Supporting information for:

Azadioxatriangulenium (ADOTA⁺): A long fluorescence lifetime fluorophore for large biomolecule binding-assays

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Figure S1: Normalized absorption and emission spectra of ADOTA-NHS in MeCN (black) and milliQ water (red). Emission quantum yield in water were determined to 41%, and the lifetime to 20.1 ns.



Figure S2: Anisotropy decays (top) and component decays (bottom) of ADOTA-*anti*IgG (left) and ADOTA-*anti*IgG-IgG (right)



Figure S3: Photostability measured by accelerated bleaching using a focused solid state laser.

Photostability: A thin and uniform film containing either ADOTA or Rhodamine 6G was prepared by spin coating. Typically a drop of uM probe in 1% aqueous PVA solution was placed on a microscope cover slip which were spun at 2000 rpm. The experimental set up used is a a confocal MicroTime 200 (Picoquant GmbH, Berlin, Germany) system coupled to an Olympus IX71 microscope (Center Valley, PA). Fluorescence photons were gathered from different places of the sample using a 60X water immersion objective (N.A 1.2, Olympus). To remove scattered light, a 488 nm long-pass filter was applied. As a light source, a pulsed laser (470 nm LDH-P-C470B) with a repetition rate of 20MHz was used. Fluorescence photons were collected with the photon-counting module (SPCM-AQR-14; PerkinElmer, Waltham, MA) and processed using the PicoHarp300 time-correlated single photon counting (TCSPC) module.