Absorption und Fluoreszenz

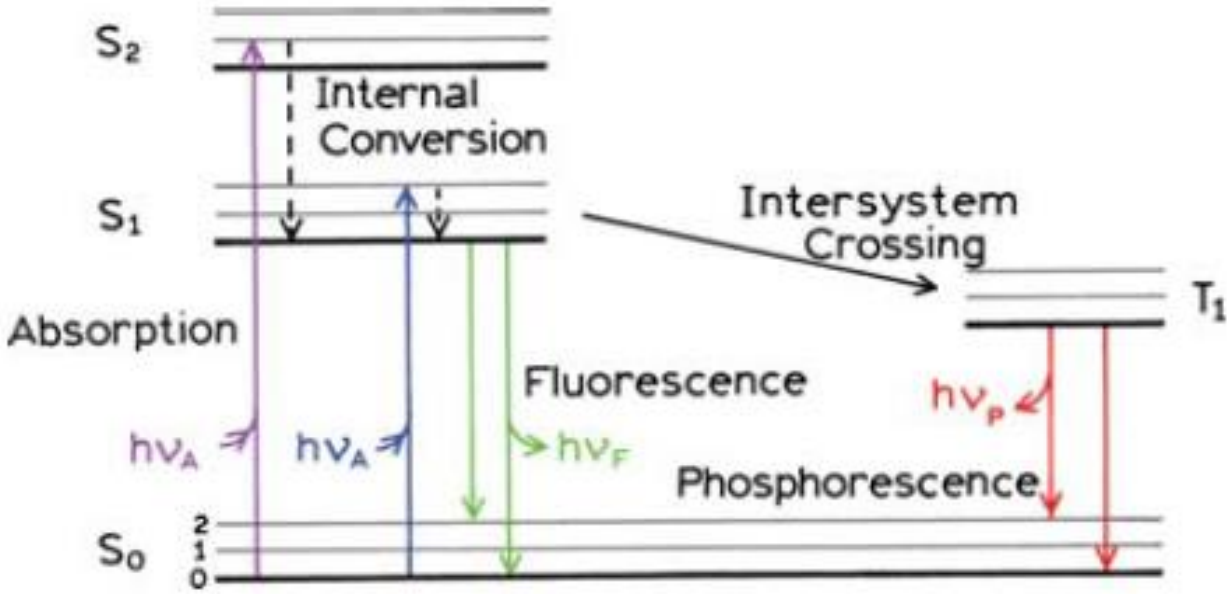


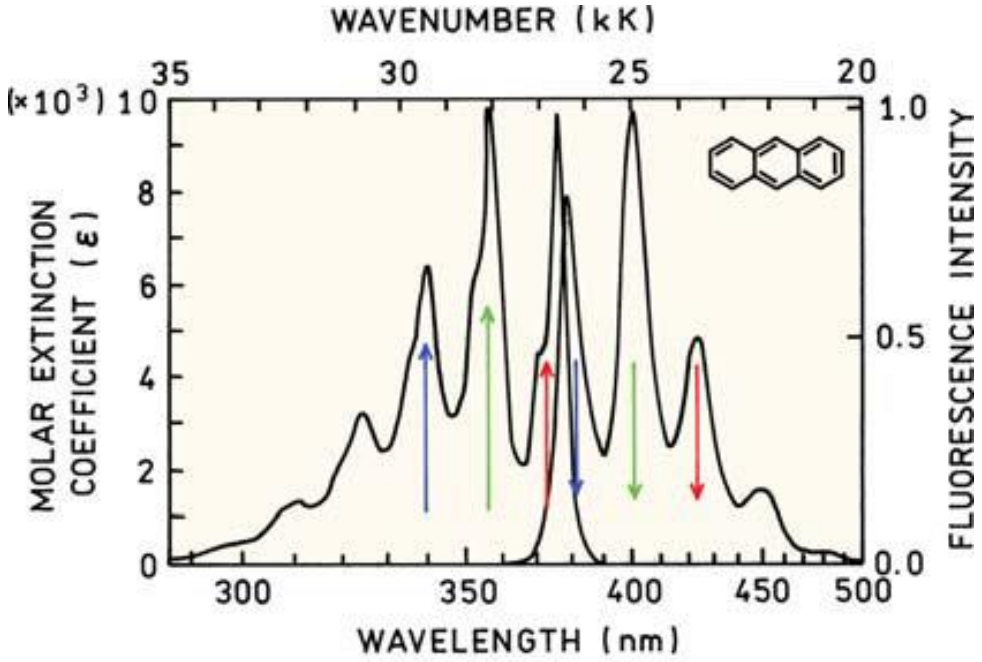
Figure 1.5. One form of a Jablonski diagram.

Figure 1.4. Professor Alexander Jablonski (1898–1980), circa 1935.

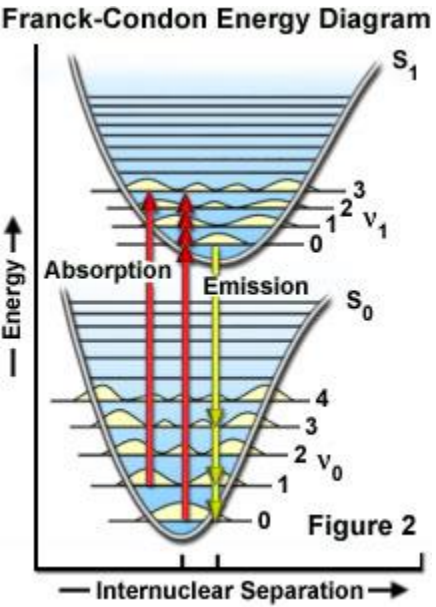
Lakowicz, Principles of Fluorescence, 3rd

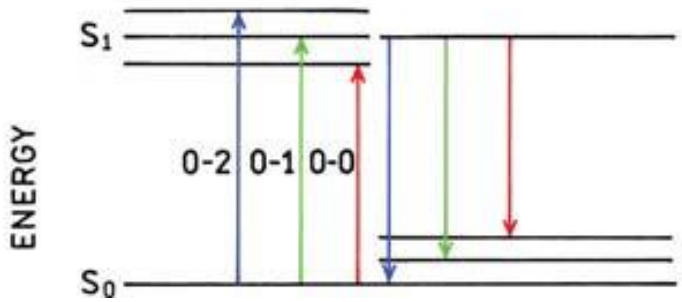
Absorption und Fluoreszenz

"mirror image rule"



