Bioconjugate Chemistry

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Tb^{3+}-tRNA for LRET Studies of Protein Synthesis

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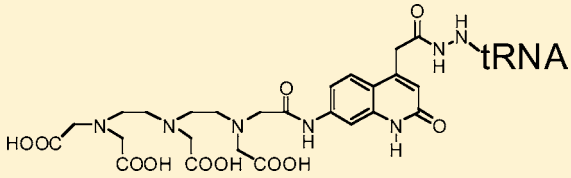
Supporting Information

ABSTRACT: When suitably labeled bulk tRNAs are transfected into cells they give rise to FRET (fluorescence resonance energy transfer) signals via binding to ribosomes that provide a measure of total protein synthesis. Application of this approach to monitoring rates of specific protein synthesis requires achieving a very high signal-to-noise ratio. Such high ratios may be attainable using LRET (luminescence resonance energy transfer) in place of FRET. Lanthanide complexes containing an antenna chromophore are excellent LRET donors. Here we describe the synthesis of a Phe-$tRNA^{Phe}$ labeled with a Tb^{3+} complex, denoted Tb^{3+}-Phe-$tRNA^{Phe}$ that, notwithstanding the bulkiness of the Tb^{3+} complex, is active in protein synthesis.



INTRODUCTION

We and our collaborators recently demonstrated that when fluorescently labeled tRNAs (fl-tRNAs), both FRET (fluorescence resonance energy transfer) donors and acceptors, are transfected into cells, they give rise to FRET signals.[1] Such signals are expected to arise when donor-labeled and acceptor-labeled tRNAs come into close proximity (<6 nm), as they do on the ribosome during the course of polypeptide elongation.[2,3] In accord with this expectation, the intensity of the FRET signal was found to correlate with the number of ribosomes engaged in protein synthesis, providing a real-time, live-cell assay for measuring rates of protein synthesis. In this prior work, fl-tRNAs were synthesized by condensing fluorescent weak-base amines, including hydrazides, with the tetrahydrouridine (THU) residues in bulk tRNA that are formed by specific reduction of dihydrouridine (DHU) residues within the D-loops of tRNA, as described.[4−6] The FRET signal generated using bulk fl-tRNAs provided a measure of the rate of total protein synthesis within transfected cells.[1]

Current efforts are focused on applying this approach to measure the intracellular rates of synthesis of specific proteins, which would be facilitated by higher signal-to-background ratios. Background interference from unbound donor and acceptor fl-tRNAs, autofluorescent compounds, and light scattering, while minimized by excitation wavelength selection and emission filters, limits the sensitivity and precision of the FRET approach. Substituting gated-detection of LRET (luminescence resonance energy transfer) for FRET offers a plausible solution to the background problem. LRET uses long-lifetime lanthanide (Ln^{3+}) donors (e.g., Tb^{3+} or Eu^{3+}) to temporally separate the delayed (>100 μs) emission of acceptors from the prompt (ns) emission of fluorescent species that are unrelated to resonance energy transfer, with reported increases in signal-to-background ratios of ≥500-fold.[7]

Ln^{3+} complexes suitable for LRET studies are composed of an "antenna" chromophore, necessary to overcome the inherently low absorbance of Ln^{3+}, attached to a multidentate chelating site for the Ln^{3+}.[7,8] Here we report the synthesis of a hydrazide derivative of such a Ln^{3+}-complex, its attachment to $tRNA^{Phe}$, and the activity of this bioconjugate, charged with Phe, both in forming a ternary complex with EF-Tu·GTP that is required for Phe-$tRNA^{Phe}$ uptake by the ribosome and in supporting synthesis of the full-length protein emerald green fluorescent protein (EmGFP).

EXPERIMENTAL PROCEDURES

Chemicals. The following reagents were purchased from Aldrich: 1,3-phenylenediamine, diethyl-1,3-acetonedicarboxylate, zinc chloride, *tert*-butyl carbazate, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDAC), diethylenetriaminepentaacetic acid (DTPA), terbium(III) chloride, and europium(III) chloride. *E. coli* $tRNA^{Phe}$ was obtained from Chemical Block (Moscow). ^{3}H-Phenylalanine (Phe) was obtained from Perkin-Elmer.

