## BIOGRAPHICAL SKETCH

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NAME: **Bassem M. Mohammed**

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

POSITION TITLE: **Postdoctoral Fellow**

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 |
|---|---|---|---|
| Cairo University, School of Pharmacy, Cairo, Egypt | B.Sc. | 7/2007 | Pharmacy |
| Virginia Commonwealth University, School of Pharmacy, Richmond, VA, USA | PhD | 4/2015 | Hemostasis & thrombosis |
| Virginia Commonwealth University, School of Pharmacy, Richmond, VA, USA | Postdoc/R25 NIH Trainee | 8/2016 | Glyco-Biology |
| Vanderbilt University Medical Center, TN, USA | Postdoc | 8/2019 | Hemostasis & thrombosis |
| Washington University School of Medicine, St. Louis, MO, USA | Scientist | 11/2020 | B-cell immunology |
| St. Louis University School of Medicine, MO, USA | Postdoc | Current | Coagulation protein structural enzymology |

### A. Personal Statement

I am a pharmacist by trade who is interested in the science of hematology. I aim to pursue an academic science career in the physiology of blood coagulation and related pathologies and promote development of new therapeutics to treat coagulopathies. I have a multidisciplinary background in pharmaceutical sciences, with specific expertise in evaluating hemostasis, studying coagulation proteins interactions and elucidating structure-activity relationships.

During my PhD training, I was part of clinical studies that investigated new treatments for hemophiliacs and trauma patients. I also contributed to preclinical studies that involved a range of animal models such as murine septic models and hemostasis models. I spent a year (2015 – 2016) at my PhD lab to finish few projects while adjusting my immigration status. Soon after, I joined the Gailani lab at Vanderbilt as my first true postdoc. There, I honed and harnessed molecular techniques used in coagulation research. I studied coagulation protein interactions, structure-activity relationships, introduced new animal models of thrombosis and hemostasis to the lab. In my second year, I was awarded an American Heart Association (AHA) grant for my work on FXI (18POST34030076). In my third year, I developed interest in antibodies as research tools for studying coagulation and as potential therapeutic modalities. I finished my AHA project ahead of time and moved to Washington University in St. Louis, where I joined the lab of a Junior PI, Dr. Ali Ellebedy for one year. This experience served two purposes: first, I witnessed firsthand a nascent lab building process; second, I deepened my immunology background. I contributed to several clinical and preclinical vaccination and immunization studies with the goal of isolating antigen-specific B cells and generating therapeutic antibodies to protect against Influenza and COVID. With the hiring freeze during COVID (2020 - 2021), the time was not right to apply for a junior faculty position. I decided to use that time to invest in my future lab direction. I was fortunate to join Dr. Enrico Di Cera lab, a worldwide renowned lab that studies structural enzymology of coagulation proteins. My plan is to finish my current project and apply for Junior faculty positions in 2023. I

contribute molecular biology and clinical assays to lab projects, while Dr. Di Cera has given me the resources to generate data on projects independent of the lab direction that I can take to my new lab. I currently study the structural enzymology of contact activation coagulation proteins.
Citations:

- **Mohammed, B.M.**, Contaifer Jr, D., Lastrapes, K.K., Martin, E.J., Mazepa, M.A., Hoffman, M., Monroe, D.M. and Brophy, D.F., 2016. Coated platelet assay: a feasible approach to a complicated science. *Haemophilia*, *22*(1), pp.e67-e70.
- **Mohammed, Bassem M.**, Qiufang Cheng, Anton Matafonov, Ingrid M. Verhamme, Jonas Emsley, Keith R. McCrae, Owen JT McCarty, Andras Gruber, and David Gailani. "A non-circulating pool of factor XI associated with glycosaminoglycans in mice." *Journal of Thrombosis and Haemostasis* 17, no. 9 (2019): 1449-1460.
- Ivanov, Ivan, Anton Matafonov, Mao-fu Sun, **Bassem M. Mohammed**, Qiufang Cheng, S. Kent Dickeson, Suman Kundu et al. "A mechanism for hereditary angioedema with normal C1 inhibitor: an inhibitory regulatory role for the factor XII heavy chain." Blood, The Journal of the American Society of Hematology 133, no. 10 (2019): 1152-1163.
- Han, J., Schmitz, A.J., Richey, S.T., Dai, Y.N., Turner, H.L., **Mohammed, B.M.**, Fremont, D.H., Ellebedy, A.H. and Ward, A.B., 2021. Polyclonal epitope mapping reveals temporal dynamics and diversity of human antibody responses to H5N1 vaccination. *Cell reports*, *34*(4), p.108682.

