Electronic Supplementary Material (ESI) for Analytical Methods.

SUPPLEMENTARY DATA

Labeling of streptavidin with acridinium

The labeling of streptavidin with acridinium by means of the Acridinium Protein Labeling Kit (Cayman Chemical, Michigan, USA) was carried out following the supplier's instructions. 50 µg of streptavidin was diluted in 300 µl of acridinium labeling solution. After adding 1 µl of acridinium to this solution (diluted 1/10 in dimethyl formamide), the mixture was incubated with agitation at room temperature (RT) for 10 min. The addition of 100 µl of acridinium quench solution stopped this reaction. After having incubated the sample with agitation for 30 min at RT, it was purified by Fast Protein Liquid Cromatography (FPLC) (Figure 1 left). The acridinium labeled streptavidin was separated from the free acridinium ester hydrolysis products on Hi-Trap desalting columns with Sephadex G-25 Superfine (GE Healthcare, Little Chalfont, UK). The elution buffer was 30 mM ammonium bicarbonate pH 7.5. The different fractions from FLPC were analyzed in a Cento XS3 LB960 microplate luminometer (BERTHOLD Technologies, Zug, Switzerland) and those fractions with labeled streptavidin were lyophilized and resuspended in 20 µl of MilliQ water.

Acridinium labeled streptavidin (ST-AC) was quantified using a NanoDrop Spectrophometer ND-1000 (Thermo Scientific, Massachusetts, USA). To verify the purification procedure, 5 µg of the collected protein were incubated with 4x loading buffer (200 mM Tris-Cl (pH 6.8), 400 mM DTT, 8% SDS, 0.4% bromophenol blue and 40% (v/v) glycerol) at 95 ºC for 2 min in a final volume of 10 µl. Samples were loaded in wells of 16% polyacrylamide SDS-PAGE minigels and after separing the protein bands using electrophoresis, gels were stained with Coomassie Brillant Blue G-250 (Bio-Rad, California, USA) following the supplier´s instructions (Figure 1 right).

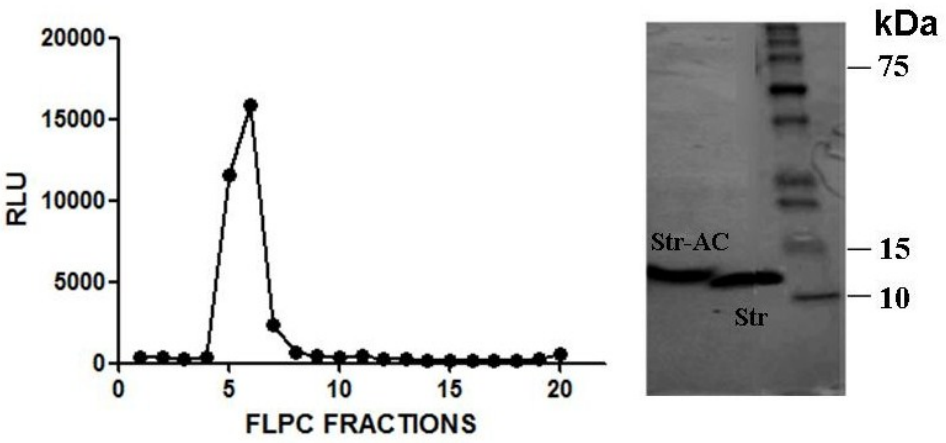

Figure S.1. Synthesis and purification of ST-AC. Left. Luminometry profile of fractions from FPLC after ST-AC synthesis. **Right**. Acrylamide gel with ST-AC (Fractions 5-7) (left), ST free (centre) and Molecular weight marker (right).

