Phosphoproteomics with *phosphomatics*

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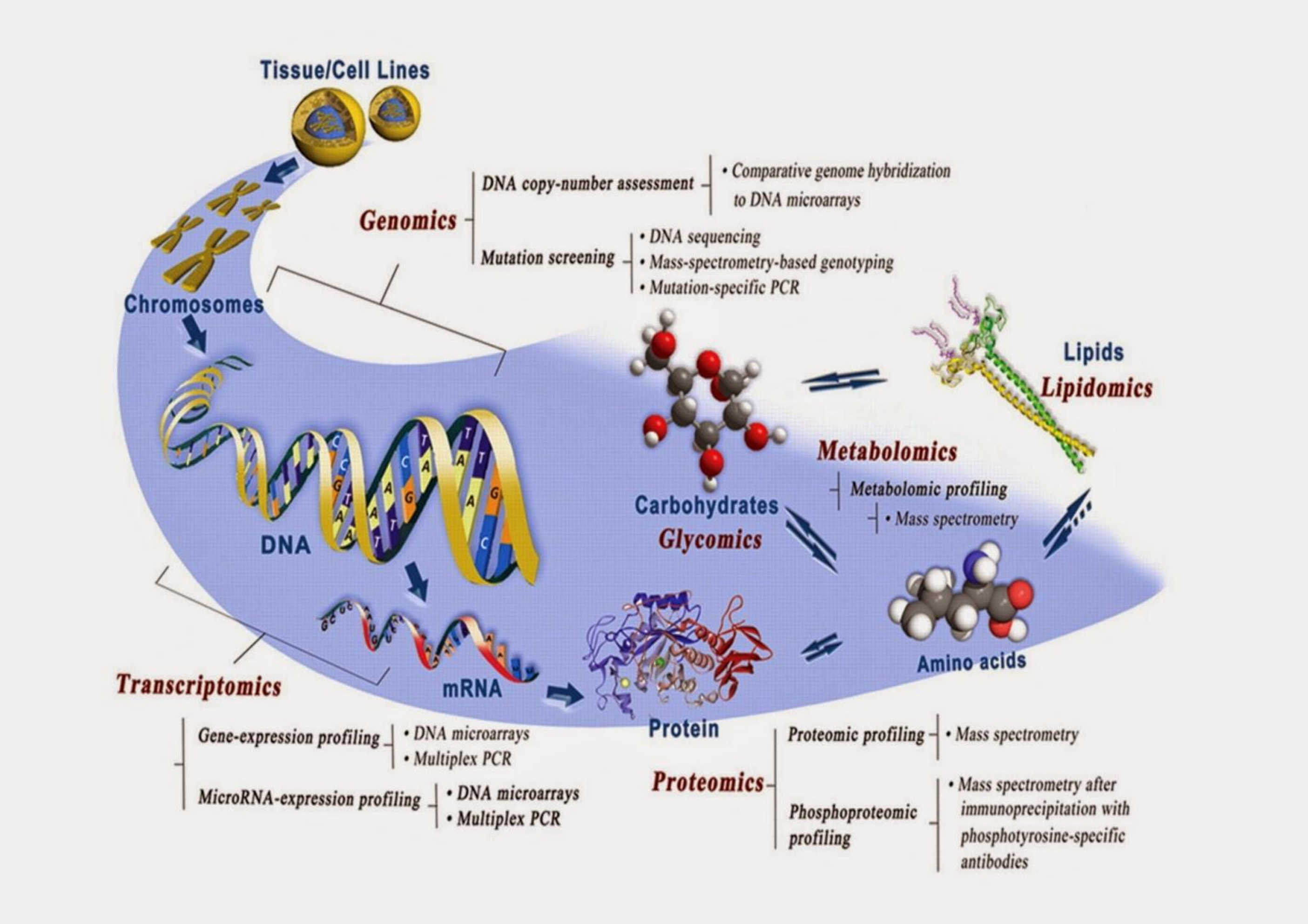
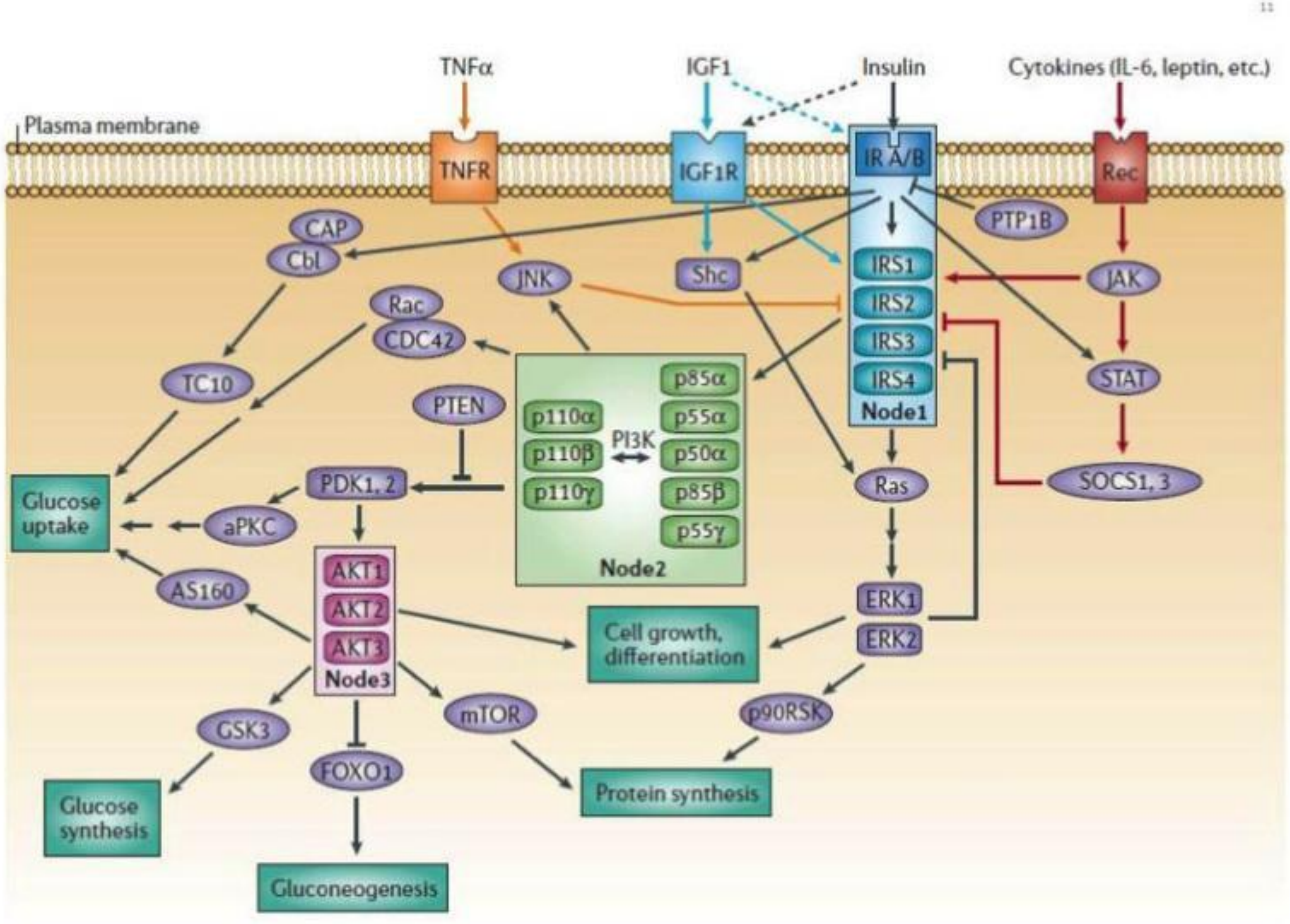
Background: ‘Omics