Given my previous accomplishments in the field of coagulation research and my current training environment (Skilled colleagues, and access to state-of-art institutional core facilities and expertise), I am confident that I can successfully complete the proposed work in this application and achieve the necessary milestone in the current stage of my career that will aid my next transition.

**B. Positions, Scientific Appointments, and Honors**

**Positions and Scientific Appointments**

| | |
|---|---|
| 2020 – Present | Post-doctoral fellow, Enrico Di Cera Lab, St. Louis University, St. Louis, MO, USA |
| 2019 – 2020 | Visiting Scientist, Ali Ellebedy Lab, Washington University, St. Louis, MO, USA |
| 2016 – 2020 | Post-doctoral fellow, Dave Gailani Lab, Vanderbilt University Medical Center, Nashville, TN, USA |
| 2015 – 2016 | Post-doctoral fellow, Donald Brophy Lab, Virginia Commonwealth University, Richmond, VA, USA |
| 2011 – 2015 | Teaching/Research assistant, Pharmaceutics, Virginia Commonwealth University, Richmond, VA, USA |
| 2015 – Present | Member, International Society for Thrombosis and Haemostasis (ISTH), USA |
| 2014 - Present | Member, American Heart Association, USA |
| 2013 - 2016 | Member, International Society for Pharmacoeconomics and Outcomes Research (ISPOR), USA |
| 2012 - Present | Member, Phi Kappa Phi Honor Society (By invitation, top 5% graduate students), USA |
| 2012 - Present | Member, Golden Key Honor Society (By invitation, top 10% graduate students), USA |
| 2007 - 2011 | Teaching/Research assistant, Cairo University, Egypt |
| 2008 - 2011 | Performance and Appraisal and Quality Assurance Unite, Member, Cairo University, Cairo, Egypt |

2010 - 2011 Center of Applied Research and Advanced Studies, Member, Cairo University, Cairo, Egypt

2007 - Present Egyptian Syndicate of Pharmacists, Member

2004 - 2006 Student Pharmacist intern, Cairo, Egypt

**Honors**

2017 Young Investigator Award, ISTH 2017, Berlin, Germany

2015 Charles T. Rector and Thomas W. Rorrer Dean's Award for Excellence in Graduate Study (Research, Teaching, and Service), Virginia Commonwealth University, School of Pharmacy, Richmond, VA, USA

2013 Best poster presentation, 16th Annual Research and Career Day, Virginia Commonwealth University, School of Pharmacy, Richmond, VA, USA

2008 Faculty of Pharmacy Honors Award, Cairo 2008 University, Egypt

2008 Egyptian Syndicate of Pharmacists Honors Award, Egypt

2008 Performance and Appraisal and Quality Assurance Unite Honors Certificate for role in Organizing and contributing to “Leading steps on the road of accreditation” Conference

**C. Contributions to Science**

**Graduate Career:** My research experience during this period concerned inflammation and coagulation. Sepsis causes 20% of global deaths. There is no current treatment other than supportive therapy. Sepsis drives the body into a systemic inflammatory response syndrome leading to coagulopathies and end organ damage. I contributed to the foundational preclinical work needed to apply for an IND for supraphysiologic doses of ascorbic acid to be used intravenously in septic patients. I was able to show the advantages of the IV route in delivering therapeutic amounts of ascorbic acid that can modulate the immune response and impact survival. Our research group continued on my work and finished the first clinical trial and published the CITRIS-ALI study results in JAMA in 2019. PMID: 31573637 PMCID: PMC6777268

