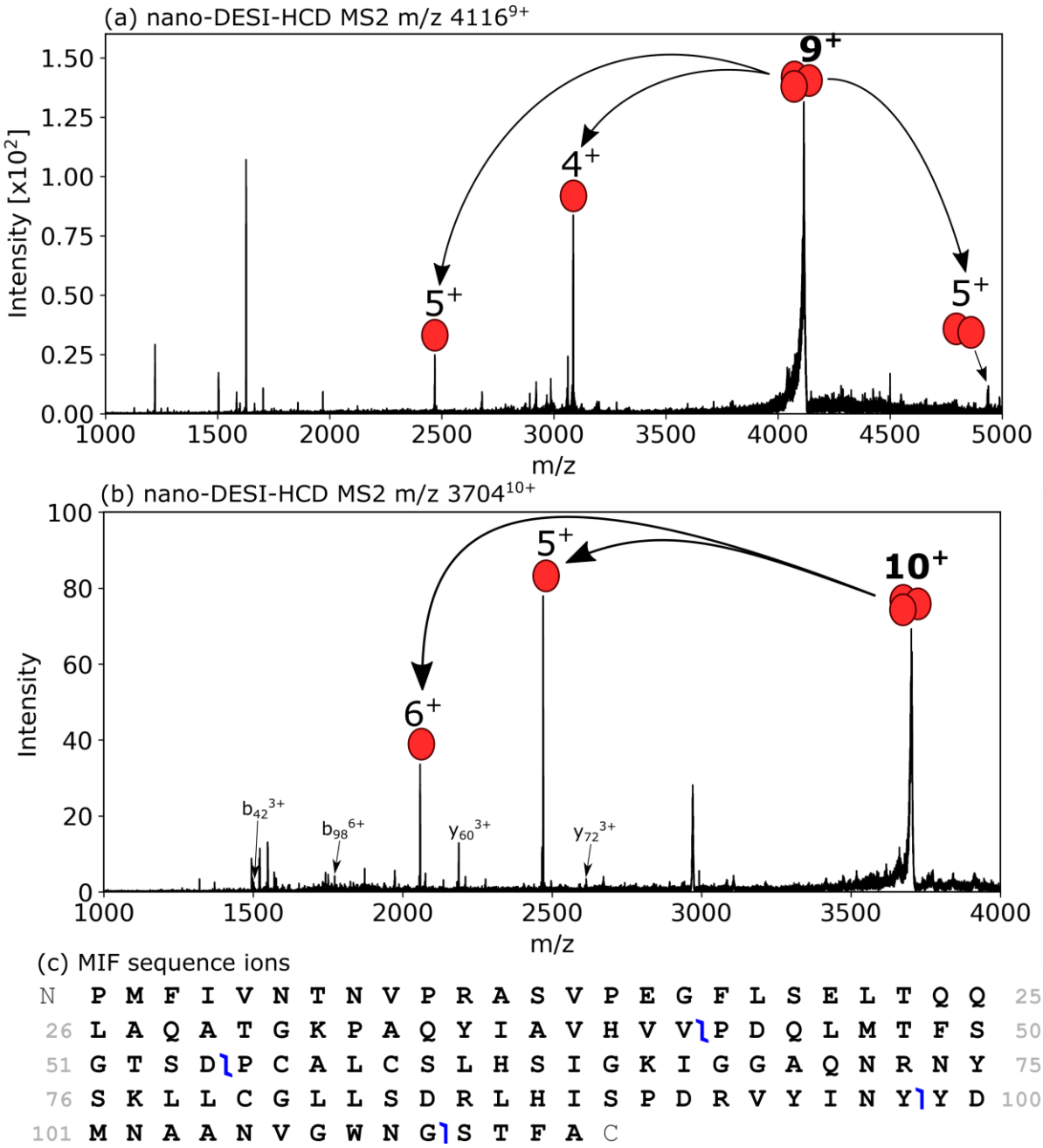


Figure S2: (a) nano-DESI-HCD MS2 of the 9+ (NCE = 45 – 46%) and (b) 10+ (NCE = 40%) charge state of macrophage inhibitory factor (MIF) homotrimer. Sequence ions are indicated for the 10+ charge state because of co-isolation of unrelated protein ion signals in the ion trap window for the 9+ charge state. Subunit ions confirm the assembly stoichiometry as homotrimeric. Mass spectra acquired on the Orbitrap Eclipse with an orbitrap resolution of 240,000 at m/z 200 using a 200 mM ammonium acetate + 0.5x CMC C8E4 solvent system. (c) Sequence ions assigned with a mass tolerance of 20 ppm. P-score = 0.054 with 4 of 6 ions explained by cleavages at D and P residues.

Table S3: Sequence ions of MIF.

Name	Theoretical Mass (Da)	Observed Mass (Da)	Mass Difference (Da)	Mass Difference (ppm)
b42	4502.3784	4502.3108	-0.0676	-15.0
b54	5781.9174	5781.8294	-0.0880	-15.2
b98	10621.4061	10621.5618	0.1557	14.7
b110	11913.9305	11914.0891	0.1586	13.3
y60	6556.2089	6556.1716	-0.0373	-5.7
y72	7835.7479	7835.6321	-0.1158	-14.8

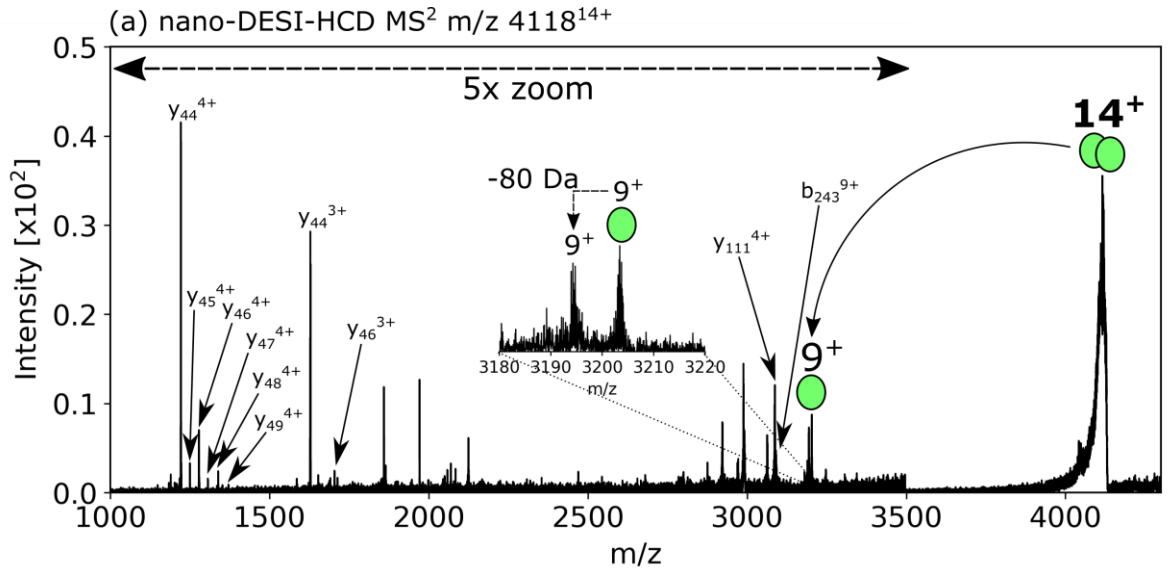


Figure S3: (a) nano-DESI-HCD MS^2 spectrum for phosphoglycerate mutase obtained with NCE = 20 – 25%. The intact mass is consistent with N-terminal acetylation (red highlight) and a single phosphorylation (blue highlight) situated towards the N-terminus. Spectrum acquired on the Orbitrap Eclipse with an orbitrap resolution of 240,000 at m/z 200 using a 200 mM ammonium acetate + 0.5x CMC C8E4 solvent system. (b) Sequence ions for PGAM1 assigned with a mass tolerance of 20 ppm. P-score = 0.067. 2 of 10 ions are explained by cleavages N-terminally to P-residues. The region I204 – P214 shows comprehensive cleavage, with the y44 ion showing as especially abundant. The precise phosphorylated residue was not determined due to the absence of identifiable b-ion signals, but is included on the Y-residue (highlighted in blue) as per annotations on Uniprot (P25113) due to the prevalence of neutral loss = 80 Da rather than 98 Da.

