Supporting information to

Norwalk virus assembly and stability monitored by mass spectrometry

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Table S1. Experimental and theoretical *m/z* values for the charge state distribution corresponding to $VP1_{80}$. To confirm that the stoichiometry was assigned correctly, theoretical *m/z* values for oligomers corresponding to ± 1 VP1 dimer are also included. The difference between the experimental and theoretical *m/z* are given in parentheses.

Experimental *m/z*	$VP1_{80}$ Theoretical	$VP1_{82}$ Theoretical	$VP1_{78}$ Theoretical
26812	26808 (< 0.1%)	26680 (0.5%)	26779 (0.1%)
26972	26970 (< 0.1%)	26836 (0.5%)	26945 (0.1%)
27136	27133 (< 0.1%)	26993 (0.5%)	27112 (< 0.1%)
27302	27299 (< 0.1%)	27153 (0.5%)	27282 (< 0.1%)
27471	27466 (< 0.1%)	27315 (0.4%)	27453 (< 0.1%)
27641	27636 (< 0.1%)	27478 (0.4%)	27627 (< 0.1%)
27813	27807 (< 0.1%)	27644 (0.4%)	27803 (< 0.1%)
27987	27981 (< 0.1%)	27811 (0.4%)	27981 (< 0.1%)
28164	28157 (< 0.1%)	27981 (0.4%)	28162 (< 0.1%)
28342	28335 (< 0.1%)	28153 (0.7%)	28344 -(<0.1%)
28523	28516 (< 0.1%)	28326 (0.7%)	28530 -(<0.1%)
28706	28698 (< 0.1%)	28502 (0.7%)	28717 -(<0.1%)
28892	28884 (< 0.1%)	28681 (0.7%)	28907 -(<0.1%)
29080	29071 (< 0.1%)	28861 (0.8%)	29100 -(<0.1%)
29271	29261 (< 0.1%)	29044 (0.8%)	29295 -(<0.1%)
29465	29454 (< 0.1%)	29229 (0.8%)	29493 -(0.1%)
29659	29649 (< 0.1%)	29416 (0.8%)	29694 -(0.1%)
29859	29846 (< 0.1%)	29606 (0.8%)	29897 -(0.1%)
30060	30047 (< 0.1%)	29798 (0.9%)	30104 -(0.1%)
30263	30250 (< 0.1%)	29993 (0.9%)	30313 -(0.1%)

The corresponding masses assuming the various stoichiometries were calculated for each *m/z* value. The average masses for $VP1_{82}$, $VP1_{80}$, and $VP1_{78}$ were found to be 4.620 ± 0.005 Mda, 4.4780 ± 0.0005 Mda, and 4.364 ± 0.004 Mda, respectively. The lowest standard deviation in mass was assuming a stoichiometry of 80 VP1 monomers, indicating that this is the correct assignment. Due to incomplete desolvation during the ESI process, theoretical *m/z* values must always be less than the experimental *m/z* values, thus a negative deviation indicates an incorrect assignment.

Table S2. Experimental and theoretical *m/z* values for the charge state distribution corresponding to $VP1_{60}$. To confirm that the stoichiometry was assigned correctly, theoretical *m/z* values for oligomers corresponding to ± 1 VP1 dimer are also included. The difference between the experimental and theoretical *m/z* are given in parentheses.

Experimental *m/z*	$VP1_{60}$ Theoretical	$VP1_{62}$ Theoretical	$VP1_{58}$ Theoretical
23126	22998 (0.6 %)	22978 (0.6%)	23020 (0.5%)
23302	23157 (0.6 %)	23131 (0.7%)	23184 (0.5%)
23464	23318 (0.6 %)	23286 (0.8%)	23351 (0.5%)
23622	23481 (0.6 %)	23444 (0.8%)	23520 (0.4%)
23785	23646 (0.6 %)	23603 (0.8%)	23692 (0.4%)
23956	23814 (0.6 %)	23765 (0.8%)	23866 (0.4%)
24127	23984 (0.6 %)	23929 (0.8%)	24043 (0.3%)
24301	24156 (0.6 %)	24095 (0.8%)	24222 (0.3%)
24480	24331 (0.6 %)	24263 (0.9%)	24405 (0.3%)
24659	24509 (0.6 %)	24434 (0.9%)	24589 (0.3%)
24839	24689 (0.6 %)	24608 (0.9%)	24777 (0.2%)
25027	24872 (0.6 %)	24783 (1.0%)	24968 (0.2%)
25214	25058 (0.6 %)	24962 (1.0%)	25161 (0.2%)
25404	25246 (0.6 %)	25142 (1.0%)	25358 (0.2%)
25601	25437 (0.6 %)	25326 (1.1%)	25558 (0.2%)
25795	25632 (0.6 %)	25512 (1.1%)	25760 (0.1%)
25999	25829 (0.7 %)	25701 (1.1%)	25966 (0.1%)
26196	26029 (0.6 %)	25893 (1.2%)	26176 (0.1%)

