CRISPR-CAS EFFECTOR COMPLEX OPTIMIZATION FOR SINGLE MOLECULE STUDIES

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CRISPR-Cas systems provide prokaryotes with adaptive immunity against viral and plasmid-borne infections which are destroyed by associated effector complexes [1]. Streptococcus thermophilus Type III-A CRISPR-Cas effector complex, called StCsm, is composed of several protein subunits and a crRNA molecule which acts as a guide against foreign transcripts [2]. StCsm complex is activated upon binding to nascent RNA transcript: it begins to shred transcribed DNA and produce signaling molecules for associated effectors [3, 4]. However, molecular mechanism of StCsm regulation by RNA has not been elucidated. This problem could be tackled by delicate single molecule studies allowing to monitor effector complex interaction with nucleic acids in real time. It is absolutely necessary to produce homogenous StCsm sample for these experiments, yet heterologous expression in E. coli yields different StCsm complexes carrying crRNAs of different sizes. During this work we have tried two approaches to purify homogenous StCsm: by using self-cleaving ribozymes to obtain crRNA of desired length, and by employing size-exclusion chromatrography for complex selection. Our results indicate that we were able to purify StCsm containing a crRNA of a distinct length. This complex will be used for single molecule experiments.

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