# On-demand dopamine receptor activation via photoresponsive nanoparticle-dopamine conjugates in differentiated SH-SY5Y and transfected HEK293 cells

## Supplementary Data

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# 1. NMR spectra

## 1.1. 3-Azidomethyl-2-hydroxy-5-nitrobenzaldehyde (4)

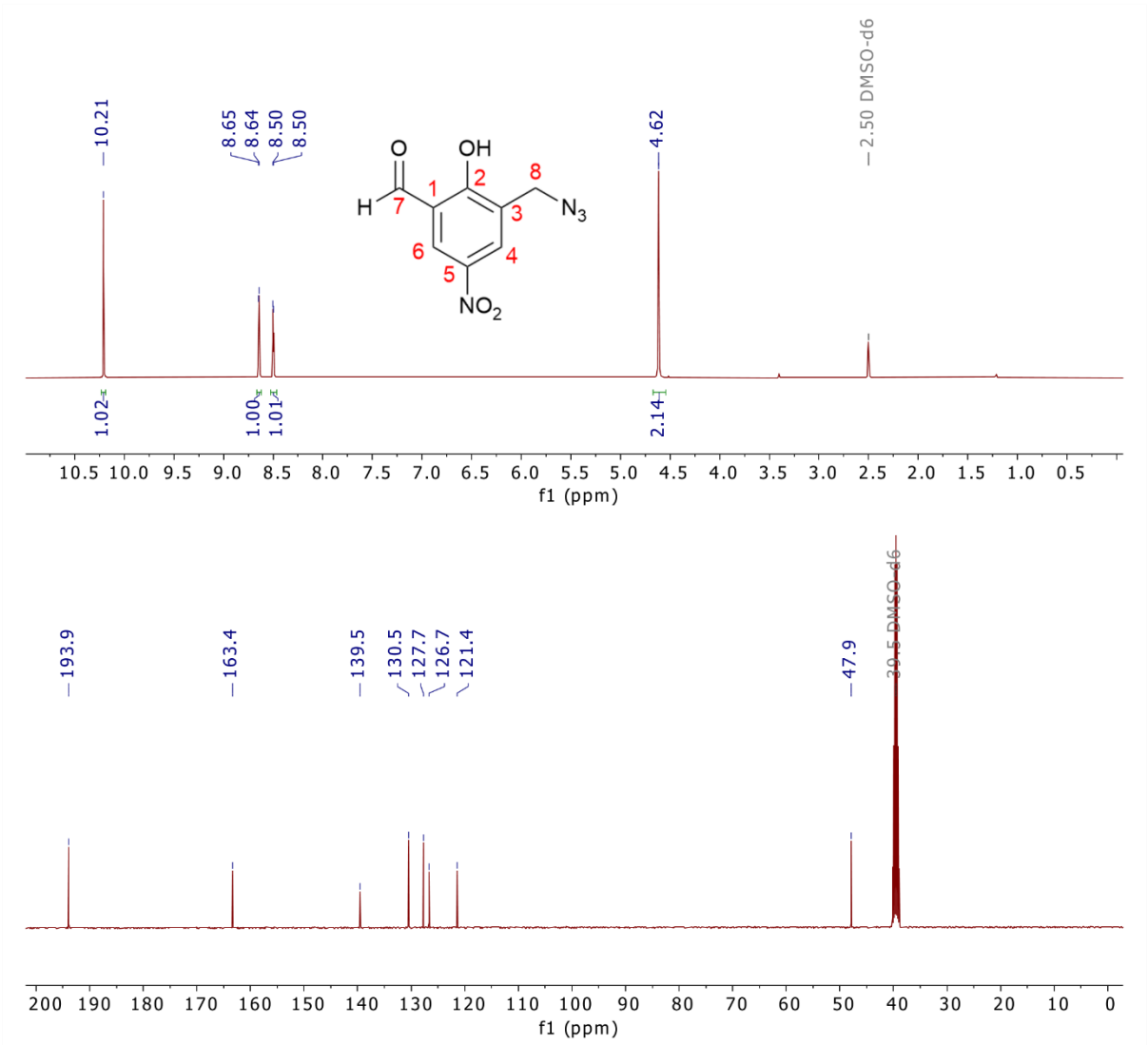


