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Codelivery of a protein toxin and an endolysosomally targeted photosensitiser via a liposomal nanocarrier: a novel strategy for light-triggered intracellular release of therapeutic macromolecules

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Calculation of the concentrations of liposomal photosensitiser and encapsulated saporin

The total number of lipid molecules per liposome in each preparation was estimated using the formula:

$$N_{lipids} = \left[4\Pi\left(\frac{d}{2}\right)^2 + 4\Pi\left(\frac{d}{2} - h\right)^2\right]_{/a}$$

where *d* is the diameter of spherical unilamellar vesicles determined by DLS, *h* is the lipid bilayer thickness and taken as 5 nm (for phosphatidylcholine liposomes[1]), and *a* is the lipid head group area.[2] For unilamellar liposomes composed of phosphatidylcholine the above equation is thus simplified to:

$$N_{lipids} = 17.69\left[\left(\frac{d}{2}\right)^2 + \left(\frac{d}{2} - 5\right)^2\right]$$

with *a* taken as 0.71 nm^2 for phosphatidylcholine.[3]

The number of liposomes per mL was then calculated using the equation:

$$N_{liposomes} = \frac{c \times N_a}{N_{lipids} \times 1000}$$

where *c* is the molar concentration of lipids and N_a is the Avogadro number.

For the calculation of the molarity of the appended TPP moieties within the liposomal nanocarrier, it is assumed that all the maleimido functions on the external layer of the liposome react with a Cys-containing Tat peptide. This is assumed to be half the total number of maleimido units,[4] since by simple distribution, half these units will be displayed on the interior of the liposome. For the available maleimides on the external surface of the liposome, 20% are therefore conjugated with a Tat peptide bearing a TPP unit (based used a 4:1 ratio of peptide **2** (Tat peptide only): **5 (**TPP-Tat peptide). This allows $c_{TPP,}$ the molarity of the TPP moieties, to be calculated as follows:

$$c_{TPP} = \frac{mol_{malemide-PEG2000-DSPE}}{Volume_{solution}} \times 0.1$$

where $mol_{malemide-PEG2000-DSPE}$ is the number of moles of maleimide-functionalised lipids used.

For the calculation of the molarity of encapsulated saporin, it is assumed that the internal volume of each liposome has a concentration of 1 mg/mL of saporin, with the molecular weight of labelled saporin taken as 32 kDa. The saporin molecules are confined to a shell (the internal volume of the liposomes)[5] defined by the formula:

$$Volume_{liposome} = \frac{4}{3}\pi\left(\frac{d}{2} - 5\right)^3$$

Using this equation to calculate the internal volume of each liposome, the concentration of encapsulated saporin can be calculated as follows:

$$c_{saporin} = \frac{Volume_{liposome} \times N_{liposomes}}{Volume_{solution} \times N_a}$$

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HPLC of peptides and porphyrin-peptide conjugates

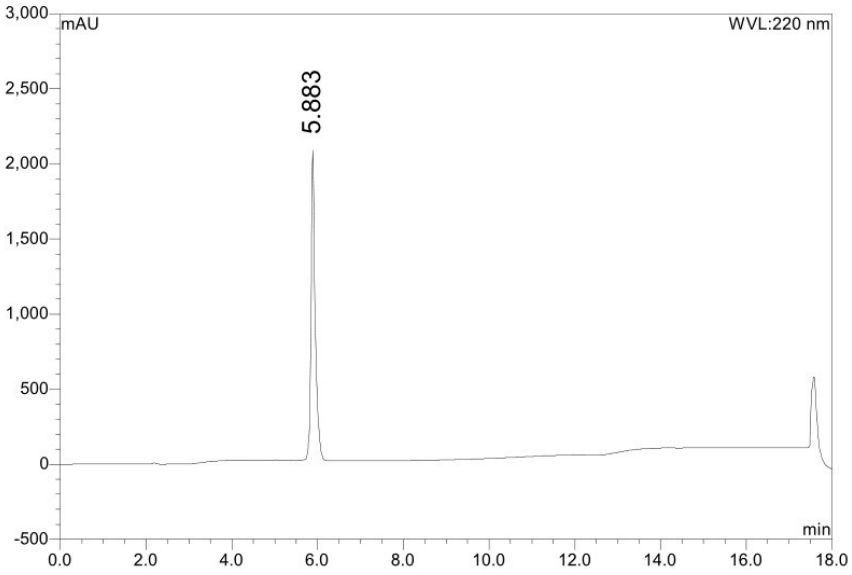

Figure 1 (a). HPLC profile of peptide **2**.

