Dr. Ankit Rai
Ramalignaswami Fellow
Assistant Professor,
Medical Biotechnology

Academic Background

- **Assistant Professor** in Medical Biotechnology at GBU, Gandhinagar, Gujarat from June 2022 – till now.
- **Ramalingaswami Fellow,** Department of Biochemistry, BHU, Varanasi, UP from Sep 2021– May 2022.
- **Postdoctoral Research** with Prof. Anna Akhmanova at Utrecht University, the Netherlands from Feb 2015 – Aug 2021.
- **Ph.D.** with Prof. Dulal Panda at IIT Bombay, Mumbai from Jan 2008 – June 2014.
- **M.Sc.** in Biotechnology from Department of Biotechnology, University of Pune, Maharashtra.
- **B.Sc.** in Chemistry, Botany, Zoology from D.D.U. Gorakhpur University, Gorakhpur, Uttar Pradesh.

Personal Information:

Date of birth: 04-07-1984
Nationality: India
Family status: Married and father of two daughters
Permanent address: H. No. 174, Ashoknagar, Basharatpur, Gorakhpur, Uttar Pradesh-273004, India
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Research Interest: Understanding the role of microtubule dynamics in autophagy and its implication in cancer and neurodegenerative diseases. During my doctoral and post-doctoral research, I gained expertise in the area of cell biology, biochemistryand biophysics. I am an expert in single-molecule analysis, high-resolution microscopy (TIRF microscopy, Spinning Disc Confocal, Electron microscopy)-based techniques, understanding drug mechanisms, protein-protein/protein-drug interactions. Currently, at GBU, I am establishing involvement of microtubule dynamics in regulating autophagy. My great interest is in setting up an in vitro reconstruction based assays for single-molecule analysis.

Post-doctoral Project:

Supervisor: Professor Anna Akhmanova, Cell Biology, Neurobiology and Biophysics, Department of Biology, Faculty of Science, Utrecht University, the Netherlands

Research Focus: Using advanced imaging approaches; I explored detailed molecular mechanisms of regulation of microtubule dynamics by microtubule-targeting cancer drugs and mammalian kinesin-4 KIF21B.

Research Results:

Microtubules are polymers of tubulin dimers, and conformational transitions in the microtubule lattice drive microtubule dynamic instability and affect various aspects of microtubule function. The exact natureof these transitions and their modulation by anti-cancer drugs such as Taxol and epothilone, which can stabilize microtubules but also perturb their growth, are poorly understood. I directly visualized the action of fluorescent Taxol and epothilone derivatives and showed that microtubules can transition to a state that triggers cooperative drug binding to form regions with altered lattice conformation. Such regions emerge at growing microtubule ends that are in a pre-catastrophe state and inhibit microtubule growth and shortening. Electron microscopy and in vitro dynamics data indicated that taxane accumulation zones represent incomplete tubes that can persist, incorporate tubulin dimers and repeatedly induce microtubule rescues. Thus, taxanes modulate the material properties of microtubules by converting destabilized growing microtubule ends into regions resistant to depolymerization (***Rai** et al., 2020**, Nature Materials***). In addition, very recently, I showed that such drug-induced defects led to frequent catastrophes and induced protofilament number mismatch. Our data suggest that structural defects within microtubule lattice can exert effects that can propagate over long distances and affect the dynamic state of the microtubule end (***Rai*** et al., 2021, ***PNAS***). Further, using in vitro reconstitution based microtubule dynamic assays, I showed that the mammalian kinesin-4 KIF21B is a processive motor that can accumulate at microtubule plus ends and induce pausing. A few KIF21B molecules are sufficient to induce strong growth inhibition of a microtubule plus-end in vitro. This property depends on non- motor microtubule-binding domains located in the stalk region and the C-terminal WD40 domain. The WD40-containing KIF21B tail displays a preference for a GTP-type over a GDP-type microtubule lattice and contributes to the interaction of KIF21B with microtubule plus ends (*van Riel* and **Rai*** et al., 2017, **Elife** *Equal first author*). In addition, I was actively involved in various collaborative projects on CLASP2, Kar-9C,CAMSAP-CKK domain proteins and microtubule targeting compounds, which are published in highly reputed scientific journals *Developmental Cell, Structure, Nature Communications, Nature Chemical Biology* and *Molecular Cell*, respectively.