a. **Mohammed, Bassem M.**, Bernard J. Fisher, Donatas Kraskauskas, Daniela Farkas, Donald F. Brophy, and Ramesh Natarajan. "Vitamin C: a novel regulator of neutrophil extracellular trap formation." Nutrients 5, no. 8 (2013): 3131-3150.
b. **Mohammed, Bassem M.**, Bernard J. Fisher, Quoc K. Huynh, Dayanjan S. Wijesinghe, Charles E. Chalfant, Donald F. Brophy, and Ramesh Natarajan. "Resolution of sterile inflammation: role for vitamin C." Mediators of inflammation 2014 (2014).
c. **Mohammed, Bassem M.**, Bernard J. Fisher, Donatas Kraskauskas, Susan Ward, Jennifer S. Wayne, Donald F. Brophy, Alpha A. Fowler, Dorne R. Yager, and Ramesh Natarajan. "Vitamin C promotes wound healing through novel pleiotropic mechanisms." International wound journal 13, no. 4 (2016): 572-584.
d. **Mohammed, Bassem M.**, Kimberly W. Sanford, Bernard J. Fisher, Erika J. Martin, Daniel Contaifer Jr, Urszula Osinska Warncke, Dayanjan S. Wijesinghe et al. "Impact of high dose vitamin C on platelet function." World journal of critical care medicine 6, no. 1 (2017): 37.

On the benign hematology front, my graduate work focused on studying strategies to treat and assess the coagulation profile in hemophilia patients (bleeding) and trauma victims (coagulopathy). The results of my work were immediately translational and helped us learn more about the usefulness of the thromboelastography and thrombin generation assays in assessing hemostasis in patients, and their response to therapy. In addition, I introduced to our laboratories common hemostasis murine models used to assess small molecule anticoagulant lead molecules.

a. **Mohammed, B. M.**, E. J. Martin, V. Salinas, R. Carmona, G. Young, and D. F. Brophy. "Failure of corn trypsin inhibitor to affect the thrombin generation assay in plasma from severe hemophiliacs." Journal of Thrombosis and Haemostasis 12, no. 9 (2014): 1558-1561.
b. White, Nathan J., Jason C. Newton, Erika J. Martin, **Bassem M. Mohammed**, Daniel Contaifer Jr, Jessica L. Bostic, Gretchen M. Brophy et al. "Clot formation is associated with fibrinogen and platelet

forces in a cohort of severely-injured Emergency Department trauma patients." Shock (Augusta, Ga.) 44, no. 0 1 (2015): 39.

c. Mehta, Akul Y., **Bassem M. Mohammed**, Erika J. Martin, Donald F. Brophy, David Gailani, and Umesh R. Desai. "Allosterism-based simultaneous, dual anticoagulant and antiplatelet action: allosteric inhibitor targeting the glycoprotein Ibα-binding and heparin-binding site of thrombin." Journal of Thrombosis and Haemostasis 14, no. 4 (2016): 828-838.

d. Brophy, D. F., E. J. Martin, **B. M. Mohammed**, J. C. Barrett, J. G. Kuhn, M. E. Nolte, B. Wiinberg et al. "Modulation of the activated protein C pathway in severe haemophilia A patients: The effects of thrombomodulin and a factor V-stabilizing fab." Haemophilia 23, no. 6 (2017): 941-947.

**Postgraduate Career:**

**Phase I (years 0 – 3):** Contact activation proteins like FXII and FXI are targets for the development of safe antithrombotic therapies that have little to no impact on normal hemostasis. I discovered a non-circulating vascular depot of murine factor FXI and elucidated the structural determinants that are unique to murine FXI and have no parallels in other species. I was awarded an AHA postdoctoral grant for that work. The result of that work informed better design of the murine models used for screening FXI-related therapeutics. In addition, I contributed multiple insights into properties of FXII in disease states like hereditary angioedema, and elucidated differences in high molecular weight kininogen across species and the role of supraphysiologic doses of FXI in improving hemostasis in hemophilic mice. I continue to contribute to ongoing work in the Gailani lab given the breadth of the research portfolio I developed over my three-year postdoc there.

