Closing Report on NKFIH-K 109486 project entitled

“Functional role, mechanism of action and exploitation of a molecular switch”

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Summary

Infectious diseases present remarkable biomedical challenge due to the appearance of resistant strains of the respective causative agents. Hence, investigations into mechanisms of pathogenicity are of considerable current interest for potential medical applications.

In the present project, we have employed a wide array of interdisciplinary state-of-the-art technologies to understand how gene expression regulation operates by focusing on a specific example of gene regulation in Staphylococcus. This regulation involves transmission of genomic elements that are responsible for transmission of virulence and toxin factors from pathogenic to non-pathogenic Staphylococcus strains, thereby rendering the originally non-pathogenic strains also pathogenic. Our studies revealed the molecular mechanism for this regulation and provided key insights to influence it. The results also shed light on a fully novel possibility to inhibit dUTPases, a key enzyme family responsible for keeping genomic integrity. Namely, we have identified a protein inhibitor of dUTPases that is characterized by nanomolar affinity and strong inhibitory capability.

The funding for this project was acknowledged in 33 international, peer-reviewed publications. 21 publications are directly related to the aims of this project, among these 9 publications appeared in D1 journals (eg Nucleic Acids Research, J. Biol. Chem, Scientific Reports, J. Am Chem Soc).

Below, some major results are summarized, based on the published journal articles. All details are thoroughly described in our published articles.

1 Mechanism of interaction between a Staphylococcal promoter and a phage dUTPase

Transfer of phage-related pathogenicity islands of Staphylococcus aureus (SaPI-s) was reported to be activated by helper phage dUTPases. This is a novel function for dUTPases otherwise involved in preservation of genomic integrity by sanitizing the dNTP pool. We investigated the molecularmechanism of the dUTPase-induced gene expression control using direct techniques. The expression of SaPI transfer initiating proteins is repressed by proteins called Stl. We found that phi11helper phage dUTPase eliminates SaPIbov1 Stl binding to its cognate DNA by binding tightly to Stl protein. We also showed that dUTPase enzymatic activity is strongly inhibited in the dUTPase:Stl complex and that the dUTPase:dUTP complex is inaccessible to the Stl repressor. Our results disprove the previously proposed G-protein-like mechanism of SaPI transfer

activation. We propose that the transfer only occurs if dUTP is cleared from the nucleotide pool, a condition promoting genomic stability of the virulence elements.

