Advancing clinical proteomics via analysis based on biological complexes

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Joint work with Wilson Wen Bin Goh

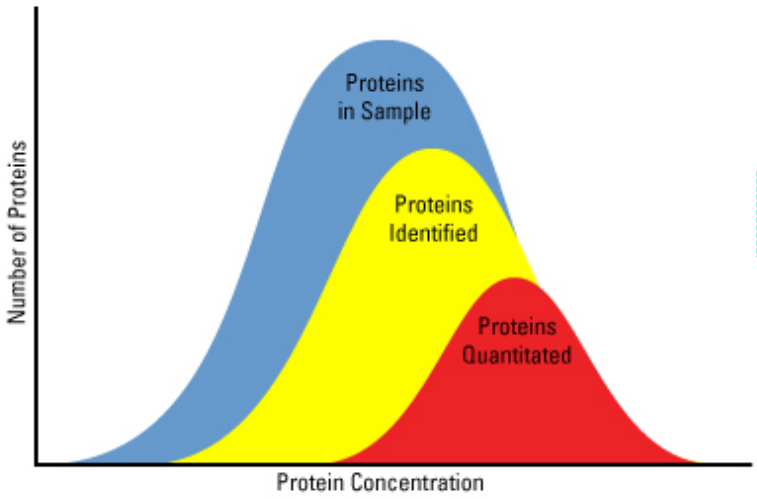
Proteomics vs transcriptomics

- **Proteomic profile**
  - Which protein is found in the sample
  - How abundant it is
- **Similar to gene expression profile. So typical gene expression profile analysis methods can be applied, except …**

- **Key differences**
  - Profiling
    - **Complexity: 20k genes vs 500k proteins**
    - **Dynamic range: > 10 orders of magnitude in plasma. Proteins cannot be amplified**
  - Analysis
    - **Much fewer features**
    - **Difficult to reproduce**
    - **Much fewer samples**
    - **Unstable quantitation**

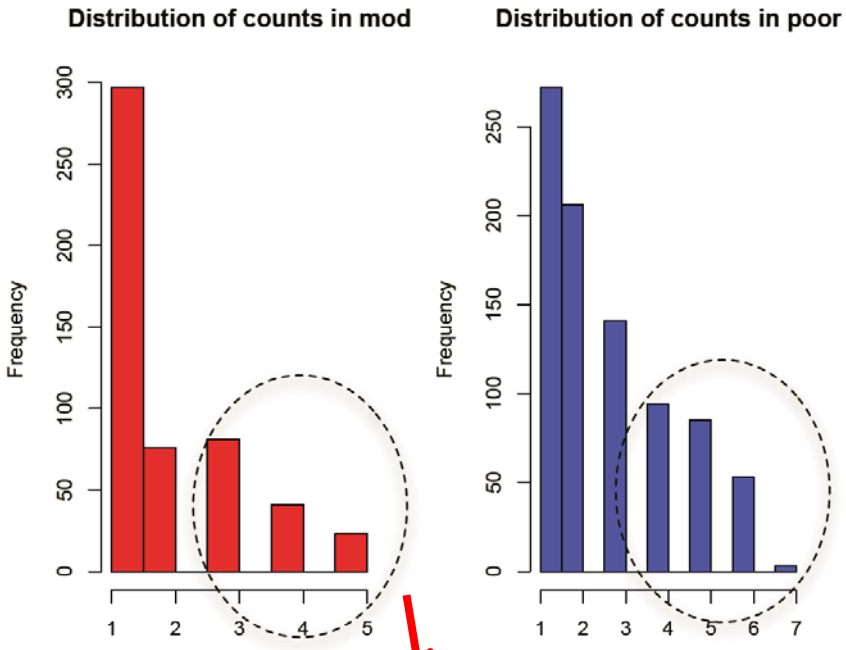
Issues in proteomics: Coverage and consistency

Technical incompleteness



Per sample

How it affects real data



Only 25 out of 800+ proteins are common to all 5 mod-stage HCC patients!

Using protein complexes to enhance proteomics: Basic ideas

An inspiration from gene expression profile analysis

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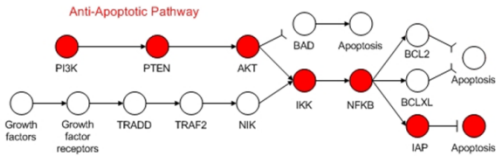
Gene Regulatory Circuits



- Each disease phenotype has some underlying cause
- There is some unifying biological theme for genes that are truly associated with a disease subtype

- Uncertainty in selected genes can be reduced by considering biological processes of the genes
- The unifying biological theme is basis for inferring the underlying cause of disease subtype

Taming false positives by considering pathways instead of all possible groups

Group of Genes

- **Suppose**
  - Each gene has 50% chance to be high
  - You have 3 disease and 3 normal samples
- **What is the chance of a group of 5 genes being perfectly correlated to these samples?**

- **Prob(group of genes correlated) = $(1/2^6)^5$**
  - Good, << $1/2^6$
- ~~**# of groups = $^{100000}C_5$**~~
- ~~**E(# of groups of genes correlated) = $^{100000}C_5 * (1/2^6)^5 = 2.6*10^{12}$**~~

# of pathways = 1000

E(# of pathways correlated) = $1000 * (1/2^6)^5 = 9.3*10^{-7}$

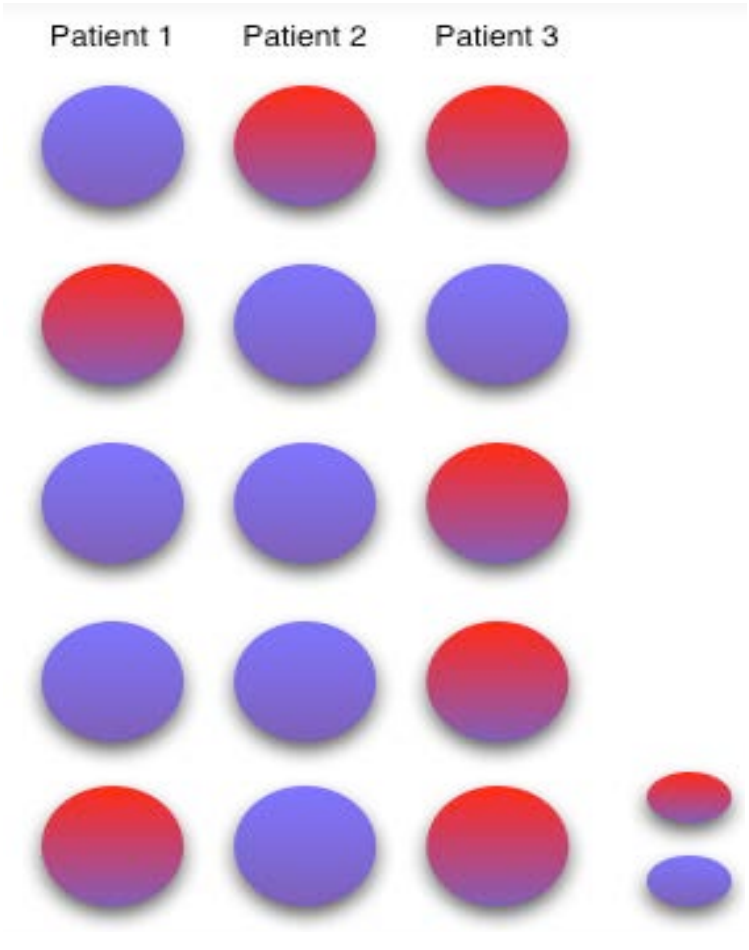
⇒ **Even more false positives?**

- **Perhaps no need to consider every group**

Microarray Workshop for Gene Expression Profiling, NUH, 23/9/2011 Copyright 2011 © Limsoon Wong

Contextualization!

Intuition



- **Suppose the failure to form a protein complex causes a disease**
  - If any component protein is missing, the complex can't form

⇒ **Diff patients suffering from the disease can have a diff protein component missing**

  - Construct a profile based on complexes?

… and some math

- **Postulate: Chance of a protein complex being present ≈ fraction of its constituent proteins being reported in the screen**

- **Suppose proteomics screen has 75% reliability; {A, B, C, D, E} is a complex; and screen reports A, B, C, D only**

⇒ **Complex has 60% (= 0.75 * 4 / 5) chance to be present**

⇒ **E has >60% chance to be present, as presence of complex implies presence of its constituents … improving coverage**

& **A, B, C, and D each has 90% (= 100% * 0.6 + 75% * 0.4) chance of being present, whereas a usual reported protein has a lower 75% chance of being present… removing noise**

Reference complexes

- **In this talk, human complexes (of size at least 5) from CORUM are used as reference complexes**

- **It is possible to use subnetworks generated from pathway and PPI databases. However these such subnetworks vary significantly depending on databases and generation algorithms used**

So I do not consider these…

Improving coverage in proteomic profiles

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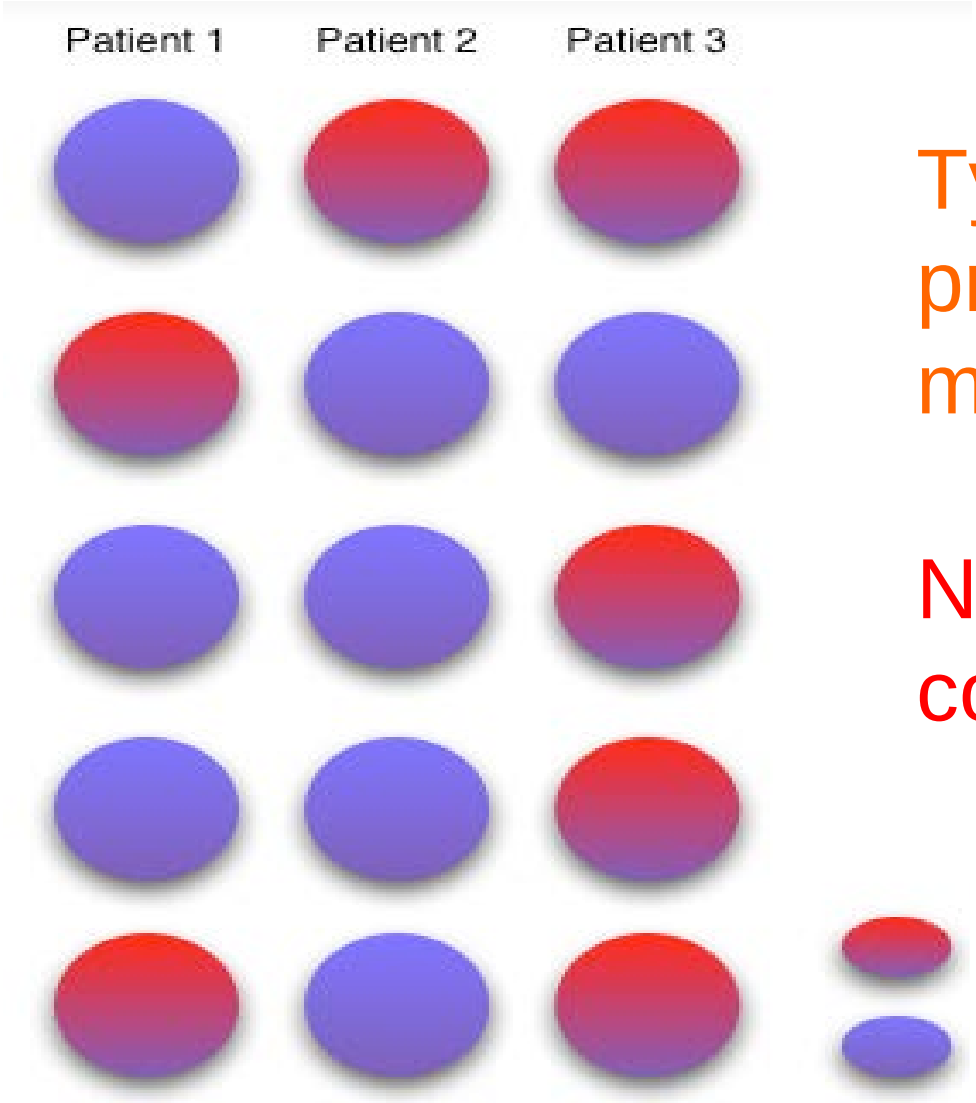

Typical proteomic profiling misses many proteins

Need to improve coverage!

