BIOGRAPHICAL SKETCH

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NAME: Anna Pluciennik

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POSITION TITLE: Assistant Professor

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Lodz, Lodz, Poland	M.Sc.	05/93	Microbiology & Genetics
University of Lodz, Lodz, Poland	Ph.D.	06/97	Biology
Texas A&M Univ. Health Sci Ctr, Houston, TX	Postdoctoral	01/98-07/01	Biochemistry
Duke University Medical Ctr., Durham, NC	Postdoctoral	10/01-10/06	Biochemistry
University of Lodz, Lodz, Poland	Habilitation (D. Sc. equivalent)	03/13	Molecular Biology

A. Personal Statement

My long-term research interests involve understanding the molecular mechanisms underlying human disease, particularly neurodegenerative disorders and cancer. My experience comprises a diverse skill set ranging from enzymology and protein biochemistry to cell biology and animal models of disease. I trained with Dr. Adam Jaworski at the University of Lodz, studying the molecular mechanisms of DNA triplet repeat expansion, a process that causes neurological disorders such as SBMA, Huntington's disease, and myotonic dystrophy type I. I received my Ph.D. for work demonstrating that CTG/CAG triplet repeat sequences undergo slippage, which result in their expansions and contractions. During my postdoctoral training with Dr. Robert D. Wells at the Institute of Biosciences and Technology, Texas A&M University in Houston, Texas, I demonstrated that CTG/CAG sequences are hotspots for genetic recombination.

I underwent further training with Dr. Paul Modrich at Duke University Medical Center in Durham NC (2015 Nobel Laurate in Chemistry) to understand the mechanisms of DNA mismatch repair, inactivation of which causes several types of cancers in humans. My work resolved two long standing questions in the field. First, I showed that the mechanism of strand discrimination in E. coli mismatch repair requires protein communication between the mismatch and the strand signal along the DNA helix contour. Next, I discovered the mechanism of eukaryotic strand discrimination by demonstrating that the orientation of the DNA-loaded replication sliding clamp PCNA directs the mismatch repair machinery to a particular strand. Next, I showed that PCNA can be loaded without orientation bias at extrahelical DNA extrusions within CTG/CAG repeats, and consequently dysregulate mismatch repair strand directionality. The latter finding provides a molecular explanation for the mutagenic involvement of mismatch repair in triplet repeat expansion in post-mitotic neurons.

Furthermore, as a researcher in the laboratory of Dr. Diane Merry at Thomas Jefferson University, my research was directed at understanding the pathological function of the CAG-encoded polyglutamine expansion within the androgen receptor in SBMA. I used a combination of biochemical, cellular, animal model approaches to demonstrate that cellular toxicity in SBMA arises (at least in part) from the polyQ-expanded-dependent dysregulation of a protein-protein interaction between AR and the deubiquitinase USP7.

My deep expertise in DNA repair pathways and my training in the field of triplet repeat instability provide a springboard for me to address questions in the intersection of these two areas. This aligns well with my long-standing interests in the mechanisms of genome instability and its impact on neurodegenerative disease. We have developed a suite of biochemical, in-cell, and proteomic approaches to investigate fundamental mechanisms of repair of repeat extrusions (structures that form during strand-slippage within long triplet repeats). Using such methods, we have recently discovered a novel role for PCNA in the activation of FAN1 nuclease function on CAG extrahelical extrusions (Phadte et al., PNAS, 2023).

Highlighted Research Support

R01-GM144553-01A1 (P.I. Pluciennik) 02/01/2023 - 12/31/2026
NIH
Crosstalk between DNA repair pathways in repeat instability.

R01 NS118082-01A1 (PI-Pluciennik) 06/30/2021 - 06/30/2026
NIH
Molecular mechanisms of triplet repeat instability in Huntington's disease.

Gies Foundation (PI-Pluciennik) 11/01/2021 - 10/31/2024
Factors that modulate FAN1 function in Huntington's disease

B. Positions, Scientific Appointments, and Honors

10/25/2021- present	Assistant Professor, Department of Biochemistry and Molecular Biology, Thomas Jefferson University, Philadelphia, PA
04/2019-10/25/2021	Research Assistant Professor, Department of Biochemistry and Molecular Biology, Thomas Jefferson University, Philadelphia, PA
10/2015-04/2019	Research Instructor, Department of Biochemistry and Molecular Biology, Thomas Jefferson University, Philadelphia, PA
01/2014-10/2015	Research Assistant A, Department of Biochemistry and Molecular Biology, Thomas Jefferson University, Philadelphia, PA
2006-2013	Senior Research Associate, Department of Biochemistry, Duke University Medical Center Durham, NC

