Supporting Information

Quantification of Cellular Deoxyribonucleoside Triphosphates by Rolling Circle Amplification and Förster Resonance Energy Transfer

Xue Qiu[1], Olivier Guittet[2], Carlos Mingoes[1], Nadine El Banna[3], Meng-Er Huang[3],*

Michel Lepoivre[2], Niko Hildebrandt[1,4]**

[1] NanoBioPhotonics (nanofret.com), Institute for Integrative Biology of the Cell (I2BC), Université Paris-Saclay, Université Paris-Sud, CNRS, CEA, Orsay 91400, France

[2] Cellular activation and signal transduction, Institute for Integrative Biology of the Cell (I2BC), Université Paris-Saclay, Université Paris-Sud, CNRS, CEA, Orsay 91400, France

[3] CNRS UMR3348, Institut Curie, PSL Research University, Université Paris-Sud, Université Paris-Saclay, Orsay 91400, France

[4] Laboratoire Chimie Organique, Bioorganique, Réactivité et Analyse (COBRA), Université de Rouen Normandie, CNRS, INSA, Mont-Saint-Aignan, France

**Corresponding authors: xue.qiu@u-psud.fr, michel.lepoivre@u-psud.fr, niko.hildebrandt@u-psud.fr*

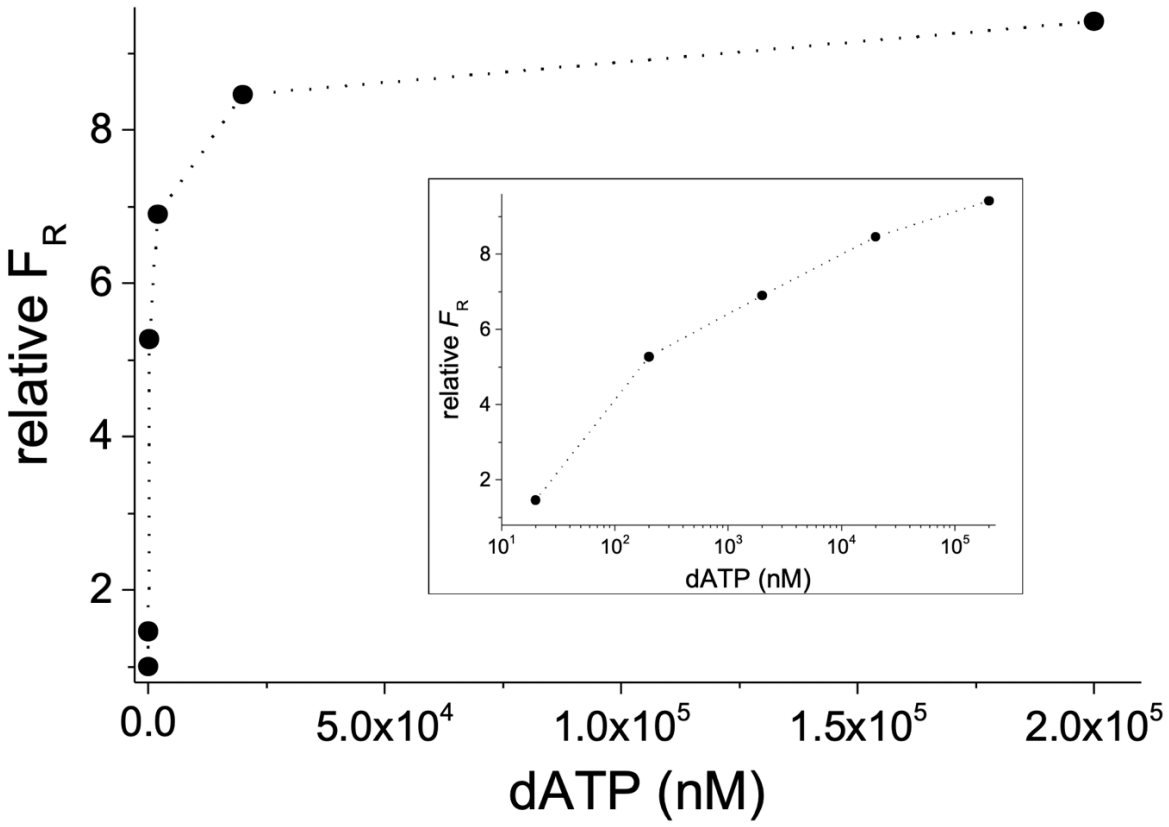

Figure S1. Preliminary dATP quantification tests revealed a dynamic range between ca. 20 nM and 200 µM (inset) with a strong linear increase in the 0 to 200 nM concentration range and an onset of saturation at ca. 2 µM.

