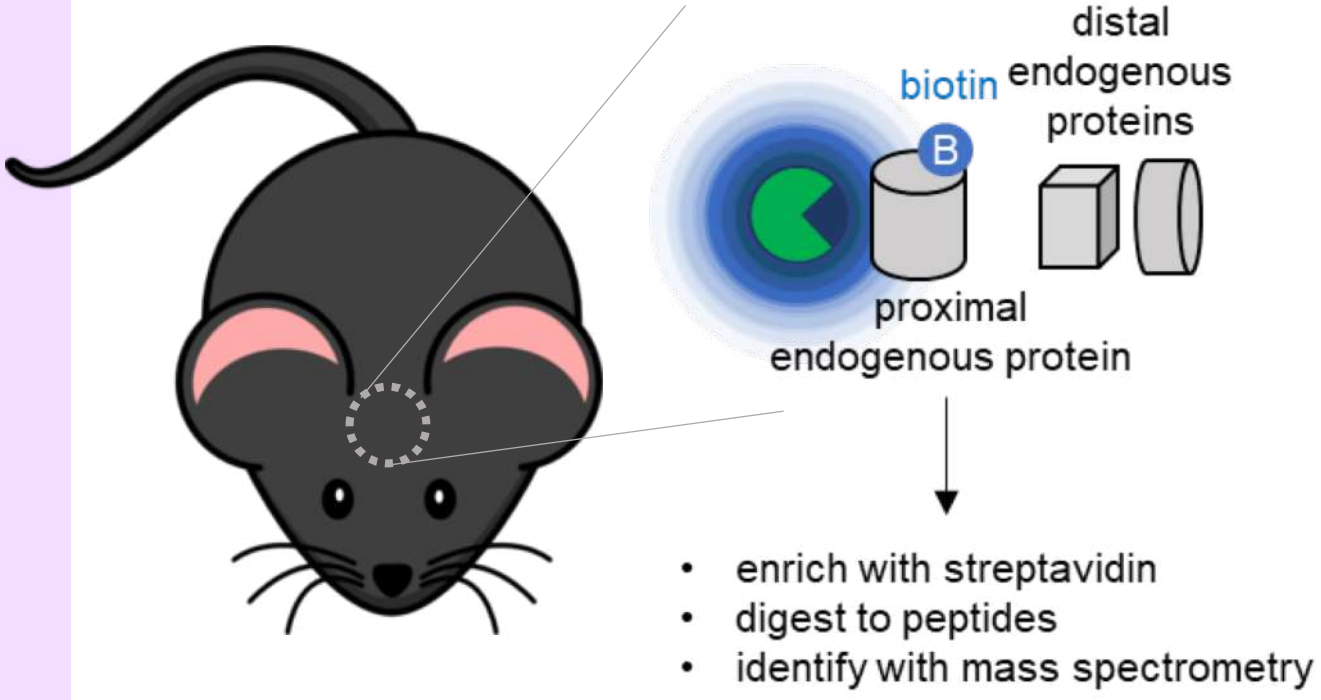
Neuro-omics Week 2:
Analysis of proximity labeling data

Shuo Han

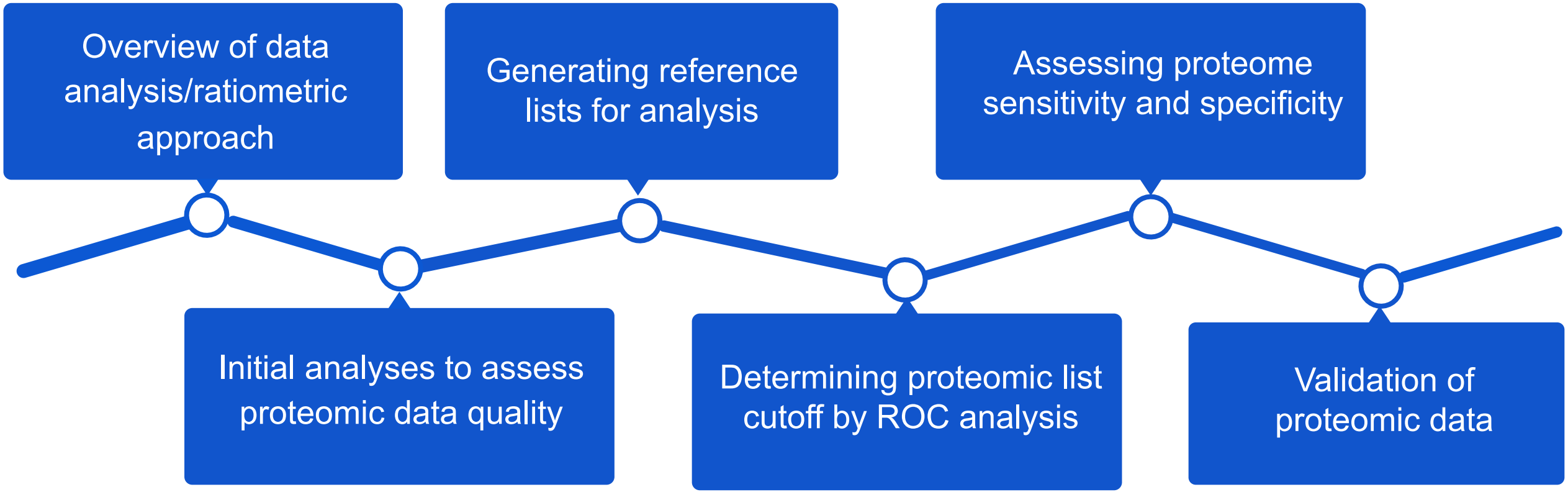
Stanford University

(Kelvin Cho, Wei Qin, Jiefu Li and Tess Branon)



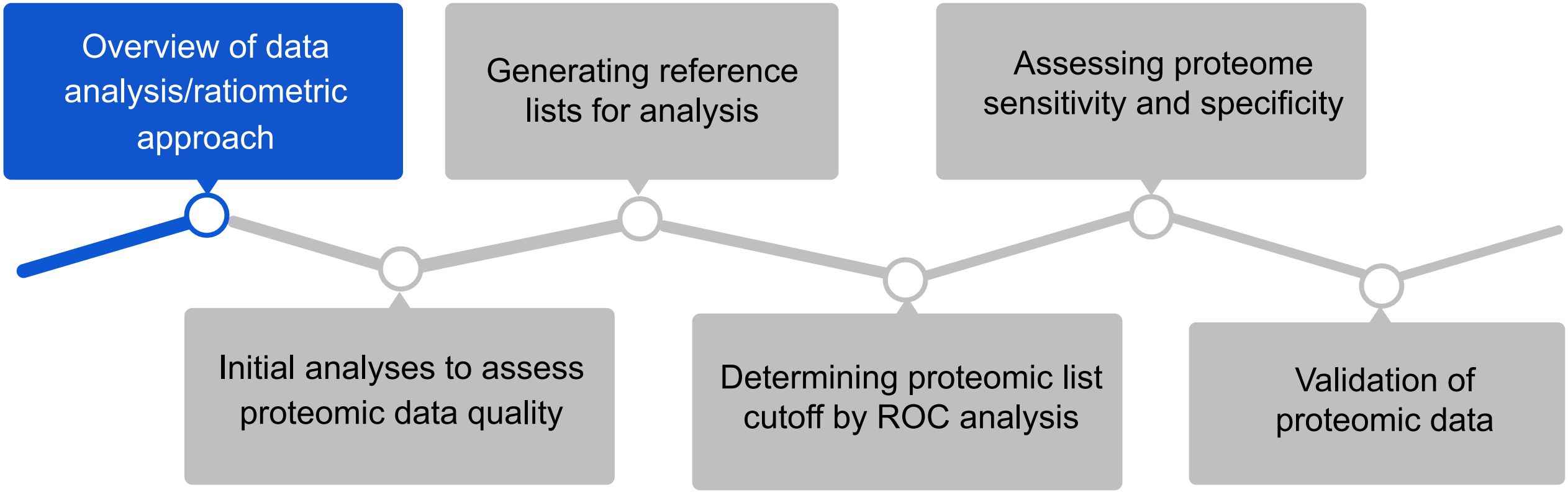
Outline

Proteomic data analysis for proximity labeling (PL) experiments:

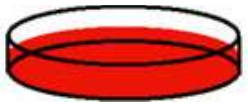
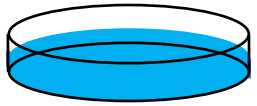
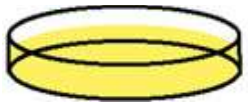
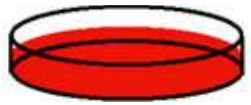
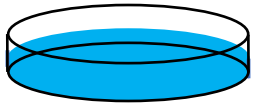
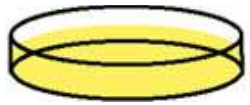


Outline

Proteomic data analysis for proximity labeling (PL) experiments:



Recap of PL proteomic experiment design

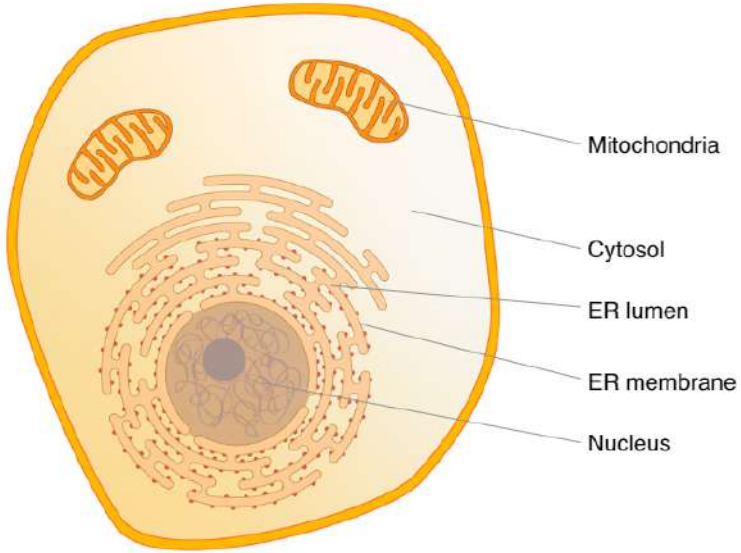
TMT label 1	TMT label 2	TMT label 3	TMT label 4	TMT label 5	TMT label 6
					
Target PL enzyme	Reference PL enzyme	omit enzyme	Target PL enzyme	Reference PL enzyme	Target PL enzyme Omit substrate

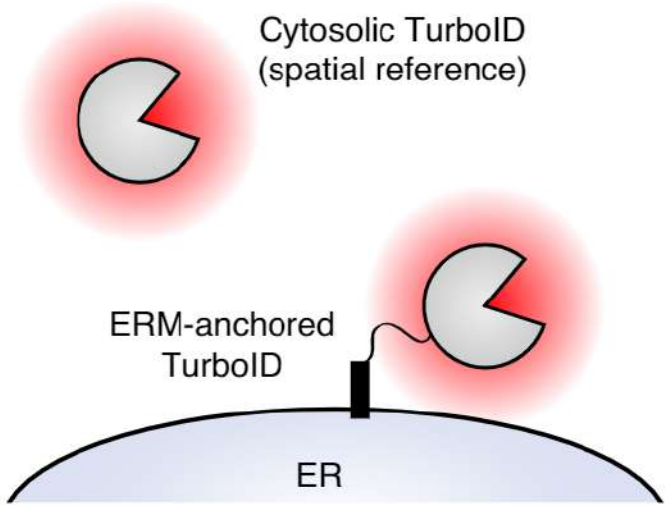
Recap of PL proteomic experiment design

TMT label 1	TMT label 2	TMT label 3	TMT label 4	TMT label 5	TMT label 6
Target PL enzyme	Reference PL enzyme	omit enzyme	Target PL enzyme	Reference PL enzyme	Target PL enzyme Omit substrate

Examples of “spatial reference” for ratiometric analysis

Mapping an open compartment- the ER membrane

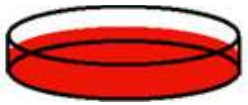
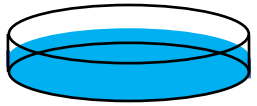
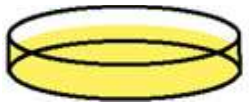
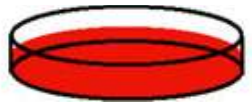
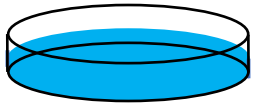
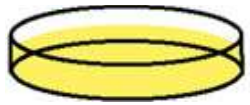




Target construct: TurboID specifically targeted to the ERM

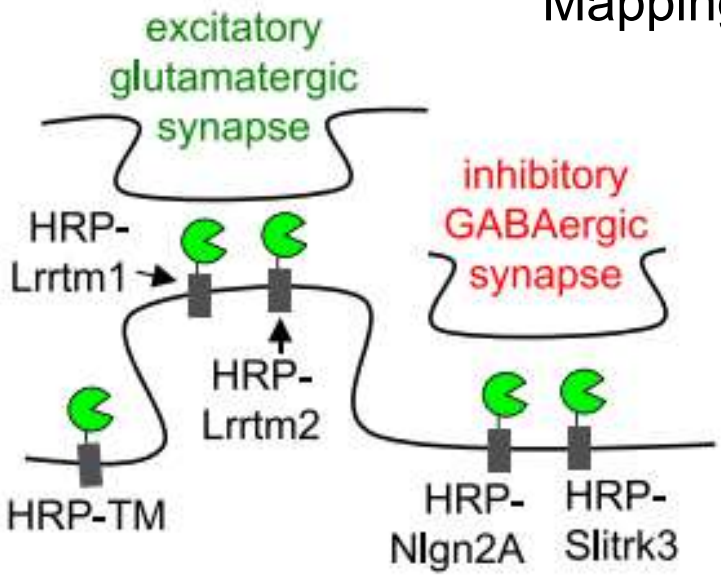
Reference construct: cytosolic TurboID (TurboID-NES)

Recap of PL proteomic experiment design

TMT label 1	TMT label 2	TMT label 3	TMT label 4	TMT label 5	TMT label 6
					
Target PL enzyme	Reference PL enzyme	omit enzyme	Target PL enzyme	Reference PL enzyme	Target PL enzyme Omit substrate

Examples of "spatial reference" for ratiometric analysis

Mapping an open compartment- the synaptic cleft



Target construct: HRP specifically targeted to the synaptic cleft

Reference construct: HRP on the entire cell surface

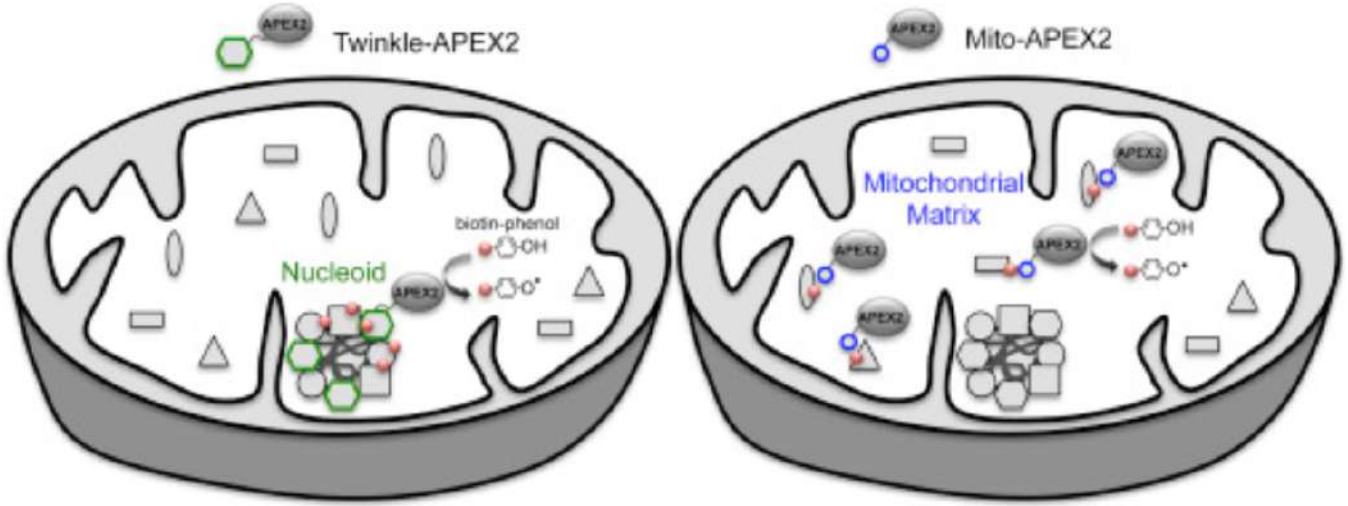
Loh et al, Cell 2016

Recap of PL proteomic experiment design

TMT label 1	TMT label 2	TMT label 3	TMT label 4	TMT label 5	TMT label 6
Target PL enzyme	Reference PL enzyme	omit enzyme	Target PL enzyme	Reference PL enzyme	Target PL enzyme Omit substrate

Examples of "spatial reference" for ratiometric analysis

Mapping a protein complex- the mitochondrial nucleoid



Han et al, Cell Chem Biol 2017

Target construct: APEX2 specifically targeted to the nucleoid

Reference construct: APEX2 everywhere in the mito matrix

Overview of experimental workflow

TMT label 1	TMT label 2	TMT label 3	TMT label 4	TMT label 5	TMT label 6
Target PL enzyme	Reference PL enzyme	omit enzyme	Target PL enzyme	Reference PL enzyme	Target PL enzyme Omit substrate

Sample lysis Streptavidin beads enrichment

On-bead trypsin digestion
TMT labeling
LC-MS/MS quantitative proteomics

(usually done by core facilities)

→ Data analysis!

Our collaborator: Steve Carr's lab at Broad Institute

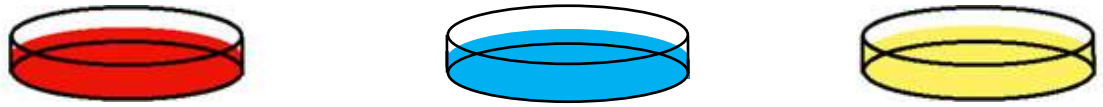
We recommend doing quantitative, ratiometric proteomics for PL

- Biotinylation extent of a protein is governed by many factors in addition to proximity to the PL enzyme: size, pH of environment, # of sterically exposed Tyr/Lys, accessibility, etc.

- Label-free proteomics only looks at the extent to which proteins are biotinylated by a single enzyme source. Does NOT exclusively reflect SPATIAL information.

We recommend doing quantitative, ratiometric proteomics for PL

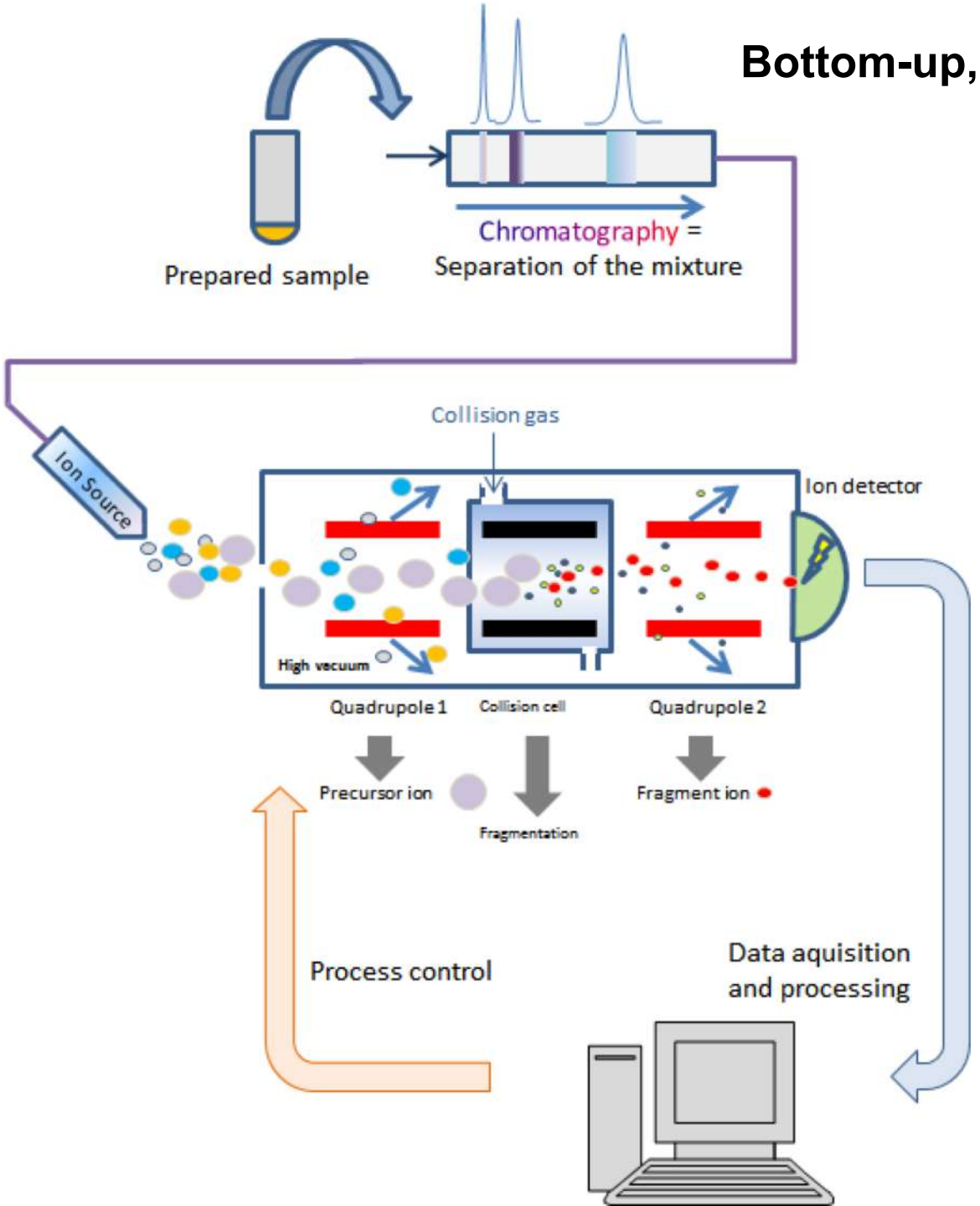
- Biotinylation extent of a protein is governed by many factors in addition to proximity to the PL enzyme: size, pH of environment, # of sterically exposed Tyr/Lys, accessibility, etc.
- Label-free proteomics only looks at the extent to which proteins are biotinylated by a single enzyme source. Does NOT exclusively reflect SPATIAL information.
- Quantitative, ratiometric approach cancels out all the other factors. The enrichment ratio reflects ONLY the spatial distance to the PL enzyme.

