

Supplemental Information for

Continuous fluorometric method for measuring β -Glucuronidase activity: comparative analysis of three fluorogenic substrates

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Procedure used for the determination of Michealis-Menten kinetic parameters.

Table S-1 Buffer systems used for pH studies.

Buffer System	pH Values (± 0.01)
Sodium Citrate/- Citric Acid	3.0, 3.6, 4.0, 4.6, 5.0
Sodium Phosphate Monobasic/- Sodium Phosphate Dibasic	6.0, 6.2, 6.4, 6.6, 6.8, 7.0, 7.2, 7.4, 8.0
Sodium Carbonate - Sodium Bicarbonate	9.2, 10.0, 10.6, 11.4

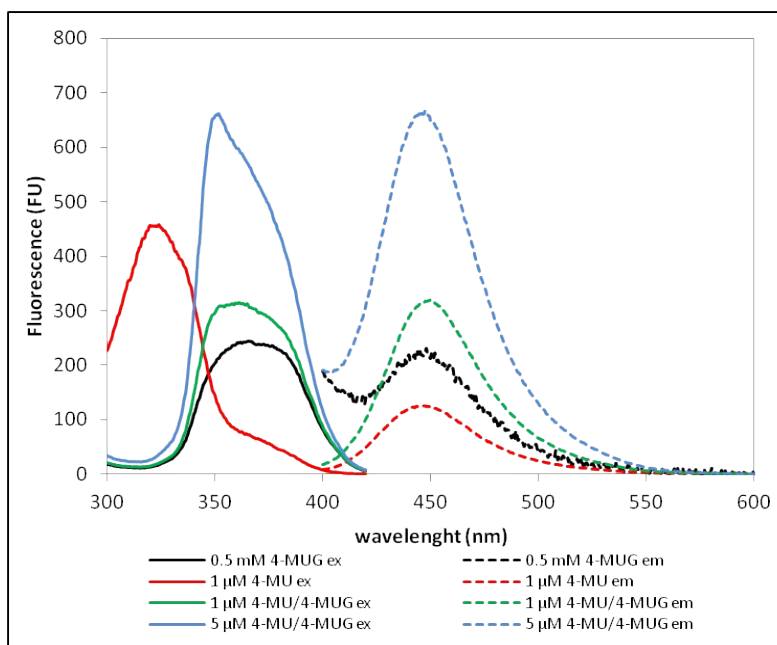


Figure S-1 Excitation and emission spectra of 4-MUG and 4-MU; 4-MU em and 4-MU ex are the spectra of 4-MU; 4-MU/4-MUG ex and 4-MU/4-MUG em are spectra of 4-MU in the presence 0.5 mM 4-MUG; concentrations of 4-MU used are shown in the legend; emission wavelength: 446 nm; excitation wavelength: 351 nm; slit width: 5 nm (ex) and 2.5 nm (em). Measurements carried out using the LSB 50 fluorometer.

