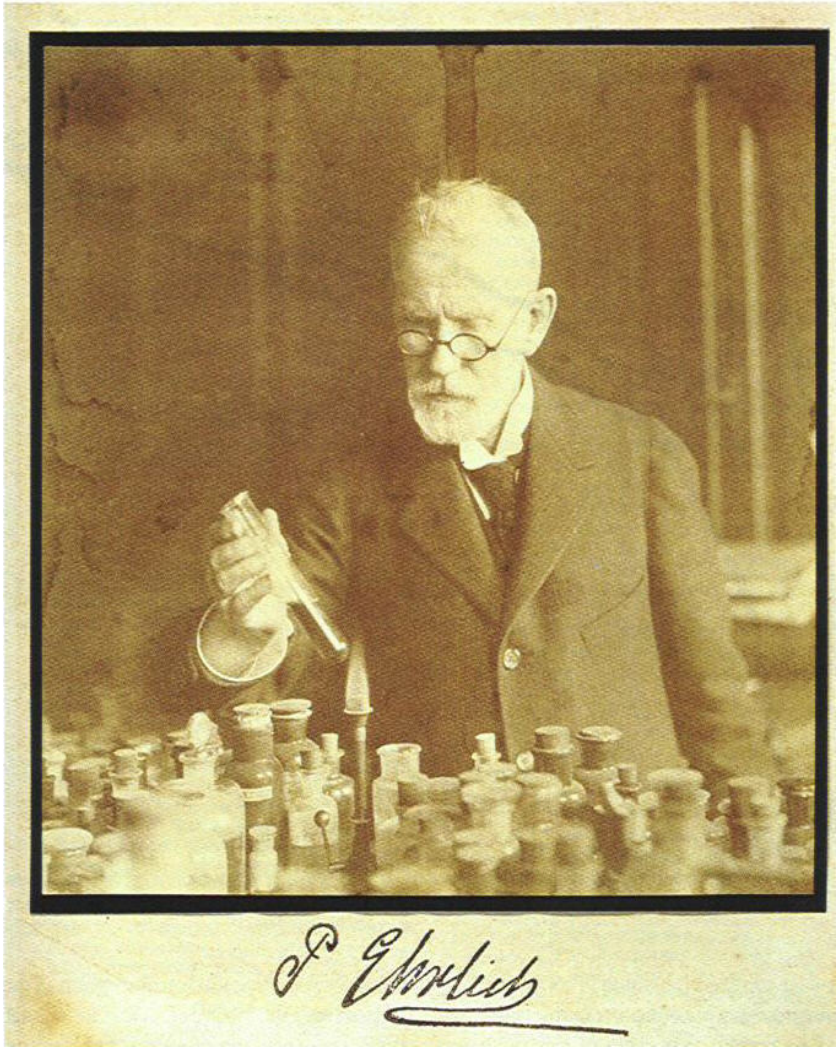


Paul Ehrlich
1854-1915
(Wellcome Library, London)

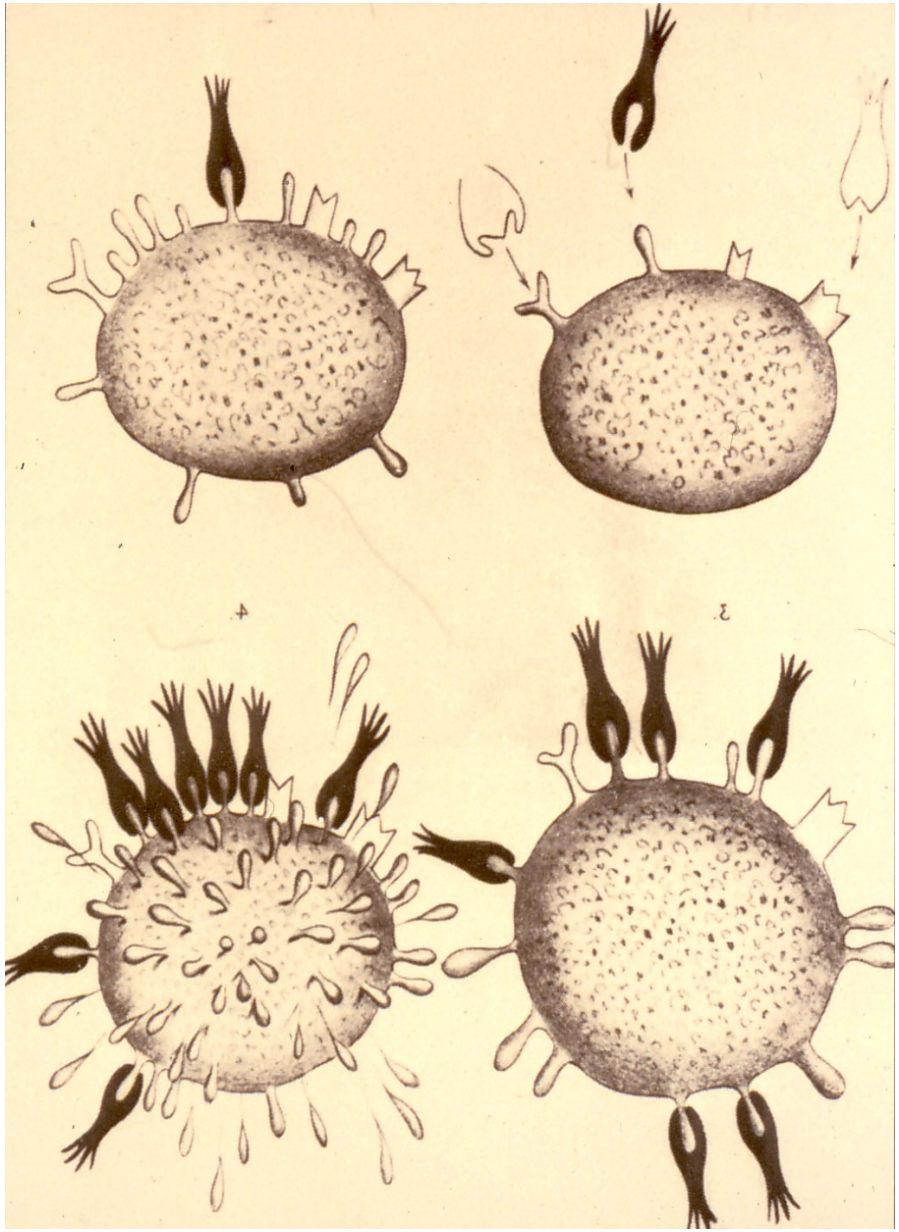
Nobel Lecture
on December 11th, 1908

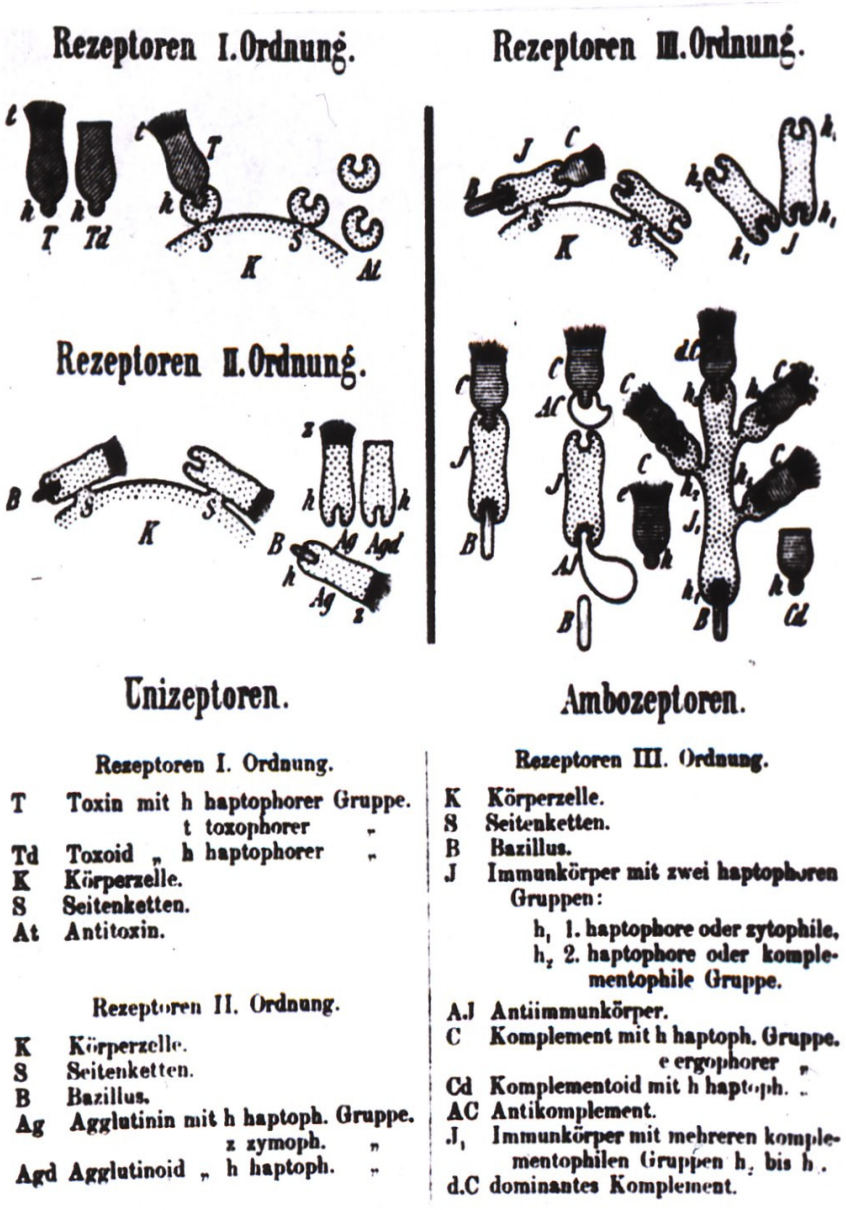
„Chemotherapy: Drugs to cure patients"
and the term „Magic Bullet"

The term "Magic Bullets" is a translation of the German word "Zauberkugeln". It is believed that Ehrlich took that term from the first German opera "Der Freischütz" ***A bűvös vadász*** (Carl Maria von Weber, 1786-1826).

In that opera a young man needed to shoot the target successfully to be able to marry his bride.

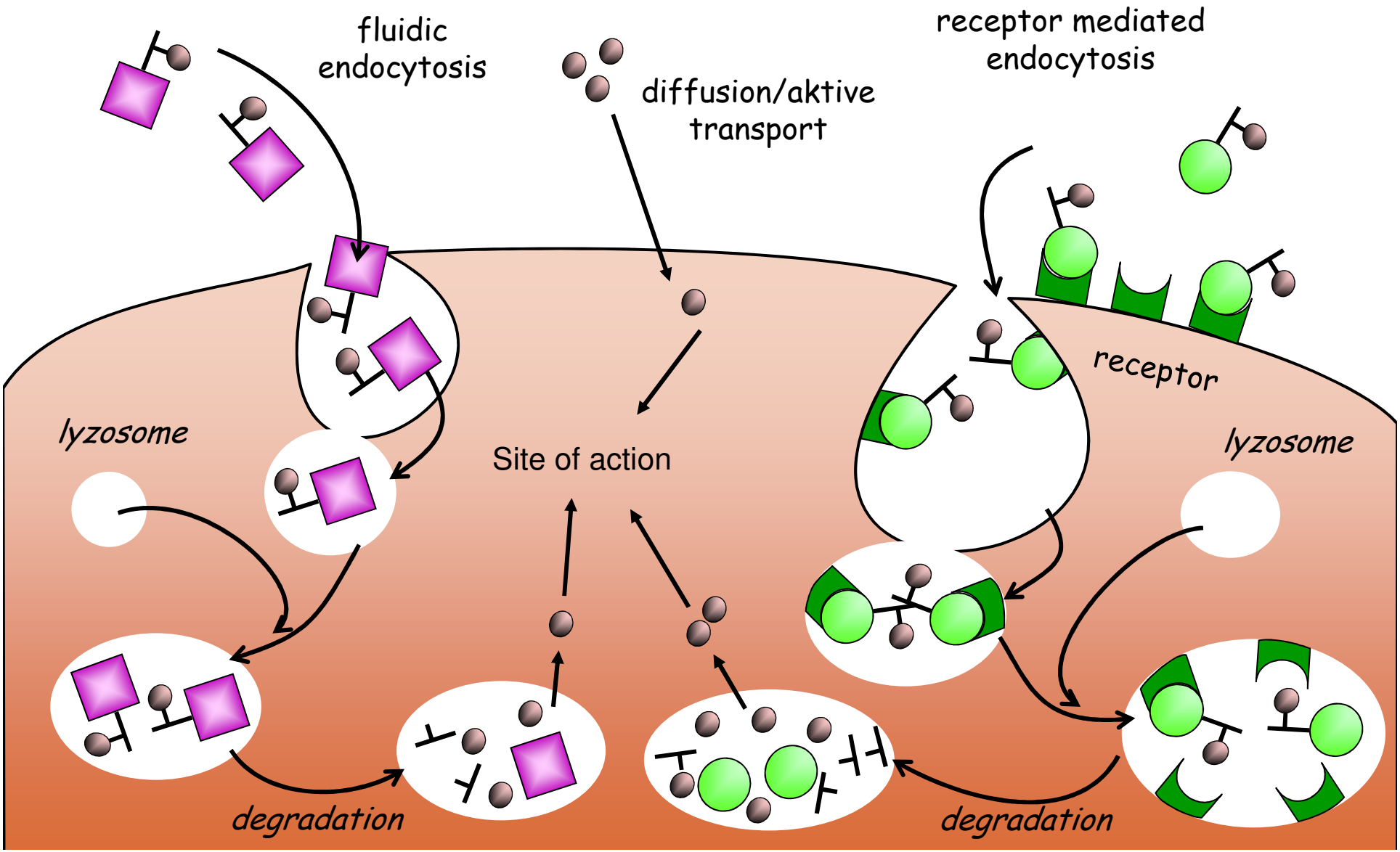
To achieve this he made a pact with the devil and got a magic bullet that would automatically hit the target.



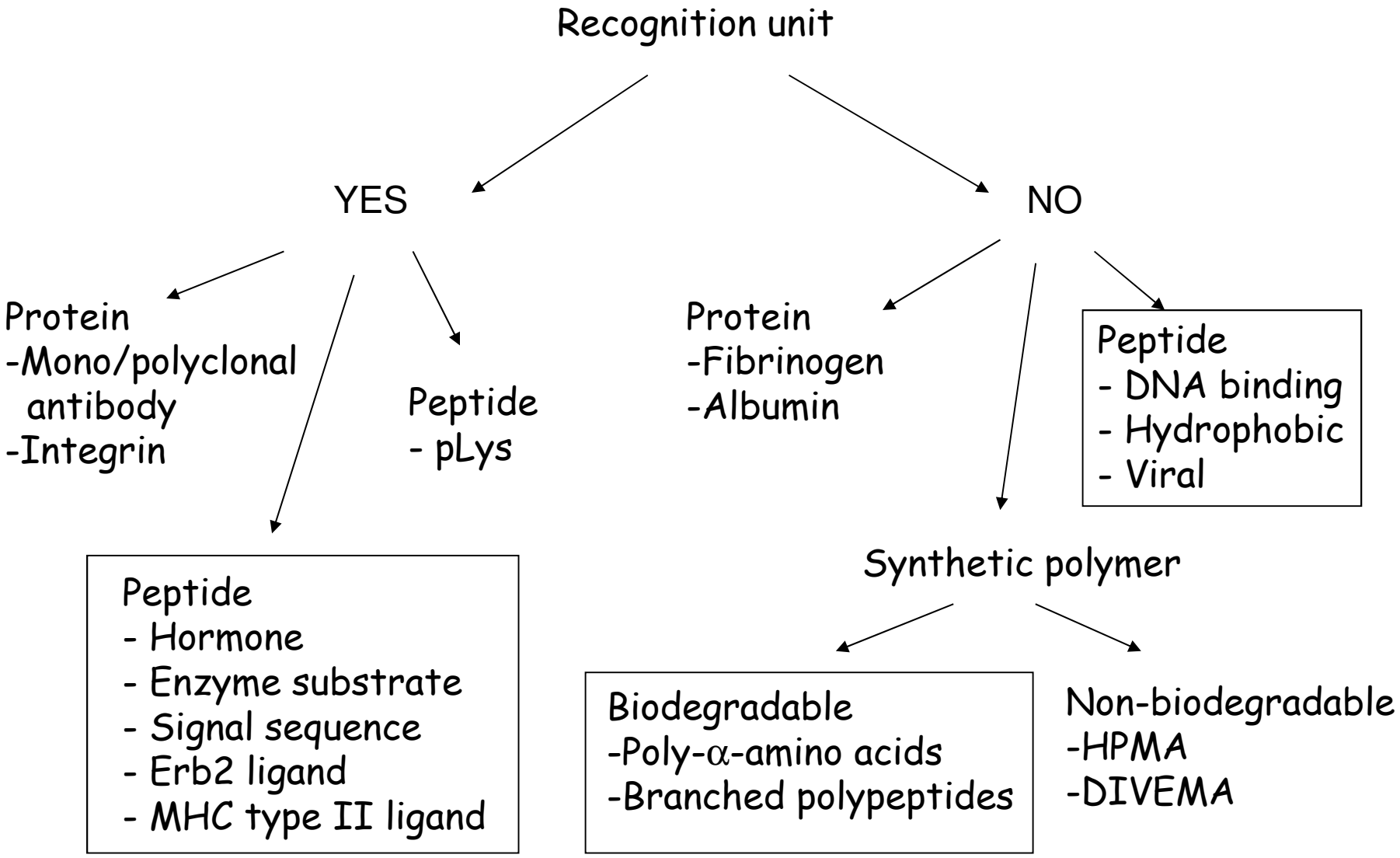


"Zauberkugeln": from the German opera "Der Freischütz" by Carl Maria von Weber.
A young man needed to shoot the target successfully to be able to marry his bride. To achieve this he made a pact with the devil and got a magic bullet that would automatically hit the target.

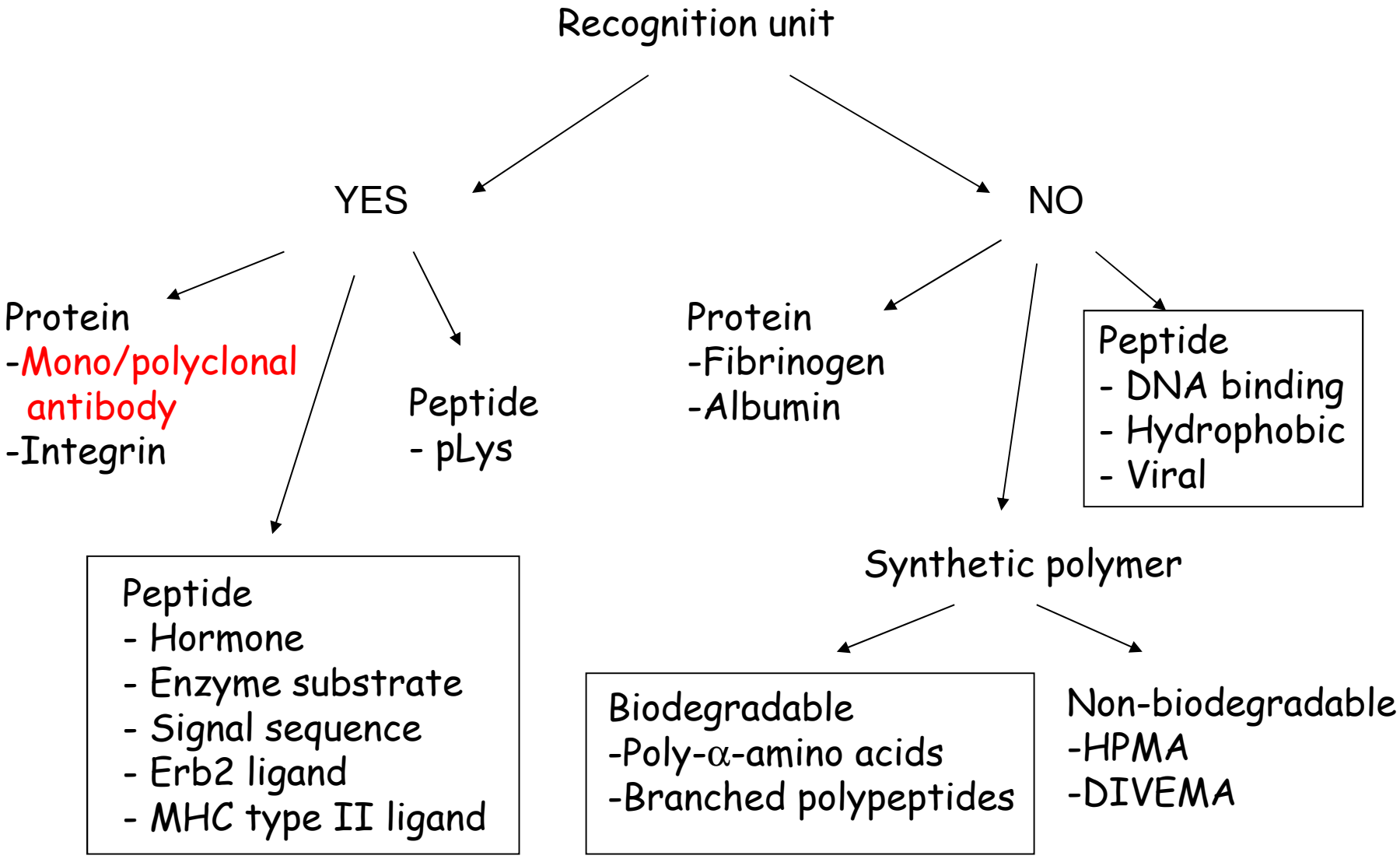
Uptake and liberation of bioactive entities

