Digvijay Singh

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EDUCATION & TRAINING

Damon Runyon fellow and Postdoctoral scientist - UC San Diego 3/2018 - Present
Advisor: Prof. Elizabeth Villa (HHMI investigator)

- Structure of the Nuclear Pore Complex & its isoforms using *in situ* tomography of cells prepared using cryo-focused ion beam milling.
- Architecture & molecular texture of phase-separated droplets.

Ph.D. in Biophysics - Johns Hopkins University School of Medicine 8/2012 - 1/2018
Advisor: Prof. Taekjip Ha (HHMI investigator)

- Single molecule imaging and biochemical assays to characterize molecular mechanisms and specificity of DNA targeting by CRISPR enzymes and DNA packaging by motor of T4 bacteriophage.
- Design and implementation of data-analysis packages for single-molecule studies.

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

- Diverse research from computational biophysics to Bio-NMR in multiple projects (total 7) during this term is outlined in section below titled Other Professional Experiences.

PUBLICATIONS (only published or in-review) [8 first/co-first] [Google-Scholar Citations > 1300]

17. **Digvijay Singh***, Elizabeth Villa. Cryo-Focused Ion Beam Milling of Cells. *In Review* (2022).
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. Media-Release *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. 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.
13. 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
12. 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
11. 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).
10. 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.
9. 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.
8. **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.
7. **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.

6. **Digvijay Singh**, Taekjip Ha. Understanding the molecular mechanism of CRISPR toolbox using single-molecule approaches. *ACS Chemical Biology* (2018).
5. 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).
4. **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.
3. 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).
2. 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).
1. 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).

SELECT PUBLIC PRESENTATIONS (only invited or selected from abstracts)

- *Playing with cellular legos:* Mapping the structure, formation and function of large cellular assemblies
  University of Virginia, USA 2022
- *Playing with cellular legos:* Mapping the structure, formation and function of large cellular assemblies
  University of Iowa, USA 2022
- *Playing with cellular legos:* Mapping the structure, formation and function of large cellular assemblies
  Oregon Health & Science University, USA 2022
- cryo-FIB milling of biological samples.
  cryo-EM workshop at Brookhaven National Lab, USA 2022
- Power of molecular structures in cellular context
  cryo-EM super group meeting, UC Boulder, USA 2022
- *In situ* architecture of yeast nuclear pore complex.
  Friends of Cell Meeting, San Diego Cluster of Institutes, USA 2022
- *In situ* architecture of yeast nuclear Pore Complex.
  National Tomography Workshop, AIIMS, India 2022
- Protocols for segmentation of tomograms and subtomogram refinements.
  National Tomography Workshop, AIIMS, India 2022
- cryo-FIB milling of biological samples.
  National Tomography Workshop, AIIMS, India 2022
- The power of molecular structures in cellular context; Nuclear Pore Complex as an example.
  Biophysical Society Meeting, San Francisco, USA 2022
- Molecular Mechanisms of CRISPR enzymes revealed by single molecule imaging and biochemical assays.
  CRISPR Workshop at CSIR-IGIB, India 2019
- DNA targeting by CRISPR-Cas at the single molecule level.
  Student Evening Seminar Series, Johns Hopkins, India 2017
- Real-time observation of DNA recognition and rejection by the RNA-guided endonuclease Cas9.
  Physics of Living Systems Conference, Arlington, USA 2015
- Real-time observation of DNA recognition and rejection by the RNA-guided endonuclease Cas9.
  Biophysical Society Meeting, Baltimore, USA 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, USA 2015

OTHER PROFESSIONAL EXPERIENCES

Visiting research assistant at 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 at Indian Institute of Technology, Kharagpur 8/2011 – 3/2012
Advisor: Prof. Swagata Dasgupta
- Computational Biophysics – Modeling of amyloid beta multimers modeling via protein structure prediction (Rosetta).

Visiting research assistant at 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 at 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 at 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 at Unilever, Bangalore, India 11/2009 – 12/2009
- Investigation of binding affinity of tea polyphenols with milk caseins.

Research Intern at 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 Positron Emission Tomography.

TEACHING, MENTORING & VOLUNTEERING EXPERIENCE

Mentor at *UC San Diego* 08/2021 - Present
- Directly mentored one student.

Mentor at *Johns Hopkins University* 08/2014 - 08/2019
- Directly mentored one undergraduate and two graduate students.

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

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

Volunteer at *ASHA for Education* 2012 - 2013
- This organization supports socia-economic upliftment of under-priviledged children. I volunteered in its multiple fundraising drives.

HONORS, AWARDS & FINALIST POSITIONS

- Finalist of Damon Runyon-Dale F. Frey Award for Breakthrough Scientists 2022
- 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
  - Chemical Science 2022-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