

**Supplementary Table 1. Secondary antibodies used in DNA–PAINT imaging.**

Target	Host	Specification and Commercial Source
Mouse	Donkey	Donkey Anti-Mouse IgG (H+L) (min X Bov, Ck, Gt, GP, Sy Hms, Hrs, Hu, Rb, Rat, Shp Sr Prot) (#715-005-151, Jackson ImmunoResearch)
Rabbit	Donkey	Donkey Anti-Rabbit IgG (H+L) (min X Bov, Ck, Gt, GP, Sy Hms, Hrs, Hu, Ms, Rat, Shp Sr Prot) (#711-005-152, Jackson ImmunoResearch)

**Supplementary Table 2. List of docking strand (PD) and corresponding imager strand (PI) used in DNA-PAINT-based imaging study.**

Docking Strand	Imager Strand
PD-1: Ab-TTTTAGGTAAAG	PI-1: CTTTACCTAA-dye
PD-2: Ab- TTTCTTCATTAC	PI-2: GTAATGAAGA-dye

**Supplementary Table 3. List of DNA-barcoded labelling agents used in DNA–PAINT imaging.**

Docking Strand	Imager Strand
Donkey-anti-mouse-PD-1	PI-1-Atto655 dye
Donkey-anti-rabbit-PD-2	PI-2-Atto655 dye

## Supplementary Information

### *Preparation of DNA-antibody conjugates*

1. Antibodies were purchased from commercial vendors (see table S1) and used for conjugation with DNA via thiol-maleimide coupling reactions.
2. Azide or any other preservatives were removed, and the antibody was buffer exchanged to phosphate buffered saline (PBS, pH 7.4) using Zeba spin columns (7000 MWCO).
3. The concentration of the antibody was measured and in a typical conjugation experiment.
4. 100  $\mu\text{g}$  antibody in 75  $\mu\text{L}$  PBS was mixed with 10 eq of maleimide-PEG2-NHS ester in 5  $\mu\text{L}$  of DMF (dimethyl formamide). The solution was then incubated at RT for 2 h.
5. Excess maleimide-PEG2-NHS cross-linker was removed from maleimide-activated antibodies using Zeba spin columns (7000 MWCO, eluent: PBS, pH 7.4) pre-equilibrated with PBS, pH 7.4.
6. In parallel, thiol-modified DNA oligos (10 nmol) were reduced using DTT in 0.1 mL H<sub>2</sub>O for 1 h at room temperature. The reduced DNA oligos were purified using NAP5 column (GE Healthcare, eluent: PBS, pH 7.4).
7. The maleimide-activated antibodies were mixed with the reduced form of their respective DNA oligos (10 eq) in PBS solution. The reaction was allowed to proceed for 12 h at 4°C.
8. DNA-antibody conjugates were purified and concentrated using Amicon Ultra Centrifugal Filter (100 kDa MWCO).

### *Preparation of DNA imager conjugates*

1. Amine-modified oligonucleotides were acquired from commercial sources (Integrated DNA technologies) and used for the coupling with NHS ester of Atto 655 fluorophore.
2. Amine-modified DNA (10 nmol, 1 mM in water) was taken in microcentrifuge tube.
3. 1 M NaHCO<sub>3</sub> solution was added to the tube for a final concentration of 0.1 M NaHCO<sub>3</sub> solution of DNA.
4. Atto 655 NHS ester (2.5 eq, 25 nmol stock in DMF) was added to the DNA solution and stirred at room temperature for 12 h.
5. The conjugated product was purified by reversed phase high performance liquid chromatography (HPLC) using TEAA buffer (buffer A: 5% acetonitrile in 95% 0.1 M TEAA, pH 7.0 and buffer B: 50% acetonitrile in 50% 0.1 M TEAA, pH 7.0) after passing through Zeba spin column.
6. Fluorophore-conjugated oligos were characterized by matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS).