Electronic Supplementary Information

Self-Assembly of Mitochondria-Specific Peptide Amphiphiles Amplifying the Lung Cancer Cell Death through Targeting the VDAC1-Hexokinase-II Complex

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SAXS modelling

To account for possible deviations from spherical entities, the scattered intensity *I* (*q*) was modelled as coming from a population of ellipsoids of rotation *I (q)* can be described in a decoupling approximation (no correlation between size/orientation and position of particles) by the following equation: [1]

$$I(q) = I(0)P(q)S^{'}(q) + B \quad (1)$$

Where $P(q) = \langle |F(q)|^2 \rangle$ (2)

$$S^{'}(q) = 1 + \beta(q)\cdot[S(q) - 1] \quad (3)$$

$$\beta(q) = |\langle F(q) \rangle|^2 / \langle |F(q)|^2 \rangle \quad (4)$$

The inner brackets $\langle\rangle$ in equations (2) and (4) represent an average weighted by the distribution of particle sizes and/or orientations, *I (0)* is the scattering at zero angle (proportional to concentration of particles, contrast, and particle volume, $P(q)$ is the form factor, $F(q)$is the amplitude of the form factor, $S(q)$ is the structure factor, and $S'(q)$ is the effective structure factor modified by the anisotropy and polydispersity of particles.

In the case of core shell ellipsoid of rotation of semiaxis *a*, *b*, *F (q)* is expressed as

$$F(q) = 3(\rho_{core} - \rho_{solvent})V_{core}\frac{\sin(qR) - qR\cos(qR)}{(qR)^3} + 3(\rho_{shell} - \rho_{solvent})V_{shell}\frac{\sin(q(R+T) - q(R+T)\cos(R+T)}{(q + (R+T)^3}$$

(5)

Where $r = [b^2\sin^2\alpha + \alpha^2\cos^2\beta]^{1/2}$ and α is the angle between the axis of ellipsoid *a* and scattering vector *q*, and *T* is the thickness of shell. A log normal distribution of *a* was used in analysis.

The excluded volume interaction calculated with the Percus-Yevick approximation for the closure relation.[2] The detailed expression for the function can be found in.[3]

Table S1 Model of ellipsoids of revolution (a, b, b) with excluded volume interaction, polydispersity sigma of an axis as log normal distribution and <a> - mean value of orbital axis.

Peptides	Concentration [mg mL^{-1}]	$< a >$ [Å]	Sigma	b [Å]
pHK-pKV	1	48±2.0	1.13±0.20	20.1±0.1
	3	41±2.0	1.03±0.08	20.1±0.1
	5	37±2.0	0.70±0.04	20.1±0.2
	10	36±0.9	0.65±0.02	20.2±0.1
Pal-pHK-pKV	1	103±3.0	1.4 fixed	42.0±0.1
	3	29±1.0	0.77±0.03	42.3±0.1
	5	31±1.0	0.59±0.02	43.0±0.1
	10	32±1.0	0.44±0.02	43 fixed

Secondary structures

The secondary structural data of the CD spectra were analyzed using a CDNN deconvolution program, which is a method based on a neural network theory to deconvolute the CD spectra into five different secondary structures (α-helix, β-sheets, β-turn, parallel, antiparallel and random coil). The obtained results are presented in the following Table S2.

Table S2 Quantitative content of peptides' conformation distributions measured by CDNN.

Secondary structure	pHK	pHK-pKV	Pal-pHK-pKV
A-helix (%)	12.8	20.1	53.9
Antiparallel β-sheet (%)	20.3	14.1	4.3
Parallel β-sheet (%)	19.1	13.7	5.2
β-turn (%)	22.3	19.8	13.5
Random Coil (%)	43.4	33.7	23.3
Total Sum (%)	117.9	101.4	100.2

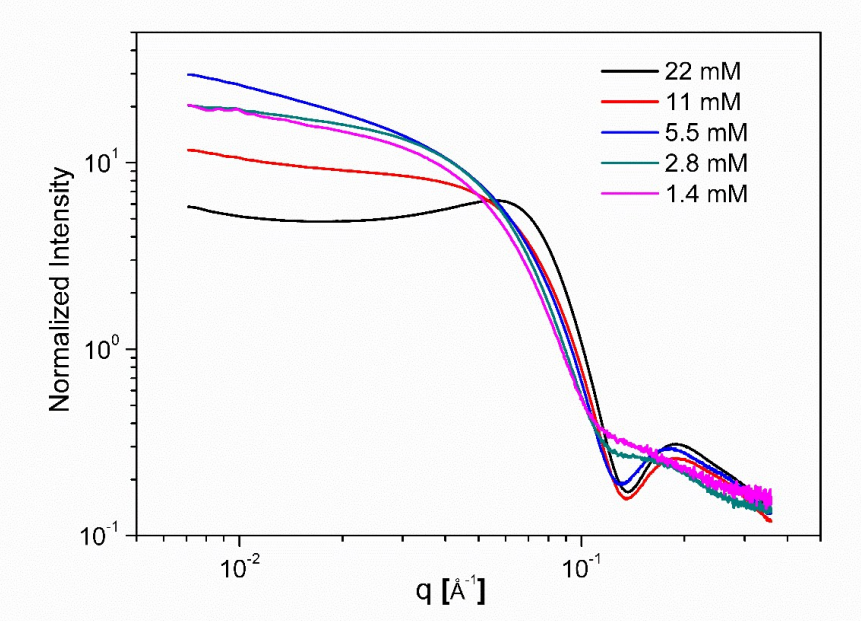

Fig. S1 SAXS patterns of aqueous solutions of Pal-pHK-pKV at high concentrations. (1.4 mM, 2.8 mM, 5.5 mM, 11 mM, 22 mM).

MTT assay

MTT measurements were performed after 24 h, 48 h, and 72 h treatments by peptide formulations. Fig. S2a shows that pHK-pKV exerts effects on the A549 cells viability at essentially higher concentrations (>12.5 μM) as compared to Pal-pHK-pKV. Notably, Pal-pHK-pKV displays a cytotoxic effect already at a very low concentration (3.13 μM Pal-pHK-pKV) (Fig. S2b).

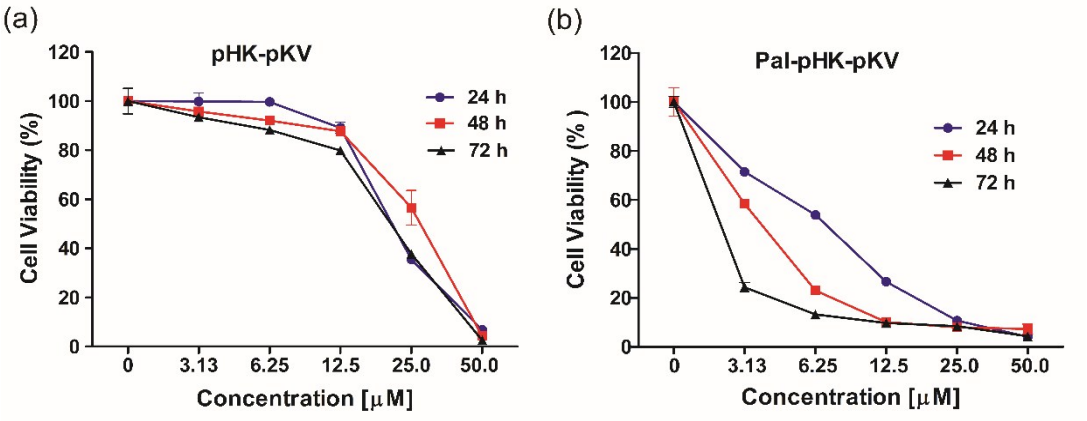

Fig. S2 Cells treated with increasing concentrations of peptides for various time intervals: a) pHK-pKV and b) Pal-pHK-pKV.

