Improving Proteomic Profile Analysis by Contextualization

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Diagnosis Using Proteomics

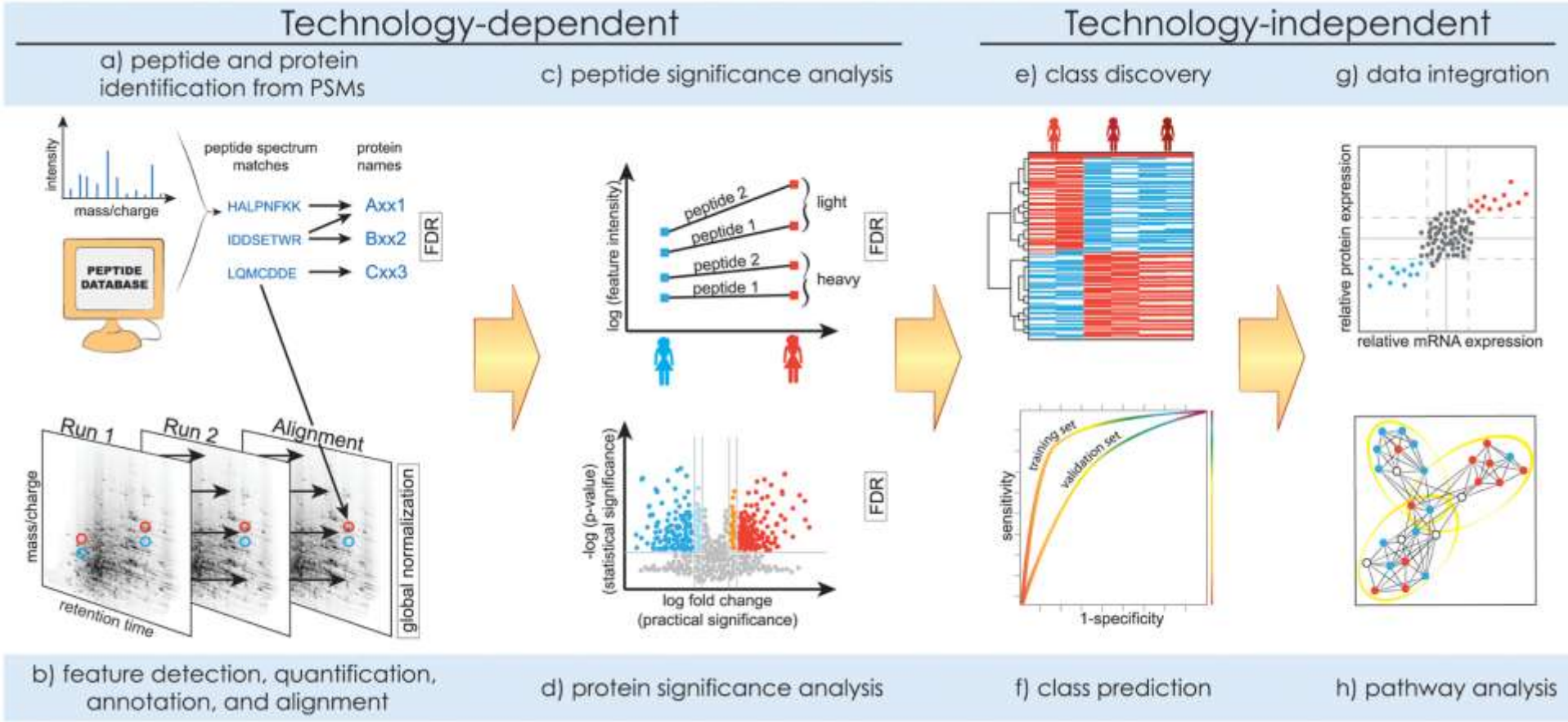

Image credit: Kall and Vitek, *PLoS Comput Biol* , 7(12): e1002277, 2011

Plan

- **Common issues in proteomic profile analysis**
- **Improving consistency**
- **Improving coverage**

Common Issues in Proteomic Profile Analysis

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Peptide & protein identification by MS is still far from perfect

- **"… peptides with low scores are, nevertheless, often correct, so manual validation of such hits can often 'rescue' the identification of important proteins."**

  Steen & Mann. **The ABC's and XYZ's of peptide sequencing**. *Nature Reviews Molecular Cell Biology*, 5:699-711, 2004

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A concrete example: Bell et al., *Nat Meth*, 6:423-430, 2009

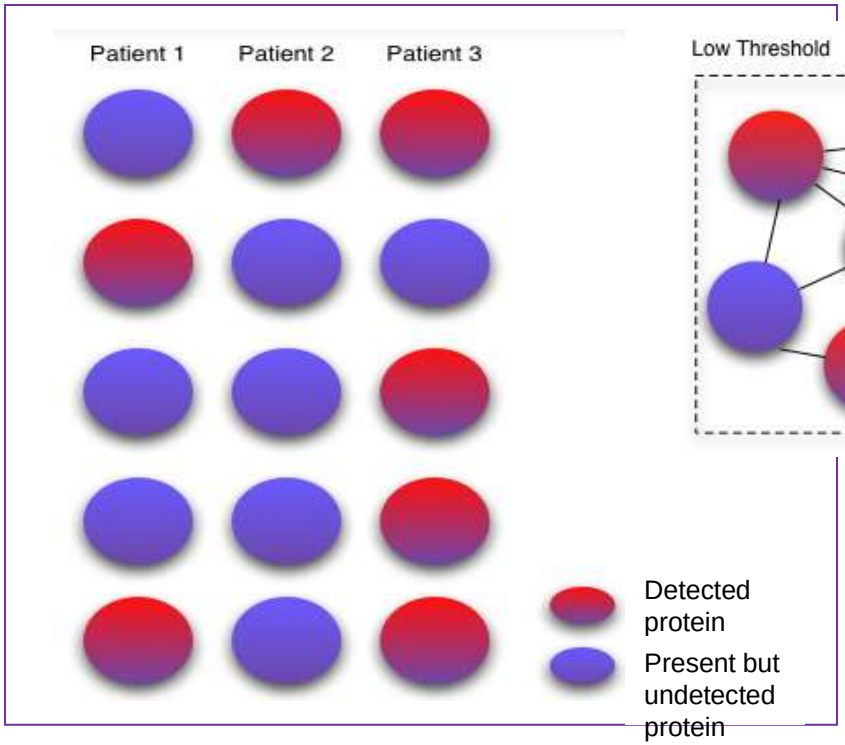
- **"… in a large-scale collaborative study by Bell et al. to assess the extent of reproducibility across different laboratories. The results were striking – only 7 out of 27 laboratories correctly reported all 20 proteins, and only 1 laboratory successfully reported all 22 unique peptides."**