Guo et al. *Nature Medicine*, *21*, 407, 2015

Missing values in a real dataset

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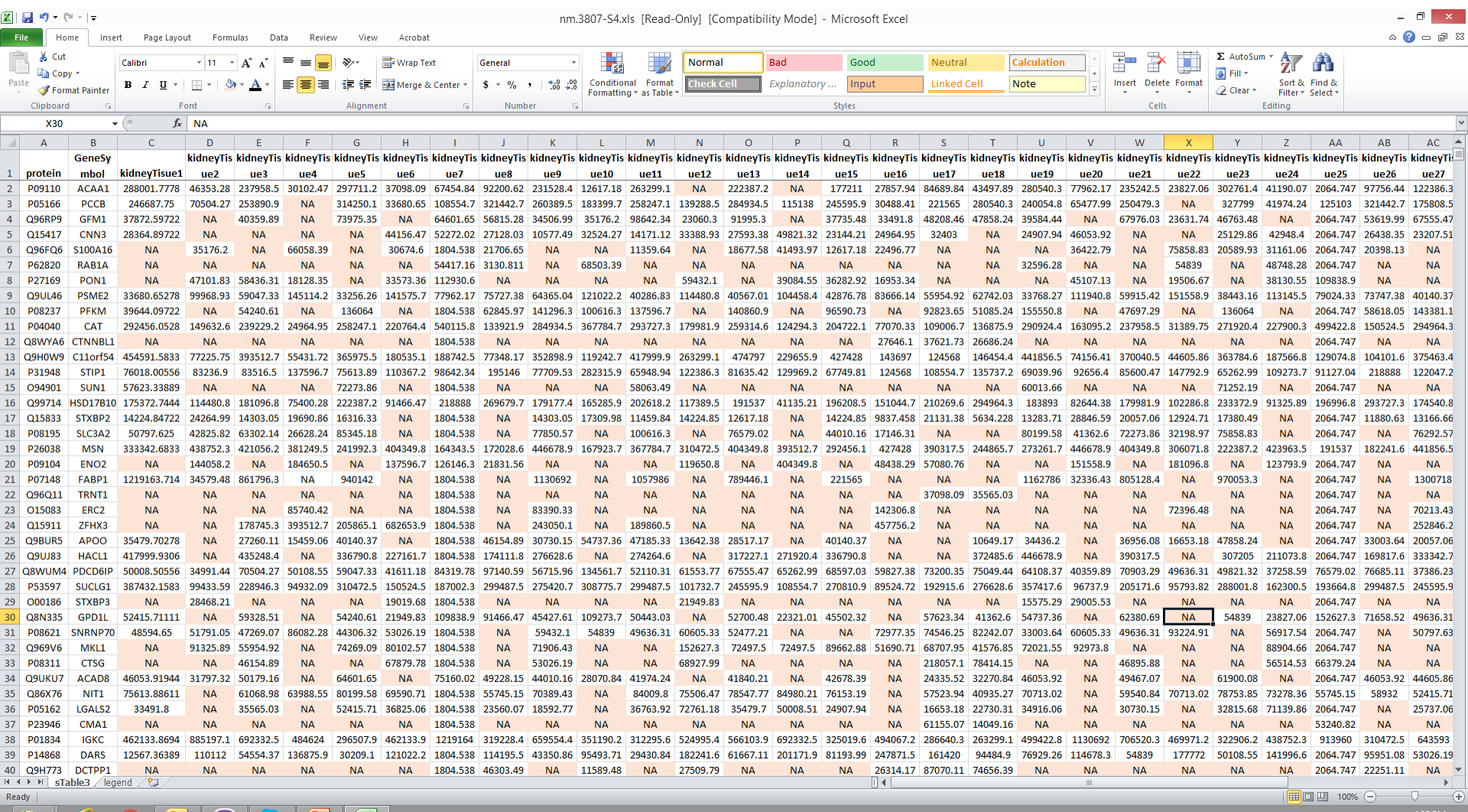


	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	AA	AB	AC
1	protein	GeneSymbol	kidneyTisue1	kidneyTis ue2	kidneyTis ue3	kidneyTis ue4	kidneyTis ue5	kidneyTis ue6	kidneyTis ue7	kidneyTis ue8	kidneyTis ue9	kidneyTis ue10	kidneyTis ue11	kidneyTis ue12	kidneyTis ue13	kidneyTis ue14	kidneyTis ue15	kidneyTis ue16	kidneyTis ue17	kidneyTis ue18	kidneyTis ue19	kidneyTis ue20	kidneyTis ue21	kidneyTis ue22	kidneyTis ue23	kidneyTis ue24	kidneyTis ue25	kidneyTis ue26	kidneyTis ue27
2	P09110	ACAA1	288001.7778	46353.28	237958.5	30102.47	297711.2	37098.09	67454.84	92200.62	231528.4	12617.18	263299.1	NA	222387.2	NA	177211	27857.94	84689.84	43497.89	280540.3	77962.17	235242.5	23827.06	302761.4	41190.07	2064.747	97756.44	122386.3
3	P05166	PCCB	246687.75	70504.27	253890.9	NA	314250.1	33680.65	108554.7	321442.7	260389.5	183399.7	258247.1	139288.5	284934.5	115138	245595.9	30488.41	221565	280540.3	240054.8	65477.99	250479.3	NA	327799	41974.24	125103	321442.7	175808.5
4	Q96RP9	GFM1	37872.59722	NA	40359.89	NA	73975.35	NA	64601.65	56815.28	34506.99	35176.2	98642.34	23060.3	91995.3	NA	37735.48	33491.8	48208.46	47858.24	39584.44	NA	67976.03	23631.74	46763.48	NA	2064.747	53619.99	67555.47
5	Q15417	CNN3	28364.89722	NA	NA	NA	NA	44156.47	52272.02	27128.03	10577.49	32524.27	14171.12	33388.93	27593.38	49821.32	23144.21	24964.95	32403	NA	24907.94	46053.92	NA	NA	25129.86	42948.4	2064.747	26438.35	23207.51
6	Q96FQ6	S100A16	NA	35176.2	NA	66058.39	NA	30674.6	1804.538	21706.65	NA	NA	11359.64	NA	18677.58	41493.97	12617.18	22496.77	NA	NA	NA	36422.79	NA	75858.83	20589.93	31161.06	2064.747	20398.13	NA
7	P62820	RAB1A	NA	NA	NA	NA	NA	NA	54417.16	3130.811	NA	68503.39	NA	NA	NA	NA	NA	NA	NA	NA	32596.28	NA	NA	54839	NA	48748.28	2064.747	NA	NA
8	P27169	PON1	NA	47101.83	58436.31	18128.35	NA	33573.36	112930.6	NA	NA	NA	NA	59432.1	NA	39084.55	36282.92	16953.34	NA	NA	NA	45107.13	NA	19506.67	NA	38130.55	109838.9	NA	NA
9	Q9UL46	PSME2	33680.65278	99968.93	59047.33	145114.2	33256.26	141575.7	77962.17	75727.38	64365.04	121022.2	40286.83	114480.8	40567.01	104458.4	42876.78	83666.14	55954.92	62742.03	33768.27	111940.8	59915.42	151558.9	38443.16	113145.5	79024.33	73747.38	40140.37
10	P08237	PFKM	39644.09722	NA	54240.61	NA	136064	NA	1804.538	62845.97	141296.3	100616.3	137596.7	NA	140860.9	NA	96590.73	NA	92823.65	51085.24	155550.8	NA	47697.29	NA	136064	NA	2064.747	58618.05	143381.1
11	P04040	CAT	292456.0528	149632.6	239229.2	24964.95	258247.1	220764.4	540115.8	133921.9	284934.5	367784.7	293727.3	179981.9	259314.6	124294.3	204722.1	77070.33	109006.7	136875.9	290924.4	163095.2	237958.5	31389.75	271920.4	227900.3	499422.8	150524.5	294964.3
12	Q8WYA6	CTNNBL1	NA	NA	NA	NA	NA	NA	1804.538	NA	NA	NA	NA	NA	NA	NA	NA	27646.1	37621.73	26686.24	NA	NA	NA	NA	NA	NA	2064.747	NA	NA
13	Q9H0W9	C11orf54	454591.5833	77225.75	393512.7	55431.72	365975.5	180535.1	188742.5	77348.17	352898.9	119242.7	417999.9	263299.1	474797	229655.9	427428	143697	124568	146454.4	441856.5	74156.41	370040.5	44605.86	363784.6	187566.8	129074.8	104101.6	375463.4
14	P31948	STIP1	76018.00556	83236.9	83516.5	137596.7	75613.89	110367.2	98642.34	195146	77709.53	282315.9	65948.94	122386.3	81635.42	129969.2	67749.81	124568	108554.7	135737.2	69039.96	92656.4	85600.47	147792.9	65262.99	109273.7	91127.04	218888	122047.2
15	O94901	SUN1	57623.33889	NA	NA	NA	72273.86	NA	1804.538	NA	NA	NA	58063.49	NA	NA	NA	NA	NA	NA	NA	60013.66	NA	NA	NA	71252.19	NA	2064.747	NA	NA
16	Q99714	HSD17B10	175372.7444	114480.8	181096.8	75400.28	222387.2	91466.47	218888	269679.7	179177.4	165285.9	202618.2	117389.5	191537	41135.21	196208.5	151044.7	210269.6	294964.3	183893	82644.38	179981.9	102286.8	233372.9	91325.89	196996.8	293727.3	174540.8
17	Q15833	STXBP2	14224.84722	24264.99	14303.05	19690.86	16316.33	NA	1804.538	NA	14303.05	17309.98	11459.84	14224.85	12617.18	NA	14224.85	9837.458	21131.38	5634.228	13283.71	28846.59	20057.06	12924.71	17380.49	NA	2064.747	11880.63	13166.66
18	P08195	SLC3A2	50797.625	42825.82	63302.14	26628.24	85345.18	NA	1804.538	NA	77850.57	NA	100616.3	NA	76579.02	NA	44010.16	17146.31	NA	NA	80199.58	41362.6	72273.86	32198.97	75858.83	NA	2064.747	NA	76292.57
19	P26038	MSN	333342.6833	438752.3	421056.2	381249.5	241992.3	404349.8	164343.5	172028.6	446678.9	167923.7	367784.7	310472.5	404349.8	393512.7	292456.1	427428	390317.5	244865.7	273261.7	446678.9	404349.8	306071.8	222387.2	423963.5	191537	182241.6	441856.5
20	P09104	ENO2	NA	144058.2	NA	184650.5	NA	137596.7	126146.3	21831.56	NA	NA	NA	119650.8	NA	404349.8	NA	48438.29	57080.76	NA	NA	151558.9	NA	181096.8	NA	123793.9	2064.747	NA	NA
21	P07148	FABP1	1219163.714	34579.48	861796.3	NA	940142	NA	1804.538	NA	1130692	NA	1057986	NA	789446.1	NA	221565	NA	NA	NA	1162786	32336.43	805128.4	NA	970053.3	NA	2064.747	NA	1300718
22	Q96Q11	TRNT1	NA	NA	NA	NA	NA	NA	1804.538	NA	NA	NA	NA	NA	NA	NA	NA	NA	37098.09	35565.03	NA	NA	NA	NA	NA	NA	2064.747	NA	NA
23	O15083	ERC2	NA	NA	NA	85740.42	NA	NA	1804.538	NA	83390.33	NA	NA	NA	NA	NA	NA	142306.8	NA	NA	NA	NA	NA	72396.48	NA	NA	2064.747	NA	70213.43
24	Q15911	ZFHX3	NA	NA	178745.3	393512.7	205865.1	682653.9	1804.538	NA	243050.1	NA	189860.5	NA	NA	NA	NA	457756.2	NA	NA	NA	NA	NA	NA	NA	NA	2064.747	NA	252846.2
25	Q9BUR5	APOO	35479.70278	NA	27260.11	15459.06	40140.37	NA	1804.538	46154.89	30730.15	54737.36	47185.33	13642.38	28517.17	NA	40140.37	NA	NA	10649.17	34436.2	NA	36956.08	16653.18	47858.24	NA	2064.747	33003.64	20057.06
26	Q9UJ83	HACL1	417999.9306	NA	435248.4	NA	336790.8	227161.7	1804.538	174111.8	276628.6	NA	NA	274264.6	NA	317227.1	271920.4	336790.8	NA	NA	372485.6	446678.9	NA	390317.5	NA	307205	211073.8	2064.747	169817.6	333342.7
27	Q8WUM4	PDCD6IP	50008.50556	34991.44	70504.27	50108.55	59047.33	41611.18	84319.78	97140.59	56715.96	134561.7	52110.31	61553.77	67555.47	65262.99	68597.03	59827.38	73200.35	75049.44	64108.37	40359.89	70903.29	49636.31	49821.32	37258.59	76579.02	76685.11	37386.23
28	P53597	SUCLG1	387432.1583	99433.59	228946.3	94932.09	310472.5	150524.5	187002.3	299487.5	275420.7	308775.7	299487.5	101732.7	245595.9	108554.7	270810.9	89524.72	192915.6	276628.6	357417.6	96737.9	205171.6	95793.82	288001.8	162300.5	193664.8	299487.5	245595.9
29	O00186	STXBP3	NA	28468.21	NA	NA	NA	19019.68	1804.538	NA	NA	NA	NA	21949.83	NA	NA	NA	NA	NA	NA	15575.29	29005.53	NA	NA	NA	NA	2064.747	NA	NA
30	Q8N335	GPD1L	52415.71111	NA	59328.51	NA	54240.61	21949.83	109838.9	91466.47	45427.61	109273.7	50443.03	NA	52700.48	22321.01	45502.32	NA	57623.34	41362.6	54737.36	NA	62380.69	NA	54839	23827.06	152627.3	71658.52	49636.31
31	P08621	SNRNP70	48594.65	51791.05	47269.07	86082.28	44306.32	53026.19	1804.538	NA	59432.1	54839	49636.31	60605.33	52477.21	NA	NA	72977.35	74546.25	82242.07	33003.64	60605.33	49636.31	93224.91	NA	56917.54	2064.747	NA	50797.63
32	Q969V6	MKL1	NA	91325.89	55954.92	NA	74269.09	80102.57	1804.538	NA	71906.43	NA	NA	152627.3	72497.5	72497.5	89662.88	51690.71	68707.95	41576.85	72021.55	92973.8	NA	NA	NA	88904.66	2064.747	NA	NA
33	P08311	CTSG	NA	NA	46154.89	NA	NA	67879.78	1804.538	NA	53026.19	NA	NA	68927.99	NA	NA	NA	NA	218057.1	78414.15	NA	NA	46895.88	NA	NA	56514.53	66379.24	NA	NA
34	Q9UKU7	ACAD8	46053.91944	31797.32	50179.16	NA	64601.65	NA	75160.02	49228.15	44010.16	28070.84	41974.24	NA	41840.21	NA	42678.39	NA	24335.52	32270.84	46053.92	NA	49467.07	NA	61900.08	NA	2064.747	46053.92	44605.86
35	Q86X76	NIT1	75613.88611	NA	61068.98	63988.55	80199.58	69590.71	1804.538	55745.15	70389.43	NA	84009.8	75506.47	78547.77	84980.21	76153.19	NA	57523.94	40935.27	70713.02	NA	59540.84	70713.02	78753.85	73278.36	55745.15	58932	52415.71
36	P05162	LGALS2	33491.8	NA	35565.03	NA	52415.71	36825.06	1804.538	23560.07	18592.77	NA	36763.92	72761.18	35479.7	50008.51	24907.94	NA	16653.18	22730.31	34916.06	NA	30730.15	NA	32815.68	71139.86	2064.747	NA	25737.06
37	P23946	CMA1	NA	NA	NA	NA	NA	NA	1804.538	NA	NA	NA	NA	NA	NA	NA	NA	NA	61155.07	14049.16	NA	NA	NA	NA	NA	NA	53240.82	NA	NA
38	P01834	IGKC	462133.8694	885197.1	692332.5	484624	296507.9	462133.9	1219164	319228.4	659554.4	351190.2	312295.6	524995.4	566103.9	692332.5	325019.6	494067.2	286640.3	263299.1	499422.8	1130692	706520.3	469971.2	322906.2	438752.3	913960	310472.5	643593
39	P14868	DARS	12567.36389	110112	54554.37	136875.9	30209.1	121022.2	1804.538	114195.5	43350.86	95493.71	29430.84	182241.6	61667.11	201171.9	81193.99	247871.5	161420	94484.9	76929.26	114678.3	54839	177772	50108.55	141996.6	2064.747	95951.08	53026.19
40	Q9H773	DCTPP1	NA	NA	NA	NA	NA	NA	1804.538	46303.49	NA	11589.48	NA	27509.79	NA	NA	NA	26314.17	87070.11	74656.39	NA	NA	NA	NA	NA	NA	2064.747	22251.11	NA