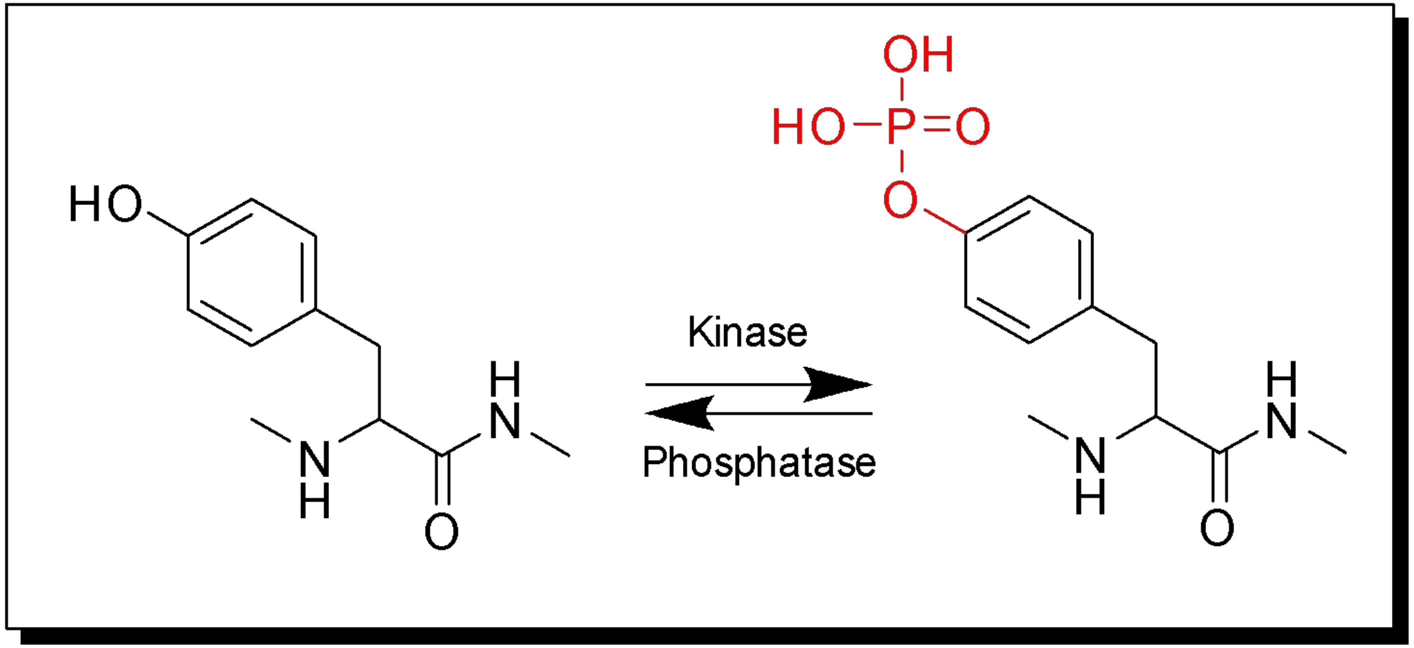
Wu *et al*. JDR, **2011,** 90, 561-572

Background: Signaling networks



De Meyts P. The Insulin Receptor and Its Signal Transduction Network (2016)

