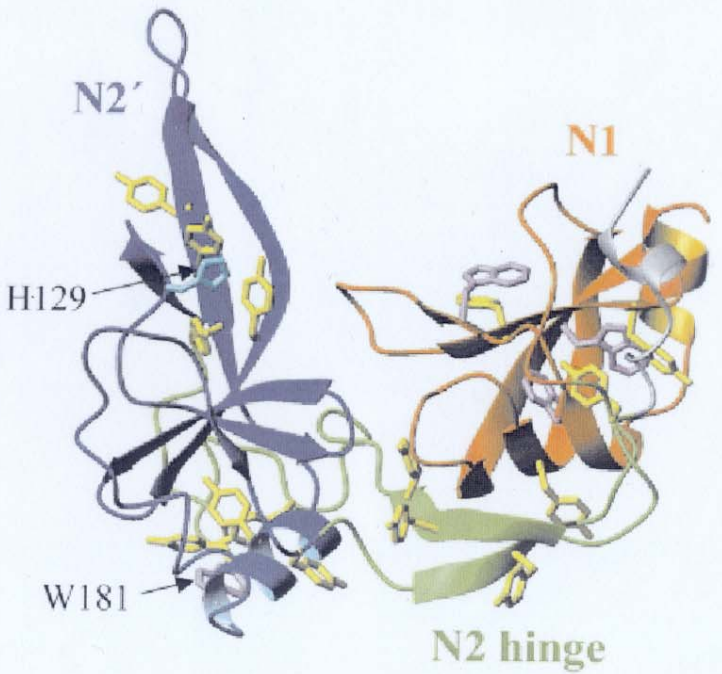
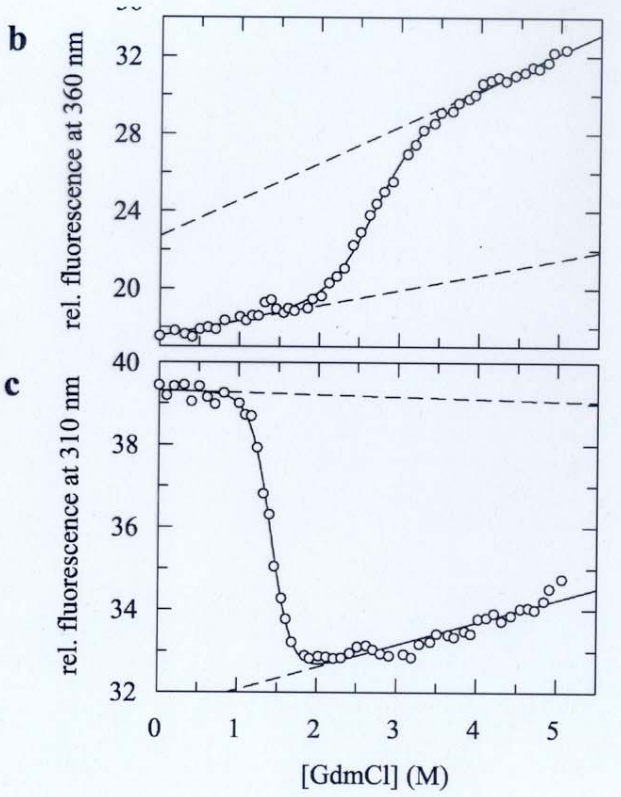
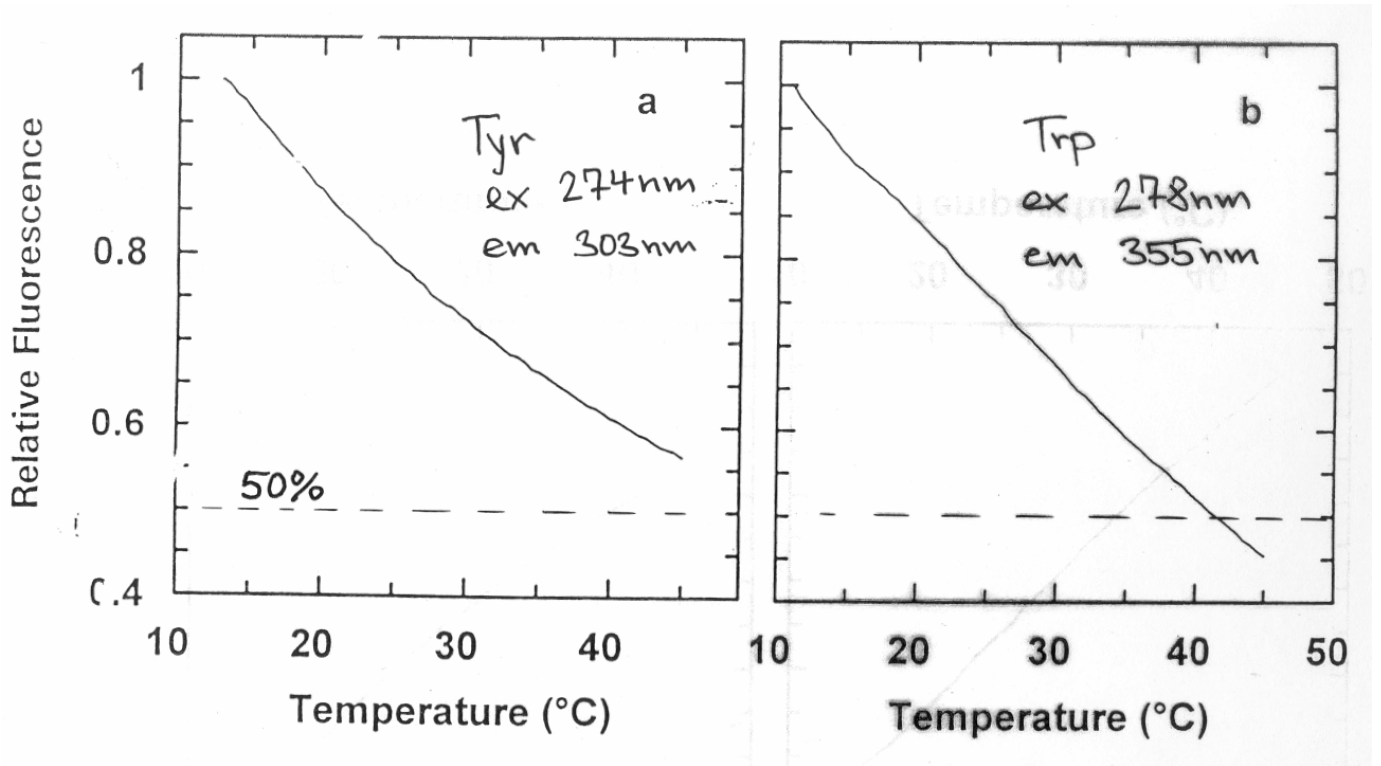
Gen-3 Protein des fd-Phagen

