BIOGRAPHICAL SKETCH

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NAME: Spies, Maria

eRA COMMONS USER NAME: MARIA_SPIES

POSITION TITLE: Professor of Biochemistry and Radiation Oncology

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
Peter the Great St. Petersburg Polytechnic University, St. Petersburg, Russia	BS	06/1994	Physics/Biophysics
Peter the Great St. Petersburg Polytechnic University, St. Petersburg, Russia	MS	04/1996	Physics/Biophysics
Osaka University, Toyonaka, Osaka, Japan	PHD	03/2000	Biological Sciences
University of California, Davis, CA	postdoc	10/2005	Biophysics

A. PERSONAL STATEMENT

I am a biophysicist with over two decades of experience in using single-molecule microscopy and spectroscopy to study macromolecular interactions and dynamics. Work in my lab focuses on the molecular machines supporting genetic integrity, DNA replication, recombination and repair. Specifically, we aim to determine how a combination of molecular associations and posttranslational modifications integrates human RAD51 recombinase, the RAD52 DNA repair protein, tumor suppressors BRCA1 and BRCA2, mismatch repair proteins and recombinational DNA helicases into a robust network of the DNA repair pathways in healthy and malignant cells. We utilize a broad spectrum of techniques from biochemical reconstitutions of DNA recombination, repair and replication reactions, to structural and single-molecule analyses of the proteins and enzymes coordinating these reactions. Our aim is to understand the coordinated and dynamic nucleoprotein transactions critical for high fidelity DNA repair and replication, to dissect the mechanisms that funnel “normal” DNA repair intermediates into “rogue” processes that destabilize the genome and lead to cancer, cell death and/or emergence of chemotherapeutic resistance, and to manipulate these processes in development of new cancer therapies.

Current projects in my lab are supported by the NIH **R35GM131704 MIRA** grant (PI: Spies), to understand the role of conformational protein dynamics in the assembly of the machinery of homologous recombination and in the activities of recombinational DNA helicases. The NIH **R01CA232425** (MPI: Spies, Malkova, Pichierri) builds upon our recent discovery (Malacaria et al 2019) of a new function of the RAD52 DNA repair protein in protecting stalled replication forks. We are developing a mechanistic understanding of the novel activities of RAD52, their importance to genome stability and the basis of the synthetic lethality between RAD52 inhibition and BRCA defects. The DoD/CDMRP **BC180227P1** (partnering PIs: Zhang, Spies) aims to define the role of MSH2/6 at different stages of basal-like breast cancers (BLBC) pathogenesis and to establish MSH2/6 as a molecular target for BLBC therapy. Finally, our NSF **1836351 EAGER** (PI: Spies) program is to develop a robust and comprehensive experimental tool combining next generation sequencing, single-molecule total internal reflection fluorescence microscopy and machine learning. All of the above mentioned projects have a significant single-molecule component.

Following works exemplify the range of my expertise in single-molecule biophysics:

1. Spies M, Bianco PR, Dillingham MS, Handa N, Baskin RJ, Kowalczykowski SC. A molecular throttle: the recombination hotspot chi controls DNA translocation by the RecBCD helicase. Cell. 2003 Sep 5;114(5):647-54. PubMed PMID: 13678587.

2. Honda M, Park J, Pugh RA, Ha T, Spies, M.‡. Single-molecule analysis reveals differential effect of ssDNA-binding proteins on DNA translocation by XPD helicase. Mol Cell. 2009 Sep 11;35(5):694-703. PubMed PMID: 19748362; PubMed Central PMCID: PMC2776038.

