BIOGRAPHICAL SKETCH

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NAME: Zhang, Cheng

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

POSITION TITLE: Associate Professor of Pharmacology and Chemical Biology

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 Science and Technology of China, Hefei, An hui, China	B.S.	07/2003	Biology
University of Science and Technology of China, Hefei, An hui, China	Ph.D.	07/2008	Biochemistry and Molecular Biology
Stanford University, Stanford, California, US	Postdoctoral fellow	10/2013	GPCR structural biology and pharmacology with Dr. Brian Kobilka

A. Personal Statement

GPCRs are important cell surface receptors that can induce complex cellular signaling pathways in response to diverse extracellular signaling molecules. They constitute more than 30% of current drug targets. My research group employs a broad range of research methods in structural biology including X-ray crystallography and cryo-electron microscopy (cryo-EM), cell biology and pharmacology to elucidate the molecular basis for the signal transduction of G protein-coupled receptors (GPCRs) involved in inflammation and neurological activities. We have determined the structures of a number of GPCRs including receptors for anaphylatoxins, prostaglandins, leukotrienes, cannabinoids, vasopressin and dopamine, which together with our results from ligand-binding assays and cell-based signaling assays provided novel insights into ligand action and receptor signaling.

Our current research focus is to determine the molecular mechanisms underlying GPCR allosteric modulation and functional selectivity using dopamine receptors and neuropeptide receptors as model receptors. In addition, we develop functional GPCR antibodies as potential therapeutics and research tools through multiple approaches including yeast display.

Ongoing projects that I would like to highlight include:

R35 GM128641
Zhang (PI)
8/01/2018-7/31/2023
Structure, pharmacology and signaling of G protein-coupled receptors (GPCRs) in inflammation

R03 TR003306
Zhang (PI)
4/1/2021 - 3/31/2022
Structure and pharmacology of GPR32 in the resolution of inflammation

R01 DA052329
Xie and Zhang (Multi-PIs)
7/1/2021 - 6/30/2026
Cannabinoid CB2 Receptor Structure and Allosteric Modulators

R01 DK116780
Vilardaga (PI). Role: co-Investigator
4/1/2018-3/31/2022
Structural Basis of PTH Receptor Function

Citations:

1. Zhuang Y, Xu P, Mao C, Wang L, Krumm B, Zhou XE, Huang S, Liu H, Cheng X, Huang XP, Shen DD, Xu T, Liu YF, Wang Y, Guo J, Jiang Y, Jiang H, Melcher K, Roth BL*, Zhang Y*, **Zhang C***, Xu HE*. Structural insights into the human D1 and D2 dopamine receptor signaling complexes. Cell. 2021 Feb 18;184(4):931-942.e18. PMID: 33571431. (*Corresponding authors)
2. Zhuang Y, Liu H, Edward Zhou X, Kumar Verma R, de Waal PW, Jang W, Xu TH, Wang L, Meng X, Zhao G, Kang Y, Melcher K, Fan H, Lambert NA, Eric Xu H, **Zhang C**. Structure of formylpeptide receptor 2-G_i complex reveals insights into ligand recognition and signaling. Nature Communications. 2020 Feb 14;11(1):885. PMID: 32060286. PMCID: PMC7021761.
3. Qi X, Liu H, Thompson B, McDonald J, **Zhang C***, Li X*. Cryo-EM structure of oxysterol-bound human Smoothened coupled to a heterotrimeric Gi. Nature. 2019; 571:279-283. PMID: 31168089. (*Corresponding authors)
4. Wang L, Yao D, Deepak RNVK, Liu H, Xiao Q, Fan H, Gong W, Wei Z, **Zhang C**. Structures of the Human PGD_2 Receptor CRTH2 Reveal Novel Mechanisms for Ligand Recognition. Mol Cell. 2018 Oct 4;72(1):48-59.e4. PubMed PMID: 30220562; PubMed Central PMCID: PMC6223628.

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2013 - 2014	Basic Life Science Research Associate, Stanford University, Stanford, CA
2014 - 2021	Assistant Professor of Pharmacology and Chemical Biology, University of Pittsburgh, School of Medicine, Pittsburgh, PA
2020 - current	Member, American Society for Biochemistry and Molecular Biology
2021 - current	Member, American Society for Pharmacology and Experimental Therapeutics
2021 - current	Associate Professor of Pharmacology and Chemical Biology, University of Pittsburgh, School of Medicine, Pittsburgh, PA

Honors

2006	David Blow Visiting Scholarship, CCP4 community, UK.
2017	Competitive Medical Research Fund (CMRF), University of Pittsburgh, USA.
2018	Maximizing Investigators' Research Award, NIH, USA.
2021	ASBMB 2021 Early Career Faculty Annual Meeting Award, ASBMB, USA.

C. Contributions to Science

1. Structural basis for the signaling and regulation of chemotactic GPCRs. My lab at the University of Pittsburgh studies a group of non-chemokine chemoattractant GPCRs that mediate chemotaxis of innate immune cells by anaphylatoxins, prostaglandin D_2, formylpeptides and leukotrienes. We have solved multiple crystal structures of two receptors, the C5a receptor (C5aR) and the prostaglandin D_2 receptor CRTH2, bound to orthosteric and allosteric ligands, and cryo-EM structures of the formylpeptide receptor 2 (FPR2)-Gi signaling complex with peptide and non-peptide agonists. The structures provided unprecedented insights into how this family of GPCRs recognize chemically diverse inflammatory molecules to induce cellular signaling

events. Based on the structures, we proposed novel strategies for developing ligands of those GPCRs as potential therapeutics with improved pharmacological properties. We also collaborated with other groups to use atomic-force microscopy (AFM) and computational simulation and docking methods to study ligand binding on these receptors.

a. Liu H, Kim HR, Deepak RNV, Wang L, Chung KY, Fan H, Wei Z, Zhang C. Orthosteric and allosteric action of the C5a receptor antagonists. Nature Structural & Molecular Biology. 2018;25(6):472-81. PMID: 29867214.

b. Wang L, Yao D, Deepak RNV, Liu H, Xiao Q, Fan H, Gong W, Wei Z, Zhang C. Structures of the human PGD_2 receptor CRTH2 reveal novel mechanisms for ligand recognition. Molecular Cell. 2018. 72, 1–12. PMID: 30220562.

c. Zhuang Y, Liu H, Edward Zhou X, Kumar Verma R, de Waal PW, Jang W, Xu TH, Wang L, Meng X, Zhao G, Kang Y, Melcher K, Fan H, Lambert NA, Eric Xu H, Zhang C. Structure of formylpeptide receptor 2-G_i complex reveals insights into ligand recognition and signaling. Nature Communications. 2020 Feb 14;11(1):885. PMID: 32060286.

d. Liu H, Deepak RNVK, Shiriaeva A, Gati C, Batyuk A, Hu H, Weierstall U, Liu W, Wang L, Cherezov V, Fan H, Zhang C. Molecular basis for lipid recognition by the prostaglandin D_2 receptor CRTH2. Proc Natl Acad Sci U S A. 2021 Aug 10;118(32). PubMed PMID: 34341104. PMCID: PMC8364189.

2. Structural basis for the signaling of dopamine receptors. Dopamine acts on five GPCRs, dopamine D1 to D5 receptors (D1-5Rs), in the central nervous system (CNS). D1R and D2R are the most abundant receptors in the CNS, representing the primary excitatory and inhibitory dopamine receptor, respectively, in all dopaminergic pathways. In collaboration with Dr. Bryan Roth and other research groups, we have determined cryo-EM structures of several D1R-G_s complexes with orthosteric and allosteric ligands and a cryo-EM structure of the D2R-G_i complex. Collectively, these structures revealed the molecular basis for ligand binding, receptor activation and G protein coupling of D1R and D2R. We are currently working on other dopamine receptors, aiming to gain a comprehensive molecular understanding of dopamine signaling through the entire dopamine receptor family.

