SCIENCES & BIOENGINEERING SCIENCES

The Research Group
Microbiology

has the honor to invite you to the public defence of the PhD thesis of

# Yifei Xu

to obtain the degree of Doctor of Bioengineering Sciences

Title of the PhD thesis:

## Expanding the genetic toolbox for *Sulfolobus acidocaldarius*, a model species of thermoacidophilic Crenarchaeota

Supervisor:
**Prof. dr. ir. Eveline Peeters (VUB)**
Co-supervisor:
**Dr. ir. Indra Bervoets (VUB)**

The defence will take place on
**Tuesday, March 31, 2026 at 5 p.m.**

VUB Etterbeek campus, Pleinlaan 2, Elsene, promotiezaal D.2.01

The defence can be followed through a live stream: Public PhD defense Yifei Xu | 会议加入 | Microsoft Teams

### Members of the jury

Prof. dr. ir. Geert Angenon (VUB, chair)
Prof. dr. Wim Vranken (VUB)
Prof. dr. Henri De Greve (VUB)
Prof. dr. ir. Marjan De Mey (UGent)
Dr. Marleen van Wolferen (University of Freiburg, DE)

### Curriculum vitae

Yifei Xu (°Chengdu, China, 13 October 1993) obtained his Bachelor's degree in Veterinary Medicine from Sichuan Agricultural University (China) in 2016. He continued his studies at the same university and received his Master's degree in Veterinary Medicine in 2019. In October 2019, he started his PhD research at the Research Group of Microbiology (MICR), Department of Bioengineering Sciences at Vrije Universiteit Brussel (Belgium), under the supervision of Prof. Eveline Peeters. His doctoral research focuses on expanding the genetic toolbox for the thermoacidophilic archaeon *Sulfolobus acidocaldarius*, including the development of improved transformation strategies, genomic integration tools, and fluorescent protein reporters. During his PhD, he supervised master's thesis students and contributed to several international conferences. His research has resulted in a peer-reviewed publication in *Frontiers in Microbiology* and multiple presentations at international scientific meetings.

### Abstract of the PhD research


Within the *Crenarchaeota*, species belonging to the *Sulfolobales* are considered important model organisms for the fundamental study of molecular biological processes in archaea and they also provide valuable evolutionary insights. In addition, they show promise as hosts for biotechnological applications due to their thermoacidophilic lifestyle, growing optimally at high temperatures between 75 and 80°C and at low pH values between 2 and 3, as well as their metabolic characteristics, such as chemoorganotrophic metabolism.

Because of its genetic stability, *Sulfolobus acidocaldarius* is regarded as a chassis species for genetic studies and for genetic engineering aimed at biotechnological applications. Over the past two decades, a suite of genetic tools has been developed for *S. acidocaldarius*, including transformation procedures for introducing exogenous DNA, shuttle plasmid vectors derived from native *Saccharolobus* viruses and plasmids and genome engineering approaches based on homologous recombination. However, many barriers still hinder progress in the use of *S. acidocaldarius* as a chassis strain. For example, although fluorescent proteins are widely used as reporter tools in a variety of host organisms — particularly in bacteria and eukaryotes — for purposes such as cellular visualization and gene expression monitoring, their use in thermophilic archaea remains limited.

This PhD study centers on *S. acidocaldarius* as a model organism, aiming to further expand and refine the genetic toolbox to facilitate future research on archaea. First, various culture media were analyzed for the growth of *S. acidocaldarius* and the conditions for electroporation-based transformation were optimized. I then employed a reporter cassette to investigate differences in expression levels at multiple genomic loci across the *S. acidocaldarius* genome, successfully identifying an ideal integration site for high expression. Finally, a panel of green fluorescent proteins was screened and a red fluorescent protein with improved thermostability was engineered. These candidate fluorescent proteins were introduced into *S. acidocaldarius* via both shuttle plasmid-based expression and chromosomal integration, followed by fluorescence detection and analysis. None of the fluorescent proteins exhibited ideal performance under the tested conditions. Nonetheless, our work provides valuable theoretical insights and methodological tools that will support further development of *S. acidocaldarius* as a chassis strain, as well as future research efforts in the field of archaea.