Supporting Information

Charvolin *et al.* 10.1073/pnas.0807132106

SI Text

Dicyclohexylcarbodiimide (DCI), 1′-*N*-hydroxybenzotriazole (HOBT), sodium hydroxide, 3-{(3-cholamidopropyl)-dimethylammonio}-1-propanesulfonate (CHAPS), triethylamine, octylamine, and isopropylamine were purchased from Aldrich, poly(acrylic acid) (PAA) and sodium methoxide from Acros, *N*-(2-aminoethyl)biotinamide hydrobromide, D-biotin succinimidyl ester (biotinyl-NHS) and Alexa Fluor 647-conjugated α-bungarotoxin from Invitrogen, hydrochloric acid, ethanol, methanol, dimethylformamide and *N*-methylpyrrolidone (NMP) from SDS, *n*-octyltetraoxyethylene (C_8E_4) from Bachem, octyl-β-D-thioglucopyranoside (OTG) and dodecyl-β-D-maltoside from Anatrace. BioBeads were purchased from Bio-Rad, Amicon filtration devices from Millipore, GammaBind Plus Sepharose and Superose 6HR columns from Amersham Biosciences, uncertified SA-coated sensor chips and HBS-N buffer from Biacore GE Healthcare, SA-coated polystyrene beads from G. Kisker GbR. All solvents were used as received. Water was purified on a Milli-Q academic system equipped with a Q-Gard1 cartridge and 2 Organex cartridges from Millipore (Milli-Q water).

Synthesis of Amphipols. Nonbiotinylated A8-35 (batch FGH20) was synthesized as described in ref. 1. Briefly, PAA was dissolved in NMP at 60 °C and octylamine (1:4 molar ratio with respect to free carboxylic acid functions) was first introduced in the reactive medium before the DCI coupling reagent. The mixture was stirred for 1 h at 60 °C, then for 3 h at room temperature. Precipitated dicyclohexylurea (DCU) was removed by filtration and the resulting filtrate heated to 50 °C and reacted with isopropylamine (4:10 molar ratio with respect to initial free carboxylic acid function) in the presence of DCI and HOBT for 1 h, then for 3 h at room temperature. Filtration followed by purification and freeze-drying afforded the expected HAPol.

Two distinct protocols were developed for the synthesis of BA8-35. Batch 1 was synthesized by grafting biotin onto the amine function carried by a functionalized version of A8-35 (UAPol) (Fig. S3), whose synthesis involves the incorporation (according to the same procedure as described above) of a short and selectively *N*-monoprotected linker (see ref. 2). UAPol (0.5 g) was reacted with 0.052 g (3 eq.) of biotinyl-NHS in 20 ml DMF at 40 °C for 2 h. The solution was cooled and poured into 200 ml of 0.1 M HCl. The resulting biotinylated APol was purified as described in ref. 1 for nonfunctionalized APols. The final product was dialyzed for 2 days against Milli-Q water and freeze-dried, yielding 0.5 g of white powder (BA8-35-1).

Batch 2 was synthesized according to the procedure described in ref. 1 and above for nonfunctionalized APols, except for the inclusion of *N*-(2-aminoethyl)biotinamide along with octylamine at the first grafting step (Fig. S4). One gram of poly(acrylic acid) ($M \approx 5{,}000$ g·mol^{-1}) was modified first with 67 mg of *N*-(2-aminoethyl)biotinamide hydrobromide in the presence of 20 mg of triethylamine and 0.48 g of octylamine and then with 0.33 g of isopropylamine. Purification and freeze-drying yielded 1.6 g of BA8-35-2 (white powder).

The chemical composition of all APol batches (summarized in Fig. 1*A* in the main text) was determined by ^{1}H and ^{13}C NMR spectroscopy (Bruker Avance 400 MHz spectrometer) as described in ref. 3. BA8-35-1 and -2 contained $\approx$0.007 and $\approx$0.014 biotin, respectively, per acrylate unit. The values tabulated in Fig. 1*A* do not add up to 100% due (*i*) to a small fraction of the carboxylates ($\approx$2%) reacting with a side-product of the coupling agent (see refs. 1 and 3) and (*ii*) as regards BA8-35-1, to the coupling reaction between the amine function of UAPol and biotinyl-NHS not reaching completion (2).

