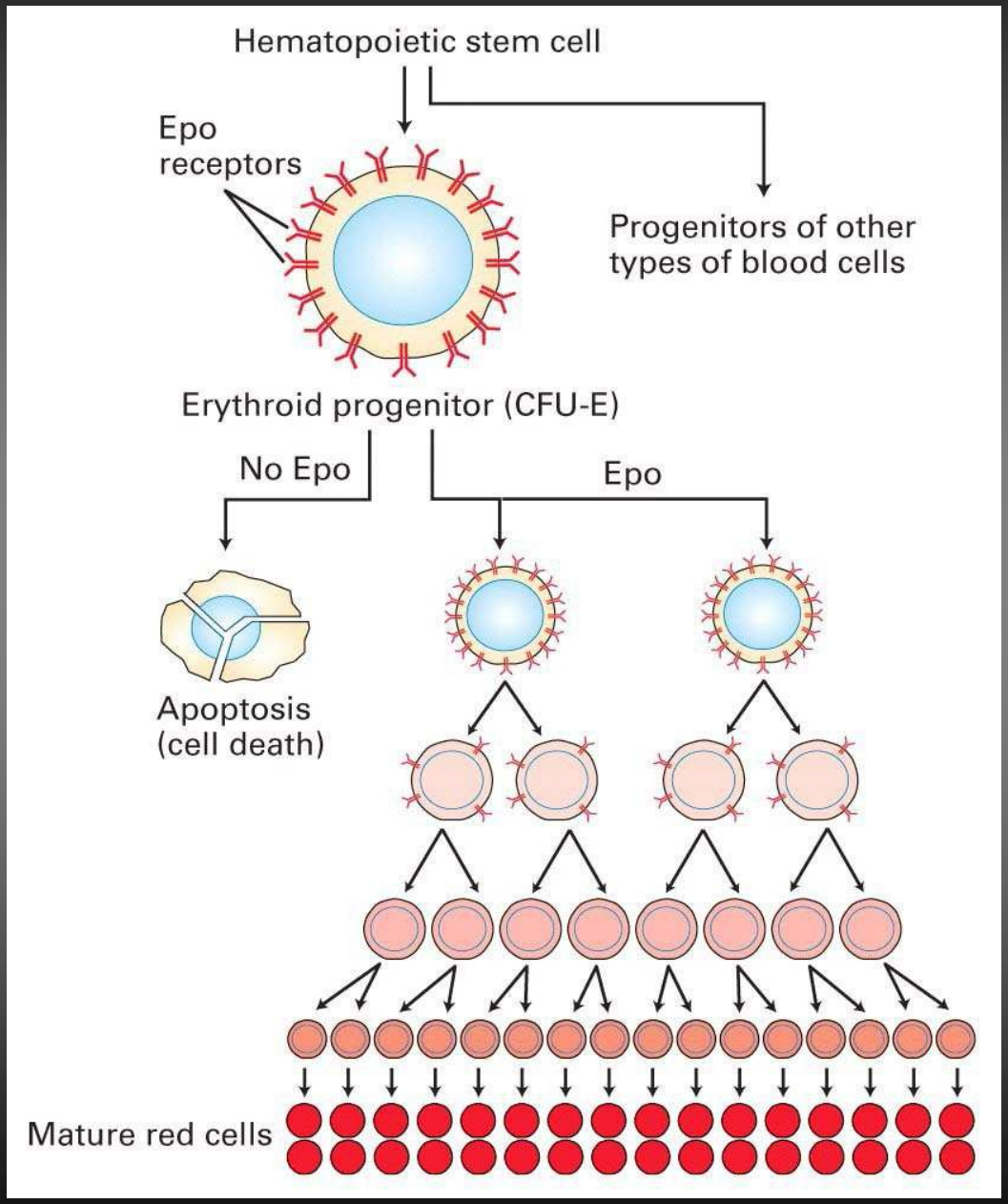

Funzioni di Epo e
molecole terapeutiche

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

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

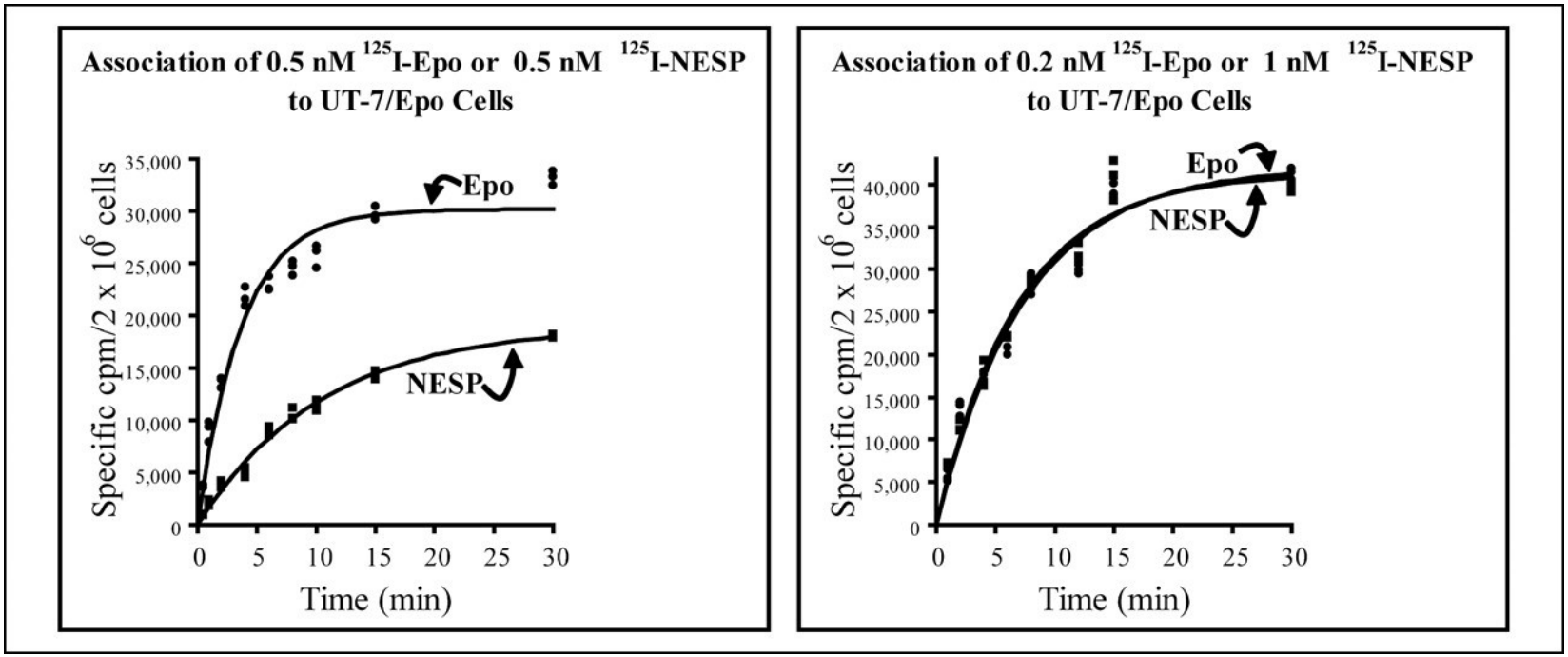
Epo contains **one** O-linked and **three** N-linked carbohydrate chains, each having 2–4 