Cell-Penetrating Peptides Escape the Endosome by Inducing Vesicle Budding and Collapse

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Supporting Information

This PDF file includes:

Other Supplementary Materials for this manuscript include the following:

Movies S1 to S3

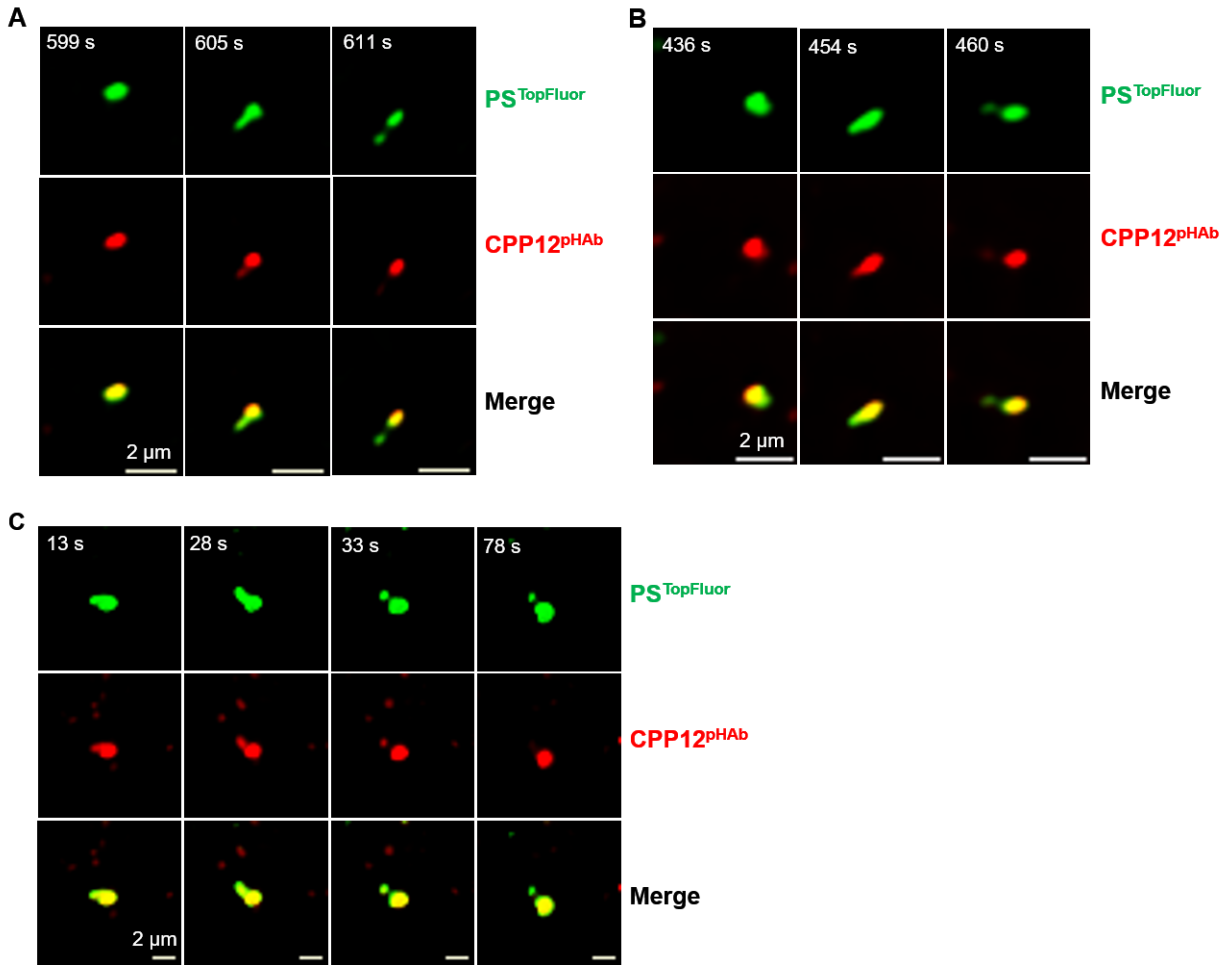


Fig. S1. Time-lapse confocal microscopic images showing three additional CPP12-induced vesicle budding and collapse events in different HeLa cells. Cells were pulse labeled with $PS^{TopFluor}$ (1.5 µM) and then incubated with $CPP12^{pHAb}$ (2.5 µM, red channel) for 10-15 min, and imaged every 6 s. The time stamp indicates the time after initiation of imaging, with some of the intermediate time-points omitted. Scale bars = 2 µm.

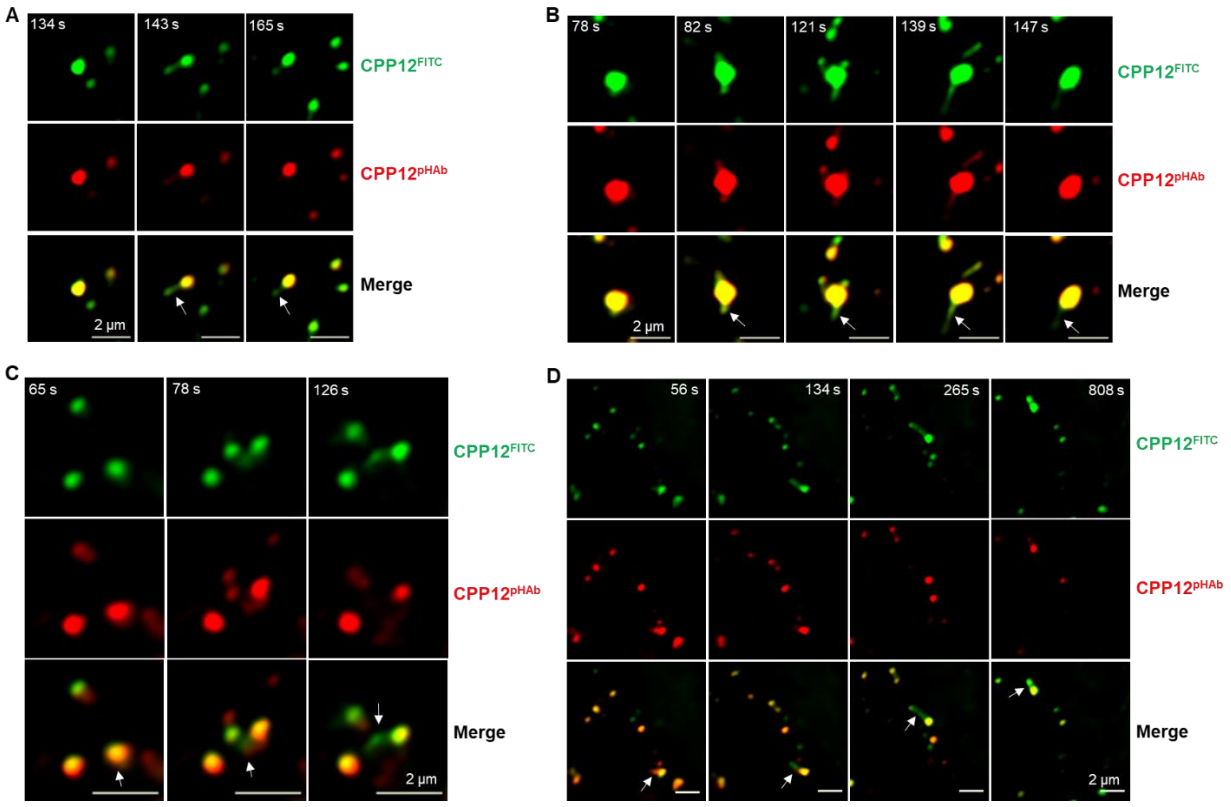


Fig. S2. Time-lapse confocal microscopic images showing four additional vesicle budding and collapse events in live cells visualized by dual-labeled CPP12. HeLa cells were incubated with $CPP12^{FITC}$ (2 µM) and $CPP12^{pHAb}$ (2 µM, red channel) for 30 min, washed and imaged every 4.5 s. The time stamp indicates the time after initiation of imaging, with some of the intermediate time-points omitted. Scale bars = 2 µm.