Assaying the Translational Activity of the Tb^{3+}-tRNA Using Cell-Free Protein Synthesis (CFPS). The CFPS kit (5prime) was utilized to synthesize EmGFP according to the manufacturer's manual, with some modifications.[9] Briefly, the cell lysate was supplemented with protease inhibitors and an inhibitor of $tRNA^{Phe}$-aminoacyl-synthetase (PheRS) and then

Received: January 31, 2013
Revised: June 4, 2013
Published: June 4, 2013

dx.doi.org/10.1021/bc400062d | *Bioconjugate Chem.* 2013, 24, 1186−1190

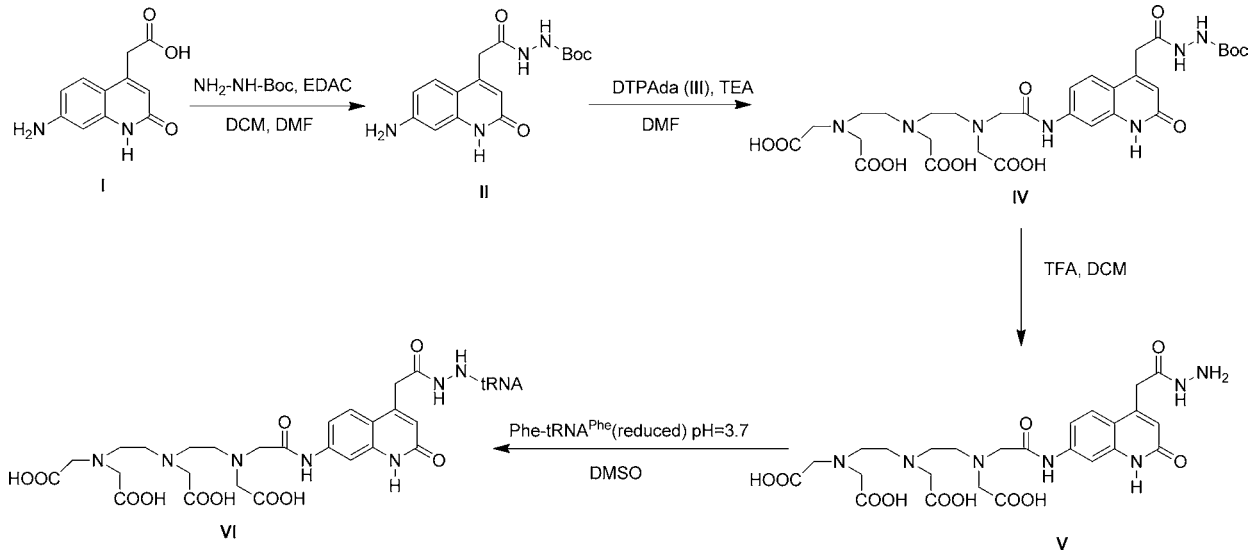


Figure 1. Synthetic scheme for **VI**.

dialyzed in order to deplete all amino acids while preventing Phe regeneration and $tRNA^{Phe}$ aminoacylation. Following dialysis, the other CFPS components were added according to the manufacturer's manual, excluding the amino acid Phe. The total ribosome concentration was estimated as 0.3 μM based on A_{260}. The ability of this $CFPS^{-Phe}$ mixture to produce EmGFP was dependent on the addition of exogenous Phe, supplied in the form of either Phe-$tRNA^{Phe}$ or Tb^{3+}-Phe-$tRNA^{Phe}$ (2 μM). The reaction was initiated by addition of 130 ng plasmid DNA per 10 μL reaction volume and fluorescence was recorded using a plate reader (EnVision, Perkin-Elmer) at 30 °C using excitation and emission wavelengths of 486 and 535 nm, respectively.

Spectra. ^{1}H NMR spectra were recorded in a Bruker DMX 300 spectrometer in deuterated solvents. ^{1}H chemical shifts were referenced to internal solvent resonances and reported relative to $SiMe_4$. Low-resolution electrospray (ESI) mass spectra were obtained with a Waters SQD equipped with an Acquity UPLC. UV−visible (UV−vis) absorption spectra were recorded using a Hewlett-Packard 8452A Diode Array Spectrophotometer. Fluorescence emission spectra were recorded using a Fluorolog-3 spectrofluorometer (Horiba Jobin Yvon). Time-resolved luminescence measurements were performed on a FS900 spectrofluorometer (Edinburgh Instruments, UK), equipped with R2658P photomultiplier tube (Hamamatsu) and a xenon flash lamp as the excitation source. Kinetic experiments were performed on a KinTek SF-300X stopped-flow spectrofluorometer.

Synthesis (Figure 1). Compounds **I** and **III** were prepared as described.[10,11]

Compound II. To a suspension of **I** (70 mg, 0.32 mmol) in DCM (2 mL) and DMF (0.8 mL) was added *tert*-butyl carbazate (85 mg, 0.64 mmol) and *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDAC) (80 mg, 0.41 mmol). The reaction was maintained for 20 h at room temperature. Solvent was removed by evaporation in vacuo. The residue was dissolved in ethyl acetate (15 mL) and extracted with a solution of saturated aqueous $NaHCO_3$ (3 × 10 mL). The organic layer was dried over anhydrous Na_2SO_4 and evaporated. The product was purified by column chromatography on silica gel using a chloroform/ethanol mixture (8:2) as eluent. Yield 62%. ESIMS (m/z) 333 $[M+H]^+$. ^{1}H NMR (300 MHz, DMSO d_6): 1.39 (s, 9H, Boc), 3.52 (s, 2H, $-CH_2CO-$), 5.74 (broad, 2H, 7 amino), 6.07 (s, 1H, 3H), 6.37 (d, 1H, 8H, J = 2.1), 6.43 (dd, 1H, 6H, J_1 = 8.7 Hz, J_2 = 2.1 Hz), 7.41 (d, 1H, 5H, J = 8.7), 9.85 (1H, broad, NH), 11.20 (1H, broad, amide). ^{13}C NMR (300 MHz, DMSO-d_6): 28.99, 44.06, 80.77, 97.40, 110.68, 111.01, 116.84, 125.92, 140.41, 146.01, 151.35, 156.50, 162.62, 170.35.

Compound III. Pyridine (1.2 mL) and acetonitrile (1.2 mL) warmed to 50 °C were mixed with a solution of DTPA (490 mg, 1.37 mmol) in acetic anhydride (0.8 mL, 8.60 mmol). After 24 h, solvent was removed by evaporation in vacuo and the residue was washed with acetic anhydride and diethyl ether. The solid was dried under vacuum. Yield 92%. ESIMS (m/z) 356 $[M-H]^-$. ^{1}H NMR (300 MHz, DMSO-d_6): 3.71 (s, 8H, terminal -NCH_2CO_2), 3.30 (s, 2H, central -NCH_2CO_2), 2.74 (t, 4H, J = 5.7 Hz, -NCH_2CH_2N-), 2.59 (t, 4H, J = 5.7 Hz, -NCH_2CH_2N-).

Compound IV. To a suspension of DTPA dianhydride **III** (54 mg, 0.15 mmol) in DMF (1 mL) was added triethylamine (0.08 mL, 0.6 mmol) under nitrogen. After 10 min a solution of **II** (15 mg, 0.04 mmol) in DMF (0.5 mL) was added and the reaction was maintained at room temperature for 3 h. A few drops of water were added to quench the reaction. The product was purified by preparative thin layer chromatography, using acetonitrile/water (7:3) as eluent. Yield 30%. ESIMS (m/z) 706 $[M-H]^-$. ^{1}H NMR (300 MHz, D_2O): 1.39 (s, 9H, Boc), 2.60−3.90 (m, 20H), 6.47 (s, 1H, 3H), 7.16 (m, 1H, 8H), 7.67−7.45 (m, 2H, 5H-6H).

Compound V. A mixture of DCM:TFA (1:1) (100 uL) was added to **IV** (2 mg, 0.003 mmol) at 0 °C and the reaction was maintained at room temperature for 2 h. **V** was used without further purification in the preparation of **VI**. The solvent was evaporated in vacuo. Quantitative yield. ESIMS (m/z) 608 $[M+H]^+$. 606 $[M-H]^-$; ε_{max}= 14800 M^{-1} cm^{-1} at 340 nm.[12]