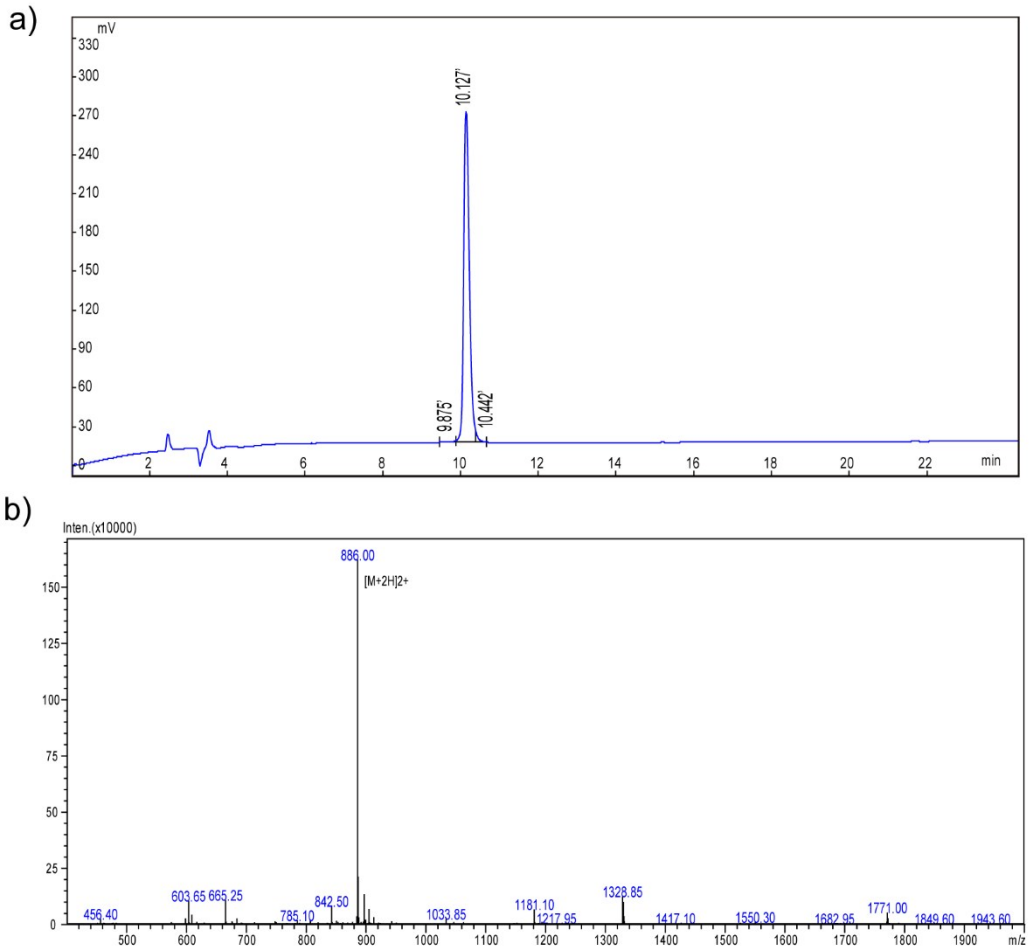

Fig. S3 (a) The purity of peptide pHK analyzed by HPLC. (b) The peptide pHK was identified using mass spectrometry (Mw = 1770.10 g · mol^{-1}).