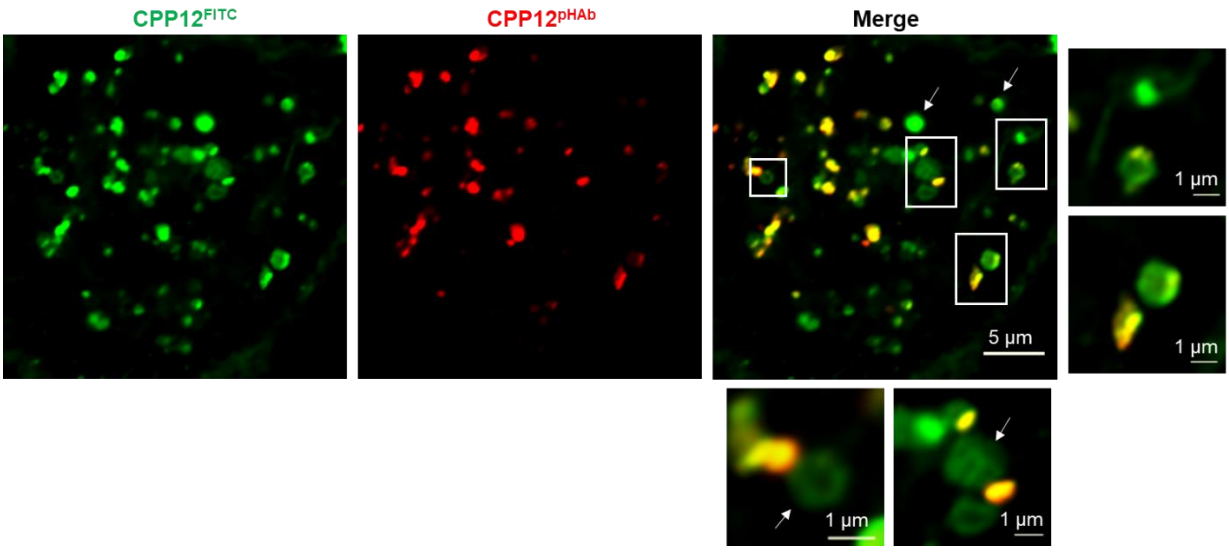


Fig. S3. Confocal microscopic images of a HeLa cell after treatment for 2 h with YM201636 (800 nM) and then for 30 min with $CPP12^{FITC}$ (2 µM, green channel) and $CPP12^{pHAb}$ (2 µM, red channel). *Left panel*, $CPP12^{FITC}$ fluorescence (total No. of fluorescent puncta = 66); *Middle panel*, $CPP12^{pHAb}$ fluorescence (total No. of fluorescent puncta = 41); *Right panel*, merge of $CPP12^{FITC}$ and $CPP12^{pHAb}$ fluorescence. Note that there is a corresponding green signal for every red fluorescence punctum, but many green fluorescence puncta (e.g., those marked by white arrows) are not matched by red fluorescence. Enlarged images of the boxed areas are shown to the right and below.

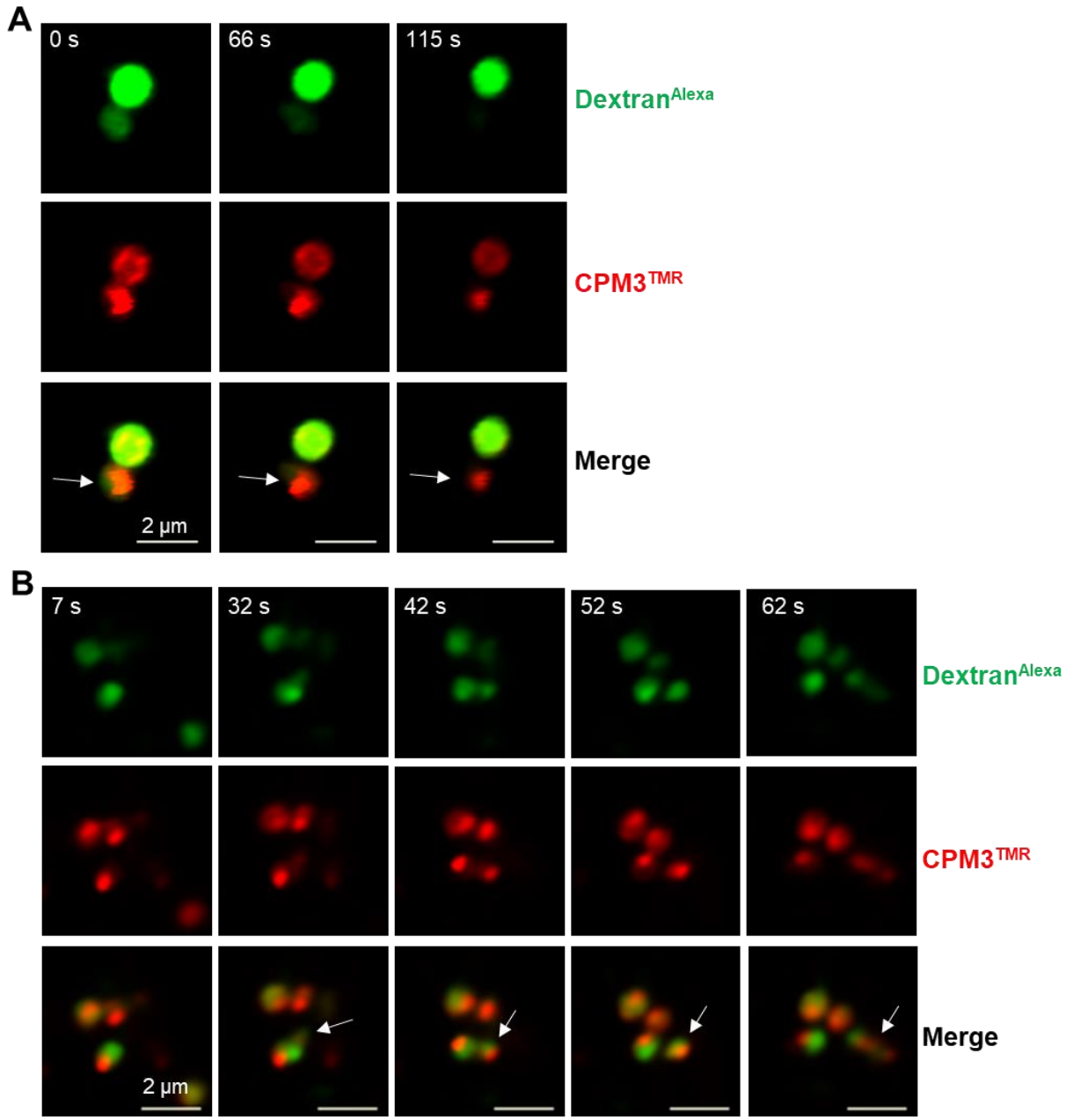
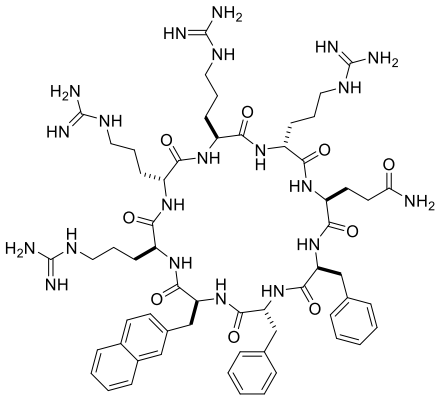