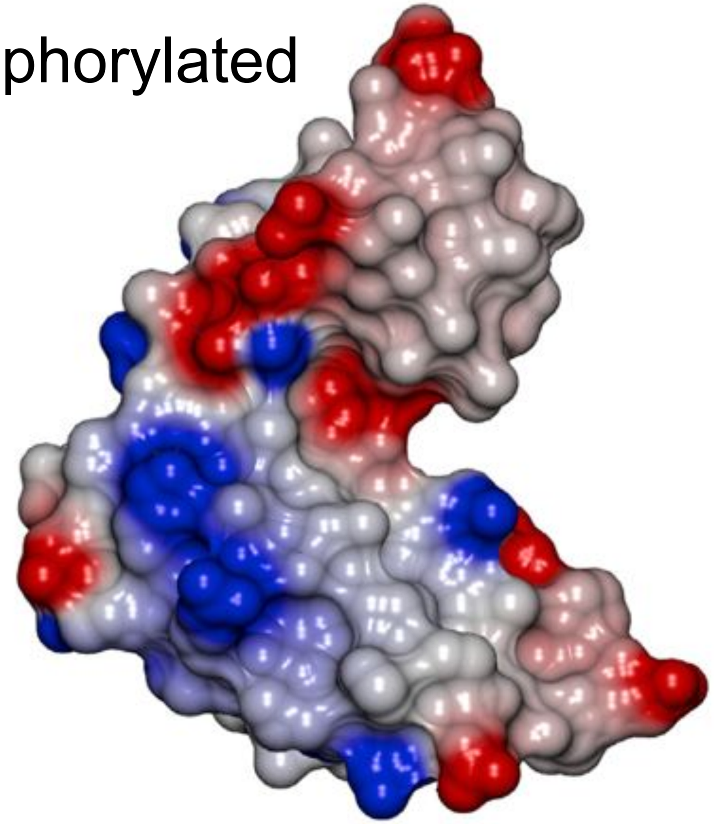
Background: Signal transduction



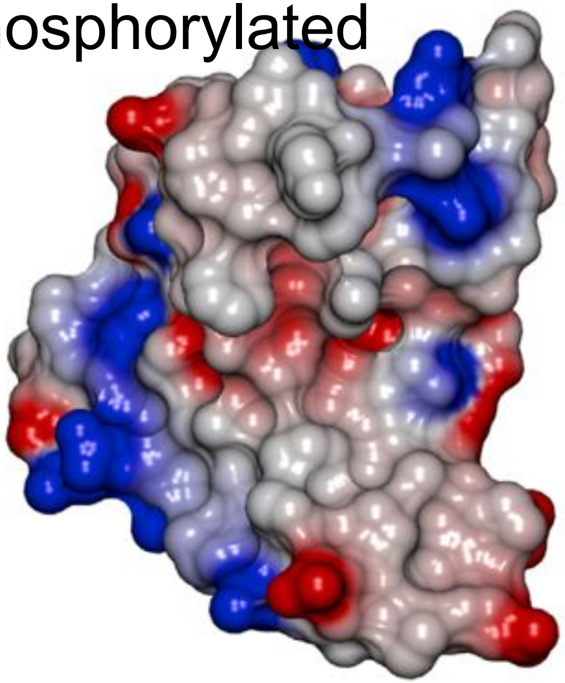
Protein phosphorylation

Cysteine string protein

Unphosphorylated



Serine 10 phosphorylated



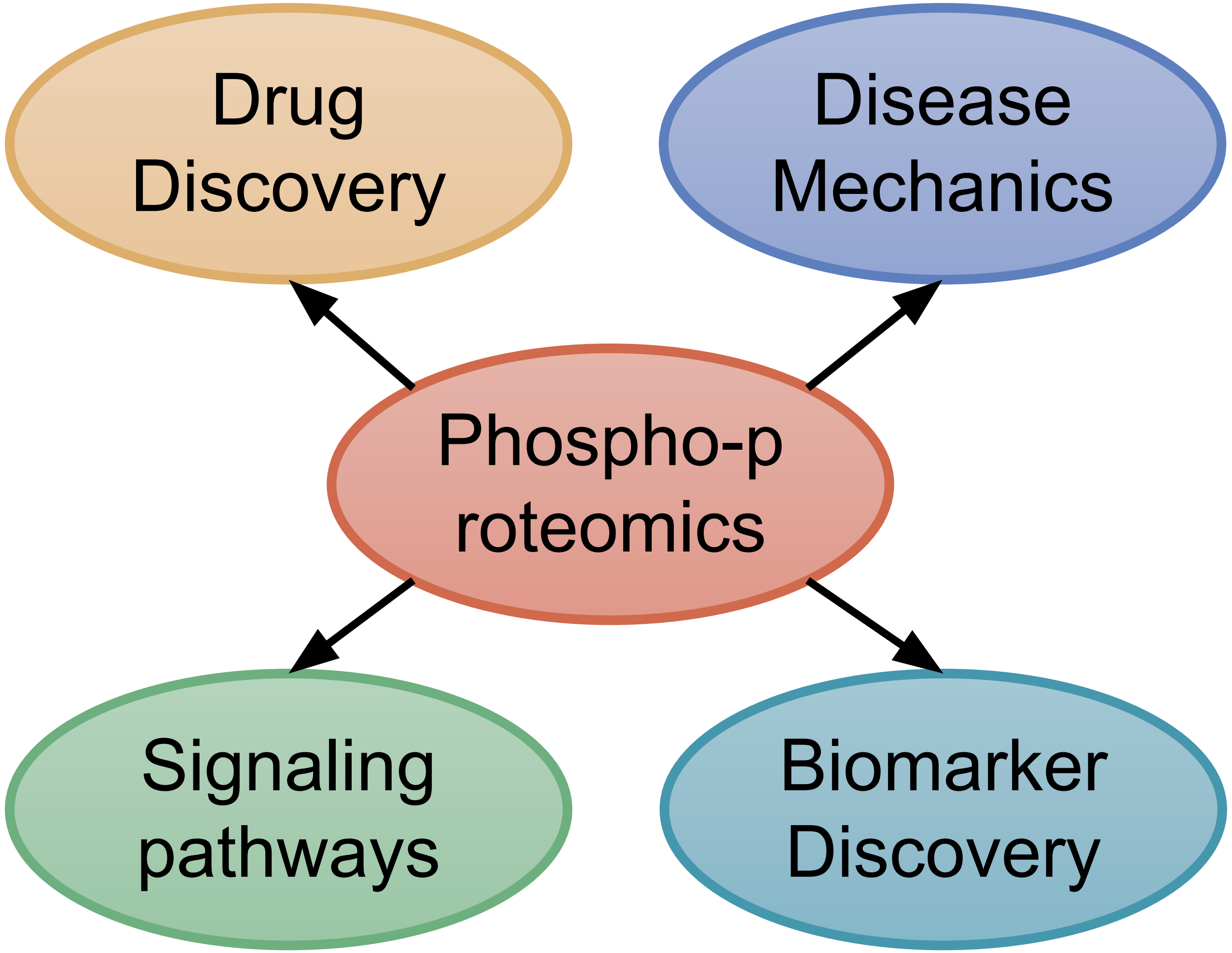
Patel et al. *Structure*, **2016**, 24, 1380–1386

Protein phosphorylation

- Very common (~75% of all proteins)
  - Occurs at Serine, Threonine and Tyrosine
- Critical in signaling transduction pathways
- Multiple phosphorylation of the same protein
  - Can give rise to different (even opposing) functions
- Can be reversible
  - Phosphatase enzymes can remove phospho groups
- Phosphorylation-induced conformational changes can control:
  - Activation state, dimerization, localisation…

Phosphoproteomics

- Global analysis of the protein phosphorylation profile of a sample
- Want to know:
  - Proteins phosphorylated
  - Site of phosphorylation
  - Quantity of phosphorylation



