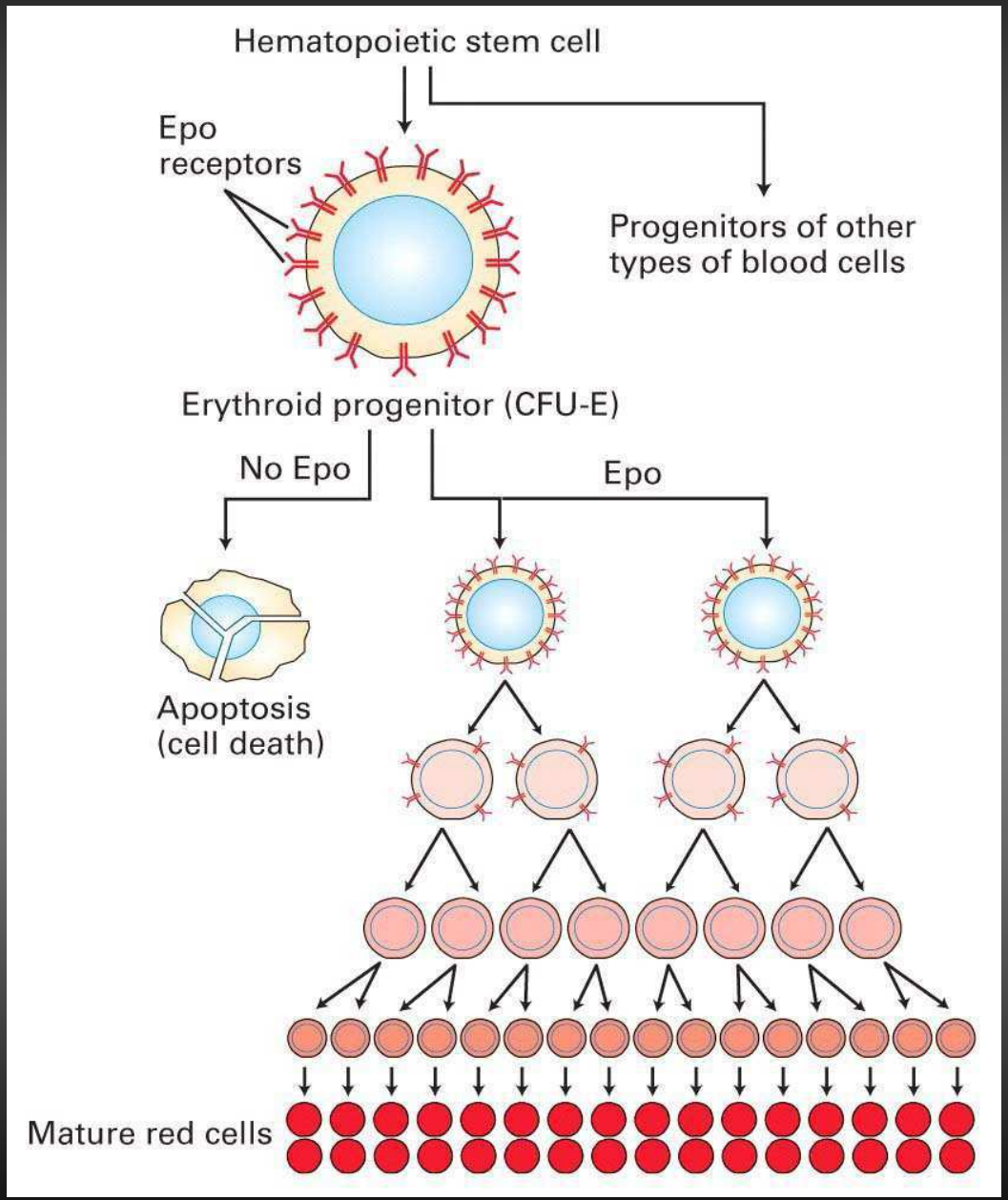

Funzioni di Epo e
molecole terapeutiche

Ruolo dell'Epo nell'eritropoiesi



EpoR è espresso sulla superficie delle cellule eritroidi (massima espressione sulle CFU-E, diminuita sugli stadi più differenziati)

Epo agisce "salvando" dall' apoptosi le cellule progenitrici eritroidi, e stimolandone la maturazione

Epo controls erythrocyte production by preventing apoptosis through activation of Janus kinase 2 (JAK2) and Stat5, which induce expression of the antiapoptotic Bcl2 family member Bcl-xl.

Epo/Bcl-xl-dependent survival is both necessary and sufficient for terminal erythroid differentiation.

Consequently, in mouse models, absence of Epo or its receptor, the Epo effector, Stat5, or the Epo/Stat5 target, Bcl-xl, results in apoptosis of erythrocyte progenitors and anemia.

Epo

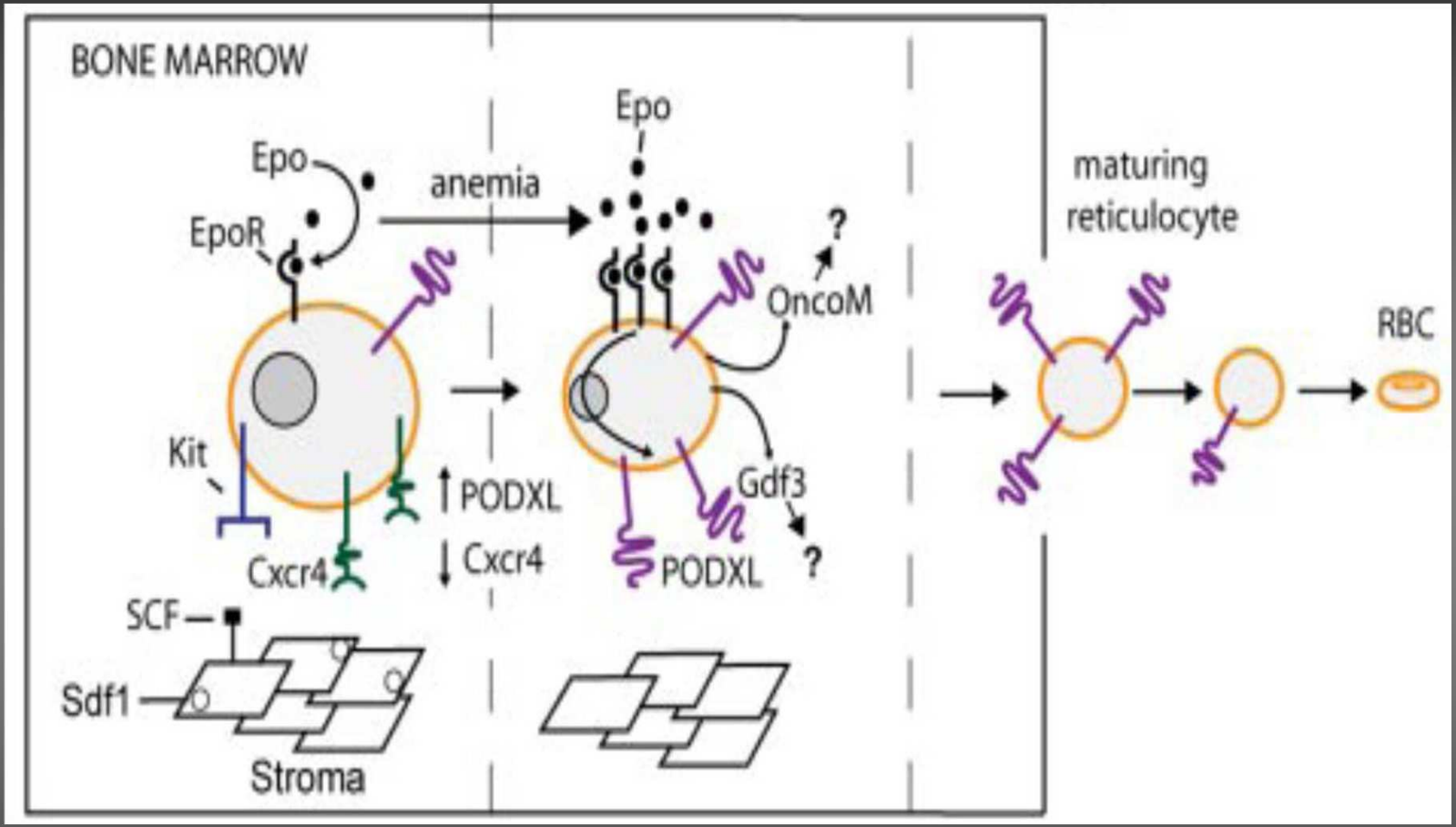
down-modulates adhesion factors

Chemokine receptor-4 (*Cxcr4) Integrin* alpha-4 (*Itga4) **mediates binding to vascular cell adhesion molecule 1 (VCAM-1), fibronectin, and paxillin**

up-modulates
growth differentiation factor-3 (*Gdf3),*
oncostatin-M (*OncoM)* – acts via JAK- Stat- heterodimeric receptor 19 and affects cell growth, differentiation,

Podocalyxin* like-1 (*PODXL)?

Model for Epo regulation of erythroid progenitor cell adhesion and migration within stromal niche



PODXL is a sulphated sialomucin, antiadhesive

Epo

down-modulates adhesion factors

Chemokine receptor-4 (*Cxcr4) Integrin* alpha-4 (*Itga4)* mediates binding to vascular cell adhesion molecule 1 (VCAM-1), fibronectin, and paxillin

up-modulates

Podocalyxin* like-1 (*PODXL)

Mucins

The amino- and carboxy-terminal regions are very lightly glycosylated, but rich in cys. The cys residues participate in establishing disulfide linkages within and among mucin monomers.

A central region formed of multiple tandem repeats of 10 to 80 residue sequences in which up to half of the aa Ser Thr. This area becomes saturated **with hundreds of O-linked oligosaccharides**.

Sialomucin - acid mucopolysaccharide containing sialic acid

Stati Patologici legati all'eritropoietina

Anemia

Inadeguata produzione endogena
(es. patologia renale)

↓

Carenza di globuli rossi

↓

Anemia

Trattamento dell'anemia

Epo ricombinante (rHuEPO)

Produzione su larga scala di Epo umana ricombinante

rHuEPO

- 34000 Da
- prodotta in cellule mammarie in cui è stato introdotto il gene dell'Epo

Novel Erythropoiesis Stimulating Protein (NESP)

NESP (darbepoetin):

- 38500 Da
- Aumentato contenuto di carboidrati, che conferiscono un aumento dell'emivita
- Somministrazione meno frequente

Epo contains **one** O-linked and **three** N-linked carbohydrate chains, each having 2–4 branches that often end in a negatively charged sialic acid.

These carbohydrate chains are not required for receptor binding in vitro or stimulation of growth of EpoR-expressing cultured cells but are **required for the in vivo bioactivity**

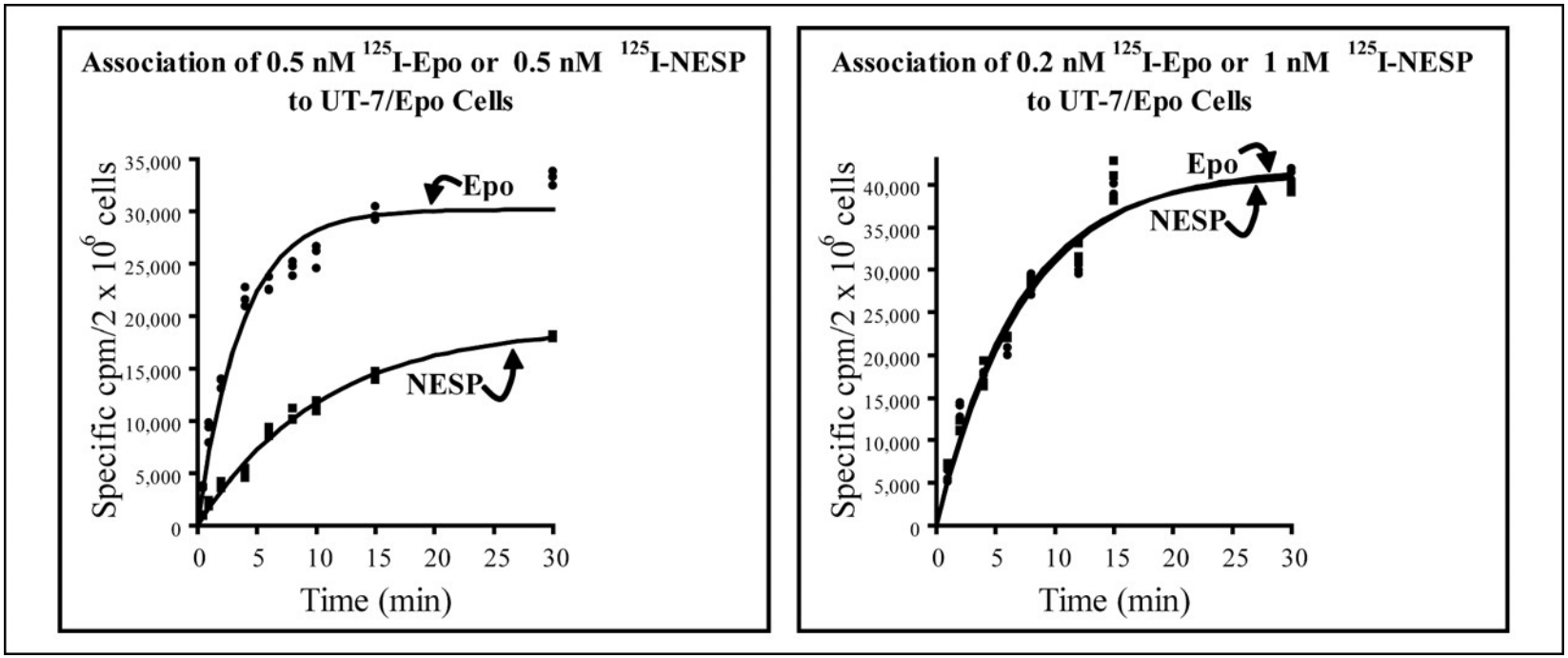
Heterogeneous branching of Epo N-linked carbohydrates results in Epo isoforms with different sialic acid contents up to a maximum of 14.

residues are mutated to provide for 2 additional N-linked glycosylation sites

Epo isoforms with higher sialic acid content have a lower affinity for EpoR but a longer serum half-life and are more effective for stimulating the production of red blood cells in vivo.

