Supporting Information

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Affinity Capturing and Surface Enrichment of a Membrane ProteinEmbedded in a Continuous Supported Lipid Bilayer

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Materials and Methods

Unless otherwise stated, all chemicals were purchased from Sigma Aldrich. All lipids were purchased from Avanti polar lipids.

Expression of full-length BACE1 (BACE)

Baculovirus containing fBACE was kindly provided by Prof. H. Danielsson, Uppsala University, Sweden (for details on generation of the viral stock, see Christeros et al[1]). The virus stock was amplified in Sf21 cells grown in SF900 II media supplemented with 10% fetal bovine serum (Life Technologies) at 27°C and 150 rpm. In brief, cells (1.5×10⁶ cells/ml) were infected at a cell-to-virus ratio of 50:1. The cells were harvested (3400×g for 15 min at 4°C) one week post infection and the supernatant was collected with a virus titer of 2×10⁸ c.f.u/ml. The virus stock was stored at -80°C. For protein production, Sf9 (or Sf21) cells were infected at mid–log (2×10⁶ cells/ml) using a cell-to-virus ratio of 1:2 and harvested 48h post infection. The cell pellet was washed twice in PBS buffer and stored at -80°C. Protein expression was analyzed by SDS-PAGE using NuPAGE gel system (Life Technologies). For Coomassie staining, the gel was transferred to Instant Blue (Expedeon). For Western blot, the PDVF membrane was blocked with 2×PBS containing BSA before incubation with primary anti-His mouse IgG (Clontech) and secondary anti-mouse IgG-alkaline phosphatase (Promega). Unbound antibodies were removed by washing in 2×PBS before development with 1-step Ultra TMB blotting solution (Thermo Fischer).

Generation of membrane preparations and purification of BACE

The cell pellet was thawed on ice, dissolved in lysis buffer (2×PBS, 1 mM EDTA and protease inhibitor cocktail, Roche) and disrupted using an UltraTurrax T25 (IKA) at 13000 rpm for 2×20 seconds. Unbroken cells were removed by centrifugation (1000×g for 10 min, Beckman Coulter). The supernatant was collected by ultracentrifugation (>100 000×g for 30 min, Beckman Coulter) and the pellet was resuspended in 2×PBS with protease inhibitor cocktail, homogenized with a dounce homogenizer. The pellet was collected once more by ultracentrifugation and dissolved in 2×PBS, 20% glycerol. Aliquots were snap frozen in liquid N₂ and stored at -80°C. Next day, the membrane prep was thawed on ice, solubilized under
stirring in 2×PBS, 2% Triton-X, protease inhibitors for 30 min at 4°C and homogenized with a dounce homogenizer. The soluble fraction was separated from the insoluble fraction by ultracentrifugation (>100 000×g for 30 min). flBACE was purified on two affinity columns based on immobilized Ni²⁺-NTA and substrate analogue inhibitor,[2] respectively. Briefly, the 5 ml HiTrap HP column (GE healthcare) was equilibrated in buffer A (2×PBS, 0.1% Triton-X) with 3% buffer B (2×PBS, 0.1% Triton-X, 0.4M imidazole pH 7.4) on an ÄKTA purifier (GE healthcare). After sample application (1.5 ml/min), the column was washed with 3% B followed by 8% B before eluting in 100% B. The eluate was pooled and the buffer was exchanged to 25 mM NaAc, 100 mM NaCl, 0.1% Triton-X, pH 4.5 using PD-10 columns (GE healthcare). A visible precipitate was formed and removed by centrifugation (4000×g for 15 min at 4°C). The sample was applied to an equilibrated column containing the substrate analogue inhibitor (Thermo Fischer) covalently coupled to NHS-activated Sepharose beads (GE healthcare). After sample application (2 ml/min), the column was washed before eluting in 2×PBS, 0.1% Triton-X, pH 7.4. Due to the limited binding capacity of the column, the flow-through was reapplied onto the column and eluted a second time. High purity was confirmed by SDS-PAGE. Protein concentration was estimated using absorbance at 280 nm (Nanodrop) and on gel using a BSA ladder of known concentrations. Aliquots were snap frozen in liquid N₂ and stored at -80°C.

