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Novel cyanine-AMP conjugates for efficient 5′ RNA fluorescent labeling by one-step transcription and replacement of [γ-32P]ATP in RNA structural investigation

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ABSTRACT

Two novel fluorescent cyanine-AMP conjugates, F550/570 and F650/670, have been synthesized to serve as transcription initiators under the T7 2.5 promoter. Efficient fluorophore labeling of 5′ RNA is achieved in a single transcription step by including F550/570 and F650/670 in the transcription solution. The current work makes fluorescently labeled RNA readily available for broad applications in biochemistry, molecular biology, structural biology and biomedicine. In particular, site-specifically fluorophore-labeled large RNAs prepared by the current method may be used to investigate RNA structure, folding and mechanism by various fluorescence techniques. In addition, F550/570 and F650/670 may replace [γ-32P]ATP to prepare 5′ labeled RNA for RNA structural and functional investigation, thereby eliminating the need for the unstable and radio-hazardous [γ-32P]ATP.

INTRODUCTION

Site-specific fluorescent labeling of RNA has many applications in biochemistry, molecular biology, structural biology and biomedicine (1–15). Fluorophores may be attached to RNA either by phosphoramidite chemistry during RNA synthesis (1–3,16), post-transcriptional fluorophore coupling with enzymatically prepared RNA (16,17), or direct fluorophore labeling during transcription (18,19). Phosphoramidite chemistry-based fluorescent labeling is commonly used to synthesize relatively small RNA molecules (<50–60 nt). When the size of RNA increases, such fluorescent-labeling procedures become impractical due to low yields of full-length RNA, high levels of impurities with short RNA fragments and high costs of chemical synthesis. On the other hand, transcription-based RNA synthesis and fluorescent labeling has no apparent RNA size restrictions; both purities and costs are essentially independent of RNA sizes. Post-transcriptional fluorescent labeling involves two essential steps: preparation of amino- or thio-derivatized RNA by transcription followed by fluorophore coupling via fluorophore-N-hydroxysuccinimide esters (NHS) or fluorophore-maleimides or fluorophore-bromides (16,17). The limitations of this method lie in (i) the scarcity of available amino- and thio-nucleotide precursors for the preparation of amino- and thio-derivatized RNA, respectively; (ii) the hydrolytic lability of fluorophore-NHS; (iii) high concentrations of fluorophore-NHS, fluorophore-maleimides or fluorophore-bromides that are required to achieve efficient fluorescent labeling of RNA; and (iv) multiple steps of manipulation of RNA samples, leading to laborious sample preparation and low RNA yields.

Direct RNA labeling at the 5′ end using the T7 2.5 promoter developed in our laboratory (18,19) only requires appropriate label-linker-AMP conjugates (adenosine 5′-monophosphate, AMP) to serve as transcription initiators. The newly developed in vitro transcription system recognizes the adenosine moiety and labels the 5′ end of RNA with high efficiency through transcription initiation (18,19). The only RNA sequence requirement is the 5′ AG. We have previously reported fluorescein-HDA-AMP (1,6-hexanediamine, HDA) for direct RNA labeling (19). For this new RNA-labeling method to be widely applicable, however, diverse fluorophores with better spectroscopic properties, such as the commonly used cyanine dye family, are highly desirable. Here, we describe the synthesis of two novel cyanine-AMP conjugates, F550/570 and F650/670, and their use to fluorescently label 5′ RNA in a single transcription step. Furthermore, we demonstrate one utility, among others, of F550/570 to replace the commonly used [γ-32P]ATP for 5′ RNA labeling in RNA structure/function/mechanism investigation.

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MATERIALS AND METHODS

Synthesis of cyanine-AMP conjugates

All reagents and chemicals were purchased from Aldrich and used as received. Synthetic procedures are shown in Scheme 1. Starting from 2,3,3-trimethyl-3H-indole-5-acetic acid 1 (20), the common intermediate 1,2,3,3-tetramethyl-indoleninium-5-acetate (2) was synthesized by methylation of 1 (20). Condensation of two molecules of 2 with one molecule of triethyl orthoformate (21) afforded the symmetrical red cyanine dye 3 with two free carboxyl groups that can be used for subsequent conjugation with AMP via a linker. Separately, condensation of one molecule of 1,3,3-triemethoxypropene (21) with two molecules of 2 produced another symmetrical blue cyanine dye 4 with two free carboxyl groups. The carboxyl groups of 3 and 4 were then activated by N,N'-dicyclohexylcarbodiimide (DCC) to form their corresponding NHS esters, 5 and 6. Finally, the intermediates 5 and 6 were individually coupled with 5'- (6-aminohexyl) adenosine phosphoramidate (HDAAMP) (19) to afford a pair of novel symmetrical cyanine-AMP conjugates, F550/570 and F650/670. Detailed description of the syntheses of 2–6, F550/570 and F650/670 are given below.

