

*Supplementary information for*

**Small molecules released from islets of Langerhans determined by liquid chromatography – mass spectrometry**

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**Table S-1: MRM conditions for all analytes and IS.**

<b>Analyte</b>	<b>Retention Time* (min)</b>	<b>Precursor Ion (m/z)</b>	<b>Product Ion (m/z)</b>	<b>Collision Energy (V)</b>	<b>RF Lens (V)</b>
ACh	1.51	146	87	15	106
ACh IS	1.51	150	91	15	101
His	3.06	260	105	25	151
His IS	3.06	266	111	26	127
Tau	3.38	230	105	15	126
Tau IS	3.38	236	111	15	105
Hyp	3.51	236	105	19	127
Hyp IS	3.51	242	111	19	93
Asn	3.65	237	105	19	94
Asn IS	3.65	243	111	19	86
Ser	3.76	210	105	16	90
Ser IS	3.76	216	111	17	81
Arg	3.93	279	105	29	167
Arg IS	3.93	285	111	30	147
Hist	3.96	216	95	18	135
Hist IS	3.96	222	111	18	118
Gln	3.98	251	105	17	118
Gln IS	3.98	257	111	18	91
$\beta$ -HSer	4.17	224	105	22	109
$\beta$ -HSer IS	4.17	230	111	20	84
Asp	4.37	238	105	27	112
Asp IS	4.37	244	111	18	90
Gly	4.55	180	105	13	97
Gly IS	4.55	186	111	14	83
Cit	4.59	280	105	22	127
Cit IS	4.59	286	111	24	97
Thr	5.04	224	105	18	108
Thr IS	5.04	230	111	20	84
Glu	5.10	252	105	16	101
Glu IS	5.10	258	111	17	87
$\beta$ -Ala	5.47	194	105	15	112
$\beta$ -Ala IS	5.47	200	111	17	77
Ala	5.80	194	105	18	96
Ala IS	5.80	200	111	17	77
Aad	6.16	266	105	18	105
Aad IS	6.16	272	111	17	95
GABA	6.33	208	105	14	93
GABA IS	6.33	214	111	17	84
$\beta$ -ABA	6.59	208	105	20	93

β-ABA IS	6.59	214	111	17	84
Pro	7.26	220	105	19	116
Pro IS	7.26	226	111	19	83
α-ABA	7.65	208	105	20	89
α-ABA IS	7.65	214	111	17	84
5HTP	8.68	325	105	25	161
5HTP IS	8.68	331	111	25	94
NAC	9.53	268	105	23	109
NAC IS	9.53	274	111	20	88
Val	9.70	222	105	18	109
Val IS	9.70	228	111	18	84
Met	9.85	254	105	21	84
Met IS	9.85	260	111	20	81
Leu	10.09	236	105	19	118
Leu IS	10.09	242	111	19	93
Orn	10.52	341	174	16	156
Orn IS	10.52	353	111	28	146
Lys	10.79	355	105	29	157
Lys IS	10.79	367	111	17	142
Ile	10.96	236	105	21	132
Ile IS	10.96	242	111	19	93
Phe	11.17	270	105	19	131
Phe IS	11.17	276	111	30	88
Trp	11.18	309	105	24	146
Trp IS	11.18	315	111	24	130
Cys	11.58	330	105	24	117
Cys IS	11.58	342	111	23	106
Kyn	11.85	417	122	18	151
Kyn IS	11.85	429	128	18	172
Tyr	12.05	390	105	26	182
Tyr IS	12.05	402	111	26	134
5HT	12.37	385	264	20	194
5HT IS	12.37	397	270	20	123
TryA	12.50	346	105	23	191
TryA IS	12.50	358	111	23	136
Epi	12.64	496	105	27	170
Epi IS	12.64	514	111	25	218
DA	12.91	466	105	25	216
DA IS	12.91	484	111	25	132

\*Retention time window was RT ± 0.50 min

**Table S-2: Human Islet Donor Information**

	<b>Donor 1</b>	<b>Donor 2</b>
Age	65	62
Race	Caucasian	Caucasian
Sex	Female	Female
Height (inches)	67	63
Weight (lbs)	160	130
BMI	25.1	23.0
HbA1c (%)	5.2	5.0

**Table S-3: Calculated resolutions for some critical pairs at the shallow, steep, and optimized (non-linear) gradients.**

<b>Analytes</b>	<b>Resolution (Rs)</b>		
	<b>Shallow</b>	<b>Steep</b>	<b>Optimized</b>
<b>Hyp Asn</b>	0.5	0.2	0.6
<b>Arg Hist</b>	0.1	0.1	0.2
<b>Gly Cit</b>	0.6	0.2	0.2
<b><math>\beta</math>-Ala Ala</b>	1.2	0.7	1.9
<b>GABA <math>\beta</math>-ABA</b>	0.9	1.6	1.9

**Table S-4: Amounts released from murine islets in response to 1 hour of static incubation in 3 and 20 mM glucose.**

Analyte \ [Glucose]	This study ( $\mu\text{mol islet}^{-1}$ )		MEKC method <sup>a</sup> ( $\mu\text{mol islet}^{-1}$ )	
	3 mM	20 mM	3 mM	20 mM
Ser	6.8 $\pm$ 0.2	3.6 $\pm$ 0.2	3.4 $\pm$ 0.2	2.8 $\pm$ 0.3
Gln	6.3 $\pm$ 0.1	2.1 $\pm$ 0.1	2.3 $\pm$ 0.2	2.7 $\pm$ 0.1
Gly	9.5 $\pm$ 0.3	5.0 $\pm$ 0.2	8.3 $\pm$ 0.7	5.7 $\pm$ 0.2
His	11.7 $\pm$ 0.1	7.4 $\pm$ 0.1	2.5 $\pm$ 0.6	1.5 $\pm$ 0.3
Ala	10.6 $\pm$ 0.1	5.3 $\pm$ 0.1	3.6 $\pm$ 0.2	6.0 $\pm$ 0.5
Glu	5.9 $\pm$ 0.2	1.0 $\pm$ 0.1	1.0 $\pm$ 0.1	0.9 $\pm$ 0.1
GABA	0.84 $\pm$ 0.01	0.25 $\pm$ 0.01	0.44 $\pm$ 0.05	0.21 $\pm$ 0.04
Val	6.6 $\pm$ 0.1	3.0 $\pm$ 0.1	3.5 $\pm$ 0.3	2.3 $\pm$ 0.4
Met	1.34 $\pm$ 0.01	0.66 $\pm$ 0.01	1.2 $\pm$ 0.1	0.75 $\pm$ 0.02
Ile	6.6 $\pm$ 0.1	1.7 $\pm$ 0.1	1.6 $\pm$ 0.1	0.7 $\pm$ 0.1
Leu	12.2 $\pm$ 0.3	4.0 $\pm$ 0.2	6.0 $\pm$ 0.4	3.4 $\pm$ 0.2
Trp	0.51 $\pm$ 0.01	0.27 $\pm$ 0.01	0.54 $\pm$ 0.04	0.30 $\pm$ 0.01
Phe	2.4 $\pm$ 0.1	1.5 $\pm$ 0.1	2.1 $\pm$ 0.1	1.5 $\pm$ 0.1
Arg	26.9 $\pm$ 1.1	4.9 $\pm$ 0.1	13.2 $\pm$ 0.8	9.2 $\pm$ 0.2

<sup>a</sup>As reported in X. Wang, L. Yi, C. Guillo and M. G. Roper, *Electrophoresis*, 2015, **36**, 1172–1178.

### Microfluidic device

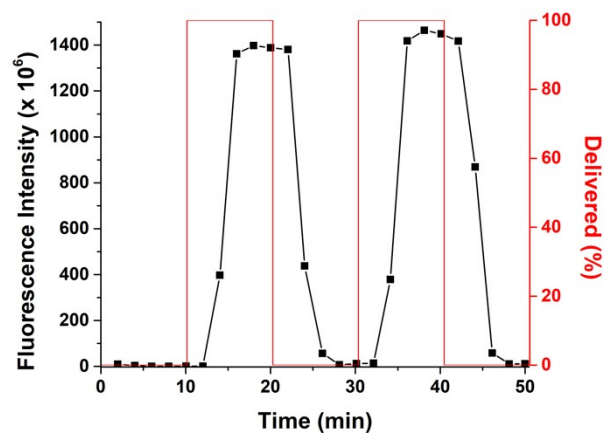
The microfluidic device was fabricated in PDMS using conventional soft lithography. The channels were in the shape of a “Y” with the islets in the center channel portion. The master mold dimensions were 450 x 90  $\mu\text{m}$  (width x height) verified using a portable surface roughness tester (SJ-310 Series, Mitutoyo, Aurora, IL). Access holes were made using a 1.26 mm diameter titanium nitride hole punch (SYNEO, Angleton, TX). The PDMS was irreversibly bonded to a 24 x 40 x 1 mm (width x length x thickness) glass coverslip (Fisher Scientific, Pittsburgh, PA) after plasma oxidation of both pieces. The islet chamber was maintained at 37°C using a thermofoil heater (Omega Engineering, Inc., Stamford, CT) placed underneath the microfluidic device and a thermocouple sensor applied adjacent to the islet chamber on top. The temperature was maintained at  $36.5 \pm 0.5^\circ\text{C}$  using a controller (Omega Engineering).

For perfusion, a pressure-driven flow system (OB1, Elvexsys, Paris, France) was used to deliver BSS containing low and high glucose concentrations to the two arms of the “Y” via 10 cm pieces of Tygon tubing (0.02” ID x 0.060” OD, Cole-Parmer North America, Vernon Hills, IL). The total flow rate in the device was maintained at  $5 \mu\text{L min}^{-1}$  using flow rate sensors (Elvexsys). The perfusate was collected via an 8 cm Tygon tubing connected to the outlet of the device. Fractions were collected every 2 min into a 96-well plate and derivatized as described in Section 2.2.

