

2D protein gel analysis tool for studying the protein phosphorylation changes associated with contraction and relaxation of vascular smooth muscle

Internship details

Where: Department of Bioengineering (GWC 643)

As: Research Assistant

Employer: Dr. Padmini Komalavilas

Requirements: Proteomics wet-lab hands-on experience and Bioinformatics programming skills in Matlab.

Phase I: Two Dimensional gel Electrophoresis

Phase II: 2D Protein gel analysis tool

# Presentation Outline

## Phase I

Introduction

Goals

Methodology and Analysis

Collecting and dissecting tissue to obtain coronary artery

Treatment of coronary artery with drugs

Tissue Pulverization

Protein Quantification

Protein Separation

Staining

Scanning

Results

# Presentation Outline

## Phase II

Introduction

Goals

Methodology and Analysis

Counting number of proteins on gel images

Displaying location and intensity of proteins

Comparing Control gel image with treated gel images

Demonstration of Application

Results

Discussion

Conclusions and Future Work

Acknowledgments

References

## Phase I: Introduction

Proteome: Proteome can be defined as the complete set of proteins that ultimately results from genome transcription in a given cell, tissue or organism.

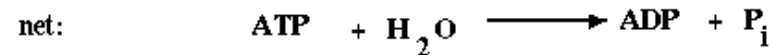
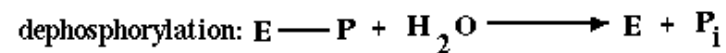
Complexity of Proteome is far beyond genome

Proteomics: Proteomics can be defined as the qualitative and quantitative comparison of proteomes under different conditions to further unravel biological processes.

## Phase I: Introduction

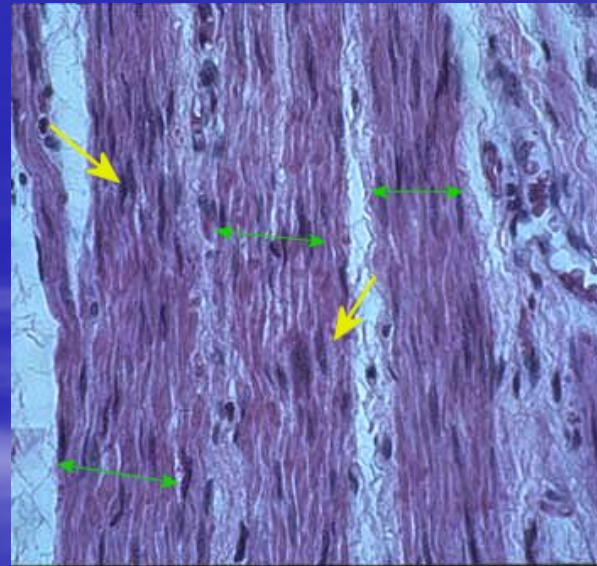
Protein phosphorylation: The chemical addition of a phosphate group (phosphate and oxygen) to a protein.

Protein phosphorylation is an important post-translational modification since it results in cellular activity and is also reversible.



## Phase I: Introduction

Smooth muscle cells are long (15  $\mu\text{m}$  to 500  $\mu\text{m}$ ) and spindle-shaped with elongated nuclei. Smooth muscle is found in the walls of many hollow organs. In a smooth muscle cell the nucleus is centrally located and that smooth muscle is more eosinophilic



*Yellow Arrow - Nuclei of Smooth Muscle Cell  
Green Arrow - Width of Smooth Muscle Tissue*

## Phase I: Goals

To study protein phosphorylation changes associated with the contraction and relaxation of vascular smooth muscle using two dimensional gel electrophoresis.

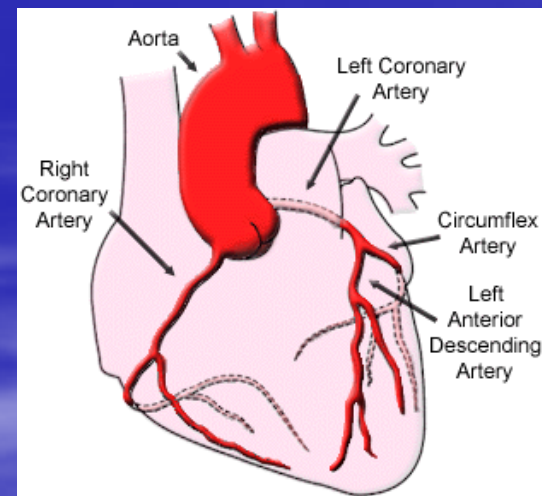
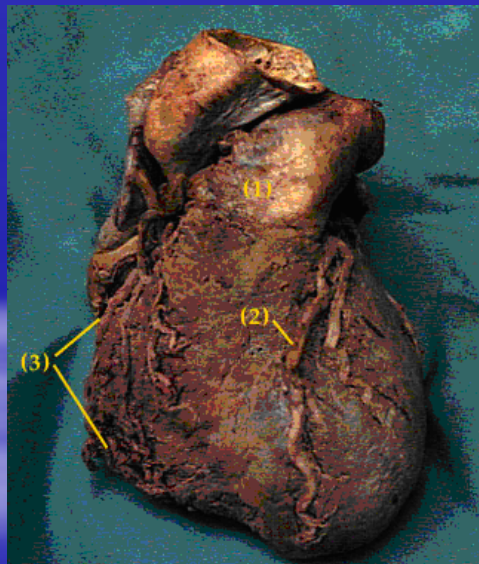
To study total proteins expressed during contraction and relaxation of vascular smooth muscle using spiro ruby stain.

