Barak Akabayov August 2, 2022

CURRICULUM VITAE

- **Personal Details**

Barak Akabayov
Work address/phone: Building 29 room 113, Department of Chemistry, Ben-Gurion University of the Negev, +972-8-6472716.
ORCID: https://orcid.org/0000-0002-3882-2742

- **Education**

B.Sc. 1999, **Bar-Ilan University**, Department of Life Sciences
M.Sc. 2001, **Bar-Ilan University**, Department of Life Sciences, Advisor: Prof. Asher Shainberg. Title of thesis: Cardiomyocytes Death Following Hypoxia - Mechanism and Ways for Treatment.
Ph.D. 2007, **Weizmann Institute of Science**, Department of Structural Biology, Advisor: Prof. Irit Sagi. Title of thesis: Structural Dynamic Insights into the Selective Catalysis of the RNA Helicase DbpA.

- **Employment History**

Since August 2014, Senior Lecturer in the Department of Chemistry at **Ben-Gurion University of the Negev**
2013-2014, Instructor for Biological Chemistry and Molecular Pharmacology, **Harvard Medical School**
2007-2013, Postdoctoral Fellow, Department of Biological Chemistry and Molecular Pharmacology, **Harvard Medical School**

- **Professional Activities**

2021 Heading the chemistry-data science program.
Since 2019 Delegate of the Israeli users' committee of SESAME (Synchrotron-light for Experimental Science and Applications in the Middle East). I have participated in SESAME Users' Committee Meeting (Allan, Jordan, Nov 28-Dec 1, 2019).
Since 2014 Member of the department committee for graduate studies.
2016-2018 Vice Chair of the SESAME Users' Committee.
Since 2017 Affiliate of the scientific board of BioRxiv (preprint server, Cold-Spring Harbor Labs).
Since 2007 Member in several scientific societies including: Biophysical Society, Biochemical Society, The Protein Society, American Society for Microbiology, Israeli Biochemistry and Molecular Biology, Israeli Biophysical Society, Israeli Chemical Society.

Reviewer of Scientific Journals
Nature publishing group Journals, FEBS Press Journals, Royal Society of Chemistry Journals, MDPI Journals.

Funding Committees
Reviewer and committee member of several funding agencies (Israeli and foreign).

- **Educational activities**

a) Courses taught

Since 2016 Proteins and Enzymes: Structure Function and Kinetics (204-1-1623) for undergraduate students, 3rd year. Lecturer, Ben-Gurion University of the Negev.
2016-2018 Biochemistry (205-1-9041) for undergraduate students, 2nd year. Lecturer, Ben-Gurion University of the Negev.
Since 2015 Topics in biochemistry and molecular biophysics (204-2-3032) for graduate students. Lecturer, Ben-Gurion University of the Negev
Since 2014 General chemistry laboratory (204-1-1543), undergraduate laboratory for Life Sciences/pharmacology, Lecturer, Ben-Gurion University of the Negev.
2012 "Molecular and Cellular Basis of Medicine", tutor in a course for medical students, Harvard Medical School.
2002 Cell Physiology course, teaching assistant, Bar-Ilan University.

b) Current research students

Four Ph.D. students: Adi Dayan (4th year Direct track, Bsore Scholarship), Shankar Bhattarai (1st year).
One Postdoc researcher: Vinay Shankar Tiwari (postdoc, 2nd year).
Four M.Sc. students (2): Sarah-Adi Eisndorfer (2nd year), Shlomi Sanens (1st year), Samuel Dhurna (1st year), Aviv Rozen (1st year).

c) Alumni

Stefan Ilic (Ph.D., 2019), Yasmin Ben-Yishay (M.Sc., 2019), Hadar Ben-Shushan (M.Sc., 2019), Shira Cohen (M.Sc., 2020), Moria Pertz (M.Sc., 2020). Meenakshi Singh (Postdoc, 2018), Rikeshwer Prasad Dewangan (Postdoc, 2018), Benjamin Tam (PhD., 2022).

- **Awards, Citations, Honors, Fellowships**

(Including all internal university support and prizes, not including research grants.)
1) Travel Grant for the 7th Congress of the Federation of the Israel Societies for Experimental Biology ("Ilanit"), Eilat, Israel, 2014.
2) Travel Grant the Polish Biochemical Society, Ukrainian Biochemical Society and Israel Society for Biochemistry and Molecular Biology, Warsaw, Poland, 2011.
3) Travel Grant for the EMBO meeting on "Helicases and NTP driven nucleic acid machines: structure-function-diseases", Arolla, Switzerland, 2005.

(b) Peer-Reviewed Instrumentation Grants

1) Measurements at Structural Biology and Genomics Technology Platform Department of Integrated Structural Biology, IGBMC. Funded by Instruct-ERIC, October 2018.
2) Since 2014, an active member in the Israeli BAG for measurements at a European Synchrotron Radiation Facility.

