BIOGRAPHICAL SKETCH

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NAME: Eisenberg, David

eRA COMMONS USER NAME (credential, e.g., agency login): EISENBERG2

POSITION TITLE: Paul D. Boyer Professor of Biochemistry & Molecular Biology

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
Harvard College, Cambridge, MA (J.T. Edsall)	A.B.	06/1961	Biochemical Sciences
Oxford University, Oxford, UK (C.A. Coulson)	D. Phil.	10/1964	Theoretical Chemistry

A. Personal Statement

Understanding biology and disease has been my career-long interest. Starting with biochemistry, computation and x-ray diffraction, I later added the tools of TEM, micro-electron diffraction, and cryoEM. I have focused increasingly on proteins associated with amyloid and prion diseases. These are diseases of protein oligomerization and fibrillation. By newly developed methods of microcrystallography and microelectron diffraction, our lab has been able to determine the atomic structures of some 200 of disease related fibril structures. In the past 5 years, we have determined structures of ~30 amyloid fibrils by cryoEM.

In laboratory training, I have supervised dozens of undergraduates, over 160 Ph.D. theses and postdoctoral fellows, most of who are carrying out research in structural and computational biology in universities, research institutes, and industries. Former lab members work in at least a dozen countries. I have coauthored ~400 research papers and reviews, and two books: a monograph on the structure and properties of water [>5000 citations], still in print after 50 years, and a text on physical chemistry for the life sciences.

I established a user-friendly facility for determination of atomic structures by x-ray and EM methods which has welcomed and helped scores of users from UCLA, other research institutions and industry.

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

MICHAEL J FOX FOUNDATION 05/17/2021 – 05/16/22 1.00 calendar months
Grant ID: MJFF-001166 $147,720 Total
Completion of Preclinical Study of a Safe and Effective Image-based Biomarker for Parkinson's Disease particles
To develop a non-radioactive diagnostic that can in principle detect the development of Parkinson's disease by MRI. This repeatable diagnostic will also enable studies of patients over time, necessary for evaluating the effectiveness of therapies.

MICHAEL J FOX FOUNDATION (PI: Sally Fraustchy) 01/01/2021 – 06/30/22 1.00 calendar months
Grant ID: MJFF-001065 $82,221.55 Total
Liganded Nanoparticles to Inhibit Alpha-synuclein (aSyn) Aggregate Deficits in Endosomal-Lysosomal and Autophagy
Genetic and sporadic Parkinson's disease (PD) cases implicate dysfunction in the endo-lysosomal

system, autophagy-lysosomal protein degradation and lysosomal biogenesis. The overarching goal is to determine whether these highly specific peptides show efficacy inPD models.

MCB 1616265 (Eisenberg) 09/01/2016 – 08/31/2022 0.20 calendar months
NSF $414,335 Total
Reversible Amyloid-Like Fibrils in Membraneless Organelles
To explore the full variety of interactions and assembly states found in membrane-less organelles by mapping the human reversible amylome.

R01 AG048120 (Eisenberg) 06/01/2019 – 05/31/2024 1.50 calendar months
NIH/NIA $1,950,000 Total
Development of Inhibitors and Diagnostics for Systemic Amyloid Diseases
We aim to further our understanding of amyloid structure, and apply this understanding to the development of new and better candidate therapeutics and diagnostics.

RF1AG065407 (Diamond) 09/15/2020 – 08/31/2024 0.50 calendar months
Seeds and Strains Derived from Tau Monomer $303,145 Total
We will oversee the characterization of tauopathy-derived brain fibrils using cryo-electron microscopy (cryoEM). His group will create micro-crystals of subdomains (i.e. local structures) of the tau protein for x-ray crystallography. This will be used to test predictions made by cross-linking mass spectrometry and other biophysical studies performed in the Joachimiak lab.

1RF1AG065407 (Kayed) 07/01/2021 – 06/31/2026 1.50 calendar months
NIH $939,220 Total
Interdisciplinary Research Network on Biologically Active Tau Aggregate Polymorphs from Alzheimer's Disease and Related Dementias
The Eisenberg lab will prepare oligomeric specimens for structural studies, including cryo-EM. Antibodies and nanobodies will be prepared for aids in specimen preparation. Cryo-EM and crystallographic structural determinations will be carried out.