Preparation of Membrane Proteins and Trapping in APols. BR, cytochrome b_6f, cytochrome bc_1, and nAChR were purified from the purple membrane of *Halobacterium salinarium* (4), from thylakoid membranes of *Chlamydomonas reinhardtii* (5), from beef heart mitochondrial membranes (6), and from the electric organ of *Torpedo marmorata* (7), respectively. tOmpA was overexpressed as inclusion bodies in *E. coli* and folded as described in refs. 8 and 9. Before trapping in APols, solutions of MPs were brought to a moderate salt concentration (100 mM NaCl) and a concentration of detergent close to its critical micellar concentration, namely 20 mM C_8E_4 for tOmpA, 15–17 mM OTG for BR, 0.2 mM dodecylmaltoside for both cytochrome b_6f and bc_1, and 10 mM CHAPS for nAChR. APols were added at weight ratios of 1:5 protein/BA8-35 for tOmpA, 1:4 (SPR experiments) or 1:2 (photocycle measurements) for BR, 1:3 for cytochrome b_6f and 1:1.5 for cytochrome bc_1. nAChR was trapped in a 3:1 mixture of BA8-35-2 and A8-35, at an overall nAChR/APol weight ratio of 1:3. The detergent was removed either by incubating MP/detergent/APol solutions with BioBeads (20 g of wet beads per gram of detergent) under stirring for 3 h at 4 °C (BR, tOmpA, nAChR), or by incubating several hours with BioBeads, followed by overnight incubation at 4 °C as a dilute solution and reconcentration on an Amicon filtration device (cutoff 50 kDa) (b_6f, bc_1). When appropriate (Fig. S1), free BA8-35 was separated from cytochrome bc_1/BA8-35 complexes by injecting 100 μL of a 15-g·l^{-1} protein solution onto a Superose 6HR column and eluting with HBS-N buffer.

Production and Purification of Antibodies. Antisera were produced by Agro-Bio. The proteins were injected to rabbits and sera collected after 49 days. Preimmune sera were collected before immunization. Antibodies were purified from sera by affinity chromatography on a GammaBind Plus Sepharose column following the manufacturer's instructions.

Immobilization of Proteins on SA Sensor Chips and Antibody Recognition. Surface plasmon resonance experiments were carried out on a Biacore 2000 instrument. Before use, SA sensor chips were docked into the instrument and preconditioned in HBS-N buffer [10 mM Hepes (pH 7.4) 150 mM sodium chloride] at 30 μL·min^{-1} by applying 3 consecutive 30-μL pulses of 1 M NaCl in 50 mM NaOH. MP immobilization was conducted at 25 °C in HBS-N running buffer flown at 10 μL·min^{-1}. Antibody recognition experiments were performed at 25 °C, at a flow rate of 10 μL·min^{-1}, by injecting 10 μL of purified antibody solution or crude sera, diluted 100 times in HBS-N buffer. Several injections of preimmune solutions were performed beforehand to minimize nonspecific binding.

Separation of Free APols from nAChR/APol Samples and Immobilization onto SA Beads. Free APol particles ($\approx$40 kDa; see ref. 1) and nAChR/BA8-35-2/A8-35 complexes were separated by size exclusion chromatography on a Superose 6HR column in a buffer comprising 100 mM NaCl, 5 mM NaH_2PO_4-Na_2HPO_4 (pH 7.4), and 1 mM EDTA (NaPh buffer). The fractions corresponding to nAChR/APol complexes were collected and concentrated on an Amicon filtration device (MW cutoff 100 kDa).

Immobilization was carried out by incubating for 1/2 h at room temperature under stirring 100 μg of 1.8-μm-diameter SA-coated polystyrene beads in 40 μL of NaPh buffer containing 0.1 μM APol-trapped nAChR, after which the beads were washed 3 times with 100 μL of NaPh buffer. To prevent nonspecific binding, this was followed by a 10-min incubation in 20 μL of BA8-35-2 solution (50 mg·l^{-1} in NaPh buffer). A negative control was made by incubating 100 μg of beads for 1/2 h with BA8-35-2 (0.25 g·L^{-1} in 40 μL of NaPh buffer), before washing them 3 times with 100 μL of NaPh buffer.