Independent user at several synchrotrons for variety of techniques.
SAXS measurements at the Cornell High Energy Synchrotron Source (CHESS), Ithaca, NY (November 2008, June 2013, November 2013) and at the National Synchrotron Light Source (NSLS), Upton, NY, USA (August 2009, April 2011, March 2012). Structural studies of the bacteriophage T7 replisome.
XAFS experiment at the NSLS (August 2009, September 2009). Conformation of the active site of T7 DNA primase.

- **Scientific Publications**

* Equally contributed
** Equal correspondence

2022
40. MolOptimizer: a molecular optimization toolkit for fragment-based screening. S-J. Viswas, D. Vilenchik, B. Akabayov (2022). Submitted.

39. Machine learning approaches to optimize small-molecule inhibitors for RNA targeting. H. Grimberg , V.S. Tiwari, B. Tam, L. Gur-Arie, D. Gingold, L. Polachek, B. Akabayov (2022). *Journal of Cheminformatics*, 14 (4).

2021

38. Inferring primase-DNA specific recognition by using a data-driven approach. A. Soffer*, S.A. Eisdorfer*, M. Ifrach, S. Ilic, A. Afek, H. Schussheim, D. Vilenchik, B. Akabayov, (2021). *Nucleic Acids Researc*h, 49(20), 11447–11458.

37. Cell-penetrating peptide conjugates of indole-3-acetic acid-based DNA primase/Gyrase inhibitors as potent antitubercular agents against planktonic and biofilm culture of Mycobacterium smegmatis. R.P. Dewangan, M. Singh, S. Ilic, B. Tam, B. Akabayov, (2021). *Chemical Biology & Drug Design*, 98(5), 722-732.

36. Molecular Dynamics Simulations of Duplexation of Acyclic Analogs of Nucleic Acids for Antisense Inhibition. R. Galindo-Murillo, J. S. Cohen, B. Akabayov (2021). *Molecular Therapy – Nucleic Acids*, 23, P527-535.

2020

35. In vivo biogenesis of a de novo designed iron-sulfur protein. B. Jagilinki, S. Ilic, C. Trncik, A. Tyryshkin, D. Pike, W. Lubitz, E. Bill, B. Akabayov, O. Einsle, J. Birrell, D. Noy, V. Nanda (2020). *ACS Synthetic Biology,* 9(12), 3400–3407.

34. Engineering Stem Cell Factor Ligands with Different c-Kit Agonistic Potencies. T. Tilayov, T. Hingaly, Y. Greenshpan, S. Cohen, B. Akabayov, R. Gazit, N. Papo, (2020). *Molecules,* 25(20):4850.

33. Dual acting small-Molecule inhibitors targeting Mycobacterial DNA replication. M. Singh*, S. Ilic*, B. Tam*, Y. Ben-Ishay, D. Sherf, D. Pappo, B. Akabayov, (2020). *Chemistry – A European Journal*, 26 (47), 10849-10860.

32. Nanobodies Targeting Prostate-specific Membrane Antigen for the Imaging and Therapy of Prostate Cancer. L. Rosenfeld, A. Sananes, Y. Zur, S. Cohen, K. Dhara, S. Gelkop, E. Ben Zeev, A. Shahar, L. Lobel, B. Akabayov, E. Arbely, N. Papo, (2020). Journal of Medicinal Chemistry. 63(14):7601-7615.

31. The Amuvatinib Derivative, N-(2H-1,3-Benzodioxol-5-yl)-4-{thieno[3,2-d]pyrimidin-4-yl}piperazine-1-carboxamide, Inhibits Mitochondria and Kills Tumor Cells under Glucose Starvation. R. Marciano, H. Ben-David, B. Akabayov, B. Rotblat, (2020). International Journal of Molecular Sciences, 21(3), 1041.

30. SIRT6 is a DNA double-strand break sensor. L. Onn, M. Portillo, S. Ilic, G. Cleitman, D. Stein, S. Kaluski, I. Shirat, Z. Slobodnik, M. Einav, F. Erdel, B. Akabayov, D. Toiber, (2020), Elife. 9: e51636.

2019

29. Discovery of small-molecule inhibitors targeting the ribosomal peptidyl transferase center (PTC) of M. tuberculosis. B. Tam, D. Sherf, S. Cohen, S. Adi Eisdorfer, M. Peretz, A. Soffer, D. Vilenchik, S.R. Akabayov, G. Wagner, B. Akabayov, (2019). *Chemical Science*, 2019, **10**, 8764-8767. [Cover: c9sc90211b]

28. DNA sequence recognition by DNA-primase using high-throughput primase profiling (HTPP). S. Ilic, S. Cohen, A. Afek, R. Gordan, D.B. Lukatsky, B. Akabayov, (2019). *JoVE*, (152), e59737.

