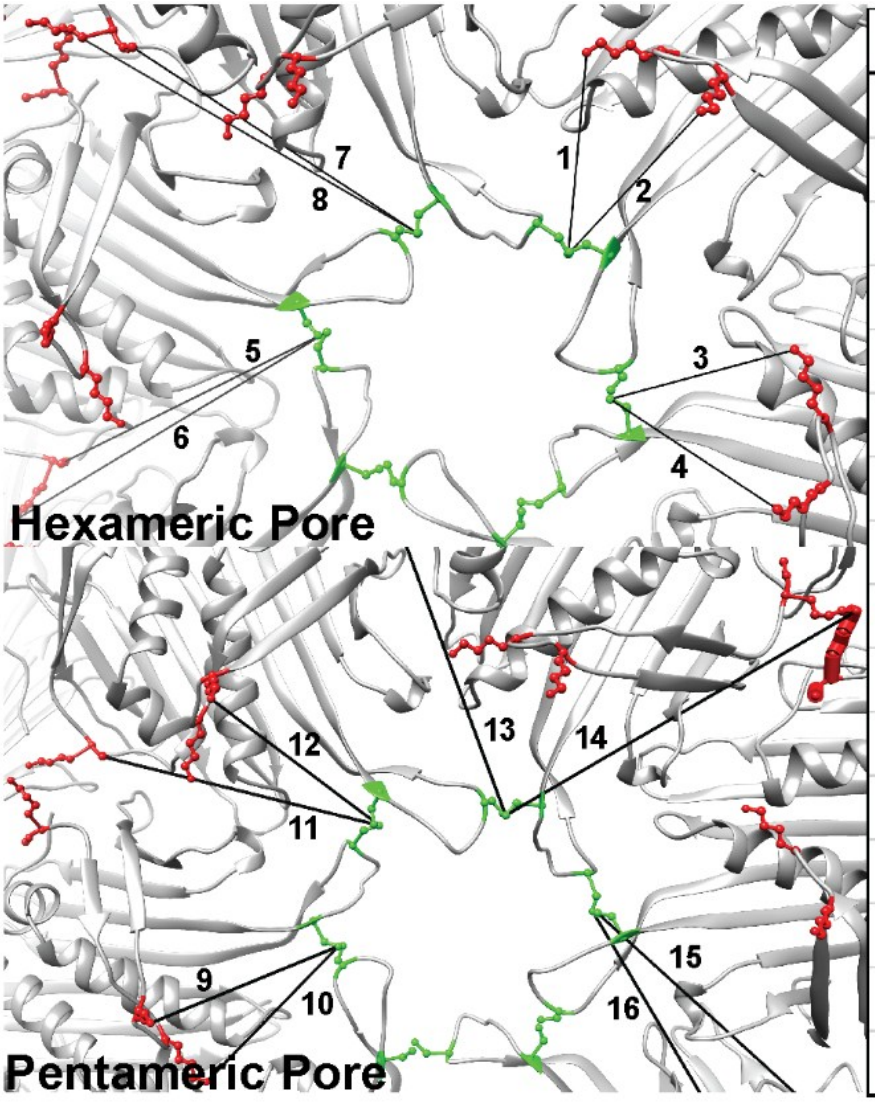
Using FRET to Measure the Time it Takes for a Cell to Destroy a Virus

Candace E. Benjamin,[a] Zhuo Chen, [a] Olivia R. Brohlin, [a] Hamilton Lee, [a] Arezoo Shahrivarkeveshahi, [a] Stefanie Boyd,[c] Duane D. Winkler,[c] and Jeremiah J. Gassensmith. [a,b,*]

Departments of [a] Chemistry and Biochemistry, [b] Bioengineering, and [c]Biological Sciences, University of Texas at Dallas, Richardson, Texas 75080, United States *gassensmith@utdallas.edu

Supplementary Figures



Label	Distance (Å)
1	21.712
2	28.323
3	20.722
4	25.417
5	32.804
6	41.922
7	32.262
8	41.199
9	25.417
10	20.722
11	32.804
12	28.323
13	41.199
14	41.012
15	24.219
16	33.03

Figure S1: Chimera rendered images of the hexameric and pentameric pores. Measurements were taken of the distances between the cysteine sulphurs and the closest amine present on either the N-terminus or the lysines. All measure measurements fall below the 4.5 nm threshold.[1]

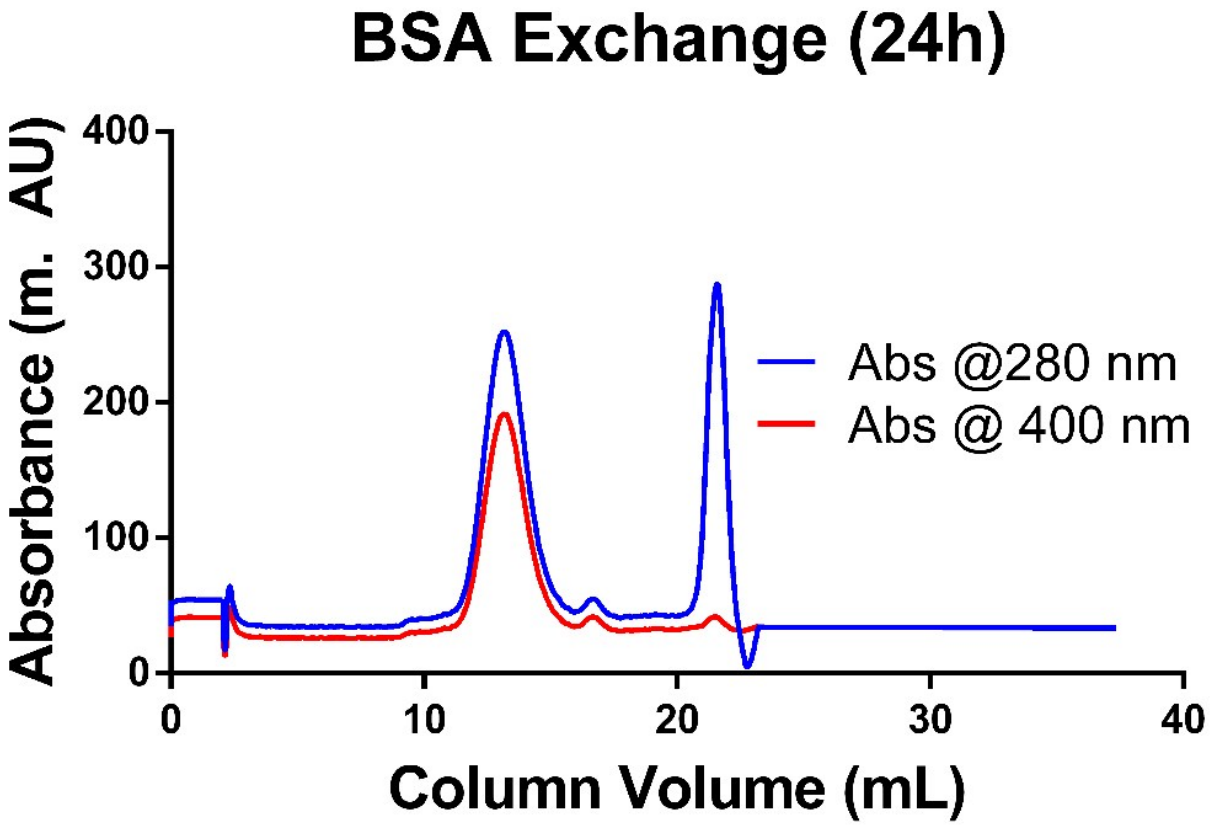


Figure S2: FPLC SEC trace of equal amounts of BSA and Qβ-FITC were incubated for 24 hours and analysed at 280 nm for proteins and 400 nm for the DB moiety. Due to the ring opening step in a pH 8.5 buffer, the DB group cannot transfer from the Qβ (~12 mL elution) to BSA (~21 mL elution).

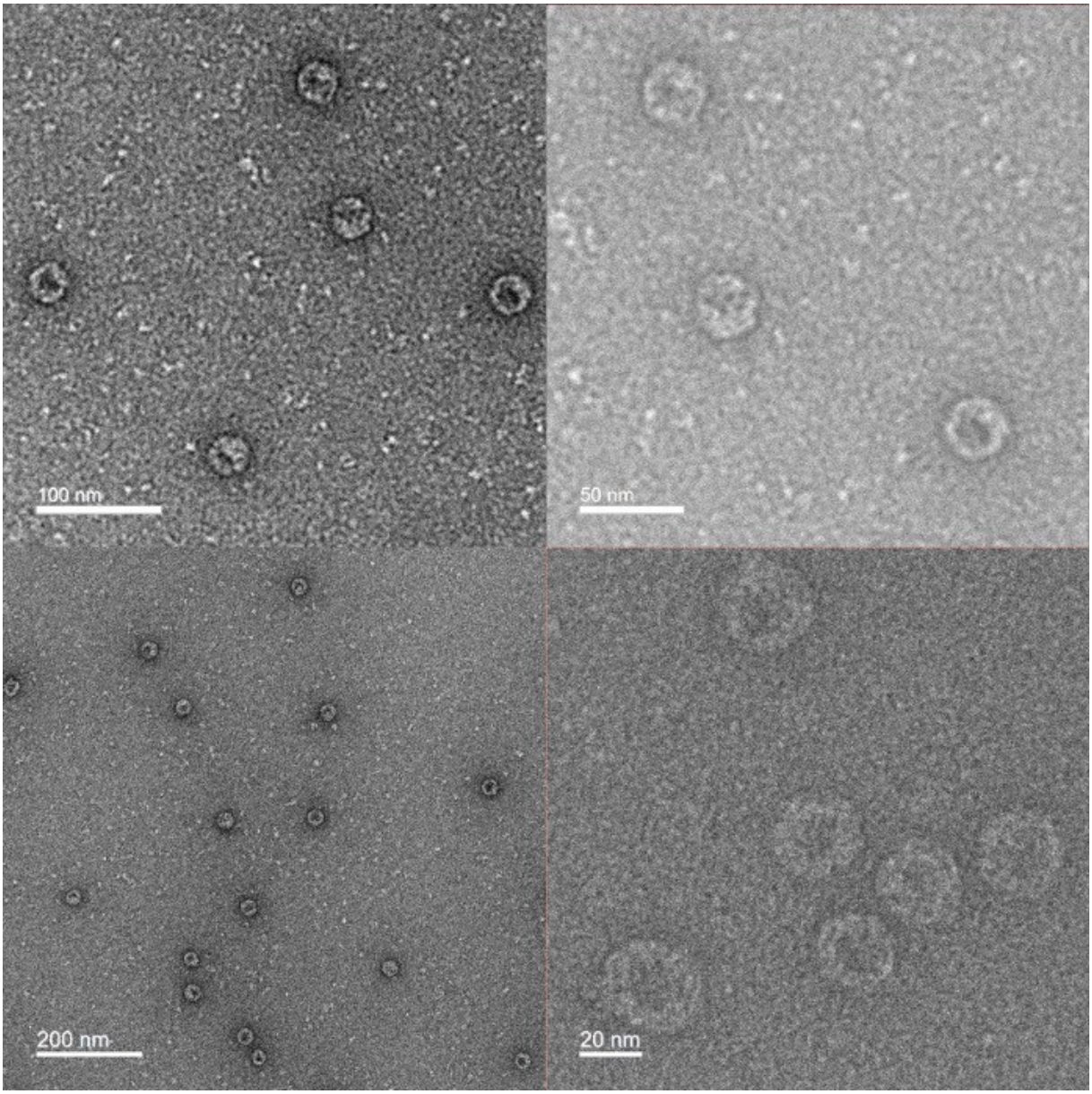


Figure S3: TEM images depicting intact VLPs after complete conjugation – no change in the VLP morphology is noted.