Anthracen





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Absorption und Fluoreszenz

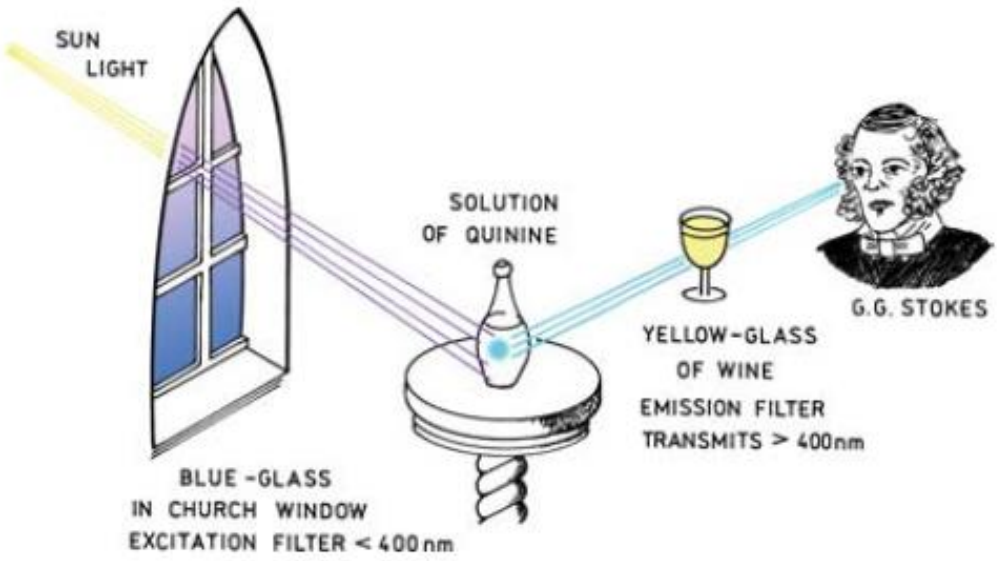
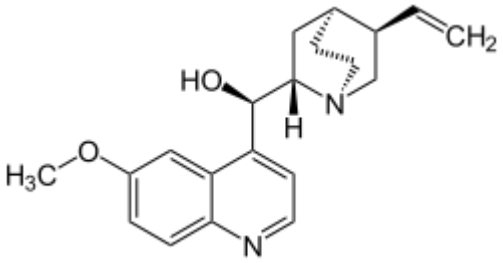
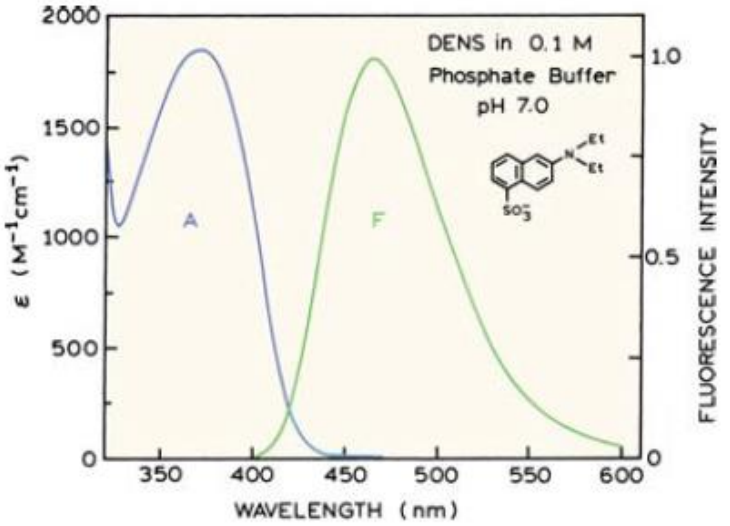


Figure 1.6. Experimental schematic for detection of the Stokes shift.

Sir George Stokes, 1819-1903





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Fluoreszenzparameter

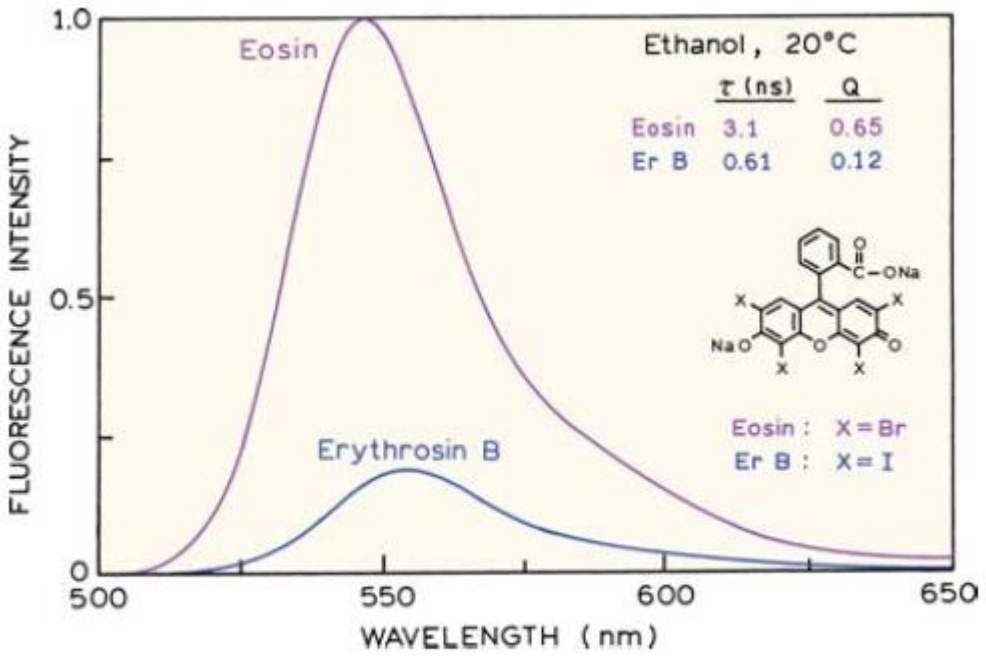


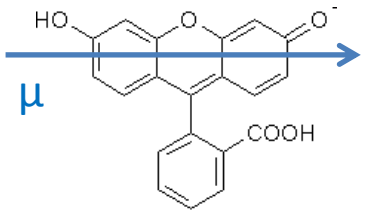
Figure 1.13. Emission spectra of eosin and erythrosin B (ErB).

- spektrale Eigenschaft von Absorption, Emission
- Quantenausbeute ϕ
- Relative Helligkeit
- Lebensdauer τ
- Anisotropie r
- Energietransfer zwischen Farbstoffen
- zeitliche Schwankung der Emission *F(t)*

Lakowicz, Principles of Fluorescence, 3rd

Messung der Fluoreszenzanisotropie

Warum?
→ Fluorophore sind Dipole!



Molekülrotation (ps – ns) und Fluoreszenzlebensdauer (ns) treten in ähnlichen Zeitskalen auf und führen zur Polarisation der Fluoreszenzemission

$\tau_{rot} << \tau_{fl}$ keine Polarisation

$\tau_{rot} \sim \tau_{fl}$

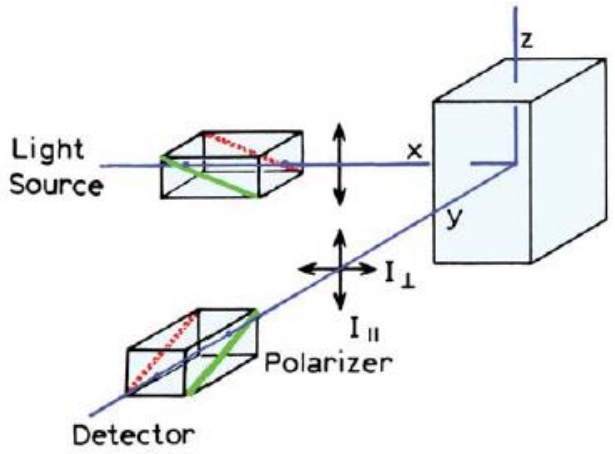


Figure 10.1. Schematic diagram for measurement of fluorescence anisotropies.

$$\Delta I = I_{parallel} - gI_{senkrecht}$$

$$P = \frac{I_{parallel} - gI_{senkrecht}}{I_{parallel} + gI_{senkrecht}}$$

$$r = \frac{I_{parallel} - gI_{senkrecht}}{I_{parallel} + 2gI_{senkrecht}}$$

$$g = \frac{i_{parallel}}{i_{senkrecht}}$$

Lakowicz, 3rd

Anwendung der Fluoreszenzanisotropie

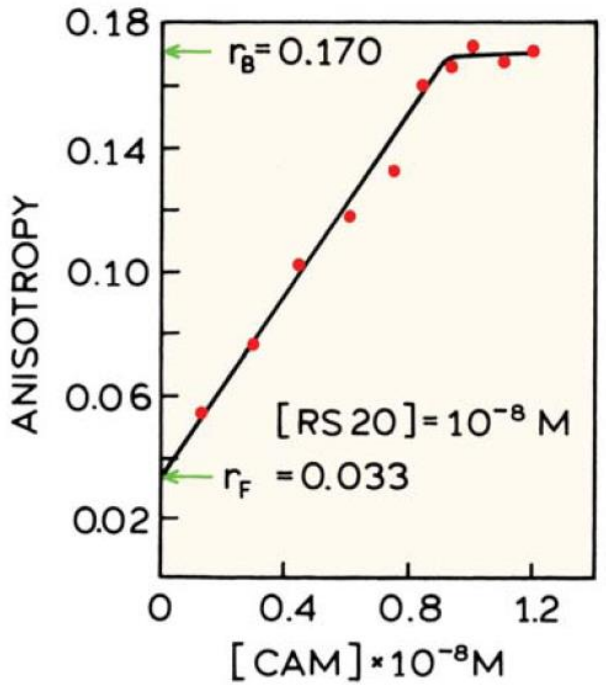


Figure 10.20. Titration of the MLCK peptide RS20 with calmodulin. Revised and reprinted with permission from [49]. Copyright © 1986, American Chemical Society.