3. Boehm, E.M., Subramanyam, S., Ghoneim, M.K.‡, Washington, M.T.‡, and Spies, M.‡, Chapter Five-Quantifying the Assembly of Multicomponent Molecular Machines by Single-Molecule Total Internal Reflection Fluorescence Microscopy (2016) Methods in Enzymology, 581, Single-Molecule Enzymology: Fluorescence-Based and High-Throughput Methods, 105-145. PMID: 27793278; PMCID: PMC5403009

4. Pokhrel, N.†, Caldwell, C.C.†, Corless, E.I., Tillison, E.A., Tibbs, J., Jocic, N., Tabei, S.M.A., Wold, M.S., Spies, M‡, and Antony, E‡. Dynamics and Selective Remodeling of the DNA Binding Domains of RPA (2019) Nature Structural and Molecular Biology 26:129-136. PMID: 30723327; PMCID: PMC6368398

(† denotes equal contribution; ‡ correspondence)

B. POSITIONS AND HONORS

Positions and Employment

1993 - 1996	Research assistant, Petersburg Nuclear Physics Institute, Gatchina, St. Petersburg, Russia
1994 - 1996	Master's thesis research with Dr. Vladislav Lanzov, Peter the Great St. Petersburg Polytechnic University and Petersburg Nuclear Physics Institute, Gatchina, St. Petersburg, Russia
1996 - 2000	Graduate student with Dr. Seiki Kuramitsu, Department of Biology, Graduate School of Science, Toyonaka, Osaka, Japan
2000 - 2005	Postdoctoral Fellow with Dr. Stephen C. Kowalczykowski, Department of Microbiology, University of California at Davis, Davis, CA, USA
2005 - 2012	Assistant Professor of Biochemistry , Department of Biochemistry, University of Illinois at Urbana-Champaign, Urbana, IL, USA
2009 - 2012	Early Career Scientist, Howard Hughes Medical Institute
2012 - 2018	Associate Professor of Biochemistry, University of Iowa, Iowa City, IA, USA
2018 -	Professor of Biochemistry and Radiation Oncology, University of Iowa, Iowa City, IA, USA

Other Experience and Professional Memberships

Member, Biophysical Society, ASBMB, American Chemical Society

2010 – 2017	The American Cancer Society DNA Mechanisms in Cancer Peer Review Panel (2010-2015 Member; 2015-2016 Vice Chair; 2017 Chair)
2013	Editor, "DNA Helicases and DNA Motor Proteins", Springer Advances in Experimental Medicine and Biology, Vol. 767, ISBN 978-1-4614-5037-5
2013 – current	PLOS ONE Academic Editor
2015	NIH MSFC study section (ad hoc)
2015	Organizer, FASEB Summer Research Conference on "Helicases and nucleic-acid based machines: from mechanism to insight into disease", July 26-31 2015, Steamboat Springs, CO
2016	Organizer, 4th Midwest Single Molecule Workshop, July 31 – August 2 2015, Iowa City, IA
2016 – 2021	Editorial board member, JBC
2016	NIH PMCB (June) and MSFB (October) study sections (ad hoc)
2016 – 2017	Editor (with Yann R Chemla, UIUC), Methods in Enzymology, "Single-molecule enzymology", Vol. 581 & 582, Print ISBN 9780128092675 & 9780128093108
2017	NIH MSFB (February, ad hoc) study section; NIH ZRG1 BCMB-D (June, ad hoc)
2017 – 2018	Editor (with Anna Malkova, U Iowa), Methods in Enzymology, "Mechanisms of DNA recombination and genome rearrangements", Vol. 600 & 601, Print ISBN 9780128144299 & 9780128139790
2018 – current	eLife board of reviewing editors
2019	Biophysical Society Molecular Biophysics Subgroup Chair
2019 – current	Biophysical Society Publications Committee
2019 – 2025	permanent member of the NIH MSFB study section

Honors

1995	St. Petersburg Administration 1995 Student Research Award, St. Petersburg Administration
1996	Master of Science "Red" Diploma with honors from Peter the Great St. Petersburg Polytechnic University (equivalent of *cum laude*), St. Petersburg, Russia
1996	Japanese Government (MONBUSHO) Graduate Scholarship
2002	American Cancer Society Postdoctoral Fellow, American Cancer Society
2009	Howard Hughes Medical Institute (HHMI) 2009 Early Career Scientist Award
2009	American Cancer Society Research Scholar
2010	Margaret Oakley Dayhoff Award in Biophysics, Biophysical Society
2015	Iowa Women of Innovation Award Finalist
2020	University of Iowa Health Care Distinguished Scholar

C. Contribution to Science

† denotes equal contribution; ‡ correspondence

1. Homologous recombination (HR) is one of the most enigmatic process in the DNA metabolism. Its central step involves the search for the homology between two DNA molecules and the subsequent exchange of the DNA strands. In all organisms this central step is orchestrated by the RecA family of the DNA strand exchange proteins, which includes eukaryotic RAD51 recombinase. Significant gaps remain in our understanding of how the HR machinery assembles, the mechanism by which RAD51 carries out the central step in HR, and how HR

is controlled by recombination mediators (e.g. human tumor suppressor BRCA2 and yeast Rad52) and antirecombinogenic DNA helicases (enzymes that use ATP to fuel DNA duplex separation and remodeling of the nucleoprotein complexes). My lab has been successful in closing these gaps through building a quantitative description of the central step in HR and its regulation. We showed, for example, that RAD51 phosphorylation by c-ABL tyrosine kinase enhances the RAD51 recombinase activity and its ability to compete with the ssDNA binding protein RPA by at least two different mechanisms (Subramanyam et al 2016), and that the ability of the FBH1 recombinational helicase to switch between pro- and antirecombinogenic functions depends on the FBH1 ubiquitylation status, which determines its interaction with RAD51 (Masuda-Ozawa et al 2013). We have advanced structural understanding of the RAD51 interaction with BRCA2 by generating an atomistic model of the RAD51-BRC4 complex, which revealed unexpected interactions between the N-terminal domain of RAD51 and BRC4 peptide (Subramanyam et al 2013). Our recent work (Pokhrel, Caldwell et al 2019) highlighted the importance of protein plasticity and conformational dynamics in the hand-off of the ssDNA from the replication protein A (RPA) to a correct downstream player in HR and DNA repair: we showed that yeast Rad52 modulates the RPA conformational dynamics to provide the landing site for Rad51 recombinase.

a. Subramanyam, S., Jones, W.T., Spies, M.‡, and Spies, M.A‡., *Contributions of the RAD51 N-terminal domain to BRCA2-RAD51 interaction* (2013) *NAR* 41(19):9020-9032 [PMID: 23935068; PMCID: PMC3799448]
b. Masuda-Ozawa T, Hoang T, Seo YS, Chen LF, Spies M. Single-molecule sorting reveals how ubiquitylation affects substrate recognition and activities of FBH1 helicase. Nucleic Acids Res. 2013 Apr 1;41(6):3576-87. PubMed PMID: 23393192; PubMed Central PMCID: PMC3616717.
c. Subramanyam, S., Ismail, M., Bhattacharya, I., and Spies, M., Tyrosine phosphorylation stimulates activity of human RAD51 recombinase. *Proc Natl Acad Sci U S A. 2016 Oct 11;113(41):E6045-E6054.* PubMed PMID: 27671650; PubMed Central PMCID: PMC5068273
d. Pokhrel, N.†, Caldwell, C.C.†, Corless, E.I., Tillison, E.A., Tibbs, J., Jocic, N., Tabei, S.M.A., Wold, M.S., Spies, M‡, and Antony, E‡. Dynamics and Selective Remodeling of the DNA Binding Domains of RPA (2019) *Nature Structural and Molecular Biology 26:129-136* [PMID: 30723327; PMCID: PMC6368398]

2. My lab has made an important contribution to understanding of the biochemical mechanism and cellular function of the DNA repair protein RAD52, whose depletion or inhibition is synthetically lethal with defects in tumor suppressors BRCA1, BRCA2 or PALB2. The following publications reflect our work towards elucidation of the RAD52 mechanisms and regulation. RAD52 is hyper-activated in the leukemia cells carrying a BCR-ABL fusion. We discovered that c-ABL or BCR-ABL phosphorylation enhances RAD52 selectivity for ssDNA and thereby promotes annealing without compromising fidelity (Honda et al 2011). We have identified a new interacting partner of RAD52, DSS1 (Stefanovie et al 2020). We showed that RAD52 binds ssDNA (and the ssDNA-RPA complex) by wrapping it around the RAD52 oligomeric rings and that this wrapped configuration promotes DNA annealing. We identified the narrow ssDNA binding groove of RAD52 ring as well as subunit-subunit interfaces within the ring as the two critical features to be targeted by the small molecule inhibitors and our FRET-based assays as robust probes for inhibitor activity (Rothenberg et al 2008, Grimme et al 2010).

