RIBOPRO

APPLICATION NOTE 01

Fluorescently Labelled SecNLuc mRNA for Quantification of Cellular Uptake and Activity

AUTHORS

Rik Oude Egberink[1], Roland Brock[1], Marco G. Drexelius[2], Félix Gayraud[2], Ines Neundorf[2]

[1]Dept. of Medical BioSciences, Radboud university medical center, Nijmegen, The Netherlands, roland.brock@radboudumc.nl

[2]Marco G. Drexelius, Félix Gayraud, Ines Neundorf, Institute of Biochemistry, Dept. of Chemistry, University of Cologne, Cologne, Germany.

INTRODUCTION

The clinical application of mRNA critically depends on delivery vehicles for efficient packaging and intracellular delivery. In the development of novel delivery vehicles both the capacity to induce cellular uptake and to enable mRNA expression are crucial. Important, in many cases, uptake does not correlate with activity as delivery vehicles may get captured inside the endolysosomal compartment due to poor endosomal release. Ideally, a reporter mRNA should enable detection of trafficking and delivery simultaneously. However, the attachment of fluorophores to nucleobases negatively impacts translation efficiency. As a consequence, for previously available fluorescently labelled mRNAs activity could only be seen when using highly active delivery vehicles, such as lipofectamine. Here, we show that a novel, highly active fluorescently labelled mRNA encoding for secreted nanoluciferase (SecNLuc) enables detection of cellular uptake and subcellular distribution by confocal microscopy in one experiment together with determination of expression by luciferase activity also for delivery systems with intermediate activity. Use of this mRNA greatly enhances the information content of experiments aiming at understanding of delivery processes and the development of new delivery vectors.

METHODS

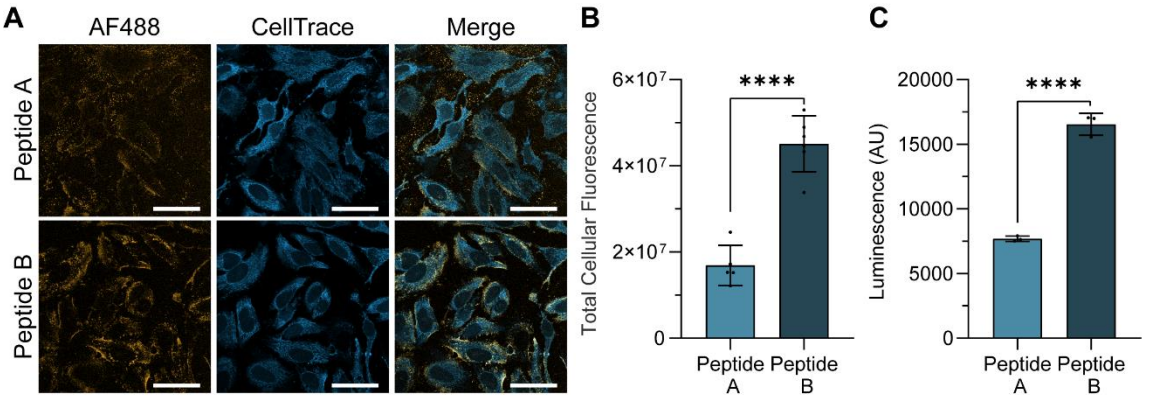
AZDye488-labelled SecNLuc mRNA (RIBOPRO) was formulated with two different cationic peptides (peptide 1 and peptide 2) by a stream method as described previously. In short, aqueous solutions of both peptide and mRNA

were dispensed simultaneously from electronically controlled pipettes with both pipette tips held in contact through a custom-made mold.[1]

One day prior to the experiment HeLa cells were seeded in Ibidi µ-slides at a density of 20,000 per well. The next day, cells were incubated with mRNA formulations containing 200 ng of AZDye488-labelled mRNA per well. After two hours of incubation, cellular uptake and subcellular distribution were determined by live-cell confocal microscopy using a Leica SP8 confocal microscope. 24 hours later, luciferase activity was determined in 50 µL of cell culture supernatant mixed 1:1 with NanoGlo luciferase substrate mixture, which was prepared according to the manufacturer's instructions. Luminescence was measured after briefly shaking the plate using the VICTOR X3Multilabel Plate Reader (PerkinElmer, Waltham, MA, USA). For quantitative analysis of uptake, the average fluorescence intensity of six individual frames was integrated using ImageJ.

RESULTS

For both delivery vectors, cells showed a predominant punctate staining of mRNAs, in line with endosomal uptake of peptide-formulations.[2] Uptake for peptide 2 was more effective than uptake of peptide 1 by a factor of 2.66. Consistent with the cellular uptake, luciferase activity in the supernatant for peptide 2 exceeded the one for peptide 1 by a factor of 2.15.



(A) Live-cell confocal microscopy of HeLa cells, incubated with peptide-mRNA nanoparticles for 2 h. Brightness and contrast of 488 channel were acquired and adjusted identically across conditions. Scale bars represent 50 µm. (B) Quantification of AZDye488-labelled mRNA as described previously.[3] Data represents the mean ± standard deviation of six individual frames. (C) Luciferase expression as measured 24 h post-transfection. Data represents the mean ± standard deviation of triplicates. ns: non-significant, **p ≤ 0.01, and ****p ≤ 0.0001.

CONCLUSIONS

Fluorescent labeling reduces the expression efficiency of mRNAs. The AZDye488-labelled mRNA that we used in this experiment exceeded the activity of previous preparations so that also for delivery vehicles that show only intermediate delivery activity, such as the peptides that we tested here, cellular uptake and expression could be determined in one single experiment.

RIBOPRO PRODUCTS

Fluorescently labelled SecNLuc mRNA

mRNA properties	
Sequence length	638nt (excluding poly A-tail)
UTRs	RIBOPROs 3' and 5' UTR
5' end	Cap1
Poly A-tail length	150nt
Fluorescent label	AZDye 488
% of labelling	5,4% (excluding poly A-tail)

REFERENCES

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3. Egberink RO, van Asbeck AH, Boswinkel M, Muradjan G, Dieker J, Brock R. Deciphering Structural Determinants Distinguishing Active from Inactive Cell-Penetrating Peptides for Cytosolic mRNA Delivery. *Bioconjug Chem.* 2023.DOI 10.1021/acs.bioconjchem.3c00346