

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: Sondek, John E.

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

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.	1985	Biochemistry
The Johns Hopkins University, Baltimore, MD	Ph.D.	1992	Biochemistry
Yale University, New Haven, CT	Post-doc	1992-96	Structural Biology

A. Personal Statement

I am focused on understanding signal transduction through 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 in order 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 include our studies on: *i*) R7-family RGS proteins, *ii*) guanine nucleotide exchange factors (GEFs) that activate 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 Faculty Director of the Structural Biology Core for the Lineberger Comprehensive Cancer Center.

B. Positions and Honors**Professional positions**

1/96 - 9/96	Research Scientist, Yale University
10/96 - 10/02	Assistant Professor of Pharmacology, UNC at Chapel Hill
10/96 - 10/02	Assistant Professor of Biochemistry & Biophysics, UNC at Chapel Hill
11/98 - present	Member, UNC Lineberger Comprehensive Cancer Center
10/02 - 12/06	Associate Professor of Pharmacology, UNC at Chapel Hill
10/02 - 12/06	Associate Professor of Biochemistry & Biophysics, UNC at Chapel Hill
12/06 - present	Professor of Pharmacology, UNC at Chapel Hill
12/06 - present	Professor of Biochemistry and Biophysics, UNC at Chapel Hill

Honors

9/81 - 9/85	Regents Scholarship, State of New York
9/81 - 9/85	Centennial Prize Scholarship, University of Rochester
9/85 - 9/86	NIH Predoctoral Fellowship, The Johns Hopkins University
3/89 - 3/92	Institute for Biophysical Research on Macromolecular Assemblies Predoctoral Fellowship, The Johns Hopkins University
1/91 - 1/92	Institutional Research Grant, The Johns Hopkins University
1/93 - 1/96	Damon Runyon - Walter Winchell Fellowship
7/99 - 6/03	Pew Scholar in the Biomedical Sciences
8/09 – 8/11	Chair, Gordon Research Conference, “Mechanisms of Cell Signaling”
2013	Winner, GlaxoSmithKline Discovery Fast Track Competition

C. Contribution to Science

For a complete list of peer-reviewed publications (91) please consult:
[http://www.ncbi.nlm.nih.gov/pubmed?term=sondek%20j\[Author\]](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. What was much less developed was our understanding of how these substitutions affected the structure and stability of proteins. What was essentially unheard of at the time was to extend similar studies to understand how proteins responds 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 small, one to two amino acid insertions or deletions in proteins. In a systematic analysis of dozen 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 in the cystic fibrosis conductance regulator (CFTR) protein – deletion of Phe508 – 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 inspired 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 also led to more facile methods for the production 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, for the production of humanize antibodies. Royalties are estimated to be in excess of 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.
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 visual signaling transduction 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 that provided an extensive understanding of the regulatory cycle of heterotrimeric G proteins at atomic-resolution. This work and similar studies by a second collaboration 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 G α subunits by GoLoco motif-containing proteins and the first structural description of the unconventional G β subunit, G β 5, 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 α 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 $\beta\gamma$ 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·AlF $_4^-$. *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 and 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 800 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. 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.
2. Snyder, J.T., Worthylake, D.K., Rossman, K.L., Betts, L., Pruitt, W.M., Siderovski, D.P., Der, C.J. and Sondek, J. (2002). Structural basis for the selective activation of Rho GTPases by Dbl exchange factors. *Nature Struct. Bio.* **9**, 468-475.
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 Asst. Prof. 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 hydrolyzed phosphatidylinositol 4,5-bisphosphate (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. Most recently, this framework has been used to explain activating mutations in PLC- γ isozymes that contribute to inflammatory diseases and cancer.

1. Gresset, A., Hicks, S. N., Harden, T. K., and Sondek, J. (2010). Mechanism of phosphorylation-induced activation of phospholipase C- β isozymes. *J. Biol. Chem.* **285**, 35836-35847. PMID: PMC2975207.
2. 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. PMID: PMC3046049.
3. Hicks, S.N., Jezyk, M.R., Gershburg, S., Seifert, J.P., Harden, T.K. and Sondek, J. (2008). General and versatile autoinhibition of PLC isozymes. *Mol. Cell* **3**, 383-394. PMID: PMC27023622.
4. Jezyk, M.R., Snyder, J.T., Gershburg, S., Worthylake, D.K., Harden, T.K. and Sondek, J. (2006). Crystal structure of Rac1 bound to its effector phospholipase C- β 2. *Nat. Struct. Mol. Biol.* **13**: 1135-40.

