Supporting Information

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SI Text

Capillary Electrophoresis. A ProteomeLab PA 800plus capillary electrophoresis system from Beckman-Coulter was used to separate FAM-labeled TAR from FAM-labeled TAR-peptide complex. Fluorescence was induced by a 488-nm Argon-ion laser and detected at 520 ± 10 nm. A bare silica-fused capillary was used, 60 cm in total length, with 50 cm from injection to the detection point, with an outer diameter of 365 μm and an inner diameter of 75 μm. The injections were done by a pressure pulse with hydrodynamic injection volumes of 46.7 nL. The electric field during the separation was 500 V/cm, with the positive charge at the inlet and ground at the outlet. The capillary temperature was maintained at 22 °C for the duration of the experiment. The run buffer was 25 mM Borax (pH 8.9). Before each injection, the capillary was rinsed by applying 50.0 psi of 100 mM HCl, 100 mM NaOH, ddH_2O, and 25 mM Borax for 4 min each; 32 Karat Software Version 9.1 (Beckman-Coulter) was used for recording the electropherograms. K_d was calculated using the areas under the peak of the complex between peptide and FAM-labeled TAR, the decay of the complex, and free TAR as described below.

Nonequilibrium Capillary Electrophoresis of Equilibrium Mixtures. In nonequilibrium capillary electrophoresis of equilibrium mixtures (NECEEM), the target, T, (in this case the peptide) and the ligand, L (FAM-labeled TAR), are incubated before injecting the mixture in the capillary, to allow complex C to form. Once an external voltage is applied, the separation of the equilibrium complex from free ligand is observed under nonequilibrium conditions; the separation is based on differential electrophoretic mobility of T, L, and C. The run buffer, however, does not contain any target or ligand species, so the mixture is no longer in equilibrium and starts to dissociate as it migrates through the capillary. The dissociation constant K_d can be directly calculated from the areas under the peaks of the free ligand, the complex, and the decay between the two

$$K_d = \frac{[T]_0\{1 + A_L/(A_D + A_C)\} - [L]_0}{1 + (A_D + A_C)/A_L},$$

where $[T]_0$ is the initial concentration of the target species, $[L]_0$ is the initial concentration of the ligand, A_L is the area under the peak of free ligand, A_D is the decay area in between the ligand and complex peaks, and A_C is the area under the peak of the complex.