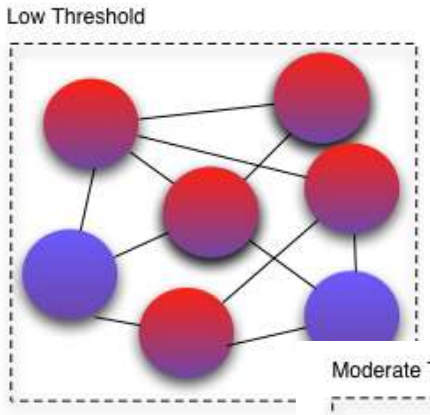
Issues in Proteomic Profiling

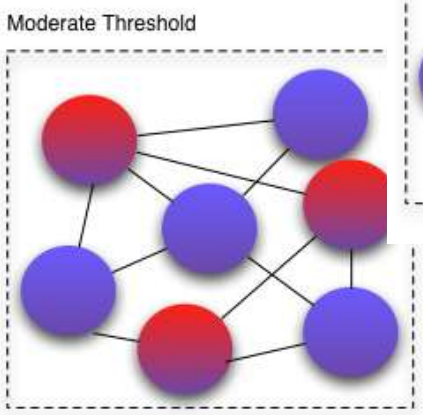
- **Coverage**
- **Consistency**

⇒ **Thresholding**

- Somewhat arbitrary
- Potentially wasteful
  - **By raising threshold, some info disappears**







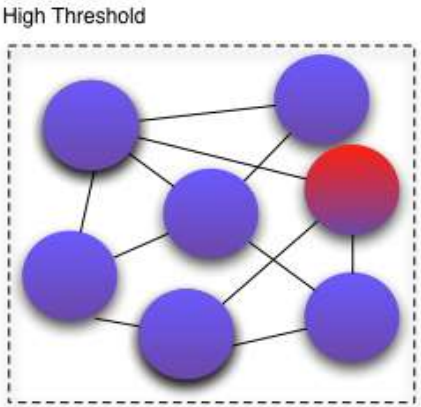
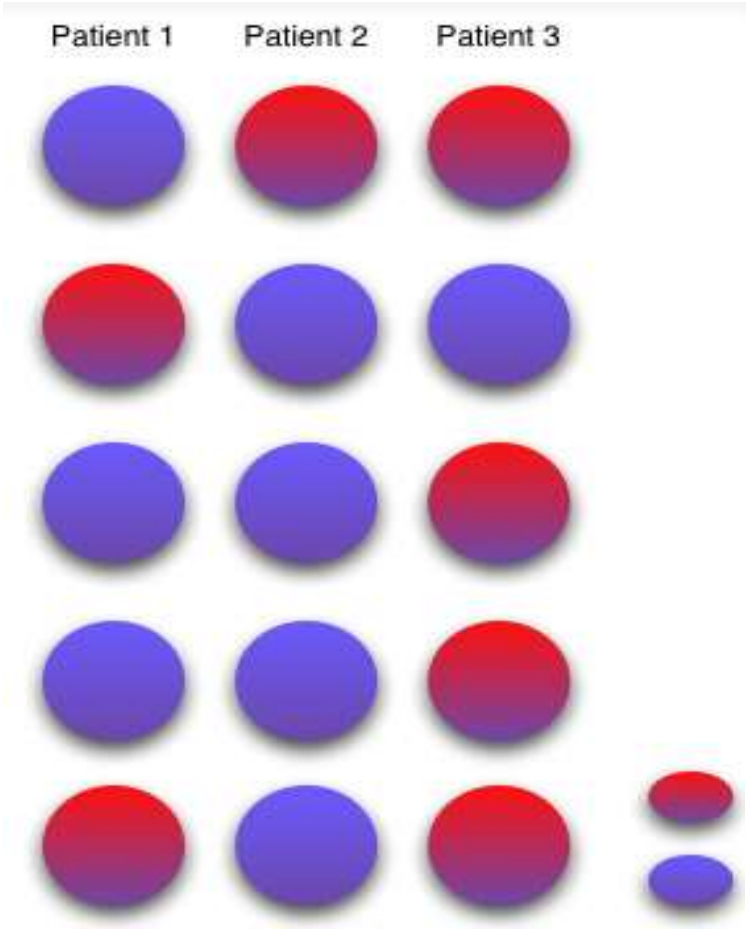


