Electronic Supplementary Material (ESI) for Chemical Communications
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Supporting information for:

Promoting strand exchange in a DNA templated transfer reaction

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Chemicals:

The MBHA resin for the solid phase synthesis was purchased from *Advanced ChemTech*. Fmoc-Gly-OH was purchased from *NovaBioChem*. The oligonucleotides were purchased from *BioTeZ* (Berlin-Buch). 6-Carboxyfluorescein was purchased from ChemPep (Florida, USA). SYBR® Gold Nucleic Acid Gel Stain (10.000× concentrate in DMSO) was purchased from *Life Technologies (Germany)*. Spermine was purchased from *Sigma*. The synthesis of PLL-*g*-dex (8K90D) was performed as previously described.[1] Further chemicals were purchased from *Acros*, *Sigma-Aldrich* and *Fluka* and used without further purification. Water was purified with a Milli-Q Ultra Pure Water Purification System, Membrapure, Germany.

Instruments:

Analytical HPLC of FAM-Gly-MESNa was performed on Agilent 1100 series instrument with a VL-quadrupol mass spectrometer (system I). The detection of signals was achieved with an UV detector at a wavelength of λ=260 nm. A *RP-C18-Gravity 3µ 124\4* from *Machery & Nagel* was used as a column (55°C). A solution of A (98.9 % H_2O, 1 % acetonitril, 0.1 % CH_3COOH) and B (98.9 % acetonitril, 1 % H_2O, 0.1 % CH_3COOH) was used as the mobile phase at a flow rate of 1 mL/min.

Preparative HPLC of FAM-Gly-MESna was performed on Agilent 1100 series instrument (column: Varian Polaris C18 A 5µ 250×100, pore size 220Å) using eluents A (98.9 % H_2O, 1 % acetonitril, 0.1 % TFA) and B (98.9 % acetonitril, 1 % H_2O, 0.1 % TFA) in a linear gradient at 6 mL/min. After removing of the eluent in vacuo, the product was dissolved in degassed H_2O.

Analytical HPLC of oligonucleotides was performed on a *Gilson Nebula Series* instrument (system II). The detection of signals was achieved with an UV detector at a wavelength of λ=260 nm. An *XBridge C18 A 5µ 4.6×250* column from *Waters* (55°C) was used. A solution of A2 (0.1 M triethylammonium acetate in H_2O, pH 7) and B2 (100 % acetonitril) was used as the mobile phase at a flow rate of 1 mL/min.

Analytical HPLC of oligonucleotides was performed on a Waters Acquity UPLC system (system III) with an *XBridge BEH 300 1.7 µ 2×100* column from *Waters* (55°C). The mobile phase was a mixture of A2 and B2 at a flow rate of 0.8 mL/min.

Preparative HPLC of DNA oligonucleotides was performed on the *Gilson Nebula Series* instrument (system IV). A Polaris C18 A 5µ 250×100 column from *Varian* (55°C) was used. The mobile phase was a mixture of A2 and B2 at a flow rate of 4 mL/min.

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Desalting of DNA oligonucleotides was performed by using an illustra NAP-5 column from *GE Healthcare*. MALDI-TOF mass spectra were recorded by using a Voyager-DE^{TM} Pro Biospectrometry Workstation of PerSeptive Biosystems using 2,4,6-trihydroxyacetophenone as matrix.

ESI-QP mass spectra were recorded on an Agilent 1100 series VL-quadrupol spectrometer.

For manual solid phase synthesis 2 mL polyethylene syringe reactors (MultiSynTech) equipped with a fritted disc were used.

UV-VIS spectroscopy for Fmoc-monitoring and concentration determination was performed on a *SmartSpec^{TM} Plus* spectrophotometer from *Biorad*. Extinction coefficients of oligonucleotides were calculated from the OligoAnalyzer from Integrated DNA Technologies using the *nearest- neighbor* method.