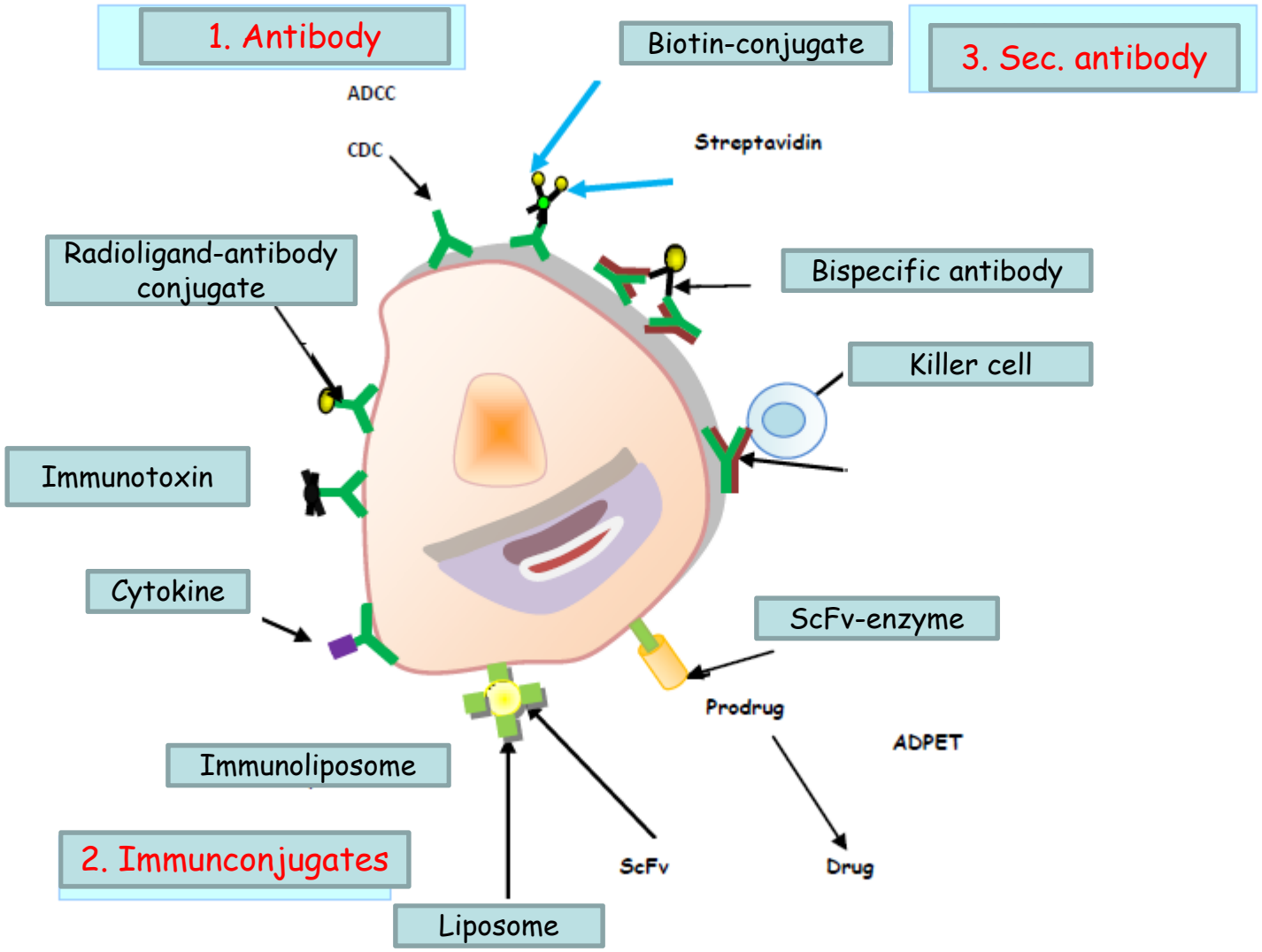


Peptide/protein based drug targeting/delivery



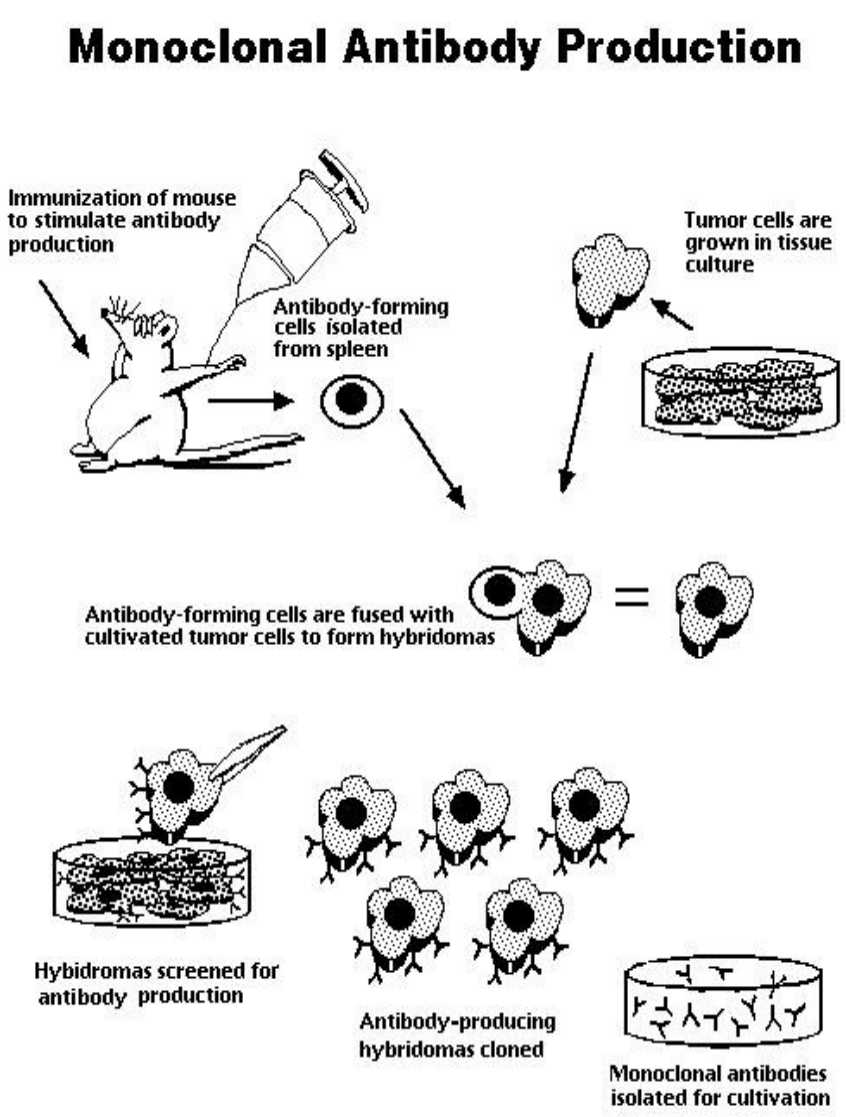
Peptide/protein based drug targeting/delivery

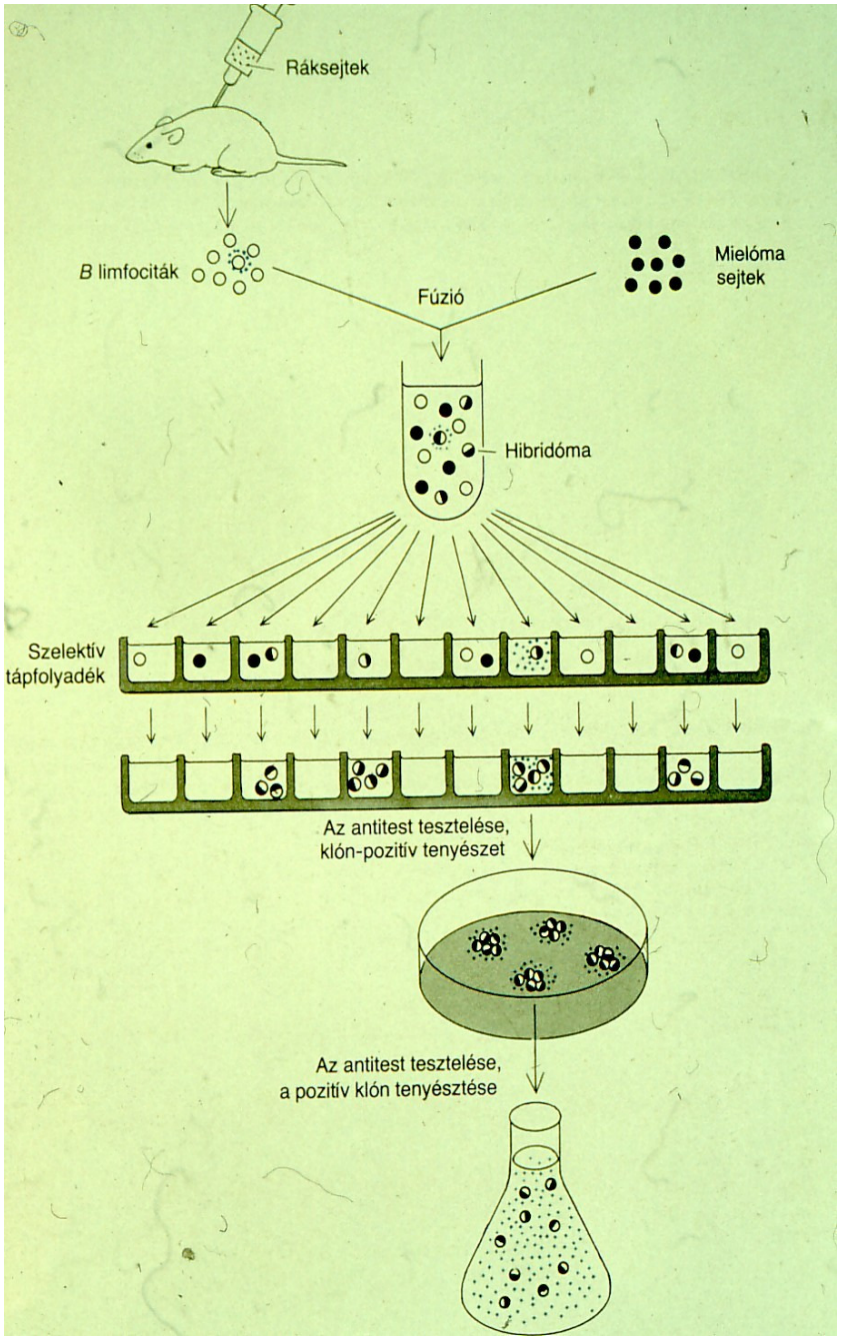




Dr. Halmos Gábor: Fejezetek a modern biofarmáciából, Budapest, 2011.

http://www.tankonyvtar.hu/hu/tartalom/tamop425/0006_1A_halmos_gabor_magyar/fejezetek_a_modern_biofarmaciabol_147_147.html

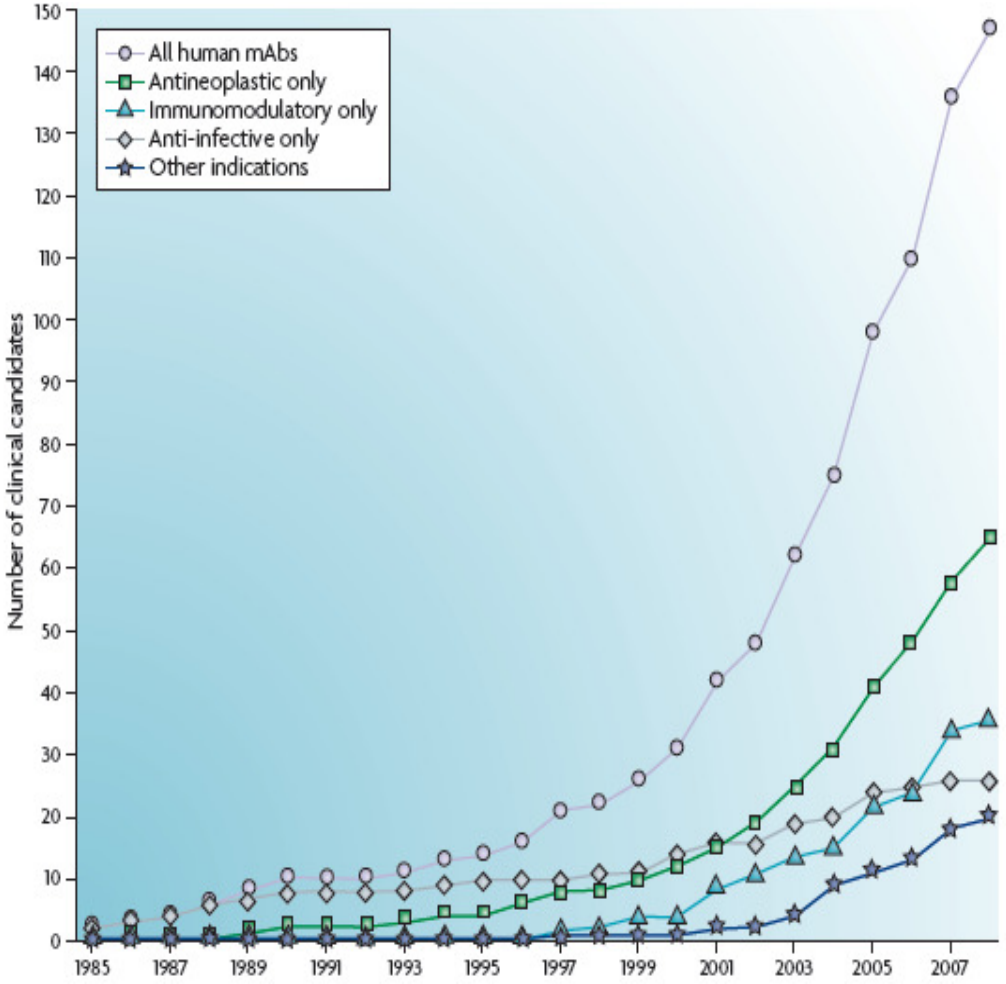




1975 First hybridoma to produce MoAb
(Nobel prize, 1984, C. Milstein, G.J.F. Köhler)

1986 First therapeutic antibody: muromomab
anti-human CD3, murine ab, kidney transplatation

Human monoclonal antibody development
1985-2008



- 40 new clinical trial
- 290 present clinical trial (December, 2010)

Aaron L. Nelson et al.

Development trends for human monoclonal antibody therapeutics.

Nature Reviews Drug Discovery (2010) doi:10.1038/nrd3229

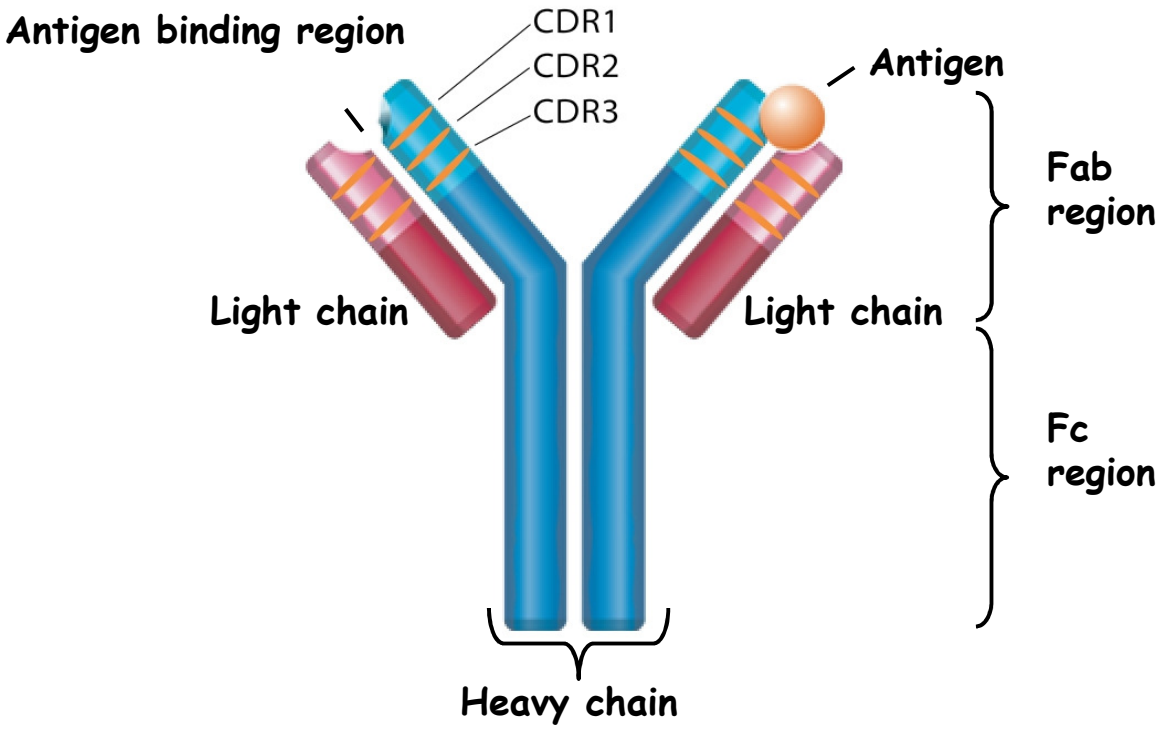
FDA* approved monoclonal antibodies for therapy

Code	Specificity	Type	Indication	Year
OKT3 (Muronomab-CD3)	CD3	mouse	transplantation	1986
ReoPro (Abciximac)	GpIIb/gpIIIa	Fab chimeric	cardiovascular disease	1994
Rituxan	CD20	chimeric	non-Hodgkin lymphoma	1997
Zenapax (Daclizumab)	CD25	humanised	transplantation	1997
Remicade (Infiximab)	TNF-a	chimeric	Crohn-disease rheumatoid arthritis	1998
Simulect (Basiliximab)	CD25	chimeric	transplantation	1998
Synagis	RSV	humanised	RSV virus	1998
Herceptin	Her-2	humanised	breast cancer (metastatic)	1998
Mylotarg	CD33	humanised toxin-linked	acute myeloid leukemia	2000
CroFab	kígyóméreg	ovine Fab	anti-rattlesnake poison	2000
DigiFab	digoxin	ovine Fab	digoxin poisoning	2001
Campath	CD52	humanised	chronic lymphoid leukemia	2001
Zevalin	CD20	murine radioligand-linked	non-Hodgkin lymphoma	2002

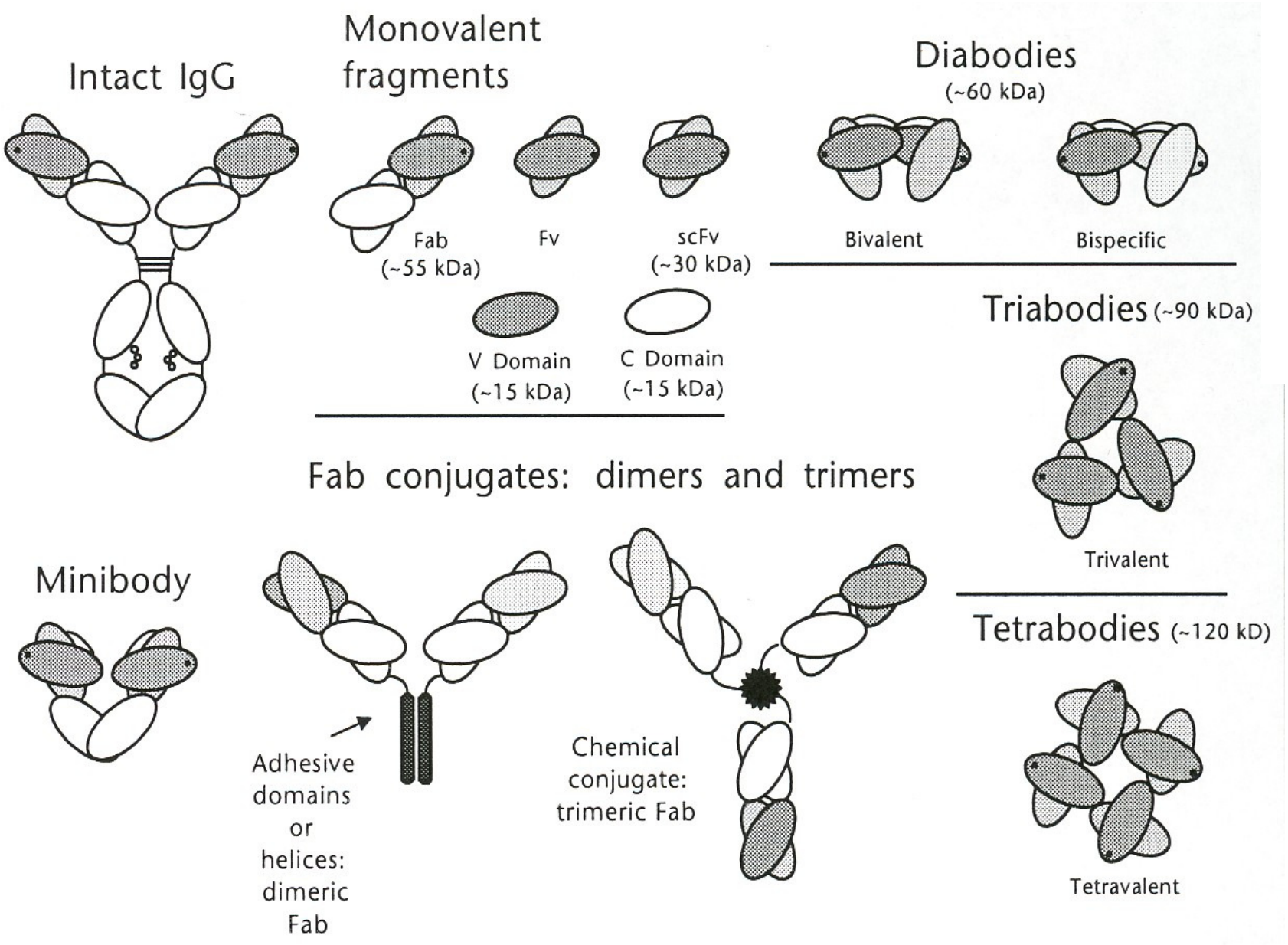
*Food and drug administration

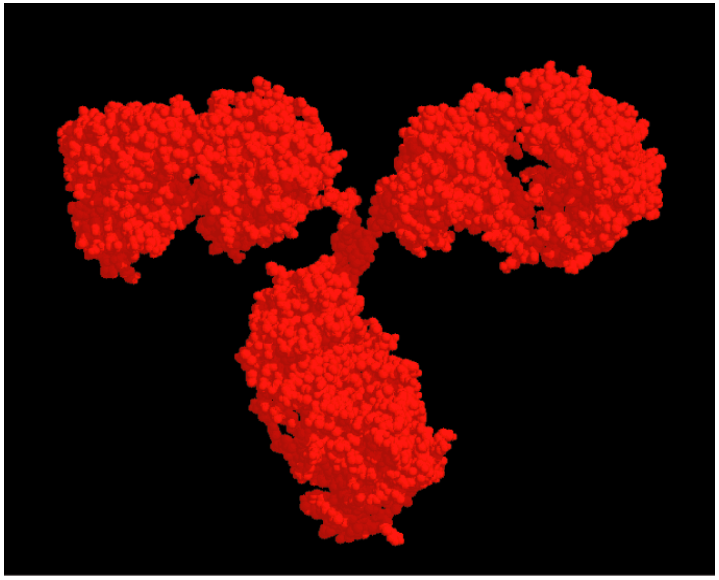
Nature Med. 9:129 (2003)

