**BIOGRAPHICAL SKETCH**

NAME: Hamza Balci

eRA COMMONS USER NAME: hbalci

POSITION TITLE: Professor

EDUCATION/TRAINING

| INSTITUTION AND LOCATION | DEGREE | Completion Date MM/YYYY | FIELD OF STUDY |
|---|---|---|---|
| Koc University, Istanbul, Turkey | BS | 06/1998 | Physics |
| University of Maryland, College Park, USA | PhD | 03/2004 | Physics |
| University of Illinois, Urbana-Champaign, USA (postdoc) | n/a | 08/2009 | Biophysics |

**A. Personal Statement**

We propose to perform cryo-electron microscopy measurements on telomeric overhangs of physiologically relevant lengths in the absence and presence of shelterin proteins that protect and maintain these overhangs. Telomeric DNA consists of a double stranded region (dsTEL) and a single stranded overhang (ssTEL). High-resolution structural studies on such overhangs are not available currently. Such telomeric overhangs can fold into multiple tandem G-quadruplex structures (GQ or G4), which protect these sites and reduce their accessibility to DNA Damage Response activators, nucleases, and telomerase. Our recent work on such systems using single molecule fluorescence methods has provided insights into the folding and accessibility of telomeric overhangs and the impact of shelterin complex on these important biological processes. We aim to complement these studies and test their outcomes with cryo-EM based structural studies. The primary purposes would be to gain an understanding of distribution of folded GQ structures and the unfolded regions throughout the overhang and the impact of shelterin on these structural features.

Our recent publications (listed below) are at the leading edge of single molecule fluorescence studies on ssTEL with physiologically relevant lengths:

I. Golam Mustafa*, Sajad Shiekh*, Keshav GC, Sanjaya Abeysirigunawardena, and **Hamza Balci**, "Interrogating Accessibility of Telomeric Sequences with FRET-PAINT: Evidence for Length-Dependent Telomere Compaction" Nucleic Acids Research (2021), 49 (6): 3371–3380

II. Sajad Shiekh*, Golam Mustafa*, Mohammed Enamul Hoque, Eric Yokie, John J. Portman, **H. Balci**, "Emerging Accessibility Patterns in Long Telomeric Overhangs", PNAS, (2022)119 (30) e2202317119

III. Sajad Shiekh*, Amanda Jack, Golam Mustafa*, Sineth G. Kodikara*, Prabesh Gyawali*, Mohammed Enamul Hoque, Ahmet Yildiz and **Hamza Balci**, "Impact of Shelterin Complex on Telomere Accessibility", Nucleic Acids Research, (2022) 50 (22): 12885–12895

Until our 2021 NAR paper (Article #I), the vast majority of single molecule measurements were on sequences that contain four GGGTTA repeats (GGGTTA sequence will be referred to as a 'G-Tract') which can form a single G-quadruplex (GQ) with a small minority of studies on constructs with 4-7 G-Tracts. We are not aware of any single molecule fluorescence measurements on ssTEL longer than eight G-Tracts (~50 nt), i.e. forming 1-2 GQs. However, in a physiological setting 2-12 GQs can form in ssTEL. In our 2021 NAR paper, we showed that using FRET-PAINT approach, it is possible to study accessibility of ssTEL that can form up to seven tandem GQs (4-28 G-Tracts). In this approach, a Cy5-labeled short PNA strand that is complementary to 7-nt of ssTEL is employed. Binding of Cy5-PNA to a surface-immobilized and Cy3-labeled partial duplex DNA construct (which has an ssTEL overhang of varying length) results in a FRET signal that depends on the location of the binding site. We analyzed binding frequency, dwell time of binding, and corresponding FRET distribution (accessibility map) to determine how accessibility of telomeric overhangs varies with ssTEL length and position within a given ssTEL (vicinity of 3'-end, intermediate, or vicinity of junction region between double and single stranded telomere). These studies showed that ssTEL form more compact structures in a length dependent manner, that the junction region is the most accessible region, and that neighboring GQs interact with positive cooperativity.

