A High-Throughput Information Added Proteomic Strategy Using Free Flow Electrophoresis and Tandem Mass Spectrometry

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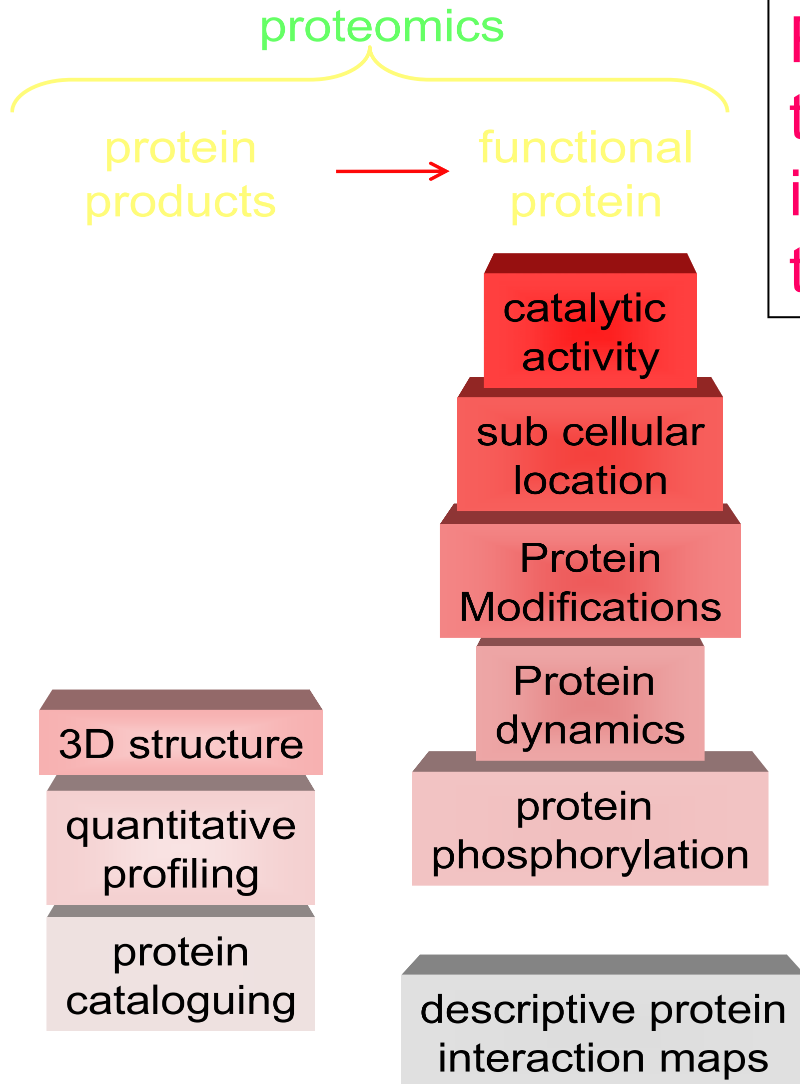
Outline

I. Introduction – Proteomics and New Strategy

II. High-throughput Information-added Proteomic Strategy (IAPS) Using Free Flow Electrophoresis (FFE) and Tandem Mass Spectrometry (MS/MS)

1. **Development - Using Yeast Nuclear Proteome**
2. **Full Evaluation - Using Whole Yeast Proteome**
3. **Application - Cataloging Whole Human Salivary Proteome**
4. **Conclusion**

I. Introduction - *Emergence of Proteomics*



Revolutionized our ability to study the collective properties of proteins inherent to function of cell and tissue on a system-wide scale.

Dynamic protein expression upon perturbation such as diseases, drugs and other environmental changes

Has great potential for identifying biomarkers of cancers and other diseases, and shows significant importance in drug discovery.

I. Introduction - *Proteomic Strategies*

Challenge to express proteins in complex biological systems

Shotgun Proteomics History:

- 2D gel + MS or MS/MS + Database Searching
- 2D HPLC + MS/MS + Database Searching

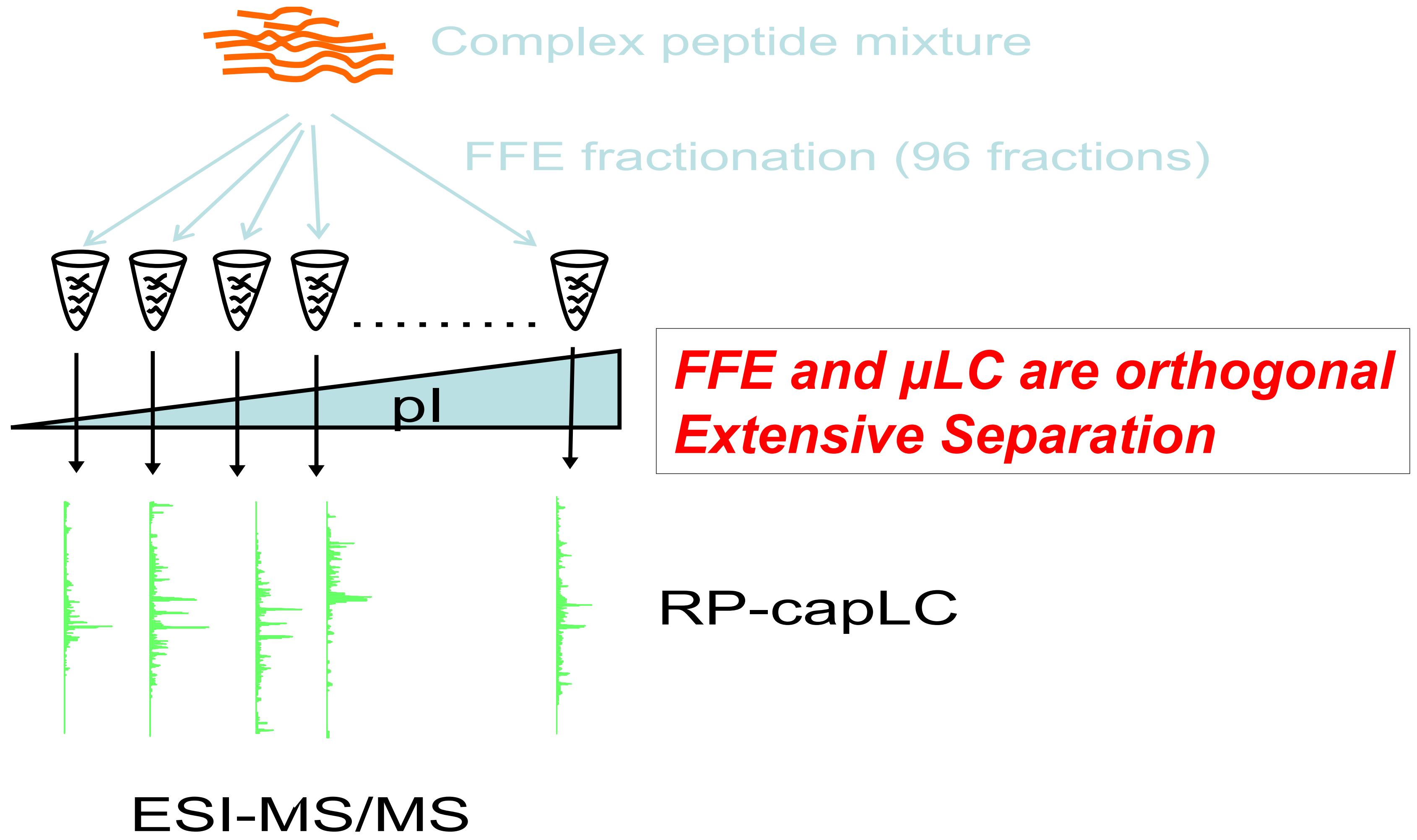
Separation (Fractionating ability) +
Identification (Database Searching Scores)

Our Strategy:

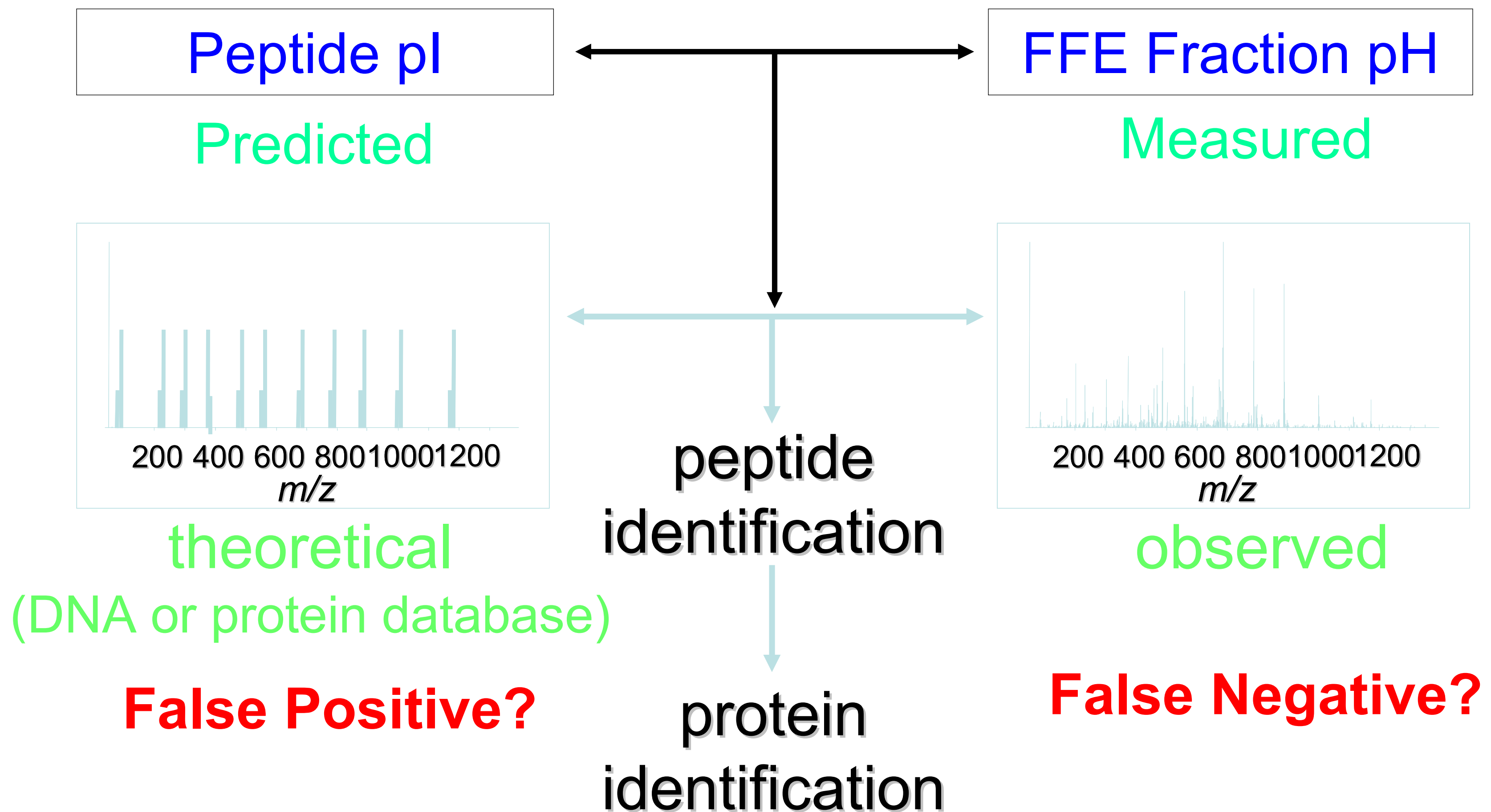
FFE + HPLC + MS/MS + Database Searching

(Extensive Separation) **(pI filter + Database Searching Scores)**

I. Introduction - *The Separation Role of FFE*



I. Introduction - *The Identification Role of FFE*

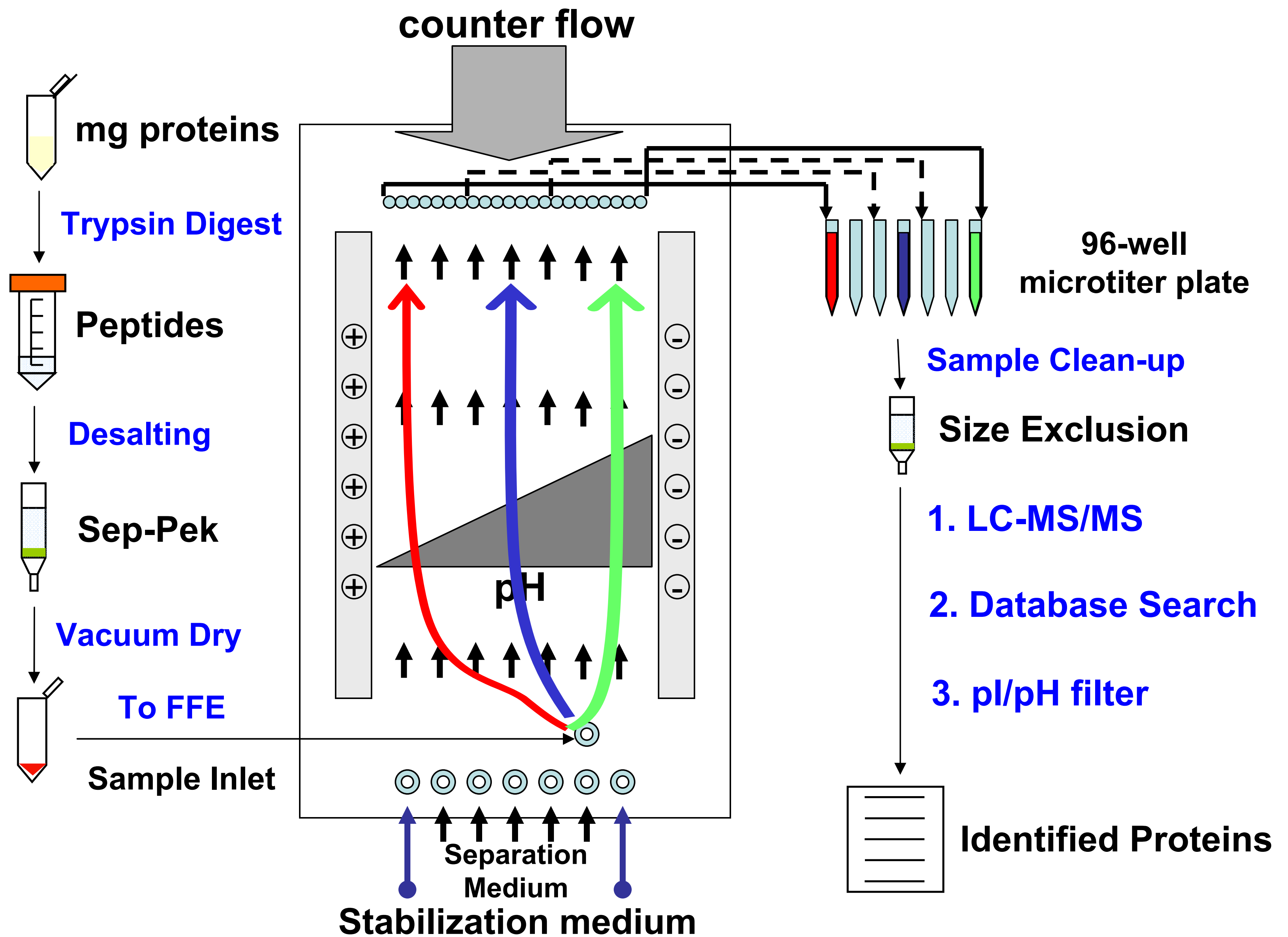


Increased Confidence for Peptide and Protein Identification

I. Introduction - *Why FFE ?*

1. Excellent fractionating ability;
2. The introduced peptide pI and pH are powerful in protein identification;
3. High-throughput;
4. Gel-free;
5. Large loading capacities and flexible loading volume;
6. High level of reproducibility

More proteins could be identified with high confidence using FFE coupled with tandem mass spectrometry and database searching



Scheme of the IAPS Proteomic Strategy

II. IAPS - *Peptide pI Prediction and pI/pH Filter*

- **pI Prediction**

  At isoelectric-point, pI = pH; where the net charge Z of peptide equals to 0 and

$$Z = \Sigma(n_i/(1 + K_i/[H^+])) – \Sigma(n_j/(1 + [H^+]/k_j))$$

K_i is the acid dissociation constant (k_a) for the conjugate acid of the basic group and k_j is that for the acidic group and n_i and n_j denote the number of such ionizable groups in a particular peptide

- **pI/pH Filter**

$$pH – \Delta pH \leq pI \leq pH + \Delta pH$$

A peptide is accepted If its pI is in the range of ±ΔpH

II.1 Development of IAPS

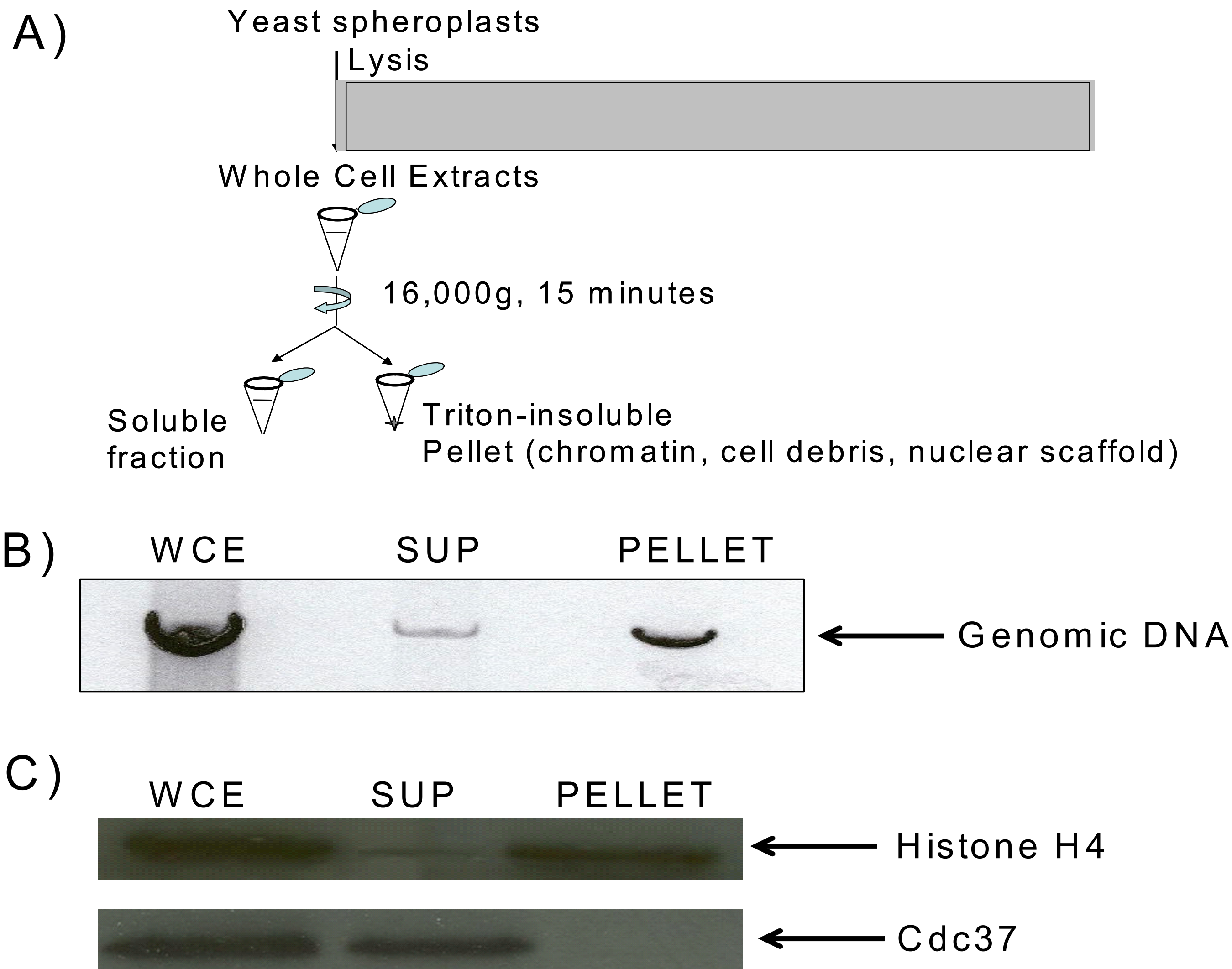
Chose yeast nuclear proteome to demonstrate the effectiveness of FFE fractionating ability and validate the use of peptide pI coupling with probability (P) score.

