

Arek Kulczyk

Mobile: 617-320-5563

Work: 848-445-4553

arek.kulczyk@rutgers.edu

<http://kulczyk-lab.cryoemcorp.com>

<https://www.linkedin.com/in/arek-kulczyk-44288110>

Institute for Quantitative Biomedicine

Dept. of Biochemistry & Microbiology

Rutgers, The State University of NJ

174 Frelinghuysen Road

Piscataway, NJ 08854, USA

EDUCATION

10/99- 04/03 **Ph.D. in Biophysics - University of Cambridge/MRC Laboratory of Molecular Biology**

- Thesis: NMR and biochemical studies of novel zinc-finger domains binding to nicked DNA.
- The project was conducted in collaboration with the Nobel Prize laureate Dr. Cesar Milstein.
- Determined a solution NMR structure of the DNA-binding domain from DNA ligase III.
- Designed and characterized the DNA ligand suitable for NMR studies and investigated its physical interactions with DNA ligase III using chemical shift mapping and other biophysical methods.
- Developed bacterial expression systems for high-level production of isotopically labeled proteins: poly(ADP-ribose) polymerase and DNA ligase III.

10/92-06/97 **M.Sc. in Molecular Biology - Jagiellonian University, Krakow, Poland**

PROFESSIONAL EXPERIENCE

09/17- Present **Assistant Professor – Rutgers University**

- My laboratory integrates structural approaches, in particular single-particle cryo-electron microscopy (cryo-EM) and single-molecule techniques to study DNA replication and repair in human mitochondria. We also develop novel correlative light and electron microscopy (CLEM) methods that allow simultaneous visualization of an enzymatic activity and structure determination.

01/13- 08/17 **Instructor in Biological Chemistry and Molecular Pharmacology - Harvard University**

- Junior faculty appointment to establish an independent research program to study DNA replication.
- Determined a structure of the ~650-kDa functional replisome assembled on DNA resembling a replication fork. The structure determined by single-particle cryo-EM provides the first structural snapshot of the entire replisome and reveals fundamental molecular mechanisms for coordination of leading- and lagging-strand synthesis.
- Obtained three grants from National Science Foundation to perform structure calculations using the eighth most powerful supercomputer in the world.
- Responsible for implementing cryo-EM software in SBGrid consortium for structural biology at Harvard.

01/05- 12/12 **Postdoctoral Fellow - Harvard Medical School**

- Developed single-molecule techniques for real-time monitoring of enzymatic activities of the replication proteins.
- Measured thermodynamic and kinetic parameters associated with assembly of the viral replication complex.
- Developed a fluorescence-based assay to monitor formation of the viral replisome.
- Measured catalytic properties of the replication proteins using a variety of enzymatic assays.
- Developed and optimized baculoviral and bacterial expression systems and purification protocols for high-level production of all components of the replication complexes.
- Optimized high-throughput screening methods for crystallization of viral and human replication enzymes.

05/03- 12/04 **Postdoctoral Fellow - University of Cambridge, UK**

- A short appointment to refine a solution NMR structure of the DNA-binding domain from DNA ligase III determined during my doctoral studies and to publish an article.

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PREDOCTORAL RESEARCH EXPERIENCE

01/99- 08/99 **Research Assistant - Northeastern University, Boston, USA**
07/97- 12/98 **Research Assistant - Jagiellonian University, Krakow, Poland**

SCHOLARSHIPS AND AWARDS

10/99- 04/03 Association for International Cancer Research Scholarship held at the University of Cambridge, UK
06/98- 12/98 Jagiellonian University Scholarship held at the University of London, UK
02/95- 07/95 ERASMUS Scholarship awarded by the European Commission and held at the University of Wolverhampton, UK

