BIOGRAPHICAL SKETCH

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NAME: Afrin, Shumaila

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

POSITION TITLE: Postdoctoral researcher

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)	END DATE MM/YYYY	FIELD OF STUDY
Aligarh Muslim University, Aligarh	BS	2012	Bichemistry
Aligarh Muslim University, Aligarh	MS	2014	Biochemistry
Aligarh Muslim University, Aligarh	PHD	2020	Biochemistry
UTSW Medical Center, Dallas, Texas	Postdoctoral Fellow	present	Structural determination of amyloid fibrils of systemic amyloidosis

A. Personal Statement

I am a postdoctoral researcher at the Centers for Alzheimer's and Neurodegenerative Diseases, University of Texas Southwestern Medical Center in the lab of Prof. Lorena Saelices. In the Saelices lab, our research focuses primarily on understanding the biopathology of transthyretin amyloidosis using structural tools like cryo-electron microscopy (cryo-EM). We also use the structural information to design diagnostic and therapeutic tools for ATTR amyloidosis. Transthyretin amyloidosis is a debilitating disease with current treatment ineffective at the late stage of the disease. Leveraging my biophysical background, my work in the Saelices lab is focused on understanding the structural variabilities associated with ATTR amyloid fibrils and their correlation with disease progression and/or phenotype. I have spent over a year optimizing amyloid fibril extraction and cryo-EM sample preparation technique. I also have experience with cryo-EM data processing software such as RELION and cryosparc and am actively involved in helical reconstruction of amyloid fibrils from ATTR patients. Our recent work demonstrates that structural polymorphism exists in ATTR amyloidosis at both patient and mutation levels. We are now determining the extent of this variability and the role of tissue type in morphological variabilities of these fibrils.

1. Nguyen,Binh A.,, Afrin,Shumaila,, Singh,Virender,, Ahmed,Yasmin,, Pedretti,Rose,, Fernandez-Ramirez,Maria Del Carmen,, Benson,Merrill D.,, Sawaya,R. Michael,, Cao,Qin,, Boyer,David,, Pope,Alexander,, Wydorski,Pawel M.,, Chhapra,Farzeen,, Eisenberg,David S.,, Saelices,Lorena,. Structural polymorphism of amyloid fibrils in cardiac ATTR amyloidosis revealed by cryo-electron microscopy. [Preprint]. 2022 June 21. DOI: 10.1101/2022.06.21.496949

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

2021 - Postdoctoral researcher, UTSW Medical Center, Dallas, TX
2015 - 2019 Ph.D research fellow, Aligarh Muslim University, aligarh

Honors

2017 - 2019 Senior Research Fellowship Award, Council of Scientific and Industrial Research (CSIR) Govt. of India

2015 - 2017 Junior Research Fellowship Award, Council of Scientific and Industrial Research (CSIR) Govt. of India

2012 - 2014 Prof. Abdul Majid Siddiqui award, Dept. of Biochemistry, AMU

2012 - 2014 M.Sc Builder fellowship, Department of Biotechnology (DBT) Govt. of India

C. Contribution to Science

1. Understanding the structural heterogeneity of transthyretin amyloidosis (ATTR) fibrils using cryo-electron microscopy. For the last 1 year, I have been actively involved in the structural determination of ATTR amyloid fibrils extracted from the heart of ATTR patients. In our recent study(1) we observe that structural polymorphism exists in ATTR amyloidosis both at the patient level (as we observed in the case of ATTR V122I mutation) as well as at the mutation level (in the case of I84S mutation), suggesting that several factors including the mutation and the patient body environment play a role in the formation of specific polymorphs in ATTR. We are now exploring structural polymorphism in a larger sample size of mutations and patients to determine the correlation between disease phenotype and specific structural morphologies. We are also exploring how these changes occur at the organ level by looking at ATTR fibrils extracted from different organs of ATTR patients.

    a. Nguyen,Binh A.,, Afrin,Shumaila,, Singh,Virender,, Ahmed,Yasmin,, Pedretti,Rose,, Fernandez-Ramirez,Maria Del Carmen,, Benson,Merrill D.,, Sawaya,R. Michael,, Cao,Qin,, Boyer,David,, Pope,Alexander,, Wydorski,Pawel M.,, Chhapra,Farzeen,, Eisenberg,David S.,, Saelices,Lorena,. Structural polymorphism of amyloid fibrils in cardiac ATTR amyloidosis revealed by cryo-electron microscopy. [Preprint]. 2022 June 21. DOI: 10.1101/2022.06.21.496949

2. As part of my early graduate work, I studied how cardiovascular drugs, specifically antiplatelet drugs ticlopidine and dipyridamole interact with transport protein serum albumin. The binding of a drug to serum albumin affects its pharmacological properties; therefore, an optimum binding affinity of drug to serum albumin is essential for its therapeutic efficacy. Moreover, drugs that bind to a common binding site on serum albumin can compete for the binding site and alter their therapeutic efficacy. I determined the binding affinity and site of ticlopidine and dipyridamole on serum albumin. Additionally, I characterized the thermodynamic parameters of the interaction. My studies also suggested that ticlopidine, at its therapeutically relevant doses, may displace bilirubin from serum albumin, which may be one of the mechanisms through which ticlopidine induced hyperbilirubinemia. I also collaborated on the study that characterized the binding site and affinity of gastrointestinal disorder drugs pirenzipine and nizatidine on serum albumin.

    a. Afrin S, Rahman Y, Alhaji Isa M, Ahmed S, Tabish M. Biophysical insights into the binding characteristics of bovine serum albumin with dipyridamole and the influence of molecular interaction with β cyclodextrin. J Biomol Struct Dyn. 2020 Jul;38(10):3046-3058. PubMed PMID: 31366288.
    b. Afrin S, Rahman Y, Tabish M. Elucidating the interaction of ticlopidine with serum albumin and its role in bilirubin displacement in vitro. J Biomol Struct Dyn. 2019 Mar;37(4):863-876. PubMed PMID: 29513159.

3. My research also explored the repurposing potential of antiplatelet rug ticlopidine by studying its interaction with DNA. Small molecules such as drugs interact with DNA mostly by binding within the grooves of DNA helices or by interacting within the basepairs. As part of a repurposing study, ticlopidine was found to inhibit Methicillin-resistant Staphylococcus aureus in synergism with antibiotics by decreasing the expression of TarO gene. My preliminary research suggested that ticlopidine, like distamycin, binds to the minor grooves of DNA in the AT-rich regions and, therefore, may disrupt transcription factor-DNA complexes, and this may explain the decrease in gene

expression observed. I also collaborated on studying the interaction between diflunisal and DNA using a similar approach. Moreover, I contributed to a study on the toxicity of iron oxide nanoparticles and its attenuation by thymoquinone both invitro and in vivo. I assessed how iron oxide nanoparticles, which have a wide range of applications, interact with DNA as well as the genotoxic implications of the interactions. My research on drug DNA interactions has contributed to developing a fundamental body of thermodynamic and structural information that can be used for the rational design of DNA binding drugs.

