Supporting Information

Sensing Cell Adhesion using Polydiacetylene-Containing Peptide Amphiphile fibres

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1. Experimental section

1.1 Materials

All starting materials were used as received. Amino acids were obtained from Novabiochem. The 2-chlorotrityl resin (200-400 mesh) was obtained from Bachem. N,N′-Diisopropylcarbodiimide (DIPCDI) was obtained from Biosolve. All other starting materials were obtained from commercial suppliers. Fetal bovine serum (FBS) was obtained from Integro (Zaandam, The Netherlands), plain Dulbecco's Modified Eagle Medium (DMEM) and trypsin/EDTA were both from PAA Laboratories (Pasching, Austria). Phenol-red free Dulbecco's Modified Eagle Medium (DMEM) and the Live/Dead® viability kit were obtained from Life Technologies (Thermo Fisher Scientific Inc., Waltham, U.S.A.). HeLa CCL-2 cells were obtained from American Type Culture Collection (ATCC, Manassas, U.S.A.). Human integrin αVβ3 protein in Triton X-100 formulation was obtained from Merck Millipore (Billerica, U. S. A.). Confocal laser scanning microscopy (CLSM) studies were performed on a Leica-microsystems (Mannheim, Germany) TCS SP2 AOBS system, installed on an.inverted motorised DM IRE2 microscope and equipped with a dry HC PL apo CS 20x/0.70 D lens. Brightfield images were obtained using the same microscope and objective. The images were acquired with a Leica DFC 420C colour- camera operated by the LAS software.

1.2 Reversed phase HPLC

Semi-preparative HPLC was performed on a Shimadzu LC-20A Prominence system (Shimadzu, 's Hertogenbosch, The Netherlands) equipped with a Gemini-NX C18 column, 110A, 150x21.2 mm, particle size 10 μm (Phenomenex). Elution of the peptides was achieved with water/acetonitrile gradient containing 0.1% trifluoroacetic acid (5-100% in 40 mins, then 20 mins at 100%, flow 6 mL/min). Samples were dissolved in acetic acid.

Analytical HPLC was performed on a Shimadzu LC-20A Prominence system (Shimadzu, 's Hertogenbosch, The Netherlands) equipped with a Gemini-NX C18 column, 110A, 150x3 mm, particle size 3 μm (Phenomenex). Elution of the peptides was achieved with water/ acetonitrile gradient containing 0.1% trifluoroacetic acid (5-100% in 50 mins, flow 0.4 mL/ min). Samples were dissolved in acetic acid at 1 mg/ml.

1.3 Liquid Chromatography Mass Spectrometry (LC-MS)

Mass spectra were recorded on a Thermo Finnigan LCQ-Fleet ESI-ion trap equipped with a C18 ReproSil column, 50x2 mm, particle size 3 μm (Screening Devices, Amersfoort, The Netherlands), elution with water/acetonitrile gradient containing 0.1% formic acid, gradient 5-100% in 20 mins. Samples were dissolved in methanol at 0.2 μM.

$^{10,12}C_{25}$-Gly-Ala-Gly-Ala-Glu-OH

ESI-ion trap m/z: $[M+H]^+$ 760.12 (calcd. 759.48)

$^{10,12}C_{25}$-Gly-Ala-Gly-Ala-Lys-OH

ESI-ion trap m/z: $[M+H]^+$ 760.56 (calcd. 758.53)

$^{10,12}C_{25}$-Gly-Ala-Gly-Ala-Ala-OH

ESI-ion trap m/z: $[M+H]^+$ 702.08 (calcd. 701.47)

$^{10,12}C_{25}$-Gly-Ala-Gly-Ala-Ser-OH

ESI-ion trap m/z: $[M+H]^+$ 718.08 (calcd. 717.47)

$^{10,12}C_{25}$-Gly-Ala-Gly-Ala-Val-OH

ESI-ion trap m/z: $[M+H]^+$ 730.12 (calcd. 729.50)

$^{10,12}C_{25}$-Gly-Ala-Gly-Ala-Gln-OH

ESI-ion trap m/z: $[M+H]^+$ 759.12 (calcd. 758.49)

$^{10,12}C_{25}$-Gly-Ala-Gly-Ala-Asp-OH

ESI-ion trap m/z: $[M+H]^+$ 746.10 (calcd. 745.46)

$^{10,12}C_{25}$-Gly-Ala-Gly-Ala-Asn-OH

ESI-ion trap m/z: $[M+H]^+$ 745.12 (calcd. 744.48)

$^{10,12}C_{25}$-Gly-Ala-Gly-Ala-Lys-Arg-Gly-Asp-Ser-NH_2

ESI-ion trap m/z: $[M+H]^+$ 1174.3 (calcd. 1172.73) $[M+H]^{2+}$ 588.00 (calcd. 587.37)

$^{10,12}C_{25}$-Gly-Ala-Gly-Ala-Lys-Asp-Gly-Ser-Arg-NH_2

ESI-ion trap m/z: $[M+H]^+$ 1174.3 (calcd. 1172.73) $[M+H]^{2+}$ 588.00 (calcd. 587.37)

1.4 Temperature dependent UV-Vis absorption spectra

UV-Vis absorption spectra of the fibre samples during heating were recorded on a Jasco V-360 spectrometer, with a temperature gradient of 5 °C/min, using a 1 cm quartz cuvette (a 0.1 mm quartz cuvette was used to measure the K-RGDS fibres). The transition temperature was determined by fitting the decrease in absorption at 647 nm with temperature using a Hill 1 function in Origin.

y = start + (end-start)*x^n / (k^n + x^n) (**Equation 1**)

Where k = the transition temperature

1.5 Transmission electron microscopy

Transmission electron microscopy (TEM) samples were prepared by floating a carbon-coated copper grid on a peptide amphiphile solution of 0.2 mg/mL for 5 min, followed by staining by floating the grid on a 1% solution of uranyl acetate in Milli-Q water for 30 seconds. Finally, the grid was floated on Milli-Q water and the excess water was removed by blotting with paper filter. The TEM samples were visualised using a JEOL 1010 transmission electron microscope set on an accelerating voltage of 60 kV.

1.6 Cell culture

HeLa cells were maintained in sterile conditions in Dulbecco's Modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS). Cells were maintained on tissue culture plastic and kept at 37°C in a humidified atmosphere of 7.5% CO_2. Cells were passaged every 2-3 days. Prior to cell viability assays or cell adhesion studies, cells within a confluent layer were detached using trypsin/EDTA.

Cells were then resuspended in phenol-red free DMEM supplemented with 10% FBS and the number of cells was counted using a standard inverted microscope and a cell counting chamber (Fuchs-Rosenthal).

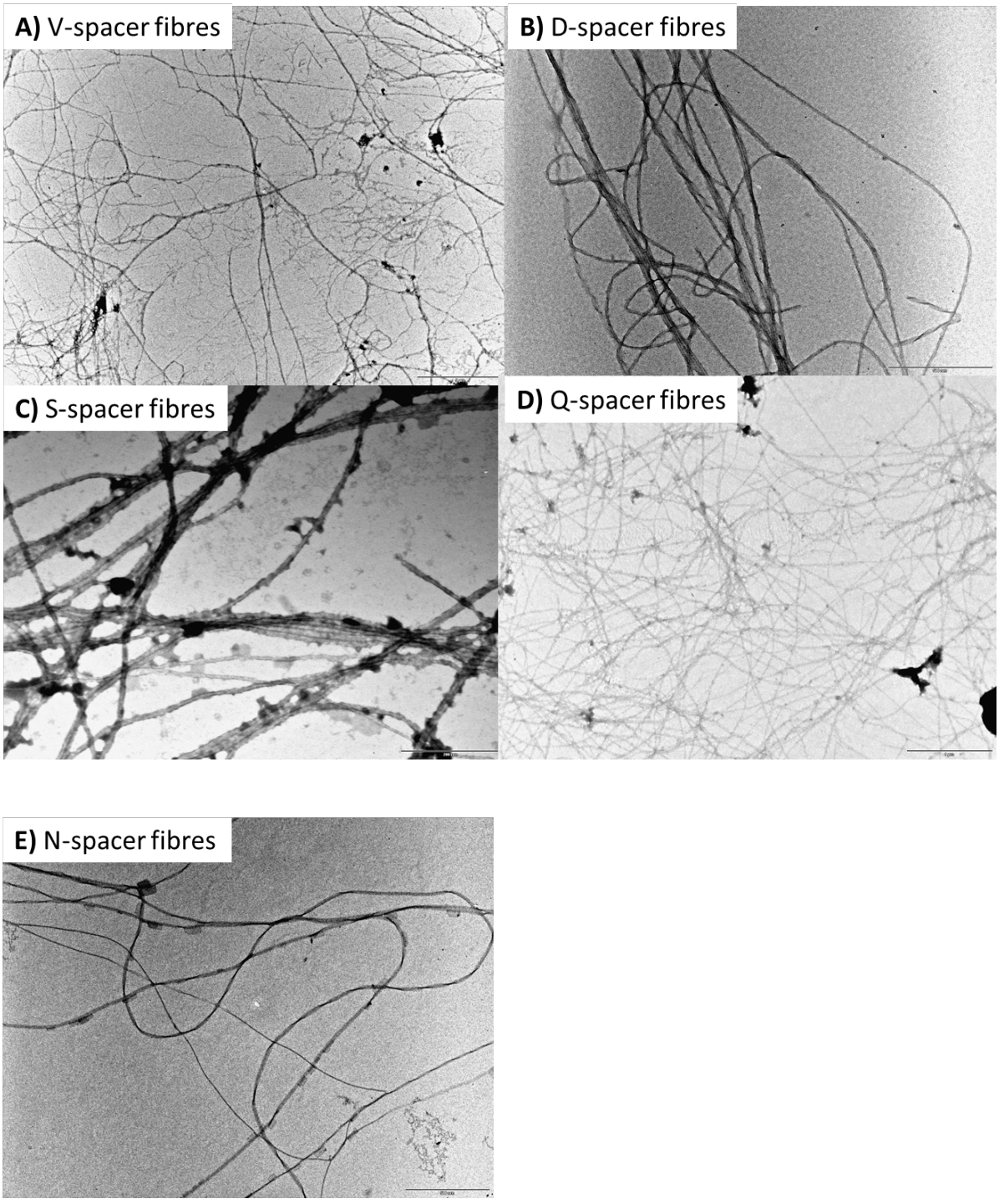
1.7 Integrin Binding

Isolated human αVβ3 integrin protein (100 nM, in 20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 2 mM $MgCl_2$, 0.2% Triton X-100) and the 6:1 K-RGDS fibre solution (100 nM (based on the RGDS content), in 20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 2 mM $MgCl_2$, 0.2% Triton X-100) were mixed 1:1 and incubated at 30 °C for 10 mins. The UV-Vis spectrum of the resulting sample was measured using a 1 mm quartz cuvette on a Varian Cary 50 spectrometer. The 50 nM fibre solution in the Triton X-100 buffer mentioned above was also measured using a Varian Cary 50. The chromatic ratio was calculated using the absorptions A_{blue} = 646 nm and A_{red} = 540 nm.

2. Supporting Figures

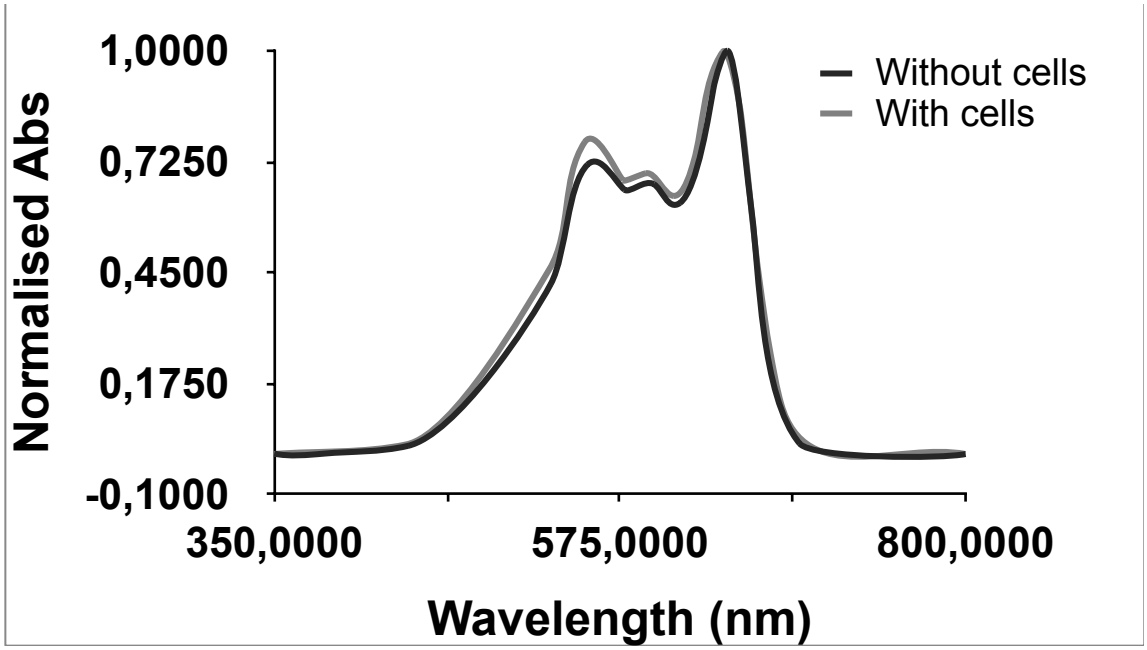
2.1 TEM Micrographs

Transmission electron micrographs of the other fibres. The scale bars are as follows, a) 6 μm b) 200 nm c) 200 nm d) 1 μm e) 500 nm.

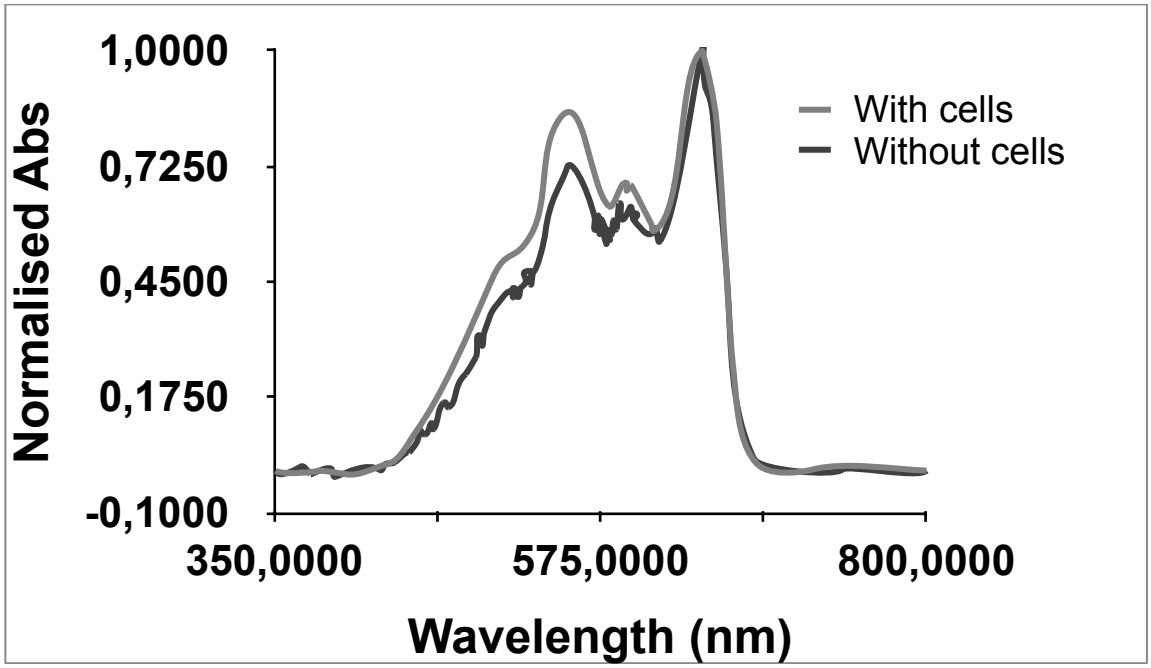


2.2 UV-Vis spectra of Fibres used to Calculate the Chromatic Ratios

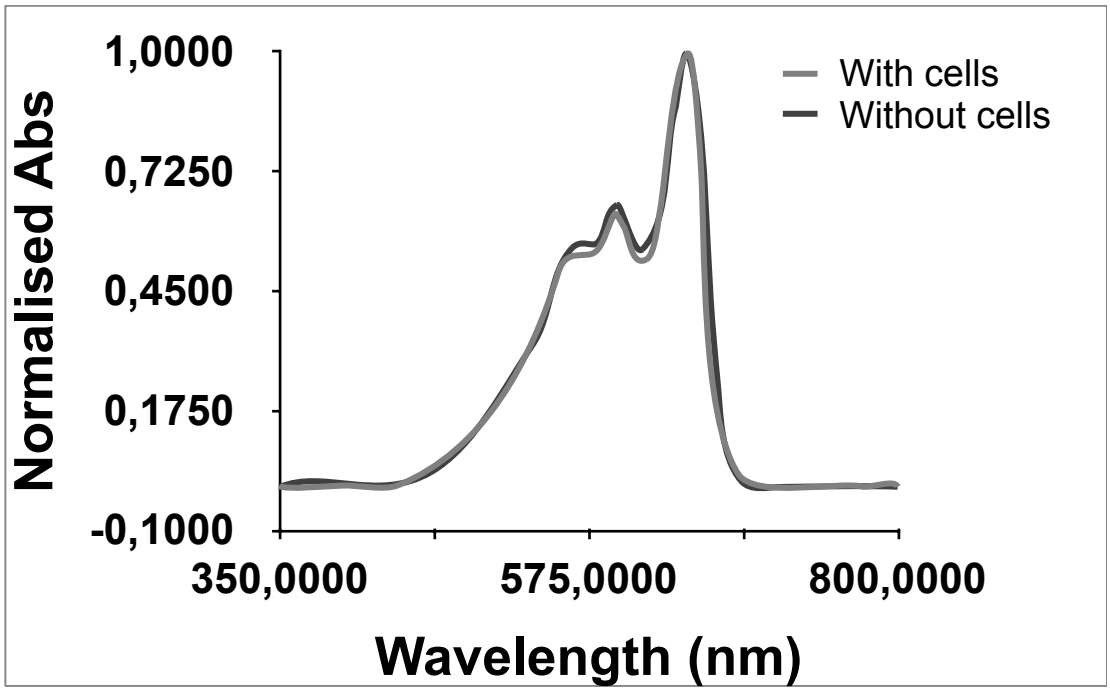
V-RGDS fibres



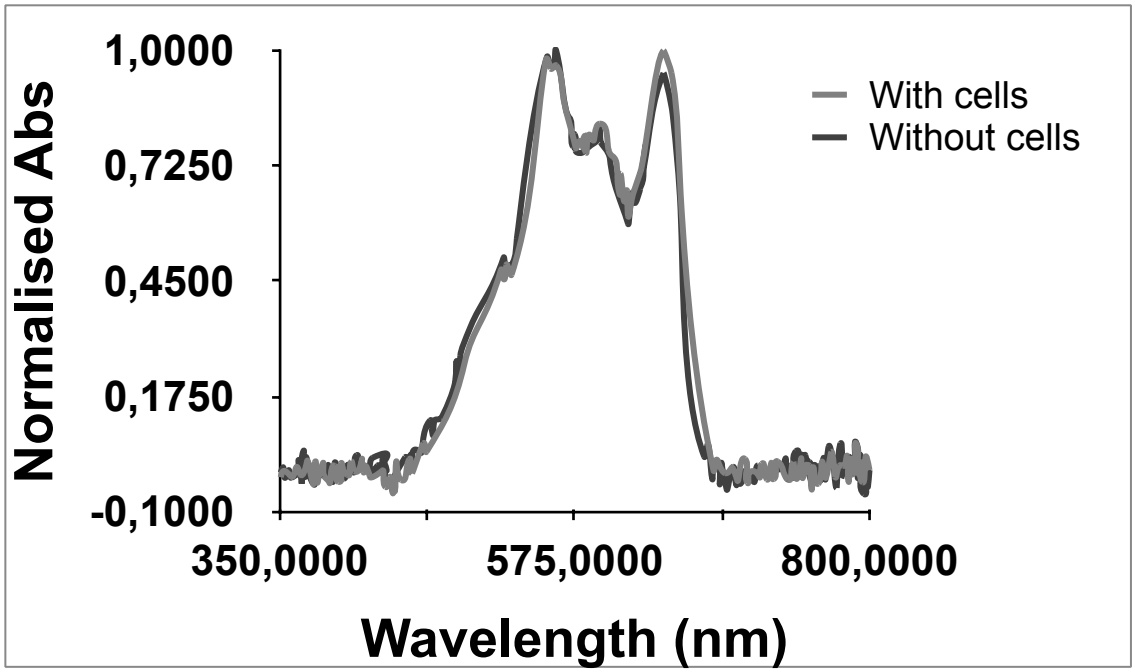
D-RGDS fibres



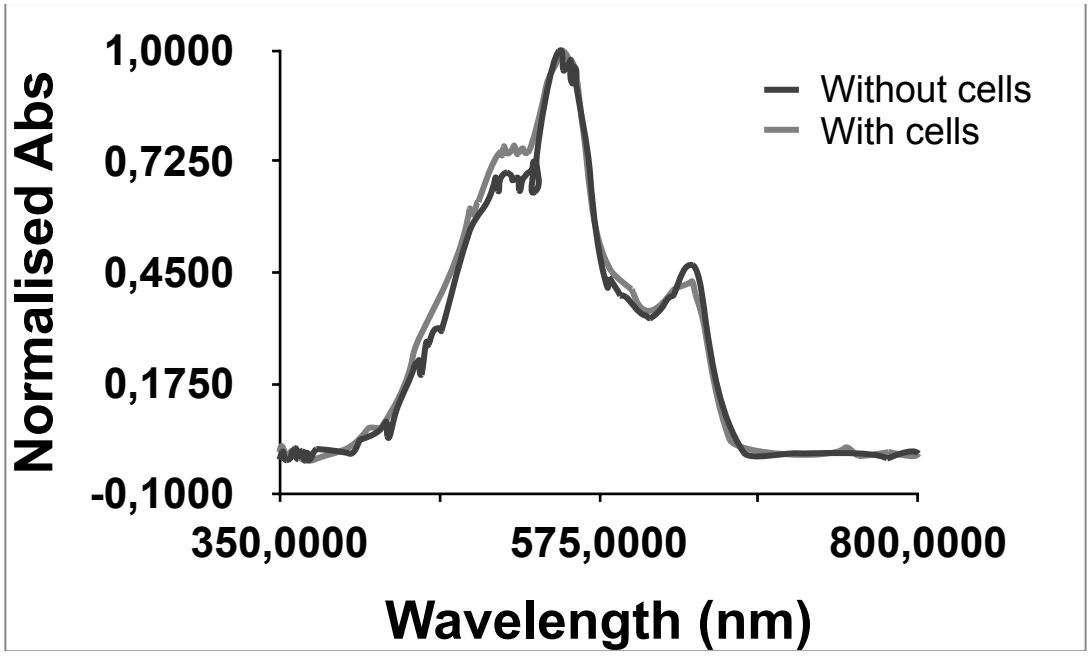
S-RGDS fibres



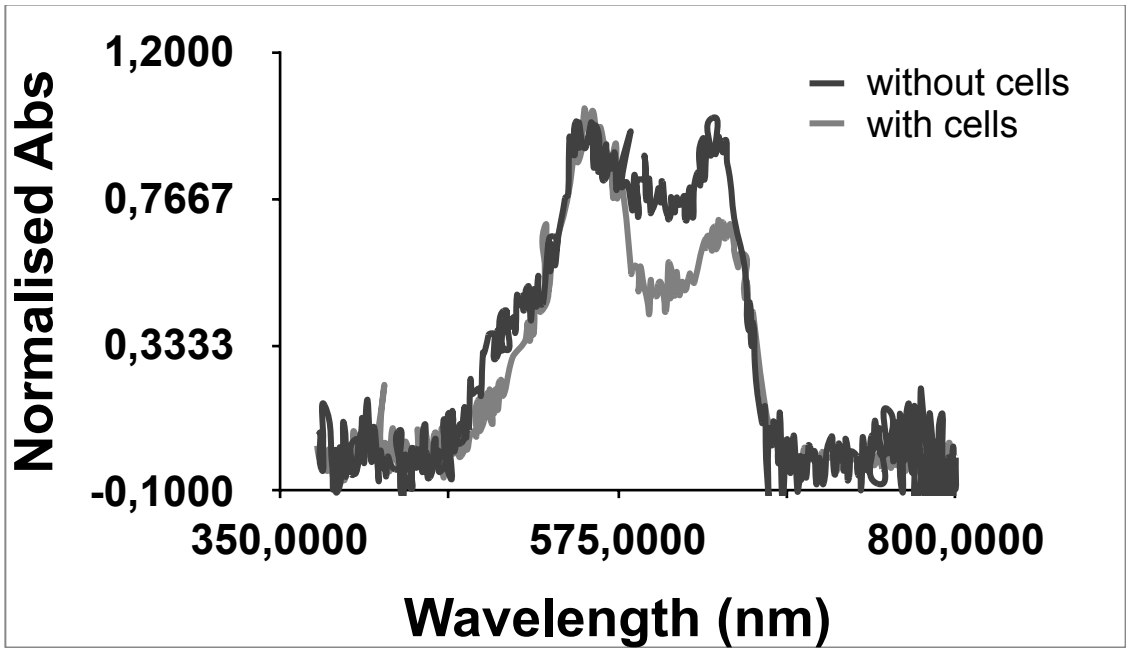
A-RGDS fibres



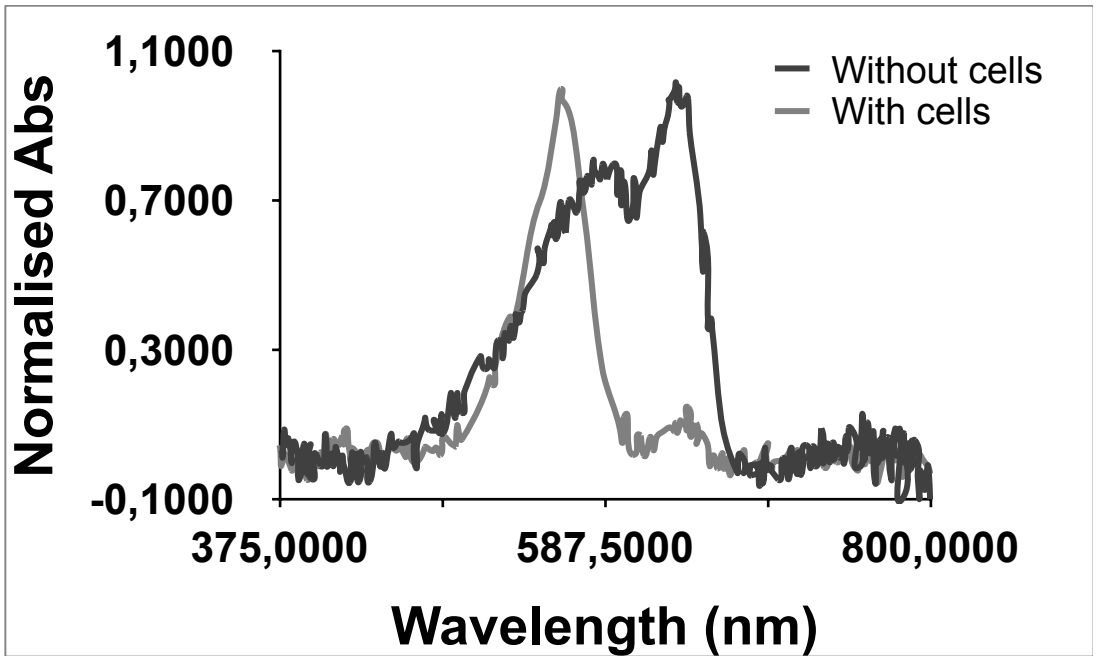
E-RGDS fibres



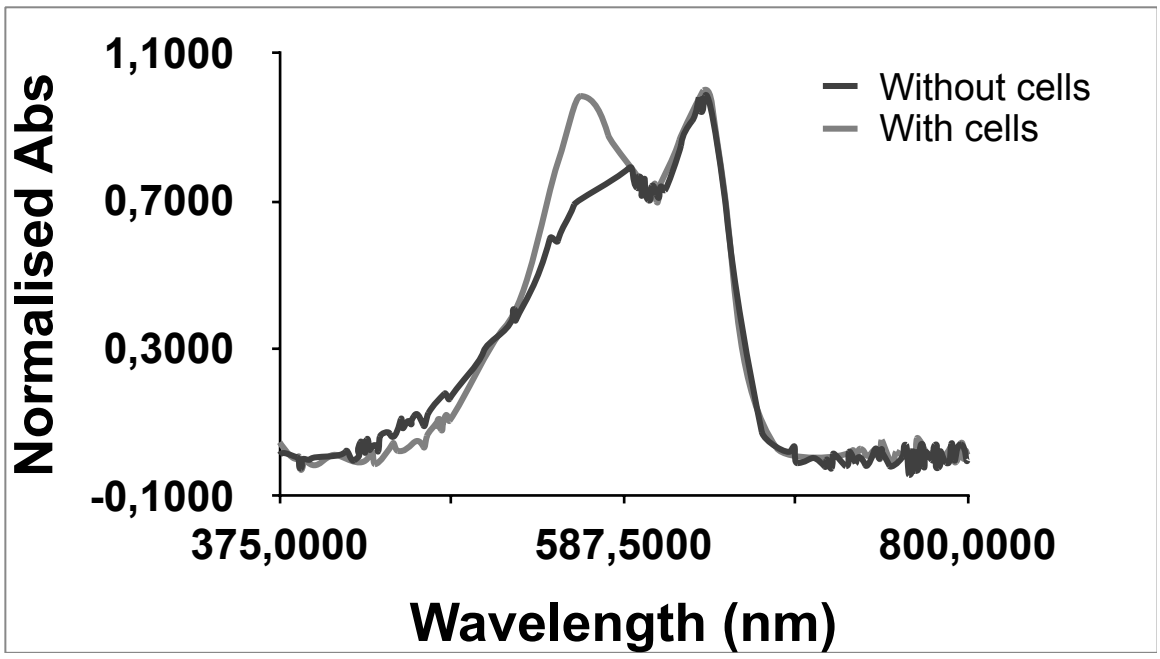
3:1 K-RGDS fibres



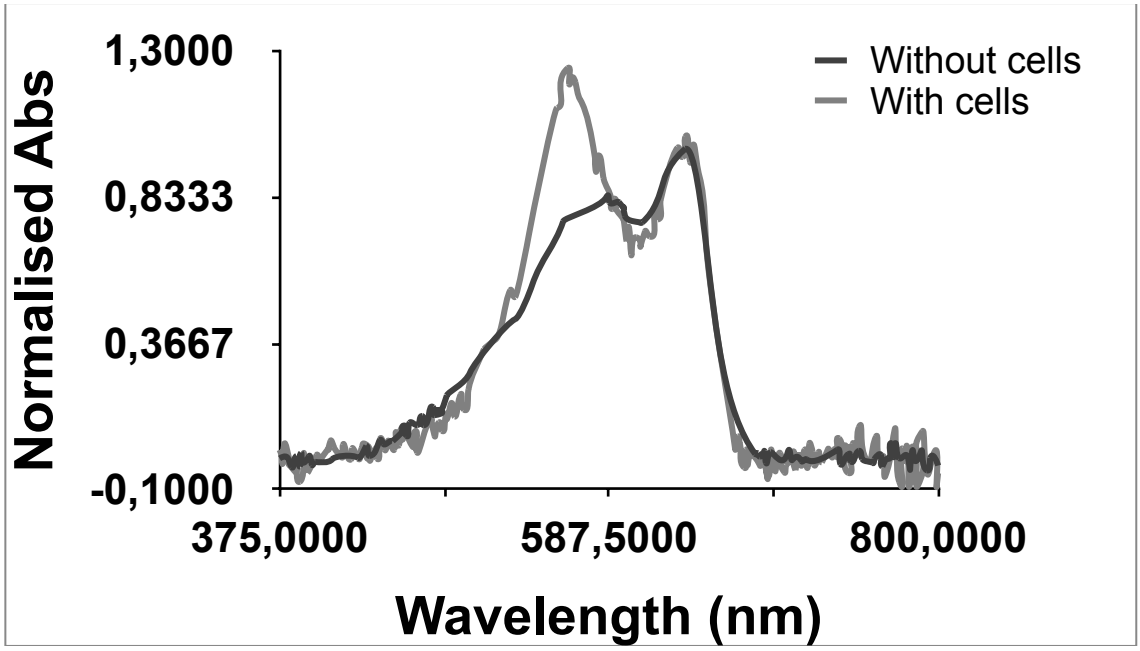
6:1 K-RGDS fibres



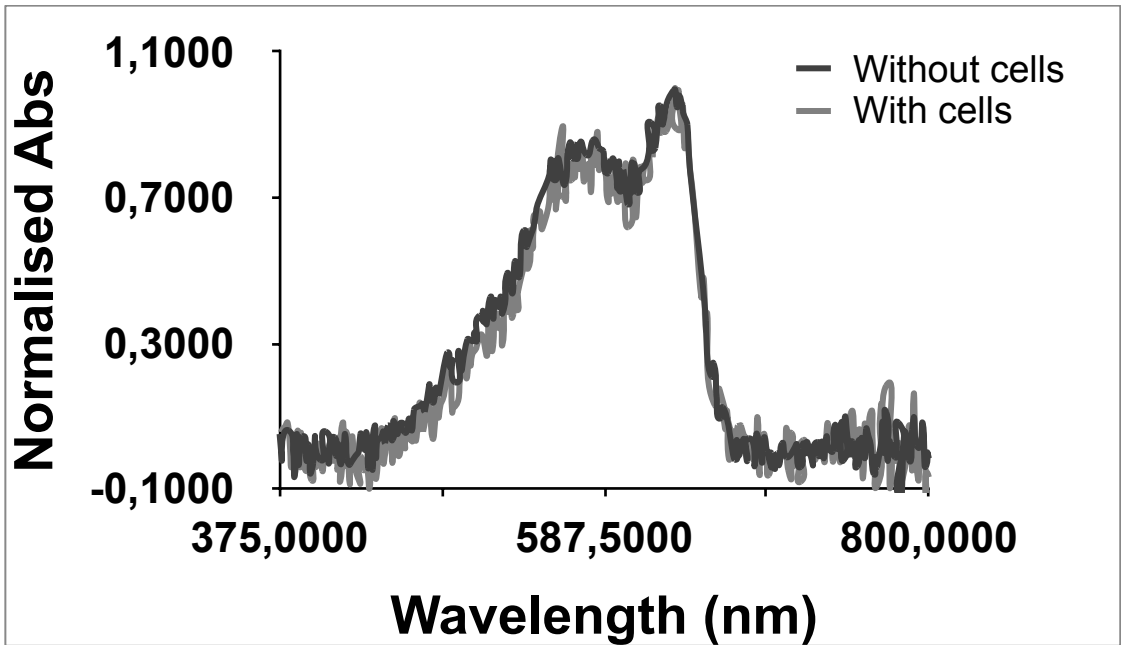
9:1 K-RGDS fibres



12:1 K-RGDS fibres

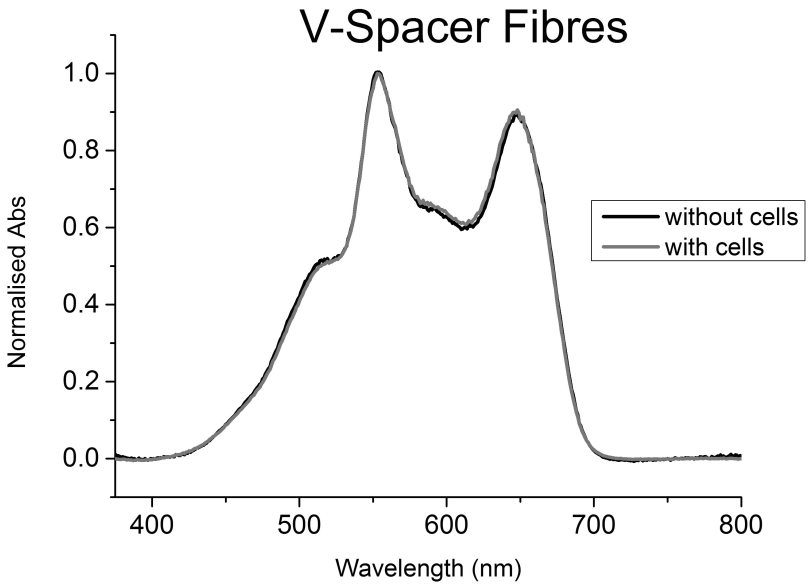


24:1 K-RGDS fibres

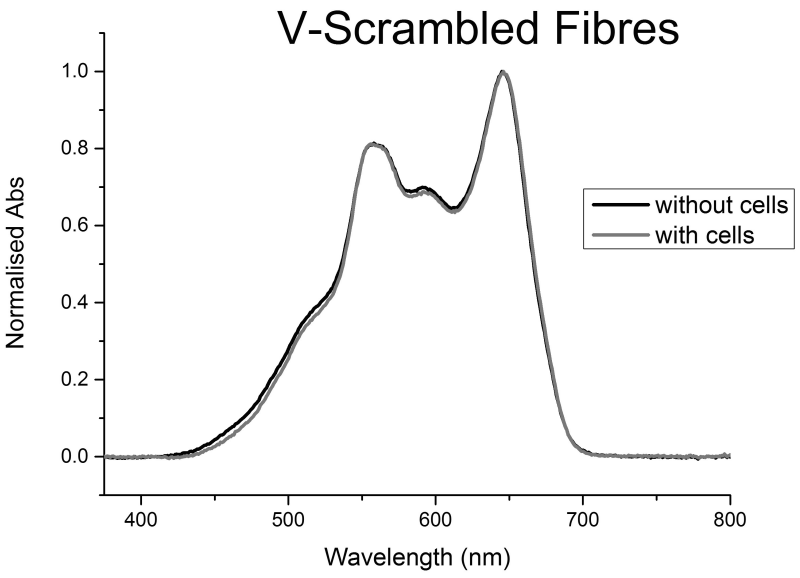


Additional UV-vis absorption spectra of the spacer- and scrambled-fibres with or without addition of cells

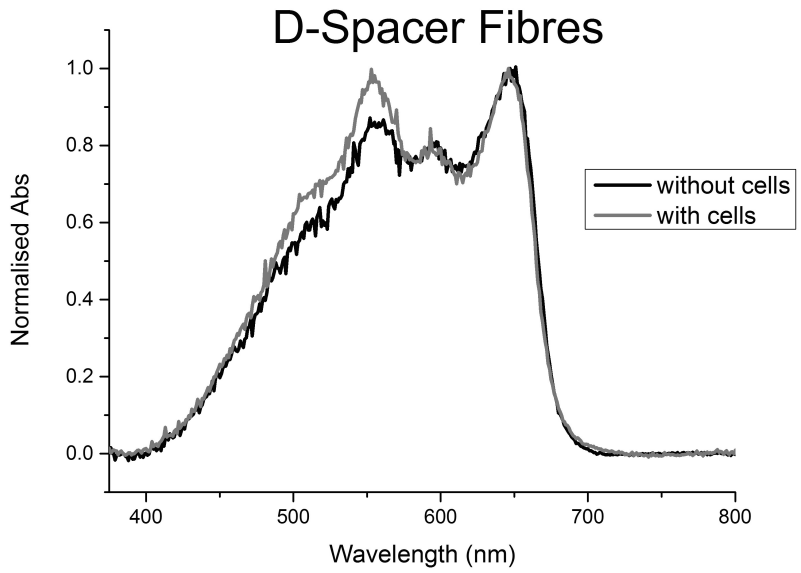
V-Spacer



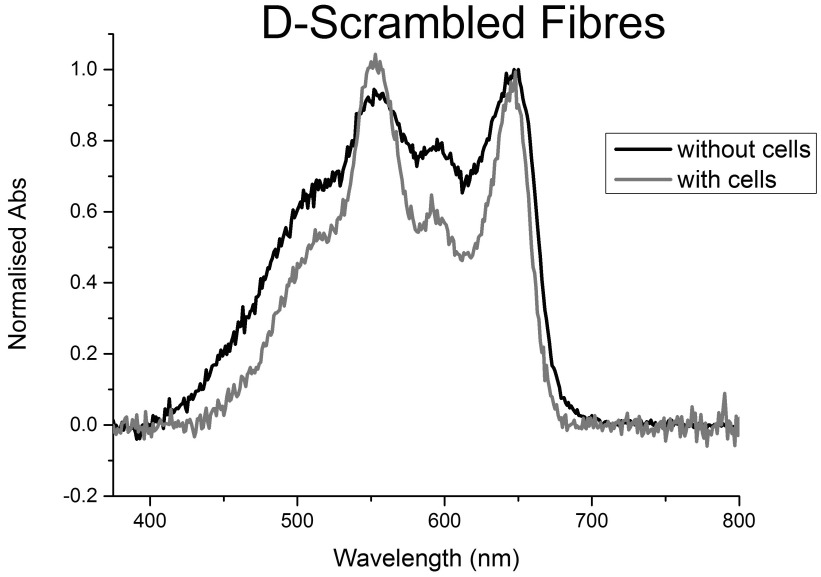
V-Scrambled



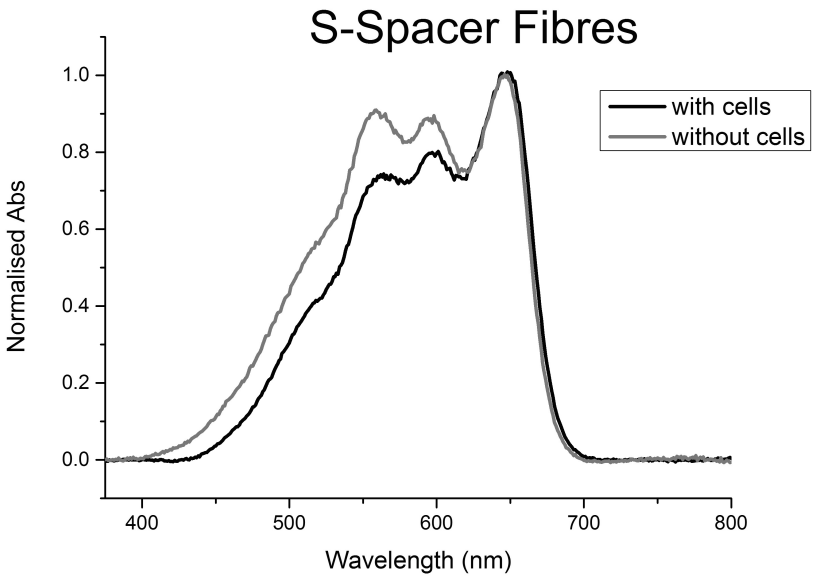
D-Spacer



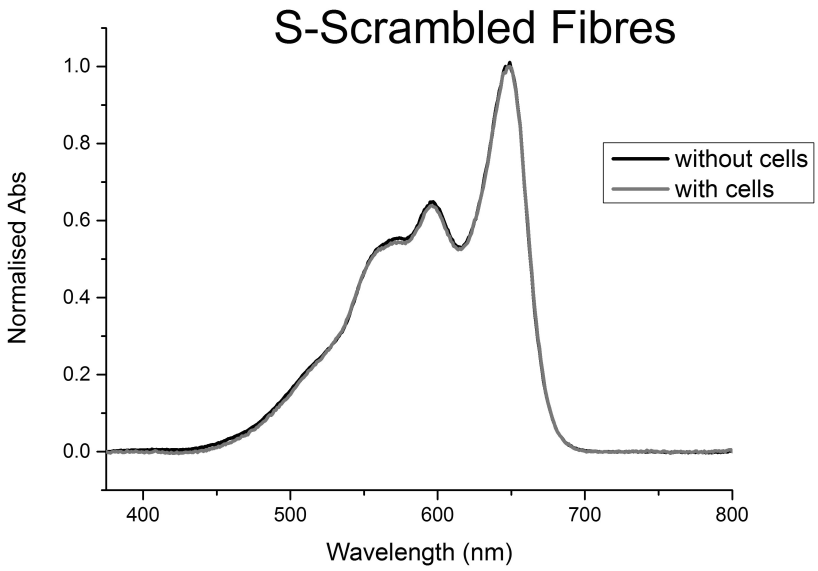
D-Scrambled



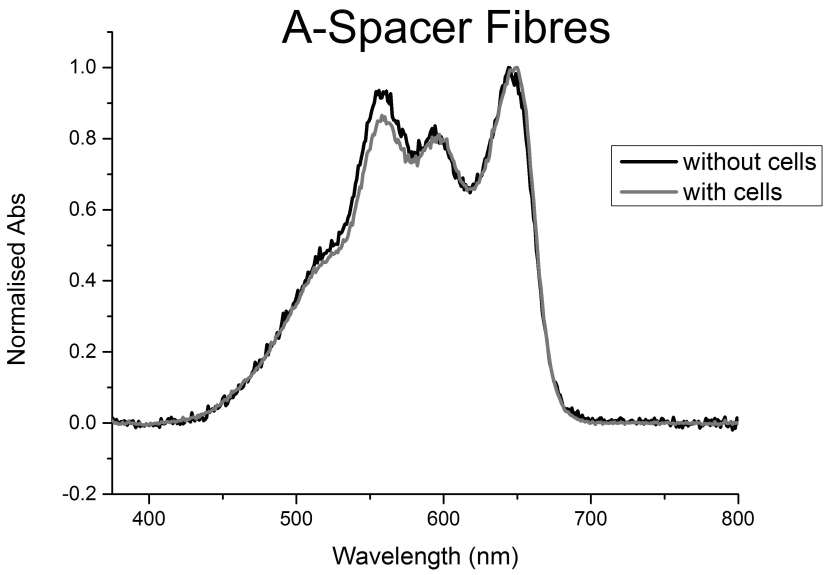
S-Spacer



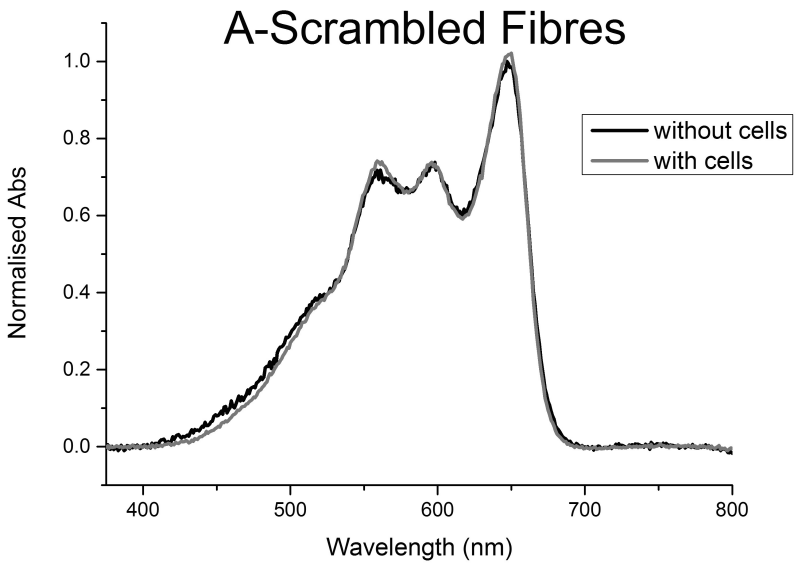
S-Scrambled



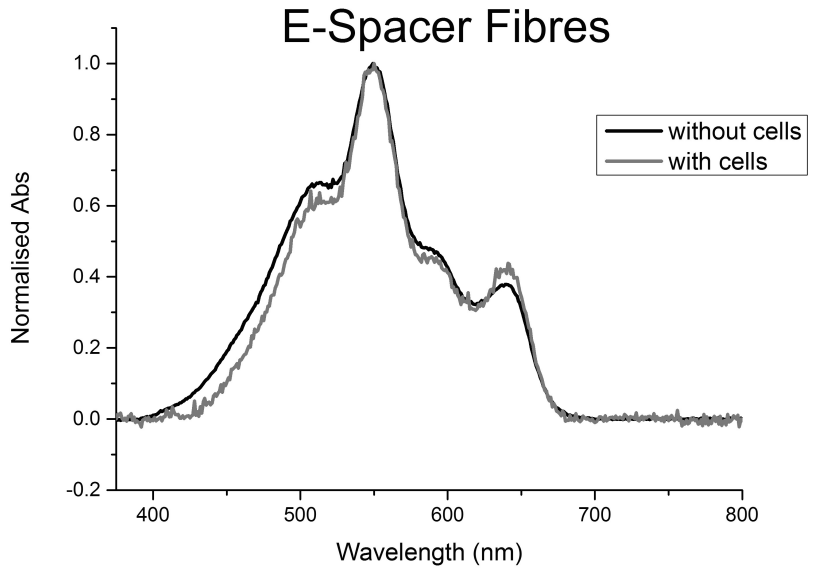
A-Spacer



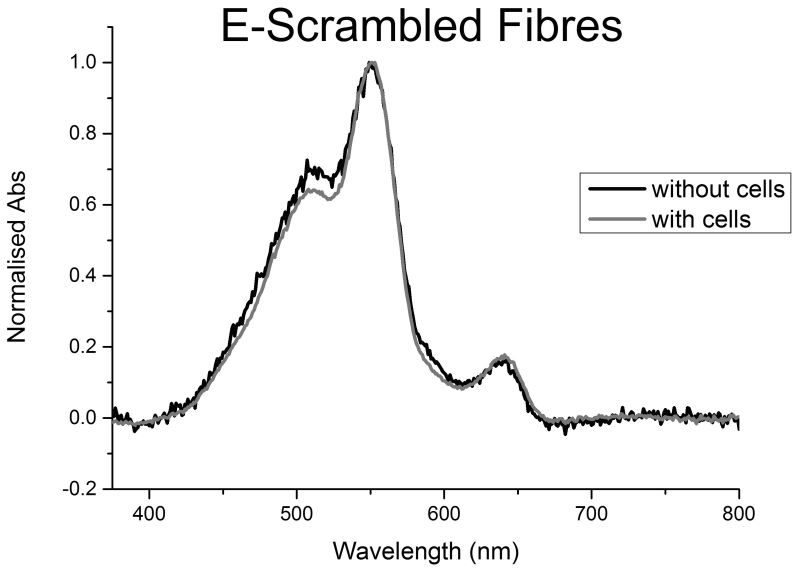
A-Scrambled



E-Spacer



E-Scrambled



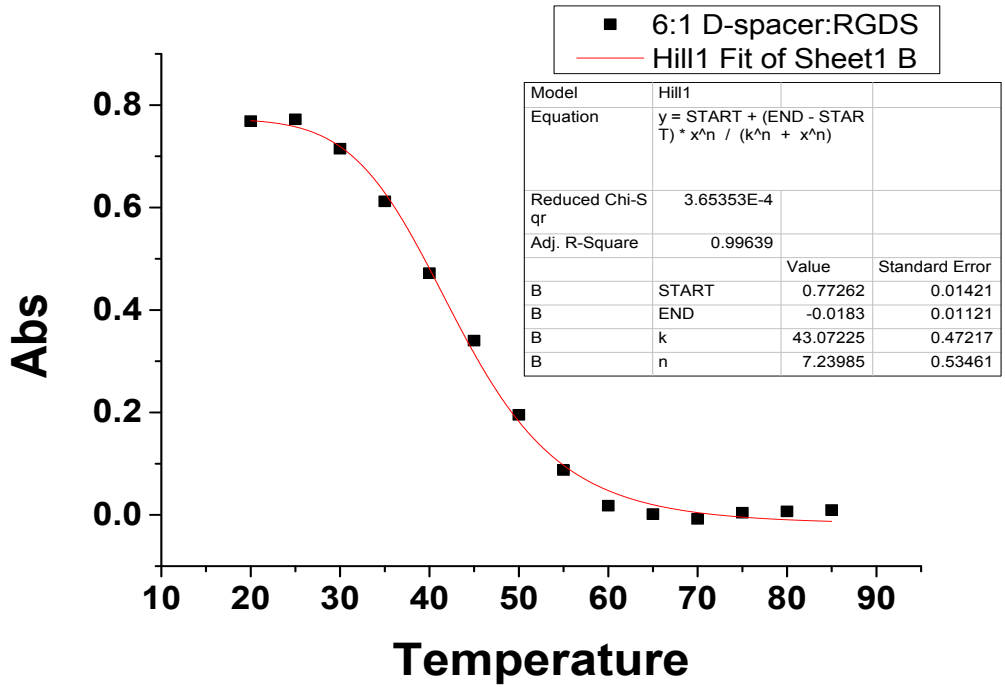
Chromatic ratios for the Spacer- and Scrambled fibres with and without cells

Fibres	Chromatic Ratio
S-spacer	9.2
S-scrambled	-1.7
A-spacer	-2.1
A-scrambled	1.9
V-spacer	-2.3
V-scrambled	1.9
D-spacer	10.4
D-scrambled	6.0
E-spacer	-6.8
E-scrambled	-7.1

Table S2.2 Chromatic ratios for the Spacer- and Scrambled fibres after addition of cells, calculated from the UV-vis absorption spectra above.

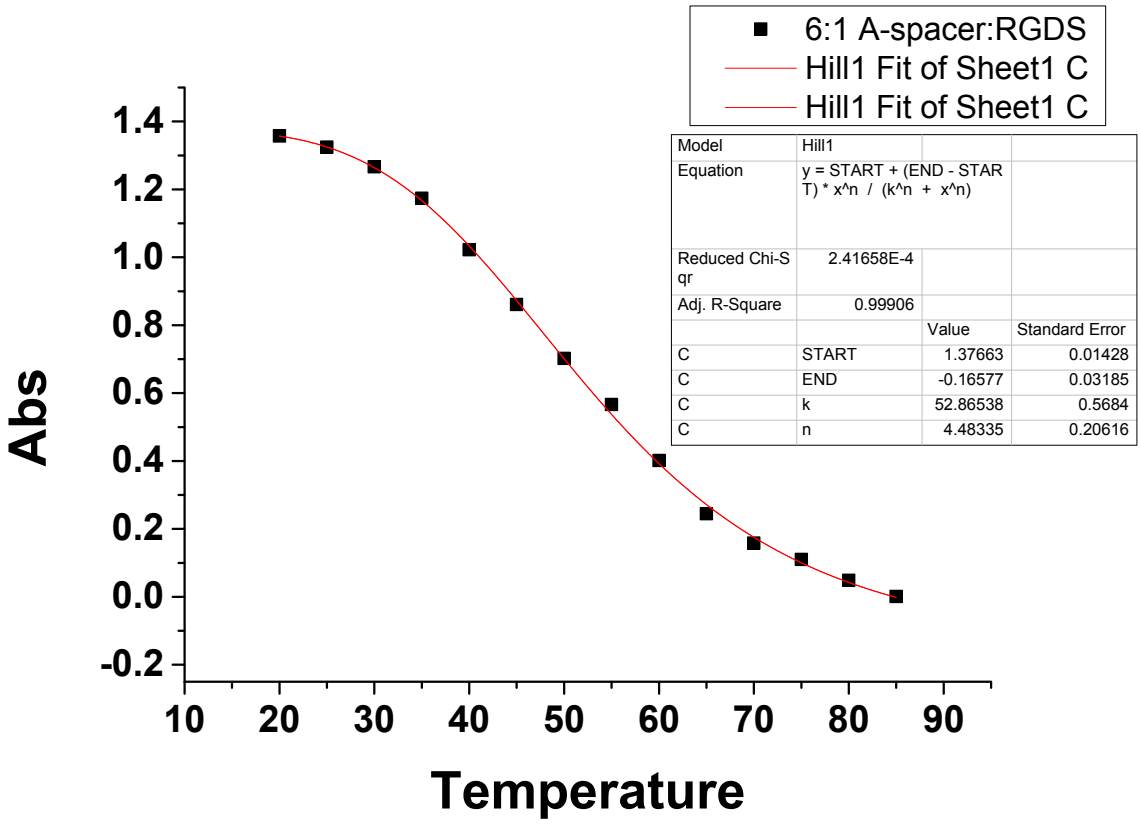
2.3 Determination of Transition Temperature

D-RGDS fibres



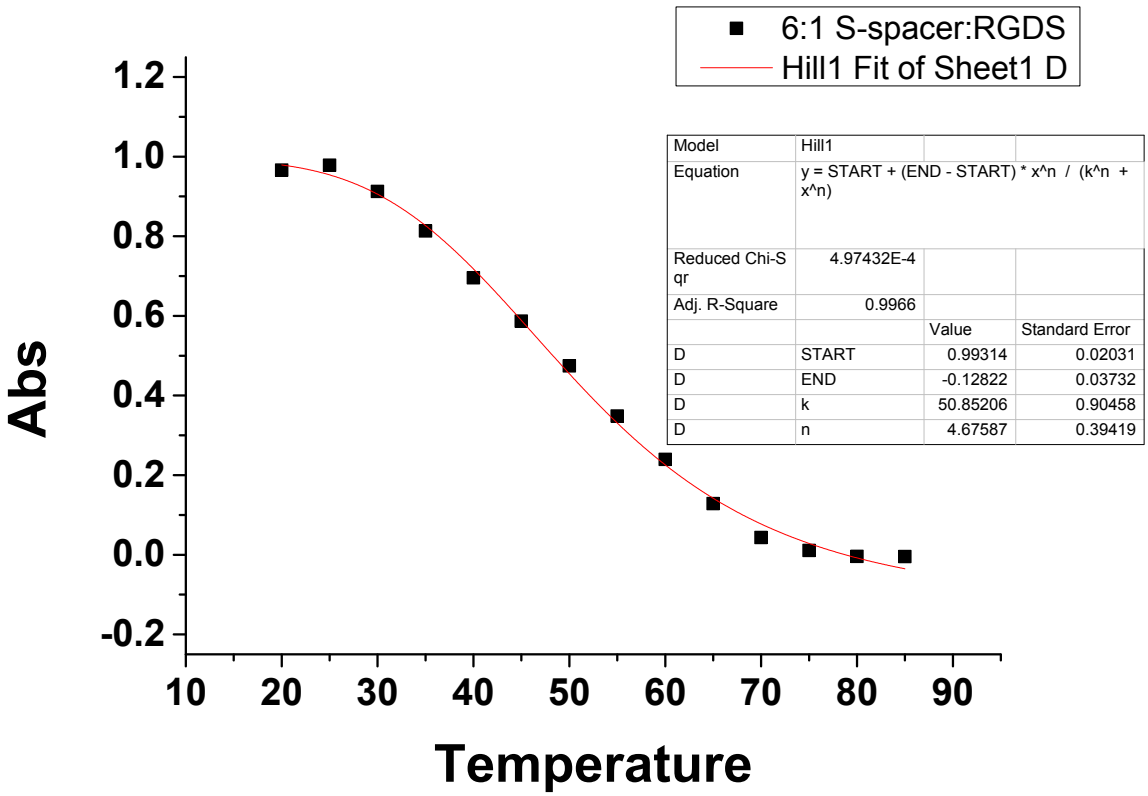
Model	Hill1		
Equation	y = START + (END - STAR T) * x^n / (k^n + x^n)		
Reduced Chi-S qr	3.65353E-4		
Adj. R-Square	0.99639		
		Value	Standard Error
B	START	0.77262	0.01421
B	END	-0.0183	0.01121
B	k	43.07225	0.47217
B	n	7.23985	0.53461

A-RGDS fibres



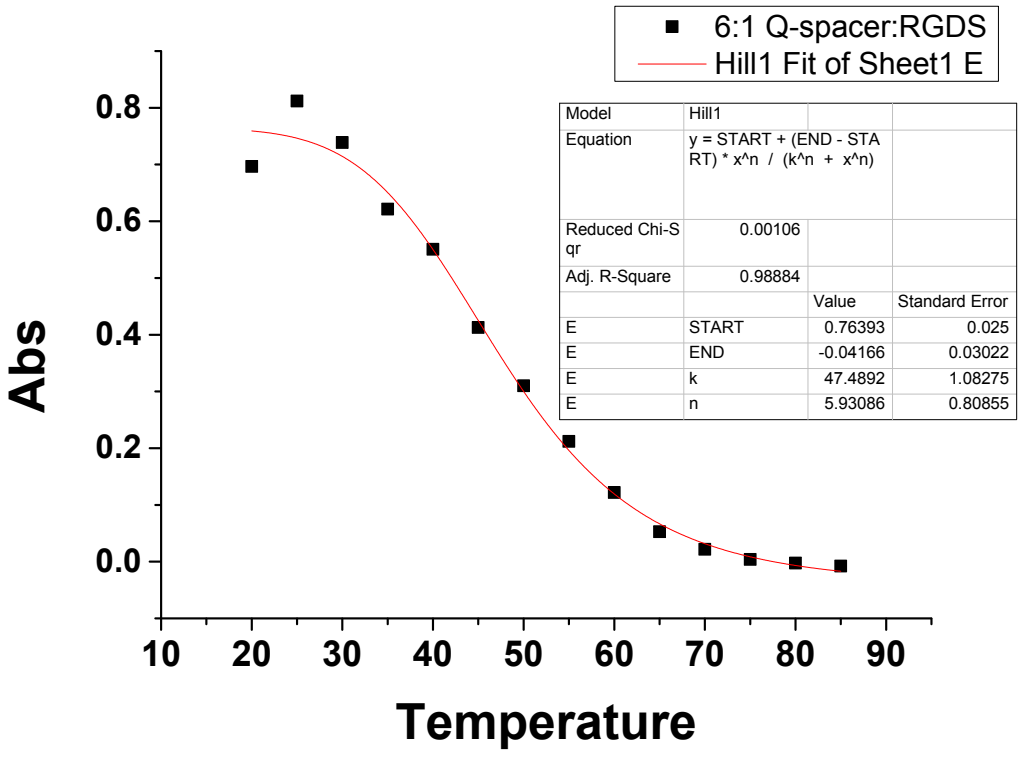
Model	Hill1		
Equation	y = START + (END - STAR T) * x^n / (k^n + x^n)		
Reduced Chi-S qr	2.41658E-4		
Adj. R-Square	0.99906		
		Value	Standard Error
C	START	1.37663	0.01428
C	END	-0.16577	0.03185
C	k	52.86538	0.5684
C	n	4.48335	0.20616

S-RGDS fibres



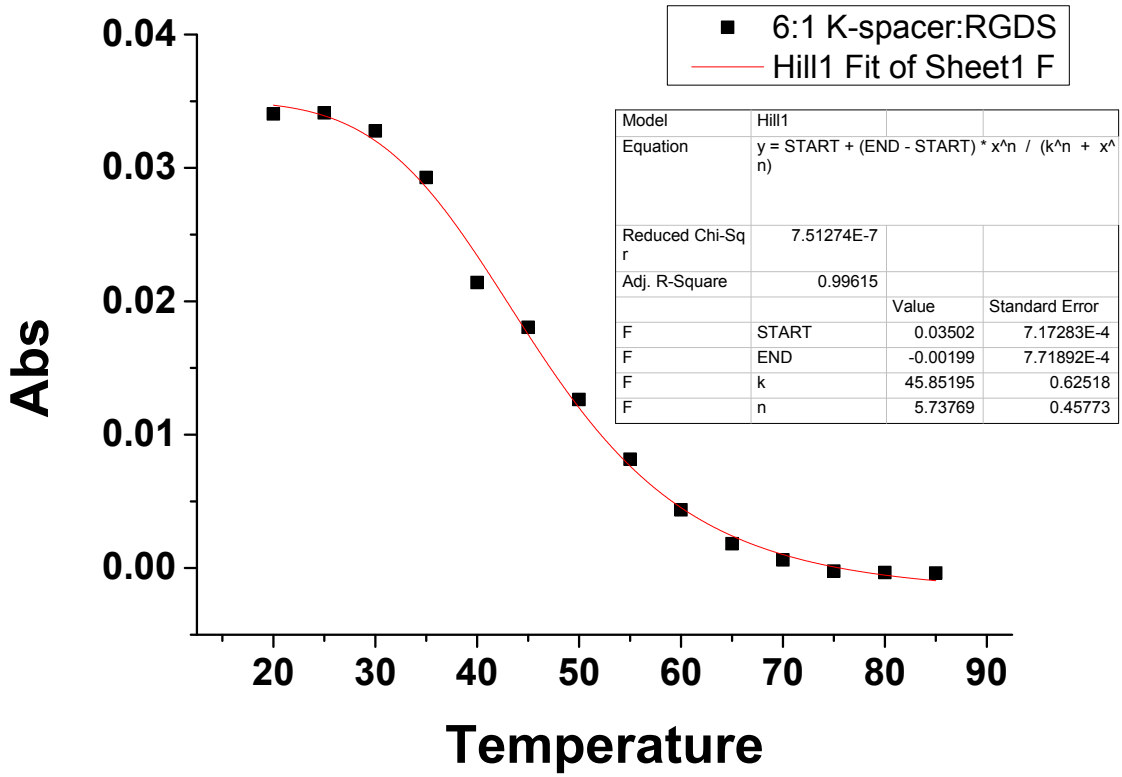
Model	Hill1		
Equation	y = START + (END - START) * x^n / (k^n + x^n)		
Reduced Chi-Sqr	4.97432E-4		
Adj. R-Square	0.9966		
		Value	Standard Error
D	START	0.99314	0.02031
D	END	-0.12822	0.03732
D	k	50.85206	0.90458
D	n	4.67587	0.39419

Q-RGDS fibres

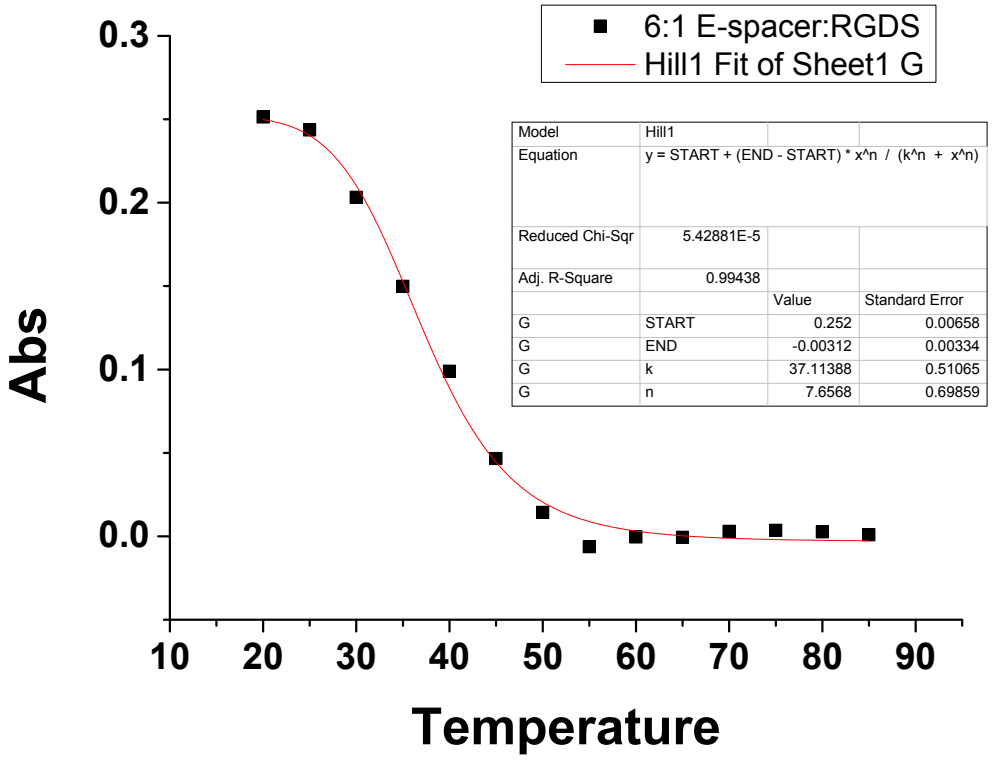


Model	Hill1		
Equation	y = START + (END - START) * x^n / (k^n + x^n)		
Reduced Chi-Sqr	0.00106		
Adj. R-Square	0.98884		
		Value	Standard Error
E	START	0.76393	0.025
E	END	-0.04166	0.03022
E	k	47.4892	1.08275
E	n	5.93086	0.80855

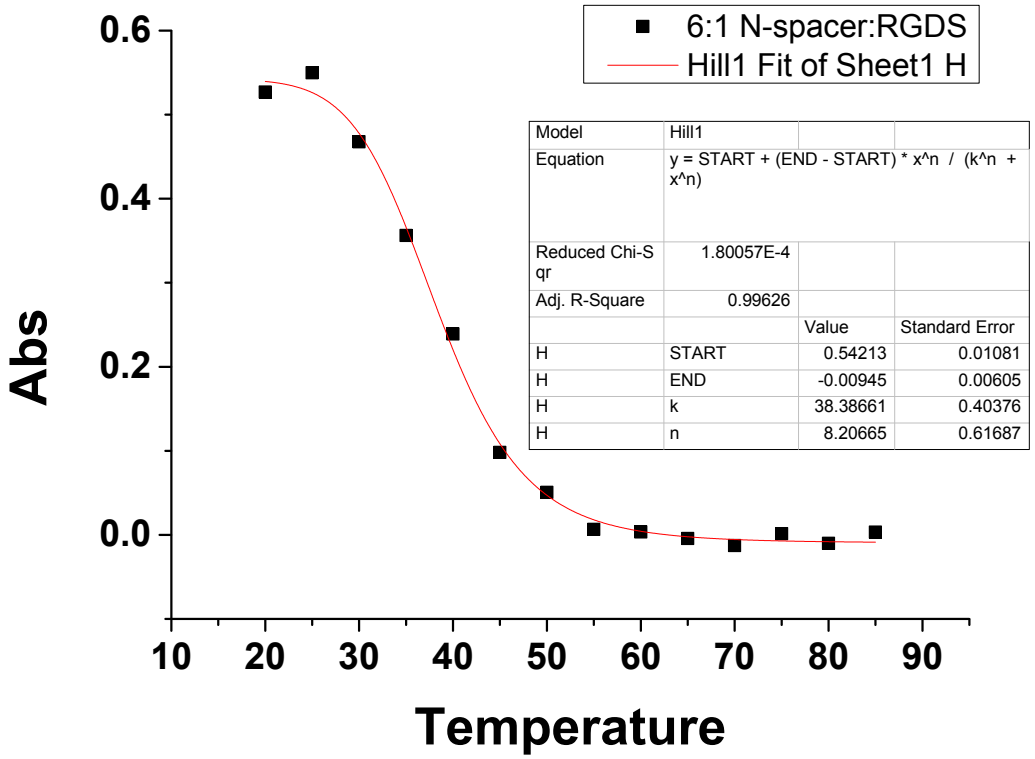
K-RGDS fibres



E-RGDS fibres

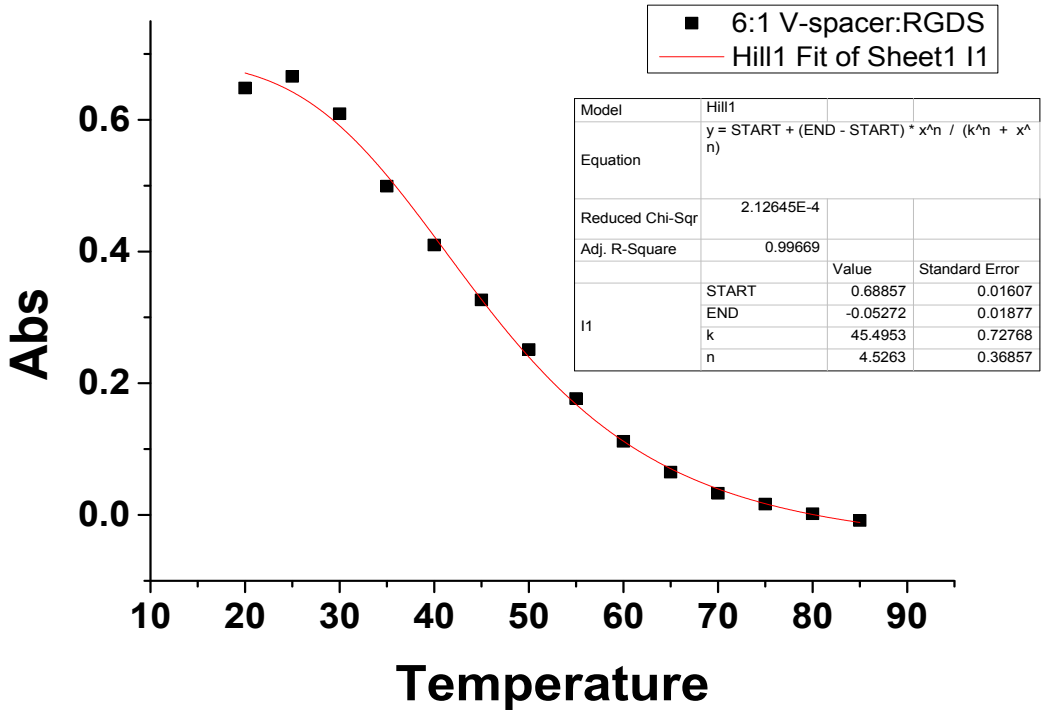


N-RGDS fibres



Model	Hill1		
Equation	y = START + (END - START) * x^n / (k^n + x^n)		
Reduced Chi-Sqr	1.80057E-4		
Adj. R-Square	0.99626		
		Value	Standard Error
H	START	0.54213	0.01081
H	END	-0.00945	0.00605
H	k	38.38661	0.40376
H	n	8.20665	0.61687

V-RGDS fibres



Model	Hill1		
Equation	y = START + (END - START) * x^n / (k^n + x^n)		
Reduced Chi-Sqr	2.12645E-4		
Adj. R-Square	0.99669		
		Value	Standard Error
I1	START	0.68857	0.01607
I1	END	-0.05272	0.01877
I1	k	45.4953	0.72768
I1	n	4.5263	0.36857

2.4 Brightfield microscopy

Brightfield images obtained by widefield microscopy. All images were captured using the same camera settings and the white balance was set using a transparant sample. The scale bar represents 150 µm.

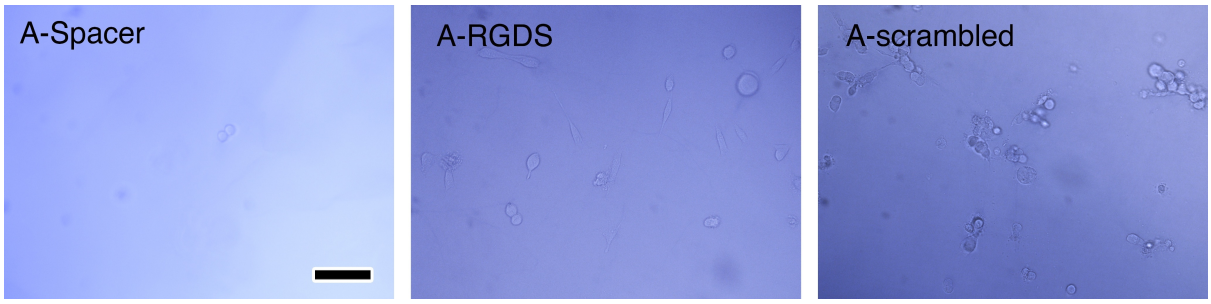


Figure S2.4.1 Brightfield micrographs of the A-fibres

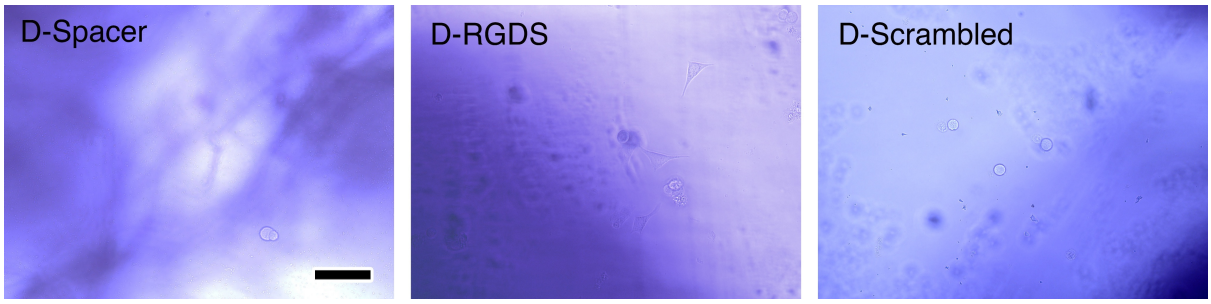


Figure S2.4.2 Brightfield micrographs of the D-fibres

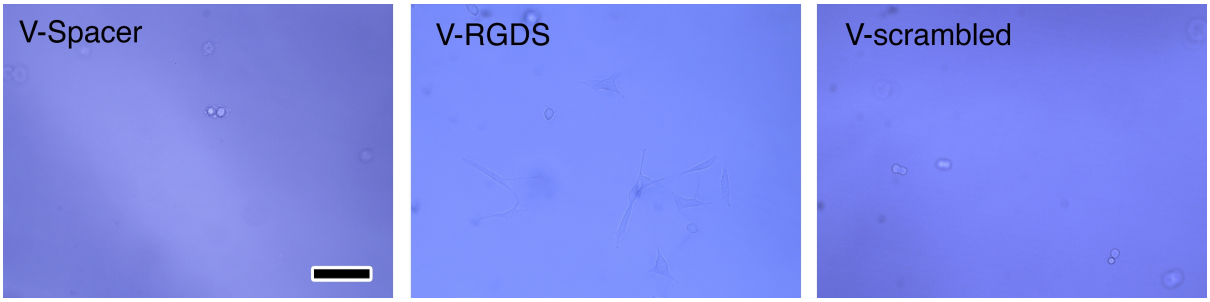


Figure S2.4.3 Brightfield micrographs of the V-fibres

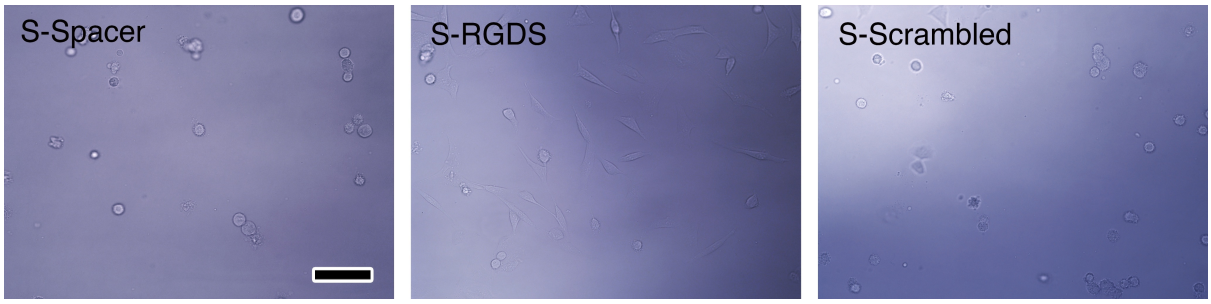


Figure S2.4.4 Brightfield micrographs of the S-fibres

Figure S2.4.5 Brightfield micrographs of the E-fibres

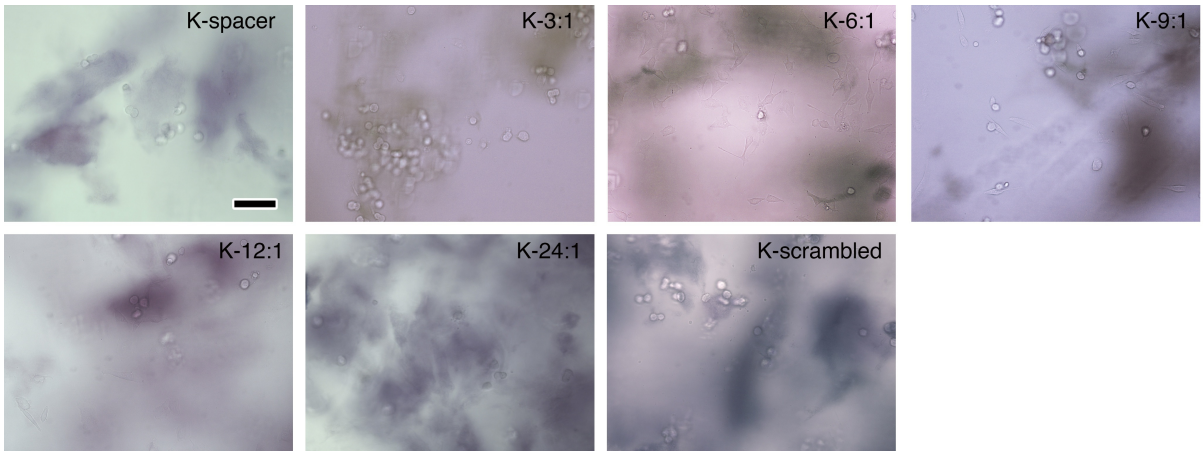


Figure S2.4.6 Brightfield micrographs of the K-fibres

2.5 Live/dead staining for cell viability

Live/dead assay data measured by confocal laser scanning microscopy. Scale bar represents 150 μm. Fluorescence images are composed from overlay images of the 488 nm excitation of calcein (green fluorescence) and the 561 nm excitation of EthD-1 (red fluorescence)

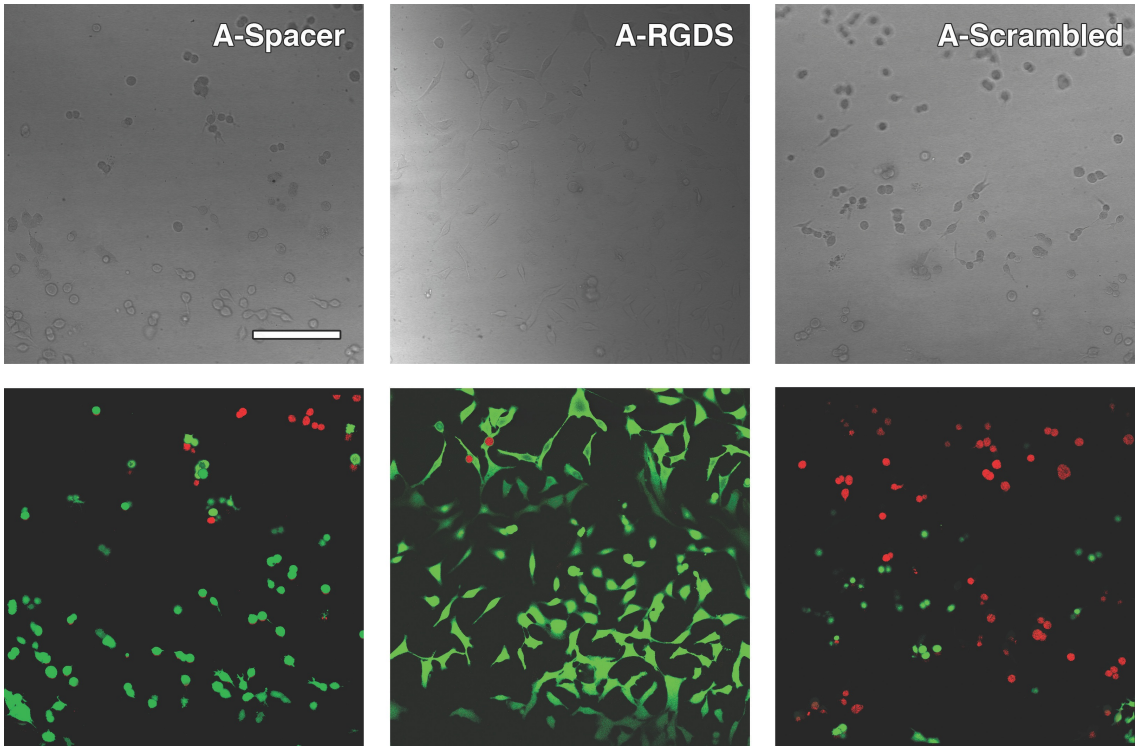


Figure S2.5.1 Confocal fluorescence laser scanning micrographs of the A-fibres. Living cells are represented as green (Calcein fluorescence) and dead cells are represented as red (EthD-1).

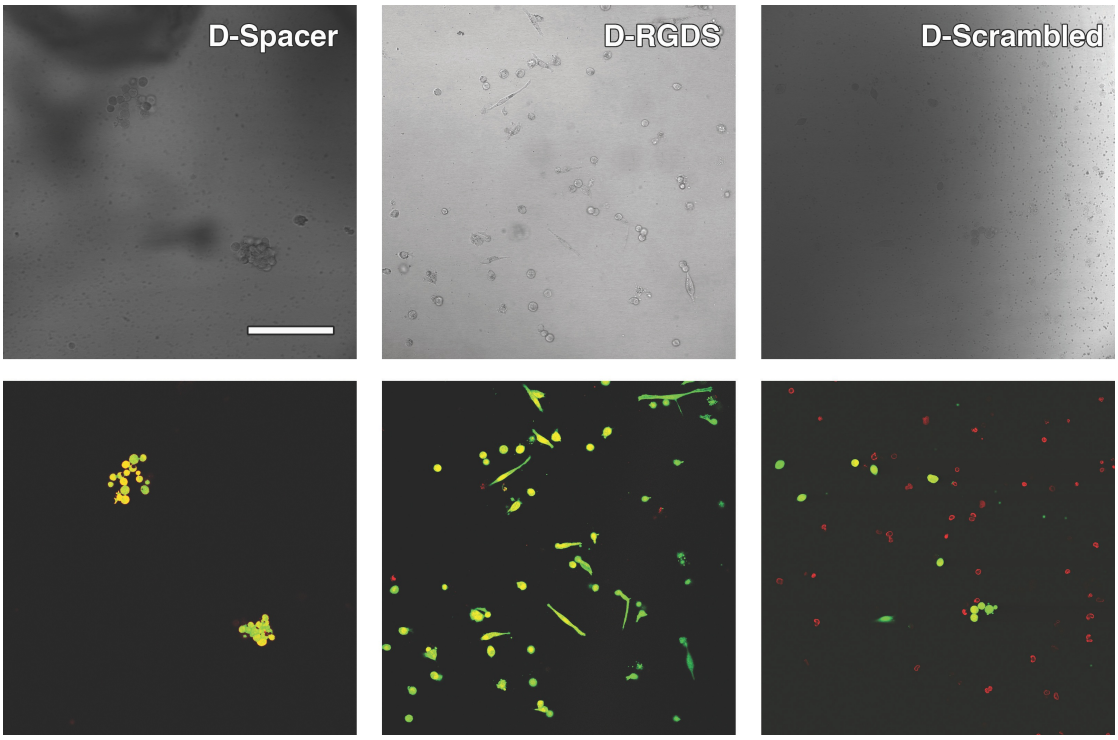


Figure S2.5.2 Confocal fluorescence laser scanning micrographs of the D-fibres. Living cells are represented as green (Calcein fluorescence) and dead cells are represented as red (EthD-1).

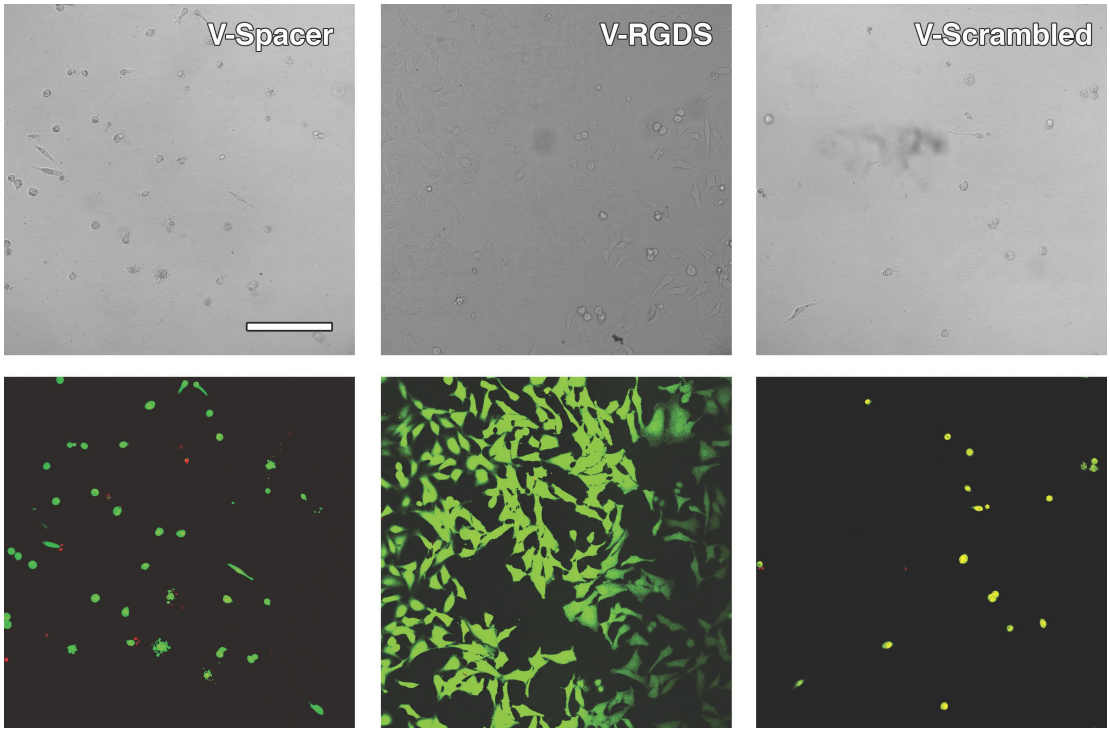


Figure S2.5.3 Confocal fluorescence laser scanning micrographs of the V-fibres. Living cells are represented as green (Calcein fluorescence) and dead cells are represented as red (EthD-1).

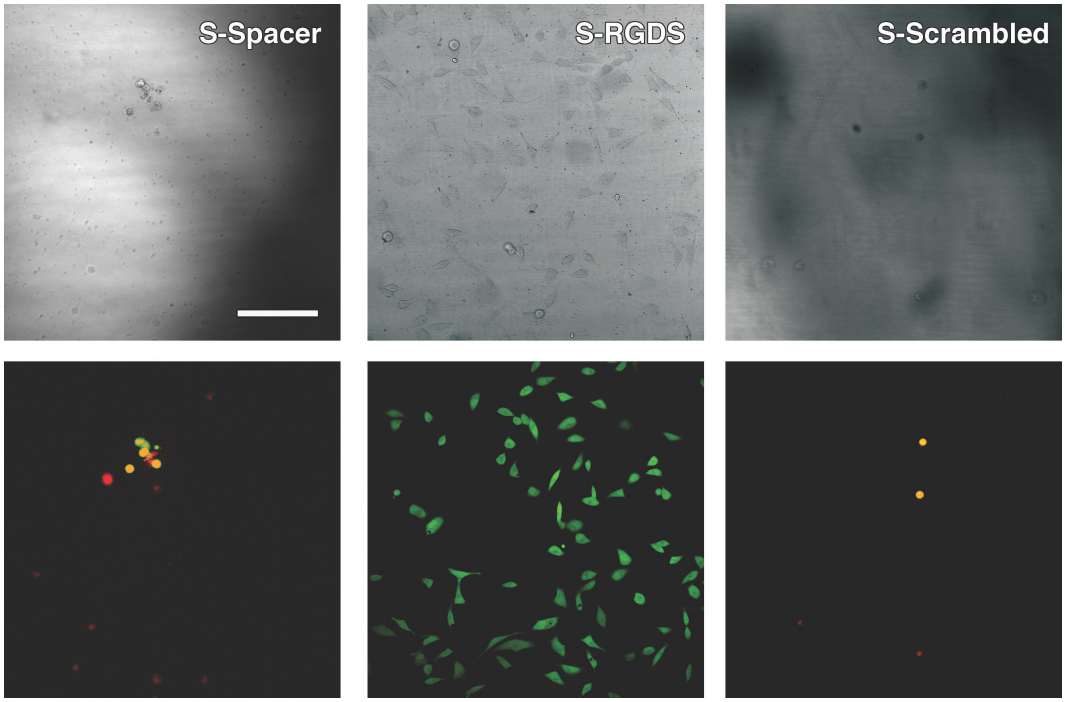


Figure S2.5.4 Confocal fluorescence laser scanning micrographs of the S-fibres. Living cells are represented as green (Calcein fluorescence) and dead cells are represented as red (EthD-1).

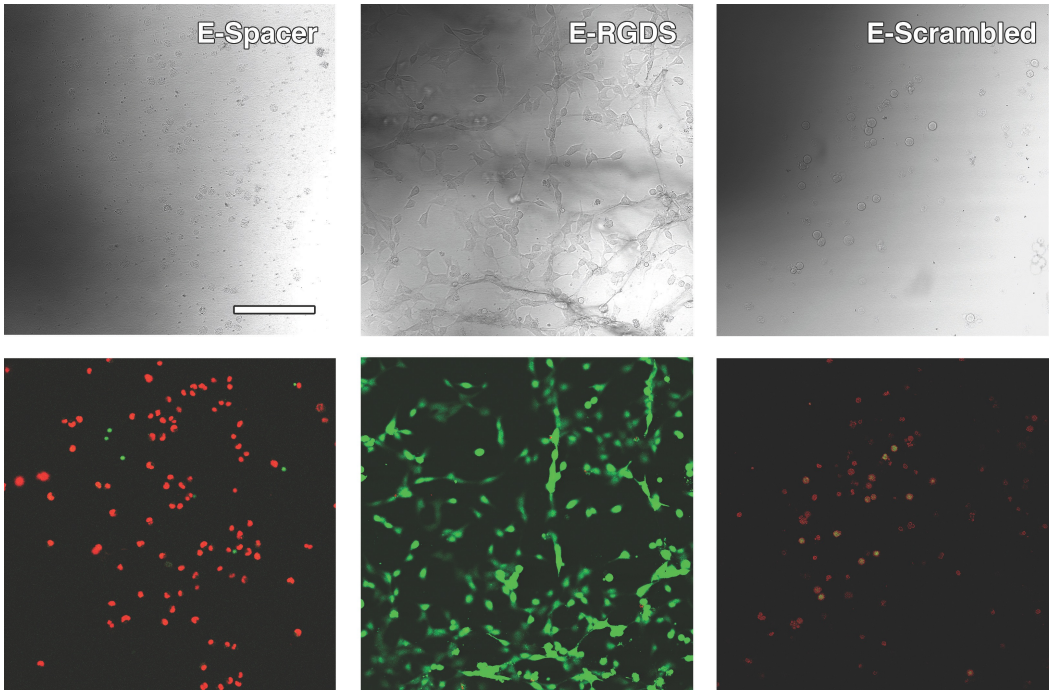


Figure S2.5.5 Confocal fluorescence laser scanning micrographs of the E-fibres. Living cells are represented as green (Calcein fluorescence) and dead cells are represented as red (EthD-1).

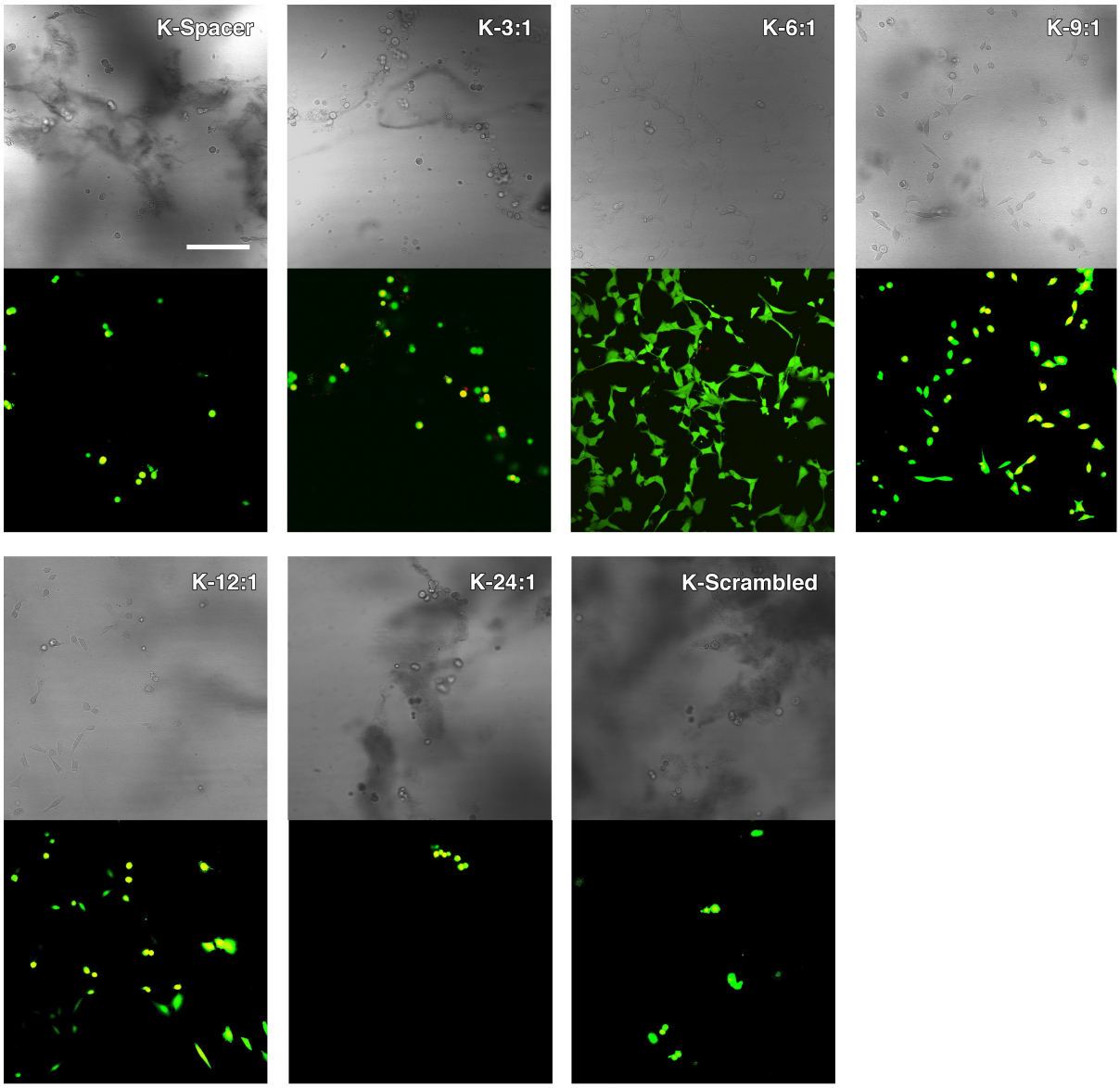


Figure S2.5.6 Confocal fluorescence laser scanning micrographs of the K-fibres. Living cells are represented as green (Calcein fluorescence) and dead cells are represented as red (EthD-1).

2.6 Additional microscopy images

The K-fibres were prepared at three different concentrations; 0.5 mg/ml; 0.75 mg/ml and the standard concentration of 1 mg/ml and used to determine the optimal concentration for cell adhesion and the thereby induced colour change in the fibres. Shown here are the phase-contrast images obtained 18 hours after the start of incubation of K-fibres with detached HeLa cells in phenol red-free DMEM. Scale bar represents 50 μm.

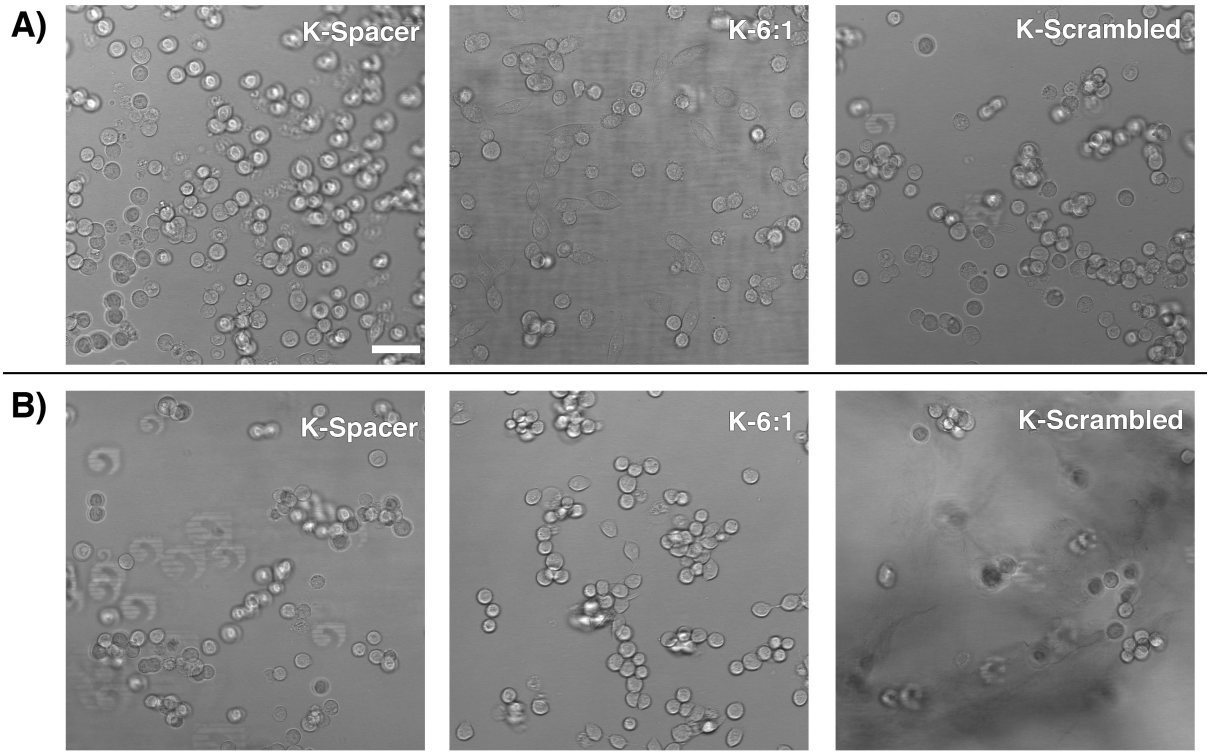


Figure S2.6.1 Phase contrast micrographs of the K-fibres at concentrations of 0.375 mg/ml (A) and 0.25 mg/ml (B).

2.7 Influence of the C-terminal amino acid

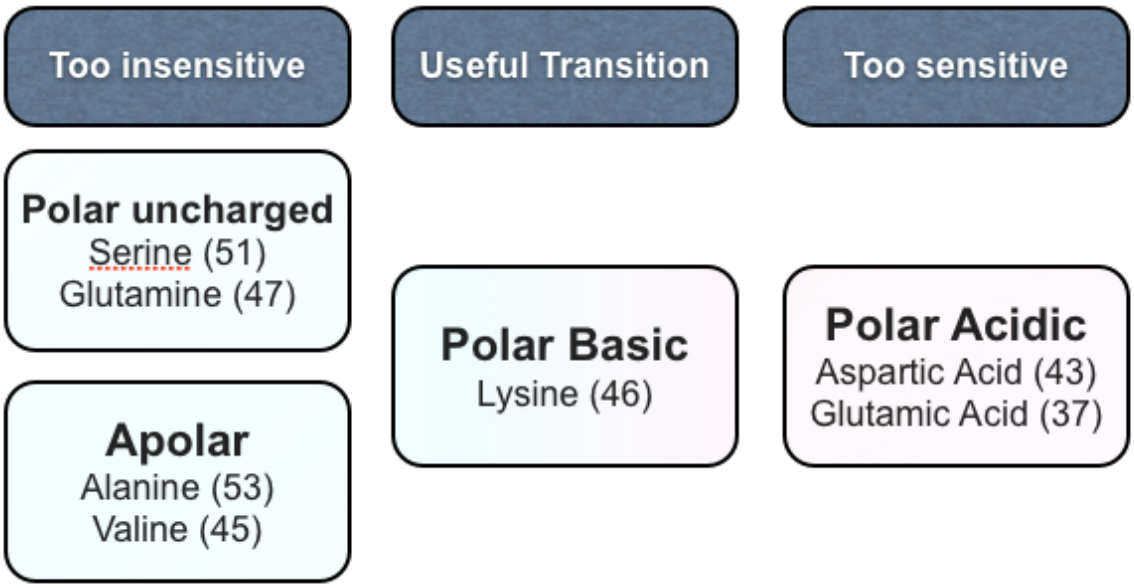


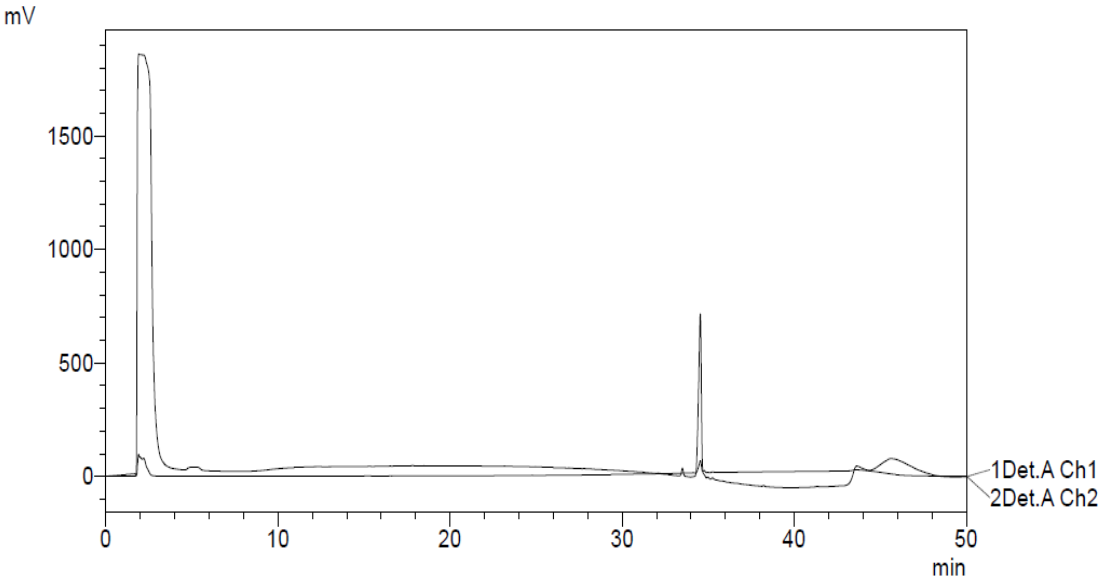
Figure S2.7.1 - grouping of the amino acid residues employed in this report to create fibres for viable cell culturing, according to their polarity and charge, sorted for the favourability of their transitions.

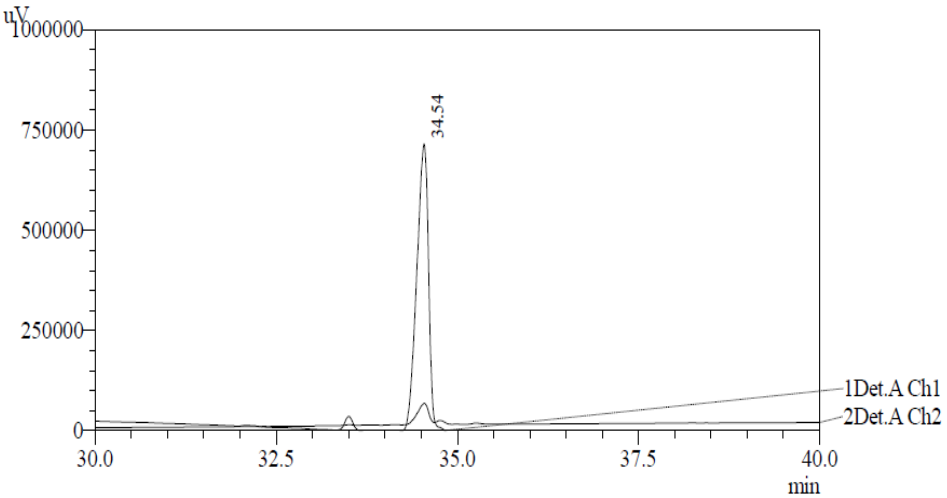
When interpreting the intrinsic contributions of the spacer amino acid residues to the aptitude of the resulting fibres to act as a sensor for cell adhesion, it may be both useful and tempting to group the amino acids used in this report based on their properties. Supporting Figure S2.7.1 shows such a grouping, with the experimentally determined transition temperatures of the fibres listed in brackets behind the spacer residues that discriminate them. A broad conclusion appears to be that uncharged residues have the propensity to result in overly stable fibres that have no switching behaviour at physiologically relevant conditions. Anionic residues, on the other hand, result in fibres that are too quick to pull the switch, so that only the cationic lysine apparently yields functional fibres.

The exact mechanism of the blue to red colour change in polydiacetylenes is still not clearly understood. However it has been established that the moieties attached to the polydiacetylene, in our case the peptide plays a central role in the colour transition as it influences the conformation of the polydiacetylene backbone, more specifically the side chain affects the rotation around the C-C bonds and thus the overlap between adjacent π-orbitals.[3,4] As the colour transition is governed by such subtle factors, it is difficult to analyse the contribution of the C-terminal amino acid to the colour change caused by cell adhesion.

