BIOGRAPHICAL SKETCH

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NAME: Kolyadko, Vladimir N

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

POSITION TITLE: Research Associate

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)	END DATE MM/YYYY	FIELD OF STUDY
Lomonosov Moscow State University, Moscow	MS	01/2010	Physics
Emanuel Institute of Biochemical Physics, Moscow	PHD	04/2017	Biophysics
Children's Hospital of Philadelphia, Philadelphia, Pennsylvania	Postdoctoral Fellow	present	Biochemistry & Structural Biology

A. Personal Statement

My research in structural biology and protein biochemistry of the blood coagulation system is focused on the molecular recognition and enzymatic activity regulation. My postdoctoral training in the Dr. Sriram Krishnaswamy laboratory at the Children's Hospital of Philadelphia and my immersion into the vibrant structural biology community at CHOP/UPENN give me perspective on the structural approach to explain complex coagulation reactions in terms of individual intermolecular interactions, binding events, and induced allosteric effects. I use X-ray crystallography and cryo-electron microscopy complemented by biophysical measurement of binding affinity and enzymatic activity to reveal the atomic details of function of coagulation factor IXa. My studies revealed that the FIXa active site exhibits latency and plasticity, which underlie its allosteric regulation mediated by the exosite in catalytic domain through reversible opening and closing of the substrate-binding pockets. Based on structural comparisons of different coagulation proteinases, such as FIXa, FXa, and thrombin, my studies indicated the exosite as a molecular surface that has unique features individual for each proteinase. Thereby, targeting the exosite can result in the high selectivity of proteinase recognition and may contribute to the mechanism of a specific proteinase-cofactor recognition in blood coagulation. Intriguingly, my studies also uncovered the paradox of a bidirectional allosteric regulation of FIXa. In the current project, I aim to study membrane-assembled complexes of FIXa by cryo-EM and address the mechanism of FVIIIa-mediated activity regulation. Utilizing my recent advances in cryo-EM of liposome-bound proteins and exploiting direct comparisons with functional studies carried out on the same liposomes, I will determine the individual contribution of the template and allosteric mechanisms in the cofactor-mediated upregulation of FIXa. I hypothesize that FVIIIa, when bound to membrane, provides an exosite for a specific substrate binding and, once the intrinsic Xase is fully assembled, FIXa possibly undergoes allosteric activation via exosite-mediated opening of the active site, which is followed by substrate cleavage. This application is seeking support in my training in the advanced methods of cryo-EM and the transition to an independent faculty position. Its outcome may bring a new understanding of enzymatic activity regulation and conceptualize the design of macromolecules with a prerequisite impact on blood clotting, in particular, targeted allosteric modulators for the treatment of coagulation-related disorders. This project may also lead to the structural studies of natural anticoagulant mechanisms that engage endothelial membrane receptors and maintain hemostatic balance in the circulation.

1. Kolyadko VN, Layzer JM, Perry K, Sullenger BA, Krishnaswamy S. An RNA aptamer exploits exosite-dependent allostery to achieve specific inhibition of coagulation factor IXa. Proc Natl Acad Sci U S A. 2024 Jul 16;121(29):e2401136121. PubMed Central PMCID: PMC11260126.

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

2021 -	Research Associate, CHILDREN'S HOSP OF PHILADELPHIA, Philadelphia, PA
2020 - 2024	Early Career Reviewer, The Journal of Biological Chemistry
2018 - 2021	Postdoctoral Fellow, CHILDREN'S HOSP OF PHILADELPHIA, Philadelphia, PA
2013 - 2015	Lab Instructor, Lomonosov Moscow State University, School of Physics, Department of Biophysics, Moscow
2012 - 2014	Research Fellow, Hemacore, LLC., R&D department, Moscow
2011 - 2011	Exchange Visitor, Waksman Institute of Microbiology, Rutgers the State University of New Jersey, Piscataway, NJ
2010 - 2017	Graduate Research Assistant, Center for Theoretical Problems of Physico-Chemical Pharmacology RAS, Moscow
2009 - 2009	Visiting Student, Max Delbrueck Center for Molecular Medicine, Berlin

Honors

2024	George W. Raiziss Postdoctoral Pilot Award, Raymond and Ruth Perelman School of Medicine, the University of Pennsylvania
2023	Abstract Award, Hemostasis and Thrombosis Research Society Scientific Symposium; Orlando, FL
2016	Top 100 Inventions in Russia - 2015, Russian Patent Office (Rospatent)
2014	Best Oral SSC Session Award, 60th Annual Meeting of the SSC ISTH; Milwaukee, WI
2014	Trainee Travel Award, XXVII International Symposium on Laboratory Hematology; the Hague, Netherlands
2014	2nd place Diploma, II Congress of Hematologists of Russia
2011	Developing World Scientists Award, XXIII Congress of the ISTH; Kyoto, Japan
2010	B.S./M.S. awarded with high honors, Lomonosov Moscow State University
2004	3rd place Diploma, Moscow City Olympiad in Physics
2003	2nd place Diploma, Moscow City Olympiad in Physics

C. Contribution to Science

1. My early scientific work was focused on the contact pathway and the phenomenon of procoagulant activity of blood platelets. Coagulation reactions are catalyzed by assemblies of protein complexes on the membranes of activated platelets and endothelial cells, containing phosphatidylserine on the extracellular side. Several contradictory mechanisms were suggested for the development of a subpopulation of procoagulant platelets. I showed that the transglutaminase-catalyzed cross-linking of procoagulant proteins may play a role in their appearance and presented this work as a poster at ISTH Congress in Kyoto in 2011. Further research demonstrated the prevalence of the transglutaminase-independent mechanisms in the formation of specific morphological features enriched in phosphatidylserine. As a part of my PhD studies, I investigated the sequence specificity in coagulation proteinases towards canonical inhibitors, with a practical goal to create a highly selective inhibitor of FXIIa that has no effect on other homologous proteinases. Highly selective inhibitor of FXIIa could benefit the storage of blood plasma for transfusion or potentially prevent blood clotting on the surfaces of medical devices upon surgical interventions. I optimized the sequence of infestin-4 inhibitor based on the sequences of FXIIa physiologic substrates and created the mutant with increased potency to FXIIa and diminished off-target effects on FXa and FIXa. I applied the mutant as a reagent in the novel diagnostics assay of thrombodynamics, which has a potential to discriminate various pathological states of the blood coagulation system and currently is utilized as a research tool to monitor anticoagulant therapy. This research formed the basis for two issued patents and was presented as oral talks at the ISLH congress and the SSC ISTH meeting in 2014.

a. Kolyadko VN, Lushchekina SV, Vuimo TA, Surov SS, Ovsepyan RA, Korneeva VA, Vorobiev II, Orlova NA, Minakhin L, Kuznedelov K, Severinov KV, Ataullakhanov FI, Panteleev MA. New Infestin-4 Mutants with Increased Selectivity against Factor XIIa. PLoS One. 2015;10(12):e0144940. PubMed Central PMCID: PMC4684401.
b. Korneeva VA, Trubetskov MM, Korshunova AV, Lushchekina SV, Kolyadko VN, Sergienko OV, Lunin VG, Panteleev MA, Ataullakhanov FI. Interactions outside the proteinase-binding loop contribute significantly to the inhibition of activated coagulation factor XII by its canonical inhibitor from corn. J Biol Chem. 2014 May 16;289(20):14109-20. PubMed Central PMCID: PMC4022879.
c. Abaeva AA, Canault M, Kotova YN, Obydennyy SI, Yakimenko AO, Podoplelova NA, Kolyadko VN, Chambost H, Mazurov AV, Ataullakhanov FI, Nurden AT, Alessi MC, Panteleev MA. Procoagulant platelets form an α-granule protein-covered "cap" on their surface that promotes their attachment to aggregates. J Biol Chem. 2013 Oct 11;288(41):29621-32. PubMed Central PMCID: PMC3795260.