Image credit: Wilson Goh

Improving Consistency in Proteomic Profile Analysis

Intuitive Example



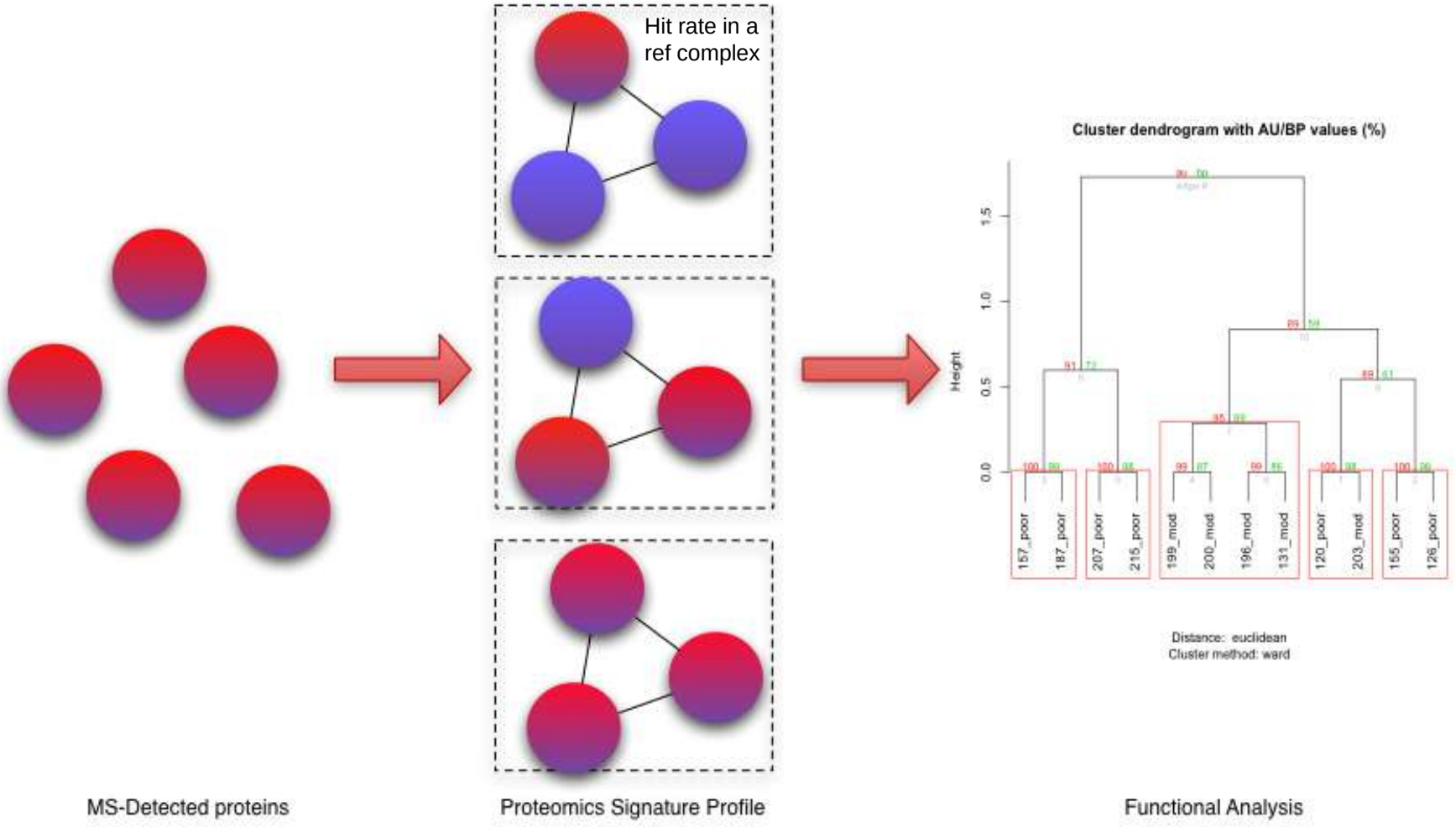
- **Suppose the failure to form a protein complex causes a disease**
  - If any component protein is missing, the complex can't form

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

  - Construct a profile based on complexes?

Goh et al. **Proteomics signature profiling (PSP): A novel contextualization approach for cancer proteomics**. *Journal of Proteome Research,*11(3):1571--1581, 2012.

