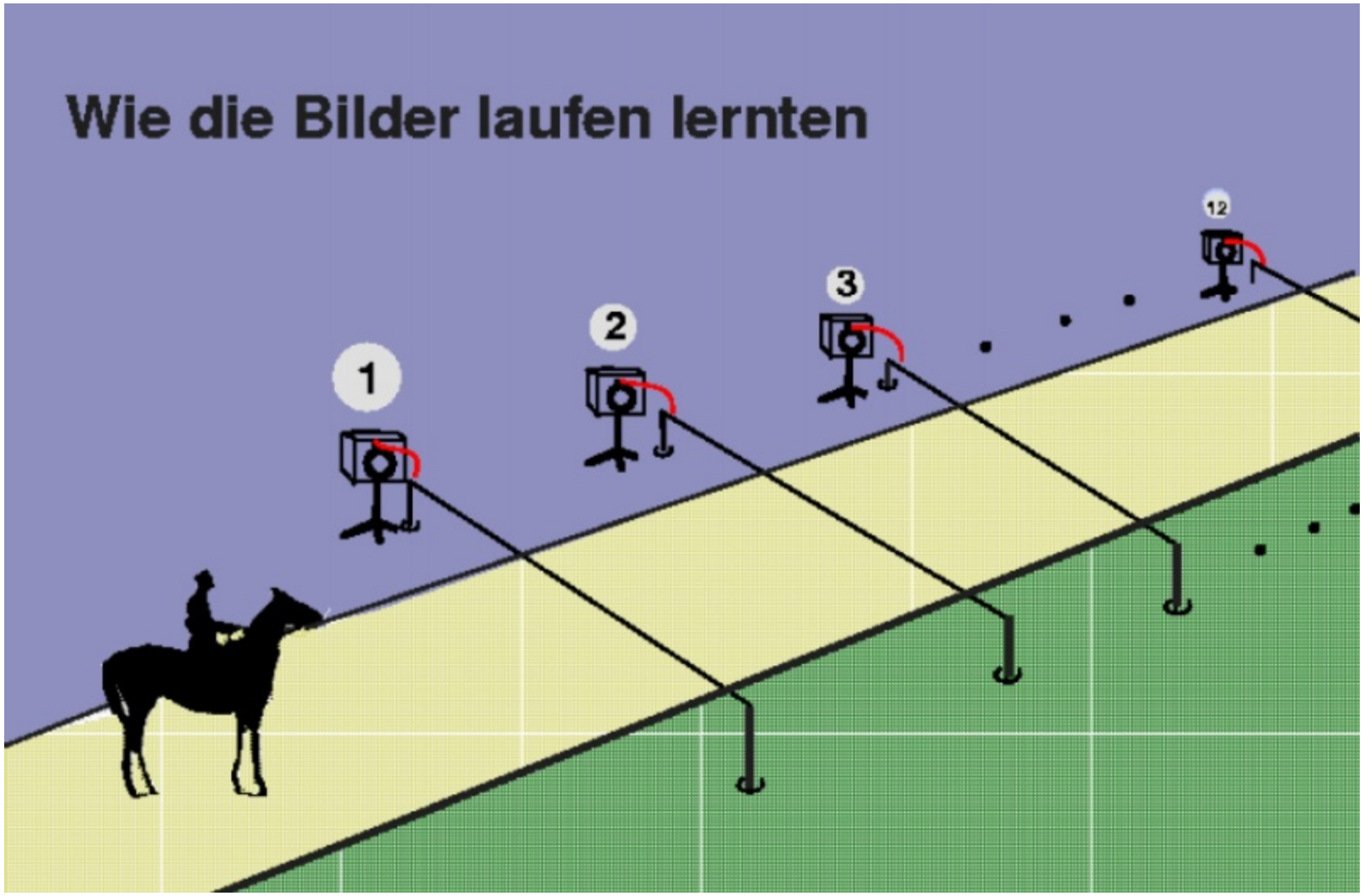
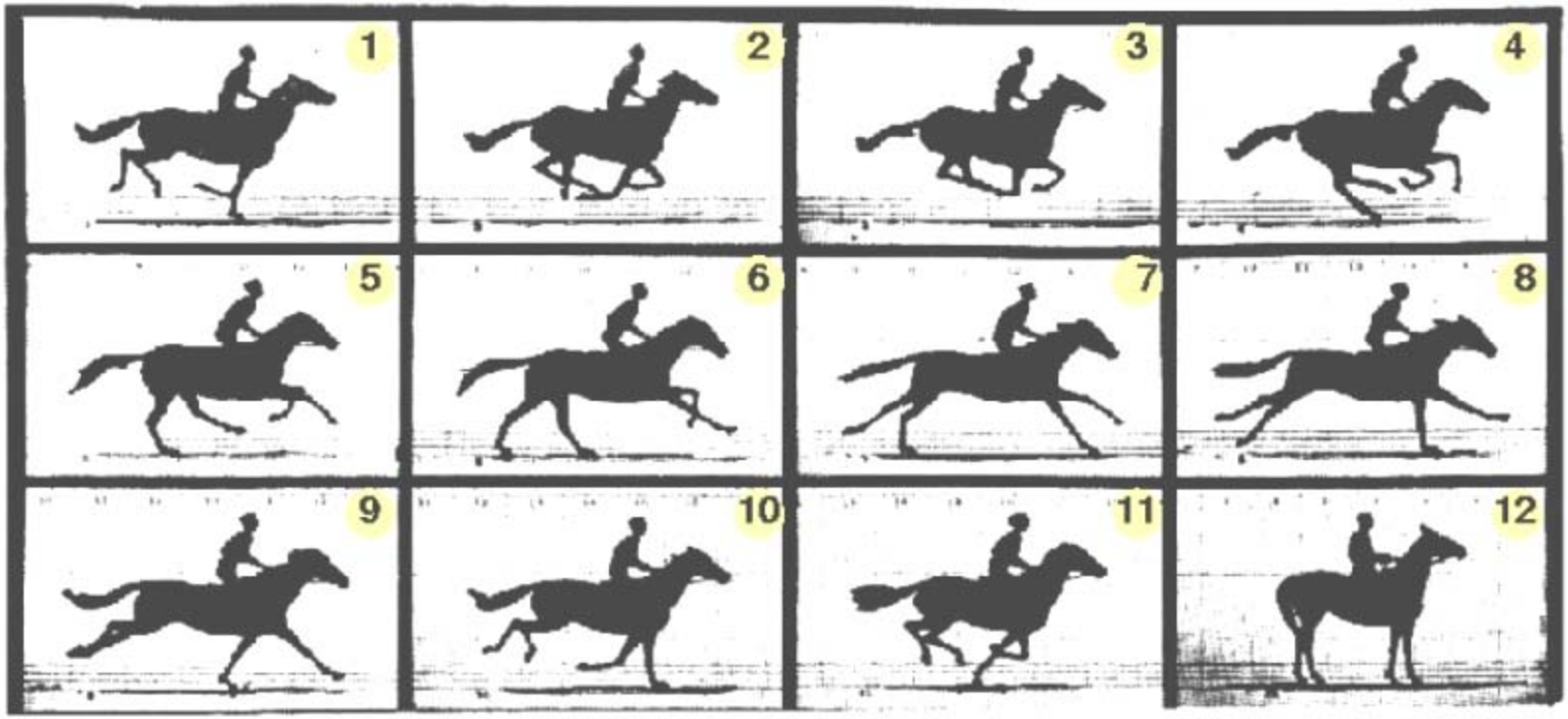
Mit Physik das Leben abbilden

Auf frischer Tat ertappt: die Beobachtung von Proteinen bei der Arbeit

Ilme Schlichting

MPI
FÜR MEDIZINISCHE FORSCHUNG

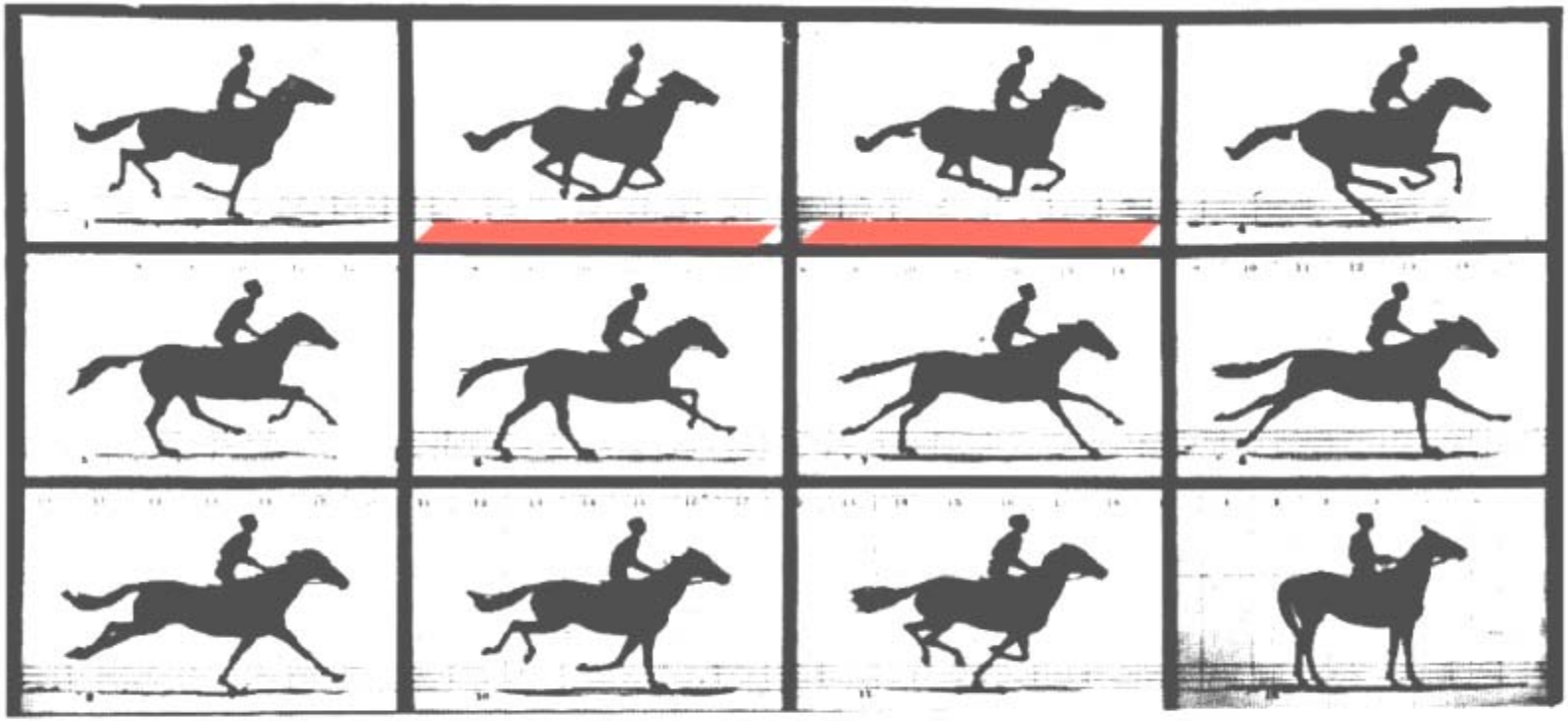

Wie die Bilder laufen lernten
1
2
3
12



THE HORSE IN MOTION.

"SALLIE GARDNER," owned by LELAND STANFORD; running at a 1.40 gait over the Palo Alto track, 19th June, 1878.

EADWEARD MUYBRIDGE. »Galoppierendes Pferd«. 1878. Albuminpapierkopie. George Eastman House, Rochester, N.Y.

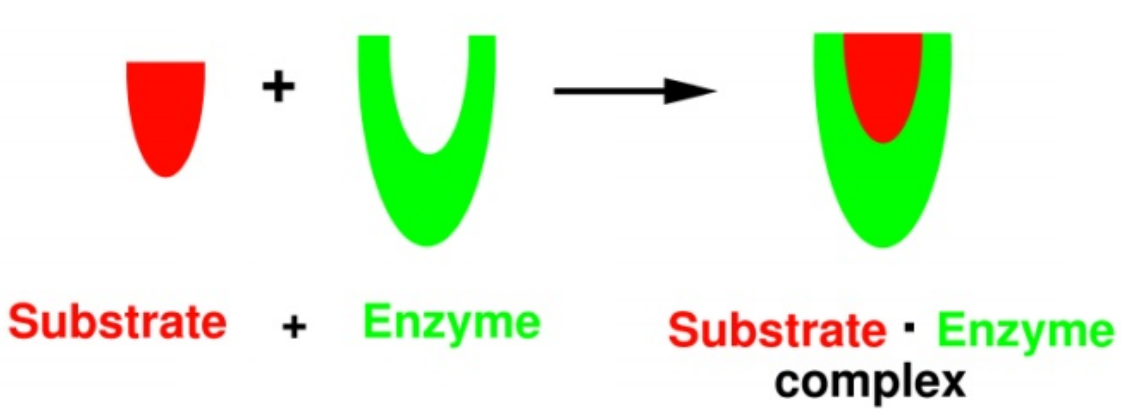


EADWEARD MUYBRIDGE. »Galoppierendes Pferd«. 1878. Albuminpapierkopie. George Eastman House, Rochester, N.Y.

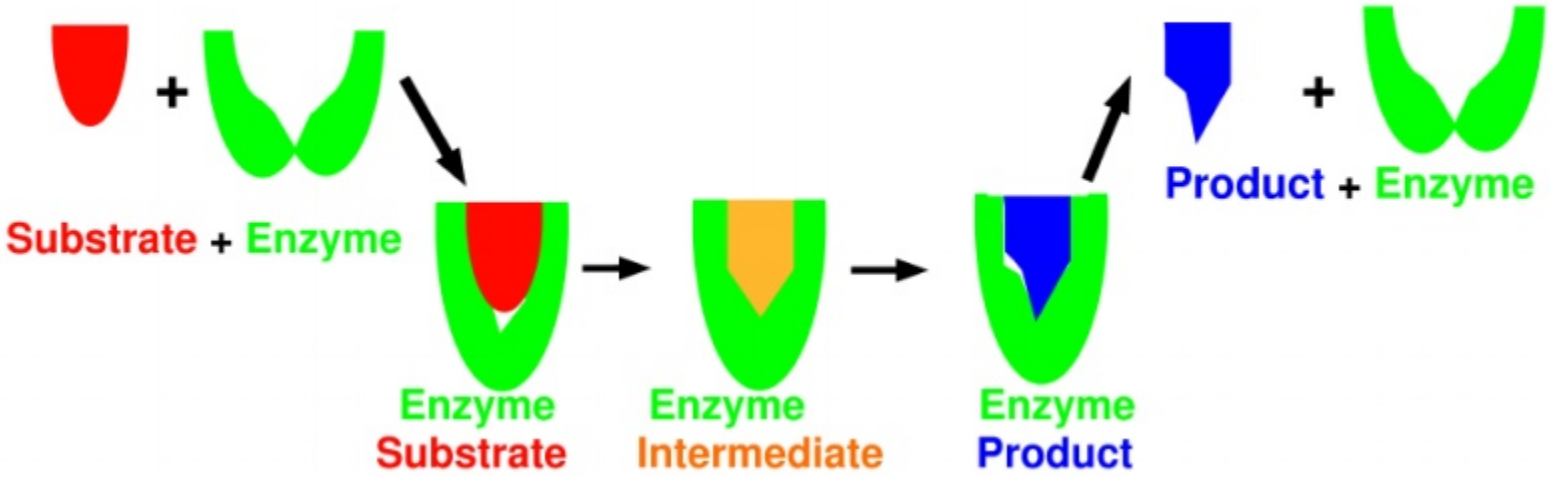
Proteine

Nanomaschinen
Motoren
Transportvehikel
Schreder
Pumpen
Kanäle
Katalysatoren

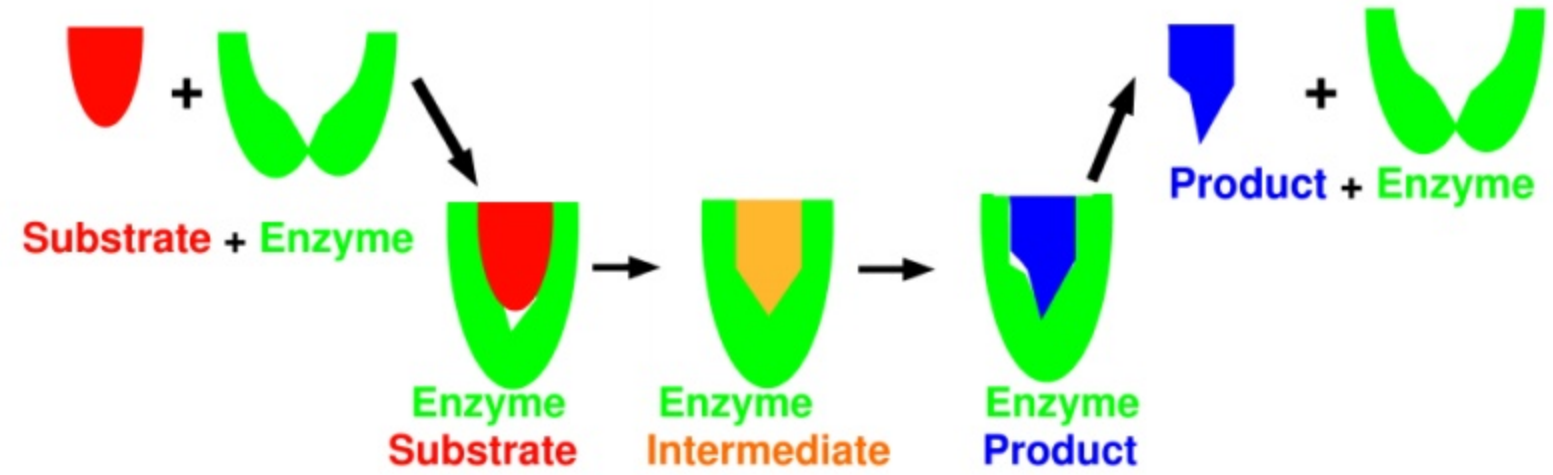
Spezifität durch Fischers Schlüssel-Schloss Prinzip