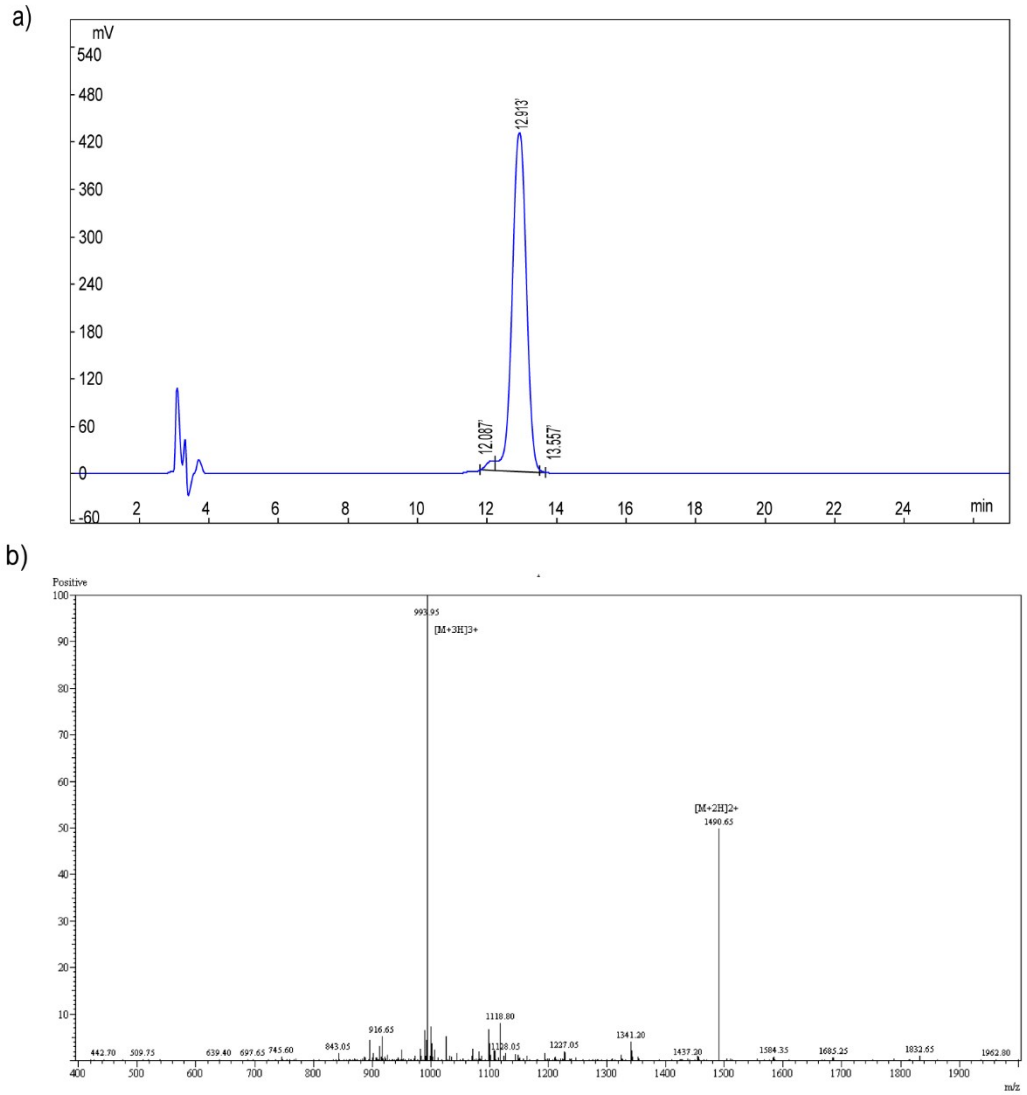

Fig. S4 (a) The purity of peptide pHK-pKV analyzed by HPLC. (b) The peptide pHK-pKV was identified using mass spectrometry (Mw = 2978.67 g · mol^{-1}).