Compound VI. Derivatization of Phe-$tRNA^{Phe}$ with the hydrazide **V** was based on a previously described protocol[5,6] involving nucleophilic substitution at THU residues. Selective reduction of the two DHU residues at positions 16 and 20 in *E. coli* $tRNA^{Phe}$ to THU residues was carried out by incubating $tRNA^{Phe}$ (2.5 mg/mL), $NaBH_4$ (10 mg/mL, added from 100

dx.doi.org/10.1021/bc400062d | *Bioconjugate Chem.* 2013, 24, 1186−1190

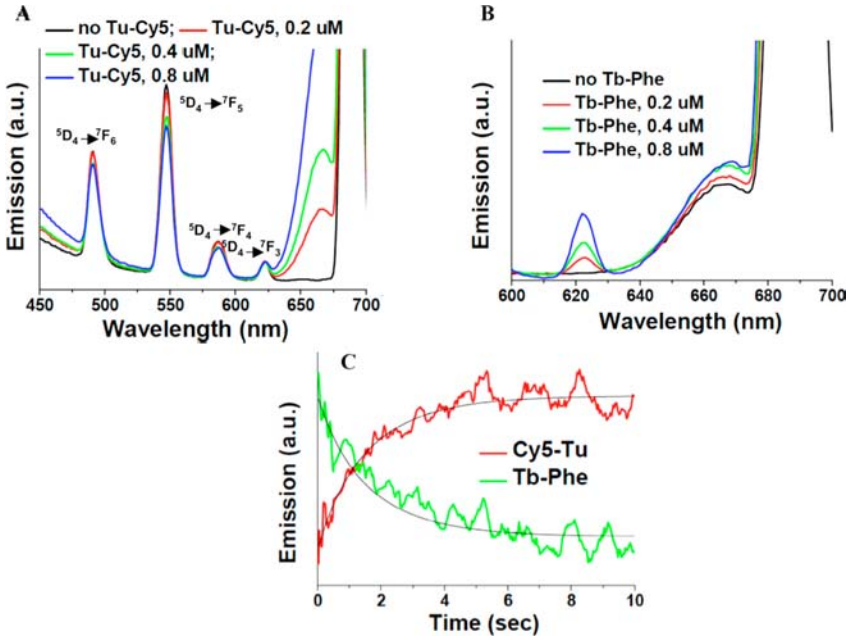


Figure 2. TC formation between EF-Tu^{Cy5}·GTP and Tb^{3+}-Phe-tRNA^{Phe}. A. Luminescence spectrum of Tb^{3+}-Phe-tRNA^{Phe} (0.2 μM) with varying amounts of EF-Tu^{Cy5} on excitation at 345 nm. B. Fluorescence spectrum of EF-Tu^{Cy5} (0.2 μM) with varying amounts of Tb^{3+}-Phe-tRNA^{Phe} on excitation at 345 nm. C. Decrease in Tb^{3+}-Phe-tRNA^{Phe} luminescence (550 ± 10 nm) and increase in EF-Tu^{Cy5} fluorescence (680 ± 10 nm) on excitation at 345 nm following rapid mixing of Tb^{3+}-Phe-tRNA^{Phe} (0.1 μM, measured as Phe), containing 0.24 Phe/tRNA, and EF-Tu^{Cy5}·GTP (0.4 μM) (final concentrations). In parts A and B, excitation frequency doubling in the Fluorolog-3 spectrofluorometer resulted in a very large peak at 690 nm. This artifact was not a problem for the KinTek SF-300X stopped-flow spectrofluorometer employed in part C.

mg/mL in 10 mM KOH) in 40 mM Tris-HCl (pH = 7.5), at 0 °C for 60 min, in a total volume of 390 μL. The reaction was quenched by lowering the pH to 4−5 by gradually adding 6 M acetic acid. Unreacted $NaBH_4$ was removed via three ethanol precipitations of tRNA^{Phe} (reduced). Aminoacylation of tRNA^{Phe}(reduced) (0.5 mg/mL) was performed in a buffer containing 75 μM ^{3}H-phenylalanine (370 cpm/pmol), 100 mM Tris-HCl (pH 8.0), 4 mM ATP (pH 7.8), 20 mM $MgCl_2$, 1 mM EDTA, 7 mM 2-mercaptoethanol, and 100 μM S100 crude synthetase in a total volume of 1.7 mL. The reaction was incubated at 37 °C during 30 min. Following phenol-chloroform extraction and ethanol precipitation, Phe-tRNA^{Phe}(reduced) was loaded onto a FPLC monoQ column. Chromatography with a gradient of 0.5−0.9 M NaCl in 50 mM NaAc (pH 5.0) yielded a preparation of Phe-tRNA^{Phe}(reduced) in which the tRNA was 30% charged with Phe. This preparation (0.7 mg) was resuspended in 11 μL of 0.2 M NaCOOH (pH 3.7) and 10 μL of DMSO. 2 μL of a DMSO solution of **V** (290 mM) was added and incubation was carried out for 150 min at 37 °C. During this period, additional 1 μL portions of **V** in DMSO solution were added at 45 and 90 min. Vacuum drying and removal of excess **V** by three rounds of ethanol precipitation gave an overall stoichiometry of labeling of 0.4 **V**/tRNA. Unlabeled Phe-tRNA^{Phe} (reduced) was removed from **V**-labeled Phe-tRNA^{Phe} by RP-HPLC using a C18/15 μm column (Waters) with a gradient of 6−21% ethanol in buffer containing 20 mM NH_4Ac (pH 5.0), 10 mM $MgAc_2$, and 400 mM NaCl, with absorption monitored at 260 and 340 nm. In this gradient, the labeled tRNA elutes after the unlabeled tRNA. This procedure yielded preparations, designated as **VI**, containing 1.3 **V**/Phe-tRNA^{Phe}, i.e., mixtures of Phe-tRNA^{Phe}s containing 1 or 2 **V**/tRNA^{Phe}.

