Electronic Supplementary Material (ESI) for Chemical Communications

Enhanced imine synthesis in water: from surfactant-mediated catalysis to host-guest mechanisms.

Kamel Meguellati,[a] Ali Fallah-Araghi,[a] Jean-Christophe Baret,[b] Abdeslam El Harrak,[c] Thomas Mangeat,[c] Carlos M. Marques,[d] Andrew D. Griffiths*[a] and Sylvain Ladame*[a,e]

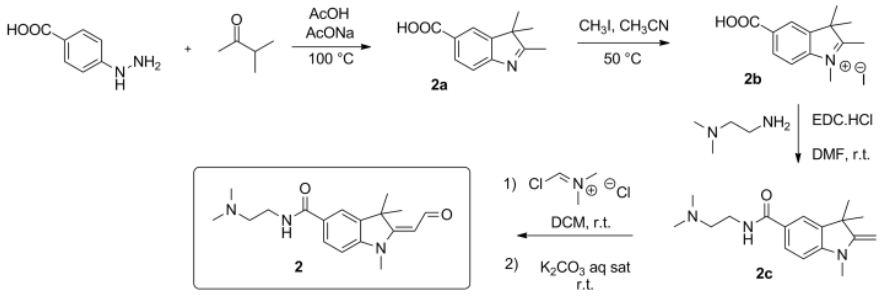
Supporting Information

1. Synthesis: Reagents and solvents were purchased from Sigma-Aldrich, Lyon, France, and used without further purification. Compounds were characterized using ^{1}H and ^{13}C NMR that were recorded on a Bruker Avance DRX 400 spectrometer at 400 and 100.6 MHz, respectively. Chemical shifts are reported as δ values (ppm) with reference to the residual solvent peaks. Products were purified by flash column chromatography on silica gel.

1.1 Synthesis of Amine 1. 2-Aminobenzothiazole (3.5 g, 23 mmol) was dissolved in acetonitrile (50 mL) and iodomethane (2 mL) was added. The solution was stirred at 45 °C for 12 hours. After cooling to room temperature, Et_2O (50 mL) was added and the white suspension was filtered. The precipitate was washed with diethyl ether and dried under vacuum. Compound **1** was obtained pure as a white solid (6.75 g, 100%, mp 227-228 °C). ^{1}H NMR (400 MHz, DMSO) δ 10.0 (s, 2 H), 8.0 (d, *J* = 7.9 Hz, 1 H), 7.69 (d, *J* = 7.9 Hz, 1 H), 7.6 (t, *J* = 7.5 Hz, 1H), 7.44 (t, *J* =7.5 Hz, 1 H), 3.75 (s, 3 H). ^{13}C NMR (100 MHz, DMSO) δ 167.9, 138.9, 127.7, 125.1, 123.4, 122.2, 122.1, 113.3, 32.2. HRMS (positive ES): *m/z*: calcd for $C_8H_9N_2S^+$: 165.0481; found 165.0475.

1.2 Synthesis of water-soluble Fisher's base aldehyde 2.

Water soluble Fisher's base aldehyde **4** was synthesized in 4 steps from commercially available 5-hydrazinobenzoic acid (see scheme below). 5-carboxylic acid 2,3,3-trimethyl-3H-indole **2a** was obtained by Fischer-Indole synthesis and subsequently quarternised with methyl iodide.[1] The resulting indolium salt **2b** was next reacted with *N*,*N*-dimethyl ethylene diamine in the presence of hydrochloride salt of EDC to afford the amide-functionalised indolenin **2c** which was finally converted into the corresponding water soluble Fisher's base aldehyde **4** via a Vilsmeier-Haack reaction.[2]