Fig. S4. (A-B) Time-lapse confocal microscopic images showing two vesicle budding and collapse events in live cells induced by CPM3. HeLa cells were pretreated with YM201636 (800 nM) for 2 h and $CPM3^{TMR}$ (2 µM, red channel) and $Dextran^{Alexa}$ (50 µg/mL, green channel) were added. After incubation for another 30-40 min, the cells were washed and imaged by live-cell confocal microscopy every 5 s. Endosomes that are undergoing or have just undergone budding and collapse are indicated by white arrows. Scale bars = 2 µm.

Fig. S5. Structures, purity, and high-resolution MS of peptides and other molecules used in this work. Note: Some of the dye-labeled peptides eluted as two separate peaks, because the commercially available dye is a mixture of 5- and 6-carboxy isomers. The mixtures of two isomers were used in all experiments.

CPP12



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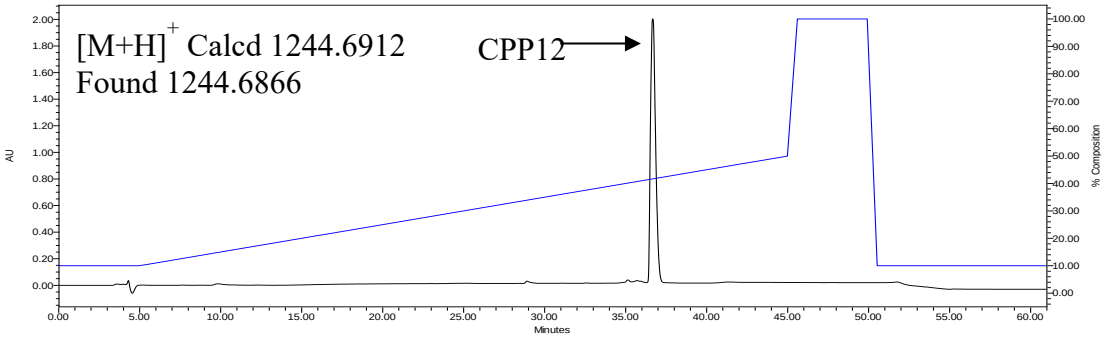

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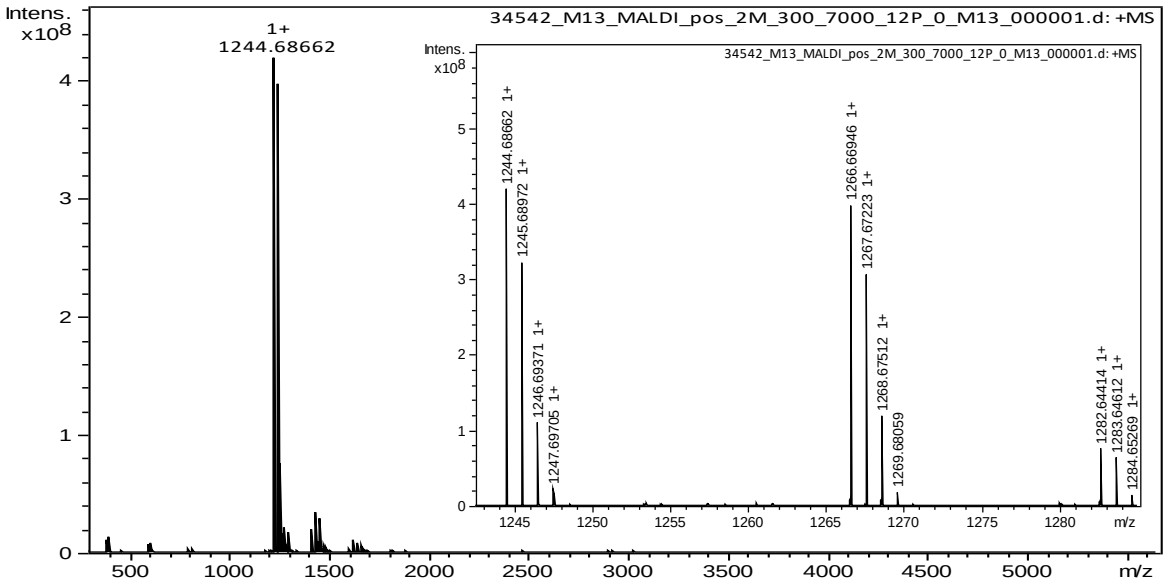
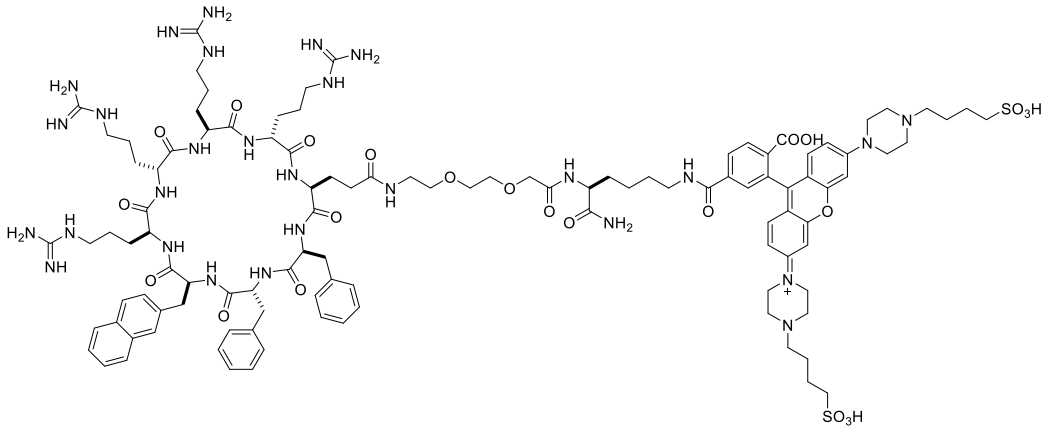


