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BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.

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NAME	POSITION TITLE
Majumder, Rinku	Associate Professor of Biochemistry
eRA COMMONS USER NAME RINKU_MAJUMDER	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Kalyani University, India	MS.	1992	Biochemistry
Jadavpur University, India	Ph.D.	1999	Biochemistry
University of North Carolina, Chapel Hill	Post-doc	1998-2003	Biochemistry

A. PERSONAL STATEMENT

My research focuses on the anticoagulant Protein S (PS). PS is a vitamin K-dependent plasma glycoprotein synthesized in the endothelium. PS deficiency is one of several known risk factors for thrombophilia and PS deficiency can increase the risk of blood clots such as Deep Vein Thrombosis (DVT) and Pulmonary Embolism (PE). In severe cases of PS deficiency, soon after birth, infants develop a life-threatening blood clotting disorder called purpura fulminans. Despite 30 years of study of this important anticoagulant, the exact role of PS is still unknown. We have recently identified a novel role of Protein S in regulating the clotting factors IXa and VIIIa independently of Activated Protein C (APC). Our recent publication in ATVB (cover page figure in October issue, 2012) demonstrated that regulation of thrombin generation via IXa/VIIIa is inhibited by Protein S. My Project aims to mechanistically define both the physiologically relevant function of PS and the specificity of PS inhibition of FIXa. We will use a multifaceted approach involving detailed in vitro, ex vivo and in vivo studies to establish that PS inhibition of FIXa is specific and physiologically significant. Based on our work, new therapeutics may be designed to target the contact point between FIX and PS, Protein S, by inhibiting hyper-functional FIX, could be used in the treatment of X-linked thrombophilia. Our project will bridge the gap in knowledge regarding the function of Protein S, and it will open a new dimension on the novel regulatory role of protein S as independent of APC and independent of tissue factor pathway inhibitor, TFPI.

B. POSITIONS AND HONORS.

Positions and Employment:

1998-2001 Post-Doctoral Fellow, University of North Carolina at Chapel Hill, Department of Biochemistry & Biophysics,

2001-2003 September, Research Associate, Dept. of Biochemistry, University of North Carolina at Chapel Hill

2003 October-2010 June, Research Assistant Professor of Biochemistry, University of North Carolina at Chapel Hill

2010 June- November 2015, Research Associate Professor of Biochemistry, University of North Carolina at Chapel Hill

2015 December- Present, Associate Professor of Biochemistry & Molecular Biology, LSU Health Science Center

Other Experience and Professional Memberships:

Professional Service:

1. Professional Journal Review: Reviewer for Blood, Journal of Thrombosis and Hemostasis, Plos One, Biochemistry, Journal of Biological Chemistry.
2. January 2013- Permanent Member of Thrombosis and Basic Science Study Section, American Heart Association
3. January 2015- March 2016- ad hoc member of the HEMA Review Committee, Department of Veterans Affairs (VA)

4. April 2016- June 2020 - **Special Government Employee** of the subcommittee for Hematology of Joint Biomedical Laboratory Research and Development and Clinical Science Research and Development Services Scientific Merit Review Board for the Department of VA

5.Co-Chaired the session “Membrane Binding and Function in Coagulation” in ISTH Meeting at Boston, 2009.

Patents:

(WO/2005/031303) Soluble Phospholipids for Use in Clotting Factor Assays
Inventors: Lentz, Barry, R.; Majumder, Rinku, Monroe, Dougald, M., (UNC Ref: OTD 03-0062), Approved in Feb, 2010

Professional Societies:

2005-Present- Member, American Society of Hematology
2008- Present- International Society of Thrombosis and Hemostasis
2012-Present- American Heart Association
2014-Present- North American Society of Thrombosis and Hemostasis

Honors and Awards:

Qualified in the nationwide (India) test, Graduate Aptitude Test for Engineering, (GATE), February 1992.

Vice Chancellor of Research Award, University of North Carolina at Chapel Hill, December 2011.

Invited Talks:

1)“Institut Jozef Stefan,” Slovenia, September 28th and 29th, 2010

1a)“Novel reagents to bypass limitations of existing clotting assays improving life of Lupus patients,” presented in Stefan Institute Colloquium, 28th September, 2010.

1b) “Regulation of Anticoagulants by Membranes in Blood Coagulation and Thrombosis,” 29th September, 2010, termed as “lecture of the Solid State Physics Department of Jozef Stefan Institute, Laboratory of Biophysics (EPR center Ljubljana), and Slovenian Biophysics Association.”