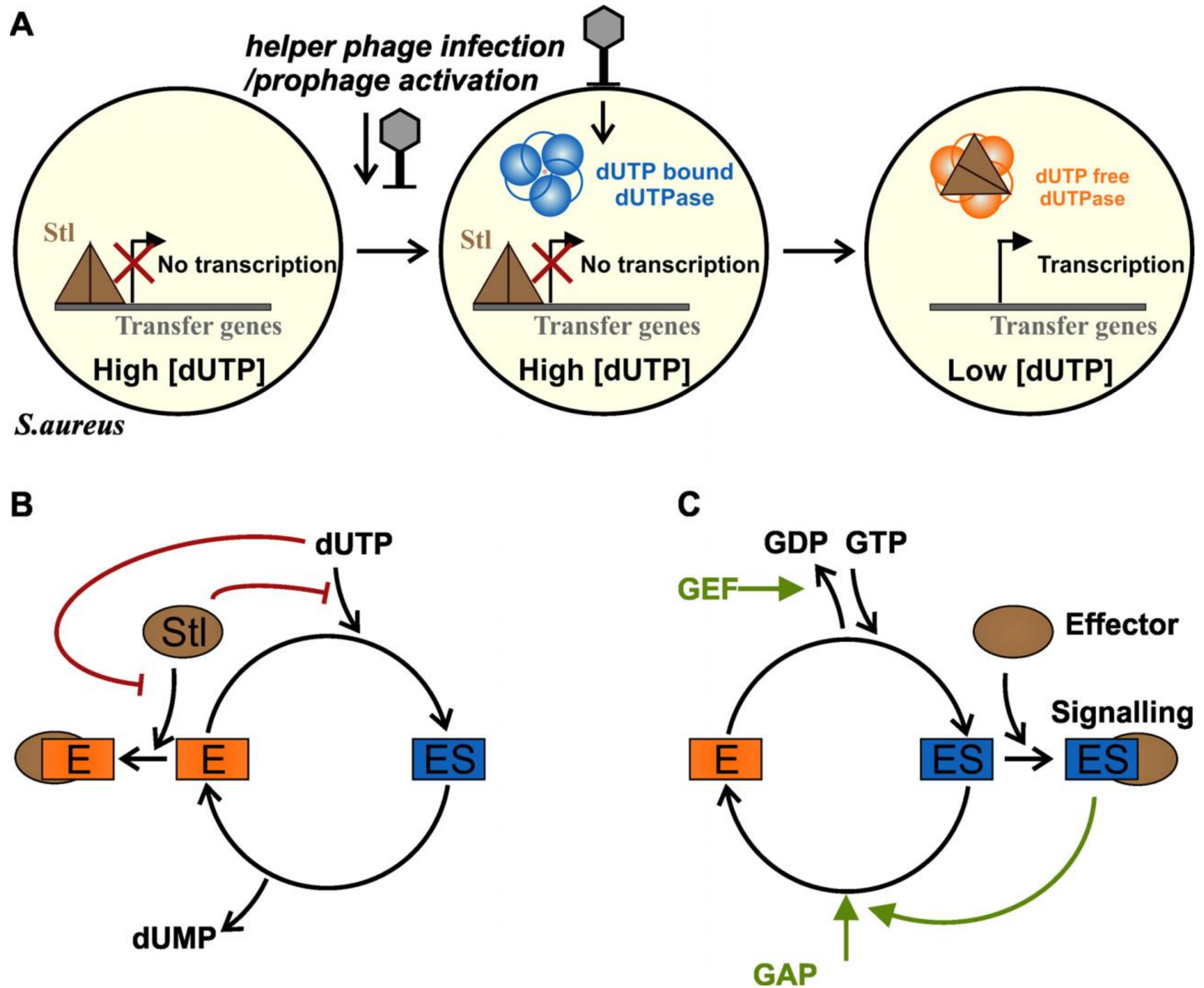

Fig. 1. Model of the mechanism of dUTPase-controlled SaPI activation. (A) Shows our novel model for dUTPase-based SaPI activation. (B) Molecular mechanism of dUTP controlled dUTPase:Stl interaction. (C) Molecularmechanism of G-protein-based switch. On panels B and C ES represents substrate bound, while E represents substrate-free enzyme (free enzyme or product bound enzyme). Red and green arrows represent inhibition and activation, respectively.

(taken from our article Szabo et al, Nucleic Acids Research, 2014 Oct 29;42(19):11912-20., entitled “Highly potent dUTPase inhibition by a bacterial repressor protein reveals a novel mechanism for gene expression control”)

2 Staphylococcal Stl binds and inhibit dUTPases from human, Drosophila and mycobacterial sources: a general scheme of proteinaceaous dUTPase inhibition

Human deoxyuridine 5′-triphosphate nucleotidohydrolase (dUTPase), essential for DNA integrity, acts as a survival factor for tumor cells and is a target for cancer chemotherapy. We discovered that the Staphylococcal repressor protein StlSaPIBov1 (Stl) forms strong complex with human dUTPase. Functional analysis reveals that this interaction results in significant reduction of both dUTPase enzymatic activity and DNA binding capability of Stl. We conducted structural studies to understand the mechanism of this mutual inhibition. Small-angle X-ray scattering (SAXS) complemented with hydrogen-deuterium exchange mass spectrometry (HDX-MS) data allowed us to obtain 3D structural models comprising a trimeric dUTPase complexed with separate Stl monomers. These models thus reveal that upon dUTPase-Stl complex formation the functional homodimer of Stl repressor dissociates, which abolishes the DNA binding ability of the protein. Active site forming dUTPase segments were directly identified to be involved in the dUTPase-Stl interaction by HDX-MS, explaining the loss of dUTPase activity upon complexation. Our results provide key novel structural insights that pave the way for further applications of the first potent proteinaceous inhibitor of human dUTPase.

