

Proteomics Course

LECTURE-19 Applications of 2-DE and DIGE



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Lecture outline

- Case studies 2DE:
 - Drug treatment in malaria parasite
 - Plasma proteome analysis of SARS
- An overview of DIGE technique
- Case study DIGE:
 - Serum proteome analysis of prostate cancer

Case study-1

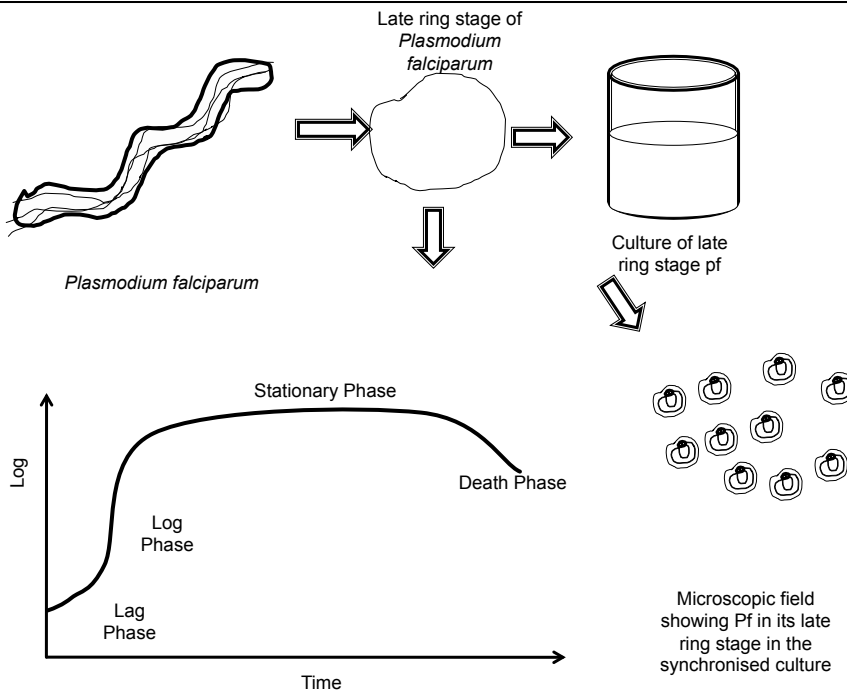
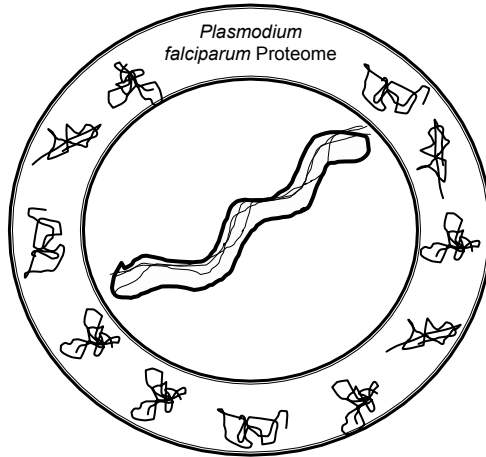
Towards a proteomic definition of CoArtem action in *Plasmodium falciparum* malaria

Makanga et al. Towards a proteomic definition of CoArtem action in Plasmodium falciparum malaria. Proteomics. 2005, 5, 1849

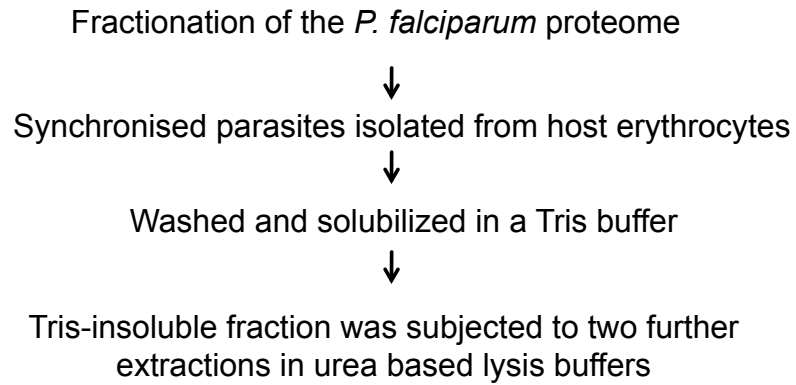
CoArtem action in *P. falciparum* malaria

- CoArtem is a combination of artemisinin-derivative artemether with lumefantrine
- Drug of choice for all cases of non-severe malaria worldwide
- Artemisinin drug action is mediated specifically through its endoperoxide moiety