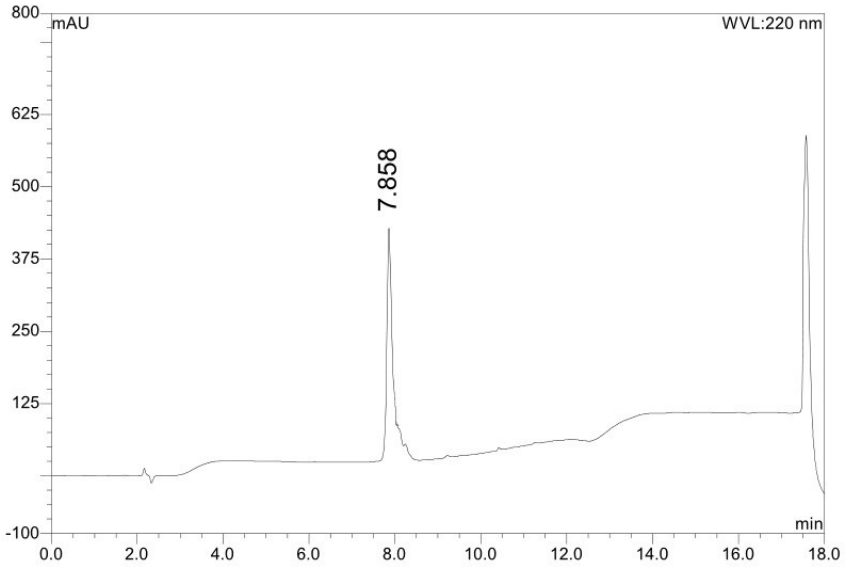

Figure 1 (b). HPLC profile of porphyrin-peptide conjugate **4**.

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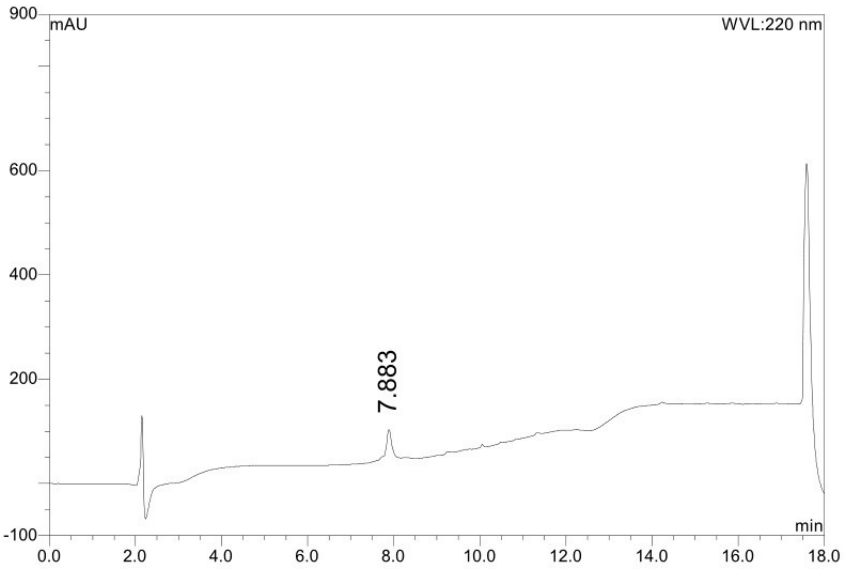

Figure 1 (c). HPLC profile of porphyrin-peptide conjugate **5**.

