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**Supplemental Information**

**Cationic Oligospermine-Oligonucleotide  
Conjugates Provide Carrier-free Splice  
Switching in Monolayer Cells and Spheroids**

**Marc Nothisen, Phanélie Perche-Létuvée, Jean-Paul Behr, Jean-Serge Remy, and Mitsuharu Kotera**

## Supplemental Information

# Cationic oligospermine-oligonucleotide conjugates provide carrier-free splice switching in monolayer cells and in spheroids

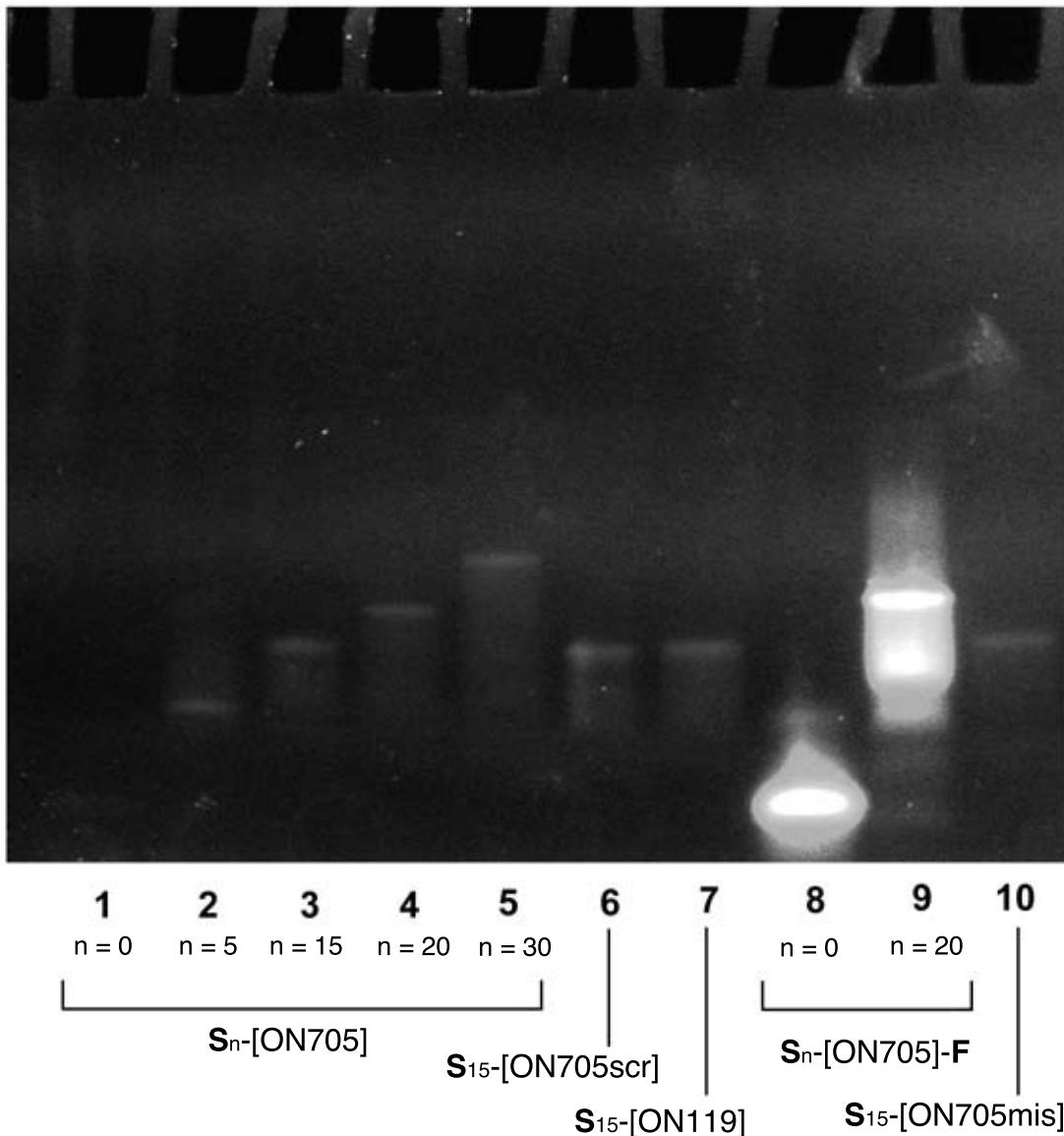
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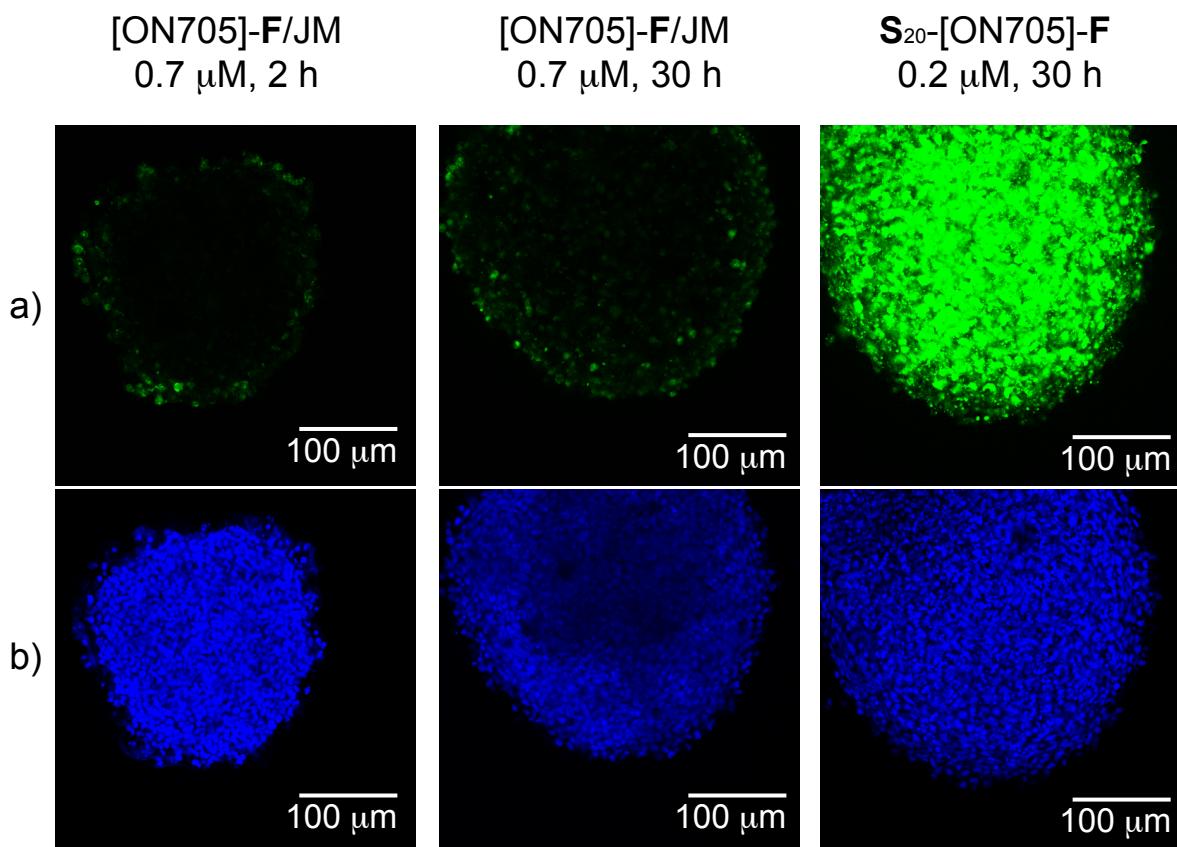
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**Figure S1.** SDS-PAGE of **S<sub>n</sub>**-SSOs. 400 pmoles of single strand oligospermine conjugates were loaded on 4-12% Bis-Tris precast gels (CriterionTM XT, Bio-Rad). Electrophoresis was carried out in XT-MES denaturing running buffer during 2 h at 75 V. Precast gels were then incubated in ethidium bromide (0.5  $\mu$ g/mL) and analyzed. Lane 1, [ON705]. Lane 2-5, **S<sub>n</sub>-[ON705]** ( $n = 5, 15, 20, 30$ ). Lane 6, **S<sub>15</sub>-[ON705scr]**. Lane 7, **S<sub>15</sub>-[ON119]**. Lane 8, [ON705]-F. Lane 9, **S<sub>20</sub>-[ON705]-F**. Lane 10, **S<sub>15</sub>-[ON705mis]**.



