Dual Surface Modification of the Tobacco Mosaic Virus: Supporting Information

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General Procedures and Materials

Unless otherwise noted, all chemicals and solvents were of analytical grade and used as received from commercial sources. Water (dd-H_2O) used in biological procedures or as reaction solvents was deionized using a NANOpure™ purification system (Barnstead, USA). UV-Vis spectroscopic measurements were conducted in quartz cuvettes using a Tidas-II benchtop spectrophotometer (J & M, Germany). Centrifugations were conducted with the following: 1) a Beckman L7-65 Ultracentrifuge using 70 mL polycarbonate tubes and 45Ti rotor, 2) an Allegra 64R Tabletop Centrifuge (Beckman Coulter, Inc., USA), 3) a Sorvall RC5C refrigerated high-speed centrifuge (Sorvall, USA), or 3) a Microfuge® 18 centrifuge (Beckman Coulter, Inc., USA).

General removal of salts and other small molecules from biological samples was achieved using BioSpin® G-25 centrifuge columns (Amersham Biosciences, USA), NAP-5™ gel filtration columns (Amersham Biosciences, USA), and μ-C18 ZipTip® columns (Millipore, USA). Prior to analysis, all TMV capsid samples were passed through 50 mg of Sephacryl™ S-300 High Resolution resin (Amersham Biosciences, USA) pre-equilibrated with the desired elution buffer and packed in BioSpin® columns using centrifugation (750 rpm, 2 min, 4 °C). Control experiments have indicated that assembled TMV capsids elute in the void volume of these columns, while dissociated monomers and small molecules are retained.

Transmission Electron Microscopy. Transmission Electron Microscopy (TEM) images were obtained at the UC-Berkeley Electron Microscope Lab using a FEI Tecnai 12 transmission electron microscope with 100 kV accelerating voltage. Protein samples were prepared for TEM analysis by submerging carbon-coated copper grids into solutions of analyte at approximately 0.2 mg/mL for 3 min, followed by rinsing with dd-H_2O. The grids were then exposed to a 1% aqueous solution of uranyl acetate for 1 min as a negative stain. After excess stain was removed by blotting, the grid was rinsed again with dd-H_2O and dried *in vacuo* until analysis.

Mass Spectrometry (MS). Protein samples at a concentration of 1-5 mg/mL were separated from small molecules using the S-300 size exclusion columns described above, after which acetic acid was added to a concentration of 66%. The resulting samples were analyzed using an Applied Biosystems LC/ESI-MS, with chromatographic separation using a reversed-phase column. Charge ladder reconstruction was accomplished using the Bayesian algorithms provided with Analyst software (Applied Biosystems). Alternatively, the acetic acid solutions were centrifuged to remove RNA and the supernatant was analyzed by MALDI-TOF mass spectrometry using a Voyager-DE PRO system from Applied Biosystems. The samples were prepared for analysis using the spot overlay method with matrix composed of saturated 2,4,6-trihydroxyacetophenone with 8 mM ammonium citrate in 2:1 MeCN:dd-H_2O.

Peptides obtained from proteolytic digests were purified using Strata C-18ETM reversed-phase columns (Phenomenex, USA). MALDI-TOF analysis was accomplished using the above instrument; samples were crystallized using the spot overlay technique with a saturated sinapinic acid matrix in 1:1 MeCN:dd-H_2O containing 0.1% TFA. Subsequently, MS/MS analyses of purified digest peptides were performed on a Q-TOF MicroTM system (Waters, USA).

High Performance Liquid Chromatography (HPLC). HPLC was performed on an Agilent 1100 Series HPLC System (Agilent Technologies, USA). Protein chromatography was achieved on reversed-phase (C3, C8, or C18 300 Å pore size) columns with a MeCN:H_2O gradient mobile phase containing either 0.1% trifluoroacetic acid or 0.1% formic acid. Analytical size exclusion chromatography was carried out on an Agilent Zorbax® GF-250 column with isocratic (1 mL/min) flow using an aqueous mobile phase (100 mM Na_2HPO_4 with 0.005% NaN_3, pH 7.4). Sample detection and spectral analysis for all HPLC experiments was achieved with inline diode array UV and fluorescence detectors.

