Quantitation of Membrane-Ligand Interactions Using Backscattering Interferometry

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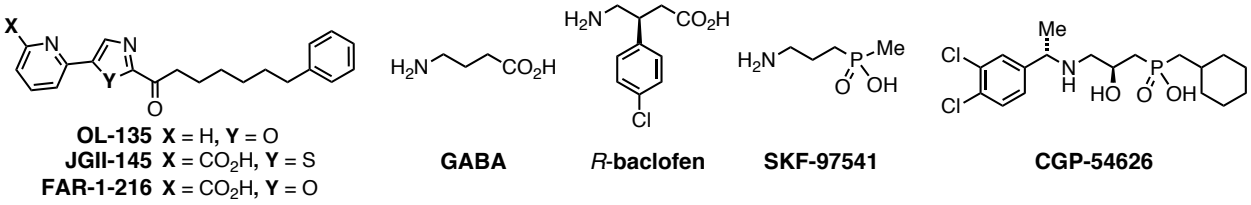
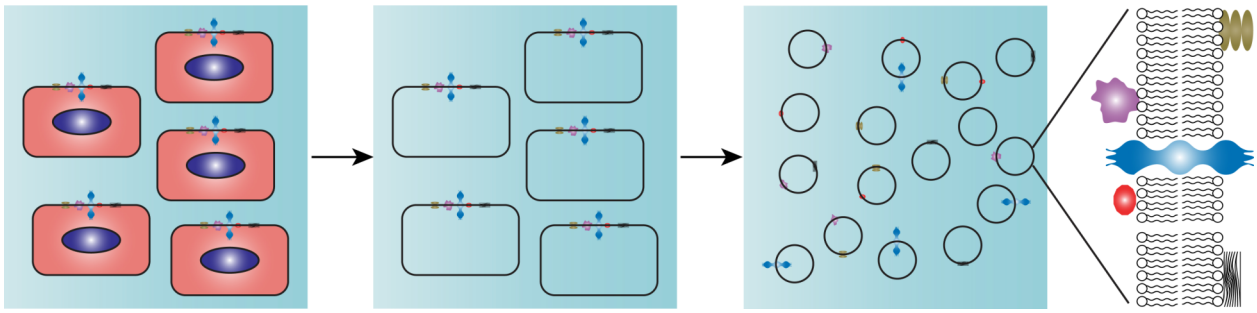
Supplementary Information



Supplementary Figure 1. Structures of small molecule ligands used in this work.



Supplementary Figure 2. Preparation of native membrane vesicles. Cells were incubated in a hypotonic solution, gently lysed, and the internal components separated from the outer membranes by centrifugation. Outer membranes were then sonicated and centrifuged to create a uniform population of SUV's containing native proteins. See references S1 and S2 for similar procedures.

Supplementary Figure 3. Western Blot of native cell membrane-derived SUV's stained for the presence of the $GABA_b$ receptor. Lanes 1-2, derived from b12.2-transformed cells; lanes 4-5, derived from CHO-K1 cells, lanes 3 and 6, bovine serum albumin.

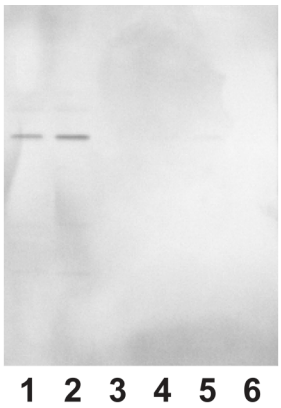


Table 1. Binding constants determined by BSI from the plots shown in Figure 2, including references to previous measurements.

Membrane-bound binding partner	Solution-phase ligand	K_d (BSI) [a]	Literature value and reference
GM1	Cholera toxin B subunit	0.13 ± 0.03 nM	K_D = 4 pM, ref. S3 [b] K_D = 20 nM, ref. S4 [c]
FAAH	OL-135	0.26 ± 0.04 nM (pH 7.4) 13 ± 7.6 pM (pH 9.0)	K_i = 4.7 nM, ref. S5
FAAH	FAR-I-216	0.13 ± 0.02 nM (pH 7.4) 0.10 ± 0.03 nM (pH 9.0)	K_i = 20 nM, ref. S6
FAAH	JG-II-145	4.1 ± 1.7 μM (pH 7.4) 310 ± 170 nM (pH 9.0)	K_i = 10 μM, ref. S7
CXCR4	SDF-1α	0.69 ± 0.33 nM	IC_{50} = 1.8 nM, ref. S8 [d]
$GABA_B$ receptor	GABA	140 ± 65 nM	IC_{50}= 140 nM, ref. S9 [e]
$GABA_B$ receptor	Baclofen	210 ± 34 nM	IC_{50} = 210, 250 nM, ref. S9, S10 [e]
$GABA_B$ receptor	SKF-97541	20 ± 7.1 nM	IC_{50} = 66 nM, ref. S10 [e]
$GABA_B$ receptor	CGP-54626	5.4 ± 1.7 nM	IC_{50} = 2.2 nM, ref. S9 [e]

(a) Error limits are derived from the statistical error of curve fitting the curves shown in Figure 2. (b) Determined by SPR. (c) Determined by fluorescence microscopy. (d) Determined by radioligand displacement on human T-lymphoblasts. (e) Determined by radioligand displacement on rat cerebral cortex extracts.

Other Label-Free Methods

The most common type of label-free measurement made on unmodified biological molecules is the optical study of intrinsically fluorescent substrates or binders, such as proteins containing tryptophan residues at or near the site of action. This method provides a relatively narrow measurement window, since the majority of substrates of interest lack the good fortune to be naturally fluorescent. Cell-based assays, such as CellKey (MDS Analytical Technologies),[S11] EPIC (Corning),[S12] xCELLigence (Roche),[S13] and BIND (SRU Biosystems)[S14] provide indirect measurements of membrane "interaction constants". Typically, impedance or waveguide interferometry-type measurements form the basis for such techniques, with the end observation being a change in cell phenotype (resistance to electrical current, morphology, etc.) after analytes of interest are added to cultures. While extremely sensitive, especially when combined with top-end image analysis, such techniques do not directly probe specific molecular interactions; further experiments, usually label-based, are required to directly relate observed morphology changes with target-ligand interactions Surface plasmon resonance (SPR) and related techniques are popular and effective[S15] but require the immobilization of the membrane and its constituents onto the plasmonic support,[S16] which may or may not be conducive to replicating a native membrane environment. Bulk-phase techniques such as isothermal

titration calorimetry can allow correlation of molecular solute interactions with enthalpic changes, however this class of techniques generally requires relatively greater amounts of reactants, has low dynamic range, and a need for precise buffer matching; conditions often unfeasible in experiments involving complex matrices.

Disconnection between Binding (K_d) and Inhibition (K_i)

Although these parameters go by different names, at least three reports have appeared for diverse proteins in which K_d and K_i differ by three orders of magnitude or more.[S17-S19] (This is approximately the difference in magnitude in these parameters observed here for FAAH, comparing BSI results to inhibition constants reported in the literature.)

SUP-T1 cells

These cells[S20] were provided as kind gift of the Torbett laboratory (The Scripps Research Institute). The negative control cell line (designated X4 -/-) were identical except for the suppression of CXCR4 levels using a specific zinc finger nuclease.[S21]

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