Spectroscopic Analyses of the Noncovalent Self-Assembly of Cyanines upon Various Nucleic Acid Scaffolds

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We utilized self-assembly of cyanine chromophores to study the conformational changes in various types of nucleic acid scaffolds: single and double stranded DNA, linear or circular DNA and RNA. We identified a chromophore that became highly fluorescent after aggregating upon nucleic acids. Fluorescence from the aggregate was instantaneous after self-assembly. Temporal emission profiles displayed a biphasic trend demonstrating kinetic dependence for assembly and disassembly. Absorption spectra of the aggregate showed a red-shifted “shoulder” peak indicative of J-aggregate. Fluorescence from J-aggregates was also red-shifted. We utilized cyanine self-assembly to quantize various nucleic acids. The limits of detection and quantization for ϕ X174 DNA were 3 and 9 fmol, respectively. We similarly determined the sensitivity for various nucleic acids and established the optimum conditions for self-assembly. Collectively, the effects of methanol, salt, and full width at half maximum for cyanine fluorescence on DNA or carboxymethylamylose scaffolds, all suggested noncovalent, electrostatic, and hydrophobic forces were involved in supramolecular self-assembly. Our results facilitate a better understanding of supramolecular self-assembly.

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Noncovalent forces are involved in the supramolecular self-assembly.^{1–4} We previously showed that a class of chromophores known as cyanines were capable of spontaneously self-assembling upon a variety of biopolymers including carbohydrates.^{5–8} Cyanines are photosensitive dyes composed of two quaternized, nitrogen-containing, and heterocyclic ring structures, that are linked by a polymethine bridge.⁹ Cyanines are characterized by high molar absorptivity, low intrinsic fluorescence and large fluorescence enhancement following self-assembly upon various templates. Supramolecular self-assembly involves reversible, noncovalent, and electrostatic (coulombic) interactions including hydrogen and/or charge bonding, van der Waals and hydrophobic forces. Cyanine self-assembly is sometimes accompanied by strong, sharp and bathochromic fluorescence, characteristic of J-aggregates.¹⁰ Cyanines are also capable of forming hypsochromic, non-fluorescent or very weakly fluorescent H-aggregates.¹⁰ Controlling and tuning the supramolecular self-assembly processes will offer insights to enable programmability to achieve desired functional nanomaterials. We therefore further investigated the self-assembling of cyanines from our molecular “library” in order to identify a chromophore that was capable of forming intensely fluorescent J-aggregates.

Our objectives were: 1) Identify cyanine chromophores from our molecular library that would be a sensitive probe for nucleic acids; 2) Correlate the form-function aspects of cyanine chemistry to self-assembly on nucleic acid scaffolds; 3) Compare the detection limits of single-stranded viral DNA, double-stranded viral DNA, double-stranded genomic DNA, and RNA, using self-assembly; 4) Optimize detection conditions for nucleic acids based upon supramolecular self-assembly

including the stability of the nucleic acids-cyanine complex; and 5) Demonstrate supramolecular self-assembly processes were involved in the aggregation of the cyanine upon nucleic acid scaffolds. Results from these investigations are described in this paper.

Experimental

Reagents and chemicals

The chemical structures of various cyanines from our molecular “library”^{5,11} are shown in Fig. 1. Stock solutions of cyanines were prepared in methanol and stored refrigerated. Single stranded, circular, ϕ X174 virion DNA (5386 bases, $M_r = 1.7 \times 10^6$ Da) from ϕ X174 *am3 cs70* bacteriophage was purchased from New England Biolabs (Ipswich, MA). Greater than 85% of the DNA molecules were circular. The double stranded bacteriophage λ DNA composed of 48502 bp ($M_r = 3.2 \times 10^7$ Da) was from Promega (Madison, WI). Unsheared *Escherichia coli* (*E. coli*) genomic DNA with an average size of 16 kb ($M_r = 1.4 \times 10^7$ Da) was purchased from Sigma Aldrich (St. Louis, MO). The DNA had been purified by equilibrium buoyant density gradient ultracentrifugation in CsCl and agarose gel (0.8%) electrophoresis was used for size determination. Genomic DNA was dissolved using TE buffer (10 mM Tris-HCl, pH 7.5, 1 mM NaCl and 1 mM EDTA). The DNA solution was stored in small aliquots at -80°C and discarded after a single use. *Saccharomyces cerevisiae* (*S. cerevisiae*, baker’s yeast) RNA (~5.3 kb, 26 S, 17 S and 5.8 S; $M_r = 18 \times 10^5$ Da) was from Sigma.

