Benefits of PEGylation: certolizumab pegol

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nanotechnology

Biomedicines and nanomedicines represent a turning point in drug therapy

A recent Report from the Pharmaceutical Research and Manufacturers of America (PhRMA) found 418 protein drugs developed through biotechnology) now being tested to treat more than 100 diseases. All of them are now in clinical trails or awaiting approval from the US Food and Drug Administration (FDA).

Among these 418 biotech drugs, there are

- 210 to treat cancer
- 50 to treat infectious disease
- 44 to treat autoimmune disorders
- 22 to treat HIV infection
- 22 to treat cardiovascular diseases

Market Forecasts for Leading Protein Drugs ($ millions)

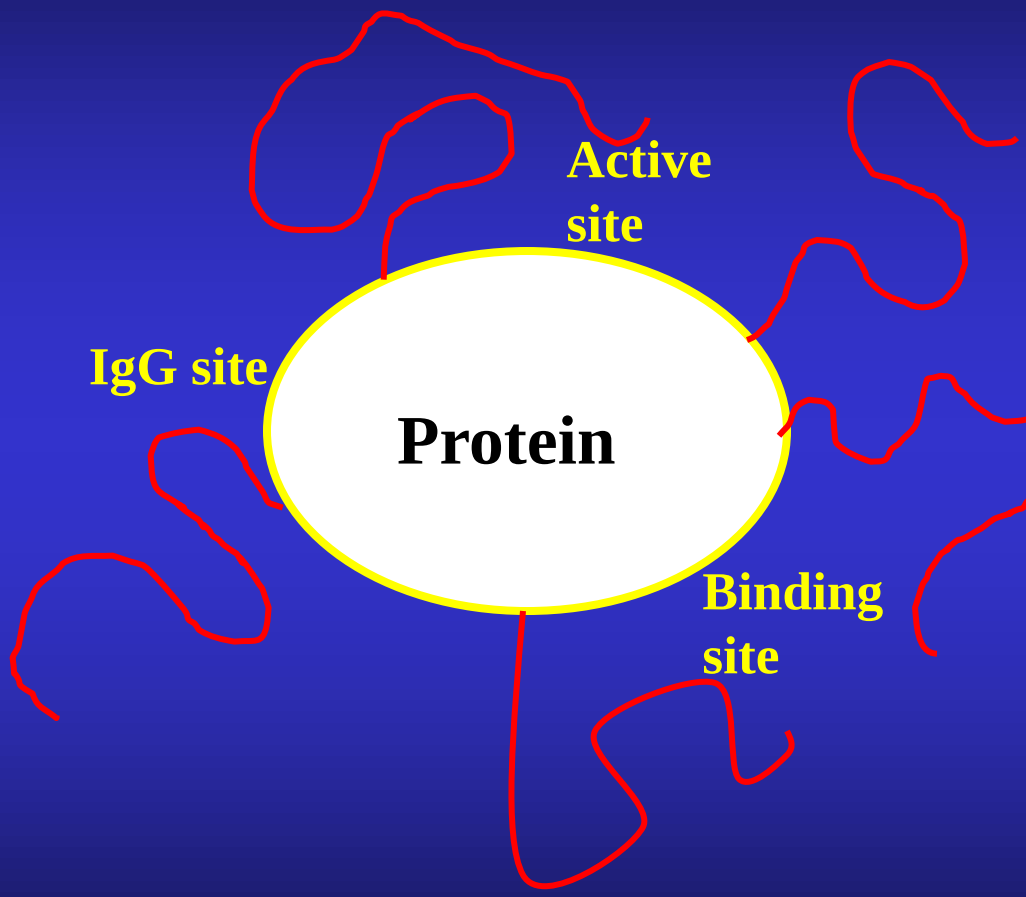
Product class	2003	2004	2005	2006	2007	2008	2009
Erythropoietins	7,763	8,136	8,473	9,268	9,777	10,893	11,779
Monoclonal antibodies	6,721	11,079	13,986	17,734	20,599	36,399	45,352
Insulin and insulin analogues	5,487	6,551	7,940	9,236	10,772	13,828	17,363
Interferons	3,935	3,692	3,825	4,050	4,392	4,983	5,828
Therapeutic hormones (including growth hormones)	3,317	3,895	4,186	4,280	4,456	4,990	5,799
Blood factors	2,354	2,598	2,595	2,679	2,998	3,215	3,432
Enzyme replacement therapies	1,057	1,203	1,374	1,533	1,713	2,111	2,738
Interleukins	219	224	229	237	250	260	273
Other therapeutic hormones	121	111	72	212	520	1,595	2,514
Total	**30,974**	**37,489**	**42,680**	**49,229**	**55,477**	**78,274**	**95,078**

Main limits to an extensive use of *peptides* and *proteins* in therapy

- Proteolytic degradation
- Rapid renal excretion
- Immunogenicity
- Antigenicity
- Aggregation
- Solubility
- Difficult formulation

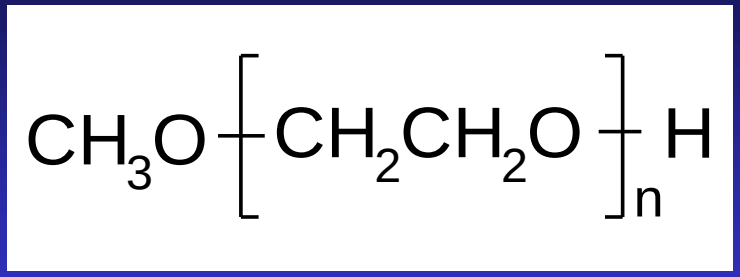
A SOLUTION: to increase the size and to change the protein surface by the covalent linking of hydrophylic polymers

Polymers may **protect proteins**, but what polymers?



The end-functionalized olygomer
m-PEG (**Davis, Abuchovski**)
overcomes the problems of the protein cross-linking

Poly(ethylene glycol), PEG



PEG, in its methoxy form, is the ideal polymer . It posses only one potentially reactive group. It is FDA approved for internal use.

Furthermore:

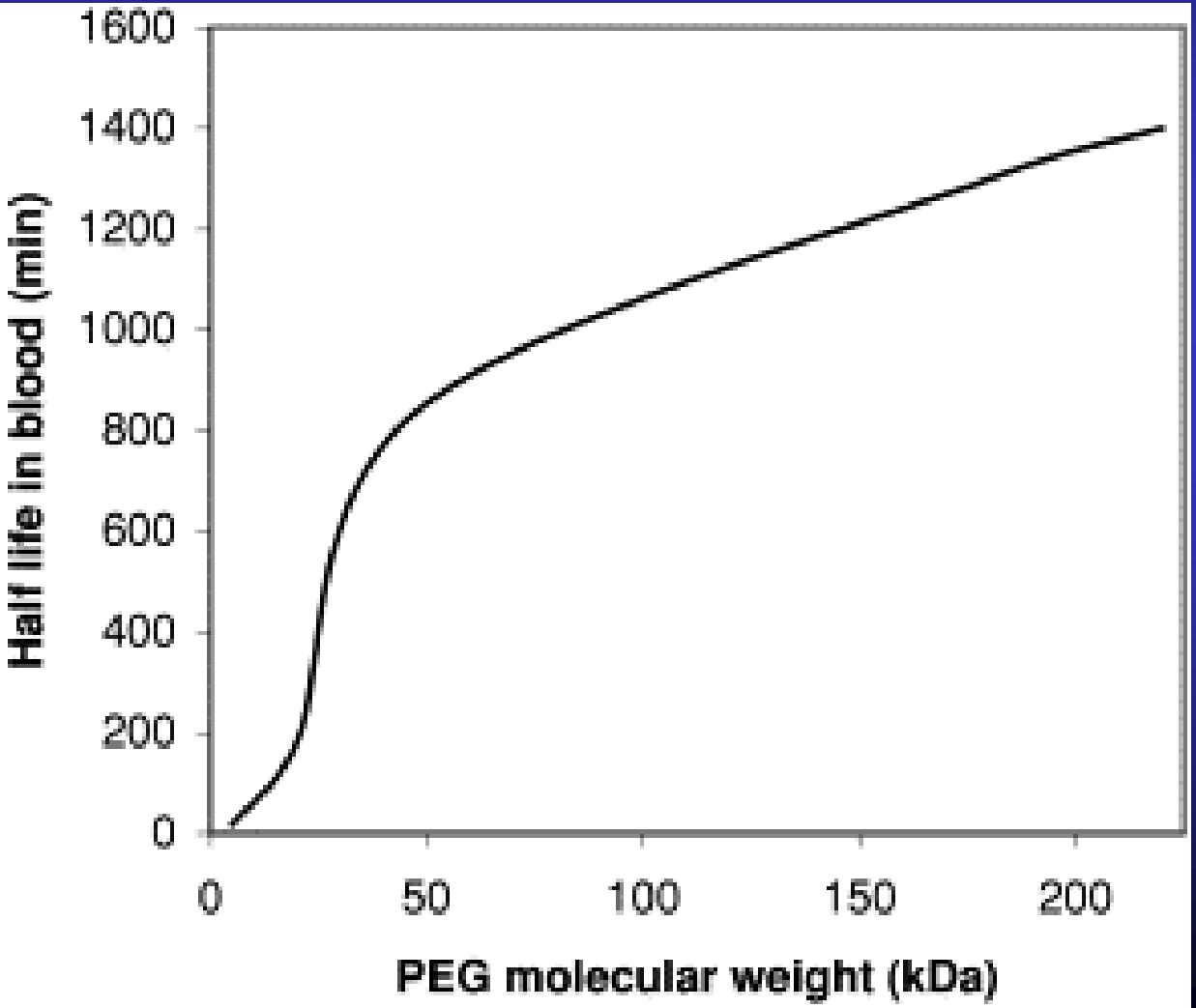
- It presents water and organic solvent solubility
- Has great flexibility
- The strong binding of water molecules to polymer oxygen units increases the PEG size, yielding decreased kidney filtration and repulsion of other proteins
- Allows for a clean chemistry
- It is non toxic and non immunogenic
- It is present in drugs as excipient and in body cure products and foods

PEG-conjugates are nanomedicines

According to the ESF, ‘ ….nanomedicines are systems working from the molecular levels using engineering devices and nanostructures (from 1 to 100nm size) to chieve a medical benefit…’

And as such PEG-conjugates must undergo specific Regulatory Guidelines regarding: characterization toxicity, reproducibility, etc, for marketing approval

Correlation plot between PEG molecular weight and half life in blood after intravenous administration. The data were obtained from the papers of Yamoka et al. and Nakaoka et al.



Pathways for PEG or PEG-conjugates escape from body

- Renal excretion = the rate is related to Mol Wt (remember the "raptation" mechanism)
- Bile excretion = prevalent for high Mol Wt
- Chain cleavage by oxidative processes = mainly related to P-450 or aldehyde dehydrogenase action
- Possible cleavage of one chain of branched PEG = by anchimeric assisted hydrolysis
- Proteolytic degradation of protein moiety = mainly carried out in liver

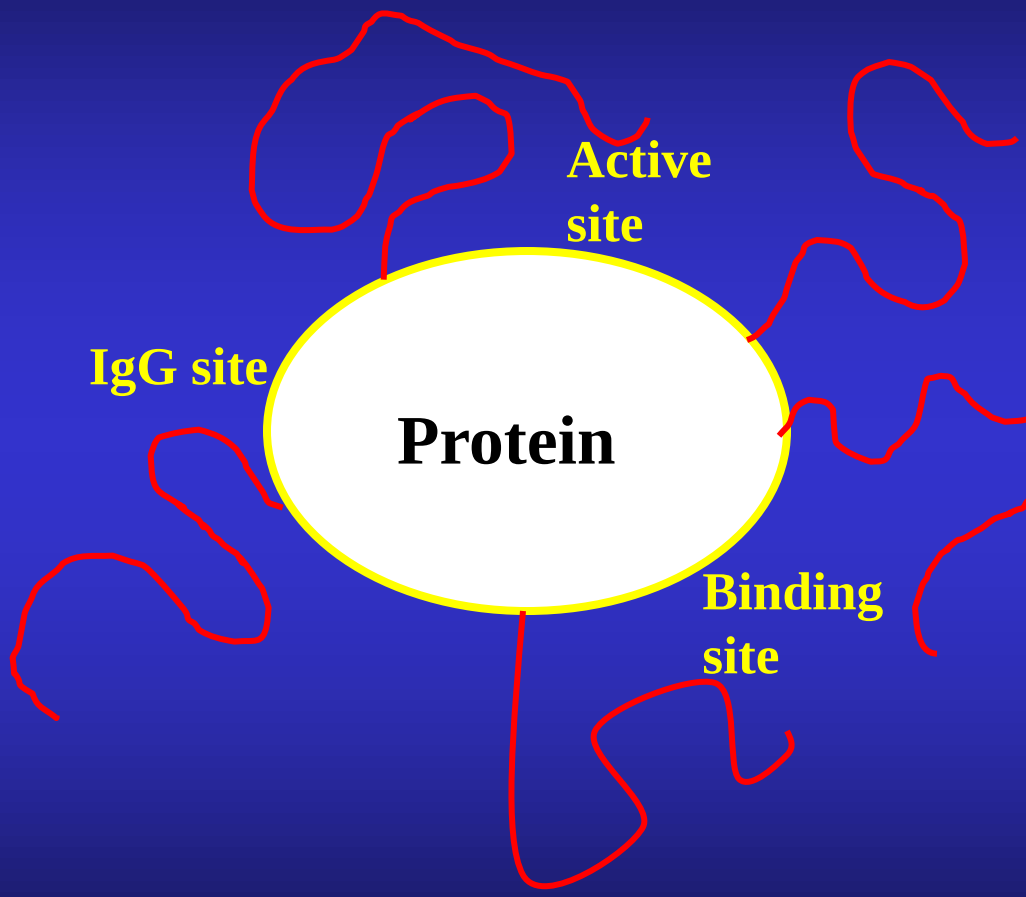
What about PEG and PEG-Protein Conjugates Safety?

•According to Bendele et al. (Toxicol. Sci. 1998), long term administration of conjugates at high doses in animals produces renal tubular vacuolation, but not associated with clinical alterations.

•According to Webster et al. (Drug. Metab. Disp. 2007), PEG itself at high doses or PEG-conjugates have a toxicological profile of very low concern and it is well tollerate at high doses following chronic and acute administration, it is excreted as such or following oxidation.

Several medicines administered i.v.(Busulfan, Etoposide,Lorazepan, Venoglobi-S, Aralast etc.) contain high doses of PEG as excipient.

Polymers may **protect proteins**, but what polymers?



The end-functionalized olygomer
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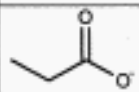
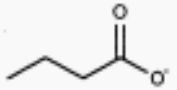
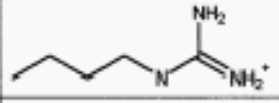
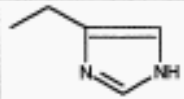
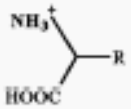
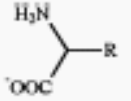
Which amino acids of a protein can be exploited for polymer binding?