a. **Mohammed, Bassem M.**, Qiufang Cheng, Anton Matafonov, Dougald M. Monroe, Joost CM Meijers, and David Gailani. "Factor XI promotes hemostasis in factor IX-deficient mice." Journal of Thrombosis and Haemostasis 16, no. 10 (2018): 2044-2049.

b. **Mohammed, Bassem M.**, Qiufang Cheng, Anton Matafonov, Ingrid M. Verhamme, Jonas Emsley, Keith R. McCrae, Owen JT McCarty, Andras Gruber, and David Gailani. "A non-circulating pool of factor XI associated with glycosaminoglycans in mice." Journal of Thrombosis and Haemostasis 17, no. 9 (2019): 1449-1460.

c. Ivanov, Ivan, Anton Matafonov, Mao-fu Sun, **Bassem M. Mohammed**, Qiufang Cheng, S. Kent Dickeson, Suman Kundu et al. "A mechanism for hereditary angioedema with normal C1 inhibitor: an inhibitory regulatory role for the factor XII heavy chain." Blood, The Journal of the American Society of Hematology 133, no. 10 (2019): 1152-1163.

d. Dickeson, S. Kent, Sunil Kumar, Mao-fu Sun, **Bassem M. Mohammed**, Dennis Phillips, James C. Whisstock, Adam J. Quek, Edward P. Feener, Ruby HP Law, and David Gailani. "A Mechanism for Hereditary Angioedema Caused by a Lysine311 to Glutamic Acid Substitution in Plasminogen." Blood (2022).

**Phase II (year 4):** Highly contagious respiratory viruses like FLU and SARS are challenging and have resulted in more pandemics than any other viruses. The recent COVID-19 pandemic has caused millions of deaths and inflicted a huge economic damage. I contributed to our fight against these pathogens by isolating FLU and SARS antigen-specific B-cell from human and animal peripheral blood samples. That was followed by synthesizing the corresponding monoclonal antibodies and characterizing them. Some of these antibodies were bought by pharmaceutical companies for further clinical development.

a. Han, Julianna, Aaron J. Schmitz, Sara T. Richey, Ya-Nan Dai, Hannah L. Turner, **Bassem M. Mohammed**, Daved H. Fremont, Ali H. Ellebedy, and Andrew B. Ward. "Polyclonal epitope mapping reveals temporal dynamics and diversity of human antibody responses to H5N1 vaccination." Cell reports 34, no. 4 (2021): 108682.

b. Su, Wen, Sin Fun Sia, Aaron J. Schmitz, Traci L. Bricker, Tyler N. Starr, Allison J. Greaney, Jackson S. Turner, **Bassem M. Mohammed** et al. "Neutralizing Monoclonal Antibodies That Target the Spike Receptor Binding Domain Confer Fc Receptor-Independent Protection against SARS-CoV-2 Infection in Syrian Hamsters." Mbio 12, no. 5 (2021): e02395-21.

**Complete List of Published Work in my bibliography:**

https://www.ncbi.nlm.nih.gov/myncbi/bassem.mohammed.1/bibliography/public/

**BIOGRAPHICAL SKETCH**

NAME: Enrico Di Cera, M.D.

eRA COMMONS USER NAME: di_cera

POSITION TITLE: Alice A. Doisy Professor and Chairman

EDUCATION/TRAINING

| INSTITUTION AND LOCATION | DEGREE | Dates | FIELD OF STUDY |
|---|---|---|---|
| Liceo Classico "M Pagano", Campobasso, Italy | HS Diploma | 1974-1979 | Classical Studies |
| Catholic University Medical School, Rome, Italy | MD | 1979-1985 | Medicine & Surgery |