Synthesis of Tb^{3+}-Phe-tRNA^{Phe}. **VI** was dissolved in water and 1.5 equiv of a 300 μM $TbCl_3$ solution, based on the stoichiometry of **V** incorporated into **VI**, was added, with vigorous stirring for 30 min at 0 °C. Excess $TbCl_3$ was removed by 4 rounds of ethanol precipitation. An analogous procedure was used to prepare Eu^{3+}-Phe-tRNA^{Phe}. Suitable control experiments demonstrated that incubation with $TbCl_3$ of tRNA^{Phe} not labeled with **V** afforded no luminescence at 550 nm on excitation at 345 nm (see Figure S1).

Cell-Free Expression of EmGFP Using the CFPS^{-Phe} Kit. The assay was performed essentially as previously described.[9] *E. coli* Phe-tRNA^{Phe}s (2 μM, measured by the amount of charged tRNA), either unlabeled or labeled with **V** and Tb^{3+}, were added to a CFPS^{-Phe} kit and EmGFP synthesis was monitored by fluorescence intensity at 535 nm.

Ternary Complex (TC) Formation. TCs were formed by combining elongation factor Tu (EF-Tu) and Phe-tRNA^{Phe} in buffer A (50 mM Tris-HCl [pH 7.5], 70 mM NH_4Cl, 30 mM KCl, 7 mM $MgCl_2$, and 1 mM DTT, 1 mM GTP, 1.5 mM phosphoenolpyruvate, and 0.5 mg/L of pyruvate kinase) as previously described[13] except that the ratio of EF-Tu/tRNA employed was 4:1 unless otherwise specified. Time-dependent TC formation was performed at 25 °C by mixing 0.1 uM of Tb^{3+}-Phe-tRNA^{Phe} with 0.4 uM of EF-Tu^{Cy5} with excitation of 345 nm. Static fluorescence was recorded with excitation of 345 nm.

RESULTS

Synthesis of the Probe, V. Compound **I** was obtained by condensing 1,3-phenylenediamine and diethyl-1,3-acetonedicarboxylate, followed by saponification of the ethyl ester according to previously reported procedure.[10] We introduced the Boc-protected hydrazide function by treatment of **I** with *tert*-butyl carbazate and *N*-(3-dimethylaminopropyl)-*N*′-ethyl-carbodiimide hydrochloride (EDAC), yielding **II**. An excess of DTPA dianhydride **III** was reacted with **II** in Et_3N and DMF at

dx.doi.org/10.1021/bc400062d | *Bioconjugate Chem.* 2013, 24, 1186−1190

room temperature for 3 h, yielding **IV**, which was purified by thin layer chromatography. Deprotection of **IV** with TFA:DCM (1:1) afforded the DTPA-based chelate hydrazide (**V**).

