**Titles of Supplementary Information**

*Article*

# Optical Fluorescence Imaging of Native Proteins Using a Fluorescent Probe With a Cell Membrane-Permeable Carboxyl Group

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**Supplementary Figure S1:** The construction of N-terminus-tagged HIV-1 Tat protein.
**Supplementary Figure S2:** Analysis of R-phycoerythrin (PE)-labeled streptavidin by SEC-HPLC with PE labeled streptavidin as the control group.
**Supplementary Figure S3:** Analysis of biotin ligase (BirA) by SEC-HPLC. The analytical SEC-HPLC profiles of BirA as the control group.
**Supplementary Figure S4:** Comparative analysis of BirA enzyme-dependent labeling of FAM 56 with a carboxyl group structure and biotin.
**Supplementary Figure S5:** LC-MS spectrum of the purified fluorescein (FAM 56)-labeled Tat.
**Supplementary Figure S6:** Mass spectrum profile of predigested fluorescein (FAM 56)-labeled Tat protein.
**Supplementary Figure Legends**

**Figure S1. The construction of N-terminus-tagged HIV-1 Tat protein.**
Schematic description of the HIV Tat protein labeled with free carboxyl group fluorescent dyes at the N-terminus. The hexahistidine (6×His)-tag was introduced in the C-terminus for Ni-affinity chromatography and carrying Avi-tag at the C-terminus for biotin labeling.

**Figure S2. Analysis of R-phycoerythrin (PE)-labeled streptavidin by SEC-HPLC with PE-labeled streptavidin as the control group.** Compared to the peaks of PE-labeled streptavidin alone, UV (280 nm, black line) detection at 5.576 min, PE-labeled streptavidin is shown with the highest peak at 5.547 min (8 L.U.) (FLD, Ex: 488 nm/Em: 575 nm, blue line). PE emissions are given in relative light units (LU).

**Figure S3. Analysis of biotin ligase (BirA) by SEC-HPLC. The analytical SEC-HPLC profiles of BirA as the control group.** SEC-HPLC of N-terminally fluorescence-labeled Tat peaks at 10.565 min (UV 280 nm, blue line, black line) and 11.388 min (FLD, Ex: 492 nm/Em: 517 nm, red line), respectively**.**

**Figure S4. Comparative analysis of BirA enzyme-dependent labeling of FAM 56 with a carboxyl group structure and biotin.** Detection of the N-terminally biotin-labeled Tat protein as the control was performed using R-phycoerythrin (PE)-labeled streptavidin alone. Positive control PE-labeled streptavidin with BirA spectrum (FLD, Ex: 488 nm/Em: 532 nm, blue line) from FAM 56-labeled Tat without BirA as negative control (FLD, Ex: 492 nm/Em: 517 nm, red line).

**Figure S5. LC-MS spectrum of the purified fluorescein (FAM 56)-labeled Tat.** HPLC-MS of purified FAM 56-labeled Tat alone (The highest peak corresponding to the retention time (RT) of 17.87 min; 751.49 *m*/*z* detected) was further analyzed by comparison with the HPLC-MS chemical matching database.

**Figure S6. Mass spectrum profile of predigested fluorescein (FAM 56)-labeled Tat protein**. The purified FAM 56-labeled Tat protein (The highest peak corresponding to the retention time of 7.21 min in Figure 4) from HeLa cells was further analyzed by LC-MS and western blot. Identification of Tat protein using LC-MS data relies on the peptide map from a Tat sequence target matching database. The following peptide sequences in the Tat protein match with the spectrum measurement. (a) RAHQNSQTHQASLSK, (b) AHQNSQTHQASLSK, and (c) LEPWKHPGSQPK, respectively.

