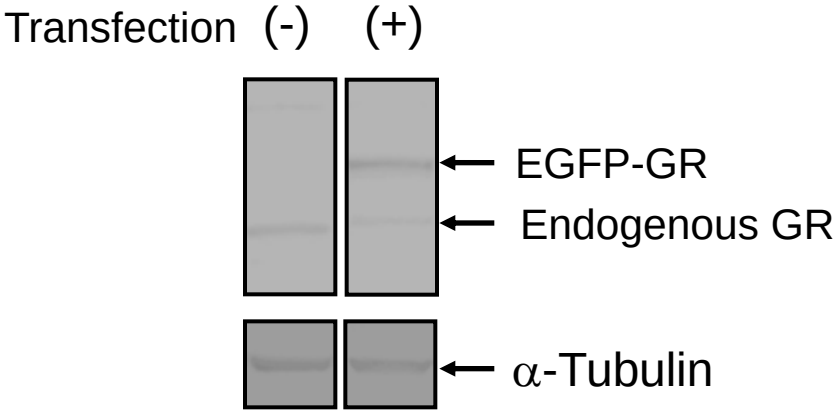
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Title	Homodimerization of glucocorticoid receptor from single cells investigated using fluorescence correlation spectroscopy and microwells
Author(s)	Oasa, Sho; Sasaki, Akira; Yamamoto, Johtaro et al.
Citation	FEBS Letters, 589(17), 2171-2178 https://doi.org/10.1016/j.febslet.2015.07.003
Issue Date	2015-08-04
Doc URL	https://hdl.handle.net/2115/62623
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Type	journal article
File Information	Supplemental figure_metho.pdf, Supplemental figure, materials and methods

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Fig. S1

(A)


Transfection (-) (+)
EGFP-GR
Endogenous GR
α-Tubulin

(B)