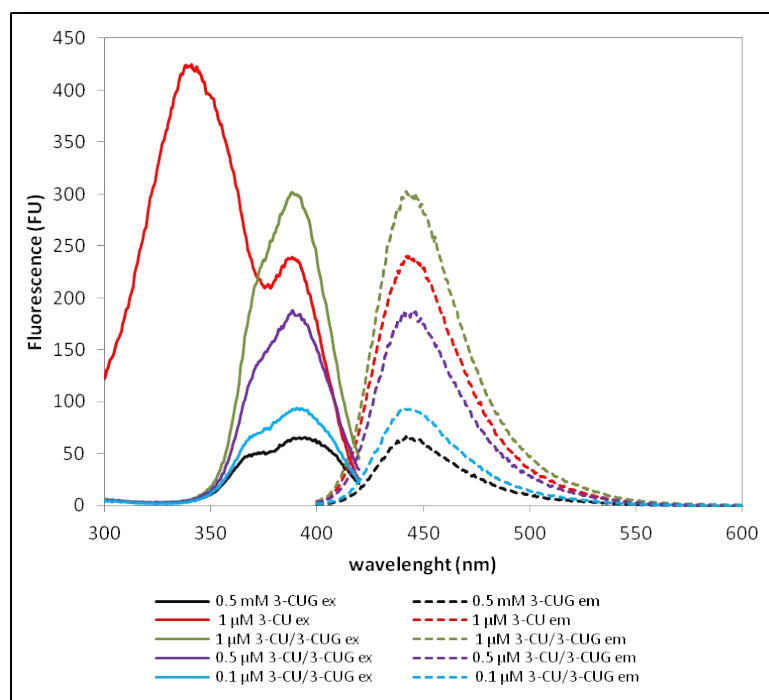


Figure S-2 Excitation and emission spectra of 3-CUG and 3-CU; 3-CU em and 3-CU ex are the spectra of 3-CU; 3-CU /3-CUG ex and 3-CU /3-CUG em are spectra of 3-CU in the presence 0.5 mM 3-CUG; concentrations of 3-CU used are shown in the legend; emission wavelength : 446 nm; excitation wavelength: 351 nm; slit width: 5 nm (ex) and 2.5 nm (em). Measurements carried out using the LSB 50 fluorometer.

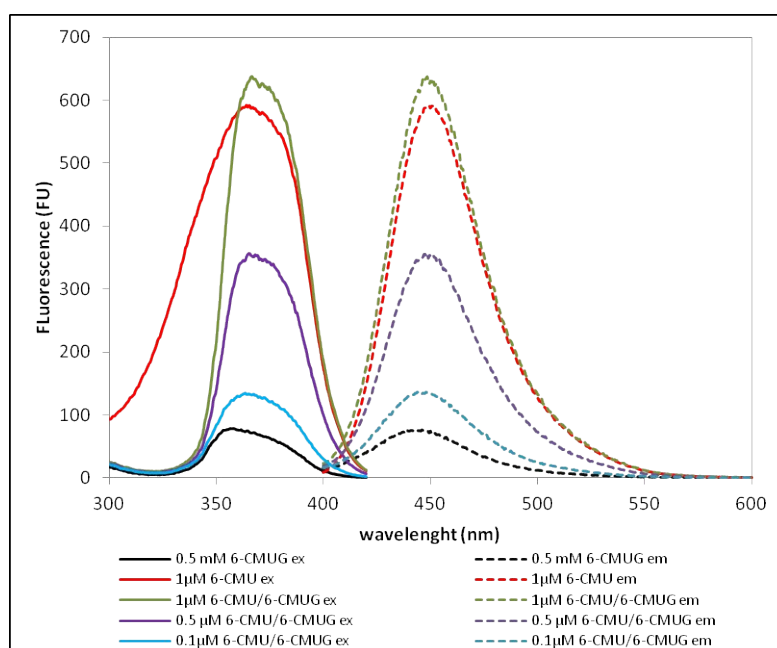


Figure S-3 Excitation and emission spectra of 6-CMUG and 6-CMU; 6-CMU em and 6-CMU ex are the spectra of 6-CMU; 6-CMU/6-CMUG ex and 6-CMU/6-CMUG em are spectra of 6-CMU in the presence 0.5 mM 6-CMUG; concentrations of 6-CMU used are shown in the legend; emission wavelength: 446 nm; excitation wavelength: 351 nm; slit width: 5 nm (ex) and 2.5 nm (em). Measurements carried out using the LSB 50 fluorometer.