**Figure S2.** Delivery of fluorescently labelled SSOs in 3D culture of HeLa pLuc/705 cells analyzed by confocal microscopy. To 2-day old HeLa pLuc/705 spheroids in serum-containing DMEM (10%) was added [ON705]-F complexed with JM (JetMessenger<sup>TM</sup>) or **S<sub>20</sub>-[ON705]-F**. Images were taken after 2 h and 30 h. a) Fluorescein (488 nm), b) Nuclei (405 nm).

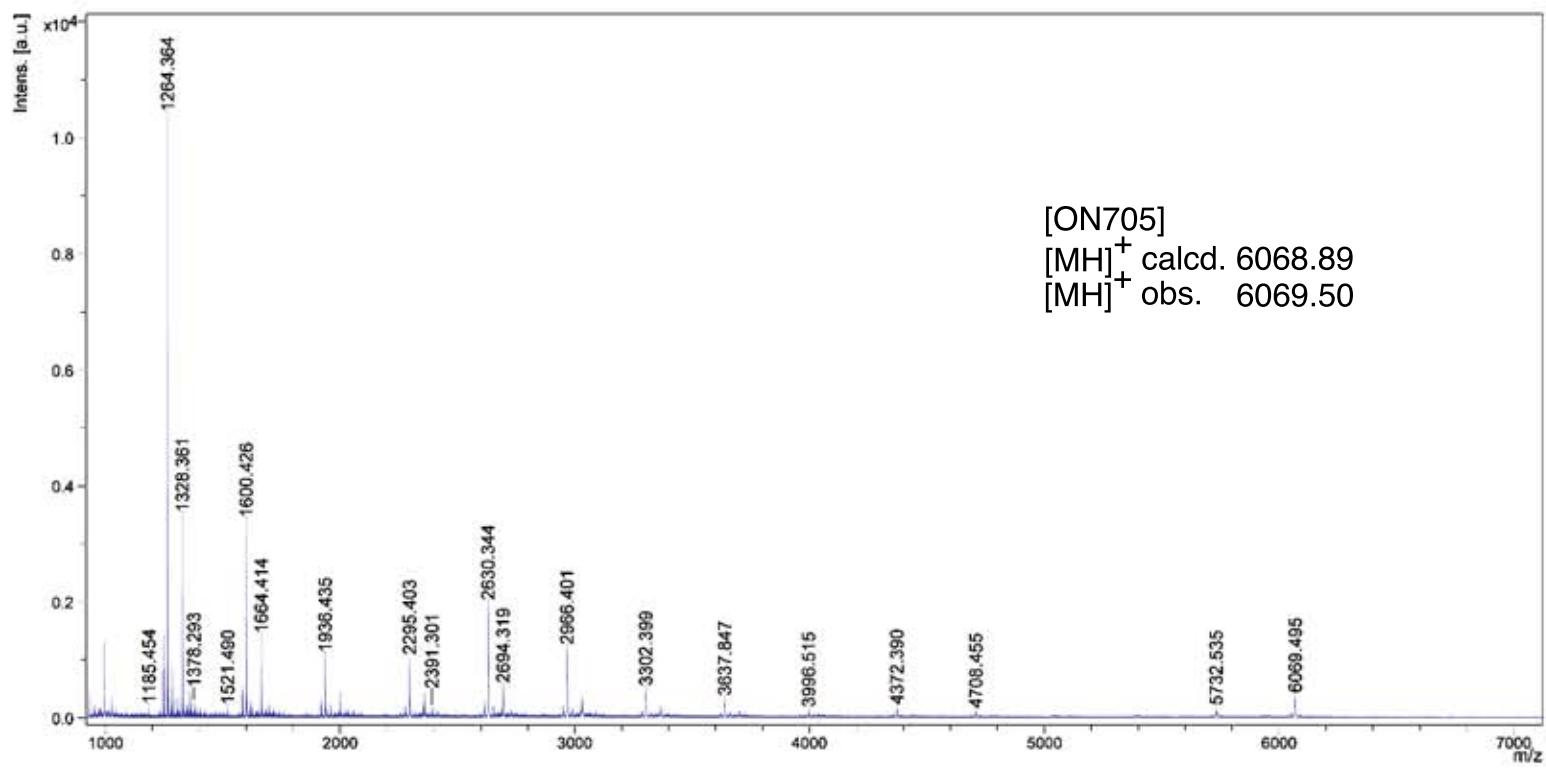


Figure S3. MALDI-TOF spectrum of [ON705]

