Electronic Supplementary Material (ESI) for Soft Matter.

Supplementary Information

TITLE

Molecular thermodynamics of receptor competition for endocytic uptake

AUTHORS

Andre C.M. DeGroot[1], Chi Zhao[1], Megan F. LaMonica[1], Carl C. Hayden[1], Jeanne C. Stachowiak[1,2]

AFFILIATIONS

[1] *Department of Biomedical Engineering, The University of Texas at Austin, Austin, TX, 78712, USA*

[2] *Institute for Cellular and Molecular Biology, The University of Texas at Austin, Austin, TX, 78712, USA*

* *To whom correspondence should be addressed: Jeanne C. Stachowiak (jcstach@austin.utexas.edu).*

SUPPLEMENTARY FIGURES

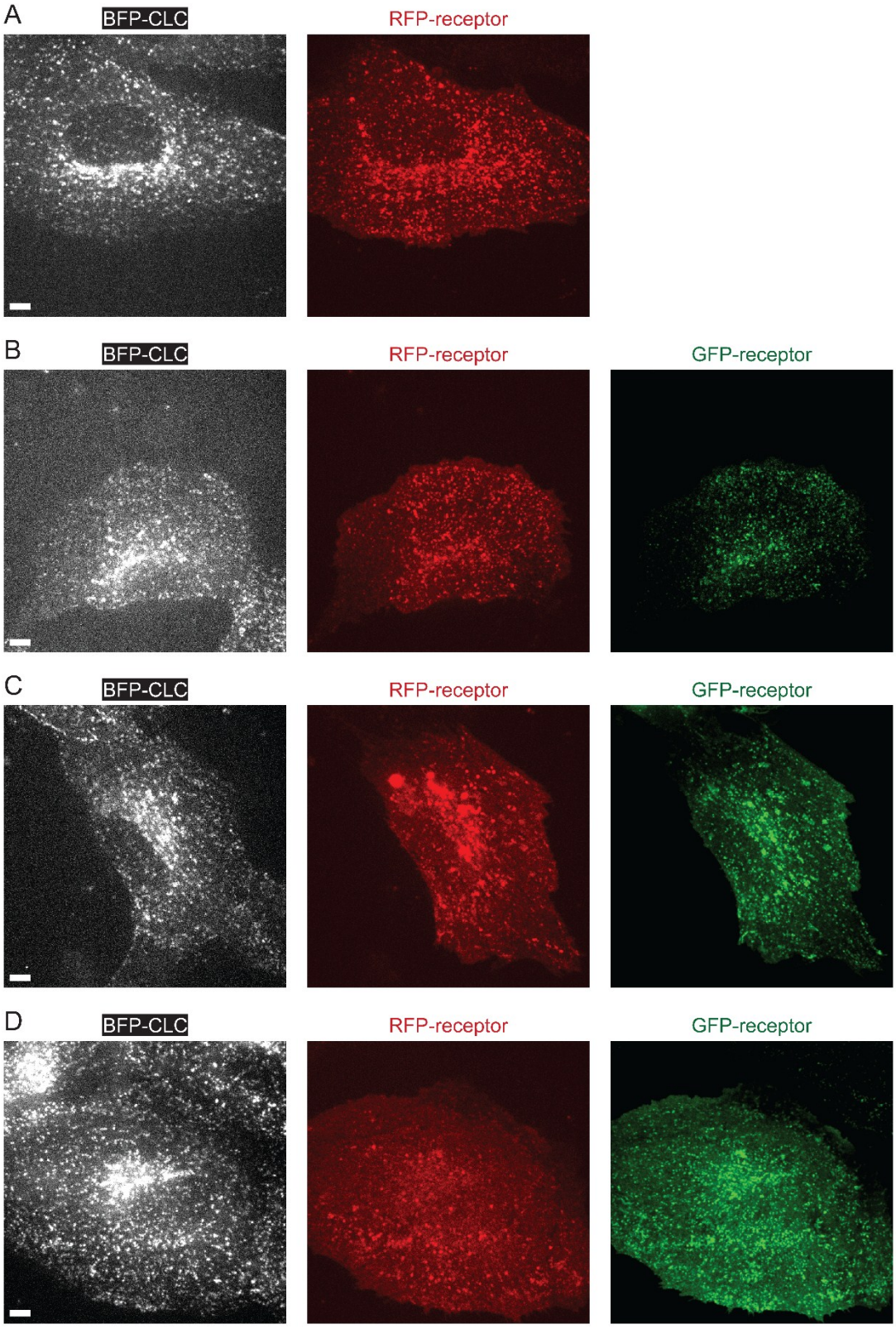

Figure S1: Full-frame images of the RPE cells stably expressing BFP-CLC shown in Figures 1 and 4. RPE cells were transiently transfected with the RFP-labeled model receptor and (A) no GFP-labeled receptor, shown in Figure 1B, (B) low levels of the GFP-labeled model receptor shown in Figure 4A, (C) medium levels of the GFP-labeled model receptor shown in Figure 4B, and (D) high levels of the GFP-labeled model receptor shown in Figure 1D and 4C. Scale bars represent 5 μm.

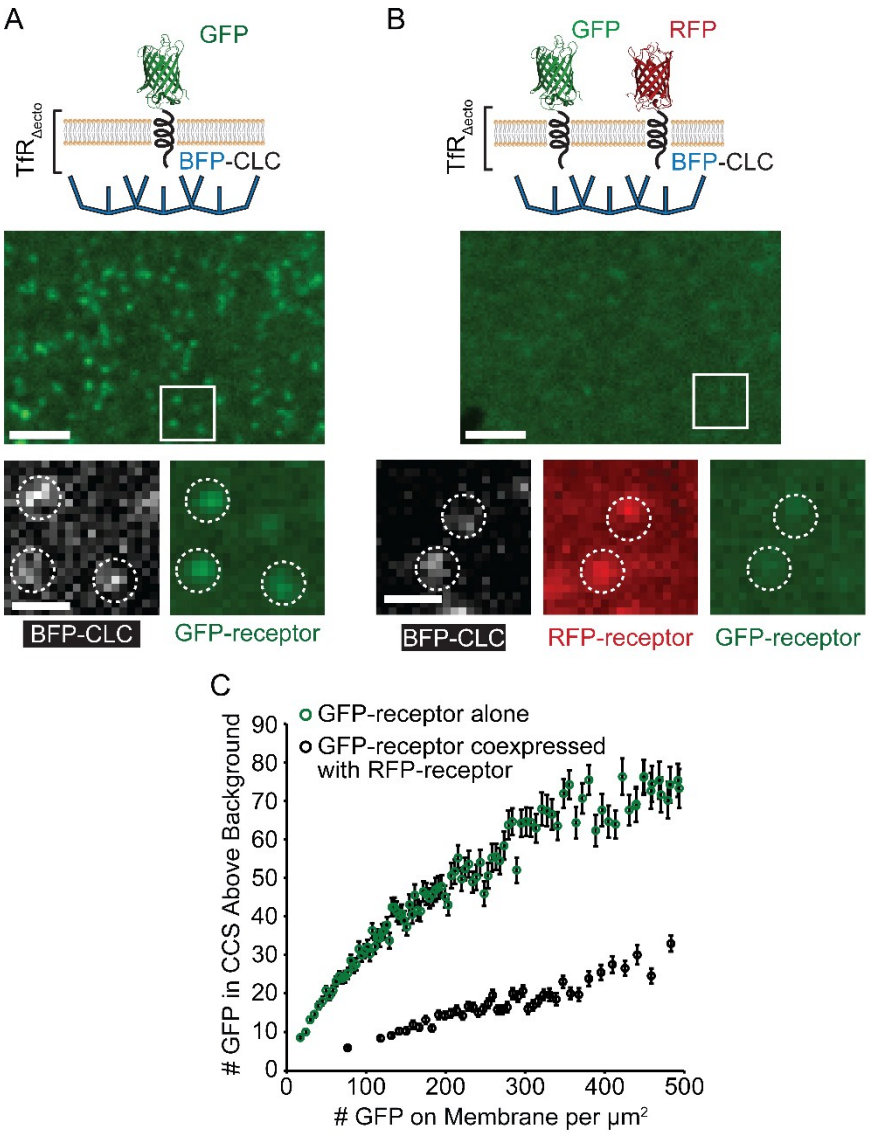

Figure S2: (A,B) Schematic (top) and spinning disc confocal image at the plasma membrane of an RPE cell (bottom) stably expressing BFP-CLC and transiently transfected with GFP-labeled receptor alone at 380 GFP/μm^2 (A) or the GFP-labeled receptor at 390 GFP/μm^2 with the RFP-labeled receptor at 1220 RFP/μm^2. All scale bars in example images represent 3 μm and in insets 1 μm. (C) Incorporation of the GFP-labeled model receptor into CCSs as a function of expression level is quantified when expressed alone and in the presence of the RFP-labeled receptor. RFP-

labeled receptor expression is constrained to 500-1500 RFP/μm^2. Each point represents measurements from 300 puncta with 29938 puncta represented by the green curve and 12697 puncta represented by the black curve. Error bars represent standard error of the mean.

