

Magnetic Levitation as a Platform for Competitive Protein- Ligand Binding Assays

Supporting Information

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General Methods. All chemicals and reagents were purchased from Sigma-Aldrich and used without further purification. PL-PEGA resin ($0.2 \text{ mmol}\cdot\text{g}^{-1}$, 300-500 μm diameter wet bead size) was produced by Varian Inc. and purchased from Agilent Technologies. The osmolality of the standard levitation solution (300 mM Gd(DTPA), and 0.05% polysorbate 20 dissolved in PBS buffer, pH 7.4) was determined by freezing point depression using an Advanced Instruments Model 3300 Osmometer. The magnets were purchased from Applied Magnets (www.magnet4less.com). The strength of the magnetic field at the surface of the magnets was measured using a handheld DC magnetometer (AlphaLab Inc, www.trifield.com). Procedures for determining the magnetic susceptibility and density of the standard levitation buffer, and quantifying the amount of protein bound per bead were performed as published previously.¹

Functionalization of PEGA Beads.

PEGA beads (0.25 g, 10% in methanol, containing $\sim 5 \mu\text{mol}$ primary amine) were rinsed three times with DMSO (5 mL) for 5 minutes. The beads were then suspended in a solution of DMSO (2 mL) containing an appropriate dye (e.g. Rhodamine isothiocyanate, 0.54 mg, $1 \mu\text{mol}$, 0.2 equiv.). After gently rocking the beads for two hours, the reaction media was removed and the beads were resuspended in a solution of DMSO (5 mL) to which the desired carboxylic acid (10 equiv., $50 \mu\text{mol}$) was added, followed by *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide (EDC, 10 equiv.), *N,N*-Diisopropylethylamine (30 equiv., $150 \mu\text{mol}$), and *N*-hydroxysuccinimide (NHS, 10 equiv.). This reaction mixture was gently rocked overnight, after which time the beads were thoroughly rinsed with DCM (2 x 10 mL), DMSO (2 x 10 mL), MeOH (2 x 10 mL), and 10 mM phosphate buffered saline, pH 7.4 (5 x 10 mL). The beads were stored in 10 mM phosphate buffered saline, pH 7.4, containing 0.1% w/v NaN_3 .

Procedure for measuring the efflux of protein from ligand-functionalized PEGA beads.

PEGA beads functionalized with 4-carboxybenzene sulfonamide as described above, were added to a centrifuge tube containing a solution of BCA (400 μM) dissolved in the standard levitation buffer (buffered 0.3 M Gd(DTPA)). These beads were gently rocked in this buffer for one week, or until the system reaches an equilibrium state as judged by the levitation height of the beads. After this time, a microcuvette was filled with a solution of a ligand of interest (e.g., 4-nitrobenzene sulfonamide) dissolved in the standard levitation solution at appropriate concentration (see Eqn. 7). A small portion of the functionalized PEGA beads (~ 15 beads) were removed from the solution of BCA and transferred to the solution of the ligand of interest. Practically, this transfer is most easily performed using a micropipette fitted with a truncated plastic tip; excess protein solution can be removed by blotting on a paper towel. After transferring the beads, the microcuvette was immediately transferred to a MagLev device. The levitation height of the beads can be monitored by eye or using a digital camera fitted with a macro lens.

Viscosity of the levitation solution.

The viscosity of the standard levitation buffer was determined by measuring the descent of a nylon sphere (nylon 6/6, purchased from McMaster Carr) through this buffer (Figure S1). We released a sphere from an initial position near the top of the solution (designated at $h = 0$), and it descended towards the bottom of the solution. Under these conditions, the motion of the sphere is described by Eqn. S1, where m (kg) is the apparent mass of the sphere in the solution, v (cm/s) is velocity, g (cm/s^2) is the acceleration due to gravity, μ ($\text{Pa}\cdot\text{s}$) is viscosity of the solution and r (cm) is the radius of the sphere.

$$m\dot{v} = -mg - 6\pi\mu r v \quad (\text{S1})$$

Together with the initial conditions, $t = 0$ s, $v = 0$ cm/s and $h = 0$ cm, this differential equation can be solved to give Eqn. S2.