Absorption spectra

Absorption measurements were taken using quartz polymethylmethacrylate cuvette (4 mL), quartz cuvette (600 μL) or polystyrene 96-microwell plates (200 μL). Cyanine

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Fig. 1 Molecular structures of the cyanines. The molecular masses of the cyanines are shown in the figure. The four cyanines were obtained in the following salt forms I^- , Br^- , PTS^- and PTS^- and had the following molecular masses: 535.3, 445.5, 605.6 and 696.5, respectively. The absorption maxima (excitation wavelength, λ) were respectively, 435, 555, 561 and 514 nm.

concentration was 10 μM final. The nucleic acids were titrated into the cyanine solution in incremental amounts with gentle mixing before taking measurements. As negative controls, absorption sweeps of “vehicle” (*i.e.*, 20% methanol and 80% water, v/v) and 10 μM cyanine dissolved in vehicle were carried out. Measurements were taken using Molecular Devices (Sunnyvale, CA) instrument equipped with a cuvette holder or in the microwell plate reader. The volume change during titrations was approximately 7% of the total volume (V_t). Consequently, no corrections were made for volume changes.

Fluorescence measurements

The cyanines were diluted using a mixture of methanol-water (20/80%, v/v). Nucleic acid solutions were also made in the same solvent. Fluorescence was measured at ambient temperature ($\sim 25^\circ C$) using a Mithras LB 940 microplate spectrofluorometer (Berthold Instruments, Oak Ridge, TN).^{5,6} The filters for instrument were purchased from Chroma Technology (Rockingham, VT). In certain experiments, a monochromator-based microplate reader (Molecular Devices) was employed to measure excitation and emission. The reaction solutions (200 μL) were dispensed in a 96-well white microplate (Perkin Elmer, Waltham, MA). Fluorescence from the J-aggregate was corrected for a small amount of intrinsic fluorescence of the cyanine monomer (<10% of maximal fluorescence of J-aggregate). Certain measurements were taken using an ultra-micro quartz cuvette (350 μL) with a FluoroMax-2 spectrofluorometer (Horiba Jobin Yvon, Edison, NJ). Fluorescence spectra were taken under conditions similar to absorption measurements. Emission scans obtained using a quartz cuvette or microplate wells were similar thus assuring validity of the data. The volume change during titrations was <10% of the total volume (V_t). Consequently, no corrections were made for volume changes.

Methanol concentration effects upon self-assembly

Each nucleic acid was tested at 25 and 50 ng. Fluorescence was measured immediately (1 to 2 min) and after 30 min at $\sim 25^\circ C$ to allow for self-assembly kinetics. Background fluorescence from 10 μM cyanine mixed with increasing concentrations of methanol was determined. The type and concentration of the scaffold ($\phi X174$ DNA, λ DNA, genomic DNA or RNA) and self-assembly kinetics influenced emission intensity. We therefore used changes in signal/background (S/B)

as an index of the effects of methanol upon cyanine self-assembly.¹² We normalized the data as % changes to S/B and compared the effects of methanol upon the four nucleic acid scaffolds.

Salt concentration effects upon self-assembly

We examined the effects of NaCl on J-aggregate fluorescence following cyanine self-assembly upon the nucleic acid scaffolds as described above for methanol.

Self assembly kinetics and stability of the self-assembled ensemble

Either 5 or 10 μM cyanine was collected upon 15 ng of $\phi X174$ DNA, 15 ng λ DNA, 15 ng *E. coli* genomic DNA or 25 ng *S. cerevisiae* RNA. Fluorescence emission was detected immediately (within 1 min) after mixing cyanine and the nucleic acids, by exciting the samples at 427 nm and measuring the emission at 470 nm. As negative control, fluorescence from six replicates of 5 and 10 μM cyanine was also measured. Timed reactions were carried out in two separate ways. In the first, the same reaction wells were excited repeatedly over time and the fluorescence emission was measured. In the second method, dedicated reaction wells were maintained for each time point. In the latter technique, reaction mixtures were excited only once and the fluorescence emission was measured. Results are presented as percent changes to S/B as described above.

