Electronic Supplementary Material (ESI) for Chemical Communications.

Supporting Information for

Inhibition of Protein Synthesis Through RNA-Based Tandem G-Quadruplex Formation

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Experimental Procedures

Reverse Transcriptase (*RTase*) Stop Assay

A double-stranded template DNA for an RNA transcription was prepared from oligonucleotides by PCR-amplification with a T7 promoter primer (5'–TAATACGACTCACTATAGAA–3') and a 3'-DNA primer (5'–TCCAACTATGTATACCTG–3'). Resulting DNAs were transcribed to single-stranded RNAs by the AmpliScribe T7 Transcription Kit (Epicentre). RNAs were purified with 8% polyacrylamide gel containing 6 M urea. With the slab gel electrophoresis analysis, a reaction mixture of template RNA (0.3 μM) and 5'-Texas Red-labeled 3'-DNA primer (0.1 μM) was heated to 80 ºC for 3 min and cooled to ambient temperature. ReverTra Ace reverse transcriptase (TOYOBO), $MgCl_2$ and dNTPs were then added to the reaction mixture and the reaction was carried out at 42 °C for 30 min. The reaction products were purified and analyzed on a Hitachi SQ5500E automated sequencer. With the capillary gel electrophoresis analysis, a reaction mixture of template RNA and 5'-fluorescein-labeled 3'-DNA primer (0.03 μM) was heated to 80 ºC for 3 min and cooled to ambient temperature. ReverTra Ace reverse transcriptase and dNTPs were then added to the reaction mixture and the reaction was performed at 42 °C for 30 min. The reaction products were purified and analyzed on an ABI3500 capillary DNA Sequencer (Life Technologies Japan Ltd.). The sequencing markers were prepared with the SequiTherm EXCEL II DNA Sequencing Kit (Epicentre) by using double template DNAs as templates.

Thermal Refolding Profile

UV melting experiments were carried out with RNA oligonucleotides in 10 mM lithium cacodylate buffer (pH 7.0) containing salts. The absorbance of the sample was monitored at 260 nm and 295 nm from 100 °C to 20 °C with a cooling rate of 0.5 °C/min. Tm values were calculated using the median method.

Circular Dichroismic Spectral Studies

CD experiments were carried out on a J-725 CD spectrometer (JASCO) using a 1.0 cm path length cell. CD spectral changes were measured in 10 mM lithium cacodylate buffer (pH 7.0) containing salts. RNAs were heated 90 ºC and slowly cooled down to the ambient temperature prior to measurements.

Cell-free Luciferase Assay in Bacterial Extracts

A double-stranded template DNA containing the *Gaussia* luciferase gene was prepared by PCR-amplification with a T7 promoter primer (5'−TAATACGACTCACTATAGGG−3') and a T7 terminator primer (5'−TATGCTAGTTATTGCTCAG−3'). Resulting DNAs were transcribed to single-stranded RNAs by the AmpliScribe T7 Transcription Kit (Epicentre). A quantified amount of mRNAs was translated in RYTS protein synthesis kit (Protein Express) at 30 ºC for 30 minutes. Reaction was terminated by an addition of RNaseA at a final concentration of 20 μg/ml. Subsequently, the PURExpress Disulfide Bond Enhancer (NEB) was used to correctly fold the luciferase according to manufacture's recommended protocol. The luciferase activity was measured with the BioLux *Gaussia* Luciferase Assay Kit (NEB).

EmGFP Assay in Bacteria Cells

Overnight cultures of BL21(DE3) cells carrying the each plasmid were diluted in MagicMedia *E. coli* Expression Medium (Invitrogen) containing 100 μg/ml carbenicillin and 100 mM KCl, and subsequently cultured for 16 h at 37 ºC. Cells were recovered by centrifugation and washed once with phosphate-buffered saline (PBS). The washed cells were again suspended in PBS, and fluorescence of EmGFP was measured by the microplate reader (excitation 395 nm and emission 509 nm). Fluorescence intensity was normalized by OD600.