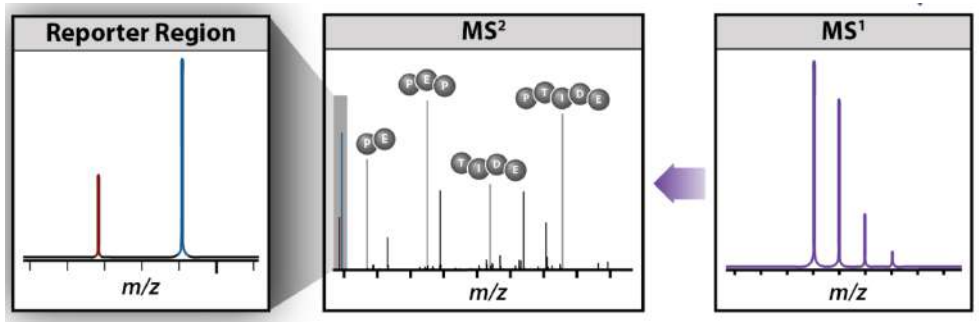


Target PL enzyme Reference PL enzyme omit enzyme

Typical quantitative proteomics methods: TMT, iTRAQ, SILAC

Bottom-up, quantitative proteomics





Some technical setups that we typically use

- **Peptide desalt**: C18 StageTips
- **Isotope labeling**: Tandem mass tag (TMT), up to 11-plex
- **MS**: Orbitrap Fusion Lumos from ThermoFisher
- **Database searching software**: Spectrum Mill MS Proteomics Workbench

Our collaborator: Steve Carr's lab at Broad Institute

Example of unprocessed data obtained from a typical experiment

Various TMT ratios

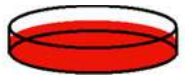
129N/130C 129N/129C

Uniprot ID Protein name Unique peptides

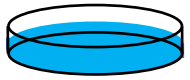
	BH	BI	BJ	BK	BL	BM	BN	BO	BP	BQ	BR	BS	BT	BU	BV	BW	BX	BY
1	129N:130C	129C:130C	130N:130C	131:130C	126:131	127N:131	127C:131	128N:131	128C:131	129N:131	129C:131	130N:131	130C:131	UniProt Ac	Species	UniProt Entry Name	Unique Peptides	
296	-0.175	1.2905		-0.275	1.4625	0.7435	0.025	0.815	1.1125	-0.0215	1.5425		0.275	Q9I7U2	DROME	CG1275, isoform C	2	
297	0.074	1.1345	0.516	-0.036	1.2145	0.8205	-0.201	1.109	0.9185	0.1325	1.2005	0.423	0.036	A1Z935	DROME	CG8632, isoform B	13	
298	0.164	-0.5725		-0.195	-0.4165	-0.9265	0.625	0.054	-0.5585	0.4835	-0.3215		0.195	C6SUX5	DROME	GM01169p (Fragment)	2	
299	1.292	0.6065	0.822	0.239	0.5005	-0.1205	1.394	0.35	0.1515	1.1625	0.4825	0.315	-0.239	Q0KIB0	DROME	CG34113, isoform O	8	
300	0.421	0.6825	-0.563	0.087	0.6235	-0.0765	0.251	1.078	0.5455	0.2335	0.5255	-0.699	-0.087	Q9W3M4	DROME	LD24308p	7	
301	0.249	0.9445	-0.564	0.038	0.9815	0.4065	0.238	-1.84	0.5085	0.1655	0.9655	-0.412	-0.038	Q7K0W1	DROME	CG8531	7	
302	0.223	1.6485	0.717	0.164	1.7905	1.2405	-0.395	1.341	1.2455	-0.0335	1.7005	0.968	-0.164	Q95T61	DROME	CG2082, isoform B	15	
303	0.496	1.3075	-0.052	0.359	1.2145	0.5115	0.057	0.407	0.6795	0.1585	1.0855	0.059	-0.359	Q0KI33	DROME	CG7956, isoform C	17	
304	0.038	2.4455	3.617	0.241	1.2575	0.6515	0.237	1.324	0.8075	-0.1165	1.3525	2.825	-0.241	Q9I7U4-5	DROME	Isoform E of Titin	7	
305	0.667	1.2305		0.237	1.2495	0.4065	0.147		0.5705	0.4315	1.0025		-0.237	Q7JRB2	DROME	CG14591, isoform A	2	
306	1.174	2.7635		1.239	1.5495	0.9375	-0.324	-0.27	1.2825	-0.0625	1.5345		-1.239	Q9NFR5	DROME	Nicotinic acetylcholine re	1	
307	1.376	1.0005		0.214	1.0915	0.2025	1.373		0.4525	1.1645	0.7965		-0.214	Q9V9V6	DROME	Kek6	1	
308	0.811	0.7795	0.692	0.099	0.6475	-0.0515	0.806	1.648	0.1155	0.6865	0.6675	0.665	-0.099	Q9VCT4	DROME	Klingon	19	
309	-0.178	0.3725		0.205	-0.2145	-0.4155	-0.07		-0.4315	-0.3805	0.1775		-0.205	Q9VPG0	DROME	CG5282	1	
310	0.573	0.4405	-1.592	0.209	0.1365	-0.3505	0.3		-0.1405	0.3665	0.2415	-1.558	-0.209	Q9W1B5	DROME	CG3209, isoform C	3	
311	0.501	-0.4005	-0.36	-0.039	-0.4815	-1.0795	0.728	-1.253	-0.7475	0.5195	-0.4795	-0.38	0.039	P45437	DROME	Coatomer subunit beta	7	
312	0.569	2.2625	-0.704	0.265	1.8975	1.0855	-0.253		1.5825	0.3905	2.0075	-0.844	-0.265	Q9W2E8	DROME	CG10320, isoform A	2	
313	0.478	0.0255		0.336	-0.1475	-0.3355	0.515	1.74	-0.2465	0.5355	-0.2835		-0.336	Q0KI85	DROME	CG34114, isoform B	3	
314	-0.295	2.5215	2.398	-0.252	2.3735	2.1265	-0.465	1.296	3.0715	-0.0225	2.5545	2.275	0.252	B7FNQ3	DROME	RE33133p	12	
315	0.047	-1.0235	-1.442	-0.158	-0.8195	-1.3325	0.62	-2.751	-1.1215	0.3665	-0.8515	-1.405	0.158	Q9VA91	DROME	40S ribosomal protein S7	8	
316	0.398	2.1145		0.282	1.9715	1.2545	-0.114		1.4995	0.1175	1.8425		-0.282	Q9VY22	DROME	CG15890	1	
317	0.013	0.2555	1.398	-0.276	0.7365	0.2695	0.266	0.281	0.6585	0.1745	0.7255	1.879	0.276	Q5LJX9	DROME	CG2893, isoform B (Fragn	4	
318	0.022	1.3765	-0.595	0.289	1.1905	0.6975	-0.428	0.892	0.9425	-0.2325	1.1625	-0.996	-0.289	Q9W0L6	DROME	CG13907	10	
319	0.848	0.4835	0.027	0.348	0.2205	-0.3955	0.551	0.048	0.1055	0.4425	0.2395	0.339	-0.348	Q94887	DROME	Neurexin-4	33	
320	-0.44	0.9195	2.345	-0.295	1.4905	0.9275	-0.424	1.121	1.2195	-0.1195	1.4015	2.036	0.295	Q8IGX6	DROME	RE09889p	9	
321	0.177	1.0195	0.01	0.075	1.0195	0.2815	0.181	0.807	0.6805	0.1405	0.9615	0.012	-0.075	Q9V8R9	DROME	Protein 4.1 homolog	76	
322	0.118	1.3635	0.628	0.145	1.3075	0.7845	-0.146	0.988	0.9285	0.0235	1.3115	0.626	-0.145	P25455	DROME	1-phosphatidylinositol 4,5	31	
323	0.368	1.5295	0.463	0.124	1.4095	0.8445	0.037	0.644	0.8955	0.2235	1.4875	0.578	-0.124	O62619-2	DROME	Isoform B of Pyruvate kin	39	
324	0.151	1.5715	1.252	0.335	1.3815	0.6815	-0.391	1.473	0.8785	-0.1695	1.3525	0.844	-0.335	Q24418	DROME	Glutamate [NMDA] recep	17	
325	0.464	1.8335	0.968	0.263	1.4685	0.9835	0.029	1.737	1.1585	0.0815	1.5145	1.127	-0.263	O62530	DROME	AP-50, isoform A	24	
326	0.621	1.6635	0.824	0.377	1.1725	0.4655	0.28		0.7075	0.2935	1.1465	0.238	-0.377	Q8IR72	DROME	CG32638	2	
327	-0.155	0.7645	-1.161	-0.269	1.0125	0.4045	0.359	2.067	0.4355	0.2615	1.0435	-0.9	0.269	Q8MQS1	DROME	GH14073p	2	
328	0.378	1.4875		0.082	1.5165	0.8395	-0.133	1.898	0.6615	0.2985	1.4155		-0.082	Q9VDV4	DROME	Anoctamin	1	

Proteomic Data - TMT Ratios

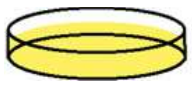
TMT label



Target PL enzyme



Reference PL enzyme



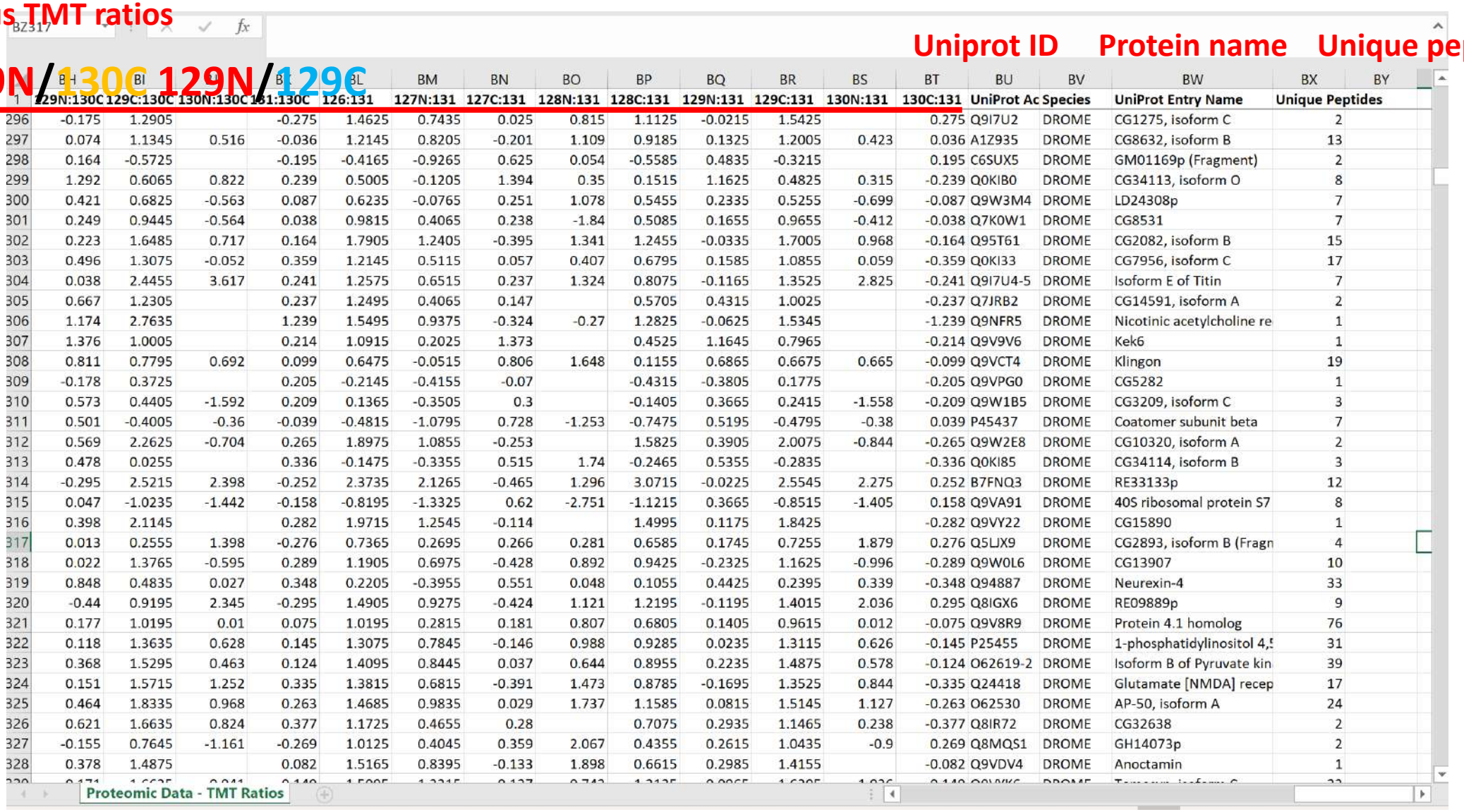
omit enzyme

Example of unprocessed data obtained from a typical experiment

Various TMT ratios

129N/130C 129N/129C

Uniprot ID Protein name Unique peptides



	BH	BI	BJ	BK	BL	BM	BN	BO	BP	BQ	BR	BS	BT	BU	BV	BW	BX	BY
1	129N:130C	129C:130C	130N:130C	131:130C	126:131	127N:131	127C:131	128N:131	128C:131	129N:131	129C:131	130N:131	130C:131	UniProt Ac	Species	UniProt Entry Name	Unique Peptides	
296	-0.175	1.2905		-0.275	1.4625	0.7435	0.025	0.815	1.1125	-0.0215	1.5425		0.275	Q9I7U2	DROME	CG1275, isoform C	2	
297	0.074	1.1345	0.516	-0.036	1.2145	0.8205	-0.201	1.109	0.9185	0.1325	1.2005	0.423	0.036	A1Z935	DROME	CG8632, isoform B	13	
298	0.164	-0.5725		-0.195	-0.4165	-0.9265	0.625	0.054	-0.5585	0.4835	-0.3215		0.195	C6SUX5	DROME	GM01169p (Fragment)	2	
299	1.292	0.6065	0.822	0.239	0.5005	-0.1205	1.394	0.35	0.1515	1.1625	0.4825	0.315	-0.239	Q0KIB0	DROME	CG34113, isoform O	8	
300	0.421	0.6825	-0.563	0.087	0.6235	-0.0765	0.251	1.078	0.5455	0.2335	0.5255	-0.699	-0.087	Q9W3M4	DROME	LD24308p	7	
301	0.249	0.9445	-0.564	0.038	0.9815	0.4065	0.238	-1.84	0.5085	0.1655	0.9655	-0.412	-0.038	Q7K0W1	DROME	CG8531	7	
302	0.223	1.6485	0.717	0.164	1.7905	1.2405	-0.395	1.341	1.2455	-0.0335	1.7005	0.968	-0.164	Q95T61	DROME	CG2082, isoform B	15	
303	0.496	1.3075	-0.052	0.359	1.2145	0.5115	0.057	0.407	0.6795	0.1585	1.0855	0.059	-0.359	Q0KI33	DROME	CG7956, isoform C	17	
304	0.038	2.4455	3.617	0.241	1.2575	0.6515	0.237	1.324	0.8075	-0.1165	1.3525	2.825	-0.241	Q9I7U4-5	DROME	Isoform E of Titin	7	
305	0.667	1.2305		0.237	1.2495	0.4065	0.147		0.5705	0.4315	1.0025		-0.237	Q7JRB2	DROME	CG14591, isoform A	2	
306	1.174	2.7635		1.239	1.5495	0.9375	-0.324	-0.27	1.2825	-0.0625	1.5345		-1.239	Q9NFR5	DROME	Nicotinic acetylcholine re	1	
307	1.376	1.0005		0.214	1.0915	0.2025	1.373		0.4525	1.1645	0.7965		-0.214	Q9V9V6	DROME	Kek6	1	
308	0.811	0.7795	0.692	0.099	0.6475	-0.0515	0.806	1.648	0.1155	0.6865	0.6675	0.665	-0.099	Q9VCT4	DROME	Klingon	19	
309	-0.178	0.3725		0.205	-0.2145	-0.4155	-0.07		-0.4315	-0.3805	0.1775		-0.205	Q9VPG0	DROME	CG5282	1	
310	0.573	0.4405	-1.592	0.209	0.1365	-0.3505	0.3		-0.1405	0.3665	0.2415	-1.558	-0.209	Q9W1B5	DROME	CG3209, isoform C	3	
311	0.501	-0.4005	-0.36	-0.039	-0.4815	-1.0795	0.728	-1.253	-0.7475	0.5195	-0.4795	-0.38	0.039	P45437	DROME	Coatomer subunit beta	7	
312	0.569	2.2625	-0.704	0.265	1.8975	1.0855	-0.253		1.5825	0.3905	2.0075	-0.844	-0.265	Q9W2E8	DROME	CG10320, isoform A	2	
313	0.478	0.0255		0.336	-0.1475	-0.3355	0.515	1.74	-0.2465	0.5355	-0.2835		-0.336	Q0KI85	DROME	CG34114, isoform B	3	
314	-0.295	2.5215	2.398	-0.252	2.3735	2.1265	-0.465	1.296	3.0715	-0.0225	2.5545	2.275	0.252	B7FNQ3	DROME	RE33133p	12	
315	0.047	-1.0235	-1.442	-0.158	-0.8195	-1.3325	0.62	-2.751	-1.1215	0.3665	-0.8515	-1.405	0.158	Q9VA91	DROME	40S ribosomal protein S7	8	
316	0.398	2.1145		0.282	1.9715	1.2545	-0.114		1.4995	0.1175	1.8425		-0.282	Q9VY22	DROME	CG15890	1	
317	0.013	0.2555	1.398	-0.276	0.7365	0.2695	0.266	0.281	0.6585	0.1745	0.7255	1.879	0.276	Q5LJX9	DROME	CG2893, isoform B (Fragn	4	
318	0.022	1.3765	-0.595	0.289	1.1905	0.6975	-0.428	0.892	0.9425	-0.2325	1.1625	-0.996	-0.289	Q9W0L6	DROME	CG13907	10	
319	0.848	0.4835	0.027	0.348	0.2205	-0.3955	0.551	0.048	0.1055	0.4425	0.2395	0.339	-0.348	Q94887	DROME	Neurexin-4	33	
320	-0.44	0.9195	2.345	-0.295	1.4905	0.9275	-0.424	1.121	1.2195	-0.1195	1.4015	2.036	0.295	Q8IGX6	DROME	RE09889p	9	
321	0.177	1.0195	0.01	0.075	1.0195	0.2815	0.181	0.807	0.6805	0.1405	0.9615	0.012	-0.075	Q9V8R9	DROME	Protein 4.1 homolog	76	
322	0.118	1.3635	0.628	0.145	1.3075	0.7845	-0.146	0.988	0.9285	0.0235	1.3115	0.626	-0.145	P25455	DROME	1-phosphatidylinositol 4,5	31	
323	0.368	1.5295	0.463	0.124	1.4095	0.8445	0.037	0.644	0.8955	0.2235	1.4875	0.578	-0.124	O62619-2	DROME	Isoform B of Pyruvate kin	39	
324	0.151	1.5715	1.252	0.335	1.3815	0.6815	-0.391	1.473	0.8785	-0.1695	1.3525	0.844	-0.335	Q24418	DROME	Glutamate [NMDA] recep	17	
325	0.464	1.8335	0.968	0.263	1.4685	0.9835	0.029	1.737	1.1585	0.0815	1.5145	1.127	-0.263	O62530	DROME	AP-50, isoform A	24	
326	0.621	1.6635	0.824	0.377	1.1725	0.4655	0.28		0.7075	0.2935	1.1465	0.238	-0.377	Q8IR72	DROME	CG32638	2	
327	-0.155	0.7645	-1.161	-0.269	1.0125	0.4045	0.359	2.067	0.4355	0.2615	1.0435	-0.9	0.269	Q8MQS1	DROME	GH14073p	2	
328	0.378	1.4875		0.082	1.5165	0.8395	-0.133	1.898	0.6615	0.2985	1.4155		-0.082	Q9VDV4	DROME	Anoctamin	1	

Proteomic Data - TMT Ratios

Spectrum Mill can export all the different TMT ratio combinations in excel format for all detected proteins

Two general approaches to data analysis

- Analysis depends on the ***nature of your experimental design*** and ***what is already known*** about the target proteome

Two general approaches to data analysis

- Analysis depends on the ***nature of your experimental design*** and ***what is already known*** about the target proteome

- If nothing is known about the target proteome and you don’t know what to expect, then employ statistical methods to determine the cutoff
  - T-tests
  - Multivariate analysis
  - Other statistical approaches

Two general approaches to data analysis

- Analysis depends on the ***nature of your experimental design*** and ***what is already known*** about the target proteome

- If nothing is known about the target proteome and you don't know what to expect, then employ statistical methods to determine the cutoff
  - T-tests
  - Multivariate analysis
  - Other statistical approaches

Example: using proximity labeling to profile a previously uncharacterized organelle contact site or map the composition of an unknown protein complex

Two general approaches to data analysis

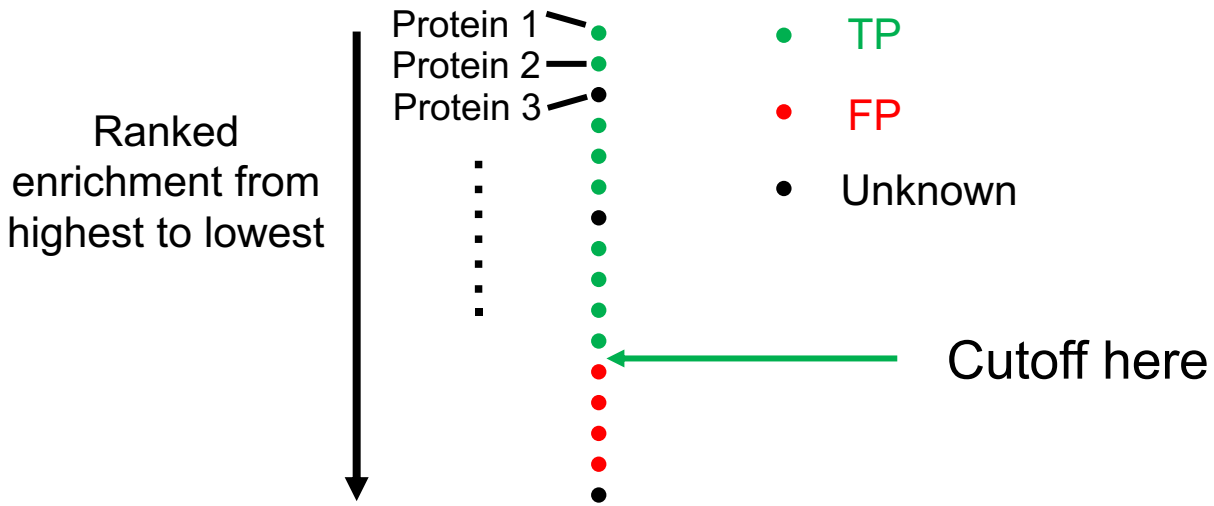
- Analysis depends on the ***nature of your experimental design*** and ***what is already known*** about the target proteome

- If an adequate amount of prior knowledge exists for the target proteome, receiver operator characteristic (ROC)-based quantitative analysis can produce highly specific proteomes

Two general approaches to data analysis

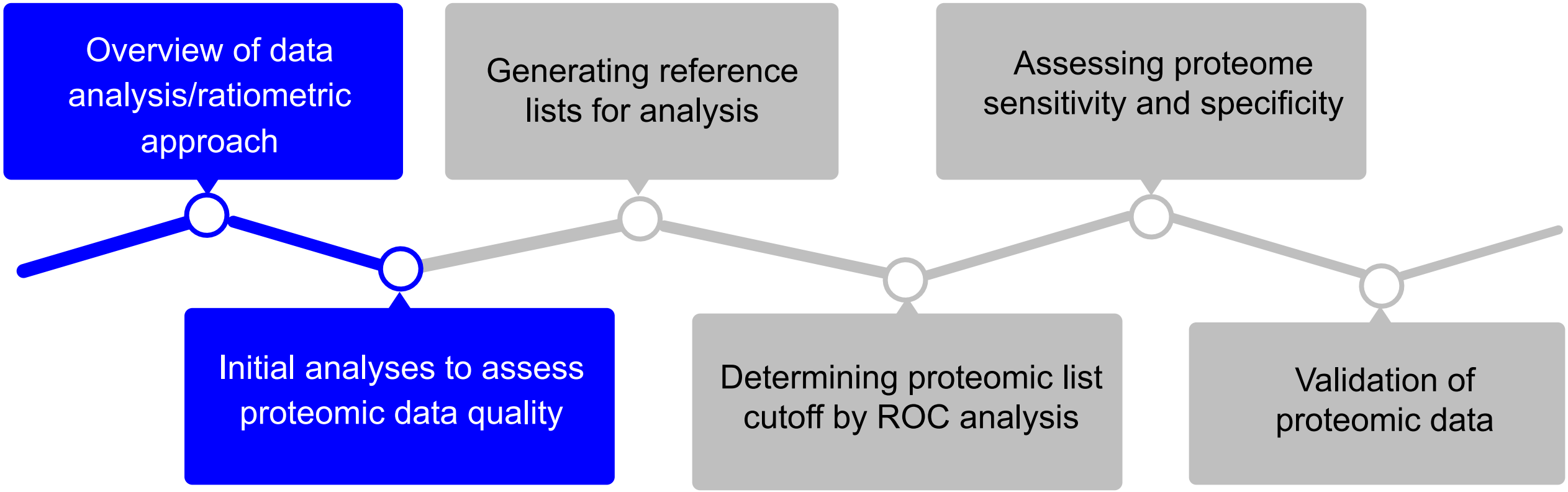
- Analysis depends on the ***nature of your experimental design*** and ***what is already known*** about the target proteome

- If an adequate amount of prior knowledge exists for the target proteome, receiver operator characteristic (ROC)-based quantitative analysis can produce highly specific proteomes
  - Need to curate a list of true positive and false positive proteins to determine cutoff



Outline

Proteomic data analysis for proximity labeling (PL) experiments:



Preliminary processing of MS data

- Remove proteins with less than 2 unique peptides
- Remove proteins that are not from species being mapped
- Remove common contaminants e.g. human keratin

BZ317

	BH	BI	BJ	BK	BL	BM	BN	BO	BP	BQ	BR	BS	BT	BU	BV	BW	BX	BY
1	129N:130C	129C:130C	130N:130C	131:130C	126:131	127N:131	127C:131	128N:131	128C:131	129N:131	129C:131	130N:131	130C:131	UniProt Ac	Species	UniProt Entry Name	Unique Peptides	
296	-0.175	1.2905		-0.275	1.4625	0.7435	0.025	0.815	1.1125	-0.0215	1.5425		0.275	Q9I7U2	DROME	CG1275, isoform C	2	
297	0.074	1.1345	0.516	-0.036	1.2145	0.8205	-0.201	1.109	0.9185	0.1325	1.2005	0.423	0.036	A1Z935	DROME	CG8632, isoform B	13	
298	0.164	-0.5725		-0.195	-0.4165	-0.9265	0.625	0.054	-0.5585	0.4835	-0.3215		0.195	C6SUX5	DROME	GM01169p (Fragment)	2	
299	1.292	0.6065	0.822	0.239	0.5005	-0.1205	1.394	0.35	0.1515	1.1625	0.4825	0.315	-0.239	Q0KIB0	DROME	CG34113, isoform O	8	
300	0.421	0.6825	-0.563	0.087	0.6235	-0.0765	0.251	1.078	0.5455	0.2335	0.5255	-0.699	-0.087	Q9W3M4	DROME	LD24308p	7	
301	0.249	0.9445	-0.564	0.038	0.9815	0.4065	0.238	-1.84	0.5085	0.1655	0.9655	-0.412	-0.038	Q7K0W1	DROME	CG8531	7	
302	0.223	1.6485	0.717	0.164	1.7905	1.2405	-0.395	1.341	1.2455	-0.0335	1.7005	0.968	-0.164	Q95T61	DROME	CG2082, isoform B	15	
303	0.496	1.3075	-0.052	0.359	1.2145	0.5115	0.057	0.407	0.6795	0.1585	1.0855	0.059	-0.359	Q0KI33	DROME	CG7956, isoform C	17	
304	0.038	2.4455	3.617	0.241	1.2575	0.6515	0.237	1.324	0.8075	-0.1165	1.3525	2.825	-0.241	Q9I7U4-5	DROME	Isoform E of Titin	7	
305	0.667	1.2305		0.237	1.2495	0.4065	0.147		0.5705	0.4315	1.0025		-0.237	Q7JRB2	DROME	CG14591, isoform A	2	

All these steps can be done by directly filtering the corresponding columns in Excel

MS data quality checks

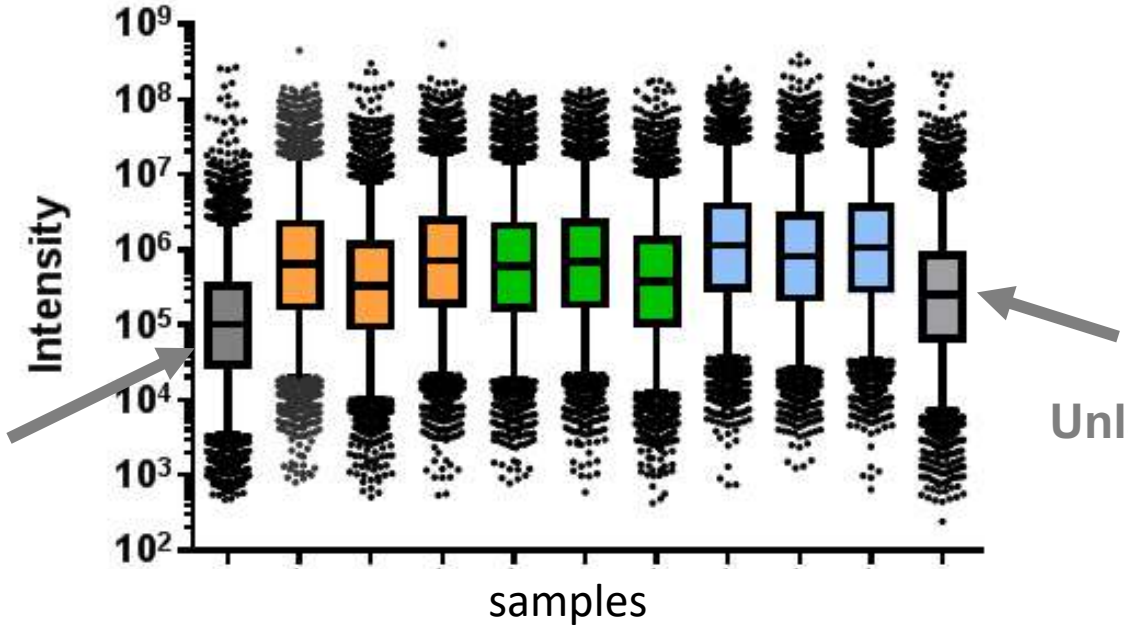
- Number of proteins detected in experimental samples should be in the thousands