How Epo is cleared from the circulation and degraded?

Net binding of 125I-Epo or 125I-NESP with UT-7/Epo cells at 37 ℃.

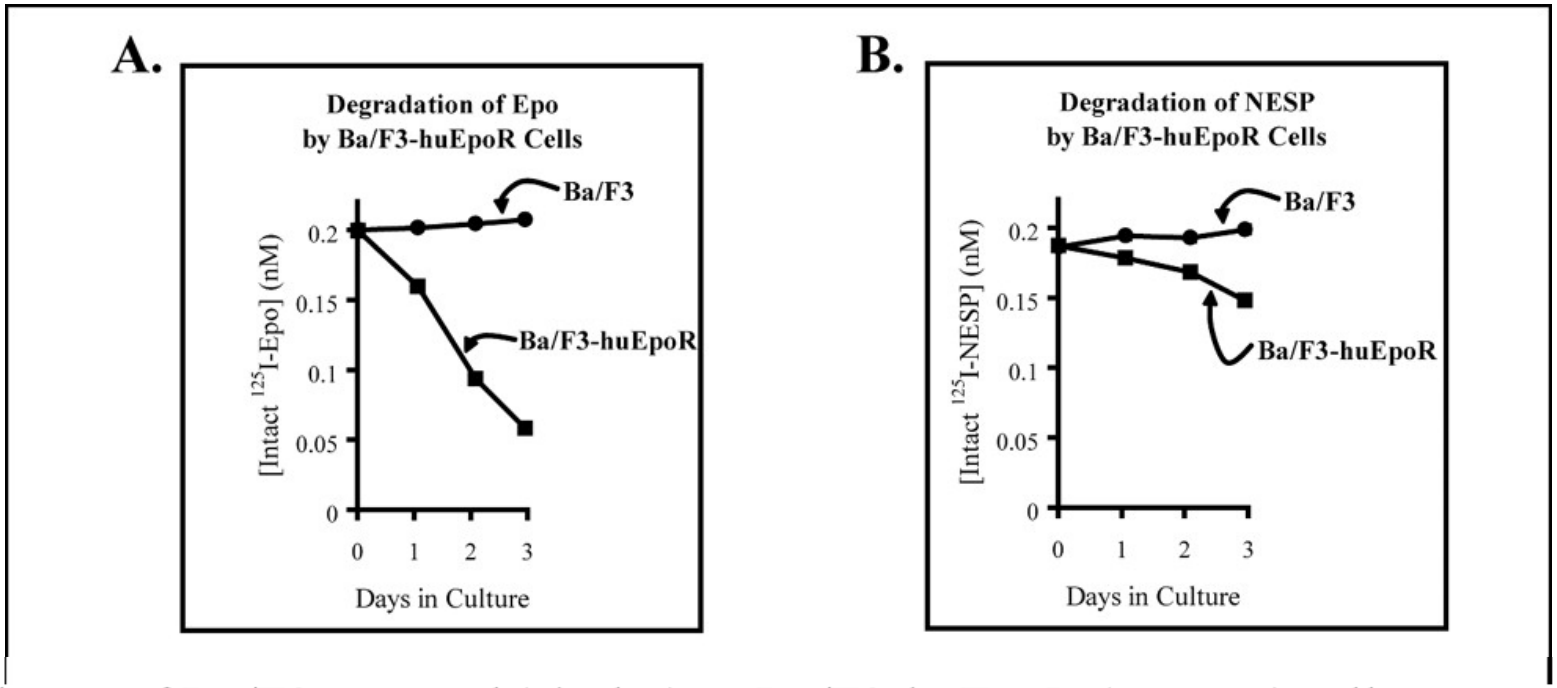


Cells were preincubated at 37 °C for 5 min with endocytosis inhibitors (0.1% sodium azide and 10 µg/ml cytochalasin B) then 125I-labeled ligand was added. Cells were collected and rapidly separated from the medium after the indicated then cell-associated radioactivity was measured. The

Gross A W , Lodish H F J. Biol. Chem. 2006;281:2024-2032

jbc

Degradation and endocytosis of Epo and NESP by Ba/F3-huEpoR cells.

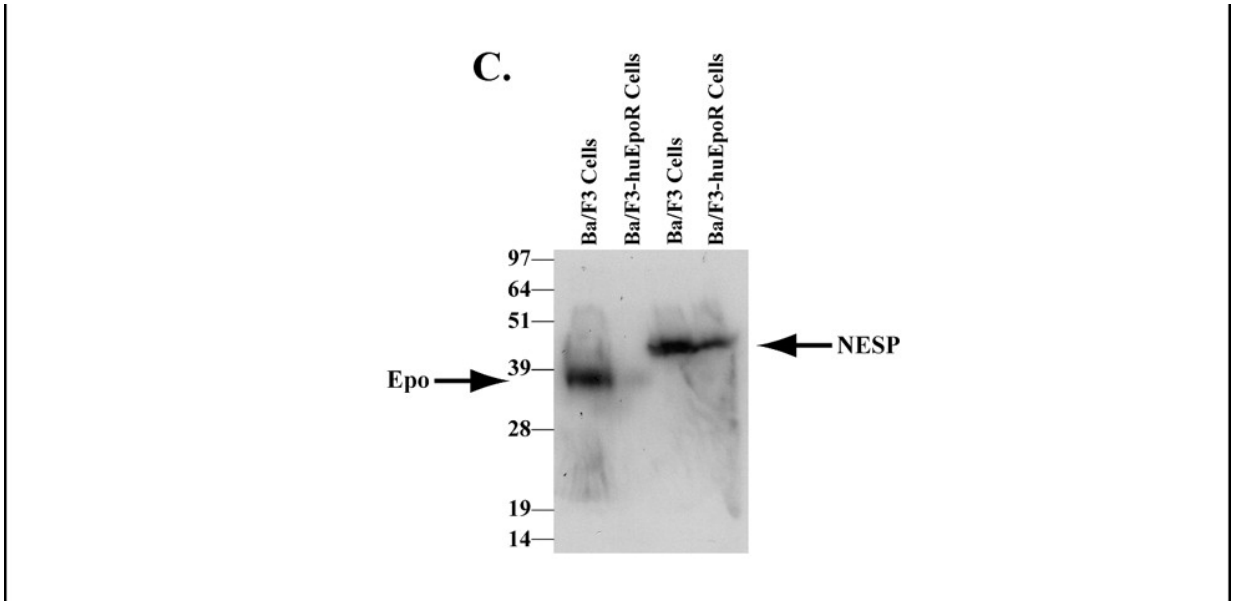


cultures of Ba/F3 parental (circles) or Ba/F3-huEpoR (squares) cells were initiated with excess IL-3 and 0.2 nm 125I-Epo (A) or 0.2 nm 125I-NESP (B)

Gross A W , Lodish H F J. Biol. Chem. 2006;281:2024-2032

jbc

Degradation and endocytosis of Epo and NESP by Ba/F3-huEpoR cells.

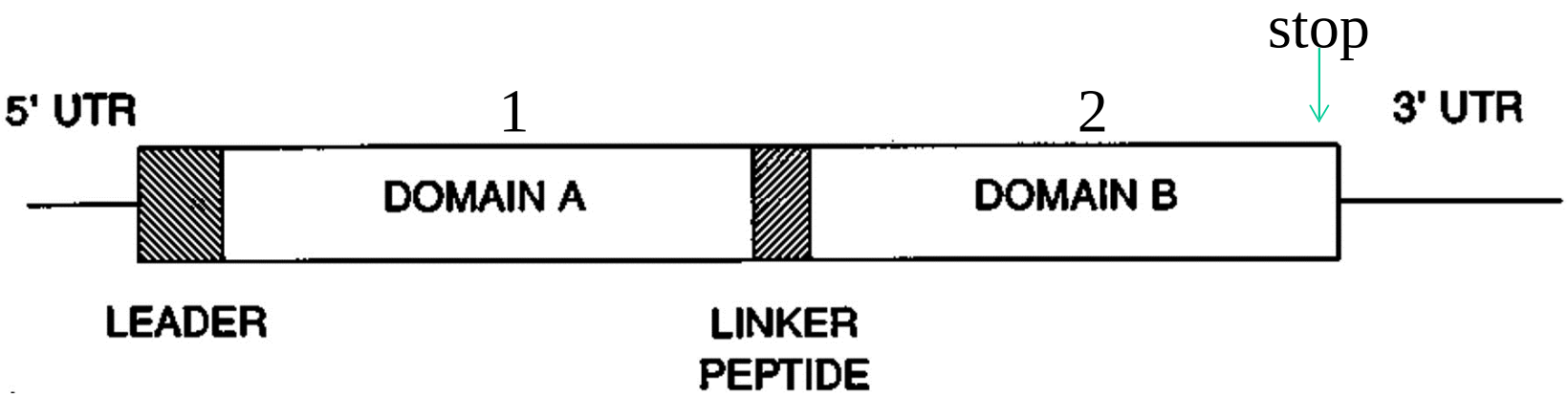


cultures of Ba/F3 parental (circles) or Ba/F3-huEpoR (squares) cells were initiated with excess IL-3 and 0.2 nm 125I-Epo (A) or 0.2 nm 125I-NESP (B)after the third day in culture, proteins precipitated by trichloroacetic acid from the media of the cultures shown in A and B were separated by SDS-PAGE and analyzed by autoradiography. The type of cells cultured with each sample is indicated at the top of each lane. The position of intact Epo and NESP proteins are indicated by arrows. Numbers indicate the size in kDa and position of prestained molecular weight markers.

Cross AR, Lodish HF. J. Biol. Chem. 2006;281:2024-2032

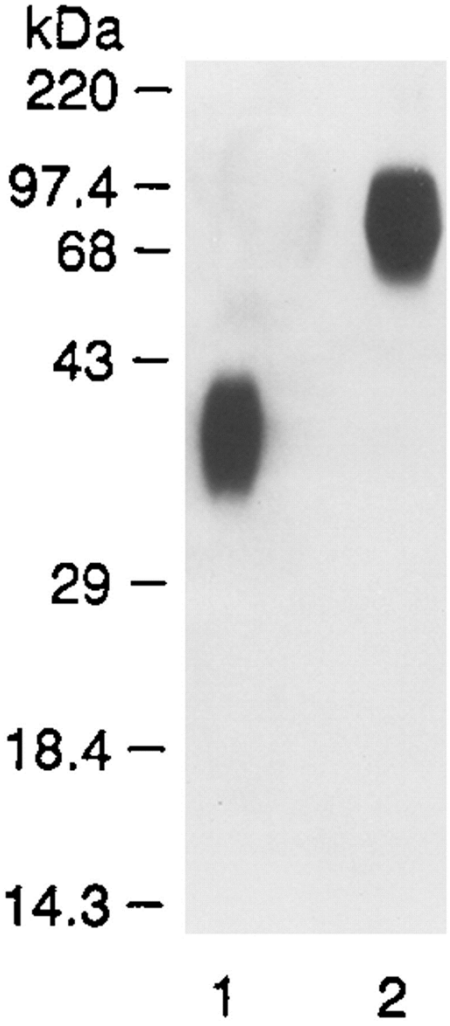
Epo-Epo” -a peptide-linked head-to-tail dimer

Diagram of cDNA encoding the Epo-Epo fusion protein.



