# Replication NAND Gate with Light as Input and Output

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## Supporting Information

## Experimental section

### 1. Peptide synthesis and characterization

Fmoc-Lys(Abz) was synthesized by coupling commercially available Fmoc-Lys-OH and Boc-2-aminobenzoic acids (Boc-2-Abz), in 1:1.1 mol ratio. The reaction was carried out in dichloromethane (DCM), using O-Benzotriazole-N,N,N',N'-tetramethyl-uronium-hexafluoro-phosphate (HBTU; 3 eq.) as coupling reagent and di-isopropyl amine (DIPEA, 20 eq.) as base. The reaction progress at room temperature was monitored by TLC [DCM:MeOH; 9:1], and typically reached completion after over night. The solvent was evaporated, the product (50% yield) was obtained after purification on silica column chromatography (DCM:MeOH), and characterized by $^{1}$H NMR.

The electrophile **E'**, containing the Abz as a donor dye, was synthesized on 2-chlorotrityl chloride resin using the Fmoc-based Chemistry. After the synthesis, the crude peptide was cleaved off the resin using AcOH/DCM/TFE (1:3:1) mixture. The C-terminus of the fully protected peptide (still as crude material) was thioesterified using N-acetylcystamine, benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) and DIPEA (6 eq. each), in DMF at room temperature. Global deprotection then took place, using the common mixture containing 95 % trifluoro acetic acid (TFA). The hydrolyzed electrophile $\mathbf{E_{hyd}}$ was synthesized in the same manner except that cleavage and global deprotection (in 95% TFA) took place at one time, right after the synthesis.

The nucleophilic peptide **N'**, containing the acceptor dye Y($NO_2$) was synthesized on solid-phase with Rink-Amide MBHA resin using the Fmoc-based chemistry. For this synthesis, Fmoc-3-Nitrotyrosine was purchased and used as is, and cleavage and global deprotection took place as above (95% TFA)

The caged nucleophile $N^{Nv}$, starting material for the synthesis of $\mathbf{T^{Nv}}$, was synthesized with the $Lys^{27}$ side chain orthogonally protected by the 4-methyltrityl (Mtt) group. After synthesis of the fully protected peptide, the Mtt group was selectively removed with 1% TFA in dry DCM, repeating 10 times for 2 minutes each time. The free ε-amine of the Lys side chain reacted with 4,5-dimethoxy-2-

nitrobenzyl formate (Nvoc-Cl) (5 mol equivalents relative to the resin loading), N-hydroxybenzotriazole (HOBT) as coupling reagent (5 eq.) and DIPEA base (10 eq.) in dry DCM containing 1M LiCl, for 3 hours and then again for 12 hours. The caged peptide, equipped with the photocleavable group at the desired position, was obtained after cleavage and global deprotection (95% TFA).

The template peptides **T**, and $\mathbf{T^{Nv}}$ were synthesized using the native chemical ligation method: The thioester peptides E, the unlabeled analog of **E'**, (1.0 eq., 3-5 mM) and the corresponding N-terminus Cys nucleophilic peptide (N or $\mathbf{N^{Nv}}$; 1.2 eq.) were dissolved in 3-(N-morpholino)propanesulfonic acid (MOPS) buffer at pH 7.7-7.8, containing 6M guanidinium hydrochloride (GdHCl) and thiophenol and benzyl mercaptan (4% v/v each). The reaction allowed to proceed at 37° C for 4-8 hours, until quenched by TFA, washed with ether and subjected to purification by HPLC.

All the studied peptides were purified by preparative HPLC using C18 reverse phase column with a step gradient of solvent A (99% water, 1% acetonitrile (ACN), 0.1% TFA) and B (90% ACN, 10% water, 0.07% TFA). The identity and purity of the peptides were analyzed by analytical HPLC (with the same solvent system for elution), MALDI-TOF MS (α-Cyano-4-hydroxycinnamic acid was used as ionization matrix) and LCMS. Molecular weights (MW) observed for all peptides were no more than ±2 off the calculated MW. The crude and pure peptide HPLC chromatograms, and the corresponding mass spectra of the peptides of **E'**, **N'**, **T** and $\mathbf{T^{Nv}}$ are given in Figure s1 below. Only peptides with 95% purity or above were used for the following kinetic FRET studies.

## 2. Following the replication experiments by FRET

Stock solutions of **E'**, **N'**, and **T** were prepared at roughly 1 mM concentration by weighting their lyophilized powders. The exact concentrations were determined prior to the experiments, using calibration curves made with from UV spectra of anthranilic acid, 2-nitrophenol, and ABA respectively. All fluorescence spectra were recorded on a Varian Cary Eclipse fluorescence spectrometer, 96-microwellplate reader (excitation slit width: 5nm). Spectra were recorded from 340 to 500 nm with an excitation wavelength of 310 nm and scan speed of 100 nm $min^{-1}$.

