1. What is antimicrobial resistance?
Antimicrobial resistance (AMR) occurs when microbes such as bacteria and viruses accumulate changes over time. They no longer respond to antimicrobial treatments such as antibiotics and antivirals. Main causes include the misuse of antimicrobials in humans, animals, and plants.

2. Why is it a global challenge?
- AMR is one of the top ten threats to global public health, food security and development. It is known as a “silent pandemic” (WHO).
- In 2019, bacterial antibiotic resistance was directly responsible for 1.27 million global deaths.
- Antibiotic resistance makes medical procedures and treatments such as surgery and cancer chemotherapy riskier.

Antibiotic discovery and resistance timeline
- Bacteria are getting faster at developing resistance to antibiotics.
- 30 years since a new class of antibiotics was last introduced.

3. Four mechanisms of resistance in bacteria
1. Modify the antibiotic target
2. Decrease uptake of antibiotics
3. Antibiotic inactivation by enzymes
4. Multidrug efflux pumps

4. Aims
How do multidrug efflux pumps work to expel antibiotics?
Can we inhibit (stop) that happening to allow antibiotics to remain inside and kill bacteria?

To tackle this biological problem we:
- Establish a multi-disciplinary academia/industry partnership
- Focus on two multidrug efflux pumps: AcrB and MdtF
- Use state-of-the-art structural biology methods

5. Structural biology methods
- Structural biology is a key tool in drug discovery which looks at proteins at a molecular level, unlocking secrets of structure, function and interactions.

Hydrogen/Deuterium eXchange Mass Spectrometry (HDX-MS)
- Tool for analysing structural features and dynamic (movement) properties of proteins

Cryogenic-electron microscopy (cryo-EM)
- Super microscope to visualise how atoms of a protein and a drug are arranged in 3D space

6. Research findings
How does AcrB expel antibiotics?
- Although structures exist for AcrB, its movement and function are unknown.
- We used HDX-MS to explore how the antibiotic (CIP) and the efflux pump inhibitor (PAβN) affect AcrB movements.

HDX-MS data summary
- CIP only
- PAβN + CIP

PAβN restricts AcrB movement
- Lower cell concentration of CIP
- Drug efflux is inhibited and bacteria die

Antibiotic susceptibility assays: PAβN enhances CIP activity
- Fluoroquinolones
- Penicillins
- Carbapenems
- Macrolides

Impact
- Efflux pumps are also found in human cells (not just in bacteria).
- The leading cause of cancer chemotherapy failure is the development of multidrug resistance by efflux pumps e.g. P-glycoproteins in human cancer cells.
- Overall, understanding how inhibitors of efflux pumps work can:
  - overcome bacterial resistance and allow us to reuse antibiotics
  - restore the sensitivity of human cancer cells toward chemotherapy drugs.