

BIOGRAPHICAL SKETCH

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NAME: Jawdat MH Al-Bassam

eRA COMMONS USER NAME (credential, e.g., agency login): JAWDAT

POSITION TITLE: Associate Professor, Molecular Cellular Biology Department, College of Biological Sciences
University of California, Davis

EDUCATION/TRAINING (*Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.*)

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
California State University, Long Beach	B.S.	09/1998	Biochemistry
Scripps Research Institute	Ph.D.	4/2004	Molecular Biophysics
Harvard Medical School; Boston, MA	Postdoctoral	04/2011	Molecular Biophysics and Structural Biology

A. Personal Statement

These projects involve the coordinated usage of biochemical, structural and biophysics approaches to elucidate fundamental soluble tubulin biogenesis, tubulin dynamic polymerization regulatory and microtubule-based motor activation mechanisms. I and my group are well suited to carry out studies described in this proposal. The studies include biochemical reconstitution of multi-subunit complexes, structural studies using cryo-EM and single particle image analysis, reconstitution of complexes using single molecule fluorescence activity assays with dynamic microtubules *in vitro* in order to test structural models. I am an expert at combining these approaches which span across multiple resolution scales to extract mechanistic knowledge. I have over twenty years of experience in molecular biology, protein biochemistry, structural biology using both x-ray crystallography and cryo-EM. I am an expert in studying microtubules, motors, and their regulation mechanisms using a variety of biophysical and biochemical techniques. I am an established investigator with significant expertise in combining biochemical reconstitution with structural biology microdiffraction x-ray crystallography, and high-resolution total internal reflection fluorescence (TIRF) microscopy to study dynamic microtubules. My group has very recently expanded towards high resolution single particle cryo-EM, as described in the preliminary data in this proposal. My group and I have developed systems incorporating all the above approaches to link atomic-level structural information to real-time functional readout on MT dynamics. Throughout my career, I have maintained successful collaborations and demonstrated high productivity in working with other groups. For the proposed work, I have established a network of long-term collaborations with three Cell biology experts with common goals using *in vivo* models.

B. Positions and Honors**Employment**

1998-2004	Graduate student, Molecular Cellular Structure and Chemistry Program Cell Biology Dept., The Scripps Research Institute, La Jolla, CA
2003-2011	Postdoctoral Fellow, Biological Chemistry and Molecular Pharmacology Dept., Harvard Medical School, Boston, MA
5/1/2011-5/1/2018	Assistant Professor, Molecular Cellular Biology Dept, College of Biological Sciences, University of California, Davis, CA
5/2/2018-present	Associate Professor, Molecular Cellular Biology Dept, College of Biological Sciences, University of California, Davis, CA

Honors:

1995-1998	California State University, Long Beach President's scholarship
1995	California State University Long Beach, Spyros Pathos IV Memorial award in Chemistry

1997	American Heart Association undergraduate summer research fellowship
1998	California State University, Long Beach, Robert Henderson Memorial award in Biochemistry
1998	California State University Long Beach College of Natural Sciences and Mathematics, Outstanding graduate of the year
2000-2003	American Heart Association Pre-doctoral Fellow
2000	The Scripps Research Institute Society of Fellows Travel award
2001	The Scripps Research Institute Graduate Program award
2002	American Society of Cell Biology Student travel award
2003	Invited speaker, 47 th annual biophysical society meeting in San Antonio
2005-2008	American Cancer Society Postdoctoral Fellow
2009	Invited speaker, Gordon Research conference in “motile and contractile systems”
2013	Invited speaker, Gordon Research conference in “motile and contractile systems”
2014	Hellman Foundation fellow
2014	Invited speaker, European Molecular Biology Laboratory conference on “Microtubules”
2014	Invited speaker Gordon Research Conference in “Cytoskeletal motor proteins”
2015	Invited speaker Gordon Research conference in “motile and contractile systems”
2016	Invited speaker, European Molecular Biology Laboratory conference on “Microtubules”
2018	Invited speaker Gordon Research Conference in “Cytoskeletal motor proteins”
2018	Invited speaker, American Society for Cell biology minisymposium “cytoskeleton tracks”
2019	Invited speaker, Gordon Research Conference in “Cytoskeletal motor proteins”
2020	Invited speaker, European Molecular Biology Laboratory conference on “Microtubules”