TECHNICAL EXPERTISE

- **Biophysical methods:** electron microscopy (EM) and cryo-electron microscopy (cryo-EM), single-molecule techniques to study protein/nucleic acids interactions, nuclear magnetic resonance (NMR) spectroscopy, X-ray crystallography, electrospray mass spectrometry (EMS), isothermal titration calorimetry (ITC), surface plasmon resonance (SPR), circular dichroism (CD), fluorescence spectroscopy (fluorescence quenching, fluorescence anisotropy), UV spectroscopy, dynamic light scattering (DLS), analytical ultracentrifugation (AUC), atomic force microscopy (AFM), confocal microscopy
- **Biochemical methods:** high-throughput screening (HTS) methods for protein crystallization, protein expression in insect cells and in bacteria, labeling of proteins with: ^{13}C , ^{15}N , ^2H , protein purification using: affinity chromatography, ion-exchange chromatography, adsorption chromatography, size-exclusion chromatography, protein concentration using different methods, dialysis, cell cultures
- **Molecular biology:** PCR, RT-PCR, restriction digest, ligation, primer and probe sequence design, cloning, mutagenesis, SDS-PAGE, chemical transformation and electroporation, chemical synthesis of DNA and RNA, extraction of DNA and RNA from cell cultures, purification of nucleic acids using PAGE and HPLC, radioactive labeling of proteins with ^{35}S , and nucleic acids with ^{32}P or ^{33}P , band shift assays, northern-, southern- and western-blotting, ELISA, electroelution, protein refolding, silver-staining, *in vitro* transcription/translation reaction using bacterial extracts or rabbit reticulocytes
- **Computing:** OS (Windows, Macintosh, LINUX and UNIX), programming languages (AWK, PERL), software for structure calculation: XPLOR, CNS, structure visualization and analysis: GRASP, INSIGHT, PROCHECK, RIBBONS, PYMOL, MOLMOL, POV-Ray, CHIMERA, sequence alignments, NMR software: XWINNMR, SPARKY, ANSIG, TALOS, EM software: RELION, IMAGIC-4D, FREALIGN, SERIAL-EM, EMAN2, APPION, LEGION, statistical analysis: MATLAB, ORIGIN, high-performance computing (HPC)

TEACHING EXPERIENCE

01/08- 06/08 **Lecturer in Biochemistry – Brandeis University, Waltham, USA**

- Responsible for developing a syllabus, teaching and grading the course: Advanced Biochemistry: Information transfer mechanisms.

01/99- 08/99 **Teaching Assistant in Microbiology - Northeastern University, Boston, USA**
07/97- 05/98 **Teaching Assistant in Molecular Biology - Jagiellonian University, Krakow, Poland**

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PROFFESIONAL MEMBERSHIPS

- American Chemical Society, American Society for Biochemistry and Molecular Biology, American Society for Microbiology, Biochemical Society, Biophysical Society, Protein Society

EDITORIAL ACTIVITIES

- Ad hoc Reviewer: Science, Molecular Cell, Nucleic Acids Research, Journal of Biological Chemistry, Journal of Molecular Biology

GRANTS

National Science Foundation and Extreme Science and Engineering Discovery Environment grants to determine a high-resolution cryo-EM structure of bacteriophage T7 replisome using high-performance computing facilities at Texas Advanced Computing Center and Pittsburgh Supercomputing Center.

01/16- 03/18 Grant # MCB150136

08/15- 03/17 Grant # MCB150101

COLLABORATIONS

I frequently collaborate with the academic and industrial partners in the USA and abroad. Past collaborations resulted in multiple scientific publications and presentations. The list of academic institutions I currently collaborate with include: Harvard University, University of Louisville, USA; Aarhus University, Denmark; Max Planck Institute of Biophysics, Germany; Jagiellonian University, Poland; University of Wollongong, Australia. Industrial collaborators included: Biogen, Cambridge, MA and Lucigen Corporation, Middleton, WI, USA.