Our results on complexation of human dUTPase:Stl and the obtained structural model based on HDX-MS together with an integrated structural biology approach and complex structure provide plausible explanations for mutual inhibition of Stl and dUTPase physiological function in their complex. On one hand, HDX results clearly delineated peptide segments around the dUTPase active site that are involved in binding to Stl and these data are in line with the observed inhibition of the dUTPase enzymatic function and competition between Stl and dUTP for binding to dUTPase. Importantly, the entrance to the dUTP accommodating beta-hairpin (ie conserved Motif 3) as well as the interaction surface for beta-gamma phosphate-chain of the substrate (ie conserved Motif 2) are both identified in our present study as involved in Stl binding. On the other hand, loss of the DNA-binding capability of Stl in its complex with human dUTPase is rationalized in light of the model resulting from SEC-SAXS measurements that necessarily involves Stl monomers hereby disrupting the functional repressor homodimer.

We conclude that proteinaceous inhibition of human dUTPase by Stl offers a novel, promising tool to investigate dUTPase function in different systems and propose further exploitation of Stl as a dUTPase-specific inhibitor. Our evidence-based structural model offers unparalleled insights into the mechanism of Stl-dUTPase complexation in general. Additionally, the model clearly delineates the peptide segments of Stl involved in interaction of the human enzyme alluding to the possibility of development of peptide based inhibitors. Since dUTPase is a key factor in genome integrity, the potentials of Stl or its derivatives to be used as a specific inhibitor cannot be underestimated.

(Figure 2 below is taken from our article Nyiri et al, Scientific Reports, (2018) 8:4326 | DOI:10.1038/s41598-018-22145-8, entitled “Structural model of human dUTPase in complex with a novel proteinaceous inhibitor”)

