Einzelmolekülstudien

Motivation

Ensemble-Messung:
- Bestimmung eines Mittelwertes des Ensembles (z.B. gemittelte Struktur von 10^{13} Protein-molekülen in einem Proteinkristall)
- gute Statistik: verlässlicher Mittelwert

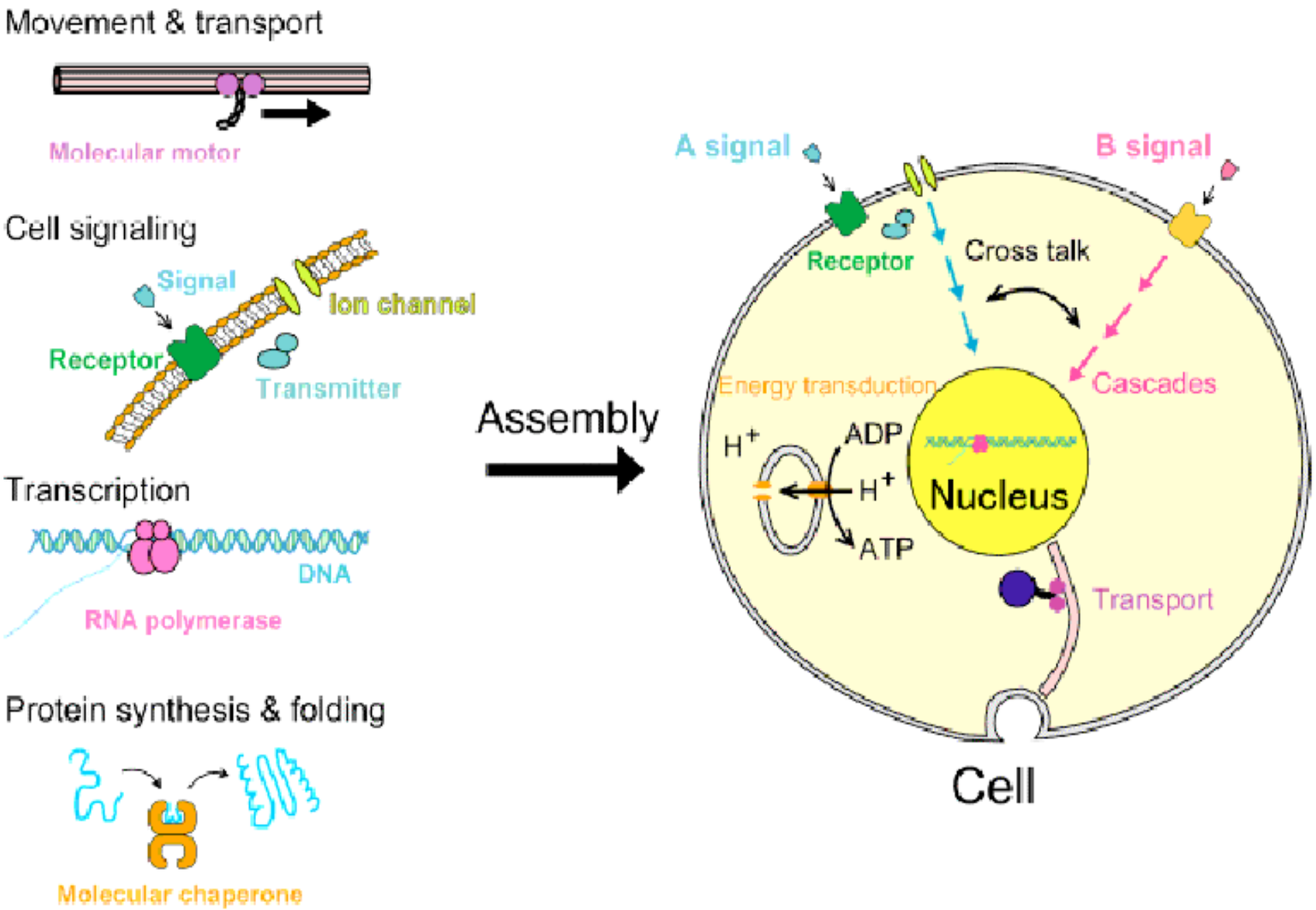
Einzelmolekülmessung:
- Bestimmung eines Messwertes der die Eigenschaft eines individuellen Moleküls widerspiegelt
- Mehrfachmessung geben weitere Information

Neue Anwendungen

statische Messungen
- Anwendung auf heterogene Proben,
- Bestimmung von Subpopulationen,Verteilungen

zeitaufgelöste Messungen
- in Proben ohne Synchronisation oder mehr Information bei „schlechter“ Synchronisation
- Beispiele: Motorproteine, Proteinfaltung, etc.

SM in der Zelle

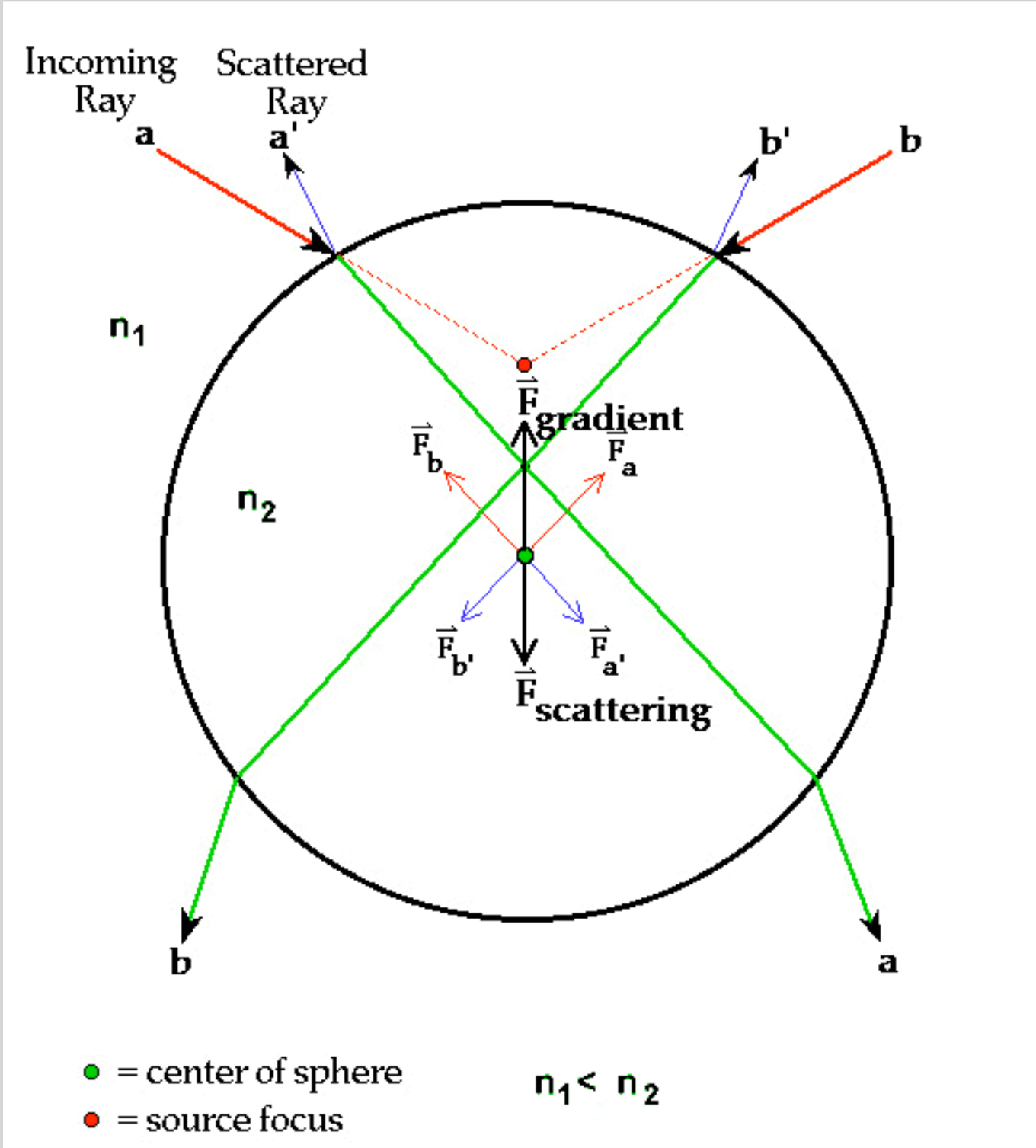


SM - Techniken

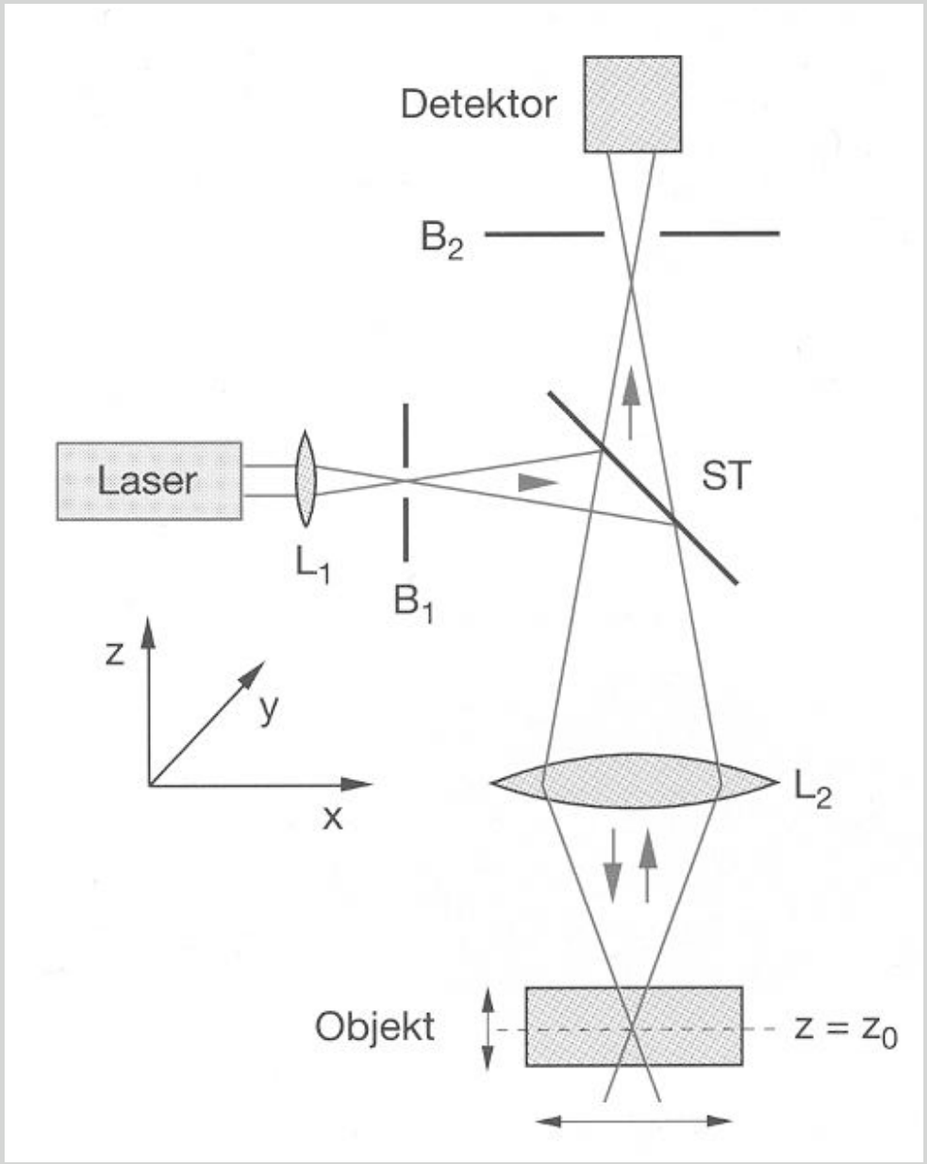
- **Optical Tweezers**
- Patch Clamp
- **Fluorescence**
- Atomic Force Microscopy (AFM)