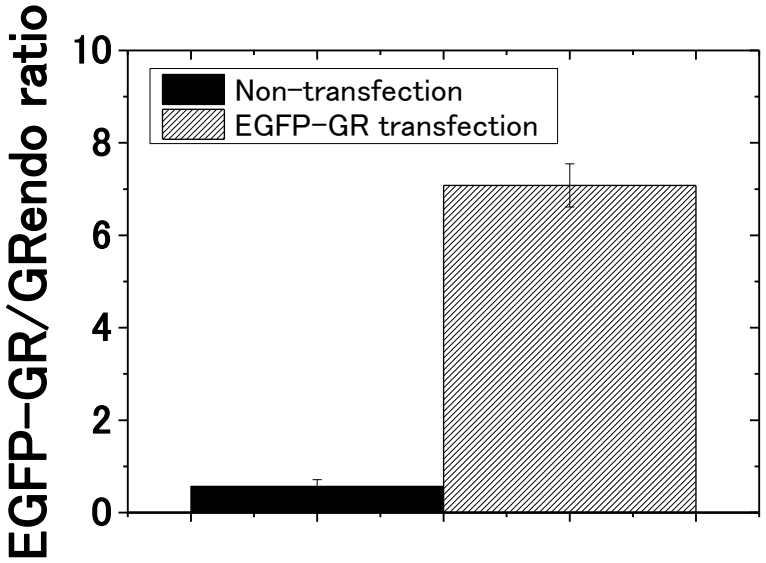

EGFP–GR/GRendo ratio
10
8
6
4
2
0
Non–transfection
EGFP–GR transfection

Fig. S2

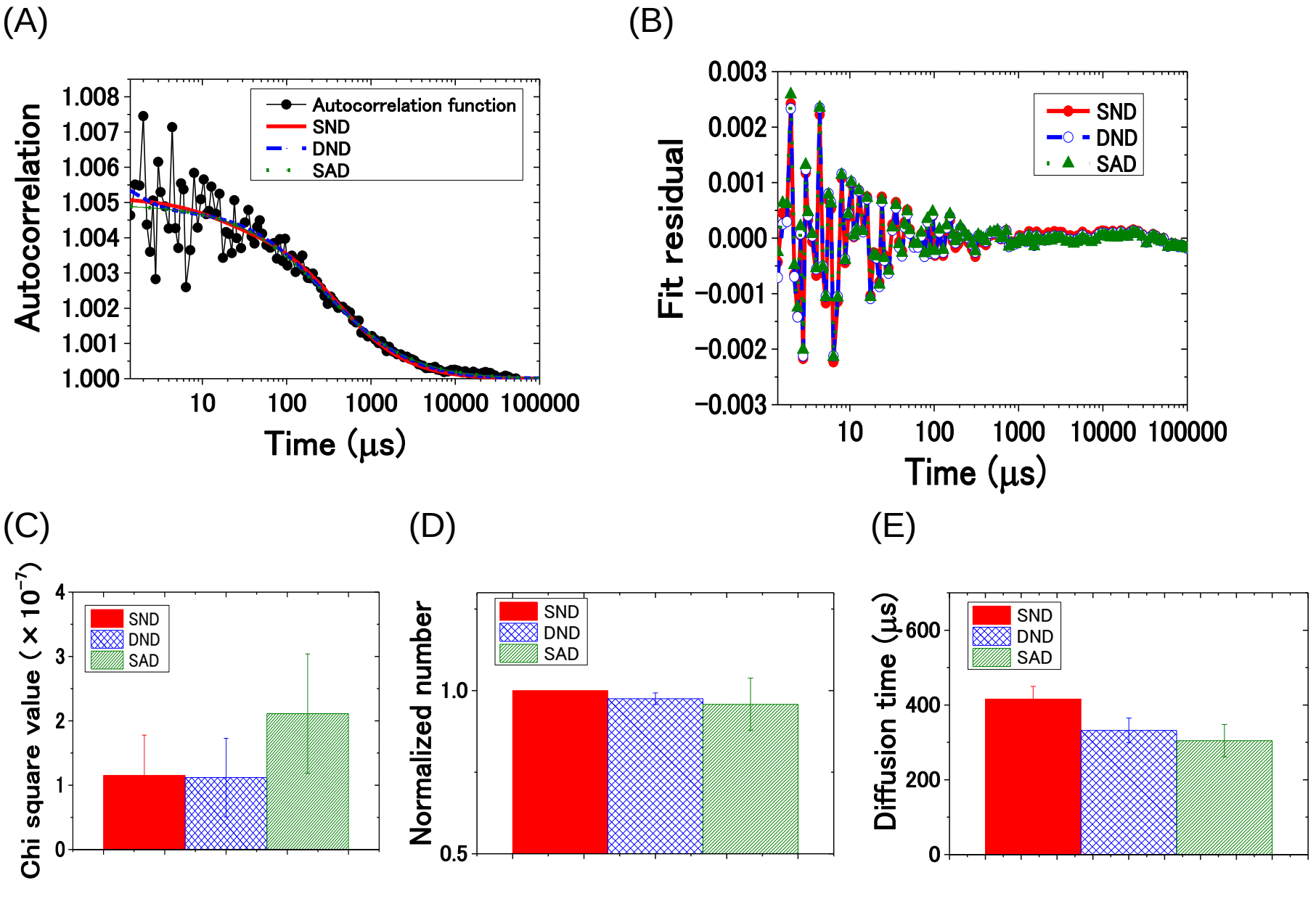

(A)
Autocorrelation
1.008
1.007
1.006
1.005
1.004
1.003
1.002
1.001
1.000
10
100
1000
10000
100000
Time (μs)
Autocorrelation function
SND
DND
SAD
(B)
Fit residual
0.003
0.002
0.001
0.000
−0.001
−0.002
−0.003
10
100
1000
10000
100000
Time (μs)
SND
DND
SAD
(C)
Chi square value (×10⁻⁷)
4
3
2
1
0
SND
DND
SAD
(D)
Normalized number
1.0
0.5
SND
DND
SAD
(E)
Diffusion time (μs)
600
400
200
0
SND
DND
SAD