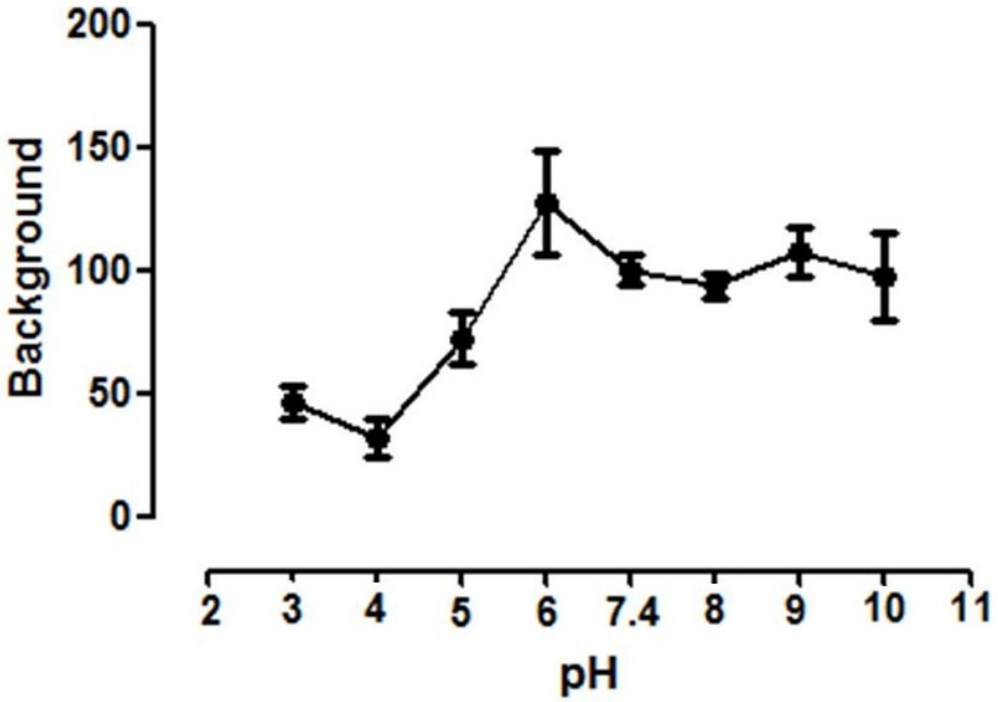

Figure S.2.Background obtained from membranes incubated with ST-AC and washed at different pHs (compared with the standard conditions at pH 7.4, taken as 100%). Wash buffer- ice-cold 50 mM Tris-HCl pH 7.4

Table S.1. Variables studied for AC (acridinium), AP (alkaline phosphatase) and HRP (horseradish peroxidase) methods. Standard conditions (STC) were 0.15 µg of ST-AP or ST-HRP or 0.2 µg of ST-AC, membranes from 50.000 cells, two washing steps and absence of detergent. The values of binding for each tag in STC were 100±3.65, 100±3.2 and 100±10.79 for AC, AP and HRP, respectively.

		ST LABELED WITH CHEMILUMINESCENT TAG (% FROM STC X±SD)		
VARIABLES TESTED		**AC**	**AP**	**HRP**
WASH NUMBER*	**1**	-	103.38±57.71	38.55±4.4
	3	-	-	82.27±5.28
	4	98.73±5.27	45.86±4.7	-
	2 + wash with 5 nM of ST	-	-	30.36±0.48
MEMBRANES FROM DIFFERENT NUMBER OF CELLS	**$10x10^4$**	255.93±9.96	180.83±22.18	161.43±14.68
	$15x10^4$	260.66±74.66	279.32±31.12	-
	$25x10^4$	-	-	262.14±4.77
MEMBRANES PREPARATION	**membranes blocked with ST**		-	131.75±43.81
	whole cell	80.92±2.76	-	-
ALTERNATIVE BINDING BUFFERS	**Citric acid buffer***	72.59±10.48	-	-
	Saline Phosphate buffer*	37.41±4.41	-	-
GHRELIN-BIOTIN REMOVED BEFORE ADDING ST	**40 min after start binding assay**	-	-	8.71±0.94
AMOUNT OF ST PER SAMPLE	**0.025 µg**	210.43±47.79	-	-
	0.05 µg	383.55±82.16	-	-
	0.075 µg	478.29±119.05	-	-
	0.1 µg	640.85±60.84	100±3.2	100±10.79
	0.15 µg	559.09±134.01	147.56±23.5	111.14±4.68
	0.2 µg	490.28±211.08	59.4±7.71	70.67±14.16
	0.25 µg	352.39±33.26	70.86±16.54	-
STREPTAVIDIN	**Effect of additional purification attempt**	70.84±0.84	-	-
	ST diluted in AP buffer	-	560,33±182.98	-
DETERGENT	**0.005% Tween-20**	190.4±11.78	-	-
	0.01% Tween-20	207.13±6.98	-	-
	0.02% Tween-20	186.19±5.23	228.76±36.09	-
	0.03% Tween-20	262.28±22.96	-	-
	0.01% TritonX-100	161.12±16.72	-	-
	0.02% TritonX-100	168.52±8.64	-	-
	0.03% TritonX-100	109.61±10.97	-	-
	0.01% Igepal	133.17±10.43	-	-
	0.02% Igepal	126.47±11.26	-	-
	0.03% Igepal	111.15±1.93	-	-

*Wash buffer- ice-cold 50 mM Tris-HCl pH 7.4

*Citric buffer- glacial acetic acid 0.4 M pH 2.5

*Saline Phosphate buffer (PBS)- 1.8 mM KH_2PO_4, Na_2HPO_4 mM, 2.7 mM KCl, 137 mM NaCl, pH 7.4

Variables studied for AP (alkaline phosphatase) and HRP (horseradish peroxidase) methods in the process of adding the chemiluminescent substrates. Standard conditions (STC) were 0.15 µg of ST-AP or ST-HRP, membranes from 50.000 cells, two washing steps and absence of detergent. The values of binding for each tag in STC were 100±3.2 and 100±10.79 for AP and HRP, respectively.

		ST LABELED WITH CHEMILUMINESCENT TAG (% FROM STC X±SD)	
VARIABLES TESTED		**AP**	**HRP**
PELLET RESUSPENDED	**in alkaline saline phosphate buffer**	248.68±44.36	-
CHEMILUMINESCENT SUBSTRATE+ALKALINE SALINE PHOSPHATE BUFFER* (in this order)	**pH 9**	347.56±34.77	-
	pH 9.5	232.89±25.75	-
	pH 10	340.6±2.44	-
ALKALINE SALINE PHOSPHATE BUFFER +CHEMILUMINESCENT SUBSTRATE (in this order)	**pH 9.5**	964.7±108.45	-
	pH 10	1667.6±44.34	-
CHEMILUMINESCENT SUBSTRATE DILUTED	**1/10**	375.19±60.71	-
	1/500	-	45.98±2.39

* Saline Phosphate buffer (PBS)- 1.8 mM KH_2PO_4, Na_2HPO_4 mM, 2.7 mM KCl, 137 mM NaCl