1. Using in-silico false positive rate analysis via reverse database searching;

   False positive % = $[2n_{reverse}/(n_{forward} + n_{reverse})] \times 100$

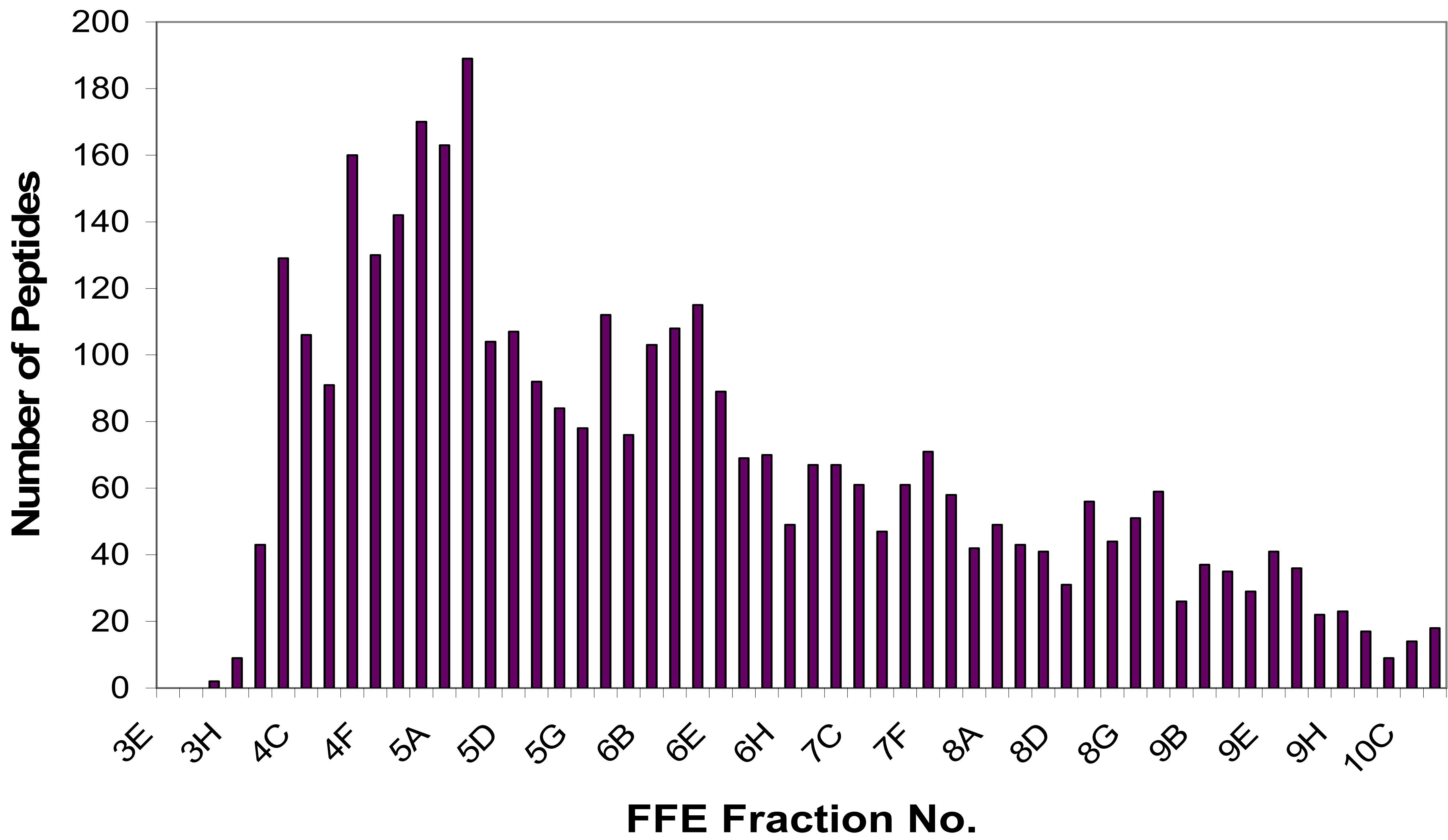
2. Protein subcellular localization information;
3. Biochemical detection of selected proteins by West-bolting test.

II.1 Development - *Separation of Yeast Nuclear Proteins*

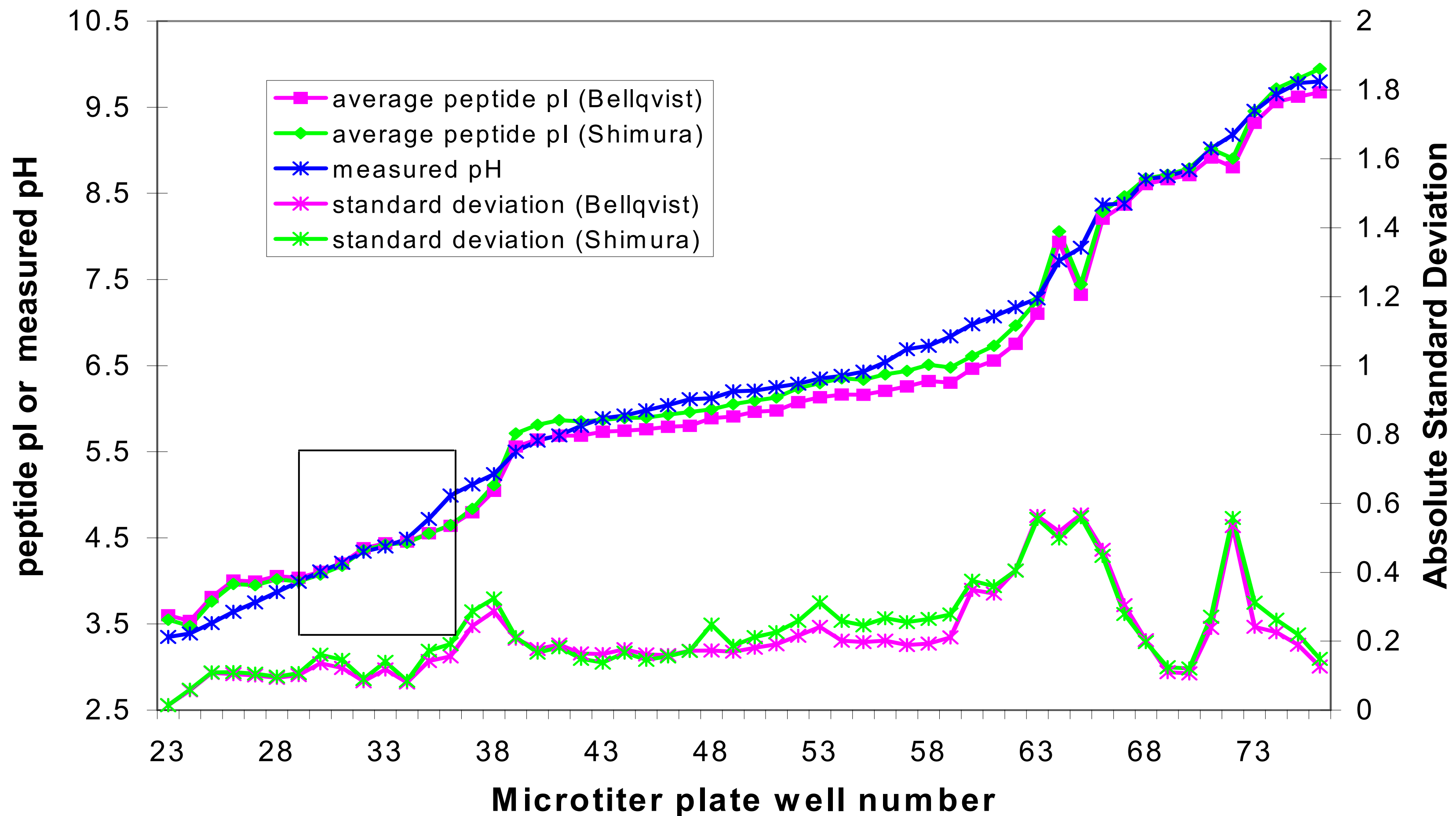


(A) Procedure for crude isolation of chromatin and associated proteins from yeast. (B) Presence of genomic DNA in whole cell extract (WCE), supernatant (SUP) containing the soluble protein fraction, and the insoluble pellet fraction (PEL). (C) Equivalent amounts of protein from whole cell extracts, supernatant, and the pellet fraction were separated by SDS-PAGE, transfer onto membrane, and analyzed by immunoblotting for chromatin-associated protein, HistoneH4p, and xytoplasmic protein, Cdc37p.