Fig. S3

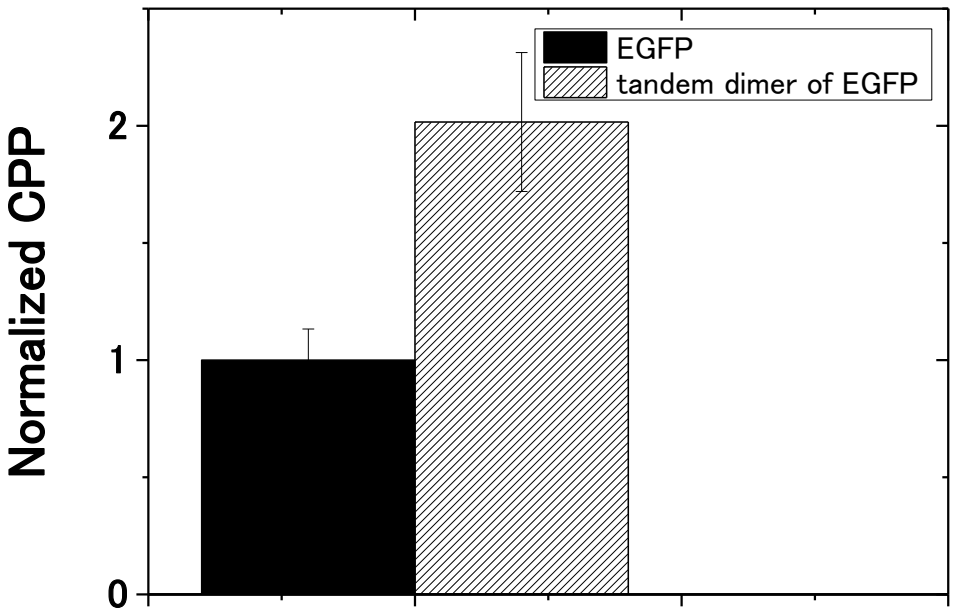


Fig. S4

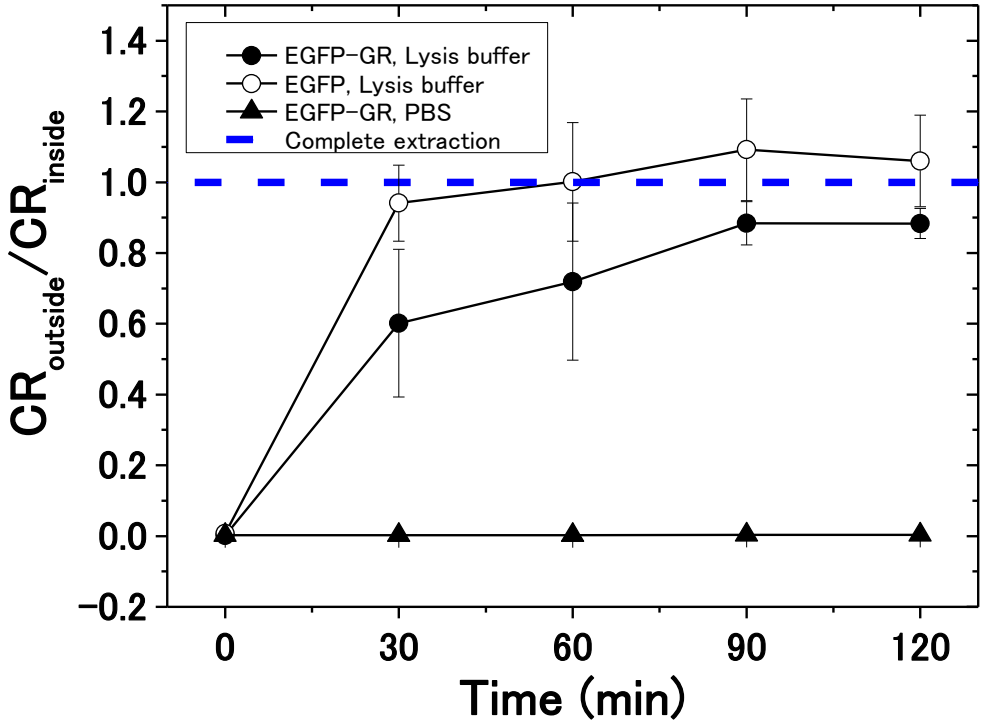

EGFP-GR, Lysis buffer
EGFP, Lysis buffer
EGFP-GR, PBS
Complete extraction
CR outside /CR inside
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