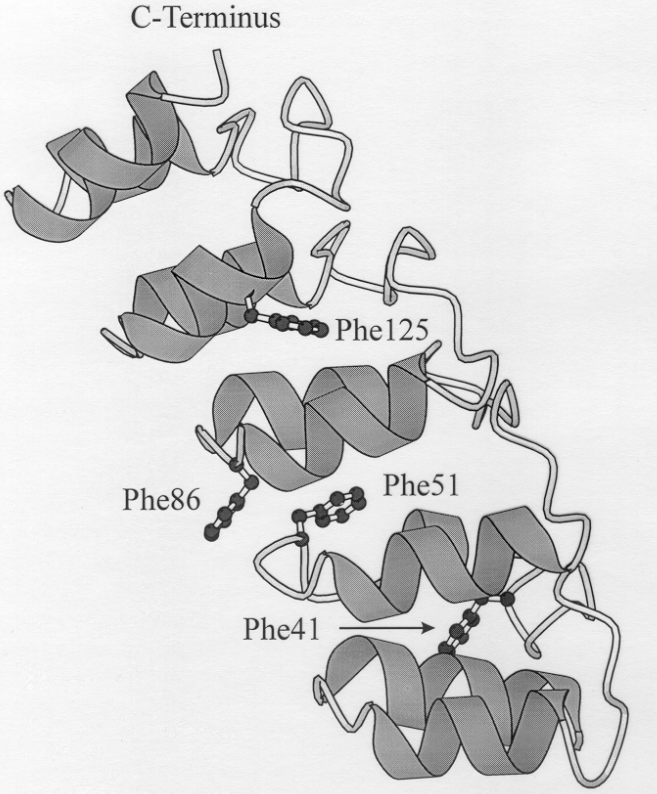




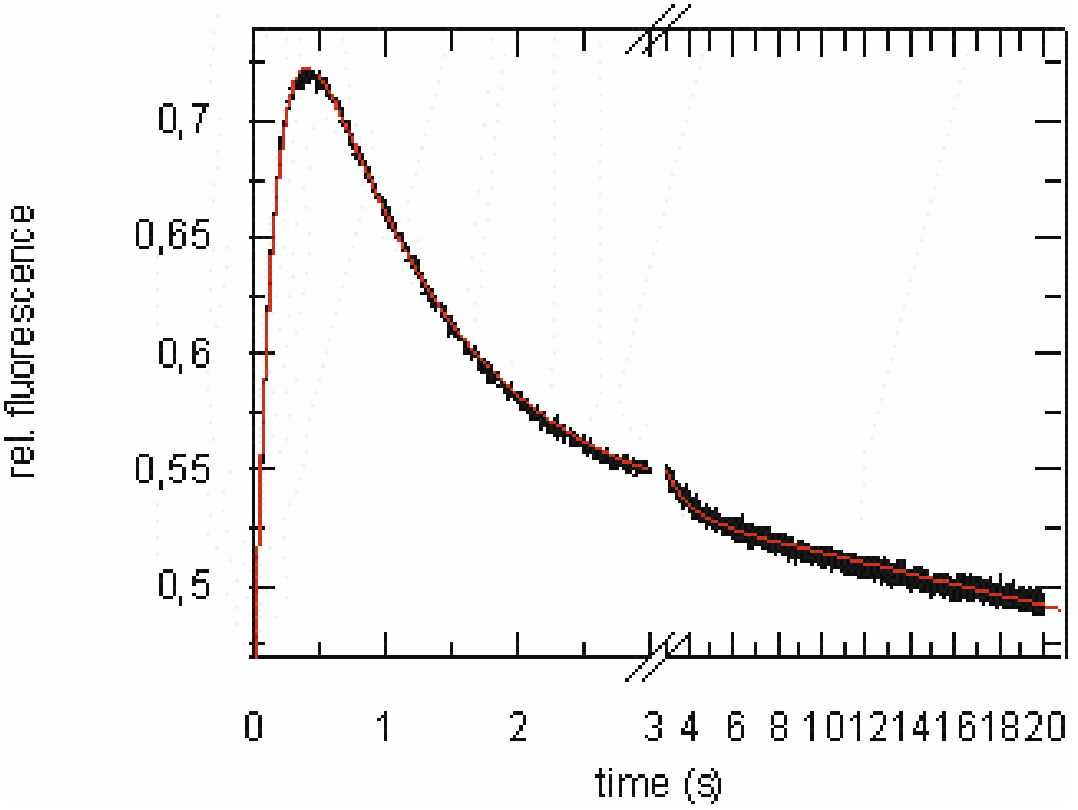
[Martin, A. & Schmid, F.X. (2003), *J. Mol. Biol.* **328**, 863-875 und **329**, 599-610.]


Relative Fluorescence
1
0.8
0.6
.4
50%
a
Tyr
ex 274nm
em 303nm
10
20
30
40
Temperature (°C)
b
Trp
ex 278nm
em 355nm
10
20
30
40
50
Temperature (°C)

