Targeting by peptide conjugates

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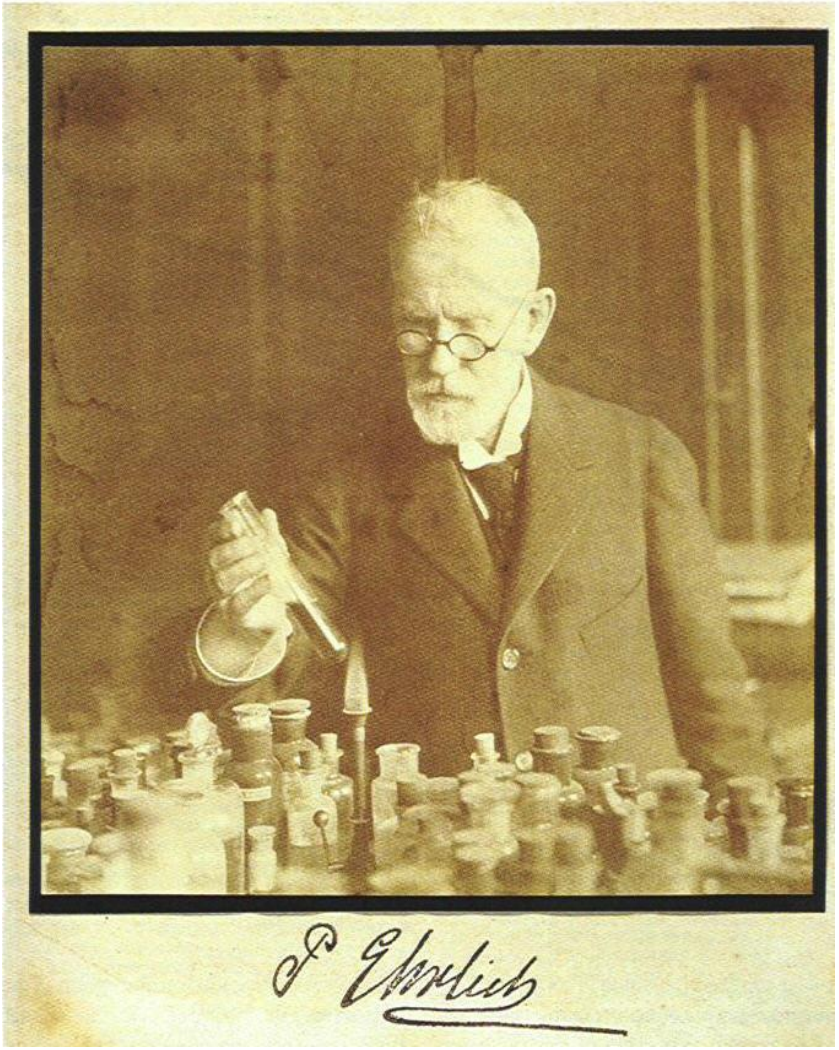
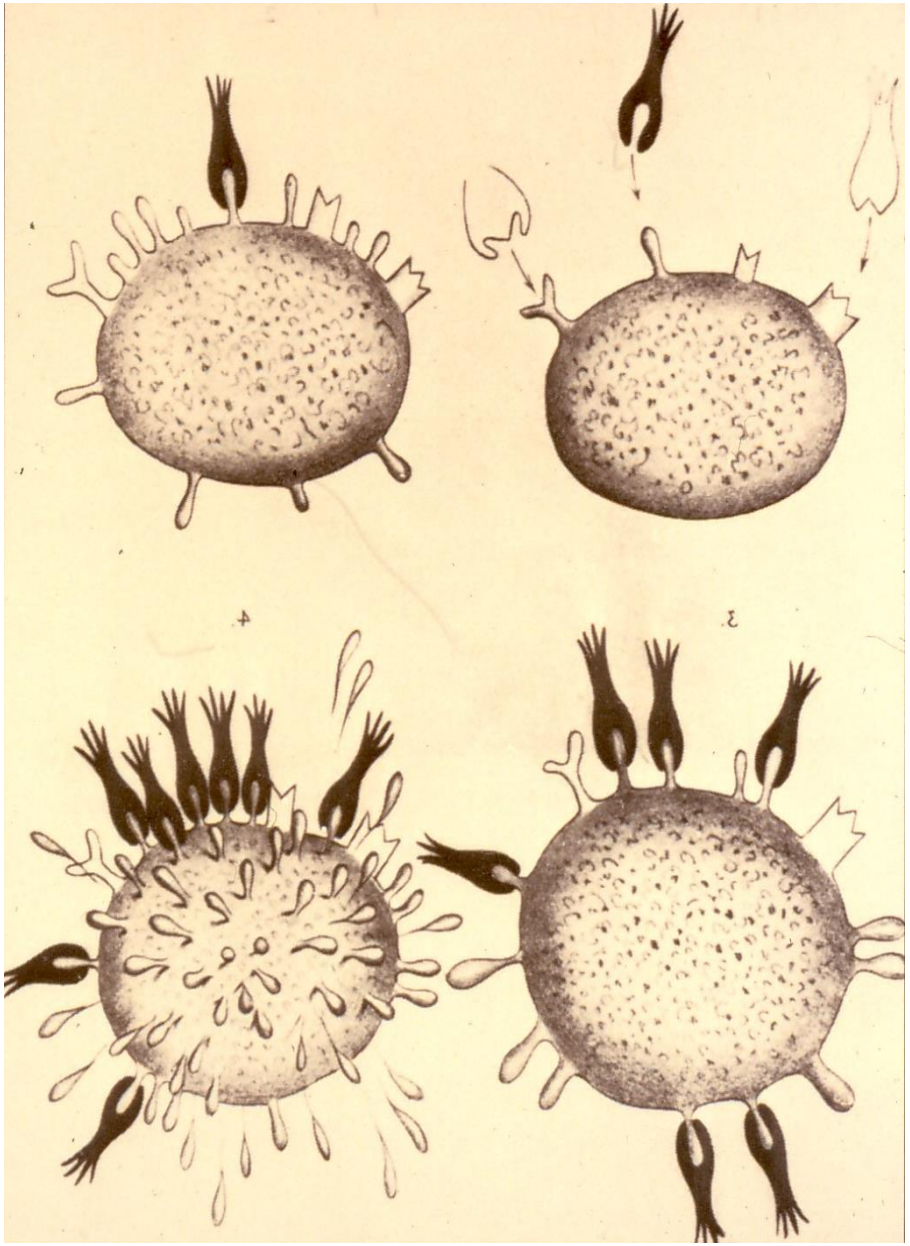
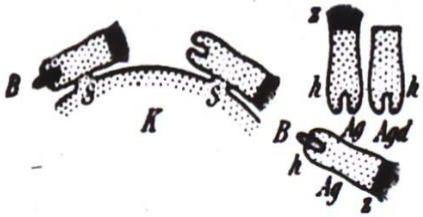


Paul Ehrlich
1854-1915
(Wellcome Library, London)

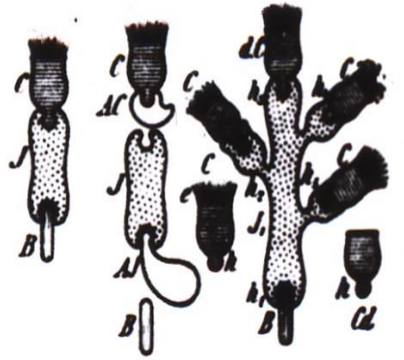


Rezeptoren I. Ordnung.

Rezeptoren II. Ordnung.



Rezeptoren III. Ordnung.



Unizeptoren.

Rezeptoren I. Ordnung.

T	Toxin mit h haptophorer Gruppe.
	t toxophorer „
Td	Toxoid „ h haptophorer „
K	Körperzelle.
S	Seitenketten.
At	Antitoxin.

Rezeptoren II. Ordnung.

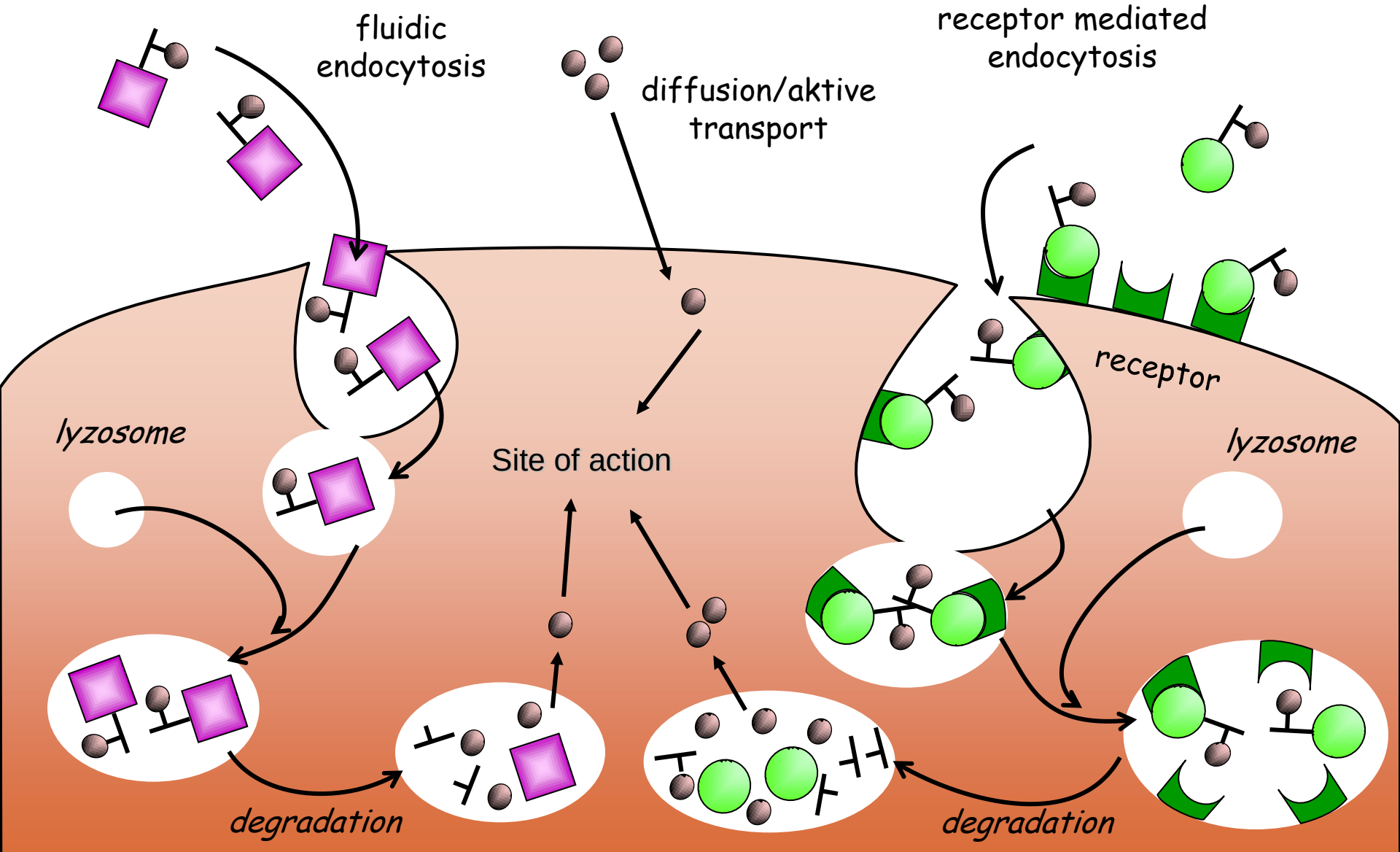
K	Körperzelle.
S	Seitenketten.
B	Bazillus.
Ag	Agglutinin mit h haptoph. Gruppe.
	z zymoph. „
Agd	Agglutinoid „ h haptoph. „

Ambozeptoren.

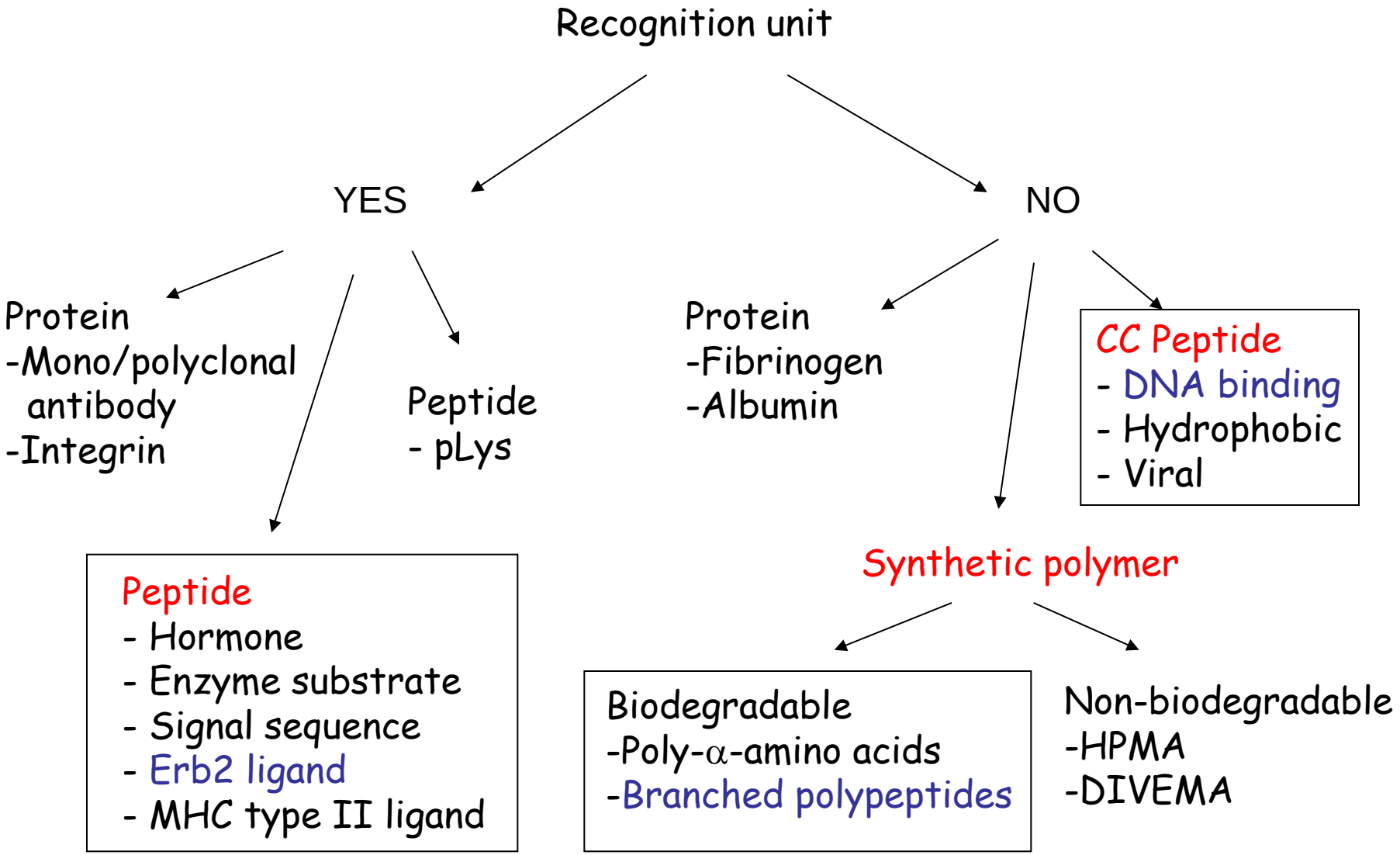
Rezeptoren III. Ordnung.

K	Körperzelle.
S	Seitenketten.
B	Bazillus.
J	Immunkörper mit zwei haptophoren Gruppen:
	h_1 1. haptophore oder zytophile,
	h_2 2. haptophore oder komplementophile Gruppe.
AJ	Antiimmunkörper.
C	Komplement mit h haptoph. Gruppe.
	e ergophorer „
Cd	Komplementoid mit h haptoph. „
AC	Antikomplement.
J_1	Immunkörper mit mehreren komplementophilen Gruppen h_2 bis h_n.
d.C	dominantes Komplement.

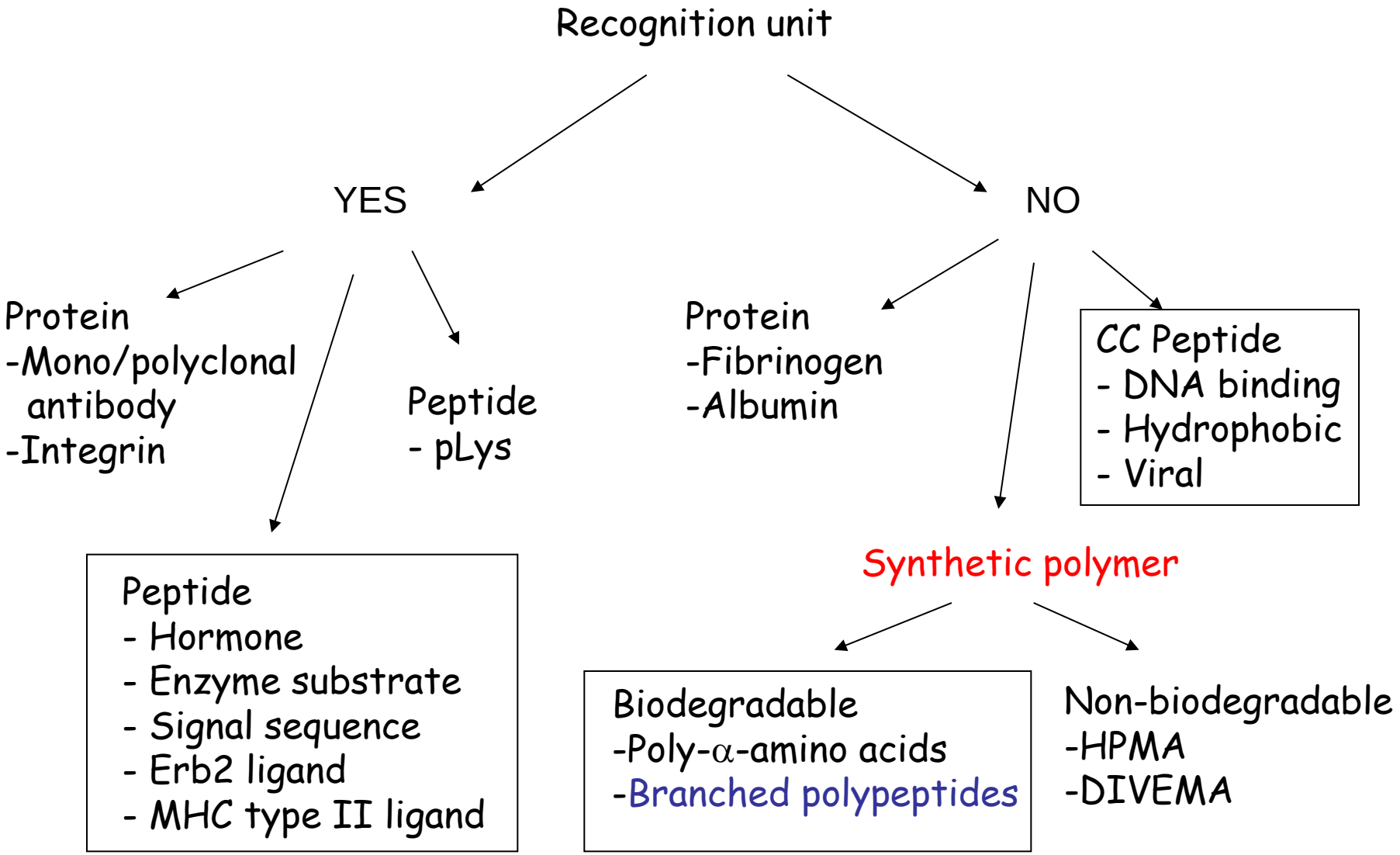
Uptake and liberation of bioactive entities



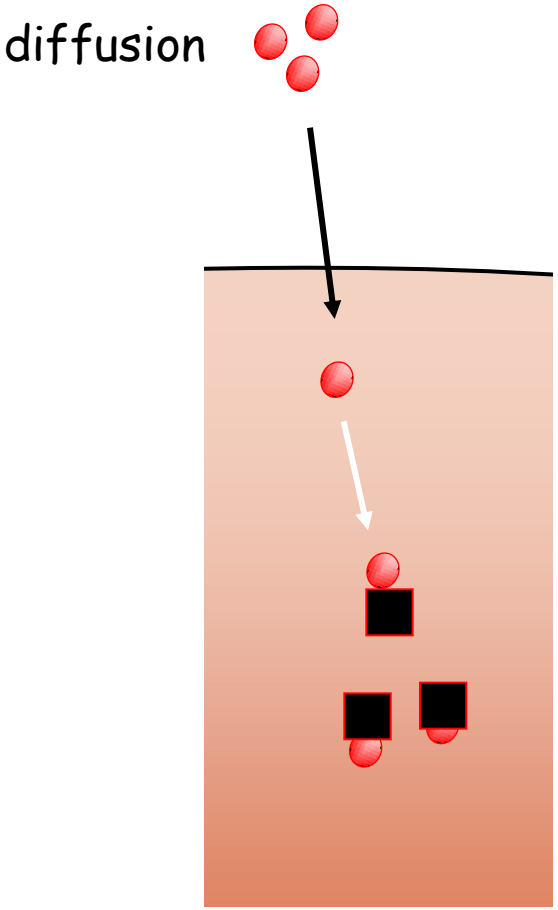
Peptide/protein based drug targeting/delivery



Peptide/protein based drug targeting/delivery



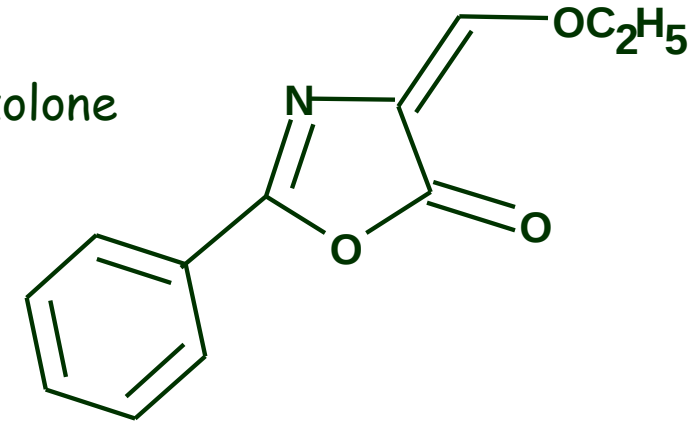
Localization of intracellulae KDEL-receptor by „reporter molecule"-peptide conjugate

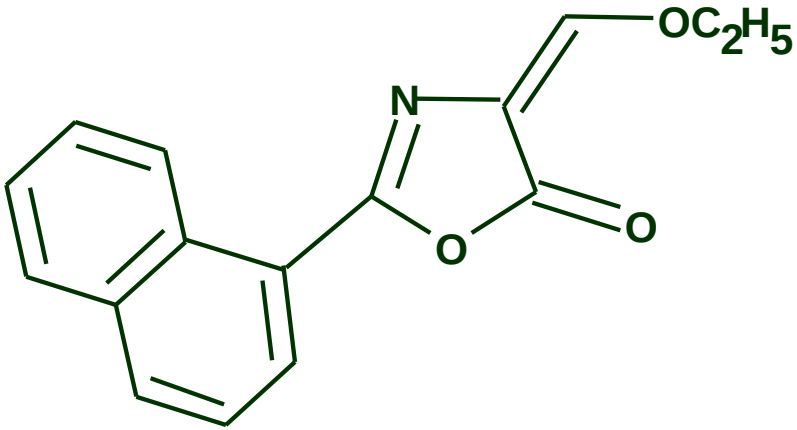


naOx – peptide conjugates

New fluorophore

4-etoximethylene-2[1]-phenyl-5(4H)-oxazolone
(phOx)



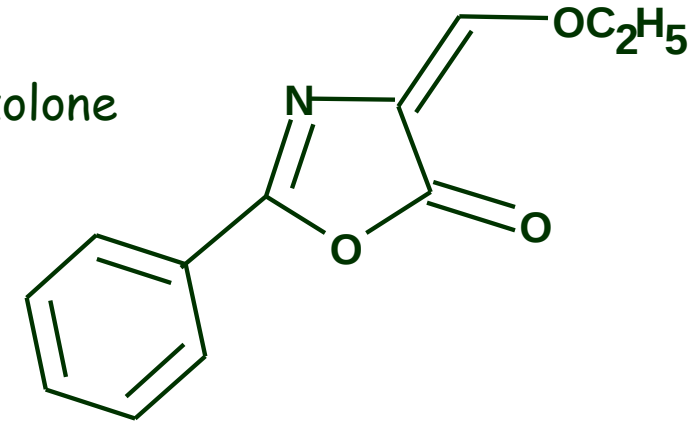


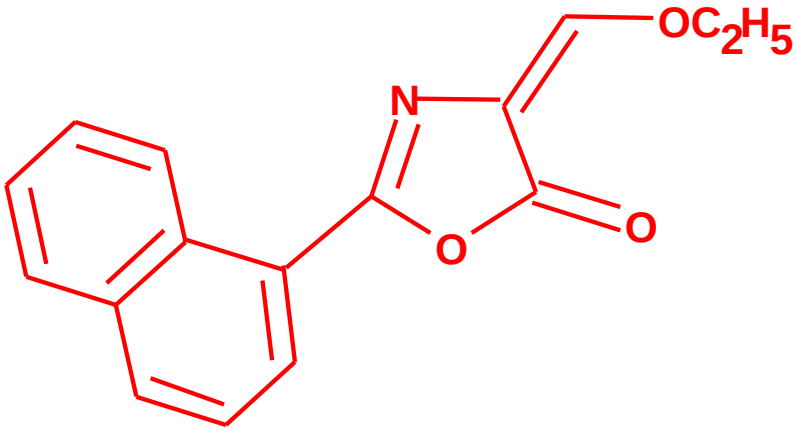
4-etoximethylene-2[1]-naftil-5(4H)-oxazolone
(naOx)

Kóczán Gy. et. al. Tetrahedron ***57****: 4589 (2001)*

New fluorophore

4-etoximethylene-2[1]-phenyl-5(4H)-oxazolone
(phOx)

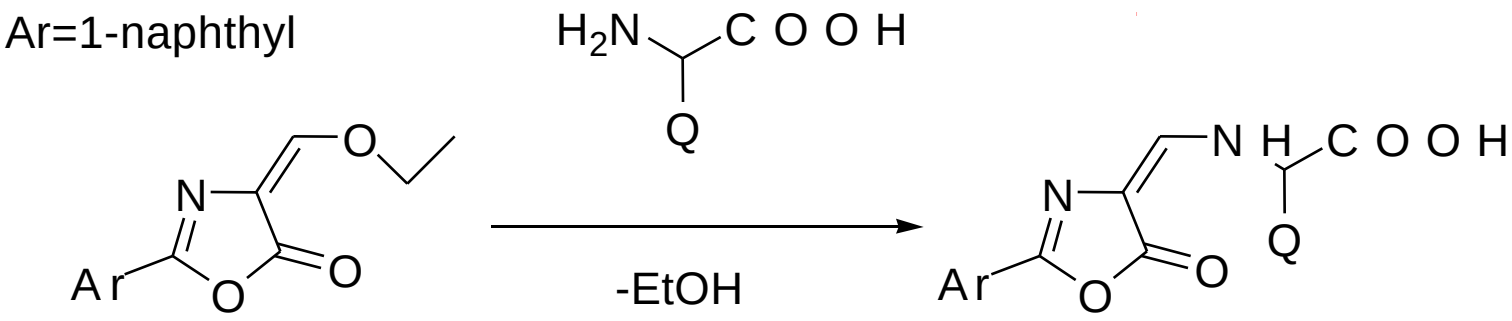


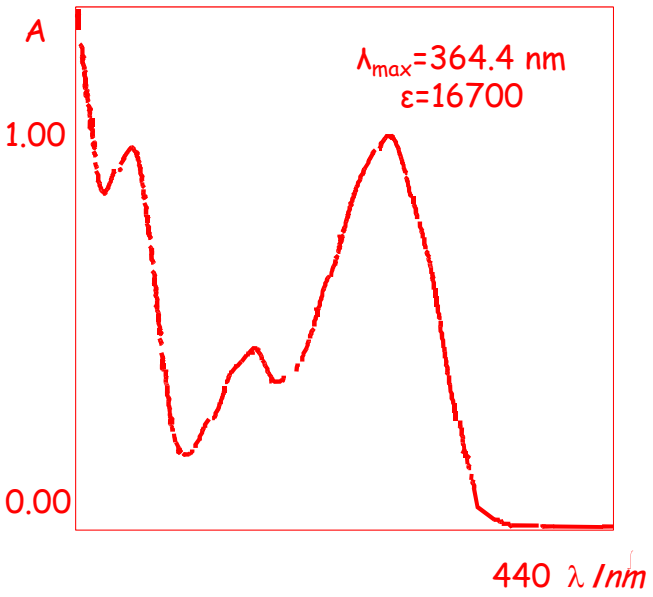


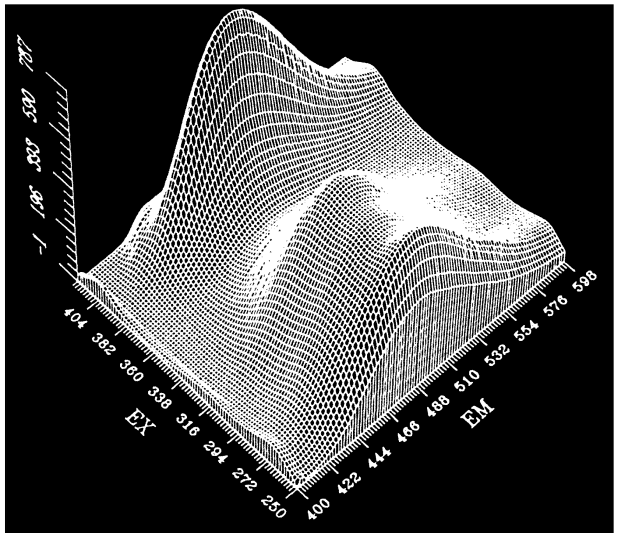
4-etoximethylene-2[1]-napthyl-5(4H)-oxazolone
(naOx)

Kóczán Gy. et. al. Tetrahedron ***57****: 4589 (2001)*

Fluorescent amino acids



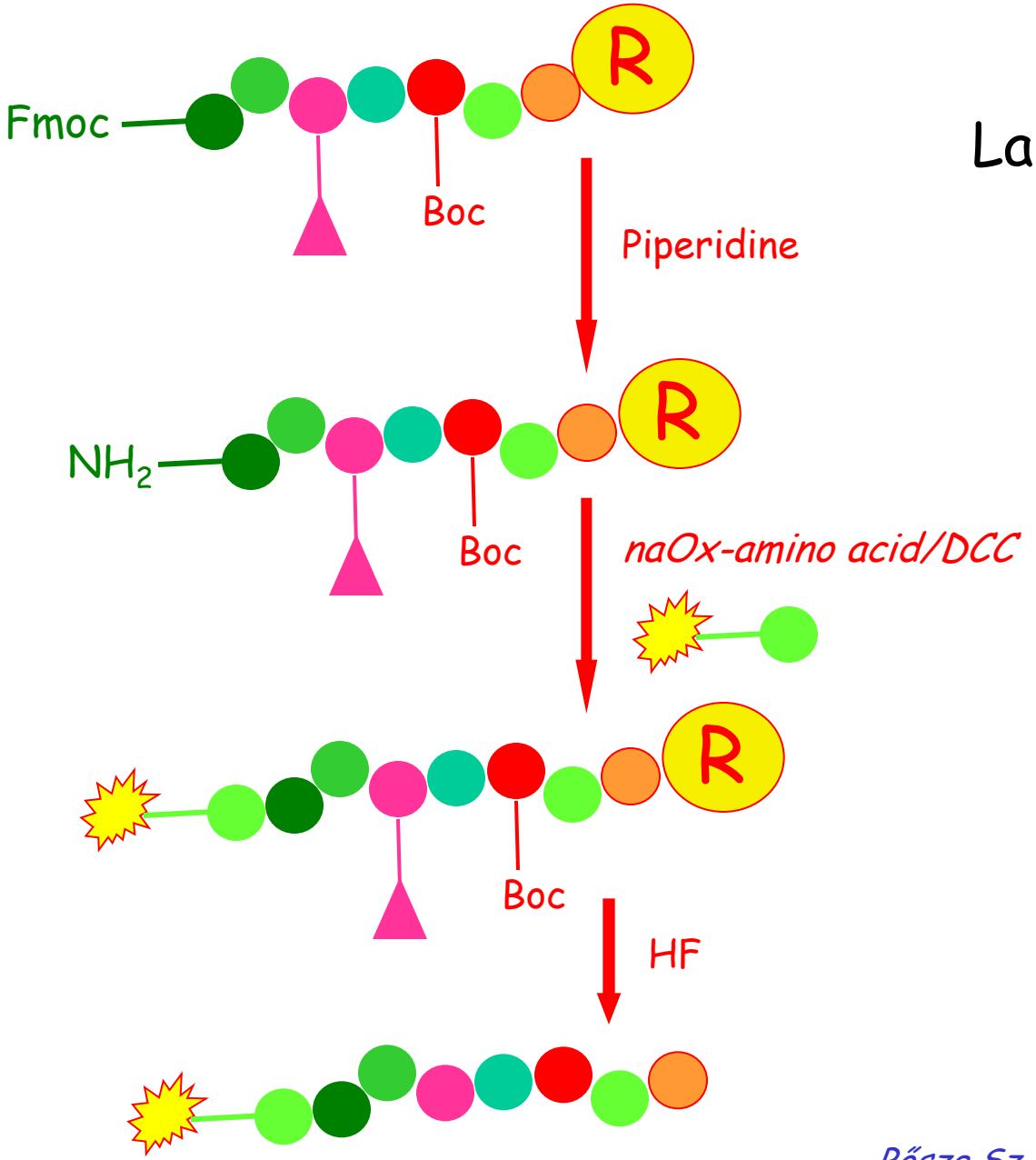




*Kóczán Gy. et. al. Tetrahedron **57**: 4589 (2001)*

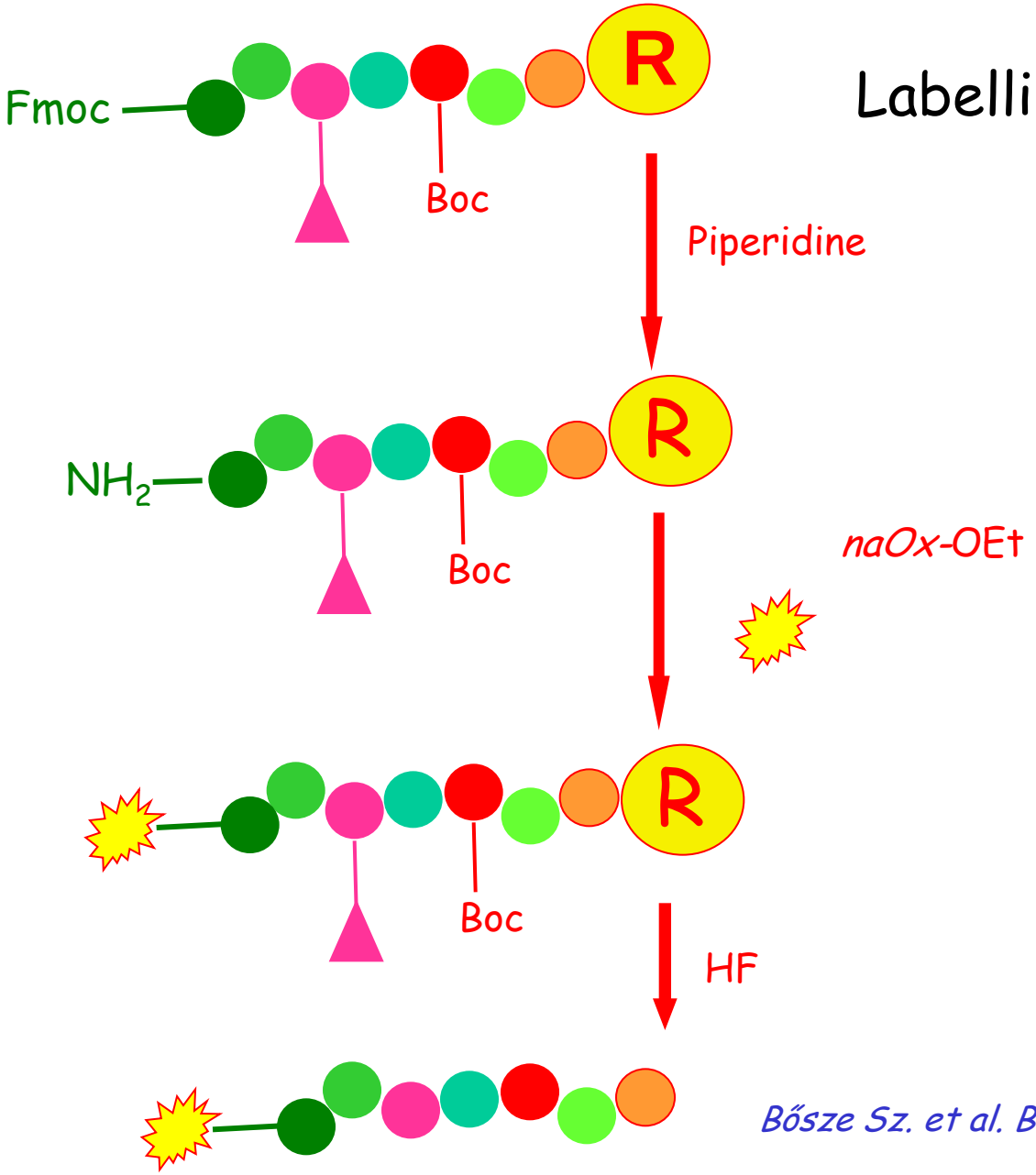
Labelling of peptides

No.1



Bősze Sz. et al. Biopolymers **79**: 489 (2005)

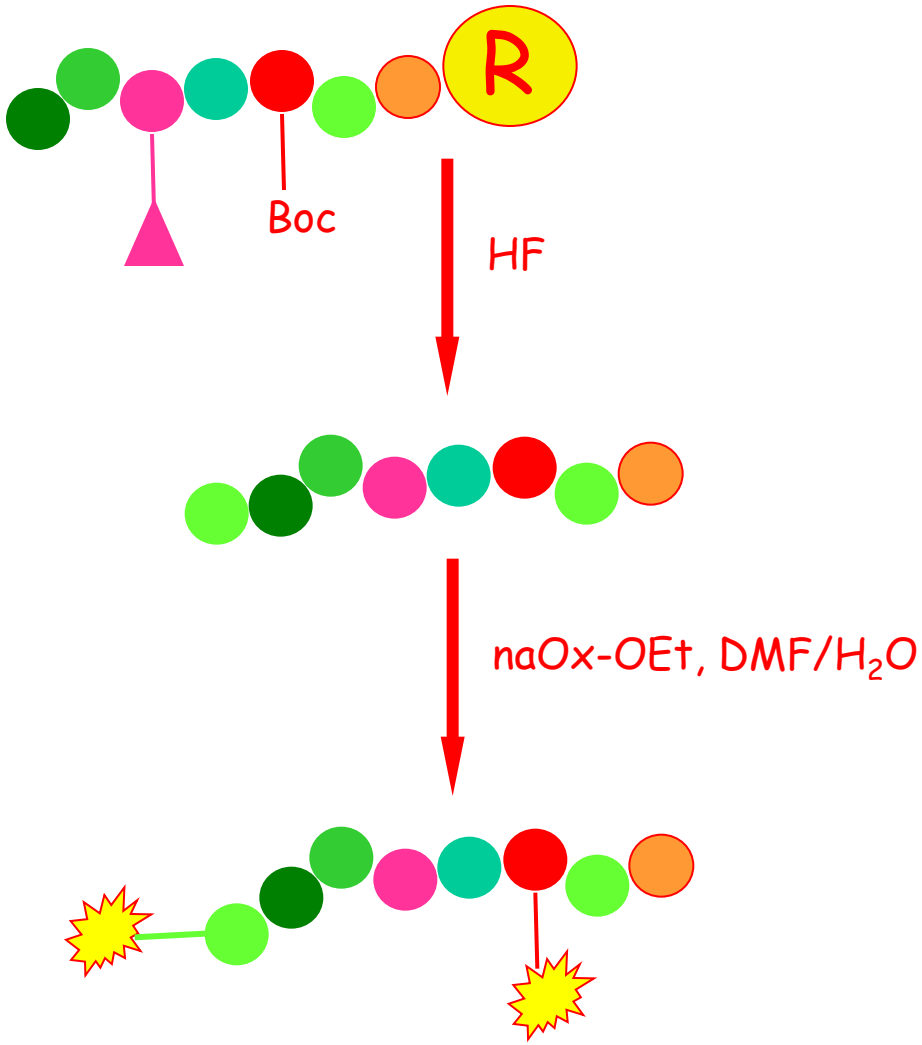
Labelling of peptides
No.2



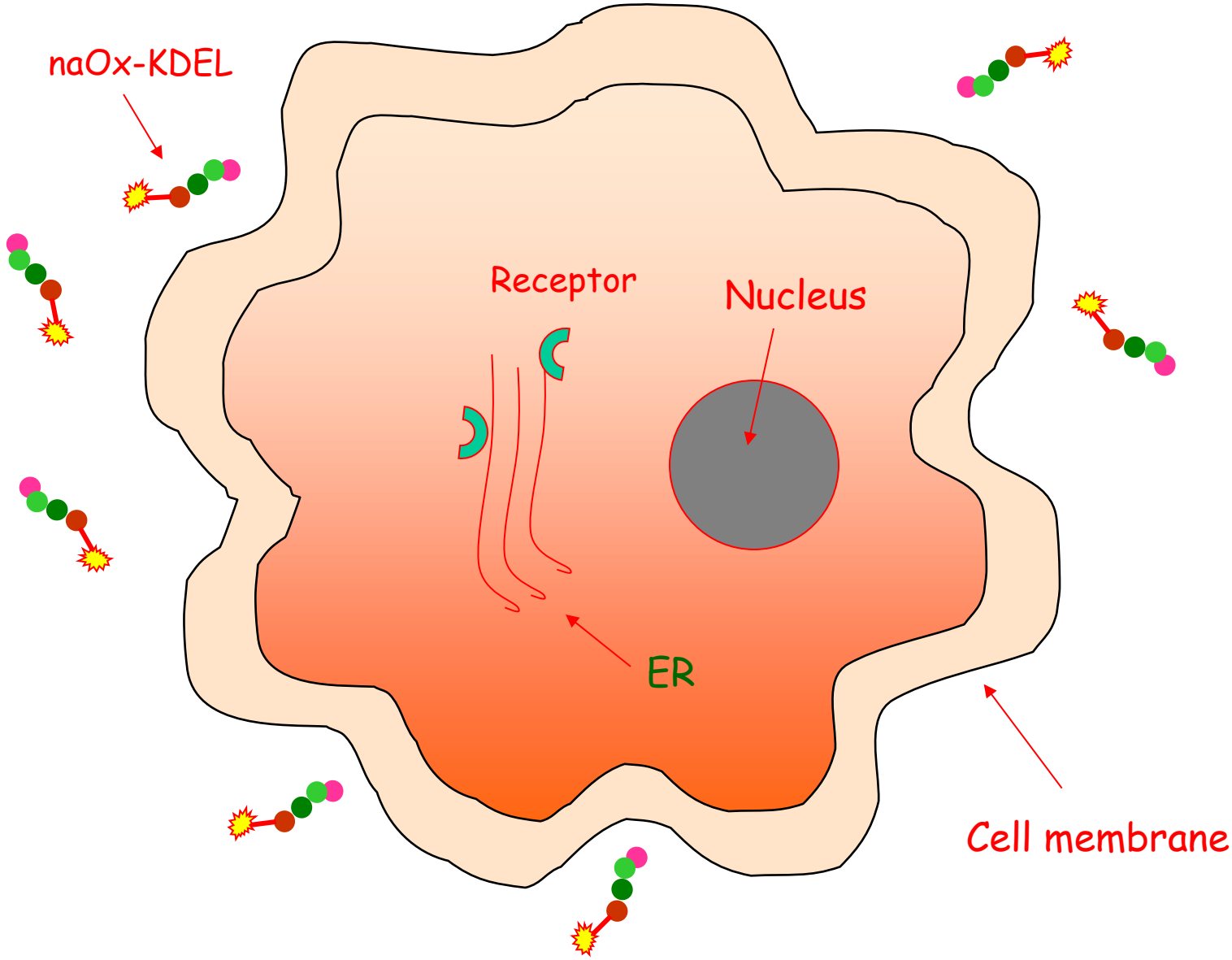
Bősze Sz. et al. Biopolymers 79: 489 (2005)