Structure of antibody

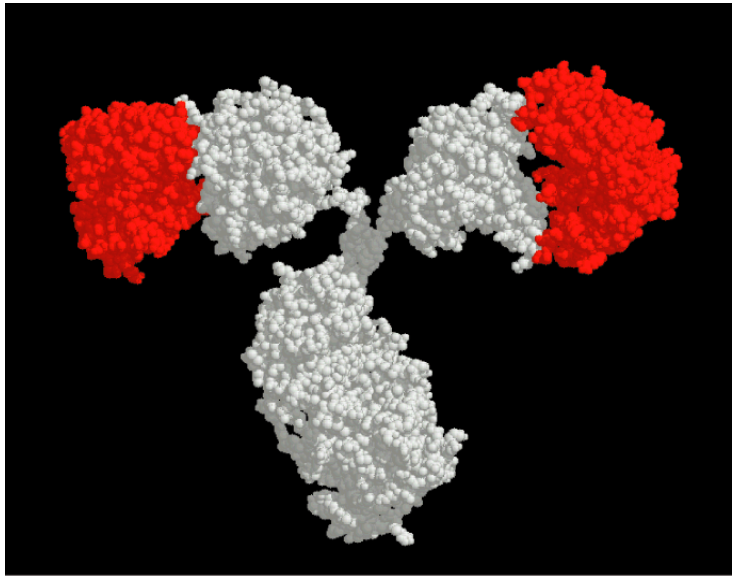


CDR: comlementarity determining regions

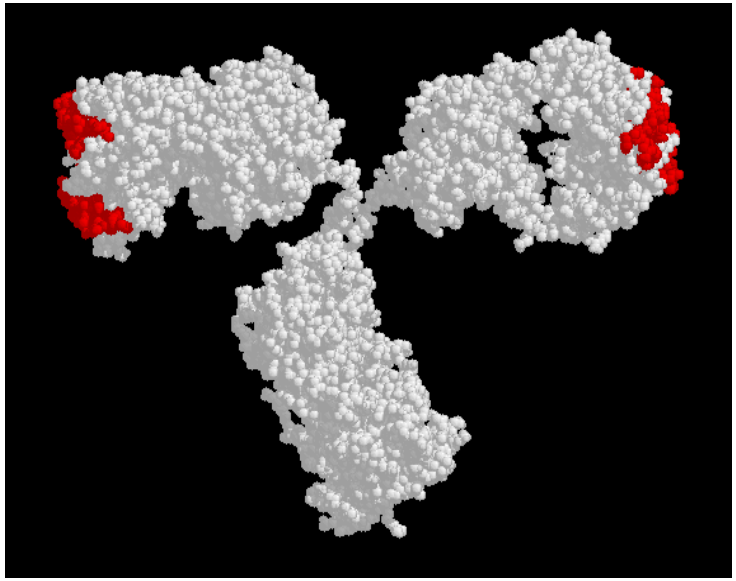

Intact IgG
Monovalent fragments
Fab (~55 kDa)
Fv
scFv (~30 kDa)
V Domain (~15 kDa)
C Domain (~15 kDa)
Diabodies (~60 kDa)
Bivalent
Bispecific
Triabodies (~90 kDa)
Trivalent
Fab conjugates: dimers and trimers
Minibody
Adhesive domains or helices: dimeric Fab
Chemical conjugate: trimeric Fab
Tetrabodies (~120 kD)
Tetravalent



Native murine monoclonal antibody



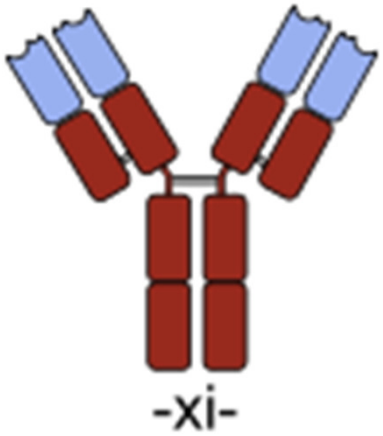
Chimeric mouse/human antibody, CH1-CH3 domains replaced



Chimeric mouse/human antibody, CH1-CH3 and CL domains replaced

First generation of therapeutical antibodies

Chimeric antibody



25% mouse

75% human

1984 Antibody chimeration[2] –

mouse Fab region + human Fc region

1994 First chimeric antibody: abciximab (anti GP IIb/IIIa antibody)

1997- Novel chimeric antibodies:

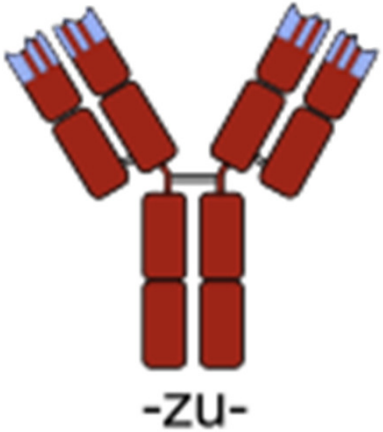
e.g. rituximab, infliximab

Efficacy

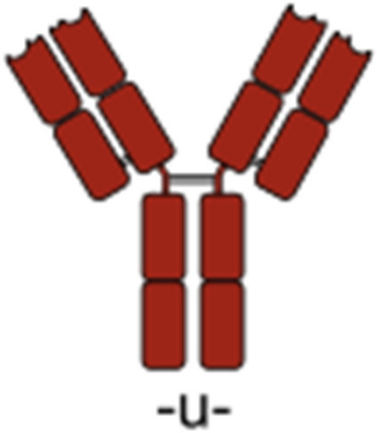
Immunogenicity – human anti-chimeric antibody (HACA) formation

1:KöhlerG, Milstein C. Nature 256. 495-497 (1975) 2: Morrison S et al. PNAS 81. 6851-6855 (1984)

Second generation of therapeutical antibodies



Humanized /
Human antitbody



95-100% human

1986 Humanization of antibodies, first time[1]

1997 The first humonized antibody: daclizumab (anti CD25)

1990 „Phage display" technology description, first time[2]

2002 The first human antibody: adalimumab (TNF inhibitor) – by phage display technology)

1994 Transgenic mice producing human IgG[3,4]

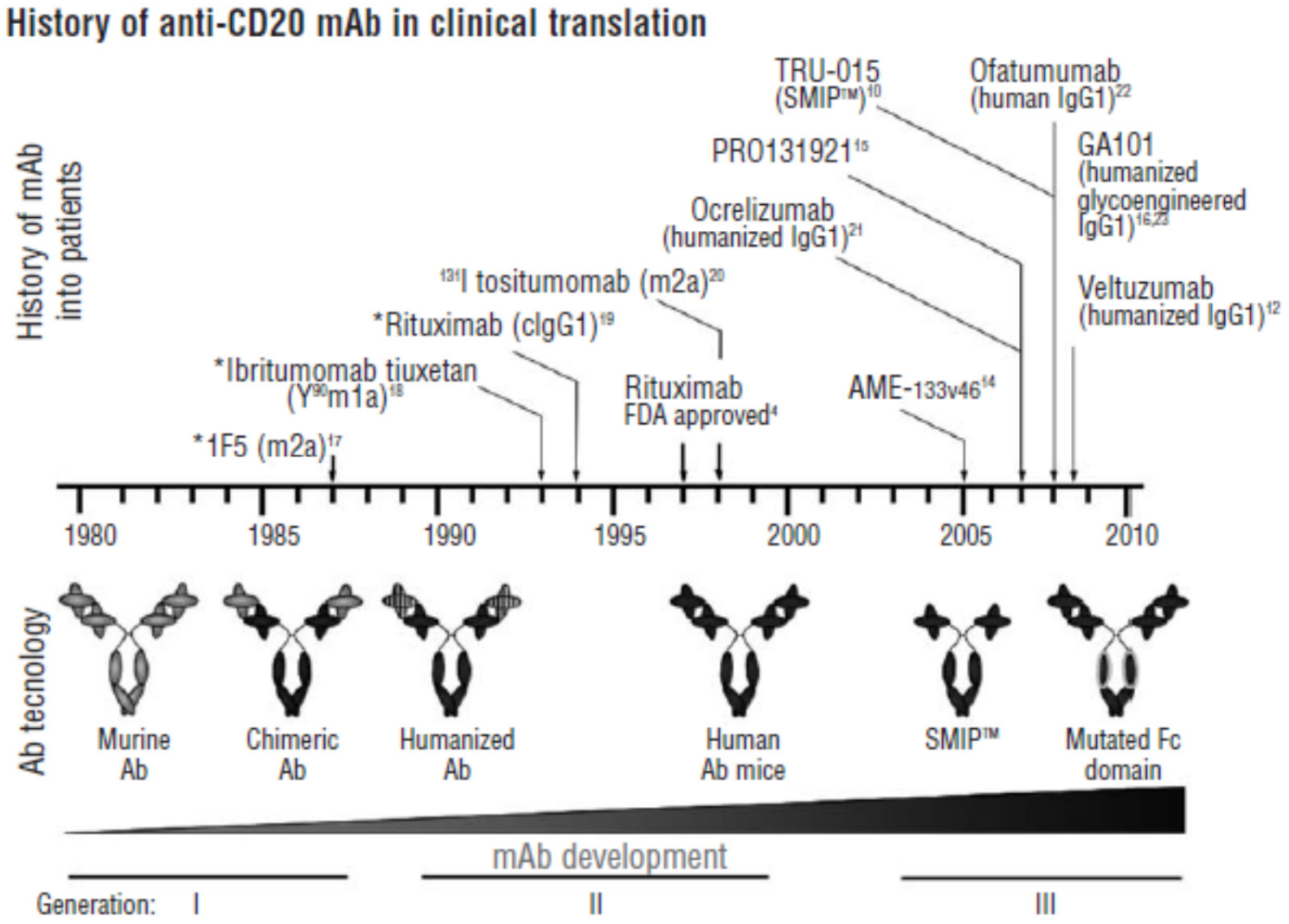
– Abgenix mouse and Medarex mouse

2009 First therapeutic human antibody produced in mice: golimumab – Medarex

Immunogenicity – human anti-human antibodies (HAHA)

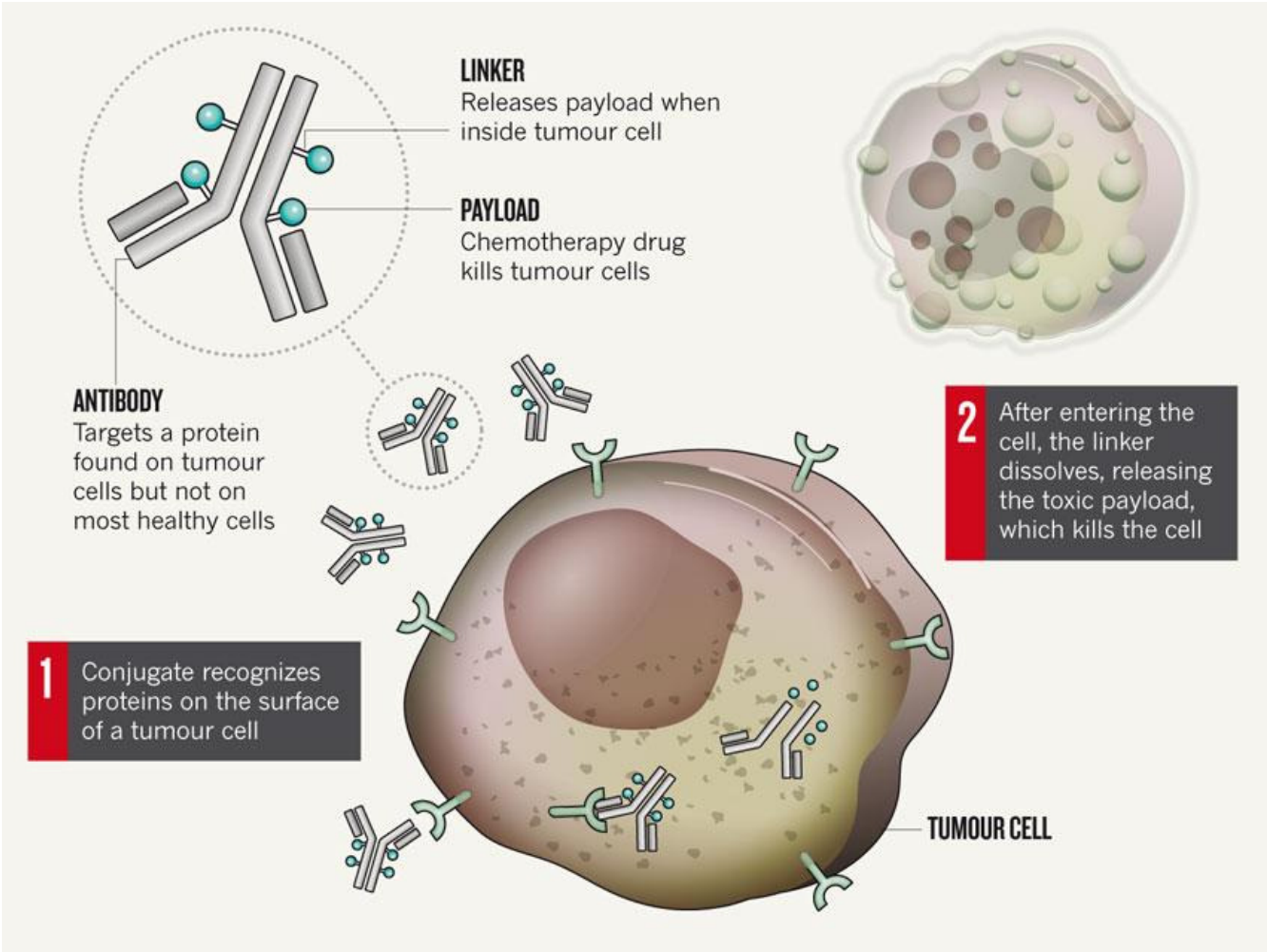
1: Jones PT. Et al. Nature 321. 522-525 (1986); 2:McCafferty et al. Nature 348. 552-554 (1990); 3:Green et al. Nature Genet. 7. 13-21. (1994); 4: Lonberg N et al. Nature 368. 856-859. (1994)

„Development" of anti-CD20 monoclonal antibodies



Sean H. Lim et al. Anti-CD20 monoclonal antibodies: historical and future perspectives. Haematologica. 2010; 95(1); 135-143

Monoclonal antibody - conjugates

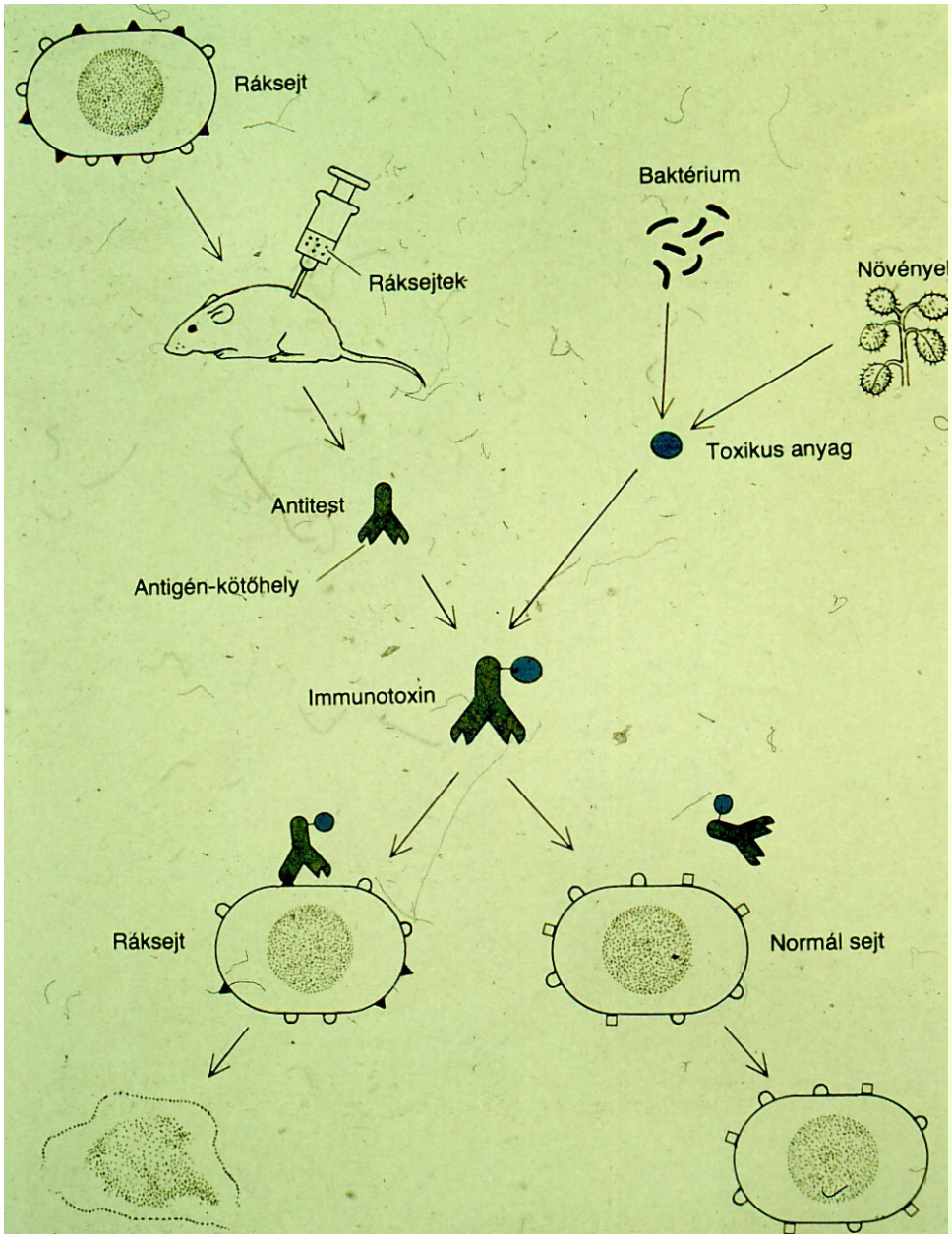


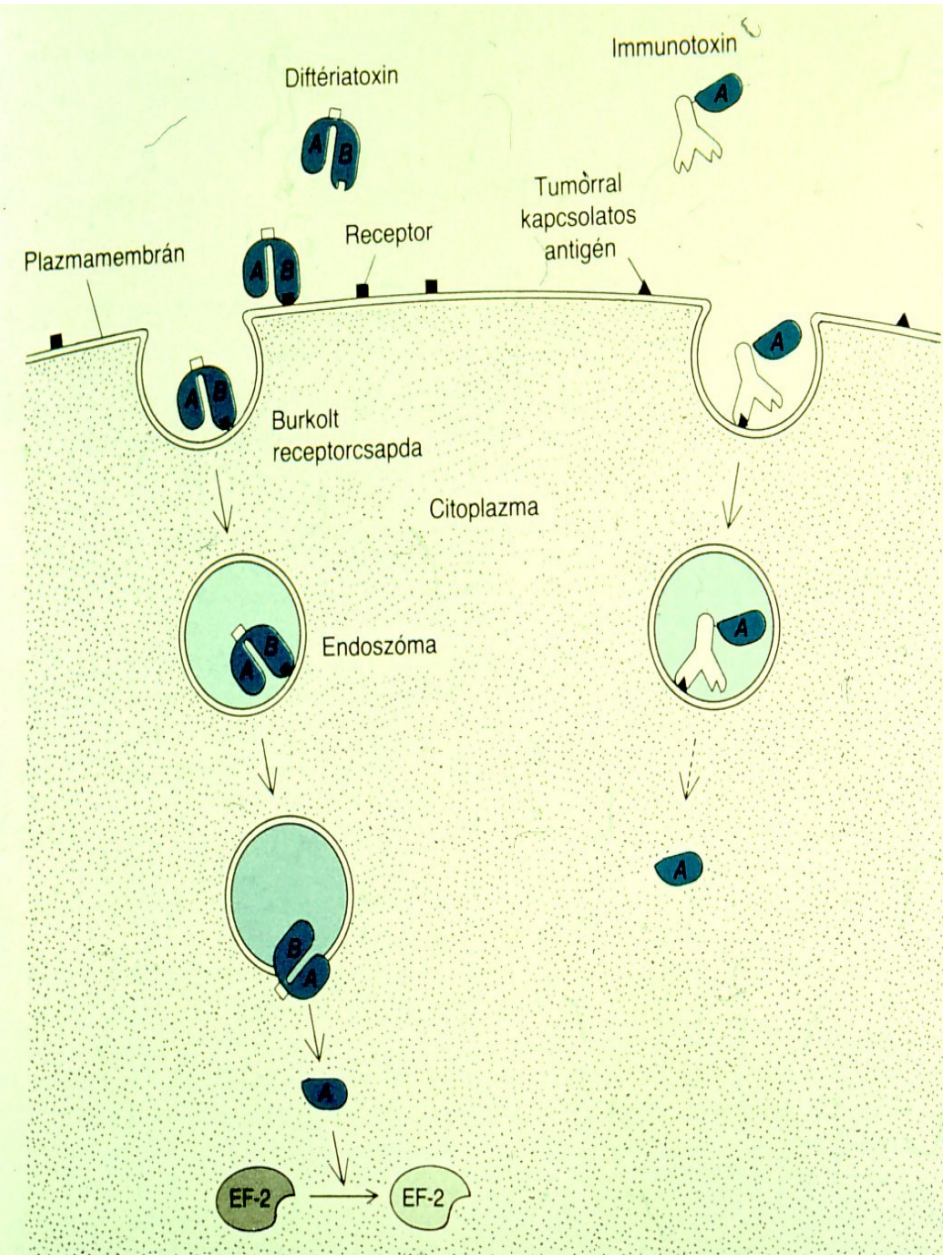
H. Ledford: Toxic antibodies blitz tumours. Nature 476, 380-381 (2011)