1R01AG070895 (Eisenberg) 02/01/22 – 01/31/27 4.00 calendar months
NIH $1,068,649 Total
Towards Treatment of Alzheimer's Disease by Targeting Pathogenic Tau and Beta-Amyloid Structures
To develop effective drugs, we take the approach that has been effective for treating cancer and HIV-AIDS: structure-based drug design by applying the powerful tools of electron microscopy and x-ray diffraction.

HHMI (Eisenberg) 09/01/18 – 08/31/23 0 calendar month
Howard Hughes Medical Institute
General Support of the Eisenberg laboratory, including salaries for PI (Eisenberg), Crystallographer, and Laboratory Manager, and Administrative Assistant.

B. Positions, Scientific Appointments, and Honors

Paul Boyer Chair of Molecular Biol.		2009-present		
Howard Hughes Medical Institute	Investigator	2001-present	Investigator	
UCLA, Los Angeles	Director	1993-2014	UCLA-DOE Institute	
UCLA, Los Angeles	Asst. Prof-Prof	1969-present	Chemistry & Biochemistry	
Caltech, Pasadena	Postdoc	1966-1969	Structural Biology	(R.E. Dickerson)
Princeton University, Princeton	Postdoc	1964-1966	Water, H-bonding	(Walter Kauzmann)

Selected Memberships and Awards
L.J. Henderson Prize, 1961 for best undergraduate thesis in Biochemical Sciences; Rhodes Scholarship, 1961-1964; Alfred P. Sloan Fellowship, 1969-1971; USPHS Career Development Award, 1972-1977; UCLA

Distinguished Teaching Award, 1975; McCoy Award of the UCLA Department of Chemistry and Biochemistry for innovative research, 1982 (with R.E. Dickerson); Guggenheim Fellowship, 1985; UCLA Faculty Research Lectureship, 1989; National Academy of Sciences, 1989; American Academy of Arts & Sciences, 1991; Pierce Award of the Immunotoxin Society, 1992; Protein Society Stein & Moore Award, 1996; American Chemical Society Repligen Award in Molecular Biology, 1998; Fellow, Biophysical Society Inaugural Year Fellow, 1999; Amgen Award of the Protein Society, 2000; Institute of Medicine 2002; American Philosophical Society, 2003; UCLA Seaborg Medal, 2004; Harvard Westheimer Medal, 2005; Harvey International Prize in Human Health, 2009; Biophysical Society, Emily Gray Award, 2009; Honorary Fellow, Queen's College, Oxford, 2010; ISMB Accomplishment by a Senior Scientist Award, 2013; Inaugural Switzer Price for Biomedical Discovery, 2014; ASBMB Bert and Natalie Vallee Award in Biomedical Science, 2015; Fellow, American Crystallographic Assoc, 2015, MBI Legacy Award, 2015; Vallee Visiting Professor, 2016; UCSF Andrew Braisted Award Lecturer, 2016; Paul Sigler Prize, Yale University, 2017. NAS Strategic Planning Committee, 2019. Passano Laureate, 2020.

C. Contributions to Science >108,000 citations, <h> = 147

1. Structural biology of the amyloid state of proteins: Prior to our atomic-resolution crystallographic studies of amyloid-forming proteins, only low-resolution information from EM and fiber diffraction were available. Papers a, b, and c describe the common spine of amyloid fibers: a pair of beta-sheets, closely mating by interdigitation of their sidechains, termed a steric zipper. Paper a was the first atomic resolution structure of the amyloid state. Paper b showed that numerous amyloid fibrils have steric-zipper spines, and classified the possible symmetries of this structural motif. Paper c reports the first identification by cryoEM structures of pathogenic fibrils of protein TM106B in the disease FTLD-TDP. Paper d reveals a new type of protein interaction—termed LARKS—between low-complexity domains, responsible for multivalent networks and gels, such as those found in membrane-less organelles.