II.1 Development - *Peptide Distribution in FFE fractions with P ≥ 0.9*

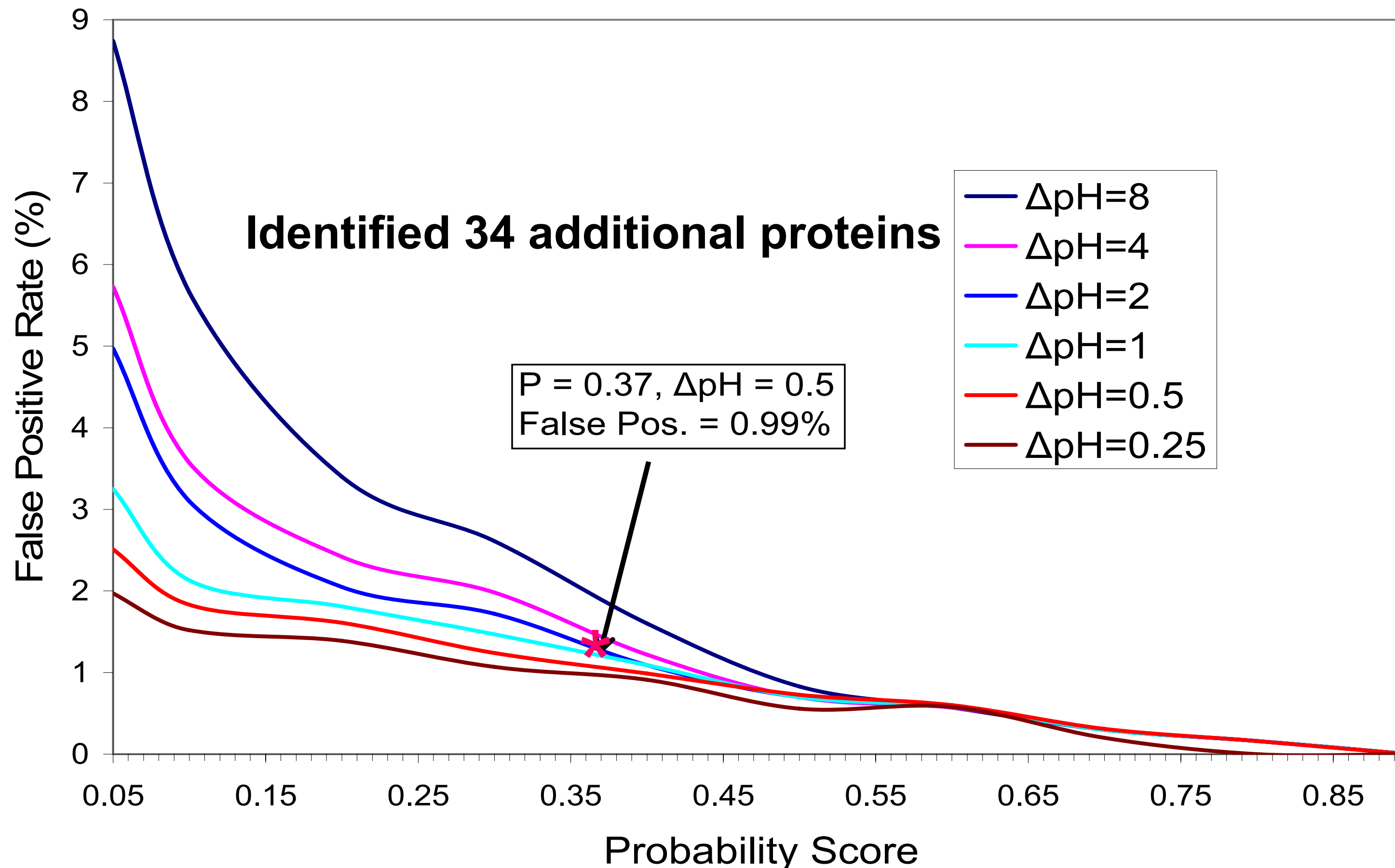


II.1 Development - *Correspondence of Measured pH and Calculated Average pI*



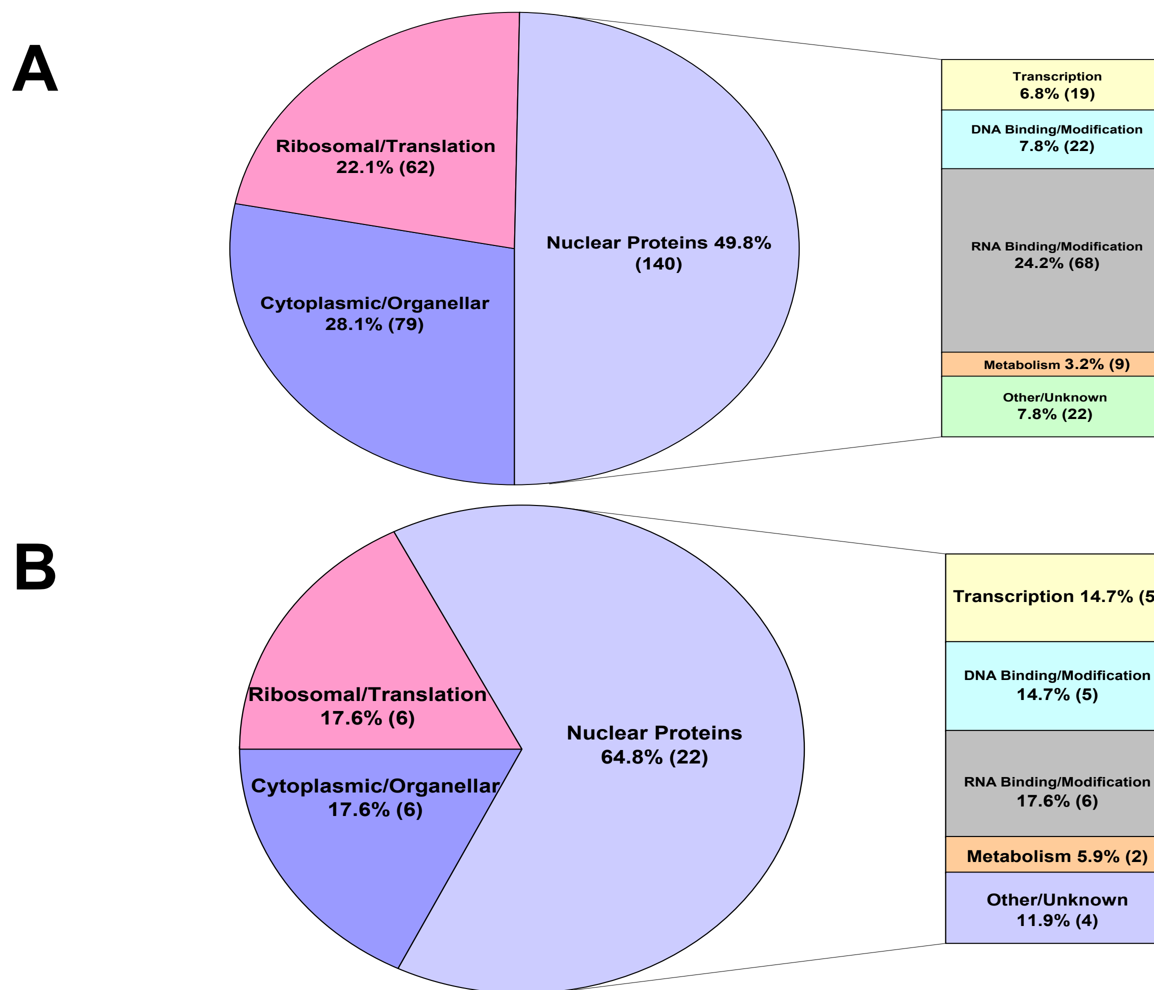
Plot of measured pH Value from each microtiter plate well versus average calculated pI of identified peptide sequences for two different pI prediction algorithms (Bellqvist[6] or Shimura[5]).

II.1 Development - *Effects of pI/pH in Protein Identification (Reducing False Positive Rate)*

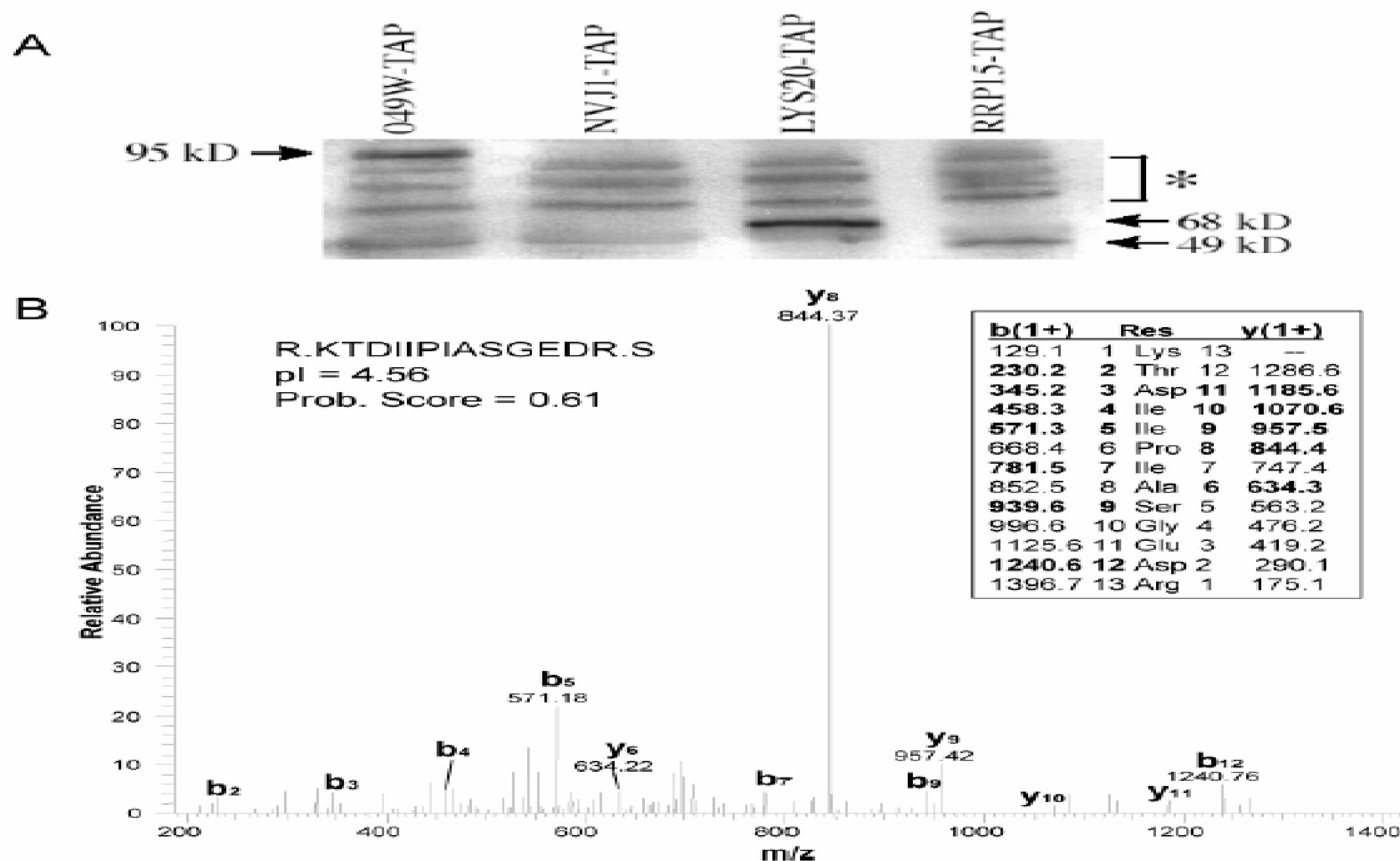


False positive rate versus probability score and the effect of peptide pI filtering. Data was from 8 acidic factions 29-36 (pH = 4~5) with most abundant peptides.