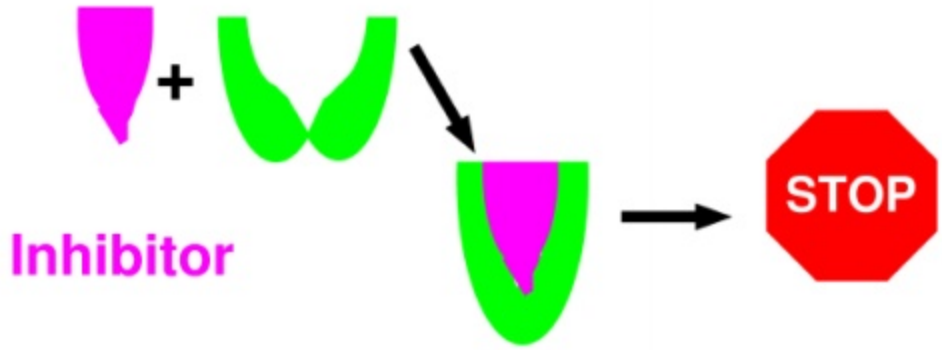


Wie entwickelt sich das Substrat zum Produkt ?

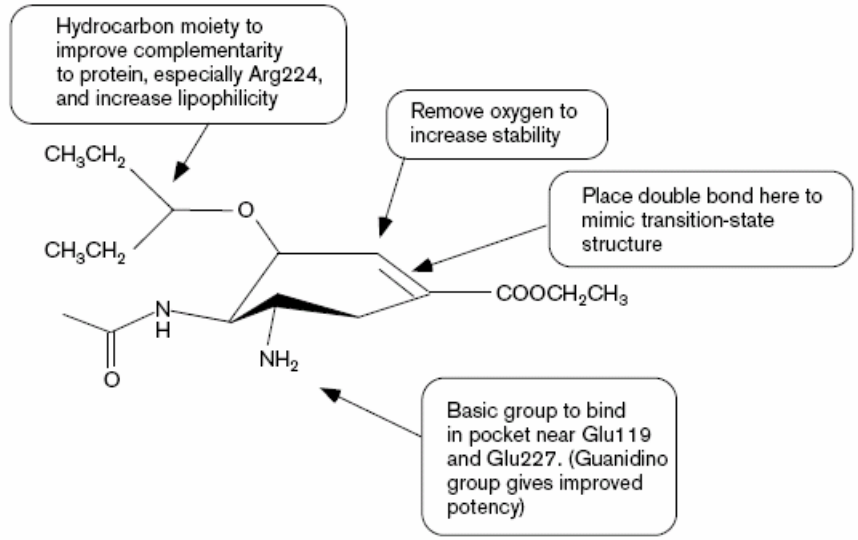


Gezielte Eingriffe in die Reaktion





z.B. Tamiflu gegen die (Vogel)grippe



Der Impakt von Strukturbiologie –
sogar auf unser tägliches Leben

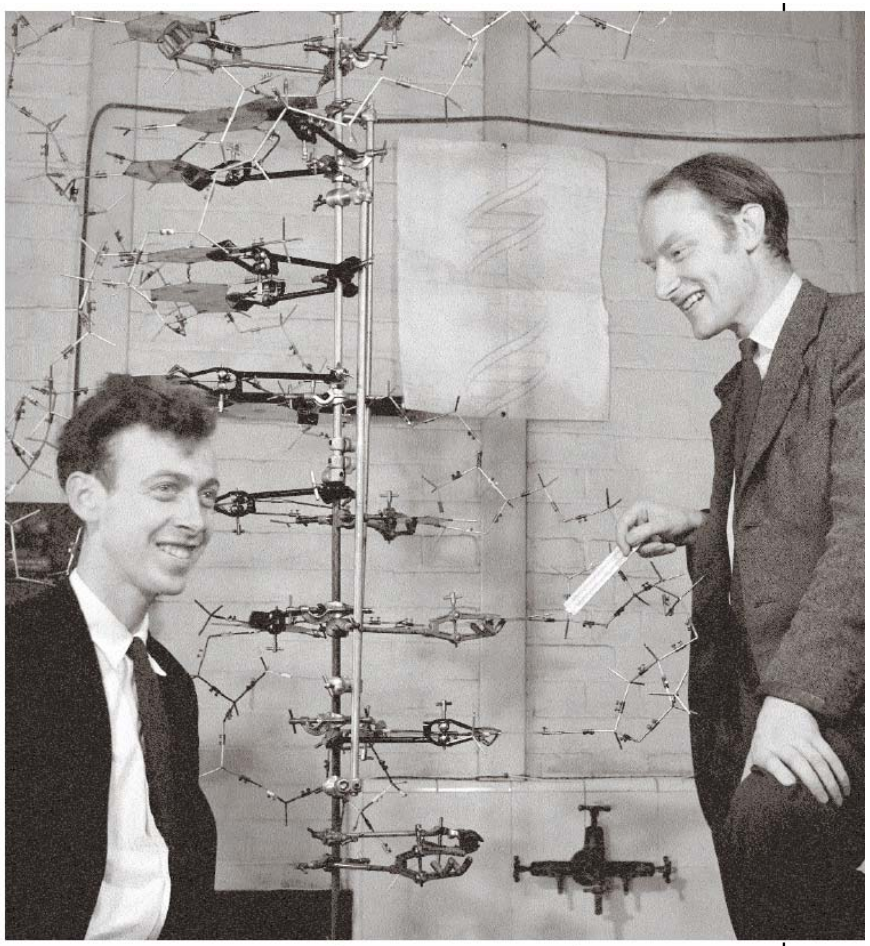
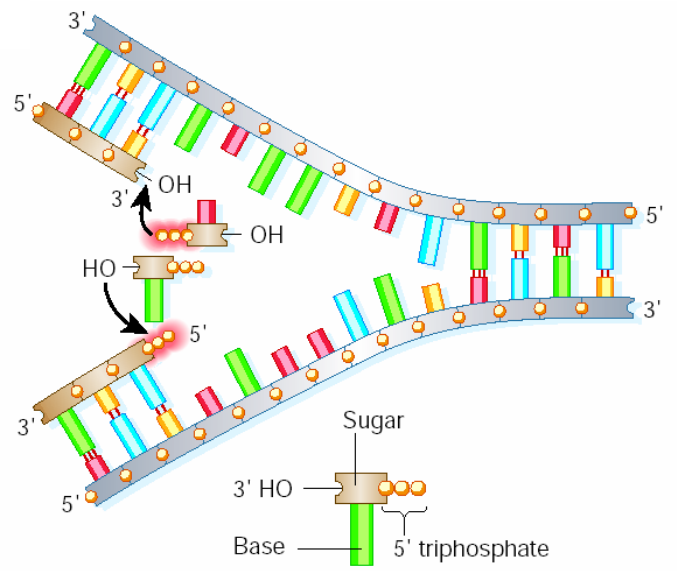


Figure 3 Anthony Barrington Brown's photograph of Watson and Crick with their model of DNA at the Cavendish Laboratory in Cambridge, 21 May 1953.

DNA

by BIJAN

. . .



Genetischer Kode enthüllt,

Molekulare Grundlage der
DNA Transkription, Replikation, Reparatur, Rekombination etabliert

⇨ ... Sequenzierung, Klonierung ...

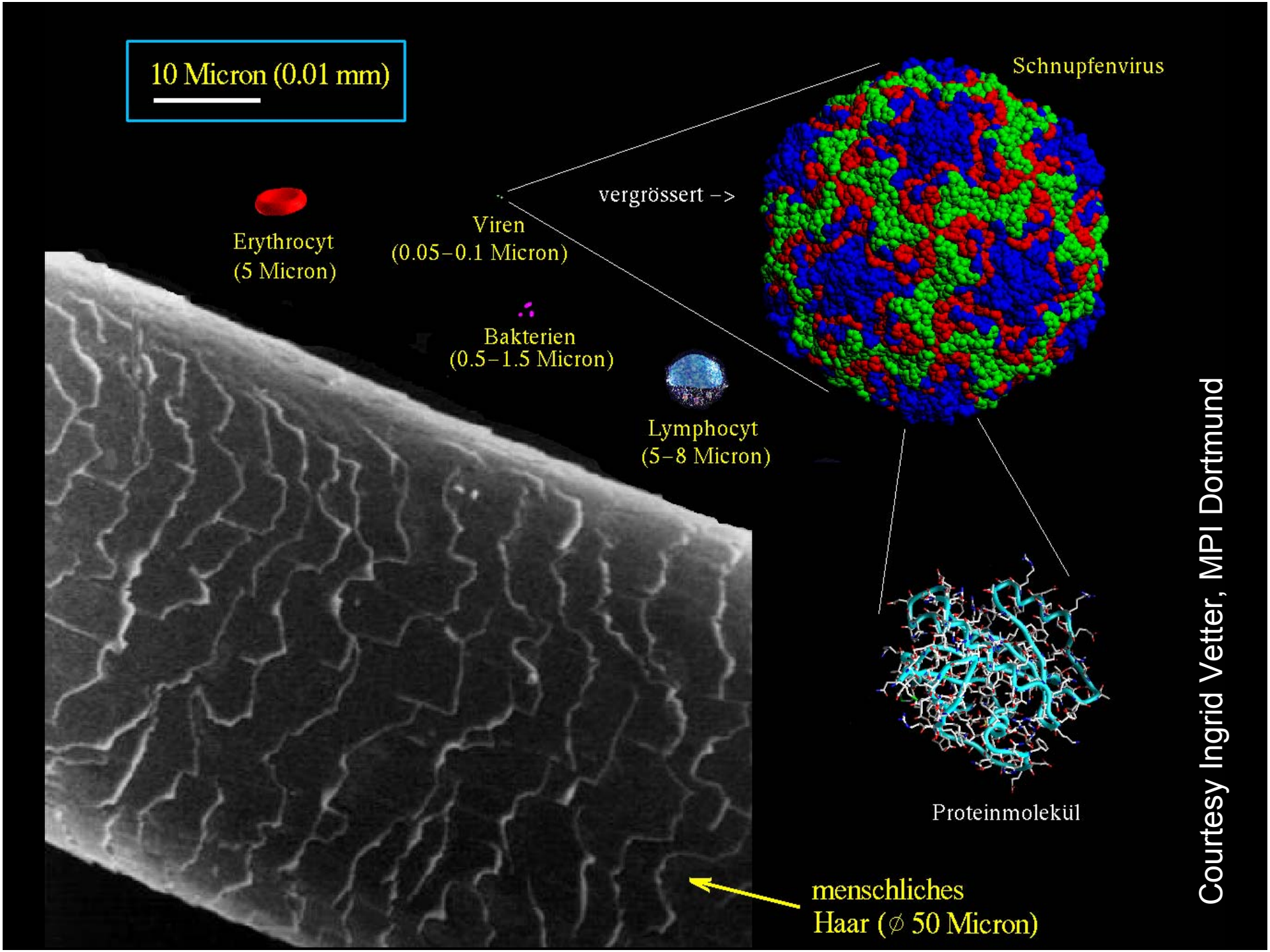
... Herstellen von veränderten Proteinen (Mutanten)

... Erste Schritte zum *de-novo* Design of Proteinen . . .

resultierend in . . .

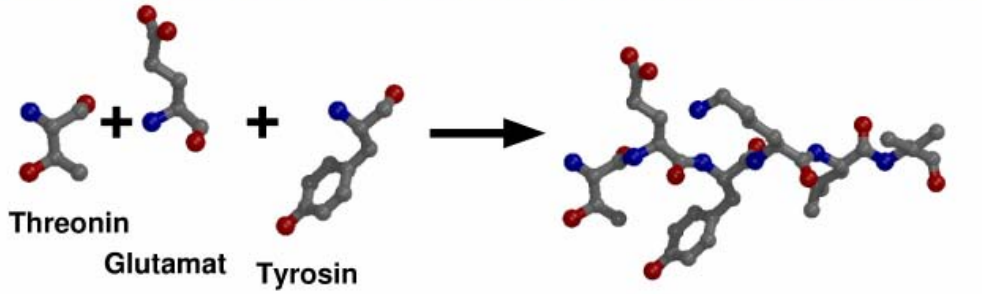
Nature ,15.2.2001

David Phillips, Royal Institution, London, 5th Nov 1965

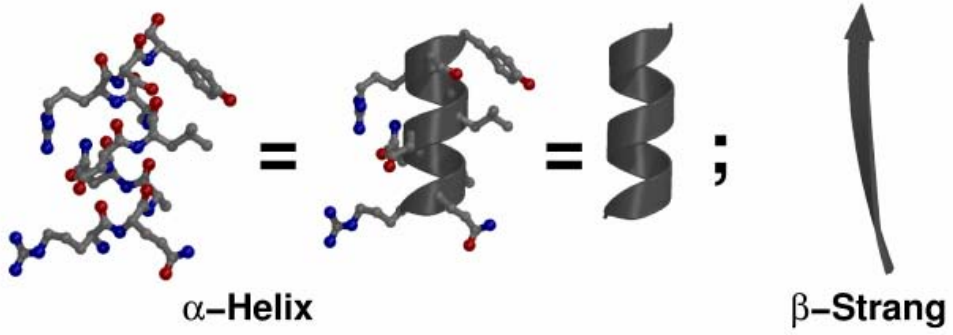

10 Micron (0.01 mm)
Schnupfenvirus
vergrössert ->
Erythrocyt
(5 Micron)
Viren
(0.05–0.1 Micron)
Bakterien
(0.5–1.5 Micron)
Lymphocyt
(5–8 Micron)
Proteinmolekül
menschliches
Haar (⌀ 50 Micron)
Courtesy Ingrid Vetter, MPI Dortmund

Aufbau von Proteinen

Lineare Ketten aus 20 verschiedenen Aminosäuren

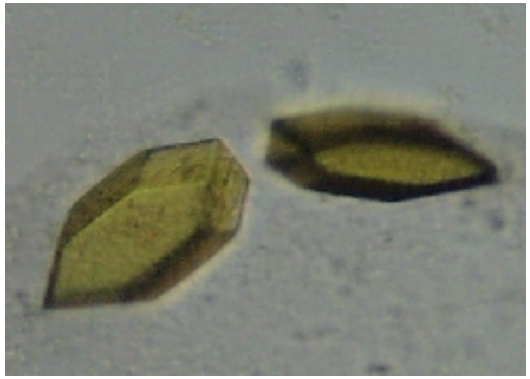


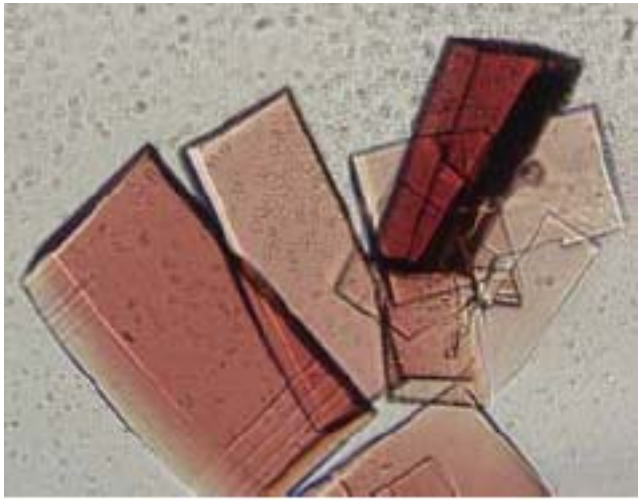
falten sich in "sekundär" Strukturelemente,

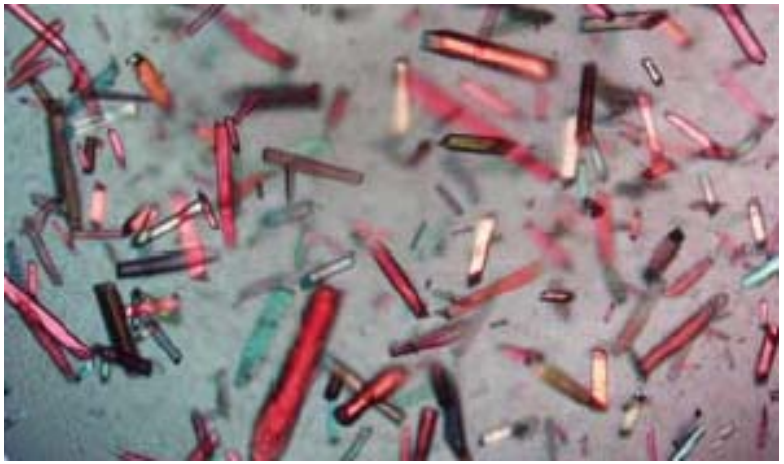


die sich räumlich anordnen

Protein Kristalle

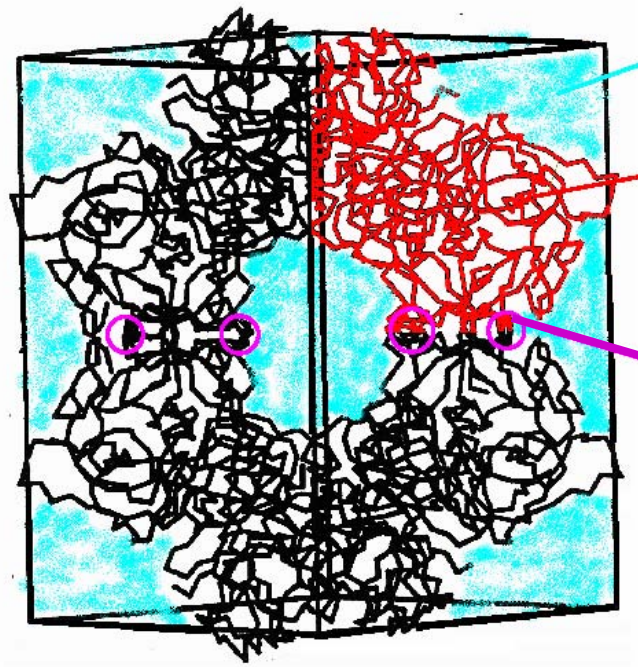






Typischerweise:
Submillimeter lang
10^{15} Moleküle

Protein Kristalle



30-80% Solvent

hohe Protein Konzentration (10-50mM)

wenige schwache Wechselwirkungen

↓

Mobilität

↓

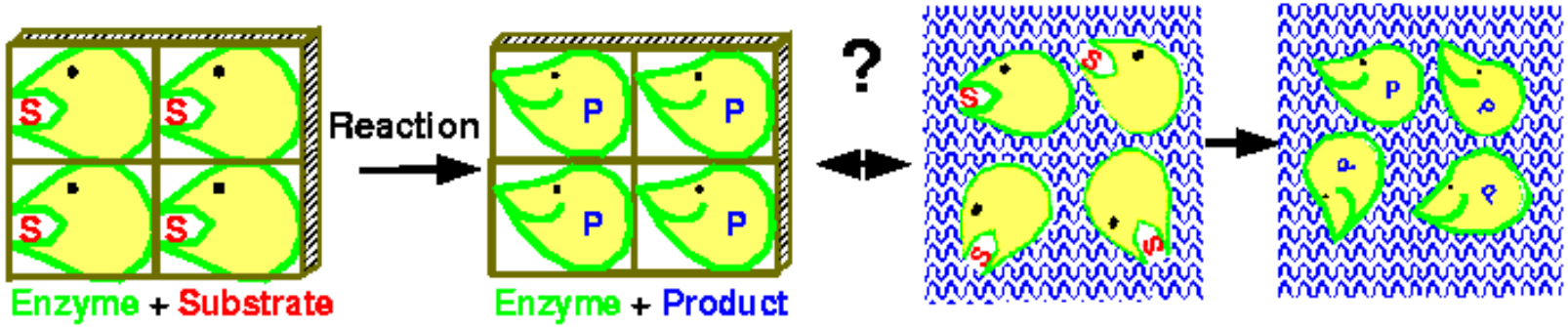
Biochemische Aktivität

Protein Kristall ~ sehr konzentrierte Lösung

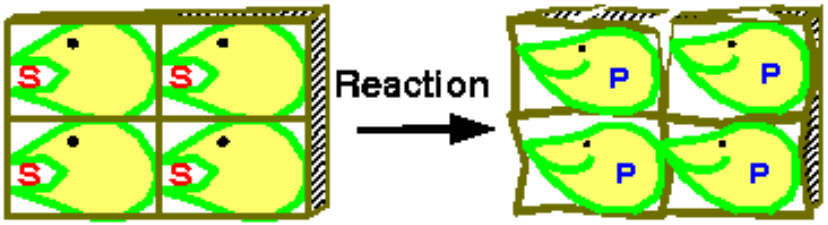
Jedoch: Lösungskanäle, räumliche Behinderung,Kristallisationsbedingungen

Wichtige Anforderungen an Kristalle

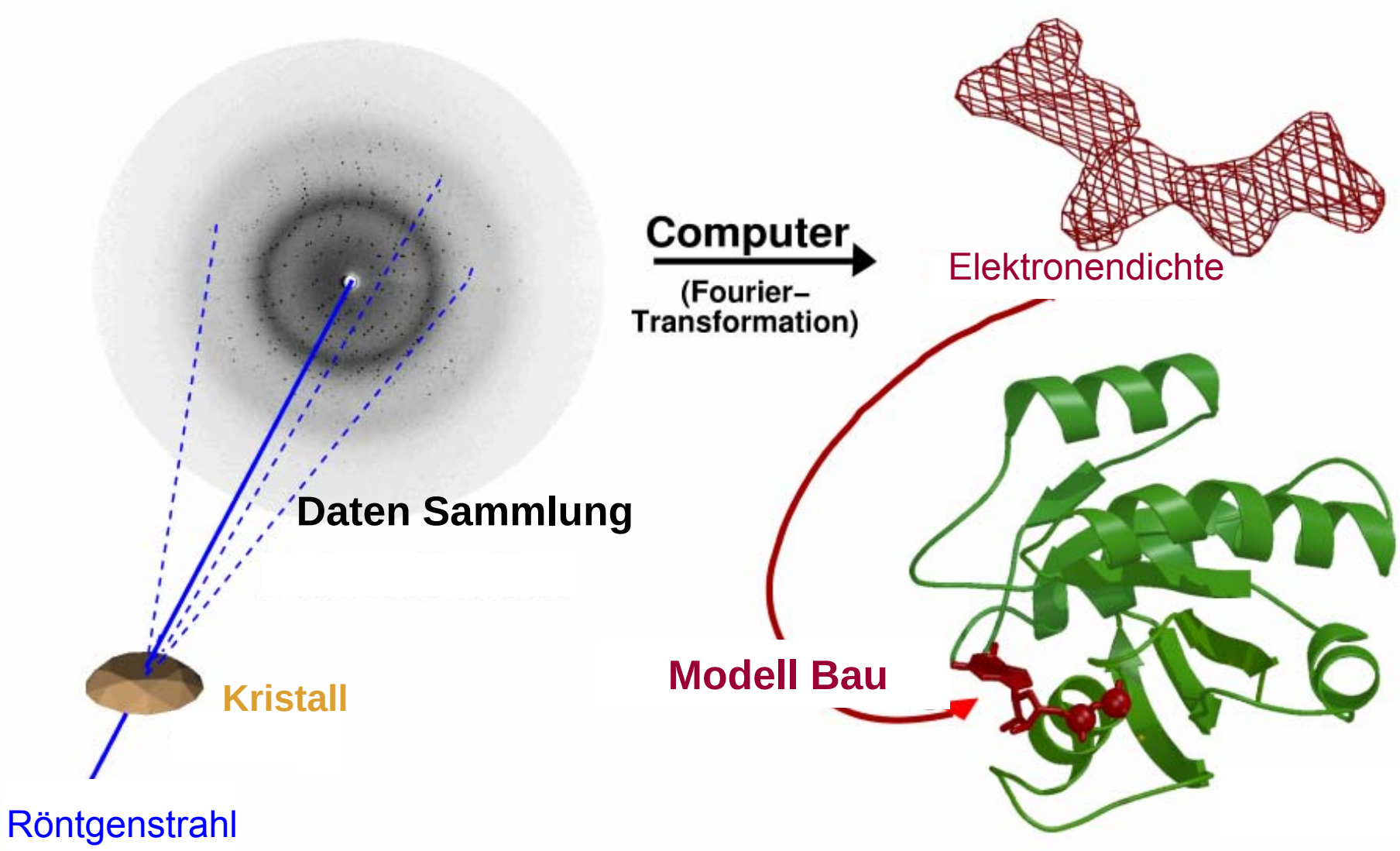
- Biochemische Aktivität



- Intaktes Gitter vor, während und nach der Reaktion

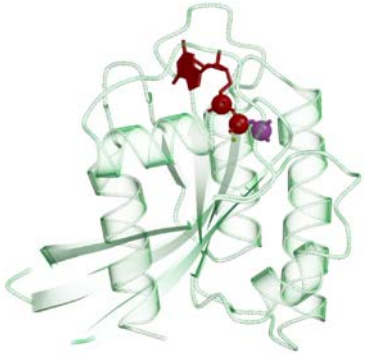


Strukturbestimmung durch Kristallographie

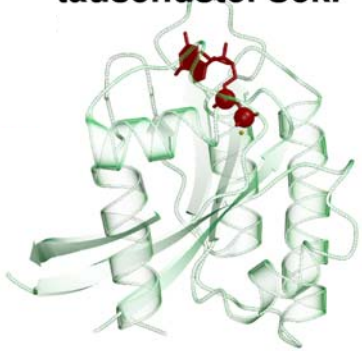


Herstellung von Kristallen dauert lange

Tage – Wochen



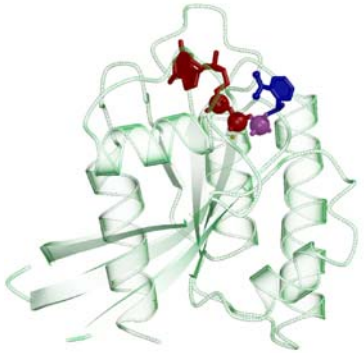
tausendstel Sek.



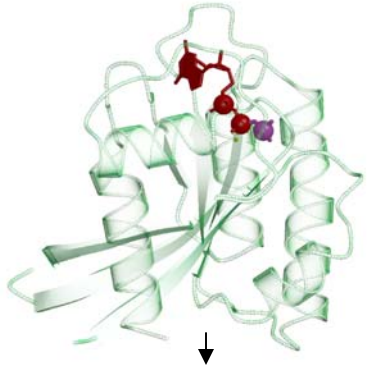
Kristallisation

Tage – Wochen

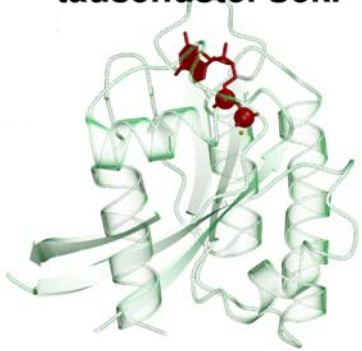
in **geschützer** Form



Enzymatische Reaktion



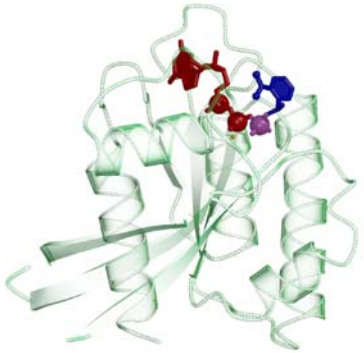
tausendstel Sek.



Kristallisation

Tage – Wochen

in **geschützer** Form

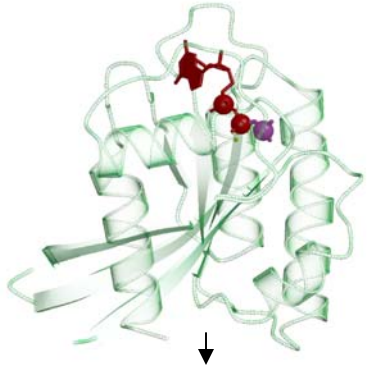


aktivieren

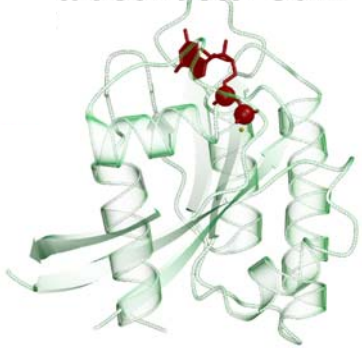
Enzymatische Reaktion

Datensammlung

typ. Stunden-Tage



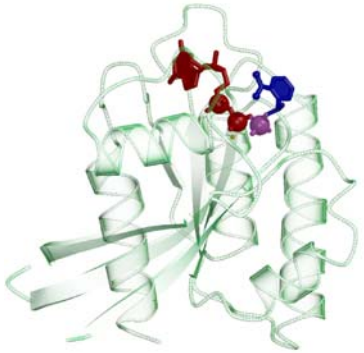
tausendstel Sek.



Kristallisation

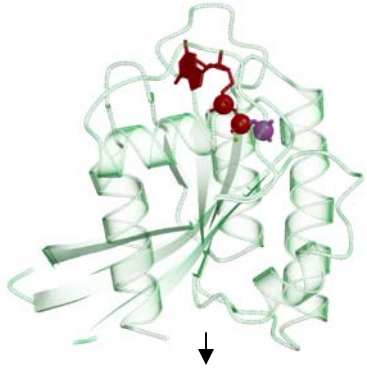
Tage – Wochen

in **geschützer** Form

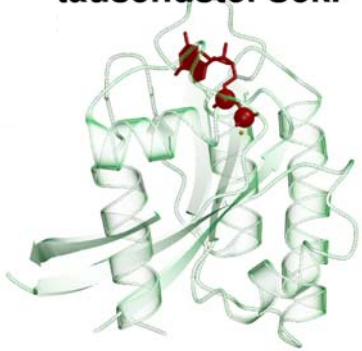


aktivieren

Enzymatische Reaktion



tausendstel Sek.