−0.2
0
30
60
90
120
Time (min)

Fig. S5

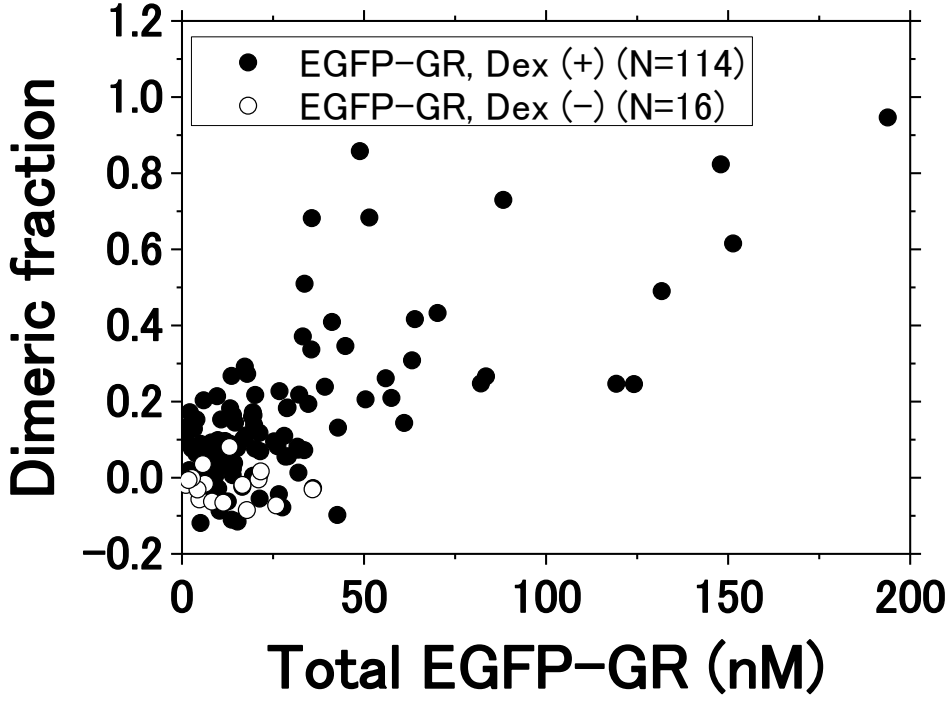

EGFP-GR, Dex (+) (N=114)
EGFP-GR, Dex (−) (N=16)
Dimeric fraction
Total EGFP-GR (nM)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
−0.2
0
50
100
150
200