Synthesis of fluorescein-glycyl-mercaptoethanesulfonate (FAM-Gly-MESNa):

A methyl benzhydrylamine hydrochloride (MBHA) resin was loaded with Fmoc-Gly-OH according to standard protocols (ca 0.3 mmol/g) (see: *Novabiochem Catalog* 2010/2011). The initial loading was performed by coupling with Fmoc-Gly-OH in a 5 µmol scale. After washing of the resin (5×1 mL DMF, 5×1 mL CH_2Cl_2, 5×1 mL DMF) unreacted amino groups were acetylated (pyridine/Ac_2O (9:1), 1 mL, 2×10 min) followed by washing and Fmoc-deprotection (DMF/piperidine (4:1), 1 mL, 2×1.5 min). Afterwards S-trityl protected mercaptopropionic acid (4 eq, 4 eq PyBOP, 4 eq NMM) was coupled to the resin for 70 min. Subsequently the resin was washed and the trityl group was deprotected by means of TFA/TES (38:1, 950 µL, 30 min). After washing with 10×1 mL CH_2Cl_2, 5×1 mL DMF Boc-Gly-OH (4 eq, 4 eq PyBOP, 8 eq. NMM, 75 min) was coupled to the resin. Afterwards the resin was washed with 5×1 mL DMF, 5×1 mL CH_2Cl_2, 5×1 mL DMF, Boc-groups were removed (TFA/*m*-cresol (95:5), 5 min, 1 mL) and 6-carboxyfluorescein (4 eq, 4 eq PyBOP, 8 eq NMM, 2×2h) was coupled. The resin was washed 5×1 mL DMF, 5×1 mL CH_2Cl_2, 5×1 mL DMF. For detachment the resin was treated with mercaptoethanesulfonate (13 eq MESNa, 7 eq DIPEA, 1×90 min, 1×30 min). The resin was extracted with water/ acetonitril (1:1). The combined filtrates were reduced in vacuo and purified by HPLC. Yield: OD_{485} = 10.1; 1.3 µmol, 26 %; HPLC-MS (*m/z*): 558.0 ($[M+H]^+$ calculated: 558.05); HPLC: t_R: 17.2 min (gradient A); $C_{25}H_{19}NO_{10}S_2$ (557.05).

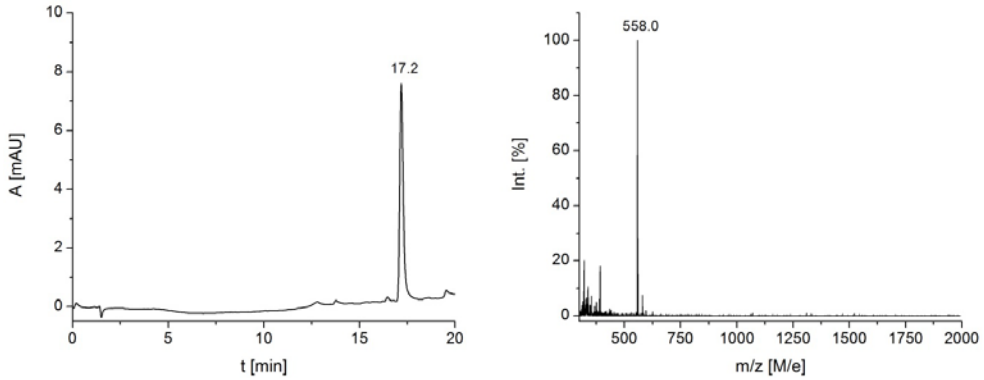
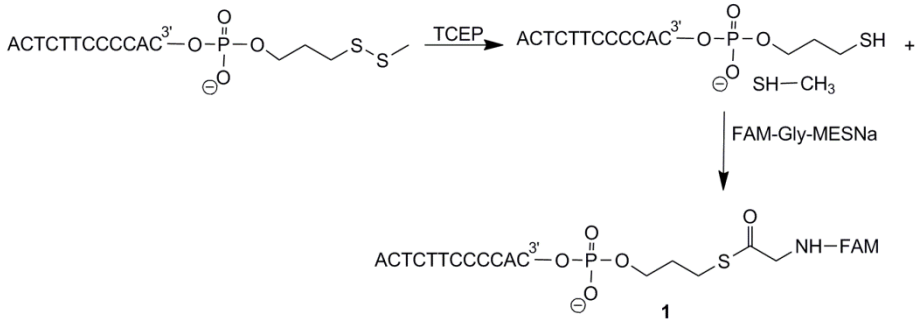


Fig. S1: HPLC-trace (left): 3-50 % eluent B in eluent A within 20 min (system I) and HPLC-MS spectrum (right) of purified FAM-Gly-MESNa.

