

Supporting Information for:
Efficient functionalization of aqueous CdSe/ZnS nanocrystals using small-molecule
chemical activators

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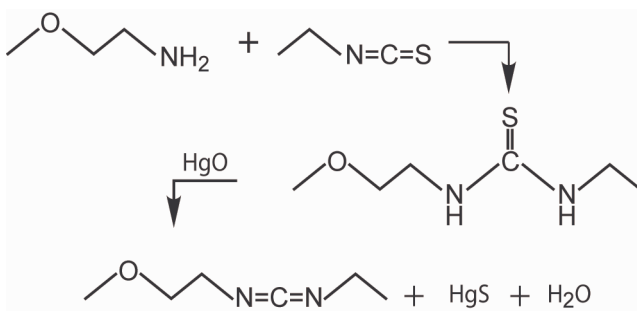
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Experimental Section

General: All chemicals were obtained from commercial sources and were used as received unless noted. Core CdSe¹⁻² and core shell CdSe/ZnS NCs³ were synthesized according to the cited references; piperazine functional rhodamine B was synthesized according to ref. 4. DMTMM was synthesized according to ref. 5. To calculate the relative ratios of dye, NC, and coupling reagent, we must know the absorption coefficient of CdSe/ZnS NCs in water. To derive this information, we first prepare the core NCs and process them via precipitation and calculate the total number of NCs using previously published protocols.⁶⁻⁷ The whole amount is then transferred to another reaction vessel for shell growth. After synthesis and assuming no loss of material, we calculate the number of NCs per gram of shell growth solution. Next, a known weight of material is processed via precipitation and water solubilized with 40% octylamine modified poly(acrylic acid),⁸ see ref. 9 for details. Generally, this method imparts water solubility with no obvious loss of NC materials (if precipitation is observed, the sample is discarded). As such, we are able to calculate the NC concentration in water and determine its extinction coefficient at any convenient wavelength.



Scheme S1 The preparation of 1-ethyl-3-(2-methoxyethyl)carbodiimide.

Preparation of 1-ethyl-3-(2-methoxyethyl)carbodiimide (EMC): In a round bottom flask 2.21 mL (25 mmol) of ethyl isothiocyanate was added into 2.0 mL of vacuum distilled 2-

methoxyethylamine (23 mmol) slowly using a syringe injector at speed 0.5 mL/hr while stirring under a N₂ atmosphere. The solution was stirred overnight under dry N₂ followed by drying under vacuum to remove the unreacted isothiocyanate. The viscous product is then frozen under atmosphere; 12 g (55 mmol) of yellow HgO was then added slowly under a strong stream of dry N₂ to prevent condensation within the reaction vessel. The flask was then allowed to slowly warm to room temperature while the mixture was triturated with a stir bar. When the color of HgO stopped changing, the product was distilled under vacuum into another vessel submerged in liquid N₂. After warming, the product was separated from the water by-product promptly using a syringe and was transferred to another vial for storage. The reaction yield in one typical preparation was found to be 23%. We have also found that vacuum-dried poly(ethylene glycol) methyl ether (750 MW) is an effective solvent for the de-sulfurization reaction; under these conditions, the reagents may be added at room temperature while the product and water by-product are distilled continuously into another vessel submerged in liquid N₂. We recommend this procedure over the solvent-free method.

NC-Rbpip Conjugation: In a typical preparation, 1 mL of a slightly acidic D.I. water suspension of ~1-3 μM polymer encapsulated CdSe/ZnS NCs was mixed with a 1-2 mg scale addition of conjugation reagent. The solution was stirred for 30 min upon which time 3 mL of a pH 8 phosphate buffer containing amine functional dye was added. The solution was stirred overnight, after which the absorption spectrum was recorded. Next, 100K Millipore concentrating filters were used to remove excess reagent and dye. After concentrating the solution to a known volume, the absorption and emission spectra of each sample were recorded.

A control sample was processed in the same manner as above; however, no coupling reagent is added. The control samples reveal that a small quantity of non-specifically adsorbing dye

remains in the sample after dialysis. This amount of non-specifically absorbing dye is subtracted from the loading levels observed in other samples when calculating coupling efficiencies.