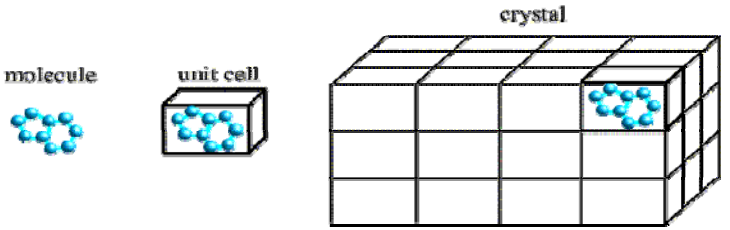
Datensammlung

typ. Stunden-Tage

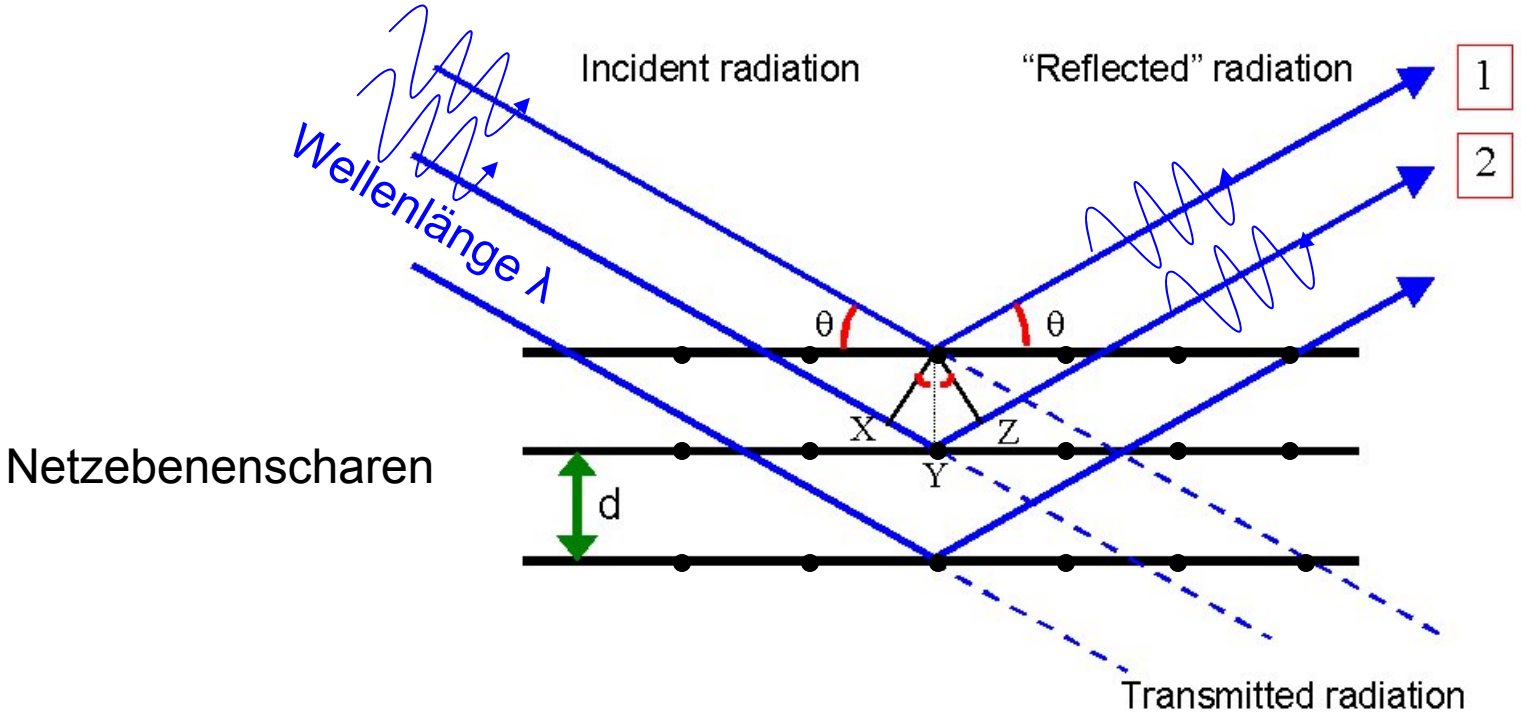
Schneller messen:
Zeitaufgelöste Kristallographie

Reaktion verlangsamen:
Kinetische Kristallographie

Bragg'sches Gesetz
Beugung am Kristallgitter



$$2 \cdot d \cdot \sin\theta = n \cdot \lambda$$



Zwei Ansätze zur Datensammlung

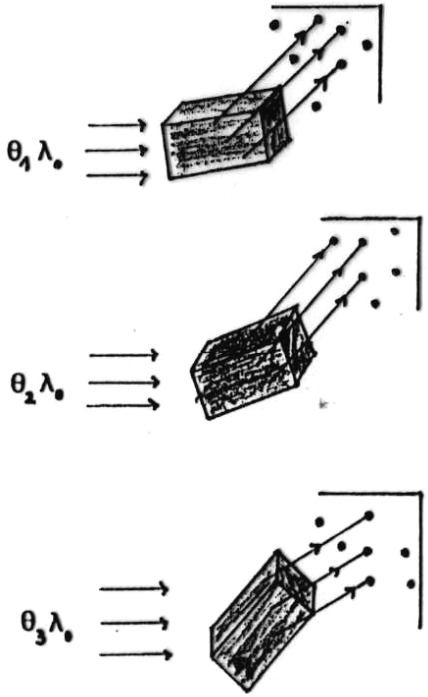
feste Wellenlänge,
rotierender Kristall

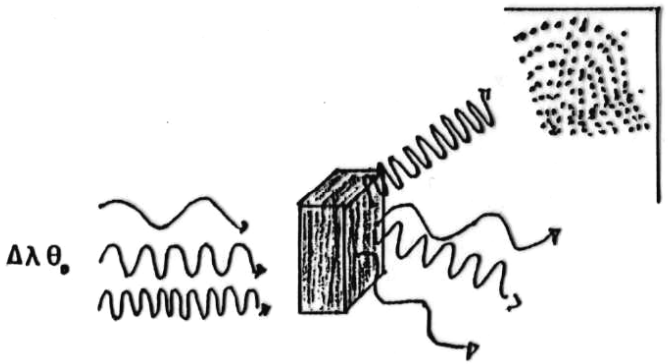
← $2 \cdot d \cdot \sin\theta = n \cdot \lambda$ →

Wellenlängenbereich,
stationärer Kristall

Monochromatische Röntgenbeugung

Polychromatische Röntgenbeugung





Typische Datensammlungszeiten mittels Synchrotronstrahlung

Minuten

ps to ms

Zwei Ansätze zur Datensammlung

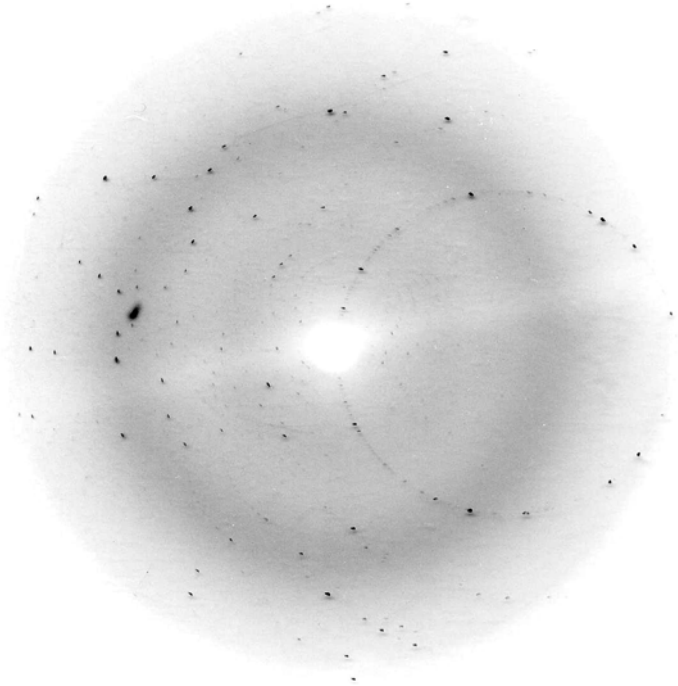
feste Wellenlänge,
rotierender Kristall

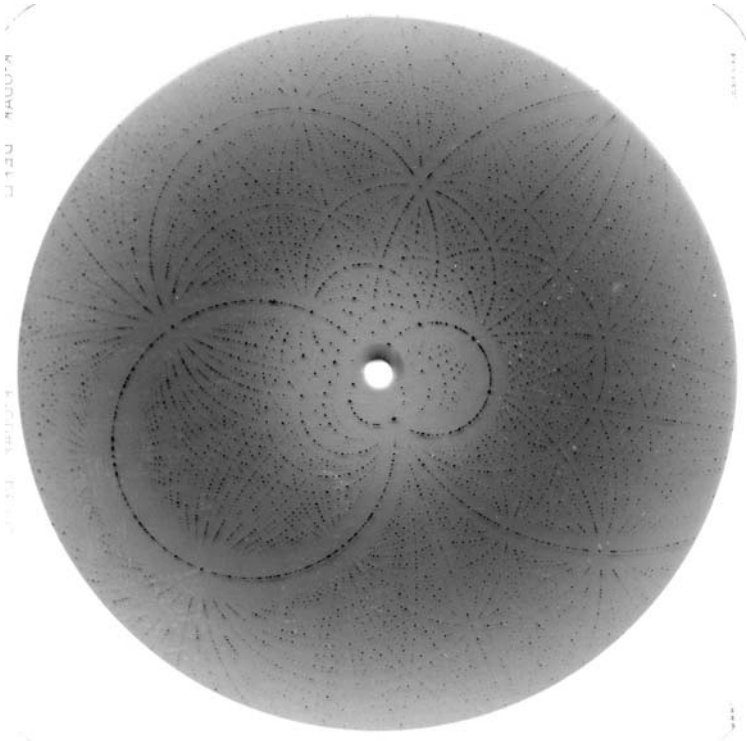
Monochromatische
Röntgenbeugung

← $2 \cdot d \cdot \sin\theta = n \cdot \lambda$ →

Wellenlängenbereich,
stationärer Kristall

Polychromatische
Röntgenbeugung





Typische Datensammlungszeiten mittels Synchrotronstrahlung

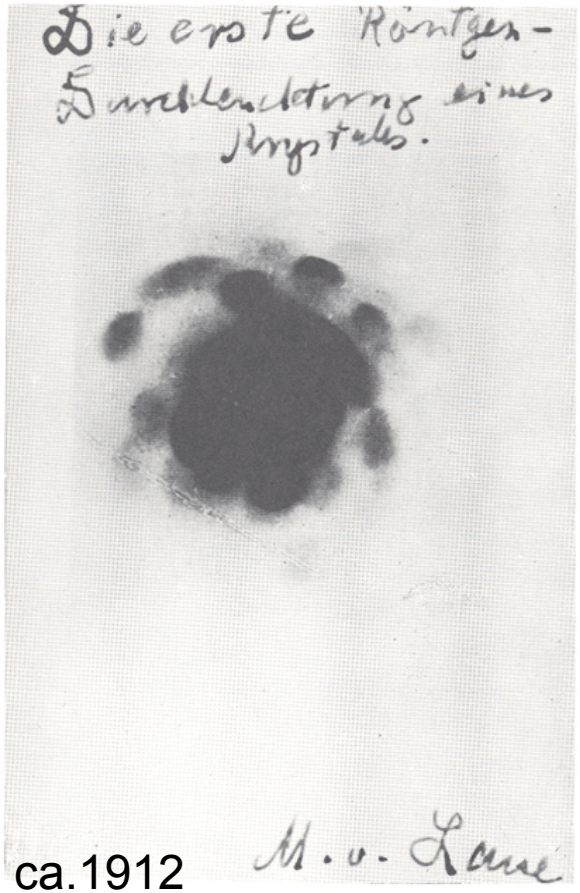
Minuten

ps to ms

Die Laue Methode

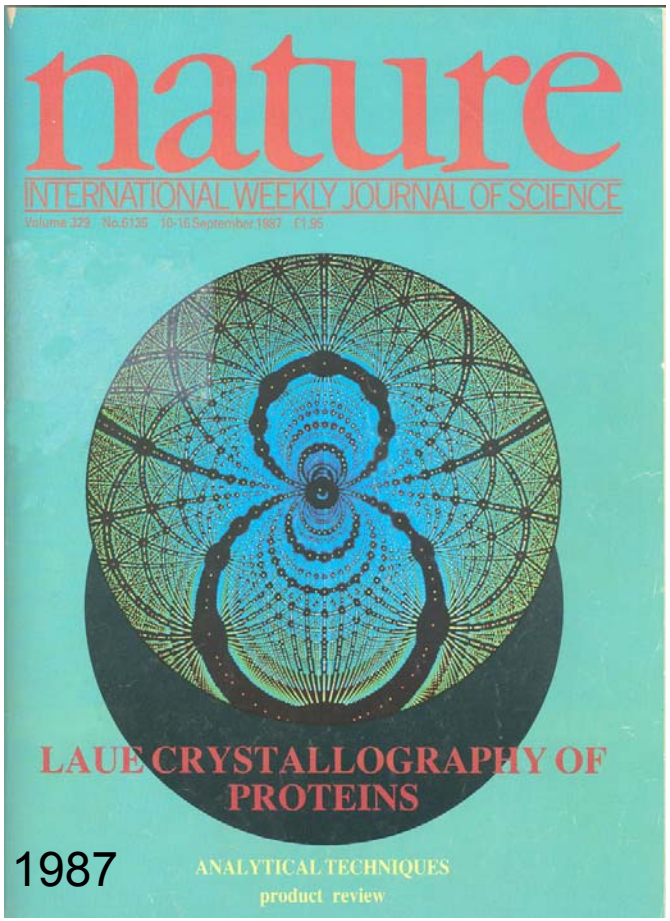
Stationärer Kristall, polychromatischer Röntgenstrahl

Bremsspektrum



ca.1912

Renaissance durch Synchrotron Speicherringe

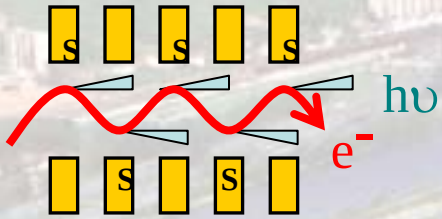


1987

Synchrotron Speicherringe
Quellen intensiver Röntgenstrahlung

Synchrotron Speicherringe
Quellen intensiver Röntgenstrahlung

Energie: 6 GeV
Max. Strom: 200 mA
Revolution frequency: 355 kHz
Zahl an Paketen: 1-992
Zeit zwischen Paketen : 2816-2.8 ns



Insertion device

844 m

Speicher Ring

"Paket"
Relativistische
Elektronen

$\delta \sim mc^2/E$

150 ps

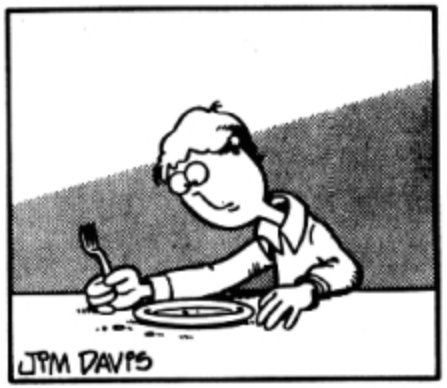
$$P(\lambda,\gamma,\psi_0,\rho,\Delta\lambda,I_B,\Delta\psi,\Delta\theta) = \int_{-\psi_0+\Delta\psi}^{+\psi_0+\Delta\psi} \frac{2}{3}\frac{e_0\Delta\lambda\Delta\theta\, I_B\rho^2}{\varepsilon_0\beta\lambda^4\gamma^4}\left[1+(\gamma\psi)^2\right]^2$$

$$\times\left[K_{2/3}\left[\xi(\lambda,\psi)\right]^2 + \frac{(\gamma\psi)^2}{1+(\gamma\psi)^2}K_{1/3}\left[\xi(\lambda,\psi)\right]^2\right]$$