C. Contributions to Science

1. During my doctoral studies with Dr. Adam Jaworski in Lodz, Poland and postdoctoral work with Dr. Robert Wells (Institute of Biosciences and Technology, Texas A&M University, Houston, Texas), I studied the molecular mechanisms of instability of repetitive DNA sequences (including CTG/CAG repeats), expansions of which cause neurodegenerative diseases like myotonic dystrophy, Huntington disease, and SBMA. This work established that strand slippage is a significant source of the genetic instabilities of triplet repeat sequences in cellular systems. In my postdoctoral studies, I discovered a novel type of CTG/CAG repeat expansion wherein massive tandem amplification of the repeat element along with flanking non-repetitive sequences occurred in cells. This mechanism may be involved in large expansions in type II neurodegenerative diseases (e.g. myotonic dystrophy), where CTG/CAG tracts expand from 10-20 repeats in normal individuals to more than 1000 repeats. Another highlight of my work was the demonstration that long CTG/CAG sequences are preferred sites for intermolecular and inter-molecular recombination.

a. Wells R.D., Parniewski P., **Pluciennik A.,** Bacolla A., Gellibolian R., and Jaworski A. (1998) Small slipped register genetic instabilities in Escherichia coli in triplet repeat sequences associated with hereditary neurological diseases. ***J Biol. Chem*.** Vol. 273, pp. 19532-19541
b. **Pluciennik A.,** Iyer R.R., Parniewski P., and Wells R.D. (2000) Tandem duplication. A novel type of triplet repeat instability. ***J Biol. Chem*.** Vol. 275 pp. 28386-28397

c. **Pluciennik A.,** Iyer R.R., Napierala M., Larson J.E., Filutowicz M., and Wells R.D. (2002) Long CTG.CAG repeats from myotonic dystrophy are preferred sites for intermolecular recombination. ***J Biol. Chem.*** Vol. 277, pp. 34074-34086
d. Napierala M., Parniewski P., **Pluciennik A.,** and Wells R.D. (2002) Long CTG.CAG repeat sequences markedly stimulate intramolecular recombination. ***J Biol Chem*****.** Vol. 277, pp. 34087-34100

2. My second postdoctoral fellowship at Duke University Medical Center involved research on the biochemistry of DNA mismatch repair. The DNA mismatch repair system corrects replication errors, ensures the fidelity of genetic recombination, and participates in cellular response to certain types of DNA damage. Mismatch repair is a postreplicative DNA repair mechanism which is directed to the newly synthesized strand. My work resolved two long standing and contentious questions in the field of mismatch repair. Working on the bacterial system, I demonstrated that mismatch repair initiation relies on transduction of the signal from the mismatch to the strand discrimination site via the contour of the DNA helix, and does not occur via DNA bending. I followed up this work with studies on the human mismatch repair system, which finally solved the long sought mechanism of strand discrimination in eukaryotes. I showed that the orientation of loaded replication sliding clamp PCNA serves to direct the endonuclease activity of MutLα to a particular strand, and in this manner acts as a strand discrimination signal in eukaryotes. These findings strongly suggest that the DNA ends on the post-replicative nascent DNA strand provide sites where PCNA is loaded in unique orientation, thus serving as a strand discrimination signal for mismatch repair. I extended these findings to triplet repeat sequences, the expansions of which underlie several neurodegenerative diseases. I showed that CTG or CAG extrahelical extrusions in closed circular DNA substrates not only provoke MutSβ-dependent mismatch repair, but also support PCNA loading and MutLα endonuclease activation on both DNA strands. Thus, DNA loops composed of CTG/CAG repeats dysregulate of mismatch repair strand directionality. Because mouse models for somatic expansion of CTG/CAG triplet repeat sequences have implicated both MutSβ and MutLα in triplet repeat expansion, and have shown that such expansions can occur in postmitotic cells, my findings provide a novel mechanism for triggering dysregulated mismatch repair on nonreplicating DNA.
My work identified novel interactions between mismatch repair proteins and the DNA repair machinery, suggesting a role for the mismatch repair system in modulation of replication fork dynamics in response to DNA lesions.

