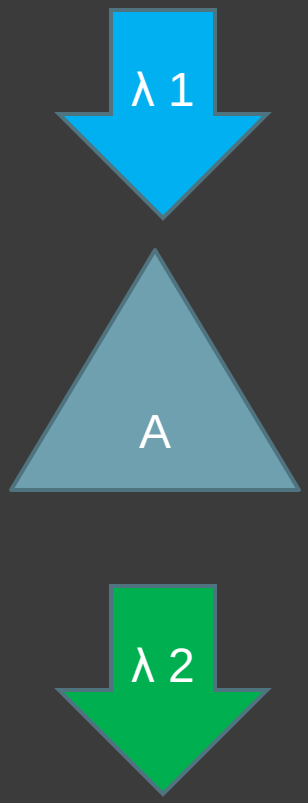
…to the left, to the right…

RESONANCE ENERGY TRANSFER

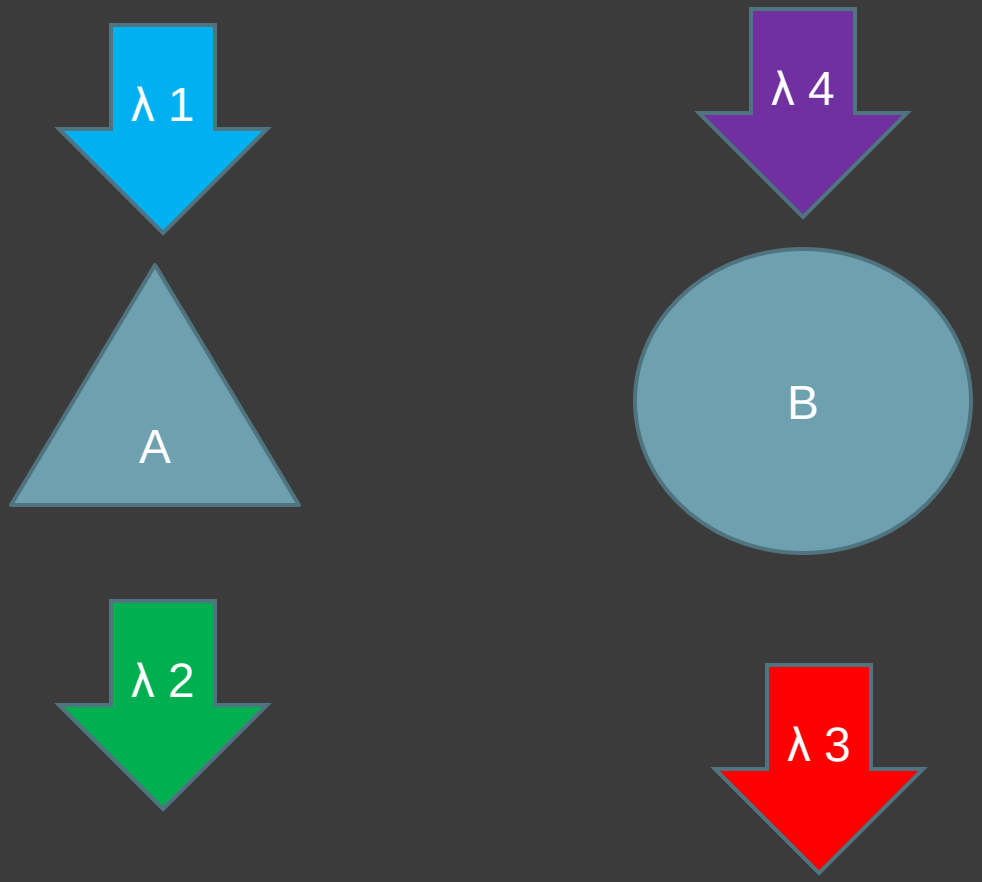
Overview: Fluoresence



Molecule “A” is excited with a light input of wavelength λ1