The corresponding masses assuming the various stoichiometries were calculated for each *m/z* value. The average masses for $VP1_{62}$, $VP1_{60}$, and $VP1_{58}$ were found to be 3.501 ± 0.005 MDa, 3.3783 ± 0.0008 MDa, and 3.255 ± 0.004 MDa, respectively. The lowest standard deviation in mass was assuming a stoichiometry of 60 VP1 monomers, indicating that this is the correct assignment. Due to incomplete desolvation during the ESI process, theoretical *m/z* values must always be less than the experimental *m/z* values, thus a negative deviation indicates an incorrect assignment.

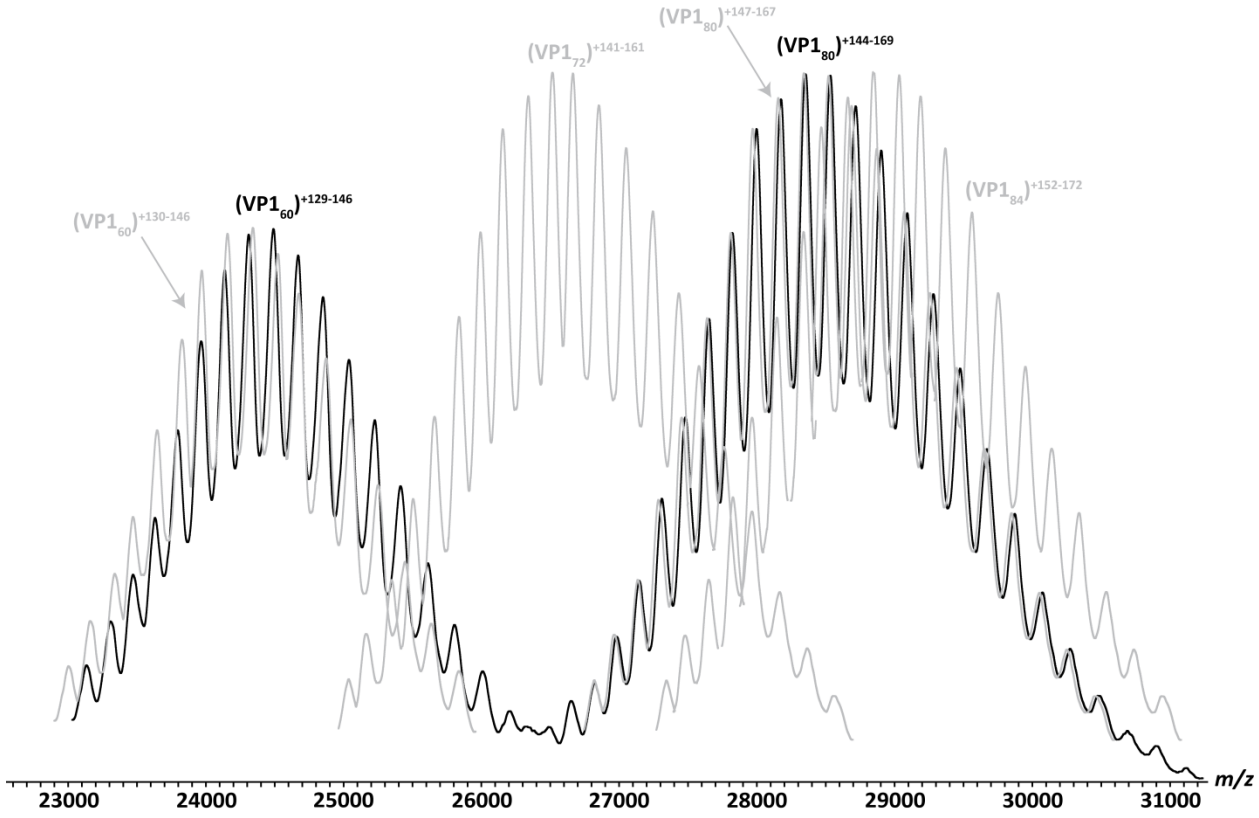

Figure S1. Representative ESI mass spectra at high desolvation energy (200V) of a solution containing 30 µM VP1 in a 250 mM ammonium acetate buffer (pH 9). Overlaid with this spectrum (light grey) are the expected charge state distributions for the $VP1_{60}$, $VP1_{80}$, $VP1_{72}$, and $VP1_{84}$ oligomers.