a. Nelson R, Sawaya MR, Balbirnie M, Madsen AO, Riekel C, Grothe R, Eisenberg D. Structure of the cross-beta spine of amyloid-like fibrils. *Nature.* **435**, 773-8 (2005). PMCID: PMC1479801 [~2300 citations]
b. Sawaya MR, Sambashivan S, Nelson R, Ivanova MI, Sievers SA, Apostol MI, Thompson MJ, Balbirnie M, Wiltzius JJ, McFarlane HT, Madsen AØ, Riekel C, Eisenberg D. Atomic structures of amyloid cross-beta spines reveal varied steric zippers. *Nature.* **447**, 453-7 (2007). PMID: 17468747 [~2260 citations]
c. Jiang, Y.X., Cao, Qin, Sawaya, M.R.…Eisenberg, D.S. Amyloid fibrils in disease FTLD-TDP are composed of TMEM106B not TDP-43. Nature (2022)
d. Michael P. Hughes, Michael R. Sawaya, David R. Boyer, Lukasz Goldschmidt, Jose A. Rodriguez, Duilio Cascio, Lisa Chong, Tamir Gonen, David S. Eisenberg. Atomic structures of low-complexity protein segments reveal kinked β-sheets that assemble into networks. *Science.* **359***, 698-701* (2018). PMCID: PMC6192703 [248 Citations]

2. Inhibition of formation of amyloid fibrils and of amyloid cytotoxicity: Dozens of human diseases are associated with amyloid fibrils. We have been able to inhibit amyloid formation both by structure-based design (papers e-h). Papers g and h report improved inhibitors of the aggregation of tau (at the root of Alzheimer's, CTE, and 25 other tauopathies) and of the intercellular prion-like spread of tau fibrils.

e. Sievers SA, Karanicolas J, Chang HW, Zhao A, Jiang L, Zirafi O, Stevens JT, Munch J, Baker D, Eisenberg D. Structure-based design of non-natural amino-acid inhibitors of amyloid fibril formation. *Nature.* **475**, 96-100 (2011). PMCID: PMC4073670 [444 citations]
f. Saelices L, Chung K, Lee JH, Benson MD, Bijzet J., Cohn W, Whitelegge, JP, Eisenberg D. Amyloid seeding of transthyretin by ex vivo cardiac fibrils: inhibition and implications, *PNAS*, **115**:E6741-E6750, (2018). www.pnas.org/cgi/doi/10.1073/pnas.1805131115
g. Seidler, PM, Boyer, DR, Rodriguez,JA, Sawaya,MR, Cascio,D, Murray,K, Gonen,T, Eisenberg,DS.. Structure-based inhibitors of tau aggregation. *Nature Chemistry*. **10**, 170-176 (2018). DOI:10.1038/NCHEM.2889 (2017). PMCID: PMC5784779 [175 Citations]
h. Seidler PM, Boyer DR, Murray KA, Yang TP, Bentzel M, Sawaya MR, Rosenberg G, Cascio D, Williams CK, Newell K, Ghetti B, DeTure MA, Dickson D, Vinters HV, Eisenberg DS* Structure-based inhibitors halt prion-like seeding by Alzheimer's disease– and tauopathy-derived brain tissue samples *J. Biol. Chem*, **294**(44):16451-16464. DOI 10.1074/jbcRA119.009688

3. Computational analysis of amino acid sequences and protein structures: As protein sequences and structures became readily available in the 1980s and 1990s, I developed new methods to extract information from sequences and structures. Paper i describes a new property of proteins—the hydrophobic moment, which has been widely applied to detect periodicities in proteins. Paper j introduced atomic solvation parameters, used subsequently by many to estimate free energy changes of protein folding and binding. Paper k introduced the Profile method for detection of distantly related protein sequences. It was later coded by others into the powerful PsiBlast algorithm. Paper l invented threading of sequences on to structures to identify new proteins having previously determined folds. This method has also been widely applied.

i. D Eisenberg, RM Weiss, TC Terwilliger. The hydrophobic moment detects periodicity in protein hydrophobicity. *Proc. Natl. Acad. Sci. U.S.A.* **81**, 140-144 (1984). PMCID: PMC344626 [1117 citations]
j. D. Eisenberg, A.D. McLachlan. Solvation energy in protein folding and binding. *Nature.* **319**,199-203 (1986). PMID: 3945310 [2330 citations]
k. M Gribskov, AD McLachlan, D Eisenberg. Profile analysis: detection of distantly related proteins. *Proc. Natl. Acad. Sci. U.S.A.* **84**, 4355-4358 (1987). PMCID: PMC305087 [1748 citations]
l. JU Bowie, R Luthy, D Eisenberg. A method to identify protein sequences that fold into a known 3D structure. *Science.* **253**, 164-170 (1991). PMID: 1853201 [3366 citations]

