# Introduction to Biophysics (II)

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# Outline

**Lecture 1: Basic concepts**

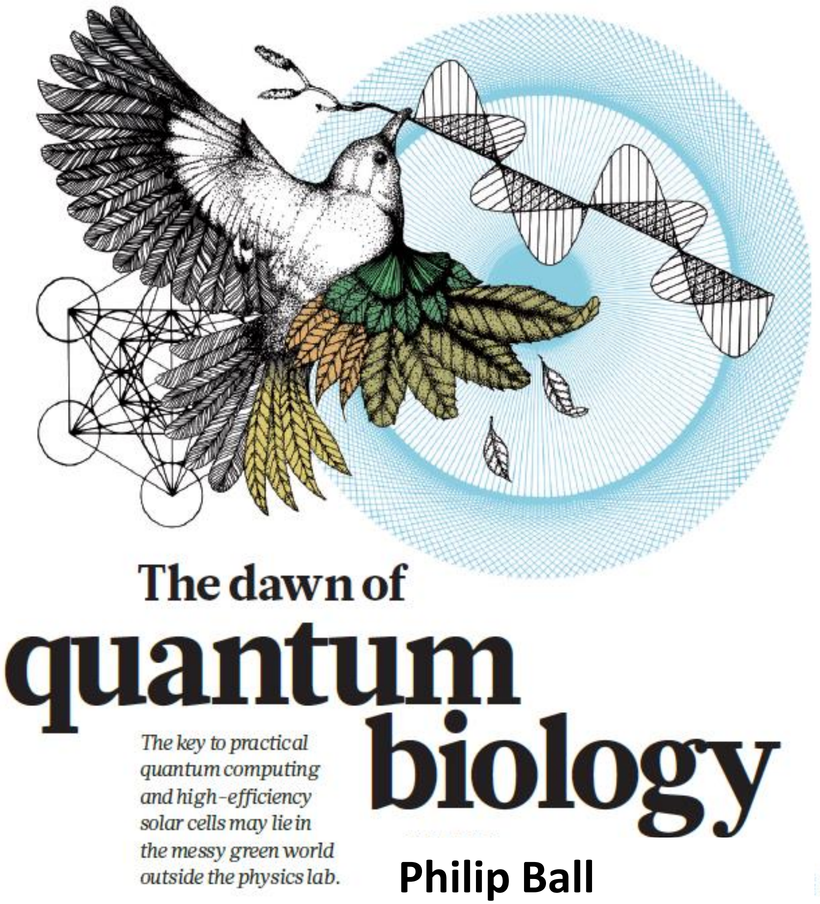
- Introduction
- The biological cell
- Central dogma of molecular biology
- Macromolecules (DNA and proteins)

**Lecture 2: Selected theoretical and experimental examples**

- Quantum biology
- Manipulation of single biomolecules
- Biomolecule-nanoparticle interactions
- Superresolution imaging
- Single-molecule spectroscopy

# 1. Quantum Biology

Quantum biology is the field of study that investigates processes in living organisms that cannot be accurately described by the classical laws of physics.




**Philip Ball**
***Nature* 27 june 2011**

**Recommendable headline review:**

Adriana Marais, …, Tjaart Krüger, Francesco Petruccione and Rienk van Grondelle,
The future of quantum biology. *J. R. Soc. Interface* **15**: 20180640 (2018)

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# 1. Quantum Biology

INTERFACE

rsif.royalsocietypublishing.org

Review

Check for updates

**Cite this article:** Marais A *et al.* 2018 The future of quantum biology. *J. R. Soc. Interface* **15**: 20180640.
http://dx.doi.org/10.1098/rsif.2018.0640

## The future of quantum biology

Adriana Marais[1], Betony Adams[1], Andrew K. Ringsmuth[2,3,†], Marco Ferretti[2], J. Michael Gruber[2], Ruud Hendrikx[2], Maria Schuld[1], Samuel L. Smith[4], Ilya Sinayskiy[1,5], Tjaart P. J. Krüger[6], Francesco Petruccione[1,5] and Rienk van Grondelle[2]

[1]Quantum Research Group, School of Chemistry and Physics, University of KwaZulu-Natal, Durban 4001, South Africa
[2]Institute for Lasers, Life and Biophotonics, Faculty of Sciences, VU University Amsterdam, De Boelelaan 1081, 1081 HV Amsterdam, The Netherlands
[3]ARC Centre of Excellence for Engineered Quantum Systems, The University of Queensland, St Lucia 4072, Australia
[4]Cavendish Laboratory, University of Cambridge, Cambridge, UK
[5]National Institute for Theoretical Physics, KwaZulu-Natal, South Africa
[6]Department of Physics, Faculty of Natural and Agricultural Sciences, University of Pretoria, Hatfield, South Africa

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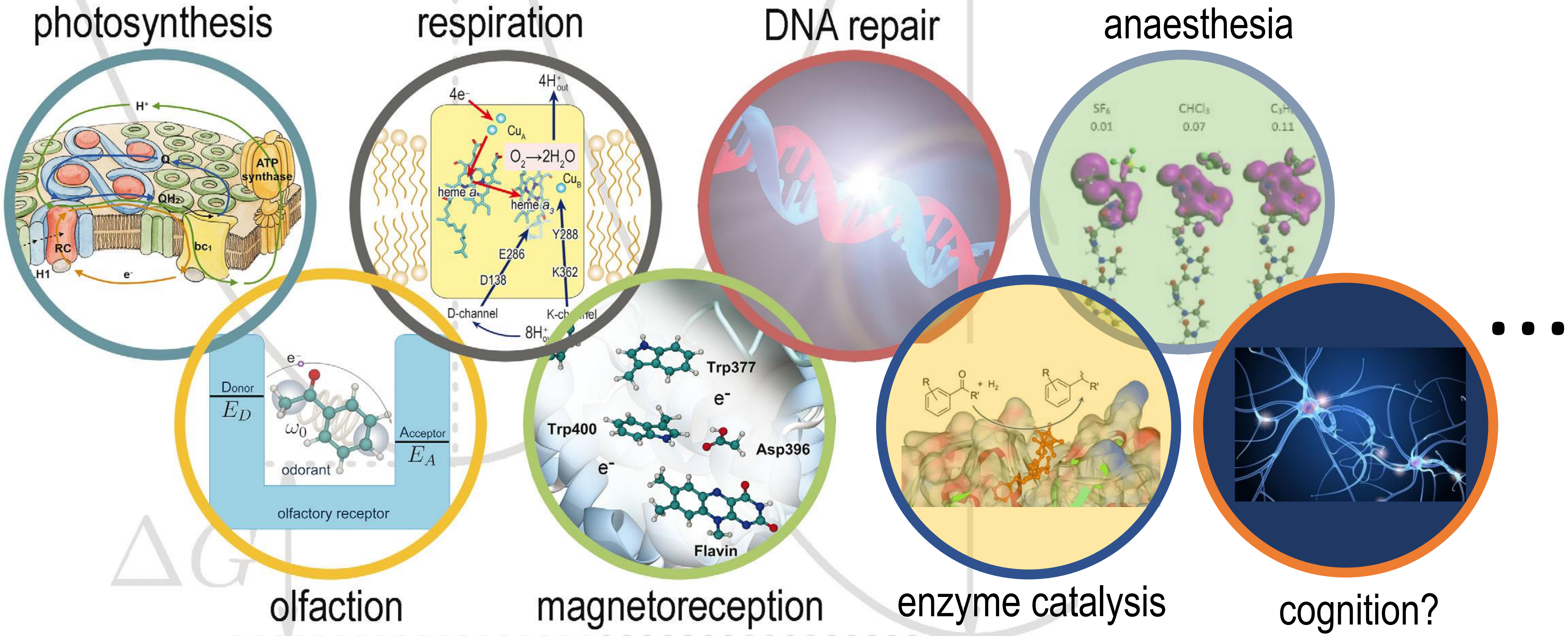
# 1. Quantum Biology

$$k_{et} = \frac{2\pi}{\hbar}|H_{AB}|^2 \frac{1}{\sqrt{4\pi\lambda k_B T}} \exp\left(-\frac{(\lambda + \Delta G)^2}{4\lambda k_B T}\right)$$

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Picture adapted from the Theoretical and Computational Biophysics Group, Beckman Institute, University of Illinois at Urbana-Champaign

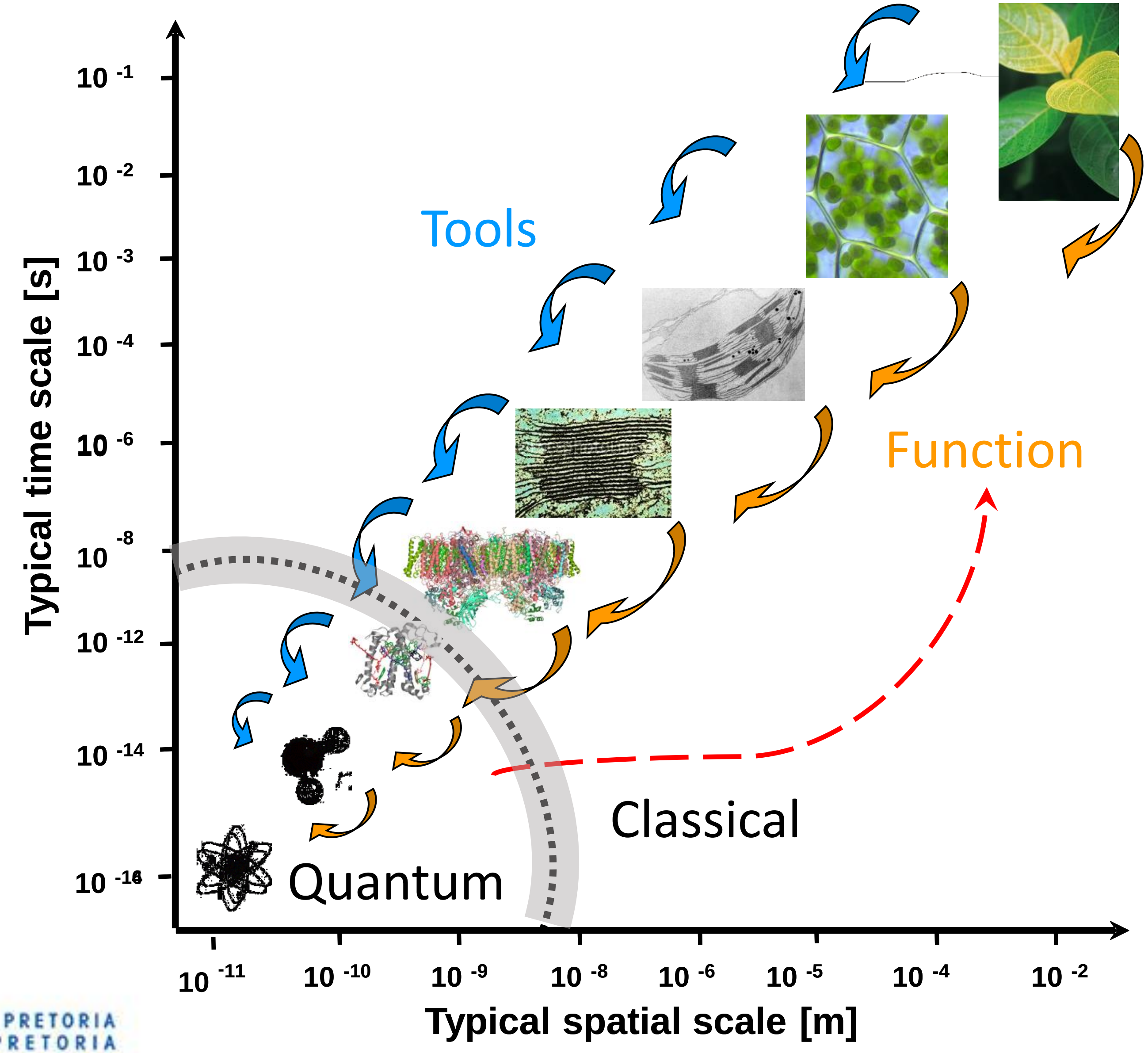
# (Long-term) applications of quantum biology

- Sensing, health, environment, information technologies, ...
- Most of the Key Enabling Technologies of the EU Horizon2020 funding programme are based on quantum mechanical phenomena and find examples in nature. For example:

| Key Enabling Quantum Technology | Research field | Examples in nature |
|---|---|---|
| Single-photon detection in a low-voltage electrochemical system | Photonics | • Eye<br>• Photoreceptors<br>• Light-harvesting complexes |
| $N_2$ fixation at room temperature and pressure | Nanotechnology | Certain bacteria |
| Chemically powered transport of ions and electrons across nanometers with low dissipation | Nanoelectronics | Transmembrane ion transport across basically every (sub)cellular membrane |
| $H_2$ from $H^+$ without noble metals at 0 V SHE | Advanced Materials | Many organisms, e.g. cyanobacteria |
| Photobiological charge separation with near-unity quantum efficiency | Photovoltaics | Photosynthetic photosystems |

# Hierarchies in Biology

From the Classical to the Quantum World




**Can non-trivial quantum effects be relevant for biological function?**

**Requires tools for studying biological structure and function at unprecedented spatial and temporal resolution**

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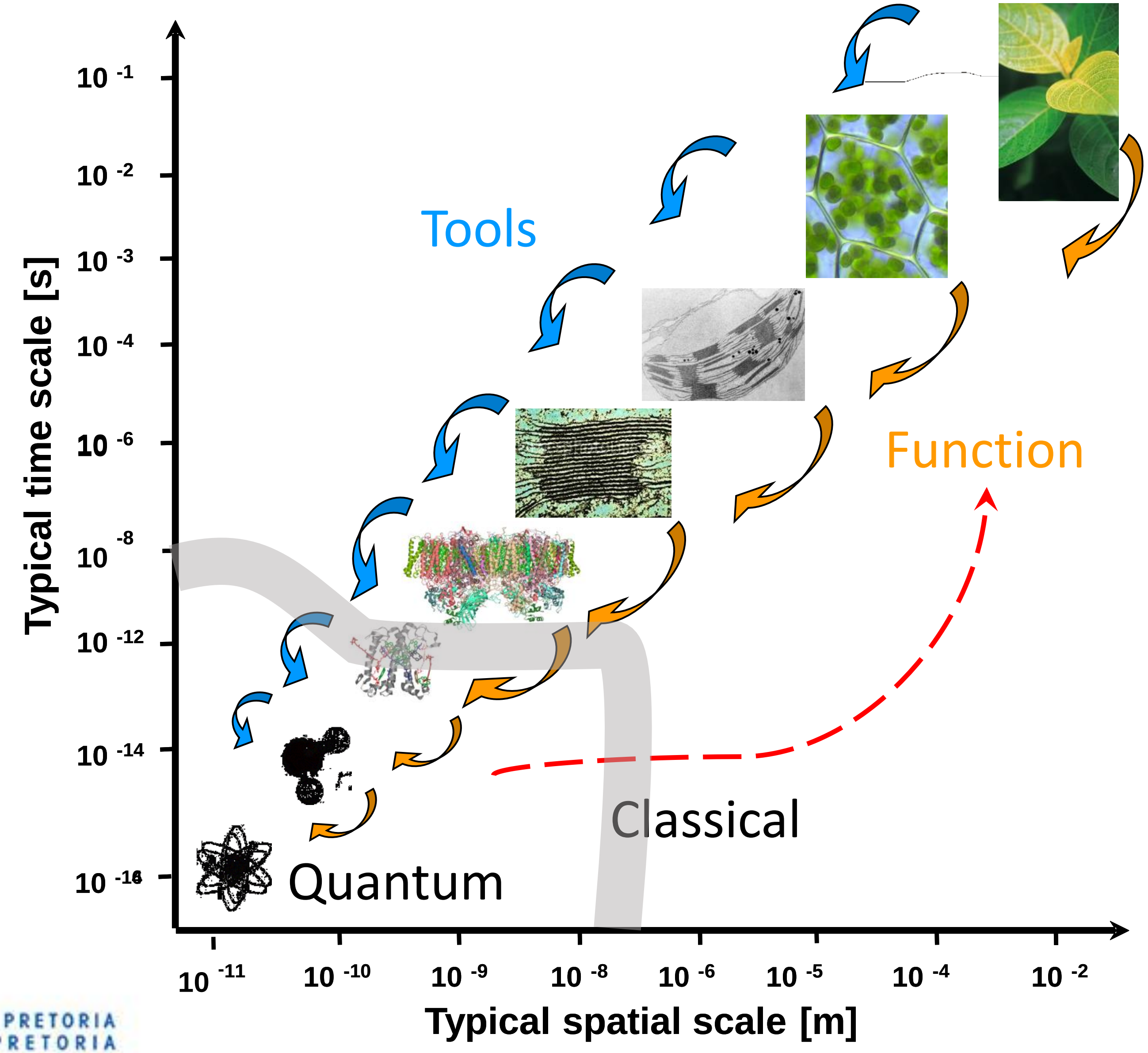
Picture adapted from Huelga & Plenio, *Contemporary Physics* 54:4 (2013). Original picture by Alipasha Vaziri

