Digvijay Singh

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Last Updated on 04/2022

EDUCATION

Johns Hopkins University School of Medicine
Ph.D., Biophysics 8/2012 - 1/2018

Indian Institute of Technology, Kharagpur
Integrated BS+MS, Chemistry 7/2007 - 4/2012

PROFESSIONAL EXPERIENCE

Damon Runyon fellow and Postdoctoral scientist
University of California, San Diego 3/2018 - Present
Damon Runyon Foundation 2/2019 - 2/2023
Advisor: Prof. Elizabeth Villa

- Development and application of cryo-electron microscopy for *In situ* structural biology for:
  - Comprehensive analysis of the *In situ* structure of the Nuclear Pore Complex and its functional isoforms.
  - Architecture & molecular texture of phase-separated droplets

Ph.D. Researcher
Department of Biophysics, Johns Hopkins University School of Medicine 1/2013 - 1/2018
(transferred from University of Illinois at Urbana-Champaign)
Advisor : Prof. Taekjip Ha

- Single molecule imaging and biochemical assays to characterize molecular mechanisms and specificity of DNA targeting by CRISPR-Cas9 and CRISPR-Cpf1 family of RNA-guided endonucleases which are being widely used for various genome engineering applications. Collaborated with Prof. Jennifer A. Doudna (UC Berkeley) and Prof. Scott Bailey (Johns Hopkins).
- Collaboration with Prof. Venigala B. Rao (CUA) & Prof. Yann Chemla (UIUC) to characterize mechanism of coordination between different subunits of bacteriophage T4 DNA packaging motor.
- Design and implementation of data-analysis packages for single molecule studies.

Visiting research assistant
University of Cambridge, UK 6/2012 – 8/2012
Advisor: Prof. Robert Best (now at NIH)

- *Theoretical Biophysics* - Construction of multi-dimensional free energy surfaces of protein folding.

Master thesis student
Indian Institute of Technology, Kharagpur 8/2011 – 3/2012
Advisor: Prof. Swagata Dasgupta

- Computational Biophysics – Modeling of amyloid beta multimers via protein structure prediction (Rosetta).

Visiting research assistant
Massachusetts Institute of Technology, USA 5/2011 – 7/2011
Advisor: Prof. Collin M. Stultz

- Computational Biophysics – Construction of structural library of intrinsically disordered amyloid beta protein, using molecular dynamics simulations, for creation of its conformational ensembles.

Visiting research assistant
Karlsruhe Institute of Technology, Germany 11/2010 – 12/2010
Advisor: Prof. Anne S. Ulrich

- Solid State NMR - Synthesis of membrane active peptides and evaluation of its alignment in lipid bilayer from NMR (Nuclear Magnetic Resonance) signals of 19F labels on the peptides.

Visiting research assistant
University of Illinois at Urbana-Champaign, USA 5/2010 – 7/2010
Advisor: Prof. Martin Gruebele

- Protein Folding - Expression and purification of FRET probe labeled protein construct for its use in fast relaxation imaging following temperature shocks.

Research Intern
Unilever, Bangalore, India 11/2009 – 12/2009

- Investigation of binding affinity of tea polyphenols with milk caseins.

Research Intern
General Electric, Bangalore, India 4/2009 – 7/2009

- Synthesis of radio labeled indoles & amides with high binding affinity to certain specific receptors found in nervous system for its use in PET (Positron Emission Tomography) imaging.

PUBLICATIONS

16. Christopher W. Akey*, **Digvijay Singh***, Christna Ouch*, Ignacia Echeverria*, Ilona Nudelman, Joseph M. Varberg, Zulin Yu, Fei Fang, Yi Shi, Junjie Wang, Daniel Salzberg, Kangkang Song, Chen Xu, James C. Gumbart, Sergey Suslov, Jay Unruh, Sue L.Jaspersen, Brian T.Chait, Andrej Sali, Javier Fernandez-Martinez, Steven J. Ludtke, Elizabeth Villa, Michael P.Rout. Comprehensive structure and functional adaptations of the yeast nuclear pore complex. *Cell* (2022). Pre-print. *Equal contribution