a. Rothenberg, E., Grimme, J. M., Spies, M. ‡ and Ha, T. ‡, *Rad52 protein mediates directionally biased homology search and DNA annealing through continuous association of two Rad52-ssDNA complexes.* (2008) PNAS 105 (51) 20274-20279
b. Grimme JM, Honda M, Wright R, Okuno Y, Rothenberg E, Mazin AV, Ha T, Spies M. Human Rad52 binds and wraps single-stranded DNA and mediates annealing via two hRad52-ssDNA complexes. Nucleic Acids Res. 2010 May;38(9):2917-30. PubMed PMID: 20081207; PubMed Central PMCID: PMC2875008.
c. Honda M, Okuno Y, Yoo J, Ha T, Spies M. Tyrosine phosphorylation enhances RAD52-mediated annealing by modulating its DNA binding. EMBO J. 2011 Jul 29;30(16):3368-82. PubMed PMID: 21804533; PubMed Central PMCID: PMC3160658.
d. Stefanovie, B. †, Hengel, S.R. †, Mlcouskova, J., Lin, Y-L, Prochazkova, J., Spirek M., Nikulenkov F., Nemecek D., Koch, B.G., Bain, F.E., Yu, L., Pasero, P., Spies, M, and Krejci, L.‡, *DSS1 interacts with and stimulates RAD52 to promote the repair of DSBs* Nucleic Acids Res. 2020 Jan 24;48(2):694-708. PubMed PMID: 31799622; PubMed Central PMCID: PMC6954417

3. An important goal of my research program is to provide a mechanistic understanding of the molecular machines that function at the intersection of DNA replication, recombination and repair. In collaboration with the group of Pietro Pichierri (ISS, Rome, Italy) we identified the new role for RAD52 in supporting recovery from the replication stress in the checkpoint deficient cells (Murfuni et al 2013). Our most exciting recent discovery concerns another novel function of RAD52 (Malacaria et al 2019). We showed that during replication stress, RAD52 can remodel stalled DNA replication forks making them refractory to reversal by molecular motors such

as SMARCAL1 and ZRANB3. We capitalized on our understanding of the RAD52 biochemistry by identifying a set of small molecules that efficiently inhibit the RAD52-ssDNA interaction *in vitro* and in cells (Hengel et al 2016). Connecting HR and DNA repair, we demonstrated how the mismatch recognition protein MSH2-MSH6 can initiate heteroduplex rejection, an important step in quality control of homologous recombination.

a. Murfuni I, Basile G, Subramanyam S, Malacaria E, Bignami M, Spies M, Franchitto A, Pichierri P. Survival of the replication checkpoint deficient cells requires MUS81-RAD52 function. PLoS Genet. 2013 Oct;9(10):e1003910. PubMed PMID: 24204313; PubMed Central PMCID: PMC3814295
b. Honda M, Okuno Y, Hengel SR, Martín-López JV, Cook CP, Amunugama R, Soukup RJ, Subramanyam S, Fishel R, Spies M. Mismatch repair protein hMSH2-hMSH6 recognizes mismatches and forms sliding clamps within a D-loop recombination intermediate. Proc Natl Acad Sci U S A. 2014 Jan 21;111(3):E316-25. PubMed PMID: 24395779; PubMed Central PMCID: PMC3903253.
c. Hengel, S.R., Malacaria, E., Bain, F.E., Diaz, A., Koch, B.G., Constantino, L.F.daS., Liping, Y., Wu, M., Pichierri, P., Spies, M.A. , and Spies, M. Small-molecule inhibitors identify the RAD52-ssDNA interaction as critical for recovery from replication stress and for survival of BRCA2 deficient cells (2016) *eLife e14740.* PubMed PMID: 27434671; PubMed Central PMCID: PMC4982760.
d. Malacaria, E., Pugliese G.M., Honda, M., Marabitti, V., Aiello, F.A., Spies, M, Franchitto, A., and Pichierri, P. ‡, RAD52 prevents excessive replication fork reversal and protects from nascent strand degradation (2019) *Nature Communication 10(1):1412* [PMID: 30926821; PMCID: PMC6441034]