Synthesis of 5'-TTACTCTTCCCCAC-(6)-carboxyfluoresceinglycyl-thioester-oligonucleotide (1):



Synthesis of the 6-carboxyfluoresceinglycyl-thioester oligonucleotide.

The 3'-methyldisulfide oligonucleotide (200 nmol, 1 mM) was dissolved in a solution of TCEP (2 mM) in buffer (200 mM NaH_2PO_4, pH 7.3). After 4h reaction time at 25°C the reaction mixture was desalted by using a Nap-column. The eluates were lyophylised. The thiol oligonculeotide (200 nmol) was dissolved in 980 µL degassed buffer (42 mM NaH_2PO_4, 2 mM TCEP, 2 mM spermine, pH 8). To the reaction mixture was added 400 µL of fluorescein-glycine-mercaptoethanesulfonate (1 µmol, 42 mM NaH_2PO_4 , 2 mM TCEP). After 48 h reaction time the solution was HPLC purified, desalted by a nap-column and lyophilized. Yield: OD_{260} = 8.77; 62 nmol, 26 %; MALDI-TOF-MS (*m/z*): 4678.5 ($[M-H]^-$ calculated: 4678.9); HPLC: t_R: 17.9 min (system II); $C_{159}H_{194}N_{42}O_{95}P_{14}S$ (4679.9).

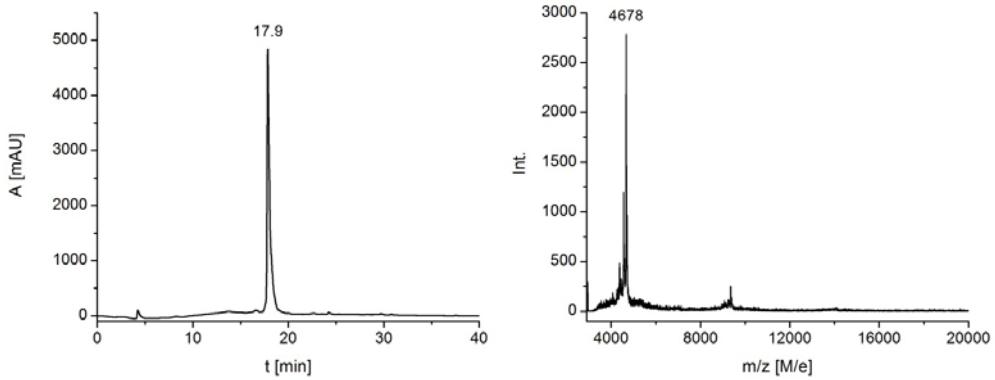


Fig. S2: HPLC-trace (left): 3-50 % B2 in A2 within 45 min (system II) and MALDI-spectrum (right) of purified **1**.

Synthesis of 5'-cysteine-aminohexyl-CCT ACA GCG C- oligonucleotide (2):

The reaction was performed in analogy to a previously published protocol.[2] To a 448 µL solution of the 5'-aminohexyl-DNA oligonucleotide (416 nmol) in buffer (200 mM NaH_2PO_4, pH 7.2) was added 688 µL of a solution of N-Fmoc-S-*tert*-butylsulfenyl cysteine succinimide in DMSO (8.7 µmol, 19 mM). Additional DMSO (1088 µL) was added to fully dissolve the reactants (final concentrations: 0.18 mM oligonucleotide, 5.8 mM N-Fmoc-S-*tert*-butylsulfenyl cysteine hydroxysuccinimide ester). After 20 h the reaction solution was concentrated in vacuo. Shaking the reaction mixture in 5 mL of ammonia solution at 37°C for 5 h was followed by evaporation of ammonia and HPLC purification. The purified product was dissolved in a TCEP-buffer solution (10 mM NaH_2PO_4, 200 mM NaCl, 2 mM, pH 7) at 0.2 mM concentration and after 4h desalted on a nap-column. Yield: OD_{260} = 14.43; 162 nmol, 40 %; MALDI-TOF-MS (*m/z*): 3254.2 ([M-H]$^-$ calculated: 3254.2); HPLC: t_R: 1.0 min (column III); $C_{104}H_{141}N_{39}O_{61}P_{10}S$ (3255.3).