27. Specific and label-free immunosensing of protein-protein interactions with silicon-based immunoFETs. I-M. Bhattacharyya*, S. Cohen*, A. Shalabny, M. Bashouti, B. Akavayov, G. Shalev, (2019). *Biosensors and Bioelectronics*, 132:143-161.

2018

26. DnaG primase- a target for the development of novel antibacterial agents. S. Ilic, S. Cohen, M. Singh, B. Tam, B. Akabayov, (2018). Special issue in *Antibiotics (Basel)*: Bacterial DNA replication and replication inhibitors. 13;7(3). pii: E72.

25. DNA sequence context controls the binding and processivity of the T7 DNA primase. A. Afek*, S. Ilic*, J. Horton, D. Lukatsky*, R. Gordan*, B. Akabayov*, (2018). *iScience*, 2(141-147).

24. NMR-fragment based virtual screening: a brief overview. M. Singh, B. Tam, and B. Akabayov, (2018). *Molecules (Special issue: Recent Advances in Biomolecular NMR Spectroscopy)*, 23(2). pii: E233.

2017

23. Modulation of RNA primer formation by Mn(II)-substituted T7 DNA primase. S. Ilic, S.R. Akabayov, R. Froimovici, R. Meiry, D. Vilenchik, A. Hernandes, H. Arthanari, B. Akabayov, (2017). *Scientific Reports*, 7(1):5797.

22. Engineering a monomeric variant of macrophage colony-stimulating factor (M-CSF) that antagonizes the c-FMS receptor. Y. Zur, L. Rosenfeld, A. Bakhman, S. Ilic, H. Hayun, A. Shahar, B. Akabayov, M. Kosloff, N. Levaot, N. Papo, (2017). *Biochemical Journal*, 20;474(15):2601-2617.

Before 2017

21. Identification of DNA primase inhibitors via a combined fragment-based and virtual screening. S. Ilic, S.R. Akabayov, H. Arthanari, G. Wagner, C.C. Richardson, B. Akabayov, (2016). *Scientific Reports*, 6:36322.

20. eIF4A augments Ago2-mediated Dicer-independent miRNA bogenesis and RNA interference. T. Yi, H. Arthanari, B. Akabayov, H. Song, E. Papadopolous, H.H. Qi, M. Jedrychowski, T. Guttler, C. Guo, R.E. Luna, S.P. Gygi, G. Wagner, (2015). *Nature Communications*, 6, 7164.

19. Human translation initiation factor eIF4G1 possesses a low affinity ATP binding site facing the ATP-binding cleft of eIF4A in the eIF4G/eIF4A complex. S.R. Akabayov, B. Akabayov, G. Wagner, (2014). *Biochemistry*, 53(41):6422-5.

Before starting BGU affiliation

18. Vanadate in structural biology. S.R. Akabayov**, B. Akabayov**, (2014). *Inorganica Chimica Acta,* 420, 16–23.

17. The interaction between eukaryotic initiation factor 1A and eIF5 retains eIF1 within scanning preinitiation complexes. R.E. Luna, H. Arthanari, H. Hiraishi, B. Akabayov, L. Tang, C. Cox, M.A. Markus, L.E. Luna, Y. Ikeda, R. Watanabe, E. Bedoya, C. Yu, S. Alikhan, G. Wagner, K. Asano, (2013). *Biochemistry,* 2013 52(52):9510-8.

16. Molecular Crowding Enhanced ATPase Activity of the RNA Helicase eIF4A Correlates with Compaction of Its Quaternary Structure and Association with eIF4G. S.R. Akabayov, B. Akabayov, C.C. Richardson, G. Wagner, (2013). *J Am Chem Soc*, 135(27), 10040-10047. (See spotlight in JACS)

15. Isolation, characterization, and aggregation of a structured bacterial matrix precursor. L. Chai, D. Romero C. Kayatekin, B. Akabayov, H. Vlamakis, R. Losick, R. Kolter, (2013). *J Biol Chem*, 288(44), 17559-17568.

14. Impact of macromolecular crowding on DNA replication. B. Akabayov, SR. Akabayov, S-J. Lee, G. Wagner, and CC. Richardson, (2013). *Nature Communications*, 4:1615.

13. An interaction between DNA polymerase and helicase is essential for the high processivity of the bacteriophage T7 replisome. AW. Kulczyk, B. Akabayov, S-J. Lee, M. Bostina, S. Berkowitz, and CC. Richardson, (2012). *J Biol Chem*, 287(46):39050-60.

12. Zinc-binding domain of DNA primase modulates binding to template DNA. S-J. Lee, B. Zhu, B. Akabayov, and CC Richardson, (2012). *J Biol Chem*, 287(46):39030-40.

