medical sciences forum

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Abstract

Characterisation and Optimisation of Anti-LexA Nanobodies Targeting the SOS-response Pathway to Fight Antibiotic Resistance †

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† Presented at the The 2nd International Electronic Conference on Antibiotics—Drugs for Superbugs: Antibiotic Discovery, Modes of Action And Mechanisms of Resistance, 15–30 June 2022 ; Available online: https://eca2022.sciforum.net/

Citation: Campagnaro, E.; Vascon, F.; Maso, L.; Chinellato, M.; Goormaghtigh, F.; Bellio, P.; Van Melderen, L.; Ruzzene, M.; Pardon, E.; Angelini, A.; et al. Characterisation and Optimisation of Anti-LexA Nanobodies Targeting the SOS-response Pathway to Fight Antibiotic Resistance. **2022**, 2, x. https://doi.org/10.3390/xxxxx

Academic Editor(s):

Published: date

Keywords: SOS response, LexA, autoproteolysis, RecA, antibiotic resistance, nanobodies

The SOS response was found to be directly involved in the onset of antibiotic resistance. Our research project represents a promising strategy to inhibit this signalling pathway blocking LexA protein autoproteolysis.

For this purpose, we initially identified three nanobodies (NbSOS1-3) that can inhibit LexA autoproteolysis. After extensive characterisation, we father optimised these Nbs by means of rational protein engineering.

Furthermore, biparatopic nanobodies (BiNbSOSs) were constructed, fusing with a flexible amino acid linker two Nbs having different recognition sites to the LexA antigen.

We chose a functional characterisation based on an integrative approach, combining mainly in vitro techniques. We performed assays based on Fluorescence Polarisation (FP) to derive the IC50 values of the NbSOSs tested. This enabled us to detect the inhibitory capacity towards the autoproteolysis of LexA. We also determined KD values of the NbSOSs by Surface Plasmon Resonance technique (SPR) in order to evaluate their binding affinity to LexA.

Finally, NbSOSs, expressed in *E. coli*, were tested to verify over time their stability and expression in presence and in absence of ciprofloxacin (a strong inducer of exogenous stress capable of activating the SOS response in bacteria).

We demonstrated that these Nbs inhibit LexA autoproteolysis with IC_{50} values in the low micromolar range. Improvements in terms of both KD, IC_{50} and expression profile were observed for rationally designed mutants as well as BiNbSOSs.

We believe that these results pave the way for novel approaches in the fight against antibiotic resistance, leaving the door open for further research in this direction.

Author Contributions:

Funding:

Institutional Review Board Statement:

Informed Consent Statement:

Data Availability Statement:

Conflicts of Interest: