## A biotin-4-fluorescein based fluorescence quenching assay for determination of biotin binding capacity of streptavidin conjugated quantum dots

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## SUPPORTING INFORMATION

Figure S1	Calibration of B4F effective concentration
Figure S2	Titration of control samples with B4F
Figure S3	Titration of biotin blocked 525 ITK SAv QDs and SAv with B4F
Figure S4	Incubation time study of SAv titration



**Figure S1. Calibration of B4F effective concentration.** Log-log plot of 1 nM SAv titrated with B4F to calibrate B4F concentration. Nominal x-intercept =  $5.5 \pm 1.0$ . Error bars represent the standard deviation of the mean of independent triplicate series.



**Figure S2. Titration of control samples with B4F.** Representative titration profiles of 1 nM carboxyl QDs with B4F as a control for zero biotin binding sites of an unfunctionalized QD, and B4F dilution series alone. A) Log-log plot of 1 nM carboxyl QDs (Qdot 705 ITK carboxyl Q21361MP) titrated with B4F. B) Log-log plot of B4F dilution series. Error bars represent the standard deviation of the mean of independent triplicate series.

## **Blocked Titrations**



Figure S3. Titration of biotin blocked 525 ITK SAv QDs and SAv with B4F. Log-log plot of 1 nM 525 ITK SAv QDs (Qdot ITK SAv conjugate Q10041MP) and 1 nM SAv blocked with 10  $\mu$ M biotin for 30 minutes, and then titrated with B4F, showing specificity of B4F for SAv with the lack of nonlinear titration behavior. Error bars represent the standard deviation of the mean of independent triplicate series.



SAv 1 nM Titration - Incubation Time Study

**Figure S4. Effect of incubation time on SAv titration.** Log-log plot of 1 nM SAv titrated with B4F and incubated from 30 minutes to overnight, confirming the accuracy and reproducibility of the assay under chosen titration conditions. Nominal x-intercept =  $6.0 \pm 0.6$ ,  $5.9 \pm 0.9$ ,  $6.1 \pm 0.8$ ,  $6.2 \pm 0.8$ , and  $7.1 \pm 0.2$  for 30 minutes, 1 hour, 2 hours, 3 hours, and overnight; respectively. Error bars represent the standard deviation of the mean of independent triplicate series.