Supporting information: Förster resonance energy transfer within the Neomycin aptamer

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Material and methods

Oligonucleotide synthesis. The synthesis of the phosphoramidite of **$Ç_m^f$** [1] was prepared as previously described, and the phosphoramidite used for incorporation of **tC_{nitro}** was purchased from Glen Research. Synthesis and purification of the labeled N1-aptamers was performed according to published procedures.[2]

Sample preparation. The neomycin aptamer was dissolved in a solution of 100 mM NaCl and 20 mM sodium cacodylate buffer at pH 7.4. Static as well as time-resolved fluorescence measurements were performed with an aptamer concentration of 1 µM (without neomycin: -Neo). For the experiments that included the ligand, neomycin was added in a 4-fold excess (4 µM: +Neo). The aptamer was prefolded prior to measurements: The aptamer solution was heated and held at 90 °C for 5 min and then cooled to room temperature for 30 min.

Steady state measurements. Static fluorescence measurements were recorded in a 10 x 2 mm UV-degree quartz cuvette using a JASCO FP 8500 fluorescence spectrometer. Offset, absorption, reabsorption, excitation, and detector corrections were performed for the obtained data.

Time-correlated single photon counting (TCSPC). The TCSPC equipment was composed of three parts: the laser diode driver PDL800-D (PicoQuant), the spectrometer FluoTime100 (FT100, PicoQuant) and the counting card TimeHarp260 Pico (TH260 P, PicoQuant). The repetition frequency range was set to 10 MHz. A pulsed LED with a FWHM of 600 ps was used for excitation at a constant wavelength of 360 nm (PLS360). The UVB390 filter was used to block the excitation light from detection.

Isothermal titration calorimetry (ITC). An iTC200 microcalorimeter was used for the ITC experiments. The sample compartment was filled with a solution of the RNA (10 µM RNA, 20 mM sodium cacodylate, 200 mM NaCl, pH 7.4). The injection syringe was filled with a neomycin solution (100 µM neomycin, 20 mM sodium cacodylate, 200 mM NaCl, pH 7.4). Before the experiment, the system was equilibrated at 20 °C for 10 min. The measurement started after an initial delay of 120 s and a 0.2 µL injection. Subsequently, 20 injections of 2 µL each were applied with a time interval of 180 s while the sample chamber was stirred at 750 rpm.

UV/vis pump-probe transient absorption experiments. The time-resolved transient absorption data were recorded with a home-built pump-probe setup, which has been described in detail elsewhere.[3] The ultra-short fundamental pulses of about 150 fs were generated by a Ti:Sa amplifier system (Clark, MXR-CPA-iSeries, repetition rate of 1 kHz, 775 nm). The excitation wavelength of 388 nm was generated by a frequency doubling (SHG) the laser fundamental (110 nJ, 220 fs). The white light pulse for detection (410 to 650 nm) was generated by focusing the laser fundamental into a CaF_2 crystal of 5 mm thickness. The pulse was split into a reference beam and a sample beam. Both white light pulses were then detected in two separate spectrographs (AMKO multimode). All experiments were performed under magic angle conditions (54.7° angle difference of pump and probe pulse polarization) to avoid anisotropy effects. Finally, the sample was measured in a fused silica cuvette with a path length of 1 mm, constantly moving in the plane perpendicular to the direction of beam propagation to prevent photodegradation.

Isothermal titration calometry (ITC)