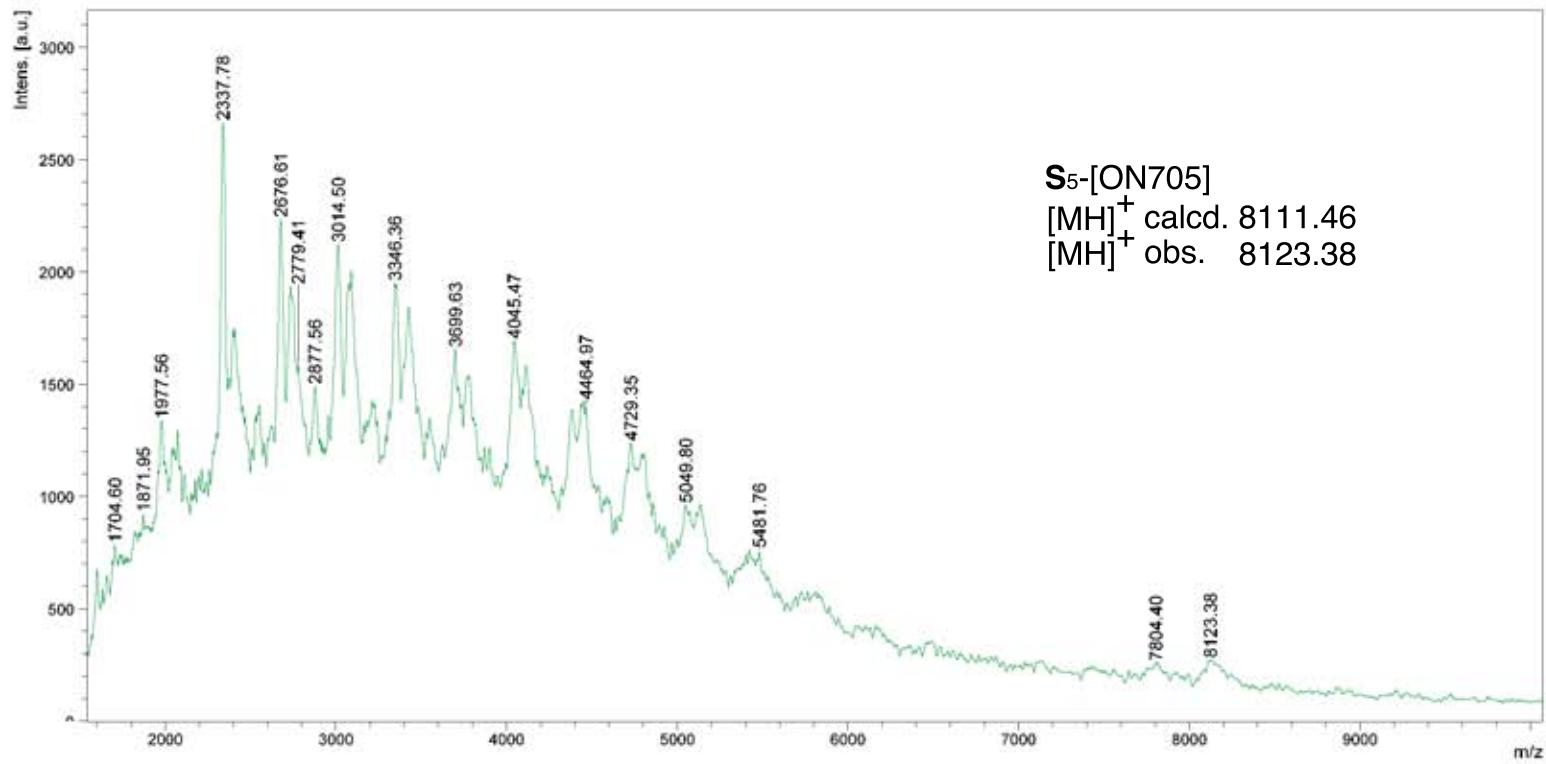


Figure S4. MALDI-TOF spectrum of S5-[ON705]

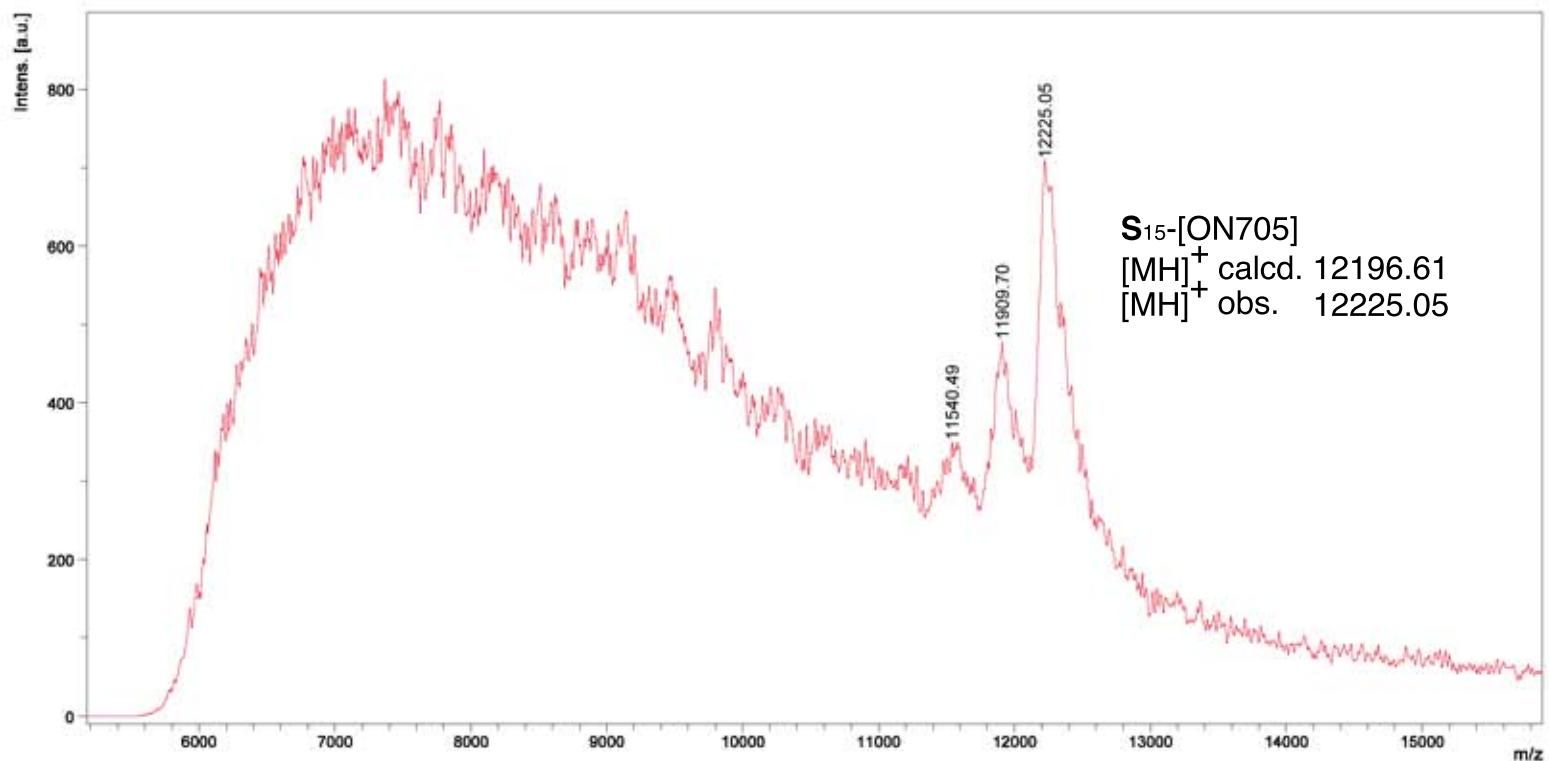


Figure S5. MALDI-TOF spectrum of S15-[ON705]