Table S4: Sequence ions of PGAM1

Name	Theoretical Mass (Da)	Observed Mass (Da)	Mass Difference (Da)	Mass Difference (ppm)
y40	4511.4647	4511.4203	-0.0444	-9.9
y42	4681.5702	4681.5313	-0.0390	-8.3
y44	4879.6707	4879.6038	-0.0669	-13.7
y45	4992.7547	4992.7020	-0.0527	-10.6
y46	5106.7977	5106.7352	-0.0624	-12.2
y47	5219.8817	5219.8276	-0.0541	-10.4
y48	5348.9243	5348.8898	-0.0345	-6.4
y49	5479.9648	5479.9110	-0.0538	-9.8
y111	12337.3724	12337.5840	0.2116	-17.1
b243	27806.1813	3090.6055	-0.2052	7.4

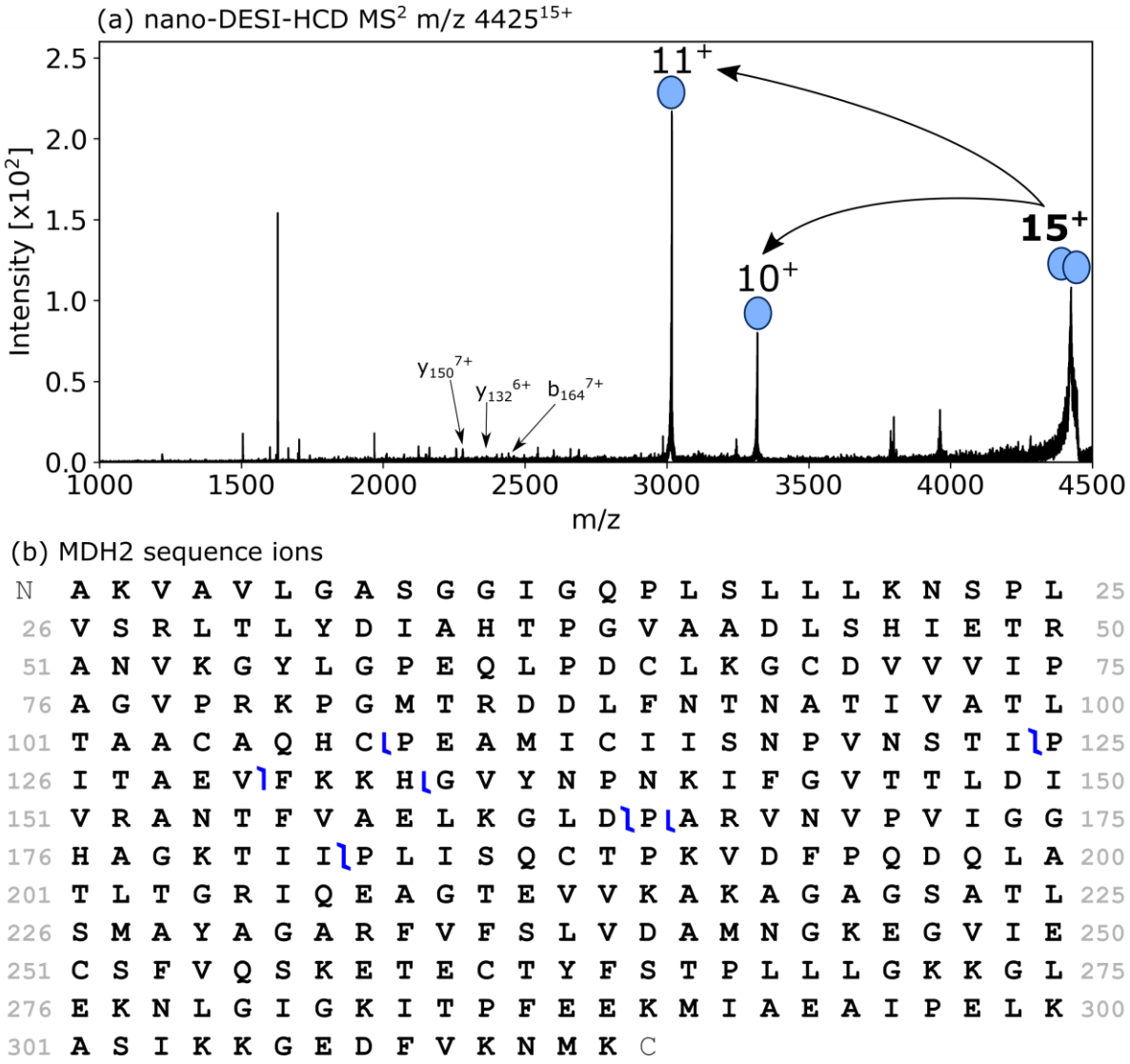


Figure S4: (a) nano-DESI-HCD MS^2 spectrum of m/z 4425^{15+}, MDH2 homodimer, showing subunit ions in 11+ and 10+ charge states (NCE = 50%) and confirming assembly stoichiometry as homodimeric. Spectrum acquired on the Orbitrap Eclipse with an orbitrap resolution of 240,000 at m/z 200 using a 200 mM ammonium acetate + 0.5x CMC C8E4 solvent system. (b) Sequence ions assigned with a mass tolerance of 20 ppm. P-score = 0.13. 7 of 9 ions are explained by cleavage at D or P residues. There is some fragment ion overlap with the MDH2 identified in the kidney by nESI (Figure S11).

Table S5: sequence ions for MDH2.

Name	Theoretical Mass (Da)	Observed Mass (Da)	Mass Difference (Da)	Mass Difference (ppm)
b124	12816.7017	12816.6559	-0.0458	-3.6
b130	13427.0343	13426.9893	-0.0450	-3.3
b164	17213.0963	17213.0173	-0.0790	-4.6
b182	18993.1434	18993.1327	-0.0107	-0.6
y132	14168.3591	14168.2997	-0.0594	-4.2
y150	15948.4062	15948.3493	-0.0569	-3.6
y180	19194.1510	19194.0491	-0.1019	-5.3
y190	20344.8008	20344.7192	-0.0816	-4.0
y206	22027.6380	22027.5247	-0.1133	-5.1

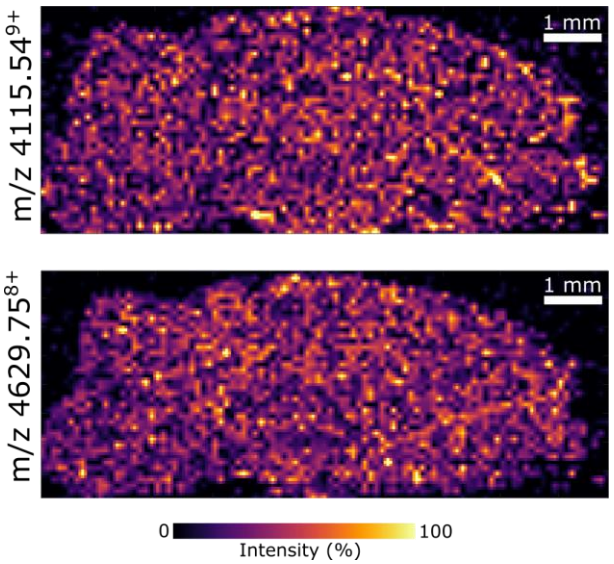


Figure S5: Ion images for 9+ and 8+ charge states of MIF showing homogenous distribution.

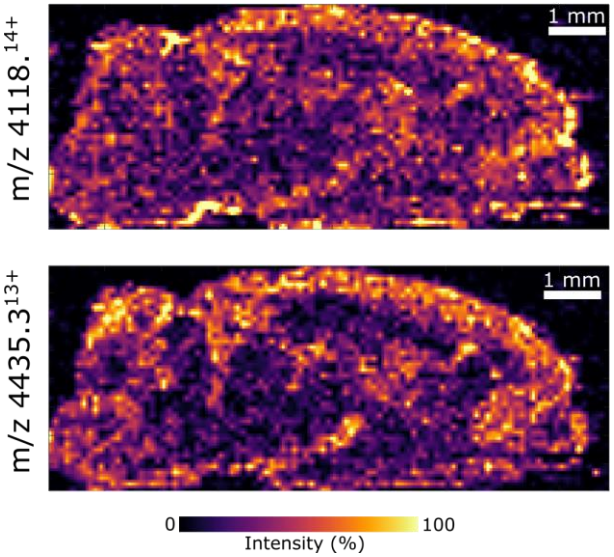


Figure S6: Ion images for 14+ and 13+ charge states of PGAM1 showing distribution in regions such as the cerebral cortex.

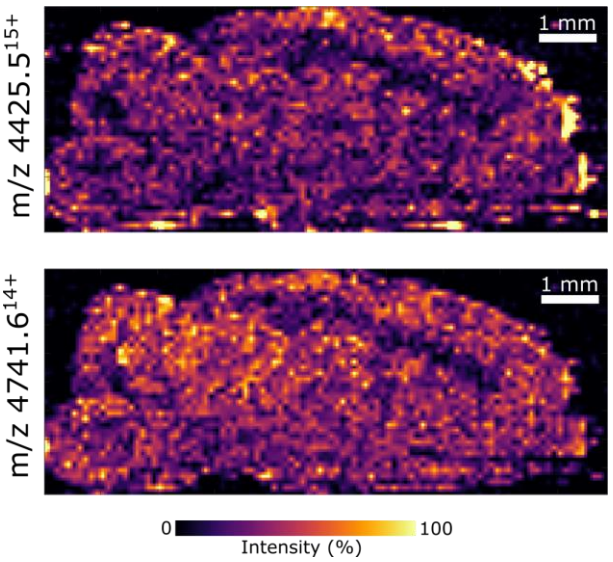


Figure S7: Ion images for 15+ and 14+ charge states of MDH2 showing distribution amongst grey matter.