a. Ansari MO, Parveen N, Ahmad MF, Wani AL, Afrin S, Rahman Y, Jameel S, Khan YA, Siddique HR, Tabish M, Shadab GGHA. Evaluation of DNA interaction, genotoxicity and oxidative stress induced by iron oxide nanoparticles both in vitro and in vivo: attenuation by thymoquinone. Sci Rep. 2019 May 6;9(1):6912. PubMed Central PMCID: PMC6502885.
b. Afrin S, Rahman Y, Sarwar T, Husain MA, Ali A, Shamsuzzaman, Tabish M. Molecular spectroscopic and thermodynamic studies on the interaction of anti-platelet drug ticlopidine with calf thymus DNA. Spectrochim Acta A Mol Biomol Spectrosc. 2017 Nov 5;186:66-75. PubMed PMID: 28614751.
c. Rahman Y, Afrin S, Husain MA, Sarwar T, Ali A, Shamsuzzaman, Tabish M. Unravelling the interaction of pirenzepine, a gastrointestinal disorder drug, with calf thymus DNA: An in vitro and molecular modelling study. Arch Biochem Biophys. 2017 Jul 1;625-626:1-12. PubMed PMID: 28558964.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
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NAME: Lorena Saelices Gómez

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

POSITION TITLE: Assistant Professor

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
University of Seville, Spain	B.Sc.	10/2005	Biology
University of Seville, Spain	Ph.D.	12/2010	Biochemical Sciences
University of California, Los Angeles	Postdoctoral Training	05/2014	Structural Biology, Amyloid
ETH Zürich, Switzerland	Postdoctoral Training	05/2015	Structural Biology, Amyloid

A. Personal Statement

My personal goal is to contribute to the understanding of the molecular basis of amyloid diseases to design effective and safe clinical tools. The focus of my laboratory is the development of peptide-based tools to inhibit or detect amyloid fibrils, with special attention to transthyretin amyloidosis (ATTR) and Alzheimer's disease (AD). ATTR is a degenerative disease characterized by the formation of amyloid fibrils made of transthyretin (TTR). These fibrils accumulate in almost every tissue, leading to organ dysfunction and death. The current treatments for familial cases are liver transplantation and protein stabilizers but are not sufficient to stop disease progression. My first study on ATTR resulted in the identification of the segments responsible for TTR aggregation and the determination of their amyloid structure by x-ray micro-crystallography (*a*). Based on the crystal structure of these segments in their amyloid form, we were able to design efficient inhibitors. I also found that *ex-vivo* ATTR amyloid fibrils catalyze fibril growth of wild-type TTR (*b*), explaining clinical observations of continuing cardiac amyloid deposition after liver transplantation. It was exciting to find that our peptide inhibitors block this seeding process. To our surprise, we found that drugs now in the clinic are not effective at halting amyloid seeding (*c*). Consistent with this, the tetramer stabilizer tafamidis does not exert significant effects when administered at late stages of ATTR. I also found that treating ATTR *Drosophila* models with peptide inhibitors rescues the healthy phenotype and reduces TTR deposition. My studies may open an avenue to novel therapeutic and diagnostic strategies (included in the International Patent Application No. PCT/US17/40103).

Although I recently started my new laboratory as an Assistant Professor at UT Southwestern, I have personally managed the progress of my research and directed a team of students and technicians for the past 9 years. I have secured funding with a prestigious DP2 New Innovator Award from the NIH, a Career Development Award from the American Heart Association, and institutional awards from UTSW. The work of my team has led to the publication of seven original papers as well as collaborative manuscripts, multiple presentations in conferences and events, and one patent. The methodological approaches and training that I acquired in my career include biochemical analysis of proteins and nucleic acids, cell biology, structure determination and analysis by cryo-electron microscopy, x-ray crystallography, and NMR, peptide design, amyloid fibril extraction, tissue staining (immunohistochemistry and die staining), fluorescence and confocal microscopy, and animal

research, among other techniques. My laboratory has determined the structures of eight ATTR fibrils extracted from patients and is finishing 4 more using cryo-electron microscopy (*d*).

Multiple collaborations have fueled the progress of my research, including well-established investigators, doctors, and professors from national and international institutions. I have had the pleasure to work with remarkable international experts such as Profs. Gunilla Westermark and Per Westermark at the University of Uppsala, Sweden, Dr. Teresa Coelho at Hospital Geral de Santo António, Porto, Portugal, or Dr. Johan Bijzet at the University Medical Center in Groningen, The Netherlands. National collaborations include Dr. Merrill Benson and his team at the University of Indiana, as well as Profs. Jeffery Kelly and Joel Buxbaum and their laboratories at the Scripps Institute in San Diego. The enviable environment at UCLA allowed me to work with outstanding professionals, such as Prof. Julian Whitelegge, Prof. Kym Faull, Dr. Sarah Dry, Prof. Kenneth Roos, and Prof. Masakazu Kamata (now at the University of Alabama, Birmingham) to name a few. At UT Southwestern I have found incredible support, starting from the Director of the Center for Alzheimer's and Neurodegenerative Diseases (CAND), Prof. Marc Diamond, and other Faculty mates such as Profs. Sarah Shahmoradian, Haiyang Yu, Lukasz Joachimiak, and Rachel Bailey. I feel fortunate to be part of UT Southwestern Medical Center, a multidisciplinary institution that offers the perfect settings for the current proposal to succeed.

Citations:

a. **Saelices L,** Johnson LM, Liang WY, Sawaya MR, Cascio D, Ruchala P, Whitelegge J, Jiang L, Riek R, Eisenberg DS (2015). Uncovering the Mechanism of Aggregation of Human Transthyretin. *J. Biol. Chem*, 290(48), 28932-43. PMC4661406

b. **Saelices** L, Chung K, Lee JH, Cohn W, Whitelegge JP, Benson MD, Eisenberg DS (2018). Amyloid seeding of transthyretin by ex vivo cardiac fibrils and its inhibition. *Proc Natl Acad Sci U S A* 115, no. 29: E6741–50

c. **Saelices L**, Nguyen BA, Chung K, Wang Y, Ortega A, Lee JH, Coelho T, Bijzet J, Benson MD, Eisenberg DS (2019). A pair of peptides inhibits seeding of the hormone transporter transthyretin into amyloid fibrils. *J Biol Chem*, 294:6130-6141.

d. Nguyen BA, Afrin S, Singh V, Ahmed Y, Pedretti R, Fernandez-Ramirez MC, Benson MD, Sawaya MR, Cao Q, Boyer D, Pope A, Wydorski PM, Chhapra F, Eisenberg DS, **Saelices L** (2022). Structural polymorphism of amyloid fibrils in cardiac ATTR amyloidosis revealed by cryo-electron microscopy. bioRxiv 2022.06.21.496949; doi: https://doi.org/10.1101/2022.06.21.496949

Ongoing and recently completed projects that I would like to highlight include:

Welch Foundation Research Award
Lorena Saelices Gómez (PI)
06/01/2022 - 05/31/2025
Cryo-EM study of the conformational switch of monomeric tau that drives its amyloid aggregation

NIH New Innovator Award - DP2-HL163810-01
Lorena Saelices Gómez (PI)
09/01/2021-08/31/2026
Closing the gap between structural biology and translational science for amyloid diseases

American Heart Association - Career Development Award # 847236
Lorena Saelices Gómez (PI)
07/2021 - 06/2024
Study of the phenotypic variability of cardiac amyloidosis to develop peptide-based diagnostic tools

UT Southwestern Start-up Funds
Lorena Saelices Gómez (PI)
06/08/2020 - 06/07/2028
The study of structural and mechanistic basis for self-assembling amyloid proteins