Synthesis of compound 2. To a 25 ml Schlenk tube was added 0.5 g (2.3 mmol) of compound 1, prepared from the published procedure (20), 1.2 ml (19.3 mmol) of iodomethane and 10 ml of acetonitrile. The reaction mixture was degassed with argon for 30 min and the tube was sealed with a cap and heated in an oil bath at 80°C for 1 h. After cooling, the reaction mixture was transferred into a flask and concentrated under vacuum to give 0.78 g (94%) of product 2.

Synthesis of compound 3. The literature procedures (20,21) were used to prepare compound 3. To 1.0 g (2.8 mmol) of compound 2, 20 ml of dry pyridine was added. While the reaction mixture was refluxing, 1.4 ml (8.4 mmol) of triethyl orthoformate was added slowly (0.4 ml per 15 min). After completion of addition, the reaction mixture was refluxed for another 2 h. Solvent was removed and the red residue was dissolved in 40 ml of methanol, followed by adding 200 ml of ethyl acetate. After concentrating to ~50 ml, another 100 ml of ethyl acetate was added, and concentrated to ~50 ml. To this suspension, 100 ml of ethyl acetate was added. The top solvent was decanted and the red residue was dried over a high vacuum to give 0.81 g (96%) of the compound 3. Mass spectrometry (MS) analysis gave the following results: C29H33N2O4+, calcd, 473.24, found 473.2 (M+).

Synthesis of compound 4. The literature procedure (21) was used to prepare compound 4. To 1.0 g (2.8 mmol) of compound 2, 28 ml of dry acetonitrile, 0.6 ml of triethylamine (TEA) and 0.2 ml of acetic acid were added. While the reaction mixture was refluxing, a solution of 1.0 g (7.6 mmol) of 1,3,3-triethoxypropene in 4.0 ml of acetonitrile was slowly added (0.5 ml per 15 min). After completion of the addition, the reaction mixture was refluxed for another 2 h. Solvent was removed and the blue/purple residue was dissolved in 40 ml of methanol, followed by adding 200 ml of ethyl acetate. After concentrating to ~50 ml, another 100 ml of ethyl acetate was added, and concentrated to ~50 ml. To this suspension, another 100 ml of ethyl acetate was added, the top solvent was decanted and the blue/purple residue was dissolved in 40 ml of methanol, followed by adding 200 ml of ethyl acetate. After concentrating to ~50 ml, another 100 ml of ethyl acetate was added, and concentrated to ~50 ml. To this suspension, another 100 ml of ethyl acetate was added, the top solvent was decanted and the red residue was dried over a high vacuum to give 0.84 g (96%) of the compound 4. The molecular peak found by MS, 499.2 (M+), is consistent with the expected formula C31H35N2O4+, 499.26.

Synthesis of 5 and 6. To 100 mg (0.16 mmol) of compound 3 or 4, 90 ml of CH2Cl2, 100 mg (0.48 mmol) of DCC, 55 mg (0.48 mmol) of NHS and 50 mg of 4-(dimethylamino)pyridine...
were added. The reaction mixture was stirred for 2 h. The urea-derivative precipitate was formed and filtered off. The filtrate was concentrated to dryness and used for the next step of coupling reaction without further purification. MS analysis gave the following results: 5—C$_{37}$H$_{39}$N$_4$O$_8^+$, calcd, 667.28, found 667.2 (M$^+$); 6—C$_{39}$H$_{41}$N$_4$O$_8^+$, calcd, 693.29, found 693.2 (M$^+$).

