Supporting Information For:

An Affinity Membrane Chromatography Method For Antibody Purification Via Nucleotide Binding Site Targeting With A Small Molecule

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SUPPORTING INFORMATION FIGURES:

Figure S1: Membrane functionalization and characterization by FTIR.

Figure S2: Acetone pulse injection on m-NBST column to assess column-packing characteristics.

Figure S3: Determination of Dynamic Binding Capacity of m-NBST column

Figure S4: SDS-PAGE demonstrating flow through collections of impurity injections

Figure S5: Effect of NaCl to antibody capture efficiency by m-NBST affinity column

Figure S6: Quant-iT™ PicoGreen dsDNA High Sensitivity Assay Kit standard curve

Figure S7: Host cell protein content standard curve using a 3rd generation CHO HCP ELISA kit from Cygnus Technologies.

Figure S8: Screening of CD20 expression for Rituximab binding on IM9 and H929 cell lines

Figure S9: Comparison of EQ Buffer Chromatograms.
Supporting Information Figures

Figure S1: Functionalization of RC membranes with tryptamine molecule. A) Immobilization of tryptamine ligand on RC membrane. B) Characterization of modified RC membranes by FTIR analysis. Peaks at 1788 cm\(^{-1}\) and 1662 cm\(^{-1}\) can confirm the carboxylation of membrane. Peaks between 1400-1600 cm\(^{-1}\) representing C-C stretches in an aromatic ring can be observed in tryptamine-functionalized membrane.

Figure S2: Acetone injection (30 μL) on m-NBST column. An acetone pulse was injected onto the m-NBST column to determine the theoretical number of plates based on peak retention time (t\(_r\)) and the width of the peak at 1/10 maximum peak intensity (W\(_b\)) plugged into the below equation to get N=107.09. For an appropriate pulse test, it is necessary to inject a sufficiently large volume of organic solvent so that it remains undiluted by the hold-up volumes. Generally, it is suggested that injection volumes is 1–2.5% of the column volumes. Therefore, we used 30
µL of acetone for 250 µL hold-up volume column, which results 12%. The HEPT (height equivalent to the theoretical plates) value is calculated by taking the column length (2 cm) and dividing it by the calculated theoretical number of plates to get HEPT = 0.019 cm. Peak asymmetry, 1.096, was determined using Agilent Technologies ChemStation LC Software.

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N = 16 \left( \frac{t_r}{W_b} \right)^2
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Figure S3: Increasing concentration of the antibody was loaded on the m-NBST column, and percent recovery yield was calculated by integrating the area under the flow trough and elution peaks. The largest amount of antibody injected on the column was 20 µg of Rituximab (10 µL of 2 mg/mL) into a column volume of 250 µL with >98% recovery yield. To calculate the 10% breakthrough dynamic binding capacity of the column, a linear equation was fitted to the recovery yield data. 10% breakthrough dynamic capacity of the m-NBST column was calculated ~0.9 g/L by using the fitted equation: \( y = -10.247x + 98.96 \) with \( R^2 = 0.96181 \).

Figure S4: Flow through (FT) fractions of impurity injections were run on a 10% SDS-PAGE gel under reducing conditions (Lines 2-7). Rituximab (Line 1), collected from elution fraction, was also run on the gel to compare with the molecular weight of the impurities. Characteristic bands
for impurities were observed at expected molecular weight; BSA (66 kDa), Supernatant (~66 KDa due to albumin), Ascites (66 kDa, 75 kDa), 373 and H929 Cell Lysates and Cell Extract (small fractions of proteins with various sizes).

**Figure S5:** A) Chromatograms illustrating the effect of NaCl concentration in the injection buffer on antibody capture efficiency by tryptamine column. B) Normalized peak integration values of the flow through and elution fractions are shown for the above injections.
Figure S6: Quant-iT™ PicoGreen dsDNA High Sensitivity Assay Kit standard curve. The amount of dsDNA present in the samples was determined based on dye fluorescence with a 485 nm excitation and 523 nm emission using the provided standard concentrations of dsDNA and by following the manufacturer recommended protocol. The data was fit by linear regression with $R^2$ value of 0.998. Data represents the means (±SD) of triplicate experiments.

Figure S7: Host cell protein (HCP) content standard curve was determined using a 3rd generation CHO HCP ELISA kit from Cygnus Technologies. The recommended high sensitivity assay as provided by the manufacturer was followed.
Figure S8: Screening of IM9 and H929 cell lines using flow through and elution fractions of purified Rituximab by m-NBST column to access CD20 expression levels. CD20 expression levels in IM9 and H929 cell lines were determined in a flow cytometer assay. A total of $1 \times 10^5$ cells/well were plated 24 h prior, and next day washed with PBS and then incubated with purified Rituximab fractions on ice for 3 h. Binding was detected using a secondary Fc specific fluorescein labeled antibody.

Figure S9: EQ Buffer injection (10 μL) chromatographs were remain the same after series of antibody and other protein injections, indicating that all samples loaded to the column were fully eluted during elution and re-equilibration time. Thus, this result validates the stability and re-usability of the m-NBST column. EQ Buffer chromatogram was used as a background and subtracted from sample injection chromatograms.