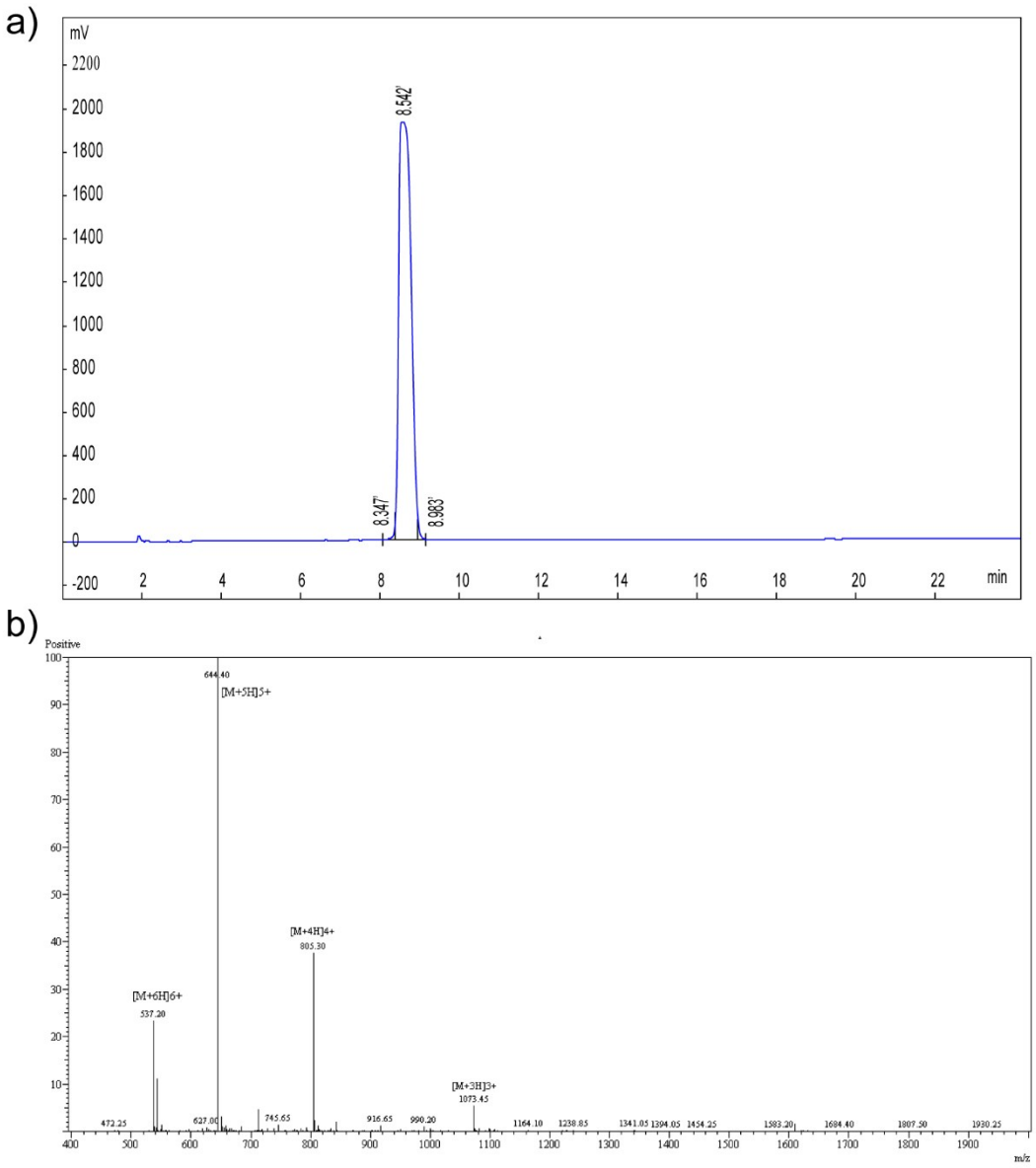

Fig. S5 (a) The purity of peptide Pal-pHK-pKV analyzed by HPLC. (b) The peptide Pal-pHK-pKV was identified using mass spectrometry (Mw = 3217.08 g · mol^{-1}).