2)“Role of Protein S in Blood Coagulation,” January, 2012, Thomas Jefferson School of Medicine, Philadelphia.

3)“Protein S, A key regulatory molecule,” April, 2012, Puget Blood Center, University of Washington at Seattle, April 2012

4) Invited as a speaker in 2015 FASEB Summer Research Conference entitled “Molecular, Structural & Clinical Aspects of Vitamin K & Vitamin K Dependent Proteins” to be held from July 12th – July 17th, 2015.

5)Invited to participate and deliver a Session Talk in the "Cardiovascular Drug Discovery & Therapy" track in “Drug Discovery & Therapy World Congress 2015" (DDTWC 2015) to be held in Boston, USA from July 22-25, 2015.

C. CONTRIBUTION TO SCIENCE

`1) Identification of a novel and crucial Apyrase that is responsible for the response of the plant *Mimosa pudica* to different stimuli- *M. pudica* is a rare plant that is able to produce rapid mechanical response when stimulated by light, touch or heat. Although understanding at the cellular level had progressed, very little was known about the biochemical origin of such processes. The apyrase from M. pudica was isolated many years back but its full characterization and role in the unique physiology had remained unknown. As a graduate student in the department of Biochemistry of Bose Institute, my work for the first time characterized this apyrase and showed that this protein was associated with polysaccharide components and that this association requires Ca^2. I was able to identify a cofactor, N5,N10-methenyl tetrahydrofolate that was associated with the apyrase. We also showed that the separation of the co-factor from the enzyme leads to its inability to increase the apyrase activity upon excitation with ultraviolet-A light, but no significant loss of basal apyrase activity. This suggested that MTHF is the crucial component of the photo-stimulation phenomenon. Our result concluded that this apyrase associated with the cofactor N5,N10-methenyl tetrahy-drofolate play an important role in the light response of M. pudica. The work behind this novel observation to decipher the mechanism of the light response of M.pudica lead to the following publications,

#1) **Rinku Ghosh (Majumder)**, PC Sen, Susweta Biswas. Mimosa pudica apyrase requires polysaccharide and Ca2+ for the activity. **Mol Cell Biochem.** 1998 Oct; 187(1-2):47-55. PMID: 9788742.

#2) **Rinku Ghosh (Majumder)**, Susweta Biswas, Siddhartha Roy. An apyrase from Mimosa pudica contains N5,N10-methenyl tetrahydrofolate and is stimulated by light. **Eur J Biochem.** 1998 Dec 15; 258(3):1009-13. PMID: 9990319

2) **Elucidation of the role of phosphatidyl serine in the regulation of prothrombinase complex during blood clotting:** Blood coagulation is essential to maintaining hemostasis, i.e., a constant environment for the cells that constitute a living organism. Defects in blood coagulation are the primary cause of death in the United States. These defects often arise from a mutated protein, which is inactive or poorly regulated. Thrombin, blood coagulation’s central regulatory molecule is produced through prothrombin proteolysis by platelet factor Xa (**FXa**), which is produced by factor VIIa (**FVIIa**) and its cofactor, tissue factor (**TF**), which is in endothelial cell membranes. Factor IXa (F**IXa**) and its cofactor factor VIIIa (**FVIIIa**) bind to membranes containing exposed PS to form a complex that enhances production of FXa. Factor Xa binds its cofactor factor Va (F**Va**) on activated platelets to form the FXa-FVa complex (prothrombinase) that increases the rate of thrombin production by ~10^{5} fold. A widely-held paradigm was that reduced reaction dimensionality provided this acceleration, but my work as a post doctoral researcher in the department of Biochemistry at UNC, Chapel Hill showed that short-chain “soluble” form of PS, C6PS to establish that PS molecules, not a membrane surface, triggers this acceleration. Our lab established for the first time that prothrombinase complex formation by FXa and FVa is regulated by phosphatidyl serine molecule. My work in 2002 was published as a breakthrough paper showing that prothrombinase assembled in the presence of membrane phophatidylserine or molecular phosphatidyl setine are equally active. My further work with human plasma proteins demonstrated the activation kinetics of different intermediates like prothrombin and meizothrombin to thrombin are similar in the presence of phosphatidylserine containing membrane or molecular soluble phophatidyl serine (C6PS). This finding eluded researchers because phosphatidylserine is located in membranes where protein structure and interactions are difficult to study. My work established that C6PS could be used as a tool in understanding important kinetic reactions of coagulation that take place on platelet membranes. I was one of the inventors of this patented technology, along with Dr. Barry Lentz, at UNC. This work resulted in multiple publications from our group.