**Figure S1.** $^{1}H$ and $^{13}C$ NMR spectra of 3-azidomethyl-2-hydroxy-5-nitrobenzaldehyde in DMSO-$d_6$.

## 1.2. 2,3,3-Trimethyl-3*H*-indole-5-carboxylic acid (5)

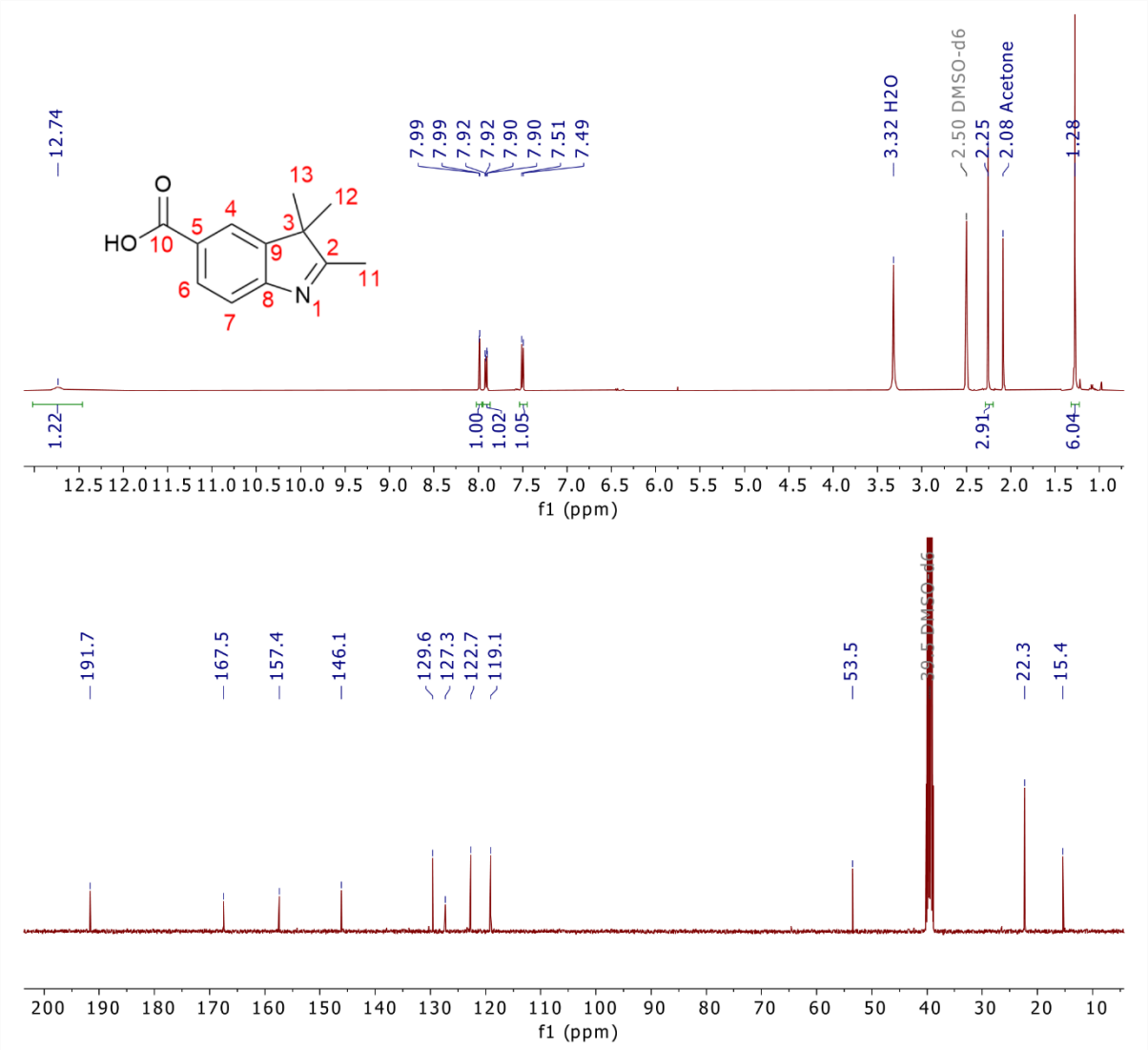


**Figure S2.** $^{1}H$ and $^{13}C$ NMR spectrum of 2,3,3-trimethyl-3*H*-indole-5-carboxylic acid in DMSO-$d_6$.

## 1.3. 5-Carboxy-1,2,3,3-tetramethyl-3*H*-indol-1-ium iodide (6)

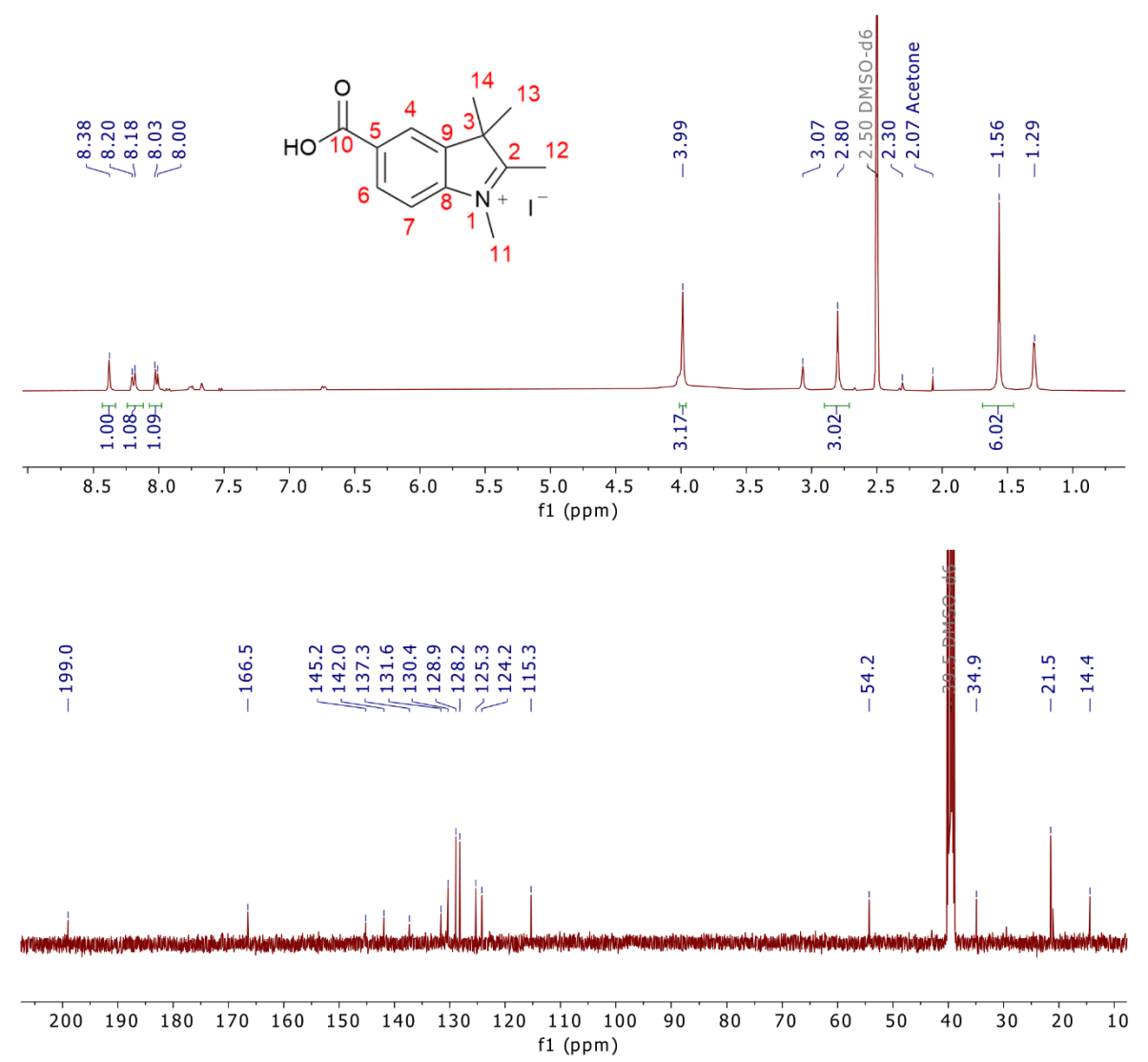


