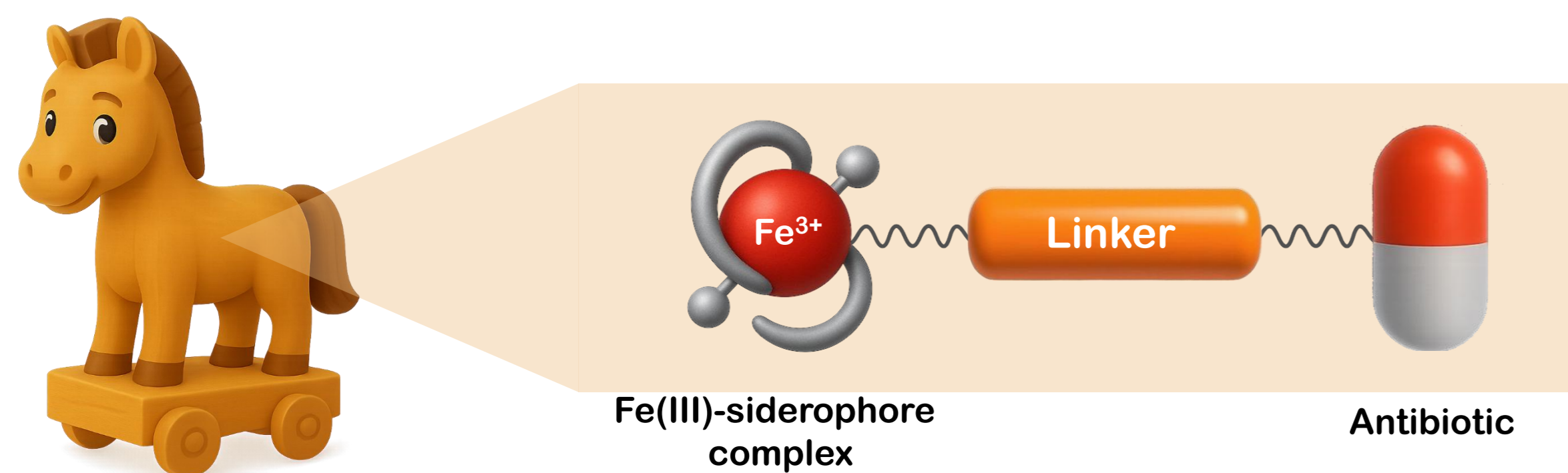


INTRODUCTION & CONTEXT

- Acinetobacter baumannii* is a **critical-priority** human pathogen (ranked by the WHO).
- It shows multidrug resistance (MDR) — low membrane permeability, efficient efflux, and rapid gene acquisition.¹
- New therapeutic strategies are urgently needed to overcome these barriers.
- Siderophores = natural bacterial "iron shuttles" → can be hijacked for drug delivery.
- The "Trojan horse" strategy²:

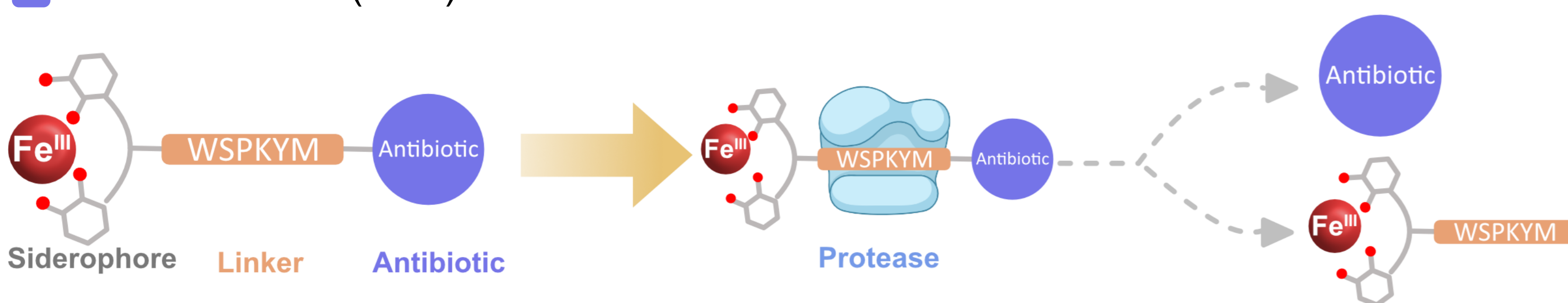
Acinetobacter baumannii



PROJECT AIM

To design and synthesize a protease-activated siderophore–antibiotic conjugate for *A. baumannii*, combining:

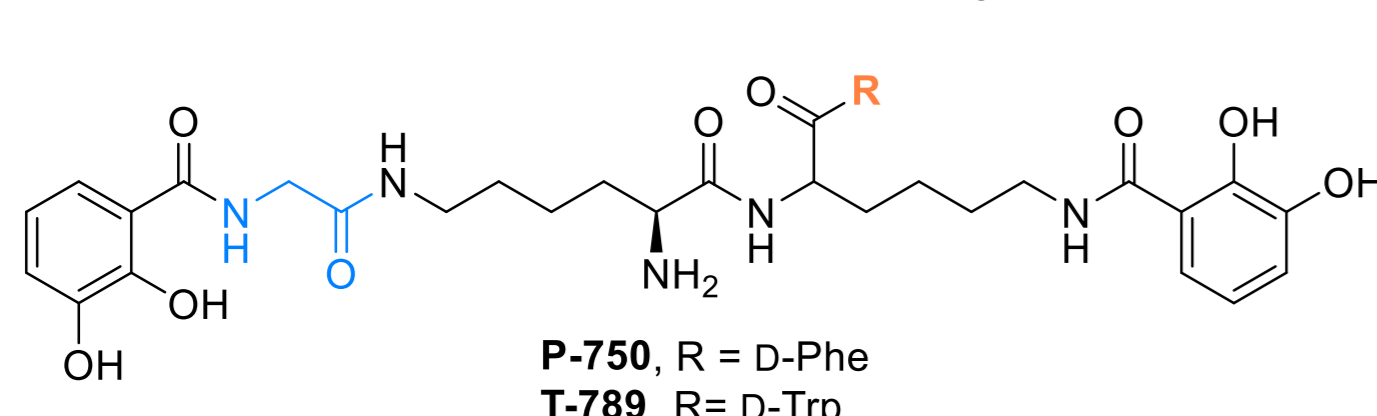
1. A simplified amonabactin siderophore analogue (AMB)
2. A protease-cleavable peptide WSPKYM
3. And norfloxacin (NFX) as antibiotic



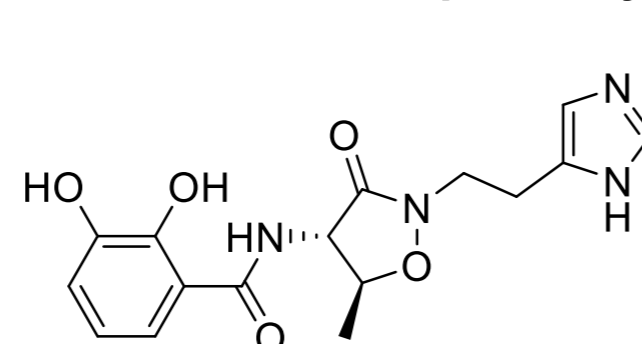
WHY AMONABACTIN?

- Amonabactin is a catechol-type siderophore similar to acinetobactin.
- Its bicatecholamide core ensures Fe³⁺ binding and receptor recognition.
- The glycine spacer improves uptake efficiency.
- Its structural simplicity makes it ideal for synthetic modification.
- Amonabactin analogues are recognized by *A. baumannii* iron transport receptors, and so, they could function as vectors for the antibiotic entry.³⁻⁵

