Supplementary Information S1 (Box) | FRET measurements in cells

Förster (or Fluorescence) Resonant Energy Transfer (FRET) is a photophysical process where a donor probe transfers its excited-state energy to a proximal probe, the acceptor, in a non-radiative way via dipole-dipole interaction. Efficient FRET requires the following conditions:

- A substantial overlap between the spectra of the donor emission and acceptor absorption.
- Close proximity (R) between the donor and the acceptor of 1-10nm (around the Förster distance, R_0)[103].
- Favorable orientation of the donor and acceptor dipoles

$$ET = \frac{R_0^6}{R_0^6 + R^6}$$

Thus, measuring FRET signals is a useful way to adequately report on tight bimolecular interactions (see Fig. 3A). For live cell imaging, chimeric proteins of interest with CFP and YFP variants are often used as donor-acceptor FRET pairs[101]. Otherwise, a large selection of synthetic fluorescent FRET pairs exists. Upon conducting careful control measurements, energy transfer (ET) can be reported in several different ways[104]. We specify below the most popular ones for cell imaging:

Sensitized emission - reported via donor and acceptor detected emissions (I_D and I_A, respectively). This technique is compatible with live cell imaging but requires a set of controls to discern emission leakage between channels and direct excitation of the acceptor (resulting in the correction factor γ)

$$ET = \frac{I_A}{I_A + \gamma I_D}$$

Acceptor photobleaching – a simpler way of measuring ET via the comparison of donor emission in the presence of the acceptor (I_{DA}) and its recovery upon acceptor photobleaching (I_D). This method is less compatible with live cell imaging due to its sequential and irreversible nature.

$$ET = 1 - \frac{I_{DA}}{I_D}$$

Donor fluorescent lifetime imaging (FLIM) – FRET quenches the donor emission, thus causing a shortening of its lifetime (τ_{DA}) in close proximity to an acceptor in respect to its unquenched lifetime lifetime (τ_D). This technique is likely the most direct measurement of ET but requires a specialized FLIM system.

$$ET = 1 - \frac{\tau_{DA}}{\tau_D}$$