PUBLICATIONS

- Kulczyk, A.W.***, Moeller, A., Meyer P., Sliz, P., Richardson, C.C*. (2017). Cryo-EM structure of the replisome reveals multiple interactions coordinating DNA synthesis. *Proceedings of the National Academy of Sciences of the USA*, 114, 1848-1856.
*Corresponding authors
- Duderstadt, K.E., Geertsema, H.J., Stratmann, S.A., Punter, C.M., **Kulczyk, A.W.**, Richardson, C.C., van Oijen, A.M. (2016). Simultaneous real-time imaging of leading- and lagging-strand synthesis reveals the coordination dynamics of single replisomes. *Molecular Cell*, 64, 1035-1047.
- Lasica, A, **Kulczyk, A.W.** et al. (2016). Structural and functional probing of PorZ, an essential bacterial-surface component of the type-IX secretion system of human oral-microbiomic *P. gingivalis*. *Scientific Reports*, 6, 37708.
- Geertsema, H.J., **Kulczyk, A.W.**, Richardson, C.C., van Oijen A. (2014). Single-molecule studies of polymerase dynamics and stoichiometry at the bacteriophage T7 replication machinery. *Proceedings of the National Academy of Sciences of the USA*, 111, 4073-4078.
- Kulczyk, A.W.***, Richardson, C.C. (2012). Molecular interactions in bacteriophage T7 priming complex. *Proceedings of the National Academy of Sciences of the USA*, 109, 9408-9413.
*Corresponding author
- Kulczyk, A.W.**, Akabayov, B., Lee, S-J. et al. (2012). An interaction between DNA polymerase and helicase is essential for the high processivity of the replisome. *Journal of Biological Chemistry*, 287(46), 39050-39060.

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7. Tran, N., Amarasiriwarden, C.J., **Kulczyk, A.W.**, Richardson, C.C., Tabor, S. (2012). Characterization of a nucleotide kinase encoded by bacteriophage T7. *Journal of Biological Chemistry*, 287 (35), 29458-29478.
8. Zhang, H., Lee, S.-J., **Kulczyk, A.W.**, Zhu, B., Richardson, C.C. (2012). Heterohexamer of 56- and 63-kD gene 4 helicase-primase of bacteriophage T7 in DNA replication. *Journal of Biological Chemistry*, 287(41), 34273-34287.
9. Akabayov, B., **Kulczyk, A.W.**, Akabayov, S., McLaughlin, L., Theile, C., Beauchamp, B., Richardson, C.C. (2011). Pyrovanadolysis: a pyrophosphorolysis-like reaction mediated by pyrovanadate, Mn²⁺, and DNA polymerase of bacteriophage T7. *Journal of Biological Chemistry*, 286(33), 29146-29157.
10. Loparo, J., **Kulczyk, A.W.**, Richardson, C.C., van Oijen A. (2011). Observing polymerase exchange by simultaneous measurements of replisome structure and function at the single-molecule level*. *Proceedings of the National Academy of Sciences of the USA*, 108, 3584-3589.
*- commentary by Shi, X., Ha, T. (2011). Seeing a molecular machine self-renew. (2011). *Proceedings of the National Academy of Sciences of the USA*, 108, 3459-3460.
11. Satapathy, A.K., **Kulczyk, A.W.**, Ghosh, S., van Oijen, A.M., Richardson, C.C. (2011). A Critical residue for coupling dTTP hydrolysis with DNA unwinding by the helicase of bacteriophage T7. *Journal of Biological Chemistry*, 286(39), 34468-34478.
12. **Kulczyk, A.W.**, Tanner N., Loparo J., Richardson C., van Oijen A. (2010). Direct observation of enzymes replicating DNA using a single-molecule DNA stretching assay. *Journal of Visual Experiments*, 37, id: 1689, doi: 10.3791/1689.
13. Akabayov, B., Akabayov, S.R., Lee, S.J., Tabor, S., **Kulczyk, A.W.**, Richardson, C.C. (2010). Conformational dynamics of bacteriophage T7 DNA polymerase and its processivity factor, *Escherichia coli* thioredoxin. *Proceedings of the National Academy of Sciences of the USA*, 107, 15033-15038.
14. Qimron, U., **Kulczyk, A.W.**, Hamdan, S.M., Tabor, S., Richardson, C.C. (2008). Inadequate inhibition of host RNA polymerase restricts T7 bacteriophage growth on hosts overexpressing udk. *Molecular Microbiology*, 67, 448-457.
15. **Kulczyk, A.W.**, Yang, J., Neuhaus, D. (2004). Solution structure and DNA binding of the zinc finger domain from DNA ligase IIIa. *Journal of Molecular Biology*, 341, 723-738.