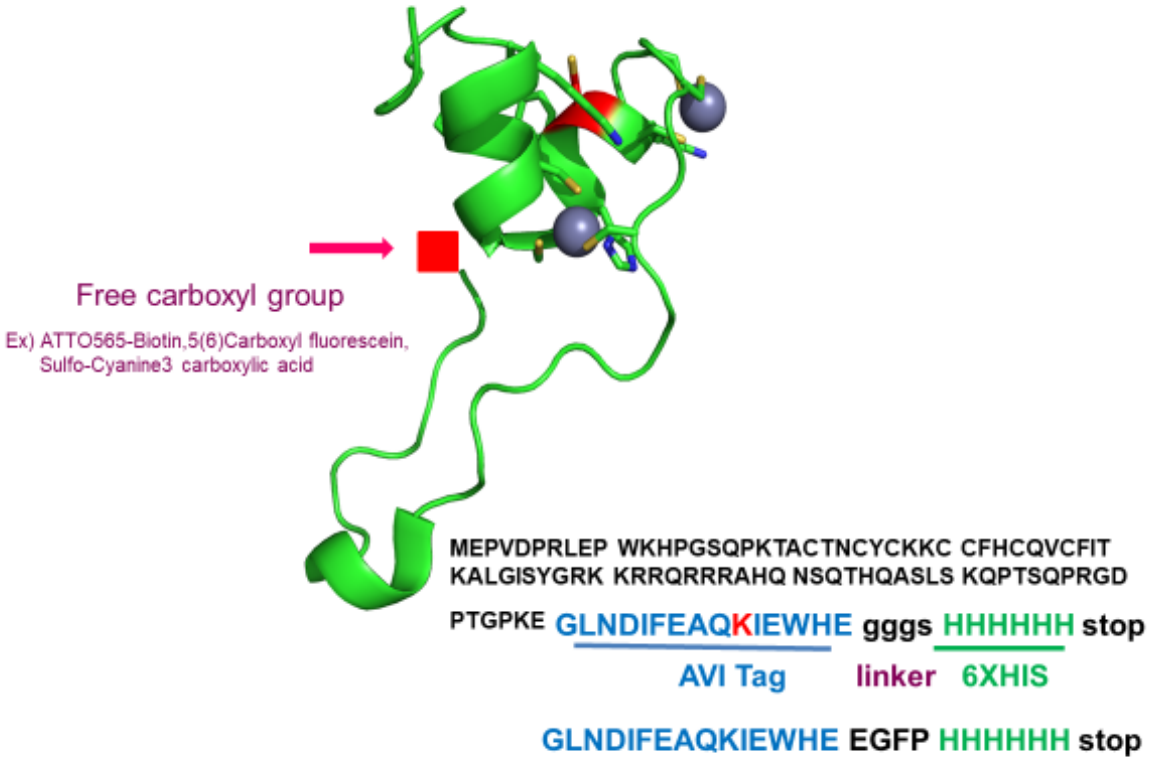
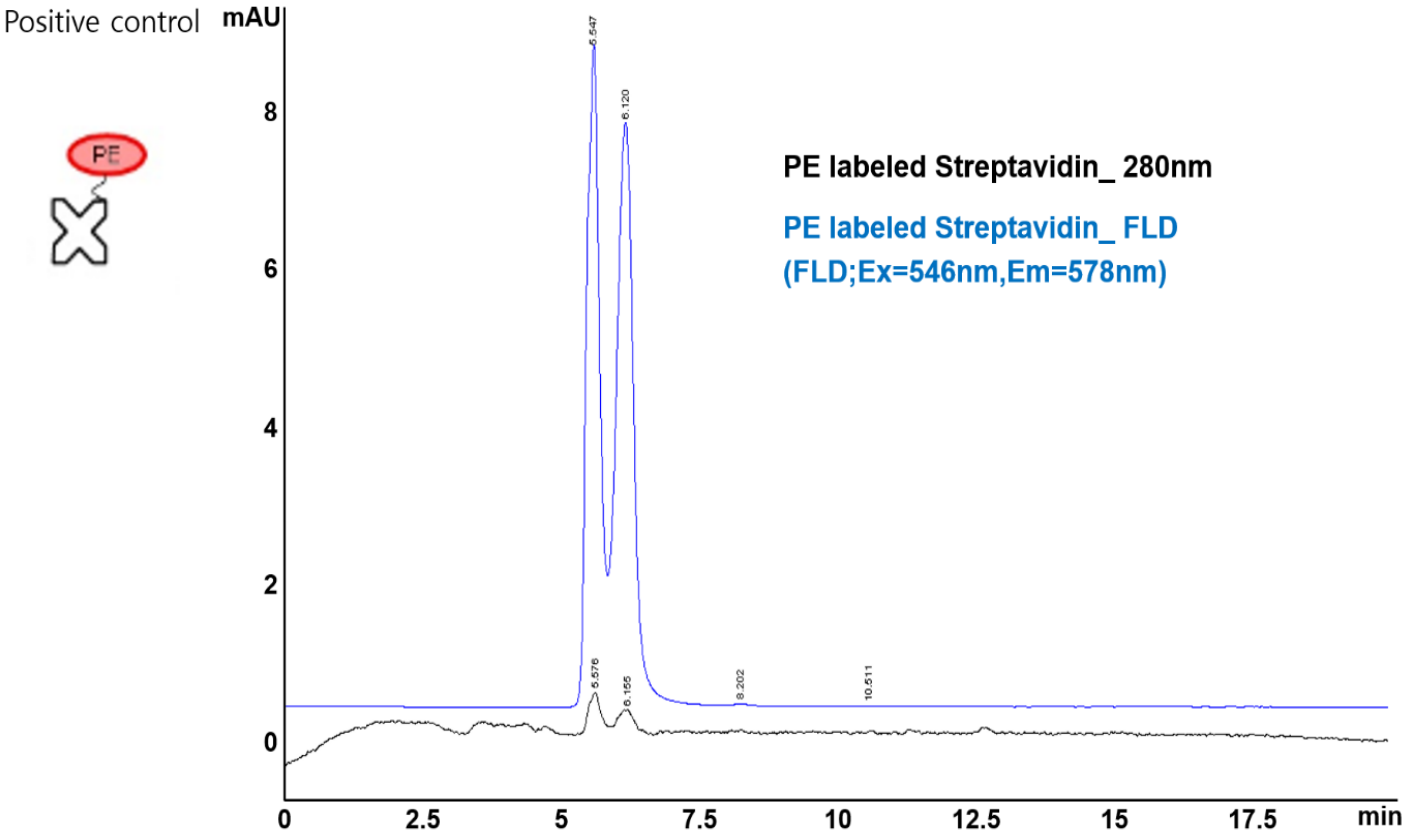



