Supporting Information

Insulin crystals grown in short peptide supramolecular hydrogels show enhanced thermal stability and slower release profile

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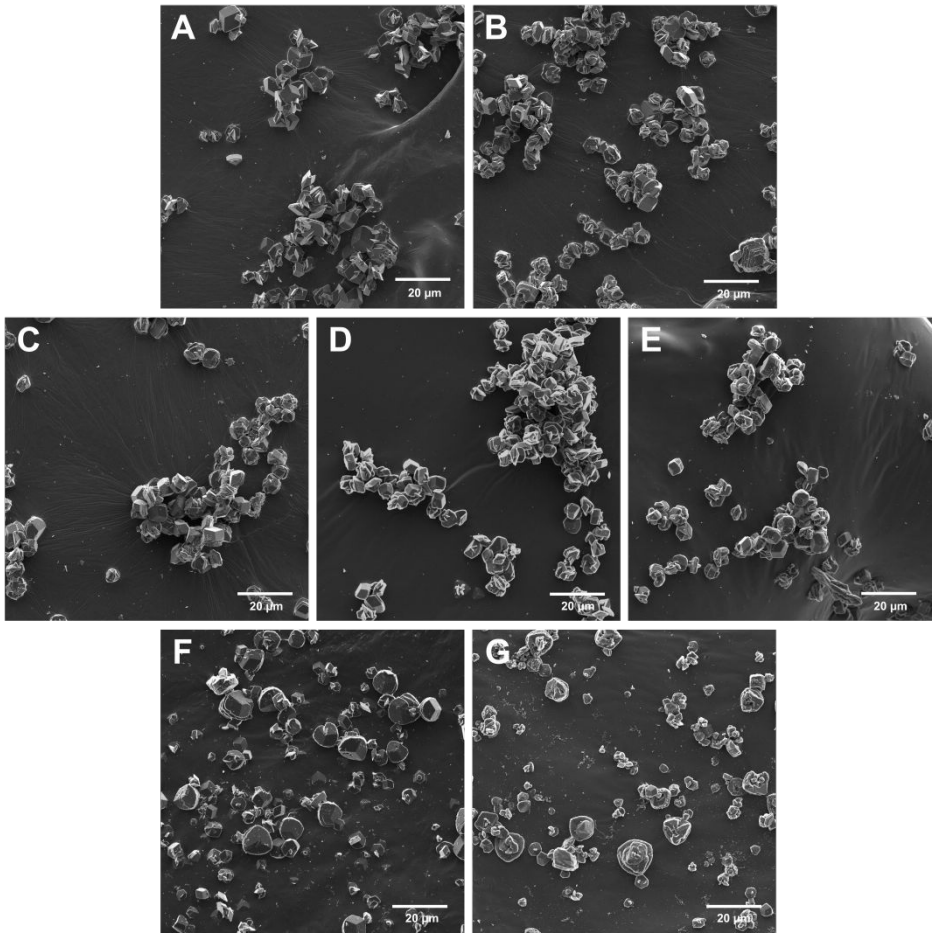


Figure S1. SEM images of insulin crystals: A) Control crystals at RT; B) Control crystals kept at 50ºC during 7 days; C) Fmoc-AA crystals kept at RT; D) Fmoc-AA crystals kept at 50 ºC during 7 days; E) Fmoc-AA crystals kept at 60 ºC during 24 hours; F) Agarose crystals kept at RT; G) Agarose crystals kept at 50 ºC during 7 days.