PAGE Analyses. For protein analysis, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out on a Mini-Protean apparatus from Bio-Rad (Hercules, CA), following the general protocol of Laemmli.[1] All protein electrophoresis samples were heated in the presence of 1,4-dithiothreitol (DTT) to ensure reduction of any disulfide bonds. Commercially available markers (Bio-Rad) were applied to at least one lane of each gel for calculation of apparent molecular masses. Visualization of protein bands was accomplished by staining with Coomassie® Brilliant Blue R-250 or Silver Stain Solution (Bio-Rad).

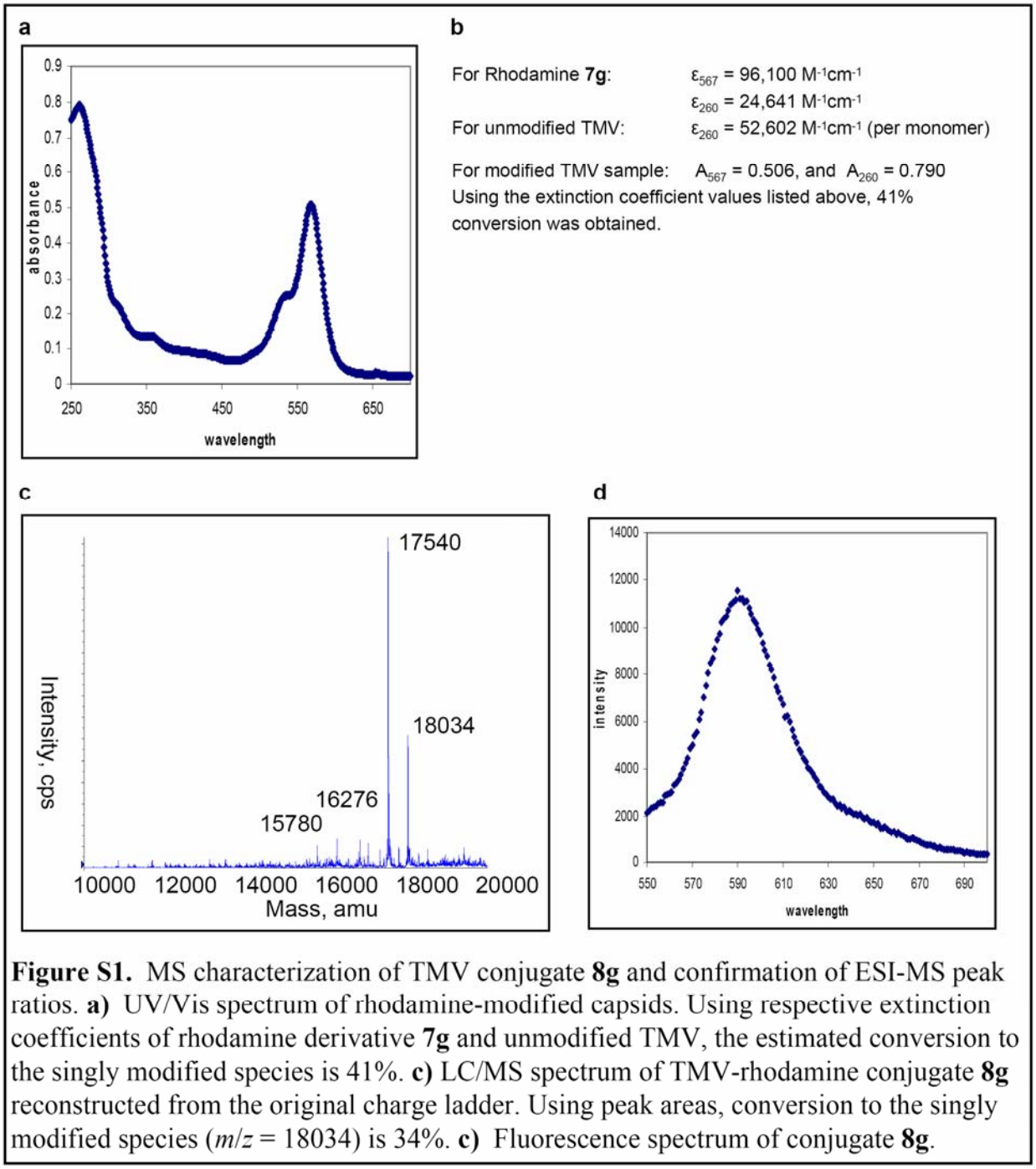

Figure S1. MS characterization of TMV conjugate **8g** and confirmation of ESI-MS peak ratios. **a)** UV/Vis spectrum of rhodamine-modified capsids. Using respective extinction coefficients of