Mass Spectra of peptides and porphyrin-peptide conjugates

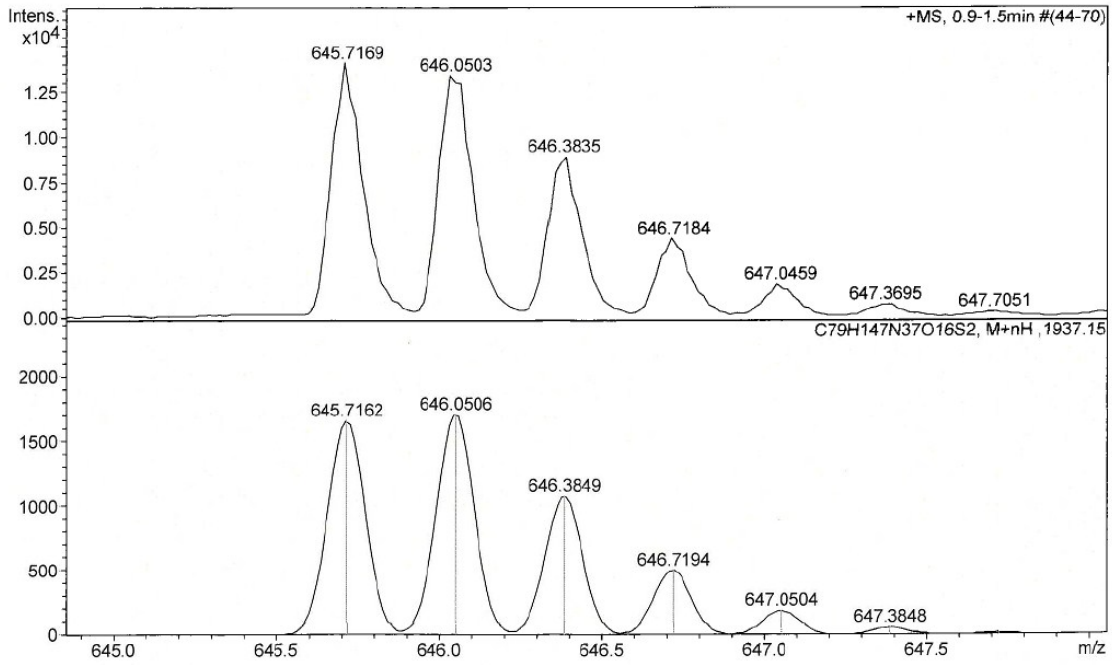

Figure 2 (a). ESI mass spectrum of peptide **2**.