FRET-based enzymatic assay

The catalytic activity of flBACE was assessed using the FRET-based TruPoint β-secretase assay kit (PerkinElmer). In short, enzyme (10 nM in Reaction buffer containing 100 mM NaAc, 1w% CHAPS, pH 4.5, as the active site of BACE will only bind substrate and substrate analogues at acidic conditions) was mixed with an equal volume of substrate peptide (0.2 µM) and incubated for 15 min. The reaction was monitored at λ_em=615 nm using excitation at λ_ex=330 nm using a Safire², microplate reader (Tecan).

Reconstitution of BACE in proteoliposomes

1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine (POPE), 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-(1′-rac-glycerol) (POPG) were dissolved in chloroform, mixed in a 3:1:1: ratio and dried under vacuum (>1h). The lipid film was rehydrated in 100 nM NaAc, 200 mM NaCl, pH 4.5, 10 mM Triton-X100 and the mixed micelles were stored at 4°C. Detergent-solubilized flBACE (0.05 mg/ml) was mixed with the micelles in a protein:lipid ratio of 1:3000 (a 150 nm liposome contains approximately 200 000 lipids) before addition of MQ-washed Biobeads SM2 (Bio-Rad) and incubated overnight with gentle mixing. The reconstitution process was monitored by dynamic light scattering (APS zetasizer, Malvern). Reconstitution efficiency was estimated using the enzymatic assay assuming that non-reconstituted BACE1 was inactive. Successful incorporation of BACE was verified with fluorescence polarization (FP) measurements using a Pherastar platereader (BMG Labtech) with appropriate filterset (540/590 nm). Polarization of the rhodamine-labeled substrate analogue (30 nM) was measured in the presence of proteoliposomes (5 mg/ml) with or without addition of the unlabeled substrate analogue (2 µM), or in empty liposomes.
**Generation of fluorescent and PEGylated liposomes**

Small unilamellar liposomes composed of 99.5 mol% 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) and 0.5 mol% N-palmitoyl-sphingosine-1-succinyl[methoxy(polyethylene glycol)5000] (PEG5kce) were produced by extrusion. Fluorescently labeled versions contained 99 wt% 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) and 1 wt% 2-(12-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)dodecanoyl-1-hexadecanoyl-sn-glycero-3-phosphocholine (NBD-PC) or 1 wt% 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl) (rhodamine-PE). Lipids dissolved in chloroform were mixed in the desired ratios and the organic solvent was evaporated under a stream of nitrogen followed by complete solvent removal under vacuum overnight. Lipid mixtures were rehydrated in PBS (10 mM sodium phosphate buffer and 150 mM NaCl, pH 7.5) at 1 mg/ml and extruded >10 times through a 100 nm polycarbonate membrane (Whatman) before stored at 4°C.

**Total internal reflection fluorescence (TIRF) microscopy**

All fluorescence micrographs were acquired with an inverted Nikon Ti-E microscope (Nikon Corporation, Tokyo, Japan), equipped with a 60× (NA = 1.49) Apo TIRF oil objective (Nikon, Melville, NY), FITC and TRITC filter sets (Semrock, Rochester, NY) and a vacuum cooled scientific CMOS camera (Neo, Andor Technology, Belfast, Northern Ireland), at a frame rate of 6 frames/min, except for FRAP measurements of lipid diffusivity where a frame rate of 0.5 frames/s was used. A mercury lamp (Super High Pressure Mercury Lamp C-SHG1, Nikon Corporation, Tokyo, Japan) was used for illumination and a perfect focus system (PFS) was used to compensate for focus drift with an accuracy of ±25 nm. Exposure times varied between 100 ms and 1 s, depending on what fluorophore and concentration that was used.

