BIOGRAPHICAL SKETCH

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NAME: Hernando José Sosa

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

POSITION TITLE: Professor of Physiology and Biophysics

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
Universidad Central de Venezuela, Caracas, Venezuela	BS/Lic	1986	Biology
Brandeis University, Waltham, MA	PhD	1994	Biophysics
The Scripps Research Institute, La Jolla CA	Postdoc	1997	
University of California, San Diego, CA	Postdoc	2000	

A. Personal Statement

I am a Professor of Biochemistry, and my research focuses on mechanistic questions of cell function related to the cytoskeleton. I have a long experience using different forms of microscopy to study cytoskeletal proteins. As a graduate student in the laboratory of Dr. H. Huxley in Brandeis University using electron microscopy and fiber X-ray diffraction, we determined that the actin and myosin filaments of striated muscle were more elastic than previously thought. As a post-doc in the laboratory of R. Milligan at Scripps we obtained the first molecular models of kinesin-microtubule complexes using cryo-electron microscopy (cryo-EM). I also developed image-analysis methods to obtain 3D reconstructions of microtubules with helical discontinuities (seams). In my laboratory we developed a fluorescence-polarization microscopy method to determine the orientation and mobility of single molecules and have used this system to elucidate the mechanism of kinesin-based motility. Most recently, we have taken full advantage of the recent advances in the cryo-electron microscopy field to solve the structure of kinesin-microtubule complexes in different functional states and have produced the highest resolution structures to date of these complexes[1, 2, 3, 4].

Citations:

1. Benoit, M.P.M.H., A.B. Asenjo, and H. Sosa. (2018) Cryo-EM Reveals the Structural Basis of Microtubule Depolymerization by Kinesin-13s. Nature Communications 9: 1662. PMC5916938. https://doi.org/10.1038/s41467-018-04044-8
2. Benoit, M.P.M.H., Asenjo A. B., Paydar, M., Kwok, B. H. and H. Sosa. (2021) Structural basis of mechanochemical coupling by the mitotic kinesin KIF14. Nature Communications. 12: 3637. PMC8206134, https://doi.org/10.1038/s41467-021-23581-3
3. Hunter, B., Benoit, P.M.H., Asenjo A.B., Doubleday, C., Trofimova, D., Frazier, C., Shoukat, I., Sosa H. and J. S. Allingham. (2022) Kinesin-8-specific loop-2 controls the dual activities of the motor domain according to tubulin protofilament shape. Nature Communications 13: 4198. PMC9300613 , https://www.nature.com/articles/s41467-022-31794-3
4. Benoit, M.P.M.H., Rao, L., Asenjo, A.B. et al. Cryo-EM unveils kinesin KIF1A's processivity mechanism and the impact of its pathogenic variant P305L. Nature Communications 15, 5530 (2024). https://doi.org/10.1038/s41467-024-48720-4

B. Positions, Scientific Appointments, and Honors

Academic appointments

1994-97. Postdoctoral Fellow, Department of Cell Biology, the Scripps Research Institute.
1997-00 Postdoctoral Fellow, University of California, San Diego.
2000-07 Assistant Professor, Dept. of Physiology and Biophysics, Albert Einstein College of Medicine.
2007-19 Associate Professor, Dept. of Physiology and Biophysics, Albert Einstein College of Medicine.
2019- Professor, Dept. of Physiology and Biophysics, Albert Einstein College of Medicine.

Honors

1984-86 Assistant Student Fellowship, Venezuelan Institute for Scientific Research (IVIC).
1988-89 Gillette Fellowship, Gillette Fellows Program, Brandeis University.