Labelling of peptides

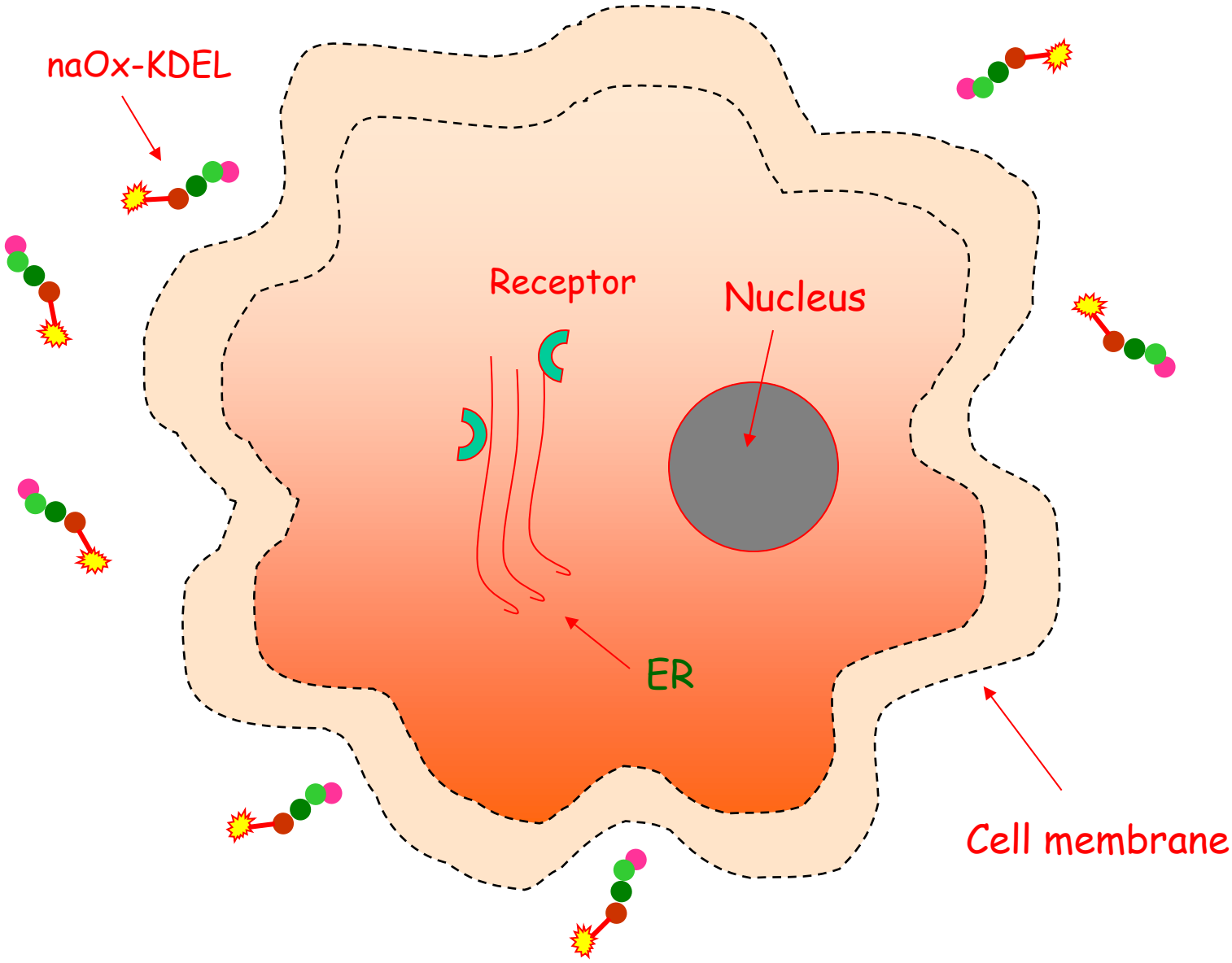
No.3

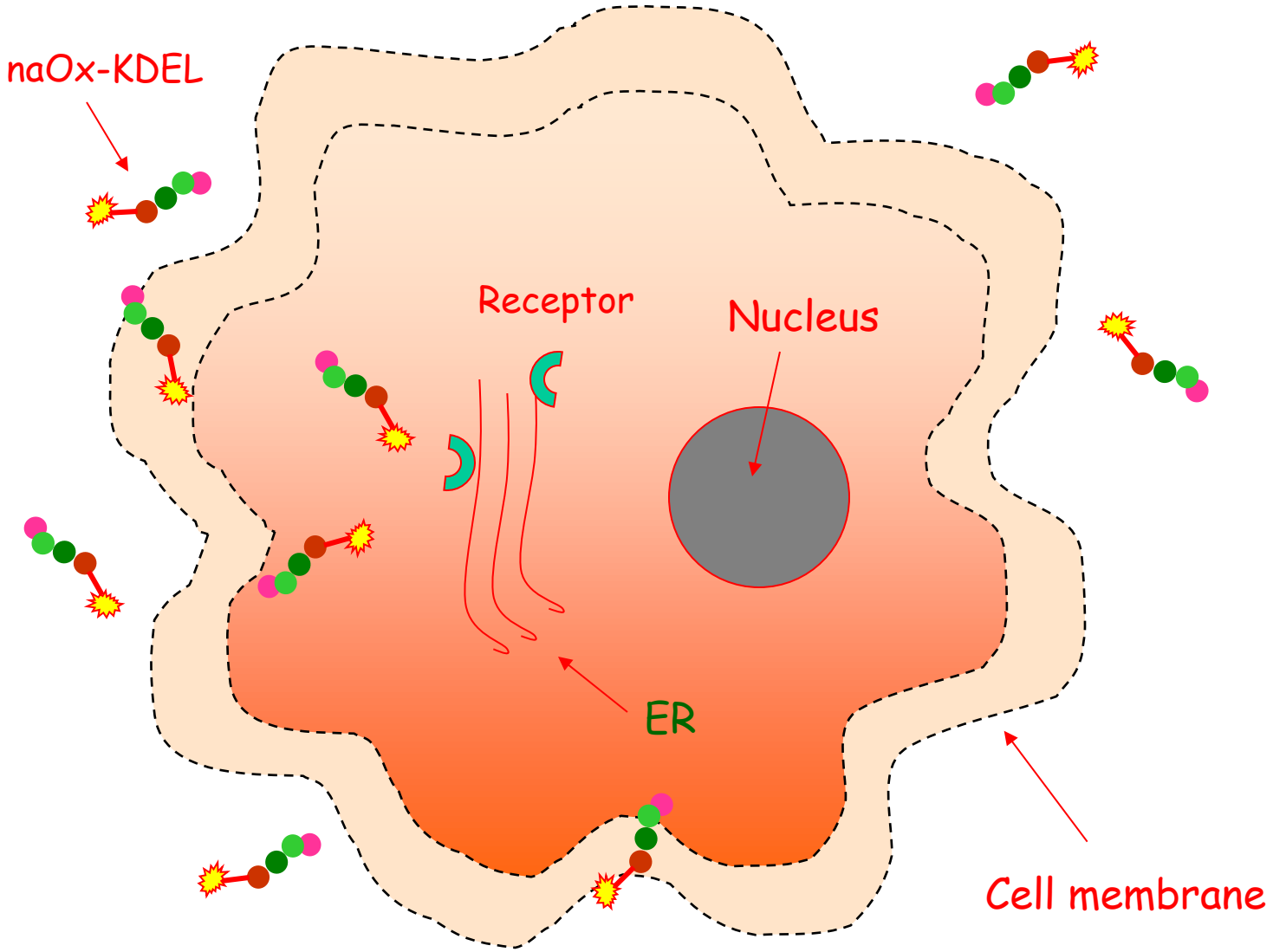


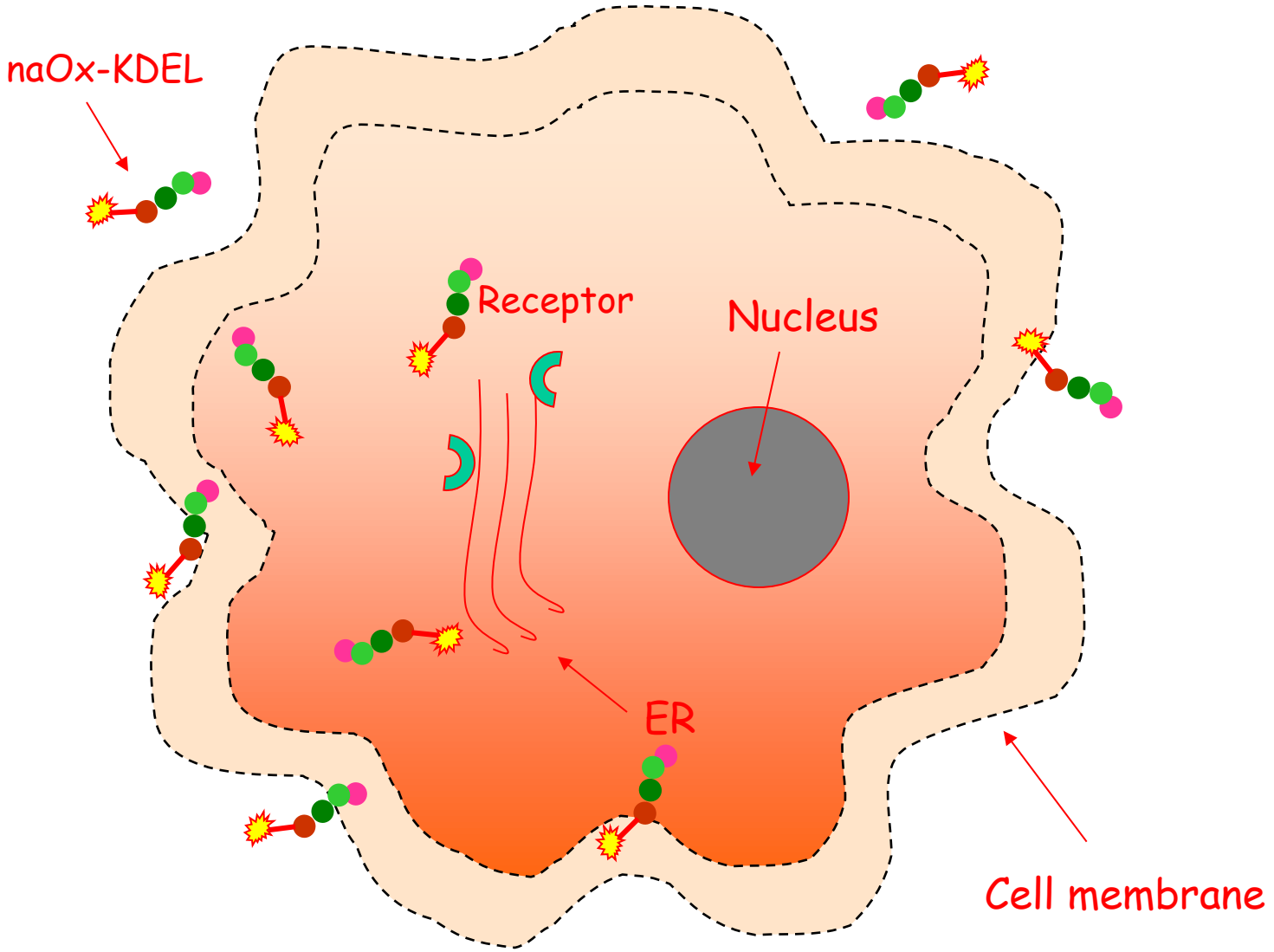
Localization of intracellular KDEL-receptor by naOx-peptide conjugate

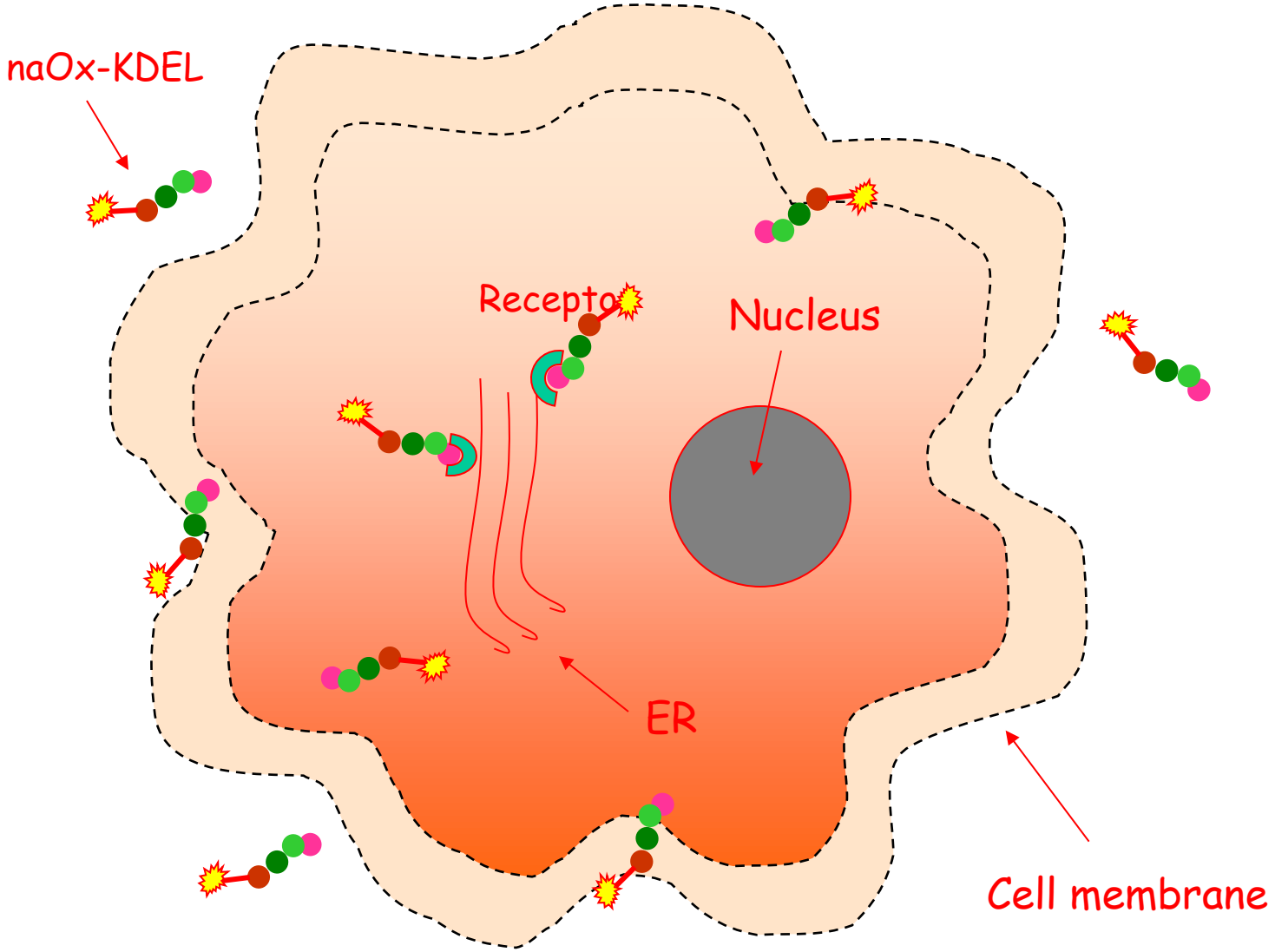


Permeabilization

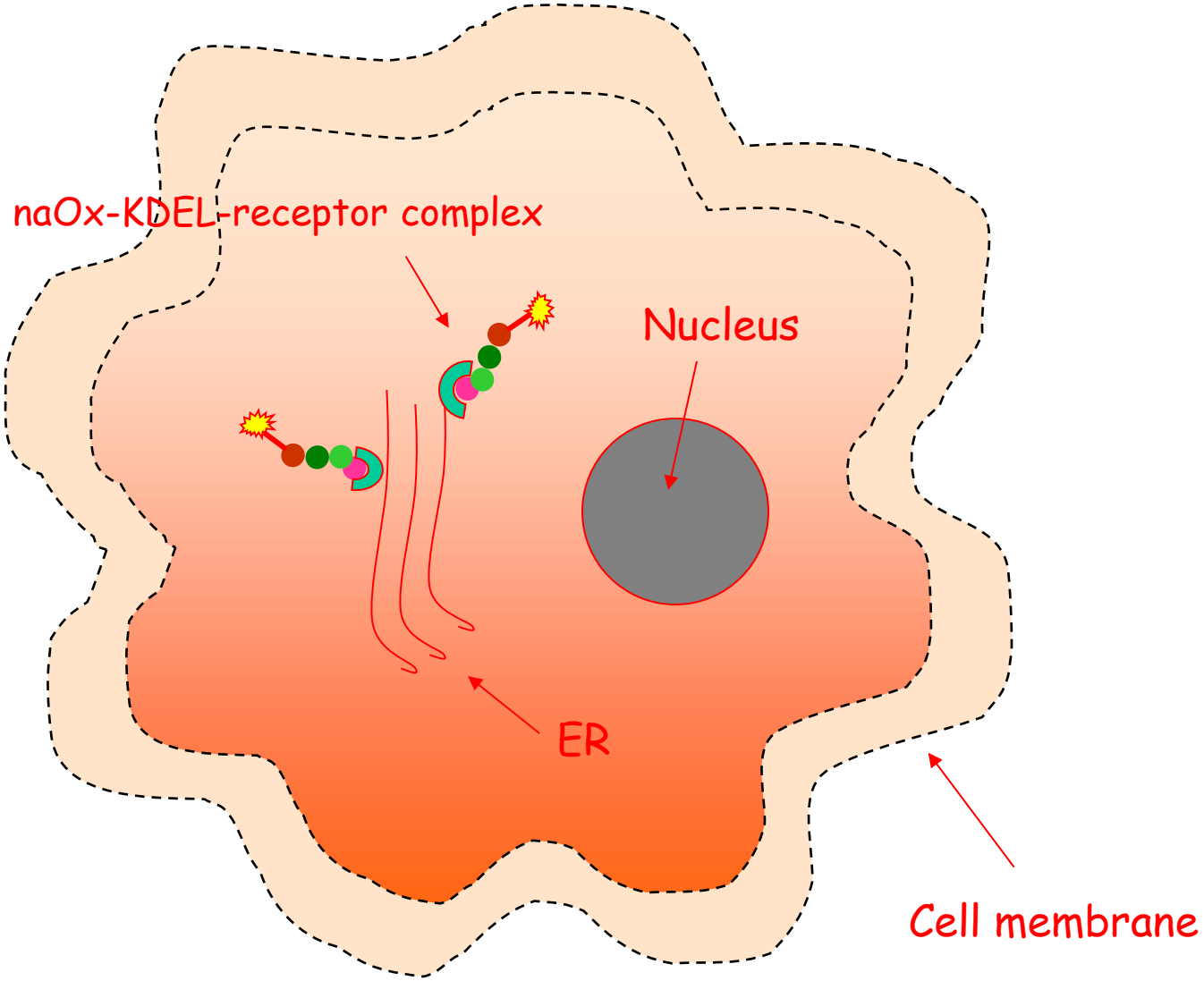



naOx-KDEL
Receptor
Nucleus
ER
Cell membrane

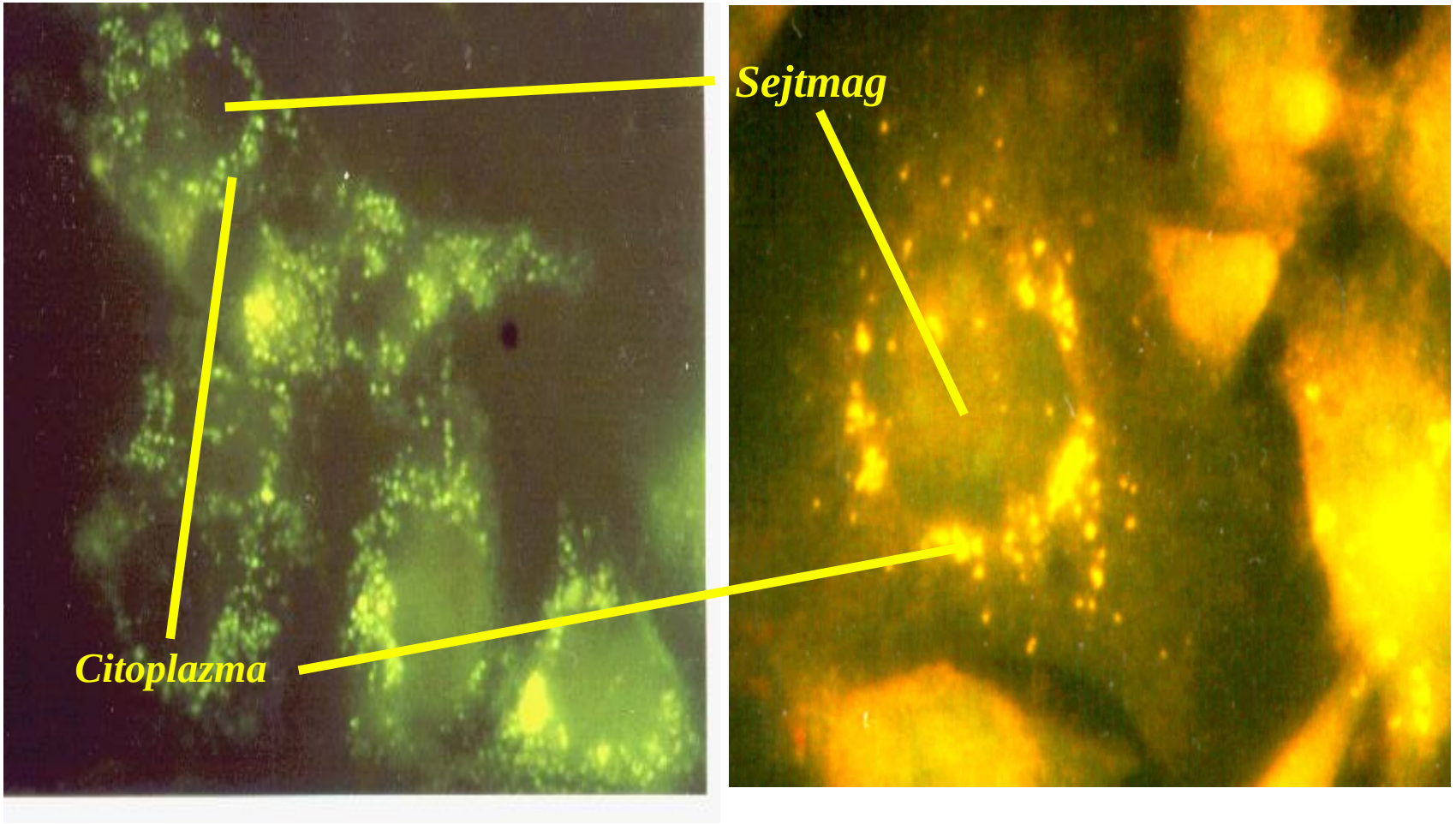

naOx-KDEL
Receptor
Nucleus
ER
Cell membrane


naOx-KDEL
Receptor
Nucleus
ER
Cell membrane

Washing

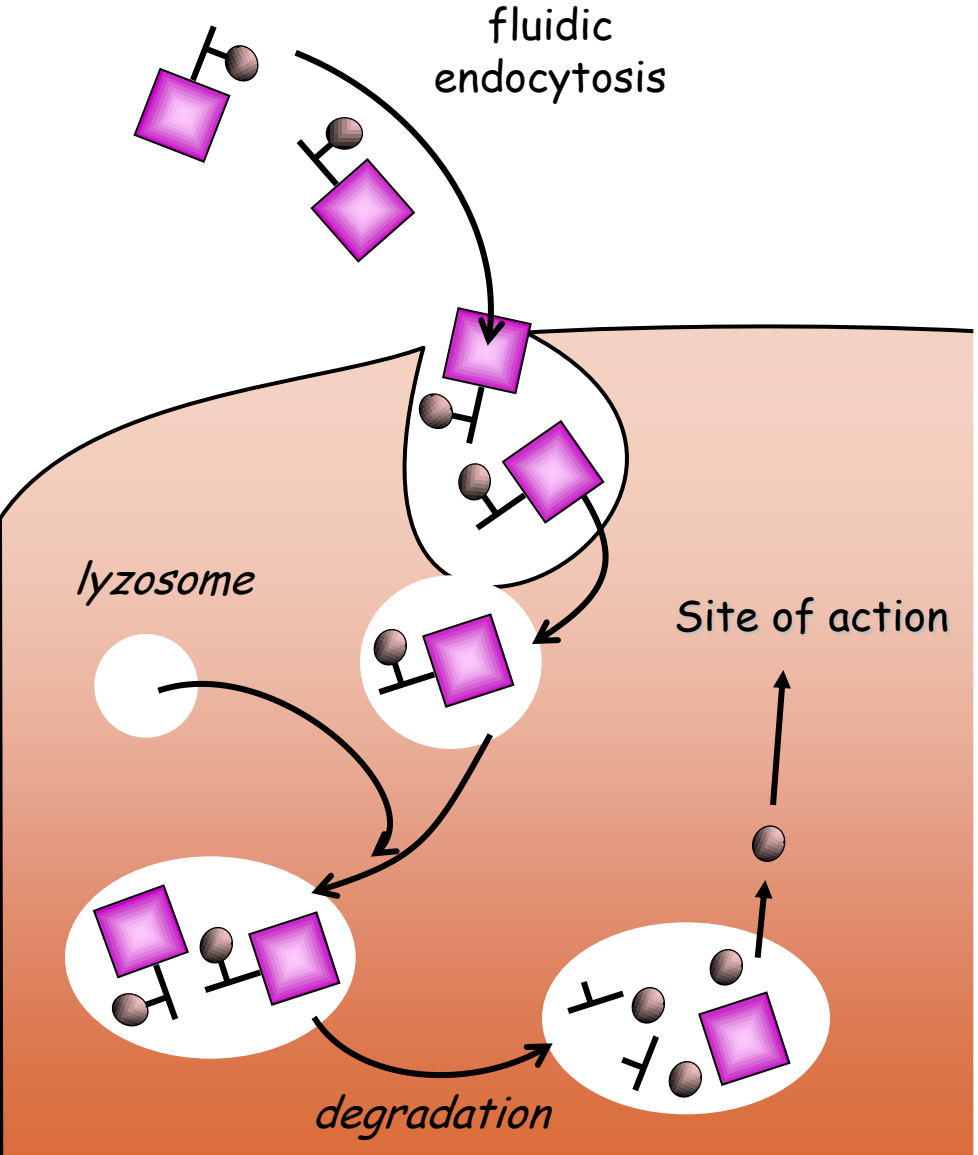


Receptor binding of naOx-KDEL peptide conjugate

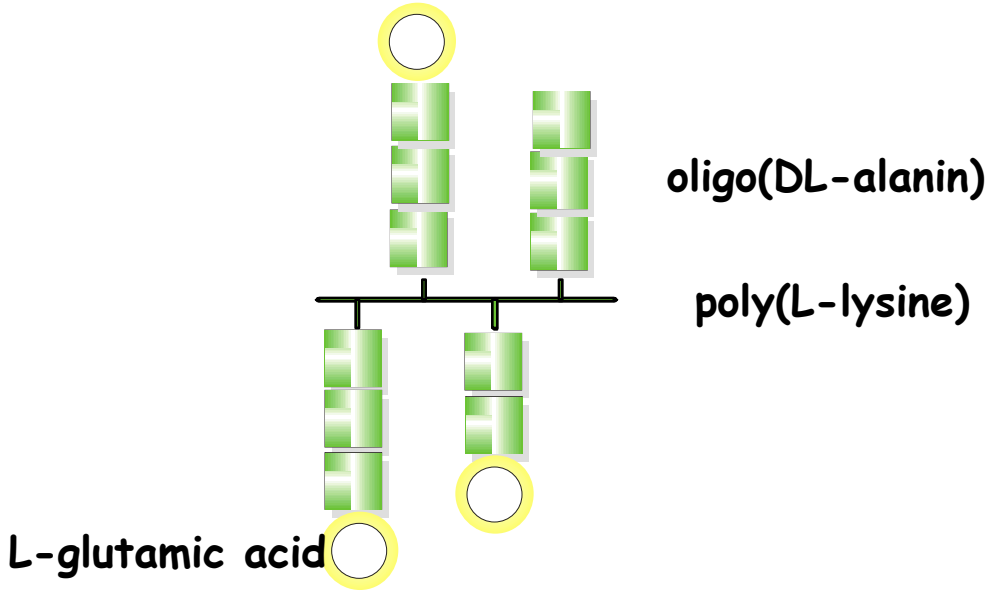


Nagy I. PhD értekezés

Uptake and liberation of bioactive entities

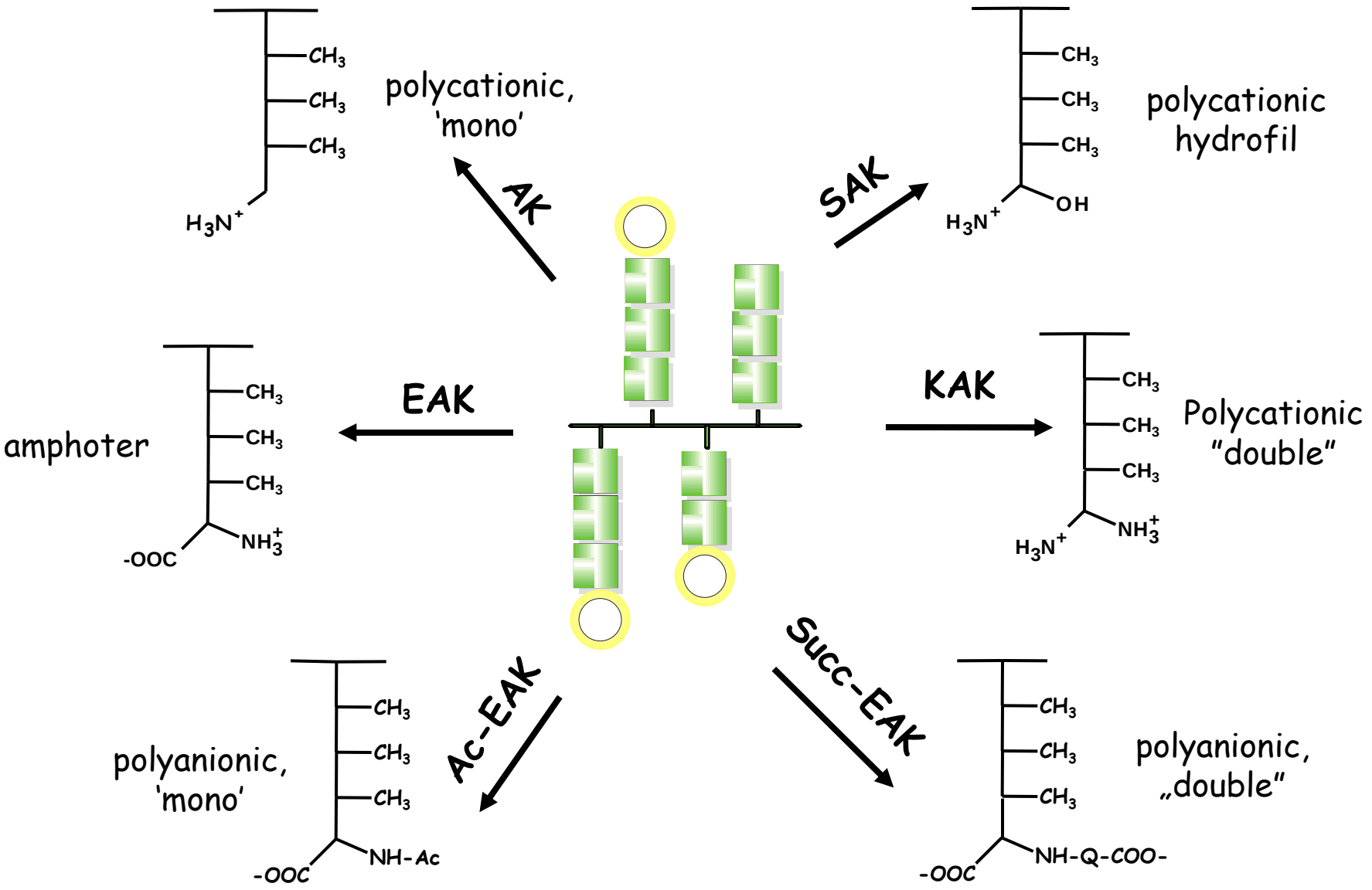


Branched chain polypeptides



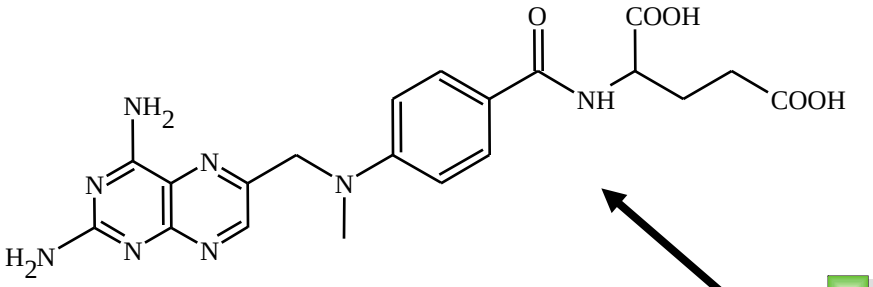
Hudecz,F.: In: Self-assembling peptide systems in biology, medicine and engineering.
(Eds.: Agelli, A., Boden, N., Zhang, S.) Kluwer Academic Publisher, The Netherlands (2001), pp. 139-160
Hudecz, F., Kóczán, Gy., Reményi, J.: In: Molecular pathomechanisms and new trends in drug research
(Eds Keri, Gy. and Toth, I.) Taylor and Francis Group, London, (2003) pp. 553-578

Branched chain polypeptides



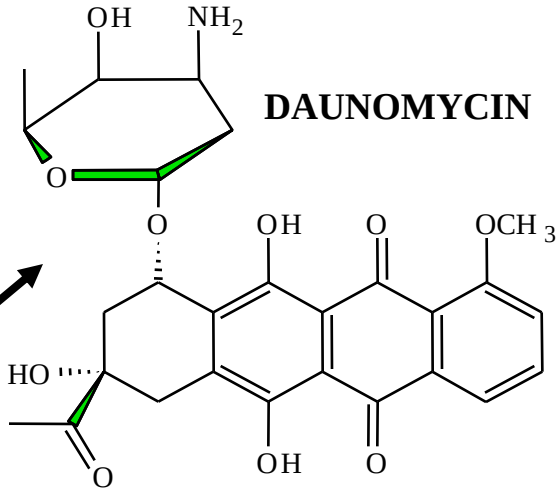
Drug-polypeptide conjugates

METHOTREXATE

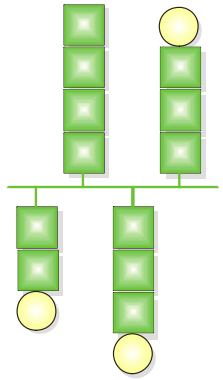


Hudecz F. et al. *Bioconjugate Chem.* **4:** 25 (1993)
Kóczán Gy. et al. *Bioconjugate Chem.* **13:** (2002)

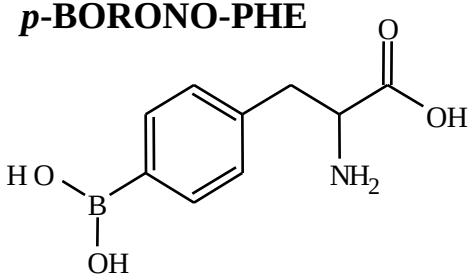
DAUNOMYCIN



Hudecz F. et al.*Bioconjugate Chem.* **3:** 49 (1992)
Gaál D., Hudecz F. *Eur.J.Cancer.* **34:** 155 (1998)

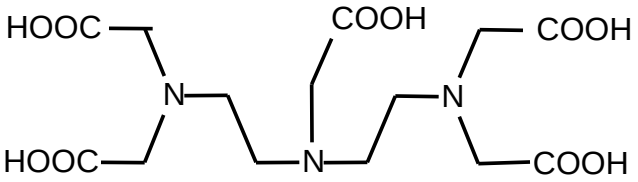


***p*-BORONO-PHE**



Mező G. et al. *J. Bio. Comp. Polymers* **11:** 263 (1996)

DIETHYLENE-TRIAMINE-PENTAACETIC ACID



Pimm MV. et al.*Int. J. Pharmaceutics* **79:** 77 (1992)
Pimm MV.et al. *J. Canc. Res. Clin. Onc.* **122:** 45 (1996)

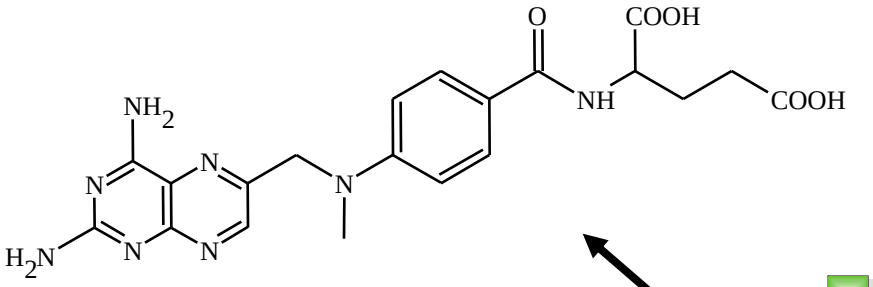
GN-RH ANTAGONIST, MI-1544

D-Trp-*D*-Cpa-*D*-Trp-Ser-Tyr-*D*-Lys-Leu-Arg-Pro-*D*-Ala

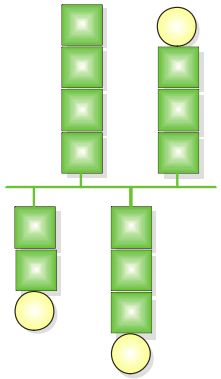
Mező, G. et al.*Bioconjugate Chem.* **7:** 642 (1996)
Vincze, B. et al. *J. Cancer Res. Clin. Onc.* **120:** 578 (1994)

Drug-polypeptide conjugates

METHOTREXATE



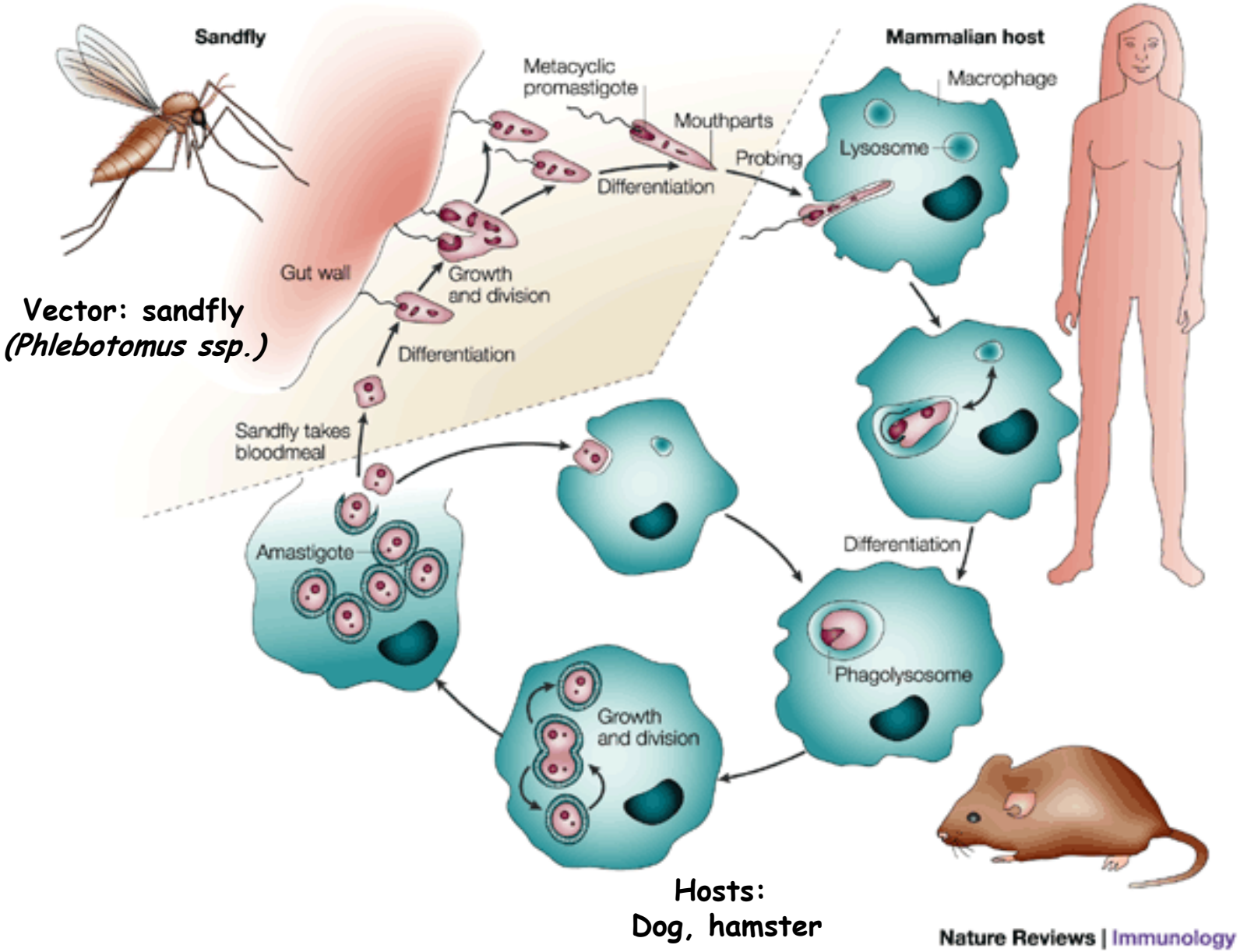
Hudecz F. et al. *Bioconjugate Chem.* **4:** 25 (1993)
Kóczán Gy. et al. *Bioconjugate Chem.* **13:** (2002)



against Leishmania infection

Antileishmania effect of methotrexate conjugates

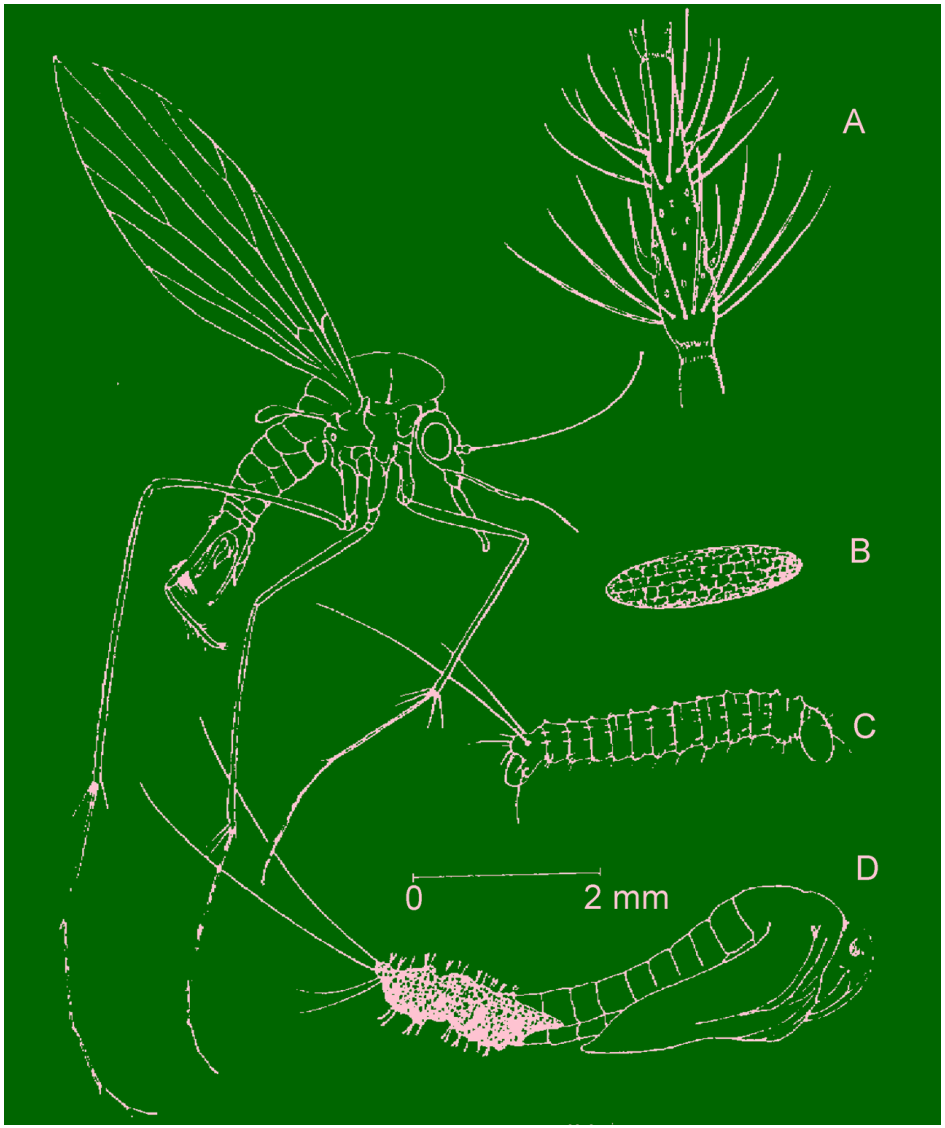
Leishmaniasis



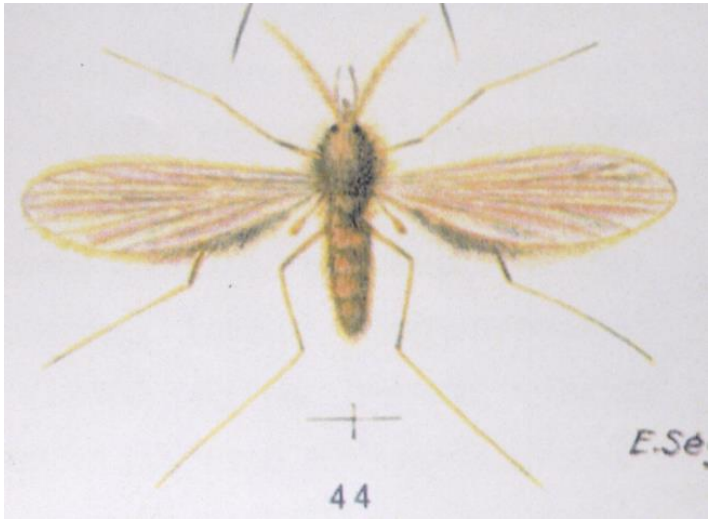
Vector: sandfly
(Phlebotomus ssp.)