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) G α subunits of heterotrimeric G proteins. The identification of inhibitors to any of these groups of proteins have immediately ramifications for the treatment of cancer as well as many other human diseases. For example, the aberrant activation of Rac1 GTPase has been implicated in the majority of cutaneous melanomas; while mutated, constitutively active G α q or G α 11 drive about 90% of uveal melanomas; and mutated, constitutively active PLC- γ 1 is found in ~20% of cutaneous T cell lymphomas. Other potential therapeutic areas include asthma that can be controlled by inhibition of G α q and rheumatoid arthritis that is exacerbated by active PLC- γ 2. 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. Charpentier, T.H., Barrett, M.O., Waldo, G.L., Harden, T.K. and Sondek, J. (2012). Methods and composition for modulating G α q signaling. U.S. provisional patent application no. 61/642,368.
2. Huang, W., Hicks, S.N., Sondek, J. and Zhang, Q. (2011). A fluorogenic, small molecule reporter for mammalian phospholipase C isozymes. *ACS Chem. Biol.* **6**, 223-228. PMID: PMC3312000.
3. Zhang, Q., Huang, W.G., Sondek, J. and Hicks, S. (2011). Fluorogenic sensors for phospholipase C isozymes. U.S. patent 8,703,437.
4. Sondek, J. and Rojas, R. (2010). Methods of identifying chemical modulators of Ras superfamily GTPases activity. U.S. patent 7,807,400.

D. Research Support

On-going research support

(Sondek) AstraZeneca 09/30/14 – 09/30/15

Identification and development of inhibitors of G α q to treat uveal melanoma

G α q is a major driver of uveal melanoma. In collaboration with AstraZeneca, we will screen their compound deck of ~2 million small molecules for lead hits that inhibit constitutively active G α q.

(Sondek) Melanoma Research Foundation 08/01/14 – 02/28/16

Fast cycling mutants of G α q and G α 11

G α q and G α 11 are substituted at either of two sites in the majority of uveal melanomas. However, a large set of less frequent, single substitutions in these proteins might similarly drive uveal melanoma. This idea will be tested with this grant.

P01-GM103723 (Hahn) NIH/NIGMS 09/30/13 – 07/31/18
Spatiotemporal dynamics of GEF-GTPase networks
Program Project designed to understand the real-time, dynamic interplay of Rho GTPases and the guanine nucleotide exchange factors that activate them using biosensors and several model cell systems. Dr. Sondek is Co-PI of Project 1: “GEF biosensors for living cells.”

A13-0866-001 (Sondek) Melanoma Research Foundation 01/25/13 – 03/31/16
Interdiction of signaling by Gαq to treat ocular melanoma
Gαq and related Gα11 are constitutively active due to mutation in approximately 85% of uveal melanomas. This project is centered on the use of structure-based peptidomimetics to inhibit the capacity of Gαq to bind effectors. No cost extension.

R01-CA161160 (Sondek / Yeh) NIH/NCI 09/01/12 – 06/30/16
Small molecule inhibition of Rho GTPase activation to probe signaling cascades
Proposal to refine high-throughput screens to identify modulators of guanine nucleotide exchange factors that activate Rho-family GTPases.

R01-GM057391 (Sondek / Harden) NIH/NIGMS 04/01/12 – 03/31/16
Regulation of phospholipase C
Project designed to understand the molecular regulation of PLCs by heterotrimeric G proteins and low-molecular weight GTPases.

R01-GM086558 (Zhang) NIH/NIGMS 09/01/11 – 08/31/16
Development of small molecule ARFGAP regulators to dissect cell signaling
QS11 is a small molecule that directly interdicts ArfGAPs. This project is designed to understand QS11 function.

Pending research support

(Bergmeier) NIH 03/30/15 – 02/28/19
Molecular and cellular regulation of the CalDAG-GEFI/Rap1B signaling module
The activation of Rap1B by CalDAG-GEFI is necessary for platelet aggregation and this collaboration with Prof. Wolfgang Bergmeier (UNC Chapel Hill) seeks to understand the regulation of these proteins in order to develop therapeutics to treat clotting disorders.

Overlap

None.

Completed research support

(Sondek) UNC/CCCNE 11/01/13 – 10/31/14
Nanoparticle-based delivery of peptides to inhibit Gαq and treat uveal melanoma
A collaboration with Profs. R. Liu and L. Huang (UNC Chapel Hill) to deliver peptides into cells that have been specifically designed to inhibit constitutively active Gαq – a major driver of ocular melanoma.

R01-GM098894 (Sondek / Zhang) NIH/NIGMS 09/26/11 – 08/31/14
High-throughput screens to identify modulators of phospholipase C isozymes
Project designed to refine high-throughput screens to identify specific and efficacious modulators of PLCs.

R01-GM081881 (Sondek) NIH/NIGMS 08/01/07-07/31/12
Gβ5/RGS proteins and GPCR signaling
Project to understand the role of Gβ5/RGS dimers in signaling downstream of G protein coupled receptors.

R01-GM062299 (Sondek) NIH/NIGMS 04/10/08 – 03/31/12
Functions and regulation of Dbl-family guanine nucleotide exchange factors
Define regulatory mechanism controlling Dbl-family exchange factors operating at biological membranes