B. Targeted Drugs –

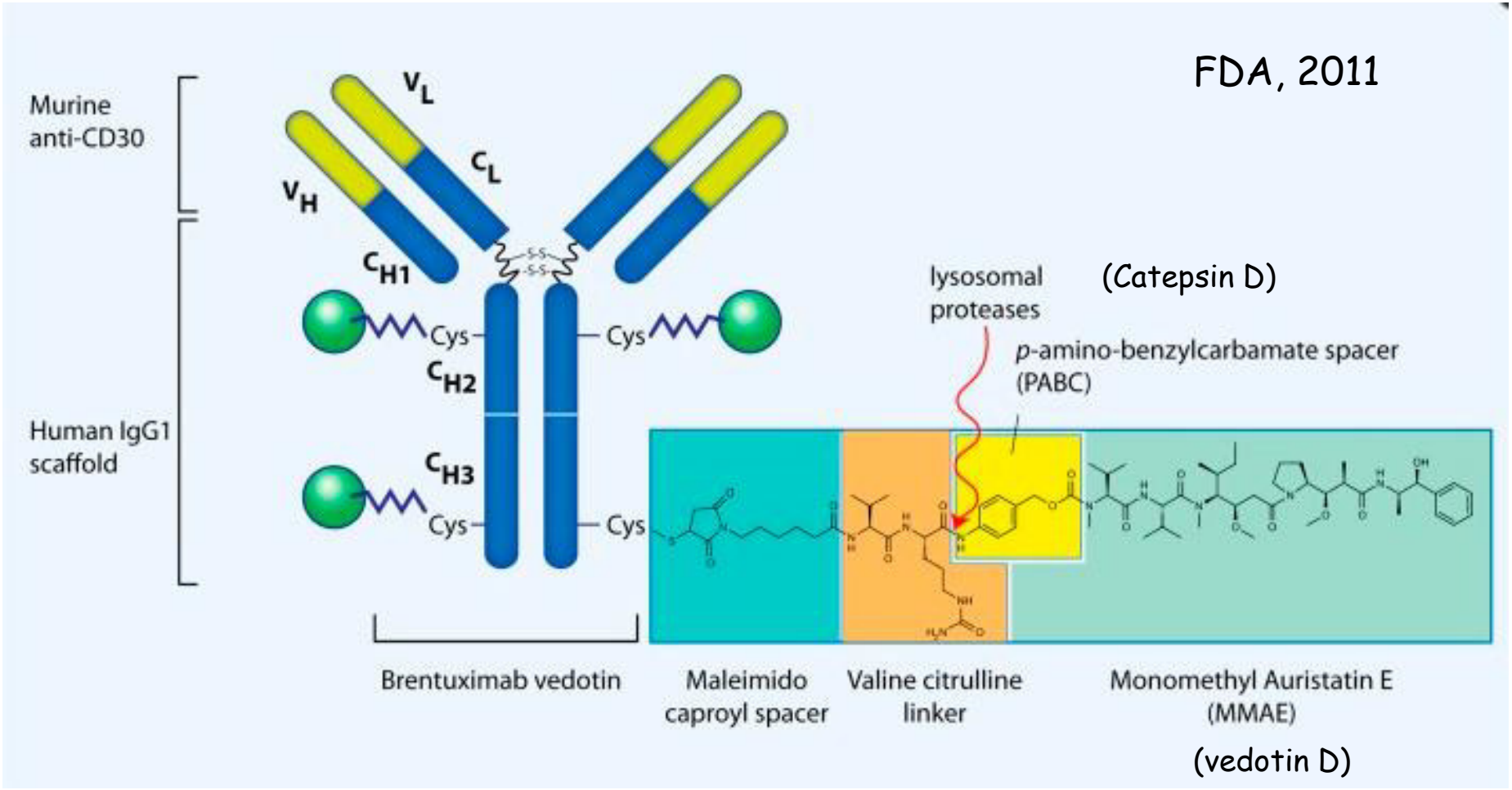
1. Tyrosine Protein Kinase Inhibitors – Imatinib, nilotinib
2. EGF receptor inhibitors – Geftinib, Erlotinib,Cetuximab
3. Angiogenesis Inhibitors – Bevacizumab, Sunitinib
4. Proteasome Inhibitor – Bortezomib
5. Unarmed monoclonal antibody – Rituximab, Trastuzumab

Monoclonal antibody - toxin conjugates



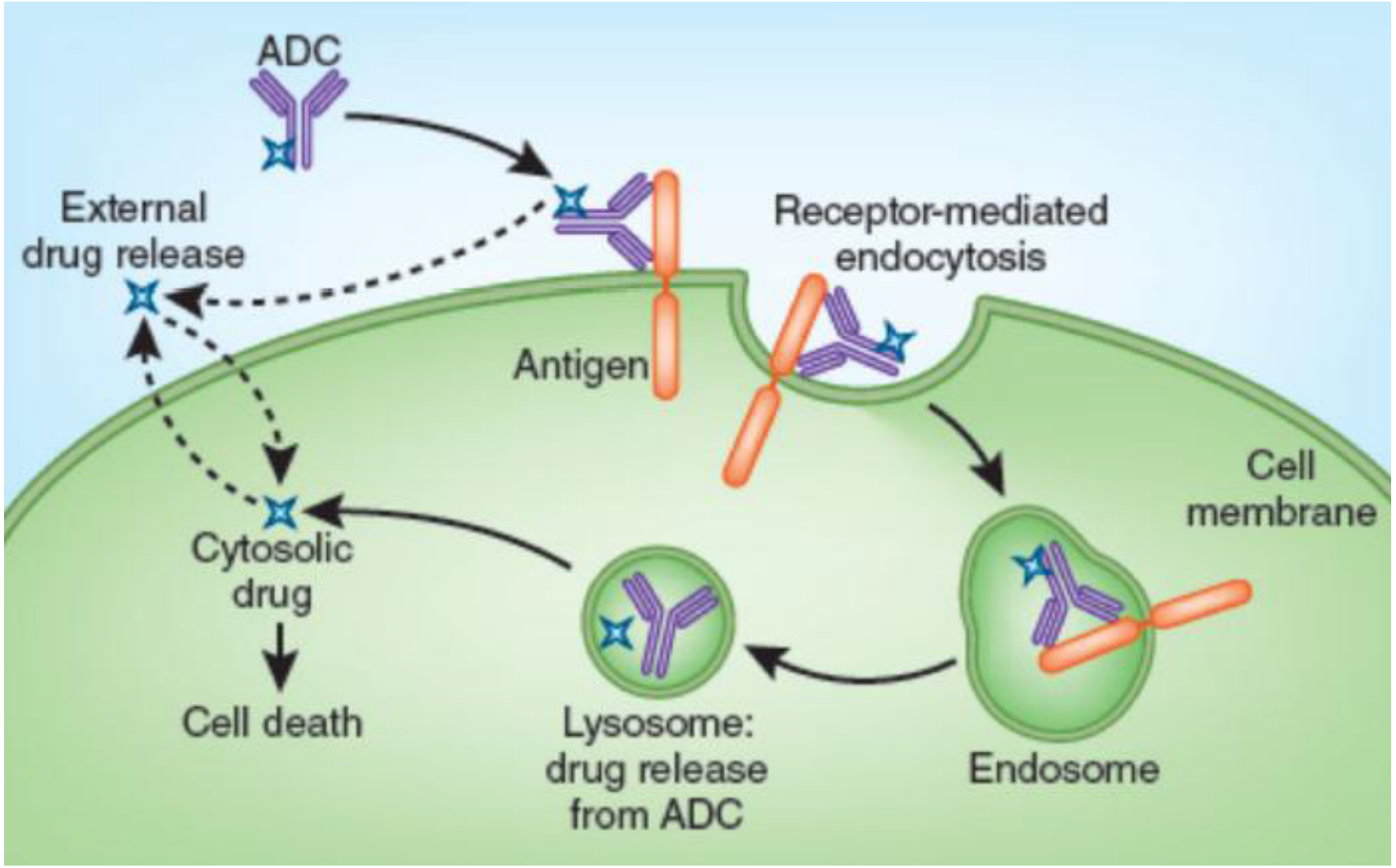


Brentuximab vedotin conjugate against CD30+ Hodgkin (HL) and non-Hodgkin lymphoma



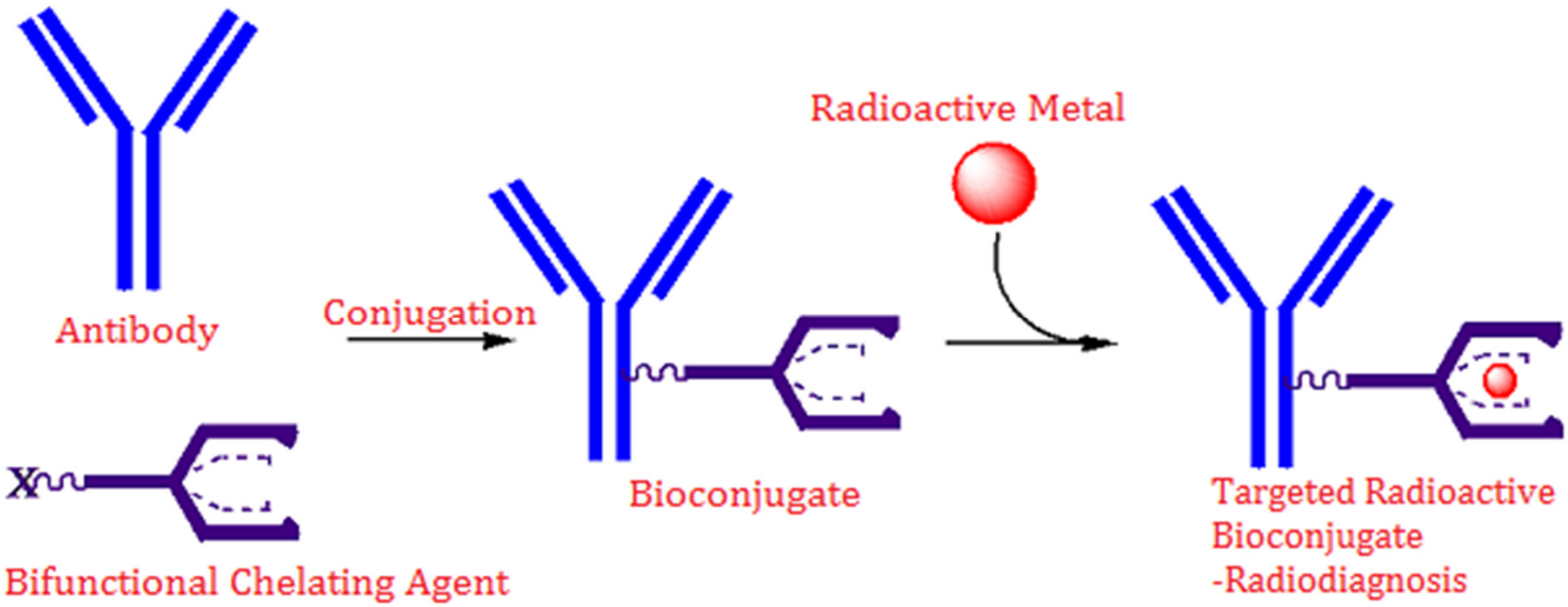
A. Wakankar, Y. Chen, Y. Gokarn, F. S. Jacobson: Analytical methods for physicochemical characterization of antibody drug conjugates, *mAbs 3:2*, 161-172; 2011.

Mechanism of action of brentuximab vedotin conjugate



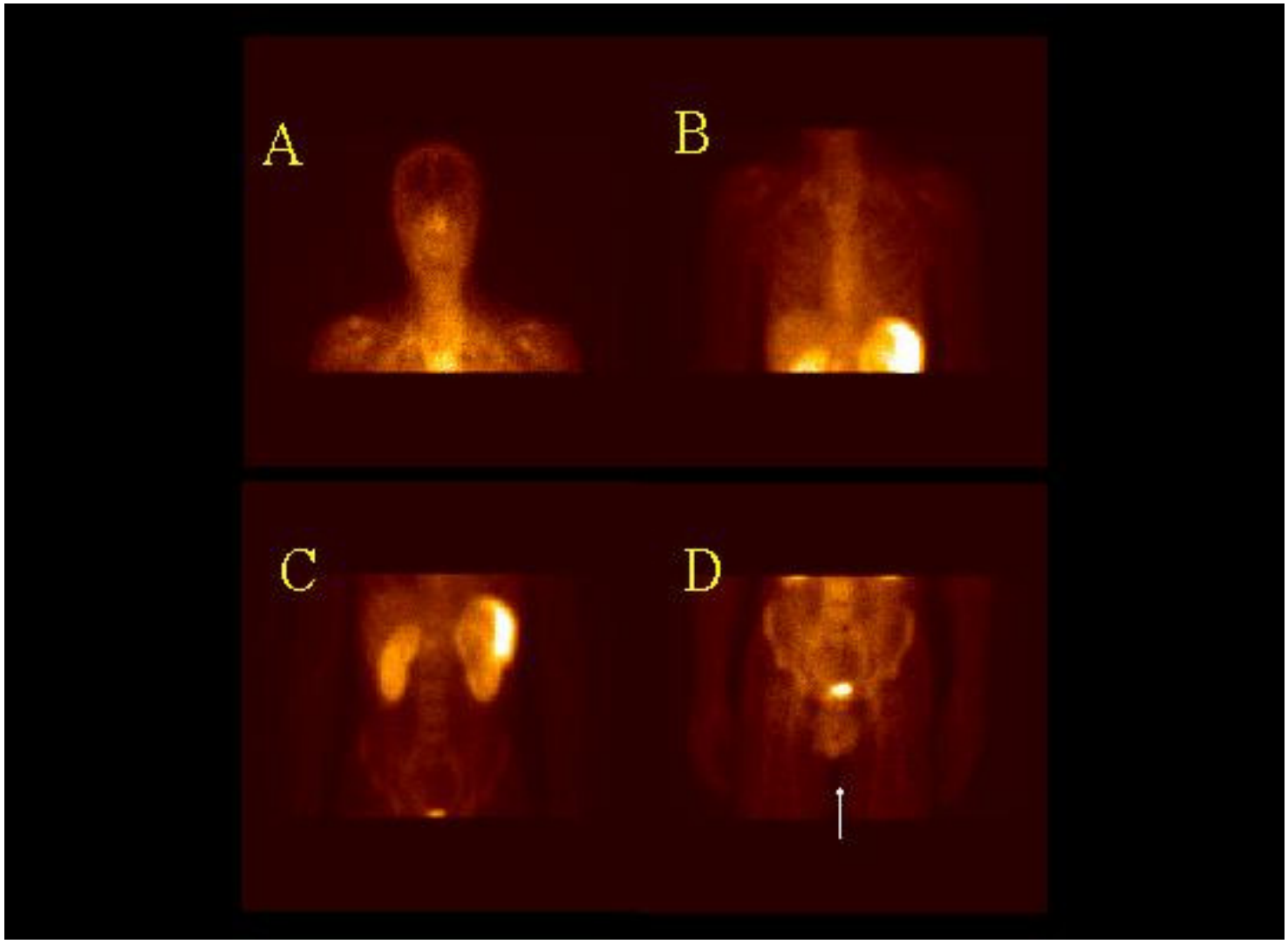
C. Vaklavas and A. Forero-Torres: Safety and efficacy of brentuximab vedotin in patients with Hodgkin lymphoma or systemic anaplastic large cell lymphoma, *Ther Adv Hematol 3(4)* 209–225, 2012.

Monoclonal antibody – radioligand conjugates



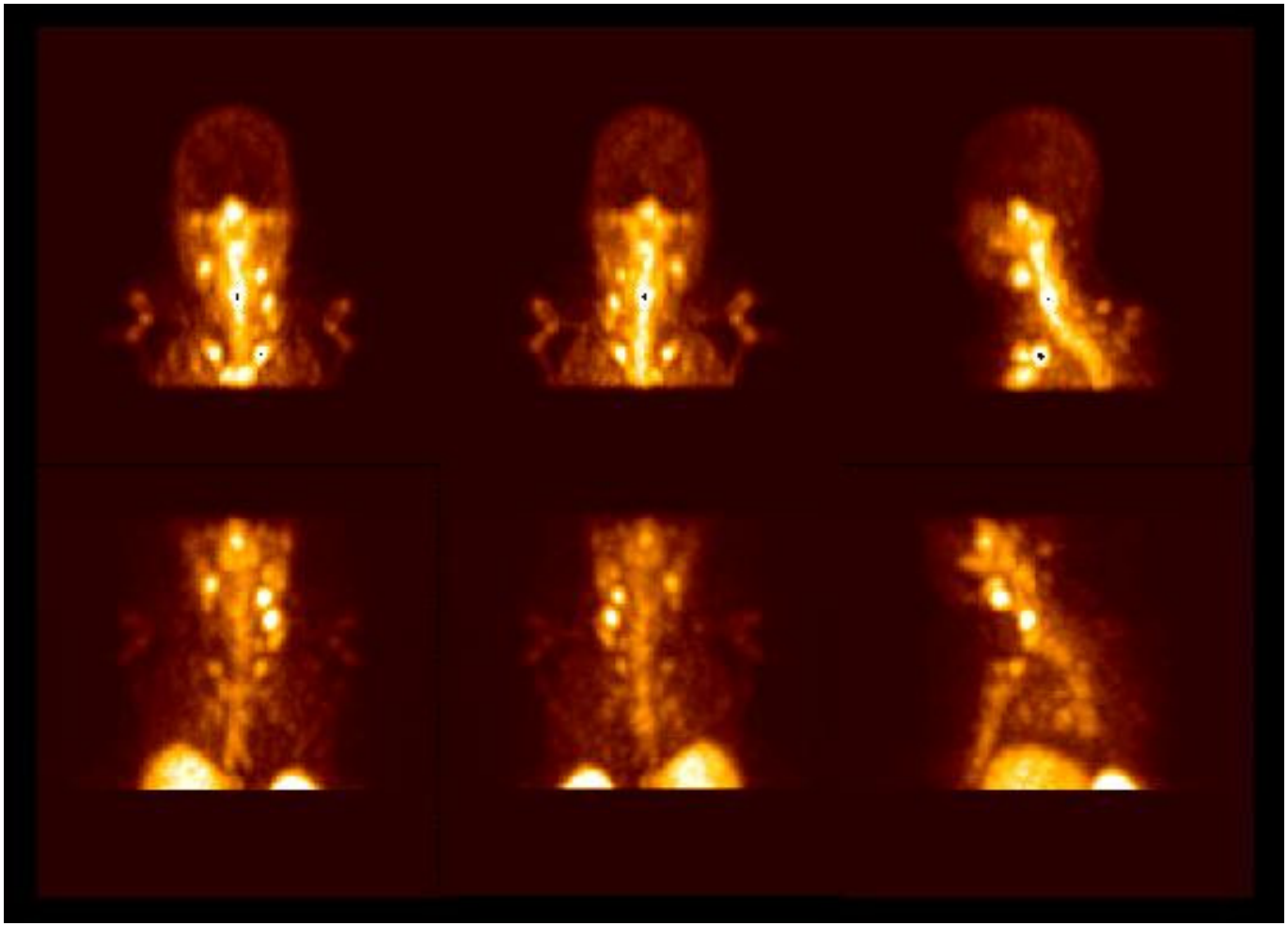
Tc^{99}, In^{111} etc.

Tc99-radiolabeled mAb against the cellular membrane of the Raji cell component of B cell lymphoma patients with non-Hodgkin's B-cell lymphoma



41 year old showing relatively normal distribution of tracer A) Nasopharynx B) Heart C) Liver, Spleen, and Kidney D) Testicles, Bladder and Bone Marrow

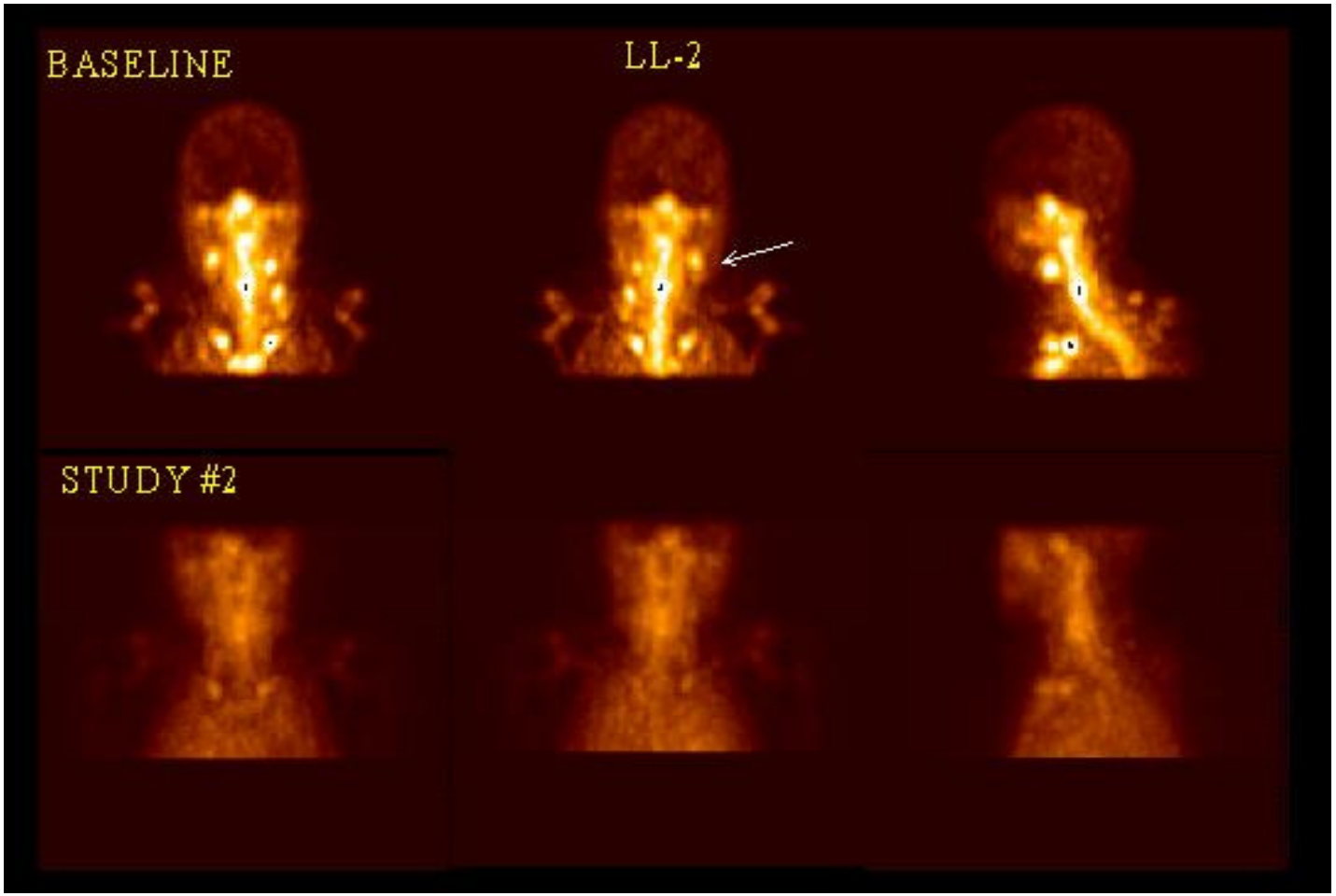
Tc99-radiolabeled mAb against the cellular membrane of the Raji cell component of B cell lymphoma patients with non-Hodgkin's B-cell lymphoma.