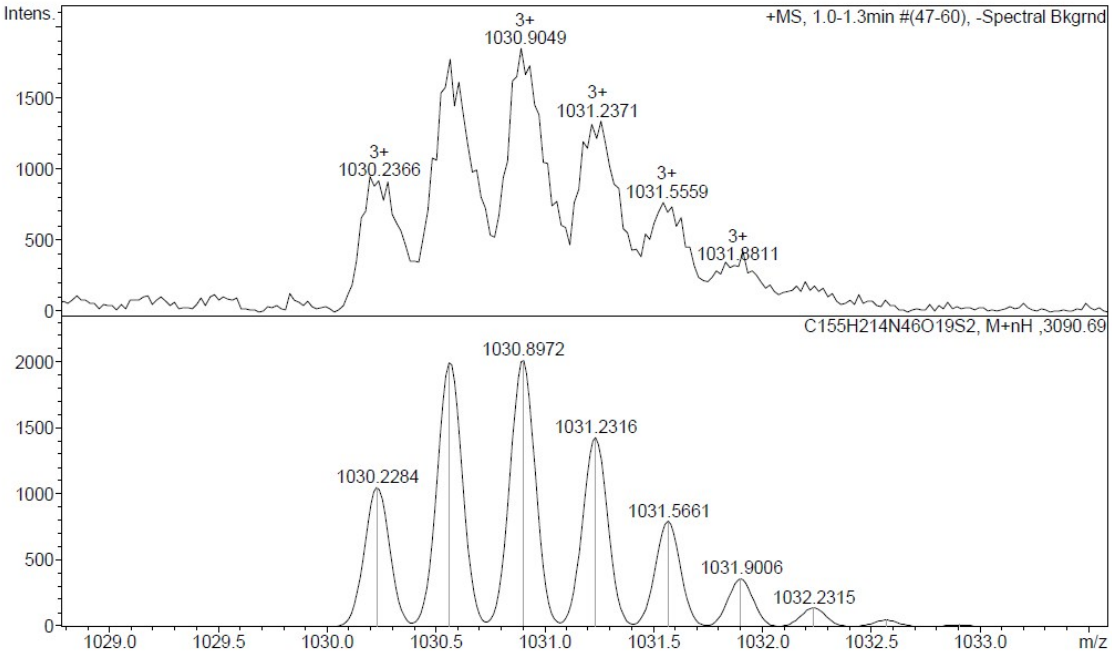

Figure 2 (b). ESI mass spectrum of porphyrin-peptide conjugate **4**.

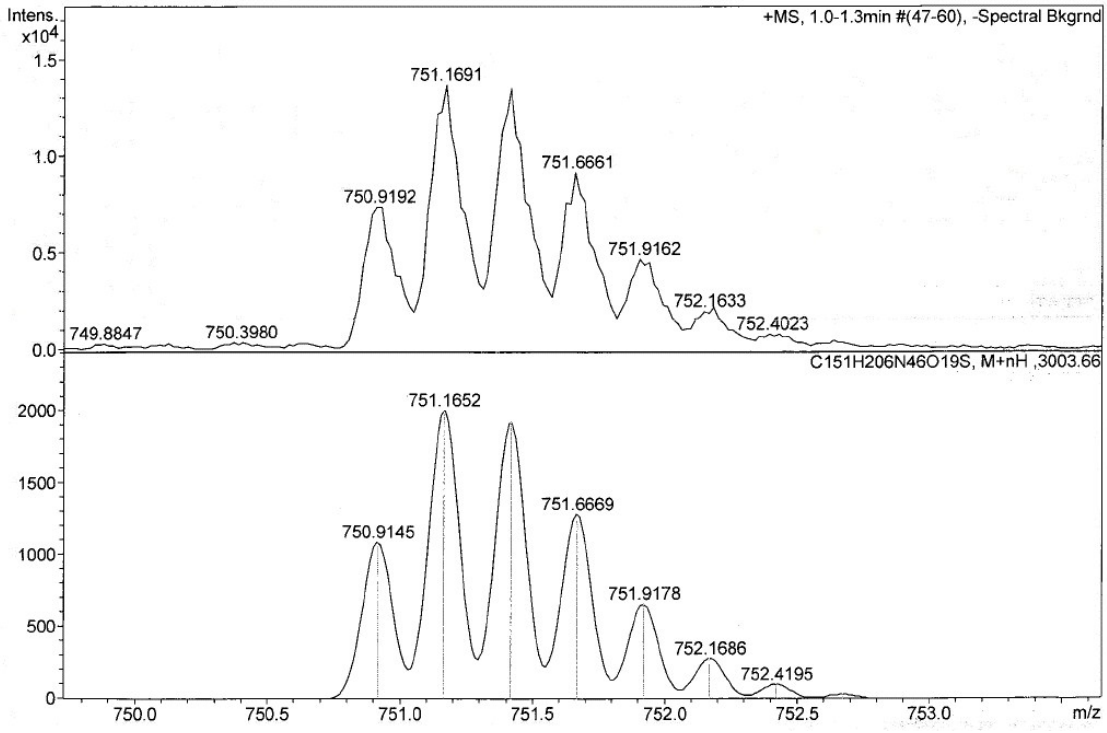

Figure 2 (c). ESI mass spectrum of porphyrin-peptide conjugate **5**.

