Supporting Information

Indicator Displacement Assays for Amino Acids Using Ni-NTA Tethered to PAMAM Dendrimers on Controlled Pore Glass

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1. Synthetic Procedures:
   i. CPG Preparation. Controlled pore glass (CPG500B), were prepared for use by refluxing in concentrated HNO₃ for 1-2 hours. The acid treated CPG were collected on a glass frit and washed with copious amount of de-ionized water until the filtrate was neutral in pH.

   ii. CPG Activation by CDI. In a typical reaction, 1,1-carbonyl diimidazole (2 g) in anhydrous 1,4-dioxane (0.4 M, 30 mL) was added to 1 g of CPG in a round bottomed flask and slowly rotated on a Buchi rotary evaporator overnight under nitrogen. The CDI activated CPG, (CPG-CDI), were collected on a medium pore glass frit and washed with 1,4-dioxane (3 x 100 mL). The collected CPG-CDI were dried under vacuum at room temperature for 30 minutes in a dessicator and used immediately for amine attachment.

   iii. PAMAM Dendrimer Attachment. Dendrimers were prepared for reaction by evaporating methanol from the commercially available methanolic PAMAM solutions, and the re-dissolving in several mL of DMSO. Dendrimers were covalently attached to CPG surfaces by adding PAMAM/DMSO solutions to CPG-CDI in a round-bottomed flask and reacting at room temperature for 3 days under nitrogen on a Buchi rotary evaporator. The mass of PAMAM needed to create a monolayer on the CPG surface was determined using the nominal dendrimer diameters and the CPG surface area.

   iv. CDI Activation of CPG-Gₓ. Dendrimer modified CPG, (CPG-Gₓ), were activated by reaction with CDI in dioxane (0.4 M) overnight in a round bottom flask with rotary mixing. The CDI activated, dendrimer modified CPG, (CPG-Gₓ-CDI), were collected on a medium porous glass frit, washed with 1,4-dioxane and dried in a vacuum dessicator at room temperature for 30-60 minutes. The CPG-Gₓ-CDI were used immediately.

   v. NTA Attachment. N,N-bis(carboxymethyl)-L-lysine, (0.032 g, 0.122 mmol) was dissolved in 10 mL of DMSO containing 3 equivalents (0.366 mmol) of triethylamine and 5 mM N-hydroxysuccinimide. This solution was added to CPG-Gₓ-CDI (1g) in a 50 mL round-bottomed flask and rotary mixed at room temperature for 2-3 days. The NTA modified product, (CPG-Gₓ-NTA), were collected on a medium porosity glass frit, washed with methanol, and dried in a vacuum dessicator.

2. Flow-cell Procedure:
   i. BPR uptake: Samples (20 mg) of modified CPG were loaded into 10 mL polypropylene chromatography columns (BioRad) and equilibrated for several hours in pH 7.1, 10 mM HEPES, 50 mM NaCl buffer. The dimensions of the CPG packing
inside the column were approximately 8x1mm. Using a flow-rate of 1 mL/min controlled by a peristaltic pump, 10 mL of 20 µM BPR solution was drawn out of a quartz cuvette, pumped through the packed column, and collected in the same cuvette, essentially creating a loop with the cuvette as the reservoir. The solution’s absorbance at 554 nm was measured with an Agilent 8453 UV-visible spectrometer every 30 seconds for 15-16 hours. The decrease in absorbance of the BPR solution with time was related to the dye uptake by the modified CPG material.

**ii. BPR release:** After 15 hours of monitoring BPR uptake, concentrated solutions of histidine were added (5-20 µL aliquots) into the quartz cuvette, and the absorbance response at 554 nm was monitored until the absorbance stabilized (20 minutes). The titration was continued until changes in absorbance were undetectable.

### 2. Metal Ion Binding Capacity by AAS

In a typical metal loading experiment, weighed samples of modified CPG were loaded into 10 mL polypropylene chromatography columns and equilibrated in pH 7.1, 10 mM HEPES for several hours. A 5 ppm solution of NiCl2 in pH 7.1, 10 mM HEPES was pumped through the column packing at a flow-rate of 0.5 mL/min using a peristaltic pump, and the effluent was collected in 5.0 mL samples as determined by a fraction collector. The effluent samples were then analyzed using a Shimadzu AA-6650 flame atomic absorption spectrophotometer for [Ni2+] until the effluent concentration reached that of the influent. At this point, the modified CPG was considered saturated with Ni2+, and the flow was stopped. To remove any non-specifically bound Ni2+, the loaded samples were washed with buffer and analyzed by AAS until the effluent was free of Ni2+. Figure S-1 shows a typical breakthrough curve for CPG-G5-NTA.

**Fig S-1:** Ni(II) breakthrough curve binding to CPG-G5-NTA, pH 7.1, 10 mM HEPES

The total Ni2+ loading capacities were calculated by summing the differences in [Ni2+] between influent and effluent for all of the collected samples, and then subtracting any metal removed in the non-specific buffer washing step.
total mg Ni$^{2+}$ = \( \sum_{i=1}^{n} (5 \text{ ppm} - x_i) \times V_{\text{sample}} \),

where \( x_i \) is the concentration of Ni$^{2+}$ in the sample, \( V_{\text{sample}} \) is the volume in liters of each collected sample, and \( n \) is the number of samples collected.

3. **Batch IDA with CPG-G₄-NTA-Ni-BPR**

   Samples of CPG-G₄-NTA-Ni-BPR (16.9 µmol Ni$^{2+}$/g) were weighed out (~4 mg) placed into 4 mL vials, and 2 mL of 20 µM BPR (pH 7.1, 10 mM HEPES, 50 mM NaCl) were added. After 2 hours of moderate shaking, the decrease in absorbance at 554 nm was measured and correlated with BPR uptake. To different vials was added 10 µL of concentrated stock substrate solution or solid substrate (depending on solubility restrictions) to achieve a final [substrate] of 400 µM. After 3 hours of moderate shaking, the spectrum of the supernatant solution was obtained, and the change in absorbance at 554 nm was related to indicator displacement by the substrate.

4. **Fig S-2:** (a) UV/vis absorption change of 20 µM BPR loading solution after uptake by 4 mg CPG-G₄-NTA-Ni. (b) After BPR displacement by 400 µM histidine.