To study phosphorylated proteins expressed during contraction and relaxation of vascular smooth muscle using phospho protein stain.

# Phase I: Methodology and Analysis

Tissue acquisition

Dissecting the tissue to obtain coronary artery



1 = pulmonary trunk; 2 = Left anterior descending coronary artery; 3 = right coronary artery with its right marginal branch.

## Phase I: Methodology and Analysis

Tissue that is not treated with drugs is called as control.

Treatment 1: Contraction is induced by treating the tissue with 1uM serotonin for 10 min.

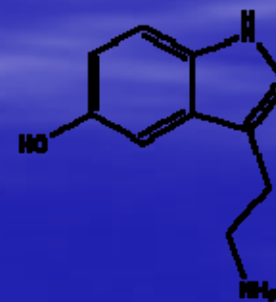
Treatment 2: Smooth muscle tissue is treated with 1uM serotonin for 5 min followed by 10uM sodium nitroprusside for 5 min to induce relaxation.

Treatment 3: Tissue is treated with 1uM serotonin followed by 100uM papaverine for 5 min to induce relaxation.

### Drug Serotonin:

The chemical name for serotonin is 5-hydroxytryptamine which is often abbreviated to 5-HT.

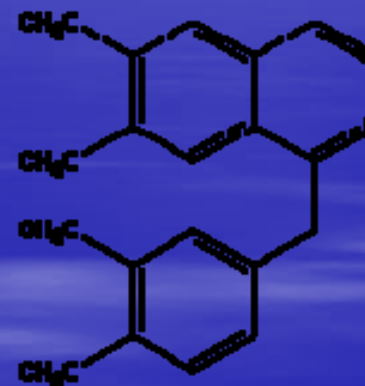
One role of this 'wonder drug' is as a neurotransmitter, allowing numerous functions in the human body which includes muscle contraction.



## Phase I: Methodology and Analysis

### Drug Papaverine

Papaverine is used to improve blood flow. It works by relaxing the blood vessels so that blood can flow more easily to the heart and through the body.



### Drug Sodium Nitroprusside

It acts by relaxation of vascular smooth muscle. Sodium nitroprusside breaks down in the blood and releases a chemical called nitric oxide (NO). Nitric oxide enters the muscle cells in the walls of the blood vessels and causes them to relax.

## Phase I: Methodology and Analysis

Tissue pulverization: After treatment with drugs tissue is frozen in liquid nitrogen and pulverized using mortar and pestle.



The tissue is solubilized in urea, dithiothreitol, CHAPS (UDC) buffer by vortexing for 30 min at room temperature. Extracted proteins are separated by centrifuging the extract at 14,000 rpm for 10 min at 4°C using a centrifuge. Supernatant are transferred to a fresh tube.

## Phase I: Methodology and Analysis

### Protein Quantification:

Proteins are quantitated using Bradford protein assay. The method is based on the proportional binding of the dye Coomassie to proteins and measuring the absorbance of the complex in a spectrophotometer and compared to known amount of standard proteins.



# Phase I: Methodology and Analysis

## Protein Quantification:

	Vol/ Standard	Vol H2O	Vol Assay mix
0	0ul	250 ul	750
0'	0ul	250 ul	750
2	10ul	240 ul	750
2'	10ul	240 ul	750
4	20ul	230 ul	750
4'	20ul	230 ul	750
6	30ul	220 ul	750
6'	30ul	220 ul	750
8	40ul	210 ul	750
8'	40ul	210 ul	750
10	50ul	200 ul	750
10'	50ul	200 ul	750

Protein	Vol of Protein	Vol H2O	Vol Assay Mix
Control 1	1ul	249	750
Control 2	1ul	249	750
5HT 1	1ul	249	750
5HT 2	1ul	249	750
5HT + SNP 1	1ul	249	750
5HT + SNP 2	1ul	249	750
5HT + Papa 1	1ul	249	750
5HT + Papa 2	1ul	249	750

## Phase I: Methodology and Analysis

### Protein Quantification:

		100ug	UDC buffer
Control	4.1 ug/ul	24.4	225.6
5HT	7.63 ug/ul	13.1	236.9
5HT + SNP	5.9 ug/ul	16.9	233.1
5HT + Papaverine	5.3 ug/ul	18.8	231.2

# Phase I: Methodology and Analysis

## Protein Separation

### Two-dimensional polyacrylamide gel electrophoresis

#### Key principles

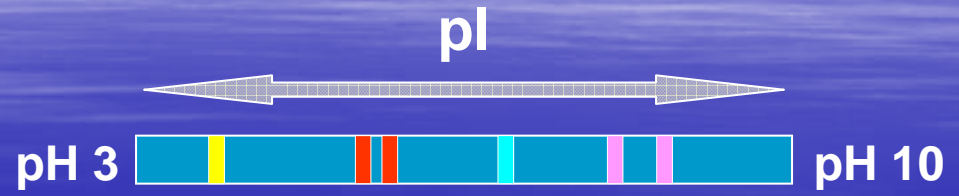
- Proteins differ from each other in terms of their mass and charge. Both these properties can be used to separate proteins by gel electrophoresis.
- The successive application of both techniques in perpendicular directions (two dimensions) provides maximum separation and allows thousands of proteins to be resolved.
- Staining the gel reveals the positions of individual proteins as spots or smudges.