"Threshold-free" Principle of PSP



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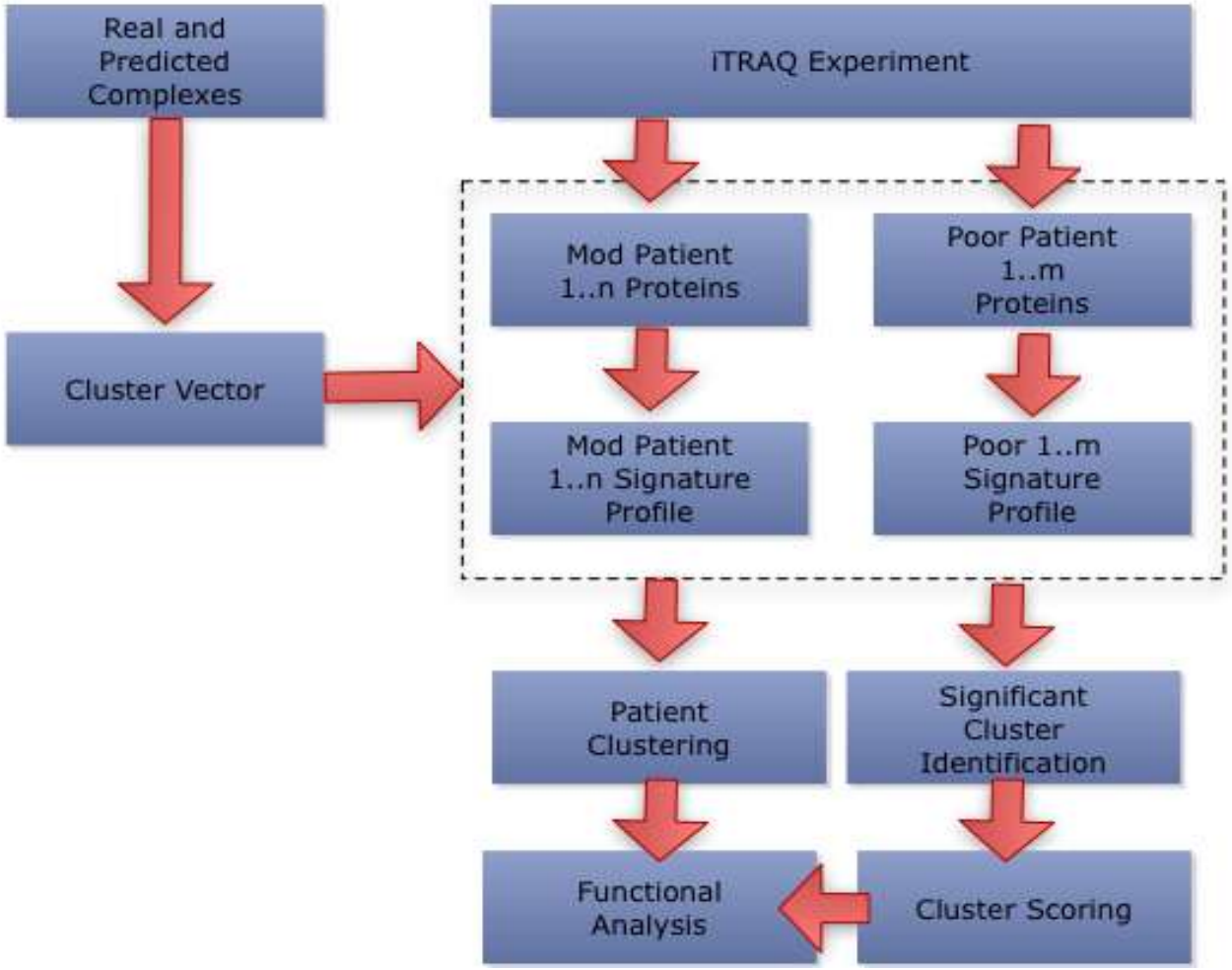
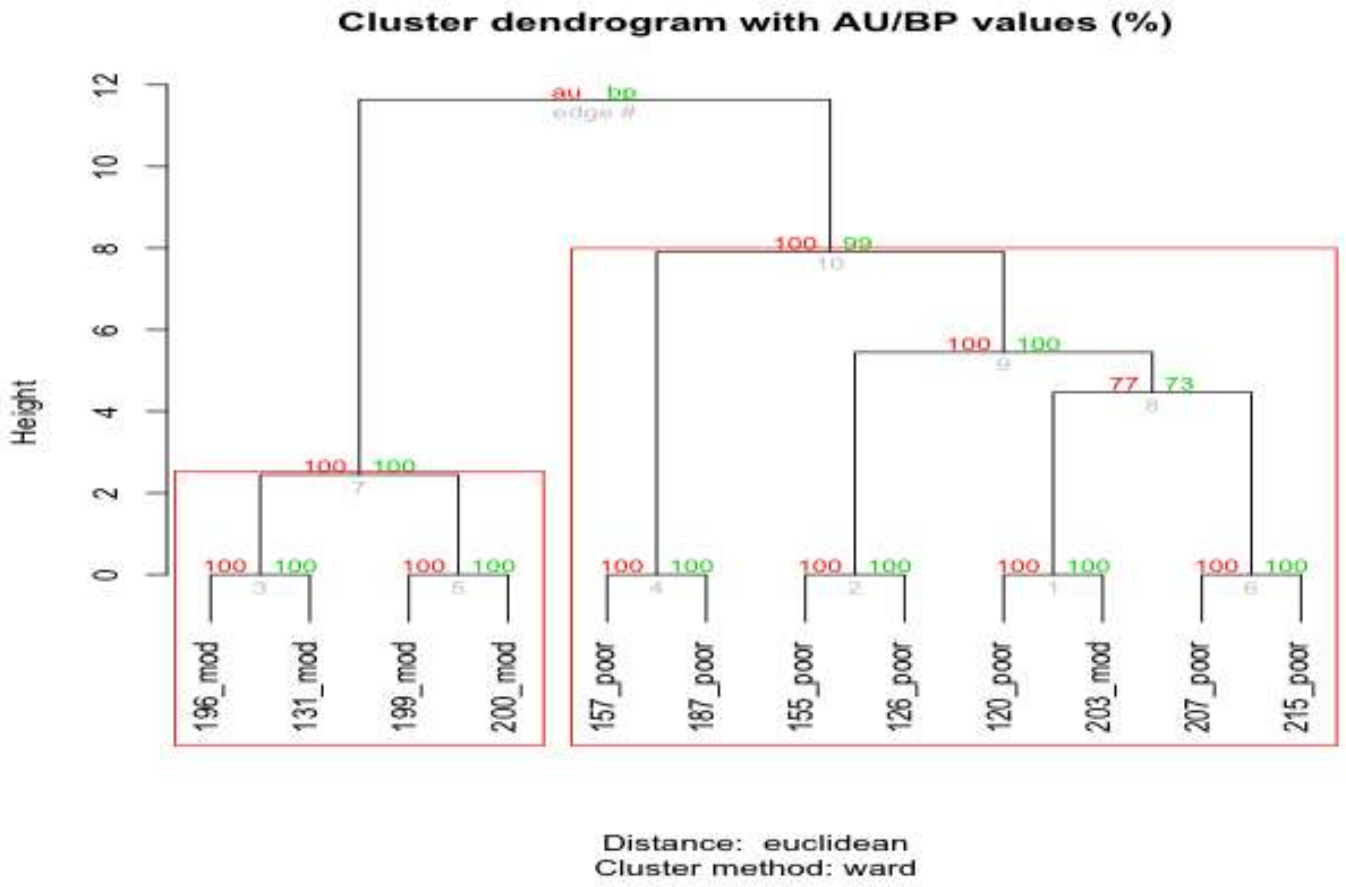
Applying PSP to a HCC Dataset



Consistency: Samples segregate by their classes with high confidence



In contrast, at the level of individual proteins, in mod-stage patients, only 25 out of over 800 proteins are common to all 5 patients. Of these 25, all are also reported in poor-stage patients. In poor-stage patients, 3 out of over 1000 proteins are common to all 7. Of these, 2 are reported in mod-stage patients.