RS20 Aminosäuresequenz (20) aus der Myosin Light Chain Kinase (MLCK), enthält ein Tryptophan

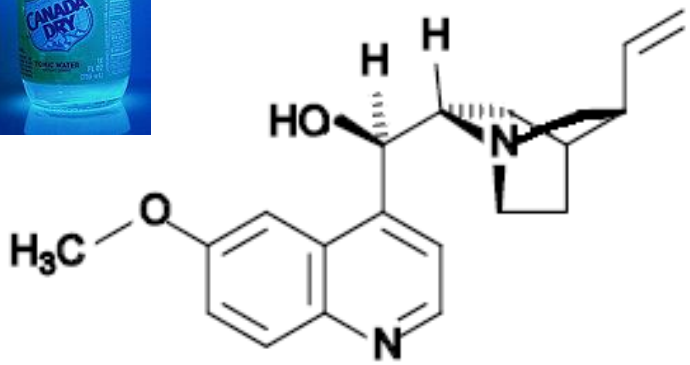
CAM Calmodulin (kein Trp)

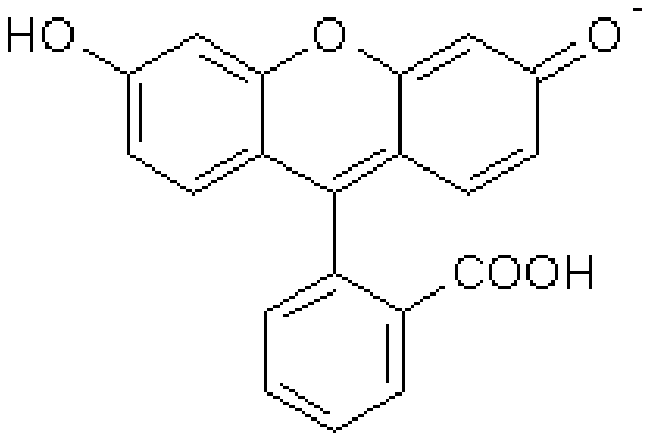
RS20 + CAM → [RS20*CAM]