69 year old with right tonsillar lymphoma and multiple positive nodes.
(A) 4-h 3D reprojection images (Ant., Post, Lat.) and (B) Comparable 24-h images

E. Tamm et al. The University of Texas-Houston Medical School, Houston, Texas

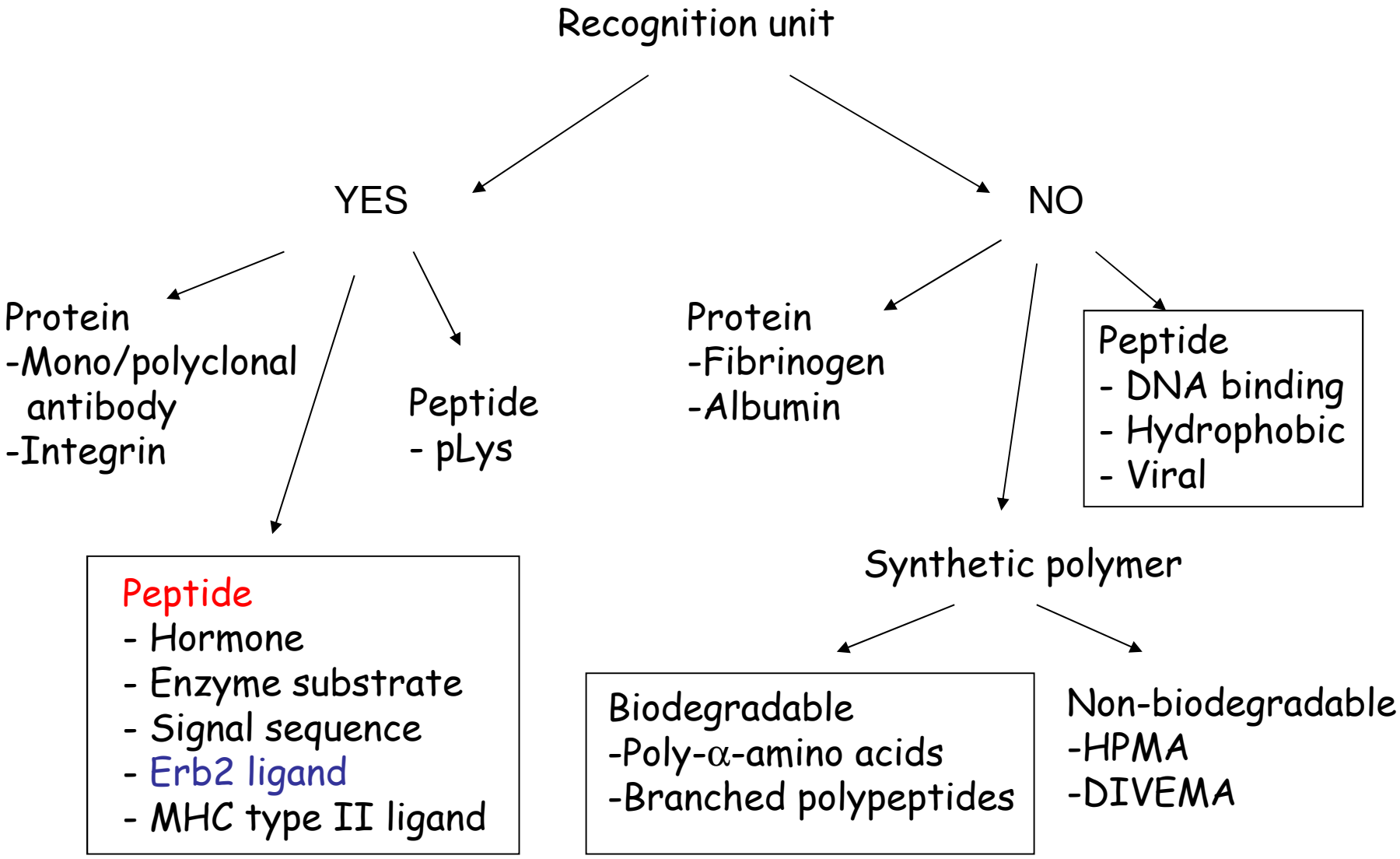
Tc99-radiolabeled mAb against the cellular membrane of the Raji cell component of B cell lymphoma of patients with non-Hodgkin's B-cell lymphoma.



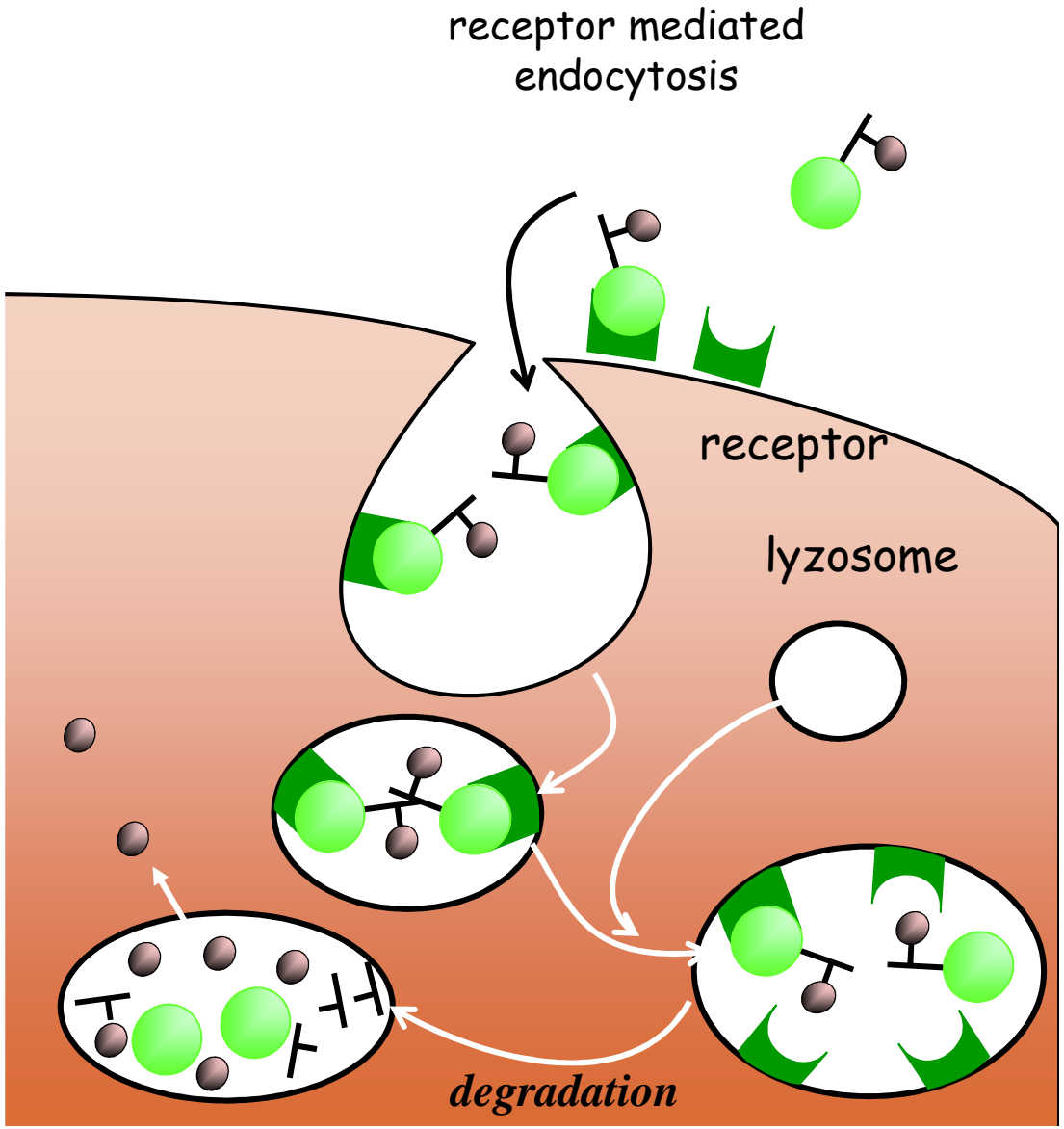
69 year old with right tonsillar lymphoma. 3D images findings on (A) Study #1 and (B) Study #2, 4 months apart.

E. Tamm et al. The University of Texas-Houston Medical School, Houston, Texas

Peptide/protein based drug targeting/delivery

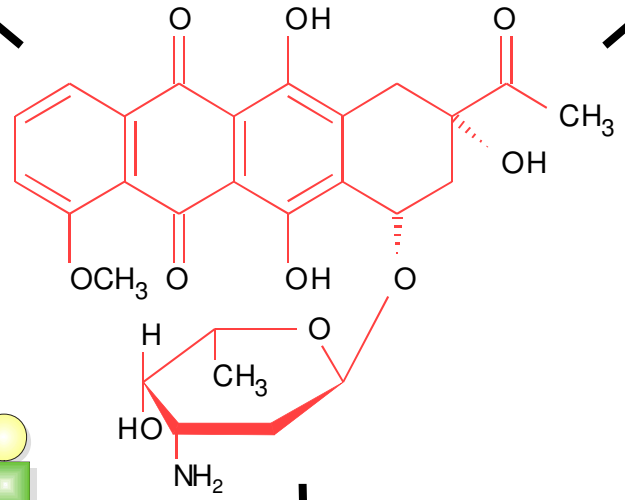


Uptake and liberation of bioactive entities

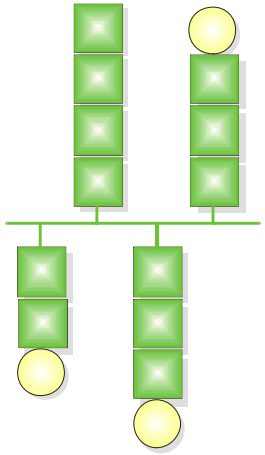


Daunomycin conjugates with oligo- or polypeptide

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

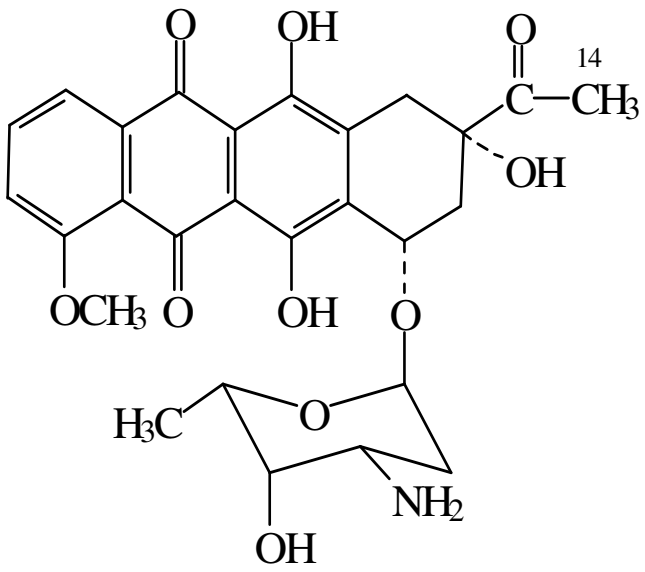


Sztaricskai F. et al.: *J Antibiotics (Tokyo),* **58**: 704 (2005)
Bánóczi Z. et al. *Archivoc* **140**, (2008)
Miklán Zs. et al. *Biopolymers* **92**: 489 (2009)

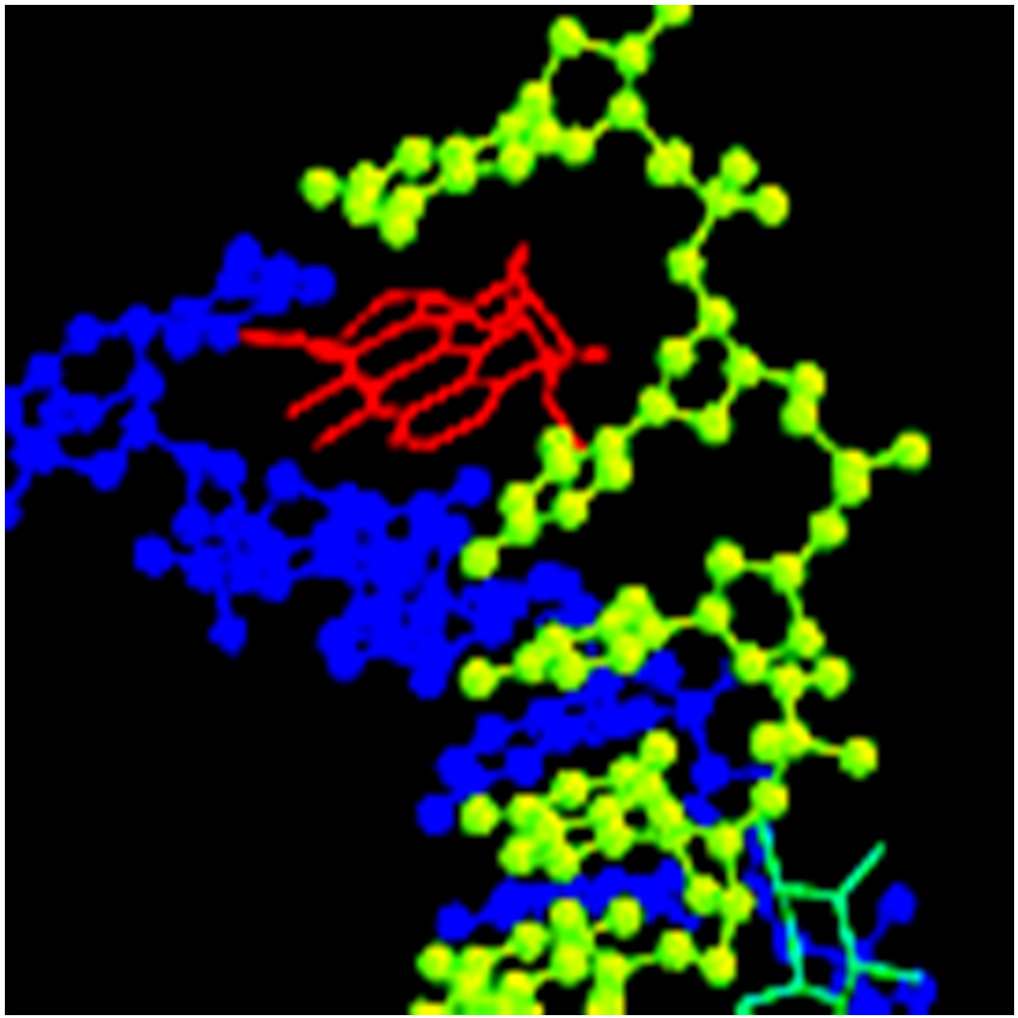


Hudecz F. et al.*Bioconjugate Chem.* **3:** 49 (1992)
Gaál D., Hudecz F. *Eur.J.Cancer.* **34:** 155 (1998)
Szabó R. et al. *Bioconjugate Chem.* **19**: 1078 (2008)
Reményi, J. et al. *Biochim. Biophys. Acta* **1798**: 2209 (2010)

Daunosamine directed intercalation into minor groove



[Frederick, 1990]

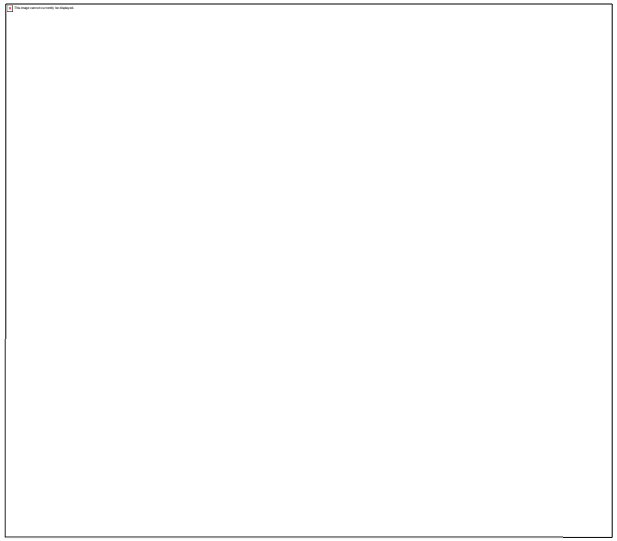


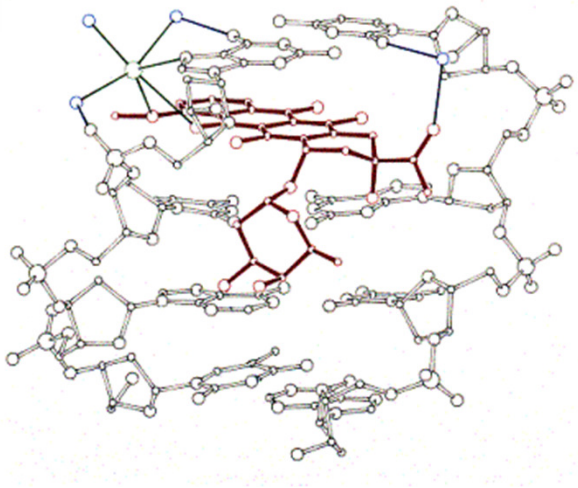
Daunomycin in tumour therapy

- Drug of anthracyclin family

- Therapeutic use:
  leukaemias (AML, CML, ALL); lymphomas, rhabdomyosarcoma, neuroblastoma

- Side effects:
  - Decreased white blood cell count
  - Cardiotoxic effect
  - Nausea and vomiting
  - Hair loss



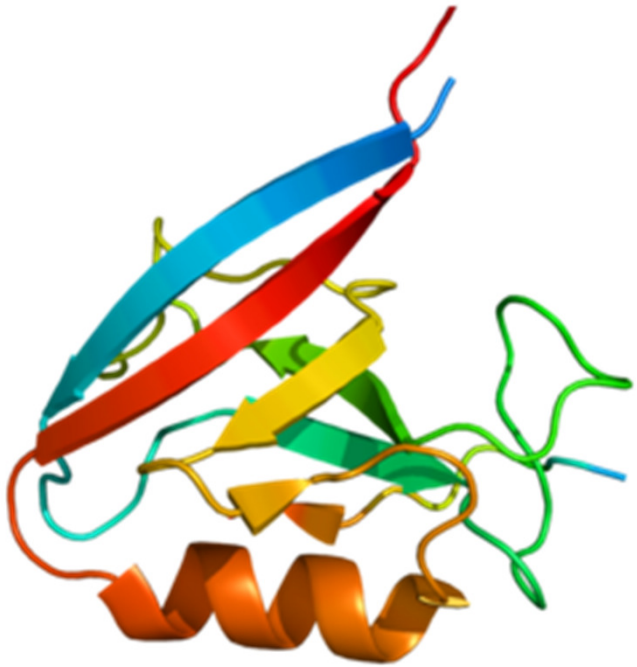


- Mechanism of action:
  - intercalating DNA,
  - stabilisation DNA-topoisomerase II complex,
  - enhancing the production of free radicals

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.