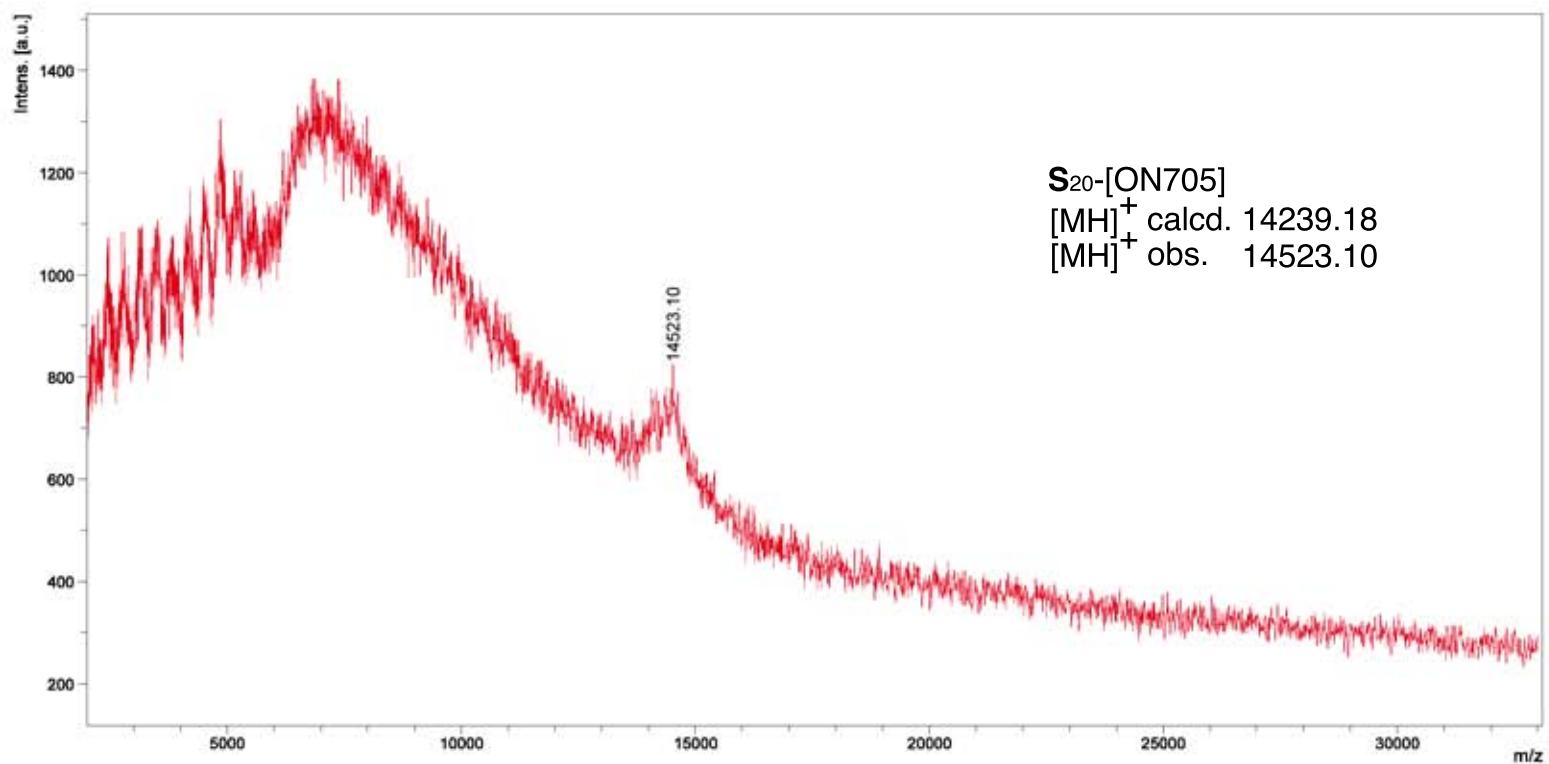


Figure S6. MALDI-TOF spectrum of S20-[ON705]