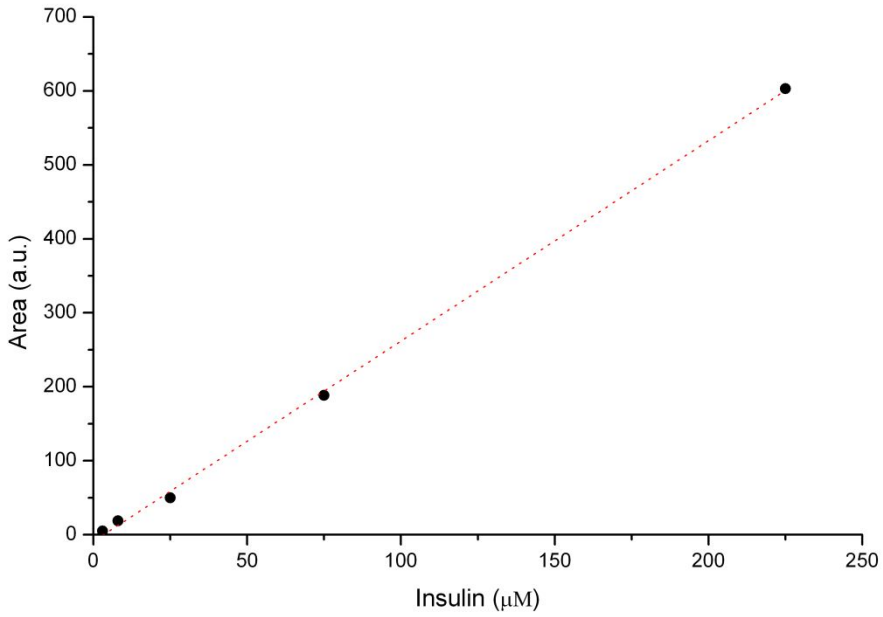
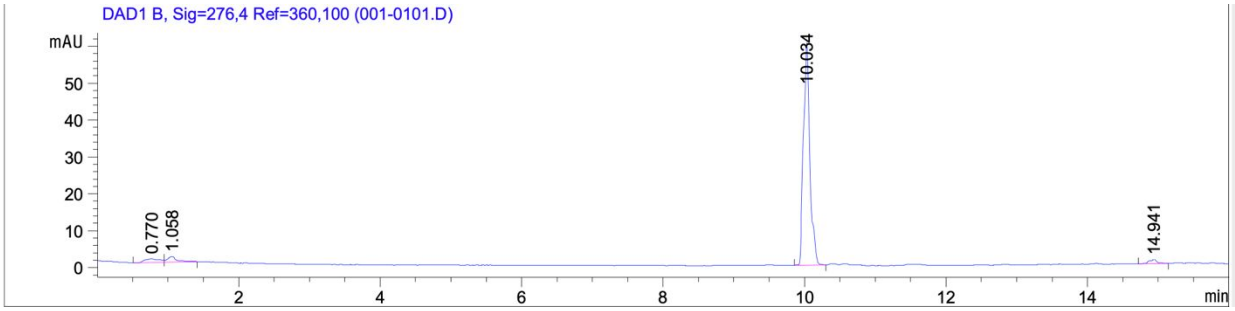


Figure S2. Calibration curve of insulin concentrations measured at 276 nm.

A)



B)