# Hierarchies in Biology

From the Classical to the Quantum World

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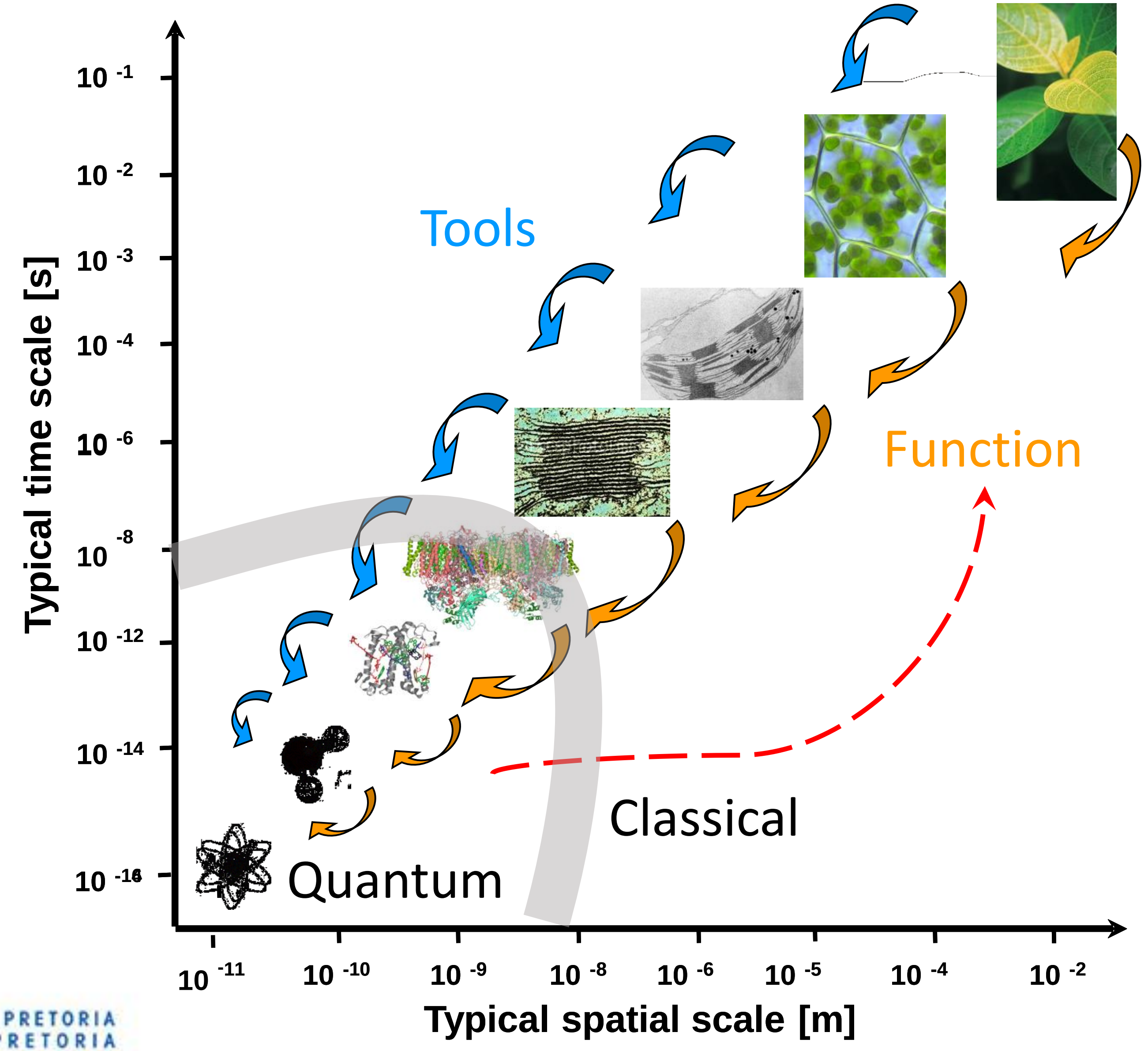


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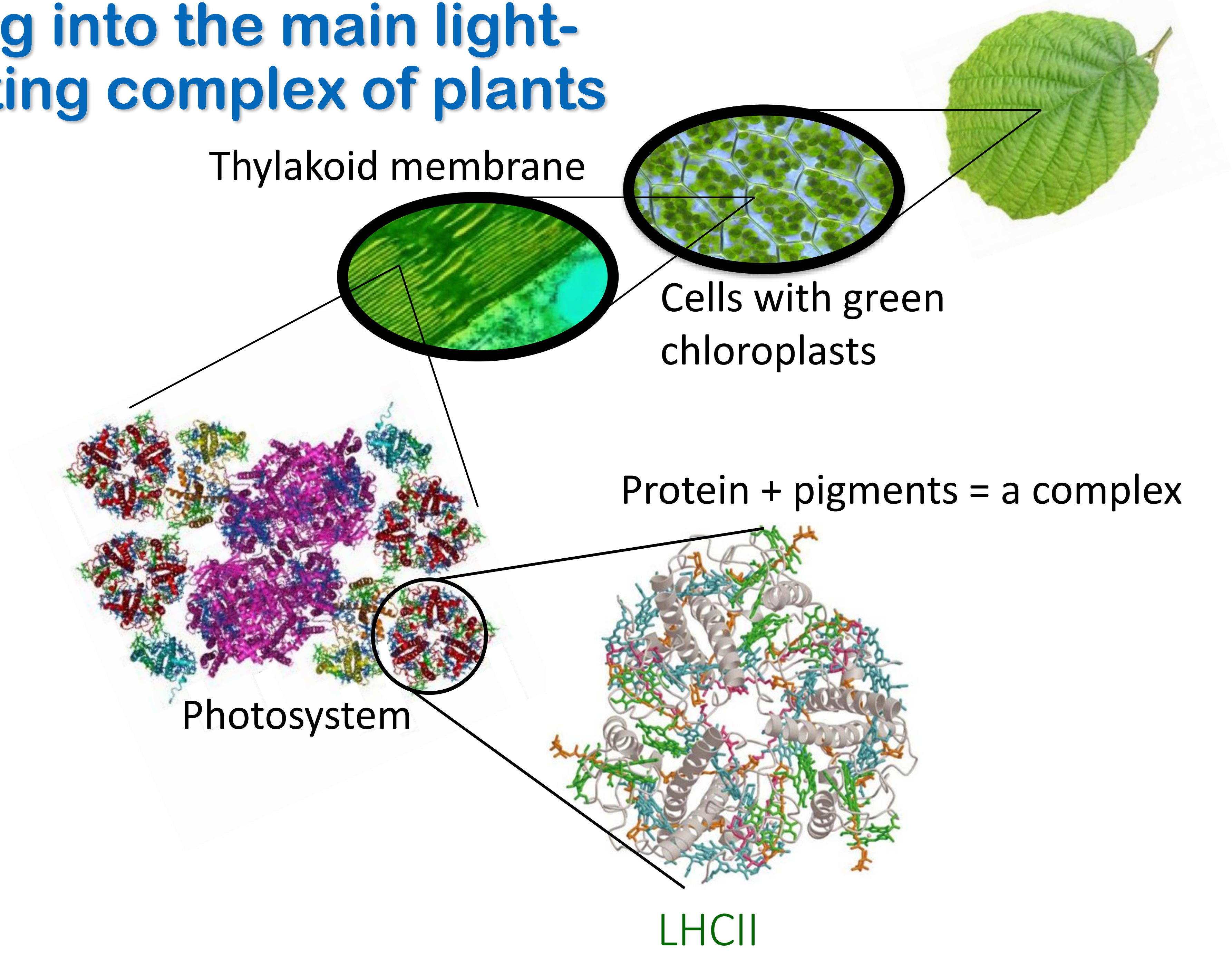
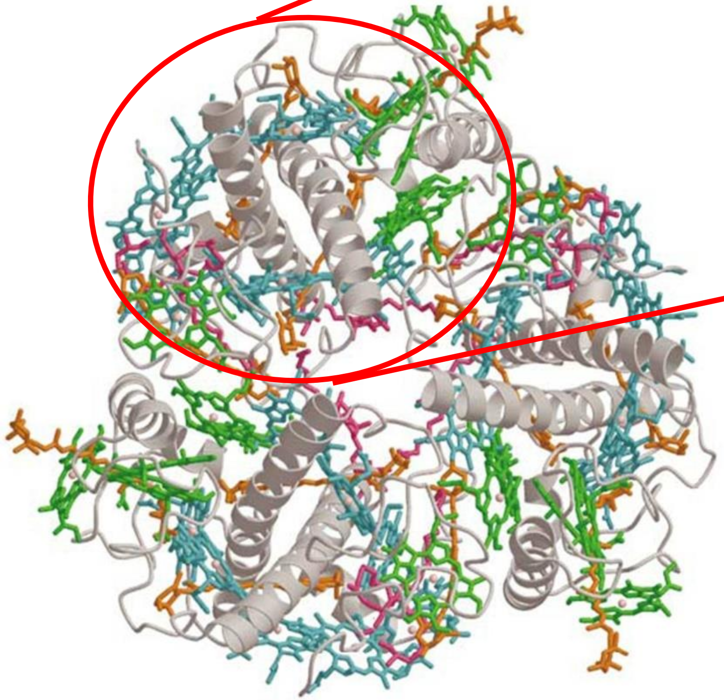
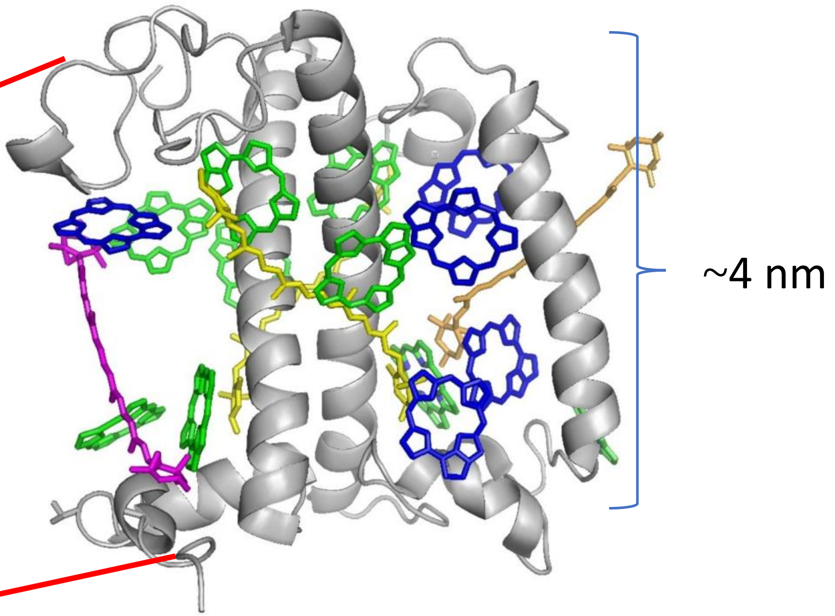
Picture adapted from Huelga & Plenio, *Contemporary Physics* 54:4 (2013). Original picture by Alipasha Vaziri

# Zooming into the main light-harvesting complex of plants



# LHCII: The main light-harvesting complex of plants



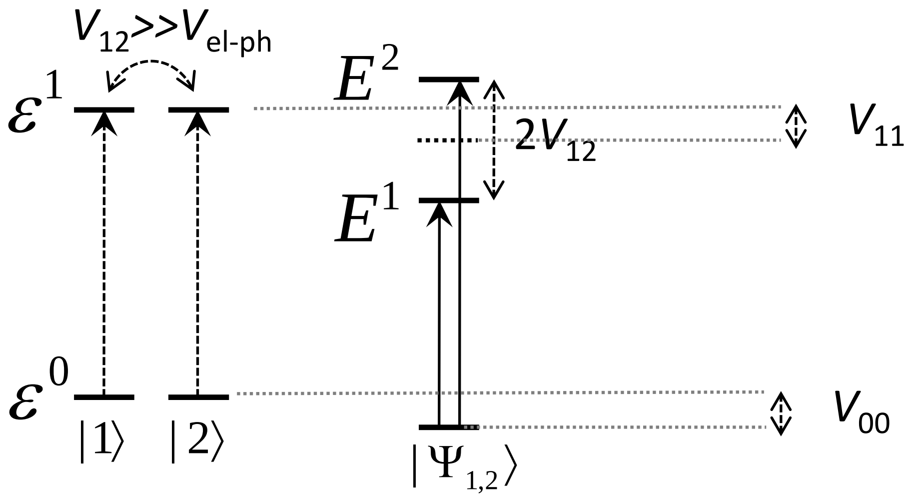



8x Chlorophyll a
6x Chlorophyll b
} Chlorophylls

2x Lutein
1x Neoxanthin
1x Violaxanthin
} Carotenoids

18 pigments/chromophores

# Molecular excitons: coherently delocalised excitations




$$\Psi_{1,2} = \frac{1}{\sqrt{2}}\left(\varphi_1^1\varphi_2^0 \pm \varphi_1^0\varphi_2^1\right)$$

Physiological benefits:

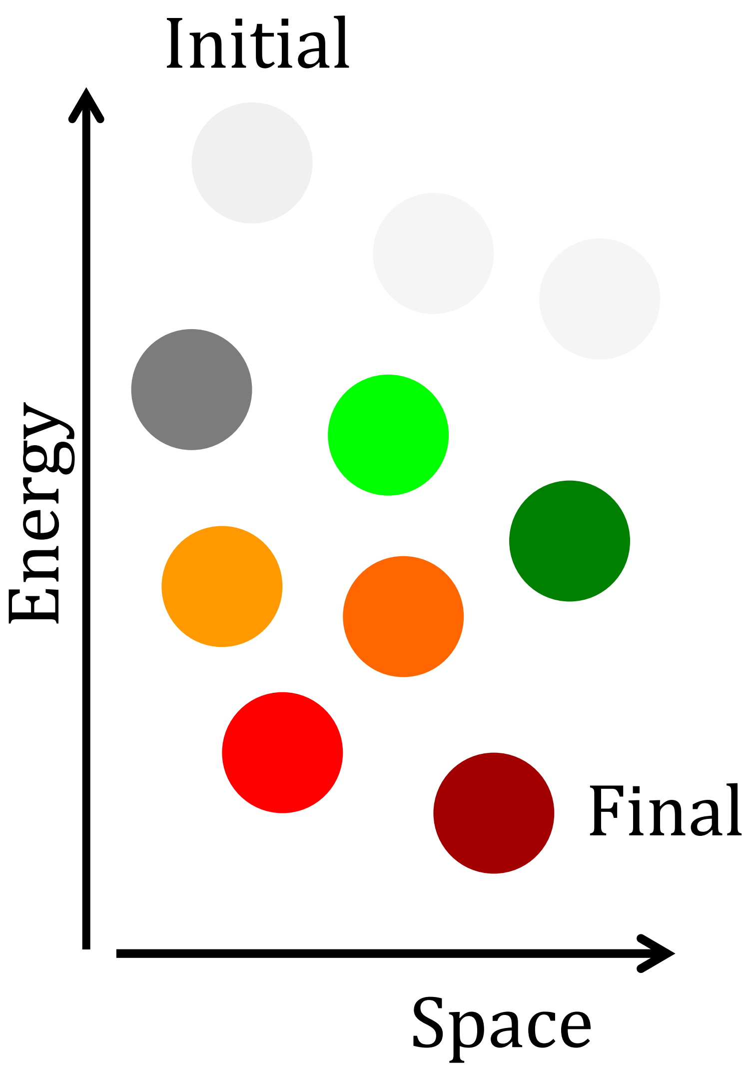
1. More efficient light absorption (Extend absorption cross-section)
2. Faster energy funnelling (Explain in next slides)
3. Faster energy transfer
4. Less trapping and loss of energy

(3–4) Compare with playing soccer with a golf ball!