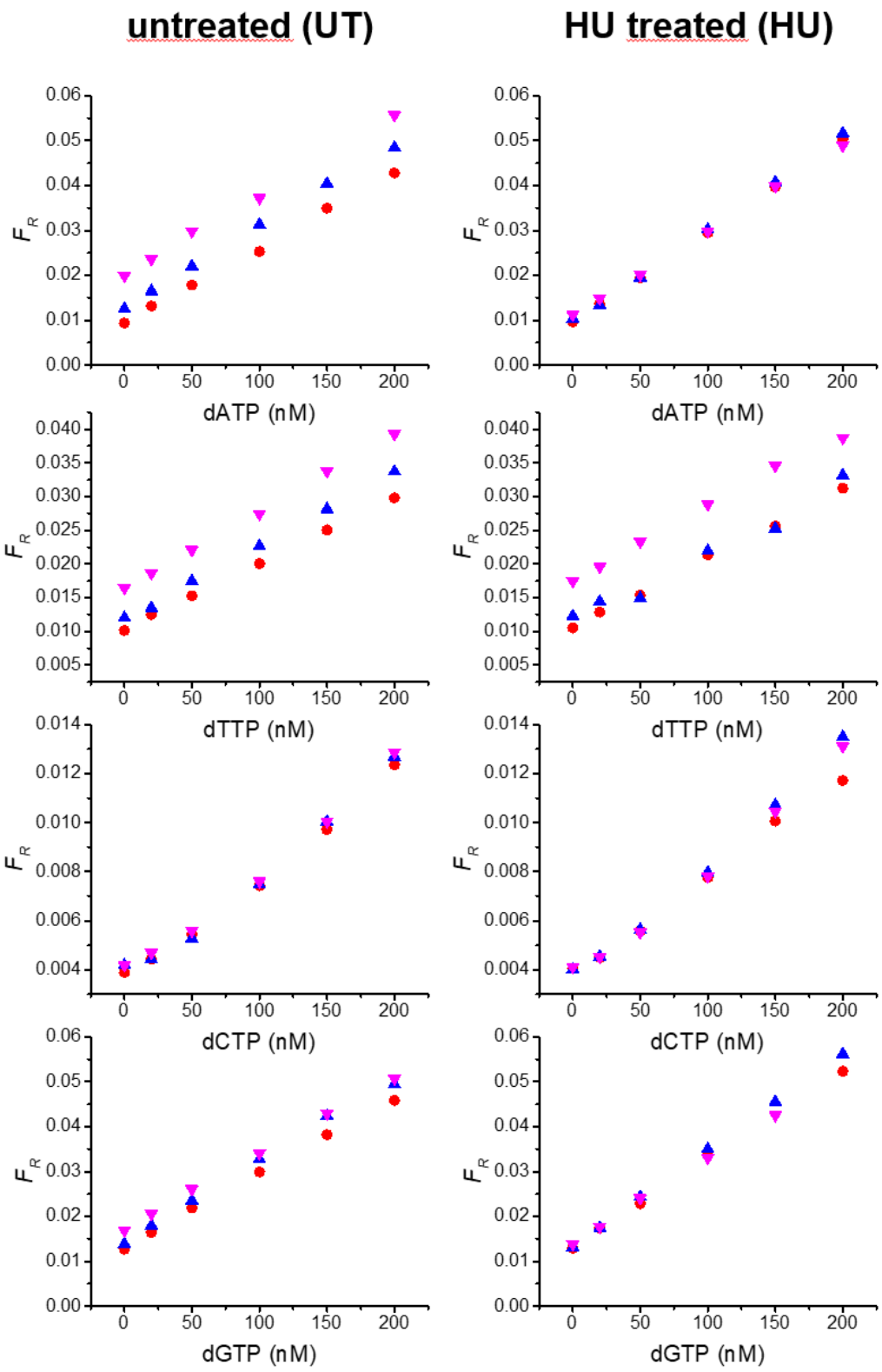

Figure S2. Calibration curves of RCA-FRET dNTP assays (dATP, dTTP, dCTP, and dGTP from top to bottom) in reaction buffer (total volume of 150 µL) including 2 µL (red), 5 µL (blue), or 15 µL (magenta) of cell extracts. The FRET ratios (F_R) were calculated from TG intensities of Cy5.5 acceptor and Tb donor PL (cf. Equation 1). Graphs show the results of cells before (left) and after (right) 24h treatment with HU.

