

Curriculum Vitae

ALEX ROJAS BIE THOMSEN

Department of Basic Science and Craniofacial Biology
345 East 24th Street, Room 921C
New York University
New York City, NY 10010, USA

Phone: (+1) 919-282-2769
email: alex.molpharm@gmail.com

EDUCATION

- **Doctor of Philosophy in Molecular Pharmacology** **2008 – 2012**
Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark
- **Master of Science in Human Biology**
Faculty of Health Sciences, University of Copenhagen, Copenhagen, Denmark **2004 – 2007**
- **Bachelor of Science in Biochemistry**
Faculty of Science, University of Copenhagen, Copenhagen, Denmark **2000 – 2004**

SCIENTIFIC EXPERIENCE

Assistant Professor **Oct 2019 – Present**
Department of Basic Science and Craniofacial Biology, New York University, New York City, NY, USA

- Studying structural architecture of GPCR signaling complexes *in situ* in metastatic melanoma cells and neurons by cryo-electron tomography.
- Investigating mechanisms underlying chemokine receptor-mediated cell migration.

Assistant Professor **May 2018 – Sep 2019**
Department of Surgery, Columbia University, New York City, NY, USA

- Studying structural architecture of GPCR signaling complexes *in situ* in metastatic melanoma cells and neurons by cryo-electron tomography.
- Investigating mechanisms underlying chemokine receptor-mediated cell migration.

Postdoctoral Scholar **Sep 2012 – Dec 2017**
Department of Medicine (Cardiology), Duke University, Durham, NC, USA
Research Mentor: Professor Robert J. Lefkowitz (Nobel Laureate)

- Discovered G protein-coupled receptor (GPCR)–G protein– β -arrestin “megaplexes” as a mechanism for sustained G protein signaling at internalized GPCRs.
- Investigated real-time cAMP kinetics by Förster resonance energy transfer (FRET) and dynamic movements within G proteins by bioluminescence resonance energy transfer (BRET) to study sustained G protein signaling across different classes of GPCRs.
- Utilized confocal microscopy and BRET to study molecular interactions between GPCRs, G protein subunits, and β -arrestin.
- Expressed, purified, reconstituted, and tested functionality of GPCR complexes *in vitro*.
- Examined the molecular architecture of GPCR complexes using negative stain electron microscopy

(EM) single particle classification analysis.

- Obtaining high-resolution structures of GPCR complexes using cryo-EM.
- Studied intercommunication between G protein signaling and β -arrestin-mediated signaling promoted by the chemokine receptor CCR7.
- Developed a high-throughput method for forming, isolating, and screening GPCR- β -arrestin complexes for structural features using negative stain EM.
- Studied β -arrestin-mediated desensitization, endocytosis, and signaling.
- Delineated the mechanism behind G protein desensitization using CRISPR knockout cells and pharmacological intervention.
- Screened small-molecule libraries to identify G protein or β -arrestin biased agonists at the β_2 -adrenergic receptor.

Visiting PhD Student

Jan 2011 – Sep 2011

Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

Research Mentor: Professor Edward M. Brown

- Studied dual regulation of fibroblast growth factor 23 (FGF23) secretion by calcium and inorganic phosphorus in calcium-sensing receptor (CaSR) and CaSR/parathyroid hormone (PTH) knockout animal models.
- Studied interactions between calcium and magnesium homeostasis mediated through CaSR using CaSR and CaSR/PTH knockout animal models.

PhD Student

Nov 2008 – Feb 2012

Department of Experimental Pharmacology, University of Copenhagen, Copenhagen, Denmark

Research Mentor: Professor Hans Bräuner-Osborne

- Investigated biased agonism at the CaSR by testing the ability of a panel of agonists to modulate cAMP production (as a measure of $G_{i/o}$ protein activity), inositol 1-phosphate accumulation (as a measure of $G_{q/11}$ protein activity), intracellular calcium concentration (as a measure of $G_{q/11}$ protein and calcium channel activity) and ERK1/2 phosphorylation.
- Investigated biased agonism at the CaSR in physiological relevant cells that express the receptor endogenously.
- Isolated, grew, and differentiated primary osteoblast cells from rat bone marrow cells or rat femoral and tibia bones.
- Examined the involvement of the CaSR in osteoblast regulation of FGF23 expression and secretion, and bone mineralization.
- Generated the first reported cell line stably expressing GRPC6A receptor, and delineation of receptor coupling specificity and signaling.