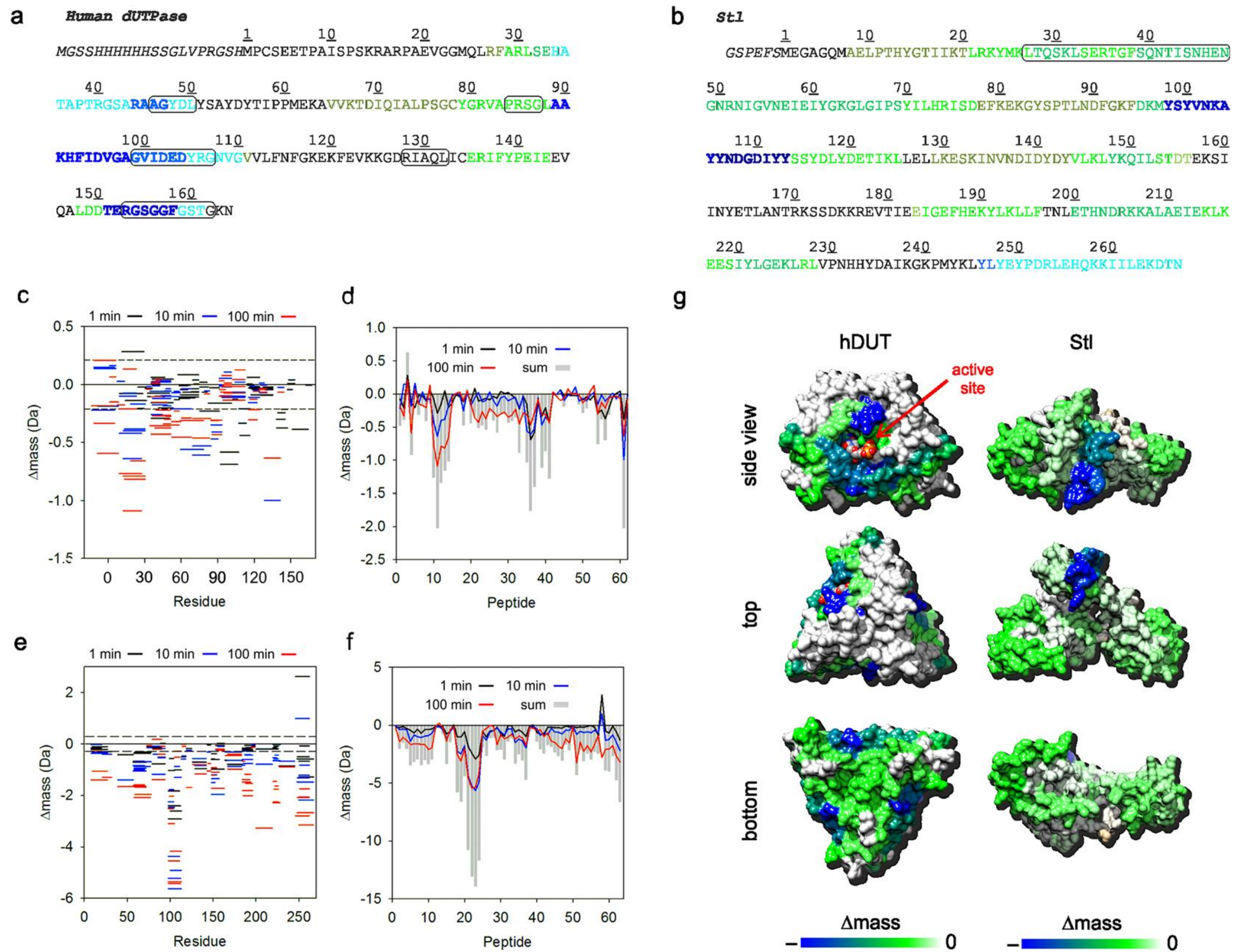

Figure 2. Representation of the hydrogen-deuterium exchange mass spectrometry results. (A,B) Sequence of human dUTPase and Stl proteins, respectively. Numbering starts at the first residue of the Uniprot sequences of the proteins (Uniprot IDs: P33316-2 and Q9F0J8 respectively). Extension compared to Uniprot sequence is in italics. Active site residues in case of dUTPase and the DNA binding motif of Stl are boxed. Sequence is colored according to HDX data (Δmass accumulated across all labelling times) applying color-scheme displayed on Panel G. (C–F) HDX-MS difference data (C and E) and associate Woods plots (D and F) for hDUT (C,D) and Stl (E,F) showing the change in isotope uptake upon complexation of the proteins. Labelling time points are indicated by different colors and the dashed lines in Panels C and E represent the 95% confidence bands. (G) Representation of the HDX-MS difference data on the surface of the human dUTPase and Stl. In case of the human dUTPase an apo state structure is shown (PDB ID: 1Q5U), the C-terminal 13 residues are omitted from the representation since the position of these residues were not resolved in the crystal structure presumably because of flexibility. Position of the substrate analogue is shown based on the structural alignment of the apo and ligand-bound structures (3EHW). The substrate analogue is shown as spheres with elemental coloring (carbon white, nitrogen blue, oxygen red, phosphorus orange) to visualize the interference of Stl and substrate binding. In case of Stl a

Phyre2 generated model is shown, which was compatible with synchrotron radiation circular dichroism and mutagenesis results obtained for the protein. Coloring is according to the scale at the bottom of the panel.

Proteins responsible for the integrity of the genome are often used targets in drug therapies against various diseases. The inhibitors of these proteins are also important to study the pathways in genome integrity maintenance. A prominent example is Ugi, a well known cross-species inhibitor protein of the enzyme uracil-DNA glycosylase, responsible for uracil excision from DNA. We found that the Staphylococcus pathogenicity island repressor protein called StlSaPIbov1(Stl) exhibits potent dUTPase inhibition in Mycobacteria. It is a clear and unequivocal further indication of a cross-species inhibitor protein for any dUTPase. We demonstrated that the Staphylococcus aureus Stl and the Mycobacterium tuberculosis dUTPase form a stable complex and that in this complex, the enzymatic activity of dUTPase is strongly inhibited. We also found that the expression of the Stl protein in Mycobacterium smegmatis led to highly increased cellular dUTP levels in the mycobacterial cell, this effect being in agreement with its dUTPase inhibitory role. In addition, Stl expression in M. smegmatis drastically decreased colony forming ability, as well, indicating significant perturbation of the phenotype. Therefore, we propose that Stl can be considered to be a cross-species dUTPase inhibitor and may be used as an important reagent in dUTPase inhibition experiments either in vitro/in situ or in vivo.