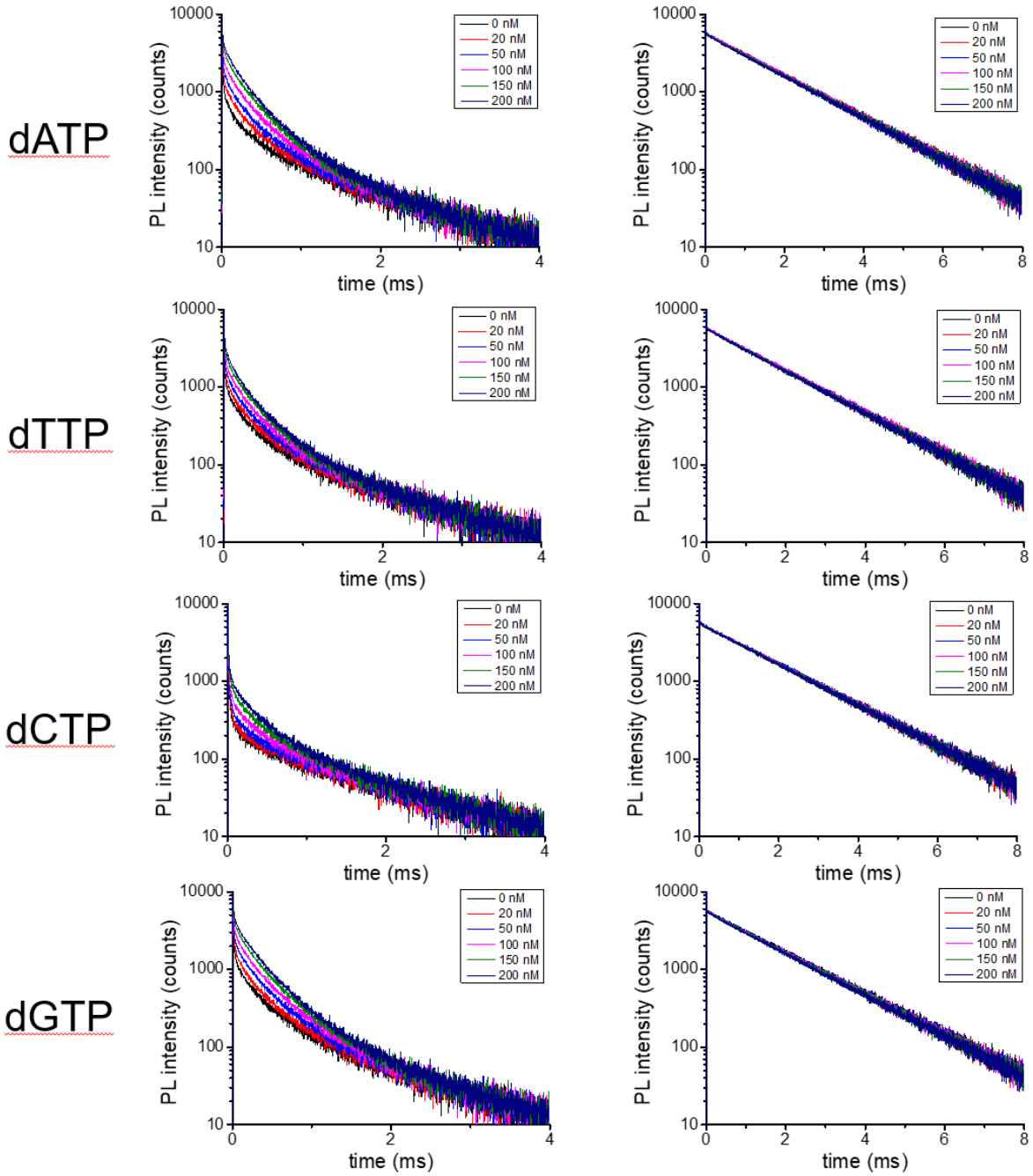

Figure S3. PL decay curves measured in the Cy5.5 (left) and the Tb (right) detection channels for different dNTP concentrations (black: 0 nM; red: 20 nM; blue: 50 nM; magenta: 100 nM; green: 150 nM; navy: 200 nM). Concentration-dependent sensitization of long-lived (milliseconds) Cy5.5 PL (left) is clearly visible in the 0 to 2 ms time range (intensities of the curves increase with concentration). Quenching of the long-lived Tb PL is much less visible because Tb-probes are contained at a high concentration inside the assays, which leads to a high Tb PL background.