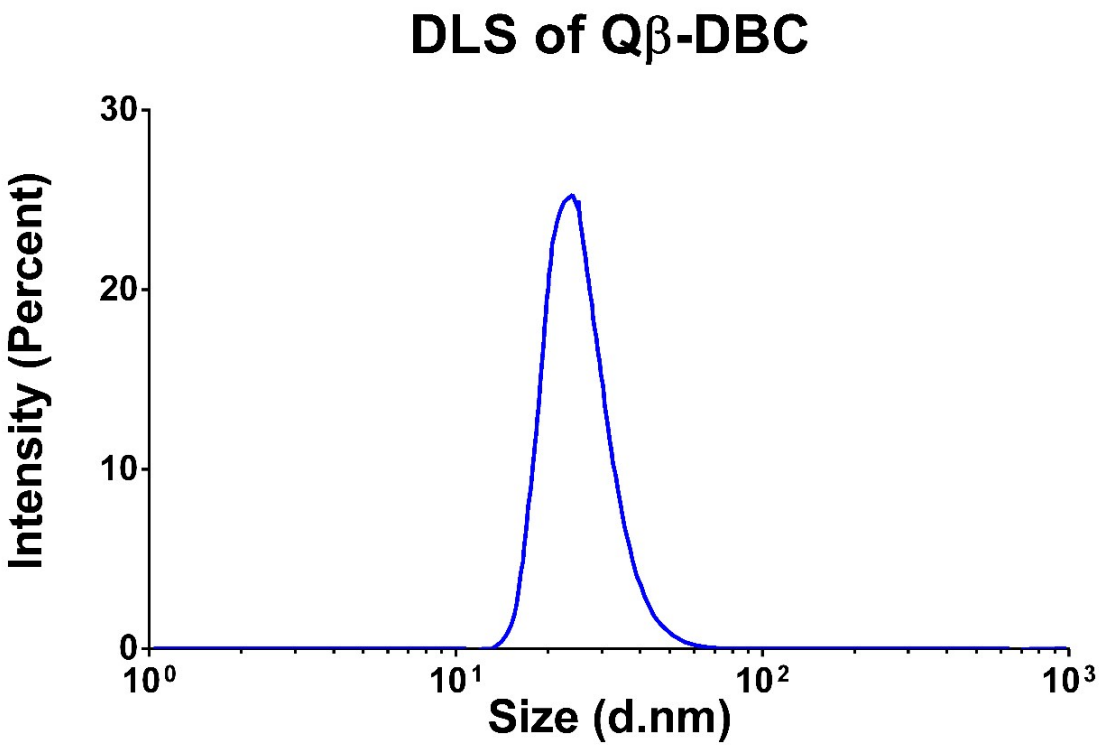


Figure S4: DLS of Qβ-DBC in 0.1 M KP Buffer pH 7 indicating a size of 32.1 ± 1.54 nm – slightly larger than the original 28 nm capsid.

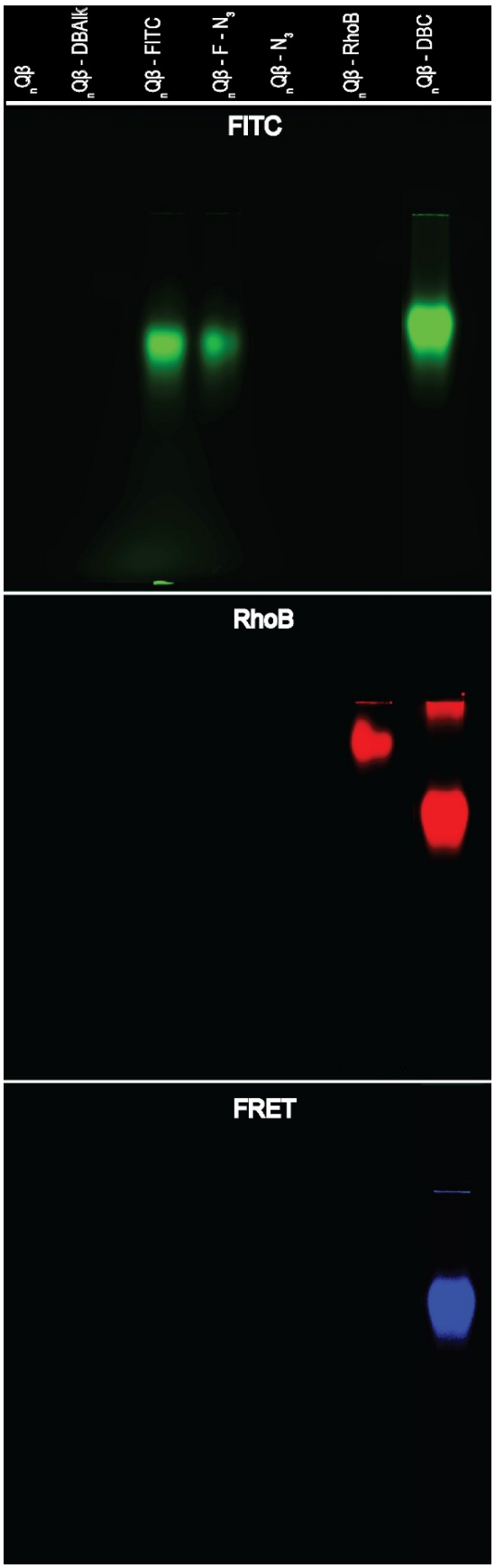


Figure S5: Agarose gel images of the Qβ conjugates under A) green (λ_{ex}: 490 nm / λ_{em}: 515 nm), B) red (λ_{ex} :520 nm/ λ_{em}: 560 nm) and C) FRET (λ_{ex}: 488 nm/ λ_{em}: 560 nm) excitation and emissions.

