Supporting Information:

Towards Larger Chemical Libraries:

Encoding with Fluorescent Colloids in Combinatorial Chemistry

Bronwyn J. Battersby[†], Darryn E. Bryant[‡], Wim Meutermans[§], Daniel Matthews[†], Mark L. Smythe[§], Matt Trau[†]*

[†]Department of Chemistry, [‡]Department of Mathematics,

[§]Centre for Drug Design and Development

The University of Queensland, St. Lucia QLD 4072, Australia

Journal: Journal of the American Chemical Society.

S1. Microscopy: The fluorescence microscope (Olympus IX70) was equipped with filters U-MWU (excitation wavelength $\lambda_{EX} = 330 - 385$ nm; emission wavelength $\lambda_{EM} > 420$ nm), U-MWB ($\lambda_{EX} = 450$ - 480 nm; $\lambda_{EM} > 515$ nm) and U-MWG ($\lambda_{EX} = 510$ - 550 nm; $\lambda_{EM} > 590$ nm). Scanning electron microscopy was performed on a Jeol 6400 SEM.

S2. Polyelectrolyte Coating of Reporters: Reporters used in this study were coated with poly(diallyldimethylammonium chloride) (PDADMAC, Aldrich; Molecular Weight (MWt) = 500 000) followed by polyacrylic acid (PAA; MWt = 250 000). Coating was

performed by soaking the reporters (3.3 mg/ml) in a 1% aqueous solution of PDADMAC for 24 hours, washing several times in Milli-Q water (by the centrifugation method), and then soaking the PDADMAC-coated reporters in a 1% aqueous solution of PAA, followed by thorough washing. The reporters were transferred gradually to DMF before use in library synthesis.

S3. A 3-Cycle Solid Phase Peptide Synthesis with Colloidal Barcoding: Polyelectrolyte-coated reporters (red emission, 5 mg/ml) were mixed with 50 mg solid support beads [Fmoc-Glu-Wang resin, 75-150 μm in diameter (Novabiochem)] in a deprotection mixture [2 ml of piperidine/dimethylformamide (1:1)] for 4 to 6 minutes. Multiple reporters (~100 – 400) attached to each bead, and the remaining reporters were washed away thoroughly using dimethylformamide (DMF). A Glycine coupling was then performed using established Fmoc methods (Alewood, P., Alewood, D., Miranda, L., Love, S., Meutermans, W., Wilson, D. *Methods in Enzymology* 1997, *289*, 14 – 29). This process was repeated with blue reporters encoding for Lysine and red/yellow reporters encoding for Alanine. (Since Glutamic acid was coupled to the resin prior to the solid phase synthesis, polyelectrolyte coated reporters (green emission) were used to code for the Glutamic acid). The colloidal barcode was read, and then the compound was cleaved using trifluoroacetic acid (1 hour) and analyzed by mass spectrometry (Fig. 3). The result showed that the compound Fmoc-Ala-Lys-Gly-Glu-OH had been synthesized abundantly and the presence of the colloidal barcode (see Fig. 2) did not interfere with the synthesis.