Optical Tweezer - Prinzip

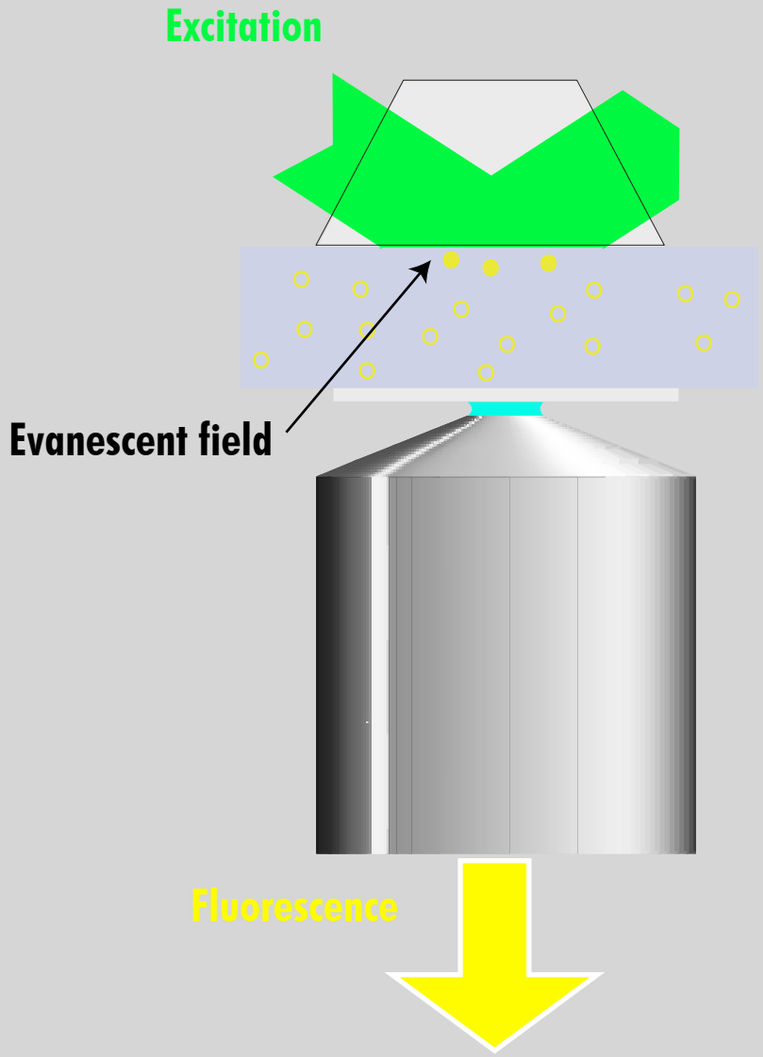
- Zwei Kräfte:
  - Gradientenkraft (Brechung)
  - Streukraft
- Erlaubt Manipulation und Kraftmessung im pN-Bereich



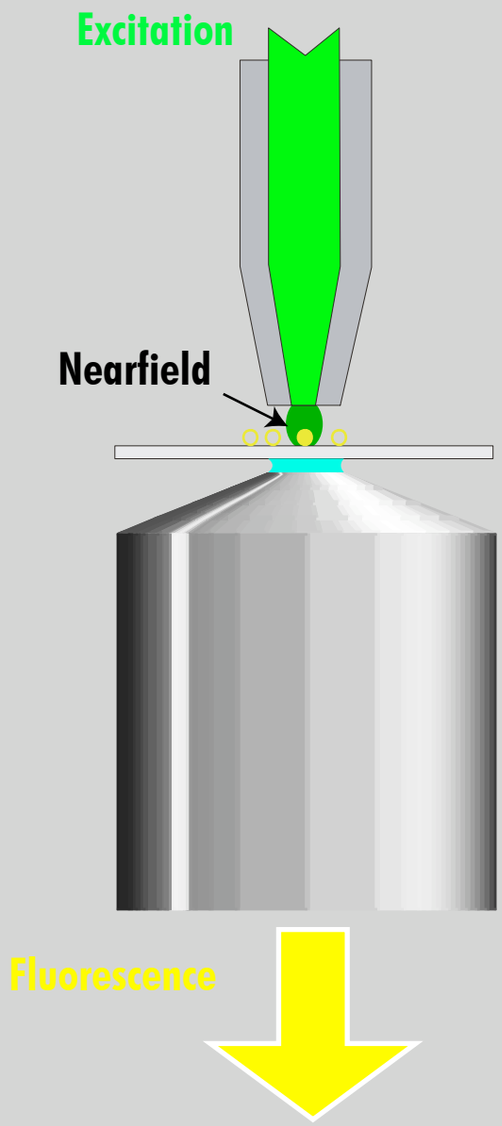
SM-Fluoreszenz Techniken



Konfokal Scanning

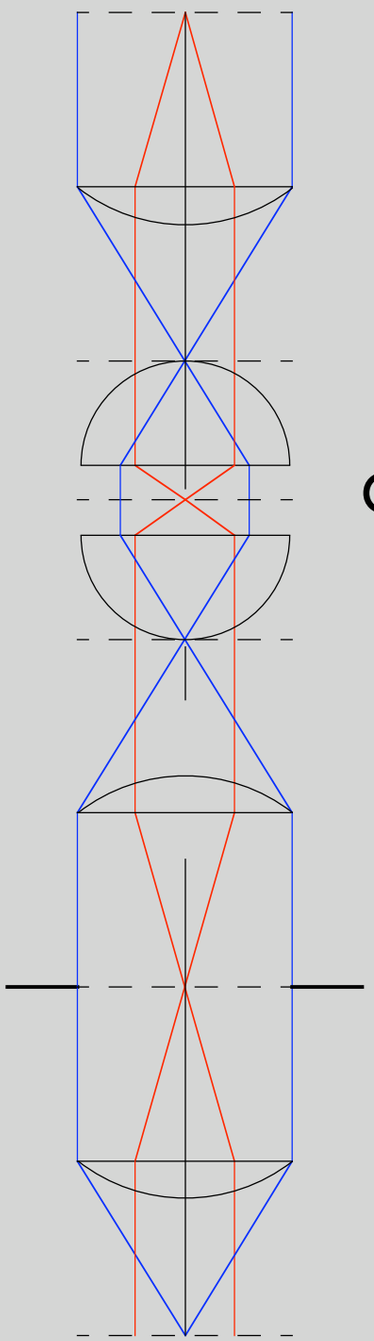


Innere Totalreflektion Imaging

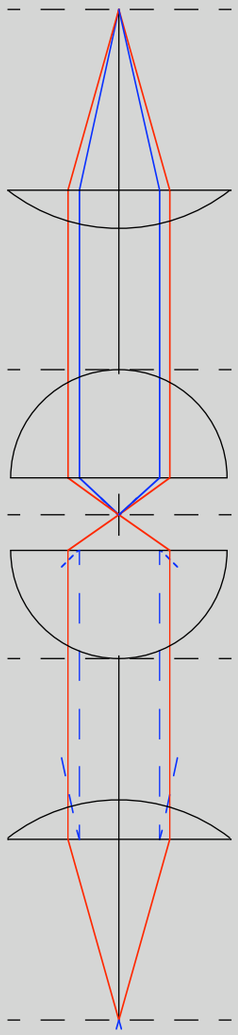


Nahfeld Scanning

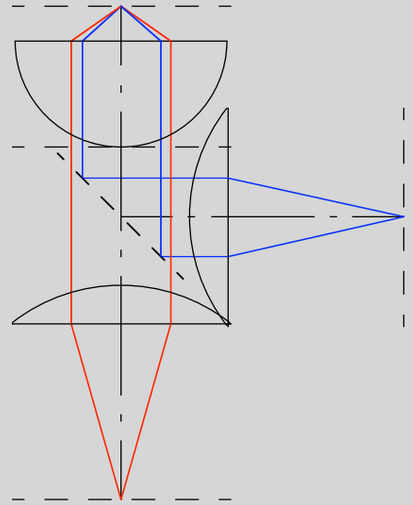
Konfokalmikroskopie



Konventionelles Mikroskop



Transmissions-Konfokalmikroskop



Reflexions-Konfokalmikroskop

Anwendung: Motorproteine

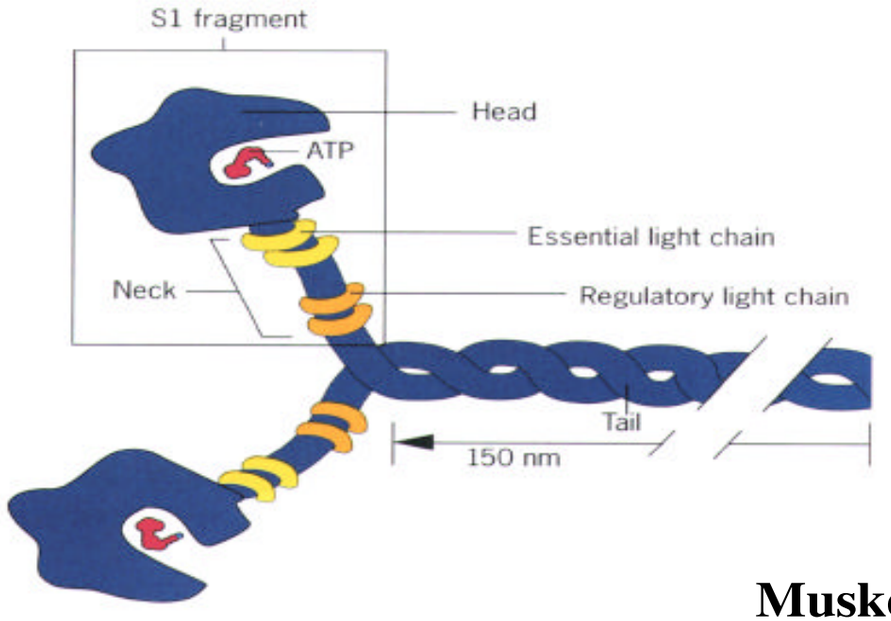
Myosin	Aktinfilament
Kinesin	Mikrotubuli
Dynein	Mikrotubuli

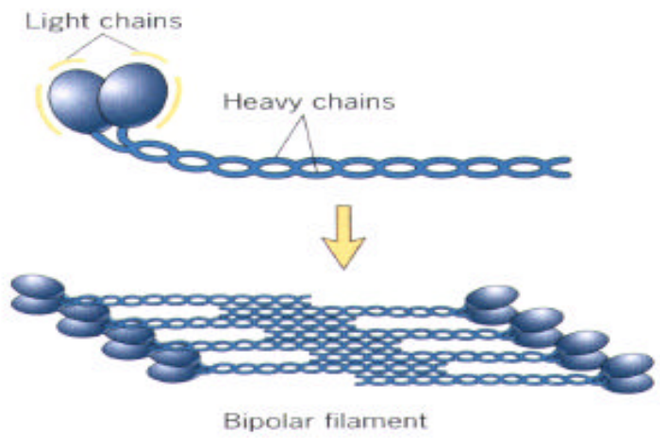
Weitere Motorproteine:

- ATPasen
- Polymerasen
- Helikasen

- gerichtete Translokation entlang eines Transportweges (Filamente)
- Umwandlung chemischer Energie (ATP- Hydrolyse) in mechanische Energie
- Geschwindigkeiten: ~ 0.02 - 8 $\mu m s^{-1}$
- Kräfte: einige Pikonewton
- Aufgaben in der Zelle: z.B. Muskelkontraktion, Transport (Vesikel, Organellen)
  Zellbewegung, Spindel (Zellteilung)

