**Table S1.** Physicochemical properties of tests solutes

| Fragment | Name | Cas Number | log D (pH 7.4) | Charge | H-Bond Donor Count (PubChem) | H-Bond Acceptor Count (PubChem) |
|---|---|---|---|---|---|---|
| 1 | Benzylamine | 100-46-9 | -0,56 | cationic | 1 | 1 |
| 41 | 5-Phenyl-2-furoic acid | 52938-97-3 | -0,35 | anionic | 1 | 3 |
| 54 | 4-(Aminomethyl)pyridine | 3731-53-1 | -1,3 | cationic | 1 | 2 |
| 57 | Histamine | 51-45-6 | -2,71 | cationic | 2 | 2 |
| 62 | 2-Phenylimidazole | 670-96-2 | 1,64 | neutral | 1 | 1 |
| 66 | 1,1,4,7,10,10-Hexamethyltriethylenetetramine | 4856-97-7 | 0,57 | neutral | 2 | 2 |
| 70 | 4-(2-Keto-1-benzimidazolinyl)piperidine | 20662-53-7 | -1,13 | cationic | 2 | 2 |
| 74 | 2,2'-(Ethane-1,2-diyl)dianiline | 34124-14-6 | 1,99 | neutral | 2 | 2 |
| 88 | Thieno[3,2-b]pyridin-7-ol | 107818-20-2 | -0,34 | neutral | 1 | 3 |
| 93 | 4,4′-Dihydroxybiphenyl | 92-88-6 | 2,26 | neutral | 2 | 2 |
| 117 | 2-Amino-3-benzyloxypyridine | 24016-03-3 | 1,53 | neutral | 1 | 3 |
| 125 | 1-Methyl-2-pyrrolecarboxylic acid | 6973-60-0 | -1,86 | anionic | 1 | 2 |
| 139 | 2-Phenyl-2-imidazoline | 936-49-2 | -0,59 | cationic | 1 | 1 |
| 150 | Salicylic acid | 69-72-7 | -0,77 | anionic | 2 | 3 |
| 153 | 2,4-Dihydroxybenzophenone | 131-56-6 | 3,16 | anionic | 2 | 3 |
| 159 | 3-Furoic acid | 488-93-7 | -1,97 | anionic | 1 | 3 |
| 161 | Sulfacetamide | 144-80-9 | -2,17 | anionic | 2 | 4 |
| 168 | 3-Cyano-6-methyl-2(1H)-pyridinone | 4241-27-4 | -0,29 | neutral | 1 | 2 |
| 169 | Sulfabenzamide | 127-71-9 | -0,62 | anionic | 2 | 4 |
| 195 | 1-Benzoylpiperidine | 776-75-0 | 1,95 | neutral | 0 | 1 |
| 209 | Biphenyl-4-carboxylic acid | 92-92-2 | 0,67 | anionic | 1 | 2 |
| 266 | Benzhydrylamine | 91-00-9 | 1,65 | cationic | 1 | 1 |
| 271 | 1,2,3,4-Tetrahydro-9-aminoacridine | 321-64-2 | 0,89 | cationic | 1 | 2 |
| 286 | (1R,2S)-(−)-2-Amino-1,2-diphenylethanol | 23190-16-1 | -0,08 | cationic | 2 | 2 |
| 288 | 2,3-Diaminopyridine | 452-58-4 | 0,12 | cationic | 2 | 3 |
| 294 | 1-(1H-Pyrrol-2-yl)ethanone | 1072-83-9 | 0,99 | neutral | 1 | 1 |
| 297 | 5-Amino-1-phenylpyrazole-4-carboxamide | 50427-77-5 | 0,83 | neutral | 2 | 3 |
| 298 | 1-Phenyl-1,2,3,4-tetrahydropyrrolo[1,2-a]pyrazine | 112758-89-1 | 2,53 | cationic | 1 | 1 |
| 302 | 4-Aminobiphenyl | 92-67-1 | 2,71 | neutral | 1 | 1 |
| 336 | trans-Ferulic acid | 537-98-4 | -1,38 | anionic | 2 | 4 |
| 346 | 2-Thiouracil | 141-90-2 | -3,6 | neutral | 2 | 2 |
| 366 | 3-Amino-2-chloropyridine | 6298-19-7 | 0,97 | neutral | 1 | 2 |
| 368 | 4-Aminopyridine | 504-24-5 | -2,41 | cationic | 1 | 2 |
| 378 | 2,6-Dichloropyridine | 2402-78-0 | 2,1 | neutral | 0 | 1 |
| 393 | 1-(4-Sulfophenyl)-3-methyl-5-pyrazolone | 89-36-1 | -4,31 | anionic | 1 | 5 |
| 411 | Ethyl 5-amino-1-phenyl-4-pyrazolecarboxylate | 16078-71-0 | 2,79 | neutral | 1 | 4 |
| 412 | 2-(carboxymethylthio)-4-methylpyrimidine | 46118-95-0 | -3,15 | anionic | 1 | 5 |
| 445 | 4-Aminohippuric acid | 61-78-9 | -4 | anionic | 3 | 4 |
| 449 | Orotic acid | 65-86-1 | -4,75 | anionic | 3 | 4 |
| 725 | Adenine | 73-24-5 | -2,06 | neutral | 2 | 4 |
| 771 | 4-Chlorophenyl methyl sulfone | 98-57-7 | 1,45 | neutral | 0 | 2 |