In response, it emits light with a wavelength of λ2

Overview: dual Fluoresence



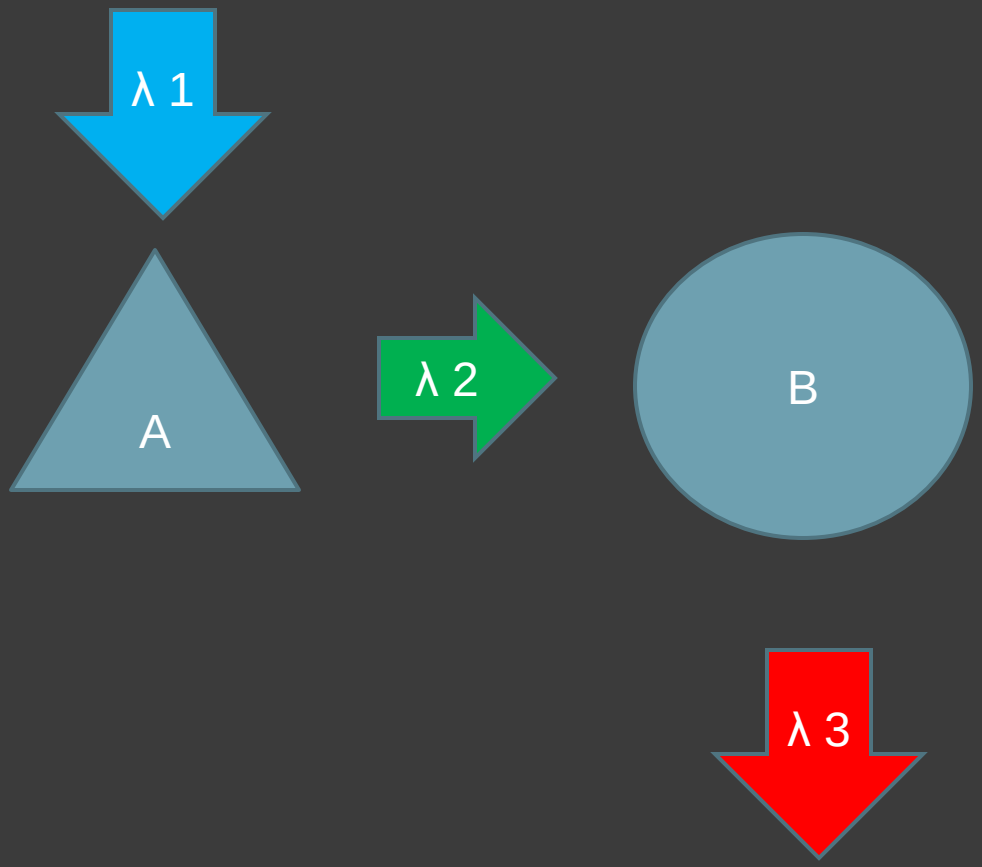
Molecule “A” is excited with a light input of wavelength λ1

