Insulin and C-peptide FRET sensor assay characteristics.

Tables S1 – S10 summarize experiments that were performed to determine basic characteristics of FRET sensor assays. Insulin and C-peptide determinations for these experiments were performed as Described in Research Design and Methods section. Between assays variation (Tables S1 and S2) was determined by measuring three samples from human islet secretion experiments five times. Each measurement was done in duplicate with independently prepared FRET sensor and calibration curve. Within assay variation (Tables S3 and S4) was determined by measuring three samples from human islet secretion experiments five times. Each measurement was done in duplicate using the same sensor mix and calibration curve. Spike and recovery (Tables S5 and S6) was assessed by adding various known amounts of insulin (or C-peptide) to five (four in the case of C-peptide) samples from human islet secretion experiments and comparing experimentally measured insulin (or C-peptide) concentrations with calculated expected concentrations. Assay linearity (Tables S7 and S8) was determined by measuring insulin (or C-peptide) in three samples from human islet secretion experiments. These samples were then serially diluted (2, 4 and 8 fold) and experimentally determined insulin (or C-peptide) concentration was compared with the expected concentration calculated from the dilution. Assay crossreactivity (Tables S9 and S10) was assessed by comparing FRET signal obtained with 10 nM human insulin (or C-peptide) with signals observed at 10 nM, 100 nM and 1000 nM of the analyte tested for cossreactivity.

Table S1. Between assay precision: insulin sensor.

Sample number	Mean (nM)	% CV
1	0.94	9.8
2	1.43	7.6
3	2.1	7.6

Table S2. Between assay precision: C-peptide sensor.

Sample number	Mean (nM)	% CV
1	3.70	8.8
2	7.43	7.0
3	14.67	4.9

Table S3. Within assay precision: insulin sensor.

Sample number	Mean (nM)	% CV
1	1.46	3.4
2	2.68	4.3
3	5.68	3.4

Table S4. Within assay precision: C-peptide sensor.

Sample number	Mean (nM)	% CV
1	0.99	5.0
2	2.09	4.8
3	4.48	3.5

Table S5. Spike and recovery: insulin sensor

Sample number	Observed concentration (nM)	Expected concentration (nM)	% of recovery
1	2.71	2.32	117
	4.89	4.32	113
	9.01	8.32	109
2	2.43	2.01	121
	4.57	4.01	114
	8.45	8.01	105
3	2.51	2.29	110
	4.58	4.29	107
	8.38	8.29	101
4	2.53	2.01	126
	4.78	4.01	119
	8.59	9.01	107
5	2.88	2.78	104
	5.04	4.78	105
	9.19	8.78	105

Table S6. Spike and recovery: C-peptide sensor

Sample number	Observed concentration (nM)	Expected concentration (nM)	% of recovery
1	2.44	2.35	104
	3.68	3.85	96
	6.52	6.84	95
2	2.71	2.44	111
	4.02	3.94	102
	7.88	6.94	113
3	1.16	1.27	92
	2.85	2.77	103
	6.50	5.77	113
4	1.17	1.24	95
	2.72	2.74	99
	7.14	5.74	124

Table S7. Assay linearity: insulin sensor

Sample number	Sample dilution (fold)	Expected concentration (nM)	Observed concentration (nM)	% of recovery
1	0	2.64	2.64	100
	2	1.32	1.38	104
	4	0.66	0.62	94
	8	0.33	0.28	85
2	0	5.38	5.38	100
	2	2.69	3.09	115
	4	1.34	1.39	104
	8	0.67	0.65	97
3	0	10.88	10.88	100
	2	5.44	5.60	103
	4	2.72	2.86	105
	8	1.36	1.21	89

Table S8. Assay linearity: C-peptide sensor

Sample number	Sample dilution (fold)	Expected concentration (nM)	Observed concentration (nM)	% of recovery
1	0	2.72	2.72	100
	2	1.36	1.24	91
	4	0.68	0.64	94
	8	0.34	0.39	115
2	0	5.68	5.68	100
	2	2.84	2.71	95
	4	1.42	1.28	90
	8	0.71	0.65	92
3	0	10.40	10.40	100
	2	5.20	5.51	106
	4	2.60	2.49	96
	8	1.30	1.12	86

Table S9. Assay crossreactivity: insulin sensor

Analyte	Concentration (nM)	Sensor response (100%)
Human insulin	10	100.0 +/- 7.2
Bovine insulin	10	187.6 +/- 4.2
	100	93.8 +/- 6.7
	1000	22.9 +/- 12.1
Porcine insulin	10	225.1 +/- 4.5
	100	134.5 +/- 32.3
	1000	29.2 +/- 0.6
Human pro-insulin	10	16.9 +/- 3.5
	100	55.4 +/- 0.4
	1000	81.9 +/- 0.7
Insulin growth factor	10	0.1 +/- 0.1
	100	0.6 +/- 0.7
	1000	1.0 +/- 0.2
Glucagon	10	0.5 +/- 0.1
	100	1.0 +/- 0.4
	1000	2.6 +/- 0.8
Human C-peptide	10	n.d.*
	100	n.d.
	1000	n.d
Canine C-peptide	10	n.d.
	100	n.d.
	1000	n.d.
Porcine C-peptide	10	n.d
	100	n.d.
	1000	n.d.
Angiotensin I	10	n.d
	100	n.d.
	1000	n.d.
Angiotensin II	10	n.d
	100	n.d.
	1000	n.d.
ACTH	10	n.d
	100	n.d.
	1000	n.d.

* Result was reported as not detectable (n.d.) when the measured signal was zero or was higher then zero but smaller then the standard deviation of the measurement.

Table S10. Assay crossreactivity: C-peptide sensor

Analyte	Concentration (nM)	Sensor response (100%)
Human C-peptide	10	100.0 +/- 4.5
Bovine insulin	10	n.d.*
	100	2.0 +/- 0.4
	1000	n.d.
Porcine insulin	10	n.d.
	100	2.1 +/- 0.4
	1000	3.1 +/-1.1
Human pro-insulin	10	9.0 +/- 1.7
	100	66.8 +/- 4.1
	1000	284.8 +/- 17.5
Insulin growth factor	10	n.d.
	100	3.1 +/- 0.3
	1000	4.1 +/- 0.5
Glucagon	10	n.d.
	100	1.4 +/- 0.4
	1000	2.0 +/- 1.2
Human insulin	10	n.d.
	100	n.d.
	1000	n.d
Canine C-peptide	10	n.d.
	100	1.4 +/- 0.3
	1000	15.0 +/- 0.1
Porcine C-peptide	10	n.d
	100	1.3 +/- 0.4
	1000	1.7 +/- 0.4
Angiotensin I	10	n.d
	100	n.d.
	1000	1.3 +/- 0.4
Angiotensin II	10	n.d
	100	n.d.
	1000	0.8 +/- 0.4
ACTH	10	n.d
	100	n.d.
	1000	1.2 +/- 0.8

* Result was reported as not detectable (n.d.) when the measured signal was zero or was higher then zero but smaller then the standard deviation of the measurement.