**Fluorescence recovery after photobleaching (FRAP) measurements**

FRAP measurements was used to determine the diffusivity of fluorescently labeled lipids and BACE bound to fluorescent substrate analogue (Rho-ELAN). A circular area (~30µm in diameter) was bleached using a diode-pumped solid state laser at 532 nm (BWN-532-100E; B&W TEK Inc., Newark, DE) for 2 s before initiating time-lapse imaging of the recovery process (6 images/min). The acquired images were analyzed using the Hankel transform method[6] and the data was fitted with a single exponential with an offset, yielding the diffusivity and immobile fraction of BACE or lipids.

**Fabrication of microfluidic channels**

The microfluidic channel was made of poly(dimethylsiloxane) (PDMS) by replica molding. The channel consisted of four channel arms in the shape of a cross. A master made of SU-8 (SU-8 2035, MicroChem Corp., Newton, MA, USA), defining the channel, was made with conventional optical lithography. The dimension of the master was characterized using an
optical surface profiler (Wyko NT 1100, Veeco Instruments Inc., Tucson, AZ, USA) to a width of 150 μm and a height of 110 μm for each channel arm. There is furthermore a $3 \times 3$ mm connection area at the end of each channel arm, where the inlet and outlet tubing are connected, with periodically spaced pillars to prevent the roof of the channel from collapsing.

The PDMS replica was made of a mixture of 10:1 Sylgard 184 and curing agent (Dow Corning, Midland, MI), which was allowed to cure for one hour at 95 °C. A glass slide (0.13-0.16 mm in thickness, Menzel-Gläser, Braunschweig, Germany) was first boiled for $>15$ min in a solution of Milli-Q™ and 2% Liqui-nox (Alconox, Whiteplaines, NY, USA) thoroughly rinsed with Milli-Q™ and dried in N₂, followed by additional drying at 150 °C for at least one hour. To obtain a strong seal between the PDMS and the glass slide, the contacting surfaces were treated with oxygen plasma (PDC-32G Plasma Cleaner, Harrick Plasma, Ithaca, NY; USA) before bonding the PDMS to the glass. Heating at 95 °C for 10 minutes improved the strength of the seal. The channels were filled with Milli-Q™ water (through capillary forces) immediately after bonding, to ensure that the surfaces remained hydrophilic. Prior to bonding the glass and the PDMS, access holes had been made in the PDMS, with a 1 mm hollow steel tube, at the four connection areas. Finally, silicon tubes (inner diameter 1.5 mm) were glued to the access holes using a silicone adhesive (Elastosil AO7, RTV-1 silicone rubber, Wacker Silicones, Munich, Germany), constituting the inlets and outlets of the device. A schematic illustration of the microfluidic channel and its dimensions are shown in Fig S1.

Figure S1. Schematic illustration of the microfluidic channel, seen from above. The average height of the channel was determined to be 113 μm with an optical profilometer.

Microfluidic setup and experimental details
To access the microfluidic device, PTFE tubing (1/16” outer diameter, 0.17 mm inner diameter, VWR International) was inserted into the silicone inlet and outlet tubes of the channel. Frictional forces hold the tubing in place. The suspensions and solutions were injected using plastic syringes and flow rates were controlled by one or up to three syringe pumps (NE-1000, New Era Pump Systems Inc., Wanatagh, NY), depending on the experiment.
Synthesis of bis-biotin-tris-NTA conjugate

Starting Materials. All chemicals were of reagent grade and used as commercially purchased without further purification. Compounds 1, 3 and 5 were synthesized according to published procedures.[3-5]

Analyses and physical measurements. Mass-spectrometric analyses were performed on a Bruker Esquire 3000 Plus MS using positive electrospray ionization (ESI) or a Sciex QSTAR XL using positive electrospray ionization and a tandem quadrupole time-of-flight mass analyzer (ESI+qTOF). For the ESI-MS analysis, the relative intensities are given in parenthesis (%). Both 1H (400 MHz) and 13C NMR (100 MHz) measurements were carried out on a 400 MHz spectrometer. The assignments in the 1D spectra were based on additional DEPT, HETCOR and COSY experiments.

2,2-dimethyl-4,15-dioxo-3,8,11-trioxa-5,14-diazaoctadecan-18-oic acid (2)

Succinic anhydride (85 mg, 0.85 mmol, 1.1 eq.) was added to a solution of 1 (0.22 g, 0.744 mmol, 1 eq.) in acetonitrile (10 mL). The mixture was stirred during 12 h at 40°C and the acetonitrile was evaporated under vacuum. The remaining oil was purified by chromatography (silica gel) eluting with CH2Cl2/MeOH 9:1 to afford 0.260 g (97%) of 4 as a yellowish oil. C15H28N2O7 ESI-MS: 349.2[M+H]+ (100%), 497.1[2M+H]2+ (43%).

Compound (4)

To a solution of 2 (0.177 g, 0.51·10⁻³ mol, 3.25 eq.) in dimethylformamide (6 mL), CDMT (0.098 g, 0.56·10⁻³ mol, 3.6 eq.) and NMM (0.17 mL, 1.5·10⁻³ mol, 9.8 eq) were added. After 15 min of stirring at room temperature, amino-biotin dimer 5 (0.143 g, 0.16·10⁻³ mol, 1 eq.) was added. The solution was stirred during 16 h at 50°C and the dimethylformamide was evaporated under vacuum. The remaining oil was purified by chromatography (silica gel) eluting with CH2Cl2/MeOH/NH3 88:10:2 to yield 0.123 g (64%) of 6 as a white foam. C55H89N11O16S2 ESI-MS: 1247.1 [M+Na]+ (100%), 635.0 [M+2Na]²⁺ (51%).
Scheme 1. Synthesis of bis-biotin-tris-NTA

**Compound (6)**

In a 5 mL round bottom flask, compound 4 (37.0 mg, 3.03·10⁻⁵ mol, 2 eq.) was dissolved in trifluoroacetic acid (0.07 mL, 9.10·10⁻⁴ mol, 30 eq.) and the inner wall of the flask was rinsed with dichloromethane (1.0 mL). The solution was stirred at room temperature during 1 h and the volatiles were removed under reduced pressure. Meanwhile, to a solution of 2 (23.1 mg, 1.51·10⁻⁵ mol, 1 eq.) in dry dimethylformamide (1.0 mL), CDMTNMM (4.7 mg, 1.71·10⁻⁵ mol, 1.1 eq.) and NMM (5 µL, 3.42·10⁻⁵ mol, 2 eq.) were added. After 15 min of stirring at room temperature, a solution of the freshly deprotected amine in dimethylformamide (1.5 mL) was
added. The mixture was stirred during 15h at 45°C and the dimethylformamide was evaporated under vacuum. The remaining oil was purified by chromatography (silica gel) eluting with CH$_2$Cl$_2$/MeOH/NH$_3$ 88:10:2 to afford 0.059 g (74%) of 7 as white foam. C$_{127}$H$_{212}$N$_{18}$O$_{37}$S$_2$ MALDI, ESI-MS: 1323.78 [M+2H]$^{2+}$ (100%)

**BisBiot-TrisNTA**

In a 50 mL round bottom flask, 7 (40 mg, ) was dissolved Phenol (100mg), TIS (100μl), ethanedithiol (100μl) and water (100μl) were freshly dissolved in trifluoroacetic acid (25 ml). The reaction mixture was stirred at room temperature for 4 hours and the volatiles were evaporated under vacuum. The oily residue was dissolved again in trifluoroacetic acid (5 ml). The product was precipitated with cold diethylether, filtered and dried.
Additional results