PhD Student

Nov 2008 – Feb 2012

LEO Pharma A/S, Ballerup, Denmark

Research Mentor: Dr. Markus Latta

- Characterized calcitonin and PTH secretion in response to stimulation of the CaSR by biased agonists in endogenous cell systems and/or animal models.
- Developed and characterized allosteric modulators at the CaSR for the treatment of secondary hyperparathyroidism.

Research Scientist
INAGEN Aps, Denmark**Feb 2008 – Nov 2008**

- Generated a library containing chemokine mutants.
- Expressed, purified, and refolded chemokines and chemokine mutants from *E. coli*.
- Screened a library of chemokine mutants to identify high affinity binders at viral encoded GPCRs.

Master Student**Mar 2006 – Aug 2007****Department of Gene and Disease, Center for Genomic Regulation, Barcelona, Spain****Research Mentor: Dr. Cristina Fillat Fonts**

- Generated adenoviral vectors expressing thymidine kinase and E-cadherin used for suicide gene therapy against pancreatic cancer.
- Validated the anti-tumor efficiency of adenoviral vectors using a panel of different pancreatic cancer cell lines and pancreatic cancer xenograph tumor models in BALB/c nude mice.

Bachelor Student**Jan 2004 – Jul 2004****Department of Clinical Biochemistry, Statens Serum Institut, Copenhagen, Denmark****Research Mentor: Dr. Paal Skytt Andersen**

- Screened for mutations in the muscle LIM protein gene in a cohort of 90 Danish hypertrophic cardiomyopathy patients and their close relatives, a total of 451 individuals, using capillary array electrophoresis-single strand conformation polymorphism.

RESEARCH INTERESTS

- **Mechanistic and Structural Studies of Endosomal G Protein Signaling Using Cryo-EM**
That some GPCRs can stimulate G protein signaling even after been internalized into endosomal compartments has become well established over the last 8-10 years. During my postdoctoral training, I discovered the mechanism behind G protein signaling by internalized GPCRs, which fundamentally changed the signaling paradigm for GPCRs. My studies revealed that some GPCRs may engage with G protein and β -arrestin simultaneously to form “megaplexes” which allow the receptor to be internalized into endosomes by β -arrestin, while maintaining its ability to activate the G protein – something previously thought to be impossible. I recently obtained a high-resolution structure of a GPCR–G protein– β -arrestin megaplex using cryo-EM that characterizes the molecular aspects of this complex in great details. Currently, I am identifying GPCRs that take part in endosomal G protein signaling and found that several chemokine receptors form megaplexes at endosomes. In addition, observations suggest that β -arrestin positively modulates sustained G protein activation at megaplex-forming GPCRs. Thus, a central avenue of one of my current independent research programs is to obtain high-resolution cellular images of chemokine receptor megaplexes using cryo-electron tomography to understand how β -arrestin positively modulate G protein signaling at endosomes for some GPCRs while blocking it for others.
- **Physiological Importance of Endosomal G Protein Signaling**
Although it is known that some GPCRs form megaplexes, which carry out sustained G protein signaling after receptor internalization, we are only starting to learn about the physiological functions of this event. Thus, key activities of my current independent research are to study physiological outcomes of this event using primary cell, organ, and animal models. The central hypothesis for my

Role: Principal Investigator

Alfred Benzon Foundation (Declined)

“Mega Complexes of the β_2 -Adrenergic Receptor, β arrestin1, and Heterotrimeric Gs Protein”