Data analysis

All data were collected in triplicate. Results are average $\pm$ standard deviation. Where not visible, error bars are masked within the symbol. Curve fitting was done using Kaleidagraph (Synergy Software, Reading, PA). The increase in fluorescence emission over various controls (cyanine “alone”, nucleic acids “alone”, vehicle “alone” and empty wells) was measured. The corrected signal was traced as relative fluorescence units (RFU).^{5,6} Limits of detection (LOD) and quantization (LOQ) were calculated as described previously.¹³

Results and Discussion

Cyanine chemistry and spectral characterization

We investigated the potential for J-aggregate formation of the various cyanines using nucleic acids as a template. The molecular structures and properties of four cyanines are shown in Fig. 1. Among these, the cyanines designated 1 and 4 were capable of forming fluorescent J-aggregates and we therefore focused on these chromophores. Since we had access to 2 and 4, we tested these as well. Until now, there is no clear understanding of the relationship between cyanine chemistry and J-aggregation potential. By testing the four cyanines with subtle differences in their structures, three out of four had the electron withdrawing chlorine substituent (Fig. 1), we hoped to better understand such relationships (*vide infra*). In order to characterize the fluorescence, we tested the cyanines using all combinations of the following filter sets: excitation filters: 320, 355, 400, 485 and 650 nm; emission filters: 400, 460, 480, 515, 530, 535 and 685 nm.

Cyanines 1 and 4 yielded moderate-to-high fluorescence as well as large S/B after self-assembly upon the various nucleic acid scaffolds. Results for 1 were obtained using excitation and emission filters 400 and 460 nm, respectively. These data are in accord with the absorption maximum for 1 (Fig. 2A). In the absence of scaffold, 1 had an absorption maximum at 425 nm. Titration with $\phi X174$ DNA resulted in a progressive decrease of the absorption of 1 monomer at 425 nm along with the appearance of a new “shoulder” peak of between 445 and 480

Fig. 2 (A) Absorption spectra of cyanine **1** titrated with increasing amounts of ϕ X174 DNA. Increasing amounts of the DNA were mixed with 10 μ M of **1**. The tracings represent decreasing absorption intensities at 425 nm upon adding the following amounts of ϕ X174 DNA: 1.3, 3.8, 8.8, 18.8, 43.8, 93.8, 143.8, and 310.8 ng DNA. The topmost solid line with the highest absorption represents the spectra of cyanine **1** in the absence of DNA. The tracings hugging the abscissa were from vehicle. (B) Absorption spectra of cyanine **4** titrated with increasing amounts of *Saccharomyces cerevisiae* (baker's yeast) RNA. Increasing amounts of the RNA were mixed with 10 μ M of cyanine **4** in a total volume of 4 mL of a mixture of 20% methanol and 80% water (v/v). The tracings represent changes in the absorption profiles upon the addition of the following amounts of RNA: 90, 180, 270, 500, 1000, 2000, 5000 and 10000 ng RNA. Both the absorption profiles of cyanine **4** (left profiles) and the J-aggregate absorption profiles (right) are shown in this figure.

nm, reflecting the bathochromicity that is characteristic of J-aggregates (Fig. 2A).¹⁰ The absorption profiles of **1** with four different nucleic acid scaffolds were similar (data not shown). Cyanines **2** and **3** did not exhibit measurable fluorescence in the presence or absence of RNA. For these compounds there was also no development of the characteristic J-aggregate absorption at wavelengths red-shifted from the monomer. For **2** there was a monotonic decrease in absorption (ascribed to monomer) as increasing amounts of RNA were added; this observation is consistent with formation of a colloidal aggregate which may separate from the solution and either precipitate or adsorb on the cuvette walls. For **3**, the monomer absorption at 561 nm decreased with addition of increasing amounts of RNA. There was also a small growth in absorption both to longer and shorter

wavelengths which may be attributed to the development of an "H" aggregate;¹⁰ the presence of isosbestic points on either side of the cyanine monomer absorption suggests that the new species remains in solution during the addition of RNA. The absorption spectrum of **4** shows clear development of J-aggregate upon addition of RNA (Fig. 2B); accompanying the change in absorption was an onset of fluorescence. The fluorescence occurred at similar wavelengths to those observed in other studies for the J-aggregate of **4** self-assembled on scaffolds such as Laponite clay.¹¹