Fig. S6

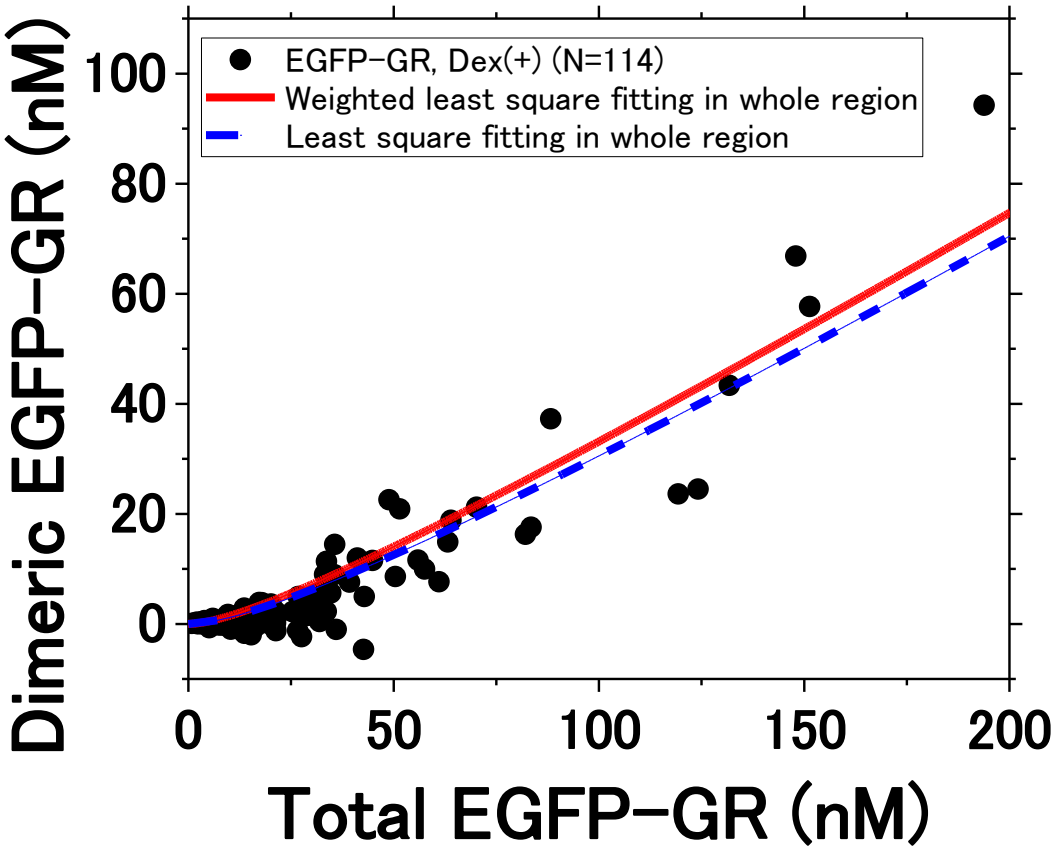
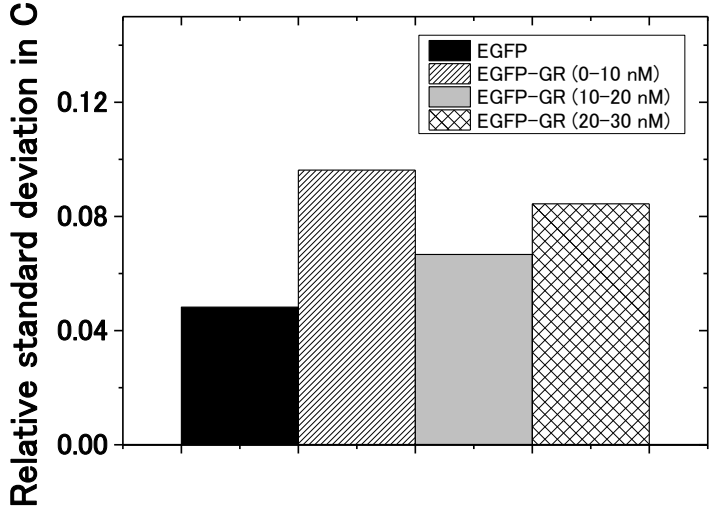
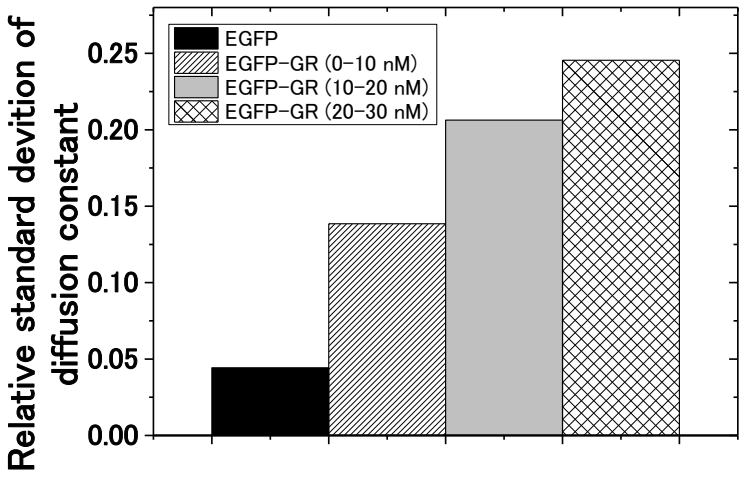