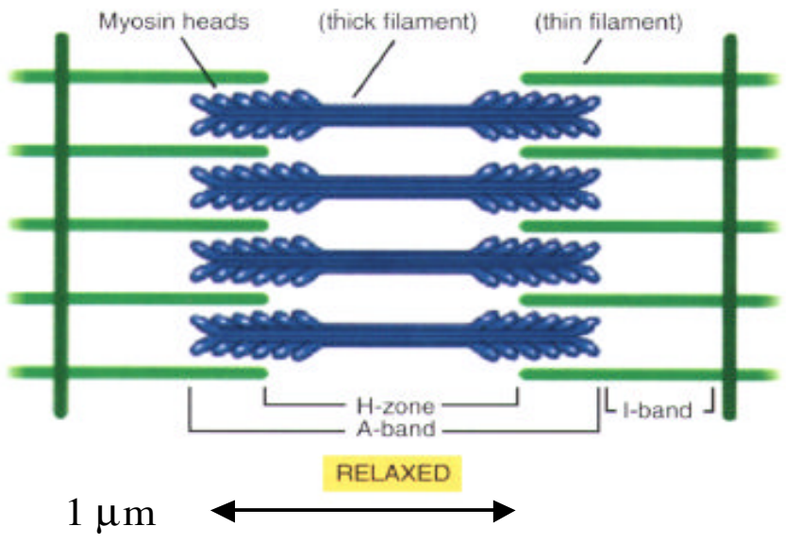
Myosin

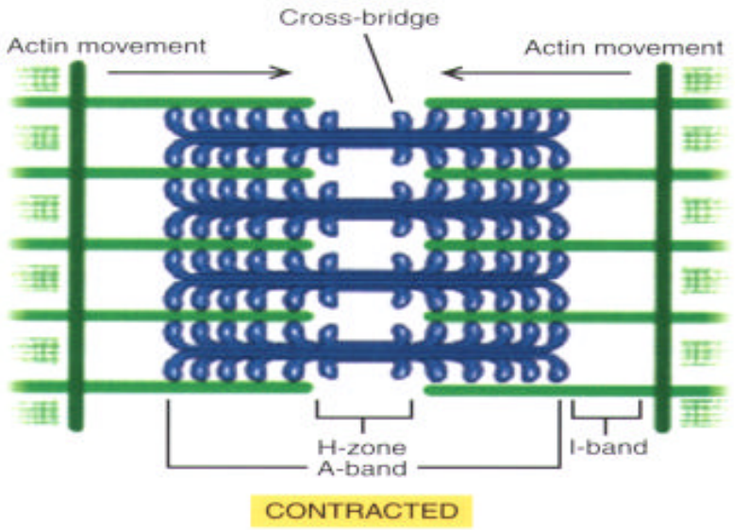




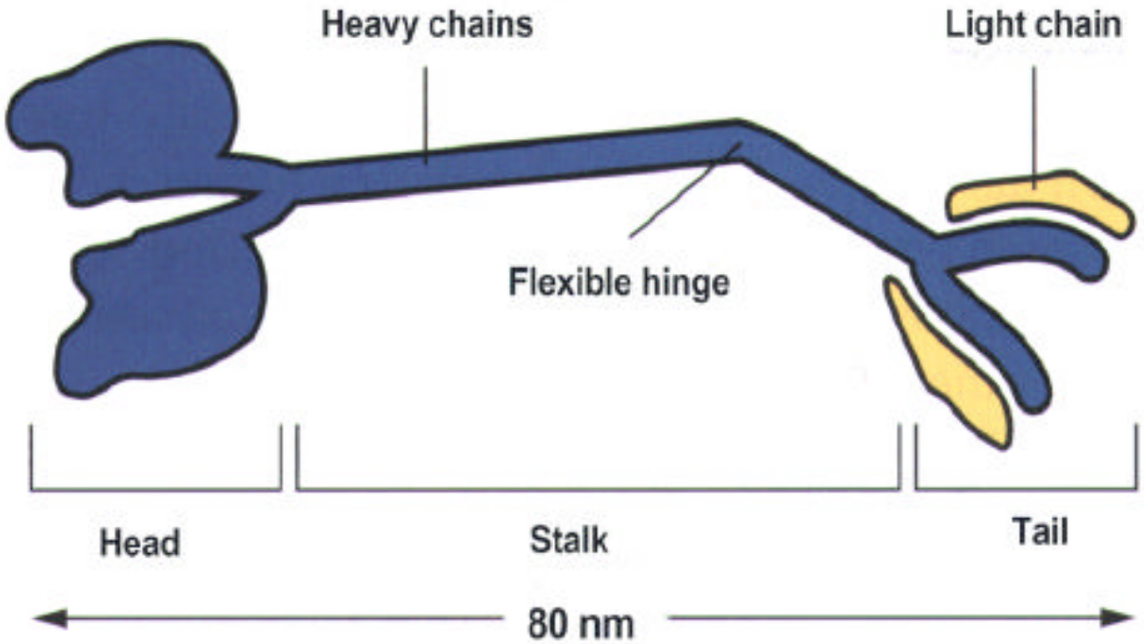
Polarität

Muskelkontraktion

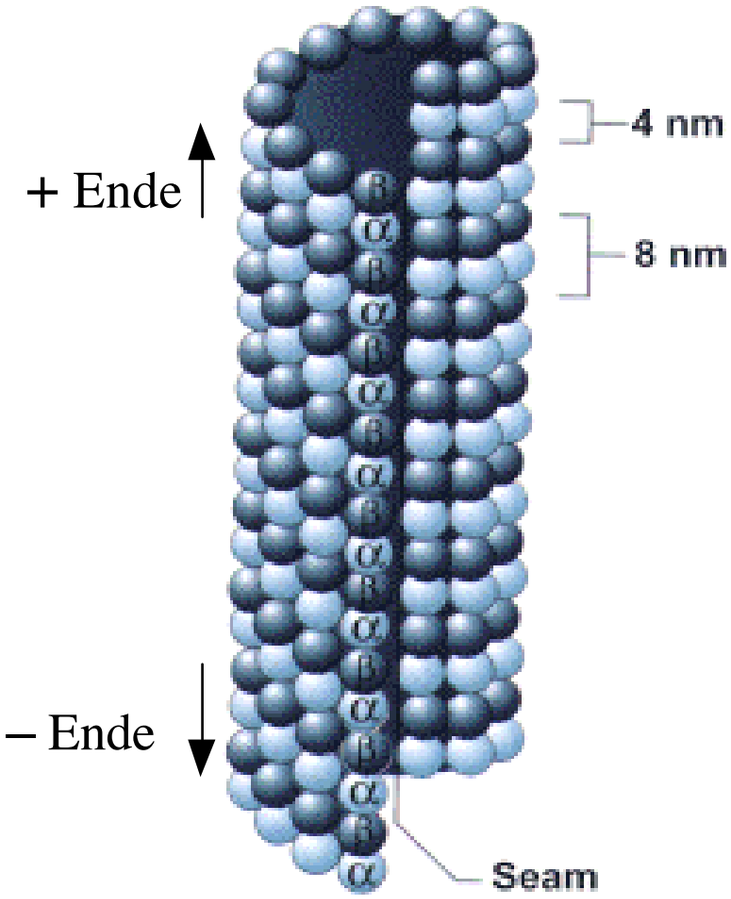




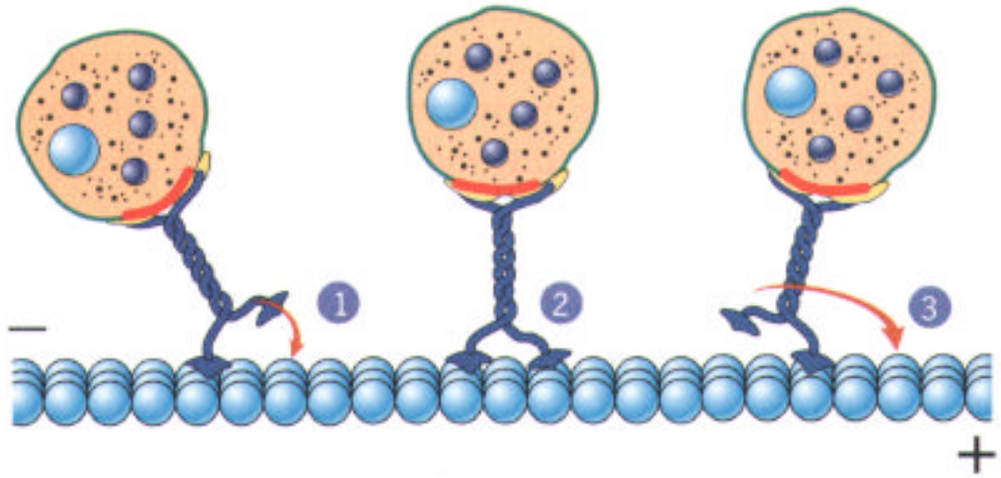
Kinesin



Mikrotubulus

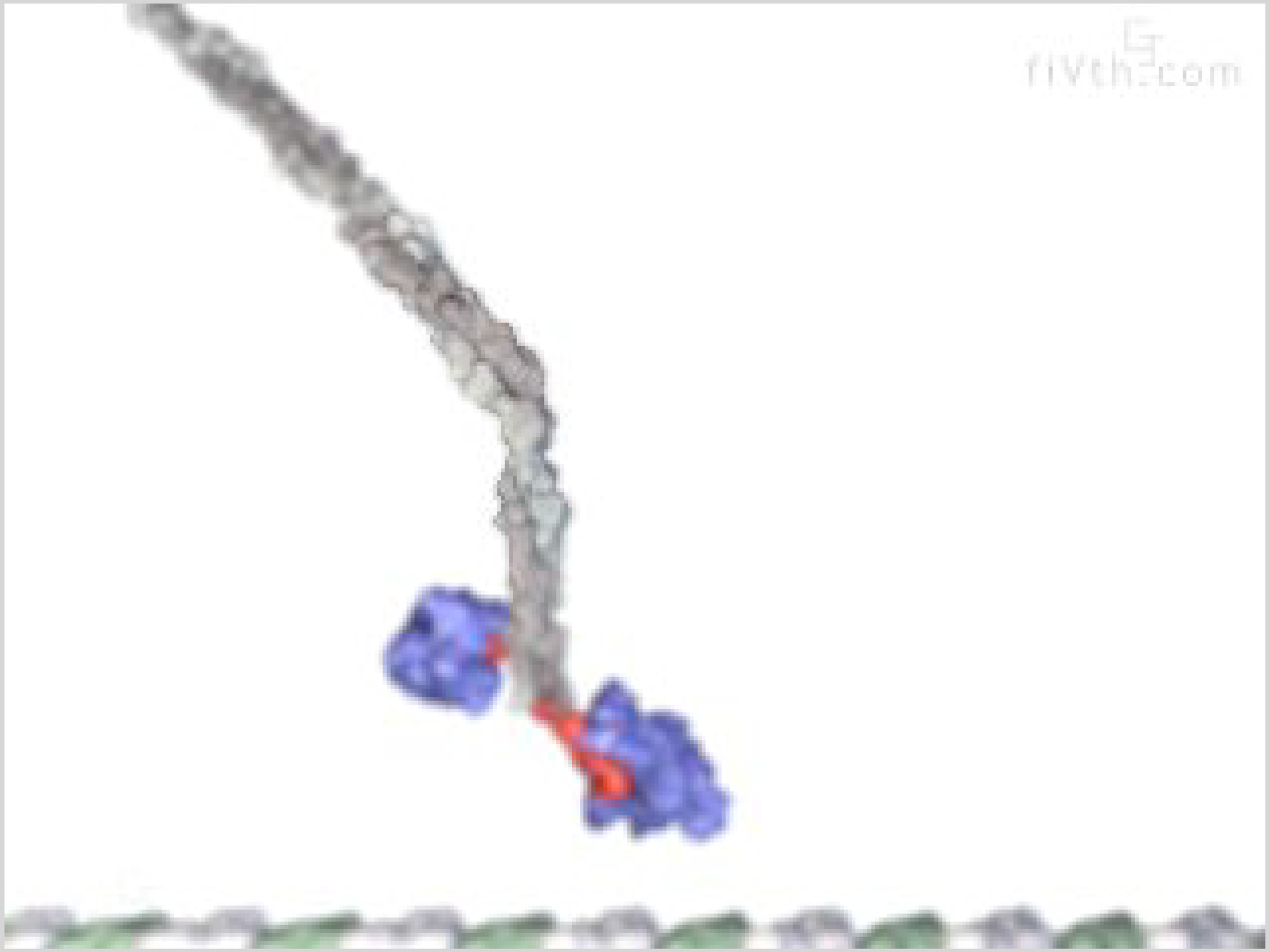