Synthesis and purification of F550/570 and F650/670. To a 300 µl aqueous solution of 370 mM HDAAMP (19), 150 µl TEA, 750 µl N,N-dimethylformide (DMF) and 20 mg of 5 or 6, which were dissolved separately in 150 µl DMF, were added. After 30 min of reaction, 1.2 ml of water was added to the sample. The resulting solution was filtered with a 0.2 µm syringe filter. Purification of F550/570 and F650/670 was achieved by semi-preparative reverse phase high-performance liquid chromatography (HPLC) (Figures 1 and 2). Isolated yields for F550/570 and F650/670 were ~20%. High-resolution MS analysis gave excellent results: F550/570—C$_{61}$H$_{85}$N$_{16}$O$_{14}$P$_2^+$, calcd, 1327.5901, found 1327.5869 (M$^+$); F650/670—C$_{63}$H$_{87}$N$_{16}$O$_{14}$P$_2^+$, calcd, 1353.6057, found 1353.5998 (M$^+$).

Spectroscopic properties of F550/570 and F650/670
UV absorbance spectra and molar extinction coefficients of F550/570 and F650/670 were obtained from a JASCO spectrometer (V-530) in 20 mM phosphate, pH 7.0. Fluorescence emission spectra were measured with an ISS PC1 fluorometer (Champaign, IL) in 20 mM phosphate, pH 7.0, under the...
excitation of 510 and 610 nm for F550/570 and F650/670, respectively. Quantum yields \((\Phi)\) of 3.4, F550/570 and F650/670 were determined by using reference fluorophores according to the following equation:

\[
\Phi_X = \Phi_R (SL_X/SL_R) (n_X/n_R)^2,
\]

where \(X\) and \(R\) stand for the sample and the reference, respectively. SL is the slope of integrated fluorescence area versus absorbance and \(n\) is the refractive index of the solvent. The reference for 3 and F550/570 was rhodamine 101 \((\Phi_R = 1\); Fluka) (22). Zinc phthalocyanine \((\Phi_R = 0.3\); Aldrich) (23) was used as the reference for quantum-yield measurements of 4 and F650/670. Fluorescence was measured at the excitation of 490 nm (for 3 and F550/570) or 600 nm (for 4 and F650/670).

**RNA 5’ labeling by F550/570 and F650/670**

Fluorescent labeling of 5’ RNA by F550/570 and F650/670 was performed under normal in vitro transcription conditions (18,19) with slight modifications: changing [ATP] from 1 to 0.25 mM and adding 2 mM of F550/570 or F650/670 to the transcription solution. The final transcription solution contained 40 mM Tris–HCl, pH 8.0, 5 mM dithiothreitol, 6 mM MgCl₂, 2 mM spermidine, 0.01% Triton X-100, 0.25 mM ATP, 1 mM each of UTP, GTP and CTP, 2 mM cAMP conjugates, the absorbance within 220–300 nm is typically used for fluorescein labeling of RNA by direct transcription (19), we sought to develop similar methods to site-specifically label 5’ RNA by cyanine dyes with the intention of bringing this simple and efficient RNA-labeling technique to broad applications in biosciences and biomedicine.

After two synthetic steps (Scheme 1), the two fluorescent cyanine dyes 3 and 4 were obtained in high yield (91%). After activation by DCC and NHS, the NHS esters 5 and 6 were readily coupled with the amino group of HDAAMP (19). Purification by reverse phase HPLC (Figures 1A and 2A) yielded pure (>96% purity) fluorescent dyes F550/570 and F650/670 (with ~20% total yields) as shown in Figures 1B and 2B. In addition to F550/570 and F650/670, there were other peaks containing cyanine dyes (Figures 1A and 2A). Their identities were not further investigated.

**RESULTS**

**Synthesis of F550/570 and F650/670**

Cyanine dyes (26) display some excellent fluorescent properties, including their high molar extinction coefficients and improved resistance to photobleaching (in contrast to the commonly used fluorescein). In addition, the color and solubility of cyanine dyes can be modulated by the number of double bonds between the two indole rings and by changing the attached chemical groups. Having successfully demonstrated 5’ fluorescein labeling of RNA by direct transcription (19), we sought to develop similar methods to site-specifically label 5’ RNA by cyanine dyes with the intention of bringing this simple and efficient RNA-labeling technique to broad applications in biosciences and biomedicine.

After two synthetic steps (Scheme 1), the two fluorescent cyanine dyes 3 and 4 were obtained in high yield (91%). After activation by DCC and NHS, the NHS esters 5 and 6 were readily coupled with the amino group of HDAAMP (19). Purification by reverse phase HPLC (Figures 1A and 2A) yielded pure (>96% purity) fluorescent dyes F550/570 and F650/670 (with ~20% total yields) as shown in Figures 1B and 2B. In addition to F550/570 and F650/670, there were other peaks containing cyanine dyes (Figures 1A and 2A). Their identities were not further investigated.

