BIOGRAPHICAL SKETCH

NAME: James Kenneth Chen

eRA COMMONS USER NAME: CHEN.JAMES

POSITION TITLE: Professor and Chair of Chemical and Systems Biology; Professor of Developmental Biology; Professor of Chemistry

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Harvard College, Cambridge, MA	A.B.	6/1991	Chemistry
Harvard University, Cambridge, MA	Ph.D.	12/1998	Chemistry and Chemical Biology
Marine Biological Laboratory, Woods Hole, MA	N/A	7/1998	Embryology
Johns Hopkins School of Medicine, Baltimore, MD	Postdoc	5/2003	Developmental Biology

A. Personal Statement

My laboratory has pioneered the use of chemical tools to interrogate the signaling pathways that underlie embryonic patterning and oncogenesis. We have discovered and/or characterized several small-molecule modulators of the Hedgehog (Hh) pathway, including the first Smoothened (SMO) antagonists (cyclopamine and SANTs 1-4), first SMO agonists (SAG and purmorphamine), first cytoplasmic dynein inhibitors (ciliobrevins), and several compounds that act at the level of GLI transcription factors (e.g., HPI-1). We have similarly identified novel genetic regulators of GLI transcription factor function, such as the putative Rho GTPase-activating protein family member ARHGAP36 and primary cilium components. In parallel with these cell-based studies, we have pursued new technologies for interrogating developmental pathways in whole organisms. For example, we have invented caged morpholino oligonucleotides that enable light- or enzyme-triggered gene silencing and used these tools to investigate the roles of T-box transcription factors in notochord, medial floor plate, and paraxial mesoderm development. We have also developed new lanthanide-based methods for ultrasensitive *in vivo* imaging.

B. Positions and Honors

Positions and Employment

Department of Chemical and Systems Biology, Stanford University
- 2003 – 2010 Assistant Professor
- 2010 – 2016 Associate Professor
- 2016 – present Professor and Chair

Department of Developmental Biology, Stanford University
- 2012 – 2016 Associate Professor
- 2016 – present Professor

Department of Chemistry, Stanford University
- 2003 – 2010 Assistant Professor (by courtesy)
- 2010 – 2016 Associate Professor (by courtesy)
- 2016 – 2019 Professor (by courtesy)
- 2019 – present Professor

Other Experience and Professional Memberships

Society Memberships
- 1989 – present Member, American Chemical Society
- 2009 – present Member, Society for Developmental Biology

2016 – present Member, International Zebrafish Society

Editorial Board Memberships

2008 – present Editorial Board Member, *Zebrafish*

2009 – present Editorial Board Member, *Cell Chemical Biology*

Peer-Review Activities (past five years)

2013 – 2015 Reviewer, NSF CAREER Award

2013 Reviewer, NIH Study Section (PAR-11-221:Collaborative Interdisciplinary Team Science in NIDDK Research Areas)

2015 Reviewer, NIH Special Emphasis Panel (PA-11-184:T32 NRSA Institutional Research Training Grants and PA-14-044: K01 Mentored Research Scientist Development Award)

2015 Reviewer, NIH Study Section (SBCB: Synthetic and Biological Chemistry B)

2018 Reviewer, NIH Special Emphasis Panel (ZRG1 BCMB-A 51R: NIH Transformative Research Awards)

2018 Reviewer, NIH Study Section (SBCA: Synthetic and Biological Chemistry A)

Consulting (past five years)

2017 – present Scientific Advisory Board Member, Vibliome Therapeutics

Stanford University Service

2003 – present Faculty Director, High-Throughput Bioscience Center

2011 – present Director, Molecular Basis of Medicine Scholarly Concentration

2013 – present Executive Committee Member, ChEM-H Institute

2014 – 2016 Executive Committee Member, School of Medicine Faculty Senate

Regional, National, and international Service

2012, 2014, 2015 Instructor, Introduction to Chemical Biology Short Course, University of São Paulo, São Paulo, Brazil

2012 – 2016 Member, Research Committee of the American Heart Association, Western States Affiliate

2017 Co-Organizer, 2017 Society for Developmental Biology West Coast Meeting

Honors and Awards

1991 – 1994 National Science Foundation Predoctoral Fellowship

1994 – 1995 American Chemical Society Organic Chemistry Graduate Fellowship

1999 – 2002 Damon Runyon-Walter Winchell Postdoctoral Fellowship

2002 – 2003 American Cancer Society Postdoctoral Fellowship

2003 W. Barry Wood, Jr. Postdoctoral Award, Johns Hopkins University

2004 – 2006 Kimmel Scholar Award

2005 – 2007 Basil O'Connor Starter Scholar Research Award, March of Dimes Foundation