branches that often end in a negatively charged sialic acid.

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

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

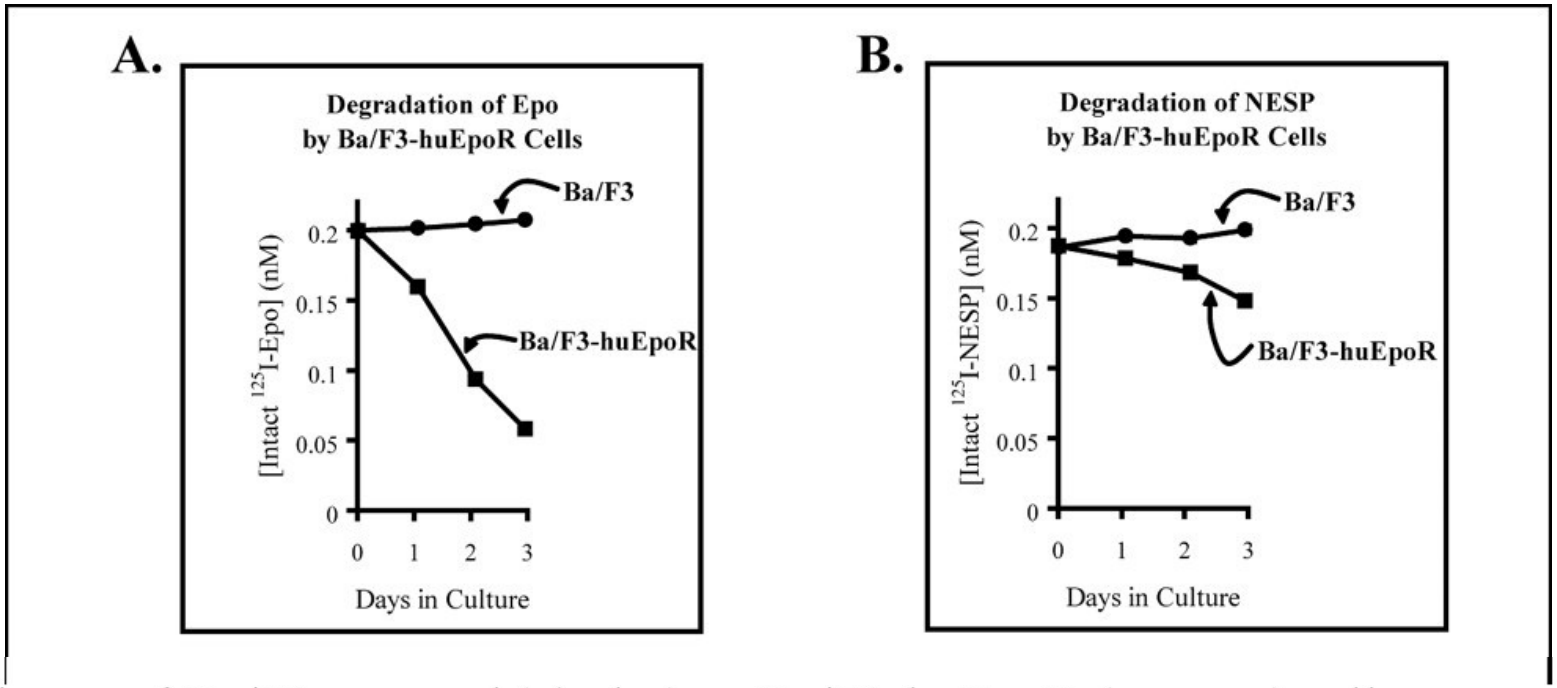
Gross A W , Lodish H F J. Biol. Chem. 2006;281:2024-2032

jbc

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?