MS data quality checks

- Number of proteins detected in experimental samples should be in the thousands
- For TMT-labeling
  - >90% peptides should have label incorporated

MS data quality checks

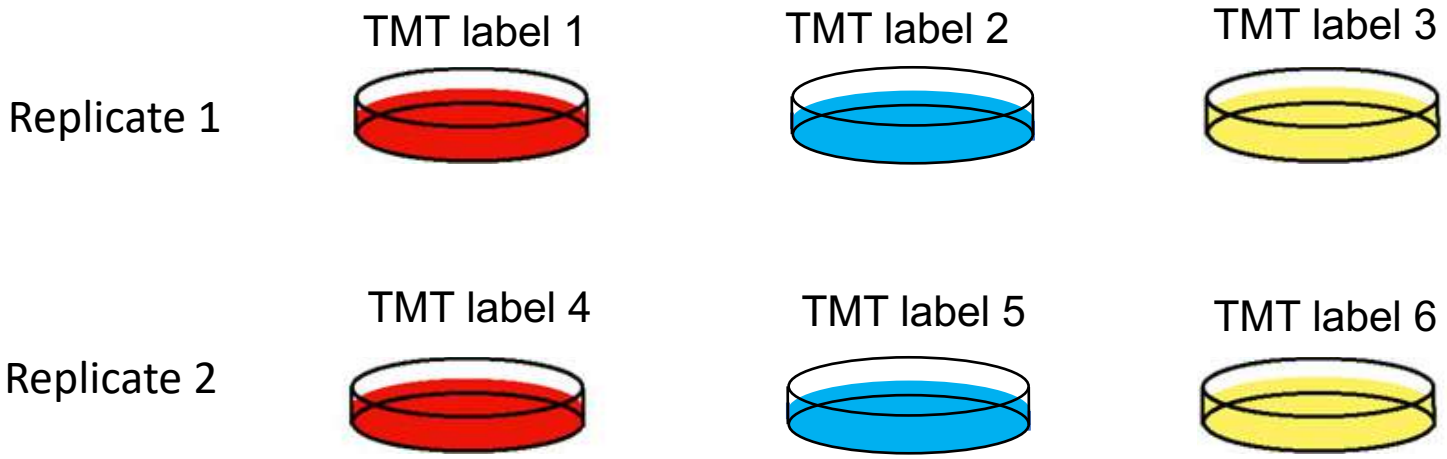
- Number of proteins detected in experimental samples should be in the thousands
- For TMT-labeling
  - >90% peptides should have label incorporated
  - TMT intensities should correlate to amount of protein in each sample

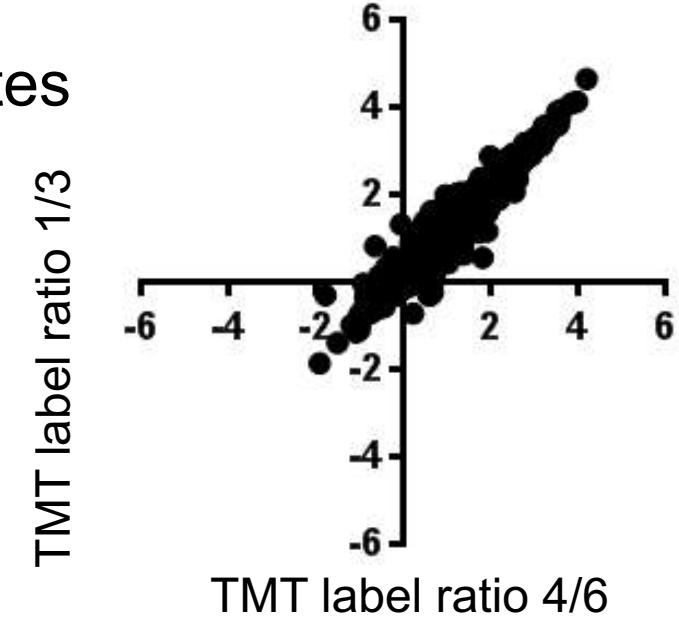


Unlabeled neg ctrl samples

MS data quality checks

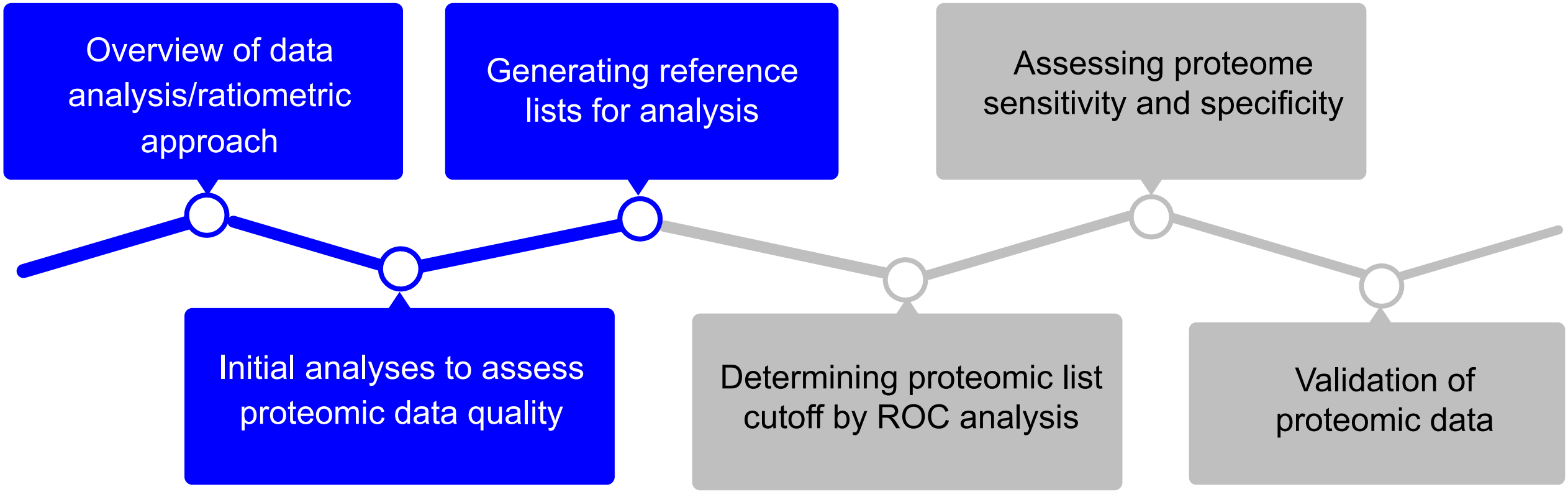
- Number of proteins detected in experimental samples should be in the thousands
- For TMT-labeling
  - >90% peptides should have label incorporated
  - TMT intensities should correlate to amount of protein in each sample
  - TMT intensities should correlate across replicates





Outline

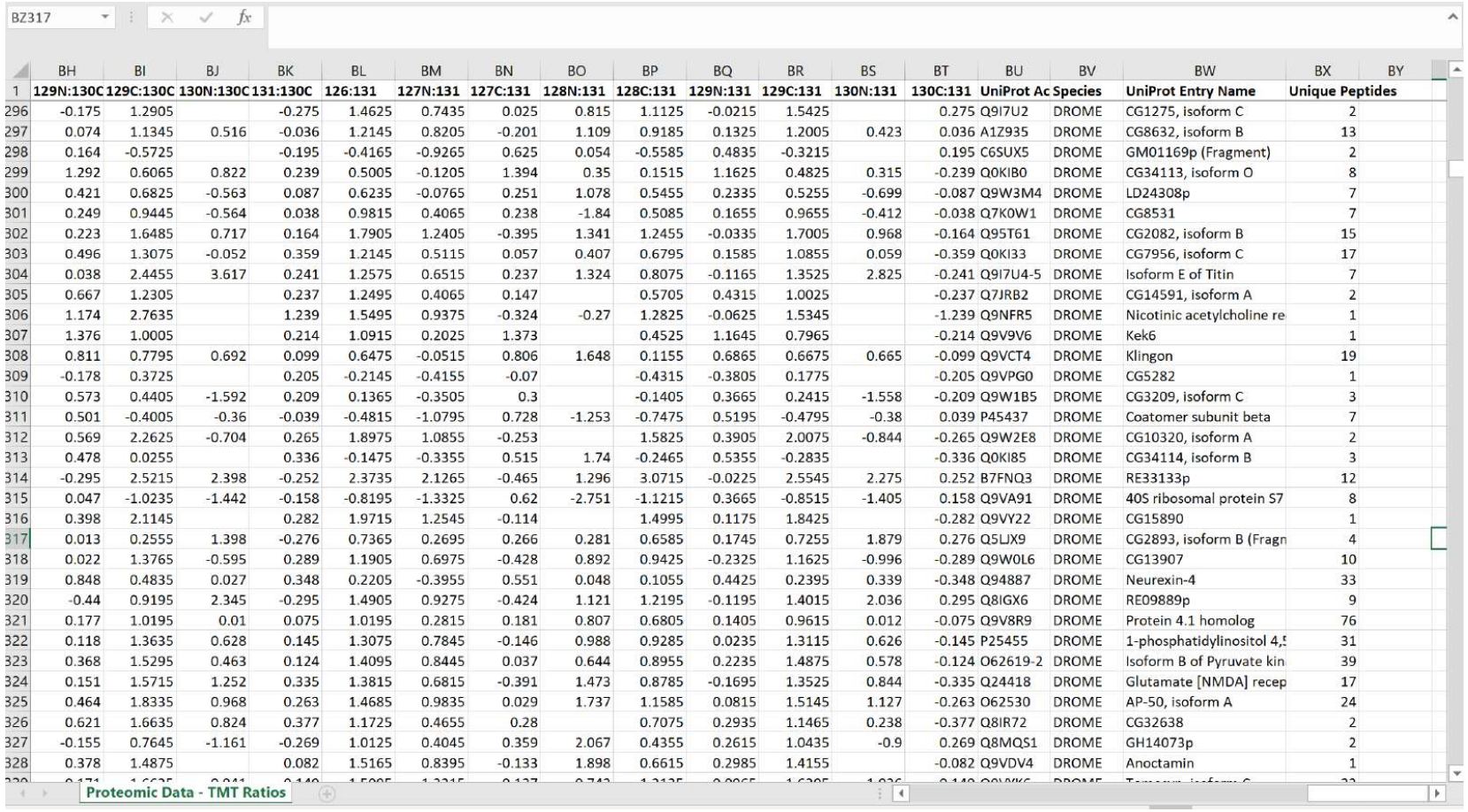
Proteomic data analysis for proximity labeling (PL) experiments:



Quantitative analysis with reference lists

- Analyze each individual TMT ratio separately, one replicate at a time.

- For a given ratio, rank the proteome data from highest to lowest. Always work with a ranked list!



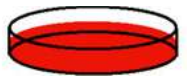
BZ317

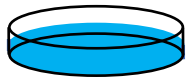
	BH	BI	BJ	BK	BL	BM	BN	BO	BP	BQ	BR	BS	BT	BU	BV	BW	BX	BY
1	129N:130C	129C:130C	130N:130C	131:130C	126:131	127N:131	127C:131	128N:131	128C:131	129N:131	129C:131	130N:131	130C:131	UniProt Ac	Species	UniProt Entry Name	Unique Peptides	
296	-0.175	1.2905		-0.275	1.4625	0.7435	0.025	0.815	1.1125	-0.0215	1.5425		0.275	Q9I7U2	DROME	CG1275, isoform C	2	
297	0.074	1.1345	0.516	-0.036	1.2145	0.8205	-0.201	1.109	0.9185	0.1325	1.2005	0.423	0.036	A1Z935	DROME	CG8632, isoform B	13	
298	0.164	-0.5725		-0.195	-0.4165	-0.9265	0.625	0.054	-0.5585	0.4835	-0.3215		0.195	C6SUX5	DROME	GM01169p (Fragment)	2	
299	1.292	0.6065	0.822	0.239	0.5005	-0.1205	1.394	0.35	0.1515	1.1625	0.4825	0.315	-0.239	Q0KIB0	DROME	CG34113, isoform O	8	
300	0.421	0.6825	-0.563	0.087	0.6235	-0.0765	0.251	1.078	0.5455	0.2335	0.5255	-0.699	-0.087	Q9W3M4	DROME	LD24308p	7	
301	0.249	0.9445	-0.564	0.038	0.9815	0.4065	0.238	-1.84	0.5085	0.1655	0.9655	-0.412	-0.038	Q7K0W1	DROME	CG8531	7	
302	0.223	1.6485	0.717	0.164	1.7905	1.2405	-0.395	1.341	1.2455	-0.0335	1.7005	0.968	-0.164	Q95T61	DROME	CG2082, isoform B	15	
303	0.496	1.3075	-0.052	0.359	1.2145	0.5115	0.057	0.407	0.6795	0.1585	1.0855	0.059	-0.359	Q0KI33	DROME	CG7956, isoform C	17	
304	0.038	2.4455	3.617	0.241	1.2575	0.6515	0.237	1.324	0.8075	-0.1165	1.3525	2.825	-0.241	Q9I7U4-5	DROME	Isoform E of Titin	7	
305	0.667	1.2305		0.237	1.2495	0.4065	0.147		0.5705	0.4315	1.0025		-0.237	Q7JRB2	DROME	CG14591, isoform A	2	
306	1.174	2.7635		1.239	1.5495	0.9375	-0.324	-0.27	1.2825	-0.0625	1.5345		-1.239	Q9NFR5	DROME	Nicotinic acetylcholine re	1	
307	1.376	1.0005		0.214	1.0915	0.2025	1.373		0.4525	1.1645	0.7965		-0.214	Q9V9V6	DROME	Kek6	1	
308	0.811	0.7795	0.692	0.099	0.6475	-0.0515	0.806	1.648	0.1155	0.6865	0.6675	0.665	-0.099	Q9VCT4	DROME	Klingon	19	
309	-0.178	0.3725		0.205	-0.2145	-0.4155	-0.07		-0.4315	-0.3805	0.1775		-0.205	Q9VPG0	DROME	CG5282	1	
310	0.573	0.4405	-1.592	0.209	0.1365	-0.3505	0.3		-0.1405	0.3665	0.2415	-1.558	-0.209	Q9W1B5	DROME	CG3209, isoform C	3	
311	0.501	-0.4005	-0.36	-0.039	-0.4815	-1.0795	0.728	-1.253	-0.7475	0.5195	-0.4795	-0.38	0.039	P45437	DROME	Coatomer subunit beta	7	
312	0.569	2.2625	-0.704	0.265	1.8975	1.0855	-0.253		1.5825	0.3905	2.0075	-0.844	-0.265	Q9W2E8	DROME	CG10320, isoform A	2	
313	0.478	0.0255		0.336	-0.1475	-0.3355	0.515	1.74	-0.2465	0.5355	-0.2835		-0.336	Q0KI85	DROME	CG34114, isoform B	3	
314	-0.295	2.5215	2.398	-0.252	2.3735	2.1265	-0.465	1.296	3.0715	-0.0225	2.5545	2.275	0.252	B7FNQ3	DROME	RE33133p	12	
315	0.047	-1.0235	-1.442	-0.158	-0.8195	-1.3325	0.62	-2.751	-1.1215	0.3665	-0.8515	-1.405	0.158	Q9VA91	DROME	40S ribosomal protein S7	8	
316	0.398	2.1145		0.282	1.9715	1.2545	-0.114		1.4995	0.1175	1.8425		-0.282	Q9VY22	DROME	CG15890	1	
317	0.013	0.2555	1.398	-0.276	0.7365	0.2695	0.266	0.281	0.6585	0.1745	0.7255	1.879	0.276	Q5LJX9	DROME	CG2893, isoform B (Fragn	4	
318	0.022	1.3765	-0.595	0.289	1.1905	0.6975	-0.428	0.892	0.9425	-0.2325	1.1625	-0.996	-0.289	Q9W0L6	DROME	CG13907	10	
319	0.848	0.4835	0.027	0.348	0.2205	-0.3955	0.551	0.048	0.1055	0.4425	0.2395	0.339	-0.348	Q94887	DROME	Neurexin-4	33	
320	-0.44	0.9195	2.345	-0.295	1.4905	0.9275	-0.424	1.121	1.2195	-0.1195	1.4015	2.036	0.295	Q8IGX6	DROME	RE09889p	9	
321	0.177	1.0195	0.01	0.075	1.0195	0.2815	0.181	0.807	0.6805	0.1405	0.9615	0.012	-0.075	Q9V8R9	DROME	Protein 4.1 homolog	76	
322	0.118	1.3635	0.628	0.145	1.3075	0.7845	-0.146	0.988	0.9285	0.0235	1.3115	0.626	-0.145	P25455	DROME	1-phosphatidylinositol 4,5	31	
323	0.368	1.5295	0.463	0.124	1.4095	0.8445	0.037	0.644	0.8955	0.2235	1.4875	0.578	-0.124	O62619-2	DROME	Isoform B of Pyruvate kin	39	
324	0.151	1.5715	1.252	0.335	1.3815	0.6815	-0.391	1.473	0.8785	-0.1695	1.3525	0.844	-0.335	Q24418	DROME	Glutamate [NMDA] recep	17	
325	0.464	1.8335	0.968	0.263	1.4685	0.9835	0.029	1.737	1.1585	0.0815	1.5145	1.127	-0.263	O62530	DROME	AP-50, isoform A	24	
326	0.621	1.6635	0.824	0.377	1.1725	0.4655	0.28		0.7075	0.2935	1.1465	0.238	-0.377	Q8IR72	DROME	CG32638	2	
327	-0.155	0.7645	-1.161	-0.269	1.0125	0.4045	0.359	2.067	0.4355	0.2615	1.0435	-0.9	0.269	Q8MQS1	DROME	GH14073p	2	
328	0.378	1.4875		0.082	1.5165	0.8395	-0.133	1.898	0.6615	0.2985	1.4155		-0.082	Q9VDV4	DROME	Anoctamin	1	

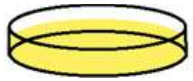
Proteomic Data - TMT Ratios

Quantitative analysis with reference lists

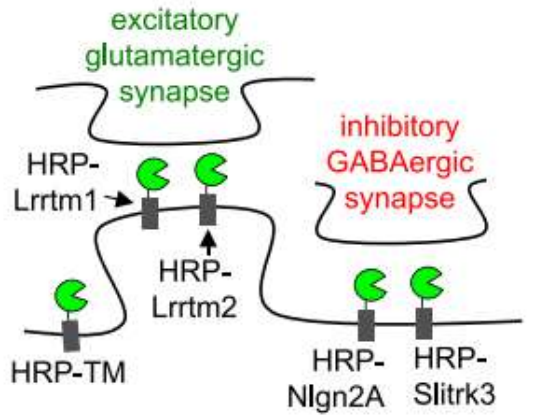
- Cutoff is determined by True-Positive (TP) and False-Positive (FP) Lists


Target PL enzyme


Reference PL enzyme


omit enzyme

- TP list: proteins that should be enriched



Loh et al, Cell 2016

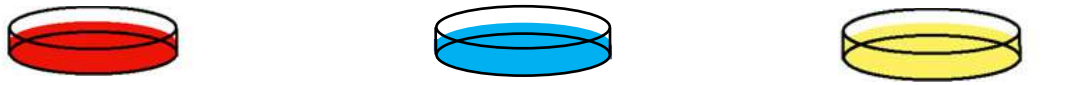
Examples:

If mapping the synaptic cleft, TP list should be previously known synaptic cleft proteins.

If mapping the interactome of Lrrtm1, TP list should be known interacting partners of Lrrtm1.

Quantitative analysis with reference lists

- Cutoff is determined by True-Positive (TP) and False-Positive (FP) Lists



Target PL enzyme Reference PL enzyme omit enzyme

- TP list: proteins that should be enriched
- FP list1: proteins that should not be labeled at all by the target enzyme

(in a different compartment separated by membrane)

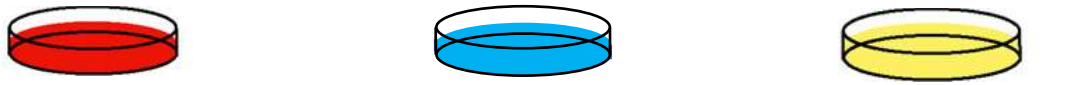
Examples:

For synaptic cleft mapping, FP list1 could be mitochondrial matrix proteins+nuclear proteins+cytosolic proteins.

For mito nucleoid complex mapping, FP list1 could be nuclear proteins+secreted proteins.

Quantitative analysis with reference lists

- Cutoff is determined by True-Positive (TP) and False-Positive (FP) Lists



Target PL enzyme Reference PL enzyme omit enzyme

- TP list: proteins that should be enriched
- FP list1: proteins that should not be labeled at all by the target enzyme
- FP list2: proteins that should be less enriched by target compared to reference (in a contiguous space as the target but less enriched)

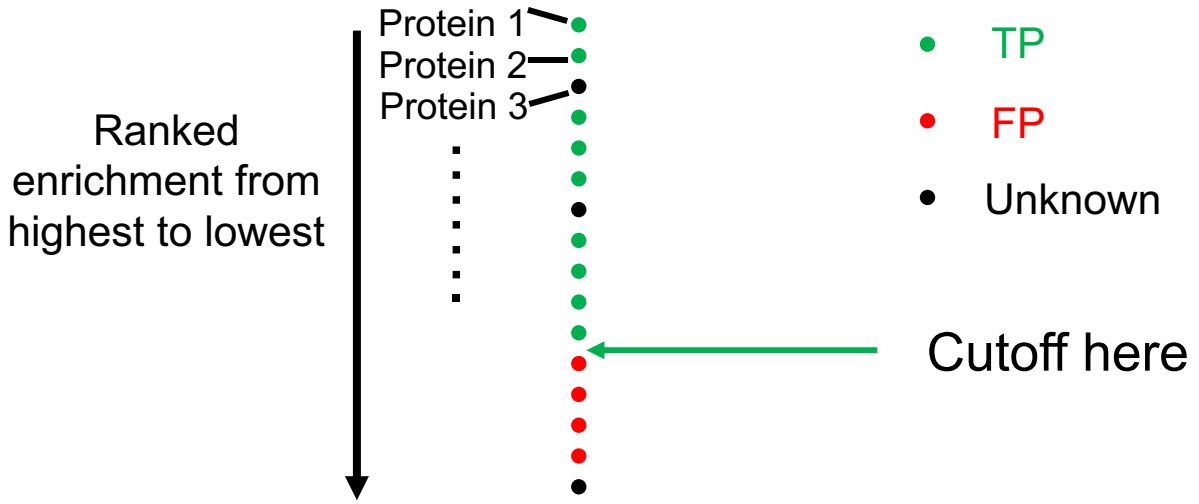
Examples:

For synaptic cleft mapping, FP list2 could be cell surface proteins that are NOT known to be synaptic.

For mito nucleoid complex mapping, FP list2 could be mito matrix proteins that are NOT known to be related to nucleoid.

Quantitative analysis with reference lists

- Proteins at the top/bottom are more likely to be true/false hits
- Cutoff is determined by True-Positive (TP) and False-Positive (FP) Lists



Resources for making reference lists

- Gene Ontology Resource

  http://geneontology.org/

Results

	Reference list	upload_1
Uniquely Mapped IDS:	20851 out of 20851	1 out of 1
Unmapped IDs:	0	0
Multiple mapping information:	0	0

xport Table XML with user input ids JSON with user input ids

isplaying all results; click here to display only significant results

Click on the numbers to see the list associated with each term

	Homo sapiens (REF)			upload_1 (▽ Hierarc	
GO biological process complete	#	#	expected	Fold Enrichment	+/
ventral midline determination	1	1	.00	> 100	+
↳regionalization	335	1	.02	62.24	+
↳pattern specification process	443	1	.02	47.07	+
↳multicellular organismal process	6985	1	.33	2.99	+
↳multicellular organism development	4906	1	.24	4.25	+
↳anatomical structure development	5301	1	.25	3.93	+
↳developmental process	5765	1	.28	3.62	+
↳ventral midline development	5	1	.00	> 100	+
↳central nervous system development	1019	1	.05	20.46	+
↳nervous system development	2203	1	.11	9.46	+
↳system development	4317	1	.21	4.83	+
mesenchymal to epithelial transition involved in metanephric renal vesicle formation	1	1	.00	> 100	+
↳metanephric renal vesicle formation	4	1	.00	> 100	+
↳renal vesicle formation	6	1	.00	> 100	+
↳renal vesicle morphogenesis	13	1	.00	> 100	+
↳morphogenesis of an epithelium	440	1	.02	47.39	+
↳epithelium development	1121	1	.05	18.60	+
↳tissue development	1763	1	.08	11.83	+
↳tissue morphogenesis	550	1	.03	37.91	+
↳anatomical structure morphogenesis	2182	1	.10	9.56	+
↳renal vesicle development	14	1	.00	> 100	+

GENEONTOLOGY Unifying Biology

PANTHER Classification System

LOGIN REGISTER CONTACT US

Home | About | PANTHER Data | PANTHER Tools | PANTHER Services | Workspace | Downloads | Help/Tutorial

PANTHER Tool for grafting sequences released!