→Bindungskonstanten und Bindungsstöchiometrien

Lakowicz, 3rd

Chinin und Fluorescein

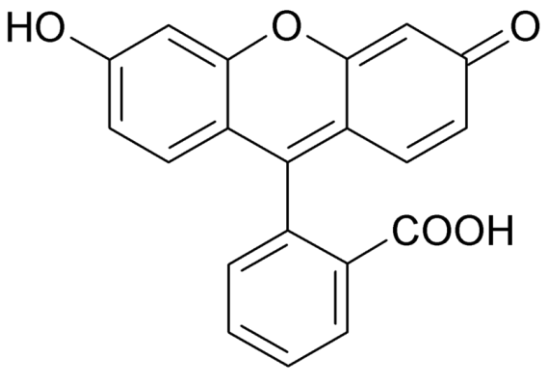




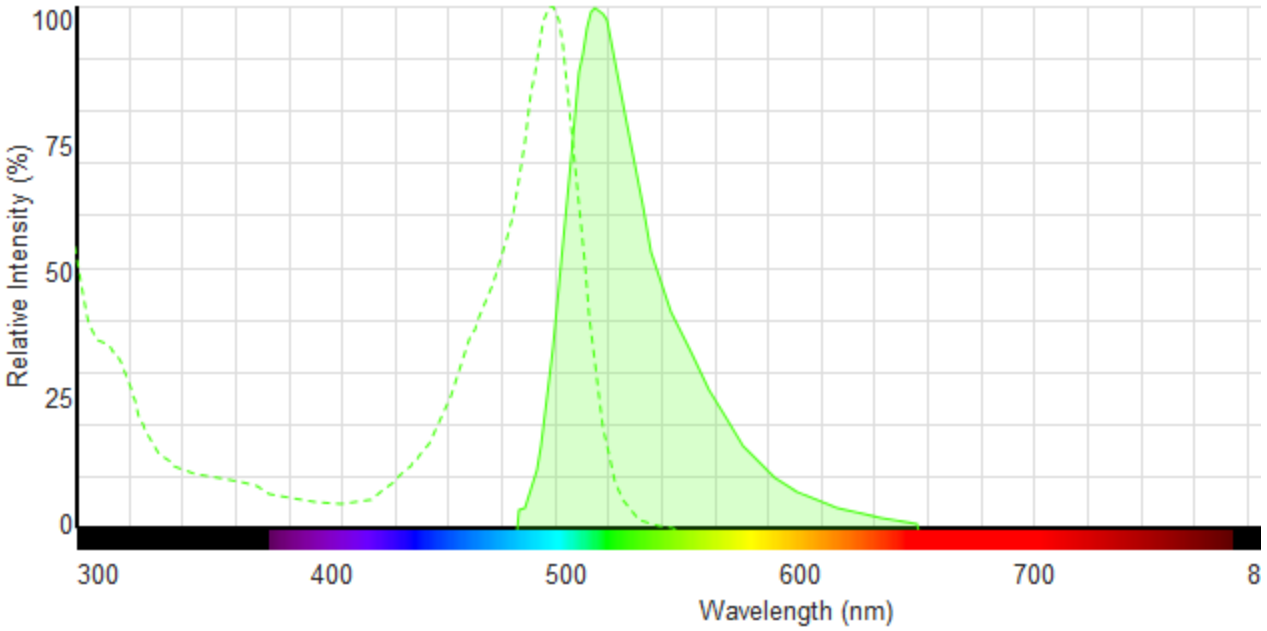
1845, Sir J. W. Herschel

1871, A. von Baeyer

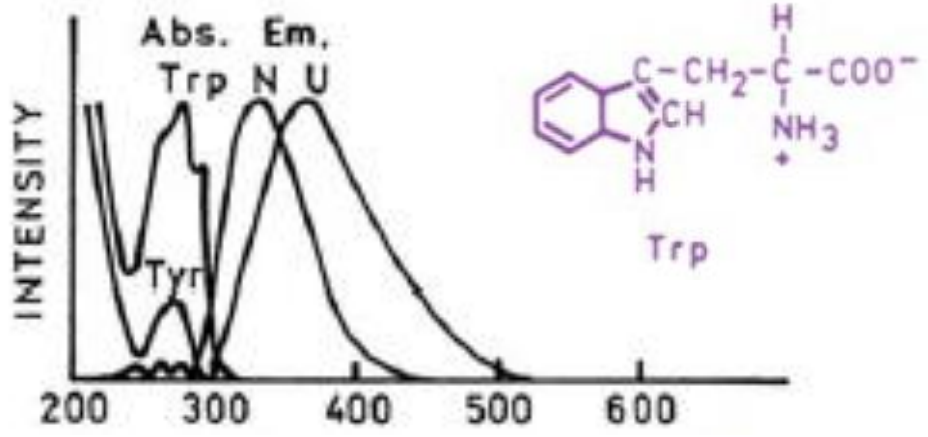
Fluorescein

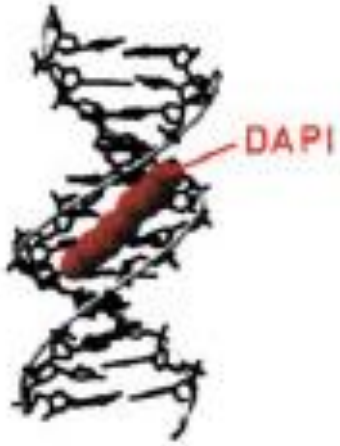


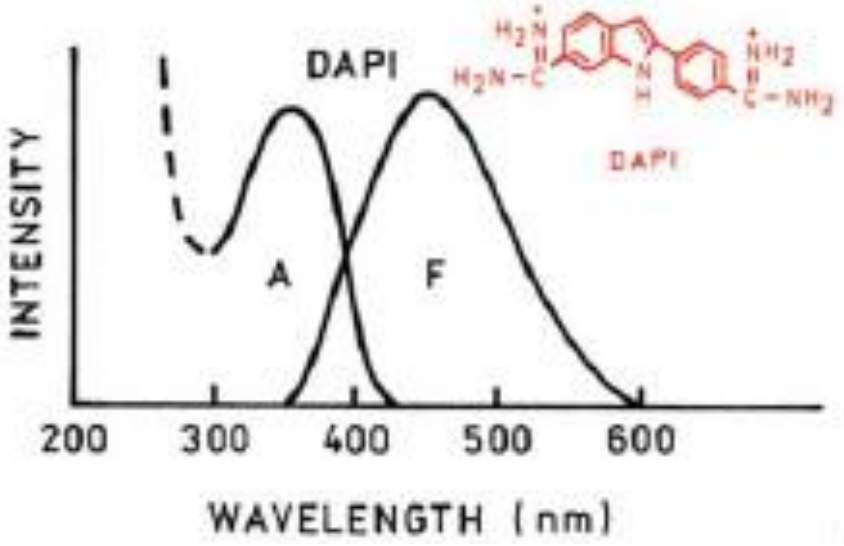
1871, A. von Baeyer



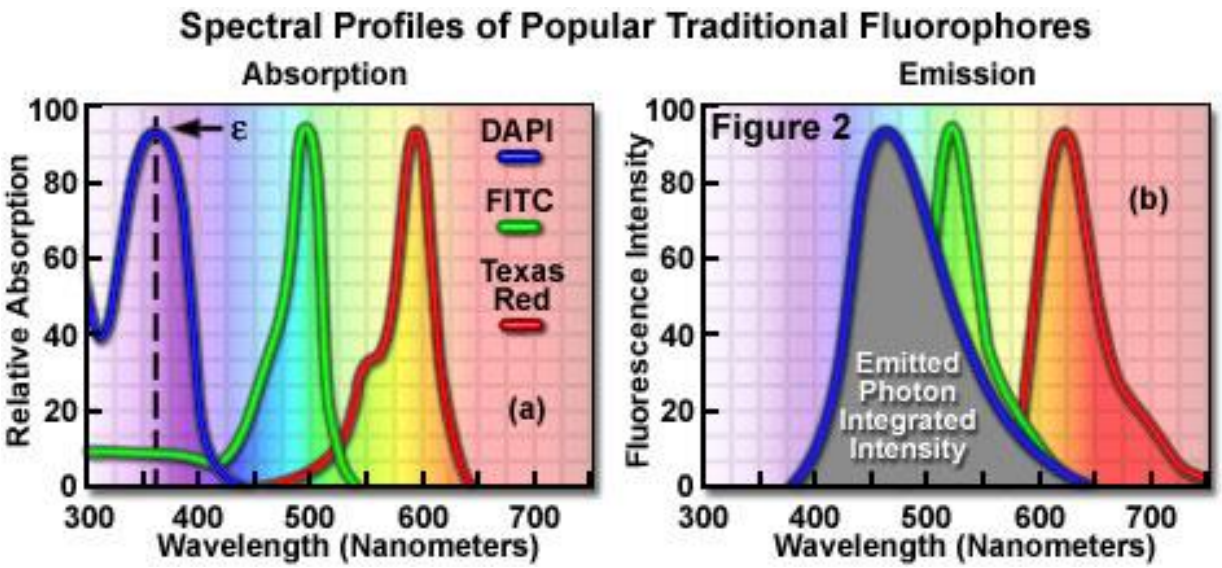
Tryptophan, DAPI



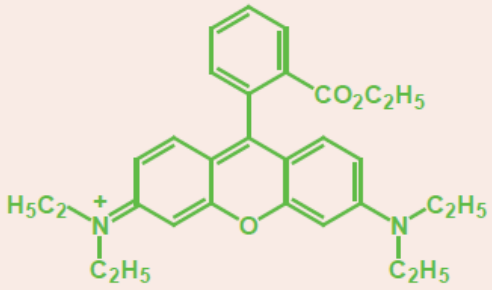




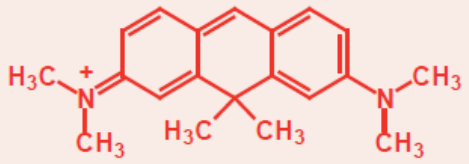
Organische Fluoreszenzfarbstoffe



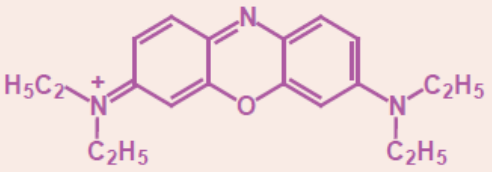
Rhodamine

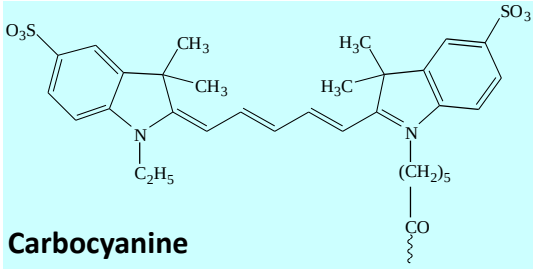


Carbopyronin



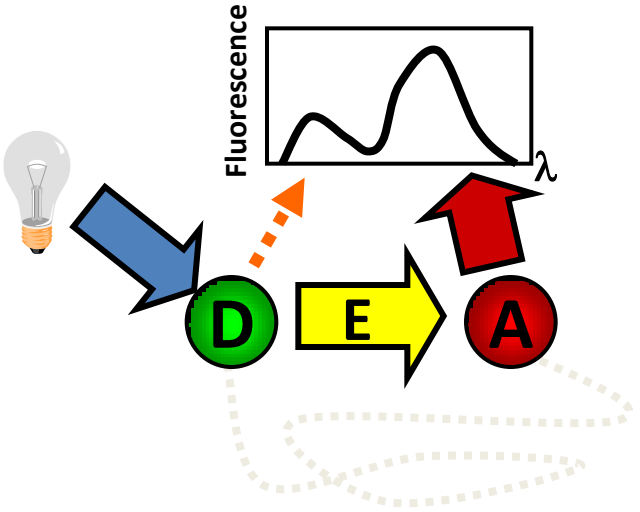
Oxazine

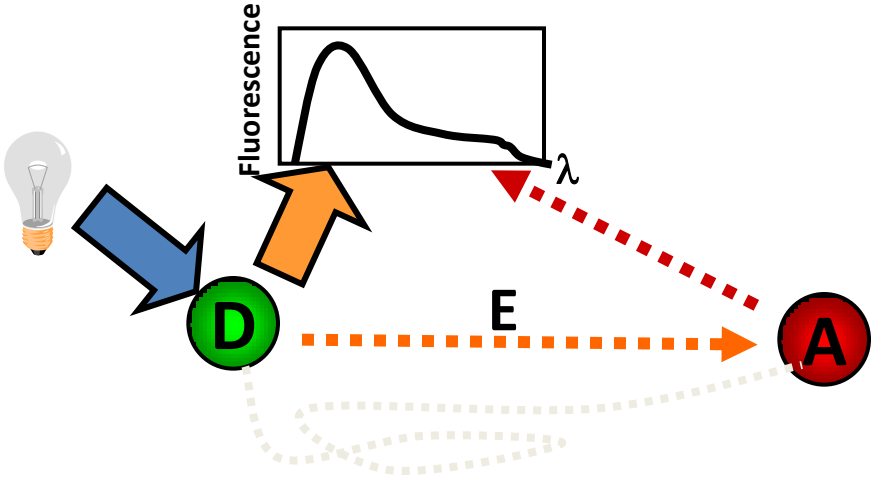




Carbocyanine

Förster Resonance Energy Transfer (FRET)