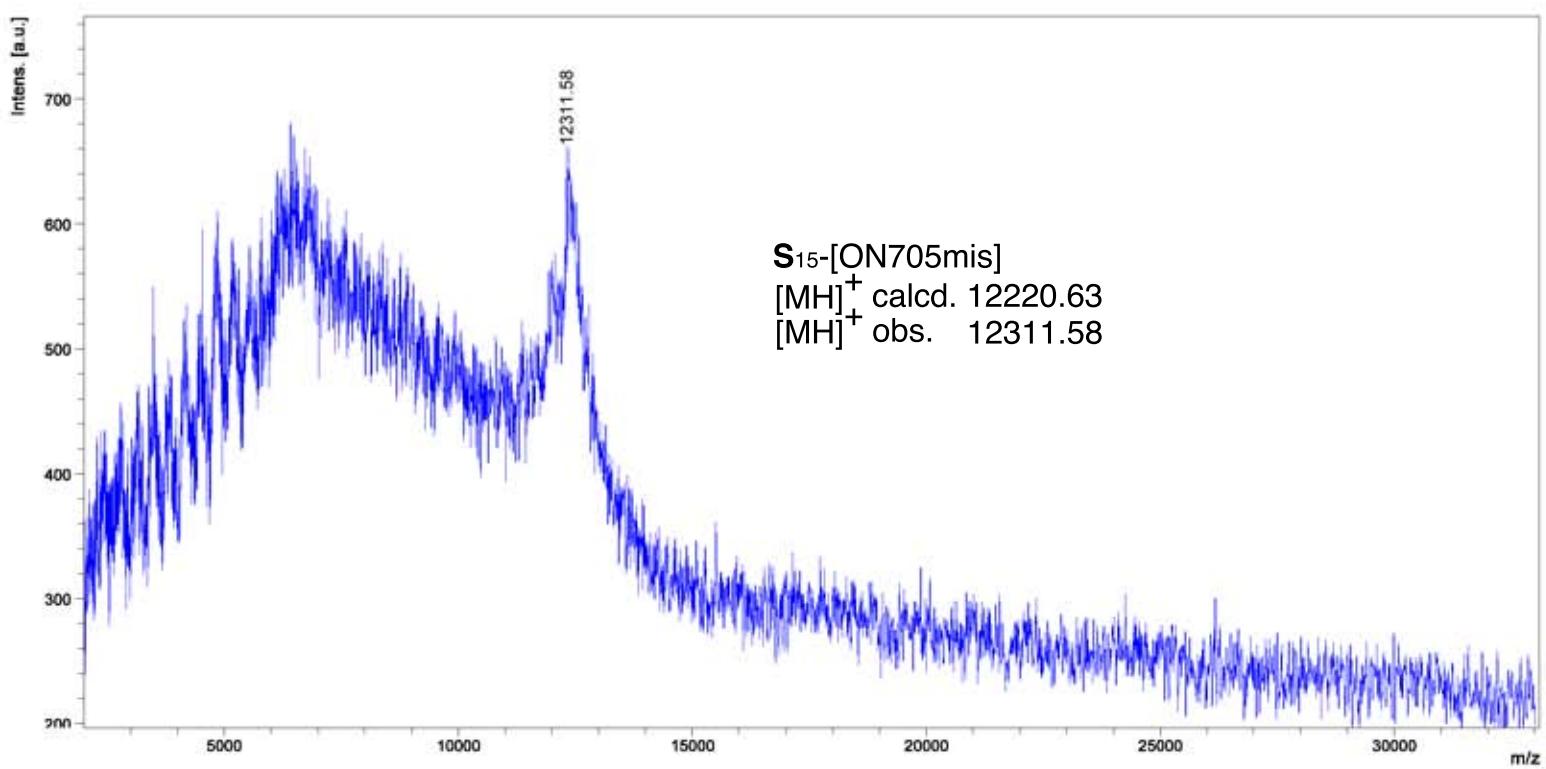


Figure S7. MALDI-TOF spectrum of S20-[ON705mis]