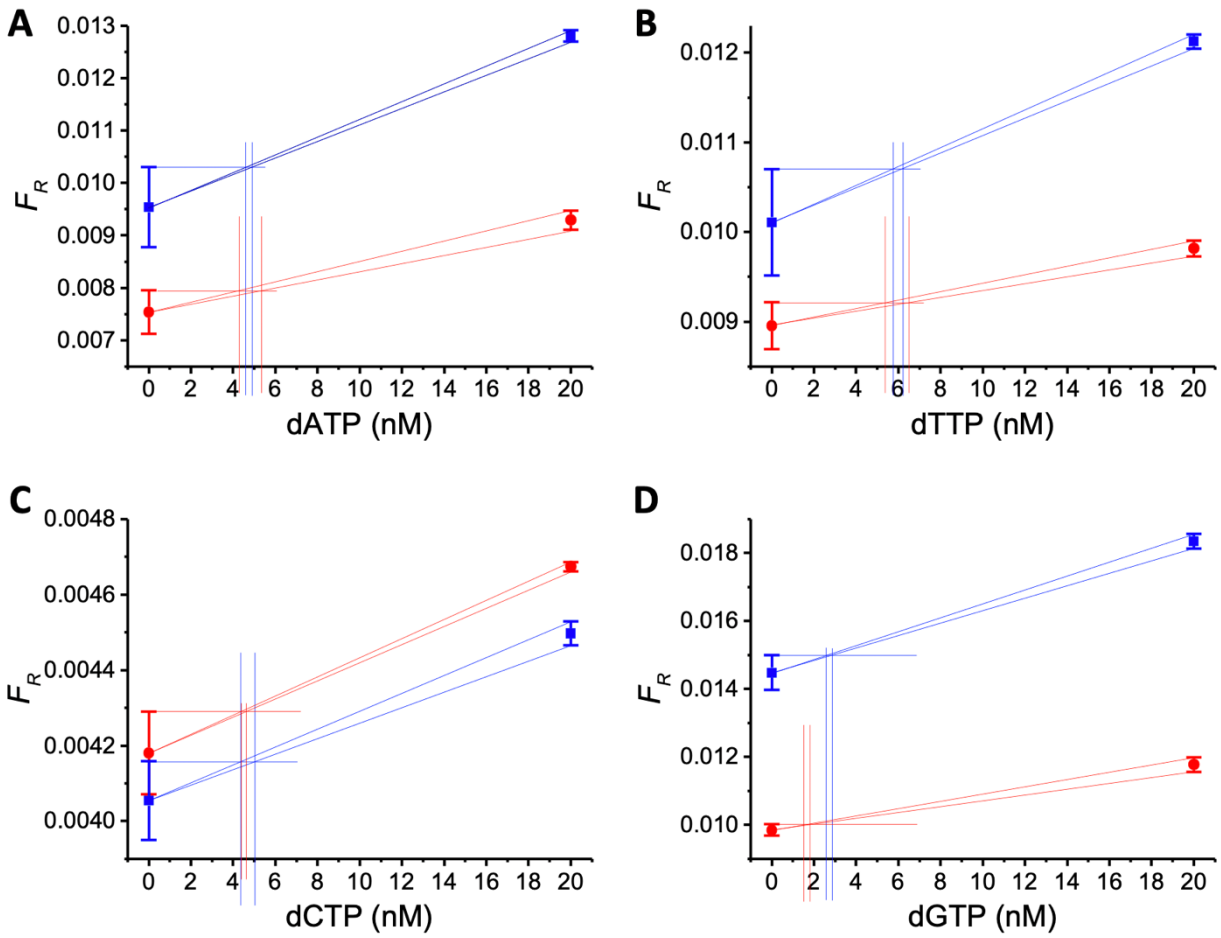

Figure S4. LODs for dATP (**A**), dTTP (**B**), dCTP (**C**) and dGTP (**D**) quantification were determined by the concentration on the calibration curve that corresponded to the F_R value of three standard deviations above the zero-concentration control (n = 30). Error bars of data points for zero dNTP concentration correspond to plus/minus three standard deviations, whereas the error bars of the data points for 20 nM dNTP concentration correspond to plus/minus one standard deviation. For LOD error estimation, two extreme calibration curves were selected.