Scientific Appointments

2019	Invited member, NIH study section panel ZGM1 TWD-7 KR
2020	Invited member, NIH study section panel ZGM1 TWD-7 MK
2021	Invited member, NIH study section panel MSFC
2021	Invited member, NIH study section panel ZGM1 TWD-7 MK
2022	Invited member, NIH study section panel ZGM1 TWD-7 MK

Professional Memberships

2000-present	Member, American Society for Cell Biology
2003-present	Member, American Biophysical Society

C. Contributions to Science

1. Biochemical and Structural studies of Microtubule Associated Proteins and Kinesin Motors: My research career started with a focus on structural mechanisms of microtubule associated proteins in neurons and studies of monomeric kinesin-3 motor proteins. I described the first cryo-EM structure of the microtubule associated proteins, MAP2 and tau, in binding along MT protofilaments and described their mechanism in stabilizing microtubule organization (Al-Bassam et al 2002). I also used biochemistry and cryo-EM to describe a structural basis for the monomeric kinesin-3 motor proteins to activate into dimerization (Al-Bassam et al 2003). In 2011, I initiated new studies of the bipolar tetrameric kinesin-5 motor to understand their novel bipolar organizations and mechanism in sliding apart antiparallel microtubules (Scholey et al 2014). We also determined the unique mechanism microtubule -motility directionality reversal in yeast kinesin-5 motors such as Cin8 (Shapira et al 2017). We recently described structural studies recently described the mechanism of disassembly of microtubule severing enzymes, Katanins (Nithianantham et al 2018).

- **Al-Bassam J.,** Ozer R.S., Safer D., Halpain S., Milligan R.A.; MAP2 and tau bind along the outer ridges of microtubule protofilaments. 2002. *J. Cell Biol.* 157: 1187-1196. PMCID: PMC2173547
- **Al-Bassam J.,** Cui Y., Klopheinstein D., Carragher, B.O., Vale R.D., Milligan, R.A. Distinct Conformations of the Kinesin Unc104 Neck Regulate a Monomer-to-Dimer Motor Transition, *J. Cell Biol.* 2003. 163: 743-753.
- Roger. B*, **Al-Bassam J*,** Milligan R.A., Halpain S. MAP2, but not tau, repeats bind and bundle f-actin, *Current Biol.* 2004. 14:363-371; PMID: 15028210.
- Scholey JE, Nithianantham S, Scholey JM, **Al-Bassam J***. "Structural Basis for the assembly of the mitotic motor Kinesin-5 into bipolar tetramers" *eLIFE*, 2014. 3:e02217. doi: 10.7554/elife.02217
- Shapira, O, Goldstein, A, **Al-Bassam J***, and Gheber L*. (2017). "A potential physiological role for bidirectional motility and motor clustering of mitotic kinesin-5 Cin8 in yeast mitosis". *J Cell Sci* 130, 725-734. *Co-corresponding.
- Singh SK, Pandey H, Al-Bassam J, Gheber L. "Bidirectional motility of kinesin-5 motor proteins: structural determinants, cumulative functions and physiological roles" *Cell Mol Life Sci.* 2018 May;75(10):1757-1771. doi: 10.1007/s00018-018-2754-7. PMID: 29397398
- **Al-Bassam J*,** Nithianantham S. Malleable folding of coiled-coils regulates kinesin-3 dimerization. *Proc Natl Acad Sci U S A.* 2018; 115:12845-12847. doi: 10.1073/pnas.1818758115.

- Nithianantham S, McNally FJ, and **Al-Bassam J*** “Structural Basis for Disassembly of Katanin Heterododecamers” *J Biol Chem.* 2018. 293:10590-10605. PMCID: PMC6036222
- Bodrug T, Wilson-Kubalek E, Thompson A, Major J, Alfieri A, Gaska I, Nithianantham S, Debs G, Gutierrez P, Gheber L, McKenney R, Sindelar C, Milligan R, Stumpff J, Forth S, Rosenfeld S, **Al-Bassam J***. “The Kinesin-5 Tail Domain Directly Modulates the Mechanochemical Cycle of the Motor for Anti-Parallel Microtubule Sliding”, *eLife.* 2020 Jan 20;9:e51131. doi: 10.7554/eLife.51131. *Corresponding author
- Nithianantham S, Iwanski M, Gaska I, Pandey H, Bodrug T, Inagaki, Major J, Brouhard GJ, Gheber L, Rosenfeld S, Forth S, Hendricks AG, **Al-Bassam J*** “The mechanochemical origins of the microtubule sliding motility within the kinesin-5 domain organization. *BioRxiv* 2021.10.12.463902; doi: <https://doi.org/10.1101/2021.10.12.463902>

2. Biochemical and Structural studies of Microtubule Polymerases: During my postdoctoral research at Harvard Medical School, and as an independent investigator at UC-Davis, I discovered two families of regulators share arrays of Tumor Overexpressed Gene (TOG) domains to recruit soluble $\alpha\beta$ -tubulins to polymerize or stabilize microtubules (Al-Bassam et al 2006), determined the first TOG domain x-ray structure and described their tubulin binding mechanism (Al-Bassam et al 2007). I and my group have reconstituted the fission yeast versions of these proteins with dynamic microtubules, showing that Alp14, the XMAP215 ortholog, is a microtubule plus-end directed microtubule polymerase, while a CLASP ortholog, is microtubule stabilizing rescue factor (Al-Bassam et al 2010, 2012). In Summary, our work in this area has fundamentally set the current knowledge area for how microtubules are polymerization is regulated. My group recently fundamentally transformed this field with rigorous biochemical and x-ray crystallographic studies describing the transitions of TOG arrays: $\alpha\beta$ -tubulin complexes as microtubule polymerases leading to a novel mechanistic model (Nithianantham et al, 2018; Cook et al, 2019).

- **Al-Bassam J**, van Breugel M, Harrison SC, Hyman A. Stu2p binds tubulin and undergoes an open-to-closed conformational change. *J Cell Biol.* 2006 Mar 27; 172(7):1009-22. PMCID: PMC2063759
- **Al-Bassam J**, Larsen NA, Hyman AA, Harrison SC. Crystal structure of a TOG domain: conserved features of XMAP215/Dis1-family TOG domains and implications for tubulin binding. *Structure.* 2007; 15(3): 355-62.
- Brouhard G, Stear J, Notzel T, **Al-Bassam J**, Kinoshita K, Harrison SC, Howard J, Hyman AA, XMAP215 is a processive microtubule polymerase that catalyzes both growth and shrinkage, *Cell.* 2008, 132:79-88.
- **Al-Bassam J***, Kim H, Brouhard G, van Oijen A, Harrison SC, Chang F*. CLASP promotes microtubule rescues by recruiting tubulin dimer to the microtubule”. *Developmental Cell*, 2010. 19: 245-258. PMCID: PMC3156696
- **Al-Bassam J***, Kim H, Flor-Parra I, Lal N, Velji H, Chang F. ” Fission yeast Alp14 is a dose dependent microtubule polymerase ”. *Molecular Biology of the Cell.* 2012. 15:2878-90. PMCID: PMC3408415
- **Al-Bassam J***, Chang F * ” Regulation of Microtubule Dynamics by TOG domain proteins XMAP215/Dis1 and CLASP”. *Trends in Cell Biology.* 2011. 21: 604-614
- **Al-Bassam J.** ”Reconstituting Dynamic Microtubule Polymerization Regulation by TOG Domain Proteins”. *Methods in Enzymology.* 2014. 540: 131-148
- Nithianantham S, Cook BD, Beans M, Guo, F, Chang FC, **Al-Bassam J.** “Structural Basis of tubulin recruitment and assembly by microtubule polymerases with Tumor Overexpressed Gene (TOG) domain arrays” *eLIFE.* pii: e38922. doi: 10.7554/eLife.38922. PMCID: PMC6251626
- Cook BD, Chang FC, Flor-Parra I, **Al-Bassam J*** “Microtubule polymerase and processive plus-end tracking activities arise from unique tubulin recruitment and self-organization by arrays of TOG domains” *Mol Biol Cell.* 2019. 30:1490-1504. PMCID: PMC6724690

3. Biochemical and Structural studies of Tubulin Biogenesis and Microtubule severing proteins: my group has uncovered a fundamental molecular mechanism for soluble $\alpha\beta$ -tubulin biogenesis and degradation processes modulated by five tubulin chaperones and Arl2 GTPase maintain their intracellular concentration. We reconstituted tubulin chaperones and Arl2 and demonstrate their shared role as cage-like multi-subunit machines that manipulate $\alpha\beta$ -tubulin polymerization through regulation of GTP hydrolysis (Nithianantham et al 2015).

