

# Transient Effects in Fluorescence Quenching Measured by 2-GHz Frequency-Domain Fluorometry

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We used harmonic-content frequency-domain fluorometry to investigate the quenching of indole fluorescence by iodide and acrylamide in aqueous solution. The time-resolved intensity decays were recovered from the frequency response of the fluorescence emission, measured over a frequency range from 10 to 2000 MHz. In the absence of collisional quenching the decay of indole in water is predominately a single exponential. The intensity decays became increasingly nonexponential when quenched by either iodide or acrylamide. We attribute the complex decays to transient effects, as predicted originally by Smoluchowski. At quencher concentrations below 0.1 M the decays are of the form  $\exp(-t/\tau - 2bt^{1/2})$ , which is known to be an approximate model. At quencher concentrations above 0.1 M this decay law does not account for the data. The data are in better agreement with the radiation model, and the diffusion coefficients and interaction radii are more reasonable. However, in aqueous solution above 0.5 M acrylamide there appear to be deviations from the radiation model. The deviations are less apparent at high iodide concentrations. For comparison with future theoretical developments, the intensity decay laws are reported up to 0.5 M iodide or 0.7 M acrylamide. Evidently, the resolution and sensitivity of the frequency-domain method are adequate to recover the complex subnanosecond decays found at high concentrations of quenchers.