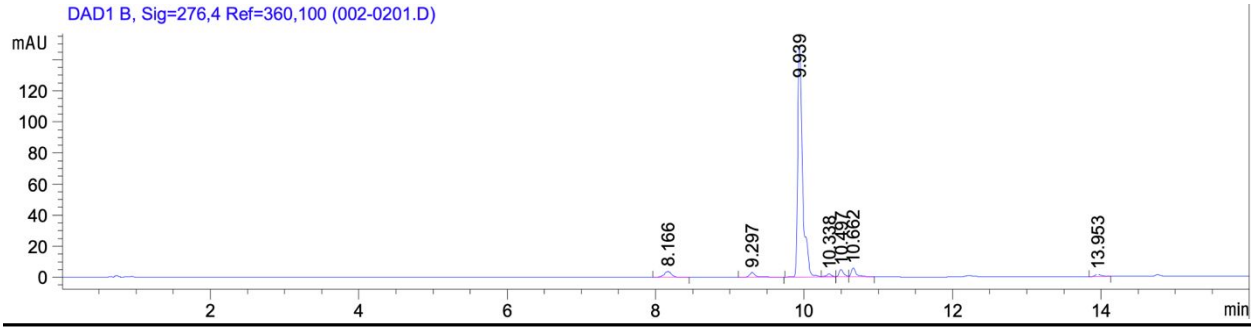
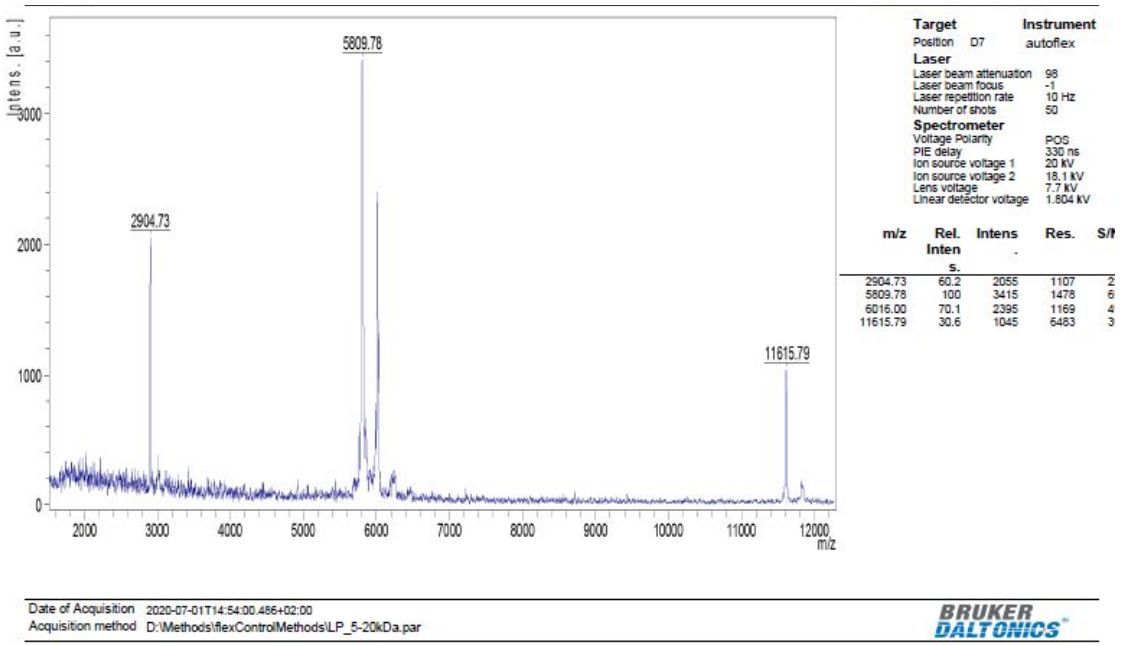


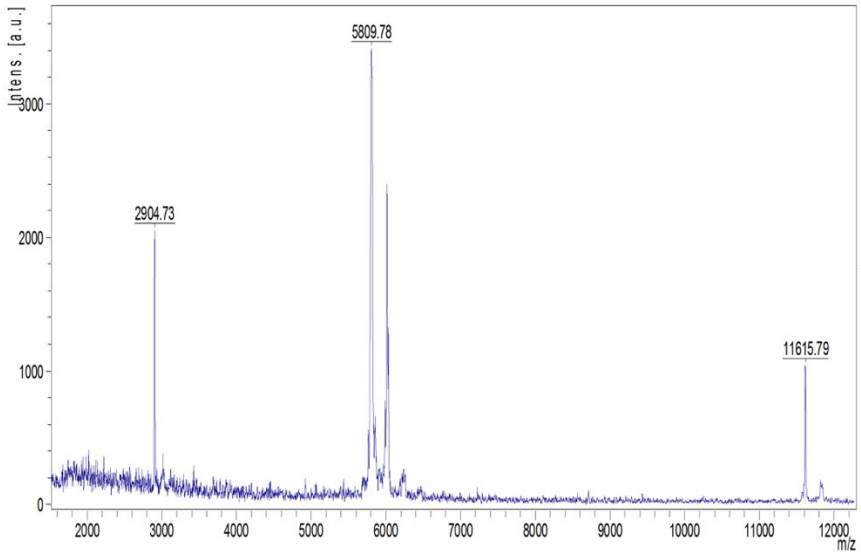
Figure S3. HPLC analysis of control crystals at RT (A) and 50 °C (B).