PANTHER GENE LIST Customize Gene list

Convert List to: -Select- Send list to: -Select-

Display: 30 items per page Refine Search

Hits 1-30 of 1121 [page: (1) 2 3 4 5 6 7 8 9 10 >>] Number of mapped ids found 1121

Species Filter: All

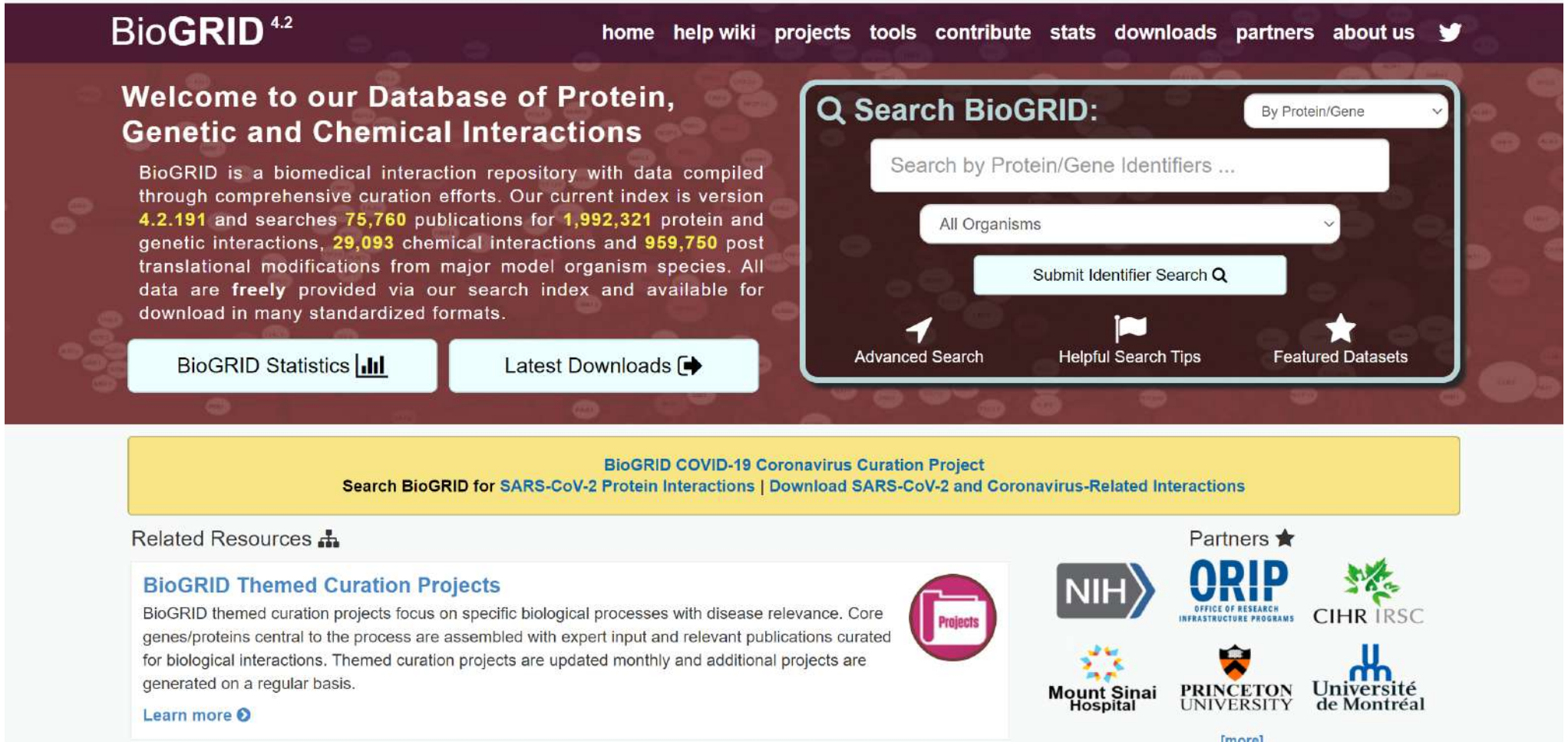
Download the list as excel

clr all		Gene ID	Mapped IDs	Gene Name Gene Symbol Ortholog	PANTHER Family/Subfamily	PANTHER Protein Class	Species
☐	1.	HUMAN|HGNC=18669|UniProtKB=Q8N3R9	HUMAN|HGNC=18669|UniProtKB=Q8N3R9	MAGUK p55 subfamily member 5 MPP5 ortholog	MAGUK P55 SUBFAMILY MEMBER 5 (PTHR23122:SF14)	nucleotide kinase	Homo sapiens
☐	2.	HUMAN|HGNC=11796|UniProtKB=P10827	HUMAN|HGNC=11796|UniProtKB=P10827	Thyroid hormone receptor alpha THRA ortholog	THYROID HORMONE RECEPTOR ALPHA (PTHR24082:SF42)	C4 zinc finger nuclear receptor	Homo sapiens
☐	3.	HUMAN|HGNC=6485|UniProtKB=O15230	HUMAN|HGNC=6485|UniProtKB=O15230	Laminin subunit alpha-5 LAMA5 ortholog	LAMININ SUBUNIT ALPHA-5 (PTHR10574:SF261)	extracellular matrix protein	Homo sapiens
☐	4.	HUMAN|HGNC=17108|UniProtKB=Q8TE57	HUMAN|HGNC=17108|UniProtKB=Q8TE57	A disintegrin and metalloproteinase with thrombospondin motifs 16 ADAMTS16 ortholog	A DISINTEGRIN AND METALLOPROTEINASE WITH THROMBOSPONDIN MOTIFS 16 (PTHR13723:SF140)	metalloprotease	Homo sapiens
☐	5.	HUMAN|HGNC=2514|UniProtKB=P35222	HUMAN|HGNC=2514|UniProtKB=P35222	Catenin beta-1 CTNNB1 ortholog	CATENIN BETA-1 (PTHR45976:SF4)	-	Homo sapiens
☐	6.	HUMAN|HGNC=1507|UniProtKB=P55212	HUMAN|HGNC=1507|UniProtKB=P55212	Caspase-6 CASP6 ortholog	CASPASE-6 (PTHR10454:SF206)	protease	Homo sapiens
☐	7.	HUMAN|HGNC=668|UniProtKB=P62745	HUMAN|HGNC=668|UniProtKB=P62745	Rho-related GTP-binding protein RhoB RHOB ortholog	RHO-RELATED GTP-BINDING PROTEIN RHOB (PTHR24072:SF0)	small GTPase	Homo sapiens
☐	8.	HUMAN|HGNC=16778|UniProtKB=Q9BYR8	HUMAN|HGNC=16778|UniProtKB=Q9BYR8	Keratin-associated protein 3-1 KRTAP3-1 ortholog	KERATIN-ASSOCIATED PROTEIN 3-1 (PTHR23260:SF3)	-	Homo sapiens
☐	9.	HUMAN|HGNC=10888|UniProtKB=Q9NPC8	HUMAN|HGNC=10888|UniProtKB=Q9NPC8	Homeobox protein SIX2	HOMEOBOX PROTEIN SIX2	homeodomain transcription factor	Homo sapiens

Resources for making reference lists

- BioGrid PPI Resource

  https://thebiogrid.org/



Download the list as excel

PTCH1

Homo sapiens

BCNS, HPE7, NBCCS, PTC, PTC1, PTCH, PTCH11, RP11-436O5.3

patched 1

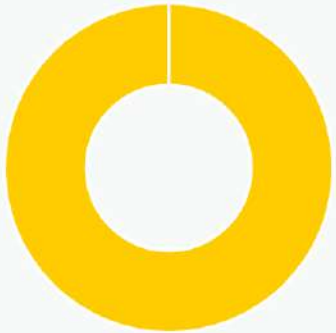
GO Process (21) | GO Function (5) | GO Component (5)

CRISPR Database | OMIM | HGNC | VEGA | Entrez Gene | RefSeq | UniprotKB | Ensembl | HPRD

Download Curated Data for this Protein

Interactor Statistics

Proteins/Genes	Publications
205	17



Physical Interactors (205)

Switch View: Interactors 205 | Interactions 272 | Network | PTM Sites 23

Showing **1** to **205** of **205** unique interactors

Filter Interactions... ADV

Interactor	Organism / Chemical Type	Aliases	Description	Evidence
SMO	H. sapiens	Gx, SMOH, FZD11	smoothened, frizzled class receptor	3 View
ABCA2	H. sapiens	ABC2, RP11-229P13.8	ATP-binding cassette, sub-family A (ABC1), member 2	2 View
ADRBK1	H. sapiens	GRK2, BARK1, BETA-ARK1	adrenergic, beta, receptor kinase 1	2 View
ARSK	H. sapiens	TSULF, UNQ630/PRO1246	arylsulfatase family, member K	2 View

Resources for making reference lists

- Organelle or protein specific databases
  e.g. MitoCarta for mitochondrial proteome

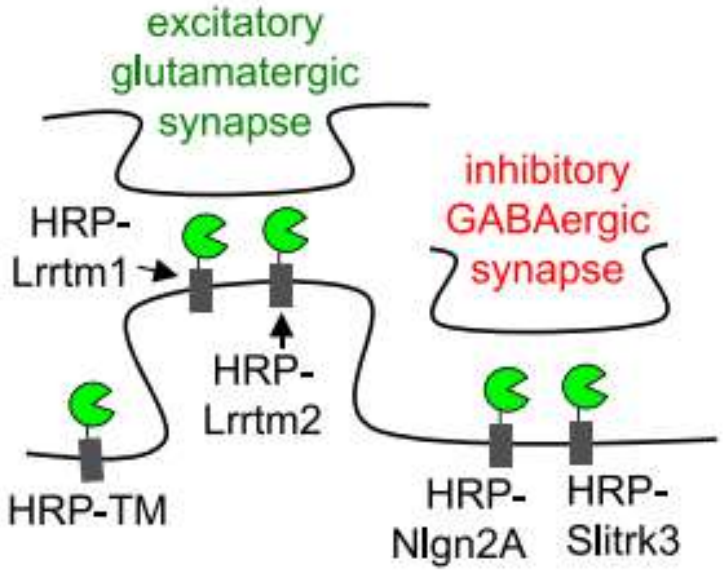
- Existing literature search (Google, Pubmed, etc)

Important:

Both the TP and FP lists should be generated ***a priori*** to be unbiased and accurate.

The criteria need to be consistent throughout. Never include or reject a protein based on the PL proteomic data.

Example of making reference lists



Loh et al, Cell 2016

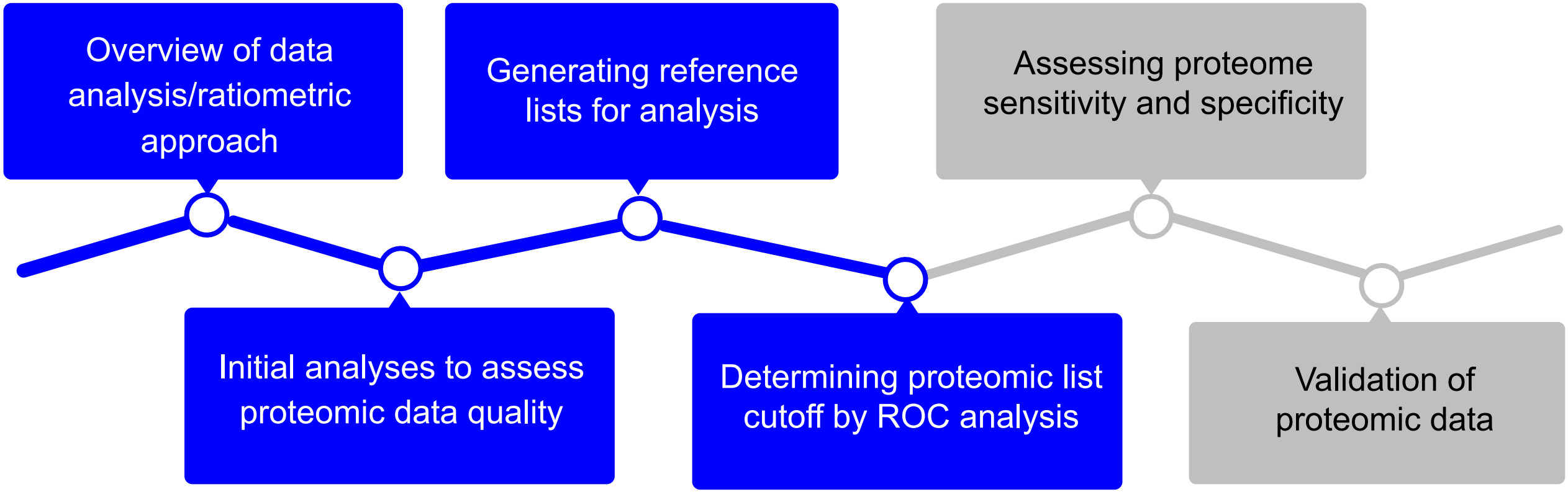
TP list: 176 known synaptic proteins based on GOCC and literature .

FP list1 (intracellular proteins that should be inaccessible to the BxxP radical): GOCC terms nucleus, mitochondria, peroxisome, lysosome, cytosol, endoplasmic reticulum, and Golgi. From this collection, removed proteins with “extracellular” annotation in GOCC, proteins present in the TP1 list, and proteins enriched in previous synapse studies by Bayés et al., 2012, Biesemann et al., 2014, Boyken et al., 2013, and Pirooznia et al., 2012.

FP list2 (non-synaptic cell surface proteins): GOCC terms: cell surface, extracellular space, extracellular region, external side of plasma membrane, extracellular matrix, extracellular vesicular, integral component of plasma membrane. From this collection, removed proteins present in the TP1 or FP1 lists, and proteins enriched in previous synapse studies by Bayés et al., 2012, Biesemann et al., 2014, Boyken et al., 2013, and Pirooznia et al., 2012.

Outline

Proteomic data analysis for proximity labeling (PL) experiments:



Calculating TPR and FPR

- Rank the proteome data by enrichment ratio from highest to lowest. Always work with a ranked list!

Calculating TPR and FPR

- Rank the proteome data by enrichment ratio from highest to lowest. Always work with a ranked list!

- Annotate the proteomic data using TP, FP lists.

Calculating TPR and FPR

- Rank the proteome data by enrichment ratio from highest to lowest. Always work with a ranked list!
- Annotate the proteomic data using TP, FP lists.
- Calculate TP and FP rate (TPR, FPR) for each ratio from high to low

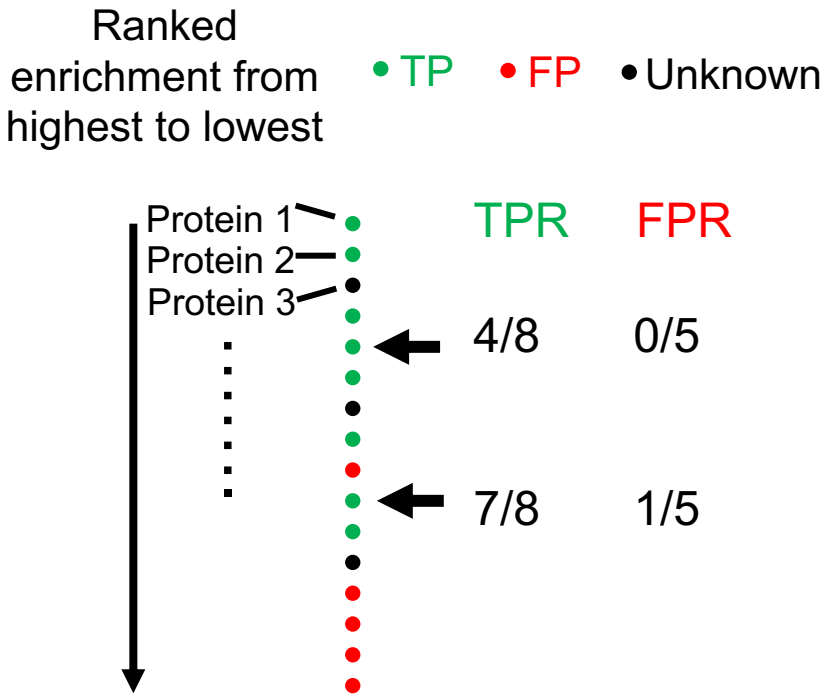
Equation:

$$\text{TPR at ratio x} = \frac{cumulative\ \#\ of\ TP\ proteins\ above\ x}{total\ \#\ of\ TP\ in\ the\ proteomic\ data}$$

$$\text{FPR at ratio x} = \frac{cumulative\ \#\ of\ FP\ proteins\ above\ x}{total\ \#\ of\ FP\ in\ the\ proteomic\ data}$$

Calculating TPR and FPR

- Rank the proteome data by enrichment ratio from highest to lowest. Always work with a ranked list!
- Annotate the proteomic data using TP, FP lists.
- Calculate TP and FP rate (TPR, FPR) for each ratio from high to low



Equation:

$$\text{TPR at ratio x} = \frac{cumulative\ \#\ of\ TP\ proteins\ above\ x}{total\ \#\ of\ TP\ in\ the\ proteomic\ data}$$

$$\text{FPR at ratio x} = \frac{cumulative\ \#\ of\ FP\ proteins\ above\ x}{total\ \#\ of\ FP\ in\ the\ proteomic\ data}$$

R10 =SUM(M2:M10)/335

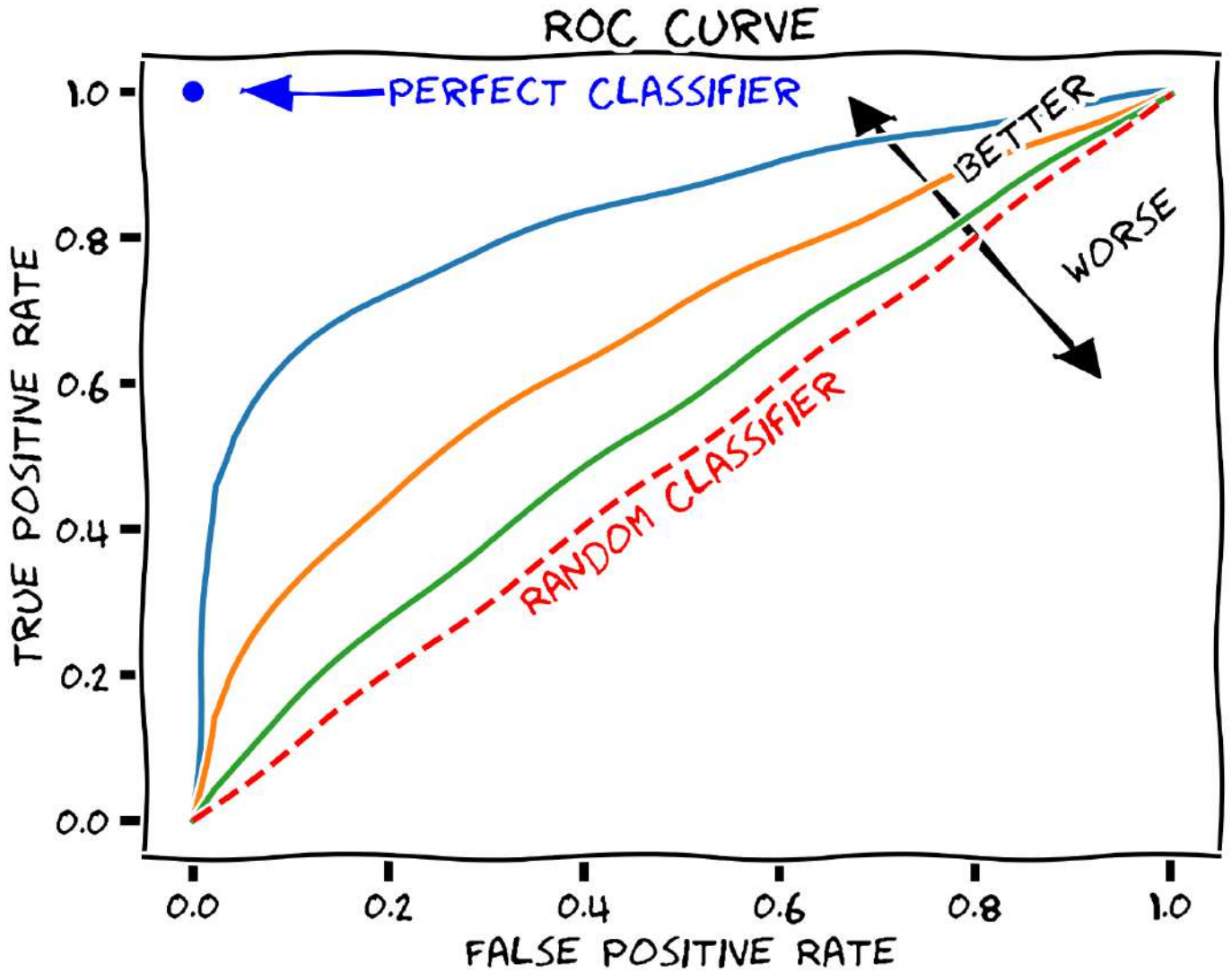
Cumulative # of TP proteins above a ratio

TP (yes=1, no=0)

	A	B	C	D	E	G	H	J	K	L	M	Q	R	S	T
1	2129C:127	126:128C	127C:130C	129N:131	id	Symbol	accession_	numPepsU	species	entry_nam	TP	FP	TPR	FPR	TPR-FPR
2	1.916	2.432	0.8635	4.2805	P10040	crb	P10040-2	2	DROME	Isoform B c	1	0	0.002985	0	0.002985
3	1.999	1.49	0.5865	1.0165	Q9V3X2	Tsp96F	Q9V3X2	3	DROME	BcDNA.LD1	1	0	0.00597	0	0.00597
4	1.551	1.481	1.8295	0.9635	P15278	Fas3	P15278-2	8	DROME	Isoform B c	1	0	0.008955	0	0.008955
5	1.154	1.397	0.6625	0.4405	A1Z6S0	mim	A1Z6S0	2	DROME	CG33558, i	1	0	0.01194	0	0.01194
6	2.224	1.383	0.2555	-0.3135	D3PK82	CG1552	D3PK82	3	DROME	RH59554p	0	0	0.01194	0	0.01194
7	1.517	1.35	0.4715	1.0275	Q9W0C1	nSyb	Q9W0C1	9	DROME	GH04664p	1	0	0.014925	0	0.014925
8	2.218	1.346	3.1485	1.6665	Q59DV8	Gfrl	Q59DV8	3	DROME	Munin	1	0	0.01791	0	0.01791
9	1.754	1.296	-0.6795	-0.2385	Q9W2L6	CG9394	Q9W2L6	35	DROME	CG9394	0	0	0.01791	0	0.01791
10	0.702	1.281	2.9915	1.5515	D0UGE6	tnc	D0UGE6	4	DROME	Tenectin is	0	0	0.01791	0	0.01791
11	1.141	1.279	0.5895	0.7735	D5A7S0	CG17734	D5A7S0	2	DROME	MIP20553p	0	0	0.01791	0	0.01791
12	1.544	1.235	0.4875	0.0625	Q7K188	CG6329	Q7K188	6	DROME	CG6329, isc	0	0	0.01791	0	0.01791
13	0.892	1.183	0.6105	0.9695	Q9VIU4	CG33116	Q9VIU4	2	DROME	CG33116	0	0	0.01791	0	0.01791
14	1.634	1.182	0.4885	0.4715	A8DZ06	CG4587	A8DZ06	57	DROME	CG4587, isc	0	0	0.01791	0	0.01791
15	1.067	1.174	0.1625	0.5235	P48613	tipE	P48613	2	DROME	Protein tipE	1	0	0.020896	0	0.020896
16	1.004	1.141	0.0405	0.1095	Q9W436	Nep1	Q9W436	10	DROME	GH03315p	1	0	0.023881	0	0.023881
17	0.305	1.138	1.0735	0.8425	Q24323	Sema2a	Q24323	2	DROME	Semaphorin	1	0	0.026866	0	0.026866
18	1.537	1.114	0.0385	0.2865	Q9VU13	CG42709	Q9VU13	6	DROME	CG17667, i	0	0	0.026866	0	0.026866
19	1.427	1.099	0.6405	0.3095	B7Z0L0	Fas1	B7Z0L0	41	DROME	Fasciclin 1,	0	0	0.026866	0	0.026866
20	1.186	1.098	1.0775	0.7135	Q9VY33	dpr8	Q9VY33	2	DROME	Dpr8	1	0	0.029851	0	0.029851
21	1.488	1.083	-0.5805	0.1375	Q7JRL9	CG31221	Q7JRL9	2	DROME	CG31221, i	0	0	0.029851	0	0.029851
22	1.098	1.07	-0.0115	0.2345	Q8IS44	Dop2R	Q8IS44-2	2	DROME	Isoform 60	1	0	0.032836	0	0.032836
23	1.2	1.062	0.6135	0.5215	Q8IQD3	CG32052	Q8IQD3	7	DROME	CG32052	0	0	0.032836	0	0.032836
24	0.491	1.06	0.0955	0.2065	A8DYJ6	side-VIII	A8DYJ6	8	DROME	CG12484, i	0	0	0.032836	0	0.032836
25	1.464	1.037	-0.2495	-0.0825	Q7KTJ7	Bsg	Q7KTJ7	15	DROME	Basigin, iso	1	0	0.035821	0	0.035821
26	1.42	1	0.2385	-0.0655	Q9W257	CG6044	Q9W257	2	DROME	CG6044, isc	0	0	0.035821	0	0.035821
27	1.041	0.995	1.5025	0.7935	Q9VDB7	CG16791	Q9VDB7	8	DROME	CG16791	0	0	0.035821	0	0.035821
28	1.368	0.977	0.4465	0.4565	Q7K0H4	stj	Q7K0H4	12	DROME	SD07723p	0	0	0.035821	0	0.035821
29	1.031	0.971	1.3585	0.9565	Q03445	GluRIA	Q03445	14	DROME	Glutamate	1	0	0.038806	0	0.038806
30	-0.09	0.957	-1.0405	-1.1565	Q7YZA2	CG7065	Q7YZA2	2	DROME	Uncharacte	0	0	0.038806	0	0.038806
31	1.024	0.957	1.5575	1.0375	Q9W3N2	dpr14	Q9W3N2	2	DROME	Dpr14	1	0	0.041791	0	0.041791

Receiver operator characteristic (ROC) analysis

Maximize the probability of enriching TP while minimizing the probability of including FP



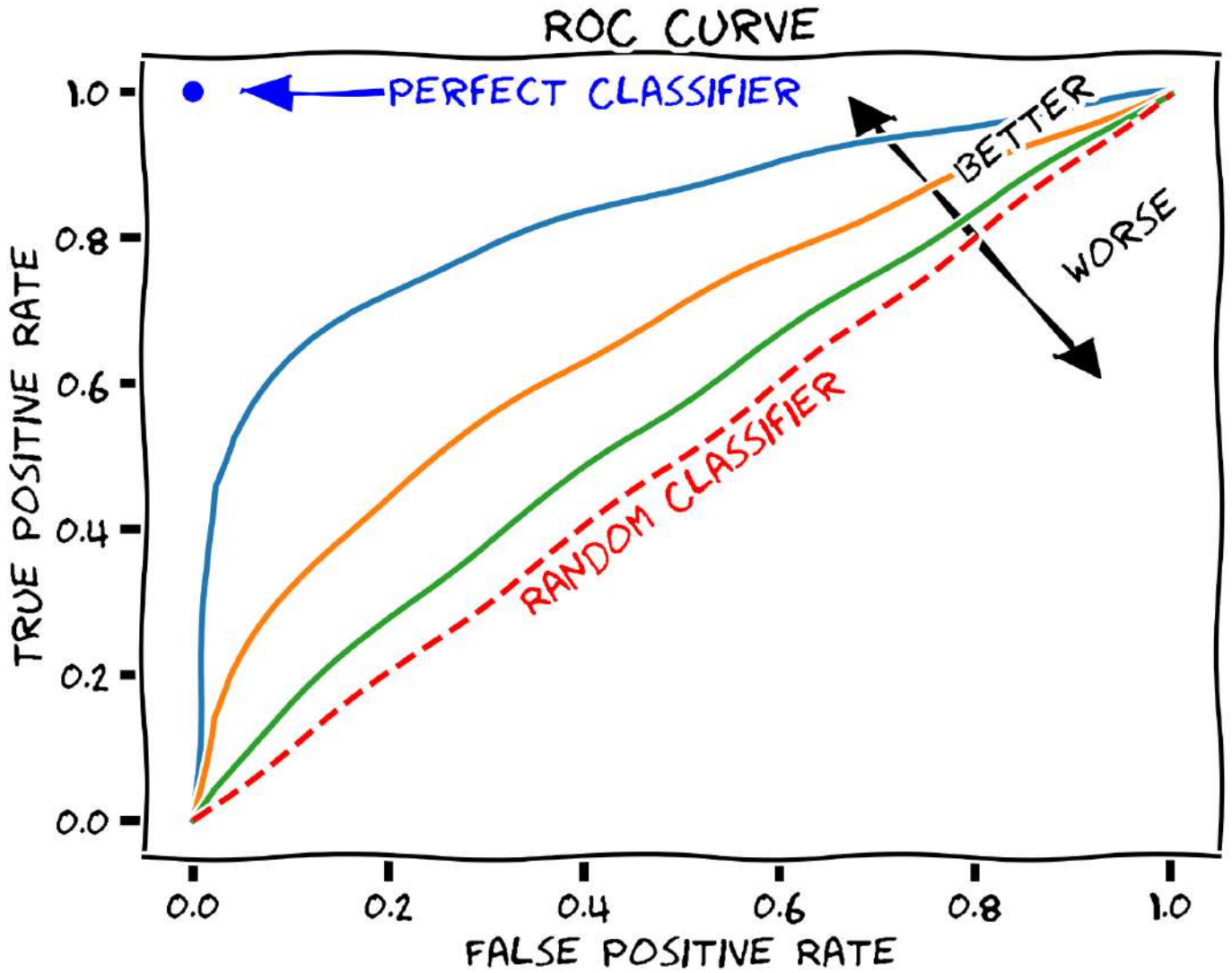
Plot FPR on X axis and TPR on Y axis at every possible cutoff on the graph.

This is the ROC curve for this TMT ratio.

Wikipedia ROC analysis

Receiver operator characteristic (ROC) analysis

Maximize the probability of enriching TP while minimizing the probability of including FP

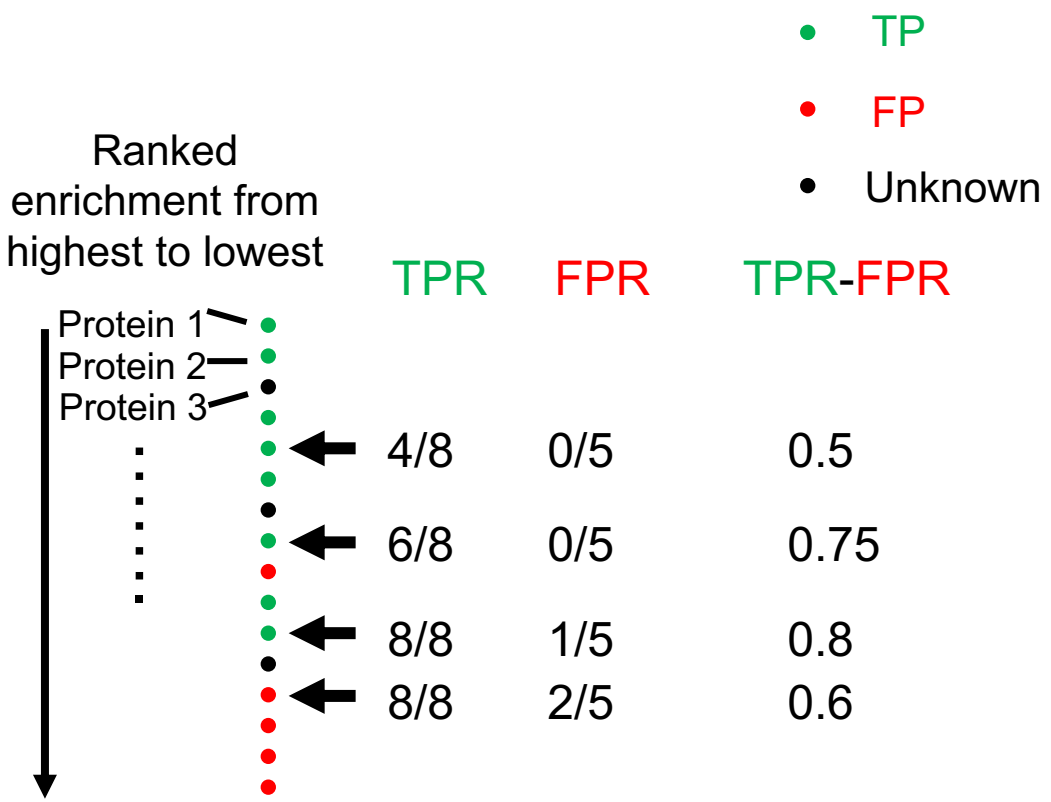


1. Always look for an upper left "elbow", which indicates that the experiment performed well.

Wikipedia ROC analysis

Receiver operator characteristic (ROC) analysis

Maximize the probability of enriching TP while minimizing the probability of including FP

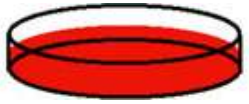


1. Always look for an upper left "elbow", which indicates that the experiment performed well.

2. Determining cutoff:
The ratio at which TPR-FPR is the greatest.

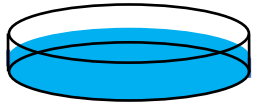
Example: ratiometric tagging for open compartments

TMT label 1



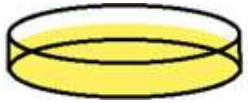
Target APEX
biotin-phenol + H_2O_2

TMT label 2

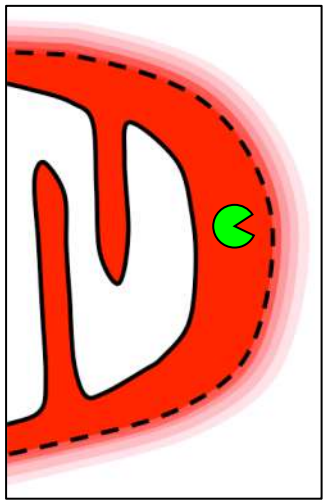


Reference APEX
biotin-phenol + H_2O_2

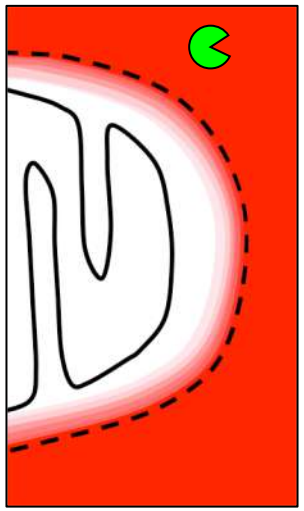
TMT label 3



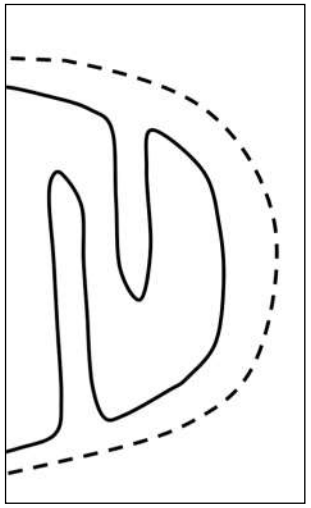
[omit APEX]



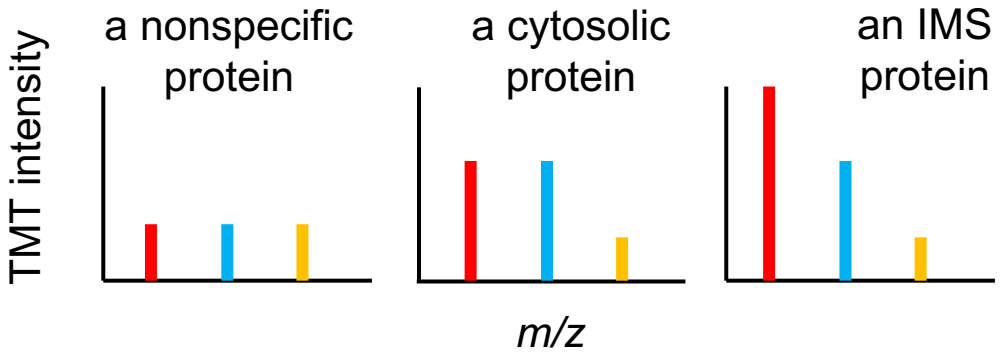
IMS-APEX



Cytosolic APEX



Neg ctrl



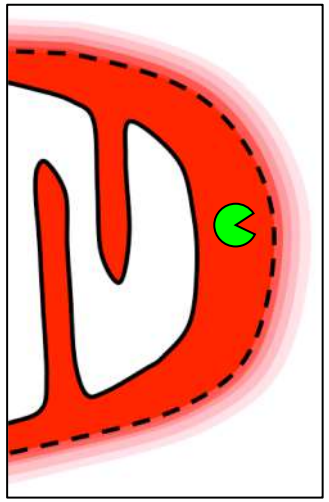
TMT ratio 1/3: Extent of biotinylation by IMS-APEX vs neg ctrl
TMT ratio 1/2: Ratio of biotinylation by IMS-APEX vs cytosolic APEX

Three lists are needed for ratiometric analysis

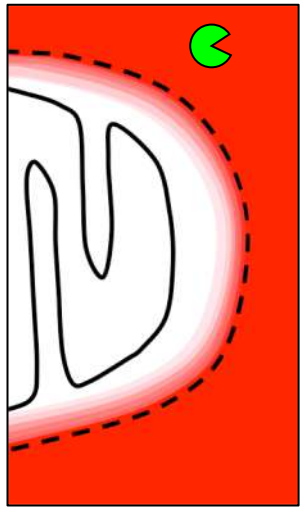
1. TP list (e.g. previously known IMS proteins)

2. FP list1 that ***should not be labeled at all*** (e.g. nuclear proteins)

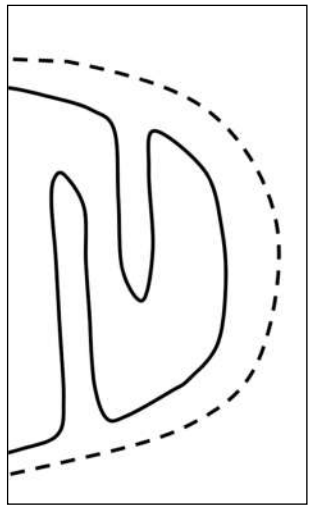
3. FP list2 that could be labeled by target enzyme, but **should not be preferentially enriched by target vs. reference**. (e.g. cytosolic proteins)



IMS-APEX



Cytosolic APEX

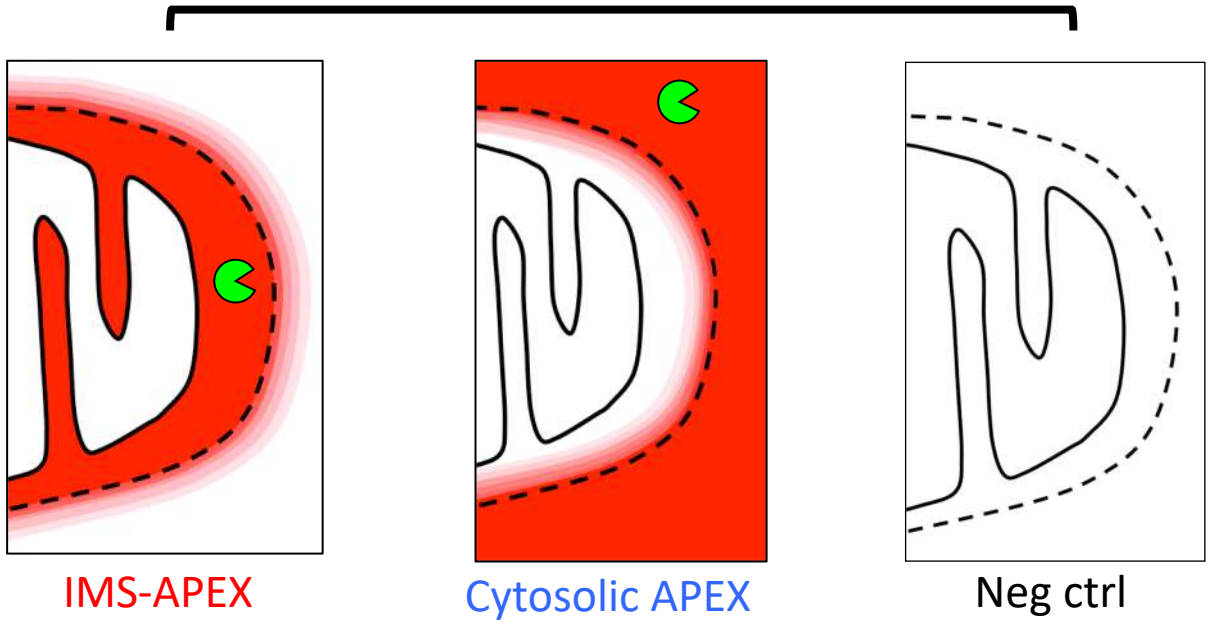


Neg ctrl

Step 1: determine target/neg ctrl cutoff

This cutoff gives a list of proteins that are enriched by target PL enzyme over nonspecific binders (i.e. proteins that are biotinylated by target).

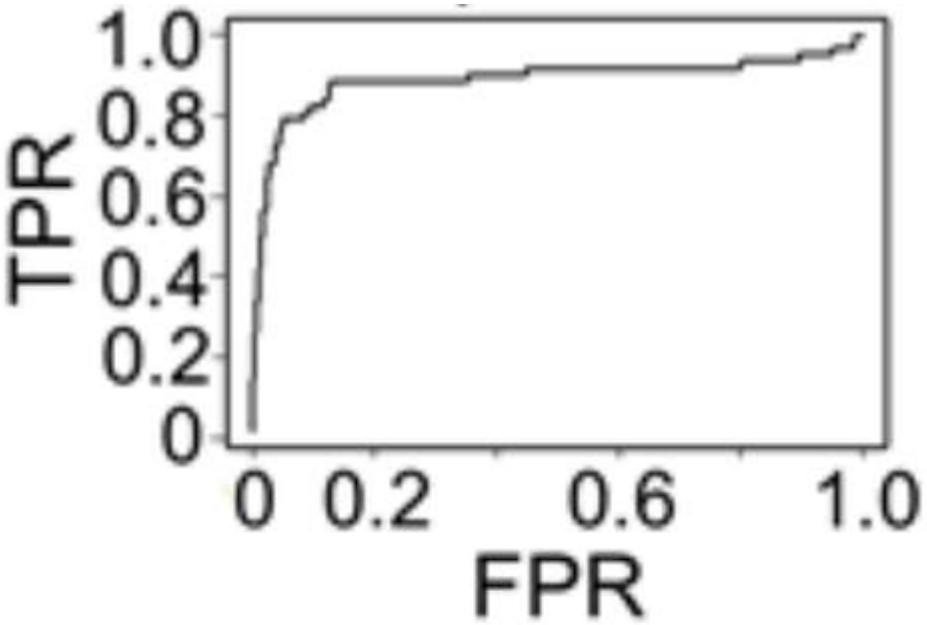
Protocol: Rank **all the data** by target vs. Neg ctrl ratio. Use TP and FP list1 for ROC analysis. **Remove the proteins below the cutoff**.

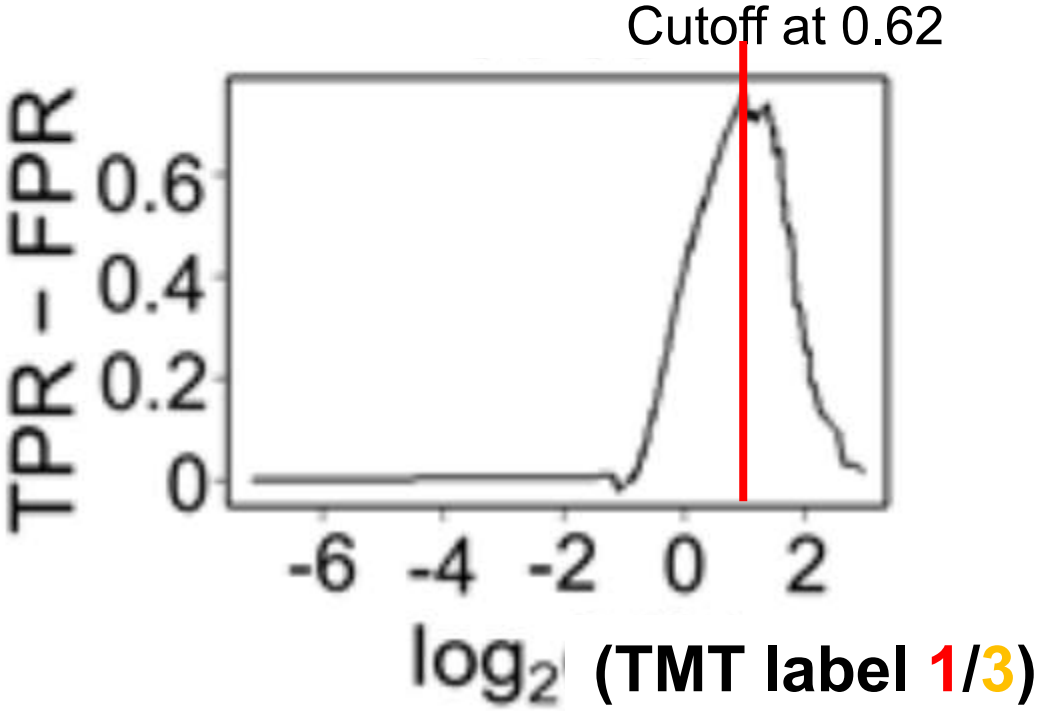


Step 1: determine target/neg ctrl cutoff

This cutoff gives a list of proteins that are enriched by target PL enzyme over nonspecific binders (i.e. proteins that are biotinylated by target).

Protocol: Rank **all the data** by target vs. Neg ctrl ratio. Use TP and FP list1 for ROC analysis. **Remove the proteins below the cutoff**.



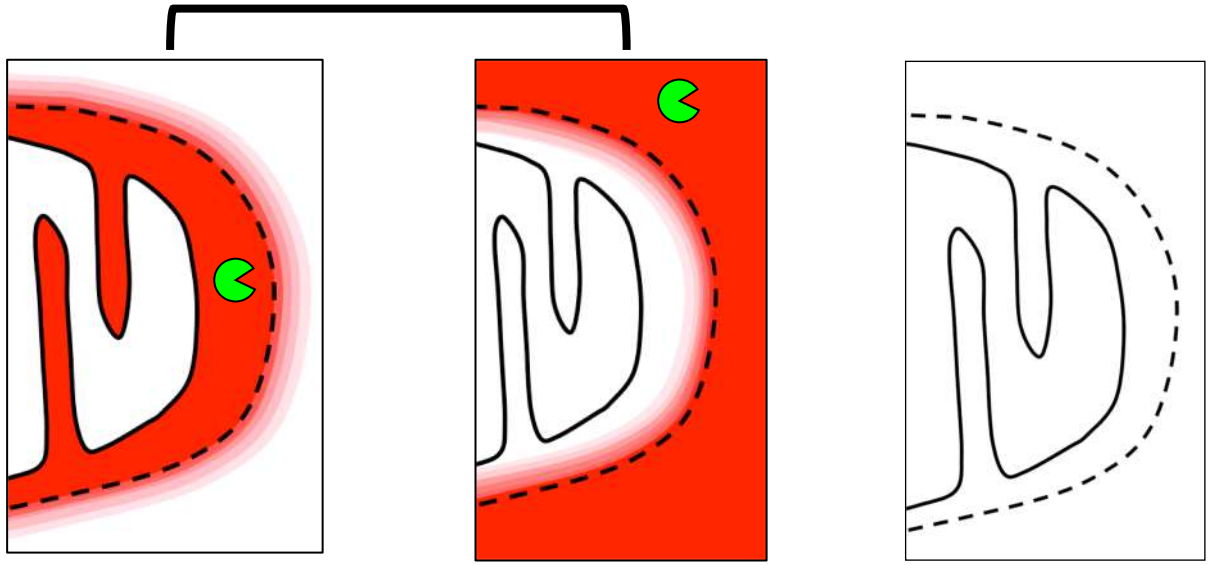


(TMT label 1/3)

Step 2: determine target/reference cutoff

This cutoff gives a list of proteins that are PREFERENTIALLY enriched by target PL enzyme over reference PL enzyme.

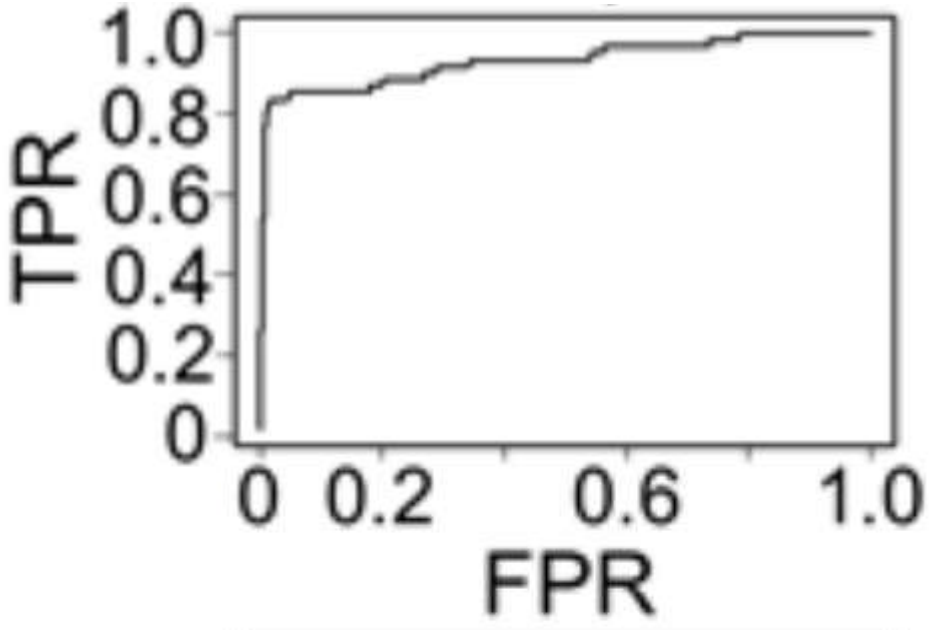
Protocol: **Remove proteins after cutoff in step 1**. For the **remaining** data, rank the proteins by target vs. reference ratio. Use TP and FP list2 for ROC analysis.

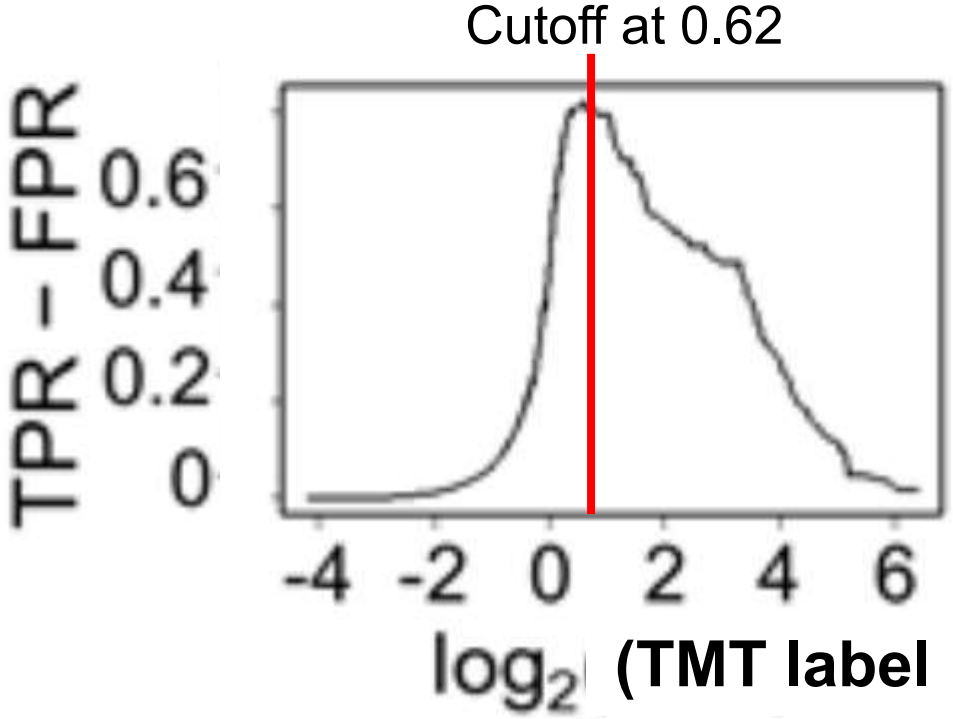


Step 2: determine target/reference cutoff

This cutoff gives a list of proteins that are PREFERENTIALLY enriched by target PL enzyme over reference PL enzyme.