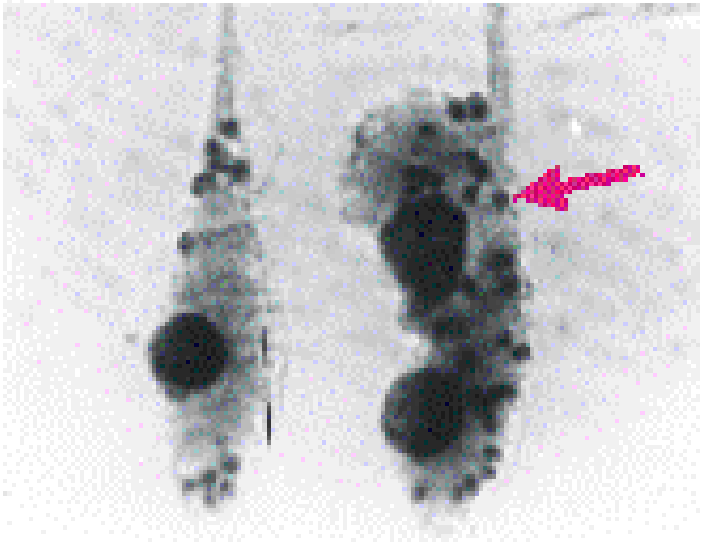
Hosts:
Dog, hamster

Leishmaniasis: parasitic tropical disease



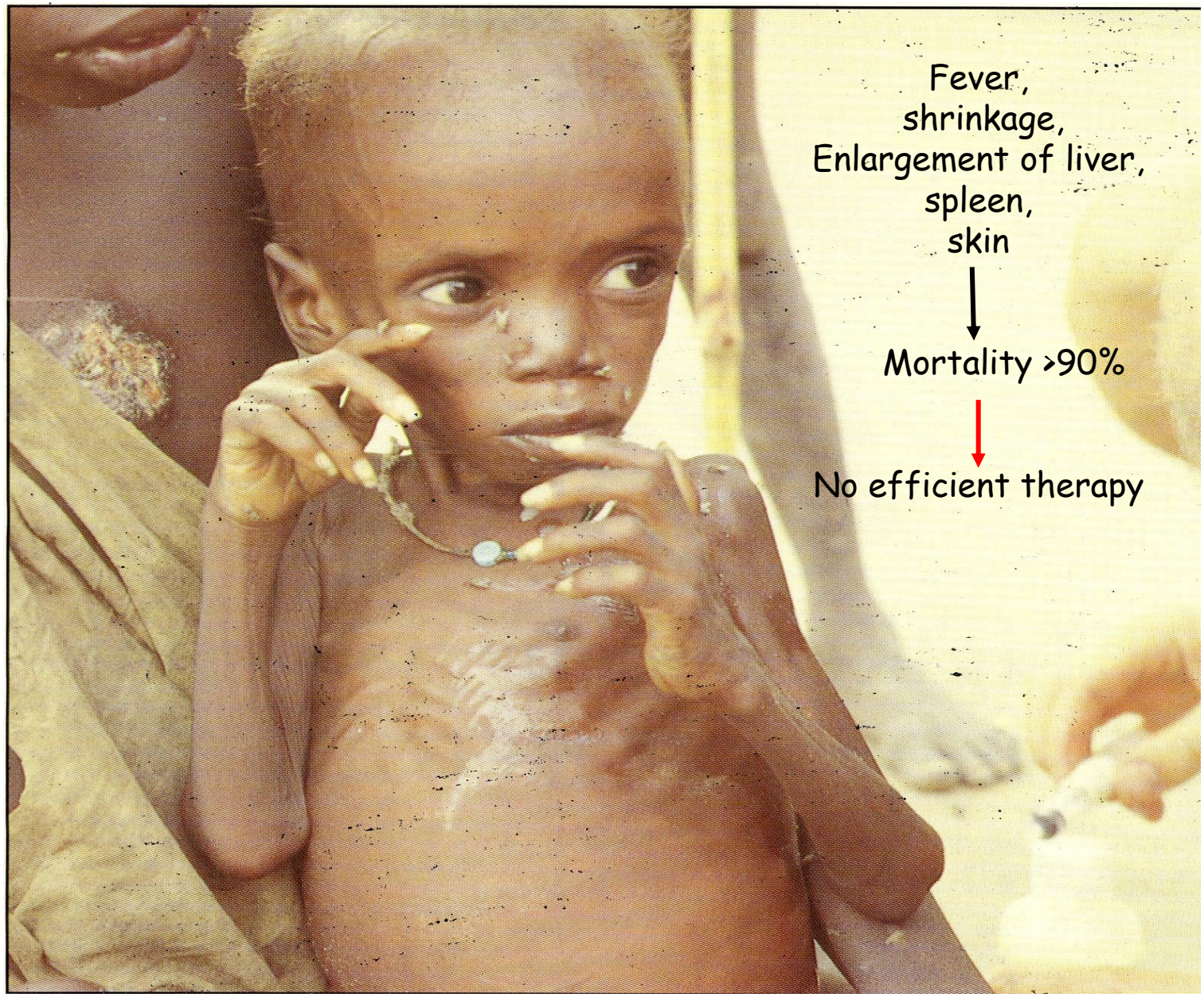
sandfly (*Phlebotomus papatassi*)





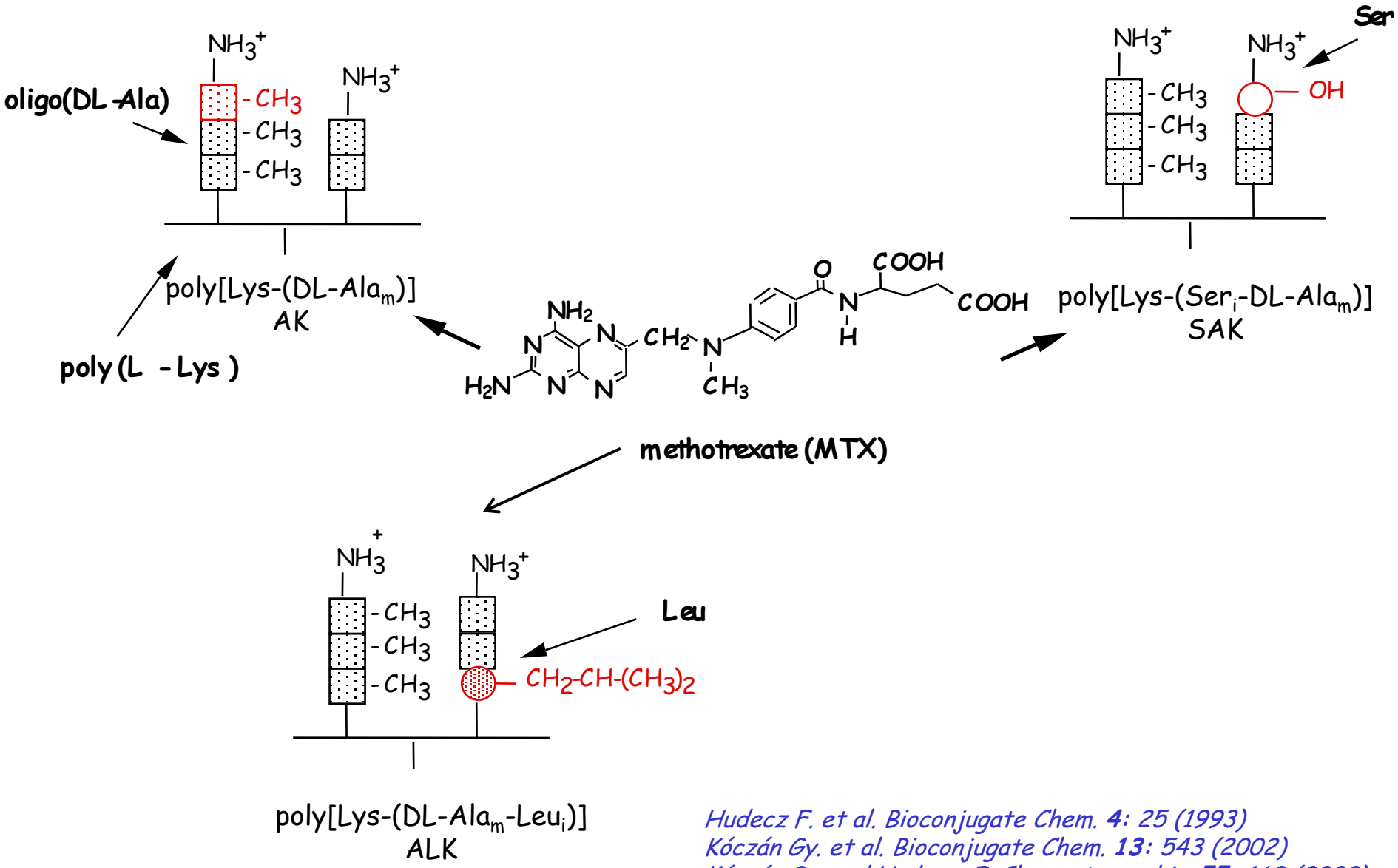
parasites in macrophage cell

Visceral Leishmaniasis, Sudan



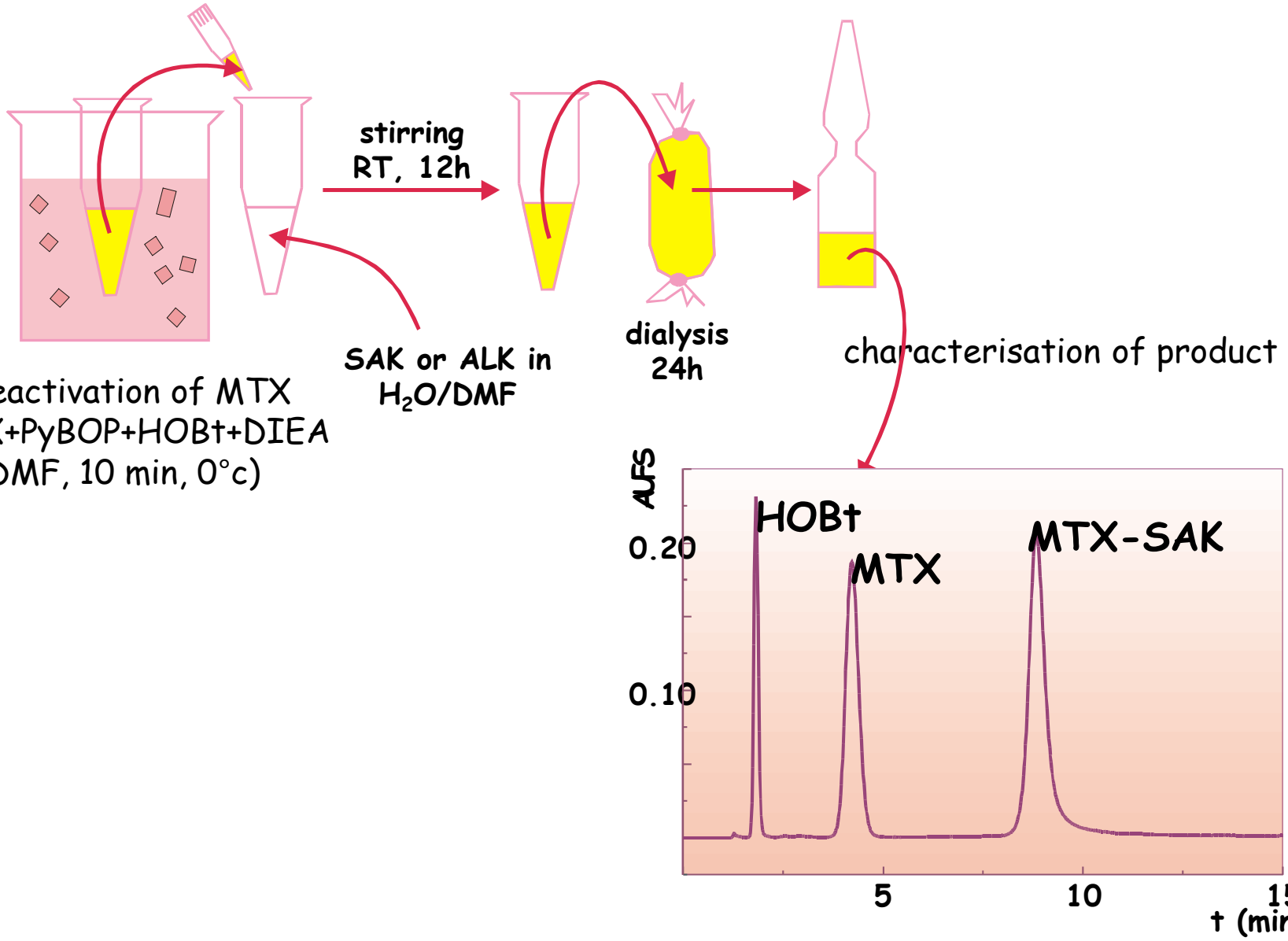
Postgraduate Doctor Africa 17: 19 (1995) photo taken by R. Wilkinson

Methotrexate-polypeptide conjugates

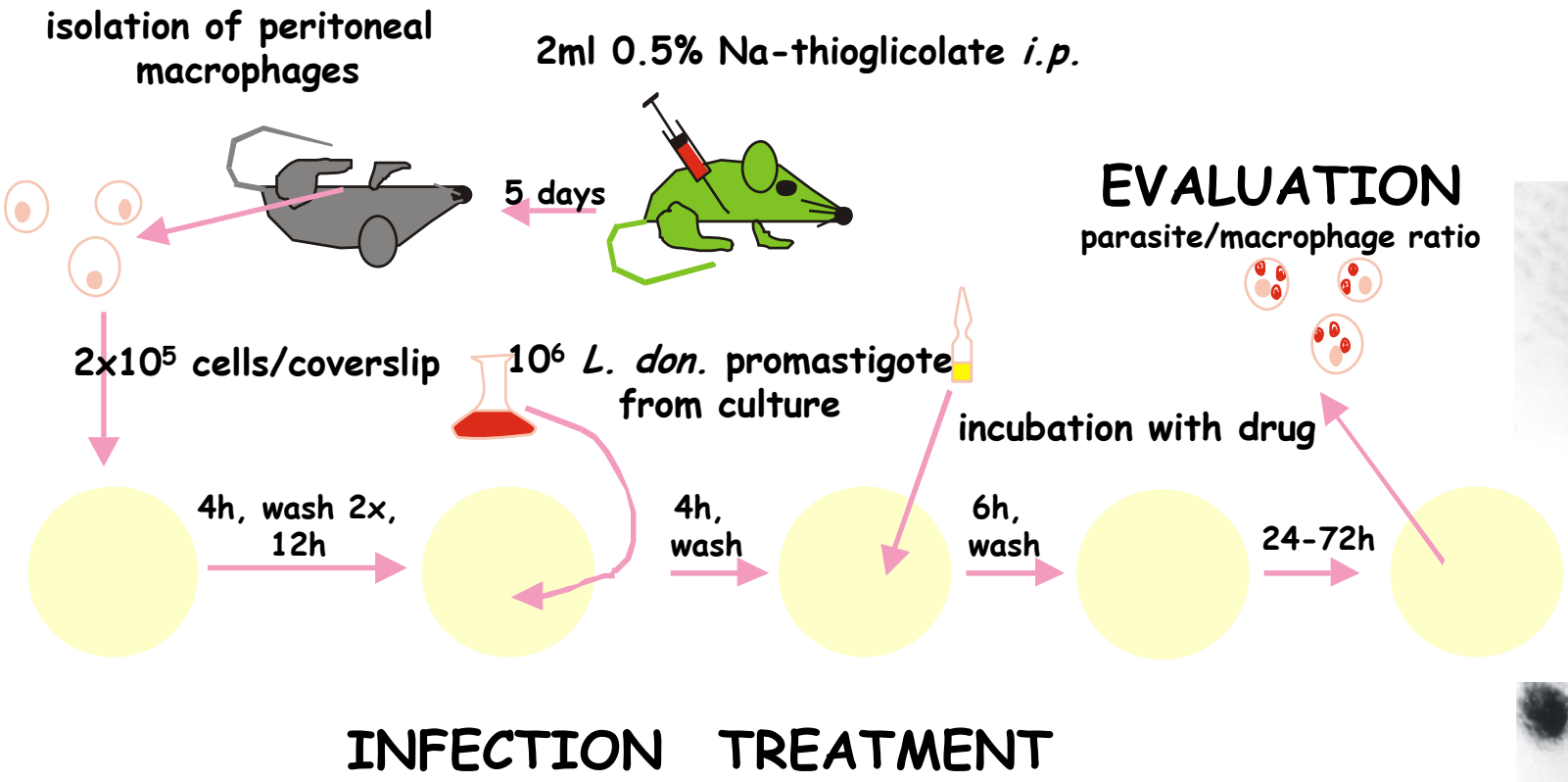


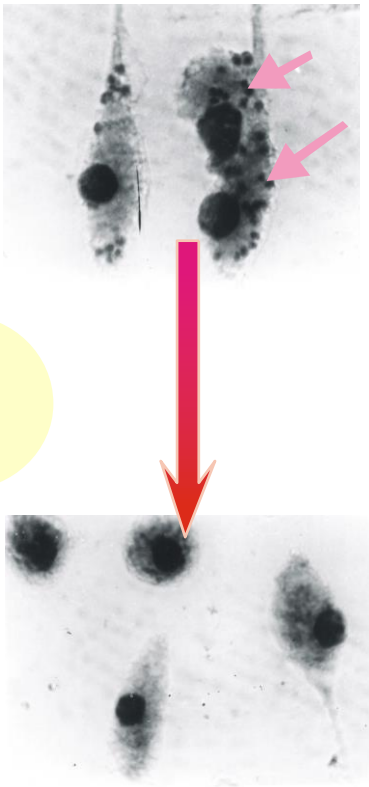
Hudecz F. et al. Bioconjugate Chem. ***4:*** *25 (1993)*
Kóczán Gy. et al. Bioconjugate Chem. ***13:*** *543 (2002)*
Kóczán Gy. and Hudecz, F. Chromatographia, ***55:*** *163 (2002)*

Synthesis of methotrexate-conjugates in practice



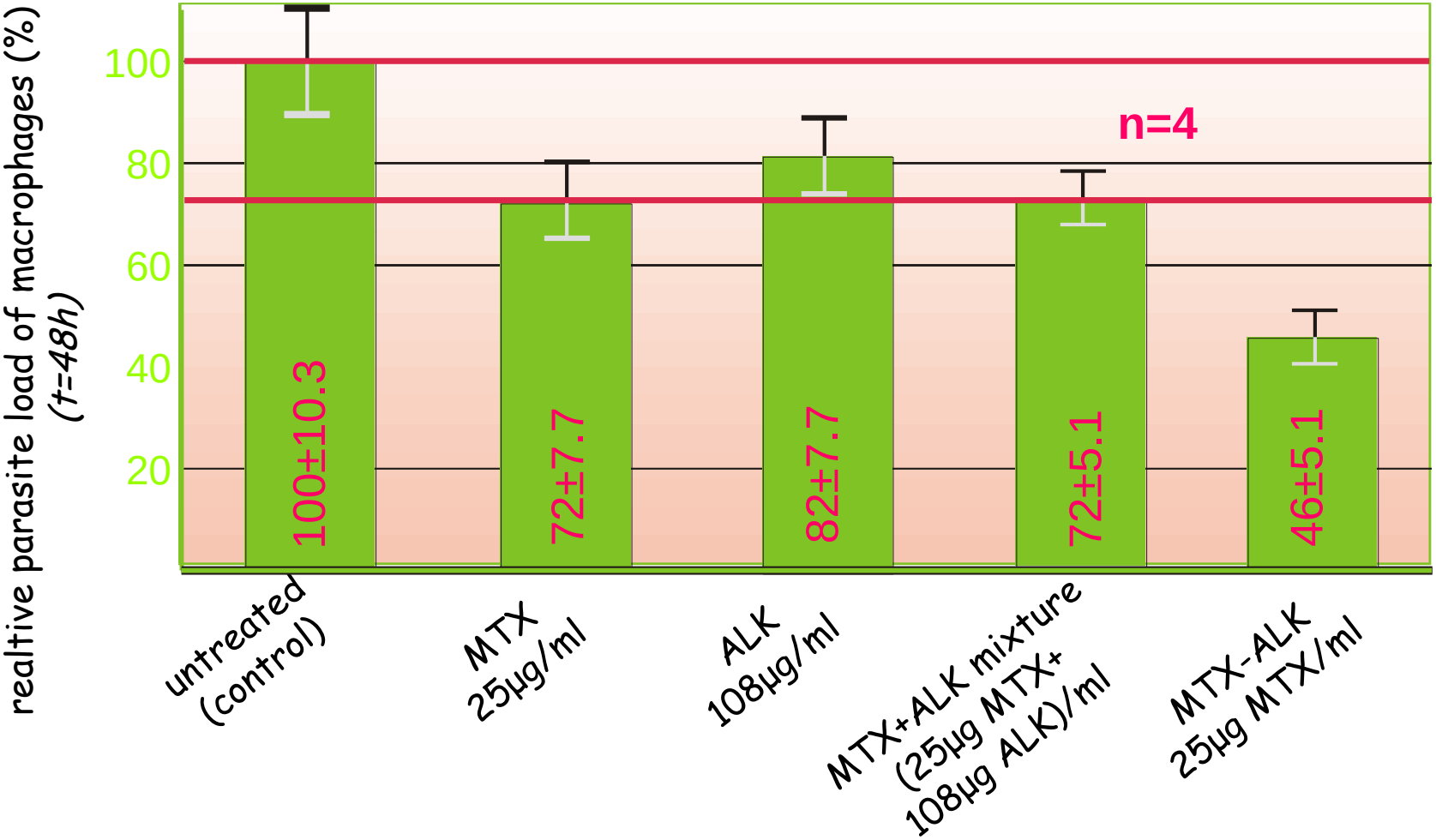
Evaluation of methotrexate-conjugates *in vitro*





Kóczán Gy. et al. Bioconjugate Chem. ***13****: 543 (2002)*

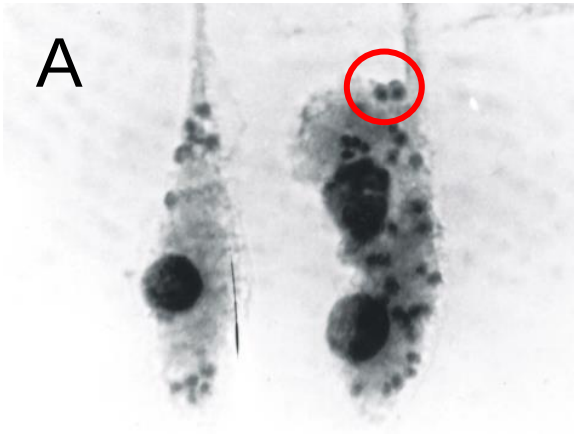
The effect of MTX-ALK conjugate *in vitro*

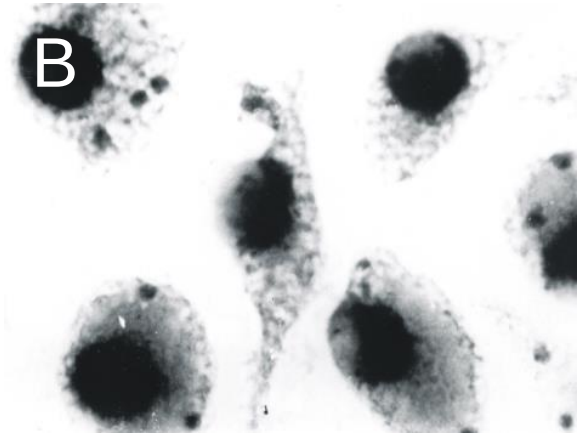


Kóczán Gy. et al. Bioconjugate Chem. ***13****: 543 (2002)*

The effect of MTX-ALK conjugate on L. donovani infected macrophages *in vitro*

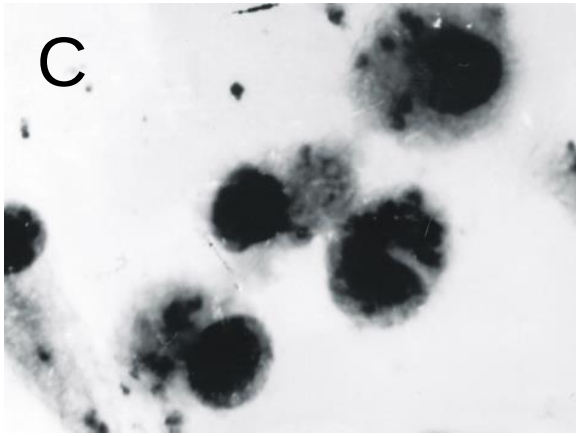
control

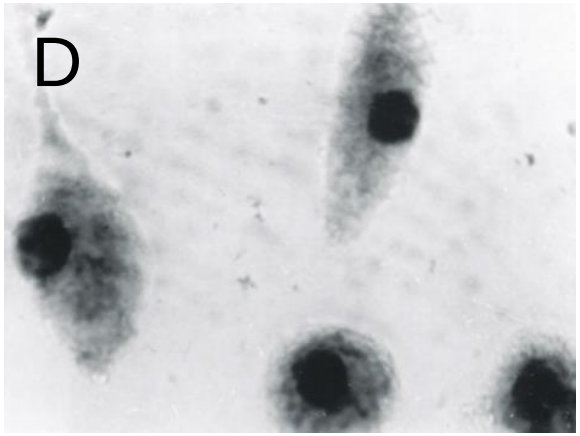




MTX

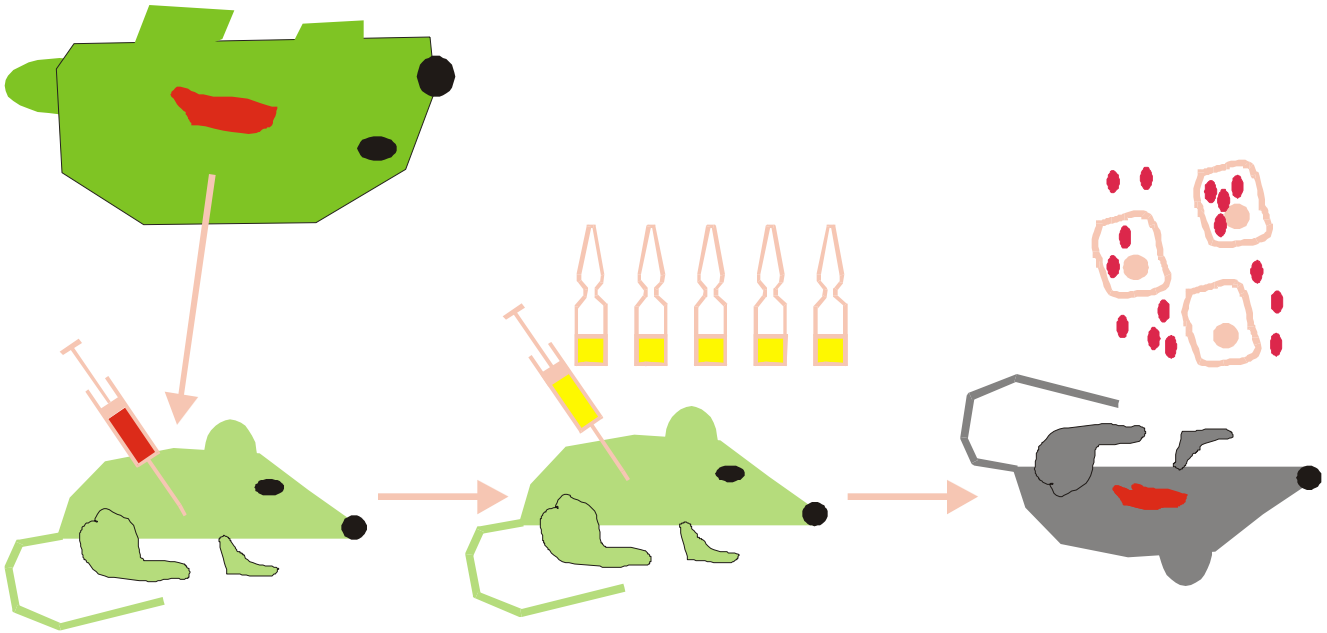
MTX plus ALK





MTX-ALK

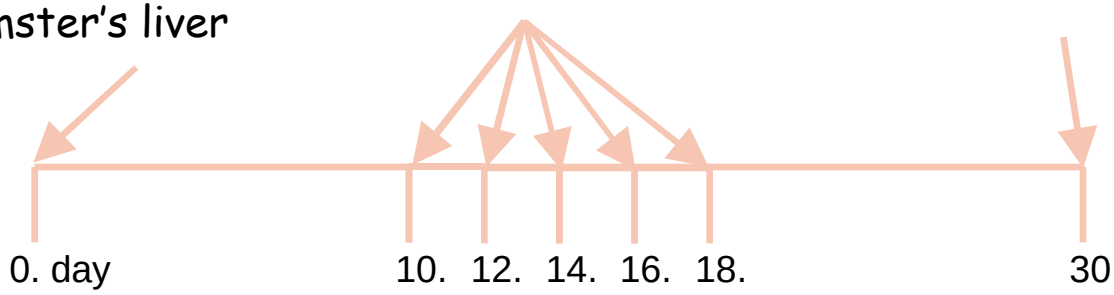
Evaluation of methotrexate-conjugates *in vivo*



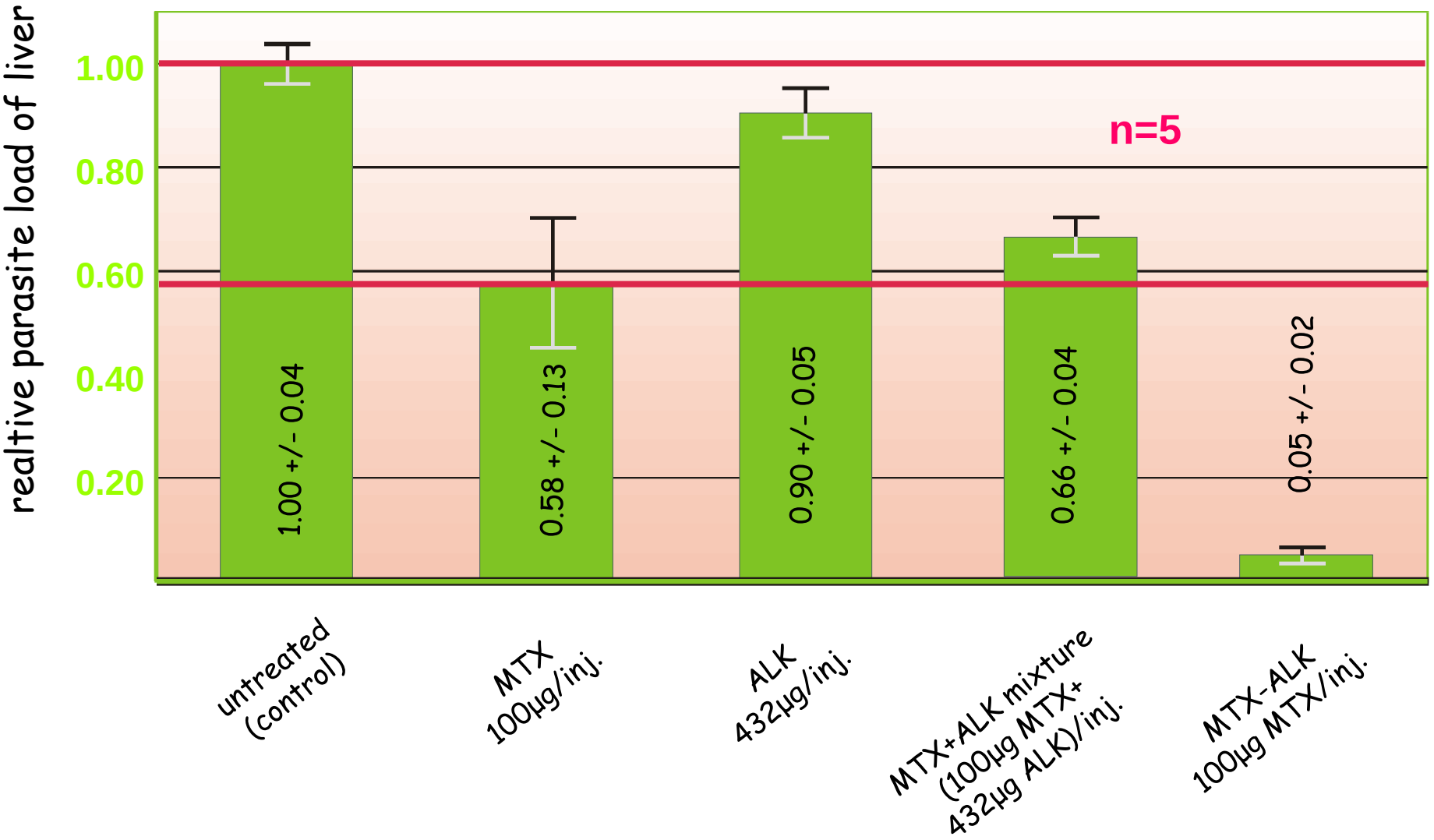
INFECTION
L. don. amastigotes
are isolated from infected
hamster's liver

TREATMENT
every 2nd day,
i.p. injection, 5x100µl

EVALUATION
counting parasite load
of liver and spleen



The effect of MTX-ALK conjugate *in vivo*



Kóczán Gy. et al. Bioconjugate Chem. ***13****: 543 (2002)*

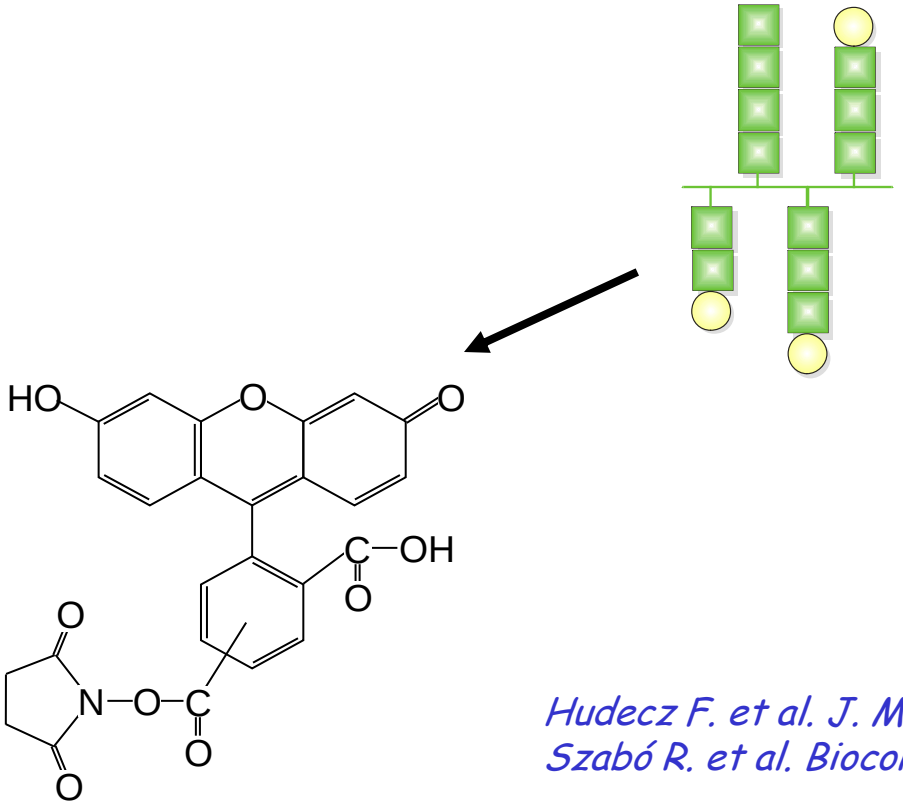
Conclusions

1. Methotrexate preserves its antileishmania activity after conjugation with branched polypeptides.

2. MTX effect in vitro as well as in vivo can be increased by conjugation to branched polypeptides.

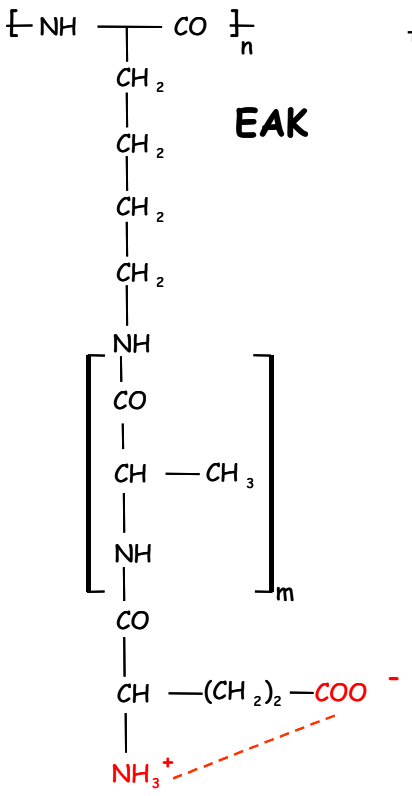
3. The antileishmania donovani activity of conjugates depends on the carrier polypeptide.

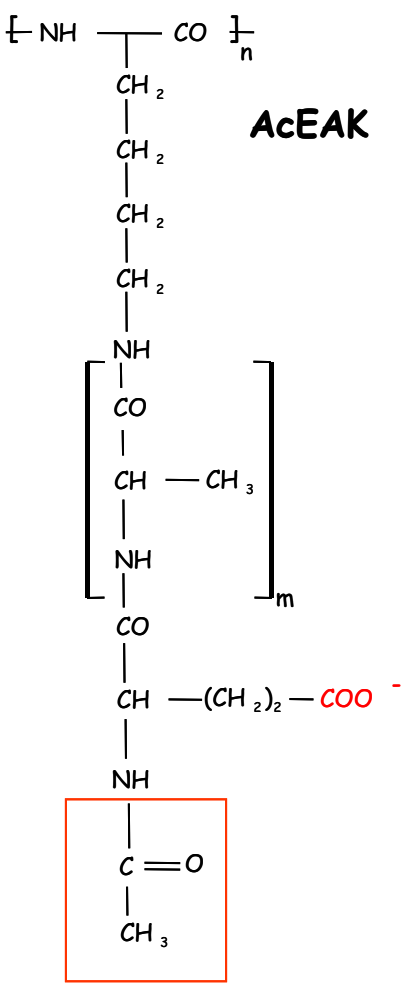
Fluorophor - polypeptide conjugates: structure – cellular uptake correlation?

