Challenges and Potential Solutions for Development of Successful Potency Assay in mRNA Therapeutics

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BUILDING LIFE

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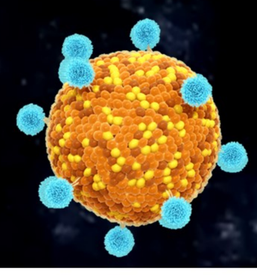
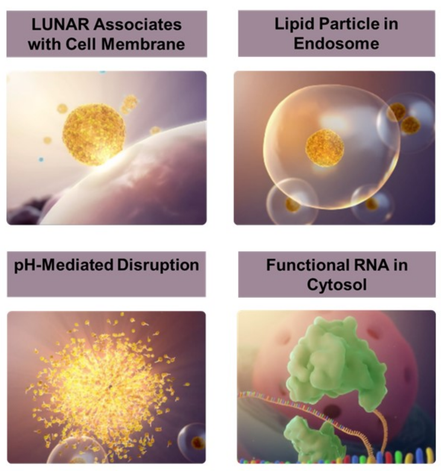


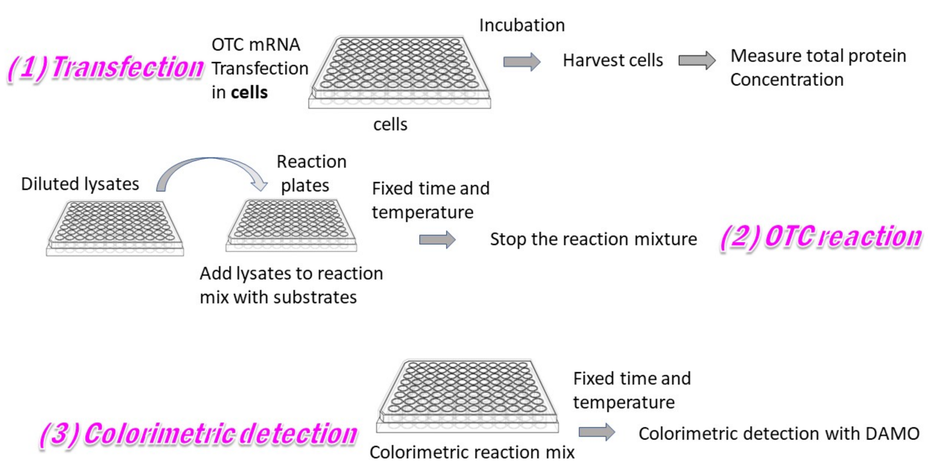
Arcturus Therapeutics is a nucleic acid medicines company focused on developing RNA therapeutics to treat rare diseases. Our proprietary LUNAR® lipid-mediated delivery technology enables the efficient delivery of any mRNA into a variety of cell types and tissues, and can be optimized for multiple routes of administration.

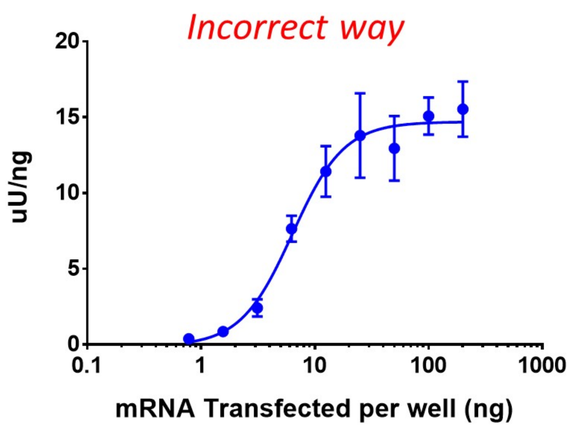


LUNAR® lipid nanoparticles carrying an mRNA payload reaches the target cell, where it fuses with the plasma membrane forming an intracellular endosome. This particle then undergoes a pH-mediated disruption that causes the breakdown of the biodegradable nanoparticle and the delivery of the mRNA into the cytoplasm. The mRNA then follows the cells endogenous translational and post-translational routes to generate the protein of interest.

LUNAR®-OTC is Arcturus' human OTC mRNA-mediated enzyme replacement therapy to treat patients suffering from ornithine transcarbamylase deficiency (OTCD). OTCD is a rare metabolic, Urea cycle disease in which the enzyme OTC does not convert Ornithine to Citrulline efficiently.

Conventional Potency Assay for Protein Biologic *May Not* Apply to mRNA Therapeutic





- mRNA drug substance is not the ultimate product *in vivo* as mRNA has to be converted to protein
- Activity of the enzyme may not linearly correlate with mRNA transfected
- ***Plateau does not represent*** maximum levels of activity of enzyme expressed in cells but rather a combination of factors – substrate depletion, enzyme inactivity, product inhibition and other non-physiological mechanisms during the reaction

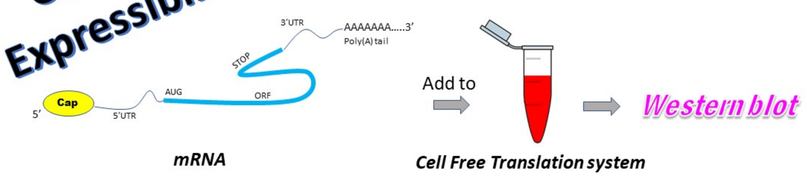
Novel ways to Measure Potency of **mRNA** Therapeutic Drug Substance