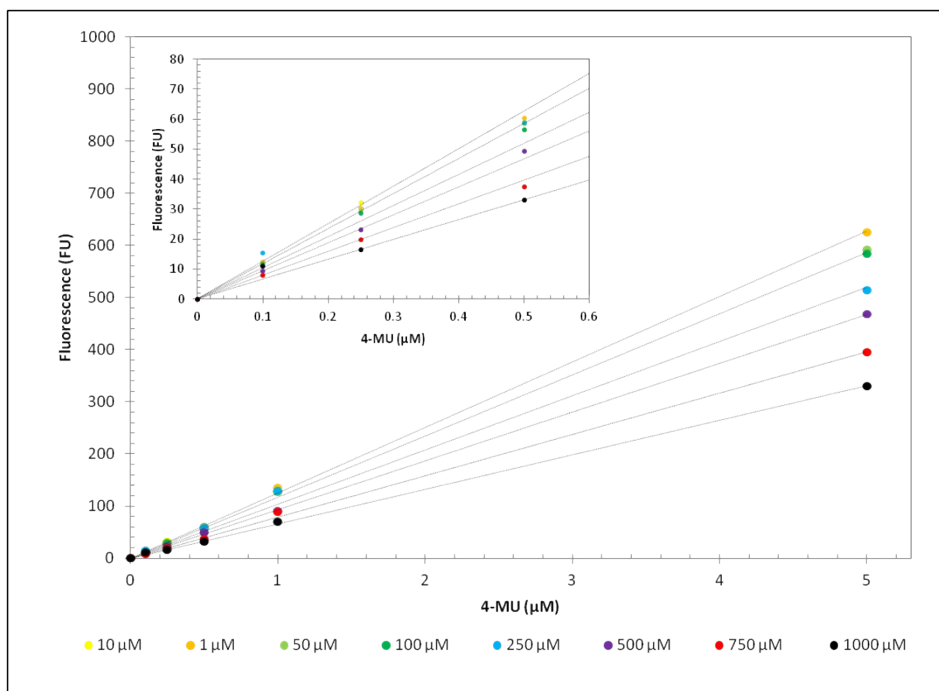


Figure S-4 Calibration curves of 4-MU in the presence of different 4-MUG concentrations (shown in the legend); λ_{ex} = 351 nm, λ_{em} 446 nm; slit widths: 5 nm (ex), 2.5 nm (em); dotted lines represent the trendlines of the linear regression model. Measurements carried out using the LSB 50 fluorometer.

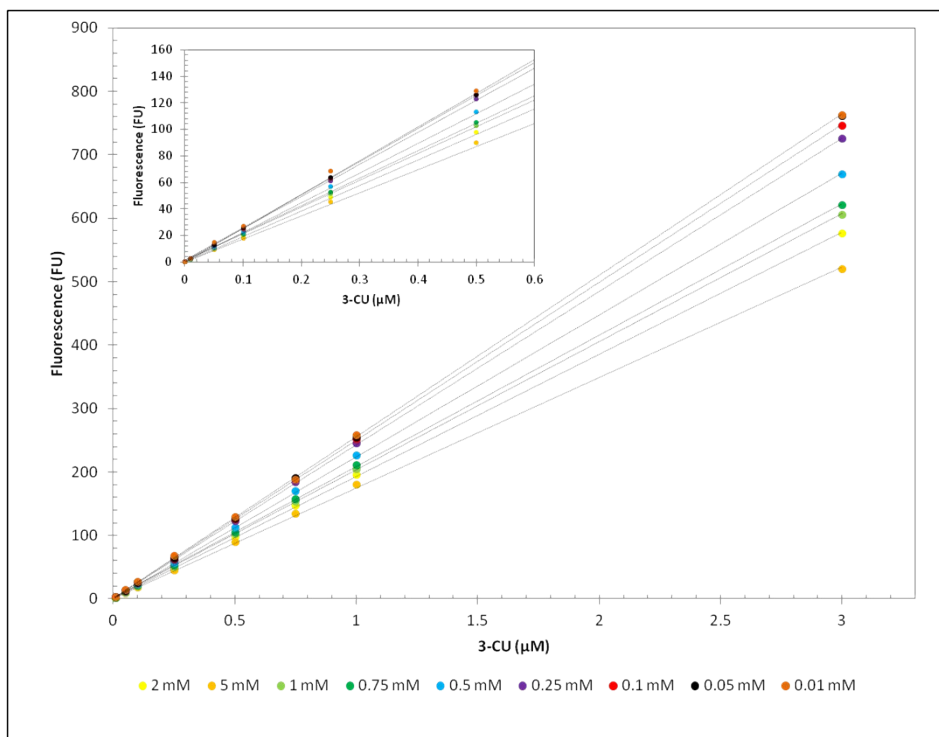


Figure S-5 Calibration curves of 3-CU in the presence of different 3-CUG concentrations (shown in the legend); λ_{ex} = 389 nm, λ_{em} 444 nm; slit widths: 5 nm (ex), 2.5 nm (em); the dotted lines represent the trendlines of the model. Measurements carried out using the LSB 50 fluorometer.