2. As a postdoc, I am studying the structure-function of the intrinsic Xase complex involving coagulation factors IXa, VIIIa, and X. This complex provides a spatial and temporal control of the hemostatic response to injury. Despite the extensive efforts to reveal the molecular mechanism of how FIXa gains its catalytic function, it is still unknown whether its cofactor acts allosterically on the proteinase active site or just serves as a template to bind and coordinate the substrate. Biochemical and structural studies in this field are limited due to the inherently low activity of FIXa, the lack of high-affinity inhibitors with known mode of action, and the requirement for membrane surfaces to bind FVIIIa. All available crystal structures of FIXa had a stabilizing substrate mimetic in the FIXa active site, which dimmed the insights into the native proteinase state. I have resolved crystal structures of FIXa (PDB IDs: 8EPK; 8EPH; 8EPC) corresponding to its conformational ensemble, which provided a direct evidence of the allosteric regulation of FIXa through its catalytic domain exosite. The first crystal structure of FIXa in the apo-state (PDB ID: 8EPH) demonstrates a latent conformation of its active site, where its substrate-binding S1 pocket is closed by Trp215 side chain. In contrast, apo-thrombin and apo-factor Xa have an open, ready to-bind-substrate conformations of the respective active site, which explains a 10-1000 lower activity of FIXa vs. thrombin or FXa. Thus, the observation of the FIXa active site in a latent conformation contributes to the field of thrombosis and hemostasis by resolving a long-standing question of the extremely low baseline activity of FIXa. I communicated this work as oral talks at the ISTH Congress in Philadelphia in 2021 and Gordon Seminar on Hemostasis in 2022. In addition, my studies recognize the exosite of coagulation proteinases as the molecular surface that exhibits a specific landscape individual for each proteinase and, therefore, can be targeted by selective ligands. Exemplified by RNA aptamers to thrombin or FIXa, such ligands show utility as potent anticoagulants that can be reversed by complementary nucleic acid sequences. Intriguingly, my studies indicate the possibility of a bidirectional allosteric regulation of FIXa by the exosite ligands, such as FVIIIa, mim8 antibody, DTRI-177 aptamer and heparin as known examples. The idea of a bidirectional activity control is also supported by the known missense mutations of Arg170, which is a part of the exosite, causing either bleeding or prothrombotic phenotypes. This idea makes the exosite an attractive target for the development of novel therapeutics.

a. Kolyadko VN, Layzer JM, Perry K, Sullenger BA, Krishnaswamy S. An RNA aptamer exploits exosite-dependent allostery to achieve specific inhibition of coagulation factor IXa. Proc Natl Acad Sci U S A. 2024 Jul 16;121(29):e2401136121. PubMed Central PMCID: PMC11260126.
b. Yu H, Kumar S, Frederiksen JW, Kolyadko VN, Pitoc G, Layzer J, Yan A, Rempel R, Francis S, Krishnaswamy S, Sullenger BA. Aptameric hirudins as selective and reversible EXosite-ACTive site (EXACT) inhibitors. Nat Commun. 2024 May 10;15(1):3977. PubMed Central PMCID: PMC11087511.

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https://www.ncbi.nlm.nih.gov/myncbi/vladimir.kolyadko.4/bibliography/public/

BIOGRAPHICAL SKETCH

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NAME: **KRISHNASWAMY, Sriram**

eRA COMMONS USER NAME: **KRISHNASWAMY**

POSITION TITLE: **Professor of Pediatrics and Stokes Investigator**

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
Syracuse University, Syracuse, N.Y.	B.S.	06/1979	Biology
Syracuse University, Syracuse, N.Y.	Ph.D.	06/1984	Biochemistry
University of Vermont, Burlington, VT	Post. Doc.	06/1987	Biochemistry