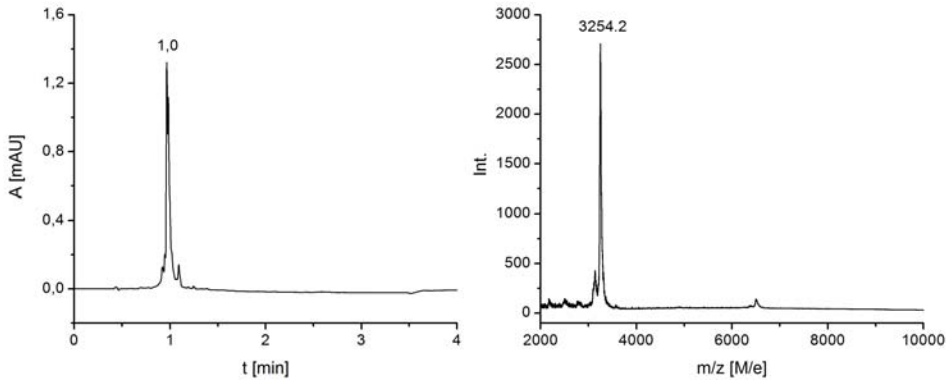


Fig. S3: UPLC-trace (left): 3-50 % B2 in A2 within 4 min (system III) and MALDI-spectrum (right) of purified **2**.

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Synthesis of 6-carboxyfluoresceinglycyl-5'-cysteine-aminohexyl-CCT ACA GCG C-oligonucleotide (4):

111 nmol of 5'-cysteine-aminohexyl-CCT ACA GCG C- oligonucleotide (**2**) and 800 nmol of fluorescein-glycyl-mercaptoethanesulfonate were lyophilized and dissolved in 800 µL TRIS-buffer (20 mM TRIS, 200 mM NaCl, pH 8.0). After the addition of 8 mg MESNa the reaction mixture was gently agitated at room temperature for two days and HPLC purified. Yield: OD_{260} = 0.66; 6 nmol, 8 %; MALDI-TOF-MS (*m/z*): 3668.3 ($[M-H]^-$ calculated: 3669.6); HPLC: t_R: 1.4 min (system III); $C_{127}H_{154}N_{40}O_{68}P_{10}S$ (3670.6).

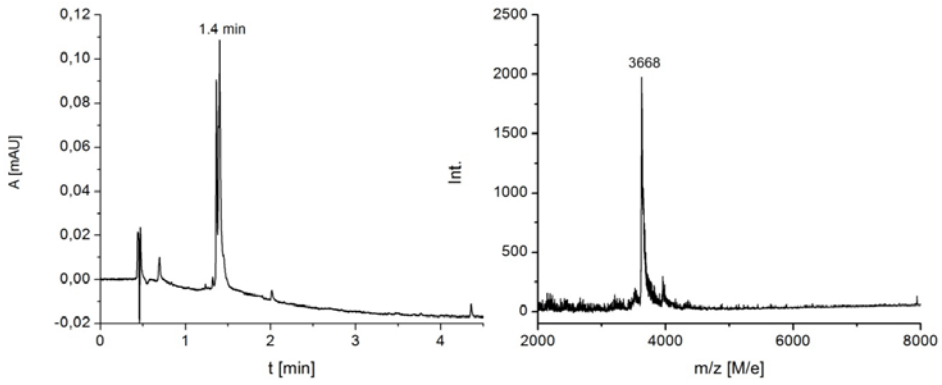

Fig. S4: UPLC-trace (left): 3-50 % B2 in A2 within 4 min (system III) and MALDI-spectrum (right) of purified **4**.