4. By employing classical and single-molecule biochemistry we made a substantial progress in determining the molecular mechanisms of the FeS cluster-containing DNA repair helicases XPD and FANCJ. We identified a secondary DNA binding site in the XPD helicase and showed that it is involved in controlling the helicase activity of XPD (Pugh et al 2012). Later Naegeli group (University of Zürich-Vetsuisse) built on this work by identifying a DNA damage verification activity located in the secondary ssDNA binding site of XPD. Our single-molecule work (Honda et al 2009) suggested that the conformational dynamics of XPD helicase auxiliary domains allows it to translocate on the ssDNA coated with the ssDNA-binding protein without displacing the latter from DNA. By combining the single-molecule observations with chemical biology, we were able to visualize this motion of the XPD domains and determined how the DNA damage recognition affects the XPD conformational dynamics (Ghoneim and Spies 2014). The dynamic interaction between human FANCJ helicase and the G-quadruplex containing DNA has been revealed in our recent study (Wu and Spies 2016).

a. Honda M, Park J, Pugh RA, Ha T, Spies M. Single-molecule analysis reveals differential effect of ssDNA-binding proteins on DNA translocation by XPD helicase. Mol Cell. 2009 Sep 11;35(5):694-703. PubMed PMID: 19748362; PubMed Central PMCID: PMC2776038.
b. Pugh RA, Wu CG, Spies M. Regulation of translocation polarity by helicase domain 1 in SF2B helicases. EMBO J. 2012 Jan 18;31(2):503-14. PubMed PMID: 22081110; PubMed Central PMCID: PMC3261565.
c. Ghoneim M, Spies M. Direct correlation of DNA binding and single protein domain motion via dual illumination fluorescence microscopy. Nano Lett. 2014 Oct 8;14(10):5920-31. PubMed PMID: 25204359; PubMed Central PMCID: PMC4189620.
d. Wu CG, Spies M. G-quadruplex recognition and remodeling by the FANCJ helicase. Nucleic Acids Res. 2016 Jun 24. pii: gkw574. PubMed PMID: 27342280; PubMed Central PMCID: PMC5062972.

5. My lab has broadened the scope of state-of-the-art single-molecule approaches, which utilize Total Internal Reflection Fluorescence Microscopy (TIRFM) to study protein-nucleic acids interactions along with tools for single-molecule analysis of the macromolecular interactions, dynamics and inhibition. To address questions of fundamental scientific importance, a number of papers highlighted in the previous sections also required technological advances. In Masuda-Ozawa et al (2013) we developed a single-molecule sorting technique, which separates, quantifies, and evaluates subpopulations of DNA repair proteins carrying their native posttranslational modifications within heterogeneous cellular milieu. Our novel TIRFM based approach reported in Ghoneim and Spies (2014) correlated the motions of the helicase modular domains with the DNA binding and damage recognition. In addition, I have also developed the framework for single-molecule analysis of non-competitive inhibitors of macromolecular interactions (Haghighat Jahromi et al 2013, Boehm et al 2016 MiE). In collaboration with Todd Washington (University of Iowa) we developed Kinetic Event Resolving Algorithm (KERA) for the analysis of dynamic multi-component complexes (Boehm et al 2016).

a. Haghighat Jahromi A, Honda M, Zimmerman SC, Spies M. Single-molecule study of the CUG repeat-MBNL1 interaction and its inhibition by small molecules. Nucleic Acids Res. 2013 Jul;41(13):6687-97. PubMed PMID: 23661680; PubMed Central PMCID: PMC3711446.
b. Qi Z, Pugh RA, Spies M, Chemla YR. Sequence-dependent base pair stepping dynamics in XPD helicase unwinding. Elife. 2013 May 28;2:e00334. PubMed PMID: 23741615; PubMed Central PMCID:

PMC3668415

c. Chen, R., Subramanyam, S., Elcock, A.H., Spies, M., and Wold, M.S.‡, *Dynamic binding of Replication Protein A is required for DNA repair* (2016) *NAR*, *44(12):5758-72* [PMID: 27131385; PMCID: PMC4937323]

d. Boehm EM, Spies M, Washington MT. PCNA tool belts and polymerase bridges form during translesion synthesis. Nucleic Acids Res. 2016 Jun 20. pii: gkw563. PubMed PMID: 27325737; PubMed Central PMCID: PMC5041468

