Supporting information

Virus like particle display of *Vibrio cholerae O*-specific polysaccharide as a potential vaccine against cholera

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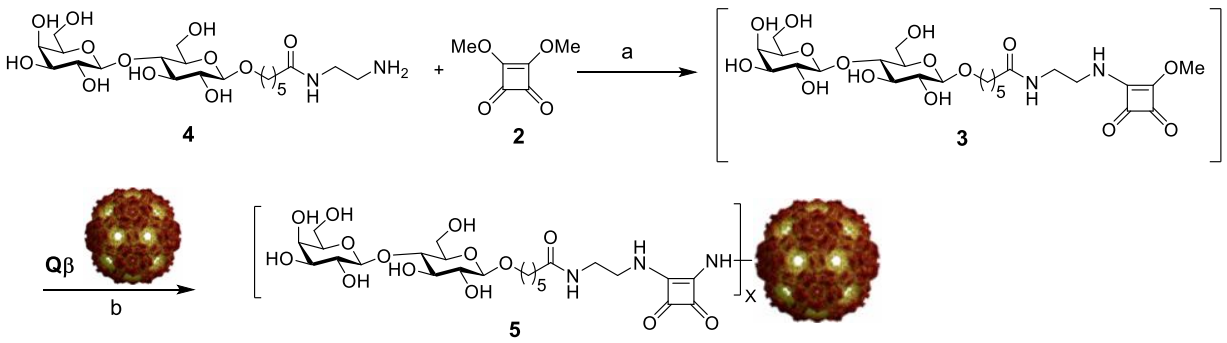

Scheme S1. Qβ conjugation to lactose squarate **3**. a) 0.5M pH 7.0 phosphate buffer, r.t., 3h, 76%; b) 0.5M borate buffer, pH 9.0, r.t., 20 h for x = 810 (14 eq **3** per Qβ monomer); 92 h for x = 1,440 (20 h at 14 eq **3** per Qβ monomer followed by another 72 h with 28 additional eq of **3**).