Service

NIH Panels:

2001 Review panel. Special study section ZRG1 SSS-U. NIH, Center for Scientific Review.
2002 Review panel. Special study section ZRG1 SSS-U 02. NIH, Center for Scientific Review.
2002 Review panel Special emphasis study section, SSS-B. NIH, Center for Scientific Review.
2003 Review panel (ad hoc). Study section BBCB. NIH, Center for Scientific Review.
2004 Review panel. Special study section ZRG1 F04B. NIH, Center for Scientific Review.
2005 Review panel. Special study section ZRG1 F04B. NIH, Center for Scientific Review.
2005 Study section MSFC. NIH, Center for Scientific Review.
2009 Review panel. Special study section ZRG1 BCMB-T 41. NIH, Center for Scientific Review.
2010 Review panel. (Ad hoc). Study section MSFC. NIH, Center for Scientific Review.
2011 Review panel. Special study section IMST-J (03) NIH, Center for Scientific Review.
2015 Special study section co-chair. ZGM1 CBB-0 (EM). Regional Consortia for High Resolution Cryo-electron Microscopy.
2011-2017 Review panel. MSFC study section member. NIH, Center for Scientific Review.
2017 Special study section. ZGM1 CBB-3 (CR) Regional Consortia for High Resolution Cryo-electron Microscopy (U24).
2019 Review Panel. Study section ZRG1 BST-T (P41 applications)
2020 Review Panel. Study section ZRG1 CB-T (55) R PAR 17-190: (R35 applications)
2023 Co-chair, Special emphasis panel. ZRG1 CDB-B 30 I, Shared Instrumentation: Electron Microscopy. NIH, Center for Scientific Review.

Other:

2001 Ad hoc reviewer for the Department of Defense (DOE).
2005-2006 Ad hoc reviewer for National Science Foundation (NSF).
2015 Reviewer. American Heart Association (AHA, Spring, PC 3 Committee).

C. Contributions to Science

1) As a graduate student in the laboratory of Dr. Hugh E. Huxley at Brandeis University, I developed EM and fiber X-ray diffraction techniques to measure the length of muscle filaments during contraction. This work allow us to obtain a better estimate of the compliance of myosin and actin filaments than was previously possible and led us to determine that the filaments were more compliant than previously thought. This finding prompted a reinterpretation of previous physiology experiments and a revision of muscle contraction models.

a) Sosa, H., D. Popp, G. Ouyang and H.E. Huxley. (1994). Ultrastructure of skeletal muscle fibers studied by a plunge quick freezing method. Myofilament length. Biophys. J. 67: 283-292.

b) Huxley, H.E., A. Stewart, H. Sosa and T. Irving. (1994). X-ray diffraction measurements of the extensibility of the actin and myosin filaments in contracting muscle. Biophys. J. 67: 2411-2421. (PMC1225358)

2) I developed a real space method to obtain 3D reconstructions of pseudo-helical filaments (microtubules with seams) as a post-doc in the laboratory of Ron A. Milligan at the Scripps Research Institute. We also produced the first atomic model of a kinesin-like protein motor domain bound to the microtubule. This model defined the kinesin-motor-domain-tubulin interface and binding configuration.

a) Sosa, H. and R.A. Milligan. (1996). Three dimensional structure of ncd decorated microtubules obtained by a Back-Projection Method. J. Mol. Biol. 260: 743-755.

b) Sosa, H., Dias, P., Hoenger, A., Whittaker, M., Wilson-Kubalek, E., Sablin, E., Fletterick, R. J., Vale, R. D., and Milligan R.A. (1997). A model for the microtubule-ncd motor protein complex obtained by cryo-electron microscopy and image analysis. Cell 90: 217-224.

3) In collaboration with Dr. Erwin Peterman, I developed a fluorescence polarization microscopy (FPM) technique that allows determining the orientation and mobility of single molecules in real time. We develop this technique when we were post-docs in the laboratories of Dr. Larry Goldstein and Dr. W.E. Moerner at UCSD and Stanford. In my laboratory, I have continued the development and use of this technique to reveal important structural features of kinesin motors. Using FPM, we determined the orientation of the neck-linker and motor domain of conventional kinesin at different point s of the ATPase cycle and during processive movement. We found how the neck-linker orientation changed in response to nucleotides and determined that the two motor domains were bound to the microtubule for most of the ATPase cycle but at limiting [ATP] one of the heads becomes mobile (tethered). These findings highlighted the importance of the relative position of the two motor domains and the connecting neck-linker domain to coordinate their catalytic activities for processive motility.