4. Methods for inferring protein interactions and functions from genome sequences. The advent of genome sequencing brought the puzzle of how to infer from this mass of information the function of proteins and the pathways and complexes formed by proteins. Our group, together with the group of Todd Yeates, worked out several methods described in papers m, n, and o. We also began a database of protein interactions described in paper o.

m. Marcotte EM, Pellegrini M, Ng HL, Rice DW, Yeates TO, Eisenberg D. Detecting protein function and protein-protein interactions from genome sequences. *Science.* **285**, 751-3 (1999). PMID: 10427000 [2148 citations]
n. Marcotte EM, Pellegrini M, Thompson MJ, Yeates TO, Eisenberg D. A combined algorithm for genome-wide prediction of protein function. *Nature.* **402**, 83-6 (1999). PMID: 10573421 [1183 citations]
o. Xenarios I, Salwínski L, Duan XJ, Higney P, Kim SM, Eisenberg D. DIP, the Database of Interacting Proteins: a research tool for studying cellular networks of protein interactions. *Nucleic Acids Res.* **30**, 303-5 (2002). PMCID: PMC99070 [2018 citations]

5. Electron microscopy and micro-electron diffraction:

p. Frank J, Goldfarb W, Eisenberg D, Baker TS. Reconstruction of glutamine synthetase using computer averaging. [The first report of TEM single particle averaging] *Ultramicroscopy*. **3**, 283-90 (1978). PMCID: PMC4167717 [279 citations]
q. Jose A. Rodriguez, Magdalena Ivanova, Michael R. Sawaya, Duilio Cascio, Francis Reyes, Dan Shi, Smriti Sangwan, Elizabeth Guenther, Lisa Johnson, Meng Zhang, Lin Jiang, Mark Arbing, Julian Whitelegge, Johan Hattne, Brent Nannega, Aaron S. Brewster, Marc Messerschmidt, Sébastien Boutet, Nicholas K. Sauter, Tamir Gonen, David Eisenberg. Structure of the toxic core of α-synuclein from invisible crystals *Nature.* **525**, 486-90 (2015). PMCID: PMC4791177 [468 citations]
r. Michael R. Sawaya, Jose Rodriguez, Duilio Cascio, Michael J. Collazo, Dan Shi, Francis E. Reyes, Johan Hattnef, Tamir Gonen, David S. Eisenberg. Ab Initio structure determination from prion nanocrystals at atomic resolution by MicroED *PNAS*, **113**, 11232-11236 (2016). 9. PMCID: PMC5056061 [83 citations]
s. de la Cruz, M. Jason; Hattne, Johan; Shi, Dan; Seidler, Paul; Rodriguez, Jose; Reyes, Francis; Sawaya, Michael R.; Cascio, Duilio; Weiss, Simon C.; Kim, Sun Kyung; Hinck, Cynthia S.; Hinck, Andrew P.; Calero, Guillermo; Eisenberg, David; Gonen, Tamir

Atomic-resolution structures from fragmented protein crystals with the cryoEM method MicroED
Nature Methods. **14**, 399-402 (2017). PCMID: PMC5376236 [128 citations]

OMB No. 0925-0001 and 0925-0002 (Rev. 03/2020 Approved Through 02/28/2023)

BIOGRAPHICAL SKETCH

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NAME: Abskharon, Romany

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

POSITION TITLE: Project Scientist

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	END DATE MM/YYYY	FIELD OF STUDY
College of Science, Assiut Unvi., Egypt	B.Sc.	09/1999	06/2002	Chemistry & Microbiology
College of Science, Assiut Unvi., Egypt	M.Sc.	09/2004	02/2008	Chemistry & Microbiology
Vrije Universiteit Brussels, Belgium (VIB Research Institute)	Ph.D.	03/2008	06/2013	Immunology & Structure Biology
National Institute of Oceanography & Fisheries, Cairo, Egypt	Lecturer	06/2013	08/2014	Bio-engineering Science
Van Andel Research Institute, Grand Rapids, MI, USA	Postdoctoral Researcher	09/2014	12/2017	Protein-misfolding in neurodegenerative disease
University of California, Los Angeles, USA	Postdoctoral Researcher	01/2018	09/2020	Structure-based design to understand and treat neurodegenerative disease
University of California, Los Angeles, USA	Project Scientist	09/2020	Present	Structure-based design to understand and treat neurodegenerative disease