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



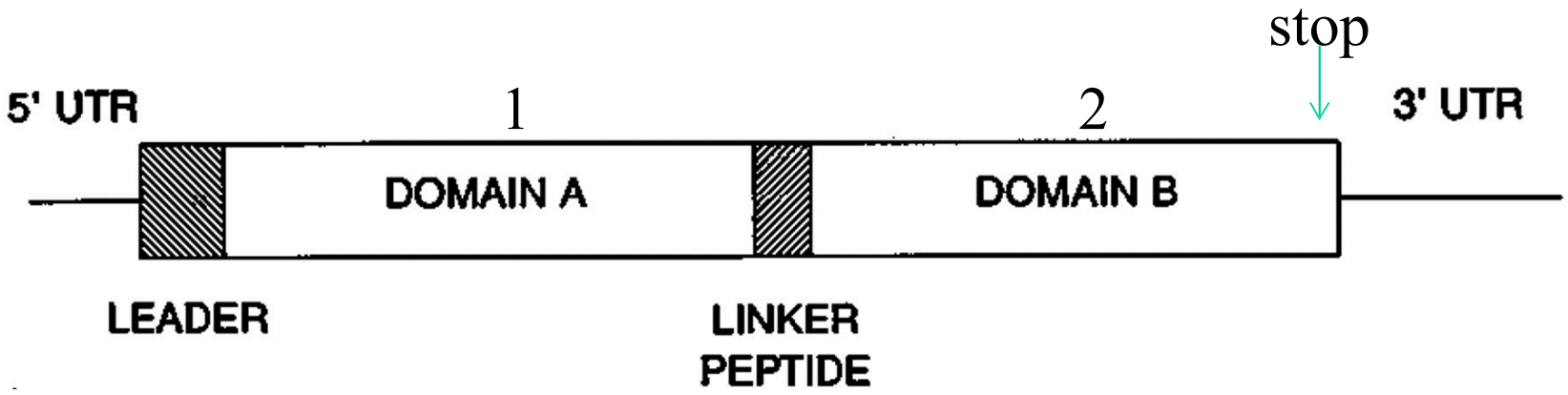
cultures of Ba/F3 parental (circles) or Ba/F3-huEpoR (squares) cells were initiated with excess IL-3 and 0.2 nm 125I-Epo (A) or 0.2 nm 125I-NESP (B)

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

jbc

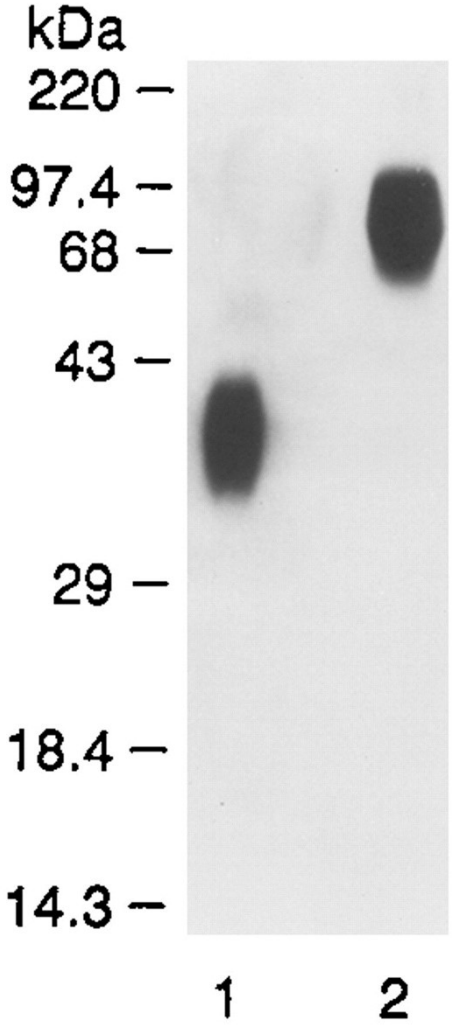
Epo-Epo” -a peptide-linked head-to-tail dimer

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

"Hormone mimicry"

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

Wrighton et al, Science 1996

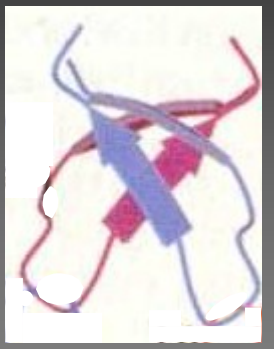
Sintesi di piccoli peptidi (20 aa) che si legano al recettore dell'Epo e lo attivano "mimano" l'effetto biologico dell'Epo