Labeling of Phe-tRNA^{Phe}(reduced) with V. *E. coli* tRNA^{Phe} has DHU residues at positions 16 and 20. Synthesis of charged, **VI** labeled tRNA^{Phe} for use in functional studies was carried out using the three-step procedure previously employed to label DHU positions, involving (1) $NaBH_4$ reduction of the DHU residues to THU to form tRNA^{Phe}(reduced); (2) charging of tRNA^{Phe}(reduced) with Phe by Phe-RS; and (3) labeling of Phe-tRNA^{Phe}(reduced) with a large molar excess of hydrazide.[5,6] Such labeling proceeds via a substitution at the THU residues to yield *N*-substituted tetrahydrocytidine (THC) residues within the tRNA. Previous work[6] showed that Cy3 hydrazide labeled Phe-tRNA^{Phe}(reduced) at both positions 16 and 20 to near equal extents (relative labeling 1.0:1.2, respectively) and we assume a similar distribution is obtained for labeling of tRNA^{Phe}(reduced) by **VI**. In the present case, the overall labeling stoichiometry was 0.4 **V**/tRNA^{Phe}. A more enriched preparation containing 1.3 **V**/tRNA^{Phe}, denoted **VI**, was obtained by separation of derivatized tRNA from underivatized tRNA by RP-HPLC. Within **VI**, 24−30% of the tRNA^{Phe} was charged with Phe.

Formation and Characterization of the Tb^{3+}-VI Complex. Treatment of **VI** with aqueous lanthanide trichlorides afforded Tb^{3+} and Eu^{3+} complexes. It was also possible to label Phe-tRNA^{Phe} with a preformed Ln^{3+}-**V** complex. However, resolution of the resulting reaction mixture by RP-HPLC was less successful than the procedure described in the Experimental Procedures. We denote the charged form of Tb^{3+}-**VI** as Tb^{3+}-Phe-tRNA^{Phe}. On excitation of the 7-aminoquinolone moiety at 345 nm, Tb^{3+}-**VI** shows the multiple luminescence bands characteristic of Tb^{3+} complexes in the range 485−620 nm, corresponding to the $^5D_4$ to ^7F_n (n = 3−6) transitions indicated (Figure 2A). The following luminescence lifetimes were measured: Tb^{3+}-**VI**: 1.06 ms, Eu^{3+}-**VI**: 0.6 ms, Tb^{3+}-Phe-tRNA^{Phe}: 1.08 ms. All lifetime measurements were well fit by single exponential decays.

Functional Assays. *Ternary Complex (TC) Formation.* Binding of aminoacyl-tRNA (aa-tRNA) to the ribosome occurs via the ternary complex aa-tRNA·EF-Tu·GTP, where EF-Tu is a translation elongation factor. We have previously shown that an EF-Tu mutant labeled with Cy5 at position 348, denoted EF-Tu^{Cy5}, retains functional activity in protein synthesis.[14] Within the TC, this position is within 34 Å and 24 Å of positions 16 and 20, respectively, of aminoacyl-tRNA [calculated from pdb1OB2], making it suitable for LRET studies with Tb^{3+}-**VI**. Formation of the TC between Tb^{3+}-Phe-tRNA^{Phe} and EF-Tu^{Cy5}·GTP is accompanied by a decrease in Tb^{3+}-Phe-tRNA^{Phe} luminescence as EF-Tu^{Cy5} is increased (Figure 2A) and an increase in EF-Tu^{Cy5} fluorescence as Tb^{3+}-Phe-tRNA^{Phe} is increased (Figure 2B). The K_d value estimated from the results in Figures 2A,B is approximately 0.3 μM. Additional evidence for LRET between Tb^{3+} and Cy5 within the TC comes from the difference in luminescence lifetimes of the TC complexes made from Tb^{3+}-Phe-tRNA^{Phe} and either EF-Tu^{Cy5}·GTP (0.45 ms, t_L) or unlabeled EF-Tu·GTP (1.08 ms, t^0_L). An estimated LRET efficiency, ϕ, equal to 0.58, could be calculated from the measured luminescence lifetime values via eq 1.

$$f = (t_L^{-1} - t_L - 1)/t_L^{-1} \quad (1)$$

The transient changes in Tb^{3+}-Phe-tRNA^{Phe} luminescence and EF-Tu^{Cy5} fluorescence (Figure 2C) on rapid mixing of Tb^{3+}-Phe-tRNA^{Phe} and EF-Tu^{Cy5}·GTP allow calculation of a second order rate constant for TC formation of 1.8×10^6 M^{-1} s^{-1}.