Synthesis of DNA probes for discrimination experiments:

Synthesis of 5'-TCTTCCCCAC-(6)-carboxyfluoresceinglycyl-thioester-oligonucleotide (5):

The synthesis was performed in analogy to the synthesis of probe **1**. Yield: OD_{260} = 1.02; 10 nmol, 14 %; MALDI-TOF-MS (*m/z*): 3469.9 ($[M-H]^+$ calculated: 3469.4); HPLC: t_R: 15.1 min (system II); $C_{159}H_{194}N_{42}O_{95}P_{14}S$ (3468.4).

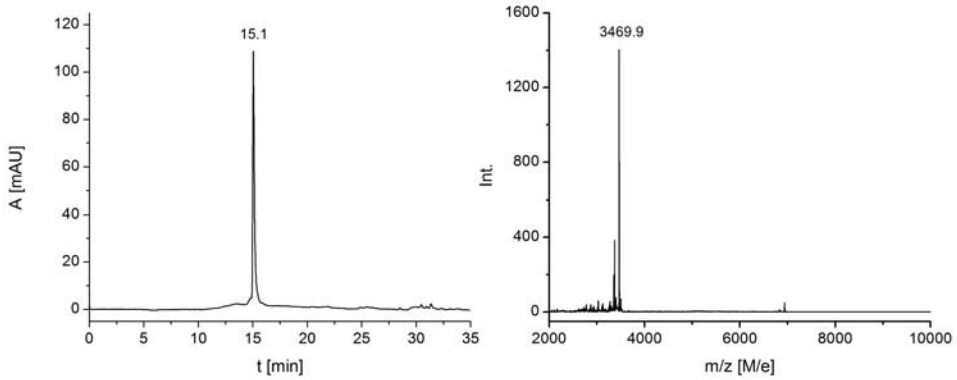

Fig. S5: HPLC-trace (left): 3-35 % B2 in A2 within 25 min (system II) and MALDI-spectrum (right) of purified **5**.

Synthesis of 5'-cysteine-aminohexyl-CCT ACA GC- oligonucleotide (6):

The synthesis was performed in analogy to the synthesis of probe **2**. Yield: OD_{260} = 13.32; 184 nmol, 46 %; MALDI-TOF-MS (*m/z*): 2637.7 ($[M-H]^+$ calculated: 2637.9); HPLC: t_R: 14.1 min (system II); $C_{85}H_{117}N_{31}O_{49}P_8S$ (2636.9).

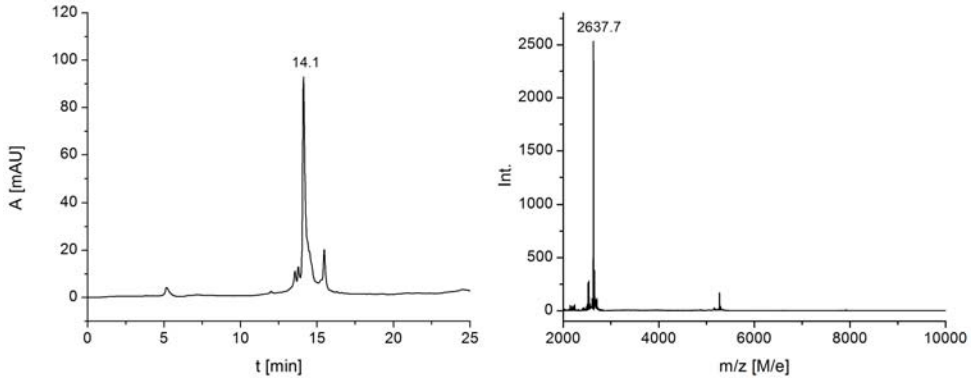

Fig. S6: HPLC-trace (left): 3-50 % B2 in A2 within 25 min (system II) and MALDI-spectrum (right) of purified **6**.

