Using azobenzene incorporated DNA aptamers to probe molecular binding interactions

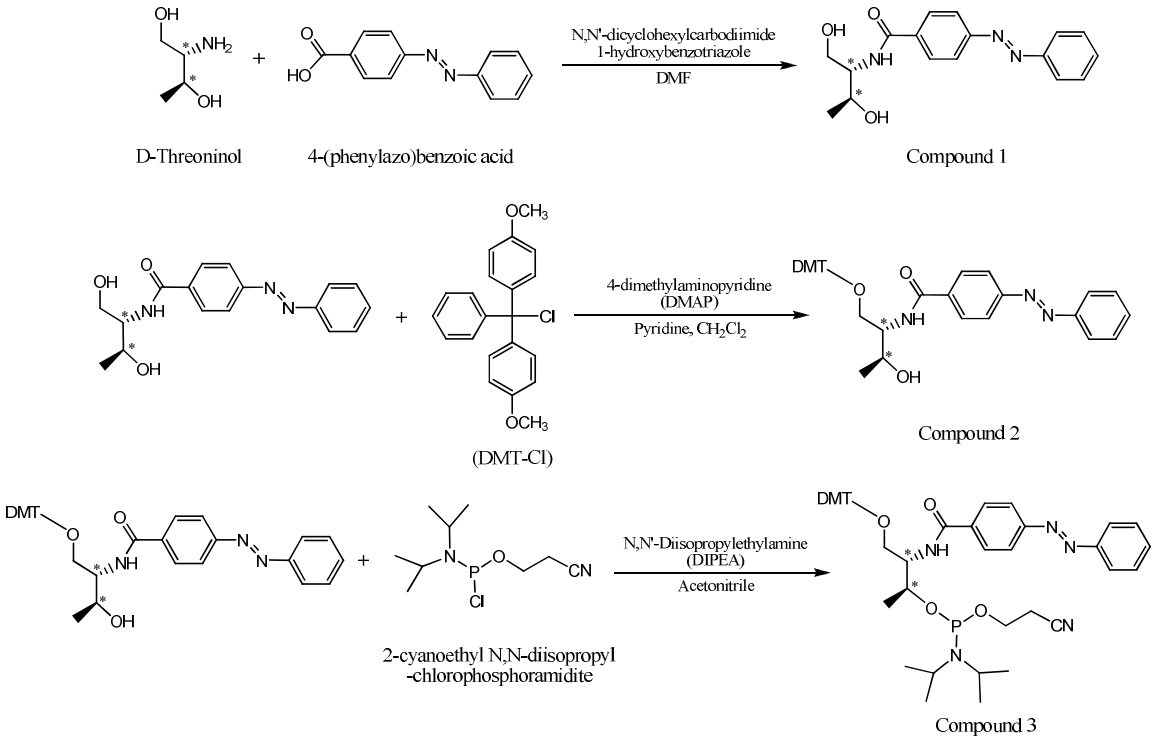
Joseph A. Phillips, Haipeng Liu, Meghan B. O'Donoghue, Xiangling Xiong, Ruowen Wang, Mingxu You, Kwame Sefah, and Weihong Tan*

Department of Chemistry and Department of Physiology and Functional Genomics, Shands Cancer Center and Center for Research at the Bio/nano Interface, University of Florida Genetics Institute and McKnight Brain Institute, University of Florida, Gainesville, FL 32611-7200. USA

*Corresponding author. Weihong Tan: Email: tan@chem.ufl.edu, Phone: (+1) 352-846-2410.

Running title: Use of azobenzene to control aptamer structure

Supporting Information



Scheme S1. Synthesis of azobenzene-tethered phosphoramidite monomer.

Derivation of dissociation constant equation:

We assume the standard equilibrium dissociation equation:

$$K_d = \frac{LR}{B} \tag{1}$$

where K_d is the dissociation equilibrium constant, L = concentration of free ligand (aptamer), R = concentration of free receptor, and B = concentration of bound receptor-ligand. In our measurement, the ratio of fluorescent signal to maximum fluorescent signal is equal to the ratio of the bound to total receptor:

$$\frac{Y}{Y_{max}} = \frac{B}{R_t} \tag{2}$$

Substituting for K_d, this equation can be written as:

$$\frac{B}{R_t} = \frac{B}{R+B} = \frac{1}{1+\frac{R}{B}} = \frac{1}{1+\frac{K_d}{L}} = \frac{L}{L+K_d}$$

Leading to:

$$Y = \frac{Y_{max}L}{L+K_d} \tag{3}$$

In our experiment, only the total ligand concentration is known, L_t = L+B, so this must be substituted into equation (3):

$$Y = \frac{Y_{max}(L_t - B)}{L_t - B + K_d} \tag{4}$$

Expanding this function about B = 0 using a Taylor series yields

$$Y = \frac{Y_{max}L_t}{L_t+K_d} - \sum_{n=1}^{\infty} \frac{Y_{max}K_d}{(L_t+K_d)^{n+1}} B^n \tag{5}$$

We can approximate this function by the first term as long as all other terms can be neglected. The second term is much less than the first term if:

$$B << \frac{L_t}{K_d}(L_t + K_d) \tag{6}$$

and all other terms are much less than the previous terms if $B << L_t+K_d$. As long as these relations are satisfied for all concentrations examined we can use the first term to fit our data. To test these relations, we can calculate B using K_d = 2 nM, the value for Sgc8c measured in this work, and an estimate of the total receptor concentration, R_t. The R_t estimate is based on fluorescence correlation spectroscopy measurements performed in our lab for Sgc8c. (Chen, Y., Munteanu, A., C., Huang, Y.-F., Phillips, J., Zhu, Z., Mavros, M., and Tan, W., (2009) Mapping receptor density on live cells by using fluorescence correlation spectroscopy. *Chem. Eur. J., 15,* 5327-5336) It was found that the target cells expressed 1,300 receptors per μm^2, which is roughly 130,000 per

cell. In our assay, 1,000,000 cells are incubated with aptamers in 1 mL solution, thus R_t = 1.3 x 10^{11} rec/mL = 0.22 nM. The equilibrium dissociation equation (1) can be solved for B, by substituting L = L_t-B and R = R_t-B which leads to a quadratic in B:

$$B^2 - B(K_d + R_t + L_t) + L_t R_t = 0 \tag{7}$$

Using the values for K_d and R_t just mentioned, and the lowest concentration of L_t used in this work, 1 nM, we can solve the quadratic equation and calculate B = 69 pM. This value is much lower than equation (6) which results in 1500 pM and L_t+K_d = 3000 pM. Therefore, under our conditions, the first term in equation (5) can be used to approximate this function, and all higher order terms can be neglected. Leading to the final form of equation (5) used for curve fitting:

$$Y = \frac{Y_{max} L_t}{L_t + K_d} \tag{8}$$