SELF-ASSEMBLED RNA ORIGAMI-BASED CODES FOR EXPLORING RNA DIVERSITY

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RNA is a key player in the transfer of information and regulation of biological processes underlined by the immense potential for RNA-based therapeutics and functional RNA nanostructures. Identifying RNA requires intricate protocols that suffer from various enzymatic biases and lead to irreversible loss of native information of RNA presence and quantity. Here we design three-dimensional molecular constructs that enable identification of native RNA at the single-molecule level using solid-state nanopore microscopy (Fig.1) [1]. Target RNA is refolded into RNA origami codes with designed sets of complementary DNA strands (Fig.1a). Each RNA code has a set of bits that correspond to the size of uncomplemented RNA structures. Nanopore microscope identification works via voltage-driven translocation of negatively charged RNA codes through a small orifice towards a positively charged electrode in an electrolyte solution (Fig.1b) [2]. It translates RNA code into a current signal with a spatial resolution comparable to complex optical microscopies with higher throughput and straightforward origami assembly (Fig.1c). We employed RNA codes and identified multiple RNA variants of messenger RNA and long non-coding RNA in human cervical adenocarcinoma. Our approach has the potential to discriminate up to 10 billion unique RNAs multiplexed pathogen detection, for vaccine byproducts, cancer diagnosis, and as an ultrarapid native RNA characterization technique.

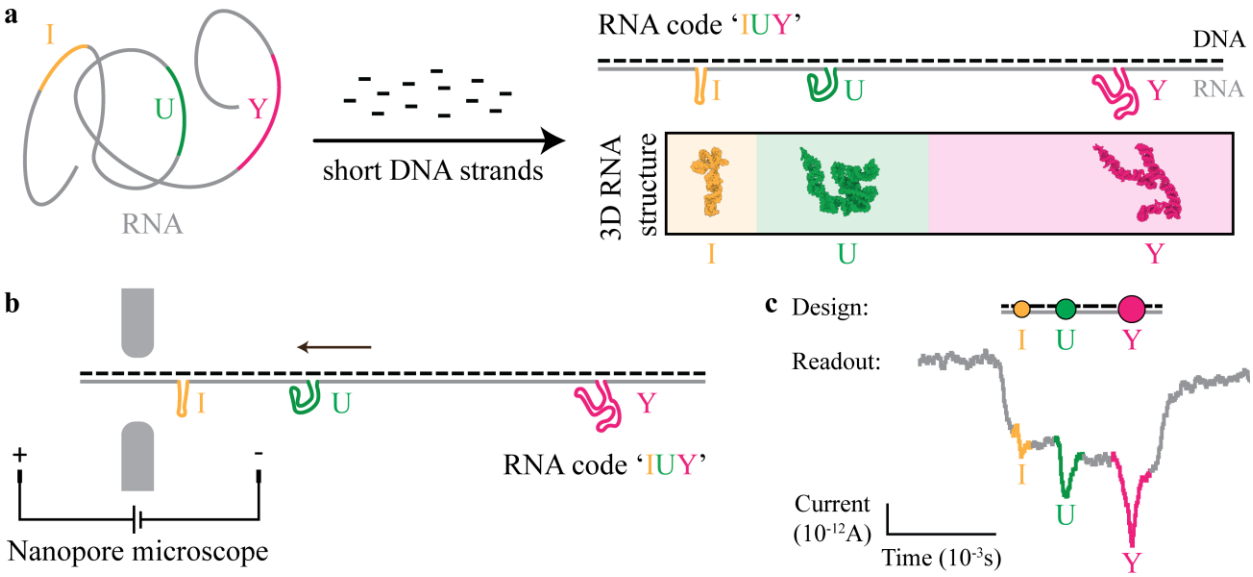

Fig. 1 Self-assembled RNA origami-based codes readout with solid-state nanopore microscopy. a) RNA is complemented with short DNA strands. Colored parts are left unpaired to self-assemble in RNA bits ('I', 'U', and 'Y') that together create a unique RNA code ('IUY'). The Three-dimensional (3D) RNA structure of each bit is designed to resemble the shape of a letter. b) RNA code 'IUY' is voltage-driven through the nanopore microscope. c) Designed RNA codes read with nanopore microscope results in the ionic current signal where downward spikes correspond to designed RNA bits.

[1] Boskovic F. and Keyser U.F., bioRxiv, 2021, https://doi.org/10.1101/2021.10.16.464631
[2] Boskovic F. and Keyser U.F., Science, **374**, 6574, 1443-1444 (2021).