11. Role of exposed cysteine located on the thioredoxin-binding region of gene 5 DNA polymerase of bacteriophage T7. N.Q. Tran*, S-J. Lee*, B. Akabayov*, DE. Johnson and CC. Richardson, (2012). *J Biol Chem*, 287(47):39732-41.

10. The C-terminal domain of eukaryotic initiation factor 5 promotes start codon recognition by its dynamic interplay with eIF1 and eIF2β. RE. Luna, H. Arthanari, H. Hiraishi, J. Nanda, P. Martin-Marcos, M. Markus, B. Akabayov, A. Milbradt, S. Hyberts, LE. Luna, M. Reibarkh, A. Farmy, H. Seo, A. Marintchev, A. Hinnebusch, J. Lorsch, K. Asano, and G. Wagner, (2012). *Cell Reports,* 1(6), 689-702.

9. Probing conformational variations at the ATPase site of the RNA helicase DbpA by high-field electron-nuclear double resonance spectroscopy. I. Kaminker, A. Sushenko, A. Potapov, S. Daube, B. Akabayov, I. Sagi and D. Goldfarb, (2011). *J Am Chem Soc,* 133(39):15514-23.

8. Pyrovanadolysis: a pyrophosphorolysis-like reaction mediated by pyrovanadate and Mn-substituted DNA polymerase of bacteriophage T7. B. Akabayov, AW. Kulczyk, SR. Akabayov, C. Thiele, LW. McLaughlin, B. Beauchamp, and CC Richardson, (2011). *J Biol Chem*, 19;286(33):29146-57.

7. Binding of Mn-deoxyribonucleoside triphosphates to the active site of the DNA polymerase of bacteriophage T7. B. Akabayov, C.C. Richardson, (2011). *Powder Diffraction,* 2(26):159-163.
6. Conformational dynamics of bacteriophage T7 DNA polymerase and its processivity factor, Escherichia coli thioredoxin. B. Akabayov**, S.R. Akabayov, S-J. Lee, A Kulczyk, S. Tabor, C.C. Richardson**, (2010). *Proc Natl Acad Sci USA*, 107 (34) 15033-15038.
5. DNA recognition by the DNA primase of bacteriophage T7: a structure-function study of the zinc-binding domain. B. Akabayov, S-J. Lee, S.R. Akabayov, S. Rekhi, B. Zhu, C.C. Richardson, (2009). *Biochemistry*, 48(8), 1763–73.
4. Key feature of the catalytic cycle of TNF-alpha converting enzyme involves communication between distal protein sites and the enzyme catalytic core. A. Solomon, B. Akabayov, M.E. Milla, I. Sagi, (2007). *Proc Natl Acad Sci USA* 104 (12): 4931-6.
3. Using softer x-ray absorption spectroscopy to probe biological systems. B. Akabayov, CJ. Doonan, IJ. Pickering, GN. George and I. Sagi, (2005). *J Synchrotron Radiat* 12 (4): 392-401.
2. RNA labeling and immobilization for nano-displacement measurements: probing three dimensional RNA structures. B. Akabayov, A. Henn, M. Elbaum, I. Sagi, (2003). *IEEE TRANS Nanobioscience*; 2(2):70-4.
1. The role of A1/A3 adenosine receptor activation in reduction of cardiomyocyte injury caused by hypoxic stress and in induction of apoptosis in rat cardiomyocyte cultures. A. Shainberg, N. Safran, N. Balas, KA. Jacobson, T. Zinman, A. Isaac, K. Schwab, B. Akabayov, V. Shneyvays, (2000). *Adv Exp Med Biol*; 486: 201-5.

(c) Conference proceedings

1. B. Akabayov, C.C. Richardson: Binding of Mn-deoxyribonucleoside triphosphates to the active site of the DNA polymerase of bacteriophage T7. Denver X-ray Conference Proceedings, Advances in X-ray Analysis, Denver, CO, 2010. (Selected by the editor in chief of Advances in X-Ray Analysis for publication in Powder Diffraction J).
2. B. Akabayov, A. Henn, G. Nautrup-Pedersen, M. Elbaum, I. Sagi: Immobilization of RNA: application to single molecule spectroscopy. Proceedings of the IEEE-EMBS topic conference on molecular, cellular and tissue engineering. Genoa, Italy, 2002

(d) Articles in popular media

Our research were covered by the popular media. For specific updates: https://akabayov-lab.org/popular-media/