Distinguished Researcher Award from the President's Research Council

B. Positions, Scientific Appointments, and Honors

Positions

2020-present	Assistant Professor, Amyloid Diseases, Center for Alzheimer's and Neurodegenerative Diseases, UT Southwestern Medical Center
2018-2020	Associate Project Scientist, Amyloidosis, Transthyretin, UCLA
2015-2018	Asst. Project Scientist, Structural Biology, Amyloid (David Eisenberg), UCLA
2014-2015	Postdoc, Structural Biology, Amyloid (Roland Riek), ETH Zürich
2012-2014	Postdoc, Structural Biology, Amyloid (David Eisenberg), UCLA
2011-2012	Postdoc, Enzymology, Structural Biology, Biochemistry, (Fco. Javier Florencio Bellido), University of Seville, Spain

Honors and awards

2022	Welch Foundation Research Award
2021	Distinguished Researcher Award from the President's Research Council
2019	Molecular Biology Institute Productivity Award
2019	The Company of Biologist Award for Scientific Meetings to organize the 2019 FASEB NextGen
2019	FASEB NextGen Award
2018	UCLA Clinical and Translational Science Institute (CTSI) Voucher Award
2018	Travel Award for The XVIth International Society of Amyloidosis 2018 Symposium.
2018	Recognized for outstanding Research and Contributions in the Subject of Neurobiology of Disease
2017	Amyloidosis Foundation David C. Seldin, MD, Ph.D. Memorial Research Grant, 2017 and 2018
2016	The Company of Biologist Award for Scientific Meetings to organize the 2016 Gordon Research Seminar
2016	Amyloidosis Foundation Research Grant, 2016
2016	Travel Award for The XVth International Society of Amyloidosis 2016 Symposium.
2016	Presentation Prize at The XVth International Society of Amyloidosis 2016 Symposium.
2012	Marie-Curie Fellowship for Postdoctoral Studies from People Programme (Marie Curie Actions) of the European Union's Seventh Framework Programme (FP7/2007-2013)
2009	Travel Fellowship for Ph.D. studies, awarded by the Government of Spain
2007	Travel Fellowship for Ph.D. studies, awarded by the Government of Spain
2006	FPI Fellowship for Ph.D. studies, awarded by the Government of Spain
2005	Honorary Collaborator of the Dept. of Genetics of University of Seville, Spain
2004	Fellowship for Undergraduate Research Training, awarded by the Government of Spain
2001	Yearly Scholarships for Undergraduate Studies, awarded by the Government of Spain

C. Contributions to Science

1. Identification of the gene responsible for cleavage of torulene in the carotenoid pathway of *Neurospora crassa*. My undergrad studies focused on the molecular mechanisms that control the production of secondary metabolites of biotechnological interest in fungi. I was assigned to lead a small research project focused on the regulation of carotenoid biosynthesis in the ascomycete *Neurospora crassa*, and its connections with the production of other secondary metabolites. This study resulted in the publication of my first paper (paper *a*), which describes the identification of *cao2*, the gene responsible for the cleavage of torulene in the carotenoid pathway. Carotenoids have attracted the attention of the industries and researchers because of their versatility. The characterization of the genetic pathway responsible for the production of carotenoids is of critical importance for future biotechnological applications, such as production of vitamin A precursors, food

coloring, and antioxidants. My work described in paper *a* marked the beginning of subsequent studies such as the one described in paper *b*, and it was included in many other manuscripts such as the review *c.*

a. **Saelices L**, Youssar L, Holdermann I, Al-Babili S, Avalos J. (2007). Identification of the gene responsible for torulene cleavage in the Neurospora carotenoid pathway. *Mol Genet Genomics,* 278(5), 527-37. PMID: 17610084
b. Estrada AF, Youssar L, Scherzinger D, Al-Babili S, Avalos J. (2008). The ylo-1 gene encodes an aldehyde dehydrogenase responsible for the last reaction in the Neurospora carotenoid pathway. *Mol. Microbiology,* 69(5), 1207-20. PMID: 18627463
c. Avalos J, Estrada AF. (2010). Regulation by light in Fusarium. *Fungal Genetics and Biology,* 47(11), 930-8. PMID: 20460165

2. Characterization of the enzymatic regulation of glutamine synthetase by protein-protein interaction in cyanobacteria. During my predoctoral period, my interest focused on enzymatic regulation by protein-protein interaction in cyanobacteria. Glutamine synthetase is a key enzyme of the metabolism of nitrogen that is finely regulated by up to two inactivating factors IF7 and IF17 in cyanobacteria. In paper *a,* we identified the binding site in IF7 and IF17 responsible for the GS regulation in *Synechocystis*. This work was followed up by paper *c,* a structural and biochemical analysis of the enzyme that describes the identification and characterization of the regulatory core of glutamine synthetase. With the support of a travel award during my Ph.D., an exhaustive training in structure analysis by x-ray crystallography in the lab of Prof. David Eisenberg at UCLA resulted in the determination of the atomic structure of the GS from *Synechocystis*, included in paper *c*. In paper *b,* we analyzed the inactivating factor IF17 by NMR and found it to be partially unfolded in its native state. This work was followed up by the biophysical study of both IF7 and IF17 in the presence of the enzyme, as shown in paper *d.* The analysis of IF7 and IF17 by NMR revealed that these unfolded proteins fold upon binding to the GS. For more publications, please visit my NCBI bibliography in the link above.

a. **Saelices L**, Galmozzi CV, Florencio FJ, Muro-Pastor MI. (2011). Mutational analysis of the inactivating factors, IF7 and IF17 from Synechocystis sp. PCC 6803: critical role of arginine amino acid residues for glutamine synthetase inactivation. *Mol Microbiology,* 82(4), 964-75. PMID: 22023175.
b. **Saelices L**, Galmozzi CV, Florencio FJ, Muro-Pastor MI, Neira JL. (2011). The inactivating factor of glutamine synthetase IF17 is an intrinsically disordered protein, which folds upon binding to its target. *Biochem,* 50(45), 9767-78. PMID: 21992216
c. **Saelices L,** Robles-Rengel R, Muro-Pastor MI, Florencio FJ. (2015). A core of three amino acids at the carboxyl-terminal region of glutamine synthetase defines its regulation in cyanobacteria. *Mol Microbiology,* 96(3), 483-96. PMID: 25626767
d. Pantoja-Uceda D, Neira JL, **Saelices L**, Robles-Rengel R, Florencio FJ, Muro-Pastor MI, Santoro J. (2016). Dissecting the Binding between Glutamine Synthetase and Its Two Natively Unfolded Protein Inhibitors. *Biochemistry*, *55*(24), 3370-82. PMID: 27232663