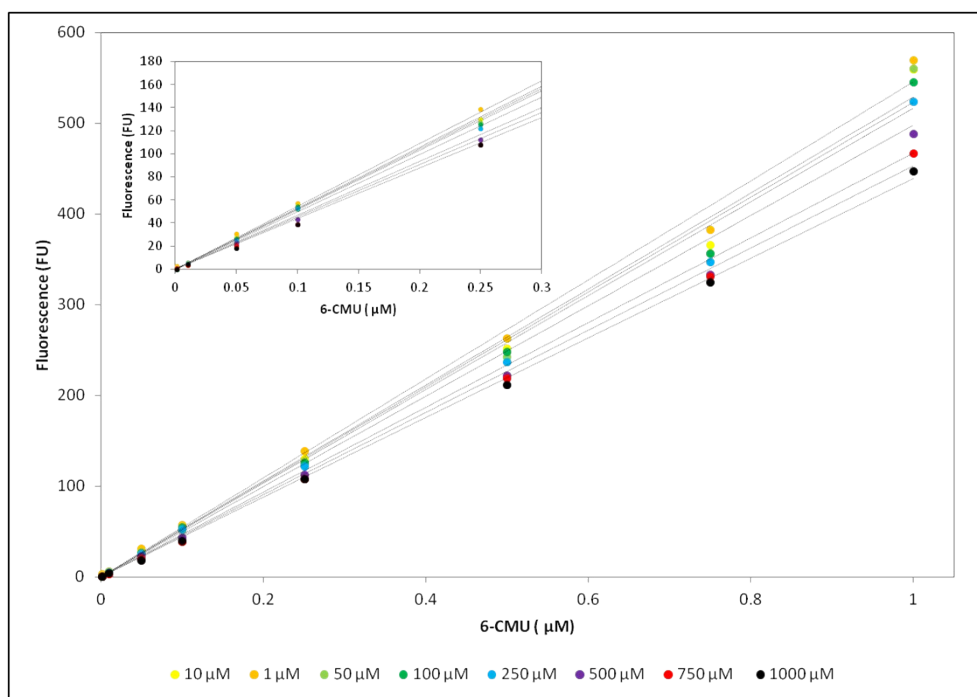


Figure S-6 Calibration curves of 6-CMU in the presence of different 6-CMUG concentrations (shown in the legend); λ_{ex} = 365 nm, λ_{em} 449 nm; slit widths: 5 nm (ex), 2.5 nm (em); the dotted lines represent the trendlines of the model. Measurements carried out using the LSB 50 fluorometer.