In the 2021 NAR paper, we studied only the constructs that have 4n G-Tracts, e.g. 4, 8,…,24,28 G-Tracts. In the next step (Article #II), we investigated the entire length scale in this range, e.g. 4, 5, 6,…,27, 28 G-tracts and observed surprising accessibility and folding frustration patterns. These studies showed that telomeric overhangs that have 4n G-Tracts (e.g. 12, 16, 20,…) are maximally frustrated have accessible sites distributed throughout the overhang. On the other hand, overhangs with 4n+2 G-Tracts (e.g. 14, 18, 22,…) are minimally frustrated and the accessible sites are concentrated at junction region between double and single stranded telomere. This study has been published at PNAS (Article #II).

In our most recent study on this system, we introduced POT1 and a 4-component shelterin (POT1-TPP1-TIN2-TRF1) and investigated how they impact the accessibility of telomeric overhangs with 4-24 G-Tracts. These measurements showed that POT1 reduces telomere accessibility by ~2.5-fold while shelterin reduces it by ~5-fold. Replacing TRF1 with TRF2 did not impact the level of protection; however, eliminating the double stranded telomere (dsTEL) or omitting TIN2 and TPP1 (while POT1 and TRF1 are kept) limits the accessibility to that provided by POT1. These results highlight the significance of the protein-mediated connection between members of shelterin that are localized on ssTEL and dsTEL and their restructuring of the telomeric overhang, particularly the junction region. This paper has been published in Nucleic Acids Research (Article #III).

**As illustrated by this brief overview, we have reached a level where high resolution structural studies could make a significant impact on our understanding of this important system. The single molecule FRET-PAINT studies have significant implications about the structural features of telomeric overhangs of physiologically relevant lengths, and cryo-EM structures would be a perfect complement to test the implications of these studies.**

My group at KSU has already published over twenty articles on GQ, protein, and small molecule interactions in some of the leading journals of the field including PNAS, Nucleic Acids Research, Biosensors and Bioelectronics, Biophysical Journal, and ACS Synthetic Biology. More than 20 graduate and undergraduate students have been trained (>1 year) in my laboratory, in addition to several others who received shorter term training (about a semester). Many of these students continued their careers in biophysics, biotechnology, or medical sciences. Being within a Physics Department, my lab has been a successful bridge for introducing Physics majors to biophysical and biomedical research fields or industries.

Before establishing my lab at KSU, I received extensive training on several single molecule microscopy methods as a post-doctoral researcher at the University of Illinois under the mentorship of Prof. Taekjip Ha (now at Johns Hopkins University) and Prof. Paul Selvin. I have constructed experimental setups (measuring fluorescence or force spectroscopy), developed new single molecule methods (3D particle tracking and super-resolution imaging), and applied these methods to various biological systems both *in vivo* and *in vitro*, which were published in respected peer reviewed journals (some are listed under Contributions to Science, Item #5).

Over the years, my group has collaborated and published with distinguished experts including Prof. Soumitra Basu and Prof. John Portman (KSU), Prof. Matthew Comstock (Michigan State Uni., East Lansing), Prof. Edwin Antony (Saint Louis University School of Medicine), Prof. Ahmet Yildiz (UC, Berkeley), Prof. Pavel Janscak (University of Zurich), Prof. Kazuo Nagasawa (Tokyo University of Agriculture and Technology, Japan), and Dr. Steve Presse (Arizona State University).

Ongoing and recently completed projects that I would like to highlight include:

NIH 1 R15GM146180
Balci (PI), S. Basu (Co-I) 05/01/2022 – 04/30/2025
Title: Targeting Promoter G-quadruplexes with CRISPR-dCas9 for Transcription Regulation

**B. Positions, Scientific Appointments, and Honors**

**Positions and Scientific Appointments**

2020 - Present Professor, Physics Department, Kent State University
2020 - Present Undergraduate Coordinator, Physics Dept, Kent State University
2015 - 2020 Associate Professor, Physics Department, Kent State University
2018 - 2019 Visiting Scholar & Marie-Curie Fellow, Bionanoscience Department, TU of Delft, Netherlands
2009 - 2015 Assistant Professor, Physics Department, Kent State University
2007 - 2009 Postdoc. Res. Assoc. U. of Illinois, Urbana-Champaign. Prof. Taekjip Ha's Lab.
2008 - 2009 Visiting Scientist on EMBO Fellowship, LMU Munich. Prof. Karl-Peter Hopfner's Lab.
2004 - 2007 Postdoc. Res. Assoc. U. of Illinois, Urbana-Champaign. Prof. Paul Selvin's Lab.
2000 - 2004 Research Assistant, University of Maryland, College Park. Prof. Richard L. Greene's Lab.
1998 - 2000 Teaching Assistant, University of Maryland, College Park