$$h = -\frac{mg}{6\pi\mu r} \left(t + \frac{m}{6\pi\mu r} e^{-\frac{6\pi\mu r}{m}t} \right) + \frac{m^2 g}{36\pi^2 \mu^2 r^2} \quad (\text{S2})$$

We used a sphere with mass ~ 2.54 mg and diameter ~ 1.59 mm. The density of the MagLev solution is 1.099 g/cm³. Using these values, Eqn. S2 was fitted to the trajectory of the sphere to give $\mu = 0.006$ Pa·s (approximately six times the viscosity of water). Note that since $6\pi\mu r t / m \gg 1$, $\exp(-6\pi\mu r t / m) \approx 0$; as a result, the relationship between height and time is approximately linear as shown in Figure S1.

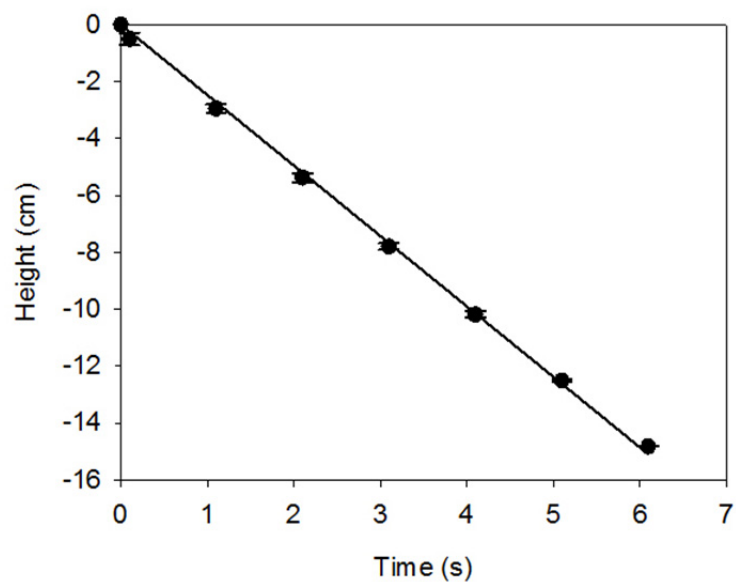


Figure S1. The rate of descent of a Nylon sphere (diameter ~ 1.59 mm) through the standard levitation buffer. These data were used to calculate the viscosity of the standard levitation buffer.

Determining the Optimal Concentration of the Soluble Ligand, $[L_S]_{sol}$

Under the standard conditions, where $[*PL]_0 \sim 3.2$ mM and $K_d^* = 85$ nM, we used Eqn. 7 to determine that the assay is optimally performed when the concentration of the soluble ligand, $[L_S]_{sol}$, is approximately 5,000 – 100,000 times the K_d of the protein-ligand complex (e.g. for a ligand with $K_d = 100$ nM, $[L_S]_{sol}$ should be 0.5 - 10 mM).

In certain situations it may be desirable to use a lower concentration of the soluble ligand. This can be accomplished either by lowering the concentration of the immobilized ligand, or by using an immobilized ligand with lower affinity for the target protein. The challenge inherent to these approaches is that they would lead to a situation where less protein would be then be bound to the beads at the start of the experiment. As a result, it would be necessary to measure a smaller overall change in density, and hence levitation height. One potential answer to this challenge, using a lower concentration of paramagnetic salt (this will increase the sensitivity of detection), is impractical: at the concentration of paramagnetic salt used (0.3 M Gd(DTPA)), the beads already require a significant period of time (~5-10 minutes) to approach the levitation height that describes their density (this pseudo-equilibration time is significantly increased when the magnetic force is lowered).

(1) Shapiro, N. D.; Mirica, K. A.; Soh, S.; Mace, C. R.; Taran, O.; Shevkoplyas, S. S.; Phillips, S. T.; Whitesides, G. M. *J. Am. Chem. Soc.* **2012**, *134*, 5637.