4-Hydrazinobenzoic acid (5.00 g, 32.9 mmol), 3-Methyl-2-butanone (4.24 g, 5.30 mL, 49.5 mmol) and NaOAc (5.40 g, 66 mmol) were dissolved in AcOH (70 mL) and the resulted brown mixture was heated to 100°C and stirred at the same temperature for 16 h. After cooling the reaction mixture to room temperature, acetic acid was removed under reduced pressure, cooled the resulted residue to 0°C and a saturated solution of K_2CO_3 (200 mL) was added slowly. The aqueous layer was extracted with CH_2Cl_2 (3 x 200 mL) before it was acidified to pH = 4 with conc. HCl at 0°C. The aqueous layer was extracted with CH_2Cl_2 (3 x 200 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated in *vaccuo* to give the title compound **2a** (6.5 g, 97%) as a brown oil.[3] The crude product was directly used in the next step without further purification. ^{1}H NMR (400 MHz, DMSO): d = 7.99 (d, *J* = 1.6 Hz, 1H), 7.91 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.50 (d, *J* = 8.0 Hz, 1 H), 2.25 (s, 3H), 1.27 (s, 6H). ^{13}C NMR (100 MHz, DMSO): d =

191.7, 167.5, 157.4, 146.1, 129.6, 127.3, 122.7, 119.1, 53.5, 22.3 (x 2), 15.3. HRMS (negative ES) *m/z*: calcd for $C_{12}H_{13}NO_2^-$ 202.086; found 202.084

To a solution of compound **2a** (6.50 g, 32.0 mmol) in acetonitrile (100 mL) iodomethane (11.3 g, 5.00 mL, 80 mmol) was added at room temperature and the resulted mixture was heated to 45°C and stirred at the same temperature for 12 h. After cooling to room temperature, Et_2O (100 mL) was added and the precipitate was filtered off. The precipitate was washed with Et_2O (3 x 30 mL) and dried under vacuum. Compound **2b** (8.03 g, 72%) was obtained as a red solid.[3] ^{1}H NMR (400 MHz, DMSO): d = 8.38 (s, 1H), 8.18 (d, *J* = 8.3 Hz, 1H), 8.02 (d, *J* = 8.5, 1H), 3.99 (s, 3H), 2.81 (s, 3H), 1.57 (s, 6H). ^{13}C NMR (100 MHz, DMSO): d = 199.0, 166.4, 145.2, 141.9, 131.6, 130.3, 124.2, 115.3, 54.2, 35.0, 21.5 (x 2), 14.5. HRMS (positive ES) *m/z*: calcd for $C_{13}H_{16}NO_2^+$ 218.118; found 218.114.

Compound **2b** (1.00 g, 2.90 mmol), *N*,*N*-dimethylethylenediamine (358 mg, 443µL, 4.06 mmol) and EDC·HCl (610 mg, 3.19 mmol) were dissolved in anhydrous DMF (45 mL) and the resulted red colored solution was stirred at room temperature for 10 hours. DMF was then evaporated off under reduced pressure and the residue was dissolved in CH_2Cl_2 (200 mL). The organic layer was washed with a saturated solution of K_2CO_3 (2 x 100 mL), dried over Na_2SO_4, filtered and concentrated in *vaccuo*. The residue was purified by column chromatography on SiO_2 (EtOAc/NEt_3 50:1, EtOAc/MeOH/NEt_3 200:5:2, 200:10:2; R_f = 0.3) to give the title compound **2c** (510 mg, 61 %) as a red oil. ^{1}H NMR (400 MHz, DMSO): d = 8.06 (t, *J* = 5.7 Hz, 1H), 7.68-7.66 (m, 2H), 6.69 (d, *J* = 8.0 Hz, 1H), 3.96 (d, *J* = 1.8 Hz, 1H), 3.95 (d, *J* = 1.8 Hz, 1H), 3.32 (q, *J* = 6.6 Hz, 2H), 3.05 (s, 3H), 2.37 (t, *J* = 7.0 Hz, 2H), 2.16 (s, 6H), 1.29 (s, 6H). ^{13}C NMR (100 MHz, DMSO): d = 166.1, 161.5, 148.4, 136.7, 127.9, 124.5, 120.9, 104.4, 75.8, 58.5, 45.3 (x 2), 43.2, 37.3, 29.6 (x 2), 28.6. HRMS (positive ES) *m/z*: calcd for $C_{17}H_{26}N_3O^+$: 288.2070; found 288.2062.

