Simplifying Progress

Increasing the productivity of IVT reaction with HPLC analytics

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Optimization of IVT reaction using PrimaS analytics

The cost of mRNA production is driven by IVT reagents, particularly the capping reagent. Optimization of mRNA yield is therefore crucial for lowering the cost of mRNA production. In order to monitor IVT reaction over time, we implemented a rapid at-line HPLC monitoring of consumption of NTPs with concomitant production of mRNA, with a sub-3 min read-out. Use of CIMac PrimaS analytical column allowed us to determine and adjust key IVT components that influence the kinetics of mRNA production and are critical for optimization of continuous addition of reagents, i.e. fed-batch IVT (Figure 1).

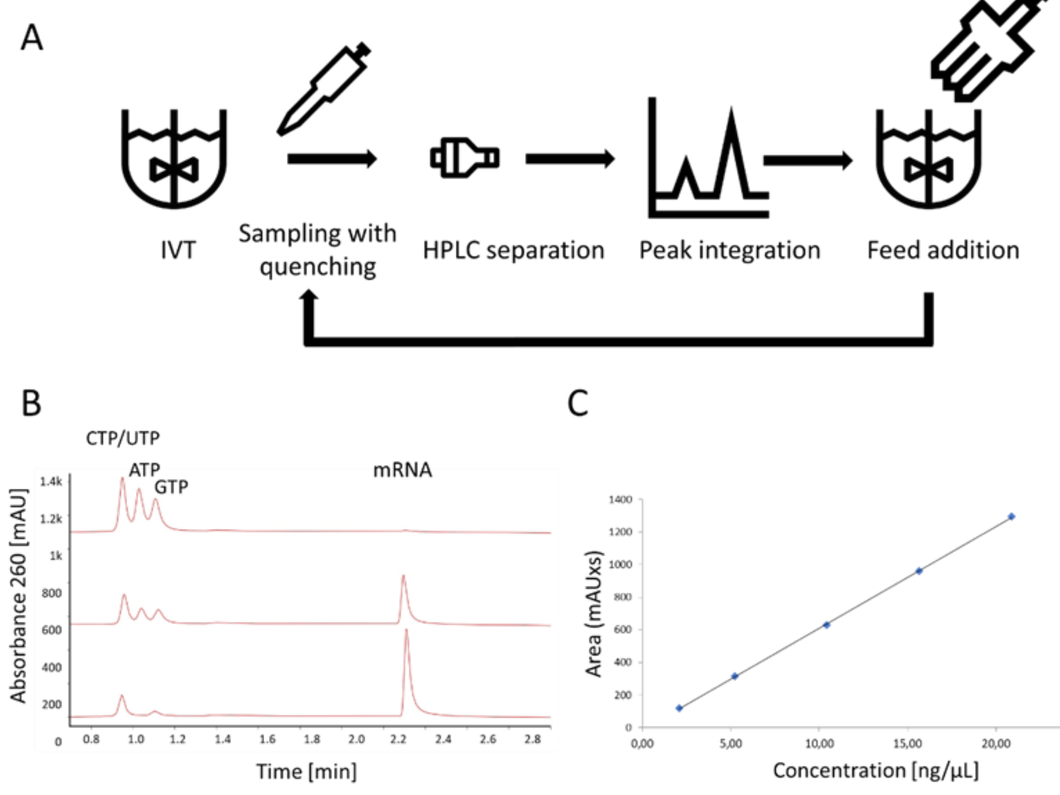

Figure 1: A) Schematic representation of IVT optimization workflow with CIMac PrimaS quantification of NTPs and mRNA in an IVT reaction. B) Representative CIMac PrimaS chromatograms from t=0, mid-point, end-point of IVT reaction. C) Calibration curve for eGFP mRNA concentration.

With at-line HPLC analytics we determined that plasmid, $MgCl_2$, NTPs as well as their ratio have the most influence on mRNA production and its rate. Increasing $MgCl_2$ conc. from 9 mM to 25 mM had a positive effect, while lower and higher conc. had an inhibitory effect on mRNA production rate (Figure 2A). Increasing pDNA conc. also resulted in increased mRNA production rate, while doubling the starting concentration of NTPs led to inhibition of transcription (Figure 2B). Therefore gradual addition (fed-batch), rather than high starting concentration of NTPs (batch), would be beneficial for increasing the productivity of the IVT.