rhodamine derivative **7g** and unmodified TMV, the estimated conversion to the singly modified species is 41%. **c)** LC/MS spectrum of TMV-rhodamine conjugate **8g** reconstructed from the original charge ladder. Using peak areas, conversion to the singly modified species (m/z = 18034) is 34%. **c)** Fluorescence spectrum of conjugate **8g**.

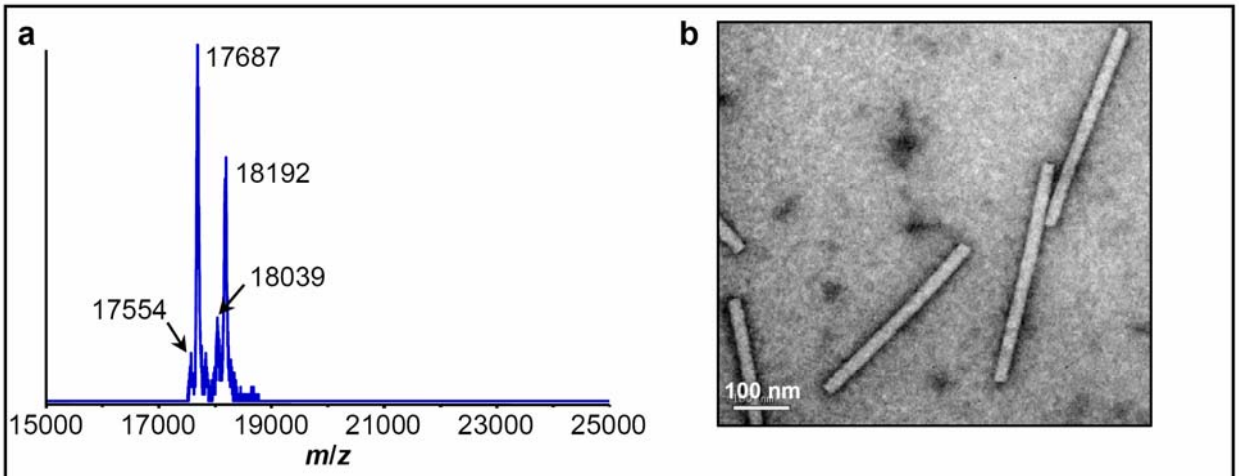

Figure S2. Characterization of TMV modified externally with *p*-aminoacetophenone and internally with **7g**. **(a)** MALDI-TOF MS. Expected masses are: unmodified TMV, m/z = 17536; externally modified, m/z = 17683; internally modified, m/z = 18029; both internal and external modifications, 18176. All mass values agree with the expected values to within 0.1%. **(b)** TEM of dual surface modified TMV rods (negatively stained with $UO_2(OAc)_2$).