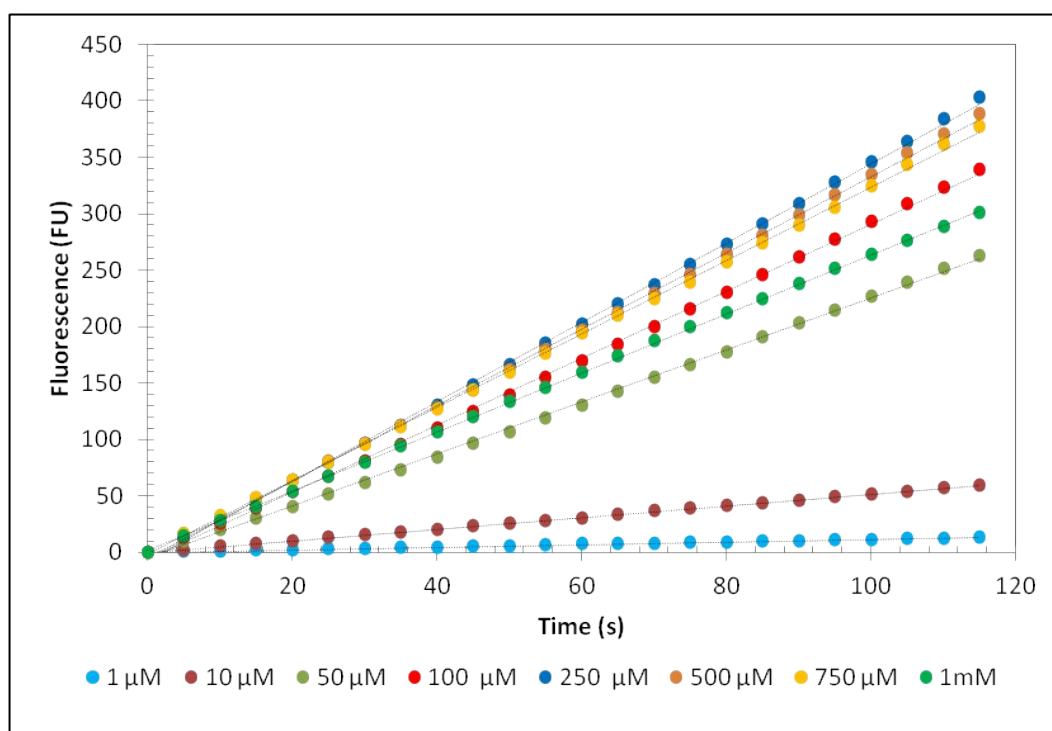


Figure S-7 Progress curves for GUS catalysed hydrolysis of different 4-MUG concentrations (shown in the legend); λ_{ex} = 351 nm, λ_{em} 446 nm; slit widths: 5 nm (ex), 2.5 nm (em); the dotted lines represent the trendlines of the model. GUS was added at a concentration of 135 ng mL⁻¹. Measurements carried out using the LSB 50 fluorometer.

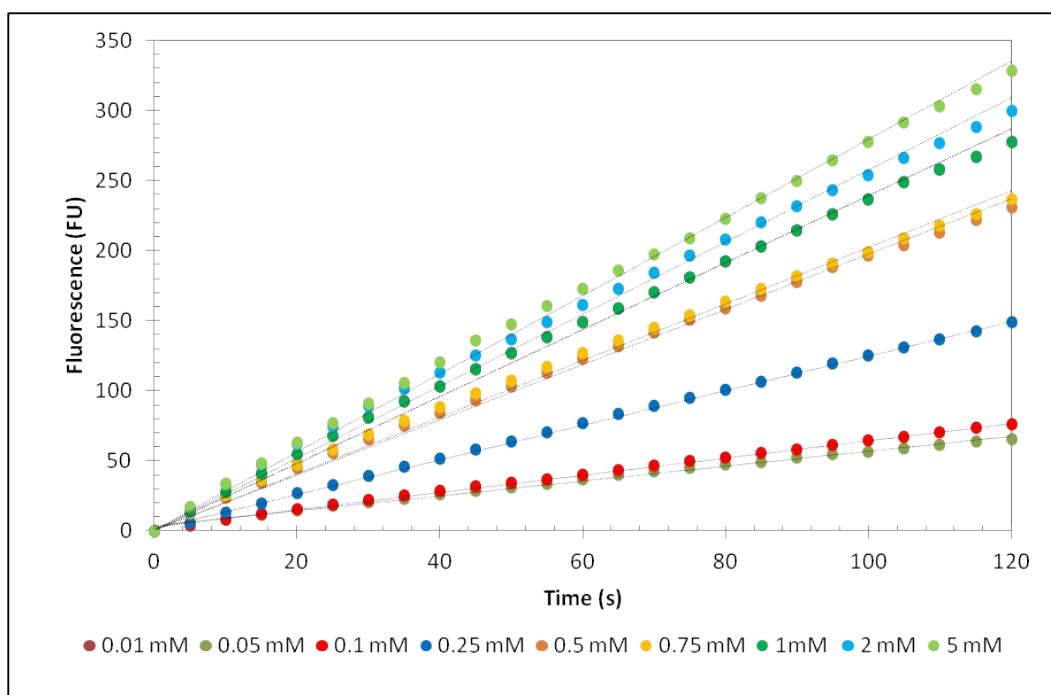


Figure S-8 Progress curves for GUS catalysed hydrolysis of different 3-CUG concentrations (shown in the legend); λ_{ex} = 389 nm, λ_{em} 444 nm; slit widths: 5 nm (ex), 2.5 nm (em); the dotted lines represent the trendlines of the model. GUS was added at a concentration of 135 ng mL⁻¹. Measurements carried out using the LSB 50 fluorometer.