Cyanine dyes were shown to interact with DNA through several modes of binding (intercalation, groove binding and supramolecular self-assembly).⁸ Cyanine **2** has a structure that is chemically similar to **1**, except for the methine bridge being two carbon atoms longer in **2** and the dichloro substituent of **1**. Likewise, **1** and **3** are also identical except for the extra two carbons in the methine bridge of **3**. Cyanine **4** has a similar conjugation length to **2** and **3** but differs in heterocycle structure and thus is not closely related structurally. These relatively small differences in chemical structures produced >100 nm shifts in absorption maxima (excitation wavelength) and also resulted in the chromophores becoming either fluorescent J-aggregates (**1** and **4**) or aggregates that were weakly or non-fluorescent (**2** and **3**). It was suggested that charged substituents on the dye could be used to control the overall structure of the J-aggregate on DNA templates.¹⁴ Since these are substituted cyanines (Fig. 1), J-aggregation may be favored due to steric and/or electrostatic interactions.⁸ From the absorption profiles (Fig. 2A), it appeared that there was only a small amount of conversion of **1** to J-aggregate. While **4** showed the most clear-cut conversion to J-aggregate among the four dyes (Fig. 2B), the fluorescence of the J-aggregate was not nearly as strong as that of the various **1**-nucleic acid complexes. Remarkably, the relatively small conversion of **1** to J-aggregates suggested by the small peak developing at 480 nm in the absorption spectra (Fig. 2A), compared to the much higher levels of J-aggregate formed when the same cyanine **1** self-assembled on a carbohydrate scaffold of carboxymethyl amylose,¹⁵ nevertheless resulted in strong J-aggregate fluorescence for the **1**-nucleic acid complex (Fig. 3). This suggested that the [1-nucleic acid] complex must have high quantum efficiency. Consequently, we focused on the details of the complexes formed between **1** and the different nucleic acids.

Detector flexibility

Experiments with a monochromator detector verified that the absorption maximum for **1** was 425 nm (Fig. 2A) and the fluorescence emission was maximal at 470 nm for the **1**- ϕ X174 DNA J-aggregate (Fig. 3). Similar results were obtained when emission spectra were collected using quartz cuvette and FluoroMax-2 fluorometer. Experiments involving **1** with the filter-based microplate reader were carried out using 400 and 460 nm excitation and emission filters, respectively. With the monochromator-detector, the excitation wavelength was set between 425 and 427 nm, usually 426 nm (ex). Fluorescence emission was monitored at 470 nm (em). We similarly optimized the absorption and emission for **1** with each of the remaining nucleic acids: λ DNA, *E. coli* DNA and *S. cerevisiae* RNA. Absorption and emission data for **1** and the various nucleic acid scaffolds were nearly identical to those shown in Figs. 2A and 3, respectively. With all scaffolds, the dominant absorption maximum for **1** monomer and **1** aggregating upon the nucleic acid templates was 425 nm along with the "shoulder" peak at 445–480 nm (Fig. 2A). The emission peak was broad in all cases, nearly 20 nm wide (463 to 486 nm), with a peak at

Fig. 3 Fluorescence emission spectra of cyanine **1** titrated with increasing amounts of ϕ X174 DNA. Increasing amounts of DNA were mixed with 10 μ M of **1** in a total volume of 100 μ L of a mixture of 20% methanol and 80% water (v/v). The tracings represent the increases in the emission intensity upon adding the following amounts of ϕ X174 DNA: 1, 5, 10, 30, 50, 100, 200 and 300 ng DNA. The topmost solid line represents fluorescence intensity in presence of 300 ng DNA. The remaining tracings below represent the fluorescent intensities obtained with progressively decreasing amounts of DNA.