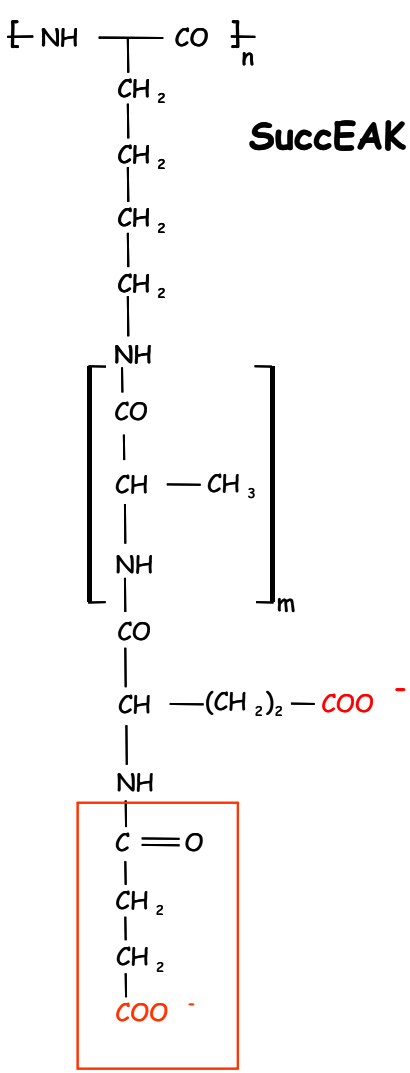


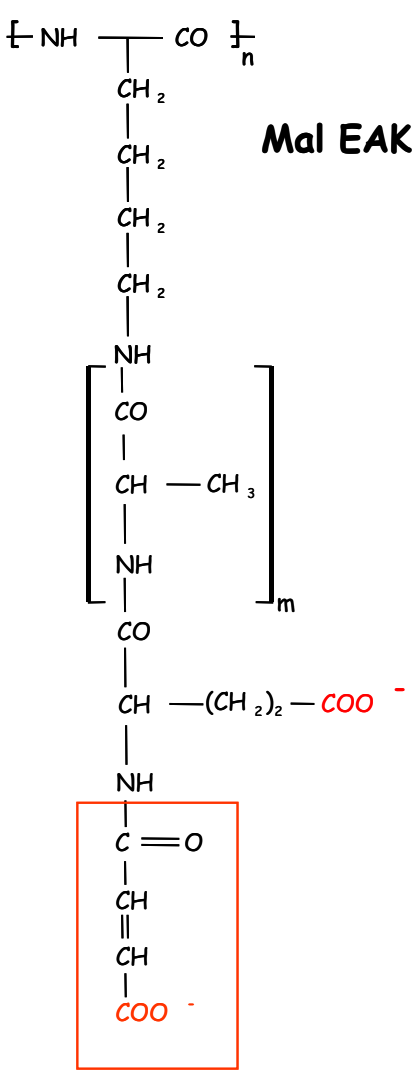
Hudecz F. et al. J. Mol. Recognition ***16:*** *288 (2003)*
Szabó R. et al. Bioconjugate Chemistry ***16:*** *1442 (2005)*

Branched chain polypeptides







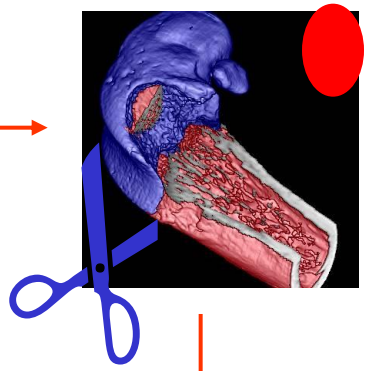


Szabó R. et al. Bioconjugate Chem. ***16:*** *1442 (2005)*

Scavenger receptor [SR-A] (+/-): bone marrow macrophages

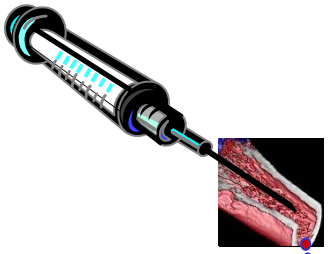
SRA +/+, SRA -/-

femur + tibia
EtOH 1 min
(sterilization)

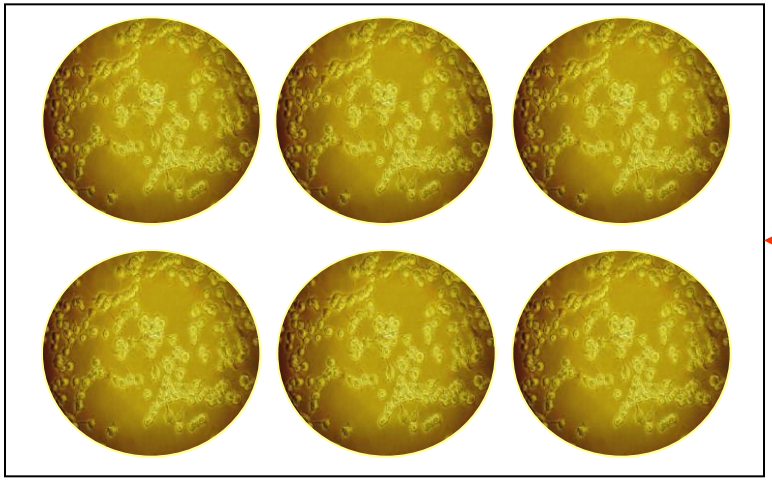


washing

1 week, 37°C
washing



adhesion

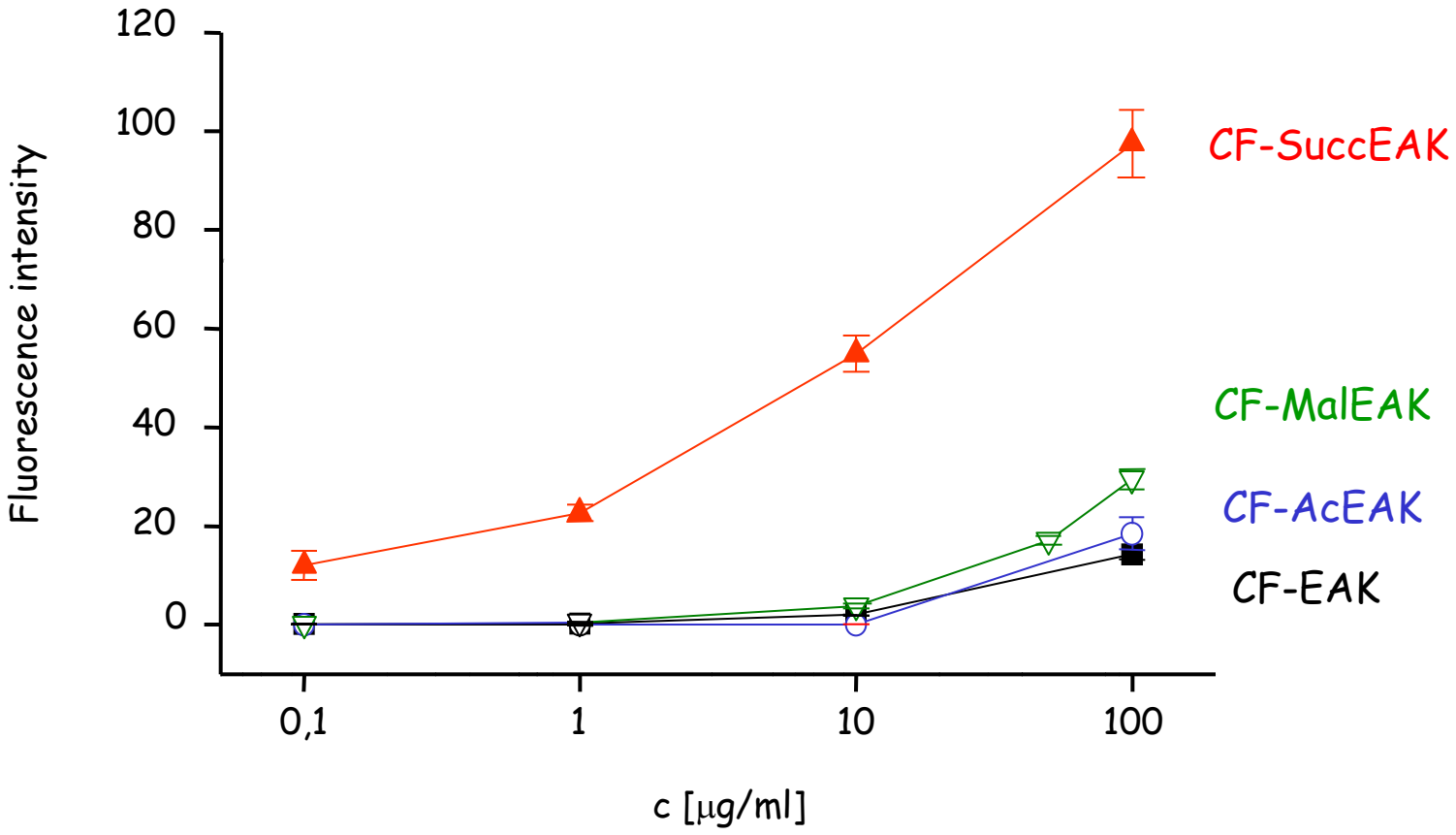


Cellular uptake studies (10^6 cells/well)

RPMI 1640
10% FCS (v/v)
50 mg/ml streptomycin
50 IU/ml penicillin
2 mM L-Gln
10 mM HEPES
15% LCM (v/v)(M-CSF)

Carrier effect: uptake of CF-polypeptides by bone marrow macrophages

(60 min)

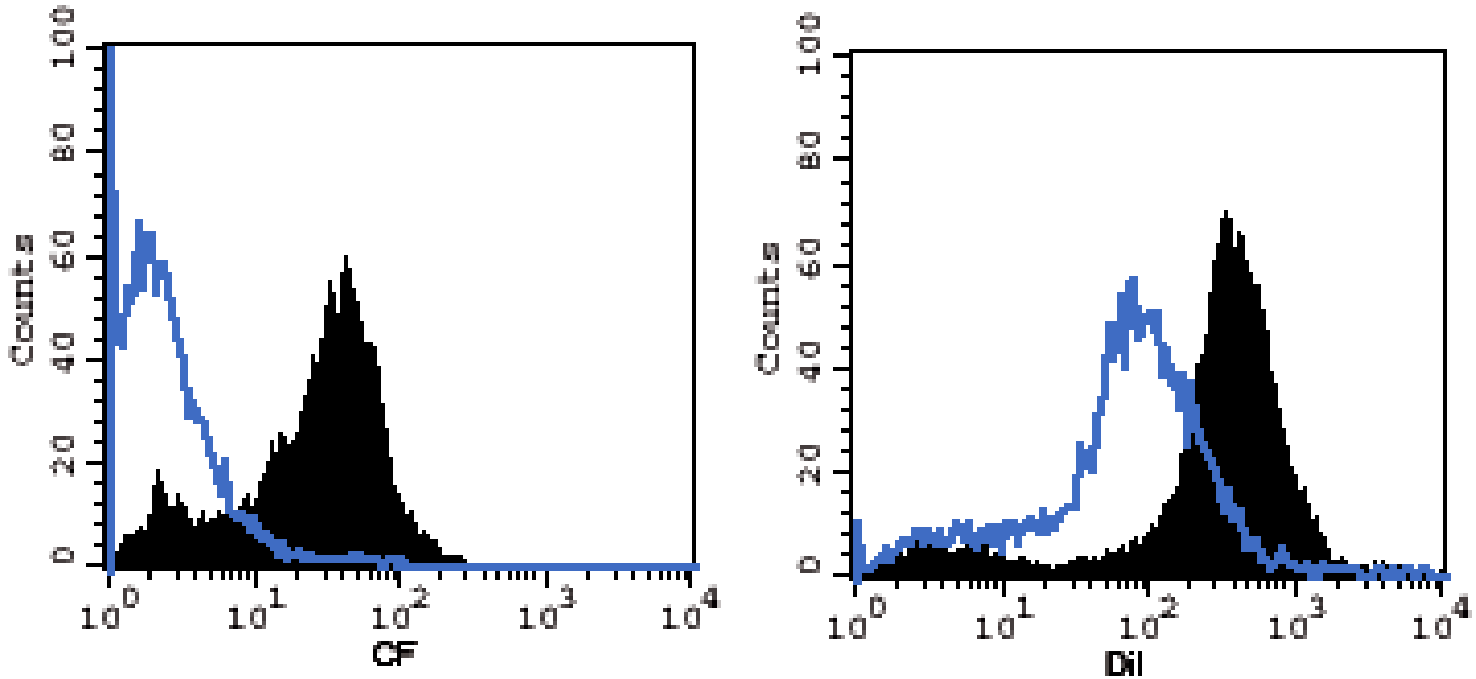


Szabó R. et al. Bioconjugate Chem. ***16:*** *1442 (2005)*

Uptake of CF-polypeptides by bone marrow macrophages: the role of SR-A receptor

CF-SuccEAK

Control: DiI-AcLDL

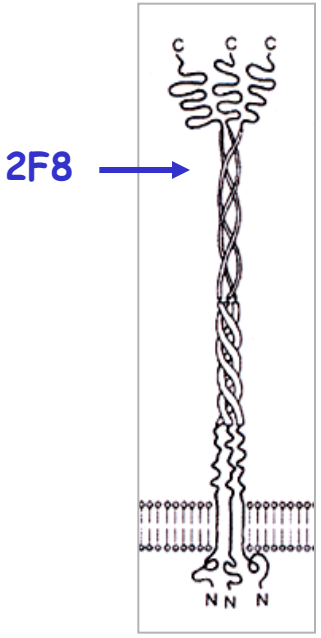


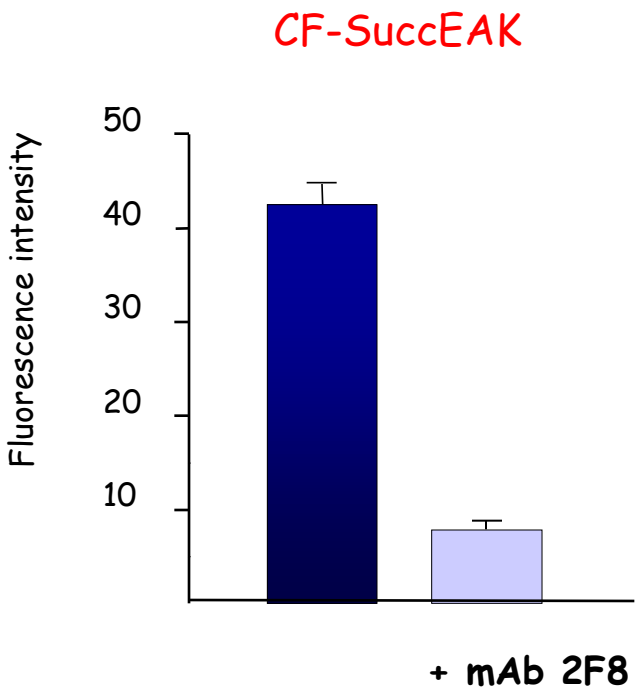
SR-A +/+

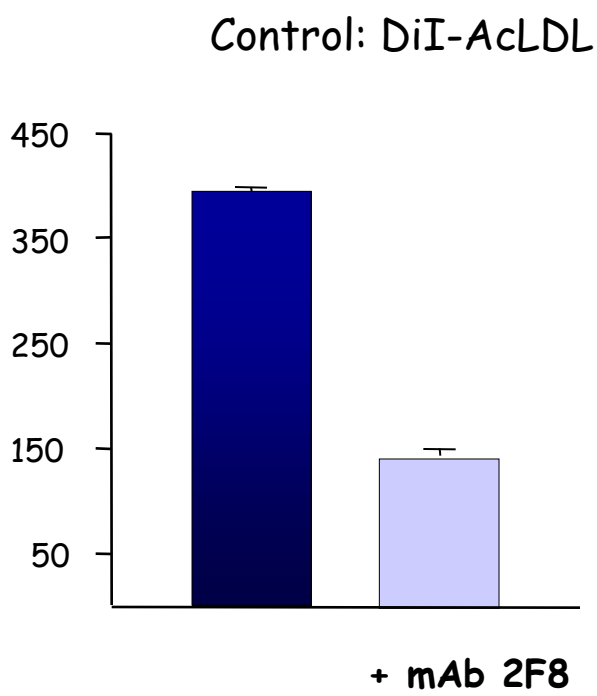
SR-A -/-

Szabó R. et al. Bioconjugate Chem. ***16:*** *1442 (2005)*

Uptake of CF-polypeptides by bone marrow macrophages: inhibition of SR-A receptor by specific antibody

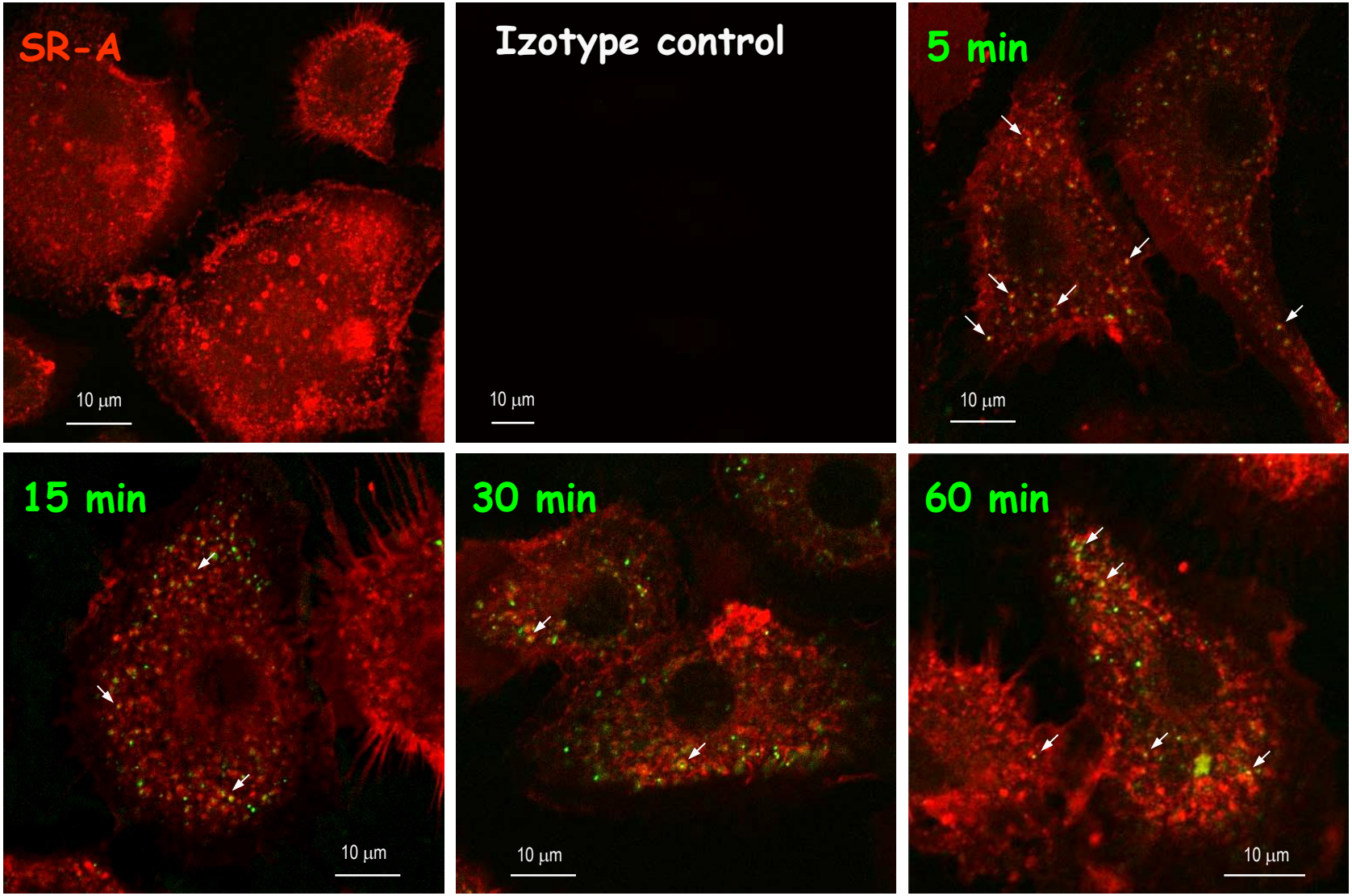






Szabó R. et al. Bioconjugate Chem. ***16:*** *1442 (2005)*

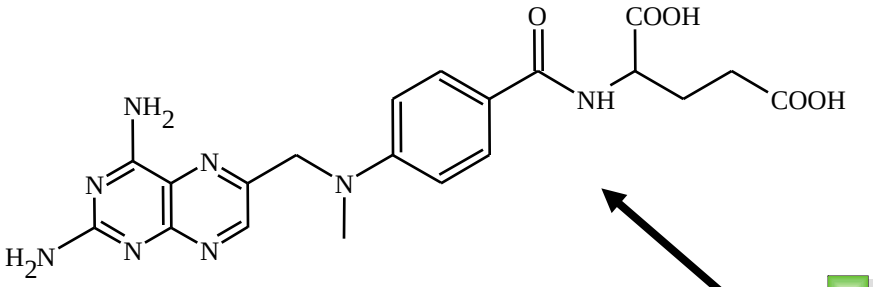
Uptake of CF-SuccEAK plus SR-A receptor complex



SR-A (2F8 + Cy3-antri-rat IgG) CF-SuccEAK

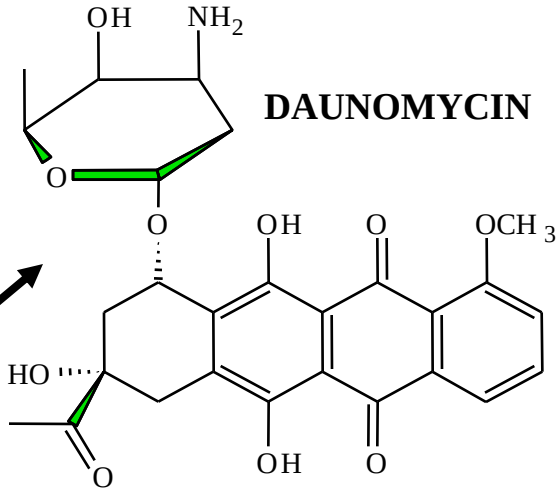
Drug-polypeptide conjugates

METHOTREXATE

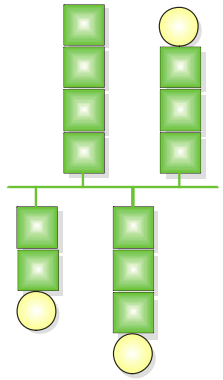


Hudecz F. et al. *Bioconjugate Chem.* **4:** 25 (1993)
Kóczán Gy. et al. *Bioconjugate Chem.* **13:** (2002)

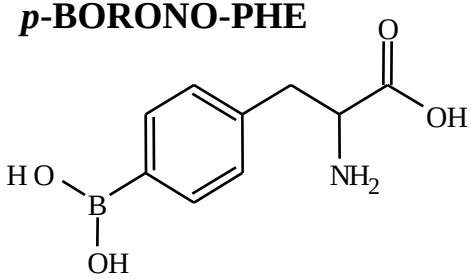
DAUNOMYCIN



Hudecz F. et al.*Bioconjugate Chem.* **3:** 49 (1992)
Gaál D., Hudecz F. *Eur.J.Cancer.* **34:** 155 (1998)

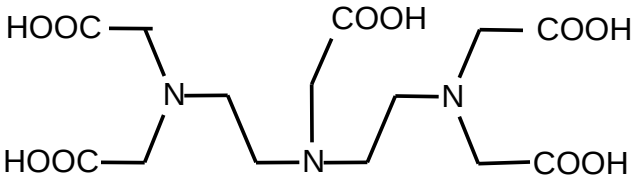


***p*-BORONO-PHE**



Mező G. et al. *J. Bio. Comp. Polymers* **11:** 263 (1996)

DIETHYLENE-TRIAMINE-PENTAACETIC ACID



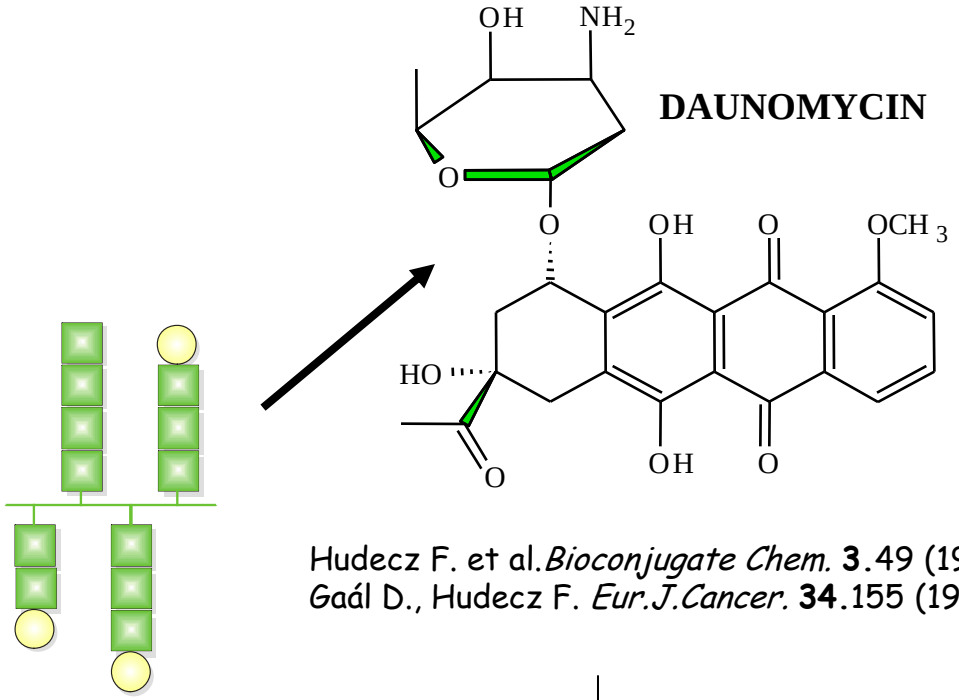
Pimm MV. et al.*Int. J. Pharmaceutics* **79:** 77 (1992)
Pimm MV.et al. *J. Canc. Res. Clin. Onc.* **122:** 45 (1996)

GN-RH ANTAGONIST, MI-1544

D-Trp-*D*-Cpa-*D*-Trp-Ser-Tyr-*D*-Lys-Leu-Arg-Pro-*D*-Ala

Mező, G. et al.*Bioconjugate Chem.* **7:** 642 (1996)
Vincze, B. et al. *J. Cancer Res. Clin. Onc.* **120:** 578 (1994)

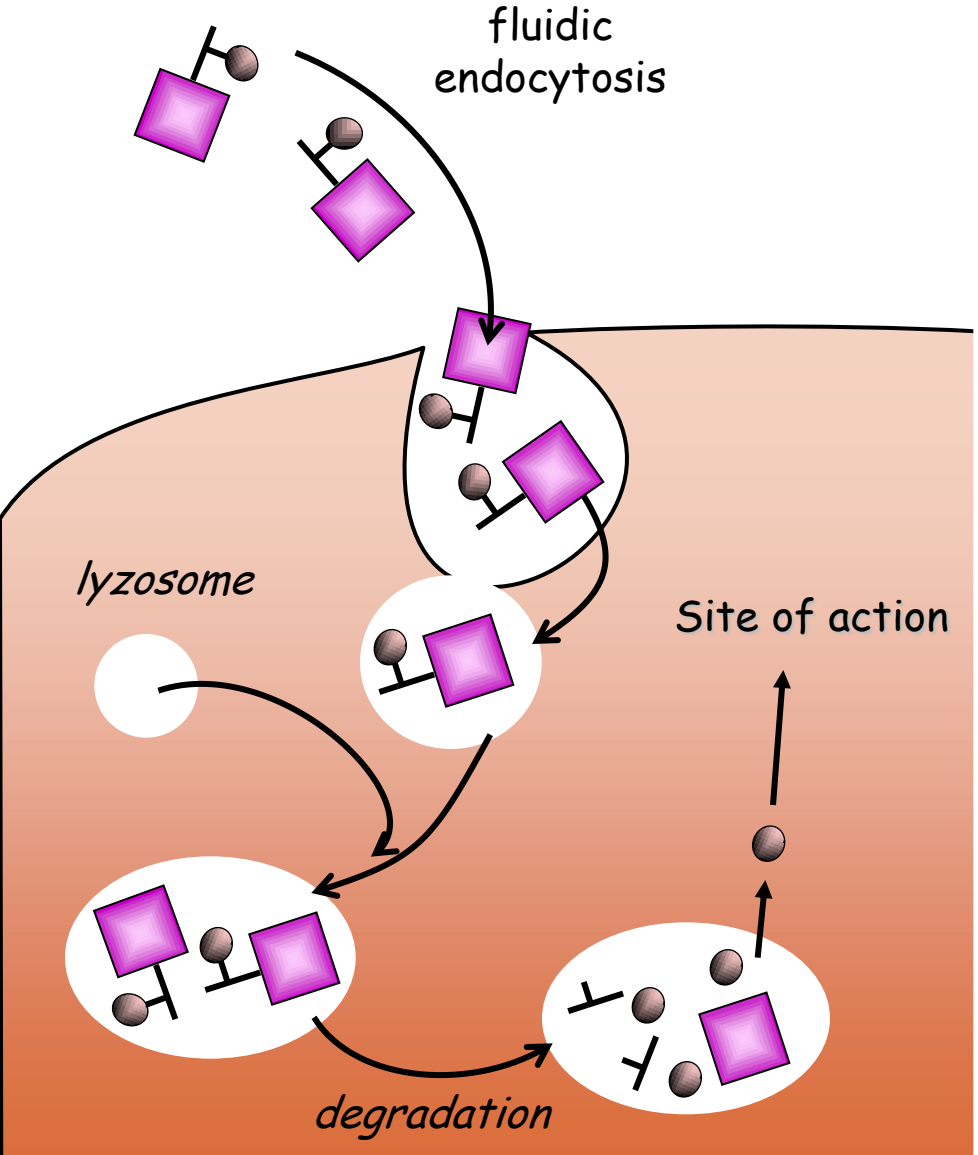
Drug-polypeptide conjugates



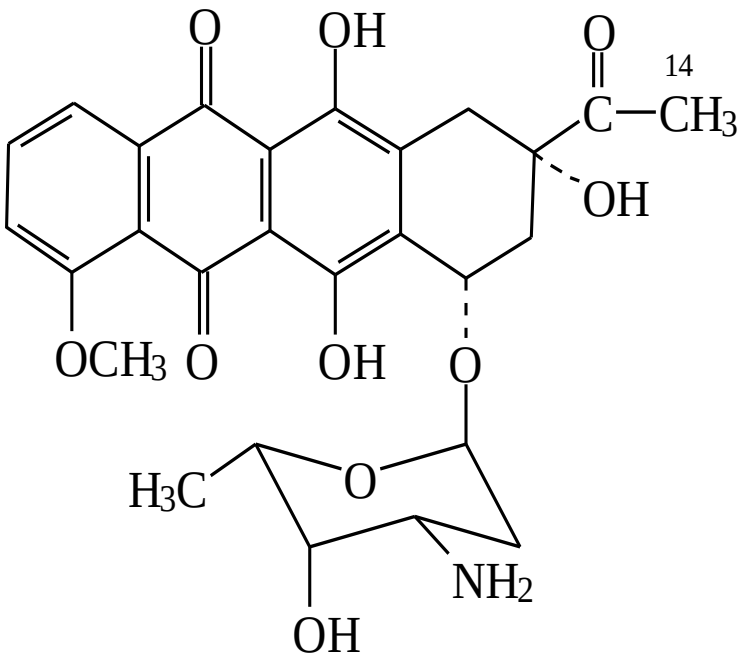
Hudecz F. et al.*Bioconjugate Chem.* **3**.49 (1992)
Gaál D., Hudecz F. *Eur.J.Cancer.* **34**.155 (1998)

Antitumour effect

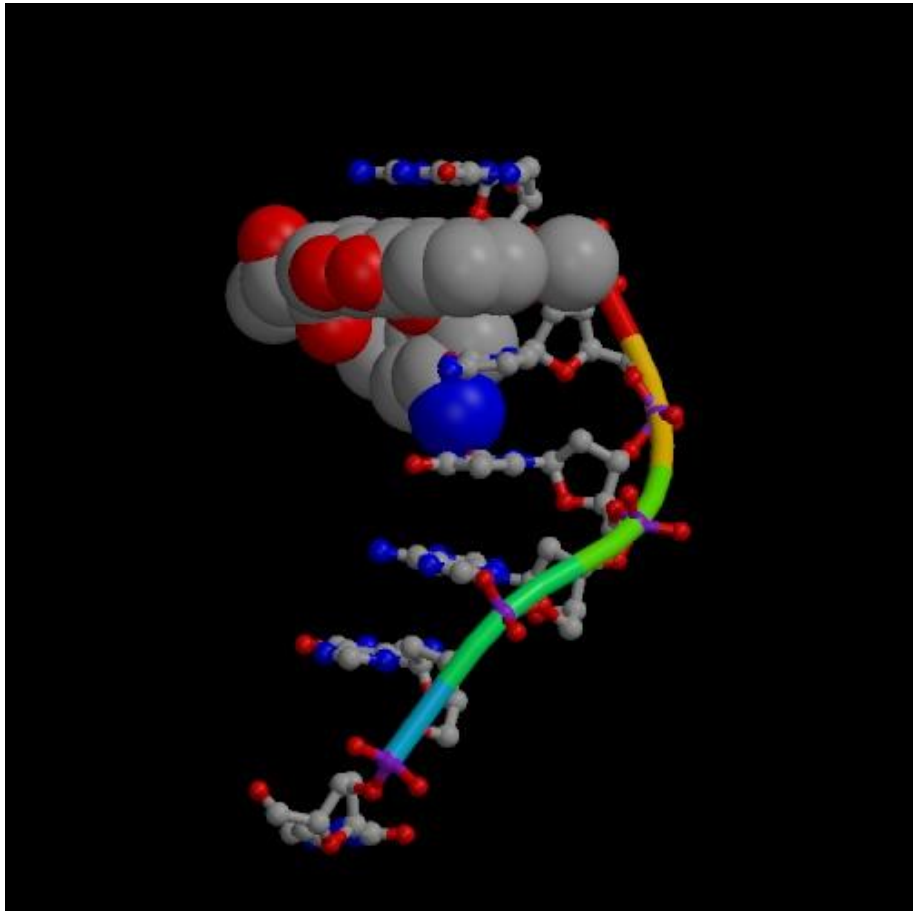
Uptake and liberation of bioactive entities



Daunosamine directed intercalation into minor groove

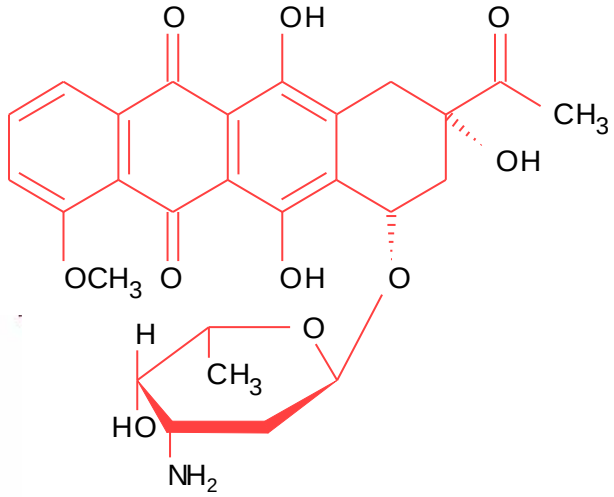


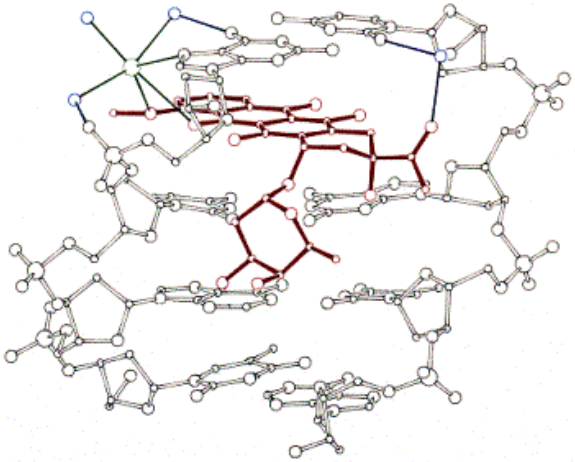
[Frederick, 1990]



Daunomycin in tumour therapy

- Drug of anthracycline family
- Therapeutic use:
  leukaemias (AML, CML, ALL); lymphomas, rhabdomyosarcoma, neuroblastoma
- Side effects:
  - Decreased white blood cell count
  - Cardiotoxicity
  - Nausea and vomiting
  - Hair loss
- Mechanism of action:
  - intercalating DNA,
  - stabilisation DNA-topoisomerase II complex,
  - enhancing the production of free radicals

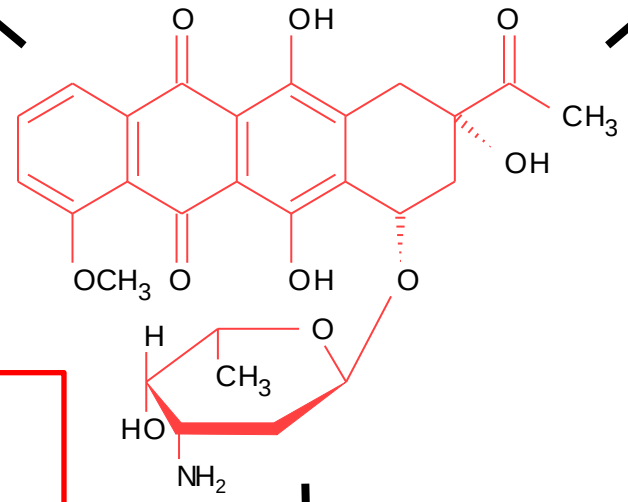




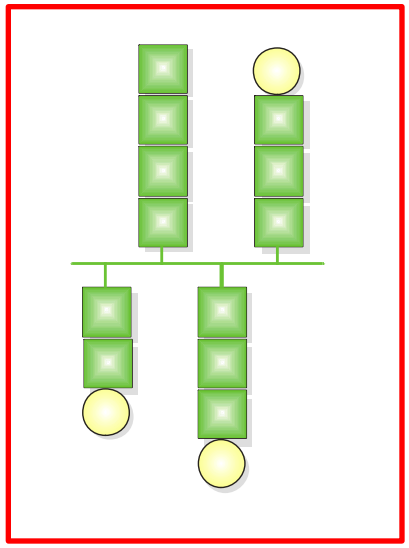
www.chemocare.com
www.cincinnatichildrens.org
Wang-Peng, J. et al, Cancer (2006) 23: 113-121
Laurent, G. et al, Blood. (2001) 98:913-24.

Daunomycin conjugates with oligo- or polypeptide

Orbán E. et al.:
Bioconjugate Chem.
92: 489-499 (2011)

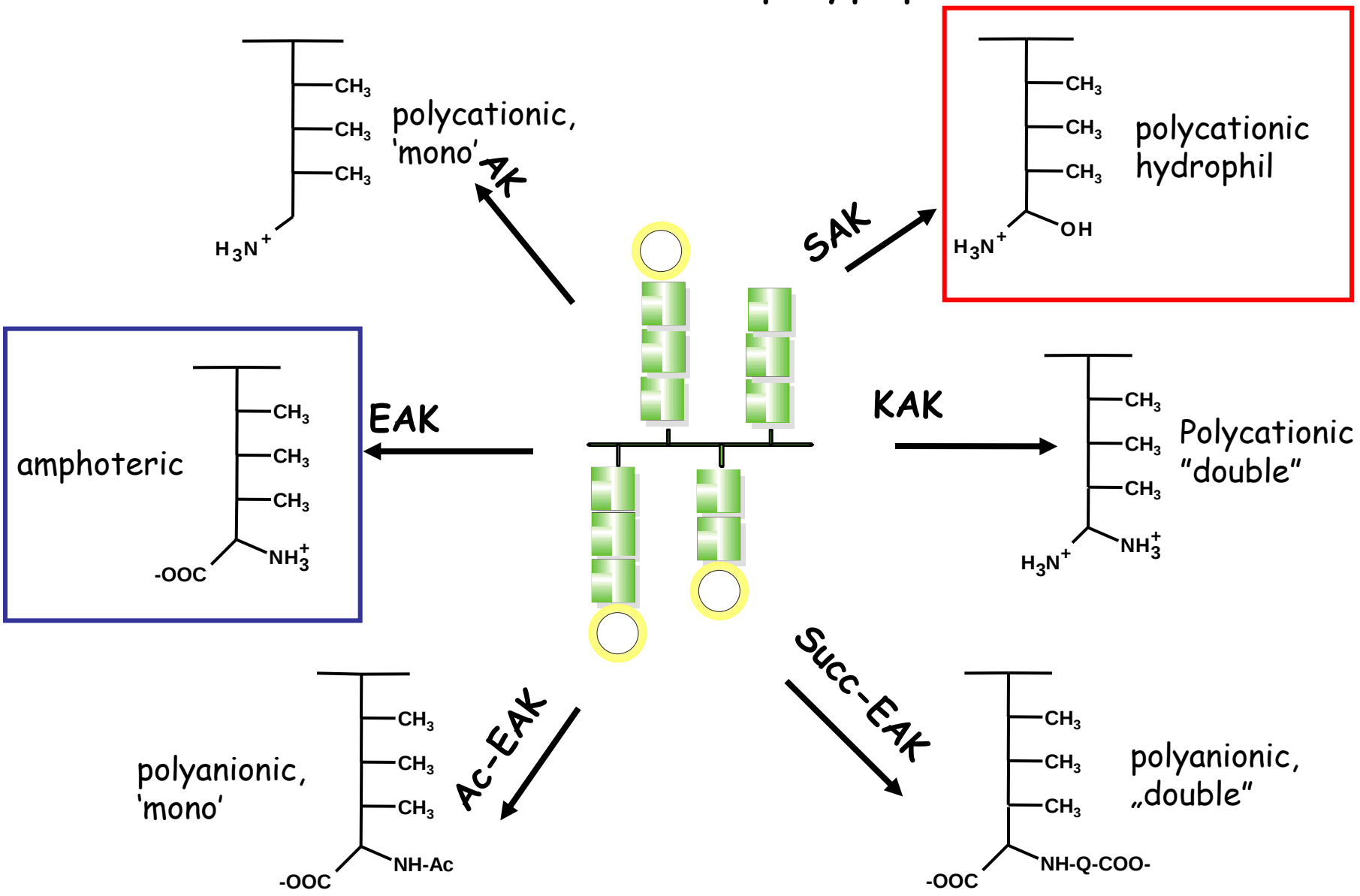


Sztaricskai F. et al.: *J Antibiotics (Tokyo),* **58**: 704 (2005)
Bánóczi Z. et al. *Archivoc* **140**, (2008)
Miklán Zs. et al. *Biopolymers* **92**: 489 (2009)

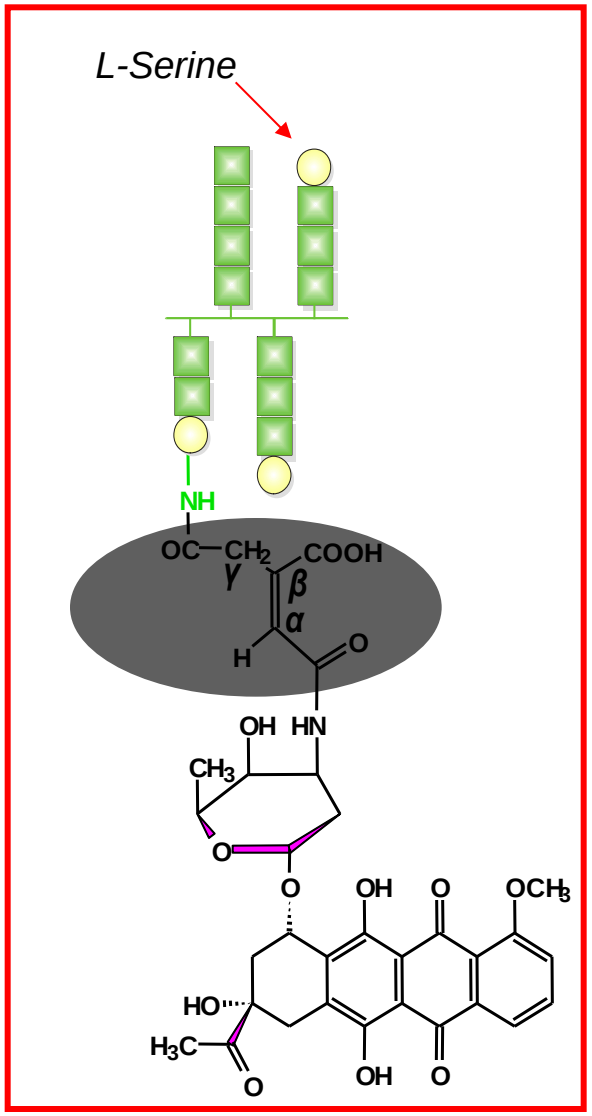


Hudecz F. et al.*Bioconjugate Chem.* **3:** 49 (1992)
Gaál D., Hudecz F. *Eur.J.Cancer.* **34:** 155 (1998)
Szabó R. et al. *Bioconjugate Chem*. **19**: 1078 (2008)
Reményi, J. et al. *Biochim. Biophys. Acta* **1798**: 2209 (2010)

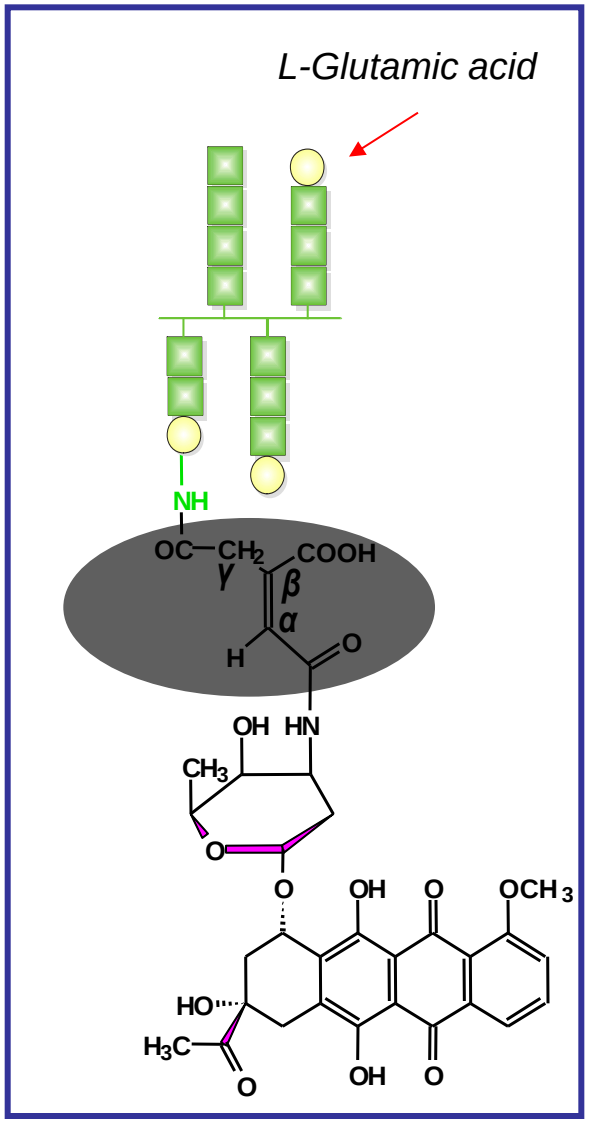
Branched chain polypeptides



Daunomycin-polypeptide conjugates



Hudecz, F. et. al.
Bioconjugate Chem. 10: 781 (1999)



Hudecz, F. et. al.
Bioconjugate Chem. 3: 49 (1992)

polypeptide

acid labile spacer

daunomycin

Toxicity of Dau and cAD-SAK polypeptide conjugate

Treatment (i.p. 1x)	Dose (mg/kg)	Dose of drug bound to polymer	Mean survival (days)	Survivors/ total	Survival (%)
Dau	1		-	7/7	100
	2		-	7/7	100
	4		-	6/7	86
	6		-	4/7	57
	8		16,0±1,7	0/7	0
	15		7,6±0,8	0/7	0
Control	-	-	-	7/7	100
cAD-SAK	180	10	-	6/6	100
Dau + SAK	6+102	6	-	2/5	40,0
SAK	102	-	-	5/5	100
Daunomycin	6	-	-	2/6	33,3
Control	-	-	-	6/6	100

Hudecz et al. J.Mol.Recognition 16: 327 (2003)

Toxicity of Dau and cAD-EAK polypeptide conjugate

Treatment (i.p. 1x)	Dose (mg/kg)	Dose of drug bound to polymer	Mean survival (days)	Survivors/total	Survival (%)
Dau	1	-	-	7/7	100
	2		-	7/7	100
	4		-	6/7	86
	6		-	4/7	57
	8		16.0±1.7	0/7	0
	15		7.6±0.8	0/7	0
Control	-	-	-	7/7	100
cAD-EAK	135	15	-	7/7	100
	205	22,5		7/7	100
	270	30		7/7	100
Dau + EAK	120+15	15	9.0±1.0	0/7	0
EAK	120	-	-	7/7	100

*Gaál D., Hudecz, F. Eur. J. Cancer **34**: 155-161 (1998)*

Antitumour effect of cAD-SAK conjugate on L1210 leukemia *in vivo*

Treatment* (i.p. 1x)	Dose (mg/kg)	Daunomycin content	Mean survival (day)	T/C (%)	Survivor/ total	Survivor (%)
cAD-SAK	180	10	11,0±1,7	105	0/5	-
Daunomycin + SAK	6+102	5	20,6±5,1	180	0/5	-
SAK	170		12,4±4,9	113,6	0/5	-
Daunomycin	6		16,4±2,8	139	0/5	-
Control			10,6±1,9	100	0/5	-

* Treatment one day after the i.p. inoculation of $5x10^6$ L1210 cells i.p. 60-day experiment

Hudecz et al. J.Mol.Recognition 16: 327 (2003)

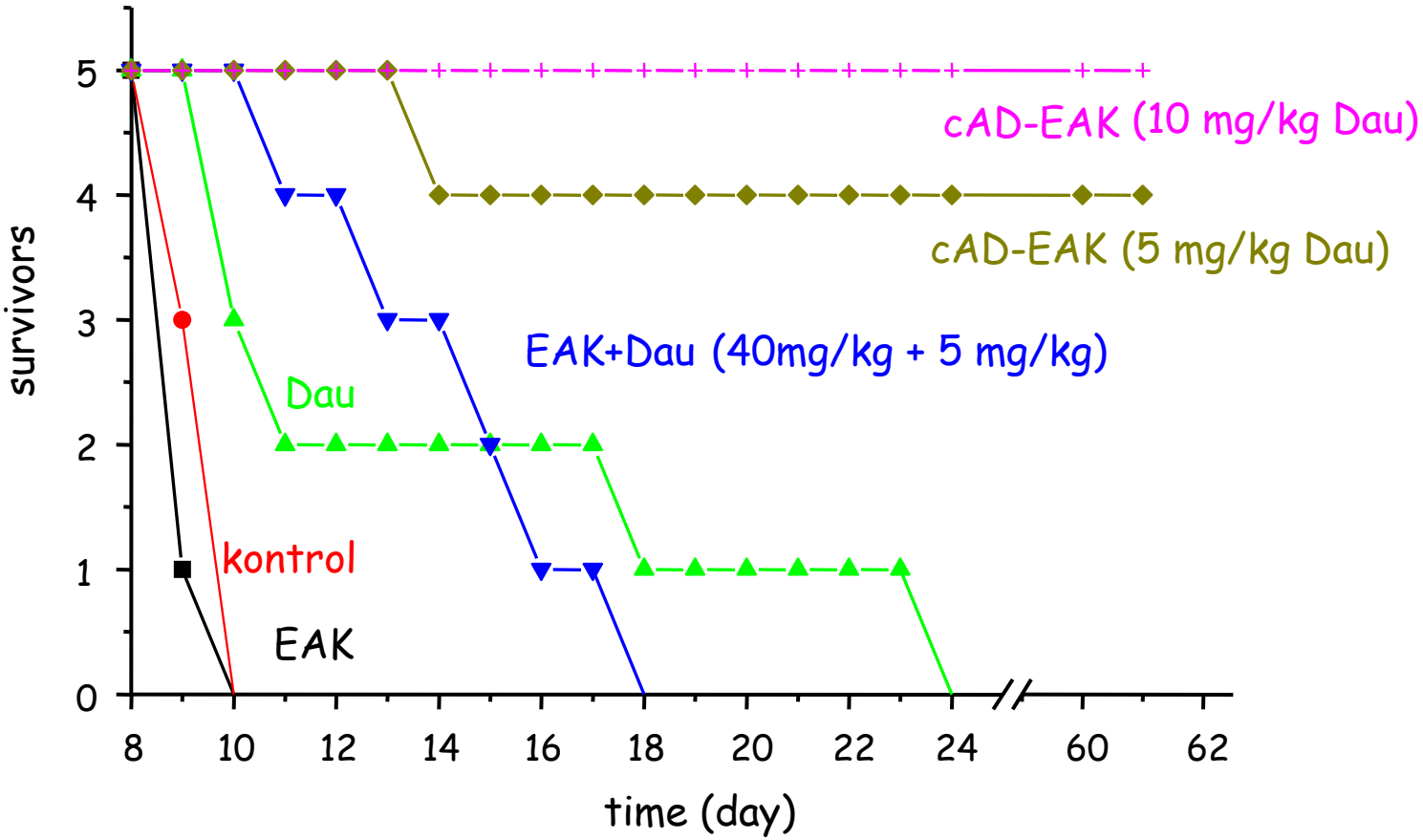
Antitumour effect of cAD-EAK conjugate on L1210 leukemia *in vivo*

Treatment* (i.p. 1x)	Dose (mg/kg)	Daunomycin content	Mean survival (day)	T/C (%)	Survivor/ total	Survivor (%)
cAD-EAK	45	5			4/5	80
	90	10			5/5	100
	4*18	2			3/5	60
Daunomycin + EAK	5+40		14.6±2.7	152	0/5	-
EAK	80		9.0±0.7	94	0/5	-
Daunomycin	5		13.2±2.2	138	0/5	-
	6		14.6±3.1	152	0/5	-
	10		7.8±0.8	81	0/5	-
	4*2 (qd)		13.4±2.9	140	0/5	-
Control			9,6±0,5	100	0/5	- -

* Treatment one day after the i.p. inoculation of $5x10^6$ L1210 cells i.p. 60-day experiment

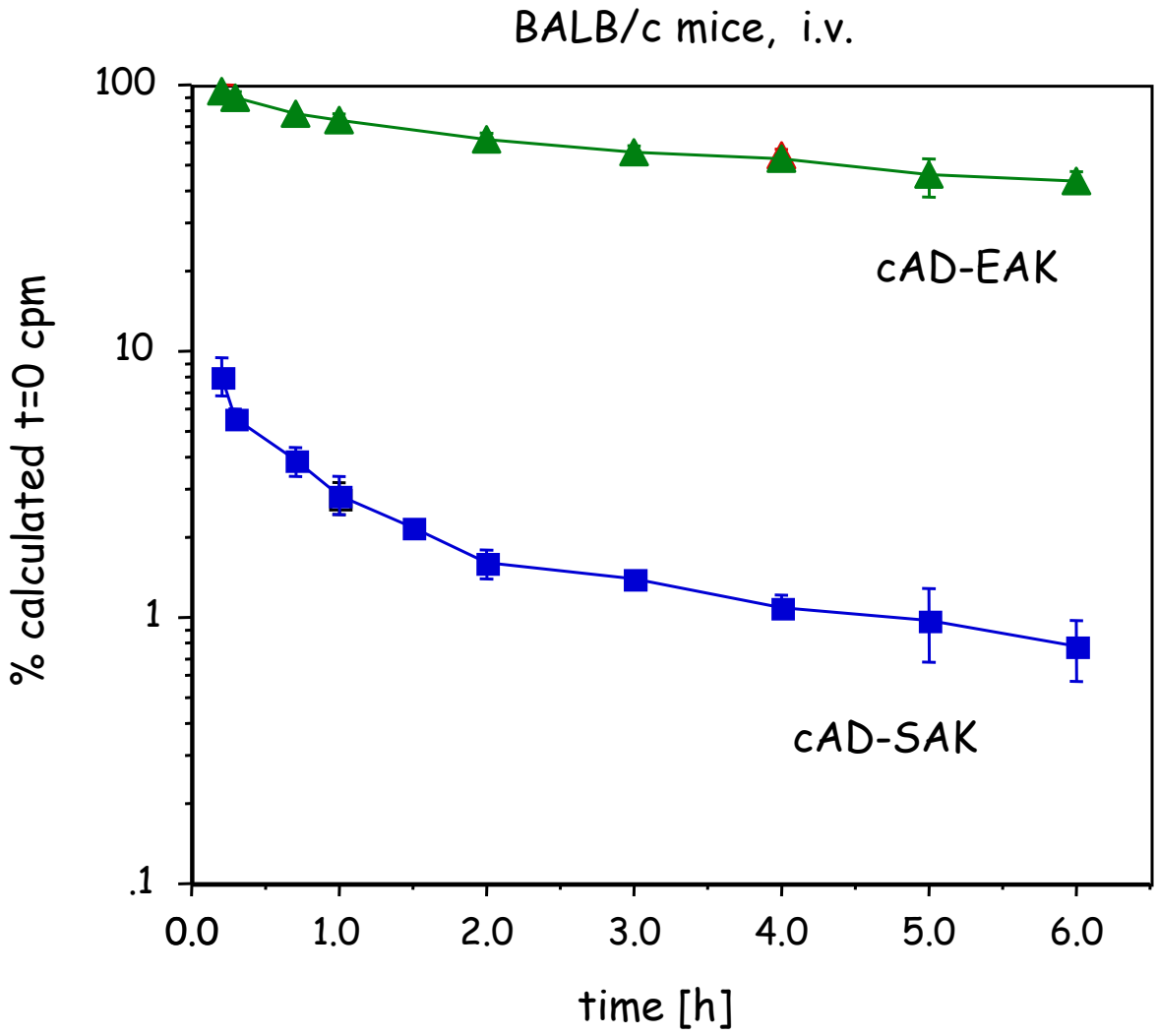
*Gaál D., Hudecz, F. Eur. J. Cancer **34**: 155-161 (1998)*

Effect of cAD-EAK conjugate on mice with L1210 leukemia

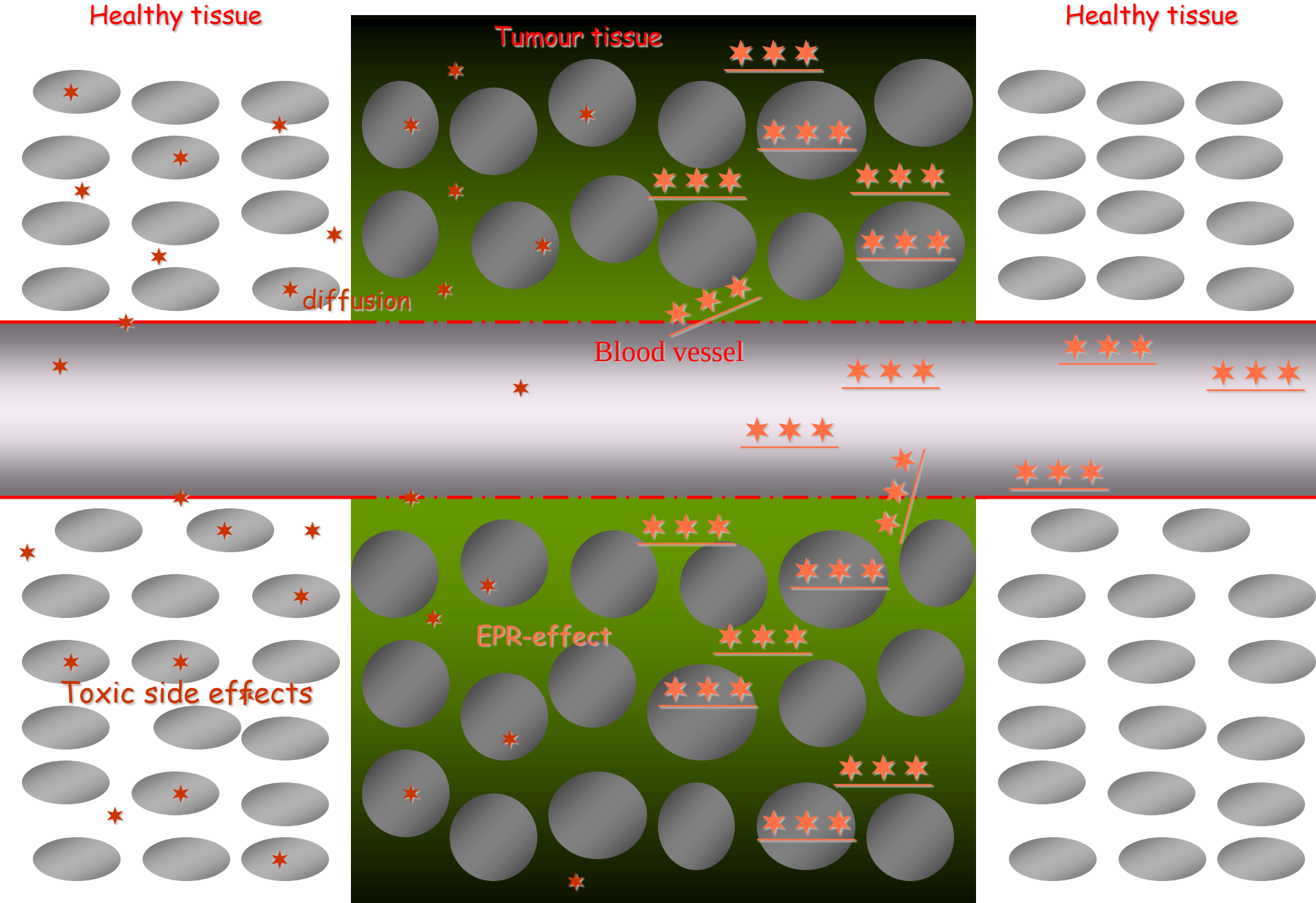


Gaál D., Hudecz, F. Eur. J. Cancer ***34****: 155-161 (1998)*

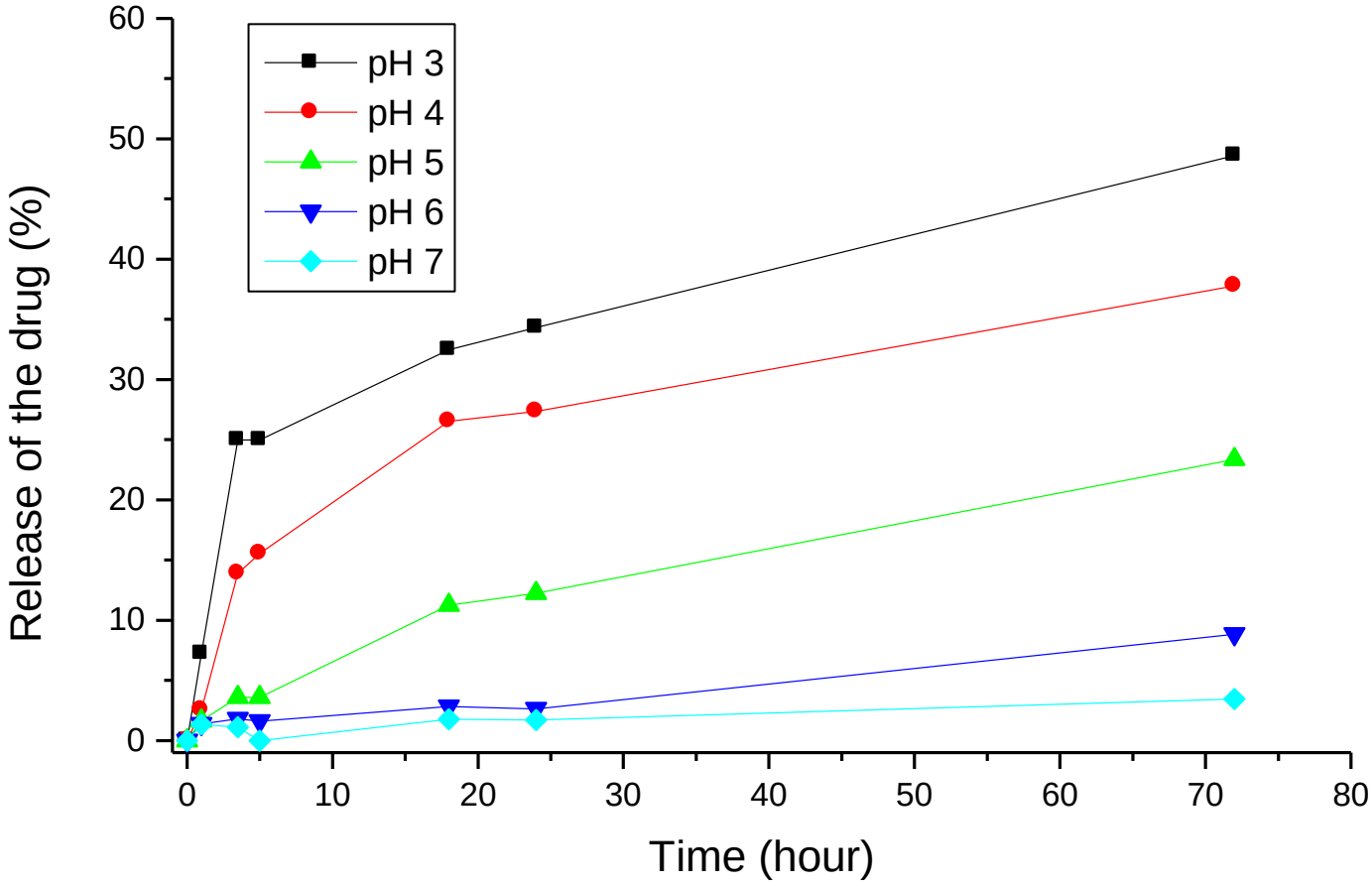
Blood clearance cAD/MTX polypeptide conjugates



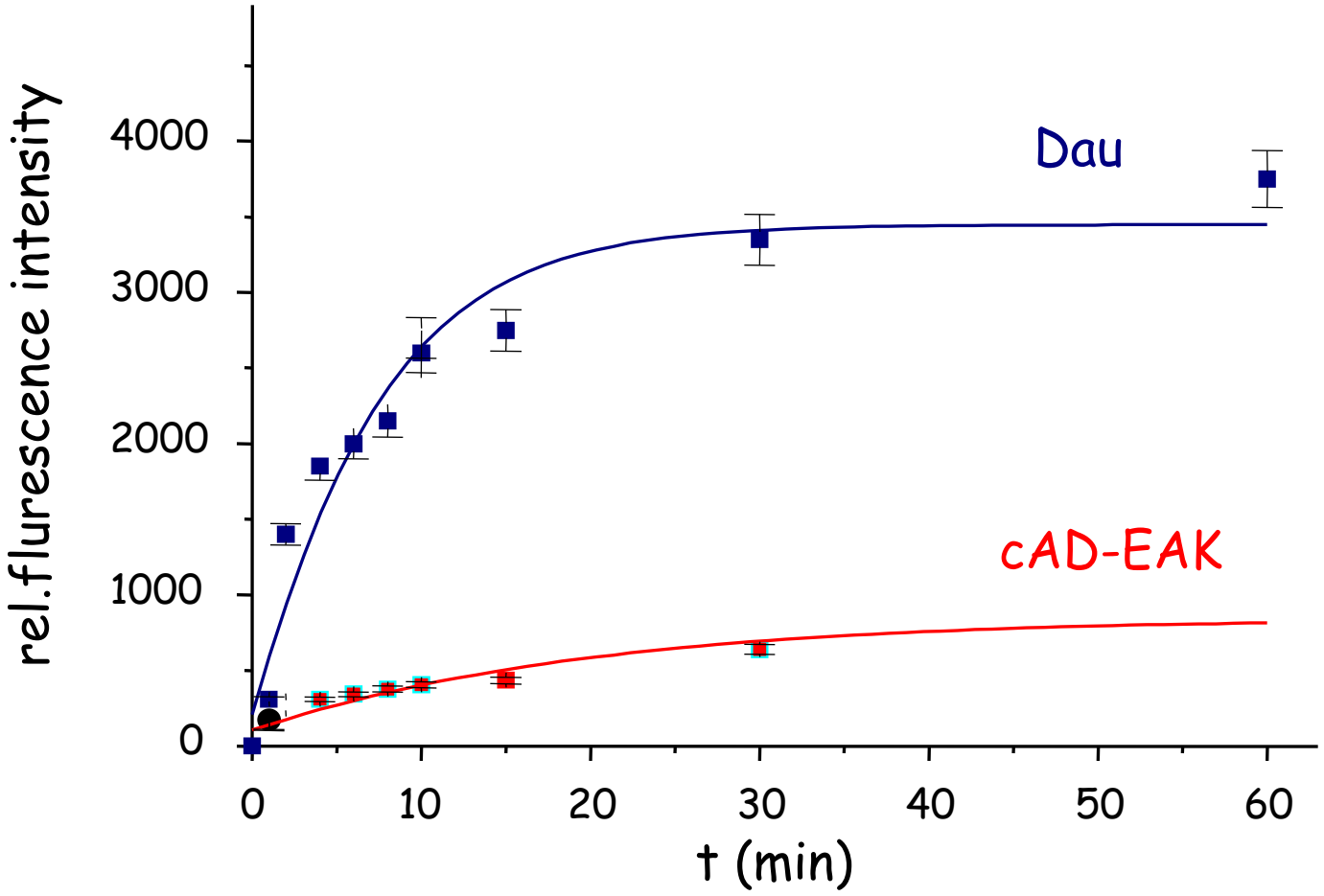
Mechanism of action



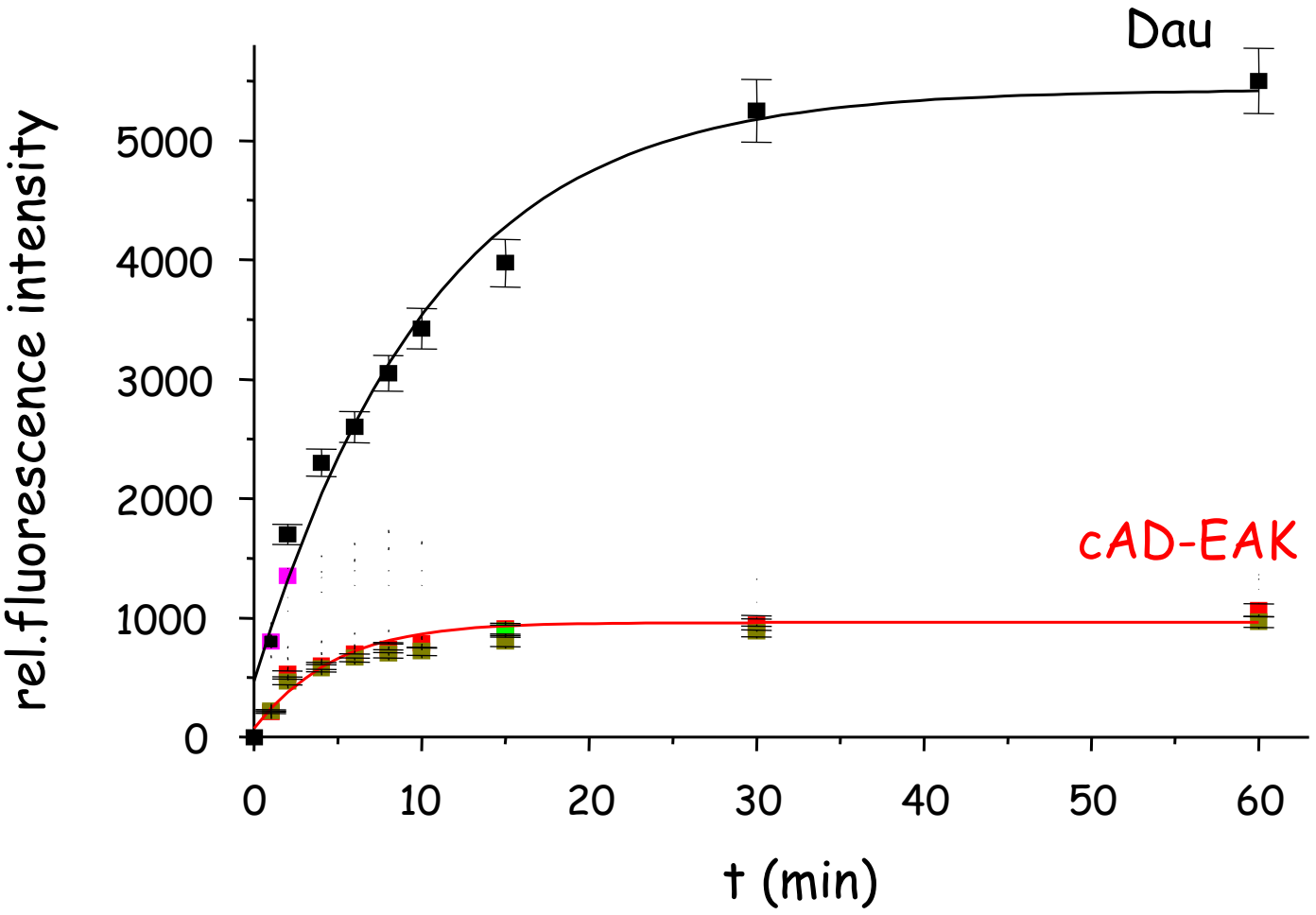
Release of drug from cAD-EAK conjugates



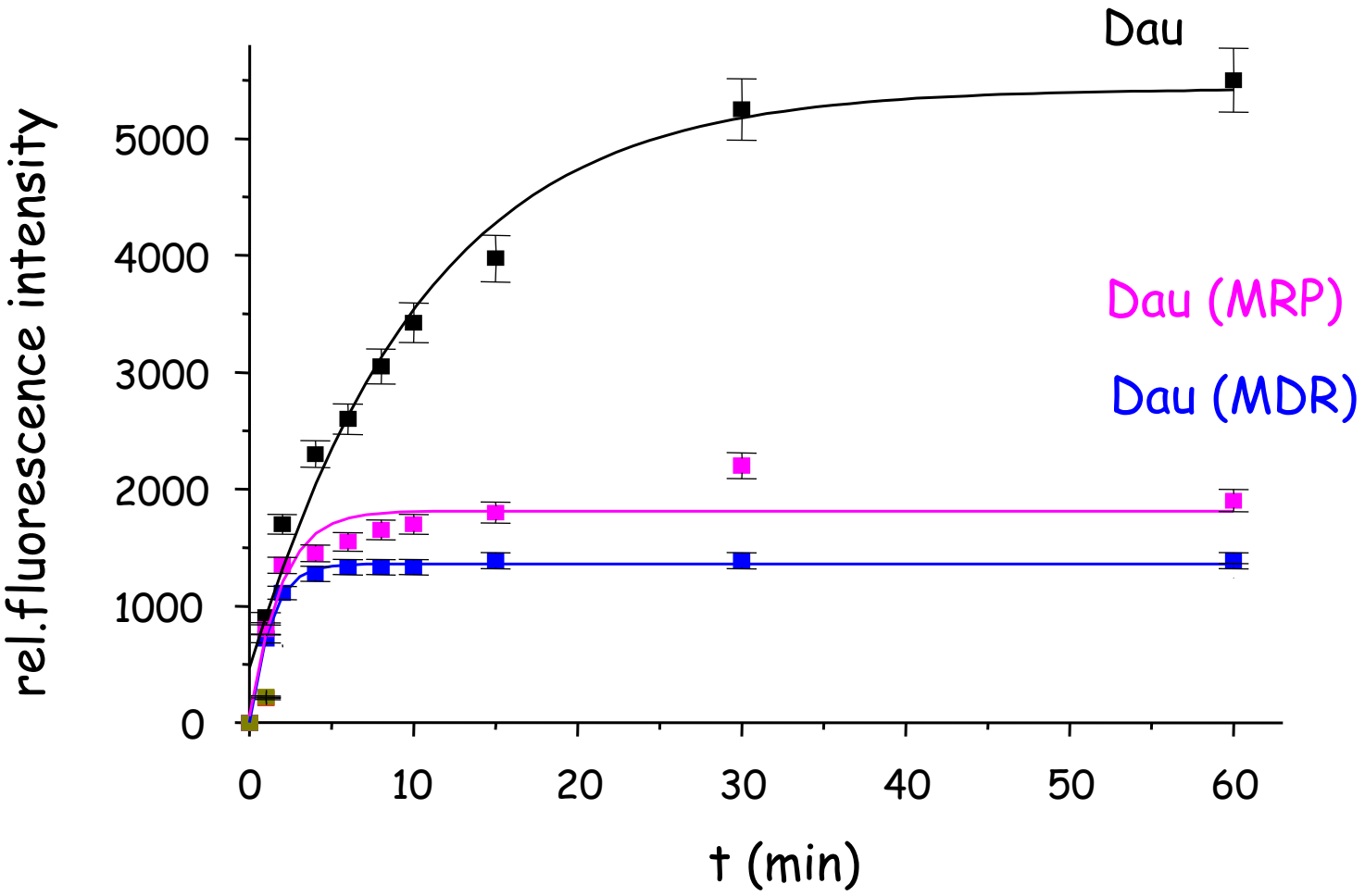
Uptake of daunomycin and cAD-EAK conjugate by L1210 cells



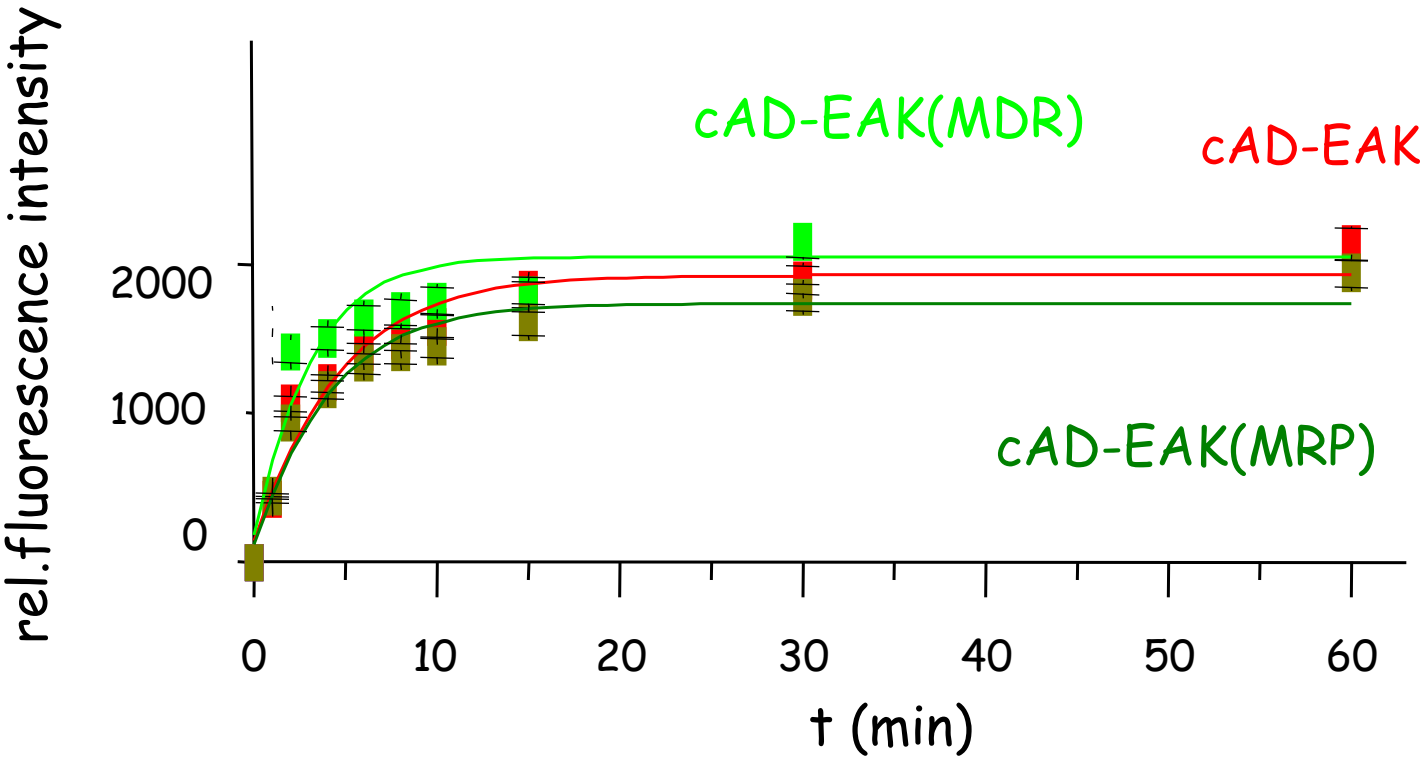
Uptake of daunomycin and cAD-EAK conjugate by sensitive HL60 cells



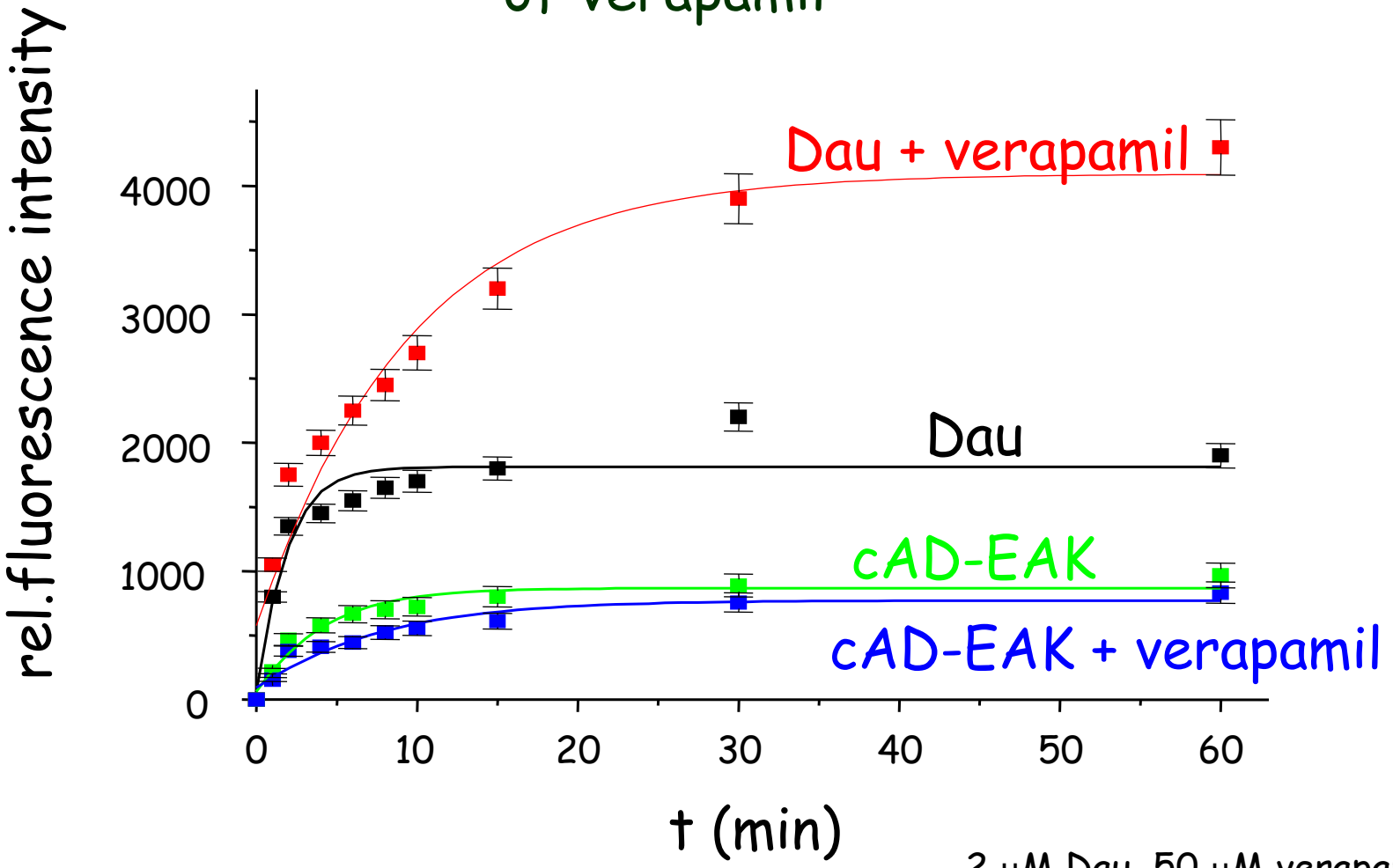
Uptake of daunomycin by sensitive and resistant (MDR1 and MRP) HL60 cells



