Supporting Information

Quantification of green fluorescent protein-(GFP-) tagged membrane proteins by capillary gel electrophoresis

Azeem Danish[#], Sang-Yong Lee[#], and Christa E. Müller[*]

PharmaCenter Bonn, Pharmaceutical Institute, Pharmaceutical Chemistry I, University of Bonn, An der Immenburg 4, D-53121 Bonn, Germany

# Both authors contributed equally to this study

[*]Address correspondence to:

Prof. Dr. Christa E. Müller, Pharmazeutisches Institut, Pharmazeutische Chemie I, An der Immenburg 4, D-53121 Bonn, Germany. Phone: +49-228-73-2301. Fax: +49-228-73-2567. E-mail address: christa.mueller@uni-bonn.de

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Table S1 Primers used for the project

Primers for pcDNA4-hSV2A-GFP

Overlaps	Oligonucleotide (upper case = annealing sequence for PCR)	Anneals	F/R
pcDNA4-HindIII	agacccaagctggctagttaagcttACCATGGAAGAGGGCTTCCG	hSV2A	Fwd
pcDNA4-XhoI	agacccaagctggctagttaagcttACCATGGAAGAGGGCTTCCG	GFP	Rev

Primers for pcDNA4-hA_{2A}-GFP

Overlaps	Oligonucleotide (upper case = annealing sequence for PCR)	Anneals	F/R
pcDNA4	agacccaagctggctagttaagcttACCATGCCCATCATGGGC	hA_{2A}	Fwd
GFP	cgcccccgtcgacGGACACTCCTGCTCCATC	hA_{2A}	Rev
hA_{2A}	aggagtgtccGTCGACGGGGGCGGCGGA	GFP	Fwd
pcDNA4	gttcgaagggccctctagactcgagTTACTTGTACAGCTCGTCCATGCCGAGAGTG	GFP	Rev

Primers for pcDNA4-mCx43

Overlaps	Oligonucleotide (upper case = annealing sequence for PCR)	Anneals	F/R
pCDNA4	agacccaagctggctagttaagcttACCATGGGTGACTGGAGC	mCx43	Fwd
eGFP	cgcccccgtcgacAATCTCCAGGTCATCAGGC	mCx43	Rev
mCx43	cctggagattGTCGACGGGGGCGGCGGA	GFP	Fwd
pcDNA4	gttcgaagggccctctagactcgagTTACTTGTACAGCTCGTCCATGCCGAGAGTG	GFP	Rev

Table S2 Quantification of GFP-tagged proteins by CE-LIF[a]

SV2A-GFP	**peak area**	**mg/ml**	**ng/ml**	**1:100 dilution (ng/ml)**	**nmol/l**	**pmol/ml**	**total amount (mg/ml)**	**pmol/mg**
Test 1	69044	0.00016295	162.95492	16295.4921	0.1393	139.3	5.00	27.9
Test 2	60554	0.00014292	142.91716	14291.7158	0.1222	122.2	5.00	24.4
Test 3	69134	0.00016317	163.16734	16316.7335	0.1395	139.5	5.00	27.9
							mean	**26.7**

A_{2A}AR-GFP	**peak area**	**mg/ml**	**ng/ml**	**1:20 dilution (ng/ml)**	**nmol/l**	**pmol/ml**	**total amount (mg/ml)**	**pmol/mg**
Test 1	2011	0.00000475	4.74628	94.9257	1.3184119	1.32	12.0	0.110
Test 2	2412	0.00000569	5.69271	113.8541	1.5813075	1.58	12.0	0.132
Test 3	2245	0.00000530	5.29856	105.9712	1.4718223	1.47	12.0	0.123
							mean	**0.121**

Cx43-GFP	**peak area**	**mg/ml**	**ng/ml**	**1:2 dilution (ng/ml)**	**nmol/l**	**pmol/ml**	**total amount (mg/ml)**	**pmol/mg**
Test 1	2341	0.00000553	5.52514	11.0503	0.1579	0.158	4.70	0.0336
Test 2	3282	0.00000775	7.74605	15.4921	0.2213	0.221	4.70	0.0471
Test 3	2692	0.00000635	6.35355	12.7071	0.1815	0.182	4.70	0.0386
							mean	**0.0398**

[a]*Example of calculation (for test 1 of SV2A-GFP determination) is given on page 4.*

Calculation example for test 1 of SV2A-GFP determination

Peak area of SV2A-GFP: **69044**

Calibration curve: y = 423700000x - 0.00002178

69044 = 423700000x - 0.00002178

x = (69044 + 0.00002178) / 423700000

x = 69044.00002178 / 423700000

x = **0.00016295 mg / ml** = **162.95492 ng / ml**

1:100 dilution

SV2A-GFP (162.95492 ng / ml) x 100

= **16295.4921 ng / ml**

Molecular weight of SV2A-GFP: 117 kDa = 117 x 1000 Da = 117000 Da = 117000 g/mol

(16295.4921 ng / ml) / (117000 g/mol)