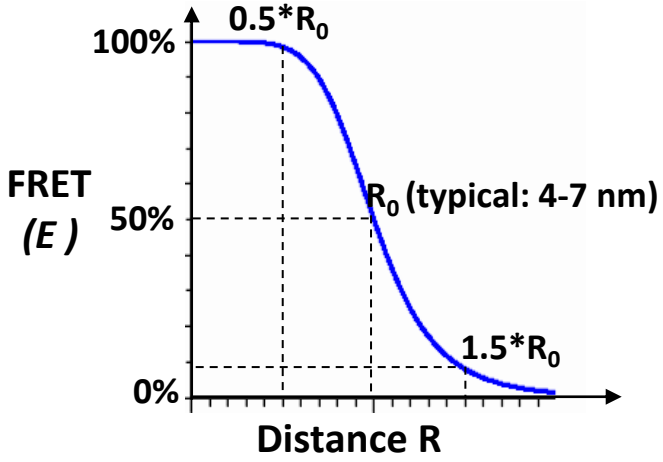




FRET Efficiency, *E*:

$$E = \frac{1}{1 + \left(\frac{R}{R_0}\right)^6}$$

R **= D-A distance**
R_0 **= Förster radius**
(D-A distance where *E* = 50%)



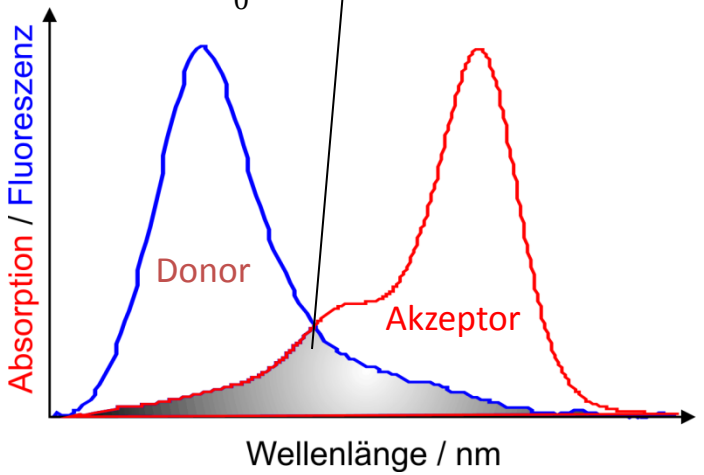
Förster Resonance Energy Transfer (FRET)

Förster-Radius:

$$R_0^6 \propto n^{-4}\kappa^2\phi_D J_D$$

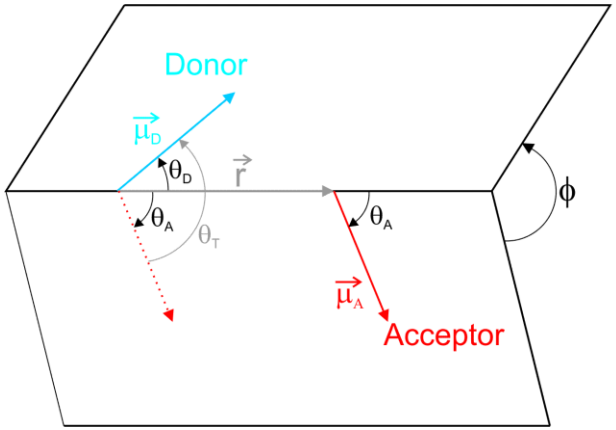
R_0 (CFP, YFP) = 4.9 nm

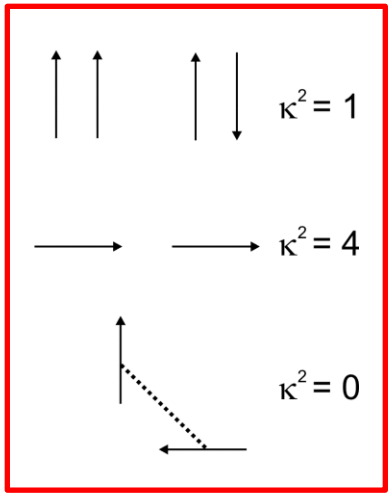
$$J_D = \int_0^\infty F_D(\lambda)\varepsilon_A(\lambda)\lambda^4 d\lambda$$



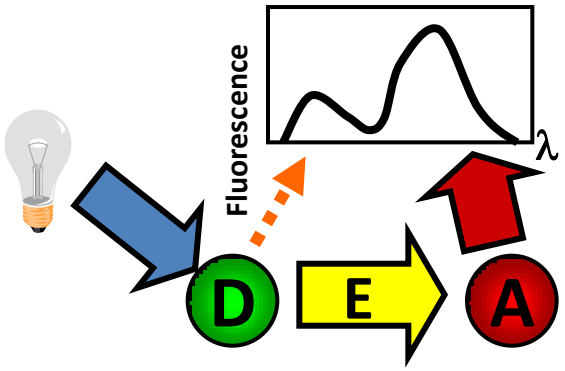
Orientierungsfaktor:

$$\kappa^2 = (\cos\theta_T - 3\cos\theta_D\cos\theta_A)^2$$
$$= (2\cos\theta_A\cos\theta_D - \cos\phi\sin\theta_A\sin\theta_D)^2$$





Messung von FRET

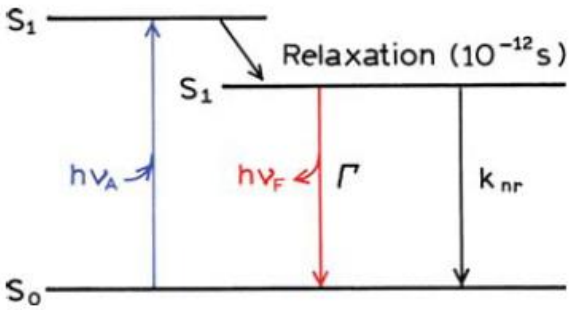


Was passiert bei FRET?

Fluoreszenzintensität des Donors *nimmt ab*

Fluoreszenzintensität des Akzeptors *nimmt zu*

Fluoreszenzlebensdauer des Donors *nimmt ab*



Bestimmung von *E*:

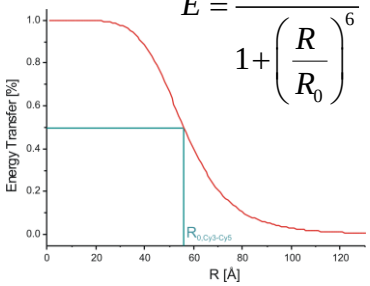
Fluoreszenzintensität des Donors in An- und Abwesenheit des Akzeptors:

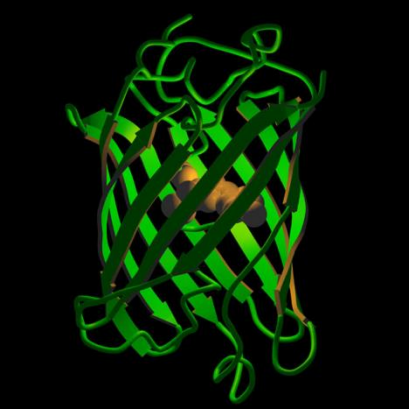
$$E = 1 - \frac{F_{DA}}{F_D}$$

Fluoreszenzlebensdauer des Donors in An- und Abwesenheit des Akzeptors:

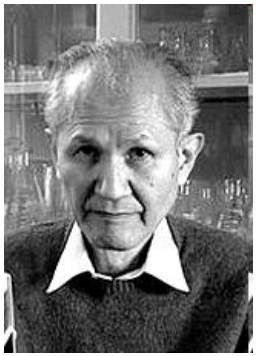
$$E = 1 - \frac{\tau_{DA}}{\tau_D}$$

$$E = \frac{1}{1 + \left(\frac{R}{R_0}\right)^6}$$

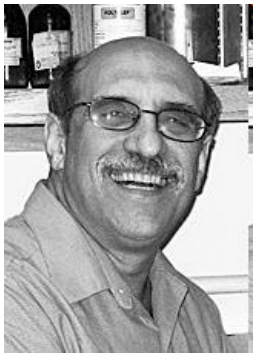




Nobel Prize in Chemistry, 2008



Osamu Shimomura



Martin Chalfie

Roger Y. Tsien

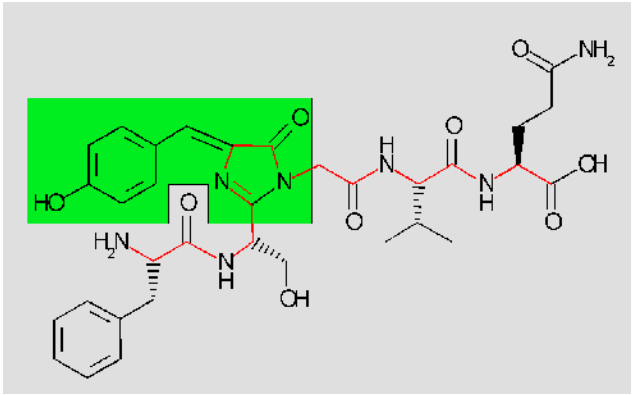
„Glowing proteins – a guiding star for biochemistry“