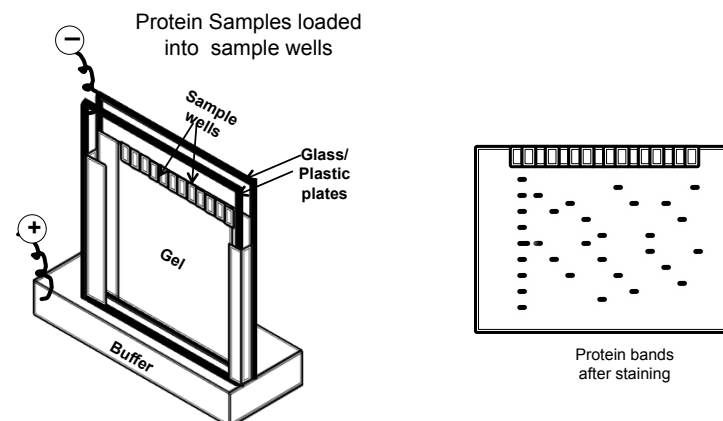
Purpose of study

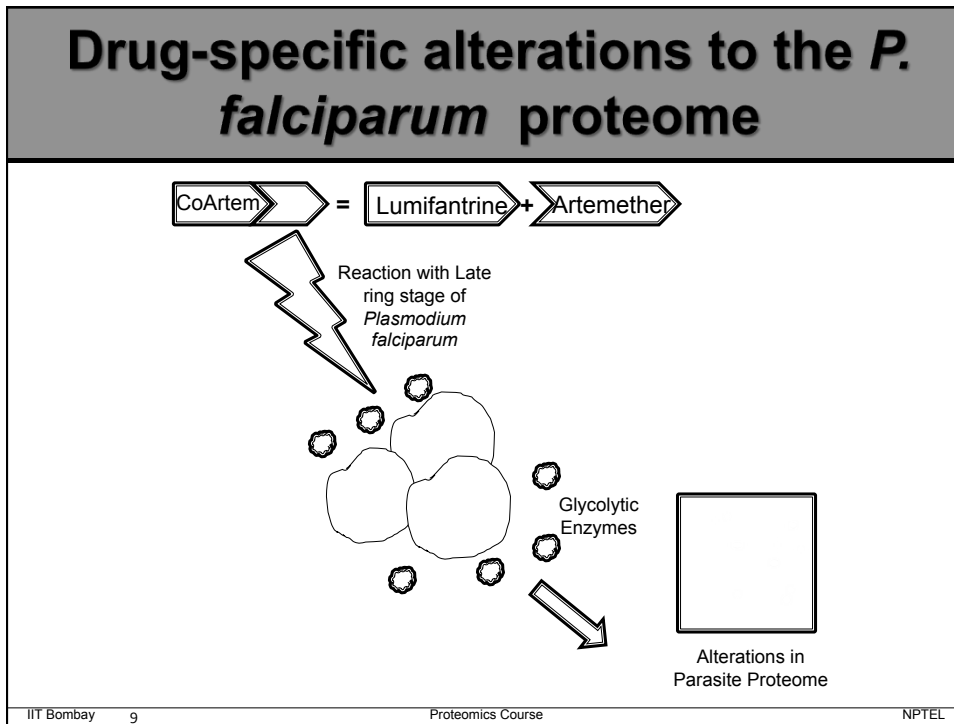


Alteration in *P. falciparum* proteome



Alteration in *P. falciparum* proteome



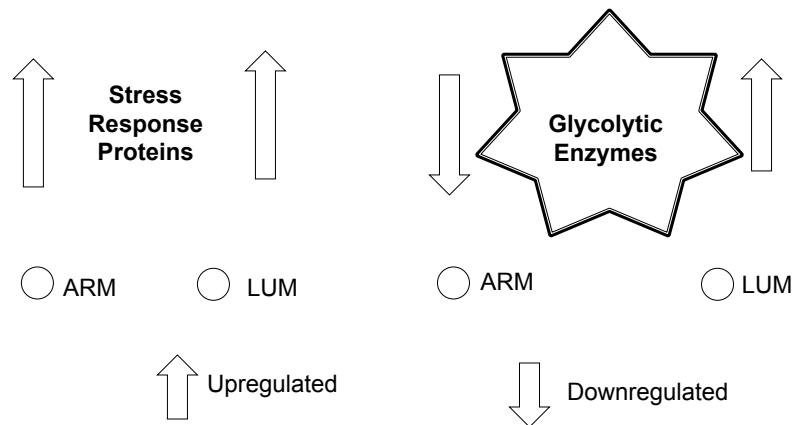


Altered protein expression levels following drug exposure

	Artemether	Lumefantrine
Membrane associated calcium binding protein	<input type="checkbox"/> U	<input type="checkbox"/> U
Aspartic proteinase (HAP)	<input type="checkbox"/> U	<input type="checkbox"/> U
HSP60, 70, 90	<input type="checkbox"/> U	<input type="checkbox"/> U
Enolase	<input type="checkbox"/> D	<input type="checkbox"/> U
Fructose biphosphate aldolase	<input type="checkbox"/> D	<input type="checkbox"/> U
Phophoglycerate kinase	<input type="checkbox"/> D	<input type="checkbox"/> U

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Altered protein expression levels following drug exposure



Major findings

- Investigation of alterations to the parasites proteome induced by the two components of CoArtem, artemether and lumefantrine
- Established relationship between pharmacologically relevant concentration and time of exposure for the two components of CoArtem

Case study-2

Plasma proteome analysis of severe acute respiratory syndrome (SARS)

Chen et al. Plasma proteome of severe acute respiratory syndrome analyzed by two-dimensional gel electrophoresis and mass spectrometry. Proc Natl Acad Sci USA. 2004, 101, 17039

Objective

- Plasma proteome analysis of severe acute respiratory syndrome (SARS)
- Technique: 2DE and Mass Spectrometry

SARS Virus –Plasma Proteome

SARS Virus

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Plasma proteome analysis

4 SARS patients → 22 plasma samples
6 Healthy Patients → 6 Healthy Controls

Protein Samples loaded into sample wells on Glass/Plastic plates containing Gel in Buffer.