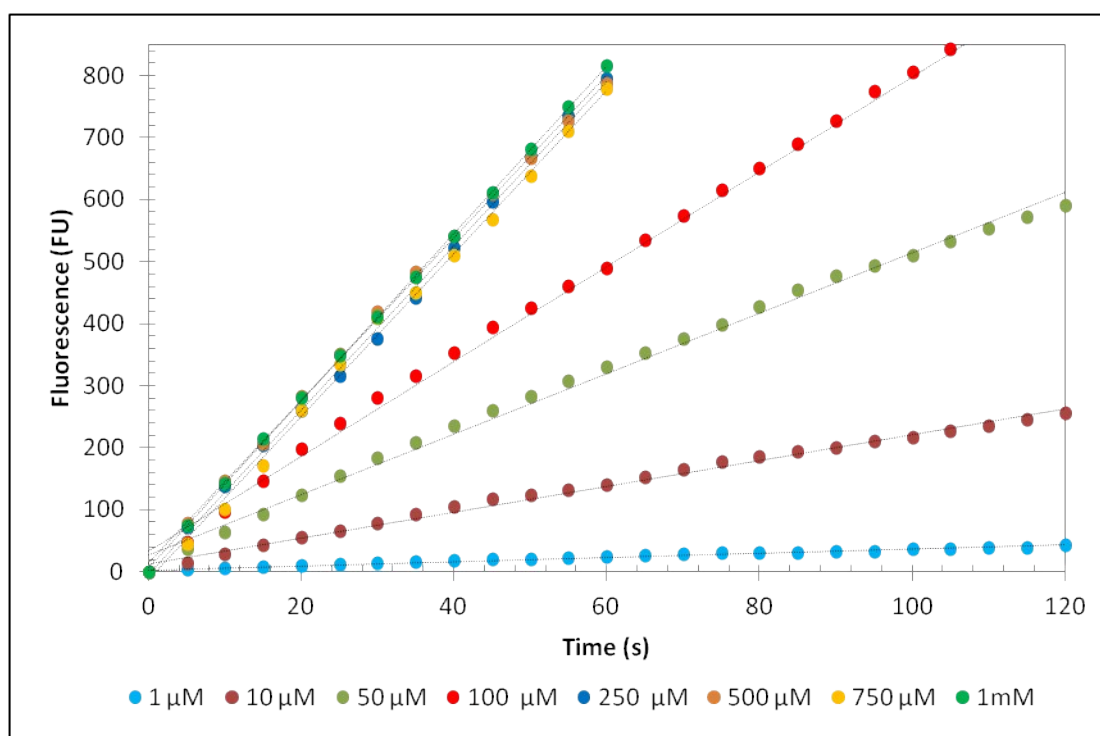


Figure S-9 Progress curves for GUS catalysed hydrolysis of different 6-CMUG concentrations (shown in the legend); λ_{ex} = 365 nm, λ_{em} 449 nm; slit widths: 5 nm (ex), 2.5 nm (em); the dotted lines represent the trendlines of the model. GUS was added at a concentration of 135 ng mL⁻¹. Measurements carried out using the LSB 50 fluorometer.