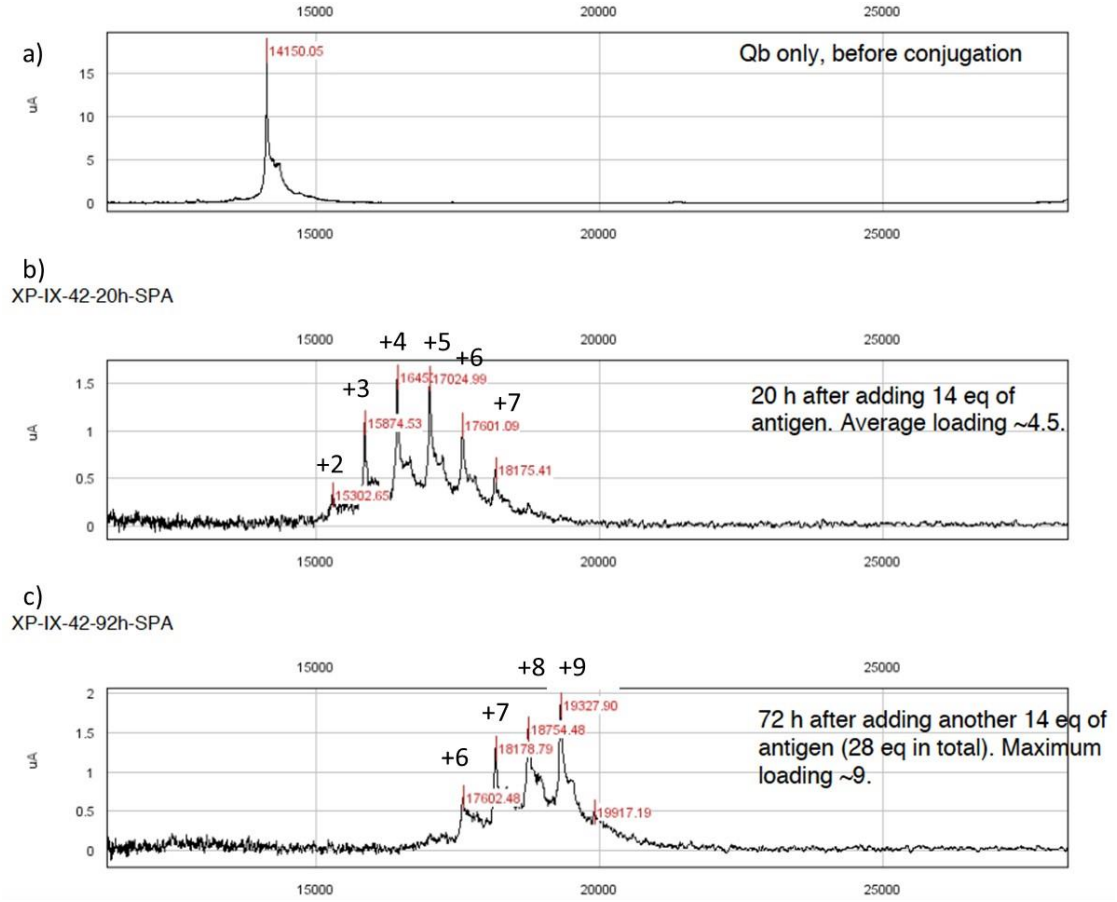

Figure S1. SELDI-TOF MS result of the conjugation of Qβ triple mutant A38K/A40C/D102C and lactose squarate **3**. a) Qβ before conjugation, b) 14 eq of **3** was added to Qβ and the reaction mixture was incubated for 20h, c) An additional 14eq of **3** was added to reaction and incubated for 72h. The mass difference between the peaks corresponds to the addition of a lactose squarate with MW of 577 Da. The average loading was calculated based on the ratio of the sum of respective antigen number of each peak multiplied by their intensity to the total intensity of all peaks.

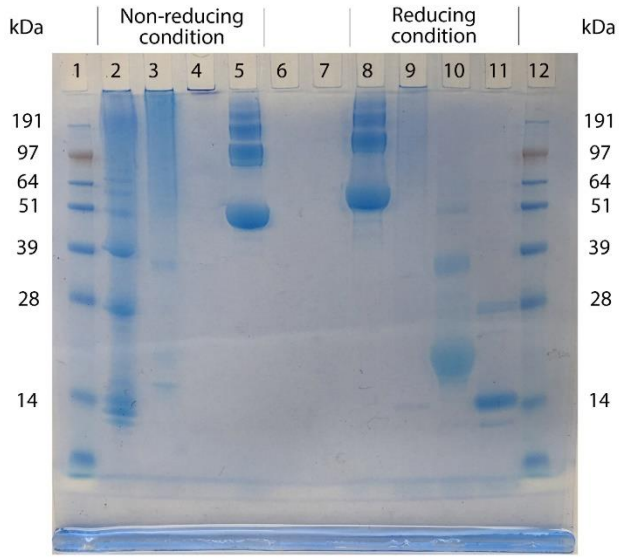

Figure S2. The SDS-PAGE of different samples under non-reducing (Lanes 1-5) and reducing (Lanes 8-12) conditions. Lanes 1, 12: molecular weight ladder; lanes 2, 11: unconjugated Qβ; lanes 3, 10: Qβ-lactose conjugate; lanes 4, 9: Qβ-OSP conjugate; lanes 5, 8: BSA. The Qβ monomer and dimer appeared at 14KDa and 28KDa under the reducing condition. The band corresponding to the Qβ-lactose **5** monomer shifted to about 19 kDa after conjugation, corresponding to the addition of about 8 lactoses per monomer. Qβ-OSP conjugate showed up as a smear at higher MW on the gel.

