BIOGRAPHICAL SKETCH

NAME: Kielkopf, Clara L.

eRA COMMONS USER NAME: ckielko1

POSITION TITLE: Professor of Biochemistry and Biophysics

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of Wisconsin, Madison	B.S. (*summa cum laude*)	08/1994	Molecular Biology
California Institute of Technology	Ph.D.	07/2000	Biology, Chemistry minor
Rockefeller University	Post-doctoral	09/2002	Biophysics

A. Personal Statement

Research: My laboratory investigates the molecular structures and dysregulation of pre-mRNA splicing factors involved in normal gene regulation and its dysfunction in cancers, particularly myelodysplastic syndromes. My group has made seminal contributions to the field's understanding of 3´ splice site recognition. Our high resolution structures reveal the critical, early-stage splicing factor U2AF2 recognizing splice site signals [*Nature Commun.* 2016]. We established phosphorylation-induced conformational changes in the splicing factor subunit SF1 and its complex with U2AF2 [*Structure* 2013]. We demonstrated that the RNA binding properties of unmodified and cancer-associated mutant SF1–U2AF2–U2AF1 ternary complexes align with the trends among affected 3´ splice site motifs [*Leukemia* 2015; *PLOS Genetics* 2016]. Our research also shows that cancer-associated mutations of U2AF2 alter RNA binding and splicing of representative pre-mRNAs [*J. Biol. Chem.* 2020]. Equipped with this insight, we identified and characterized a small molecule modulator of U2AF2 as proof-of-concept for a pharmacological means to manipulate pre-mRNA splicing [*Cell Chem. Biol.* 2021]. An ongoing goal is to determine the molecular architecture of multi-subunit U2AF splicing factor complexes using cryogenic electron microscopy and orthogonal methods. Through my investigations, I seek to not only expand our fundamental understanding of RNA biology but also to uncover potential therapeutic targets for addressing human diseases.

Mentoring: Beyond my scientific endeavors, I am dedicated to mentoring the next generation of scientists and promoting diversity in STEM fields. Nine pre-doctorates have completed Ph.D. training in my laboratory and achieved successful scientific careers in academia and biotechnology settings. It has been my pleasure to mentor several underrepresented postdoctoral scholars with successful careers, and I am an enthusiastic mentor of undergraduate research. I teach in three Ph.D. level courses each year and direct a core course of our PhD programs. I chaired the Biophysics PhD Program Admissions Committee for three years, served on the committee for five years and now serve on the Biochemistry PhD Program Admissions Committee.

Ongoing projects that I would like to highlight include:

R01 GM070503
Role: Principal Investigator
08/01/2022 – 07/31/2026
Molecular Recognition during pre-mRNA Splicing

Edward P. Evans Foundation
Role: Principal Investigator
09/01/2020-02/29/2024 (no cost extension)
Cryo-electron microscopy structures of mutant U2AF1-containing ribonucleoproteins associated with MDS

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2007-present Assistant, Associate (2009, with tenure), and Professor (2017) of Biochemistry and Biophysics, University of Rochester Medical Center (URMC)
2019-present Member, Wilmot Cancer Institute (WCI) Program in Genetics, Epigenetics and Metabolism
2010-present Co-director, Structural Biology and Biophysics Facility (with Prof. Joseph Wedekind)
2008-present Member, Center for RNA Biology, URMC
2007-present Member, Graduate Training Program in Biochemistry, Molecular, and Cell Biology, URMC
2007-present Member, Graduate Training Program in Biophysics and Structural Biology, URMC

2005-2007 Member, Graduate Training Program in Chemistry and Biology, Johns Hopkins University (JHU)
2004-2007 Joint Appointment in Oncology: JHU
2002-2007 Assistant Professor of Biochemistry and Molecular Biology, JHU
2002-2007 Member, Graduate Training Program in Computational and Molecular Biophysics, JHU
2000-2002 Postdoctoral Research with Prof. Stephen K. Burley, Rockefeller University
1994-2000 Graduate research with Prof. Douglas C. Rees, California Institute of Technology
1993-1994 Undergraduate research with Prof. Ivan Rayment, University of Wisconsin-Madison

Honors

2005 Faculty Research Initiative Award, Johns Hopkins University
2005 Prostate Cancer Foundation Award
2004 Kimmel Scholar Award
2004 Basil O'Connor Award
2015, 2017, 2020 Edward P. Evans Foundation Research Award
2009 Cover illustration for CSHL RNA processing meeting abstract book
2003 Faculty Innovation Award, Johns Hopkins University
2002 Faculty Development Award, Johns Hopkins University
2000-2002 American Cancer Society Postdoctoral Fellowship
1994-1997 National Science Foundation Pre-Doctoral Fellowship
1994 General and Molecular Biology Honors, University of Wisconsin-Madison
1994 *Suma cum laude:* Graduating class rank 1/3000, University of Wisconsin-Madison
1993 Hilldale Summer Research Fellowship, University of Wisconsin-Madison