Drug Discovery

Disease Mechanics

Phospho-proteomics

Signaling pathways

Biomarker Discovery

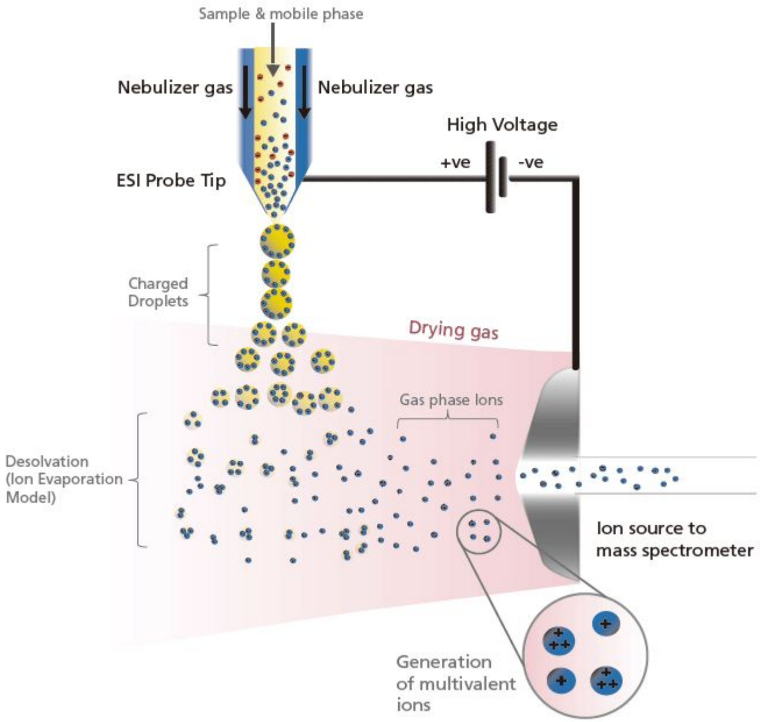
Phosphoproteomics: Experiments

Mass Spectrometry

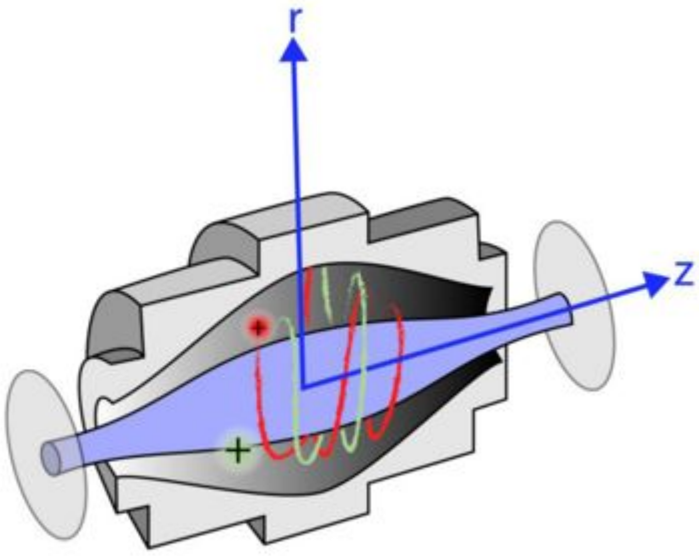
Generate ions

Measure *m/z*

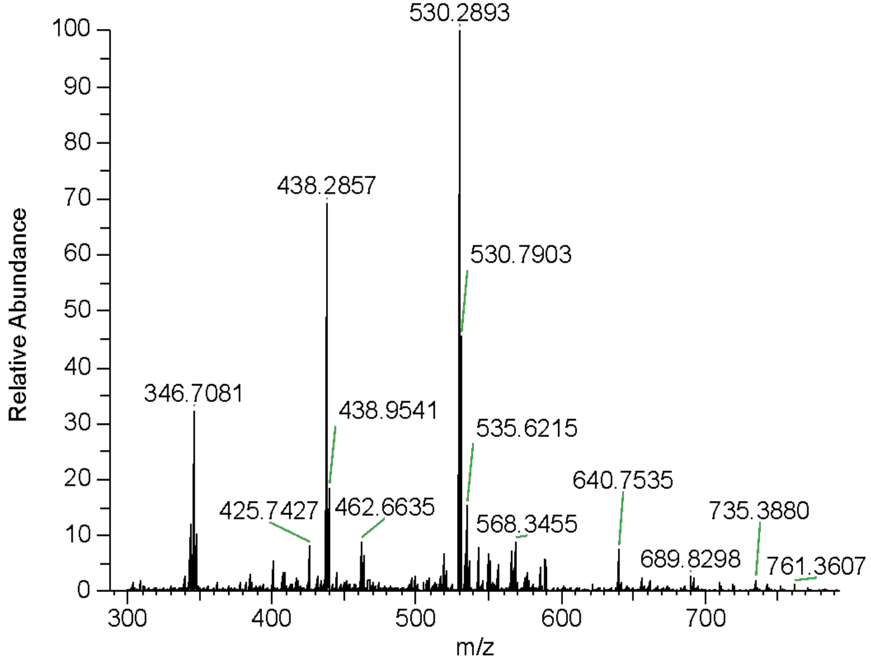
Produce
‘mass spectrum’



Shimadzu: Interfaces for LCMS



Savaryn JP et al. *Proteomics*. **2016**, 16, 2435



Mass spectrometry for proteomics

- Measuring the mass of a peptide is not enough!
- Eg: The *m/z* of a peptide 1+ peptide is measured at **698.358**
  - From this, we may be able to deduce that the peptide contains:

    1xS, 1xV, 1xA, 1xH, 1xR, 1xE
  - But in which order?

    SEVAHR, ESVAHR, VSEAHR, EVSAHR, VESAHR,

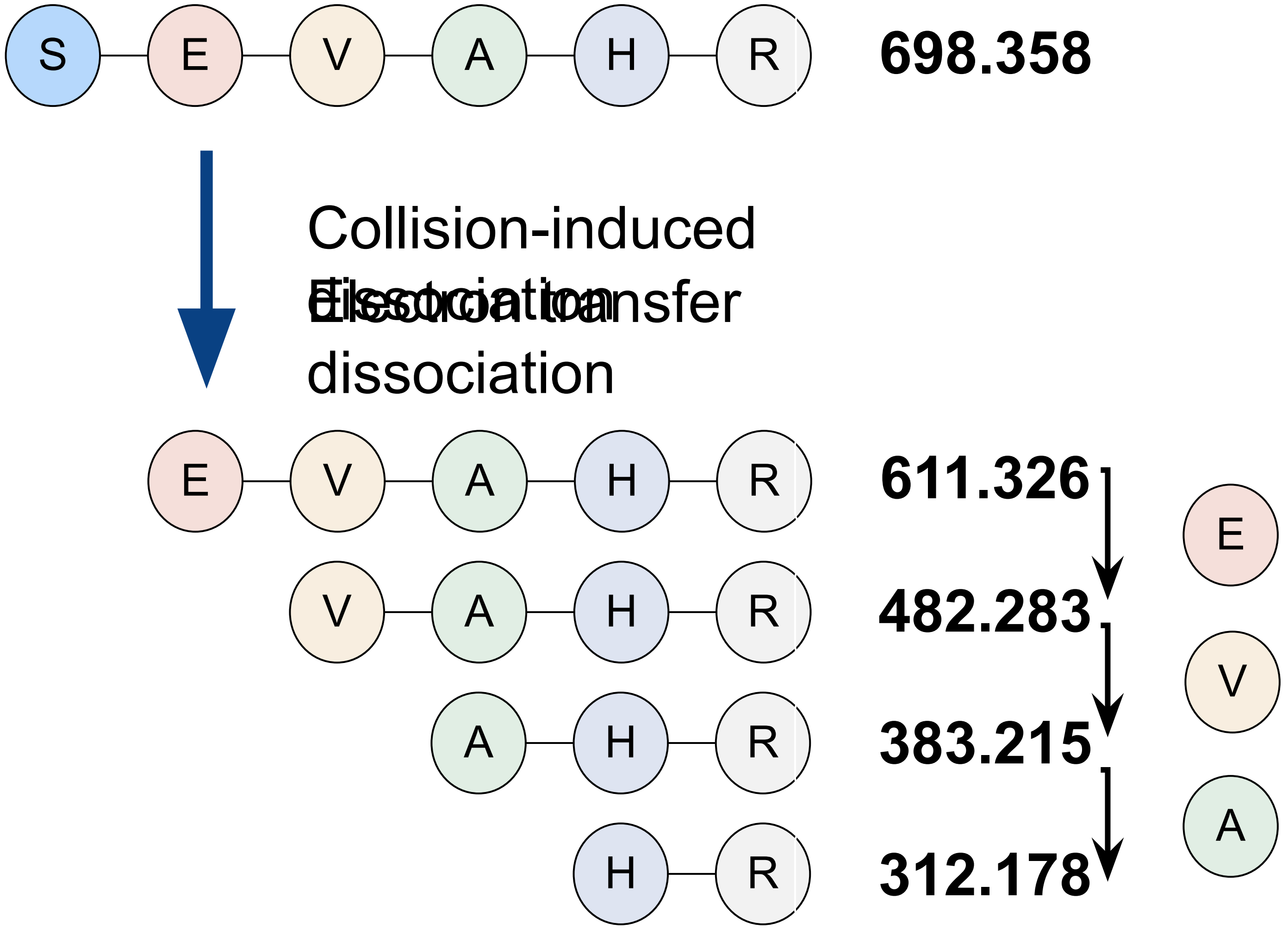
    AESVHR, EASVHR, SAEVHR, EAVSHR, AEVSHR….
- How can we differentiate between these?

