Biophysical Journal, Volume *117*

Supplemental Information

Detecting and Controlling Dye Effects in Single-Virus Fusion Experiments

Robert J. Rawle, Ana M. Villamil Giraldo, Steven G. Boxer, and Peter M. Kasson

Supporting Information for
Detecting and controlling dye effects in single-virus fusion experiments

Robert J. Rawle, Ana M. Villamil Giraldo, Steven G. Boxer, Peter M. Kasson

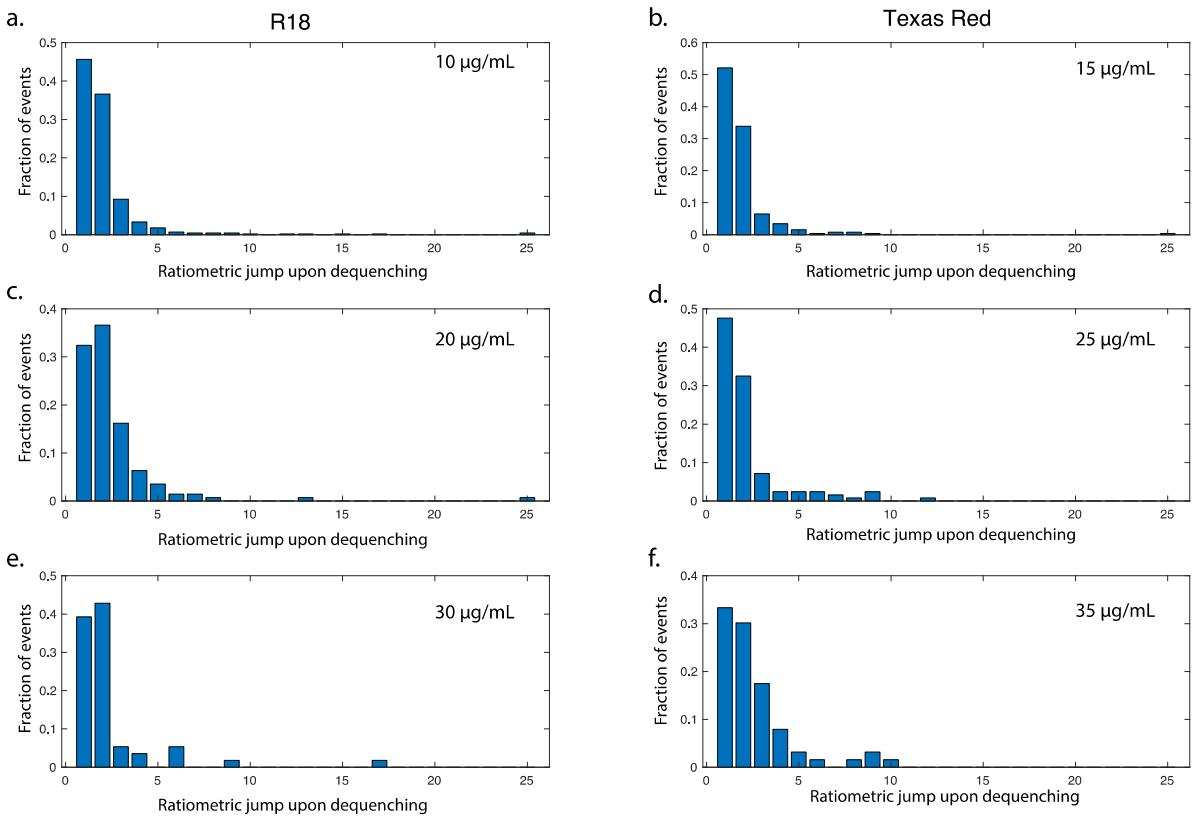


Figure S1. Ratiometric intensity increase upon lipid mixing for different labeling conditions. Intensity traces of single influenza virus spots were analyzed for fusion, and the intensity ratio after versus before each identified dequenching event was calculated. Histograms of these ratios, thus representing the distribution of dequenching jumps across the population of fusing labeled virions, are plotted for viruses labeled with R18 at 10 µg/mL (a), 20 µg/mL (c), and 30 µg/mL (e) and for Texas Red at 15 µg/mL (b), 25 µg/mL (d), and 35 µg/mL (e).

