Supporting Information

Development of a Photo-crosslinkable Diaminoquinazoline Inhibitor for Target Identification in *Plasmodium Falciparum*

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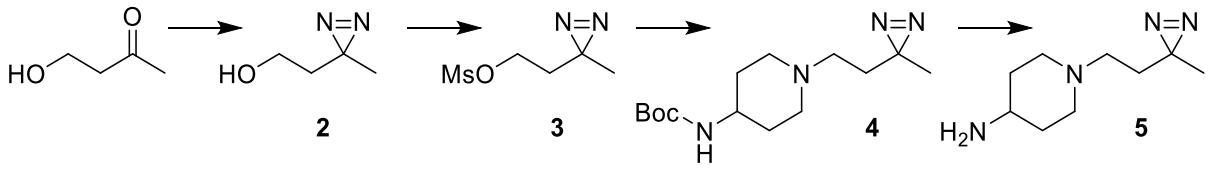
Table of contents

Synthesis of Probe 1

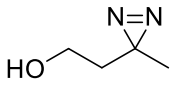
General Procedures

All reactions were performed under an atmosphere of dry nitrogen unless otherwise stated. Column chromatography was carried out using Fluorochem 60 silica gel (230–400 mesh, 0.040 0.063 mm). Thin layer chromatography (TLC) was performed on aluminium plates using Merck Kiesegel 60 F254 (230–400 mesh) fluorescent treated silica which were visualised under ultraviolet light (254 nm), or by staining with potassium permanganate or ninhydrin solution as appropriate. All ^{1}H and ^{13}C NMR spectra were recorded using Bruker 400 MHz spectrometers or Bruker AV 500 MHz at Imperial College London Department of Chemistry NMR Service. Chemical shifts (δ) are quoted in units of parts per million (ppm) downfield from tetramethylsilane and are referenced to a residual solvent peak. Coupling constants (J) are given in Hertz (Hz). High and low-resolution mass spectrometry (EI and ESI) were recorded on Micromass Platform II and Micromass AutoSpec-Q spectrometers.



Scheme S1. Synthesis of diazirine-containing side-chain.

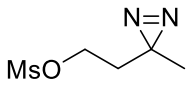
2-(3-methyl-3*H*-diaziren-3-yl)ethanol (2)



Synthesised by published procedures. Spectroscopic data correspond with those reported.[1] Ammonia (20 mL) was condensed at -78 °C into a flask containing 4-hydroxy-2-butanone (2.0 mL, 23.2 mmol) and the mixture was stirred at -78 °C for 4 h. Hydroxylamine-*O*-sulfonic acid (3.15 g, 27.9 mmol) was dissolved in methanol (20 mL) and the solution added to the reaction over 30 min at -78 °C. The mixture was allowed to slowly warm to room temperature and stirred for 16 h. The slurry was diluted with methanol (30 mL), filtered and washed further with methanol (2 x 15 mL). Triethylamine (6.5 mL, 46.6 mmol) was added to the filtrate, followed by iodine until the reddish-brown colour persisted, and the solution was stirred at room temperature for 2 h. The solvent was evaporated, and the residue diluted with diethyl ether (150 mL), washed with aqueous sodium thiosulfate solution (10%, 150 mL) and hydrochloric acid (1M, 100 mL), dried over magnesium sulfate and concentrated *in vacuo* to yield the product as a yellow solid (1.08 g, 46%), which was used in the next step without further purification.

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.56 (t, *J* = 6.3, 2H), 1.67 (t, *J* = 6.2, 2H), 1.10 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 57.8, 37.0, 24.2, 20.3

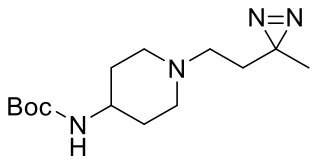
2-(3-methyl-3*H*-diaziren-3-yl)ethyl methanesulfonate (3)



Synthesised by published procedures.[2] A solution of **2** (0.60 g, 5.99 mmol) in dichloromethane (40 mL) was cooled to 0 °C. Methanesulfonyl chloride (0.7 mL, 9.04 mmol) and triethylamine (1.3 mL, 9.33 mmol) were added and the solution allowed to warm to room temperature and stirred for 5 h. The solution was washed with hydrochloric acid (1M, 50 mL), saturated aqueous sodium hydrogen carbonate solution (50 mL) and brine (50 mL). The organic layer was then dried over magnesium sulfate and concentrated *in vacuo* to yield the product as a yellow solid (930 mg, 87%), which was used in the next step without further purification.