(chloromethylene)dimethylammonium chloride (798 mg, 6.24 mmol) was dissolved in CH_2Cl_2 (30 mL) and stirred at room temperature for 15 min. Then a solution of compound **2c** (300 mg, 1.04 mmo) in CH_2Cl_2 (5 mL) was added dropwise. After stirring the reaction mixture for 2 h, the solvent was removed in *vaccuo*. The residue was dissolved in THF (30 mL) and a saturated solution of K_2CO_3 (30 mL) was added carefully. After stirring the mixture for 12 h at room temperature, THF was removed under reduced pressure and the aqueous layer was extracted with CH_2Cl_2 (3 x 20 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated in *vaccuo*. The residue was purified by column chromatography on SiO_2 (EtOAc/MeOH/NEt_3 9.5:0.5:1, R_f = 0.2) to give the title compound **2** (280 mg, 86%) as a yellow oil. ^{1}H NMR (400 MHz, DMSO): d = 9.92 (d, *J* = 8.9 Hz, 1H), 8.38 (t, *J* = 5.6 Hz, 1H), 7.90 (d, *J* = 1.6 Hz, 1H), 7.82 (dd, *J* = 8.3, 1.6 Hz, 1H), 7.14 (d, *J* = 8.3 Hz, 1H), 5.35 (d, *J* = 8.6 Hz, 1H), 3.42 (q, *J* = 6.3 Hz, 2H), 3.27 (s, 3H), 2.62 (t, *J* = 6.4 Hz, 2H), 2.35 (s, 6H), 1.61 (s, 6H). ^{13}C NMR (100 MHz, DMSO): d = 185.8, 173.0, 165.7, 145.8, 139.0, 128.2, 127.9, 120.9, 107.9, 99.5, 58.5, 56.1 (x 2), 45.3, 37.5, 28.9, 18.5 (x 2). HRMS (positive ES) *m/z*: calcd for $C_{18}H_{26}N_3O_2^+$: 316.2020; found 316.2016.

2. Fluorescence spectroscopy. Imine **3** formation was followed in a 384-well plate using a fluorescence plate reader (SpectraMax M5, Molecular Devices). Excitation and emission were measured from above (Top Read) with λ_{exc} = 480 nm and λ_{em} = 520 nm at 25°C. Reactions were also monitored in a quartz cuvette (5 mm path length) on a Jobin Yvon Fluorolog 3.22 instrument. The excitation and emission bandwidths were each 5 nm.

3. Imine quantification. Imine **3** was quantified by simultaneously monitoring the formation of the fluorescent imine **3** (λ_{exc} = 480 nm and λ_{em} = 520 nm) and the disappearance of the weakly fluorescent aldehyde **2** (λ_{exc} = 340 nm and λ_{em} = 400 nm). A mixture of amine **1** and aldehyde **2** (10 mM each) was incubated in 0.1% w/w SDS at 25°C. After 2h, equilibrium was reached which corresponded to a 5% conversion of aldehyde **2** (Figure S1). Hence, the concentration of imine **3** at equilibrium was 0.5 mM (5% of 10 mM) which corresponds to 450 Rfu at λ_{exc} = 480 nm and λ_{em} = 520 nm.