A. Personal Statement

Research in my laboratory focuses on the enzymology, protein chemistry and physical biochemistry of the reactions of blood coagulation. Our goal is to employ physical measurements to establish the thermodynamic and kinetic features of the membrane-dependent assembly of coagulation enzyme complexes. We then apply this information to strategies designed to uncover the mechanistic bases for enzyme complex function. The two main themes of investigation relate to the basis by which distinctive substrate specificity is achieved by the structurally homologous proteinases of coagulation and the mechanisms by which cofactor proteins profoundly enhance reaction rate. Our findings in these two areas have substantially changed the entrenched thinking in the field and have established a role for exosite-dependent substrate recognition as a central determinant of coagulation enzyme function. Our more recent studies establish an unexpected role for the zymogen to proteinase transition and the ability of the coagulation proteinases to interconvert between zymogen-like and proteinase-like states in the regulation of enzyme function. Our main areas of investigation are 1) The enzymology of prothrombinase; 2) Coagulation enzyme complex assembly and function on natural membranes and in vivo; 3) Mechanisms of proteinase regulation; 4) Structural biology using x-ray crystallography, small angle x-ray scattering and cryo-EM to understand structure-function correlates in the coagulation enzymes. Papers or review articles relevant to these major areas are cited below.

Ongoing research projects I would like to highlight:
P01 HL139420-06 (Program Director: Krishnaswamy, S) 09/01/18-06/30/29
NIH/NHLBI
Hemostasis and Thrombosis: Chemistry, Biology and Physiology
Project 1*: Macromolecular Substrate Recognition in Blood Coagulation* (Project Lead: Krishnaswamy, S)
The aims of this project are directed towards understanding the basis for substrate specificity and cofactor function in prothrombinase and the other membrane-dependent enzyme complexes of coagulation.
Core A*: Administrative Core* (Core Director: Krishnaswamy, S)
This core provides administrative support and oversight to the program.
Core B*: Molecular Biology, Protein Expression and Structural Biology* (Core Director: Krishnaswamy, S)
This core provides support for recombinant protein expression, molecular biology, x-ray crystallography, SAXS, cryo-EM and HDX to the program.

Citations:

a) **Krishnaswamy S**. Exosite-driven substrate specificity and function in coagulation. *J Thromb Haemost* 2005;3:54-67. [[PMID15634266]]
b) Ivanciu L, **Krishnaswamy S**, Camire RM. New insights into the spatio-temporal localization of prothrombinase in vivo. *Blood* 2014;124:1705-14. [PMC4162104]

c) **Krishnaswamy S**. The transition of prothrombin to thrombin. *J Thromb Haemost* 2013;11:265-76 [PMC3713535]
d) Gunaratne R*, Kumar S*, Frederiksen JW, Stayrook S, Lohrmann J, Perry K, Bompiani-Myers KM, Thalji NK, Ho MD, Arepally G, Camire RM, **Krishnaswamy S**, Sullenger BA. Multimodal inhibition of Factor X/Xa for potent, reversible anticoagulation. *Nat Biotech* 2018;36:606-13. [PMC6349032]

B. Positions, Scientific Appointments and Honors

Positions and employment

2023- Member, Institute for Structural Biology, Univ. of Pennsylvania
2014- Professor (Secondary), Department of Biochemistry and Biophysics, Univ. of Pennsylvania
2011- Professor, Department of Pediatrics, Univ. of Pennsylvania, Philadelphia, PA
1998-2011 Associate Professor, Department of Pediatrics, Univ. of Pennsylvania, Philadelphia, PA
1998- Stokes Investigator, Children's Hospital of Philadelphia, Philadelphia, PA
1997-1998 Associate Professor, Dept. of Medicine, Emory University, Atlanta, GA
1990-1997 Assistant Professor, Dept. of Medicine, Emory University, Atlanta, GA
1989-1990 Assistant Professor, Dept. of Biochemistry, University of Vermont
1987-1989 Research Assistant Professor, Dept. of Biochemistry, University of Vermont