Uptake of cAD-EAK conjugate by sensitive and resistant (MDR1 and MRP) HL60 cells

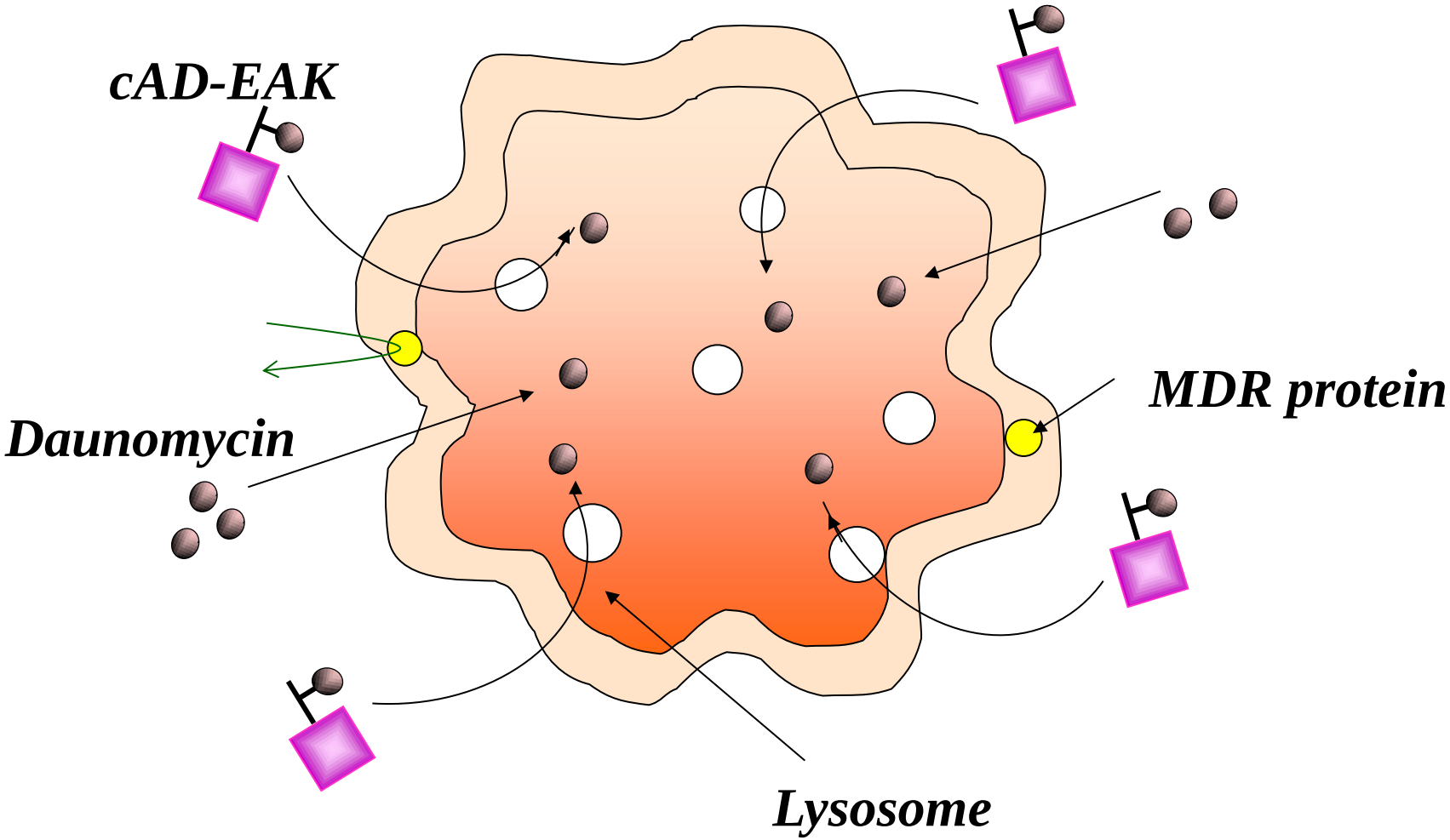


Uptake of daunomycin and cAD-EAK conjugate by HL60/MRP1 cells (f=0.61) in the absence or presence of verapamil

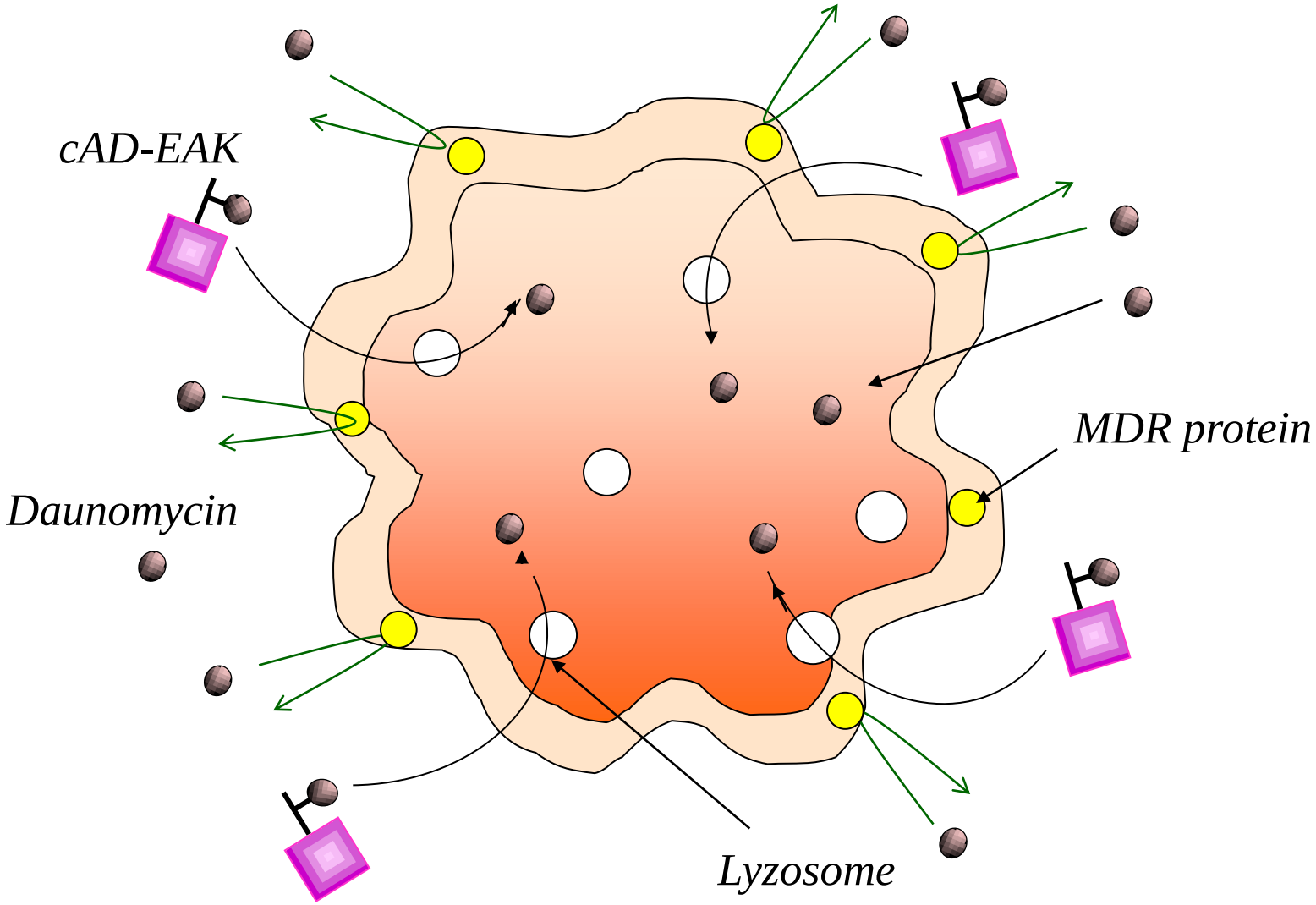


2 μM Dau, 50 μM verapamil

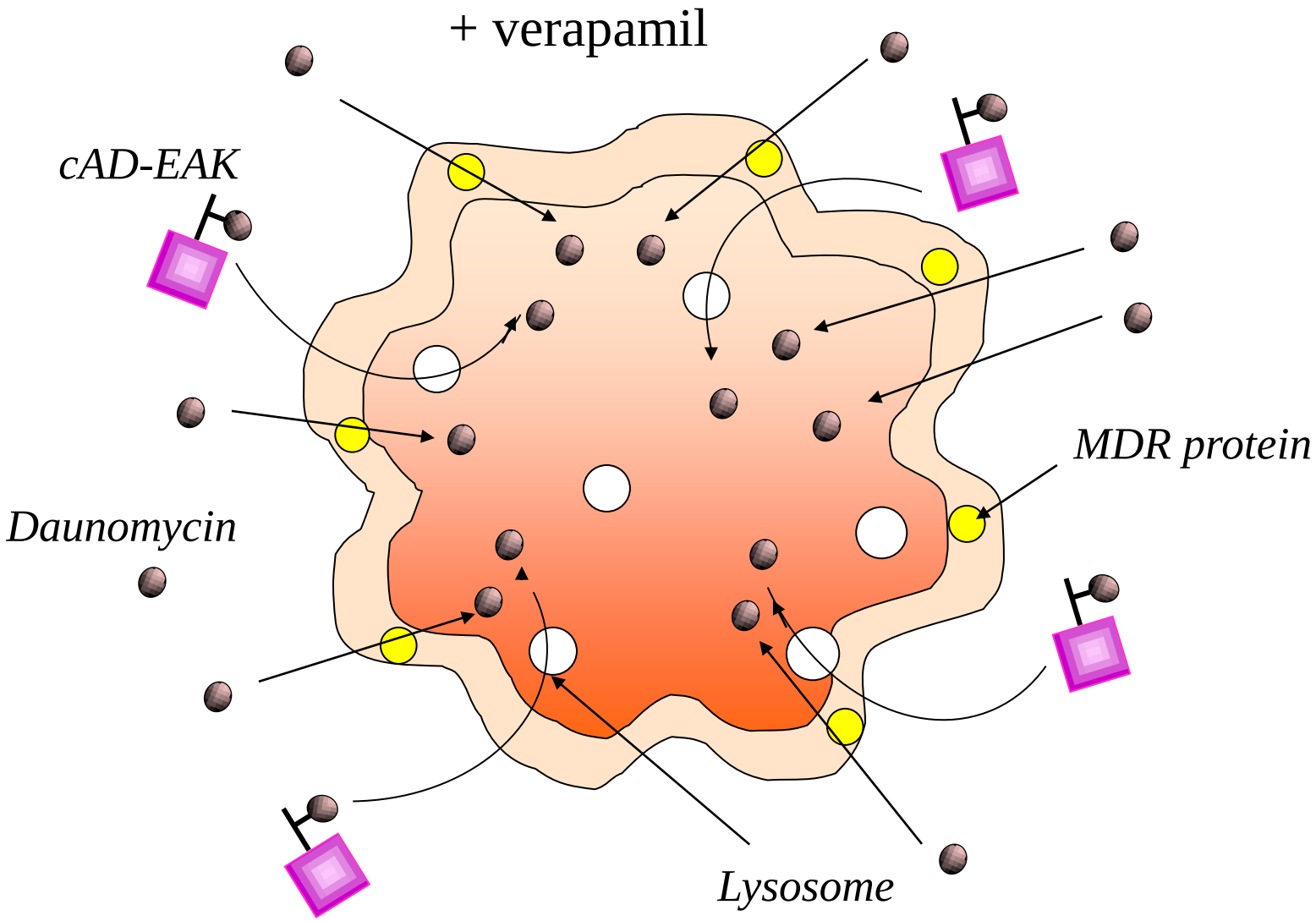
Uptake of daunomycin and cAD-conjugates by sensitive tumour cells



Daunomycin „resistant" tumor cell

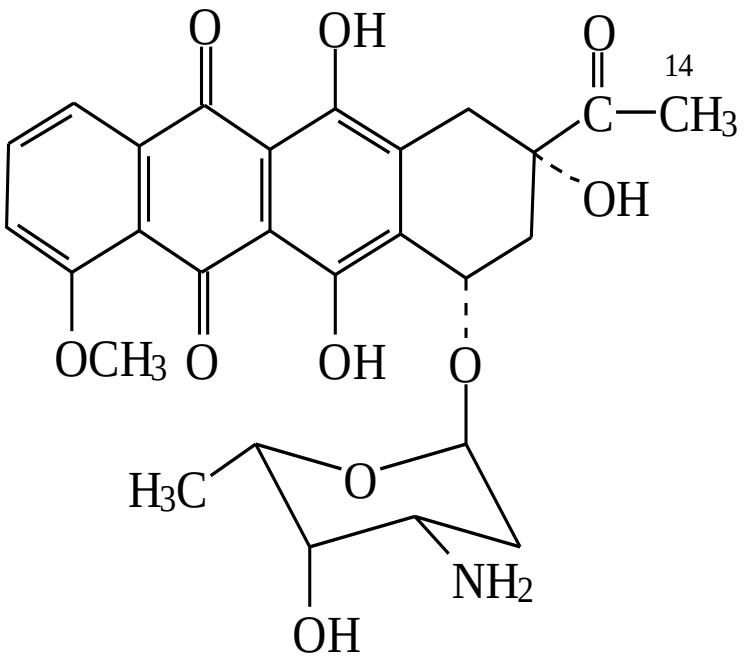


Daunomycin „resistant" tumor cell

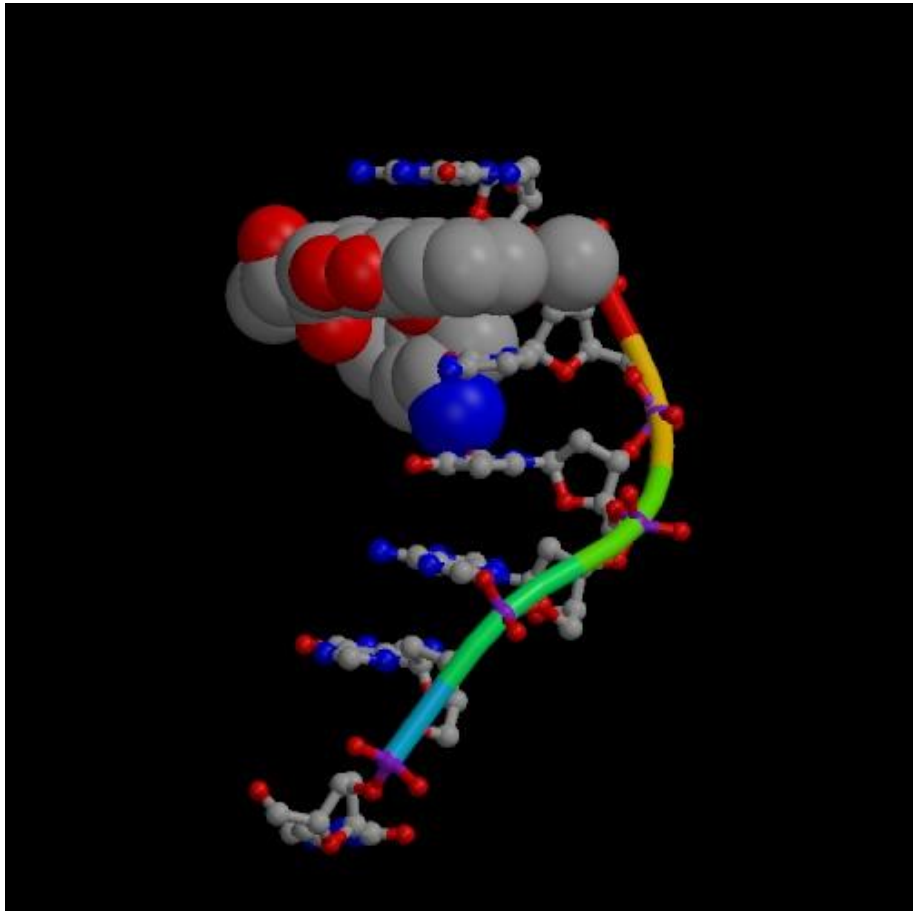


Verapamil = Daunomyin uptake

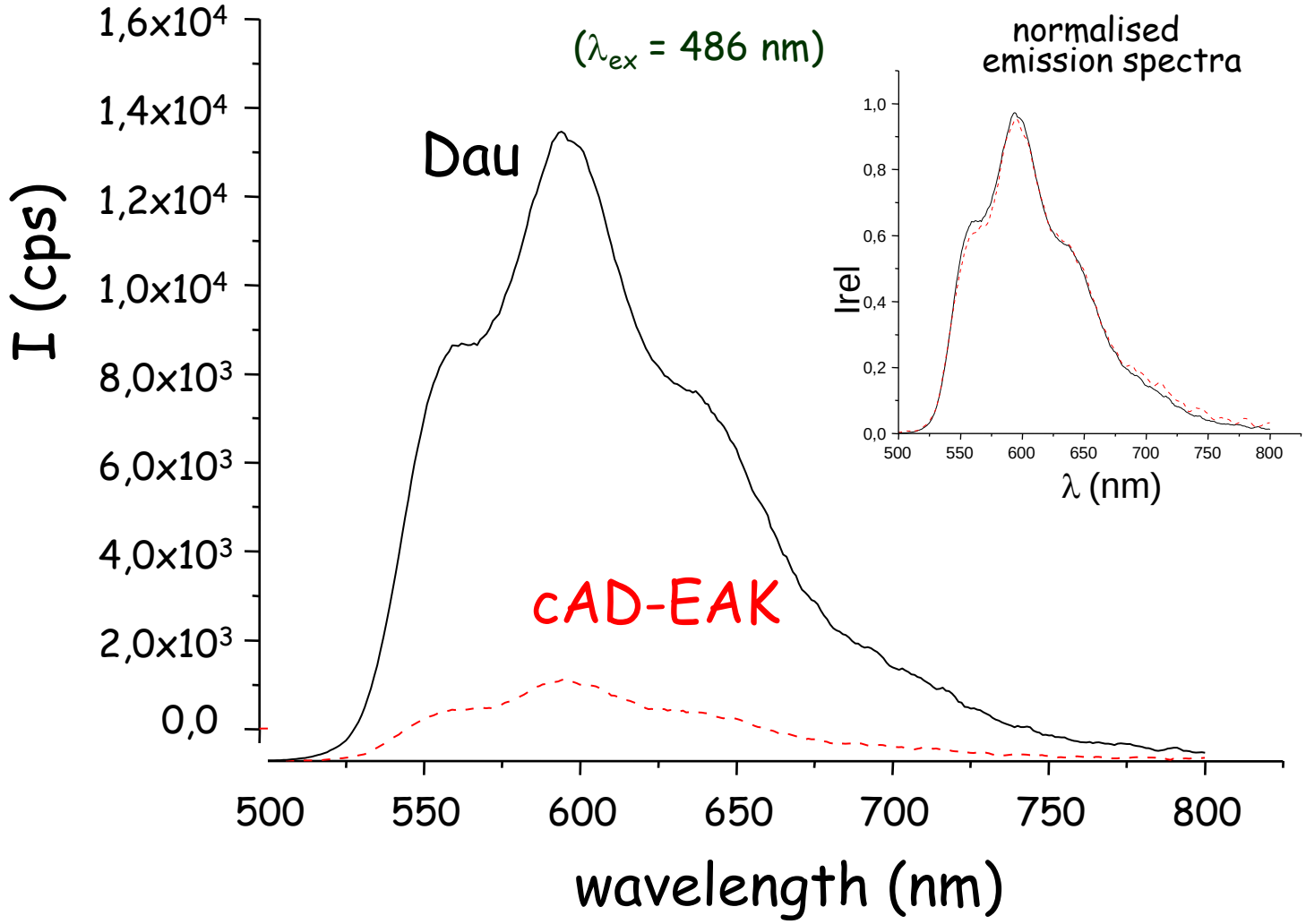
Daunosamine directed intercalation into minor groove



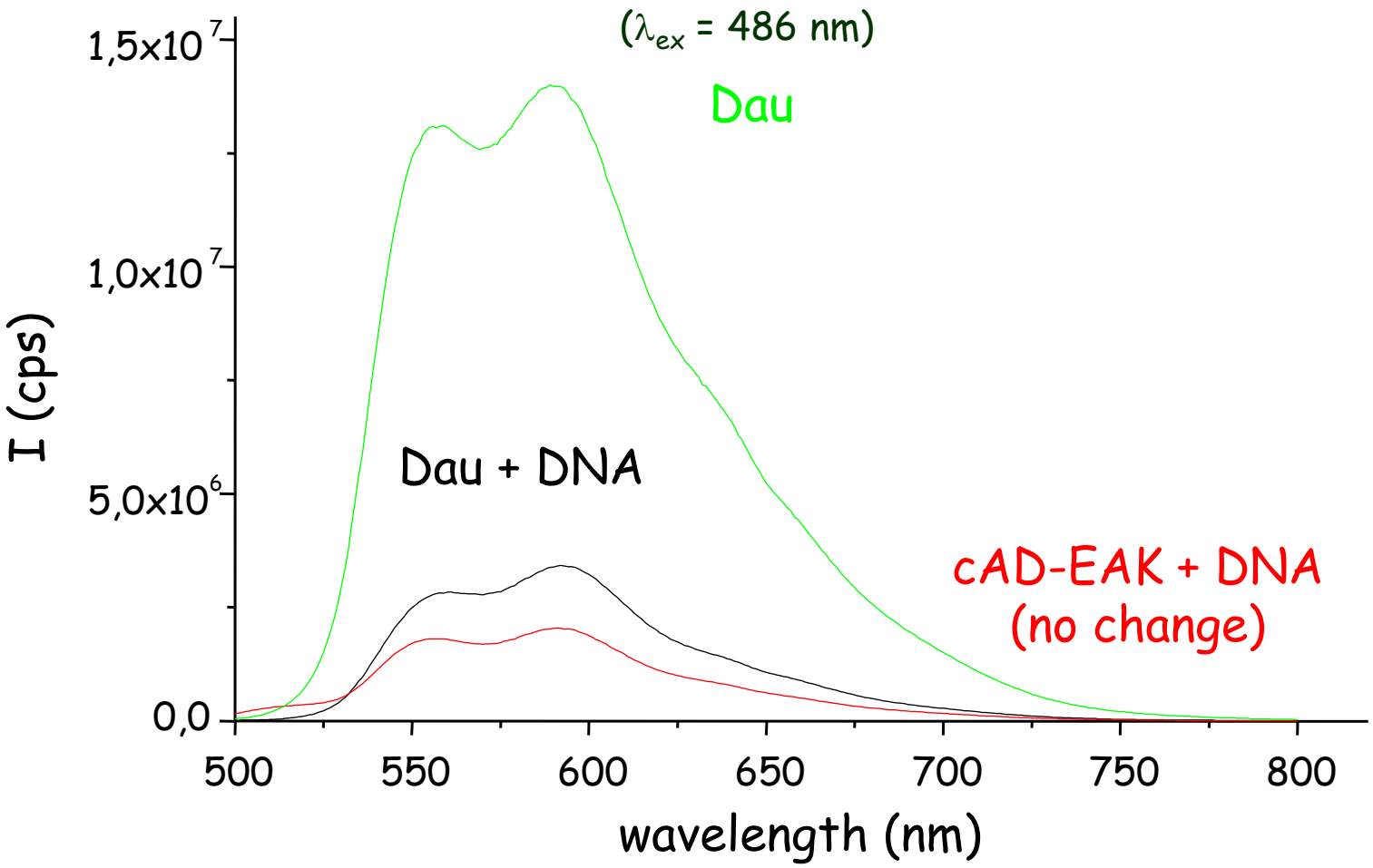
[Frederick, 1990]



Fluorescence spectra of daunomycin and cAD-EAK conjugate

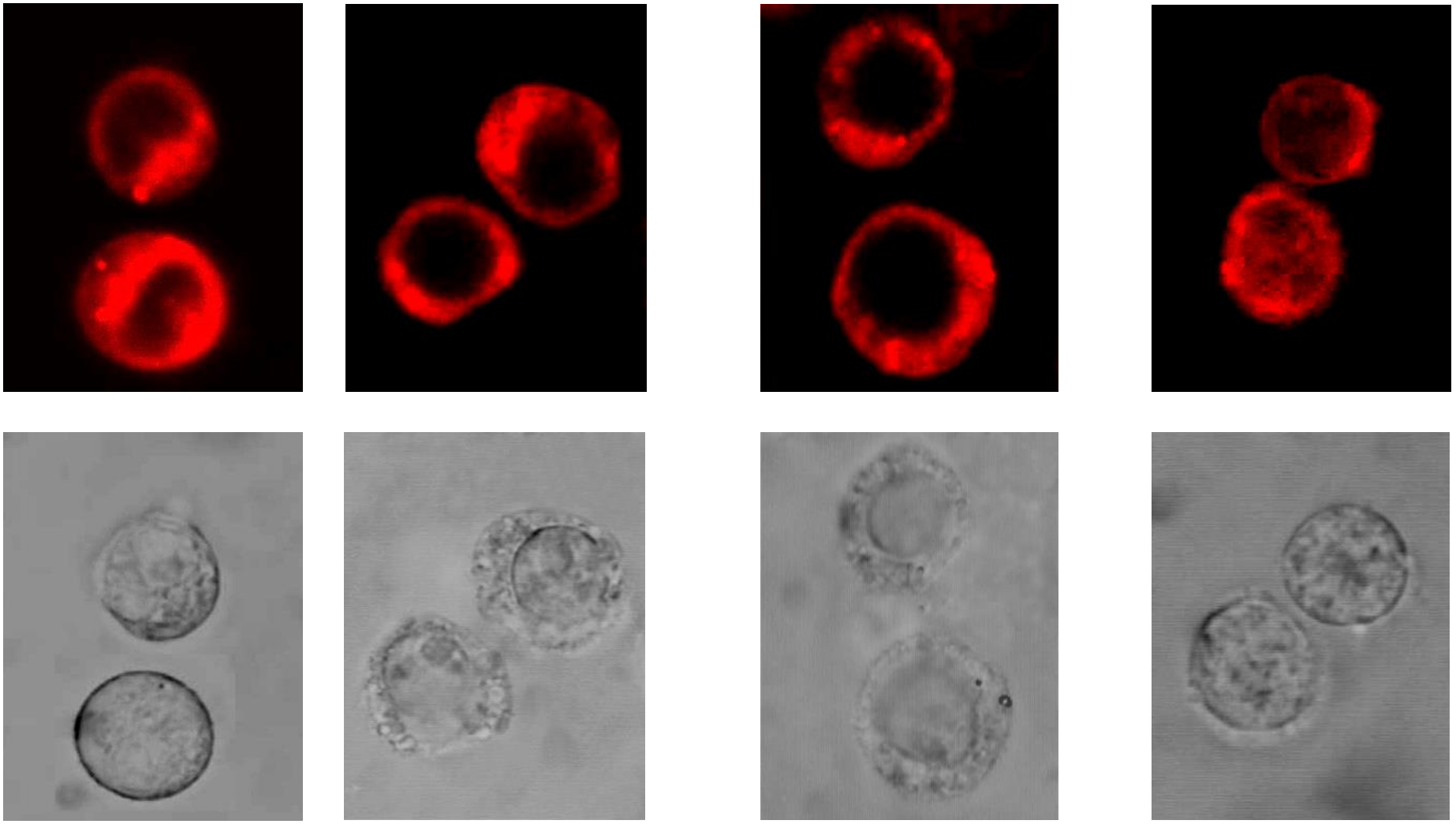


Fluorescence spectra of daunomycin and cAD-EAK conjugate in the absence or presence of DNA

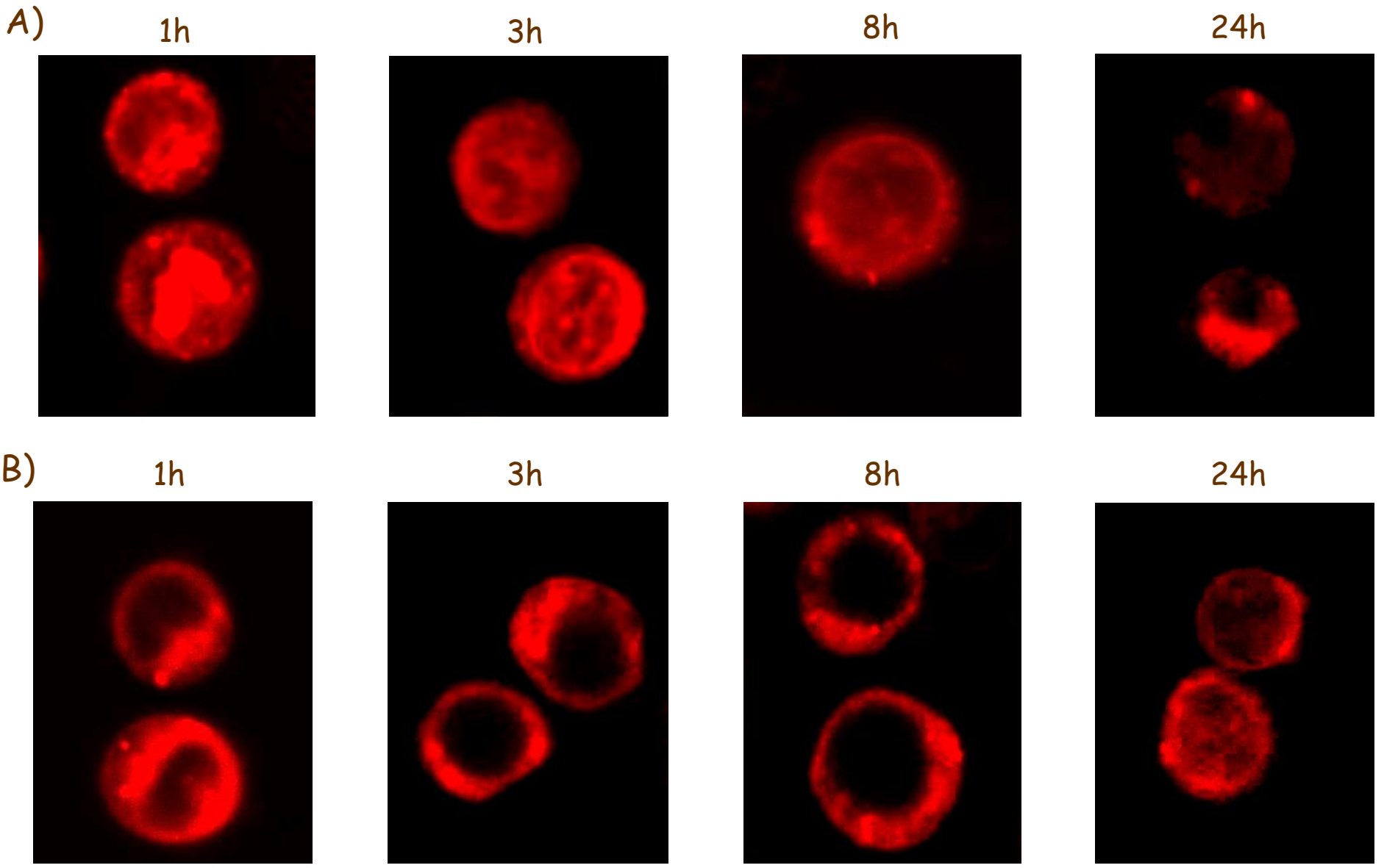


Time dependent localization of cAD-EAK conjugate (daunomycin: 2 μM) in HL-60/sensitive cells (f=0.13)

1h 3h 8h 24h

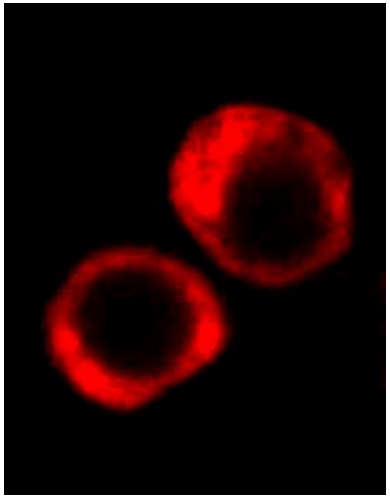


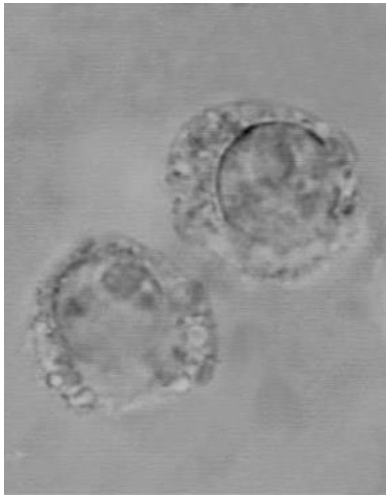
Time dependent localization of daunomycin (2 μM) (A) and cAD-EAK conjugate (daunomycin: 2 μM) (B) in HL-60/sensitive cells (f=0.13)



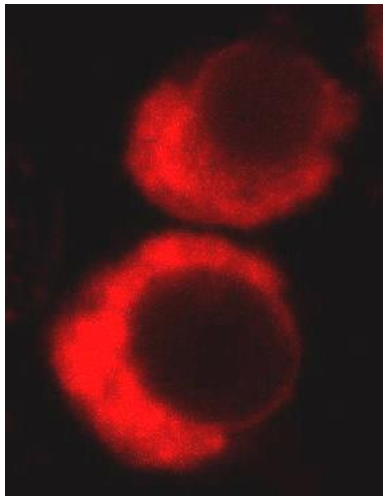
Localization of cAD-EAK conjugate (daunomycin: 2 μM) in sensitive and resistant cells (incubation: 3h)

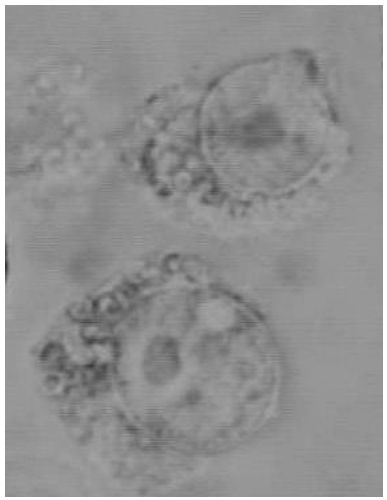
HL-60/sensitive (f=0.13)



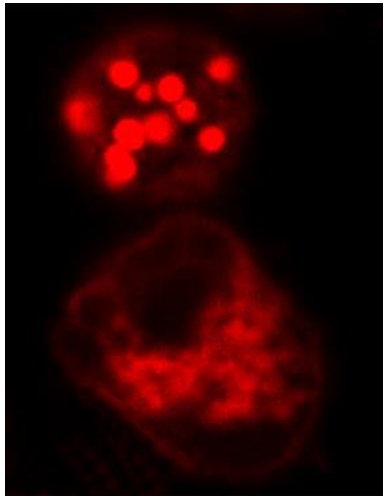


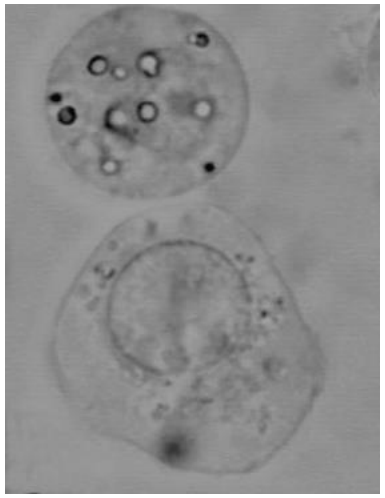
HL-60/MDR1 (f=0.90)





HL-60/MRP1 (f=0.61)

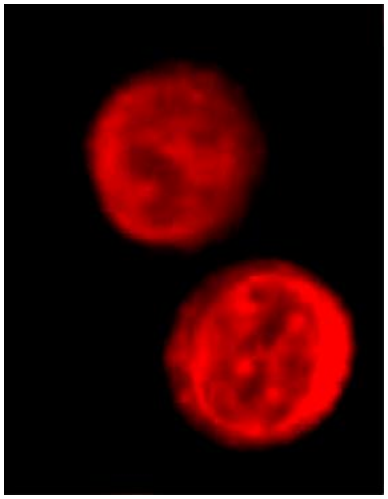




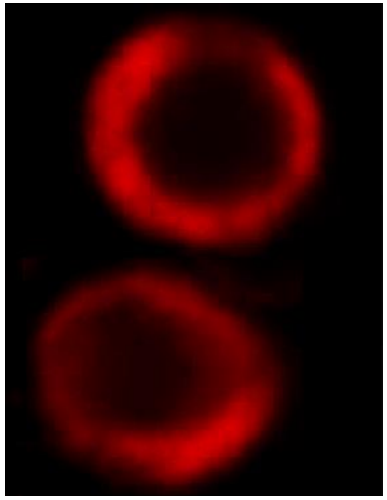
Localization of daunomycin (2 μM) (A) and cAD-EAK conjugate (daunomycin: 2 μM) (B) in sensitive and resistant cells (3h)

A)

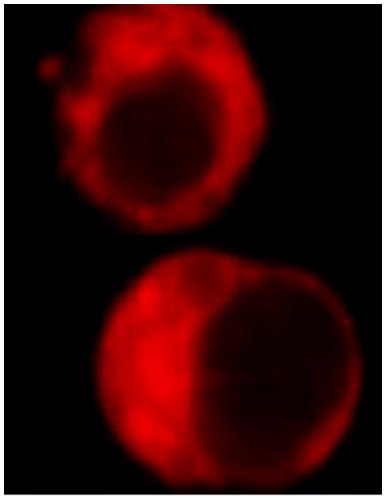
HL-60/sensitive (f=0.13)



HL-60/MDR1 (f=0.90)

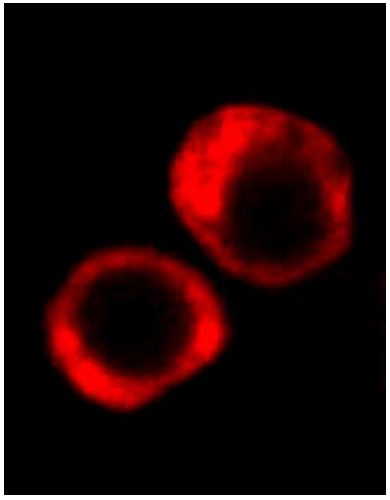


HL-60/MRP1 (f=0.61)

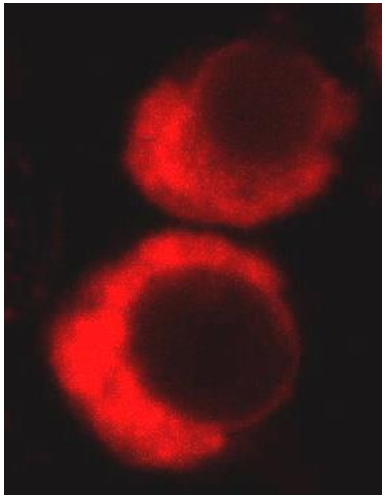


B)

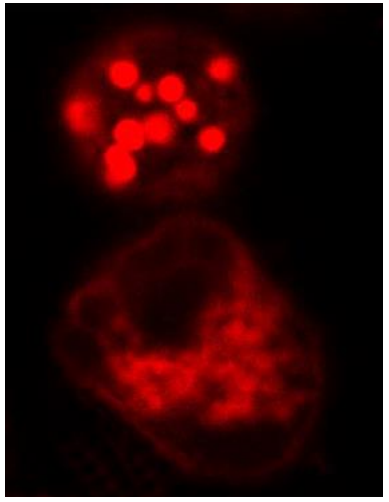
HL-60/sensitive (f=0.13)



HL-60/MDR1 (f=0.90)



HL-60/MRP1 (f=0.61)



Conclusions

1. Daunomycin conjugated with polycationic (SAK) or amphoteric (EAK) polypeptide exhibit no *in vivo* toxicity in mice at 10 mg/kg dose.

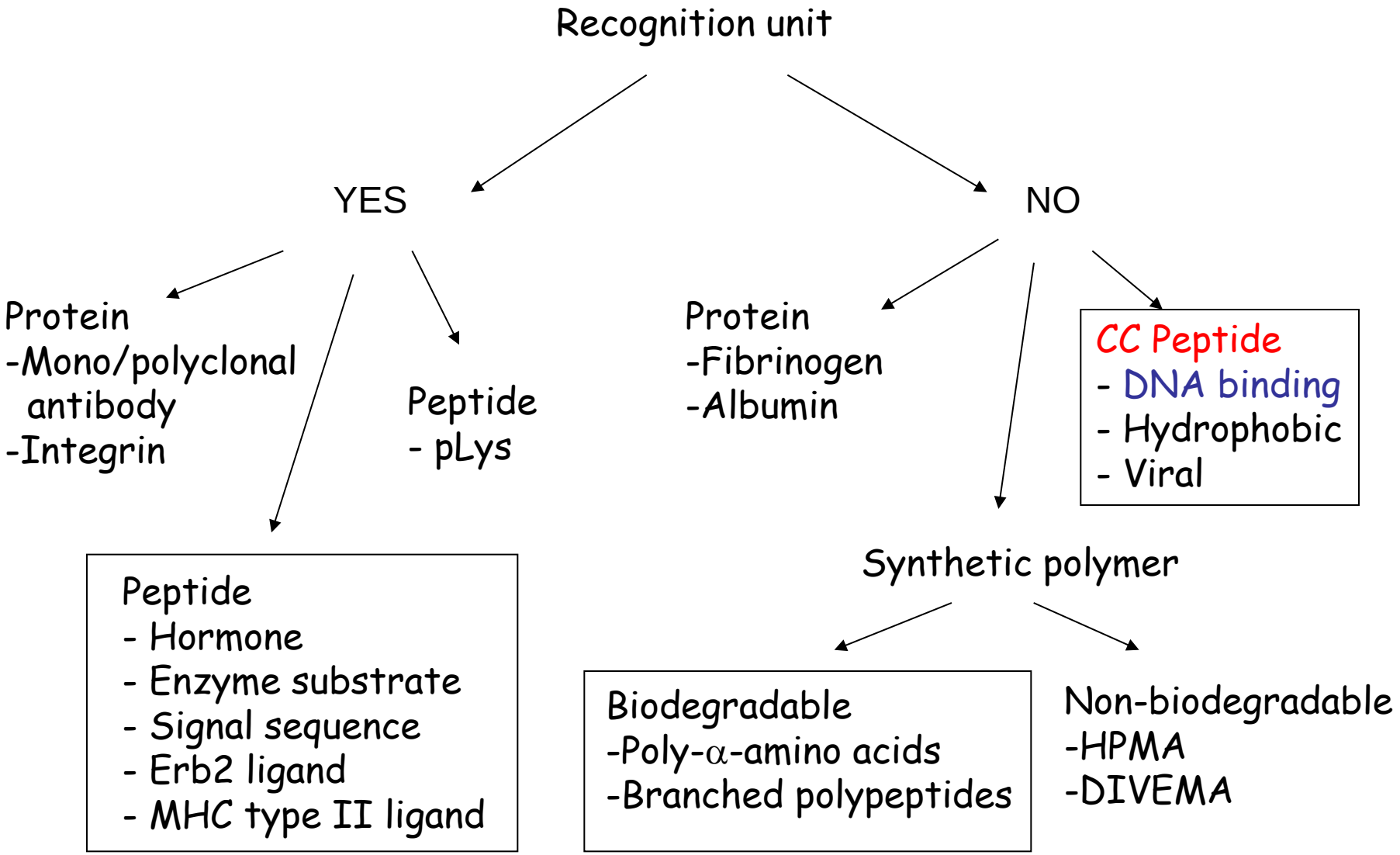
2. The antitumour effect of daunomycin-polypeptide conjugate depends on the nature of the polypeptide (cAD-EAK vs. cAD-SAK).