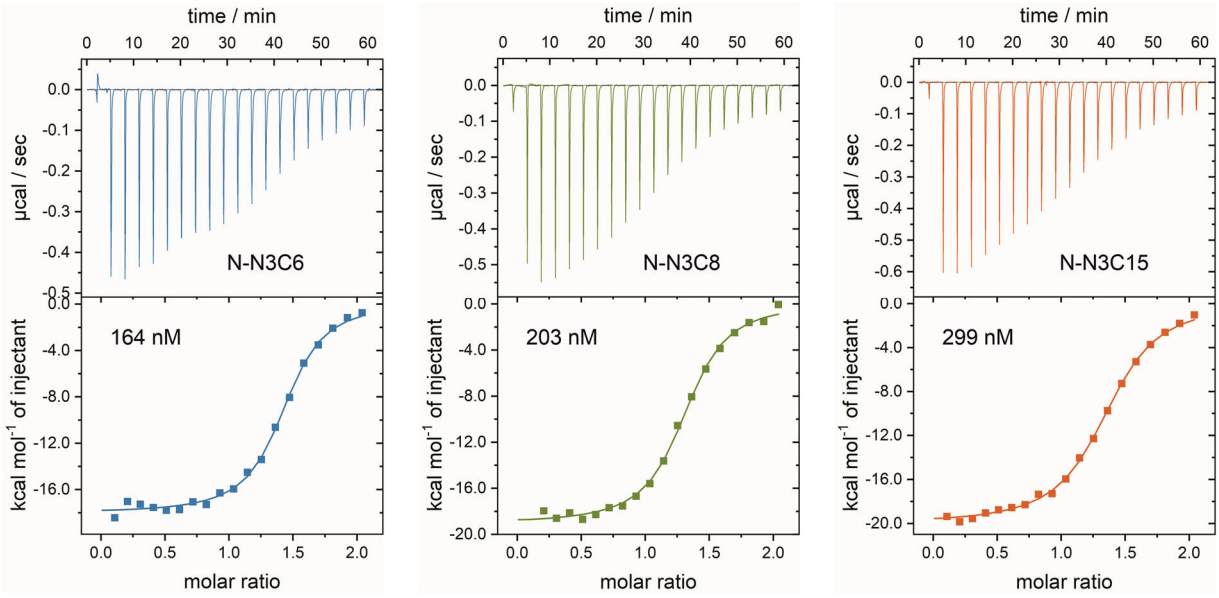

Figure S1 ITC data of **N-N3C6** (blue), **N-N3C8** (green), **N-N3C15** (orange) with the respective K_D values

The determined K_D values (Figure S1) indicate that the binding affinity is comparable to donor only labeled aptamers (Table S1).[4] This comparability further supports the assumption that the data are equivalent to those of the donor only labeled aptamer, regardless of the implementation of the **tC$_{nitro}$**.

Table S1 Dissociation konstants (K_D) values for the unlabeled N1-aptamer as well as for the singly and doubly labeled N1-aptamers.[2]

	K_D [nM]
N1 (unlabeled)	6
N-C6	375
N-C8	318
N-C15	480
N-N3C6	164
N-N3C8	203
N-N3C15	299

Table S2 Lifetimes obtained from the TCSPC experiments. Shown are the lifetimes of the multiexponential decay and their percentage weighting, resulting in the averaged amplitude weighted lifetime τ_{DA}.

	Neo	τ_1 [ns]	τ_2 [ns][a]	τ_3 [ns]	τ_{DA} [ns][a]
N-N3C6	-	0.0147 (99.02%)	0.4731 (0.98%)	/	0.0218
	+	0.0279 (59.08%)	0.2632 (40.92%)	/	0.1242
N-N3C8	-	0.1155 (84.97%)	0.7995 (13.28%)	5.340 (1.75%)	0.2980
	+	0.177 (31.16%)	0.7903 (35.56%)	2.264 (33.29%)	1.0898
N-N3C15	-	0.1236 (28.17%)	1.7673 (64.01%)	3.272 (7.83%)	1.4221
	+	0.3505 (10.88%)	1.1577 (13.46%)	1.905 (75.66%)	1.6353

[a] τ_{DA} corresponds to the averaged amplitude weighted fluorescence lifetime of donor in presence of the acceptor.

