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: Huang, Xin-Yun

eRA COMMONS USER NAME (credential, e.g., agency login): XYHUANG1

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
Wuhan University, China	B.S.	5/1983	Cell Biology
Sun Yatsen University, China (CUSBEA Program)	Certificate	6/1984	English
University of Houston, Houston, TX	Ph.D.	5/1988	Biochemistry and Biophysics
Columbia University, New York, NY	Postdoc	9/1991	Biochemistry and Molecular Biophysics
Harvard University, Cambridge, MA	Res. Assoc.	6/1994	Biochemistry and Molecular Biology

A. Personal Statement

My laboratory has been investigating the cellular signaling mechanisms by G-protein-coupled receptors and heterotrimeric G-proteins for more than 28 years. For this specific research project, we have contributed to the understanding of the activation of GPCRs and G-proteins. We have solved the X-ray crystal structure of a physiologically relevant oligomeric state of a GPCR, β_1-adrenergic receptor. Using systematic mutagenesis studies in vitro and in cells, we have demonstrated that Linker 2 of Gs is critical for Gs-activation by β_1-adrenergic receptor. We have solved the cryo-EM structures of the complexes of β_1-adrenergic receptor with Gs, Gi and a chimera Gi/s mutant, as well as the complexes of β_1-adrenergic receptor with Gs bound with a full agonist (isoproterenol), a partial agonist (dobutamine), and an antagonist (cyanopindolol). We have also solved the cryo-EM structures of the complexes of LPA, LPA receptor 1 and Gi, of S1P, S1P receptor 1 and Gi, as well as of Siponimod, S1P receptor 1 and Gi.

I have been actively involved in teaching and training graduate students and postdoctoral fellows. I have been participating in teaching in the graduate school and the medical college at Cornell. I have trained 23 PhD students and 34 postdoctoral fellows.

Ongoing projects that I would like to highlight include:

R01 GM138676
Huang (PI)
08/01/20-05/31/24
Molecular Basis of β_1-Adrenergic Receptor Function

Four publications directly related to this project:

a. Huang, J., Chen, S., Zhang, J.J., and **Huang, X.-Y.** 2013. Crystal structure of oligomeric beta1-adrenergic G protein-coupled receptors in ligand-free basal state. Nature Structural & Molecular Biology 20: 419-425. PMCID: PMC361857.

b. Su, M., Zhu, L., Zhang, Y., Paknejad, N., Dey, R., Huang, J., Lee, M-Y., Williams, D., Jordan, K.D., Eng, E.T., Ernst, O.P., Meyerson, J.R., Hite, R.K., Walz, T., Liu, W., and **Huang, X.-Y.** 2020. Structural Basis of the Activation of Heterotrimeric Gs-Protein by Isoproterenol-Bound β 1-Adrenergic Receptor. Mol. Cell 80: 59-71. PMID: 32818430 PMCID: PMC7541785. DOI: 10.1016/j.molcel.2020.08.001.

c. Alegre, K.O., Paknejad, N., Su, M., Lou, J.S., Huang, J., Jordan, K.D., Eng, E.T., Meyerson, J.R., Hite, R.K., and **Huang, X.-Y.** 2021. Structural basis and mechanism of activation of two different families of G proteins by the same GPCR. Nature Structural & Molecular Biology 28:936-944. PMID: 34759376. PMCID: PMC8719444. DOI: 10.1038/s41594-021-00679-2

d. Liu, S., Paknejad, N., Zhu, L., Kihara, Y., Ray, M., Chun, J., Liu, W., Hite, R.K., and Huang, X.-Y. 2022. Differential activation mechanisms of lipid GPCRs by lysophosphatidic acid and sphingosine 1-phosphate. Nature Communications. Feb 8;13(1):731. doi: 10.1038/s41467-022-28417-2.

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2001- Professor, Department of Physiology and Biophysics, Cornell University Weill Medical College

1998-2001 Associate Professor, Department of Physiology and Biophysics, Cornell University Weill Medical College, New York City, New York.

1994-1998 Assistant Professor, Department of Physiology and Biophysics, Cornell University Weill Medical College, New York City, New York.

1991-1994 Associate Research Scientist, Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts.

1988-1991 Postdoctoral Fellow, Department of Biochemistry and Molecular Biophysics, Columbia University College of Physicians & Surgeons, New York City, New York.