- **Lectures and Presentations at Meetings and Invited Seminars**

(a) Invited lectures

1. Cardioprotective effect of adenosine derivatives: a significant decrease of [Ca2+] in hypoxia. Annual meeting of Israeli subsection of the international society for heart research. Tel-Aviv, 2000.
2. A possible mechanism for cardioprotection against ischemia by adenosine receptor agonists. The annual symposium of Israel society of cardiology, Technion, Haifa, 2001.
3. Time-resolved freeze-quench X-Ray absorption spectroscopy. Application to the spectroscopic studies of bacteriochlorophyll and related systems. The Avron Minerva Center for Photosynthesis meeting at the Hebrew University, 2004.
4. X-ray absorption fine structure (beamline design and specifications). 3rd SESAME Users Meeting, Antalya, Turkey, 2004.
5. Structure function and dynamics of DbpA. “Exploring Protein Structure” meeting in Haifa University, Haifa, Israel, 2005.
6. Pyrophsphorolytic and pyrovanadolytic activities of the T7 DNA polymerase. 1st North American Core Shell Spectroscopy Conference (& the 59th Denver X-ray Conference), Denver, CO. 2010.

7. Impact of macromolecular crowding on DNA replication. The 8th Parnas Conference organized by the Polish Biochemical Society, Ukrainian Biochemical Society and Israel Society for Biochemistry and Molecular Biology, Warsaw, Poland (awarded travel fellowship), 2011.
8. Faculty of Chemistry, Weizmann Institute of Science, Structure-Function Studies of a Replisome, 2011.
9. Pyrovanadolytic Activity of the T7 DNA Polymerase. The 8th Vanadium Symposium, Crystal City, VA, 2012.
10. Bar-Ilan University Medical School, Structure-function Studies of a Replisome. 23 December 2013.
11. Tel-Aviv University, Joint Seminar Department of Biochemistry and Molecular Biology and Department of Molecular Microbiology and Biotechnology, 31 December 2013.
12. Macromolecular Crowding alters the binding properties of proteins, their activities, and their structures. The 7th Congress of the Federation of the Israel Societies for Experimental Biology, Eilat, Israel, (awarded travel fellowship), 2014.

Starting BGU affiliation

13. Using a novel hybrid method for the design of a new class of antibiotics to target DnaG primase of Mycobacterium tuberculosis. Different approaches in structural biology. Israeli Crystallographic Association (ICA) annual meeting 2017.
14. Development of anti-tuberculosis agents by using a combined NMR-fragment based screening and virtual screening. Emerging Trends on Drug Design and Development-2018, Indian Institute of Technology, BHU, Varanasi, India. January 18-20, 2018.
15. Development of anti-tuberculosis agents by using NMR-fragment-based and virtual screening. Israel Chemical Society (ICS) Meeting, Tel Aviv, February 13-14, 2018.
16. Application of Synchrotron-Based Techniques in Biochemical Sciences. Israel-Synchrotron Based Research in the Israeli Scientific Community (ESRF workshop), The Israel Academy of Sciences and Humanities, Jerusalem, March 21, 2018.
17. Using a Combined NMR-fragment based Screening and Virtual Screening for the Development of Anti-tuberculosis Agents. Drug Discovery Science and Technology Meeting, Jinan, China, November 6-8, 2018.
18. Inhibitors targeting the ribosomal peptidyl transferase center of Mycobacterium tuberculosis. Biochemical Society, Translation UK, Glasgow, July 3-5, 2019.
19. Development of antibacterial agents that target the ribosomal PTC of M. tuberculosis. New frontiers in structure-based drug discovery. Florence, Italy. 23-25 September 2019.
20. Targeting noncoding RNA: NMR fragment-based design Targeting RNA Europe Congress. Frankfurt, Germany. November 26-27, 2019 (Fully reimbursed).
21. A machine-learning approach to model and predict Okazaki fragment start sites on phage T7 genome. Confirmed speaker at the Israeli Chemical Society, International Convention Center, Jerusalem, December 24, 2020.
22. Reconfiguring primase DNA-recognition sequences by using a data-driven approach. Israel Data Science Initiative: IDSI 2022. Ein Gedi, January 3-6, 2022.
23. MMCS2022. Confirmed speaker at the 3rd Molecules Medicinal Chemistry Symposium, Rome, Italy, 27-29/7/2022.

24. MCS-ICS. Expanding the chemical space of a hit molecule. Invited speaker for the 18th Annual Meeting of The Medicinal Chemistry Section of the Israel Chemical Society (MCS-ICS), Weizmann Institute of Science, July 31st , 2022.

I have been invited for few future international conferences (talks and discussion panels)

(b) Presentation of papers at conferences/meetings (poster presentation).