a. Zhuang Y, Xu P, Mao C, Wang L, Krumm B, Zhou XE, Huang S, Liu H, Cheng X, Huang XP, Shen DD, Xu T, Liu YF, Wang Y, Guo J, Jiang Y, Jiang H, Melcher K, Roth BL*, Zhang Y*, Zhang C*, Xu HE*. Structural insights into the human D1 and D2 dopamine receptor signaling complexes. Cell. 2021 Feb 18;184(4):931-942.e18. PMID: 33571431. (*Corresponding authors)

b. Zhuang Y, Krumm B, Zhang H, Zhou XE, Wang Y, Huang XP, Liu Y, Cheng X, Jiang Y, Jiang H, Zhang C, Yi W, Roth BL, Zhang Y, Xu HE. Mechanism of dopamine binding and allosteric modulation of the human D1 dopamine receptor. Cell Res. 2021 May;31(5):593-596. PMID: 33750903.

3. Molecular mechanisms underlying signaling of neuropeptide GPCRs. GPCRs for peptide hormones regulate diverse behaviors and physiological functions including appetite, energy homeostasis and motivation. We have determined cryo-EM structures of two neuropeptide GPCRs, the vasopressin V2 receptor (V2R) and the ghrelin receptor GHSR, bound to endogenous and synthetic agonists. Together with data from functional assays, our structures revealed unique binding mechanisms for the cyclic arginine vasopressin peptide and the lipidated ghrelin peptide. They also provided important molecular insights into how synthetic agonists of these two GPCRs mimic endogenous peptide agonists to activate their receptors. Our results are expected to facilitate development of novel agonist drugs for these two receptors.

a. Wang L, Xu J, Cao S, Sun D, Liu H, Lu Q, Liu Z, Du Y, Zhang C. Cryo-EM structure of the AVP-vasopressin receptor 2-G_s signaling complex. Cell Res. 2021 Mar 4. PMID: 33664408.

b. Liu H, Sun D, Myasnikov A, Damiaan M, Baneres JL, Sun J, Zhang C. Structural basis of human ghrelin receptor signaling by ghrelin and the synthetic agonist ibutamoren. Nature Communications. 2021 Nov 4, 12: 6410.

4. Structural and pharmacological characterization of cannabinoid receptors. In collaboration with Dr. Xiang-qun Xie's group and other research groups, we have determined a cryo-EM structure of human cannabinoid receptor CB2 and Gi signaling complex. Structural analysis together with computational studies suggested the structural determinants for distinguishing CB2 agonists and antagonists and revealed significant differences between the two cannabinoid receptors, CB1 and CB2, in receptor activation, ligand recognition

and G protein coupling. The results paved the road for our future studies to design novel CB2 ligands including allosteric modulators, which hold promise for treating numerous inflammatory diseases and pain.

a. Xing C, Zhuang Y, Xu T, McDonald J, Feng Z, Zhou E, Chen M, Wang L, Meng X, Xue Y, Wang J, Liu H, McGuire T, Zhao G, Melcher K, Zhang C*, Xu E*, Xie X*. Cryo-EM Structure of Human Cannabinoid Receptor CB2-Gi Signaling Complex. Cell. 2020 Feb 20;180(4):645-654.e13. PMID: 32004460. (*Corresponding authors)

5. Structural characterization of a Smoothened signaling complex. Smoothened (SMO) is an oncoprotein that transduces the Hedgehog signal from the tumor repressor Patched-1 to the glioma-associated oncogene transcription factors GLI. It belongs to the Class-F GPCRs, which also include Frizzled receptors. In collaboration with Dr. Xiaochun Li at the UT Southwestern Medical Center, we solved the structure of SMO and Gi signaling complex with a sterol agonist by cryo-EM. This is the first structure of a Class-F GPCR signaling complex. The structure revealed a new sterol ligand binding site in the 7-transmembrane domain, a novel receptor activation mechanism and a new binding mode of Gi protein that is different from Class-A GPCRs. Our work provides innovative insights into Hedgehog signaling and the activation of Class-F GPCRs.

a. Qi X, Liu H, Thompson B, McDonald J, Zhang C*, Li X*. Cryo-EM structure of oxysterol-bound human Smoothened coupled to a heterotrimeric Gi. Nature. 2019; 571:279-283. PMID: 31168089. (*Corresponding authors).