Mass spectrometry for proteomics

Measure peptide ‘intact’ *m/z*

Fragment peptide ion

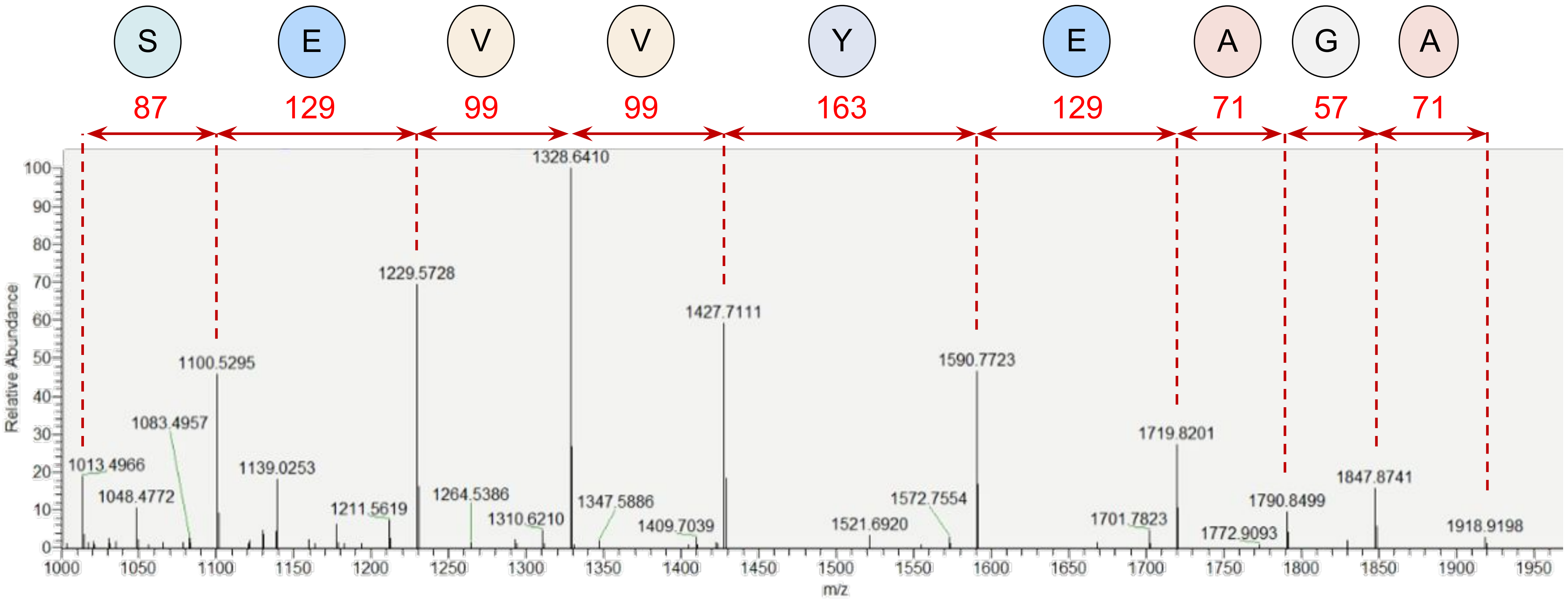
Measure *m/z* of fragments



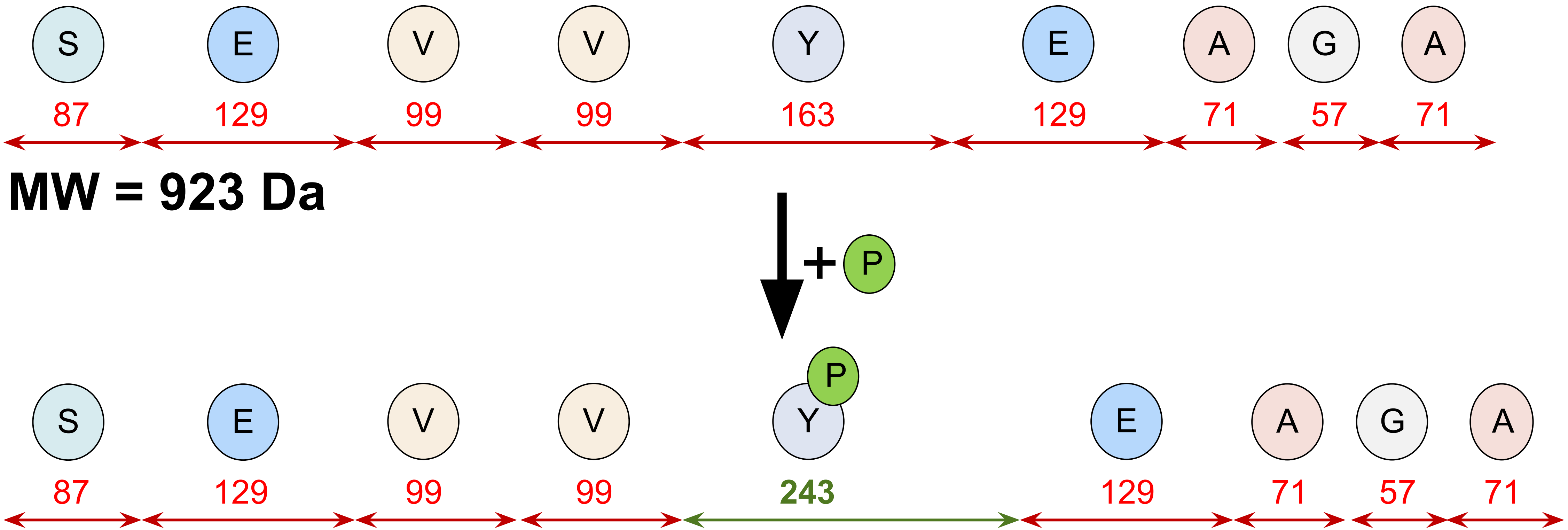
Collision-induced dissociation

Electron transfer dissociation

Mass spectrometry for proteomics



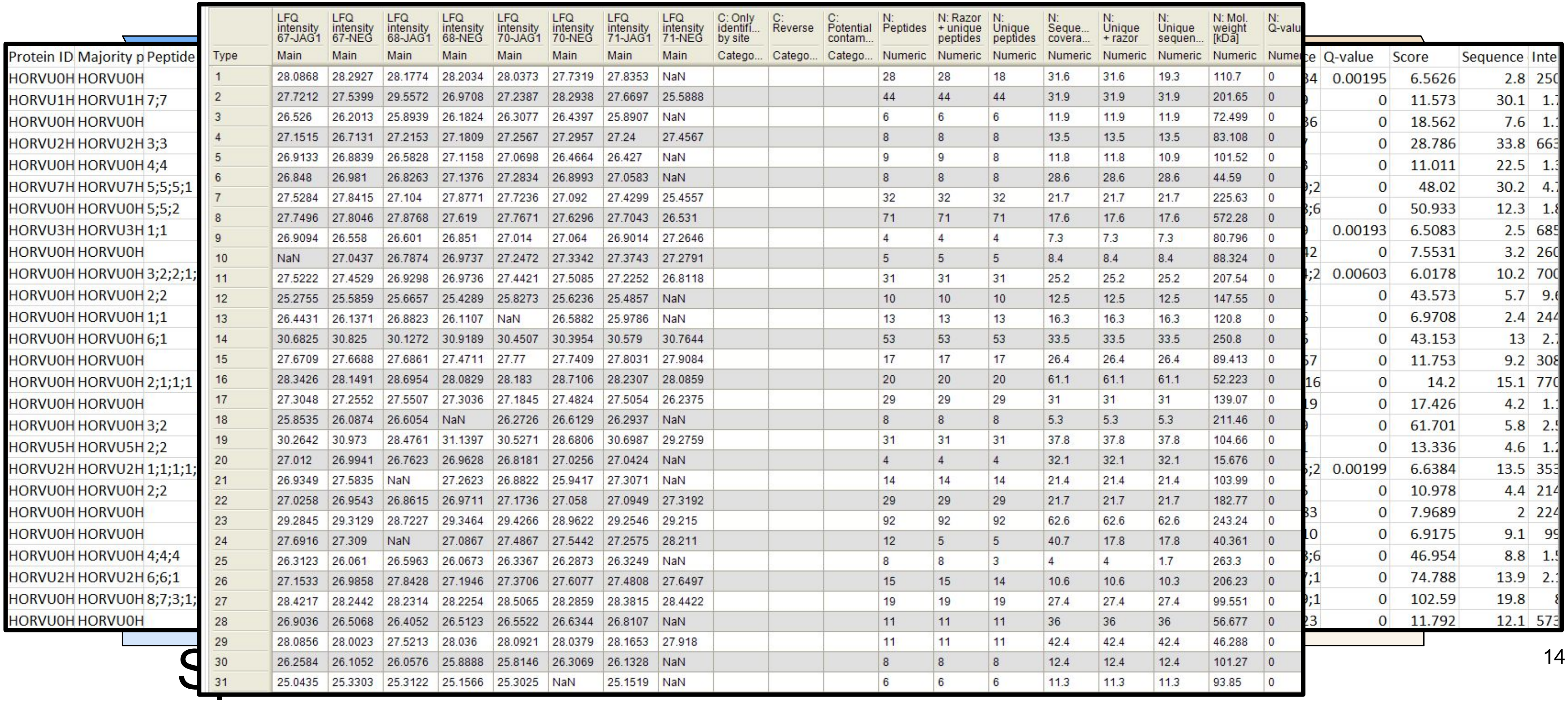
Phosphorylated peptides



MW = 923 Da

MW = 1003 Da

Post-acquisition data analysis



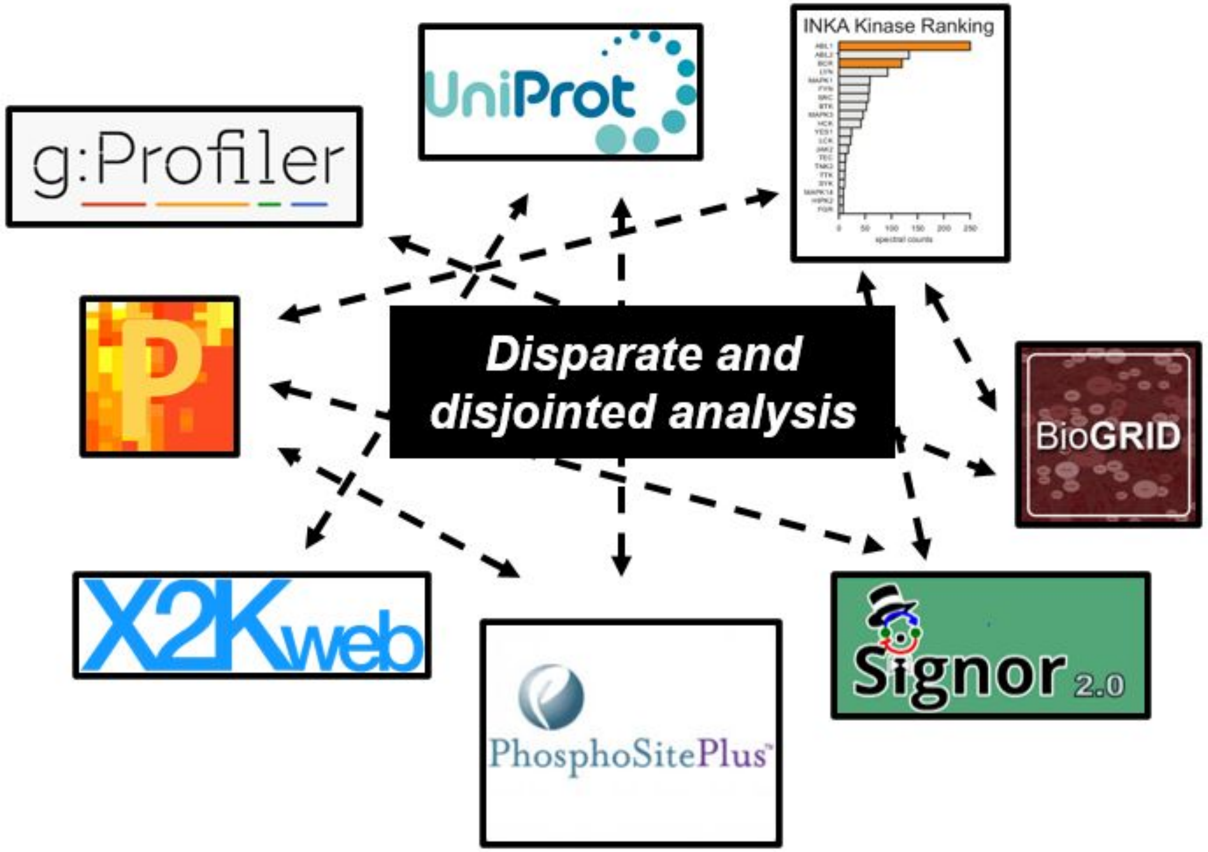
Protein ID	Majority p	Peptide
HORVU0H	HORVU0H	
HORVU1H	HORVU1H	7;7
HORVU0H	HORVU0H	
HORVU2H	HORVU2H	3;3
HORVU0H	HORVU0H	4;4
HORVU7H	HORVU7H	5;5;5;1
HORVU0H	HORVU0H	5;5;2
HORVU3H	HORVU3H	1;1
HORVU0H	HORVU0H	
HORVU0H	HORVU0H	3;2;2;1;
HORVU0H	HORVU0H	2;2
HORVU0H	HORVU0H	1;1
HORVU0H	HORVU0H	6;1
HORVU0H	HORVU0H	
HORVU0H	HORVU0H	2;1;1;1
HORVU0H	HORVU0H	
HORVU0H	HORVU0H	3;2
HORVU5H	HORVU5H	2;2
HORVU2H	HORVU2H	1;1;1;1;
HORVU0H	HORVU0H	2;2
HORVU0H	HORVU0H	
HORVU0H	HORVU0H	
HORVU0H	HORVU0H	4;4;4
HORVU2H	HORVU2H	6;6;1
HORVU0H	HORVU0H	8;7;3;1;
HORVU0H	HORVU0H	

Type	LFQ intensity 67-JAG1	LFQ intensity 67-NEG	LFQ intensity 68-JAG1	LFQ intensity 68-NEG	LFQ intensity 70-JAG1	LFQ intensity 70-NEG	LFQ intensity 71-JAG1	LFQ intensity 71-NEG	C: Only identifi... by site	C: Reverse	C: Potential contam...	N: Peptides	N: Razor + unique peptides	N: Unique peptides	N: Seque... covera...	N: Unique + razor	N: Unique sequen...	N: Mol. weight [kDa]	N: Q-valu
	Main	Main	Main	Main	Main	Main	Main	Main	Catego...	Catego...	Catego...	Numeric	Numeric	Numeric	Numeric	Numeric	Numeric	Numeric	Nume
