

Decoding pain sensitization

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GPR35 tunes sensory neuron ex-
citability through the elusive
Nav1.9 channel

Margaux Theys

Thesis submitted to fulfil the requirements for
the degree of Doctor in Health Sciences

Academic year 2025-2026

Doctoral advisory committee

Prof. Dr. Alain Labro

Department of Basic and Applied Medical Sciences, Physiology
Group, Ghent university, Belgium

Prof. Dr. Patrick Wouters

Department of Anesthesiology and Perioperative Medicine,
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Molecular Physiology and Neurophysics Group

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Molecular Physiology and Neurophysics Group

margaux.theys@ugent.be

ORCID: 0000-0002-0058-104X

research.ugent.be/web/person/margaux-theys-0/

Decoding pain sensitization

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GPR35 tunes sensory neuron ex-
citability through the elusive
Nav1.9 channel

Margaux Theys

Thesis submitted to fulfil the requirements for
the degree of Doctor in Health Sciences

Academic year 2025-2026

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Group, Ghent university, Belgium

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Molecular Physiology and Neurophysics Group

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Department of Basic and Applied Medical Sciences, Molecular Physiology and Neurophysics Group, Ghent university, Belgium

Faculty of Medicine and Pharmaceutical Sciences, Center for Neurosciences (C4N), Vrije Universiteit Brussel, Belgium

Department of Pharmaceutical Sciences, Experimental Pharmacology, Vrije Universiteit Brussel, Belgium

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CONTACT

Margaux Theys

Faculty of Medicine and Health sciences

Department of Basic and Applied Medical Sciences
Molecular Physiology and Neurophysics Group

margaux.theys@ugent.be

ORCID: 0000-0002-0058-104X

research.ugent.be/web/person/margaux-theys-0/

Decoding pain sensitization

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GPR35 tunes sensory neuron ex-
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Molecular Physiology and Neurophysics Group

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CONTACT

Margaux Theys

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Department of Basic and Applied Medical Sciences
Molecular Physiology and Neurophysics Group

margaux.theys@ugent.be

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Molecular Physiology and Neurophysics Group

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GPR35 tunes sensory neuron ex-
citability through the elusive
Nav1.9 channel

Margaux Theys

Thesis submitted to fulfil the requirements for
the degree of Doctor in Health Sciences

Academic year 2025-2026

Doctoral advisory committee

Prof. Dr. Alain Labro

Department of Basic and Applied Medical Sciences, Physiology
Group, Ghent university, Belgium

Prof. Dr. Patrick Wouters

Department of Anesthesiology and Perioperative Medicine,
Department of Basic and Applied Medical Sciences,
Ghent University, Belgium

Examination committee

Prof. Dr. Patrick Calders – Chair

Department of Rehabilitation Sciences,
Ghent University, Belgium

Prof. Dr. Christophe Stove – Secretary

Department of Bioanalysis, Laboratory of Toxicology,
Ghent University, Belgium

Prof. Dr. Luc De Baerdemaeker

Department of Anesthesiology and Perioperative Medicine,
Department of Basic and Applied Medical Sciences,
Ghent University Hospital, Belgium

Prof. Dr. Ruslan Dmitriev

Department of Human Structure and Repair, Tissue Engineering
and Biomaterials Group, Ghent University, Belgium

Prof. Dr. Filip Van Petegem

Department of Biochemistry and Molecular Biology,
University of British Columbia, Canada

Prof. Dr. Katharina Zimmermann

Klinik für Anästhesiologie am Universitätsklinikum Erlangen,
Friedrich-Alexander Universität, Germany

Short Curriculum Vitae

2020 – 2025: PhD in Health Sciences

University of Ghent, Belgium

2020 – 2024: Fellowship Fundamental Research

Research Foundation Flanders (FWO)

2018 – 2020: M.S. in Biomedical Sciences – Major Neuroscience

University of Ghent, Belgium

2015 – 2018: B.S. in Biomedical Sciences

University of Ghent, Belgium

2019: SCORE International Research Exchange

CEBio, Porto Velho, Brazil

2017 – 2019: Honours Programme in Life Sciences

University of Ghent, Belgium

Publications

Theys M., Vander Cruyssen J., Salvatierra J., Bosmans F. (in review). GPR35 modulates Nav1.9 to shape sensory neuron excitability. *PNAS*.

Theys M., De Waele J., Garud S., Willegems K., Van Petegem F., Bosmans F. (2025). A robust expression system reveals distinct gating mechanisms and calmodulin regulation of Nav1.9 channels. *Science Advances*.

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*Co-first authors

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Summary

The voltage-gated Na⁺ channel Na_v1.9 is expressed in peripheral nociceptors and highly sensitive to inflammatory mediators. However, its investigation is hampered by unresolved technical challenges, leaving its role in pain elusive and its therapeutic potential untapped. This dissertation addresses this gap through two main aims: (1) to establish a reproducible platform for heterologous Na_v1.9 expression, and (2) to investigate regulation of Na_v1.9 by the G protein-coupled receptor GPR35, an emerging drug target in inflammatory diseases.

The first aim led to the development of a reliable, cost-effective protocol for Na_v1.9 expression in heterologous systems. This approach facilitates pharmacological screening and accelerates characterization of disease-associated variants, as demonstrated with the congenital insensitivity to pain mutation *p.L881P* and its mouse analogue. The protocol requires only standard laboratory equipment and provides a fully transparent methodology, lowering technical barriers for the broader research community. We also demonstrate this method can be used to gain insights into the relationship between the distinctive gating properties of Na_v1.9 and its sequence, identifying the pre-IQ region as a key determinant of channel availability.

The second aim examined Na_v1.9 regulation by GPR35. Using immunofluorescence and proximity ligation assays, we show that GPR35 co-localizes with Na_v1.9 in dorsal root ganglia. Functional assays revealed context-dependent modulation: receptor activation enhanced Na_v1.9 currents under baseline conditions, but had the opposite effect in PGE₂-sensitized neurons. Recordings from GPR35-deficient mice showed altered channel kinetics and depolarized

resting membrane potentials, consistent with constitutive modulation of neural excitability. Moreover, cGMP accumulation was sufficient to potentiate Na_v1.9 currents in a GPR35-dependent manner, suggesting cGMP may be a more relevant agonist than previously recognized.

Together, these studies establish a tractable framework for Na_v1.9 research and identify GPR35 as a novel regulator of its activity. By addressing key technical and mechanistic gaps, the work not only advances the field's understanding of Na_v1.9 gating and function but also provides new entry points for ligand screenings and probing its role in nociception.

Promotor

Prof. Dr. Frank Bosmans

Department of Basic and Applied Medical Sciences, Molecular Physiology and Neurophysics Group, Ghent university, Belgium

Faculty of Medicine and Pharmaceutical Sciences, Center for Neurosciences (C4N), Vrije Universiteit Brussel, Belgium

Department of Pharmaceutical Sciences, Experimental Pharmacology, Vrije Universiteit Brussel, Belgium

Funding

- FWO fundamental research fellowship 1106221N
- Research Foundation–Flanders grant G000220N
- ERA-NET Neuron grant GOH8120N

An electronic version of the thesis can be obtained via UGent Biblio or upon request.

CONTACT

Margaux Theys

Faculty of Medicine and Health sciences

Department of Basic and Applied Medical Sciences
Molecular Physiology and Neurophysics Group

margaux.theys@ugent.be

ORCID: 0000-0002-0058-104X

research.ugent.be/web/person/margaux-theys-0/