Vesikeltransport



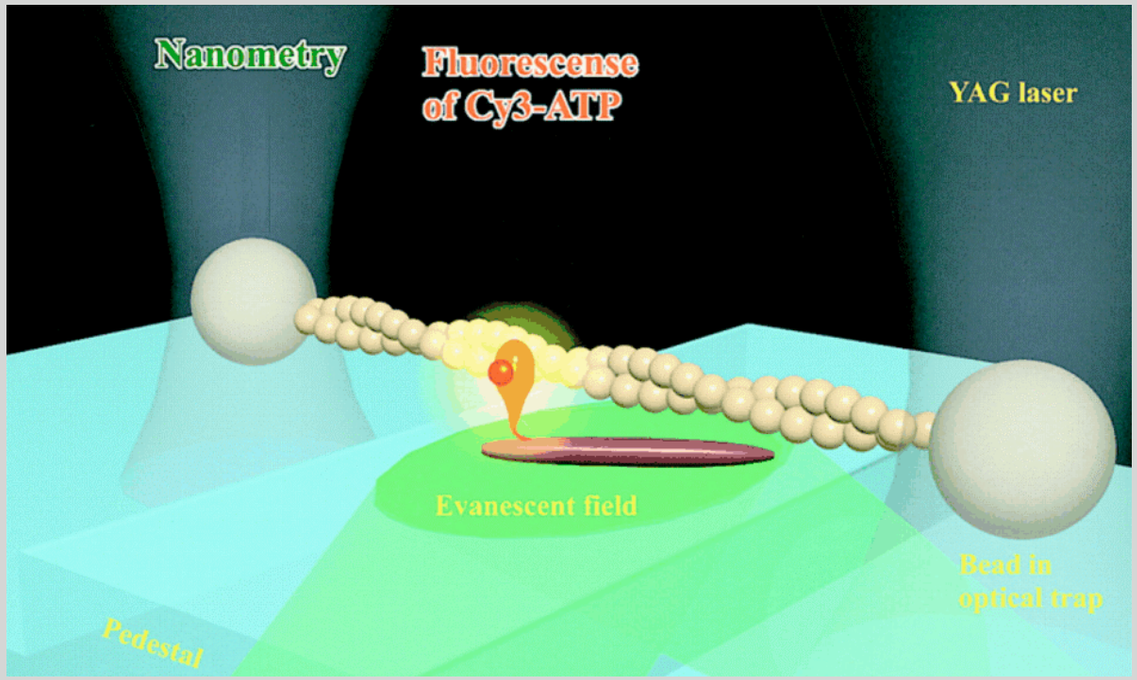
Hand-over-Hand Modell

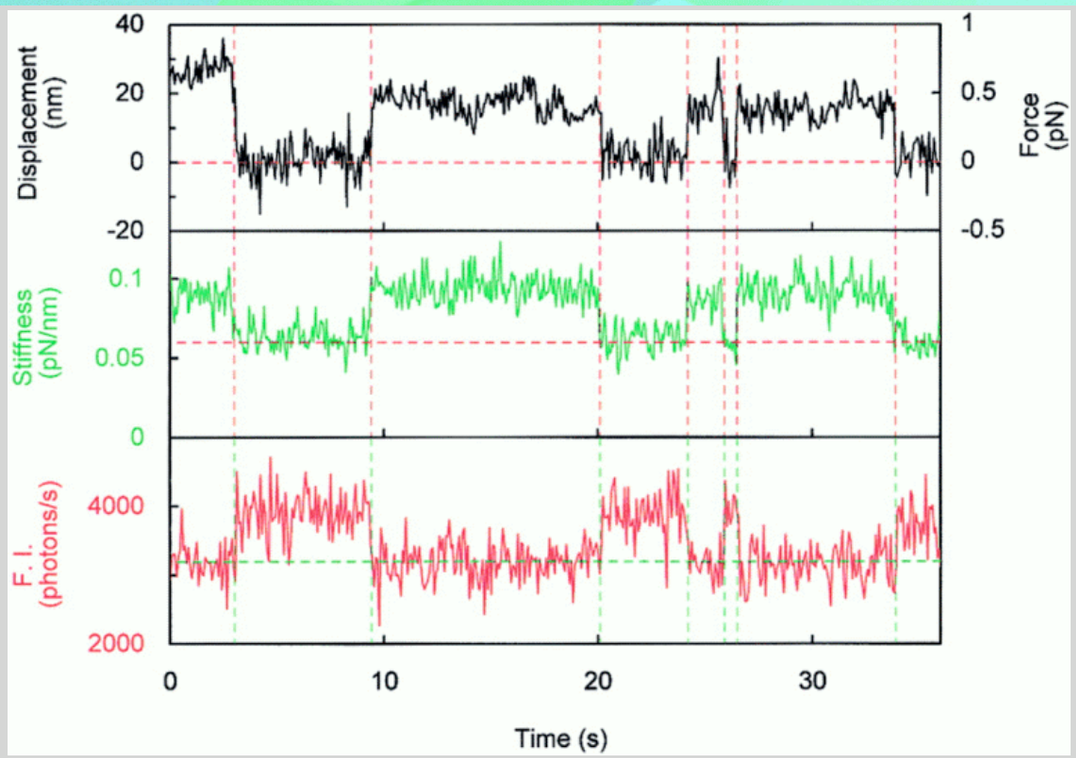
Kinesin



Beobachtung mit Tweezer + Fl.

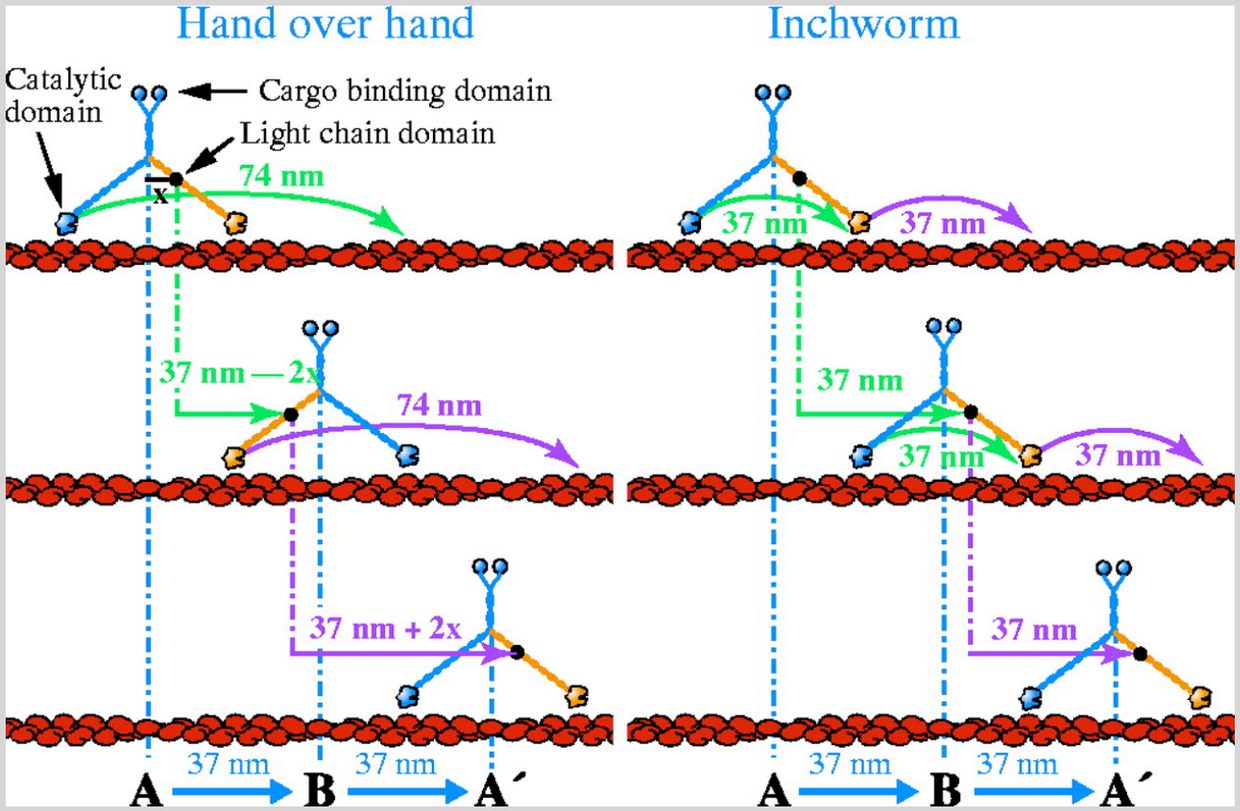
- Motor auf Deckglas immobilisiert
- Aktinfilament mit Beads an den Enden
- ATP-Bindung mit fluoreszenzmarkiertem ATP verfolgt