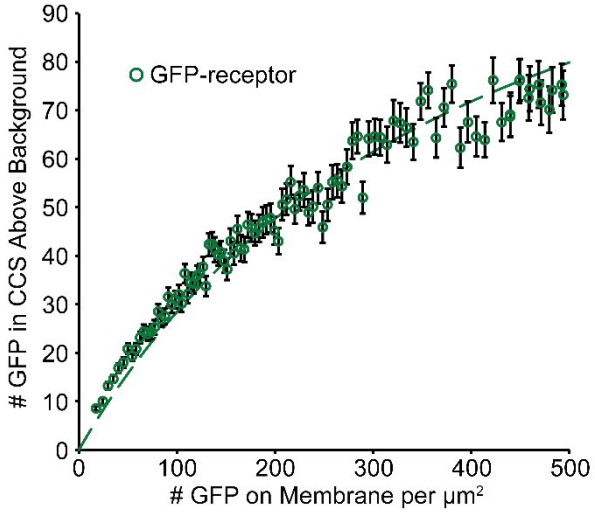

Figure S3: Model fit to the internalization curve of the GFP-labeled receptor expressed alone. The was performed simultaneously for the data in Figure 3B and S3. Each point represents measurements from 300 puncta with 29938 total puncta represented. Error bars represent standard error of the mean.

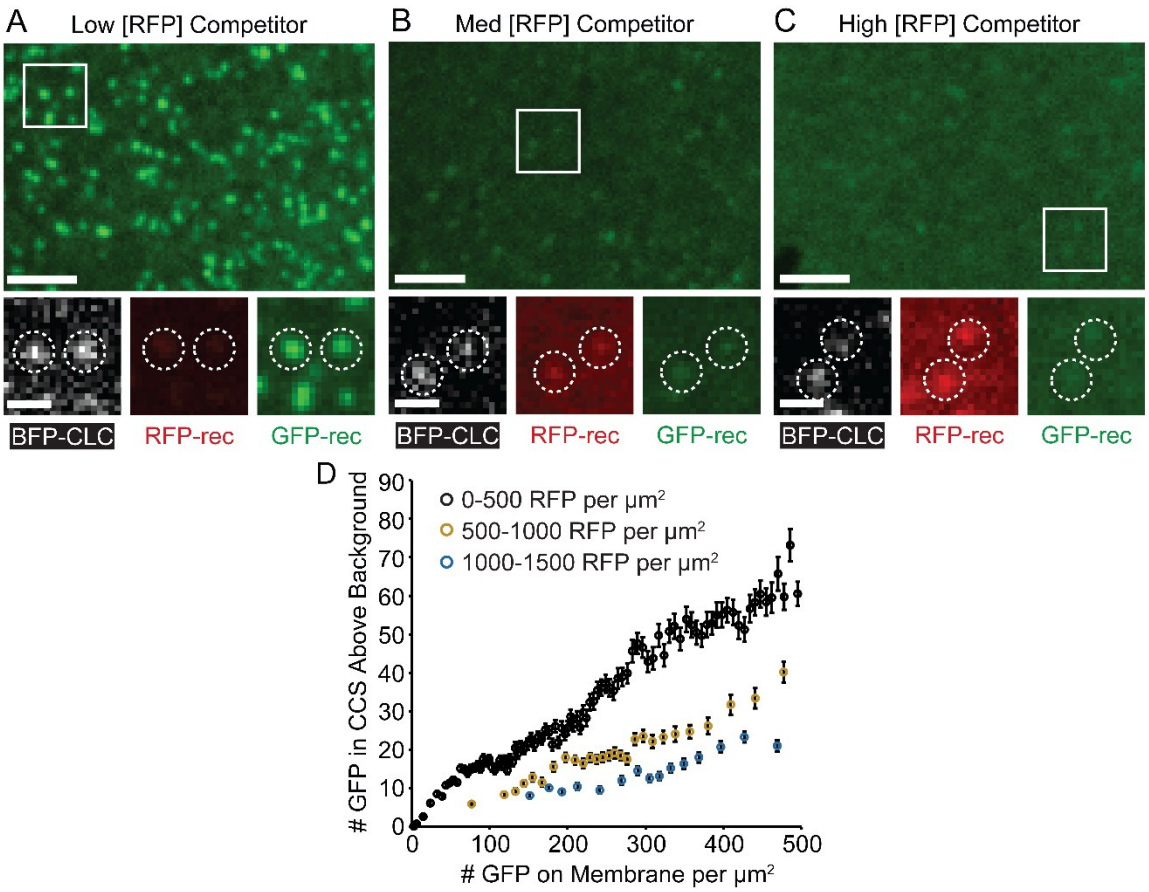

Figure S4: (A-C) Spinning disc confocal images at the plasma membrane surface of RPE cells stably expressing BFP-CLC and transiently transfected with the GFP-labeled receptor and RFP-labeled receptor. The concentration of the GFP-labeled model receptor and RFP-labeled competitor receptor is 420 GFP/μm^2 and 210 RFP/μm^2 (A), 300 GFP/μm^2 and 810 RFP/μm^2 (B), and 390 GFP/μm^2 and 1220 RFP/μm^2 (C). All scale bars in example images represent 3 μm and in insets 1 μm. (D) GFP-labeled receptor incorporation into CCSs is analyzed when expressed in the absence of competitor and in the presence of the RFP-labeled receptor ranging from 0-1500 RFP/μm^2. Each point represents measurements from 300 puncta with 28022 puncta represented by the black curve, 8085 puncta represented by the yellow curve, and 4612 puncta represented by the blue curve. Error bars represent standard error of the mean.

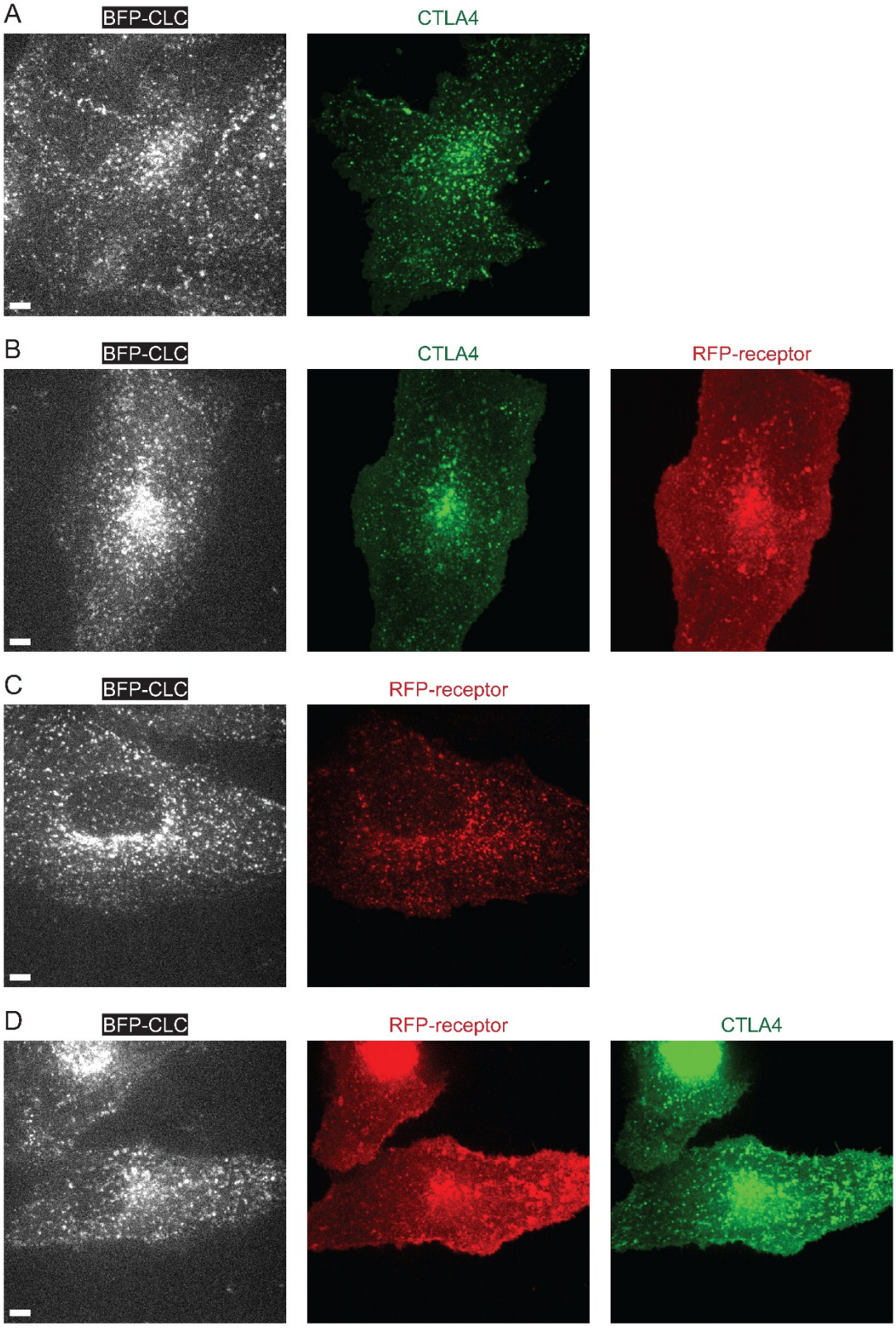
A
BFP-CLC
CTLA4
B
BFP-CLC
CTLA4
RFP-receptor
C
BFP-CLC
RFP-receptor
D
BFP-CLC
RFP-receptor
CTLA4

Figure S5: Uncropped images of the RPE cells stably expressing BFP-CLC shown in Figures 5 and 6. (A,B) RPE cells were transiently transfected with CTLA4-GFP (A) alone, shown in Figure 5A, or (B) with the RFP-labeled receptor in Figure 5B. (C,D) RPE cells were transiently transfected with the RFP-receptor (A) alone, shown in Figure 6A, or (B) with CTLA4-GFP shown in Figure 6B. Scale bars represent 5 μm.