Specific proteinaceous inhibitors of key factors in this process have high potential for deciphering pathways of DNA metabolism and repair. We investigated whether the staphylococcal Stl repressor may interact with not only bacterial but also eukaryotic dUTPase. We provide experimental evidence for the formation of a strong complex between Stl and Drosophila melanogaster dUTPase. We also find that dUTPase activity is strongly diminished in this complex. Our results suggest that the dUTPase protein sequences involved in binding to Stl are at least partially conserved through evolution from bacteria to eukaryotes.

3 A generally applicable molecular switch system

We have developed a generally applicable system to reveal the mechanism of the interaction between Stl and its cognate DNA within the cellular environment. Our unbiased approach combines random mutagenesis with high-throughput analysis based on the lac operon to create a well-characterized gene expression system. Our results clearly indicate that, in addition to a previously implicated helix-turn-helix segment, other protein moieties also play decisive roles in the DNA binding capability of Stl. Structural model-based investigations provided a detailed understanding of Stl:DNA complex formation. The robustness and reliability of our novel test system were confirmed by several mutated Stl constructs, as well as by demonstrating the interaction between Stl and dUTPase from the Staphylococcal phi11 phage. Our system may be applied to high-throughput studies of protein:DNA and protein:protein interactions.

Figure 3 and 4 below are taken from our article Suranyi et al, Viruses 2018, 10, 168; doi:10.3390/v10040168 entitled "Exploiting a Phage-Bacterium Interaction System as a Molecular Switch to Decipher Macromolecular Interactions in the Living Cell"

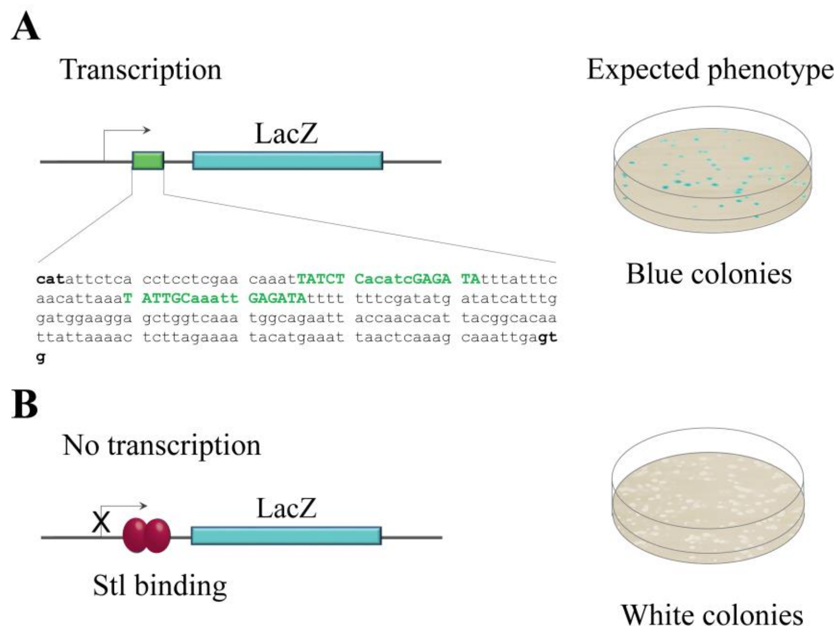

Figure 3. The design of the switch system (A) In the absence of Stl-binding to its recognition sequence, lacZ can be expressed in the cell leading to blue colonies in the experimental setup. The exact sequence from the SaPIbov1 genome (13733 – 13933) cloned into the lacZ promoter is shown. Specific Stl binding sites are labeled with green, conserved sites are shown with capital letters. (B) Stl binding to the promoter inhibits lacZ expression, leading to white colonies in the experimental setup.