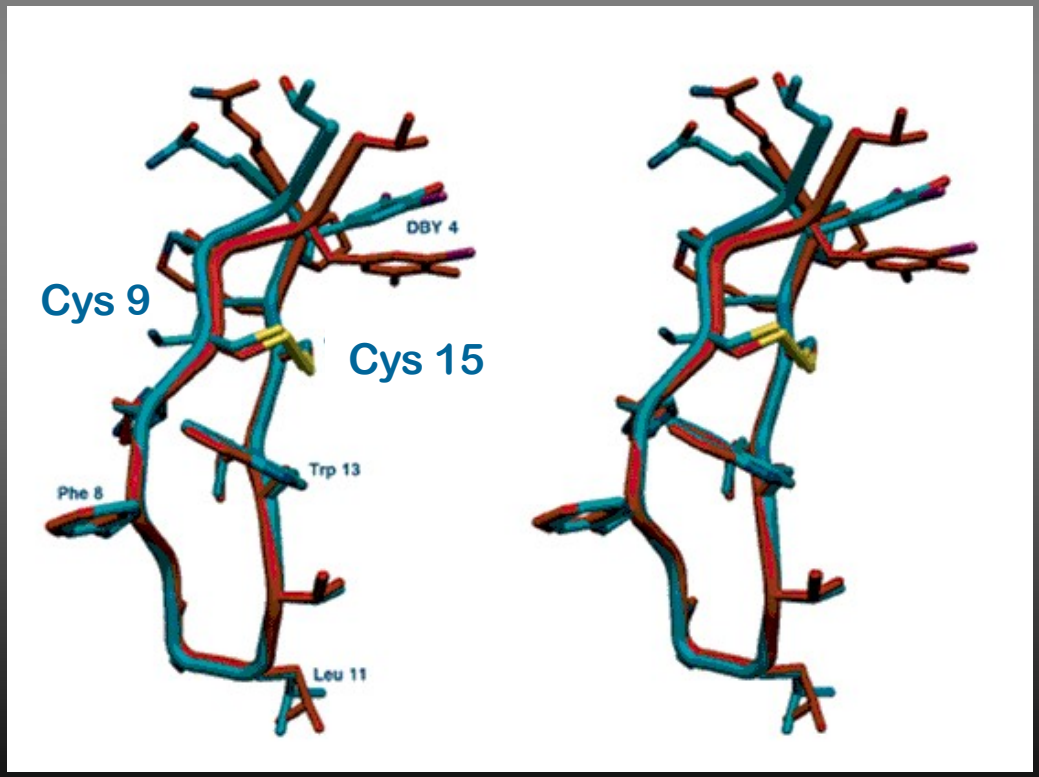
EMP1

Eritropoietina

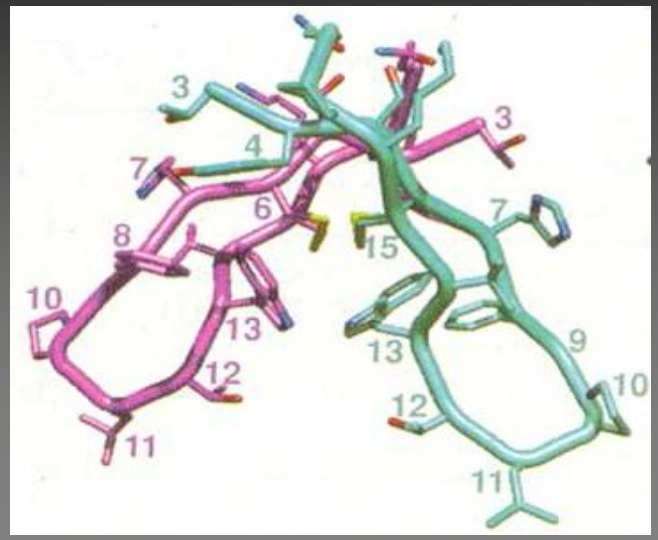
EMP1 (EPO mimetic peptides (EMPs)