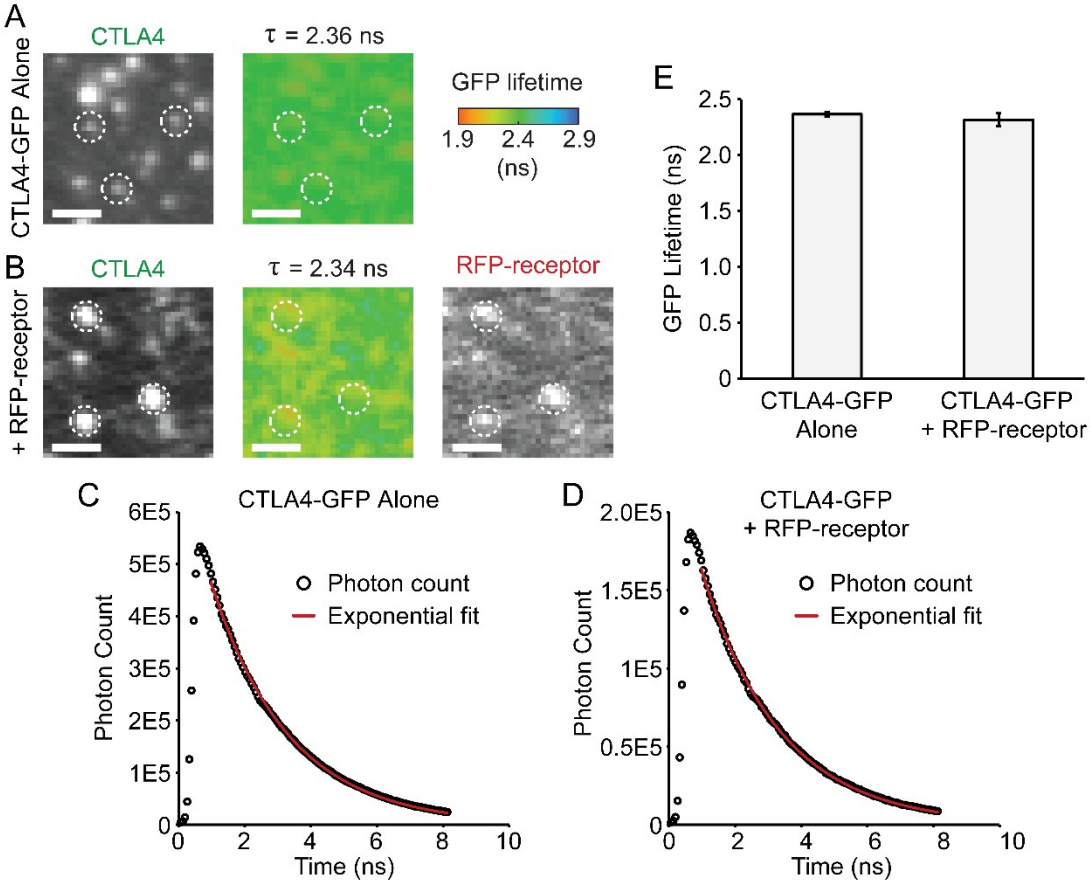

Figure S6: Time-correlated single photon counting microscopy yields fluorescence lifetime measurements for CTLA4-GFP when expressed alone (A) and when co-expressed with the RFP-labeled receptor (B). All scale bars represent 2 μm. (C,D) Corresponding photon counting histograms are shown for CTLA4-GFP expressed alone (C) and when co-expressed with the RFP-labeled receptor (D). (E) Exponential fits to this data for 8 cells expressing CTLA4-GFP alone and 12 cells expressing CTLA4-GFP with the RFP-labeled receptor reveal an average fluorescence lifetime of approximately 2.4 ns for each condition (C). Collectively these data indicate that the presence of the RFP-labeled model receptor has no measureable impact on the lifetime of CTLA4-GFP, suggesting that there is no significant FRET effect.

SUPPLEMENTARY MOVIES

Supplementary Movie 1: Example TIRF microscopy movie of an untransfected RPE cell stably expressing BFP-CLC. Images were taken at 3 second intervals over the course of 10 minutes.

Supplementary Movie 2: Example TIRF microscopy movie of an RPE cell stably expressing BFP-CLC and transiently expressing the GFP-labeled and RFP-labeled model receptors. Images were taken at 3 second intervals over the course of 10 minutes.

Supplementary Movie 3: Example TIRF microscopy movie of fluorescence recovery after photobleaching (FRAP) for an RPE cell stably expressing BFP-CLC and transiently expressing the GFP-labeled model receptor. Images were taken at 2 second intervals over the course of 2 minutes.