68,182 USD

2015

Role: Principal Investigator

Sapere Aude: Young Elite Researcher 2013 Award

“High-Resolution Structure of the β_2 -Adrenergic Receptor bound to β -arrestin1 using Fusion Proteins”

183,113 USD

2012 – 2014

Role: Principal Investigator

Danish Council for Independent Research

“Development of a Cell Free Approach for Determining the G Protein or β -arrestin Bias of Ligands at 7 Transmembrane Receptors”

395,589 USD

2012 – 2014

Role: Principal Investigator

Alfred Benzon Foundation (Declined)

“Development of a Cell Free Approach for Determining the G Protein or β -arrestin Bias of Ligands at 7 Transmembrane Receptors”

73,529 USD

2012 – 2013

Role: Principal Investigator

Oticon Fonden

“Regulation of Phosphorous Homeostasis by the Calcium-Sensing Receptor”

2,407 USD

2011

FARMA Travel Grant

“Regulation of Renin Secretion from the Juxtaglomerular Apparatus by the Calcium-Sensing Receptor”

5,185 USD

2011

Drug Research Academy Equipment and Material Grant

“Differential Signaling of the Calcium-Sensing Receptor”

5,091 USD

2010

Beckett Fonden

“Regulation of Phosphorous Homeostasis by the Calcium-Sensing Receptor”

10,020 USD

2009

Centre for Genomic Regulation Student Fellowship

3,973 USD

2006 – 2007

Hotelejer Anders Månsson og Hustru Hanne Månssons Legat

2,724 USD

2006

Peter og Emma Thomsens Legat

18,750 USD

2005

LAUNGUAGES

- Danish: Native language
- English: Fluent (speaking, reading, writing)

- Spanish: Fluent (speaking), intermediate (reading, writing)
- Portuguese: Fluent (speaking), intermediate (reading and writing)

PEER-REVIEWED PUBLICATIONS

20. Nguyen A*, **Thomsen ARB***, Cahill III TJ*, Huang R, Huang L-Y, Clarke OB, Masoudi A, Ben-Hail D, Samaan F, Dandey VP, Tan YZ, Hong C, Mahoney JP, Triest S, Little IV J, Chen X, Sunahara R, Steyaert J, Yu Z, des Georges A, Lefkowitz RJ (2019). Structure of an Endosomal Signaling GPCR–G Protein– β -Arrestin Mega-Complex. *Nat Struct Mol Biol* (in press). [*co-first authors]
19. **Thomsen ARB**, Jensen DJ, Hicks GA, Bunnnett NW (2018). Therapeutic Targeting of Endosomal GPCRs. *Trends Pharmacol Sci* 39:879-91.
18. Zhang DL, Sun YJ, Ma ML, Wang YJ, Lin H, Li RR, Liang ZL, Gao Y, Yang Z, He DF, Lin A, Mo H, Lu YJ, Li MJ, Kong W, Chung KY, Yi F, Li JY, Qin YY, Li J, **Thomsen ARB**, Kahsai AW, Chen ZJ, Xu ZG, Liu M, Li D, Yu X, Sun JP (2018). Gq activity- and β -arrestin-1 scaffolding-mediated ADGRG2/CFTR coupling are required for male fertility. *Elife* 7. pii: e33432.
17. Cahill III TJ*, **Thomsen ARB***, Tarrasch JT, Plouffe B, Nguyen A, Yang F, Bassoni D, Gavino B, Lamerdin J, Triest S, Huang L-Y, Shukla A, Kahsai AW, Berger B, Little IV J, Antar A, Blanc A, Qu C-X, Chen X, Kawakami K, Inoue A, Aoki J, Steyaert J, Sun J-P, Bouvier M, Skiniotis G, Lefkowitz RJ (2017). Distinct Conformations of GPCR– β -Arrestin Complexes Mediate Desensitization, Signaling and Endocytosis. *Proc Natl Acad Sci USA* 114:2562-67. [*co-first authors]
16. Liu CH, Gong Z, Liang ZL, Liu ZX, Yang F, Sun YJ, Ma ML, Wang YJ, Ji CR, Wang YH, Wang MJ, Cui FA, Lin A, Zheng WS, He DF, Qu CX, Xiao P, Liu CY, **Thomsen ARB**, Cahill III TJ, Kahsai AW, Yi F, Xiao KH, Xue T, Zhou Z, Yu X, Sun J-P (2017). Arrestin-biased AT1R agonism induces acute catecholamine secretion through TRPC3 coupling. *Nat Commun* 8:14335.
15. **Thomsen ARB***, Plouffe B*, Cahill III TJ*, Shukla AK, Tarrasch JT, Dosey AM, Kahsai AW, Strachan RT, Pani B, Mahoney JP, Huang L-Y, Breton B, Heydenreich FM, Sunahara RK, Skiniotis G, Bouvier M, Lefkowitz RJ (2016). GPCR–G Protein– β -arrestin Super-Complex mediates Sustained G Protein Signaling. *Cell* 166:907-19. [*co-first authors] (Highlighted in *Trends Biochem Sci* and *Angew Chem Int Ed*, Recommended by Faculty of 1000)
14. Kahsai AW, Wisler JW, Lee J, Ahn S, Cahill III TJ, Dennison SM, Staus DP, **Thomsen ARB**, Anasti KM, Pani B, Wingler LM, Desai H, Bompiani KM, Strachan RT, Qin X, Alam SM, Sullenger BA, Lefkowitz RJ (2016). Conformationally Selective RNA Aptamers Allosterically Modulate the β 2-Adrenoceptor. *Nat Chem Biol* 12:709-16.
13. Strachan RT, Sun JP, Rominger DH, Violin JD, Ahn S, **Thomsen ARB**, Zhu X, Kleist A, Costa T, Lefkowitz RJ (2014). Divergent Transducer-Specific Molecular Efficacies Generate Biased Agonism at a G Protein-Coupled Receptor (GPCR). *J Biol Chem* 289:14211-24.