**A. Personal Statement**

My independent research career started at Washington University in 1990 and remains focused on the structural enzymology of coagulation factors. The approach uses a combination of conventional (rapid kinetics, protein engineering, X-ray crystallography, NMR) and state-of-the-art (smFRET, cryo-EM) biophysical techniques that make our laboratory uniquely positioned to tackle unsolved problems in the field. An additional strength of the laboratory is the long standing emphasis on the theoretical underpinnings of ligand binding and allostery started during my training as a postdoc with Drs Stanley Gill and Jeffries Wyman. My work has been continuously funded by the NIH since 1994 and has so far generated a total of $20M+ in grant money, 200 peer-reviewed articles, 50 review and invited articles, 4 monographs, 80+ X-ray structures of thrombin, prethrombin-2, prethrombin-1, prothrombin and thrombin complexes with fragments of protein C, PAR1, PAR3 and PAR4, and the cryo-EM structures of coagulation factors V and Va. Our recent focus on cryo-EM is emerging as a new strength of the lab, benefiting from our collaboration with Dr. James Fitzpatrick at the Washington University Center of Cellular Imaging (WUCCI). We have established a partnership with the WUCCI that gives faculty at Saint Louis University access to all the cryo-EM resources of the WUCCI with the same priority, technical assistance and costs as Washington University faculty.

Currently funded projects:

1. R01 HL049413-25 (Di Cera); 06/01/2020 – 05/31/2024; Title: **Structure and dynamics of prothrombin**. The proposed research project focuses on the structure and dynamics of prothrombin free and bound to prothrombinase studied by NMR and cryo-EM.
2. R01 HL139554-04 (Di Cera); 05/01/2018 – 04/30/2022; Title: **Molecular mechanism of protein C activation.** The proposed research project focuses on the thrombomodulin-dependent activation of protein C by thrombin as a key regulatory feedback loop of the coagulation response.
3. R01 HL139554-05 (Di Cera); 05/01/2022 – 04/30/2026; Title: **Structural enzymology of protein C.** The proposed research project focuses on the cryo-EM investigation of protein C free and bound to the thrombin-thrombomodulin complex.
4. R01 HL147821-03 (Di Cera); 06/01/2019 – 05/31/2023; Title: **Allosteric equilibria of thrombin and its precursors**. The proposed research project focuses on the structural determinants of the allosteric E*-E equilibrium of thrombin and prethrombin-2.

Notable contributions from our work during the past three years include:

1. Protein engineering of thrombin for anticoagulant activity and development of the mutant W215A/E217A that recently concluded Phase II (NCT03963895) for the treatment of thrombotic complications and stroke: Protein C activator AB002 rapidly interrupts thrombus development in baboons and appears safe in a first-in-human trial. Tucker EI, Verbout NG, Markway BD, Wallisch M, Lorentz CU, Hinds MT, Shatzel JJ, Pelc LA, Wood DC, McCarty OJT, Di Cera E, Gruber A (2020) Blood 135, 689-699.
2. Pioneering application of cryo-EM to the analysis of coagulation factors: Cryo-EM structures of human coagulation factors V and Va. Ruben EA, Rau MJ, Fitzpatrick JAJ, Di Cera E (2021) Blood 137, 3137-3144; Cryo-EM structure of the prothrombin-prothrombinase complex. Ruben EA, Summers B, Rau MJ, Fitzpatrick JAJ, Di Cera E (2022) Blood, submitted for publication.

3. Elucidation of the general kinetic signatures of conformational selection and induced fit as mechanisms of ligand binding in biological macromolecules: Mechanisms of ligand binding. Di Cera E (2020) Biophys Rev 1, 011303.

Our current efforts on the structural enzymology of coagulation factors and their complexes focus on the molecular basis of the interaction of prothrombin with prothrombinase leading to thrombin generation in the penultimate step of the coagulation cascade (supported by HL049413-25) and the interaction of protein C with the thrombin-thrombomodulin complex triggering the feedback loop that controls the coagulation response (supported by HL139554-04 and it continuation HL139554-05). These cofactor-assisted interactions are critical to hemostasis and are now mature for a deep structural investigation based on cryo-EM, as a continuation of our recent success in solving the structures of factors V, Va and prothrombinase free and bound to prothrombin. Studies on the structure and dynamics of thrombin and its zymogen precursors are supported by HL147821-03. They focus on the molecular basis of the zymogen to protease transition using NMR, X-ray structural biology, enzymology and protein engineering.

We produce all critical reagents that are essential to the performance of our work. Over the years, we have made these reagents freely available upon request to dozens of investigators in the US and around the world. The environment in our laboratory offers a unique combination of expertise for the training of students and postdoctoral fellows interested in biophysical approaches to proteins of biological interest.