Rat kidney:

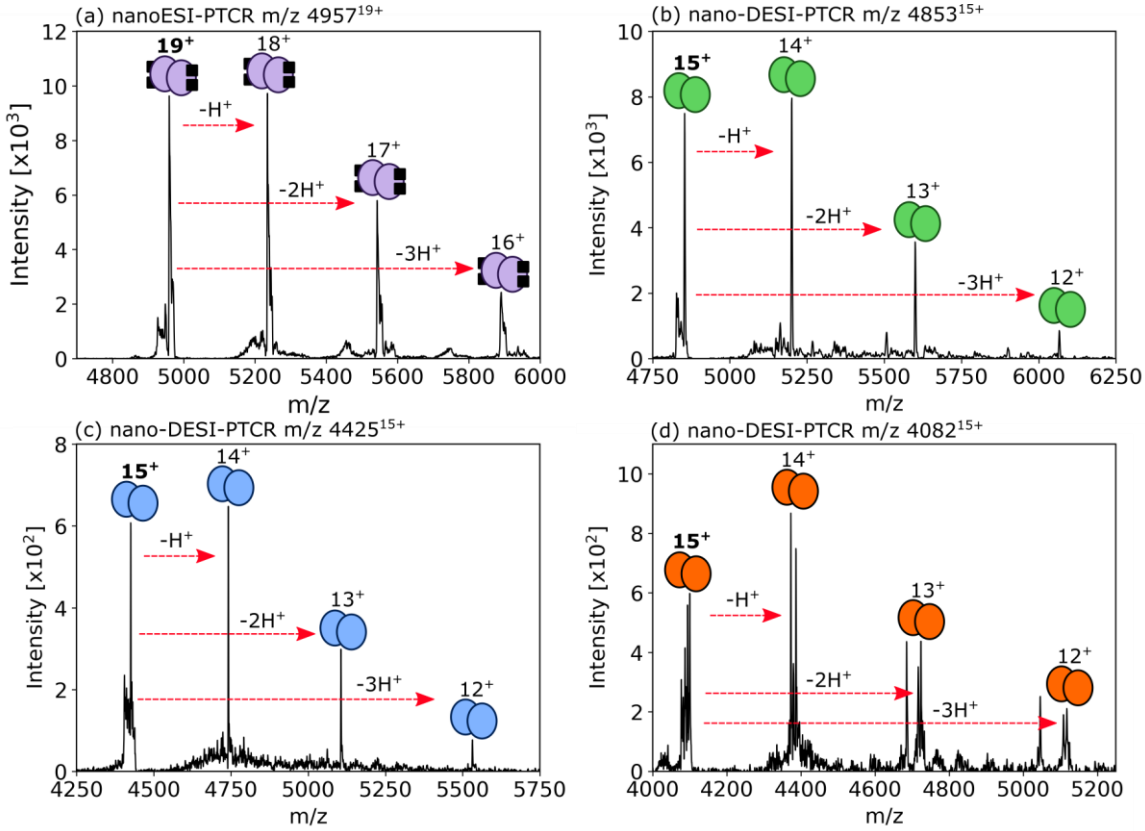
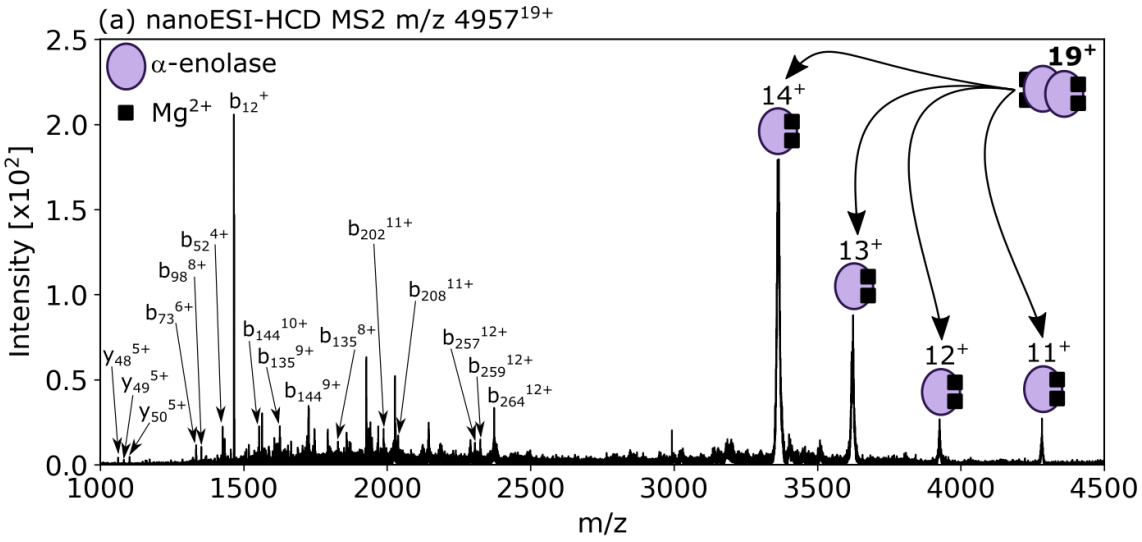


Figure S8: Determination of intact mass of kidney protein assemblies. (a) nanoESI-PTCR MS^2 spectrum of m/z 4957 indicating precursor ions in the 19+ charge state. (b) nano-DESI-PTCR MS^2 spectrum of m/z 4853 indicating precursor ions in the 15+ charge state (approx. 72.8 kDa). (c) nano-DESI-PTCR MS2 spectrum for m/z 4425 indicating precursor ions in the 15+ charge state (approx. 66.3 kDa). (d) nano-DESI-PTCR MS2 spectrum for m/z 4082 indicating precursor ions in the 15+ charge state (approx. 61.3 kDa). PTCR spectra were acquired with an orbitrap resolution of 7500 at m/z 200.



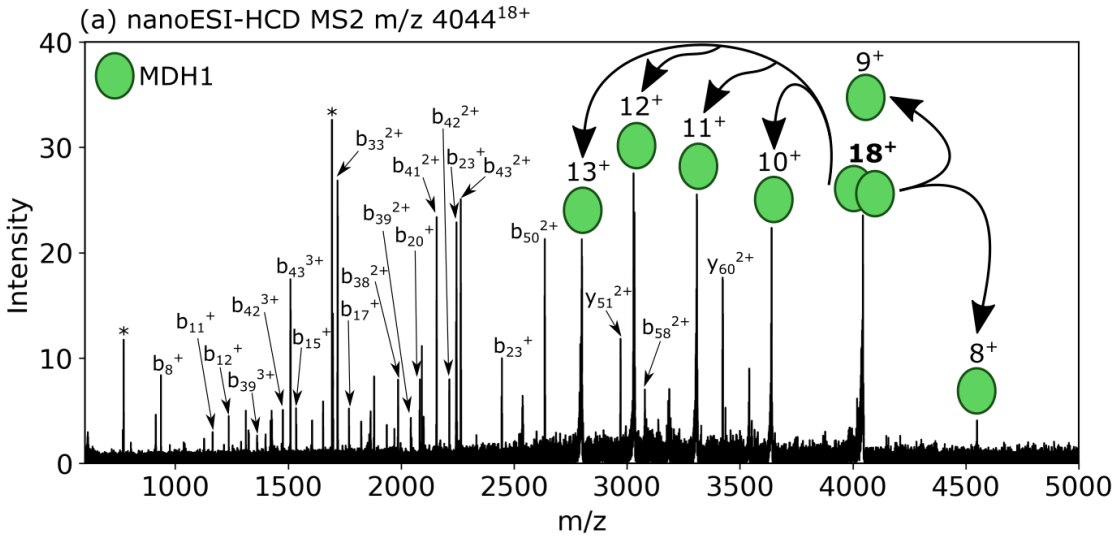
(b) α-enolase sequence ions

N	S	I	L	K	I	H	A	R	E	I	F	D⌉	S	R	G	N	P	T	V	E	V	D	L	Y	T	25
26	A	K	G	L	F	R	A	A	V	P	S	G	A	S	T	G	I	Y	E	A	L	E	L	R	D	50
51	N	D⌉	K	T	R	F	M	G	K⌉	G	V	S	K	A	V	E⌉	H	I⌉	N	K	T	I	A⌉	P	A	75
76	L	V	S	K	K	L	N	V	V⌉	E	Q	E	K	I	D	Q	L	M	I	E	M⌉	D⌉	G⌉	T	E	100
101	N⌉	K	S	K	F	G	A	N	A	I	L	G	V	S	L	A	V	C	K⌉	A	G⌉	A	V⌉	E⌉	K⌉	125
126	G	V	P	L	Y	R	H	I	A	D⌉	L	A	G	N⌉	P	E	V	I	L⌉	P	V⌉	P	A	F	N	150
151	V	I	N	G	G	S	H	A	G	N	K	L	A	M	Q	E	F	M	I	L	P	V	G	A	S	175
176	S	F	R	E	A	M	R	I	G	A	E	V	Y	H	N	L	K	N	V	I	K	E	K	Y	G	200
201	K	D⌉	A	T	N	V	G	D⌉	E	G	G	F	A	P	N	I	L	E	N	K	E	A	L	E	L	225
226	L	K	S	A	I	A	K	A	G	Y	T	D	Q	V	V	I	G	M	D	V	A	A	S	E	F	250
251	Y	R	A	G	K	Y	D⌉	L	D⌉	F	K	S	P	D⌉	D	A	S	R	Y	I	T	P	D	Q	L	275
276	A	D	L	Y	K	S	F	I	K	D	Y	P	V	V	S	I	E	D	P	F	D	Q	D	D	W	300
301	D	A	W	Q	K	F	T	A	T	A⌊	G	I	Q	V	V	G	D	D	L	T	V	T	N	P	K	325
326	R	I	A	K	A	A	G⌊	E	K	S	C	N	C	L	L	L	K	V	N	Q	I	G	S	V	T	350
351	E	S	L	Q	A	C	K	L	A	Q	S	N	G	W	G	V	M	V	S	H	R	S	G	E	T	375
376	E	D	T	F	I⌊	A	D	L⌊	V⌊	V⌊	G	L	C	T	G	Q	I	K	T	G	A	P	C	R	S	400
401	E	R	L	A	K	Y	N	Q	I	L	R	I	E	E	E	L	G	S	K	A	K	F	A	G	R	425
426	S	F	R	N	P	L	A	K	C																	

Figure S9: (a) nanoESI-HCD MS^2 spectrum of α-enolase homodimer (NCE = 40%) showing subunits and sequence ions. The subunit ions are consistent with the intact assembly being homodimeric. Spectrum acquired on the Orbitrap Eclipse using 200 mM aqueous ammonium acetate + 2x CMC LDAO and an orbitrap resolution of 120,000 at m/z 200. (b) sequence ions of N-terminally acetylated alpha-enolase assigned with a mass tolerance of 20 ppm. P-score = 0.00043. 13 of 31 ions are explained by cleavages at D and P residues.