In response, it emits light with a wavelength of λ2

Molecule “B”, is excited with a light input of wavelength λ 4

Which in turn emits ligth in wavelength λ 3

Overview: FRET



Molecule “A” is excited with a light input of wavelength λ1

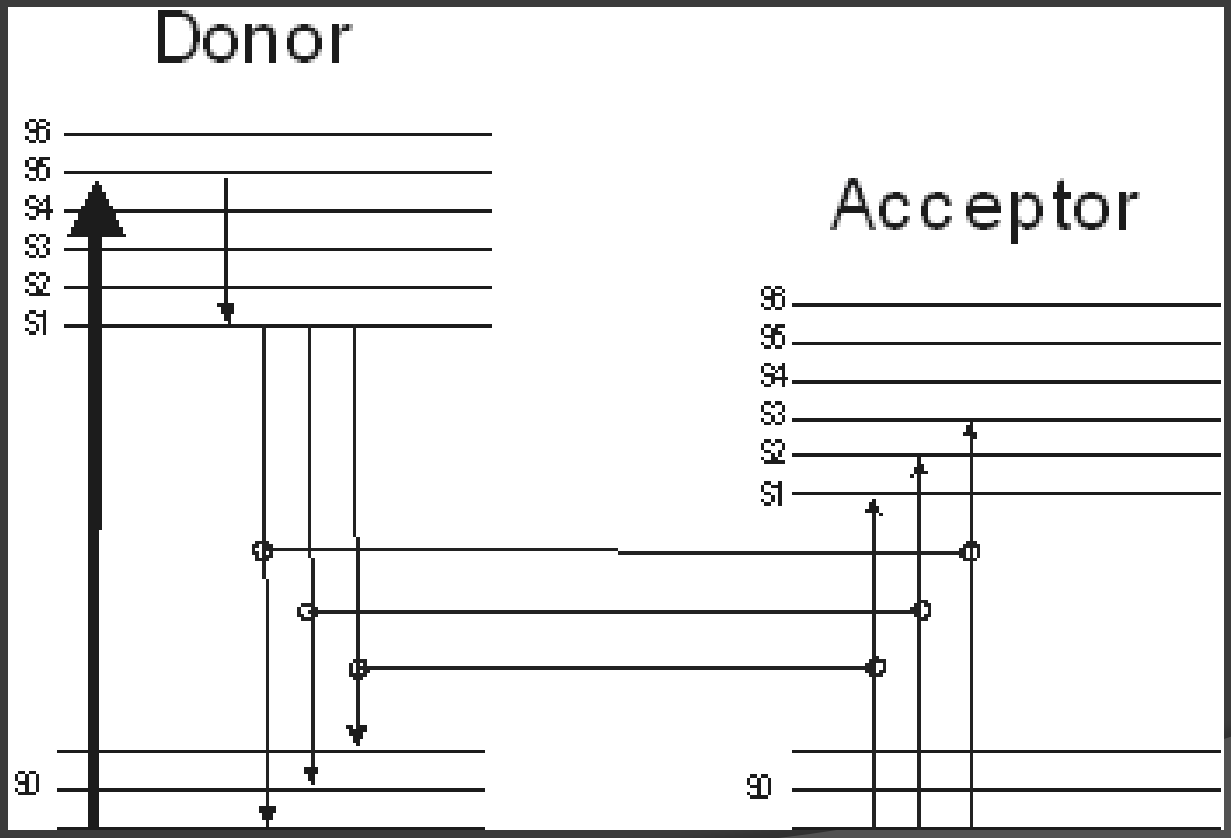
In response, it emits light with a wavelength of λ2

If $\lambda 2 = \lambda 4$
And $\Delta d < 10nm$

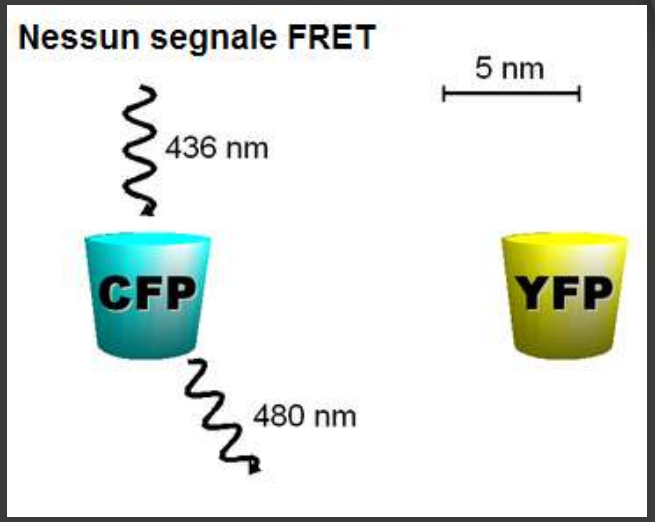
This light is captured by nearby protein “B”

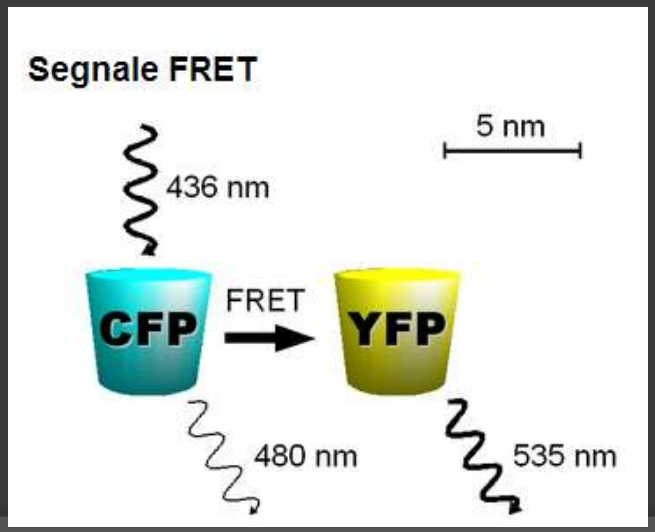
Which in turn emits light in wavelength λ 3

Electric Explanation



xFP example

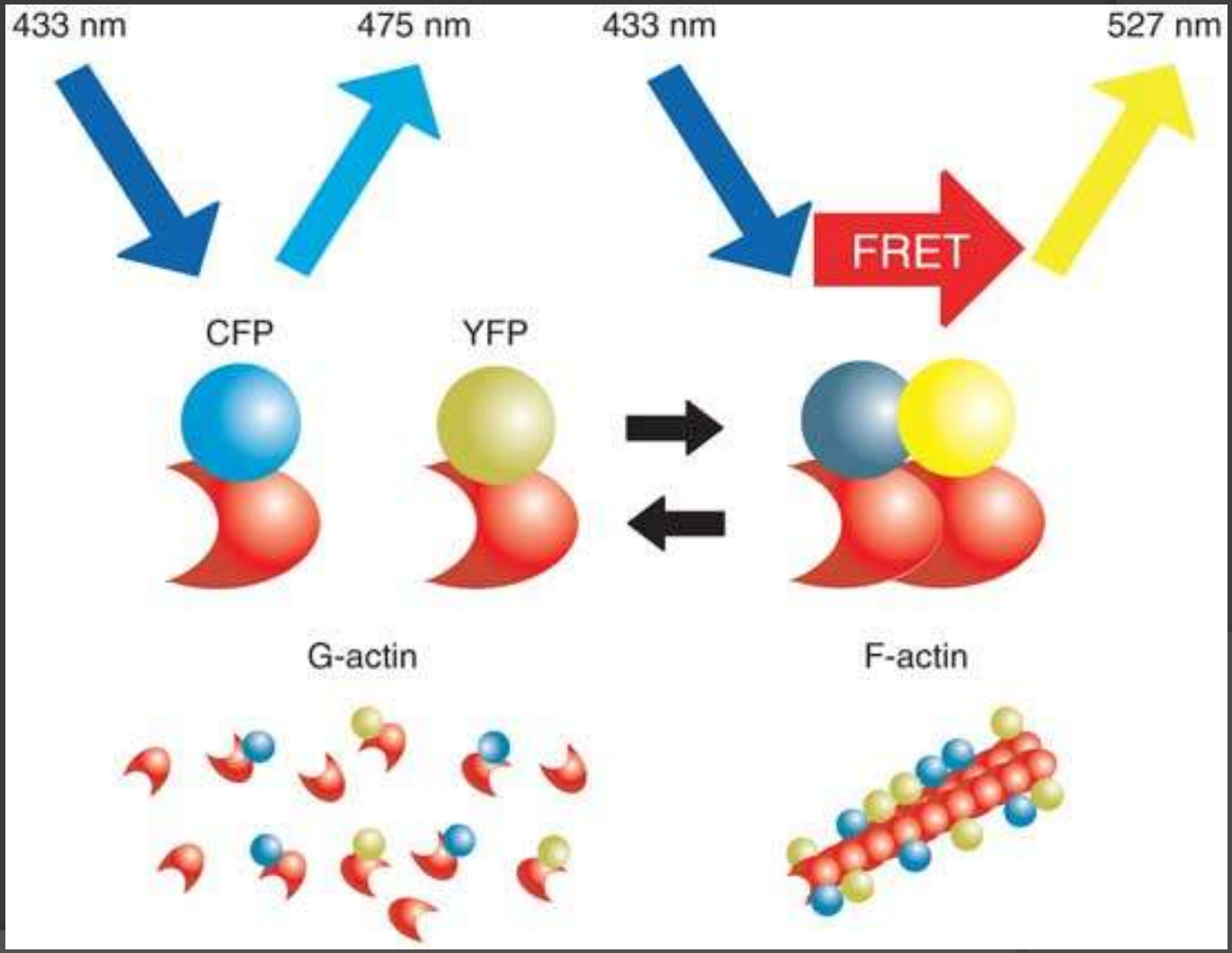

Nessun segnale FRET
5 nm
436 nm
CFP
YFP
480 nm


Segnale FRET
5 nm
436 nm
CFP
FRET
YFP
480 nm
535 nm

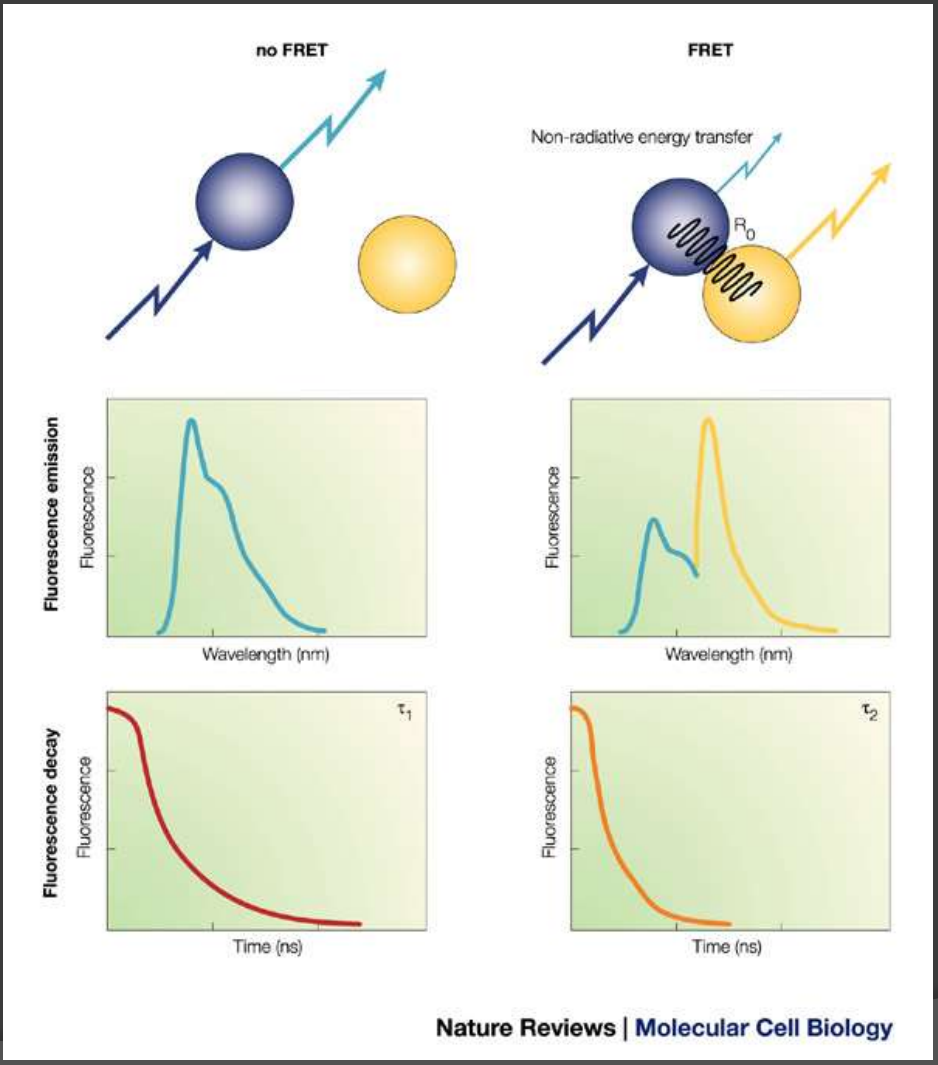
Polymerisation Application

If there is polymersiation, then we see yellow.

If not, we see blue.