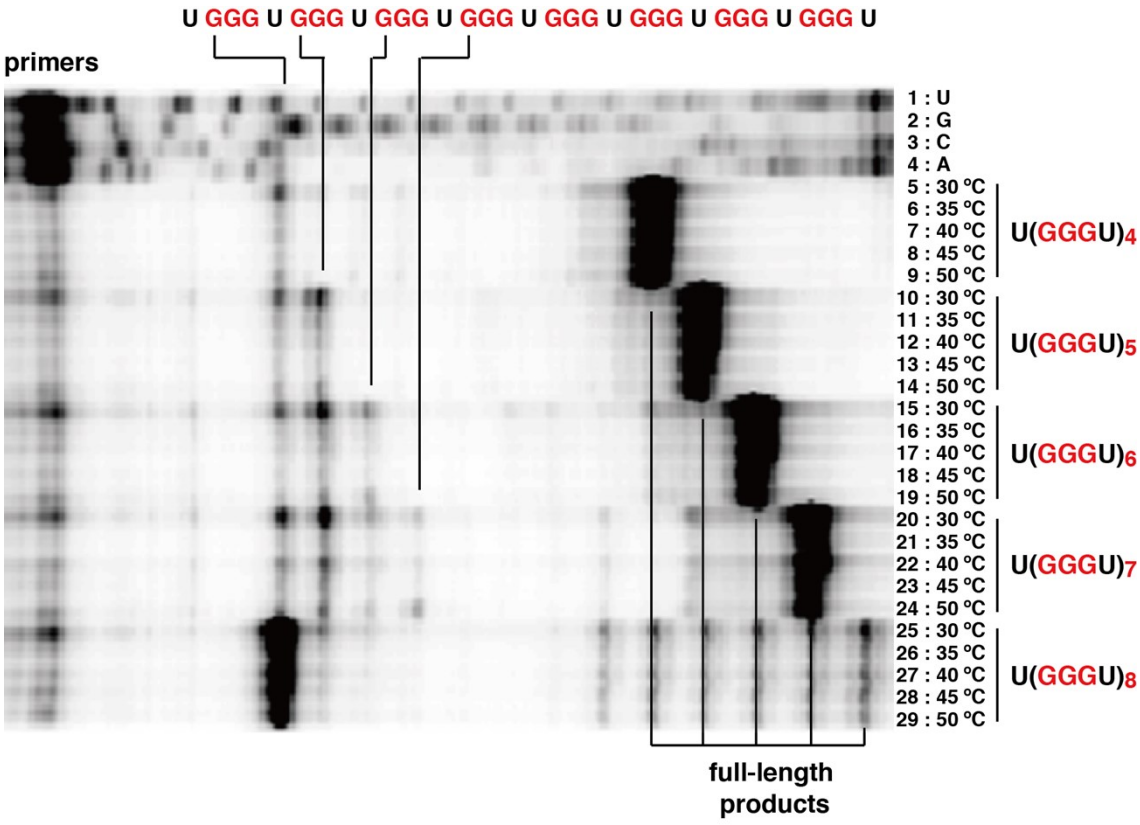

Figure S1. Interruption of *RTase*-mediated DNA synthesis at different reaction temperatures on RNA templates containing four to eight GGG repeat sequences in the presence of 50 mM LiCl. The lane markers U, G, C, and A indicate the bases on the template strand.

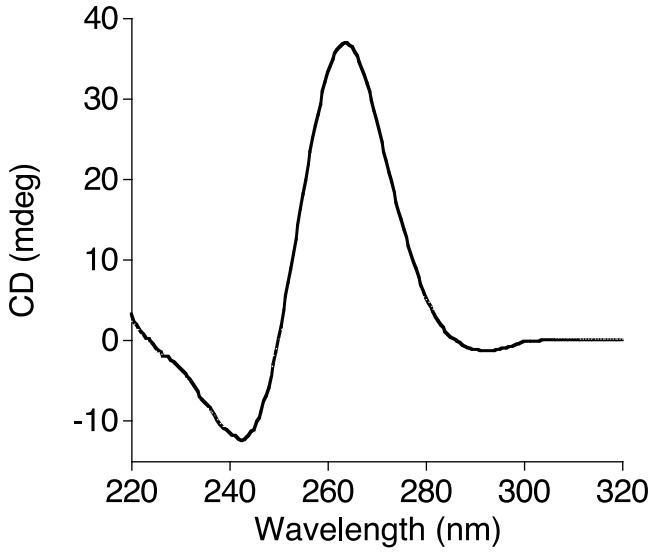

Figure S2. CD spectra of **rODN17** in 10 mM lithium cacodylate buffer (pH 7.0) containing 50 mM KCl at 25 °C.

