Supporting Information

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Toward the Construction of Universal Small-Molecule Microarrays: Immobilization of Complex Natural Products on Glass Slides Using a Photoaffinity Reaction and an Application for Detecting Protein-Small Molecule Interactions

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Materials and Methods: AC slides [fluoresin-imprinted DNA microarray TYPE1 slides (φ 1.0 mm high-density amine coated well slides)] were purchased from Matsunami Glass Ind., Ltd. (No. S117806; Osaka, Japan). All commercially available chemicals for chemical synthesis were used without further purification unless otherwise noted. Anhydrous DMF was purchased from Aldrich (Milwaukee, WI, USA). N-tert-butoxycarbonyl-2,2'-ethylenedioxy-bis(ethylamine) was prepared according to the procedure in ref. 7. [3-(trifluoromethyl)-3H-diazirin-3-yl]benzoic acid was prepared according to the procedure in ref. 8. All chemical reactions were monitored by thin layer chromatography (TLC) using 0.25-mm precoated silica gel plate 60F₂₅₄ Art. 5715 (E. Merck, Darmstadt, Germany). Yields refer to chromatographically and spectroscopically pure compounds. ¹H-NMR spectra were measured on JEOL JNM-AL400 and JNM-AL300 spectrometers. Electrospray ionization mass spectra were obtained on Perkin-Elmer SCIEX API 2000 mass spectrometer.
Mouse monoclonal anti-digoxin clone DI-22-FITC conjugate was purchased from Sigma (Saint Louis, MO, USA). Streptavidin-Alexa<sub>633</sub> conjugate was purchased from Molecular Probes, Inc. (Eugene, OR, USA). Monoclonal mouse anti-cyclosporin A clone CSZ.22 was purchased from HyTest Ltd. (Turku, Finland). The human FKBP12 gene was cloned from Jurkat cDNA libraries and subcloned into pRSET C vector (purchased from Invitrogen, Carlsbad, CA, USA). (His)<sub>6</sub>-FKBP12 was expressed in <i>E. coli</i> and purified according to the reported procedure, which can be found under http://www.schreiber.chem.harvard.edu/home/protocols/SMP_text.html. (His)<sub>6</sub>-FKBP12-Alexa<sub>488</sub> conjugate was prepared from purified (His)<sub>6</sub>-FKBP12 using an Alexa<sub>488</sub> protein labeling kit (Molecular Probes, Inc.) according to the recommended protocol. Anti-cyclosporin A-Alexa<sub>532</sub> conjugate was prepared from anti-cyclosporin A using an Alexa<sub>532</sub> protein labeling kit (Molecular Probes, Inc.) according to the recommended protocol.

**Synthesis of photoaffinity linker 1:**

![Chemical structure of linker 1](image)

To a mixture of N-tert-butoxycarbonyl-2,2'-ethylenedioxy-bis(ethylamine) (73.8 mg 297 µmol) and 4-[3-(trifluoromethyl)-3H-diazirin-3-yl]benzoic acid (17.9 mg, 72.0 µmol), N,N-dimethylaminopyridine (3.1 mg, 25 µmol) in THF (1 mL) was added 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (24.1 mg, 126 µmol). The resultant mixture was stirred for 17 h at room temperature in the dark. The reaction mixture was concentrated in vacuo, and purified on a small pipette column [Kanto silica gel 60N (spherical, neutral), CHCl<sub>3</sub>:MeOH = 25:1] to afford [2-(2-{4-(3-Trifluoromethyl-3H-diazirin-3-yl)-benzoylamino}-ethoxy)-ethoxy]-ethyl carbamic acid tert-butyl ester (27.6 mg, 83% from 4-[3-(trifluoromethyl)-3H-diazirin-3-yl]benzoic acid) as a colorless oil: ¹H-
NMR (300 MHz, CD$_3$OD) $\delta = 7.91$ (d, $J = 8.0$ Hz, 2H), 7.33 (d, $J = 8.0$ Hz, 2H), 3.53-3.68 (m, 8H), 3.49 (t, $J = 5.7$ Hz, 2H), 3.21 (t, $J = 5.7$ Hz, 2H), and 1.41 (s, 9H). ESI-MS $m/z$ 461.3 [M+H]$^+$. This material was dissolved in CH$_2$Cl$_2$ (1 mL) and trifluoroacetic acid (150 µL), and was stirred for 1 h at room temperature. The solution was concentrated in vacuo, and purified on a small pipette column (Nacalai CosmoSil 140C18-OPN, 0 → 50% aq. MeOH) to afford a trifluoroacetic acid salt of 1 (21.1 mg, 74%) as a colorless oil: $^1$H-NMR (400 MHz, CD$_3$OD) $\delta = 7.89$ (d, $J = 8.6$ Hz, 2H), 7.34 (d, $J = 8.6$ Hz, 2H), 3.72-3.63 (m, 8H), 3.58 (t, $J = 4.8$ Hz, 2H), 3.07 (t, $J = 5.6$ Hz, 2H). ESI-MS $m/z$ 361.1 [M+H]$^+$. 

**Preparation of PALC slides:**

AC slides were treated with a solution of $N,N'$-disuccinimidyl carbonate (1.0 M) and $N,N$-diisopropylethylamine (DIPEA, 1.0 M) in DMF overnight at room temperature. The resultant slides were rinsed briefly with EtOH, washed successively with EtOH, Milli-Q water, EtOH, and Milli-Q water (10 min each), centrifuged (480 x g, 1 min), then dried in vacuo. The resultant succinimidyl slides were treated with a solution of the trifluoroacetic acid salt of 1 (100 mM) and DIPEA (500 mM) in DMF in the dark overnight at room temperature. The slides were washed briefly with EtOH and Milli-Q water, and centrifuged (480 x g, 1 min). The slides were then blocked with a solution of ethanolamine (1.0 M) in DMF for 1 h at room temperature. The blocked slides were washed with EtOH and Milli-Q water, centrifuged (480 x g, 1 min), and dried in vacuo.
**Immobilization of small molecules on PALC slides:** Small-molecule solutions (0.2 µL each) were spotted on PALC slides. The slides were pre-dried in an incubator at 30 °C, then dried completely *in vacuo* overnight. The dried slides were irradiated at 365 nm for 30 min using a Super-light model LS-D3 lamp (Irie Seisakusyo Co., Ltd., Tokyo, Japan). Subsequently, the slides were rinsed briefly with EtOH, washed successively with EtOH, DMF, THF, EtOH, and Milli-Q water (1 h each), and centrifuged (400 x g, 1 min).

**Detection of biotin-streptavidin interaction:** The slide on which biotin was immobilized was probed with streptavidin-Alexa<sub>633</sub> conjugate (3.7 µg/mL) in a reaction buffer (38 mM Tris·HCl, pH 7.5, 77 mM NaCl, 0.05% Tween 20, 1% skim milk) for 1 h at room temperature. The slide was washed (4 x 5 min) with TBST buffer (38 mM Tris·HCl, pH 7.5, 77 mM NaCl, 0.05% Tween 20), rinsed briefly with Milli-Q water, and centrifuged (400 x g, 1 min). The slide was scanned by using a DNA scope IV fluorescence slide scanner.