3. Structural study of ATTR fibrils and structure-based design of specific peptide-inhibitors. My postdoctoral studies aimed to characterize the mechanism of protein aggregation that leads to the formation of amyloid fibrils by the human blood protein transthyretin (TTR) causing transthyretin amyloidosis (ATTR). ATTR is characterized by the abnormal formation of amyloid fibrils mainly made of TTR, which accumulates in almost every organ thereby leading to organ dysfunction and death. The current treatments for ATTR are not sufficient to stop disease progression when intervened at late stages. My research furthers the understanding of the molecular basis of TTR aggregation and proposes a tentative explanation of this lack of efficacy at late stages. Paper *a* describes the identification of the TTR segments responsible for the formation of amyloid fibrils. Based on the crystal structure of these segments in their amyloid form, we were able to design efficient inhibitors (paper *a*). We discovered that fibrils present in the organs of ATTR patients can catalyze, or *seed*, fibril formation of soluble TTR, thereby accelerating deposition (paper *b*). Our peptide inhibitors inhibited this seeded fibril, whereas TTR stabilizers used currently in the clinic do not hinder this process (paper *c*). These findings provide an explanation to the lack of efficacy of TTR stabilizers when administered at late stages of ATTR, whose progression may be driven by amyloid seeding of preformed fibrils rather than *de novo* nucleation of soluble TTR. I studied these peptide inhibitors in two Drosophila models of ATTR, showing a significant reduction of TTR deposition and an improvement of overall mobility. Our peptide inhibitors represent a novel therapeutic strategy that fills a gap in the field of amyloidosis, by

targeting amyloid seeding, and are included in International Patent - Application No. PCT/US17/40103. Finally, the most recent study of my laboratory reveals unprecedented structural polymorphism of amyloid fibrils extracted from ATTR amyloidosis patients (paper *d*)

a. **Saelices L,** Johnson LM, Liang WY, Sawaya MR, Cascio D, Ruchala P, Whitelegge J, Jiang L, Riek R, Eisenberg DS (2015). Uncovering the Mechanism of Aggregation of Human Transthyretin. *J. Biol. Chem*, 290(48), 28932-43. PMC4661406
b. **Saelices** L, Chung K, Lee JH, Cohn W, Whitelegge JP, Benson MD, Eisenberg DS (2018). Amyloid seeding of transthyretin by ex vivo cardiac fibrils and its inhibition. *Proc Natl Acad Sci U S A* 115, no. 29: E6741–50
c. **Saelices L**, Nguyen BA, Chung K, Wang Y, Ortega A, Lee JH, Coelho T, Bijzet J, Benson MD, Eisenberg DS (2019). A pair of peptides inhibits seeding of the hormone transporter transthyretin into amyloid fibrils. *J Biol Chem*, 294:6130-6141.
d. Nguyen BA, Afrin S, Singh V, Ahmed Y, Pedretti R, Fernandez-Ramirez MC, Benson MD, Sawaya MR, Cao Q, Boyer D, Pope A, Wydorski PM, Chhapra F, Eisenberg DS, **Saelices L** (2022). Structural polymorphism of amyloid fibrils in cardiac ATTR amyloidosis revealed by cryo-electron microscopy. Preprint available on bioRxiv 2022.06.21.496949; doi: https://doi.org/10.1101/2022.06.21.496949

4. Inhibition of amyloid-beta aggregation by naturally occurring peptides in Alzheimer's disease models. My recent studies focused on the inhibition of aggregation of amyloid-beta peptides by naturally occurring peptides, derived from neuroprotective molecules, such as TTR. As explained above, TTR is an amyloidogenic protein that causes systemic ATTR. However, TTR can also serve as a neuroprotective agent in the brain. Previous studies have shown that TTR binds to amyloid-beta, inhibits its aggregation, and exerts neuroprotection in animal models. In my recent studies, I found that the tetrameric functional form of TTR needs to dissociate into monomers to exert such protection (*a*). Based on structural analysis, I identified the minimum segment of TTR that can mimic its inhibitory effect. Now I aim to use this information to develop therapeutic options for Alzheimer's disease patients.

a. Cao Q, Anderson DH, Liang W, Chou J, **Saelices** L. (2020). The inhibition of cellular toxicity of amyloid-beta by dissociated transthyretin. *JBC*, 295(41):14015-14024.
Preprint available on *BioRxiv*. https://doi.org/10.1101/852715

Full list of my published work:
https://www.ncbi.nlm.nih.gov/myncbi/lorena.saelices%20gomez.1/bibliography/public/

BIOLOGICAL SKETCH

NAME: Binh Nguyen

POSITION TITLE: Lab Instructor

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	Date	FIELD OF STUDY
University of Otago, New Zealand	B.Pharm.	03/2002 - 05/2005	Pharmacy
University of Otago, New Zealand	Ph.D.	07/2007 - 05/2012	Molecular microbiology
School of Medicine, Vietnam National University of Hochiminh city, Viet Nam	Lecturer training program	05/2013 - 10/2013	Biochemistry in medicine

A. Personal Statement

I am currently working at Centers for Alzheimer's and Neurodegenerative Diseases, University of Texas Southwestern Medical Centers. My main research objective is to investigate the mechanism of systemic amyloid formations using cryogenic electron microscopy. 3 years into this field, I have mastered several laboratory skill-sets including protein analysis using biochemical tools, electron microscopy, cryo-electron microscopy, amyloid fibrils extraction, helical reconstruction, model building and validation. In addition to my previous laboratory experience such as biochemical skills in DNA manipulation, protein expression and purification, cell biology in general, and a basic level of x-ray crystallography and protein structural determination, I am very confident that my research aim is within reached. Here are my recent publications:

(1) **Binh A. Nguyen**, Shumaila Afrin, Virender Singh, Yasmin Ahmed, Rose Pedretti, Maria Del Carmen Fernandez-Ramirez, Merrill D. Benson, R. Michael Sawaya, Qin Cao, David Boyer, Alexander Pope, Pawel M. Wydorski, Farzeen Chhapra, David S. Eisenberg, Lorena Saelices (2022). Structural polymorphism of amyloid fibrils in cardiac ATTR amyloidosis revealed by cryo-electron microscopy. Biorxiv, https://doi.org/10.1101/2022.06.21.496949.

(2) Abskharon, R., Sawaya, M. R., Boyer, D. R., Cao, Q., **Nguyen, B. A.**, Cascio, D., & Eisenberg, D. S. (2022). Cryo-EM structure of RNA-induced tau fibrils reveals a small C-terminal core that may nucleate fibril formation. Proceedings of the National Academy of Sciences, 119(15), e2119952119.

(3) Cao, Q., Boyer, D. R., Sawaya, M. R., Abskharon, R., Saelices, L., **Nguyen, B. A.**, et al & Eisenberg, D. S. (2021). Cryo-EM structures of hIAPP fibrils seeded by patient-extracted fibrils reveal new polymorphs and conserved fibril cores. Nature Structural & Molecular Biology, 28(9): 724-730.

(4) Saelices, L., **Nguyen, B.A.**, Chung, K., Wang, Y., Ortega, A., Lee, J.H., Coelho, T., Bijzet, J., Benson, M.D. and Eisenberg, D.S., (2019). A pair of peptides inhibits seeding of the hormone transporter transthyretin into amyloid fibrils. *Journal of Biological Chemistry*, 294(15), pp.6130-6141.

B. Positions and Honors

Positions

12/2011 – 06/2012	Research Assistant, Molecular Microbiology Lab, Department of Oral Sciences, University of Otago, New Zealand.
10/2013 – 08/2017	Medical Biochemistry Lecturer, Biochemistry and Molecular Biology Department, School of Medicine, Vietnam National University of Hochiminh city, Viet Nam.
2014 – 2017	Collaborated researcher at the Research Center of Genomics and Reproductive Health, School of Medicine, Vietnam National University of Hochiminh city, Viet Nam.
2017 – 2020	Research Staff Associate, Chemical Biology Department, University of California Los Angeles, CA, USA.
2020 – now	Lab Instructor, Center of Alzheimer's and Neurodegenerative Disease, UTSW, Dallas, TX, USA.