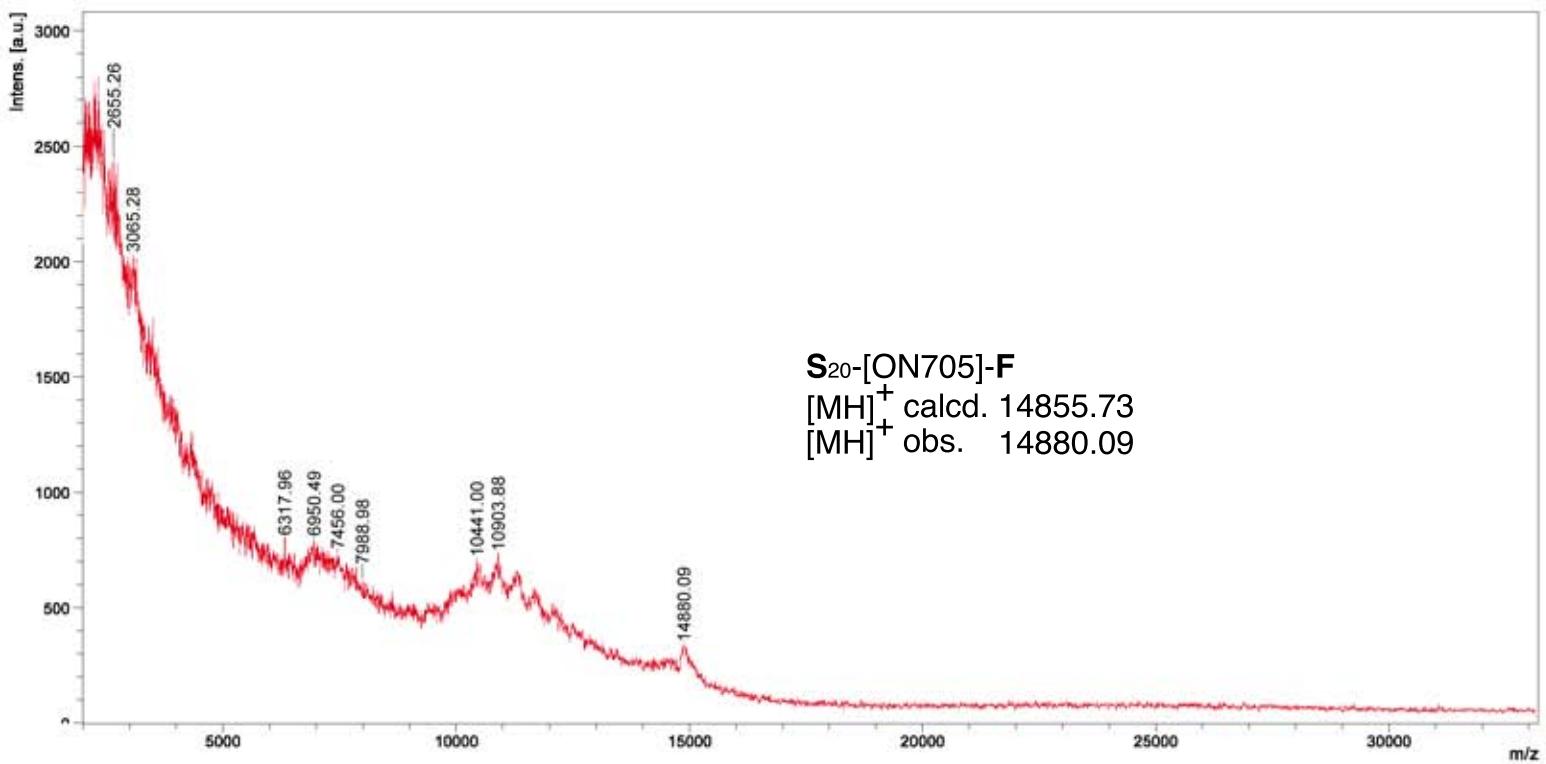


Figure S8. MALDI-TOF spectrum of S20-[ON705]-F

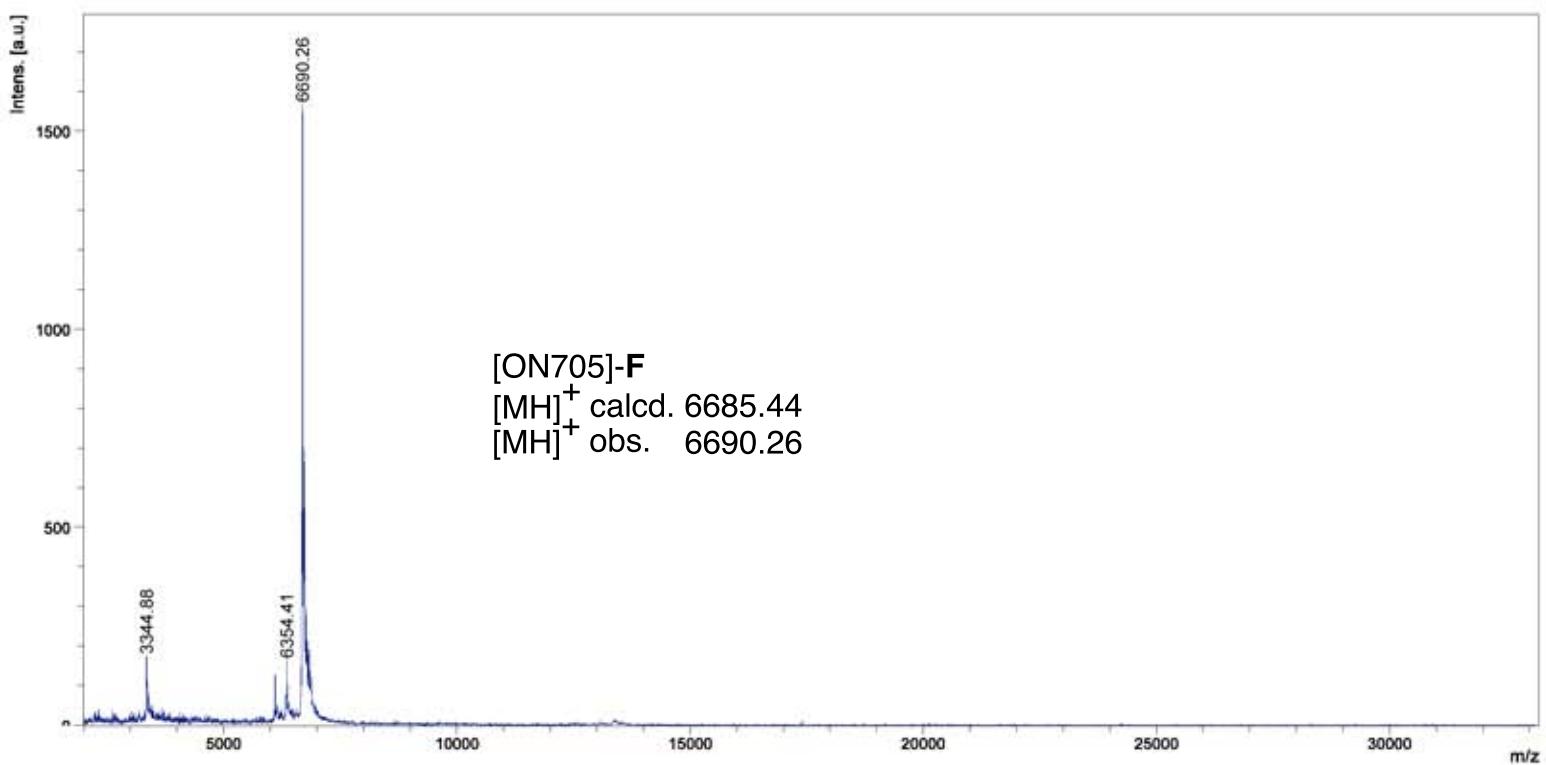