a) Sosa, H., Peterman, E. J. G., Moerner, W. E., and Goldstein, L. S. B. (2001). ADP-Induced Rocking of the Kinesin Motor Domain Revealed by Single-Molecule Fluorescence Polarization Microscopy. Nature Struct. Biol. 8: 540-544.

b) Asenjo, A, Weinberg, Y. and Sosa H. (2006). Nucleotide Binding and Hydrolysis Induces a Disorder-Order Transition in the Kinesin Neck-Linker Region. Nature Struct. & Mol. Biol. 13: 648-654.

c) Asenjo A. B. and Sosa H. A Mobile Kinesin-Head Intermediate in the ATP-waiting State. (2009) Proc. Natl Acad. Sci. USA. 106: 5657-5662. (PMC2667011).

d) Chatterjee, C., Benoit, M. P., DePaoli V., Diaz-Valencia, J. D. Asenjo, A. B. Gerfen G.J., Sharp D.J. and H. Sosa. (2016). Distinct interaction modes of the kinesin-13 motor domain with the microtubule. Biophys. J. 110: 1593–1604. (PMC4833770)

4) We discovered in my laboratory that the kinesin13 motor domain has an additional tubulin binding site that induces the formation of a distinct spiral assembly consisting of a curved tubulin protofilament with bound kinesin-13 motor domains wrapped around a microtubule. This additional tubulin binding site promotes microtubule bundling and helps focusing microtubules at spindle poles in-vivo. The spirals assemblies also allowed using cryo-EM and helical 3-D reconstruction methods to obtain a 3D structure of a microtubule depolymerization intermediate (curved tubulin protofilament kinesin-13 motor domain complex). Most recently we have pushed cryo-EM structural analysis methods of these and other kinesin MT complexes to near atomic resolution (2.6 - 4.0 Å). These studies are providing an unprecedent amount of structure-function information on the kinesin-microtubule system and are revealing how different kinesins are adapted for motile or microtubule depolymerization activities.

a) Tan, D. Asenjo, A.B., Mennella V., Sharp, D.J. and Sosa, H. (2006), Kinesin-13s Form Rings Around Microtubules. J. Cell Biol. 175: 25-31. (PMC2064489)

b) Zhang, D., A.B. Asenjo, M. Greenbaum, L. Xie, D.J. Sharp, and H. Sosa, A second tubulin binding site on the kinesin-13 motor head domain is important during mitosis. PLoS ONE, 2013. 8(8): p. e73075. (Free full text).

c) Benoit, M.P.M.H., A.B. Asenjo, and H. Sosa. (2018) Cryo-EM Reveals the Structural Basis of Microtubule Depolymerization by Kinesin-13s. Nature Communications 9: 1662. PMC5916938

Complete List of Published Work in:

https://pubmed.ncbi.nlm.nih.gov/?term=Sosa+H+and+(Asenjo+or+Benoit+or+Peterman+or+Chretien+or+Kielian+or+Sharp+or+Moerner+or+Milligan+or+Goldstein+or+Huxley+or+Padron+or+Argibay+not+Baden)

BIOGRAPHICAL SKETCH

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NAME: Ana Belen Asenjo

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

POSITION TITLE: Research 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
Universidad Central de Venezuela, Caracas, Venezuela	BS/Lic	1987	Biochemistry
Brandeis University, Waltham, MA	PhD	1994	Cell & Molecular Biology
University of California, San Diego, CA	Postdoc	1994	Genetics and Cell Biology

A. Personal Statement

I have a strong interest in understanding how biological enzymes work and developing and improving methods and protocols to study them. Since my undergraduate years I have used a variety of biochemical and biophysical techniques to determine the mechanism of action of key biological molecules such as lipoproteins, opsins, taste receptors and molecular motors. I have accumulated expertise in a wide range of areas such as cell biology, molecular biology, protein chemistry, fluorescence microscopy and electron microscopy. This expertise is very well suited for the structure and functional analysis we do in the Sosa's laboratory. I now oversee, design or perform many of the experiments carried in the laboratory and I am also responsible for training all new personnel coming to the laboratory.