Other experience and professional memberships

Member (ex-officio), ISTH Committee on Awards and Lectures, 2021-2022
Member (ex-officio), ISTH Council, 2020-2022
Member, Annual Congress Committee, ISTH, 2018-2022
Member, Editorial Board, Blood, 2015-2018
Member, Scientific Subcommittee on Hemostasis, American Society of Hematology, 2017-2019, Chair 2019
Member, Editorial Board, Journal of Biological Chemistry, 2008-2013, 2015-2020
Member, Committee on Appointments and Promotions, Pediatrics, University of Pennsylvania, 2016-2023
Member, Editorial Board, Journal of Thrombosis and Haemostasis, 2015
Member, Brinkhous Award Committee, ATVB, American Heart Association, 2012-2016
Chair, Program Committee, ISTH 2009, Coagulation and Fibrinolysis (Basic Sciences)
NIH, NHLBI U54, R35, LRP, Catalyze Special Emphasis Panels 2011, 2013, 2016, 2022
Member, 2005-2009, Ad Hoc 2010-2012, NIH, Heart, Lung and Blood Program Project Study Section (HLBP)
Ad Hoc Member and periodic Chair, NIH HT/HTBT Study Section SEPs, 2004-2012, 2019, 2020, 2025
Member 1999-2003, Ad Hoc 1998, 2004, 2005, NIH Hem 1/HT Study Section
Member, 1999-2003, Chair 2002-2003, American Heart Association, Mid-Atlantic Peer Review Committee
Ad Hoc Member, NHLBI Program Project Ad Hoc Review Committees, 1995-2016
Member, ASH Scholar Study Section, American Society of Hematology, 2004, 2010-2011
Member, Affiliate Study Group A, American Heart Association, 1997
Member, Thrombosis Study Section, American Heart Association, 1992-1997

Honors

Keynote Speaker, 2022 Earl Davie Symposium, University of British Columbia
Congress President, 2021 Congress of the ISTH, Philadelphia
Beutler Lecture and Prize, American Society of Hematology, 2019
14th Mosesson Lecture, Blood Center of Wisconsin, Milwaukee, 2015
BACH Investigator Recognition Award, ISTH, 2015
Sol Sherry Distinguished Lecture in Thrombosis, American Heart Association, 2014
Faculty Mentor Award, Children’s Hospital of Philadelphia, 2014
ATVB Special Recognition Award in Thrombosis, American Heart Association, 2013
7th Stuart Stone Memorial Lecturer, FASEB Conference “Proteases in Hemostasis and Vascular Biology”, 2011
Gordon Research Conference on Hemostasis, Vice Chair 2010, Chair 2012
FASEB Summer Conference, Proteases in Hemostasis and Vascular Biology, Vice Chair 2005, Chair 2007
Kenneth Brinkhous Young Investigator Prize in Thrombosis, American Heart Association, 1989
Alexander Gourevitch Award for Excellence in Graduate Research, Syracuse University, 1984

C. Contributions to Science

1. Physical studies of prothrombinase assembly. Since my postdoctoral work and my first faculty position, I have been committed to the use of physical biochemistry, fluorescence spectroscopy, rapid kinetics and equilibrium binding measurements to resolve the mechanisms underlying the membrane-dependent assembly of prothrombinase. Our commitment in this area for the past 25 years has been a driving force in moving the field past inferences dominated by measurements of enzyme function to the development, maturation and testing of models of enzyme complex assembly that have, thus far, stood the test of time. We have been able to resolve most of the stepwise binding and rate constants and have also established a major role for linkage in the membrane-mediated interactions between Xa and Va. These advances have moved the field beyond thinking along the lines of receptor-mediated interactions, they provide the physical bases for structure-function studies and serve as a paradigm for the understanding of how the coagulation enzymes assemble.

    a) **Krishnaswamy S.** Prothrombinase complex assembly. Contributions of protein-protein and protein-membrane interactions toward complex formation. *J Biol Chem* 1990;265:3708-18. [PMID: 2303476]
    b) Boskovic DS, Troxler T, **Krishnaswamy S.** Active site-independent recognition of substrates and product by prothrombinase. A fluorescence resonance energy transfer study. *J Biol Chem* 2004;279:20786-93. [PMID14988397]
    c) Buddai SK, Lu G, Layzer J, Rusconi CP, Sullenger BA, Monroe D, **Krishnaswamy S**. An anticoagulant RNA aptamer that inhibits proteinase-cofactor interactions within prothrombinase. *J Biol Chem* 1010;285:5212-23 [PMC2820749]
    d) Soule, E.E., Yu, H., Olson, L., Naqvi, I, Kumar, S., **Krishnaswamy, S.,** Sullenger, B.A. (2022) Generation of an Anticoagulant Aptamer that Targets Factor V/Va and Disrupts the FVa-Membrane Interaction in Normal and COVID-19 Patient Samples. Cell Chem. Biol., doi.org/10.1016/j.chembiol.2022.01.009