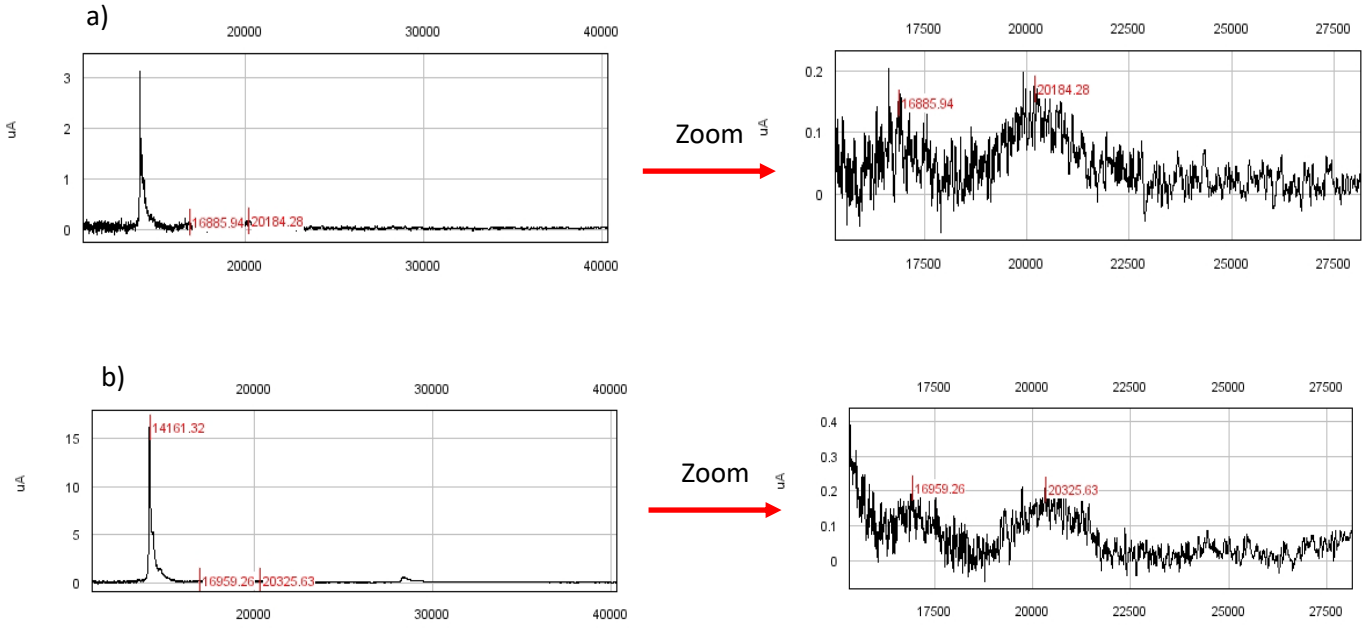

Figure S3. SELDI-TOF MS results of conjugation of a) wild type Qβ or b) mutant Qβ to squarate-OSP. The broad weak peak could be observed at ~20 KDa for Qβ-OSP conjugates, which correlated to conjugate **6** with 1 copy of OSP loaded.

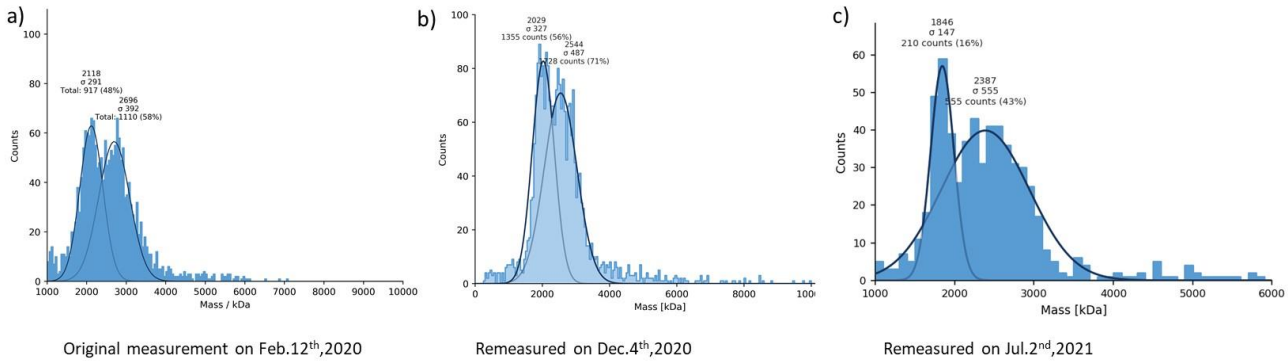

Figure S4. The MP result of Qβ triple mutant A38K/A40C/D102C. The same Qβ sample which was measured several times over time of this study showed a decrease of the MW. The right peak shifted from 2696 KDa in (a) to 2544KDa (b) and 2387KDa in (c).

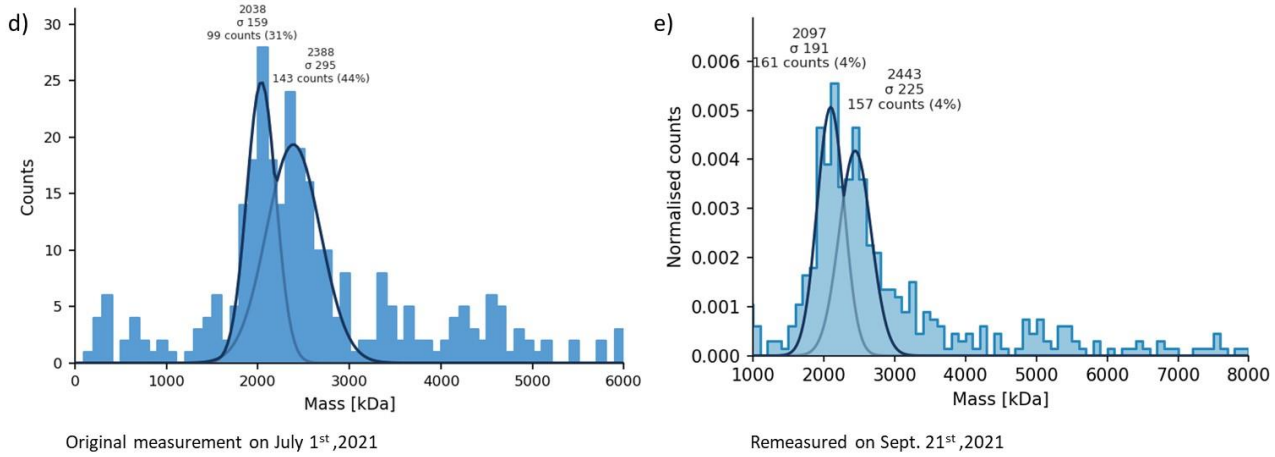

Figure S5. The MP result of Qβ without RNA. The measurement of Qβ sample without RNA was repeated after three months. The MW of right peak was 2388 KDa in (d) and 2443 KDa in (e) respectively.