Kinetic experiments were carried out on 96-microwellplate, with each well containing a total volume of 75 μL. All experiments were performed by reacting 100 μM of **E'** and' **N** at 25 ºC, in the presence of specific amount of the template **T**, in 240 mM MOPS buffer at pH 7.2, and in presence of tris(2-carboxyethyl)phosphine hydrochloride (TCEP; 2 mM) as reducing agent. The reactions

were initiated by automatically adding the buffer to the microwell. The first time points were taken immediately (in less than 15 sec), and subsequent fluorescence spectra were recorded at various time intervals as indicated in Figure 2 in the manuscript, and in Figure s2 below. The 420 nm fluorescence value at each time point was used to calculate the quenching due to energy transfer from donor to acceptor ($F_0$-F). The asymptotic value of F =30 AU was empirically estimated for the 100% conversion, which was correlated with the formation of 100 μM of **T'**, and thus used to linearly calculate the product formation at each time point. The fluorescence spectra of reactions in the presence of high concentrations of **T** ([**T**] >10 μM) showed an additional maximum at ~355 nm. Control measurements of solutions containing only **T** (40 and 80 μM) confirmed that this second maximum was coming from **T** itself (Fig. s2e) and not from any by-product of the studied reaction.

3. **Following light-induced replication experiments by light.** Stock solutions of reactants (**E'** and **N'**) and templates (**T** or $\mathbf{T^{Nv}}$) were prepared by weighing lyphoilized peptides into Eppendorf tubes and dissolving in Millipore water to yield 0.5 – 4 mM solutions. Experiment solutions were prepared by mixing aqueous solutions containing 100 μM of **E'** and **N'**, TCEP (2 mM) as reducing agent, and the desired amounts of the corresponding template peptide in the dark (covered with aluminum foil). Reactions in the dark allowed to equilibrate for 30 minutes and the reactions were initiated by the addition of MOPS buffer at pH = 7.2 yielding a total volume of 75 μL. We have shown previously that shining light on $\mathbf{T^{Nv}}$ for >8 minutes results in the release of more than 90% of the Nvoc group from $\mathbf{T^{Nv}}$, thus producing the equivalent amount of **T** readily available for efficient template-assisted ligation reactions. Reactions 'in light' were thus illuminated for 15 minutes (365 nm; Tungsten lamp at 8W/cm$^2$) prior to the experiment, and followed as described above. All experiments were repeated at least two times and showed small variations (> 5%) in product formation between consecutive runs.