**Figure S1.** The construction of N-terminus-tagged HIV-1 Tat protein. Schematic description of the HIV Tat protein labeled with free carboxyl group fluorescent dyes at the N-terminus. The hexahistidine (6×His)-tag was introduced in the C-terminus for Ni-affinity chromatography and carrying Avi-tag at the 8-terminus for biotin labeling.

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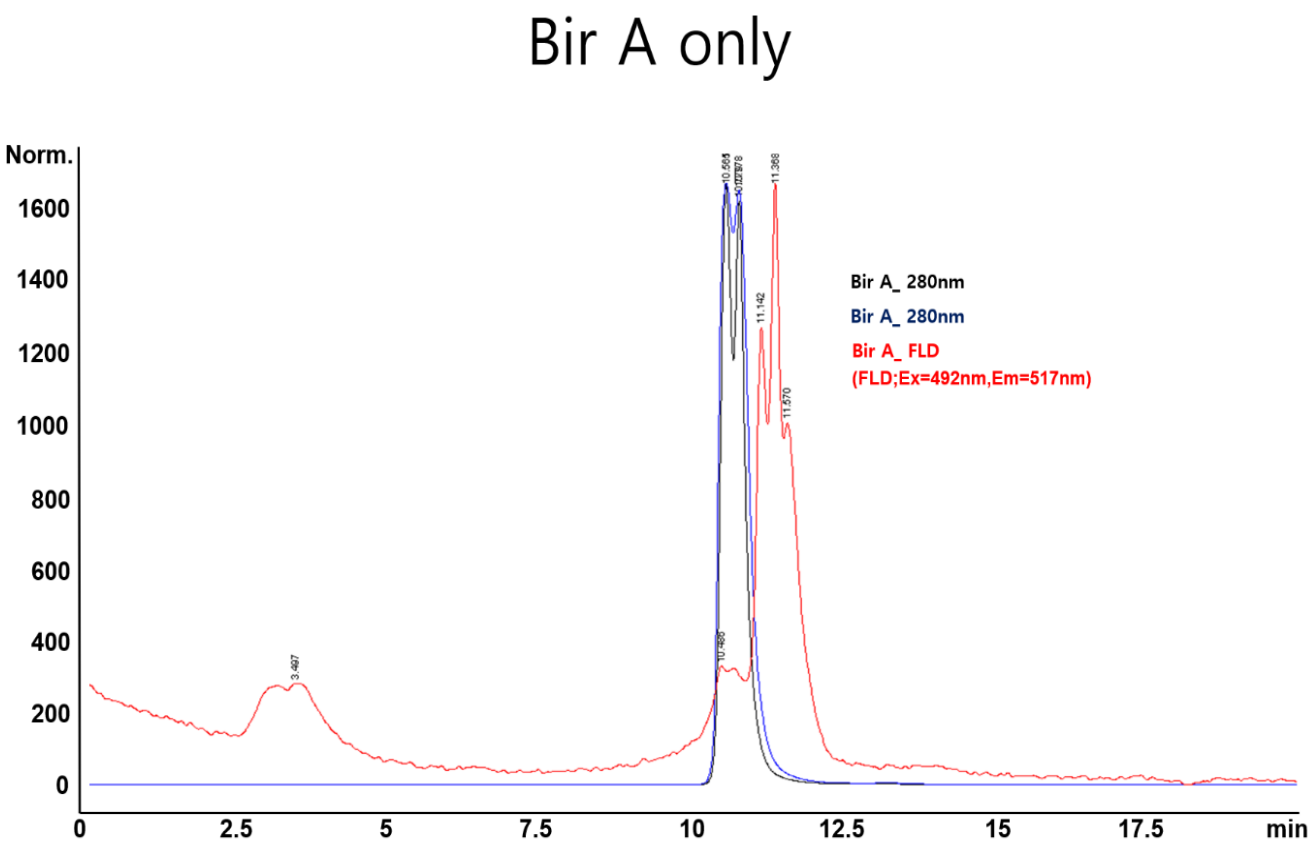