2. Enzymology of prothrombin activation. When I began as a postdoctoral fellow, the process of prothrombin activation was viewed as a mature, fully worked out and not worthy of more attention. I was advised to work on other problems. This myth quickly fell away with some of the first experiments that I did documenting the ordered activation of prothrombin via the formation of meizothrombin which was previously not considered to occur. In the last 30 years, we have made major advances in understanding the process of prothrombin activation, we have uncovered the previous unappreciated complexity associated with satisfactorily interpreting the kinetics of product formation when cleavage is required at two (or more) sites in the substrate. Appropriate recombinant tools to resolve this problem have turned many of the widely accepted ideas in the 1980s-2005 on their heads. We have also made major advances in understanding how membrane binding by prothrombin and its activation intermediates affects function causing major revisions to ideas that have predominated since 1984. This has been a very productive area for scientific advances and continues to be so.

    a) **Krishnaswamy S**, Church WR, Nesheim ME, Mann KG. Activation of human prothrombin by human prothrombinase. Influence of factor Va on the reaction mechanism. *J Biol Chem* 1987;262:3291-9. [PMID3818642]
    b) Orcutt SJ, **Krishnaswamy S**. Binding of substrate in two conformations to human prothrombinase drives consecutive cleavage at two sites in prothrombin. *J Biol Chem* 2004;279:54927-36. [PMI15494418]
    c) Bradford HN, Miccuci JA, **Krishnaswamy S**. Regulated Cleavage of Prothrombin by Prothrombinase. Repositioning a cleavage site reveals the unique kinetic behavior of the action of prothrombinase on its compound substrate. *J Biol Chem* 2010;285:328-38. [PMC2804180]
    d) Bradford HN, Orcutt SJ, **Krishnaswamy S**. Membrane binding by prothrombin mediates its constrained presentation to prothrombinase for cleavage. *J Biol Chem* 2013;288**:**27789-800 [PMC3784695]

3. Exosite-dependent substrate recognition. Mechanisms underlying protein substrate specificity lie at the heart of the coagulation reactions. This becomes an important problem it raises the question of how these structurally homologous proteinases of coagulation achieve such distinctive specificities (an enzymological question) and how determinants of specificity can be exploited for the development of therapeutic antithrombotics. Unfortunately, the field has been dominated by active site-centric thinking drawing from

ideas developed in early studies with trypsin and chymotrypsin. These ideas have been persistent despite the fact there has been little evidence to support any peptidyl-sequence preference for cleavage site specificity beyond the P1 arginine for prothrombinase. We have made major strides in establishing the importance of exosite-binding and not active site docking in determining the action of prothrombinase on prothrombin. These studies, which began in the late 90s, have resulted in a series of new ideas and concepts underlying the determinants of substrate specificity and enzyme function.

a) Orcutt S, Pietropaolo C, **Krishnaswamy S**. Extended interactions with the macromolecular substrate enforce affinity and specificity in prothrombinase function. *J Biol Chem* 2002;277:46191-6. [PMID12370181]
b) Hacisalihoglu A, Panizzi P, Bock, PE, Camire RM, **Krishnaswamy S**. Restricted active site docking by enzyme-bound substrate enforces the ordered cleavage of prothrombin by prothrombinase. *J Biol Chem* 2007;282:32974-82. [PMC2292459]
c) Basavaraj, M.G. and **Krishnaswamy, S.** (2020) Exosite binding drives substrate affinity for the activation of coagulation factor X by the intrinsic Xase complex. *J. Biol. Chem.* **295,** 15198-15207 doi:10.1074/jbc.RA120.015325. Highlighted as Editors Pick by Oliva, M.L.V, Dreveny, I., Elmsley, J. *J. Biol. Chem.* **295,** 15208-15209
d) Yu, H., Kumar, S., Frederiksen,J.W., Kolyadko, V.N., Pitoc, G., Layzer, J., Yan, A., Rempel, R., Francis, S., **Krishnaswamy, S**.*, Sullenger, B.A.* (Co-corresponding authors) (2024) Aptameric hirudins as potent, selective, and reversible EXosite-ACTive site (EXACT) inhibitors. Nature Comm. 15, 3977 https://doi.org/10.1038/s41467-024-48211-6