CPP12^{pHAb}



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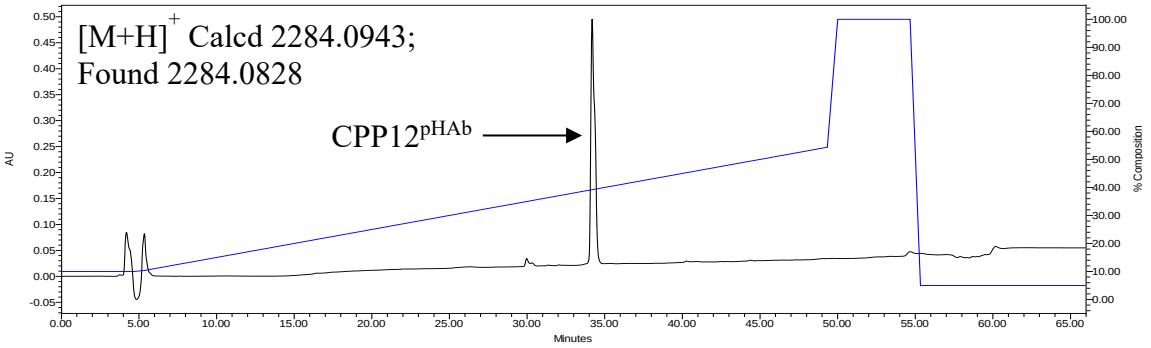

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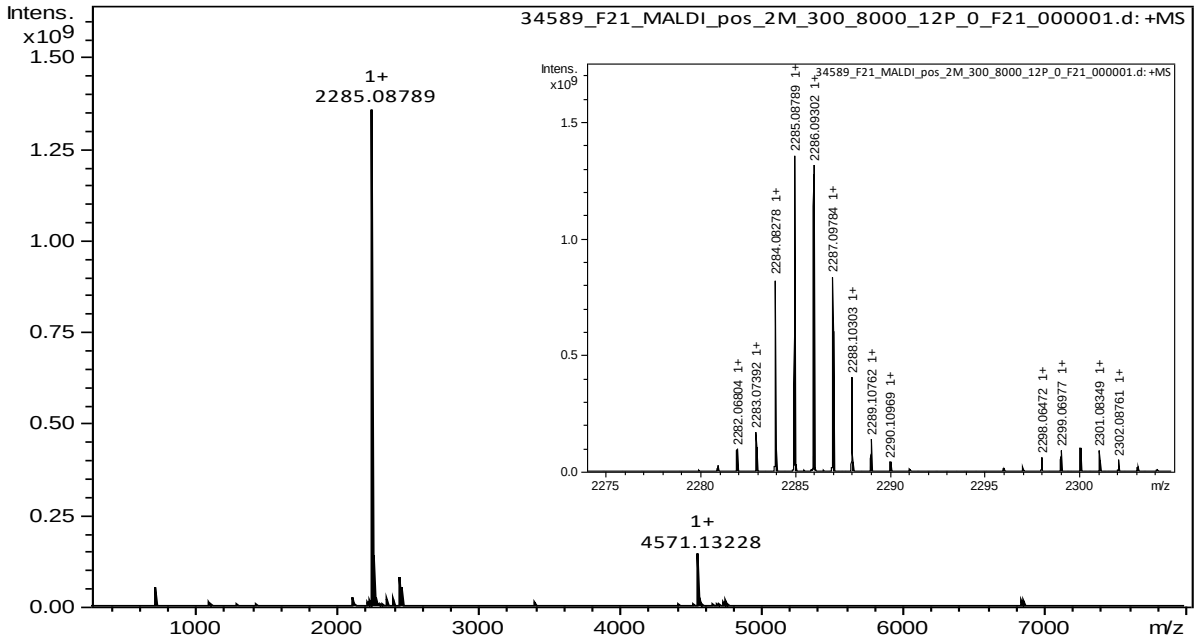
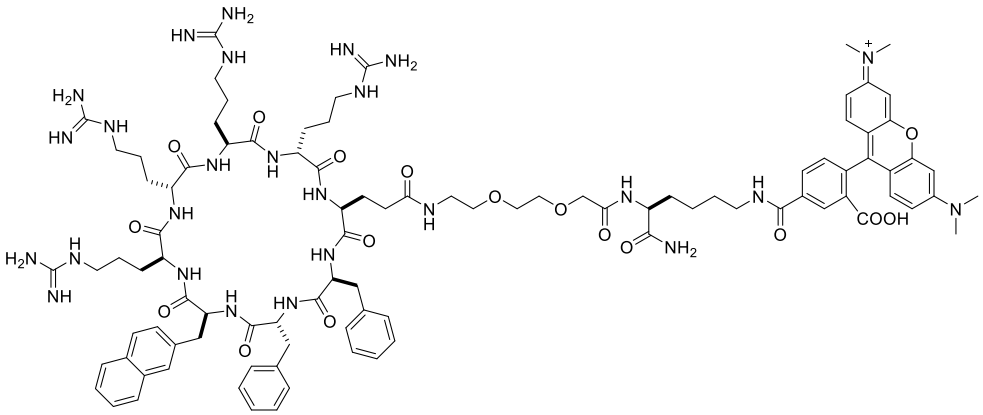


CPP12^{TMR}



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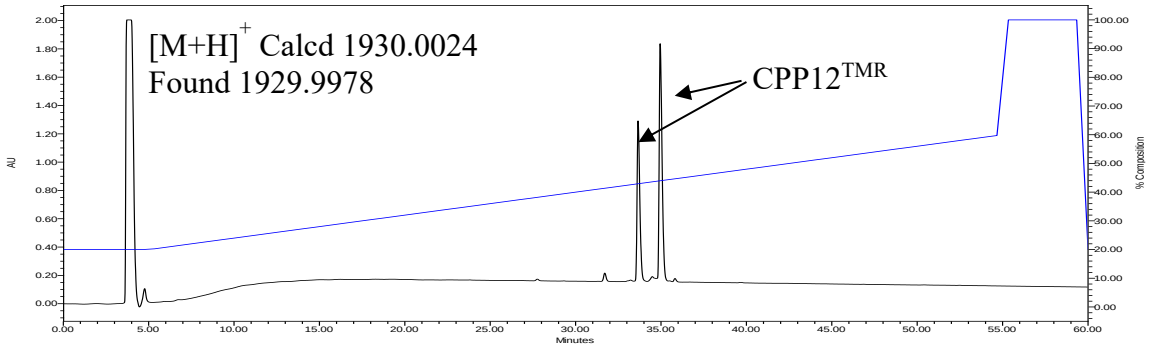