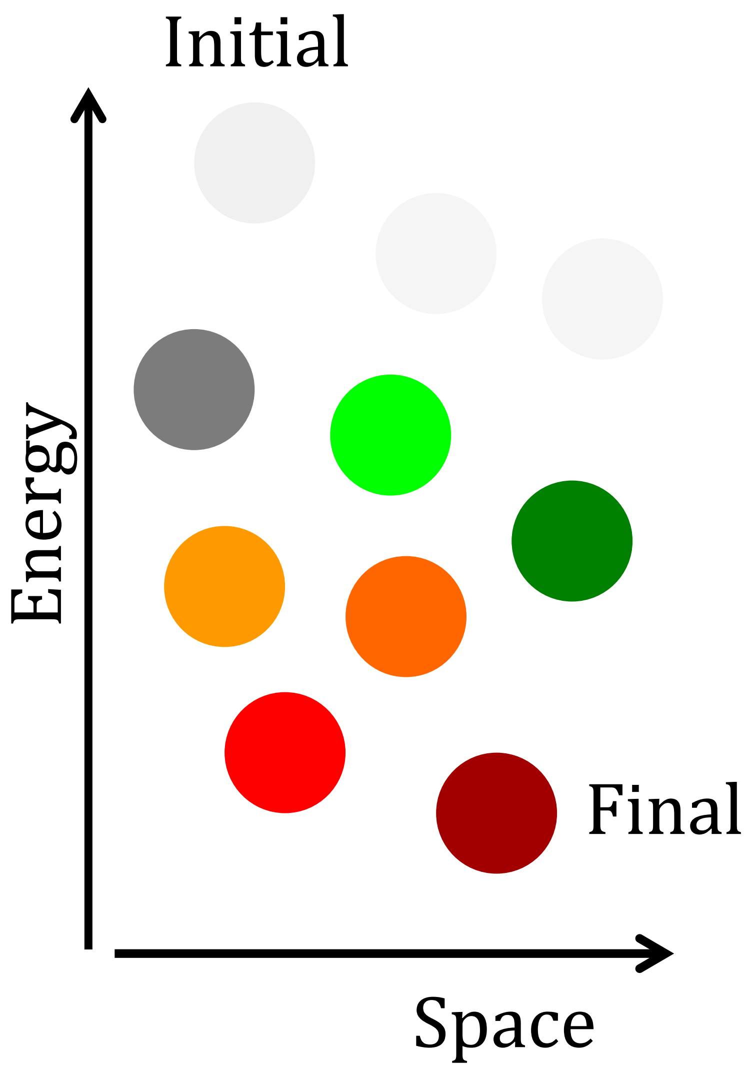


Van Grondelle & Novoderezhkin, *Phys Chem Chem Phys* 2010, 12, 7352–7365

# Exciton relaxation

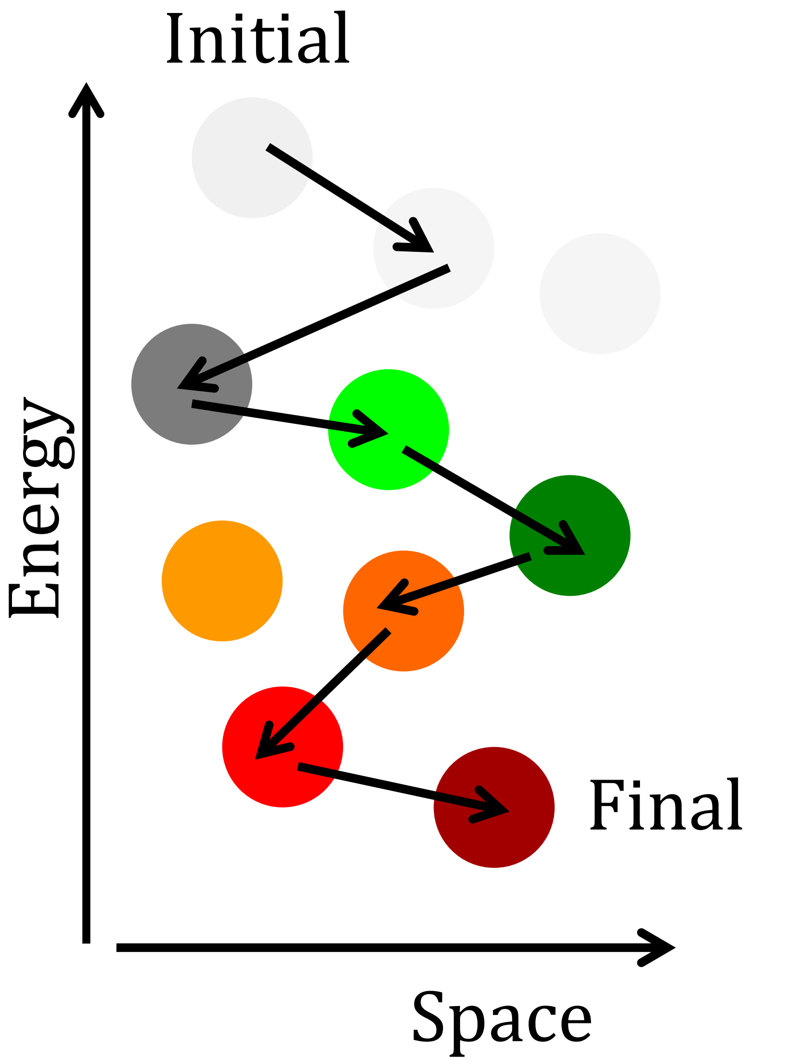



Individual pigments

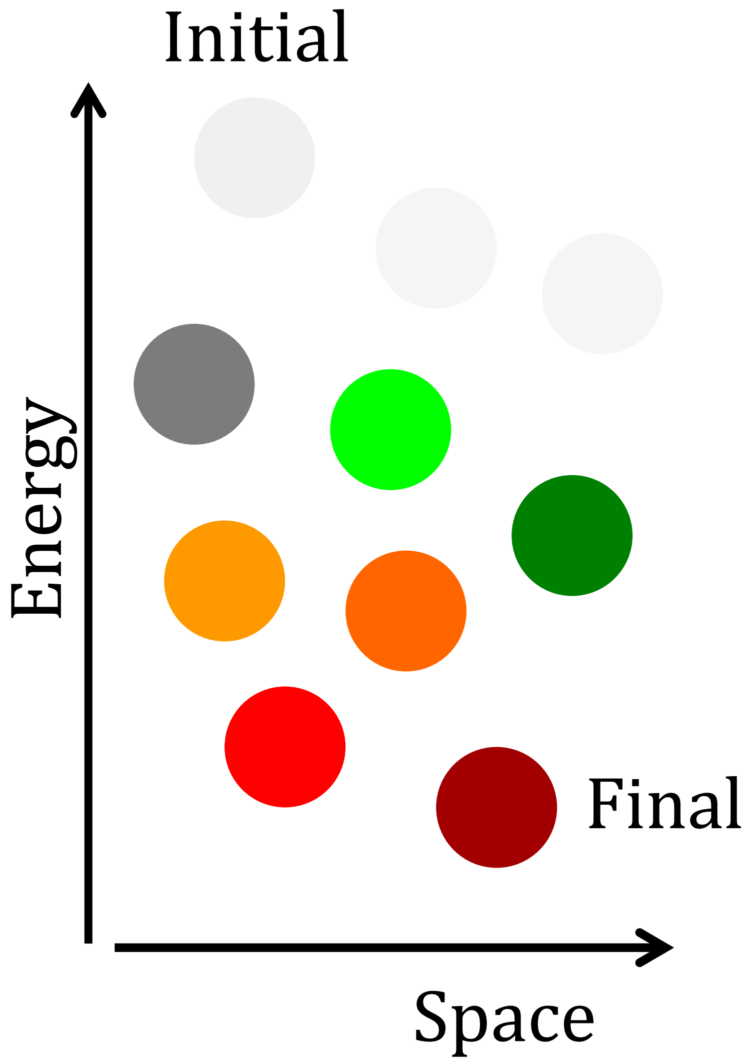



Excitons

# Exciton relaxation

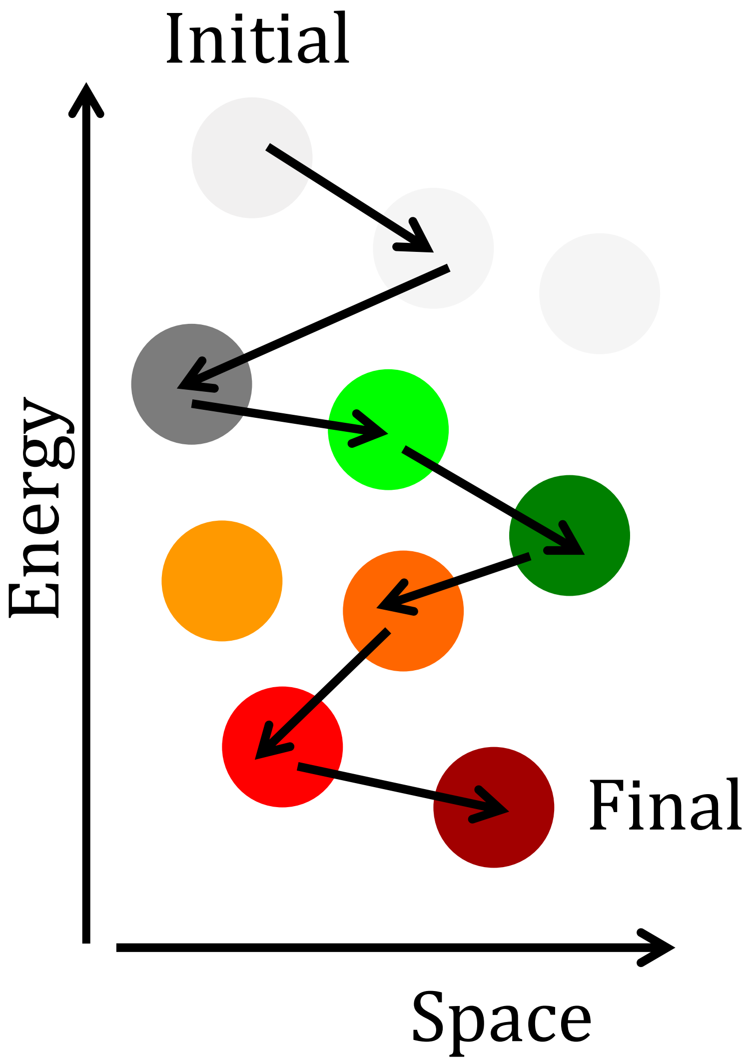



Individual pigments

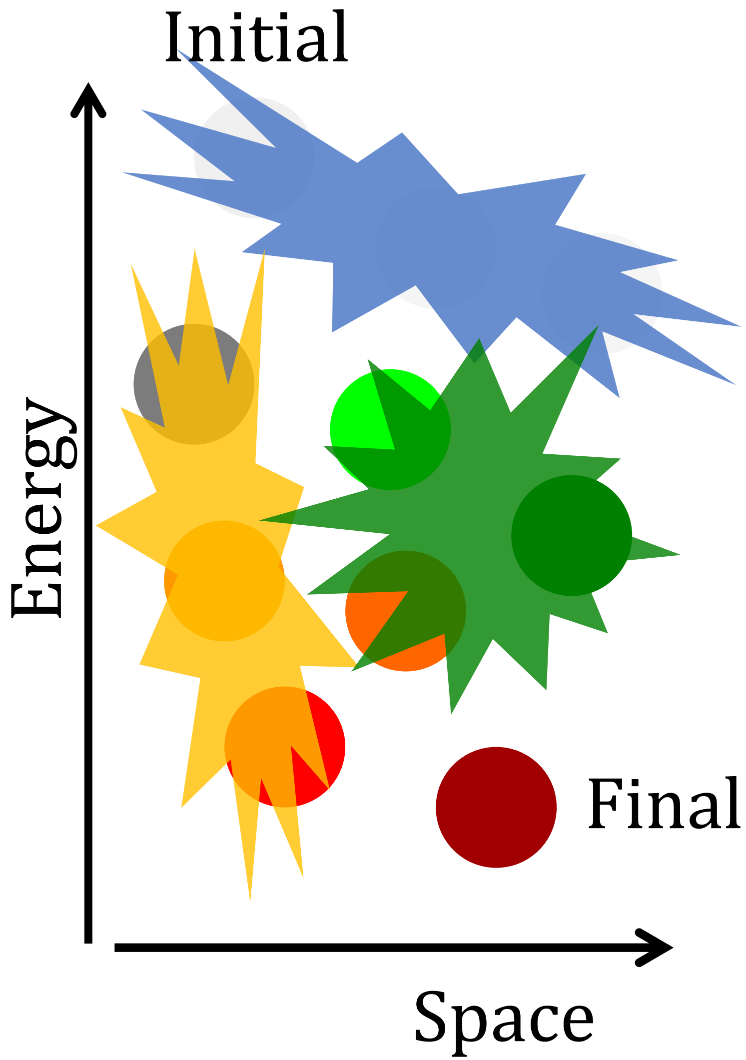



Excitons

# Exciton relaxation

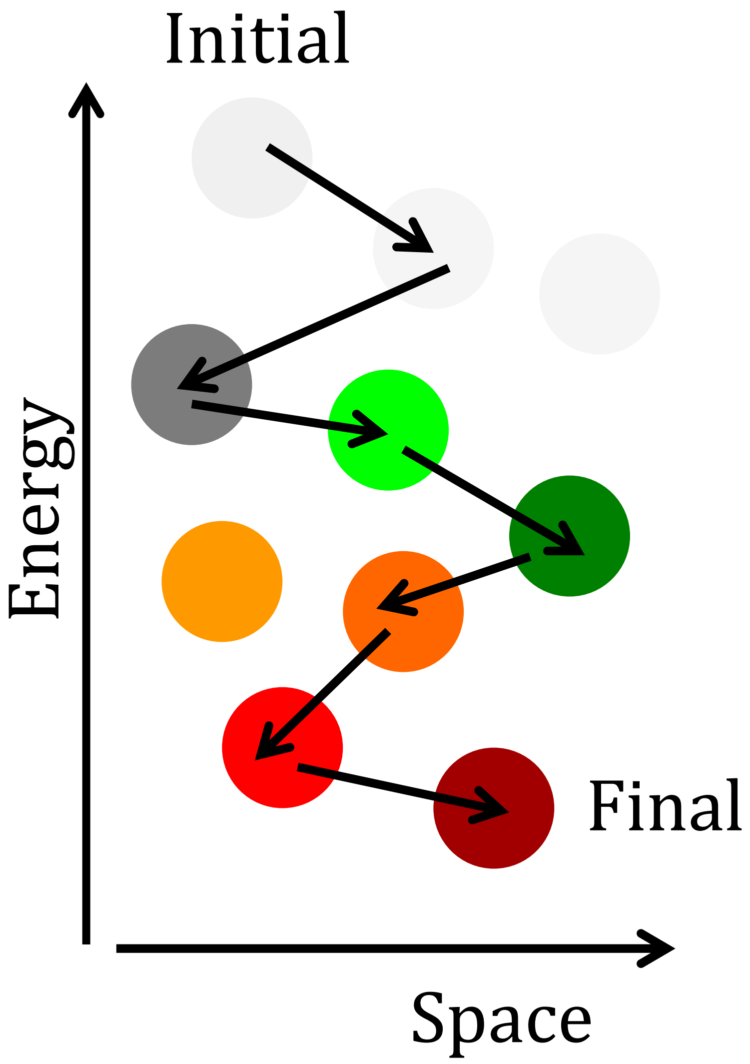



Individual pigments

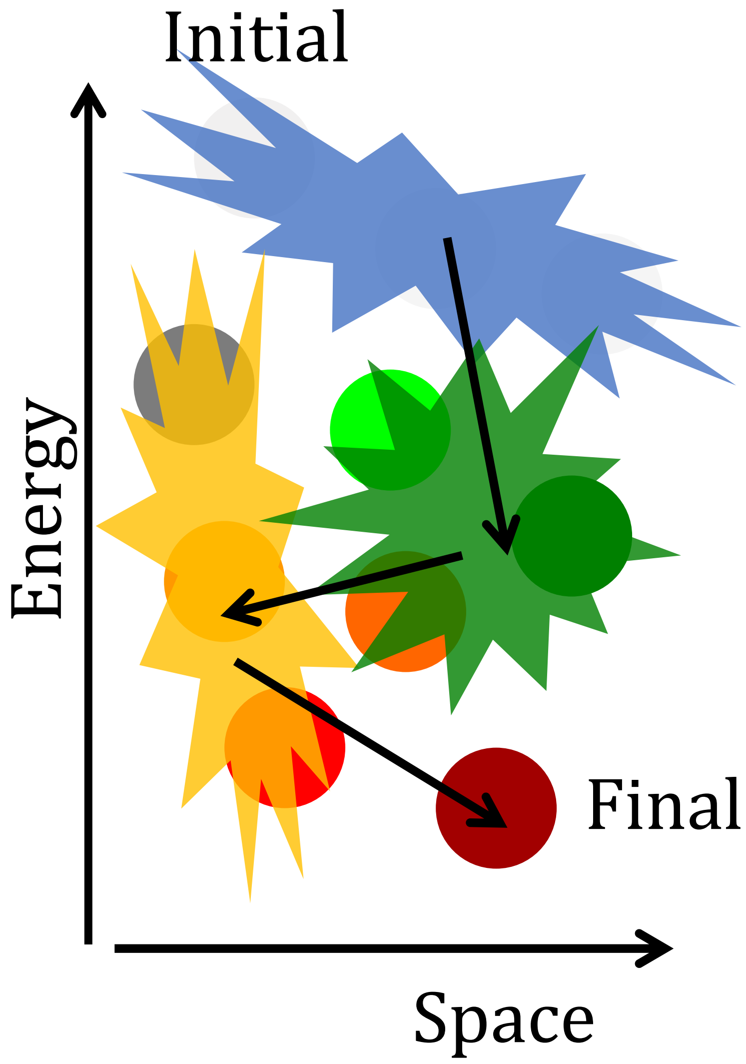



Excitons

# Exciton relaxation




Individual pigments




Excitons

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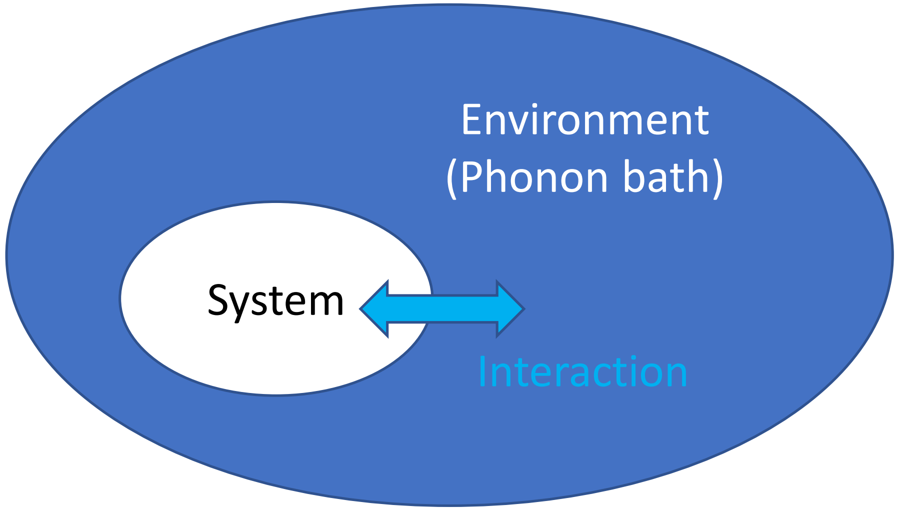
# Calculating the Hamiltonian

## of a light-harvesting complex

### Open Quantum Systems approach:




Every real-life quantum system is coupled to an environment

# Calculating the Hamiltonian
## of a light-harvesting complex

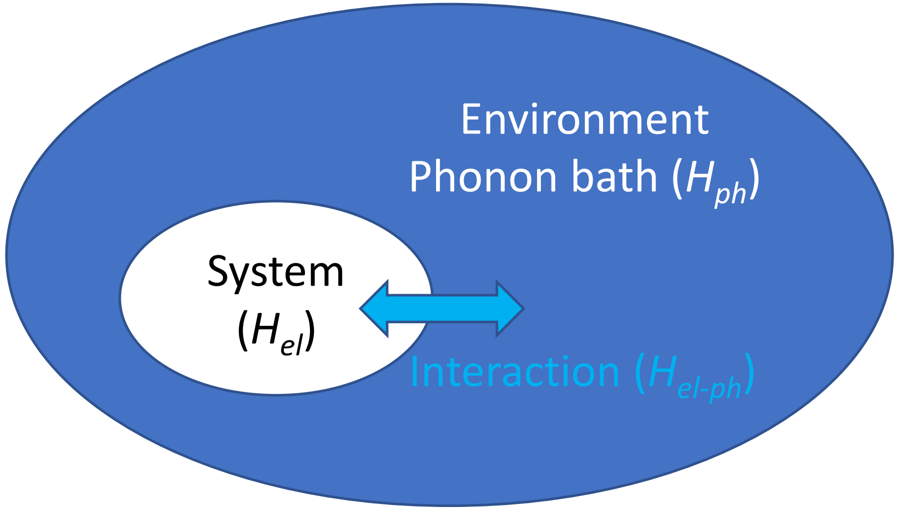
Coupling of transition dipoles

Full Hamiltonian: $H = H_{\text{el}} + H_{\text{el–ph}} + H_{\text{ph}}$

where $$H_{\text{el}} = \sum_{i} \epsilon_i \left| e_i \right\rangle \left\langle e_i \right| + \sum_{i \neq j} J_{ij} \left| e_i \right\rangle \left\langle e_j \right|$$

and $$H_{\text{el–ph}} = \sum_{i} g_{i\xi} \hat{q}_{\xi} \left| e_i \right\rangle \left\langle e_i \right|$$

Coupling between nuclear modes and excitons



# Calculating the Hamiltonian
## of a light-harvesting complex

$$FL(\omega) \propto \omega^3 \sum_i P_i \chi_i(\omega)$$

Steady-state population ($P_i$)

Fluorescence lineshape ($\chi_i(\omega)$)

$$\chi_i(\omega) = |\mu_i|^2 \int_0^\infty d\tau e^{-i(\omega-\omega_i+\lambda_i)\tau - g_{ii}^*(\tau) - \frac{\Gamma_i}{2}\tau}$$

Dipole strength ($\mu_i$)