SUPPLEMENTARY MODELING SECTION

Thermodynamic Analysis

A simple equilibrium thermodynamic approach was developed to predict the average number of a receptor species inside a CCS, n, which has a total number of Ω_p binding sites available for receptors as described previously [1]. Briefly, these uniformly sized sites can be either occupied by a single receptor or unoccupied. Ω sites surround the CCS, which represent the plasma membrane area per CCS. The total number of receptors within this combined region ($\Omega + \Omega_p$) is N, which includes n receptors inside the CCS, such that there are a total of $N - n$ receptors outside the CCS. Here, we assume that n receptors inside the CCS and $N - n$ receptors outside the CCS are distributed randomly among the available sites. The internal energy per receptor of species within the CCS is $\varepsilon_{n_{CCS}}$, while the energy of each receptor on the surrounding plasma membrane surface is $\varepsilon_{n_{mem}}$. For receptors which are incorporated into CCSs via adaptor protein binding interactions, $\varepsilon_{n_{CCS}}$ is less than $\varepsilon_{n_{mem}}$ owing to biochemical affinity. In this way, receptors entering the CCS reduce the overall enthalpy of the system.

To consider the case of two receptor species on the plasma membrane, a second species denoted by M can be introduced such that m receptors exist within the CCS with M total receptors and therefore $M - m$ receptors outside the CCS on the surrounding membrane surface. The internal energy per receptor of species M within the CCS is $\varepsilon_{m_{CCS}}$, while the energy of each receptor on the surrounding plasma membrane surface is $\varepsilon_{m_{mem}}$.

Defining the number of microstates within each state, n, m: Assuming the system is in thermodynamic equilibrium, the various states of the system form a canonical ensemble such that the relative probability of each state is Boltzmann distributed. The states of the system are defined by the number of receptors within the CCS, n, m, from 0 to Ω_p. Each unique arrangement of the receptors within each state comprise a microstate. The number of ways of arranging n receptors in Ω_p sites within an endocytic structure are then:

$$\frac{\Omega_p!}{n!(\Omega_p - n)!} \qquad \text{Equation S1}$$

The number of ways of arranging m receptors into $\Omega_p - n$ unfilled sites within the CCS is:

$$\frac{(\Omega_p - n)!}{m!(\Omega_p - n - m)!} \qquad \text{Equation S2}$$

The number of ways of arranging $N-n$ receptors in Ω sites outside the endocytic structure is:

$$\frac{\Omega!}{(N-n)!(\Omega-(N-n))!}$$ Equation S3

Similarly, the number of ways of arranging $M-m$ receptors in $\Omega-(N-n)$ sites outside the CCS is:

$$\frac{(\Omega-(N-n))!}{(M-m)!(\Omega-(N-n+M-m))!}$$ Equation S4

Defining the enthalpy of each state, n, m: The enthalpy of n receptors within the CCS and $N-n$ receptors outside the CCS are given by

$$\varepsilon_{tot}=\varepsilon_{n_{mem}}(N-n)+\varepsilon_{n_{CCS}}(n)+\varepsilon_{m_{mem}}(M-m)+\varepsilon_{m_{CCS}}(m)=n\Delta\varepsilon_n+N\varepsilon_{n_{mem}}+m\Delta\varepsilon_m+M\varepsilon_{m_{mem}}$$

Equation S5

Determining the free energy of the system: Boltzmann's equation can be used to relate the number of microstates available to the system, W, to the entropy of the system, S:

$$S=k_B\ln W$$ Equation S6

We can then write an expression for the free energy of this system as a function of enthalpic and entropic terms in terms of Ω, Ω_p, N, n, $\Delta\varepsilon_n$, $\varepsilon_{n_{mem}}$, M, m, $\Delta\varepsilon_m$, and $\varepsilon_{m_{mem}}$:

$$G=n\Delta\varepsilon_n+N\varepsilon_{n_{mem}}+m\Delta\varepsilon_m+M\varepsilon_{m_{mem}}-k_BT\ln\left[\left(\frac{\Omega_p!}{n!(\Omega_p-n)!}\times\frac{(\Omega_p-n)!}{m!(\Omega_p-n-m)!}\right)\times\left(\frac{\Omega!}{(N-n)!(\Omega-N+\right.\right.$$

Equation S7

Simplifying this expression yields:

$$G=n\Delta\varepsilon_n+N\varepsilon_{n_{mem}}+m\Delta\varepsilon_m+M\varepsilon_{m_{mem}}-k_BT\ln\left[\left(\frac{\Omega_p!}{n!m!(\Omega_p-n-m)!}\right)\times\left(\frac{\Omega!}{(N-n)!(M-m)!(\Omega-N+n\right.\right.$$