- 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



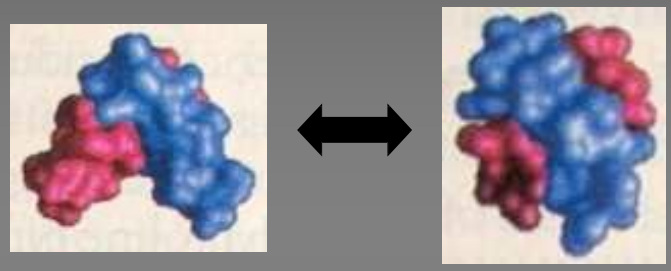


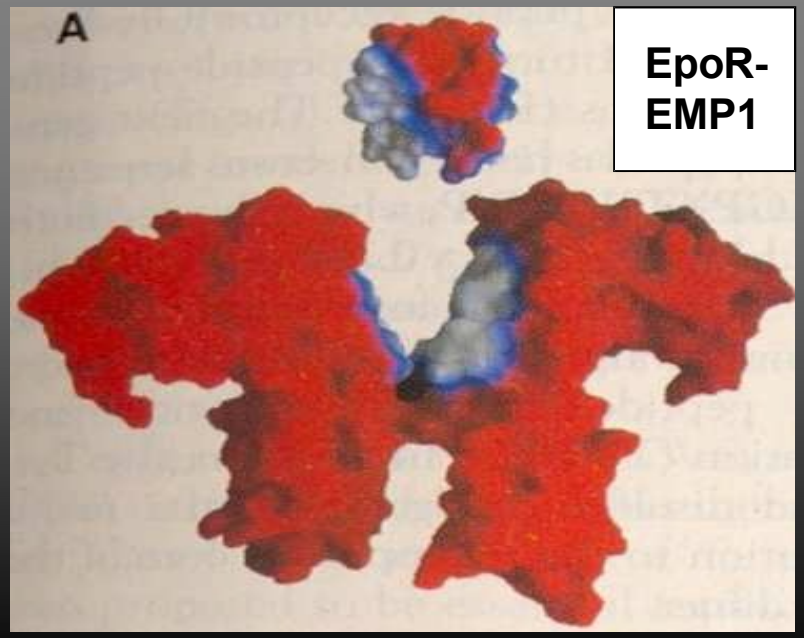
Complesso EpoR-EMP1



EMP1 dimerizza per legarsi a EpoR

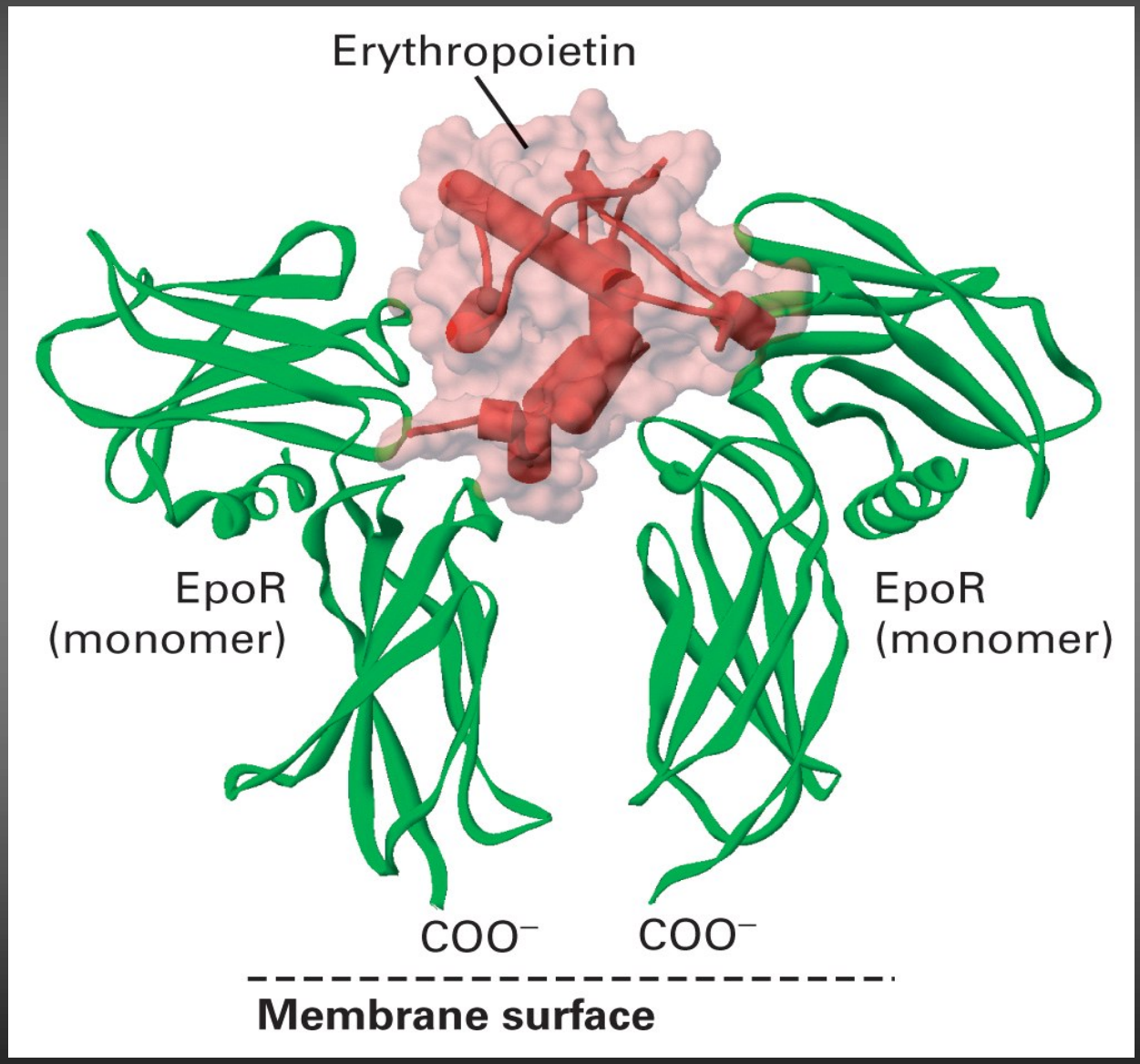
Struttura dimerica molto forte, stabilizzata da 4 legami idrogeno