| Fragment | Name | Cas Number | log D (pH 7.4) | Global charge | H-Bond Donor Count (PubChem) | H-Bond Acceptor Count (PubChem) |
|---|---|---|---|---|---|---|
| 1 | Benzylamine | 100-46-9 | -0,56 | 1 | 1 | 1 |
| 41 | 5-Phenyl-2-furoic acid | 52938-97-3 | -0,35 | -1 | 1 | 3 |
| 54 | 4-(Aminomethyl)pyridine | 3731-53-1 | -1,3 | 1 | 1 | 2 |
| 57 | Histamine | 51-45-6 | -2,71 | 1,8 | 2 | 2 |
| 62 | 2-Phenylimidazole | 670-96-2 | 1,64 | 0,3 | 1 | 1 |
| 66 | 1,1,4,7,10,10-Hexamethyltriethylenetetramine | 4856-97-7 | 0,57 | 0 | 2 | 2 |
| 70 | 4-(2-Keto-1-benzimidazolinyl)piperidine | 20662-53-7 | -1,13 | 1 | 2 | 2 |
| 74 | 2,2'-(Ethane-1,2-diyl)dianiline | 34124-14-6 | 1,99 | 0 | 2 | 2 |
| 88 | Thieno[3,2-b]pyridin-7-ol | 107818-20-2 | -0,34 | 0 | 1 | 3 |
| 93 | 4,4′-Dihydroxybiphenyl | 92-88-6 | 2,26 | 0 | 2 | 2 |
| 117 | 2-Amino-3-benzyloxypyridine | 24016-03-3 | 1,53 | 0 | 1 | 3 |
| 125 | 1-Methyl-2-pyrrolecarboxylic acid | 6973-60-0 | -1,86 | -1 | 1 | 2 |
| 139 | 2-Phenyl-2-imidazoline | 936-49-2 | -0,59 | 1 | 1 | 1 |
| 150 | Salicylic acid | 69-72-7 | -0,77 | -1 | 2 | 3 |
| 153 | 2,4-Dihydroxybenzophenone | 131-56-6 | 3,16 | -0,5 | 2 | 3 |
| 159 | 3-Furoic acid | 488-93-7 | -1,97 | -1 | 1 | 3 |
| 161 | Sulfacetamide | 144-80-9 | -2,17 | -1 | 2 | 4 |
| 168 | 3-Cyano-6-methyl-2(1H)-pyridinone | 4241-27-4 | -0,29 | -0,3 | 1 | 2 |
| 169 | Sulfabenzamide | 127-71-9 | -0,62 | -1 | 2 | 4 |
| 195 | 1-Benzoylpiperidine | 776-75-0 | 1,95 | 0 | 0 | 1 |
| 209 | Biphenyl-4-carboxylic acid | 92-92-2 | 0,67 | -1 | 1 | 2 |
| 266 | Benzhydrylamine | 91-00-9 | 1,65 | 1 | 1 | 1 |
| 271 | 1,2,3,4-Tetrahydro-9-aminoacridine | 321-64-2 | 0,89 | 1 | 1 | 2 |
| 286 | (1R,2S)-(−)-2-Amino-1,2-diphenylethanol | 23190-16-1 | -0,08 | 1 | 2 | 2 |
| 288 | 2,3-Diaminopyridine | 452-58-4 | 0,12 | 0,5 | 2 | 3 |
| 294 | 1-(1H-Pyrrol-2-yl)ethanone | 1072-83-9 | 0,99 | 0 | 1 | 1 |
| 297 | 5-Amino-1-phenylpyrazole-4-carboxamide | 50427-77-5 | 0,83 | 0 | 2 | 3 |
| 298 | 1-Phenyl-1,2,3,4-tetrahydropyrrolo[1,2-a]pyrazine | 112758-89-1 | 2,53 | 0,7 | 1 | 1 |
| 302 | 4-Aminobiphenyl | 92-67-1 | 2,71 | 0 | 1 | 1 |
| 336 | trans-Ferulic acid | 537-98-4 | -1,38 | -1 | 2 | 4 |
| 346 | 2-Thiouracil | 141-90-2 | -3,6 | -0,2 | 2 | 2 |
| 366 | 3-Amino-2-chloropyridine | 6298-19-7 | 0,97 | 0 | 1 | 2 |
| 368 | 4-Aminopyridine | 504-24-5 | -2,41 | 1 | 1 | 2 |
| 378 | 2,6-Dichloropyridine | 2402-78-0 | 2,1 | 0 | 0 | 1 |
| 393 | 1-(4-Sulfophenyl)-3-methyl-5-pyrazolone | 89-36-1 | -4,31 | -1 | 1 | 5 |
| 411 | Ethyl 5-amino-1-phenyl-4-pyrazolecarboxylate | 16078-71-0 | 2,79 | 0 | 1 | 4 |
| 412 | 2-(carboxymethylthio)-4-methylpyrimidine | 46118-95-0 | -3,15 | -1 | 1 | 5 |
| 445 | 4-Aminohippuric acid | 61-78-9 | -4 | -1 | 3 | 4 |
| 449 | Orotic acid | 65-86-1 | -4,75 | -1 | 3 | 4 |
| 725 | Adenine | 73-24-5 | -2,06 | 0 | 2 | 4 |
| 771 | 4-Chlorophenyl methyl sulfone | 98-57-7 | 1,45 | 0 | 0 | 2 |