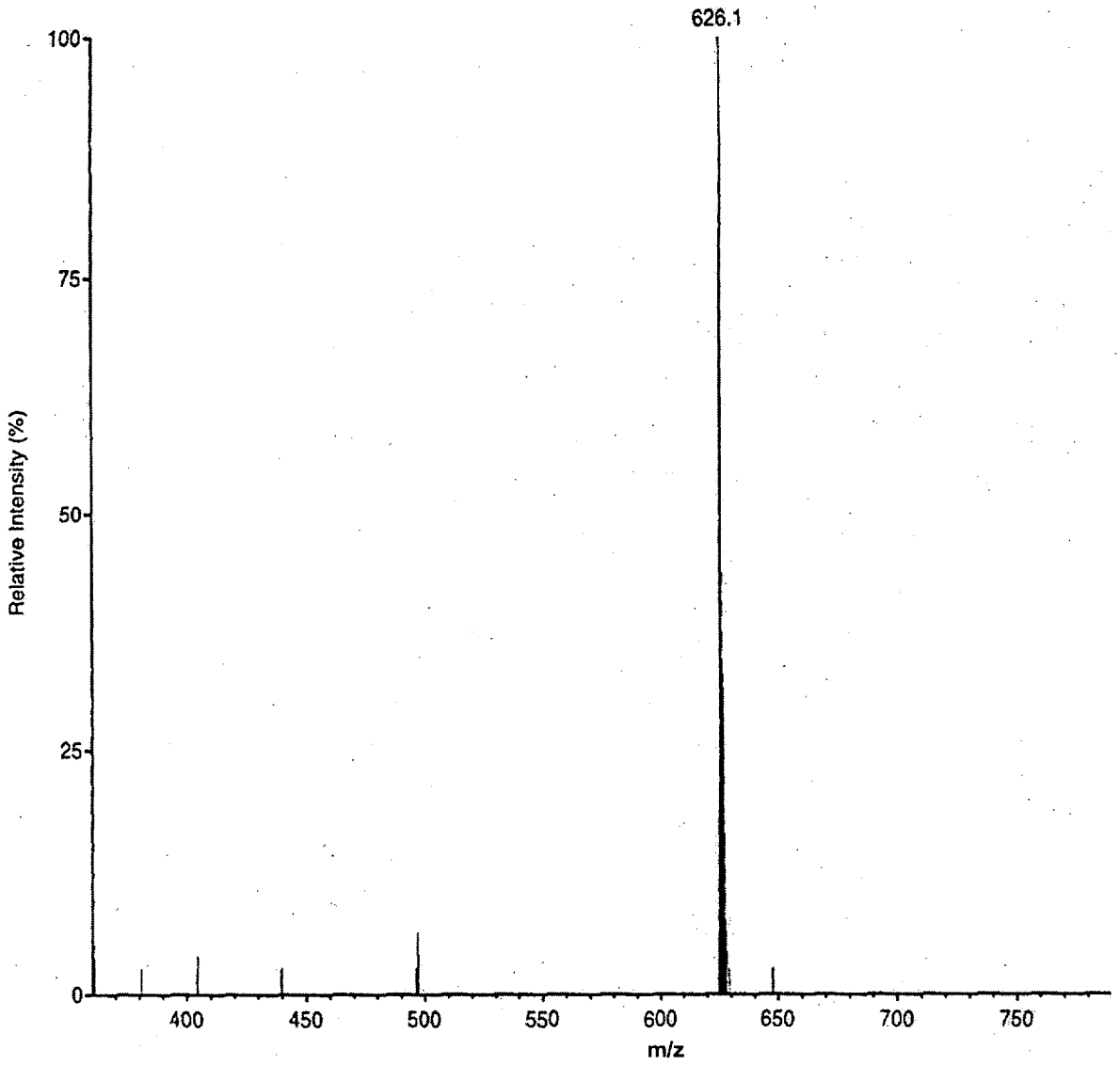


Fig. 3. Mass spectrum of the compound, Fmoc-Ala-Lys-Gly-Glu-OH, cleaved off solid supports after solid phase peptide synthesis with colloidal barcoding.

S4. Colloidal Barcoding of a 100-Compound Library Prepared by the Split and Mix Technique:

A solid phase peptide library was synthesized using twenty different amino acids over two synthesis cycles (Table 1). Twenty different types of silica reporter (Table 1) were required to barcode the library. Each type of reporter contained one or more fluorescent dyes and one type of reporter was attached to the solid support beads during each of the 20 reactions in the split-synthesis process. Most of the reporters were custom synthesized in our Laboratory using the method described by van Blaaderen A. and Vrij, A. *Langmuir* 1992, *8*, 2921-2931. Three types of commercial silica microspheres (4 μm, Micromod GmbH) were also used for colloidal barcoding the library.