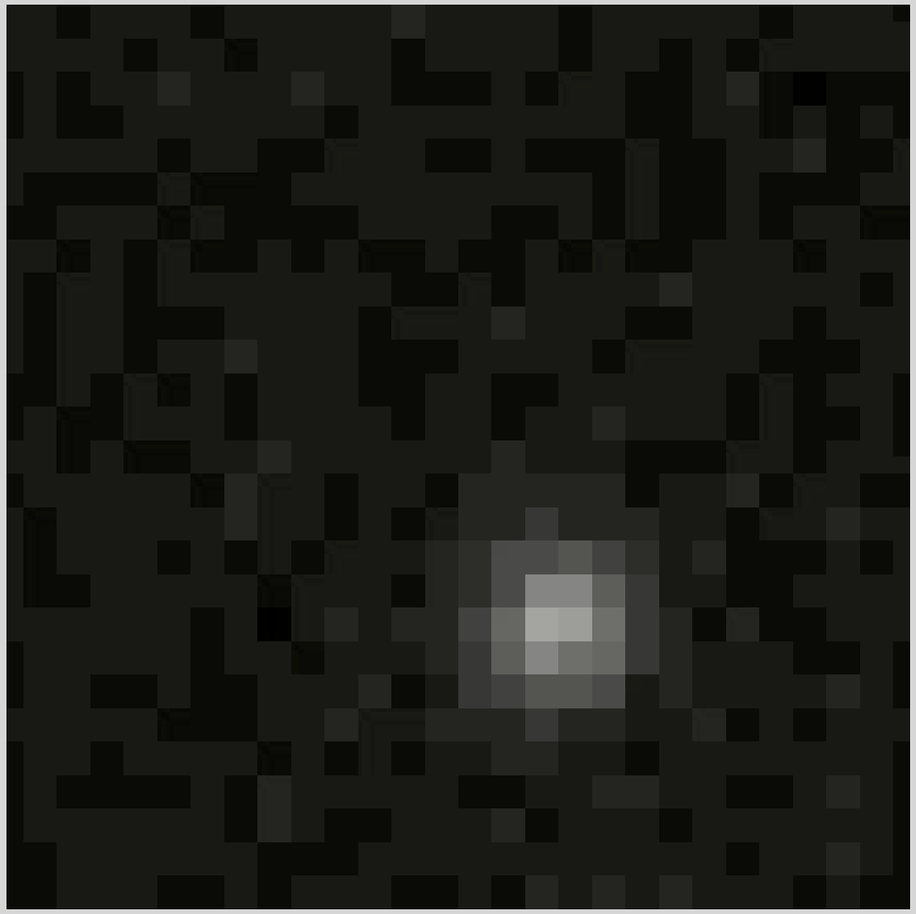




Ishijima et al. Cell, 1998

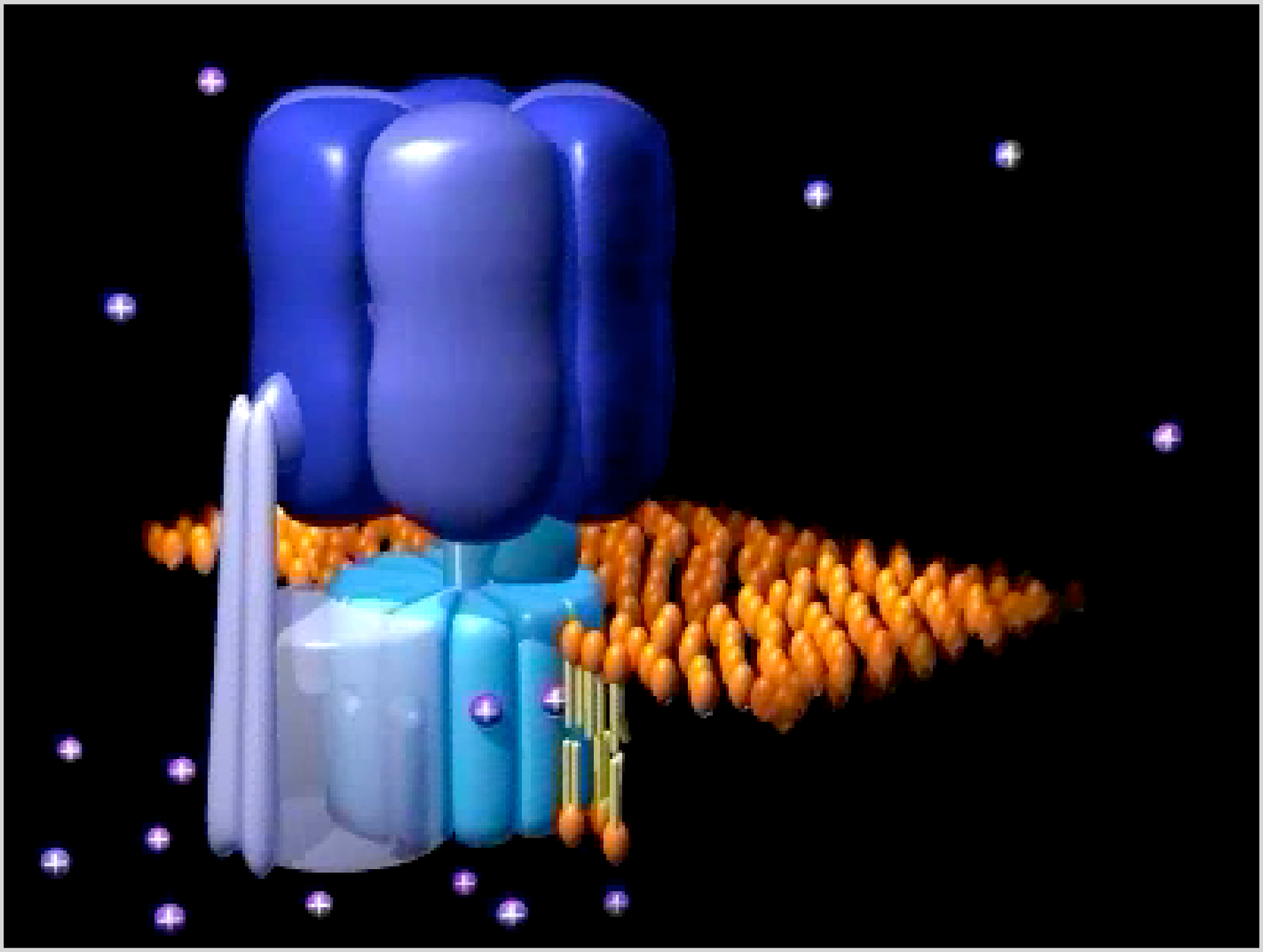
Beobachtung mit Fluoreszenz



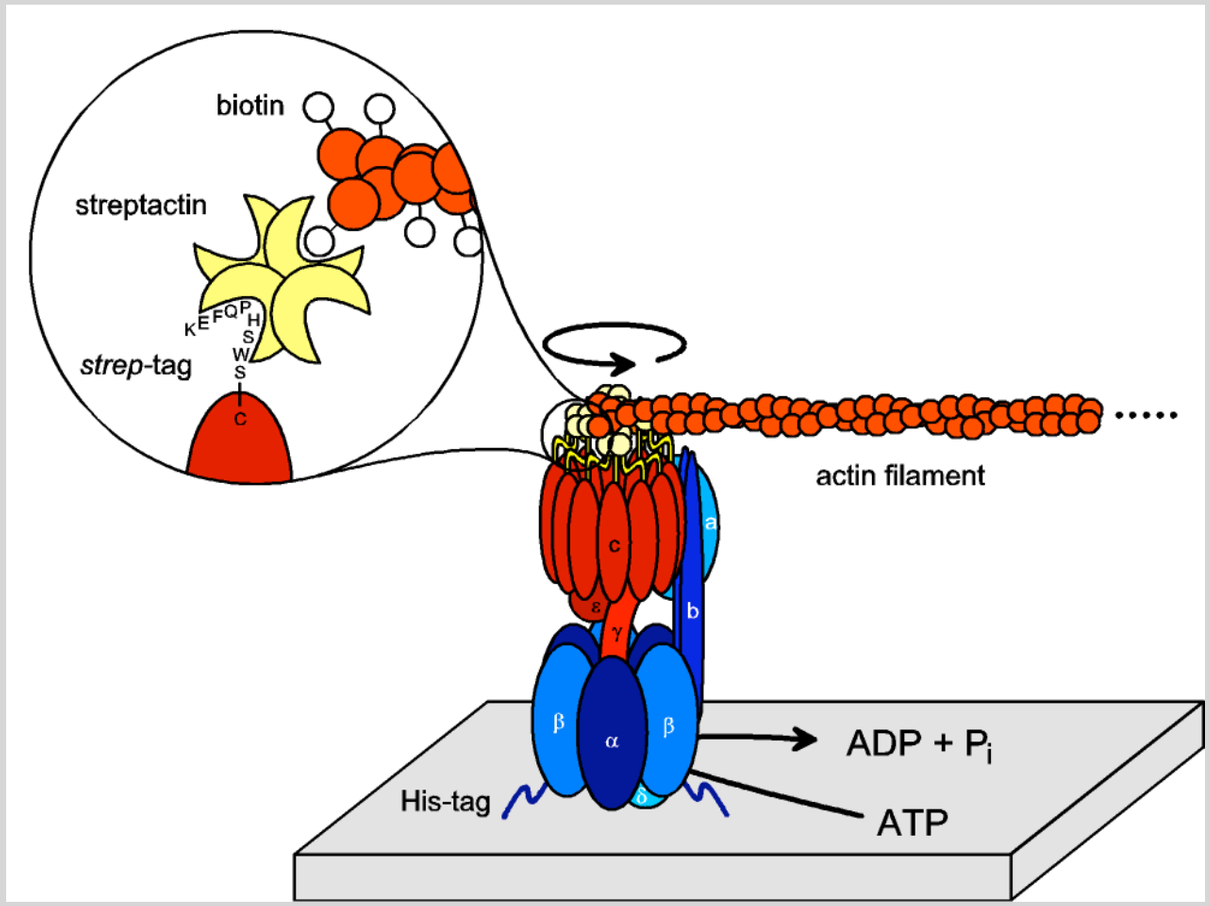


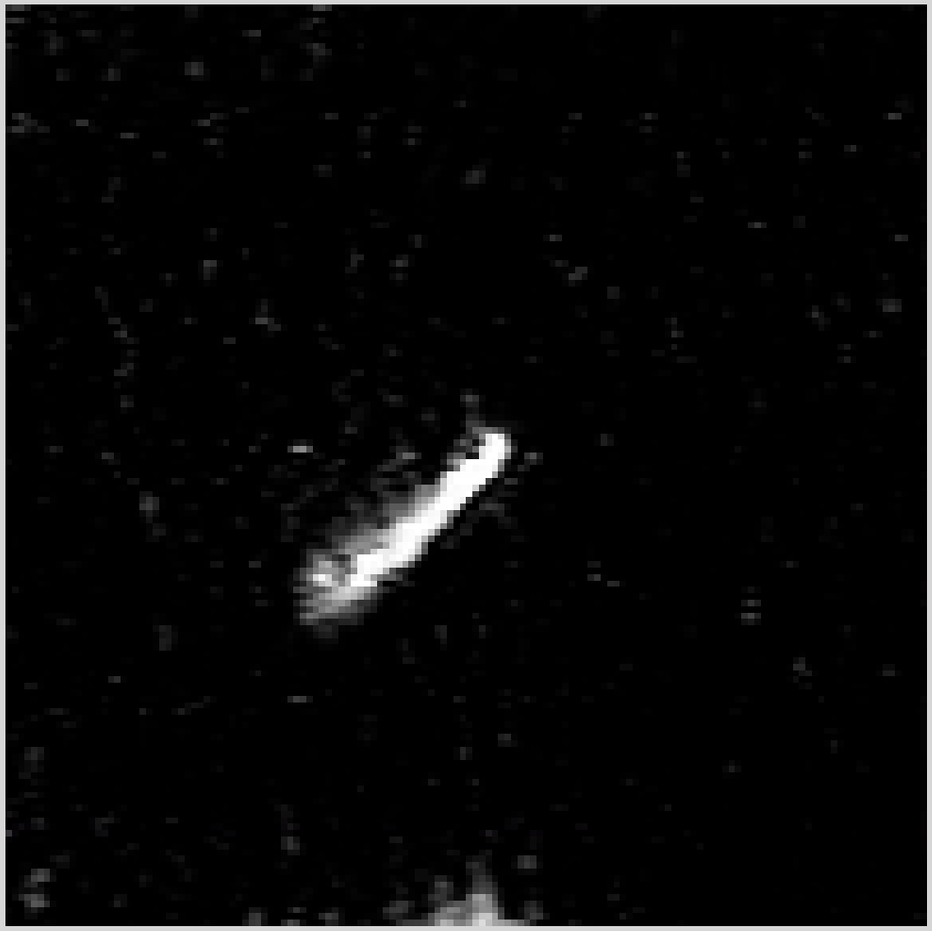
- Hand-Over-Hand: Verschieden weite Schritte
- Inchworm: Gleich weite Schritte
- Auflösung: 230 nm, Positioniergenauigkeit: 2 nm

