Casting Light on RNA Delivery: A NanoBRET based RNA Delivery Quantification Method utilizing Fluorescent Base Analogues

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Background: RNAs offer huge potential for therapeutic applications. Despite promising characteristics, few RNA-based pharmaceutical products are available. Due to unreliable and/or labor-intensive methods for cytosolic RNA delivery quantification, accurate results for RNA delivery are scarce. A quick and reliable method is needed to accurately measure RNA delivery. This project focusses on establishing a method to quantify cytosolic RNA delivery in real time. This way valuable data can be generated to help rational design of RNA based drug formulations. Method: Cytosolically delivered RNA is detected via a dCas13a-NanoLuc fusion protein. While dCas13a serves as the means for affinity, the addition of NanoLuc allows for NanoBRET measurements. Herein, the RNA of interest is fluorescently labeled. Eventually the fluorophores will be co-transcriptionally incorporated as fluorescent base analogues. For initial testing ROX-5 is used instead, which is conjugated to the RNA via SPAAC. Fluorescent RNA is delivered into dCas13a-NanoLuc expressing cells via a vector of interest. After addition of NanoLuc substrate, the BRET ratio will correlate to delivery success. Results: The dCas13a-NanoLuc fusion protein has been successfully expressed in Hek-293 cells. Fluorescently labeled model RNA has been generated which is currently used for preliminary testing in cell lysate. Conclusion: We explore cytosolic RNA delivery via a NanoBRET based system. Affinity and proximity are provided using dCas13a with an appropriate gRNA. This NanoBRET approach will allow for sensitive, real-time analysis of cytosolic RNA delivery. This method will eventually help the design of RNA based pharmaceutical formulations