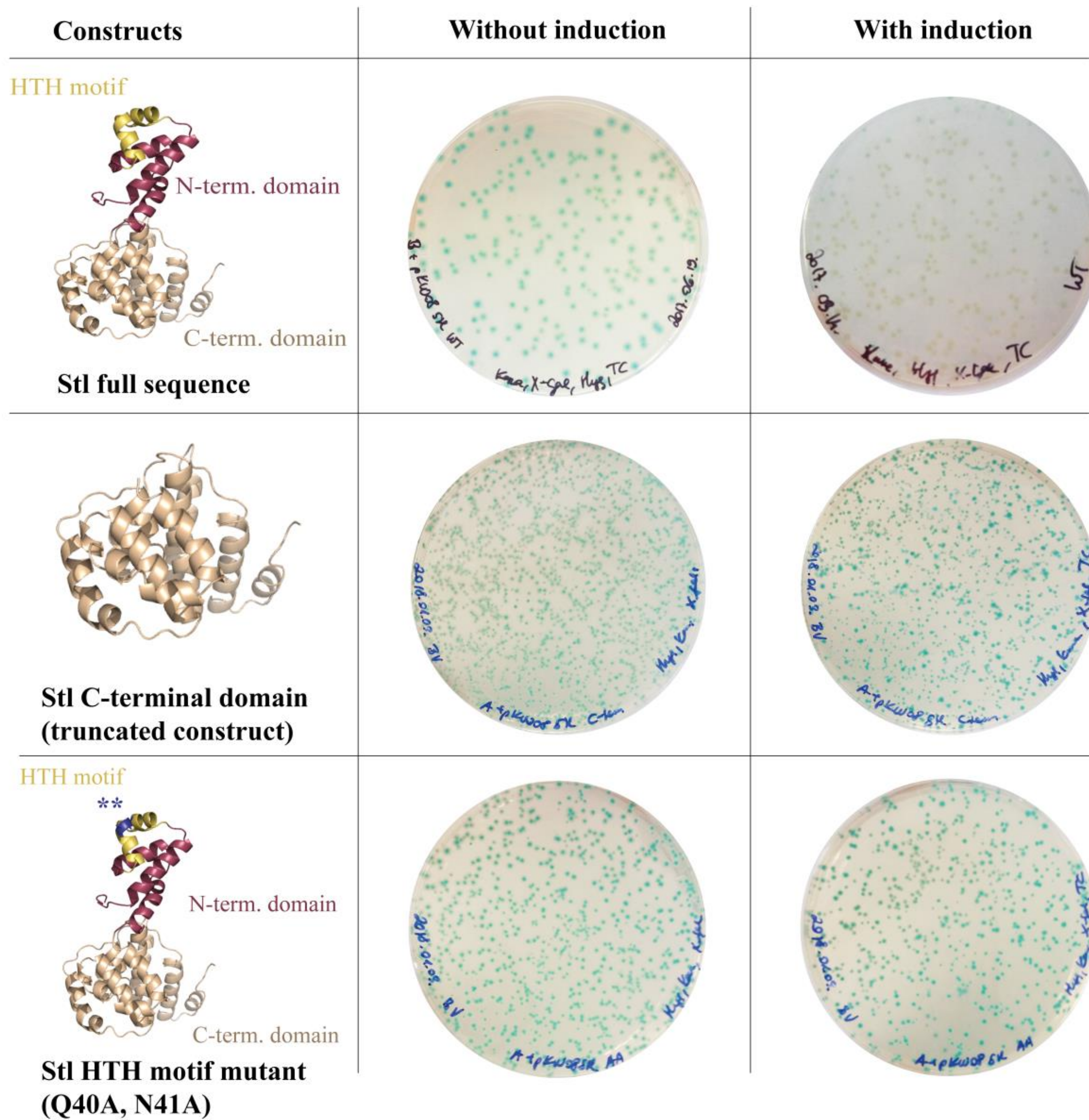

Figure 4. Validation of the switch system: To test the system functionality, several Stl constructs with various DNA-binding abilities were used. Without induction, i.e., in the absence of the Stl expression inducer tetracycline, cells form blue colonies in all cases. Upon the induction of the full-length wild-type Stl, white colonies are formed as Stl is able to fully repress the lacZ gene. Both the truncated Stl C-terminal domain construct and the HTH motif mutant Stl have diminished DNA-binding ability and, as expected, their expression in M. smegmatis leads to the formation of blue colonies. Note that the difference in the number of colonies between variants represents the transformation efficiency.