Complete List of Published Work in MyBibliography:
https://www.ncbi.nlm.nih.gov/myncbi/1Xqbv7vTlyh5w/bibliography/public/

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: Vilardaga, Jean-Pierre

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

POSITION TITLE: Professor of Pharmacology & Chemical Biology

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
Academy of Nice, France	baccalaureat	06/1983	Mathematics
University of Nice, France	B.S.	09/1985	Physics
University of Nice, France	B.S.	09/1986	Chemistry
University of Nice, France	M.S.	06/1987	Molecular Chemistry
University Free of Brussels (ULB), Belgium	M.S.	10/1990	Molecular Biology
University Free of Brussels (ULB), Belgium	Ph.D.	02/1996	Molecular Pharmacology
University of San Francisco (UCSF), USA	Postdoctoral	10/1998	Molecular Endocrinology

A. Personal Statement

My work integrates computational, structural, molecular, and biochemical techniques using cell and *in vivo* mouse studies. The outcome of these investigations is revealing new and fundamental information about the mechanisms of the Structural and Molecular Basis of GPCR regulation of Ca^{2+} homeostasis. This endeavor serves as a foundation to understand how class B and C GPCRs regulate biomolecule homeostasis pathways that are critically important to health and disease.

Calcium homeostasis is quintessential in numerous physiological processes from bone formation to nerve transmission and cardiac contractility. The parathyroid hormone (PTH) receptor (PTHR) and calcium-sensing receptor (CaSR) are the major G protein-coupled receptors (GPCRs) regulating the maintenance of essential elements in calcium homeostasis – serum Ca^{2+}, PTH, Vitamin D, and phosphate ions. The disorders in mechanisms regulating calcium homeostasis are associated with significant human morbidity including bone diseases, kidney stones, and soft tissue calcification that are very common and pose serious health threats. Current treatments have multiple caveats.

My laboratory has engineered optical systems (FRET) for studying signaling processes for the PTHR as well as other GPCRs in real time and in live cells, and have thereby made a series of breakthrough observations that have led to new paradigms of GPCR signaling and regulation mechanisms in endosomes. We discovered that the PTHR is one of the first GPCRs found to sustain cAMP signaling after internalization of the PTH–receptor complex in endosomes, a process with both physiological and disease relevance. Recently, my lab has regrouped and assembled an international consortium that applied state-of-the-art cryoelectron microscopy to reveal near-atomic 3Å resolution of the PTHR in complex with the Gs protein. These results give us invaluable mechanistic insights into PTHR activation and provide a framework for the proposed studies that will permit a rational development of therapies in the kidney–bone axis in mineral diseases.

This track record demonstrates that I have the specific training and expertise in GPCR signaling, and leadership to serve as a PI on this new R01 proposal aimed at studying PTHR druggability.

Ongoing projects that I would like to highlight include:

R01DK116780	Vilardaga, Jean-Pierre	4/1/2018 - 3/31/2022	2.70 calendar
NIH/DK		Total Costs: $1,605,707	

Structural Basis of PTH Receptor Function
These studies will provide new insights into how PTHR activate G proteins and the structural mechanism differentiating the action of PTH, PTHrP and LA-PTH.

R01DK122259	Vilardaga JP; Chang	9/12/2019 - 8/31/2024	3.00 calendar
NIH/DK		Total Costs: $970,307	

Regulation of Parathyroid Functions By G-Protein Coupled Receptors
These studies will help to better understand mechanisms of PTH regulation and secretion and might have clinical importance in pathological conditions such as PTG disorders.