3. Daunomycin-peptide conjugate is effective against sensitive and MDR resistant L1210/HL60 tumour cells.

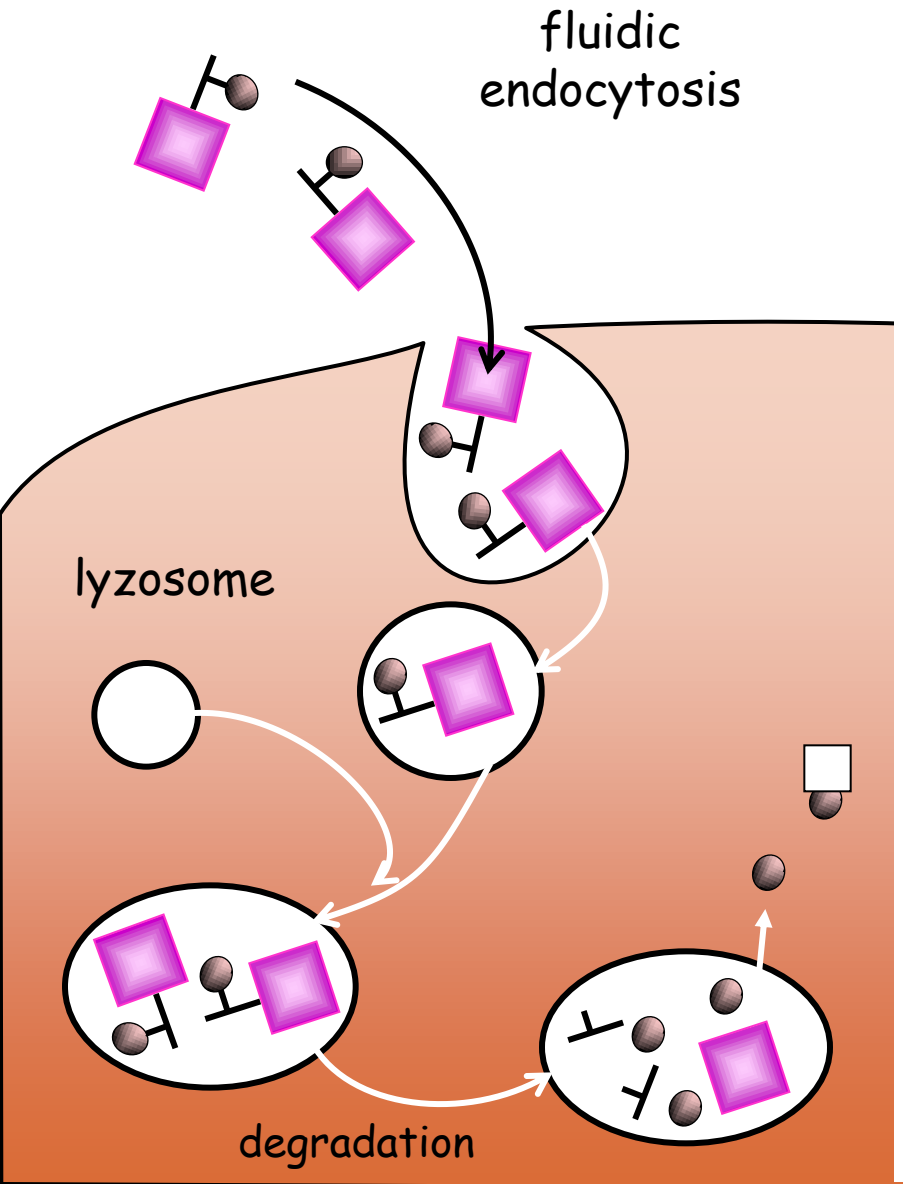
4. Daunomycin-peptide conjugate is taken up by active transport (endocytosis) both in sensitive and resistant HL60 tumour cells.

5. Daunomycin-peptide conjugate is not a ligand of MDR/MRP proteins.

Peptide/protein based drug targeting/delivery

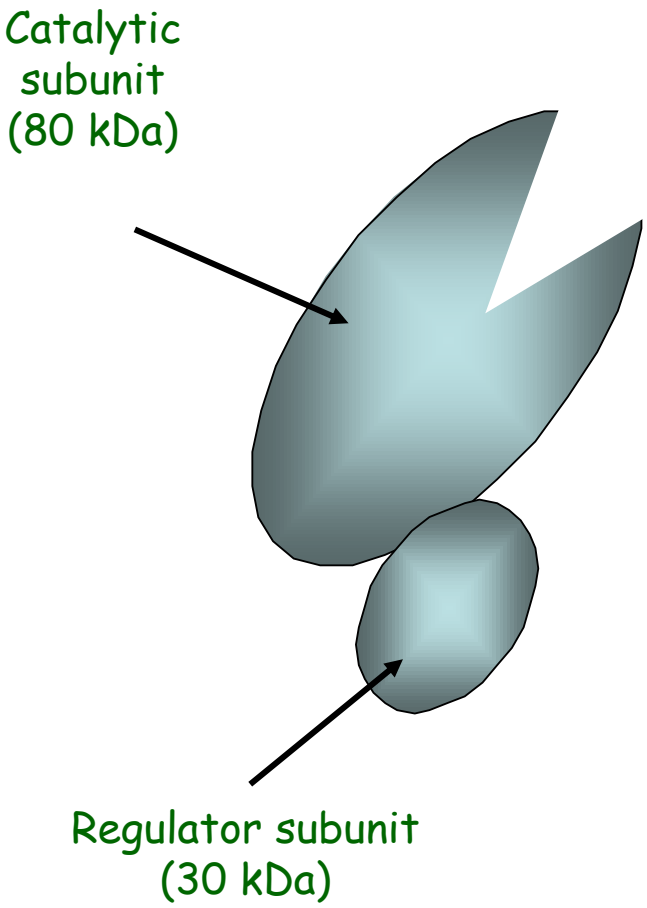


Uptake and liberation of bioactive entities



penetratin – enzyme activator

Calpains



Intracellular enzymes

Superfamily of
Ca^{2+} dependent cysteine proteases

Ca^{2+} signal induced cleavage of specific proteins involved in signaling cascades

In mammals m-calpain and μ-calpain are constitutively expressed in all tissues

Calpains can be activated by different pathways

Large number of substrates

Calpastatin (complexed with calpain)

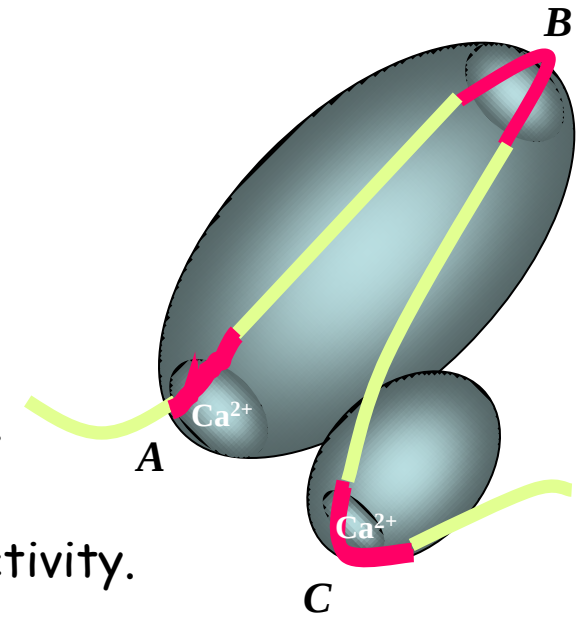
Endogenous specific inhibitor of calpains.

Protein with 110 kD.

Three highly conserved regions: A, B and C.

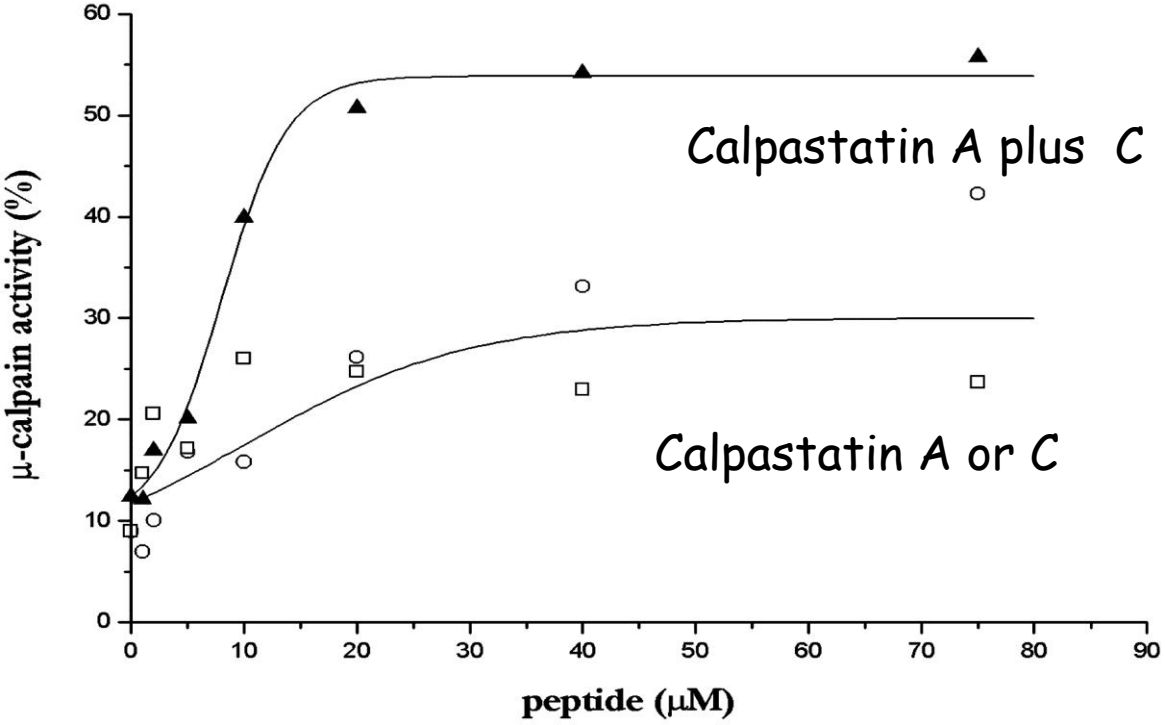
Region B is responsible for the inhibitory activity.

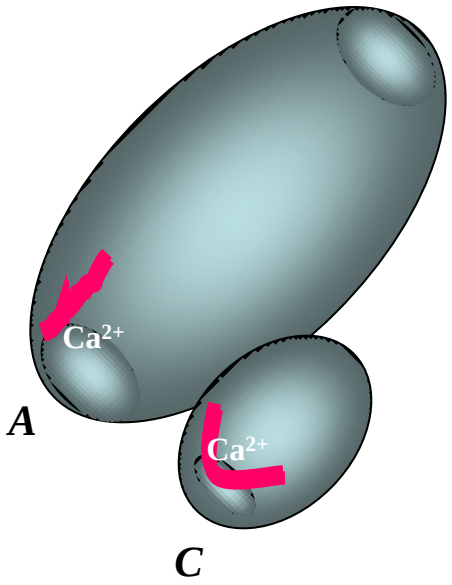
Regions A and C (Ca^{2+}-binding domains) activate calpain *in vitro*.



Tompa, P. et al., *J. Biol. Chem.*, 2002, 277, 9022-9026.

Activation of calpain *in vitro* by calpastatin fragments

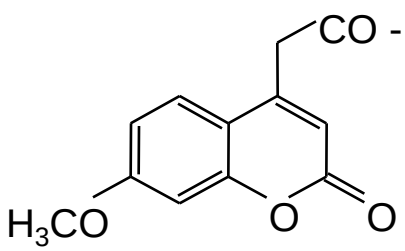




Tompa, P., Hudecz, F. et al., *J. Biol. Chem.* 2002, 277 9022-9030.

Intracellular activation of calpain *in vitro* by calpastatin fragments in COS7 cells

- Peptides do not penetrate cells;
- Conjugation with penetratin;
- Linkage between: amide, thioether or disulphide;
- Labeling with fluorophores



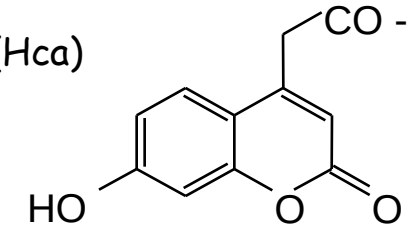
4-(7-hydroxycoumaryl)-acetic acid (Hca)

λ_{ex}= 360 nm, λ_{em} = 480 nm

RQIKIWFQQNRRMKWKKC-NH_2

S - S

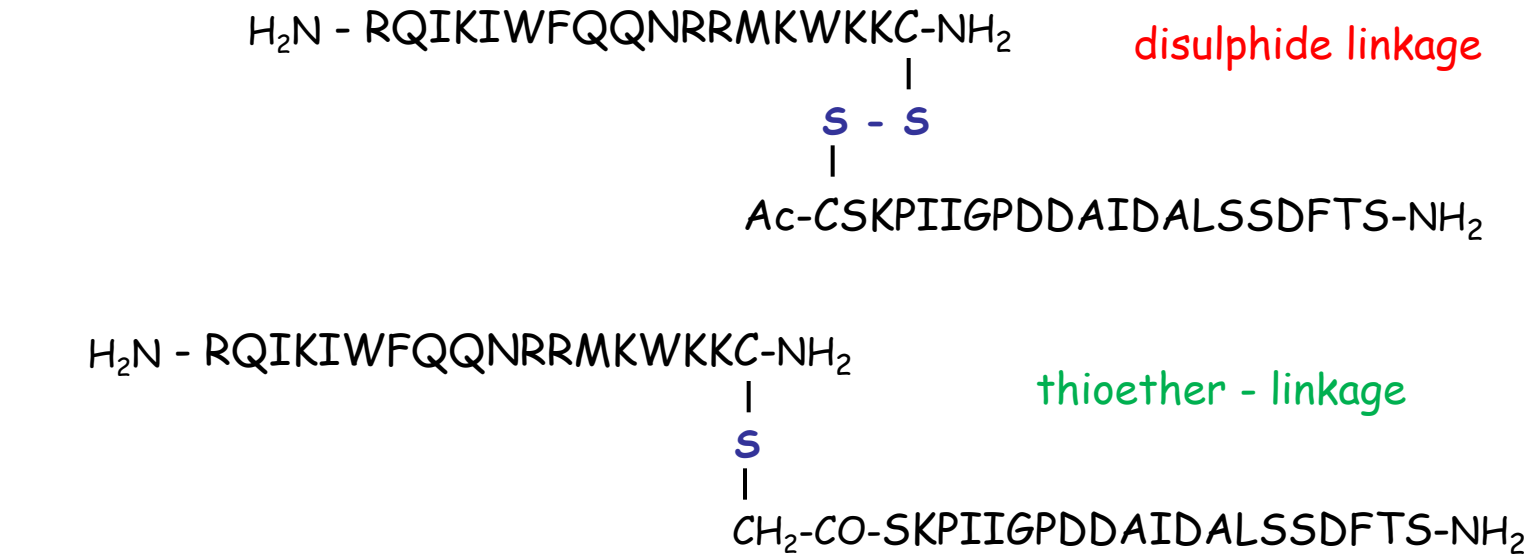
Ac-CSKPIIGPDDAIDALSSDFTS-NH_2



4-(7-methoxycoumaryl)-acetic acid (Mca)

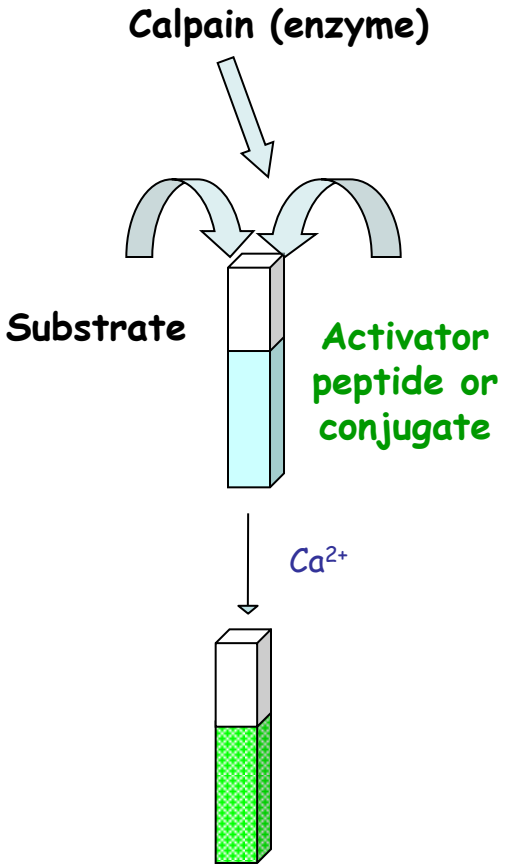
λ_{ex}= 320 nm, λ_{em} = 400 nm

Penetratin – calpastatin C peptide conjugates

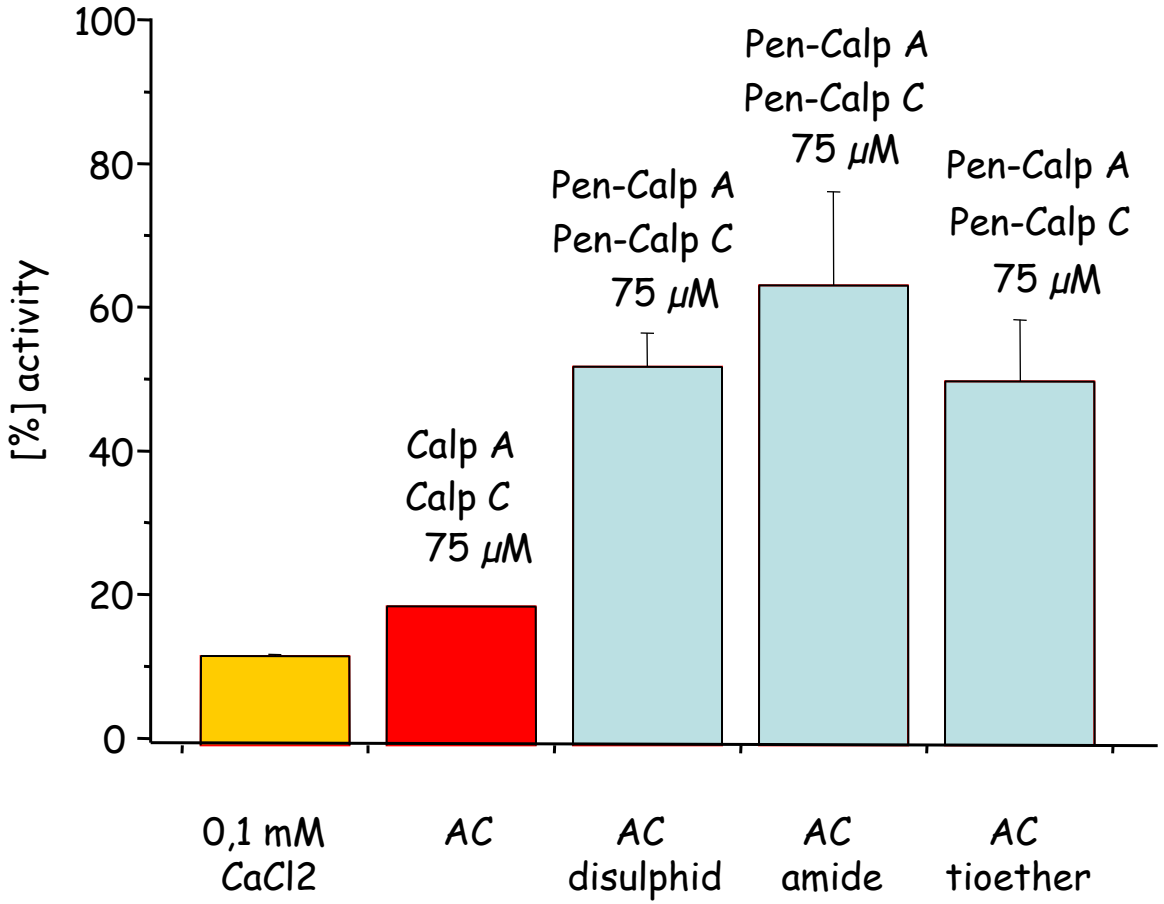


Bánóczi, Z. et al. Bioconjugate Chemistry 18: 130-137 (2007)

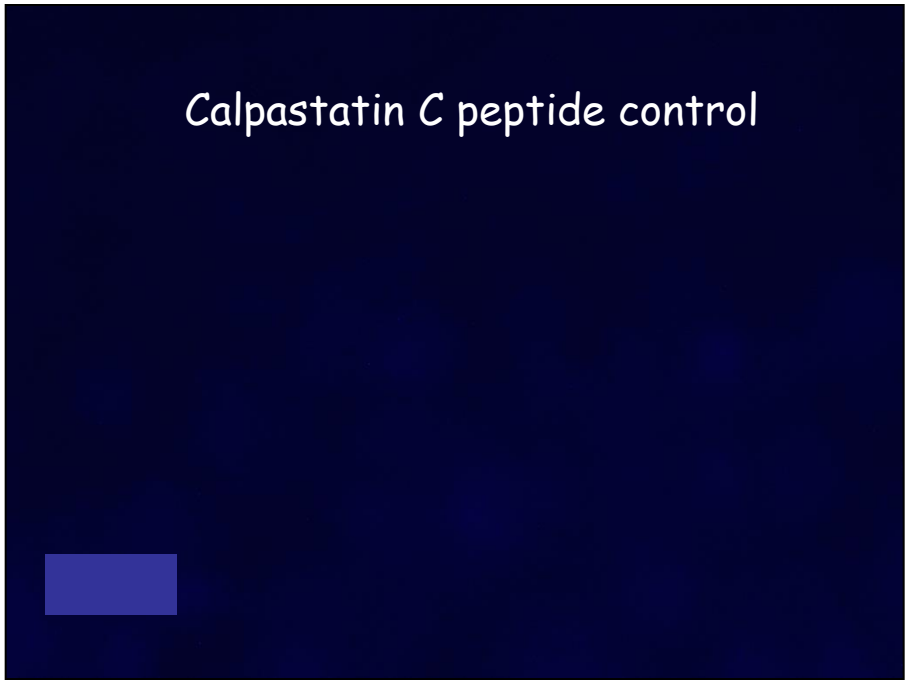
Activation of calpastatin conjugates on isolated enzyme

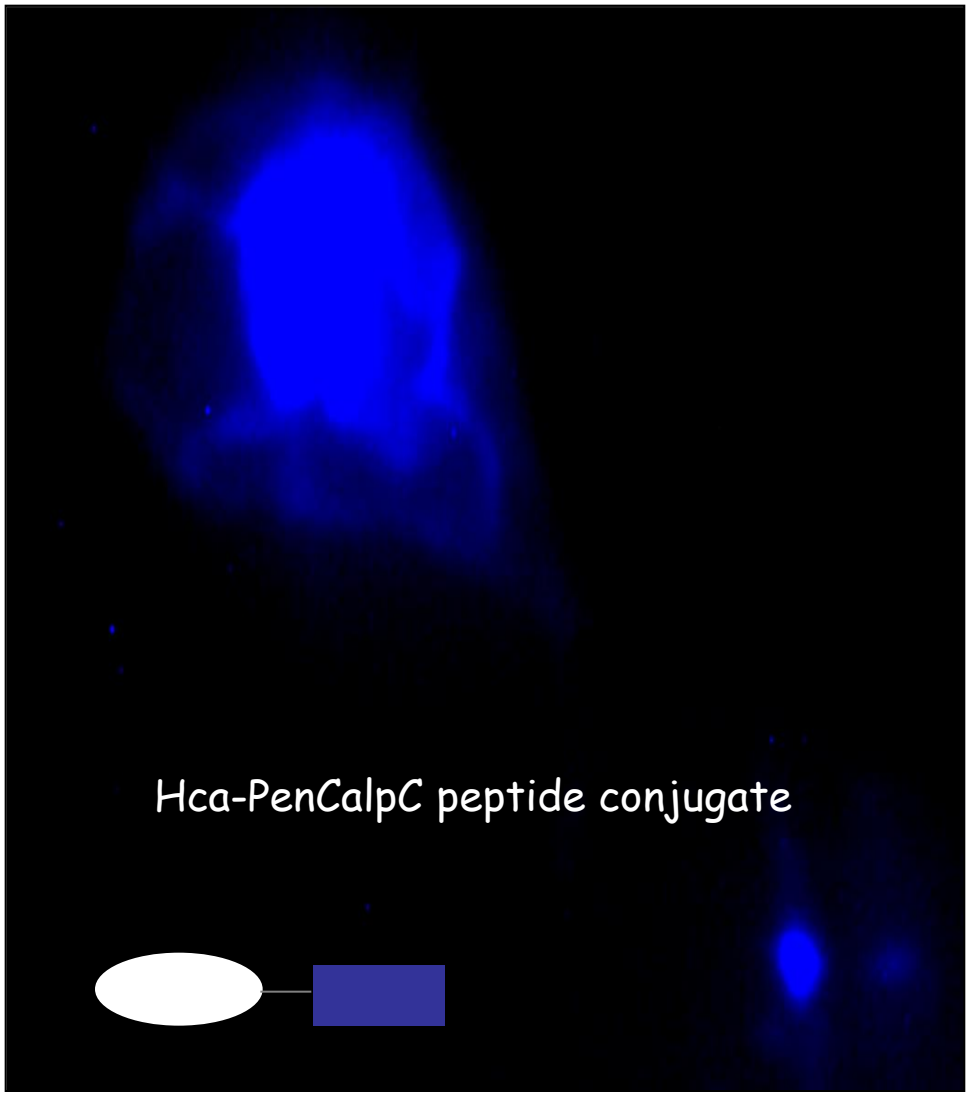


$\lambda_{ex.}$ = 380 nm, $\lambda_{em.}$ = 460 nm



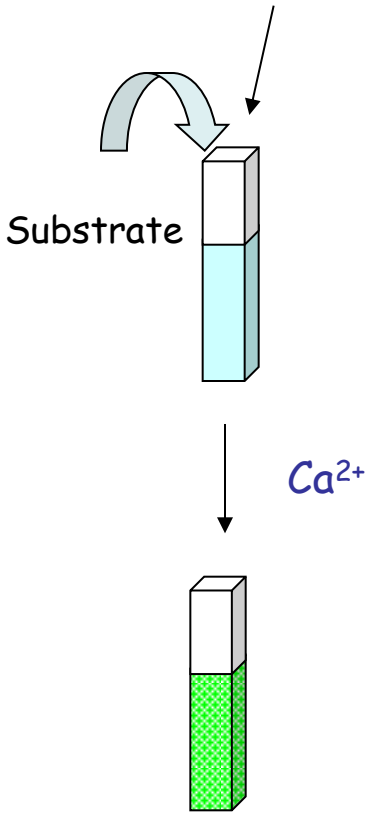
Uptake of Hca-PenCalp C conjugate by COS-7 cells



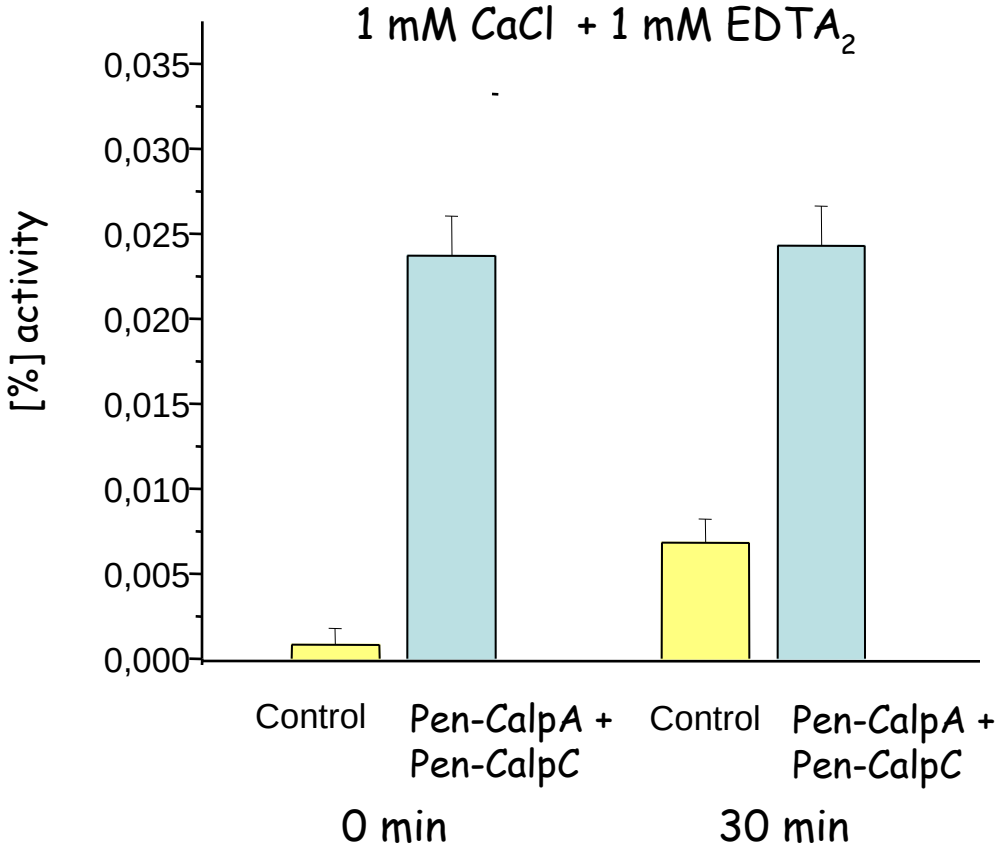


Activation of calpain *in vitro* by calpastatin conjugates in COS7 cell lysate

Cell lysate treated by conjugates



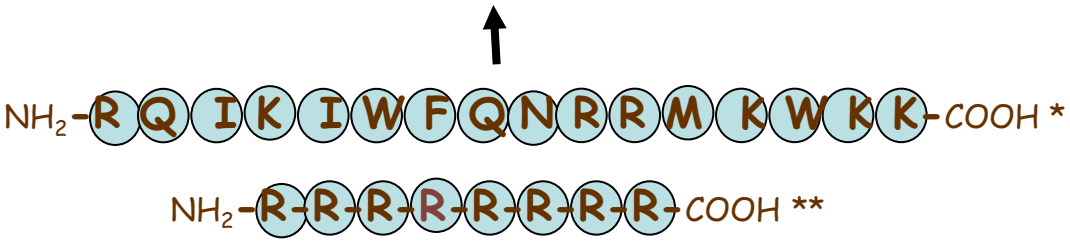
λ_g = 380 nm, λ_e = 460 nm



Calpain related – conjugates: promising tools for the analysis of calpain function

SUBSTRATE PEPTIDE

TPLKSPPPSPR
TAMRA-TPLKSPPPSPRK(cf)
DABCYL-TPLKSPPPSPRE(EDANS)



ACTIVATOR PEPTIDE(S)

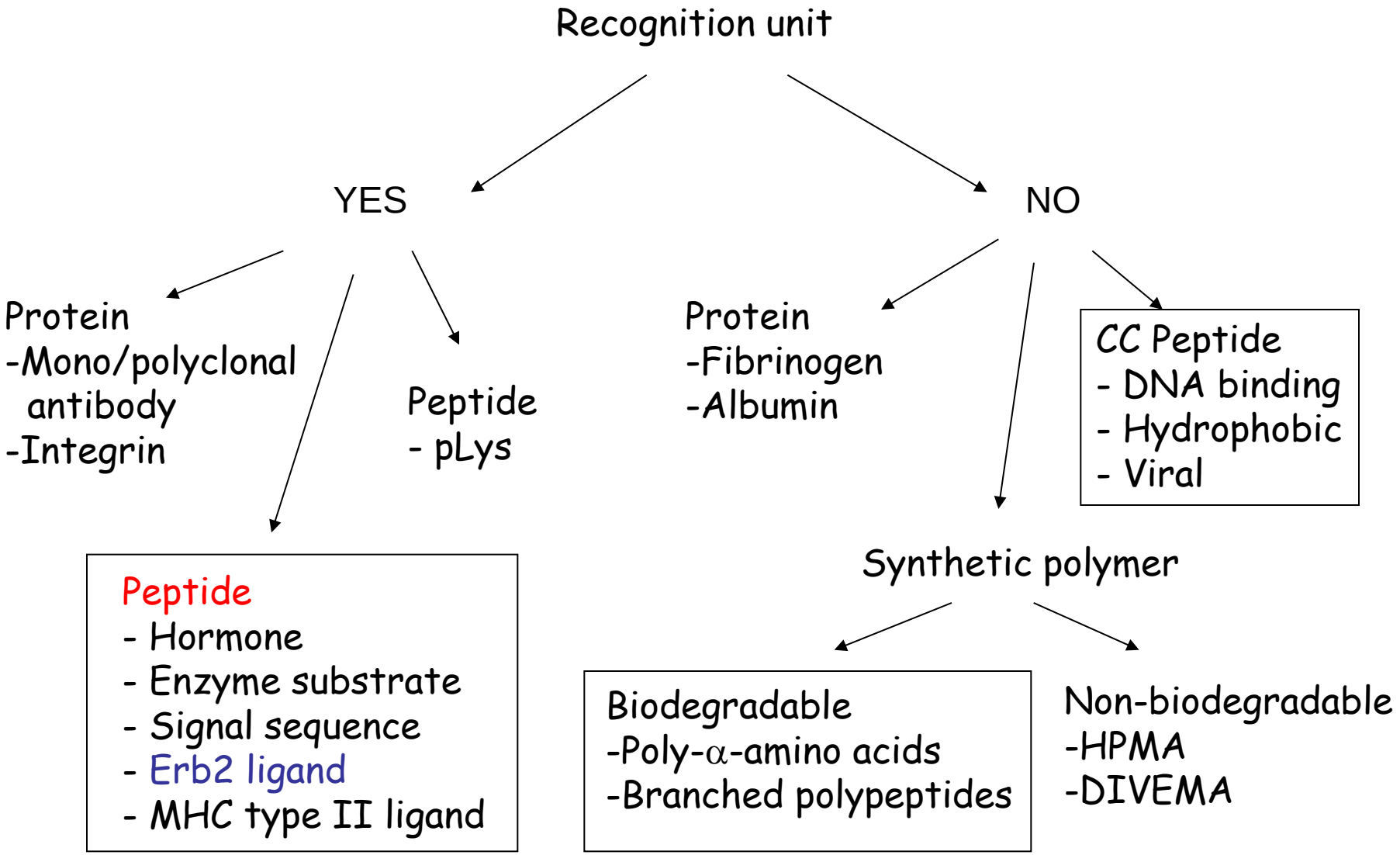
SKPIIGPDDAIDALSSDFTS-NH_2
SGKSGMDAALDDLIDTLGG-NH_2

INHIBITOR PEPTIDE(S)

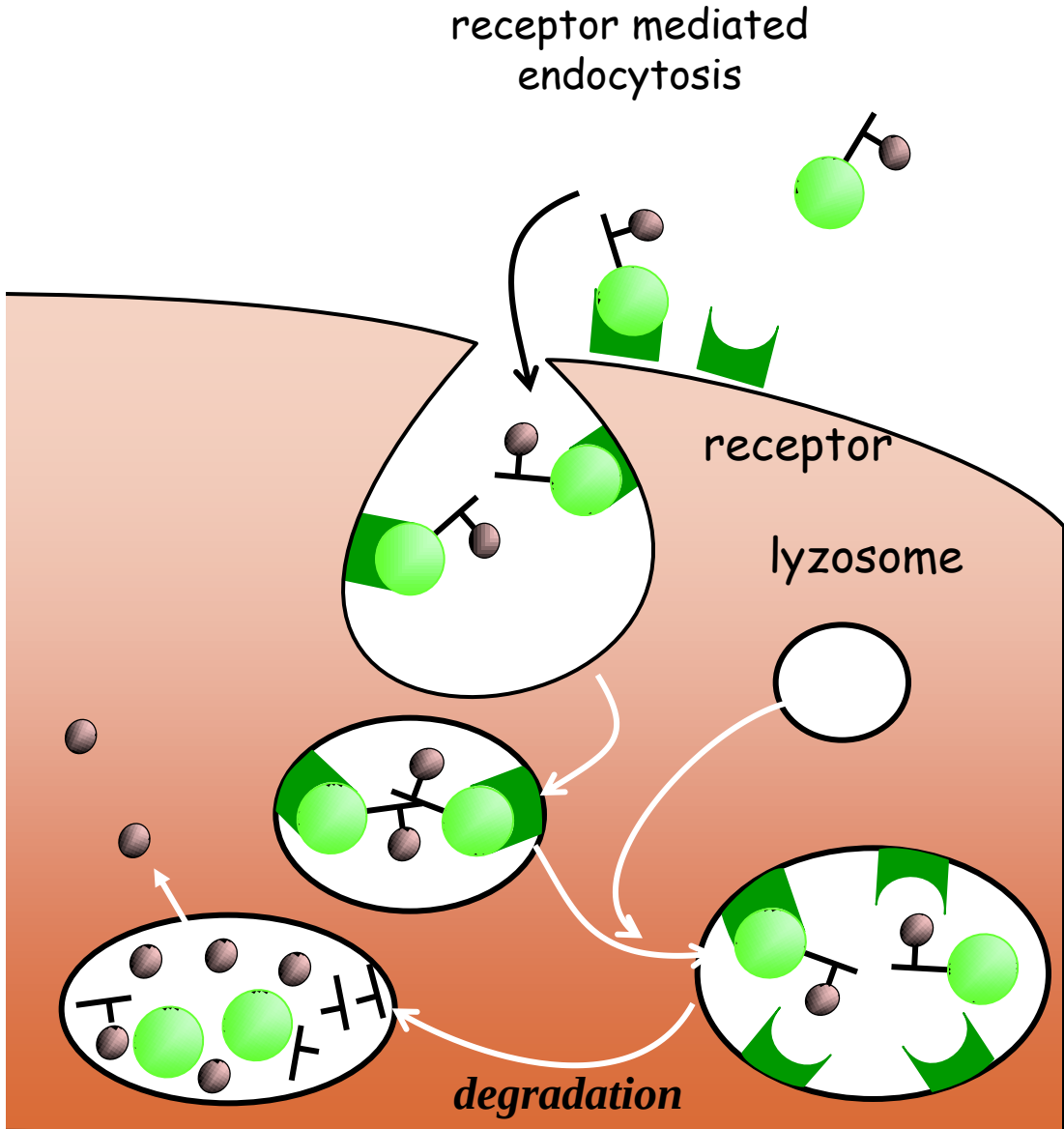
Ac-TPLaGlySPPPS
Ac-TXLaGlySPPPS
X = S or W

Bánóczi, Z. et al. Bioconjugate Chemistry 18: 130-137 (2007)
Bánóczi, Z. et al. Bioconjugate Chemistry 19: 1378-1381 (2008)

Peptide/protein based drug targeting/delivery

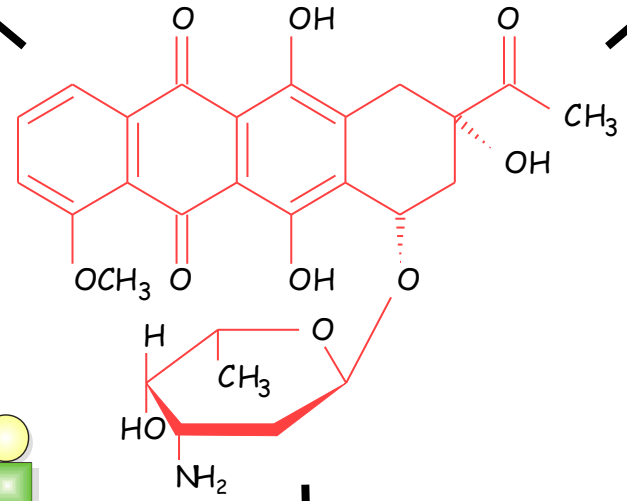


Uptake and liberation of bioactive entities

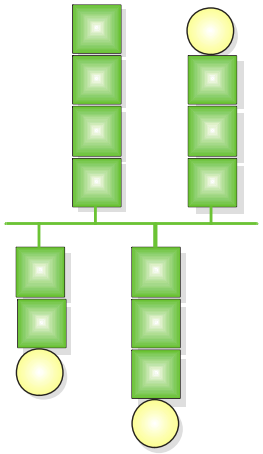


Daunomycin conjugates with oligo- or polypeptide

Orbán E. et al.:
Bioconjugate Chem.
22.489 (2011)

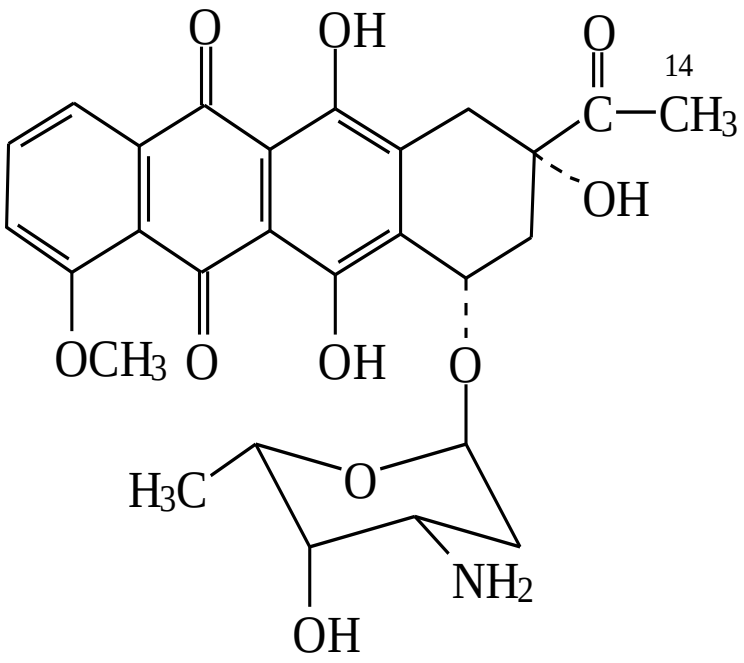


Sztaricskai F. et al.: *J Antibiotics (Tokyo),* **58**: 704 (2005)
Bánóczi Z. et al. *Archivoc* **140**, (2008)
Miklán Zs. et al. *Biopolymers* **92**: 489 (2009)