Exciton energies ($\omega_i$)

Stokes shift ($\lambda_i$)

Lineshape function ($g_{ii}^*(\tau)$)

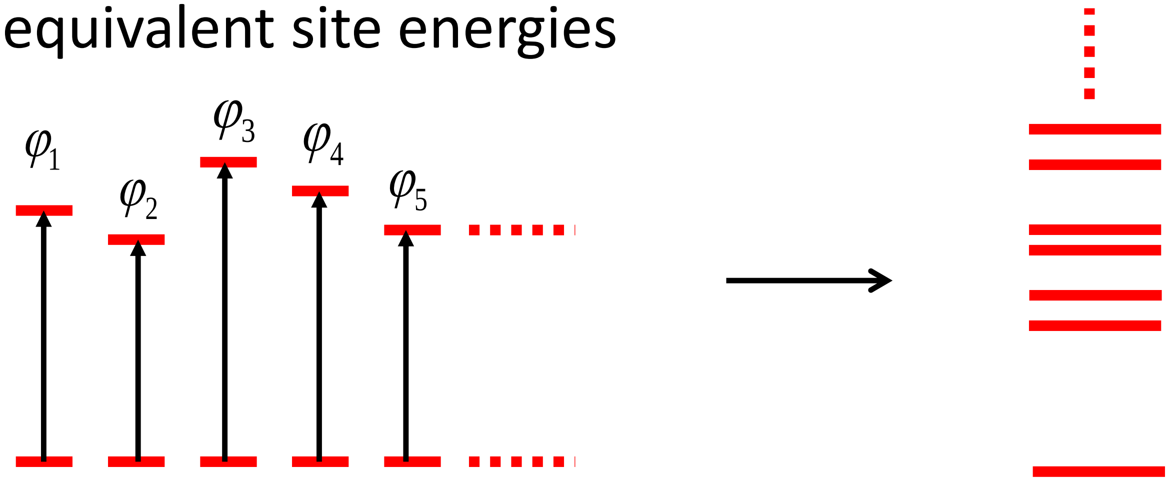
Decay ($\Gamma_i$)

Depend on $H_{\mathrm{el}}$

# Excitons in LHCII

1) 42 interacting chlorophylls

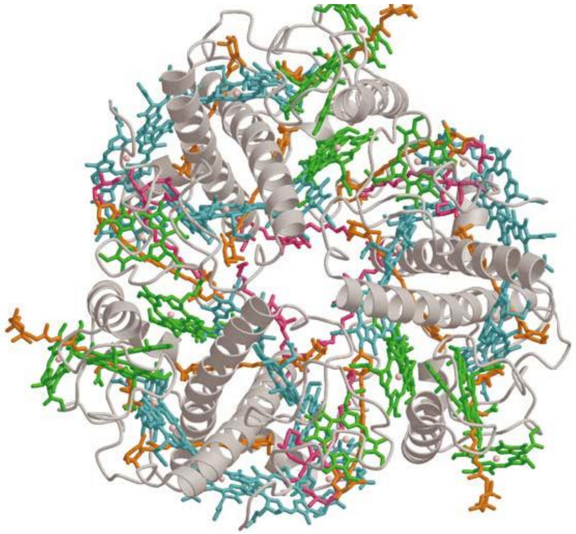
2) Non-equivalent site energies



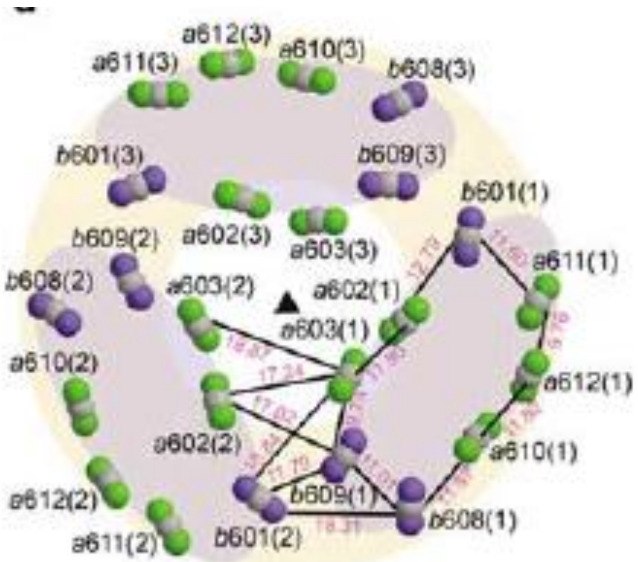

3) Complicated spectral density

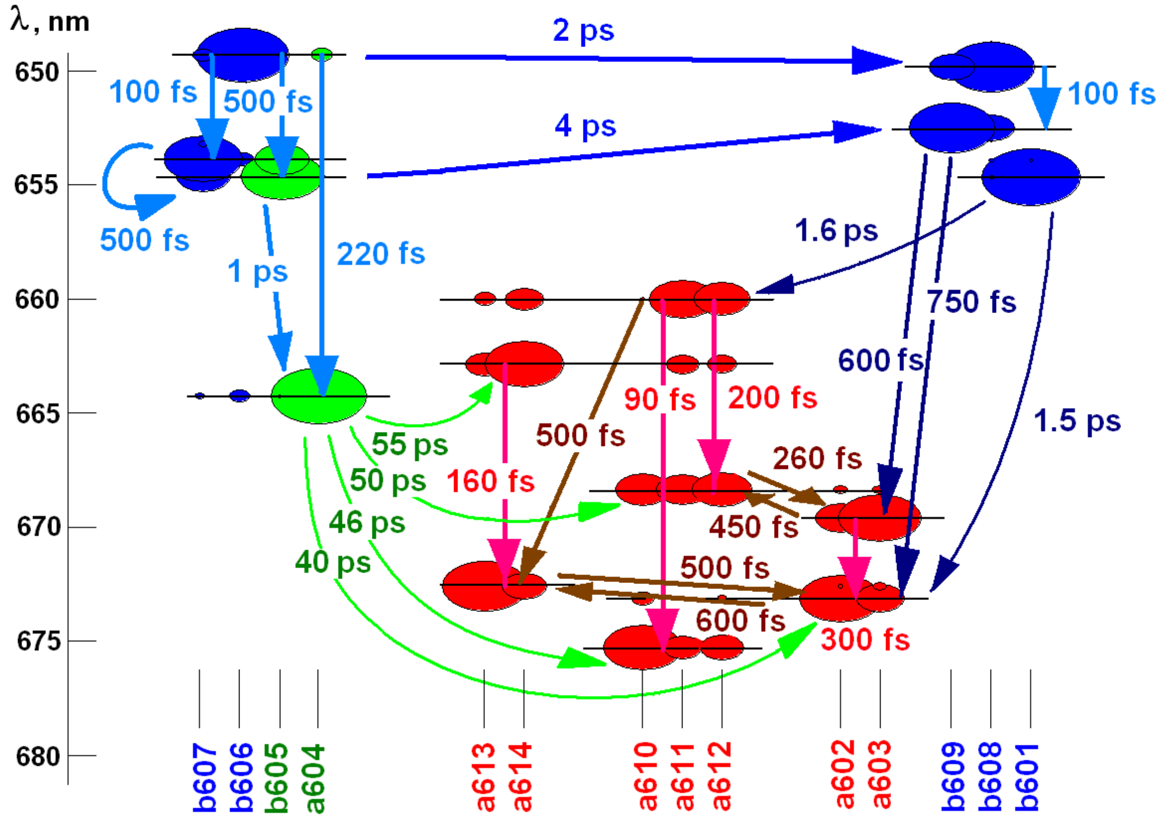
4) Static disorder (time-dependent site energies and couplings)

# Example: Unraveling energy transfer in LHCII



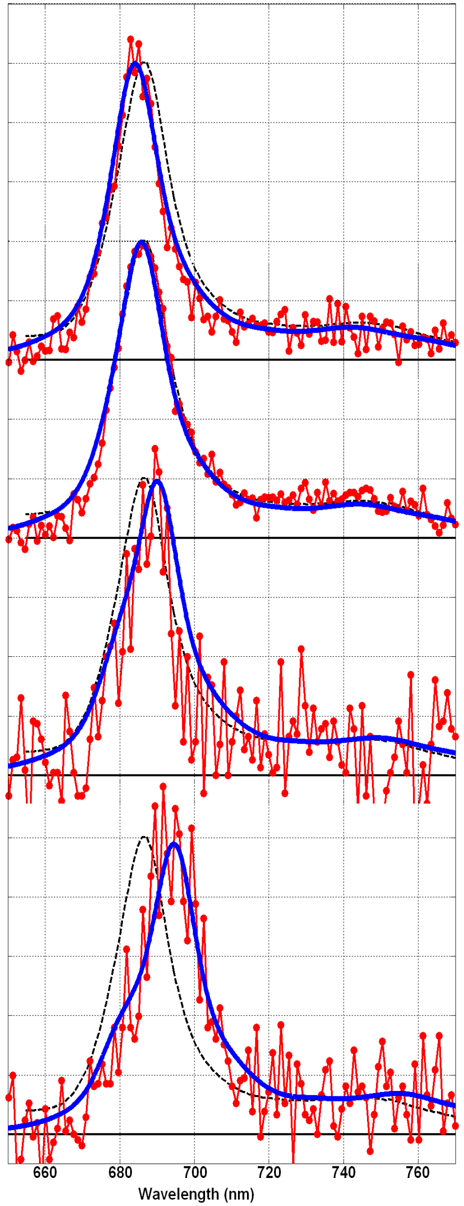
Liu et al. *Nature* 2004

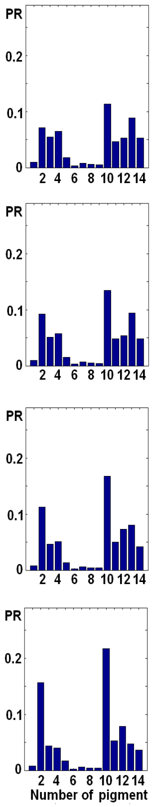


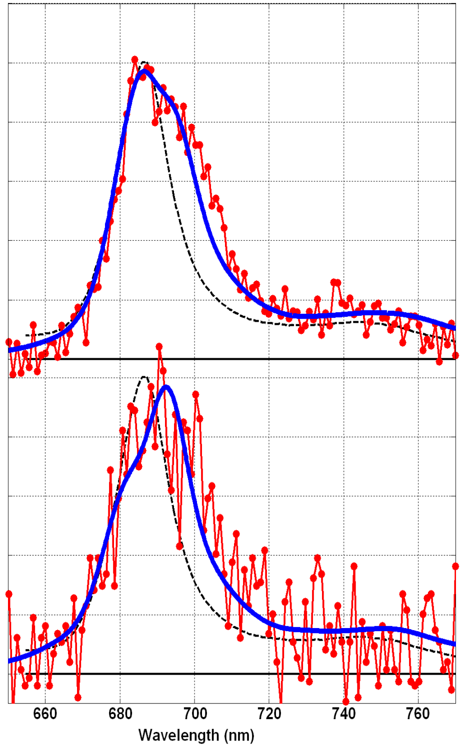



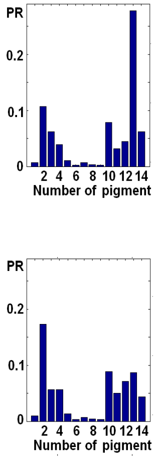

van Grondelle & Novoderezhkin, *Phys Chem Chem Phys* 2006

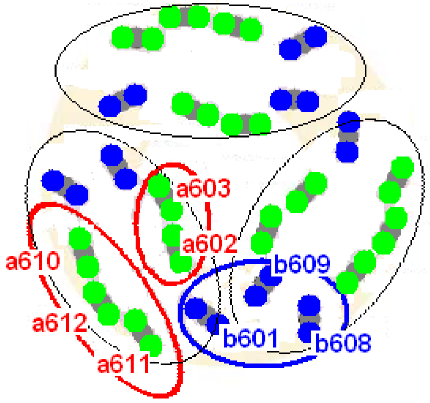
# Single realisations of the disorder to fit fluorescence spectra of single LHCII complexes.

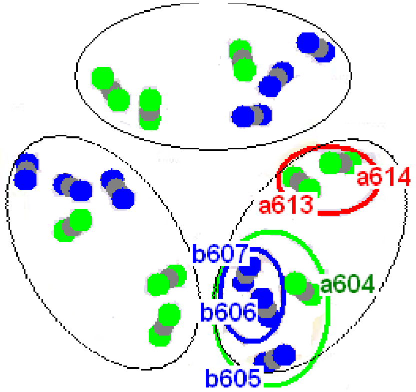


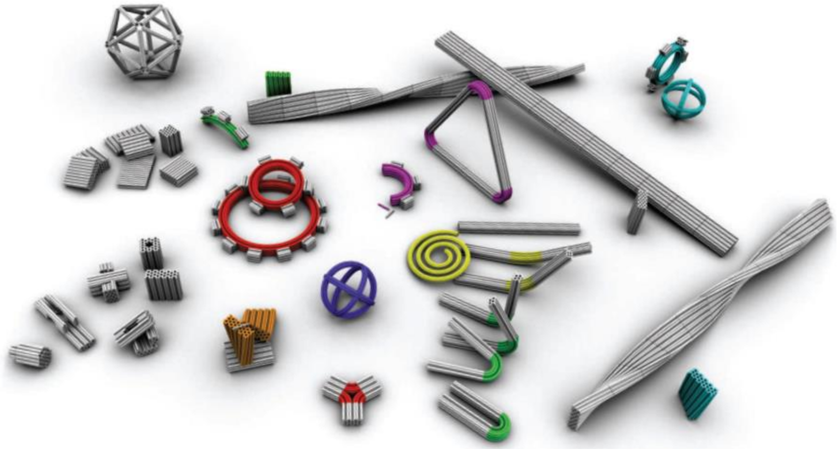

$$PR_n = \sum_k p_k \left(c_n^k\right)^4$$

Krüger *et al. Biophys J* 98:3093 (2010)

# Experimental examples

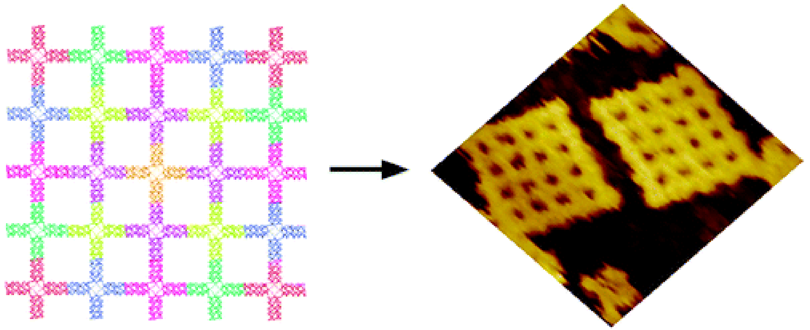
# 1. Manipulation of single biomolecules:
## (i) DNA Origami

Complex, self-assembling nanostructures

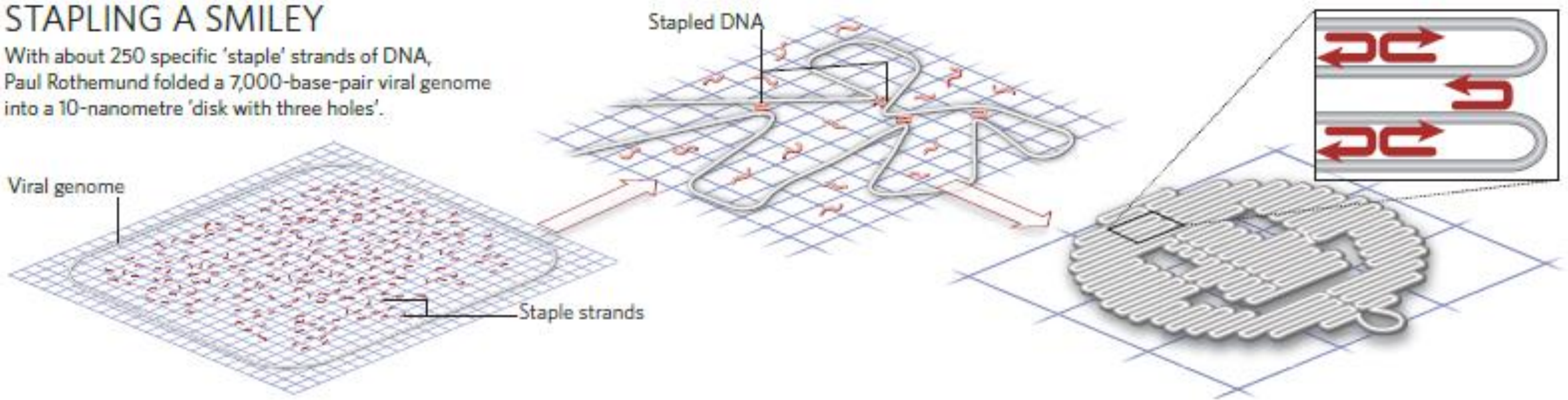


Sanderson *Nature* 464:158–159 (2010)

Self-assembly of DNA nanoarrays



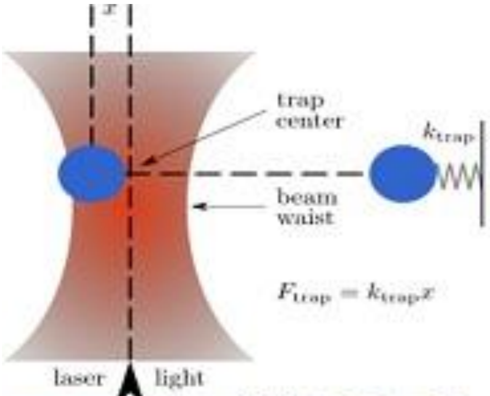
Liu, Ke & Yan, *JACS* 127:17140-17141 (2005)




Rothemund *Nature* 440:297–302 (2006)