# Phase I: Methodology and Analysis

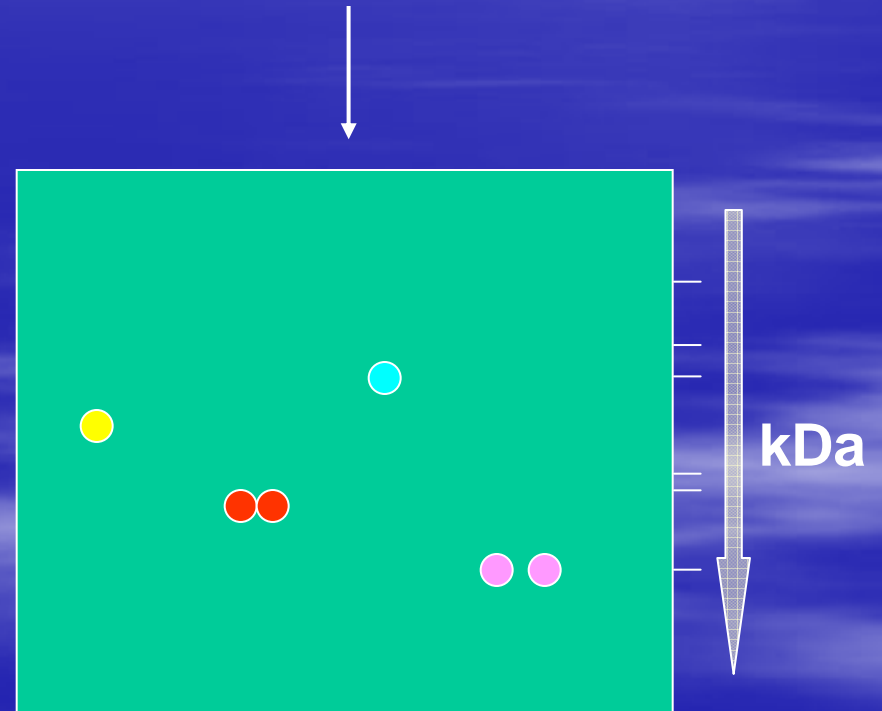
## Protein Separation

### Two-dimensional polyacrylamide gel electrophoresis

**1st dimension**  
Separation by charge  
(isoelectric focussing)



**2nd dimension**  
Separation by molecular weight  
(SDS-PAGE)



## Phase I: Methodology and Analysis

### First Dimension Methodology of a 2D Gel

It uses a procedure called isoelectric focusing, which separates polypeptide chains depending on the surrounding pH and the charge of the protein (negative or positive).



## Phase I: Methodology and Analysis

### Strip Equilibration

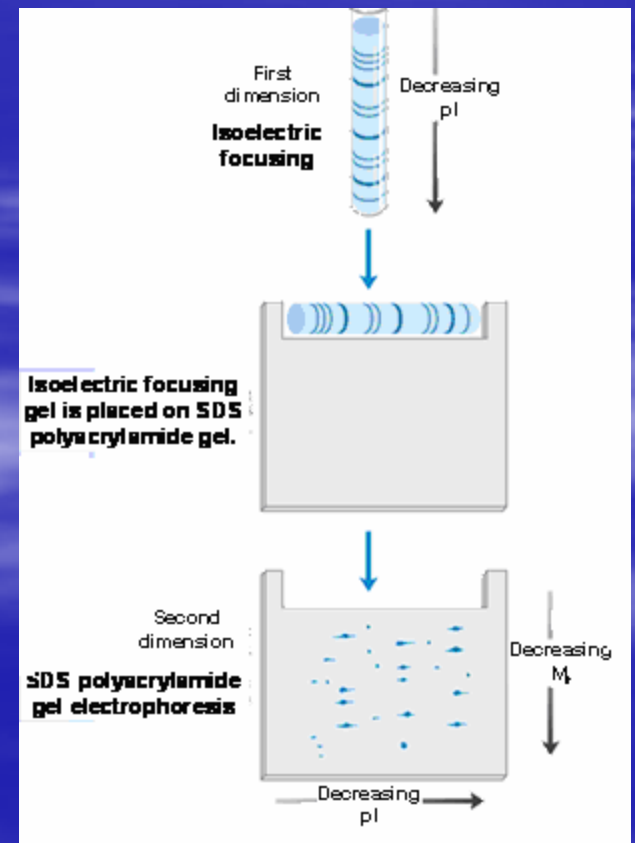
#### Equilibration Solutions

Solution 1	10mL Stock Re-equilibration Buffer + 100mg DTT
Solution 2	10mL Stock Re-equilibration Buffer + 250mg iodacetamide

# Phase I: Methodology and Analysis

## Second Dimension

The second dimension is usually an SDS-polyacrylamide gel electrophoresis, which allows the separation of the proteins based on their molecular size



## Phase I: Methodology and Analysis

### Staining

Pro-Q® Diamond phosphoprotein gel stain

#### **Staining procedure**

- 1 Fix the gel
- 2 Wash the gel
- 3 Stain the gel
- 4 Destain the gel
- 5 Wash the gel

#### **Imaging and Documenting the Gel**

## Phase I: Methodology and Analysis

### Staining

SYPRO Ruby protein gel stain

#### **Staining procedure**

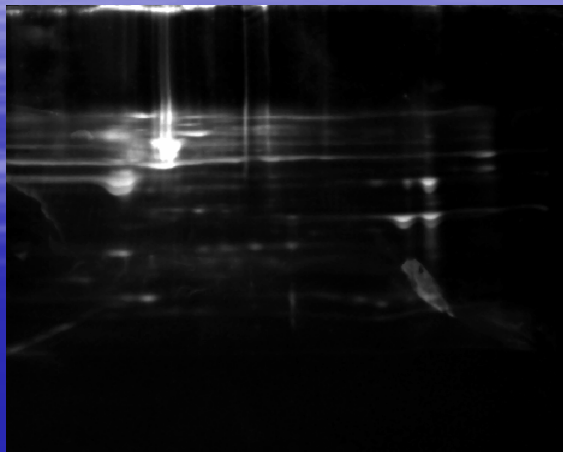
- 1 Fix the gel
- 2 Wash the gel
- 3 Stain the gel
- 4 Destain the gel
- 5 Wash the gel