Motor: F_0F_1 ATPase

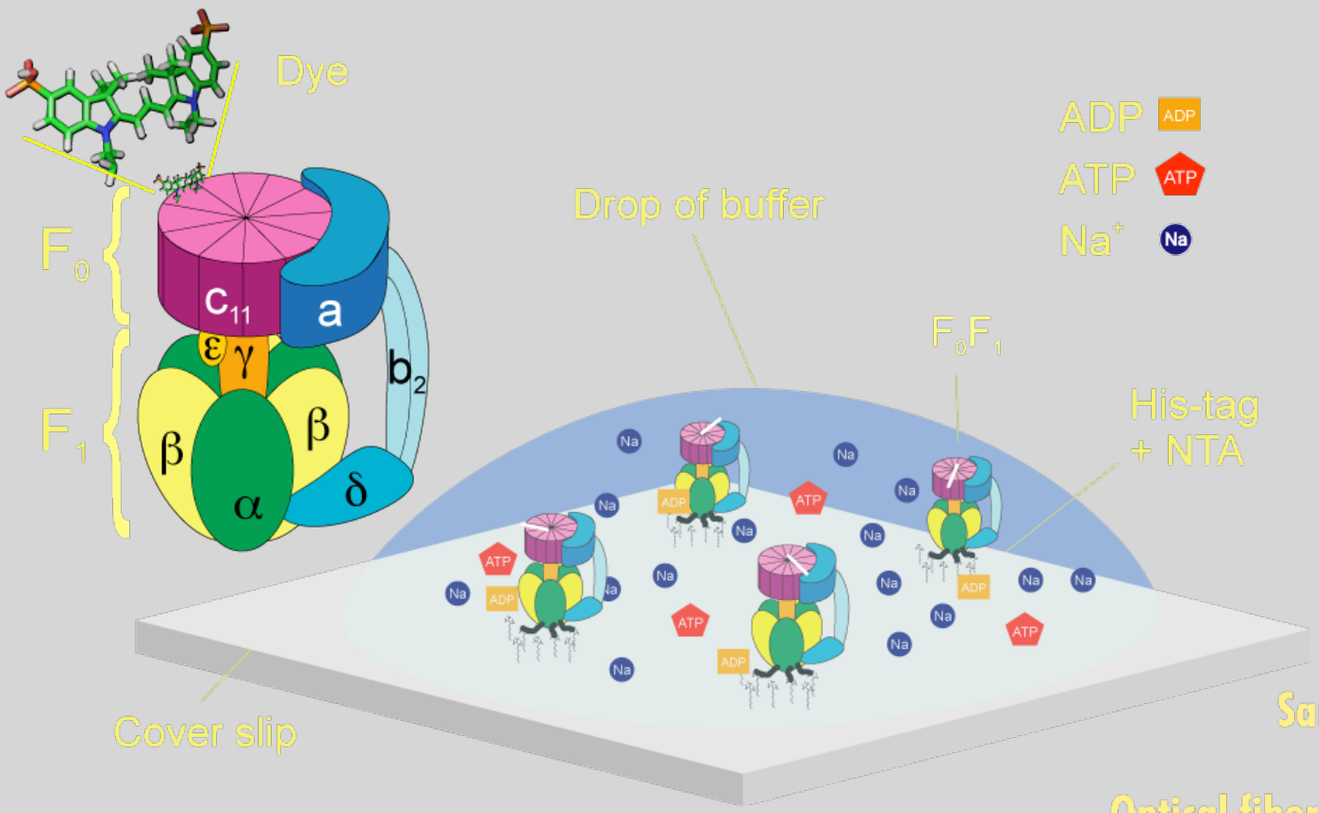


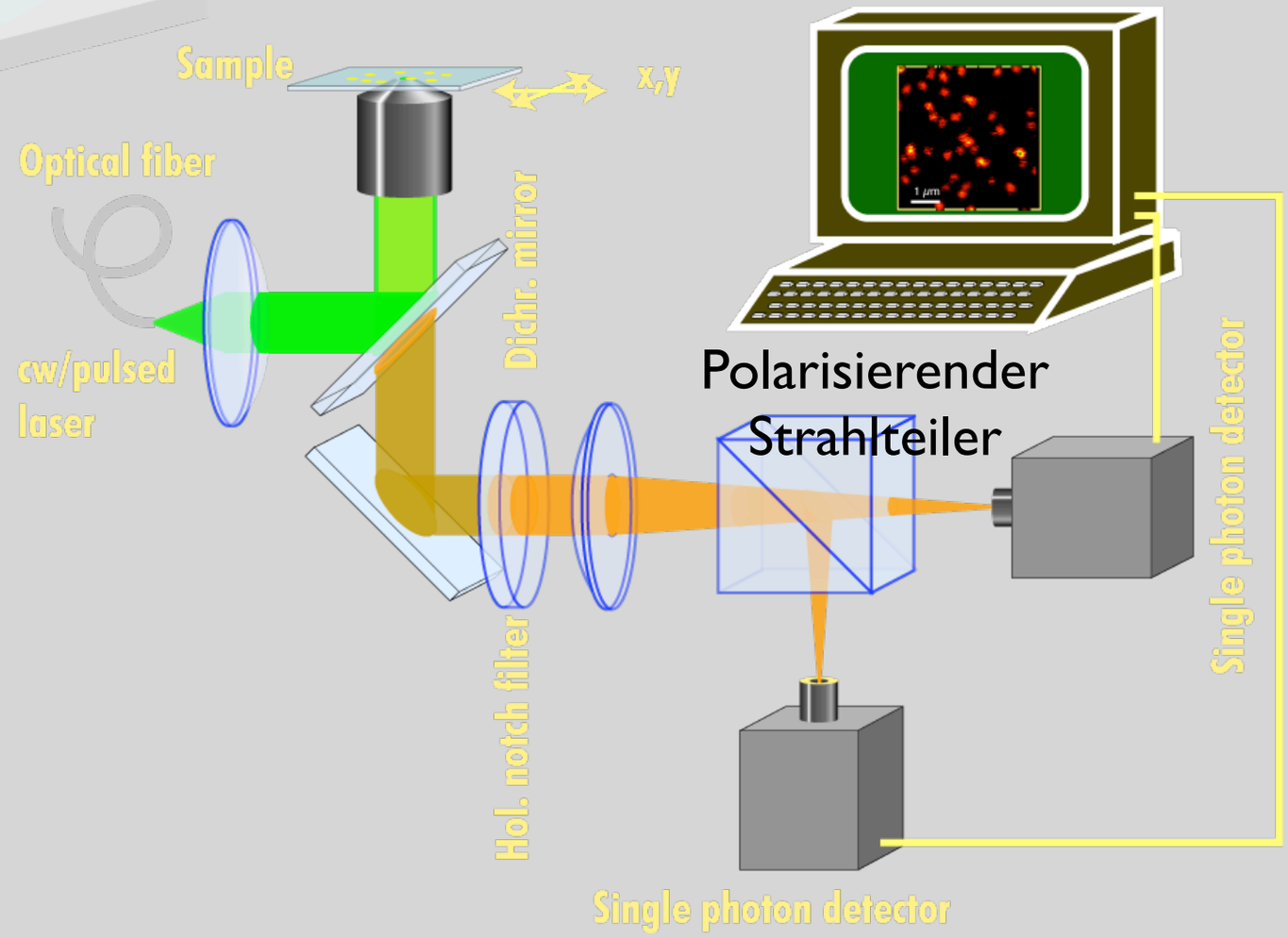
Fluoreszenz - Videomikroskopie





Polarisationsaufgelöste Detektion



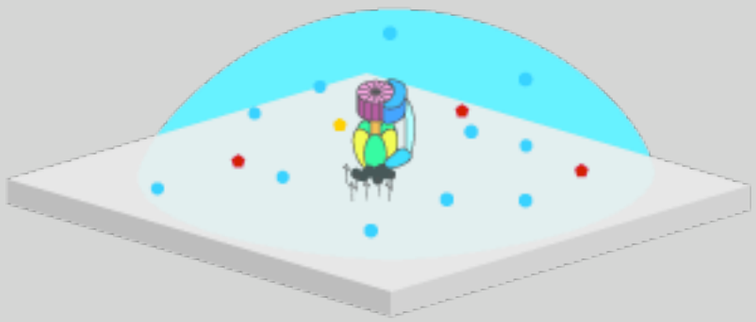


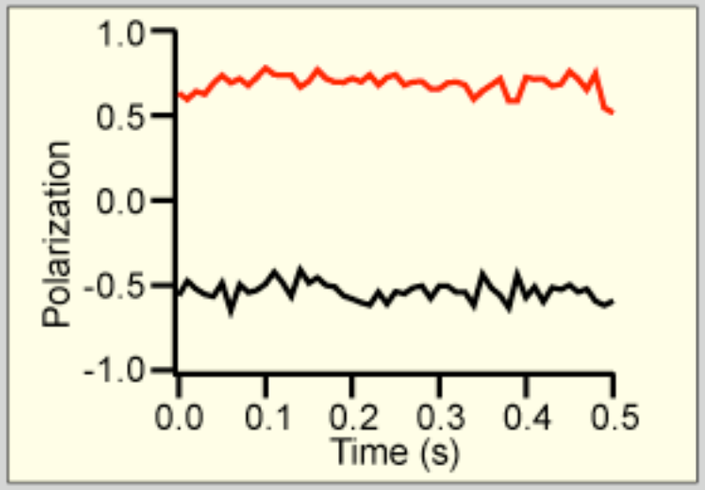
Rotation bei der ATP Hydrolyse

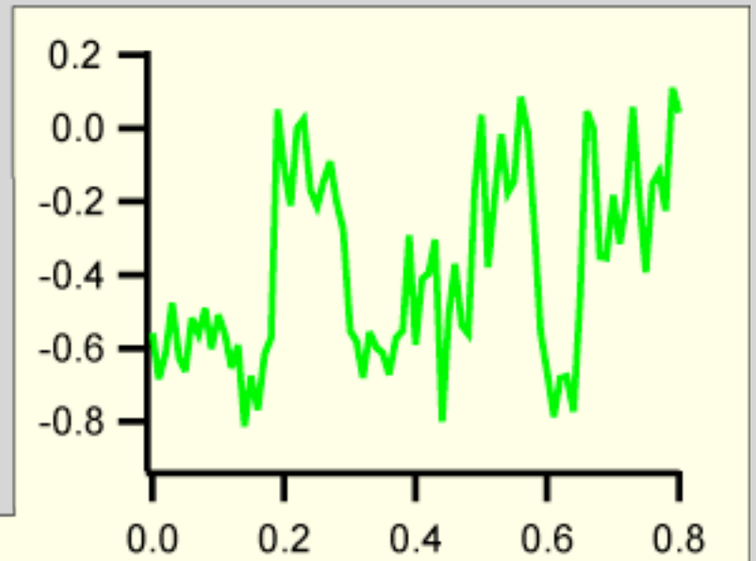
Polarization: $P = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + I_{\perp}}$

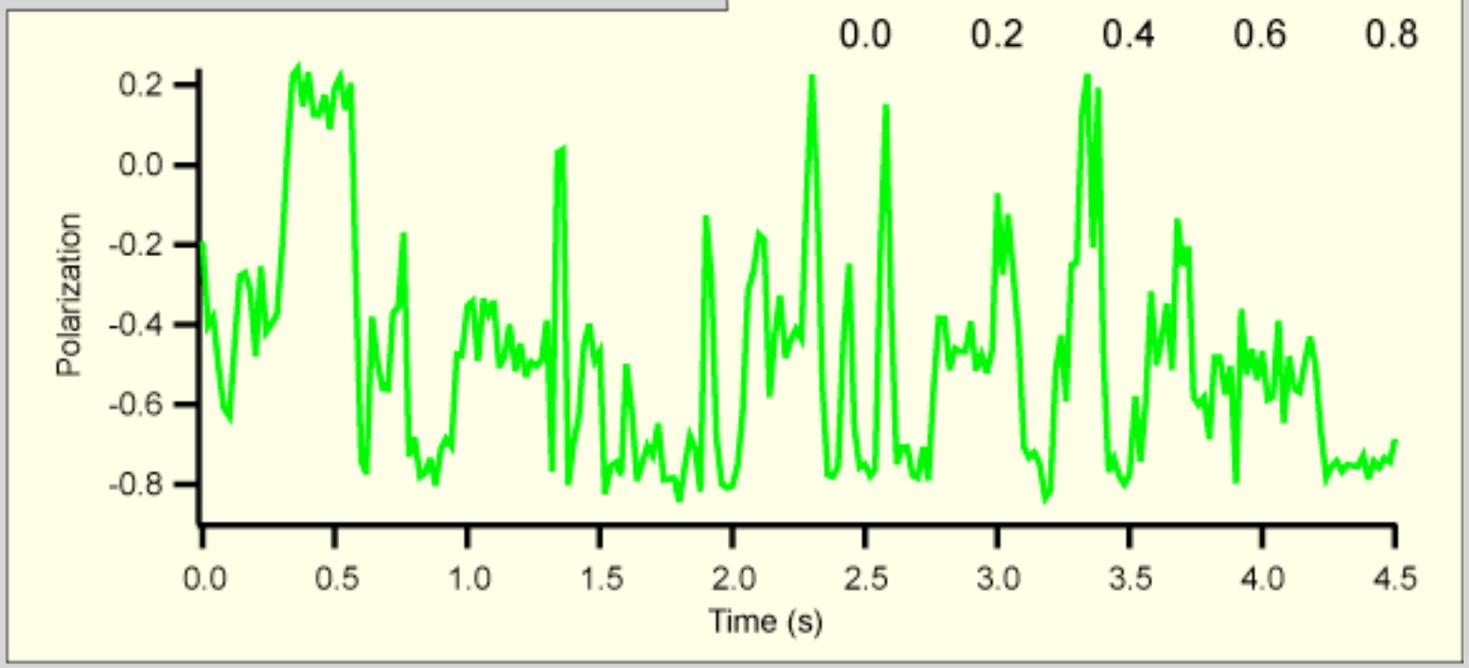
No Na^+
low ATP (~0.5µM)