R01HD100468	John Wysolmerski	8/1/2020 - 7/31/2023	0.96 calendar
NIH/DK		Total Costs: $98,478	

Heterodimerization of the Calcium-sensing receptor with the GabaB receptors in the breast
FRET experiments in live cells will be performed in Dr. Vilardaga lab to determine the rate limiting reaction that regulates signal transduction through the CaSR/$GABA_{B1}$R heteromer.

R21AI148776	Xiao; Randhawa	6/3/2020 - 5/31/2022	0.60 calendar
NIH/DK		Total Costs: $38,194	

Enhanced Diagnosis of Antibody-Mediated Kidney Rejection by Machine Learning and Hybrid Targeted-Shotgun Proteomics
The central hypothesis is that LC-MS/MS can be used to define disease-specific biomarkers using a discovery data set, which can then be followed up by a validation data set to determine if LC-MS/MS based tests can be implemented in clinical practice.

SCIENTIFIC OVERLAP STATEMENT
There is no overlap among the current projects.

Relevant publications for this project:

a. Alex D. White, Karina A. Peña, Lisa J. Clark, Zhiqiang Cheng, Shi Liu, Fei Fang, Frédéric G. Jean-Alphons, Chia-Ling Tu, Nicholas Szeto, Asuka Inoue, Thomas J. Gardella, Samuel H. Gellman, Kunhong Xiao, Wenhan Chang, and **Vilardaga, JP***. Regulation of parathyroid hormone receptor biological effectiveness via location bias in cAMP signaling. *Science Signaling* (2021) *in press*.

b. Clark, L.J., Krieger, J., Bondarenko, V., White, A.D., Lei, S., Fang, F., Li, H., Jean-Alphonse F.G.,Tang, P., Gardella, T.J., Xiao, K., Sutkeviciute, I., Coin, I., Bahar, I*., **Vilardaga, JP*.** Allosteric interactions in the parathyroid hormone GPCR–arrestin complex formation. *Nat. Chem. Biol.* (2020) 16:1096-1104. PMCID: PMC7502484.

c. Alex D. White, Frederic G. Jean-Alphonse, Shi Liu, Gabriele M. Konig, Asuka Inoue, Samuel H. Gellman, Evi Kostenis, and **Vilardaga, JP***. Gq/11-dependent regulation of endosomal cAMP generation by parathyroid hormone class B GPCR. *Proc. Natl. Acad. Sci. USA* (2020) 117: 7455-7460. PMCID: PMC7132270.

d. Zhao, L.H., Shanshan Ma, S., Sutkeviciute, I., Shen, D.D., Zhou, E., W. de Waal, P., Kang, Y., Clark, L.J., Jean-Alphonse, F.G., White, A.D., Jiang, I., Melcher, K., Wang, M.W., **Vilardaga, JP***, Xu*, E.H., Zhang Y*. Structure and dynamics of the active human parathyroid hormone receptor-1. *Science* (2019) 363:148-153. PMCID: PMC6929210.

B. Positions, Scientific Appointments, and Honors

Positions, Scientific Appointments

1998 – 2004 Instructor in Pharmacology, University of Würzburg, Germany
2004 – 2005 Assistant Professor of Pharmacology, University of Würzburg, Germany

2005 – 2008 Assistant Professor of Medicine, Harvard Medical School, USA
2008 – Present Consultant of Medicine, Massachusetts General Hospital, USA
2008 – 2011 Assistant Professor of Pharmacology, University of Pittsburgh, USA
2011 – 2015 Associate Professor of Pharmacology (tenure), University of Pittsburgh, USA
2016 – Present Professor of Pharmacology & Chemical Biology (tenure), University of Pittsburgh, USA