Reporters were synthesised from the monomers tetraethyl orthosilicate (TEOS, Aldrich) and the dye-coupling agent 3-aminopropyltrimethoxysilane (APS, Aldrich). The fluorescent dyes used were: Alexa 430 (A430, Molecular Probes), Alexa 350 (A350, Molecular Probes), fluorescein isothiocyanate (FITC, Sigma), quinolizino-substituted fluorescein isothiocyanate (QFITC, Sigma), Naphthofluorescein (Molecular Probes).

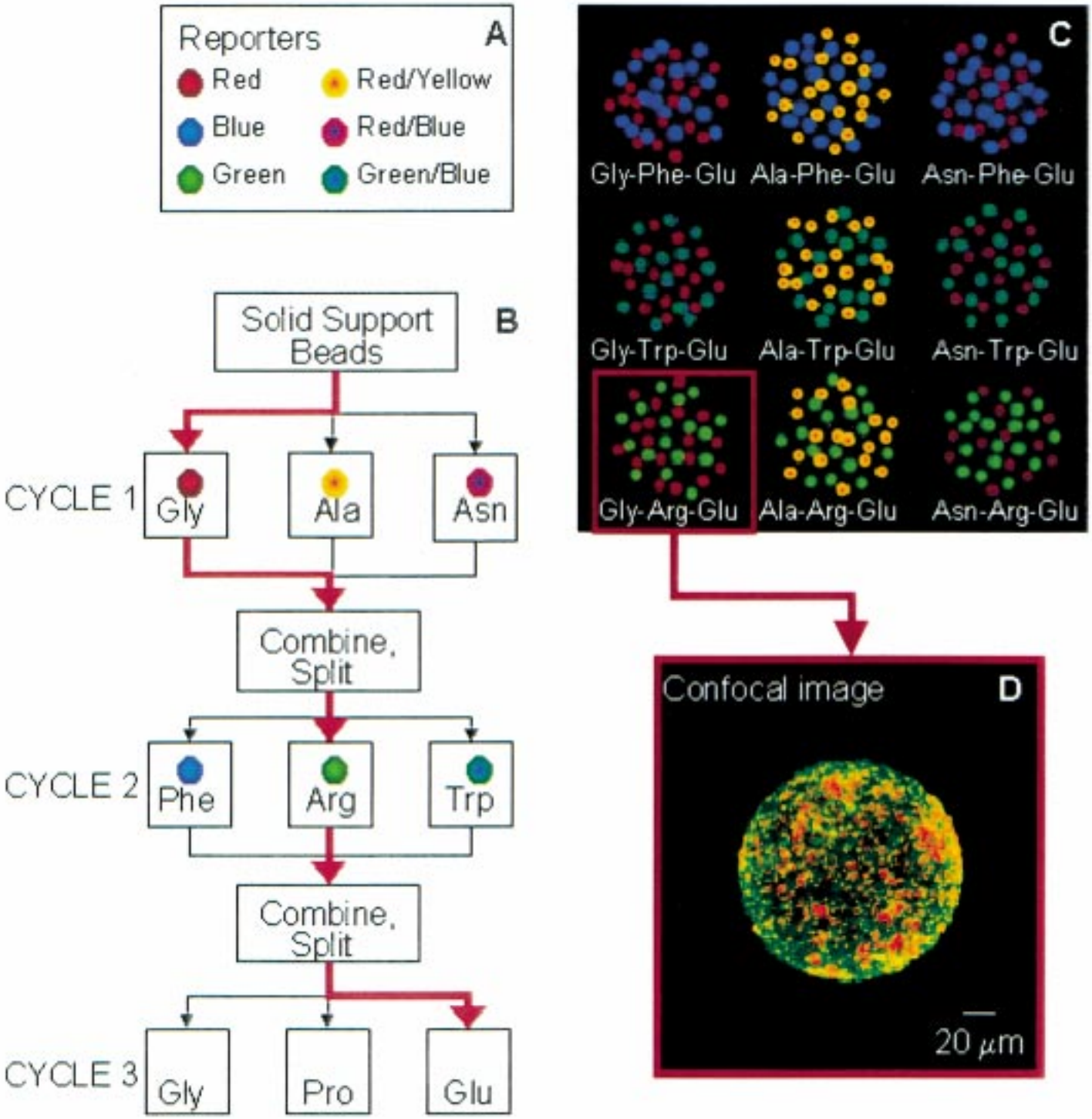
The twenty different types of reporter were coated with polyelectrolytes (described in Section S2). For each of the 10 coupling reactions in the first Cycle, a 300 μL solution containing reporters (3.3 mg/ml in DMF) was mixed with a 100 mg portion of swelled solid support beads [Fmoc-Gly-Wang resin, 75-150 μm in diameter (Novabiochem)] in the deprotection mixture [2 - 5 ml of piperidine/dimethylformamide (1:1)] for 4 to 6 minutes. Each of the 10 portions received a different type of reporter as shown in Table 1. Multiple reporters (~50 - 200) attached to each bead, and the remaining reporters were washed away thoroughly using dimethylformamide (DMF). A peptide coupling (using different amino

acids for each portion) was then performed using established Fmoc methods (Alewood, P., Alewood, D., Miranda, L., Love, S., Meutermans, W., Wilson, D. *Methods in Enzymology* 1997, *289*, 14 – 29). The beads were combined and split into 10 equal portions. For each of the 10 coupling reactions in the second Cycle, a 500 μL solution containing reporters (3.3 mg/ml in DMF) was mixed with each portion in the deprotection mixture for 4 to 6 minutes. Each of the 10 portions received a different type of reporter from the remaining 10 reporter types not used in the first cycle. Multiple reporters again attached to each bead, and the remaining reporters were washed away thoroughly using DMF. The second peptide coupling (see Cycle 2 in Table 1) was then performed on each of the 10 portions of beads using Fmoc chemistry. The colloidal barcodes were viewed and detected by fluorescence microscopy.

Table 1: Scheme for colloidal barcoding of a 100-member peptide library.

Cycle	Reaction Number	Amino Acid Coupled	Type of Reporter	Reporter Diameter/μm
1	1	Glycine	FITC	0.5
1	2	Alanine	FITC / Naphthofluorescein	0.5
1	3	Leucine	A430	0.5
1	4	Isoleucine	FITC / QFITC	0.5
1	5	Proline	A350	0.5
1	6	Valine	A350 / QFITC	0.5
1	7	Methionine	QFITC	0.5
1	8	Phenylalanine	A350 / A430	0.5
1	9	Tryptophan	Naphthofluorescein	0.5
1	10	Tyrosine	A430 / QFITC	0.5
2	11	Histidine	DAPI / aminofluorescein	4.0
2	12	Glutamine	DAPI / aminofluorescein / RhodamineB	4.0
2	13	Cysteine	DAPI / RhodamineB	4.0
2	14	Serine	FITC / Naphthofluorescein / QFITC	0.5
2	15	Threonine	Naphthofluorescein / QFITC	0.5
2	16	Asparagine	A350 / FITC / Naphthofluorescein	0.5
2	17	Glutamic acid	A430 / Naphthofluorescein / QFITC	0.5
2	18	Aspartic acid	A350 / Naphthofluorescein	0.5
2	19	Arginine	A430 / Naphthofluorescein	0.5
2	20	Lysine	A350 / A430 / Naphthofluorescein	0.5

