Small vertical movement of a K^{+} channel voltage sensor measured with luminescence energy transfer

Selvin, et al., doi:10.1038/nature03819

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Voltage-gated potassium (Kv) channels

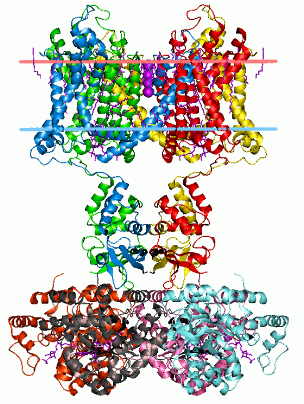


Figure : Kv channels respond to transmembrane voltage.

Crucial roles in

- neuron firing,
- muscle contraction,
- hormone release, etc.

Controversy arose

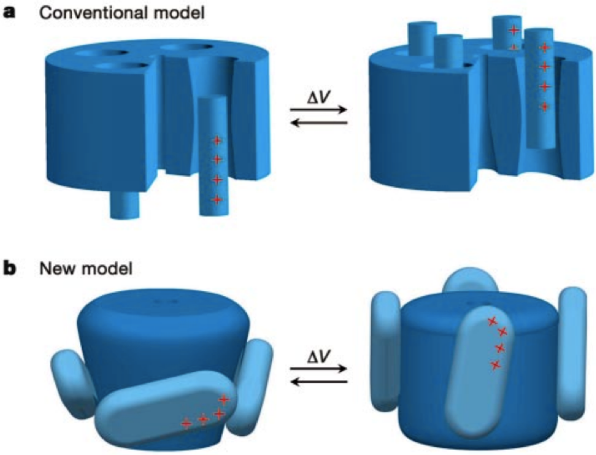


Figure : The conventional model vs the paddle model. (doi:10.1038/nature01580, 2003)

Structure of Kv channel

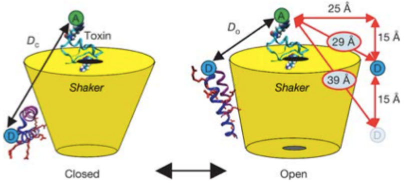


Figure : Left: The isolated voltage sensor. (doi:10.1038/nature01580, 2003) Right: Diagram of paddle model (doi:10.1038/nature03819, 2005)

Magnitude of displacement as the litmus test

- The conventional model predicts small (~2Å) movement
- Paddle model predicts large (~10Å) movement
- LRET (to be explained) measurement
  - donors attached to S4, S3b sites
  - acceptor attached to channel using scorpion venom
- Scorpion venom has no effect on the "paddles."

Pyramid geometry

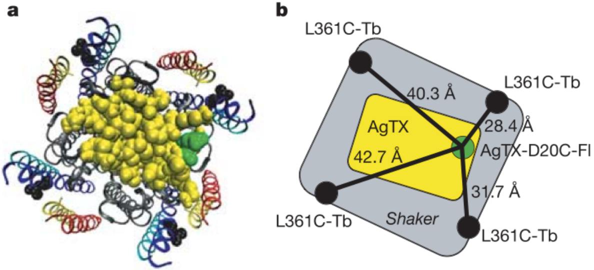


Figure : The 4 donors are attached to S4, S3b sites, while the acceptor is attached to the pore.

Reigning champion

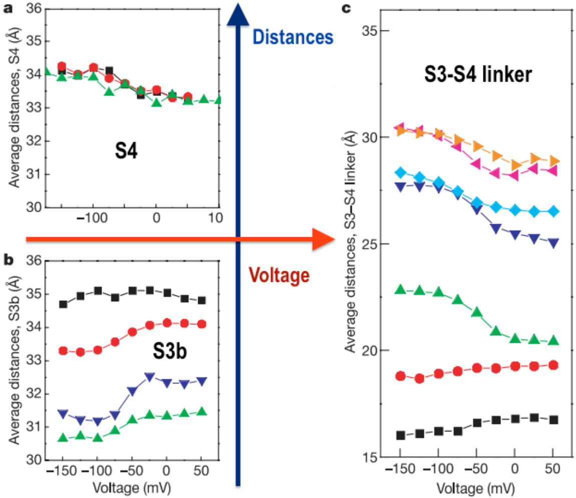
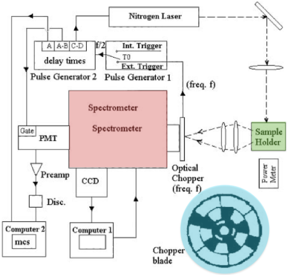


Figure : Selvin, doi:10.1038/nature03819

Lanthanide-Based Resonance Energy Transfer



Lanthanide-Based Resonance Energy Transfer

Paul R. Selvin

(Invited Paper)

The crucial component

We are to measure distances ~1Å *in vivo* via common optical instruments!