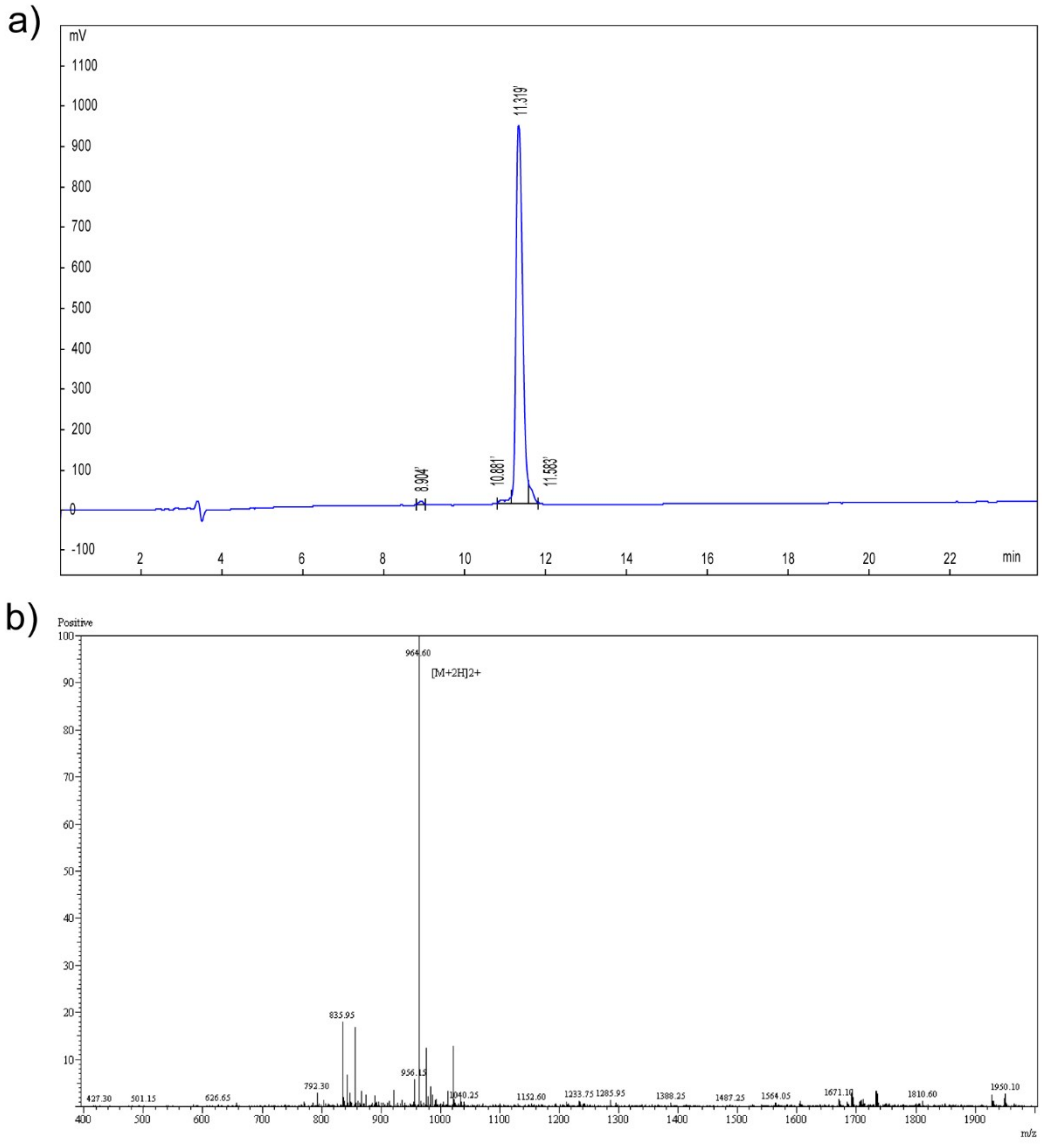

Fig. S6 (a) The purity of peptide pHK-AMC analyzed by HPLC. (b) The peptide pHK-AMC was identified using mass spectrometry (Mw = 1927.27 g · mol^{-1}).