### Microfluidic device characterization

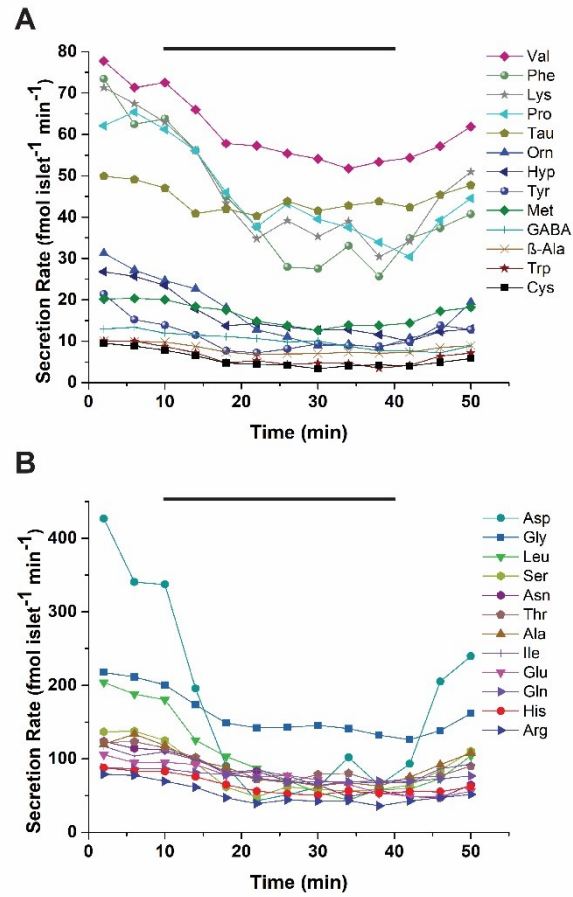
To determine the response time of the system, 500 nM fluorescein in BSS was delivered to the device while the other reservoir contained BSS only. A stepwise change of BSS and fluorescein every 10 min was performed, with fractions collected every 2 min into a

96-well plate and analyzed using a plate reader (SpectraMax iD5, Madison, WI) with excitation and emission at  $485 \pm 20$  nm and  $535 \pm 25$  nm, respectively. The results from this experiment are shown in **Figure S-1**.

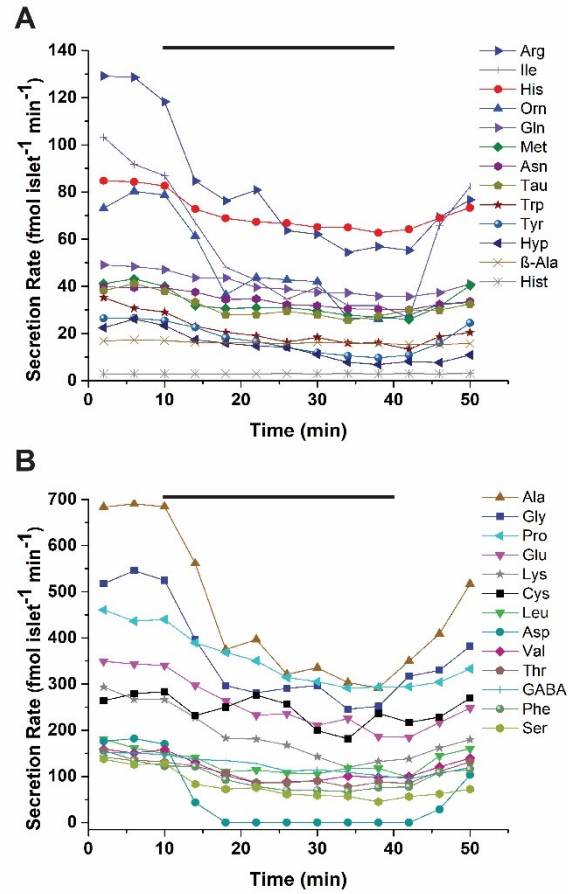


**Figure S-1. Microfluidic device dynamics.** Low (0 nM) and high (500 nM) concentrations of fluorescein were delivered to the device using a pressure-driven flow system in a pattern shown by the red line (right y-axis). Fractions were collected every 2 min into a 96-well plate and the fluorescence intensities are plotted in black (left y-axis).





**Figure S-2. Secretion profiles of small molecules from murine islets.** A second experiment was performed to measure secretion profiles from 25 murine islets. Secretions from murine islets are shown with the low (**A**) and high (**B**) concentration analytes separated for ease in viewing. The time that 20 mM glucose was delivered is shown by the bars on top of the graphs.



**Figure S-3. Secretion profiles of small molecules from human islets.** A second experiment using 25 islets from Donor 2 was performed. The same perfusion protocol as described in the text for **Figure 4** was used with secretion rates for the low (**A**) and high (**B**) concentration analytes shown. The time that 20 mM glucose was delivered is shown by the bars on top of the graphs.