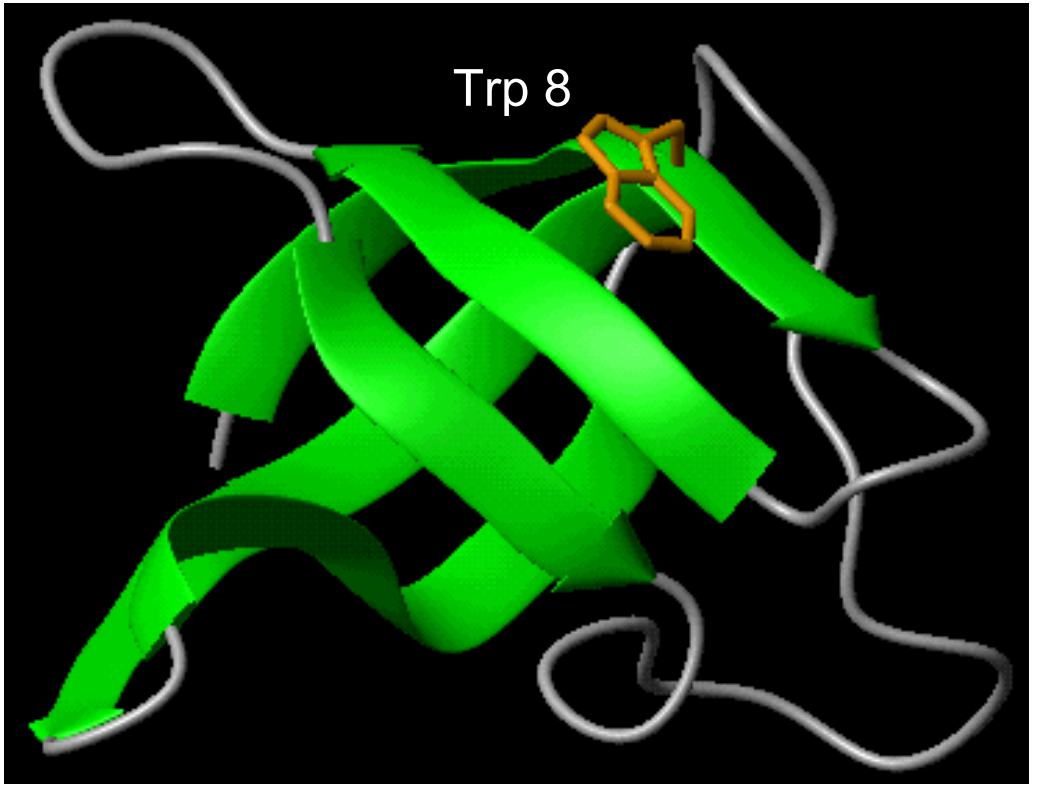
CDK-Inhibitor P19^{INK4d}



Rückfaltung von P19-H96W
(6M Harnstoff -> 0.45M Harnstoff)



Kälteschockprotein CspB



DNA-Fragment Y-Box25:

5‘-ATCCTACTGATTG
GCCAAGGTGCTG-3‘

Zeeb, M., Balbach, J., (2003)
Protein. Science, **12**, 112-123.

Isotherme Titration von CspB mit ssDNA (Y-Box 25)

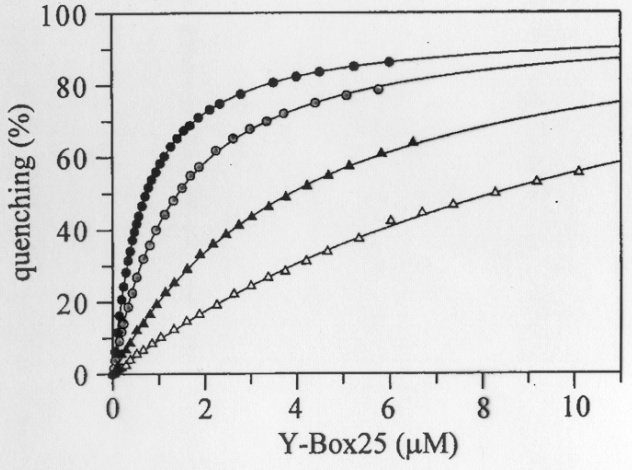


Figure 7. Quenching effect on the Trp8 fluorescence of CspB upon binding to Y-Box25. The hyperbolic binding isotherms of 0.25 μM wild-type CspB (open circles), G35A (filled circles), F38A (open triangles), and G54P (filled triangles) depict different Y-Box25 binding affinities in 50 mM sodium cacodylate/HCl, 100 mM KCl, pH 7.0 at 15°C. The resulting K_D values from fits using Equation 1 (solid lines) are given in Table 1.

Table 1. *Dissociation constants of CspB/Y-Box25 complexes and thermodynamic stability of the respective CspB variant*

Protein	$K_D \cdot 10^{-6}$ (M)	T_m (°C)	ΔG_U (15°C) (kJ/mole)
Wild type	3.9 ± 0.1	55.7 ± 0.1	13.1 ± 0.4
F9A[a]	15.1 ± 0.6	34.0 ± 0.2	2.9 ± 0.1
K13Q[a]	21.6 ± 0.2	55.3 ± 0.2	12.7 ± 0.7
F15A[a]	27.2 ± 0.8	36.2 ± 0.1	4.7 ± 0.1
F15Y[a,c]	1.9 ± 0.1	55.5 ± 0.1	14.0 ± 0.6
F17A[a]	48.4 ± 1.6	41.2 ± 0.3	6.7 ± 0.4
F27A[a]	17.0 ± 0.3	47.9 ± 0.6	8.2 ± 1.1
H29Q[a]	27.5 ± 1.0	48.7 ± 0.3	9.1 ± 0.8
F30A[a]	40.8 ± 0.6	54.5 ± 0.1	13.4 ± 0.4
F30W[a,c]	1.4 ± 0.1	57.8 ± 0.1	14.1 ± 0.2
● G35A[b]	1.8 ± 0.1	48.5 ± 0.1	9.1 ± 0.2
G35P[b]	26.1 ± 0.8	44.2 ± 0.3	6.7 ± 0.5
△ F38A[a]	35.4 ± 1.1	56.8 ± 0.1	13.4 ± 0.6
G54A[b]	3.2 ± 0.1	46.2 ± 0.3	7.4 ± 0.6
▲ G54P[b]	12.1 ± 0.3	36.0 ± 1.1	2.5 ± 0.4
P58A[d]	4.4 ± 0.1	49.6 ± 0.2	9.4 ± 0.4