ErbB2 receptor and peptide LTVSPWY as ligand

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



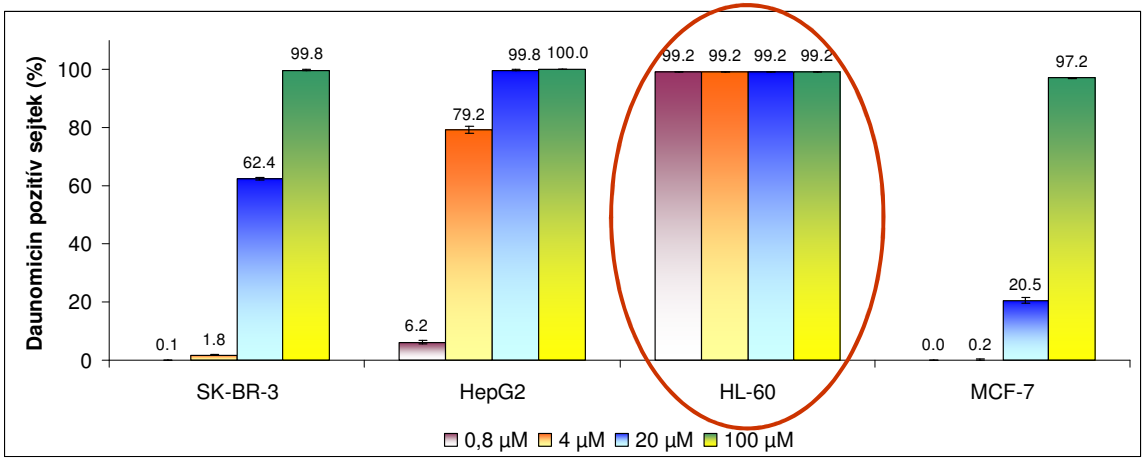
http://www.genenames.org

Cytotoxicity:

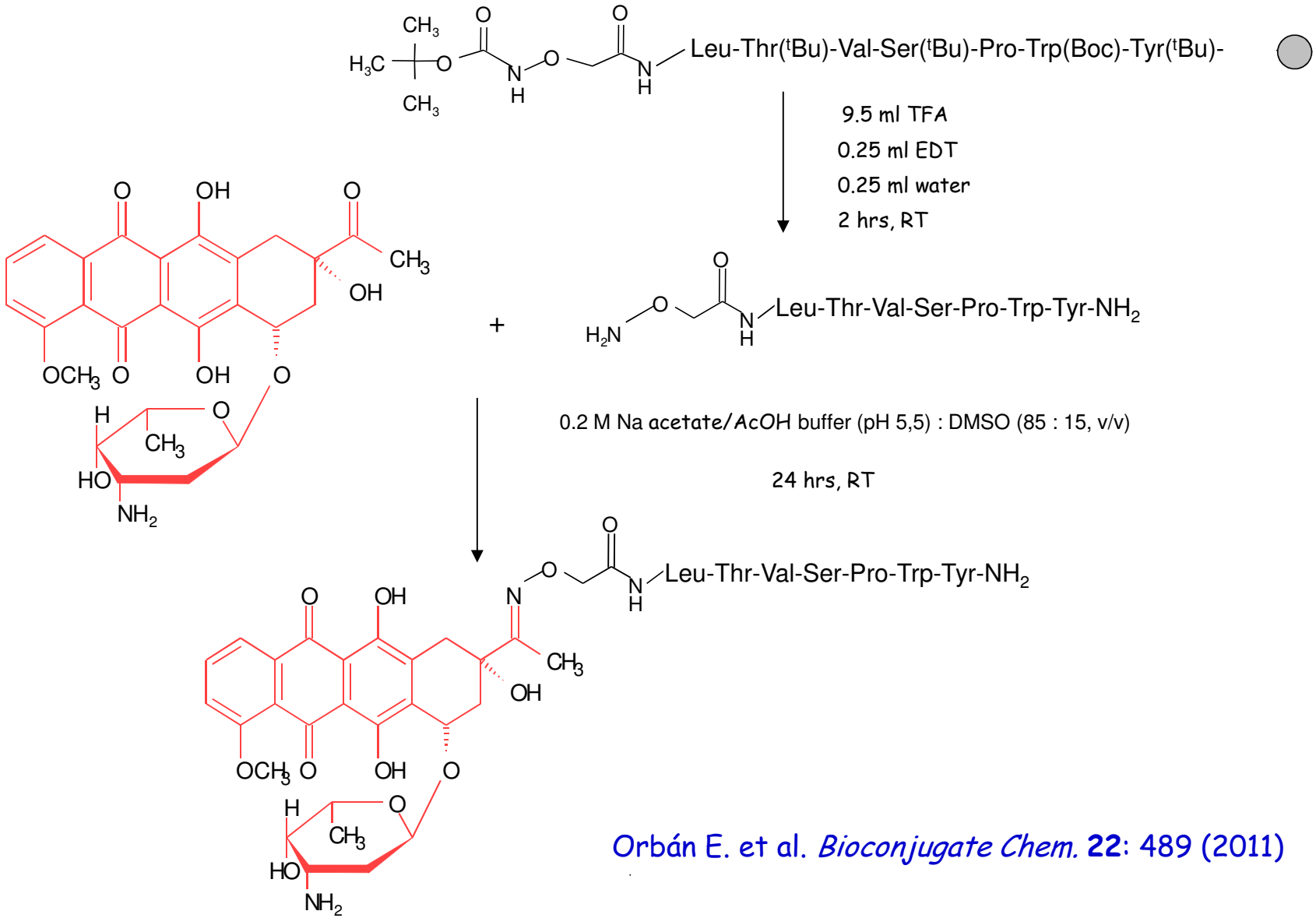
IC_{50} : 0,53 +/- 0.12 (HL-60)

IC_{50} : 37,9 +/- 2,83 (SK-BR-3)

Cellular uptake (FACS):

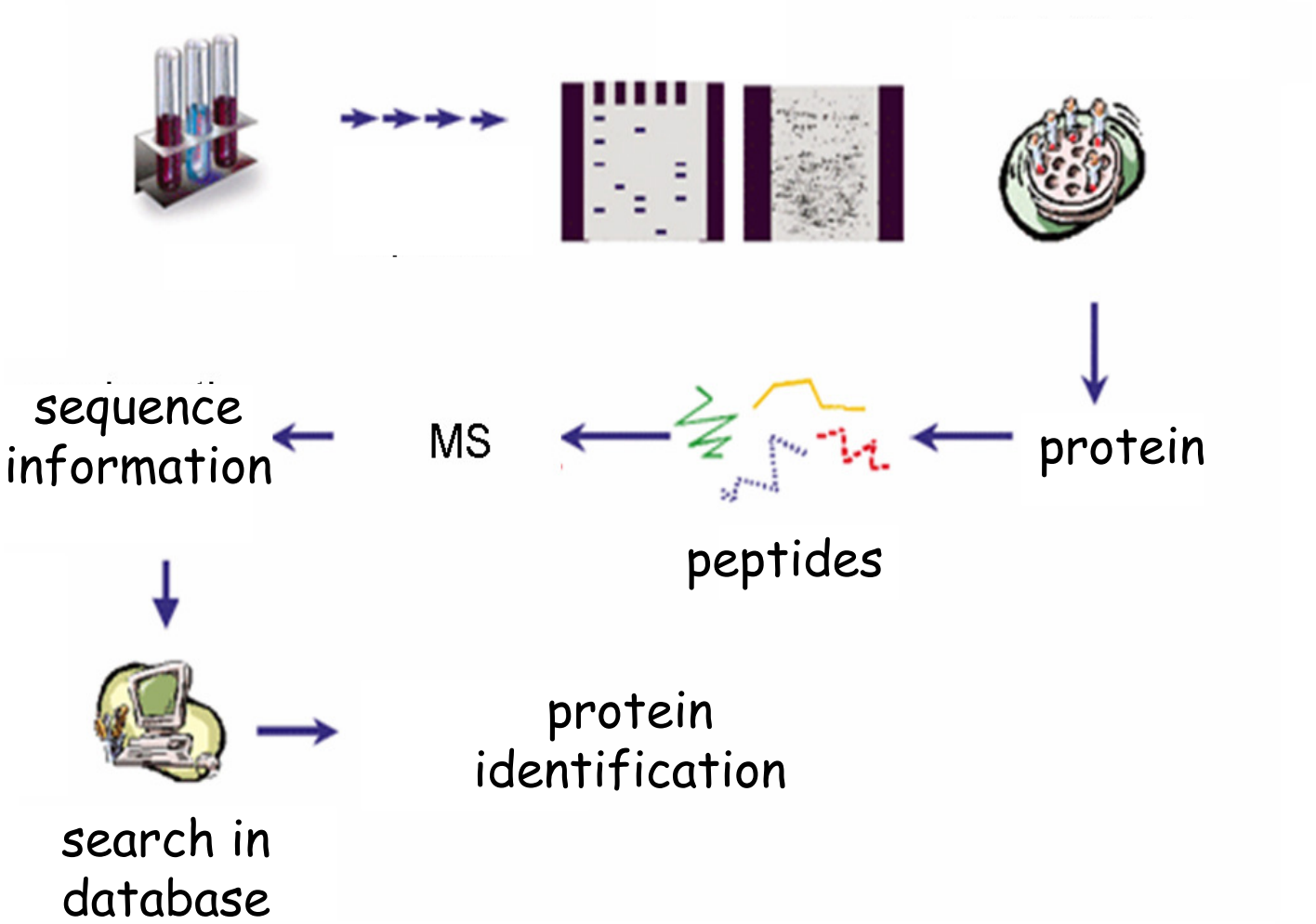


Synthesis of Dau-oligoarginine conjugates with oxime bond



Orbán E. et al. *Bioconjugate Chem.* **22**: 489 (2011)

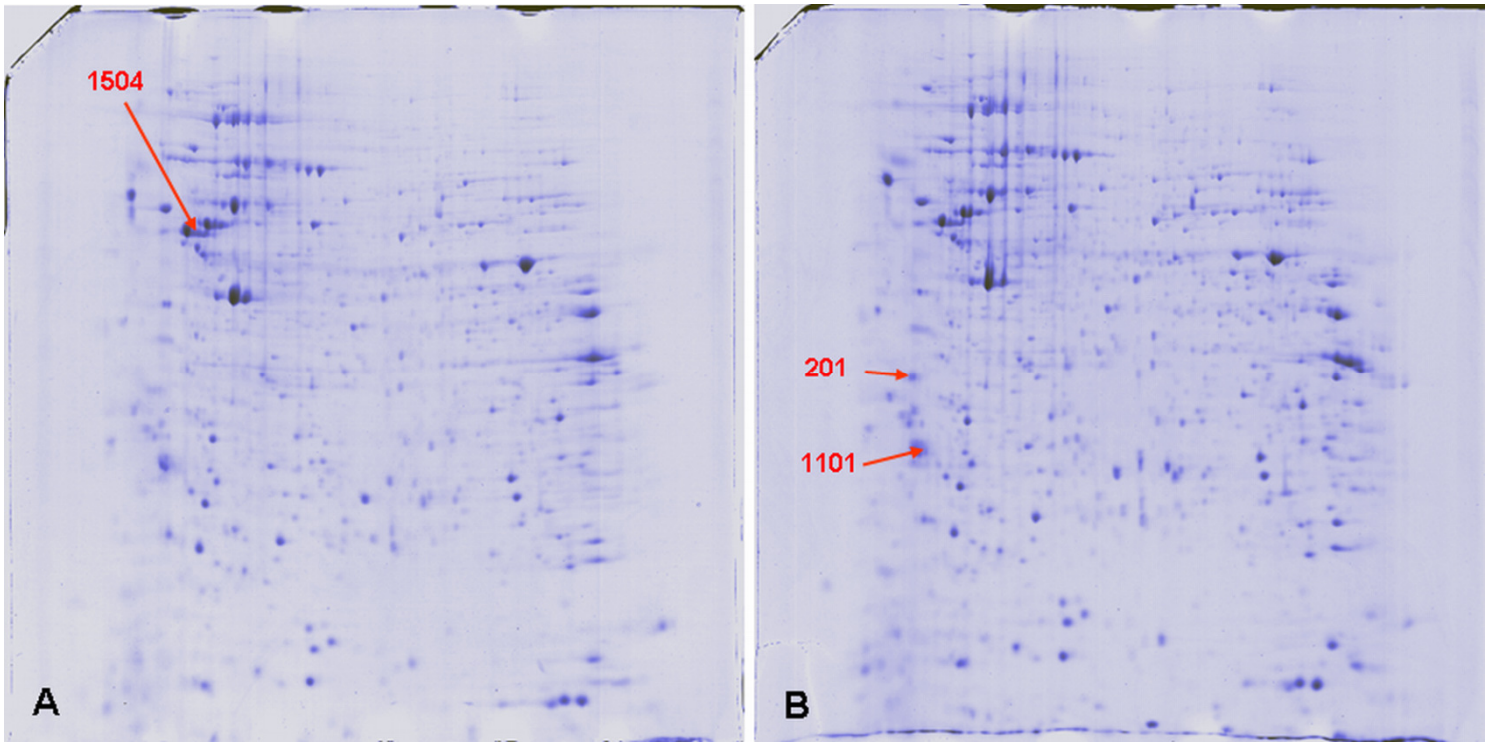
Analysis of protein expression profile



HL-60 cells: protein expression profile after treatment with daunomycin (A) or Dau=Aoa-LTVSPWY-NH_2 conjugate (B)

A: daunomycin, c = 0.024 μM

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



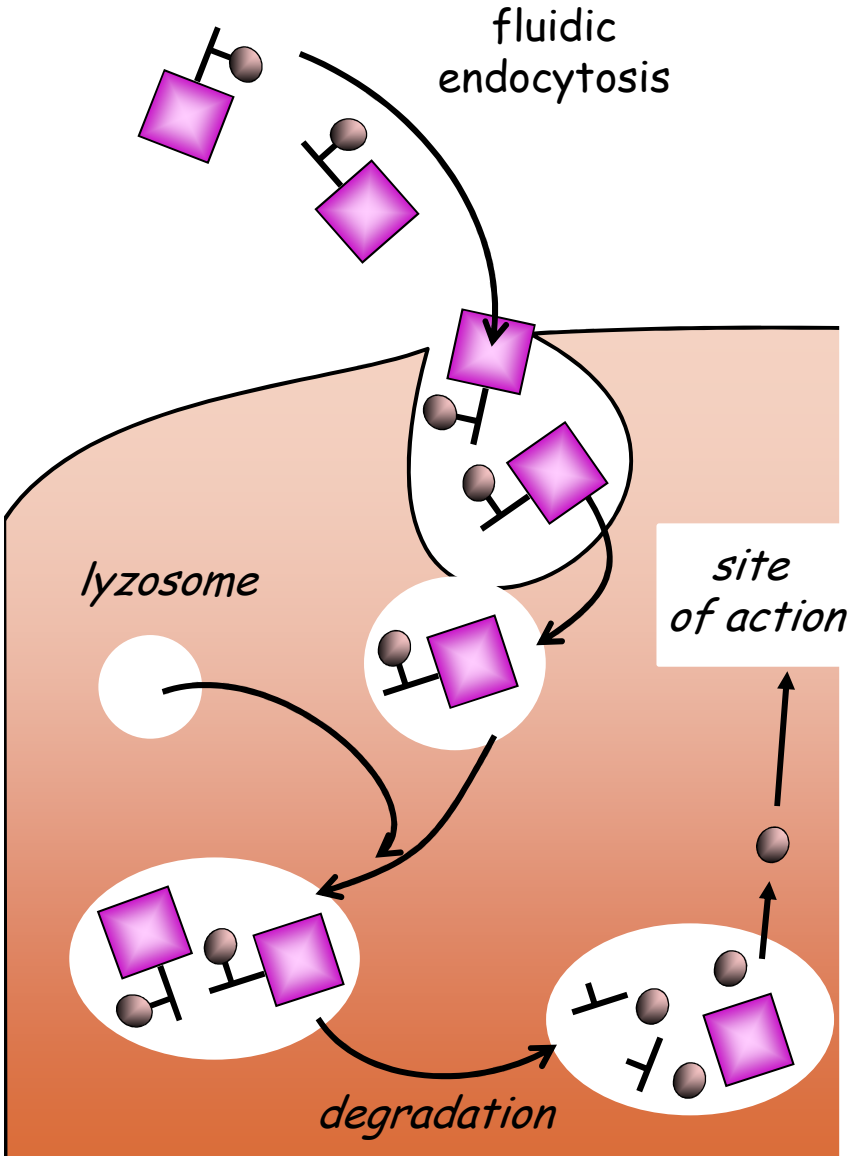
- tubulin beta chain (lower)
- proliferating cell nuclear antigen (higher)
- protein kinase C inhibitor protein 1 (higher)

Treatment: 24 hrs

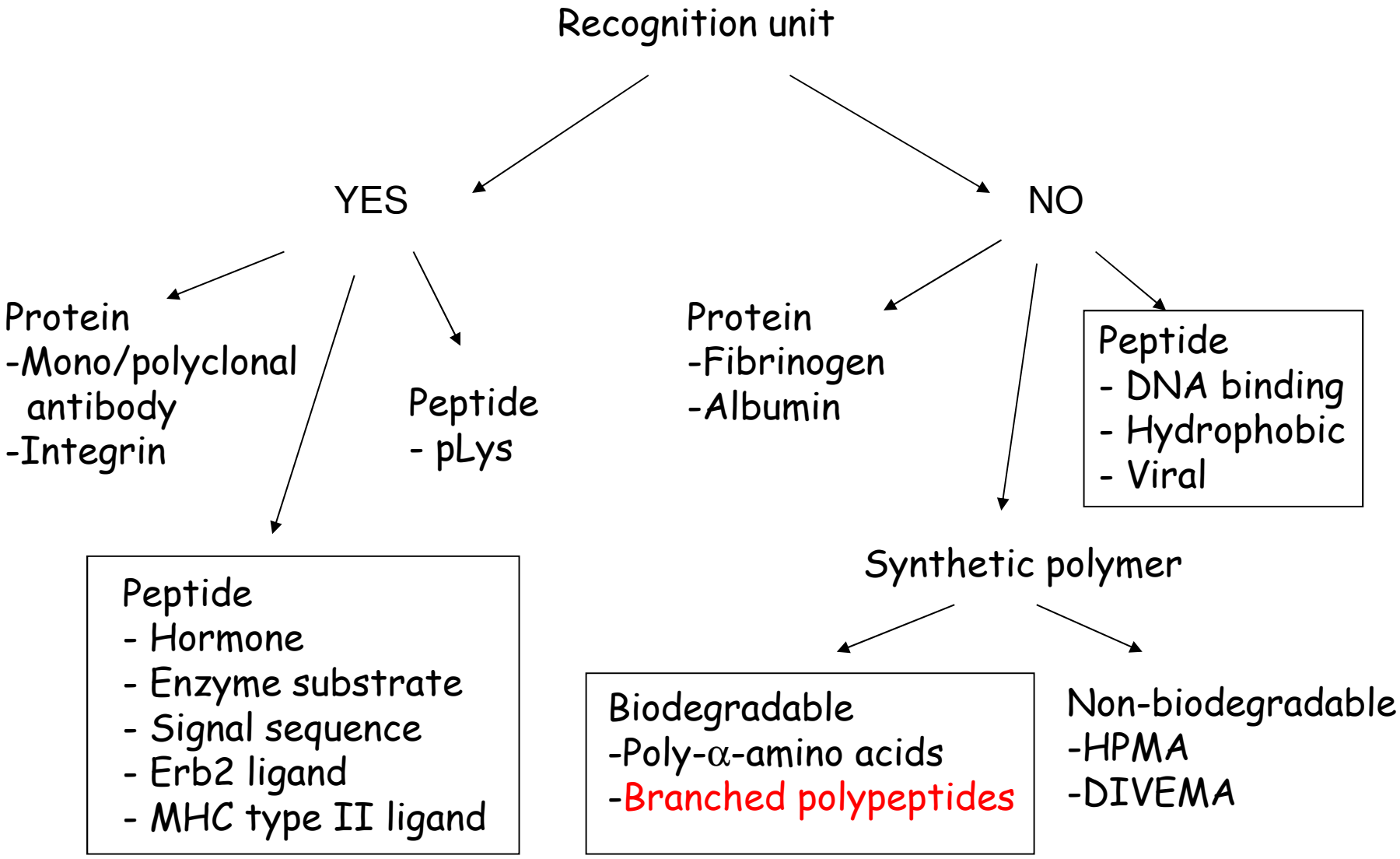
Conclusion

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

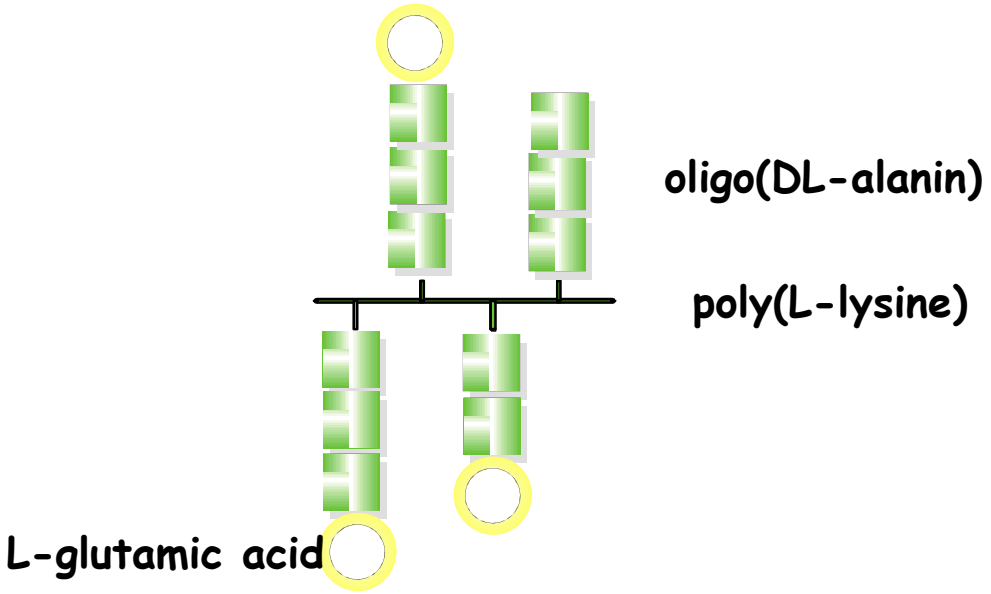
Uptake and liberation of bioactive entities



Peptide/protein based drug targeting/delivery



Branched chain polypeptides



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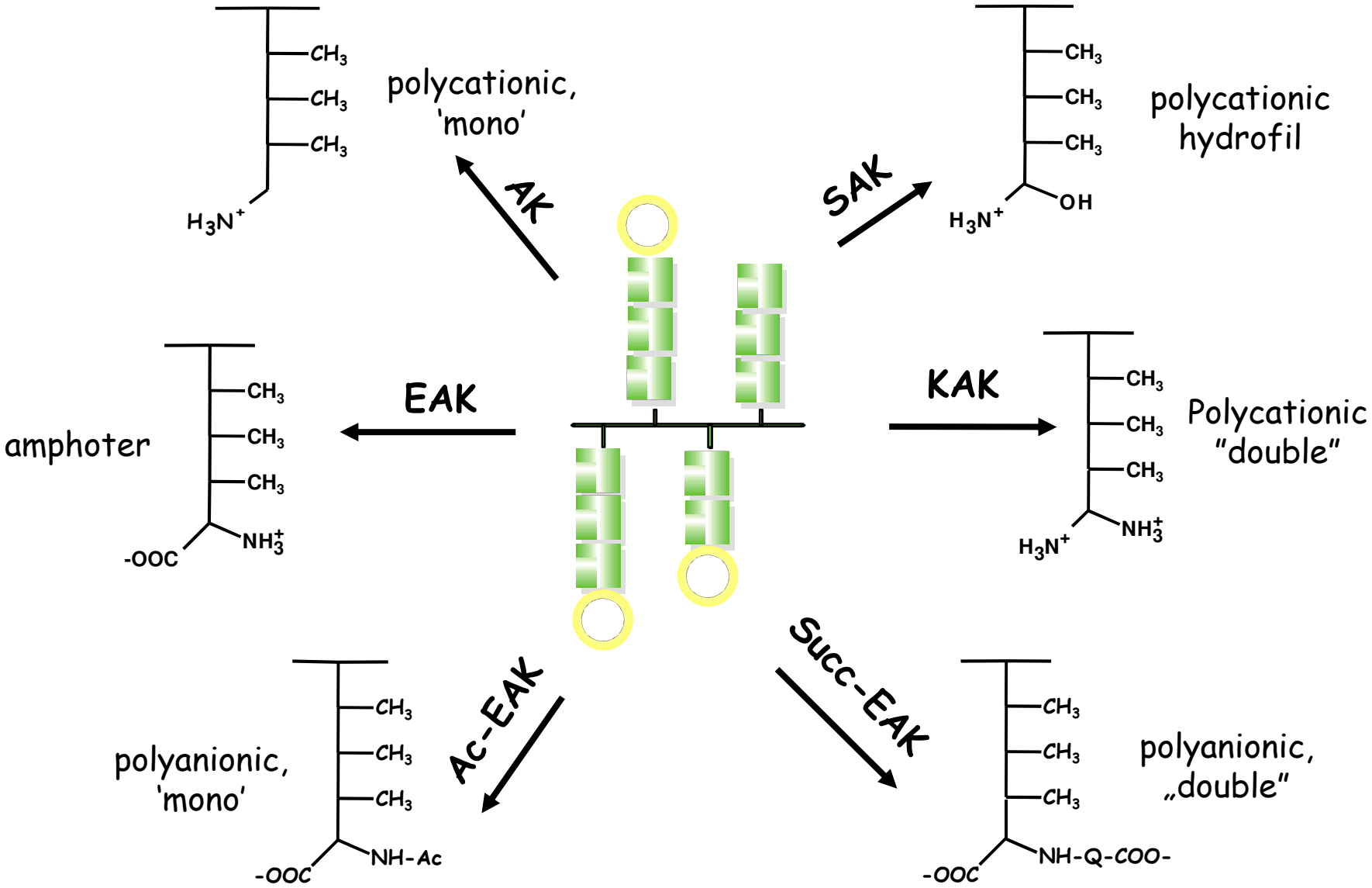
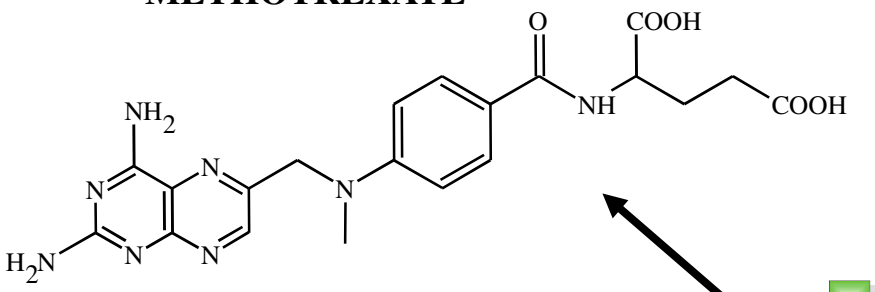
Branched chain polypeptides