Synthesis of 6-carboxyfluoresceinglycyl-5'-cysteine-aminohexyl-CCT ACA GC-oligonucleotide (7):

The synthesis was performed in analogy to the synthesis of probe **4**. Yield: OD_{260} = 0.59; 6.3 nmol, 63 %; MALDI-TOF-MS (*m/z*): 3053.7 ($[M-H]^+$ calculated: 3053.2); HPLC: t_R: 17.6 min (system II); $C_{108}H_{130}N_{32}O_{56}P_8S$ (3052.3).

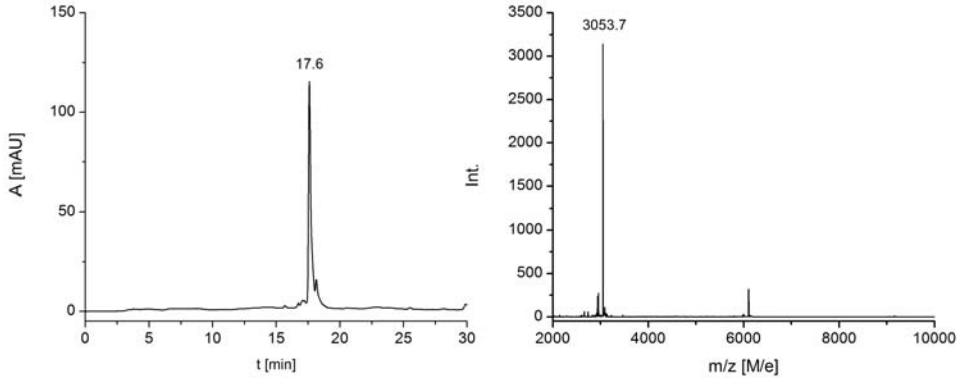

Fig. S7: HPLC-trace (left): 3-30 % B2 in A2 within 25 min (system II) and MALDI-spectrum (right) of purified **7**.

T_m measurements:

Melting experiments were performed on a Carey 100 Bio spectrometer from *Varian*. Measurements were performed by using 1 ml cuvettes 114-QS from *Hellma*. Spermine or αPLL-g-Dex was added from prefabricated stock solutions in the buffer (10 mM NaCl, 10 mM NaH_2PO_4, 2 mM TCEP, pH 7.4). RasT and the corresponding DNA oligonucleotide were added to 1 mL buffer. Changes in the absorption at 260 nm were measured four times at a temperature change from 20°C to 80°C at a heating rate of 0.5°C/min.

Transfer reactions:

The reaction buffer was degassed and the pH adjusted to 8 or 7.4 (as indicated) by dropwise addition of 2 mM NaOH solution. The transfer reactions were carried out while avoiding unnecessary exposure to oxygen. Additives such as spermine or PLL-*g*-Dex (8K90D) were each added to the buffer before the oligonucleotides were dissolved. Aqueous 2 µM oligonucleotide probe solutions were prepared using the buffer with or without the indicated additive. The solutions of reactive oligonucleotides **1** and **2** were combined in an Eppendorf tube followed by the addition of the appropriate amount of the RasT template for the indicated time. For the reaction analysis aliquots (11 µL) were withdrawn and 1 µL of a RasT complementary DNA (100 µM) was added to stop the reaction followed by instant freezing of the sample. 10 µL of formamide loading buffer (FLB) containing 1 µM of reference fluoresceine-DNA (20 mer) were added to perform separation by gel electrophoresis.

Gel electrophoresis:

Polyacrylamide gel electrophoresis (20% PAGE) was performed under denaturing conditions (7 M urea) and a 17 × 18 cm 20 % gel (acrylamide/ bisacrylamine (19:1)). To each of the samples

formamide loading buffer was added and incubated for 5 min at 90°C. For the electrophoresis a voltage of 300 V was applied until the samples reached the separation gel and then 500 V were applied for three hours. Separation of DNA probes (8mer Cys-DNA and 10 mer FAM-Gly-DNA) for discrimination experiments was performed on a 25 % polyacrylamide gel for six to seven hours at 500V.