Other Experience and Professional Memberships

2021 NIH ZRG1 IMST-U(02) study section
2019 NIH ZRG1 Cell Biology-J study section
2010-2014 Co-Director, Graduate Program in Physiology, Biophysics and Systems Biology, Weill Graduate School of Medical Sciences of Cornell University
2009 Chairman, NIH NIGMS ZGM1 Panel
2007 DOD CDMRP IMP-3 Peer Review Panel
2005 NIH Cellular Signaling and Dynamics Study Section
2004- Reviewer for Wellcome Trust
2004- Reviewer for the National Science Foundation of China
2004- Reviewer for the Research Grants Council of Hong Kong
2002-2007 Editorial Board, Journal of Biological Chemistry
1999-2004 NIH Biochemistry Study Section
1996, 1998, 2000: External Reviewer, Alberta Cancer Board (Canada)

Honors

2012- Fellow, AAAS
2001-2003 Charles H. Leach Foundation Scholar
2000-2004 Irma Hirschl Career Scientist Award
2000-2003 Established Investigator, American Heart Association
1999-2003 Research Scholar, American Cancer Society
1995-1998 Beatrice F. Parvin Investigator of the American Heart Association
1994-1997 Cornell Scholar

C. Contributions to Science

1. My laboratory was among the first groups to investigate the cellular signaling pathways from G-protein-coupled receptors to protein tyrosine phosphorylation and the MAPK pathways. At that time, the cellular signaling community was mostly on the signaling from growth factor receptor tyrosine kinases (such as EGFR) to the MAPK pathways, and the GPCR/G-protein filed was on the classical cAMP and calcium pathways. We used genetic and biochemical approaches to identify signaling components from GPCRs to MAPKs. Our work on G-protein signaling has contributed to the revelation of new signaling pathways, especially the stimulation of protein tyrosine phosphorylation by G-protein-coupled receptors and G-proteins. It is now well accepted that GPCRs, like receptor tyrosine kinases, can modulate physiological and pathological functions through cellular protein tyrosine phosphorylation.
    a. Wan, Y., Kurosaki, T., and **Huang, X.-Y.** 1996. Tyrosine kinases in activation of the MAP kinase cascade by G-protein-coupled receptors. Nature 380:541-544.
    b. Bence, K., Ma, W., Kozasa, T., and **Huang, X.-Y.** 1997. Direct stimulation of Bruton's tyrosine kinase by Gq-protein α-subunit. Nature 389:296-299.
    c. Ma, Y.-C., and **Huang, X.-Y.** 1998. Identification of the binding site for Gqα on its effector Bruton's tyrosine kinase. Proc. Natl. Acad. Sci. USA 95: 12197-12201.
    d. Jiang, Y., Ma, W., Wan, Y., Kozasa, T., Hattori, S., and **Huang, X.-Y.** 1998. The G protein Gα12 stimulates Bruton's tyrosine kinase and a rasGAP through a conserved PH-BM domain. Nature 395: 808-813.

2. We have contributed to the understanding of the connections between heterotrimeric G-proteins to small GTPases, as well as the understanding of the regulation of actin cytoskeletal reorganization and cell migration by GPCRs and by G-proteins. Small GTPases are critical regulators of actin cytoskeletal reorganization, and some GPCRs could regulate cell morphological changes. We have shown that GPCR- and G-protein-induced tyrosine phosphorylation of GEFs for small GTPases could increase the GEF activity and activate small GTPases such as Rac and RhoA.
    a. Aghazadeh, B., Lowry, W., **Huang, X.-Y.,** and Rosen, M. 2000. Structural basis for relief of autoinhibition of the Dbl homology domain of proto-oncogene Vav by tyrosine phosphorylation. Cell 102: 625-633.
    b. Ma, Y.-C., Huang, J., Ali, S., Lowry, W., and **Huang, X.-Y.** 2000. Src tyrosine kinase is a novel direct effector of G proteins. Cell 102: 635-646.
    c. Zheng, B., Ma, Y.-C., Ostrom, R.S., Lavoie, C., Gill, G.N., Insel, P.A., **Huang, X.-Y.**, and Farquhar, M. G. 2001. RGS-PX1, a GAP for Gα_s and sorting nexin in vesicular trafficking. Science 294: 1939-1942.
    d. Guo, D., Tan, Y.-c., Wang, D., Madhusoodanan,K.S., Zheng, Y., Maack, T., Zhang, J.J., and **Huang, X.-Y.** 2007. A Rac-cGMP signaling pathway. Cell 128: 341-355. PMCID: PMC1965458.

3. We have contributed to the cellular signaling mechanisms controlling tumor cell migration and invasion. We have shown that fascin is essential for tumor cell migration, invasion, and metastasis. We have solved the X-ray crystal structures of the wild-type fascin and four fascin mutants to define the active and inactive configurations of fascin, and have revealed the structural basis for the conformational changes of fascin during the actin-bundling process. We have identified the actin-binding sites on fascin and have shown that point mutations in any one of these actin-binding sites abolish the actin-bundling activity of fascin in vitro and filopodial formation in cells. We have used cryo-electron tomography and analyzed the in vitro reconstituted unconstrained three-dimensional bundles formed by fascin and actin filaments. To identify fascin inhibitors, we have screened small-molecule chemical libraries and have identified small molecule hits that inhibit the biological function of fascin and tumor cell migration, invasion, and metastasis. We have used biophysical methods to investigate the direct interaction between the small-molecule inhibitors and fascin. We have solved the X-ray crystal structure of a complex of fascin and an inhibitor. We have chemically optimized the fascin inhibitor hits and obtained potent fascin inhibitors. We have performed in vitro biochemical, cell-based, and animal model studies with the optimized fascin inhibitors for their efficacy and toxicities. IND-enabling GLP toxicology studies in rats and dogs for a lead fascin inhibitor showed that fascin inhibitors are safe with a clean toxicology profile (no toxicity was observed). FDA-approved Phase 1 clinical trials have been completed, and our fascin inhibitor NP-G2-044 is safe for cancer patients and with

antitumor efficacy. Phase 2 clinical trials are ongoing. We hope these fascin inhibitors can be used to benefit the cancer patients.