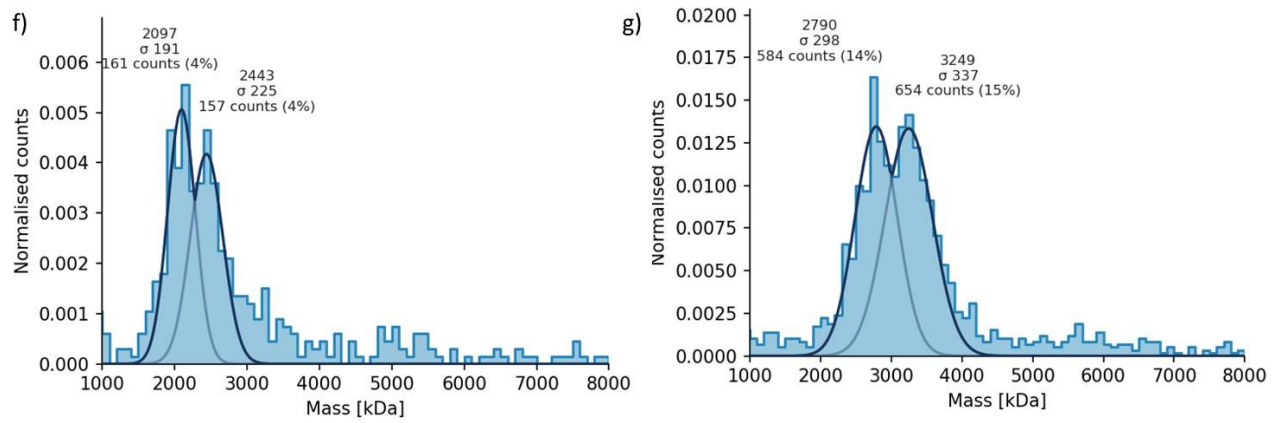

Figure S6. The MP result of wild-type Qβ without RNA f) before and g) after conjugation with lactoside **3**. The right peak shifted from 2,443 KDa to 3,249 KDa, which suggests the conjugation of about 7 lactosides per Qβ monomer on average.

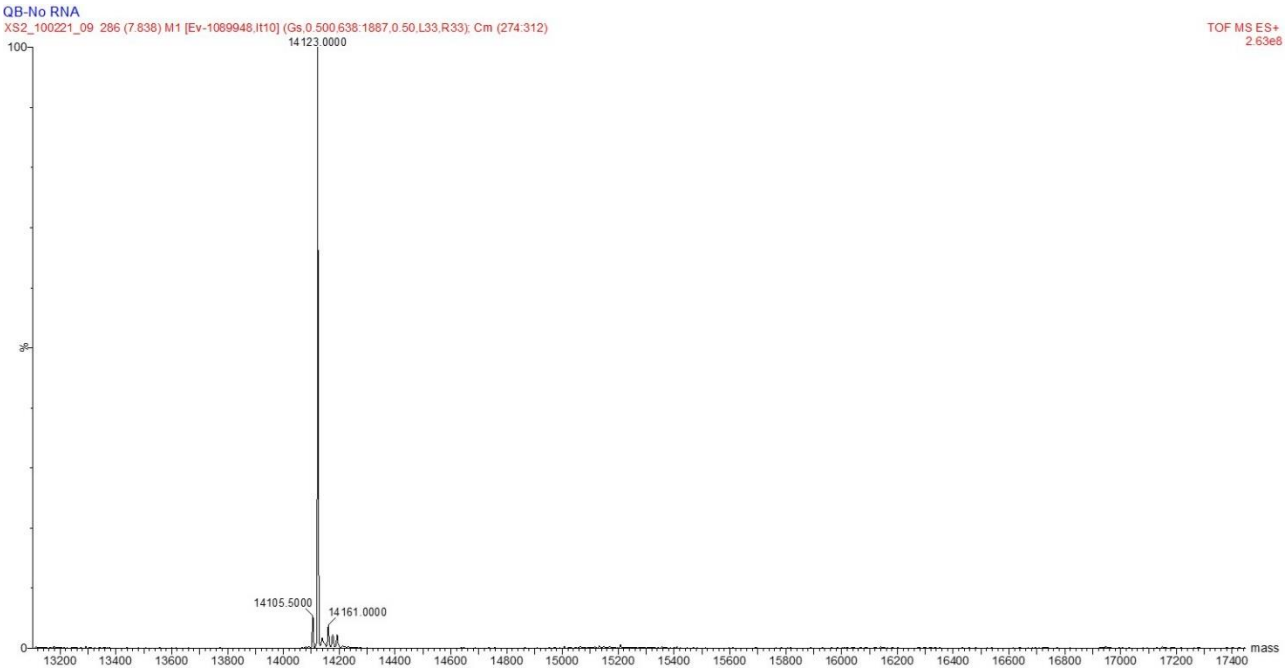

Figure S7. Mass spectrum of wild-type Qβ without RNA.