EmGFP Synthesis. We recently described a real-time coupled transcription−translation assay that continuously monitors formation of the fluorescent protein Emerald GFP (EmGFP) and can be manipulated, so that EmGFP formation is totally dependent on exogenously added Phe-tRNA^{Phe}, through use of a so-called CFPS^{-Phe} kit.[9] Here we use this assay to demonstrate that Tb^{3+}-Phe-tRNA^{Phe} displays activity in supporting EmGFP synthesis that is roughly comparable to that of native Phe-tRNA^{Phe} (Figure 3). The assay was carried out under conditions in which the extent of EmGFP synthesis is roughly proportional to added Phe-tRNA^{Phe} concentration. It is possible that the lower amount of EmGFP synthesized with Tb^{3+}-Phe-tRNA^{Phe} reflects a functional heterogeneity of this sample. For example, the labeling stoichiometry is 1.3 **VI**/tRNA, and it is possible that the 2:1 labeled tRNA is less active than either of the 1:1 complexes.

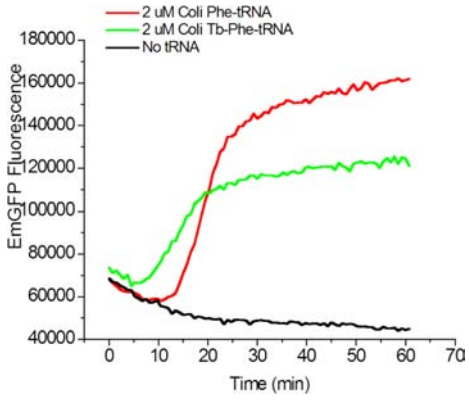


Figure 3. Phe-tRNA^{Phe}-dependent cell-free protein synthesis of EmGFP. Experiments were performed using the CFPS^{-Phe} mixture, supplemented with 2 μM of the indicated tRNAs, initiated with 130 ng plasmid DNA. Recordings of EmGFP fluorescence were performed every 45 s using the plate reader, set to 30 °C using excitation and emission wavelengths of 486 and 535 nm, respectively.

DISCUSSION

This work demonstrates that Phe-tRNA^{Phe} derivatized with the rather bulky luminescent Tb^{3+} complex **VI** at THU positions formed by selective $NaBH_4$ reduction of DHUs at residues 16 and 20 within the D-loop retains high functionality in protein synthesis (Figure 3). Such retention of functionality is not completely unexpected. Positions 16 and 20 are both quite far from the site of EF-Tu interaction with charged tRNAs, accounting for the readiness with which Tb^{3+}-Phe-tRNA^{Phe} reacts with EF-Tu to form a ternary complex (Figure 2C), and proximal to the variable loop of tRNA, within which insertions of up to 19 nucleotides are well-tolerated by the ribosome during protein translation.

The success of the current work opens up the prospect that Tb^{3+}-Phe-tRNA^{Phe}, or molecules like it, can be used to monitor LRET signals in live cells that are generated by juxtaposition on the ribosome of a tRNA labeled with **VI** and a suitable fl-tRNA, labeled, for example, with Cy5, that serves as an LRET acceptor. The antenna in **VI**, an aminooxoquinoline, was chosen based on prior work showing it to have excellent optical

and stability properties for LRET experiments.[15] More recently, other antennas have been described that have superior optical properties,[16,17] but are somewhat larger than 7-aminoquinolones. Further work will determine whether tRNAs containing Ln^{3+} complexes of such antennas will demonstrate functionality comparable to that found with Tb^{3+}-Phe-$tRNA^{Phe}$.

■ ASSOCIATED CONTENT

Supporting Information

A figure comparing luminescence observed for **VI** and unmodified $tRNA^{Phe}$ on incubation with Tb^{3+}. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was funded by NIH grant GM090404 to BSC and by a Human Frontier Science Program fellowship to GR. We thank Profs. Larry Miller (UIC) and Sergei Vinogradov (Penn) for helpful discussions.

■ REFERENCES

(1) Barhoom, S., Kaur, J., Cooperman, B. S., Smorodinsky, N. I., Smilansky, Z., Ehrlich, M., and Elroy-Stein, O. (2011) Quantitative single cell monitoring of protein synthesis at subcellular resolution using fluorescently labeled tRNA. *Nucleic Acids Res. 39*, e129.

(2) Blanchard, S. C., Kim, H. D., Gonzalez, R. L., Jr, Puglisi, J. D., and Chu, S. (2004) tRNA dynamics on the ribosome during translation. *Proc. Natl. Acad. Sci. U. S. A. 101*, 12893−8.