Figure S9. MALDI-TOF spectrum of [ON705]-F

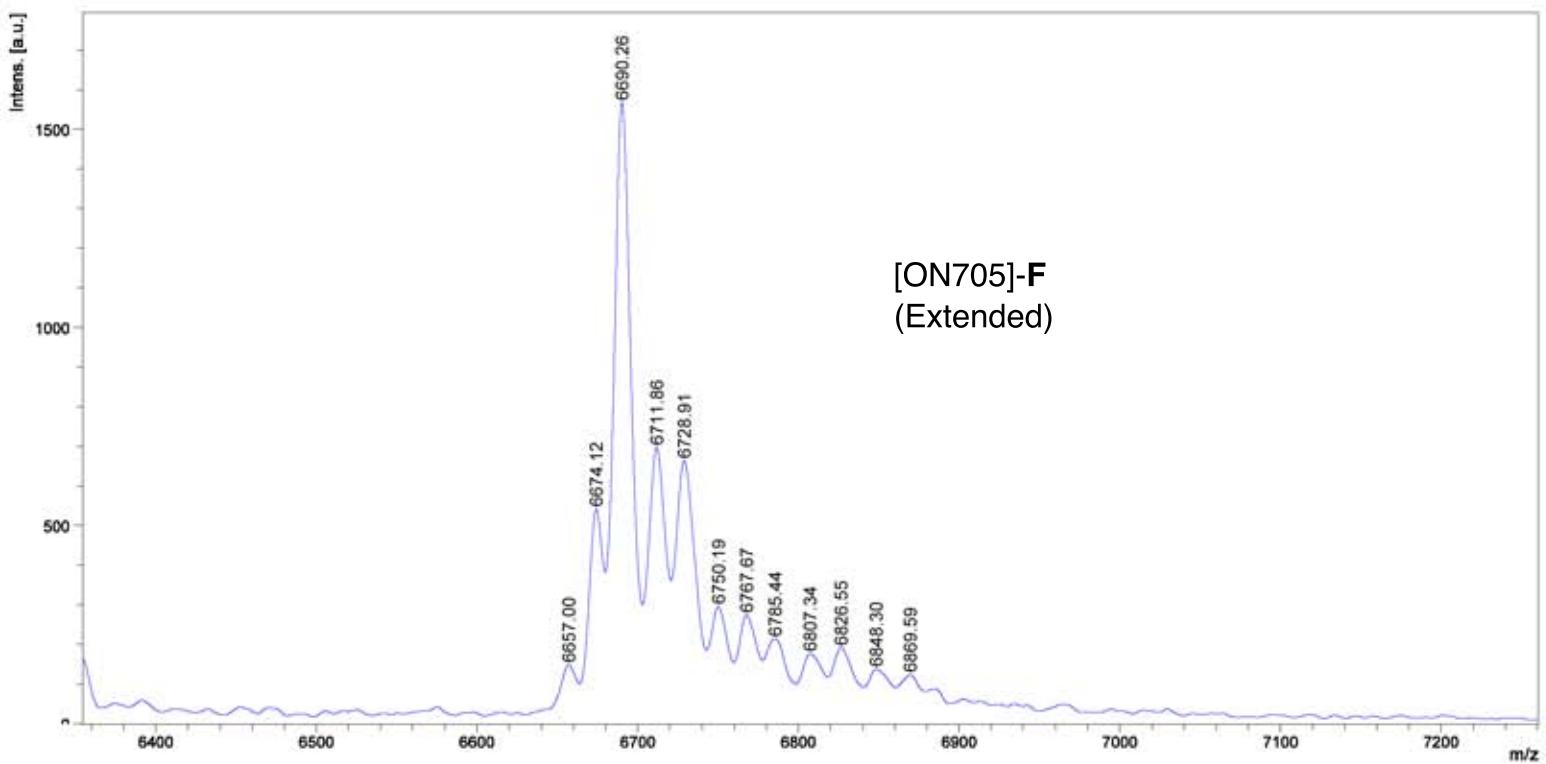


Figure S10. MALDI-TOF spectrum of [ON705]-F (extended)