[a] CspB revealed at this positions extreme line broadening in the NMR titration experiment.
[b] CspB revealed at this positions strong chemical shift changes in the NMR titration experiment.
[c] Substitution based on thermophilic cold-shock proteins from *B. caldolyticus* and *T. maritima*.
[d] Substitution to increase the number of possible backbone conformations of loop β4–β5.

Assoziations- und Dissoziationsraten von CspB/ssDNA

CspB variant	$k_{on.}$ 10^8 ($M^{-1}s^{-1}$)	k_{off} (s^{-1})
wild type	2.4 ± 0.1	2.4 ± 0.1
F15Y	2.5 ± 0.1	2.2 ± 0.1
K13Q	1.1 ± 0.1	5.5 ± 0.2
F15A	1.1 ± 0.1	36 ± 2
F27A	1.6 ± 0.1	103 ± 2
H29Q	1.7 ± 0.1	35 ± 2
F30A	3.1 ± 0.2	177 ± 3
F38A	2.4 ± 0.1	137 ± 4
G35P	1.5 ± 0.1	73 ± 1
P58A	1.5 ± 0.1	1.4 ± 0.7

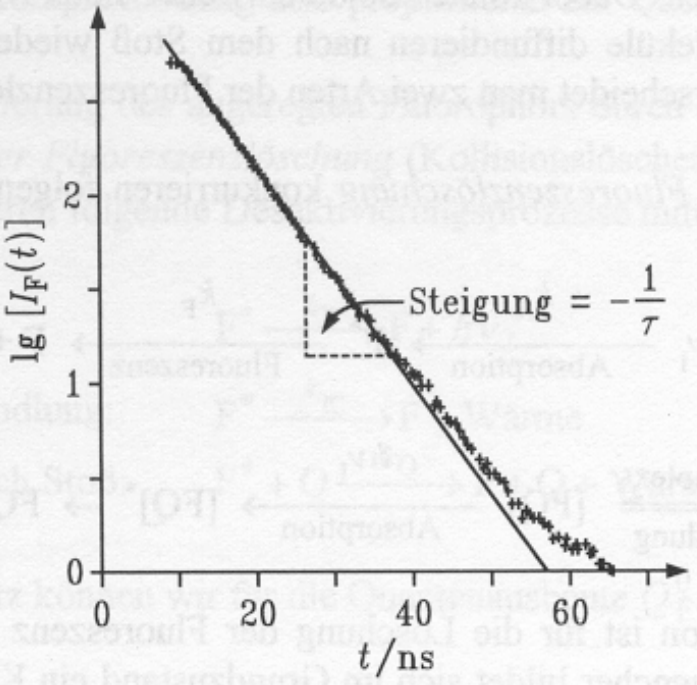


Abb. V.77:
Halblogarithmische Darstellung der Fluoreszenzabnahme der Y-Base in Hefe-t-RNA^{Phe}. Nach der elektronischen Anregung durch den Anregungspuls nimmt die Fluoreszenz in dieser Darstellung linear mit der Zeit ab (nach: C.R. Cantor, T. Tao, in *Procedures in Nucleic Acid Research*, Vol. 2, S. 31, Haper & Row, New York, 1971).