We can create highly multifunctional NCs using this method and as demonstrated in our previous report;¹⁰ at the request of a reviewer, we have estimated the water-solubilizing polymer to NC ratio to augment these results. First, ~8 nmol of CdSe/ZnS NCs were processed twice via precipitation and then weighed (4.2 mg). Next, we solubilized the sample with an excess of 40% octylamine modified poly(acrylic acid) polymer (2910 Da MW) and dispersed the material into water; we note of utmost importance that the NCs dispersed very well and that there was no visible loss of NCs. Excess polymer was removed with dialysis using 100K MW concentrating filters from Millipore; the sample was then dried and weighed (12.6 mg). The weight of the remaining NC-coating polymers (8.4 mg) allows us to calculate the ratio of ~360 polymers:NC. Assuming that the original polymer contains 25 repeat units (as per the manufacturer's specifications) and 40% are coupled to octylamine, we calculate a COOH:NC ratio of ~5400.

Instrumentation: UV/Vis absorbance spectra were taken using a Varian Cary 300 Bio UV/Vis Spectrophotometer. Fluorescence emission spectra were taken using a customized Fluorolog (HORIBA Jobin Yvon) modular spectrofluorometer. The absorbance of all solutions was kept near or below 0.1 OD at the excited wavelength to avoid inner-filter effects.

Cell Culture: MDCKII cells were cultured in Dulbecco's Modified eagle Media (DMEM, Invitrogen, Inc.) supplemented with 10% FBS, 2 mM L-glutamine, 100 unit / mL penicillin and 100 mg / mL of streptomycin at 37 °C and 5% CO₂. Cells were passaged using 0.05% trypsin / 0.03% EDTA solution (Invitrogen, Inc.).

Preparation of NC-labeled cells for microscopic imaging. Cells (~80% confluent) were trypsinized and re-seeded into Lab-TekTM, 8-well chambered slides (Thermo-Fisher Scientific,

Inc.) at a concentration of ~20,000 cells/well and incubated overnight at 37 °C and 5% CO₂. NC's were diluted into complete medium to the desired concentration; the cells were incubated in the NC solution for 1h 37 °C and 5% CO₂. Prior to imaging, the cells were washed twice with phosphate buffered and fresh, complete medium was added.

Fluorescence microscopy. Epi-fluorescence microscopy of adherent live cells was performed using an Axiovert 200 (Carl Zeiss, Inc.) equipped with a 63× EC plan Neofluar oil immersion objective (NA=1.25). Excitation illumination was provided by a 100 W Hg lamp. Excitation and emission light were selected by appropriate band-pass filters from Chroma Technologies, Inc.: HQ545/30(ex.), HQ610/75(em.). Images (12-bit, grayscale) were detected using a Zeiss Axiocam MRM CCD camera, and captured with Zeiss Axiovision 4.6 software. Images were adjusted for brightness and contrast using NIH image J (v.1.34) and prepared for publication using Adobe Photoshop 5.5.