**Honors**

2023 The Students Choice Best Advisor Award, Society of Physics Students, KSU Chapter
2018 - 2019 Marie-Curie Individual Fellowship, European Commission

2017 Excellence in Advising Award, Society of Physics Students, KSU Chapter
2015 URC, Research Activity Support including Undergraduate Students, KSU
2013 URC, Research Activity Support including Undergraduate Students, KSU
2013 College of Arts and Sciences Research Resources Award, KSU (2013)
2013 Burroughs Wellcome Fund Collaborative Research Travel Grant (2013)
2011 - 2014 Farris Family Innovation Award for Early Achievement (2011)
2011 ICAM-KSU International Travel Award (2011)
2011 University Undergraduate Research Scholars Program Award (2011)
2008 European Molecular Biology Organization Short-Term Fellowship (2008)
2000 - 2001 Finalist for Dean's Award for Excellence in Teaching, U of Maryland, College Park
1994 - 1998 Vehbi Koc Scholar, Koc University, Istanbul Turkey (1994-1998)

**C. Contributions to Science**

Authors marked with * were graduate students under my supervision and those marked with ** were undergraduate students under my supervision.

**1- Targeting GQ forming sequences with Cas9/dCas9:** This is a relatively recent effort in my laboratory; however, one which we will work on more extensively in the next few years. We have recently published a paper where we reported single molecule studies (Article #2 below) and another paper where we reported cellular assays about the interactions of CRISPR-Cas9 or nuclease-dead Cas9 (dCas9) with potentially GQ forming sequences (PQS) (Article #1 below). In Article #1, we demonstrated that dCas9 can used to target a PQS in promoter region of tyrosine hydroxylase gene and modulate (up or down regulate) transcription of the gene. This system provides a system where a transient regulation of transcription is achieved in a sequence specific manner, without introducing mutations in the genome or using small molecules that only have structural specificity. In this article, we also demonstrated that Cas9-mediated DNA cleavage is impacted in a strand-specific manner (i.e., G-rich vs. C-rich strand) when a PQS is targeted. In Article #2, we demonstrated that depending on their location and stability, GQs can impact the conformational diversity and dynamics of the complex formed by CRISPR-Cas9 and the target DNA strand.

I. Mohammed Enamul Hoque, Golam Mustafa*, Soumitra Basu, **Hamza Balci**, "Encounters Between Cas9/dCas9 and G-quadruplexes: Implications for Transcription Regulation and Cas9-Mediated DNA Cleavage", ACS Synthetic Biology (2021), 10, 5, 972–978
PI's grant(s) that supported this work: NIH 1R15GM123443.

II. **Hamza Balci**, Viktorija Globyte, Chirlmin Joo, "Targeting G-quadruplex Forming Sequences with Cas9", ACS Chemical Biology (2021), 16, 4, 596–603
PI's grant(s) that supported this work: Marie-Curie Fellowship from the European Commission (795567)