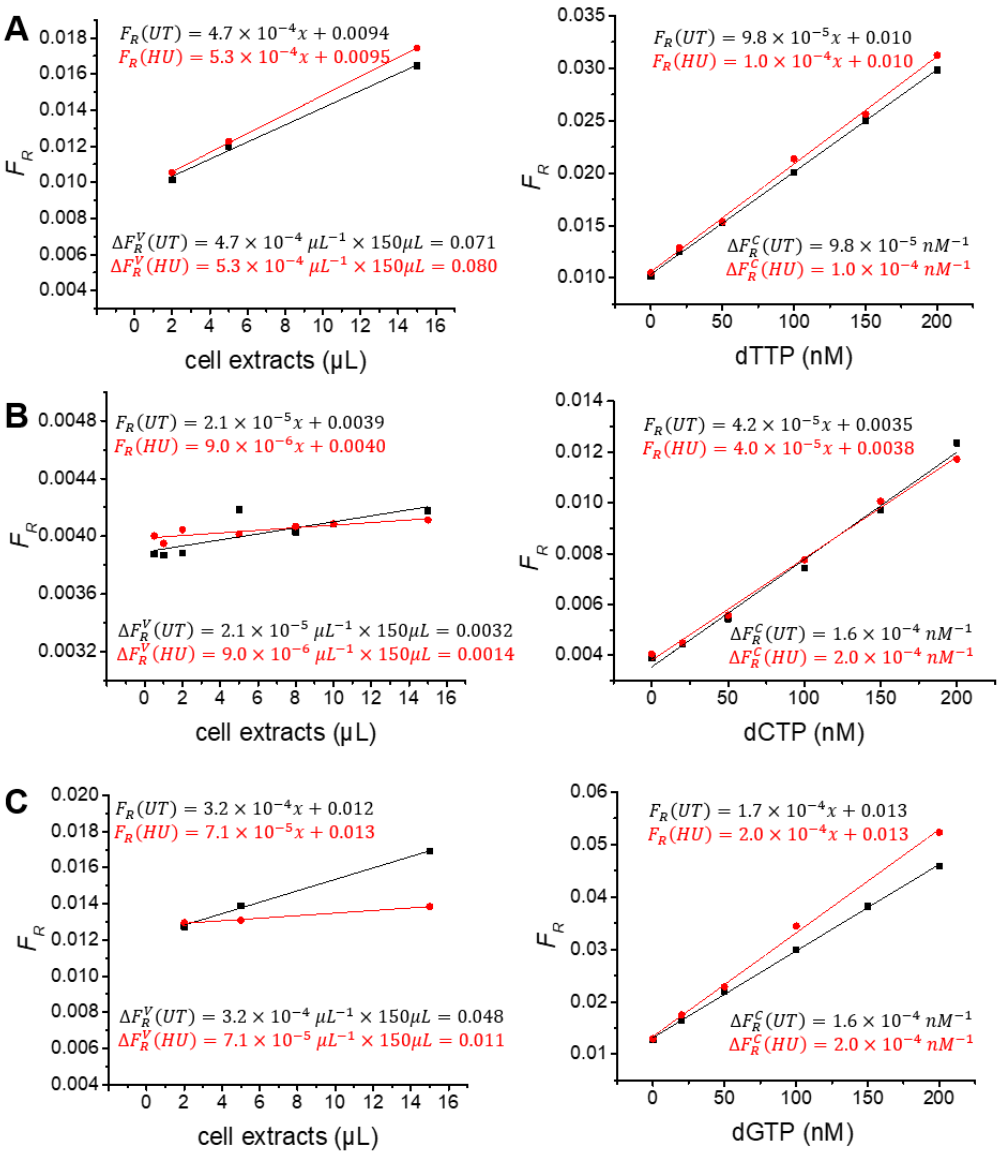

Figure S5. RCA-FRET assay calibration curves for dTTP (**A**), dCTP (**B**), and dGTP (**C**) inside CEM-SS cells before (UT: untreated) and after (HU) 24h of treatment with HU. **Left:** F_R as a function of the volume fractions of cell extracts inside the entire measuring volume for the determination of ΔF_R^V (*i.e.*, slopes of the linear fits multiplied by the total measuring volume of 150 µL). **Right:** F_R as a function of dNTP concentration for the determination of ΔF_R^C (*i.e.*, slopes of the linear fits). Functions of the linear fits and calculation of ΔF_R^V and ΔF_R^C are shown inside the graphs.