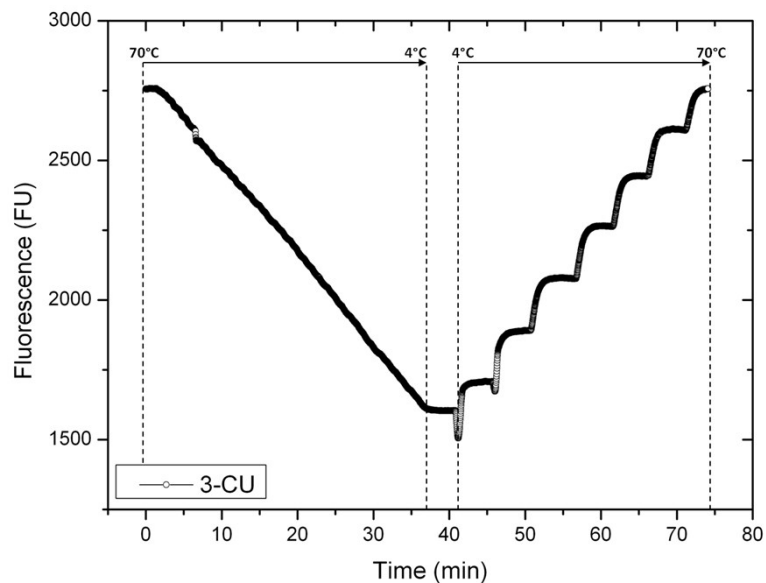


Figure S-10 Temperature dependent fluorescence intensity of 3-CU at pH 6.8. Constant temperature decrease at a rate of $2\text{ }^{\circ}\text{C min}^{-1}$ from $70\text{ }^{\circ}\text{C}$ to $4\text{ }^{\circ}\text{C}$ (left) and stepwise temperature increase from $4\text{ }^{\circ}\text{C}$ to $70\text{ }^{\circ}\text{C}$ with 5 min equilibrium steps (right); the first step used in this case was from $4\text{ }^{\circ}\text{C}$ to $10\text{ }^{\circ}\text{C}$ after which all the subsequent steps were $10\text{ }^{\circ}\text{C}$ each; $\lambda_{\text{ex}}=385\text{ nm}$, $\lambda_{\text{em}}445\text{ nm}$.

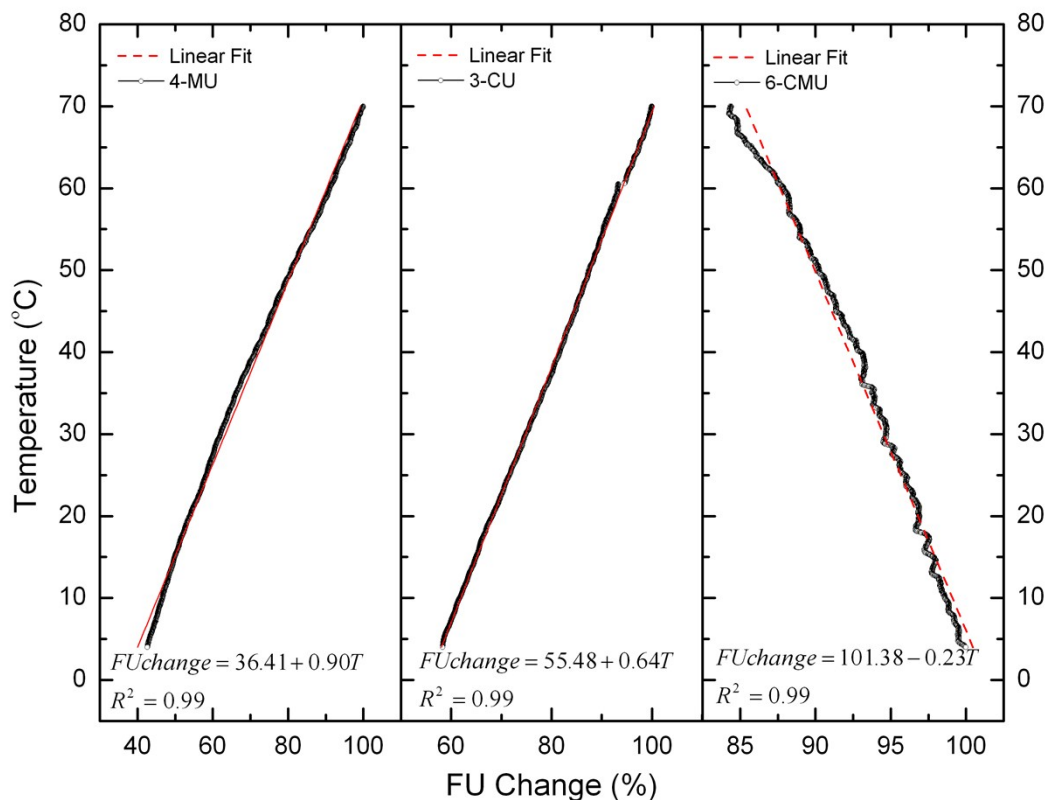


Figure S-11 Temperature dependent fluorescence intensity of 4-MU, 3-CU and 6-CMU at pH 6.8. A constant temperature decrease rate of $2\text{ }^{\circ}\text{C min}^{-1}$ from $70\text{ }^{\circ}\text{C}$ to $4\text{ }^{\circ}\text{C}$ was used to collect the data (example shown in Fig S-10). Equations corresponding to the linear fitting of the data are shown in each panel; $\lambda_{\text{ex}} / \lambda_{\text{em}}$ used were: $364 / 447$ (4-MU), $385 / 445$ (3-CU), $369 / 452$ (6-CMU).