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UV spectra

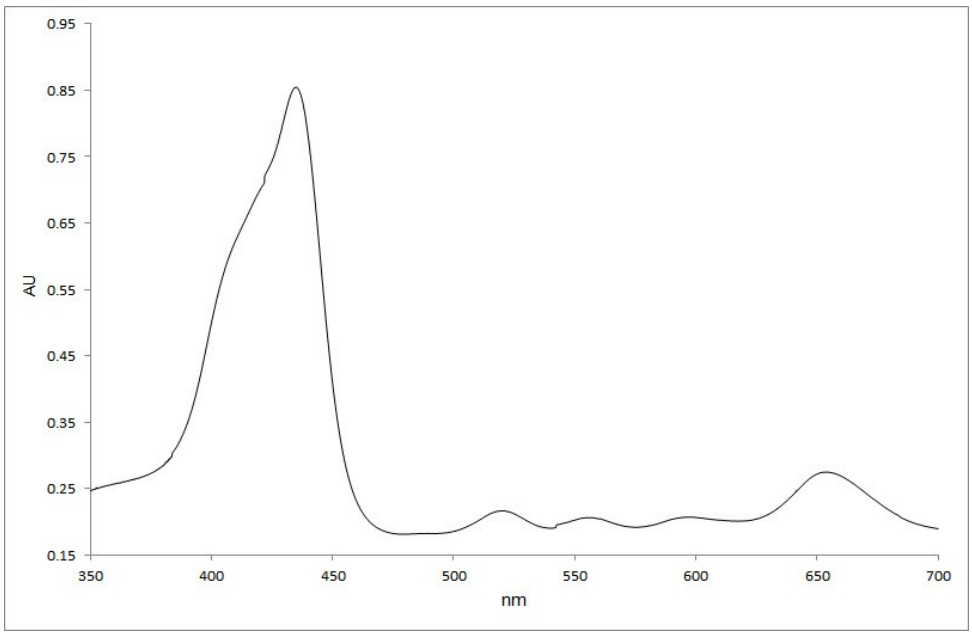

Figure 3 (a). UV-visible spectrum of porphyrin-peptide conjugate **4**.

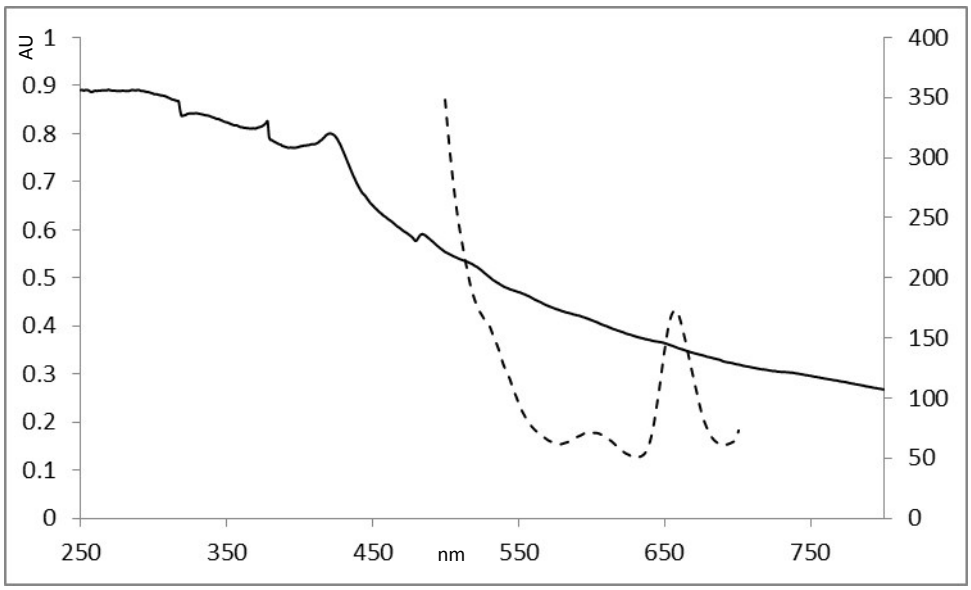

Figure 3 (b). UV-visible (solid line) and fluorescence (dotted line) spectra in HEPES buffer for TPP-Tat liposomes ($\lambda_{exc.}$ = 420 nm).

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