Table S6: sequence ions for α-enolase

Name	Theoretical Mass (Da)	Observed Mass (Da)	Mass Difference (Da)	Mass Difference (ppm)
b12	1464.8089	1464.7992	-0.0096	-6.6
b52	5698.9382	5698.9239	-0.0143	-2.5
b59	6547.4073	6547.3621	-0.0452	-6.9
b66	7217.7723	7217.6770	-0.0953	-13.2
b68	7467.9153	7467.8704	-0.0449	-6.0
b73	7995.2220	7995.1307	-0.0913	-11.4
b84	9143.9501	9143.8183	-0.1318	-14.4
b96	10631.6501	10631.5405	-0.1096	-10.3
b97	10746.6770	10746.5348	-0.1423	-13.2
b98	10813.0164	10813.0884	-0.0720	6.7
b101	11147.8317	11147.6309	-0.2007	-18.0
b119	12934.8444	12934.7204	-0.1240	-9.6
b121	13062.9030	13062.7301	-0.1729	-13.2
b123	13233.0085	13232.8609	-0.1476	-11.2
b124	13362.0511	13361.8760	-0.1751	-13.1
b125	13490.1460	13489.9080	-0.2380	-17.6
b135	14622.2267	14622.1430	0.0837	-5.7
b139	14966.9298	14966.8227	-0.1071	-7.2
b144	15518.2616	15518.1042	-0.1575	-10.1
b146	15714.3828	15714.2580	-0.1248	-7.9
b202	21855.3286	21855.1922	0.1364	-6.2
b208	22399.7612	22399.4078	-0.3534	-15.8
b257	27657.3870	27657.4242	-0.0372	1.3
b259	27885.4986	27885.5358	-0.0372	1.3
b264	28459.7730	28459.8066	-0.0336	1.2
y48	5304.8451	5304.7491	-0.0960	-18.1
y49	5409.7373	5409.7063	0.0310	-5.7
y50	5508.8058	5508.7558	0.0500	-9.1
y53	5802.1301	5802.0912	-0.0389	-6.7
y101	10993.6499	10993.7733	0.1233	11.2
y123	13197.8776	13197.7840	-0.0936	-7.1



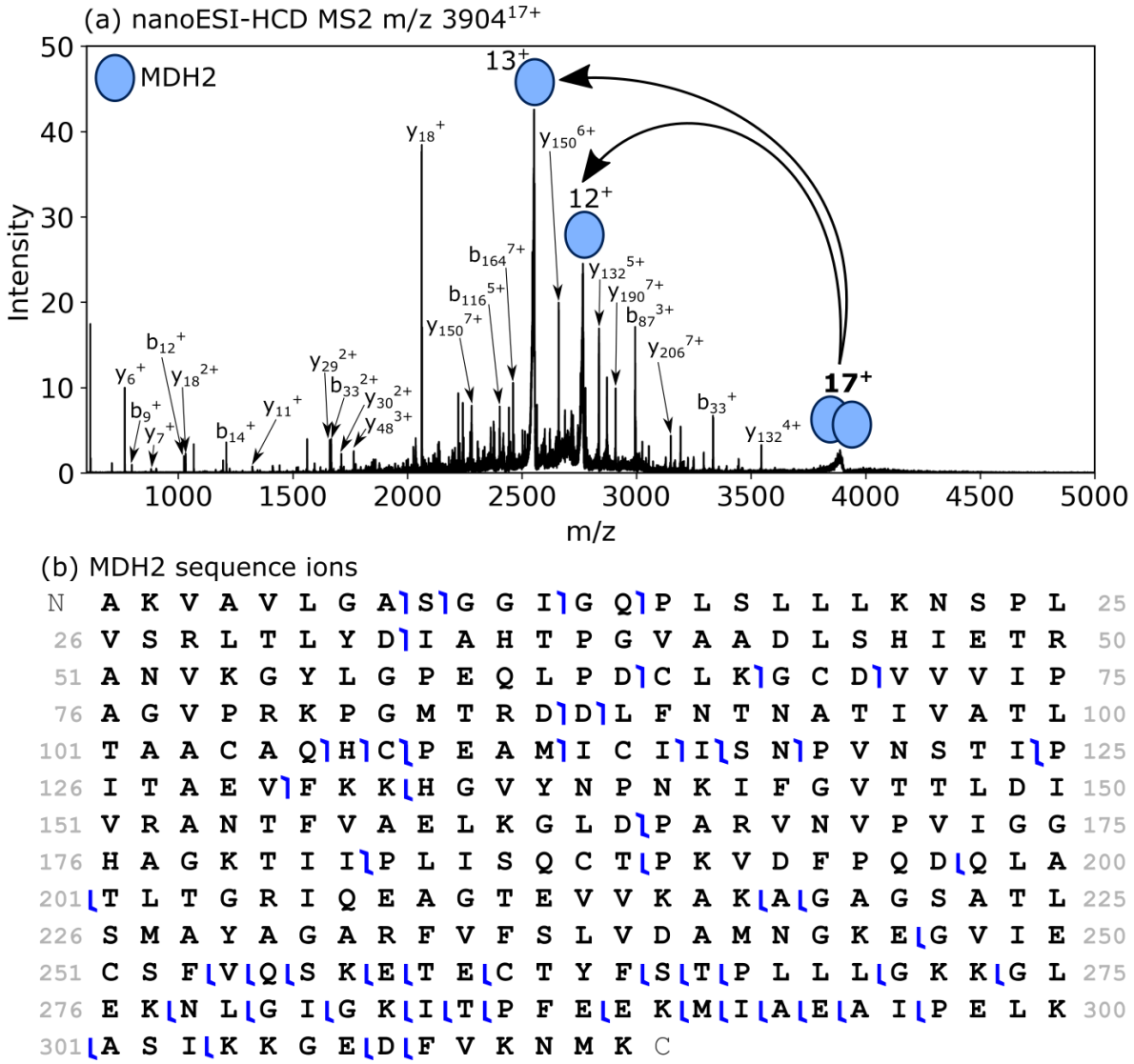
(b) MDH1 sequence ions

N	S	E	P	I	R	V	L	V⌉	T	G	A⌉	A⌉	G	Q	I⌉	A⌉	Y⌉	S	L	L⌉	Y	S	I⌉	G	N	25
26	G	S	V	F	G	K	D⌉	Q⌉	P	I⌉	I⌉	L⌉	V⌉	L⌉	L⌉	D⌉	I⌉	T⌉	P	M	M	G	V	L	D⌉	50
51	G	V	L	M	E	L	Q	D⌉	C	A	L	P	L	L	Q	D	V	I	A	T	D	K	E	E	V	75
76	A	F	K	D	L	D	V	A	V	L	V	G	S	M	P	R	R	E	G	M	E	R	K	D	L	100
101	L	K	A	N	V	K	I	F	K	S	Q	G	A	A	L	E	K	Y	A	K⌉	K	S	V	K	V	125
126	I	V	V	G	N⌉	P	A	N	T	N	C	L	T	A	S	K	S	A	P	S	I	P	K	E	N	150
151	F	S	C	L	T	R	L	D	H	N	R	A	K	S	Q	I	A	L	K	L	G	V	T	A	D	175
176	D	V	K	N	V	I	I	W	G	N	H	S	S	T	Q	Y	P	D	V	N	H	A	K	V	K	200
201	L	Q	G	K	E	V	G	V	Y	E	A	L	K	D	D	S	W	L	K	G	E	F	I	T	T	225
226	V	Q	Q	R	G	A	A	V	I	K	A	R	K	L	S	S	A	M	S	A	A	K	A	I	S	250
251	D	H	I	R	D	I	W	F	G	T	P	E	G	E	F	V	S	M	G	V	I	S	D⌊	G	N	275
276	S	Y	G	V	P	D⌊	D⌊	L	L	Y	S	F	P	V	V	I	K	N	K	T	W	K	F	V	E	300
301	G	L	P	I	N	D⌊	F	S	R	E	K	M	D	L	T	A	K	E	L	T	E	E	K	E	T	325
326	A	F	E	F	L	S	S	A	C																	

Figure S10: (a) nanoESI-HCD MS^2 spectrum of MDH1 homodimer showing subunits and sequence ions. Subunit ions confirm the assembly stoichiometry as homodimeric. The 9+ monomer signal overlaps the 18+ precursor ion signal. Spectrum acquired on the QE-HF with an orbitrap resolution of 120,000 at m/z 200. (b) sequence ions of N-terminally acetylated MDH1 assigned with a mass tolerance of 20 ppm. P-score = 3e-15. 11 of 27 ions are explained by cleavage at D or P residues. Data were acquired on the QE-HF, NCE= 30%, 200 mM aqueous ammonium acetate + 2x CMC LDAO.