Amonabactin siderophore system



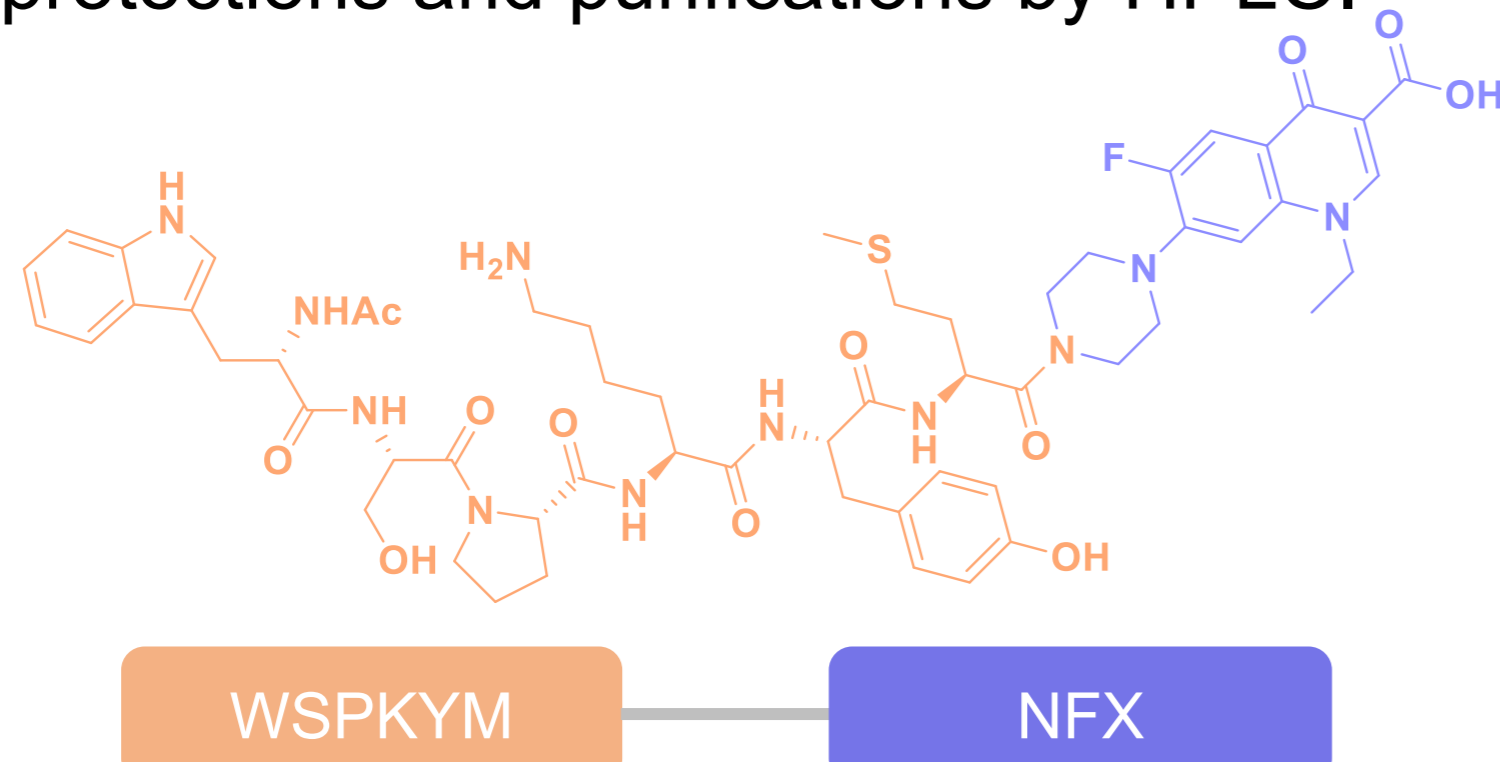
Acinetobactin siderophore system



EXPERIMENTAL APPROACH

1. Synthesis of conjugate WSPKYM – NFX⁶

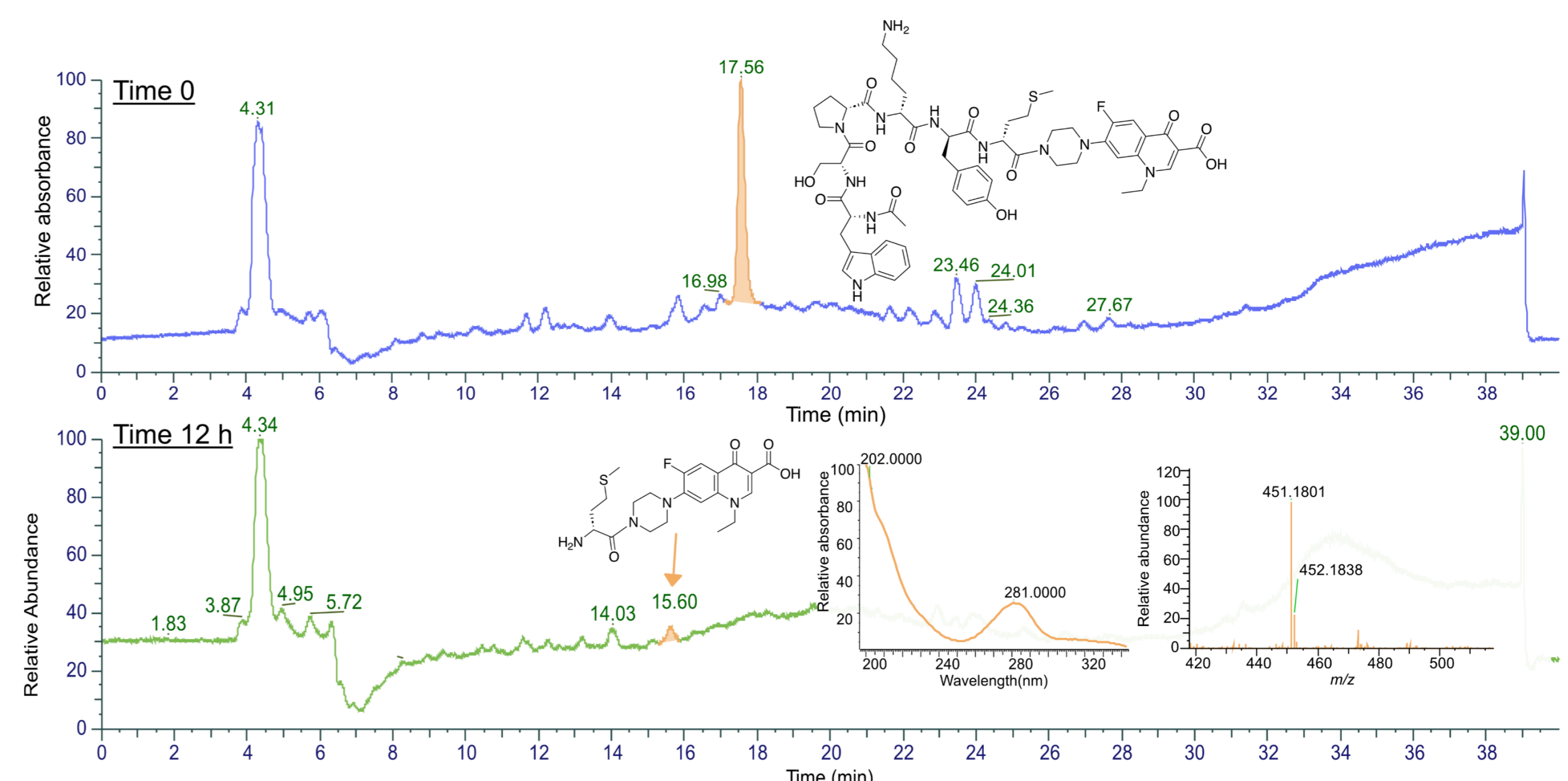
- Linker was synthesized by **SPPS** under inert atmosphere.
- C-carboxylic acid terminal was activated with **NHS** and coupled to NFX.
- Last steps were deprotections and purifications by HPLC.