Sytkowski A J et al. J. Biol. Chem. 1999;274:24773-24778

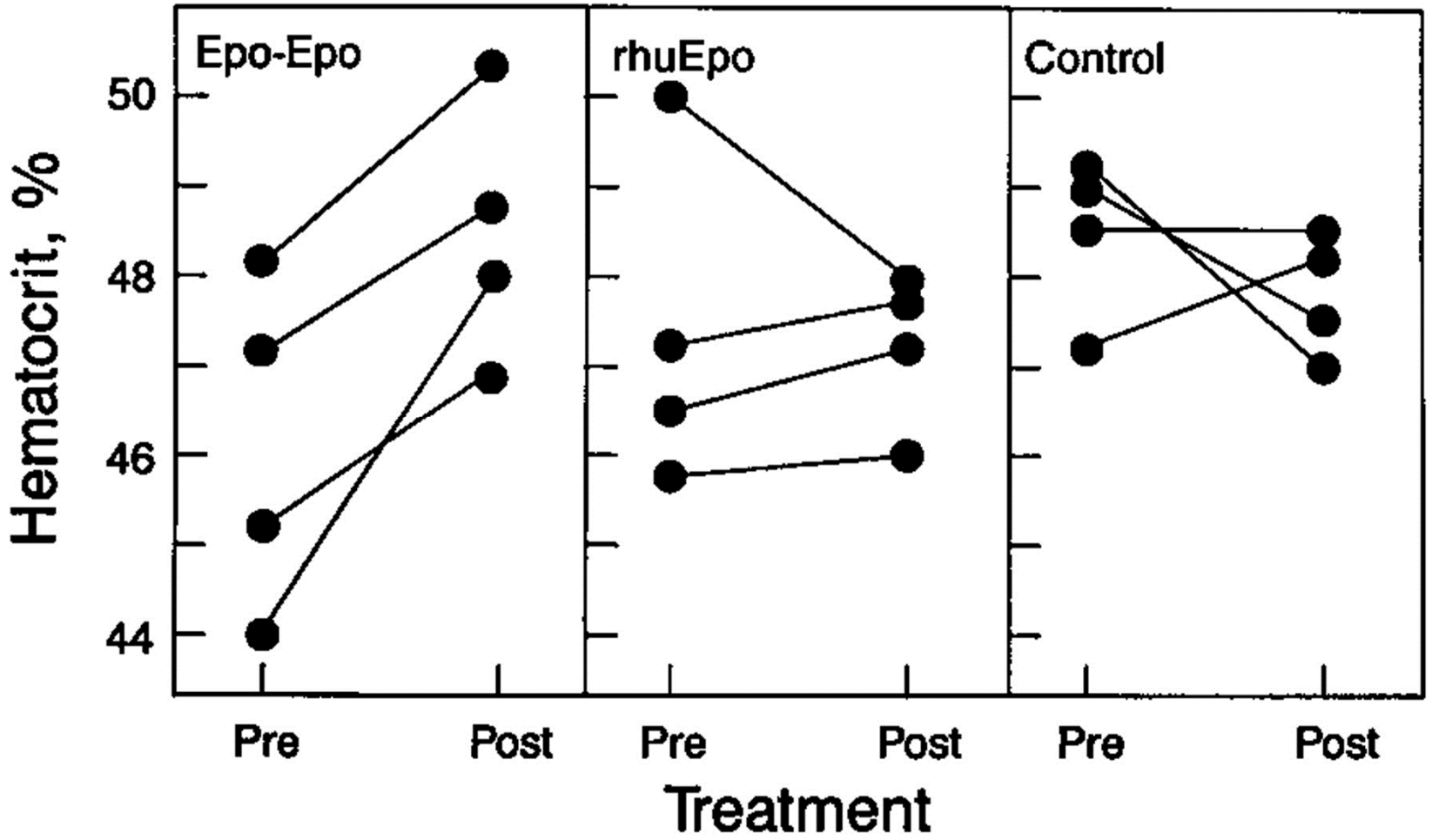
Western blot of purified recombinant Epo (lane 1) and the supernatant of COS1 cells transfected with Epo-Epo cDNA (lane 2).



Sytkowski A J et al. J. Biol. Chem. 1999;274:24773-24778

jbc

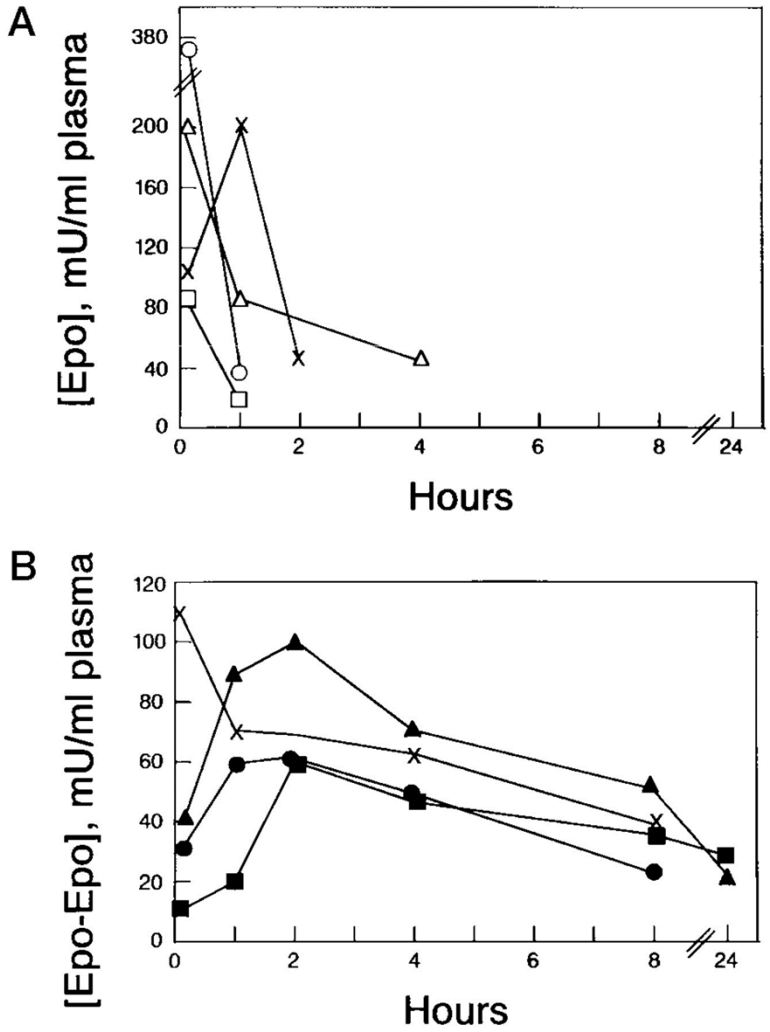
In vivo efficacy of Epo-Epo compared with that of conventional Epo .



Sytkowski A J et al. J. Biol. Chem. 1999;274:24773-24778

jbc

Pharmacokinetics of Epo (A) and Epo-Epo (B) in mice.



Sytkowski A J et al. J. Biol. Chem. 1999;274:24773-24778

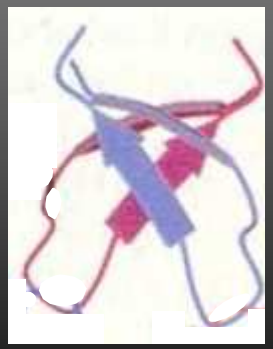
"Hormone mimicry"

Una piccola molecola può "mimare" la funzione di un grande ORMONE POLIPEPTIDICO

Wrighton et al, Science 1996

Sintesi di piccoli peptidi (20 aa) che si legano al recettore dell'Epo e lo attivano "mimano" l'effetto biologico dell'Epo

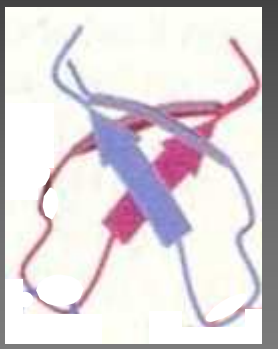
EMP1

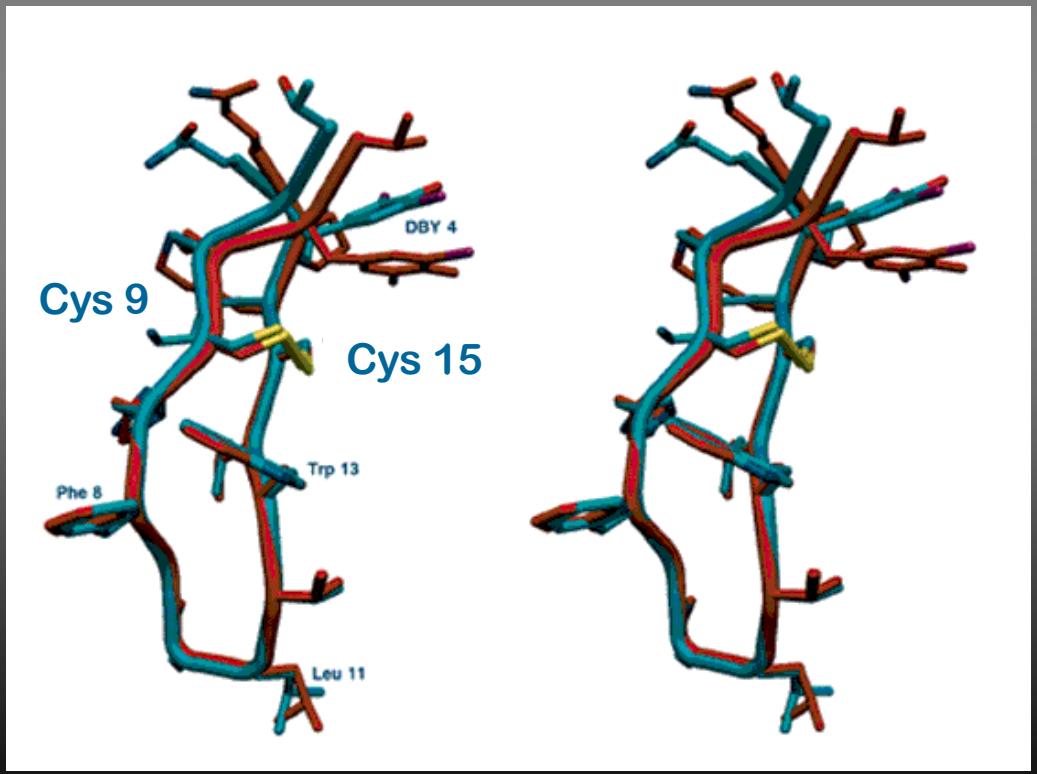


Eritropoietina

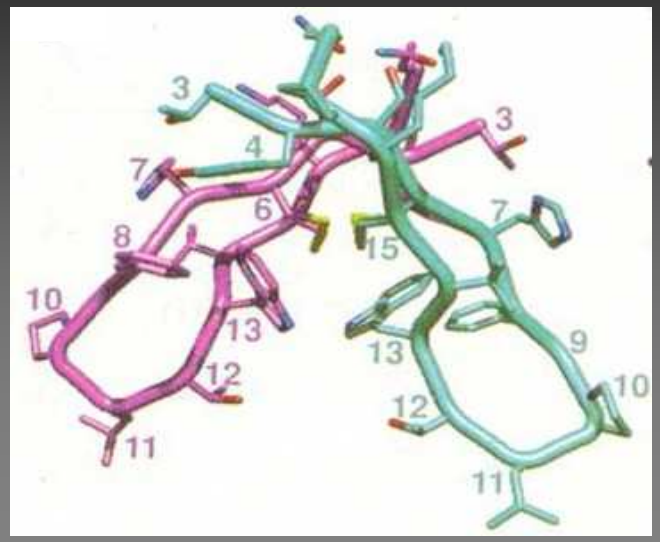
EMP1 (EPO mimetic peptides (EMPs)

- Peptide di 20 aa (2 kDa): GGTYSCHFGPLTWVCKPQGG
- Struttura: 2 corti ß-foglietti uniti da un ponte disolfuro
- Sintesi: ottenuto da una libreria di peptidi random prodotti in sistema fagico (phage display); selezionato mediante saggi di legame alla porzione extracellulare di EpoR