1	28.0868	28.2927	28.1774	28.2034	28.0373	27.7319	27.8353	NaN				28	28	18	31.6	31.6	19.3	110.7	0
2	27.7212	27.5399	29.5572	26.9708	27.2387	28.2938	27.6697	25.5888				44	44	44	31.9	31.9	31.9	201.65	0
3	26.526	26.2013	25.8939	26.1824	26.3077	26.4397	25.8907	NaN				6	6	6	11.9	11.9	11.9	72.499	0
4	27.1515	26.7131	27.2153	27.1809	27.2567	27.2957	27.24	27.4567				8	8	8	13.5	13.5	13.5	83.108	0
5	26.9133	26.8839	26.5828	27.1158	27.0698	26.4664	26.427	NaN				9	9	8	11.8	11.8	10.9	101.52	0
6	26.848	26.981	26.8263	27.1376	27.2834	26.8993	27.0583	NaN				8	8	8	28.6	28.6	28.6	44.59	0
7	27.5284	27.8415	27.104	27.8771	27.7236	27.092	27.4299	25.4557				32	32	32	21.7	21.7	21.7	225.63	0
8	27.7496	27.8046	27.8768	27.619	27.7671	27.6296	27.7043	26.531				71	71	71	17.6	17.6	17.6	572.28	0
9	26.9094	26.558	26.601	26.851	27.014	27.064	26.9014	27.2646				4	4	4	7.3	7.3	7.3	80.796	0
10	NaN	27.0437	26.7874	26.9737	27.2472	27.3342	27.3743	27.2791				5	5	5	8.4	8.4	8.4	88.324	0
11	27.5222	27.4529	26.9298	26.9736	27.4421	27.5085	27.2252	26.8118				31	31	31	25.2	25.2	25.2	207.54	0
12	25.2755	25.5859	25.6657	25.4289	25.8273	25.6236	25.4857	NaN				10	10	10	12.5	12.5	12.5	147.55	0
13	26.4431	26.1371	26.8823	26.1107	NaN	26.5882	25.9786	NaN				13	13	13	16.3	16.3	16.3	120.8	0
14	30.6825	30.825	30.1272	30.9189	30.4507	30.3954	30.579	30.7644				53	53	53	33.5	33.5	33.5	250.8	0
15	27.6709	27.6688	27.6861	27.4711	27.77	27.7409	27.8031	27.9084				17	17	17	26.4	26.4	26.4	89.413	0