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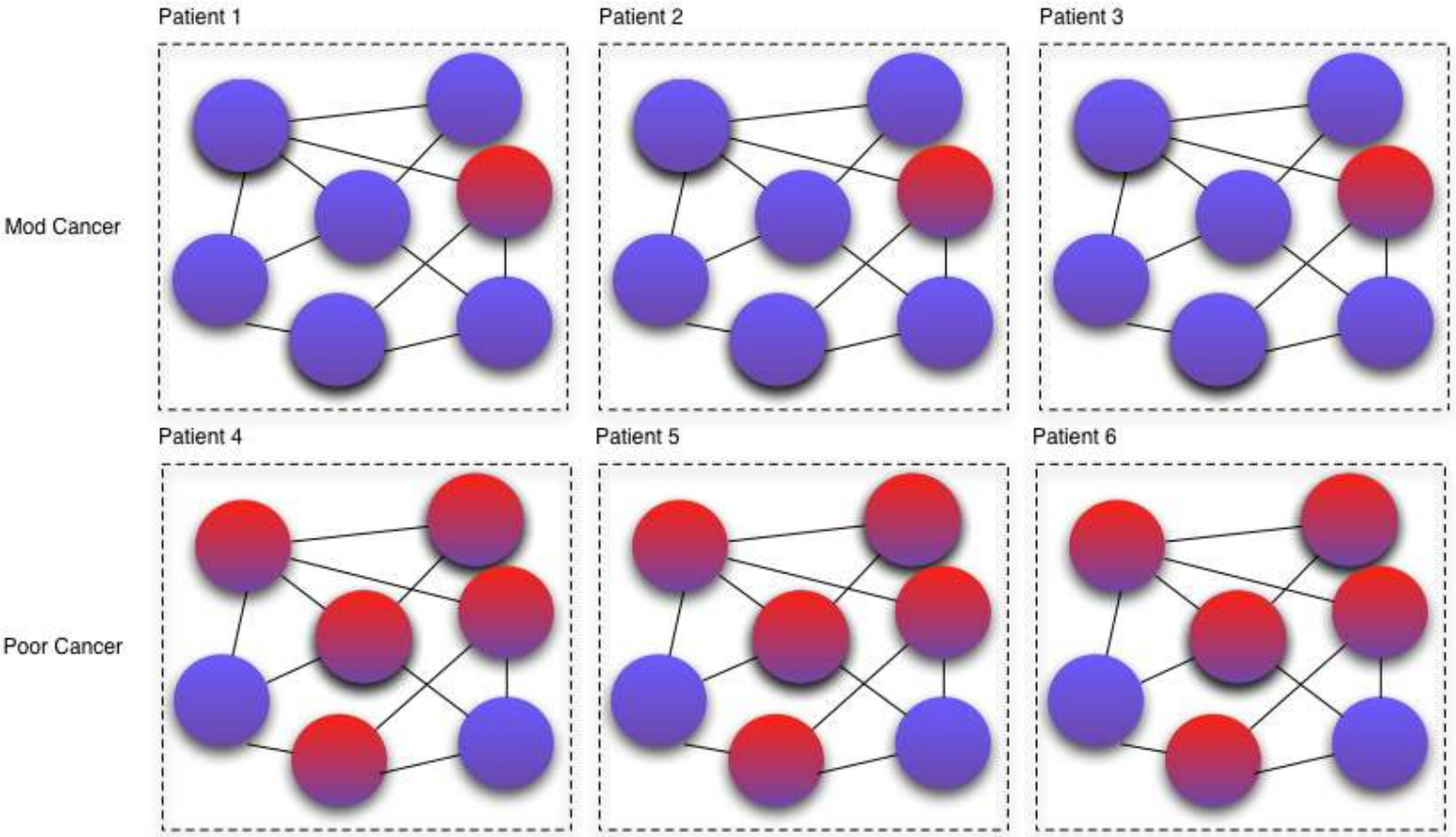
Feature Selection



$$t\_score = \frac{\bar{HA} - \bar{HB}}{S_{HA,HB}\sqrt{\frac{1}{n} + \frac{1}{m}}}$$

where

$$S_{HA,HB} = \sqrt{\frac{(m-1)S_{HA}^2 + (n-1)S_{HB}^2}{m+n-2}}$$

Top-Ranked Complexes

Cluster_ID	p_val	mod_score	poor_score	cluster_name
5179	0.000300541	0.513951977	3.159758312	NCOA6-DNA-PK-Ku-PARP1 complex
5235	0.000300541	0.513951977	3.159758312	WRN-Ku70-Ku80-PARP1 complex
1193	0.000300541	0.513951977	3.159758312	Rap1 complex
159	0	0	2.810927655	Condensin I-PARP-1-XRCC1 complex
2657	0.008815869	0	2.55616281	ESR1-CDK7-CCNH-MNAT1-MTA1-HDAC2 complex
3067	0.00911641	0	2.55616281	RNA polymerase II complex, incomplete (CDK8 complex), chromatin structure modifying
1226	0.013323983	0.715352108	2.420592827	H2AX complex I
5176	0	0.513951977	2.339059313	MGC1-DNA-PKcs-Ku complex
1189	0	0.513951977	2.339059313	DNA double-strand break end-joining complex
5251	0	0.513951977	2.339059313	Ku-ORC complex
2766	0	0.513951977	2.339059313	TERF2-RAP1 complex

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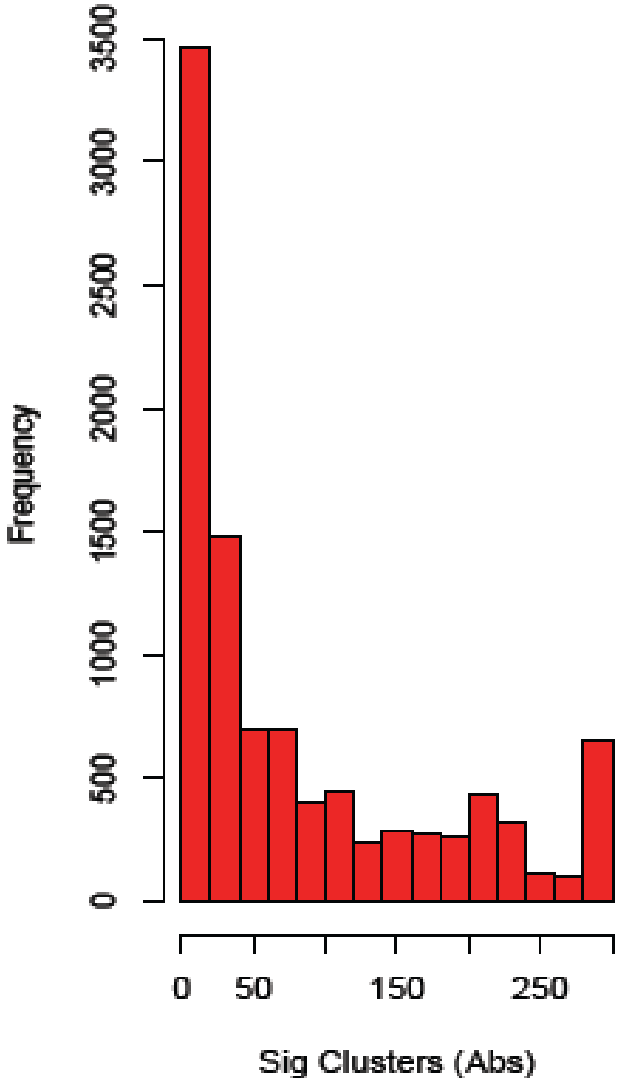
Top-Ranked GO Terms

GO ID	Description	No. of clusters
GO:0016032	viral reproduction	36
GO:0000398	nuclear mRNA splicing, via spliceosome	34
GO:0000278	mitotic cell cycle	28
GO:0000084	S phase of mitotic cell cycle	28
GO:0006366	transcription from RNA polymerase II promoter	26
GO:0006283	transcription-coupled nucleotide-excision repair	22
GO:0006369	termination of RNA polymerase II transcription	22
GO:0006284	base-excision repair	21
GO:0000086	G2/M transition of mitotic cell cycle	21
GO:0000079	regulation of cyclin-dependent protein kinase activity	20
GO:0010833	telomere maintenance via telomere lengthening	20
GO:0033044	regulation of chromosome organization	19
GO:0006200	ATP catabolic process	18
GO:0042475	odontogenesis of dentine-containing tooth	18
GO:0034138	toll-like receptor 3 signaling pathway	17
GO:0006915	apoptosis	17
GO:0006271	DNA strand elongation involved in DNA replication	17

Goh et al. **Enhancing utility of proteomics signature profiling (PSP) with pathway derived subnets (PDSs), performance analysis and specialized ontologies**. *BMC Genomcs,* 14:35, 2013.