Monitoring Ligand Binding to Immobilized nAChR. Beads (7.5 μg) coated either with nAChR/BA8-35-2/A8-35 or with pure BA8-35-2 were incubated for 1 h at room temperature with 10 nM Alexa Fluor 647-conjugated α-bungarotoxin (BTx-647) in NaPh buffer. The same quantity of nAChR-coated beads was preincubated for 20 min at room temperature with 1 μM nonfluorescent α-bungarotoxin (BTx) and further incubated for 1 h with a mixture of 10 nM BTx-647 and 1 μM BTx. The suspensions of beads (typically 300 μL) were transferred to a sample holder closed at the bottom with a 0.17-mm-thick glass coverslip and allowed to settle for $\approx$20 min. Fluorescence images were recorded using an SP5 confocal microscope (Leica Microsystems) with a 63.0× 1.20w HCX PL APO CS objective (Zeiss). Fluorescence was excited by a laser at 633 nm and detected in the 642- to 768-nm range. No background coming from the beads was detectable in our experimental conditions. To ensure a reproducible positioning of the confocal plan, the distance from the surface was reset to 0.9 μm (which corresponds approximately to the center of the beads) before each new image, using the reflection signal of the surface and a piezoelectrically controlled z-stage. For kinetic experiments, 7.5 μg of beads were suspended in 100 μL of buffer and transferred to the holder. At $t = 0$, 200 μL of a 15 nM solution of BTx-647 were added without mixing (so as to minimize bead movements). Images were recorded at different times over a period of $\approx$1 h. Each image was taken in a new area of the sample to avoid an underestimation of the amount of BTx-647 bound due to photobleaching.

Fluorescence Analysis and Evaluation of Ligand Binding Kinetics. To evaluate the overall fluorescence intensity per bead, raw images (1,024 × 1,024, 12 bits) were imported in Igor Pro 5.03 (WaveMetrics) and filtered with a 3 × 3 median filter to reduce the noise. The beads were located by applying an appropriate threshold value to the images, thus defining regions of interest (ROIs). The threshold value was chosen so that the minimal ROI dimensions matched that of an individual bead. ROIs that corresponded to aggregates of beads were rejected after visual inspection. Then, the average intensity was calculated for each selected ROI and corrected for background, yielding the fluorescence intensity distribution of the bead population.

To estimate ligand-binding kinetics, average intensities of bead populations were calculated for each image taken at increasing times after addition of BTx-647. The contribution of nonspecific binding was evaluated from the BA8-35 sample to be $y(t) = 0.69 \cdot t$, where t is the time in minutes, and subtracted from each value. The results were fitted using a first-order reaction model, $y(t) = y(0)(1 - e^{-k_{obs} \cdot t})$, where $y(t)$ is the fluorescence intensity, k_{obs} is the first-order reaction constant, and t is the time.

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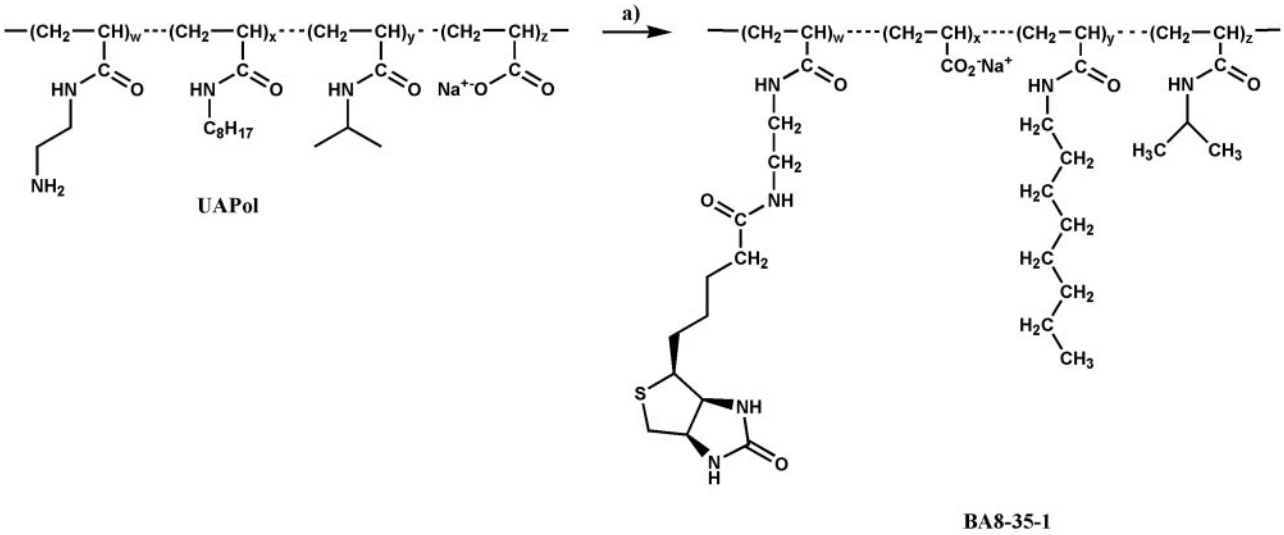

Fig. S1. Synthesis of BA8-35-1. Biotinyl-NHS active ester was allowed to react with an amino-functionalized A8-35 (UAPol; see ref. 2) a) Biotinyl-NHS, DMF, 40 °C, 2 h.