Webb-Robertson, *JPR*, 14(5):1993-2001, 2015

Missing values are not mostly due to low-abundance proteins

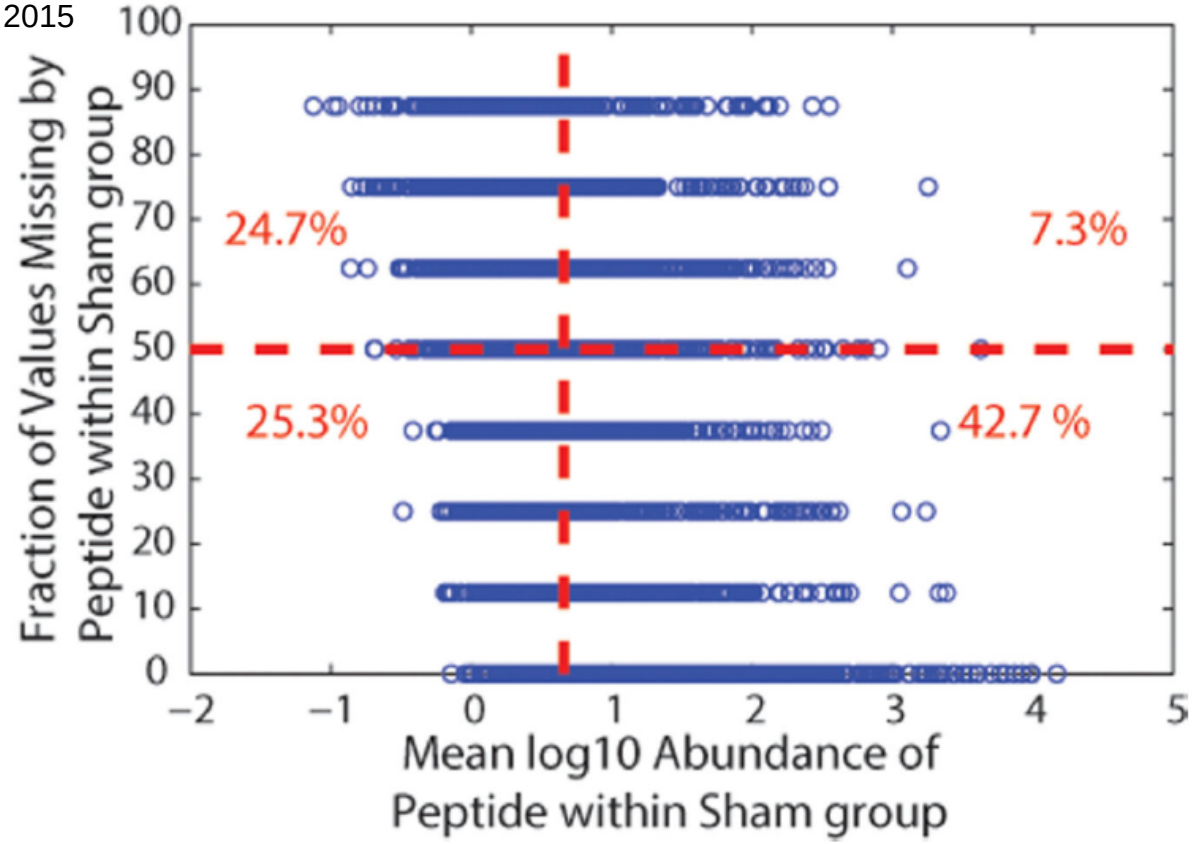


Figure 1.
Average $\log_{10}$ intensity as measured by peptide peak area in the control group versus fraction of missing values and peptide counts associated with bins corresponding to the fraction of missing data comparing phenotypes and exposures for datasets from (A) human plasma and (B) mouse lung. The control group for the human plasma is the normal glucose tolerant (NGT) samples, and the sham group for the mouse lung is the regular weight mice with no lipopolysaccharide (LPS) exposure. The vertical red line represents median average intensity, and the horizontal red line represents the point that 50% of the values are missing.

Webb-Robertson, JPR, 14(5):1993-2001, 2015

Current imputation methods don't work very well

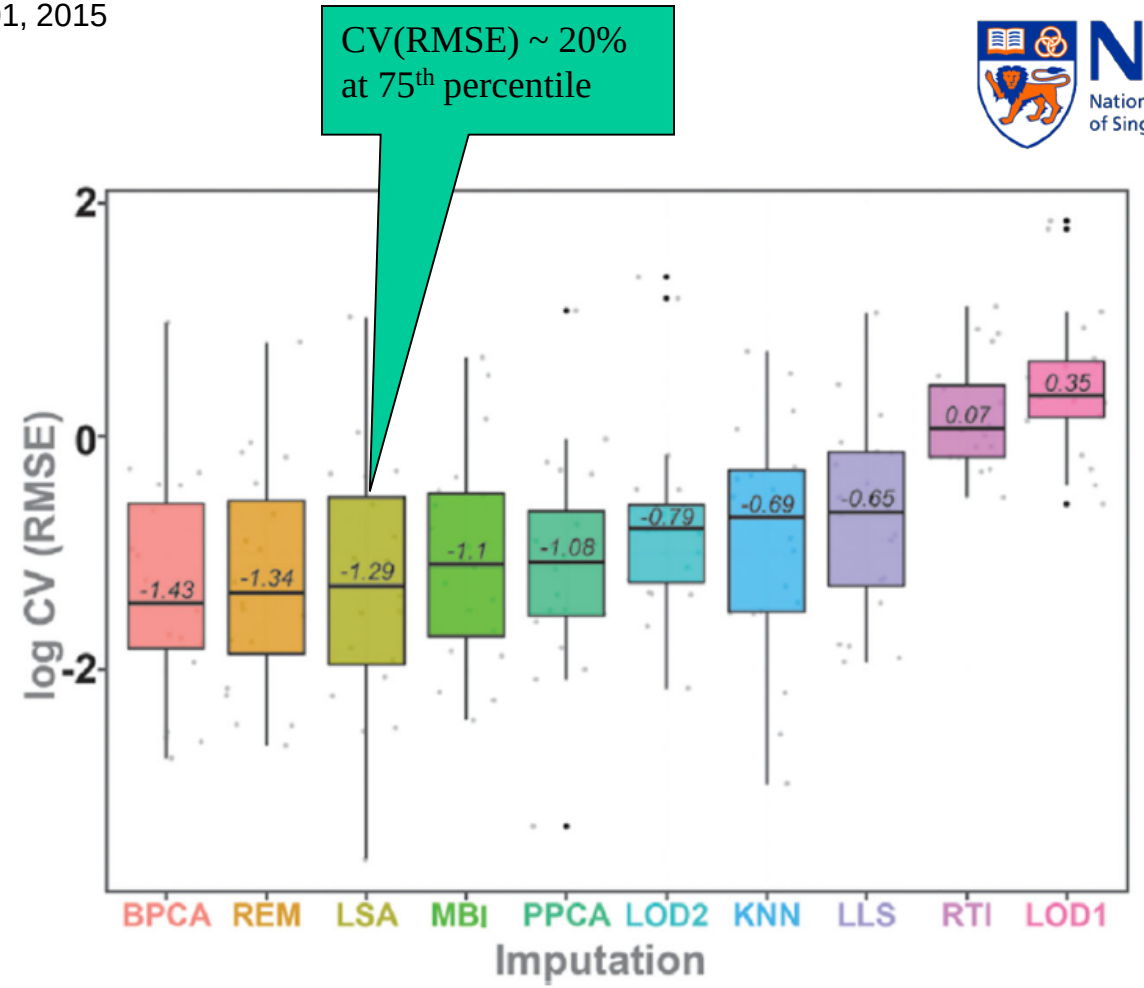


Figure 2.
Boxplot of the average $\log_{10}$ CV(RMSE) for the imputed dilution series datasets (Table 1) at the (A) peptide and (B) protein levels. The lower line represents the 25th percentile, the upper line of the box represents the 75th percentile, and the inner line corresponds to the median $\log_{10}$ CV(RMSE).

Goh et al. **Comparative network-based recovery analysis and proteomic profiling of neurological changes in valporic acid-treated mice**. *JPR*, 12(5):2116--2127, 2013

FCS

- **Rescue undetected proteins from high-scoring protein complexes**

- **Procedure:**
  - Score a protein complex based on proportion of its member proteins being reported in the screen
  - A complex is declared significant if this proportion is much higher than chance
  - Unreported proteins in a significant complex are predicted to be present

- **Shortcoming: Many complexes are not known**

Li et al. **Network-assisted protein identification and data interpretation in shotgun proteomics**. *Mol. Syst. Biol., 5:*303, 2009.

CEA

- **Generate cliques from PPIN**
- **Rescue undetected proteins from cliques containing many high-confidence proteins**

- **Reason: Cliques in a PPIN often correspond to proteins at the core of complexes**

- **Shortcoming: Cliques are too strict**

$\Rightarrow$ **Use more powerful protein complex prediction methods**

Goh et al. **A Network-based pipeline for analyzing MS data---An application towards liver cancer**. *Journal of Proteome Research*, 10(5):2261--2272, 2011

PEP

- **Map high-confidence proteins to PPIN**
- **Extract immediate neighbourhood & predict protein complexes using CFinder**
- **Rescue undetected proteins from high-ranking predicted complexes**

- **Reason: Exploit powerful protein complex prediction methods**

- **Shortcoming: Hard to predict protein complexes**
  - Do we need to know all the proteins a complex?