16	28.3426	28.1491	28.6954	28.0829	28.183	28.7106	28.2307	28.0859				20	20	20	61.1	61.1	61.1	52.223	0
17	27.3048	27.2552	27.5507	27.3036	27.1845	27.4824	27.5054	26.2375				29	29	29	31	31	31	139.07	0
18	25.8535	26.0874	26.6054	NaN	26.2726	26.6129	26.2937	NaN				8	8	8	5.3	5.3	5.3	211.46	0
19	30.2642	30.973	28.4761	31.1397	30.5271	28.6806	30.6987	29.2759				31	31	31	37.8	37.8	37.8	104.66	0
20	27.012	26.9941	26.7623	26.9628	26.8181	27.0256	27.0424	NaN				4	4	4	32.1	32.1	32.1	15.676	0
21	26.9349	27.5835	NaN	27.2623	26.8822	25.9417	27.3071	NaN				14	14	14	21.4	21.4	21.4	103.99	0
22	27.0258	26.9543	26.8615	26.9711	27.1736	27.058	27.0949	27.3192				29	29	29	21.7	21.7	21.7	182.77	0
23	29.2845	29.3129	28.7227	29.3464	29.4266	28.9622	29.2546	29.215				92	92	92	62.6	62.6	62.6	243.24	0
24	27.6916	27.309	NaN	27.0867	27.4867	27.5442	27.2575	28.211				12	5	5	40.7	17.8	17.8	40.361	0
25	26.3123	26.061	26.5963	26.0673	26.3367	26.2873	26.3249	NaN				8	8	3	4	4	1.7	263.3	0
26	27.1533	26.9858	27.8428	27.1946	27.3706	27.6077	27.4808	27.6497				15	15	14	10.6	10.6	10.3	206.23	0
27	28.4217	28.2442	28.2314	28.2254	28.5065	28.2859	28.3815	28.4422				19	19	19	27.4	27.4	27.4	99.551	0
28	26.9036	26.5068	26.4052	26.5123	26.5522	26.6344	26.8107	NaN				11	11	11	36	36	36	56.677	0
29	28.0856	28.0023	27.5213	28.036	28.0921	28.0379	28.1653	27.918				11	11	11	42.4	42.4	42.4	46.288	0
30	26.2584	26.1052	26.0576	25.8888	25.8146	26.3069	26.1328	NaN				8	8	8	12.4	12.4	12.4	101.27	0
31	25.0435	25.3303	25.3122	25.1566	25.3025	NaN	25.1519	NaN				6	6	6	11.3	11.3	11.3	93.85	0