Insulin control



$(M+2H)^{+2}$ = 2,904; $(M+1H)^{+1}$ = 5,808 and $(2M+1H)^{+1}$ = 11,615 – impurity = 6,016. This impurity, that appears in most of the samples, might be assigned to the byproduct formed by the reaction of insulin monomer with the matrix adduct sinapinic acid (3,5-dimethoxy-4-hydroxycinammic acid). This type of reaction has been previously observed using the same Mass spectrometry technique, see C. K. Fagerquist et al. *J. Am. Soc. Mass Spectrom*. 2012, **23**, 2102-2114.

Control crystals



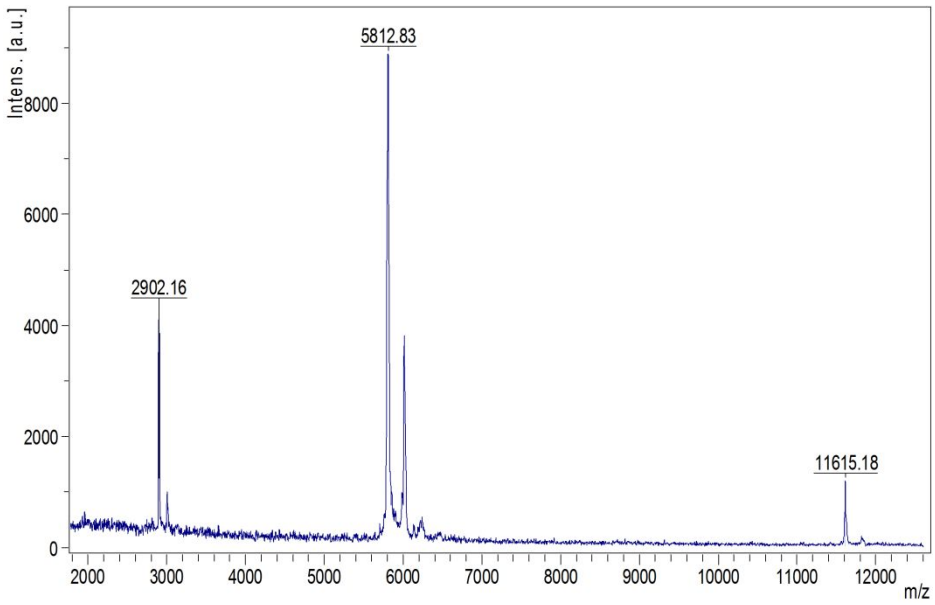
Target		Instrument
Position	D7	autoflex
Laser		
Laser beam attenuation		98
Laser beam focus		-1
Laser repetition rate		10 Hz
Number of shots		50
Spectrometer		
Voltage Polarity		POS
PIE delay		330 ns
Ion source voltage 1		20 kV
Ion source voltage 2		18.1 kV
Lens voltage		7.7 kV
Linear detector voltage		1.804 kV

m/z	Rel. Intens.	Intens.	Res.	S/N
2904.73	60.2	2055	1107	22
5809.78	100	3415	1478	69
6016.00	70.1	2395	1169	49
11615.79	30.6	1045	6483	39

Date of Acquisition 2020-07-01T14:54:00.486+02:00
Acquisition method D:\Methods\flexControlMethods\LP_5-20kDa.par

BRUKER DALTONICS

Control crystals 50°C



Target		Instrument
Position	P6	autoflex
Laser		
Laser beam attenuation		55
Laser beam focus		-1
Laser repetition rate		10 Hz
Number of shots		100
Spectrometer		
Voltage Polarity		POS
PIE delay		330 ns
Ion source voltage 1		20 kV
Ion source voltage 2		18.1 kV
Lens voltage		7.7 kV
Linear detector voltage		1.804 kV

m/z	Rel. Intens.	Intens.	Res.	S/N
2896.51	12.9	1142	819	10
2902.16	13.4	1188	734	13
5812.83	100	8890	614	105
6014.11	42.3	3760	5036	45
11615.18	13.5	1200	3151	27