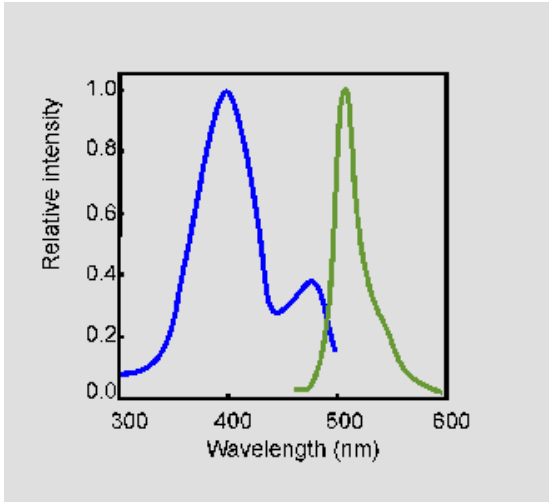
	wtGFP
M	27 kDa (238 aa)
Absorption	395/470 nm
Emission	509
ϕ	~0.7
τ	3.2 ns
ε	12000/27500 $(cmM)^{-1}$

Serin (65)

Tyrosin (66)

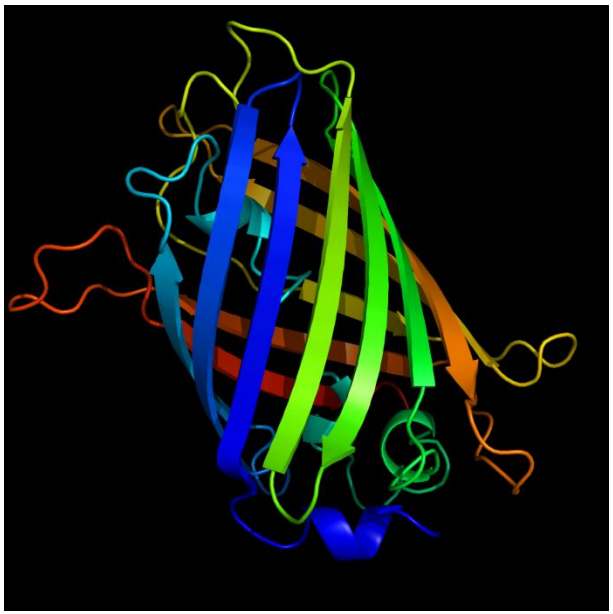
Glycin (67)



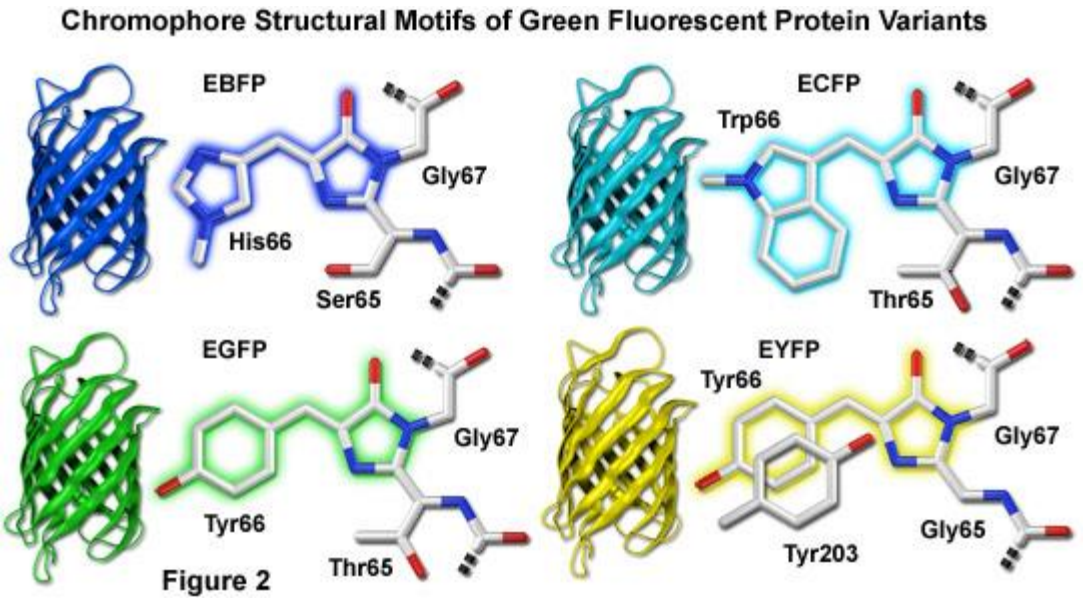


http://www.cryst.bbk.ac.uk/PPS2/projects/jonda/

Fluoreszierende Proteine



wtGFP (Ser – Tyr – Gly)



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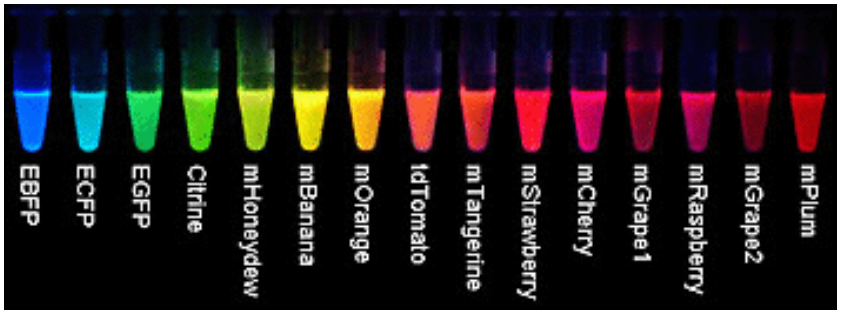


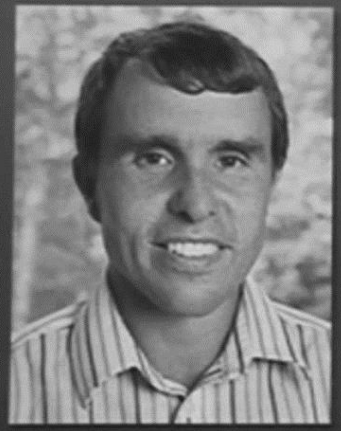
mFruit

LIVE

The Nobel Prize in Chemistry 2014

Nobelpriset i kemi 2014

KUNGL. VETENSKAPS-AKADEMIEN
THE ROYAL SWEDISH ACADEMY OF SCIENCES



Eric Betzig
Janelia Farm Research Campus, Howard Hughes Medical Institute, Ashburn, VA, USA

Stefan W. Hell
Max Planck Institute for Biophysical Chemistry, Göttingen, German Cancer Research Center, Heidelberg, Germany