**BACE reconstitution in liposomes**

Full-length BACE was successfully expressed in Sf21 cells, solubilized in Triton-X100 and purified using dual affinity purification (columns containing Ni²⁺-NTA and the immobilized substrate analogue inhibitor) as confirmed by a single band on the SDS-PAGE gel (Fig. S2A). The enzymatic activity of the protein was confirmed by digestion of a FRET-peptide substrate (data not shown). BACE was subsequently reconstituted in liposomes consisting of PC, PE and PG in a 3:1:1 molar ratio with a lipid:protein ratio of 3000:1 which yielded approximately 30-40 proteins per liposomes (reconstitution efficiency was estimated to 50±20% using the enzymatic assay). Reconstitution was achieved by slow detergent removal using Biobeads SM2 from a mixture of detergent-solubilized protein and mixed micelles leading to the formation of proteoliposomes. The process was monitored by dynamic light scattering (Fig. S2B) and the size of the final proteoliposomes was determined to 170±70 nm. Protein incorporation was confirmed by fluorescence polarization (FP) measurements using a rhodamine-labeled substrate analogue (Rho-PI, Fig. S2C).

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**Figure S2A.** SDS-PAGE of purified BACE (arrow). **B.** Dynamic light scattering measurement before (dotted line) and after (solid line) liposome formation by detergent removal. **C.** Fluorescence polarization measurement using a fluorescent peptide inhibitor (Rho-PI). The retained polarization of the peptide upon binding to BACE-liposomes can be eliminated by competition with high concentration of the unlabeled peptide. The peptide does not bind unspecifically to empty liposomes.

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**Confirmation of functional bis-biotin-tris-NTA conjugate**

BACE proteoliposomes (0.5 mg/ml) was adsorbed on clean glass cover slides (boiled for >15 min in a solution of Milli-Q™ and 2% Liqui-nox, Alconox, Whiteplaines, NY, USA, thoroughly rinsed with Milli-Q™ and dried in N₂), together with a small fraction (1 µg/ml) of NBD-PC liposomes to follow the rupture process. A poly(dimethylsiloxane) (PDMS, 10:1 mixture, Sylgard 184 and curing agent, Dow Corning, Midland, MI, USA) slab with four punched holes was placed on top of the glass slide, forming four wells in which complementary experiments could be performed simultaneously. The surface was rinsed with HEPES buffer.
(10 mM HEPES, 100 mM NaCl, pH=7.4) before addition of bis-biotin-tris-NTA conjugate in HEPES buffer + 10 mM Ni^{2+} to one of the wells. Finally, the surface was rinsed before addition of streptavidin-FITC (10 ug/ml) and imaged (Fig. S3). As a control experiment the procedure was repeated in a second well, lacking the bis-biotin-tris-NTA conjugate

**Figure S3.** Functional validation of the bbtrisNTA conjugate. A. Chemical structure of the Bis-biotin-tris-NTA conjugate (bbtrisNTA) and schematic illustration of assay. B. Normalized average intensity from FITC-labeled streptavidin bound to SLB containing reconstituted 6xHis-tagged BACE in the presence and absence of bbtrisNTA after reference subtraction.

**Accumulation of BACE in PEG-supported lipid bilayer (PEG-SLB) using hydrodynamic forces**

A 6-way selection valve (Upchurch Scientific, Oak Harbor, WA, USA) was connected to the channel through connection area 1 (see Figure S1), respectively, in order to be able to switch between the different suspensions and solutions (liposomes, substrate and buffers). A PEG-supported lipid bilayer (PEG-SLB) was formed by adding BACE proteoliposomes and PEGylated liposomes (1:1 ratio, dissolved in 10 mM Tris, 100 mM NaCl, 1 mM EDTA, pH 7.4) together with 0.1 wt% fluorescently labeled POPC liposomes through channel arm 1 at 10 μL/min, while simultaneously flowing Tris buffer through channel arm 4 at 20 μL/min and keeping channel arms 2 and 3 open. Using the buffer counter flow ensures SLB formation solely in the left part of the microfluidic cross channel. After SLB formation and removal of excess liposomes by rinsing with Tris buffer, the valves connected to arms 2 and 3 were closed. The PEG-SLB and BACE was moved from left to right by applying a bulk flow of Tris buffer (set to 200 μL/min) from inlet 1 to outlet 4. After driving the SLB for 15 min, fluorescent Rho-PI (2 μM, dissolved in 100 mM NaAc, 200 mM NaCl, pH 4.5) was added (at 10 μL/min) to the BACE-containing SLB, followed by rinsing with Tris buffer in order to visualize the BACE and confirm a preserved APP binding pocket.