Goh et al. *International Journal of Bioinformatics Research and Applications*, 8(3/4):155--170, 2012

MaxLink

- **Map high-confidence proteins ("seeds") to PPIN**
- **Identify proteins that interact many seeds but few non-seeds**
- **Rescue these proteins**

- **Reason: Proteins interacting with many seeds are likely to be part of the same complex as these seeds**

- **Shortcoming: Likely to have more false-positives**

Goh et al. **Comparative network-based recovery analysis and proteomic profiling of neurological changes in valporic acid-treated mice**. *JPR*, 12(5):2116--2127, 2013

NUS National University of Singapore

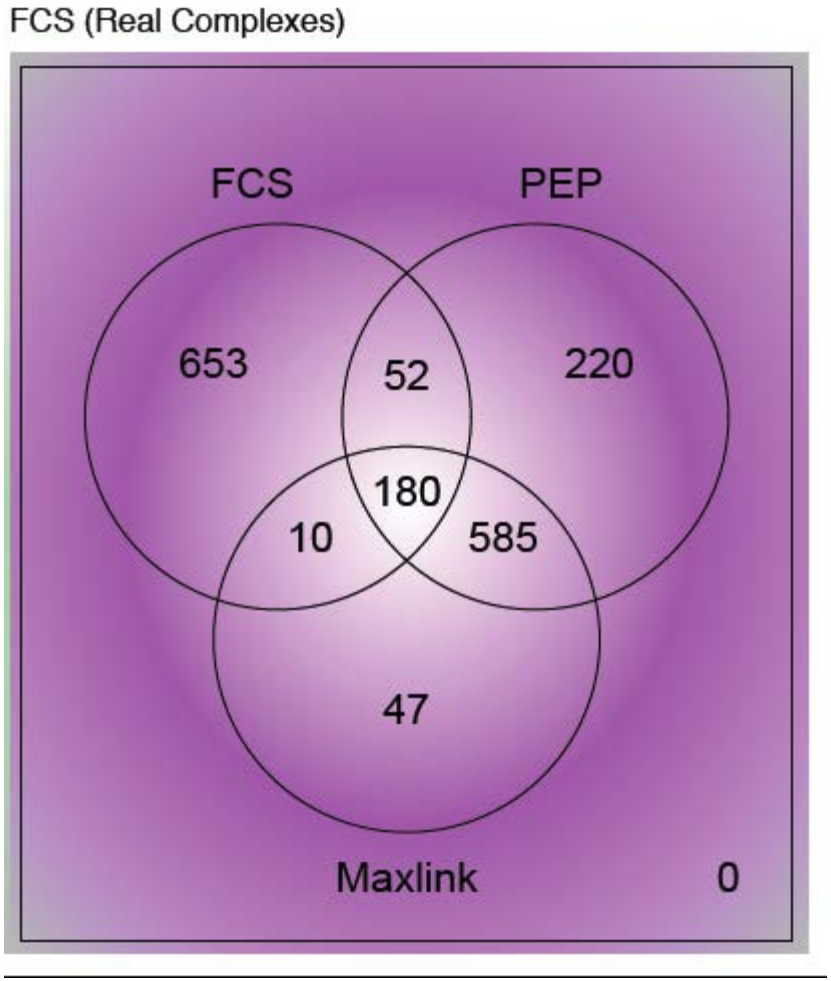
Experiment

- **Valporic acid (VPA)-treated mice vs control**
  - VPA or vehicle injected every 12 hours into postnatal day-56 adult mice for 2 days
  - Role of VPA in epigenetic remodeling

- **MS was scanned against IPI rat db in round #1**
  - 291 proteins identified
- **MS was scanned against UniProtkb in round #2**
  - 498 additional proteins identified

- **All recovery methods ran on round #1 data and the recovered proteins checked against round #2**

Moderate level of agreement of reported proteins between various recovery methods



Performance comparison

Method	Novel Suggested Proteins	Recovered proteins	Recall	Precision
PEP	1037	158	0.317	0.152
Maxlink	822	226	0.454	0.275
FCS (predicted)	638	224	0.450	0.351
FCS (complexes)	895	477	0.958	0.533

- **Looks like running FCS on real complexes is able to recover more proteins and more accurately**

Another validation experiment

- **If there are technical replicates, they should have reported the same proteins. So we can run FCS on one replica, and see whether the predicted missing proteins show up in other replicas**
- **If there are multiple biological replicates (i.e. patients of the same phenotype), we can run FCS on one of them, and check on the others**
- **Proteomics data used: Renal cancer**
  - Guo et al. *Nature Medicine*, 21(4):407-413, 2015
  - 6 pairs of normal vs cancer ccRCC tissues
  - SWATH in duplicates

>20% of predicted missing proteins are supported by ≥1 reported peptide in replicates

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C Strategy 3 (complex to proteins in the peptide list)

Sample	N T1-> N T2	N T2 -> N T1	C T1-> C T2	C T2 -> C T1
1	0.212 0 984 209	0.210 0 937 197	0.198 0 823 163	0.182 0 911 166
2	0.213 0 936 199	0.216 0 889 192	0.205 0 904 185	0.202 0.001 918 185
3	0.212 0 972 206	0.196 0 950 186	0.218 0 849 185	0.249 0 840 209
4	0.224 0 943 211	0.233 0 948 221	0.197 0.002 925 182	0.222 0 930 206
5	0.188 0.002 912 171	0.235 0 964 227	0.185 0 877 162	0.209 0 904 189
6	0.224 0 883 198	0.246 0 977 240	0.227 0 886 201	0.249 0 927 231

Note: Treating proteins supported by ≥1 peptide as reported increases verified proteins by 10x, & reported proteins by 2x

And >80% are supported by significant complexes in replicates

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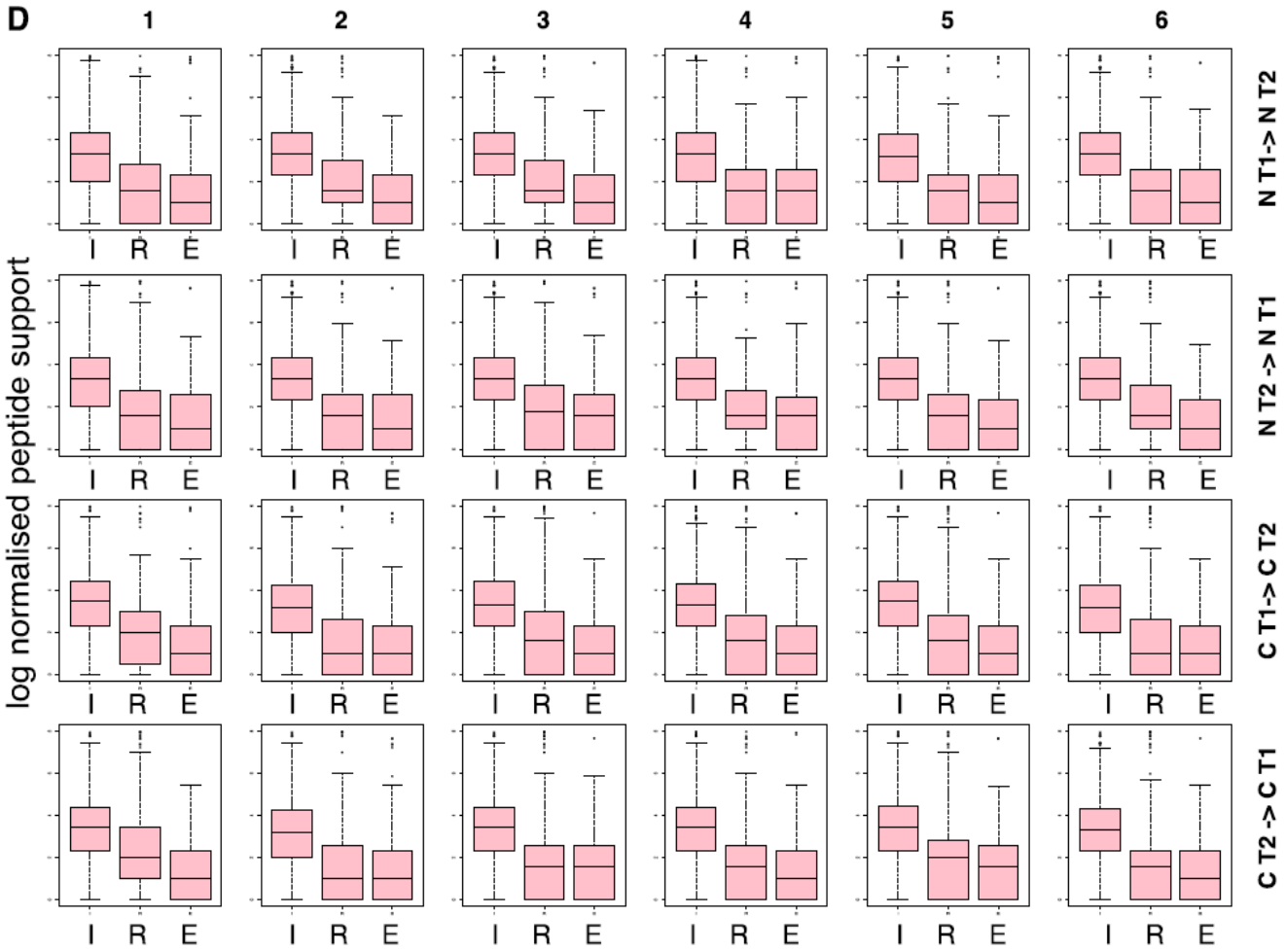
B Strategy 2 (complex to complex)

Sample	N T1-> N T2	N T2 -> N T1	C T1-> C T2	C T2 -> C
1	0.872 0 985 859	0.916 0 937 858	0.938 0 823 772	0.845 0 911 770
2	0.878 0 936 822	0.935 0 889 831	0.895 0 904 809	0.888 0 918 815
3	0.892 0 972 867	0.916 0 950 870	0.879 0 849 746	0.899 0 840 775
4	0.875 0 943 825	0.895 0 948 848	0.836 0 925 773	0.842 0 930 783
5	0.871 0 912 794	0.853 0 964 822	0.851 0 877 746	0.846 0 904 765
6	0.907 0 883 801	0.832 0 977 813	0.915 0 886 811	0.904 0 927 838

High level of consistency at the level of complexes

Recovered proteins are more reliable than excess ones



The y-axis is the number of supporting peptides (0 – 8) per protein. The 3 barplots in each box are labelled I R E
I - identified (proteins in batch 1), R - recovered (proteins in batch 2), E - excess (proteins neither observed nor predicted missing)

~20% FCS-predicted missing proteins are supported by peptides in replicate. Can we do better?

Recall this postulate:

Chance of a complex being present ≈ fraction of its protein members being correctly reported in screen

Presence of complex implies presence of all member proteins

PROTREC: Rank predicted missing proteins by

Prob(Protein p is present but unreported) = $\text{Max}_{\text{complex C contains p}}$ Prob(p is present | C is present) * Prob(C is present) + Prob(p is present | C is absent) * Prob(C is absent)

227 significant complexes by FCS
corresponding to 1319 proteins
334 are from this screen.
So missing proteins is 985

N1_T12

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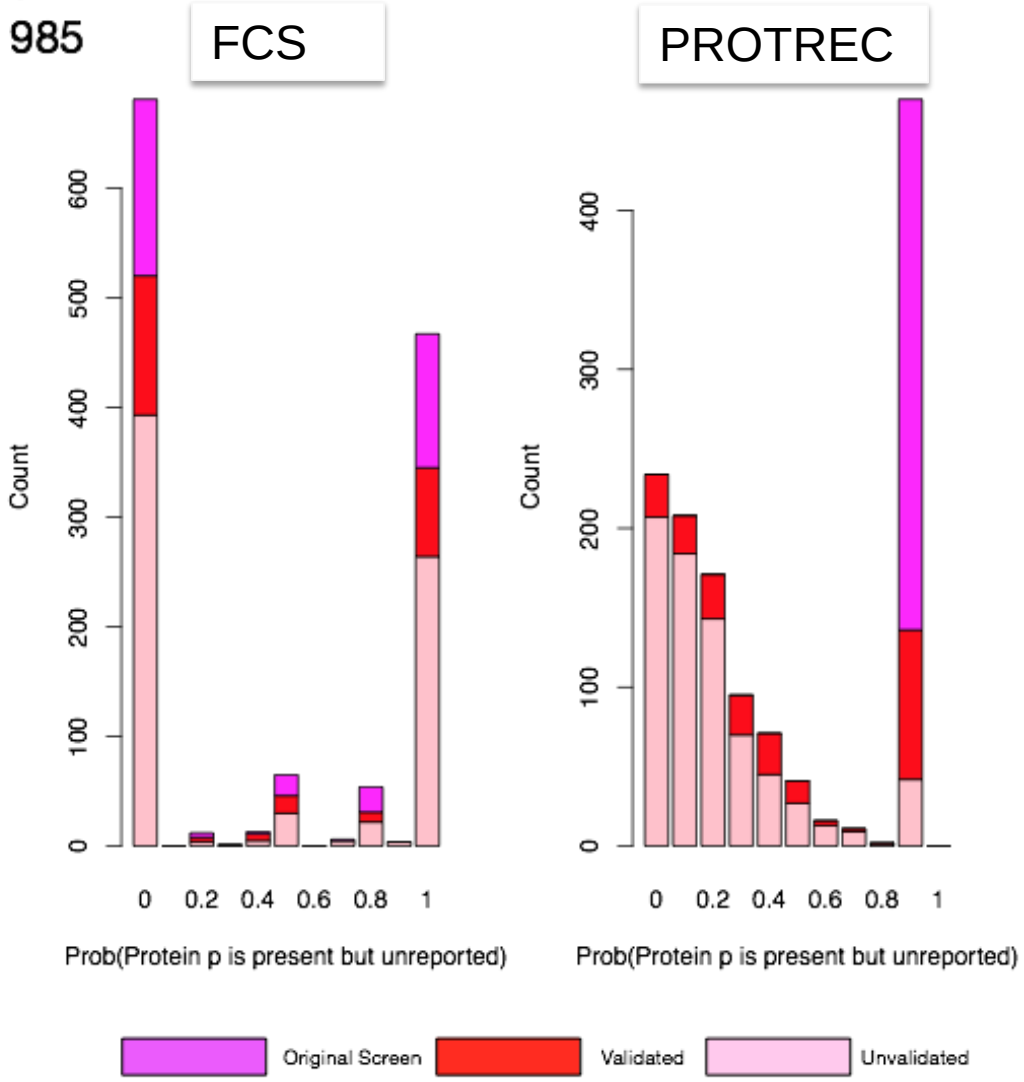
Much improved precision