William E. Moerner
Stanford University, Stanford, CA, USA

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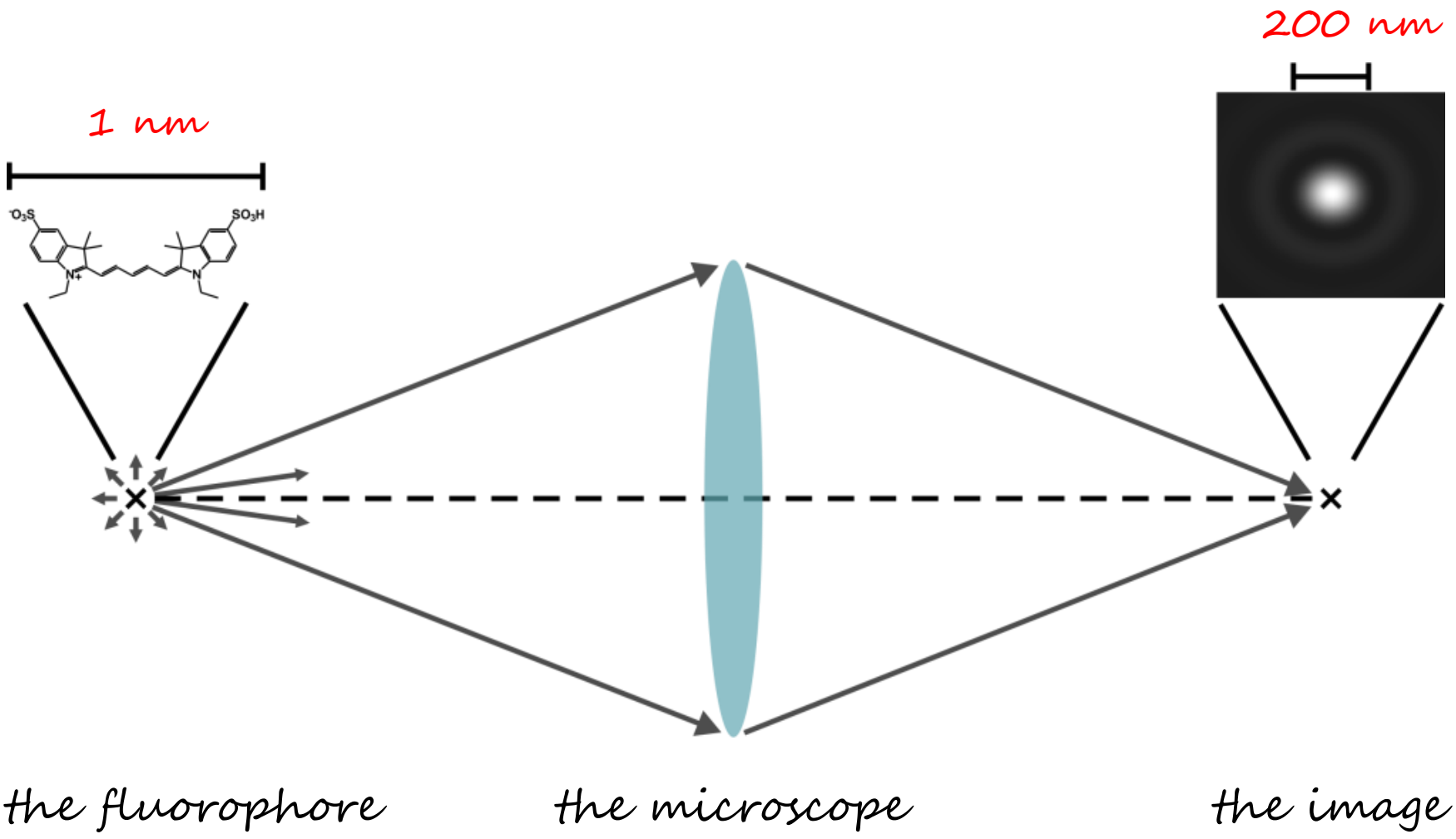
A simplified view of a microscope



fluorophore

image

structure

image

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February 1, 1995 / Vol. 20, No. 3 / OPTICS LETTERS

Proposed method for molecular optical imaging

E. Betzig

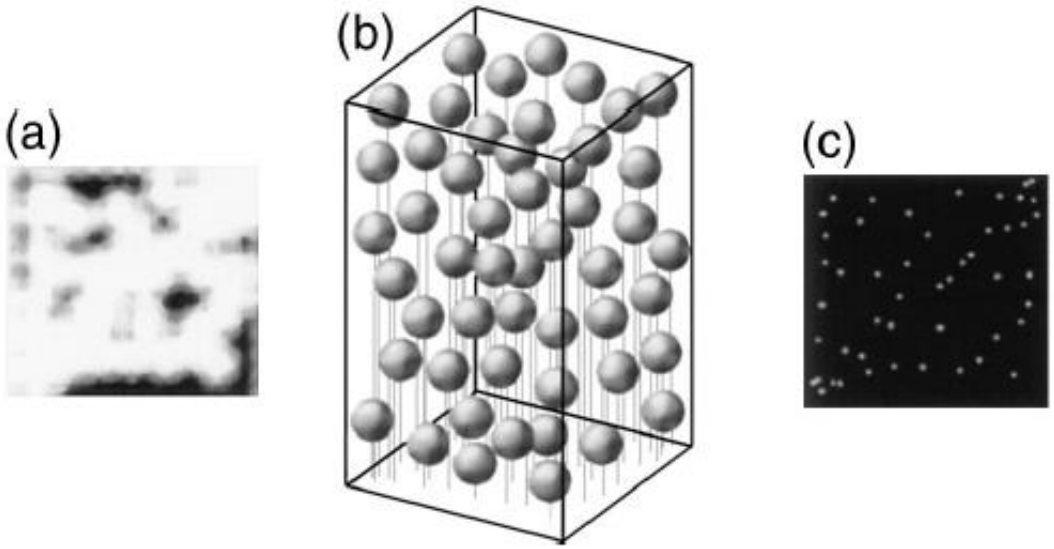
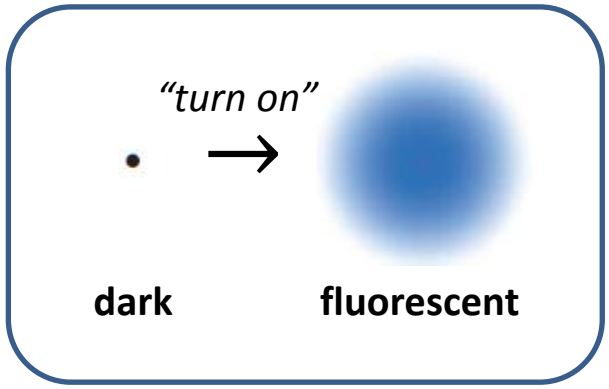
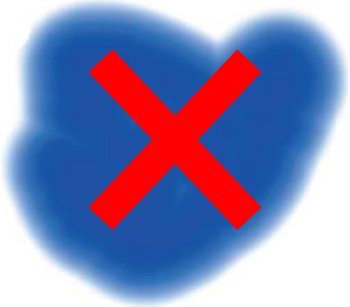
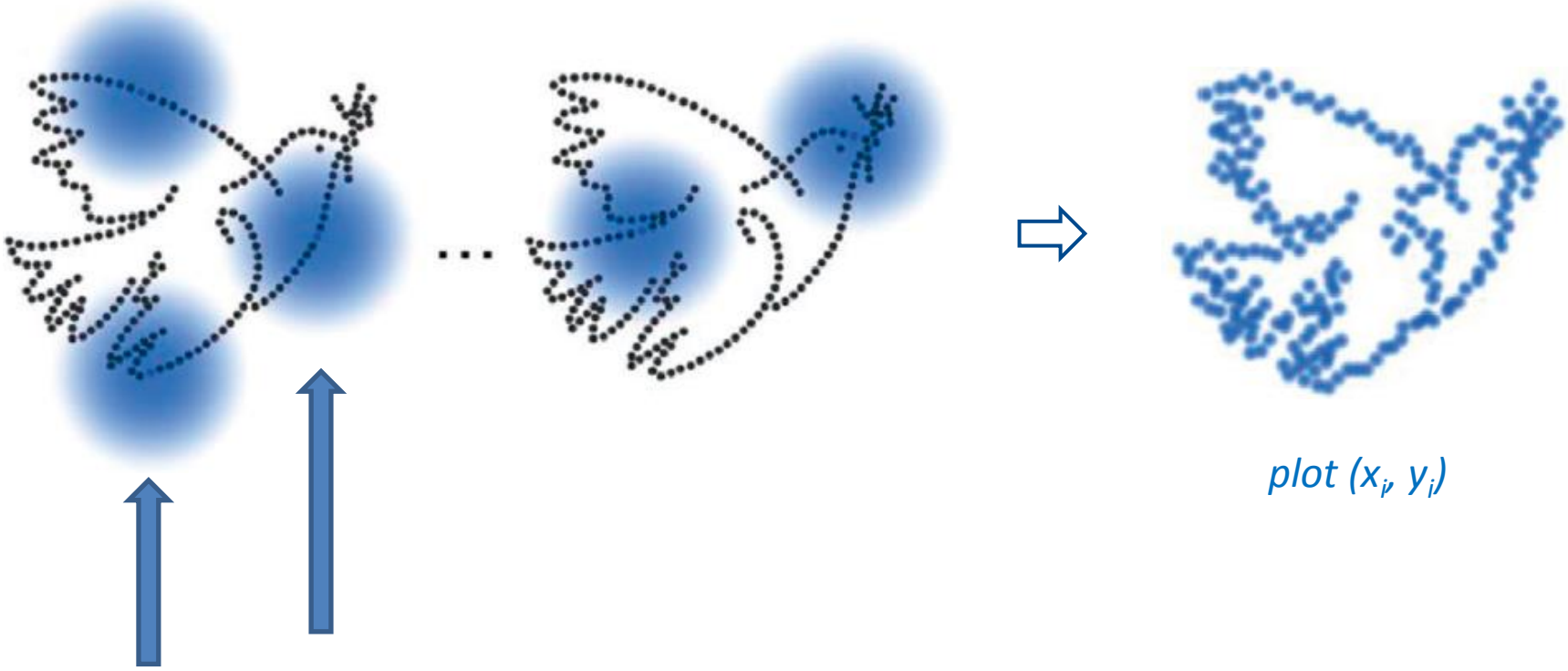