Honors

2002	Recognition for academic achievement in Chemistry, an offer to study the Bachelor of Science with Honours degree in Chemistry, University of Otago.
2016	Appreciation Award for organizing the Science and Medicine Conference 2-16, VNU-HCM, Vietnam.
2017	Recognition Award for organizing the international meeting on "Clinical Research and Clinical Trials 2017" between School of Med, VNU-HCM and Korea University, Korea.
2016 & 2017	Outstanding Contribution Awards at Institutional level, School of Medicine, VNU-HCM, Vietnam.

C. Contributions to Science

1. Optimizing the concentration of a pair of peptides inhibiting the formation of transthyretin (TTR) amyloid fibrils *in-vivo*. From previous publications, Dr. Lorena Saelices and colleagues have identified the segment from TTR protein which is responsible for the aggregation of amyloid fibrils. The team was then able to design and optimize the corresponding peptide inhibitors. The peptides have been shown to effectively inhibit amyloid formation using the recombinant TTR seeded by *ex-vivo* ATTR fibrils, whereas commercially available TTR tetramer stabilizers were not effective (4).

2. Application of cryoEM, helical reconstruction and model building to determine amyloid structures. For the last 2 years, we were able to solve seven structures of transthyretin fibrils. Based on these structural details, we are developing diagnostic tools with high specificity and sensitivity to early diagnose ATTR amyloidosis patients; we are also investigating the drug to treat these fibrils in ATTR amyloidosis patient at late stage (1).

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. DO NOT EXCEED FIVE PAGES.

NAME: Singh, Virender

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

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)	START DATE MM/YYYY	COMPLETION DATE MM/YYYY	FIELD OF STUDY
Ambala College of Engineering & Applied Research, Ambala, India	B.TECH	07/2004	12/2008	Biotechnology
Indian Institute of Technology Kanpur, Kanpur, Uttar Pradesh, India	M.TECH (joint with PhD)	07/2011	06/2019	Biophysics
Indian Institute of Technology Kanpur, Kanpur, Uttar Pradesh, India	PhD	07/2011	06/2019	Biophysics and Structural Biology
Case Western Reserve University, Cleveland, Ohio, USA	Postdoctoral Scholar	02/2019	present	Biophysics and Structural Biology

A. Personal Statement

The focus of the current project is to understand the structural heterogeneity of ATTR aggregates implicated in TTR related cardiomyopathy amyloidosis and polyneuropathy.Earlier I worked on understanding role of tau aggregate polymorphism in tauopathies including Alzheimer's disease. The specific aim is to ascertain connection between presence of cofactors and pathological mutations and aggregate structural polymorphism. The project involves expression and purification of tau variant dGAE, seeding with tau aggregates isolated form AD and FTDP-17 patient brain tissue, aggregation kinetics using Thioflavin T assay, aggregate morphology with atomic force microscopy and structure elucidation using mass spectrometry and cryo-electron microscopy (cryo-EM). This work will be done under the supervision of Prof. Witold Surewicz at Case Western Reserve University. This research will utilize my training in protein expression and purification, atomic force microscopy and mass spectrometry (backbone hydrogen/deuterium (H/D) exchange, histidine H/D exchange and hydroxyl radical footprinting). I have enhanced the method to isolate tau aggregates from patient brain tissue. Currently I am optimizing cryo-EM conditions to elucidate the structure of the brain derived as well as dGAE/dGAE P301L tau aggregates. I have extensive experience working on intrinsically disordered proteins including tau. At the start of my postdoctoral training, I worked on a project looking at the implication of metal ions, specifically zinc, on the liquid-liquid phase separation behavior of tau, now published in the *Journal of Biological Chemistry*. During my PhD, I worked on polyglutamine protein aggregation implicated in Huntington's disease. To accomplish my research goals, I have incorporated concepts of biophysics, structural biology (NMR, fluorescence, infrared and circular dichorism spectroscopy), analytical chemistry as well as chemical biology and biochemistry. I have undergone rigorous training during my PhD, continuing on in my postdoc. Hence, I am confident that I will be able to address the questions raised in this research proposal and I look forward to achieving them and furthering my scientific goals.

1. BA Nguyen, S Afrin, **V Singh**, Y Ahmed, R Pedretti et al. Structural polymorphism of amyloid fibrils in cardiac ATTR amyloidosis revealed by cryo-electron microscopy. **bioRxiv, 2022.** **doi: https://doi.org/10.1101/2022.06.21.496949**

2. **Singh V**, Xu L, Boyko S, Surewicz K, Surewicz WK. Zinc promotes liquid-liquid phase separation of tau protein. *J Biol Chem*. 2020 May 1;295(18):5850-5856. PubMed PMID: 32229582; PubMed Central PMCID: PMC7196643.
3. **Singh V**, Patel KA, Sharma RK, Patil PR, Joshi AS, Parihar R, Athilingam T, Sinha N, Ganesh S, Sinha P, Roy I, Thakur AK. Discovery of Arginine Ethyl Ester as Polyglutamine Aggregation Inhibitor:

Conformational Transitioning of Huntingtin N-Terminus Augments Aggregation Suppression. *ACS Chem Neurosci*. 2019 Sep 18;10(9):3969-3985. PubMed PMID: 31460743.

4. **Singh V**, Deepak RNVK, Sengupta B, Joshi AS, Fan H, Sen P, Thakur AK. Calmidazolium Chloride and Its Complex with Serum Albumin Prevent Huntingtin Exon1 Aggregation. *Mol Pharm*. 2018 Aug 6;15(8):3356-3368. PubMed PMID: 29979597.
5. **Singh V**, Sharma RK, Athilingam T, Sinha P, Sinha N, Thakur AK. NMR Spectroscopy-based Metabolomics of Drosophila Model of Huntington's Disease Suggests Altered Cell Energetics. *J Proteome Res*. 2017 Oct 6;16(10):3863-3872. PubMed PMID: 28871787.

B. Positions and Honors

Positions and Employment

2008 - 2010 Executive (Quality Control), Biocon, Bangalore, India
2016 - 2017 Research Associate , Indian Institute of Technology Kanpur, Kanpur
2019 - Postdoctoral Scholar , Case Western Reserve University, Cleveland, OH

Other Experience and Professional Memberships

2012 - 2012 Co-coordinator, Annual Department Foundation Day Symposium Biological Sciences and Bioengineering, Indian Institute of Technology Kanpur, India
2015 - 2017 Organizer of the department painting competition Biological Sciences and Bioengineering, Indian Institute of Technology Kanpur, India
2016 - 2017 Department placement coordinator, Indian Institute of Technology Kanpur
2017 - Life Member, National Magnetic Resonance Society (NMRS), India
2020 - Member of Early Career Reviewer board, Journal of Biological Chemistry
2020 - Member, Harvard Business Review Advisory Council
2020 - Peer reviewer: Advanced Science (1), Scientific reports (1), British Journal of Pharmacology (1) and PeerJ (3)

Honors

2011 - 2012 Ranked among top 98.7 percentile in all India (Fellowship 192,000 INR/year) Graduate Aptitude Test in Engineering 2011 - Biotechnology
2012 - 2016 Ranked 24th in all India joint CSIR-UGC National Eligibility Test (336,000 INR/year), Council of Scientific & Industrial Research and University Grants Commission
2016 - 2017 Institute fellowship (336,000 INR/year), Indian Institute of Technology Kanpur, India
2017 Travel grant - $1300, European Molecular Biology Organization
2017 International Travel award - $900, Science and Engineering Research Board, Department of Science and Technology (DST), India
2018 International Travel award - $1000, Indian Council of Medical Research
2018 **Sailife-NOST Best Thesis Award** 2018 (PhD Thesis). Received a certificate of recognition and award of INR 10,000, National Organic Symposium Trust and Sai Life Sciences
2018 Shortlisted for **Building Bharat-Boston Biosciences (B4)** fellowship (Harvard University), Department of Biotechnology within the Government of India and Lakshmi Mittal and Family South Asia Institute at Harvard University - *declined*,

