Dr. Saurabh Awasthi

Ramalingaswami Fellow, Department of Biotechnology
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Research Interests: Nanopores (synthetic and biological), Single-molecule characterization, Protein misfolding and aggregation in aging-related neurodegeneration, Amyloid oligomers, Intrinsically disordered proteins, Developing biomarker sensing approaches for neurodegeneration, Identifying therapeutics to prevent amyloid toxicity, Disease associated post-translational modifications

Education

- **Ph.D.** (2012-2016)- SASTRA University, School of Chemical and Biotechnology, Thanjavur, Tamil Nadu, India.
- **M.Sc. Biotechnology** (2009-2011)- Bangalore University, Bangalore, India.
- **B.Sc. Biotechnology** (2006-2009)- Bangalore University, Bangalore, India.

Professional Experience

- **Ramalingaswami Fellow-** National Institute of Pharmaceutical Education and Research (NIPER-Raebareli), Lucknow, U.P. India
  (March 2023-present)

- **Group Leader-** Adolphe Merckle Institute, University of Fribourg, Fribourg-1700, Switzerland **(March 2022-Feb 2023)**

- **Postdoctoral Fellow-** Adolphe Merckle Institute, University of Fribourg, Fribourg-1700, Switzerland
  (August 2017-Feb 2022)

Awards/Honors

- Awarded **DBT-Ramalingaswami Fellowship,** 2022**.**
- Founder Chancellor's Award for the **Best Ph. D. Dissertation** in Sciences for the year 2016.
- Best out-going student award for "**First Rank**" in M.Sc. (2009-2011) batch.
- Nominated **Reviewer** for the area of **Non-Neuroimaging Biomarkers** by the Alzheimer's Association International Conference (AAIC) 2022, San Diego, USA.
- Invited to give a **lecture** for the Swiss NanoConvention 2022 in Fribourg, Switzerland.
- Invited as a **Speaker** for the 5th Edition of World Nanotechnology Conference, virtually, 2022.

- Invited as a **Speaker** for the Nanopore Weekly Meeting, virtually, 2023.

Sponsored Research Projects

1. Michael J. Fox Foundation for Parkinson's Research (MJFF), USA-17924 02/2020-04/2021
Role: **Co-PI**
Amount: USD 196,004 (INR 1.5 Cr) Status: Completed

2. Swiss National Science Foundation (SNSF)-CRSK-3_195960 04/2021-03/2022
Role: **PI**
Amount: CHF 99,772 (INR 80 Lakhs) Status: Completed

3. Department of Biotechnology-Ramalingaswami Fellowship, India (Starting from 2023)
Role: **PI**
Amount: INR 1.2 Cr (Fellowship + Research grant) Status: Not yet started

Key Technical Skills/Training

- Nanopores
- Single-molecule mass analysis (Mass photometry) of proteins
- Electron microscopy (TEM and SEM)
- Small and macromolecule crystallography
- Physiological (lipid-bilayer) and synthetic surface coatings
- DNA isolation, purification, and sequencing
- Protein purification, HPLC, FPLC
- Circular Dichroism (CD) Spectropolarimetry
- Liposomes

List of Publications (Total **19** Publications**+5 in preparation/review**, **9** Conference presentations and **4** workshops). (https://scholar.google.co.in/citations?user=_iHivQQAAAAJ&hl=en

Publications as Corresponding Author (3)