e. **Pluciennik A.,** and Modrich P. (2007) Protein roadblocks and helix discontinuities are barriers to the initiation of mismatch repair. ***Proc. Natl. Acad. Sci. USA*****.** Vol. 104, pp.12709-12713
f. **Pluciennik A.,** Burdett V., Lukianova O., and Modrich P. (2009) Involvement of the Beta Clamp in Methyl-Directed Mismatch Repair *in vitro*. ***J Biol. Chem*****.** Vol. 284 pp. 32782-32791 PMCID: PMC2781695
g. **Pluciennik, A.**[1], Dzantiev, L. [1], Iyer, R. R., Constantin, N., Kadyrov, F. A., and Modrich, P. (2010) PCNA Clamp Function in the Activation and Strand Direction of MutLα Endonuclease. ***Proc. Natl. Acad. Sci. USA*****.** Vol. 107, pp. 16066-16071, *joint first authors* PMCID: PMC2941292
h. **Pluciennik, A.,** Burdett V., Baitinger C., Iyer R. R., Shi K., and Modrich P. (2013) Extrahelical Triplet Repeat Elements Support PCNA Loading and MutLα Endonuclease Activation. ***Proc. Natl. Acad. Sci. USA*****.** Vol. 110, pp. 12277-12282 PMCID: PMC3725108

3. I was involved studying molecular mechanisms of spinal and bulbar muscular atrophy, caused by an expansion of a polyglutamine tract in the androgen receptor (AR). Disease pathology is characterized by muscle atrophy and by loss of motor neurons from the spinal cord and brainstem. I contributed to a study which determined that the mutant AR forms soluble aggregates as a full-length protein and is only later proteolyzed within maturing inclusions, in a proteasome activity-dependent manner, to produce mature inclusions that contain only the amino-terminal portion of the AR. I was also involved in the discovery that an interdomain interaction (the N/C interaction) of the mutant AR is required for aggregation and toxicity in a mouse model of SBMA. I also contributed to a study demonstrating that the nuclear export of polyQ-expanded AR is impaired and that promoting nuclear export of polyQ-expanded AR reduces its aggregation and toxicity.

I also demonstrated that cellular toxicity in SBMA arises (at least in part) from the polyQ-expanded-dependent dysregulation of a protein-protein interaction between AR and the deubiquitinase USP7. Our study showed that knockdown of USP7 resulted in suppression of disease phenotypes in SBMA and spinocerebellar ataxia type 3 (SCA3) fly models, and monoallelic knockout of Usp7 ameliorated several motor deficiencies in transgenic SBMA mice. Our findings have implications for the exploration of USP7 as a therapeutic target for the treatment of SBMA.

i. Heine, E.M., Berger, T.R., **Pluciennik, A.,** Orr, C.R., and Merry, D.E. (2015) Proteasome-Mediated Proteolysis of Polyglutamine-Expanded Androgen Receptor is a Late Event in SBMA Pathogenesis. ***J Biol. Chem.*** Vol. 290, pp. 12572-12584 PMCID: PMC4432278
j. Zboray, L., **Pluciennik, A.,** Curtis, D., Liu, Y., Berman-Booty, L.D., Kesler, C.T., Berger, T.R., Gioeli, D., Paschal, B.M., and Merry, D.E. (2015) Preventing the Androgen Receptor N/C Interaction Delays Disease Onset in Mouse Model of SBMA. ***Cell Rep.*** Vol. 13, pp. 2312-2323 PMCID: PMC4684905
k. Arnold, F.J., **Pluciennik, A.,** and Merry, D.E. (2019) Impaired Nuclear Export of Polyglutamine-Expanded Androgen Receptor in Spinal and Bulbar Muscular Atrophy. ***Sci Rep.*** Vol. 9, pp. 119 PMCID: PMC6333819
l. **Pluciennik A.**, Liu Y., Molotsky E., Marsh G.B., Ranxhi B., Arnold F.J., St.-Cyr S., Davidson B., Pourshafie N., Lieberman A.P., Gu W., Todi S.V. and Merry D.E. (2021) Deubiquitinase USP7 contributes to the pathogenicity of spinal and bulbar muscular atrophy. ***J Clin Invest***. Vol. 131, pp. e134565 PMCID: PMC7773404

Complete List of Published Work in MyBibliography:

https://www.ncbi.nlm.nih.gov/myncbi/anna.pluciennik.1/bibliography/public/

BIOGRAPHICAL SKETCH

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NAME: Fenglin Li

eRA COMMONS USER NAME (credential, e.g., agency login): FXL044

POSITION TITLE: Research Specialist

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
Shenyang Agricultural University, Liaoning, China	B.S.	07/2015	Biological Safety
Shenyang Agricultural University, Liaoning, China	M.S.	07/2018	Natural Products

A. Personal Statement

My research journey began with my Master's thesis at Shenyang Agricultural University in China, where I specialized in analyzing the structures of chemical compounds derived from microbial metabolisms. This work involved the use of mass spectrometry (MS), nuclear magnetic resonance (NMR), and chromatography. Driven by a profound interest in disease-relevant biological macromolecules and structural biology, I set out on a long-term academic career with the overarching goal of improving lives through impactful biomedical research.