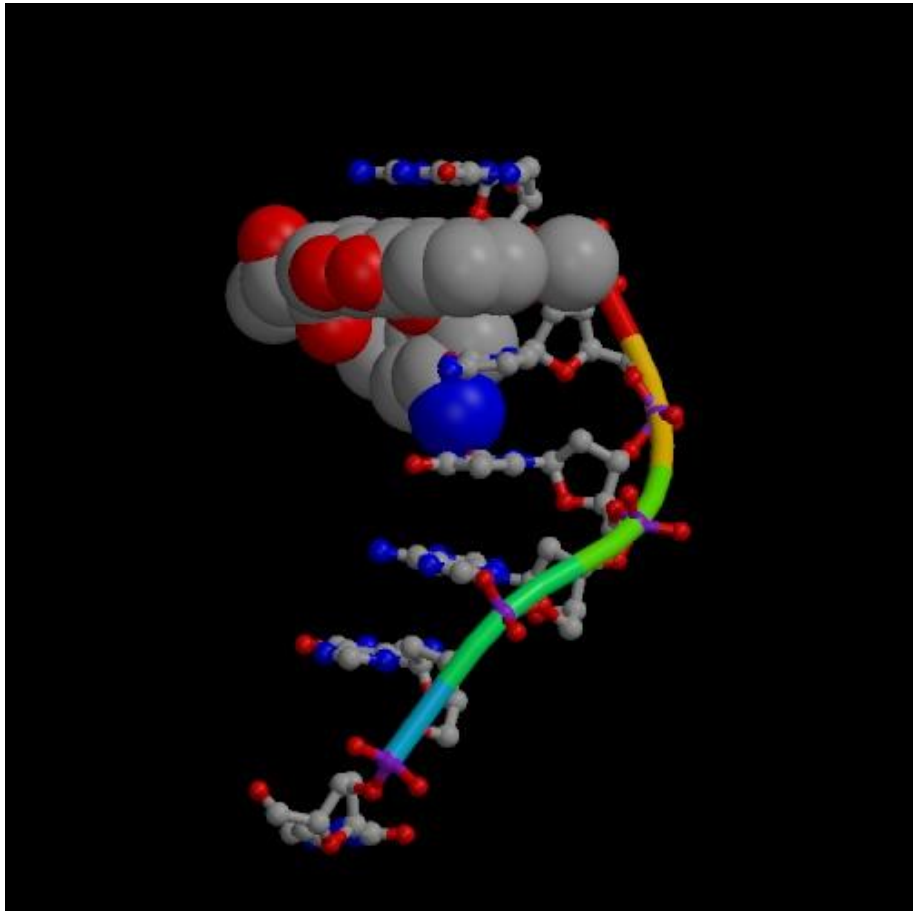


Hudecz F. et al.*Bioconjugate Chem.* **3:** 49 (1992)
Gaál D., Hudecz F. *Eur.J.Cancer.* **34:** 155 (1998)
Szabó R. et al. *Bioconjugate Chem.* **19**: 1078 (2008)
Reményi, J. et al. *Biochim. Biophys. Acta* **1798**: 2209 (2010)

Daunosamine directed intercalation into minor groove

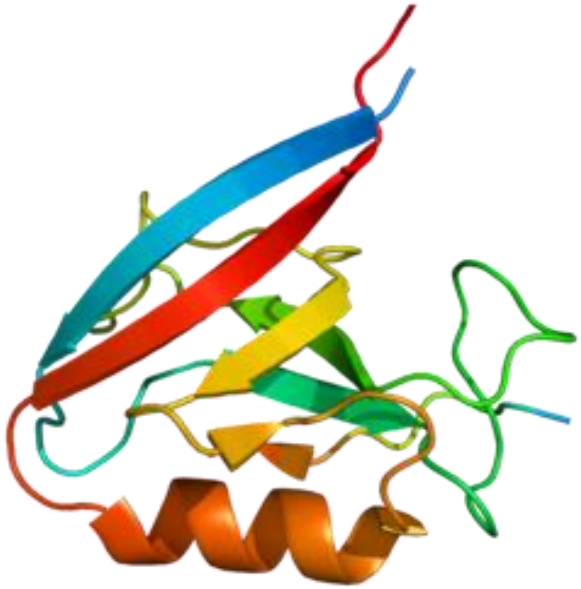


[Frederick, 1990]



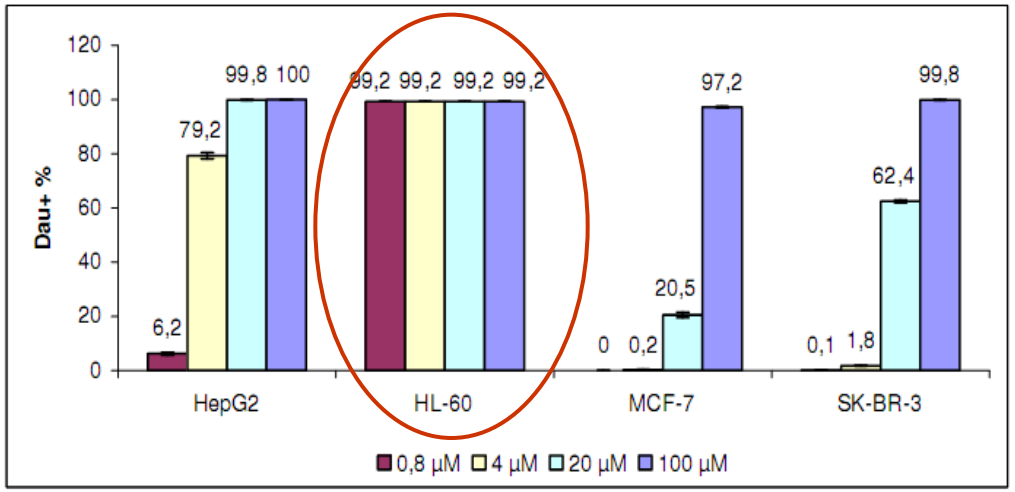
In vitro cytotoxicity and uptake of Dau=Aoa-LTVSPWY-amide conjugate

- ErbB2: overexpressed by certain cell lines (e.g. SK-BR-3)
- ErbB2: ligand: binding and internalization (e.g. breast cell lines)



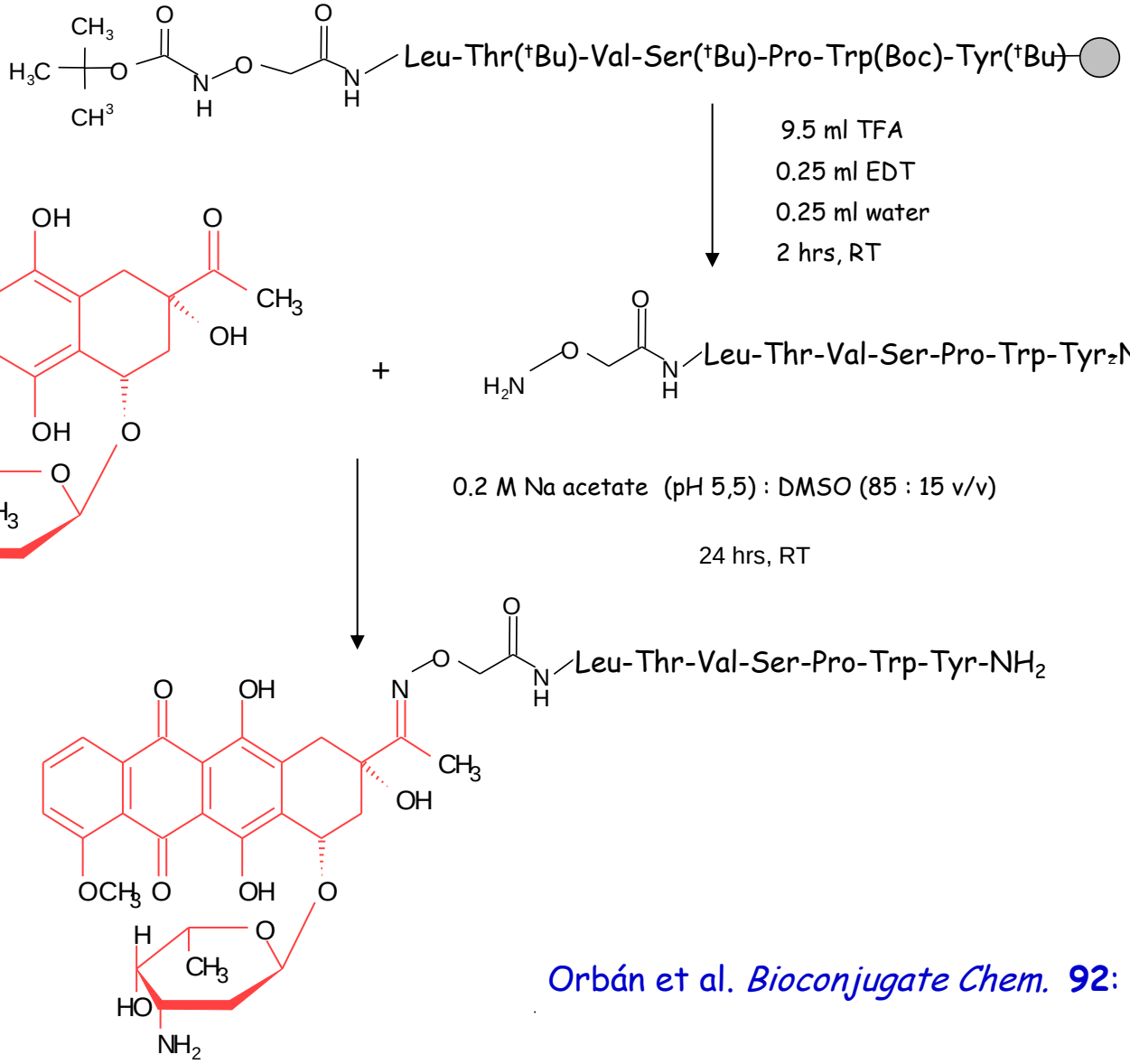
http://www.genenames.org

In vitro cytotoxicity		
Cell line	IC_{50} ± s.d. (μM)	
	Conjugate	Dau
HepG2	3.07 ± 0,02	0.66 ± 0.21
HL-60	0.53 ± 0.12	0.05 ± 0,03
MCF-7	7.42 ± 0.5	0.18 ± 0.09
SK-BR-3	37.9 ± 2.64	3.64 ± 0.52



Cellular uptake c = 0.8 – 100 μM, 90 min

Synthesis of Dau-heptapeptide conjugate with oxime bond



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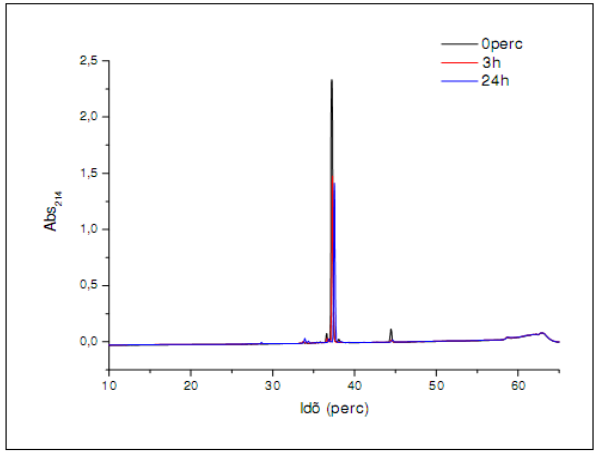
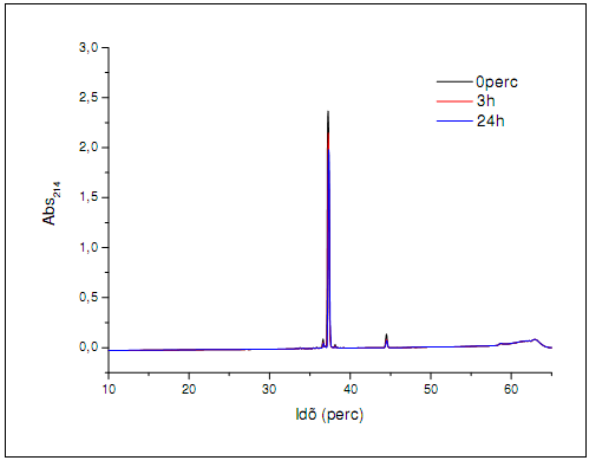
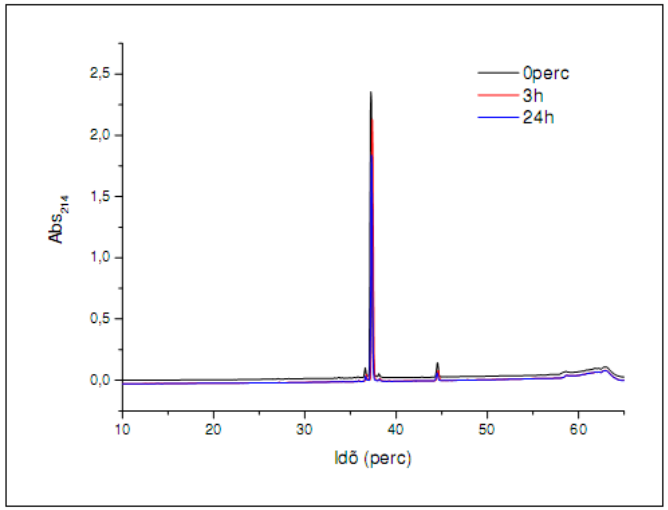
Stability of of Dau=Aoa-LTVSPWY-amide conjugate with oxime bond



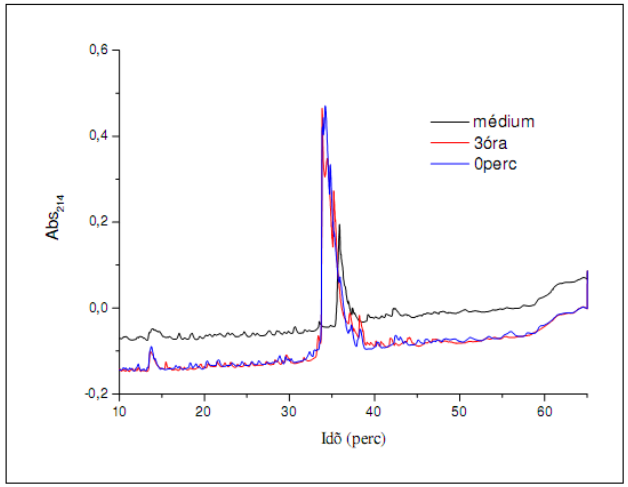
Analytical RP-HPLC chromatogram
in 0.1 M Na citrate buffer, pH 2.5



Analytical RP-HPLC chromatogram
in 0.1 M Na citrate buffer, pH 5.0



Analytical RP-HPLC chromatogram
in 0.1 M Na citrate buffer, pH 7.0



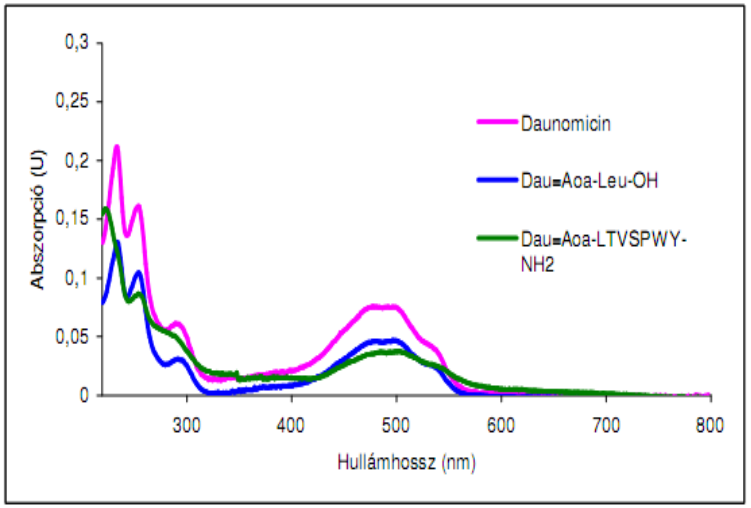
Analytical RP-HPLC chromatogram
after incubation in RPMI-1640 medium with 10% sera

Characteristics of Dau=Aoa-LTVSPWY-amide conjugate with oxime bond

Compound	MS[a] [M]		R_t[b] (min)
	Calc.	Measd.	
Dau=Aoc-LTVSPWY-NH_2	1224,3	1224,3	27,0
Dau	527,5	n.a.	34,9

[a] SELDI-MS
[b] HPLC, Column: Supelcoil LC-18-DB (C18, 120 A, 5 μm, 4,6 x 250 mm), gradient elution: 0-5 min 5% eluent B, 5-50 min 90% eluent B, where eluent A: 0.1 % TFA in water, eluent B: 0.1% TFA in AcN-water (80-20 v/v %)

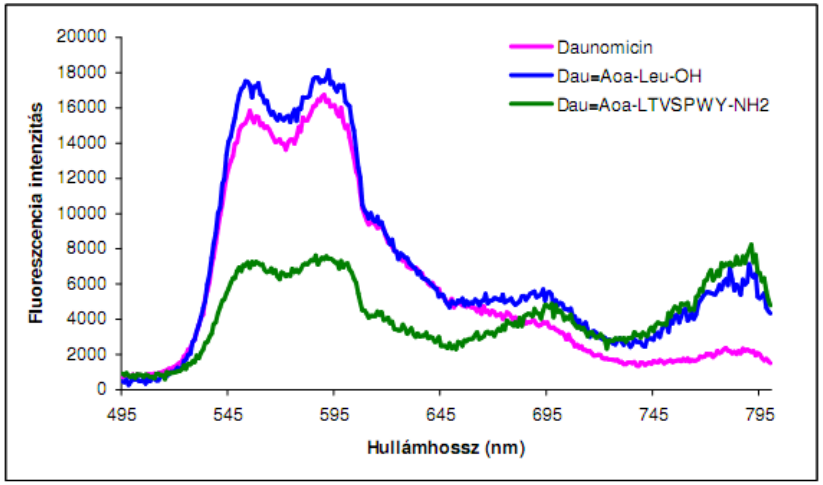
Absorbtion spectra



in 0.1 M Tris buffer, pH 7.4
c = 1,8 x 10^{-5} M (Dau)

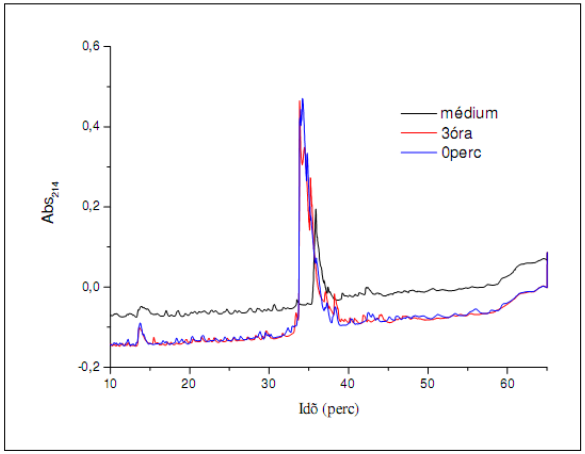
Emission spectra

λ_{ex} = 473 nm



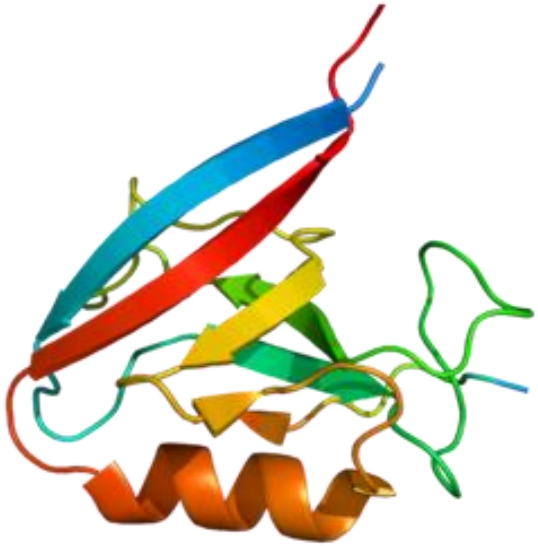
in 0.1 M Tris buffer, pH 7.4
c = 1,8 x 10^{-5} M (Dau)

In vitro cytotoxicity and uptake of Dau=Aoa-LTVSPWY-amide conjugate

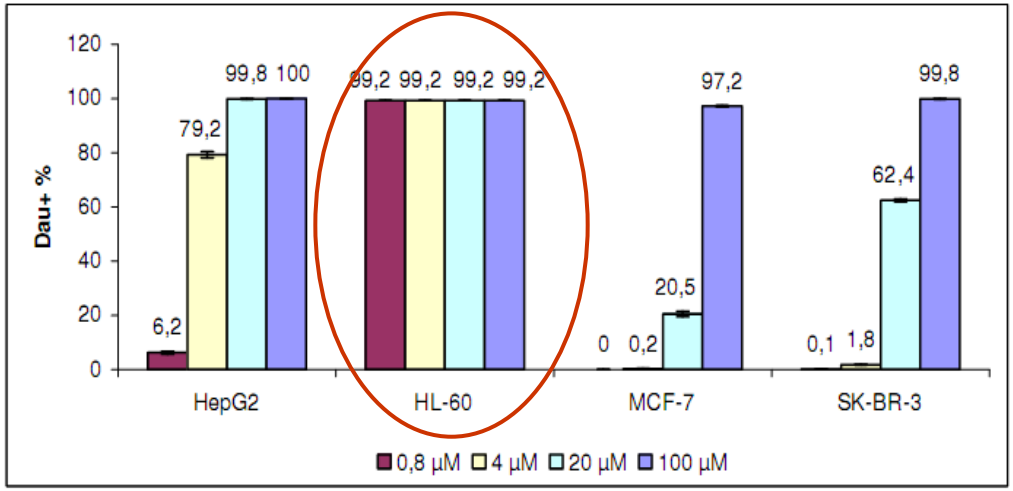


Stability in RPMI-1640 medium with 10% sera

In vitro cytotoxicity		
Cell line	IC_{50} ± s.d. (μM)	
	Conjugate	Dau
HepG2	3.07 ± 0,02	0.66 ± 0.21
HL-60	0.53 ± 0.12	0.05 ± 0,03
MCF-7	7.42 ± 0.5	0.18 ± 0.09
SK-BR-3	37.9 ± 2.64	3.64 ± 0.52

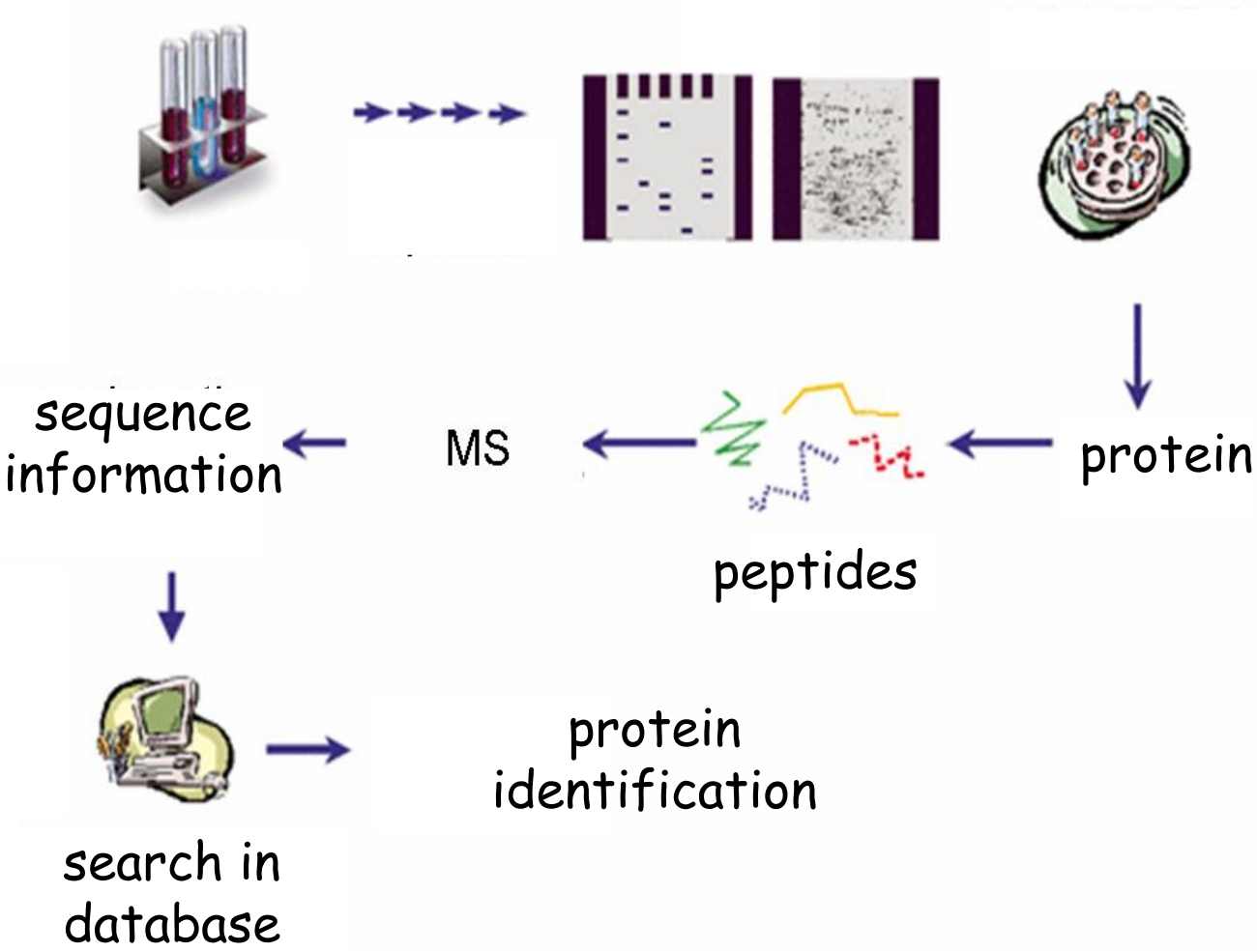


http://www.genenames.org

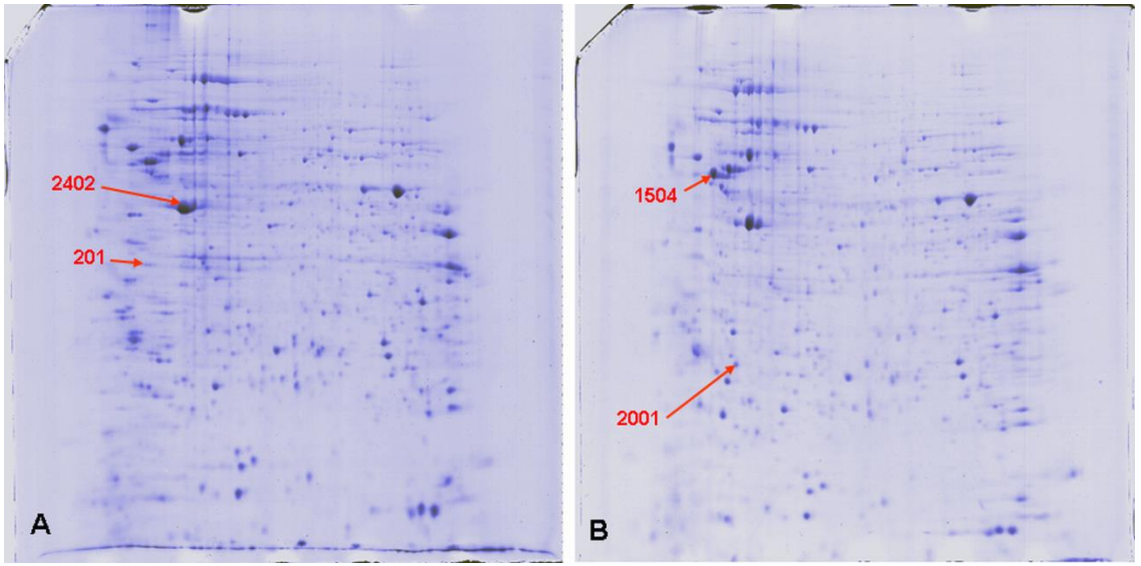


Cellular uptake c = 0.8 – 100 μM, 90 min

Analysis of protein expression profile



Protein expression profile of non-treated (A) and Dau-treated HL-60 cells (B).



t = 24 hr

Identified proteins	Average protein expression level in	
	Controll	Dau
Actin, cytoplasmic 1 (Beta-actin)	11178.5	1615.8
Proliferating cell nuclear antigen (PCNA) (Cyclin)	1440.7	171.5
Ran-specific GTPase-activating protein (Ran-binding protein 1)	789.7	1648.5
Tubulin beta chain (Tubulin beta-5 chain)	1337.6	9713.9

Arrows and spot numbers show the significantly different spots on the gel where expression level was higher.

Protein expression profile of non-treated (A) and Dau-treated HL-60 cells (B).

Identified Protein	Spot number	Average level in		Fold-change	Mascot score	M_r(Da)	pI
		Control	Dau				
Proliferating cell nuclear antigen (PCNA) (Cyclin)	201	1440.7	171.5	0.12	2111	28768.78	4.57
Tubulin beta chain (Tubulin beta-5 chain)	1504	1337.6	9713.9	7.26	11510	49670.82	4.78
Ran-specific GTPase-activating protein (Ran-binding protein 1) (RanBP1)	2001	789.7	1648.5	2.09	560	23310.12	5.19
Actin, cytoplasmic 1 (Beta-actin)	2402	11178.5	1615.8	0.14	17877	41736.73	5.29

Spot number: for the identification on the gel.

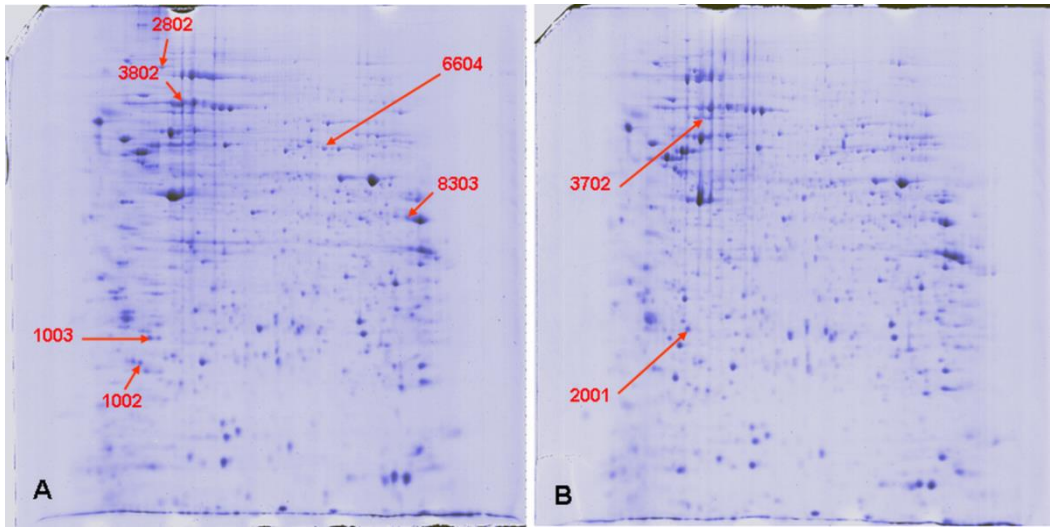
Proteins with different expression level were identified after tryptic in-gel digestion using OrbiTrap nano-LC-MS/MS mass spectrometry and MASCOT database. **Average levels of the protein**: calculated by PDQuest 8.0 software.

Fold change: the ratio of the average protein expression level in the conjugate and Dau-treated samples.

M_r : the theoretical molecular weight

pI : the theoretical isoelectric point of the identified protein.

Protein expression profile of non-treated (A) and Dau=Aoa-LTVSPWY-NH_2 conjugate-treated HL-60 cells (B)



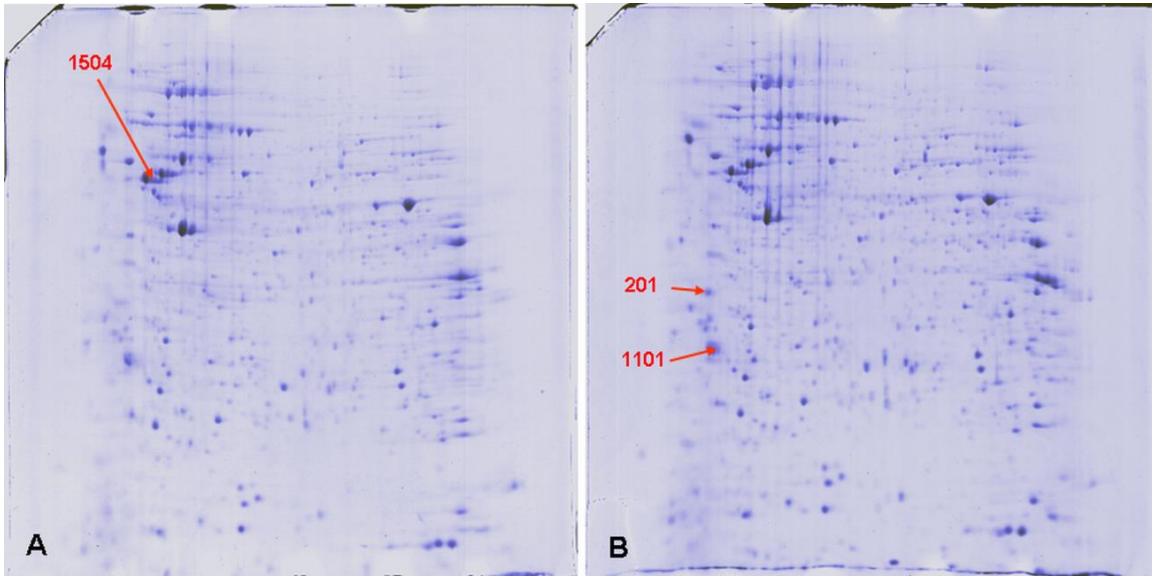
t = 24 hr

Identified proteins	Average protein expression level in	
	Control	Dau=Aoa-LTVSPWY-NH_2
D-3-phosphoglycerate dehydrogenase (EC 1.1.1.95) (3-PGDH)	1067.9	526.0
Fructose-bisphosphate aldolase A (EC 4.1.2.13) (Muscle-type aldolase)	2105.1	999.3
Heat shock cognate 71 kDa protein (Heat shock 70 kDa protein 8)	6507.8	5.6
Plastin-2 (L-plastin) (Lymphocyte cytosolic protein 1) (LCP-1)	482.3	1065.6
Ran-specific GTPase-activating protein (Ran-binding protein 1)	789.7	1805.0
Rho GDP-dissociation inhibitor 2 (Rho GDI 2) (Rho-GDI beta) (Ly-GDI)	2253.9	5.6
Transitional endoplasmic reticulum ATPase (Valosin-containing protein) (VCP)	994.4	271.7
Translationally-controlled tumor protein (TCTP) (p23) (Histamine-releasing factor)	1406.2	5.6

Protein expression profile of non-treated (A) and Dau=Aoa-LTVSPWY-NH_2 conjugate-treated HL-60 cells (B)

Identified Protein	Spot number	Average level in		Fold-change	Mascot score	M_r(Da)	pI
		Control	Dau=Aoa-LTVSPWY-NH_2				
Translationally-controlled tumor protein (TCTP) (p23) (Histamine-releasing factor) (HRF)	1002	1406.2	5.6	0.004	3665	19595.34	4.84
Rho GDP-dissociation inhibitor 2 (Rho GDI 2) (Rho-GDI beta) (Ly-GDI)	1003	2253.9	5.6	0.002	3288	22988.01	5.10
Ran-specific GTPase-activating protein (Ran-binding protein 1) (RanBP1)	2001	789.7	1805.0	2.29	560	23310.12	5.19
Transitional endoplasmic reticulum ATPase (TER ATPase) (15S Mg(2+)-ATPase p97 subunit) (Valosin-containing protein) (VCP)]	2802	994.4	271.7	0.27	2657	89321.80	5.14
Plastin-2 (L-plastin) (Lymphocyte cytosolic protein 1) (LCP-1) (LC64P)	3702	482.3	1065.6	2.21	4924	70288.39	5.29
Heat shock cognate 71 kDa protein (Heat shock 70 kDa protein 8)	3802	6507.8	5.6	0.001	10972	70898.09	5.37
D-3-phosphoglycerate dehydrogenase (EC 1.1.1.95) (3-PGDH)	6604	1067.9	526.0	0.49	4131	56650.5	6.29
Fructose-bisphosphate aldolase A (EC 4.1.2.13) (Muscle-type aldolase) (Lung cancer antigen NY-LU-1]	8303	2105.1	999.3	0.47	1143	39420.02	8.30

Protein expression profile of Dau-treated (A) and Dau=Aoa-LTVSPWY-NH_2 conjugate-treated HL-60 cells (B)



t = 24 hr

Identified proteins	Average protein expression level in	
Proliferating cell nuclear antigen (PCNA) (Cyclin	171.5	2165.8
14-3-3 protein gamma (Protein kinase C inhibitor protein 1) (KCIP-1)	157.7	1814.0
Tubulin beta chain (Tubulin beta-5 chain	9713.9	1981.3

A = Dau, c = 0.024 μM

B = Dau=Aoa-LTVSPWY-NH_2 conjugate c = 9 μM

Arrows and spot numbers show the significantly different spots on the gel where expression level was higher.

Protein expression profile of Dau-treated (A) and Dau=Aoa-LTVSPWY-NH_2 conjugate-treated HL-60 cells (B)

		Average level in					
Identified Protein	Spot number	Dau	Dau=Aoa-LTVSPWY-NH_2	Fold-change	Mascot score	Mr(Da)	pI
Proliferating cell nuclear antigen (PCNA) (Cyclin)	201	171.5	2165.8	12.6	2111	28768.78	4.57
14-3-3 protein gamma (Protein kinase C inhibitor protein 1) (KCIP-1)	1101	157.7	1814.0	11.5	3116	28302.59	4.80
Tubulin beta chain (Tubulin beta-5 chain)	1504	9713.9	1981.3	0.2	11510	49670.82	4.78

Spot number: for the identification on the gel.

Proteins with different expression level were identified after tryptic in-gel digestion using OrbiTrap nano-LC-MS/MS mass spectrometry and MASCOT database. **Average levels of the protein**: calculated by PDQuest 8.0 software.

Fold change: the ratio of the average protein expression level in the conjugate and Dau-treated samples.

M_r : the theoretical molecular weight

pI : the theoretical isoelectric point of the identified protein.

Comparison of protein expression profiles of Dau- and Dau-peptide conjugate- and non-treated HL-60 cells: an interpretation

	Average level in				
Identified Protein	Control	Dau	Fold-change	Dau=Aoa-LTVSPWY-NH_2	Fold-change
Proliferating cell nuclear antigen (PCNA) (Cyclin)	1440.7	171.5 ↓	0.12	2165.8 ↑	12.6
Tubulin beta chain (Tubulin beta-5 chain)	1337.6	9713.9 ↑	7.26	1981.3 ↓	0.2
Ran-specific GTPase-activating protein (Ran-binding protein 1)	789.7	1648.5	2.09	No change	
Actin, cytoplasmic 1 (Beta-actin)	11178.5	1615.8	0.14	No change	
14-3-3 protein gamma (Protein kinase C inhibitor protein 1) (KCIP-1)		157.7	No change	1814.0	11.5

1. Cyclin and tubulin beta-5 are involved in both processes.
2. Ran-binding protein 1 and actin are involved in Dau action.
3. 14-3-3 protein gamma is involved in Dau-conjugate action.

Conclusions

1. The expression level of several proteins altered due to the treatment with the free drug (Dau) or its conjugate in comparison with proteins from untreated cells.

2. After treatment with Dau for 24 h, the expression levels of cytoskeletal as well as cell-cycle regulatory proteins (four) have been changed.

3. Three proteins were identified, whose expression was lower (tubulin beta chain) or markedly higher (proliferating cell nuclear antigen and protein kinase C inhibitor protein 1) after administration of HL-60 cells with Dau-peptide conjugate vs free drug. These proteins are cytoskeletal proteins or involved in signalisation or metabolism.

Conclusions

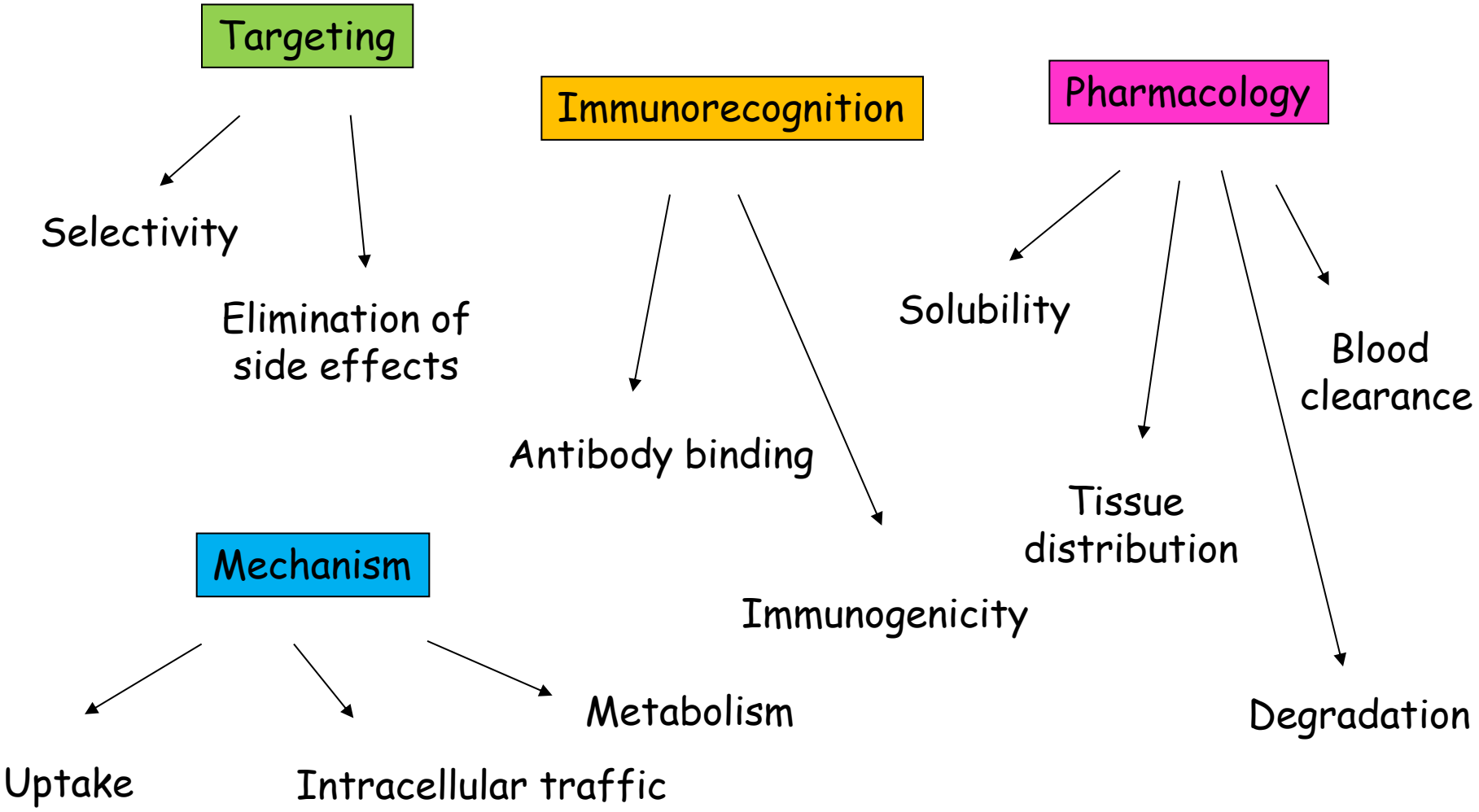
1. Branched polypeptide – methotrexate conjugates could maintain or even enhance *in vitro* and *in vivo* anti Leishmania donovani effect as compared to free drug.

2. Branched polypeptide – daunomycin conjugates could maintain or even enhance in vitro and in vivo anti Leishmania donovani effect as compared to free drug.

3. Penetratin - enzyme activator/inhibitor/substrate conjugate could be utilized for the analysis the function of intracellular enzymes.

4. Erb2 ligand peptide – daunomycin conjugate could be used to identify target proteins and identify novel pathways.

Peptide/protein conjugation based alteration of relevant biological properties



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