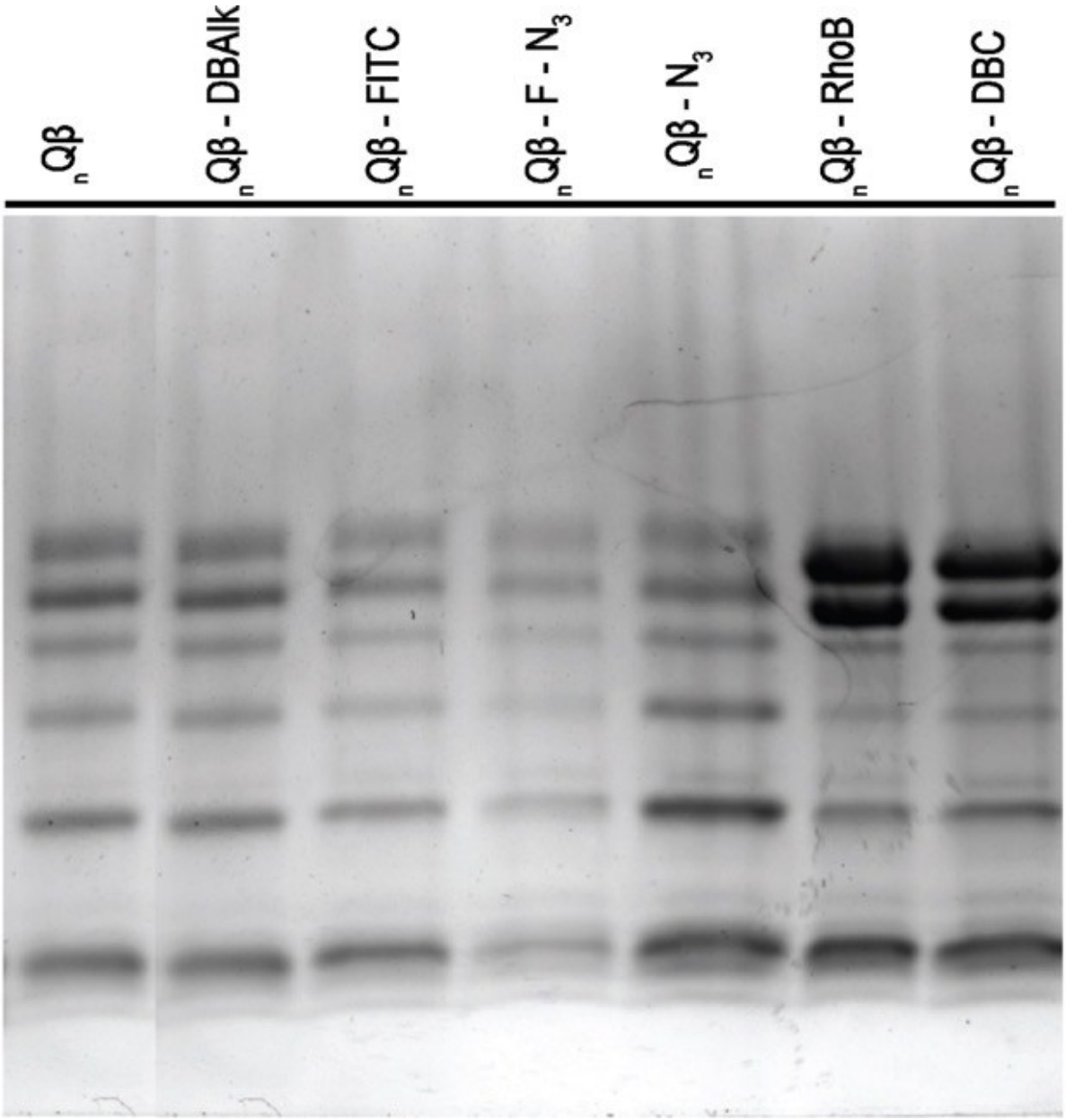


Figure S6: Coomassie stained non-denaturing SDS-PAGE of gels shown in Figure 1D.