Table S7: sequence ions for MDH1

Name	Theoretical Mass (Da)	Observed Mass (Da)	Mass Difference (Da)	Mass Difference (ppm)
b8	935.5440	935.5474	0.0034	3.6
b11	1164.6505	1164.6491	0.0014	-1.2
b12	1235.6874	1235.6807	-0.0066	-5.4
b15	1533.8515	1533.8561	0.0047	3.0
b16	1604.8886	1604.8936	0.0051	3.2
b17	1767.9519	1767.9542	0.0023	1.3
b20	2081.1520	2081.1421	-0.0100	-4.8
b23	2444.3315	2444.2989	-0.0326	-13.3
b32	3305.7295	3305.7335	0.0039	1.2
b33	3433.7881	3433.7672	-0.0209	-6.1
b35	3643.9249	3643.9112	-0.0137	-3.8
b36	3757.0090	3756.9844	-0.0246	-6.6
b37	3870.0930	3870.0817	-0.0114	-2.9
b38	3969.1615	3969.1603	-0.0011	-0.3
b39	4082.2455	4082.2462	0.0007	0.2
b40	4195.3296	4195.3127	-0.0169	-4.0
b41	4310.3565	4310.3656	0.0091	2.1
b42	4423.4406	4423.4107	-0.0299	-6.8
b43	4524.4883	4524.4536	-0.0347	-7.7
b50	5267.8229	5267.7479	-0.0750	-14.2
b58	6153.2495	6153.1801	-0.0694	-11.3
b120	12992.9401	12992.9429	0.0028	0.2
b130	14016.5860	14016.6045	-0.0185	1.3
y27	3136.5274	3136.5137	-0.0137	-4.4
y51	5938.0793	5938.0832	0.0039	0.7
y52	6053.1063	6053.0942	-0.0121	-2.0
y60	6842.4356	6842.3604	-0.0752	-11.0



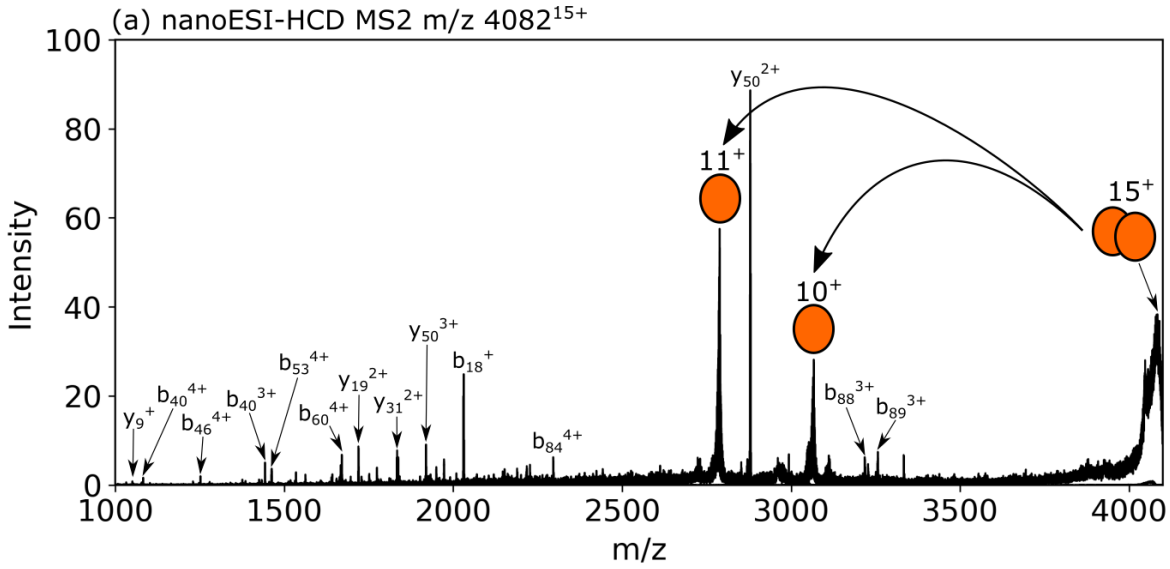
N	A	K	V	A	V	L	G	A⌉	S⌉	G	G	I⌉	G	Q⌉	P	L	S	L	L	L	K	N	S	P	L	25
26	V	S	R	L	T	L	Y	D⌉	I	A	H	T	P	G	V	A	A	D	L	S	H	I	E	T	R	50
51	A	N	V	K	G	Y	L	G	P	E	Q	L	P	D⌉	C	L	K⌉	G	C	D⌉	V	V	V	I	P	75
76	A	G	V	P	R	K	P	G	M	T	R	D⌉	D⌉	L	F	N	T	N	A	T	I	V	A	T	L	100
101	T	A	A	C	A	Q⌉	H⌉	C⌊	P	E	A	M⌉	I	C	I⌉	I⌊	S	N⌉	P	V	N	S	T	I⌊	P	125
126	I	T	A	E	V⌉	F	K	K⌊	H	G	V	Y	N	P	N	K	I	F	G	V	T	T	L	D	I	150
151	V	R	A	N	T	F	V	A	E	L	K	G	L	D⌊	P	A	R	V	N	V	P	V	I	G	G	175
176	H	A	G	K	T	I	I⌊	P	L	I	S	Q	C	T⌊	P	K	V	D	F	P	Q	D⌊	Q	L	A	200
201	⌊T	L	T	G	R	I	Q	E	A	G	T	E	V	V	K	A	K⌊	A⌊	G	A	G	S	A	T	L	225
226	S	M	A	Y	A	G	A	R	F	V	F	S	L	V	D	A	M	N	G	K	E⌊	G	V	I	E	250
251	C	S	F⌊	V⌊	Q⌊	S	K⌊	E⌊	T	E⌊	C	T	Y	F⌊	S⌊	T⌊	P	L	L	L⌊	G	K	K⌊	G	L	275
276	E	K⌊	N	L⌊	G	I⌊	G	K⌊	I⌊	T⌊	P	F	E⌊	E	K⌊	M⌊	I⌊	A⌊	E⌊	A	I⌊	P	E	L	K	300
301	⌊A	S	I⌊	K	K	G	E⌊	D⌊	F	V	K	N	M	K	C											

Figure S11: (a) nanoESI-HCD MS^2 spectrum of MDH2 showing subunits and sequence ion signals. Subunit ions confirm the assembly stoichiometry as homodimeric. Spectrum acquired on the QE-HF with an orbitrap resolution of 120,000 at m/z 200. (b) sequence ions of MDH2 assigned with a mass tolerance of 20 ppm. P-score = 2.8e-44. 21 of 61 ions are explained by cleavage at D or P residues. Data were acquired on the QE-HF, NCE = 26%, in 200 mM aqueous ammonium acetate + 2x CMC LDAO.

Table S8: sequence ions for MDH2

Name	Theoretical Mass (Da)	Observed Mass (Da)	Mass Difference (Da)	Mass Difference (ppm)
b8	709.4486	709.4516	0.0030	4.2
b9	796.4807	796.4837	0.0031	3.9
b12	1023.6076	1023.6007	-0.0070	-6.8
b14	1208.6877	1208.6928	0.0051	4.2
b33	3331.9231	3331.8772	-0.0459	-13.8
b64	6582.5965	6582.6395	0.0431	6.5
b67	6926.7847	6926.7558	-0.0289	-4.2
b70	7201.8423	7201.7894	-0.0529	-7.3
b87	8974.8506	8974.8402	-0.0104	-1.2
b88	9089.8775	9089.8829	0.0054	0.6
b106	10893.7964	10893.7466	-0.0498	-4.6
b107	11030.8553	11030.8523	-0.0030	-0.3
b108	11133.8645	11133.7957	-0.0688	-6.2
b112	11562.0375	11561.9269	-0.1105	-9.6
b115	11891.2148	11891.1617	-0.0531	-4.5
b116	12004.2988	12004.2738	-0.0250	-2.1
b118	12205.3738	12205.3202	-0.0536	-4.4
b124	12816.7017	12816.5978	-0.1039	-8.1
b130	13427.0343	13426.8845	-0.1497	-11.2
b164	17213.0963	17212.9301	-0.1662	-9.7
b182	18993.1434	18992.9855	-0.1579	-8.3
y6	765.4207	765.4236	0.0029	3.8
y7	880.4477	880.4459	-0.0017	-2.0
y11	1322.7019	1322.6998	0.0021	-1.6
y14	1593.8548	1593.8408	-0.0141	-8.8
y18	2061.1292	2061.1107	-0.0185	-9.0
y20	2245.2504	2245.2596	0.0092	4.1
y21	2374.2930	2374.2635	-0.0295	-12.4
y22	2445.3301	2445.3249	-0.0051	-2.1
y23	2558.4141	2558.4003	-0.0138	-5.4
y24	2689.4546	2689.4514	-0.0033	-1.2
y26	2946.5922	2946.5802	-0.0119	-4.1
y29	3319.7559	3319.7565	0.0006	0.2
y30	3420.8036	3420.7723	-0.0314	-9.2
y31	3533.8877	3533.8903	0.0026	0.7
y33	3719.0041	3718.9926	-0.0115	-3.1
y35	3889.1096	3889.0749	-0.0348	-8.9
y37	4116.2366	4116.2203	-0.0163	-4.0
y41	4543.4797	4543.4546	-0.0251	-5.5
y44	4856.6911	4856.6697	-0.0214	-4.4
y48	5292.9960	5292.9648	-0.0312	-5.9
y49	5394.0437	5394.0297	-0.0140	-2.6

y50	5481.0757	5481.0439	-0.0319	-5.8
y54	5995.2643	5995.2021	-0.0622	-10.4
y56	6225.3546	6225.3254	-0.0292	-4.7
y57	6354.3972	6354.3919	-0.0053	-0.8
y59	6569.5242	6569.4830	-0.0412	-6.3
y60	6697.5827	6697.5305	-0.0522	-7.8
y61	6796.6512	6796.6061	-0.0451	-6.6
y68	7531.9773	7531.9224	-0.0549	-7.3
y96	10334.3242	10334.2450	-0.0792	-7.7
y97	10405.3613	10405.2900	-0.0712	-6.8
y114	12187.3612	12187.2128	-0.1484	-12.2
y117	12499.5409	12499.4693	-0.0717	-5.7
y125	13425.9907	13425.9533	-0.0375	-2.8
y132	14168.3591	14168.3513	-0.0078	-0.5
y150	15948.4062	15948.3854	-0.0208	-1.3
y181	19331.2099	19331.1069	-0.1029	-5.3
y190	20344.8035	20344.8329	-0.0294	1.4
y198	21157.2036	21157.0005	-0.2031	-9.6
y206	22027.6406	22027.6861	-0.0455	2.1