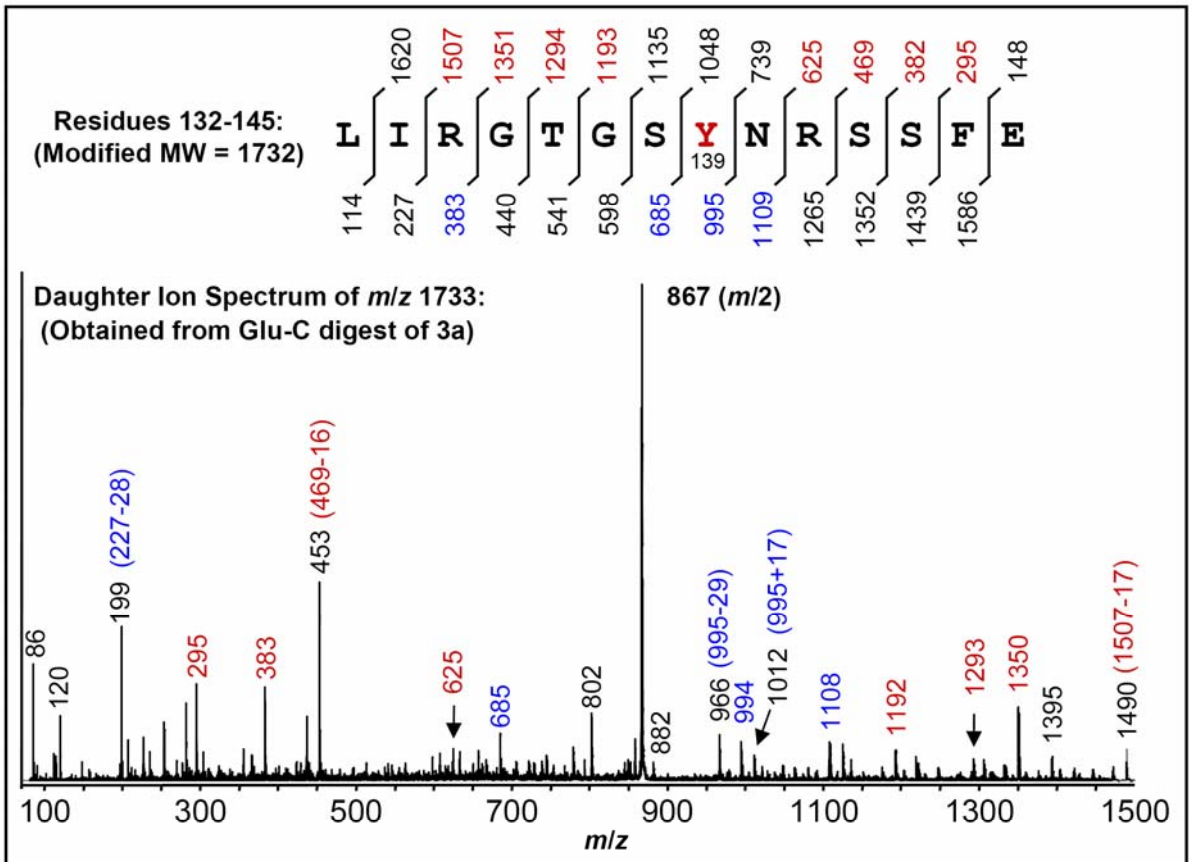

Figure S3. Confirmation of modification site for product **3a**. After digestion with endoproteinase Glu-C, the modified peptide was identified using ESI-MS. The MS/MS spectrum for the isolated modified fragment is shown. Tyrosine 139 was identified as the modification site.