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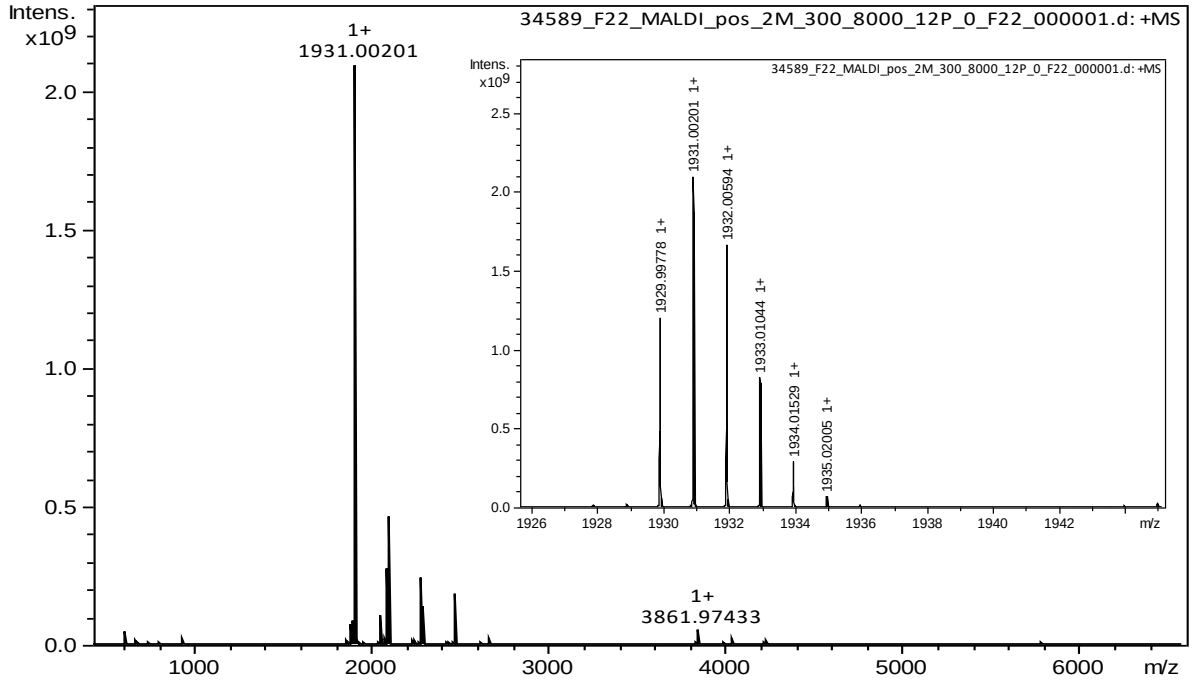
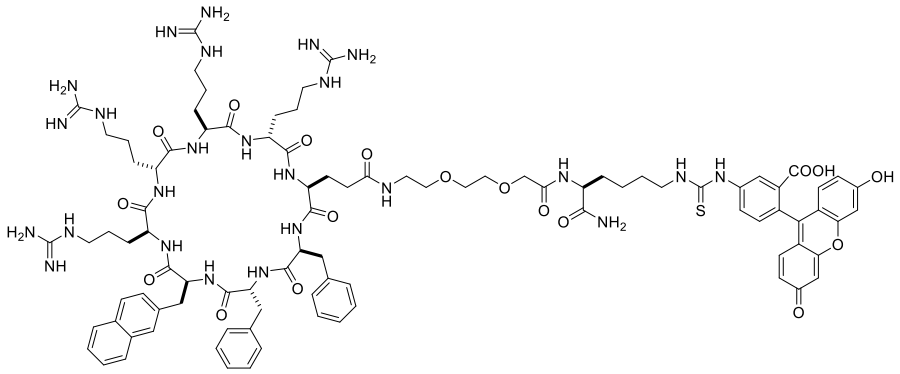


CPP12^{FITC}



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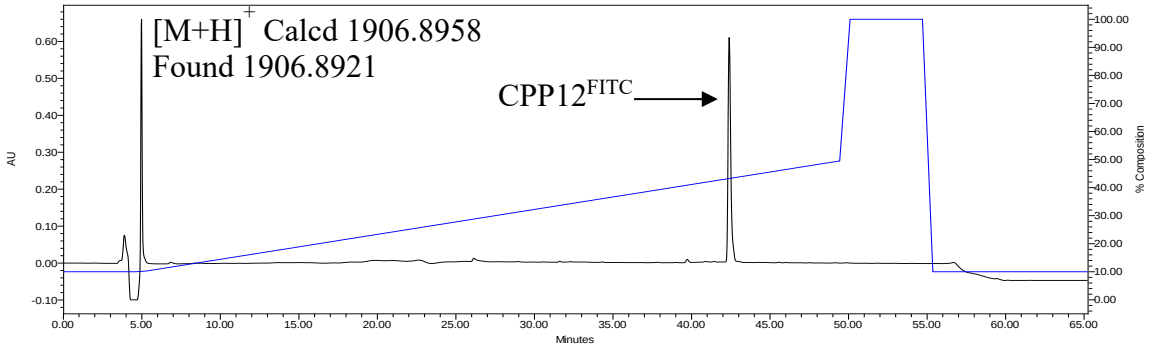

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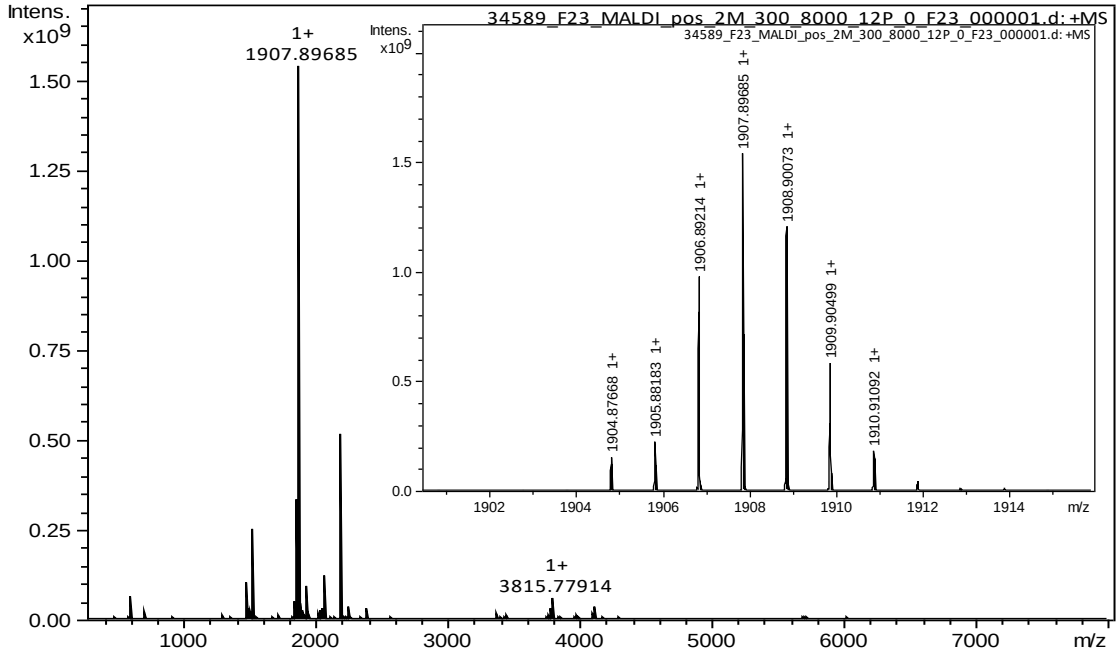
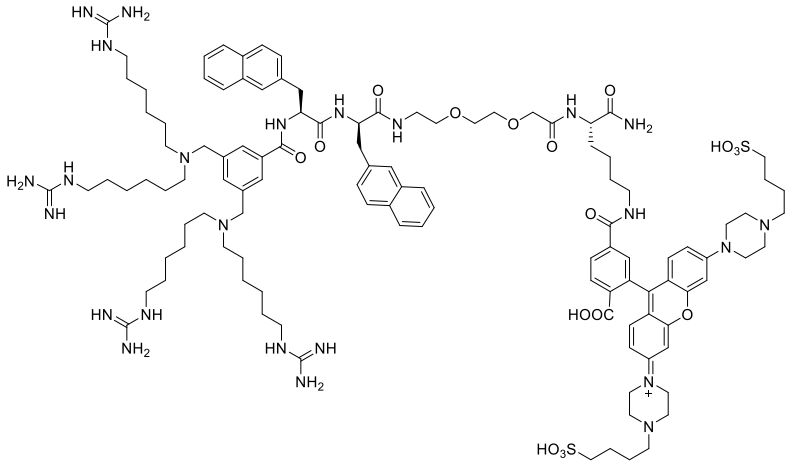


