Sensing Caspase 3 Activity with Quantum Dot-Fluorescent Protein Assemblies

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Supplementary Information.

QD synthesis

550 nm emitting QDs coated were synthesized and made water soluble with dihydrolipoic acid (DHLA) as described previously [1].

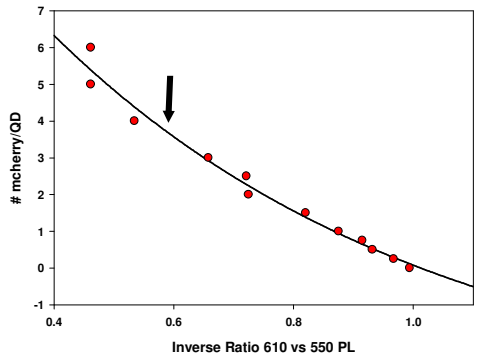
mCherry purification.

A license for using the mCherry gene sequence was purchased from Stratagene (La Jolla, CA). Plasmids encoding mCherry with and without the modified caspase 3 cleavage sequences in the commercial pRSetB vector (Invitrogen, Carlsbad CA) were expressed in Rosetta 2 (DE3) cells (Invitrogen). Briefly, cells were transformed and colonies were immediately grown overnight at 37°C in 250 mls LB media supplemented with 100 μg/ml carbamicillin and 34 μg/ml chloramphenical and 2% glucose for promoter suppression. Overnight cultures were spun down and resuspended in 2X LB with carbamicillin/ chloramphenical and 1mM IPTG and allowed to induce mCherry expression for 24 hours. Cultures turned a dark pink color with time, indicative of mCherry expression.

500 mls of cell culture was harvested and resuspended in 25 mls of PBS (0.1 M sodium phosphate and 0.15 M NaCl pH 7.4) with a small amount of sodium azide, lysozyme and protease inhibitor present. Cells were sonicated for cell lysis and centrifuged several times to remove cell debris. The reddish color supernatant was bound to 15 mls of PBS equilibrated Ni-NTA resin (Qiagen, Valencia CA) for 1.5 hrs with shaking. The Ni-NTA column was washed two times with 50 mls PBS supplemented with sodium azide and protease inhibitor and eluted in PBS supplemented with 300 mM imidizole. Protein eluate was dialyzed 2X in 4L PBS and quantified by the mCherry chromophore spectral absorbance at 587 nm.

Caspase Assay FRET Calibration.

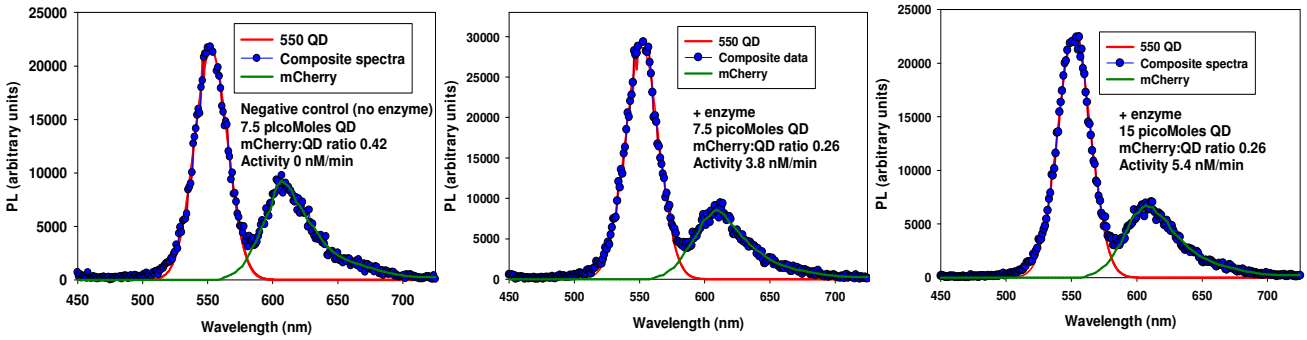
Calibration curves were created using 15 pmols/QD per reaction and titrating in varying amounts of respective mCherry/QD in 0.4X PBS pH 8.0 and measuring the subsequent fluorescent emission. A calibration curve was then generated using the inverse ratio of the mCherry peak PL (610 nm) to the QD peak PL (550 nm) at each concentration. A representative calibration curve is show in supplementary figure 1. A concentration of approximately 3.5 mCherry substrates per QD was chosen in the data shown in Supplementary Figure 1 as this is the concentration where a shift in FRET efficiency after proteolysis would be most detectable. Calibration equations (fits to the data) were used to correlate the changes in FRET efficiency into number of mCherry cleaved per QD during an assay as described previously[2].