A. Personal Statement

My overall goal as a scientist is to use structure-based design, structure biology and immunological approaches to understand and treat neurodegenerative diseases. During my career, I published 25 papers, and currently have three under preparation. As graduate student under the supervision of **Prof. Jan Steyaert**, I solved the first crystal structure of full-length human prion protein in complex with a nanobody. In this project, I had successes in generating, selecting and characterizing a number of nanobodies against prion proteins. I have also developed an innovative expression system for production of soluble prion proteins in *E. coli*. During my first postdoctoral-experience at Center for Neurodegenerative Science (Van Andel Research Institute), I focused on studying nanobodies as potential therapeutic approaches for protein misfolding-induced neurodegenerative diseases, such as Parkinson's disease and prion disease.

In 2018, I was honored to join **Prof. David Eisenberg's lab,** and as a senior postdoctoral researcher, my goal is to use structure-based design and structure biology to further understand and treat neurodegenerative

diseases. I initiated four projects: **In the first project**, I designed and engineered a single-chain antibody (scFv-M204), that inhibits seeding by tau oligomers and pathological extracts from donors with Alzheimer's disease (AD) and Chronic Traumatic Encephalopathy (CTE). Remarkably, the scFv-M204 antibody binds to oligomeric tau, but not to recombinant monomers or fibrils (a patent has been accepted and a paper has been published in JBC). Additionally, I expressed and purified several monoclonal antibodies that specifically bind to certain regions of tau protein from hybridoma cell lines. **In my second project**, I used cryo-EM in determining the structures of full-length fibrils of tau protein both seeded by fibrils purified from autopsied Alzheimer's disease (AD) brains and by unseeded fibrils. In both samples, tau fibrils are generated using a new method in which RNA is used as a natural co-factor for aggregation (One paper has been published in PNAS and second manuscript in preparation). **In the third project**, I employed the structure-based approach to design a panel of synthetic nanobody inhibitors to block the prion-like spread by extracts from autopsied brains of patients with AD and progressive supranuclear palsy (PSP) (a patent and manuscript are in preparation). **In the fourth project**, Determine the structure of recombinant tau oligomers and AD brain extracted oligomers in complex with antibody fragments (Fabs and nanobodies). Since 2018, my collaborations with various Eisenberg lab members have led to five manuscripts as first author (two published and three under preparation as first author) and many other papers under revision as co-author.

a) **Abskharon R.**, Sawaya MR., Boyer DR., Cao Q, Nguyen BA., Cascio D., Eisenberg DS (2022). Cryo-EM structure of RNA-induced tau fibrils reveals a small C-terminal core that may nucleate fibril formation. PNAS. 119(15):e2119952119. PMID: 35377792.

b) Jiang YX., Cao Qin., Sawaya MR., **Abskharon R**., Ge P., DeTure M., Dickson D., Fu J., Loo R., Loo J., Eisenberg D.S. (2022). Amyloid fibrils in disease FTLD-TDP are composed of TMEM106B not TDP-43. Nature. 605(7909):304-309. PMID: 35344984.
Cao Q., Boyer DR., Sawaya MR., **Abskharon R**., Saelices L., Nguyen BA., Lu J., Kandee F., Eisenberg DS., (2021). Cryo-EM structures of hIAPP fibrils seeded by patient-extracted fibrils reveal new polymorphs and conserved fibril cores. Nat Struct Mol Biol. 28(9):724-730. PMID: 34518699.

c) **Abskharon R**., Seidler PM., Sawaya MR., Cascio D., Yang P., Philipp S., Williams CK., Newell KL., Ghetti B, DeTure MA., Dickson DW., Vinters HV., Felgner PL., Nakajima R., Glabe CG., Eisenberg DS (2020). Crystal structure of a conformational antibody that binds tau oligomers and inhibits pathological seeding by extracts from donors with Alzheimer's disease. J. Biol. Chem. 295:10662-10676. PMCID: PMC7397112.