CPM3^{pHAb}



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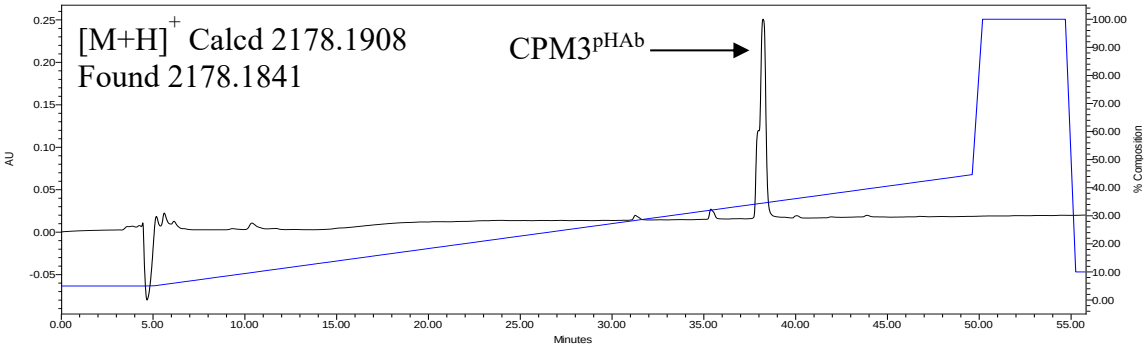

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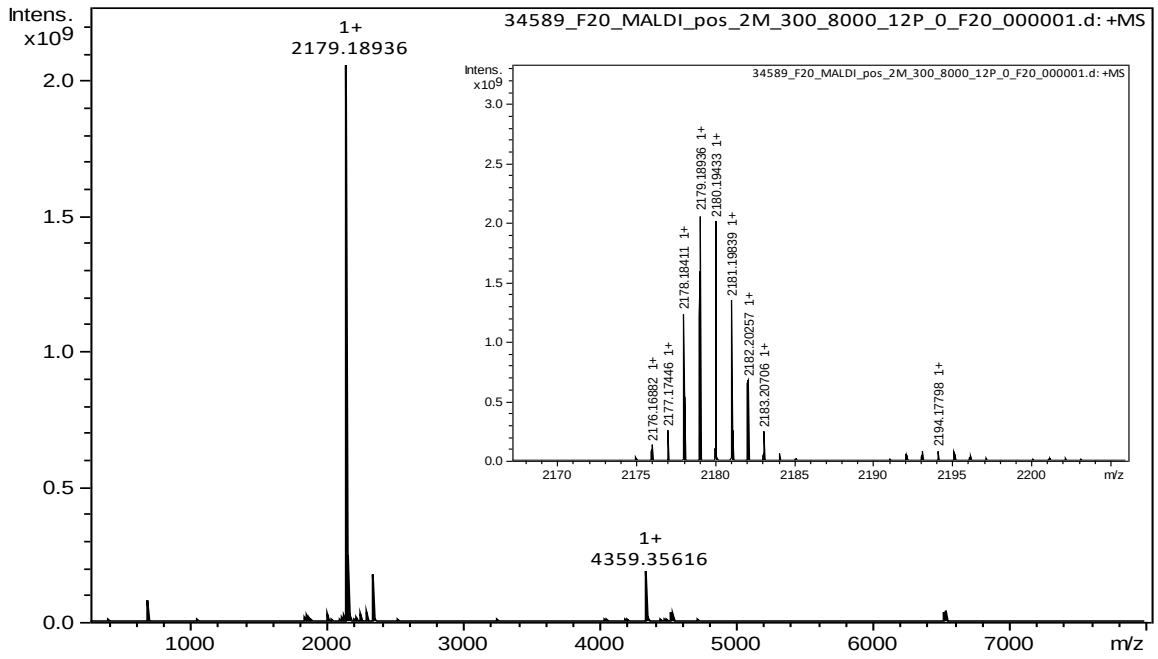
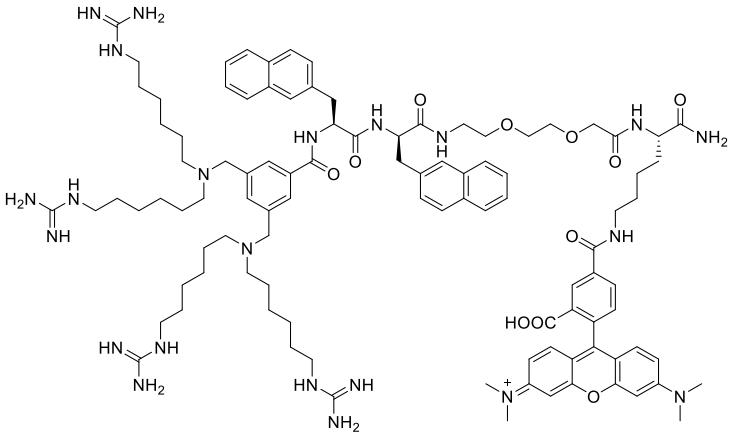


CPM3^{TMR}



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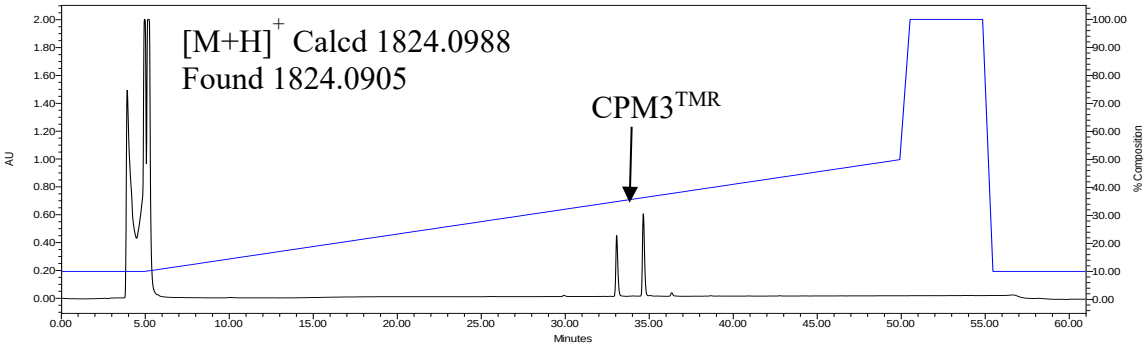