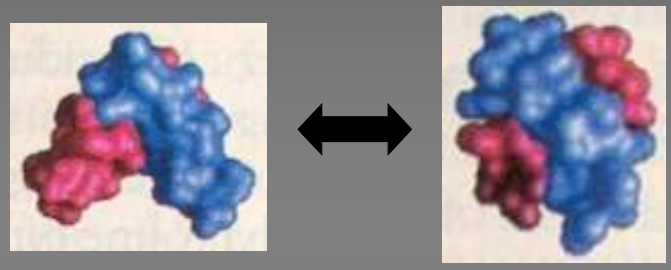


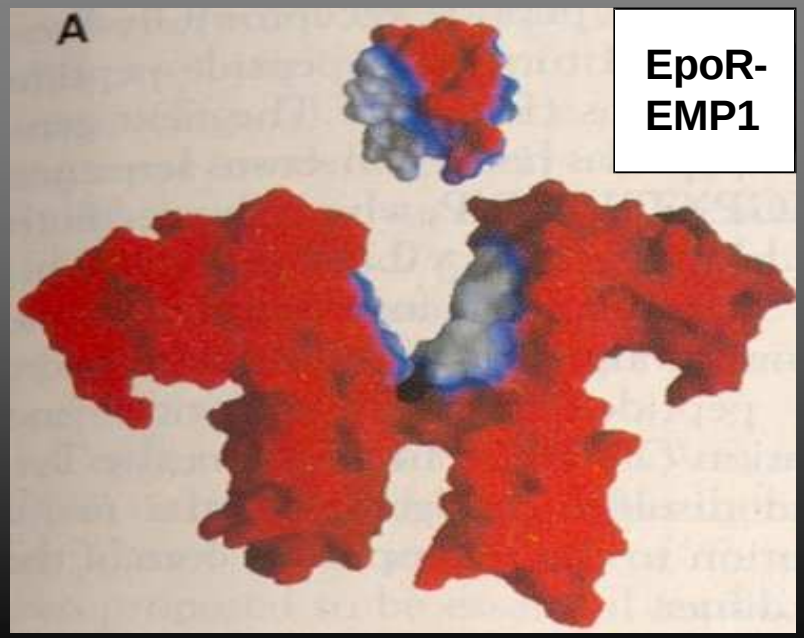
Complesso EpoR-EMP1



EMP1 dimerizza per legarsi a EpoR

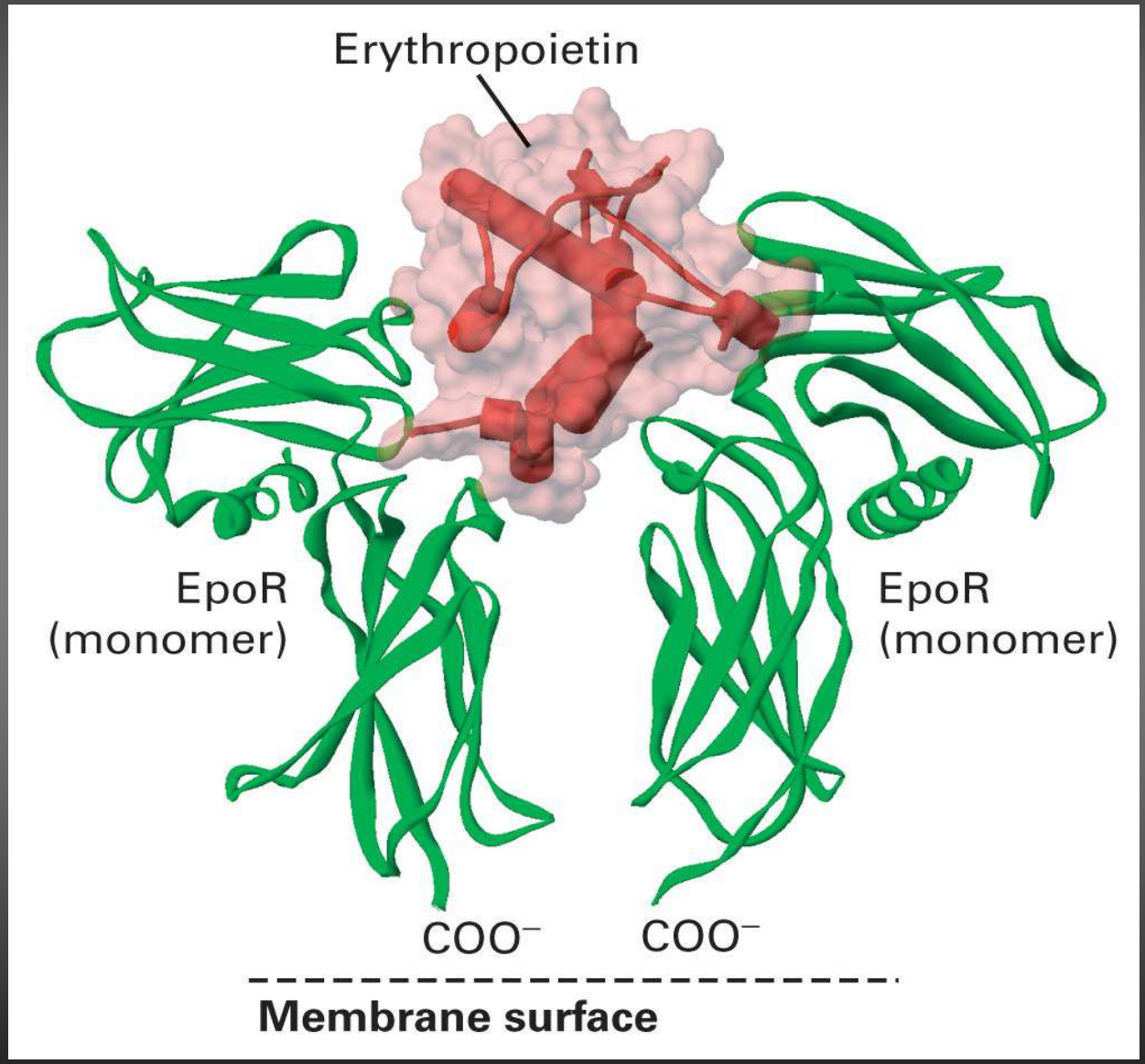
Struttura dimerica molto forte, stabilizzata da 4 legami idrogeno



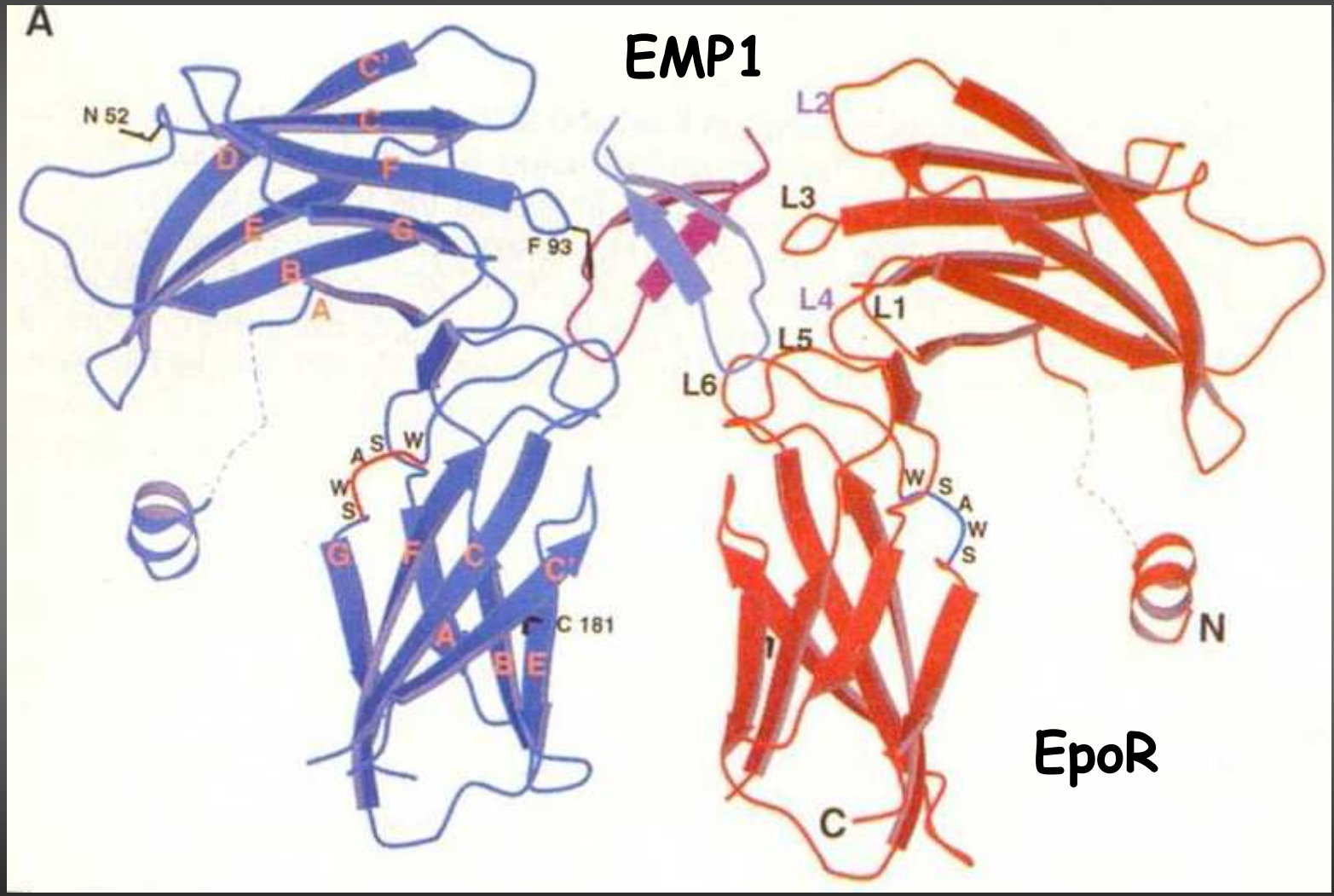


Ogni monomero di EMP1 interagisce sia con l'altro monomero che con EpoR

Erythropoietin-Epo Receptor complex

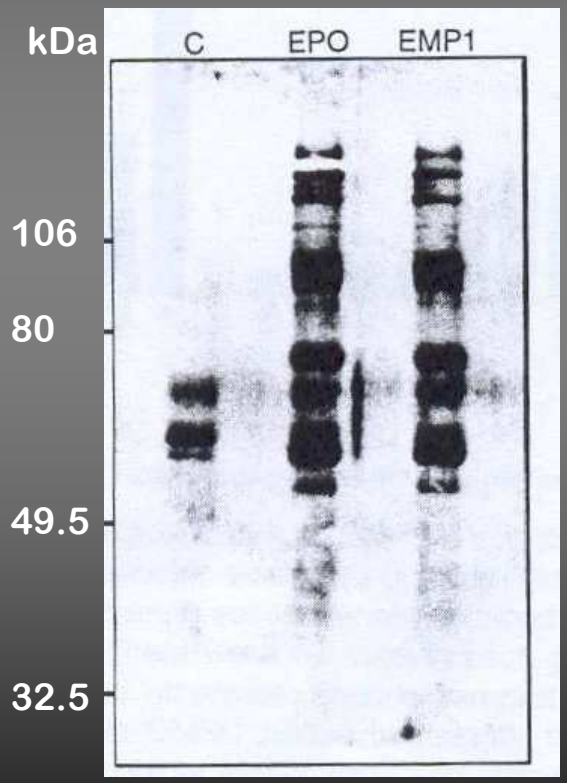


Complesso EpoR-EMP1



EMP1 stimola l'eritropoiesi attraverso la *stessa via di trasduzione del segnale* indotta da Epo

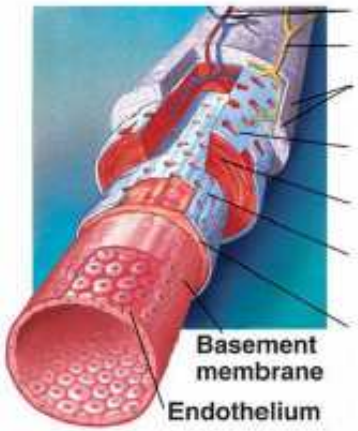
Western blot (anticorpo anti-fosfoTyr)

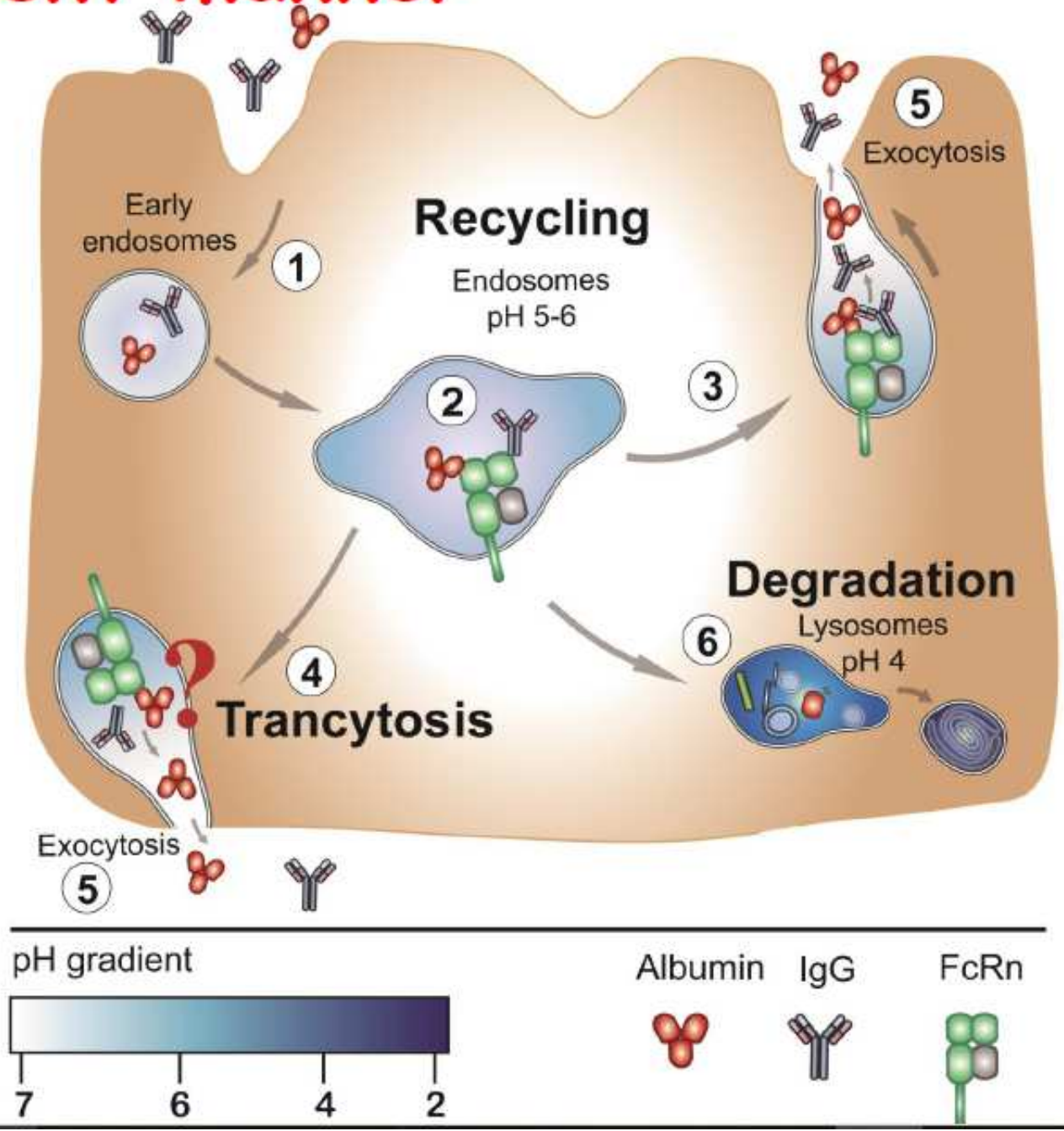


Cellule stimolate con EMP1 e con Epo presentano lo stesso pattern di fosforilazione