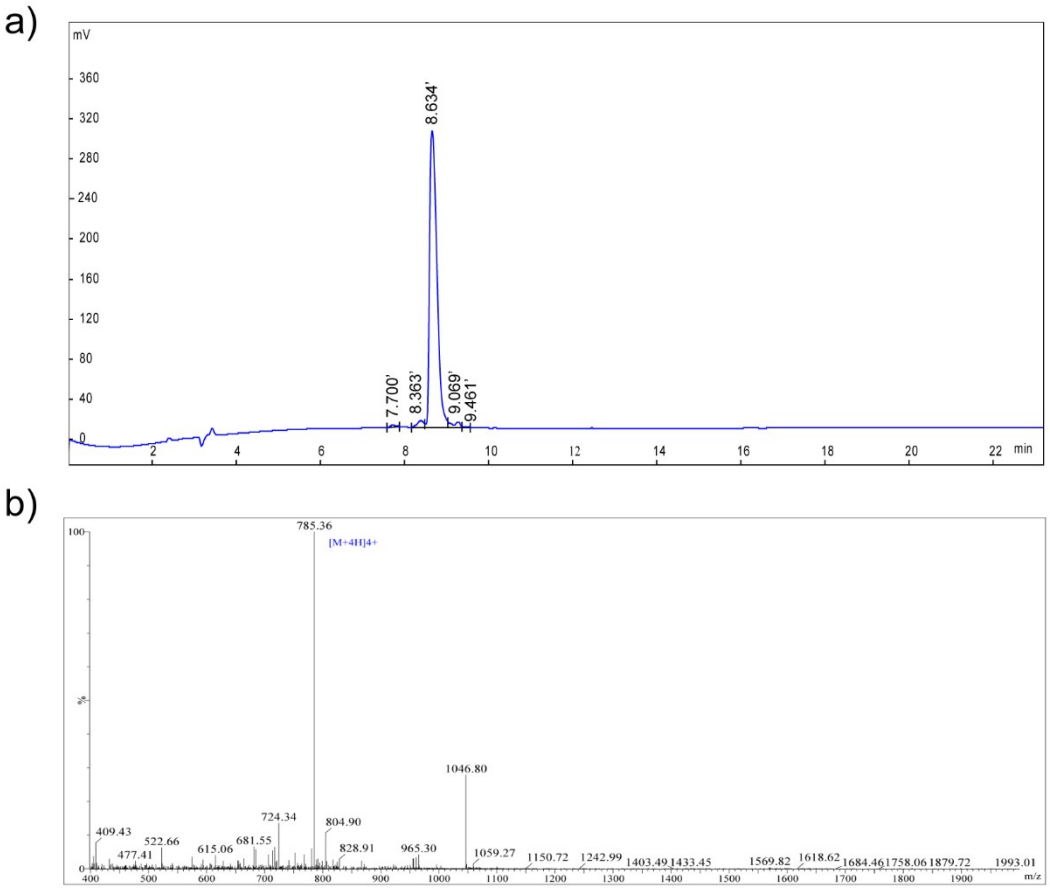

Fig. S7 (a) The purity of peptide pHK-pKV-AMC analyzed by HPLC. (b) The peptide pHK-pKV-AMC was identified using mass spectrometry (Mw = 3135.84 g · mol^{-1}).