B. Positions and Honors

Positions and Employment

2004 -2008 Researcher assistant, College of Science, Assiut University, Egypt.
2008 - 2013 PhD student, Vrije Universiteit Brussels, Brussels, Belgium (Supervisor: Dr. Jan Steyaert).
2013 - 2014 Lecturer, National Institute of Oceanography & Fisheries, Cairo, Egypt.
2014 - 2017 Postdoctoral Fellow, Van Andel Research Institute, Grand Rapids, MI, USA.
2018 - 2020 Postdoctoral Fellow, University of California, Los Angeles (Supervisor: Dr. David Eisenberg).
2020 - Present Project Scientist, University of California, Los Angeles (Supervisor: Dr. David Eisenberg).

Honors

2011 RAMC Award for the best poster presentation from RAMC (Recent Advances in Macromolecular Crystallization) Strasbourg, France.
2012 HERCULES Award for the best poster presentation from the School of Neutrons & Synchrotron Radiation for Science, Grenoble, France.
2012 Prion Award, for the best poster presentation from NeuroPrion committee at the international annual Prion conference, Amsterdam, Netherland.
2016 APSPR Award, Asia Pacific Society of Prion Research (APSPR) travel Award, Tokyo, Japan.
2019 State Encouragement Awards in Biological Science, from Egyptian Academy of Scientific Research and Technology.
2020 Scientific Creativity and Innovation "Makram Mehanna" Award, from the Coptic Orthodox Cultural Center.
2020 First class Medal of Science and Arts, from Arab Republic of Egypt (from the president of Egypt).

Other Experience and Professional Memberships

2010 NATO ASI, 10th Course: Biophysics and Structure. Organized by Stanford School of Medicine in Ettore Majorana Center for Scientific Culture in Erice, Sicily (EMFCSC), Italy.

2010 The BCA/CCP4 Protein Crystallography Summer School. Organized by British crystallographic community in Oxford University Diamond Light Source, UK.

2012 HERCULES European School of Crystallography: One-month course, coordinated by the Université Grenoble, EMBL, and ESRF, at European Synchrotron Radiation Facility (Grenoble, France), Swiss Light Source / Paul Scherrer Institute in Villigen, Switzerland and SOLEIL (St Aubin, France).

2016 Member of Asia Pacific Society of Prion Research (APSPR).

2019 Member of Antibody Society.

2019 Member of Alzheimer's Association.

C. Contribution to Science

1. Graduate Career:

The focus of my dissertation work was on the structural investigation of prion proteins using nanobody-aided crystallography. I determined the first structure of full-length prion protein in complex with a nanobody. Nanobodies are single domain antibodies derived from Camelidae. These nanobodies stabilize particular conformers of prions, making this aggregating protein amenable to X-ray crystallography. I have had successes in generating, selecting and characterizing a large number of nanobodies against prion proteins**.** I also developed an innovative expression system for production of soluble prion proteins in *E. coli*. Prior to my work, production of recombinant PrP was only achieved by refolding protocols. I discovered that the co-expression of two different PrP^{C} (normal form of prion) with the human Quiescin Sulfhydryl OXidase (QSOX), a human chaperone with thiol/disulfide oxidase activity, in the cytoplasm of *E. coli* produces soluble recombinant PrP. Furthermore, I discovered that QSOX inhibits human prion propagation in the cell-free protein misfolding cyclic amplification and inhibits murine prion propagation in scrape-infected neuroblastoma cells. I also determined QSOX preferentially binds PrP^{Sc} from prion-infected human or animal brains but not PrP^{C} from uninfected brains. My finding provides a useful tool for prion diagnosis. My study indicates that QSOX plays a role in prion formation, which opens new venues for developing therapeutic targets in prion or other neurodegenerative diseases. Indeed, I successfully utilized nanobody-assisted X-ray crystallography to solve the very first structures of the full-length human prion protein (PrP) and its C-terminal truncated version and solved the structure at 1.5 Å resolutions. The new structure provides the first structural elements leading to the disease-causing conversion. To determine whether this critical epitope is important to preventing and/or treating prion diseases, I used nanobody-assisted crystallography, a power tool to unveil local structural features of intrinsically disordered proteins. My solved structure supports the notion that the conserved palindromic sequence mediates β-enrichment in the PrP^{C} monomer as one of the early events in prion formation. I discovered that Nb484 is a unique crystallization chaperone for mouse prion (1.2 Å) and other pathological human mutants such as. V210I (1.5 Å) and E219K (1.5 Å).