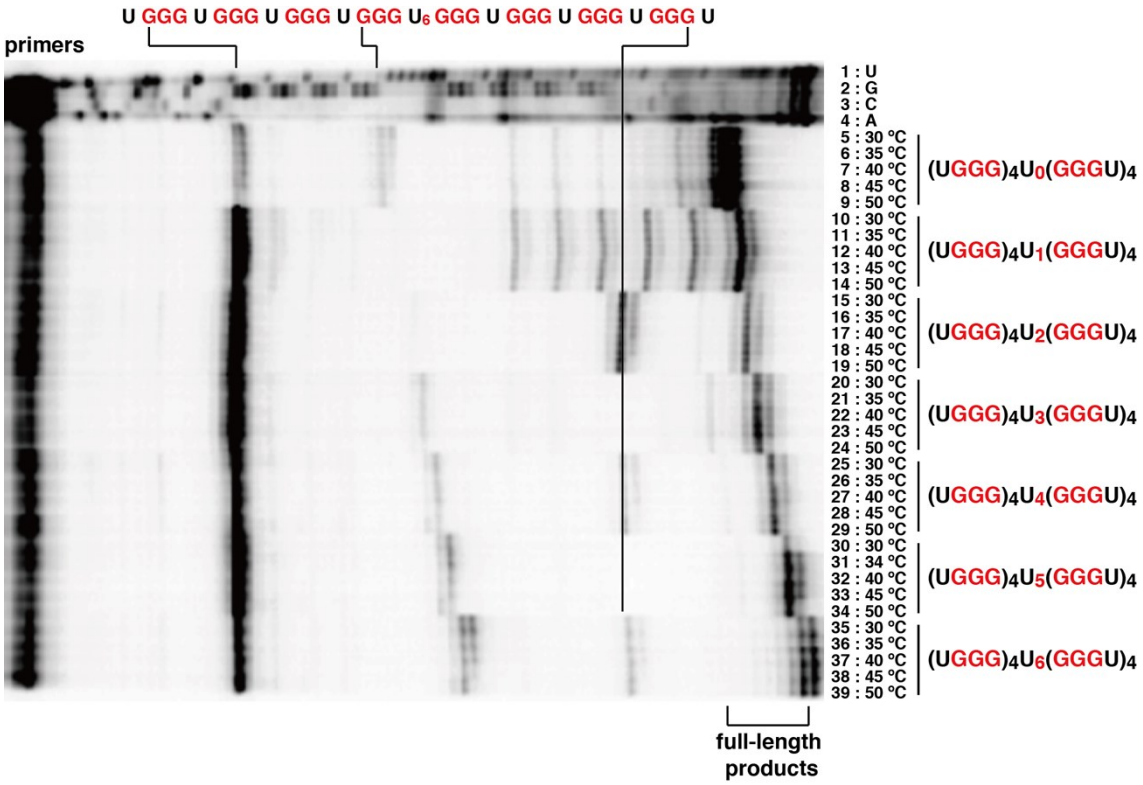

Figure S3. Interruption of *RTase*-mediated DNA synthesis at different reaction temperatures on RNA templates containing uracil bases between each consecutive four GGG repeat sequences in the presence of 50 mM LiCl.