## B. Positions, Scientific Appointments and Honors

### Positions

2010- Alice A. Doisy Professor and Chairman, Edward A. Doisy Department of Biochemistry and Molecular Biology, Saint Louis University (SLU) School of Medicine (SOM), St Louis, MO
2018-21 Interim Vice Dean for Research, SLU SOM, St Louis, MO
2018-19 Deputy Vice President for Research, SLU, St Louis, MO
2006-10 Roy and Diana Vagelos Professor, Washington University Medical School (WUMS), St Louis, MO
2004-10 Professor of Internal Medicine, WUMS, St Louis, MO
1998-10 Professor of Biochemistry and Molecular Biophysics, WUMS, St Louis, MO
1995-97 Director, Molecular Biophysics Graduate Program, WUMS, St Louis, MO
1994-98 Associate Professor of Biochemistry and Molecular Biophysics, WUMS, St Louis, MO
1990-94 Assistant Professor of Biochemistry and Molecular Biophysics, WUMS, St Louis, MO
1988-90 Ricercatore, Catholic University Medical School, Rome, Italy
1986-88 Research Associate, University of Colorado, Boulder, CO (with Profs SJ Gill and J Wyman)
1983-86 Research Assistant, University of Rome, Rome, Italy (with Prof M Brunori)

### Scientific Appointments

2022- Member, Hemostasis Thrombosis Blood Cell and Transfusion (HTBT) NIH Study Section.
2019- Editor-in-Chief: Biomolecular Concepts
2003- Editorial Board: Italian Journal of Biochemistry (2003-07), Journal of Biological Chemistry (2003-08; 2012-17), Cellular and Molecular Life Sciences (2008-09), IUBMB Life (2008-11), Biomolecular Concepts (2009-19), Science Advances (2019), Biophysical Chemistry (2020-)
1998-12 Consultant: Eli Lilly (1998-99), Johnson & Johnson (2000-08), Humagene (2006-08), Verseon (2010-12)
1995-22 Ad hoc Reviewer, NIH Study Sections: MetalloBiochemistry (1995), Physiological Chemistry (1997), Hematology 1 (2000, 2003-04), Cancer Biology (2001), Erytrocyte and Leukocyte Biology (2006-08), Hemostasis and Thrombosis (2007-09, 2020), Vascular and Hematology (2009, 2014, 2017), NHLBI Conference Grants (2012), Hemostasis Thrombosis Blood Cells and Transfusion (2021-22), Macromolecular Structure and Function D (2021)
1995-19 Editor: Biophysical Chemistry
1994-03 Conference Chair: 8$^{th}$ Gibbs Conference on Biological Thermodynamics, Carbondale, IL (1994); FASEB Conference on 'Thrombin and Vascular Medicine', Whitefish, MT (2001); FASEB Conference on 'Proteases and Vascular Biology', Saxton's River, VT (2003)

### Honors

2022: State-of-the-Art Lecture, XXXVI ISTH Congress, London, UK
2019: Fellow, American Association for the Advancement of Science

2019: Core Program Graduation Address, SLU SOM, St Louis, MO
2018: Keynote Lecture, FEBS Advanced Summer School, Nove Hrady, Czech Republic
2016: Marina Diana Mercurio Award, SIBPA
2015: Fellow, St Louis Academy of Science
2015: Outstanding St Louis Scientist Award, St Louis Academy of Science
2015: Brown and Williamson Lecture, University of Louisville, Louisville, KY
2014: Plenary Lecture, XXII SIBPA Congress, Palermo, Italy
2012: Gary K. Ackers Lecture, 26th Gibbs Conference on Biothermodynamics, Carbondale, IL
2011: Pioneering Biology Discovery Lecture, University of Texas at Galveston, Galveston, TX
2010: Edward A. Doisy Lecture, SLU SOM, St Louis, MO
2010: Alice A. Doisy Professorship in Biochemistry and Molecular Biology
2009: Plenary Lecture, 64th Calorimetry Conference, Santa Fe, NM
2007: State-of-the-Art Lecture, XXI ISTH Congress, Geneva, Switzerland
2006: Roy and Diana Vagelos Professorship in Biochemistry and Molecular Biophysics
2005: Stuart R. Stone Lecture, FASEB Conference on 'Proteases in Hemostasis', Saxton's River, VT
2000: Visiting Professor, Alpha Omega Alpha Honor Medical Society, U Michigan, East Lansing, MI
1999: Keynote Lecture, Molecular Biophysics Program, UCSD, San Diego, CA
1999: Dompe'-Amgen Biotec Award
1998-99: Visiting Scientist, Eli Lilly and Co
1993-98: Established Investigator Award in Thrombosis, American Heart Association and Genentech
1988: Medicina Award, Italian Medical Association
1986: Young Investigator Award, Italian Society of Biophysics and Molecular Biology
1985: Laurea *cum Laude* in Medicine and Surgery