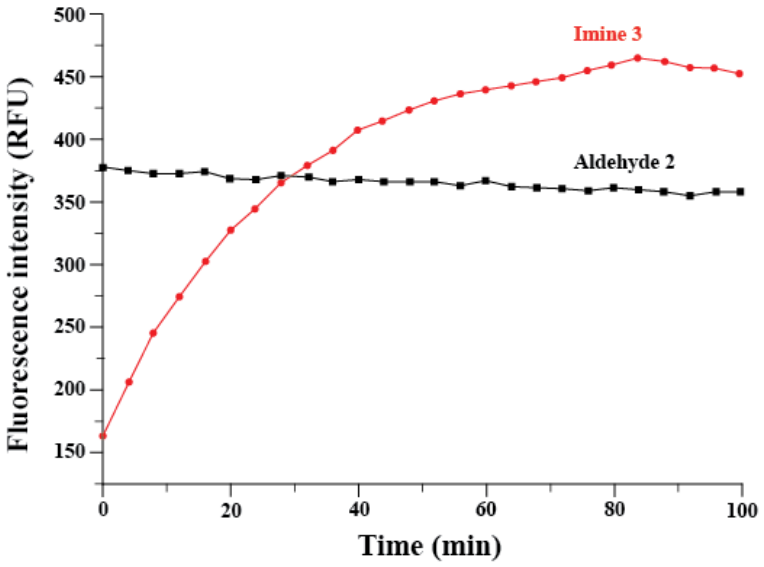


Figure S1. Formation of imine **3** and disappearance of aldehyde **2**, monitored by fluorescence spectroscopy. Aldehyde **2** and Imine **3** exhibit absorption maxima at 340 and 480 nm and emission maxima around 400 and 520 nm, respectively.

4. Reaction of imine formation in bulk solvent.

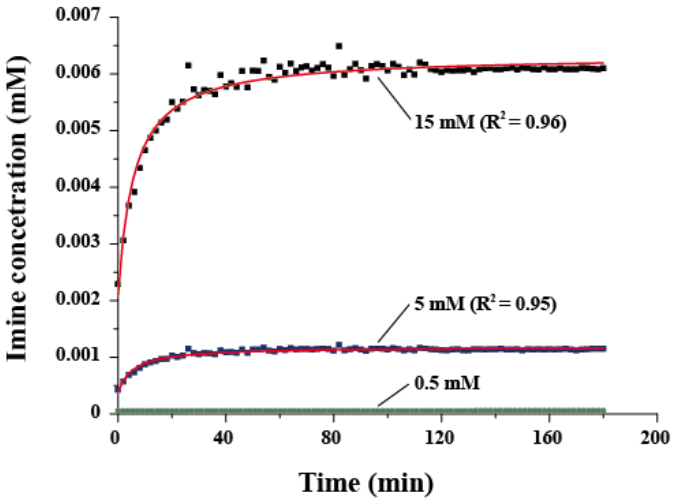


Figure S2. Kinetics of formation of imine **3** in bulk solvent (water, pH=7) at 25°C from a stoichiometric mixture of 0.5 mM (red), 5 mM (blue) and 15 mM (black) of amine **1** and aldehyde **2**. Reactions were monitored by fluorescence spectroscopy (λ_{em} = 520 nm). The curves were fitted to the integrated rate-equation for a reversible bimolecular reaction[4] and R^2 values are shown.

5. Calculation of equilibrium and rate constants. The equilibrium constant K_{eq} for the reaction of imine formation were determined from the concentration of imine **3** in the plateau of the plot of imine **3** concentration *vs*. time.

$$K_{eq} = \frac{[\text{Imine}]}{([\text{Amine}]_0 - [\text{Imine}])([\text{Aldehyde}]_0 - [\text{Imine}])}.$$

Where [Imine] is the concentration of imine **3** at equilibrium, and $[\text{Amine}]_0$ and $[\text{Aldehyde}]_0$ are the initial concentrations of amine **1** and aldehyde **2**, respectively. The second order rate constant k_1 was determined from the initial rate v_0 of the plot of imine **3** concentration *vs*. time.