4. Zymogen to proteinase transition and allostery. Our interest in the zymogen to proteinase transition, grounded in studies of prothrombin activation, has come to the fore in the last 10 years. Initial findings indicated that this was a key process in the sequential presentation of the bonds in prothrombin for cleavage by prothrombinase. We were particular struck by the unexpected ability to manipulate this transition for the purposes of testing ideas related to prothrombin activation. Since then, our studies have established that although thrombin formation results from irreversible cleavage, the product can reversibly interconvert between zymogen-like and proteinase-like forms depending on the occupation of anion binding exosite 1 and anion binding exosite 2 by natural ligands. This provides a new framework for the consideration of thrombin allostery that has been dominated by the confusing lexicon of “slow”, “fast”, “E”, “E*” and Na^+ bound and free states of thrombin all with dubious relevance to physiology. Our work not only provides a simple framework based on physical studies for the problem of proteinase allostery but also reveals new approaches to understand proteinase regulation.

   a) Kamath P, Huntington JA, **Krishnaswamy S**. Ligand binding shuttles thrombin along a continuum of zymogen-like and proteinase-like states. *J Biol Chem* 2010;285:28651-8. [PMC 2937891]
   b) Bradford HN, **Krishnaswamy S**. Meizothrombin is an unexpectedly zymogen-like variant of thrombin. *J Biol Chem* 2012;287:30914-25 [PMC3436291]
   c) Bradford HN, **Krishnaswamy S**. The fragment 1 region of prothrombin facilitates the favored binding of fragment 12 to zymogen and enforces zymogen-like character in the proteinase. *J Biol Chem* 2016;291**:**1114-23 [PMC4900261]
   d) Bradford, HN, **Krishnaswamy S**. Occlusion of anion binding exosite 2 in meizothrombin explains its impaired ability to activate factor V. *J Bio Chem* 2019;294:2422-35 [PMC6378960]

5. The other coagulation enzymes and biological insights. Over the years, we have exploited every opportunity to draw from our experimental strategies developed for studies with prothrombinase to investigate key features of the other coagulation enzymes and to study these reactions in more biologically relevant settings. Thus far, the emphasis has been on the extrinsic Xase and to a limited extent the thrombin-thrombomodulin complex. Much of this work has shed new and unexpected light on these enzymes and would not like have been investigated were it not for concepts, tools and strategies developed with prothrombinase.

a) Lu G, Chhum S, **Krishnaswamy S**. The affinity of protein C for the thrombin-thrombomodulin complex is determined in a primary way by active site-dependent interactions. *J Biol Chem* 2005;280:15471-8. [PMID15705565]
b) Lechtenberg BC, Murray-Rust TA, Johnson DJD, Adams TE, **Krishnaswamy S**, Camire RM, Huntington JA. Crystal structure of the prothrombinase complex from the venom of Pseudonaja textilis. *Blood* 2013;122**:**2777-83. [PMC3798893]
c) Thalji NK, Ivanciu L, Davidson R, Gilmotty PA, **Krishnaswamy S**, Camire RM. A rapid pro-hemostatic approach to overcome direct oral anticoagulants: hemostatic efficacy and unexpected mechanism of action. *Nat Med* 2019;22:924-32. [PMID27455511]
d) Kolyadko, V.N., Layzer, J.M., Perry, K.H., Sullenger, B.A., **Krishnaswamy, S.** (2024) An RNA Aptamer Exploits Exosite-Dependent Allostery to Achieve Specific Inhibition of Coagulation Factor IXa. *Proc. Natl. Acad. Sci. USA,* **121**, e2401136121 DOI: https:/doi.org/10.1073/pnas.2401136121

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