Wrighton et al., Science 1996, 273:458-463

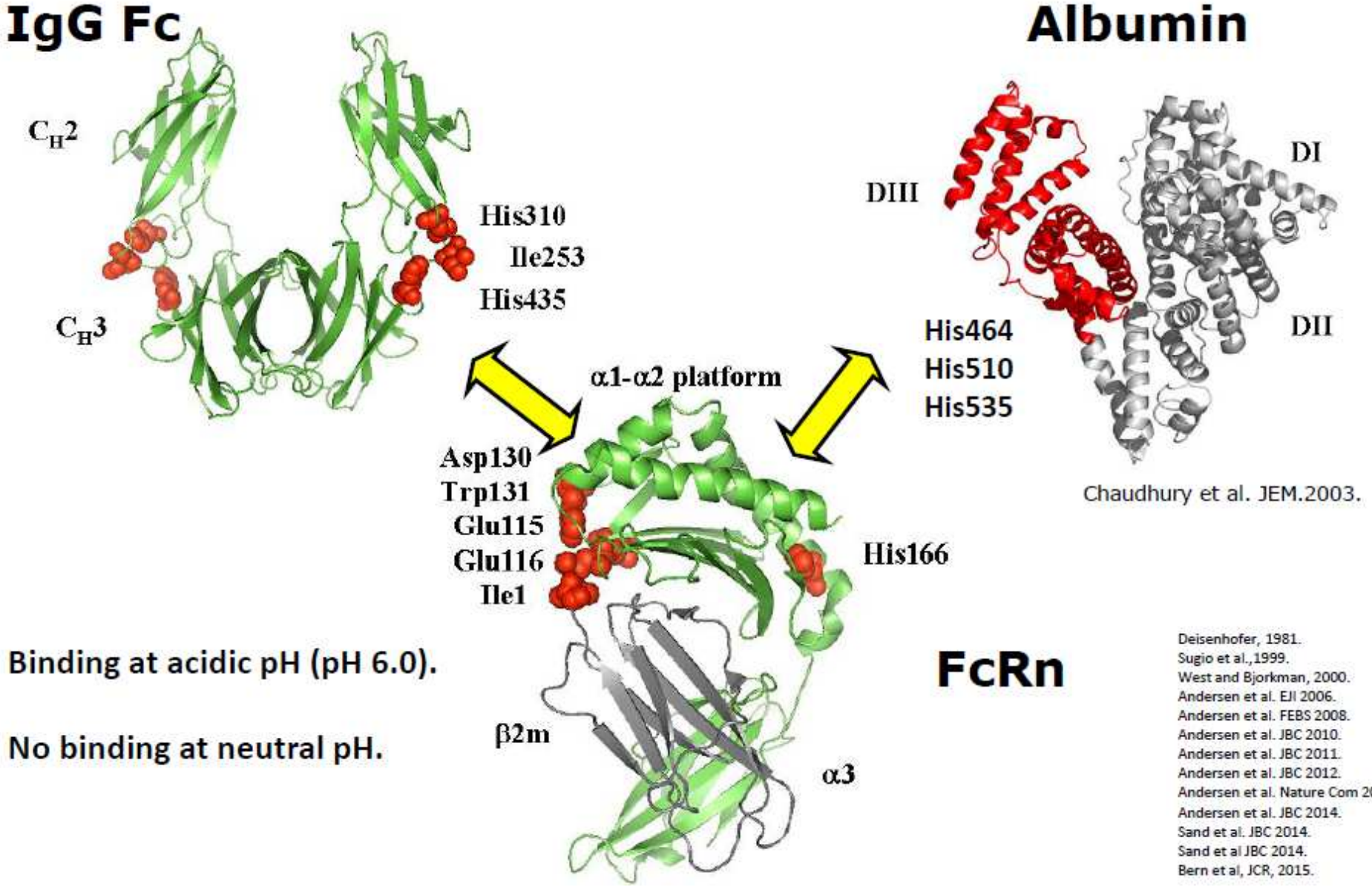
FcRn recycles its ligands in a pH dependent manner





Sand et al, Front Immunol, 2014.

FcRn binds IgG and albumin in a pH dependent fashion simultaneously

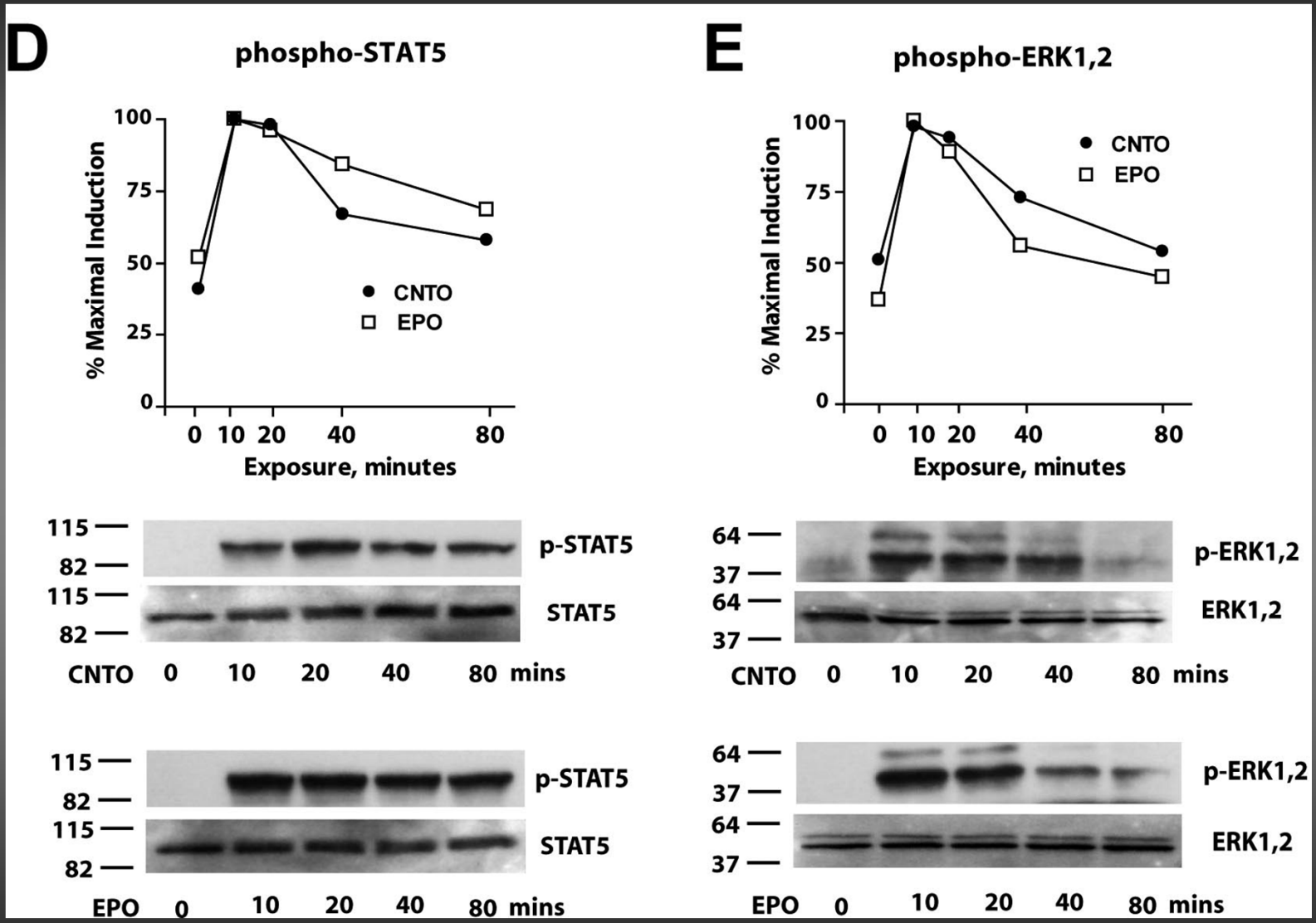


Chaudhury et al. JEM.2003.

- Binding at acidic pH (pH 6.0).
- No binding at neutral pH.

Deisenhofer, 1981.
Sugio et al.,1999.
West and Bjorkman, 2000.
Andersen et al. EJI 2006.
Andersen et al. FEBS 2008.
Andersen et al. JBC 2010.
Andersen et al. JBC 2011.
Andersen et al. JBC 2012.
Andersen et al. Nature Com 2012.
Andersen et al. JBC 2014.
Sand et al. JBC 2014.
Sand et al JBC 2014.
Bern et al, JCR, 2015.

CNTO 530 activates known EPO signal transduction pathways



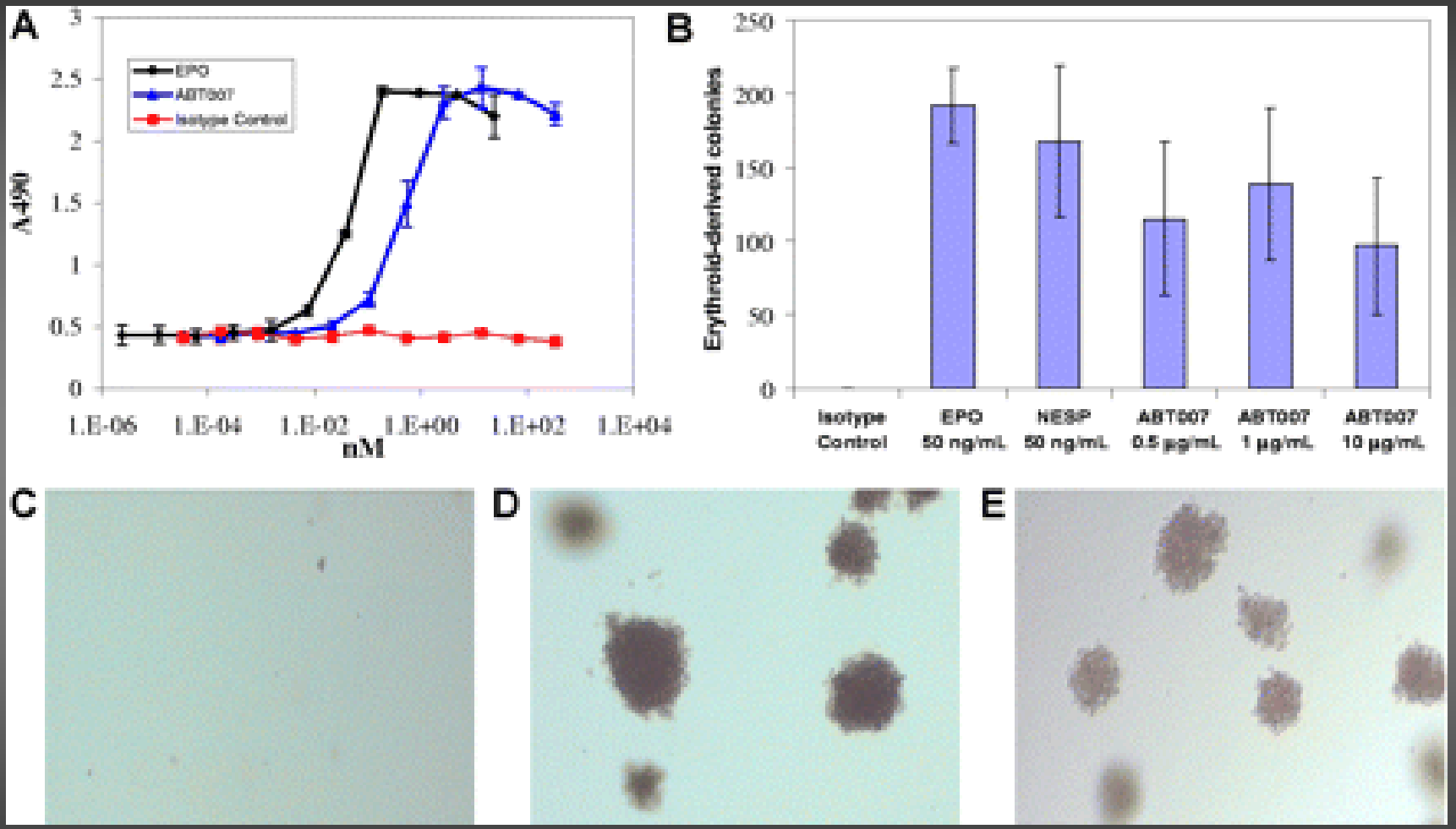
CNTO 530 is a dimeric EMP fused to a human lgG4 Fc

"Hormone mimicry"

EMP1 è la dimostrazione che una molecola di 20 aa può mimare la funzione di un ormone

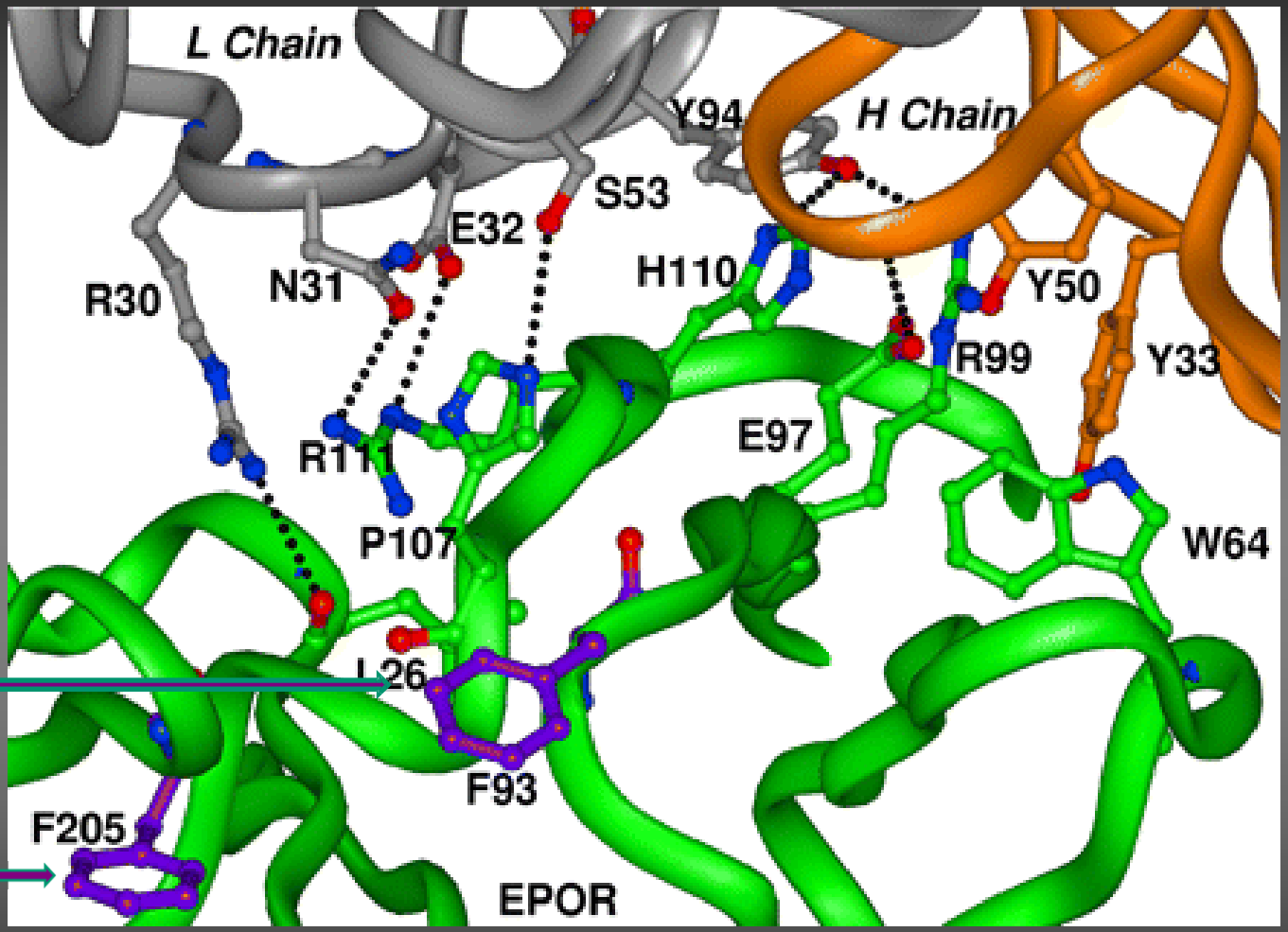
- Stimolando la stessa via di trasduzione del segnale (JAK, STAT...)
- Senza avere nessuna omologia di sequenza o struttura con l'ormone

A potent erythropoietin-mimicking human antibody



ABT007 stimulates in vitro erythropoiesis

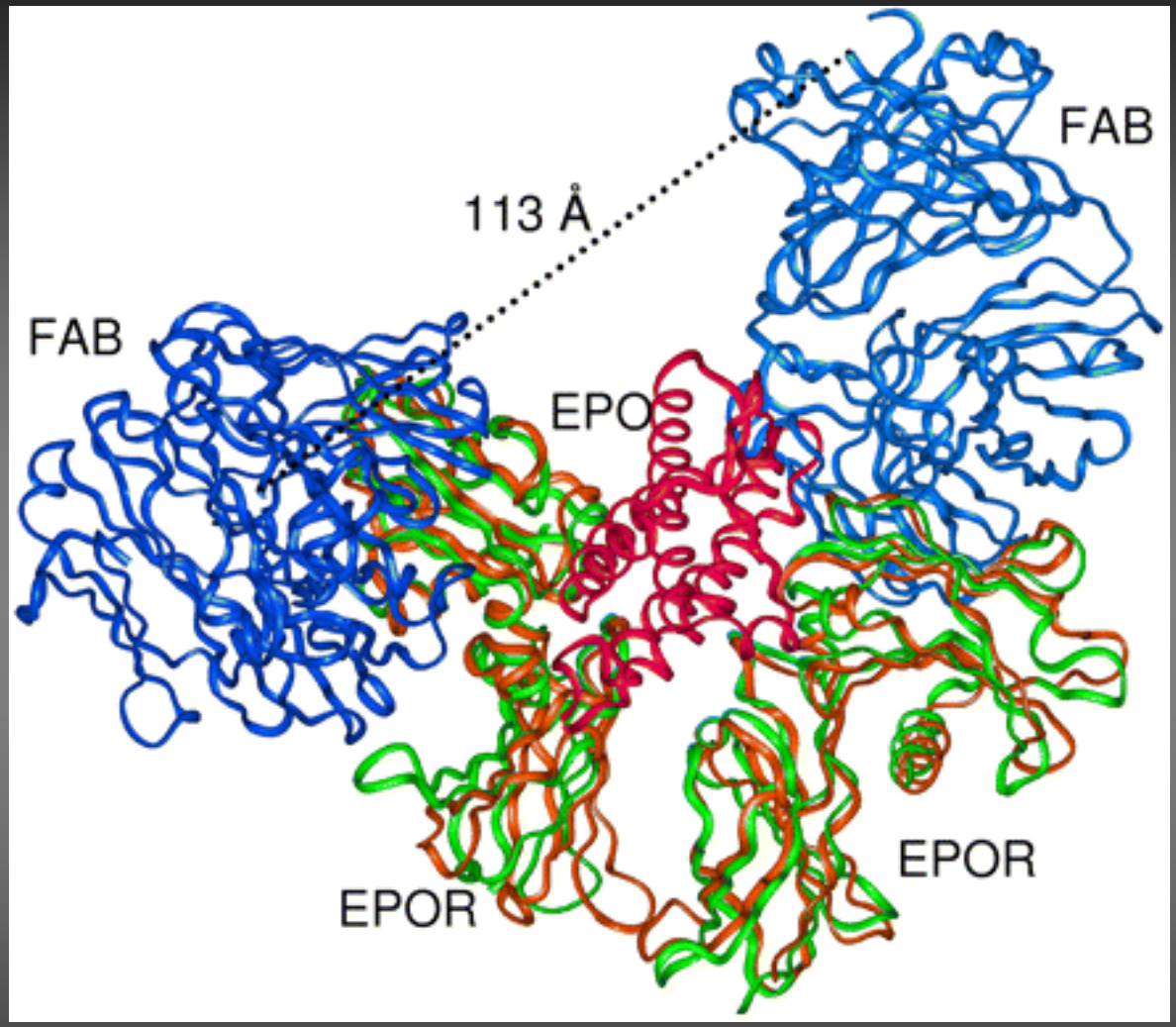
The antibody interacts through a novel binding site



Epo binding

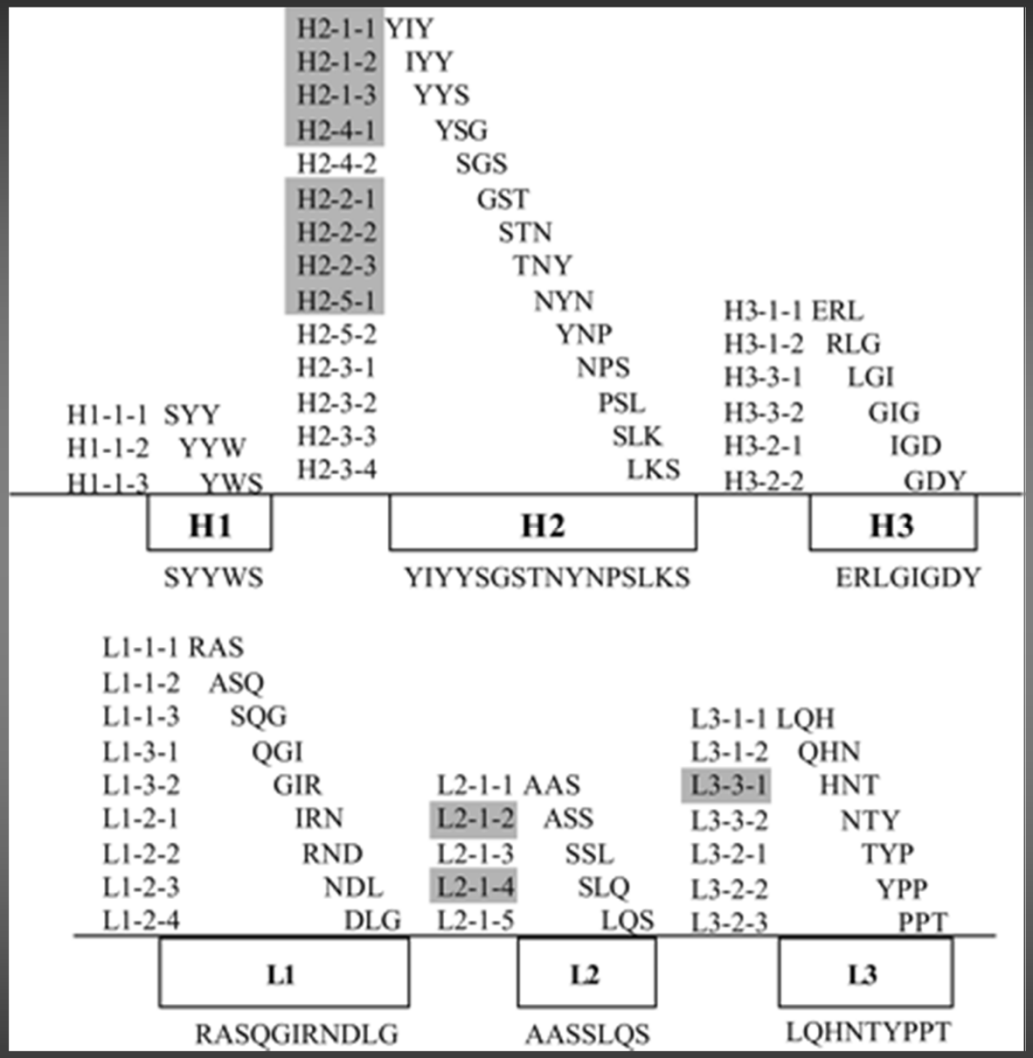
F93 and F205 of EPOR, highlighted in purple, are key residues involved in binding EPO and are not involved in Fab binding.

Comparison of the Fab-EPOR complex with the EPO-activated EPOR

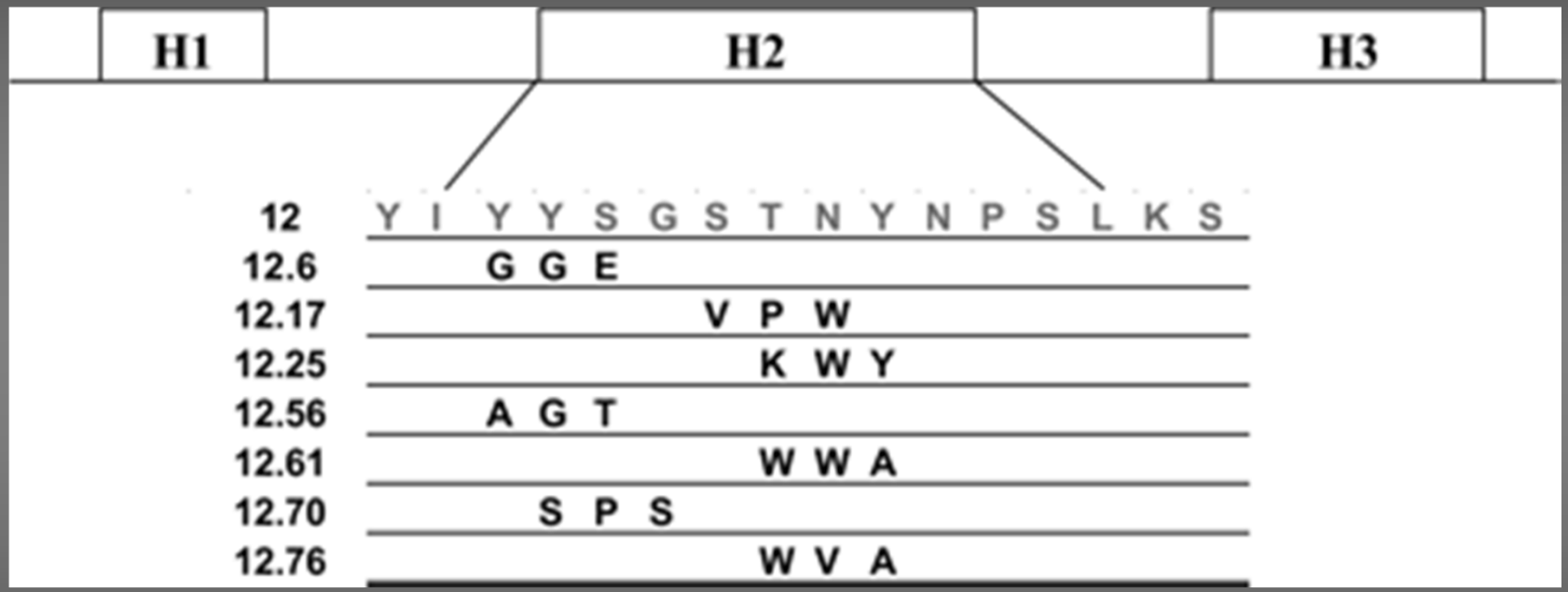


A model of activation based on a conformation induced onto EPOR by ABT007 in a 2:1 ratio that is different from that caused by EPO.