ORCID ID: https://orcid.org/0000-0002-7375-8037
Google scholar: http://scholar.google.com/citations?user=zD-FavIAAAAJ&hl=en
myNCBI: http://www.ncbi.nlm.nih.gov/myncbi/browse/collection/47141158/?sort=date&direction=descending

D. ADDITIONAL INFORMATION: RESEARCH SUPPORT

Ongoing Research Support

NIH/NIGMS
R35GM131704 (PI: Spies) 04/01/2019-03/31/2024
Assembly and Dynamics of Molecular Machines in Genome Maintenance
Major Goals: To understand the role of conformational protein dynamics in the assembly of the machinery of homologous recombination and in the activities of recombinational DNA helicases

NIH/NCI
R01 CA232425 (MPI: Spies, Malkova, Pichierri) 04/01/2019-03/31/2024
The role of human RAD52 protein in genome stability
Major Goals: To develop a mechanistic understanding these two novel activities of RAD52, their importance to genome stability and the basis of the synthetic lethality between RAD52 inhibition and BRCA defects.

DOD/CDMRP
BC180227P1 (partnering PIs: Zhang, Spies) 04/01/2018-3/31/2022
Modulating Cancer Genetics for Immune Regulation and Breast Cancer Therapy
Major Goals: This project aims to define the role of MSH2/6 at different stages of BLBC pathogenesis and to establish MSH2/6 as a molecular target for BLBC therapy

NSF
1836351 EAGER (PI: Spies) 08/01/2018-07/31/2020
G4-TIRFM, a platform for massively parallel analysis of non-canonical DNA structures
Major Goals: to develop a robust and comprehensive experimental tool for the analysis and comparison of the potential structures that can form within the G-rich sequences, as well as the features of G4s that affect specific cellular processes.

PNCC (Pacific Northwest Center for Cryo-EM)
51258 (PI: Spies; co-PI Schnicker) 02/01/2020 - 01/31/2022
Determination of RAD52 and replication fork DNA complex
Major Goals: This proposal provides us with up to 480 hours annual Titan Krios and 200kV Talos Artica access at the PNCC. Our objective is to visualize the RAD52-DNA fork structure at atomic resolution.

University of Iowa Center for Biocatalysis and Bioprocessing Pilot Grant
(PIs: M Spies, MA Spies) 07/29/2019-07/28/2020
Targeting RAD51 DNA repair protein for cancer therapy: development of a combined experimental/computational workflow
Major Goals: to initiate a drug-discovery campaign targeting human RAD51 recombinase, an important player in homology-directed DNA repair.

NIH/NIEHS
R01 ES029203 (PI: Freudenthal) 09/15/2018 - 06/30/2023
APE1 Cleavage Mechanisms During DNA Repair (Subaward number: ZAF00030 with KUMC)
Major Goals: Personnel in the Spies' lab provides expertise and aiding the members of Dr. Bret Freudenthal's lab during the collection of single molecule TIRFM data.
Role: Collaborator

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. DO NOT EXCEED FIVE PAGES.

NAME: Razzaghi, Mortezaali

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

POSITION TITLE: Postdoctoral research scholar with Prof. Maria Spies

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
University of Tabriz, Tabriz, East Azerbaijan	BS	04/2004	Chemistry
University of Tabriz, Tabriz, East Azerbaijan	MS	02/2007	Chemistry
Southern Illinois University Edwardsville (SIUE), Edwardsville, Illinois	MS	12/2013	Chemistry
The University of Iowa, Iowa City, Iowa	PHD	05/2020	Chemistry