- Nithianantham S, Le S, Seto E, Ti S, Yue A, Jia W, Leary J, Corbett KD, Moore JK, **Al-Bassam J***. “Tubulin cofactors and Arl2 are cage-like chaperones that regulate the soluble $\alpha\beta$ -tubulin pool for microtubule dynamics”. *eLIFE.* 2015 Jul 24;4. doi: 10.7554/eLife.08811. PMCID:PMC4574351
- **Al-Bassam J***. Revisiting the tubulin cofactors and Arl2 in the regulation of soluble $\alpha\beta$ -tubulin pools and their effect on microtubule dynamics. *Molecular Biology of the Cell.* 2017 28(3);359-363. doi: 10.1091/mbc.E15-10-0694. PMCID: PMC5341719

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support

National Institutes of Health (5R01-GM110283)

1/1/2015-12/31/2024

“Mechanisms of Tubulin Dimer Regulatory Pathways and Their Impact on Microtubule Function”

Role : PI

BIOGRAPHICAL SKETCH

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NAME: Bharti Singal

eRA COMMONS USER NAME (credential, e.g., agency login): BSINGAL

POSITION TITLE: Postdoctoral Scholar

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
HansRaj College, University of Delhi, India	B.Sc.	07/2011	Life Sciences
SPM College, University of Delhi, India	B. Ed.	07/2012	Education
Jamia Millia Islamia University, Delhi, India	M.Sc.	07/2015	Bioinformatics
Nanyang Technological University, Singapore	Ph.D.	07/2020	Biological Sciences (Structural Biology and Biochemistry)

A. Personal Statement

I am a postdoctoral Scholar in structural biology at UC-Davis working with Dr Jawdat Al-Bassam with a focus on gaining expertise in cryo-EM and single particle image analysis methods. I have more than six years of training and expertise in using computational and experimental biochemistry and structural biology approaches. I started my research career with in utilizing bioinformatics analysis and in-silico drug discovery focusing on infectious diseases and cancer progression during Masters degree training. I further built the computational biology expertise with PhD doctoral training working with Dr Gerhard Grüber with expertise in biochemistry and experimental structural biology focusing on X-ray crystallography and NMR spectroscopy. I investigated the structure and mechanism of a crucial protein required to regulate stringent alarmone with the help of biochemical enzymatic studies. During doctoral work, I gained expertise in various biophysical techniques such as SAXS, ITC, DLS. I have gained expertise in x-ray crystallographic structure determination and model building methodology by working on three structures during my Ph.D. As postdoctoral scholar working with Dr Jawdat Al-Bassam, my work is majorly focused on utilizing cryo-electron microscopy (Cryo-EM) and single particle image analysis to study the structures and mechanism of microtubule plus-end polymerization regulatory proteins and the mechanisms of activation of the microtubule-based motors from the Kinesin super family. During the past eighteen months of training, I have gained expertise in protein expression in bacteria and insect cells, biochemical purification and reconstitution of multi-protein complexes, sample preparation and vitrification of single particle samples using GP2, methods of grid screening using Thermofisher Glacios and I am trained in the latest tools used in cryo-EM image processing such as Relion, cryosparc and neural network methods for particle picking. I have gained extensive expertise in model building and validation using Phenix, ccpem and coot. I have thorough experience in structure visualization and refinement tools like chimera and pyMol.

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

2012-2014	Elementary Biology Teacher, Maxfort School, Rohini, Delhi, India
2014-2015	Research Intern, Deshbandhu College, University of Delhi, India
2016-2020	Graduate Student, Department of Structural Biology and Biochemistry, College of Biological

2020-Present Sciences, Nanyang Technological University, Singapore
Postdoctoral Scholar, Department of Molecular and Cellular Biology, College of Biological Sciences, University of California, Davis, CA, USA

Honors/Awards:

2015 Talk - International Conference on Mathematical and Computational Biology, IIT Kanpur, India
2016 Gold Medalist, M.Sc. Bioinformatics first rank holder, Jamia Millia Islamia University, India
2016-2020 Nanyang Technological University Doctoral Research Scholarship
2019 Gordon Research Conference allowance by Nanyang Technological University
2022 Recognized among Top 75 women in STEAM by Red Dot Foundation, Office of Principal Scientific Advisor- Government of India and British High Commission.