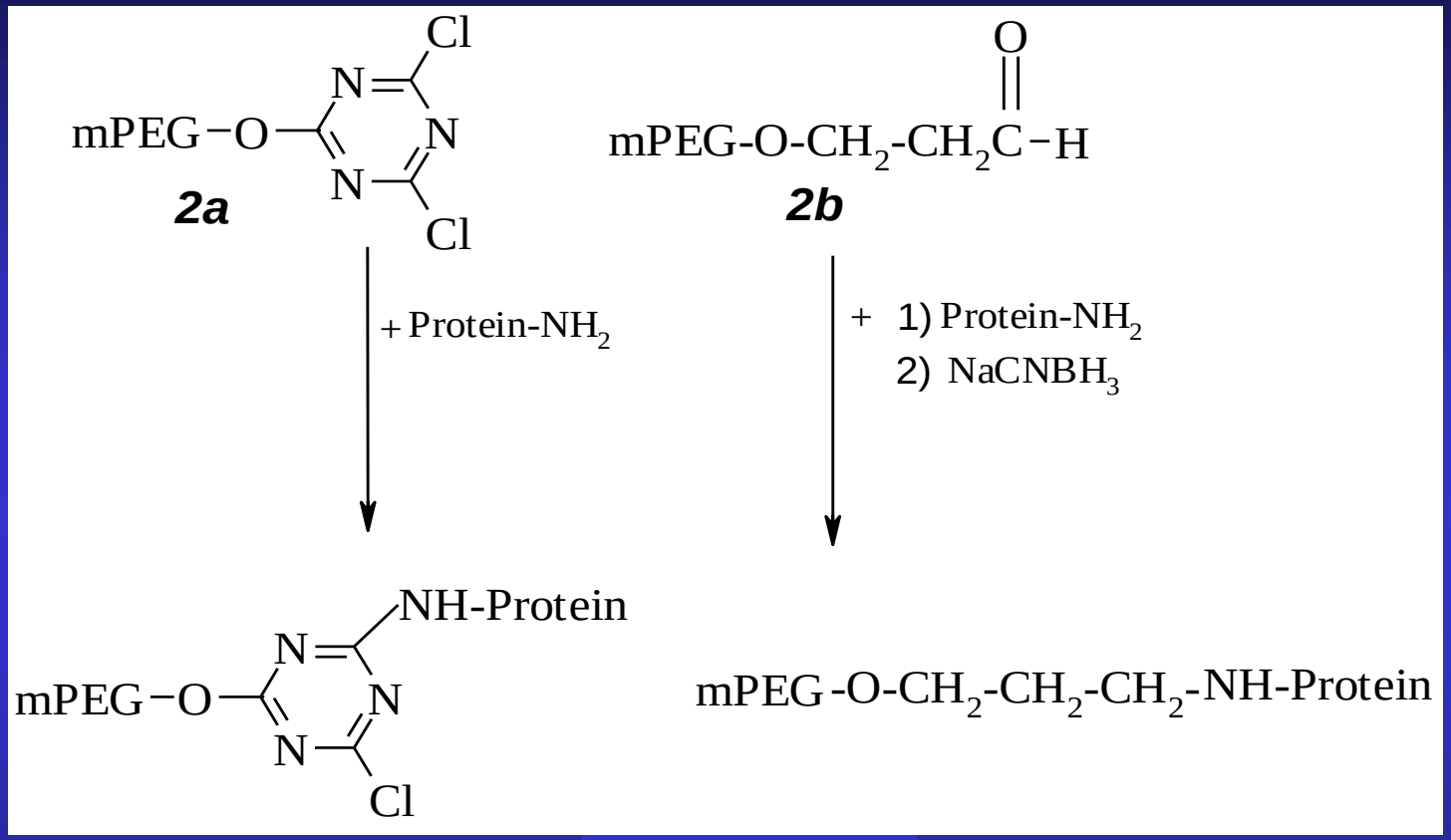
Amino acid	Structure	pKa
Lysine		9.3-9.5
Aspartic acid		3.7-4
Glutamic acid		4.2-4.5
Arginine		>12
Cysteine		8.8-9.1
Tyrosine		9.7-10.1
Serine Treonine		
Asparagine Glutamine		
Histidine		6.7-7.1
α-carbonyl; *C*-terminus		2.1-2.4
α-amine; *N*-terminus		7.6-8

The conjugate is composed of:

PEG-------Linker-------Protein

and each must be properly characterized_

Example of PEG chemical linkage to the protein –NH2



But herogeneous protein conjugates are obtained

Common limitations : multiple isomers

α-interferon, random amino PEGylation

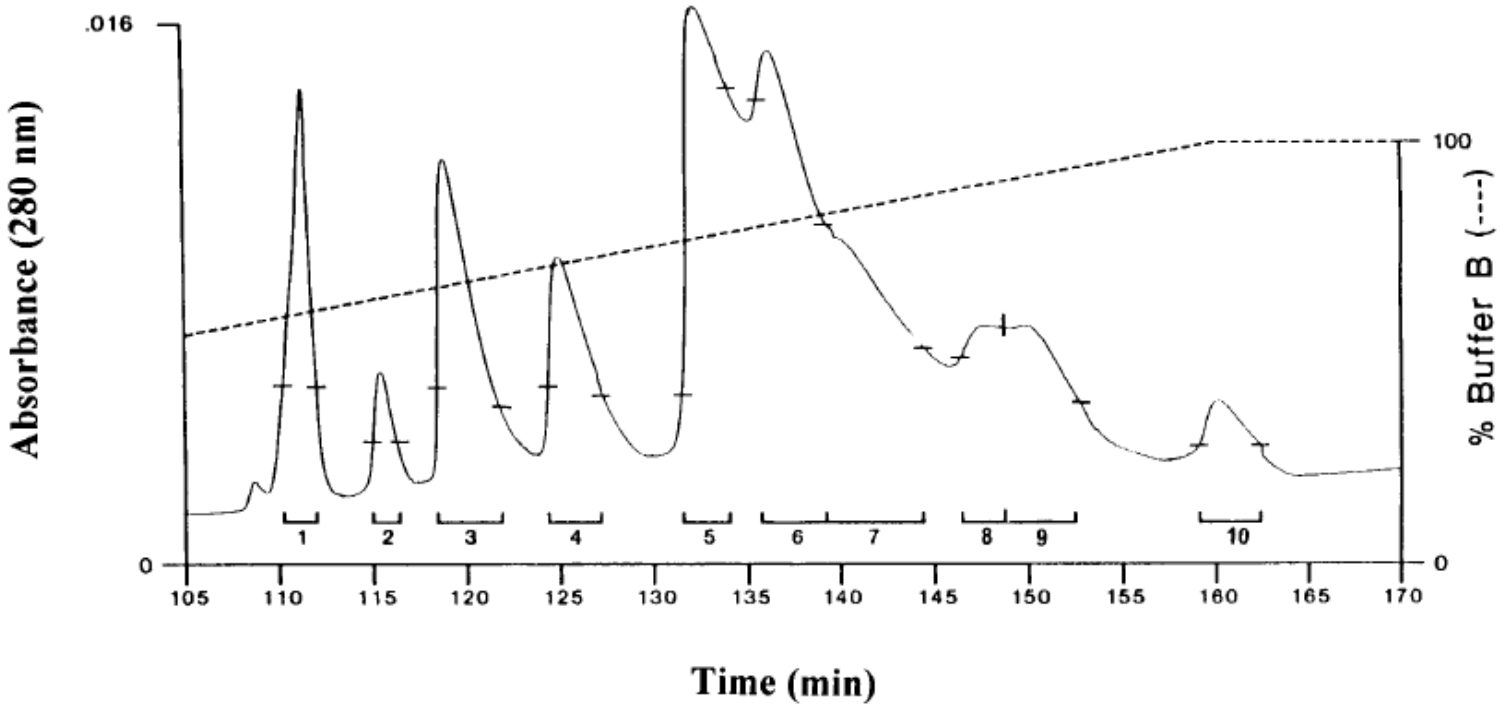


FIG. 1. Preparative SP-HPLC of 9 mg of PEG-IFN. The peak area between the two adjacent bars represents the pool collected for analysis.

:polydispersity of PEG

How can we get a site specific PEGylation?

>By changing the reaction conditions (pH, temperature, solvent,etc.)