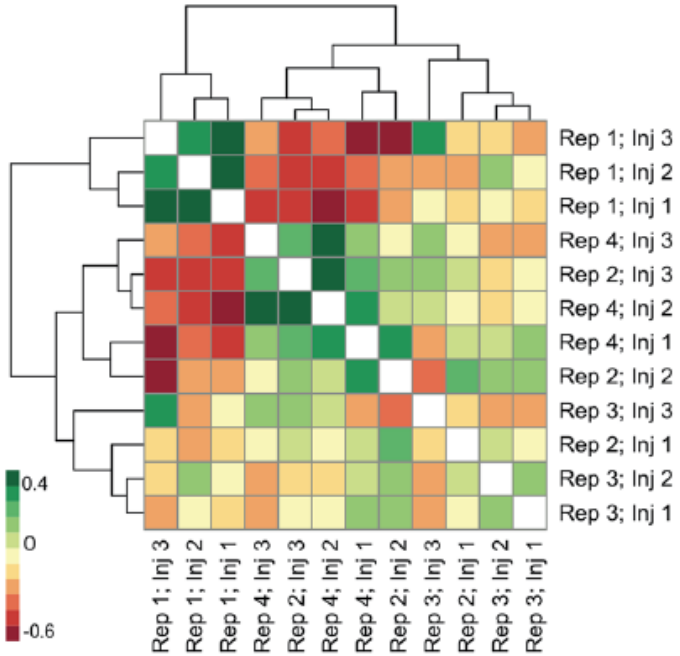


Improving consistency in proteomic profile analysis

Proteomic profiles generally not consistent, even for technical replicates

- **A human kidney tissue**
  - Guo et al. *Nature Medicine*, 21(4):407-413, 2015
  - Digested in quadruplicates
  - Analyzed in triplicates

- **Clustering by proteins**
  - Correlation betw replicates is not good (~0.4)
  - Technical replicates of the same biological replicate are not tightly clustered



Goh et al. **Quantitative proteomics signature profiling based on network contextualization**. *Biology Direct*, 10:71. 2015

qPSP

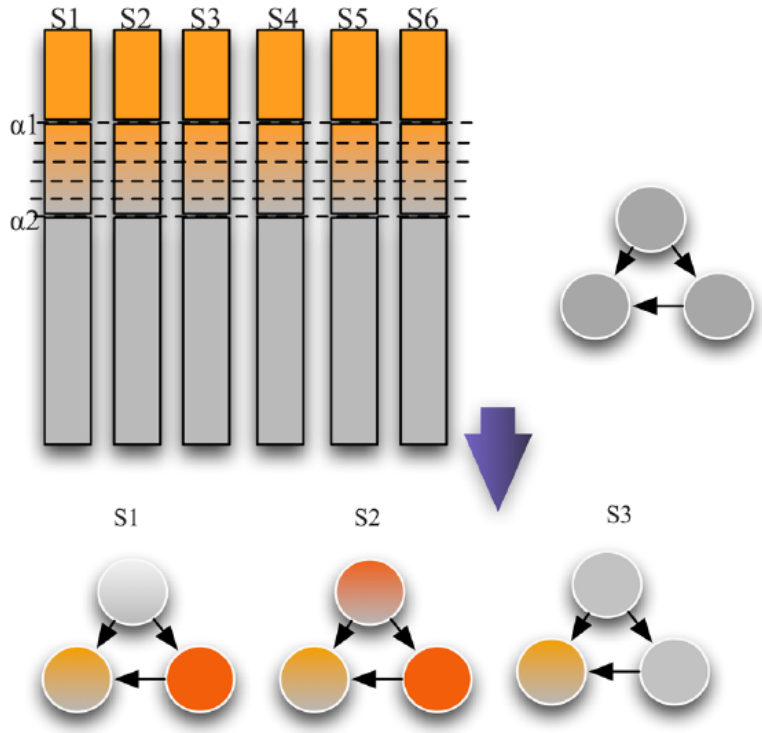
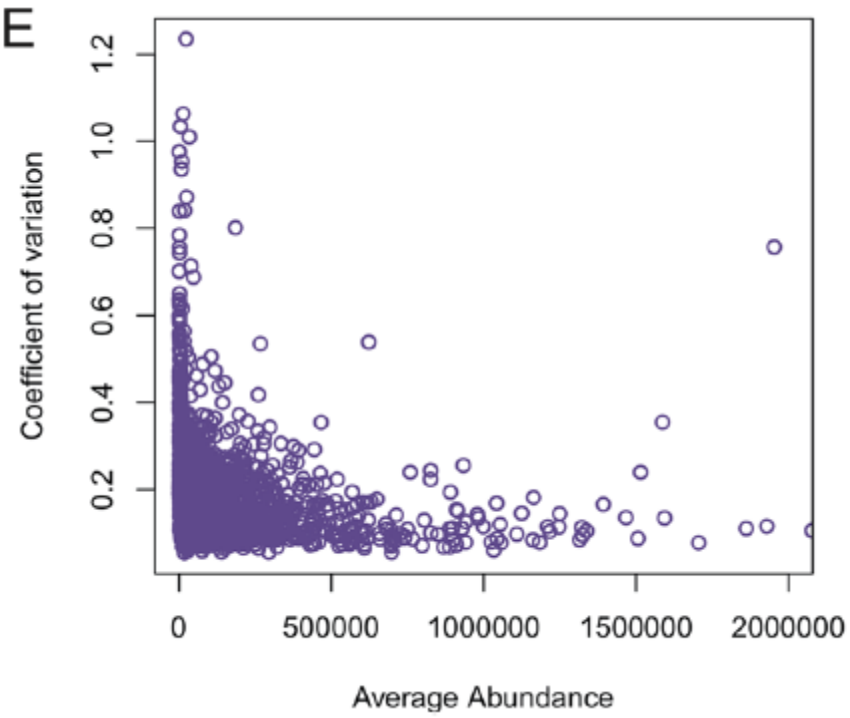


Fig. 1 Schematic demonstrating qPSP's fuzzification procedure. First, alpha1 at top 10 % was defined. An alpha2 was defined from top 10-20 %. To place less confidence in the lower-scoring alpha2, proteins that fall within this range were grouped into 5 bins with descending weights. The modulated hit-rates for each sample could then be used for generating each sample's proteomic signature profile

- **Features are complexes**
- **Feature values are fuzzy weighted proportion of proteins in a complex**
  - $\text{score}(C,S_i) = \Sigma_{p \in C} \, fs(p,S_i) \,/\, |C|$
- **Complex C is significant if $\{\text{score}(C,S_i) \mid S_i \in A\}$ is very different by t-test from $\{\text{score}(C,S_i) \mid S_i \in B\}$**

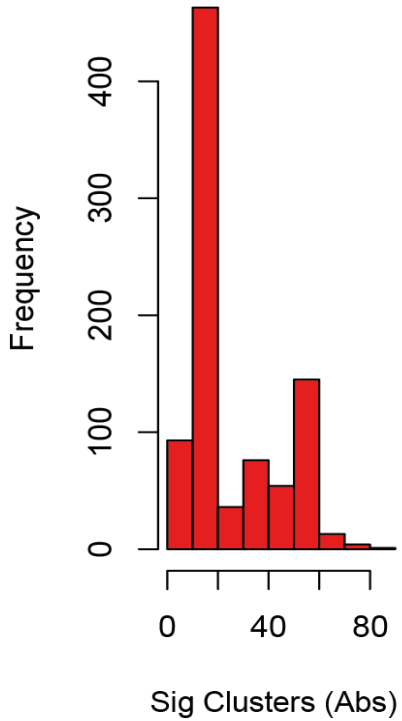
Justification for fuzzy scoring

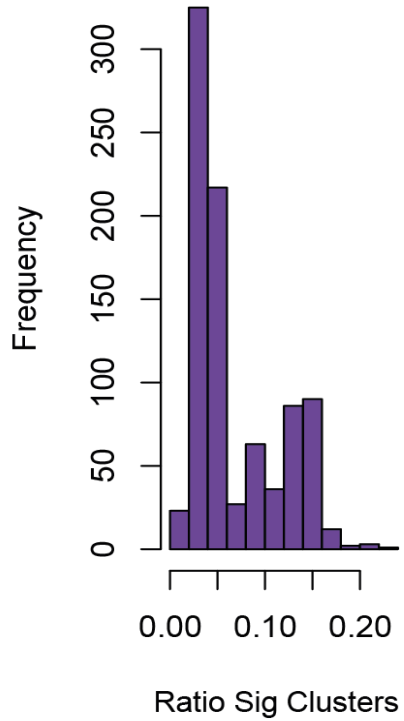


- **Low-abundance proteins have very high coefficient of variation; they thus are very noisy**
- **Fuzzy scoring mitigates this**

False-positive rate analysis

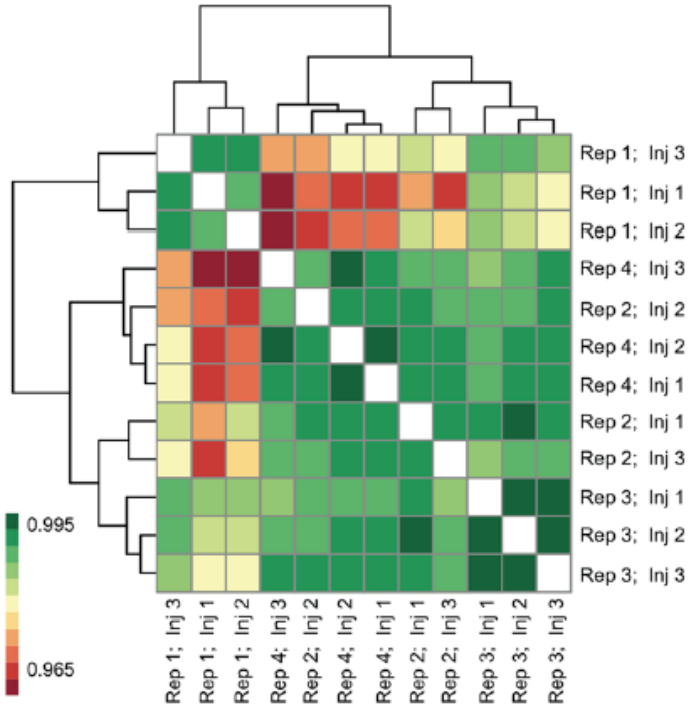
- **12 kidney controls randomly assigned into two groups of equal size, and qPSP analysis performed many rounds**
- **# of significant clusters (5% FDR) determined each round**
- **False-positive rate well within the expectation levels**
  - Sig Clusters (Abs)
    - **Expect: 19, Observed: 16**
  - Sig Clusters (Ratio)
    - **Expect: 0.05, Observed: 0.04**





Consistency of qPSP

- **Clustering of benchmarking control data based on protein complexes (i.e. qPSP)**
  - Correlation betw replicates is >0.95
    - **Cf. 0.4 based on proteins**
  - Technical replicates are better clustered