470 nm (Fig. 3), representing a Stokes shift of 45 nm compared to **1** monomer peak absorption. At 100 ng of each nucleic acid scaffold, the emission intensities of **1** J-aggregates were similar, being 1.5 to 3.0 $\times 10^4$ RFU. System flexibility was thus demonstrated by generating similar data with the three different types of instruments (two different microplate readers and a fluorometer).

Evidence for supramolecular self-assembly of cyanine **1** on nucleic acid scaffolds

It was previously reported that methanol inhibited the aggregation of cyanine dyes by disrupting the electrostatic and hydrophobic interactions.¹⁴ Salt (NaCl) was also found to influence the aggregation of cyanine dyes on DNA scaffolds in a concentration- and temperature-dependent manner.¹⁴ It is clear from Fig. 4, that 20%(v/v) methanol is both necessary and sufficient for maximal fluorescence from **1** J-aggregate. The increase in fluorescence between 5 to 20% methanol might be due to increased solubility of the cyanine in the solvent, thus enhancing J-aggregate formation. On the other hand, higher concentrations of methanol inhibited the self-assembly by disrupting the hydrophobic interactions between the chromophore and the scaffold. It is also possible that at high concentrations of methanol, the nucleic acids might be coming out of solution, thereby disrupting or decreasing the amount of J-aggregate, resulting in fluorescence loss. This manifested in the biphasic curve shown in Fig. 4. The concentration of methanol to effect 50% reduction in fluorescence intensity (IC_{50}) was approximately the same for all four nucleic acid scaffolds, being 30–35% (v/v).

With the exception of RNA, salt uniformly inhibited self-assembly and decreased the fluorescence intensity (Fig. 5). Unlike methanol profiles (Fig. 4), the strength of NaCl inhibition was different with different scaffolds (Fig. 5). Interaction between **1** and λ DNA was most sensitive to ionic strength, whereas interactions of **1** with the other three nucleic acid scaffolds were more resistant to salt (Fig. 5). The NaCl concentrations for 50% reduction in fluorescence intensity (IC_{50}) with the nucleic acid scaffolds were: λ DNA = 10 mM; ϕ X174

Fig. 4 Effects of methanol concentration on the self-assembly of cyanine **1**. The tracing with open circles is ϕ X174 DNA, closed circles λ DNA, open squares *Escherichia coli* genomic DNA and closed squares *Saccharomyces cerevisiae* RNA.

DNA and genomic DNA = 100 mM; and RNA = 75 mM. The dose-response profile of ϕ X174 DNA (double stranded, circular DNA) and *E. coli* genomic DNA (linear, double stranded DNA) was nearly identical (Fig. 5). Salt effects could be rank-ordered as follows (least-to-most susceptible): ϕ X174 DNA = genomic DNA > RNA > λ DNA. The effect upon RNA was a departure from the rest of the nucleic acids, since it required ~ 25 mM NaCl for maximum fluorescence, declining thereafter. On the other hand, there was no stimulatory effect of NaCl upon the aggregation of **1** with the remaining nucleic acid scaffolds (Fig. 5).

The salt permits neutralization of the cationic charge density of the aggregate and allows electrostatic screening between the anionic nucleic acids and the cationic dye.¹⁴ Unlike earlier reports,^{16,17} we excluded a direct inhibitory effect of NaCl upon fluorescence, due to different perturbations of the ensembles (Fig. 5). Consistent with the data of Fang *et al.*,¹⁷ some decrease in *S/B* was due to an increase in intrinsic fluorescence of monomeric **1** in the presence of NaCl. The salt and methanol sensitivities of λ DNA suggested a self-assembly mechanism based upon electrostatic (coulombic) and hydrophobic forces. With the remaining nucleic acid scaffolds, both self-assembly and intercalation modes of interactions might be operative. Considering the effects of methanol and NaCl together, we speculate that non-covalent, reversible, and supramolecular self-assembly is the dominant mechanism without excluding a small extent of intercalation taking place between **1** and the various nucleic acids.

Changes in temperature and ionic strength were crucial for inter-conversion between fluorescent J-aggregate and non-fluorescent or minimally fluorescent H-aggregate.¹⁴ As reported previously, cyanine exists as monomer, H-aggregate and J-aggregate depending upon the temperature. Between the temperature range of 5 and 35°C, cyanines formed J-aggregates predominantly.¹⁴ Thermal stability of the J-aggregate was also dependent on the dye concentration.¹⁴ Since our experiments were conducted at 22°C, within the window of J-aggregate-forming temperatures, and due to the high dye-to-DNA ratio employed in our studies (*vide infra*), we believe that **1** was undergoing supramolecular self-assembly upon the nucleic acid scaffolds.

The full width at half maximum (FWHM) can be used to compare the spectral profiles obtained under different conditions. We obtained the fluorescence spectra for **1** self-assembling upon ϕ X174 DNA and carboxymethylamylose

Fig. 5 Effects of salt concentration on the self-assembly of cyanine **1**. The tracing with open circles is ϕ X174 DNA, closed circles λ DNA, open squares *Escherichia coli* genomic DNA and closed squares *Saccharomyces cerevisiae* RNA.

(CMA) templates. Based upon spectroscopic and circular dichroism data, we previously demonstrated that supramolecular self-assembly was involved in the interaction of **1** and CMA.¹⁵ We found that the FWHM values for the fluorescence emission of **1**-CMA and **1**- ϕ X174 DNA ensembles were nearly identical (~ 19 nm). Taken together, our data suggested that supramolecular self-assembly was involved in the interactions of **1** with nucleic acids.

Cyanine **1** optimization for detecting nucleic acids

The optimum concentration for detecting and quantizing ϕ X174 virion DNA was 2.5 – 5 μ M of **1** (Fig. 6). Similar biphasic trends were observed during dose-response titrations with the other nucleic acids, where the optimum concentration of **1** was 5 – 10 μ M. This high dye-to-DNA is characteristic as reported previously⁸ and is similar to the 5 μ M concentration of a different cyanine optimized for detecting nucleic acids by Fang *et al.*¹⁷ As noted above, aggregation is influenced by several parameters including the type and concentration of the nucleic acid and the dye, ionic strength, pH and temperature.¹⁴ A biphasic curve was observed when **1** self-assembled upon hyaluronic acid.⁶ Two reasons might contribute to the decline in fluorescence with increasing concentrations of **1** (Fig. 6). The first is the “inner filter” effect arising from re-absorption of the emitted radiation perhaps by the scaffold. Inner filter effect may also arise from high concentrations of the absorbing chromophore. Therefore, the intensity of excitation light is not constant throughout the dye-DNA ensemble. Consequently, only a small portion of the excitation light reaches the ensemble, leading to diminished photon emission. A second possibility might be the perturbation of the J-aggregate at high ratios of **1** to scaffold.⁶ Between 100 to 500 μ M of **1**, “self-absorption” (self-quenching) effect became prominent (Fig. 6).

DNA quantization using cyanine **1**

We next constructed a calibration curve of ϕ X174 DNA with 5 μ M of **1** (Fig. 7). We calculated limit of detection (LOD) of 3 fmol DNA and a limit of quantitation (LOQ) of 9 fmol (30 and 90 pM; 50 and 150 ppb, respectively). Linearity of dose-response and the overlap between data and curve fit were good (Fig. 7). The sensitivity for single stranded DNA were comparable to those reported using double stranded DNA, where detection limits are usually lower.¹⁸ Our assay can be miniaturized to ultra-high-throughput screening (uHTS) with

Fig. 6 Optimization of cyanine **1** concentration for the detection and quantization of ϕ X174 DNA. Increasing concentrations of **1** were mixed with 450 pM ϕ X174 virion DNA in a total volume of 100 μ L (45 fmol DNA). Other details are described under the Experimental section of the paper.