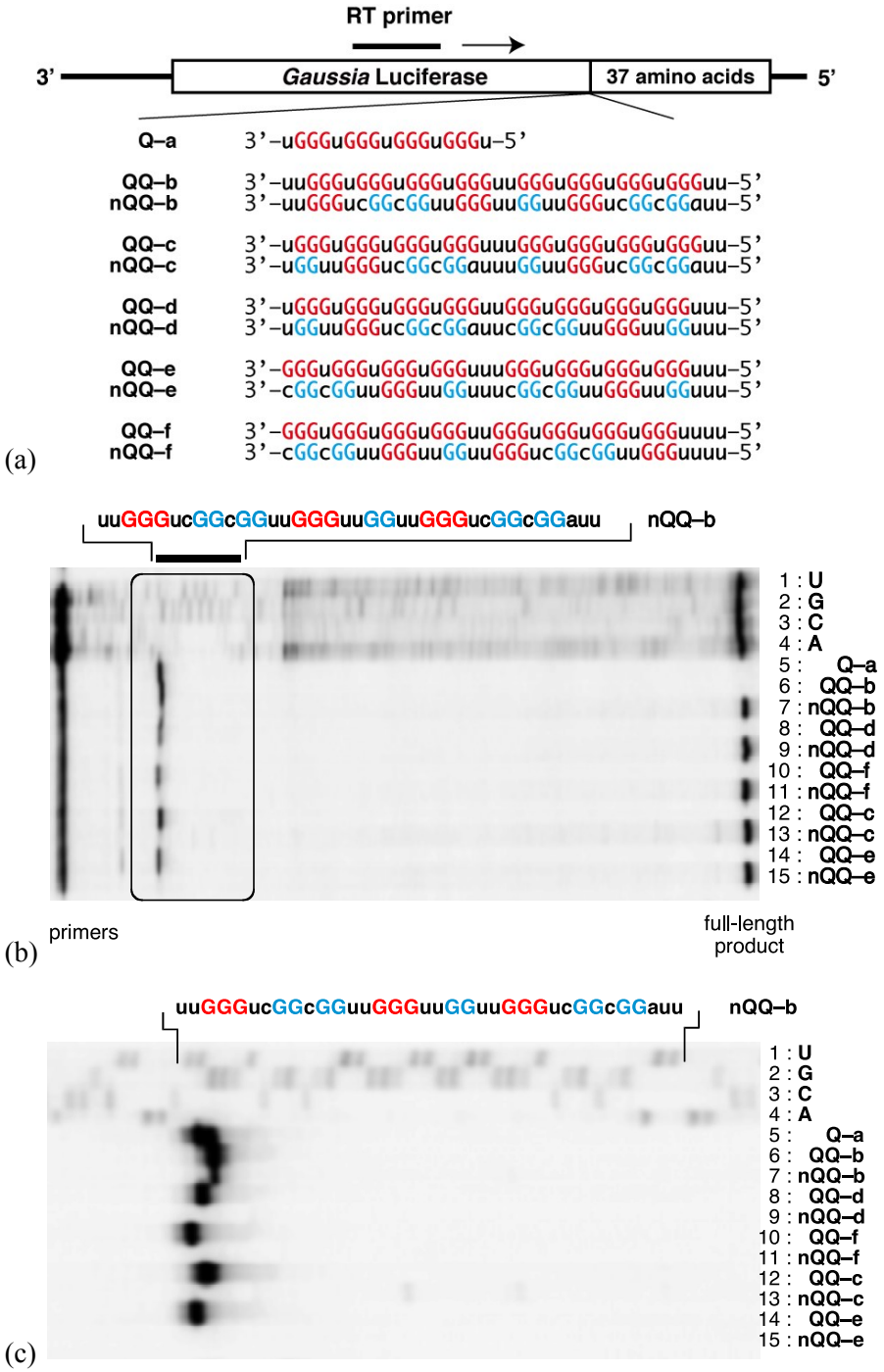

Figure S4. (a) A schematic illustration of the *RTase* stop assay for RNA templates containing a series of guanine repeat sequences between the 37 amino-acids leader sequence and the GLuc coding-sequence. The contiguous GGG and GG repeat sequences are indicated with red and blue letters, respectively. (b, and c) Interruption of *RTase*-mediated DNA synthesis on RNA templates containing a series of guanine repeat sequences in the presence of 100 mM KCl. (c) An enlarged view of the boxed region of figure (b). The lane markers U, G, C, and A indicate

the bases on the template strand of the nQQ–b.