Drug-polypeptide conjugates

METHOTREXATE



Hudecz F. et al. *Bioconjugate Chem.* **4:** 25 (1993)
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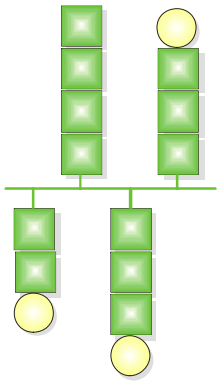
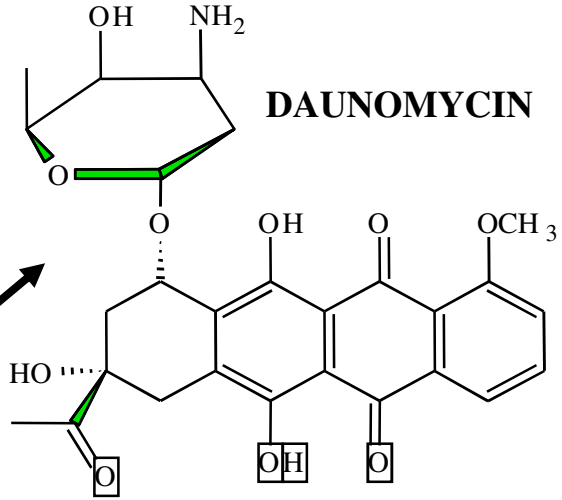
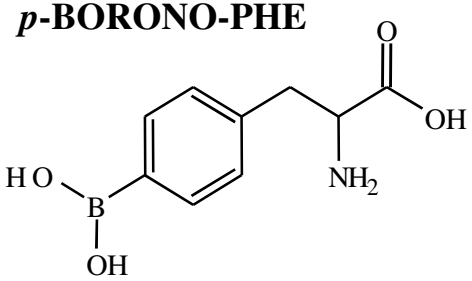


DAUNOMYCIN



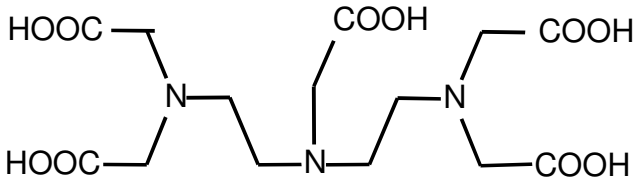
Hudecz F. et al.*Bioconjugate Chem.* **3:** 49 (1992)
Gaál D., Hudecz F. *Eur.J.Cancer.* **34:** 155 (1998)

***p*-BORONO-PHE**



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

DIETHYLENE-TRIAMINE-PENTAACETIC ACID



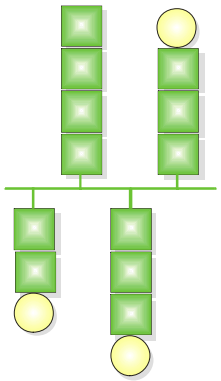
Pimm MV. et al.*Int. J. Pharmaceutics* **79:** 77 (1992)
Pimm MV.et al. *J. Canc. Res. Clin. Onc.* **122:** 45 (1996)

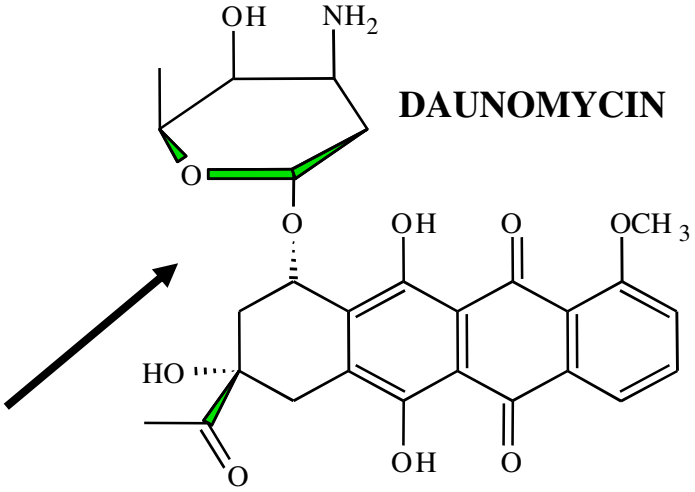
GN-RH ANTAGONIST, MI-1544

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

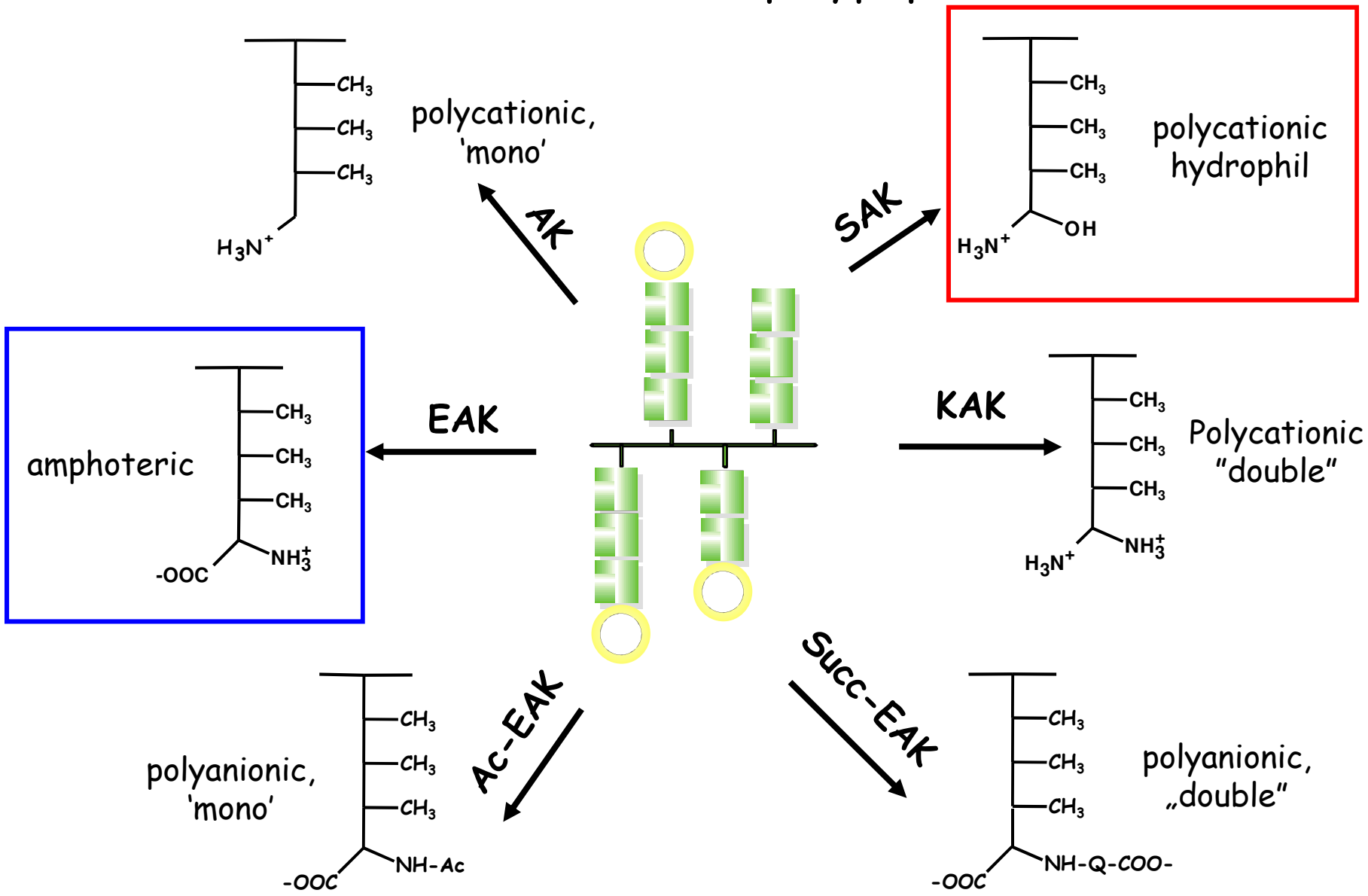




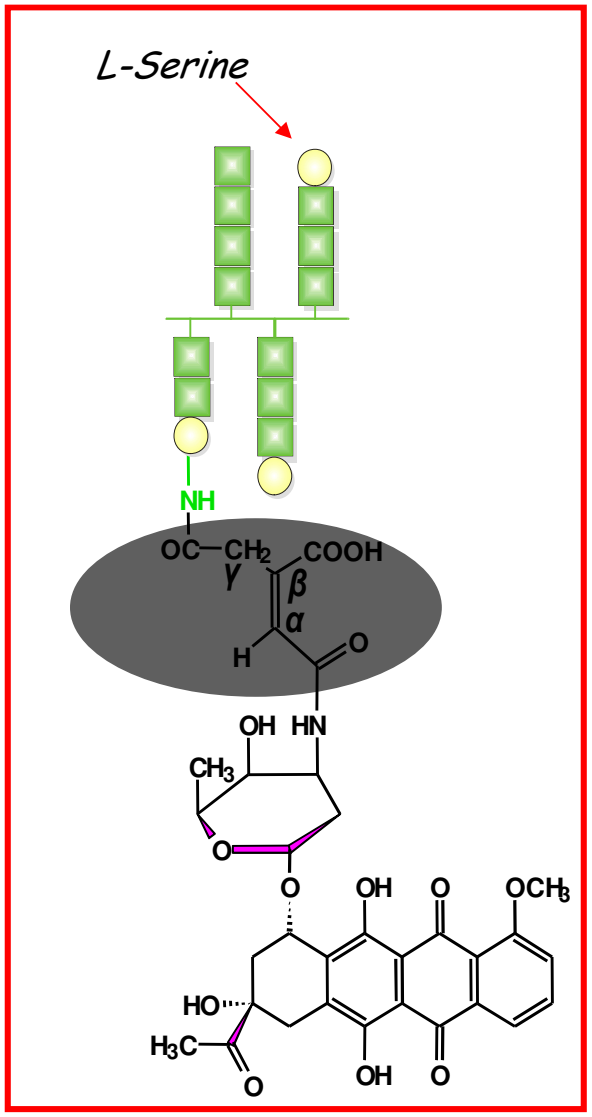
Hudecz F. et al.*Bioconjugate Chem.* **3**.49 (1992)
Gaál D., Hudecz F. *Eur.J.Cancer.* **34**.155 (1998)

Antitumour effect

Branched chain polypeptides



Daunomycin-polypeptide conjugates



Hudecz, F. et. al.
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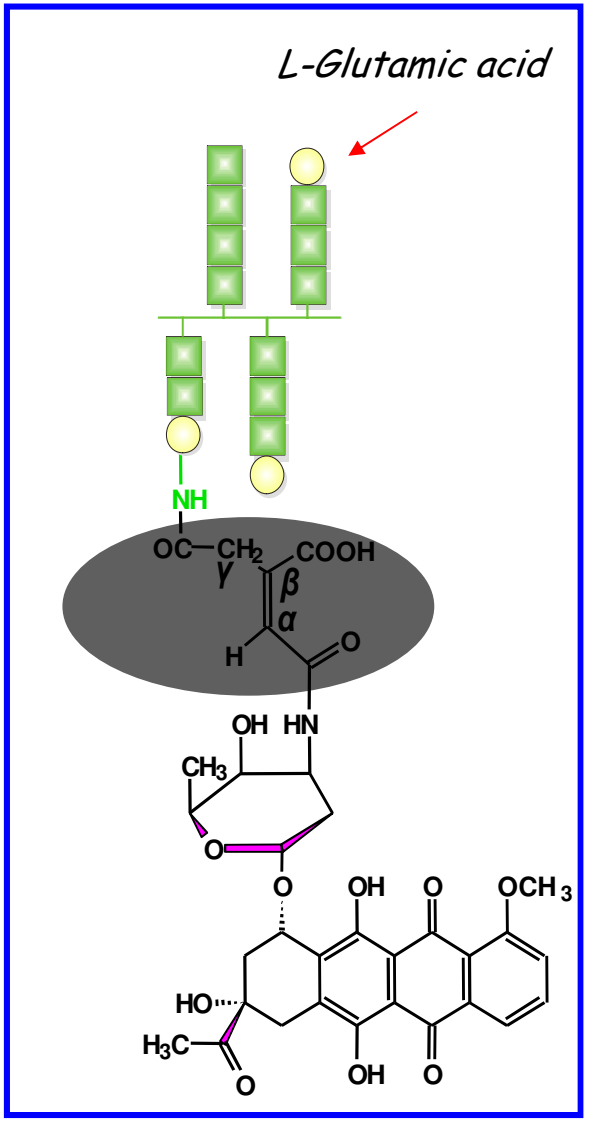


Hudecz, F. et. al.
Bioconjugate Chem. 3: 49 (1992)

polypeptide

acid labile spacer

daunomycin

In vivo 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)

In vivo 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. and Hudecz,F. Eur.J.Cancer. 3: 49 (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)

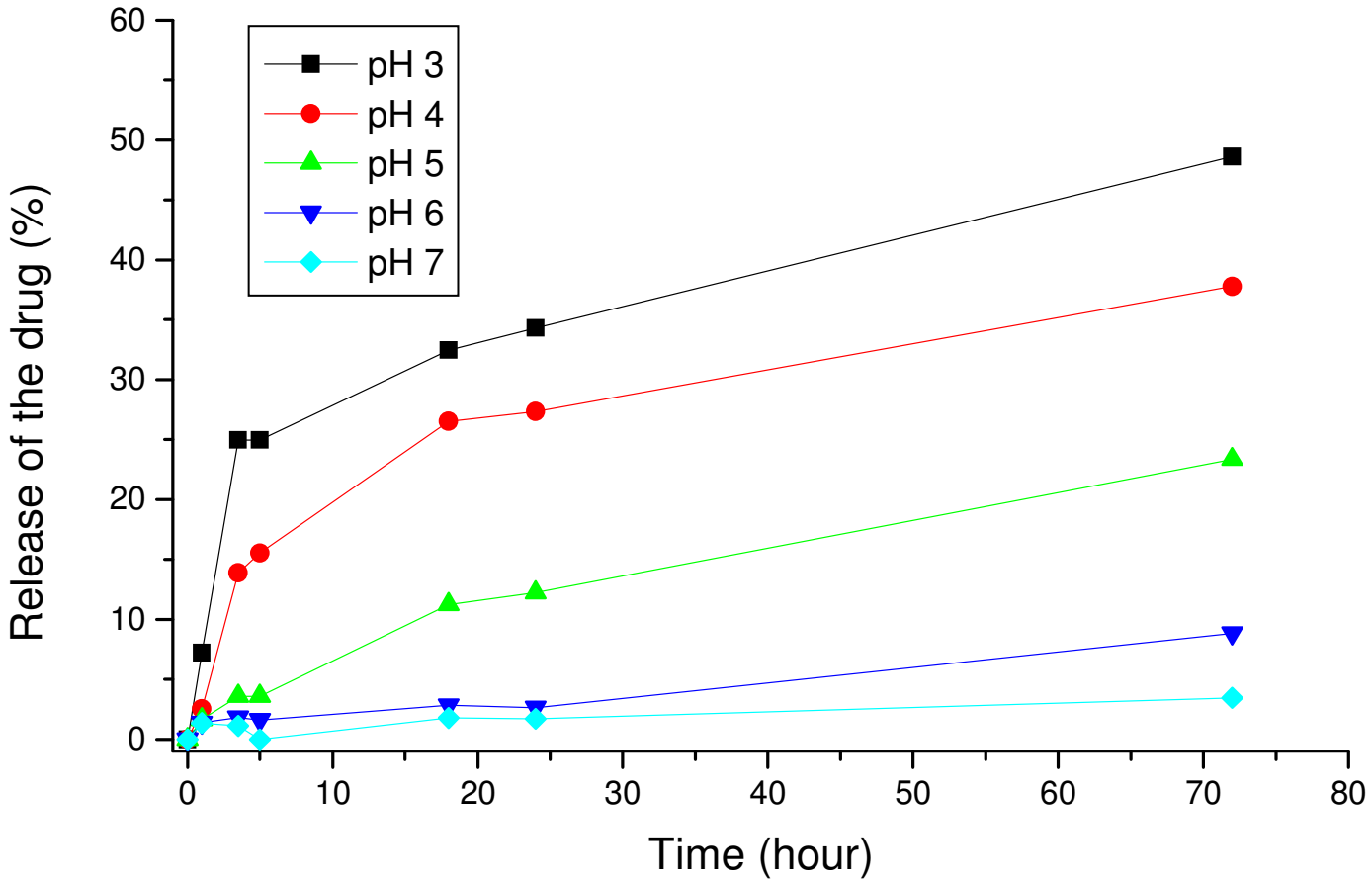
Antitumour effect of cAD-EAK conjugate on L1210 leukemia *in vivo*

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	- -

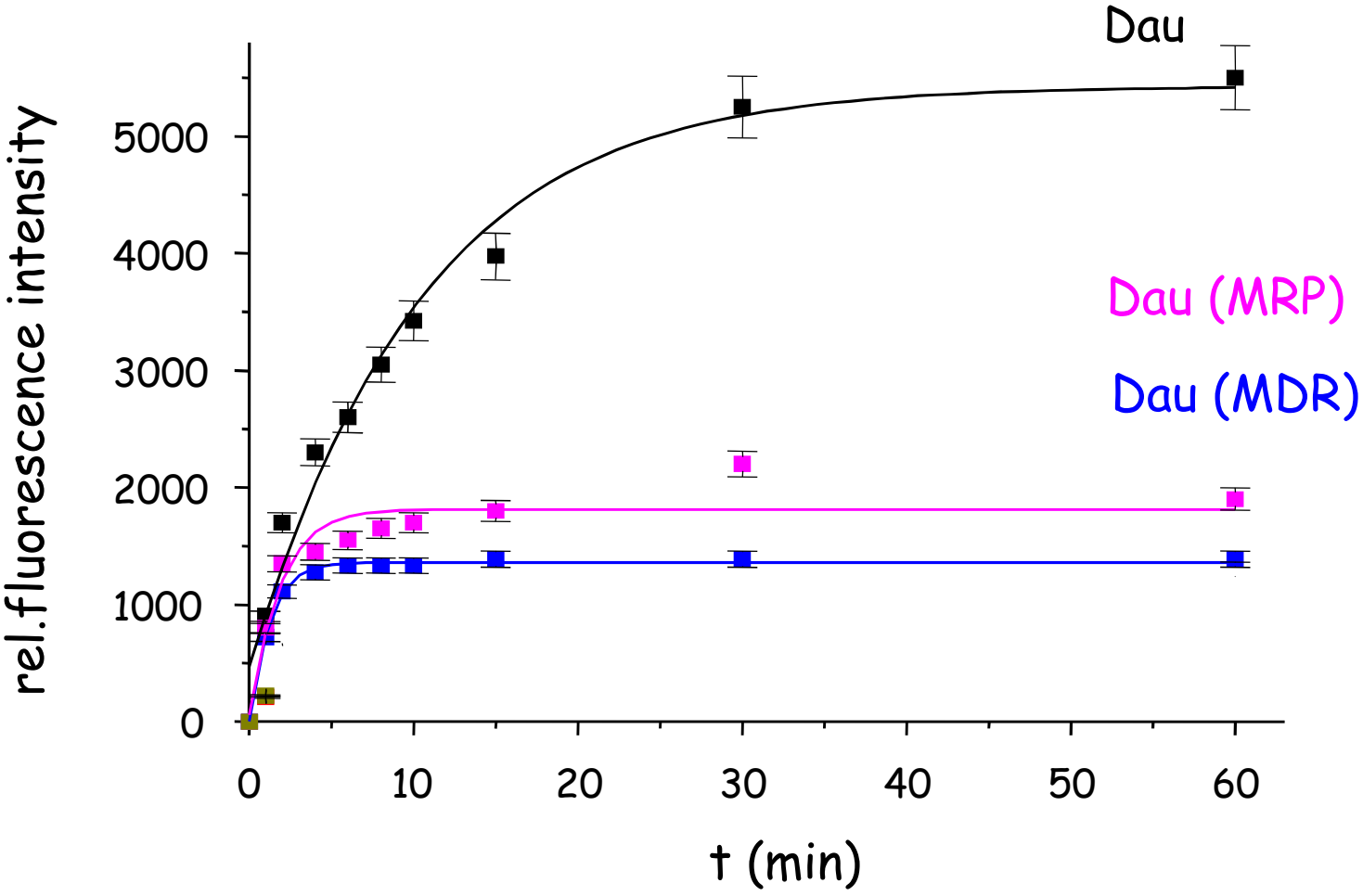
* Treatment one day after the i.p. inoculation of $5x10^6$ L1210 cells i.p. 60-day experiment

Gaál,D. and Hudecz,F. Eur.J.Cancer. 3: 49 (1998)

