Title:

Orientation and Distance Measurements using FRET and rigid fluorogenic RNA aptamers

Authors:

Sunny Jeng[a], Robert J Trachman III[b], Lynda Troung[b], Jay R. Knutson[b], Ilenia Manuguerra[c], Guido Grossi[c], Ebbe Andersen[c], Adrian R. Ferré-D'Amaré[b] and **Peter J. Unrau**[a]

[a]Department of Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, BC V5A 1S6, Canada.
[b]Biochemistry and Biophysics Center, National Heart, Lung, and Blood Institute, 50 South Drive MSC 8012, Bethesda, MD 20892-8012, USA.
[c]Interdisciplinary Nanoscience Center, Aarhus University, 8000 Aarhus C, Denmark.

Abstract:

The angle dependence of fluorescence resonance energy transfer (FRET) has many useful applications for the study of conformationally active RNA structures. Angle dependent FRET has been difficult to study as it is hard to ensure that the donor and acceptor dipoles of the fluorescent system precisely track the orientation of the structure under study. Chemically coupling fluorophore reporters directly to nucleic acid partially solves this problem, but the fluorophores are not fully oriented[1]. Guided by crystal structures of RNA Spinach[2,3] and Mango I[4] and the orthogonal fluorophore binding potential of these two aptamers[5], it was recently demonstrated that a fluorogenic aptamer-based FRET signal can be modulated by a small molecule, or invader nucleic acid strands[6]. Structural data, however, suggest the fluorophore binding core of Mango I is flexibly connected to external sequence[4] presumably precluding precise angular measurements.

Recently we developed three new RNA Mango aptamers[7]. Like Mango I, all of these aptamers connect to external RNA helices. Using Broccoli/DFHBI-1T as a donor and the far red-shifted Mango III/YO3-Biotin complex as an acceptor, we measured FRET efficiency using an RNA duplex of variable length between the two aptamers. FRET was dependent on the length of the joining RNA duplex, and oscillated in intensity precisely with the predicted twist of the duplex. In contrast, replacing Mango III with Mango I resulted in a FRET signal that was substantially rotationally averaged. This finding is consistent with a recent crystal structure of Mango III (Trachman et al., in press) that indicates that the fluorophore binding domain of Mango III is rigidly connected to its closing helix. As aptamer-tagged RNA constructs can be transcribed in living cells, and since the fluorogenic dyes used are cell permeable and non-toxic, we believe that rigid fluorogenic aptamers offer the prospect of building reliable biological FRET reporter systems.

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