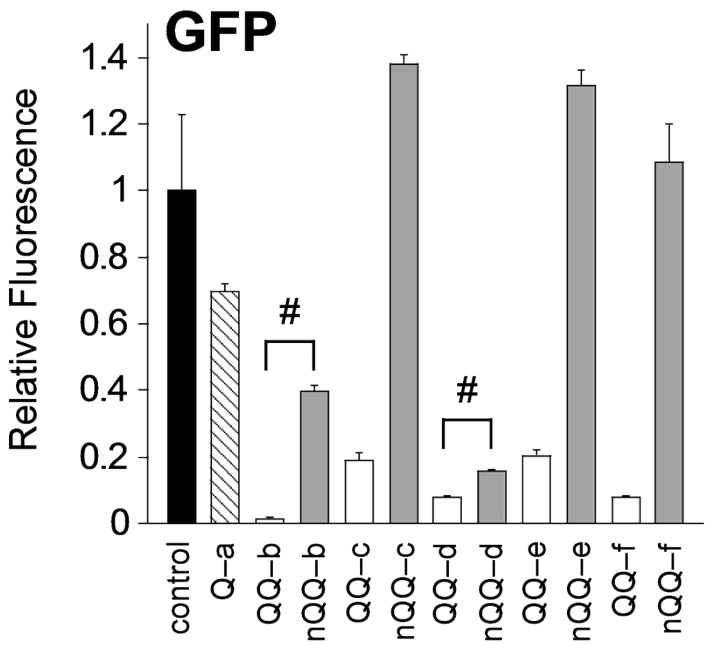

Figure S5. Expression of EmGFP in *E. coli* strain BL21 (DE3). All data were normalized relative to the fluorescence of the control, and fluorescence intensities were normalized by OD_{600}. #: The expression of these proteins resulted in the formation of inclusion bodies with a weak fluorescence. All data were averages of three independent measurements and the error bars represent S.D.

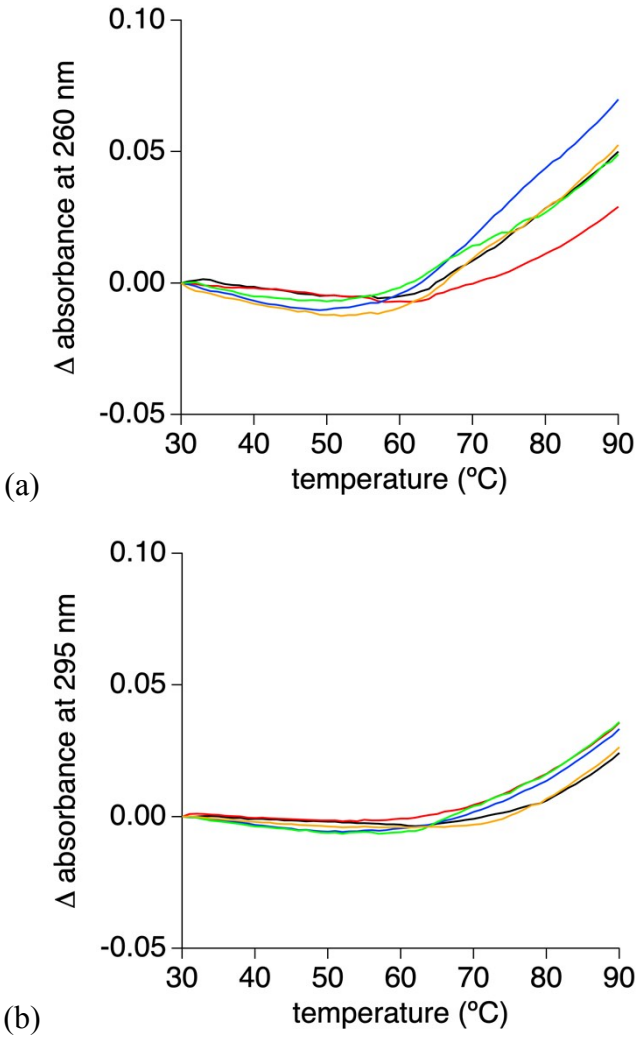

Figure S6. Biophysical analyses of G-quadruplexes in guanine-repeat RNA oligonucleotides. Annealing UV profiles of oligonucleotides of ORN-n-QQ-b (black), ORN-n-QQ-c (red), ORN-n-QQ-d (blue), ORN-n-QQ-e (green), and ORN-n-QQ-f (orange) detected at (b) 260 and (c) 295 nm in 10 mM lithium cacodylate buffer (pH 7.0) containing 50 mM KCl. Experiments were performed at 1.3 μM ODN strand concentration.