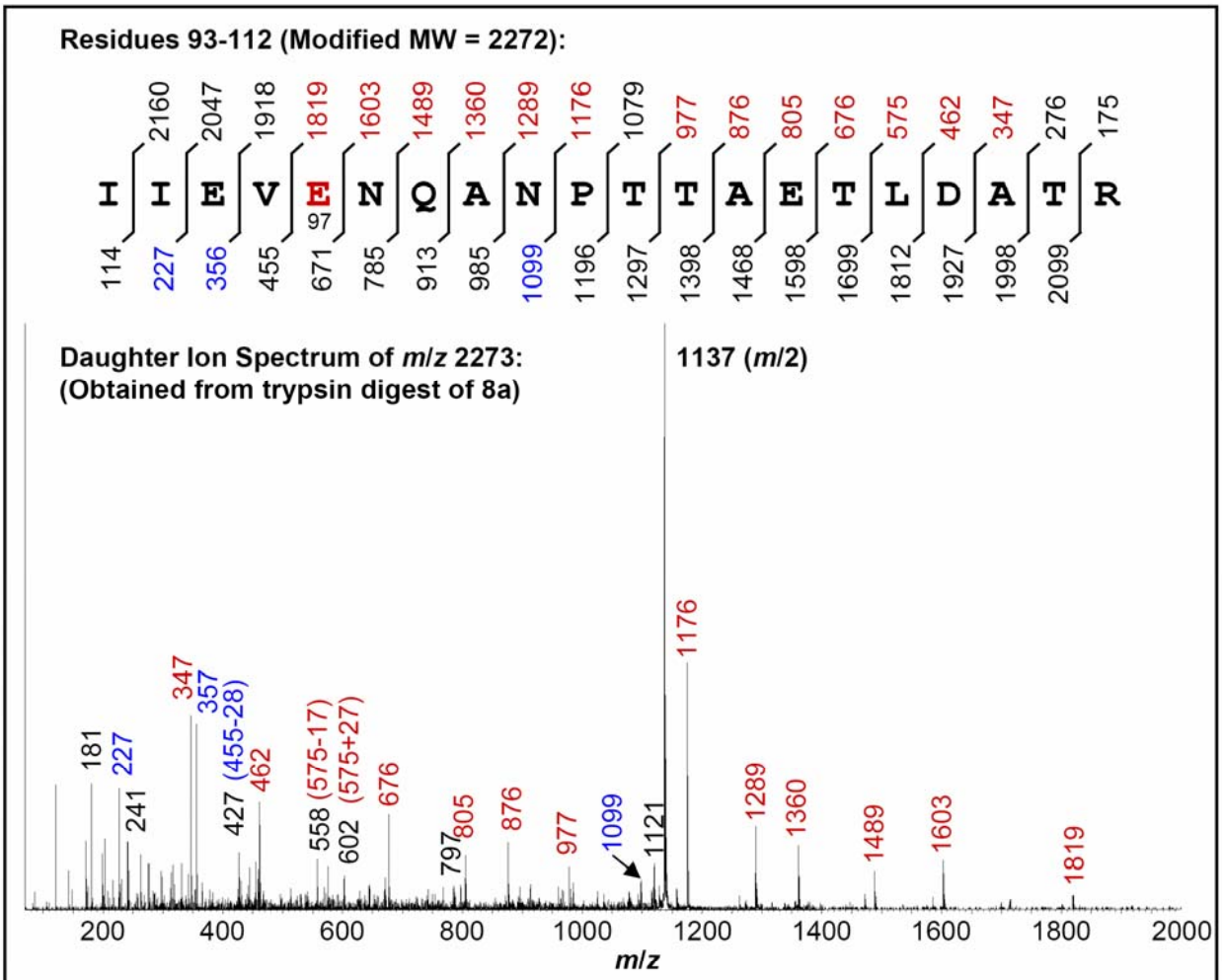

Figure S4. Confirmation of modification site for product **8a**. After digestion with trypsin, the modified peptide was identified using ESI-MS. The MS/MS spectrum for the isolated modified fragment is shown. Glu 97 was identified as the modification site. Similar data were obtained for Glu 106.

[1] Laemmli, U. K. *Nature* **1970**, *227*, 680.

[2] Chapman, S.N. In *Methods in Molecular Biology*, Foster, G.D., Taylor, S.C., Ed.; *Vol. 81: Plant Virology Protocols: From Virus Isolation to Transgenic Resistance*; Humana Press Inc., Totowa, NJ, 1998, pp. 123-129.

[3] Nguyen, T.; Francis, M.B. *Org. Lett.* **2003**, *5*, 3245-3248.