Single-molecule IVTT assay

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Methods

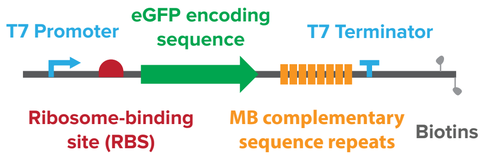
Optical trap setup

Three fluorescence color channels optical trap setup[1] is used in this experiments to image the IVTT reaction teking place in a six-channel microfluidic chip

Molecular beacons

A molecular beacon is an oligonucleotide functionalized with a commercially available fluorophore and a quencher at its two termini[2]. In a solution, it forms a hairpin and brings the two close together, which quenches the fluorophore. Once bound to a complementary sequence on an mRNA, it extends, which enables to use the fluorophore signal as an mRNA probe. Multiple MB repeats are usually used to improve the S/N ratio[3]

DNA construct



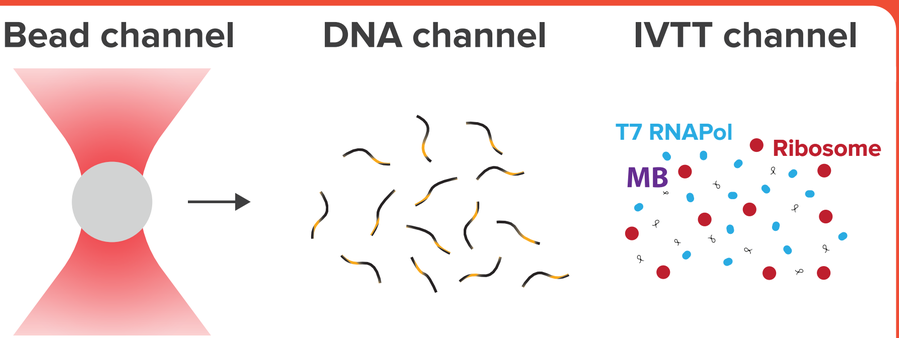
Imaging alternatives

Alternatively, mango aptamer[4] can be used to image transcription and SunTag[5] can be employed to observe translation without protein maturation-induced delay

Experimental procedure

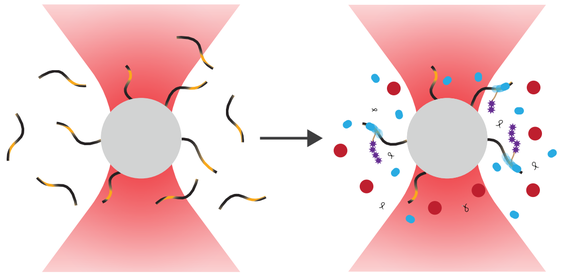
1. Assembling the system

Streptavidin-coated polystyrene bead is trapped with the optical tweezer. It is then moved into the DNA channel to bind a few biotinylated dsDNA containing molecular beacon (MB) **complementary sequence** repeats



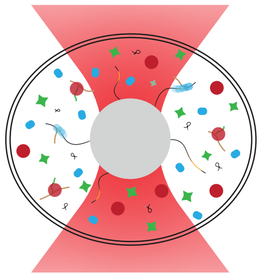
2. Monitoring single transcription events

Transcription starts in the IVTT channel. Multiple **molecular beacons** bind to the newly produced mRNA and fluoresce, ensuring high S/N ratio

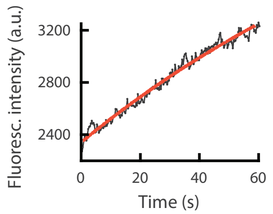


3. Following translation

Over time **eGFP** produced by the ribosomes maturates. To prevent the diffusion of the mRNA and eGFP and to mimic the confined cellular conditions, the system is encapsulated in the liposome from the start

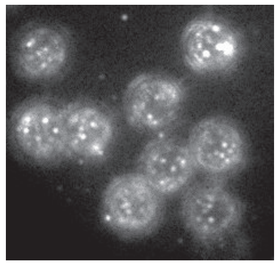


First results



Top: exponential fit of the fluorescence increase over time in a mixture of MB and mRNA with 2x MB complemntary sequence repeats

Right: PS beads with DNA constructs attached to their surface in a solution with T7 RNA polymerase and MB



Next steps

Assess the performance of a 32x MB repeat DNA construct and whether MB provide the sufficient single-molecule resolution

Optimize encapsulation of DNA-coated PS beads into liposomes using cDICE[6]

Outlook

Compare the performance of different IVTT systems (cell lysate, PUREfrex2.0, PUREfrex, etc.)

Quantitatively describe transcription and translation in a single DNA copy limit

@NotYetDrBogatyr

References

1 - Nature Communications, (2015), 1-12, 6(1)
2 - Nature Biotechnology, (1996), 303-308, 14(3)
3 - Scientific Reports, (2017), 1-11, 7(1)
4 - ACS Chemical Biology, (2014), 2412-2420, 9(10)
5 - Cell, (2014), 635-646, 159(3)
6 - bioRxiv, (2021), 2021.02.24.432456

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