A. Personal Statement

My experience in physical biochemistry and protein X-ray crystallography will allow me to contribute to my current project. Currently I am postdoctoral research scholar at the Prof. Maria Spies lab. My first project is structural studies (X-ray crystallography and cryo-EM) of the RAD52 DNA repair protein and other proteins involved in the DNA replication fork protection. I am also working on the drug discovery effort targeting BRCA-deficient cancer. RAD52 is synthetically lethal with BRCA-deficient cancer cells and I will explore to find new potent and efficient small-molecule inhibitors using high throughput screening of NCI's library of natural products fractions. The training in cryo-EM will help me to broaden my structural biology expertise, and that I will learn new approaches of structure/mechanisms-based drug discovery and cutting-edge biophysics. I will also get training in single-molecule technique in Prof. Spies lab to investigate the mechanism, structure and activity of the human mismatch repair protein MSH2/6 and its role in the pathology of basal like breast cancer. To achieve my goals, I trained using Vitrobot for cryo-EM grid plunging and plunged samples of RAD52 alone and in complex with DNA and DSS1. I also collected X-ray diffraction data for N-terminal domain of RAD52 and planning to co-crystallize the diffracting crystal with inhibitors discovered in our lab. I audited the Radiation Biology class (FRRB 5000:0001 Spr21) which taught me the molecular and biological effects of ionizing radiation and its therapeutic applications in cancer therapy. My career goal is becoming an expert in my field and applying my expertise in human disease especially cancer research.

B. Positions and Honors

Positions and Employment

2011 - 2013 Graduate teaching and research assistant, Southern Illinois University Edwardsville, Edwardsville, IL

2011 - 2013 Master's thesis research with Prof. Yun Lu, Southern Illinois University Edwardsville (SIUE), Edwardsville, IL

2013 - 2015 Graduate teaching assistant, The University of Iowa, Iowa City, IA

2013 - 2020 Graduate student with Dr. Mishtu Dey, Department of Chemistry, The University of Iowa, Iowa City, IA

2016 - 2019 Graduate research assistant, The University of Iowa, Iowa City, IA

2020 - Postdoctoral research scholar with Prof. Maria Spies, Department of Biochemistry, The University of Iowa, Iowa City, IA

Other Experience and Professional Memberships

2004 - 2007 Member, Iranian Polymer Society (IPS)

2013 - 2016 Member, American Chemical Society (ACS)
2019 - Member, The American Society for Biochemistry and Molecular Biology (ASBMB)

Honors

2012 Graduate Teaching Assistant Award, Southern Illinois University Edwardsville
2013 Graduate Research Award, Southern Illinois University Edwardsville
2016 - 2016 Department of Chemistry Graduate Fellowship, 2016 (summer), University of Iowa

C. Contribution to Science

1. My academic training has involved utilizing biophysical and structural techniques in order to characterize and determine the structure of important enzymes of metabolic pathways. During my graduate studies I have become very knowledgeable in molecular biology methods having spent time working on multiple constructs of different proteins for expression, purification, and crystallization. I applied various methods of crystallization in order to solve different issues that can be unique to each protein. In addition to crystallization, I have performed size exclusion chromatography coupled with multiple angle light scattering and small angle X-ray scattering (SEC-MALS-SAXS) to study biomolecular interactions in solution. One of my projects involved posttranslational modifications of pyruvate kinase muscle 2 (PKM2) which is critical for tumor metabolism and a key glycolytic enzyme. PKM2 is in tetrameric form in normal cell but it becomes monomer/dimer in cancerous cells. We explored the mutant or phosphorylation and acetylation mimics of PKM2 which disrupt the oligomeric state of enzyme. The results of this project published in following journals which I am first co-author. I also studied a bacterial enzyme DddL which belongs to cupin superfamily DMSP lyases. These enzymes catalyze the cleavage of DMSP to generate DMS and acrylate using different transition metal ions as cofactor. DddL is unique among six distinct DMSP lyases where it has higher (hexamer) oligomeric state and along with DddY it is one of the most active DMSP lyase. The third project I involved during my PhD program is studying post-translational modification of proline residues by Prolyl-4-hydroxylase enzyme. Prolyl hydroxylation is a very common post-translational modification and plays many roles in eukaryotes such as collagen stabilization, hypoxia sensing, and controlling protein transcription and translation. In this study, we examine if BaP4H (Bacillus anthracis Prolyl 4-Hydroxylase) targets BaEFTu (Bacillus anthracis elongation factor Tu) for proline hydroxylation and find that it indeed modifies Pro54 of BaEFTu in addition to six other minor hydroxylation sites.

    a. Nandi S, Razzaghi M, Srivastava D, Dey M. Structural basis for allosteric regulation of pyruvate kinase M2 by phosphorylation and acetylation. J Biol Chem. 2020 Sep 28;PubMed PMID: 32989054.
    b. Srivastava D, Razzaghi M, Henzl MT, Dey M. Structural Investigation of a Dimeric Variant of Pyruvate Kinase Muscle Isoform 2. Biochemistry. 2017 Dec 19;56(50):6517-6520. PubMed PMID: 29182273.
    c. Schnicker NJ, Razzaghi M, Guha Thakurta S, Chakravarthy S, Dey M. Bacillus anthracis Prolyl 4-Hydroxylase Interacts with and Modifies Elongation Factor Tu. Biochemistry. 2017 Oct 31;56(43):5771-5785. PubMed PMID: 28981257; PubMed Central PMCID: PMC5735995.

2. My master's thesis studies were modeling enzymatic reactions in the solution and investigating the reaction mechanisms using powerful mechanistic tool kinetic isotope effects (KIE). We determined the secondary kinetic isotope effects for the hydride transfer reactions from aliphatic alcohols to two carbocations (NAD+ models) in acetonitrile. The results suggest that the hydride transfer takes place by tunneling and that the rehybridizations of both donor and acceptor carbons lag behind the H-tunneling. This is quite contrary to the observations in alcohol dehydrogenases where the importance of enzyme motions in catalysis is manifested.

    a. Maharjan B, Raghibi Boroujeni M, Lefton J, White OR, Razzaghi M, Hammann BA, Derakhshani-Molayousefi M, Eilers JE, Lu Y. Steric effects on the primary isotope dependence of secondary kinetic isotope effects in hydride transfer reactions in solution: caused by the isotopically different tunneling ready state conformations?. J Am Chem Soc. 2015 May 27;137(20):6653-61. PubMed PMID: 25941865.
    b. Kashefolgheta S, Razzaghi M, Hammann B, Eilers J, Roston D, Lu Y. Computational replication of the abnormal secondary kinetic isotope effects in a hydride transfer reaction in solution with a motion

assisted H-tunneling model. J Org Chem. 2014 Mar 7;79(5):1989-94. PubMed PMID: 24498946; PubMed Central PMCID: PMC3985929.

c. Hammann B, Razzaghi M, Kashefolgheta S, Lu Y. Imbalanced tunneling ready states in alcohol dehydrogenase model reactions: rehybridization lags behind H-tunneling. Chem Commun (Camb). 2012 Nov 28;48(92):11337-9. PubMed PMID: 23082319.

3. My first master's degree is in chemistry where I studied the detection of volatile organic compounds like ethyl acetate, acetone, ethanol and methyl ethyl ketone using quartz crystal nanobalance (QCN) as a powerful mass sensitive sensor. In this study we modified the surface of quartz disc in QCN sensor with polyethylene glycol and determined the analytes concentrations in the range of 4-35 mg/L for acetone and 4-70 mg/L for other vapors. The principal component analysis was also utilized to process the frequency response data of the organic vapors. Using principal component analysis, it was found that over 95% of the data variance could still be explained by use of two principal components (PC1 and PC2). Subsequently, the successful discrimination of ethyl acetate and other compounds was possible through the principal component analysis of the transient responses of the PEG-modified QCN sensor (https://doi.org/10.1080/10934520902958492).

D. Additional Information: Research Support and/or Scholastic Performance

Academic record – PhD The University of Iowa	
Course title	Grade
Introduction to Biocatalysis	A^-
Mechanisms of Organic Reactions	A^-
Chemical Kinetics	A^-
Biophysical Chemistry II	B^-
Spectroscopic Methods in Organic Chem	C^+
GPA	3.30
Academic record – Master's degree Southern Illinois University Edwardsville	
Advanced analytical chemistry	A
Advanced topics in organic chemistry	B
Advanced inorganic chemistry	A
Advanced physical chemistry	B
Advanced organic chemistry	A
Organic spectral analysis	A
NMR operation, experiment design and analysis	A
GPA	3.84