Schwinger Gleichung

Probleme bei zeitaufgelösten Messungen

Probleme bei zeitaufgelösten Messungen



1) Zeitauflösung der Datensammlung

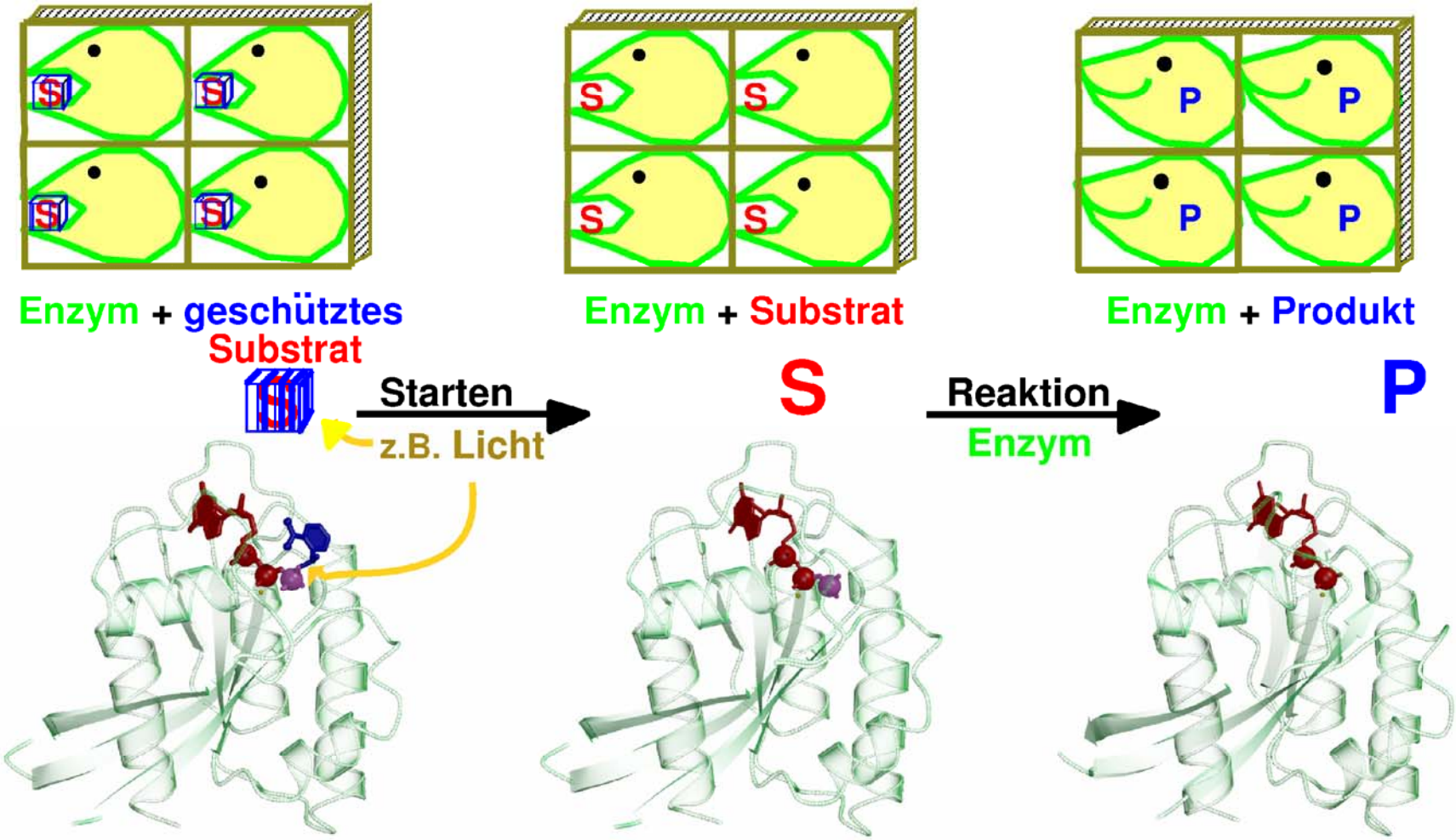
Probleme bei zeitaufgelösten Messungen

1) Zeitauflösung der Datensammlung

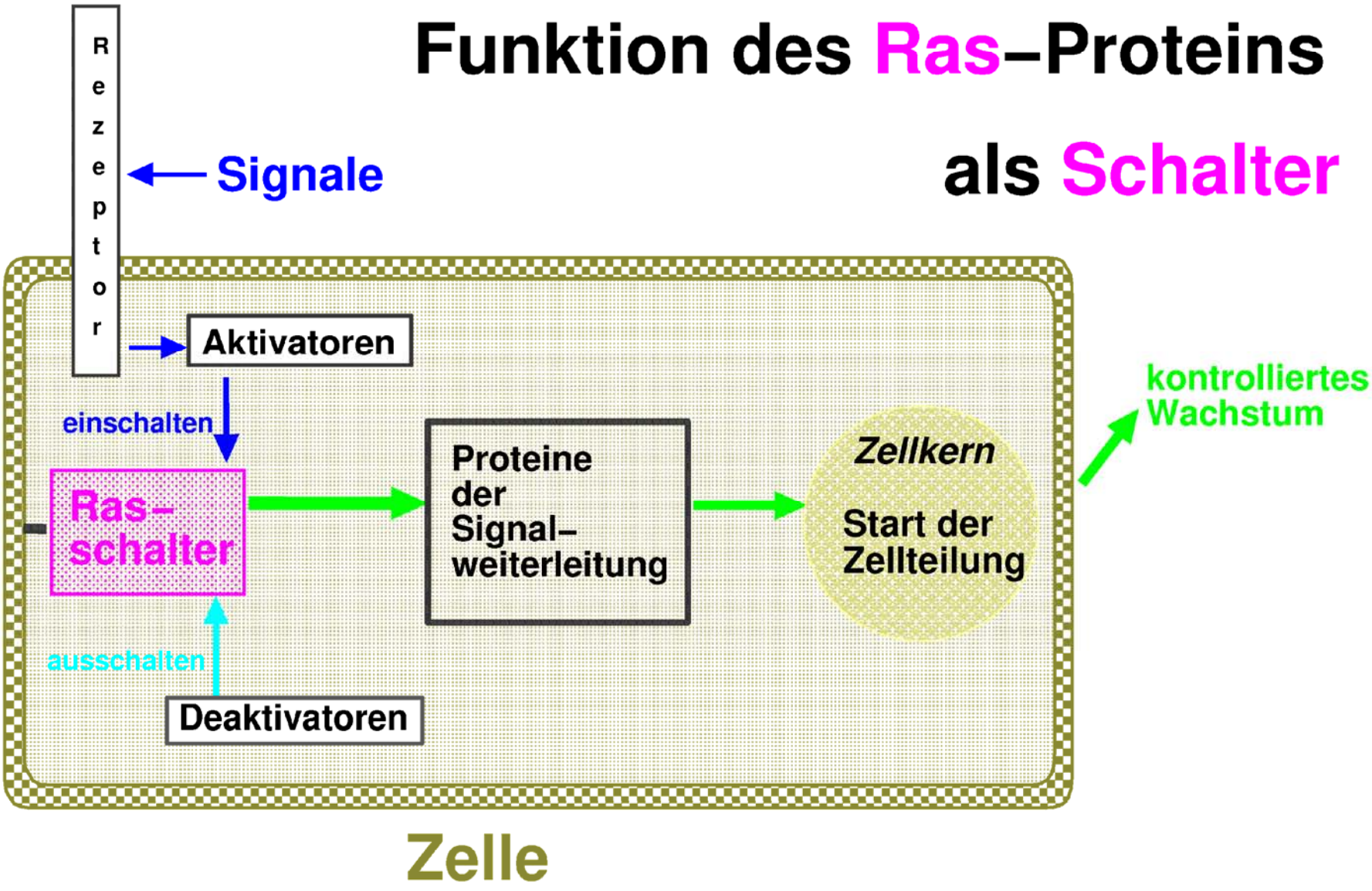
Probleme bei zeitaufgelösten Messungen

1) Zeitauflösung der Datensammlung
2) Starten der Reaktion

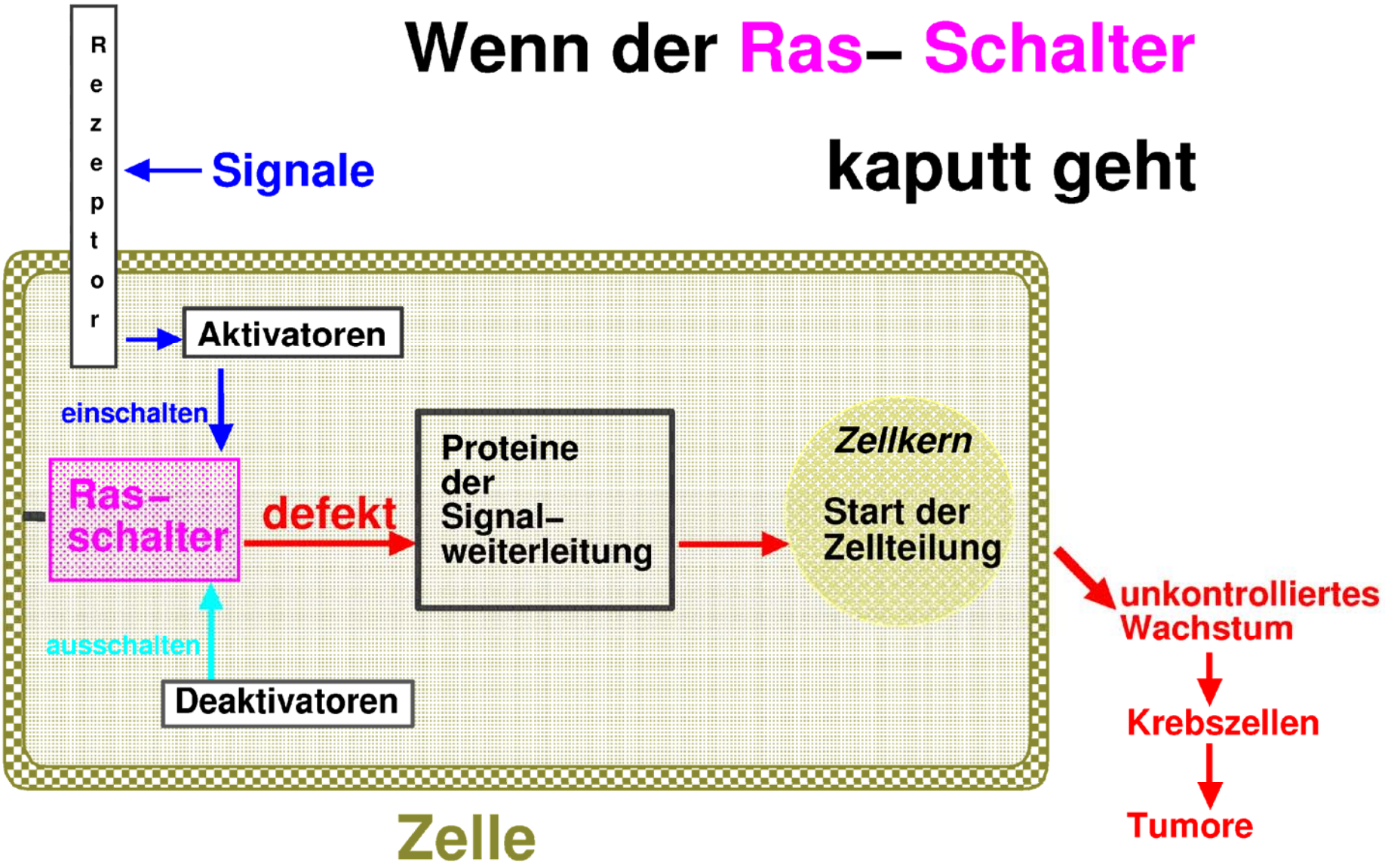
Das Prinzip


S
S
S
S
Enzym + geschütztes Substrat
Starten
z.B. Licht
S
S
S
S
Enzym + Substrat
Reaktion
Enzym
P
P
P
P
Enzym + Produkt
P