Gel Analysis:

Gel analysis was performed on a *Kodak Image Station 4000 MM Pro*. Excitation filter is 470 nm and the emission filter at 535 nm. The analysis of the gels and integration of gel bands was performed by using the Kodak Molecular Imaging Software.

Adjustment of charge ratio:

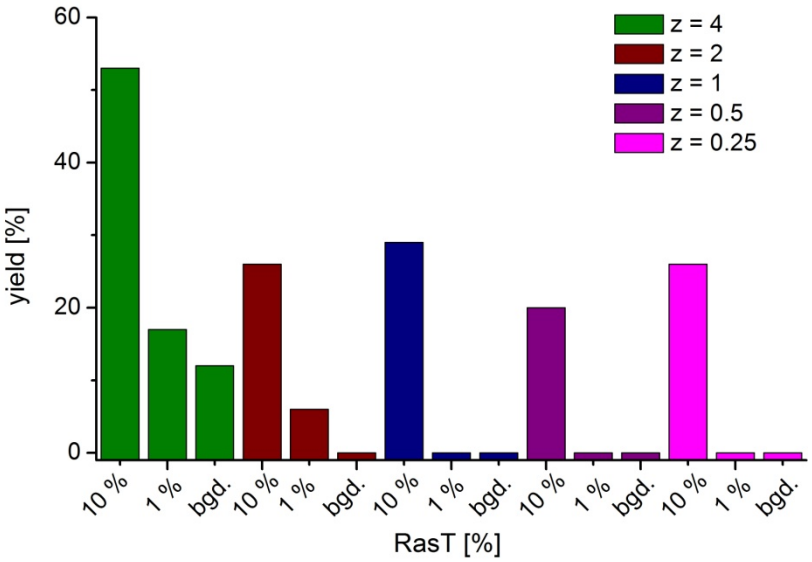

Fig. S8: Yields of transfer product **4** as a function of template load when αPLL-g-Dex was added at different charge ratios (z). Conditions: 2 µM **1**, 2 µM **2**, 12- 220 µM αPLL-g-Dex (220 µM, z = 4; 110 µM, z = 2; 55 µM, z = 1; 27.5 µM, z = 0.5; 12 µM; z = 0.25), 2 mM TCEP, 42 mM NaH_2PO_4, pH 8, 25°C, 4 h.

Gels of Transfer Reactions at turnover conditions:

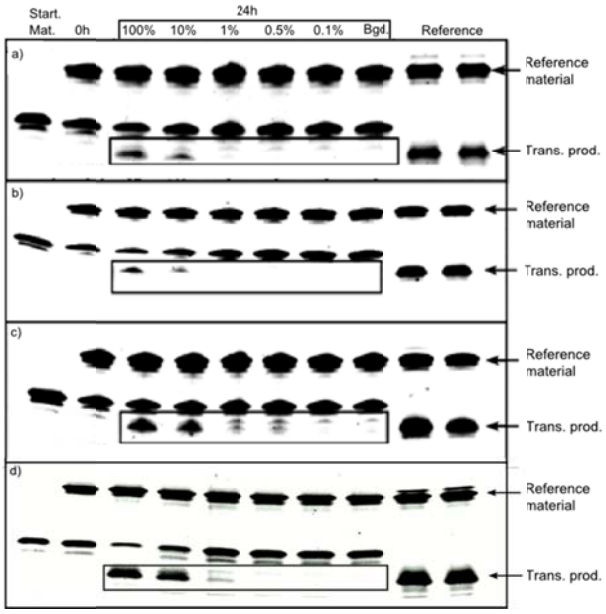

Fig. S9: Analysis of the acyl transfer reactions at varied loads of RasT template a) in absence of additives, b) with 100 µM spermine c) with 48 µM αPLL-g-Dex or d) with 48 µM polydextran and 1 µM reference DNA included as by-stander DNA on a 20 % polyacrylamide gel. Conditions: 1 µM **1**, 1 µM **2**, 10 mM NaCl, 10 mM NaH_2PO_4, pH 7.4, 25°C, 24h.