Equation S8

Applying the Stirling approximation to the resulting equation we obtain:

$$G=n\Delta\varepsilon_n+N\varepsilon_{n_{mem}}+m\Delta\varepsilon_m+M\varepsilon_{m_{mem}}-k_BT(\Omega_p\ln\Omega_p-\Omega_p+\Omega\ln\Omega-\Omega-n\ln n+n-m\ln m+m-(\Omega$$
$$\ln(\Omega_p-n-m)+(\Omega_p-n-m)-(N-n)\ln(N-n)+(N-n)-(M-m)\ln(M-m)+(M-m)$$
$$-(\Omega-N+n-M+m)\ln(\Omega-N+n-M+m)+(\Omega-N+n-M+m)$$

Equation S9

This expression can be further simplified:

$$G = n\Delta\varepsilon_n + N\varepsilon_{n_{mem}} + m\Delta\varepsilon_m + M\varepsilon_{m_{mem}} - k_BT(\Omega_p \ln \Omega_p + \Omega \ln \Omega - n \ln n - m \ln m - (\Omega_p - n - m)\ln(\Omega_p - (N-n)\ln(N-n) - (M-m)\ln(M-m) - (\Omega - N + n - M + m)\ln(\Omega - N + n - M + m)$$

Equation S10

Minimization of free energy with respect to n: Next, we minimize free energy with respect to n:

$$\frac{\partial G}{\partial n} = 0 = \Delta\varepsilon_n - k_BT\big(-\ln n + \ln(\Omega_p - n - m) + \ln(N-n) - \ln(\Omega - N + n - M + m)\big)$$

Equation S11

Simplifying this expression yields:

$$\frac{\partial G}{\partial n} = 0 = \Delta\varepsilon_n - k_BT \ln \frac{(\Omega_p - n - m)(N-n)}{n(\Omega - N - M + n + m)}$$

Equation S12

Taking the exponential we have:

$$e^{\frac{\Delta\varepsilon_n}{k_BT}} = \frac{(\Omega_p - n - m)(N-n)}{n(\Omega - N - M + n + m)}$$

Equation S13

This equation can be rearranged:

$$e^{\frac{\Delta\varepsilon_n}{k_BT}} \times \frac{n}{N-n} = \frac{\Omega_p - n - m}{\Omega - N - M + n + m}$$

Equation S14

Under the assumptions that $n \ll N \ll \Omega$ and $m \ll M \ll \Omega$, this equation becomes:

$$e^{\frac{\Delta\varepsilon_n}{k_BT}} \times \frac{n}{N} = \frac{\Omega_p - n - m}{\Omega}$$

Equation S15

Minimization of free energy with respect to m: We can perform the same minimization of free energy with respect to m:

$$\frac{\partial G}{\partial m} = 0 = \Delta\varepsilon_m - k_BT\big(-\ln m + \ln(\Omega_p - n - m) + \ln(M-m) - \ln(\Omega - N + n - M + m)\big)$$

Equation S16

Simplifying the expression yields:

$$\frac{\partial G}{\partial m} = 0 = \Delta\varepsilon_n - k_BT \ln \frac{(\Omega_p - n - m)(M-m)}{m(\Omega - N - M + n + m)}$$

Equation S17

Taking the exponential we have:

$$e^{\frac{\Delta\varepsilon_m}{k_BT}} = \frac{(\Omega_p - n - m)(M-m)}{m(\Omega - N - M + n + m)}$$

Equation S18

This equation can be rearranged:

$$e^{\frac{\Delta\varepsilon_m}{k_BT}} \times \frac{m}{M-m} = \frac{\Omega_p - n - m}{\Omega - N - M + n + m}$$ Equation S19

Under the assumptions that $n \ll N \ll \Omega$ and $m \ll M \ll \Omega$, this equation becomes:

$$e^{\frac{\Delta\varepsilon_m}{k_BT}} \times \frac{m}{M} = \frac{\Omega_p - n - m}{\Omega}$$ Equation S20

Expected value of n: Setting left sides of Equation S15 and Equation S20 equal to one another:

$$e^{\frac{\Delta\varepsilon_n}{k_BT}} \times \frac{n}{N} = e^{\frac{\Delta\varepsilon_m}{k_BT}} \times \frac{m}{M}$$ Equation S21

Rearranging to solve for m:

$$m = e^{\frac{\Delta\varepsilon_n - \Delta\varepsilon_m}{k_BT}} \times \frac{Mn}{N}$$ Equation S22

Substituting Equation S22 into Equation S15:

$$e^{\frac{\Delta\varepsilon_n}{k_BT}} \times \frac{n}{N} = \frac{\Omega_p - n - e^{\frac{\Delta\varepsilon_n - \Delta\varepsilon_m}{k_BT}} \times \frac{Mn}{N}}{\Omega}$$ Equation S23

