Optimizing protein V untranslated region sequence in M13 phage for increased production of single-stranded DNA for origami

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ABSTRACT

DNA origami requires long scaffold DNA to be aligned with the guidance of short staple DNA strands. Scaffold DNA is produced in *Escherichia coli* as a form of the M13 bacteriophage by rolling circle amplification (RCA). This study shows that RCA can be reconfigured by reducing phage protein V (pV) expression, improving the production throughput of scaffold DNA by at least 5.66-fold. The change in pV expression was executed by modifying the untranslated region sequence and monitored using a reporter green fluorescence protein fused to pV. In a separate experiment, pV expression was controlled by an inducer. In both experiments, reduced pV expression was correlated with improved M13 bacteriophage production. High-cell-density cultivation was attempted for mass scaffold DNA production, and the produced scaffold DNA was successfully folded into a barrel shape without compromising structural quality. This result suggested that scaffold DNA production throughput can be significantly improved by reprogramming the RCA in *E. coli*.

INTRODUCTION

DNA origami, in which a long single-stranded DNA (ssDNA) scaffold is folded into a custom-nanoscale shape with the guidance of multiple short DNA staple strands, introduces a versatile but deterministic molecular pegboard with a resolution of a few nanometers (∼5 nm) (1–7). In particular, the exotic modularity of staple strands can integrate regularly arrayed and highly dense ssDNA handles into two- or three-dimensionally parallelized double helices (i.e. DNA origami), which in turn promotes their transformative applications, including as nanomachines (8–10), drug delivery systems (11–13), and in nanophotonics (14–16). Nevertheless, performing DNA origami and its relevant translation into practical applications has not yet become popular, mainly because of the limited access to and high cost of obtaining sufficient amounts of scaffold. The high synthesis cost for staple DNA production, which is another major obstacle, has been effectively addressed through biotechnological production of phagemids with self-excising DNAzyme cassettes (i.e., encoding all staple and scaffold DNA on one phagemid) (17). However, this well-established biotechnological mass production protocol has been limited to relatively small DNA origami based on 3200 bases (€0.18/mg); relatively long single-stranded DNA (7000–8600 bases) can still be viewed as a commodity scaffold in overall DNA origami society. For example, most DNA origami studies, reported thus far, have depended on only one scaffold DNA (i.e. 7249 bases; p7249), mainly because it is commercially available. Nevertheless, such biotechnological production strategy is not yet elucidated for this commodity scaffold. The scaffold DNA from the M13 phage can be produced in *Escherichia coli* harboring F pili. To customize the production of scaffold DNA with efficient throughput, high cell density fermentation (*E. coli*) with optimization of variables (e.g. temperature, media composition, and freeing regiments) has previously been proposed (18–24). However, to facilitate the rapid uptake of DNA origami for newcomers and experts alike, scaffold DNA production, which enables improved throughput, needs to be further diversified without compromising DNA folding quality.

The M13 phage is a virus infecting *E. coli* and has its own replication system independent to the host's system, called rolling circle amplification (RCA) (18). When the

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