3. HPLC traces

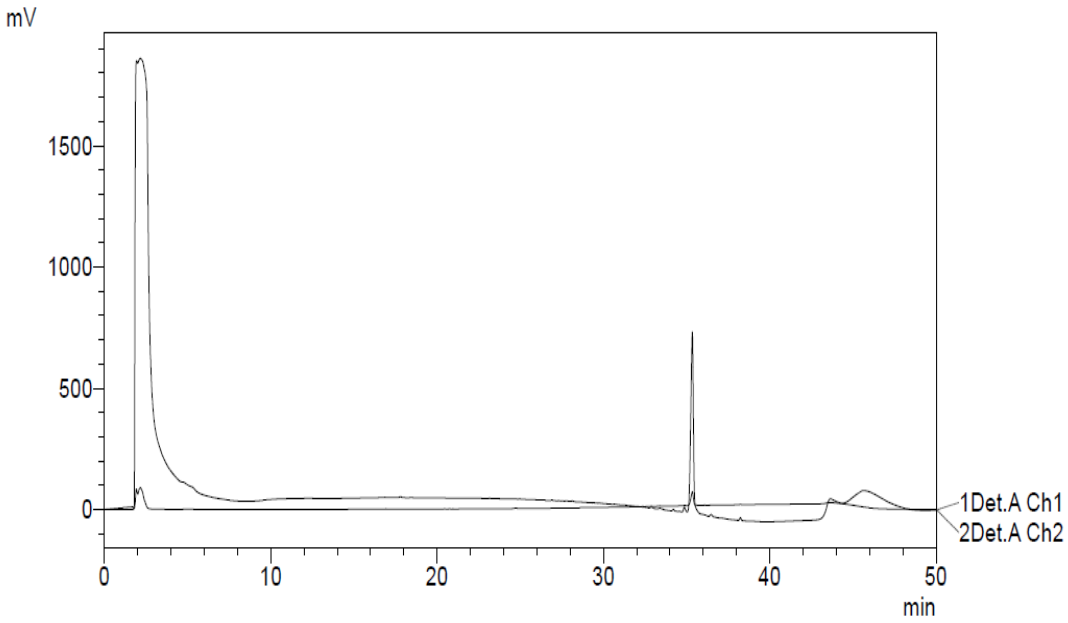
$^{10,12}C_{25}$-Gly-Ala-Gly-Ala-Glu-OH

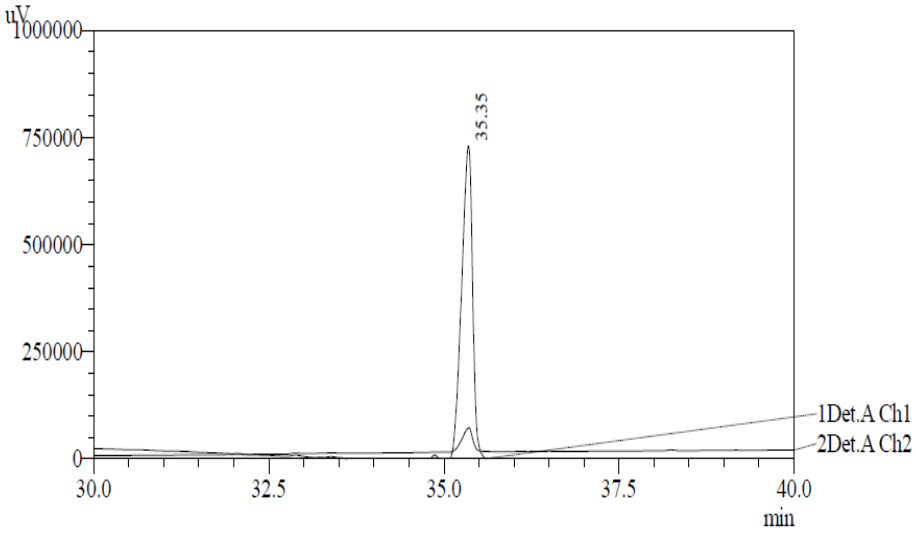




1 Det.A Ch1 / 214nm
2 Det.A Ch2 / 254nm

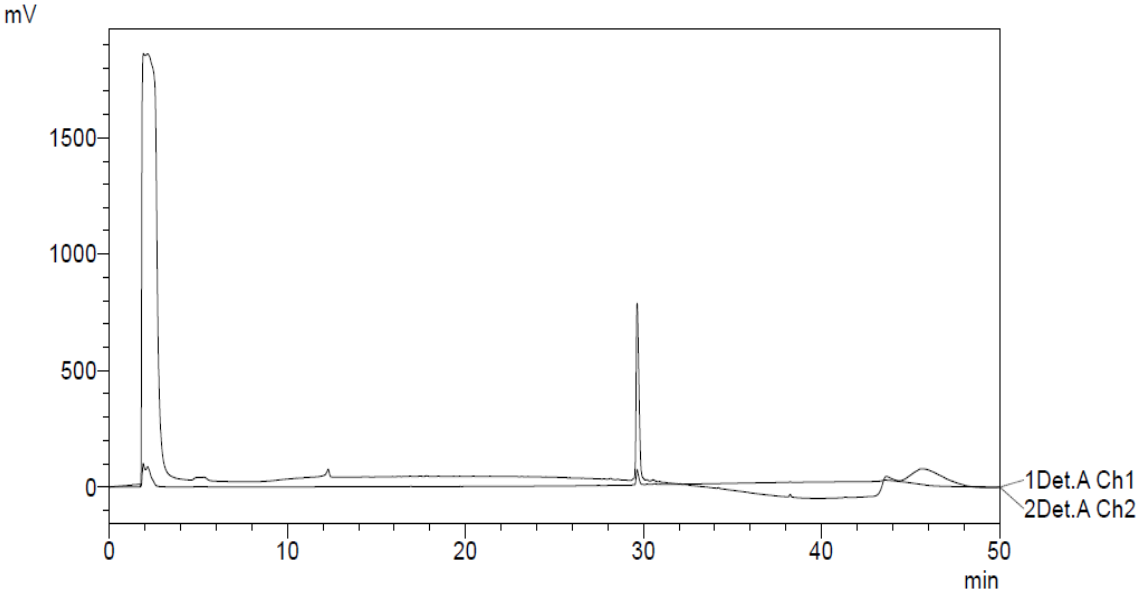
$^{10,12}C_{25}$-Gly-Ala-Gly-Ala-Ala-OH

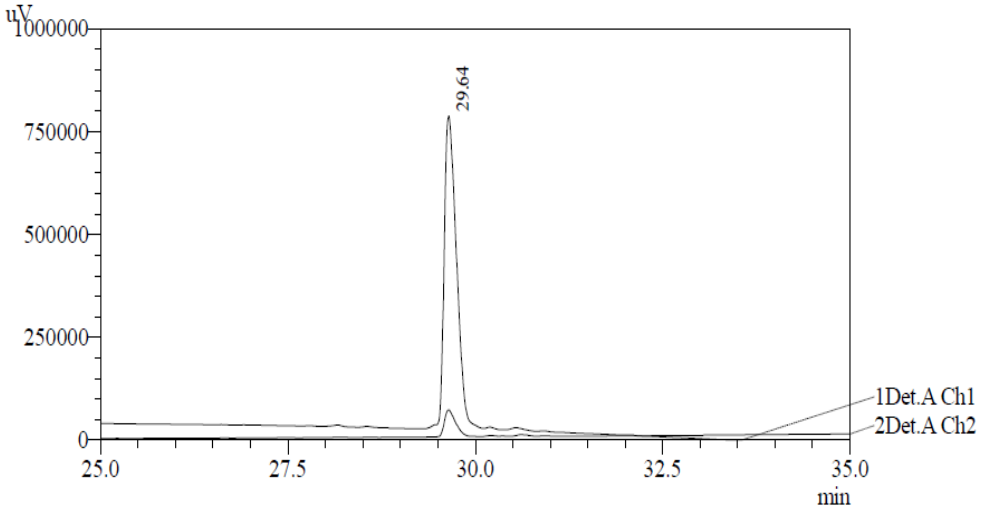




1 Det.A Ch1 / 214nm
2 Det.A Ch2 / 254nm

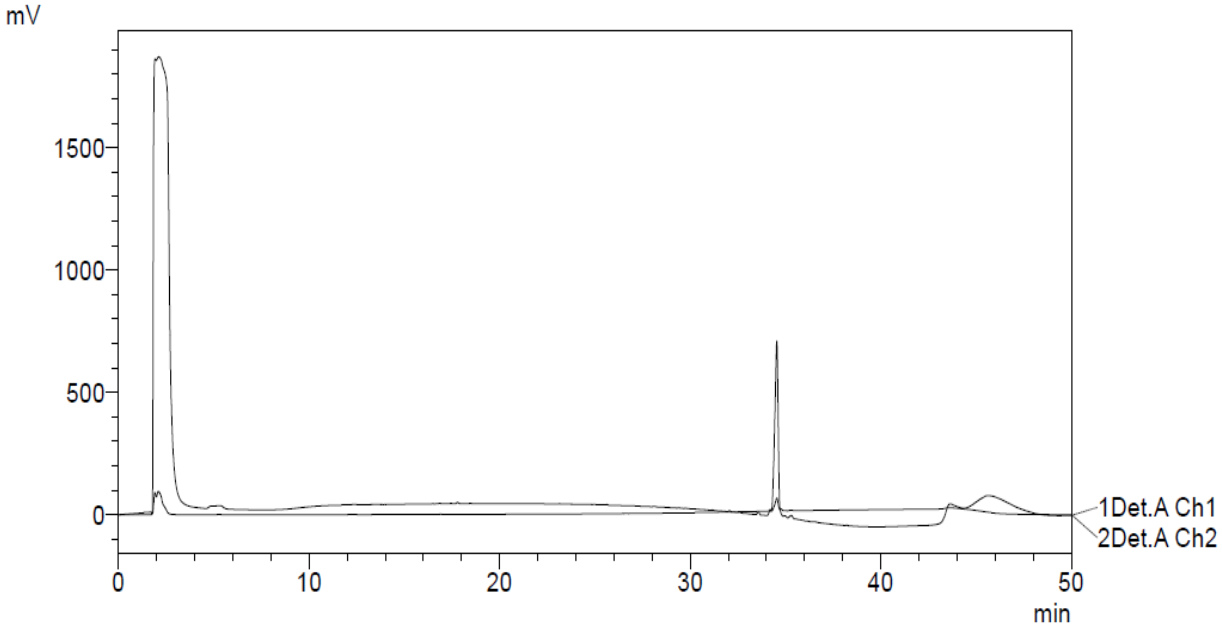
$^{10,12}C_{25}$-Gly-Ala-Gly-Ala-Lys-OH

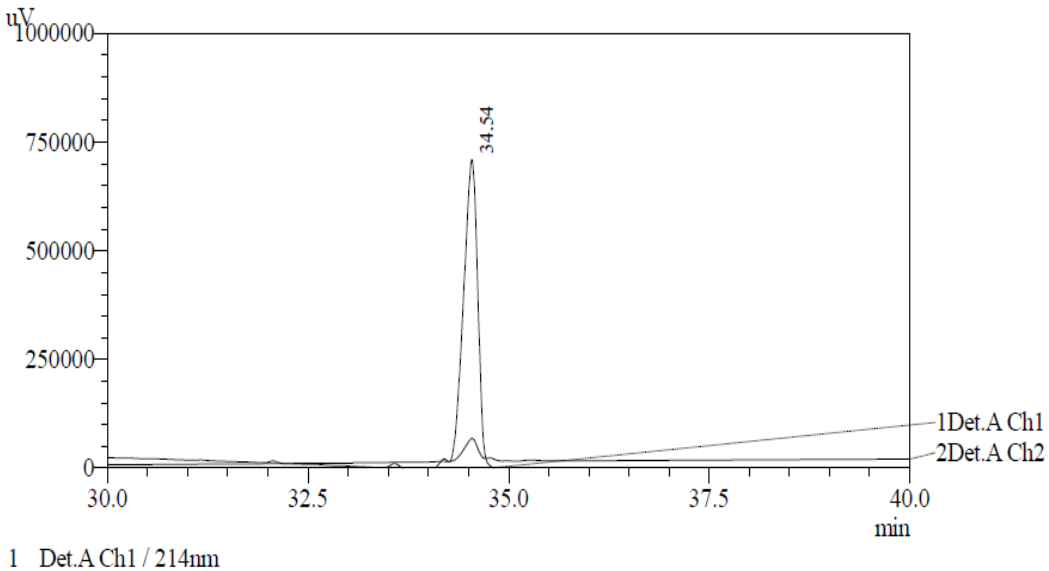




1 Det.A Ch1 / 214nm
2 Det.A Ch2 / 254nm

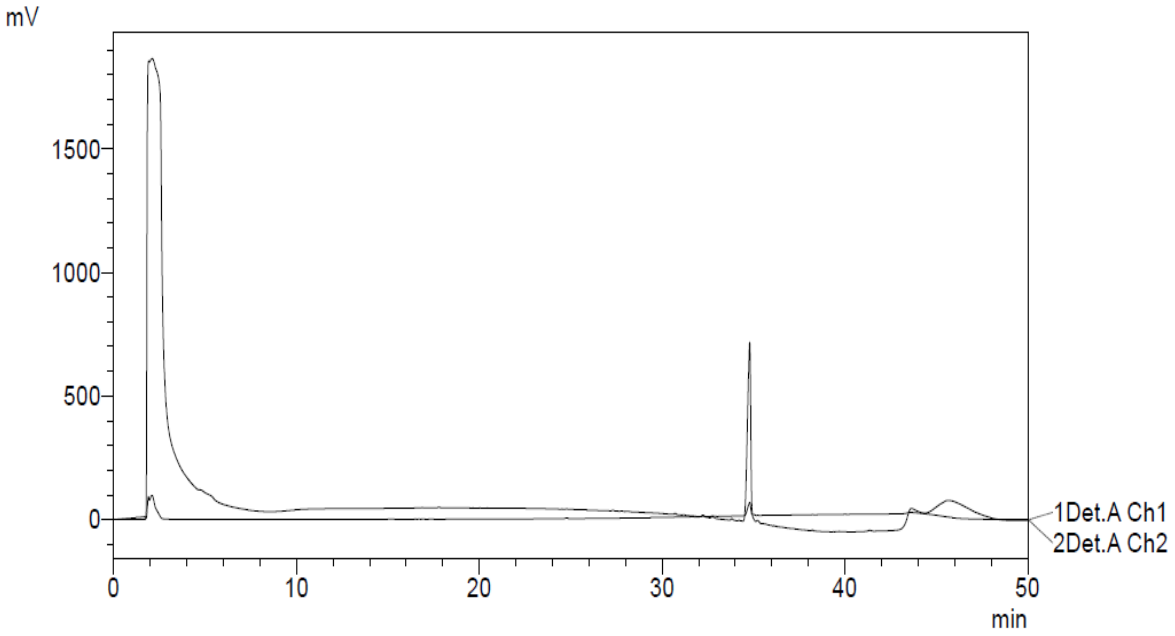
$^{10,12}C_{25}$-Gly-Ala-Gly-Ala-Asp-OH

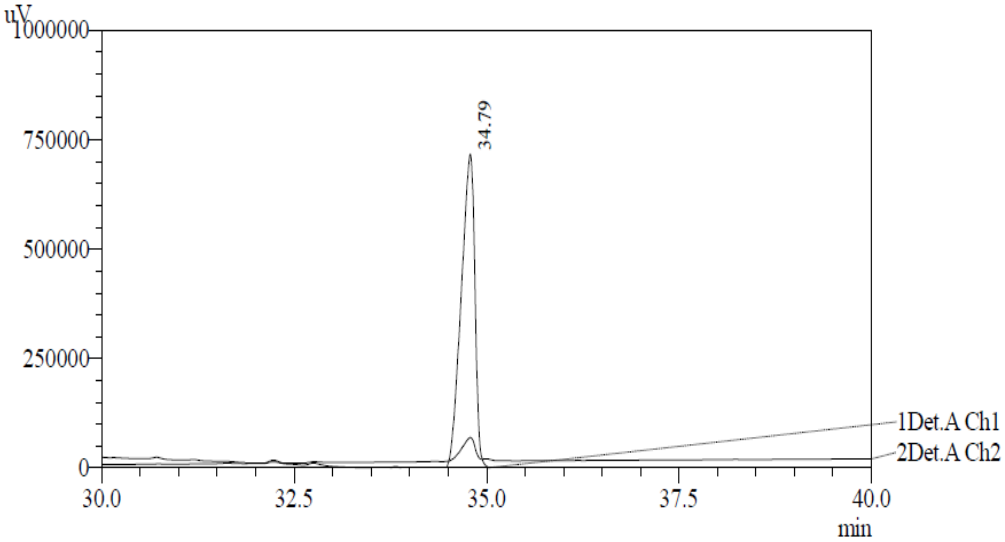




1 Det.A Ch1 / 214nm
2 Det.A Ch2 / 254nm

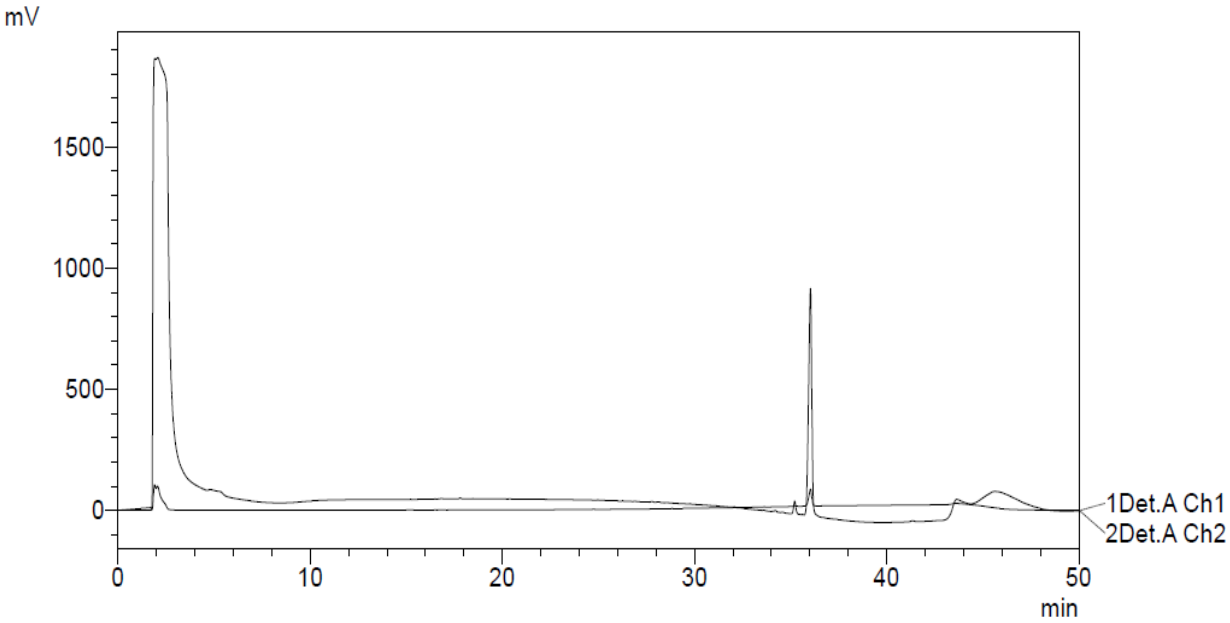
$^{10,12}C_{25}$-Gly-Ala-Gly-Ala-Ser-OH

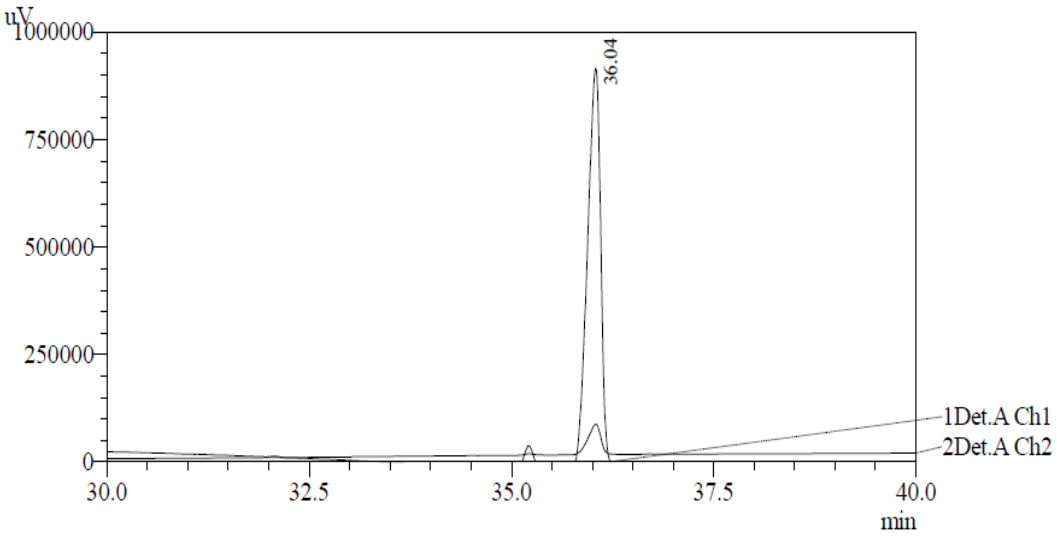




1 Det.A Ch1 / 214nm
2 Det.A Ch2 / 254nm

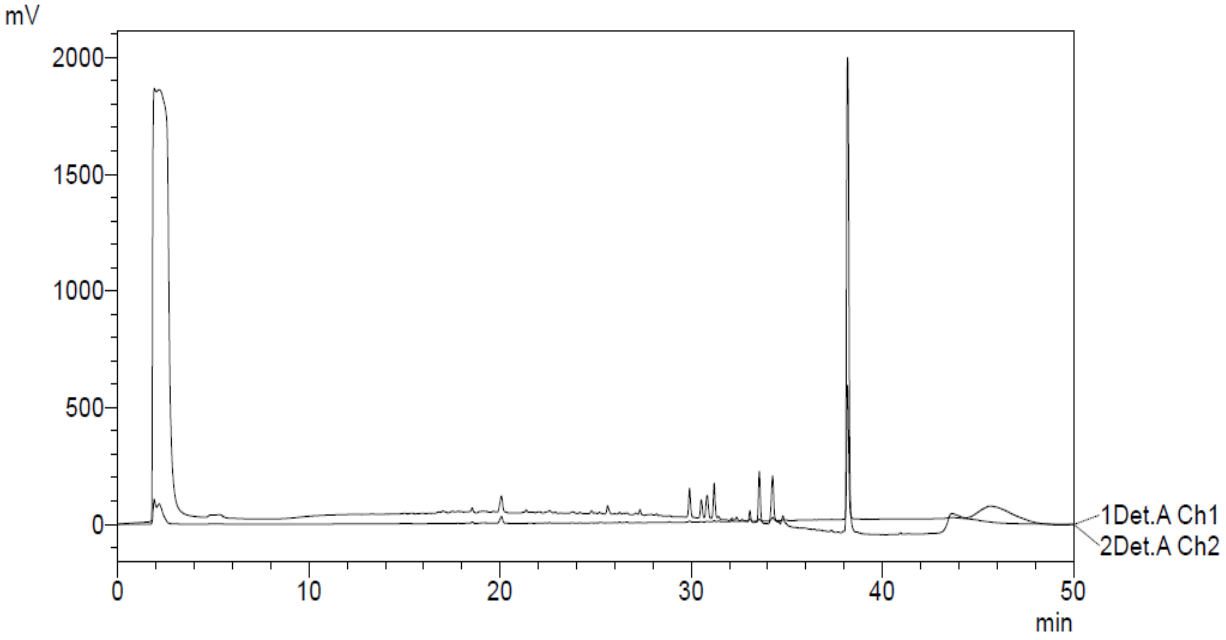
$^{10,12}C_{25}$-Gly-Ala-Gly-Ala-Val-OH

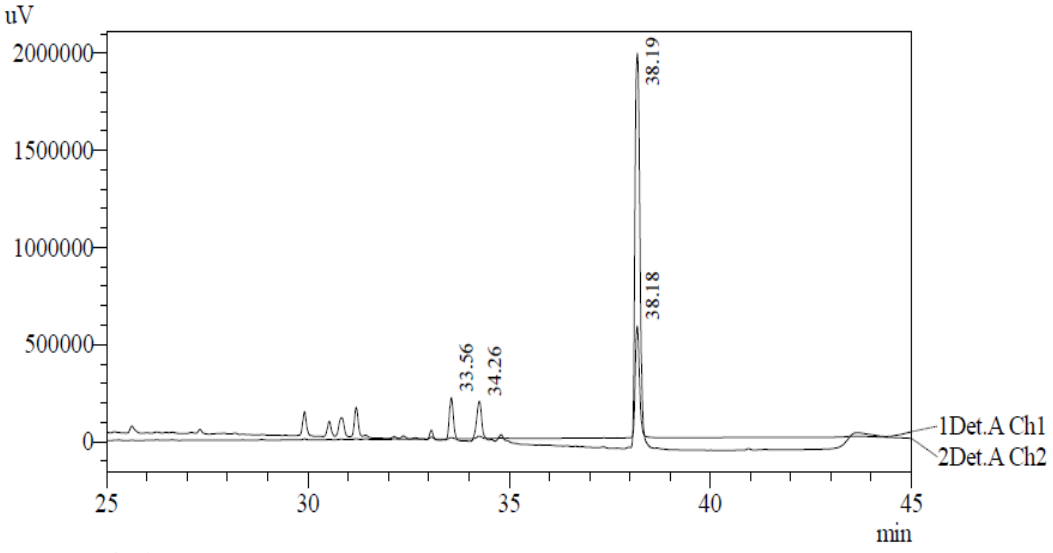




1 Det.A Ch1 / 214nm
2 Det.A Ch2 / 254nm