Since 2019, I have been conducting research at Thomas Jefferson University in Philadelphia, USA. Over time, my focus has evolved to encompass the analysis of macromolecular structures, utilizing advanced techniques such as cryogenic electron microscopy (cryo-EM) and crystallography. This evolution has broadened my horizons within the field of structural biology.

During the initial four years in Dr. Gino Cingolani's lab, my research pursued two primary objectives: (1) catalyzing the development of viable phage therapies to combat numerous bacterial diseases globally, and (2) elucidating the fundamental biological mechanisms of nucleocytoplasmic communication mediated by nuclear transport receptors (NTRs) through nuclear pore complexes (NPCs). These endeavors resulted in various publications.

In October 2023, I transitioned into Dr. Anna Pluciennik's lab, marking a new chapter in my scientific journey. In this role, my primary focus is on identifying specific DNA repair mechanisms targeting Huntington's disease, a hereditary disorder. This transition represents a significant shift in my research direction, providing an exciting opportunity for exploration and contribution to the field.

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

12/2022 – current	Research Specialist	Thomas Jefferson University, PA, USA
09/2019 – 11/2022	Research Assistant	Thomas Jefferson University, PA, USA
03/2019 – 09/2019	Laboratory Technician	Chinese Academy of Sciences, Liaoning, China
10/2023 – current	Member	Society of Women Engineers

Honors

06/2023	Poster Presenter, XXVII Biennial Conference on Phage/Virus Assembly, Manchester, UK
06/2023	Poster Presenter, FASEB Virus Structure and Assembly Conference, MA, USA

C. Contributions to Science

(1) Phage therapy offers a promising approach to combat bacterial pathogens while circumventing concerns related to antibiotic resistance. Through the lens of structural biology, I sought to unravel the structural intricacies of protein machinery during phage infections, with a particular emphasis on their dynamic conformational changes. I have undertaken in-depth explorations of two major bacteriophage families: (a) *Podoviridae* and (b) *Myoviridae*, distinguished by their unique tail structures and genome delivery mechanisms.

(a) With regards to *Podoviridae*, phages known for their short tails for infection, I sought to characterize the structure of *Shigella* phage which infect the gram- negative pathogen *Shigella* that causes shigellosis. My work revealed *Shigella* phage Sf6's complete *in-situ* structure by cryo-EM, exposing a mechanism for infection that involves symmetry-mismatched interfaces. In particular, I noted that the mismatch between the capsid's 5-fold vertex and the tail machinery's 6-fold central tunnel is essential for Sf6's genome-delivery. The design principles I explored in Sf6's infection machinery is likely conserved across all P22-like *Podoviridae.*

Additionally, my research involved the prototypical *Podoviridae* T7 in which I assisted in structurally characterizing genome delivery machinery called the DNA- ejectosome. In contrast to my research of *in situ* Sf6, we worked with *in vitro* reconstituted protein assemblies that, upon ejection into host membrane, create a tunnel for genome translocation. Our high-resolution structure of the DNA- ejectosome sheds light on one of the many ways phages evade bacterial defenses.

(b) With regards to *Myoviridae,* phages known for their long, non-contractile tails, my research focused on *Pseudomonas* phage E217 which is clinically-tested to eradicate the cystic fibrosis-associated pathogen *Pseudomonas aeruginosa*. By cryo-EM, I solved the complete viral architecture composed of ~45 MDa of protein. I explored conformational changes of genome delivery machinery between pre- ejection and post-ejection states and unveiled E217's recognition of the host O- antigen as receptor. I hope that this work aids clinical trial development involving *Pseudomonas* Myo-phages for phage therapy by exploiting their knack for infection as effective biomedicines.