Improving mRNA yield with fed-batch approach to IVT

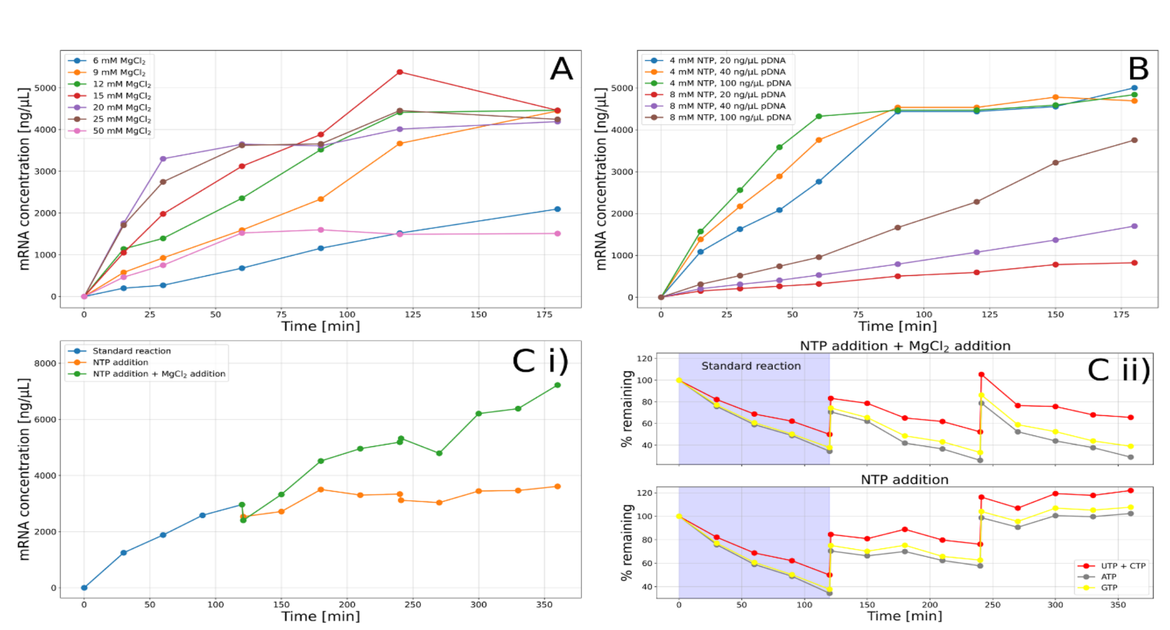

Figure 2: Effect of key IVT components on the kinetics of mRNA production. Impact of A) initial Mg^{2+} conc. (6-50 mM) and B) pDNA conc. (20, 40, 100 ng/µL) with 4 or 8 mM NTP on an IVT reaction. C) NTP-Mg^{2+} complex addition caused significantly higher mRNA conc. (i) in comparison to addition of NTPs alone that led to accumulation of NTPs (ii).

Fed-batch approach was tested in the presence and absence of Mg^{2+} ions (Figure 2C). Reaction with addition of NTPs did not increase mRNA yield, while addition of NTP-Mg^{2+} resulted in significantly higher mRNA concentration, suggesting that Mg^{2+} was the limiting factor for the progression of mRNA production. Fed-batch procedure was then optimized (Figure 3). IVT with 8 mM each NTP, 30 mM $MgCl_2$ and 100 ng/µL pDNA as starting conditions with bolus feedings of 3 mM each NTP and 9 mM $MgCl_2$ reached mRNA concentrations of up to 10 mg/ml in 3 hrs, demonstrated with two constructs (eGFP and Cas9).

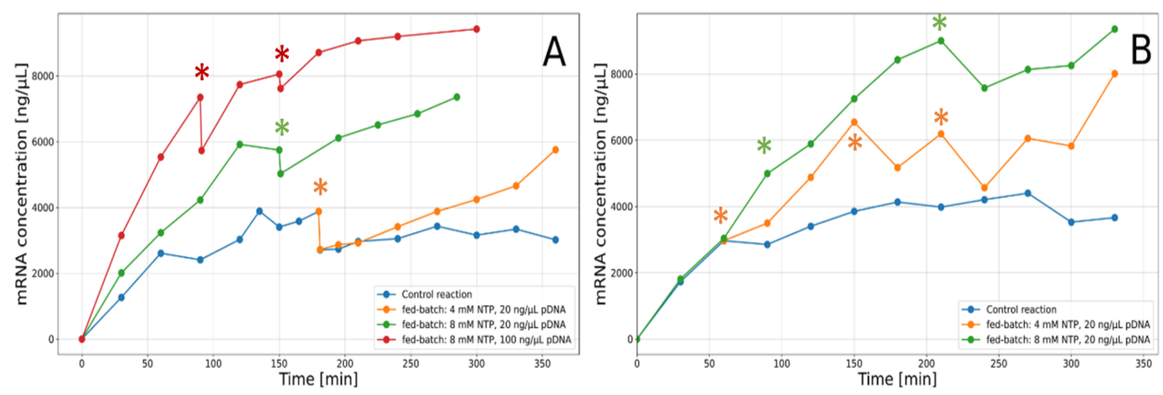

Figure 3: Fed-batch procedure optimization. Fed-batch was tested on A) smaller (950 nt) mRNA construct eGFP and B) larger (4495 nt) mRNA construct Cas9. Bolus addition timepoints marked with asterisks.

Conclusions

- Rapid at-line HPLC analytics using CIMac PrimaS™ column enables an insight into kinetics of mRNA production.
- Reaction can be sampled at high frequency and analysed at-line.
- Effects of IVT reaction components, e.g. pDNA, $MgCl_2$, NTPs on mRNA production rate can be studied.
- Fed-batch approach can improve mRNA yield from 4-6 mg/mL to 10-12 mg/mL.