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.16 (t, *J* = 6.2, 2H), 3.09 (s, 3H), 1.82 (t, *J* = 6.3, 2H), 1.13 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 64.4, 52.6, 37.7, 34.3, 19.9. **HRMS** (EI) *m/z* found $[M+NH_4]^+$ 196.0765, $C_5H_{14}N_3O_3$ requires 196.0756

tert-butyl (1-[2-(3-methyl-3*H*-diaziren-3-yl)ethyl]piperidin-4-yl)carbamate (4)

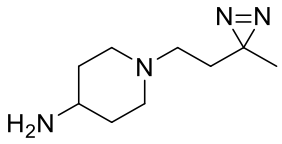


To a solution of **3** (900 mg, 5.1 mmol) and 4-(*N*-Boc-amino)-piperidine (1.01 g, 5.0 mmol) in acetonitrile (50 mL), potassium carbonate (2.11 g, 15 mmol) and sodium iodide (382 mg, 2.5 mmol) were added, and the mixture was heated to reflux for 3 h. The mixture was dissolved in dichloromethane (80 mL) and filtered. The filtrate was washed with water (100 mL) and brine (100 mL), dried over magnesium sulfate, concentrated *in vacuo* and purified by column

chromatography eluting with dichloromethane:methanol (97:3). The product was collected as a pale orange solid (921 mg, 65%).

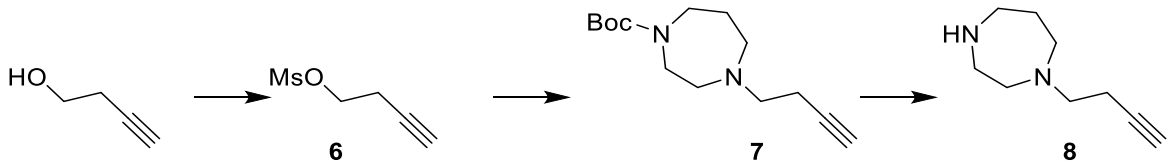
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.55-3.39 (m, 1H), 2.85 – 2.73 (m, 2H), 2.30-2.21 (m, 2H), 2.09-2.01 (m, 2H), 1.94 (d, *J* = 12.6, 2H), 1.54-1.49 (m, 2H), 1.46 (s, 9H), 1.42-1.36 (m, 2H), 1.04 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 155.2, 79.3 53.4, 52.8 (2C), 52.3, 47.7, 32.6 (2C), 32.3, 28.4 (3C), 20.0

1-[2-(3-methyl-3*H*-diaziren-3-yl)ethyl]piperidin-4-amine (5)



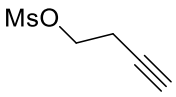
To a solution of **4** (900 mg, 3.2 mmol) in dichloromethane (12 mL), trifluoroacetic acid (12 mL) was added dropwise. The solution was stirred for 4 h, basified to pH 10 with aqueous sodium hydroxide (1M), and the organic layer separated. The aqueous layer was extracted with dichloromethane (2 x 50 mL) and the combined organic layers dried over magnesium sulfate and concentrated *in vacuo* to yield the product as a brown oil (552 mg, 95%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 2.87-2.77 (m, 2H), 2.72-2.60 (m, 1H), 2.30-2.22 (m, 2H), 2.06-1.93 (m, 2H), 1.87-1.77 (m, 2H), 1.55-1.48 (m, 2H), 1.46-1.33 (m, 2H), 1.04 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 53.4, 52.8, 52.4 (2C), 48.7, 35.9 (2C), 32.3, 20.0



Scheme S2. Synthesis of alkyne-containing side-chain.

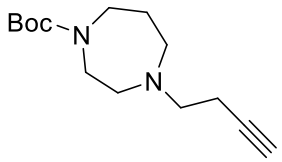
But-3-ynyl methanesulfonate (6)



Synthesised by published procedures. Spectroscopic data correspond with those reported.[3] 3-Butyn-1-ol (1.51 mL, 19.9 mmol) was dissolved in dichloromethane (25 mL) and triethylamine (4.2 mL, 30.1 mmol) was added. A solution of methanesulfonyl chloride (2.3 mL, 29.7 mmol) in dichloromethane (5 mL) was added dropwise and the mixture stirred for 16h at room temperature. The reaction was quenched with hydrochloric acid (2M, 20 mL) and the organic phase separated. The aqueous layer was extracted with dichloromethane (20 mL) and the organic layers combined, washed with hydrochloric acid (1M, 30 mL), saturated sodium bicarbonate solution (30 mL) and brine (30 mL), dried over magnesium sulfate, and concentrated *in vacuo* to yield alkyne **6** as a yellow-orange oil (2.96 g, 91%) which was used in the next step without further purification.