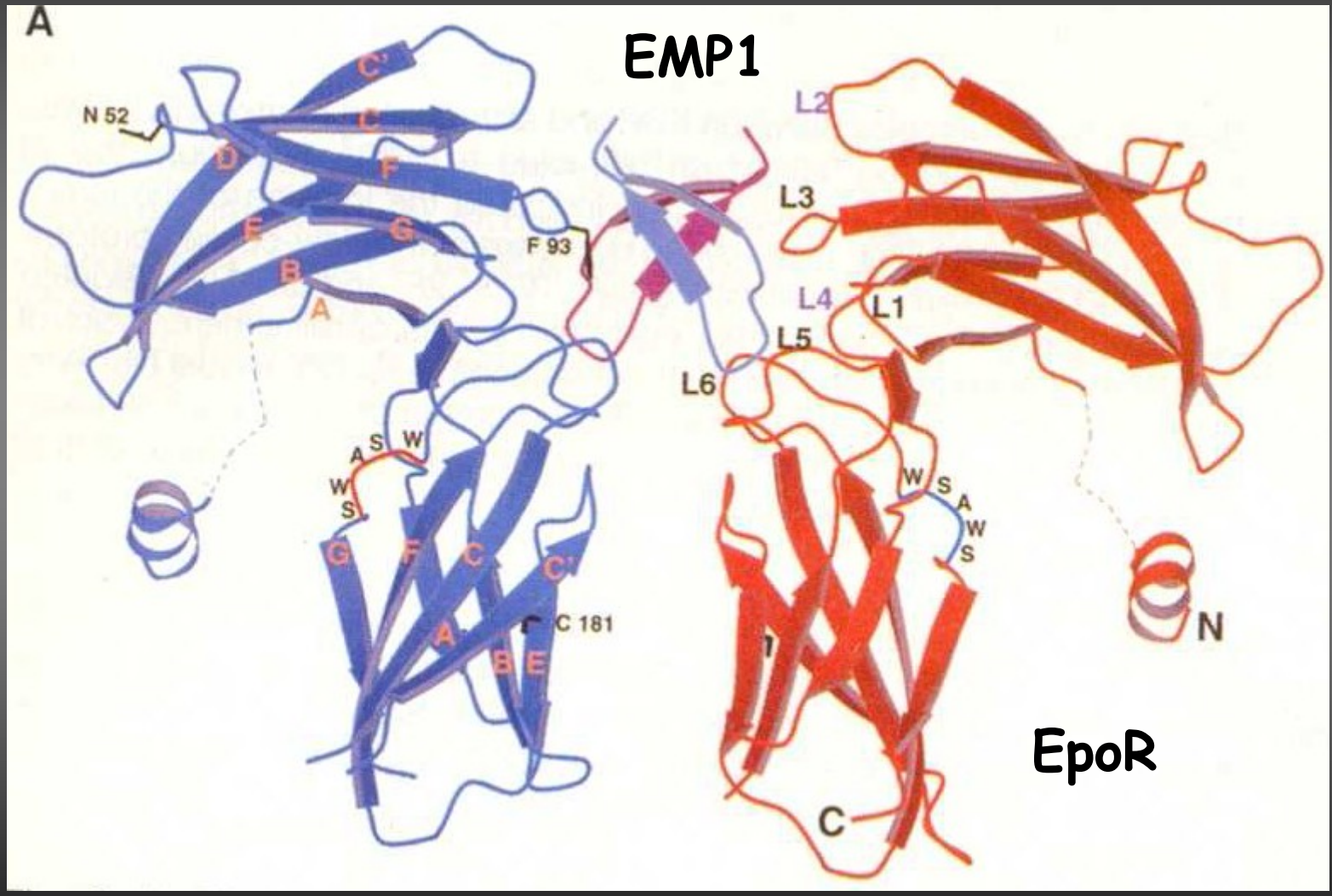


Ogni monomero di EMP1 interagisce sia con l'altro monomero che con EpoR

Erythropoietin-Epo Receptor complex

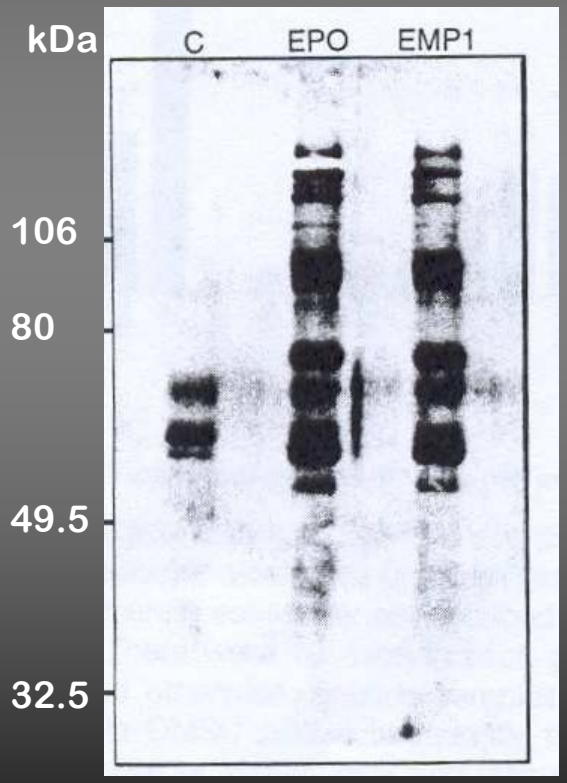


Complesso EpoR-EMP1



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