# 1. Manipulation of single biomolecules:
## (ii) Optical tweezers

**Nobel Prize in Physics 2018**




**Arthur Ashkin**

**Affiliation at the time of the award: Bell Laboratories, Holmdel, NJ, USA**

**Prize motivation: "for the optical tweezers and their application to biological systems."**

**Prize share: 1/2**

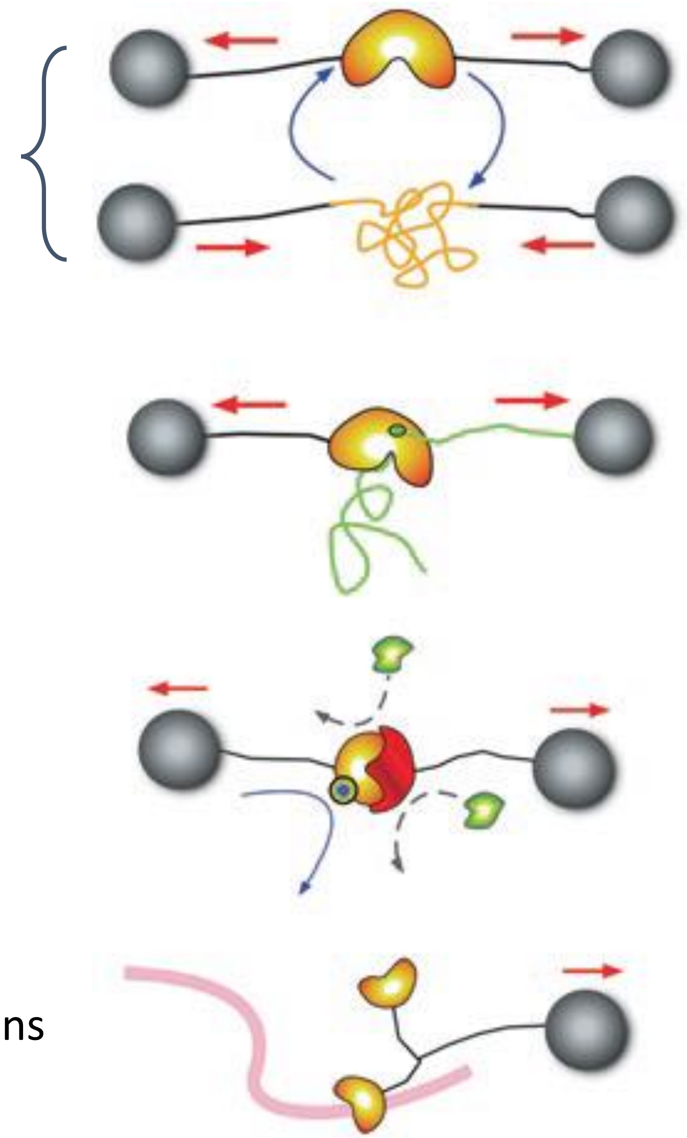
# 1. Manipulation of single biomolecules:
## (ii) Optical tweezers

protein unfolding and folding

DNA binding proteins

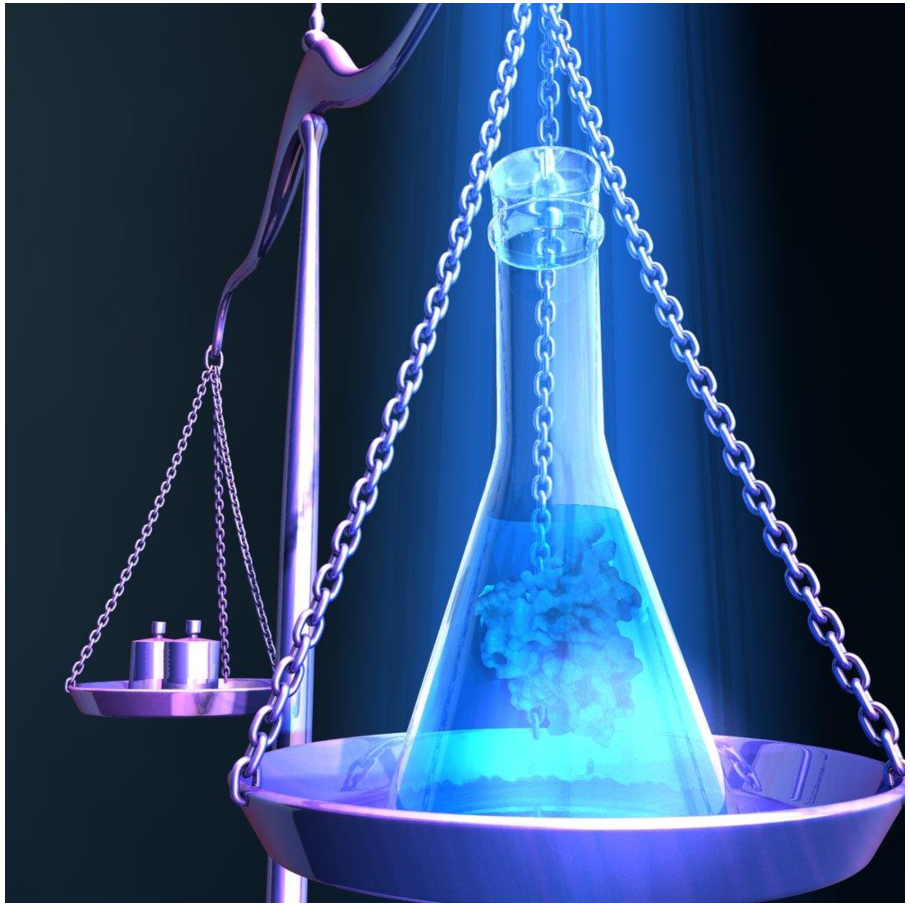
ligand-receptor bonds

cytoskeletal motor proteins



Dufrêne et al. (2011) Nature Methods

# 1. Manipulation of single biomolecules:
## (iii) Weighing a single protein with light



Young, G. et al. *Science* **360**, 423–437 (2018)

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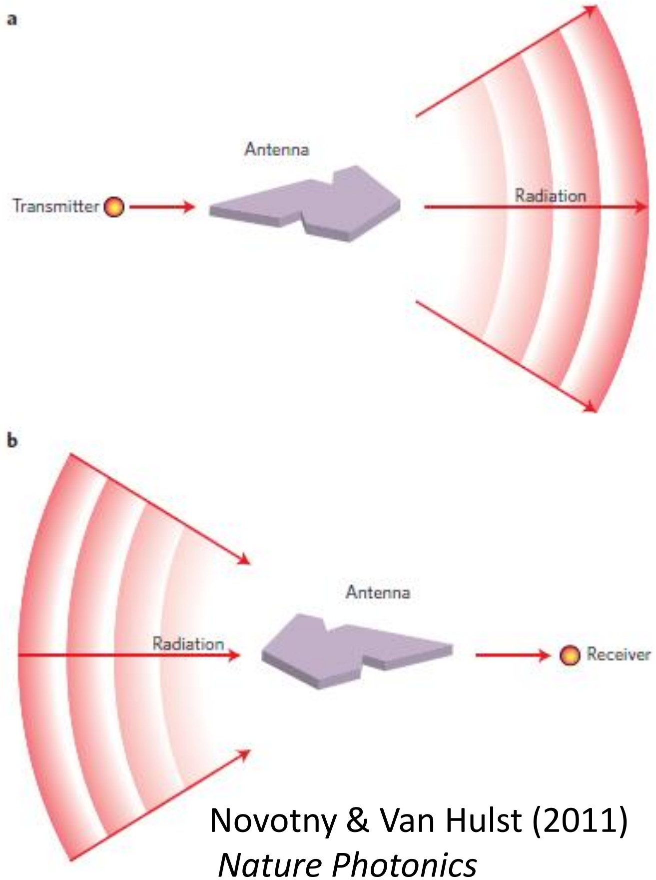
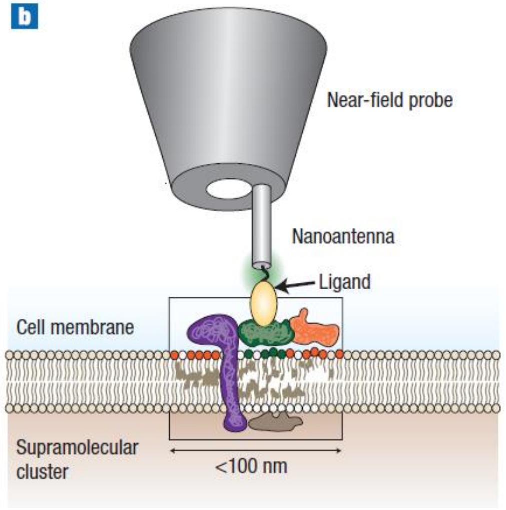
# 1. Manipulation of single biomolecules:
## (iv) Metallic nanostructures

Localisation & enhancement of optical radiation at the nm scale




Novotny & Van Hulst (2011) *Nature Photonics*



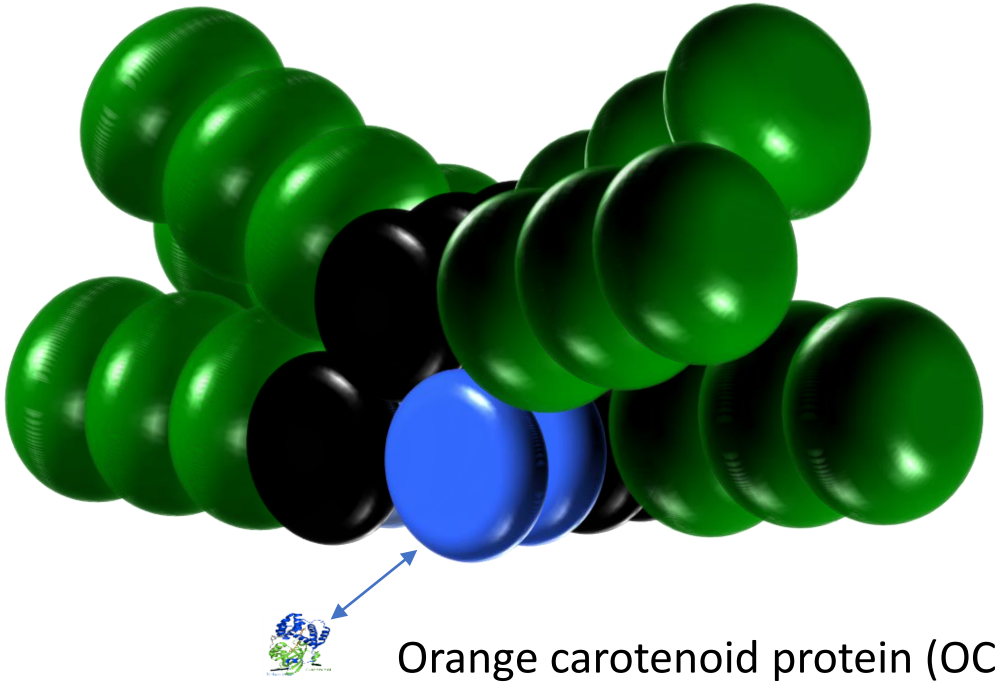

Garcia-Parajo (2008) *Nature Photonics*

**Some Applications**:
- Biosensors
- Optical imaging (~10 nm)
- Single particle tracking in live cells
- Photothermal therapy
- Solar cells (Light harvesting & energy conversion)
- Nanoscale optical circuitry
- Enhanced photoabsorption & emission

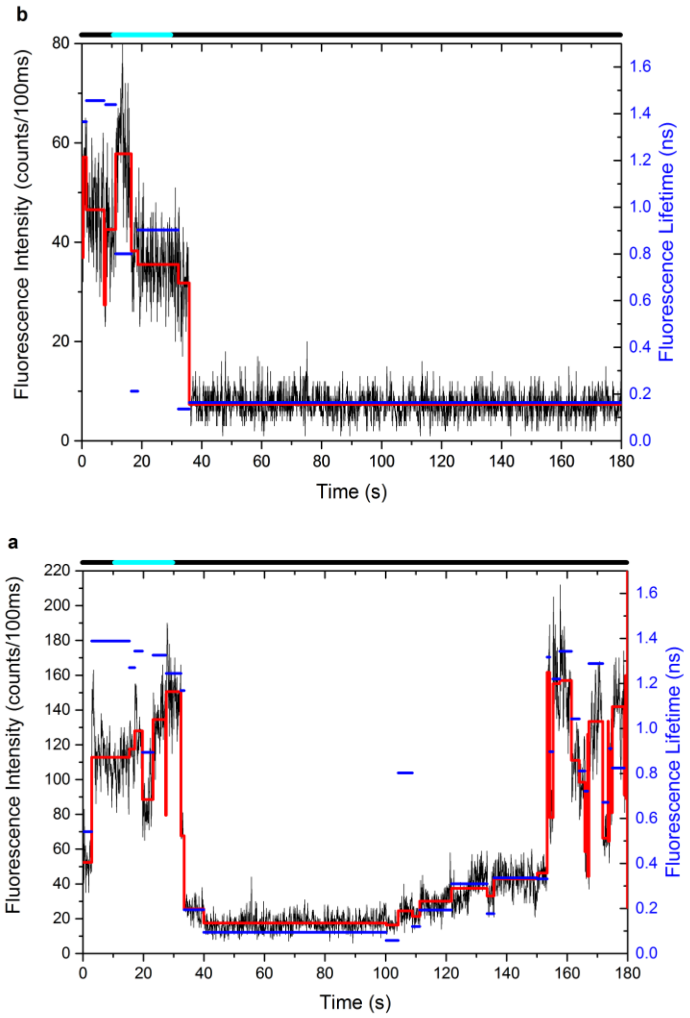
# 1. Manipulation of single biomolecules:

## (v) Using other biomolecules

Phycobilisome



Orange carotenoid protein (OCP)




Gwizdala, Botha, Wilson, Kirilovsky, van Grondelle & TPJK, “Switching an individual phycobilisome off and on” *J Phys Chem Lett* 9:2426-2432 (2018)

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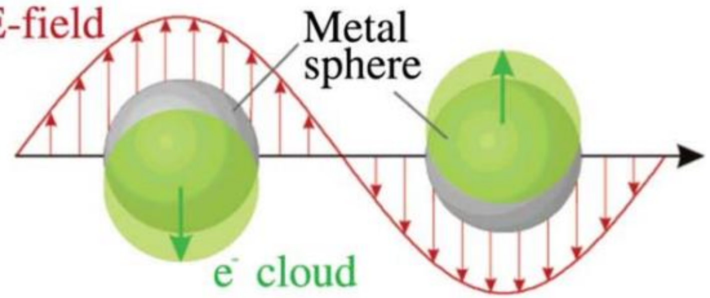
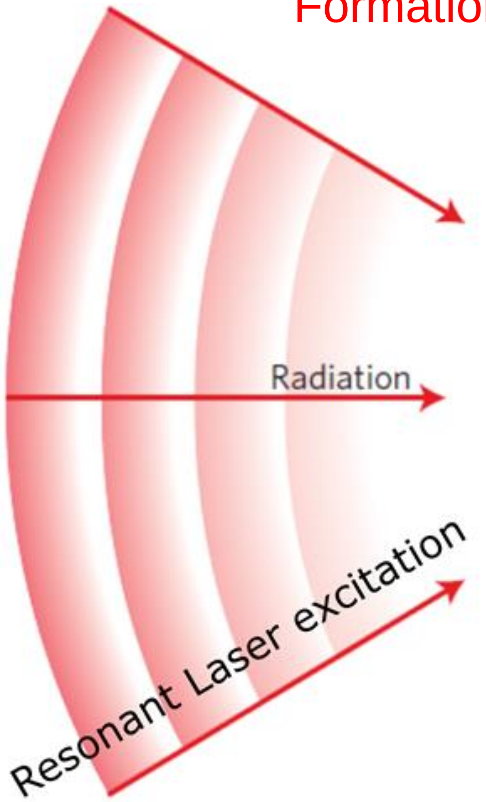
# 2. Single biomolecule–nanoparticle interactions:
## LHCII + Gold nanorods (AuNRs)