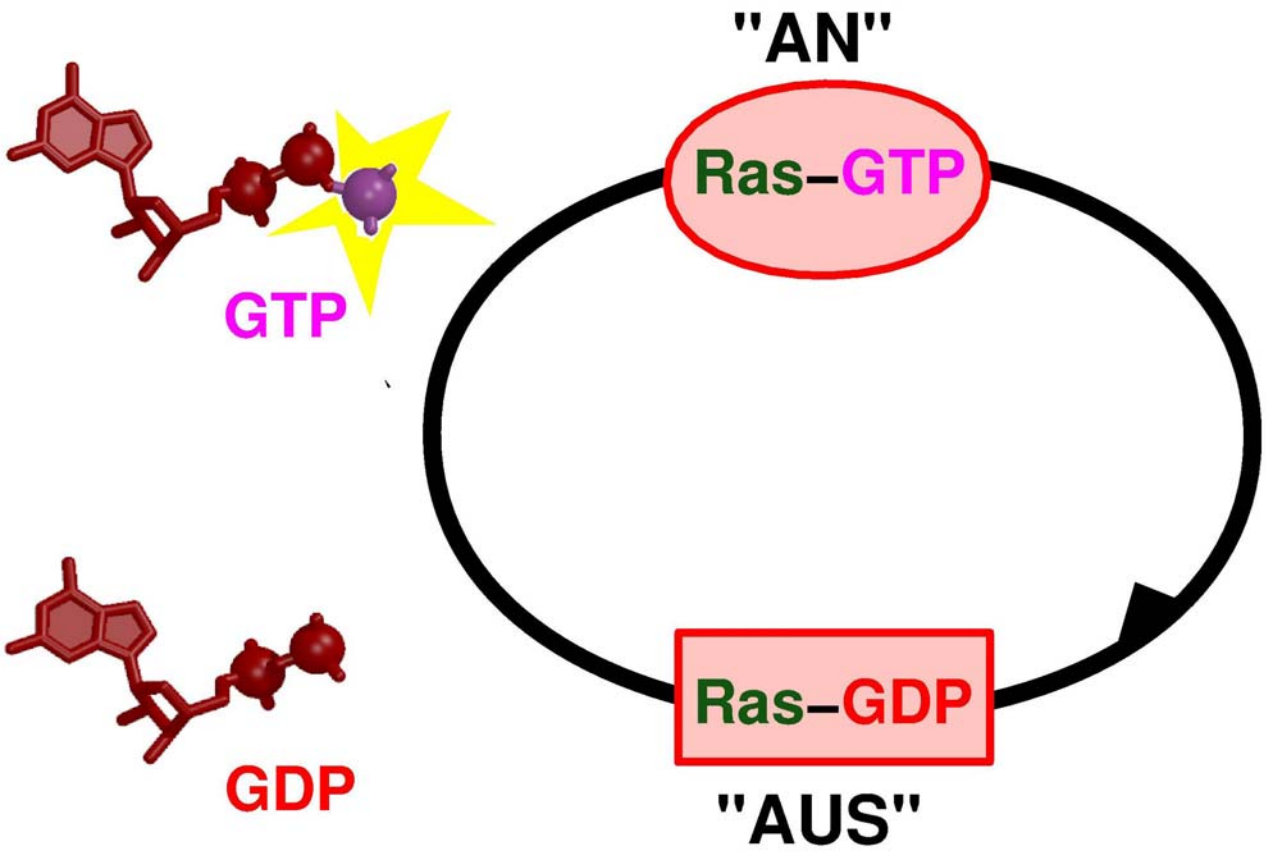
Funktion des Ras–Proteins als Schalter



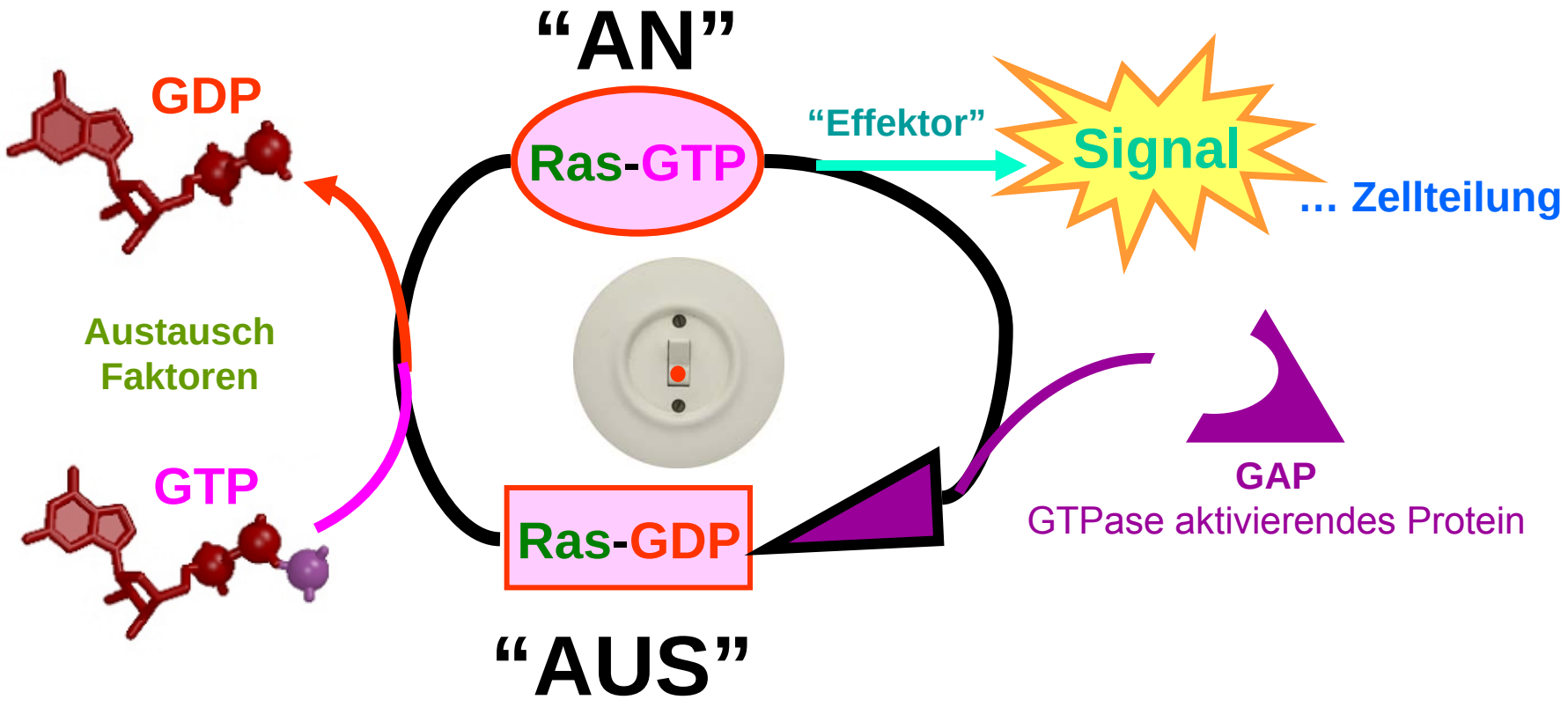
Wenn der Ras– Schalter kaputt geht



Das Ras Protein ist ein molekularer Schalter

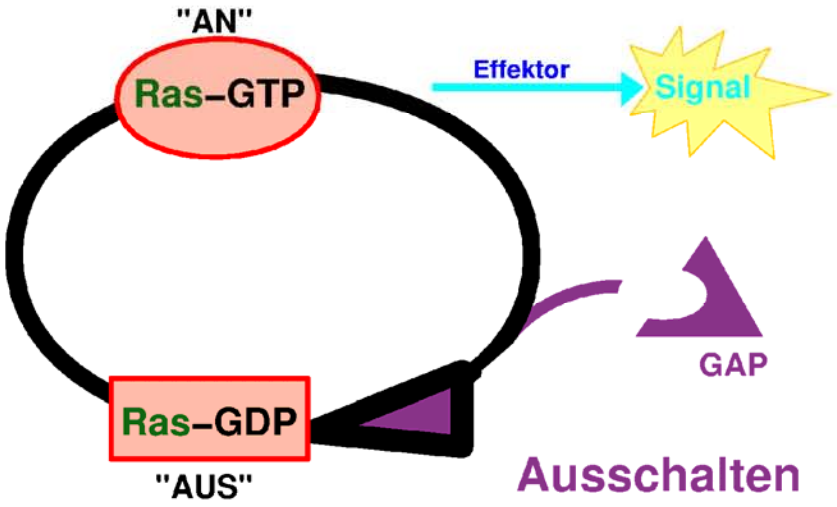


Der molekulare Schalter Ras - Schaltkreis

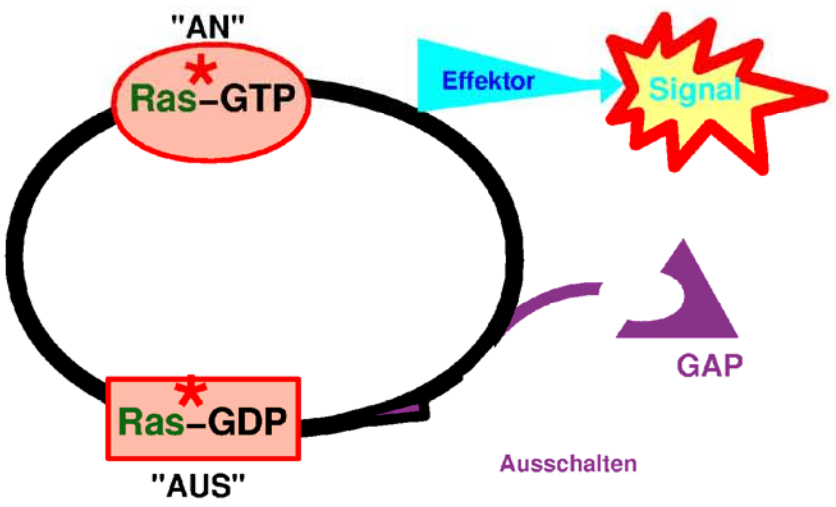


Molekulare Schalter ausser Kontrolle

- **Normale Zelle**



- **Krebszelle, mutiertes Ras**



30% aller menschlichen Tumoren enthalten mutiertes Ras*

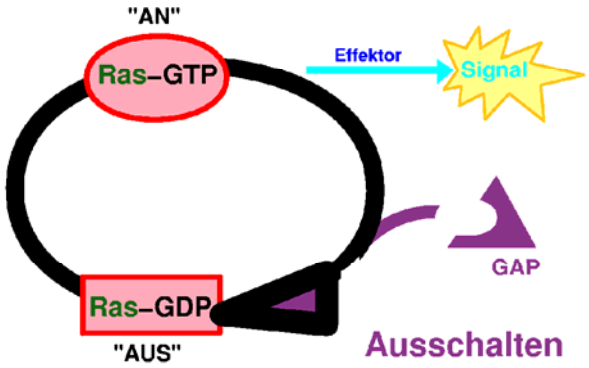
Fragestellung

1.) Unterschiede — Ras–GTP — ? — Ras–GDP — Schalter

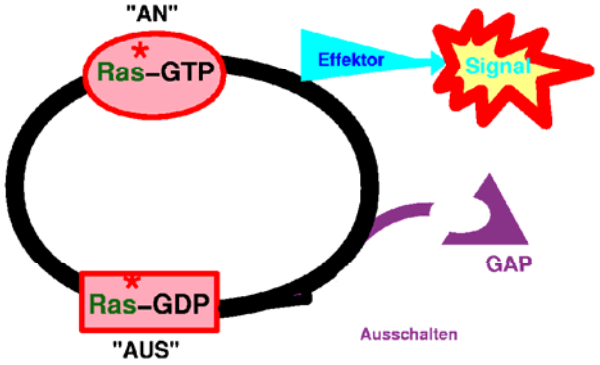
2.) Mechanismus — Ras–GTP — ? → — Ras–GDP — ausschalten

3.) Unterschiede — Ras–GTP — ? — Ras–GTP* — Krebs

● Normale Zelle

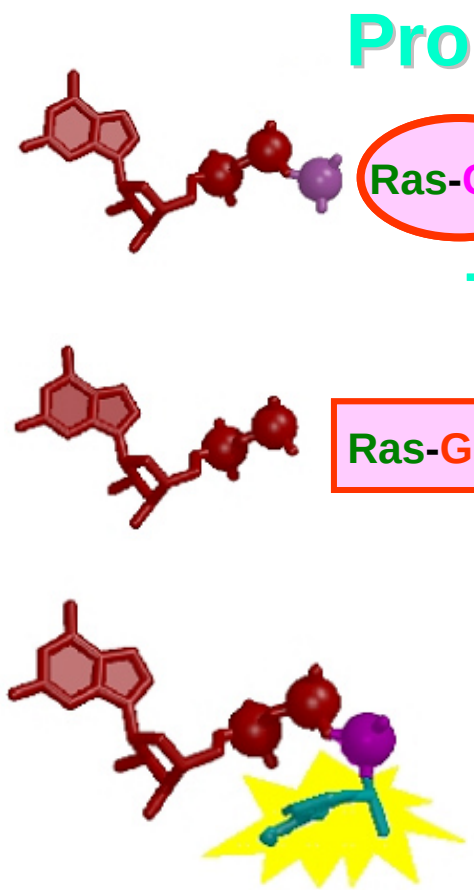


● Krebszelle, mutiertes Ras



Untersuchung der strukturellen Unterschiede:

Ras-GTP ↔ Ras-GDP



Problem:

Ras-GTP

$T_{1/2}$ = 20min

Ras-GDP

Kristallisation von

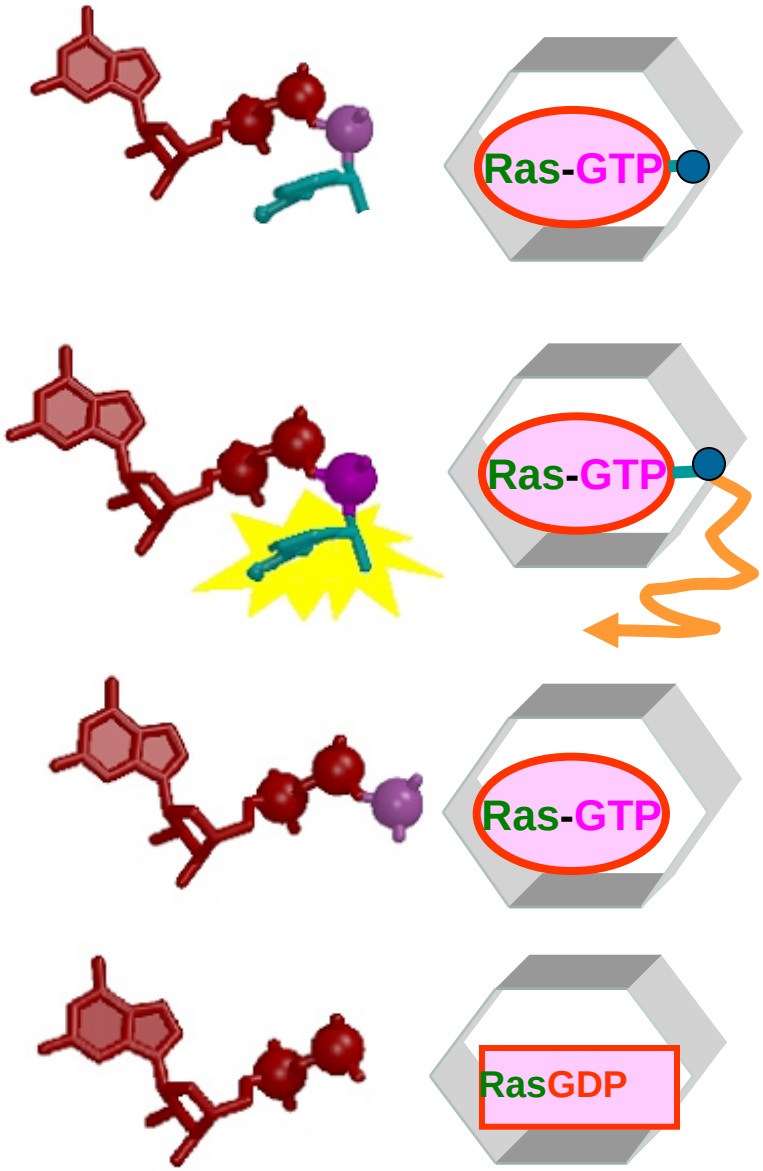
Ras-GTP

nicht direkt möglich

Lösung:

GTP durch lichtempfindliche Schutzgruppe vor Abbau schützen

Vorgehensweise



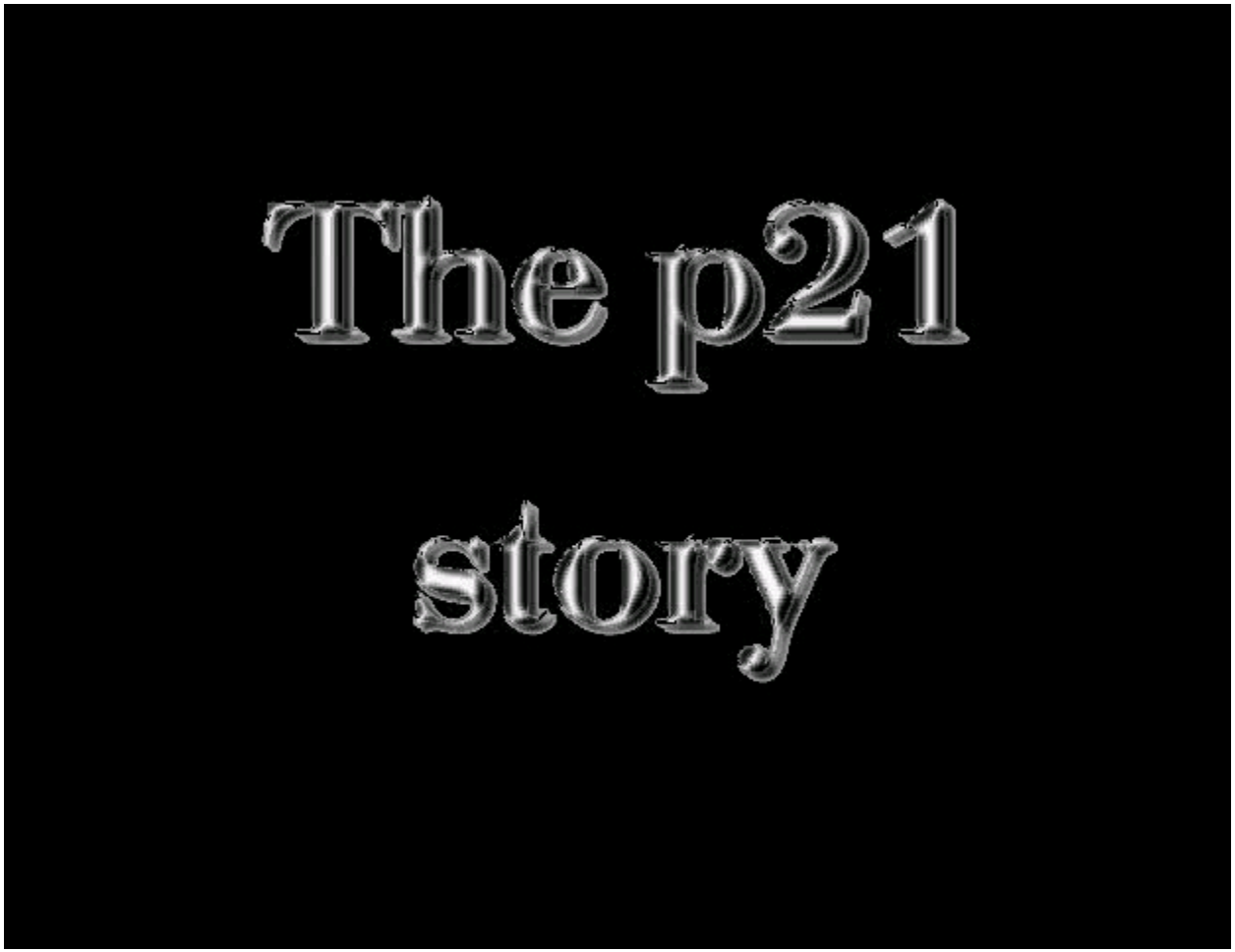
Kristallisation von
Ras-GTP-Schutzgruppe
im Dunklen