Fig. 7 Quantization of ϕ X174 DNA using the supramolecular self-assembly of cyanine **1**. Increasing amounts of ϕ X174 DNA were reacted with 5 μ M **1**. The closed circles data were collected with 355 nm (ex) and 460 nm (em) filters (left, Y-1 axis). The open diamonds data were obtained with 400 nm (ex) and 460 nm (em) filters (right, Y-2 axis). The cross symbols data were obtained using the monochromator-based instrument with 426 nm (ex) and 470 nm (em) (left, Y-1 axis). Linearity (r^2) of the dose-response curves were: open diamonds, 0.99192; closed circles, 0.99830; crosses, 0.99675.

1536-wells using the principles described by us previously.¹² The sensitivity for other nucleic acids ranged between 25 to 50 ng/mL (LOD) and 75 to 150 ng/mL (LOQ), with the exception of RNA, where LOQ was 250 ng/mL. Whereas **1** was equally sensitive toward single stranded, double stranded, linear and circular DNA, it was less sensitive for RNA. The reasons for this may relate to structural differences of the chromophore and/or the scaffold (*vide supra*).

As summarized by Skripinets *et al.*,¹⁹ PicoGreen was the most sensitive with a detection limit of 0.25 ng/mL DNA, followed by ethidium bromide (10 ng/mL), methylene blue (30 ng/mL), and finally, Tb(III)-1,10-phenanthroline (100 ng/mL). A microplate assay utilizing SYBR Green I reported an LOD of 2000 ng/mL for λ DNA²⁰ relative to our LOD of 25 – 50 ng/mL.

Fig. 8 Kinetics of the supramolecular self-assembly of cyanine 1. Cyanine 1 was mixed with *S. cerevisiae* RNA (closed squares), ϕ X174 DNA (open circles), λ DNA (closed circles) and *E. coli* genomic DNA (open squares). Changes to the fluorescence intensity were measured over time.

Huang *et al.*,²¹ reported LOD of 13 to 60 ng/mL for calf thymus DNA, fish sperm DNA and yeast RNA. They also reported LOD of 84 – 99 ng/mL for the same nucleic acids while using a copper-phenol method.²² More recently, Fang *et al.*,¹⁷ reported LOD of 3 – 3.5 ng/mL using a cyanine dye to detect DNA by light scattering. These reports constitute the benchmarks for our assay.

Self-assembly kinetics and photostability

Results from the reaction wells that were repeatedly excited and wells that were dedicated for each time point were similar. However, due to a doubling of the background during multiple excitation/emission cycles, the *S/B* was reduced. In order to segregate the photostability of the dye and the ensemble, only the data obtained from reaction wells that were interrogated for a single time point was used for analysis. Fluorescence was detected immediately upon mixing dye and the scaffold (<1 min) (Fig. 8). All the scaffolds required at least 15 min for optimum self-assembly. Highest increase in *S/B* was noted for RNA resulting in nearly 4-fold brighter fluorescence. Other nucleic acid scaffolds showed an increase in *S/B* over the 15 min window, albeit to lower extents. After 15 min, there was a progressive decline in the emission, resulting in a biphasic temporal curve (Fig. 8). At 2 h, DNA-dye ensemble fluorescence dropped steeply reaching levels that were less than the initial fluorescence. By contrast, at 2 h the RNA-dye ensemble was twice as bright compared to $t = 1$ min. These data are indicative of the relative stabilities of the various ensembles and hint at the assembly-disassembly processes taking place during dye-nucleic acid interactions. The increase in fluorescence intensity over time from the ensembles was rank ordered as follows: RNA > genomic DNA > λ DNA > ϕ X174 DNA (Fig. 8).

Conclusions

In summary, we describe the supramolecular self-assembly of a highly fluorescent cyanine J-aggregate upon various nucleic acid scaffolds. We describe several factors driving the assembly and dis-assembly of the cyanine. We present evidence for the non-covalent, reversible, electrostatic (coulombic) and hydrophobic interactions that are involved in supramolecular self-assembly. We used the fluorescence from the J-aggregate to detect and quantize various types of nucleic acids. We suspect that the

binding of cyanine to the various nucleic acid scaffolds involves subtle changes to the conformation of the nucleic acids and also the existence of a competition between the chromophore and other nucleic acid-binding ligands such as polyamines.²³ Studies are in progress to investigate these aspects modulating the self-assembly.

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