Application to renal & colorectal cancers

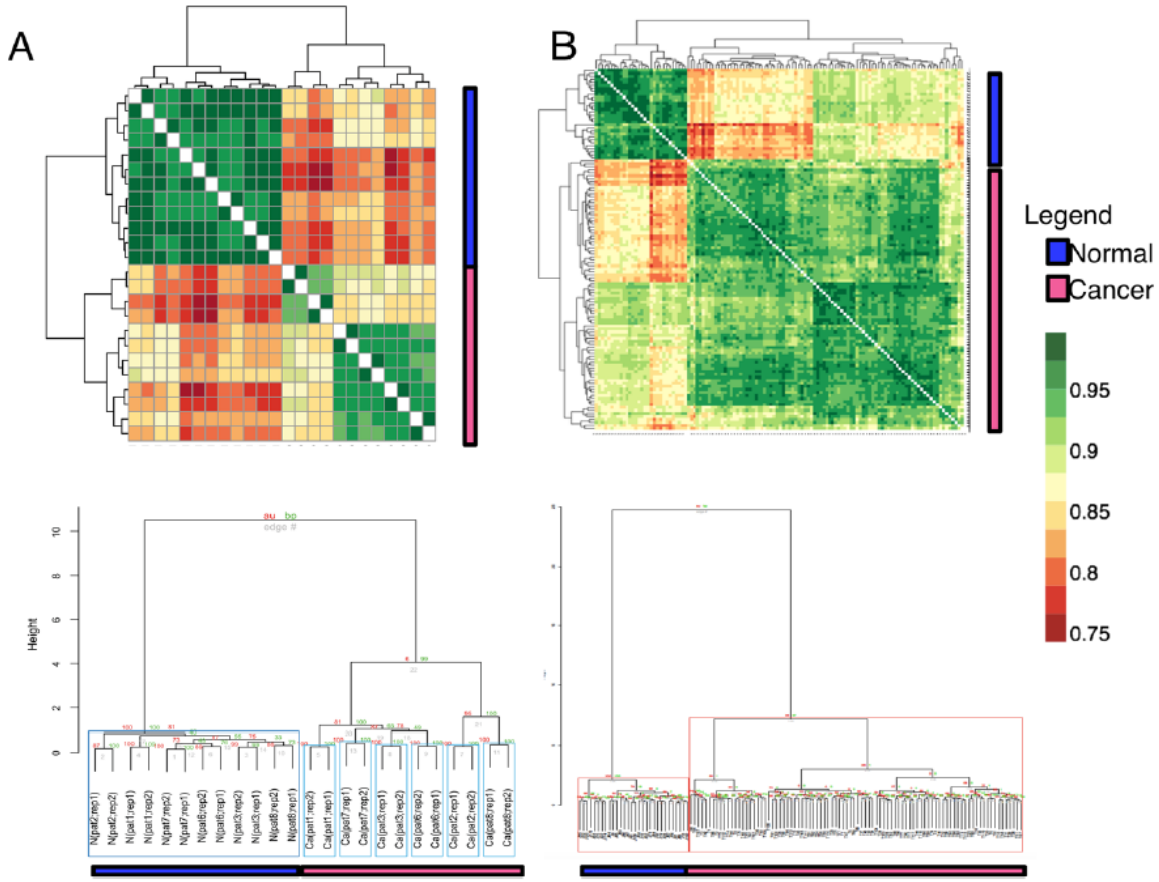
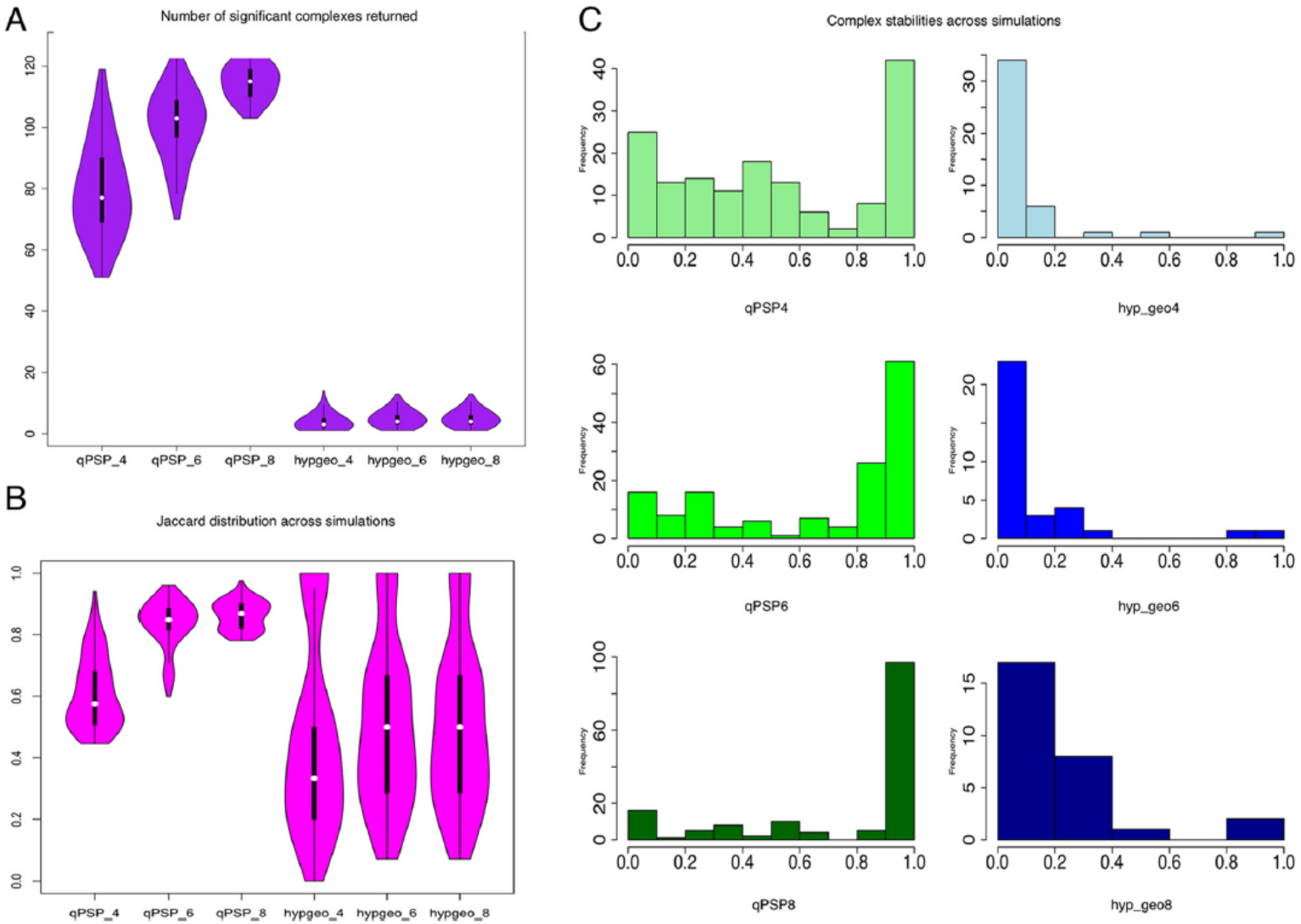


Fig. 3 qPSP strongly discriminates sample classes for renal cancer (**a**) and colorectal cancer (**b**). Clustered similarity maps at the top row showed specific and consistent segregation of non-cancer and cancer samples. The trees below the heatmaps are from bootstrap analysis (PVCLUST), which demonstrates that the discrimination between sample classes based on qPSP hit-rates is highly stable

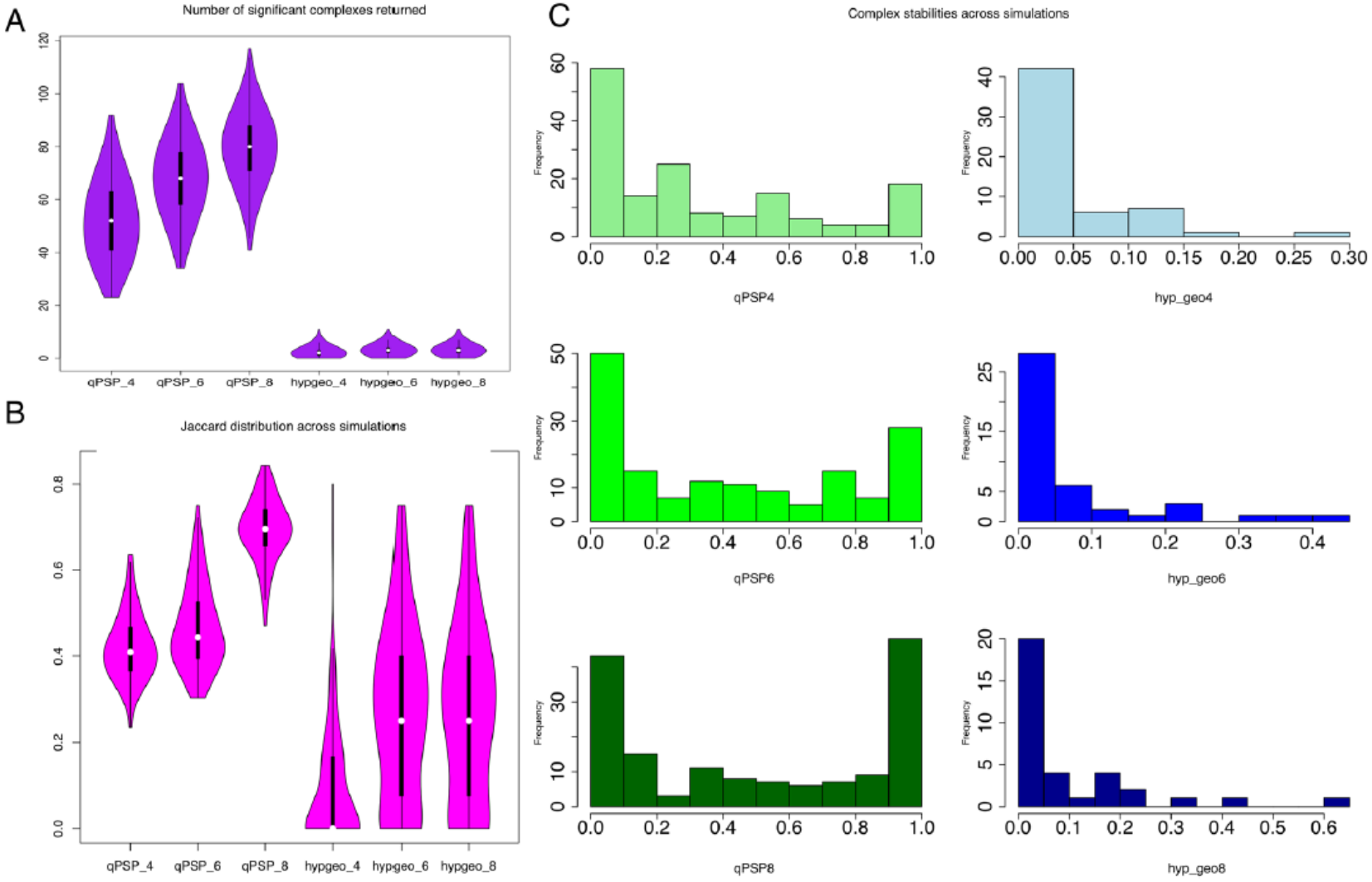
Comparing qPSP to HE

- **Hypergeometric enrichment (HE)**
  - A complex is significant if, based on the hypergeometric test, it has a larger-than-chance intersection with the list of t-test significant proteins
- **Data used**
  - Renal cancer, Guo et al. *Nature Medicine*, 21(4):407-413, 2015
  - Colorectal cancer, Zhang et al. Nature, 513(7518):382-387, 2014
- **Evaluation**
  - Generate subsamples of size 4, 6, 8
  - Run a method on a subsample; check agreement of the selected complexes betw diff runs

Stability of qPSP – Renal cancer



Stability of qPSP – Colorectal cancer



Aspects to improve for qPSP

- **Low-abundance proteins are ignored**
- **The performance, especially feature-selection stability, on colorectal cancer is not as good as that on renal cancer**
- **Precision/recall not evaluated**

Further improving consistency, as well as catching significant low-abundance complexes

ESSNet, adapted for proteomics

- Let g_i be a protein in a given protein complex
- Let p_j be a patient
- Let q_k be a normal

- Let $\Delta_{i,j,k} = Expr(g_i,p_j) - Expr(g_i,q_k)$

- Test whether $\Delta_{i,j,k}$ is a distribution with mean 0

- Null hypothesis is "Complex C is irrelevant to the difference between patients and normals, and the proteins in C behave similarly in patients and normals"

- No need to restrict to most abundant proteins

$\Rightarrow$ Potential to reliably detect low-abundance but differential proteins

Lim et al. **A quantum leap in the reproducibility, precision, and sensitivity of gene expression profile analysis even when sample size is extremely small**. *JBCB*, 13(4):1550018, 2015

Five methods to compare with

- **Network-based methods**
  - Hypergeometric enrichment (HE)
  - Direct group analysis (DG), similar to GSEA
  - qPSP
  - PFSNET, Lim & Wong. *Bioinformatics*, 30(2):189--196, 2014

- **Standard t-test on individual proteins (SP)**

Langley & Mayr, *J. Proteomics*, 129:83-92, 2015

Simulated data

- **Simulated datasets from Langley and Mayr**
  - D.1.2 is from study of proteomic changes resulting from addition of exogenous matrix metallopeptidase (3 control, 3 test)
  - D2.2 is from a study of hibernating arctic squirrels (4 control, 4 test)
- **Both D1.2 and D2.2 have 100 simulated datasets, each with 20% significant features**
  - Effect sizes of these differential features are sampled from one out of five possibilities (20%, 50%, 80%, 100% and 200%), increased in one class and not in the other
- **Significant artificial complexes are constructed with various level of purity (i.e. proportion of significant proteins in the complex)**
  - Equal # of non-significant complexes are constructed as well