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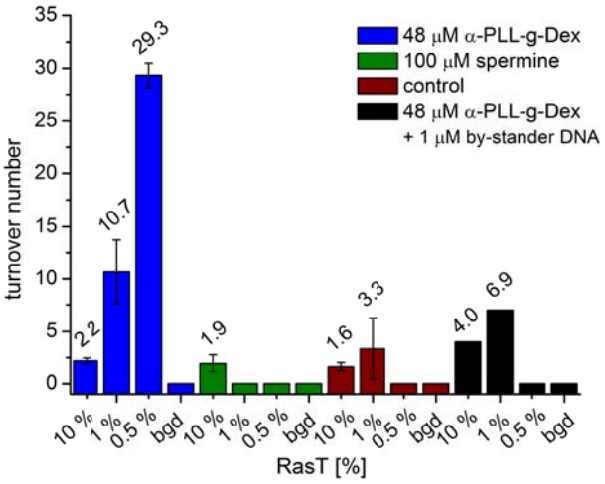

Fig. S10: Effect of additives on the turnover in RasT template in the acyl transfer reaction between **1** and **2** at varied loads of RasT template. Conditions: 1 µM **1**, 1 µM **2**, 10 mM NaCl, 10 mM NaH_2PO_4, 100 µM spermine when added, 48 µM αPLL-g-Dex when added, 1 µM by-stander DNA when added, pH 7.4, 25°C, 24 h.

Single base mismatch discrimination

Probes

5, 5'-TCTTCCCCAC-3'O-PO_2^--OC_3H_6-S-CO-CH_2-NH-CO-FAM

6, $NH_2CH(CH_2SH)CO$-NH-C_6H_{12}-O-PO_2^--5'O-CCTACAGC-3'

RasT template

3'-TTGAGAAGGGGTGTGGATGTCGCG-5'

RasG template

3'-TTGAGAAGGGGTGTGGAGGTCGCG-5'

competitor DNA

5'-CCTCCAGC-3'

Fig. S11: Sequences of the oligonucleotides used for studying the reactions on single base mismatched template **RasG**.

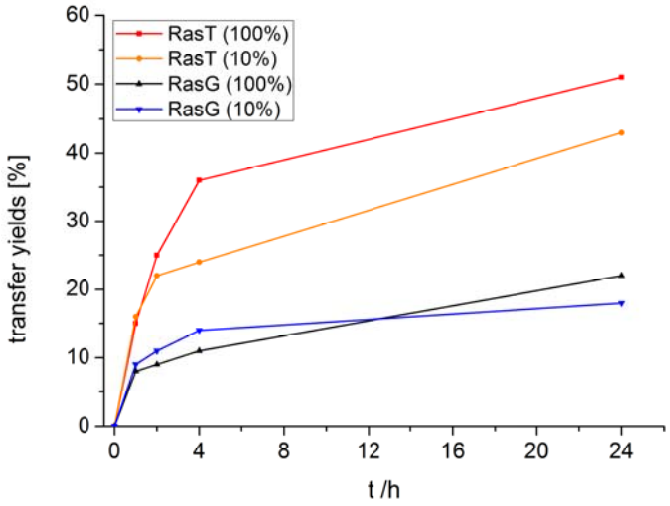

Fig. S12: Kinetic analysis of acyl transfer reaction between **5** and **6** in presence of match (**RasT**) or mismatch (**RasG**) DNA template and four equivalents of competitor DNA. Conditions: 1 µM **5**, 1 µM **6**, 4 µM competitor DNA, 10 mM NaCl, 10 mM NaH_2PO_4, 48 µM αPLL-g-Dex, pH 7.4, 25°C. Analysis was performed on a 25 % polyacrylamide gel.

Literature:

1 H. Watanabe, A. Ferdous, M. Katoh, T. Ishihara, T. Akaike, A. Maruyama, *Bioconjugate Chem.*, 1998, **9**, 292-299.

2 M. Lovrinovic and C. M. Niemeyer, *Biochem. Biophys. Res. Commun.*, 2005, **335**, 943–948.