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An Enzyme Cascade Enables Production of Therapeutic Oligonucleotides in a Single Operation

AUTHORS

Hubert CASAJUS / THE UNIVERSITY OF MANCHESTER, JOHN GARSIDE BUILDING, SALFORD
Ewan MOODY / THE UNIVERSITY OF MANCHESTER, JOHN GARSIDE BUILDING, MANCHESTER
Richard OBEXER / THE UNIVERSITY OF MANCHESTER, JOHN GARSIDE BUILDING, MANCHESTER
Florian NICKL / THE UNIVERSITY OF MANCHESTER, JOHN GARSIDE BUILDING, MANCHESTER
Reynard SPIESS / THE UNIVERSITY OF MANCHESTER, JOHN GARSIDE BUILDING, MANCHESTER
Corresponding author : Sarah LOVELOCK / sarah.lovelock@manchester.ac.uk

PURPOSE OF THE ABSTRACT

Therapeutic oligonucleotides are short DNA analogues that selectively bind to target mRNA through Watson-Crick base pairing and regulate the production of disease related proteins [1]. Following the award of the 2006 Nobel Prize for RNA interfering technology and recent FDA approvals of several RNA-based therapeutics for the treatment of rare diseases, there has been significant investment into therapeutic oligonucleotides as a new drug modality. There are currently more than 160 oligonucleotide products in clinical trials including those for population based indications, in fact Inclisiran a cholesterol-lowering drug was approved in 2020, which has the potential to treat 30 million patients in the USA alone [2].

The increase in the number of potential therapeutics creates a significant manufacturing challenge, as current synthetic approaches are not scalable or sustainable. Existing chemical methods use complex building blocks containing multiple protecting groups (resulting in poor atom economy) and chromatographic purification of the final molecule is required. Syntheses are performed using solid supports which restricts the process to 10 kg batches making it unsuitable for large-scale applications (>100 kg). Moreover, the process uses prohibitively large volumes of acetonitrile (1000 kg per kg of oligonucleotide) which presents a threat to the security of oligonucleotide supply, given recent world shortages of acetonitrile [3,4].

Our group has developed an alternative PCR-based biocatalytic platform for oligonucleotide synthesis that requires no protecting groups, is atom economical and works under aqueous conditions. A combination of polymerases and endonucleases are used to amplify a catalytic template using unprotected deoxynucleotide triphosphate monomers. The target oligonucleotide is produced in a single operation, in high purity and with complete control of stereoselectivity (for molecules containing phosphorothioate linkages). Our methodology is showcased through the synthesis of the phosphorothioate modified therapeutic Vitravene as a single stereoisomer in high yield and purity.

FIGURES

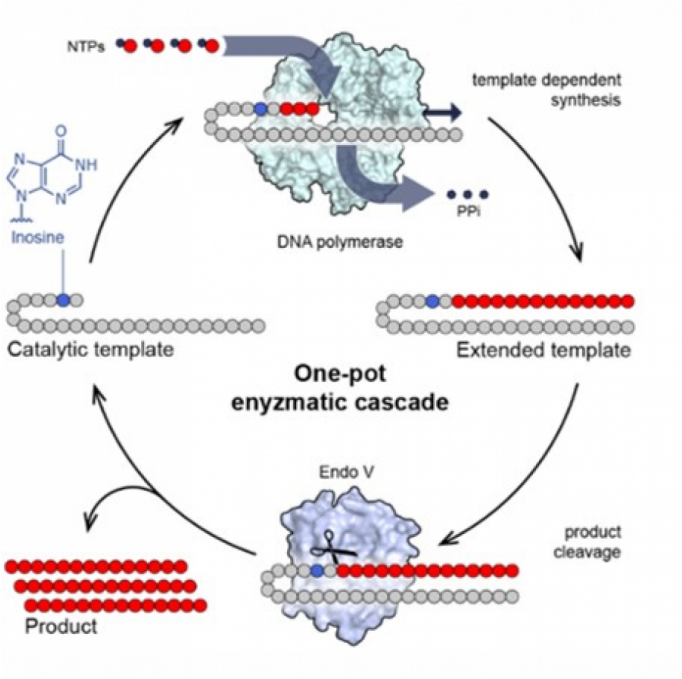

FIGURE 1

A one-pot enzymatic cascade for oligonucleotide synthesis

FIGURE 2

KEYWORDS

Therapeutic oligonucleotides | Enzyme cascade | Biocatalytic oligonucleotides synthesis

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