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.34 (t, *J* = 6.7, 2H), 3.08 (s, 3H), 2.69 (td, *J* = 6.7, 2.7, 2H), 2.09 (t, *J* = 2.7, 1H). **HRMS** (ESI) m/z found $[M+H]^+$ 149.0269, $C_5H_9O_3S$ requires 149.0272

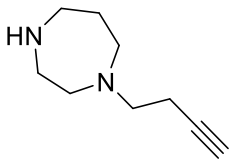
***tert*-Butyl 4-(but-3-ynyl)-1,4-diazepane-1-carboxylate (7)**



To a solution of **6** (1.43 g, 9.7 mmol) and 1-Boc-hexahydro-1,4-diazepine (2.0 mL, 10 mmol) in acetonitrile (20 mL), potassium carbonate (4.00 g, 29 mmol) and sodium iodide (750 mg, 5.0 mmol) were added, and the mixture was heated to reflux for 3 h. The mixture was dissolved in dichloromethane (80 mL), filtered, washed with water (100 mL) and brine (100 mL), dried over magnesium sulfate, and concentrated *in vacuo*. The crude product was purified by column chromatography eluting with dichloromethane:methanol (95:5) to provide the product as a yellow oil (1.47 g, 60%).

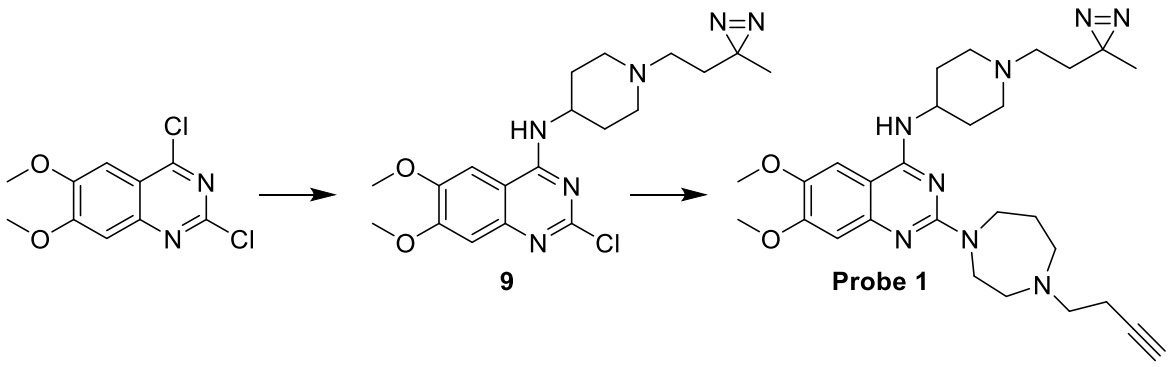
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.54-3.41 (m, 4H), 2.80-2.64 (m, 6H), 2.43-2.30 (m, 2H), 2.04-1.97 (m, 1H), 1.91-1.78 (m, 2H), 1.48 (s, 9H)

1-(but-3-ynyl)-1,4-diazepane (8)



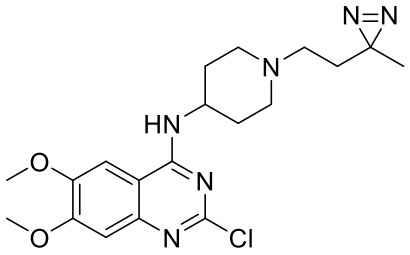
To a solution of **7** (1.3 g, 5.15 mmol) in dichloromethane (12 mL), trifluoroacetic acid (12 mL) was added dropwise. The solution was stirred for 4 h, basified to pH 10 with aqueous sodium hydroxide (1M), and the organic layer separated. The aqueous layer was extracted with dichloromethane (2 x 50 mL) and the combined organic layers dried over magnesium sulfate and concentrated *in vacuo* to yield the product as an orange oil (730 mg, 93%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.10-3.03 (m, 4H), 2.85-2.78 (m, 6H), 2.39 (td, *J* = 7.4, 2.7, 2H), 2.00 (t, *J* = 2.7, 1H), 1.88 (p, *J* = 5.9, 2H). **HRMS** (ESI) m/z found $[M+H]^+$ 153.1390, $C_9H_{17}N_2$ requires 153.1392



Scheme S3. Synthesis of probe **1**.

2-chloro-6,7-dimethoxy-N-(1-[2-(3-methyl-3H-diaziren-3-yl)ethyl]piperidin-4-yl)quinazolin-4-amine (9)

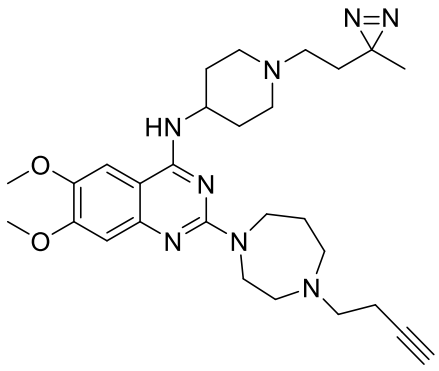


2,4-dichloroquinazoline (711 mg, 2.7 mmol) was dissolved in anhydrous tetrahydrofuran (30 mL). Amine **5** (500 mg, 2.7 mmol) was then added, followed by *N,N*-diisopropylethylamine (1.45 mL, 8.3 mmol). The reaction mixture was left to stir at 30 °C for 20 h, and concentrated *in vacuo*. The resulting residue was dissolved in dichloromethane (100 mL),washed with water (100 mL) and brine (100 mL), dried over magnesium sulfate, concentrated *in vacuo* and purified by column chromatography eluting with a gradient of dichloromethane:7N

ammonia in methanol (99:1 → 97:3) to yield the product as a pale yellow solid (168 mg, 15%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.13 (s, 1H), 6.84 (s, 1H), 5.47 (d, *J* = 7.8, 1H), 4.35-4.23 (m, 1H), 4.00 (s, 3H), 3.97 (s, 3H), 2.93-2.82 (m, 2H), 2.36-2.28 (m, 2H), 2.23-2.13 (m, 4H), 1.69-1.57 (m, 2H), 1.56-1.50 (m, 2H), 1.06 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 159.1, 156.2, 155.0, 149.0, 148.1, 107.3, 106.7, 99.6, 77.2, 56.4, 56.3, 52.8, 52.2 (2C), 48.3, 32.2 (2C), 25.0, 20.0.

2-[4-(but-3-yn-1-yl)-1,4-diazepan-1-yl]-6,7-dimethoxy-*N*-(1-[2-(3-methyl-3*H*-diaziren-3-yl)ethyl]piperidin-4-yl)quinazolin-4-amine (Probe 1)



Quinazoline **9** (100 mg, 0.25 mmol) and amine **8** (120 mg, 0.79 mmol) were dissolved in toluene (5 mL), and the mixture was heated to reflux for 18 h. The solvent was removed *in vacuo* and the crude product was purified by column chromatography eluting with a gradient of dichloromethane:7N ammonia in methanol (100:0 → 98:2) to yield probe **1** as a pale-yellow oil (5.0 mg, 4%), which was >99% pure by LC-MS analysis.

^{1}H NMR (400 MHz, MeOD) δ 7.44 (s, 1H), 6.94 (s, 1H), 4.17-4.13 (m, 1H), 3.93 (s, 3H), 3.91 (s, 3H), 3.86 (t, J = 6.3, 2H), 3.06-2.98 (m, 2H), 2.89 (t, J = 5.0, 2H), 2.80-2.70 (m, 6H), 2.47-2.34 (m, 4H), 2.28 (t, J = 2.7, 1H), 2.19-2.08 (m, 4H), 2.08-1.95 (m, 2H), 1.77-1.73 (m, 2H),

1.62-1.54 (m, 2H), 1.06 (s, 3H). **HRMS** (ESI) m/z found $[M+H]^+$ 521.3370, $C_{28}H_{41}N_8O_2$ requires 521.3352

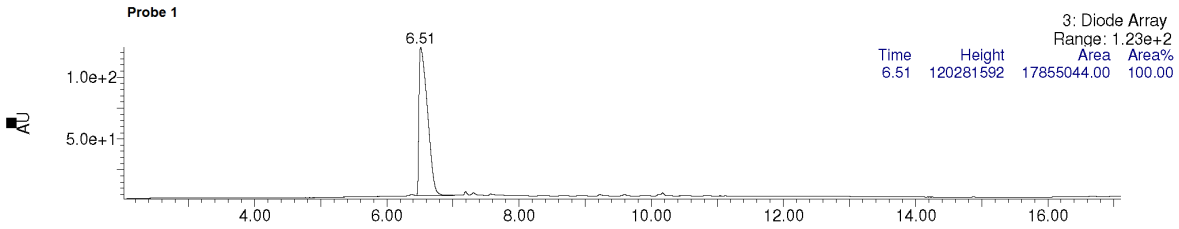


Figure S1. The Purity of the probe **1** was assessed by LC-MS analysis on a Waters HPLC system equipped with a 2767 autosampler, a 515 pump, a 3100 mass spectrometer with ESI, and a 2998 Photodiode Array Detector (detection at 200-600 nm). The system was fitted with a Waters XBridge C18 column (4.6 mm × 100 mm). The flow rate was 1.2 mL/min with an 18 min runtime. A gradient of water/acetonitrile 95:5 to 2:98, both containing 0.1% of formic acid, was used as the mobile phase. The purity of the final product was 100%.

In Vitro *P. falciparum* Parasite Killing Activity

Compounds were tested against drug sensitive *P. falciparum* 3D7 strain parasites using a three-day SYBR Green I based assay. Parasites were cultured at 2% hematocrit with an initial parasitemia of 0.5–0.8% in RPMI 1640 containing 0.5% Albumax. Compounds were initially screened at a range of 7 concentrations starting from 2 μM (2-time serial dilutions) in duplicate wells in 100 μL cultures in a 96-well format, and concentration range was then adapted in a second and third experiment when needed. Parasites were allowed to grow with probe **1** or BIX-01294 for three days before 100 μL of SYBR Green I lysis buffer (20mM Tris pH 7.5, 5 mM EDTA, 0.008% saponin, 0.08% Triton X-100, 2x SYBR Green I) were added. Lysed parasite samples were measured for total SYBR Green I fluorescence (λ_{ex}: 488 nm, λ_{em}: 520 nm), and the obtained data fitted to a standard inhibition curve with variable Hill slope. Values are quoted as the mean ± standard deviation from replicate experiments.

Cell Culture and Lysate Preparation

Plasmodium falciparum 3D7 cultures were maintained at ~2% haematocrit in RPMI-1640-HEPES medium with 0.5% Albumax II, 0.25% sodium bicarbonate, 50 mg L^{-1} hypoxanthine and 25 mg L^{-1} gentamicin. Parasites were incubated at 37 °C in an atmosphere of 5% oxygen, 5% carbon dioxide and 90% nitrogen. Parasites were harvested at 30-40 hours post invasion (trophozoite/schizont stage) at ~6-8% parasitaemia.[4] For saponin lysis, parasites were first spun down (800 *g*, 5 min) and the supernatant removed. Parasites were lysed with 0.075% saponin in PBS at room temperature for 10 min, and then pelleted (2800 *g*, 4 °C, 10 min). The supernatant was removed, and the pellet washed three times in cold PBS (2800 *g*, 4 °C, 10 min). The pellets were stored at -80 °C.

Parasites were lysed by nitrogen cavitation.[5] Saponin pellets were thawed on ice, and then suspended in an equal volume of cold PBS with EDTA-free protease inhibitors (Roche cOmplete, 1 tablet in 40 mL PBS). The suspension was added to a Parr 439 45 mL cell disruption vessel at 4 °C, the chamber of sealed and a nitrogen pressure of 1500 psi maintained for 1 h. The pressure was released, the suspension centrifuged (10,000 *g*, 4 °C, 10 min) and the lysate-containing supernatant collected. The protein concentration of the lysate was measured using a NanoDrop (Thermo Fisher), diluted with cold PBS to 3 mg mL^{-1} and stored at -80 °C.

Mass Spectrometry Analysis

LC-MS/MS runs were performed on an Easy nLC-1000 system coupled to a QExactive mass spectrometer *via* an easy-spray source (all Thermo Fisher Scientific) as previously described.[6,7] 3 µL injections of peptide sample were separated on a reverse phase Acclaim PepMap RSLC column (50 cm x 75 µm inner diameter, Thermo Fisher Scientific) across a 2 h acetonitrile gradient containing 0.1% formic acid, using a flow rate of 250 $nL \cdot min^{-1}$. The

QExactive was operated in data-dependent mode with survey scans acquired at a resolution of 75,000 at *m/z* 200 (transient time 256 ms). Up to 10 of the most abundant isotope patterns with a charge of +2 or higher from the survey scan were selected with an isolation window of 3.0 *m/z* and fragmented by higher-energy collision dissociation (HCD) with normalized collision energies of 25. The maximum ion injection times for the survey scan and the MS/MS scans (acquired with a resolution of 17 500 at *m/z* 200) were 20 and 120 ms, respectively. The ion target value for MS was set to 10^6 and for MS/MS to 10^5, and the intensity threshold was set to 8.3×10^2.

The data were processed with MaxQuant version 1.5.7.4,[8,9] and the peptides were identified from the MS/MS spectra searched against the *Plasmodium falciparum* Swissprot+TrEMBL database (September 2017) using the Andromeda search engine. Cysteine carbamidomethylation was selected as a fixed modification and methionine oxidation as a variable modification. Up to two missed cleavages were allowed. Peptides and proteins were identified utilising a 0.01 false discovery rate (FDR), with "Unique and razor peptides" mode selected (razor peptides are uniquely assigned to protein groups and not to individual proteins). Other parameters were used as pre-set in the software. Data was analysed using Perseus version 1.5.6.0[10] and Microsoft Office Excel 2016. Peptides categorised by MaxQuant as 'potential contaminants', 'only identified by site' or 'reverse' were filtered, and the processed LFQ intensities transformed in Log2(LFQ). Two valid values were required for identification. Missing values were replaced from a normal distribution with width 0.3 and downshift 1.8. (Table S1). Correlations between duplicates are shown in Figure S3. identified genes in terms of their viability to disruption by transfection, using the PhenoPlasm database (http://phenoplasm.org/).[11] For GO term enrichment analysis, the essential proteins significantly identified by probe **1** (Table S1) were input into PANTHER (http://www.pantherdb.org) [12-14] to analyse them according to their protein class (Figure S4).

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE[15] partner repository with the dataset identifier PXD008164.

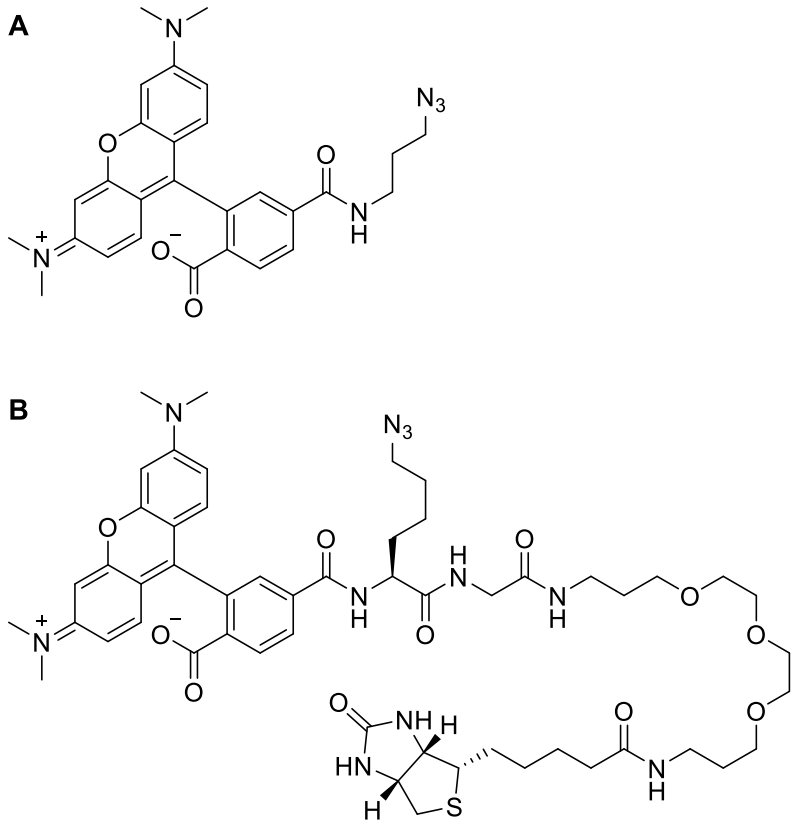


Figure S2. Structure of capture reagents for copper-catalysed click-chemistry. (A) TAMRA-Azide (AzT) (B) Azide-TAMRA-Biotin (AzTB)[6,16].

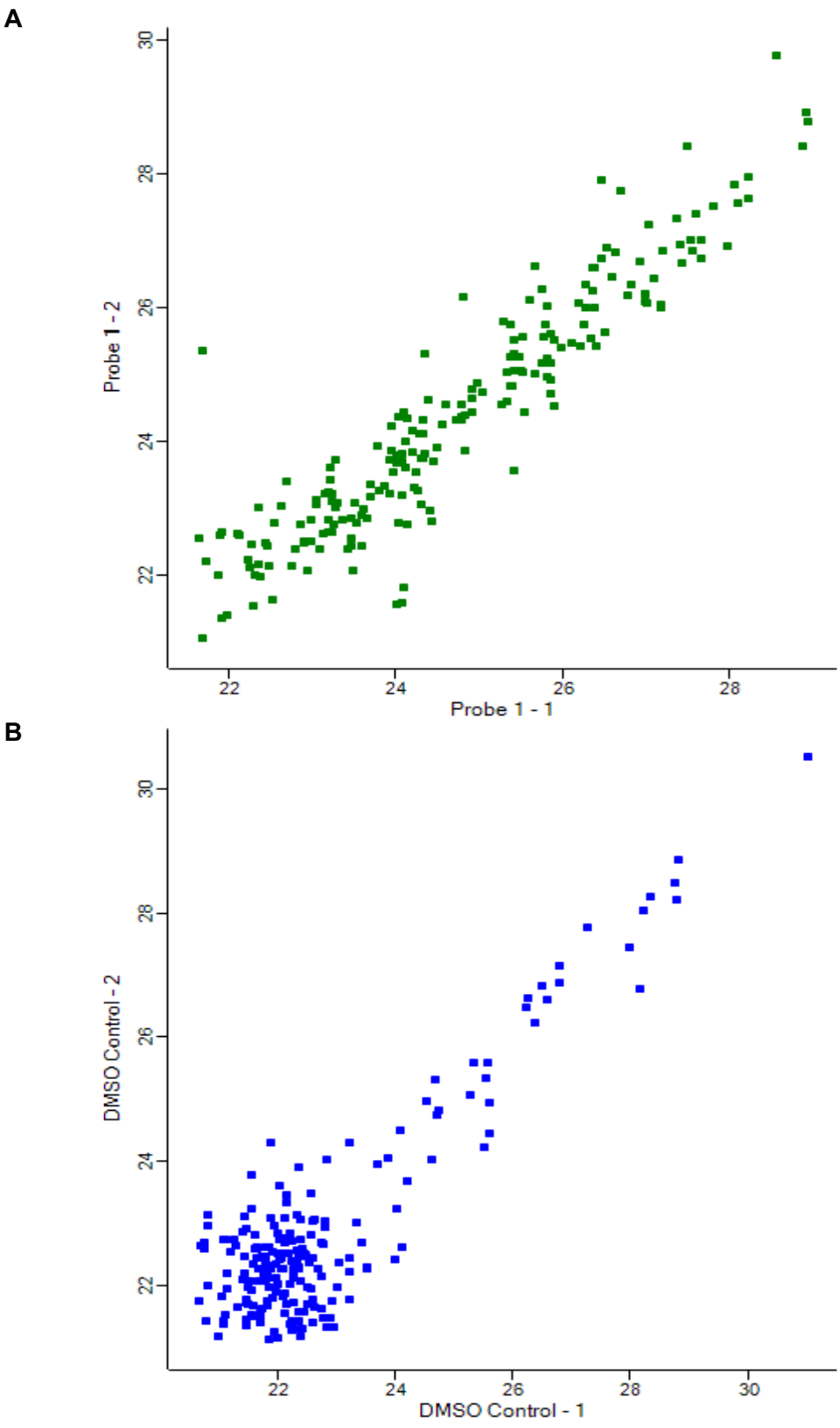


Figure S3. Scatter plots to show correlation of duplicate samples containing (A) probe **1**, and (B) DMSO controls, for avidin pull-down and target ID. Axes are log2(LFQ intensity) after missing values replaced from normal distribution (width 0.3 and downshift 1.8)

