Supporting Information

Traceless Immobilization of Analytes for High-Throughput Experiments with SAMDI Mass Spectrometry

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Materials & Methods

Reagents. All reagents were obtained from Sigma-Aldrich, unless otherwise noted. Disulfides used to form self-assembled monolayers were purchased from ProChimia Surfaces (Sopot, Poland) or Chemtos (Round Rock, TX). The P450 CYP2C9*1 enzyme was purchased from Corning, (Tewksbury, MA). Deionized (DI) water was prepared by a Millipore filtration unit and used for all experiments.

Solid Phase Peptide Synthesis of Photoaffinity linkers. Peptide synthesis was performed according to standard protocols. MBHA-FMOC-Rink Amide Resin was placed in a column with filters plugged. The FMOC was deprotected with 20% piperidine in dimethylformamide (DMF) for 20 min; the solvent was filtered with a vacuum manifold. The resin was then washed 5 times with DMF. A solution contained 4:4:8 parts of amino acid, benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP), and N-methyl morpholine was prepared and applied to the resin for 30 min. The solutions were then filtered; the resin was washed five times with DMF, and then the process was repeated. After the last step of the coupling, a cleavage cocktail was applied to the resin containing 95% trifluoroacetic acid (TFA), 2.5% H_2O, and 2.5% triethylsilane (TES), and the resin was incubated for 2 hours. The solution was filtered with cotton to remove the resin and the remaining solution was evaporated under a stream of nitrogen. The residues were purified with liquid extraction with diethyl ether, dried with nitrogen, and lyophilized overnight. The first coupling step was for a FMOC-cysteine (Trt), the second for FMOC-Lys$(Me)_3$-OH chloride, and the last for the photoaffinity group with a carboxylic acid. The diazirine group used was 4-[3-(trifluoromethyl)-3H-diaziren-3-yl]benzoic acid (TDBA), the benzophenone group used was (RS)-2-(3-benzoylphenyl)-propionic acid, and the aryl azide group used was 4-azidobenzoic acid. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance III 500 MHz spectrometer.

Preparation of Array Plates. Stainless steel plates (18 X 18 mm) were washed in hexanes, ethanol, DI water, ethanol again, and then dried under nitrogen. The plates were modified by evaporation of 5 nm of titanium at a rate of 0.02 nm sec^{-1} (Electron Beam, Thermionics Laboratory Inc. Hayward, CA) at a pressure of 1–5 x 10^{-6} mTorr through an aluminum mask with holes in the geometry of a standard 384-well array with 2.8 mm circles. A layer of 35 nm of gold was then deposited at 0.05 nm sec^{-1}. The plates were stored under vacuum until use.

Monolayer formation. The gold-coated plates were immersed in an ethanolic solution of two alkyl-disulfides (0.2 mM) where one end was functionalized with a maleimide group and the other with a tri(ethylene glycol) group in a 1:4 ratio for 16 hours at room temperature. The chips were then washed with ethanol, DI water, ethanol again, and then dried under a stream of nitrogen. A solution of the photoaffinity linker (100 µM in 100 mM tris buffer, pH 7.5) was applied to the plates for 30 min at 37°C to immobilize the 3-trifluoromethyl-3-phenyl-diazirine (TPD) group to the monolayer array.

Photoimmobilization of Molecules. Small volumes of solutions of molecules or reaction mixtures (1 μL) were transferred onto the plates having an array of monolayers presenting the TPD group. The solutions were dried over air or in a vacuum desiccator. The plates were placed under a UV lamp sealed with nitrogen gas for 10 min at 1 J/cm^{-2}. The UV lamp used was the UVP Cross-linker 1000L with 365 nm tubes. After irradiation, the plates were rinsed with ethanol, DI water, and ethanol again. Then the MALDI-matrix, 10 mg/mL solution of 2,4,6-trihydroxyacetophenone in acetone, was applied to the monolayer for analysis with the AB Sciex 5800 MALDI-TOF/TOF mass spectrometer in the reflector positive mode.

Enzyme Reactions. Reactions of CYP2C9-mediated oxidation of tolbutamide were performed in 15-μL reaction mixtures containing tolbutamide (25−1250 μM), 100 mM tris buffer, pH 7.5, P450 CYP2C9*1 (0.4 μM) and the NADPH-regenerating system (1.3 mM NADP+, 3.3 mM glucose-6-phosphate, 3.3 mM magnesium chloride, and 0.4 U/mL glucose-6-phosphate dehydrogenase). Mixtures were preincubated at 37 °C for 5 min and the reactions were initiated by the addition of an NADPH-regenerating system and incubated at 37 °C. Reactions were terminated at various time points (0, 30, 60 min) by the addition of HCl (3M, 5 μL). Proteins were removed by pelleting with high-speed centrifugation at 10,000 g for 5 min. The supernatants were extracted for tolbutamide and hydroxy-tolbutamide with diethyl ether. The extracted organic layer was reduced to a residue that was reconstituted in acetonitrile: water for analysis with TI-SAMDI.

Chemical Reactions. The Suzuki-Miyaura coupling reaction was performed by combining 2-bromobenzonitrile (125 mM, final concentration), potassium (4-methyl-phenyl)trifluoroborate (150 mM), and K_2CO_3 (125 mM) in 4 mL of ethanol:water (1:1, v/v). The catalyst, $Pd(OAc)_2$ (1 mol %), was added to initiate the reaction. During the course of the reaction, samples (100 μL) were removed at various time points and quenched with addition of formic acid (10 μL). The catalyst was removed by filtration with cotton and diatomite, and the reaction mixtures were stored at -20°C until analysis. A standard molecule, 4'-methyl-2-biphenylcarboxylic acid (125 mM), having a similar structure to the product was added in equal volumes to the quenched sample. The sample was then ready for TI-SAMDI analysis.

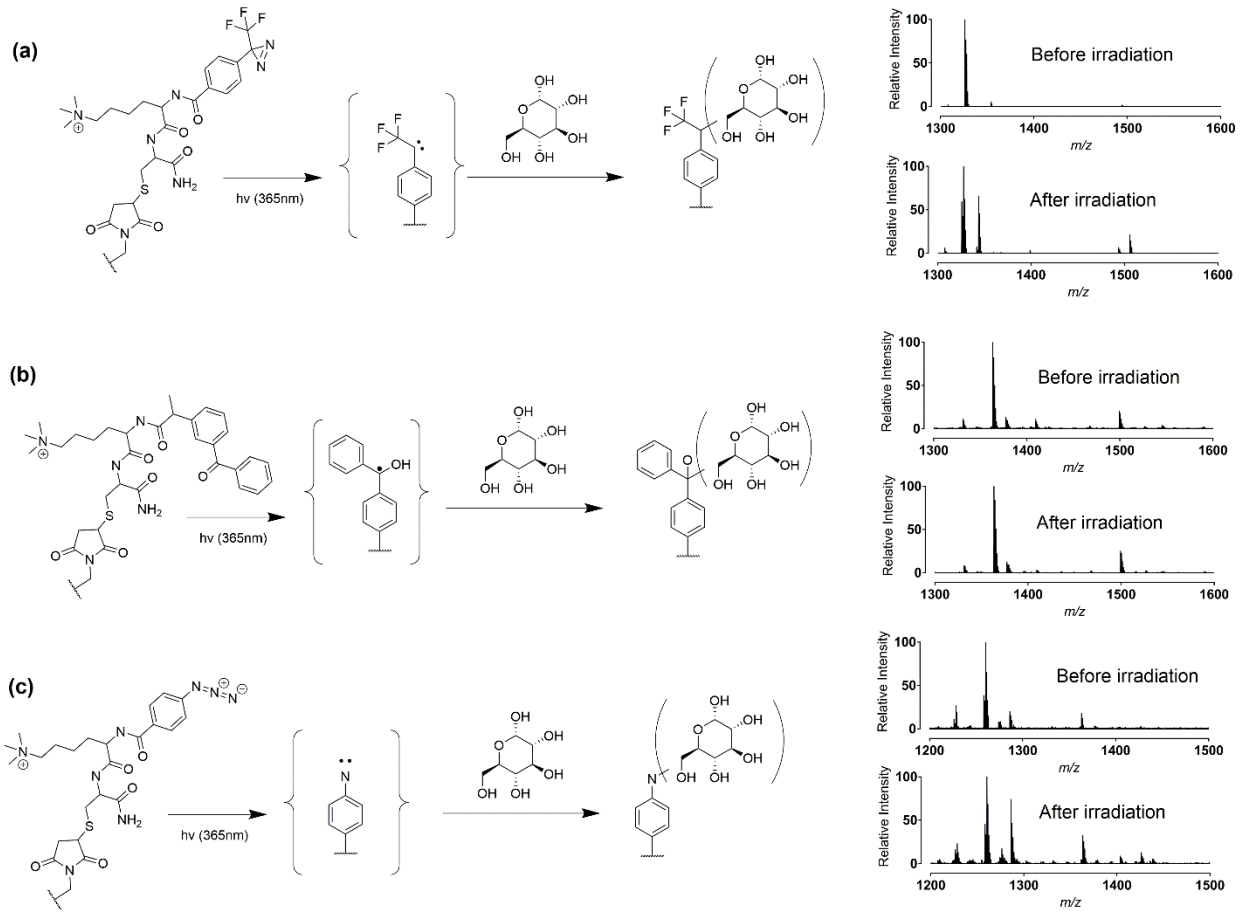

Figure S.1. Comparison of three photoimmobilization strategies for glucose. Spectra are shown for monolayers presenting each of the three photoaffinity groups; (a) diazirine; (b) benzophenone; (c) aryl azide, before and after irradiation to immobilize glucose. (a) The diazirine-terminated alkanethiolate appears at m/z 1325 (after loss of nitrogen and conversion to carbene during the MALDI experiment) and showed the expected peak after immobilization of glucose (m/z 1505) The byproducts are due to reaction with water (m/z 1341) and 2,4,6-trihydroxyacetophenone (m/z 1493), the MALDI matrix. (b) The benzophenone group (m/z 1363) showed no reaction with glucose after irradiation, (c) and the aryl azide group (m/z 1261) showed inefficient immobilization of glucose with many byproducts.

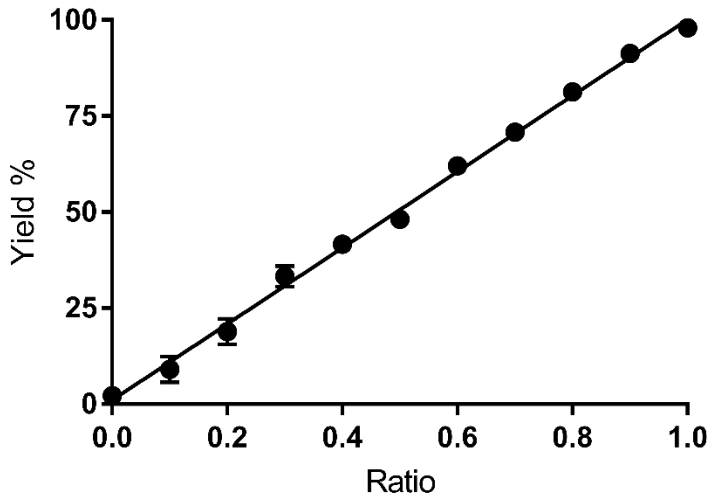

Figure S.2. A calibration curve for tolbutamide and hydroxy-tolbutamide. A series of solutions having a range of hydroxy-tolbutamide to tolbutamide ratios, at a constant total concentration (1mM) were prepared, photoimmobilized as described in the earlier, and analyzed by SAMDI MS. The measured fractions of hydroxy-tolbutamide (determined from the peak intensity for hydroxy-tolbutamide divided by the sum of the intensities for hydroxy-tolbutamide and tolbutamide) were linearly related to the actual fractions, demonstrating that these molecules had similar immobilization and ionization efficiencies.

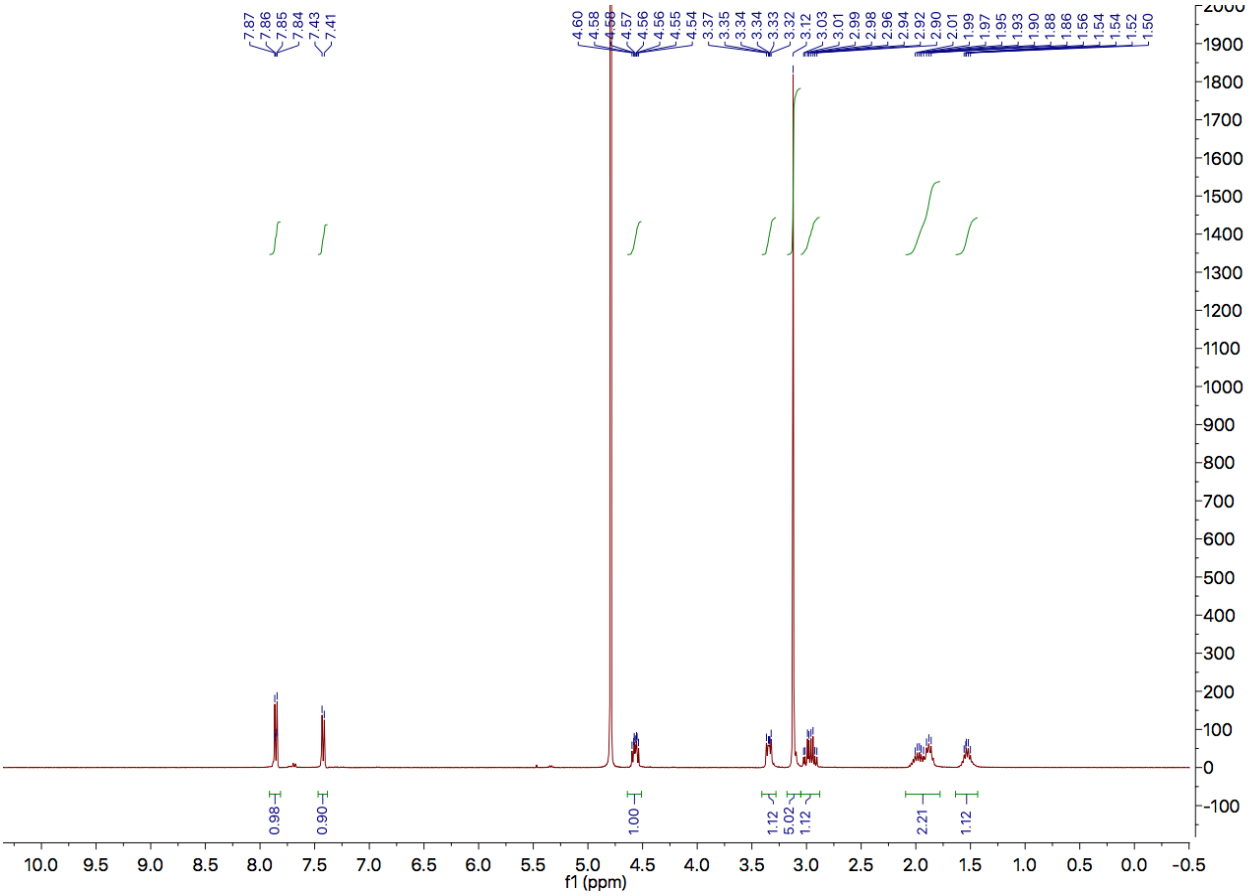

Figure S.3. ^{1}H-NMR spectrum of photoaffinity linker.

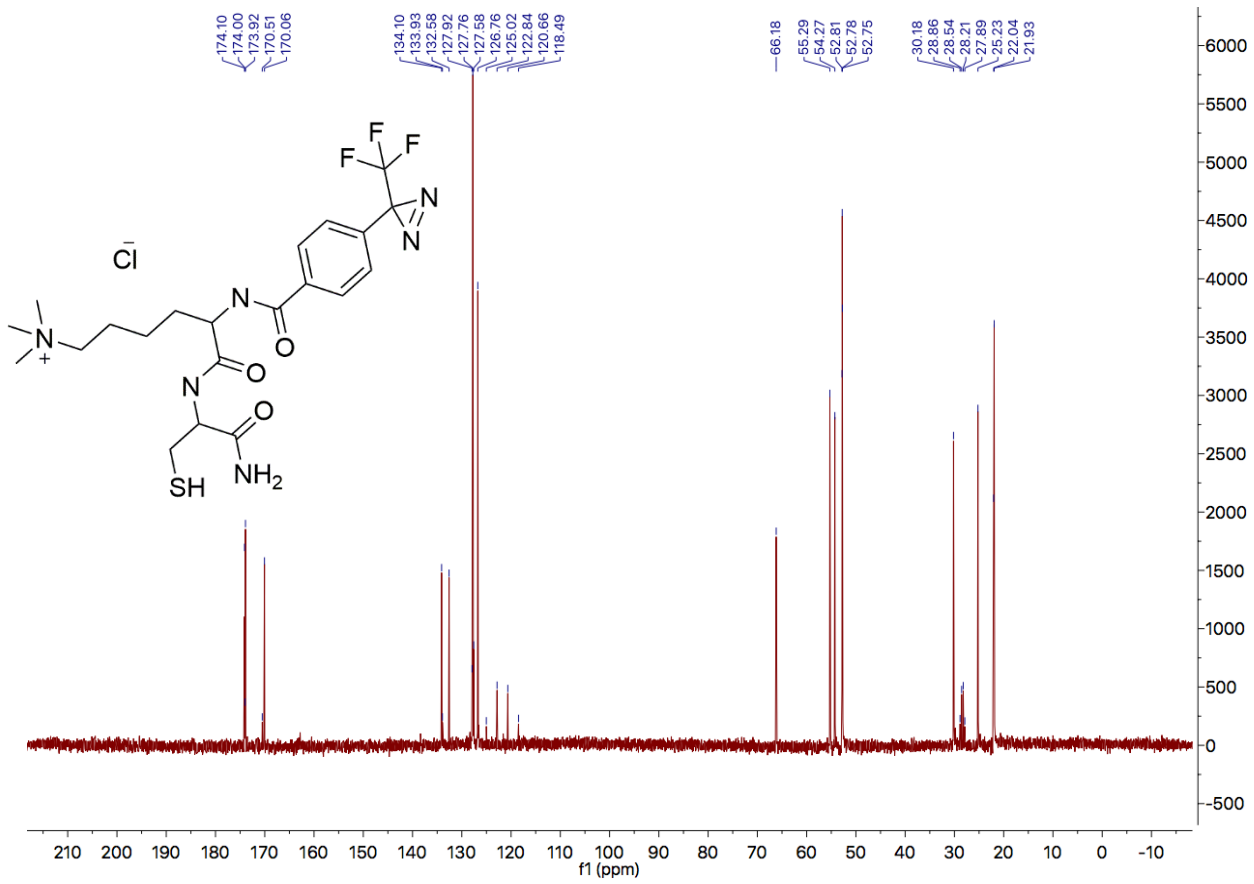

Figure S.4. ^{13}C-NMR spectrum of photoaffinity linker.