False Positive Rate Analysis



- **Divide 7 poor patients into 2 groups**
  - Significant complexes produced by PSP here are false positives
- **Repeat many times to get dull distribution**
  - Median = 40, mode = 6
- **Cf. 523 complexes in CORUM (size ≥4) used in PSP. At p ≤ 5%, 523 * 5% ≈ 27 false positives expected**

Improving Coverage in Proteomic Profile Analysis

FCS

- **Rescue undetected proteins from high-scoring protein complexes produced by e.g. FCS**

- **Why?**

  Let A, B, C, D and E be the 5 proteins that function as a complex and thus are normally correlated in their expression. Suppose only A is not detected and all of B–E are detected. Suppose the screen has 50% reliability. Then, A's chance of being false negative is 50%, & the chance of B–E all being false positives is $(50\%)^4$=6%. Hence, it is almost 10x more likely that A is false negative than B–E all being false positives.

- **Shortcoming: Databases of known complexes are still small**

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

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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. **A network-based maximum link approach towards MS identifies potentially important roles for undetected ARRB1/2 and ACTB in liver cancer progression**. *IJBRA*, 8(3/4):155--170, 2012.

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MaxLink

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

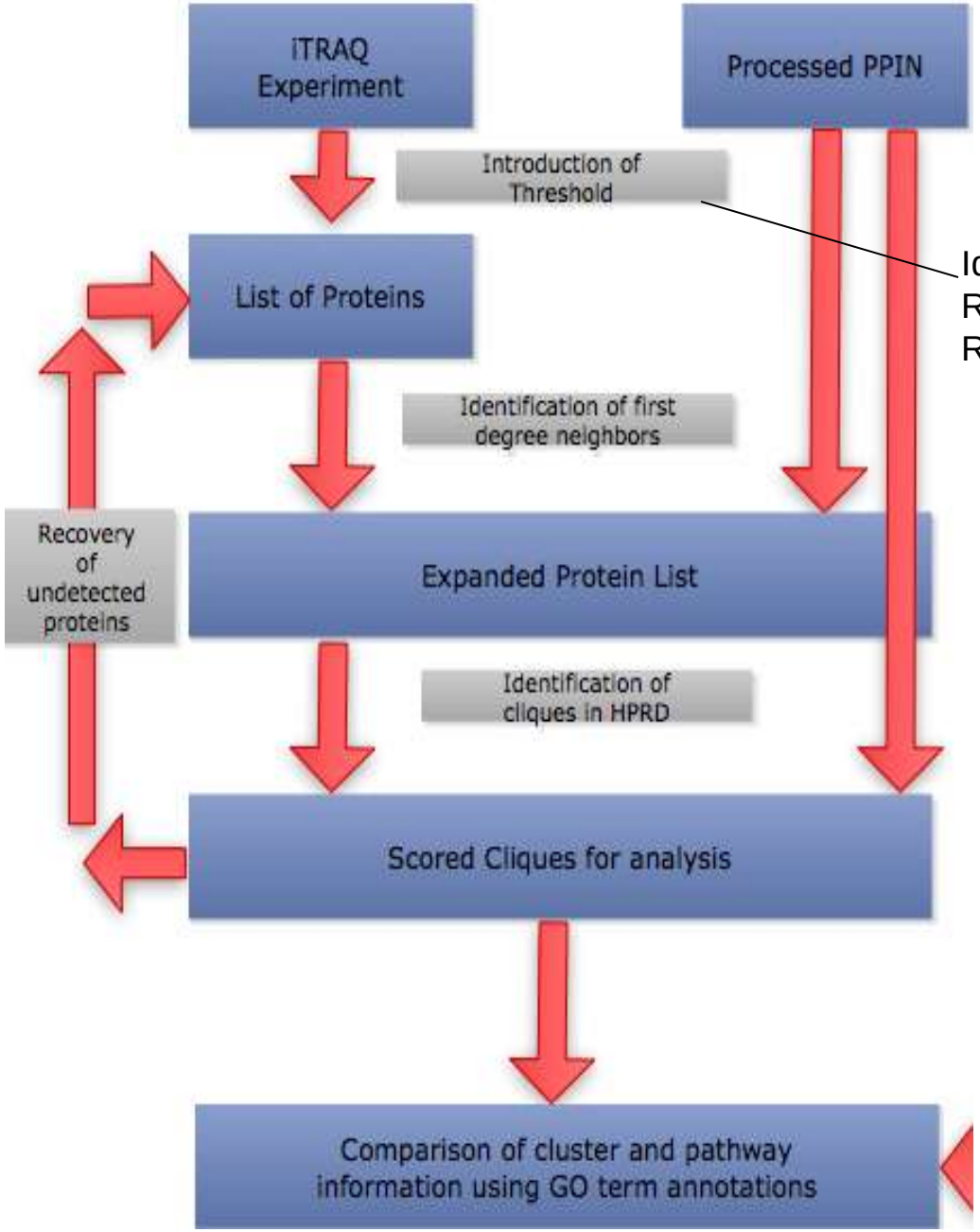
- **Reason: Proteins interacting with many seeds are likely to be part of the same complex as these seeds**