Protein bands after staining

38 Spots selected for Protein Identification

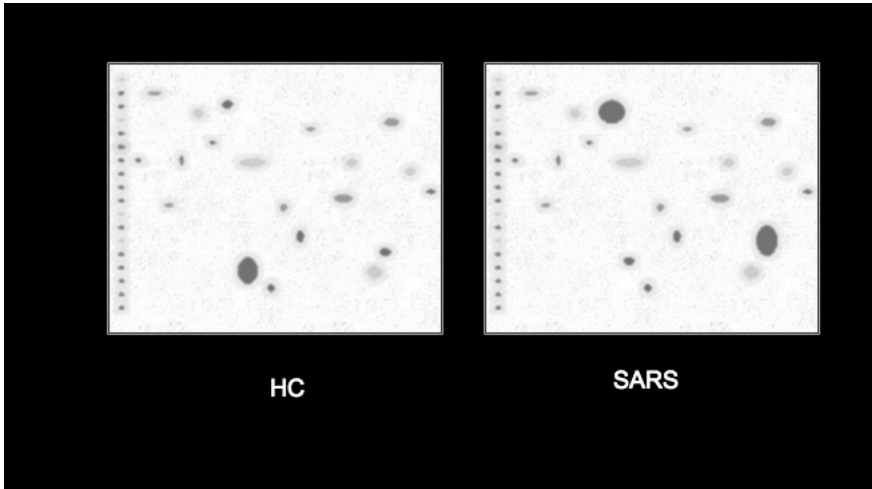
Trypsin

385 Proteins with >2 fold change excised

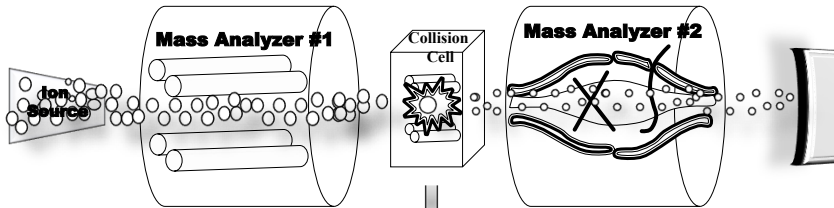
Trypsin Digestion

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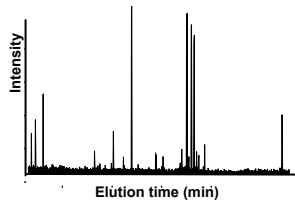
Comparative proteomic analysis



MS identification of proteins



LC-MS Data Analysis



Your name: Email:

Search title:

Database (s):

Taxonomy:

Fixed modification:

Variable modification:

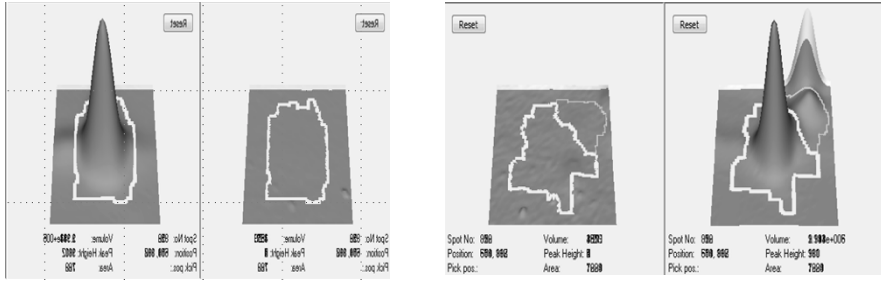
Peptide tol: Da # C¹³: MS/MS tol: Da

Peptide:

Data format:

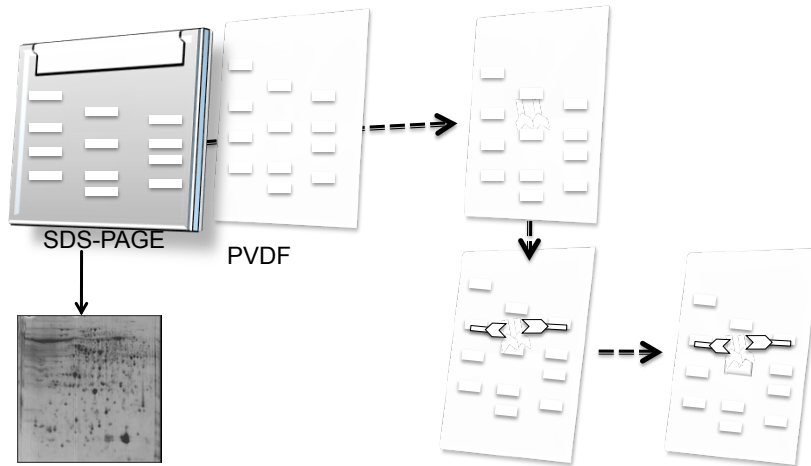
Instrument:

MS identification of proteins

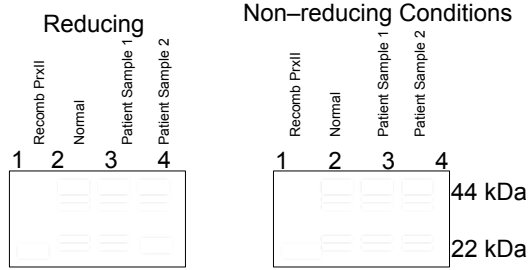


Differentially expressed proteins

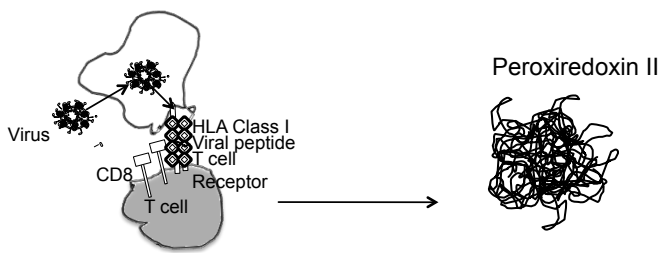
Western blotting

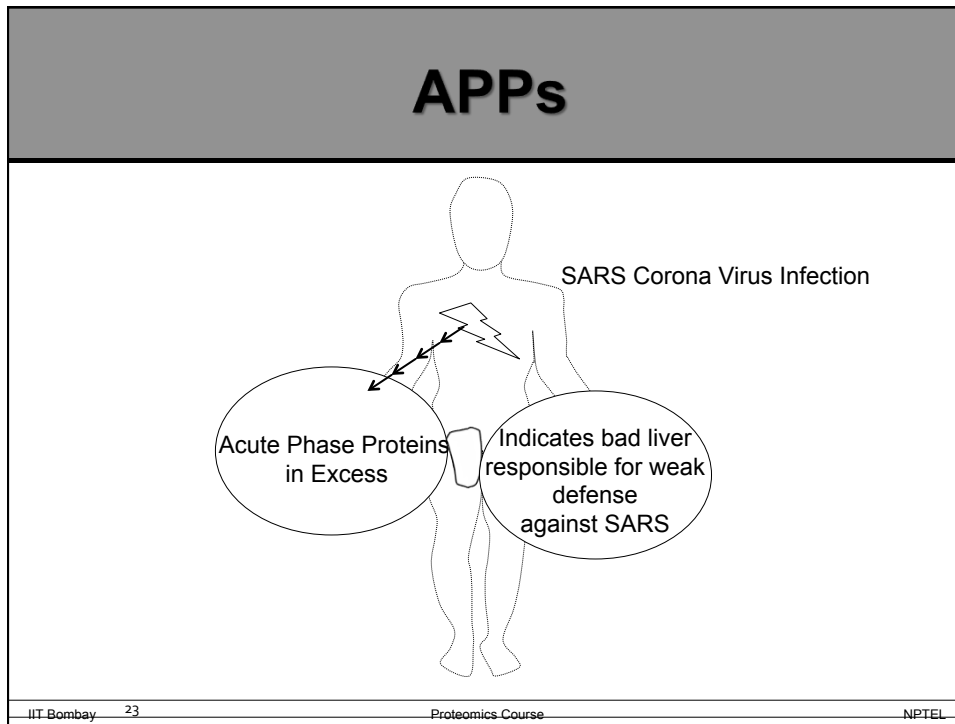


Western blot analysis of PrxII

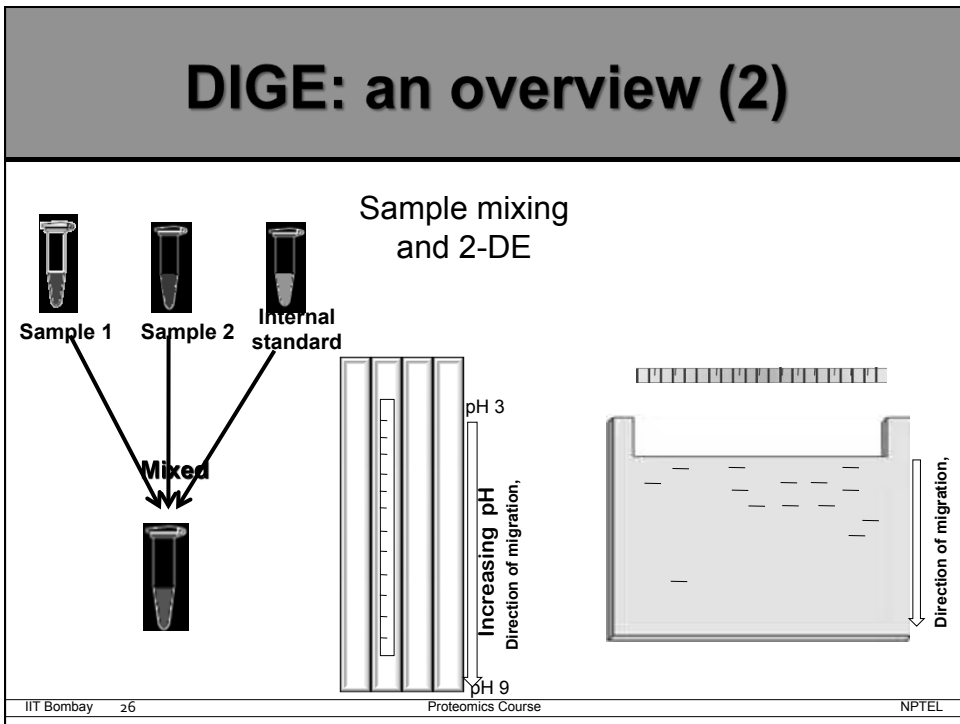
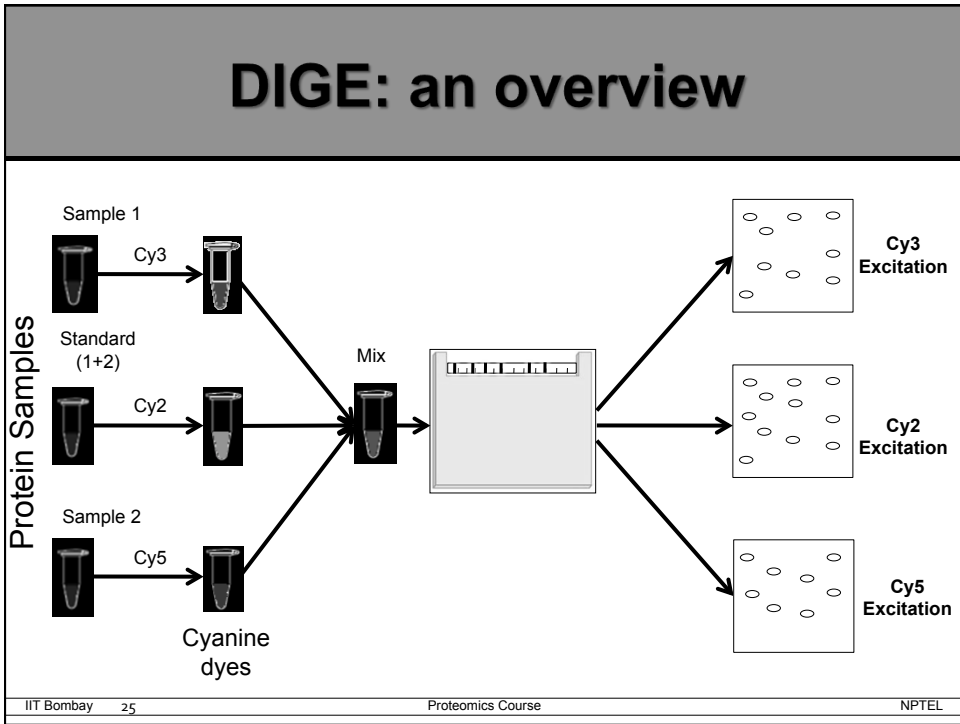