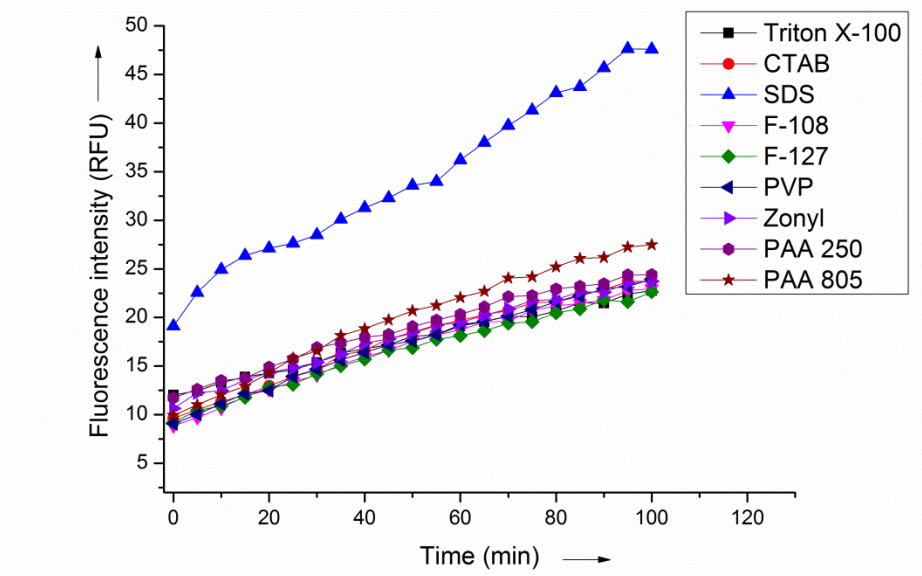
The first order rate constant k_{-1} for the reaction in bulk was calculated from K_{eq} and k_1;

$k_{-1} = k_1 / K_{eq}$.

6. Effect of surfactants on the apparent equilibrium constant (K_{eq}) and apparent second order rate constant (k_1) of imine formation

Reactions were carried out with stoichiometric quantities of amine 1 and aldehyde 2 (15 mM each) and in the presence of 0.03% or 0.8% w/w surfactant or polymer and monitored by fluorescence spectroscopy (λ_{em} = 520 nm) in a microtiter plate over 4-5 hours. The surfactants and polymers tested were sodium dodecylsulfate (SDS), Triton X-100, cetyl trimethylammonium bromide (CTAB), Pluronic F-108, Pluronic F-127, Polyvinylpyrrolidone (PVP), Zonyl and Polyacrylic acids (PAA250 and PAA805).

a) In the presence of 0.03% w/w surfactant



b) In the presence of 0.8% w/w surfactant

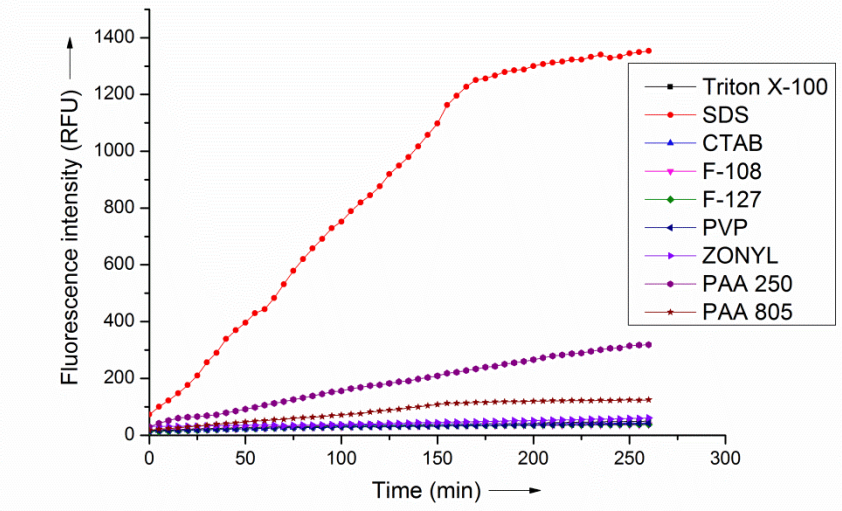


Figure S3. Formation of imine **3** from a stoichiometric mixture of aldehyde **2** and amine **1** (15 mM each) in the presence of 0.03% w/w surfactant (top, a) or 0.8% w/w surfactant (bottom, b). λ_{exc} = 480nm, λ_{em} = 520 nm.

