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Supporting Information

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for

SPOT Synthesis of Peptide Arrays on Self-Assembled Monolayers and their Evaluation as Enzyme Substrates

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Preparation of the amine-terminated self-assembled monolayers on gold

A disposable 64-wells gold surface (Applied Biosystems) was cleaned with a 5:1 solution of concentrated sulphuric acid and hydrogen peroxide (caution: very oxidizing agent) and thoroughly rinsed with deionised water, ethanol and dried under a stream of nitrogen. Self-assembled monolayers on gold surfaces were prepared by spotting a DMSO solution (final concentration 0.1 mg/mL, 0.4 µL per spot) of a 1:1 mixture of carboxylic acid-terminated and tri(ethylene glycol)-terminated alkanethiols (ProChimia, chemical formulae $HS(CH_2)_{17}(OCH_2CH_2)_6OCH_2CO_2H$ and $HS(CH_2)_{17}(OCH_2CH_2)_3OH$, respectively) into each well and left overnight to react before rinsing with DMSO, ethanol and drying as above. The terminal carboxylic acid was then activated for 1 h at RT by spotting a freshly prepared DMF solution of EDC (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, 0.2 M) and NHS (*N*-hydroxysuccinimide, 0.05 M). After rinsing and drying as above, a DMF solution of *N*-Fmoc diaminobutane (Aldrich, 100 mM) was spotted and left to react overnight at RT. Following washing steps as above, the gold slide was dipped into a 20% solution of piperidine in DMF for 10 min, then rinsed and dried as above.

General procedure for SPOT synthesis.

Coupling conditions: 10 mg of Fmoc amino acid were dissolved in 0.25 mL of a solution containing 52 mg/mL of PyBOP and 36 µL/mL of DIPEA in dry DMF and allowed to react for 5 min at RT. 0.4 µL of the above solution was then manually spotted in each well of the slide prepared as above using an Eppendorf pipette, and the slide was then placed at 37 °C in a sealed container. After 1 h, the surface was rinsed with DMF, ethanol and dried under a stream of nitrogen.

Fmoc deprotection: removal of the Fmoc was carried out by dipping the slide into a 20% solution of piperidine in DMF for 10 min at RT, followed by rinsing and drying.

Cleavage of the side chain protecting group: the slide was dipped into a 50% solution of trifluoroacetic acid in DCM and allowed to stand for 4 h at RT before rinsing and drying.

Enzymatic reactions on gold surfaces.

Thermolysin-catalysed hydrolysis: each well of the slide was incubated overnight at 37°C with a solution of thermolysin (2 mg/mL in potassium phosphate buffer, pH 7.5).

Thermolysin-catalysed peptide-bond formation: each well of the slide was incubated overnight at 37°C with a saturated solution of Fmoc amino acid (about 50 mg/mL) and thermolysin (2 mg/mL) in potassium phosphate buffer, pH 7.5.

ppGalNAcT2: Incubation was performed by spotting onto each well an assay mixture of the enzyme ppGalNAcT2, UDP-GalNAc and $MnCl_2$ in AMPD buffer pH 7.4 as previously described,[1] and incubated overnight at 37°C.

Bovine b1,4-GalT: each well was coated with a mixture of β1,4-GalT, UDP-Gal and $MnCl_2$ in Tris-HCl buffer pH 8.0 [2] and incubated overnight at 37°C.

MALDI-ToF MS analysis.

Each well of the surface was coated with a solution of THAP (2,4,6-trihydroxyacetophenone, 10 mg/mL in acetone), and the target was loaded in to a Voyager-DE STR Biospectrometry MALDI-ToF mass spectrometer (PerSeptive Biosystems) operating with a 337 nm nitrogen laser. Mass spectra were acquired either using reflector or

[1] N. Laurent, J. Voglmeir, A. Wright, J. Blackburn, N. T. Pham, S. C. C. Wong, S. J. Gaskell, S. L. Flitsch, *ChemBioChem* **2008**, *9*, 883-887

[2] Z.-I. Zhi, N. Laurent, A. K. Powell, R. Karamanska, M. Fais, J. Voglmeir, A. Wright, J. M. Blackburn, P. R. Crocker, D. A. Russell, S. L. Flitsch, R. A. Field, J. E. Turnbull *ChemBiochem* **2008**, *9*, 1568-1575.

linear ToF, positive ion mode using an accelerating voltage of 20kV and an extraction delay of 200 ns (reflector mode) or 225 ns (linear mode).

Thermolysin-catalysed peptide bond formation.

Table S1: *m/z* detected after incubation of immobilised Phe or Leu with a mixture of thermolysin and Fmoc amino acid (for clarity, only the m/z of the sodium adducts of the mixed disulfide formed between the peptide-terminated and the tri(ethylene glycol)-terminated alkanethiols are shown).

	Immobilised amino acid	Fmoc amino acid added	Product	*m/z* detected
1	Phe	FmocLeu	FmocLeuPhe	1604
2	Phe	FmocPhe	Phe FmocPhePhe	1269 1638
3	Phe	FmocAla	Phe FmocAlaPhe	1269 1561
4	Phe	FmocGly	Phe	1269
5	Leu	FmocLeu	FmocLeuLeu	1570
6	Leu	FmocPhe	Leu FmocPheLeu	1235 1604
7	Leu	FmocAla	Leu FmocAlaLeu	1235 1528
8	Leu	FmocGly	Leu	1235

Muc1 array:

Table S2 provides a list of Muc1-derived peptides array with the masses detected before and after enzymatic glycosylation and the relative ratios glycopeptides/peptide. For clarity, the signals for the mixed disulfide formed between the peptide-terminated and the tri(ethylene glycol)-terminated alkanethiols were omitted from the table but were also considered for calculating the ratios glycopeptide/peptide.

	m/z peptide		m/z glycopeptide		Ratio
Peptide	M+H	M+Na	M+H	M+Na	glycopeptide / peptide
AHGVTSAPA	1891	1913	2094	2116	1.0
AHGVTPAPA	1901	1923	2104	2126	1.5
AHGVTRAPA	1960	1982	2163	2185	0.65
AHGVTDAPA	1919	1941	2122	2144	1.0
AHGVTIAPA	1917	1939	2120	2142	1.1
AHGVTGAPA	1861	1883	2064	2086	0.88
AHGVTMAPA	1935	1957	2138	2160	0.67
AHGVTHAPA	1941	1963	2144	2166	0.98
AHGVTFAPA	1951	1973	2154	2176	0.58
AHGVTYAPA	1967	1989	2170	2192	0.77
AHGVTQAPA	1932	1954	2135	2157	0.84
AHGVTVAPA	1903	1925	2106	2128	1.2
AHGVTEAPA	1933	1955	2136	2158	1.1
AHGVTTAPA	1905	1927	2108	2130	0.79
AHGVTNAPA	1918	1940	2121	2143	0.63
AHGVTAAPA	1875	1897	2078	2100	1.2
AHGSTSAPA	1878	1900	2081	2103	1
AHGPTSAPA	1888	1910	2091	2113	1.9
AHGRTSAPA	1947	1969	2150	2172	0.10
AHGDTSAPA	1906	1928	2109	2131	0
AHGITSAPA	1904	1926	2107	2129	0.67
AHGGTSAPA	1848	1870	2051	2073	1.1
AHGMTSAPA	1922	1944	2125	2147	0
AHGHTSAPA	1928	1950	2131	2153	0.53
AHGFTSAPA	1938	1960	2141	2163	0
AHGYTSAPA	1954	1976	2157	2179	0
AHGQTSAPA	1919	1941	2122	2144	0
AHGVTSAPA	1891	1913	2094	2116	1.0
AHGETSAPA	1920	1942	2123	2145	0
AHGTTSAPA	1892	1914	2095	2117	*
AHGNTSAPA	1905	1927	2108	2130	0.10
AHGATSAPA	1862	1884	2065	2087	1.2

*: the peptide AHGTTSAPA resulted in a mixture of mono- and di-glycosylated peptides after incubation with the ppGalNAcT2 and was therefore not included.