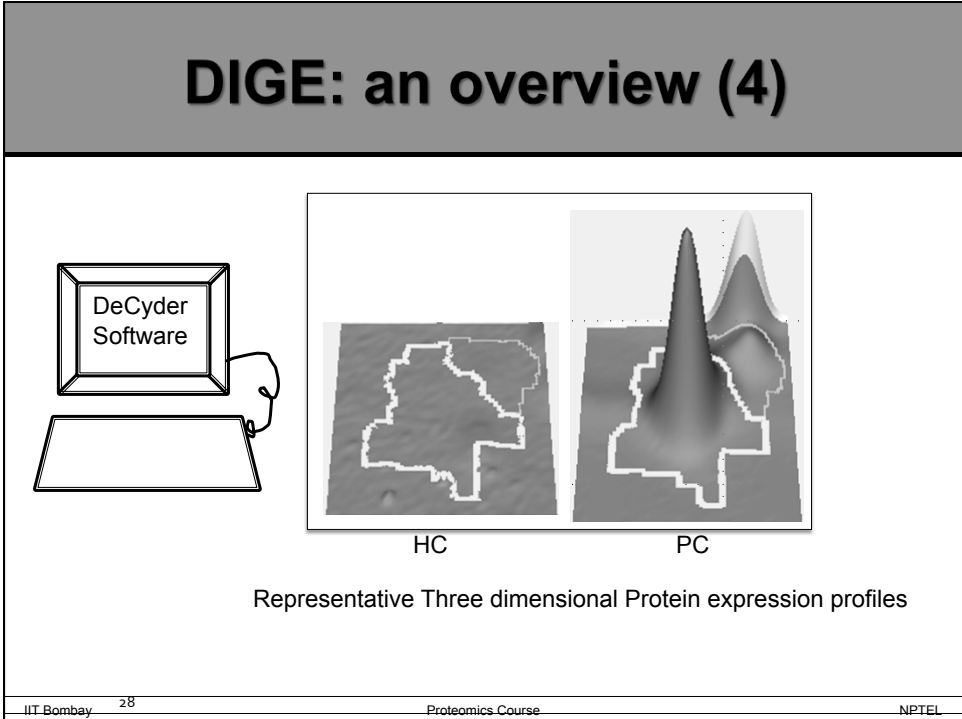
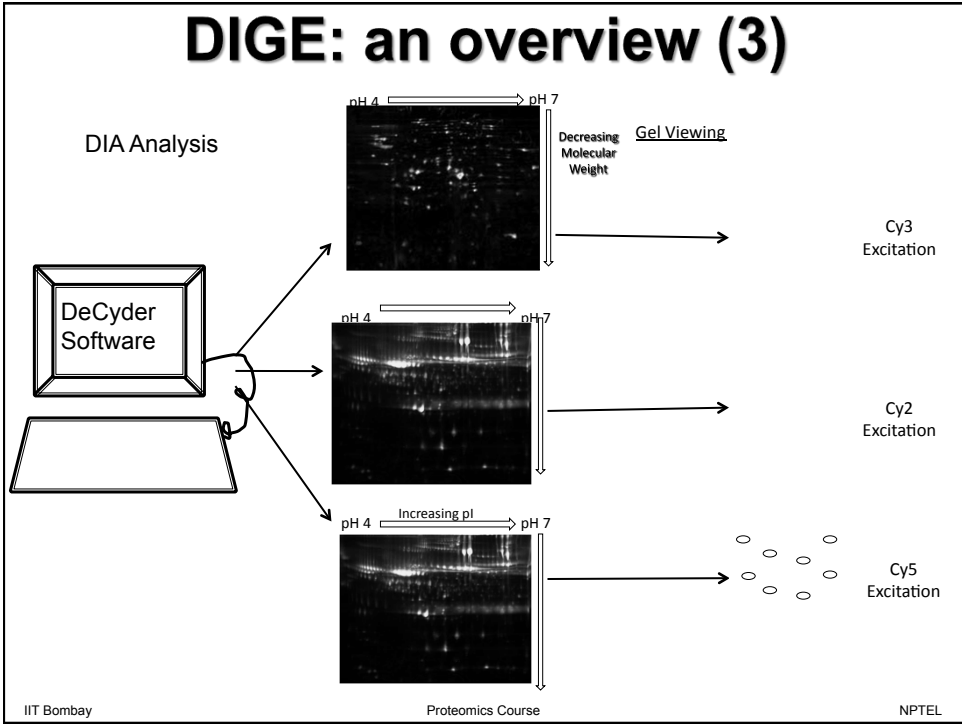
Peroxiredoxin II - Biomarker