1. Saurabh Awasthi, Pongsatorn Sriboonpeng, Cuifeng Ying, Jared Houghtaling, Ivan Shorubalko, Sanjin Marion, Sebastian James Davis et al. Polymer Coatings to Minimize Protein Adsorption in Solid-State Nanopores. **Small Methods** 4, 11 (2020): 2000177. (**IF- 15.36**)
Abstract: Nanopore-based resistive-pulse recordings represent a promising approach for single-molecule biophysics with applications ranging from rapid DNA and RNA sequencing to "fingerprinting" proteins. Based on advances in fabrication methods, solid-state nanopores are increasingly providing an alternative to proteinaceous nanopores from living organisms; their widespread adoption is, however, slowed by nonspecific interactions between biomolecules and pore walls, which can cause artifacts and pore clogging. Although efforts to minimize these interactions by tailoring surface chemistry using various physisorbed or chemisorbed coatings have made progress, a straightforward, robust, and effective coating method is needed to improve the robustness of nanopore recordings. Here, covalently attached nanopore surface coatings are prepared from three different polymers using a straightforward "dip and rinse" approach and compared to each other regarding their ability to minimize nonspecific interactions with proteins. It is demonstrated that

polymer coatings approach the performance of fluid lipid coatings with respect to minimizing these interactions. Moreover, these polymer coatings enable accurate estimates of the volumes and spheroidal shapes of freely translocating proteins; uncoated or inadequately coated solid-state pores do not have this capability. In addition, these polymer coatings impart physical and chemical stability and enable efficient and label-free characterization of single proteins without requiring harsh cleaning protocols between experiments.

2. Saurabh Awasthi, Cuifeng Ying, Jiali Li, Michael Mayer, Simultaneous determination of size and shape of single α-synuclein oligomers in solution. **ACS Nano** (**In Revision)** (**IF-18.02**)
Abstract: Soluble oligomers of amyloid-forming proteins are implicated as toxic species in the context of several neurodegenerative diseases. Since the size and shape of these oligomers influences their toxicity, their biophysical characterization is essential for a better understanding of the structure-toxicity relationship. Amyloid oligomers are difficult to characterize by conventional approaches due to their heterogeneity in size and shape, their dynamic aggregation process, and their low abundance. This paper demonstrates that resistive-pulse measurements using polymer-coated solid-state nanopores enable single-particle level characterization of the size and shape of individual αSyn oligomers in solution within minutes. A comparison of the resulting size distribution with single-particle analysis by transmission electron microscopy and mass photometry reveals that nanopore-based characterization agrees well with both methods, while providing better size resolution and elucidating that αSyn samples are composed of stable oligomer sub-populations that contain multiples of approximately 12 monomers (i.e., 12-, 24-, 48-, 60-, 84-mers). Applying the unique capability of nanopores to approximate particle size and shape to picomolar concentrations of αSyn oligomers in the putatively toxic size range, revealed shapes that agree well with previous estimates by cryo-EM with the added advantage that nanopore-based analysis occurs rapidly, in solution, and has the potential to become a widely accessible technique.

3. Louise Bryan, Saurabh Awasthi, Yuanjie Li, Peter Niraj Nirmalraj, Jerry Yang, Michael Mayer, Site-specific C-terminal fluorescent labeling of Tau protein. **ACS Omega** 2022, 7, 50, 47009–47014 **(IF-4.13)**
Abstract: Formation of Tau protein aggregates in neurons is a pathological hallmark of several neurodegenerative diseases, including Alzheimer's disease. Fluorescently labeled Tau protein is therefore useful to study the aggregation of these pathological proteins and to identify potential therapeutic targets. Conventionally, cysteine residues are used for labeling Tau proteins; however, the full-length Tau isoform contains two cysteine residues in the microtubule-binding region, which are implicated in Tau aggregation by forming intermolecular disulfide bonds. To prevent the fluorescent label from disturbing the microtubule binding region, we developed a strategy to fluorescently label Tau at its C-terminus while leaving cysteine residues unperturbed. We took advantage of a Sortase A-mediated transpeptidation approach to bind a short peptide (GGGH6-Alexa647) with a His-tag and a covalently attached Alexa 647 fluorophore to the C-terminus of Tau. This reaction relies on the presence of a Sortase recognition motif (LPXTG), which we attached to the C-terminus of recombinantly expressed Tau. We demonstrate that C-terminal modification of Tau protein results in no significant differences between the native and C-terminally labeled Tau monomer with regard to aggregation kinetics, secondary structure, and fibril morphology.