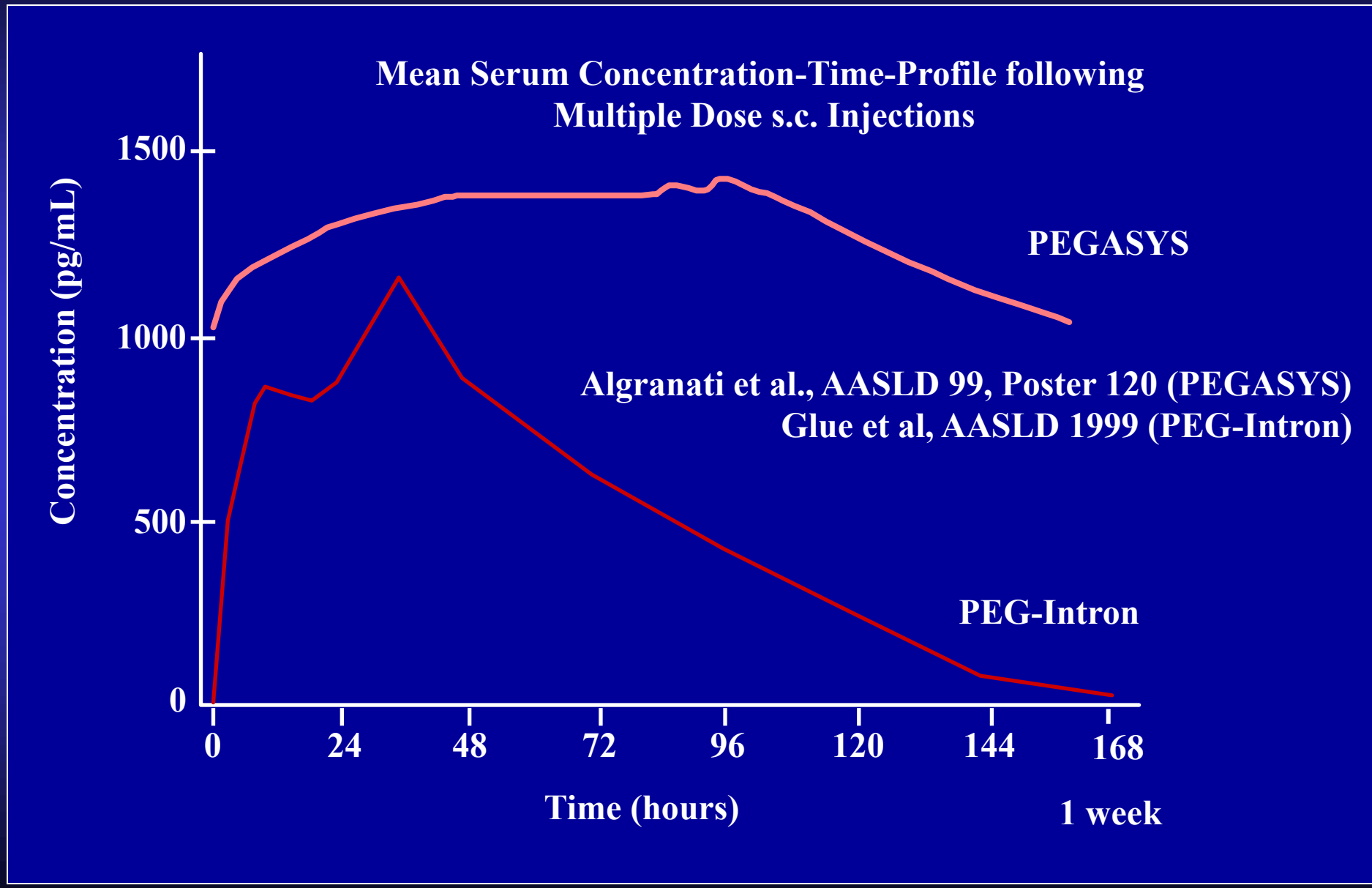
>By changing the structure of PEG

>By using enzymes in the conjugation

PEG conjugates on the market

PEG CONJUGATES	***Type of PEGylation***	***Year to market***	***Disease***
With Proteins			
PEG-asparaginase (*Oncaspar*®)	Random, linear PEG 5 kDa	1994	Acute lymphoblastic Leukemia
PEG-adenosine deaminase (*Adagen*®)	Random, linear PEG 5 kDa	1990	Severe combined immunodeficiency disease (SCID)
PEG-interferon α2a (*Pegasys*®)	Random, Branched PEG 40 *k*Da	2002	Hepatitis C
PEG-interferon α2b (*PEG-Intron*®)	Random, linear PEG 12 *k*Da	2000	Hepatitis C and clinical trials for cancer, multiple sclerosis, HIV/AIDS.
PEG-G-CSF (*pegfilgrastim*, *Neulasta*®)	Selective, linear PEG 20 *k*Da	2002	Treating of neutropenia during chemotherapy
PEG-growth hormone receptor antagonist (*Pegvisomant*, *Somavert*®)	Random, linear PEG 5 *k*Da (genetic modified protein)	2002	Acromegaly
PEG-Anti-TNF-Fab (*Certolizumab, Cimzia)*	PEG 20kDa	2008	Rheumatoid arthritis -Crohn's disease
With Oligonucleotides			
Branched PEG-anti-VEGF aptamer (*Pegaptanib, Macugen*™)	Selective, branched PEG 40 *k*Da	2004	Macular degeneration (age-related)

Comparison of Pegasys and PEG-Intron pharmacokinetics

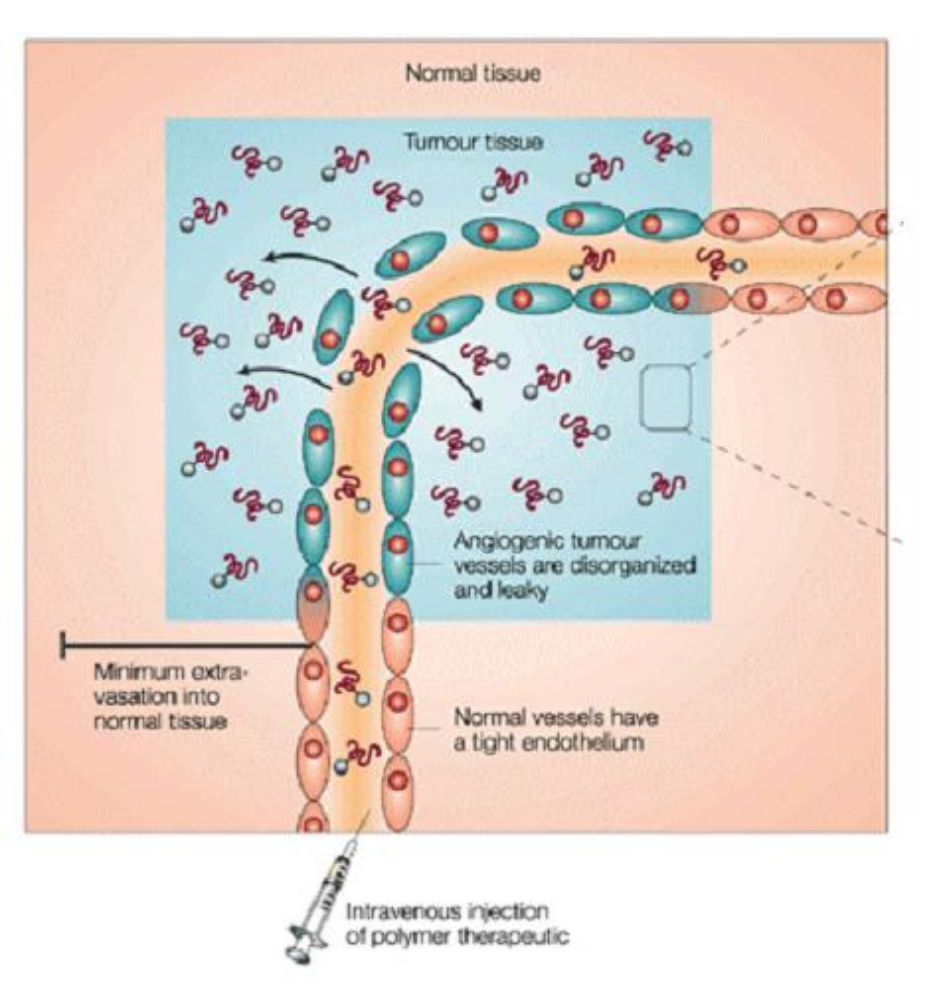


Biochemical and chemical properties of the two PEGylated interferons

PK parameter	IFNα	PEGASYS®	PEG-INTRON®
$t_{1/2}$ (h)	6-9	72-96	32-40
Clearance	6000 mL/h	60-100 mL/h	725 mL/h
Absorption half-life (h)	2.4	50	4.6

