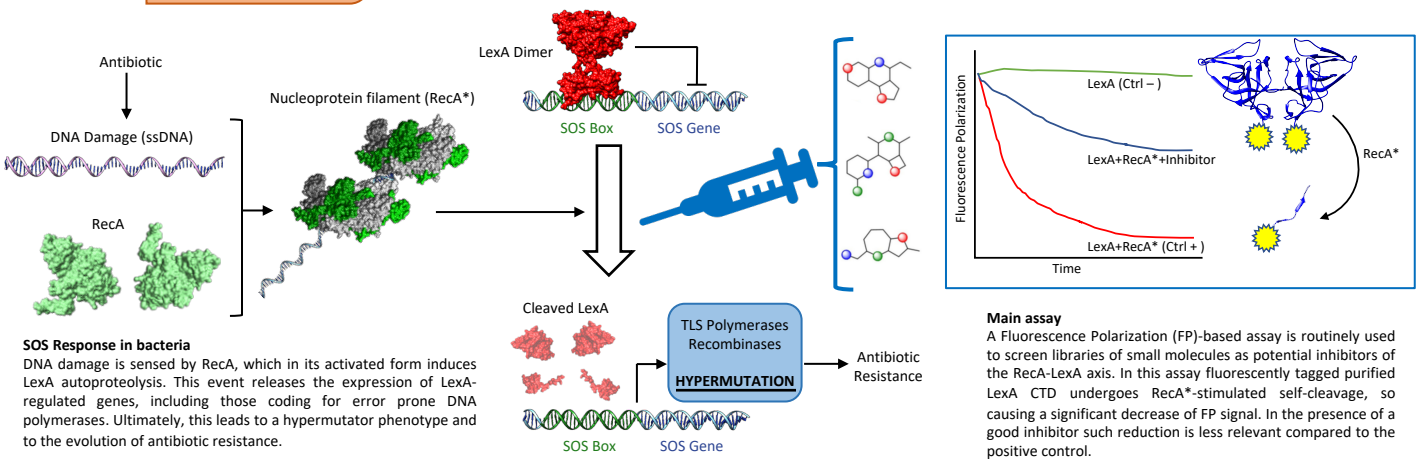
Discovery and preliminary characterization of a novel inhibitor of the SOS response in *Pseudomonas aeruginosa*

Filippo Vascon*[1], Monica Chinellato[2], Filippo Genovese[3], Zhanna Romanyuk[1], Sofia De Felice[1], Donatella Tondi[3], Povilas Kavaliauskas[4], Laura Cendron[1]

* Correspondence to *filippo.vascon@phd.unipd.it*

[1] Department of Biology, Università degli Studi di Padova, Padova, Italy
[2] Department of Medicine, Università degli Studi di Padova, Padova, Italy
[3] Department of Life Sciences, Università degli Studi di Modena e Reggio Emilia, Modena, Italy
[4] Department of Medicine, Weill Cornell Medicine of Cornell University, New York, USA

