Appendix

Estimation of hEGFR-EGF dissociation constants and proportions of high- and low-affinity receptors at the surface of A431 cells.

1.) Estimating the K_D for high- and low-affinity binding

The occupation of hEGFR binding sites at the membrane surface can be calculated using the equation

$$[Occ_{[EGF]}] = \frac{[EGF][LA]}{[EGF] + K_D^L} + \frac{[EGF][HA]}{[EGF] + K_D^H}$$

Where K_D^L and K_D^H are the dissociation constants of low- and high-affinity sites and $[LA]$, $[HA]$ and $[Occ_{[EGF]}]$ are the fractions of the surface receptors that bind EGF with low-affinity, high-affinity, and the total number of receptors occupied by a given $[EGF]$, respectively.

From the experimentally observed binding of fluorescently labelled EGF to cells with and without mAb 2E9 (which blocks low-affinity binding only) and PMA (which abolishes high-affinity binding) treatments (main text Fig. 2A) we estimate that ~10% of cell surface receptors can bind EGF with high affinity, hence $[HA] = 0.1$ and $[LA] = 0.9$.

For $[EGF] = 1$ nM, $[Occ_{[EGF]}]$ is

$$[Occ_1] = \frac{1 \times 0.9}{1 + K_D^L} + \frac{1 \times 0.1}{1 + K_D^H}$$

And for [EGF] = 100 nM

$$[Occ_{100}] = \left(\frac{100 \times 0.9}{100 + K_D^L} + \frac{100 \times 0.1}{100 + K_D^H}\right) = \frac{90}{100 + K_D^L} + \frac{10}{100 + K_D^H}$$

From the published dissociation constant of mAb 2E9 (K_D = 32 nM, (1)) EGF-binding to 86% of low affinity receptors is blocked after treatment with 200 nM of this antibody (Fig. 2A). This is the fraction of EGF binding we observed to be blocked by mAb 2E9 in cells treated with PMA (Main text, Fig. 2A). The fraction of low-affinity binding sites that EGF can bind after mAb 2E9 treatment is therefore = 0.14 $[LA]$. From this, the total receptor occupation fraction after mAb 2E9 treatment, $[Occ^A_{[EGF]}]$, is

$$[Occ^A_{[EGF]}] = \left(\frac{[EGF] \times 0.14[LA]}{[EGF] + K_D^L} + \frac{[EGF] \times [HA]}{[EGF] + K_D^H}\right)$$

Fig. 2A in the main text shows that the number of receptors occupied after exposure to 1 nM EGF is 1/9 of those occupied by 100 nM EGF. This figure also shows that the number of receptors occupied after exposure to 1 nM EGF is 1.8 times the number occupied by the same concentration in cells pre-treated with 2E9 (Fig. 2A). From this,

$$[Occ_1] = \frac{1}{9}[Occ_{100}]$$

$$[Occ_1] = 1.8\,[Occ_1^A]$$

Substituting the values of [EGF] in both equations we get,

$$\left[\frac{0.9}{1+K_D^L}+\frac{0.1}{1+K_D^H}\right]=\frac{1}{9}\left(\frac{90}{100+K_D^L}+\frac{10}{100+K_D^H}\right)$$

and

$$\left[\frac{0.9}{1+K_D^L}+\frac{0.1}{1+K_D^H}\right]=\ 1.8\ \left[\frac{0.14\times 0.9}{1+K_D^L}+\frac{0.1}{1+K_D^H}\right]$$

These equations can each be simplified to give,

$$K_D^L = \frac{9(K_D^H\ +\ 1)}{K_D^H}$$

$$K_D^L = 7.4 + 8.4K_D^H$$

Solving these, we get $K_D^H \sim 1$ nM and $K_D^L \sim 16$ nM. These values are comparable to previous results in A431 cells where $0.3 < K_D^H < 2$ nM and $5 < K_D^L < 36$ nM (1-5).

Fig, 2A also shows that the number of receptors occupied after exposure to 1 nM EGF is half that when receptors are pre-treated with mAb 2E9 and exposed to 100 nM EGF

$$[Occ_1] = \frac{1}{2}[Occ_{100}^A]$$

Which substituting the values of [EGF] gives,

$$\frac{0.9}{1+K_D^L}+\frac{0.1}{1+K_D^H}=\frac{1}{2}\left(\frac{12.6}{100+K_D^L}+0.1\right)$$

$K_D^H \sim 1$ nM and $K_D^H \sim 16$ nM are also solutions of this equation showing that the results in Fig. 2A are consistent.

2.) Fraction of occupied receptors that are high-affinity after pre-treatment with 200 nM mAb 2E9 and exposure to 100 nM EGF

From the estimated K_D values it follows that

The fraction of high-affinity sites occupied by 100 nM EGF is $\frac{100}{100+1} = 0.99$

The fraction of low-affinity sites occupied by 100 nM EGF is $0.14 \times \left(\frac{100}{100+16}\right) = 0.12$

The fraction of occupied receptors that are high-affinity for this labelling approach is therefore

$$\frac{(0.99 \times 0.1)N}{(0.99 \times 0.1)N + (0.12 \times 0.9)N} = 0.47$$

3.) Fraction of occupied receptors that are high affinity after exposure to 1 nM EGF

From the estimated K_D values it follows that

The fraction of high-affinity sites occupied by 1 nM EGF is $\frac{1}{1+1} = 0.5$

The fraction of low-affinity sites occupied by 1 nM EGF is $\frac{1}{1+16} = 0.059$

The fraction of occupied receptors that are high-affinity for this labelling approach is therefore

$$\frac{(0.5 \times 0.1)N}{(0.5 \times 0.1)N + (0.059 \times 0.9)N} = 0.48$$

Therefore the fraction of ligand bound receptors that are high-affinity after treatment with mAb 2E9 and 100 nM EGF (47%) or by 1 nM EGF (48%) are similar; consistent with the similar short distances for these two labelling conditions obtained by FRET measurement (Fig. 3A and 3B).

References

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