Quantum Madness



Depending on the Quantum Yield, we will see spectrum contamination…

Down the Rabbit Hole

E	quantum yield
kET	rate of energy transfer
kF	radiative decay (!radiation)
Ki	rate constants of other deexitation

$$E = \frac{k_{ET}}{k_f + k_{ET} + \sum k_i}$$

E	quantum yield
r	separator distance
Ro	Förster distance (distance at which transfer rate is 50%)

$$E = \frac{1}{1 + (r/R_0)^6}$$

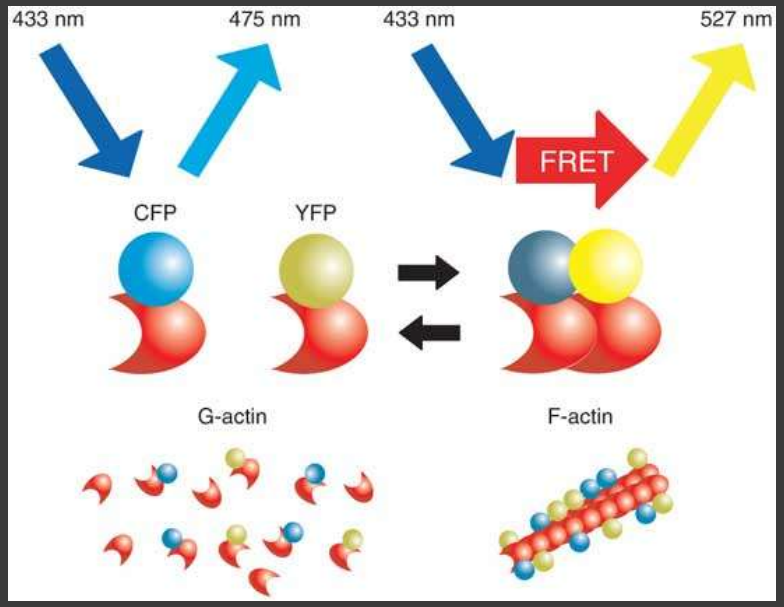
Ro	Förster distance
Qo	E in the abscence of the other
K	dipole orientation factor (one per dipole)
J	spectral overlap
n	refractive index
Na	Avogadro's number

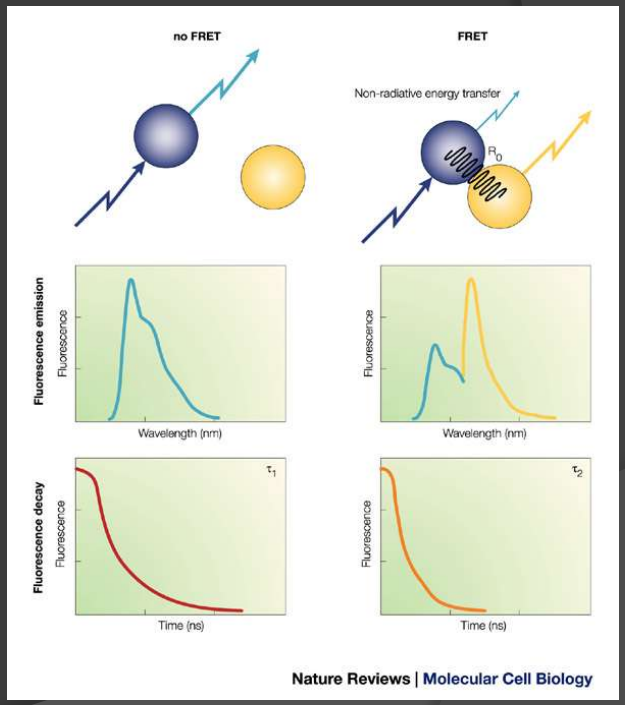
$$R_0^{\,6} = \frac{9\,Q_0\,(\ln 10)\kappa^2\,J}{128\,\pi^5\,n^4\,N_A}$$

Quantum What?!

$$\Phi = \frac{\#\ \text{photons emitted}}{\#\ \text{photons absorbed}}$$

Probability of emission per excitation event.





So...

- For quite low quantum yield, we will have some slight spectral pollution.
- For normal quantum yield values, our efficiency will be average (think firefly).
- For good quantum yield values, our system would be quite efficient.

And what about BRET ?!

- Bioluminesent
- Resonance
- Energy
- Transfer

Sources

- http://www.biotek.com/resources/articles/fluorescence-resonance-energy-transfer.html
- http://en.wikipedia.org/wiki/Förster_resonance_energy_transfer
- http://www.nature.com/nrm/journal/v4/n7/fig_tab/nrm1153_F2.html
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