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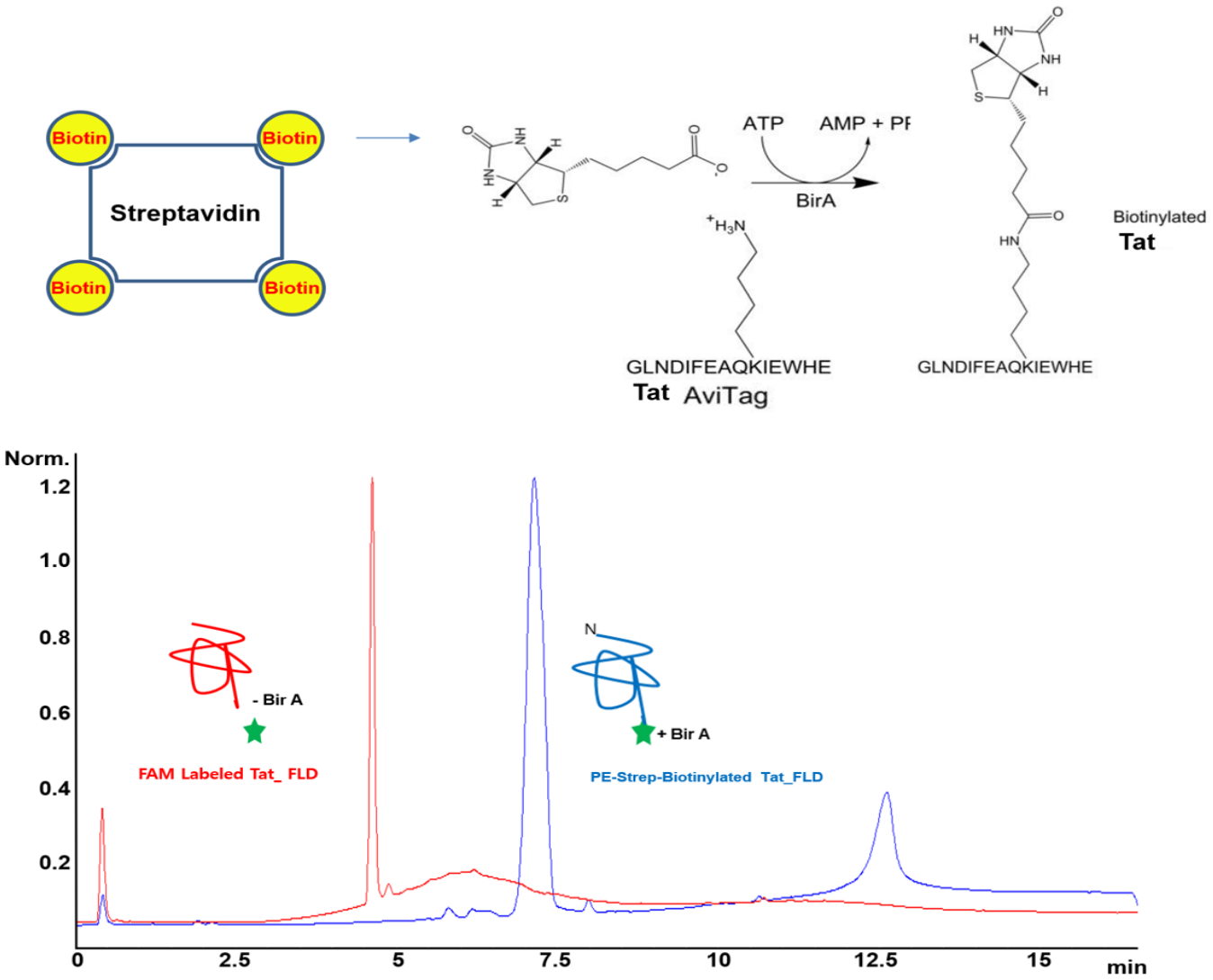