1. Predicting Okazaki fragments start sites. Applied Bioinformatics in Life Sciences (3rd edition), Leuven, Belgium 13-14 February 2020.
2. Remastering specific DNA recognition by DNA primase. EMBO conference DNA replication, Heidelberg, Germany 7-10 May 2018.
3. Using a novel hybrid method to target DnaG primase for the design of new class of Mycobacterium tuberculosis antibiotics. EMBO conference Tuberculosis September Paris, France, 19-23 2016.
4. Developing inhibitors that target bacterial DNA replication. ASM Microbe, Boston, MA, June 16-20 2016.
5. Small molecule inhibitors for M. tuberculosis DnaG. The Second Israeli-American Kavli Frontiers of Science Symposium, Jerusalem, February 23-25, 2015.
6. Macromolecular crowding alters the binding properties of protein complexes, their activities, and their structures. The Protein Society meeting, Boston, MA, 2013.
7. Conformational dynamics of bacteriophage T7 DNA polymerase and its processivity factor, Escherichia coli thioredoxin. Keystone Symposia on DNA Replication and Recombination, Keystone, CO, 2011.
8. Pyrophophorolysis of DNA primer by T7 DNA polymerase: a structure-function study. Biophysical Meeting, San-Francisco, CA, 2010.
9. Conformational dynamics in bacteriophage T7 DNA polymerase: insights from low to high resolution. Biophysical Meeting, Boston, MA, 2009.
10. Rational design for mutation analysis of the phage T7 gene 5 protein. Keystone Symposia on DNA Replication and Recombination, Santa Fe, NM, February 10-15, 2008.
11. The metal ion in the RNA helicase DbpA may act as a single atom turnover switch during catalysis. The EMBO meeting on “Helicases and NTP driven nucleic acid machines: structure-function-diseases”, Arolla, Switzerland, 2005 (awarded poster prize).
12. RNA engineering for single molecule spectroscopy. The 21st Annual Conference and Technical Workshop of the Israel Vacuum Society (IVS) Dan Hotel, Tel-Aviv, 2002.
13. Hypoxia: signaling and biochemical modifications in cardiomyocytes and the attempt for cardioprotection. The Annual Meeting of the Israel Society for Physiology and Pharmacology, Male-Ha’Chamisha, 2000.

(d) Seminar presentations at universities and institutions

1. Seminar in the Department of Life Sciences, Ben-Gurion University of the Negev, December 15, 2014.
2. Seminar in the National Institute of Biotechnology of the Negev, Ben-Gurion University of the Negev, May 20, 2015.
3. Seminar in the Department of Biotechnology Engineering, Ben-Gurion University of the Negev, January 17, 2016.

4. Small-molecule inhibitors for bacterial replication and translation. Department of Structural Biology, Weizmann Institute of Science (June 25 2019).
5. Small-molecule inhibitors for bacterial replication and translation. Department of Microbiology and Molecular Genetics, The Hebrew University-Hadassah Medical School, Jerusalem (June 17 2019).
6. NMR-fragment based Screening and Virtual Screening, MIGAL Galilee Research Institute (May 19, 2019).
7. Development of anti-tuberculosis agents by using NMR-fragment-based and virtual screening Department of Biochemistry and Microbiology Fermentation Webinar, Rutgers University, New-Jersey (zoom), March 9, 2021.
8. Using NMR-fragment based and computational chemistry for the development of novel antibacterial agents targeting the ribosomal PTC of M. tuberculosis. Department Seminar at seminar at DSEEP, Ben-Gurion University of the Negev, Sede Boqer (zoom), April 6, 2021.
9. Schulich Faculty of Chemistry, Technion - Israel Institute of Technology, January 24, 2022.
10. Nanocenter seminar at the Hebrew University. Specific primase DNA recognition, April 24, 2022.
11. Chemical Biology seminar, Hebrew University. Reconfiguring DNA recognition by a primase, June 23, 2022.

- **Patents**

1. Inventor, “lead compounds/inhibitors for bacterial DNA replication”, US Patent 11,339,189.
2. Inventor, “Inhibitors for bacterial ribosomal peptidyl transferase center”, PCT submitted with BGN, 2019 (filed a US provisional patent application, Serial Number 62/789,570, on January 8, 2019 (BGU-P-087-US).
3. Inventor, 2022, Patent application (continuation-in-part, CIP).

- **Research Grants**

1. 2018-2022, Israel Science Foundation (ISF): 4 years, Grant No. 1023/18
2. 2017-2019, the IMTI (TAMAT) / Israel Ministry of Industry– KAMIN Program, Grant No. 59081
3. 2017-2019, US-Israel Bi National Science Foundation (BSF-startup grant, Grant No. 2016142)
4. 2018, Instruct integrative biology (Instrumentation peer reviewed grant). Funded trips for measurements at the synchrotron.
5. 2019-2020, the IMTI (TAMAT) / Israel Ministry of Industry– KAMIN Program (3rd year), Grant No. 65890
6. 2020-2022, Water Authority, ministry of energy
7. 2021-2023, Ministry of Defense, Grant No. 4441137465

- **Meetings and Courses**

Chairing session in Israeli Biophysical Society meeting, Technion, 2015.
Chairing session in the Medicinal Chemistry Branch of ICS (Virtual Meeting), June 23 2021.