**Figure S3.** $^{1}H$ and $^{13}C$ NMR spectrum of 5-carboxy-1,2,3,3-tetramethyl-3*H*-indol-1-ium iodide in DMSO-$d_6$.

1.4. 8-(Azidomethyl)-1’,3’,3’-trimethyl-6-nitrospiro[chromene-2,2’-indoline]-5’- carboxylic acid (7)

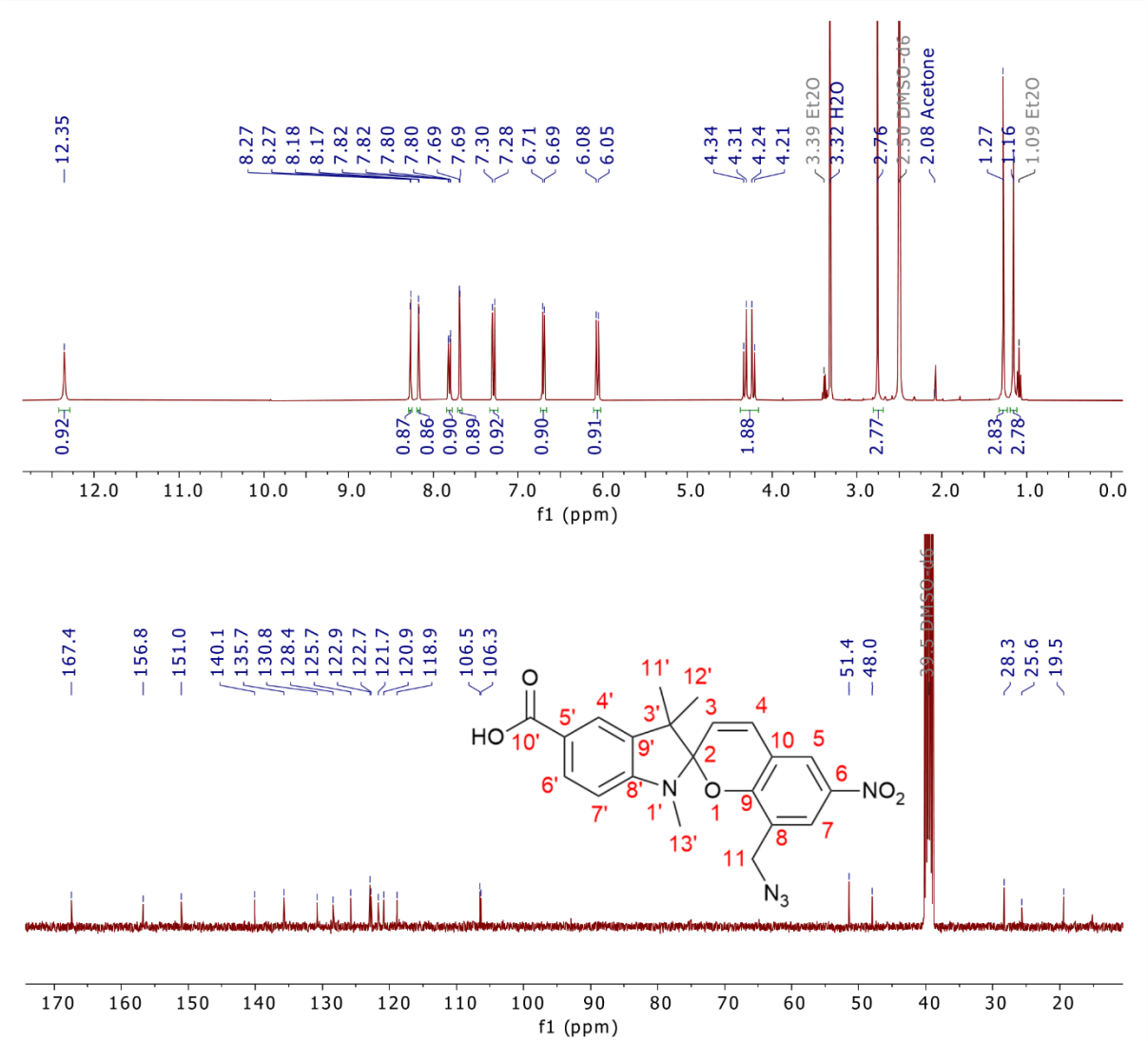


**Figure S2.** $^{1}H$ and $^{13}C$ NMR spectrum of 8-(azidomethyl)-1’,3’,3’-trimethyl-6-nitrospiro[chromene-2,2’-indoline]-5’- carboxylic acid in DMSO-$d_6$.

## 2. SiNP synthesis and fabrication

### 2.1. SiNP characterisation

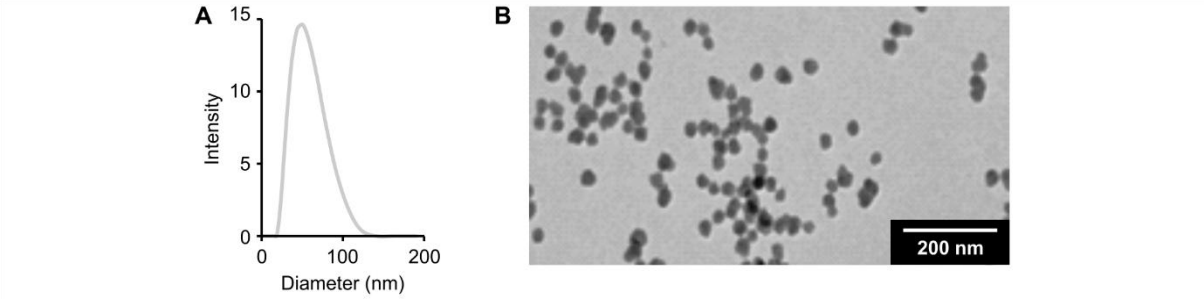


**Figure S5.** Unmodified silica nanoparticle (SiNP) characterisation via DLS (**A**) and TEM (**B**). The mean SiNP diameter is 45.9 nm ± 0.4 nm (PDI = 0.12 ± 0.01) and 33 ± 0.9 nm by DLS and TEM, respectively.

### 2.2. SiNP modification with spiropyran-dopamine conjugates

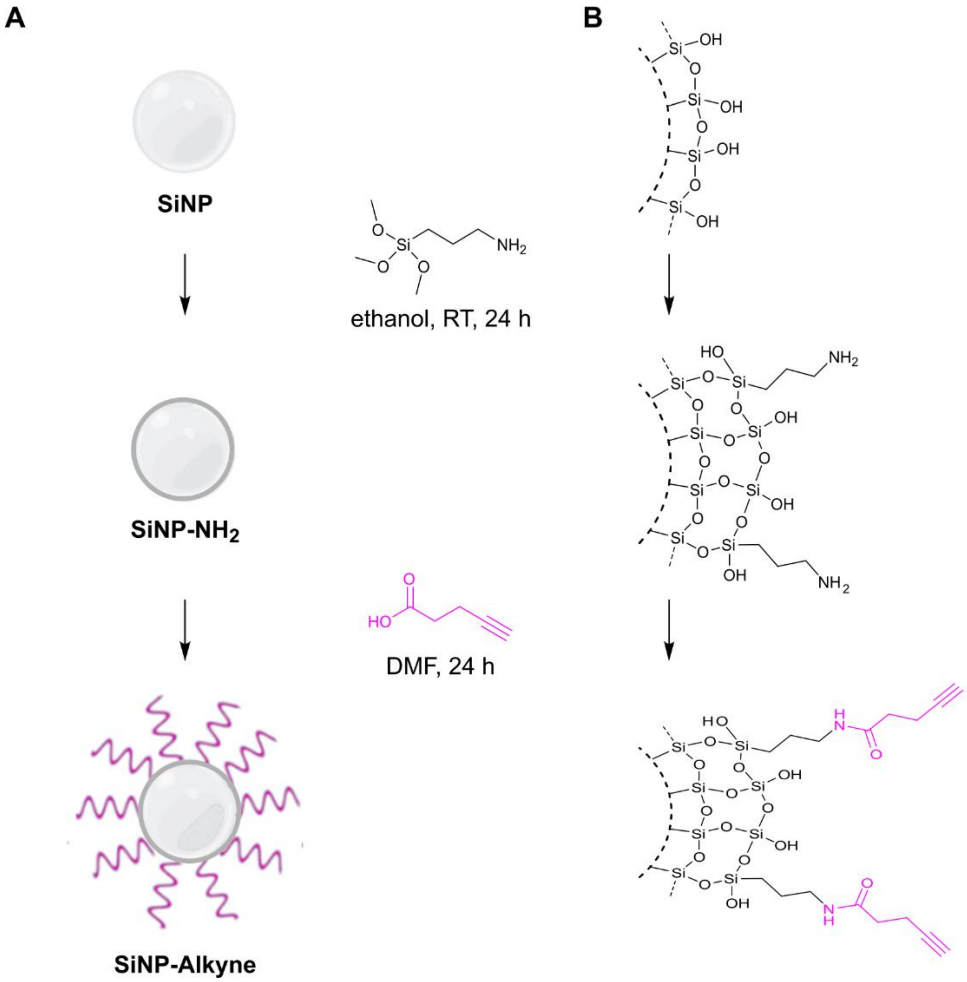


**Figure S6.** Surface modification steps to prepare alkyne terminated silica nanoparticles as precursors for the preparation of nanoparticles functionalised with dopamine-spiropyran conjugates. Silica nanoparticles (SiNP) are reacted with 3-(aminopropyl)trimethoxysilane (APTMS) to introduce amine groups on the particle surface (SiNP-$NH_2$). SiNP-$NH_2$ are reacted with pentynoic acid to introduce alkyne functionalities on the particle surface (SiNP-Alkyne). A. Schematic presentation of the synthesis procedure. B. Chemical functionalisation occurring at each particle modification step. Partly created in BioRender. Alghamdi, H. (2026) https://BioRender.com/c3yahay.