ORN-n-QQ-b: 5’-AGGCGGCUGGGUUGGUUGGGUUGGCGGCUGGGU-3’

ORN-n-QQ-c: 5’-AGGCGGCUGGGUUGGUUUAGGCGGCUGGGUUGGU-3’

ORN-n-QQ-d: 5’-UGGUUGGGUUGGCGGCUUAGGCGGCUGGGUUGGU-3’

ORN-n-QQ-e: 5’-UGGUUGGGUUGGCGGCUUUGGUUGGGUUGGCGGC-3’

ORN-n-QQ-f: 5’-UGGGUUGGCGGCUGGGUUGGUUGGGUUGGCGGC-3’

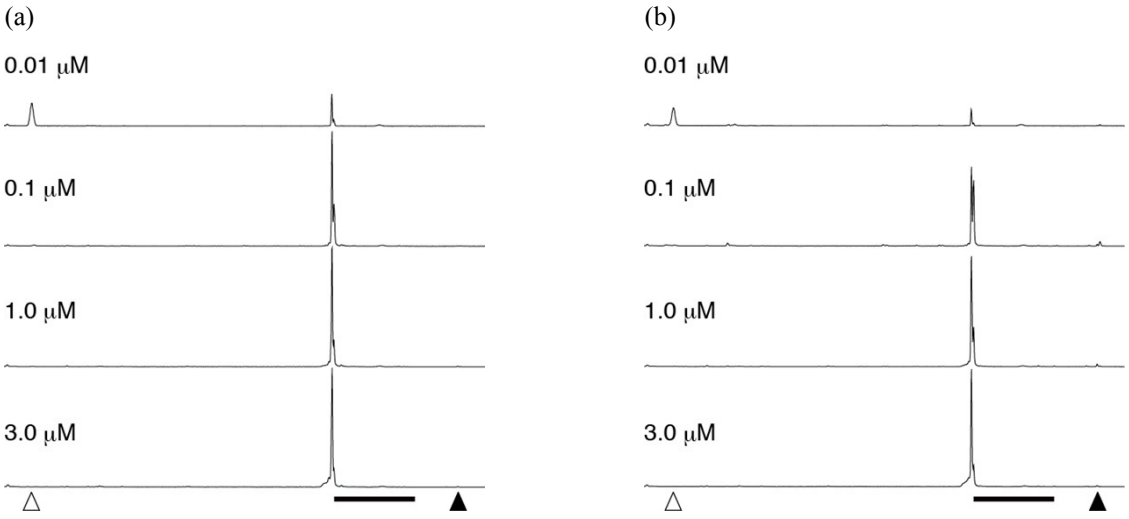

Figure S7. Concentration independent tandem G-quadruplex formation in the RNA template containing $U(GGGU)_8$ repeats. Capillary electrophoresis chromatograms for fragment analysis of the elongated primers against templates in the presence of (a) 50 mM KCl, and (b) 50 mM LiCl. From top to bottom, concentrations of RNA templates are 0.01, 0.1, 0.3, and 3.0 μM, respectively, while the concentration of labeled-primer was kept constant at 0.03 μM. Open and black triangles indicate the position of the non-elongated primer and the fully elongated primer, and bar indicates the $U(GGGU)_8$ repeat region.

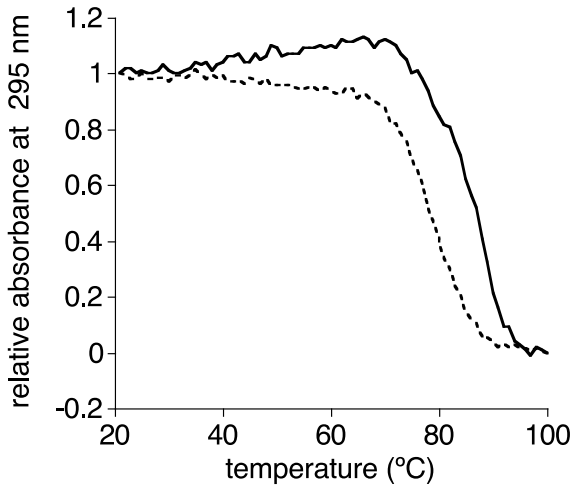

Figure S8. Annealing UV profiles of **rODN34** (solid line) and **rODN17** (dashed line) in 10 mM lithium cacodylate buffer (pH 7.0) containing 1 mM KCl and 49 mM LiCl. All experiments were carried our at 2.5 μM **rODN17** and at 1.3 μM **rODN34** strand concentration.