C. Contribution to Science

1. **Targeting metabolite amyloids (M.Tech thesis)**: My M.Tech research focused on the understanding of the Phenylalanine (Phe) self-assembly towards amyloid. Project build on the observation that Phe forms amyloid like fibrils (metabolite amyloid) implicated in Phenylketonuria, an inborn metabolic disorder. We proposed an alternative strategy to alter the self-assembly of Phe to non-fibrillar pathway with the help of enantiomer D-Phe. Using HPLC and fluorescence-based assays, I determined the aggregation kinetics of Phe. With NMR spectroscopy, I demonstrated the self-assembly of small molecule Phe in solution and recorded DOSY-NMR spectra which enabled the calculation of the diffusion coefficient as a factor of Phe concentration in solution. With the help of Thioflavin T fluorescence and scanning electron microscopy, I

showed that D-Phe prevents amyloid-like fibril formation by modulating the seeding process. This is the first report to show alteration of metabolite amyloid and resulted in first publication from our lab.

a. **Singh V**, Rai RK, Arora A, Sinha N, Thakur AK. Therapeutic implication of L-phenylalanine aggregation mechanism and its modulation by D-phenylalanine in phenylketonuria. *Sci Rep*. 2014 Jan 27;4:3875. PubMed PMID: 24464217; PubMed Central PMCID: PMC3902384. Google Scholar citations 78.

2. **Metabolic signatures of Huntington's disease, polyglutamine aggregation and aggregation inhibitors (PhD thesis)**: My PhD research contributions focused on the polyglutamine (polyQ) aggregation involved in Huntington's disease (HD). My thesis consist two parts: (1) Evaluation of aggregation inhibitors and (2) characterization of Drosophila HD metabolome. For this project, working with Drosophila, I characterized an HD model using fluorescence, confocal and scanning electron microscopy for suitability for an NMR metabolomics study. Results from this research are highly relevant as they provided new details into the altered energy metabolism in HD using Drosophila model. I also discovered two categories of Huntingtin exon1 peptide aggregation inhibitors. Calmidazolium Chloride, a hydrophobic molecule binds to hydrophobic face of N-terminus of Htt peptide (NT17) to prevent its polyQ aggregation. Arginine derivative Arginine ethyl ester (AEE) directly binds to NT17 to prevent its aggregation. I used a combination of techniques i.e. HPLC, infrared spectroscopy, circular dichroism and fluorescence spectroscopy to characterize aggregation inhibition by molecules. Also, I developed a nano-formulation for a hydrophobic drug. My PhD thesis resulted in 3 peer-reviewed publications and 2 Indian patent applications (1 granted; Application no. 201611003335 -pending).

    a. Thakur AK, **Singh V**., inventors. Arginine derivatives as polyglutamine aggregation inhibitors. India 357388. 2021 February 3.

b.

c. Thakur AK, **Singh V**., inventors. A drug screening process and a drug formulation for Huntington's disease. India 346555. 2020 September 11.

d. **Singh V**, Patel KA, Sharma RK, Patil PR, Joshi AS, Parihar R, Athilingam T, Sinha N, Ganesh S, Sinha P, Roy I, Thakur AK. Discovery of Arginine Ethyl Ester as Polyglutamine Aggregation Inhibitor: Conformational Transitioning of Huntingtin N-Terminus Augments Aggregation Suppression. *ACS Chem Neurosci*. 2019 Sep 18;10(9):3969-3985. PubMed PMID: 31460743. News highlight in IndiaBioscience.

e. **Singh V**, Deepak RNVK, Sengupta B, Joshi AS, Fan H, Sen P, Thakur AK. Calmidazolium Chloride and Its Complex with Serum Albumin Prevent Huntingtin Exon1 Aggregation. *Mol Pharm*. 2018 Aug 6;15(8):3356-3368. PubMed PMID: 29979597.

f. **Singh V**, Sharma RK, Athilingam T, Sinha P, Sinha N, Thakur AK. NMR Spectroscopy-based Metabolomics of Drosophila Model of Huntington's Disease Suggests Altered Cell Energetics. *J Proteome Res*. 2017 Oct 6;16(10):3863-3872. PubMed PMID: 28871787.

3. **Small molecular weight Hydrogelator Fmoc-Phe**: This is extension of my M.Tech project on phenylalanine self-assembly. This project started with serendipitous discovery of Fmoc conjugation to phenylalanine in phosphate buffer. We pursued it further and optimized reaction conditions, then characterized the Fmoc-Phe self-assembly to hydrogel. With the help of NMR, we showed the involvement of hydrophobic interaction in the gelation process. Further, we found it to be active against Gram-positive bacteria. For the first time, we associated the change in surface tension by Fmoc-Phe to its ability to disrupt the cell membrane integrity. We also showed alteration in the levels of ROS, GSH and betaine that resulted in oxidative and osmotic stress contributing to its antibacterial activity. We published these findings in Soft Matter (2018) where I am co-corresponding author. This work is further extended to the development of Fmoc-Phe hydrogel with aztreonam with broad spectrum anti-microbial action. Lastly we studied Fmoc-Phe action on biofilm formation, which is now published in The Journal of Antibiotics.

a. Singh H, Gahane A, **Singh V**, Ghosh S, Thakur A. Antibiofilm activity of Fmoc-phenylalanine against Gram-positive and Gram-negative bacterial biofilms. *J Antibiot*. 2021/02; doi: 10.1038/s41429-021-00409-2.

b. Gahane AY, **Singh V**, Kumar A, Kumar Thakur A. Development of mechanism-based antibacterial synergy between Fmoc-phenylalanine hydrogel and aztreonam. *Biomater Sci*. 2020 Mar 31;8(7):1996-2006. PubMed PMID: 32073033.

c. Gahane AY, Ranjan P, **Singh V***, Sharma RK, Sinha N, Sharma M, Chaudhry R, Thakur AK*. Fmoc-phenylalanine displays antibacterial activity against Gram-positive bacteria in gel and solution phases. *Soft Matter*. 2018 Mar 28;14(12):2234-2244. PubMed PMID: 29517792. *corresponding author

d. **Singh V**, Sharma R, Sinha N, Thakur A. Optimization of Ion-Dependent Green Synthesis of Fmoc-Amino Acids in Phosphate Buffer. *ChemistrySelect*. 2017 December 19; 2(35):11826-11831.

e. **Singh V**, Snigdha K, Singh C, Sinha N, Thakur AK. Understanding the self-assembly of Fmoc-phenylalanine to hydrogel formation. *Soft Matter*. 2015 Jul 14;11(26):5353-64. PubMed PMID: 26059479.

4. **Product development (2018-2019)**: I have participated in clinical immersion program jointly organized by Indian Institute of Technology Kanpur and King George's Medical University Lucknow, India. We identified a growing risk of cervical cancer in Indian population and non-availability of affordable screening platforms in the market. As part of the technical team, I helped in designing the portable endoscope for gynecological examination and cervical cancer screening. Our team successfully secured early stage funding of $70,000 from Biotechnology Ignition Grant Scheme (BIG) – BIRAC for prototype development and testing.

   a. Sethi R, Pandey D, **Singh V**, Kumar A., inventors. A device and method for gynaecological examination and cervical cancer screening. India 201811032508. 2018 August 30.