#### **Imaging and Documenting the Gel**

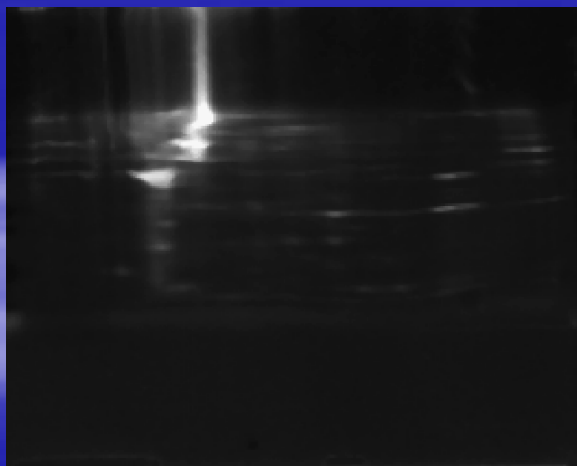
# Phase I: Results

Spyro ruby stain

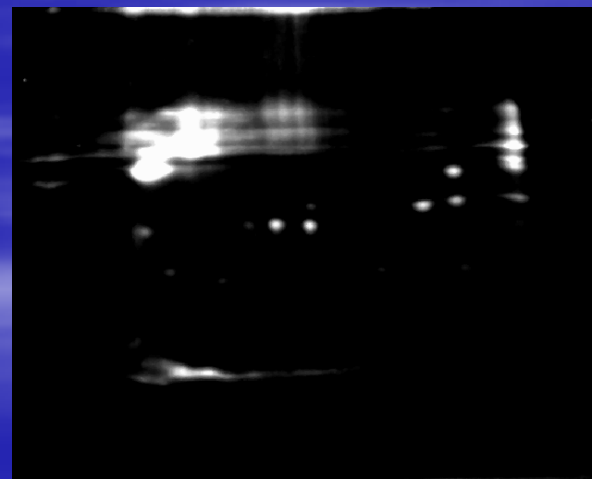
Control



5HT



5HT + SNP

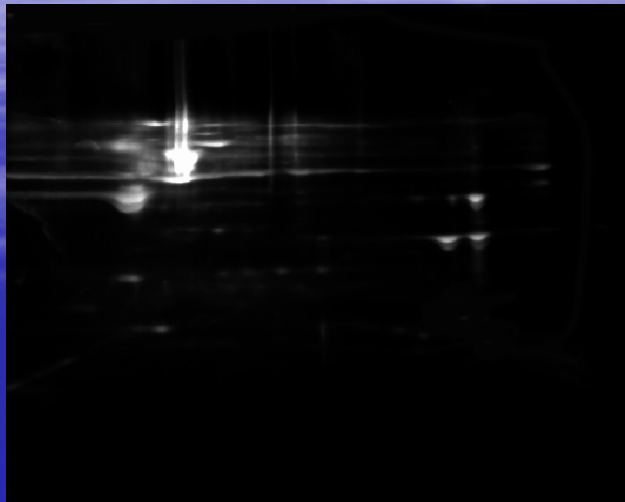


5HT + Papaverine

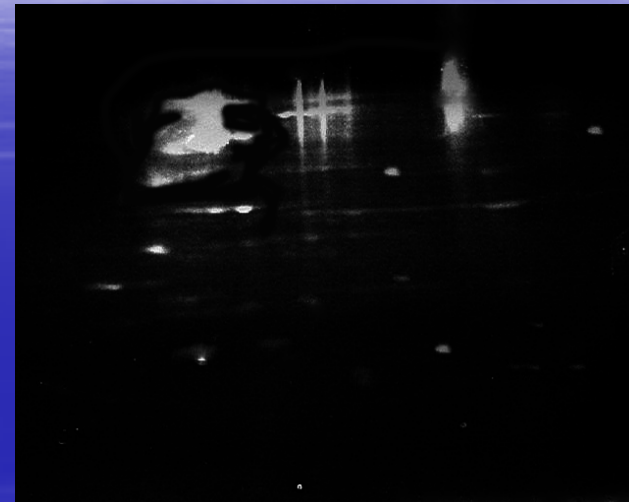
# Phase I: Results

Phospho Protein stain

Control



5HT



5HT + SNP



5HT + Papaverine

## Phase II: 2D Protein Analysis tool

To develop a 2D Protein analysis tool to obtain total count of proteins along with the location and intensity of proteins.

To compare two two-dimensional (2-D) protein gels by location of proteins using 2D protein analysis tool.

