RaMoA: Combination of Raman micro-spectroscopy with deep learning for rapidly predicting the Mechanism of Action of an antimicrobial and its potential novelty

C. Madras[a], C. Constanza[a], B. Courbon[a], L. Thibon[a], H. Dugua[a], Z. Sedaghat[a], D. Abedini[a], A. Novoloaca[a], C. Couturier[a], D. Mercer[a], C. Guyard[a], C. Védrine[a], L. Alibaud[a], S. Dixneuf[a]

[a]BIOASTER, 40 avenue Tony Garnier, 69007 Lyon, France

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Background. The discovery of new antimicrobial agents, natural or synthetic, displaying innovative mechanisms of action (MoA) is urgently needed to tackle the emergence and spreading of antimicrobial resistance. Determining the precise MoA of novel antimicrobial drug candidates is highly desirable to predict the potential for toxicity, yet it can be a time-consuming and expensive process. Moreover, a rapid, robust, inexpensive, and versatile tool able to determine the MoA or suspect a novel MoA of a drug candidate would be most favourable to help lead candidate selection.

Methods and results. We propose an innovative technology to classify the MoA of an antimicrobial and detect its novelty. RaMoA is based on the combination of Raman micro-spectroscopy and Deep Learning. In combination with proper spectra pre-processing, Raman micro-spectroscopy enables a label-free chemical fingerprinting of the treated bacteria, to reveal phenotypic responses to antibiotics. Deep Learning techniques are powerful tools to extract discriminative features from complex spectra and classify them. We assess the performance of the RaMoA technology for a wild type *E. coli* strain treated with 15 antibiotic molecules representing the 5 main inhibition classes (i.e. Cell Wall synthesis inhibitors, Cell Membrane inhibitors, Proteins synthesis inhibitors, DNA and RNA synthesis inhibitors). First, a 1D Convolutional Neural Network classifier is used to detect the mode of action of known antibiotics with 93% accuracy at spectrum-level and 100% at molecule-level. Secondly, we show how AutoEncoders can be used for the novelty assessment of the MoA of a candidate antibiotic.

Conclusion. We successfully demonstrate that RaMoA is a very promising (fast and label-free) tool for MoA detection of new antimicrobial candidates, and that it is very complementary to morphological-profiling-based technologies such as Bacterial Cytological Profiling[1] (i.e. label-based) or HoloMoA[2] (i.e. label-free), in terms of readouts and interpretability.

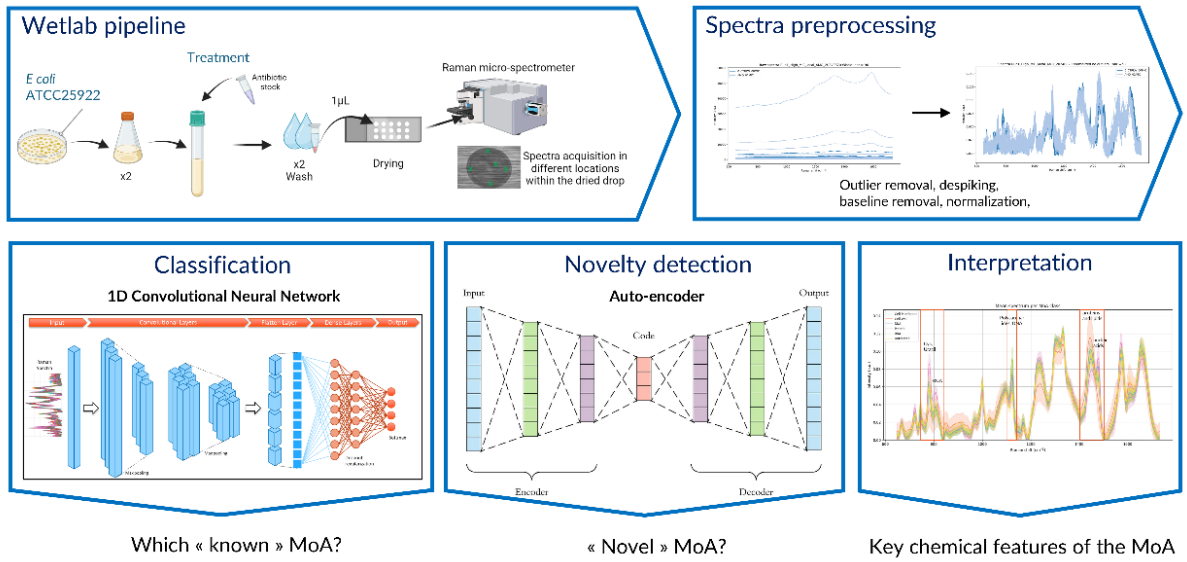

Figure 1. RaMoA pipeline to predict the MoA and novelty of MoA of antimicrobial candidates

[1] Nonejuie P, Burkart M, Pogliano K, Joe Pogliano J. 2013. Bacterial cytological profiling rapidly identifies the cellular pathways targeted by antibacterial molecules. PNAS 110:16169-16174

[2] Sedaghat Z, Courbon B et al. 2025. HoloMoA: Fast holography and deep-learning-based tool for the novelty detection of mechanism of action of antimicrobial candidates. In preparation for submission to Antimicrobial Agents and Chemotherapy.