## 2.3. Modification of SiNPs with dopamine

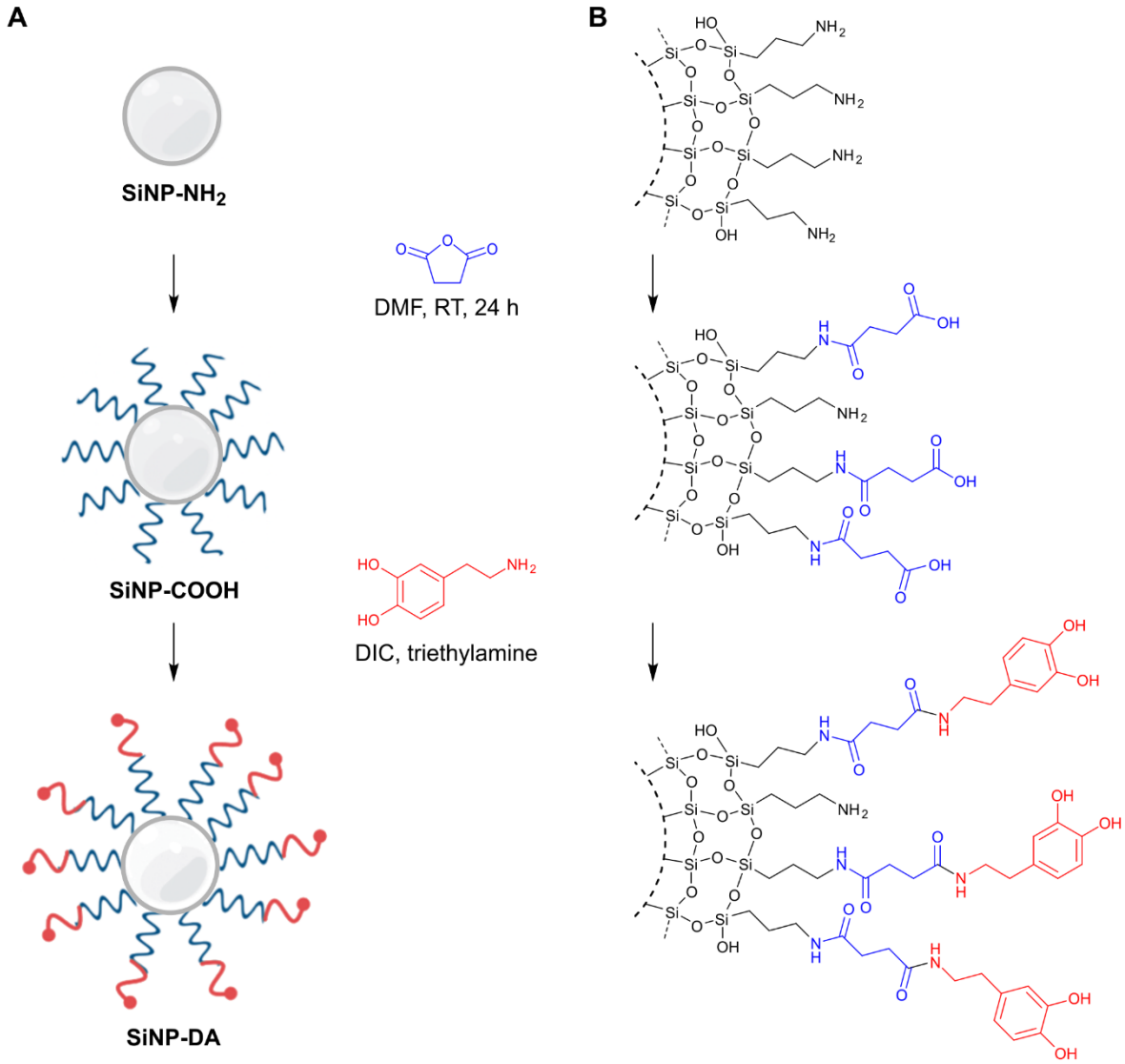


**Figure S7.** Surface modification steps to prepare dopamine functionalised nanoparticles. Amine functionalised particles (SiNP-$NH_2$) are reacted with succinic anhydride to introduce carboxylic acid groups on the particle surface (SiNP-COOH). Subsequently, dopamine is attached to the COOH groups to yield nanoparticles functionalised with dopamine (SiNP-DA). A. Schematic presentation of the synthesis procedure. B. Chemical functionalisation occurring at each particle modification step. Partly created in BioRender. Alghamdi, H. (2026) https://BioRender.com/c3yahay.

## 3. Interpretation of Infrared spectra for SiNP-SP-DA preparation

IR spectra for the SiNP surface modification to form SiNP-SP-DA are shown in Figure 3C. Unmodified SiNPs show typical peaks for silica. The strong peak at 1049 $cm^{-1}$ and adjacent shoulder and the medium peak at 950 $cm^{-1}$ are assigned to asymmetric Si-O-Si and Si-O-H stretching vibrations while the signal at 793 $cm^{-1}$ is caused by symmetric Si-O-Si stretching.[1] The introduction of amine groups (modification of SiNP with APTMS to yield SiNP-$NH_2$) is accompanied by the appearance of weak peaks at 2862 $cm^{-1}$ and 2945 $cm^{-1}$ assigned to $CH_2$ stretching vibrations. The peak at 1457 $cm^{-1}$ could be assigned either to C-H bending or symmetric $NH_3^+$ deformation, while the peak at 1651 $cm^{-1}$ corresponds to asymmetric $NH_3^+$ deformation. The presence of a very broad but weak feature from 2500 – 3500 $cm^{-1}$ indicates the presence of water and supports the suggestion that protonated $NH_3^+$ groups exist on the particle surface. Peaks around 3300 $cm^{-1}$ for N-H stretching that were previously reported for pure APTES[2] are not observed. This is consistent with previous reports where the absence of these peaks was attributed to the weak dipole moment and low quantities of amine groups in monolayers.[2]