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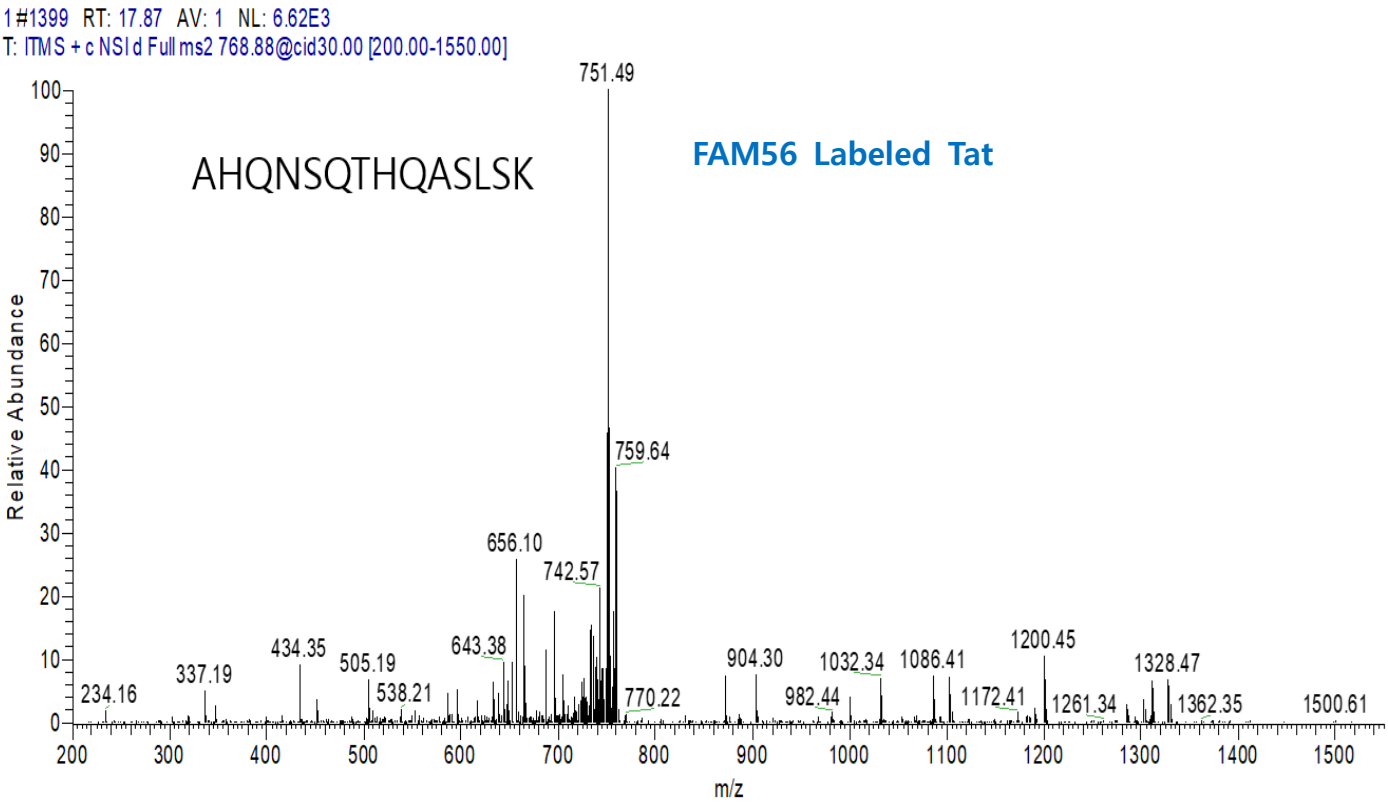


