Abstract

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

Enrica Campagnaro 1, Filippo Vascon 1, Lorenzo Maso 1, Monica Chinellato 2, Frédéric Goormaghtigh 3,4, Pierangelo Bellio 5, Laurence Van Melderen 4, Maria Ruzzene 6, Els Pardon 7,8, Alessandro Angelini 9, Giuseppe Celenza 5, Jan Steyaert 7,8 and Donatella Tondi 10, Laura Cendon 1,*

1 Department of Biology, Università degli Studi di Padova, Padova, Italy; enrica.campagnaro.1@studenti.unipd.it (E.C.); filippo.vascon@gmail.com (F.V.); lorenzo.maso@unipd.it (L.M.)
2 Department of Medicine, Università degli Studi di Padova, Padova, Italy; monica.chinellato@studenti.unipd.it (M.C.)
3 Biozentrum, University of Basel, CH4056 Basel, Switzerland; frederic.goormaghtigh@unibas.ch (F.G.)
4 Faculté des Sciences, Université Libre de Bruxelles (ULB), 12 rue des Professeurs Jeener et Brachet, B 6041 Brussels, Belgium; laurence.van.melderen@ulb.be
5 Dipartimento di Scienze Cliniche Applicate e Biotecnologiche, Università degli Studi dell’Aquila, Via Vetoio Coppito, 67100 L’Aquila, Italy; pierangelo.bellio@univaq.it (P.B.); giuseppe.celenza@univaq.it (G.C.)
6 Dipartimento di Scienze Biomediche, Università degli Studi di Padova, Via Ugo Bassi 58/b, 35131 Padova, Italy; maria.ruzzene@unipd.it
7 Vrije Universiteit Brussel (VUB), Pleinlaan 2, 1050 Brussels, Belgium; Els.Pardon@vub.ac.be (E.P.); jan.steyaert@vub.be (J.S.)
8 VIBVUB Center for Structural Biology, Pleinlaan 2, 1050 Brussels, Belgium
9 Dipartimento di Scienze Molecolari e Nanosistemi, Università Ca Foscari, via Torino 155, Mestre, 30172 Venice, Italy; alessandro.angelini@unive.it
10 Department of Life Sciences, Università degli Studi di Modena e Reggio Emilia, Modena, Italy; donatella.tondi@unimore.it
* Correspondence: laura.cendon@unipd.it

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 further 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: