Supplementary Material (ESI) for Chemical Communications

Supporting Information:

Peptide modification confirmation via ^{1}H NMR:

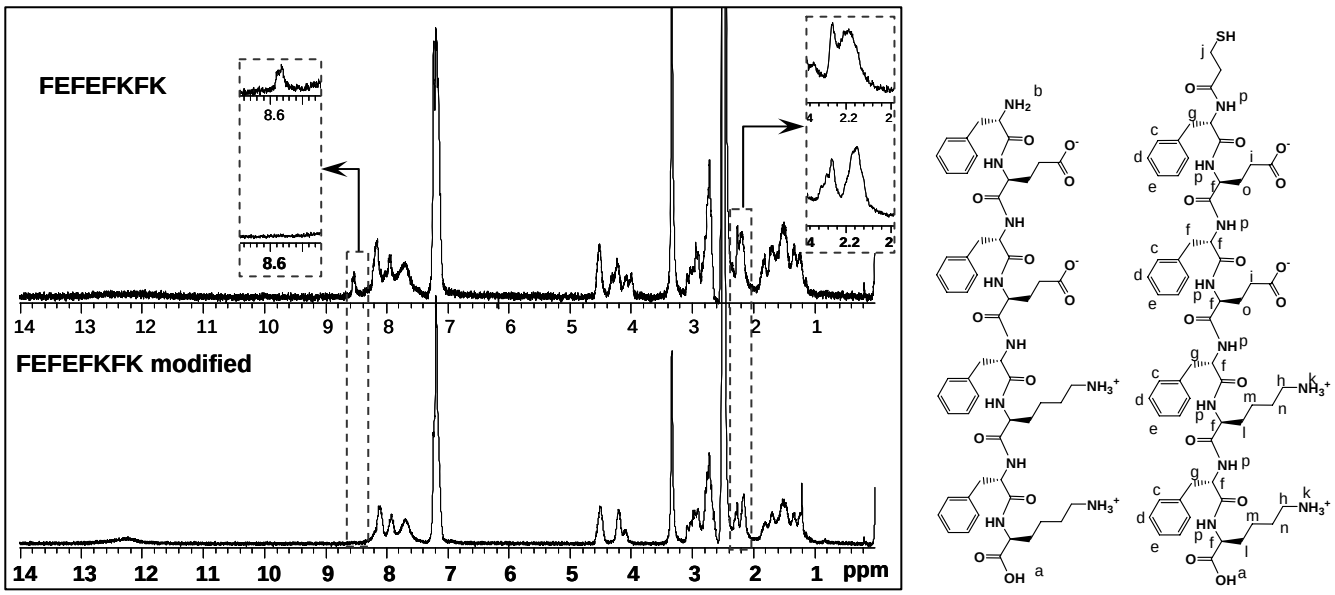


Figure 1: ^{1}H-NMR for the unmodified and modified peptides

	Group type	Peak [ppm]	Assignment
1	COOH	12.34	a
2	NH_2 (backbone – end – present only in unmodified peptide)	8.63	b
3	Aromatic rings	7.2	c, d, e
4	CH (backbone)	4.0-4.6	f
5	CH_2 (next to aromatic rings)	3.3	g
6	CH_2 (next to NH_2 side chains)	2.85-3.15	h
7	CH_2 (next to COOH side chains)	2.85-2.5	i
8	DMSO-d6	2.5	
9	CH_2 (next to SH – present only in modified peptide)	2.35	j
10	NH_2 (side chains)	2.0-2.2	k
11	CH (side chains)	1.0-2.0	l, m, n, o
12	NH (backbone)	7.25-8.5	p

Table 1: 1H NMR peaks assigned to groups

All peaks present in the ^{1}H NMR spectra (400 MHz Bruker) for both the pure and modified peptides are assigned in Table 1. The most relevant of these are 8.63 ppm and 2.35 ppm. The peak at 8.63 ppm corresponds to the free NH_2 end group in the pure, unmodified peptide. As expected, this group is present only in the original peptide and not in the modified peptide. The end group of the modified peptide is SH and the peak at 2.35 ppm in the modified peptide is attributed to its neighbouring CH_2 group. These results suggest the successful modification of the peptide.