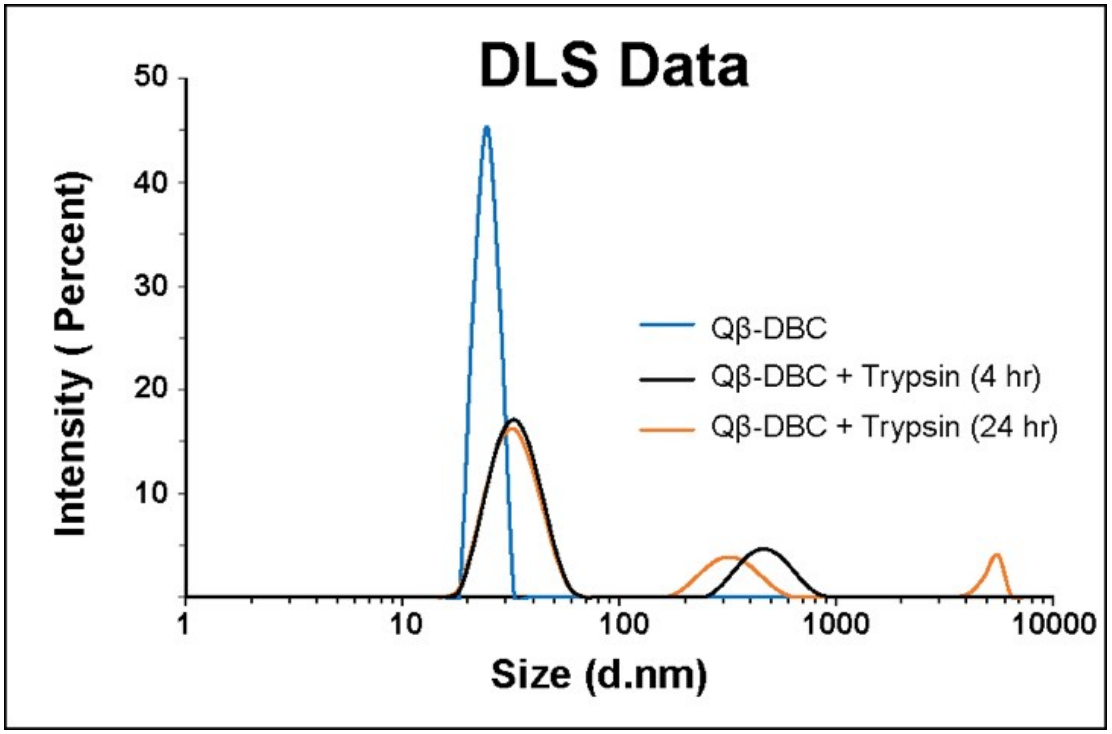


Figure S7: DLS data after incubation with 0.1 mg/mL trypsin showing increasing aggregation as time progresses.

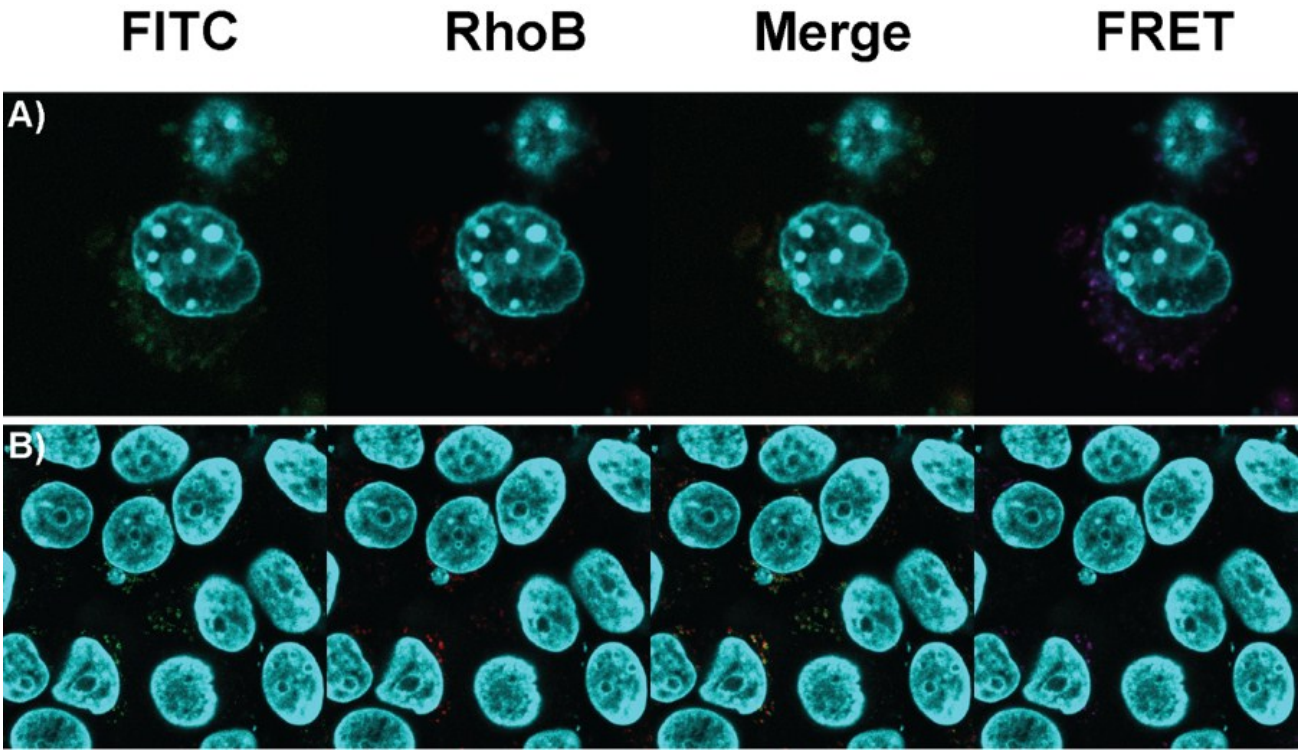


Figure S8: Unprocessed cell images referenced in Figure 3a. The remaining fluorophore emission is quite weak in both RAW cells (top) and MCF-7 (bottom).