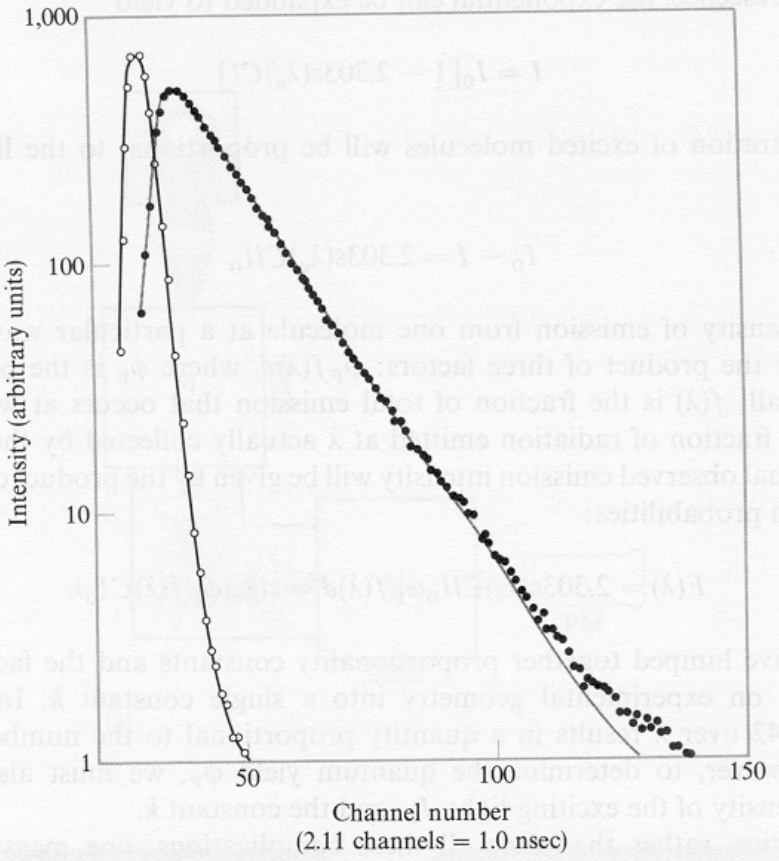
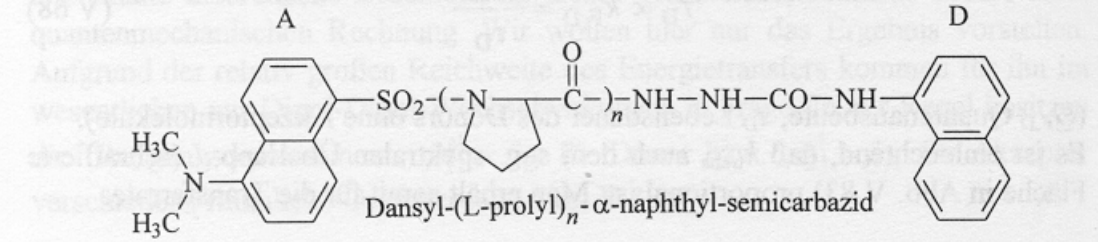


Figure 8-14

Fluorescence decay of the Y base in yeast $tRNA^{Phe}$. The black line shows the exciting pulse. The gray line through the fluorescence observations was generated from a knowledge of the shape of the exciting pulse and the assumption that the excited singlet state decays as a single exponential with $\tau_F = 6.2$ nsec. [After C. R. Cantor and T. Tao, in *Procedures in Nucleic Acid Research*, vol. 2 (New York: Harper & Row, 1971), p. 31.]