## Phase II: Methodology

Image segmentation using thresholding

### **Hard thresholding**

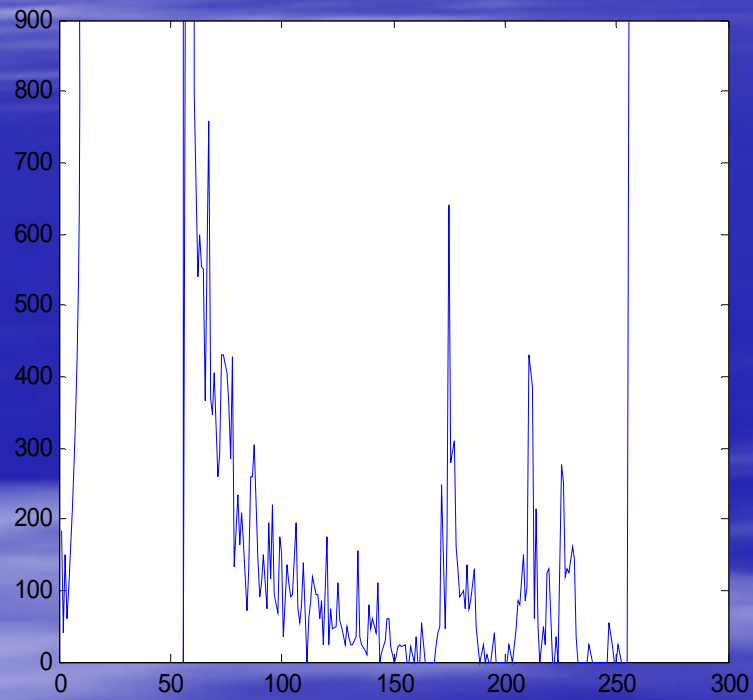
$$\begin{aligned}\tilde{\theta}_j &= y_j^* & |y_j^*| \geq \lambda, \\ &= 0 & |y_j^*| < \lambda.\end{aligned}$$

### **Soft thresholding**

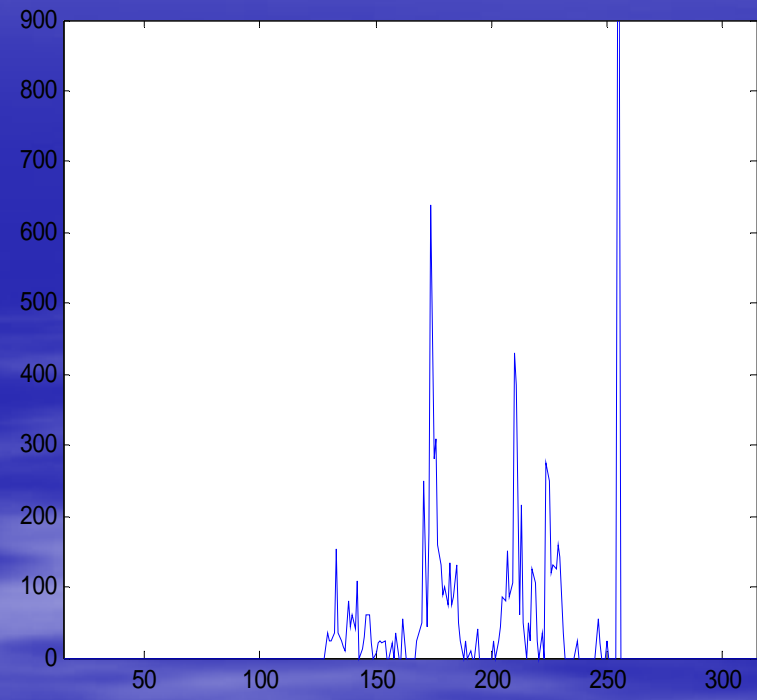
$$\hat{\theta}_j = \text{sign}(y_j^*) (|y_j^*| - \lambda)_+.$$

## Phase II: Methodology

Before Thresholding



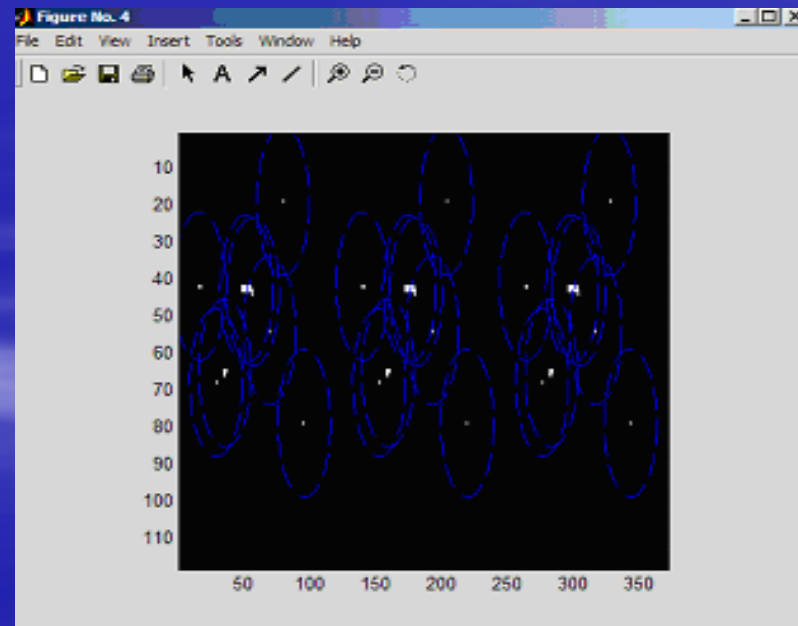
After Thresholding



## Phase II: Methodology

### Counting number of proteins

Fastcentroid function returns the centroid coordinates for the connected components (iind, jind) and the number of pixels of each component (numberofpixels). This software was developed by Marcelino Sanchez Gonzalez in May 2002.