a. Chen, L., Yang, S., Jakoncic, Zhang, J.J., and **Huang, X.-Y.** 2010. Migrastatin analogues target fascin to block tumor metastasis. Nature 464: 1062-1066.
b. Huang, F., Han, S., Xing, B., Huang, J., Liu, B., Bordeleau, F., Reinhart-King, C.A., Zhang, J.J., and Huang, X.-Y. 2015. Targeted inhibition of fascin function blocks tumor invasion and metastatic colonization. Nature Communications 6: 7465 doi: 10.1038/ncomms8465. PMID: 26081695.
c. Huang, J., Dey, R., Wang, Y., Jakoncic, J., Kurinov, I., and **Huang, X.-Y.** 2018. Structural insights into the induced-fit inhibition of fascin by a small-molecule inhibitor. J. Mol. Biol. 430(9):1324-1335. doi: 10.1016/j.jmb.2018.03.009.
d. Wang, Y., Song, M., Liu, M., Zhang, G., Zhang, X., Li, M.O., Ma, X., Zhang, J.J., and **Huang, X.-Y**. 2021. Fascin inhibitor increases intratumoral dendritic cell activation and anti-cancer immunity. Cell Reports 35:108948. doi: 10.1016/j.celrep.2021.108948. PMCID: PMC8050791.

4. We have revealed the signaling mechanisms by which $G\alpha_{13}$ participates in the control of actin cytoskeletal reorganization, cell shape changes, and cell migration. We have demonstrated that $G\alpha_{13}$ is required for GPCR- and RTK-induced cell migration. We have shown that $G\alpha_{13}$ is critical for adult angiogenesis as well as for embryonic angiogenesis in mouse models. We have revealed that $G\alpha_{13}$ regulates actin cytoskeletal reorganization by controlling the disassembly of dorsal ruffles, leading to the control of cell migration. Furthermore, we have shown that $G\alpha_{13}$ functions together with tyrosine kinase Abl to remodel actin cytoskeleton and cell migration. Additionally, we have shown that Ca^{2+} influx through ORAI1 and STIM1 is critical for cell migration.
    a. Shan, D., Chen, L., Wang, D., Tan, Y.C., Gu, J.L., and **Huang, X.-Y.** 2006. The G protein G13 is required for growth factor-induced cell migration. Developmental Cell 10: 707-718.
    b. Singhvi, A., Liu, B., Friedman, C.J., Fong, J., Lu, Y., **Huang, X.-Y.**, Shaham, S. 2016. A glial K/Cl transporter controls neuronal receptive ending shape by chloride inhibition of an rGC. Cell 165:936-948. doi: 10.1016/j.cell.2016.03.026. PMID:27062922.
    c. Wang, L., Wang, D., Xing, B., Tan, Y.C., Huang, J., Liu, B., Syrovatkina, V., Espenel, C., Kreitzer, G., Guo, L., Zhang, J.J., and **Huang, X.-Y**. 2017. G-Protein Gα13 Functions with Abl Kinase to Regulate Actin Cytoskeletal Reorganization. J Mol Biol. 2017 Dec 8;429(24):3836-3849. doi: 10.1016/j.jmb.2017.10.020.
    d. Yang, S., Zhang, J.J., and **Huang, X.-Y.** 2009. Orai1 and STIM1 are critical for breast tumor cell migration and metastasis. Cancer Cell 15: 124-134.

5. I discovered SL2, the RNA trans-spliced leader in C. elegans. This led to the discovery of operons in nematodes. Nowadays, SL2 is widely used in the scientific community for polycistronic expression of multiple genes together in nematodes. Also, my laboratory discovered a peptide ligand (named Stunted) for the G-protein-coupled receptor (named Methuselah) which controls the lifespan in Drosophila. Later studies by other labs demonstrate that the Stunted ligand is a circulating insulinotropic peptide, and that the Stunted-Methuselah ligand-receptor system modulates physiological insulin levels in response to nutrients.
    a. **Huang, X.-Y.,** and Hirsh, D. 1989. A second trans-spliced RNA leader sequence in C. elegans. Proc. Natl. Acad. Sci. USA 86: 8640-8644.
    b. Cvejic, S., Zhu, Z., Felice, S.J., Berman, Y., and **Huang, X.-Y.** 2004. The endogenous ligand *Stunted* of the GPCR *Methuselah* extends lifespan in Drosophila. Nature Cell Biology 6: 540-546.
    c. McGarrigle, D. and **Huang, X.-Y.** 2007. *Methuselah* antagonist extends lifespan. Nature Chemical Biology 3: 371-372.

Complete List of Published Work in MyBiBliography: https://www.ncbi.nlm.nih.gov/myncbi/xin-yun.huang.1/bibliography/public/.