EGFP-GR, Dex(+) (N=114)
Weighted least square fitting in whole region
Least square fitting in whole region
Dimeric EGFP-GR (nM)
Total EGFP-GR (nM)
0
20
40
60
80
100
0
50
100
150
200

Fig. S7

(A)


Relative standard deviation in CPP
0.00
0.04
0.08
0.12
EGFP
EGFP-GR (0-10 nM)
EGFP-GR (10-20 nM)
EGFP-GR (20-30 nM)

(B)


Relative standard devition of diffusion constant
0.00
0.05
0.10
0.15
0.20
0.25
EGFP
EGFP-GR (0-10 nM)
EGFP-GR (10-20 nM)
EGFP-GR (20-30 nM)

Supplemental figure legends

Fig. S1 Comparison of the amounts of exogenous EGFP-GR and endogenous GR.

(A) The images of bands that originated from the exogenous EGFP-GR and endogenous GR or α-tubulin were cropped from the membranes blotted using an anti-GR or anti-α-tubulin antibody. (B) The amounts of exogenous EGFP-GR and endogenous GR were 0.56±0.14 and 7.07±0.47 in nontransfected cells and EGFP-GR expressing cells, respectively. The average and standard deviation were calculated from three-independent experiments. Solid bar, nontransfected HeLa cells; Shaded bar, EGFP-GR-expressing HeLa cells.

Fig. S2 Comparison of fitting models to cellular EGFP measurement.

The fitting models between a single component with normal diffusion (SND), double components with normal diffusion (DND) and a single component with anomalous diffusion (SAD) were compared in EGFP measurement in living HeLa cells. (A) Typical autocorrelation functions and fitting curves. Black plots, Autocorrelation function; Red line, Fitting curve of SND; Blue dashed line, Fitting curve of DND; Green dotted line, Fitting curve of SAD. (B) Fit residuals. Red line with filled circle, Fitting curve of SND; Blue dashed line with open circle, Fitting curve of DND; Green dotted line with filled triangle, Fitting curve of SAD. (C) The chi square value of the autocorrelation function and fitting curves. (D) The number of molecules. (E) The diffusion time. The diffusion time of the first component is shown for DND because almost all fractions were in the first component (above 90%). Although the diffusion times of DND and SAD were shorter than that of SND, there were no significant differences among the fitting models for the chi square value and number of molecules. Therefore, the fitting model using a single component with normal diffusion (SND) was selected. The data number was 3 for calculation of the average value and standard deviation of each fitting model. Filled red bar, SND; Meshed blue bar, DND; Shaded green bar, SAD.

Fig. S3 comparison of CPP between EGFP and tandem dimer of EGFP.

Plasmids of EGFP and the tandem dimer of EGFP (0.1 μg) were transfected into HeLa cells. The CPP of EGFP and the tandem dimer of EGFP were measured by FCS using whole HeLa cell lysate. The CPP of EGFP and the tandem dimer of EGFP were 11.3±1.5 kHz and 22.9±6.7 kHz, respectively. The average value and standard deviation were calculated from five independent experiments. Filled bar, EGFP; Shaded bar, tandem dimer of EGFP.

Fig. S4 The efficiency of EGFP-GR extraction into lysis buffer.