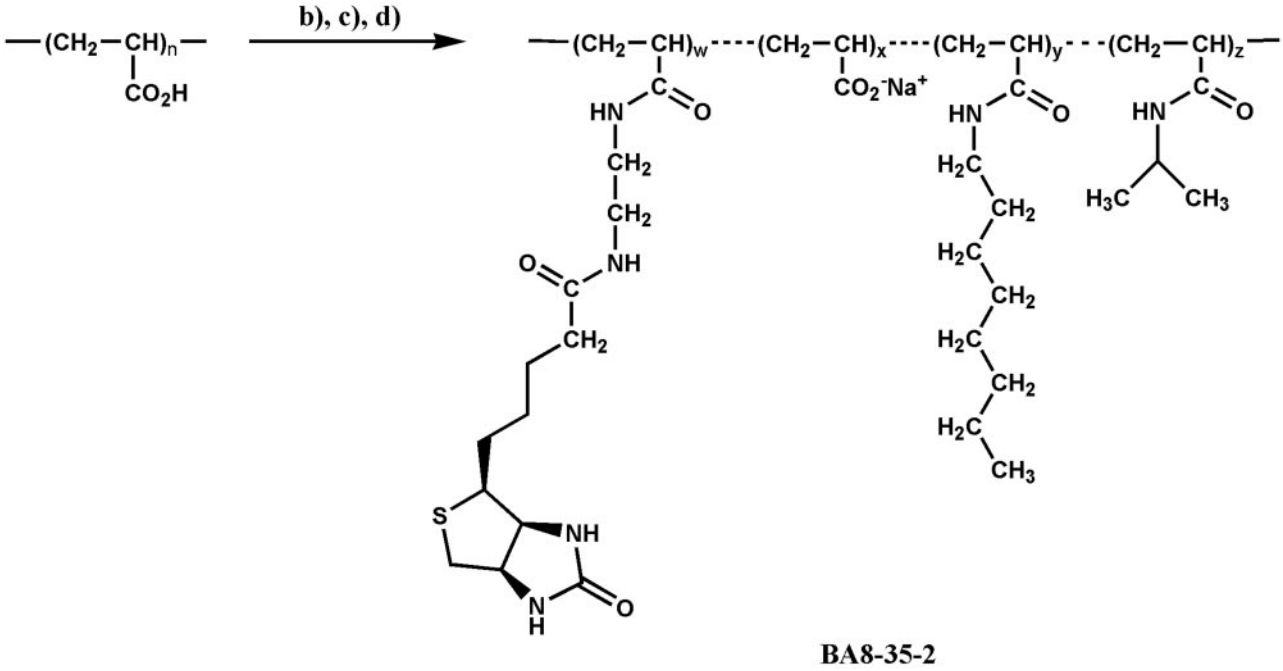

Fig. S2. Synthesis of BA8-35–2. An amino-functionalized biotin was directly grafted on the polymer b) *N*-(2-aminoethyl)biotinamide/DCC, then octylamine/DCC, NMP, 1 h at 60 °C, then 2 h at room temperature; c) isopropylamine/HOBT/DCC, NMP, 50 °C, 1 h at 50 °C and then 2 h at room temperature; d) NaOH.