(b) omega-amidase sequence ions

N	S	T	F	R	L	A	L	I	Q	L	Q	V	S	S	I	K	S	D⌉	N	I	T	R	A	C	S	25
26	L	V	R	E	A	A	K	Q	G	A	N	I⌉	V⌉	S	L⌉	P	E⌉	C	F⌉	N⌉	S⌉	P	Y	G⌉	T	50
51	N⌉	Y	F⌉	P	E	Y	A	E⌉	K⌉	I⌉	P	G	E	S	T	K	K	L	S	E	V⌉	A	K⌉	E⌉	N	75
76	S⌉	I	Y⌉	L⌉	I⌉	G⌉	G	S	I⌉	P	E	E	D⌉	D⌉	G	K	L	Y	N	T	C	A	V	F	G	100
101	P	D	G	N	L	L	V	K	H	R	K	I	H	L	F	D	I	D	V	P	G	K	I	T	F	125
126	Q	E	S	K	T	L	S	P	G	D	S	F	S	T	F	D⌉	T	P	Y	C	R	V	G	L	G	150
151	I	C	Y	D⌊	M	R	F	A	E	L	A	Q	I	Y	A	R	R	G	C	Q	L	L	V	Y	P	175
176	G	A	F	N	M	T	T	G	P	A	H	W	E	L	L	Q	R	A	R	A	V	D	N	Q	V	200
201	Y	V	A	T	A	S	P	A	R	D	E	K	A	S	Y	V	A	W	G	H	S	T	V	V	D	225
226	⌊P	W	G	Q	V	L	T	K	A	G	T	E	E	T	I	L	Y	S	D⌊	I	D⌊	L	K	K	L	250
251	S	E	I	R	Q	Q	I	P	I	L	K	Q	K	R	A	D⌊	L	Y	S	V	E	S	K	K	P	275

Figure S12: (a) nano-ESI-HCD MS2 spectrum showing subunit signals and sequence ions of omega-amidase. Subunit ions confirm the assembly stoichiometry as homodimeric.. (b) Sequence ions of N-terminally acetylated omega-amidase automatically assigned with a mass tolerance of 20 ppm. P-score = 3.3e-15. 14 of 31 ions are explained by cleavage at D or P residues. Data were acquired on the Orbitrap Eclipse, (NCE = 46 – 55%) using a 200 mM ammonium acetate + 0.5x CMC C8E4 solvent system.

Table S9: sequence ions for omega-amidase

Name	Theoretical Mass (Da)	Observed Mass (Da)	Mass Difference (Da)	Mass Difference (ppm)
b18	2029.1208	2029.1123	-0.0085	-4.2
b37	4025.1844	4025.1479	-0.0365	-9.1
b38	4124.2528	4124.2083	-0.0444	-10.8
b40	4324.3689	4324.3262	-0.0427	-9.9
b42	4550.4642	4550.4378	-0.0265	-5.8
b44	4800.5418	4800.4908	-0.0510	-10.6
b45	4914.5847	4914.5425	-0.0423	-8.6
b46	5001.6168	5001.5996	-0.0172	-3.4
b49	5318.7543	5318.7069	-0.0475	-8.9
b51	5533.8449	5533.7869	-0.0580	-10.5
b53	5843.9767	5843.9466	-0.0301	-5.1
b58	6433.2151	6433.1711	-0.0439	-6.8
b59	6561.3100	6561.2365	-0.0735	-11.2
b60	6674.3941	6674.3311	-0.0630	-9.4
b71	7830.0076	7829.9635	-0.0441	-5.6
b73	8029.1397	8029.0828	-0.0568	-7.1
b74	8158.1823	8158.1063	-0.0759	-9.3
b76	8359.2572	8359.2038	-0.0534	-6.4
b78	8635.4046	8635.3360	-0.0686	-7.9
b79	8748.4887	8748.3975	-0.0912	-10.4
b80	8861.5727	8861.4898	-0.0830	-9.4
b81	8918.5942	8918.5110	-0.0831	-9.3
b84	9175.7317	9175.6589	-0.0729	-7.9
b88	9645.8966	9645.8248	-0.0719	-7.4
b89	9760.9236	9760.8289	-0.0947	-9.7
b141	15494.8528	15494.6840	-0.1688	-10.9
y9	1049.5760	1050.6445	-1.0685	-6.8
y29	3436.0293	3435.9857	-0.0436	-12.7
y31	3664.1403	3664.0969	-0.0434	-11.8
y50	5753.1770	5753.1382	-0.0389	-6.8
y121	13657.1237	13656.9793	-0.1443	-10.6

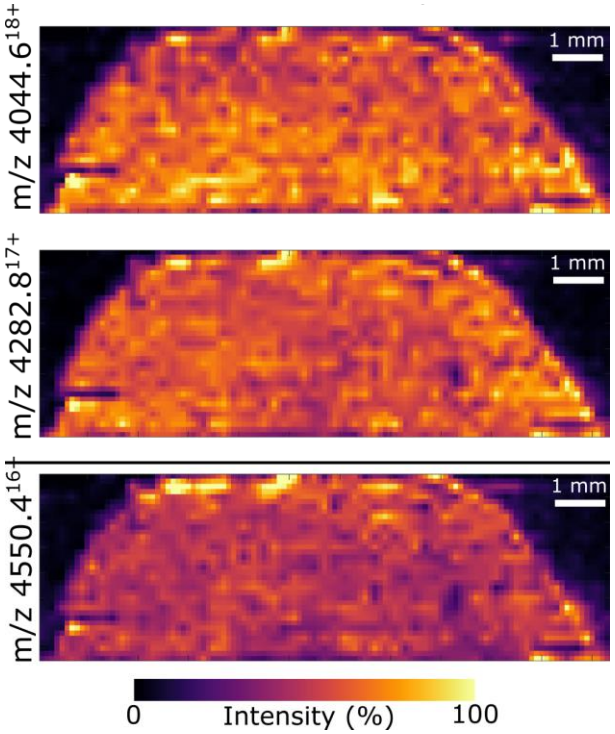


Figure S13: ion images for the 18+, 17+ and 16+ charge states of the MDH1 homodimer showing homogenous distribution.

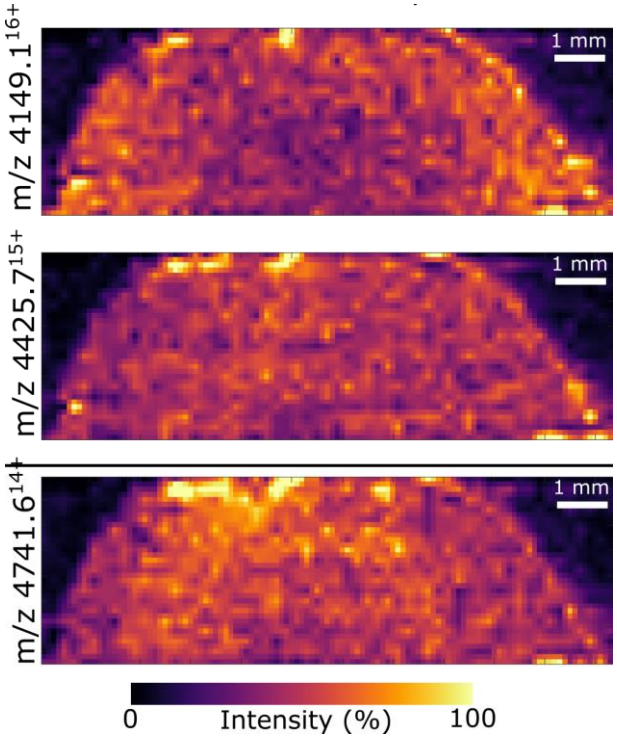


Figure S14: ion images for the 16+, 15+ and 14+ charge states of MDH2 showing homogenous distribution.

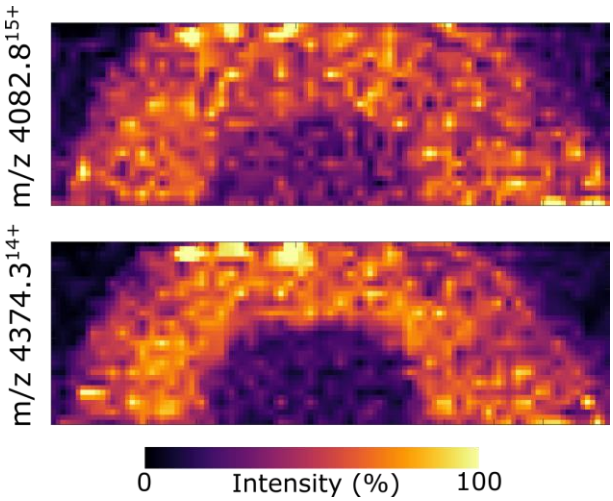


Figure S15: ion images for 15+ and 14+ charge states of omega-amidase homodimer showing distribution throughout cortex tissue.