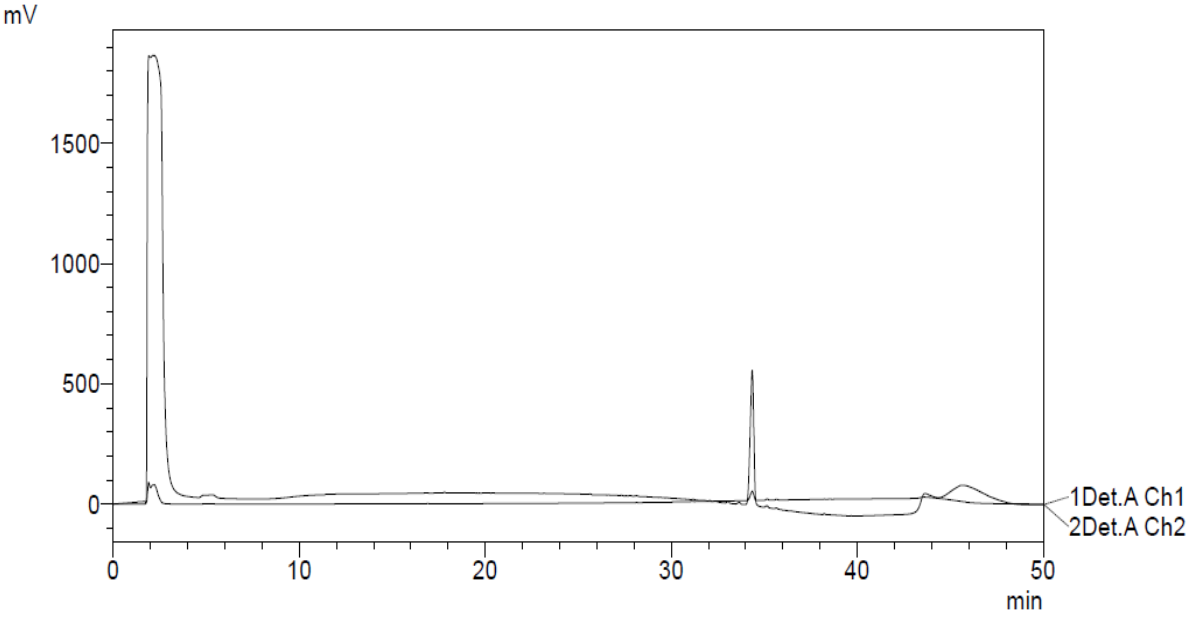
$^{10,12}C_{25}$-Gly-Ala-Gly-Ala-Asn-OH

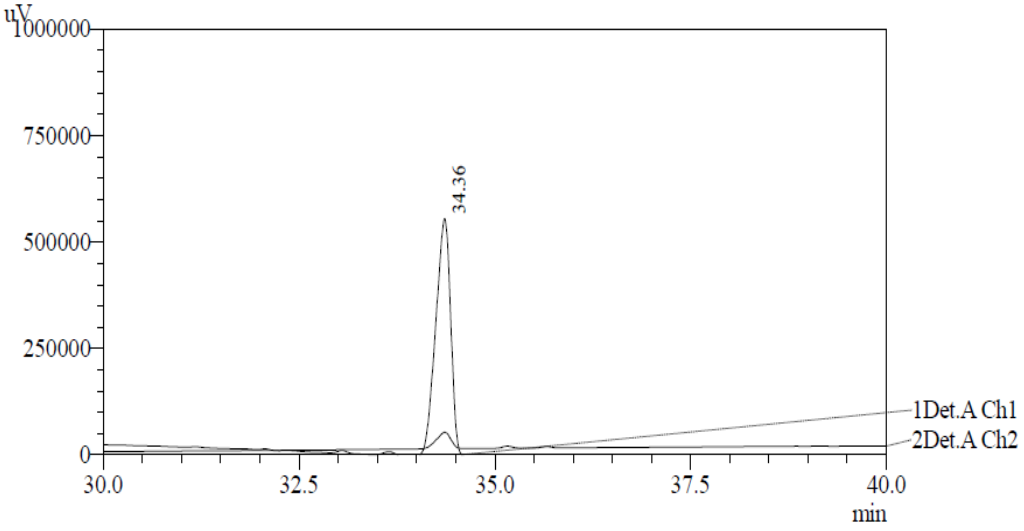




1 Det.A Ch1 / 214nm
2 Det.A Ch2 / 254nm

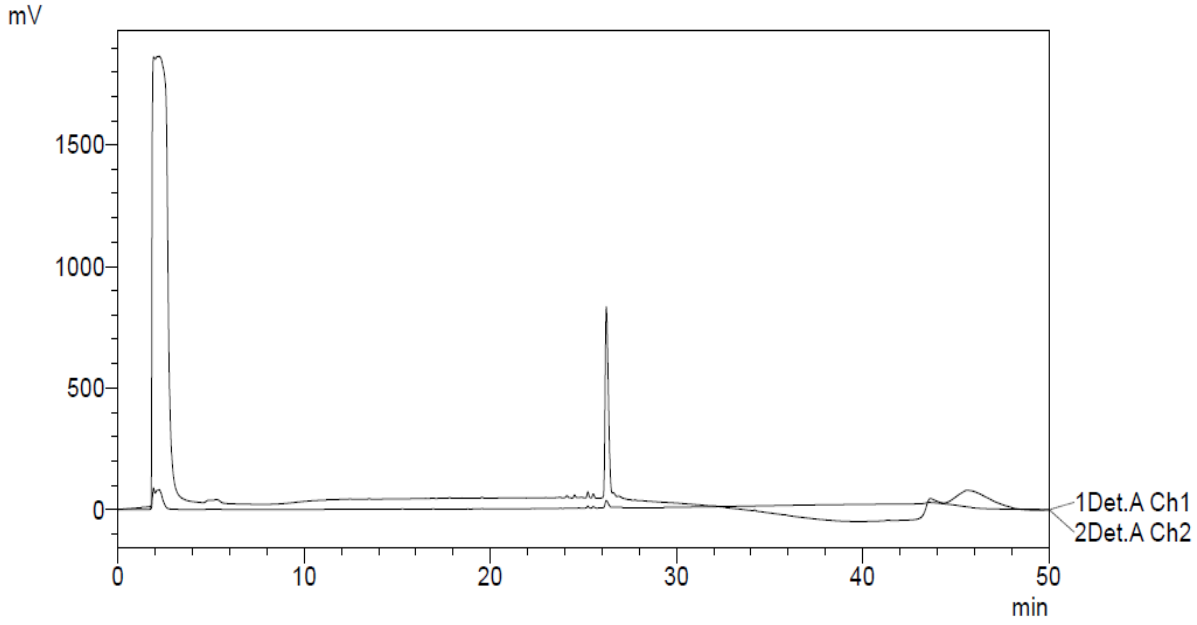
$^{10,12}C_{25}$-Gly-Ala-Gly-Ala-Gln-OH

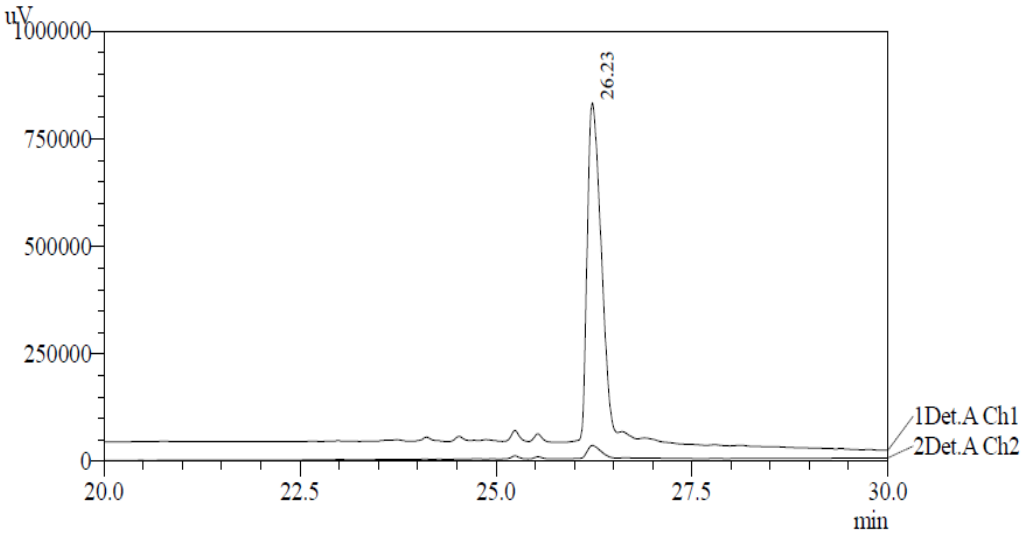




1 Det.A Ch1 / 214nm
2 Det.A Ch2 / 254nm

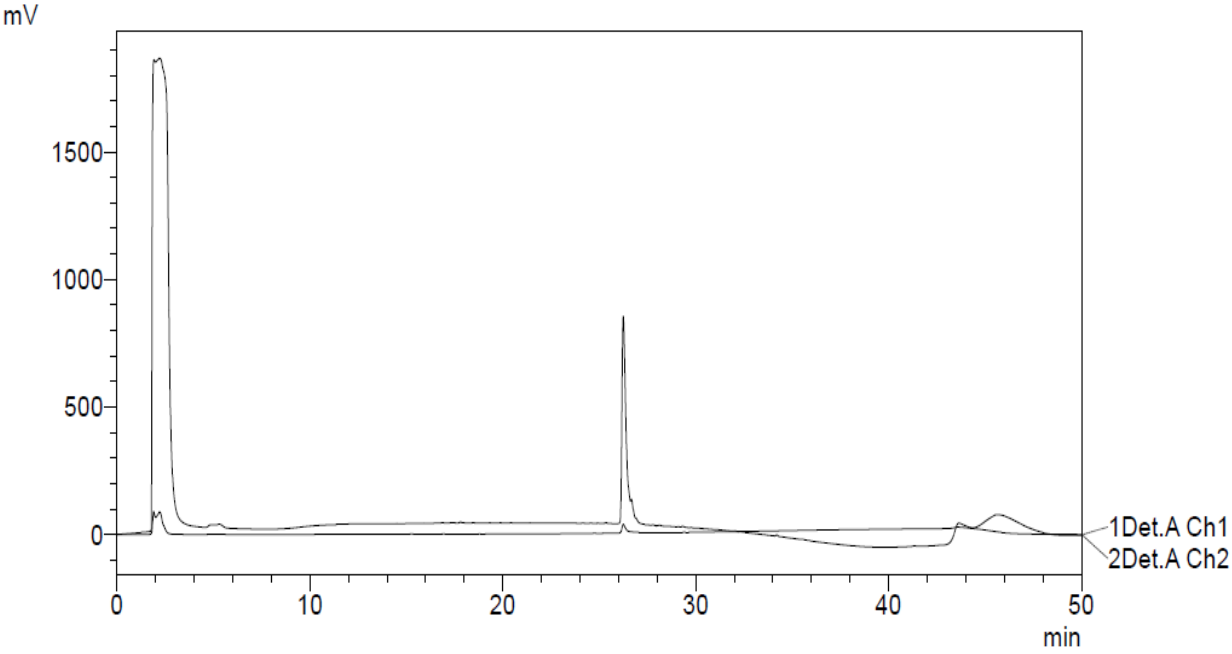
$^{10,12}C_{25}$-Gly-Ala-Gly-Ala-Lys-Arg-Gly-Asp-Ser-NH_2

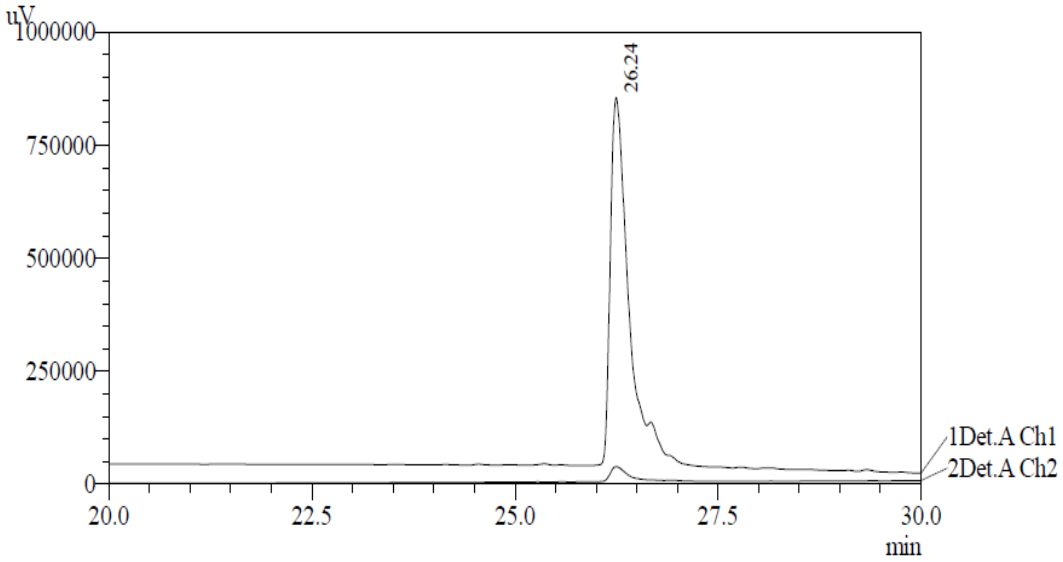




1 Det.A Ch1 / 214nm
2 Det.A Ch2 / 254nm

$^{10,12}C_{25}$-Gly-Ala-Gly-Ala-Lys-Asp-Gly-Ser-Arg-NH_2





1 Det.A Ch1 / 214nm
2 Det.A Ch2 / 254nm

4. References

1. E. Kaiser, R.L. Colescott, C.D. Bossinger, P.I. Cook, *Anal. Biochem.*, 1970, **34**, 595-598
2. D. H. Charych, J. O. Nagy, N. Spevak, M. D. Bednarski, *Science,* 1993, **261**, 585
3. R. W. Carpick, D. Y. Sasaki, M. S. Marcus, M. A. Eriksson, A. R. Burns, *J. Phys. Condens. Matter*, 2004, **16**, 679–697
4. H. Eckhardt, D. S. Boudreaux, R. R. J. Chance, *J. Chem. Phys.*, 1986, **85**, 4116–4119