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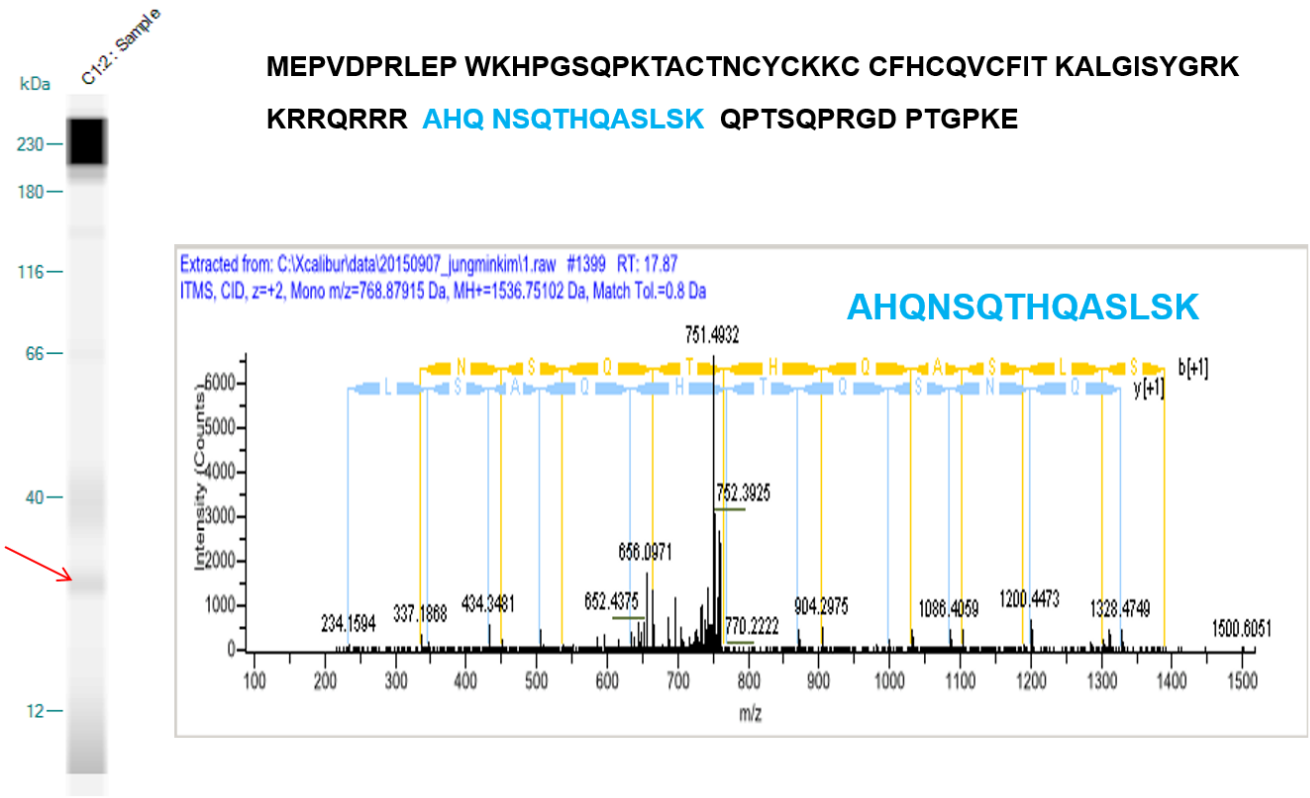
**Sequence of HIV Tat protein.**

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