BIOGRAPHICAL SKETCH

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NAME: Sondek, John E.

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POSITION TITLE: 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 Rochester, Rochester, NY	B.S.	05/1985	Biochemistry
The Johns Hopkins University, Baltimore, MD	Ph.D.	05/1992	Biochemistry
Yale University, New Haven, CT	Post-doc	10/1996	Structural Biology

A. Personal Statement

I am focused on understanding signal transduction cascades controlled by heterotrimeric G proteins ($G\alpha\beta\gamma$) and small GTPases related to Ras. I primarily use biophysical techniques to dissect these cascades at atomic and molecular resolution to understand and manipulate cellular processes controlled by heterotrimeric G proteins and small GTPases. I was involved in the determination of the first atomic-resolution structures of transducin, the heterotrimeric G protein that controls human vision. More recent work in this vein includes our studies on: *i*) R7-family RGS proteins, *ii*) guanine nucleotide exchange factors (GEFs) that active Rho-family GTPases, and *iii*) phospholipase C isozymes that are directly activated by $G\alpha$-GTP subunits and $G\beta\gamma$ dimers. Among other work, we are currently using these studies to produce biosensors to monitor these signaling cascades in real-time in cells and guide drug discovery programs to treat uveal and cutaneous melanomas.

I am committed to training our next generation of scientists. In the past six years, I have trained seven graduate students, including one M.D.-Ph.D. student, as well as seven post-doctoral associates. Of note, eight of this cadre obtained independent fellowships while under my mentorship. In the past, I have served as the Director of Graduate Studies for the Molecular and Cellular Biophysics Training Program at UNC-Chapel. In addition to service teaching, I volunteer to teach under-represented minorities through the Biophysical Society Summer Course in Biophysics.

Structural biology is a mainstay of my research. This research requires extensive infrastructure that is best supported through multi-user facilities. To support these facilities, I currently serve as Director of the UNC Center for Structural Biology.

Ongoing and completed research projects from the past three years that I would like to highlight include:

P30-CA016086 (Earp) NIH/NCI 06/01/97 – 11/30/25
Structural biology
Sub-project of parent award in support of the Lineberger Comprehensive Cancer Center. Award supports personnel associated with the UNC Center for Structural Biology as a Shared Resource.

R01-CA258993 (Zhang/Sondek/Pearce) NIH/NCI 05/01/21 – 04/30/25
A high-throughput platform to identify selective allosteric inhibitors of the PLC-gamma isozymes
Project to identify inhibitors of PLC-γ1 and -γ2 for the treatment of leukemias and lymphomas.

21-1815 (Zhang/Sondek) Cure Alzheimer's Fund 03/01/21 – 02/28/23
Small molecule activators of PLC-γ2 as novel therapeutic for Alzheimer's disease
Identification of small molecules that specifically activate PLC-γ2 and mimic the enhanced phospholipase activity of the protective variant, PLC-γ2 (P522R).

R01-GM057391 (Sondek) NIH/NIGMS 05/01/98 – 05/31/22
Regulation of phospholipase C
Project focused on the molecular regulation of PLCs with special emphasis on the PLC-γ isozymes.

R01-MH112205 (Roth) NIH/NIMH 01/01/17 – 12/31/21
Molecular details of psychoactive drug action
Structural analyses of the serotonin (5-$HT2_A$, -$HT2_B$) and dopaminergic receptors (D2 and D4).

R01-GM120291 (Sondek/Liu) NIH/NIGMS 09/01/16 – 08/31/21
Inhibition of small GTPases and G proteins to treat human disease
GTPases that are constitutively active contribute to a variety of diseases, most notably cancer. This project seeks to develop new methodologies using directed evolution to produce peptidomimetic inhibitors of overly active GTPases that will be specifically targeted to tumors.

(Sondek) Leukemia and Lymphoma Society 07/01/19 – 06/30/21
PLC-γ isozymes: unexploited drug targets for the treatment of leukemia and lymphoma
Proposal to identify inhibitors of PLC-γ1 and -γ2 for the treatment of leukemias and lymphomas.

R01-HL130404 (Bergmeier) NIH/NHLBI 07/01/16 – 06/30/21
Rap signaling platelet homeostasis and vascular hemostasis
Project designed to understand the reciprocal regulation of Rap1 by CalDAGGEF-1 and Rasa3 required for platelet aggregation.