Other Experience and Professional Memberships
2006 – Present Member, ASBMB (American Society for Biochemistry & Molecular Biology)
2009 – Present Member, ASBMR (American Society for Bone & Mineral Research)
2009 – Present Member, Endocrine Society
2011 – Present Member, ASPET
2011 – 2013 Member, Biophysical Society
2012 NIH Peer Review Committee, Small Business: Biological Chemistry, Biophysics, and Drug Discovery
2014 NIH Peer Review Committee, Cell Biology Integrated Review Group
2014 – Present Chair of the International Union of Basic and Clinical Pharmacology (IUPHAR) subcommittee on parathyroid hormone receptors
2022 – 2027 *Journal of Biochemical Chemistry*, Editorial Board

Honors
1990 Erasmus Exchange program of the European community
1991 University Fellowship Fellowship for pre-doctoral studies of the Free University of Brussels, Belgium
1992 Science Plan Grant from the European Community for PhD studies at the Free University of Brussels, Belgium
2003 Ramon y Cajal Award from the Spanish government
2006 Young Investigators Award Advances in Mineral Metabolism and the American Society for Bone and Mineral Research (AIMM/ASBMR)
2006 Session Chair Annual meeting of the ASBMR
2013 Session Chair Annual meeting of the Endocrine Society
2016 Session Chair Annual meeting of the Endocrine Society

C. Contributions to Science

1. Contribution to the pharmacology of the PTH receptor. Focusing on the parathyroid hormone receptor (PTHR) signaling at UCSF under the supervision of Dr. Nissenson, I began postdoctoral studies by investigating the molecular basis underlying PTHR signaling. I continued this work at the University of Wurzburg (Germany) with Dr. Lohse by developing optical techniques (FRET) to examine the role of PTHR dynamics in cellular signaling, focusing on receptor's conformational changes induced upon hormone binding. Important findings from these studies were the determination of kinetics of receptor activation and deactivation in live cells, and the identification of transmembrane helical rearrangements within the receptor, which are critical for G proteins activation and signal propagation.
   a. **Vilardaga JP**, Lin I, Nissenson RA. (2001) Analysis of parathyroid hormone/secretin receptor chimeras differentiates the role of functional domains in the PTH/PTH-related peptide (PTHrP) P receptor on hormone binding and receptor activation. *Mol Endocrinol*. 15:1186-1199
   b. **Vilardaga JP**, Frank M, Krasel C, Dees C, Nissenson RA, Lohse MJ. (2001) Differential conformational requirements for activation of G proteins and the regulatory proteins arrestin and G protein-coupled receptor kinase in the G protein-coupled receptor for parathyroid hormone (PTH)/PTH-related protein. *J Biol Chem*. 276:33435-33443.
   c. **Vilardaga JP**, Krasel C, Chauvin S, Bambino T, Lohse MJ, Nissenson RA. (2002) Internalization determinants of the parathyroid hormone receptor differentially regulate β-arestin/receptor association. *J Biol Chem.* 277:8121-8129.
   d. **Vilardaga JP**, Bünemann M, Krasel C, Castro M, Lohse MJ. (2003) Measurement of the millisecond activation switch of G protein-coupled receptors in living cells. *Nat Biotechnol* 21:807-812.
2. Optical Approches to study signaling mechanisms of GPCRs. Following my move at MGH/Harvard and later at PITT as an Assistant Professor, my laboratory combined FRET with biochemical and pharmacological approaches to examine the role of GPCR dynamics in cellular signaling to uncover several fundamental

mechanisms including: (1) mechanism of parathyroid hormone binding to its receptor; (2) direct measure of ligand efficacy differentiating full, partial, and inverse agonists acting at GPCRs; and (3) existence of a trans-conformational spread between two different brain GPCRs as a means to integrate the action of multiple extracellular stimuli (norepinephrine and morphine) in cellular signaling.