Publications as First Author/co-author (18)

4. Ying, Cuifeng, Edona Karakaci, Esteban Bermudez-Urena, Alessandro Ianiro, Ceri Foster, Saurabh Awasthi, Anirvan Guha et al. Watching Single Unmodified Enzymes at Work. **ArXiv Preprint**

arXiv:2107.06407 (2021).

5. Saurabh Awasthi, R Preethy, NT Saraswathi, Nordihydroguaiaretic acid prevents glycation induced structural alterations and aggregation of albumin. **International Journal of Biological Macromolecule**. (2019) 87:1-6. (**IF-8.02**)
6. Cuifeng Ying, Jared Houghtaling, Olivia, Anirvan Guha, Peter Nirmalraj, Saurabh Awasthi, Jianguo Tian, Michael Mayer, Formation of single nanopores with diameters of 20-50 nm in silicon nitride membranes using laser-assisted controlled breakdown. **ACS Nano**. (2018) 14:1-9. (**IF-18.02**)
7. Saurabh Awasthi, Aravind Ravi and NT Saraswathi. Troxerutin imparts preservative effects on albumin by preventing Maillard reaction mediated early and advanced glycation modification. **Journal of Bimolecular Structure and Dynamics**. (2017) 14:1-9. (**IF-5.23**)
8. Saurabh Awasthi**,** Kamatchi Sankaranarayanan and NT Saraswathi, Advanced glycation end products induce differential structural modifications and fibrillation of albumin. **Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy**. (2016) 163:60-7. (**IF-4.83**)
9. Saurabh Awasthi and NT Saraswathi, Non-enzymatic glycation mediated structure–function changes in proteins: case of serum albumin. **RSC Advances**. (2016) 6 (93), 90739-90753. (**IF-4.03**)
10. Saurabh Awasthi and NT Saraswathi, Vanillin restrains non-enzymatic glycation and aggregation of albumin by chemical chaperon like function. **International Journal of Biological Macromolecule**. (2016) 87:1-6. (**IF-8.02**)
11. Saurabh Awasthi and NT Saraswathi "Carbonyl scavenging and chemical chaperon like function of essential amino acids attenuates non-enzymatic glycation of albumin. **RSC Advances.** (2016) 6, 24557-24564. (**IF-4.03**)
12. Saurabh Awasthi**,** NT Saraswathi, Sinigrin, a major glucosinolate from cruciferous vegetables restrains non-enzymatic glycation of albumin. **International Journal of Biological Macromolecule**. (2015) 83:410-5. (**IF-8.02**)
13. Saurabh Awasthi and NT Saraswathi, Silybin, a flavonolignan from milk thistles seeds restrains the early and advanced glycation end products modification of albumin. **RSC Advances**. (2016) 5: 87660-87666. (**IF-4.03**)
14. Saurabh Awasthi, NT Saraswathi, Elucidating the molecular interaction of sinigrin, a potent anticancer glucosinolate from cruciferous vegetables with bovine serum albumin: effect of methylglyoxal modification. **Journal of Biomolecular Structure and Dynamics**. (2015) 14:1-9. (**IF-5.23**)
15. Saurabh Awasthi, N Arul Murugan, NT Saraswathi, Advanced glycation end products modulate structure and drug binding properties of albumin. **Molecular Pharmaceutics**. (2015) 12: 3312-22. (**IF-5.36**)
16. Saurabh Awasthi, and NT Saraswathi. (2015), Crystal structure of Alanine-Copper (II) complex to understand the mechanism of salt induced prebiotic oligomerization of amino acids. **Crystal Research and Technology**, (2015) 50: 304-311. (**IF-1.59**)
17. Saurabh Awasthi, S. K. Gayathiri, R. Ramya, Duraichelvan R, Dhason A, and NT Saraswathi. Advanced glycation modified human serum albumin evokes alterations in membrane and eryptosis in erythrocytes. **Applied Biochemistry and Biotechnology**. (2015), 1013-1024. (**IF-3.09**)
18. Dhananjaya K., Sibi G., Mallesha H., Ravikumar KR, Saurabh Awasthi, *In silico* studies of daidzein and genistein with human estrogen receptor α. **Asian Pacific Journal of Tropical Biomedicine**. (2012), 2, S1747–S1753. (**IF-1.51**)
19. G Sibi, Saurabh Awasthi, K Dhananjaya, H Mallesha and KR Ravikumar, Comparative studies of Plumeria species for their phytochemical and antifungal properties against *Citrus sinensis* pathogens. **International Journal of Agricultural Research**, (2012), 7: 324-331.