Representative Professional Activities (in past five years)

2023 Ad-hoc Reviewer: Board of Scientific Counselors review of National Institute of Environmental Health Sciences, Division of Intramural Research, National Institutes of Health (NIH)
2023 Biochemistry and Biophysics Faculty Search Committee
2022-present MedSac Steering Committee, University of Rochester School of Medicine & Dentistry (URMC)
2022-present Biochemistry and Molecular Biology PhD Program Admissions Committee, URMC
2022 Co-Chair, Protein-Nucleic Acids Session of the annual American Crystallographic Association (ACA) meeting, Portland, OR
2022 U of R Sproull and Provost Fellowship Review Committee
2017, 2021 (virtual), 2022 Edward P. Evans Foundation Research Grant Review
2019-present URMC Electron Microscopy Facility Committee
2019 WCI Faculty Search Committee
2018-2021 F1000 – Peer-elected Faculty Member
2019-2021 URMC Faculty Professionalism Council
2020 Ad-hoc Reviewer: Biological Chemistry and Biophysics Conflict of Interest Panel, NIH
2020 RNA Salon Student Presentation Awards – Judge
2018-2020 URMC Minority Diversity Liaison Committee
2019 Biology Faculty Search Committee, Wilmot Cancer Institute, U. Rochester
2019 Nomination Committee for cryoEM Special Interest Group, ACA

Journal Article Reviews: *ACS Chemical Biology, Biochemistry, Biophysical Journal, Genes and Development, Cell Chemical Biology, Genes and Genomics, Journal of the American Chemical Society, Journal of Biological Chemistry, Journal of Molecular Biology, Molecular Cell, Nature, Nature Communications, Nature Structural and Molecular Biology, Nucleic Acids Research*, *PLoS Genetics, PLoS One, PNAS, Proteins, RNA, Science Reports, Structure.*

Professional Memberships: American Chemical Society, American Crystallographic Association, American Society for Biochemistry and Molecular Biology, Biophysical Society, RNA Society.

Representative extramural presentations (in past five years)

2023 Invited Speaker, American Chemistry Society Symposium on Drugging Pre-mRNA Splicing
2022 Invited Speaker, WCI Hematology Cluster
2022 Invited Speaker, WCI GEM Cluster
2021 Invited Speaker, Steenbock Symposium: SpliceCon 2021, U. Wisconsin-Madison, WI (virtual)
2020 Invited Speaker, Quantitative Medicine Seminar Series, Rutgers the State University, NJ (virtual)

2020 Invited Speaker, RNA Collaborative Seminar Series (inter-institutional) (virtual)
2019 Speaker selected from abstracts, ACA Annual Meeting, Covington, KY
2019 Invited Speaker, Splicing Factor Mutations in Cancer Workshop, New Haven, CT

C. Contribution to Science

Complete List of Published Work in My Bibliography:

https://www.ncbi.nlm.nih.gov/myncbi/clara.kielkopf.1/bibliography/public/

Specific Contributions to the Field:

1. Structure and MDS-related dysregulation of U2AF1 (also called U2AF^{35}). An S34F mutation of U2AF^{35} is prevalent among myelodysplastic syndromes (MDS) and leukemias. We developed methods to produce nearly full length U2AF^{35} complexes with splicing factor partners, in sufficient quantities for structural biophysics. We compared the RNA affinities of wild-type (WT) U2AF^{35} with an S34F mutation that is prevalent among MDS and lung adenocarcinomas, for representative affected splice sites. We found that relative to wild-type U2AF^{35}, the S34F-mutation modulated the binding affinity for splice site RNAs, often in agreement with sequences of S34F-affected splice sites. This result shows a direct, and potentially treatable, molecular consequence of the MDS-relevant U2AF^{35} S34F mutation. We identified and characterized a small molecule targeting the U2AF2 (also called U2AF^{65}) subunit, which modulated pre-mRNA splicing and selectively killed cells expressing mutant U2AF^{35}. This work provided a molecular mechanism for the U2AF1 mutant to contribute to the progression of MDS.
   a. Okeyo-Owuor T, White BS, Chatrikhi R, Mohan DR, Kim S, Griffith M, Ding L, Ketkar-Kulkarni S, Hundal J, Laird KM, **Kielkopf CL**, Ley TJ, Walter MJ, Graubert TA (2015) U2AF1 mutations alter sequence specificity of pre-mRNA binding and splicing. *Leukemia* 29:909-917. PMID: 25311244; PMCID: PMC4391984.
   b. Fei DL, Motowski H, Chatrikhi, R, Prasad S, Yu, J, Gao J, **Kielkopf CL,** Bradley RK, Varmus H. (2016) Splicing factor U2AF1 inhibits splicing associated with a recurrent U2AF1 mutant in human lung cancers and is required for cell survival. *PLOS Genetics,* 12:e1006384. PMID: 27776121; PMCID: PMC5077151.
   c. Warnasooriya C, Feeney CF, Laird KM, Ermolenko DN, **Kielkopf CL.** A splice site-sensing conformational switch in U2AF2 is modulated by U2AF1 and its recurrent myelodysplasia-associated mutation. *Nucleic Acids Res*. 2020; 48: 5695-5709. PMID: 32343311; PMCID: PMC7261175.
   d. Chatrikhi R, Feeney CF, Pulvino MJ, Alachouzos G, MacRae AJ, Falls Z, Rai S, Brennessel WW, Jenkins JL, Walter MJ, Graubert TA, Samudrala R, Jurica MS, Frontier AJ, **Kielkopf CL.** (2021) A synthetic small molecule stalls pre-mRNA splicing by promoting an early-stage U2AF2-RNA complex. *Cell Chem. Biol.* 28:1145-1157.e6. PMID: 33689684; PMCID: PMC8380659.
2. Polypyrimidine tract signals recognition by the key pre-mRNA splicing factor, U2AF2 (also called U2AF^{65}). Our seminal structures of U2AF^{65} detailed the arrangement of its RNA recognition motifs (RRM1 and RRM2) bound to polypyrimidine tract RNAs. Small-angle X-ray scattering and crystal structures of U2AF2 bound to cytidine-substituted polypyrimidine (Py) tracts showed local and global inter-RRM structural changes adapting to the different Py tracts. Most recently, crystal structures complemented by molecular dynamics and CLIP-seq revealed distinct RNA conformations of nucleotide variations at the center of the Py tract adapt to a U2AF^{65} scaffold. Altogether, this work provides a basis for relating U2AF^{65}–Py tract interactions to natural splice site sequences and their defects in human genetic disease.
   a. Sickmier EA, Frato KE, Paranawithana S, Shen H, Green MR and **Kielkopf CL.** (2006) Structural basis of polypyrimidine tract recognition by the essential splicing factor U2AF^{65}. *Mol. Cell* 23:49-59. PMID: 16818232; PMCID: PMC2043114.
   b. Jenkins JL, Agrawal AA, Gupta A, Green MR, **Kielkopf CL.** (2013) U2AF^{65} adapts to diverse pre-mRNA splice sites through conformational selection of specific and promiscuous RNA recognition motifs. *Nucleic Acids Res.* 41:3859-73. PMID: 23376934; PMCID: PMC3616741.
   c. Agrawal AA, Salsi E, Chatrikhi R, Henderson S, Jenkins JL, Green, MR, Ermolenko DN, **Kielkopf CL.** (2016) An extended U2AF^{65}-RNA binding domain recognizes the 3´ splice site signal. *Nature Commun*. 7:10950. PMID: 26952537; PMCID: PMC4786784.
   d. Glasser E, Maji D, Biancon G, Puthenpeedikakkal AMK, Cavender CE, Tebaldi T, Jenkins JL, Mathews DH, Halene S, **Kielkopf CL.** (2022) Pre-mRNA splicing factor U2AF2 recognizes distinct conformations

of nucleotide variants at the center of the pre-mRNA splice site signal. *Nucleic Acids Res.* 50:5299-5312. PMID: 35524551; PMCID: PMC9128377.