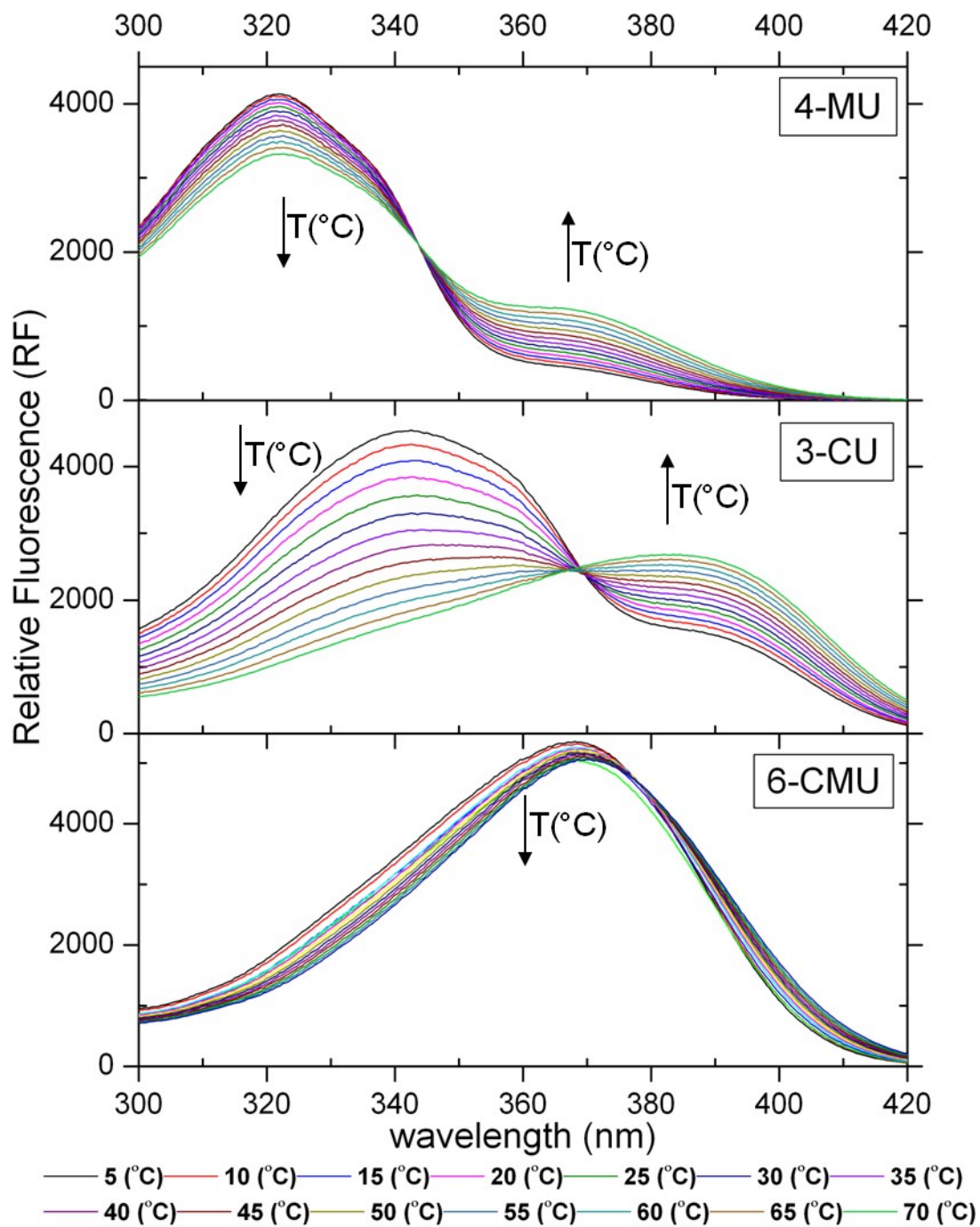


Figure S-12 Temperature dependent excitation spectra of 4-MU, 3-CU and 6-CMU at pH 6.8; λ_{em} used were: 447 (4-MU), 445 (3-CU), 452 (6-CMU).