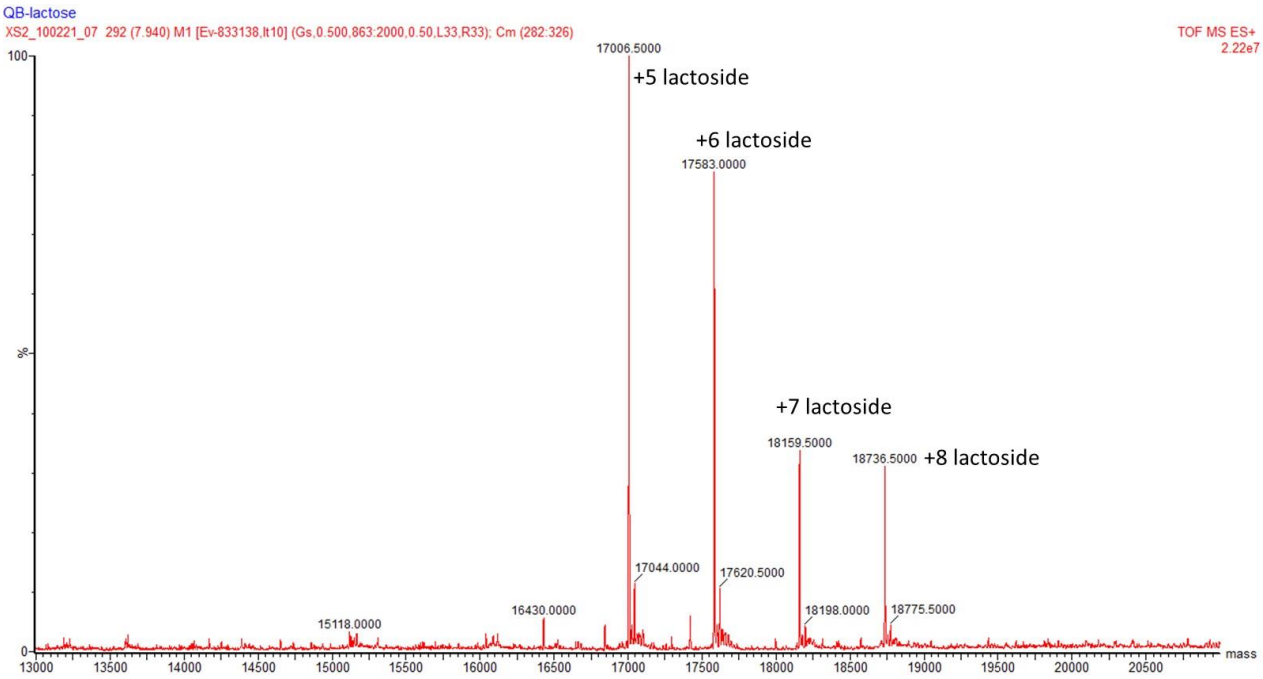

Figure S8. Mass spectrum of wild-type Qβ without RNA after conjugation to lactoside. The average loading of lactoside is 6 per Qβ monomer.