Spectroscopy

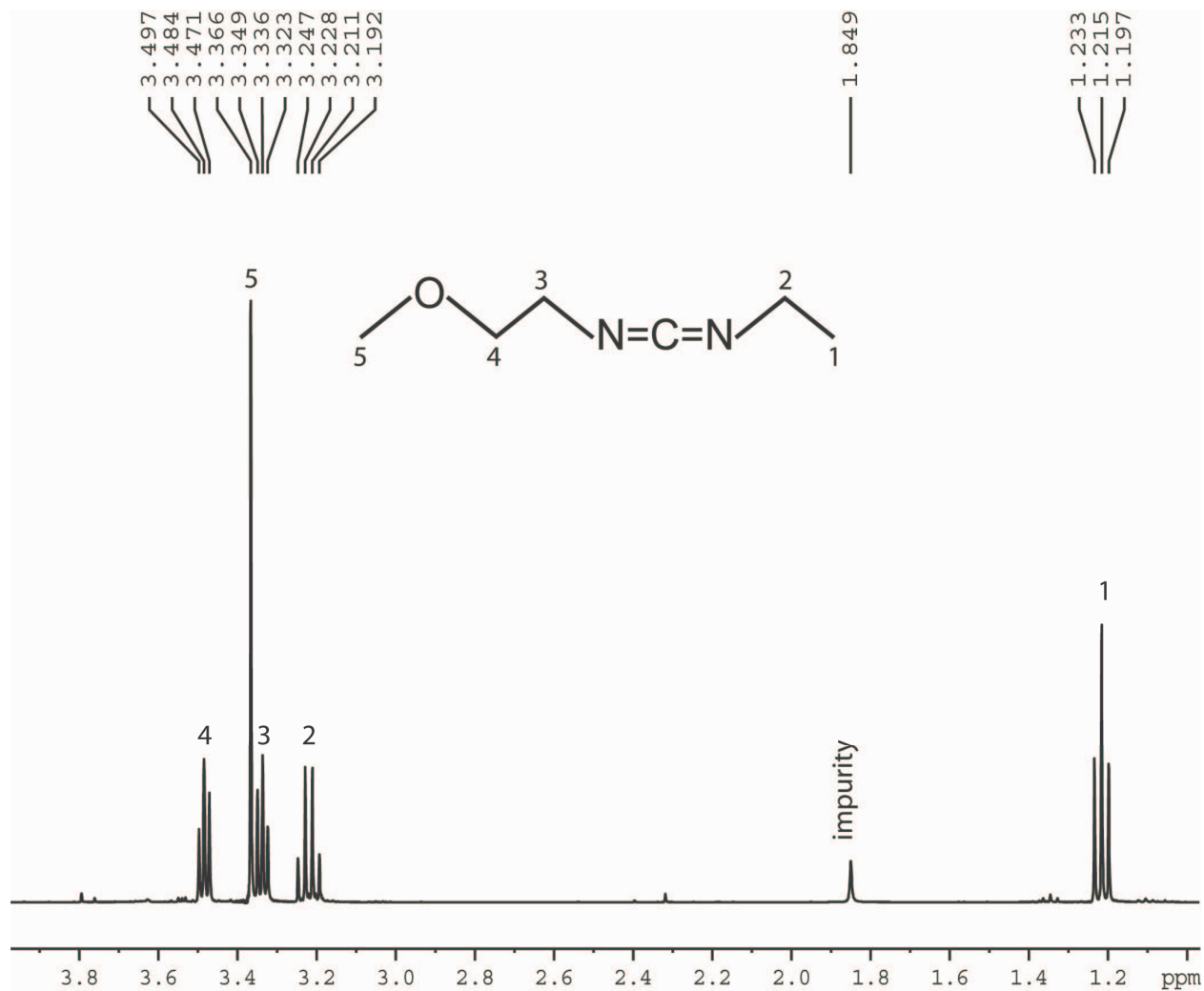


Fig. S1. ¹H NMR spectrum of 1-ethyl-3-(2-methoxyethyl)carbodiimide in a CDCl₃ solvent taken with a 400MHz Bruker Avance spectrometer. A slight impurity is observed at 1.849 ppm.