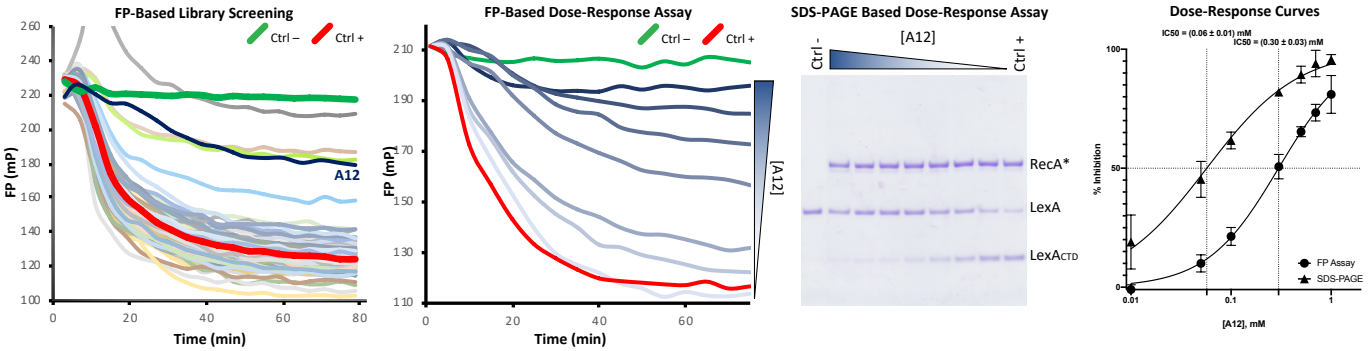
Introduction



SOS Response in bacteria
DNA damage is sensed by RecA, which in its activated form induces LexA autoproteolysis. This event releases the expression of LexA-regulated genes, including those coding for error prone DNA polymerases. Ultimately, this leads to a hypermutator phenotype and to the evolution of antibiotic resistance.

Main assay
A Fluorescence Polarization (FP)-based assay is routinely used to screen libraries of small molecules as potential inhibitors of the RecA-LexA axis. In this assay fluorescently tagged purified LexA CTD undergoes RecA*-stimulated self-cleavage, so causing a significant decrease of FP signal. In the presence of a good inhibitor such reduction is less relevant compared to the positive control.

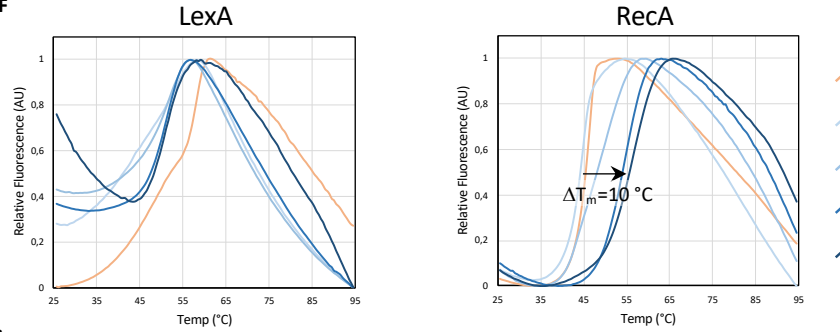
Library screening and hit validation



From a first screening of 400 small molecules against *P. aeruginosa* SOS system, 5 compounds showed promising inhibitory potentials, one of which was confirmed to be a true positive (compound "A12").
Two *in vitro* techniques (namely FP and SDS-PAGE) were used to validate A12 inhibitory potential and to construct dose-response curves, obtaining a half maximal inhibitory concentration in the range 60-300 μM.

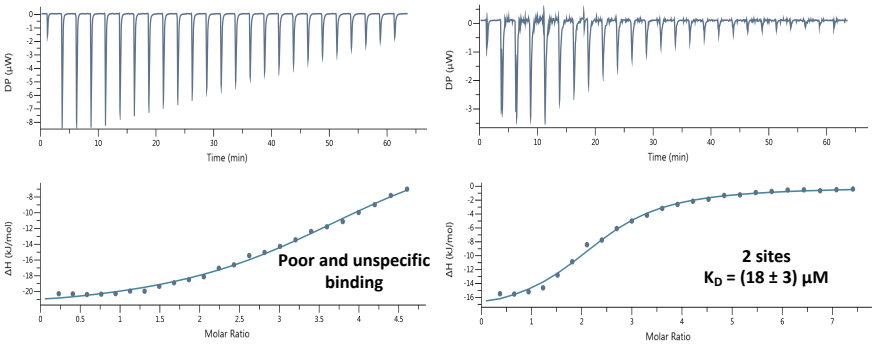
Target identification

DSF



RecA is the main target of A12
Differential Scanning Fluorimetry showed a significant increase in thermal stability of RecA as A12 concentration was raised. Conversely, LexA melting temperature showed slight, negative variations.
From Isothermal Titration Calorimetry studies, 2 binding sites for A12 at 18 μM K_D were calculated for RecA, while LexA showed poor and unspecific binding to A12.

ITC



Future work

- Determination of A12 effect on RecA: does it alter ATP binding or filament assembly?
- X-Ray crystallography of RecA-A12 complexes
- Synthesis and screening of A12-based sub-libraries
- Antimicrobial susceptibility testing and mutagenesis rate measurement on bacterial cultures