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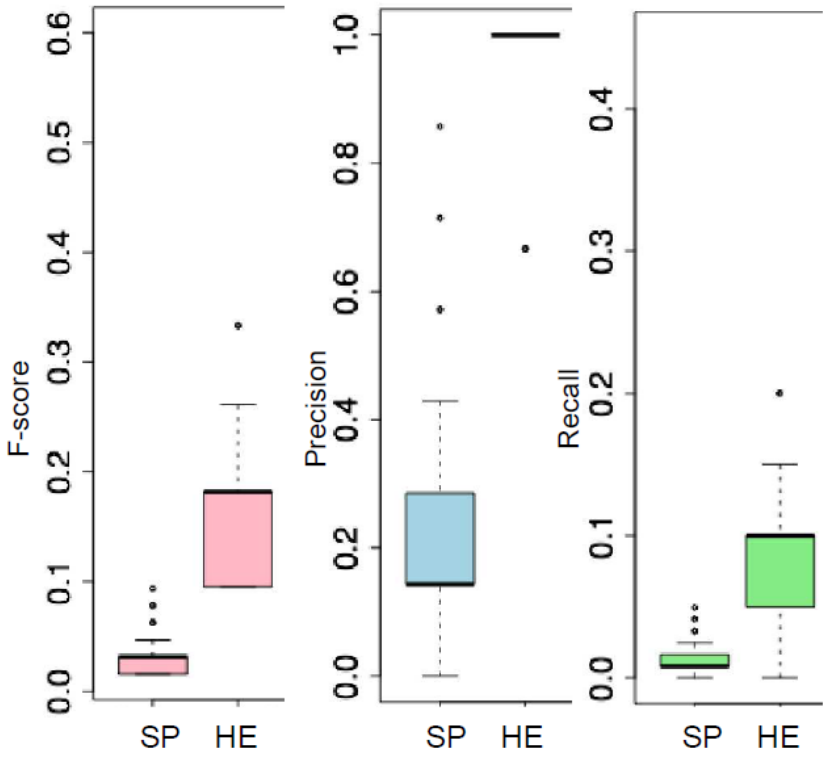
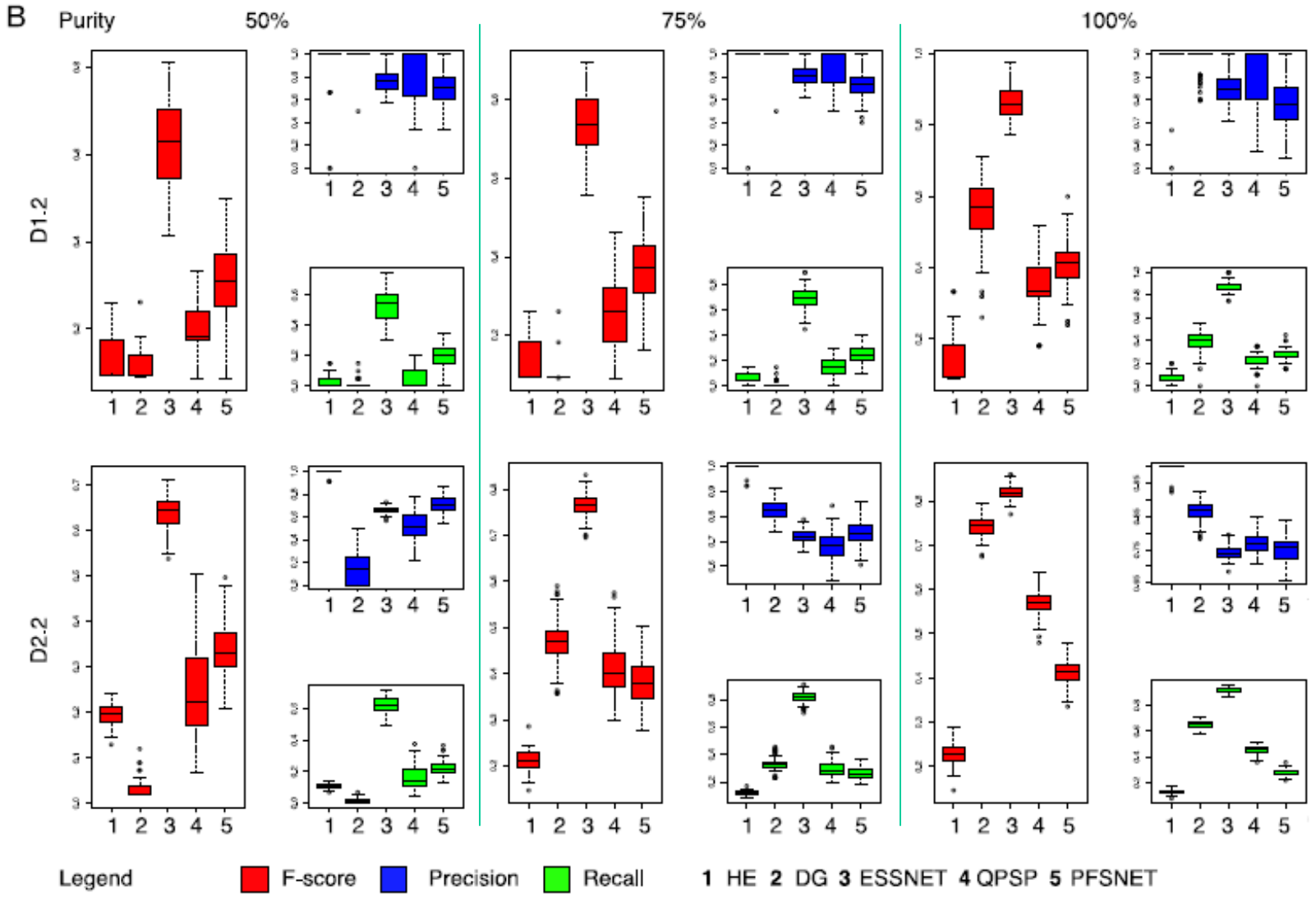
SP shows poor performance on simulated data

Can network-based methods do better?



Supplementary Figure 1 Single protein (SP) precision-recall performance on D1.2. The f-score (pink), precision (blue) and recall (green) shows that SP performs abysmally on simulated data. HE is shown next to SP as a reference.

ESSNET shows excellent recall/precision on simulated data



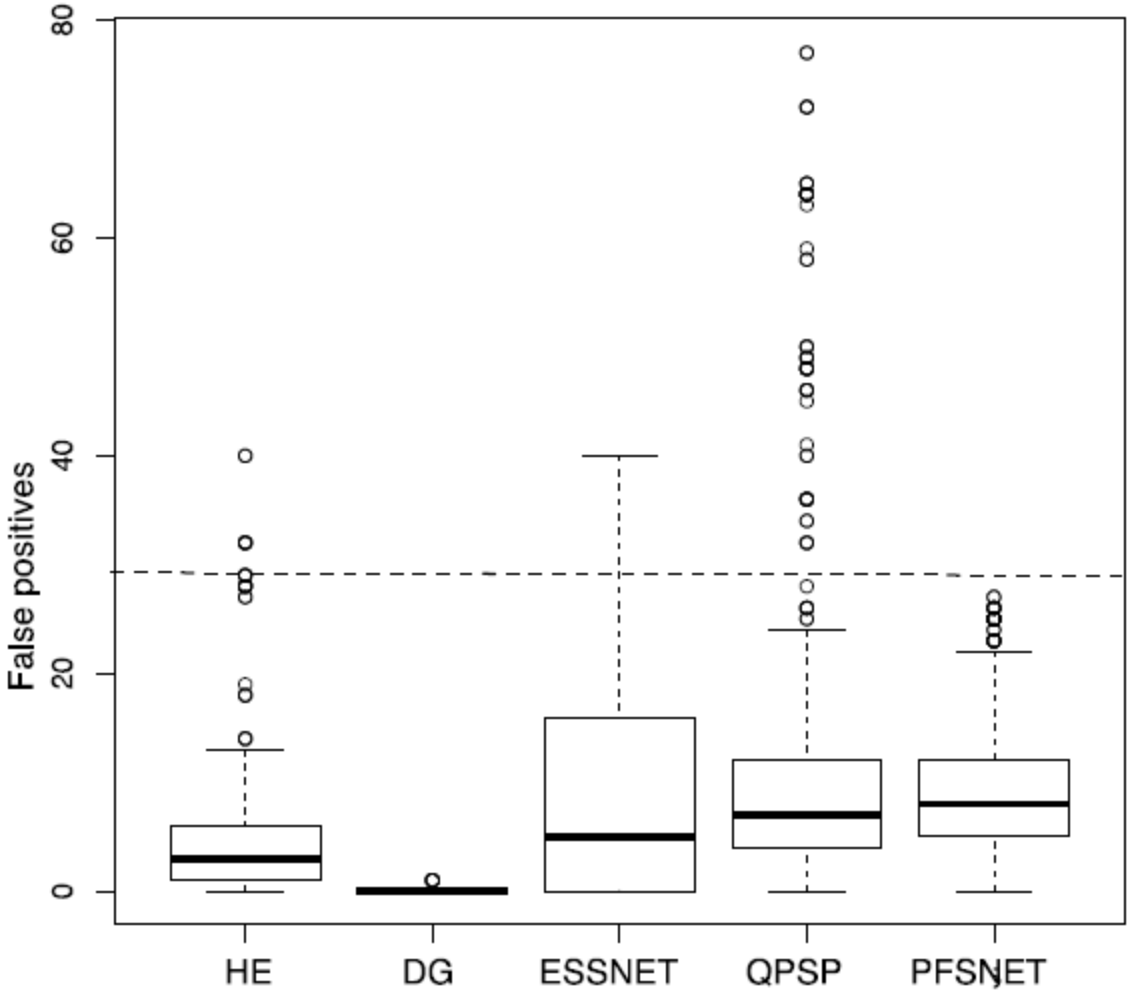
Guo et al. *Nature Medicine*, 21(4):407-413, 2015

Renal cancer control data (RCC)

- **12 runs originating from a human kidney tissue digested in quadruplicates and analyzed in triplicates**

- **Excellent for evaluating false-positive rates of feature-selection methods**
  - Randomly split the 12 runs into two groups. Report of any significant features between the groups must be false positives

All methods control false positives well



Dash line corresponds to expected # of false positives at alpha 0.05 (~30 complexes)

Guo et al. *Nature Medicine*, 21(4):407-413, 2015

Renal cancer data (RC)

- **12 samples are run twice so that we have technical replicates over 6 normal and 6 cancer tissues**

- **Excellent opportunity for testing reproducibility of feature-selection methods**
  - A good method should report similar feature sets between replicates

- **Can also test feature-selection stability**
  - Apply feature-selection method on subsamples and see whether the same features get selected

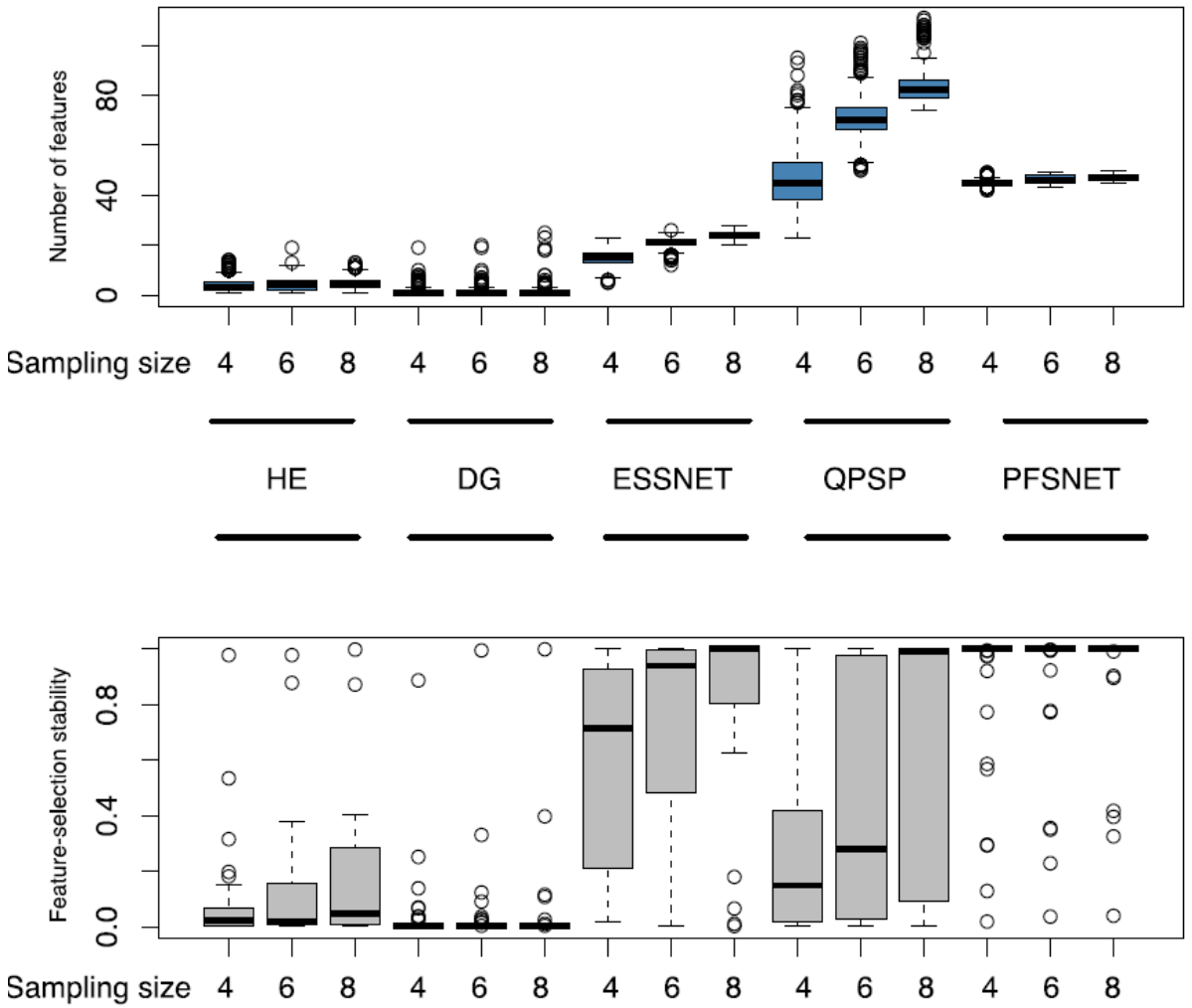
ESSNET & PFSNET show excellent reproducibility

Number of terms	HE	DG	ESSNET	QPSP	PFSNET
Replicate 1	4	1	35	86	45
Replicate 2	6	2	29	75	46
Overlaps	0.25	0.5	0.83	0.66	0.94

HE	DG	ESSNET	QPSP	PFSNET	
1	0.5	0.71	0.86	0.71	HE
	1	1	1	1	DG
		1	0.93	0.98	ESSNET
			1	0.90	QPSP
				1	PFSNET

This table is computed on by applying the methods on the full RC dataset

ESSNET & PFSNET show excellent stability

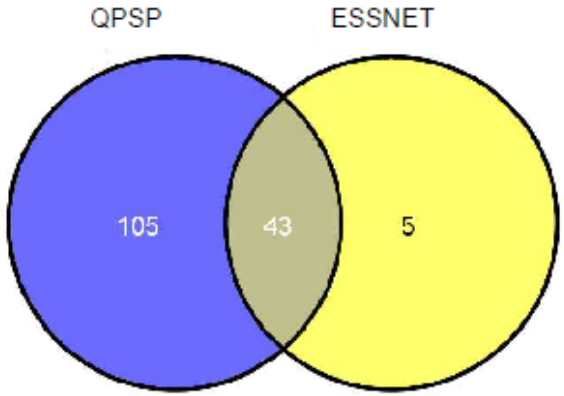


ESSNET & PFSNET show excellent stability

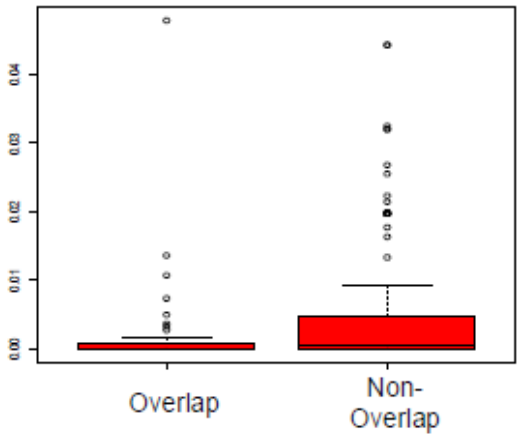
	4	6	8	Mean
HE	0.022	0.016	0.047	0.030
DG	0.001	0.001	0.002	0.001
ESSNET	0.714	0.941	1.000	0.885
QPSP	0.149	0.282	0.991	0.470
PFSNET	1.000	1.000	1.000	1.000

ESSNET can assay low-abundance complexes that qPSP cannot

A



B



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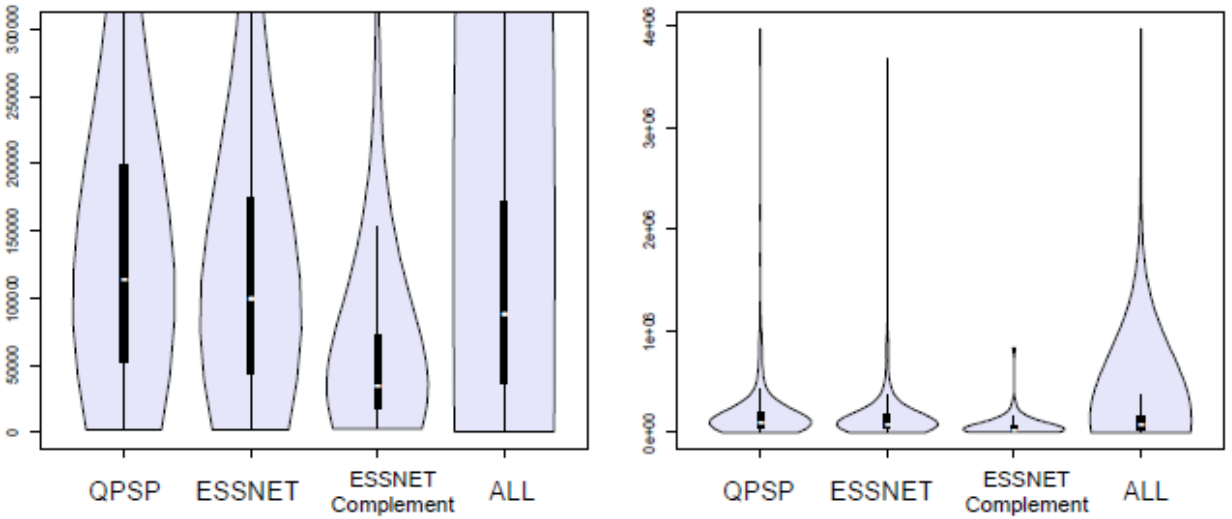
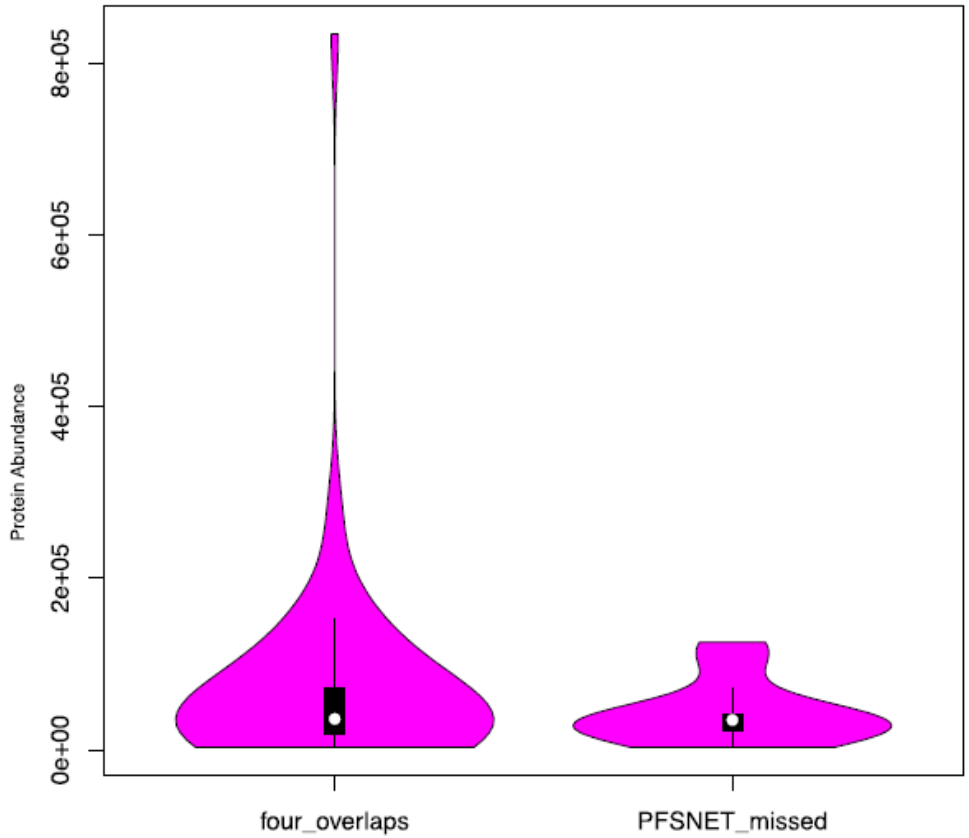


A: QPSP-ESSNET significant-complex overlaps

B: P-value distribution for overlapping and non-overlapping QPSP complexes.

C: Sampling abundance distribution. The left panel is a zoom-in of the right. The y-axis is the protein abundance while the four categories are the distribution of abundances of complexes found in QPSP, ESSNET, ESSNET unique (complement), and all proteins in RC.

ESSNET can assay low-abundance complexes that PFSNET cannot



Of the 5 ESSNET-unique complexes, PFSNET can detect 4; the missed complex consists entirely of low-abundance proteins.

If p-value threshold is adjusted by Benjamini-Hochberg 5% FDR, PFSNET can detect only 3 of the 5 ESSNET-unique complexes while ESSNET continues to detect them all.

Concluding remarks

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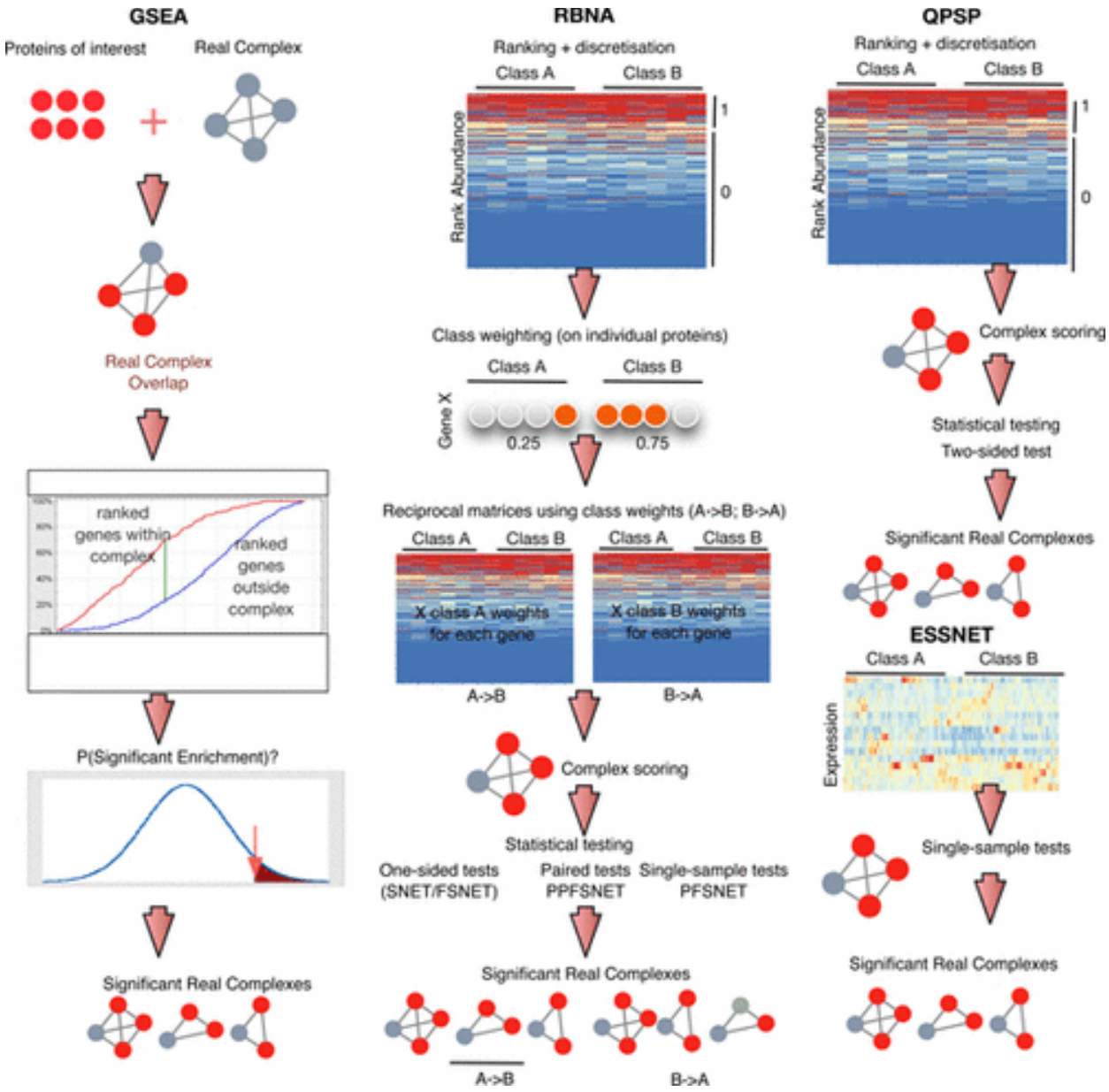
In conclusion…

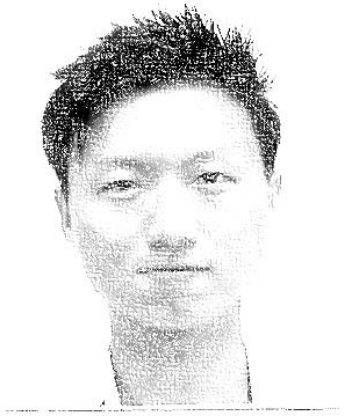
Contextualization (into complexes) can deal with coverage and consistency issues in proteomics



NetProt

Goh & Wong. **NetProt: Complex-based feature selection**. *JPR*, 16(8):3102-3112, 2017

Acknowledgements



Wilson Goh

- **Singapore Ministry of Education**

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