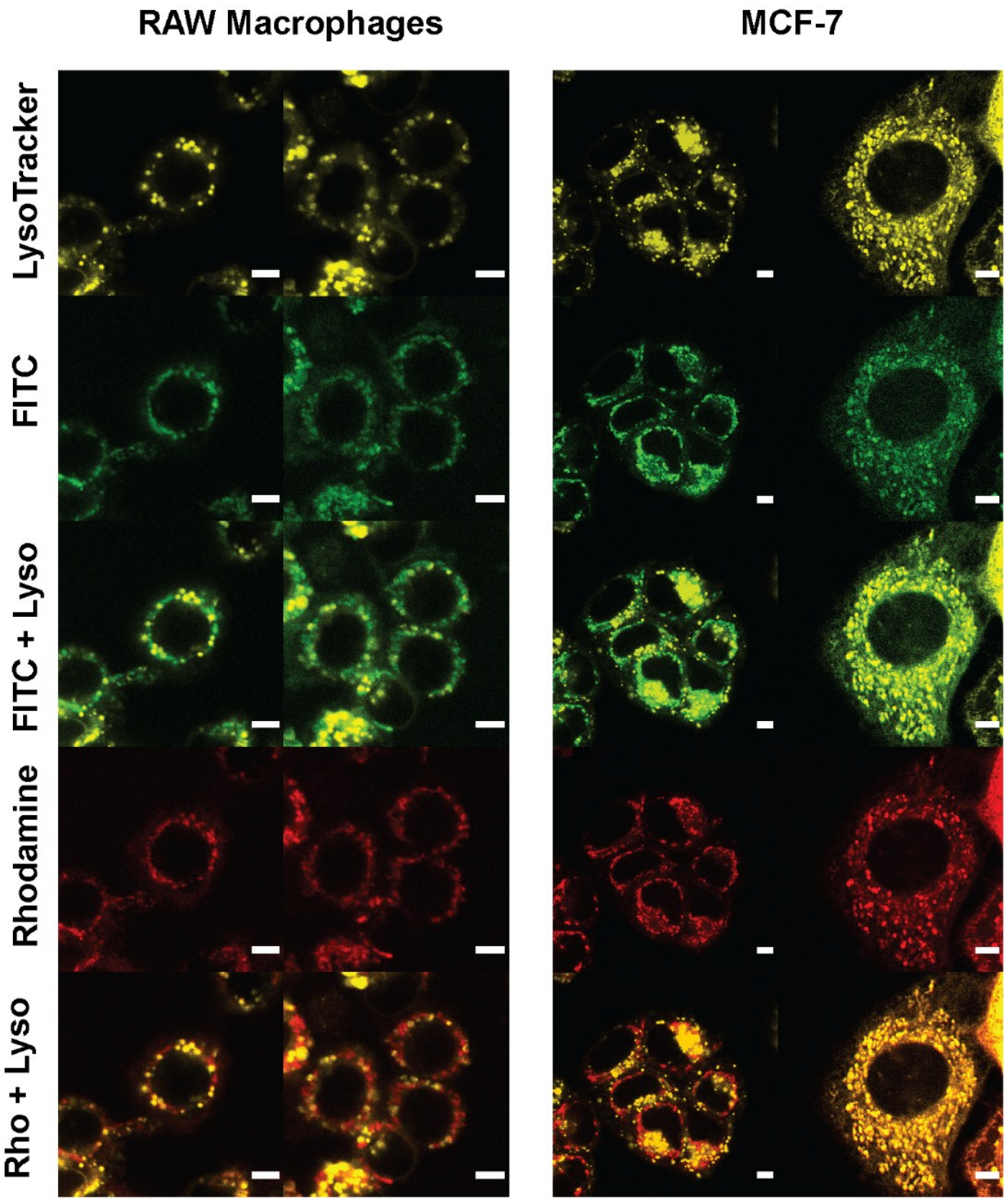


Figure S9: Confocal images showing colocalization with LysoTracker at 4h (left) and 8h (right) of the respective cell lines. Pearson's coefficients were calculated as follows – (left to right) 0.68, 0.82, 0.79 and 0.92. Scale bars are 5 μm.

Additional Material Characterization

FPLC

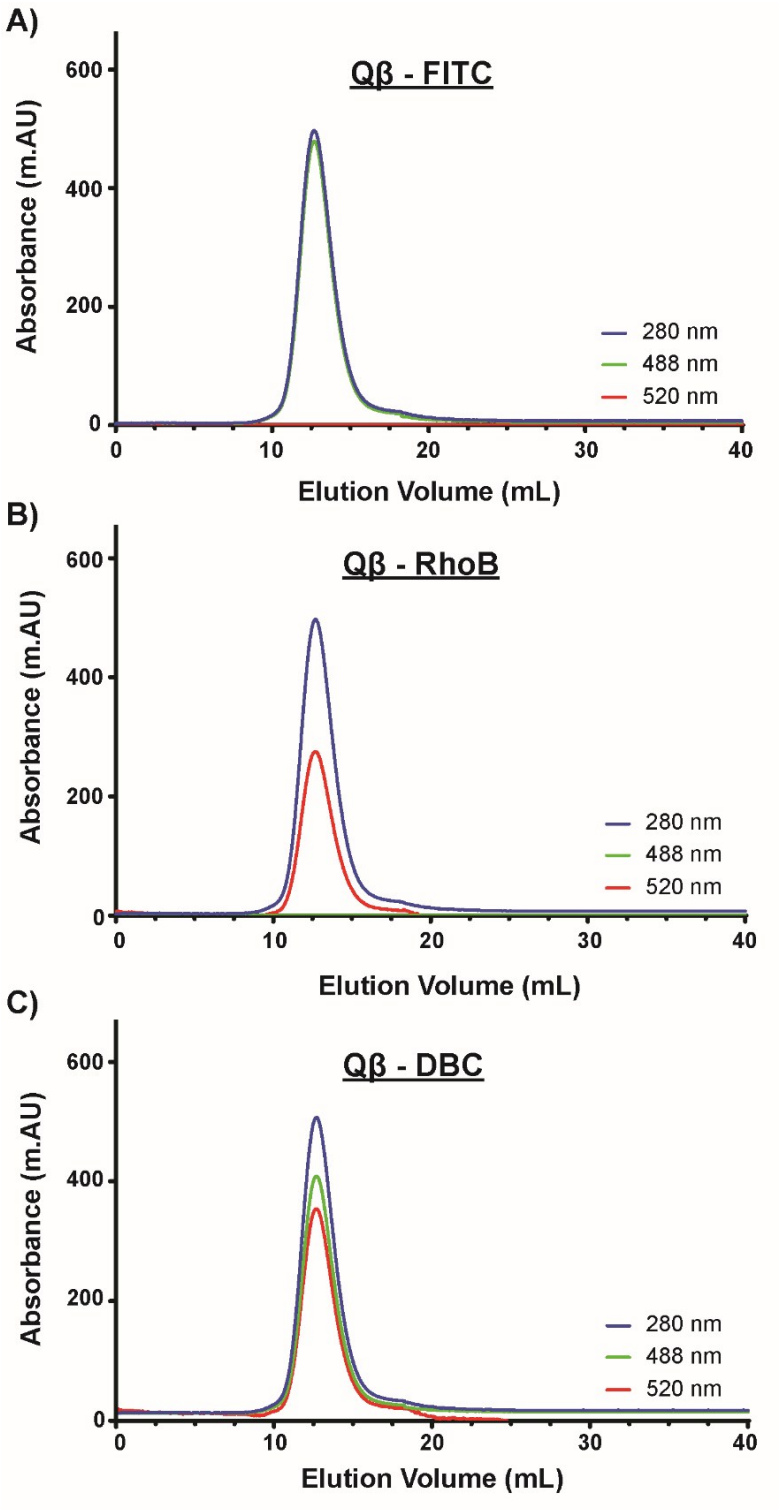


Figure S10: FPLC Traces of A) Qβ-FITC, B) Qβ-Rhodamine and C) Qβ-DBC after conjugation of dyes and spin column purification. Each trace shows a single peak of elution indicating a single product and signals in accordance with the attached fluorophores. (λ_{abs} 280 nm, protein; 488 nm, FITC; 520 nm, Rhodamine B).