2005 – 2008 Terman Fellow, Stanford University

2005 Astellas USA Foundation Award

2006 Stanford University Faculty Fellow

2006 – 2008 Brain Tumor Society Award/Rachel Molly Markoff Research Chair

2008 – 2011 American Cancer Society Research Scholar Award

2008 – 2013 NIH Director's Pioneer Award

2009 Nature Biotechnology SciCafe Award for Outstanding Research Achievement

2013 – 2015 Innovation Award, Alex's Lemonade Stand Foundation

2013 – 2017 NSF INSPIRE Award

2019 Rocek Lectureship in Chemical Biology, University of Illinois at Chicago

C. Contributions to Science

1. **Chemical technologies for *in vivo* biology.** As a scientist deeply interested in both chemistry and developmental biology, I have explored how chemical tools can advance our understanding of tissue formation. My laboratory invented the first caged morpholino oligonucleotides (MOs), which enable light-inducible inhibition of RNA splicing and translation in whole organisms. Our strategy was to tether the 25-base RNA-targeting MO to a shorter complementary MO through a photocleavable linker, thereby generating an oligonucleotide hairpin that resists RNA binding. We next developed the first cyclic caged MOs, demonstrating that the MO activity can be conformationally controlled through oligonucleotide curvature. Using this approach, we also designed and synthesized the first caged MO that can be enzymatically

activated, enabling tissue-specific gene silencing in transgenic animals that express the triggering enzyme. Most recently, we collaborated with Prof. Pehr Harbury (Stanford) to develop new methods for ultrasensitive, autofluorescence-free imaging of lanthanide-based probes. I am the corresponding author for the four papers listed below and three other studies in this area. I also co-authored a study describing light-inducible protein degradation in zebrafish embryos.

a. Shestopalov, I. A., Sinha, S., and **Chen, J. K.** (2007) Light-controlled gene silencing in zebrafish embryos. *Nat. Chem. Biol*. 3: 650-651. PMCID: PMC3288381
b. Yamazoe, S., Shestopalov, I. A., Provost, E., Leach, S. D., and **Chen, J. K.** (2012) Cyclic caged morpholinos: Conformationally gated probes of embryonic gene function. *Angew. Chem. Int. Ed.* 51: 6908-6911. PMCID: PMC3434341
c. Yamazoe, S., McQuade, L. E., and **Chen, J. K.** (2014) Nitroreductase-activated caged morpholino oligonucleotides for in vivo gene silencing. *ACS Chem. Biol.* 9: 1985-1990. PMCID: PMC4168795
d. Cho, U., Riordan, D. P., Ciepla, P., Kocherlakota, K. S., **Chen, J. K.***, and Harbury, P. B.* (2018) Ultrasensitive optical imaging with lanthanide lumiphores. *Nat. Chem. Biol.* 14: 15-21. PMCID: PMC5726931.

2. **Mechanistic studies of vertebrate development and regeneration.** A major strength of our research program is our ability to go beyond proof-of-concept demonstrations for new technologies and conduct in-depth biological investigations. For example, we have used caged MOs and zebrafish models to study how T-box transcription factors such as No tail-a (Ta; the zebrafish ortholog of Brachyury) and T-box 16 (Tbx16) drive key aspects of early development. Our unconventional approach has: (1) revealed dynamic changes in Ta function during notochord development and identified new regulators of notochord vacuolization; (2) uncovered a role for Tbx16 in collinear *hox* gene activation, supporting a new model for anterior-posterior somite patterning; and (3) led to our discovery that the two T-box proteins regulate the morphogenetic movements of medial floor plate progenitors (and not their specification as previously believed). Most recently, we have used mouse models to study the roles of homeodomain-interacting protein kinase 4 (HIPK4) in spermiogenesis. I am the corresponding author on the four papers listed below and two additional zebrafish-related research articles. I have also co-authored five other studies of zebrafish development and a clinical report on a novel missense GLI3 variant.

a. Shestopalov, I. A., Pitt, C. L. W., and **Chen, J. K.** (2012) Spatiotemporal resolution of the Ntla transcriptome in axial mesoderm development. *Nat. Chem. Biol.* 8: 270-276. PMCID: PMC3288381
b. Payumo, A. Y., Walker, W. J., McQuade, L. E., Yamazoe, S., and **Chen, J. K.** (2015) Optochemical dissection of T-box gene-dependent medial floor plate development. *ACS Chem. Biol.* 10: 1466-75. PMCID: PMC4672996
c. Payumo, A. Y., McQuade, L. E., Walker, W. J., Yamazoe, S., and **Chen, J. K.** (2016) Tbx16 regulates *hox* gene activation in mesodermal progenitor cells. *Nat. Chem. Biol.* 12: 694-701. PMCID: PMC4990471
d. Crapster, J. A.*, Rack, P. G., Hellmann, Z. J., Behr, B., Li, Y., Lin, J., Zeng, H., and **Chen, J. K.*** (2019) HIPK4 is essential for mammalian spermiogenesis. *bioRxiv* 703637.