a. **Vilardaga JP**, Steinmeyer R, Harms G, Lohse MJ. (2005) Molecular basis of inverse agonism in a G protein-coupled receptor. *Nat Chem Biol* 1:25-28.
b. Castro M, Nikolaev OV, Palm D, Lohse MJ, **Vilardaga JP***. (2005) Turn-on switch in parathyroid hormone receptor by a two-step parathyroid hormone binding mechanism. *Proc. Natl. Acad. Sci. USA* 102:16084-16089.
c. Nikolaev OV, Hoffmann C, Bünemann M, Lohse MJ, **Vilardaga JP***. (2006) Molecular basis of partial agonism at the neurotransmitter α_{2A}-adrenergic receptor and G_i-protein heterotrimer. *J. Biol. Chem*. 2006, 281:24506-24511.
d. **Vilardaga JP***, Nikolaev OV, Lorenz K, Ferrandon S, Zhuang Z, Lohse MJ. (2008) Conformational cross-talk between α_{2A}-adrenergic and μ-opioid receptors control cell signaling. *Nat Chem Biol* 4:126-131.

3. Studies of PTH- and β_2-adrenergic receptors in Bone Biology. Extending my research efforts on the role of GPCR dynamics in bone biology and in collaboration with Dr. Masaki Noda (University of Tokyo), we discovered that osteopontin (a cytokine and one of the major non-collagenous extracellular matrix proteins of bone) regulates the sympathetic control of bone mass by directly regulating the duration of β_2-adrenergic receptor (β_2AR) signaling in bone cells. Keeping with the function of the β_2AR in bone, we recently uncovered that the osteo-anabolic action of PTH is regulated by the sympathetic system via the β_2AR receptor, indicating an unexpected functional interaction between the PTH-receptor and the β_2AR. In collaboration with my colleagues at MGH, Drs, Gardella and Potts, we showed that certain PTH analogs that stabilize the endosomal location of the PTHR also prolong calcemic response (blood Ca^{2+}) when injected in mice.

   a. Okazaki M, Ferrandon S, **Vilardaga JP**, Bouxsein ML, Potts JT, Gardella TJ. (2008) Prolonged signaling at the parathyroid hormone receptor by peptide ligands targeted to a specific receptor conformation. *Proc. Natl. Acad. Sci. USA* 105:16525-16530. PMCID: PMC2571912

   b. Nagao M, Feinstein TN, Ezura Y, Hayata T, Notomi T, Saita Y, Hanyu R, Hemmi H, Izu Y, Takeda S, Wang K, Rittling S, Nakamoto T, Kaneko K, Kurosawa H, Karsenty G, Denhardt DT, **Vilardaga JP***, Noda M*. (2011) Sympathetic control of bone mass regulated by osteopontin. *Proc. Natl. Acad. Sci. USA* 108:17767-17772. PMCID: PMC3203767.

   c. Hanyu R, Wehbi VL, Hayata T, Moriya S, Feinstein TN, Yoichi Ezura Y, Nagao M, Saita Y, Hemmi H, Notomi T, Nakamoto T, Schipani E, Takeda S, Kaneko K, Kurosawa H, Karsenty G, Kronenberg KM, **Vilardaga JP***, Noda, M*. (2012) Anabolic action of PTH regulated by the β_2-adrenergic receptor. *Proc. Natl. Acad. Sci. USA* 2012 109:7433-7438. PMCID: PMC3358909.

4. Endosomal cAMP production by the PTH receptor: mechanisms and consequences. Our studies revealed the new concept that certain GPCRs mediate G-protein signaling not only from the plasma membrane, but also from endosomal membranes. This model proposes that following ligand binding and activation, cell surface GPCRs internalize and redistribute into early endosomes where cAMP production is regulated by noncanonical actions of β-arrestins (maintain the signal ON) and the retromer complex (turns the signal OFF). This new and unexpected process is drastically changing how we think about GPCR signaling and regulation, and how we study drugs that target this receptor family. In the case of the PTH-receptor, we recently found that PTH analogs promoting endosomal Gs/cAMP signaling in cells, also prolong hypercalcemic and hypophosphatemic responses when injected into mice. These effects suggest potential medical applications for certain forms of hypoparathyroidism, and for which action in vivo of injected PTH(1–34) is too short-lived.