= 0.1393 (ng/ml x mol/g)

= **0.1393 nmol/ml**

= **139.3 pmol/ml**

Total protein concentration according to the Bradford method: **5.00 mg/ml**

The calculated concentration of SV2A in the cell homogenate:

(139 pmol/ml)/(5.00 mg/ml protein) = 139 pmol/5.00 mg protein

= **27.9 pmol/mg protein**

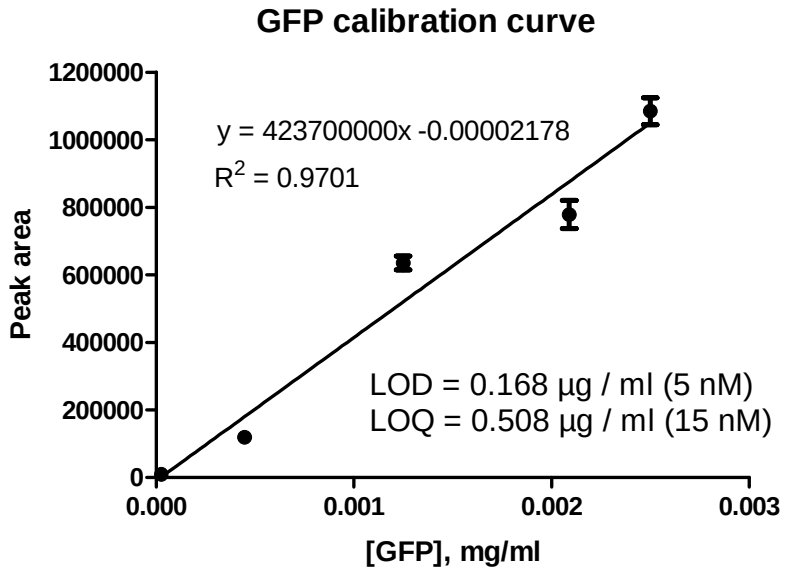

Fig. S1 Calibration for GFP determined by CGE-LIF. Free GFP (in mg/ml) is plotted on the X-axis and the measured peak area is shown on the Y-axis. Data points are means ± SD of three independent experiments. LOD: limit of detection; LOQ: limit of quantification.

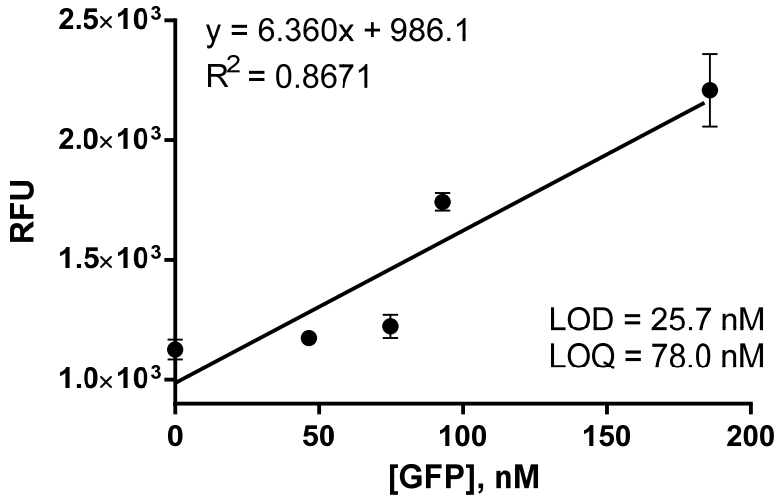

Fig. S2 Calibration curve for GFP determined in a fluorimeter. The calibration curve was obtained by plotting the free GFP concentration in nM on the X-axis versus the corresponding fluorescence intensity on the Y-axis. The fluorescence of free GFP was measured in a black solid-bottom 96-well plate using a fluorescence plate reader (Mithras LB940, Berthold

Biotechnologies, Bad Wildbad, Germany**)**. Data points represent means ± SD of three independent measurements. LOD: limit of detection, LOQ: limit of quantification, RFU: relative fluorescence units.