Professional Memberships:

2020 Student Membership, Biophysical Society
2021-2022 Early Career Member, American Society for Biochemistry and Molecular Biology

C. Contributions to Science

1. Structural and Mechanistic insights into the Rel protein of *Mycobacterium tuberculosis*: During my doctoral training I started working on the mechanism responsible in eliciting stringent response in Mycobacteria upon nutrient starvation. With the help of X-ray crystallography, I described the first structure of active Rel protein from *Mtb* (*MtRel*) which exhibits both hydrolase and synthetase activity for hyperphosphorylated Guanine ((p)ppGpp). Prior to this work there was no structure available for the bifunctional class of RelA/SpoT family proteins (Singal et. al 2017). To further understand the interactions between tRNA and *MtRel* protein, I carried NMR spectroscopy studies and identified the residues involved in interacting with deacylated tRNA which is in abundance during nutrient starvation (Shin and Singal et al 2021). Considering multiple hypothesis of activation and autoinhibition of *MtRel* protein, NMR spectroscopy structural and titration studies were conducted with aliphatic amino acids. This study further reflected the importance of dimerization of *MtRel* by ACT domain and proposed a mechanism to sense the deficiency of amino acids in the cell during starvation (Shin and Singal et. al 2021). These studies not only solidified the understanding of *MtRel* bifunctional role but also brought new light to the regulation of *MtRel*.

- Singal, B., Balakrishna, A.M., Nartey, W., Manimekalai, M.S.S., Jeyakanthan, J. and Grüber, G., 2017. Crystallographic and solution structure of the N-terminal domain of the Rel protein from Mycobacterium tuberculosis. *FEBS letters*, 591(15), pp.2323-2337.
- Shin, J.*, Singal, B.*, Sony Subramanian Manimekalai, M., Wei Chen, M., Ragunathan, P. and Grüber, G., 2021. Atomic structure of and valine binding to the regulatory ACT domain of the Mycobacterium tuberculosis Rel protein. *The FEBS Journal*, 288(7), pp.2377-2397.
- Shin, J.*, Singal, B.*, Grüber, A., Wong, D.M.K., Ragunathan, P. and Grüber, G., 2021. Atomic structure of the regulatory TGS domain of Rel protein from Mycobacterium tuberculosis and its interaction with deacylated tRNA. *FEBS letters*, 595(24), pp.3006-3018.

2. Other contributions

- Singh, A.*, Singal, B.*, Nath, O. and Singh, I.K., 2015. Functional annotation and classification of the hypothetical proteins of Neisseria meningitidis H44/76. *Am. J. of Biosci. and Bioeng*, 3, pp.57-64.
- Singh, I.K., Kumari, P., Mittal, P., Kumar, A., Singal, B., Hasan, G.M., Aggarwal, R., Kamal, M.A., Singh, A. and Hassan, M., 2021. Emerging Therapeutic Approaches to COVID-19. *Current Pharmaceutical Design*, 27(31), pp.3370-3388.
- Singal, B. and Chhibber-Goel, J., Accepted, To be published in Nov. 2022. Aminoacyl-tRNA synthetases as malaria drug targets: a structural biology perspective in Drug Development for Malaria: Novel Approaches for Prevention and Treatment. *Wiley*.
- Singal, B. and Chhibber-Goel, J., Accepted, To be published in Nov. 2022. The imminent threat of antimalaria drug resistance in Drug Development for Malaria: Novel Approaches for Prevention and Treatment. *Wiley*.

(* - authors contributed equally)

D. Scholastic performance during doctoral studies

YEAR	COURSE TITLE	GRADE
NANYANG TECHNOLOGICAL UNIVERSITY		
2016	Foundation Course in Molecular and Cellular Biology	A+
2016	Practical Course in Multidimensional NMR Spectroscopy	A-
2017	Informational Biology	A+
2017	Graduate Seminar Course	A+
2017	University Teaching for Teaching Assistants	P

Except for the University Teaching for Teaching Assistants course, Nanyang Technological University graduate courses are graded A (excellent) to F (fail). P states Pass.