But othe properties are improved as well in PEG-conjugated proteins as:
solubility, stability, loss of immunogenicity, loss of aggregation propensity etc.
and body distribution

Tumour targeting by EPR effect (Maeda 2000)



The same tissue accumulation was observed to take place in different ***inflammed tissues*** *(Garrod 2006, Wang 2004 etc.) and to be probably at the basis of improved targeting in various diseases as rheumatic artritis, Cronh's disease and psoriasis*

What what is the case of antibodies as drugs?

Pharmacokinetics of IgG, Fab', di-Fab' and PEG-Fab'

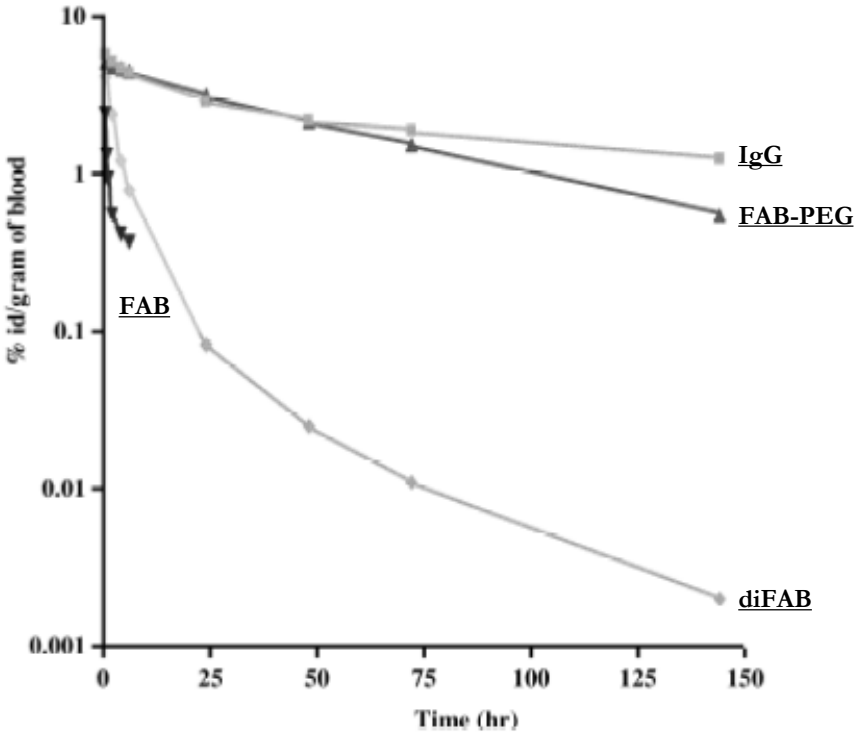


Fig. 5. Pharmacokinetics of ^{125}I-labelled conjugates in rats; comparison of IgG, Fab′, diFab′ and site-specifically attached PEG-Fab′. Pharmacokinetic clearance in rats, of PEGylated Fab′ compared to the parent IgG, non-PEGylated Fab′ and chemically dimerised Fab′. ■, IgG; ▲, Fab′-PEG (40-kDa); ♦, DFM (diFab′ maleimide); ▼, Fab′.

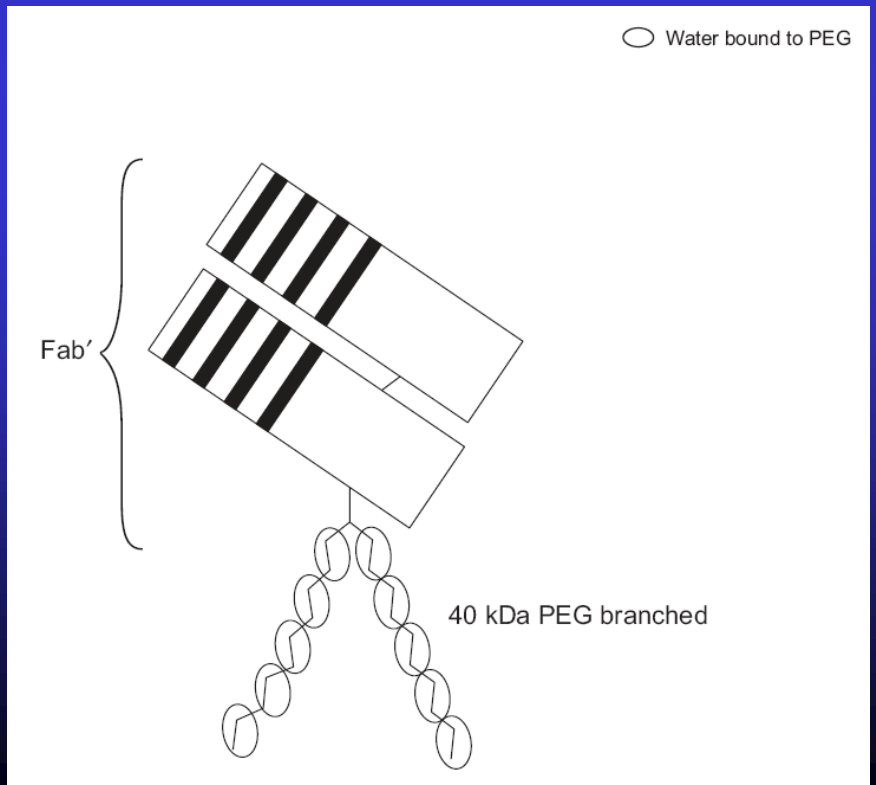
The full antibodies size is sufficient for EPR effect, this is the case of the anti-TNFα

Infliximab:……Chimeric mouse-human mAb

Adalimumab:……Human IgG mAb

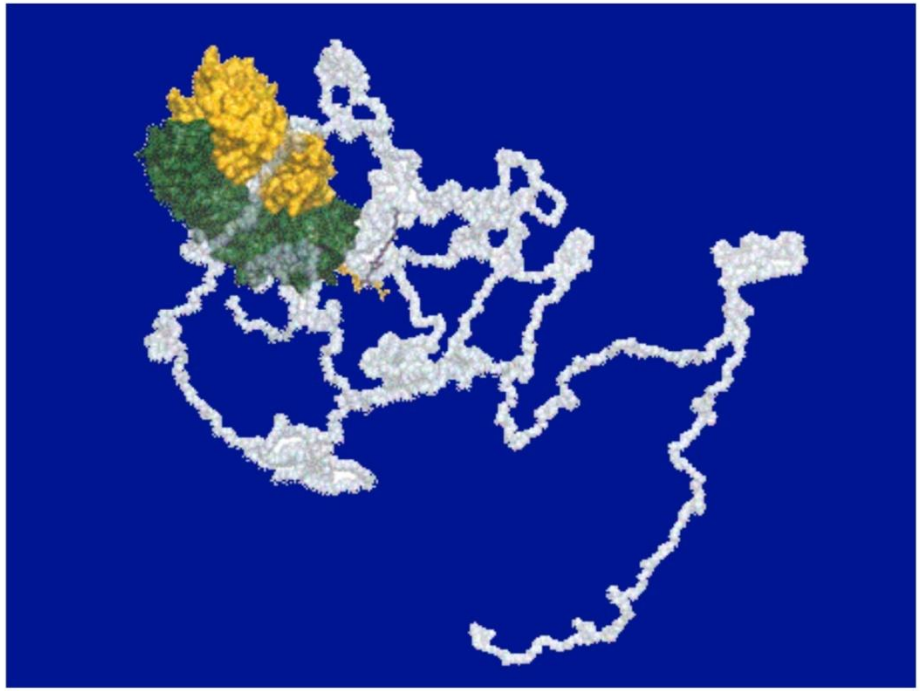
while

***Certulizumab pegol*:….Humanized Fab' fragment-PEG reaches the needed size thanks to the contribution of the bound polymer**



