BIOGRAPHICAL SKETCH

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NAME: Majumder, Rinku

eRA COMMONS USER NAME (credential, e.g., agency login): RINKU_MAJUMDER

POSITION TITLE: Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

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

A. Personal Statement

My research centers on Protein S (PS), a vitamin K-dependent plasma glycoprotein produced in endothelial cells and a critical regulator of coagulation. PS deficiency is a known risk factor for thrombophilia, leading to conditions such as deep vein thrombosis (DVT), pulmonary embolism (PE), and, in severe neonatal cases, purpura fulminans. Despite decades of study, the precise mechanisms of PS function remained unclear.

My expertise encompasses protein–lipid interactions, coagulation biochemistry, and therapeutic development using in vivo and in vitro systems. I have received continuous NIH funding and serve on multiple NIH and VA review panels.

My laboratory discovered that Protein S (PS) directly inhibits coagulation factors IXa and VIIIa independently of activated protein C (APC), a previously unrecognized function that we first reported in Arteriosclerosis, Thrombosis, and Vascular Biology (cover article, October 2012). We further mapped the PS-binding region on FIXa and identified a double mutant (K132A/R170A) that fails to bind PS, leading to accelerated clot formation in hemophilia B mouse models. Building on this foundation, I have recently expanded my research focus into cancer biology. In collaboration with Dr. Sam Majumder, we demonstrated that PS has potential as a dual-function therapeutic agent, capable of both inhibiting tumor progression and preventing thrombotic events. Our studies showed that intra-tumoral administration of PS resulted in a dose-dependent reduction in tumor volume and mass in a PANC-1 xenograft model using immunocompromised C57BL/6 nude mice. These findings have motivated my ongoing efforts to establish PS as a novel therapeutic with both antitumor and antithrombotic efficacy.

Current Research Support:

National Institutes of Health 1R01HL151613-01A1, 01/15/2021- 01/14//2026, “A Mechanistic Study to elucidate the role of Protein S in elevating the risk of Thrombosis in Obese, Pre-menopausal women”, **Rinku Majumder, PI**, $650,000 yearly.

National Institutes of Health 1R01HL163018-01, 04/01/2022-03/31/2026, “Protein S Regulates Blood Coagulation by Inhibiting Factor IXa, **Rinku Majumder, PI,** $530,622 yearly.

Completed Research Support:

National Institutes of Health 1R01HL118557-01A1, 09/01/2014-01/31/2021,), "A Novel Regulatory Role of Protein S in Blood Coagulation," Rinku Majumder, PI, $250,000 yearly

American Society of Hematology Bridge Funding, 05/01/2019-04/30/2021, Rinku Majumder, PI, $200,000.

Leveraging Innovation for Technology Transfer (LIFT2) Grant, The Board of Supervisors of Louisiana State University, 05/15/2020-05/15/2021, "Development of Peptide Mimic of Protein S, a Physiological Anticoagulant for Antithrombotic Therapy", Rinku Majumder, PI, $50,000 yearly.

LSU Health Science Center Alumni Foundation, COVID-19 Intramural Research Program, 10/07/2020-10/06/20221, Rinku Majumder, PI, $6,000 yearly.

National Institutes of Health STTR R41 GM083393-01A2, 05/1/2009-04/30/2010," Novel Reagents to Bypass Limitations of Existing Coagulation Assays, Steve Burgess, PI, Rinku Majumder, co-PI at the academic level (50% effort), $241,675.

Corrigan Endowment (UNC Institutional funding), 12/15/2010-11/30/2011, "Regulation of Factor IXa/VIIIa by Protein S independent of APC," Rinku Majumder, PI.

National Institutes of Health 2R01 HL72827 05A, 8/15/2009-5/31/2013, "Lipid Regulation of Thrombin Generation," Barry Lentz, PI, Rinku Majumder, co-PI (50% effort, 2009-2010, 90% effort, 2010-2013), $250,000 yearly.