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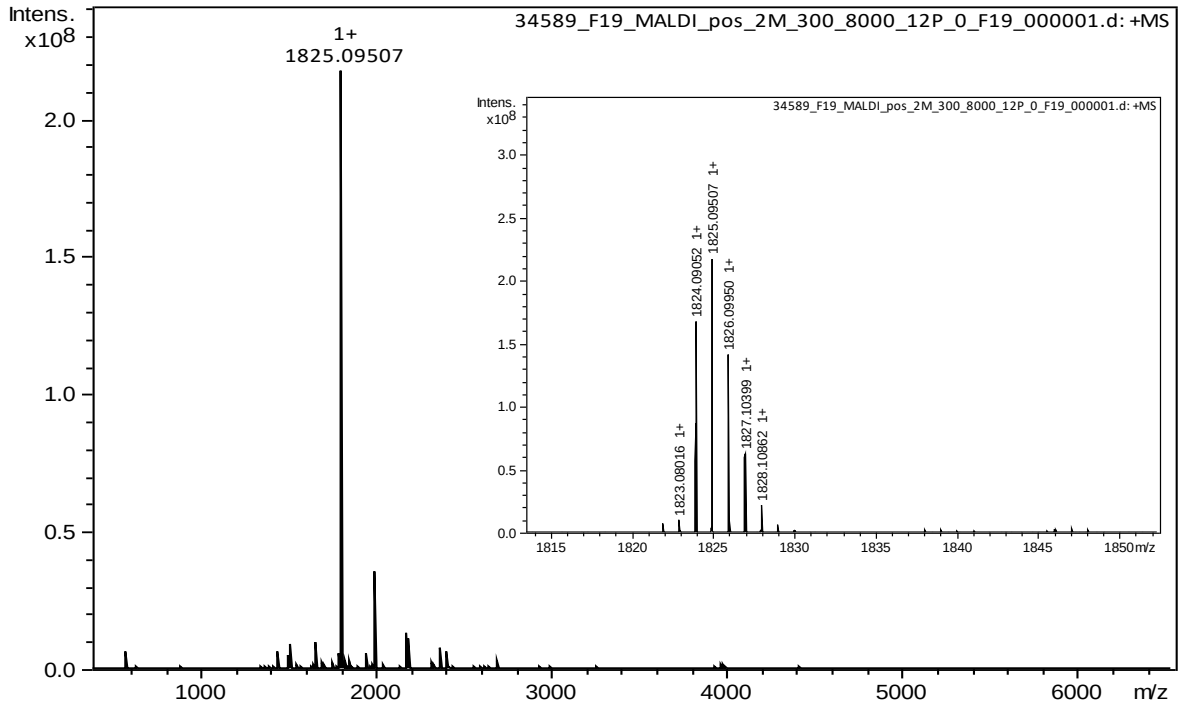
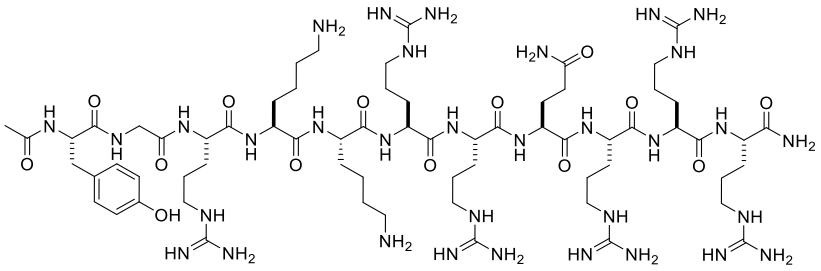


Tat



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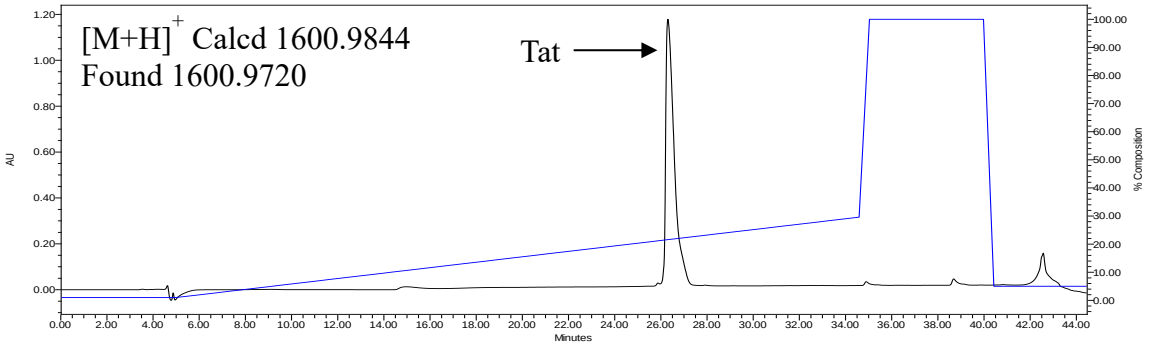

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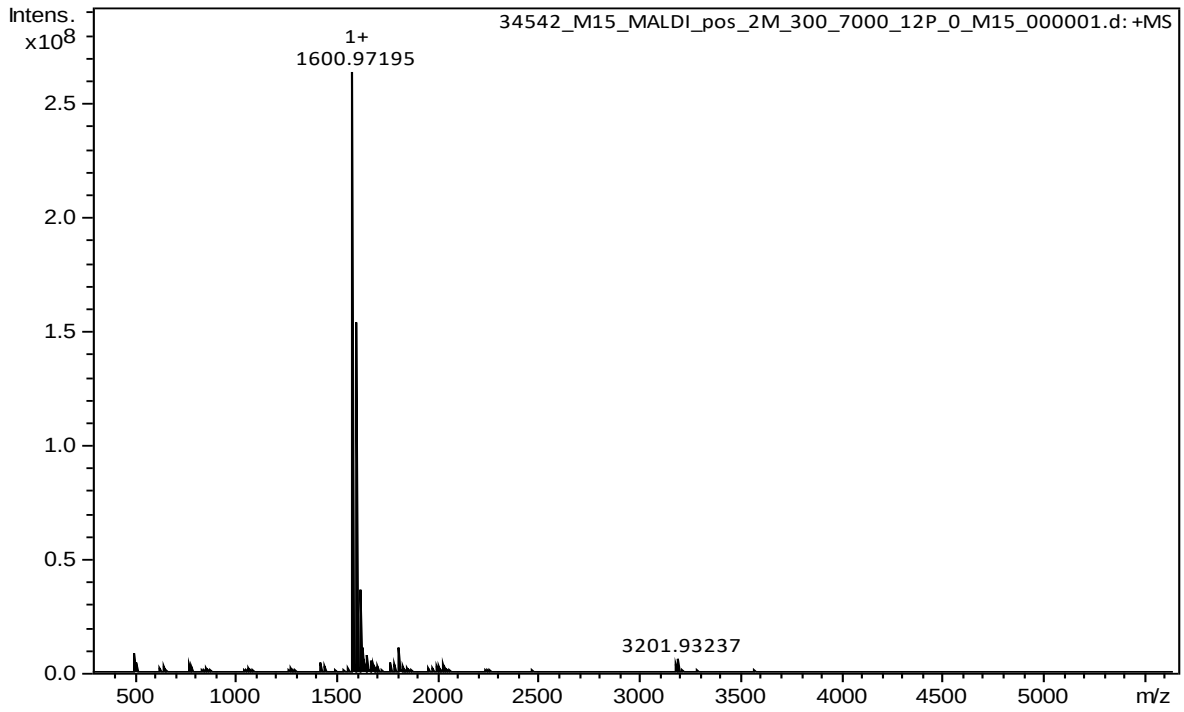
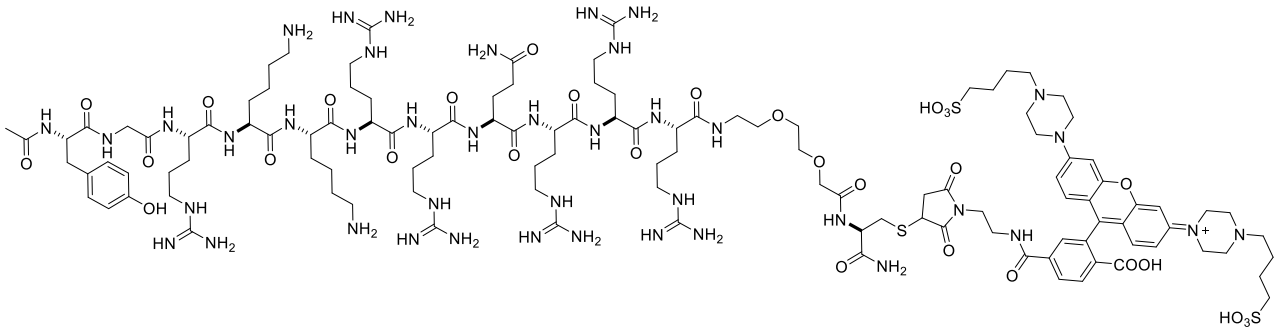


Tat^{pHAb}



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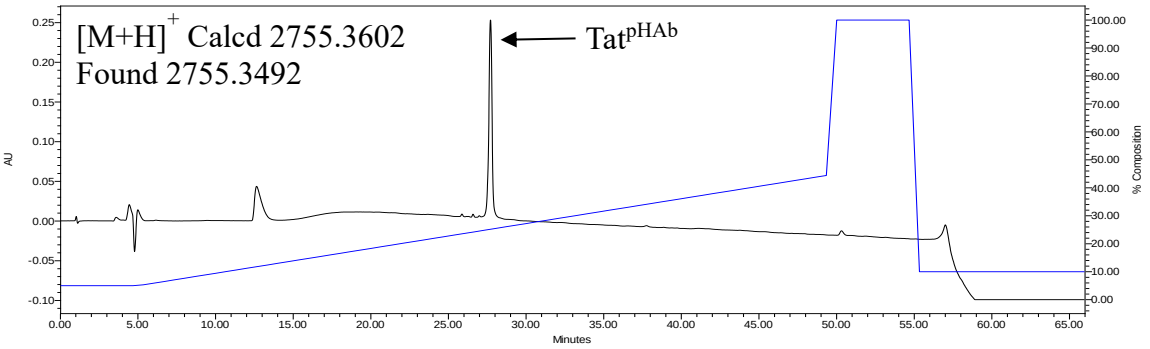

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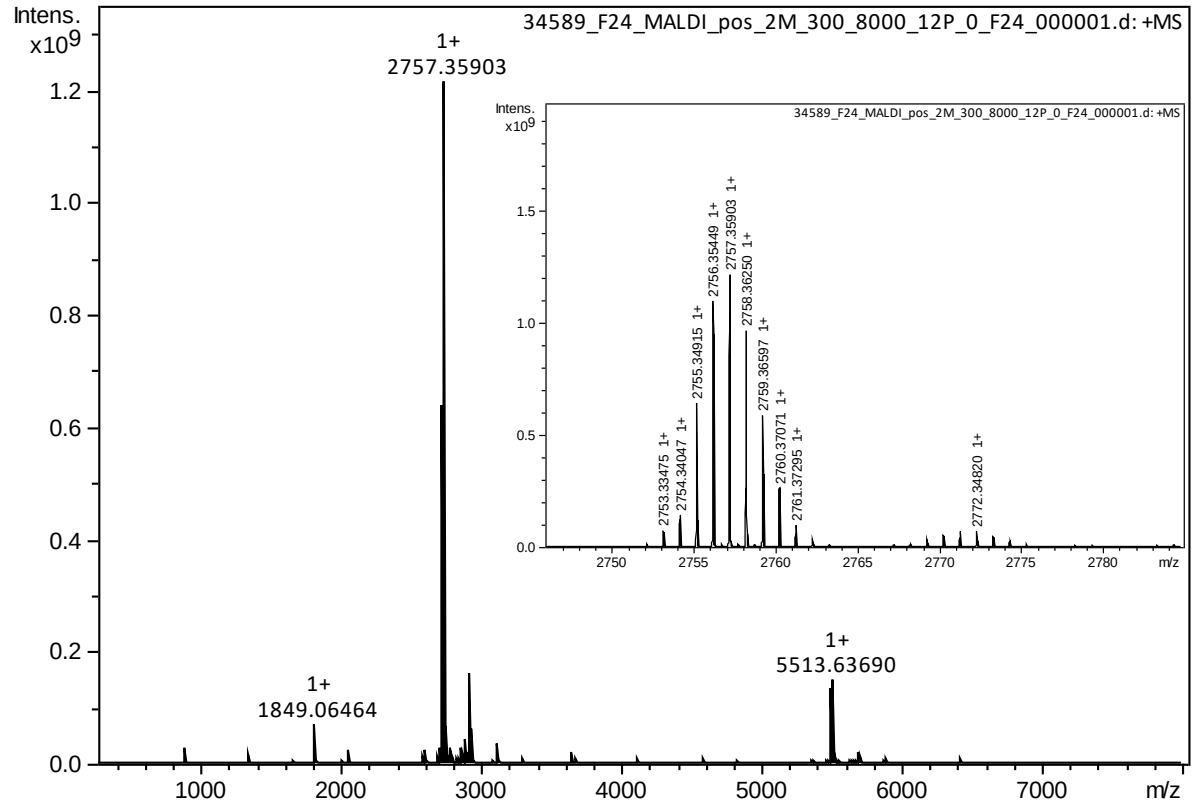

Movie S1. **A CPP12-induced vesicle budding and collapse event in a HeLa cell**. Cells were incubated with $PS^{TopFluor}$ (1.5 μM, green channel) for 15 min on ice, washed with DPBS, treated with $CPP12^{pHAb}$ (2.5 μM, red channel) for 10 min, and imaged by confocal microscopy every 6 s. The video consists of time-lapse images of a single endosome (boxed in Fig. 1C) from 119-150 s after initiation of imaging. Panels from left to right represent the merge, $PS^{TopFluor}$ (green), and $CPP12^{pHAb}$ signals (red), respectively. The endosome split into two vesicles of unequal sizes at 137 s; at 143 s, the smaller vesicle traveled to the upright corner and collapsed, while the larger vesicle remained intact. Scale bars = 2 μm.

Movie S2. **A vesicle budding and collapse event visualized with dual labeled CPP12**. HeLa cells were treated simultaneously with $CPP12^{FITC}$ (2 μM, green channel) and $CPP12^{pHAb}$ (2 μM, red channel) for 30 min, washed, and imaged by confocal microscopy every 4.5 s. The video consists of time-lapse images of a small cytoplasmic volume (in Fig. 2) from 721-843 s after initiation of imaging. Scale bars = 1 and 20 μm.

Movie S3. A CPP12-induced vesicle budding and collapse event from an enlarged endosome. HeLa cells were pretreated with YM201636 (800 nM) for 2 h, and $CPP12^{TMR}$ (2 μM, red channel) and $Dextran^{Alexa}$ (50 μg/mL, green channel) were added. After incubation for another 30-40 min, the cells were washed and imaged by live-cell confocal microscopy every 17 s. The video consists of time-lapse images of a small cytoplasmic volume (in Fig. 3E) between 0-405 s after initiation of imaging. Panels from left to right represent the merge, $Dextran^{Alexa}$ (green), and $CPP12^{TMR}$ signals (red), respectively. Scale bars = 2 μm.