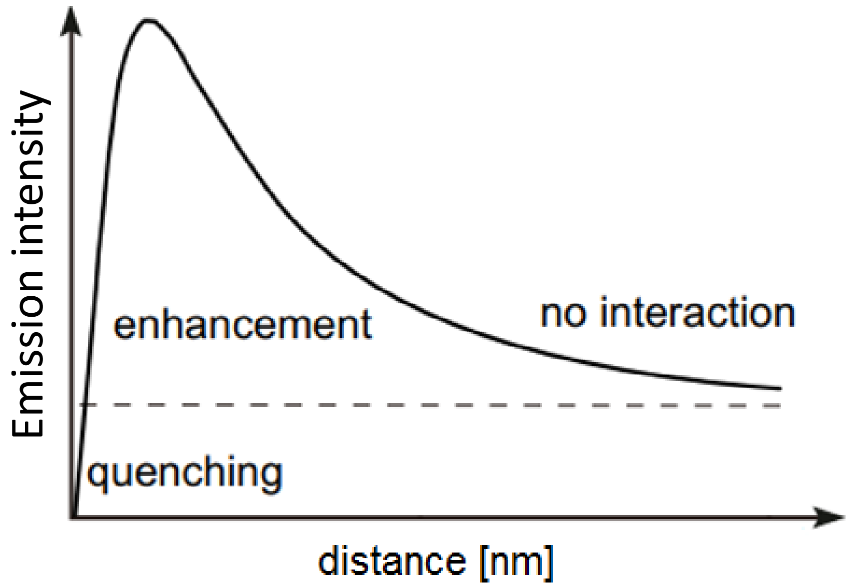
$d < \lambda$




Formation of localised surface plasmon resonances









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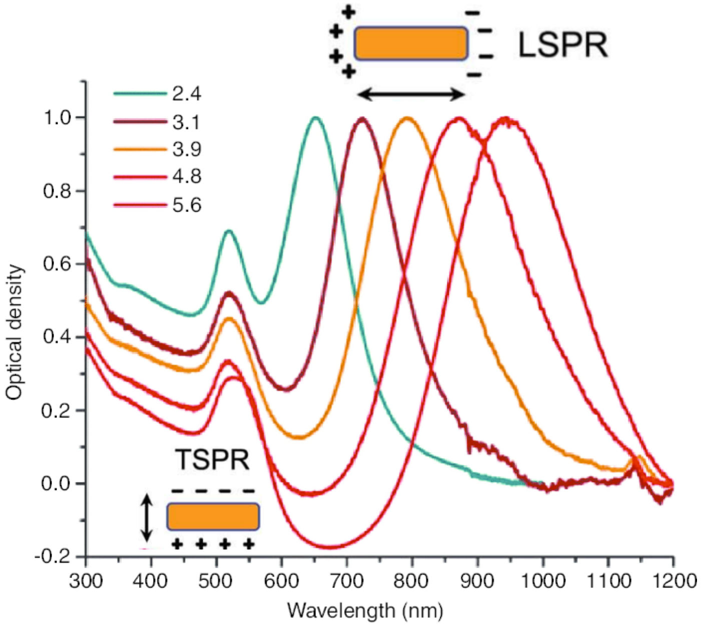
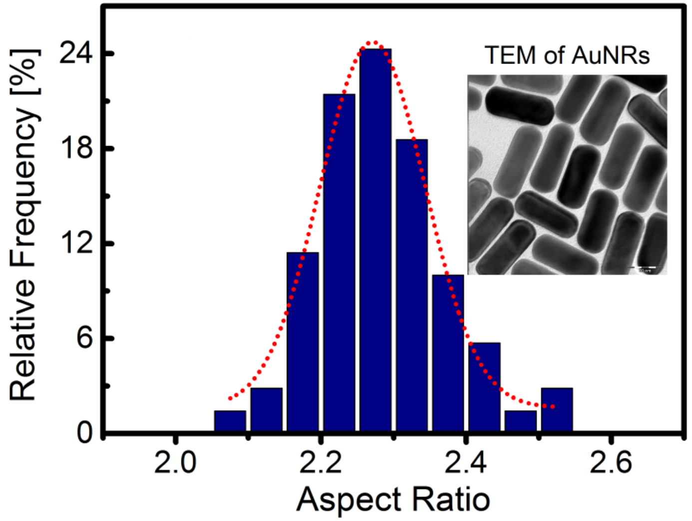
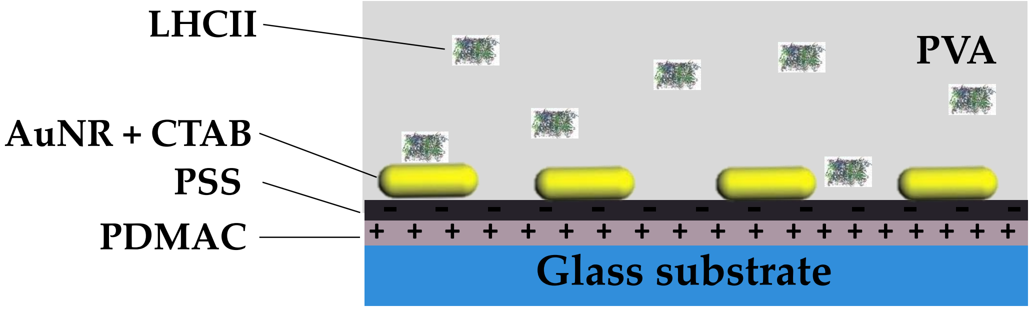
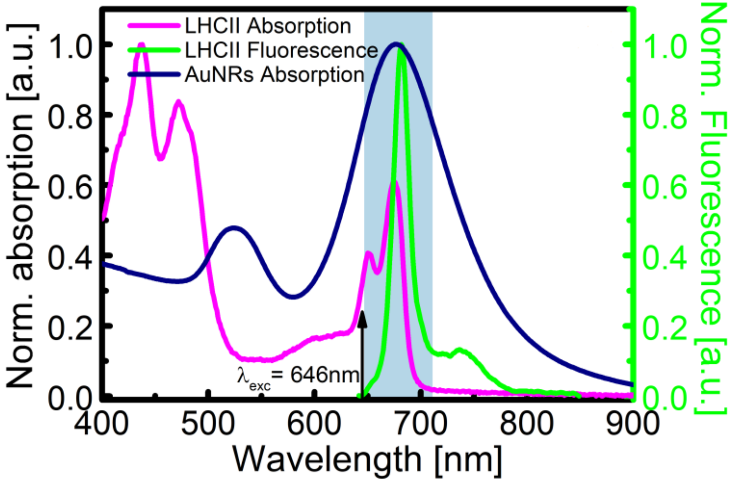
# 2. Single biomolecule–nanoparticle interactions: LHCII + Gold nanorods (AuNRs)

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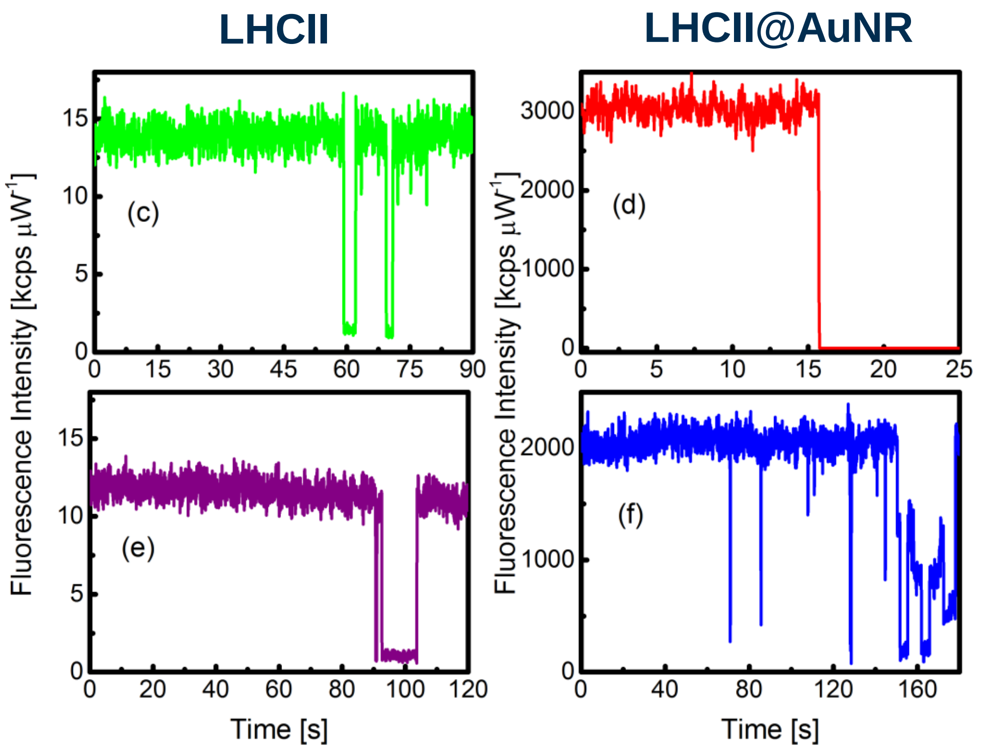
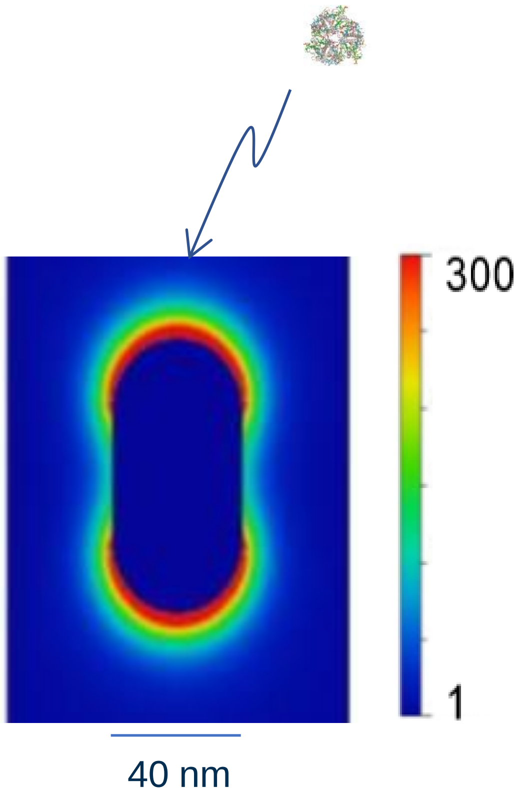
F. Kyeyune, J.L. Botha, B. van Heerden, P. Maly, R. van Grondelle, M. Diale and T.P.J. Krüger. *Nanoscale*, **11**, 15139-15146 (2019);

# 2. Single biomolecule–nanoparticle interactions:
## LHCII + Gold nanorods (AuNRs)

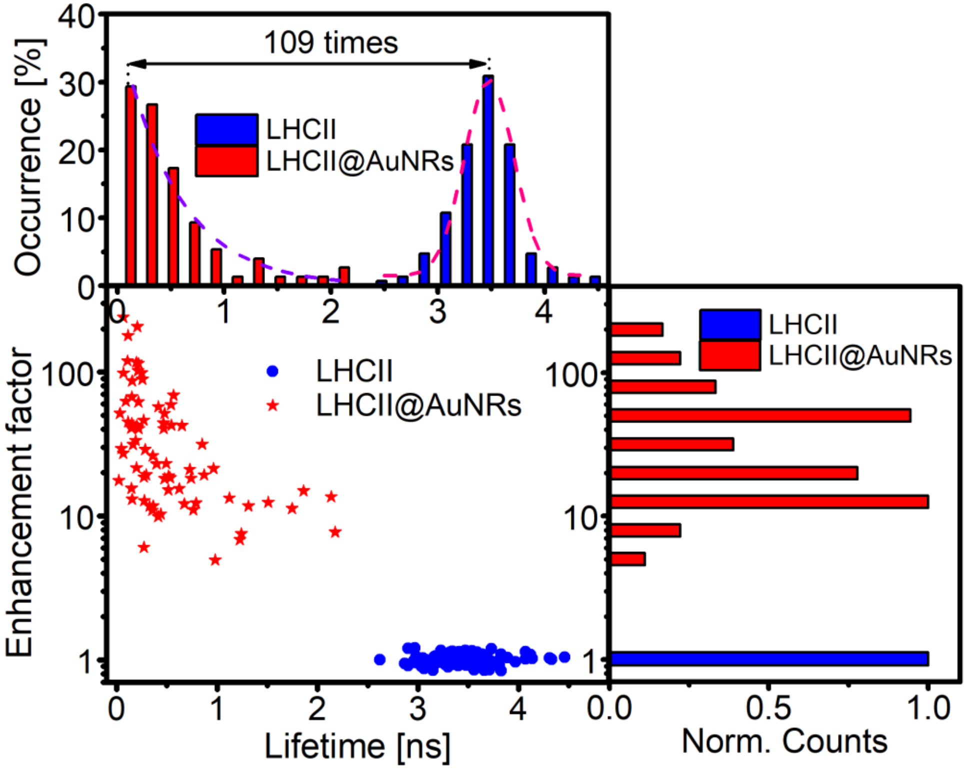


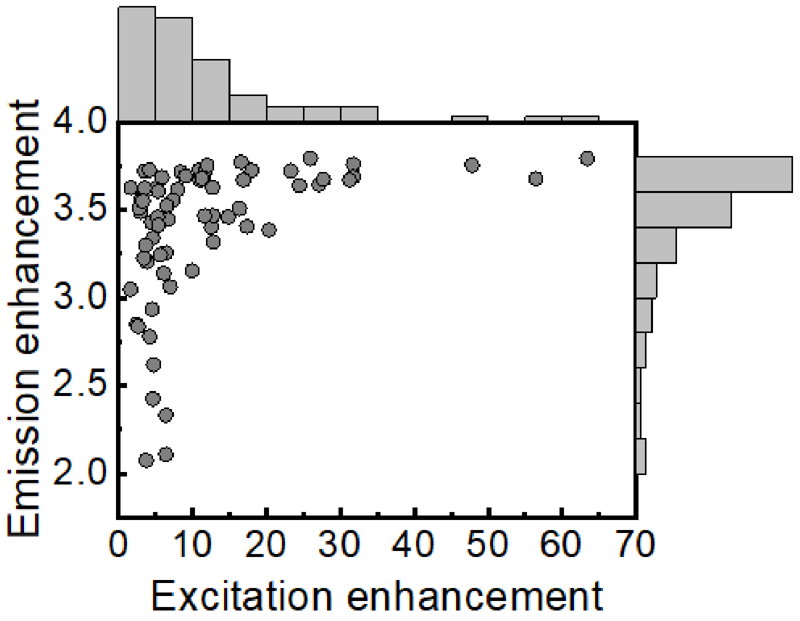




F. Kyeyune, J.L. Botha, B. van Heerden, P. Maly, R. van Grondelle, M. Diale and T.P.J. Krüger. *Nanoscale*, **11**, 15139-15146 (2019);
L.C. Ugwuoke, T. Mancal, T.P.J. Krüger. *Plasmonics* (2019).

# 2. Single biomolecule–nanoparticle interactions:
## LHCII + Gold nanorods (AuNRs)

**Drastic tuning of the radiative and nonradiative rates!**




F. Kyeyune, J.L. Botha, B. van Heerden, P. Maly, R. van Grondelle, M. Diale and T.P.J. Krüger, *Nanoscale*, 11: 15139-15146 (2019)

L.C. Ugwuoke, T. Mančal and T.P.J. Krüger. *J Appl Phys* 127: 203103 (2020)

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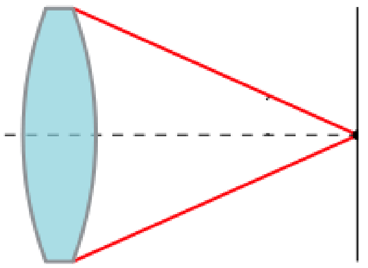
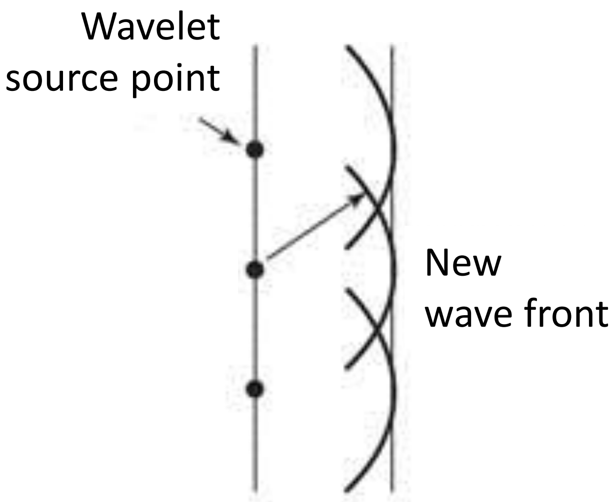
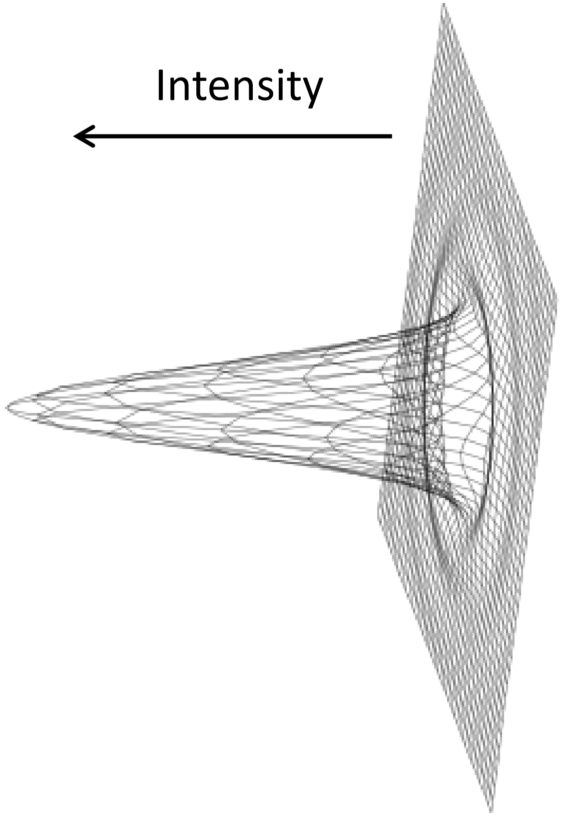
# 3. Superresolution imaging / Nanoscopy





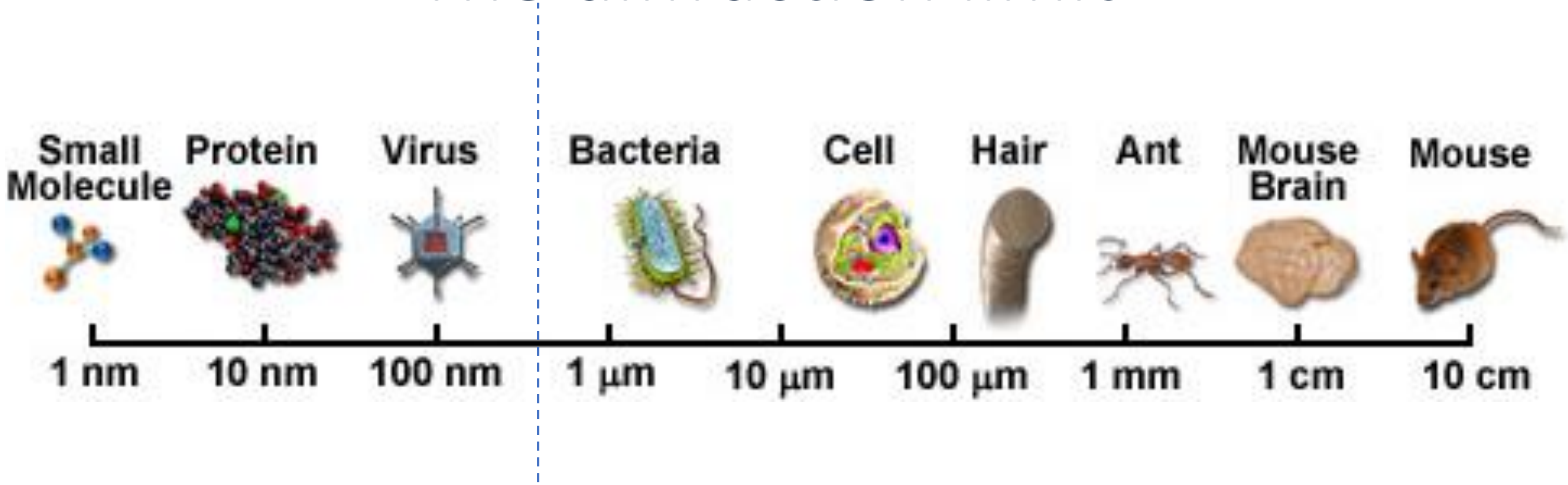

Huygens’ Principle




Fraunhofer Diffraction

# 3. Superresolution imaging / Nanoscopy

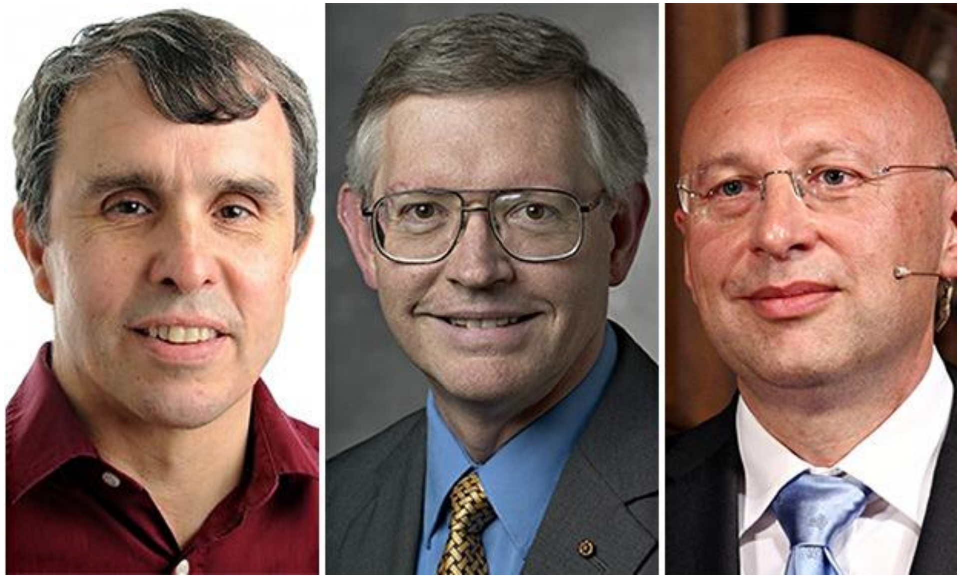
## The diffraction limit




Abbè’s diffraction limit ~ $\lambda/2$
I.e. ~200 nm with blue light (400 nm)