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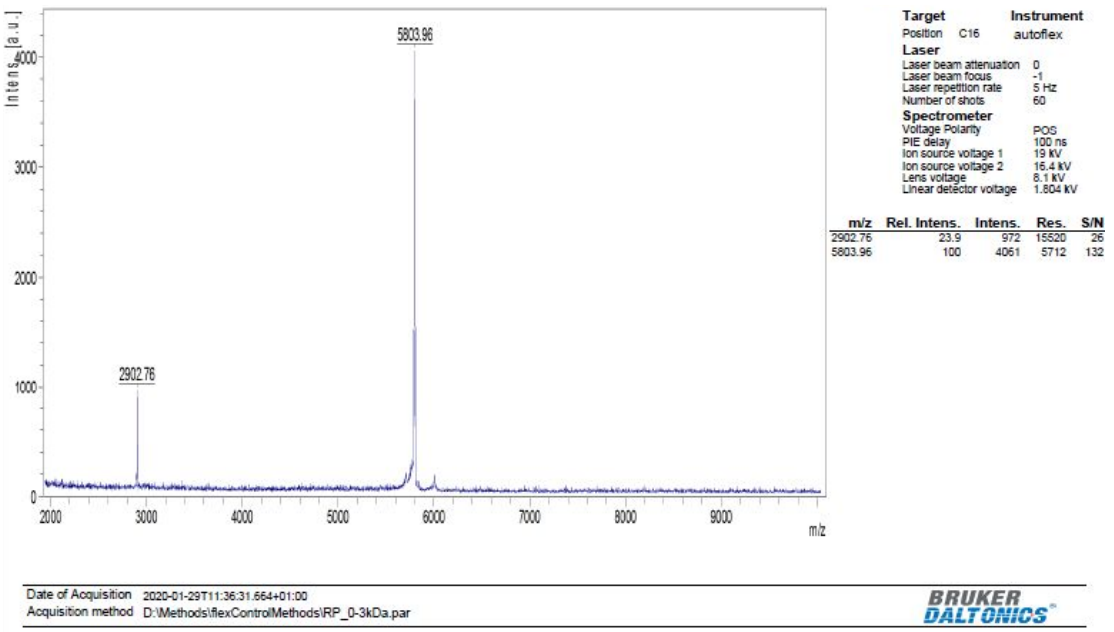
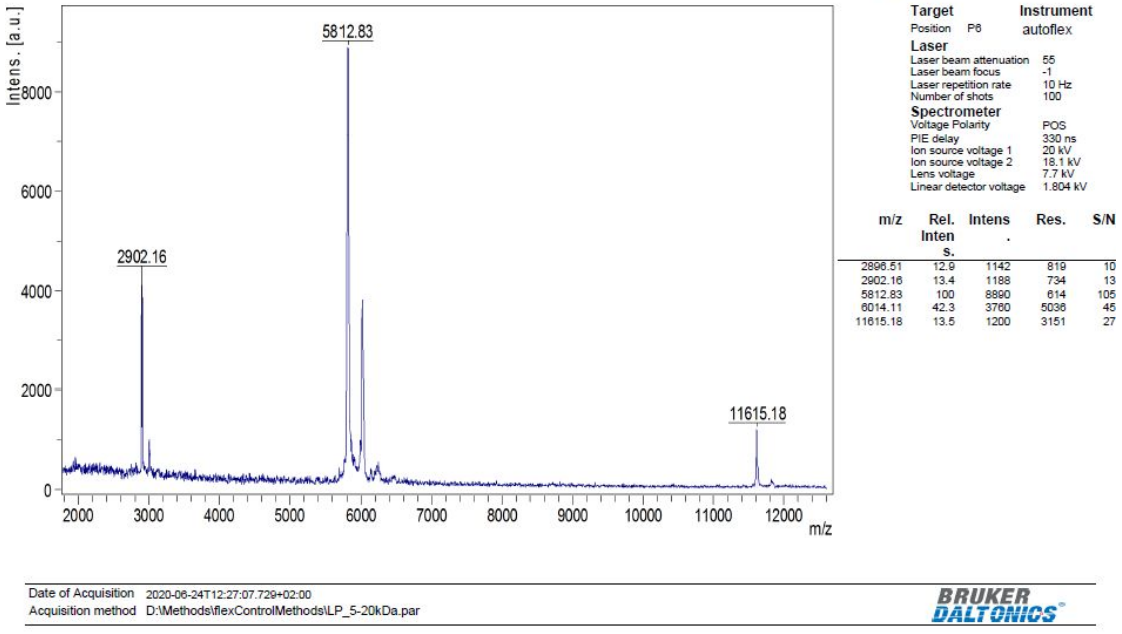
BRUKER DALTONICS