## Phase II: Methodology

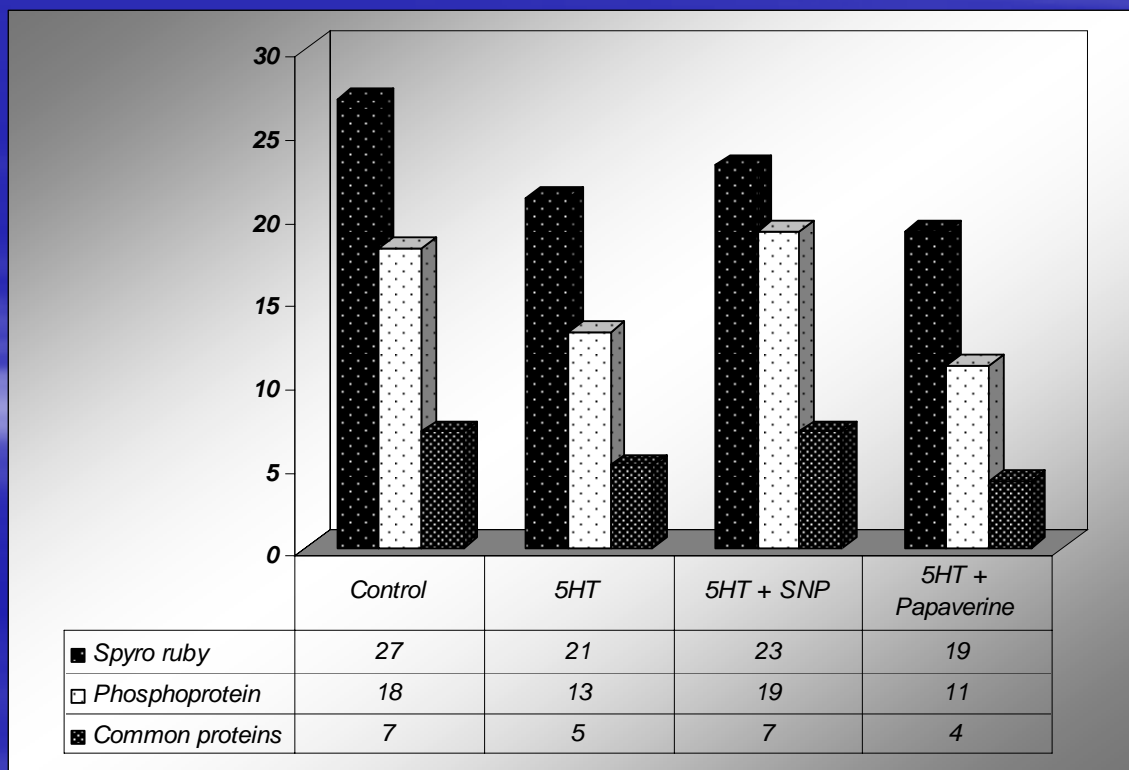
Finding similar proteins in control and treated gel images

The matching criterion is based on the Euclidean distances. The straight line distance between two points

$$dist = \sqrt{\sum_{k=1}^n (p_k - q_k)^2}$$

## Phase II: Results

	Spyro ruby	Phosphoprotein	Common proteins
Control	27	18	7
5HT	21	13	5
5HT + SNP	23	19	7
5HT + Papaverine	19	11	4