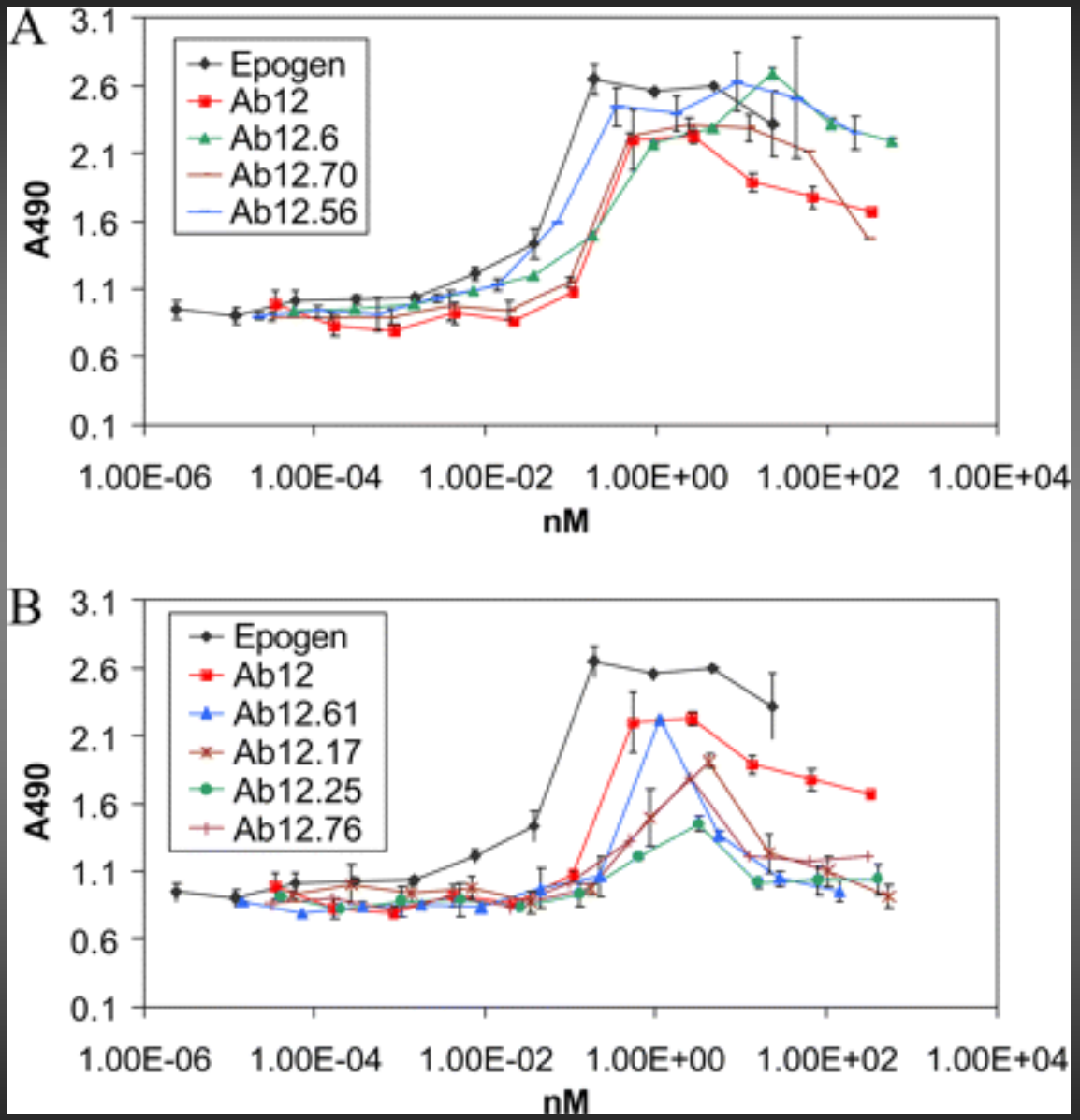
Ab12 scFv CDR VH and VL yeast libraries



Ab12 CDR H2 variants



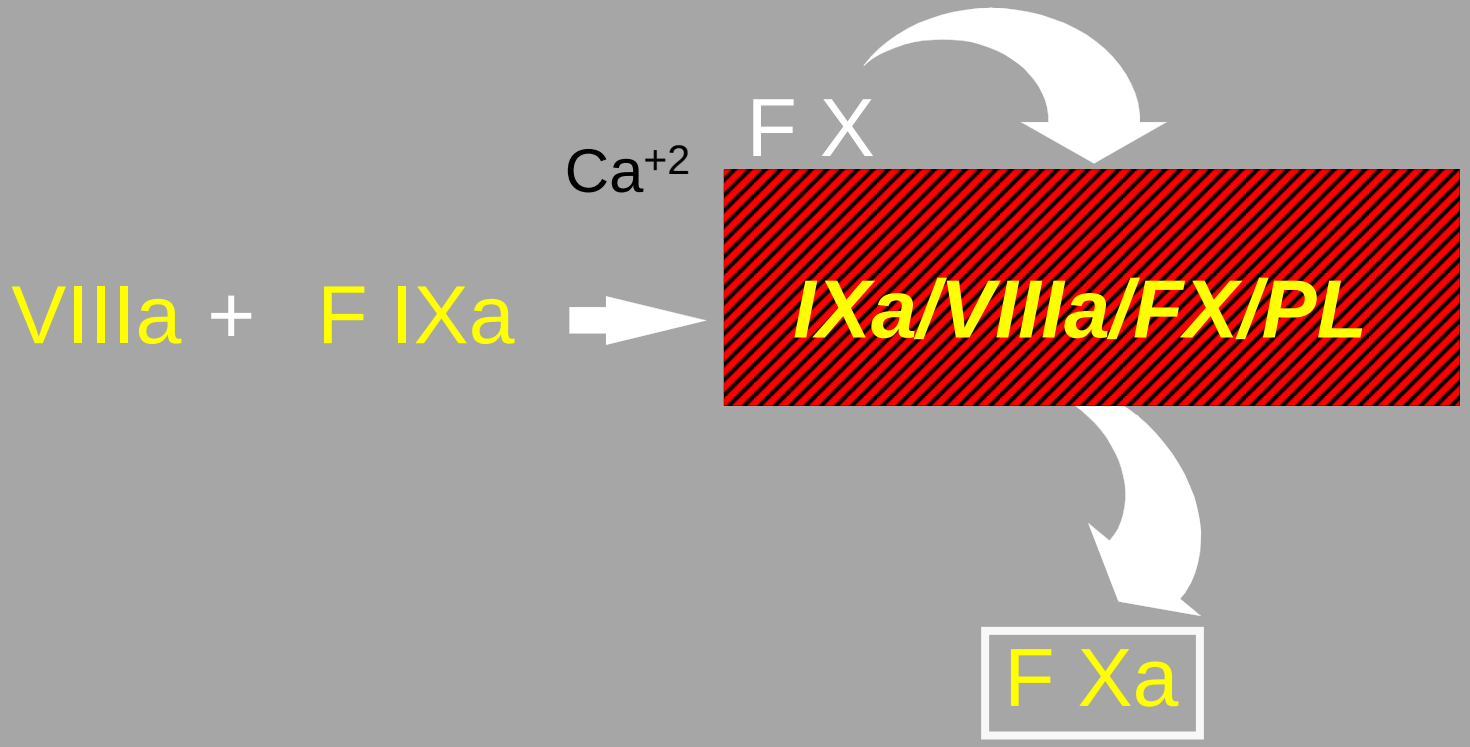
EPO-dependent cell proliferation activity of Ab12 variants



activity of Ab12 variants correlates inversely with Kd

COAGULATION CASCADE

Tenase Complex

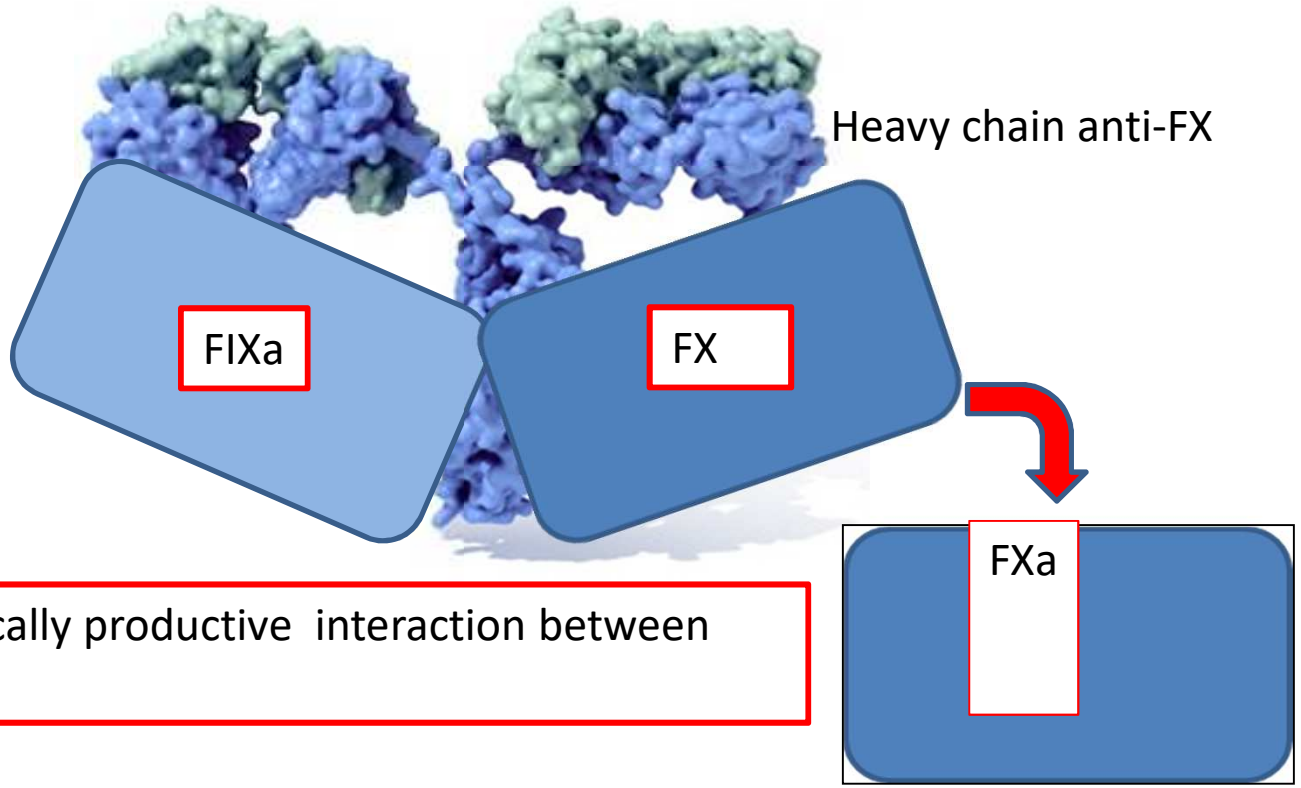


Humanized asymmetric antibody mimicking FVIIIa function

Emicizumab (ACE910)

Heavy chain anti-FIXa

Heavy chain anti-FX



Maintaining a catalytically productive interaction between FIXa and FX

potential to help overcome some of the clinical challenges faced in haemophilia

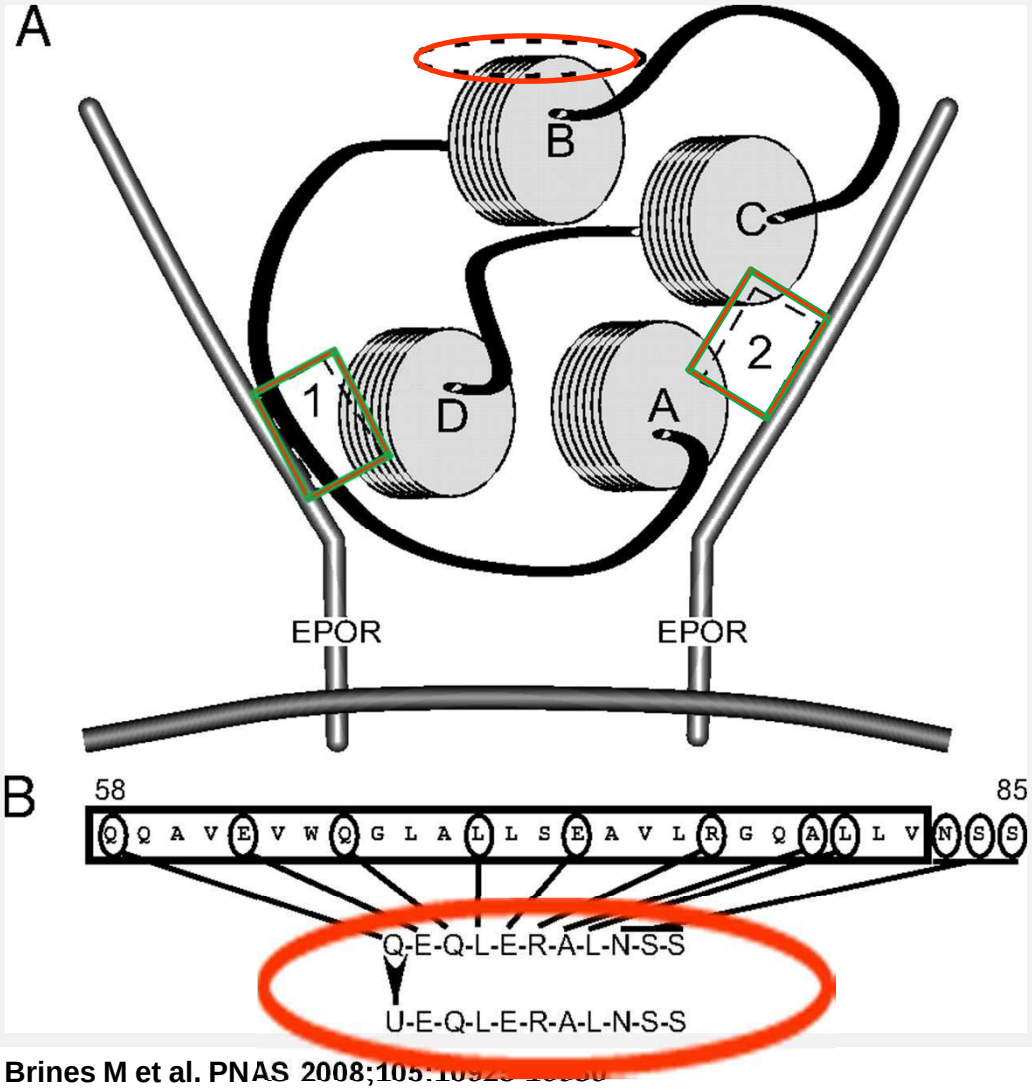
EPO's tissue-protective actions have been shown to be mediated by a tissue-protective receptor complex consisting of the EPO receptor and the β common-receptor (CD131) subunit that is also used by GM-CSF, IL-3, and IL-5.