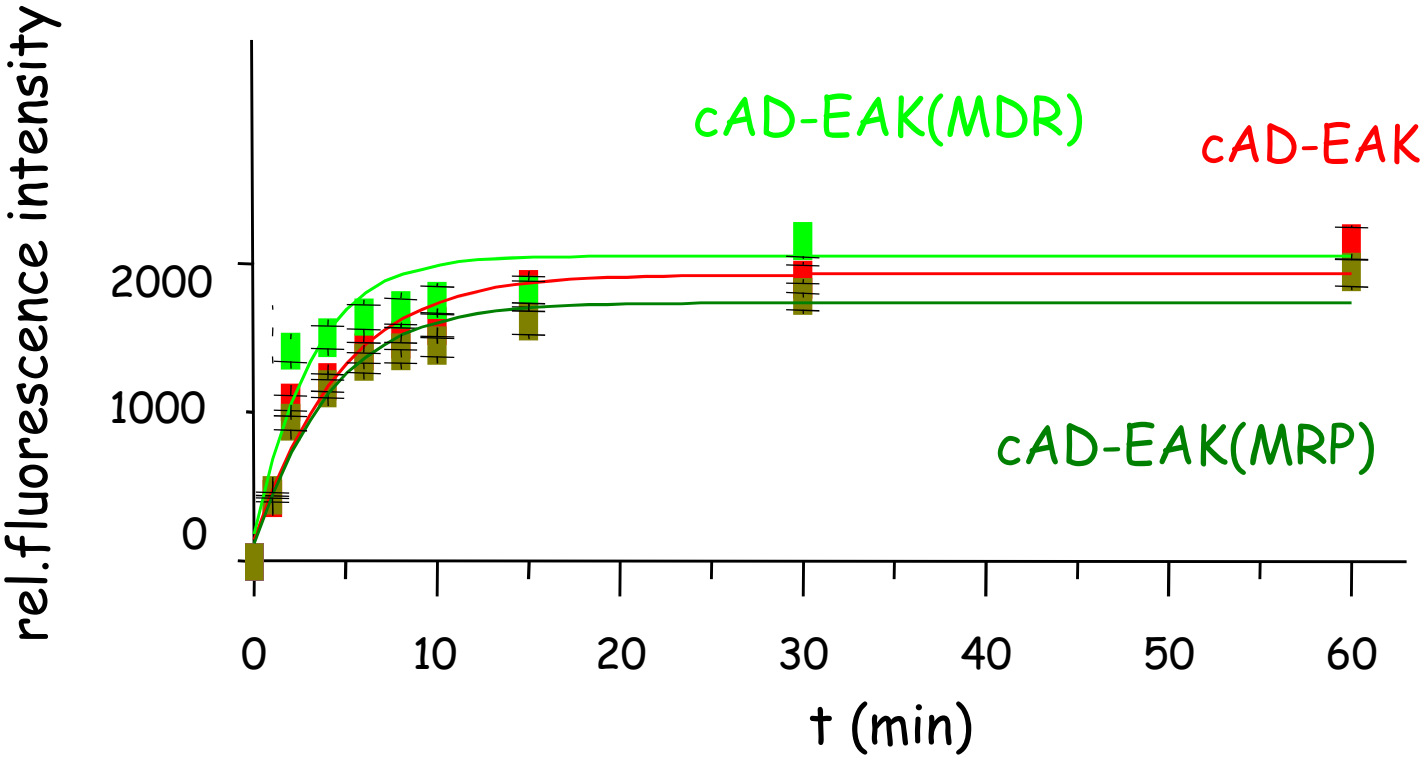
Release of daunomycin from cAD-EAK conjugates



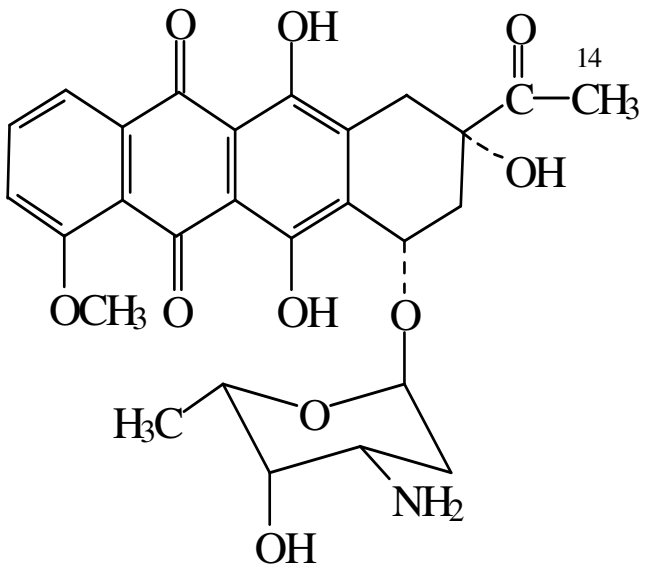
Uptake of daunomycin and cAD-EAK conjugate by sensitive and resistant (MDR1 and MRP) HL60 cells



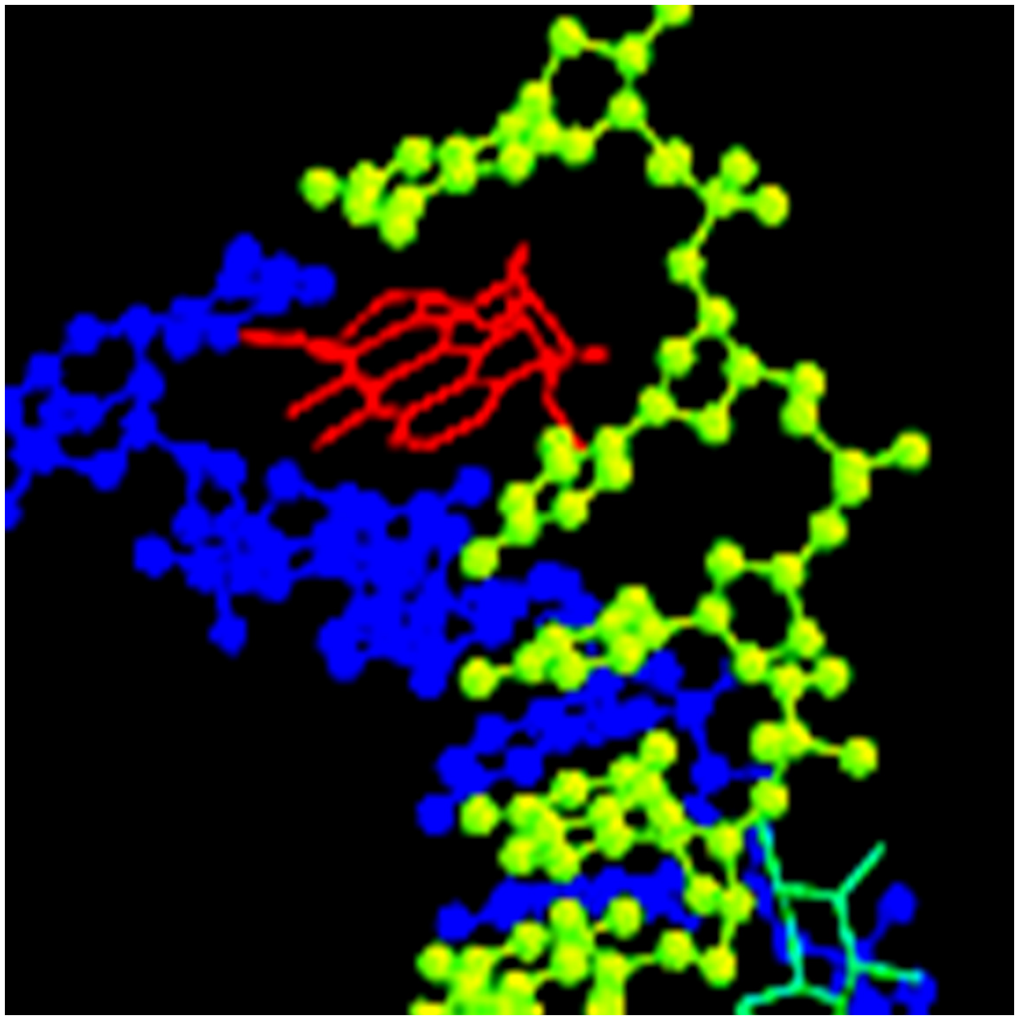
Uptake of daunomycin and cAD-EAK conjugate by sensitive and resistant (MDR1 and MRP) HL60 cells



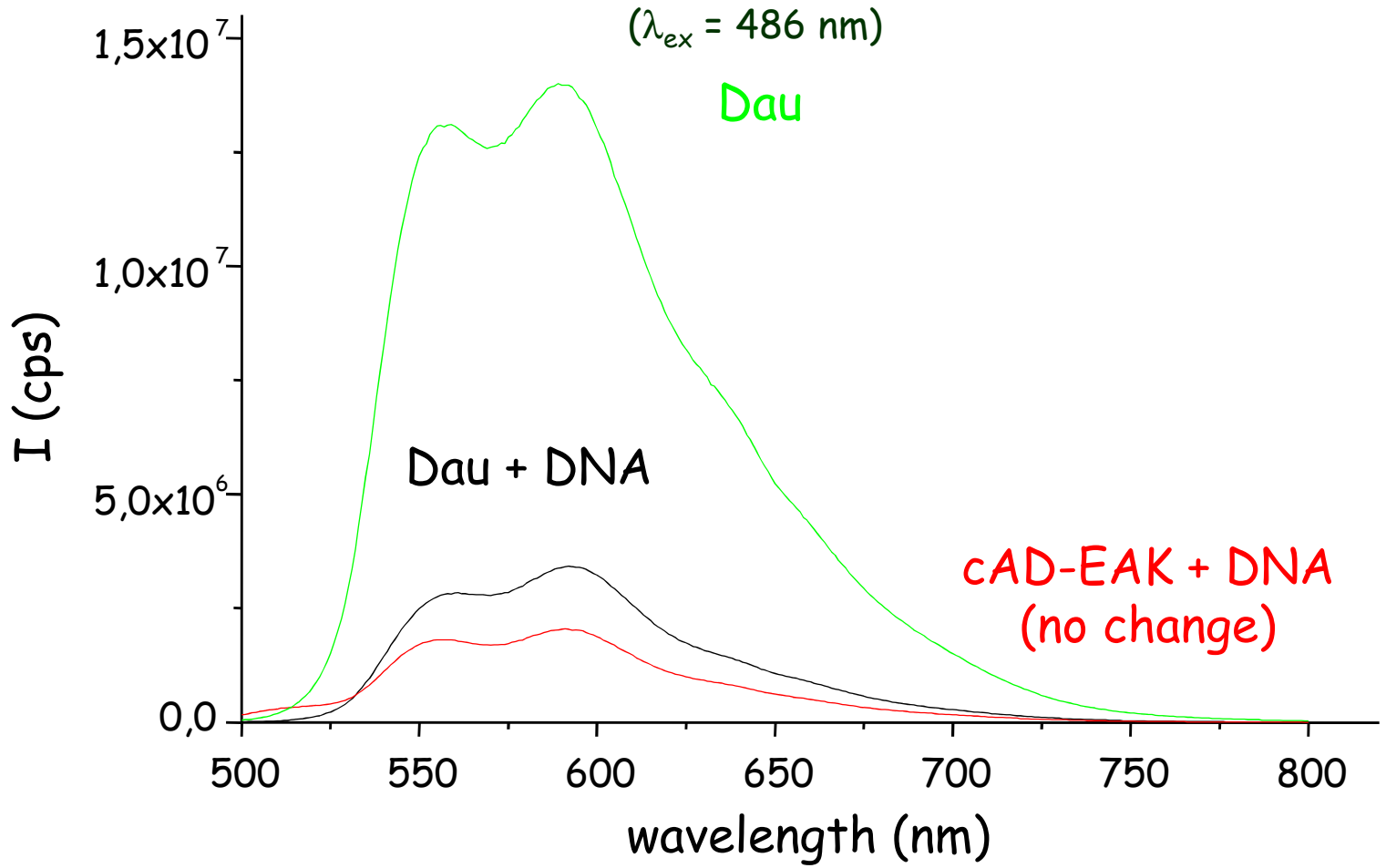
Daunosamine directed intercalation into minor groove



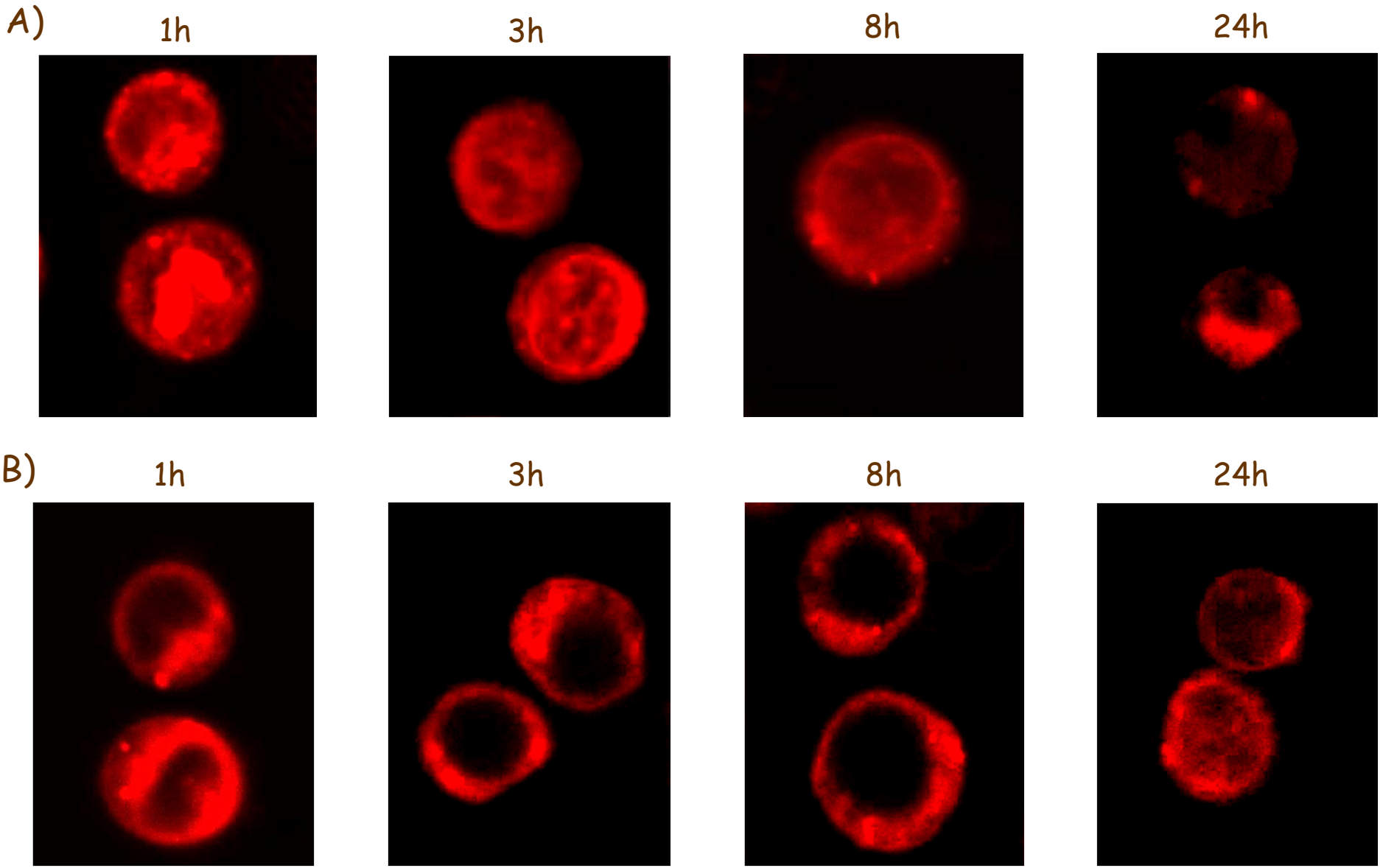
[Frederick, 1990]



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



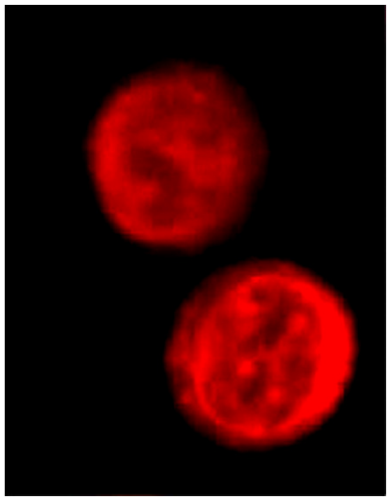
Time dependent localization of daunomycin (2 μM) (A) and cAD-EAK conjugate (daunomycin: 2 μM) (B) in HL-60/sensitive cells (f=0.13)



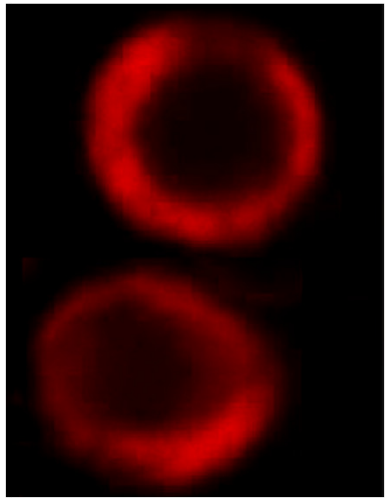
Localization of daunomycin (2 μM) (A) and cAD-EAK conjugate (daunomycin: 2 μM) (B) in sensitive and resistant cells (3h)

A)

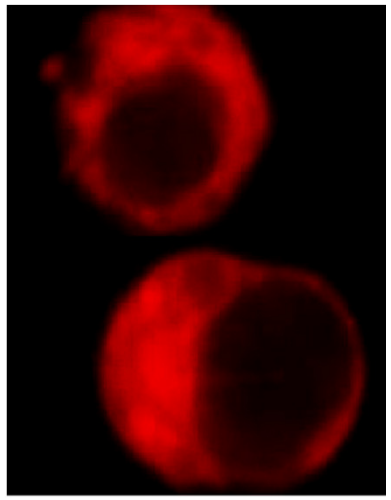
HL-60/sensitive (f=0.13)



HL-60/MDR1 (f=0.90)

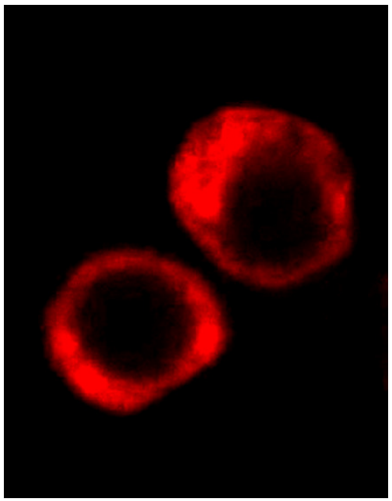


HL-60/MRP1 (f=0.61)

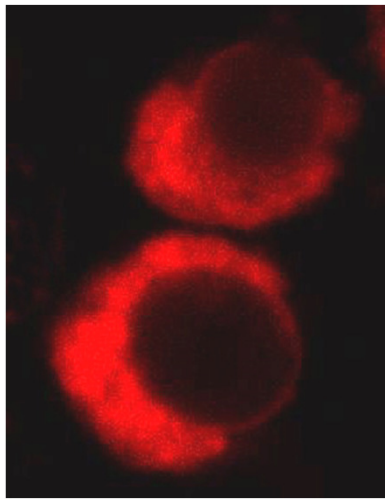


B)

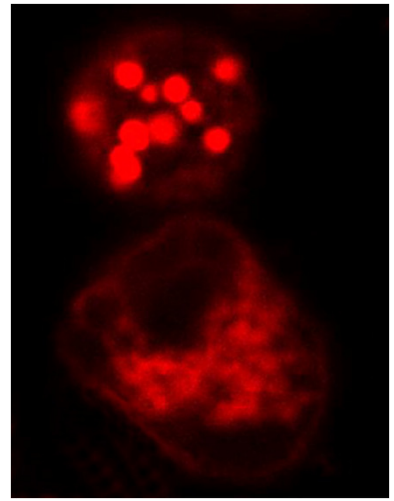
HL-60/sensitive (f=0.13)



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).

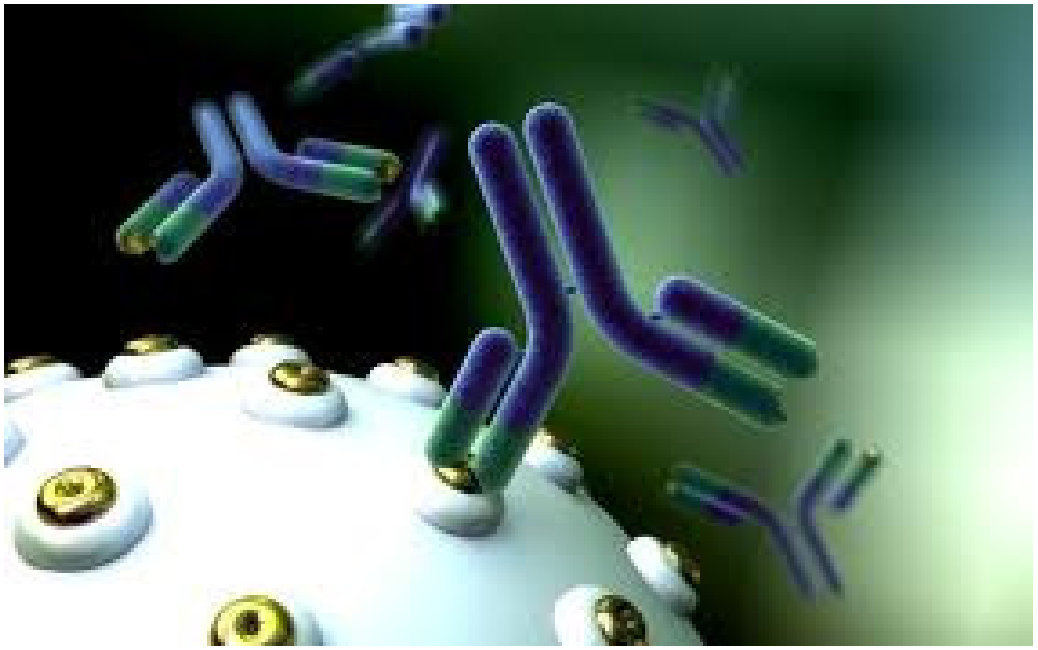
3. Daunomycin-peptide conjugate is effective against sensitive and MDR resistant L1210/HL60 tumour cells.

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.

6. Daunomycin-peptide conjugate is present in the cytosol of sensitive/resistant tumour cells.

Acknowledgements



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