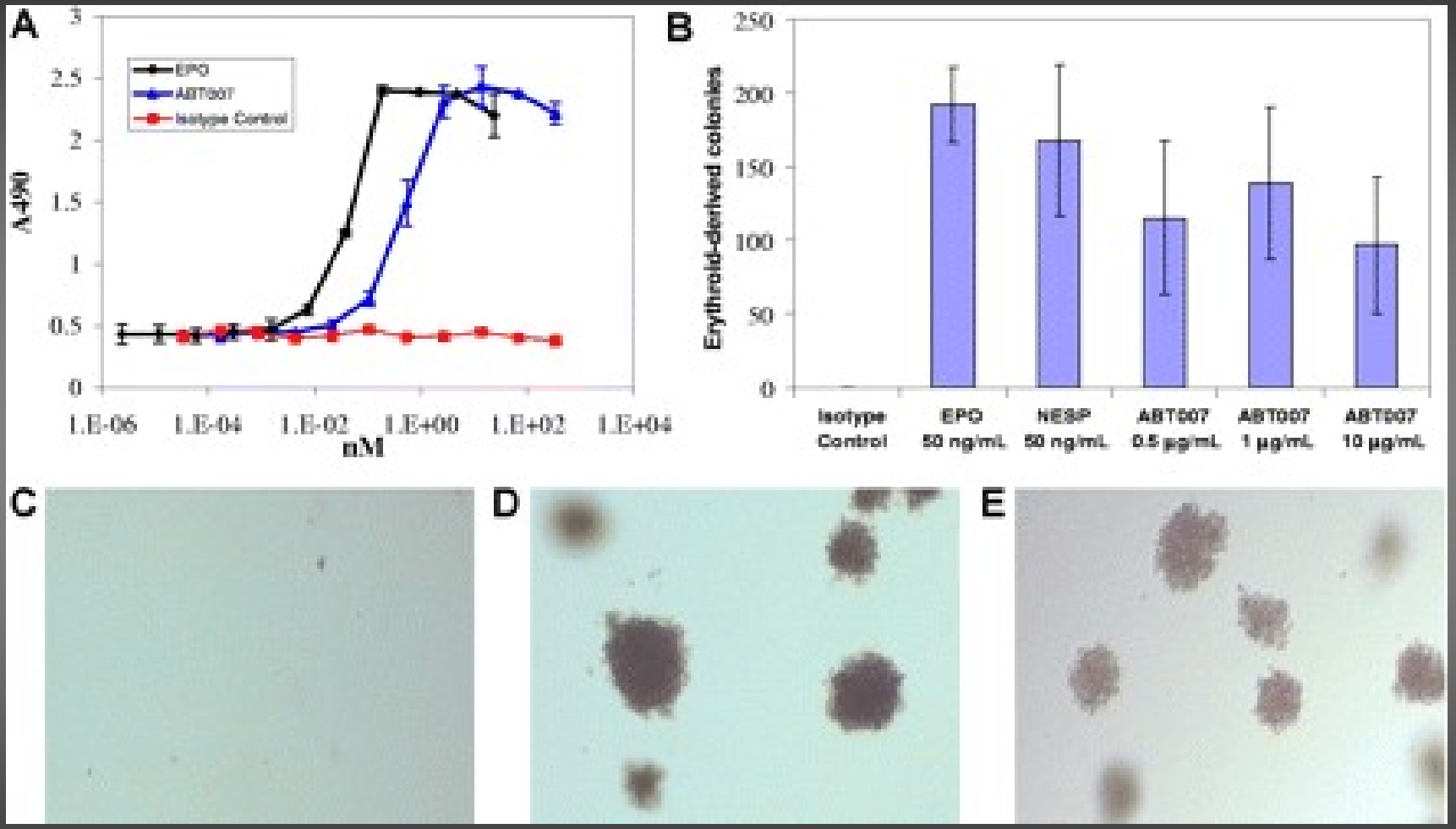
Wrighton et al., Science 1996, 273:458-463

"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



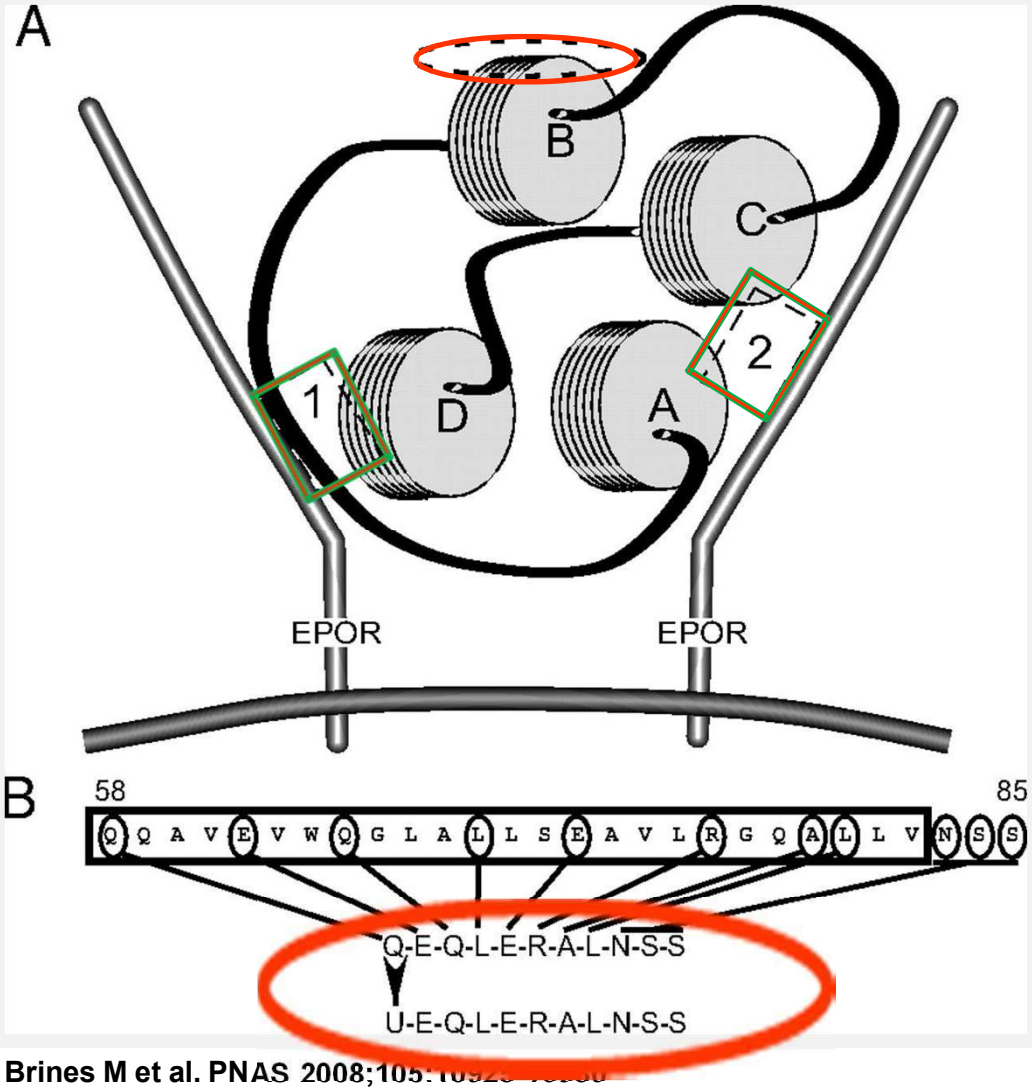
ABT007 stimulates in vitro erythropoiesis