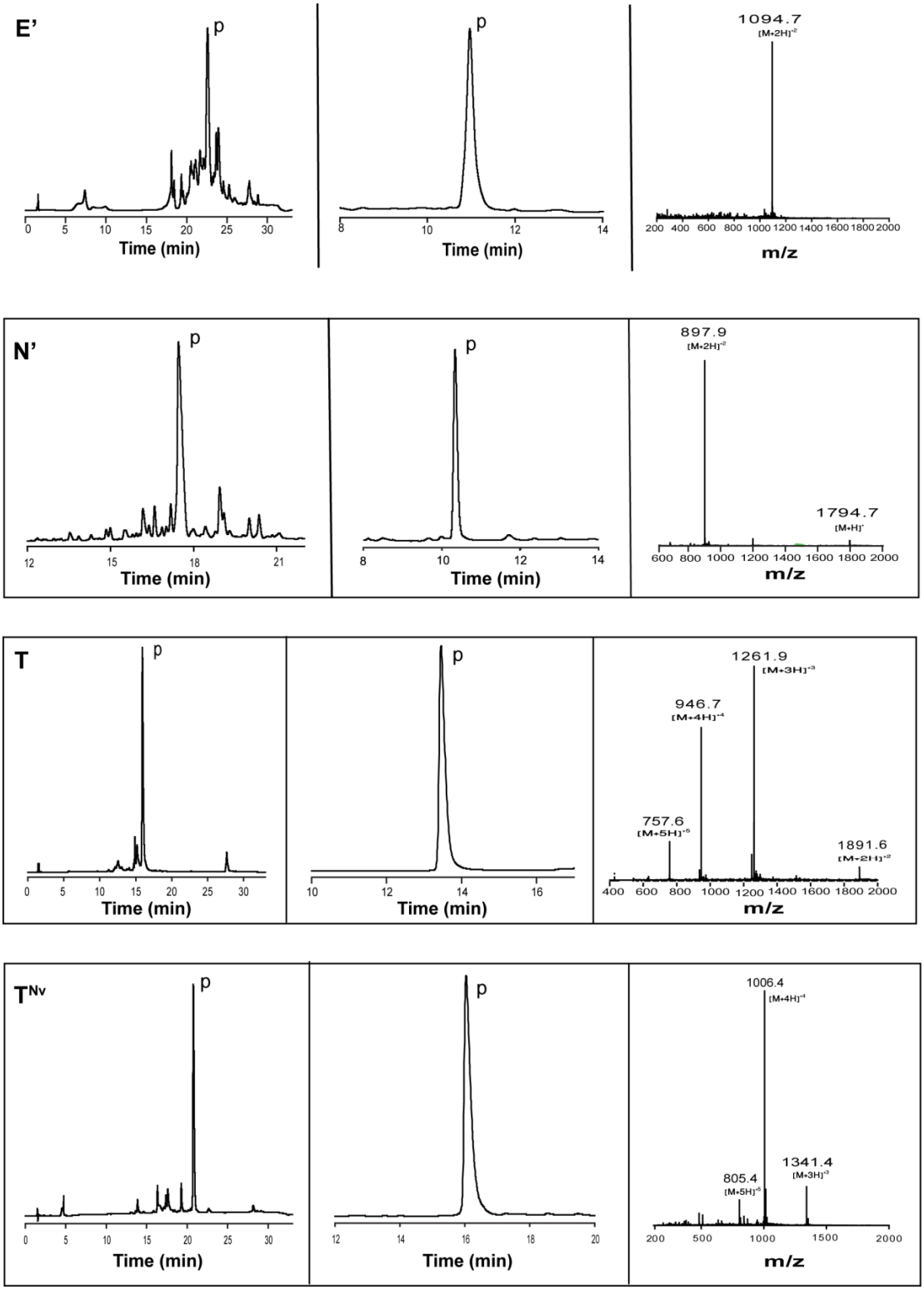


**Figure s1**: Peptide characterization by HPLC and LCMS. Peptides name are shown to the left of each panel. For each of the peptides, the panels show the HPLC chromatograms of the crude (left) and pure (middle) peptide and LCMS spectrum obtained for the pure peptide (right).

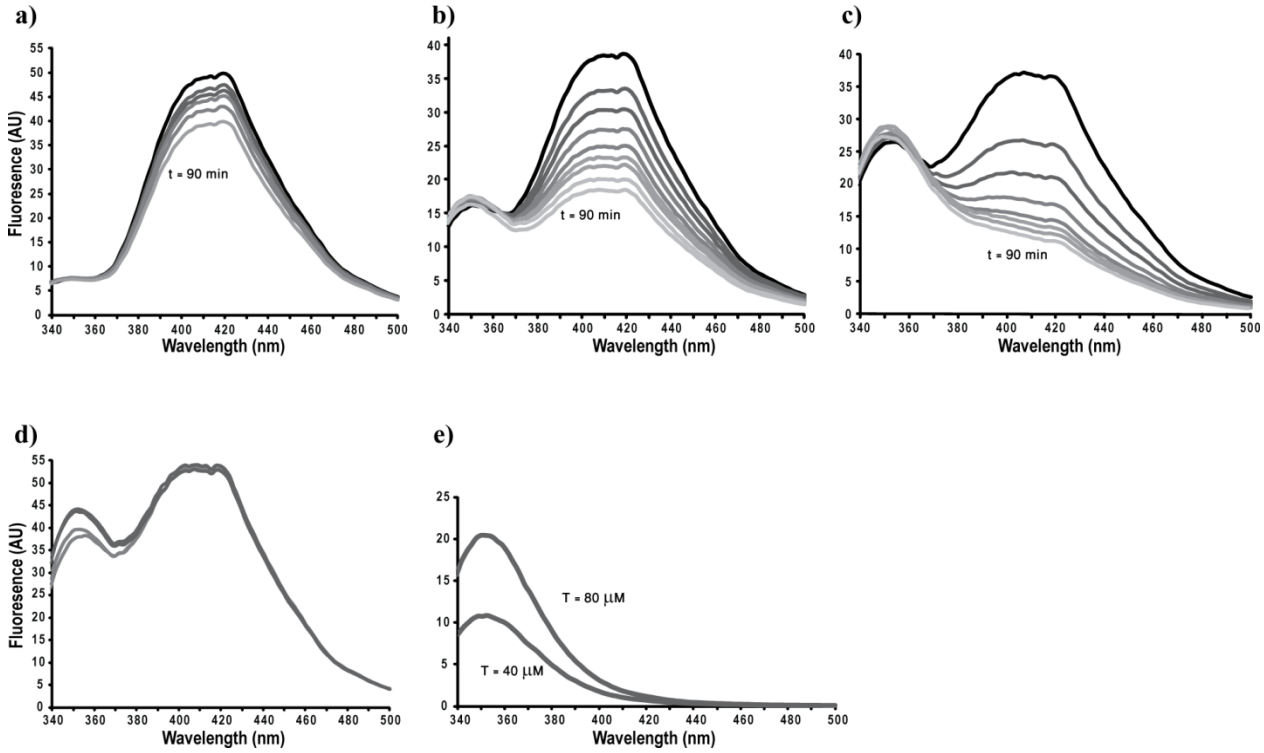


**Figure s2**: a - c) Fluorescence spectra obtained at different times (0 to 90 min) for reaction mixtures containing **E'**, **N'** (100 µM each) and 11 (a), 44 (b), and 87 (c) µM of **T**. d) Fluorescence spectra obtained at different times for reaction mixtures containing $\mathbf{E_{hyd}}$, **N'** (100 µM each) and 87 µM of **T**. These measurements served as control, and showed that the intermediate complex has a short life time that does not induce any fluorescence quenching. e) Fluorescence spectra obtained for 40 and 80 µM of **T**. These measurements were carried out to confirm that the second fluorescence maximum at ~355 nm was coming from **T** itself and not from any by-product of the studied reaction.