Citations:

1. Benoit, M.P.M.H., A.B. Asenjo, and **H. Sosa**. (2018) Cryo-EM Reveals the Structural Basis of Microtubule Depolymerization by Kinesin-13s. Nature Communications 9: 1662. PMC5916938, http://www.ncbi.nlm.nih.gov/pmc/articles/pmc5916938/

2. Benoit, M.P.M.H., Asenjo A. B., Paydar, M., Kwok, B. H. and **H. Sosa**. (2021) Structural basis of mechanochemical coupling by the mitotic kinesin KIF14. Nature Communications 12: 3637. PMC8206134, https://doi.org/10.1038/s41467-021-23581-3

B. Positions, Scientific Appointments, and Honors

Academic appointments

1994-1997	Postdoctoral Research Scientist, Laboratory of Dr. Charles Zuker, Howard Hughes Medical Institute Department of Biology. UCSD.
1998-2000	Senior Staff Scientist, Seashell Technology, LLC., La Jolla CA.
2000-2004	Research Associate, Dept. of Physiology and Biophysics, Albert Einstein College of Medicine.
2004-2012	Associate, Dept. of Physiology and Biophysics, Albert Einstein College of Medicine.

2012- Research Assistant Professor. Dept. of Biochemistry, Albert Einstein College of Medicine

Honors

1984-87 Assistant Student Fellowship, Venezuelan Institute for Scientific Research (IVIC).

C. Contributions to Science

1) As a graduate student at Brandeis University I purified for the first time the pigments (opsins) responsible for animal color vision and determine their absorption spectrum. To solve the problem of very low purification yields from natural sources I chemically synthesized the genes for the three human color pigments, and cloned them into a eukaryotic-expression system for purification. Using site directed mutagenesis we identified the amino acids that are responsible of the green and red human color vision discrimination and identified a key chloride binding site in the green/red color pigments that modulates their absorption spectra.

   a) Oprian D.D., **Asenjo A.B.**, Lee N., and Pelletier, S. 1991. Design, Chemical Synthesis, and Expression of Genes for the three Human Color Visual Pigments. Biochemistry 30:11367-11372.

   b) Wang Z., **Asenjo A.B.**, and Oprian D.D. 1993. Identification of the Chloride Binding Site in Human Red and Green Visual Pigments. Biochemistry 32:2125-2130.

   c) **Asenjo A.B.**, Rim J., and Oprian D.D. 1994. Molecular Determinants of Human Red/Green Color Discrimination. Neuron. 12:1131-1138.

2) As a postdoctoral fellow in Professor Zuker laboratory at UCSD I started the effort that led to the cloning of the molecular components of the taste transduction system. For this I conducted a genetic screen for Drosophila mutants in the taste transduction pathway and identified 20 mutants in the taste pathway. Using a second strategy I did single cell libraries from taste receptor cells, using cDNA substation against non taste tissue we identified multiple clones that were specific for the taste receptors cells. By *insitu* hybridization we identified three specific genes for taste cells.

   a) Zuker C. S., Adler J. E., **Asenjo A.B**. and Lindemeir. 1998. Genes Preferentially Expressed in Mammalian Taste-Receptor Cells. Record of Invention UC Case # 98-122-1.

3) As senior research scientist at Seashell Technology I developed Plasmon resonance particles associate with RNA for use *in situ* hybridization. With this technology we were able to label several RNAs in a cell with a distinct fluorescence spectrum for simultaneous colocalization..

   a) S. J. Oldenburg, J. J. Mock, J. Glass, **A.B. Asenjo**, C. Genick, D. R. Smith, D. A. Schultz, S. Schultz. (2002) "Metal Nanoparticles for Biodetection".Proceedings of SPIE, 4810, 36-41

4) I contributed to elucidate the walking mechanism of kinesin-1 and how it coordinates the catalytic activity of its two motor domains by determining the orientation of the neck--linker and motor domains during processive movement using fluorescence polarization microscopy.

   a) **Asenjo, A.B**., Kronh, N. and Sosa H. (2003). Configuration of the two kinesin motor domains during ATP hydrolysis. Nat. Struct. Mol. Biol. 10: 836-842.

   b) **Asenjo, AB.**, Weinberg, Y. and Sosa, H. (2006) Nucleotide binding and hydrolysis induces a disorder-order transition in the kinesin neck-linker region. Nat. Struct. Mol. Biol. 13: 648-654.

microtubules.

c) **Asenjo, AB** and Sosa, H (2009). A Mobile Kinesin-Head Intermediate During the ATP-waiting State. PNAS 106: 5657-5662.