15. Li Dai*, **Digvijay Singh***, Suoang Lu, Vishal Kottadiel, Reza Vafabakhsh, Marthandan Mahalingam, Yann R Chemla, Taekjip Ha, Venigalla B Rao. A viral genome packaging ring-ATPase is a flexibly coordinated pentamer. *Nature Communications* (2021). Pre-print. *Co-first

14. **Digvijay Singh**, Yanbo Wang, John Mallon, Olivia Yang, Jingyi Fei, Anustup Poddar, Damon Ceylan, Scott Bailey, Taekjip Ha. Mechanism of improved specificity of engineered Cas9s revealed by single molecule analysis.. *Nature Structural and Molecular Biology.* (2018). Pre-print.

13. **Digvijay Singh**, John Mallon, Anustup Poddar, Yanbo Wang, Ramreddy Tipanna, Olivia Yang, Scott Bailey, Taekjip Ha. Real-time observation of DNA target interrogation and product release by RNA-guided endonuclease CRISPR-Cpf1. *PNAS* (2018). Pre-print.

12. Ikenna Okafor*, **Digvijay Singh***, Yanbo Wang*, Minhee Jung, Haobo Wang, John Mallon, Scott Bailey, Jungjoon K. Lee, Taekjip Ha. Single molecule analysis of effects of non-canonical guide RNAs and specificity-enhancing mutations on Cas9-induced DNA unwinding. *Nucleic Acids Research* (2019). Pre-print. *Co-first.

11. **Digvijay Singh**, Samuel H. Sternberg, Jingyi Fei, Jennifer A. Doudna, Taekjip Ha. Real-time observation of DNA recognition and rejection by the RNA-guided endonuclease Cas9. *Nature Communications* (2016). Pre-print.

10. **Digvijay Singh**, Taekjip Ha. Understanding the molecular mechanism of CRISPR toolbox using single-molecule approaches. *ACS Chemical Biology* (2018).

9. Jingyi Fei, **Digvijay Singh**, Qiucen Zhang, Seongjin Park, Divya Balasubramanian, Ido Golding, Carin K. Vanderpool, Taekjip Ha. Determination of in vivo target search kinetics of regulatory non-coding RNA. *Science* (2015).

8. Haiyang Yu, Shan Lu†, Kelsey Gasior†, **Digvijay Singh**, Olga Tapia, Sonia Vazquez-Sanchez, Divek Toprani, Melinda S. Beccari, John R. Yates, Sandrine Da Cruz, Jay M. Newby, Miguel Larfaga, Amy S. Gladfelter, Elizabeth Villa, and Don W. Cleveland. TDP-43 and HSP70 phase separate into anisotropic, intranuclear liquid spherical annuli. *Science* (2020). Pre-print.†Equal Contribution

