

ENGINEERING 2'-O-mRNA METHYLTRANSFERASES FOR INDUSTRIAL BIOCATALYSIS

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Eukaryotic messenger RNA (mRNA) are universally modified at their 5' end into a cap 0 structure consisting of an *N*⁷-methylguanosine and an inverted 5'-5' triphosphate bridge linking the penultimate nucleoside. Multicellular eukaryotes possess the capacity to further modify this cap by 2'-O-methylating the ribose of the penultimate nucleotide producing a so-called cap 1 structure¹. This methylation seems to be a molecular signature for the discrimination between self and non-self mRNA². In order to escape the innate immune system of the infected cell, some viruses have also evolved the ability to methylate their cap structures¹.

By analogy, therapeutic mRNAs must be non-immunogenic in order to restore or supplement the function of altered genes by mRNA-based therapy³. In this context, we propose to exploit the capacity of *Vaccinia virus* to produce non-immunogenic mRNAs. More specifically, VP39 is a 39 kDa-enzyme directly involved in the mRNAs' post-transcriptional modifications. It catalyses the 2'-O-methylation in the 5' cap structure producing the cap 1 mRNA and acts by heterodimerisation as a processivity factor with the poly(A) RNA polymerase⁴. However, the low expression level of VP39 in *Escherichia coli* (*E. coli*) as well as its low *in vitro* catalytic efficiency have so far limited its use for industrial biocatalysis.

Here, the two above-mentioned limitations are tackled by complementary approaches: *i*) we use a Split-GFP⁵ strategy coupled with ultrahigh throughput screening to select for higher soluble expression in *E. coli* and *ii*) we design smart libraries seeking to directly improve the catalytic turnover of the enzyme.

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