7. Calculation of standard Gibbs free energy. The reaction can be treated as a simple thermodynamic system

$$K_{eq} = \frac{k_1}{k_{-1}} = e^{-\left(\frac{\Delta G^{o}_{eq}}{RT}\right)} = e^{-\left(\frac{\Delta G^{o\ddagger}_{f} - \Delta G^{o\ddagger}_{r}}{RT}\right)}$$

where R is the gas constant and T is the absolute temperature. From equilibrium thermodynamics, the difference in standard Gibbs free energy between the reactants and product, ΔG^{o}_{eq}, of the reaction can be calculated from

$$\begin{aligned}\Delta G^{o}_{eq} &= G^{o}_{(\text{product})} - G^{o}_{(\text{reactants})} \\ &= G^{o}_{(\text{imine 3})} - (G^{o}_{(\text{amine 1})} + G^{o}_{(\text{aldehyde 2})}) \\ &= -RT\ln K_{eq}\end{aligned}$$

From transition state theory, the difference in standard Gibbs free energy between the reactants and the transition state (TS), $\Delta G^{o\ddagger}_{f}$, and between the product and the TS, $\Delta G^{o\ddagger}_{r}$, of the reaction can be calculated from

$$\begin{aligned}\Delta G^{\circ\ddagger}_{f} &= G^{\circ}_{(\text{TS})} - G^{\circ}_{(\text{reactants})} \\ &= G^{\circ}_{(\text{TS})} - (G^{\circ}_{(\text{amine 1})} + G^{\circ}_{(\text{aldehyde 2})}) \\ &= -RT\ln\left(\frac{k_1 h}{\kappa k_B T}\right) \\ \Delta G^{\circ\ddagger}_{r} &= G^{\circ}_{(\text{TS})} - G^{\circ}_{(\text{product})} \\ &= G^{\circ}_{(\text{TS})} - G^{\circ}_{(\text{imine 3})} \\ &= -RT\ln\left(\frac{k_{-1} h}{\kappa k_B T}\right)\end{aligned}$$

where h is the Planck constant, κ is the transmission coefficient and k_B is the Boltzmann constant. The transmission coefficient, κ, is generally close to 1.0 for simple reactions and was assumed to be 1.0.

8. Effect of SDS concentration on the apparent equilibrium constant (K_{eq}) and apparent second order rate constant (k_1) of imine formation.

Table S1 Effect of SDS concentration on the apparent equilibrium constant (K_{ea}) and apparent second order rate constant (k_1) of imine formation[a]

SDS concentration (fraction of CMC)	K_{eq} (M^{-1})	k_1 ($M^{-1}.s^{-1}$)	ΔG°_{eq} (kJ.mol^{-1})	$\Delta G^{\circ\ddagger}_{f}$ (kJ.mol^{-1})	$\Delta G^{\circ\ddagger}_{r}$ (kJ.mol^{-1})
0	2.62×10^{-02}	1.43×10^{-5}	8.88	98.9	90.1
1	5.10×10^{-02}	1.32×10^{-3}	7.25	87.9	80.7
3	1.52×10^{-01}	1.54×10^{-3}	-1.02	87.6	88.6

[a]Reactions were carried out with stoichiometric quantities of amine **1** and aldehyde **2** (15 mM each). The difference in standard Gibbs free energy between the reactants and product, ΔG°_{eq}, between the reactants and the transition state (TS), $\Delta G^{\circ\ddagger}_{f}$, and between the between the product and the TS, $\Delta G^{\circ\ddagger}_{r}$, of the reaction in bulk and in droplets were calculated from K_{eq} and k_1 using equilibrium thermodynamics and transition state theory.