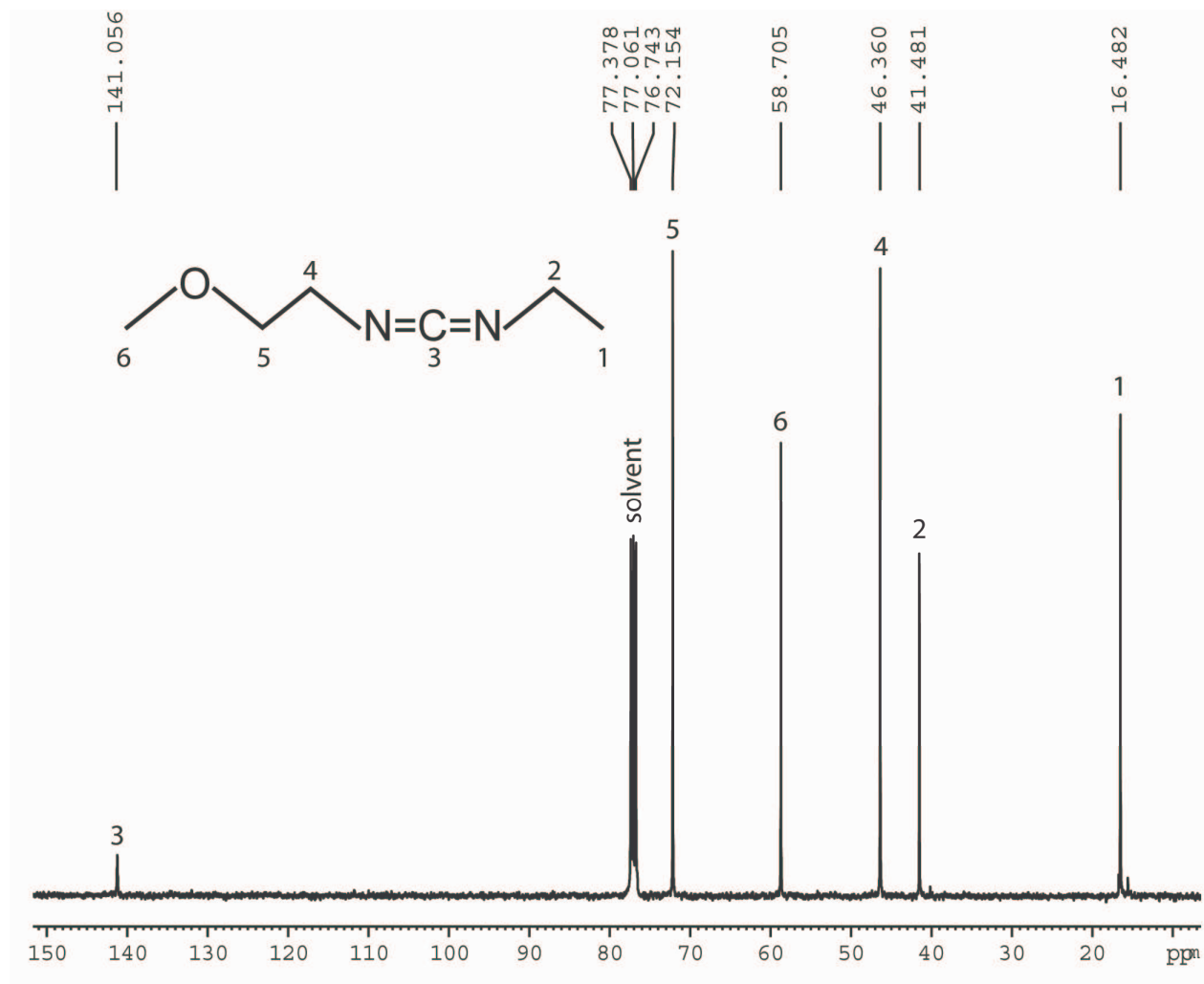


Fig. S2. ^{13}C NMR spectrum of 1-ethyl-3-(2-methoxyethyl)carbodiimide in a CDCl_3 solvent taken with a 400MHz Bruker Avance spectrometer.