EXPERIMENTAL APPROACH

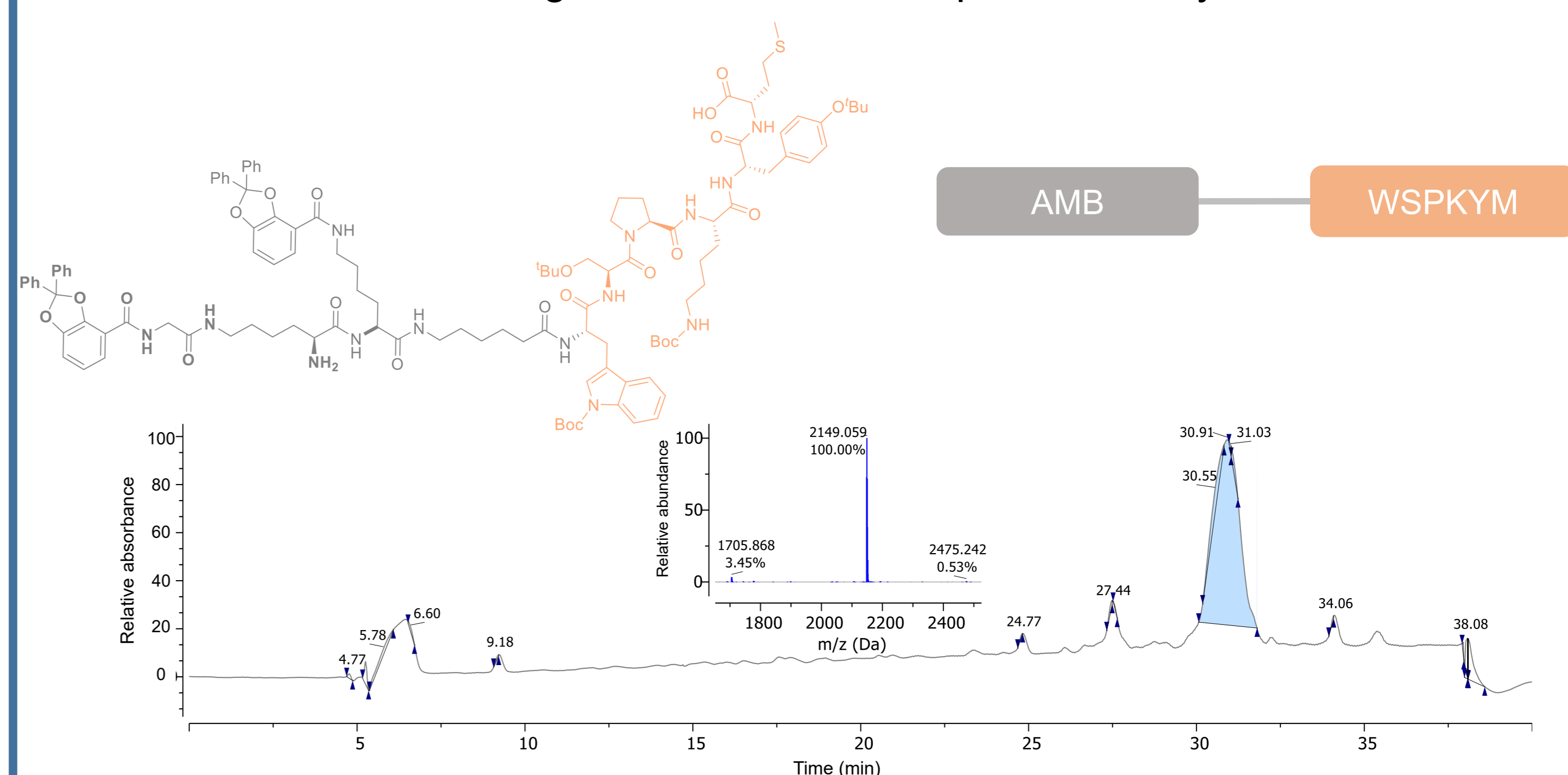
2. Protease cleavage assay

- Model conjugate was incubated with a **periplasmic protease extract** from *A. salmonicida*.
- HPLC/MS** of the product displayed disappearance of hexapeptide and appearance of free NFX → **confirmed cleavage**.