- **Additional activities**

<u>Outreach activities</u>
Teaching science class in elementary school.

- **Synopsis of research, including reference to publications and grants in above lists**

Overview:
My overarching goal is to understand DNA replication and protein translation at the molecular level and to use this information for biomedical applications. In my research lab at BGU we are utilizing innovative biophysical tools and approaches to assess the structural nature and the biomolecular interactions in protein-protein and protein-nucleic acid complexes. From the results we learn how these interactions determine and impact biological catalysis of these domains in vivo.

The imminent need for the development of new antibacterial drugs has led us to develop inhibitors targeted against components in crucial molecular biology domains in bacterial cell, such as DNA replication and protein translation.

I. Studies of DNA replication

Mycobacterium Tuberculosis is a pathogenic bacterium and the causative agent of tuberculosis, which infects a third of the world population and kills more than 1.5 million people worldwide every year. **The long-term goal of my laboratory is to study the DNA replication in *M. tuberculosis*, which is performed by the replisome, a multi enzyme complex that synthesizes DNA.** Although DNA replication has been studied extensively in many model organisms, the organization of the protein components at the replication fork of *M. tuberculosis,* and their activities are poorly understood. Understanding the basic mechanisms that regulate DNA replication in *M. tuberculosis*is critical for the development of new therapeutic approaches to control bacterial proliferation. To date, the minimal *M. tuberculosis* replisome arrangement that is required for coordinated DNA synthesis is not known, and the role that many protein components play at the replication fork, as well as the interactions between them, are still largely unexplored. Mounting evidence collected over the past few years supports the idea that DNA replication in mycobacteria is unique and may be coordinated differently from that found in other bacteria as well as in humans.

In filling these **knowledge gaps**, my lab is aiming to shed light on the activities at the replication fork of *M. tuberculosis*. In particular, we will unravel the sequence on the DNA that is recognized by the DnaG primase, an essential enzyme that binds to a specific DNA sequence on the genome and performs catalytic activity important for normal DNA replication. Revealing the primase binding signature on genomic DNA was made possible through protein-DNA binding microarray containing massive amount of DNA sequences with their assigned binding scores to the primase (**A. Afek and S. Ilic et al., 2019, *iScience***). In a new research paper (**A. Soffer et al., 2021, *Nucleic Acid research***), we sought to leverage advances in data science and in machine learning to analyze DNA-primase interactions. Machine-learning algorithms were used for characterization of specific DNA sequences that lead to DNA sequence recognition which is an essential step in the recruitment of DNA polymerase on the DNA replication fork. We hope to discover the minimal RNA size that is required to initiate polymerase activity and discover the importance of these new primase-DNA recognition sequences to DNA replication.

Since the methods for data acquisition and data analysis were developed on the bacteriophage T7 system (which serves as the simplest model for DNA replication, see **S. Ilic et al., *Sci Rep* 2016**) this paves the way for studies of the complex and unexplored system of DnaG from *M. tuberculosis*. We have already obtained large amounts of highly purified *M. tuberculosis* DnaG and we are using data science tools and approaches to explore the specific recognition site of DnaG primase on the *M. tuberculosis* genome (**work in progress**).

Similarly to the studies on DnaG primase, we are characterizing other DNA replication components of *M. tuberculosis*, such as PolIII protein components DnaB helicase and SSB. We are currently evaluating the processivity of PolII to determine the *in vitro* rate of DNA synthesis by the DNA polymerase α subunit. Structure determination of the DNA replication components of *M. tuberculosis* will provide insights, at atomic resolution, into key activities at the replication fork. Complementary structural approaches, such as NMR and SAXS, provides insights into the key interactions that regulate DNA synthesis in the

leading and lagging DNA strands (**A. Dayan and B. Akabayov, manuscript in preparation**) and the maturation process of DnaB helicase (**S. Cohen and B. Akabayov manuscript in preparation**).

II. Developing inhibitors for DNA replication of *M. tuberculosis*

In addition to basic research, my lab develops inhibitors that target the DNA replication machinery in *M. tuberculosis.* We developed a drug discovery approach designated as NMR-fragment based virtual screening to discover inhibitors for DNA primase of bacteriophage T7. Proof of concept for the workflow has already yielded five potent small-molecule inhibitors (**Ilic S. et al., *Sci Rep* 2016**, PCT patent filed with BGN, 2016 **WO-2018/073828**). Three small molecules were found to inhibit the related *M. tuberculosis* DnaG primase. Intrigued by these recent results we synthesized derivatives with improved binding/inhibitory properties that target DnaG primase and GyrB-gyrase, two essential enzymes in *M. tuberculosis* (**M. Singh et al., *Chemistry –A European Journal* 2020**). As a strategy to improve the penetrability of these indole-based molecules into bacterial cells the inhibitors were conjugated to cell penetrating peptides (**R.P. Dewangan et al, *Chemical Biology and Drug Design* 2020**).