**2- Impact of Small molecules on Helicase-Mediated GQ Unfolding and Intermolecular GQ Formation:** Stabilizing telomeric GQs with small molecules is known to inhibit telomerase activity, which makes such small molecules potential anti-cancer drugs. These small molecules have also been extensively used to demonstrate the role of GQs in promoter regions in transcription regulation, where introducing small molecules has generally resulted in reduced mRNA and protein expression. Furthermore, GQ-stabilizing small molecules have been shown to result in elevated levels of truncated transcription and genomic breaks. The standard method to screen GQ stabilizing small molecules is to quantify their effect on thermal melting temperatures ($\Delta T_m$) of GQs. However, in a cellular setting, GQs are destabilized by helicases and DNA binding proteins. Therefore, we performed single molecule assays to quantify the impact of three prominent small molecules on the activity of BLM helicase in unfolding telomeric GQ. Even though these small molecules were reported to result in very different $\Delta T_m$ for telomeric GQ, their impact on BLM activity was similar: they all reduced BLM-mediated GQ unfolding by 2-3 fold. It is possible that BLM disrupts the stacking interactions between the GQ and small molecule before unfolding the GQ, making $\Delta T_m$ an insufficient parameter to characterize the impact of small molecules on helicase-GQ interactions. These results illustrate the need for more comprehensive screening assays for these anti-cancer drug candidates. In another study, we investigated the impact of these small molecules, which are designed primarily to stabilize intramolecular GQ, on intermolecular GQ formation by two different strands of DNA. This study was motivated by bioinformatic studies that identified hundreds of thousands of sites in the human genome where complementary strands had 1-3 G-rich repeats in proximity, which could combine to form an intermolecular GQ. We demonstrated that these small molecules could enhance intermolecular GQ formation by an order of magnitude when one of the strands has three GGG-repeats while the other has one GGG repeat. The impact was smaller (~2-fold) when each strand had two GGG-repeats. These studies also provided insights about designing "split-GQ" sensors that have been used for detecting chemicals, ions, or particular nucleic acid sequences. The following articles are on these studies:

I. Parastoo Maleki*, G. Mustafa*, P. Gyawali*, J. B. Budhathoki*, Yue Ma, Kazuo Nagasawa, **H. Balci** "Quantifying the impact of small molecule ligands on G-quadruplex stability against Bloom helicase", Nucleic Acids Research, (2019) 47 (20): 10744–10753
PI's grant(s) that supported this work: NIH 1R15GM123443 & 1R15GM109386

II. P. Gyawali*, Keshav GC, Yue Ma, Sanjaya Abeysirigunawardena, Kazuo Nagasawa and **H. Balci** "Impact of Small Molecules on Intermolecular G-Quadruplex Formation", Molecules, 24 (8), 1570, (2019)
PI's grant(s) that supported this work: NIH 1R15GM123443 & 1R15GM109386

**3- Liquid Crystalline Phase Formation in Highly Concentrated DNA Solutions:** The local DNA concentration in chromosomes, viral capsids, and sperm nuclei is on the order of hundreds of mg/mL, concentrations at which DNA can form highly ordered lyotropic liquid crystalline (LC) phases. In vitro, short double-stranded DNA (dsDNA) fragments (~6–20 bp, significantly shorter than persistence length of dsDNA) also exhibit certain LC phases, but not others. By introducing a flexible single stranded "gap" within the dsDNA structure, it is possible to attain layered ("smectic") phases which could be used for various biological applications. Synchrotron small/wide angle X-ray scattering (SAXS/WAKS) and polarization optical microscopy measurements were used in these studies. For example, using such a design we were able to calculate the base stacking energies between AT-AT, AT-GC, or GC-GC base pairs at physiologically relevant DNA concentrations which are about three orders of magnitude higher than those reached in a typical UV-Vis thermal melting assay. The energies we measured in these SAXS measurements (performed at Brookhaven National Lab, NSLS-II) were somewhat smaller than those obtained in single molecule measurements, although consistent trends were observed. In a current study, we are investigating the impact of end-fraying and base-to-base stacking on the stability of the stacking interactions between different duplexes and how this stability depends on the type of base and ionic conditions of the environment, again at physiologically relevant DNA concentrations and using high-resolution SAXS experiments. The following articles are on these studies:

I. Sineth G. Kodikara*, P. Gyawali*, J. T. Gleeson, A. Jakli, S. Sprunt, and **Hamza Balci**, "Stability of End-to-End Base Stacking Interactions in Highly Concentrated DNA Solutions", Langmuir (2023) 39, 13, 4838–4846
PI's grant(s) that supported this work: NSF DMR-1904167.

II. Prabesh Gyawali*, R. Saha, S. G. Kodikara*, Ruipeng Li, Masafumi Fukuto, James T. Gleeson, Gregory P. Smith, Noel A. Clark, Antal Jakli, **Hamza Balci**, and Samuel Sprunt, "Smectic-B phase and temperature-driven smectic-B to -A transition in concentrated solutions of "gapped" DNA", Physical Review Research (2022) 4, 033046
PI's grant(s) that supported this work: NSF DMR-1904167.

