FACULTEIT DIERGENEESKUNDE
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UNIVERSITEIT GENT

# INVITATION PUBLIC DEFENSE

## The efficacy of self-amplifying RNA vaccines against H5N1 influenza and the impact of pre-existing immunity against the replicase of self-amplifying RNA

**Xiaole Cui**

**23rd October 2025**

PROMOTORS

Prof. dr. Niek N Sanders
Faculty of Veterinary Medicine, UGent

Dr. Zifu Zhong
Faculty of pharmaceutical sciences, UGent

## Curriculum Vitae

Xiaole Cui was born on 16 August 1991 in Yuncheng, China. He obtained a BSc in Veterinary Medicine from Northwest A&F University, China, in 2015. In 2018, he received his MSc in Veterinary Medicine from the Graduate School of the Chinese Academy of Agricultural Sciences, where his research focused on the impact of gene mutations on the pathogenicity of avian influenza A virus. Since February 2021, Xiaole has been conducting his doctoral research in the laboratory of Professor Niek Sanders at Ghent University, focusing on the development of self-amplifying mRNA vaccines against avian influenza A virus and investigating the immune response to self-amplifying mRNA replicase.

## Where?

The defense will take place on **Thursday 23rd Oct at 15.00h**

**Auditorium D-entrance 19**

Faculty of Veterinary Medicine
Ghent University, Campus Merelbeke
Salisburylaan 133, Merelbeke

The defense will be followed by a reception, to which you are all kindly invited.

## How to attend?

If you would like to attend, please register before 16th Oct, by email to Xiaole.cui@ugent.be

## Members of the Jury

Prof. dr. Kristien Van Reeth
Chairman of the Jury
Faculty of Veterinary Medicine, UGent

Prof. dr. Kathlyn Laval
Secretary of the Jury
Faculty of Veterinary Medicine, UGent

Prof. dr. Herman Favoreel
Faculty of Veterinary Medicine, UGent

Dr. João Portela Catani
Department of Biochemistry and microbiology, VIB-UGent

Dr. Francis Combes
Biotechnology and Nanomedicine, SINTEF research institutes

## Summary

Since the 1990s, mRNA technology has been explored as a vaccine platform, achieving its first widespread success during the COVID-19 pandemic in 2020. Self-amplifying mRNA (saRNA), typically derived from alphaviruses, offers significant advantages over conventional mRNA by inducing stronger immune responses at lower doses. By 2023, saRNA-based COVID-19 vaccines received emergency use authorization in India, and later also full approval in Japan and the EU, underscoring their transformative potential in modern vaccinology.

The key advantages of saRNA include: (1) dose-sparing due to its self-amplifying property, (2) prolonged antigen expression, driving more robust and sustained immune responses. These features position saRNA as a promising platform for next-generation vaccines.

Chapter 1 provides a general introduction relevant to this thesis and covering three main areas: (1) an overview of current influenza vaccines and the advantage of different platforms, (2) the structure, translation, and delivery mechanisms of mRNA, and (3) the innate and adaptive immune response induced by mRNA or saRNA vaccines.

Chapter 2 outlines the scientific aims of this thesis: (1) to optimize antigen design of H5N1 hemagglutinin (HA) protein, (2) to assess safety and biodistribution profiles of the saRNA vaccines in murine models, and (3) to investigate pre-existing immunity against the saRNA replicase and the consequences of anti-replicase immunity on subsequent saRNA vaccination schedule using a different antigen.

Chapter 3 presents the first research project, which focuses on the development of saRNA vaccines against avian influenza H5N1. We first optimized the N/P ratio (cationic lipid: mRNA) to achieve optimal delivery efficiency. Next, we compared the antibody and T cell responses induced by saRNA vaccines encoding different parts of the HA protein. The secreted full-length HA induced the highest levels of antibody responses and was therefore selected to determine the optimal injection dose. Subsequently, we compared the secreted full-length HA with the membrane anchored HA, which showed that the membrane anchored form induced stronger cellular and humoral immune responses. Finally, we evaluated the biosafety of saRNA vaccine by measuring the biodistribution of H5 saRNA vaccine and monitoring body weight after intramuscular injection.

Although saRNA vaccines work efficiently in mice, they also exhibit some limitations. The major shortcoming is that the non-structural proteins, which form the RNA replicase that drives the amplification of the saRNA , can potentially elicit adaptive immune responses. This anti-replicase immunity may impair the efficacy of subsequent saRNA vaccines or gene therapies.

Chapter 4 presents the second research project, which examined the occurrence and influence of anti-replicase immunity on saRNA vaccination. Here, we first found that the saRNA platform induces both antibody and T cell responses against the replicase, and that luciferase expression of saRNA was negatively affected by this immunity in mice T cells responses. We then evaluated whether this immunity impacts the efficacy of saRNA vaccination. While antibody responses to new antigens encoded by a second saRNA vaccine schedule were unaffected, new antigen-specific Th1 cell responses were impaired, and this impairment was not attenuated by elongating the interval between two different saRNA vaccination schedules. Further analysis revealed that antibody responses as well as T-cell responses against the replicase may play a key role in suppressing T cell responses to subsequent vaccines. Finally, a viral challenge experiment showed that the saRNA vaccine maintained complete protection despite pre-existing anti-replicase immunity.

Chapter 5 provides the general discussion. In this final chapter, we summarize and discuss the key findings from our research, including (1) the HA antigen design and selection, (2) the advantage of membrane anchored antigen compared to secreted one, (3) cellular immunity and vaccine platforms, (4) impact of pre-existing immunity to the saRNA replicase on subsequent saRNA vaccine schedules, (5) potential strategies to mitigate pre-existing immunity challenges, (6) mechanisms of saRNA-induced immune responses to intracellular antigens, (7) the efficacy of self-amplifying RNA vaccination, (8) the limitation of our research and (9) future perspectives on saRNA vaccine design and development.