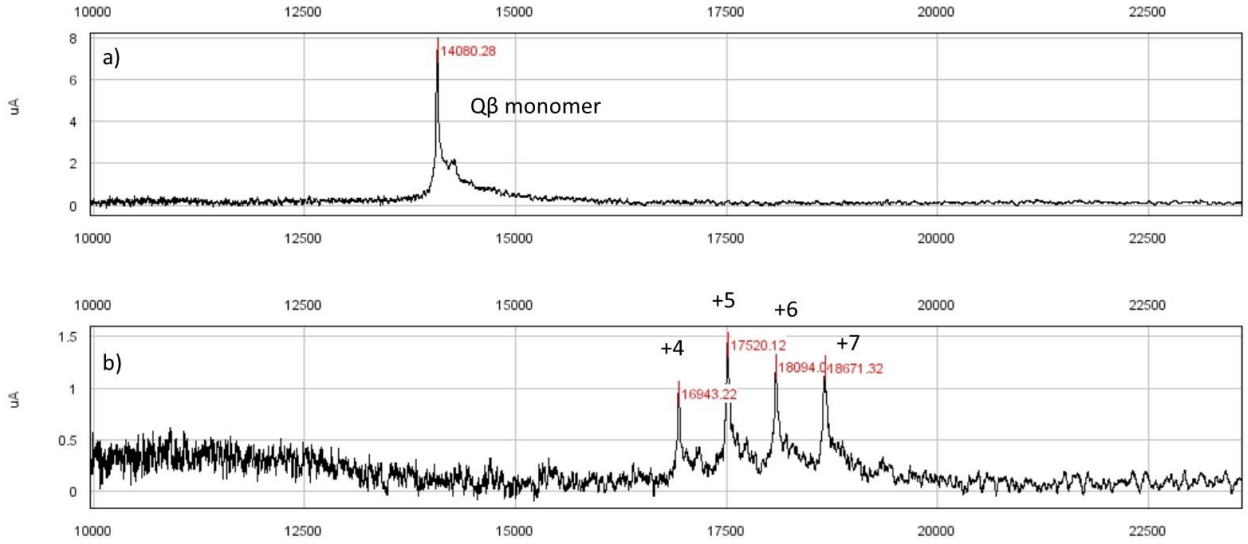

Figure S9. SELDI-TOF MS result of a) wild type Qβ without RNA or b) wild type Qβ without RNA conjugated to compound **3**. The average loading of lactoside is about 6 per Qβ monomer.

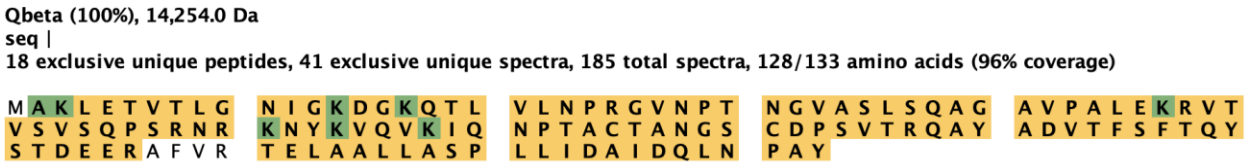

Figure S10. Sequence coverage of wild type Qβ conjugated with lactoside **3**, from MS sequencing following trypsin digestion. AA found in sequencing are highlighted yellow, AA modified with lactoside **3** are highlighted green. Alanine functionalization was on the N-terminus. Sequence coverage was 96%.

Conjugation Site	SUM TIC of Peptides Containing Modified AA	SUM TIC of All Peptides Containing Indicated AA	% Functionalized	Ave. Number of Lactoside 3/Capsid Each AA Contributes
N-Terminus	1211768.95	159180.95	13.1	23.6
K3	1211768.95	551373.41	45.5	81.9
K14	221370.02	179417.34	81.0	145.9
K17	5360909.39	4860382.58	90.7	163.2
K47	442203.76	4234.08	1.0	1.7
K61	19769.33	17206.6	87.0	156.7
K64	57881.63	57881.63	100.0	180.0
K68	137070.3	137070.3	100.0	180.0
			Ave. # of Lactoside **3**/capsid	933.0
			Ave. # of Lactoside **3**/monomer	5.2

Table S1. TIC from sequencing of wild type Qβ conjugated with lactoside **3**, from MS sequencing following trypsin digestion. The functionalization percentage was calculated based on the ratio of TIC of glycan modified peptide over the sum of TIC corresponding to all peptides containing the amino acid residues.