S5. Colloidal barcoding of a 27-Compound Library Prepared by the Split and Mix Technique: Six different types of silica reporter (1 μm in diameter; Microcaps GmbH, Germany) were used to barcode the library in Fig. 4 (Fig. 4a). Each type of reporter contained one of the following fluorescent dyes or dye combinations: Rhodamine (red-emission), aminofluorescein (green emission); DAPI (blue emission); combined Rhodamine and aminofluorescein, combined Rhodamine and DAPI and combined DAPI and aminofluorescein. One type of reporter was attached during each of the six peptide coupling reactions that were performed in Cycles 1 and 2 of the split-synthesis process (Fig. 4b). See Section S4 for colloidal barcoding and peptide coupling methods. The amino acids were: Glycine (Gly); Alanine (Ala); Asparagine (Asn); Phenylalanine (Phe); Arginine (Arg); Tryptophan (Trp); Proline (Pro); Glutamic acid (Glu). Fig. 4c shows a schematic of the nine beads present in the Glutamic acid (Glu) portion after Cycle 3. The beads present at each of the three peptide coupling reactions in Cycle 3 followed one of nine possible paths through the Cycles 1 and 2. The path followed by each bead is determined by its attached reporters. Each type of reporter codes for a reaction and for its place in the sequence of reactions. The reporter combinations create a colloidal barcode that determines the structure of the chemical compound synthesized on any bead. A confocal microscopy image (Fig. 4d) reveals the colloidal barcode of a bead that followed the red path indicated in Fig. 4b. Shown in Figure 5 is a mass spectrum of the 9 compounds cleaved off solid supports from the Glutamic acid portion.


Reporters
A
Red
Red/Yellow
Blue
Red/Blue
Green
Green/Blue
Solid Support Beads
B
CYCLE 1
Gly
Ala
Asn
Combine, Split
CYCLE 2
Phe
Arg
Trp
Combine, Split
CYCLE 3
Gly
Pro
Glu
C
Gly-Phe-Glu
Ala-Phe-Glu
Asn-Phe-Glu
Gly-Trp-Glu
Ala-Trp-Glu
Asn-Trp-Glu
Gly-Arg-Glu
Ala-Arg-Glu
Asn-Arg-Glu
Confocal image
D
20 μm