Ph.D. Project:

PhD Supervisor: Professor Dulal Panda, IIT Bombay, India

Thesis Title: Anti-proliferative mechanism of action of tubulin-targeting agents CXI-benzo-84, BCFMT and TN-16.

Research Focus: Exploring the interaction of small molecule inhibitors with tubulin and understanding the role of microtubule dynamics in signal transduction and resistance development towards microtubule inhibitors.

Research Results:

Microtubules, the dynamic polymers of eukaryotic cytoskeleton, play important roles in several cellular functions including cell division, cell differentiation, cell migration, intracellular trafficking and cell signaling cascades. Several clinically used anticancer drugs target microtubules network and block the cell cycle progression in mitosis, which eventually activates the cell death program. Though microtubule targeting drugs are found to be very successful in cancer chemotherapy, the development of resistance against the existing drugs limits the use of known tubulin-targeting drugs in cancer chemotherapy.

In my PhD thesis, using cell-based screening approaches, potential anticancer agents CXI- benzo-84 [***Rai** et al., **BiochemPharmacol**. 2013;86(3):378-91*] and BCFMT [***Rai** et al., **PLoSOne**. 2012;7(8):e44311*] were discovered using a large subset of benzimidazole and rhodanine derived scaffolds as a starting point. Benzimidazole derivative CXI-benzo-84 and rhodanine derivative BCFMT displayed their anticancer

activity by depolymerizing microtubules, inhibiting cell cycle progression in mitosis and accumulating spindle assembly checkpoint proteins at the kinetochores, which subsequently helped in activation of apoptotic cell death pathways in several types of cancer cells including highly metastatic and drug-resistant cells. I have developed a colchicine-resistant variant of MCF-7 cell line by the gradual increment of colchicine, which showed more than 8-fold resistance towards colchicine as compared to the parent MCF-7 cells. Time-lapse imaging of microtubules in live cells showed that the stability of individual microtubules in MCF-7_{Col30} cells increased as compared to the parent MCF-7 cells indicating that resistance was due to changes in the microtubule cytoskeleton [***Rai** et al., **Biochem Pharmacol**. 2017;15;132:38-47*]. Further, using TN16, a known tubulin targeting agent, I have found thatNF-κB interacts with microtubules in cells, and suppression of microtubule dynamics stimulates the NF- κB signaling cascade [***Rai et al., BiochemPharmacol.** 2015;93(3):277-89*]**.** In addition, I have worked on several collaborative projects in which I have investigated the antiproliferative mechanism of action o different compounds using in vitro and cellular techniques. These works resulted in 9 publications, on which I am a co-author and two patents are also filled.

M.Sc. Project: Evaluation of nutrient starvation model and role of antimycobacterial drugs in *Mycobacterium smegmatis*, **under the guidance of Dr. Dhiman Sarkar, National Chemical Laboratory (NCL) Pune, India**

Research publications in peer-reviewed journals (25 published, Scopus h index: 17, citations: 793, Goggle scholar h-index 18, citations: 1057)

1) **Rai A**, Liu T, Katrukha EA, Estévez-Gallego J, Paterson I, Díaz JF, Kapitein LC, Moores CA, Akhmanova A. Lattice defects induced by microtubule-stabilizing agents exert a long-range effect on microtubule growth by promoting catastrophes. **Proc Natl Acad Sci U S A. 2021** Dec 21;118(51):e2112261118.

   Impact factor: 12.78

2) Gao L, Meiring JCM, Heise C, **Rai A**, Müller-Deku A, Akhmanova A, Thorn-Seshold J, Thorn-Seshold O. Photoswitchable epothilone-based microtubule stabilisers allow GFP-imaging-compatible, optical control over the microtubule cytoskeleton. **Angew Chem Int Ed Engl. 2021** Dec 13. doi: 10.1002/anie.202114614. Online ahead of print. PMID: 34902214

   Impact factor: 16.82

3) Saraon P, Snider J, Schormann W, **Rai A,** Radulovich N et. al….Chemical genetics screen identifies COPB2 tool compounds that alters ER stress response and induces RTK dysregulation in lung cancer cells. **J Mol Biol. 2021** Nov 19;433(23):167294.