II.1 Development - *Effects of pI/pH in Protein Identification (Identified More Proteins with High Confidence)*



Subcellular distribution of proteins identified from 8 acidic fractions (A) at P>0.9; (B) additional 34 proteins at P ≥ 0.37 and ΔpH = ±0.5.



b(1+)	Res		y(1+)
129.1	1	Lys 13	--
230.2	**2**	Thr 12	1286.6
345.2	**3**	Asp **11**	**1185.6**
458.3	**4**	Ile **10**	**1070.6**
571.3	**5**	Ile **9**	**957.5**
668.4	6	Pro **8**	**844.4**
781.5	**7**	Ile 7	747.4
852.5	8	Ala **6**	**634.3**
939.6	**9**	Ser 5	563.2
996.6	10	Gly 4	476.2
1125.6	11	Glu 3	419.2
1240.6	**12**	Asp 2	290.1
1396.7	13	Arg 1	175.1

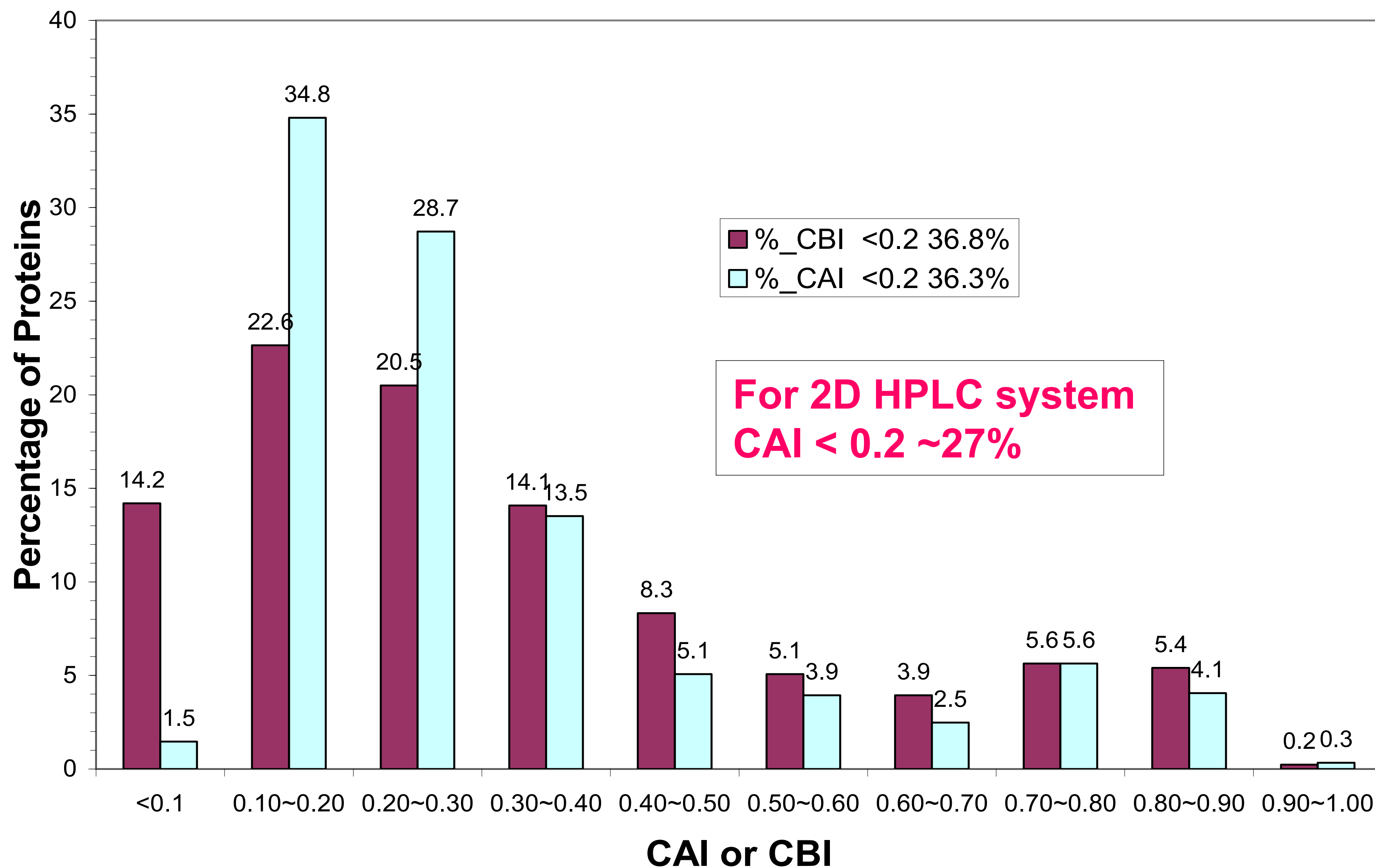
(A) Immunodetection of TAP-tagged versions of four different proteins,Yer049Wp, Nvj1p, Lys20p and Rrp15p, that had been identified by a single, partially tryptic peptide at P ≥ 0.37 and ΔpH = ±0.5. With the exception of Nvj1-TAP, remaining proteins were identified by presence of bands at expected molecular weights as indicated by arrowheads. (* denotes nonspecific background bands.) (B) Representative MS/MS spectrum of partially tryptic peptide derived from the protein Rrp15. The single-charged y and b ions detected are indicated in bold in the ion list and the corresponding peaks are labeled in the spectrum.

II. 2 Evaluation of IAPS

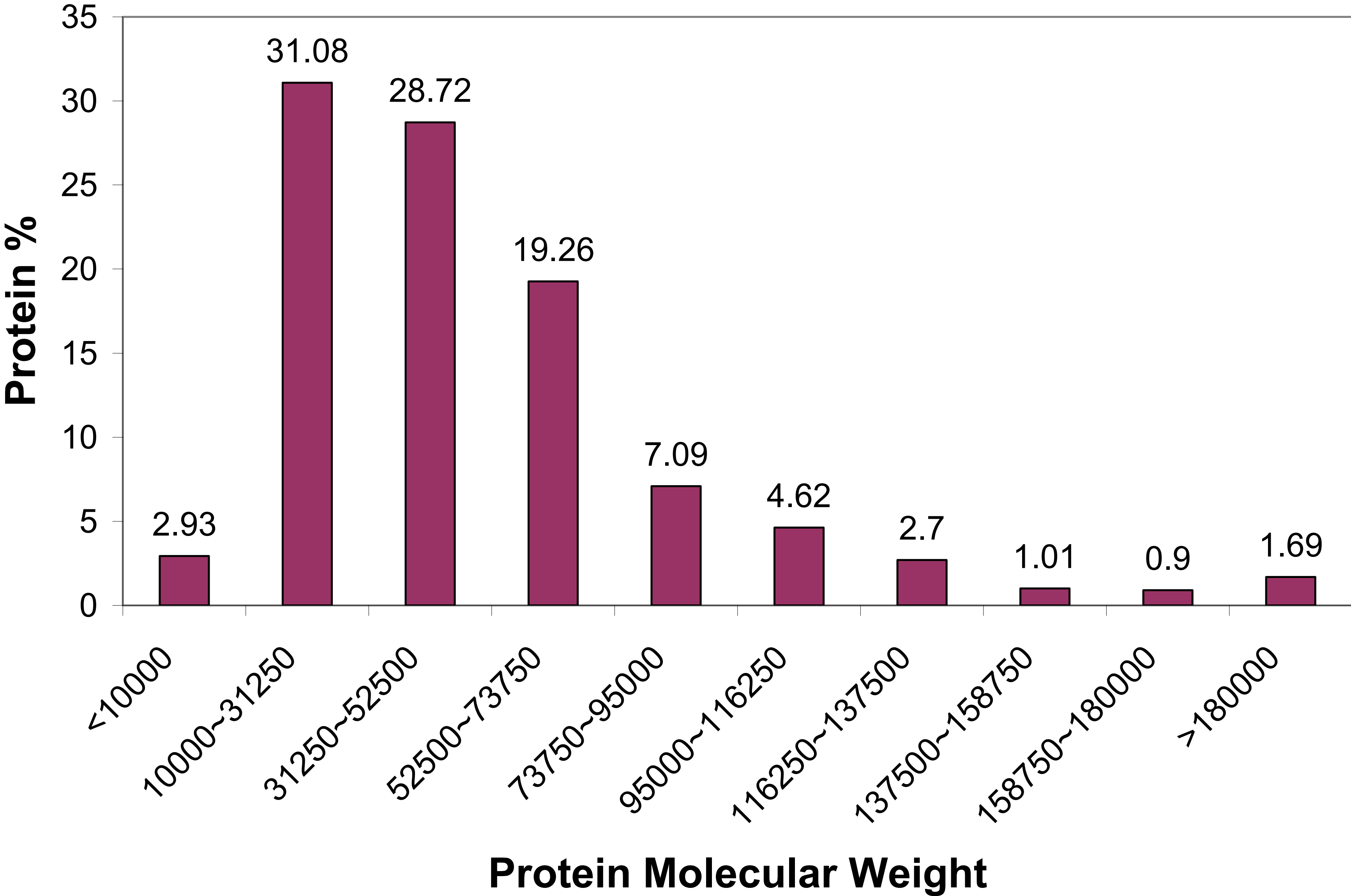
Using whole yeast lysate to evaluate the IAPS strategy because yeast proteome is well annotated and easily compare with other proteomic strategies.