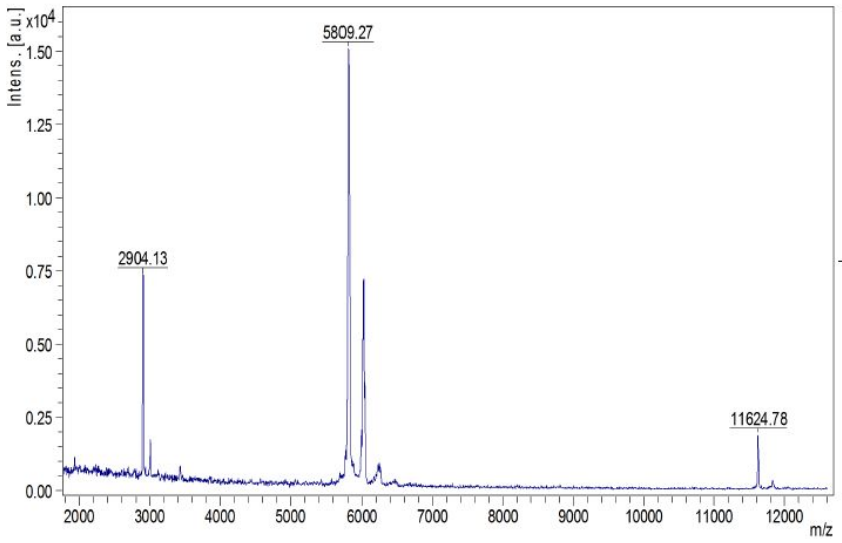
Fmoc-AA crystals RT



Fmoc-AA crystals 50°C



Fmoc-AA crystals 60°C



Target Instrument
Position L6 autoflex
Laser
Laser beam attenuation 55
Laser beam focus -1
Laser repetition rate 10 Hz
Number of shots 100
Spectrometer
Voltage Polarity POS
PIE delay 330 ns
Ion source voltage 1 20 kV
Ion source voltage 2 18.1 kV
Lens voltage 7.7 kV
Linear detector voltage 1.804 kV

m/z	Rel. Intens.	Intens.	Res.	S/N
2904.13	48.4	7290	6611	39
5798.02	62.0	9345	1540	97
5809.27	100	15075	1589	158
6019.69	45.6	6880	3415	73
11624.78	12.6	1895	1180	39