1)Mou Banerjee§, **Rinku Majumder§** , Gabriel Weinreb§ and Barry R. Lentz: Role of procoagulant lipids in human prothrombin activation. 2: Soluble phosphatidyl serine up regulates and directs factor Xa to appropriate peptide bonds in prothrombin. **Biochemistry,** 2002, 41(3): 950-957. (§- authors contributed equally to this work).

2)**Rinku Majumder,** Gabriel Weinreb, Xin Zhai & Barry R.Lentz. Soluble Phosphatidylserine Triggers Assembly in Solution of a Prothrombin Activating Complex in the Absence of a Membrane Surface. **J Biol Chem.** 2002, 277(33), 29765-73.
3)G.E.Weinreb§, K. Mukhopadhyay§, **Rinku Majumder§,** and B.R. Lentz. The Cooperative Roles of Membranes and Factor Va in the Membrane-Bound Prothrombinase Complex. **J. Biol. Chem.,** 2003, 278(8): 5679-84. (§- authors contributed equally to this work).
4)**Rinku Majumder,** Jiangfang Wang and Barry R.Lentz: Functional and structural consequences of water-soluble phosphatidylserine binding to bovine factor Xa. **Biophysical Journal**, 2003, 84 (2), 1238- 1251.
5)**Rinku Majumder,** Mary Ann Quinn-Allen, William H. Kane and Barry R.Lentz. The phosphatidylserine binding site of the factor Va C2 domain accounts for membrane binding but does not contribute to the assembly or activity of a human factor Xa-factor Va complex. **Biochemistry,** 2005, 44(2):711-718.
6)**Rinku Majumder,** G. E. Weinreb and Barry. R. Lentz. Efficient thrombin generation requires molecular phosphatidylserine, not a membrane surface. **Biochemistry,** 2005, 44(51):16998-7006.
7)**Rinku Majumder,** Mary Ann Quinn-Allen, William H. Kane & Barry R. Lentz. A Phosphatidylserine Binding Site in Factor Va C1 Domain Regulates both Assembly and Activity of the Prothrombinase Complex, **Blood,** 2008,112 (7): 2795-2802.
8) **Rinku Majumder,** Xiaoe Liang, Mary Ann Quinn-Allen, William H. Kane and Barry R. Lentz. Modulation of prothrombinase assembly and activity by phosphatidylethanolamine, J. Biol. Chem. 2011, 286: 35535-3554.

3) **Lipid regulation of clotting factors at different stages of blood coagulation:** When I became an Assistant Professor in the Department of Biochemistry at UNC, I focused my research on lipid regulation of all phases of human blood coagulation. The role of different lipids in different phases of coagulation was unexplored and my research questions were unique. Initiation of blood clotting occurs when endothelial membranes are disrupted in regions of tissue damage and trauma, producing small lipid-sheathed vesicles or endothelial microparticles (**MPs**). Phosphatidic acid (**PA**) plays an important role in endothelial cell membranes and is likely a moderately significant component of endothelial MPs. *Amplification* and *propagation* are accompanied by phosphatidylserine (**PS**) and phosphatidylethanolamine (**PE**) exposure on platelet MPs that appear during platelet activation. My work showed for the first time that FVIIa that is associated with initiation is regulated mainly by molecular phosphatidic acid. However, FIXa/VIIIa that is required for propagation and amplification is regulated by molecular PS and PE. Our hypothesis was that PS, PE, and PA bind to serine proteases and their cofactors and induce conformational changes that regulate their activities at different stages of blood coagulation. The experiments performed with molecular lipids (soluble forms of the lipids) supported our hypothesis. This work has not only enabled the coagulation field to understand lipid regulation of clotting factors at different stages of clotting, but the work has been instrumental in developing therapeutics based on the mechanism of the regulation. We have published significant papers and several manuscripts are in different stages of review.

1)Tilen Koklic, Rima Chattopadhyay, **Rinku Majumder** and Barry R. Lentz: Factor Xa dimerization competes with prothrombinase complex formation on platelet-like membrane surfaces. Biochem J. 2015 Apr 1;467(1):37-46.
2)**Rinku Majumder,** Tanusree Sengupta, Daud Cole, Rima Chattopadhyay, Subir Biswas, Dougald Monroe & Barry R. Lentz: Regulation of Clotting FIXa by Phosphatidylserine, Plos One , 2014, 9(6): e100006.
3)Tilen Koklic§, **Rinku Majumder**§ & Barry R. Lentz: Ca2+ Switches the Effect of PS-containing Membranes on Factor Xa from Activating to Inhibiting in the Range of Plasma Ca2+ Concentration.Biochemical Journal, 2014, 462 (3): 591-601. (§- both authors contributed equally)
4)Kinshuk Raj Srivasatava, **Rinku Majumder**, William H. Kane, Mary Ann Quinn-Allen, and Barry R. Lentz: Phosphatidylserine and FVa Regulate FXa Structure. Biochemical Journal, 2014, 459(1): 229–239(Selected as a Podcast)

4) **Role of the anticoagulant Protein S in maintaining hemostasis:** More recently, my research has focused on development of new, more effective therapeutics for thrombotic diseases and Hemophilia. My group was the first to discover a previously unknown function for Protein S, an anticoagulant that, despite 30 years of research by others, remained poorly characterized. We discovered that Protein S inhibits Factor IXa, which, in turn, inhibits thrombin formation. [This work was featured on the cover of the October, 2012 issue of Arteriosclerosis, Thrombosis, and Vascular Biology]. This newly recognized function of Protein S is the basis for creating new hemostasis therapies, as described below.. PS deficiency is one of several risk factors for thrombophilia, and PS deficiency can increase the risk of abnormal blood clotting such as Deep Vein Thrombosis and Pulmonary Embolism.. The inhibition of thrombin generation via Factor IXa by Protein S is being exploited to more

effectively treat Hemophilia B. Hemophilia B results from a deficiency in FIXa. Factor IXa converts prothrombin to thrombin, which forms the blood clot at an injured site. Hemophilia B is treated by infusion of FIXa to replace the missing or inactive Factor IXa in affected individuals. However, the infused FIXa has a limited therapeutic lifetime, likely in part because it is inhibited by Protein S. Thus, our studies are directed towards developing agents that inhibit the activity of Protein S. Such agents are expected to increase the therapeutic efficacy of FIXa replacement therapy for Hemophilia B patients. We are using mouse models to assess whether one inhibitor of Protein S, *i.e*., a specific Protein S antibody, prolongs FIXa therapy. We are taking an opposite approach with Protein S to develop treatments for thrombosis, diseases that result from inappropriate activation of blood coagulation. For example, thrombophilia is an X-linked disorder that results from hyperactive FIXa. We are investigating the precise mechanism by which PS inhibits FIXa with the goal of creating more effective PS-based inhibitors to alleviate thrombophilia and other hypercoagulation disorders. Our lab is currently investigating the binding site/s in FIXa for PS by using in vivo, ex vivo and in vitro methods to determine the mechanism of inhibition of FIXa by PS. Because we needed mouse FIXa to continue our animal studies, we were successful in purifying Protein S from mouse plasma by using a novel FIX Select column that is specifically designed to purify human proteins. This unique method of purification recently resulted in a publication (1). This work has resulted in couple of papers and at least 2-3 papers are currently being written.

1)Vijaya Satish Pilli, Will Plautz, Dougald Monroe & **Rinku Majumder:** A Novel One- Step Purification of Mouse Factor IX. Thrombosis Research.2016 .139: 125–126
2)Rima Chattopadhyay, Tanusree Sengupta & **Rinku Majumder** : Inhibition of Intrinsic Xase by Protein S - a novel regulatory role of Protein S independent of Activated Protein C, Arteriosclerosis, Thrombosis, and Vascular Biology, 2012, 32(10) : 2387-2393. (Selected as a cover page figure)

D. RESEARCH SUPPORT:

Current:

1R01HL118557-01A1 09/01/2014- 07/31/2018
A Novel Regulatory Role of Protein S in Blood Coagulation
Role: PI

Completed Research:

2 R01 HL72827 05A Barry Lentz (PI) 8/15/2009 - 5/31/2013

Lipid Regulation of Thrombin Generation
Role: Co-PI

R41 GM083393 S. Burgess(PI STTR 05/1/2009 -04/30/2010

Identification and Characterization of a Novel Lipid Molecule for Membrane-free Coagulation Reactions
Role: Co-PI

Institutional Funding Rinku Majumder (PI)
12/15/2010-11/30/2011

(Corrigan Endowment) Regulation of Factor IXa/VIIIa by Protein S independent of APC
Role: PI