Applications of 2D-DIGE





Mass spectrometry

LC MS/MS

Mass Analyzer #1 Collision Cell Mass Analyzer #2

LC-MS Data Analysis

Mass Spectra - PC

Your name: Email:

Search title:

Database(s): Enzyme:

Taxonomy:

Fixed modifications:

Variable modification:

Peptide tol.: Da # C¹³: MS/MS tol.: Da

Peptide charge: Monoisotopic Average

Data file:

Data format: Precursor:

Instrument: Start search...

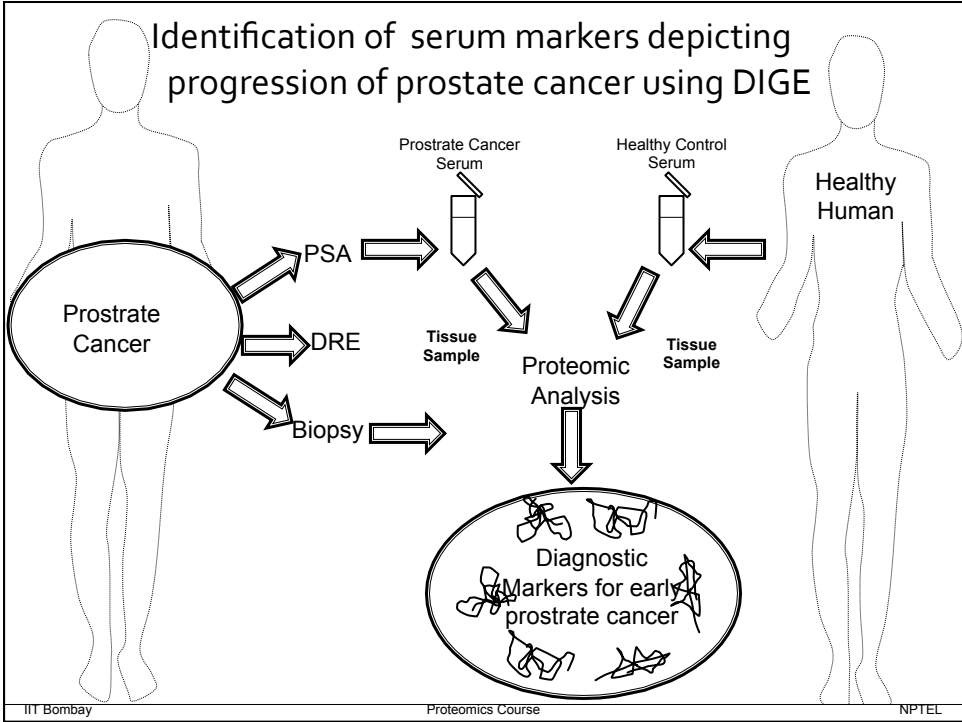
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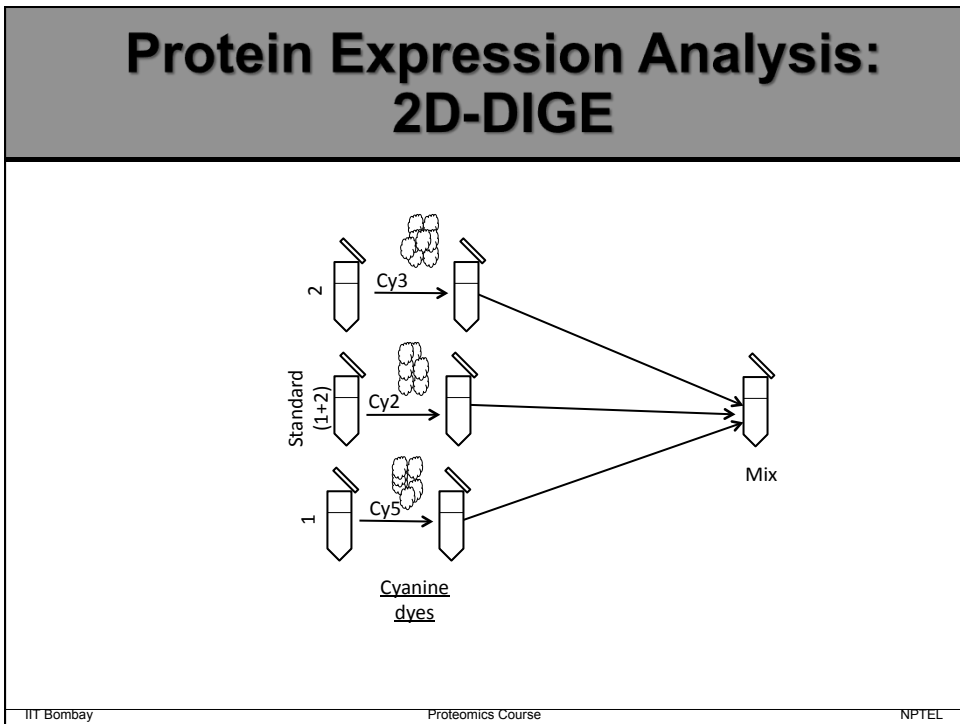
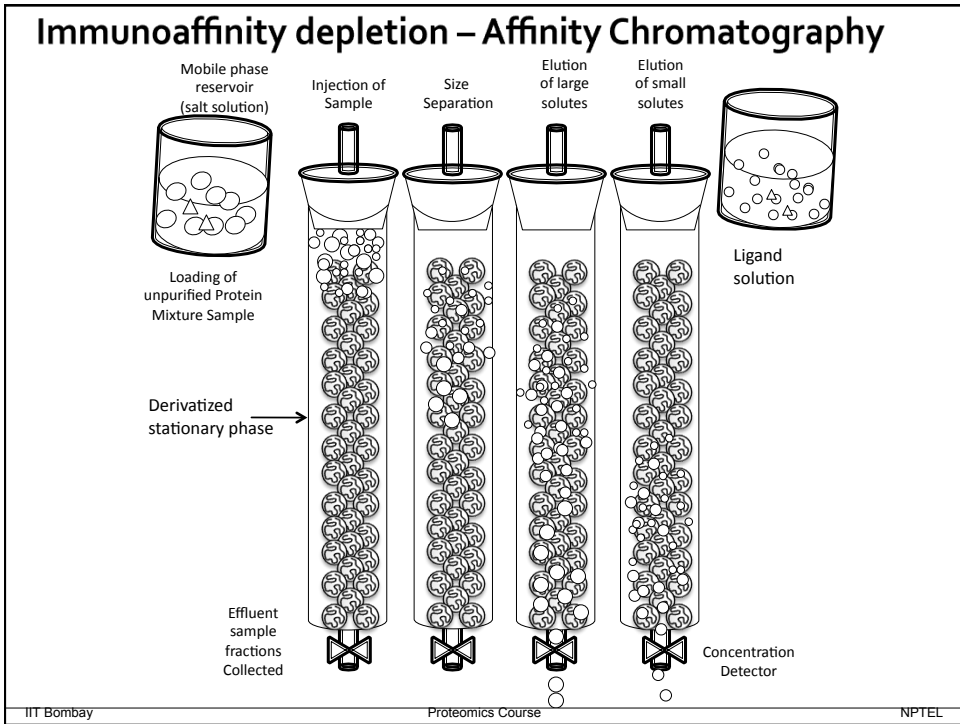
Applications of 2D-DIGE