Conference Abstract Publications

1. Saurabh Awasthi and Michael Mayer, Single particle mass photometry provides size distributions of tau oligomers in CSF samples revealing strong differences between samples from Alzheimer's patients and healthy controls. **Alzheimer's & Dementia (2022)**.
2. Edona Karakaci, Cuifeng Ying, Saurabh Awasthi, Esteban Bermuúdez-Urenña, Michael Mayer, Interrogating conformational dynamics of single-proteins in a plasmonic hotspot. **Biophysical Journal**, (2022), 121, 182a.
3. Yuanjie Li, Saurabh Awasthi, Louise Bryan, Peter N Nirmalraj, Michael Mayer, Sortase A-mediated site-specific labeling of Tau protein. **Biophysical Journal**, (2022), 121, 353a.
4. Saurabh Awasthi and Michael Mayer, Single-particle characterization of tau oligomers in solution. **Alzheimer's & Dementia (2021)** 17, e051821.
5. Jiali Li, C Ying, Saurabh Awasthi, T Kalkus, MC Acharjee, Michael Mayer, Protein Trapping in a Nanopore Well. **Biophysical Journal**. (2020) 118 (3), 157a.
6. Saurabh Awasthi, J Houghtaling, C Ying, A Fennouri, I Shorubalko, M Calame, MC Acharjee, J Li, Michael Mayer, Nanopores to Interrogate the Conformational Ensembles of Intrinsically Disordered Proteins on a Single-Molecule Level. **Biophysical Journal**. (2020) 118 (3), 214a.
7. Saurabh Awasthi, and NT Saraswathi, Understanding the mechanism of antidiabetic activity and efficacy of functional foods against advanced glycation end products: *Nigella sativa* and *Moringa oleifera*. **Planta Medica** (2013), 79-PN8.

Workshops

1. Workshop on "Big data analytics" organized by SASTRA University on December 6, 2014.
2. Workshop on "Application of x-ray crystallography for three-dimensional structure determination" organized by SASTRA University, Thanjavur on 27th November 2014.
3. Workshop on "Computational chemistry and bio-molecular simulations" organized by SASTRA University on 9th December 2013.
4. Workshop on "Ligand-protein docking and computer-aided drug design" organized by Swiss Institute of Bioinformatics on 3-5 November 2020, Lausanne, Switzerland.

Professional Memberships

1. Alzheimer's Association International Society to Advance Alzheimer's Research and Treatment (ISTAART)- Annual
2. Biophysical Society (BPS) (www.biophysics.org)- Annual
3. Indian Society of Chemists and Biologists (ISCB)- Lifetime
4. Michael J. Fox Foundation's "Parkinson's Disease Research Exchange Consortium."

Journal Reviewing & Editing

Reviewer- Frontiers in Pharmacology (IF-5.81)

Chemistry: An Asian Journal-Wiley (IF- 4.56)

Medicinal Chemistry Research-Springer (IF-1.96)

Frontiers in Nutrition (IF-6.59)

Research Topic Editor- Frontiers in Molecular Biosciences (IF-5.24)