   a. Ferrandon S, Feinstein TN, Castro C, Bouley R, Potts JT, Gardella TJ, and **Vilardaga JP*.** (2009) Sustained cyclic AMP by Parathyroid Hormone receptor endocytosis. *Nat Chem. Biol.* 5: 734-742. PMCID: PMC3032084
   b. Feinstein TN, Wehbi VL, Ardura JA, Wheleer DS, Ferrandon S, Gardella TJ, and **Vilardaga JP***. (2011) Retromer terminates the generation of cAMP by internalized parathyroid hormone receptor. *Nat Chem. Biol*. 7: 278-284. PMCID: PMC3079799.

c. Gidon A, Al-Bataineh MM, Jean-Alphonse FG, Stevenson H, Watanabe T, Louet C, Khatri A, Calero G, Pastor-Soler NM, Gardella TJ, **Vilardaga JP***. (2014) Endosomal GPCR signaling turned off by negative feedback actions of PKA and v-ATPase. *Nat Chem. Biol.,* 10: 707-711. PMCID: PMC4138287
d. **Vilardaga JP***, Jean-Alphonse FG, Gardella TJ. (2014) Endosomal generation of cAMP in GPCR signaling. *Nat Chem. Biol.* 10: 700-706. (review). PMCID:PMC4417940.

5. Structural and cellular Pharmacology of the PTH receptor. We are extending our research program to determine the structural and cellular basis by which PTHR functions and activates G proteins in response to PTH, and PTH analogs. Our recent studies revealed: a) a fundamental mechanism underlying the coordinated regulation of PTHR signaling by the β_2AR; and b) that extracellular Ca^{2+} acts a positive allosteric modulator of PTHR signaling that can be involved in regulating calcium homeostasis.
   a. Jean-Alphonse FG, Wehbi VL, Chen Jingming, Noda M, Taboas JM, Xiao K, **Vilardaga JP***. β_2-adrenerigic receptor control of endosomal PTH receptor signaling via G$\beta\gamma$. *Nat Chem Biol* (2017) 13:259-261. PMCID:PMC5322277.
   b. Alex D. White, Fei Fang, Frederic G. Jean-Alphonse, Lisa J. Clark, Hyun-Jung An, Hongda Liu, yang Zhao, Shelley Reynolds, Sihoon Lee, Kunhong Xiao, Ieva Sutkeviciute and **Vilardaga JP***. Ca^{2+} allostery in PTH-receptor signaling. *Proc. Natl. Acad. Sci. USA* (2019) 116 (8):3294-3299. PMCID:PMC6386702
   c. Li-Hua Zhao[1], Shanshan Ma[1], Ieva Sutkeviciute[1], Dan-Dan Shen, X. Edward Zhou, Parker W. de Waal, Yanyong Kang, Lisa Clark, Fredreric G. Jean-Alphonse, Alex D. White, I Jiang, Karsten Melcher, Ming-Wei Wang, **Vilardaga JP***, H. Eric Xu[*], Yan Zhang[*]. Structure and dynamics of the active human parathyroid hormone receptor-1. *Science* (2019) 363:148-153. PMCID:PMC6929210.
   d. Lisa J. Clark, James Krieger, Vasyl Bondarenko, Alex D. White, Saifei Lei, Fei Fang, Hongchun Li, Frederic Jean-Alphonse, Pei Tang, Thomas J. Gardella, Kunhong Xiao, Ieva Sutkeviciute, Irene Coin, Ivet Bahar*, **Vilardaga JP***. Allosteric interactions in the parathyroid hormone GPCR-arrestin complex formation. *Nat Chem. Biol.* (2020) 16:1096-1104. PMCID:PMC7502484

D. Publications and Citations

Total of 87 papers (58 original articles, 24 Reviews, 5 textbook chapters); > 6900 citations; H-index:43; (based on Google Scholar)
Complete List of Published Work in MyBibliography:
http://www.ncbi.nlm.nih.gov/sites/myncbi/jean-pierre.vilardaga.1/bibliography/40440082/public/