

Extending the affinity range of Weak Affinity Chromatography for the identification of weak ligands targeting membrane proteins

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Supplementary Material S1 :

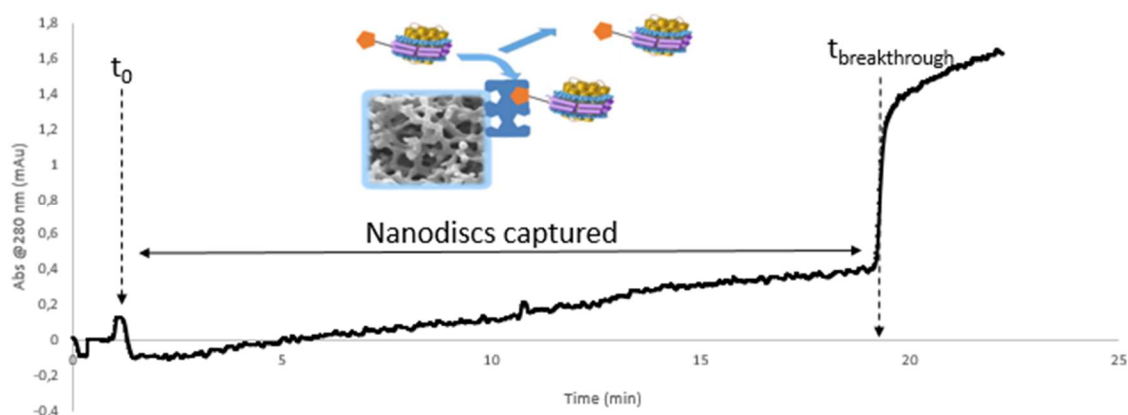


Figure S1. UV monitoring (@280 nm) of the capture of biotinylated AA_{2A}R nanodiscs by the streptavidin monolith. AA_{2A}R nanodiscs (2.6 μ M) solubilized in 50 mM HEPES pH 7.4, 150 mM NaCl; streptavidin monolith $l = 8.5$ cm i.d 75 μ M; applied pressure 12 MPa (0.14 cm.s⁻¹); $t_{capture} = t_{breakthrough} - t_0 = 18.3$ min; amount of nanodiscs captured = 1.7 pmol/cm.

Supplementary Material S2 :

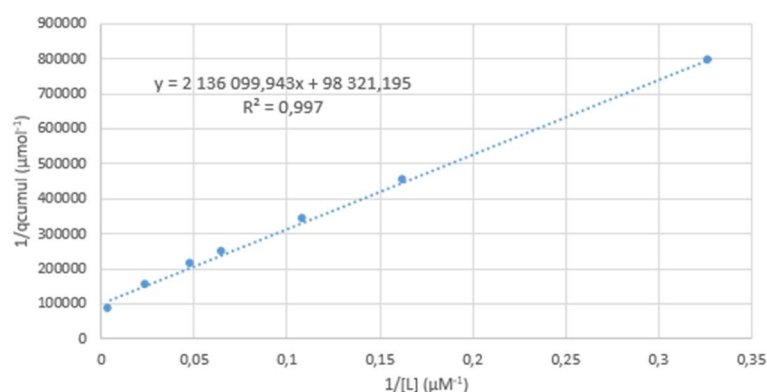
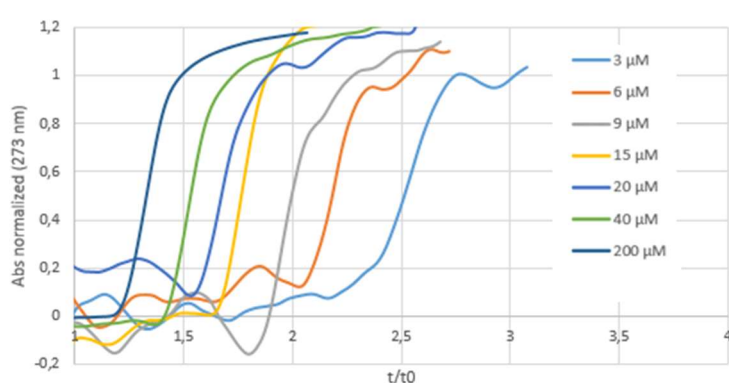