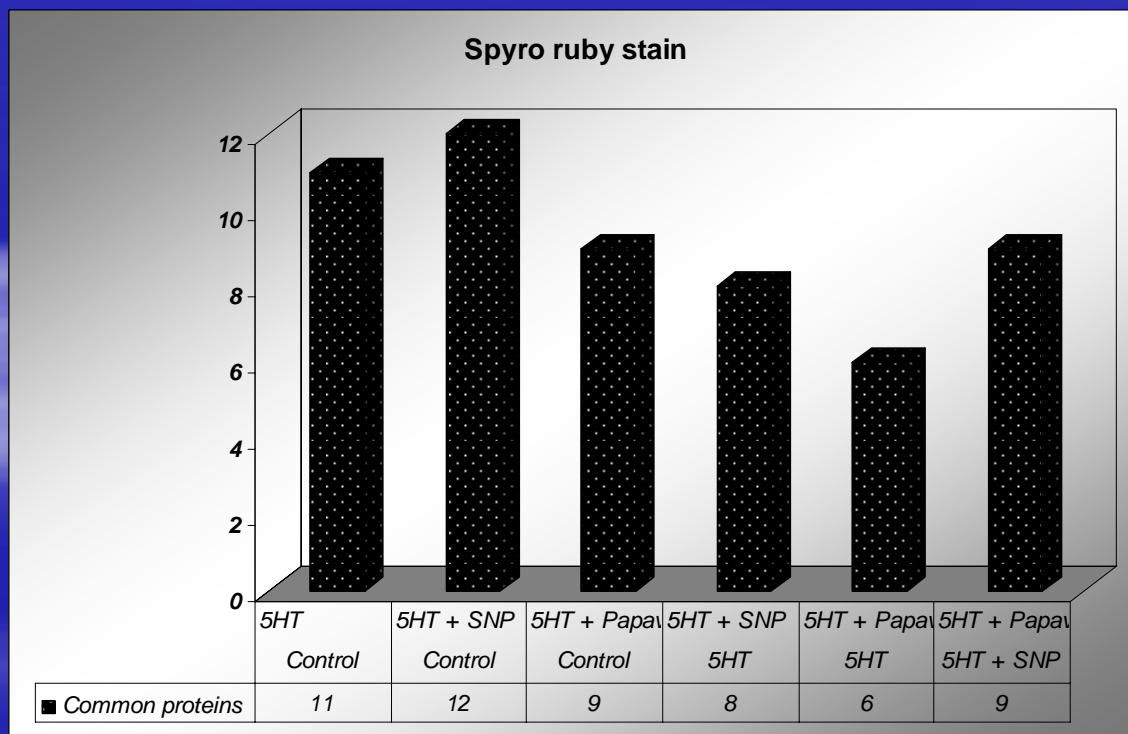


# Phase II: Results

Spyro ruby stain

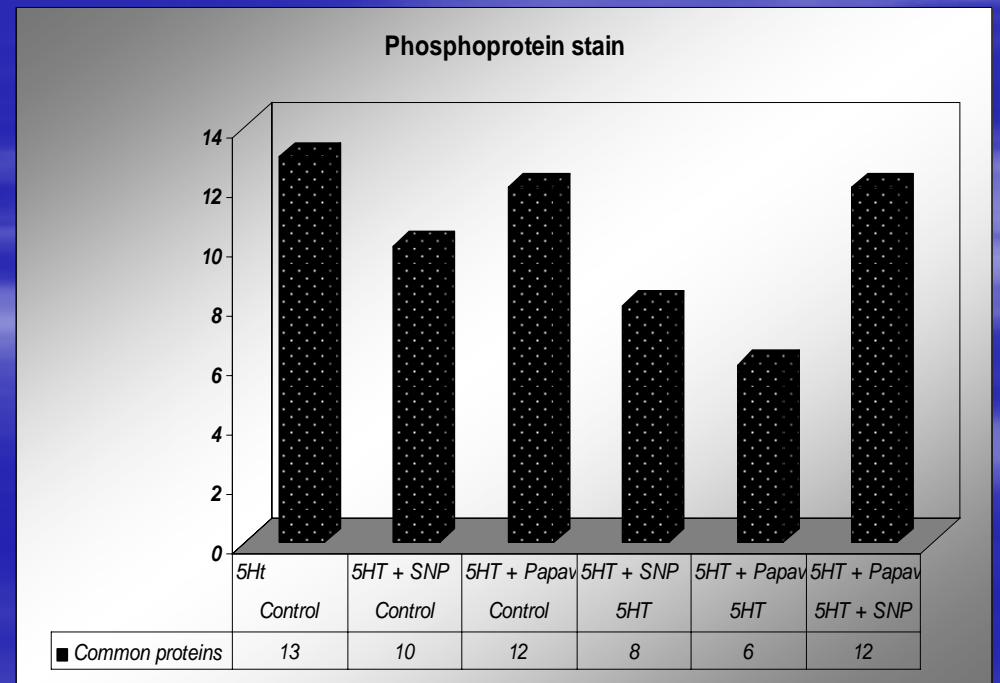
Common proteins

Control	5HT	11
Control	5HT + SNP	12
Control	5HT + Papaverine	9
5HT	5HT + SNP	8
5HT	5HT + Papaverine	6
5HT + SNP	5HT + Papaverine	9



## Phase II: Results

	Phosphoprotein stain	Common proteins
Control	5Ht	13
Control	5HT + SNP	10
Control	5HT + Papaverine	12
5HT	5HT + SNP	8
5HT	5HT + Papaverine	6
5HT + SNP	5HT + Papaverine	12



## Conclusion

There is minimal change in the total number of proteins picked up by spyruby stain during control, contraction and relaxation of vascular smooth muscle.

There is decrease in the total number of phosphorylated proteins picked up by phosphor protein stain during control, contraction and relaxation of vascular smooth muscle when compared to the total number of proteins.

## Discussion

Significance of the project

Limitations of 2-D gel electrophoresis.

Limitations of 2-D Protein gel analysis tool

## Future work

To develop second version of 2D protein gel analysis tool which can match proteins based on the intensity

To study phosphorylated proteins by using anti-phosphoamino acid antibodies.

## References

<http://arxiv.org/ftp/cs/papers/0305/0305048.pdf>

<http://www.reindeergraphics.com/tutorial/index.shtml>

<http://www.csse.uwa.edu.au/~pk/Research/MatlabFns/Match/matchbycorrelation.m>

<http://www.csse.uwa.edu.au/~pk/Research/MatlabFns/>

## Acknowledgments

I would like to express my sincere gratitude to Dr. Padmini Komalavilas for suggesting the project and providing valuable Guidance.