1. How many proteins can be identified from whole yeast lysate?
   > 1500 proteins with high confidence **(false positive%<1%)**
2. Biased to certain classes of proteins?
   Unbiased to *low abundance*, *high/low molecular weight*, *high/low pI*, and *membrane-associated* proteins
3. Compatible to reagents for quantification?
   Yes. Compatible to iCAT, PIC and iTRAQ reagents
4. Excellent for identifying post-translational proteins?
   Under investigation - phosphorylation, acelylation, oxidation, etc.

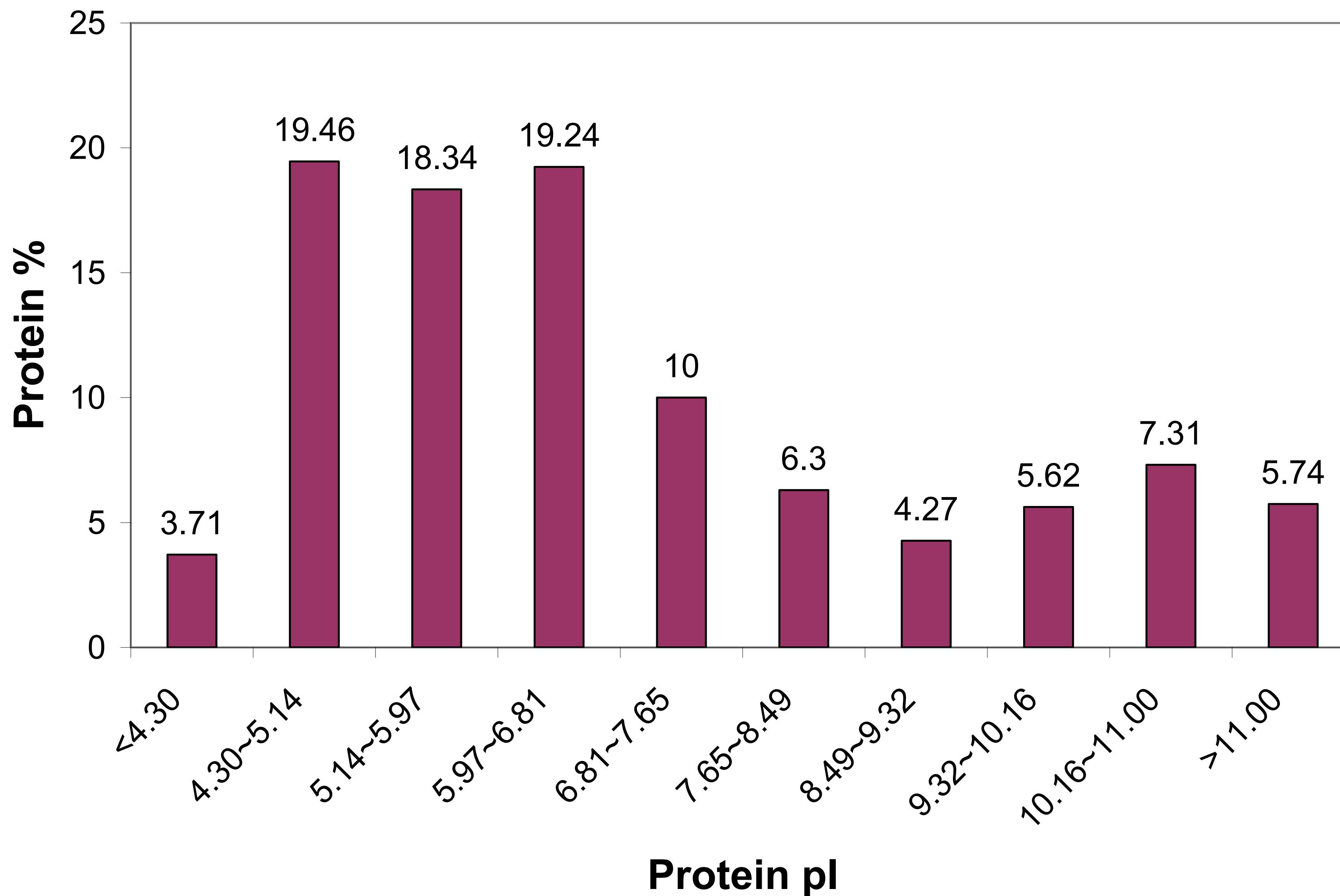
II.2 Evaluation - *CBI/CAI Distribution*



II.2 Evaluation - *Molecular Weight Distribution*



II.2 Evaluation - *Protein pI Distribution*



II.3 Application - *Human Salivary Proteome*

- Potential Clinic Fluids for Oral Cancer and other Diseases
- Oral Cancer is 6th largest, >30,000 cases per year in USA
- Challenge for completely protein expression - large dynamic range

  2D gel + MS or MS/MS methods -- less than 100 proteins

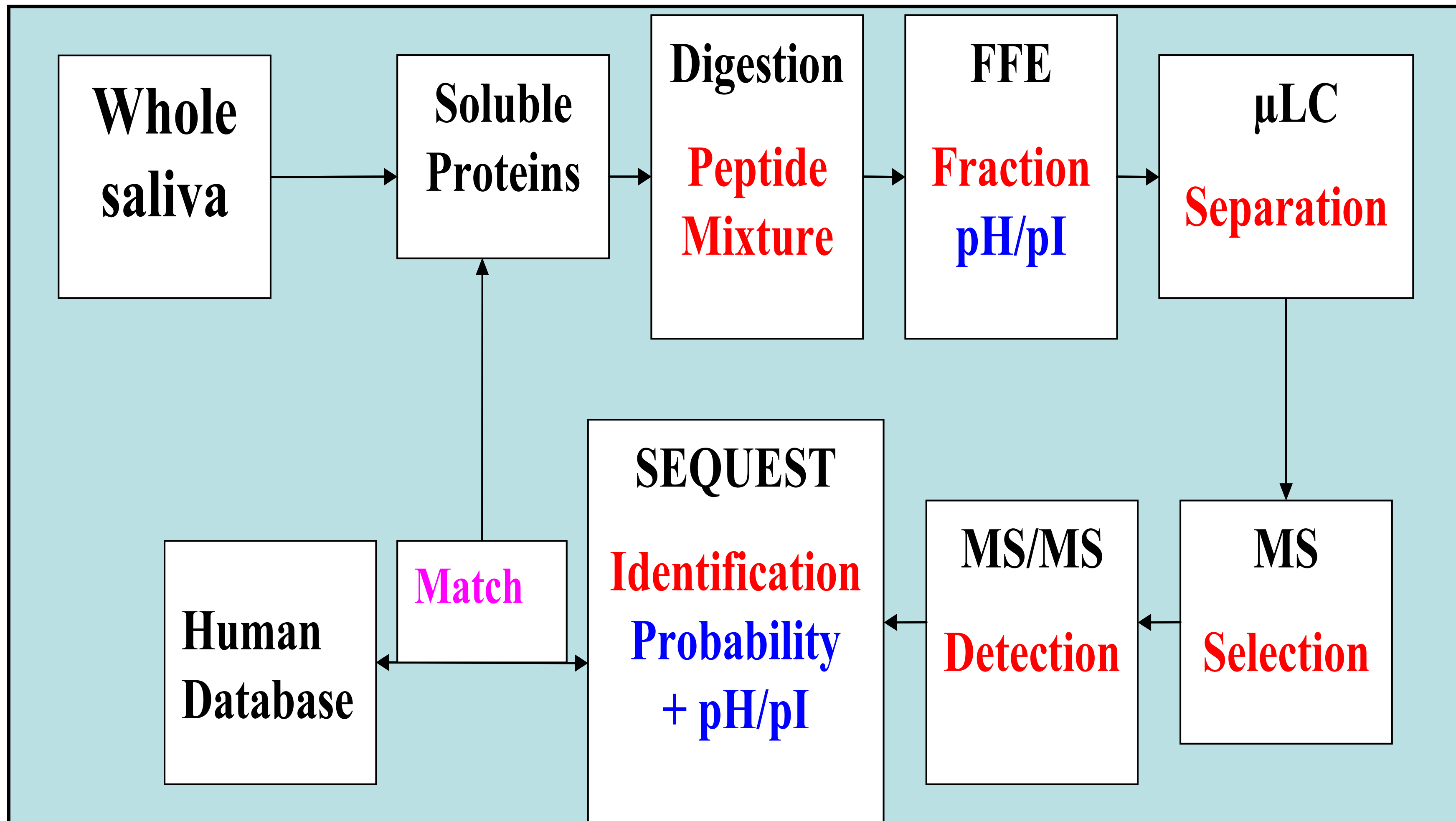
  2D HPLC + MS/MS -- 102 proteins with high confidence

Our Strategy :