Research Papers:

a) **Abskharon R**., Giachin G., Wohlkonig A., Soror S.H., Pardon E., Legname G., Steyaert J. (2014). Probing the N-Terminal β-Sheet Conversion in The Human Prion Protein Bound To A Nanobody, Journal of the American Chemical Society, 136; 3: 937-944.

b) Yuan J.*, Zhan A.Y.*, **Abskharon R*.**, Xiao X., Martinez M.C., Knealeg G., Jacqueline M., Lehmann S., Castillaj J., Steyaert J., Kong Q., Petersen R.B., Wohlkonig A., Zou W.Q. (2013). Recombinant Human Prion Protein Inhibits Prion Propagation in vitro, Scientific Reports- Nature Publishing group, 9; 3: 2911. PMCID: PMC3793212. ***Co-first author.**

c) **Abskharon R**., Ramboarin S., Hassan H.E., Gad W., Apostol M.I., Giachin G., Legname G., Steyaert J., Messens J., Soror S.H., Wohlkonig A. (2012). A novel expression system for production of soluble prion proteins in E. coli, Microbial Cell Factories, 10; 11:6. PMCID: PMC3283519. **Flagged highly accessed paper on Biomed central.**

d) **Abskharon R**., Soror S.H., Pardon E., Hassan H.E., Legname G., Steyaert J., Wohlkonig A. (2011). Combining in situ proteolysis and microseed matrix screening to promote crystallization of PrPC-nanobody complexes, Protein Engineering, Design, and Selection, 24;9: 737-41.

2. First Postdoctoral Career:

I focused on generating nanobodies as therapeutic approaches for protein-misfolding induced neurodegenerative diseases, such as Parkinson's disease and prion disease. I used a prion disease model to investigate the therapeutic values of nanobodies generated against PrP^{Sc}, the misfolded prion protein (PrP) that causes prion diseases. I have screened and characterized 35 nanobodies against different PrP confomers, including the normal prion protein (PrP^{C}), the misfolded PrP intermediate (PrPI) and the misfolded diseased causing PrP^{Sc}. While most of the nanobodies have high binding affinity for the normal prion protein (PrP^{C}), some are able to discriminate between the misfolded (PrPI and PrP^{Sc}) and normal (PrP^{C}) form. Interestingly, one of the nanobodies, Nb196, binds strongly to a misfolded neurotoxic PrP species, cytosolic PrP, both *in vitro* in test tubes and *ex vivo* in cultured cells and able to decrease the prion infectivity by 99%. Our data suggested that Nanobodies can assist in investigating the structural mechanism that governs the PrP misfolding. I also established two Nanobodies libraries by immunizing alpaca with various amyloid fibrils of prion and A-syn proteins.

Research Papers:

- **a)** Wang F, Wang X, **Abskharon** R, Ma J. (2018). Prion infectivity is encoded exclusively within the structure of proteinase K-resistant fragments of synthetically generated recombinant PrPSc. Acta Neuropathol Commun. 24,6(1):30. PMCID: PMC5921397.
- **b)** **Abskharon R**., Dang J., Elfarash A., Wang Z., Shen P., Zou S.L., Hassan S., Wang F., Fujioka H., Steyaert J., Mulaj M., Surewicz K. W, Castilla J., Wohlkonig A., Zou W.Q. (2017). Soluble polymorphic bank vole prion proteins induced by co-expression of quiescin sulfhydryl oxidase in E. coli and their aggregation behaviors. Microbial Cell Factories. 16:170. PMCID: PMC5628483.
- **c)** Zhan A.Y*., **Abskharon R***., Yuan J., Martinez C.M., Xiao X., Jacquelin M., Lehmann S., Steyaert J., Kong Q., Petersen B.R., Wohlkonig A., Zou W.Q. (2016). Quiescin-sulfhydryloxidase inhibits prion formation in vitro, Aging (Albany NY), 8, 12: 3419–3429. PMCID: PMC5270677. ***Co-first author**
- **d)** **Abskharon R**., Wang F., Vander Stel J.K., Sinniah K., Ma J. (2016). The role of the unusual threonine string in the conversion of prion protein, Scientific Reports- Nature Publishing Group, 6, 38877. PMCID: PMC5159806.

