

PROTEOMIC QUESTIONS

(ANSWERS DUE BY EMAIL July 18th at 10:00am US EST, 10:00pm in Chengdu)

1. What are two distinctive characteristics of proteins that are used in 2D gel electrophoresis?
2. What is the relationship to pH and pI of an amino acid?
3. There is a protein with pI value of 7.5. If this protein is placed in pH 4, what would be the electric charge of this protein (electric negative or electric positive)? Which side of electrode would this protein migrate under electric field?

4. For the labeling of protein sample with Cy-dyes, please fill the blank with appropriate numbers that we went through during class (**please watch the video of Lab 1**)

Sample Labeling: Label 25µg protein with cydye (Samples are in 4µg/µl concentration)

Sample	sample vol	500mM Tris-HCl (pH8.8)	Cy dye	10mM Lysine	2X SB Equal to reaction volume	Final Rx volume
Sample A (_____)	µl	1µl	Cy3-1µl	1.2µl	(_____)µl	(_____)µl
Sample A (_____)	µl	1µl	Cy3-1µl	1.2µl	(_____)µl	(_____)µl

Incubate on ice for 30 min Incubate on ice for 10 min

If you have understood the labeling reaction and can fill out those blanks, you should able to do blanks below

(please note that different amount of proteins used and protein concentration are differ from example shown above)

Sample Labeling: Label 50µg protein with cydye (Samples are in 6 µg/µl concentration)

Sample	sample vol	500mM Tris-HCl (pH8.8)	Cy dye	10mM Lysine	2X SB Equal to reaction volume	Final Rx volume
Sample A (_____)	µl	1µl	Cy3-1µl	1.2µl	(_____)µl	(_____)µl
Sample A (_____)	µl	1µl	Cy3-1µl	1.2µl	(_____)µl	(_____)µl

5. Referring to the “**Proteomics Analysis Protocol**” in handout, **Part II, section IV- Buffers and Reagent Section.**

Prepare 210 ml of 10% SDS-polyacrylamide gel solution.

ddH₂O:

1.5M Tris-HCl (pH 8.8):

Protogel (30% PA):

10% SDS:

10% APS:

TEMED:

6. What is the colour of **Cy3** dye- stained proteins. (**video of Lab 2**)

At what wavelength of light does **propyl-Cy3** maximally absorb?

7. What is a Mass Spectrometer (what does it do)? How are the results expressed?