3. **Chemical modulators of the Hedgehog pathway**. Since I first learned of the plant-derived teratogen cyclopamine, I have been interested in small-molecule modulators of developmental signaling pathways. As a postdoctoral fellow with Prof. Philip Beachy (then at Johns Hopkins School of Medicine), I discovered that cyclopamine directly inhibits Smoothened (SMO), a transmembrane receptor in the Hedgehog (Hh) pathway. I subsequently identified the first synthetic SMO antagonists (SANTs 1-4) and agonists (SAG and purmorphamine). Our work has helped advance the development of Hh pathway-targeting therapeutics, some of which are now being used to treat advanced basal cell carcinoma and medulloblastoma. More recently, my laboratory has focused on Hh pathway inhibitors that act downstream of SMO (e.g., HPIs 1-4, JK184, etc.). Among these compounds are the first specific chemical antagonists of cytoplasmic dyneins 1 and 2 (ciliobrevins), which we discovered through a high-throughput phenotypic screen and characterized in collaboration with Prof. Tarun Kapoor (Rockefeller University). We recently developed ciliobrevin analogs with greater potency and dynein 2 selectivity, enhancing their utility as mechanistic probes. I have authored eleven studies of chemical Hh pathway modulators, including the four listed below.

a. Sinha, S. and **Chen, J. K.** (2006) Purmorphamine activates the Hedgehog pathway by targeting Smoothened. *Nat. Chem. Biol.* 2: 29-30.
b. Hyman, J. M., Firestone, A. J., Heine, V. M., Zhao, Y., Ocasio, C. A., Han, K., Sun, M., Rack, P. G., Sinha, S., Wu, J. J., Solow-Cordero, D. E., Jiang, J., Rowitch, D. H., and **Chen, J. K.** (2009) Small-

molecule inhibitors reveal multiple strategies for Hedgehog pathway blockade. *Proc. Natl. Acad. Sci. U. S. A*. 106: 14132-14137. PMCID: PMC2721821

c. Firestone, A. J., Weinger, J. S., Maldonado, M., Barlan, K., Langston, L. D., O'Donnell, M. D., Gelfand, V. I., Kapoor, T. M.*, and **Chen, J. K.*** (2012) Small-molecule inhibitors of the AAA+ ATPase motor cytoplasmic dynein. *Nature* 484: 125-129. PMCID: PMC3321072

d. See, S. K., Hoogendoorn, S., Chung, A. H., Ye, F., Steinman, J. B., Sakata-Kato, T., Miller, R. M., Cupido, T., Zalyte, R., Carter, A. P., Nachury, M. V., Kapoor, T. M., and **Chen, J. K.** (2016) Cytoplasmic dynein antagonists with improved potency and isoform selectivity. *ACS Chem. Biol.* 11: 53-60. PMCID: PMC4715766

4. **Genetic regulators of the Hedgehog pathway.** In addition to the chemistry-driven studies described above, I have pursued the identification of new Hh pathway regulators through genetic screens. My laboratory collaborated with Prof. Matthew Scott (Stanford) to conduct a focused siRNA screen, through which we identified neuropilins as positive regulators of the Hh pathway. Investigations by other laboratories have subsequently implicated neuropilins in medulloblastoma, the most common pediatric brain tumor. My research group also completed a genome-scale cDNA overexpression screen, leading to our discovery of ARHGAP36 as a potent GLI transcription factor activator. This atypical Rho GTPase-activating protein acts downstream of SMO, traffics through and requires the primary cilium, and functions in a neural-specific manner *in vivo*. Consistent with its oncogenic potential, ARHGAP36 is upregulated over 100-fold in GLI-dependent murine medulloblastomas that have acquired resistance to SMO antagonists, and it is highly expressed in certain human medulloblastomas and neuroblastomas. Most recently, I collaborated with Profs. Maxence Nachury and Michael Bassik (Stanford) to conduct a genome-wide CRISPR-based screen for Hh signaling modulators. Our study has yielded the most comprehensive compendium of pathway regulators to date, including several new ciliopathy genes.