The lifetimes (τ_1, τ_2, τ_3) contributing to the multiexponential decay and the averaged lifetime τ_{DA} are given in Table S1. For samples **N-N3C8** and **N-N3C15**, three lifetimes are needed to fit the data correctly. Only two are needed for **N-N3C6**, which is due to the very high FRET efficiency. The lifetimes were obtained by performing a reconvolution fit (equation S1).

$$I(t) = \int_{-\infty}^{t} IRF(t') \sum_{i=1}^{n} A_i e^{-\frac{t-t'}{\tau_i}} \, dt' \qquad \textbf{(S1)}$$

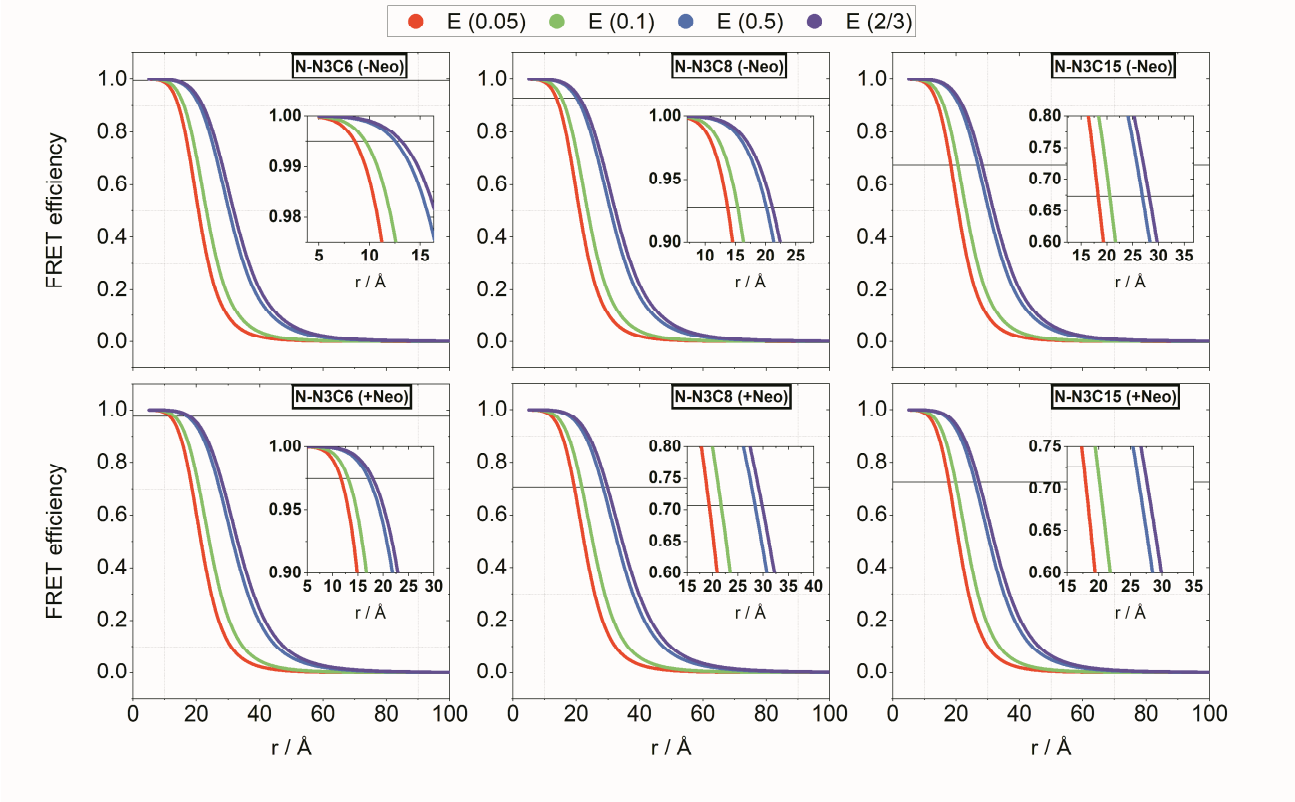

Figure S2 Plot of FRET efficiency E against distance r for samples **N-N3C6**, **N-N3C8**, **N-N3C15** (-/+ Neo). The horizontal gray lines indicate the measured FRET efficiencies E_{trf}. Fluorescence quantum yields of donor alone and κ^2 of 0.05, 0.1, 0.5, and 2/3 were used as input.

