# Digvijay Singh

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## EDUCATION & TRAINING

**K99, Damon Runyon, Visible Molecular Cell & Postdoctoral fellow - UC San Diego** 3/2018 - Present

*Advisor: Prof. Elizabeth Villa (HHMI investigator)*

- Co-led an interdisciplinary team of scientists to determine the in-cell structure and model of the Nuclear Basket, which had been elusive for over 30 years, resulting in a publication in *Cell*.
- Co-led an interdisciplinary team of scientists to determine the structure and molecular model of the Nuclear Pore Complex, leading to a highly cited publication in *Cell*.
- Authored a chapter in a book on cryo-electron tomography, intended to be an authoritative resource by experts. And revitalized a project detailing the steps of in-cell structural biology using cryo-focused ion beam milling, culminating in a highly cited publication.
- Determined structures of condensates, including those related to SARS-CoV-2.

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

*Advisor: Prof. Taekjip Ha (HHMI investigator)*

- Led teams & collaborated with experts, including Nobel laureate Prof. Doudna, to uncover molecular mechanisms of CRISPR enzymes & T4 bacteriophage, resulting in multiple high-impact publications and advancing genome engineering, earning multiple honors.
- Advanced Super Resolution Microscopy and single-molecule techniques through collaboration with interdisciplinary teams, contributing to multiple high-profile publications.

**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-print) [9 first/co-first] [Google-Scholar citations > 2000]

19. **Digvijay Singh***, Neelesh Soni*, Joshua Hutchings*, Ignacia Echeverria, Farhaz Shaikh, ..., Michael Rout, Andrej Sali, Elizabeth Villa. The Molecular Architecture of the Nuclear Basket. *Cell* (in press). Pre-print *Equal Contribution
18. **Digvijay Singh***, Elizabeth Villa. Cryo-Focused Ion Beam Milling of Cells. *Springer book on Cryo-Electron Tomography* (2023).
17. Barak Raveh, Roi Eliasian, Shaked Rashkovits, Daniel Russel, Ryo Hayama, Samuel Sparks, **Digvijay Singh**, ..., Elizabeth Villa, Michael Rout, David Cowburn, Andrej Sali. Integrative spatiotemporal map of nucleocytoplasmic transport. *Biorxiv* (2024).
16. Christopher Akey*, **Digvijay Singh***, Christna Ouch*, Ignacia Echeverria*, ..., Elizabeth Villa, Michael 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, ..., 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, ..., 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**, ..., 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**, ..., 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*, ..., 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, ..., 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, ..., 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**, ..., 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).

...: Please refer to the link for complete author list.

## FUNDING

- NIH K99-R00 Pathway to Independence Award (Score: 14), National Institute of Health. 2023 - 2028
  Role : Principal Investigator (PI)
- Damon Runyon Fellowship, Damon Runyon Cancer Foundation 2019 - 2023
  Role: Principal Fellow
- Virtual Molecular Cell Consortium (VMCC) Fellow, UC San Diego. 2018 - 2019
  Role: Principal Fellow

## SELECT PUBLIC PRESENTATIONS (only invited or selected from abstracts)

- 3D Electron Microscopy-Gordon Research Conference, USA 2023
- MesaWide Cryo-Electron Microscopy (cryo-EM) Meeting, USA 2023
- Biophysical Society Meeting, San Diego, USA 2023
- Southern California cryo-EM symposium, USA 2022
- Cryo-EM workshop at Brookhaven National Lab, USA 2022, 2023
- Cryo-EM supergroup meeting, UC Boulder, USA 2022
- Friends of Cell Meeting, San Diego Cluster of Institutes, USA 2022
- National Tomography Workshop (3 lectures & workshops each year), AIIMS, New Delhi, India 2022, 2023
- Biophysical Society Meeting, San Francisco, USA 2022
- CRISPR Workshop at CSIR-IGIB, New Delhi, India 2019
- Student Evening Seminar Series, Johns Hopkins, USA 2017
- Physics of Living Systems Conference, Arlington, USA 2015
- Biophysical Society Meeting, Baltimore, USA 2015
- 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)*
- Generated theoretical models of multi-dimensional energy landscapes of protein folding.

**Master thesis student** at Indian Institute of Technology, Kharagpur 8/2011 – 3/2012
*Advisor: Prof. Swagata Dasgupta*
- Modeled amyloid beta multimers using protein structure prediction (Rosetta). Awarded the highest grade.

**Visiting research assistant** at Massachusetts Institute of Technology 5/2011 – 7/2011
*Advisor: Prof. Collin M. Stultz*
- Generated a computational library of amyloid beta using molecular dynamics to study its role in neurodegeneration.

**Visiting research assistant** at Karlsruhe Institute of Technology, Germany 11/2010 – 12/2010
*Advisor: Prof. Anne S. Ulrich*
- Synthesized membrane-active peptides for NMR studies on their alignment in membranes and antibacterial action.

**Visiting research assistant** at University of Illinois at Urbana-Champaign, USA 5/2010 – 7/2010
*Advisor: Prof. Martin Gruebele*
- Expressed & purified protein constructs for imaging their folding inside cells after temperature-induced unfolding.

**Research Intern** at Unilever 11/2009 – 12/2009
- Investigated the binding affinity of tea polyphenols with milk casein for its impact on popular tea products.

**Research Intern** at General Electric 4/2009 – 7/2009
- Synthesized radio-labeled antidepressants for analyzing their brain distribution through Positron Emission Tomography.

## TEACHING, MENTORING & VOLUNTEERING EXPERIENCE

**Mentor** at *UC San Diego* 08/2021 - Present
- Directly mentored one student (who went onto MD program at UCSF), one undegraduate and one high school student (as an ENLACE program (intended to foster scientific exchange between the USA and latin America) instructor.

**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 upliftment of underprivileged children. I volunteered in its multiple fundraising drives.

## OTHER HONORS, AWARDS & FINALIST POSITIONS

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