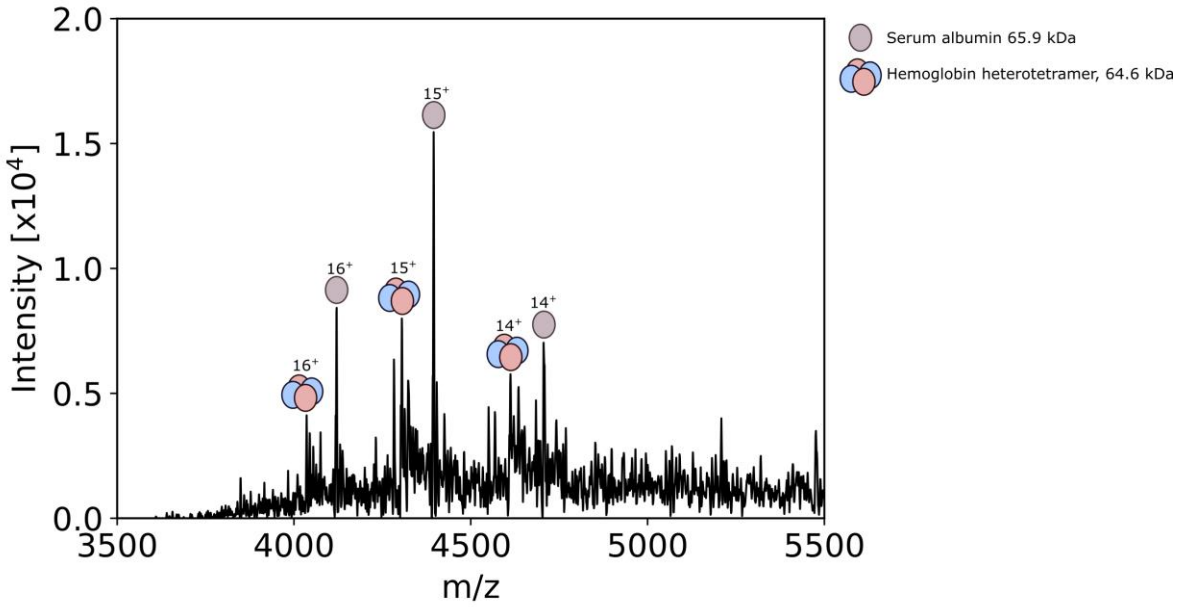


Figure S16: native mass spectrum composed of the average signals from 12 scans in a vascular region by nano-DESI. Signals for serum albumin and intact hemoglobin heterotetramer dominate the spectrum.

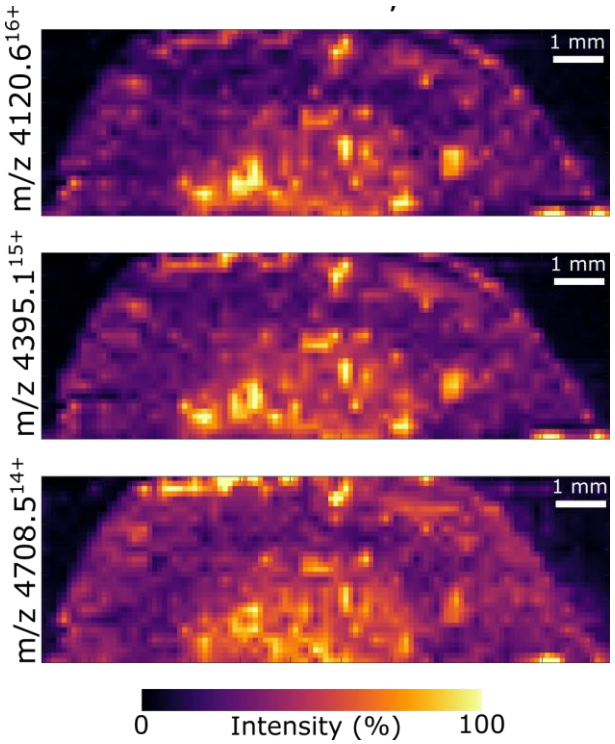


Figure S17: ion images for 16+, 15+ and 14+ charge states of serum albumin showing distribution in vascular regions and the medulla.

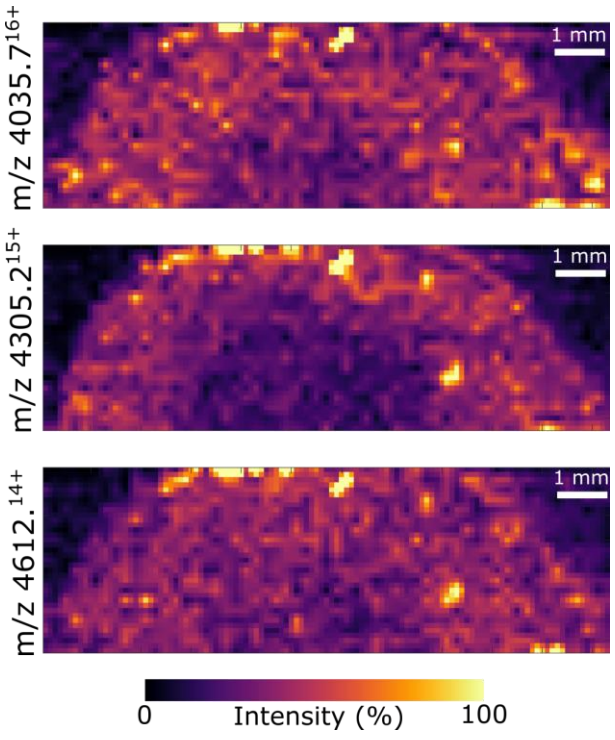


Figure S18: ion images for 16+, 15+ and 14+ charge states of hemoglobin heterotetramer showing distribution in blood vessels.

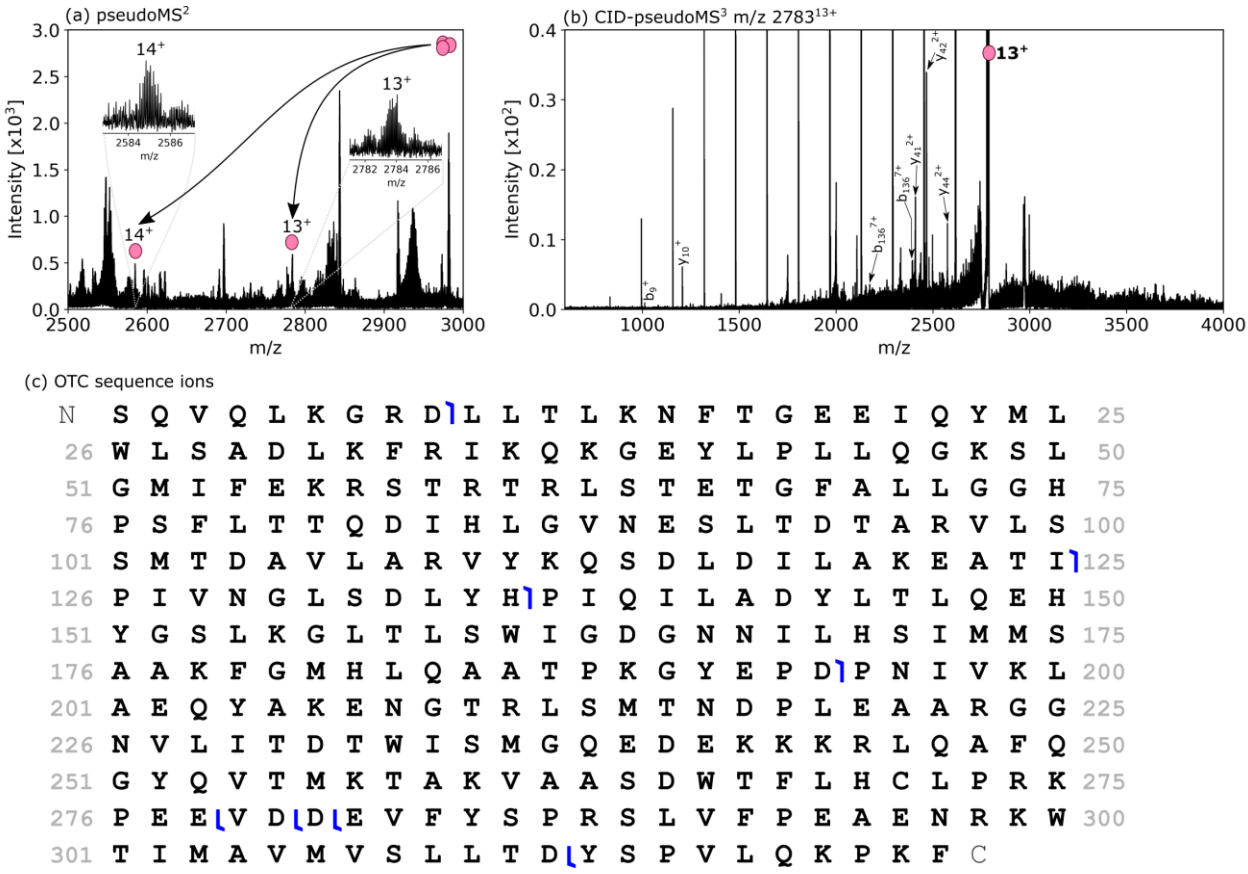


Figure S19: (a) nanoESI-pseudoMS^2 by in-source fragmentation enabled detection of isotopically resolved OTC monomers consistent with the intact assembly being a homotrimer (Data were acquired on the Orbitrap Eclipse at orbitrap resolution = 500,000 at m/z 200). (b) NanoESI-pseudoMS^3 by isolation and CID fragmentation of the 13+ monomer provided main sequence product ions from OTC. P-score = 0.15. 8 of 10 sequence ions are explained by cleavage at D or P residues. The regular intense peaks are attributable to product ions from carbohydrate precursor ions co-isolated with OTC 13+ which give a repeating neutral loss of -162Da. (c) Sequence ions assigned with a mass tolerance of 20 ppm.