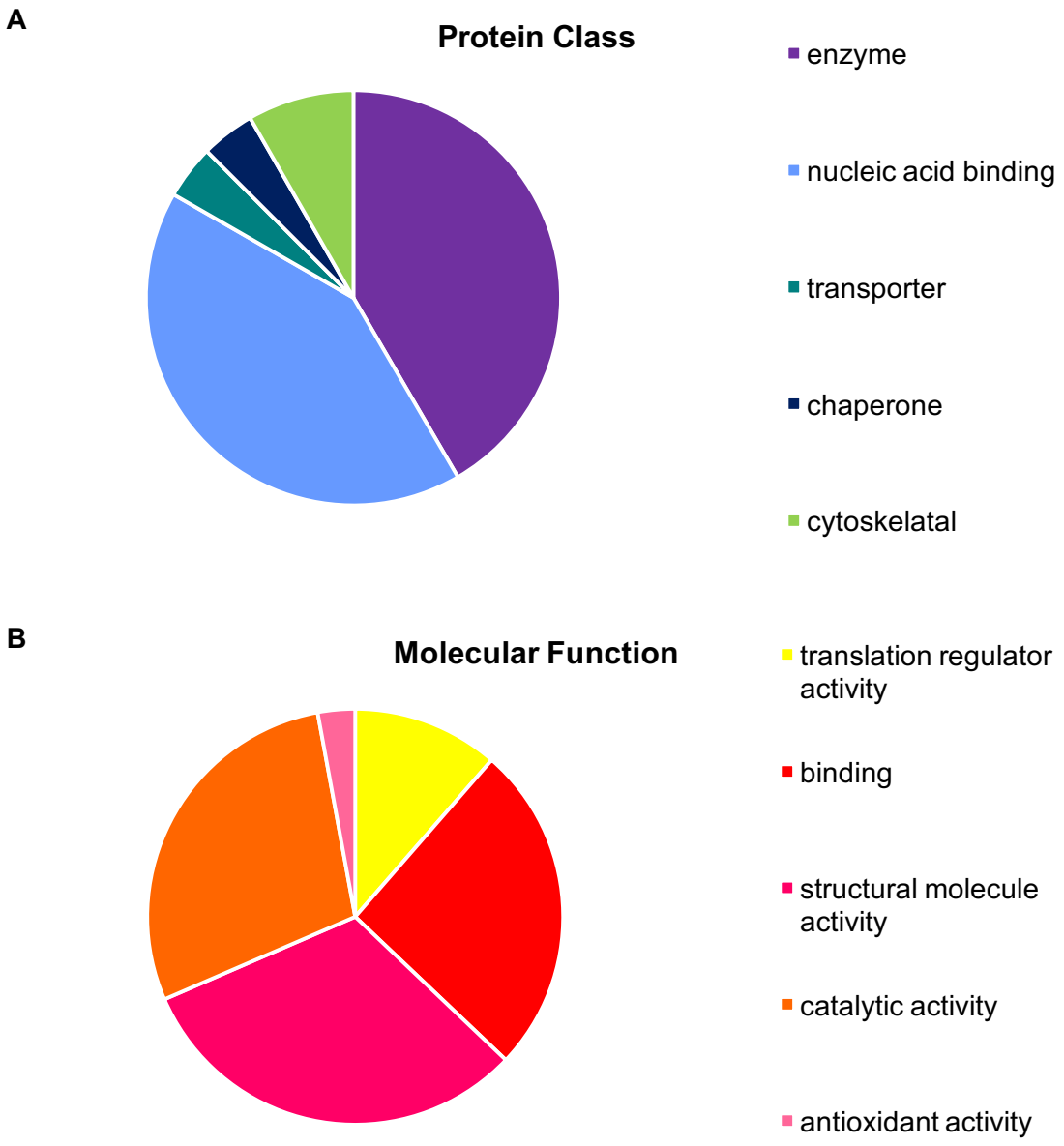


Figure S4. Analysis of protein functional class analysis of the 38 essential significant targets engaged by probe **1** (Table S1), based on gene ontology (GO) annotations using the protein analysis through the evolutionary relationships (PANTHER) classification system, which includes both experimental data and bioinformatics algorithms. (A) Analysis of protein class. (B) Analysis of molecular function.

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