Key Publications:

1) Hope, Wilson, Aliah Paaysse, Narender Kumar, Brian Cooley, Pratyayadipta Rudra, Adrianne Dorosey, Diana Polania-Villanueva, Sabyasachi Chatterjee, Maisa Janbain, Maria Velez and Rinku Majumder. Protein S antibody as an adjunct therapy for hemophilia B. Blood Adv. 2024 Jan 23;8(2):441-452. PubMed PMID: 37773781.
2) Rinku Majumder, T. Nguyen. (2021) Protein S: function, regulation, and clinical perspectives. Curr Opin Hematol. 2021 Sep 1;28(5):339-344. Pubmed PMID: 34224431.
3) Will E. Plautz, Vijaya Satish Pilli, Brian C. Cooley, Rima Chattopadhyay, Pamela R. Westmark, Todd Getz, David Paul, Wolfgang Bergmeier, John P. Sheehan and Rinku Majumder. Anticoagulant Protein S Targets the Factor IXa Heparin-Binding Exosite to Prevent Thrombosis. Arterioscler Thromb Vasc Biol. 2018 Apr; 38(4):816-828. PubMed PMID: 29419409.
4) Rima Chattopadhyay, Tanusree Sengupta and Rinku Majumder: Inhibition of Intrinsic Xase by Protein S - a novel regulatory role of Protein S independent of Activated Protein C. Arterioscler Thromb Vasc Biol. 2012 Oct; 32(10):2387-2393. PubMed PMID: 22904276. (Selected as a cover page figure)

B. Positions, Scientific Appointments, and Honors

Academic Employment

1998 – 2001	Post-Doctoral Fellow, University of North Carolina at Chapel Hill, Department of Biochemistry & Biophysics
2001 – Sept 2003	Research Associate, Dept. of Biochemistry, University of North Carolina at Chapel Hill
Oct 2003 – June 2010	Research Assistant Professor of Biochemistry, University of North Carolina at Chapel Hill
June 2010 – Nov 2015	Research Associate Professor of Biochemistry, University of North Carolina at Chapel Hill
Dec 2015 – June 2021	Associate Professor of Biochemistry & Molecular Biology, LSU Health Science Center
July 2021- Present	Professor of Biochemistry & Molecular Biology, LSU Health Science Center
August 2022-Present	Professor of Interdisciplinary Oncology, LSU Health Science Center

Honors and Awards

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

Vice Chancellor of Research Award, University of North Carolina, 2011.

Invited Chair at the 9th Symposium in Hemostasis, April 2018. The session chaired was "The Clotting Factor Countdown".

Invited Chair at the 58th American Society of Hematology Conference, November 2017. The session chaired was “Blood Coagulation and Fibrinolytic Factors: Factor VIII/IX and Hemophilia.”
Invited Chair at the 60th American Society of Hematology Conference, November 2018. The session chaired was “Blood Coagulation and Fibrinolytic Factors: Structural Biology of Coagulation Proteins.”
Invited Chair at the North American Society of Hematology Symposium, May 2019. The session chaired was, “Targeting the Platelet: Promising New Therapies, Sometimes Unwanted Side Effect”.
American Society of Hematology Bridge Grant Award, 2019
Invited Chair at the ISTH 2021 Congress, July 2021. The season chaired was, “Regulation of Activated Protein C and Factor V”.
Invited Platform Speaker at the 10th Symposium on Hemostasis: Coagulation, Platelets and Transfusion Medicine, April 2022.
Invited to present **State of the Art Lecture** in ISTH 2022 Congress, July 2022.
Invited to present in the seminar series at Children’s Hospital of Philadelphia, PA, April 2024.
Invited Chair at the International Society on Thrombosis and Hemostasis (ISTH) Congress, June 2024 in the SSC session “Physiological Anticoagulants and Thrombophilia”.
Other

NIH Study Sections
Hemostasis, Thrombosis, Blood Cells and Transfusion (HTBT) Study Section, NHLBI, R01. Standing Member, 2021-Present.
Hemostasis, Thrombosis, Blood Cells and Transfusion (HTBT) Study Section, NHLBI, R01. Ad hoc reviewer, 2020-2021.
Hemostasis and Thrombosis (HT) Study Section, NHLBI, R01. Ad hoc reviewer, 2016-2020.
Special Emphasis Panel/Scientific Review Group 2017/05 ZRG1 VH-B (02) M, NHLBI- R01. Ad hoc reviewer, 2017 – present.
ZRG1 VH-C (80) A Study Section (Vascular and Hematology AREA application review), NHLBI, R15. Ad hoc reviewer, 2016-present.