The conversion of SiNP-$NH_2$ to SiNP-alkyne via amide bond formation between the amines and pentynoic acid shows a strong peak at 1648 $cm^{-1}$ attributed to the C=O stretching of the amide. The conversion of amines to amides is further supported by a significant reduction of the intensity of the peak at 1547 $cm^{-1}$ compared to the spectrum from SiNP-$NH_2$. Subsequent addition of SP to form SiNP-SP shows signals from the COOH and $NO_2$ groups of the SP derivative. The broad peak from 3000 – 3600 $cm^{-1}$ is assigned to O-H stretching vibrations, peaks at 1438 $cm^{-1}$ and 1406 $cm^{-1}$ to O-H bending vibrations and the peak at 1316 $cm^{-1}$ to C-OH stretch vibration from the COOH group. These assignments also match the peaks found for the COOH group in the IR spectrum of SP-COOH in ESI, Figure S9. The N-O stretching vibration from the $NO_2$ group of SP on SiNP-SP is assigned to the peak at 1389 $cm^{-1}$ while signals from the cyclic ether are assigned to C-O vibrations at 1256 $cm^{-1}$ and 1102 $cm^{-1}$. The 1256 $cm^{-1}$ peak likely includes a contribution from the C-N stretch vibration of the tertiary amine. These assignments are in line with previous observations on spiropyran conjugated to carbon nanoparticles.[3] The absence of a signal around 2100 $cm^{-1}$ for the stretch vibration of the azide further supports successful conjugation of SP to the particles via CuAAC. It should be noted that the peak positions of SP are known to change when transitioning to the MC form,[4] and that the data shown here likely represents a mixture of the SP and MC form.

After conversion of the COOH group to an amide bond to form SiNP-SP-DA, the intensity of the COOH peak at 3000-3600 $cm^{-1}$ reduces significantly while the intensity of the C=O stretching signal from the amide at 1655 $cm^{-1}$ increases significantly. Together with a notable presence of aromatic peaks at 2930 $cm^{-1}$ and 2865 $cm^{-1}$ that are also present in the SiNP-DA sample (ESI, Figure S9) and have been reported before for dopamine,[5] this data suggest successful particle modification with SP-DA.

## 4. Characterisation of dopamine functionalised nanoparticles

To attach dopamine to the nanoparticles via its $NH_2$ functionality, the $NH_2$ groups on the nanoparticles were first converted to COOH groups via reaction with succinic anhydride.[1] Successful conversion was supported by a decrease in the particle surface charge (Figure S8, SiNP-COOH) compared to the surface charge of SiNP-$NH_2$. Note that the surface charge measured for SiNP-$NH_2$ is slightly different to that reported in Figure 3 because they originate from different batches. The COOH functionalised nanoparticles were then reacted with dopamine; the resulting particles (SiNP-DA) showed an increase of the surface charge of the particles (Figure S8).

In the FTIR spectra (Figure S9), formation of SiNP-COOH from SiNP-$NH_2$ leads to the appearance of the O-H signals at 3400 $cm^{-1}$ from COOH. This signal disappears when DA is conjugated to SiNP-COOH while a new signal at 1735 $cm^{-1}$, assigned to the C=O stretch vibration for the ester confirms the dopamine conjugation.

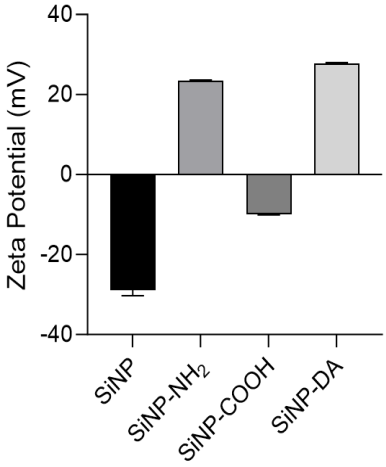


**Figure S8.** Zeta potential of SiNPs measured at different stages of the surface modification procedure to obtain SiNP-DA. Data represents the mean of 3 measurements. Errors represent standard deviations.