Supplementary Figure 1: Representative calibration curve for mCherry containing the caspase 3 substrate 1 cleavage sequence. Inverse ratios of 610 to 552 PL were obtained from the FRET measurements where the amount of mCherry per QD was varied as indicated. The curve was used to determine the appropriate ratio of mCherry:QD to use as substrate that would be capable of transducing a significant change in FRET efficiency following proteolysis. Here it is 3.5 mCherry/QD as denoted by the arrow. This curve was also used to correlate changes in FRET efficiency into the number of mCherry cleaved/QD following exposure to the enzyme in the assay.

Caspase Assay

mCherry-substrate proteins were assembled in 0.4X PBS pH 8.0 and self-assembled at room temperature for about 15 minutes. Reactions were aliquoted into 0.5 ml PCR tubes at concentrations ranging from 7.5 to 50 pmols of QD per reaction. Caspase 3 (Calbiochem, San Diego, CA) was diluted in 0.4X PBS pH 8.0 to a stock concentration of 3.25 units/µl and 65 units was added per 100 µl to the remaining reactions. One sample contained no caspase was used as a negative control. Samples were incubated for 30 minutes at 30°C. 5 µl of a 7.5 mg/ml stock of α-iodoacetamide in 0.4X PBS pH 8.0 was added to halt the reaction. Samples were transferred to a 96-well plate and emission spectra were read on Tecan Safire Dual Monochromator Multifunction Microtiter Plate Reader (Tecan, U.S.A.).



Supplementary Figure 2: Representative composite and deconvoluted component emission spectra from assay data for the negative control (7.5 picoMoles QD, no enzyme) and samples containing 7.5 and 15 picoMoles of QD self-assembled with substrate 1-mCherry (~3.5 mCherry/QD) exposed to enzyme. Note the difference in mCherry to QD acceptor:donor ratio in the absence and presence of enzyme. Data were converted into activity or velocity by comparison to the calibration curve as described. Note that although the same ratio is seen for the 7.5 and 15 picoMoles QD substrates, the latter has a higher velocity or activity due to the larger amount of substrate present for the same enzyme concentration.

Data Analysis

The ratio of donor:acceptor PL at 610 nm and 550 nm is used to determine the average number of mCherry/QD in the negative control by direct comparison to the calibration curve above. For each assay point, the same process indicates the remaining ratio of mCherry/QD following proteolysis and by comparison to the negative control, the ratio cleaved during proteolysis is derived (see Supplementary Figure 2). This ratio is then multiplied by the total concentration of QD used at each point to determine the total amount of mCherry cleaved per reaction point. The values are then converted into concentration and divided by the time (30 mins) to obtain the enzymatic velocity which is presented in units of nM mCherry/cleaved per min. Fitting the data with the Michaelis-Menton equation (Equation 2) allows us to estimate the corresponding Michaelis constants K_M and maximal velocity V_{max}.

References

(1) Mattoussi, H.; Mauro, J. M.; Goldman, E. R.; Anderson, G. P.; Sundar, V. C.; Mikulec, F. V.; Bawendi, M. G. *J. Am. Chem. Soc.* **2000**, *122*, 12142-12150.

(2) Medintz, I.L.; Clapp, A.R.; Brunel, F.M.; Tiefenbrunn, T.; Uyeda, H. T.; Chang, E.L.; Deschamps, J.R.; Dawson, P.E.; Mattoussi, H. *Nat. Mat*. **2006**, *5*, 581-589.