# 3. Superresolution imaging / Nanoscopy

**Nobel Prize in Chemistry 2014**



Eric Betzig WE Moerner Stefan Hell

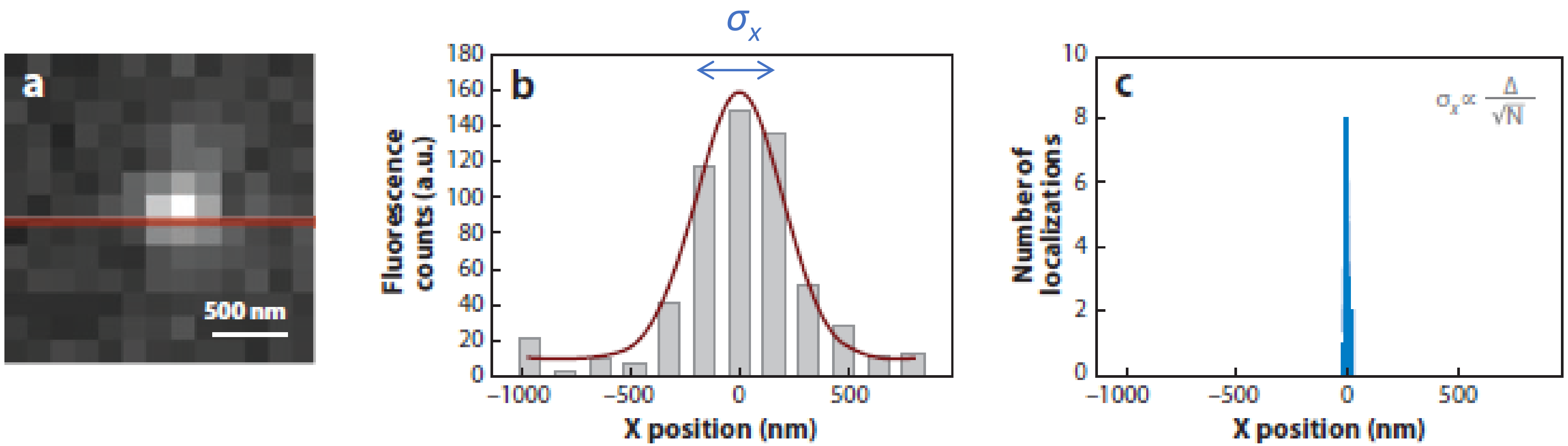
**Stochastic localisation microscopy (PALM/STORM)** — Eric Betzig, WE Moerner

**Deterministic depletion (STED)** — Stefan Hell

Both techniques can give a resolution down to ~10 nm using light!

# 3. Superresolution imaging / Nanoscopy

STORM/PALM: Single molecule localisation with ~10 nm precision




**Localisation ≠ resolution**

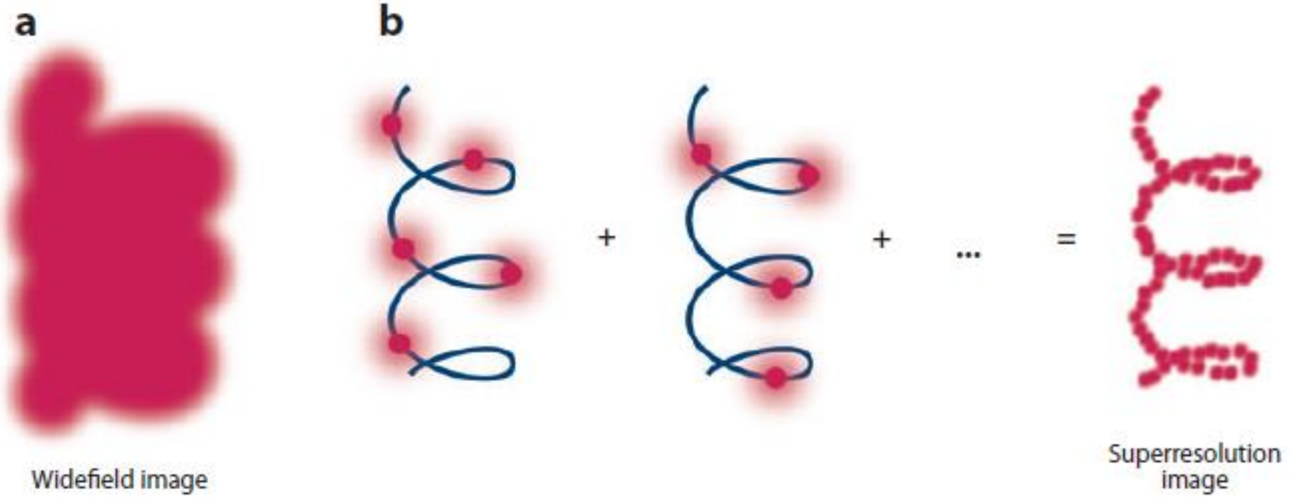
$$\sigma_x \approx \frac{1}{\sqrt{N}} \frac{\lambda}{2NA} = \frac{r_{Abbe}}{\sqrt{N}}$$

$$NA = n \sin\theta$$

Thompson MA, Lew MD, Moerner WE
*Annu Rev Biophys*. 41:321-342 (2012)

# 3. Superresolution imaging / Nanoscopy

## Principle of STORM/PALM:
## Stochastic (de)activation of sparse subsets

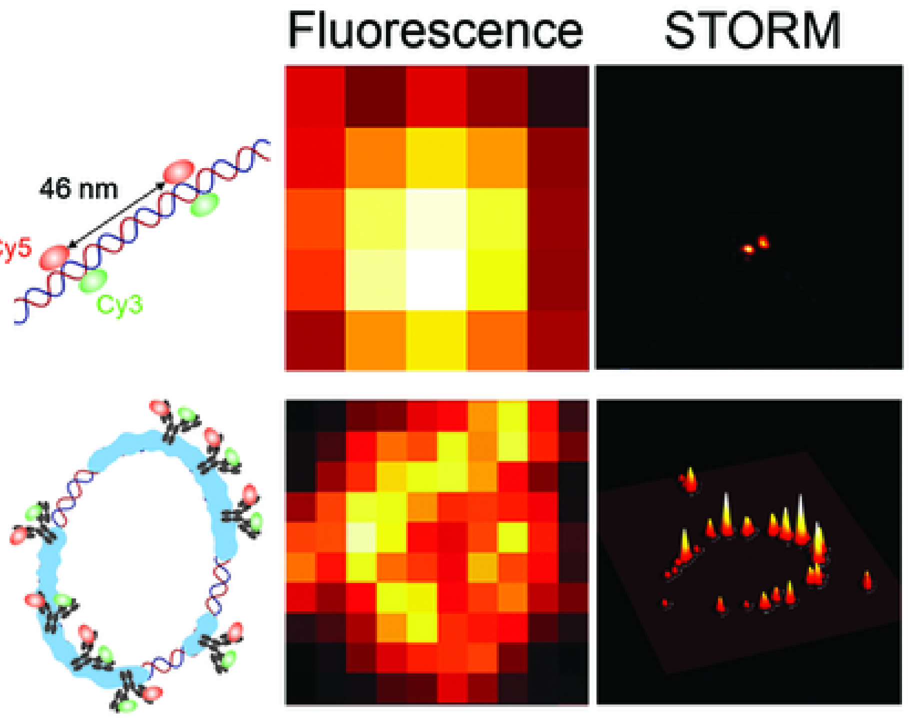



Thompson MA, Lew MD, Moerner WE
*Annu Rev Biophys*. 41:321-342 (2012)

# 3. Superresolution imaging / Nanoscopy

## STORM: STochastic Optical Reconstruction Microscopy

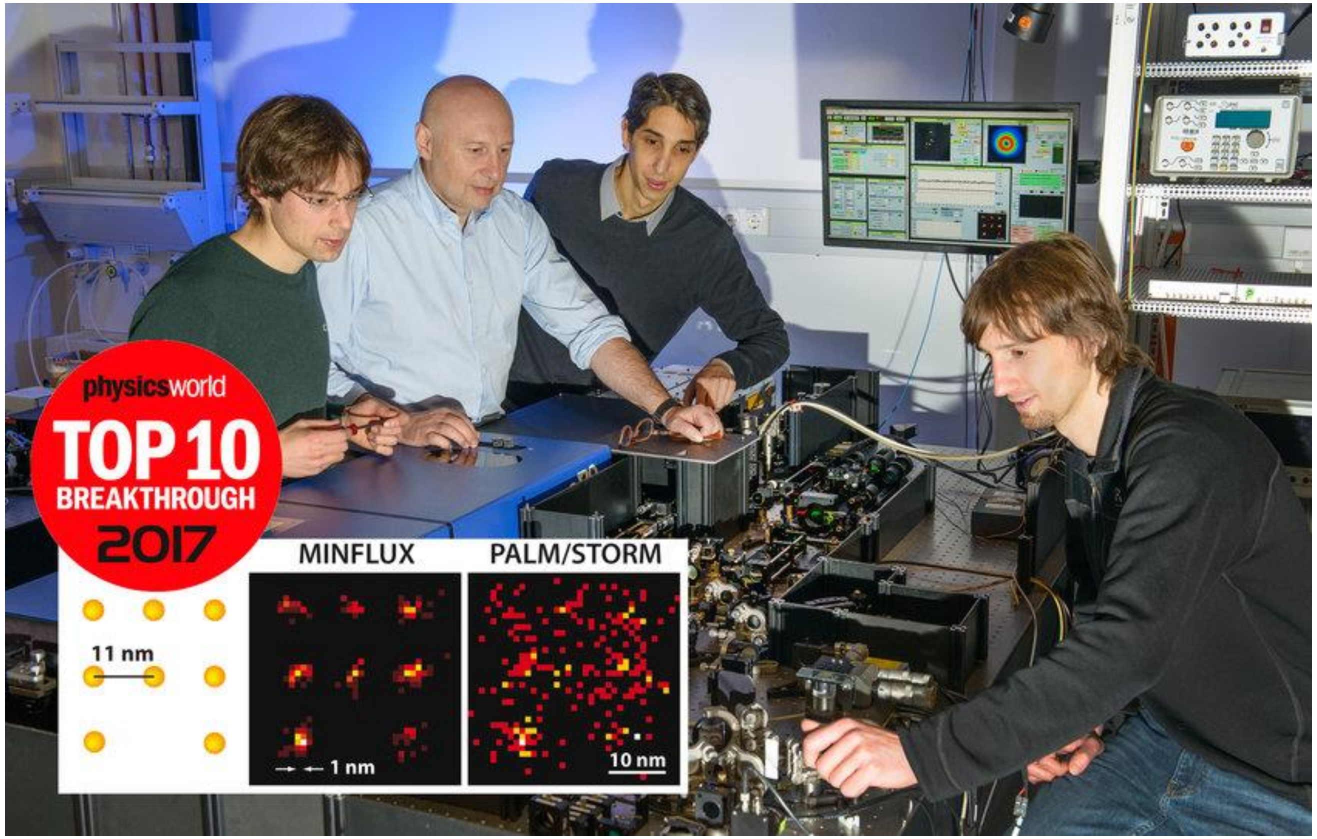
(Wide-field) (Reconstruction)




Rust et al. *Nature Methods* 3:793 (2006)

# 3. Superresolution imaging / Nanoscopy

MINFLUX combines the two approaches to yield a resolution of 1 nm!



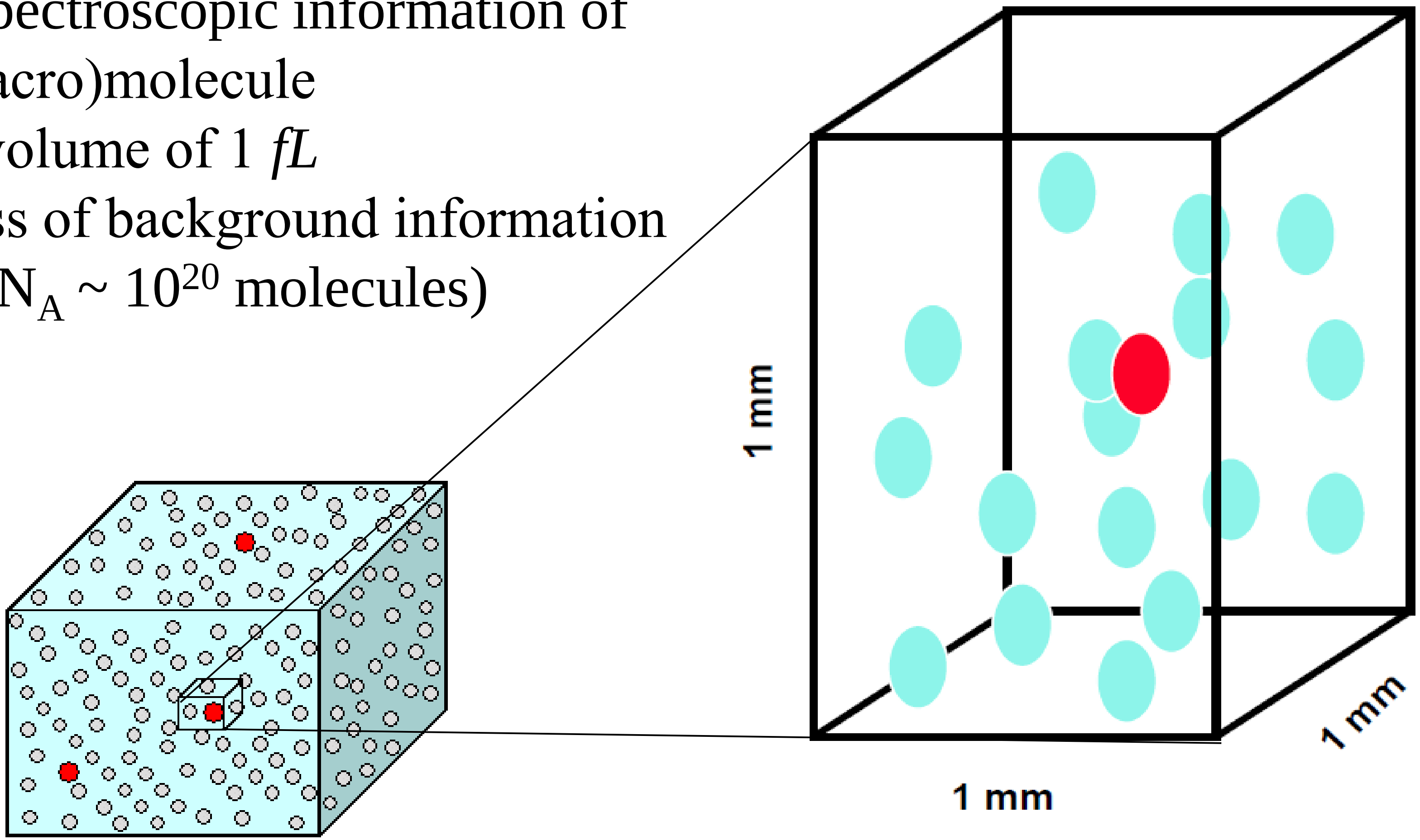

Balzarotti et al. *Science* 355:606-612 (2017)

# 4. Single molecule spectroscopy

**The challenge:**

Find spectroscopic information of

- 1 (macro)molecule
- in a volume of 1 *fL*
- excess of background information ($10^{-3}$ $N_A$ ~ $10^{20}$ molecules)