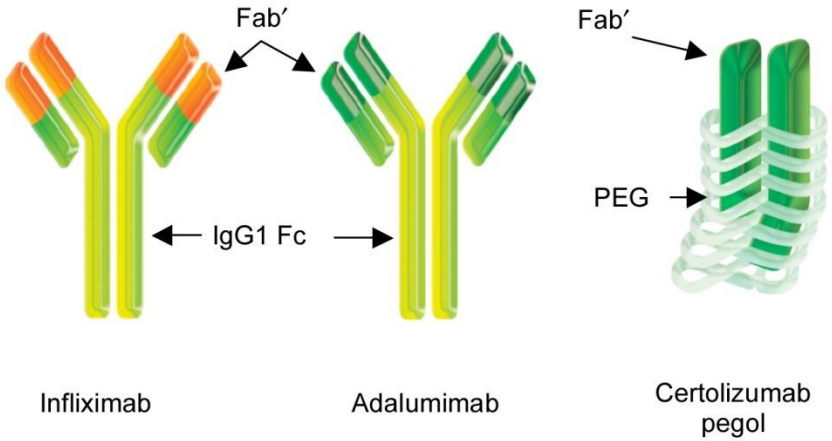
Vision of different anti-TNFα agents

a.



From crystallography

b.



From chemistry

Comparative characteristics and pharmacology of anti-TNFα agents

Characteristics and parameters	Agent		
	Infliximab	Adalumimab	Certolizumab pegol
Structure	Chimeric mouse-human IgG1 mAb	Recombinant human IgG1 mAb	PEGylated humanized anti-Fab′ fragment of an anti-TNFα mAb No Fc region
Conjugate	None	None	PEG2*: 2 × 20 kDa chains
Route of administration	Intravenous	Subcutaneous	Subcutaneous
Dose	3–5 mg/kg at weeks 0, 2 and 6, then 8-weekly Can be increased to 10 mg/kg or frequency increased	40 mg every second week (or weekly if necessary) (RA). An induction of up to 160 mg followed by 80 mg 2 weeks later is required for CD. An 80-mg initial dose followed by 40 mg 1 week later is required for psoriasis	400 mg every 2 weeks for first 4 weeks, then every 4 weeks
Half-life (days)	7.7–9.5	10–20	14
Major biomarkers affected	CRP, inflammatory cytokines, anti-CCP, RF, MMPs, bone and cartilage markers, regulatory T-cells	CRP, inflammatory cytokines, anti-CCP, RF, MMPs	CRP

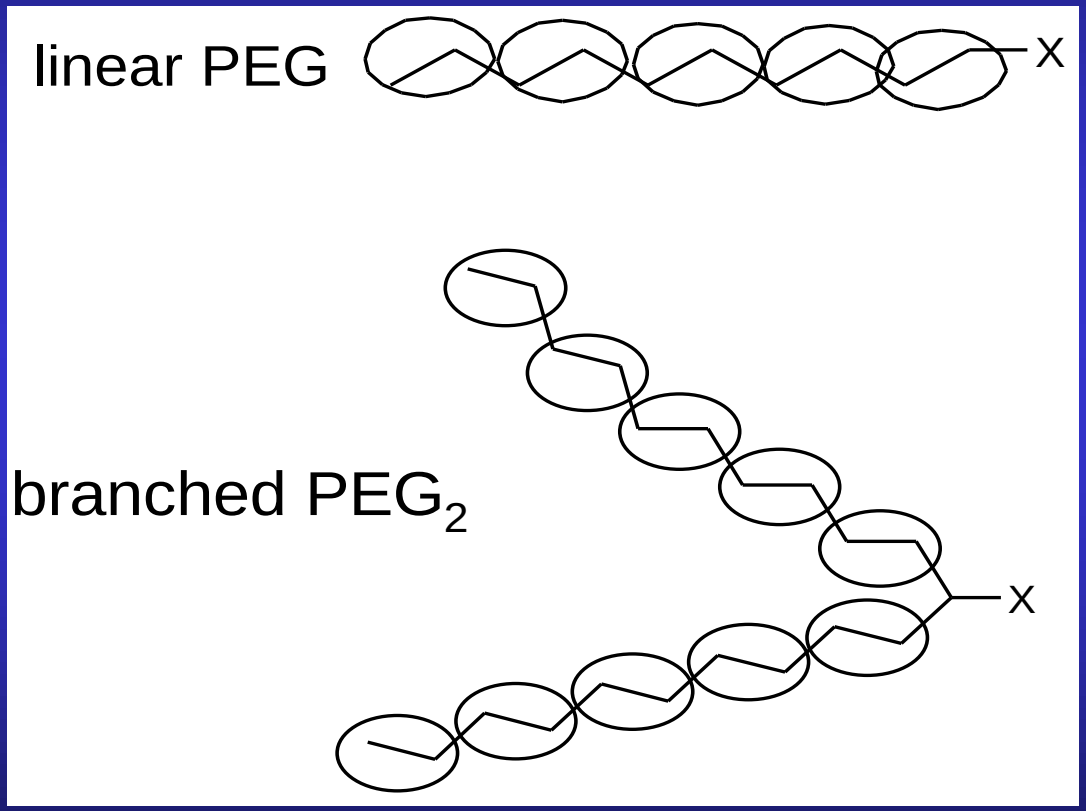
CCP, cyclic citrullinated peptide; CD, Crohn's disease; CRP, C-reactive protein; Ig, immunoglobulin; mAb, monoclonal antibodies; MMPs, matrix metalloproteinases; PEG, polyethylene glycol; RA, rheumatoid arthritis; RF, rheumatoid factor; TNF, tumour necrosis factor.