5) I have contributed to our understanding of the molecular mechanism of microtubule depolymerization by members of the kinesin-13 family by 1) Co-discovering of a characteristic unusual form of assembly among kinesins (a tubulin-kinesin-13 complex wrapped around microtubules). 2) Discovered of a novel tubulin binding site on the kinesin-13 MD that helps bundling microtubules at the spindle poles during mitosis. 2) Providing the cryo-em structure of kinesin-13-microtubule depolymerization intermediate (kinesin-13 motor domain in complex with curved tubulin) that revealed a major structural change on the tubulin heterodimer induced by kinesin-13 binding. In addition, we have resolved other Kinesins Microtubules complexes to near atomic resolution (2.6 to 4.0 Å). These studies provide inside to how a common core of kinesin motor adapts to walk and depolymerized microtubules

a) Tan, D., **Asenjo, A.B**., Mennella, V., Sharp, D.J. and Sosa H. (2006) Kinesin-13s form rings around microtubules. J. Cell Biol. 175:25-31.

b) Zhang, D., **A.B. Asenjo**, M. Greenbaum, L. Xie, D.J. Sharp, and H. Sosa, A second tubulin binding site on the kinesin-13 motor head domain is important during mitosis. (2013) PLoS ONE, 8(8): p. e73075.

c) **Asenjo, A.B.**., C. Chatterjee, D. Tan, V. DePaoli, W. J. Rice, R. Diaz-Avalos, M. Silvestry, and H. Sosa, Structural model for tubulin recognition and deformation by kinesin-13 microtubule depolymerases. Cell Reports, 2013. 3(3): p. 759-768.

d) Arora, K., Talje L., **Asenjo, A.B.,**Andersen P., Atchia K., Joshi M., Sosa H., Allingham J.S. and Kwok B.H. (2014). Kif 14 Binds Tightly to Microtubules and Adopts a Rigor-Like Conformation Journal of Molecular Biology, 426:2993-2996

e) Chatterjee, C.,Benoit, P.M.H., De Paoli, V., Diaz-Valencia, J.D., **Asenjo, A.B**., Gerfen, G. J., Sharp, D.J., and Sosa H. Distinct interaction modes of the kinesin-13 motor domain with the microtubules. Biophysical J.(2016), 110:1593-1604.

f) Benoit, M.P.M.H**., A.B. Asenjo**, and H. Sosa. (2018) Cryo-EM Reveals the Structural Basis of Microtubule Depolymerization by Kinesin-13s. Nat Commun. 11;9(1):2748. doi: 10.1038/s41467-018-04858-6.PMID: 29992962 9: 1662.

g) Benoit, M.P.M.H., Asenjo A.B., Paydar M.,Dhakal S., Kwok B.H. and H. Sosa. Structural Basis of mechano-chemical coupling by the mitotic KIF14. Nat Commun. 2021 2021 Jun 15;12(1):3637. doi: 10.1038/s41467-021-23581-3.PMID: 34131133.

h) Hunter B, Benoit MPMH, **Asenjo AB**, Doubleday C, Trofimova D, Frazer C, Shoukat I, Sosa H, Allingham JS. 2022 Kinesin-8-specific loop-2 controls the dual activities of the motor domain according to tubulin protofilament shape.Nat Commun. Jul 20;13(1):4198. doi: 10.1038/s41467-022-31794-3.PMID: 35859148

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https://www.ncbi.nlm.nih.gov/pubmed/?term=asenjo++ab