The plot of FRET efficiencies as a function of the relative distance between donor and acceptor clearly shows that the change in efficiency is greatest around the Förster radius (Figure S2). Especially for the lower measured efficiencies, this explains the deviations of the measured values from the simulated values for the efficiencies of the energy transfer. Since RNA structures are very flexible and the simulated values are based on the NMR structure of the N1-aptamer, which does not take into account the influence of the incorporation of $\mathbf{tC_{nitro}}$ or $\mathbf{Ç_m^f}$, it is not surprising that these values differ.

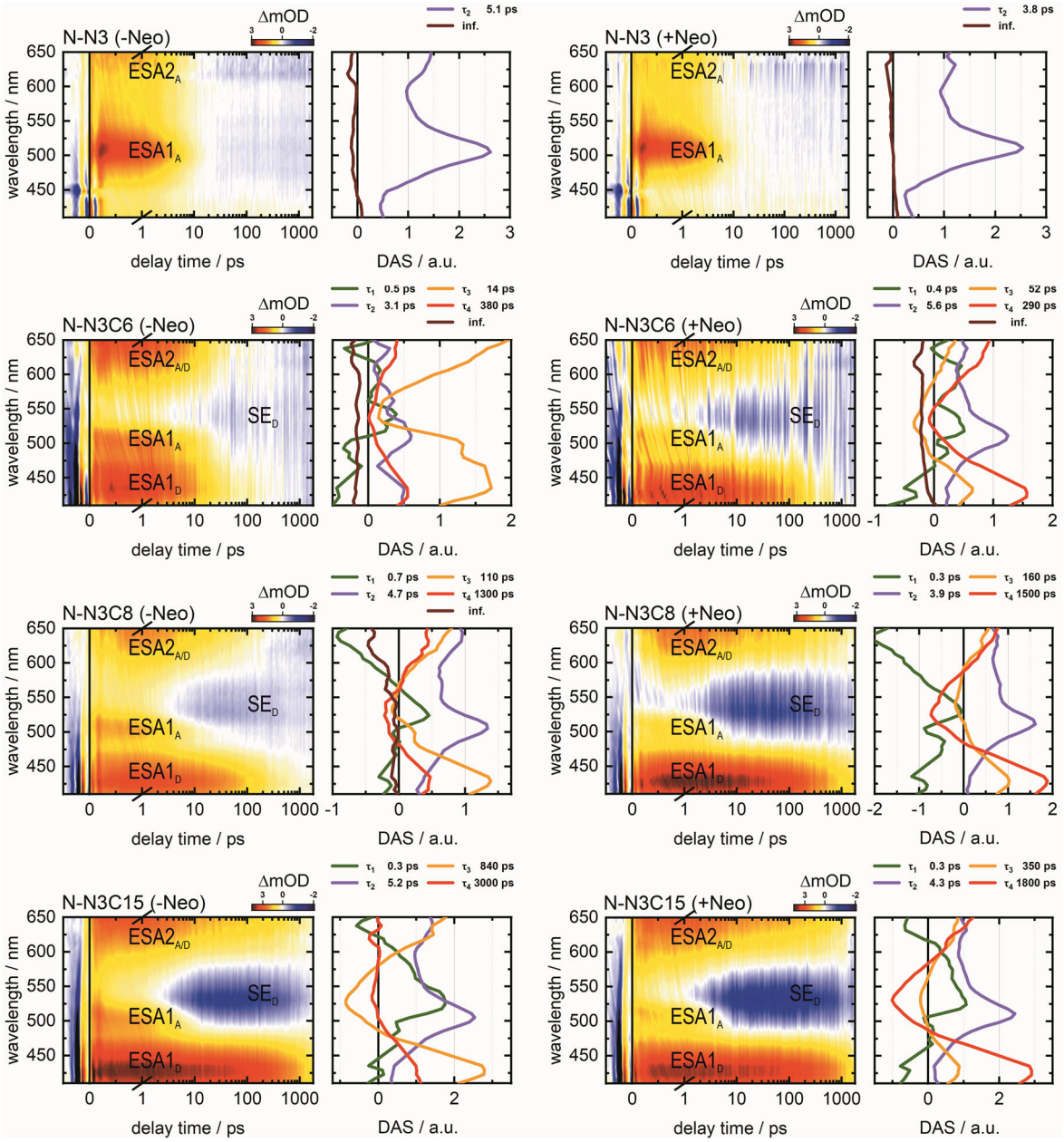

Figure S3 Transient absorptions maps and Decay associated spectra (DAS) of the samples **N-N3**, **N-N3C6**, **N-N3C8** and **N-N3C15**: left column without neomycin, right column with neomycin, top to bottom **N-N3**, **N-N3C6**, **N-N3C8**, **N-N3C15**. τ_1 for the depature of the Franck Condon region is shown in green, τ_2 for the decay of the directly excited tC_{nitro} is shown in violett, τ_3 for the first emitting state of $Ç_{mf}$ is shown in orange, τ_4 for later emitting states is shown in red and the infinity lifetimes shown in brown.

Figure S3 shows all transient absorption measurements including the respective DAS with all determined lifetimes.

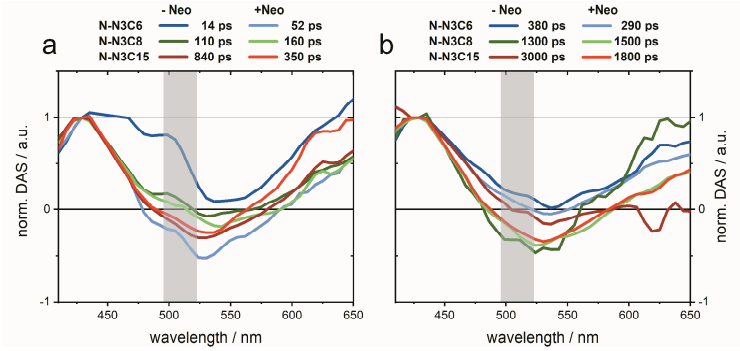