The ratio of fluorescent intensity (Count Rate [CR]) between inside and outside the cell was measured by FCS after cell lysis. When all EGFP and EGFP-GR molecules were extracted from the cell, the ratio of CR inside the cell to that outside the cell reached 1.0 (Blue dashed line). Open circles indicate the extraction of EGFP by lysis buffer as a positive control and filled triangles show extraction of EGFP-GR by PBS as a negative control. Filled circles show extraction of EGFP-GR by lysis buffer. These results suggested that 90 min lysis was an effective extraction time. Data number was 10 for calculation of the average value and standard deviation.

Fig. S5 Calculation of dimeric fraction of EGFP-GR.

Dimeric fractions of EGFP-GR in the presence and absence of Dex after cell lysis were calculated by equation (17) and normalized CPP of EGFP-GR. Open symbol, EGFP-GR without Dex treatment; Filled symbol, EGFP-GR with Dex treatment. The numbers of data were 16 and 114 in the absence and presence of Dex, respectively.

Fig. S6 Comparison of fitting methods for the dissociation constant of homodimerization of EGFP-GR.

The fitting methods between least-squares fitting and weighted least-squares fitting in whole range were compared to determine the dissociation constant for homodimerization of EGFP-GR. The weighting factor of the each data point for fitting was the inverse of the number of data points within window$\pm$2.5 nM from each data point. The similar dissociation constant was obtained by the other windows from $\pm$1 to $\pm$5 nM (data not shown). Black filled symbol, EGFP-GR in the presence of Dex; Red solid line, Weighted least-squares fitting in whole region; Blue dashed line, Least-squares fitting in whole region. Data number was 114.

Fig. S7 The heterogeneity of homodimerization and complex formation.

(A) Relative standard deviations of normalized CPP of EGFP and EGFP-GR. **(B)** Relative standard deviations of diffusion constants of EGFP and EGFP-GR. The relative standard deviation of EGFP-GR with different concentrations of EGFP-GR, 0 to 10 nM, 10 to 20 nM and 20 to 30 nM was calculated from the average value and standard deviation of CPP and the diffusion constant in microwells obtained from single-cell data shown in Fig. 4C and 4E. Filled black bar, EGFP; Shaded bar, EGFP-GR (0-10 nM); Filled gray bar, EGFP-GR (10-20 nM); Meshed bar; EGFP-GR (20-30 nM). Number of data was 5.

Supplemental materials and methods

Western blot analysis.

To estimate the amount of exogenous EGFP-GR and endogenous GR in HeLa cells, western blots were performed. Whole cell lysates from HeLa cells which non-transfected of expressed EGFP-GR were separated by SDS-PAGE and transferred to PVDF membranes (Bio Rad). The membranes were blotted with a polyclonal anti-GR antibody (ab3579, abcam) or anti-α-tubulin antibody (upstate cell signaling solutions) in Can Get Signal solution 1 (TOYOBO), subsequently horseradish peroxidase (HRP)-conjugated anti-mouse or anti-rabbit antibody (Jackson ImmunoResearch) in Can Get Signal solution 2 (TOYOBO). All blots were visualized with the ECL kit (GE Healthcare) and ImageQuant LAS4000-mini (Fuji film).

The amount ratio of exogenous EGFP-GR and endogenous GR ($R_{\mathrm{EGFP\text{-}GR,\ endoGR}}$) was estimated as follows;

$$R_{\mathrm{EGFP-GR,\ endoGR}} = \frac{I_{\mathrm{EGFP-GR}}}{I_{\mathrm{endoGR}} \times TE}$$

where $I_{EGFP\text{-}GR}$ is band intensity of EGFP-GR normalized that of α-tubulin, I_{endoGR} is band intensity of endogenous GR normalized that of tubulin and *TE* show the transfection efficiency of EGFP-GR (TE = 0.34±0.08) estimated from LSM images, because EGFP-GR was not expressed in all HeLa cells by transient transfection. All intensity of bands were quantified by ImageJ (NIH).