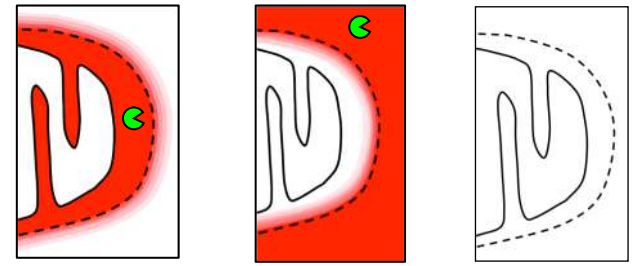
Protocol: **Remove proteins after cutoff in step 1**. For the **remaining** data, rank the proteins by target vs. reference ratio. Use TP and FP list2 for ROC analysis.



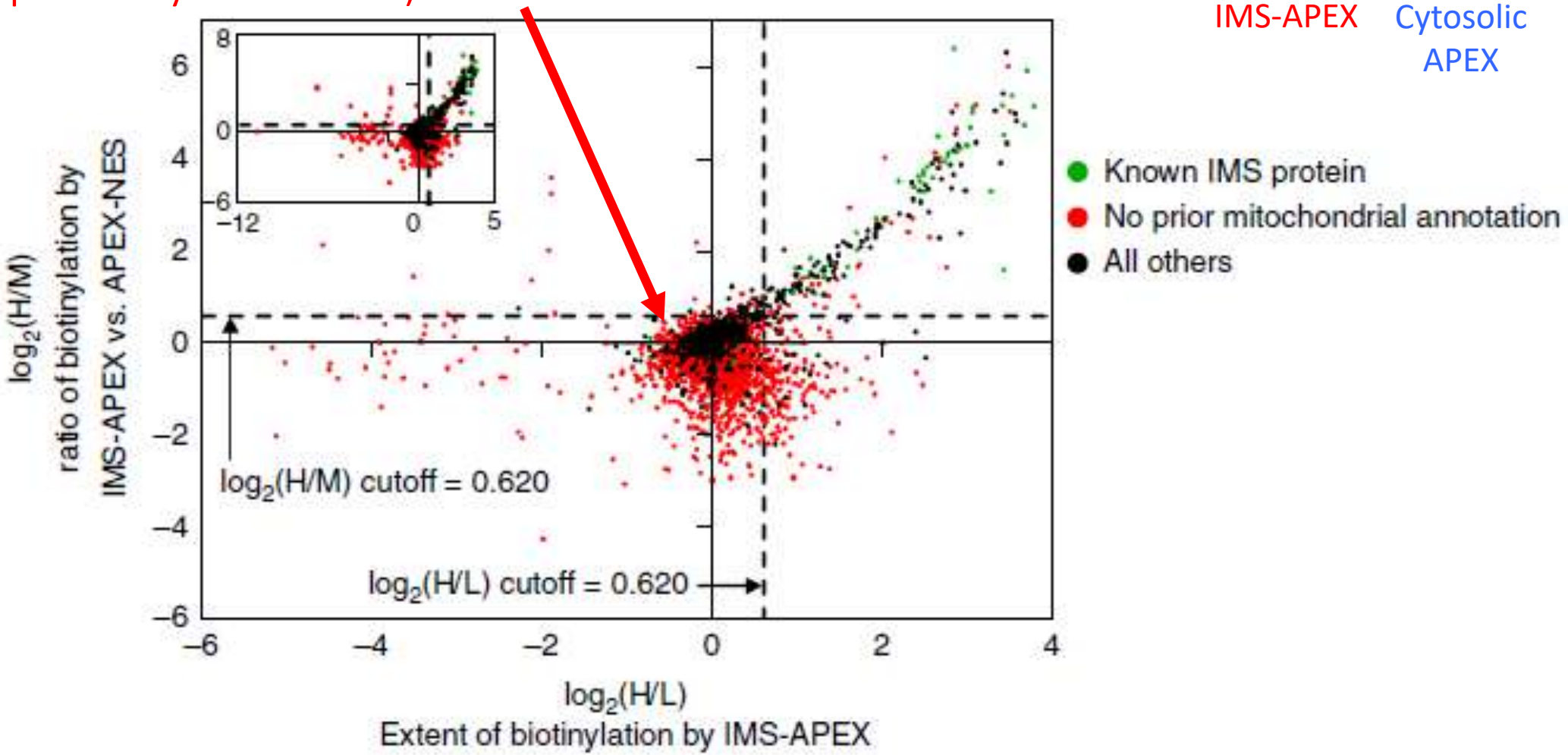


Three-state analysis

e.g. nuclear proteins, ER lumen proteins, etc (separated by a membrane)

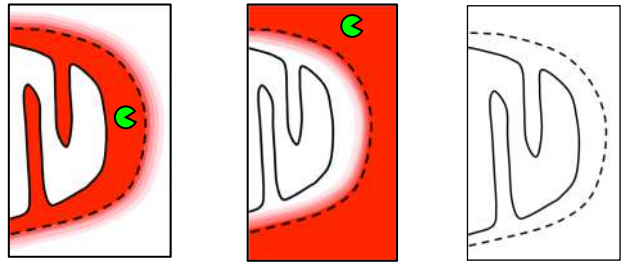


IMS-APEX | Cytosolic APEX | Neg ctrl

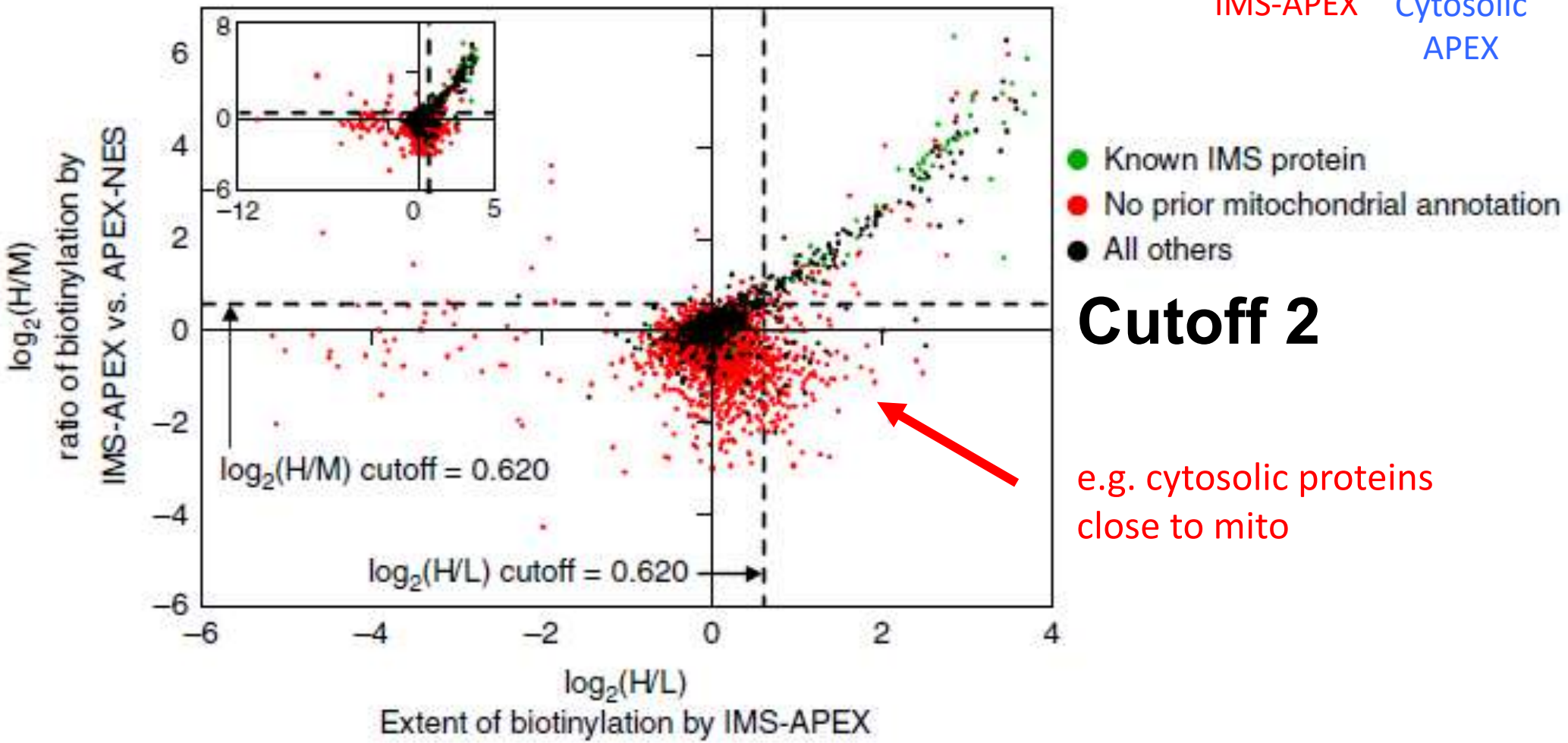


More examples: Hung et al. Mol Cell 2014; Loh et al Cell 2016; Han et al Cell Chem Biol 2017; Hung et al Elife 2017

Three-state analysis



IMS-APEX | Cytosolic APEX | Neg ctrl



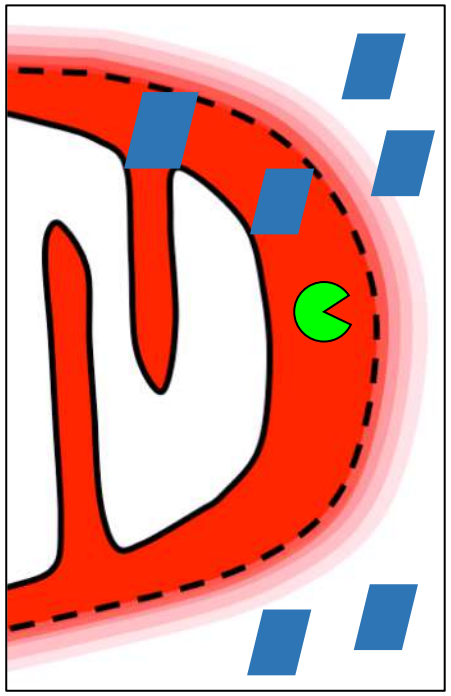
Cutoff 2

e.g. cytosolic proteins close to mito

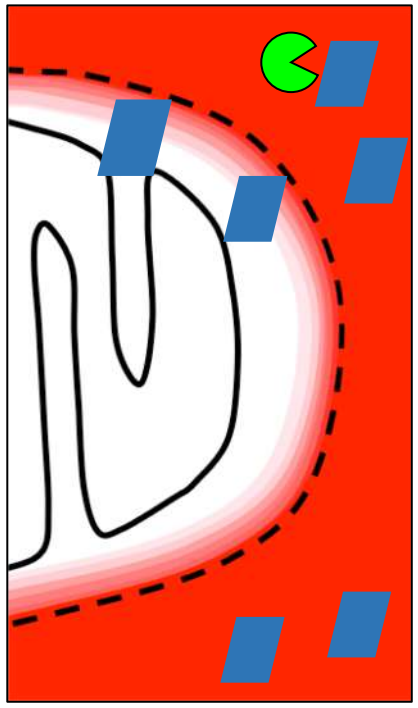
More examples: Hung et al. Mol Cell 2014; Loh et al Cell 2016; Han et al Cell Chem Biol 2017; Hung et al Elife 2017

Limitations of ratiometric approach

Ratiometric approach (filtering by target vs. reference) may result in exclusion of dual-localized proteins, reducing coverage/sensitivity.



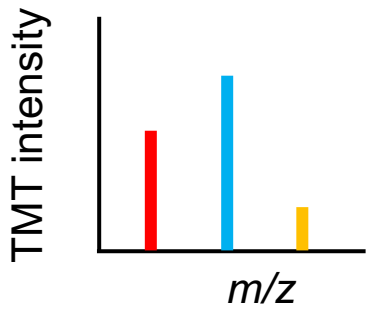
IMS-APEX



Cytosolic APEX

Example of an IMS protein that is filtered out by ratiometric approach

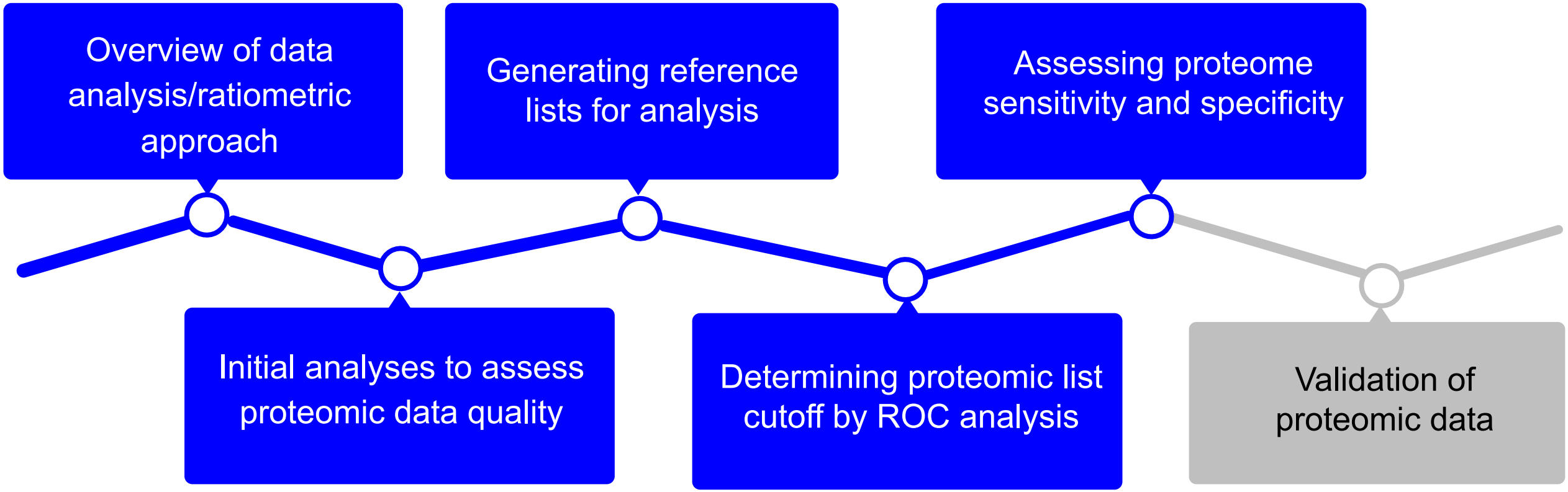
a dual-localized protein



Such proteins will pass cutoff 1, but not cutoff 2.

Outline

Proteomic data analysis for proximity labeling (PL) experiments:



Determining *sensitivity* of proteomic datasets

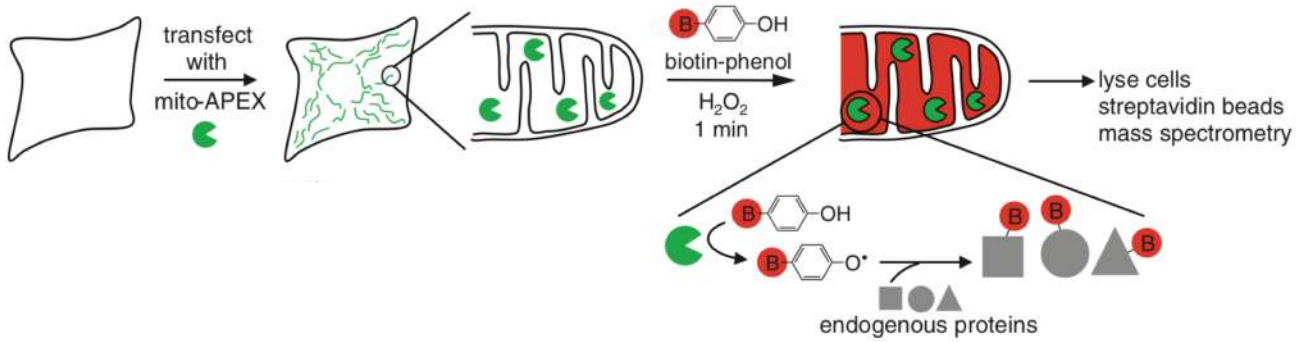
- The sensitivity of a dataset is defined as the fraction of true positive proteins recovered

Determining *sensitivity* of proteomic datasets

- The sensitivity of a dataset is defined as the fraction of true positive proteins recovered

$$\text{Sensitivity} = \frac{\text{\# true positive proteins detected}}{\text{\# total true positive proteins}}$$

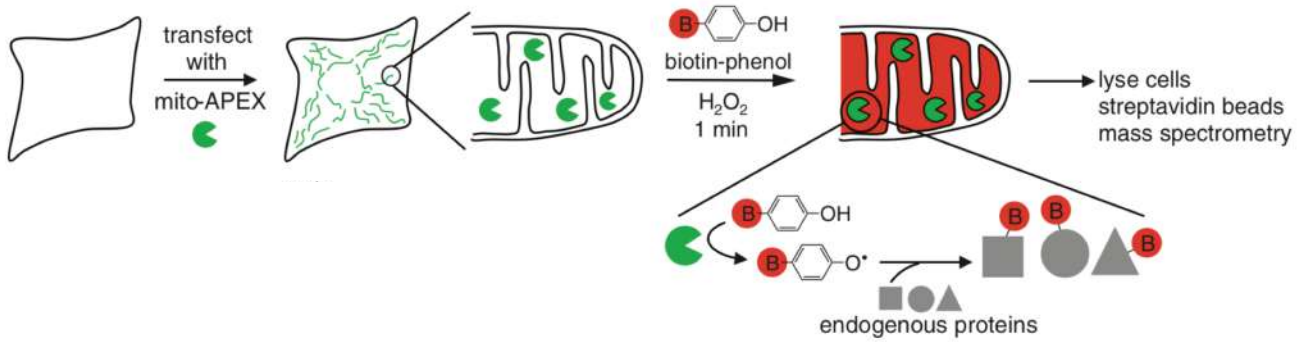
Sensitivity analysis of mito matrix proteome (APEX)



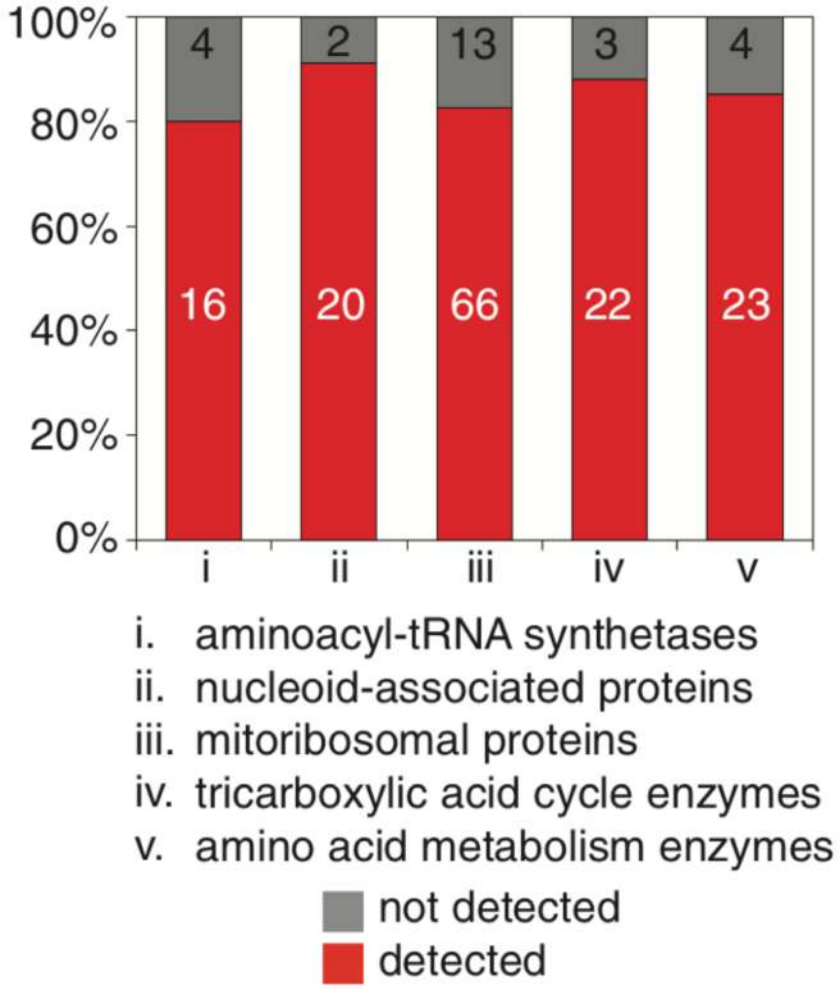
- Compare list of detected proteins to groups of well-known mitochondrial matrix proteins

Rhee et al. *Science*, 2013.

Sensitivity analysis of mito matrix proteome (APEX)



- Compare list of detected proteins to groups of well-known mitochondrial matrix proteins



Rhee et al. *Science*, 2013.

Determining *specificity* of proteomic datasets

- The specificity of a dataset is a measure of how well the proteomic hits align with target localization/functions

$$\text{Specificity} = \frac{\text{\# of proteins in the final list with prior annotation}}{\text{\# total proteins in the final list}}$$

Determining *specificity* of proteomic datasets

- The specificity of a dataset is a measure of how well the proteomic hits align with target localization/functions

$$\text{Specificity} = \frac{\text{\# of proteins in the final list with prior annotation}}{\text{\# total proteins in the final list}}$$

  - Based on previous literature
  - Based on previous annotations/databases
  - Gene ontology analyses
  - Clustering analyses

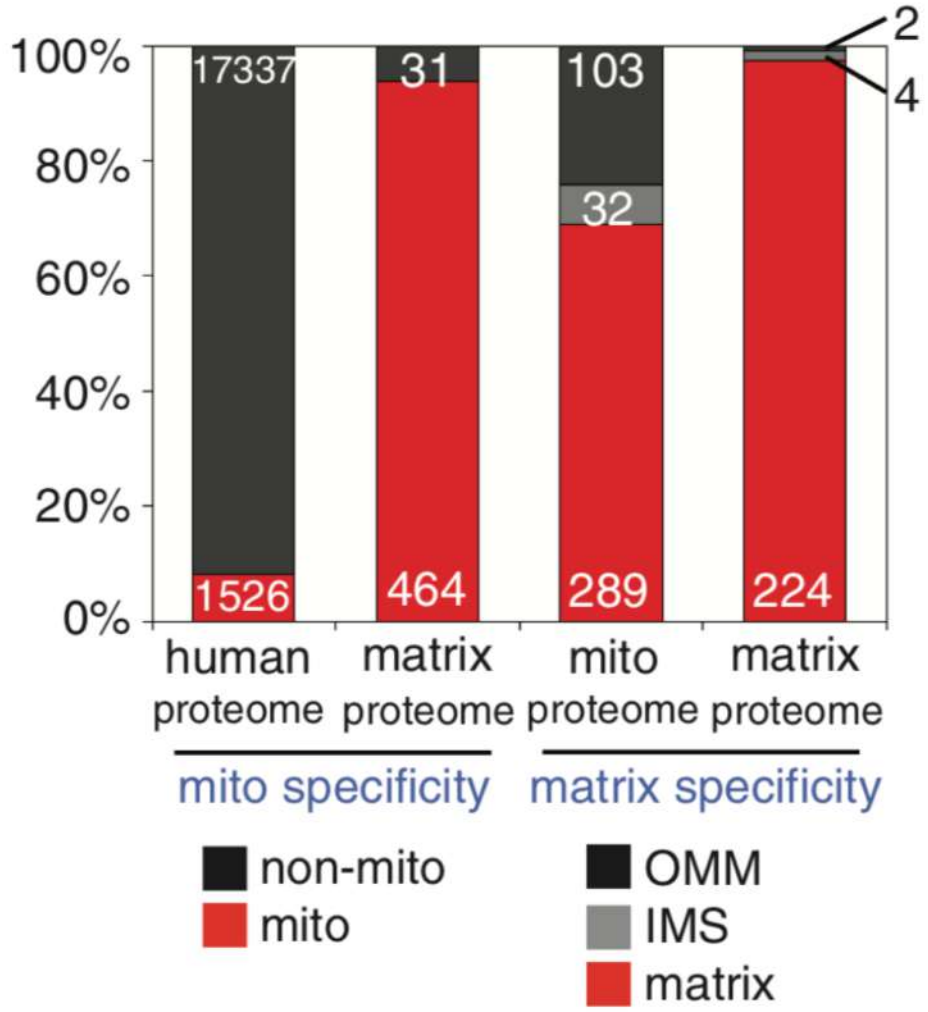
  This generally represents a lower bound and the uncharacterized hits are potentially new biological discoveries.

Gene ontology analyses

- Can search single genes or lists of genes simultaneously
- GO terms can be distinguished by biological process, molecular function, or cellular component
- GO terms can be sorted by species

www.geneontology.org

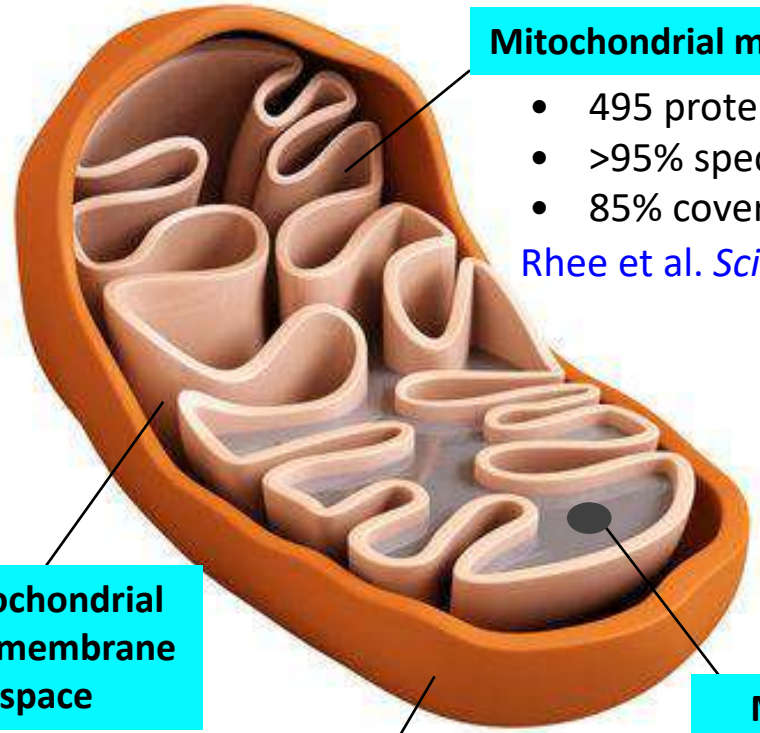
Specificity analysis of mito matrix proteome (APEX)



- APEX-generated proteome is highly specific for mitochondrial matrix proteins

Rhee et al. *Science*, 2013.