Table S10: sequence ions for OTC.

Name	Theoretical Mass (Da)	Observed Mass (Da)	Mass Difference (Da)	Mass Difference (ppm)
b9	1011.5464	1011.5478	-0.0014	-5.5
b125	13992.4582	13992.4066	-0.0516	-3.7
b136	15201.0772	15200.8124	-0.2648	-17.4
b194	21537.2621	21537.1460	-0.1161	-5.4
y10	1205.6808	1205.6773	-0.0035	-2.9
y41	4815.5172	4815.4878	-0.0294	-6.1
y42	4930.5441	4930.5140	-0.0301	-6.1
y44	5144.6394	5144.6696	0.0302	5.9

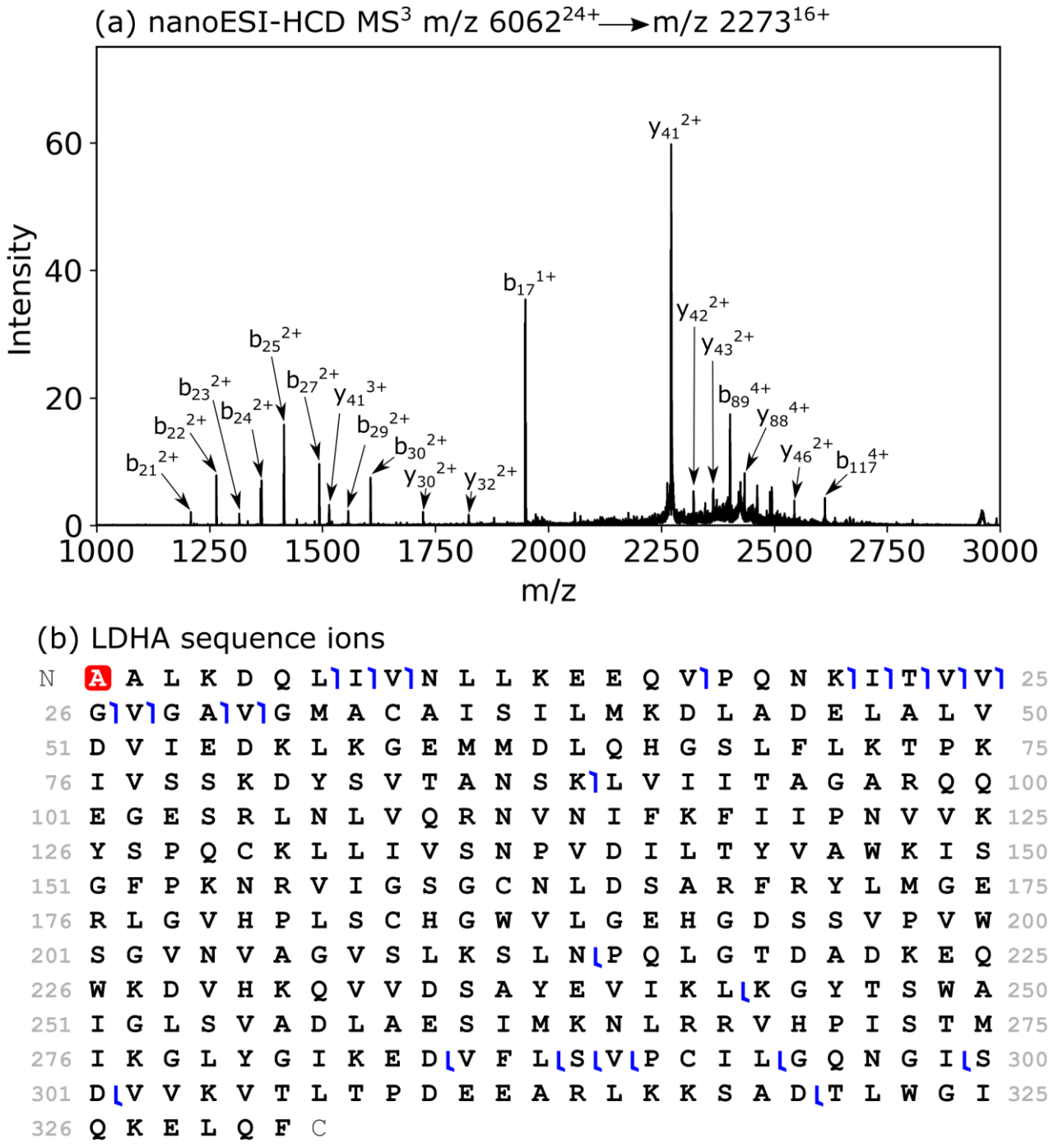


Figure S20: (a) nanoESI-HCD MS3 of LDHA. Tetramers were dissociated to monomers by HCD (NCE = 40%) then the monomer was isolated and dissociated by HCD (NCE = 60%). (b) LDHA sequence ions assigned within a mass tolerance of 20 ppm. P-score = 3.5e-10. 6 of 24 sequence ions were the result of cleavage at the C-terminus of D or N-terminus of P.

Table S11: Sequence ions for LDHA

Name	Theoretical Mass (Da)	Observed Mass (Da)	Mass difference (Da)	Mass Difference (ppm)
b7	781.4334	781.4385	-0.0051	-2.8
b8	894.5174	894.5222	-0.0048	-2.8
b17	1947.1040	1947.112	-0.0080	-3.4
b9	993.5859	993.5897	-0.0038	-3.5
b21	2414.3532	2414.36	-0.0064	-3.4
b22	2527.4374	2527.443	-0.0058	-3.5
b23	2628.4850	2628.492	-0.0070	-2.9
y11	1361.7343	1361.737	-0.0025	-3.5
b24	2727.5534	2727.561	-0.0074	-2.6
b25	2826.6218	2826.626	-0.0046	-3.5
b26	2883.6432	2883.649	-0.0056	-3.1
b27	2982.7118	2982.716	-0.0042	-3.5
y41	4539.4048	4539.411	-0.0059	-3.5
b29	3110.7702	3110.772	-0.0014	-4.2
b30	3209.8388	3209.842	-0.0028	-3.7
y30	3441.8872	3441.883	0.0044	-5.5
y32	3643.9460	3643.971	-0.0246	2.7
b17	1947.1041	1947.105	-0.0006	-3.4
y37	4113.1746	4113.189	-0.0146	0.0
y41	4539.4046	4539.403	0.0018	-3.6
y42	4638.4730	4638.463	0.0096	-5.2
y43	4725.5050	4725.524	-0.0194	1.0
b89	9601.1289	9601.1	0.0285	-6.0
y88	9727.2029	9727.279	-0.0759	4.8
y46	5084.7260	5084.689	0.0370	-10.1
y117	13047.9071	13047.92	-0.0161	-1.6

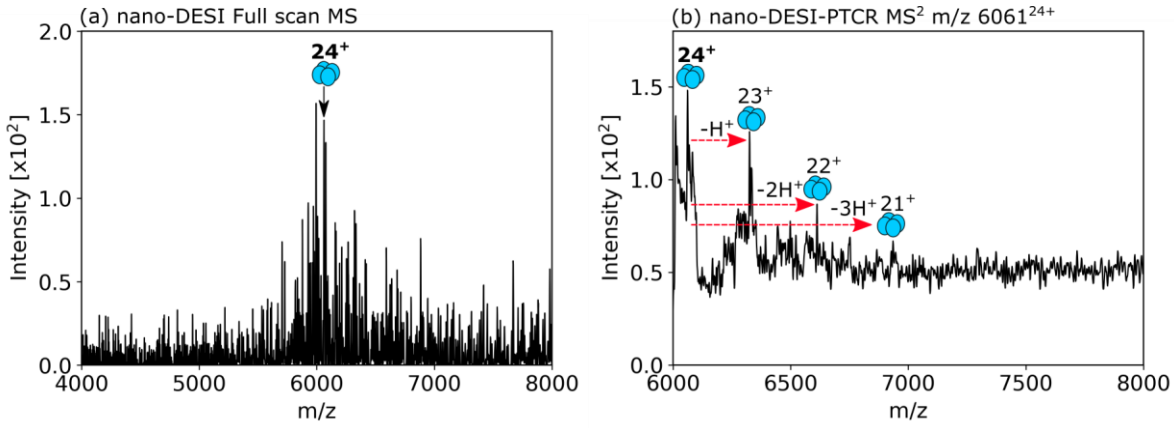


Figure S21: nano-DESI analysis of LDHA in kidney tissue (a) full scan mass spectrum with 24+ charge state detected and (b) PTCR MS2 spectrum of m/z 6061^{24+} enabling confirmation of charge state and intact mass. The data is much noisier than the equivalent data obtained with nanoESI. Data were acquired on the Orbitrap Eclipse at an orbitrap resolution of 7500 at m/z 200.

References

(1) Randall, E. C.; Bunch, J.; Cooper, H. J. Direct analysis of intact proteins from Escherichia coli colonies by liquid extraction surface analysis mass spectrometry *Anal Chem* **2014**, *86*, 10504-10510.