Abspaltung der Schutzgruppe durch Licht

GTP Komplex

Verfolgen der enzymatischen Reaktion im Kristall

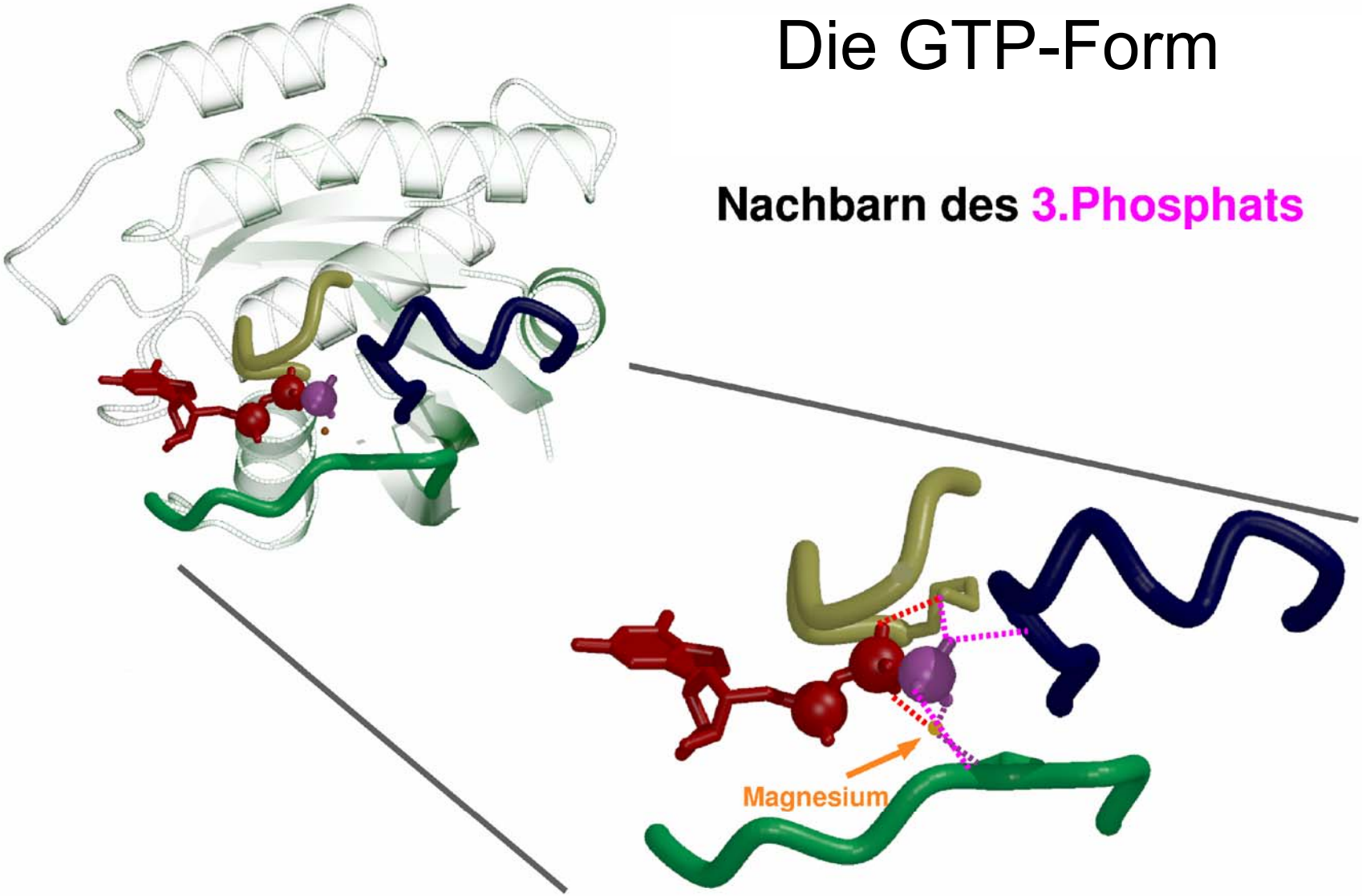
GDP Komplex



(p21 ist der ursprüngliche Name des Ras Proteins)

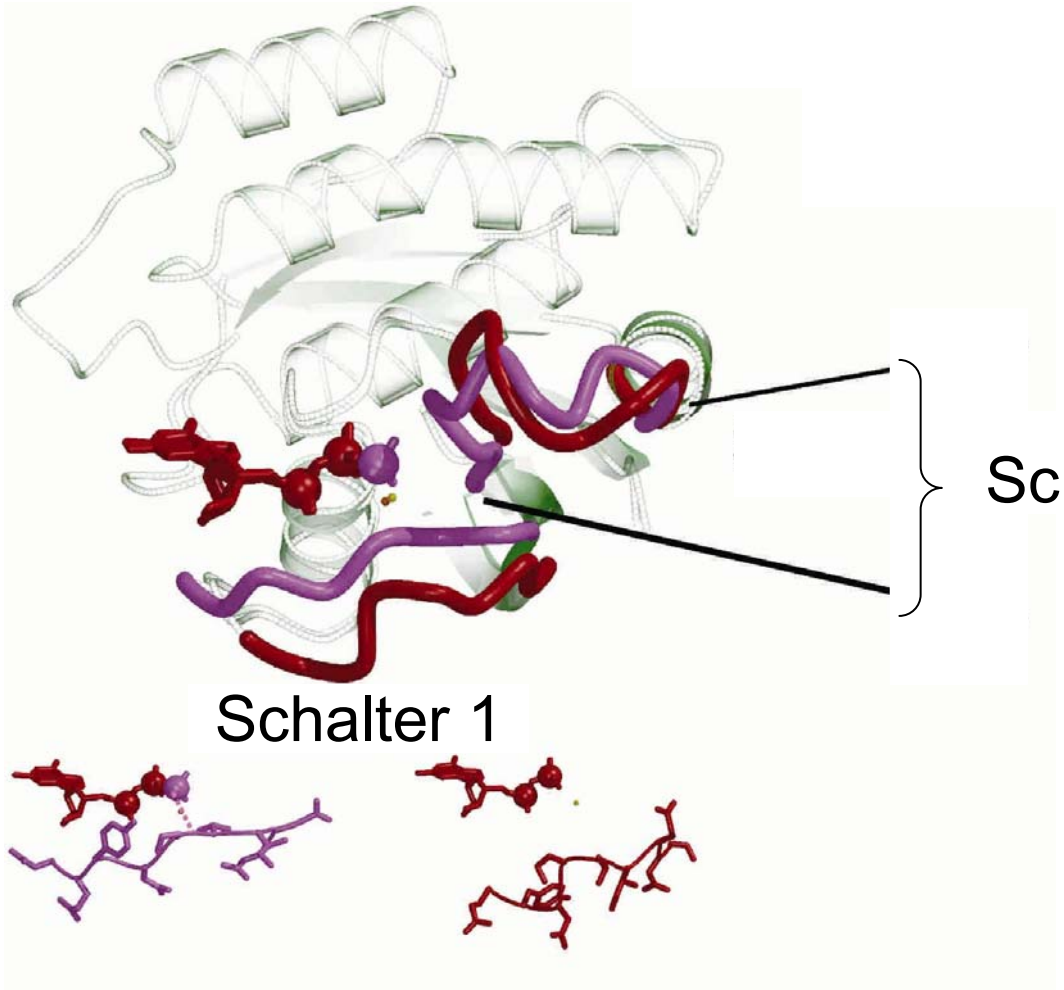
Die GTP-Form

Nachbarn des 3.Phosphats



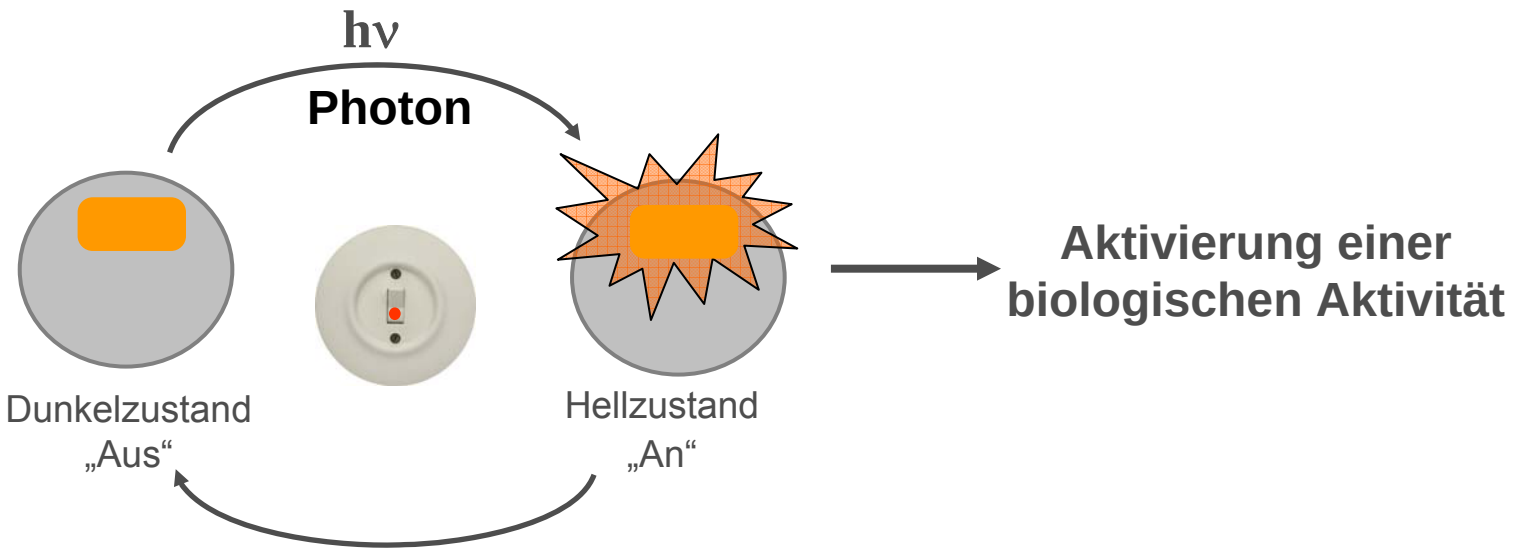
Konsequenzen des Verlusts des 3. Phosphats

- Änderungen in den Schalterregionen,
die mit Effektor-Molekülen wechselwirken

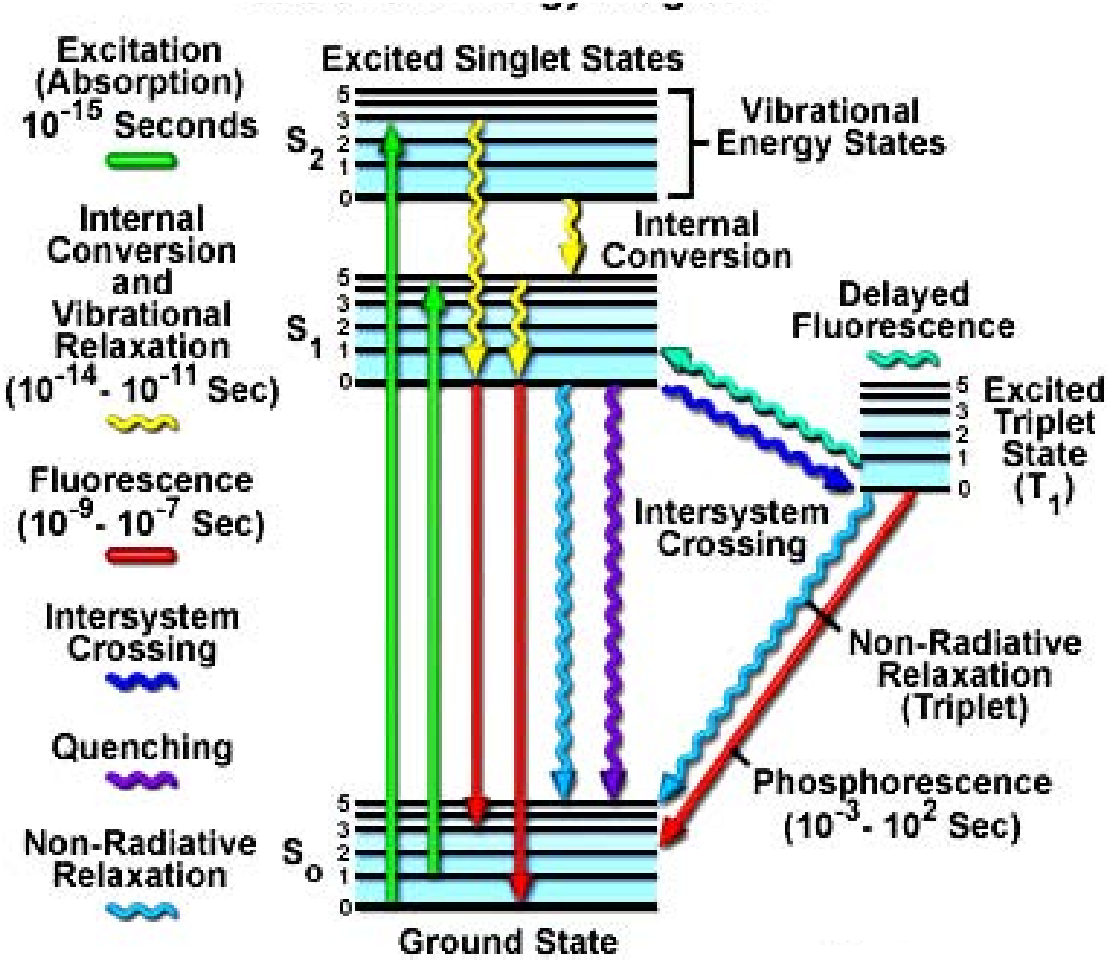


Molekulare Lichtschalter

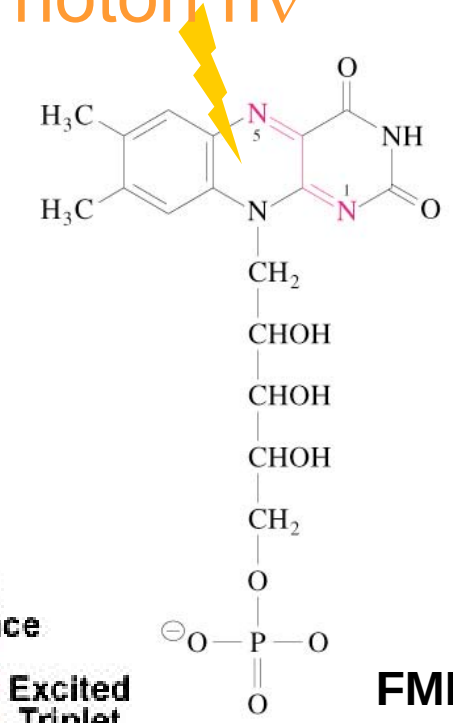
- Sehen
- Circadianer Rhythmus (Tag/Nacht)
- Bakterielle Phototaxis
- Planzen Wachstum und Bewegung



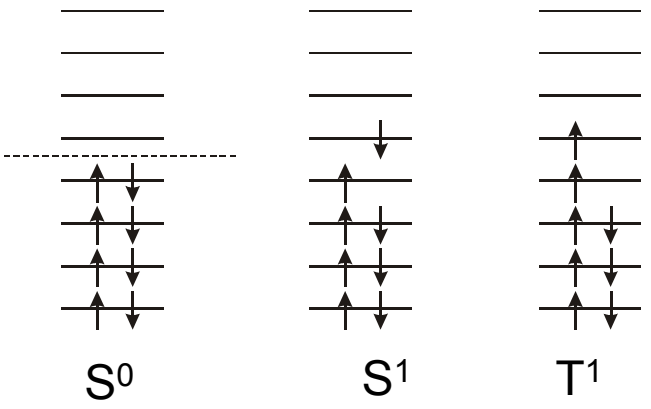
Photon Absorption durch Flavin

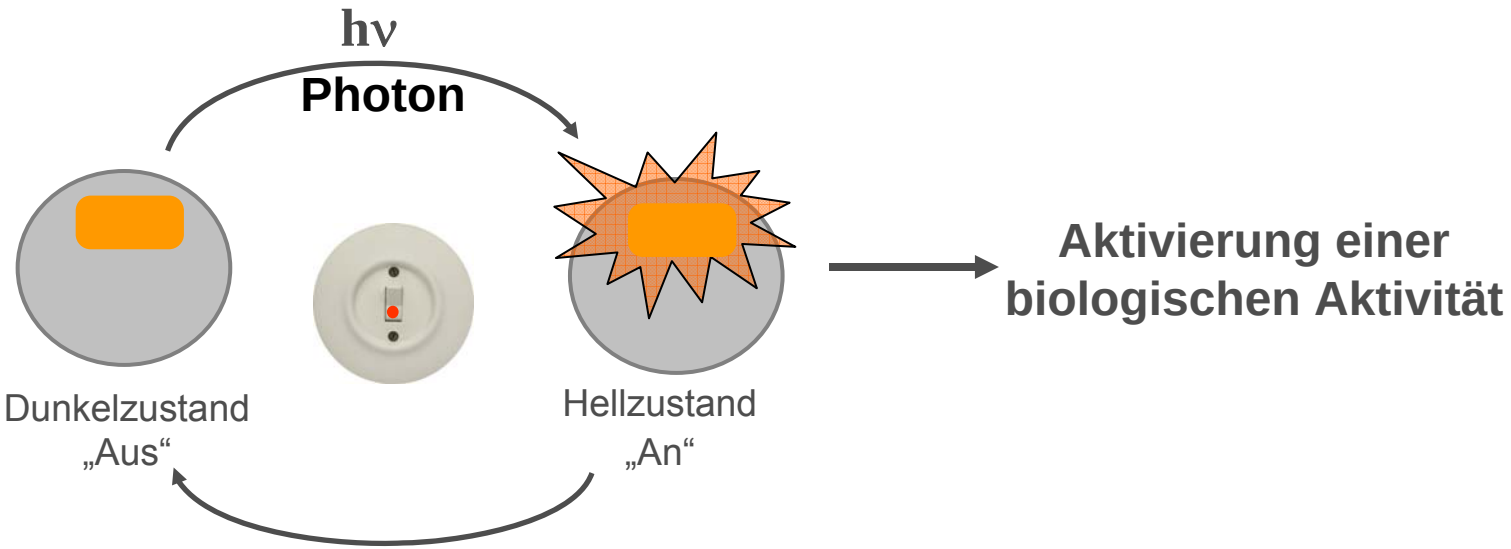


http://www.olympusmicro.com/primer/java/jablonski/jabintro/index.html



Richard Kuhn
Nobel Preis 1938





- Spektroskopie → Photozyklus
- Quantenchemie → Reaktivitäten, elektronische- und Spinzustände, Selektivitäten
- Raumstrukturen im Hell- und Dunkelzustand
- Dynamik
- Aktivierung der Effektordomäne
- Regulation des Schalters
- Design neuer Schalter → Zellbiologische Anwendungen

Röntgenkristallographie an Makromolekülen

Ziel
Strukturaufklärung von Makromolekülen, mit dem Ziel, den der biologischen Funktion unterliegenden chemischen Mechanismus zu verstehen.