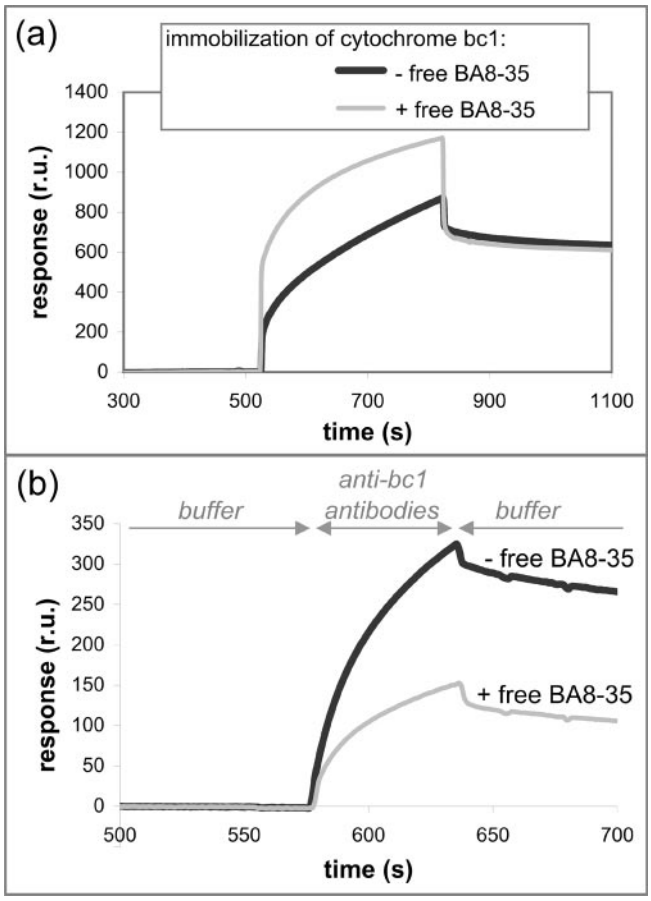

Fig. S3. Effect on SPR measurements of eliminating free BA8-35 from membrane protein/BA8-35 preparations. (*a*) Samples of cytochrome bc_1 trapped in BA8-35 and separated (black line) or not (gray line) from excess A8-35 by size exclusion chromatography were immobilized by flushing 50 μL of each solution over an SA sensor chip at the same concentration of protein (2 μM in NaPh buffer). (*b*) The 2 flow cells were then challenged with anti-bc_1 antibodies (1/100 dilution in NaPh buffer).

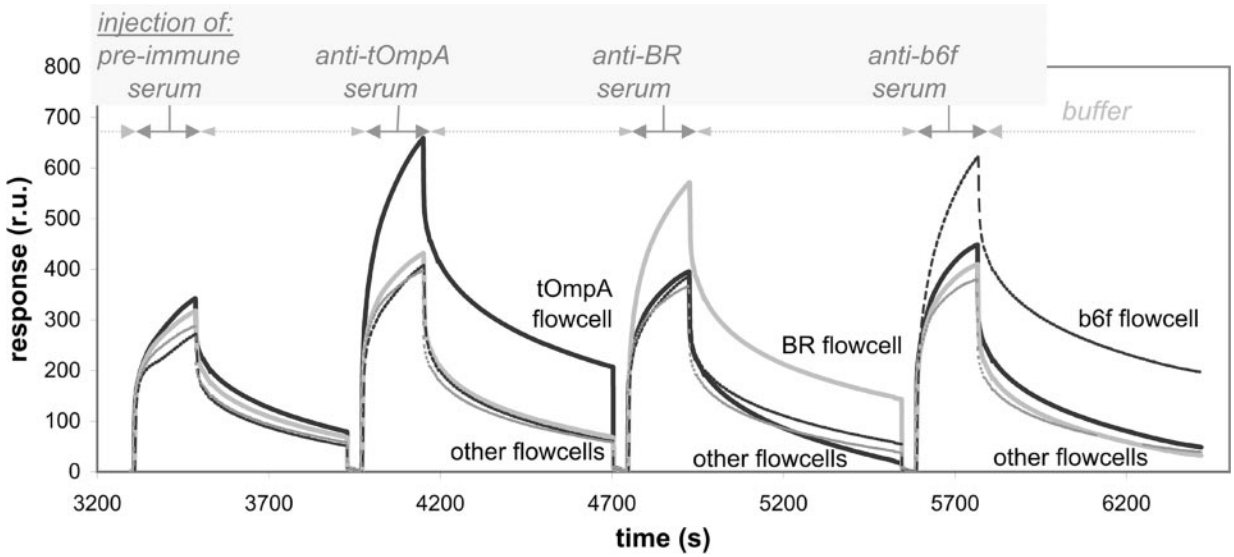

Fig. S4. Recognition by crude sera of immobilized membrane protein/BA8-35 complexes. Postimmune crude sera (30 μL) raised against tOmpA, BR or cytochrome b_6f were flushed over flow cells onto which BA8-35-1 (dashed thin gray line), tOmpA/BA8-35-1 (black line), BR/BA8-35-1 (gray line) or cytochrome b_6 *f*/BA8-35-1 complexes (dashed thin black line) had been immobilized. Sera were used at 1/100 dilution in NaPh buffer. Preimmune serum was injected several times over flow cells before postimmune sera to minimize nonspecific binding (data not shown).