**C. Contributions to Science**

1. My scientific career started with an interest in allostery and linkage thermodynamics, as a result of my training with Profs Stanley Gill and Jeffries Wyman, two pioneers in ligand binding thermodynamics and allosteric theory. I developed the theory of site-specific effects in ligand binding with application to the energetics of site-directed mutations in macromolecular systems. I used the rigor of this approach in my early work on thrombin.
    a. Thermodynamic theory of site-specific binding processes in biological macromolecules. Di Cera E. (1995, Cambridge University Press, Cambridge, UK). Paperback edition published in 2005.
    b. Site-specific analysis of mutational effects in proteins. Di Cera E. (1998) Adv Protein Chem 51, 59-119.
    c. Site-specific dissection of substrate recognition by thrombin. Vindigni A, Dang QD, Di Cera E. (1997) Nat Biotechnol 15, 891-895.
    d. Defining epitopes: it is not as easy as it seems. Greenspan NP, Di Cera E. (1999) Nat Biotechnol 17, 936-937.

2. My work on thrombin started with the discovery of the allosteric effect of $Na^+$. In 1995, we showed that $Na^+$ binding to thrombin is needed to efficiently cleave fibrinogen but is dispensable for activation of the anticoagulant protein C and identified the $Na^+$ binding site in collaboration with Prof Tulinsky. This was the first $Na^+$ binding site to be documented in any protein by X-ray crystallography and impacted similar investigations of trypsin-like proteases by other investigators. A $Na^+$ binding site analogous to that of thrombin has been identified in other clotting (fVIIa, fIXa, fX, activated protein C) and complement (MASPs, C1r, C1s) factors by several groups in the US and Europe. The $Na^+$ binding site is now recognized as a key structural determinant of allosteric proteases and an evolutionary marker for enzymes of blood clotting and immune response. The work on $Na^+$ binding to thrombin has also served as a framework for the analysis of monovalent cation activation of enzymes in general.
    a. An allosteric switch controls the procoagulant and anticoagulant activities of thrombin. Dang QD, Vindigni A, Di Cera E. (1995) Proc Natl Acad Sci USA 92, 5977-5981.
    b. The $Na^+$ binding site of thrombin. Di Cera E, Guinto ER, Vindigni A, Dang QD, Ayala YM, Wuyi M, Tulinsky A. (1995) J Biol Chem 270, 22089-22092.
    c. Residue 225 determines the $Na^+$-induced allosteric regulation of catalytic activity in serine proteases. Dang QD, Di Cera E. (1996) Proc Natl Acad Sci USA 93, 10653-10656. PMID: 8855234
    d. Role of $Na^+$ and $K^+$ in enzyme function. Page MJ, Di Cera E. (2006) Physiol Rev 86, 1049-1092.

3. The discovery of the differential effect of $Na^+$ on the procoagulant (fibrinogen) and anticoagulant (protein C) substrates of thrombin suggested that the enzyme could be re-engineered to become selective for protein C.