***PEG2 is linked to the Fab cysteine thiol group.**

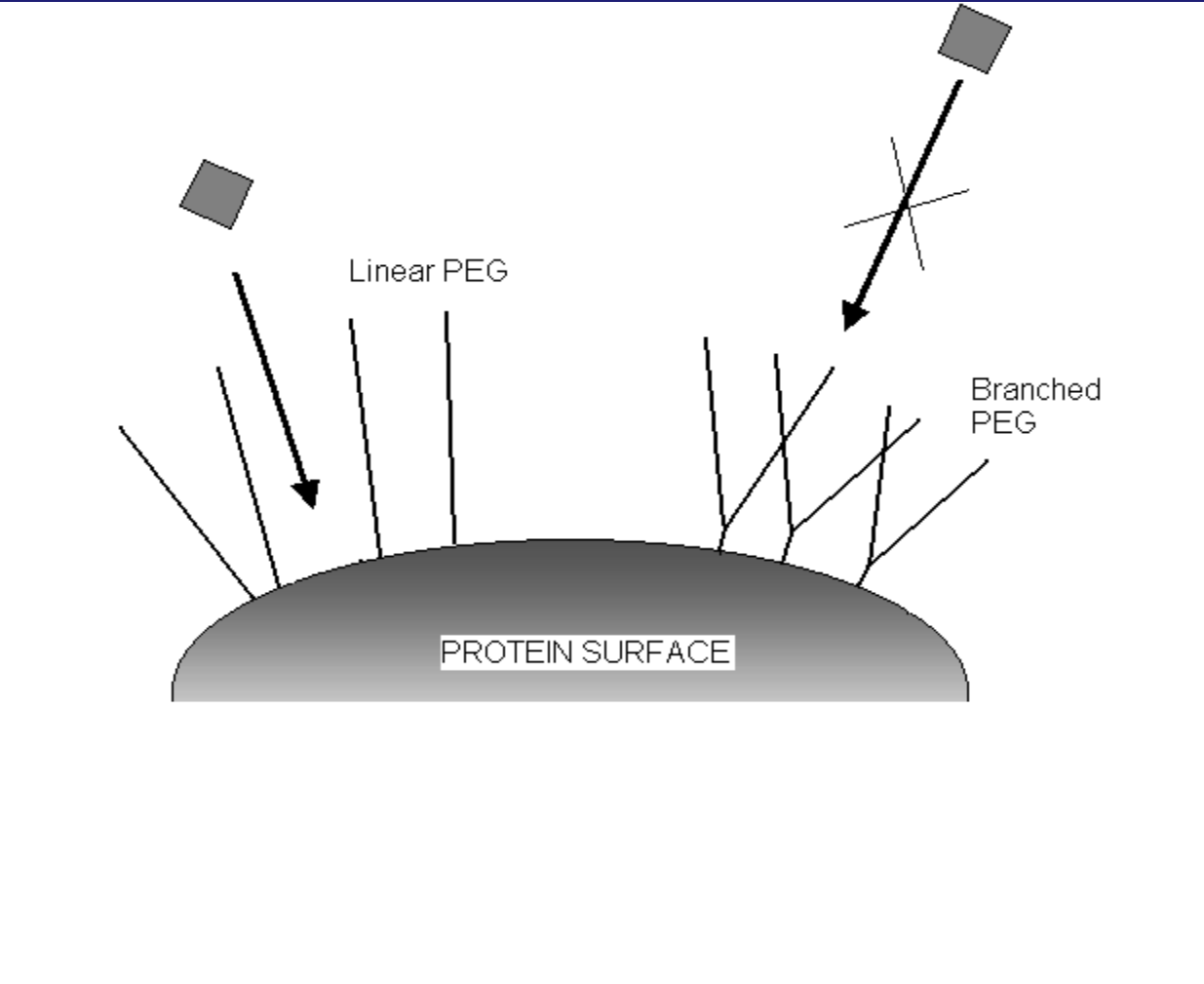
Certulizumab pegol: Properties-1

>Fc region absent in the product
>Fab' fragment with a reactive –SH, for specific PEG conjugation in the hinge region, is obtained by recombinant gene technology
>Fab' fragment is conveniently produced by E Coli
>The short ipervariable complementary determinant region of Fab' derives from a murine mAb
>The position of conjugation allows for the binding and neutralization of TNF
>It binds both soluble and membrane bound TNF
>A branched PEG 40k, constituted by two chains of 20k bound together, is linked to a single point of Fab'

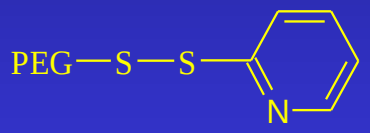
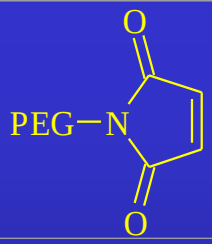
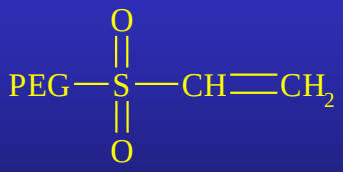
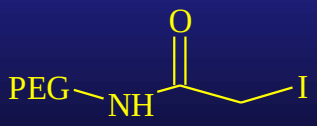
Different architecture of PEG



The PEG structure (linear or branched) plays also a role in the biological behaviour of conjugates



PEGs reactive towards a thiol group

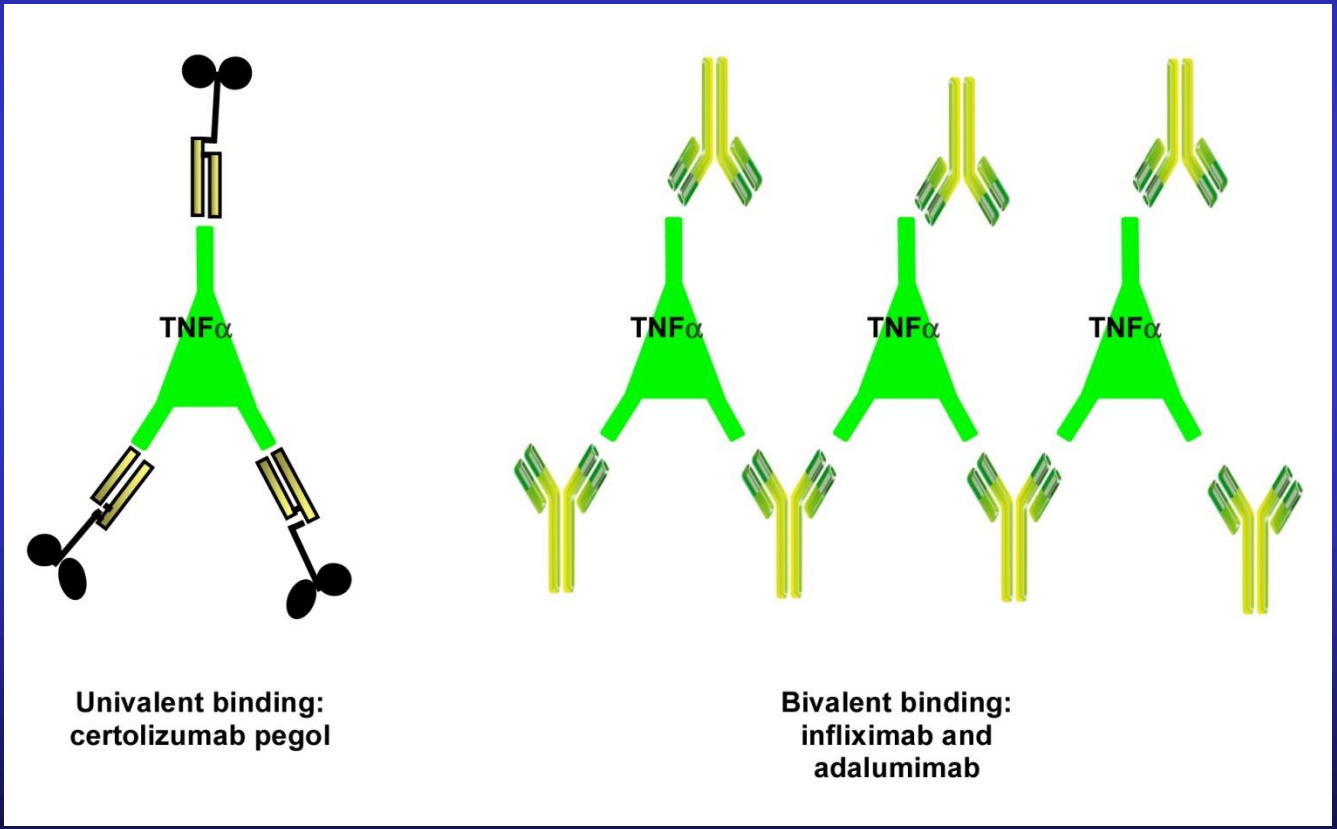
Structure	Thioreactive PEGs	Properties
	PEG-pyridildisulphide	The most specific towards thiol but yields a cleavable linkage by a reducing agent also *in vivo*.
	**PEG-maleimide**	Give stable linkage by double bond addition but may also react with amines at pH > 8.
	PEG-vinylsulfone	
	PEG-iodo acetamide	Less reactive, not much used.