Figure S4 Decay associated spectra (DAS) of the samples **N-N3C6**, **N-N3C8** and **N-N3C15** (-/+Neo). a) DAS spectra for the first emitting state of **Çmf**. b) DAS spectra for the second emitting state of **Çmf**.

Normalized DAS of the first and second emitting state of $\mathbf{Ç_m^f}$ (Figure S4) The DAS are comparable for all samples and differ only slightly in their amplitudes. Gray line around 505 nm indicates the wavelength at which the excited state of $\mathbf{tC_{nitro}}$ decays after energy transfer.

Table S3 Lifetimes of the first (τ_3) and second (τ_4) emitting state of $\mathbf{Ç_m^f}$, the percentages of the respective lifetimes and the averaged lifetimes ($\boldsymbol{\tau_{DA(TAS)}}$) based on the percentages of the first and second emitting state.

	Neo	τ_3 [ps]	τ_4 [ps]	τ_3 [%]	τ_4 [%]	$\tau_{DA(TAS)}$ [ps]
N-N3C6	-	14	380	73.79%	26.21%	110
	+	52	290	27.73%	72.27%	224
N-N3C8	-	110	1300	74.70%	25.30%	411
	+	160	1500	34.95%	65.05%	1032
N-N3C15	-	840	3000	72.83%	27.17%	1427
	+	350	1800	22.40%	77.60%	1475

Table S3 shows the lifetimes of the first and second emitting states as well as the percentages of the respective lifetimes and the averaged lifetimes based on the percentages of the first and second emitting state. To determine the percentage of lifetimes, the corresponding DAS of the first and second emitting states were integrated in the range from 416 nm to 435 nm and the integrals were put in relation to each other.

References

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