TEM

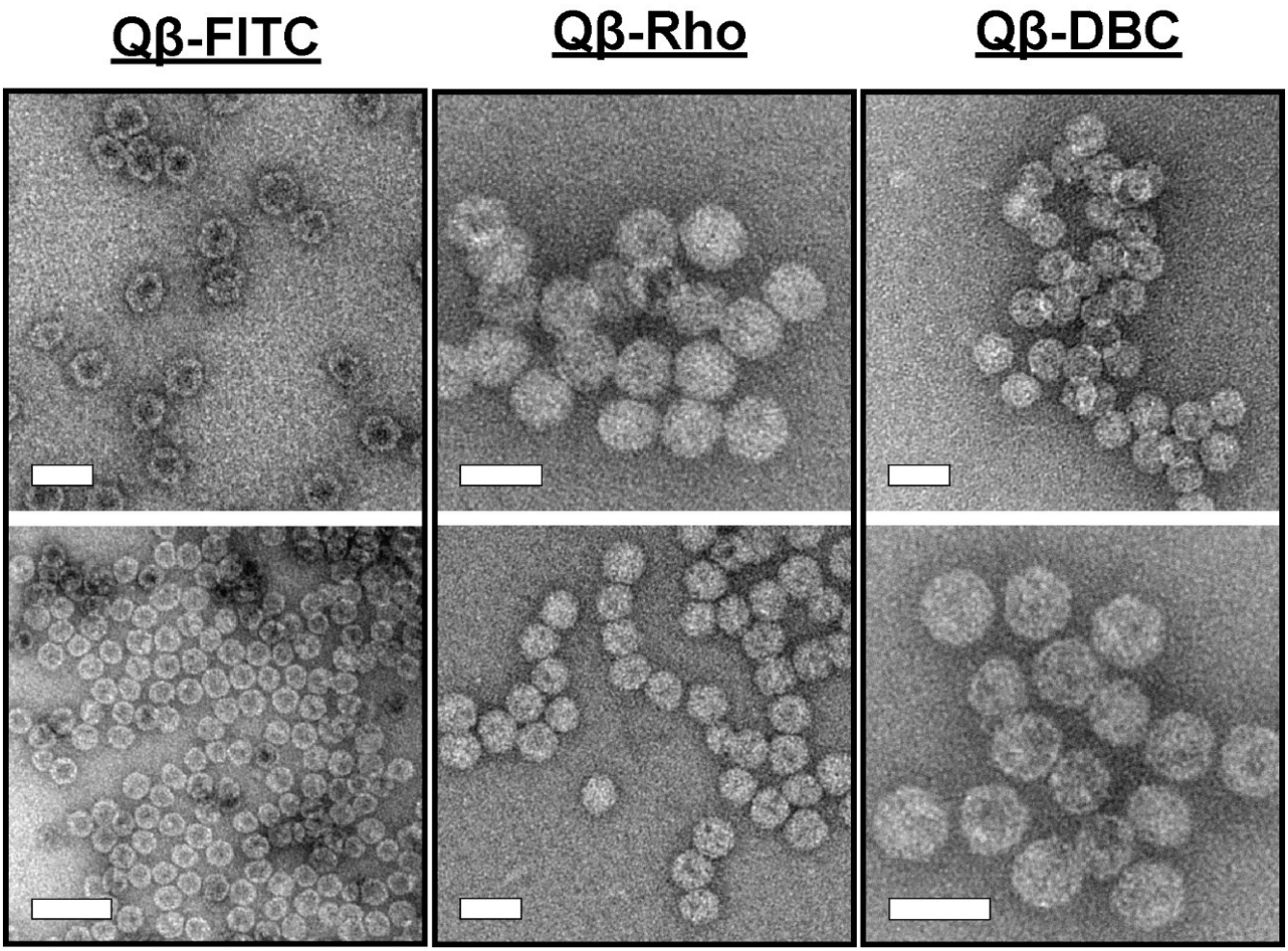
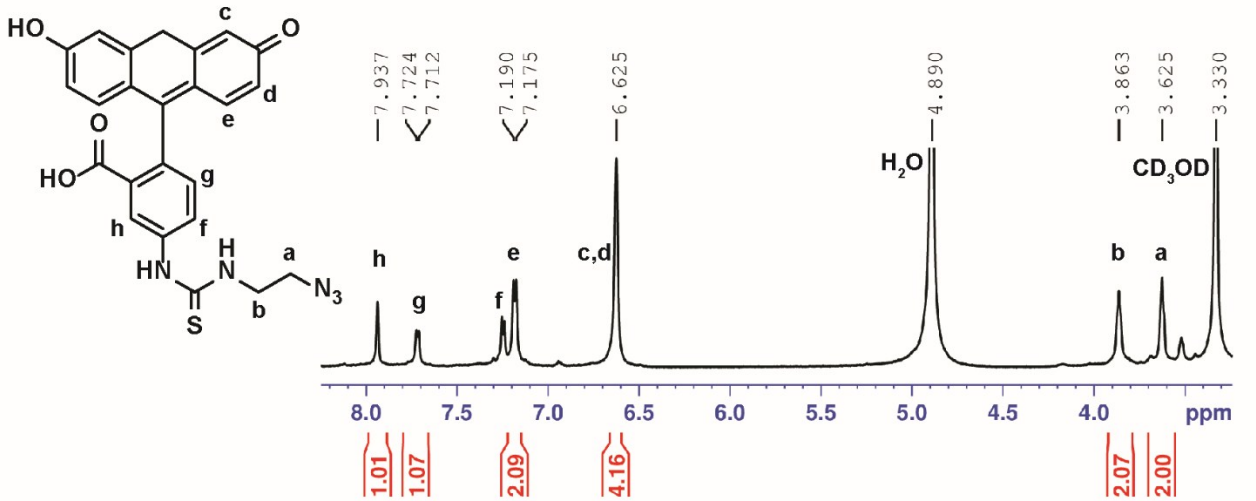


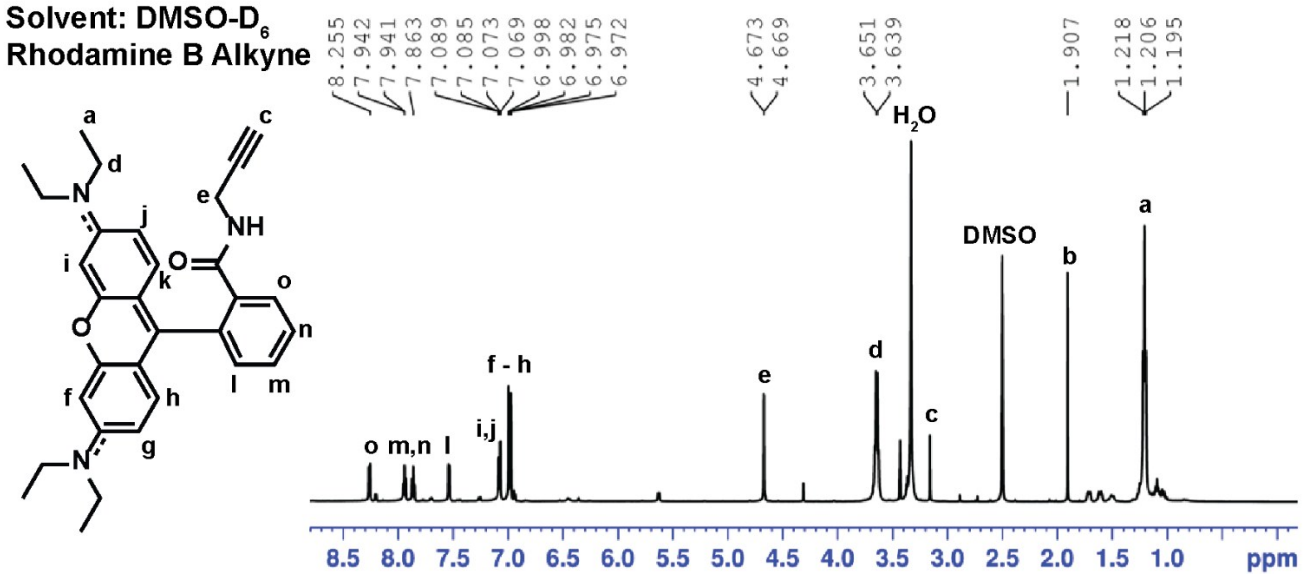
Figure S10: TEM images of VLPs after addition and purification of each new dye. All scale bars are 50 nm with the exception of the bottom left image which is 100 nm.

NMR

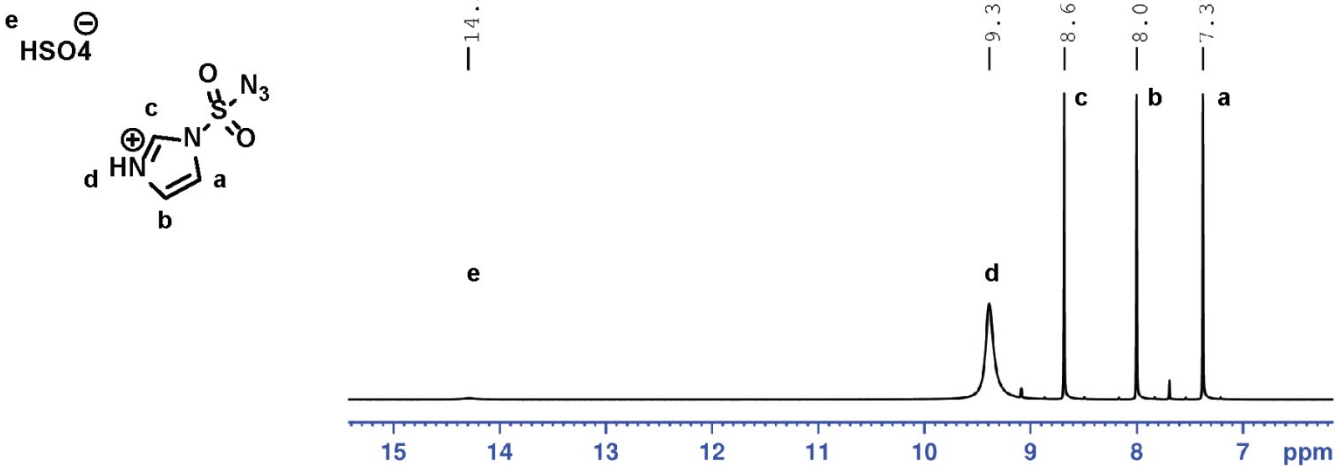
^{1}H NMR
Solvent: CD_3OD
FITC-N_3



^{1}H NMR
Solvent: DMSO-D_6
Rhodamine B Alkyne



^{1}H NMR
Solvent: DMSO-D_6
Diazo Transfer Reagent



References

1. Y. Li, M. S. Budamagunta, J. Luo, W. Xiao, J. C. Voss and K. S. Lam, *ACS Nano*, 2012, **6**, 9485-9495.