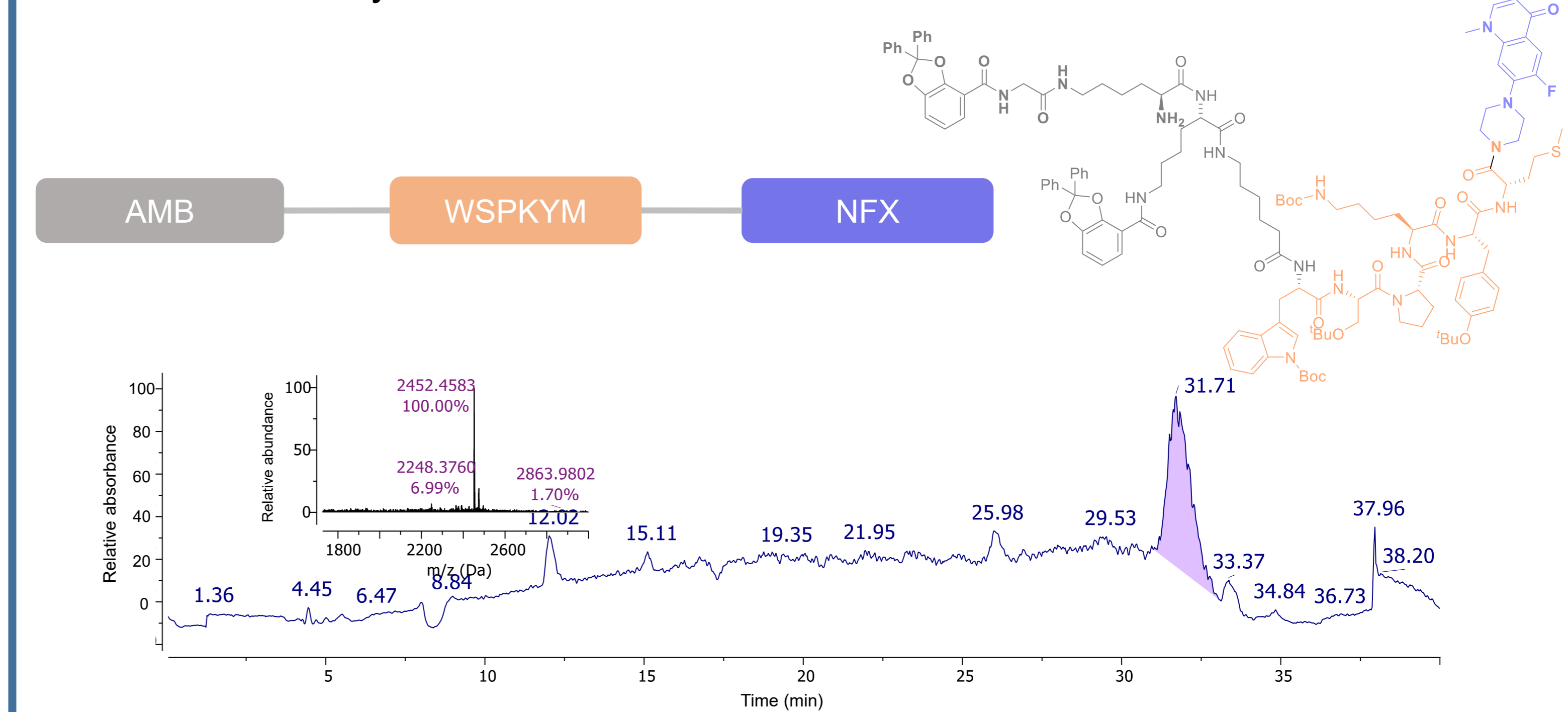


3. Synthesis of conjugate AMB – WSPKYM – NFX

- Solution-phase synthesis** of AMB.
- Coupling with the WSPKYM by **SPPS**.
- AMB-WSPKYM** cleavage from the resin and purification by HPLC.



- Final coupling with NFX (using the coupling agent **HATU**).
- Confirmation by **HPLC/MS**.



FUTURE WORK

- Scale-up of synthesis of the conjugate.
- Biological testing under iron restriction.
- Evaluation of antimicrobial activity and selectivity.

CONCLUSIONS

Successful synthesis of the protease-activated Amonabactin–WSPKYM–NFX conjugate for *A. baumannii* were achieved. The WSPKYM linker was confirmed to be cleaved by periplasmic enzymes, enabling antibiotic release. This modular platform offers adaptability to other antibiotics and represents a promising "Trojan horse" strategy to overcome antibiotic resistance.