3. U2AF2 defects contributing to human disease. Considering the MDS-associated mutation of U2AF1, we asked whether U2AF2 is mutated in cancers. We identified clusters of cancer-associated mutations at the U2AF2 – RNA interface and confirmed that representative mutations alter RNA binding and splicing. Point mutations in Py tracts also dysregulate splicing of specific transcripts, leading to inherited human disease. Guided by our structural understanding, we engineered a synthetic $U2AF^{65}$ protein that specifically improved binding and splicing of representative disease-casing mutations in Py tracts. This work establishes a molecular mechanism for U2AF2 defects to contribute to human disease and cancers.
   a. Agrawal AA, McLaughlin KJ, Jenkins JL, **Kielkopf CL.** (2014) Structure-guided $U2AF^{65}$ variant improves recognition and splicing of a defective pre-mRNA. *PNAS* 111:17420-17425. PMID: 25422459; PMCID: PMC4267390.
   b. Glasser E, Agrawal AA, Jenkins JL, **Kielkopf CL**. (2017) Cancer-Associated Mutations Mapped on High-Resolution Structures of the U2AF2 RNA Recognition Motifs. *Biochemistry.* 56:4757-4761. PMCID: PMC6005654.
   c. Maji D, Glasser E, Henderson S, Galardi J, Pulvino MJ, Jenkins JL, **Kielkopf CL.** (2020) Representative cancer-associated U2AF2 mutations alter RNA interactions and splicing. *J. Biol. Chem.* 295:17148-17157. PMID: 28850223; PMCID: PMC7863893.
4. An SF3B1 hub for UHM-containing splicing factors. We discovered domains of U2AF1 and U2AF2 bind short linear motifs of protein ligands (ULMs) and represent a greater family of "U2AF Homology Motif" (UHM)-containing splicing factors [*Cell* 2001; *RNA* 2016]. We established that the MDS-relevant splicing factor SF3B1 (also called SF3b155) offers five ULMs as a molecular hub for regulation of splice sites by UHM-containing proteins, including $U2AF^{65}$, CAPERα, and Tat-SF1.
   a. Thickman KR, Swenson M, Gryczynski Z and **Kielkopf CL.** (2006) Multiple $U2AF^{65}$ binding sites within SF3b155: Thermodynamic and spectroscopic characterization of protein-protein interactions by pre-mRNA splicing factor complexes. *J. Mol. Biol.* 356:664-683. PMID: 16376933; PMCID: PMC2043113.
   b. Loerch S, Maucuer A, Manceau V, Green MR, and **Kielkopf CL.** (2014) Cancer-relevant splicing factor CAPERα engages the essential splicing factor SF3b155 in a specific ternary complex. *J. Biol. Chem.* 289:17325-17337. PMID: 24795046; PMCID: PMC4067167.
   c. Loerch S, Leach JR, Horner SW, Maji D, Jenkins JL, Pulvino MJ, **Kielkopf CL.** (2019) The pre-mRNA splicing and transcription factor Tat-SF1 is a functional partner of the spliceosome SF3B1 subunit via a U2AF homology motif interface. *J. Biol. Chem*. 22:2892-2902. PMID: 30567737; PMCID: PMC6393619.
   d. Galardi JW, Bela VN, Jeffery N, He X, Glasser E, Loerch S, Jenkins JL, Pulvino MJ, Boutz PL, **Kielkopf CL.** (2022) A UHM – ULM interface with unusual structural features contributes to U2AF2 and SF3B1 association for pre-mRNA splicing. *J Biol Chem.* 298:102224. PMID: 35780835; PMCID: PMC9364107.
5. Structure and regulation of Splicing Factor-1 (SF1). Before association with SF3B1 during spliceosome assembly, $U2AF^{65}$ forms a ternary complex with SF1, which in turn recognizes the BPS of the pre-mRNA. The SF1-interacting region of $U2AF^{65}$ is a UHM similar to that found in $U2AF^{35}$. We showed that specific binding of the $U2AF^{65}$ UHM to SF1 is conferred by an extension of the minimal ULM by a coiled-coil SF1 interface. By homology with the $U2AF^{65}$ UHM, we identified a UHM-containing kinase (KIS or UHMK1) that specifically phosphorylates an SPSP motif in SF1. This SPSP motif of SF1 isolated from human cells is predominately in the phosphorylated state. SF1 phosphorylation triggers local folding of an arginine claw surrounding the SPSP motif and enhances phosphorylated SF1 association with $U2AF^{65}$.
   a. Manceau, V, Swenson, M, LeCaer, JP, Sobel, A, **Kielkopf, CL** and Maucuer, A. (2006) Major phosphorylation of SF1 on adjacent Ser-Pro motifs enhances interaction with $U2AF^{65}$. *FEBS J.* 273:577-587. PMID: 16420481; PMCID: PMC1949809.
   b. Gupta A, Jenkins JL and **Kielkopf CL.** (2011) RNA induces conformational changes in the SF1/$U2AF^{65}$ splicing factor complex. *J. Mol. Biol.* 405:1128-1138. PMID: 21146534; PMCID: PMC3037027.
   c. Wang, W, Maucuer, A, Gupta, A, Manceau, V, Bauer, WJ, Kennedy, SD, Wedekind, JE, Green, MR, and **Kielkopf, CL.** (2013) Structure of phosphorylated SF1 bound to $U2AF^{65}$ in an essential splicing factor complex. *Structure* 21:197-208. PMID: 23273425; PMCID: PMC3570649.
   d. Chatrikhi R, Wang W, Gupta A, Loerch S, Maucuer A, **Kielkopf CL.** (2016) SF1 phosphorylation enhances specific binding to $U2AF^{65}$ and reduces binding to 3'-splice site RNA. *Biophysical J.* 111:2570-2586. PMID: 28002734; PMCID: PMC5192697.