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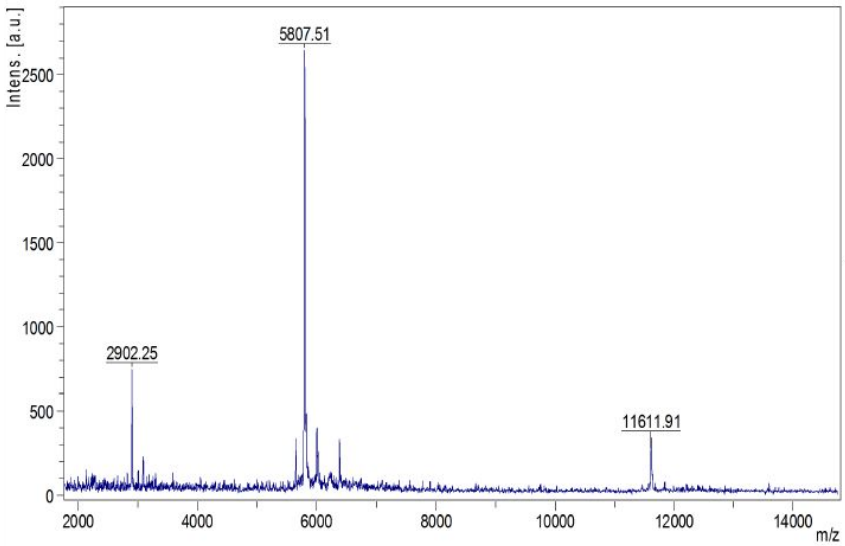
BRUKER DALTONICS

Agarose crystals RT



Target Instrument
Position O3 autoflex
Laser
Laser beam attenuation 100
Laser beam focus -1
Laser repetition rate 10 Hz
Number of shots 50
Spectrometer
Voltage Polarity POS
PIE delay 330 ns
Ion source voltage 1 20 kV
Ion source voltage 2 18.1 kV
Lens voltage 7.7 kV
Linear detector voltage 1.804 kV

m/z	Rel. Intens.	Intens.	Res.	S/N
2902.25	28.2	745	2875	2
5807.51	100	2645	1997	7
6009.91	13.8	365	5934	1
11611.91	9.83	260	7247	1

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BRUKER DALTONICS

Agarose crystals 50 °C

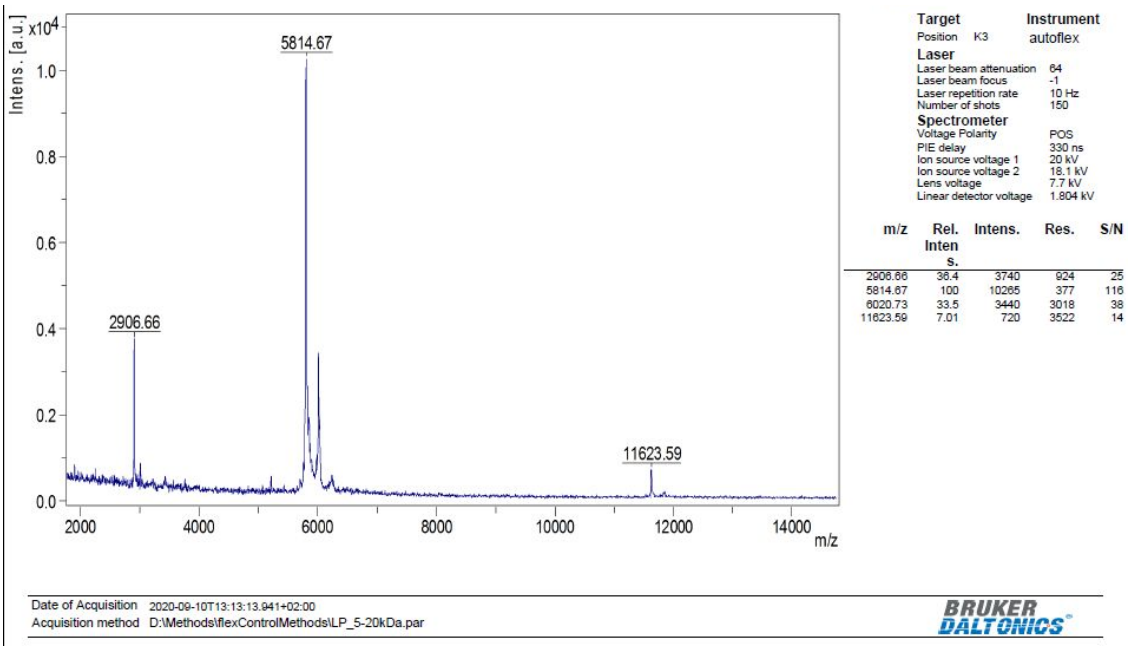

Figure S4. Mass spectra of insulin samples.