APEX organelle proteome sensitivity and specificity



Mitochondrial matrix

- 495 proteins
- >95% specificity
- 85% coverage

Rhee et al. *Science* 2013

Mitochondrial intermembrane space

- 127 proteins
- >94% specificity
- 65% coverage

Hung et al. *Molecular Cell* 2014

Mitochondrial outer membrane

- 137 proteins
- >84% specificity
- 53% coverage

Hung et al. *eLife* 2017

Mitochondrial nucleoid (mtDNA)

- 37 proteins
- >81% specificity
- 62% coverage

Han et al. *Cell Chemical Biology* 2017

ER lumen

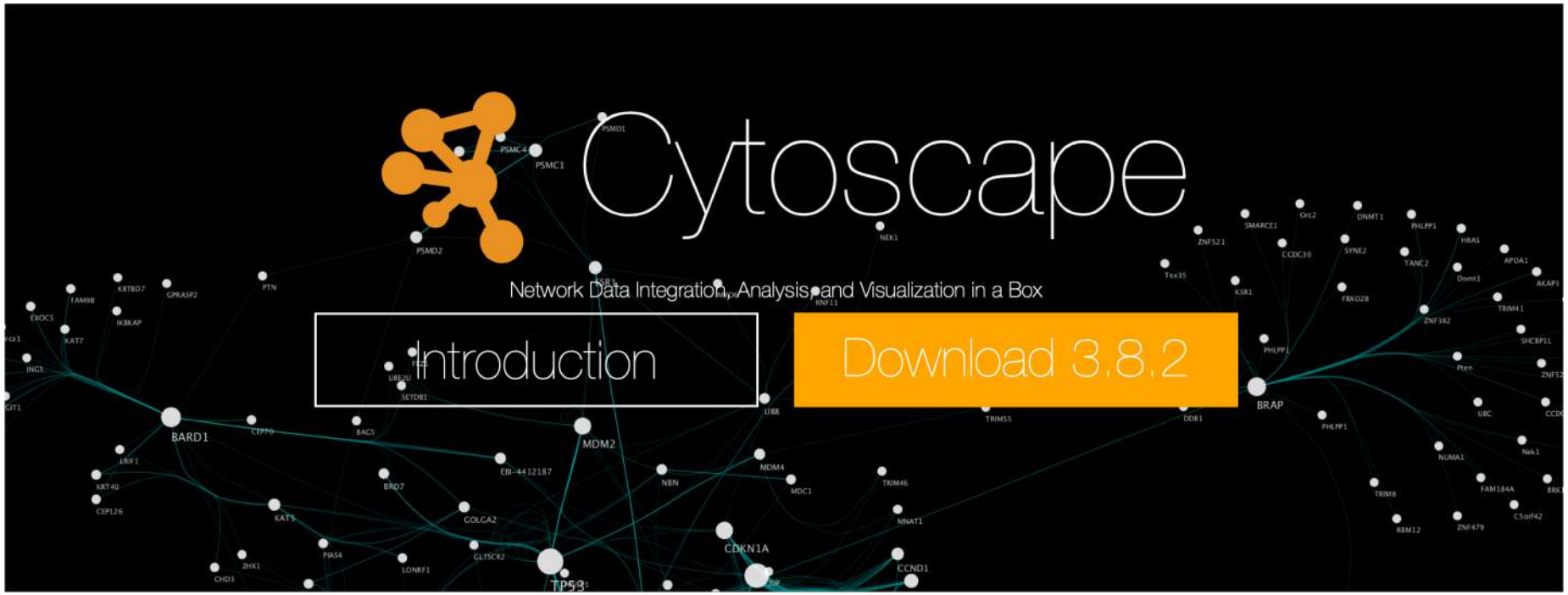
- 338 proteins
- >98% specificity
- 63% coverage

ER membrane

- 634 proteins
- >89% specificity
- 44% coverage

Hung et al. *eLife* 2017

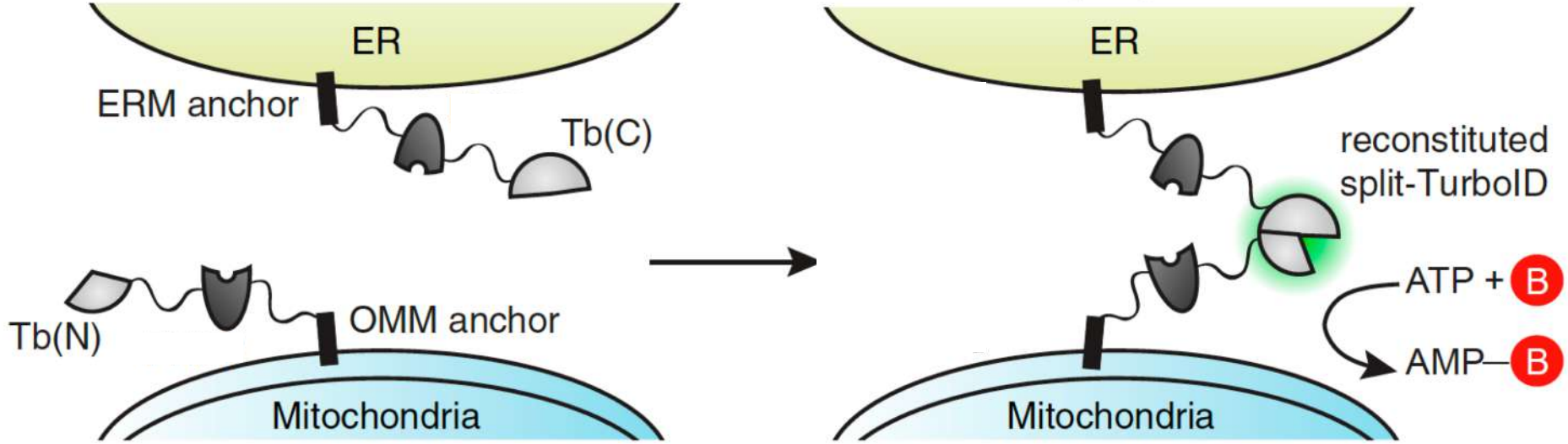
Cytoscape is a useful tool for simple clustering



- Tool for data integration, analysis, and visualization
- Markov clustering using protein-protein interaction scores from the STRING (search tool for the retrieval of interacting genes/proteins) database

www.cytoscape.org

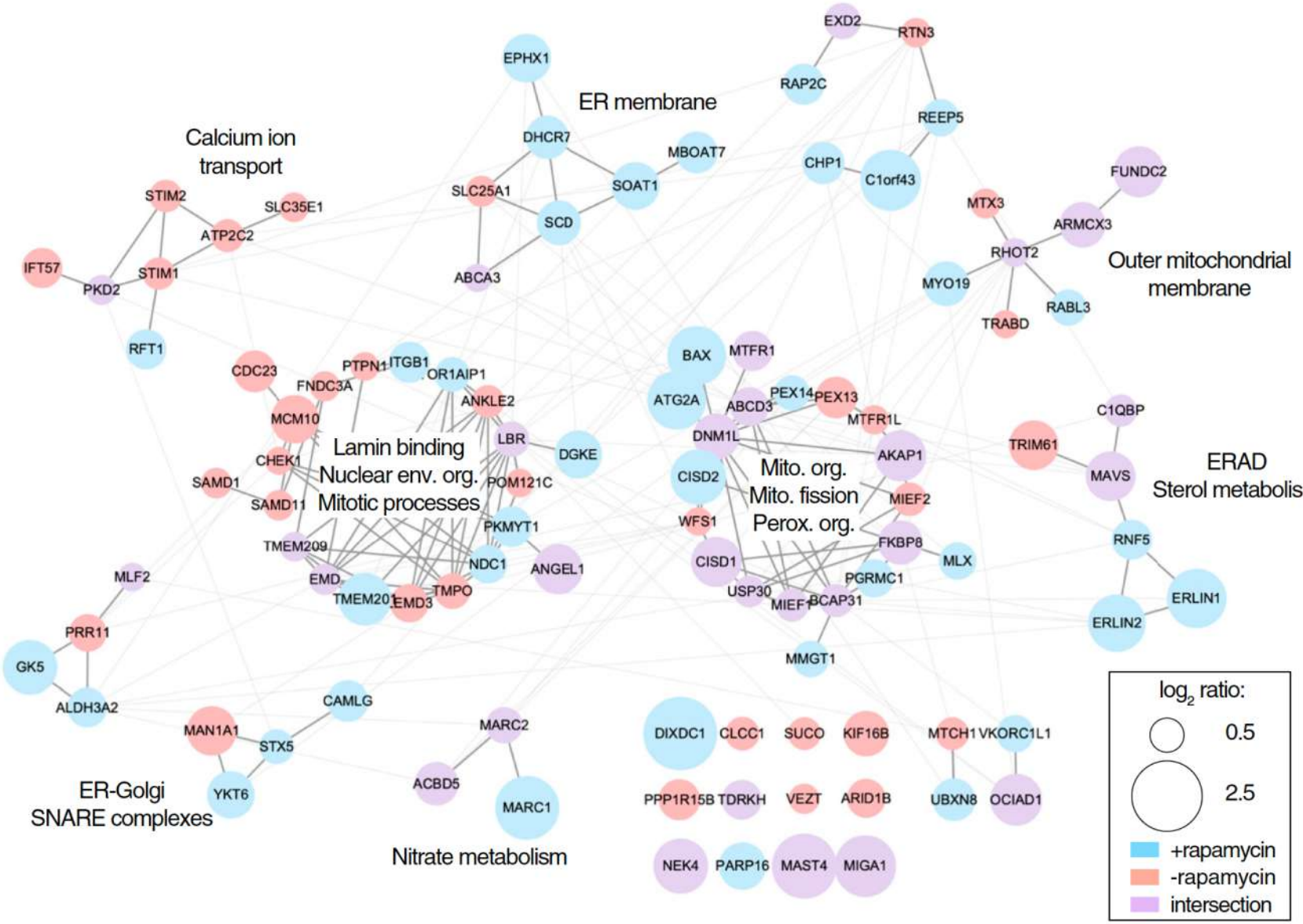
Clustering proteomic hits reveals different functions



- Split-TurboID is targeted to the endoplasmic reticulum and outer mitochondrial membranes
- Reconstitution occurs specifically at organelle contact sites

Cho et al. *PNAS*, 2020.

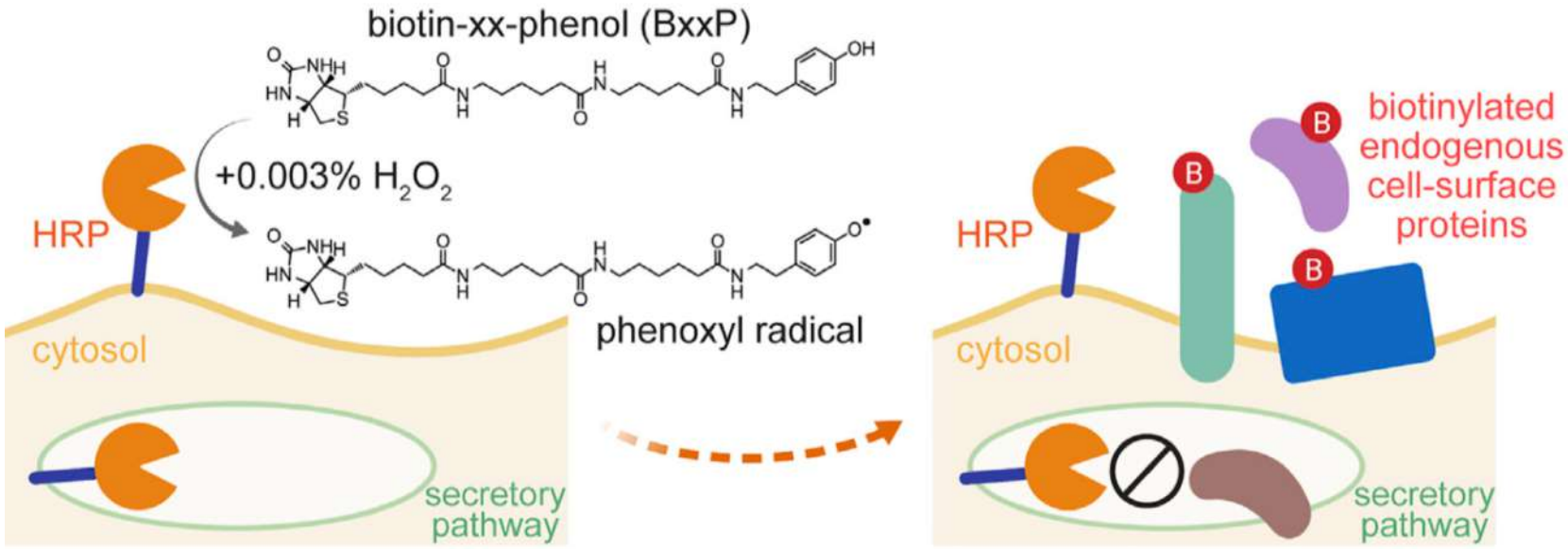
Clustering proteomic hits reveals different functions



- Clustering followed by GO term analysis shows both previously known and unknown functions of ER-mito contact sites

Cho et al. *PNAS*, 2020.

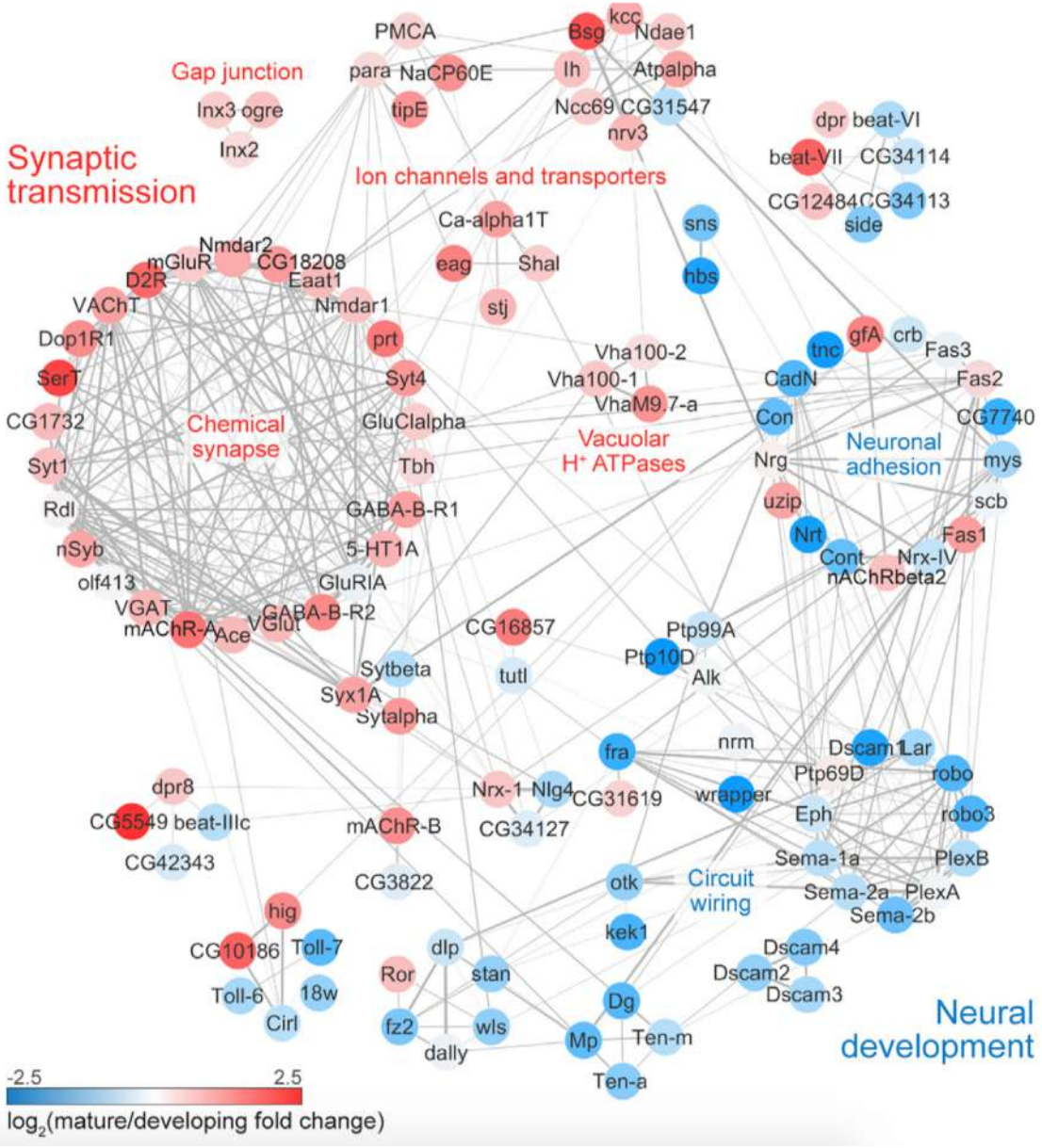
Clustering proteomic hits reveals different functions



- HRP for cell surface labeling of olfactory projection neurons in fly brains (performed in both developing pupae and adults)
- BxxP is a membrane impermeant substrate for proximity labeling

Li*, Han* et al. *Cell*, 2020.

Clustering proteomic hits reveals different functions

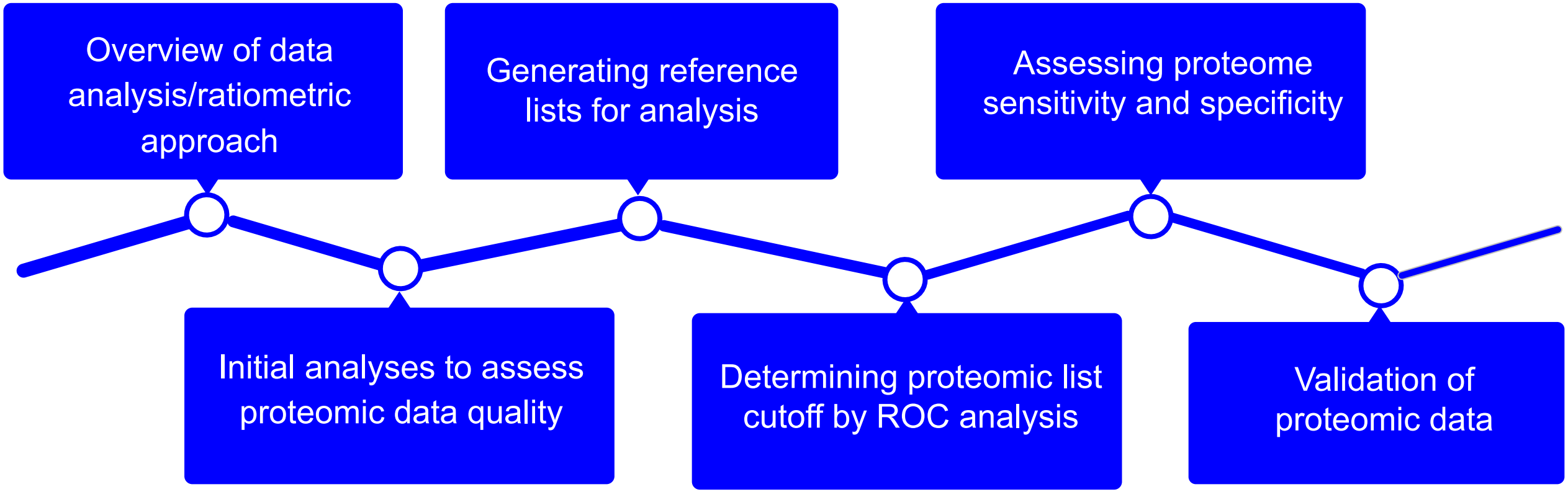


- TMT-based quantification of proteomic hits shows changes in projection neuron cell surface proteomes in development and maturation

Li*, Han* et al. *Cell*, 2020.

Outline

Proteomic data analysis for proximity labeling (PL) experiments:



Validation of proteomic hits

- After specificity analysis, you will get a list of proteomic hits without prior annotations.

  Novel hits v.s. false positives?

Validation of proteomic hits

- After specificity analysis, you will get a list of proteomic hits without prior annotations.

  Novel hits v.s. false positives?

- Use orthogonal strategies for validation.

  For subcellular proteome mapping: Imaging, biochemical purification…

  For interactome mapping: IP, proximity ligation assay…

Validation of proteomic hits

- After specificity analysis, you will get a list of proteomic hits without prior annotations.

  Novel hits v.s. false positives?

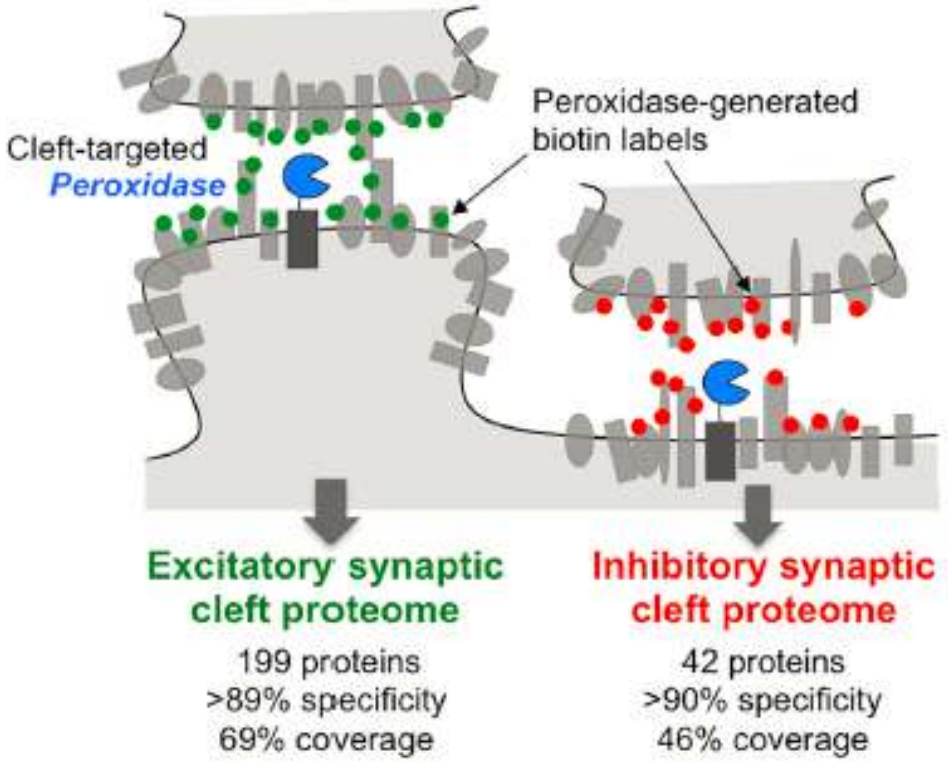
- Use orthogonal strategies for validation.

  For subcellular proteome mapping: Imaging, biochemical purification…

  For interactome mapping: IP, proximity ligation assay…

- The selection of hits for validation could be guided by the availability of commercial antibodies and transgenes for recombinant expression.