High Na^+ (2mM)
low ATP (~0.5µM)



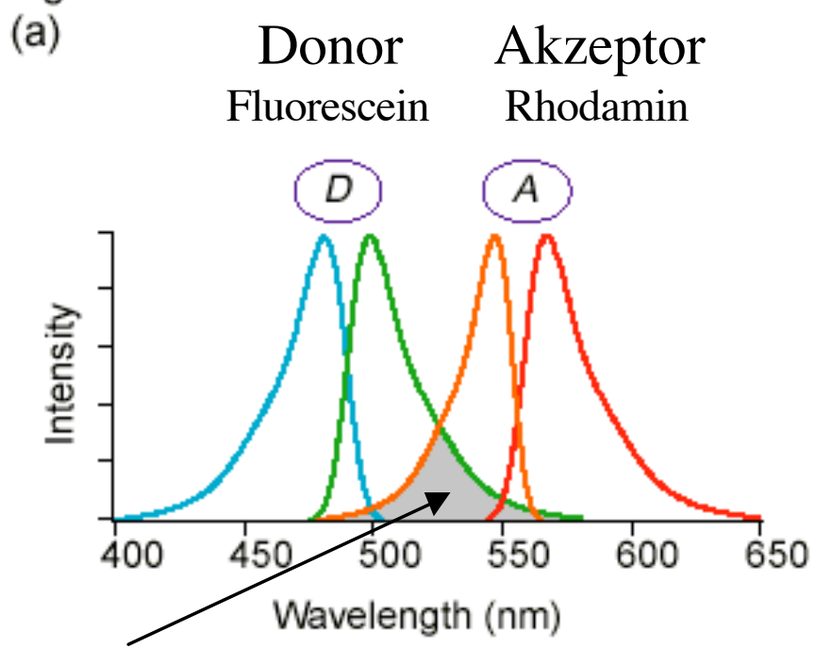




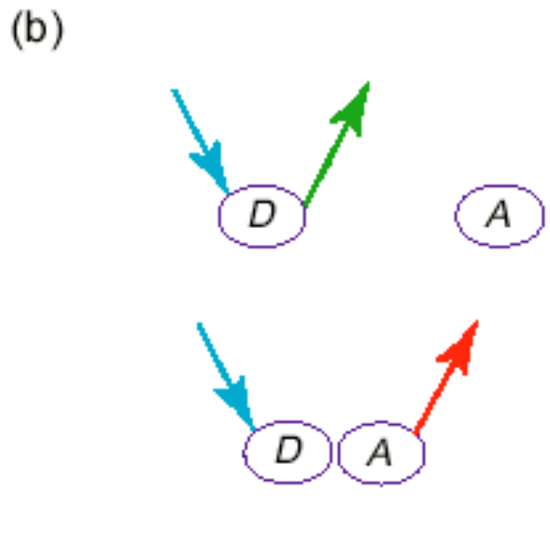


Einzelmolekül - FRET

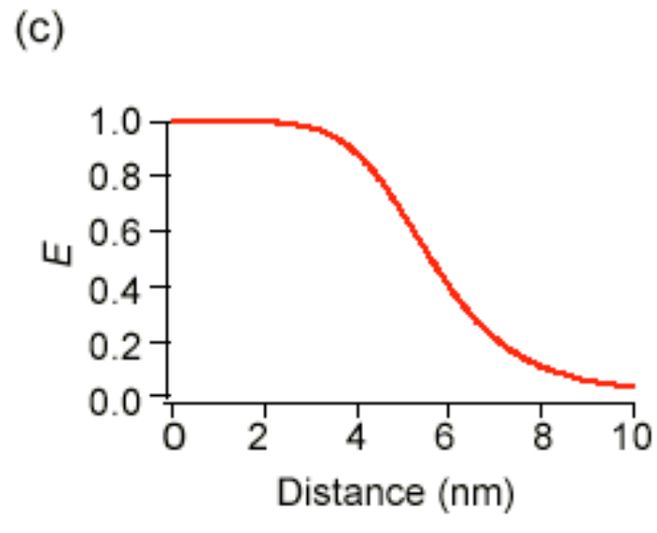
Fig. III



Energieüberlapp

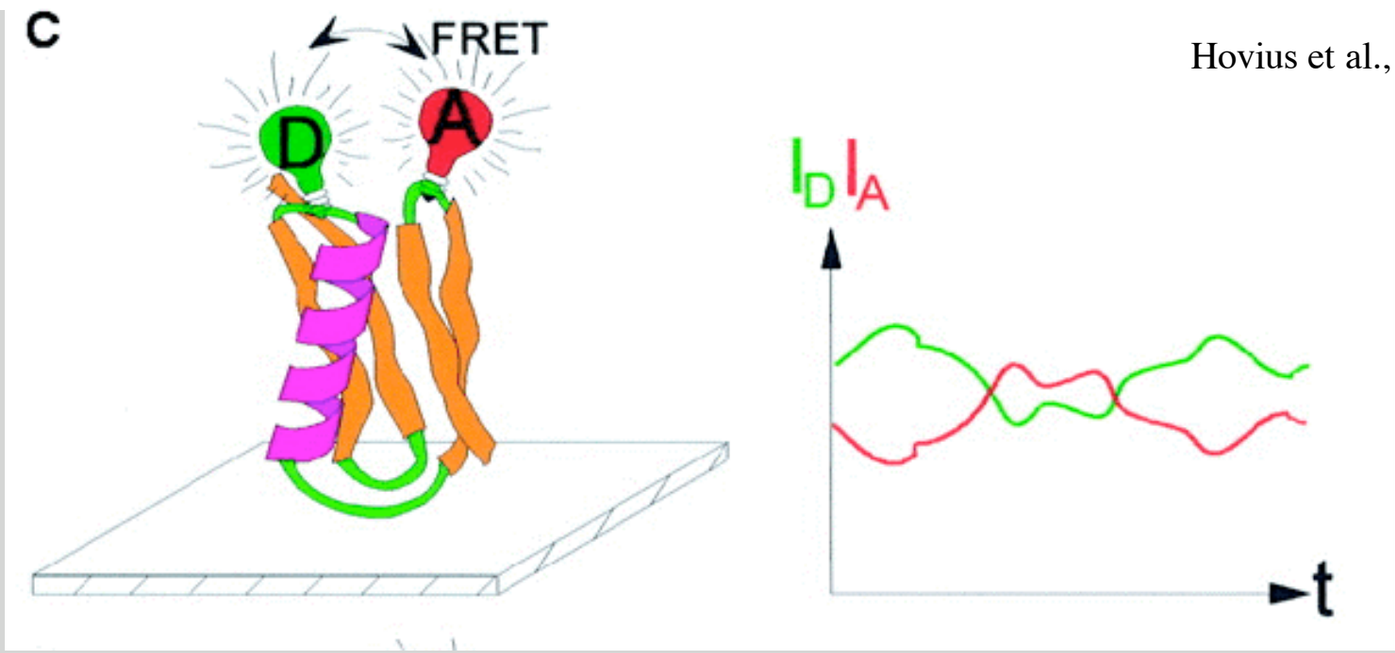


$$E = 1 - \frac{I_{DA}}{I_D} \quad [5]$$



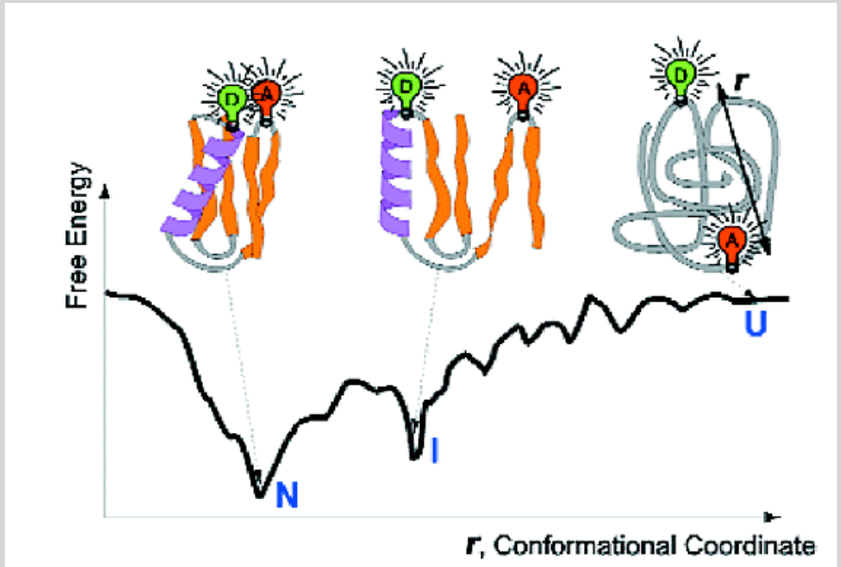
$$E = \frac{R_0^6}{R_0^6 + R^6} \quad [6]$$