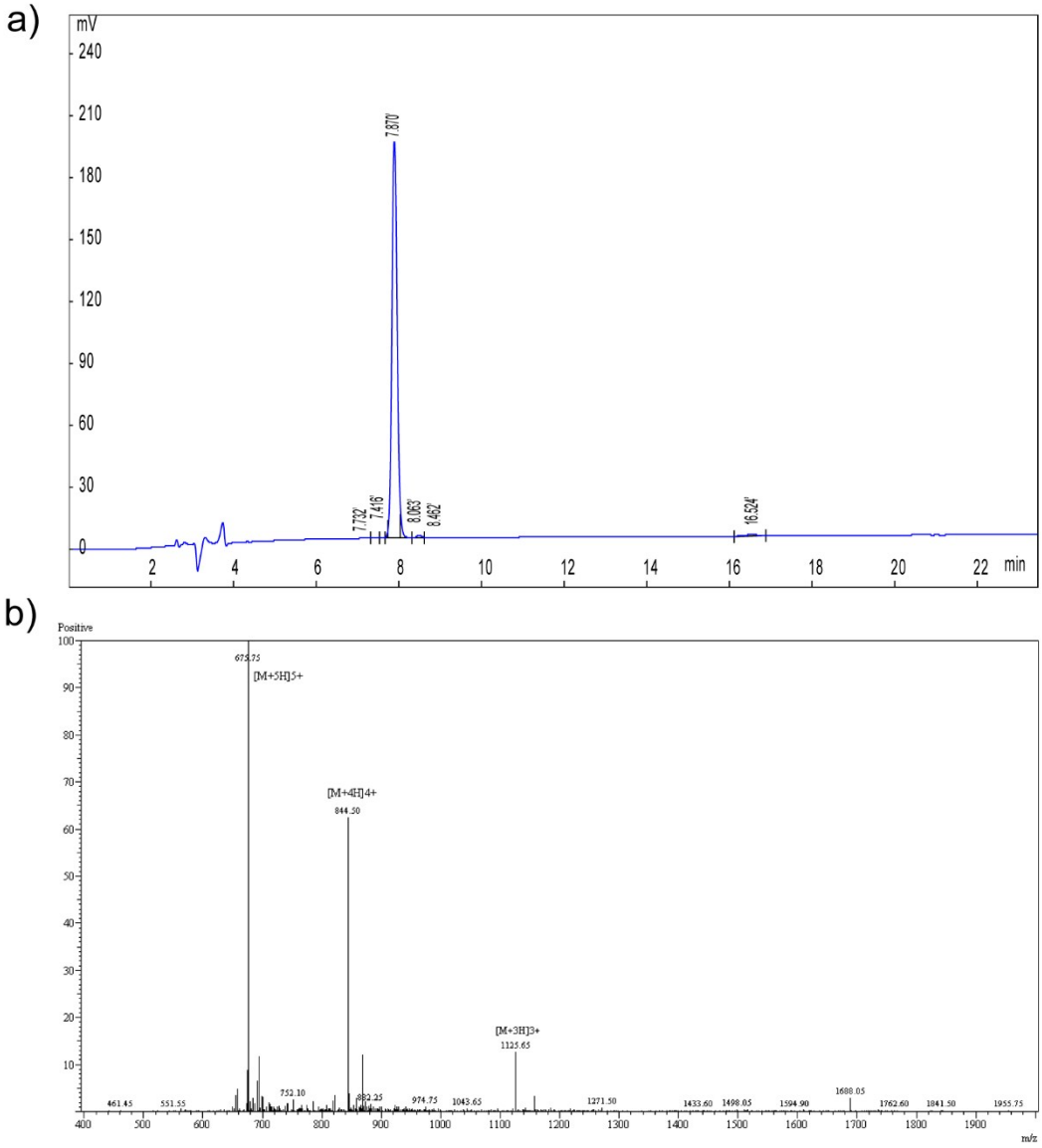

Fig. S8 (a) The purity of peptide Pal-pHK-pKV-AMC analyzed by HPLC. (b) The peptide Pal-pHK-pKV-AMC was identified using mass spectrometry (Mw = 3374.26 g · mol^{-1}).

References

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2. D. J. Kinning and E. L. Thomas, *Macromolecules*, 1984, **17**, 1712-1718.
3. M. Kotlarchyk and S. H. Chen, *Adv. Colloid Interface Sci.*, 1997, **70**, 171-210.