B. Positions, Scientific Appointments, and Honors

Professional positions

07/14 - present	Faculty Director, UNC Center for Structural Biology
12/06 - present	Professor of Biochemistry and Biophysics, UNC at Chapel Hill
12/06 - present	Professor of Pharmacology, UNC at Chapel Hill
10/02 - 12/06	Associate Professor of Biochemistry & Biophysics, UNC at Chapel Hill
10/02 - 12/06	Associate Professor of Pharmacology, UNC at Chapel Hill
11/98 - present	Member, UNC Lineberger Comprehensive Cancer Center
10/96 - 10/02	Assistant Professor of Biochemistry & Biophysics, UNC at Chapel Hill
10/96 - 10/02	Assistant Professor of Pharmacology, UNC at Chapel Hill
01/96 - 09/96	Research Scientist, Yale University

Honors

2013	Winner, GlaxoSmithKline Discovery Fast Track Competition
08/09 - 08/11	Chair, Gordon Research Conference, "Mechanisms of Cell Signaling"
07/99 - 06/03	Pew Scholar in the Biomedical Sciences
01/93 - 01/96	Damon Runyon - Walter Winchell Fellowship
01/91 - 01/92	Institutional Research Grant, The Johns Hopkins University
03/89 - 03/92	Institute for Biophysical Research on Macromolecular Assemblies Predoctoral Fellowship, The Johns Hopkins University
09/81 - 09/85	Regents Scholarship, State of New York
09/85 - 09/86	NIH Predoctoral Fellowship, The Johns Hopkins University
09/81 - 09/85	Centennial Prize Scholarship, University of Rochester

C. Contribution to Science

For a complete list of peer-reviewed publications (104) please consult:
http://www.ncbi.nlm.nih.gov/pubmed?term=sondek%20j[Author]

Protein plasticity and humanized antibodies
In the early 1980's it became routine to introduce single substitutions into proteins using oligonucleotide site-directed mutagenesis to understand how proteins evolved. What was essentially unheard of at the time was to extend similar studies to understand how proteins respond to insertion and deletions. In general, insertions and

deletions were assumed to be either deleterious if introduced into blocks of secondary structure or essentially neutral if they occurred in loops. For my Ph.D. work with Dr. David Shortle at Johns Hopkins University, I sought to quantify the energetic and structural effects of one to two amino acid insertions or deletions in proteins. In a systematic analysis of dozens of mutant proteins, we found *that insertions and deletions were remarkably well tolerated in model proteins*; changes in stability were comparable to the equivalent substitution at flanking sites. Indeed, insertions into secondary structural elements often led to register shifts or bulges as determined by protein crystallography and we coined the phrase, *"α-aneurysm"* for the first identification of the structural equivalent of a β-bulge in α-helices. Additional work led us to conclude that the most common mutation, deletion of Phe508, in the cystic fibrosis conductance regulator (*CFTR*) protein contributes to cystic fibrosis through localized rearrangements of the surrounding β-sheets that reduce stability but not function. It has since become clear that deletion of Phe508 results in misfolding and poor intracellular trafficking of the CFTR protein due to lack of stability, but once trafficked correctly, will function normally.

My graduate work was inspired by earlier studies in protein evolution that clearly pointed toward roles of insertions and deletions in the expansion of protein families and the creation of new functions. In particular, I was motivated by earlier indications that the natural process of somatic hypermutation incorporated insertions and deletion during the directed evolution of mature antibodies. Our work in this field produced more facile methods for the creation of large numbers of mutant proteins using site-directed mutagenesis. Indeed, this work led to an *international patent* that was licensed to the publicly traded, mid-cap biotechnology company, Morphosys AG, to produce *humanize antibodies*. Royalties are estimated to be more than one million dollars and emphasize the importance of this work to the pharmaceutical industry.

1. Sondek, J. and Shortle, D. (1999). Synthesis of diverse and useful collections of oligonucleotides. U.S. patent 5,869,644. dddd
2. Keefe, L.J., Sondek, J., Shortle, D. and Lattman, E.E. (1993). The α aneurysm: A structural motif revealed in an insertion mutant of staphylococcal nuclease. *Proc. Natl. Acad. Sci. U.S.A.* **90**, 3275-3279.
3. Sondek, J. and Shortle, D. (1992). A general strategy for random insertion and substitution mutagenesis: Substoichiometric coupling of trinucleotide phosphoramidites. *Proc. Natl. Acad. Sci. U.S.A.* **89**, 3581-3585.
4. Sondek, J. and Shortle, D. (1990). Accommodation of single amino acid insertions by the native state of staphylococcal nuclease. *Proteins: Struct., Func., and Gen.* **7**, 299-305.