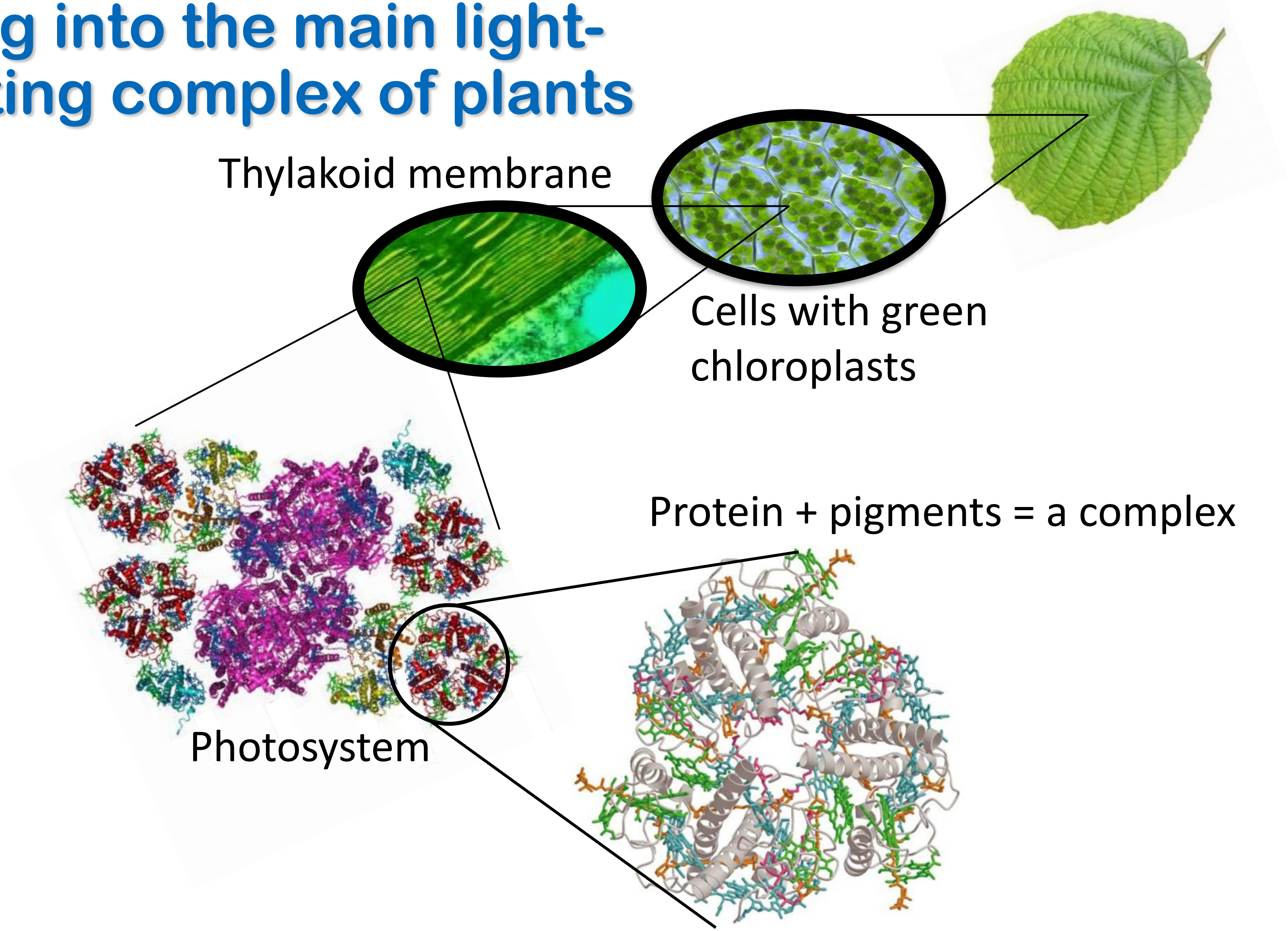


# 4. Single molecule spectroscopy

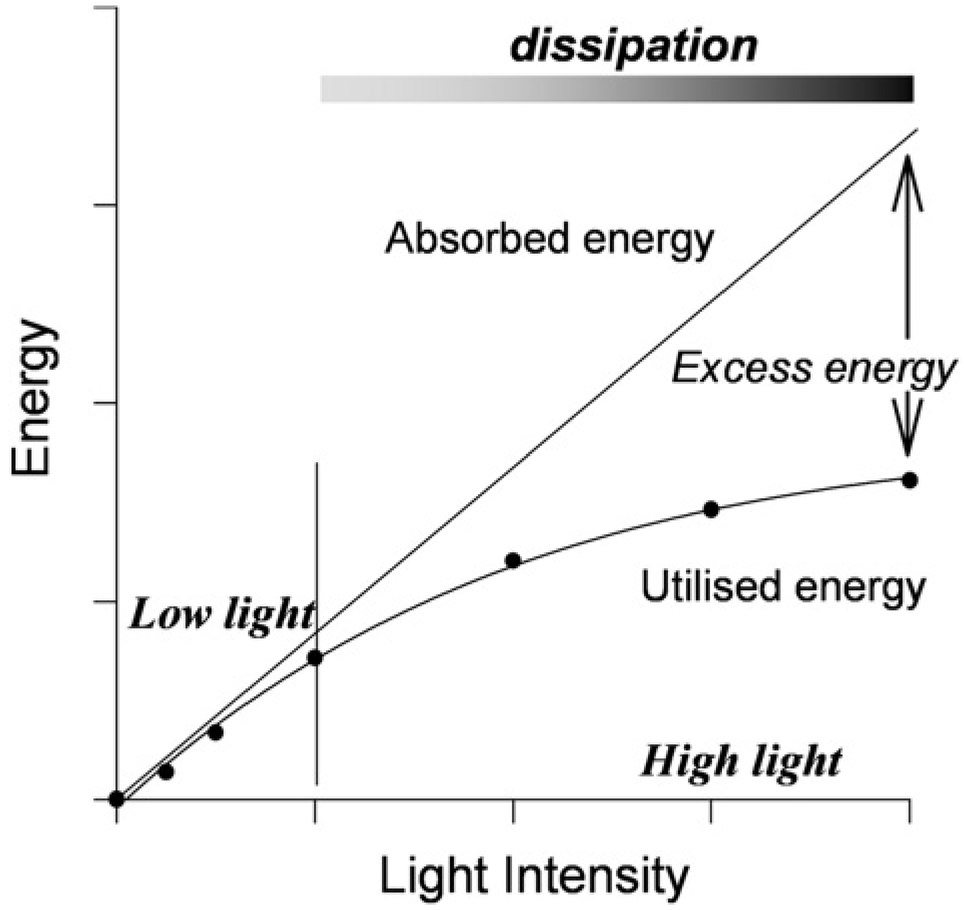
**Review papers**


1. T.P.J. Krüger and R. van Grondelle, "Design principles of natural light-harvesting as revealed by single molecule spectroscopy." *Physica B: Condensed Matter*, 480:7-13 (2016) https://doi.org/10.1016/j.physb.2015.08.005
2. T.P.J. Krüger and R. van Grondelle, "The role of energy losses in photosynthetic light harvesting" *J Phys B: At Mol Opt Phys* 50: 132001 (2017) https://iopscience.iop.org/article/10.1088/1361-6455/aa7583/
3. J.M. Gruber, P. Maly, T.P.J. Krüger and R. van Grondelle, "From isolated light-harvesting complexes to the thylakoid membrane:a single-molecule perspective" *Nanophotonics* 7:81-92 (2018) https://doi.org/10.1515/nanoph-2017-0014

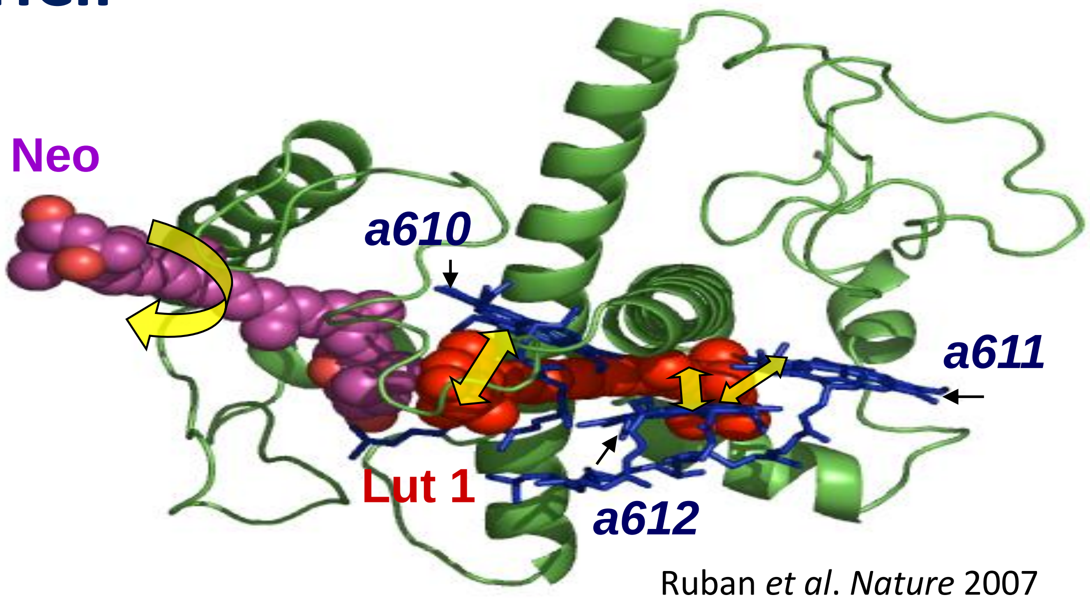
# Zooming into the main light-harvesting complex of plants



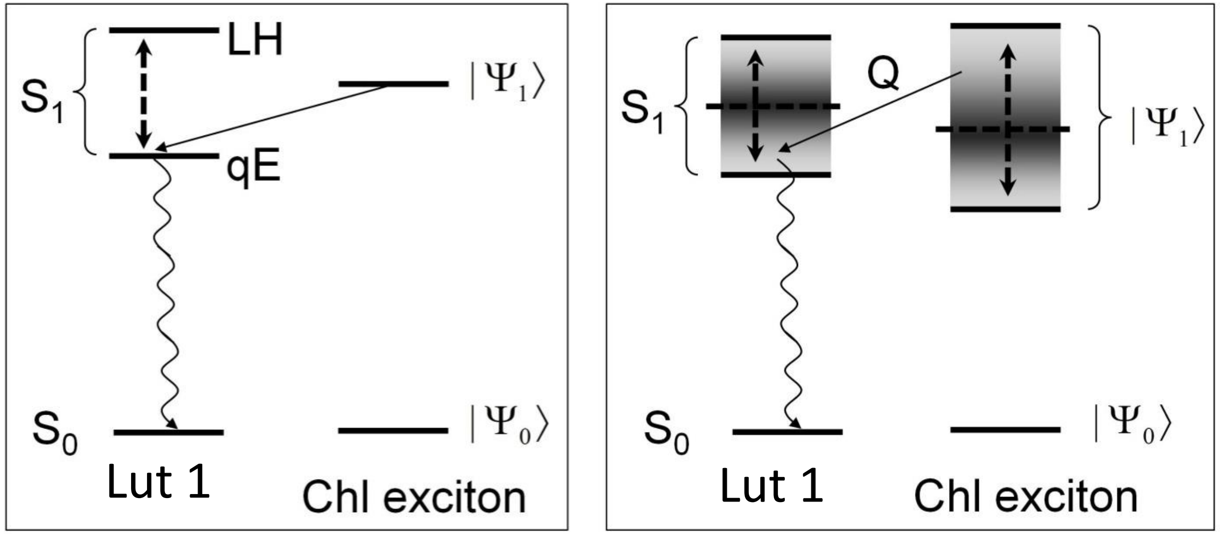
# Non-photochemical Quenching (NPQ):



# NPQ mechanism in LHCII




Ruban *et al*. *Nature* 2007

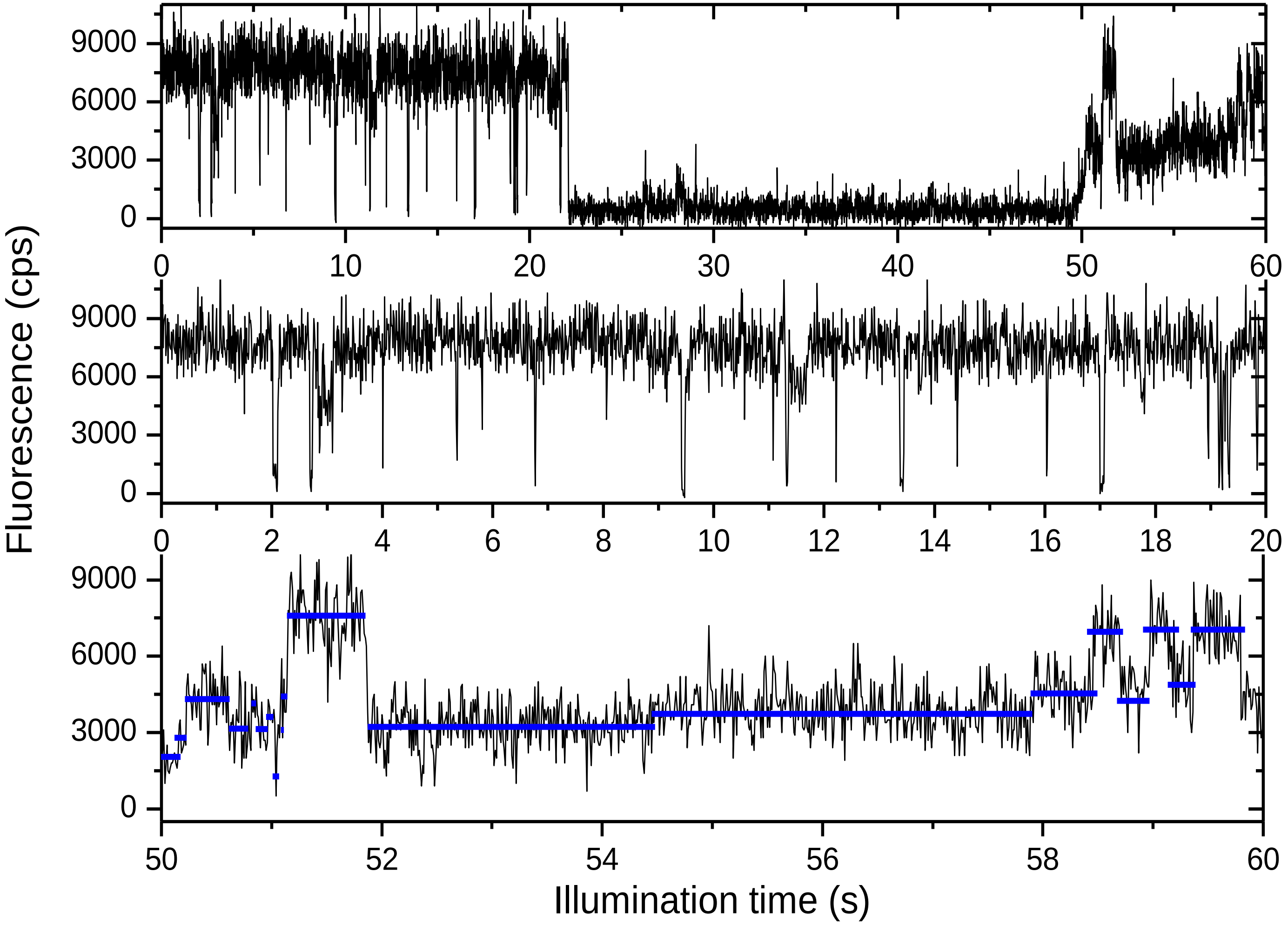



**Is LHCII a controlled switch?**



Krüger *et al.* How Protein Disorder Controls Nonphotochemical Quenching, *In*: Advances in Photosynthesis and Respiration, Including Biofuels and Related Processes, Springer, 2014

# Fluorescence intermittency ("blinking")




Krüger *et al.* *J Phys Chem* (2011a)
Valkunas *et al.*, *J Phys Chem Lett* 3:2779 (2012)
Chmeliov *et al.*, *New J Physics* 15 (2013)

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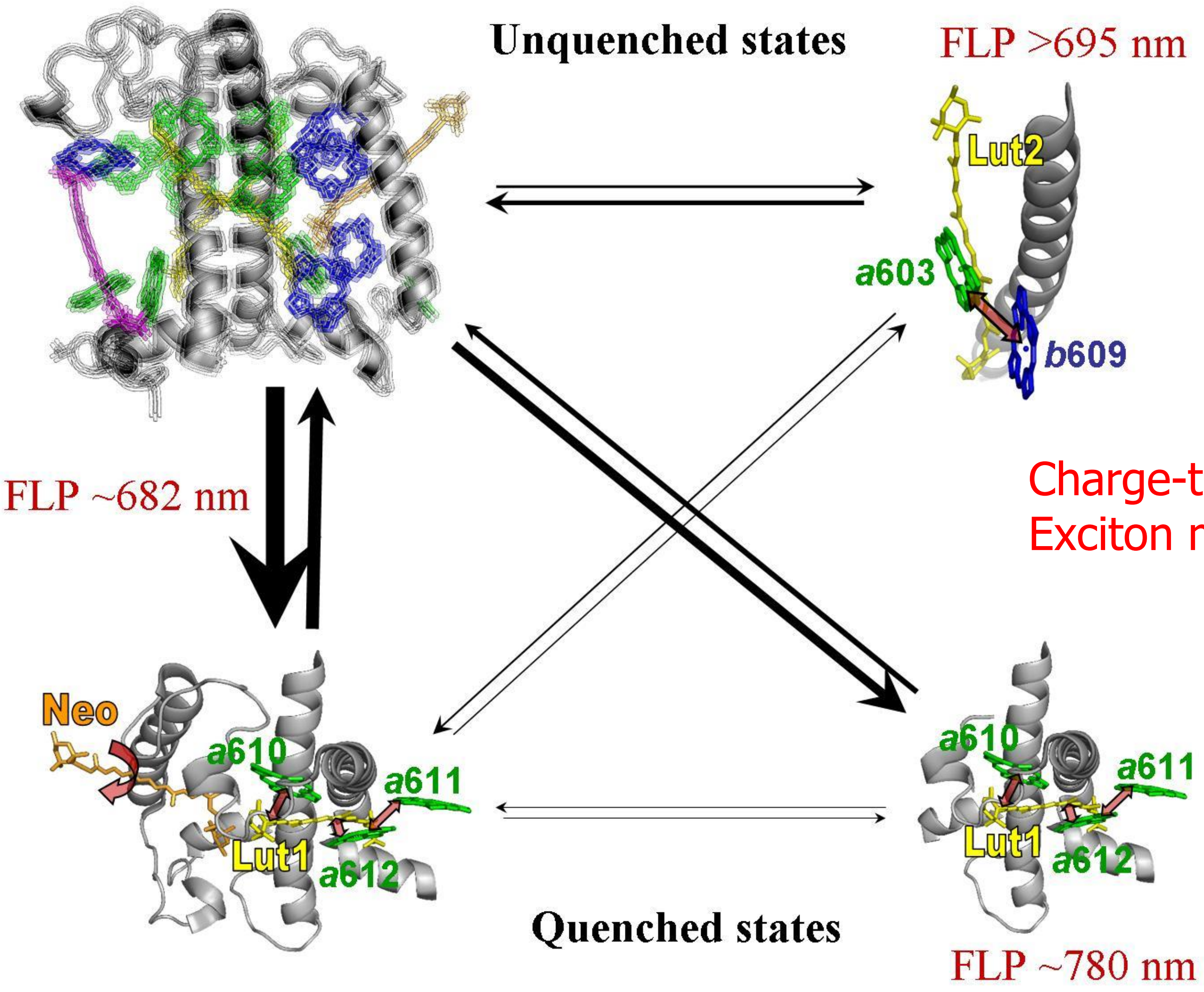
# Environmental control over the intrinsic protein disorder




Charge-transfer-
Exciton mixing

Krüger *et al. Biophys J* 2012; 2013; *BBA* 2014