Peptide functionalisation confirmation by MALDI-Tof:

FEFEFKFK

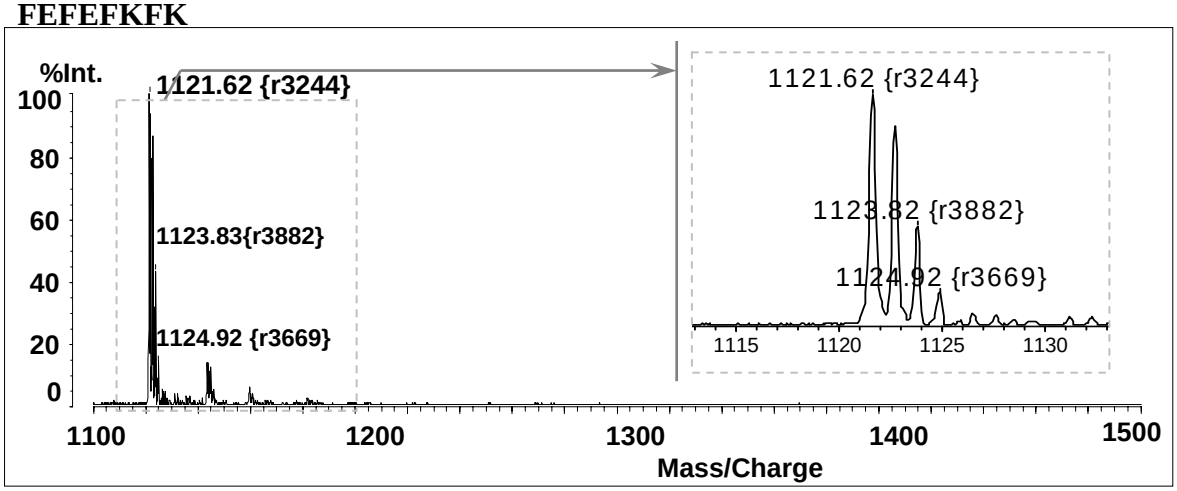


Figure 2: MALDI-Tof of the unmodified peptide (FEFEFKFK)

FEFEFKFK modified

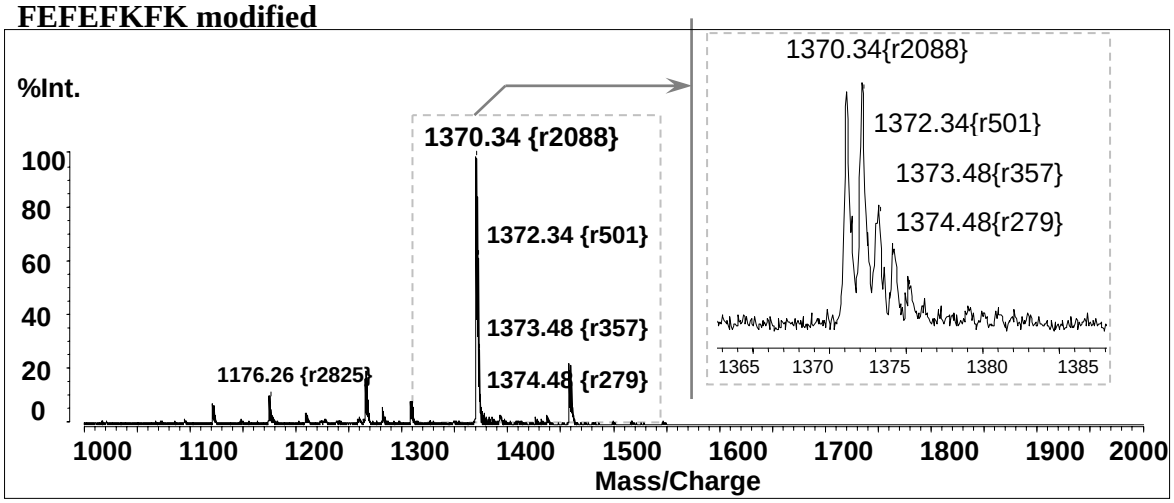


Figure 3: MALDI-Tof of the modified peptide (mod FEFEFKFK)

The results given in figures 2 and 3 show a significant difference in the molecular weight of the two peptides (the modified peptide is ~249 g mol^{-1} heavier that the unmodified peptide) confirming functionalisation. These results were obtained from Kratos PC Axima CFR V.2.4.1 operated in reflection mode.