**Polymer-supported lipid membranes on functionalized surfaces**

Two 6-way selection valves (Upchurch Scientific, Oak Harbor, WA, USA) were connected to the channel through connection area 1 and 2 (Figure S1), respectively, in order to be able to switch between the different suspensions and solutions (liposomes, polymers, proteins and buffers). A PEG-SLB (POPC) was formed by adding PEGylated POPC liposomes labeled with 0.1 wt% NBD-POPC (dissolved in 10 mM Tris, 100 mM NaCl, 1 mM EDTA, pH 7.4) through
channel arm 1 at 10 μL/min, while simultaneously flowing Tris buffer through channel arm 4 at 50 μL/min and keeping channel arms 2 and 3 open. Using the buffer counter flow ensures SLB formation solely in the left part of the microfluidic cross channel. After SLB formation and removal of excess liposomes by rinsing with Tris buffer, Rhodamine labeled PLL-g-PEG (PLL-g-PEG-Rh, 1 μg/mL) was added through channel arm 4 at 30 μL/min, while simultaneously flowing Tris buffer through channel arm 1 at 30 μL/min for about 1 min. By then stepwise changing the flow rates of the two injecting syringe pumps attached to channel arm 1 and 4 and injecting for about 1 min for each step, a PEG-g-PEG-Rh coverage ladder was built up (Fig S4). The very last step was added at 20 μg/mL and until saturation was reached. The coverage of the different steps could then be estimated by comparing the fluorescence intensity to that of the saturated coverage. After rinsing with Tris buffer, the valves connected to arms 2 and 3 were closed. The SLB could now be moved from left to right by applying a bulk flow of Tris buffer (set to 200 μL/min) from inlet 1 to outlet 4. The PEG-SLB stopped its motion at the third step, corresponding to 40% of saturated coverage. While the second step corresponding to 25% of the saturated coverage, the maximum coverage allowing PEB-SLB motion is between 25% and 40%.

The same procedure was repeated for streptavidin conjugated to Fluorescein Isothiocynate (streptA-FITC), with some exceptions. The PEG-SLB was labeled with rhodamine-PE. There was no need for formation of a coverage ladder, since the PEG-SLB moved across a layer of streptA-FITC also at saturated surface coverage, which however, is far from a complete monolayer according to QCM-D data shown in Fig S5. The procedure was also repeated for a POPC SLB (lacking PEG) and the SLB could not be moved across the functionalized surfaces.

![Figure S4A. Fluorescence micrographs (190×190 um²) of PLL-g-PEG coverage ladder before and after formation and lateral movement of the SLB. The PEG-supported SLB is able to stretch over the absorbed polymer layer up to 25% of full coverage before further movement is prevented.](image)

**Figure S4A.** Fluorescence micrographs (190×190 um²) of PLL-g-PEG coverage ladder before and after formation and lateral movement of the SLB. The PEG-supported SLB is able to stretch over the absorbed polymer layer up to 25% of full coverage before further movement is prevented.