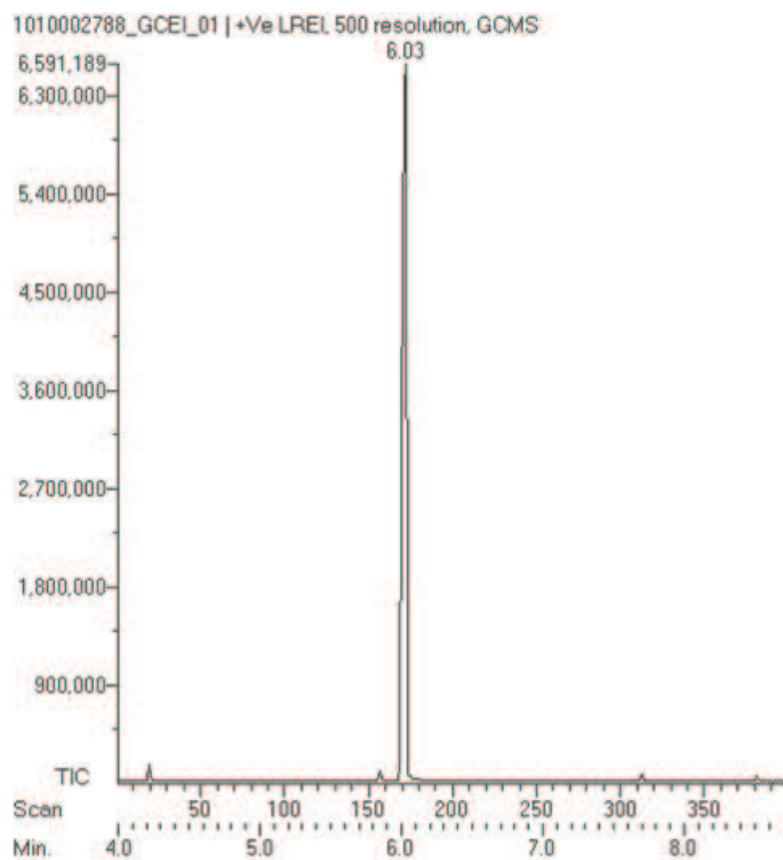


Fig. S3. Gas chromatogram of 1-ethyl-3-(2-methoxyethyl)carbodiimide, taken with a JEOL GCMate II.

1010002700_GCC1_01 | +Ve LRFCL 500 resolution, GCMG
Scan: 168-173(163-164) TIC=3233438 (Max.Inten: 605191) Base=57.7%FS #ions=128 RT=6.01

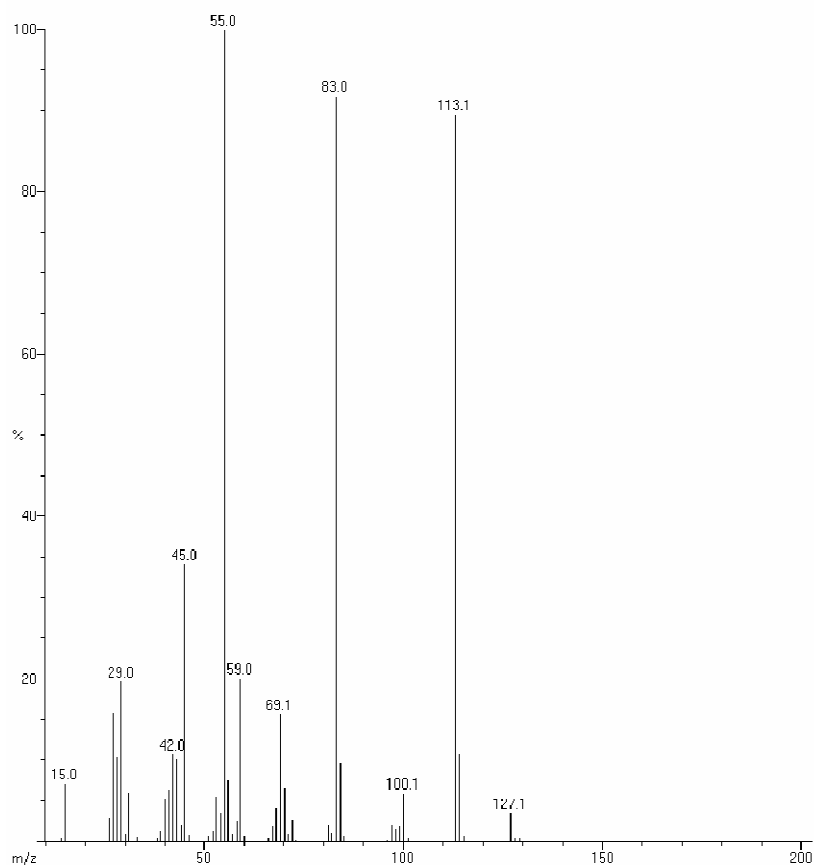


Fig. S4. ESI mass spectrum of 1-ethyl-3-(2-methoxyethyl)carbodiimide, taken with a JEOL GCMate II. The formula represent the intact molecule minus (–) the fragment indicated. The fragmented masses are assigned as:

| Formula | Cal. m/z | Exp. m/z |
|--|----------|----------|
| $[\text{C}_6\text{H}_{12}\text{ON}_2 - \text{H}]^+$ | 127.0 | 127.1 |
| $[\text{C}_6\text{H}_{12}\text{ON}_2 - \text{CH}_3]^+$ | 113.0 | 113.1 |
| $[\text{C}_6\text{H}_{12}\text{ON}_2 - \text{CH}_2\text{OCH}_3]^+$ | 83.0 | 83.0 |
| $[\text{C}_6\text{H}_{12}\text{ON}_2 - \text{CH}_2\text{CH}_2\text{OCH}_3]^+$ | 69.0 | 69.1 |
| $[\text{C}_6\text{H}_{12}\text{ON}_2 - \text{N}=\text{C}=\text{NCH}_2\text{CH}_3]^+$ | 59.0 | 59.0 |
| $[\text{C}=\text{NCH}_2\text{CH}_3]^+$ | 55.0 | 55.0 |
| $[\text{CH}_2\text{OCH}_3]^+$ | 45.0 | 45.0 |
| $[\text{CH}_2\text{CH}_3]^+$ | 29.0 | 29.0 |
| $[\text{CH}_3]^+$ | 15.0 | 15.0 |

Table S1. Fragmentation analysis of 1-ethyl-3-(2-methoxyethyl)carbodiimide.

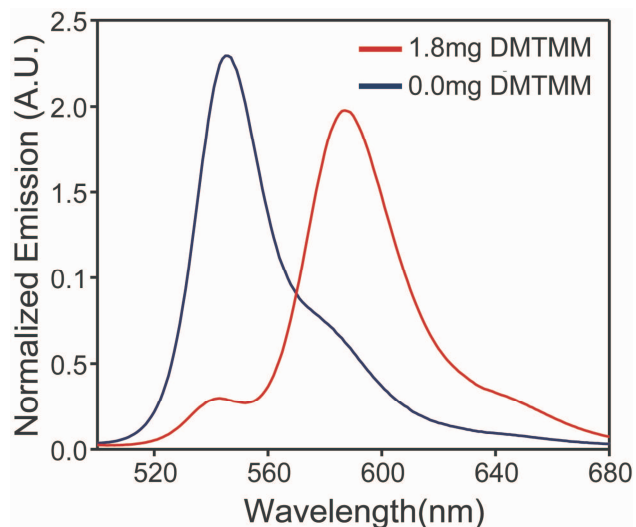


Fig. S5. Normalized emission spectra of Rbpip-CdSe/ZnS conjugated with DMTMM after dialysis. Energy transfer confirms close contact of the two chromophores. The control sample shows emission from non-specifically adsorbing dye.

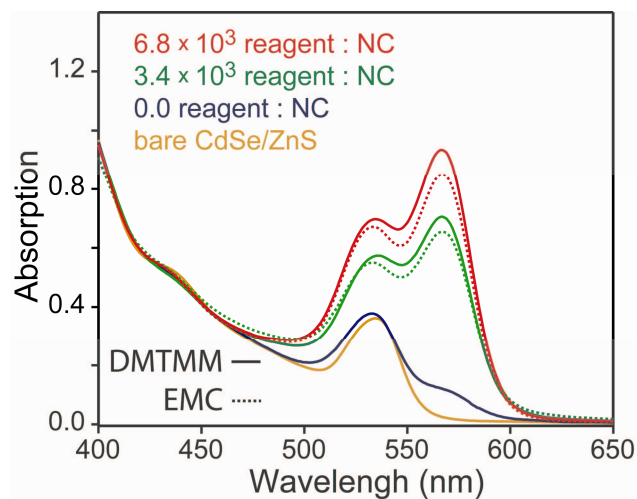


Fig. S6. Absorption spectra of CdSe/ZnS NCs and Rbpip dye after dialysis as a function of coupling reagent exposure. The reaction yields are summarized in Table 2. The starting dye to NC ratio is ~20:1.

| Reagent | Reagent:NC (by mol) | Yield (%) ^a | Rbpip:NC ^a |
|---------|---------------------|------------------------|-----------------------|
| DMTMM | 6.8×10^3 | 73 | 14:1 |
| EMC | 6.8×10^3 | 61 | 9.6:1 |
| DMTMM | 3.4×10^3 | 45 | 7.8:1 |
| EMC | 3.4×10^3 | 40 | 7.1:1 |
| Control | 0 | NA | 1.0:1 |

Table S2. Reaction yield data. a. The amount of dye that appears to non-specifically adhere to the control has been subtracted from the yield and dye:NC ratio. This alters the yield with respect to the original dye:NC ratio. The EMC data presented here is not from the same dataset used in the main text.

References

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