Supplementary information

Figure 1
Histogram of ensemble FRET efficiencies measured by FLIM in A431 cells labelled at 4 °C with a 1:2 mixture of 100 nM $EGF^{Alexa546}$ and $EGF^{Alexa647}$ (Förster radius for this pair is 7.4 nm), before and after adding 1% paraformaldehyde for 10 minutes.

Figure 2
The images show one frame (one polarisation only) from a sequence of images taken of EGF-Cy3 and EGF-Cy5 in HeLa cells, together with the white light transmission image. Note the molecules labelled A and B. Cy3 fluorescence is observed in the bottom-left quadrant, Cy5 fluorescence in the top-left quadrant. Raw pixel intensities for this frame are 13340 for A and 9136 for B in the Cy5 channel and 8375 and 672 in the Cy3 channel. This suggests that two levels of FRET are also seen in HeLa cells.

Figure 3
Video microscopy (raw data) of receptor displacement in live A431 cells labelled for 20 minutes at 18 ºC with 0.1 nM EGF^{Cy3} diluted in anti-internalisation buffer. The four quadrants are of the same field of view but differ in spectral and polarisation content. Cy3 emission is imaged to the two quadrants on the right; any signal on the left is autofluorescence or bleedthrough in the 660-700 nm range. Diagonally-opposite quadrants have the same emission polarisation, adjacent quadrants have orthogonal polarisations.