Signaling by G proteins

Dr. Heidi Hamm was an excellent biochemist at the University of Illinois at Chicago with expertise studying vision while Dr. Paul Sigler was an established crystallographer at the University of Chicago when they established a collaboration to understand visual signal transduction. This collaboration led to the first atomic-resolution structure of a heterotrimeric G protein alpha subunit when Dr. Joe Noel determined the structure of active transducin in 1994. David Lambright and I quickly followed this initial work with a series of papers in *Nature* describing structures of additional states of transducin including the intact heterotrimer. These papers provided an *extensive understanding of the regulatory cycle of heterotrimeric G proteins at atomic resolution.* This work and similar studies by the groups of Drs. Alfred Gilman and Stephen Sprang at the University of Texas Southwestern Medical Center were *foundational in establishing our current understanding of signaling by G proteins*.

I continue to study signaling by G proteins. My early work at UNC Chapel Hill included a detailed study of inhibition of Gα subunits by GoLoco motif-containing proteins and the first structural description of the unconventional Gβ subunit, Gβ5, bound to an essentially full-length RGS9.

The papers listed below have been cumulatively cited over 2200 times according to the Web of Science (Thomson Reuters).

1. Kimple, R.J., Kimple, M.E., Betts, L., Sondek, J. and Siderovski, D.P. (2002). Structural determinants for GoLoco-induced inhibition of G nucleotide release. *Nature* **416**, 878-881.
2. Sondek, J., Bohm, A., Lambright, D.G., Hamm, H.E. and Sigler, P.B. (1996). Crystal structure of a G protein βγ dimer at 2.1 Å. *Nature*, **379**, 369-374.
3. Lambright, D.G., Sondek, J., Bohm, A., Skiba, N.P., Hamm, H.E. and Sigler, P.B. (1996). The 2.0 Å crystal structure of a heterotrimeric G protein. *Nature*, **379**, 311-319.
4. Sondek, J., Lambright, D.G., Noel, J.P., Hamm, H.E. and Sigler, P.B. (1994). GTPase mechanism of G proteins from the 1.7 Å crystal structure of transducin α·GDP·AlF4-. *Nature* **372**, 276-279.

Activation of Rho GTPases

He was not even a graduate student in my lab, but Mr. Kent Rossman asked me so many questions about the purification and structural biology of Dbl-family proteins that we would eventually collaborate on over twenty papers related to these proteins. Dbl-family proteins comprise the major set of activators of Rho GTPases in humans and together with Dr. David Worthylake, Kent and I described the first atomic-resolution complex of a Dbl-family protein in complex with a Rho-family GTPases. This work was *essential for understanding how Dbl-family proteins directly activate Rho GTPases* and was published in Nature in 2000. Our subsequent work in this field would *detail how Dbl-family protein are regulated by membranes* where they operate. We would also go on to explain the *structural rules that dictate the specific pairings* between the 70 human Dbl-family proteins and the three major Rho-family GTPases: RhoA, Rac1 and Cdc42.

Our review of this field in 2005 has been cited over 1,000 times based on Thomson Reuters' Web of Science, placing it in the top 1% of cited papers in the field of cell biology and indicative of our major impact in this area.

I continue to study Dbl-family proteins and our most recent work is focused on the creation of FRET-based biosensor of Dbl-family proteins using general design principles based on core regulation of these proteins. These biosensors are being used to map the spatiotemporal activation of Dbl-family proteins in cells to be correlated with similar work monitoring Rho GTPases.

1. Marston, D.J., Vilela, M., Huh, J., Ren, J., Azoitei, M.L., Glekas, G., Danuser, G., Sondek, J. and Hahn, K.M. (2020). Multiplexed GTPase and GEF biosensor imaging enables network connectivity analysis. *Nature Chem. Biol.* **16**, 826-833.
2. Rossman, K.L., Der, C.J. and Sondek, J. (2005). GEF means GO: Turning on Rho GTPase with guanine nucleotide exchange factors. *Nat. Rev. Mol. Cell. Biol.* **6**, 167-180.
3. Rossman, K.L, Worthylake, D.K., Snyder, J.T., Siderovski, D.P., Campbell, S.L. and Sondek, J. (2002). A crystallographic view of interactions between Dbs and Cdc42: PH domain-assisted guanine nucleotide exchange. *EMBO J.* **21**, 1315-1326.
4. Worthylake, D.K., Rossman, K.L. and Sondek, J. (2000). Crystal structure of Rac1 in complex with the guanine nucleotide exchange region of Tiam1. *Nature* **408**, 682-688.