Q-value	Score	Sequence	Inte
0.00195	6.5626	2.8	250
0	11.573	30.1	1.7
0	18.562	7.6	1.1
0	28.786	33.8	663
0	11.011	22.5	1.3
0	48.02	30.2	4.7
0	50.933	12.3	1.8
0.00193	6.5083	2.5	685
0	7.5531	3.2	260
0.00603	6.0178	10.2	700
0	43.573	5.7	9.6
0	6.9708	2.4	244
0	43.153	13	2.7
0	11.753	9.2	308
0	14.2	15.1	770
0	17.426	4.2	1.1
0	61.701	5.8	2.5
0	13.336	4.6	1.2
0.00199	6.6384	13.5	353
0	10.978	4.4	214
0	7.9689	2	224
0	6.9175	9.1	99
0	46.954	8.8	1.5
0	74.788	13.9	2.1
0	102.59	19.8	8
0	11.792	12.1	573

Post-acquisition data analysis

- Many different software applications needed for data analysis
- Different output/input data formats
- Hosted on different websites
- Difficult to train new researchers
- Requires specialist knowledge



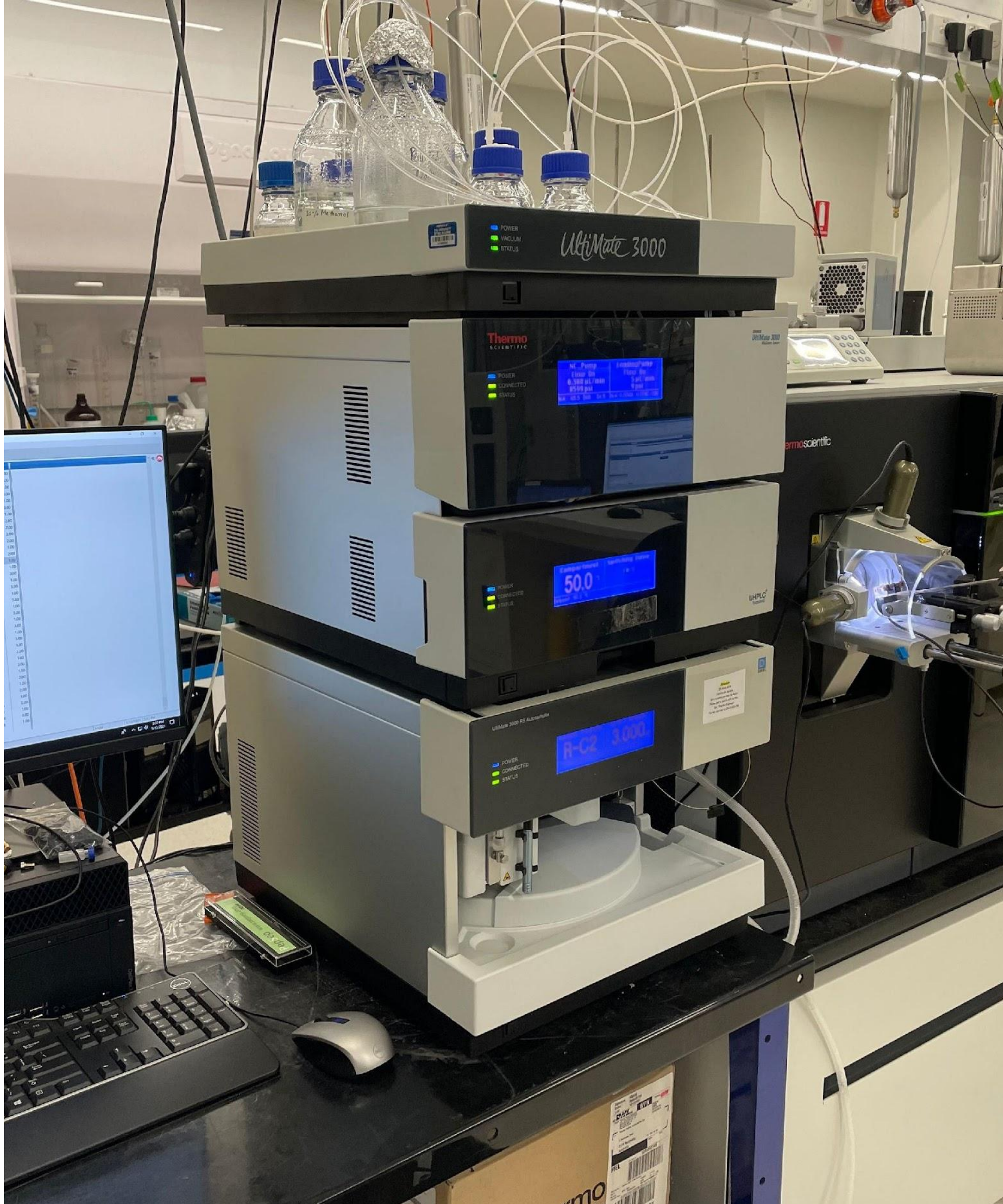
Disparate and disjointed analysis

Phosphomatics

- Single web-based software platform
- Compatible with output from many protein search applications
- Centralises multiple tools:
  - Statistical analysis (univariate + multivariate)
  - Ligand libraries
  - Over-representation analysis (GO/Pathways)
  - Kinase-substrate enrichment analysis
- Tracks results for later review/reuse
- Ease of training new researchers

Demonstration

www.phosphomatics.com



Directions

- Species coverage
- Data import pre-set options
- Statistical methods
- Analysis methods

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