12. Nørskov-Lauritsen L, **Thomsen ARB**, Bräuner-Osborne H (2014). G Protein-Coupled Receptor Signaling Analysis using Homogenous Time-Resolved Förster Resonance Energy Transfer (HTRF®) Technology. *Int J Mol Sci* 15:2554-72.
11. Wisler JW, Xiao K, **Thomsen ARB**, Lefkowitz RJ (2014). Recent Developments in Biased Agonism. *Curr Opin Cell Biol* 27:18-24.
10. Jacobsen SE*, Nørskov-Lauritsen L*, **Thomsen ARB***, Smajilovic S, Wellendorph P, Larsson NH, Lehmann A, Bhatia VK, Bräuner-Osborne H (2013). Delineation of the GPRC6A Receptor Signaling Pathways using a Mammalian Cell Line Stably Expressing the Receptor. *J Pharmacol Exp Ther* 347:298-309. [*co-first authors]
9. Johansson H, Cailly T, **Thomsen ARB**, Bräuner-Osborne H, Sejer Pedersen D (2013). Synthesis of the Calcilytic Ligand NPS 2143. *Beilstein J Org Chem* 9:1383-7.
8. Quinn SJ, **Thomsen ARB**, Egbuna O, Pang J, Baxi K, Karapils A, Goltzman D, Pollak M, Brown EM (2013). CaSR-mediated Interactions Between Calcium and Magnesium Homeostasis in Mice. *Am J Physiol Endocrinol Metab* 304:E724-33.
7. Quinn SJ*, **Thomsen ARB***, Pang JL, Kantham L, Bräuner-Osborne H, Pollak M, Goltzman D, Brown EM (2012). Interactions between Calcium and Phosphorus in the Regulation of the Production of Fibroblast Growth Factor 23 *In Vivo*. *Am J Physiol Endocrinol Metab* 304:E310-20. [*co-first authors]
6. **Thomsen ARB**, Worm J, Jacobsen SE, Stahlhut M, Latta M, Bräuner-Osborne H (2012). Strontium is a Biased Agonist of the Calcium-Sensing Receptor in Rat Medullary Thyroid Carcinoma 6-23 Cells. *J Pharmacol Exp Ther* 343:638-49.
5. **Thomsen ARB**, Smajilovic S, Brauner-Osborne H (2012). Allosteric Modulation and Differential Signaling of the Calcium-Sensing Receptor. *Curr Drug Targets* 13:1324-35.
4. **Thomsen ARB**, Hvidtfeldt M, Brauner-Osborne H (2011). Biased Agonism of the Calcium-Sensing Receptor. *Cell Calcium* 51:107-16.
3. Gloriam DE*, Wellendorph P*, Johansen LD*, **Thomsen ARB**, Phonekeo K, Brauner-Osborne H (2011). Chemogenomic Discovery of Novel Allosteric Antagonists at the GPRC6A Receptor. *Chem Biol* 18:1489-98. [*co-first authors]
2. Garcia-Rodríguez L, Abate-Daga D, **Rojas A**, González JR, Fillat C (2011). E-cadherin Contributes to the Bystander Effect of TK/GCV Suicide Therapy and Enhances its Antitumoral Activity in Pancreatic Cancer Models. *Gene Ther* 18:73-81.
1. Andersen PS, Havndrup O, Bundgaard H, Hougs L, Sørensen KM, Larsen LA, Hedley P, **Thomsen ARB**, Moolman-Smook J, Christiansen M (2009). Sarcomere Gene Mutations in Hypertrophic Cardiomyopathy: Diagnostic Yield, Interpretation and Clinical Usefulness. *Hum Mutat* 30:363-70.