Proteomic analysis of synaptic clefts

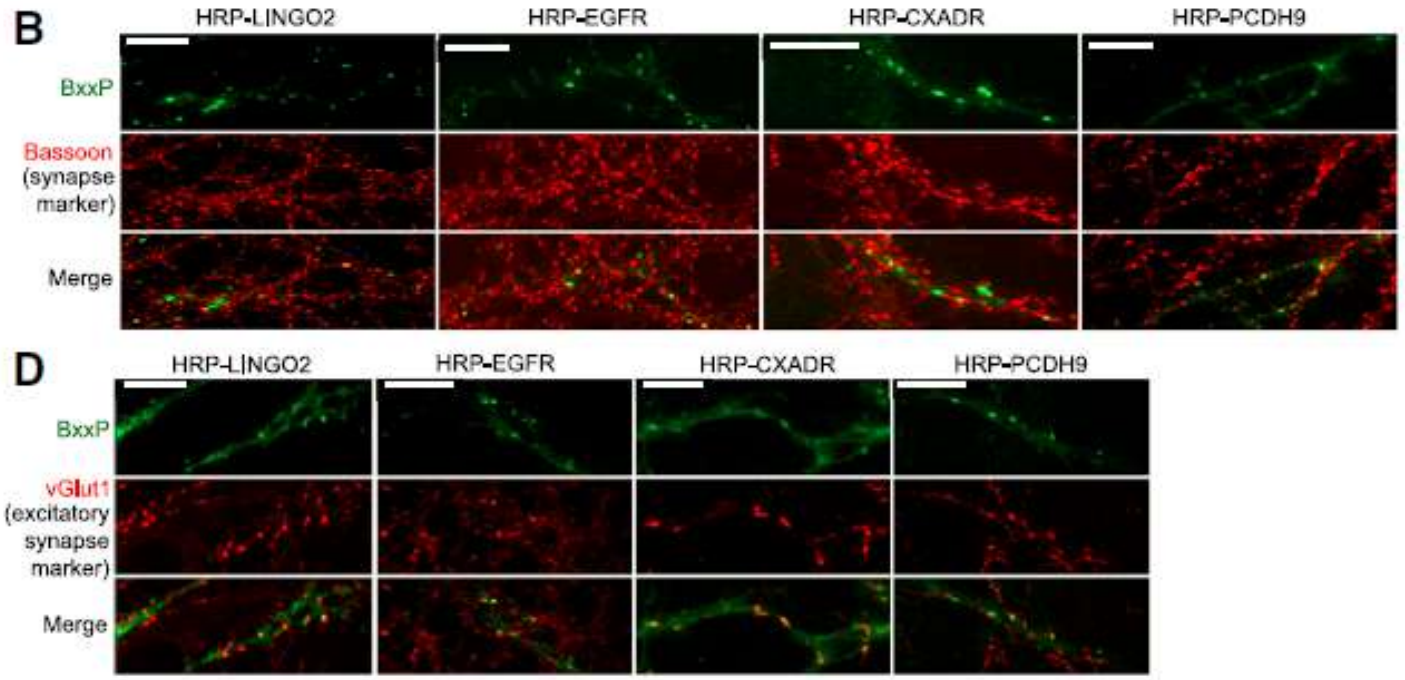


33 proteins with no previous literature assigning them to synapses

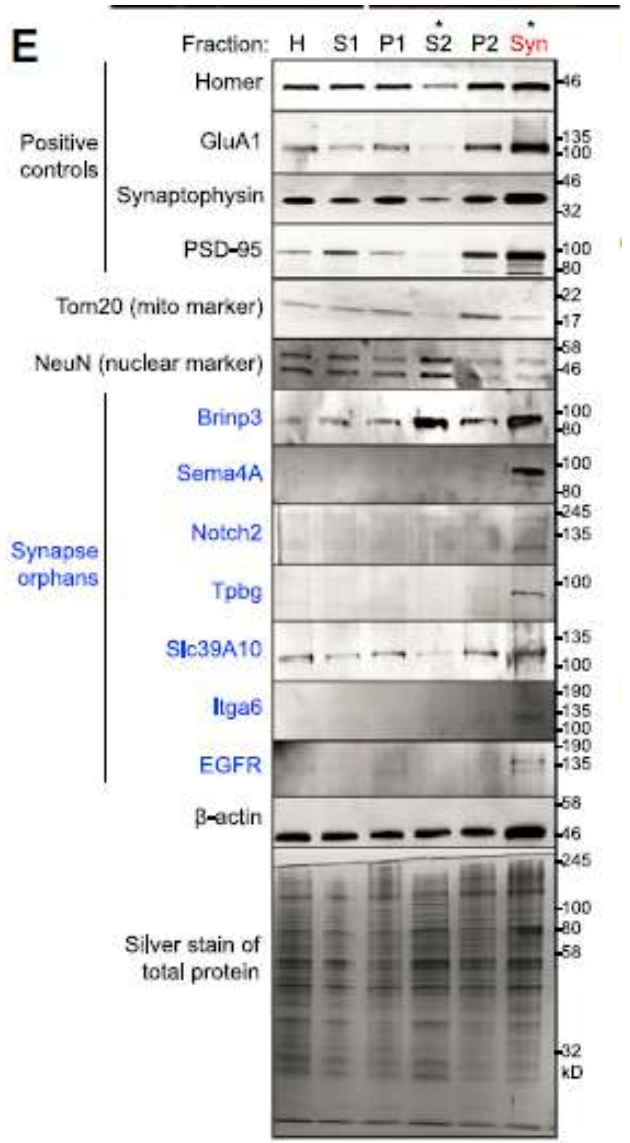
14 proteins selected for validation

Loh et al. *Cell* 2016

Validation of new synaptic proteins



Fluorescence imaging



Purification of synaptosomes

Validation of new synaptic proteins

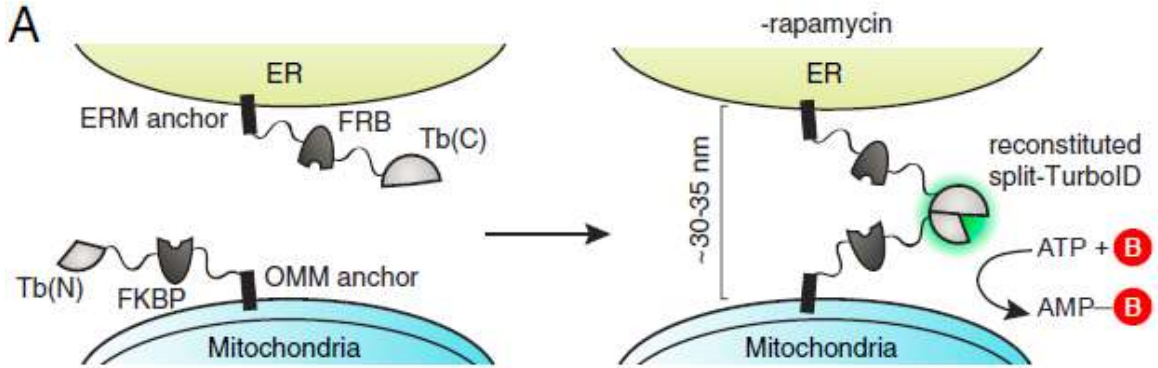
Synapse orphans	Evidence for synaptic localization		
	Imaging with Bassoon	Imaging with vGlut1	Immunoblot of synaptosomes
LINGO2	Figure 5B	Figure 5D	
EGF receptor	Figure 5B	Figure 5D	Figure 5E
CXADR	Figure 5B	Figure 5D	
Protocadherin-9 (PCDH9)	Figure 5B	Figure 5D	
BRINP3			Figure 5E
Semaphorin-4A (SEMA4A)			Figure 5E
NOTCH2	Figures 5G,H		Figure 5E
Trophoblast glycoprotein (TPBG)			Figure 5E
Zinc transporter ZIP10 (SLC39A10)			Figure 5E
Integrin alpha-6 (ITGA6)			Figure 5E

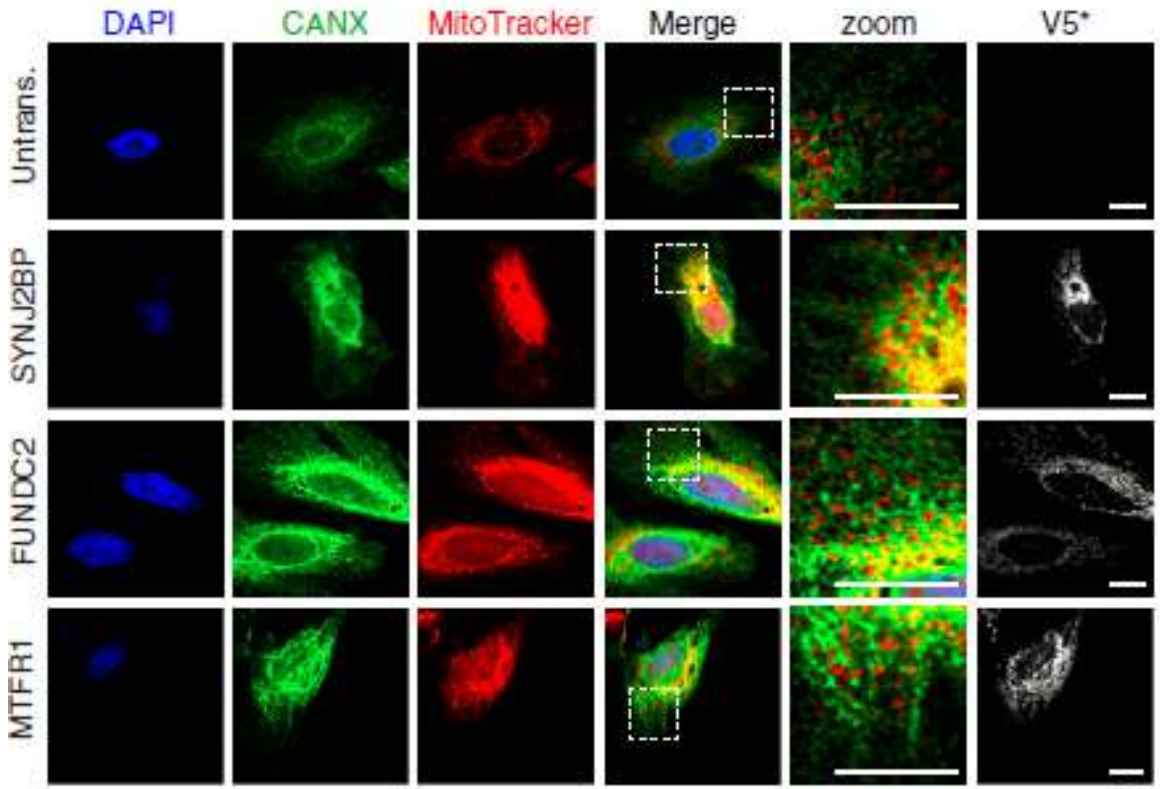
- 10 hits with positive validation.
- 2 hits were inconclusive.

(non-specific antibody for Notch3 and HRP tag disruption of surface trafficking for Matn2)

- 2 hits with negative results.

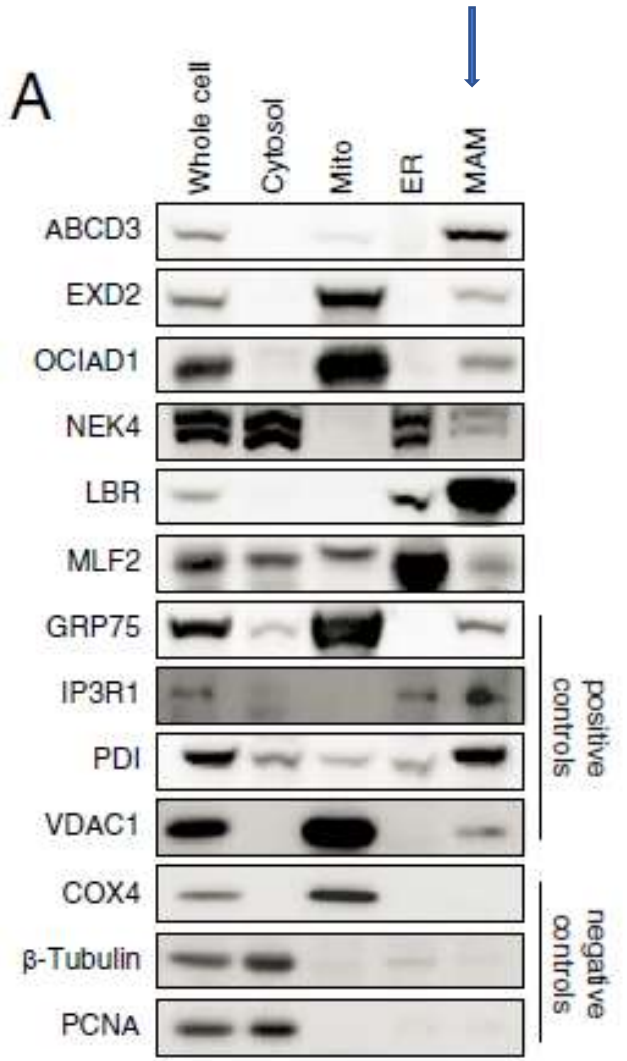
Validation of novel proteins at mito-ER contact sites





Fluorescence imaging

Cho et al. *PNAS* 2020



Purification of mitochondria-associated membranes

Functional validation

Table 2. RNAi Screen in VM PNs and ORNs: Genes, Molecular Features, Phenotypes, and Penetrance

Gene	Human Ortholog	Molecular Feature	RNAi Phenotype	% Phenotypic Penetrance (n)
CG7166	–	immunoglobulin and fibronectin domains	ORN trajectory error and dorsal mistargeting	74.1 (58), 38.9 (54)
CG7466/Megf8	*MEGF8*	epidermal growth factor domain	ORN ventral mistargeting	62.0 (50), 40.6 (64)
CG7749/kug	*FAT3*	cadherin repeat	PN ventral mistargeting	98.0 (52), 62.0 (50)
CG17839	–	immunoglobulin and fibronectin domains	PN lateral mistargeting	48.1 (52), 37.5 (32)
CG33087/LRP1	*LRP1*	low-density lipoprotein receptor	PN and ORN local mistargeting	41.7 (48), 36.5 (52), 65.4 (52), 39.3 (28)
CG34353	*LSAMP*	immunoglobulin and fibronectin domains	ORN posterior mistargeting	22.9 (48), 21.2 (52)
CG2054/Cht2	*CHIA*	chitinase	ORN medial mistargeting	83.3 (54), 21.4 (42)
CG3036	–	anion transporter	ORN posterior mistargeting	34.0 (50), 40.3 (62)
CG3921/bark	–	scavenger receptor	PN and ORN local mistargeting	73.1 (52), 23.9 (46)
CG4645	*YIPF1*	Yip domain	ORN medial mistargeting	50.0 (50), 98.1 (54)*
CG6113/Lip4	*LIPM*	lipase	PN ventral mistargeting	92.0 (50), 30.0 (30)
CG6821/Lsp1γ	–	hemocyanin domain	global disruption	100.0 (24), 56.0 (50)
CG8460	*CHID1*	chitinase	ORN dorsal mistargeting	69.6 (56)
CG9565/Nep3	*ECE1*	neprilysin peptidase	ORN ventral mistargeting	68.5 (54), 60.7 (56)
CG9796/GILT1	*IFI30*	thiol reductase	PN and ORN local mistargeting	83.3 (36), 87.0 (54)
CG14234	*TMEM198*	–	PN and ORN local mistargeting	68.9 (58), 100.0 (58)*
CG14446/dtn	*TMEM132E*	–	ORN dorsal mistargeting	70.4 (54), 51.9 (52)
CG31998	–	–	ORN dorsal mistargeting	70.0 (60), 50.0 (58)
CG34380/smal	*DDR2*	coagulation factor	PN random mistargeting	39.6 (48), 26.9 (52)
CG43737	–	–	ORN dorsal and PN random mistargeting	28.9 (52), 83.3 (54)

Human orthologs were searched by the FlyBase Homologs search tool. Only orthologs consistently identified by four or more databases are listed. Molecular features were searched through FlyBase and UniProt. The top 6 proteins in the table belong to families of classic wiring molecules based on their structural domains; the bottom 14 proteins come from molecular families not previously linked to neural development. The phenotypic penetrance of each RNAi is listed, with the number of antennal lobes examined in parentheses. An antennal lobe image of each RNAi is included in Figure S5.
*, two cases where pan-neuronal RNAi was lethal and *PN-GAL4* was used instead to drive RNAi expression.

Li*, Han* et al. *Cell*, 2020.

Summary: proximity labeling data analysis

- The ratiometric approach can produce highly specific proteomes using proper spatial specificity controls
- True positive and negative lists should be generated based on prior knowledge of the proteome of interest
- Sensitivity and specificity analyses can inform on the quality of the proteome
- Novl proteomic hits can be validated (imaging, western blots, functional assays, etc.) for making new biological discoveries.
- Useful PL protocol resources: Hung et al. *Nature Protocols* 2016 and Cho et al. *Nature Protocols* 2020

PROTOCOL

Spatially resolved proteomic mapping in living cells with the engineered peroxidase APEX2

Victoria Hung[1], Namrata D Udeshi[2], Stephanie S Lam[1], Ken H Loh[1], Kurt J Cox[1], Kayvon Pedram[1], Steven A Carr[2] & Alice Y Ting[1,2]

[1]Department of Chemistry, Massachusetts Institute of Technology (MIT), Cambridge, Massachusetts, USA. [2]Broad Institute of MIT and Harvard, Cambridge, Massachusetts, USA. Correspondence should be addressed to A.Y.T. (ating@mit.edu).

Published online 11 February 2016; doi:10.1038/nprot.2016.018

nature protocols

PROTOCOL

https://doi.org/10.1038/s41596-020-0399-0

Check for updates

Proximity labeling in mammalian cells with TurboID and split-TurboID

Kelvin F. Cho[1,2,8], Tess C. Branon[3,8], Namrata D. Udeshi[4], Samuel A. Myers[4], Steven A. Carr[4] and Alice Y. Ting[2,5,6,7]✉

Protocol | Published: 02 November 2020

Proximity labeling in mammalian cells with TurboID and split-TurboID

Kelvin F. Cho, Tess C. Branon, Namrata D. Udeshi, Samuel A. Myers, Steven A. Carr & Alice Y. Ting

Nature Protocols (2020) | Cite this article

Supplementary information

Reporting Summary

Supplementary Table 1

Human proteome of proteins, annotated by whether each protein was previously detected in a PL proteomic experiment from our lab (regions include: mitochondrial matrix[6,15], mitochondrial intermembrane space[76], mitochondrial nucleoid[77], ER membrane[6,7,78], outer mitochondrial membrane[7,78], ER-mitochondria contact sites[7,78], nucleus[6], synaptic cleft[20], and cytosol[6,7,78]). For each protein, the compartment(s) in which they were detected are listed.

Supplementary Table 2

Compilation of data from previous PL proteomic mapping experiments performed by our lab, categorized by organelle/region of interest (each tab is a different subcellular compartment). In each tab, the relevant studies and corresponding enrichment ratios (SILAC, TMT, or iTRAQ) for proteins detected above the respective cutoffs are provided. Data are included for the mitochondrial matrix[6,15] (Tab 1), mitochondrial intermembrane space[76] (Tab 2), mitochondrial nucleoid[77] (Tab 3), ER membrane[6,7,78] (Tab 4), outer mitochondrial membrane[7,78] (Tab 5), ER-mitochondria contact sites[7,78] (Tab 6), nucleus[6] (Tab 7), synaptic cleft[20] (Tab 8), and cytosol[6,7,78] (Tab 9).

- Compilation of proteomic mapping data of subcellular compartments from previous Ting lab studies

Supplementary tables in Cho et al. *Nature Protocols* 2020 contain aggregated proteomic data from previous Ting lab studies

Tab	Compartment	Studies	Enzyme	Details/Definitions
1	mitochondrial matrix	Rhee, H. W. *et al.* Proteomic mapping of mitochondria in living cells via spatially restricted enzymatic tagging. *Science.* **339**, 1328–1331 (2013).	APEX	30m BP, 1m H2O2; SILAC; negatives: omit APEX (rep 1) and omit BP/H2O2 (rep 2)
		Branon, T. C. *et al.* Efficient proximity labeling in living cells and organisms with TurboID. *Nature Biotechnology* **36**, 880–898 (2018).	BioID	18h biotin (BioID); TMT; 126: BioID (mito matrix); 127: omit biotin (rep 1); 130: BioID (mito matrix); 131: omit ligase (rep 2)
			TurboID	10m biotin (TurboID); TMT; 128: TurboID (mito matrix); 129: omit biotin (rep 1); 129: TurboID (mito matrix); 126: omit ligase (rep 2)
			miniTurbo	10m biotin (miniTurbo); TMT; 130: miniTurbo (mito matrix); 131: omit biotin (rep 1); 127: miniTurbo (mito matrix); 126: omit ligase (rep 2)
2	mitochondrial intermembrane space (IMS)	Hung, V. *et al.* Proteomic Mapping of the Human Mitochondrial Intermembrane Space in Live Cells via Ratiometric APEX Tagging. *Mol. Cell* **55**, 332–341 (2014).	APEX	30m BP, 1m H2O2; SILAC; H: IMS-APEX, M: APEX-NES, L: omit APEX
3	mitochondrial nucleoid	Han, S. *et al.* Proximity Biotinylation as a Method for Mapping Proteins Associated with mtDNA in Living Cells. *Cell Chem. Biol.* **24**, 404–414 (2017).	APEX	30m BP, 1m H2O2; TMT; 126, 129: Twinkle APEX (Rep 1, 2); 127, 130: Mito-APEX (Rep 1, 2); 128: omit H2O2 (rep 1); 131: omit APEX (rep 2)
		Hung, V. *et al.* Proteomic mapping of cytosol-facing outer mitochondrial and ER membranes in living human cells by proximity biotinylation. e*Life* **6**, (2017).	APEX	30m BP, 1m H2O2; SILAC; H: ERM-APEX, M: APEX-NES, L: omit APEX (rep 1); L: omit H2O2 (rep2)

Compartments:

- Mito matrix
- Mito IMS
- Mito nucleoid
- ER membrane
- Outer mito membrane
- ER-mito contacts
- Nucleus
- Synaptic cleft
- Cytosol

Supplementary tables in Cho et al. *Nature Protocols* 2020 contain aggregated proteomic data from previous Ting lab studies

Uniprot Accession	Gene Names	Hung et al, 2017		Cho et al, 2020		
		Log2 SILAC Ratio (H/M) Rep 1	Log2 SILAC Ratio (H/M) Rep 2	Log2 TMT Ratio (127C/126C) Rep 1	Log2 TMT Ratio (127C/127N) Rep 2	[BioID] Log2 (127N/
Q9NRG9;Q9NRG9-2;F8VZ44;H3BU82	AAAS	0.407730429	0.339073894	1.19	1.365	0.70
Q9NY61	AATF					
Q99758	ABCA3			1.039	1.169	
P33527	ABCC1					1.01
E7EUE1;P28288;F5GYC1;P28288-2	ABCD3	0.181320587	0.203804229	1.205	1.314	1.76
Q8NFV4	ABHD11					1.44
Q8N2K0-2	ABHD12					1.09
Q9BV23	ABHD6	0.638164182	0.507966575			0.93
Q9H3P7	ACBD3	0.84980351	0.565230436	0.999	1.14	1.46
Q5T8D3-3;Q5T8D3-2;Q5T8D3;Q5T8D3-4;B7Z2A7;Q5T8E0	ACBD5	0.617811456	0.700803556	1.01	1.039	0.81
S4R3H4	ACIN1					
Q86TX2	ACOT1					
P49753	ACOT2					
Q8N9L9	ACOT4					

- Corresponding ratios (SILAC, TMT, etc.) are listed for the proteins that were detected in the indicated study

Supplementary tables in Cho et al. *Nature Protocols* 2020 contain aggregated proteomic data from previous Ting lab studies

Identifiers | **1st compartment detected** | **2nd compartment detected** | **3rd compartment detected** | **…**

UniProt Accession ID	Gene ID	Gene Name	Protein Name	Compartment 1	Reference 1	Compartment 2	Reference 2	Compartment 3	Reference 3	Compartment 4	R
Q9NRG9	8086	AAAS	Aladin	ERM	Tab 4 (Hung et al, 2017)	ERM	Tab 4 (Branon et al, 2018) (BioID)	ERM	Tab 4 (Branon et al, 2018) (TurboID 10m)	ERM	Tab 4 2018
Q86V21	65985	AACS	Acetoacetyl-CoA synthetase	nucleus	Tab 7 (Branon et al, 2018) (TurboID)	nucleus	Tab 7 (Branon et al, 2018) (miniTurbo)	cytosol	Tab 9 (Hung et al, 2017)		
P22760	13	AADAC	Arylacetamide deacetylase								
Q6P093	344752	AADACL2	Arylacetamide deacetylase-like 2								
Q5VUY0	126767	AADACL3	Arylacetamide deacetylase-like 3								
Q5VUY2	343066	AADACL4	Arylacetamide deacetylase-like 4								
Q8N5Z0	51166	AADAT	Kynurenine/alpha-aminoadipate aminotransferase, mitochondrial								
			Alpha- and gamma-adaptin-binding		Tab 7 (Branon et al,		Tab 7 (Branon et al,		Tab 7 (Branon et al,		Tab 9

- In the 2nd spreadsheet, you can search by protein name and see whether we have detected it before, and if so, in which cellular compartment; references to the first sheet for specific data

Supplementary tables in Cho et al. *Nature Protocols* 2020 contain aggregated proteomic data from previous Ting lab studies

Identifiers | **1st compartment detected** | **2nd compartment detected** | **3rd compartment detected** | **…**

UniProt Accession ID	Gene ID	Gene Name	Protein Name	Compartment 1	Reference 1	Compartment 2	Reference 2	Compartment 3	Reference 3	Compartment 4	R
Q9NRG9	8086	AAAS	Aladin	ERM	Tab 4 (Hung et al, 2017)	ERM	Tab 4 (Branon et al, 2018) (BioID)	ERM	Tab 4 (Branon et al, 2018) (TurboID 10m)	ERM	Tab 4 2018
Q86V21	65985	AACS	Acetoacetyl-CoA synthetase	nucleus	Tab 7 (Branon et al, 2018) (TurboID)	nucleus	Tab 7 (Branon et al, 2018) (miniTurbo)	cytosol	Tab 9 (Hung et al, 2017)		
P22760	13	AADAC	Arylacetamide deacetylase								
Q6P093	344752	AADACL2	Arylacetamide deacetylase-like 2								
Q5VUY0	126767	AADACL3	Arylacetamide deacetylase-like 3								
Q5VUY2	343066	AADACL4	Arylacetamide deacetylase-like 4								
Q8N5Z0	51166	AADAT	Kynurenine/alpha-aminoadipate aminotransferase, mitochondrial								
			Alpha- and gamma-adaptin-binding		Tab 7 (Branon et al,		Tab 7 (Branon et al,		Tab 7 (Branon et al,		Tab 9

P57105	55333	SYNJ2BP	Synaptojanin-2-binding protein	mitochondrial IMS	Tab 2 (Hung et al, 2014)	ERM	Tab 4 (Hung et al, 2017)	OMM	Tab 5 (Hung et al, 2017)	ER-mito contacts	Tab

- In the 2nd spreadsheet, you can search by protein name and see whether we have detected it before, and if so, in which cellular compartment; references to the first sheet for specific data

Acknowledgement

Dr. Alice Ting
Dr. Liqun Luo

Panelists: Kelvin Cho, Boxuan Zhao, Wei Qin, Jiefu Li, Tess Branon

PROTOCOL

Spatially resolved proteomic mapping in living cells with the engineered peroxidase APEX2

Victoria Hung[1], Namrata D Udeshi[2], Stephanie S Lam[1], Ken H Loh[1], Kurt J Cox[1], Kayvon Pedram[1], Steven A Carr[2] & Alice Y Ting[1,2]

[1]Department of Chemistry, Massachusetts Institute of Technology (MIT), Cambrid
Massachusetts, USA. Correspondence should be addressed to A.Y.T. (ating@mit.edu

Published online 11 February 2016; doi:10.1038/nprot.2016.018

nature protocols

PROTOCOL

https://doi.org/10.1038/s41596-020-0399-0

Check for updates

Proximity labeling in mammalian cells with TurboID and split-TurboID

Kelvin F. Cho[1,2,8], Tess C. Branon[3,8], Namrata D. Udeshi[4], Samuel A. Myers[4], Steven A. Carr[4] and Alice Y. Ting[2,5,6,7]