Figure S2. Frontal affinity chromatography experiments implemented to study the interaction between caffeine and immobilized AA₂R- Caffeine solutions (solubilized in acetate buffer 20mM pH=7.4) are percolated at increasing concentrations ranging from 3 to 200μM on a poly(GMA-co- EDMA) monolith column functionalized with NDs; . Column dimension : l=8.5 cm, i.d = 75 μM; applied pressure 12 MPa (0.14 cm.s⁻¹).

Supplementary Material S3 :

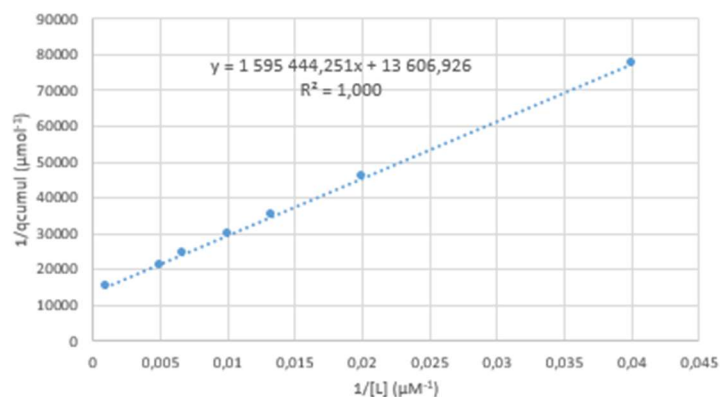
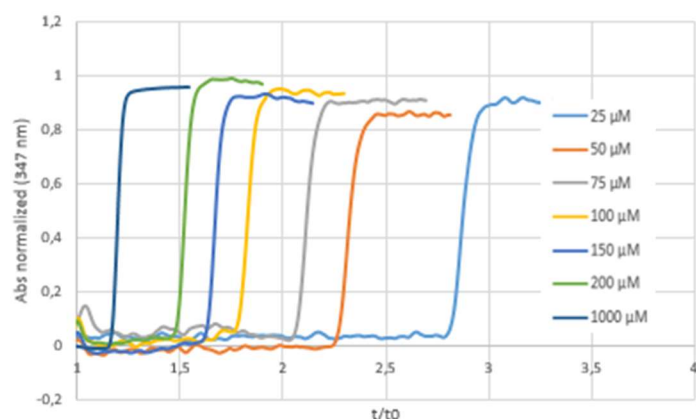


Figure S3. Frontal affinity chromatography analysis of the interaction between HABA and immobilized streptavidin on poly(GMA-co- EDMA) monolith column. Column dimension : l=8.5 cms i.d = 75 μM; applied pressure 12 MPa (0.14 cm.s⁻¹).

Supplementary Material S4 :

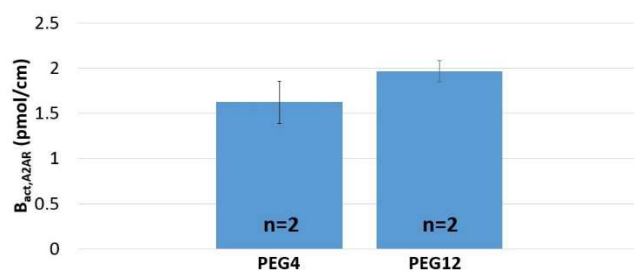


Figure S4. Comparison of the number of active binding sites determined on monolithic capillary columns using AA₂R nanodiscs functionalized with biotin-PEG4 and biotin-PEG 12 linkers.