

**Supplementary Table 1. Comparison of protein identification and characterization results in pTop and MSPATHfinder for human ovarian tumor lysate sample.** Running times for deconvolution and database search, and the number of proteins, proteoforms, and protein-spectrum matches (PrSMs) identified by pTop and MSPATHfinder at 1% FDR. The search for internal cleaved proteoforms was disabled in MSPATHfinder because pTop v1.2 is able to search only non-cleaved proteoforms. Detailed parameter settings used in MSPATHfinder and pTop are in Supplementary Table 2 and 3, respectively.

		pTop v1.2	MSPATHfinder
Run Time (minutes)	Deconvolution	19.4	52.9
	Database Search	48.5	33.6
	Total	67.9	86.5
#Proteoform-Spectrum Matches (PrSMs)		351	392
#Proteins		37	45
#Proteoforms		67	78

**Supplementary Table 2. Parameter settings for ProMex, MSDeconv+, and ICR-2LS.**

	ProMex	MSDeconv+		ICR-2LS	
Min. charge	2	1		2	
Max. charge	60	30		50	
Min. mass	2000 Da	N/A		2000 Da	
Max. mass	50000 Da	50000 Da		50000 Da	
Tool-specific parameters	N/A	M/Z error tolerance	0.02	MaxFit	0.2
				SignalToNoiseThreshold	2
		Signal/Noise Ratio	1	PeakBackgroundRatio	2
				MinIntensityForScore	10

**Supplementary Table 3. Parameter settings for MSPATHfinder, MS-Align+ (TopPIC v0.9.1), ProSightPC v3.0, and pTop v1.2**

	MSPATHfinder		MS-Align+ (TopPIC v0.9.1)		ProSightPC v3.0	pTop v1.2		
Search mode*	multiple internal cleavages		N/A		Biomarker discovery	N/A		
Precursor error	10 ppm		15 ppm		10 ppm	± 3.2 Da		
Fragment error	10 ppm		15 ppm		10 ppm	± 10 ppm		
Max. mass	50000 Da		N/A		N/A	50000 Da		
Min. mass	2000 Da		N/A		N/A	N/A		
Variable modifications	Oxidation at Met Dehydro at Cys Acetyl at Protein N-terminal		N/A		N/A	Oxidation[M] Dehydro[C] Acetyl[ProteinN-term]		
Max. modifications per sequence	4		N/A		N/A	4		
Tool-specific parameters	Min. sequence length	21	Cysteine protecting group	no modification	N/A	Precursor Detection	Isolation Width	10
	Max. sequence length	300					Mixture Spectra	checked
	Min. precursor ion charge	2	Max. unexpected PTM mass	10 <sup>6</sup> Da		Deconvolution	Max. charge	30
	Max. precursor ion charge	60					Max. mass	50000 Da
	Min. product ion charge	1	Max. unexpected PTMs	2		M/Z tolerance	20 ppm	
	Max. product ion charge	20	Cutoff type	EVALUE		S/N Ratio	1.5	
	tagSearch*	1 (enable)	Cutoff value	0.01				

\*In the comparison with pTop, SearchMode and tagSearch in MSPATHfinder were set to no-cleavage and 0 (disable), respectively