III. P. Gyawali*, R. Saha, M. Salamonczyk, P. Kharel, S. Basu, R. Li, M. Fukuto, G. P. Smith, J. T. Gleeson, A. Jakli, **H. Balci**, and S. Sprunt, " Mono and bilayer smectic liquid crystal ordering in dense solutions of "gapped" DNA duplexes", PNAS (2021) 118 (12) e2019996118
PI's grant(s) that supported this work: NSF DMR-1904167.

**4- Development and Application of Novel Single Molecule Methods:** Even though the work mentioned in this section is more than five years old, it is relevant for the proposed tethered particle motion assay and stoichiometry determination by photobleaching steps, which require familiarity with particle tracking algorithms and experience with building optical microscopy setups. I worked as a postdoctoral scientist in Prof. Paul Selvin's laboratory on developing and applying high resolution microscopy methods to elucidate different aspects of cargo carrying motor proteins such as myosin V, myosin VI, and kinesin. In one of these studies, we demonstrated one of the earliest superresolution methods for measuring the separation between the motor domains of a myosin VI dimer when attached to actin. Sequential photobleaching of two eGFP molecules which were attached to Myosin VI motor domains was used to measure a separation of 30 nm between these domains using visible light fluorescence microscopy (Balci *et al.* Biophys Jour 2005). In addition, we developed a bi-focal imaging-based 3D particle tracking method and demonstrated its functionality both *in vitro* and *in vivo* systems using fluorescent and non-fluorescent markers (Toprak and Balci et al. Nanoletters 2007). I also worked in the laboratory of Prof. Taekjip Ha, as a postdoctoral scientist, where we combined single molecule FRET with triangulation ideas to determine the conformational state of Rep helicase-DNA complex in different nucleotide states (Balci et al. Biophys Jour. 2001). Using this novel approach, we were able to elucidate the conformation of the 2B domain of Rep helicase in four different nucleotide states. In addition, I worked on building and automating an optical trap and fluorescence microscope setup along with a graduate student in the Ha lab. This instrument was used to image, in real time, the movement of UvrD helicase on a stretched ssDNA and also while it unwinds dsDNA (Lee and Balci et al. Nature Comm. 2013). Various aspects of translocation and dsDNA unwinding activity of UvrD were measured at the single molecule or single dimer level. More recently, we developed a FRET-based force spectroscopy method where force generated by short, looped DNA is used maintain nucleic acid secondary structures under tension (Mustafa et al. Biosensors and Bioelectronics, 2018). With this

method, tens of nucleic acid secondary structures were maintained under tension and studied simultaneously. The following articles are examples of the efforts summarized in this section:

I. G. Mustafa*, C.Y. Chuang, W. A. Roy, M. M. Farhath, N. Pokhrel, Y. Ma, K. Nagasawa, E. Antony, M. J. Comstock, S. Basu, **Hamza Balci**, "A Force Sensor that Converts Fluorescence Signal into Force Measurement Utilizing Short Looped DNA", Biosensors and Bioelectronics (2018) 121, 34-40.
PI's grant(s) that supported this work: NIH 1R15GM123443 & 1R15GM109386.

II. Kyung Suk Lee, **Hamza Balci**, Haifeng Jia, Timothy M. Lohman, Taekjip Ha, Direct imaging of single UvrD helicase dynamics on long single-stranded DNA" Nature Communications, (2013) PMID: **23695672**

III. **H. Balci**, S. Arslan, S. Myong, T. M. Lohman, Taekjip Ha, "Single Molecule Nano-Positioning: Structural Transitions of a Helicase-DNA Complex during ATP Hydrolysis" Biophysical Journal, (2011), 101, 4, 976-984.

IV. Erdal Toprak[#], **Hamza Balci**[#], Benjamin H. Blehm, Paul R. Selvin, "Three-Dimensional Particle Tracking via Bifocal Imaging" Nano Lett., (2007), 7 (7), 2043-2045. [#]**Equal Contribution**

Complete List of Published Work in MyBibliography:
http://www.ncbi.nlm.nih.gov/sites/myncbi/hamza.balci.1/bibliography/49488464/public/?sort=date&direction=ascending