   a. Hillman, R. T., Feng, B. Y., Ni, J., Woo, W.-M., Milenkovic, L., Hayden Gephart, M. G., Teruel, M. N., Oro, A. E., **Chen, J. K.**, and Scott, M. P. (2011) Neuropilins are positive regulators of Hedgehog signal transduction. *Genes Dev*. 25: 2333-2346. PMCID: PMC3222900

   b. Rack, P. G., Ni, J., Payumo, A. Y., Nguyen, V., Crapster, J. A., Novestadt, V., Kool, M., Jones, D. T. W., Mich, J. K., Firestone, A. J., Pfister S. M., Cho, Y.-J., and **Chen, J. K.** (2014) Arhgap36-dependent activation of Gli transcription factors. *Proc. Natl. Acad. Sci. U. S. A*. 111: 11061-11066. PMCID: PMC4121834

   c. Breslow, D. K.*, Hoogendoorn, S., Kopp, A. R., Morgens, D. W., Vu, B. K., Han, K., Li, A., Hess, G. T., Bassik, M C., **Chen, J. K.***, and Nachury, M. V.* (2018) A CRISPR-based screen for Hedgehog signaling provides insights into ciliary function and ciliopathies. *Nat. Genet*. 50: 460-471. PMCID: PMC5862771.

Complete List of Published Work (67 peer-reviewed and 9 non-peer-reviewed articles):

My Bibliography
http://www.ncbi.nlm.nih.gov/sites/myncbi/james.chen.1/bibliography/40444676/public/?sort=date&direction=descending

D. Research Support

Ongoing Research Support

R35 GM120730 (Chen) 4/1/18 – 3/31/23
NIH/NIGMS
Title: Chemical tools for developmental biology
The goals of this project are to develop molecular probes for studying the mechanisms that regulate tissue formation and regeneration.
Role: PI

N/A (Flynn) 11/1/17 – 10/31/20
Male Contraception Initiative
Title: Development of selective HIPK4 kinase inhibitors as viable non-hormonal male contraceptives
The goal of this project is to develop Type 2 inhibitors of HIPK4 and evaluate their effects on spermiogenesis.
Role: Co-I

Translational Research Program Pilot Grant (Chen) 7/1/19 – 6/30/20
Stanford SPARK
Title: Small-molecule inhibitors of colorectal cancer metabolism
The goal of this project is to develop ALDH1B1-selective antagonists and evaluate their efficacy against colorectal cancer.
Role: PI

N/A (Chen) 8/19/19 – 8/18/21
Stanford Cancer Institute-Goldman Sachs Foundation
Title: Targeting cancer stem cell metabolism with aldehyde dehydrogenase antagonists
The goal of this project to develop chemical inhibitors of ALDH1 enzymes and evaluate their efficacy against cancer stem cells.
Role: PI

R61 HD099720 (Chen) 9/15/19 – 8/31/21
NIH/NICHD
Title: Development of allosteric HIPK4 inhibitors as non-hormonal male contraceptives
The goal of this project is to develop Type 3 and 4 inhibitors of HIPK4.
Role: PI

Completed Research Support

R21 HD078385 (Chen) 9/22/14 – 8/31/17
NIH/NICHD
Title: Chemical genetic dissection of HIPK4-dependent Hedgehog pathway activation
The goal of this project is to determine the substrates of HIPK4 and their roles in Hh pathway regulation.
Role: PI

NSF INSPIRE Award (Chen) 10/1/13 – 9/30/17
NSF
Title: Lanthanide-based probes for visualizing RNAs and proteins in live organisms
The goal of this project is to develop lanthanide-based reagents for *in vivo* imaging.
Role: PI

R01 GM113100 (Chen) 7/1/15 – 3/31/18
NIH/NIGMS
Gli1-selective inhibitors of the Hedgehog signaling pathway
The goals of this project are to determine the target of a chemical inhibitor of Gli1 function, develop analogs with improved pharmacological properties, and evaluate their efficacy in murine models of medulloblastoma.
Role: PI

P50 GM107615 (Ferrell) 9/30/13 – 6/30/18
NIH/NIGMS
Title: Systems biology of collective cell decisions
The primary aim of this center grant is to understand the systems-level basis for cellular decision making. Our project goal is to study collective cellular processes during embryogenesis with morpholino-based tools.
Role: Co-I

R01 GM108952 (Chen) 8/1/14 – 7/31/18
NIH/NIGMS
Title: Development of lariat-shaped caged morpholinos for optochemical gene regulation
The goal of this project is to develop new strategies for the optical control of morpholino oligonucleotides.
Role: PI

R01 GM112728 (Deiters) 9/1/15 – 7/31/18
NIH/NIGMS
Chemically triggered morpholino antisense oligonucleotides
The goals of this project are to develop small-molecule- and enzyme-triggered morpholinos to achieve spatiotemporal control of organismal gene function in optically inaccessible tissues.
Role: Co-I