5. **Metals and tau liquid-liquid phase separation (Postdoc project)**: Tau is a microtubule-binding protein that, under pathological conditions, self-assembles into neurofibrillary tangles, a hallmark of tauopathies including Alzheimer's disease (AD). Recently our lab showed the capability of tau to undergo liquid–liquid phase separation (LLPS) under crowding condition to form dynamic liquid droplets driven by electrostatic interactions. Growing evidence indicates altered metal homeostasis in AD and that metals like zinc enhance tau aggregation. Using turbidimetry measurement by light scattering and fluorescent microscopy imaging, I found that zinc, but not other metals, can potentiates the tau LLPS even under non-crowded conditions. Replacing cysteines abrogates this LLPS induction by zinc. With the help of Fluorescence recovery after photobleaching using confocal microscopy, I show that droplets maintain a dynamic nature for hours. These results offer new insights into the relationship between irregular homeostasis of zinc and the possible pathogenic mechanisms of AD.

   a. **Singh V**, Xu L, Boyko S, Surewicz K, Surewicz WK. Zinc promotes liquid-liquid phase separation of tau protein. *J Biol Chem*. 2020 May 1;295(18):5850-5856. PubMed PMID: 32229582; PubMed Central PMCID: PMC7196643. **Editors' picks** article. Featured in JBC news.

Complete List of Published Work in My Bibliography:
https://www.ncbi.nlm.nih.gov/myncbi/virender.singh.1/bibliography/public/

Google scholar profile: https://scholar.google.com/citations?user=0wyRCucAAAAJ&hl=en&oi=ao

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. DO NOT EXCEED FIVE PAGES.

NAME: María del Carmen Fernández Ramírez

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

POSITION TITLE: Postdoctoral Researcher

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date	FIELD OF STUDY
Francisco de Vitoria University, Madrid, Spain	Academic Degree	09/2010	Management & Administration of Biotech. Companies
Francisco de Vitoria University, Madrid, Spain	BSc	09/2010	Biotechnology
Autonomous University of Madrid, Spain	MSc	03/2012	Neuroscience
Cajal Institute-CSIC, Madrid, Spain	Trainee student	03/2015	Structural Biology/Neuroscience
Cajal Institute-CSIC, Madrid, Spain	PhD	05/2019	Structural Biology/Neuroscience
Cajal Institute-CSIC, Madrid, Spain	Postdoctoral training	08/2019	Structural Biology/Neuroscience
UT Southwestern Medical Center, Dallas, USA	Postdoctoral training	Present	Structural Biology/Neuroscience

A. Personal Statement

The primary interest in my scientific career has been the understanding of the structural features that determine the folding and misfolding processes of amyloid proteins in relation with its function and pathology, with the final purpose of point at a target for pharmacological intervention. I mainly focused on the first steps of amyloidogenesis by recording and analyzing the fluctuations and conformations of monomers, using atomic force microscopy (AFM). I pursue that my scientific career continue in the same direction, with the main focus on pathological amyloidogenic proteins. Also, I plan to continue using and learning high resolution techniques such as cryogenic electron microscopy, due to its contribution to fibril structural determination and its potential contribution in the study of amyloidogenic monomers. Additionally, I am interested in developing and applying new strategies to improve the study of these single molecules.

Attracted by the problems that conventional neuroscience is not able to solved, I started to study tau and other neurotoxic and functional amyloids by biophysical methods during my MSc project in the laboratory of the Dr. Mariano Carrión who is an expert in the nanomechanics of proteins. Along these stages, I have acquired skills in cloning, expression and purification of recombinant proteins. I used basic cell culture techniques and I performed biochemical and biophysical studies by bulk (circular dichroism and transmission electron microscopy) and AFM-based single-molecule force spectroscopy (AFM-SMFS) in order to monitor protein aggregation of intrinsically disordered proteins (IDPs), to understand the beginning of their amyloidogenic pathway and to find the structural differences between pathological and functional amyloids. Another important part of my work during these years was the development of a new strategy to study the conformational polymorphism of amyloid proteins by AFM-SMFS. The purpose was to facilitate the use of this high-resolution technique to non-expert scientists in the field.

Along these years, I collaborated on other projects within my previous laboratory, and with other groups from the "MisingLink" Consortium (EU Joint Programme – Neurodegenerative Disease Research JPND), in which I participated. Additionally, I supervised the final grade/MSc projects of three students. Thanks to these experiences I worked with the proteins β-amyloid, TDP-43 and the functional amyloids Orb2/ApCPEB, acquiring a wider view of the amyloidogenic processes in the brain. This made me improve my abilities in experimental design, data analysis, interpretation and discussion of results. I disseminated my results by attending to conferences with poster and oral presentations and writing chapters of books and research articles (1-4). Moreover, I collaborated in the writing of proposals for national and European grants. After my PhD, I worked as a postdoctoral researcher for a short period of three months in the same laboratory.

Continuing with these inquisitiveness, I joined the last year to the Dr. Diamond and Dr. Saelices labs, since they are expert in tau protein and biophysics of amyloids, respectively. They are supporting me to continue my studies on tau monomer, learning Cryo-EM as a new technique and developing new strategies to address the analysis of monomeric IDPs.

1. Hervás R, Li L, Majumdar A, **Fernández-Ramírez MdC**, Unruh JR, Slaughter BD, Galera-Prat A, Santana E, Suzuki M, Nagai Y, Bruix M, Casas-Tintó S, Menéndez M, Laurents DV, Si K, Carrión-Vázquez M (2016). Molecular Basis of Orb2 Amyloidogenesis and Blockade of Memory Consolidation. PLoS Biol. 14(1): e1002361.
2. **Fernández-Ramírez MC**, Hervás R, Galera-Prat A, Laurents DV and Carrión-Vázquez M (2018). Efficient and simplified nanomechanical analysis of intrinsically disordered proteins. Nanoscale. 10(35):16857-16867.
3. Hervás R*, **Fernández-Ramírez MC***, Galera-Prat A, Suzuki M, Nagai Y, Bruix M, Menéndez M, Laurents DV, Carrión-Vázquez M. Divergent CPEB Prion-Like Domains Reveal Different Assembly Mechanism for a Generic Amyloid Fold. Under revision in BMC Biology. 10.1101/2020.05.19.103804.
4. **Fernández-Ramírez MC**, Hervás R, Menéndez M, Laurents DV, Carrión-Vázquez M. Tau amyloidogenesis begins with a loss of conformational polymorphism. bioRxiv. 10.1101/2020.06.18.158923. **Co-corresponding author**.

B. Positions and Honors

Positions

2021-Present Postdoctoral Researcher in Dr. Diamond and Dr. Saelices Labs. UT Southwestern Medical Center. Dallas, Texas, USA.

2015-2019 Researcher Member of the "MisingLink" consortium from EU Joint Programme – Neurodegenerative Disease Research (JPND). Protein Nanomechanics of the Nervous System. Dr. Mariano Carrión. Cajal Institute. Madrid, Spain.

Jun-Aug 2019 Postdoctoral researcher. Protein Nanomechanics of the Nervous System. Dr. Mariano Carrión. Cajal Institute. Madrid, Spain.