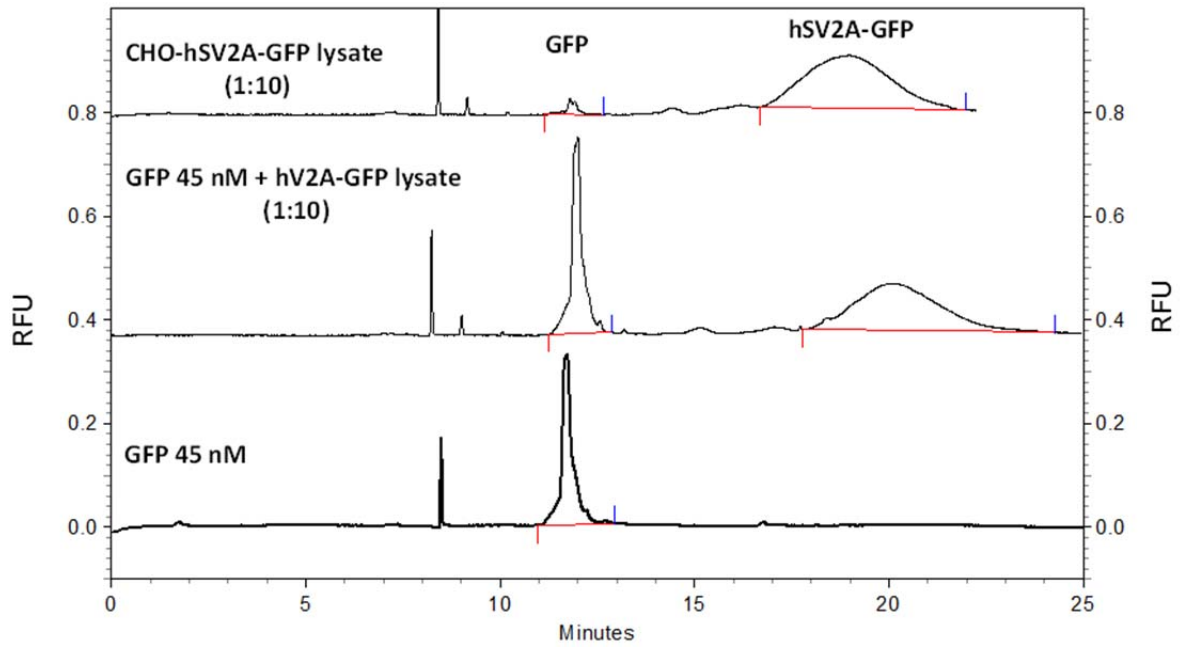

Fig. S3 Separation of free GFP (standard) and SV2A-GFP in a cellular homogenate. RFU: relative fluorescence units.

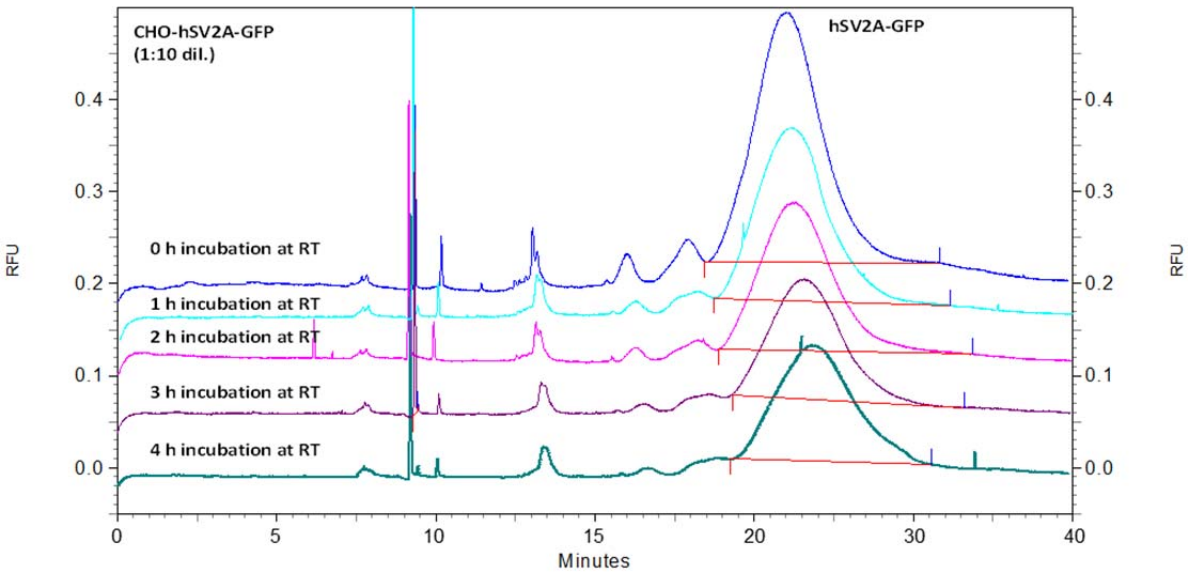

Fig. S4 Effect of SDS (1%) in sample buffer on peak area of SV2A-GFP measured after different incubation times. RT: room temperature, RFU: relative fluorescence units.