Regulation of phospholipase C isozymes

I started as an Assistant Professor at UNC-Chapel Hill in 1996 and immediately began a collaboration with Dr. Ken Harden (UNC-Chapel Hill) to understand the biology of phospholipase C (PLC) isozymes and their activation by heterotrimeric G proteins. Our collaboration lasted until 2014 when Dr. Harden retired. During this period, we published 25 papers together describing the regulation of PLC isozymes. PLCs hydrolyze phosphatidylinositol 4,5-bisphospate (PIP_2) to produce the second messengers inositol 1,4,5-trisphosphate and diacylglycerol. PIP_2 as well as its second messengers control a plethora of downstream events including levels of intracellular calcium and the activation of PKC isozymes. Furthermore, humans possess 13 distinct PLC isozymes that are activated by a myriad of inputs including Ras- and Rho-family GTPases, heterotrimeric G proteins and receptor tyrosine kinases. Therefore, PLCs occupy an important *nexus between extracellular stimuli and intracellular responses including migration and proliferation*. Our work focused on understanding the regulation of PLCs isozymes and in 2008, we formulated a *coherent framework that explained the near-universal autoinhibition of PLC isozymes as well as the capacity of diverse inputs to activate these phospholipases*. This framework has withstood the test of time and continues to be used by many researchers in this field. More recently, this framework has been used to explain activating mutations in PLC-γ isozymes that contribute to *inflammatory diseases and cancer*.

1. Hussain, M., Cummins, M.C., Endo-Streeter, S., Sondek, J., and Kuhlman, B. (2021). Designer proteins that competitively inhibit Gαq by targeting its effector site. J Biol Chem, 101348. PMCID: PMC8633581
2. Hajicek N, Keith NC, Siraliev-Perez E, Temple BRS, Huang W, Zhang Q, Harden TK and Sondek J (2019). Structural basis for the activation of PLC-γ isozymes by phosphorylation and cancer-associated mutations. *eLife* **8**, e51700. PMCID: PMC7004563.
3. Charpentier, T.H., Waldo, G.L., Barrett, M.O., Huang, W. Zhang, Q., Harden, T.K. and Sondek, J. (2014). Membrane-induced allosteric control of phospholipase C-β isozymes. *J. Biol. Chem.* **289**, 29545-29557. PMCID: PMC4207972.
4. Waldo, G. L., Ricks, T. K., Hicks, S. N., Cheever, M. L., Kawano, T., Tsuboi, K., Wang, X., Montell, C., Kozasa, T., Sondek, J. and Harden, T. K. (2010). Kinetic scaffolding mediated by a phospholipase C-β and Gq signaling complex. *Science* **330**, 974-980. PMCID: PMC3046049.

New targets to treat cancer

Our structural and biophysical studies often require us to develop new methods to produce and assay pure proteins. Consequently, these methods and reagents are often easily adaptable to high-throughput screens. In recent years, we have *developed and patented high-throughput screens* to identify chemical modulators of: i) the Ras superfamily of GTPases, ii) phospholipase C isozymes, and iii) Gα subunits of heterotrimeric G proteins. The *identification of inhibitors to any of these proteins have immediate ramifications for the treatment of cancer as well as many other human diseases*. For example, the aberrant activation of Rac1 GTPase is implicated in most cutaneous melanomas. Similarly, mutated and constitutively active Gαq or Gα11 drive about 90% of uveal melanomas while constitutively active PLC-γ1 contributes to ~20% of cutaneous T cell lymphomas. Other potential therapeutic areas include asthma that can be controlled by inhibition of Gαq and rheumatoid arthritis that is exacerbated by active PLC-γ2. We have enlisted the help of several large screening operations: GlaxoSmithKline, AstraZeneca, and the NIH-funded Molecular Libraries Production Centers Network to screen these targets. In addition, I have co-founded a biotech firm to commercialize these technologies.

1. Huang, W., Carr, A.J., Hajicek, N., Sokolovski, M., Siraliev-Perez, E., Hardy, P.B., Pearce, K.H., Sondek, J., and Zhang, Q. (2020). A high-throughput assay to identify allosteric inhibitors of the PLC-γ isozymes operating at membranes. Biochemistry *59*, 4029-4038. PMCID: PMC8081235
2. Huang W, Wang X, Endo-Streeter S, Barrett M, Waybright J, Wohlfeld C, Hajicek N, Harden TK, Sondek J and Zhang Q (2018). A membrane-associated, fluorogenic reporter for mammalian phospholipase C isozymes. *J. Biol. Chem.* **293**, 1728-1735. PMCID: PMC5798302.
3. Charpentier, T.H., Waldo, G.L., Lowery-Gionta, E.G., Krajewski, K., Strahl, B.D., Kash, T.L., Harden, T.K. and Sondek, J. (2016). Potent and selective peptide-based inhibitors of the heterotrimeric G protein, Gαq. *J. Biol. Chem.* **291**, 25608-25616. PMCID: PMC27742837.
4. Sondek, J., Harden, T.K., Waldo, G.L., Barrett, M.O. and Charpentier, T.H. (2016). Methods and compositions for modulating Gαq signaling. U.S. provisional patent application no. 14/398,678.