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.

helix B-surface peptide (HBSP)

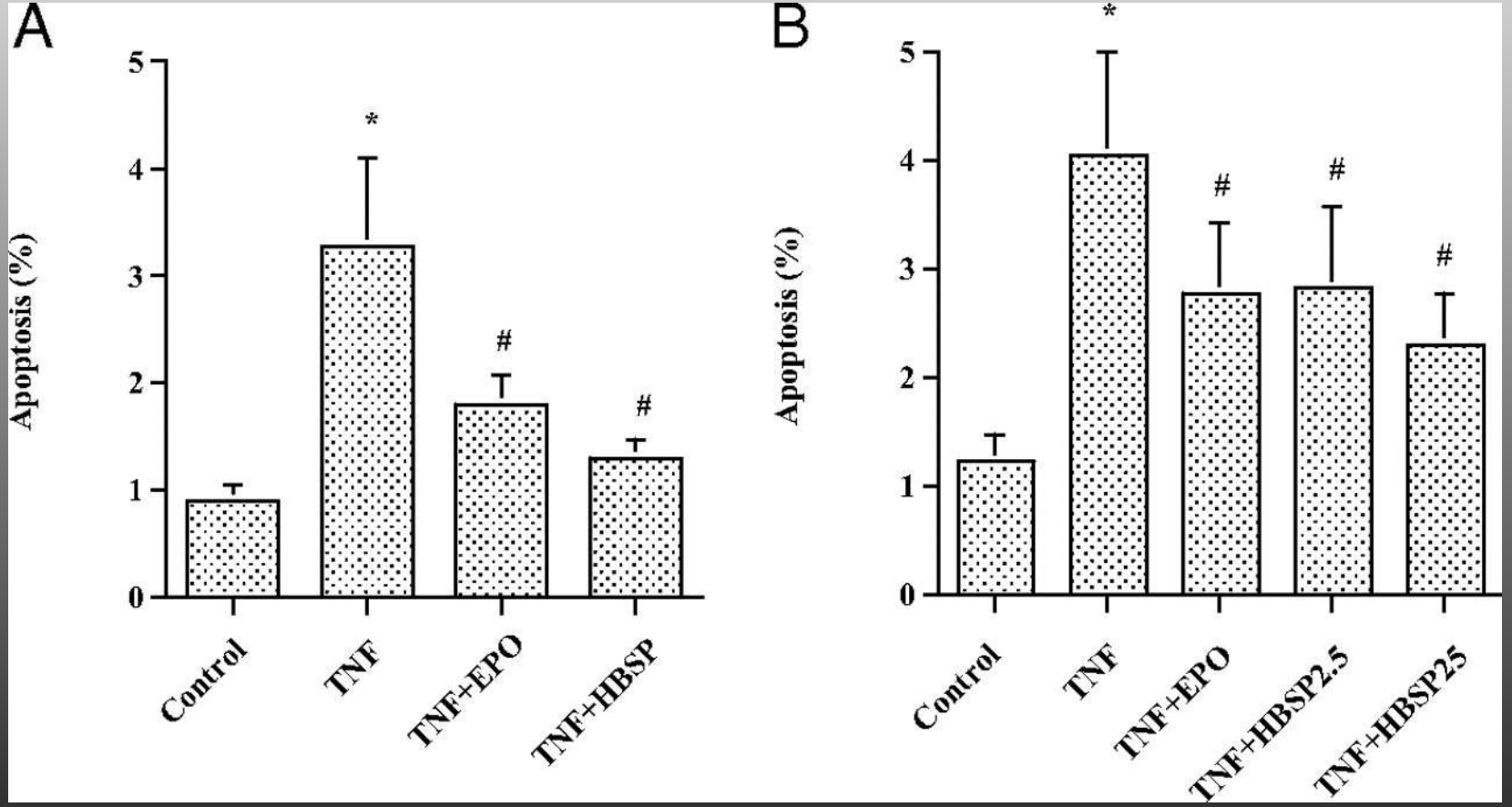
This peptide is composed of 11 amino acids (QEQLERALNSS) derived from the aqueous face of helix B of EPO and exhibits tissue-protective activities

Structure of EPO indicating tissue protective domains and sequences.



Brines M et al. PNAS 2008;105:10925–10930

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



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