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

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

An Experiment: PEP

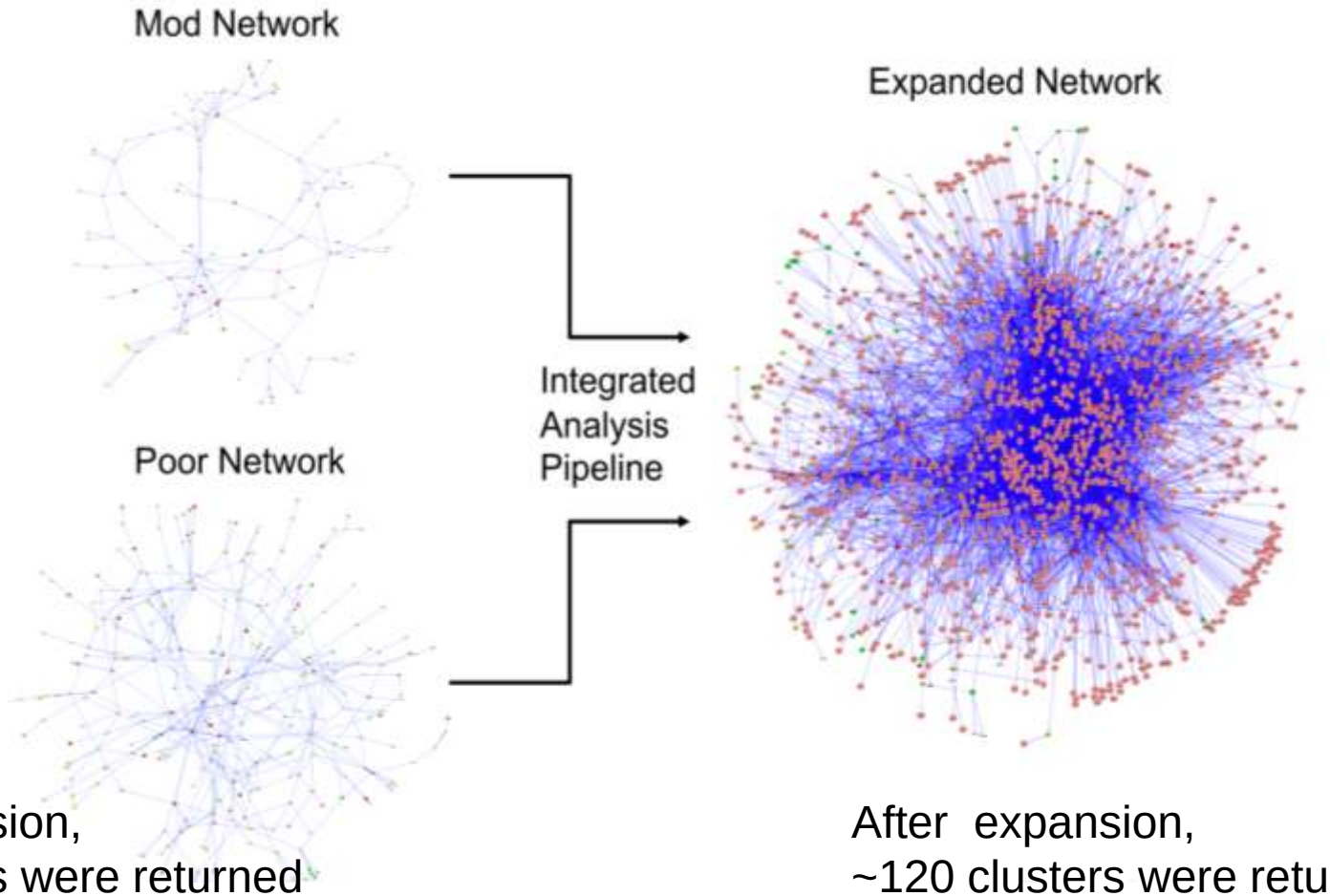
- **HCC (Hepatocellular carcinoma)**
  - Classified into 3 phases: differentiated, moderately differentiated and poorly differentiated
- **Mass Spectrometry**
  - iTRAQ (Isobaric Tag for Relative and Absolute Quantitation)
  - Coupled with 2D LC MS/MS
  - Popular because of ability to run 8 concurrent samples in one go



Identify the “seeds”
Ratio < 0.8 and > 1.25 for Mod (min 3 patients)
Ratio < 0.8 and > 1.25 for Poor (min 4 patients)

PEP Workflow

Expansion to include neighbors greatly improves coverage



W/o expansion,
4 k3 cliques were returned

After expansion,
~120 clusters were returned

"Validation" of Rescued Proteins

- **Direct validation**
  - Use the original mass spectra to verify the quality of the corresponding y- and b-ion assignments
  - Immunological assay, etc.

- **Indirect validation**
  - Check whether recovered proteins have GO terms that are enriched in the list of seeds
  - Check whether recovered proteins show a pattern of differential expression betw disease vs normal samples that is similar to that shown by the seeds

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Returning to Mass Spectra

- **Test set: Several proteins (ACTR2, CDC42, GNB2L1, KIF5B, PPP2R1A, PKACA and TOP1) from top 34 clusters not detected by Paragon**

- **The test: Examine their GPS and Mascot search results and their MS/MS-to-peptide assignments**

- **Assessment of MS/MS spectra of their top ranked peptides revealed accurate y- and b-ion assignments and were of good quality ($p < 0.05$)**

$\Rightarrow$ **In silico expansion verified**

Another Experiment: Comparison

- **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**
  - 396 proteins identified
- **MS was scanned against UniProtkb in round #2**
  - 393 additional proteins identified