   Impact factor: 5.469

4) **Rai A**, Liu T, Glauser S, Katrukha EA, Estévez-Gallego J, Rodríguez-García R, Fang WS, Díaz JF, Steinmetz MO, Altmann KH, Kapitein LC, Moores CA, Akhmanova A. Taxanes convert regions of perturbed microtubule growth into rescue sites. **Nature Materials. 2020** Mar;19(3):355-365. PubMed PMID: 31819210.

   Impact factor: 47.656

5) Peronne L, Denarier E, **Rai A**, Prudent R, Vernet A, Suzanne P, Ramirez-Rios S, Michallet S, Guidetti M, Vollaire J, Lucena-Agell D, Ribba AS, Josserand V, Coll JL, Dallemagne P, Díaz JF,

Oliva MÁ, Sadoul K, Akhmanova A, Andrieux A, Lafanechère L. Two Antagonistic Microtubule Targeting Drugs Act Synergistically to Kill Cancer Cells. **Cancers (Basel). 2020** Aug 6;12(8):E2196. PubMed PMID: 32781579.

Impact factor: 6.32

6) Jost M, Chen Y, Gilbert LA, Horlbeck MA, Krenning L, Menchon G, **Rai A**, Cho MY, Stern JJ, Prota AE, Kampmann M, Akhmanova A, Steinmetz MO, Tanenbaum ME, Weissman JS. Pharmaceutical- Grade Rigosertib Is a Microtubule-Destabilizing Agent. **Mol Cell. 2020** Jul 2;79(1):191-198.e3. PMID: 32619469.

Impact factor: 19.33

7) Saraon P, Snider J, Kalaidzidis Y, Wybenga-Groot LE, Weiss K, **Rai A**, Radulovich N, Drecun L, Vučković N, Vučetić A, Wong V, Thériault B, Pham NA, Park JH, Datti A, Wang J, Pathmanathan S, Aboualizadeh F, Lyakisheva A, Yao Z, Wang Y, Joseph B, Aman A, Moran MF,Prakesch M, Poda G, Marcellus R, Uehling D, Samaržija M, Jakopović M, Tsao MS, Shepherd FA, Sacher A, Leighl N, Akhmanova A, Al-Awar R, Zerial M, Stagljar I. A drug discovery platform to identify compounds that inhibit EGFR triple mutants. **Nat Chem Biol. 2020**, May;16(5):577-586. PubMed PMID: 32094923.

Impact factor: 16.29

8) Rashid A, Naaz A, **Rai A**, Chatterji BP, Panda D. Inhibition of polo-like kinase 1 suppresses microtubule dynamics in MCF-7 cells. **Mol Cell Biochem**. **2020** Feb;465(1-2):27 36. PubMed PMID: 31782084.

Impact factor: 3.842

9) Atherton J, Luo Y, Xiang S, Yang C, **Rai A**, Jiang K, Stangier M, Vemu A, Cook mAD, Wang S, Roll-Mecak A, Steinmetz MO, Akhmanova A, Baldus M, Moores CA. Structural determinants of microtubule minus end preference in CAMSAP CKK domains. **Nat Commun. 2019** Nov 20;10(1):5236.PubMed PMID: 31748546.

Impact factor: 17.69

10) Naaz A, Ahad S, **Rai A**, Surolia A, Panda D. BubR1 depletion delays apoptosis in the microtubule-depolymerized cells. **Biochem Pharmacol. 2019** Apr;162:177-190. Epub 2018 Nov22. PubMed PMID: 30468712.

Impact factor: 6.1

11) Aher A, Kok M, Sharma A, **Rai A**, Olieric N, Rodriguez-Garcia R, Katrukha EA, Weinert T, Olieric V, Kapitein LC, Steinmetz MO, Dogterom M, Akhmanova A. CLASP Suppresses Microtubule Catastrophes through a Single TOG Domain. **Dev Cell. 2018** Jul 2;46(1):40-58.e8. PubMed PMID: 29937387.