Figure : Selvin, *Selected Topics in Quantum Electronics*, Dec 1996, doi: 10.1109/2944.577339

Short and fast decays

- Two electrons in the ground state $S = 0$
- Fluorescence: singlet-singlet ($S = 0$)
  - short lifetime $\sim 1\mathrm{ns}$
- Phosphorescence: singlet-triplet ($S = 1$)
  - much longer lifetime $\sim 1\mathrm{ms}$
- Vibrations broaden the spectra and dissipate energy

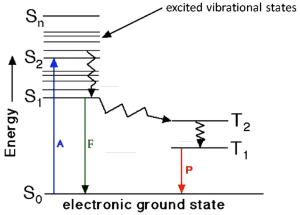


Figure : Jablonski diagrams of fluorescent and phosphorescent emissions.

Elements of wonder: the Lanthanides

- High-spin atoms
  - in general $S > 1$
  - fluorescence or phosphorescence?
  - luminescence!
  - long-lived $\sim 1\text{ms}$
- Valence electrons in f-orbitals
  - the f-orbitals are "buried inside"
  - also weak absorbers

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The donor and the acceptor

- The donor comprises 3 parts
  - the lanthanide ion (blue in figure), which will transfer energy to the acceptor
  - the antenna (red in figure), which receives energy from the laser and pass it on to the ion
  - the chelate, which holds and protects the ion
- The acceptors are the usual organic dyes

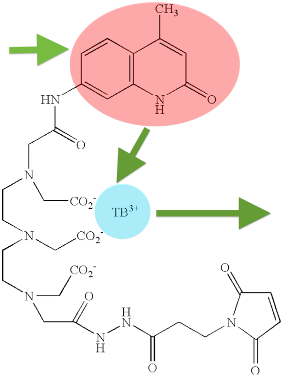

Figure : Selvin, et al.

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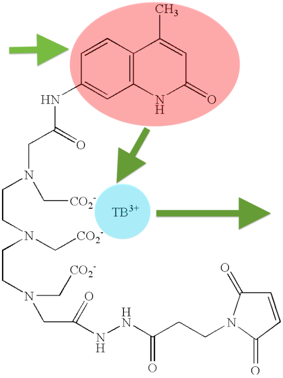

Figure : Selvin, et al.

Energy transfer via donor-acceptor coupling

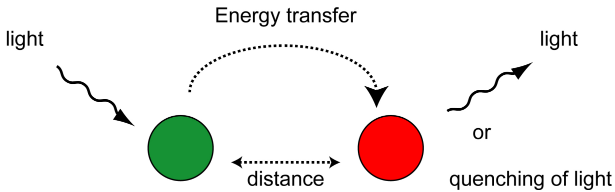

Figure : The donor receives energy from the laser then transfers a virtual photon to the acceptor which emits a photon of a different frequency.

- Within an effective range $R_0 \sim \lambda$, the donor may transfer energy to the acceptor
  - The matrix element is dipole-dipole coupling
  - The rate of energy transfer is therefore $\propto 1/R^6$

Efficiency of energy transfer is distance-dependent

- The excited donor loses energy and decays back to ground state in essentially two ways.

Efficiency of energy transfer

$$\begin{aligned}\mathcal{E} &= \frac{\#(\text{energy transfer})}{\#(\text{energy transfer}) + \#(\text{direct emission})} \\ &= \frac{k_{\mathrm{DA}}}{k_{\mathrm{DA}} + k_{\mathrm{D}}} = \frac{1}{1 + k_{\mathrm{D}}/k_{\mathrm{DA}}}\end{aligned}$$

- k_{D} is the decay rate of a single donor
- k_{DA} is the decay rate of donor-acceptor pair
- The rate k_{DA} is distance dependent and $k_{\mathrm{D}}/k_{\mathrm{DA}} = (R/R_0)^6$

Energy transfer efficiency is related to lifetimes

- The donor-acceptor coupling will also modify donor lifetime

$$\tau_{\mathrm{D}} \xrightarrow{\text{donor-acceptor coupling}} \tau_{\mathrm{DA}}$$

  - τ_{D} donor lifetime
  - τ_{DA} modified lifetime

The energy transfer efficiency can also be written as

$$\mathcal{E} = \frac{\Delta\tau}{\tau_{\mathrm{D}}} = \frac{\tau_{\mathrm{D}} - \tau_{\mathrm{DA}}}{\tau_{\mathrm{D}}} = 1 - \frac{\tau_{\mathrm{DA}}}{\tau_{\mathrm{D}}}$$

- The lifetimes can be accurately measured by fitting curves.
- Therefore, we will obtain the distance R from the data of lifetimes!

Greater the overlap, longer the ruler!

- The effective range provides us a length scale, the size of the ruler.
- The R_0 is related to spectral overlap J, $R_0^6 \propto J$

Normalized spectral overlap

$$J = \frac{\int \epsilon_A(\lambda) f_D(\lambda) \lambda^4 d\lambda}{\int f_D(\lambda) d\lambda}$$

- ϵ_A is the absorption spectrum of the acceptor
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How to improve the accuracy?

- Sources of noise
  - The background, autofluorescent emissions $\sim 1\text{ns}$
  - The unpaired donors, lifetime $\sim 1\text{ms}$, same order as that of the donor-acceptor pair
- The former can be separated from the donor-acceptor emission through their significantly different lifetimes!

Failure in the latter

However, the unpaired cannot be distinguished by the minor differences in lifetimes!

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LRET is insensitive to incomplete labeling

- We may distinguish the emissions from donors and donor-acceptor pairs through "colors"

Different Spectral Properties

- The acceptors are organic molecules whose spectra spread out
- Lanthanide donor spectra are sharply spiked

- Therefore, we are able to filter out the donors by looking at the "gaps."

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Spectra of the donor and acceptor

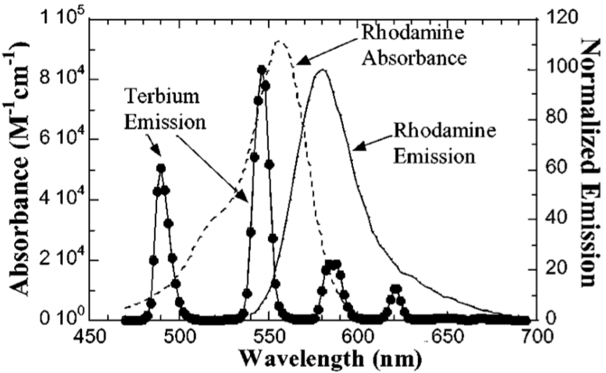

Figure : The donor (with a lanthanide ion) spectrum is sharply spiked and exhibits dark regions. There is a good overlap between donor emission and acceptor absorbance.

LRET Setup

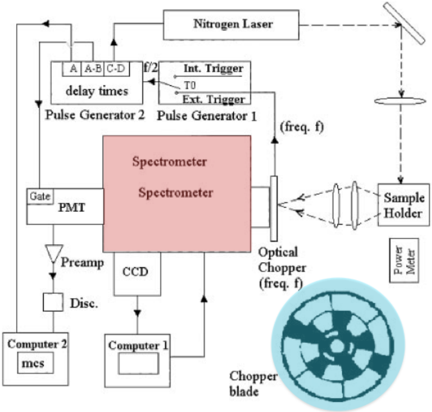

Figure : The chopper (blue) is synchronized with the laser pulses and the spectrometer (red) is tuned to the dark regions of the donor spectrum.

Cited 111 times in total

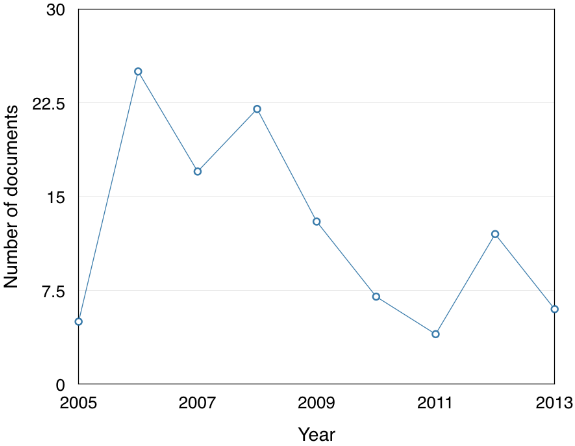

Figure : Citation data obtained from *Scopus*

An interesting and important work

Our Comments

- The paper is well-written with clearly defined concepts and motivation.
- The LRET is mature, whose accuracy has been verified.
- The use of AgTX (venom) unambiguously resolves the controversy.
- Important in cellular biology, neuroscience, medical science.
- Niche paper, yet, easy to understand.

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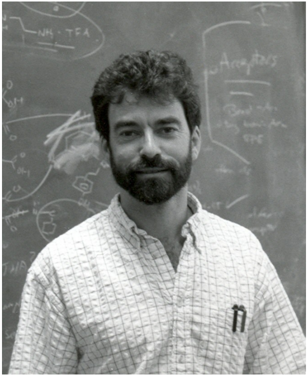
Medical application

- **hERG potassium channels and cardiac arrhythmia** (doi:10.1038/nature04710, 2006)
- hERG, the gene for the subunit of K^+ channel
- A blockage of hERG K^+ channel causes arrhythmia
  - mutations in the hERG gene
  - or by drugs
- The S4 domain moves in response to changes in membrane potential

Dr. Selvin is very nice!



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