Peptides purity evaluation by HPLC:

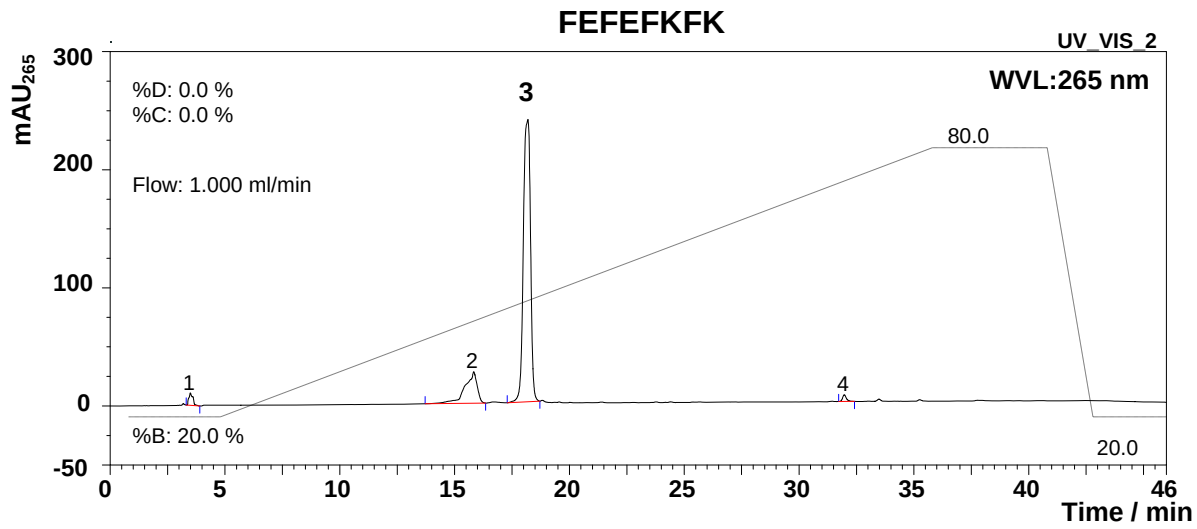


Figure 4: HPLC of the unmodified peptide (FEFEFKFK)

No.	Ret.Time	Area	Height	Rel.Area
	min	*mAU*min*	*mAU*	*%*
1	3.489	2.1274	10.554	2.02
2	15.83	16.1914	26.66	15.35
3	18.191	86.0258	239.09	**81.57**
4	31.972	1.1242	5.717	1.07
Total		105.469	282.02	100