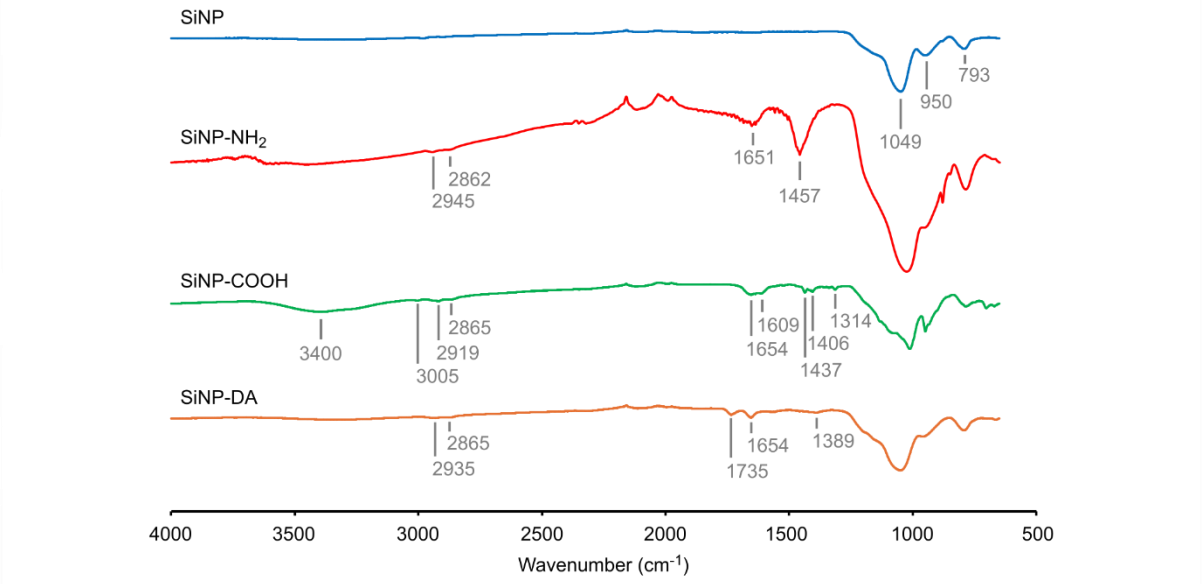


**Figure S9.** IR spectra of the nanoparticles obtained at each modification step to prepare SiNP-DA. Plain SiNPs (blue), amine modified SiNP (SiNP-$NH_2$, red), carboxylic acid modified SiNPs (SiNP-COOH, green) and dopamine modified SiNPs (SiNP-DA, orange).

## 5. Cell viability of SH-SY5Y and HEK293 cells

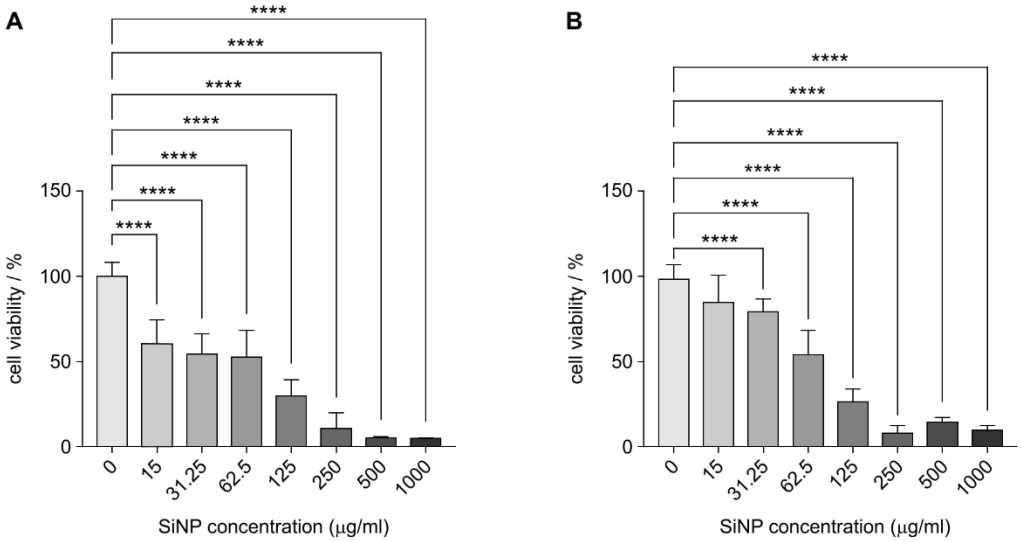


**Figure S10.** Cell viability of SH-SY5Y (A) and CRE/DRD1 transfected HEK293 (B) cells in the presence of unmodified SiNPs at concentrations ranging from 15 μg/ml to 1000 μg/ml measured with an MTT cytotoxicity assay after 24 h of cell culture. The data is shown as mean ± standard deviation (n = 3). Statistical difference from the unexposed control group was determined by one way ANOVA followed by a Tukey test. The asterisk indicates significance of **** $P < 0.0001$.

## 6. Activation of DRD1/CRE Co-transfected HEK293 cells

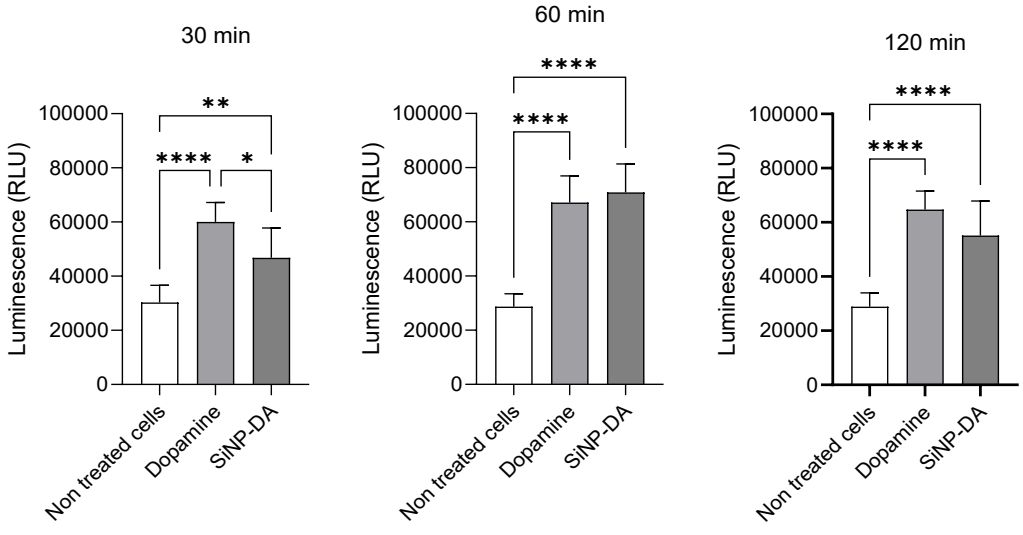


**Figure S11.** Activation of dopamine receptor as a result of different exposure times to DA and SiNP-DA determined using luciferase luminescence in CRE/DRD1 transfected HEK263 cells measure via one glow luciferase assay. [DA] = 1 mM; [SiNP-DA] = 64 μg/ml; exposure times: 30 min, 60 min, 120 min. Values are reported as mean ± SD of 3 biological repeats (n = 3). Statistical signifcance was tested using an ANOVA with a Tukey post test. The asterisk indicates significance of **** $P < 0.0001$, ** $P < 0.01$ and * $P < 0.05$