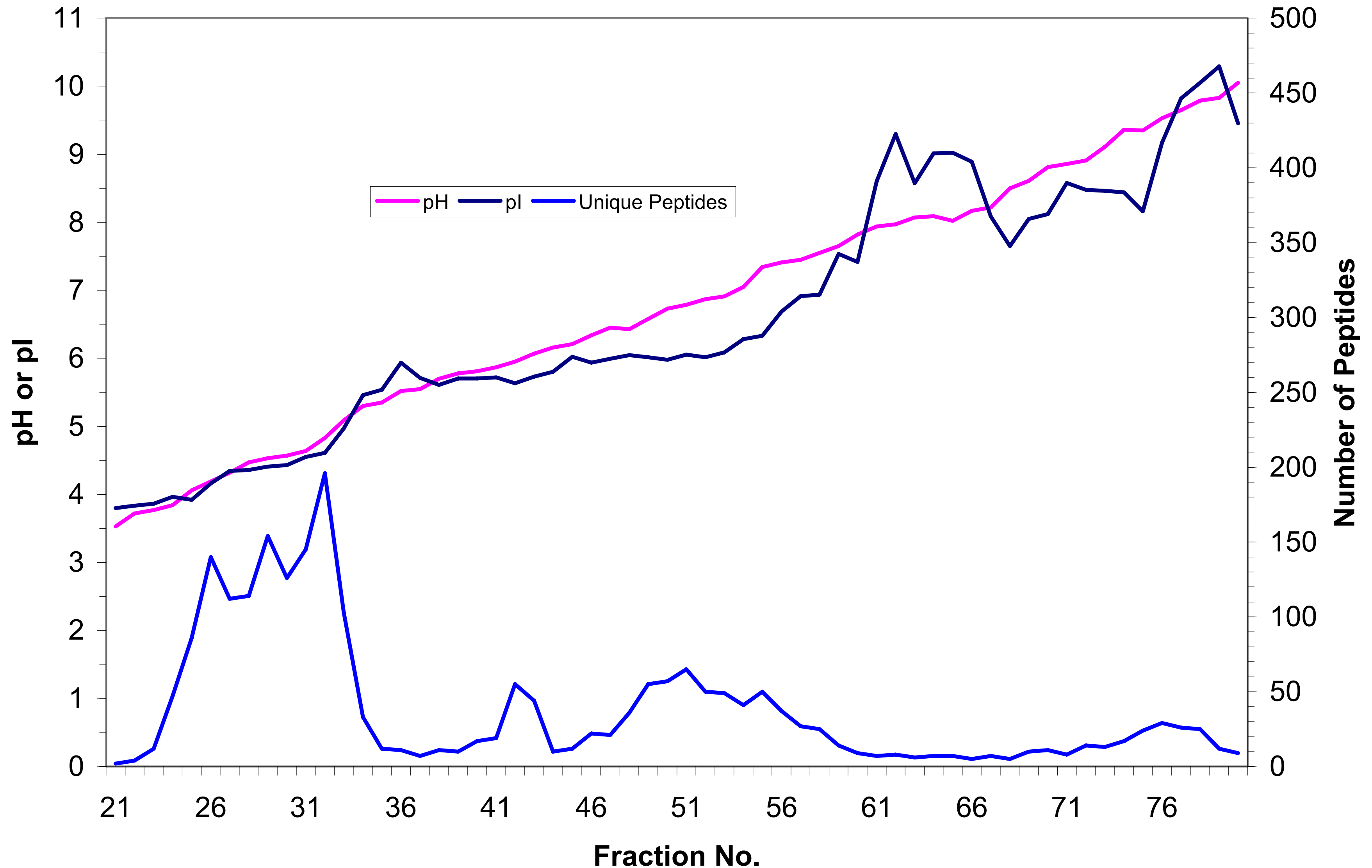
437 proteins with high confidence (false% ≤ 1%)

diverse in their most likely subcellular localization and in their most likely biochemical functions.

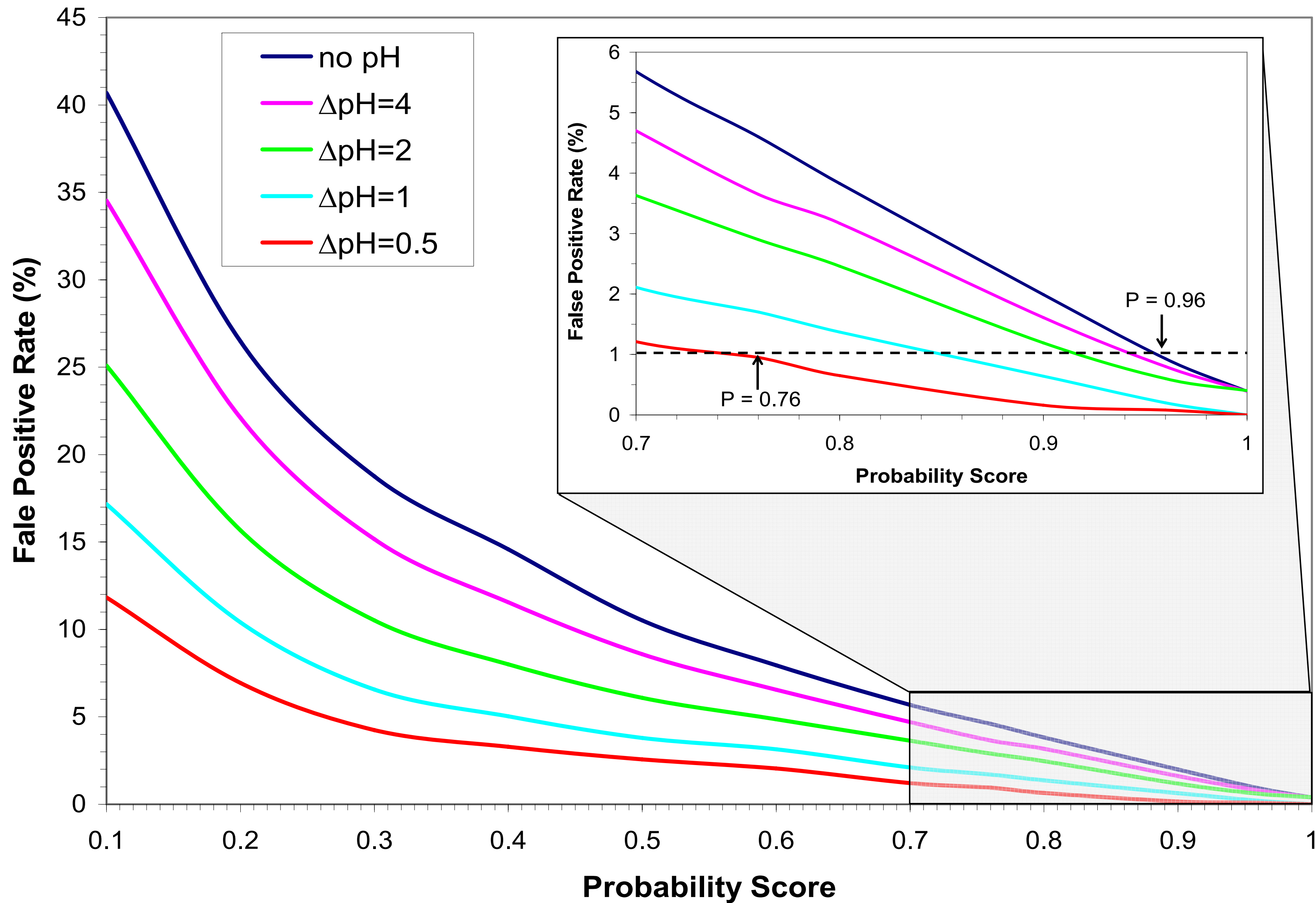
II.3 Application - Experimental Procedure



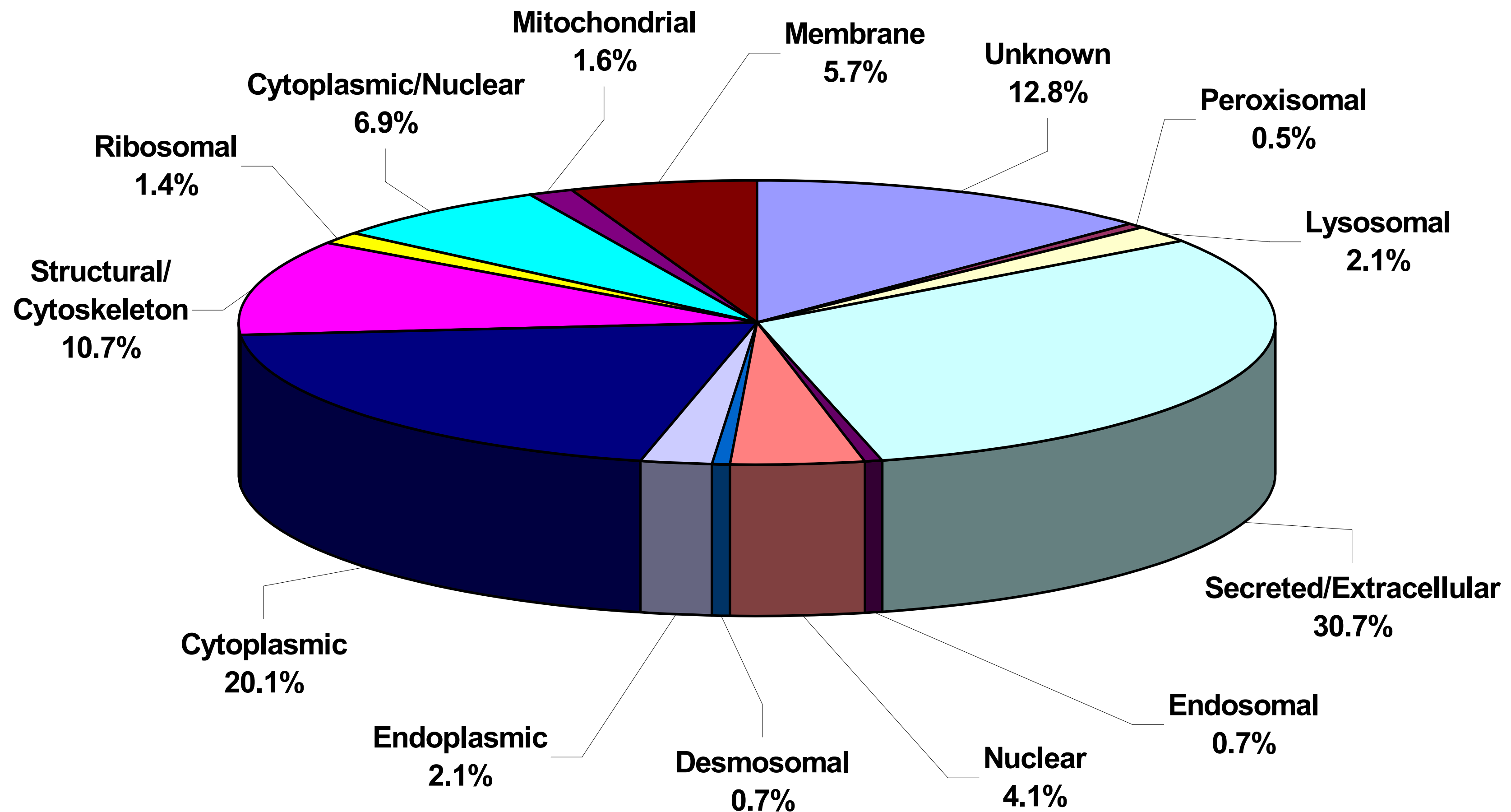
II.3 Application - *pI/pH and Peptide Distribution*



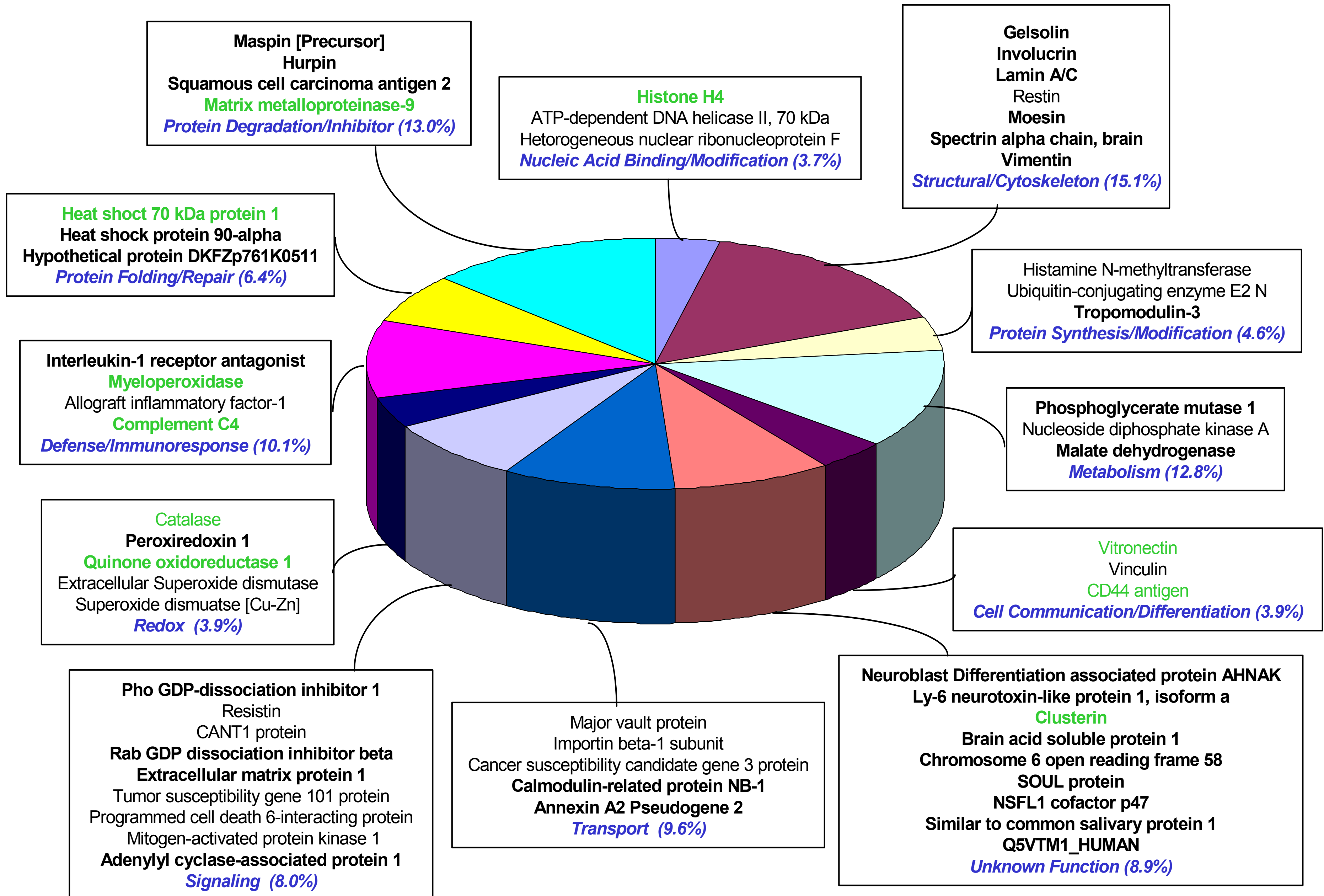
II.3 Application - *Effects* of *pI/pH filter*



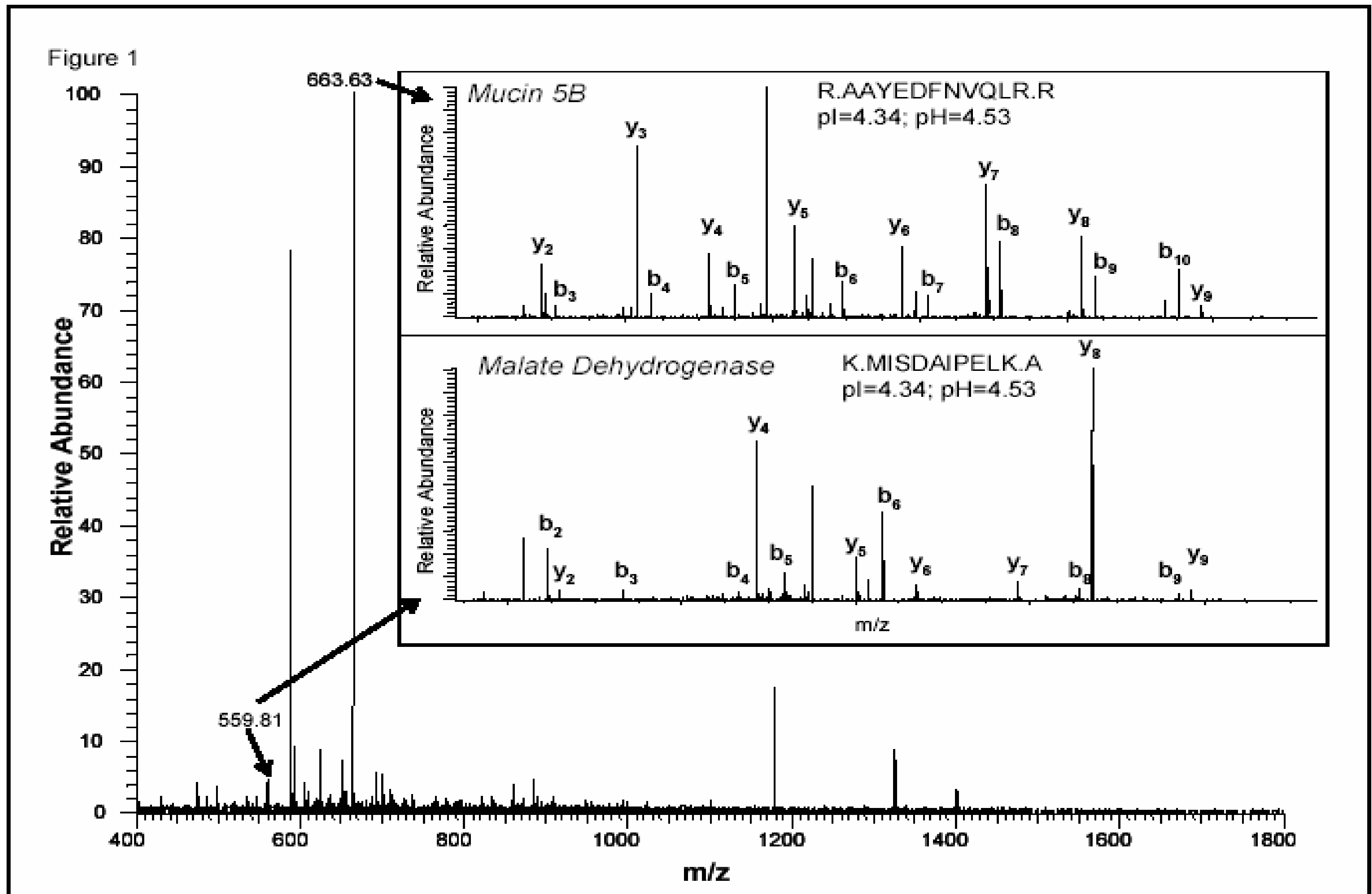
II. 3 Application - *Subcellular Distribution*



II. 3 Application - *Functional Distribution*



II. 3 Application - *Example of High Sensitivity*



II. 4 *Conclusions*

- IAPS strategy takes both advantages of extensively fractionating ability and introduced pI/pH information from FFE. It's high sensitive, high confident, high-throughput and unbiased to any class of proteins;
- The introduced pI and pH information is powerful in protein identification, which minimizes both false positive and false negative, more proteins can be identified with high confidence;
- The strategy is effective for profiling proteins from complex biological resources such as Human saliva, demonstrating it's a general and powerful tool for mass spectrometry based proteomics;
- The strategy is friendly to chemical reagents for quantitative analysis such as iCAT, PIC and iTRAQ reagents.

Challenges: FFE Resolution (± 0.5 pH unit?); pI prediction in pH=6.5~8; pI shift on post-translational modification (PTM); Automation

Acknowledgements

Dr. Griffin's Lab

Dr. Timothy J. Griffin

Dr. Sri Bandhakavi

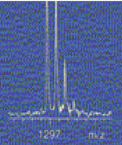
Mikel Roe

Dr. Nelson Rhodus
Dental School

Dr. Robert J. Griffin
Therapeutic Radiology-Radiation Oncology

Dr. John V. Carlis
Computer Science and Engineering

Mass Spectrometry
Consortium for the Life Sciences



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Funding
American Cancer Society
Minnesota Medical Foundation