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

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Introduction

The SOS response is one of the most conserved pathways among bacterial species. It represents a global response to DNA damage in which the cell cycle is interrupted, and DNA repair and mutagenesis are induced. The activation depends on the interaction between two proteins, **RecA** and **LexA**. The former is a recombinase that acts as a DNA damage sensor. The latter is a bifunctional protein composed of the NTD and the CTD domains. Under normal conditions, LexA acts as a transcriptional repressor of SOS genes. In *E. coli*, such genes are involved in the onset of mutations related to antimicrobial resistance mechanisms. Instead, in case of DNA lesions, single-filament DNA fragments (ssDNA) accumulate and promote ATP-dependent oligomerisation of RecA on the ssDNA itself. This process leads to the activation of RecA (RecA*). The ssDNA/RecA* complex induces the autoproteolysis of LexA. Consequently, the NTD domain detaches from DNA with the derepression of SOS genes (Figure 1). The inhibition of the SOS response represents a promising strategy to delay the onset of bacterial resistance. Our approach for suppressing the SOS response is based on anti-LexA camelid-derived nanobodies (Maso et al., Structure, 2022, under revision). Following llama immunisation and phage display technology, we identified three anti-LexA nanobodies (**NbSOS1-3**) that block the self-cleavage of LexA and the SOS response activation. Crystal structures demonstrated that NbSOS1-3 recognise the CTD domain and trap LexA in an inactive state (Figure 2).

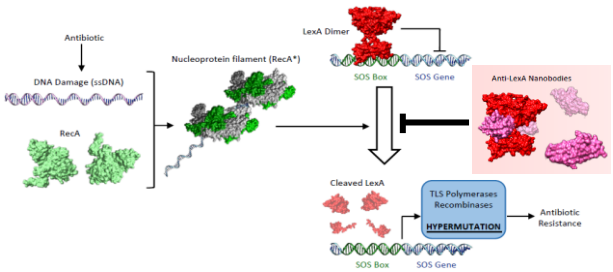

Figure 1. SOS pathway.

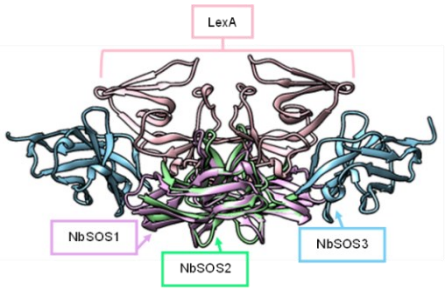

Figure 2. Crystal structures of NbSOSs and LexA.

Studies and experiments

The recombinant production and purification of these Nbs allowed us to achieve a high degree of purity for these proteins, which is necessary for their fine biochemical characterisation.

Fluorescence Polarisation (FP) Studies

To screen the Nb library and characterise *in vitro* the inhibitory activity on LexA self-cleavage of emerging hits, a Fluorescence Polarisation-based assay was set up by tagging LexA CTD with a fluorophore, so that its autoproteolysis could be followed. NbSOS1-3 inhibit FlAsH CTD LexA autoproteolysis with **IC_{50}** values ranging from **0.7** to **1.9 µM** (Figure 3).

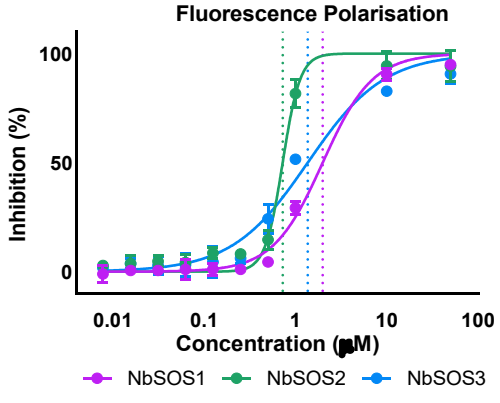

Figure 3. FP-based *in vitro* LexA autoproteolysis assay in the presence of NbSOSs at different concentrations. (Maso et al., Structure, 2022, under revision)

Surface Plasmon Resonance (SPR) Studies

The binding studies, performed with the SPR technique, allowed us to obtain the K_D values, essential to evaluate the binding affinity of the Nbs to LexA (the uncleavable mutant S119A was immobilised on chip surface). All Nbs showed sub-micromolar binding affinities towards the antigen (**K_D = 0.08 – 0.2 µM**) (Figure 4).

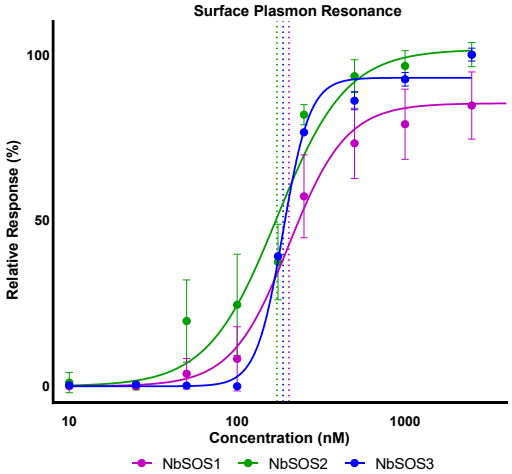

Figure 4. SPR steady state affinity determination of NbSOSs for LexA.

Time Expression Analysis

Finally, we conducted a Western Blot experiment to follow the expression of NbSOSs in *E. coli* in absence and in presence of ciprofloxacin. The results demonstrated that NbSOS1 is better than NbSOS2 in terms of expression and stability in both the conditions we tested (Figure 5).

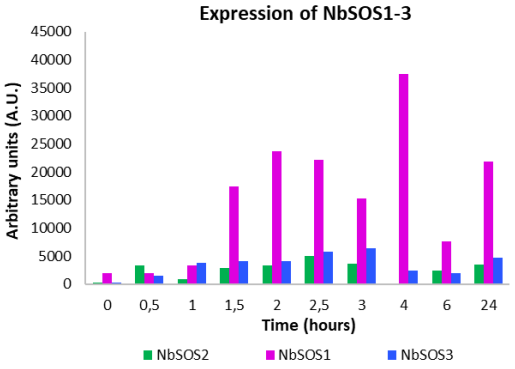

Figure 5. Levels of expression of NbSOSs during 24 hours in *E. coli*.

Ongoing work and future perspectives: NbSOSs improvement

We designed the mutagenesis of NbSOS1 to transfer the different features of NbSOS2 to it. In fact, NbSOS2 turns out to be the most promising as far as affinity and inhibitory capacity of LexA autoproteolysis are concerned. Therefore, we added to NbSOS1 an additional salt bridge, peculiar to NbSOS2, which appears to improve the interaction between the Nb and LexA. From molecular dynamics simulations, the mutated NbSOS1 is predicted to be able to improve the affinity towards the LexA target. Furthermore, we are constructing biparatopic nanobodies (BiNbSOSs) by fusing two Nbs having different recognition sites on the LexA antigen.

Conclusions

We characterised anti-LexA nanobodies that act as inhibitors of LexA in *E. coli*. We suggest that they might be effective in preventing the activation of SOS response upon antibiotic treatment, so reducing the accumulation of new mutations and the onset of resistance. This point would constitute a good step forward in the fight against antibiotic resistance and rehabilitation of ineffective antibiotics.