Multi-plexed proteome analysis with neutron-encoded stable isotope labeling in cells and mice

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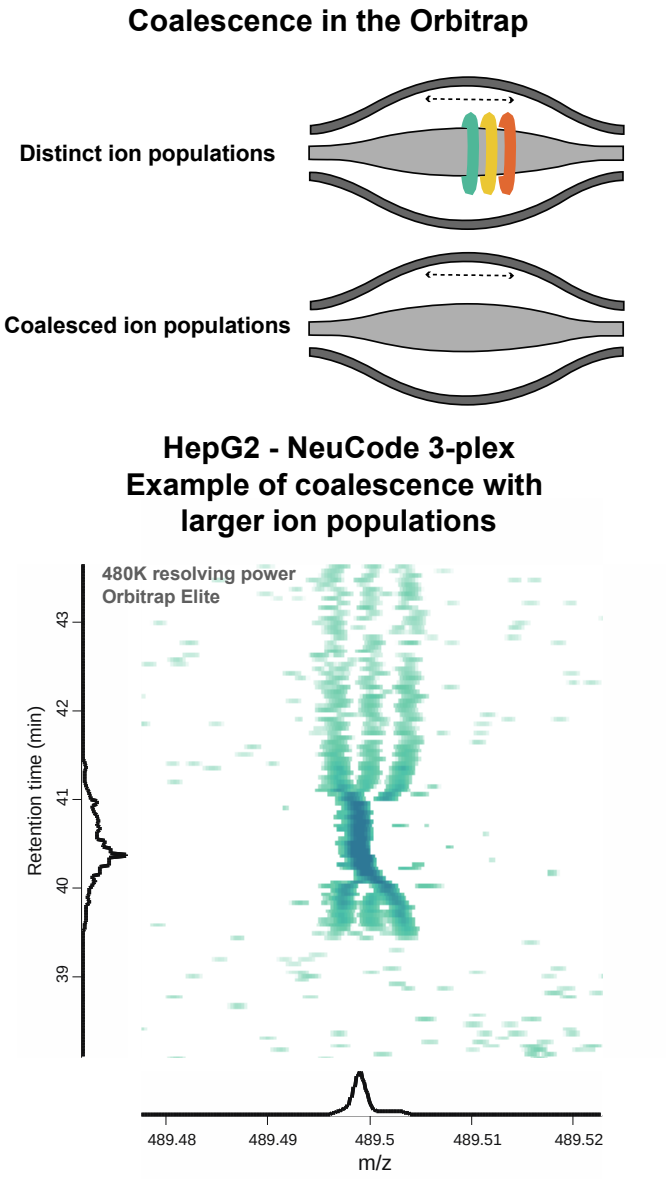
Supplementary Material



Supplementary Figure 1. High-abundance ion populations in the Orbitrap can exhibit space charge induced frequency shifts resulting in coalescence of the observed m/z.

Coalescence is more likely to occur with NeuCode peaks due to the close mass-to-charge but will occur in proportion to the abundance of the ion population. Exampled in this figure is the observed coalescence of the NeuCode channels with increased ion intensity. Reduction of the automatic gain control (AGC) target will limit the ions permitted into the Orbitrap mass analyzer and thus minimize the coalescence of NeuCode peaks (see ?Troubleshooting).

Supplementary Table 1. Orbitrap Elite method settings for 240,000 MS^1 resolving power.

Scan Event	Method Setting	Value
Scan Event 1	Analyzer	FTMS
	Mass Range	Normal
	Resolution	240000
	Scan Type	Full
	Polarity	Positive
	Data Type	Profile
	Scan Range	300-1250 m/z
Scan Event 2-21	Analyzer	Ion Trap
	Mass Range	Normal
	Scan Rate	Rapid
	Data Type	Centroid
	Dependent Scan	Yes
	Mass determined from	Scan Event 1
	Activation Type	CID
	Default charge state	2
	Minimum Signal Required	500
	Isolation width m/z	2
	Normalized collision energy	35
	Activation Q	0.25
	Activation time (ms)	5
	Dynamic Exclusion	Enabled
	Repeat count	1
	Repeat duration (s)	45
	Exclusion list size	500
	Exclusion duration	45
	Exclusion mass width low	25 ppm
	Exclusion mass width high	10 ppm

Supplementary Table 2. Orbitrap Fusion or Fusion Lumos method settings for 240,000 MS^1 resolving power.

Experiment	Method Parameter	Value
Experiment 1	Cycle time (sec)	2
	MS OT	
	Detector Type	Orbitrap
	Orbitrap Resolution	240000
	Mass Range	Normal
	Use Quadrupole Isolation	True
	Scan Range (m/z)	350-1100

RF Lens (%)	30
AGC Target	1.0e6
Maximum Injection Time (ms)	100
Microscans	1
Data Type	Profile
Polarity	Positive
Source Fragmentation	Disabled
Use EASY-IC	False
Monoisotopic Precursor Selection (MIPS) Filter	
Monoisotopic Peak Determination	Peptide
Relax restrictions when too few precursors are found	True
Exclude undetermined Charge States	True
Charge State Filter	
Include charge state(s)	2-6
Include undetermined charge states	False
Include charge states 25 and higher	False
Dynamic Exclusion Filter	
Exclude after n times	1
Exclusion duration (sec)	5
Mass Tolerance	m/z
Low	0.55
High	1.55
Exclude Isotopes	False
Perform dependent scan on single charge state per precursor only	False
Decisions	
Data dependent mode	TopSpeed
Precursor Priority	Most Intense
Number of Scan Event Types	1
Scan Event Type 1	ddMS2 IT HCD
MS_n Level	2
Isolation Mode	Quadrupole
Use isolation m/z offset	False
Activation Type	HCD
HCD Collision Energy (%)	30
Stepped Collision Energy	False
Detector Type	Ion Trap
Scan Range Mode	Define m/z range
Ion Trap Scan Rate	Turbo
Scan Range (m/z)	200-1200
AGC Target	1.0e4
Injection ions for all available parallelizable time	False
Maximum Injection Time (ms)	15
Microscans	1
Data Type	Centroid