Fig. 1. (a) Field of discrete features as conventionally imaged in m spatial dimensions with a broad PSF. (b) Same features after isolation in $m + n$ dimensions on the basis of n distinguishing optical characteristics. (c) Final image reconstructed at resolution $\delta\mathbf{x}$ given by the uncertainty in the measured position of each isolated feature. In general, $|\delta\mathbf{x}| \ll$ PSF.







plot (x_i, y_i)

determine the center of mass (x, y)

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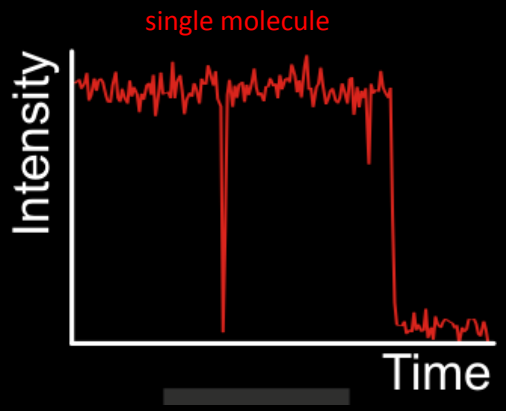
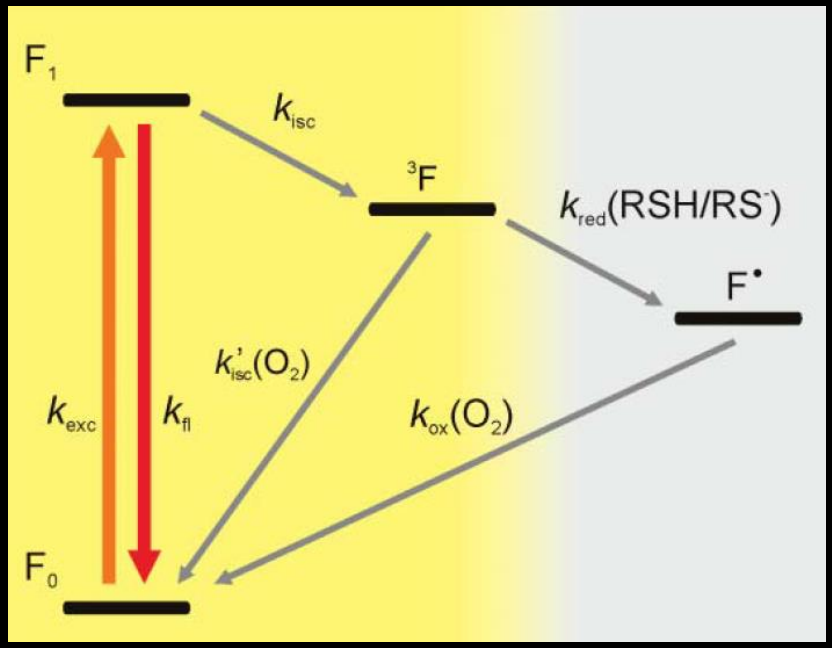
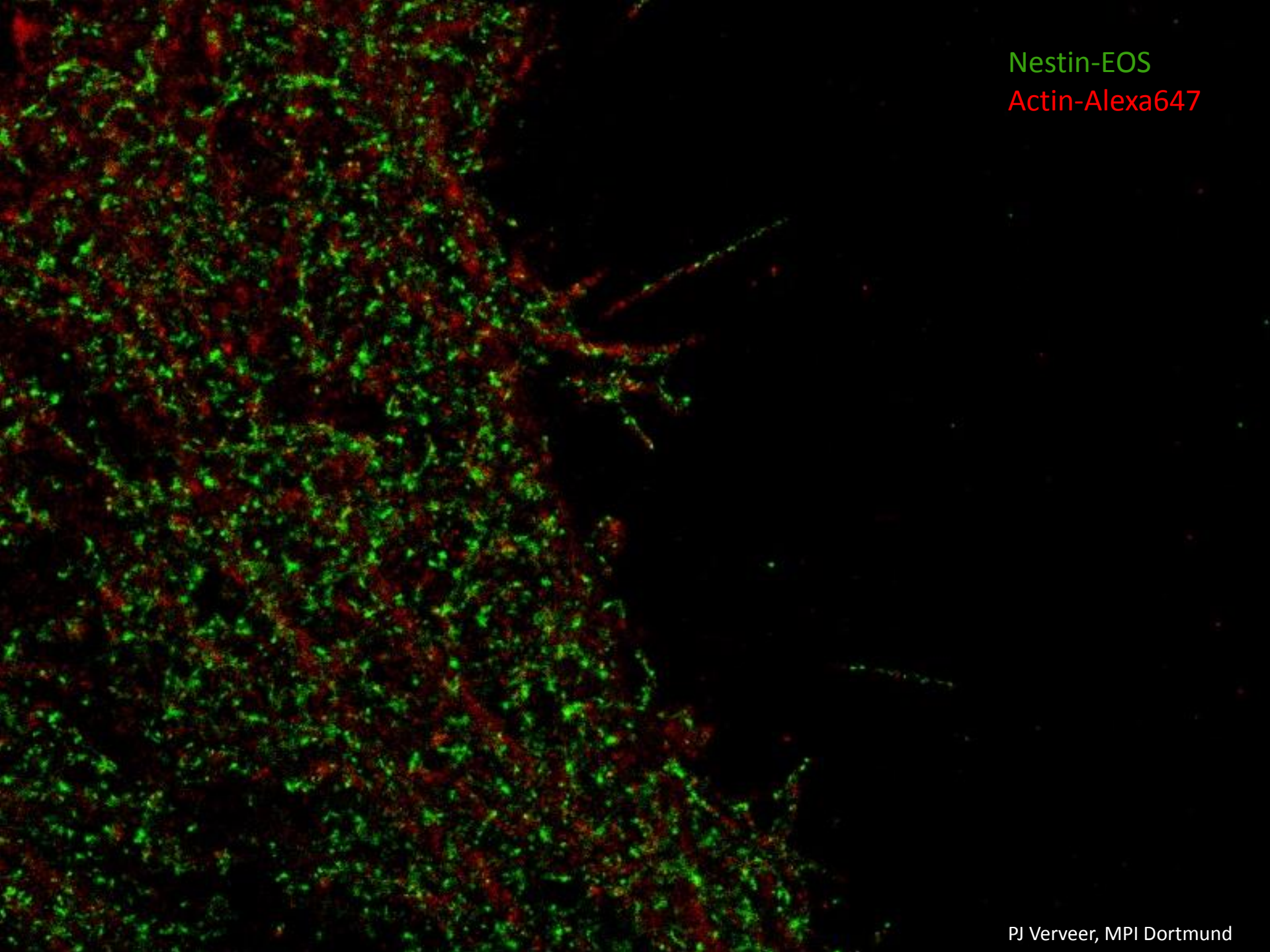
Operating conventional fluorophores as photoswitches



PBS



PBS + thiol



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