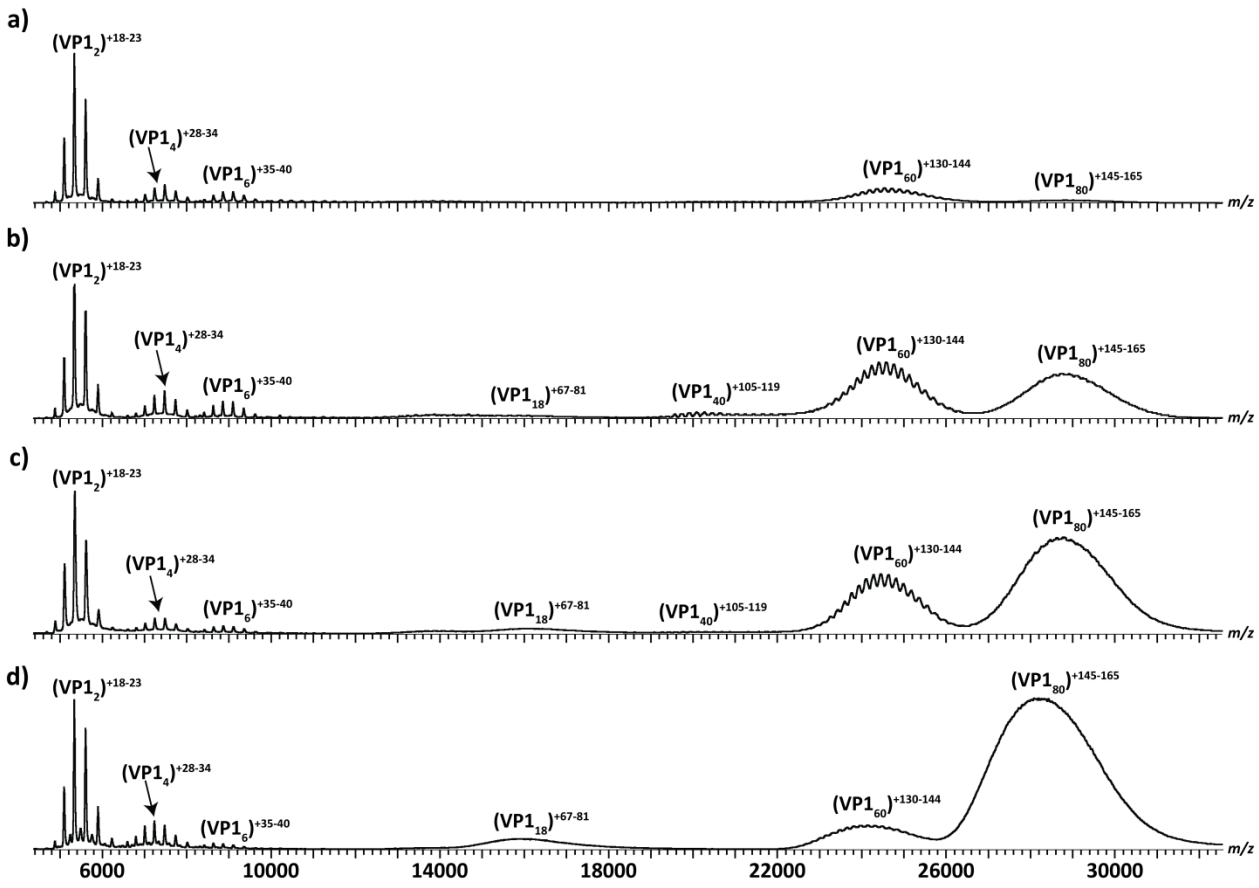

Figure S2. Representative ESI mass spectra demonstrating the effect of protein concentration on the oligomeric state of VP1. Spectra were obtained from aqueous solutions of VP1 in a 250 mM ammonium acetate buffer (pH 9) at concentrations of a) 16 µM, b) 23 µM, c) 40 µM and d) 80 µM.