Impact factor: 13.42

12) Jost M, Chen Y, Gilbert LA, Horlbeck MA, Krenning L, Menchon G, **Rai A**, Cho MY, Stern JJ, Prota AE, Kampmann M, Akhmanova A, Steinmetz MO, Tanenbaum ME, Weissman JS. Combined CRISPRi/a-Based Chemical Genetic Screens Reveal that Rigosertib Is a Microtubule- Destabilizing Agent. **Mol Cell. 2017** Oct 5;68(1):210-223.e6. PubMed PMID: 28985505

Impact factor: 19.33

13) Kumar A, Manatschal C, **Rai A**, Grigoriev I, Degen MS, Jaussi R, Kretzschmar I, Prota AE, Volkmer R, Kammerer RA, Akhmanova A, Steinmetz MO. Short Linear Sequence Motif LxxPTPh Targets Diverse Proteins to Growing Microtubule Ends. **Structure. 2017** Jun 6;25(6):924-932.e4. PubMed PMID: 28552577.

Impact factor: 5.871

14) van Riel WE*, **Rai A***, Bianchi S, Katrukha EA, Liu Q, Heck AJ, Hoogenraad CC, Steinmetz MO, Kapitein LC, Akhmanova A. Kinesin-4 KIF21B is a potent microtubule pausing factor. **Elife. 2017** Mar 14;6. pii: e24746. PubMed PMID: 28290984.

***equal first author. Impact factor: 8.713**

15) **Rai A**, Kapoor S, Naaz A, Kumar Santra M, Panda D. Enhanced stability of microtubulescontributes in the development of colchicine resistance in MCF-7 cells. **Biochem Pharmacol. 2017** May 15;132:38-47. PubMed PMID: 28242250.

Impact factor: 6.1

16) **Rai A**, Kapoor S, Singh S, Chatterji BP, Panda D. Transcription factor NF-κB associates with microtubules and stimulates apoptosis in response to suppression of microtubule dynamics in MCF-7 cells. **Biochem Pharmacol. 2015** Feb 1;93(3):277-89. PubMed PMID: 25536174.

Impact factor: 6.1

17) Singh D, Bhattacharya A, **Rai A**, Dhaked HP, Awasthi D, Ojima I, Panda D. SB-RA-2001 inhibits bacterial proliferation by targeting FtsZ assembly. **Biochemistry. 2014** May 13;53(18):2979-92. PubMedPMID: 24749867.

Impact factor: 3.321

18) **Rai A**, Gupta TK, Kini S, Kunwar A, Surolia A, Panda D. CXI-benzo-84 reversibly binds to tubulin at colchicine site and induces apoptosis in cancer cells. **Biochem Pharmacol. 2013** Aug 1;86(3):378-91. PubMed PMID: 23747346.

Impact factor: 6.1

19) **Rai A**, Surolia A, Panda D. An antitubulin agent BCFMT inhibits proliferation of cancer cells and induces cell death by inhibiting microtubule dynamics. **PLoSOne. 2012**;7(8):e44311. PubMed PMID: 22952952.

Impact factor: 3.752

20) Pathak RK, Hinge VK, Mahesh K, **Rai A**, Panda D, Rao CP. Cd2+ complex of a triazole-based calix[4]arene conjugate as a selective fluorescent chemosensor for Cys. **Anal Chem. 2012** Aug 7;84(15):6907-13. PubMed PMID: 22834792.

Impact factor: 8.008

21) Pathak RK, Tabbasum K, **Rai A**, Panda D, Rao CP. A Zn2+ specific triazole based calix[4]arene conjugate (L) as a fluorescence sensor for histidine and cysteine in HEPES buffer milieu. **Analyst. 2012** Sep 7;137(17):4069-75. PubMed PMID: 22822479.

Impact factor: 5.227

22) Pathak RK, Tabbasum K, **Rai A**, Panda D, Rao CP. Pyrophosphate sensing by a fluorescent Zn2+ bound triazole linked imino-thiophenyl conjugate of calix[4]arene in HEPES buffer medium: spectroscopy, microscopy, and cellular studies. **Anal Chem. 2012** Jun 5;84(11):5117-23. PubMed PMID: 22551314.