Many thanks to

Dr. Lokesh Joshi

Dr. Kirkmann Liff

Mr. Ashutosh Kulkarni

Mr. Vinod Swarna

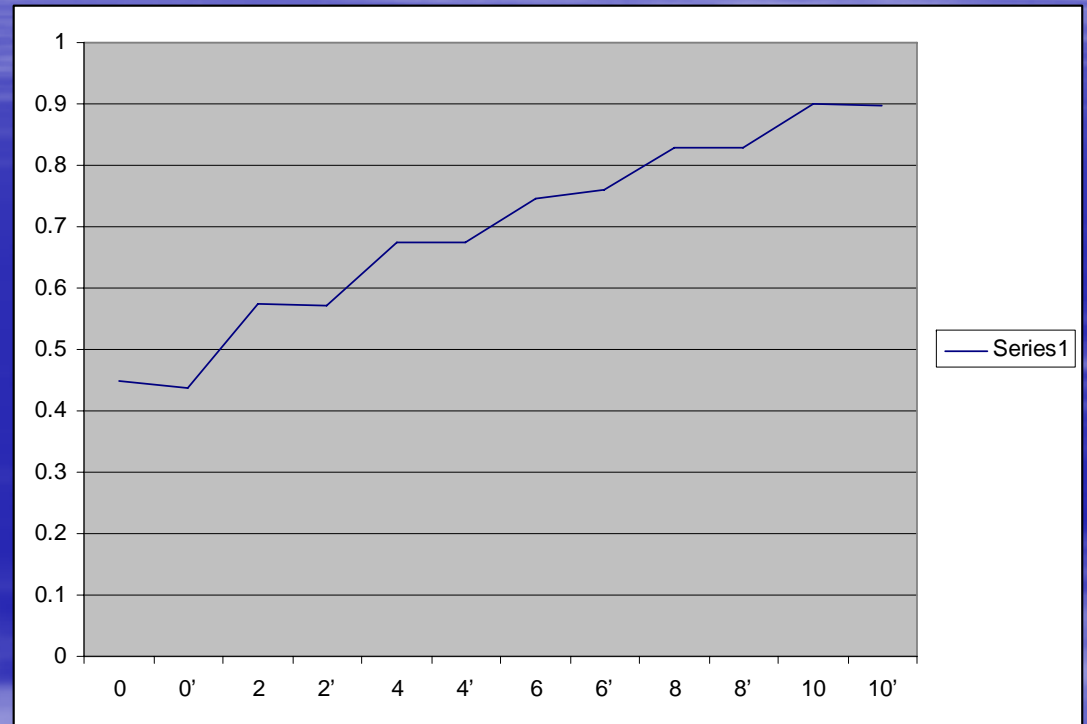


*Any Questions*



## Phase I: Methodology and Analysis

ID	U <sub>g</sub>	Net A
Blank		
1	0	0.450
2	0'	0.436
3	2	0.573
4	2'	0.572
5	4	0.673
6	4'	0.675
7	6	0.745
8	6'	0.760
9	8	0.828
10	8'	0.828
11	10	0.900
12	10'	0.898



## Phase I: Methodology and Analysis

Control 1	0.684
Control 2	0.666
5HT 1	0.861
5HT 2	0.880
5Ht + SNP 1	0.773
5HT + SNP 2	0.784
5HT + Papa 1	0.739
5HT + Papa 2	0.742

		100ug	UDC buffer
Control	4.1 ug/ul	24.4	225.6
5HT	7.63 ug/ul	13.1	236.9
5HT + SNP	5.9 ug/ul	16.9	233.1
5HT + Papa	5.3 ug/ul	18.8	231.2

# MATLAB APPLICATION

Please Input Image

control.jpg

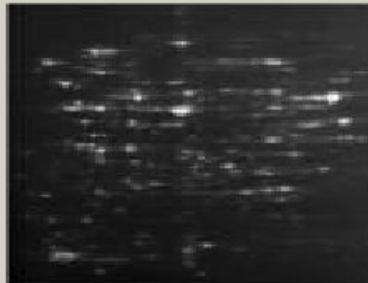
Load Image



77.74

Apply

View Location



No. of Proteins 83

Location and intensity

1	31	85	34
2	178	1	82
3	202	2	83
4	206	135	253

Please Input Image

treated.bmp

Load Image



77.44

Apply

view location



No. of Proteins 40

Location and intensity

1	46	77	30
2	196	22	114
3	31	31	117
4	160	26	87
5	76	27	181

Similar proteins based on location with reference to image 1

similar proteins location

1	13	15	18	5	6
31	37	51	46	44	141
85	70	69	72	32	30
34	112	153	84	147	8

Close



MATLAB

E:\matlab\pallavimatab\...

GUIdemo

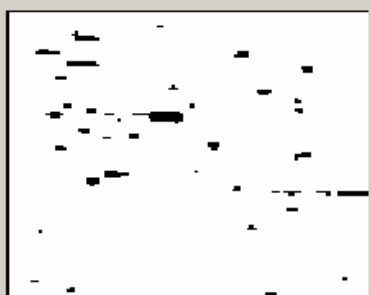
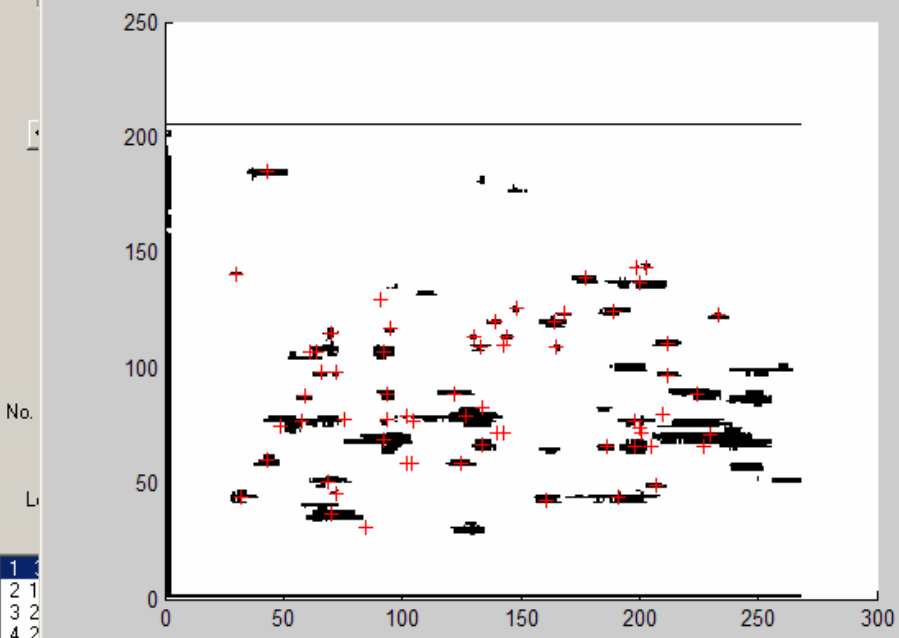
4:00 PM



GUIDemo

Figure No. 4

File Edit View Insert Tools Window Help



Similar proteins based on location with reference to image 1

similar proteins location

1	13	15	18	5	6
31	37	51	46	44	141
85	70	69	72	32	30
34	112	153	84	147	8

Close