Rearranging:

$$e^{\frac{\Delta\varepsilon_n}{k_BT}} = \frac{\left(\Omega_p - n - e^{\frac{\Delta\varepsilon_n - \Delta\varepsilon_m}{k_BT}} \times \frac{Mn}{N}\right)N}{\Omega n}$$ Equation S24

Solving for n:

$$n\Omega e^{\frac{\Delta\varepsilon_n}{k_BT}} = \Omega_p N - nN - e^{\frac{\Delta\varepsilon_n - \Delta\varepsilon_m}{k_BT}} \times Mn$$ Equation S25

$$n\Omega e^{\frac{\Delta\varepsilon_n}{k_BT}} + nN + e^{\frac{\Delta\varepsilon_n - \Delta\varepsilon_m}{k_BT}} \times Mn = \Omega_p N$$ Equation S26

$$n = \frac{\Omega_p N}{\Omega e^{\frac{\Delta\varepsilon_n}{k_BT}} + N + Me^{\frac{\Delta\varepsilon_n - \Delta\varepsilon_m}{k_BT}}}$$ Equation S27

Expected value of m: Solving for n in Equation S21:

$$n = e^{\frac{\Delta\varepsilon_m - \Delta\varepsilon_n}{k_B T}} \times \frac{Nm}{M}$$ Equation S28

Substituting Equation S28 into Equation S20:

$$e^{\frac{\Delta\varepsilon_m}{k_B T}} \times \frac{m}{M} = \frac{\Omega_p - e^{\frac{\Delta\varepsilon_m - \Delta\varepsilon_n}{k_B T}} \times \frac{Nm}{M} - m}{\Omega}$$ Equation S29

Rearranging:

$$e^{\frac{\Delta\varepsilon_m}{k_B T}} = \frac{\left(\Omega_p - e^{\frac{\Delta\varepsilon_m - \Delta\varepsilon_n}{k_B T}} \times \frac{Nm}{M} - m\right)M}{\Omega m}$$ Equation S30

Solving for m:

$$m\Omega e^{\frac{\Delta\varepsilon_m}{k_B T}} = \Omega_p M - e^{\frac{\Delta\varepsilon_m - \Delta\varepsilon_n}{k_B T}} \times Nm - mM$$ Equation S31

$$m\Omega e^{\frac{\Delta\varepsilon_m}{k_B T}} + e^{\frac{\Delta\varepsilon_m - \Delta\varepsilon_n}{k_B T}} \times Nm + mM = \Omega_p M$$ Equation S32

$$m = \frac{\Omega_p M}{\Omega e^{\frac{\Delta\varepsilon_m}{k_B T}} + M + Ne^{\frac{\Delta\varepsilon_m - \Delta\varepsilon_n}{k_B T}}}$$ Equation S33

Here we see that the incorporation of species N into CCSs, Equation S27, takes an identical form to the incorporation of species M into CCSs, Equation S33. These expressions depend on Ω_p, Ω, the expression of the species of interest, the expression of the competing species, the binding energy of the species of interest, and the relative binding energies of the two species. As discussed in the main text, in the limit of no competitor, Equation S27 becomes:

$$n = \frac{\Omega_p N}{\Omega e^{\frac{\Delta\varepsilon_n}{k_B T}} + N}$$ Equation S34

This expression is identical to the expression for the average number of species N incorporated into CCSs in the absence of a competitor developed previously [1].

Considering the case of equal binding energies with respect to the internalization of species N, $\Delta\varepsilon_n = \Delta\varepsilon_m = \Delta\varepsilon$, we see that the internalization of the species of interest depends on the relative expression of each receptor. Under these conditions, Equation S27 becomes:

$$n = \frac{\Omega_p N}{\Omega e^{\Delta\varepsilon/k_B T} + N + M}$$ Equation S35

We can then rearrange to solve for the denominator:

$$\Omega e^{\Delta\varepsilon/k_B T} + N + M = \frac{\Omega_p N}{n}$$ Equation S36

Considering the case of equal binding energies with respect to the internalization of species M, $\Delta\varepsilon_n = \Delta\varepsilon_m = \Delta\varepsilon$, Equation S33 becomes:

$$m = \frac{\Omega_p M}{\Omega e^{\Delta\varepsilon/k_B T} + M + N}$$ Equation S37

We can then rearrange to solve for the denominator:

$$\Omega e^{\Delta\varepsilon/k_B T} + M + N = \frac{\Omega_p M}{m}$$ Equation S38

Setting the right side of Equation S36 and Equation S38 equal and rearranging yields:

$$\frac{n}{m} = \frac{N}{M}$$ Equation S39

SUPPLEMENTARY REFERENCES

1 A. C. M. DeGroot, D. J. Busch, C. C. Hayden, S. A. Mihelic, A. T. Alpar, M. Behar, and J. C. Stachowiak, *Biophys. J.*, 2018, **114**, 1377-1388.