Table 2: HPLC results for the original peptide and the purity percentage

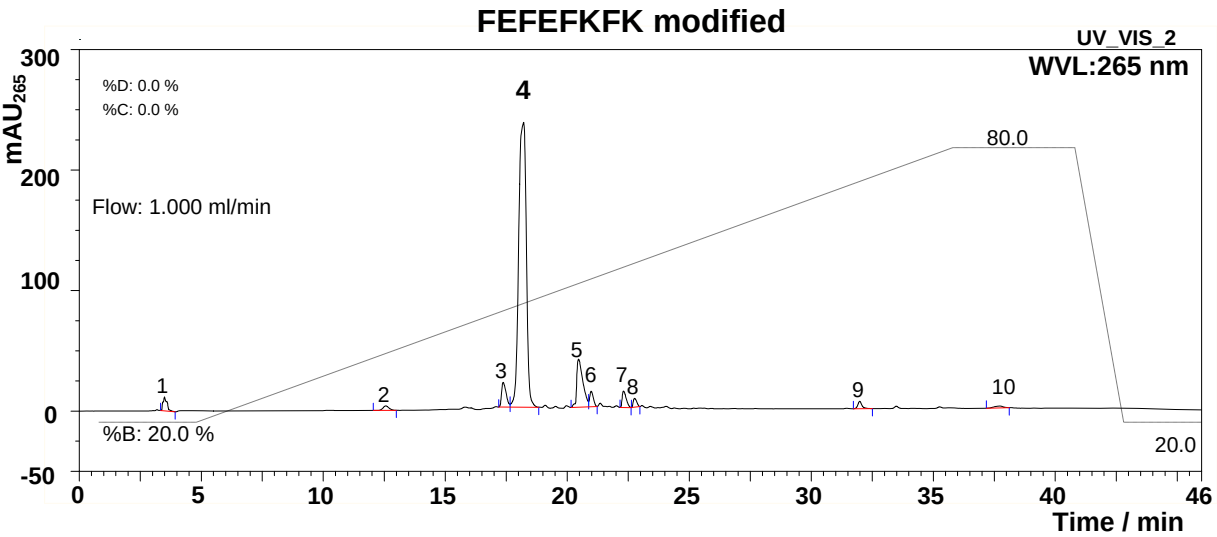


Figure 5: HPLC of the modified peptide (mod FEFEFKFK)

No.	Ret.Time	Area	Height	Rel.Area
	min	*mAU*min*	*mAU*	*%*
1	3.493	2.1686	11.343	1.93
2	12.562	1.0444	3.719	0.93
3	17.372	4.4374	20.486	3.94
4	18.207	85.2175	236.288	**75.74**
5	20.464	11.7165	39.817	10.41
6	20.98	2.252	12.812	2
7	22.307	2.6062	13.666	2.32
8	22.762	1.2377	7.28	1.1
9	31.989	1.1651	6.086	1.04
10	37.734	0.6698	1.575	0.6
Total:		112.515	353.072	100

Table 3: HPLC results for the modified peptide and the purity percentage

The HPLC results show a high purity in both the unmodified and modified peptides; 81 % and 76 % respectively, confirming the MALDI results. These results were obtaining using reverse phase HPLC where buffer A was HPLC grade water with 0.1% trifluoroacetic acid (TFA) and buffer B was HPLC grade acetonitrile with 0.1% TFA. A gradient of 20-80% of B was used with a sample injection of 100 µL. The following set up was employed: Dionex P680 HPLC pump, with a Dionex ASI 100 automated sample injector, a Dionex UVD170U detector. The column is a Macherey-Nagel EC250/4.6 Nucleosil 100-5 C18 column.

Confirmation of conjugation by solid state NMR:

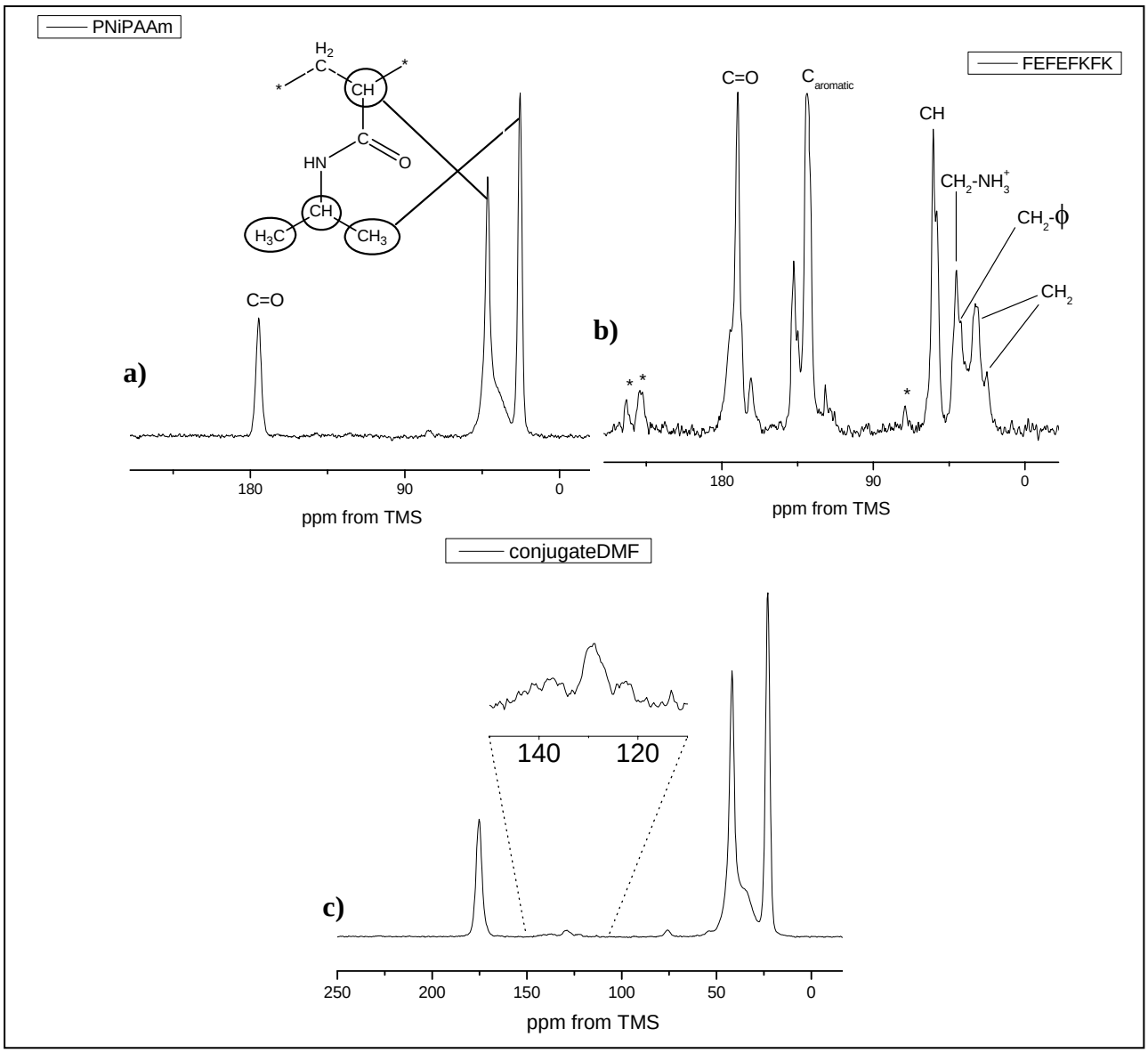


Figure 6: Solid state NMR: a) pure polymer, b) pure peptide and c) the conjugate.

After synthesis and purification the conjugate was analysed by solid state NMR using the non-conjugated polymer and the peptide as standards. Spectra are shown in Figure 6 where peaks have been identified. For the conjugate (Figure 6c), supplementary peaks were found in the range of 110-150 ppm, that corresponds to the phenyl rings present in the peptide. These results confirmed the presence of the peptide in the conjugate structure. NMR spectra were recorded at 9.4 T using a Bruker DSX-400 spectrometer, in ^{1}H to ^{13}C cross-polarisation magic angle spinning mode.

Quantification pf peptide/NIPAAm ratio in conjugate by ^{1}H NMR and UV:

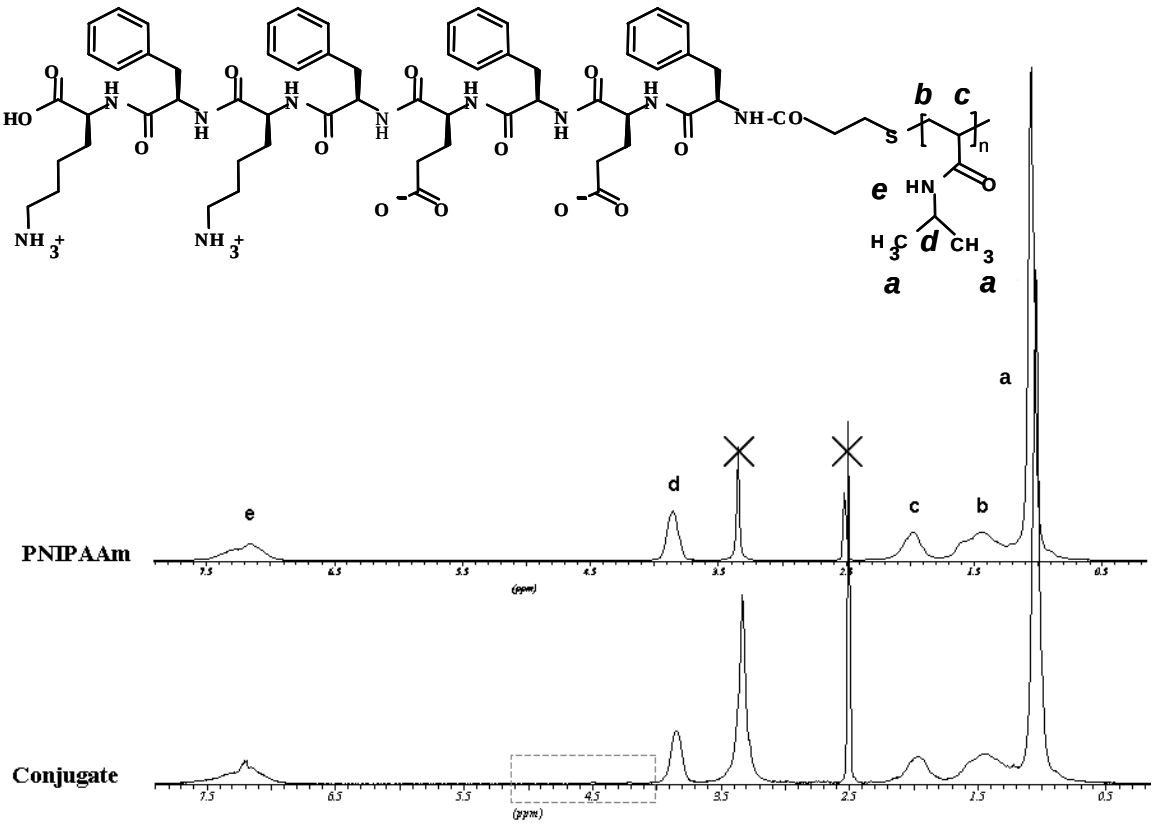


Figure 7: ^{1}H NMR spectra for pure polymer and for the conjugate

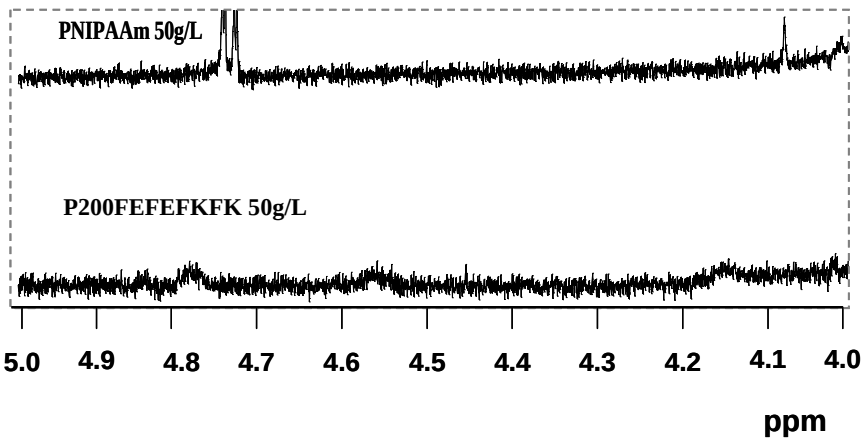


Figure 8: Magnified ^{1}H NMR spectra for pure polymer and for the conjugate

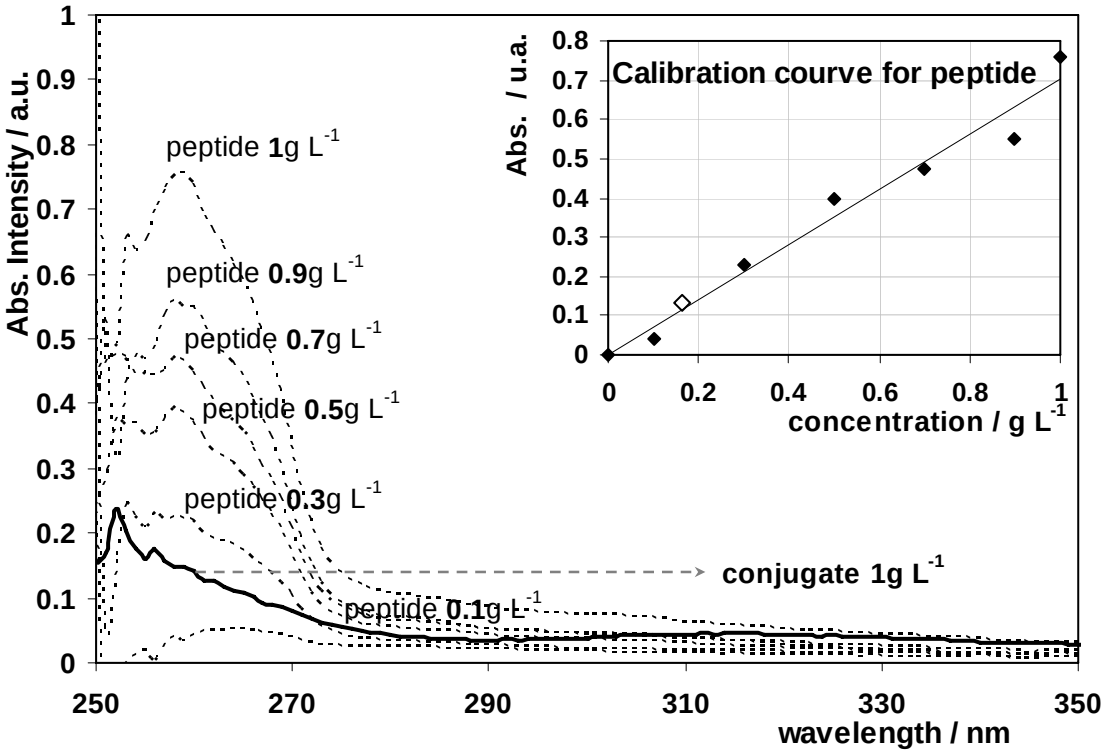
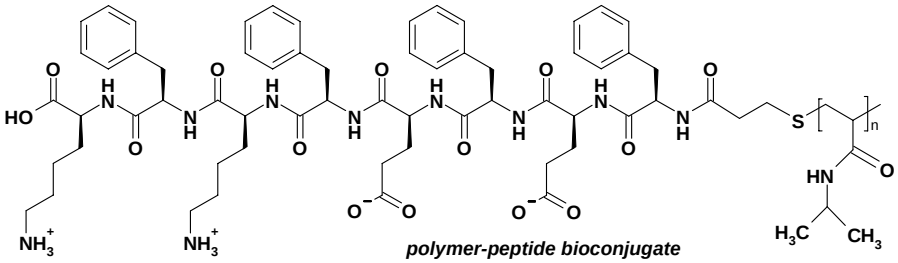


Figure 9: UV spectra for unmodified peptide solutions with varying concentrations (dotted lines) and for a conjugate solution at 1 g L^{-1}. Insert: calibration curve (closed diamonds) obtained for the unmodified peptide and conjugate absorbance (open diamond).

In order to quantify the amount of peptide attached at the end of the polymeric chain, two methods were used: proton NMR and UV. Peak assignment in the ^{1}H NMR spectra is shown in Figure 7 and the region of interest for quantification is magnified in Figure 8. The peaks at 4.1, 4.55 and 4.8 ppm correspond to the C<u>H</u> from the backbone of the peptide and are very distinctive. These peaks were used for quantification taking the C<u>H</u>$_3$ groups of polymeric chain as a reference point. The 1H NMR spectra were recorded on a Bruker, 400MHz spectrometer, using 16 scans for time averaging. The absorbance at 258 nm was used to confirm the quantification of amount of peptide by UV. A calibration curve was build using the modified peptide and was then subsequently used to quantify the amount of peptide present in the conjugate. Spectra were recorded on a Shimadzu 2501 double beam spectrometer.

Graphical Content Entry



polymer-peptide bioconjugate