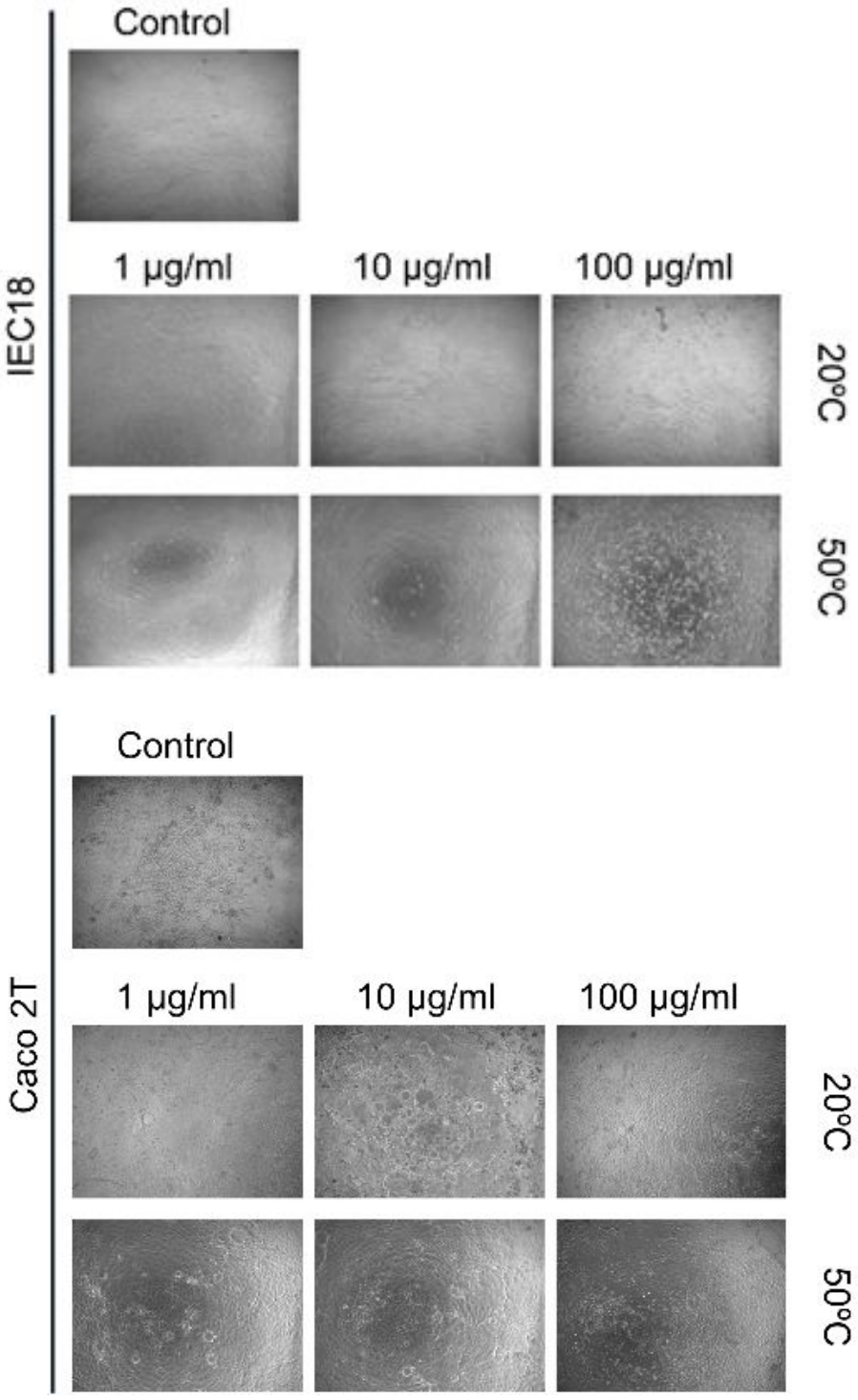

Figure S5. Contrast phase microscopy of the two cell lines used to study the cytotoxicity.