Case study-3

2D-DIGE Strategy To Identify Serum Markers for Progression of Prostate Cancer

Byrne et al. 2D-DIGE as a Strategy To Identify Serum Markers for the Progression of Prostate Cancer
J Proteome Research. 2009, 8, 942-57





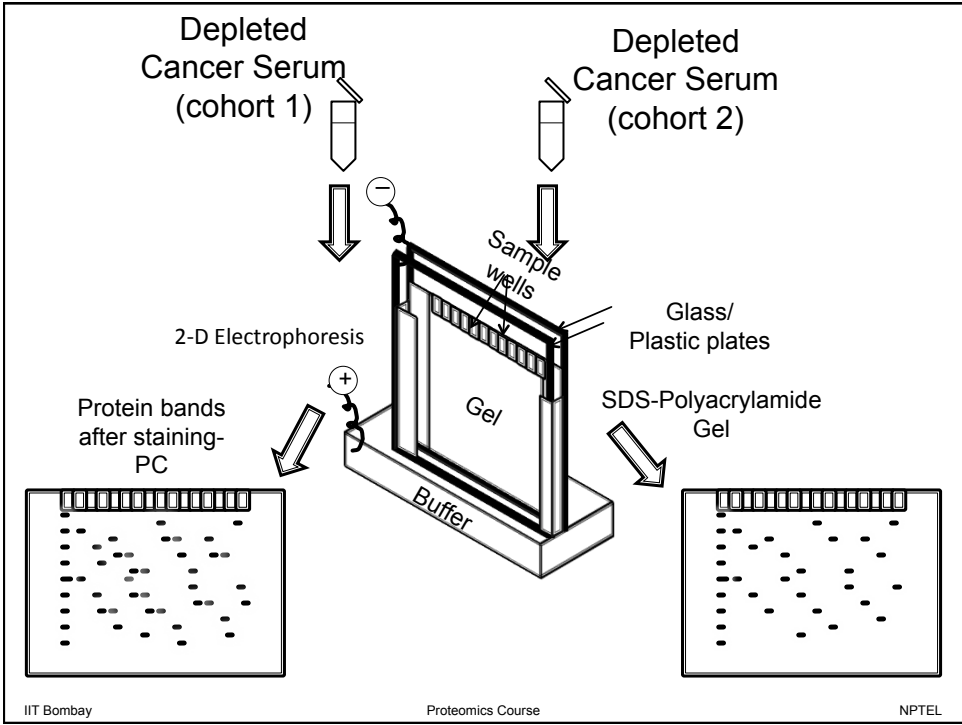
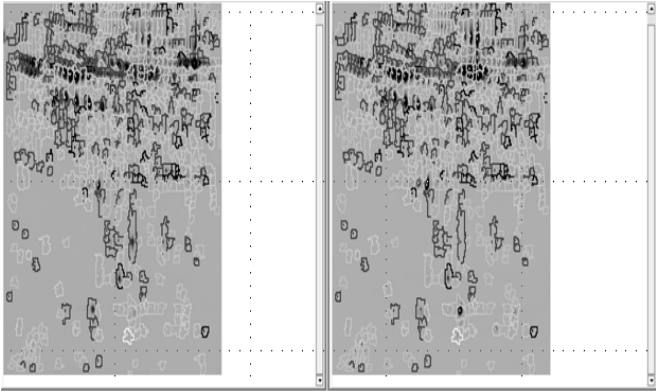