Trainee experience

2011-2015 Protein Nanomechanics of the Nervous System. Dr. Mariano Carrión. Cajal Institute. Madrid, Spain.

Jul-Sept 2009 Department of Biochemistry. Prof. Ian M. Willis. Albert Einstein College of Medicine. New York, USA.

Honors and awards

2005/2006 Academic Excellence Scholarship. Francisco de Vitoria University

2006/2007 FIDES Foundation Scholarship. Francisco de Vitoria University.

2007 Summer Language Course scholarship. Government of Spain.

2007/2008 FIDES foundation scholarship. Francisco de Vitoria University.

2008 Summer Language Course scholarship. Government of Spain.

C. Contributions to Science

ORCID ID:
https://orcid.org/0000-0002-3265-5242

1. Protein Nanomechanics applied to the single-molecule study of amyloidogenic proteins.

Since I started my undergraduate period, I focused in the Atomic Force Microscopy-based Single-Molecule Force Spectroscopy as a versatile tool with the potential to shed light to key aspects from Intrinsically Disordered Proteins (IDPs). As a result of an extensive review of the bibliography, I participated in the publishing of two book chapters. There, the concepts and possibilities of this methodology were explained and discussed. Also, the relevance of the approach and the possibility of its combination with other techniques were emphasized, due to the issues that AFM-SMFS can address depend on this additional factors (1, 2). My interest in contributing to the toolbox of protein nanomechanics for the study of IDPs, led me to the development of a new strategy that provides several advantages and solves most of the problems associated to previous strategies. Thus, the analysis of the data is more efficient because the quick identification of valid recordings, the generation of contamination-free signals and the unambiguous identification of all complex and irregular mechanical patterns typical from amyloidogenic proteins. Also, this simplified version allows that non-experts in the field can address the nanomechanical study of IDPs and opens the door to the automated data analysis (3).

1. Hervás R, Galera-Prat A, Gómez-Sicilia A, Losada-Urzáiz F, **Fernández-Ramírez MC**, Fernández-Bravo D, Santana E, Barrio-García C, Melero C and Carrión-Vázquez M (2012). *The nanomechanics of ordered and disordered proteins*. In: Oberhauser A (eds) AFM studies on the mechanical properties of proteins. Springer-Verlag, Heidelberg, 1-47.
2. Hervás R, **Fernández-Ramírez MC**, Abelleira-Hervás L, Laurents DV and Carrión-Vázquez M (2013). *Nanomechanics of neurotoxic proteins: insights at the start of the neurodegeneration cascade*. In: Uversky, V.N. & Lyubchenko, A.K. (eds) Bionanoimaging: Insights into Protein Misfolding and Aggregation. Elsevier, Oxford.
3. **Fernández-Ramírez MC**, Hervás R, Galera-Prat A, Laurents DV and Carrión-Vázquez M (2018). *Efficient and simplified nanomechanical analysis of intrinsically disordered proteins*. Nanoscale. 10(35):16857-16867.

2. Molecular mechanisms and structural features of pathological amyloidogenic proteins.

During my PhD, in the laboratory of Nanomechanics of Proteins, I applied biophysical and biochemical methods to unravel the underlying molecular mechanisms of amyloidogenic proteins involved in neurodegeneration. My aim was to find the first event that triggers the pathological aggregation or, alternatively, to identify the responsible region of the protein which would represent a novel target to prevent aggregation. The aggregation of the protein TDP-43 is implicated in Amyotrophic Lateral Sclerosis. A segment rich in Q/N was identified as the starter of the process and its interference by the peptide QBP1 was able to block it (1). I also addressed the monomer behavior of tau protein, involved in several neurodegenerative disorders including Alzheimer's disease. I identified the first steps of tau aggregation. The critical conformational change, contrary to other amyloids previously studied, was the loss of its conformational polymorphism. This mechanism starts by unfolding tau monomers thereby exposing the amyloid-prone segments. However, the rearrangements caused depend on the context, which may

explain the distinct morphologies found in tau deposits. My results point to an alternative strategy for pharmacological intervention (2).
Since I started my current postdoc, I am determining the structure of the fibrils that transthyretin is able to form in ATTR patients. I am contributing in the understanding of the polymorphism associated to the aggregation of this protein in vivo (3).

1. Monpeán M, Ramírez de Mingo D, Hervás R, **Fernández-Ramírez MDC**, Carrión-Vázquez M, Laurents DV (2019). Molecular mechanism of the inhibition of TDP-43 amyloidogenesis by QBP1. Arch Biochem Biophys. 675:108113.
2. **Fernández-Ramírez MC**, Hervás R, Menéndez M, Laurents DV, Carrión-Vázquez M. Tau amyloidogenesis begins with a loss of conformational polymorphism. bioRxiv. 10.1101/2020.06.18.158923.
3. Nguyen BA, Afrin S, Singh V, Ahmed Y, Pedretti R, **Fernández-Ramírez MDC**, Benson MD, Sawaya RM, Cao Qin, Boyer D, Pope A, Wydorski PM, Chhapra F, Eisenberg DS, Saelices L. Structural polymorphism of amyloid fibrils in cardiac ATTR amyloidosis revealed by cryo-electron microscopy.
doi: https://doi.org/10.1101/2022.06.21.496949

3. Molecular mechanisms in the aggregation of functional amyloids.

Parallel to the aforementioned questions, I contributed to the understanding of how neural functional amyloids self-assemble and which are the differences with those that are pathological. With this objective, I studied the amyloidogenesis of Orb2 and ApCPEB and found that they share some aggregation features with pathological amyloids along the aggregation cascade. However, I found key different features that could explain their harmlessness. In vitro, they also form toxic olimeric species but they are very transient and evolve very quickly to non-toxic mature fibrils (1, 2). Additionally, my results indicate that ApCPEB has an aggregation mechanism based on coiled-coil formation, which provides an additional step for amyloidogenesis regulation in the cell (2).

1. Hervás R, Li L, Majumdar A, **Fernández-Ramírez MdC**, Unruh JR, Slaughter BD, Galera-Prat A, Santana E, Suzuki M, Nagai Y, Bruix M, Casas-Tintó S, Menéndez M, Laurents DV, Si K, Carrión-Vázquez M (2016). Molecular Basis of Orb2 Amyloidogenesis and Blockade of Memory Consolidation. PLoS Biol. 14(1): e1002361.
2. Hervás R*, **Fernández-Ramírez MC***, Galera-Prat A, Suzuki M, Nagai Y, Bruix M, Menéndez M, Laurents DV, Carrión-Vázquez M. Divergent CPEB Prion-Like Domains Reveal Different Assembly Mechanism for a Generic Amyloid Fold. BMC Biology. 19(1):43.

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

Scholastic Performance

DATE	COURSE TITLE
8th-11th Oct. 2018	"Advanced + Applied Statistics. Experiment design and Multivariate Analysis". CSIC. 20 hours.
11th May-12th June 2015	"Adobe Indesign CS5 On Line". CSIC. 40 hours
20th Ap.-22nd May 2015	"Specific English: Meetings-Advanced". CSIC. 30 hours.
16th-19th Jan. 2012	SENC Course: "XX Introduction Course to Stereological Techniques in Histology and Neurobiology". Autonomous University of Madrid, Madrid. 30 hours.
Nov. 2011-Feb. 2012	Officially accredited title for animal experimentation (category C). Autonomous University of Madrid, Madrid. 80 hours.