Slide EPO1

helix B-surface peptide (HBSP)

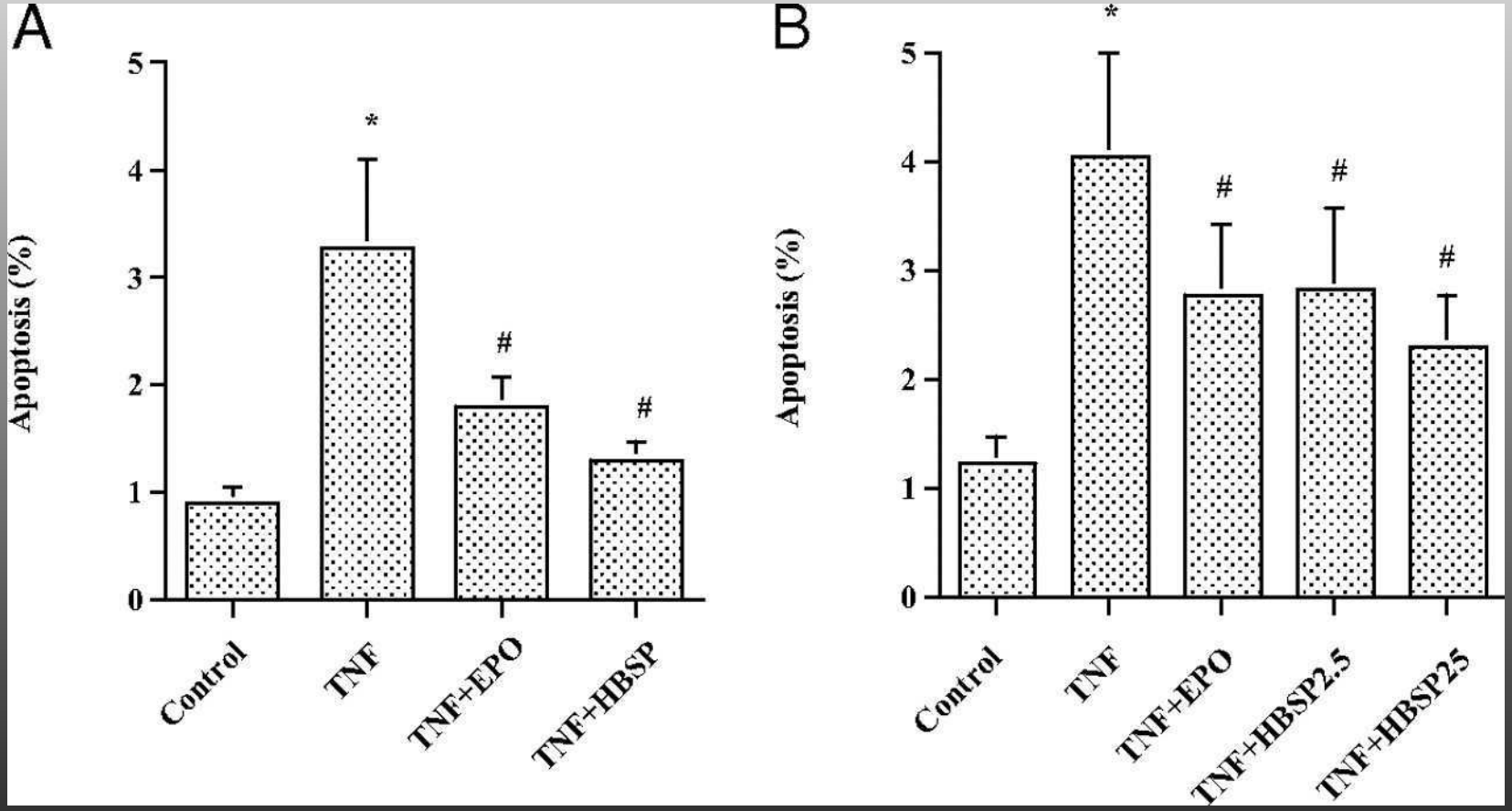
This peptide is composed of 11 amino acids (QEQLERALNSS) derived from the aqueous face of helix B of EPO and exhibits tissue-protective activities

Structure of EPO indicating tissue protective domains and sequences.



Brines M et al. PNAS 2008;105:

Effect of HBSP on TNF-α-induced cardiomyocyte apoptosis.

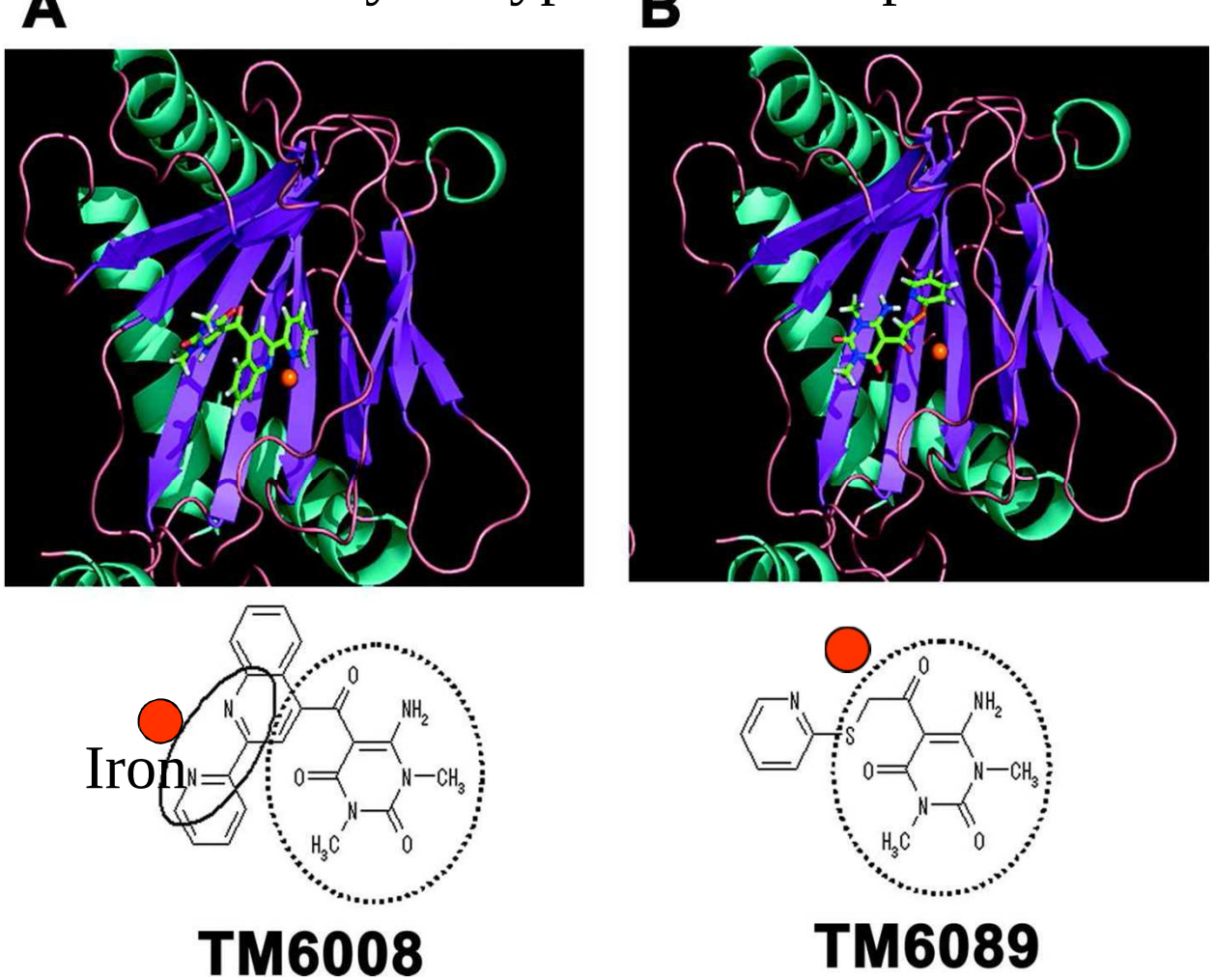


Ueba H et al. PNAS 2010;107:14357-14362

HIF prolyl hydroxylase inhibition results in endogenous erythropoietin induction, erythrocytosis

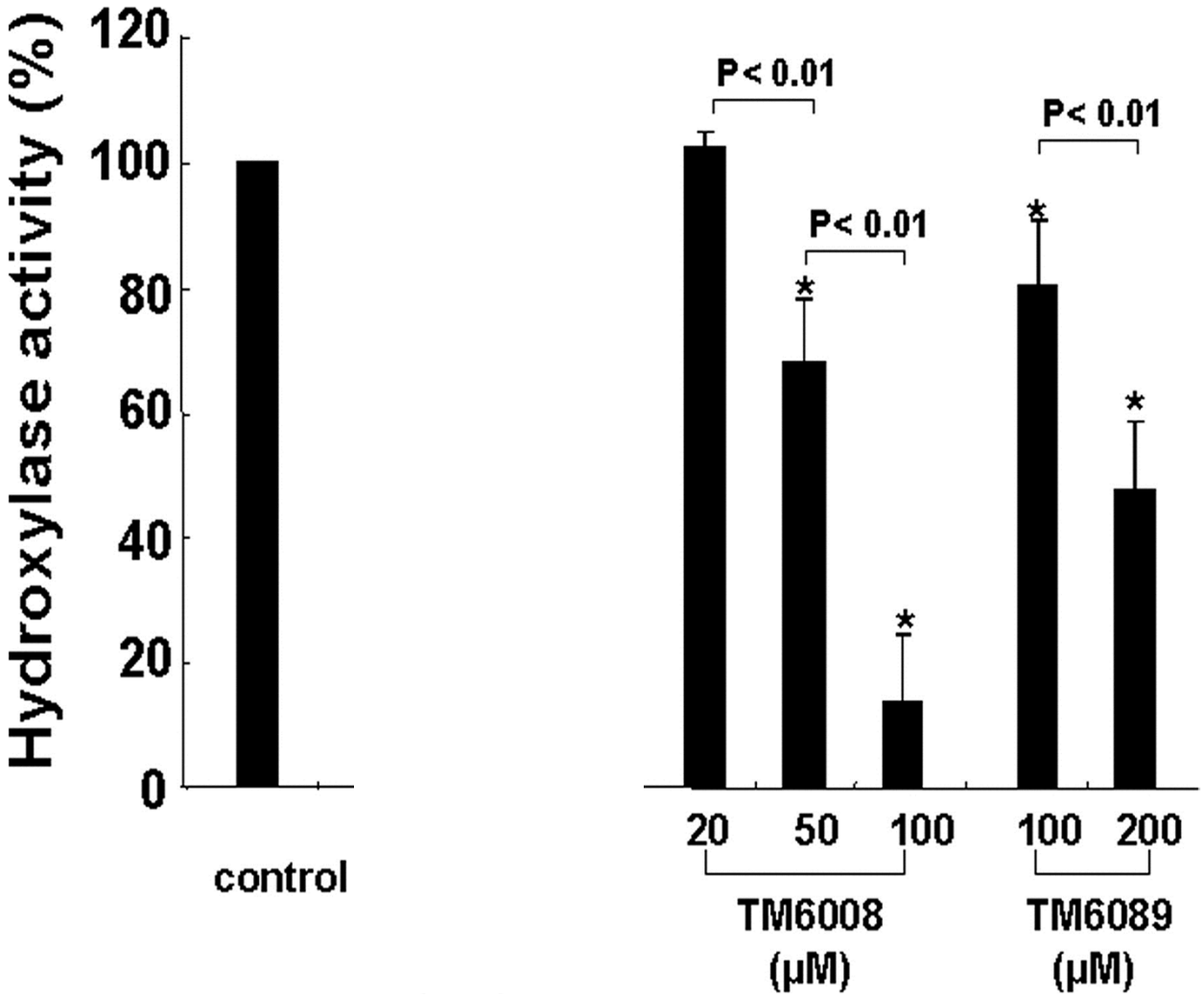
Figure 3. The predicted binding modes of TM6008 (A) and TM6089 (B) in PHD2.

PHD produces trans-4-hydroxyproline in the presence of Fe(II)



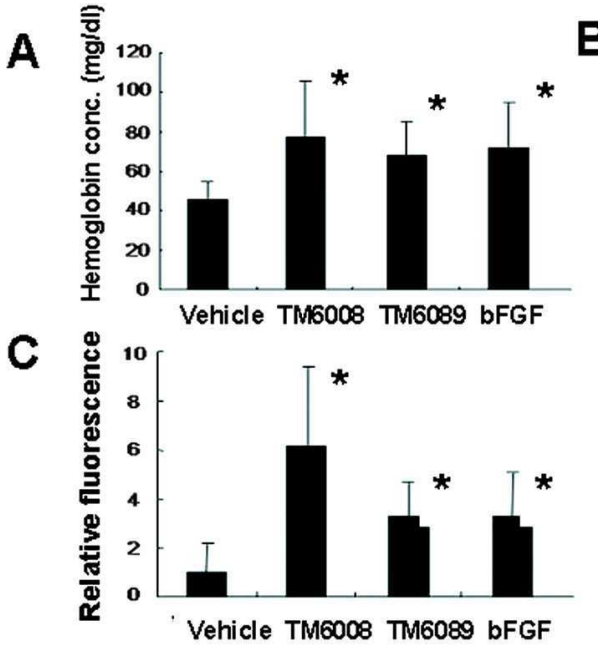
Nangaku M et al. Arterioscler Thromb Vasc Biol 2007;27:2548-2554

Figure 2. Inhibition of PHD activity.



Nangaku M et al. Arterioscler Thromb Vasc Biol 2007;27:2548-2554

Figure 4. Stimulation of angiogenesis in the mouse



Nangaku M et al. Arterioscler Thromb Vasc Biol 2007;27:2548-2554

Prosegue nel file PHD