**UV absorbance and fluorescent properties of F550/570 and F650/670**

Ultraviolet and visible spectra of F550/570 and F650/670 (Figure 3, Ab lines) show additive contribution from both the adenosines and the cyanine cores. For both cyanine-AMP conjugates, the absorbance within 220–300 nm is
contributed mainly by the adenosine moieties. The absorbance between 450 and 580 nm of F550/570, with \( \lambda_{\text{max}} \) at 550 nm, is purely due to the cyanine dye 3 core. Similarly, for F650/670, the absorbance between 580 and 700 nm (\( \lambda_{\text{max}} = 650 \text{ nm} \)) originates from the cyanine dye 4 core. Molar extinction coefficients measured in 20 mM phosphate buffer, pH 7.0, are \( \epsilon_{550} = \sim 130 \text{ 000 M}^{-1} \text{ cm}^{-1} \) and \( \epsilon_{650} = \sim 210 \text{ 000 M}^{-1} \text{ cm}^{-1} \) for F550/570 and F650/670, respectively. Fluorescence emission spectra of F550/570 and F650/670 are marked as Em curves in Figure 3. F550/570 fluoresces between 550 and 700 nm, with emission \( \lambda_{\text{max}} = 570 \text{ nm} \). Under excitation, F650/670 emits fluorescence within 640–700 nm (\( \lambda_{\text{max}} = 670 \text{ nm} \)). Within the visible range (440–780 nm), both the absorption spectra and fluorescence emission spectra of F550/570 and F650/670 are similar to those of common cyanine dyes, Cy3 and Cy5, respectively (26). The quantum yields (from three sets of measurements) of F550/570 and F650/670 are 0.16 ± 0.03 and 0.47 ± 0.06, respectively, compared with 0.11 ± 0.03 and 0.27 ± 0.04 for their corresponding precursors 3 and 4. Therefore, the attachment of two adenosines and HDA linkers at the opposite ends of the cyanine cores increases their quantum yields, but has no apparent effects on other spectroscopic properties of the cyanine dye within the visible range.

**Figure 3.** UV–visible absorption spectra (Ab) and fluorescence emission spectra (Em) of F550/570 and F650/670. The spectra were measured in 20 mM phosphate buffer, pH 7.0. All spectra were normalized to 1 at their \( \lambda_{\text{max}} \). The \( \lambda_{\text{max}} \) difference between excitation and emission is 20 nm for both F550/570 and F650/670.

Fluorescent labeling of 5′RNA by F550/570 and F650/670

Fluorescent labeling of 5′RNA is achieved by simply including F550/570 or F650/670 in transcription solutions under the T7 class II promoter ϕ2.5 (18,19). One of the two adenosines within F550/570 or F650/670 initiates transcription, resulting in 5′RNA labeling by the cyanine dyes. Although there are two identical adenosines within F550/570 or F650/670, the probability of both adenosines initiating transcription to produce head-to-head joined RNA via F550/570 or F650/670 is low due to the high concentration ratios of F550/570 (or F650/670) over transcribed RNA molecules (i.e. mM versus μM). To confirm the prediction, purified F550/570-RNA (TES33, 32P-labeled) was added to the transcription solution in the absence of [α-32P]ATP and F550/570. No head-to-head joined RNA dimer was observed by phosphorimaging after PAGE. Because neither F550/570 nor F650/670 contains a nucleoside 5′-triphosphates, the cyanine dyes cannot be incorporated into internal RNA positions by T7 RNA polymerase. Figure 4 shows 5′RNA (TES33) labeling by F550/570 and F650/670. Three parallel transcription experiments (with [α-32P]ATP and F550/570) were carried out in the absence of the internal radiolabel) were carried out in the absence of the cyanine dyes (lane 1) or in the presence of F550/570 (lane 2) or F650/670 (lane 3). Phosphorimaging based on 32P (Figure 4A) revealed an additional shorter RNA band in lanes 2 and 3. Fluorescence scanning of the same gel under the excitation with the 532 nm green laser (Figure 4B) shows only a single RNA band in lane 2, whose location overlaps with that of the upper band of lane 2 in Figure 4A. Under the excitation of a 633 nm red laser (Figure 4C), scanning of the same gel displays another single RNA band in lane 3, whose location superimposes with that of the upper band of lane 3 in Figure 4A. Taken together, the three different scanings of the same gel based on excitation by 32P (Figure 4A), 532 nm photons (Figure 4B) and 633 nm photons (Figure 4C) indicate fluorescent labeling of RNA by F550/570 (lane 2) and F650/670 (lane 3) during transcription. The labeling yields were 60 ± 5% and 35 ± 5% for F550/570 and F650/670, respectively. Total RNA yields for F550/570- and F650/670-labeled RNA were 110 ± 30% and 90 ± 30% of the control RNA (in the absence of dye-AMP). In a different set of labeling experiments with varying RNA sizes (100–500 nt), 15 independent transcriptions gave labeling yields of 78 ± 3% and 55 ± 3% for F550/570 and F650/670, respectively. The respective total RNA yields relative to the control RNA were 150 ± 30% and 110 ± 30%. Therefore, F550/570 and F650/670 appear to stimulate transcription initiation under the transcription

