Supporting Information

Quantum-dot-based Nanosensor for RRE IIB RNA-Rev Peptide Interaction Assay

Chun-yang Zhang and Lawrence W. Johnson*
Department of Chemistry, York College and The Graduate Center, The City University of New York, Jamaica NY 11451, USA

Experimental Methods

1. Material

Biotinylated 34-mer RRE IIB RNA (5'-Biotin-GGU CUG GGC GCA GCG CAA GCU GAC GGU ACA GGC C-3') with more than 95% purity was obtained from Dharmacon, Inc. (Lafayette, CO). Cy5-labeled 22-amino acidarginnine-rich peptide (Cy5-Thr-Arg-Gln-Ala-Arg-Arg-Asn-Arg-Arg-Arg-Arg-Trp-Arg-Glu-Arg-Gln-Arg) was purchased from Phoenix Pharmaceuticals, Inc. (Belmont, CA). Streptavidin-coated 605QDs were obtained from Quantum Dot Corp., (Hayward, CA). Neomycin B was purchased from Sigma (St. Louis, MO). All other reagents and buffers were obtained from Sigma and used without further purification.

2. RRE RNA –Rev Interaction

RRE RNA concentrations were determined by measuring the absorbance at 260 nm using an extinction coefficient of 322.9 $mM^{-1}cm^{-1}$. The Cy5-labeled Rev concentration was determined spectroscopically at 649 nm using an extinction coefficient of 250 $mM^{-1}cm^{-1}$. The interaction of RRE RNA with Rev was performed at 20 ºC in the standard buffer solution containing 140 nM NaCl, 5mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$

and 20 mM HEPES (pH7.4). Three replicate samples were prepared at each concentration. The 605QD fluorescence and Cy5 fluorescence were measured following 15 min. incubation with streptavidin-coated 605QD.

3. Steady-State Fluorescence Measurements of Rev-RRE RNA Interaction

The fluorescence of 605QD and Cy5 was measured at 20 ºC on a fluorescence spectrometer (LS50B, PerkinElmer, Inc., Wellesley, MA). Emission spectra were recorded over the wavelength range of 550-725 nm using a scan rate of 200 nm/min and an excitation wavelength of 488 nm. An excitation and emission bandwidth of 2.5 nm was used. The temperature was controlled with thermostated bath. For every point, three measurements were made, and their average values were used for calculation. The dissociation constant for Rev binding to RRE RNA was determined by following both the decrease in fluorescence of 605QD at 605 nm and the increase in the fluorescence of Cy5 at 670 nm as a fixed concentration of RRE RNA was titrated with increasing amounts of Rev.

The dissociation constant (K_d) of the Rev-RRE complex was obtained by fitting the data of 605QD fluorescence intensity with equation 1

$$F = F_0 - \frac{(F_0 - F_f)}{2\times[R]} \times \left\{([R] + K_d + [L]) - \sqrt{([R] + K_d + [L])^2 - 4\times[R]\times[L]}\right\} \qquad (1)$$

where F_0 and F_f are the initial and final fluorescence intensities of 605QD, respectively, [R] is the total RRE concentration, [L] is the total concentration of Rev.

The dissociation constant was also calculated by fitting the data of Cy5 fluorescence intensity using equation 2

$$F = F_0 + \frac{(F_f - F_0)}{2 \times [R]} \times \left\{ ([R] + K_d + [L]) - \sqrt{([R] + K_d + [L])^2 - 4 \times [R] \times [L]} \right\} \qquad (2)$$

where all terms are the same as in equation 1, except that F_0 and F_f are the initial and final fluorescence intensities of Cy5, respectively.

When equation 1 or 2 was used to determine the dissociation constant, the value of [R] was held fixed, and the values of K_d, F_0 and F_f were allowed to vary to obtain the best fit to the data [1].

The errors were estimated by the standard uncertainties of the data from the best-fit theoretical curves. This method assumes that the standard uncertainty of the measurement is approximated by the standard deviation of the points from the fitted curve [2].

References

[1] Müller, B.; Restle, T.; Reinstein, J.; Goody, R.S.; *Biochemistry* 1991, 30, 3709-3715.

[2] Flannery, B.P.; Teukolsky, S.A.; Vetterling, W.T.; Numerical Recipes in Fortran, 2nd ed., Cambridge University Press, Cambridge, U.K. 1992.