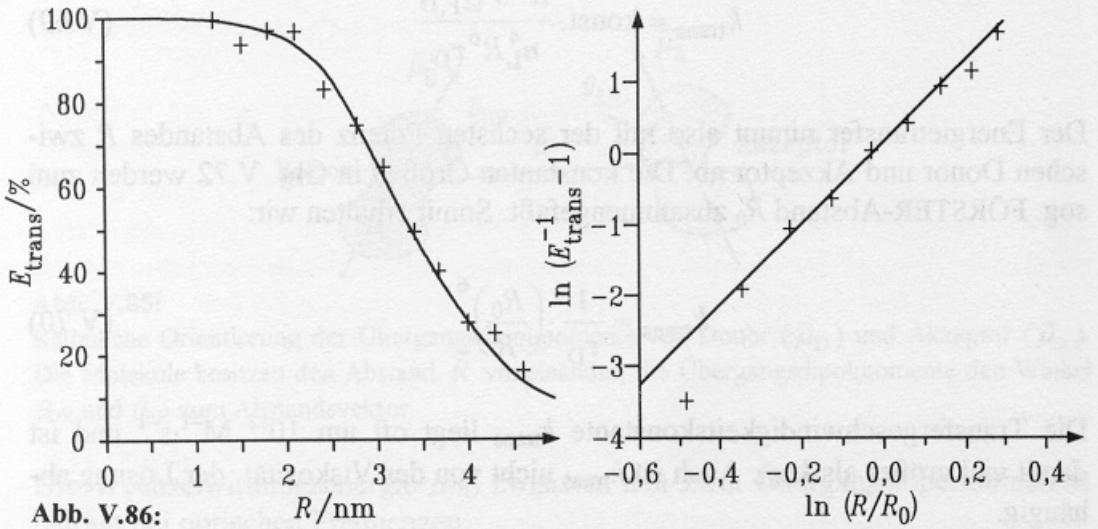


Abb. V.86:
Transfereffizienz der α-Naphthylgruppe auf die Dansylgruppe in dem Oligopeptid Dansyl-(L-prolyl)$_n$-α-naphthyl-semicarbazid für n = 1 bis n = 12. Der Donor-Akzeptorabstand variiert zwischen 12 Å (n = 1) und 46 Å (n = 12). Die durchgezogene Linie (links) ist für einen FÖRSTER-Radius von 3,46 nm berechnet. Aus der doppelt logarithmischen Darstellung (rechts) erhält man eine Steigung von 5,9 ± 0,3 (nach: L. Stryer, R. Haugland, Proc. Natl. Acad. Sci. USA **58** (1967) 719).