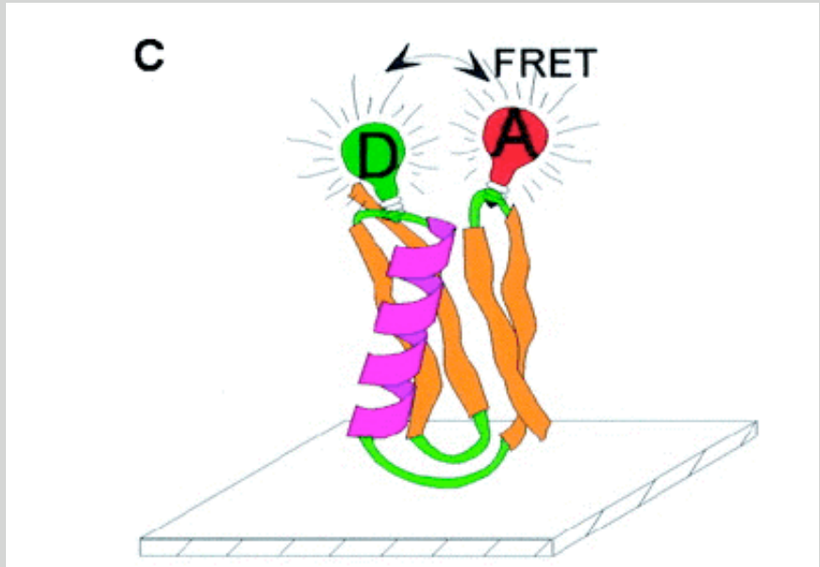
acological Sciences



Hovius et al., TIBS, 2000

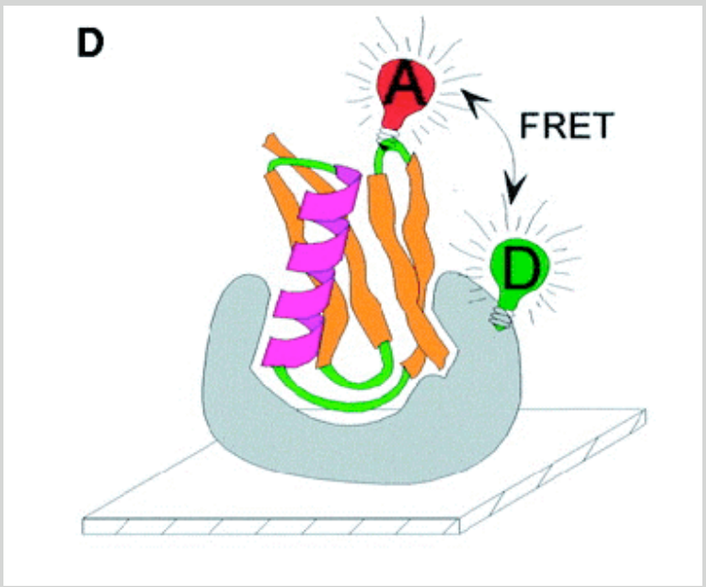
Anwendungen spFRET





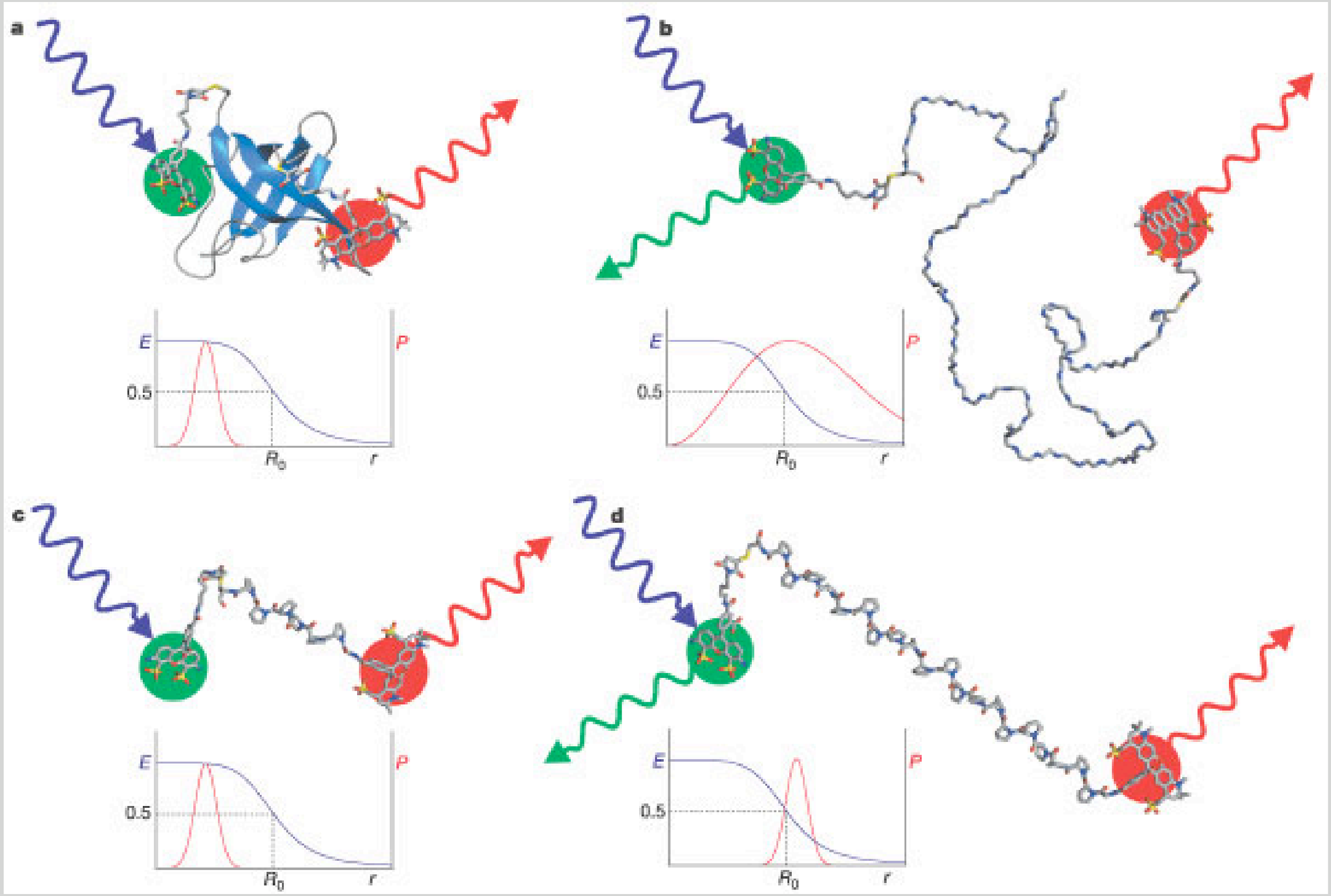
Proteinfaltung

Konformationsänderung



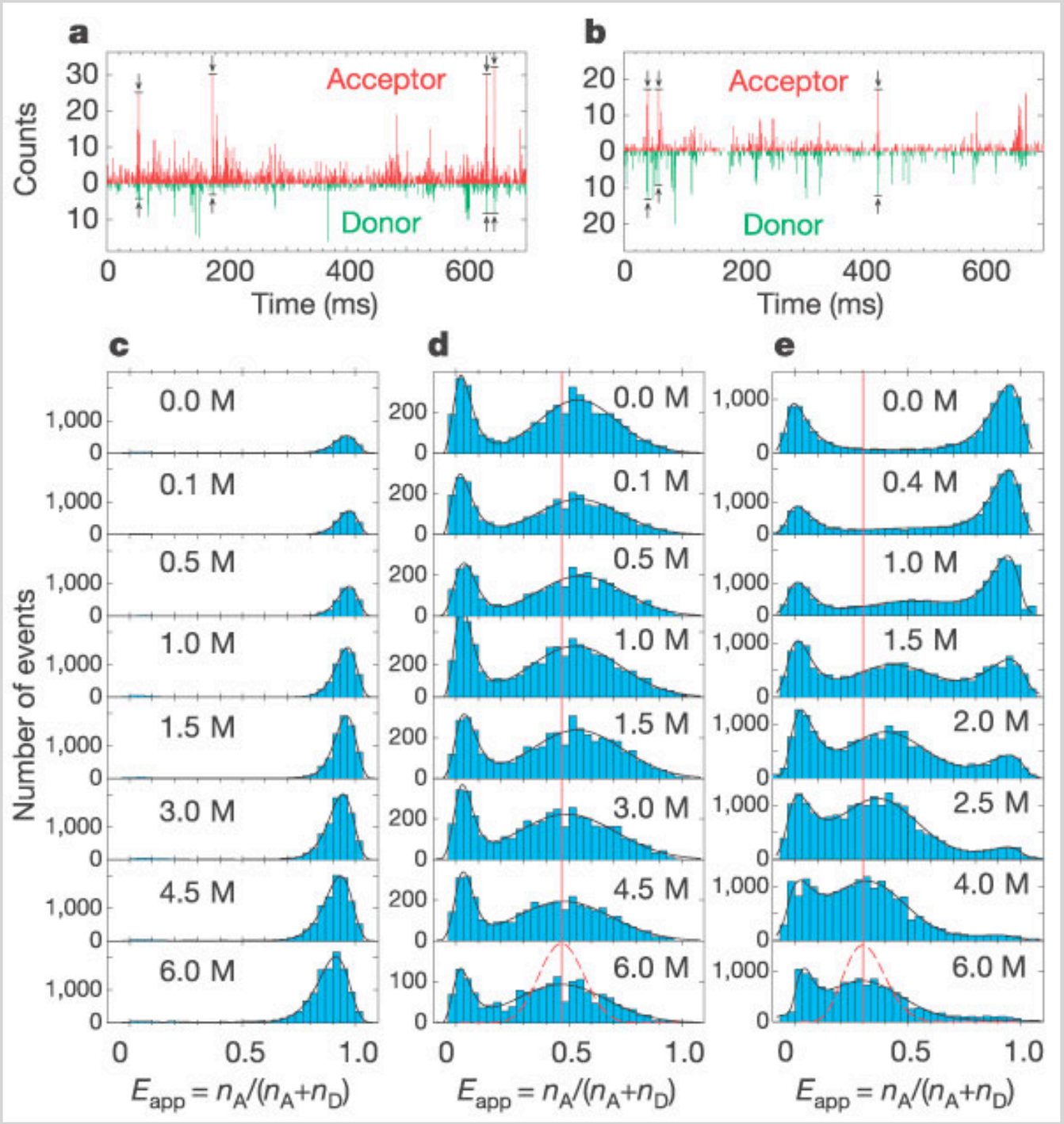
Protein-Protein/Substrat Wechselwirkung

Anwendung auf Proteinfaltung



Schuler *et al.*, *Nature* **419**, 743 - 747 (17 October 2002); doi:10.1038/nature01060

Anwendung auf Proteinfaltung

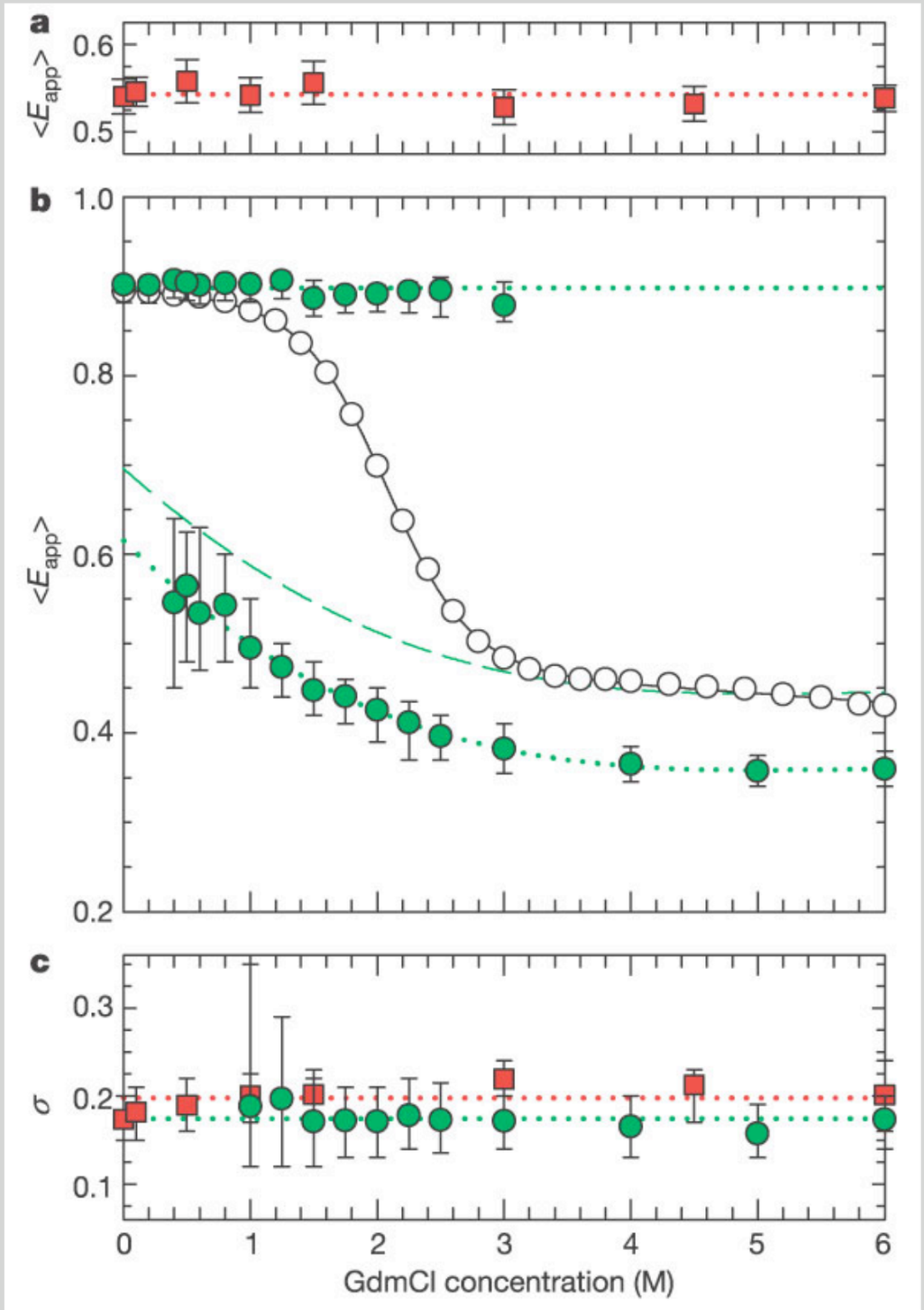


Zeitspuren

Effizienz - Histogramme

$(Pro)_6$ $(Pro)_{20}$ Csp*Tm*

Anwendung auf Proteinfaltung



$(Pro)_{20}$

Csp*Tm*