(3) Chen, C., Stevens, B., Kaur, J., Cabral, D., Liu, H., Wang, Y., Zhang, H., Rosenblum, G., Smilansky, Z., Goldman, Y., and Cooperman, B. S. (2011) Single molecule fluorescence measurements of ribosomal translocation dynamics. *Mol. Cell 42*, 367−77.

(4) Wintermeyer, W., Schleich, H. G., and Zachau, H. G. (1979) Incorporation of amines or hydrazines into tRNA replacing wybutine or dihydrouracil. *Methods Enzymol. 59*, 110−21.

(5) Pan, D., Qin, H., and Cooperman, B. S. (2009) Synthesis and functional activity of tRNAs labeled with fluorescent hydrazides in the D-loop. *RNA 2*, 346−54.

(6) Kaur, J., Raj, M., and Cooperman, B. S. (2011) Fluorescent labeling of tRNA dihydrouridine residues: Mechanism and distribution. *RNA 17*, 1393−400.

(7) Rajapakse, H. A., Gahlauta, N., Mohandessia, S., Yu, D., Turner, J. R., and Miller, L. W. (2010) Time-resolved luminescence resonance energy transfer imaging of protein−protein interactions in living cells. *Proc. Natl. Acad. Sci. U.S.A. 107*, 13582−7.

(8) Rajapakse, H. E., Reddy, D. R., Mohandessi, S., Butlin, N. G., and Miller, L. W. (2009) Luminescent terbium protein labels for time-resolved microscopy and screening. *Angew. Chem., Int. Ed. Engl. 48*, 4990−2.

(9) Rosenblum, G., Chen, C., Kaur, J., Cui, X., Goldman, Y. E., and Cooperman, B. S. (2012) Real-time assay for testing components of protein synthesis. *Nucleic Acids Res.*, DOI: 10.1093/nar/gks232.

(10) Reddy, D. R., Pedró Rosa, L. E., and Miller, L. W. (2011) Luminescent trimethoprim-polyaminocarboxylate lanthanide complex conjugates for selective protein labeling and time-resolved bioassays. *Bioconjugate Chem. 22*, 1402−9.

(11) Ardestani, M. S., Arabzadeh, A. J., Heidari, Z., Hosseinzadeh, A., Ebrahimi, H., Hashemi, E., Mosayebnia, M., Shafiee-Alavidjeh, M., Alavi, A., Babei, M. H., Rahmim, A., Ebrahimi, S. E. S., and Amanlou, M. (2010) Novel and facile methods for the synthesis of DTPA-monoamide: a new completely revised strategy in the radiopharmaceutical chemistry. *J. Radioanal. Nucl. Chem. 283*, 447−55.

(12) Krasnoperov, L. N., Marras, S. A. E., Kozlov, M., Wirpsza, L., and Mustaev, A. (2010) Luminescent probes for ultrasensitive detection of nucleic acids. *Bioconjugate Chem. 21*, 319−27.

(13) Pan, D., Kirillov, S. V., and Cooperman, B. S. (2007) Kinetically competent intermediates in the translocation step of protein synthesis. *Mol. Cell 25*, 519−29.

(14) Cooperman, B. S., Goldman, Y. E., Chen, C., Farrell, I., Kaur, J., Liu, H., Liu, W., Rosenblum, G., Smilansky, Z., Stevens, V., and Zhang, H. (2011) Mechanism and Dynamics of the Elongation Cycle, in *Ribosomes: Structure, Function and Dynamics* (Rodnina, M.V. et al., Eds.) pp 339−48, Springer Verlag.

(15) Li, M., and Selvin, P. R. (1997) Amine-reactive forms of a luminescent diethylenetriaminepentaacetic acid chelate of terbium and europium: attachment to DNA and energy transfer measurements. *Bioconjugate Chem. 8*, 127−32.

(16) Moore, E. G., Samuel, A. P., and Raymond, K. N. (2009) From antenna to assay: lessons learned in lanthanide luminescence. *Acc. Chem. Res. 42*, 542−52.

(17) Xu, J., Corneillie, T. M., Moore, E. G., Law, G. L., Butlin, N. G., and Raymond, K. N. (2011) Octadentate cages of Tb(III) 2-hydroxyisophthalamides: a new standard for luminescent lanthanide labels. *J. Am. Chem. Soc. 133*, 19900−10.