PATENTS

1. *Invention Disclosure Pending: Thomsen ARB*, Cahill TJ, Lefkowitz RJ (2014). Novel Method for Isolation and Characterization of Receptor-Transducer Protein Complexes from Crude Cell Lysates. Number 4497.

CONFERENCE PRESENTATIONS

12. Endosomal GPCR Signaling – Unexplored Corners of the GPCR World. **Thomsen ARB**. University of Michigan, Ann Arbor, MI, USA. **(Invited speaker)**
11. Endosomal GPCR Signaling – Unexplored Corners of the GPCR World. Drug Research Academy (DRA) Lecture. **Thomsen ARB**. University of Copenhagen, Copenhagen, Denmark. **(Invited speaker)**
10. Exploration of Endosomal GPCR Signaling using Electron Microscopy. **Thomsen ARB**. Drug Discovery Chemistry 2019, San Diego, CA, USA. **(Invited speaker)**
9. Exploration of Endosomal GPCR Signaling using Electron Microscopy. **Thomsen ARB**. Drug Discovery on Target 2018, Boston, MA, USA. **(Invited speaker)**
8. Biophysical Basis for Sustained G Protein Signaling by Internalized G Protein-Coupled Receptors. **Thomsen ARB**. GPCR-Targeted Drug Design Conference 2017, San Diego, CA, USA. **(Invited speaker, presentation highlighted in *Genetic Engineering & Biotechnology News*)**
7. Biophysical Basis for Sustained G Protein Signaling by Internalized G Protein-Coupled Receptors. **Thomsen ARB**, Plouffe B, Cahill III TJ, Shukla AK, Tarrasch JT, Dosey AM, Kahsai AW, Strachan RT, Pani B, Mahoney JP, Huang L, Breton B, Sunahara RK, Skiniotis G, Bouvier M, Lefkowitz RJ. 3rd GPCR Targeted Screening Conference 2016, Berlin, Germany. **(Oral presentation prize winner)**
6. Biophysical Basis for Sustained G Protein Signaling by Internalized G Protein-Coupled Receptors. **Thomsen ARB**, Plouffe B, Cahill III TJ, Shukla AK, Tarrasch JT, Dosey AM, Kahsai AW, Strachan RT, Pani B, Mahoney JP, Huang L, Breton B, Sunahara RK, Skiniotis G, Bouvier M, Lefkowitz RJ. 3rd GPCR Targeted Screening Conference 2016, Berlin, Germany. **(Poster presentation)**
5. Super Complexes of the β_2 -Adrenergic Receptor. **Thomsen ARB**. GPCR Workshop 2013, Maui, HI, USA. **(Oral presentation)**
4. Delineation of the GPRC6A Receptor Signaling Pathways Using a Mammalian Cell Line Stably Expressing the Receptor. **Thomsen ARB**, Jacobsen SE, Nørskov-Lauritsen L, Smajilovic S, Wellendorph P, Larsson NHP, Lehmann A, Bhatia VK, Bräuner-Osborne H. GPCR Workshop 2013, Maui, HI, USA. **(Poster presentation)**
3. Differential Signaling of the Calcium-Sensing Receptor in Calcitonin Secreting 6-23 cells, **Thomsen ARB**, Drug Research Academy Summer Conference, University of Copenhagen, Copenhagen, Denmark, 2010. **(Oral presentation)**