## 7. Molecular docking of dopamine into $D_1R$

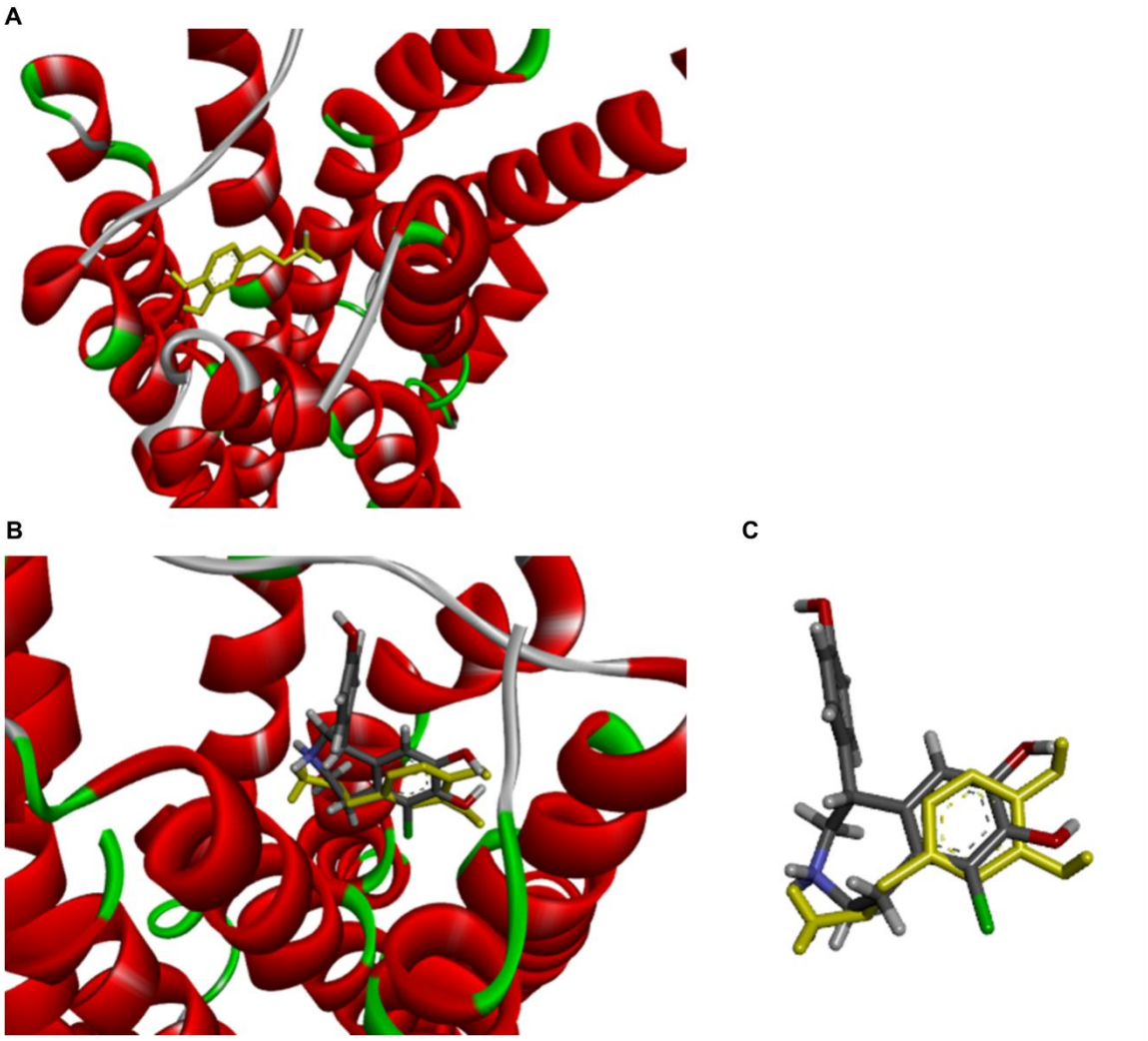


**Figure S12.** Homology model of $D_1R$ showing (A) dopamine (yellow) inside the $D_1R$ binding site and (B) the overlap between docked dopamine (yellow) and the co-crystalised ligand G3C (grey). **C** is a magnification of the dopamine / G3C overlap from **B**.

## 8. References


1. I. Petreanu, V. C. Niculescu, S. Enache, C. Iacob and M. Teodorescu, *ANALYTICAL LETTERS*, 2023, **56**, 390-403.
2. R. M. Pasternack, S. Rivillon Amy and Y. J. Chabal, *Langmuir*, 2008, **24**, 12963-12971.
3. B. Liao, P. Long, B. Q. He, S. J. Yi, B. L. Ou, S. H. Shen and J. Chen, *JOURNAL OF MATERIALS CHEMISTRY C*, 2013, **1**, 3716-3721.
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