- **Fenglin Li**, Chun-Feng Hou, Ravi K. Lokareddy, Francesca Forti, Federica Briani, Gino Cingolani. High-resolution cryo-EM structure of the *Pseudomonas* bacteriophage E217. ***Nature Communications*** 14 (1):4052, 2023.
- **Fenglin Li**, Chun-Feng Hou, Ruoyu Yang, Richard Whitehead 3rd, Carolyn M Teschke, Gino Cingolani. High-resolution cryo-EM structure of the *Shigella* virus Sf6 genome delivery tail machine. ***Science Advances*** Dec 9;8(49):eadc9641, 2022.
- Nicholas A Swanson, Ravi K Lokareddy, **Fenglin Li**, Chun-Feng David Hou, Sebastian Leptihn, Mikhail Pavlenok, Michael Niederweis, Ruth A Pumroy, Vera Y Moiseenkova-Bell, Gino Cingolani. Cryo-EM structure of the periplasmic tunnel of T7 DNA-ejectosome at 2.7 Å resolution. ***Molecular Cell***. 81(15):3145-3159.e7, 2021.

(2) My research in the field of NTRs, particularly importins α1/β, has been equally illuminating. α1/β plays a crucial role in facilitating the intracellular transport of various proteins. Dysfunctions in α1/β have been linked to neurodegenerative disorders, cancer, and viral infections. Throughout independent and collaborative projects, my exploration of α1/β has centered around 2 sub-aims: (a) solving α1/β's transport machinery at a molecular-level, and (b) understanding how α1/β accommodates both small and large cargoes during nucleocytoplasmic transport.

(a) Regarding the first sub-aim, I characterized the binding of α1/β with two transport factors, nucleoporins (Nup) and RanGTP. Nups are NPCs that can house hydrophobic phenylalanine-glycine (FG) motifs which

capture NTRs during NPC passage. After traversing NPCs, NTRs release cargoes upon RanGTP-binding in the nucleus. By *in vitro* assembly, I complexed α1/β with FG-containing Nup fragments called NupFG (~25 kDa) and traced the key binding motifs to uncharged spacers of importin β. I assisted in examining α1/β/RanGTP structure, however it contained only density of β/RanGTP; α is absent. This aligns with the hypothesis of α1 self-inhibits by binding to the major nuclear localization signal (NLS) binding site via its importin-beta-binding (IBB) domain to release cargo.

(b) Regarding the second sub-aim, I contributed to the studies of α1/β's interactions with both small and large cargoes, specifically TAR DNA-binding protein 43 (TDP-43) and Hepatitis B Virus (HBV), respectively.

TDP-43, a prion-like protein, is implicated in conditions like amyotrophic lateral sclerosis and frontotemporal dementia when mislocalized to the cytoplasm. Our biochemical and crystallographic approaches revealed that α1/β recognizes TDP-43's bipartite NLS with extensive contacts at the minor NLS-binding site. Mechanistically, this NLS binding induces steric clashes with the C-terminus of importin α1, disrupting the TDP-43 N-terminal domain dimerization interface which may push TDP-43 towards aggregation as seen in disease. I explored α1/β's ability to accommodate the large cargo HBV which causes chronic infections targeting the liver. By cryo-EM, I helped solve the NLS of the HBV capsid and revealed that up to 30 copies of α1/β heterodimers can simultaneously bind to a single HBV capsid. Additionally, it was found that phosphorylation of HBV capsid protein induced a compaction of its C-terminal domain, favoring α1/β recruitment. A key novelty of this work is that it represents the first molecular-level insights into HBV's nuclear entry.

- Ruoyu Yang, Ying-Hui Ko, **Fenglin Li**, Ravi K. Lokareddy, Chun-Feng Hou, Christine Kim, Shelby Klein, Santiago Antolínez, Carolina Pérez-Segura, Martin F. Jarrold, Adam Zlotnick, Jodi A. Hadden-Perilla [*] and Gino Cingolani [*]. Structural basis for nuclear import of Hepatitis B Virus (HBV) nucleocapsid core. ***Science Advances***. 2023. *In Press*.
- Steven G. Doll, Hamed Meshkin, Alexander J. Bryer, **Fenglin Li**, Ying-Hui Ko , Ravi K. Lokareddy , Richard Gillian , Kushol Gupta , Juan R. Perilla and Gino Cingolani. Recognition of the TDP-43 Nuclear Localization Signal by importin a1/b. ***Cell Reports***, 39(13):111007, 2022.

Complete List of Published Work in Google Scholar:
https://scholar.google.com/citations?user=OiOWdQcAAAAJ&hl=en&oi=ao