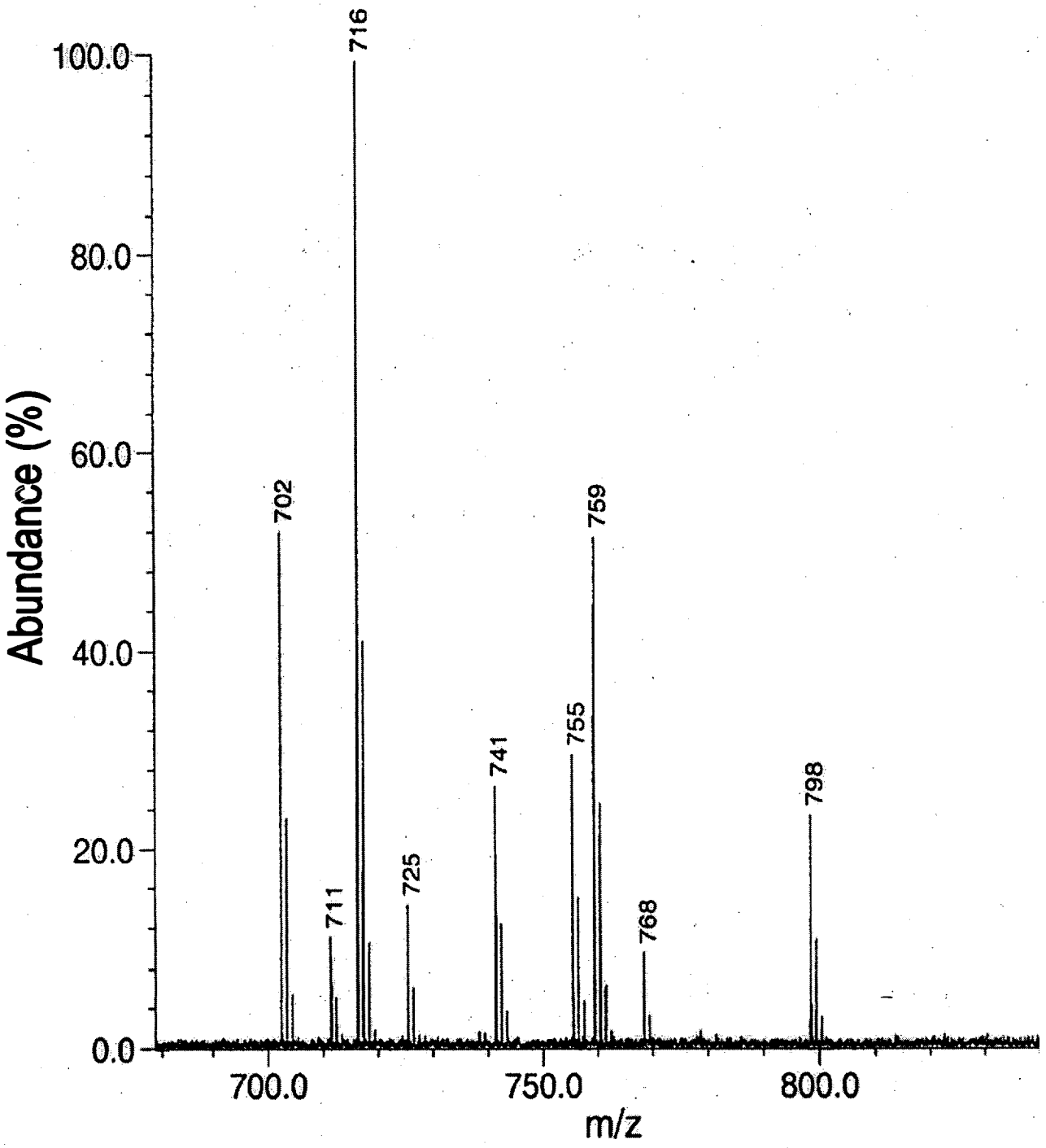


Figure 5: Mass spectrum of compounds cleaved from the Glutamic acid portion after the third cycle in the split and mix synthesis described in Fig. 4. The nine different colloidal barcodes from this portion were read, the compounds cleaved from these solid support beads and analyzed using mass spectrometry. The chemical structure of these compounds corresponded with those decoded from the colloidal barcode.

S6. Solvent Resistance. Reporter adhesion and exchange was tested in the presence of various solvents and reagents. Experiments involved mixing two differently barcoded (red and green) solid support portions prior to immersion in the following solvents/reagents. A 100 mg portion of aminomethylated solid support beads (Peptide Institute, 0.26 mmol/g) was barcoded with red reporters by mixing the resin with 0.25 ml of 10 mg/ml polyelectrolyte-coated red reporters in DMF and washing thoroughly to remove excess reporters. A second 100 mg portion of beads was barcoded using the same method, but with green reporters. The two portions were mixed together in DMF, washed with DCM/methanol and dried under nitrogen gas. 10 mg of the dry resin was added to each of the following solvents (0.3 ml) and reagents and examined by fluorescence microscopy after 1 hour and after a 24 hour period. The tests involved DMF, tetrahydrofuran (THF), acetonitrile, ethylacetate, methanol, trifluoroacetic acid (TFA) and dichloromethane (DCM) at 25°C; all previous except DCM at 50°C; TFA at 100°C for 5 minutes; DMF/diisopropyl ethylamine; methanol/sodium methoxide ($NaOCH_3$) at 25°C and 50°C; THF/sodium hydride (NaH) at 25°C; methanol/sodium cyanoborohydride; dichloromethane/tetrakis (triphenylphosphine) palladium(0) [$Pd(PPh_3)_4$]; dichloromethane/trifluoroacetic acid (4:1); dichloromethane/acetic acid; DMF/5-nitro-2-hydroxybenzaldehyde; DCM/pyridine dichromate; dichloromethane/ $Pd(PPh_3)_4$/diethylazodicarboxylate; DMF/Fmoc-Gly-OH/ diisopropylethylamine/2-(1H-benzotriazole-1-yl)-1,1,3,3 tetramethyluronium hexafluorophosphate (HBTU); 1M NaCl. The colloidal barcode remained intact in all cases with very few occurrences of red reporters on green barcoded beads or green reporters on red barcoded beads. In two cases (THF/NaH and DCM/pyridine dichromate), the solid supports were not resistant to the chemicals and split apart, but the reporters remained adhered.