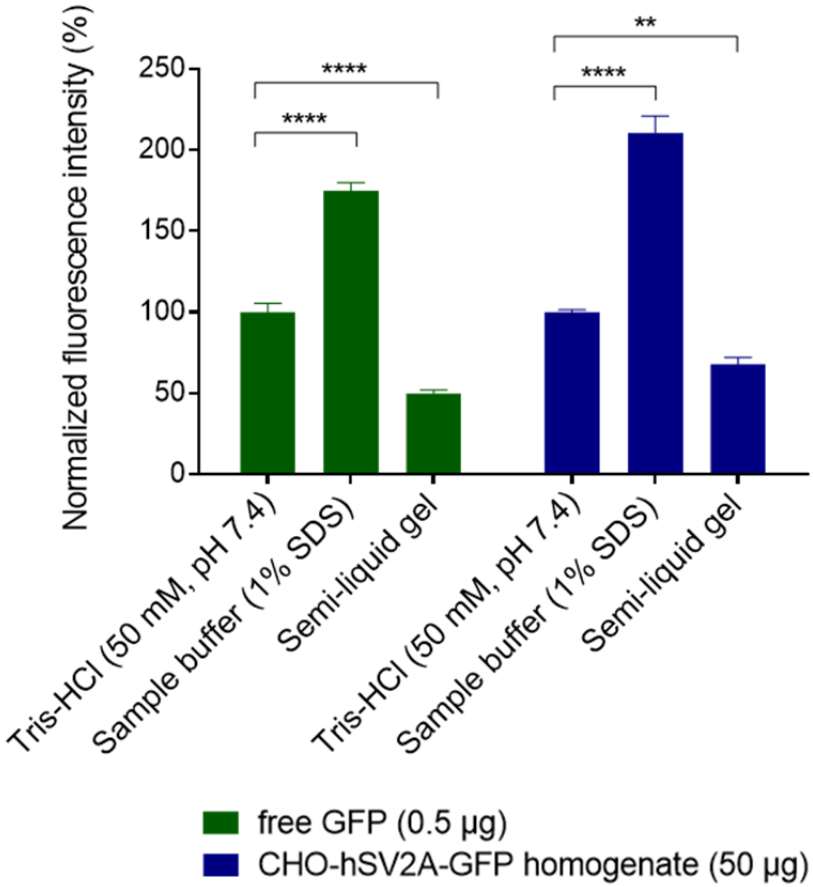

Fig. S5 Effect of 1% SDS in sample buffer and semi-liquid gel on fluorescence intensity of free GFP and SV2A-GFP homogenate. Fluorescence intensity was measured using a microplate fluorimeter (Mithras LB940, Berthold Biotechnologies GmbH, Bad Wildbad, Germany) at room temperature using excitation (485/14 nm) and emission (535/25 nm) filters. Data is normalized versus respective controls (protein in Tris-HCl 50 mM, pH 7.4). Data shown are means ± SEM of three individual experiments. Statistical analysis was performed by one-way-ANOVA with Dunnett's test for multiple comparisons, where control (Tris-HCl) was compared with sample buffer and semi-liquid gel. ****, $p < 0.0001$; **, $p = 0.0060$.

Calculation of B_{max} values for SV2A-GFP from radioligand binding data

K_D and B_{max} calculations:

For homologous competition binding assays:

$$K_D = K_i = IC_{50} - RL$$

K_D: equilibrium dissociation constant (nM)

K_i: equilibrium inhibition constant (nM)

IC_{50}: half maximal inhibitory concentration (nM)

RL: measured concentration of radioligand (nM)

K_D = 83.5 nM – 2.0 nM = 81.5 nM

$$B_{max} \text{ (cpm)} = \text{(Top - Bottom) Specific Binding} / (RL / (K_D + RL))$$

B_{max}: maximum number of binding sites (cpm)

Top: total binding of radioligand (cpm)

Bottom: non-specific binding of radioligand (cpm)

B_{max} (cpm) = (2839 - 437) / (2.0 / (81.5 + 2.0)) = 100283 cpm

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B_{max} (fmol/mg) = B_{max} (cpm) * 100 / efficiency (%) x 2.2 x specific activity (Ci/mmol) x protein (mg)

Specific activity of [^{3}H]BRV = 94 Ci/mmol

Scintillation counter efficiency = 52%

Specific activity cpm/fmol = 108.5 cpm/fmol

Protein amount = 0.2 mg

B_{max} (fmol/mg) = B_{max} (cpm) x 100 / efficiency (%) x 2.2 x specific activity (Ci/mmol) x protein (mg)

B_{max} (fmol/mg) = 100283 x 100 / (52 x 2.2 x 94 x 0.2) = 4663 fmol/mg protein