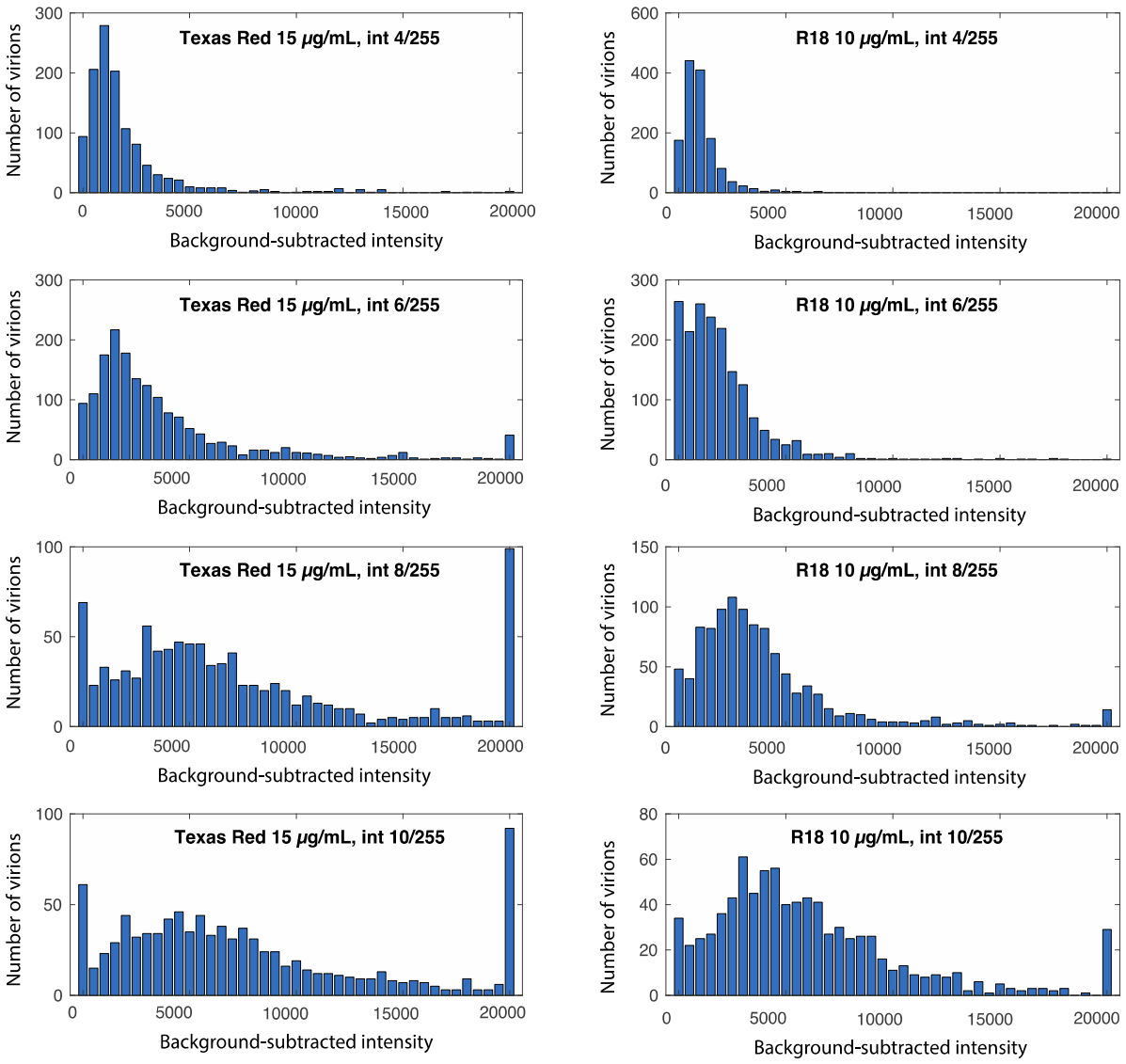


Figure S2. Starting intensity distributions of Texas-Red-labeled and R18-labeled viral particles at different illumination levels. As expected, intensities increase slightly with illumination, although the intensity distribution for R18 is perturbed less than the corresponding one for Texas Red. Intensity values are taken 4s after pH drop. Absolute numbers of particles undergoing lipid mixing out of those identified are as follows:

R18 10 µg/mL:

illumination 4/255: 571 dequenched of 1396 identified

illumination 6/255: 869 dequenched of 1743 identified

illumination 8/255: 349 dequenched of 1036 identified

illumination 10/255: 278 dequenched of 847 identified

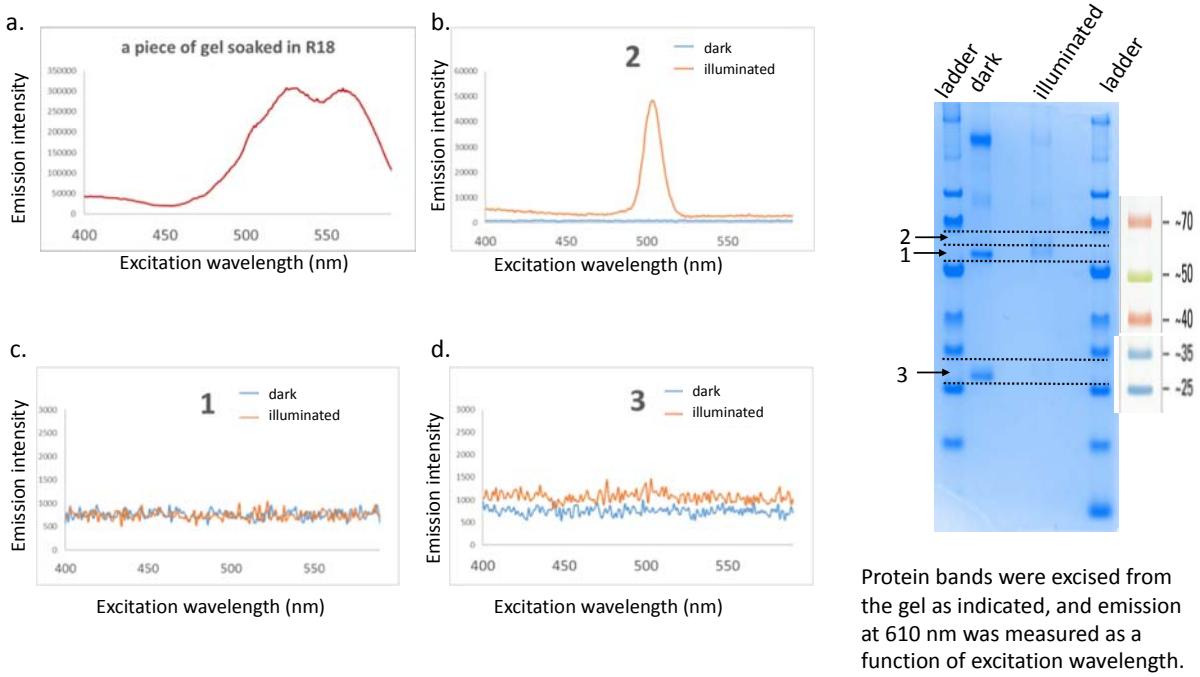
Texas Red 15 µg/mL:

illumination 4/255: 382 dequenched of 1171 identified

illumination 6/255: 539 dequenched of 1668 identified

illumination 8/255: 302 dequenched of 949 identified

illumination 10/255: 289 dequenched of 936 identified.



Protein bands were excised from the gel as indicated, and emission at 610 nm was measured as a function of excitation wavelength.

Figure S3. Fluorescence spectra of R18 and photolabeled viral protein. Influenza virus was labeled with R18, illuminated, detergent-solubilized, and the proteins separated via SDS-PAGE. The indicated bands in the illuminated sample were excised, and fluorescence excitation spectra were measured as indicated (emission at 610 nm as a function of excitation wavelength). Bands are compared to gel soaked in R18 dye (a). The illuminated band at 55 kDa showed a gel-shift, and the shifted portion of this band (b), but not the unperturbed portions (c, d) showed fluorescence with an excitation wavelength slightly blue-shifted from unmodified R18. Each lane on the gel contained viral sample containing 10 µg of viral protein prior to labeling. We do not have sufficient information to assign the nature of the chemical change, but the excitation shift demonstrates a fluorescent product that is chemically modified from the R18 reactant.

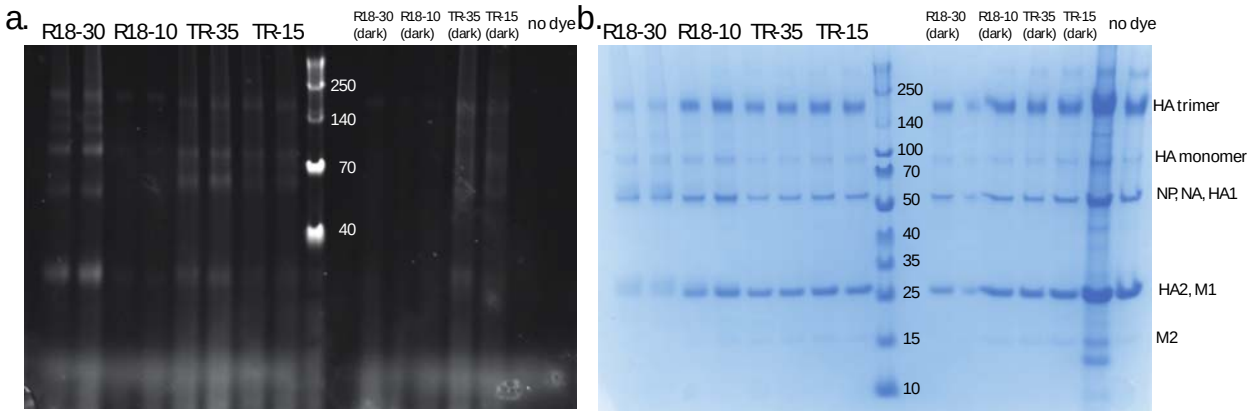


Figure S4. Photolabeling and gel shift of R18-labeled influenza virus. Shown are fluorescence imaging and Coomassie staining of viral protein under different labeling and illumination conditions. Virus was labeled with the designated dye: R18 or Texas Red (TR) and amount in µg/mL, either illuminated or kept in the dark, and then detergent-solubilized and separated via SDS-PAGE. Shown are fluorescence imaging of the gel (a) using bandpass filters for excitation 520−545 nm and emission 577–613 nm and subsequent Coomassie staining (b). These gels were quantitated in Figure 4. Each lane on the gel contained viral sample containing 10 µg of viral protein prior to labeling. Bands are labeled with major viral proteins that migrate at those molecular weights. In influenza viral extract, several viral proteins can co-migrate, and these are indicated by comma-separated lists.