Impact factor: 8.008

23) Pathak RK, Hinge VK, **Rai A**, Panda D, Rao CP. Imino-phenolic-pyridyl conjugates of calix[4]arene(L1 and L2) as primary fluorescence switch-on sensors for Zn2+ in solution and in HeLa cells and the recognition of pyrophosphate and ATP by [ZnL2]. **Inorg Chem. 2012** May 7;51(9):4994-5005. PubMed PMID: 22519733.

Impact factor: 5.436

24) Naik PK, Santoshi S, **Rai A**, Joshi HC. Molecular modelling and competition binding study of Br-noscapine and colchicine provide insight into noscapinoid-tubulin binding site. **J Mol Graph Model. 2011** Jun;29(7):947-55. PubMed PMID: 21530342.

Impact factor: 2.942

25) Balakrishna MS, Suresh D, **Rai A**, Mague JT, Panda D. Dinuclear copper(I) complexes containing cyclodiphosphazane derivatives and pyridyl ligands: synthesis, structural studies, and antiproliferative activity toward human cervical and breast cancer cells. **Inorg Chem. 2010** Oct 4;49(19):8790-801. PubMed PMID: 20812680.

Impact factor: 5.436

Patents:

1) M.S. Balakrishna, D. Panda and **A. Rai.** "Antiproliferative activity of dinuclearcopper(I) complexes containing cyclodiphosphazane derivatives towards human cervical breast cancer cells." Patent application no: 2458/MUM/2010 dated 3rd September 2010.

2) A. Surolia, **A. Rai** and D. Panda. "Rhodanine compounds as tubulin binding anticancer agent and their application in treating cancers" India Patent application number 1167/DEL/2011 dated April 20, 2010, PD003017-IN-SC.

Conference/Symposium/Talk:

1) Presented **a talk** "Visualization of microtubule drug interactions in real time at the level of single filaments" at the Annual Dutch meeting on molecular and cellular Biophysics (Dutch Biophysics–NOW) held during 2-3 October 2017, at Veldhoven, the Netherlands.

2) Presented **a poster** "Visualization of microtubule-paclitaxel interactions in real time at the level of single filaments" at **EMBO | EMBL Symposium: Microtubules: From Atoms to Complex Systems,** held during 27-30 May 2018, at EMBL Heidelberg, Germany.

3) Presented **a poster** "microtubule stabilizing agents induce propagating microtubule lattice alterations which promote microtubule rescue" at **EMBO | EMBL Symposium: Microtubules: From Atoms to Complex Systems,** held during 29 May-1 June 2016, at EMBL Heidelberg, Germany.

4) Presented **a poster** "DRB perturbed the interaction between Casein Kinase 2 and MT, depolymerized MTs and induced apoptosis in MCF-7 cells", **51st Annual Meeting of American Society for Cell**

Biology, held during Dec. 03-07, 2011, at Denver, Colorado, USA.

5) Presented **a poster** entitled "Nuclear import of NF-kB and pERK coordinately induced apoptosis in MCF-7 cells in response to MT damage." **53rd Annual Meeting of American Society for Cell Biology,** held during Dec. 14-18, 2013, at New Orleans, LA, USA.

6) Attended a workshop in **Molecular Motors, Tracks and Transport** organized at Pondicherry, India during 23rd to 28th January, 2010

7) Volunteered in **International Symposium on Emerging Areas in Biosciences and Bioengineering** (ISEABB 09) at the Department of Biosciences and Bioengineering, Indian Institute of Technology Bombay, Mumbai.

Academic Achievements/Awards:

- DBT Ramalingaswami Re-entry Fellowship 2020-2021.
- Award for Excellence in 2014 from IIT Bombay, India for my thesis work.
- Selected for M.Sc. by Combined M.Sc. Biotechnology Entrance Examination 2005 conducted by JNU, India.
- Qualified All India National Eligibility Test (NET) 2007, Examination India.
- Qualified GATE, (2006 AIR-546, 2007 AIR-381), India.
- Scholarship during M.Sc. from Department Of Biotechnology, Govt. Of India.
- Awarded JRF-Scholarship for pursuing PhD in life science by the Council of Scientific and IndustrialResearch, Government of India.
- Financial assistant to participate in ASCB conference in 2011 from Department of Biotechnology, Government of India and in 2013 from DST, Government of India.
- ASCB travel award 2013 for attending the 53rd Annual Meeting of American Society for Cell Biology.

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