Different assays for different stages of development

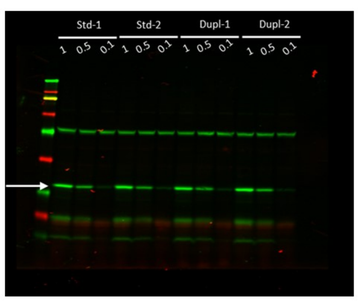
Potency for mRNA therapeutic defined as follows:

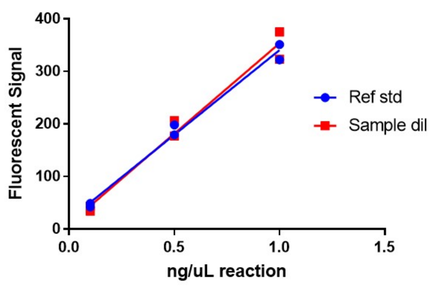
1. Expressibility of the protein (Cell-Free Translation)
2. Stability of expressed protein (In Cell Translation)
3. Activity of expressed protein (Cell based activity)

Cell-free Expressibility



- Cell-Free Translation system as a Potency test for Expressibility
- Identify Conditions where the protein expression is linearly dependent on mRNA concentration

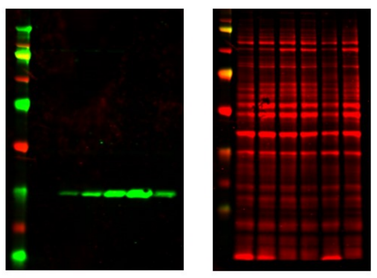


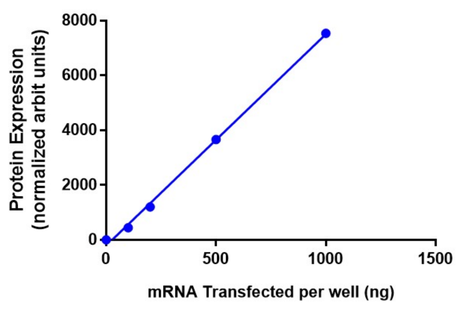


Relative Potency= **106.6%**

Cell-based Expressibility

***In vitro* protein expression determined by Western blotting**

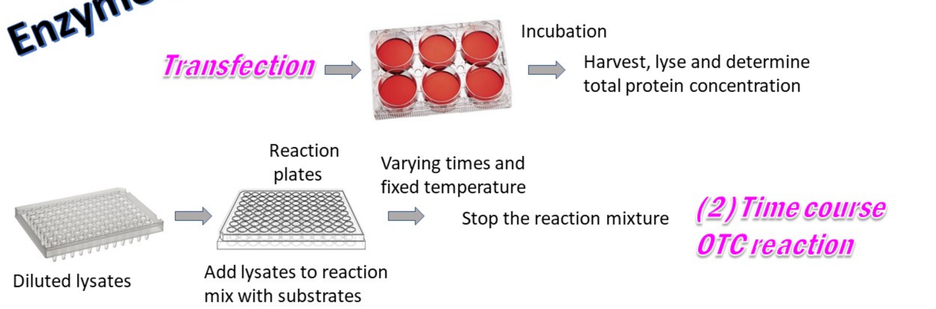


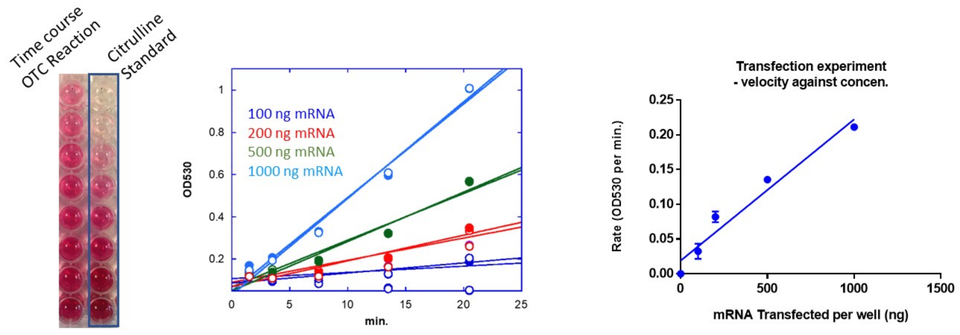


- Cellular protein expression was evaluated by transfecting immortalized human cell line with OTC mRNA
- Degree of expression was linearly dependent on amount of mRNA transfected

Enzyme Activity

Potency determined by measuring initial velocity in a steady-state experimental set up





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Conclusions

- mRNA Therapeutics require development of potency assays early during the pre-clinical stage
- Conventional potency methods for protein biologic may not apply directly to mRNA therapeutic drug substance
- Cell-based potency assays have to be carefully evaluated to make sure that the read-outs correspond to the actual potency of the drug substance and not an artifactual value
- mRNAs can be evaluated for potency at level of protein expression (cell-free), cellular protein expression/stability and enzyme activity *in vitro*