7. Felix R. Wagner*, Reika Watanabe*, Ruud Schampers, **Digvijay Singh**, Hans Persoon, Miroslava Schaffer, Peter Fruhstorfer, Jürgen Plitzko, Elizabeth Villa. Preparing samples from whole cells using focused-ion-beam milling for cryo-electron tomography. *Nature Protocols* (2020).*Co-first.
6. Yanbo Wang, John Mallon, Haobo Wang, **Digvijay Singh**, Myung Hyun Jo, Boyang Hua, Scott Bailey, and Taekjip Ha. Real-time observation of Cas9 postcatalytic domain motions. *PNAS* (2020).
5. Shan Lu*, Qiaozhen Ye*, **Digvijay Singh**, Elizabeth Villa, Don W. Cleveland, Kevin D. Corbett. The SARS-CoV-2 Nucleocapsid phosphoprotein forms mutually exclusive condensates with RNA and the membrane-associated M protein. *Nature Communications* (2020). Pre-print. *Co-first
4. Apratim Dhar, Kirdhar Girdhar, **Digvijay Singh**, Simon Ebbinghaus and Martin Gruebele. Different protein stability and folding kinetics in the nucleus, endoplasmic reticulum, and cytoplasm of living cells. *Biophysical Journal* (2011).
3. Boyang Hua, Kyu Young Han, Ruobo Zhou, Hajin Kim, Xinghua Shi, Sanjaya C. Abeysirigunawarden, Ankur Jain, **Digvijay Singh**, Vasudha Aggarwal, Sarah A. Woodson, Taekjip Ha. An improved surface passivation method for single-molecule Studies. *Nature Methods* (2014).
2. Anustup Poddar, Muhammad S Azam, Tunc Kayikcioglu, Maksym Bobrovskyy, Jichuan Zhang, Xiangqian Ma, Piyush Labhsetwar, Jingyi Fei, **Digvijay Singh**, Zaida Luthey-Schulten, Carin K. Vanderpool, Taekjip Ha. Effects of individual base-pairs on in vivo target search and destruction kinetics of small RNA. *Nature Communications* (2021). Pre-print.
1. Boyang Hua, Yanbo Wang, Kyu Young Han, Seongjin Park, **Digvijay Singh**, Jin H. Kim, Wei Cheng, Taekjip Ha. Single-molecule centroid localization algorithm improves the accuracy of fluorescence binding assays. *Biochemistry* (2018).

HONORS, AWARDS & FINALIST POSITIONS

- Damon Runyon Fellowship 2019 - 2023
- Virtual Molecular Cell Consortium (VMCC) Postdoctoral Fellow, UCSD 2018 - 2019
- Biophysical Society Education Travel Award 2017
- Finalist of International Howard Hughes Medical Institute fellowship 2015
- Johns Hopkins Biophysics department nominee for international Weintraub Award 2018
- INSPIRE fellowship by Government of India 2008 - 2012

PROFESSIONAL SERVICES

- Reviewer for:
  - Nature Structural and Molecular Biology 2018-Present
  - Proceedings of the National Academy of Science 2017-Present
  - Nature Communications 2019-Present
  - Journal of Biological Chemistry 2019-Present
  - Biochemistry 2018-Present
  - ACS Omega 2019-Present
  - Scientific Reports 2018-Present
  - Cellular and Molecular Life Sciences 2018-Present
  - Biophysics of RNA-Protein Interactions by Springer Books 2018
  - Frontiers in Molecular Neuroscience 2018-Present
  - Frontiers in Neuroscience 2019-Present
  - Journal of Visualized Experiments. 2018-Present

TEACHING & MENTORING EXPERIENCE

Mentor
Johns Hopkins University 08/2014 - 08/2019

- Directly mentored one undergraduate and three graduate students.

Instructor
Center for the Physics of Living Cells (CPLC) summer schools, University of Illinois 2013 - 2015
- Taught & designed single molecule microscopy experimental modules.

Teaching assistant
Department of Physics, University of Illinois 2014-2015
- Advanced Biophysics course (smFRET module)

SELECTED PUBLIC PRESENTATIONS

- "The power of molecular structures in cellular context; Nuclear Pore Complex as an example".
  Biophysical Society Meeting. 2022
- "Molecular Mechanisms of CRISPR enzymes revealed by single molecule imaging and biochemical assays".
  CRISPR Workshop at CSIR-IGIB, New Delhi (crispr.igib.res.in). 2019
- "Role of co-factors in Rhodopsin: An action potential story of vision".
  Wednesday Morning Seminar Series, Biophysics, JHMI. 2017
- "DNA targeting by CRISPR-Cas at the single molecule level".
  Student Evening Seminar Series, Biophysics. 2017
- "Real-time observation of DNA recognition and rejection by the RNA-guided endonuclease Cas9".
  Physics of Living Systems Conference, Arlington, VA. 2015
- "Real-time observation of DNA recognition and rejection by the RNA-guided endonuclease Cas9".
  Biophysical Society Meeting. 2015
- "Real-time observation of DNA recognition and rejection by the RNA-guided endonuclease Cas9".
  Center for Physics of Living Cells Symposium, University of Illinois 2015