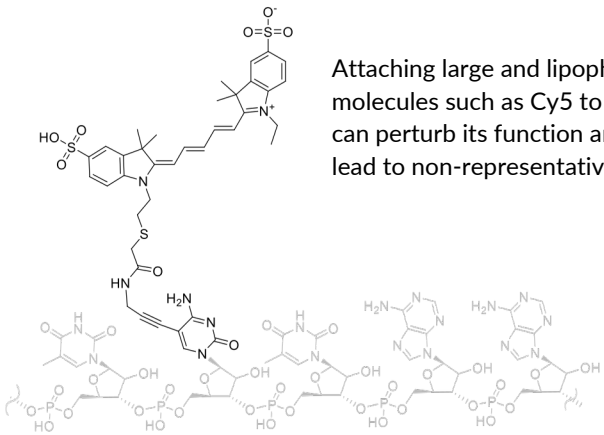
Stealth Labeled mRNA

LANTERNA

How do LanteRNA's technologies compare to the competition?

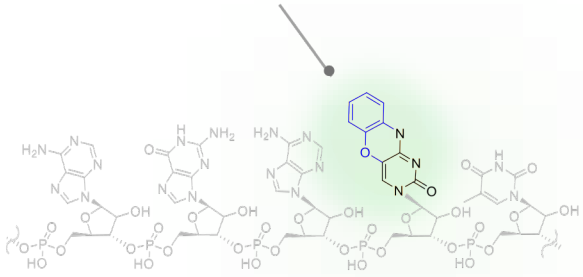
Fluorescence-based methods are essential for RNA therapeutics and vaccine development, as they allow for direct RNA visualization in live cells and tissue. However, to facilitate detection, the non-emissive RNA requires labeling. Here, we investigate the effect on polymerase processivity and RNA translatability when labeling mRNA with Cy5 versus LanteRNA's Stealth Label tCO.



Attaching large and lipophilic molecules such as Cy5 to RNA can perturb its function and lead to non-representative data.

VS.

LanteRNA's small and integrated labels are highly fluorescent, while having a minimal impact on the structure and function of RNA.



A series of in vitro transcription (IVT) reactions with 0%, 5%, 10%, 20%, or 40% CTP substituted for either cytosine analog tCOTP, or Cy5-CTP were assembled. The reactions were run using a standard T7 polymerase and a DNA template coding for mCherry. The resulting 1 kb labeled RNA was then purified using silica spin columns and analyzed by UV-vis spectroscopy and gel electrophoresis. Based on the absorption data, the degree of labeling in the RNA was determined (Fig. 1).

For a perfect cytosine triphosphate analog, which has a reactivity that is equal to that of CTP, the labeling degree with respect to C positions in the RNA should equal the fraction of label added to the reaction, resulting in a slope of 1 in Fig. 1 (dashed line). This data shows that tCOTP outperforms Cy5-CTP by a factor of 4–5 in terms of T7 polymerase compatibility in the IVT reaction. Having a reactivity close to 1 allows for straightforward tuning of labeling degree, a good labeling economy, and unperturbed IVT yields.

The labeled RNAs, after capping and tailing, were transfected into live human hepatocytes (Huh-7 cells) using a common lipofection protocol. After 24 h exposure, the cells were washed, detached, and analyzed using flow cytometry, probing for the reporter protein. The mCherry intensity from the cells exposed to the labeled mRNAs, normalized to that of the unlabeled mRNA, was then plotted as a function of incorporation degree (Fig. 2).

The difference in protein production for the two labeling technologies is striking, with the Cy5-mRNA producing very little protein at any practically useful incorporation degree. In sharp contrast, ca 50% mRNA translatability is retained when substituting 2% of all bases in the mRNA for tCO. At this labeling degree, LanteRNA's translating tCO-mRNA can be applied in a variety of fluorescence microscopy-based techniques to study e.g. mRNA delivery, without mixing in unlabeled mRNA in the formulation. Thus, using Stealth Labeled mRNA, users can be sure that the signal they are observing comes from the RNA that's being translated.

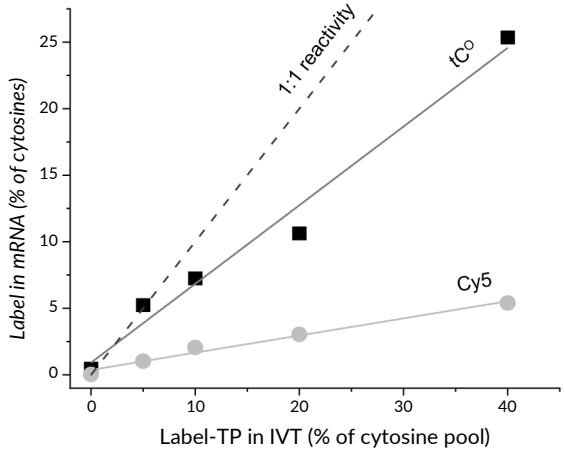


Figure 1. Fraction of Cy5 (circles) or tCO (squares) in the RNA, as a function of the fraction of triphosphate added to the IVT reaction.

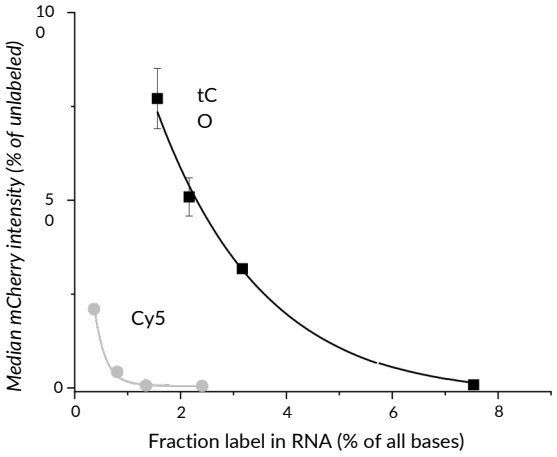


Figure 2. *In cellulo* protein production as a function of labeling degree for mRNA labeled with Cy5 (circles) or tCO (squares).