Veterans Affairs
Ad hoc member of the HEMA Review Committee, Department of Veterans Affairs, 2021-Present
Ad hoc member of the HEMA Review Committee, Department of Veterans Affairs, 2015-2016
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 Veteran Affairs, 2016-2020.

Reviewer for Blood, Journal of Thrombosis and Hemostasis, PLOS ONE, Biochemistry, Journal of Biological Chemistry, Science Reports, Journal of Theoretical Biology and Cardiovascular Toxicology.
Abstract Reviewer for American Society of Hematology Meeting, 2016-present
Abstract Reviewer for International Society of Thrombosis and Hemostasis Meeting, 2022 February-Present.

C. Contributions 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 tetrahydrofolate plays 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)**, 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. PubMed PMID: 9990319.
2) **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. PubMed PMID: 9788742.

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 ~$10^{\mathbf{5}}$ 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) **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 Oct 14; 286: 35535-3554. PubMed PMID: 21859710; PubMed Central PMCID: PMC3195639.
2) **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 Oct 1; 112 (7): 2795-2802. PubMed PMID: 18587009; PubMed Central PMCID: PMC2556615.
3) **Rinku Majumder,** Gabriel E. Weinreb and Barry. R. Lentz. Efficient thrombin generation requires molecular phosphatidylserine, not a membrane surface. Biochemistry. 2005 Dec 27; 44(51):16998-7006. PubMed PMID: 16363813.
4) **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 Jan 18; 44(2):711-718. PubMed PMID: 15641797.

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) Tanusree Sengupta, Tilen Koklic, Barry R. Lentz and **Rinku Majumder**: Phosphatidylserine and phosphatidylethanolamine regulate the structure and function of FVIIa and its interaction with soluble tissue factor. Bioscience Reports. 2021 Feb 26; 41(2): BSR20204077. PubMed PMID: 33479740.
2) 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. PubMed PMID: 25572019.
3) **Rinku Majumder,** Tilen Koklic, Tanusree Sengupta, Daud Cole, Rima Chattopadhyay, Subir Biswas, Dougald Monroe and Barry R. Lentz: Soluble Phosphatidylserine Binds to Two Sites on Human Factor IXa in a Ca^{2+} Dependent Fashion to Specifically Regulate Structure and Activity. PLoS One , 2014; 9(6): e100006. PubMed PMID: 24979705; PubMed Central PMCID: PMC4076177.

4) Role of the anticoagulant Protein S in maintaining hemostasis: More recently, my research has focused on the 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 resulted in a publication (3).

1) **Rinku Majumder**, T. Nguyen. (2021) Protein S: function, regulation, and clinical perspectives. Curr Opin Hematol. 2021 Sep 1;28(5):339-344. Pubmed PMID: 34224431

2) Vijaya Satish Pilli, Arani Datta, Sadaf Afreen, D. Catalano, Gyongyi Szabo and **Rinku Majumder**. Hypoxia downregulates protein S expression. Blood. 2018 Jul 26;132(4):452-455 (Selected as a featured article on the cover page) PubMed PMID: 29784640.

3) Will E. Plautz, Vijaya Satish Pilli, Brian C. Cooley, Rima Chattopadhyay, Pamela R. Westmark, Todd Getz, David Paul, Wolfgang Bergmeier, John P. Sheehan and **Rinku Majumder.** Anticoagulant Protein S Targets the Factor IXa Heparin-Binding Exosite to Prevent Thrombosis. Arterioscler Thromb Vasc Biol. 2018 Apr; 38(4):816-828. PubMed PMID: 29419409.

4) Rima Chattopadhyay, Tanusree Sengupta and **Rinku Majumder**: Inhibition of Intrinsic Xase by Protein S - a novel regulatory role of Protein S independent of Activated Protein C. Arterioscler Thromb Vasc Biol. 2012 Oct; 32(10):2387-2393. PubMed PMID: 22904276. (**Selected as a cover page figure**)

Complete list of Publication: https://pubmed.ncbi.nlm.nih.gov/?term=Rinku+Majumder&sort=date