**Affinity capture of BACE in a PEG-supported lipid bilayer**
Two 6-way selection valves (Upchurch Scientific, Oak Harbor, WA, USA) were connected to the channel through connection area 1 and 2 (see Figure S1), respectively, in order to be able to switch between the different suspensions and solutions (liposomes, polymers, proteins, chelator, substrate and buffers). The two-dimensional affinity capture surface is formed as a stripe in the center of the four-arm crossing (see Figure 4A), by first injecting streptavidin conjugated to Fluorescein Isothiocyanate (streptA-FITC, 4 μg/mL) or PLL-g-PEG conjugated to Rhodamine or NTA (1:1 ratio dissolved in 10 mM Ni²⁺, 10 mM Tris, 100 mM NaCl buffer, Surface Solutions, Zürich, Switzerland) through arm 2, while running Tris buffer (10 mM tris, 100 mM NaCl, 1 mM EDTA) through arm 1 and 4. All flows were set to 10 μL/min. The streptavidin injection was followed by injection of bisbiotin-trisNTA (2 μM, dissolved in 10 mM Ni²⁺, 10 mM HEPES, 100 mM NaCl, pH 7.4 buffer), following the same injection pattern and building up the affinity capture stripe. The 6-way selection valve connected to arm 2 was turned and flow rates were increased to 30 μL/min, in order to rinse. Next step was to add BACE proteoliposomes and PEGylated liposomes (1:1 ratio) together with 0.1 wt% fluorescently labeled POPC liposomes through channel arm 1 at 10 μL/min, while simultaneously flowing Tris buffer through channel arm 4 at 70 μL/min and keeping channel arms 2 and 3 open. Using the buffer counter flow ensures SLB formation from the BACE/PEG liposome mixture solely in channel arm 1 of the microfluidic cross channel. After rinsing with Tris buffer, the valves connected to arms 2 and 3 were closed. The PEG-SLB and BACE was either moved across the stripe directly from left to right by applying a bulk flow of Tris buffer (set to 200 μL/min) from inlet 1 to outlet 4, or another turn of PEGylated POPC liposomes were added in front of the existing PEG-SLB (as an attempt to reduce unfavorable edge effects that may arise as the BACE accumulates in the front of the PEG-SLB), prior to the flow-induced motion of PEG-SLB and BACE. After driving the PEG-SLB for at least 1 hour, fluorescent Rho-PI (2 μM, dissolved in 100 mM NaAc, 200 mM NaCl, pH 4.5) was added (at 10 μL/min) to the BACE-containing SLB, followed by rinsing with Tris buffer in order to visualize the BACE and confirm a preserved APP binding pocket. Results for bbtrisNTA are shown in Figure 4B-D and PLL-g-PEG-monoNTA are shown in Fig. 4D and Fig. S5. Fig. S6 shows control experiment for bbtrisNTA (see main text).
**Figure S5A.** Schematic illustration of experimental procedure for affinity captures of 6×His-tagged BACE on NTA-functionalized PLL-g-PEG. 

**B.** Fluorescence micrograph overlay (200×90 µm²) of rhodamine-labeled substrate analogue bound to BACE (red) on adsorbed PLL-g-PEG-NTA-Ni²⁺ (grey). 

**C.** Intensity profile across channel (dotted line in B) in the presence of PLL-g-PEG-NTA (blue) and after subtracting the unspecific component of the signal (red). The signals have been subtracted from the rhodamine-labeled PLL-g-PEG signal (grey dashed line).

**Figure S6A.** Fluorescence micrograph (270×170 µm²) of rhodamine-labeled substrate analogue bound to BACE. 

**B.** Intensity profile (red) across channel (thick dotted line in B). Positive (light grey) and negative control (dark grey) intensity profiles are included for comparison.

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**Quartz crystal microbalance with dissipation (QCM-D)**

Streptavidin adsorption to SiO₂ was monitored by QCM-D. Prior to mounting the SiO₂-coated QCM-D crystals (AT-cut, f₀ = 5 MHz, Q-Sense AB, Sweden) were cleaned in 10 mM sodium...
dodecyl sulfate (SDS), rinsed in Milli-Q and UV-ozone treated for 10 min. Measurements were performed using a QCM-D E system (Q-Sense AB, Sweden). Streptavidin was added at 10 μg/ml. The result shows a final frequency shift of about -7 Hz (Fig S7B).

**Figure S7A.** Intensity profile across the microfluidic channel for a layer of adsorbed StreptA-FITC as a PEG-SLB moves across it. Dashed line I indicate the StreptA-FITC layer edge and dashed line II indicate the edge of the PEG-SLB. **B.** Changes in QCM resonance frequency, Δf, as Streptavidin is adsorbed to SiO₂ compared to a Streptavidin monolayer bound to a SLB containing biotinylated lipids[7].

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