- *Nano-FAC data processing for column characterization (protein specific interactions (active binding sites, affinity (dissociation constant)) and non-specific interactions)*

Nano-FAC experiments were used to determine the quantity of ligand capture at different ligand concentrations ($q_{captured}$). In staircase method, increasing concentrations of ligand were continuously injected into the nanocolumn (Figure S1 A). This allows to determine the cumulative captured quantities ($q_{captured\ cumul.}$) of ligand at each concentration step (n).

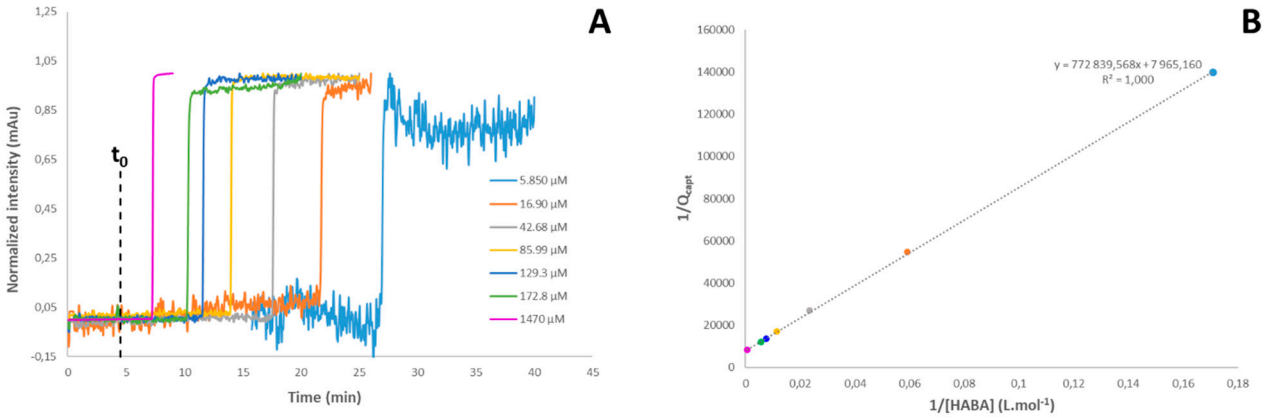


**Figure S1.** Frontal affinity chromatograms of 4'-Hydroxyazobenzene-2-carboxylic acid (HABA) on streptavidin-functionalized poly(DHPMA-co-MBA) monolith. A) Breakthrough curves of HABA at different concentrations in phosphate buffer (67mM, pH 7.4); UV detection at 347 nm; short injection of DMSO 0.1 % in water was used to mark the dead time. B) Plot of the reciprocal of the quantity of the specific captured quantity of HABA minus the non-specific captured quantity of HABA ($1/(cumulQ_{capt} - (K_{NS} \times [HABA]))$) ($\mu mol^{-1}$) versus the reciprocal of HABA concentration (1/[HABA]) ($L.mol^{-1}$)

If only specific interactions are observed, the plot of $\mathbf{1/q_{Cumul\ captured\ (n)}}$ vs 1/[L]n is linear. A deviation from linearity in the high concentration region of the plot indicates the presence of a non-specific interaction contribution. For unspecific interactions (if any), $\boldsymbol{q_{Cumul\ captured\ (n)}}$ is the sum of the amounts bound by specific interactions $\frac{B_{act} \times [L]_n}{K_d + [L]_n}$ and the amount of ligand captured by non-specific interactions $\boldsymbol{K_{non-specific} \times [L]_n}$, leading to equation S1:

$$\boldsymbol{q_{Cumul\ captured\ (n)} = \frac{B_{act} \times [L]_n}{K_d + [L]_n} + K_{non-specific} \times [L]_n} \qquad \text{(Equation S1)}$$

Assuming Knon-specific is constant, equation S1 can be written as follows:

$$\boldsymbol{\frac{1}{q_{captured} - K_{non-specific} \times [L]_n} = \frac{K_d}{B_{act}} \times \frac{1}{[L]_n} + \frac{1}{B_{act}}} \qquad \text{(Equation S2)}$$

The excel solver function is used to compute the $K_{non\text{-}specific}$ factor for which the best $r^2$ value is reached.

In case of presence of non-specific interaction, to determine the number of active site ($B_{act}$) and dissociation constant properly the plot of $1/[q_{\ cumul\ Capt\ (n)} - (K_{NS} \times [L]_n)]$ vs $1/[L]_n$ was used (figure S1B right).