Importantly, we have synthesized a new generation of specific inhibitors for the *M. tuberculosis* Gyrase with superior efficacy (improvement of two orders of magnitude **B. Tam, V.S. Tiwari, H. Grimberg, and B. Akabayov, manuscript in preparation**).

III. Developing inhibitors for protein translation of *M. tuberculosis*

In a tangential project, we have developed new lead compounds that target gene translation in *M. tuberculosis*. Gene translation is an essential event in the cell cycle of every bacterium; it is performed by the ribosome, a molecular machine composed of two subunits that are made up of RNA molecules and proteins. The bacterial ribosome fulfills most of the requirements of a potential drug target and the active site of the ribosome, the peptidyl transferase center (PTC) has already proven to be a hotspot for antibiotic binding. It seems eminently reasonable to extrapolate these findings to *M. tuberculosis* (including resistant strains). We therefore used a fragment-based screening workflow in which the first step was the novel exploitation of NMR transverse relaxation times to identify fragment molecules that bind specifically to RNA hairpin 91 in the ribosomal PTC of *M. tuberculosis*.

This initial screening was followed by computational optimization of the fragment molecules into larger molecules with drug-like properties. Specifically, a virtual filtration followed by a high-throughput docking procedure yielded drug-sized molecules. We trained various machine-learning models for predicting the docking binding free energy (ΔG_{bind}) as a function of geometric features extracted from each of the above molecules. As superior inhibitors, the machine-learning model predicted two molecules that exhibited IC_{50} values superior to that of chloramphenicol, an antibiotic drug that acts on the ribosomal PTC. Findings of this study were recently published (**B. Tam et al., *Chemical Science* 2019**, a US provisional patent application is filed, Serial Number **62/789,570, BGU-P-087-US**). Intrigued by these recent results we synthesized derivatives with improved binding/inhibitory properties. Our studies will yield new anti-tuberculous agents and will provide new tools for fragment-based lead discovery.

We are currently using state-of-the-art data science approaches to further optimize our phenyl-thiazol based molecules, to synthesize them in the lab and to verify our optimization models (**H. Grimberg et al., *J. Cheminformatics* 2022**).

- **Lab progress**

I established my research lab in the Chemistry Department in 2014. Our studies are multidisciplinary by nature, we are using tools taken from the fields of biochemistry, structural biology, bacterial genetics, and microbiology to answer complex scientific

questions. We use data science approaches to construct models and to raise testable hypotheses. Our experimental approaches are used to verify the computational models and predictions.

Active group: I have an active multi-disciplinary team of individuals with background on chemistry, chemical biology, biochemistry, and data science. Several students have already graduated: a Ph.D. student, and four M.Sc. students. Two former postdocs are holding academic positions in India.

Grants: I have obtained competitive grants (BSF and ISF) and the following: Water authority, Israel Ministry of Defense, Kamin, Kamin 3rd year, and Instruct instrumentation grants that funds measurements in Europe. My research group is part of the Israeli blocked allocation group (BAG) for the European synchrotron.

Publications: My lab has published twenty papers (including collaborative, reviews, submitted, and under revision) and two patents with chemistry@bgu affiliation, among them 9 as last and corresponding author (with students). I have been invited to give 14 national and international seminars in universities and leading conferences (several others were canceled during the last year due to the COVID-19 crisis).

Teaching statement: As a teacher I would like to guide the students how to answer complex biological questions using the basic principles learned in the class and through current examples from the literature. I believe that establishing a strong background in the subject matter is a key component in achieving the goals of the course.
My personal style is based on the following principles:

1) Effective communication - I will make sure that the communication with the students is effective in a way that they are actively participating in the learning process. I will encourage work in groups so that students will be able to learn from each other and also will develop better ideas together. I find the technique of learning in small groups to be effective in encouraging students to participate actively in discussions even those who usually don not tend to do so in large classroom settings.
2) Learning through examples - I will use case studies from current published papers to convey important messages even when teaching basic courses. I believe that teaching through examples of the current literature would encourage the students to practice the basic materials learned in class. The basic knowledge that is acquired in the class should be a prerequisite when dealing with more complex concepts.
3) Fair and flexible assessments - I will make sure that the evaluation process is fair and consists of many parameters that truly reflect the achievements but also would consider the sincere efforts of the students during the entire course.
4) Real time feedback and two way communication - Through multiple performance assessment methods including quizzes, assignments, and participation in discussions I will encourage the students to engage from different perspectives. That would allow me to estimate the general level and interest among the students, as well as applying changes if necessary.