Approximate molecular weights (kDa) of anti-TNFα/TNFα immune complexes at differing molar ratios (ligh scattering values, kD)

Parameter	Agent		
	Infliximab	Adalumimab	Certolizumab pegol
TNFα alone	48	33	34
Antibody alone	289	286	417
Antibody:TNFα ratio 1:1	**2727**	**2304**	**613**
Antibody:TNFα ratio 4:1	559	277	581

Certulizumab pegol: Properties-2

>Certulizumab does not cross-link to TNFα trimer, as adulimumab and infliximab, to yield complexes with proinflammatory effect as PMNs degranulation and superoxide formation



Certulizumab pegol: Properties-3

- **A strong binding takes place between certulizumab pegol and TNFα**

Affinities of infliximab, adalimumab, and certolizumab pegol for soluble TNFα

Parameter	Agent		
	Infliximab	Adalumimab	Certolizumab pegol
Association rate constant, k_a ($M^{-1}s^{-1}$)	**1.01** ± 0.06 × 10^6	**0.724** ± 0.30 × 10^6	**1.22** ± 0.09 × 10^6
Dissociation rate constant, k_d (s^{-1})	2.30 ± 0.34 × 10^{-4}	1.14 ± 0.12 × 10^{-4}	1.09 ± 0.13 × 10^{-4}
Equilibrium constant, K_D (k_d/k_a, pM)	**227**.2	**157**.4	89.3

Certulizumab pegol: Properties-4

>The absence of Fc region prevents complement fixation and cell lysis, as well as elimination by FcRn mediated transport

>The non reactivity with FcRn seems to reduce also transport to milk and crossing of placenta

>On the contrary of infliximab and adalimumab, certulizumab pegol does not induce antibody-dependent cell mediated cytotoxicity, complement-dependent cytotoxicity or apoptosis in cells bearing membrane-bound TNFα

Certulizubab pegol: Properties-5

>The high hydrodinamic volume of PEG allows for a long retention time in blood and inflammed tissues

>It is distributed to the major organs, but not brain

>There is no evident accumulation over time and repeated administrations

>NMR studies demonstrated the elimination, at 84 days, of over 73% in urine and 18% in faeces

>SDS studies demonstrated that in urine PEG of 40k is present. The polymer is no further metabolized, while FAB' is released

Pharmacokinetic parameters of certolizumab pegol in humans and primates (cynomolgus monkeys) after intravenous or subcutaneous administration

Parameter	Humans	Primates
Central volume of distribution, V_1 (mL/kg)	45 (44–47)	46 (44–48)
Total body clearance, CL (mL/h/kg)	0.17 (0.16–0.18)	0.29 (0.27–0.31)
Peripheral volume of distribution, V_2 (mL/kg)	29 (25–33)	31 (26–37)
Intercompartmental clearance, CLd (mL/h/kg)	0.69 (0.52–0.90)	1.1 (0.8–1.5)
Elimination half-life, $t_{½}$ (h)	**313 (284–345)**	**192 (174–212)**
Bioavailability, F	1 (0.9–1.1)	1.1 (1.0–1.3)
Absorption rate constant, Ka (h^{-1})	0.018 (0.015–0.020)	0.11 (0.08–0.16)

Summarizing, preclinical studies showed :

	Complex Format.	Affinity TNF	Cyt.Secr. Inhibit(3)	CDC ADCC	Apoptos. (1)	Neutroph death (2)
Certulizumab	-	+++	+++	-	-	-
Adalimumad	+++	++	++	+++	+++	+++
Infliximab	+++	++	++	+++	+++	+++
Etanercept	-	+++	+/-	++	++	++

CDC, Complement dependent cytotoxicity; ADCC, Antibody dependent cytotoxicity
(1)of activated lynphosites and monocytes, (2) degranulation (3) following anti-TNF incubation and lipopolysacc. stimulation

Certulizumab pegol: Properties-6

Several phase II and III clinical trials (PRECISE 1 and 2, DMARG, RAPID 1 and 2) demonstrated that:

- **>Certulizumag pegol is convenient for both monotherapy or in combination with MTX**
- **>It presents an acceptable safety profile**
- **>Significantly reduces the signs of RA**
- **>It inhibits the progression of structural damages**
- **>The onset of benefit certulizumab pegol is rapid**
- **>The majority of adverse events are mild to moderate**
- **>The discontinuation due to adverse event is low, below 6%**
- **>Low incidence of pain or reaction at injection site**
- **>Minimal incidence of infections, similar to other anti-TNFα agents**

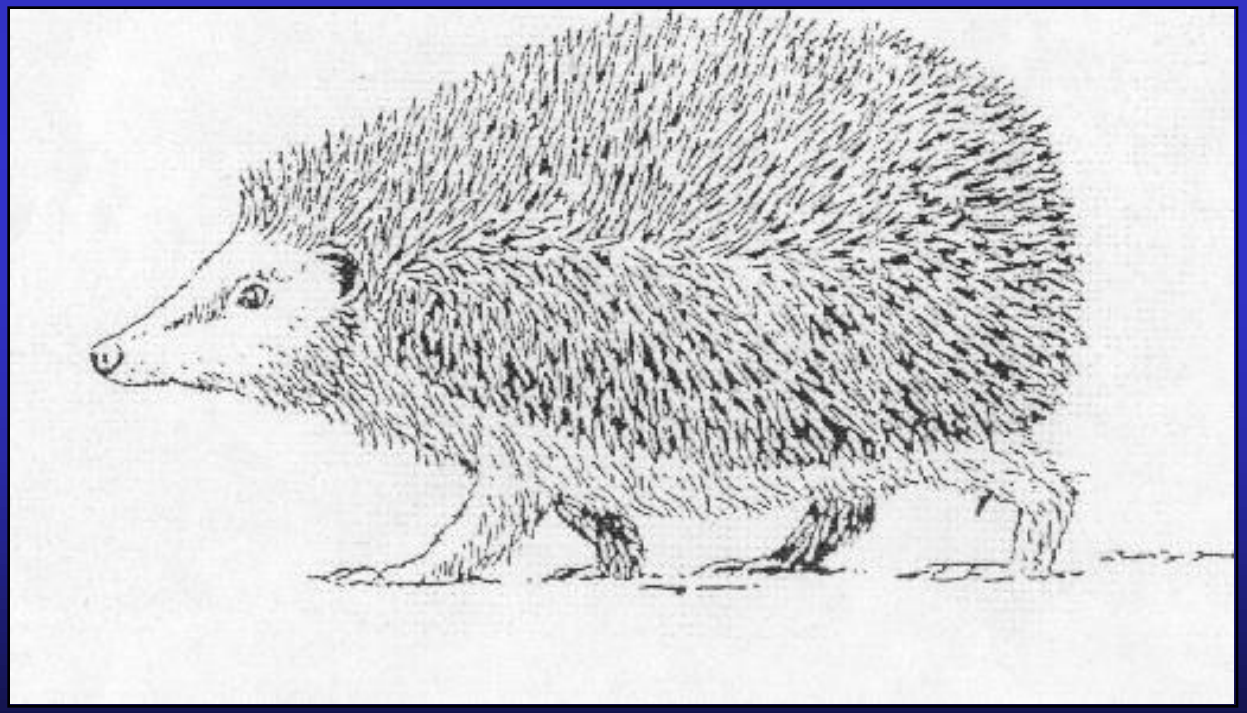
But we must also underline the cost of these nanomedicines, a problem that however is becoming common not only for the new proteins as drugs but for all of the drugs in general, due to:

>>The research investments
>>The increasing requirements by FDA or EMEA

WE can say that now aspirin would not be approved by Regulatory Authorities

To conclude

PEG is a wonderful and versatile tool for ***protecting*** and ***improving*** the properties of different drugs, as in nature spines are protecting the hedge horn, and we may be confident that this technique will give further important applications in the near future



Thanks