3. Second Postdoctoral Career: I joined **Dr. David Eisenberg's laboratory** in 2018 as a senior postdoctoral fellow with the aims of **a)** Using structure-based design to develop synthetic nanobody inhibitors of pathological tau aggregation, and **b)** Determine the high-resolution molecular structures of tau oligomers using tau-specific antibodies. For this project, I developed an expression system to produce a single chain antibody (scFv) in bacteria that specifically binds to tau oligomers. Furthermore, this antibody shows a potential effect for inhibiting tau aggregation and seeding by AD brain patients' samples. Additionally, I established the expression and the purification of several monoclonal antibodies that specifically bind to certain regions of Tau protein from hybridoma cell lines. When I joined the **Eisenberg laboratory,** I had the opportunity to work with many exceptional scientists in structure-based design field. This allowed me to design a panel of *de novo* nanobody inhibitors for tau seeding by extracts from autopsied brains of patients with Alzhimer's disease and other tauopathies.

Research Papers:

- **a)** **Abskharon R.**, Sawaya MR., Boyer DR., Cao Q, Nguyen BA., Cascio D., Eisenberg DS (2022). Cryo-EM structure of RNA-induced tau fibrils reveals a small C-terminal core that may nucleate fibril formation. PNAS. 119(15):e2119952119. PMID: 35377792.
- **b)** Jiang YX., Cao Qin., Sawaya MR., **Abskharon R**., Ge P., DeTure M., Dickson D., Fu J., Loo R., Loo J., Eisenberg D.S. (2022). Amyloid fibrils in disease FTLD-TDP are composed of TMEM106B not TDP-43. Nature. 605(7909):304-309. PMID: 35344984.
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4. **Other publications from my undergraduate carrier in Egypt**
   a) Hassan SH., Van Ginkel S., Hussein M., **Abskharon R**., Oh S. (2016). Toxicity assessment using different bioassays and microbial biosensors. Environment International, 92, 106–118.
   b) **Abskharon R**., Hassan S., Kabir M.H., Qadir S.A., Gad El-Rab S., Wang M.H. (2009). The Role of Antioxidants Enzymes of E. coli ASU3, a Tolerant Strain to Heavy Metals Toxicity, in combating oxidative stress. World Journal of Microbiology and Biotechnology, 26; 2: 241-247. DOI: 10.1007/s11274-009-0166-4.
   c) Hassan S.H., **Abskharon R**., Gad-Elrab S., Ahmed Shoreit A. (2008). Characterization of heavy metal resistant strain Pseudomonas aeruginosa isolated from polluted sites in Assiut, Egypt, Journal of Basic Microbiology, 48; 168–176.
   d) **Abskharon R**, Hassan SH, Gad-Elrab SM, Shoreit A (2008). Heavy metal resistant of E. coli isolated from wastewater sites in Assiut city, Egypt. Bulletin of Environmental Contamination and Toxicology 81; 309–315.

Complete List of Published Work in My Bibliography
https://pubmed.ncbi.nlm.nih.gov/?term=Abskharon&sort=date

Selected Conferences:

1. **Poster presentation:** Atomic structures of a single chain antibody that binds and inhibits seeding by tau oligomers. Antibody engineering and therapeutics conference 2019 take place at Marriott Marquis San Diego, California, December 9th - ,13th 2019.
2. **Poster presentation**: Atomic structures of a single chain antibody that binds and inhibits seeding by tau oligomers. Advances in Protein Science – Structure & Function, Engineering & Design A scientific conference, hosted by Amgen in 1050 Rancho Conejo Blvd Thousand Oaks, CA 91320, 10th August 2018.
3. **Poster presentation:** The influence of Prnpb polymorphisms and the conserved 4-threonine stretch of alpha-helix 2 on prion protein conversion, Tokyo, Japan. May 2016.
4. **Poster presentation:** Aglycosylated recombinant prion protein inhibits prion propagation in vitro. PRION 2013, in Banff, Alberta, Canada from May 2013.
5. **Poster presentation**: Quiescin-sulfhydryl oxidase inhibits prion formation in vitro. PRION 2013, in Banff, Alberta, Canada from May 2013.
6. **Oral presentation:** Crystal Structure of a full-length Human PrP/Nanobody complex. Prion, 2012, Amsterdam, Netherlands.

NEWS:

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

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