2. Differential Signaling of the Calcium-Sensing Receptor, **Thomsen ARB**, Day of Research at the Faculty of Pharmaceutical Sciences, University of Copenhagen, Copenhagen, Denmark, 2010. **(Poster presentation)**
1. Physiological Studies of the Calcium-Sensing Receptor using Gene Knock-Down Techniques and Pharmacological Interventions, **Thomsen ARB**, LEO Pharma A/S Research Day, LEO Pharma A/S, Ballerup, Denmark, 2009. **(Oral presentation)**

REFEREE FOR SCIENTIFIC JOURNALS

- *Proceedings of the National Academy of Sciences of the United States of America*
- *Journal of Pharmacology and Experimental Therapeutics*
- *Molecular and Cellular Endocrinology*
- *Scientific Reports*

MENTORING/SUPERVISION OF POSTDOCTORAL TRAINEES AND STUDENTS

Columbia University

- **Nicole A. Perry**, Postdoctoral Research Scientist, Columbia University **2019-Present**
“Endosomal Chemokine Receptor Signaling as Basis for Metastasis in Malignant Melanoma”
- **John Little IV**, Research Assistant, Columbia University **2019-Present**

Duke University

- **Anthony Nguyen**, MD/PhD Student, Duke University **2016 – 2017**
“High-Resolution Structure of the GPCR–G Protein– β -arrestin Megaplex”
- **Thomas J. Cahill III**, MD/PhD student, Duke University **2014 – 2017**
“Depicting GPCR– β -arrestin Conformations”
- **Adi Blanc**, Undergraduate student, Duke University **2014 – 2015**
- **Benjamin Berger**, Undergraduate student, Duke University **2016 – 2017**
- **Albert Antar**, Undergraduate student, Duke University **2016 – 2017**
- **Jack Little IV**, Undergraduate student, Duke University **2016 – 2017**

University of Copenhagen

- **Stine E. Jacobsen**, MSc student, University of Copenhagen **2011 – 2012**
“Cellular Signaling of GRPC6A”
- **Karina Phonekeo**, MSc student, University of Copenhagen **2010 – 2011**
“Structure-Activity Relationships of N-Benzyl Phenethylamines as 5-HT_{2A/2C} Agonists”

- **Maja Hvidtfeldt**, MSc student, University of Copenhagen **2010 – 2011**
“Agonist Promoted ERK1/2 Signaling by the Calcium-Sensing Receptor”

REFERENCES

- **Professor Robert J. Lefkowitz, Duke University Medical Center**
Phone: (+1) 919 684 5001
Email: lefko001@receptor-biol.duke.edu
- **Professor Hans Bräuner-Osborne, University of Copenhagen**
Phone: (+45) 35 33 44 69
Email: hbo@sund.ku.dk
- **Professor Edward M. Brown, Brigham and Women’s Hospital, Harvard Medical School**
Phone: (+1) 617 732 5363
Email: embrown@bwh.harvard.edu