**Figure 4.** RNA fluorescent labeling by F550/570 and F650/670 under the T7 ϕ2.5 promoter (18,19). All RNA was also internally 32P-labeled by [α-32P]ATP. After transcription, RNA samples were fractionated by PAGE. Lane 1, normal transcription; lanes 2 and 3, transcription in the presence of F550/570 and F650/670, respectively. (A) 32P-phosphorimaging reveals total RNA bands in different transcription experiments. (B) Scanning of the same gel under the excitation of a 532 nm laser shows only F550/570-labeled RNA. (C) Under excitation with a 633 nm laser, only F650/670-labeled RNA is visible. The RNA sequence was that of a thioester-synthesizing ribozyme TES33, 92 nt (24). RNAs from ~5 μl transcription were used for the gel.
conditions. In a typical experiment, ~20 μg of dye-labeled RNA can be prepared from 100 μl transcription.

Since no transcription-based methods would produce 100% labeled RNA, isolation of labeled RNA from unlabeled (normal) RNA may be required for its applications. Owing to their relatively large sizes, F550/570- and F650/670-labeled RNA displays significant migration retardation (5–6 nt difference) by PAGE. This added property of F550/570- and F650/670-labeled RNA may be exploited to achieve high purity levels of fluorescent RNA by PAGE. To establish the RNA size-PAGE resolution relationship, five different sizes of RNA (100, 200, 300, 400 and 500 nt) were labeled by both 32P and F550/570 (F650/670) and run 30 cm on a large sequencing gel (42 × 35 × 0.08 cm²). As shown in Figure 5A, gel-running time varied from ~4 h for an RNA of 100 nt to ~18 h for the 500 nt RNA. Separation between dye-labeled RNA and unlabeled RNA ranged from 12 to 3 mm when RNA increased from 100 to 500 nt (Figure 5B). Therefore, F550/570- and F650/670-labeled RNA (up to 500 nt, depending on the skill of the experimenter) can be separated from unlabeled RNA by 8% PAGE. In one experiment, we purified an F550/570-labeled 120-nt RNA to >95% purity using 8% denaturing PAGE (42 × 35 × 0.08 cm²) after running for 4 h at 45 V/cm.

Figure 5. Resolution of dye-labeled RNA of different sizes by 8% PAGE (42 × 35 × 0.08 cm²). Each RNA was run 30 cm from the origin under either constant voltage or constant wattage. (A) The relationship of RNA size and gel-running time under two different conditions. (B) Relative electrophoretic mobility of different RNA sizes to xylene cyanol (XC) and resolution between dye-labeled RNA and unlabeled RNA.

DISCUSSION
Our approach for direct fluorescent labeling of RNA by F550/570 and F650/670 during transcription offers the following distinct advantages over the phosphoramidite chemistry method and post-transcriptional fluorescent labeling. First, site-specific RNA fluorescent labeling is achieved in a single step of transcription, greatly reducing the RNA sample handling time and RNA loss. Typically, fluorophore-labeled RNA can be made available in common laboratories within 2–4 h. Second, there is no apparent RNA size restriction; fluorescently labeled small and large RNAs can be prepared by the same transcription method with similar yields, purities and costs. The commonly used in vitro transcription under the T7 66.5 promoter produces G-initiated RNAs. The same RNA sequences can be used for fluorescence labeling under
the T7 62.5 promoter by adding an extra A before the first G. Third, F550/570 and F650/670 are chemically stable, allowing laboratories to maintain steady stocks without constant purchase of fresh supplies. The current finding will make site-specifically fluorophore-labeled RNA readily available for a variety of applications in biochemistry, structural biology and nucleic acids-based clinical diagnostics. In particular, fluorophore-labeled large RNAs prepared by the current method may be appealing to biochemists and structural biologists to investigate RNA structure, folding and mechanism by various fluorescence techniques such as fluorescence resonance energy transfer (6–9,13) and single-molecule kinetics (10,15,30). Without an apparent restriction on RNA sequence (except for the 5' AG) and size, the described fluorophore labeling by F550/570 and F650/670 can be easily applied to large RNAs such as the group I and group II introns, the RNase P RNA, spliceosomal RNAs, ribosomal RNAs and artificially selected functional RNAs (aptamers and ribozymes).

