

# Design and structure analysis of fusion proteins of bovine DNA exotransferase and *E. coli* SSB protein

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This work aimed to design a model of a fusion protein from bovine DNA exotransferase and single-stranded DNA-binding protein from *E. coli* (EcSSB), choose the orientation of domains, find out optimal linker between two protein genes, and test stability of the fusion protein. The hypothesis of the study is that an additional DNA-binding domain attached to TdT will provide an increase in the activity and stability of the enzyme, as well as create new ways of controlling exotransferase activity *in vitro*, which is of particular importance for the creation of an enzymatic method for *de novo* DNA synthesis.

At the first stage, we modeled fusion proteins with the addition of EcSSB at the N and C terminals of the enzyme. As a result of the study, it was proved that EcSSB at the N terminal of the enzyme does not interact with DNA passing through the DNA-affinity domain of the enzyme and, will practically does not effect on enzyme activity (Fig.). In this case, the addition of EcSSB at the C terminal of the enzyme probably has a positive effect on the enzyme activity.

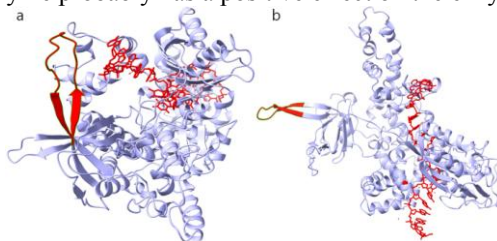


Fig. Model of fusion protein, the DNA and DNA-affinity domain of EcSSB are highlighted in red: a – fusion at the C terminal of the enzyme; b – fusion at the N terminal of the enzyme

Further we designed fusion proteins with various linkers, which are most often used to create fusion polymerases [1, 2]. The primary validation of the obtained models was carried out using the Ramachandran map and the QMEAN index. Further validation of the models was carried out by the molecular dynamics method. According to the results research we found out that the linkers GGGSGGGSGGGGS and GGGSGGGS have the best stability indicators (minor fluctuations of RMSD and the total number of hydrogen bonds), as well as the smallest gyration radius (6.0 angstroms), coupled with high mutual mobility of DNA-affinity domains of proteins.

## References

- [1] S. Sun, L. Geng, Y. Shamoo. Prot. Struct. Func. Bioinf. (2006) 65 (1):238.
- [2] T. L. Chisty, D. Quaglia, M. R. Webb. PLOS ONE. (2018) 13 (2):20.