Book Chapters:

16. **Kulczyk, A.W.**, Richardson, C.C. (2016). The Replication System of Bacteriophage T7. *The Enzymes*, 39, 89-136.

Conference Proceedings:

17. Duderstadt K.E., Punter C.M., **Kulczyk A.W.**, Richardson C.C., van Oijen A. (2014). Simultaneous imaging of leading- and lagging-strand synthesis reveals distinct operational modes of single replication machines. *Biophysical Journal*, 106(2), 273a.
18. **Kulczyk, A.W.**, Richardson, C.C. (2013). Single-molecule studies of the replisome structure and dynamics. *Protein Science*, 22, 69.
19. Satapathy, A.K., **Kulczyk, A.W.**, Ghosh, S., Richardson, C.C. (2011). Coupling dTTP hydrolysis with DNA unwinding by the helicase of bacteriophage T7. *The FASEB Journal*, 25:880.6.

LECTURES AND PRESENTATIONS

Invited Lectures:

- European Physical Society meeting, Podlesice, Poland, 2017
- European Physical Society meeting: Biomolecules and Nanostructures, Krakow, Poland, 2015
- International Biophysics Congress, Brisbane, Australia, 2014
- Keystone Symposium on DNA Replication and Recombination, Banff, Canada, 2013

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Talks at Research Seminars:

- SBGrid Workshop on EM Data Processing, Harvard Medical School, 2017
- Center for Integrative Proteomics Research, Rutgers University, 2017
- Seminar on DNA repair and replication, Harvard University, 2016
- Interdisciplinary Nanoscience Center, University of Aarhus, Denmark, 2015
- The 6th Brazil School for Single Particle Cryo-EM, Sao Paulo, Brazil, 2014
- AstraZeneca, Waltham, USA, 2012
- Harvard Medical School, BCMP, Boston, USA, 2012
- Brazilian Synchrotron Light Laboratory, Sao Paulo, Brazil, 2012
- Biogen Idec, Cambridge, USA, 2011
- McGill University, Facility for Electron Microscopy Research, Montreal, Canada, 2010
- Harvard Medical School, BCMP, Boston, USA, 2004
- Harvard University, DMCB, Cambridge, USA, 2004
- Northwestern University, Evanston, USA, 2004
- University of California, Berkeley, USA, 2004
- The Scripps Research Institute, La Jolla, USA, 2004
- University of Cambridge, MRC Laboratory of Molecular Biology, UK, 2003
- Northeastern University, Department of Biology, Boston, USA, 1999
- Jagiellonian University, Institute of Molecular Biology, Krakow, Poland, 1998
- University of London, Queen Mary and Westfield College, UK, 1998

Poster Presentations at Conferences:

- American Academy of Arts and Sciences, Cambridge, USA, 2016
- Biological Chemistry and Molecular Pharmacology Symposium, Cape Cod, USA, 2016
- Symposium on Cryo-EM, Houston, USA, 2016
- National Center for Macromolecular Imaging Workshop on Single Particle Reconstruction, Structural Variability and Modeling, Houston, USA, 2016
- American Academy of Arts and Sciences Symposium, Cambridge, USA, 2015
- Cryo-EM 3D Image Analysis Symposium, Tahoe City, USA, 2014
- Maintenance of Genome Stability Meeting, St. Kitts, 2014
- American Academy of Arts and Sciences, Cambridge, USA, 2013
- Symposium of the Protein Society, Boston, USA, 2013
- Keystone Conference on DNA Replication and Recombination, Keystone, USA, 2011
- Maintenance of Genome Stability Meeting, Antigua, 2010
- American Academy of Arts and Sciences, Cambridge, USA, 2006
- International Crystallographic Association Meeting, Chicago, USA, 2004
- XXth International Conference on Magnetic Resonance, Toronto, Canada, 2002
- 15th International Meeting on NMR Spectroscopy, Durham, Scotland, 2001
- EMBO Meeting on Structure Determination of Biological Macromolecules by solution NMR, Heidelberg, Germany, 2001