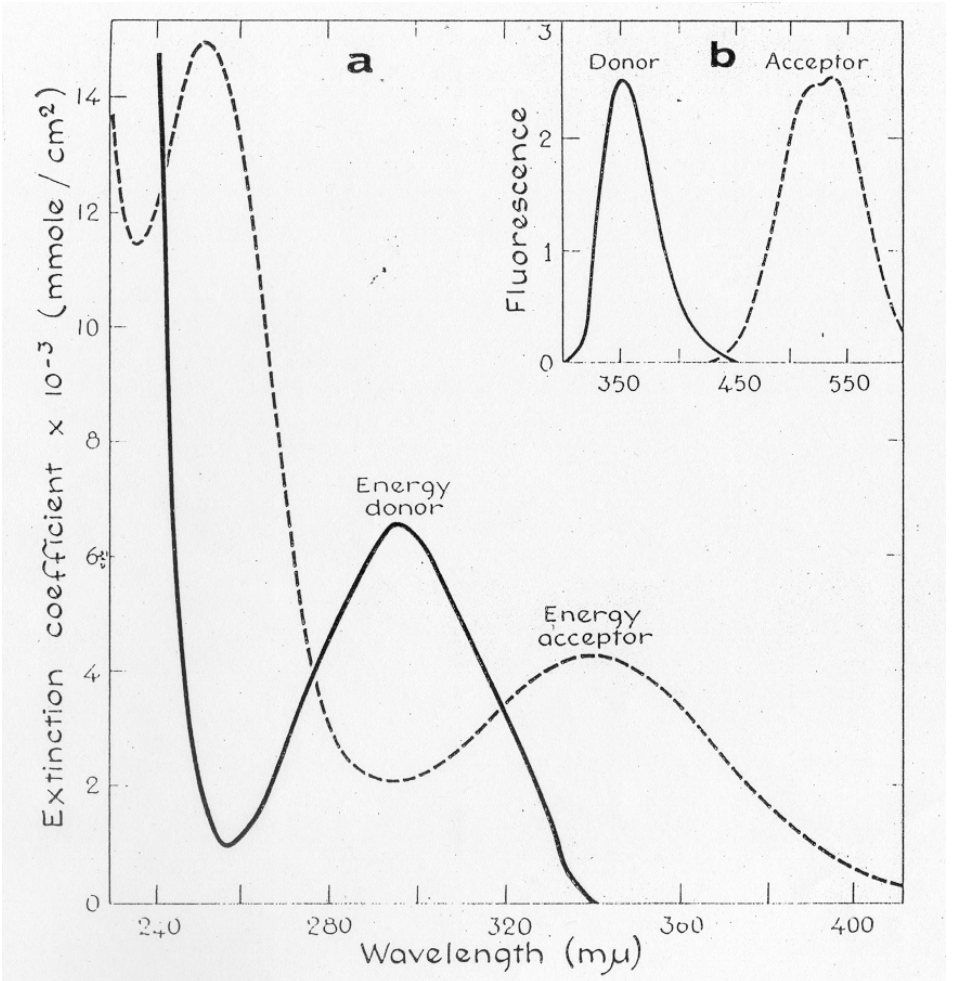
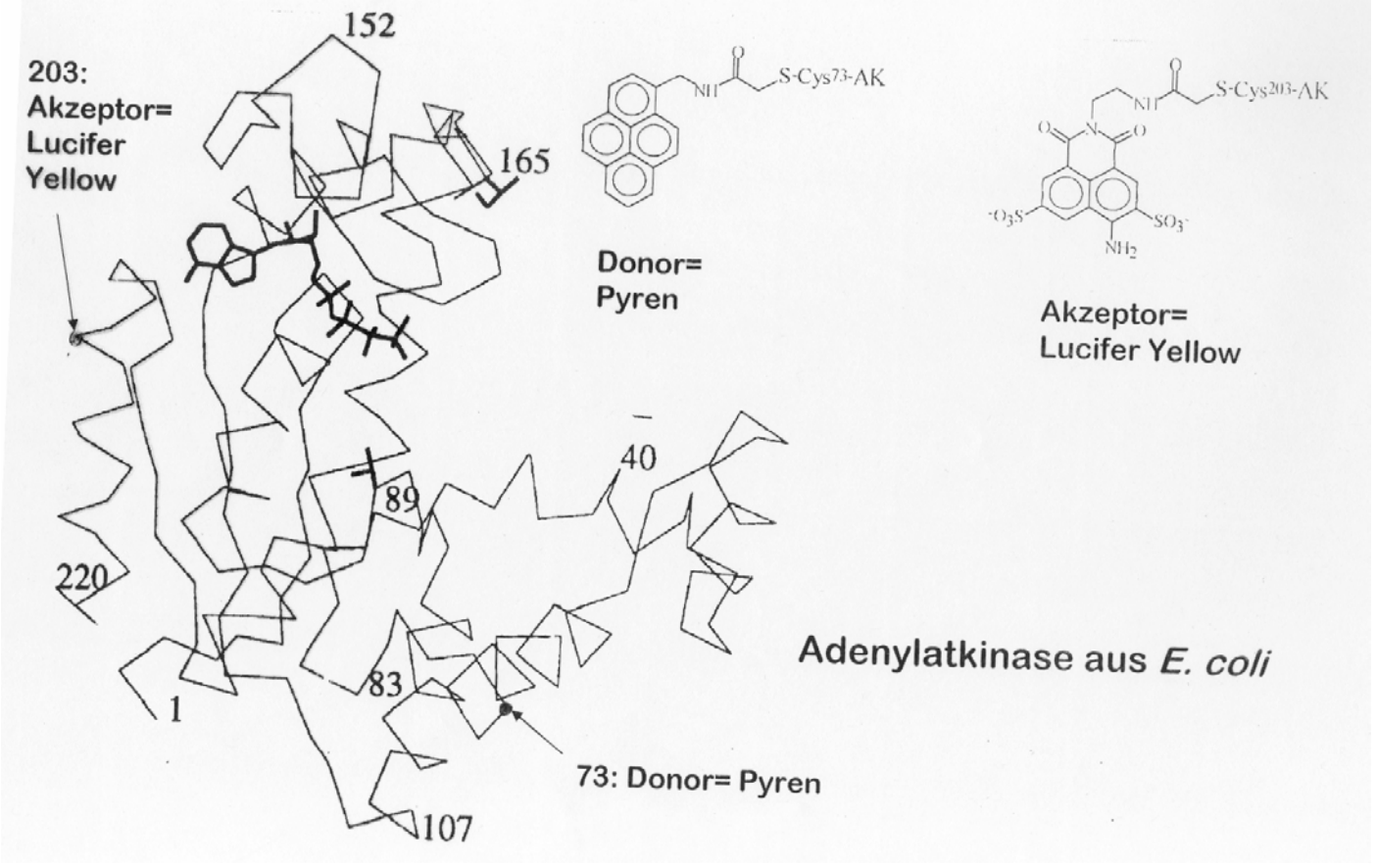


FIG. 2.—(*a*) Absorption spectrum of the energy donor (1-acetyl-4-(1-naphthyl) semicarbazide, ———) and the energy acceptor (dansyl-L-prolyl-hydrazide, - - - - -) in ethanol; (*b*) emission spectrum of the energy donor (———) and the energy acceptor (- - - - -) in ethanol.


152
203:
Akzeptor=
Lucifer
Yellow
165
S-Cys73-AK
Donor=
Pyren
S-Cys203-AK
Akzeptor=
Lucifer Yellow
40
89
220
83
1
73: Donor= Pyren
107
Adenylatkinase aus E. coli

Adenylatkinase

Donor Pyren
Akzeptor Lucifer Yellow

Veränderungen der Abklingzeiten
während der Faltung

Ratner, Sinev & Haas,
J. Mol. Biol. 299 (2000) 1383-91