Anwendungen
1. Zell- und Molekularbiologie
2. Chemie und Physikalische Chemie
3. Medikamenten Entwicklung

Vorteile
1. Reife Disziplin auf hohem technischem Niveau, erlaubt statische und zeitaufgelöste Messungen
   Röntgenstrukturen: ~36,000 (>5,000 in 2006), NMR Strukturen: ~6,000
2. Keine Einschrankung an die Grösse des zu untersuchenden Moleküls

   Synchrotronstrahlungsquellen

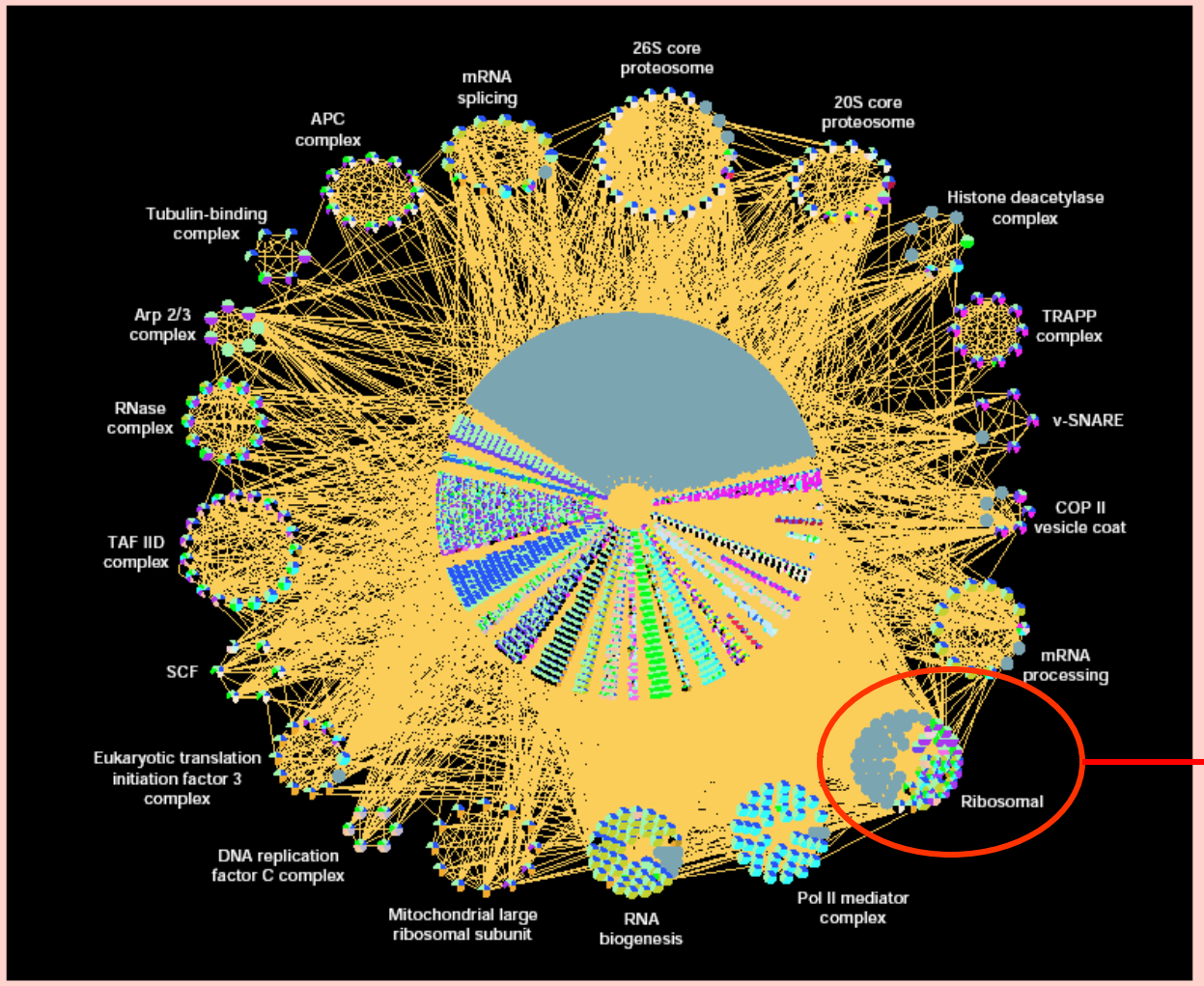
Kann Proteinkristallographie noch verbessert werden?

1. Keine Erfordernis einer **kristall**inen Probe.
2. Vermeidung des **“Phasenproblems”** durch direkte Messungen

“Cryo-Elektronenmikroskopie” mit “Einzelpartikelrekonstruktion” erfüllt diese Bedingungen bereits.
Das Problem des Strahlenschadens existiert jedoch auch dann noch.

Sind aus biologischer Sicht weitere technische Entwicklungen nötig?

Ja, um den Herausforderungen durch Supramolekulare-Komplexe zu begegnen



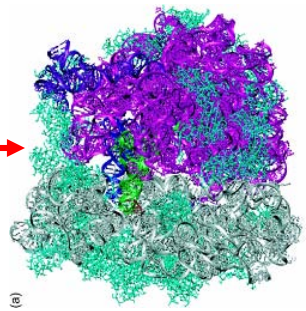
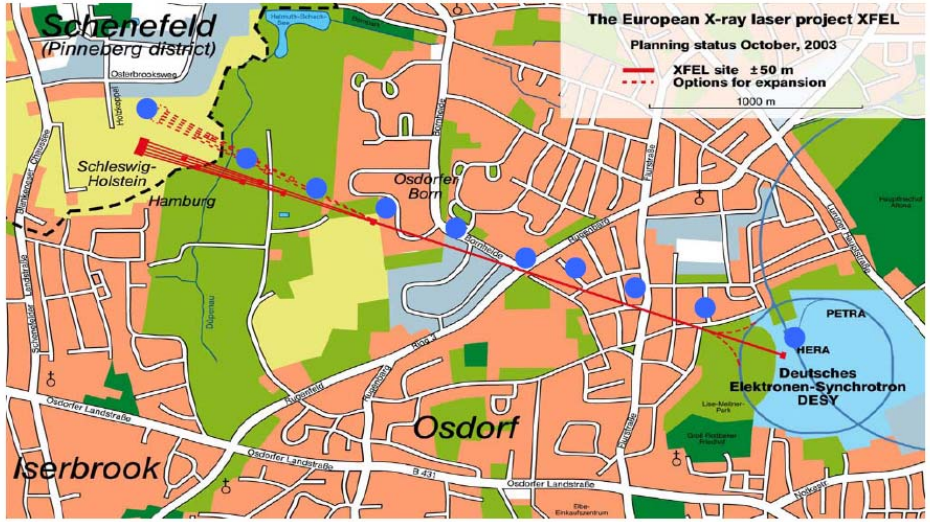


Figure 2 Visualization of combined, large-scale interaction data sets in yeast. A total of 14,000 physical interactions obtained from the GRID database were represented with the Osprey network visualization system (see http://biodata.mshri.on.ca/grid). Each edge in the graph represents an interaction between nodes, which are coloured according to Gene Ontology (GO) functional annotation. Highly connected complexes within the data set, shown at the perimeter of the central mass, are built from nodes that share at least three interactions within other complex members. The complete graph contains 4,543 nodes of ~6,000 proteins encoded by the yeast genome, 12,843 interactions and an average connectivity of 2.82 per node. The 20 highly connected complexes contain 340 genes, 1,835 connections and an average connectivity of 5.39.

Tyers & Mann, Nature 422, 193-197, 2003

X-FEL

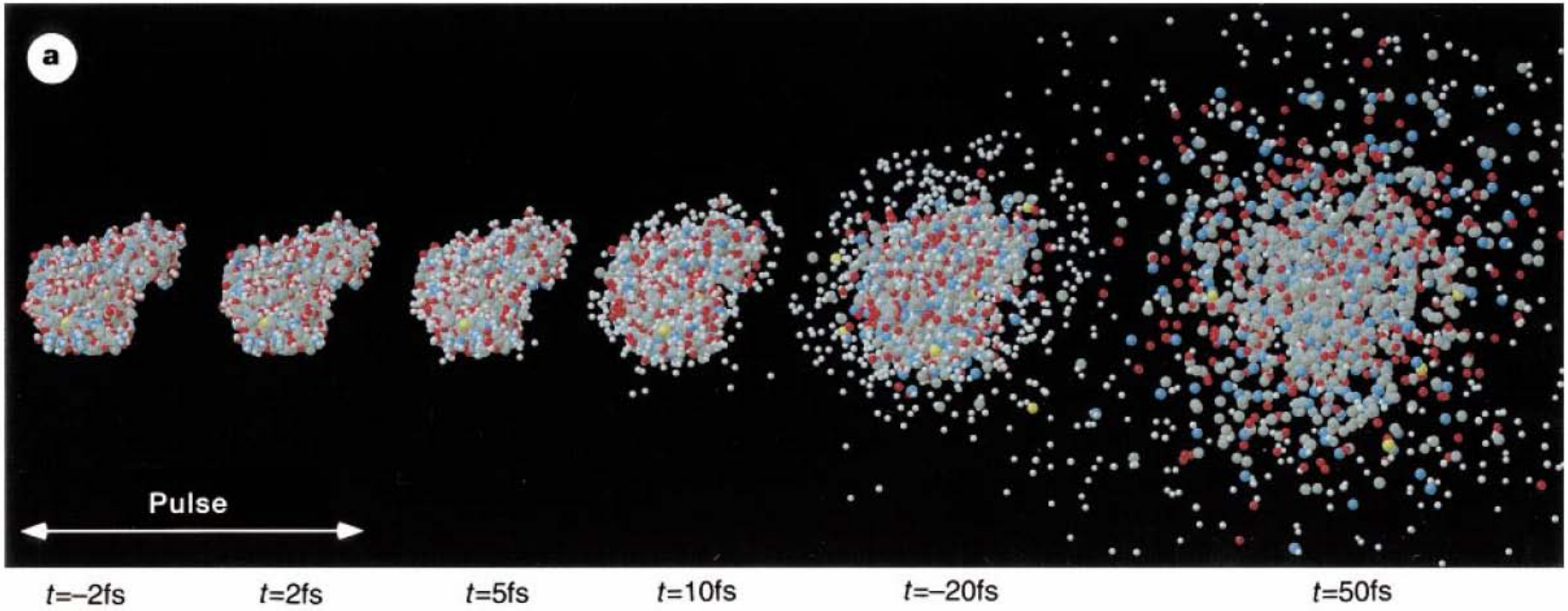
Freie-Elektronen-Laser, der nach dem SASE-Prinzip arbeitet
(self-amplified spontaneous emission)
Gesamtlänge der Anlage: ca. 3,4 km
Länge des Beschleunigertunnels: ca 2,1 km
Tiefe unter der Erde: ca. 6 - 38 m
2 unterirdische Experimentierhallen mit je 10 Messplätzen, 2 Ausbaustufen
Wellenlänge der Röntgenstrahlung: 6-0,085 nm, bzw. 10-20 GeV)
Länge der Lichtpulse: unter 100 Femtosekunden (fs)
Projektierter Start 2013, Standort Hamburg



Potential for biomolecular imaging with femtosecond X-ray pulses

Richard Neutze*, Remco Wouts*, David van der Spoel*, Edgar Weckert†‡ & Janos Hajdu*

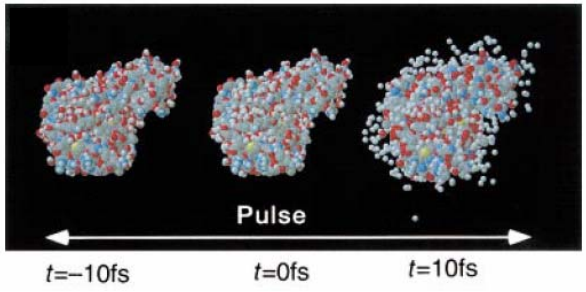
Henderson limit may be extended at very high dose rates and very short exposure times



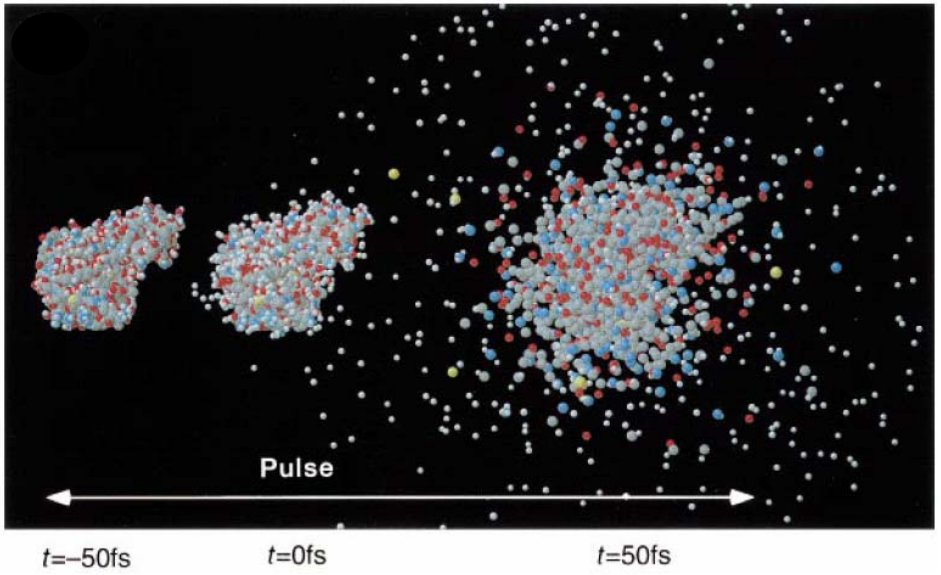
Nature **406**:752-757. 2000

Hit it hard and fast

10fs HW-HM pulse



50fs HW-HM pulse



Nature **406**:752-757. 2000

X-FEL - the best thing since sliced bread?

Scientific and Programmatic Recommendations – U.S.

1999, Leone BESAC Subpanel Report concluded:
"**Novel, Coherent Light Sources**" …
"Given currently available knowledge and limited funding resources, the **hard X-ray region** (8-20 keV or higher) is **identified as the most exciting potential area for innovative science….**

. . . Should this new femtosecond window in imaging provide a path to high-resolution structural information without the need for macroscopic crystals, its impact on structural biology would be tremendous.

Nature **406**:752-757. 2000