Poster

Insight into structure and function of a *Klebsiella* phage capsular depolymerase: from a serendipitous finding to the design of active mini-enzymes against *K. pneumoniae*

Mario Privitera[1,2], Barbara Maciejewska[3], Flavia Squeglia[1], Agnieszka Latka[3], Sebastian Olejniczak[3], Paulina Switala[3], Alessia Ruggiero[1], Daniela Marasco[4], Eliza Kramarska[1], Zuzanna Drulis-Kawa[3], Rita Berisio[1]

[1]Institute of Biostructures and Bioimaging, CNR, Napoli, Italy, [2]University of Campania Luigi Vanvitelli, Caserta, Italy, [3]Department of Pathogen Biology and Immunology, Institute of Genetics and Microbiology, University of Wrocław, Wrocław, Poland, [4] Department of Pharmacy, University of Naples Federico II, Napoli, Italy

mario.privitera@unicampania.it

Antimicrobial-resistance (AMR) stands nowadays as a pressing concern in public health. A group of six pathogens is named with the acronym 'ESKAPE' because of their ability to evade the biocidal action of traditional clinically used antibiotics [1,2]. Structural insight of molecular factors which play a key role in AMR is fundamental to deeply understand their mechanisms on and develop effective new drugs. The bacterium *Klebsiella pneumoniae* exploits various instruments to evade the immune system response and antibiotic attacks during infection. One such strategy involves its cell envelope, that includes polysaccharide layers acting as protective shield against stress and external substances.

Virion-associated depolymerases are large trimeric and multi-domain proteins that constitute the phage arsenal to degrade the polysaccharidic barriers in outer membrane of their bacterial host [3]. Thus, as recombinant proteins, they are endowed with outstanding potential in biotechnology and medicine [4]. In this study [5], we elucidated the structural and functional features of the capsular depolymerase KP34gp57 from the Klebsiella phage KP34. Based on the crystal structure and site-directed mutagenesis, we localized the key catalytic residues in an intra-subunit deep groove. Moreover, we engineered several N- and C-terminally truncated versions of KP34gp57 to dissect the role of each domain in the enzyme stability and catalytic activity. Serendipitously, our studies revealed C-terminally trimmed KP34gp57 variants that did not trimerize and were sufficiently stable to preserve full catalytic activity as monomers [6]. The development of trimmed monomeric and fully active phage depolymerases is innovative in the field, as no previous example exists apart from bacterial enzymes. Mini phage depolymerases can be optionally combined within chimeric enzymes to extend their activity range, facilitating their use in standalone treatments.

[1] Mulani M.S., Kamble E.E., Kumkar S.N., Tawre M.S., Pardesi K.R. (2019) *Front Microbiol.* 10:539.

[2] De Oliveira D.M.P., Forde B.M., Kidd T.J., Harris P.N.A., Schembri M.A., Beatson S.A., Paterson D.L., Walker M.J. (2020) *Clin Microbiol Rev.* 33(3).

[3] Majkowska S.G., Łątka A., Berisio R., Maciejewska B., Squeglia F., Romano M., Lavigne R., Struve C., Drulis-Kawa Z. (2016)

Viruses. 8(12):324.

[4] Majkowska-Skrobek G, Latka A, Berisio R, Squeglia F, Maciejewska B, Briers Y, Drulis-Kawa Z. (2018) *Front Microbiol.*

9:2517.

[5] Maciejewska B., Squeglia F., Latka A., **Privitera** M., Olejniczak S., Switala P., Ruggiero A., Marasco D., Kramarska E., Drulis- Kawa Z., Berisio R. (2023) *mBio* 14(5).

[6] Squeglia F, Maciejewska B, Łątka A, Ruggiero A, Briers Y, Drulis-Kawa Z, Berisio R. (2020) *Structure* 28(6):613-624.