Image analysis



MS identification of proteins

LC-MS Data Analysis

Intensity

Elution time (min)

Your name: Email:

Search title:

Database (s):

Taxonomy:

Fixed modification:

Variable modification:

Peptide tol: Da # C: MS/MS tol: Da

Peptide:

Data format:

Instrument:

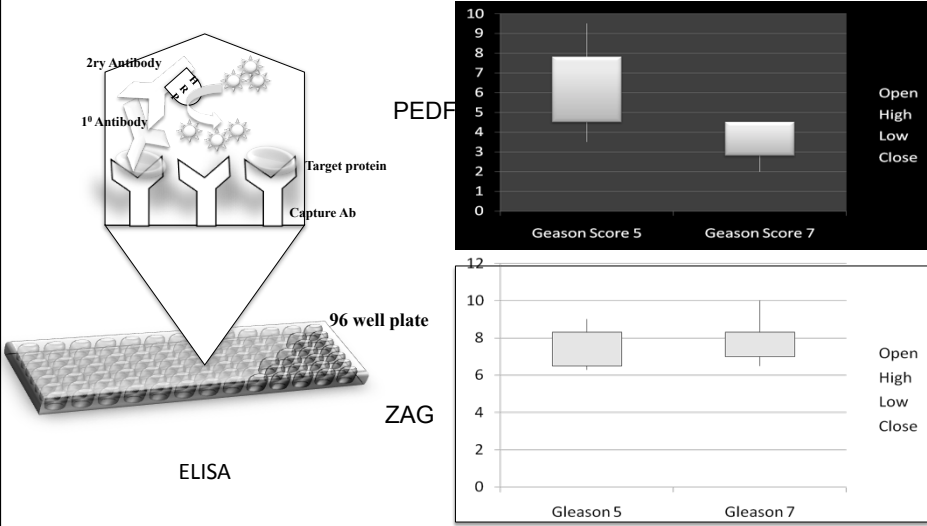
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Differential protein expression in Gleason7 vs Gleason 5

	DeCyder	Progenesis
Pigment epithelium-derived factor	<input type="text" value="D"/>	<input type="text" value="D"/>
Zinc- α 2-glycoprotein	<input type="text" value="U"/>	<input type="text" value="U"/>
Ficolin 3	<input type="text" value="D"/>	<input type="text" value="D"/>
Apolipoprotein A-II	<input type="text" value="U"/>	<input type="text" value="U"/>

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Validation: ELISA – PEDF, ZAG

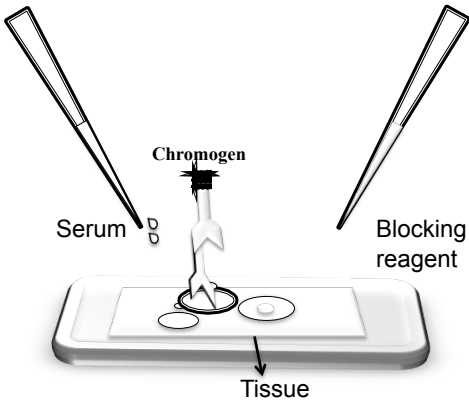


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Validation: Immunohistochemistry



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Conclusions

- Serum markers reflective of pathological grade and stage are beneficial for identification of appropriate treatment strategies
- Confirmed differential expression of PEDF and ZAG by various validation techniques
- PEDF a potential marker of early stage prostate cancer prediction

Summary

- Two dimensional electrophoresis (2DE and 2D-DIGE) can be applied for various applications
- Case studies –
 - Drug treatment in malaria parasite
 - Plasma proteome analysis of SARS
 - Serum proteome analysis of prostate cancer

REFERENCES

- Makanga et al. Towards a proteomic definition of CoArtem action in Plasmodium falciparum malaria. *Proteomics*. 2005, 5, 1849
- Chen et al. Plasma proteome of severe acute respiratory syndrome analyzed by two-dimensional gel electrophoresis and mass spectrometry. *Proc Natl Acad Sci USA*. 2004, 101, 17039
- Byrne et al. 2D-DIGE as a Strategy To Identify Serum Markers for the Progression of Prostate Cancer. *J Proteome Research*. 2009, 8, 942-57
- Issaq H, Veenstra T. Two-dimensional polyacrylamide gel electrophoresis (2D-PAGE): advances and perspectives. *Biotechniques*. 2008, 44, 697-8, 700.

REFERENCES

- John F. Timms, Rainer Cramer Professor, PROTEOMICS. Difference gel electrophoresis. Volume 8, Issue 23-24, pages 4886–4897, No. 23-24 December 2008.
- Ray et al. Serum proteome analysis of vivax malaria: An insight into the disease pathogenesis and host immune response. *Journal of Proteomics*. Volume 75, Issue 10, 6 June 2012, Pages 3063–3080