We and other groups have devoted substantial efforts toward this goal. Our thrombin mutant W215A/E217A (WE) has been tested, in collaboration with Dr Andras Gruber, Dr John Griffin, Dr Jay Degen and others, in a number of animal models relevant to thrombosis, acute and chronic inflammation, arthritis, myocardial reperfusion and stroke. Importantly, WE is at least as effective and safer than heparin and tPA. WE has been patented, completed a Phase I trial with Fast Track Designation by the FDA and completed Phase II testing.

a. Rational engineering of catalytic activity and specificity in a serine protease. Dang QD, Guinto ER, Di Cera E. (1997) Nat Biotechnol 15, 146-149.
b. Relative antithrombotic and antihemostatic effects of protein C activator versus low molecular weight heparin in primates. Gruber A, Marzec UM, Bush LA, Di Cera E, Fernandez JA, Berny MA, Tucker EI, McCarty OJT, Griffin JH, Hanson SR. (2007) Blood 109, 3733-3740.
c. Protein C activator AB002 rapidly interrupts thrombus development in baboons and appears safe in a first-in-human trial. Tucker EI, Verbout NG, Markway BD, Wallisch M, Lorentz CU, Hinds MT, Shatzel JJ, Pelc LA, Wood DC, McCarty OJT, Di Cera E, Gruber A (2020) Blood 135, 689-699.
d. Antithrombotic thrombin variants. Gruber A, Hanson SR, Di Cera E. US Patent 6,706,512 (03.16.04). Expression of thrombin variants. Di Cera E, Gruber A, Gandhi PS, Pelc LA, Pozzi N, Wood DC. US Patent 8,940,297 (01.27.15). IND 18154 (05.16.18). Phase I NCT03453060 (05.30.18-11.28.18). FDA Fast Track designation (06.07.18). Phase II NCT03963895 (07.10.19-12.31.20).

4. Our experimental and theoretical interest in mechanisms of ligand binding has led to the discovery of a pre-existing, allosteric equilibrium between open (E) and closed (E*) conformations of the active site of trypsin-like proteases. The Protein Data Bank documents 50+ structures that support this equilibrium as a general property that impacts all functional aspects of the protease, including the Huber-Bode mechanism of zymogen to protease conversion. The E*-E equilibrium rationalizes the widely different range of activities found in this enzyme family, the cofactor-induced enhancement of catalytic activity, the effect of many natural and site-directed mutations and zymogen autoactivation found in some members of the coagulation and complement cascades. The E*-E equilibrium is an example of conformational selection and reflects the inherent dynamic nature of protein structure. Recent theoretical analysis of conformational selection has revealed unexpected general properties of this mechanism. Our contributions in this area have impacted our understanding of molecular recognition in biological macromolecules and the rigorous analysis of experimental data.
    a. Allostery in trypsin-like proteases suggests new therapeutic strategies. Gohara DW, Di Cera E (2011) Trends Biotechnol 29, 577-585. PMCID: PMC3191250
    b. Conformational selection in trypsin-like proteases. Pozzi N, Vogt AD, Gohara DW, Di Cera E (2012) Curr Opin Struct Biol 22, 421-431. PMCID: PMC3423485
    c. Interplay between conformational selection and zymogen activation. Chakraborty P, Acquasaliente L, Pelc LA, Di Cera E (2018) Sci Rep 8, 4080. PMCID: PMC5840343
    d. Mechanisms of ligand binding. Di Cera E (2020) Biophys Rev 1, 011303. PMCID: PMC32855236.

5. Our functional work over the years has been complemented by a growing emphasis on structural biology. Following our success in the crystallization of prethrombin-1, we have solved the first and several structures of prothrombin and uncovered the unanticipated plasticity of its architecture. Additional studies by smFRET have revealed alternative conformations of prothrombin that interact differently with prothrombinase and other biological targets. Closed and open forms of prothrombin have been resolved by X-ray crystallography and their different role during activation by prothrombinase has been elucidated. Important differences between zymogen and protease following the Huber-Bode mechanism of activation have been identified by $^{19}$F NMR studies of thrombin and prethrombin-2. More recently, we have pioneered the cryo-EM analysis of coagulation factors and solved the structures of factors V, Va and prothrombinase free and bound to prothrombin. Cryo-EM structures of protein C free and bound to the thrombin-thrombomodulin complex are currently under refinement. This new focus of the laboratory will significantly advance our understanding of structure-function relationships of coagulation factors and their complexes.
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