PNK-catalyzed phosphorylation by [γ-32P]ATP is a common procedure to prepare [5',32P]RNA that is required in various studies of structure/function/mechanism (27–29). However, such a 32P-based RNA labeling method may present a series of problems for the experimenter. First, 32P radioactivity quickly diminishes with time due to its short half-life. In addition, RNA labeling efficiency by [γ-32P]ATP actually decreases much faster than the radiodecay of 32P, probably due to ATP damage caused by strong 32P radioactivity. Therefore, usable [γ-32P]ATP has an even shorter half-life (<14 days). Frequent supply of fresh [γ-32P]ATP is necessary for efficient 5' RNA labeling by 32P and PNK. Unless it is used by several experimenters in a sizable laboratory or shared among different laboratories, a substantial amount of [γ-32P]ATP is wasted due to its fast radiodecay. Second, [5',32P]RNA may slowly lose its function during storage as a result of structural damage by its 32P radioactivity. Accordingly, it may be necessary to frequently prepare freshly 32P-labeled RNA before a previous preparation is fully

![Figure 6. Ribozyme reaction site mapping by F550/570-labeled RNA. RNA from 10 μl transcription was used for the analysis. (A) The secondary structure of ACT3 (25). It is difficult to label the 5' end by [γ-32P]ATP and PNK due to its recessed 5' end. (B) The secondary structure of ACT3 (25). It is difficult to label the 5' end by [γ-32P]ATP and PNK due to its recessed 5' end. (B) PAGE analysis of Neutravidin column-eluted RNA fragments (lane 1), along with an RNase T1-digested ladder of the same F550/570-labeled RNA. RNA fragments were generated by partial lead hydrolysis of biocytinCoA-reacted RNA (F550/570-labeled). After electrophoresis, the gel was scanned directly by an Amersham Typhoon phosphorimager under the excitation of a 532 nm laser. (C) Fluorescence intensity profile of lane 1 in (B). (D) An illustration of the reaction site mapping. Controlled lead-induced RNA hydrolysis randomly cuts the RNA to all possible fragments, except for the reactive site where the 2' OH is blocked by biocytin. The eluted RNA sample from Neutravidin chromatography contains all biocytin-tagged fragments, C51 and above. None of the untagged RNA fragments (U49 and below) is retained by the column. Therefore, PAGE analysis and F550/570-based phosphorimaging can detect all RNA fragments equal to or longer than C51. The reaction site is one nucleotide below C51, i.e. U50.](http://nar.oxfordjournals.org/Downloaded from http://nar.oxfordjournals.org/ at The University of Southern Mississippi on October 13, 2016)
consumed. Third, some RNA (such as the one shown in Figure 6A) may present difficulties for efficient labeling by PNK and [γ-32P]ATP. Finally, 32P is a source of radio-hazard. Its use requires user training and strict workplace safety regulations.

Our newly developed F550/570 and F650/670 offer solutions to the above [γ-32P]ATP-based RNA labeling problems. Regardless of RNA structure, the 5′ end of RNA can be readily labeled by F550/570 and F650/670, if the RNA has AG at its 5′ end. If the subject RNA does not possess a 5′ AG sequence, AG can be added to the 5′ end for the purpose of facilitating 5′ fluorescent labeling by F550/570 or F650/670. With phosphorimagers/fluorimagers becoming widely accessible, the RNA-labeling advantages offered by F550/570 and F650/670 over traditional 32P-based methods may be fully realized.

The cyanine dye-AMP conjugates F550/570 and F650/670 described in this report have been made available by AdeGenix Inc. (Monrovia, CA). Detailed application notes can be found at www.adegenix.com.

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