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

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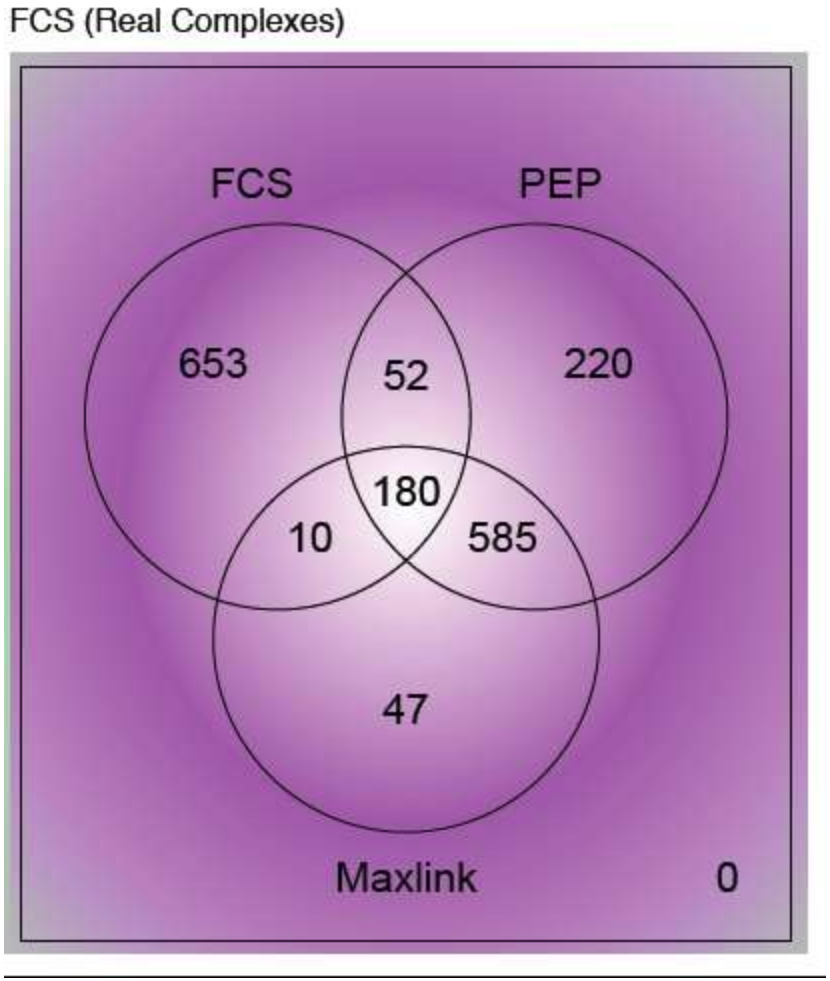
Moderate level of agreement of reported proteins between various recovery methods



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Performance Comparison

Method	Novel Suggested Proteins	Recovered proteins
PEP	375	158
Maxlink	910	226
FCS (predicted)	678	224
FCS (complexes)	789	775

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

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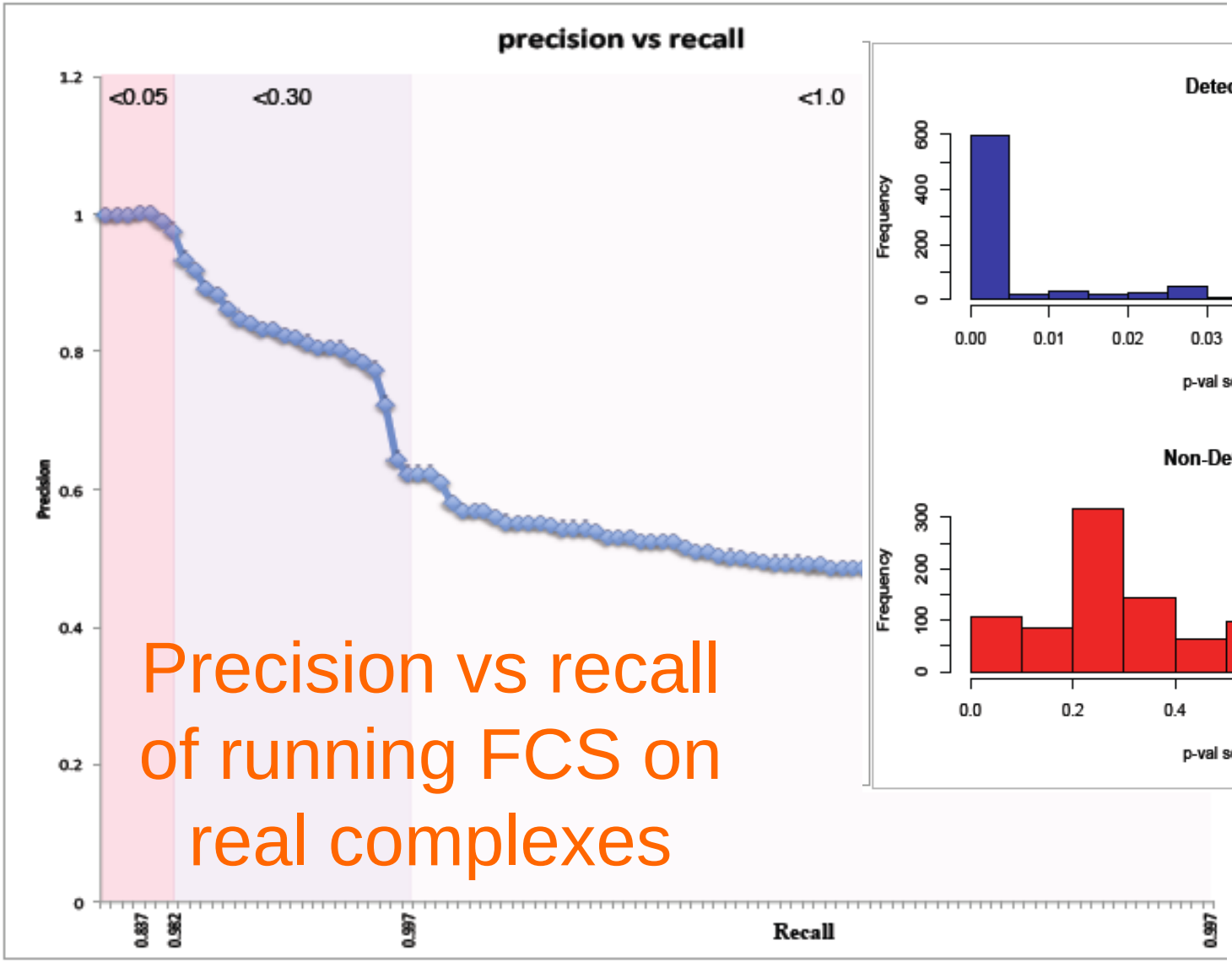

Precision vs recall of running FCS on real complexes

Remarks

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What have we learned?

- **Contextualization (into complexes and pathways) can deal with consistency issues in proteomics**
- **GO term analysis also indicates that context-based methods select clusters that play integral roles in cancer**
- **Context-based methods reveal many potential clusters and are not constrained by any prior arbitrary filtering which is a common first step in conventional analytical approaches**

Acknowledgements & References

- This talk is based on joint work with

Wilson Goh

[PSP] Goh et al**. Proteomics signature profiling (PSP): A novel contextualization approach for cancer proteomics**. *Journal of Proteome Research,*11(3):1571--1581, 2012.

[PEP] 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

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