9. Dynamic Light Scattering. The ability of each surfactant and polymer (CTAB, Triton X-100, SDS, F-127, F-108, Zonyl, PVP, PAA250, PAA805) to form micelles at a 0.8% w/w concentration in water was tested by Dynamic Light Scattering (DLS) studies using a Zetasizer Nano-S instrument from Malvern Instruments. The measurements were made in a standard low volume disposable cuvette, by backscatter at 173°. The experimental parameters were adjusted to work at 0.3 mm from the cuvette wall (to avoid multiple scattering) and with the appropriate attenuator to collect between 400 and 700 counts per second on the detector. The correlation function was registered for each solution and the Zetasizer software uses algorithms to extract the decay rates for a number of size classes to produce a size distribution. To avoid disturbing the molecular self-organisation, the solutions where not filtered, and to reduce the dust scattering contribution we consider the number size distribution. At 0.03% w/w concentration none of the surfactant formed micelles, but at 0.8% w/w concentration all surfactants formed detectable micelles except for F-108 and PAA250 and PAA805 which are not self-organised in solution at this concentration.

10. DNA-induced reaction of imine formation monitored by fluorescence spectroscopy. The ability of dsDNA, ssDNA, dNTPs and deoxyribonucleotides to catalyse the fluorogenic reaction of imine **3** formation was examined by fluorescence spectroscopy (Jobin Yvon Fluorolog 3.22 instrument)

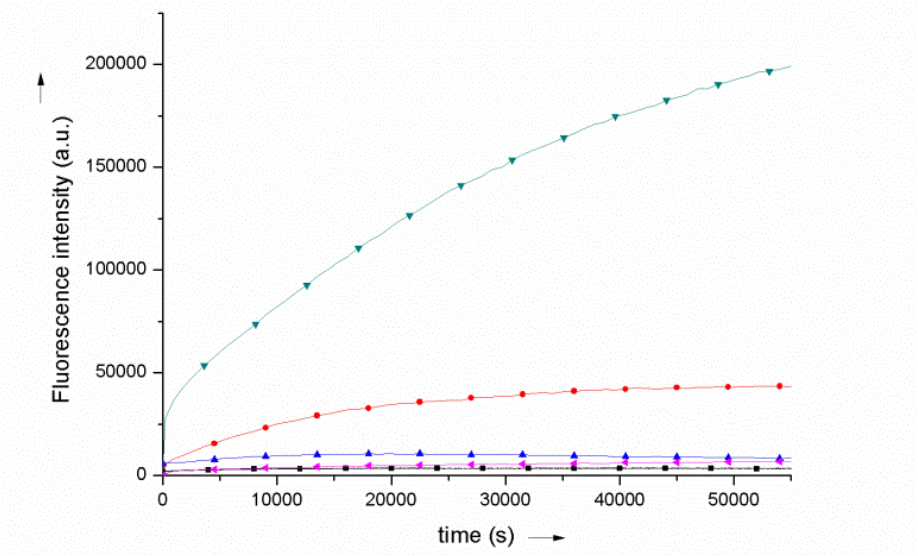


Figure S4. Formation of imine **3** from a stoichiometric mixture of aldehyde **2** and amine **1** (500 µM each) in water (pH 7) in the absence (black) and in the presence of 60 µM dsDNA (green), 600 µM dNTPs (red), 600 µM deoxyribonucleotide mix (blue) and 60 µM ssDNA (purple). λ_{exc} = 480nm, λ_{em} = 520 nm.

11. References.

1 Adapted from W. Pham, W. F. Lai, R. Weissleder and C. H. Tung, *Bioconjugate Chem.*, 2003, **14**, 1048.

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