Università degli Studi di Ferrara

Facoltà di Scienze MM.FF.NN.

Laurea specialistica in
Scienze biomolecolari e cellulari

Ciclossigenasi (COX): struttura e ruolo

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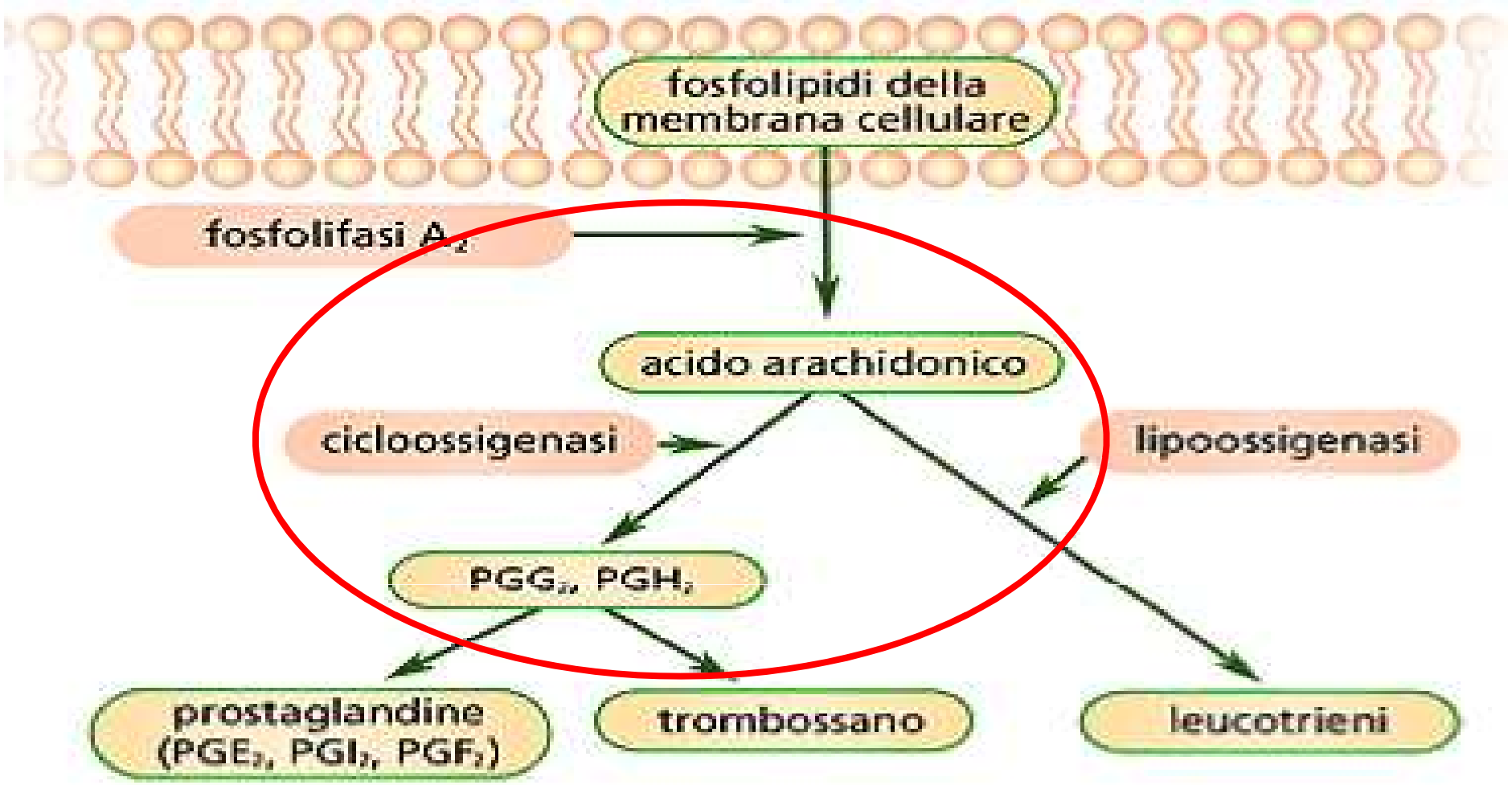
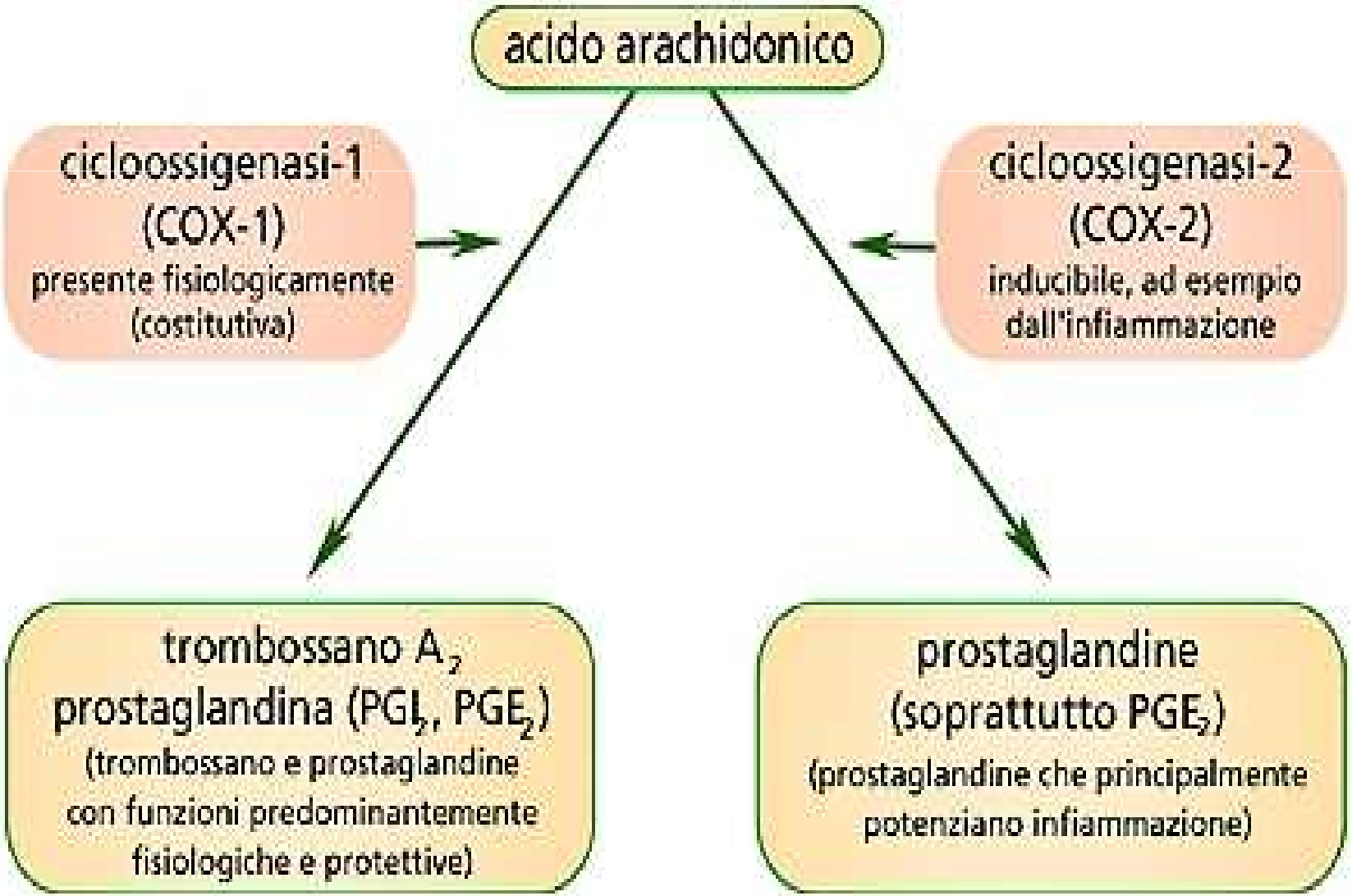

fosfolipidi della membrana cellulare
fosfolifasi A_2
acido arachidonico
cicloossigenasi
lipoossigenasi
PGG_2, PGH_2
prostaglandine (PGE_2, PGI_2, PGF_2)
trombossano
leucotrieni

Le isoforme

COX 1 - COX 2


acido arachidonico
cicloossigenasi-1
(COX-1)
presente fisiologicamente
(costitutiva)
cicloossigenasi-2
(COX-2)
inducibile, ad esempio
dall'infiammazione
trombossano A_2
prostaglandina (PGI_2, PGE_2)
(trombossano e prostaglandine
con funzioni predominantemente
fisiologiche e protettive)
prostaglandine
(soprattutto PGE_2)
(prostaglandine che principalmente
potenziano infiammazione)

Differenze fra le due isoforme

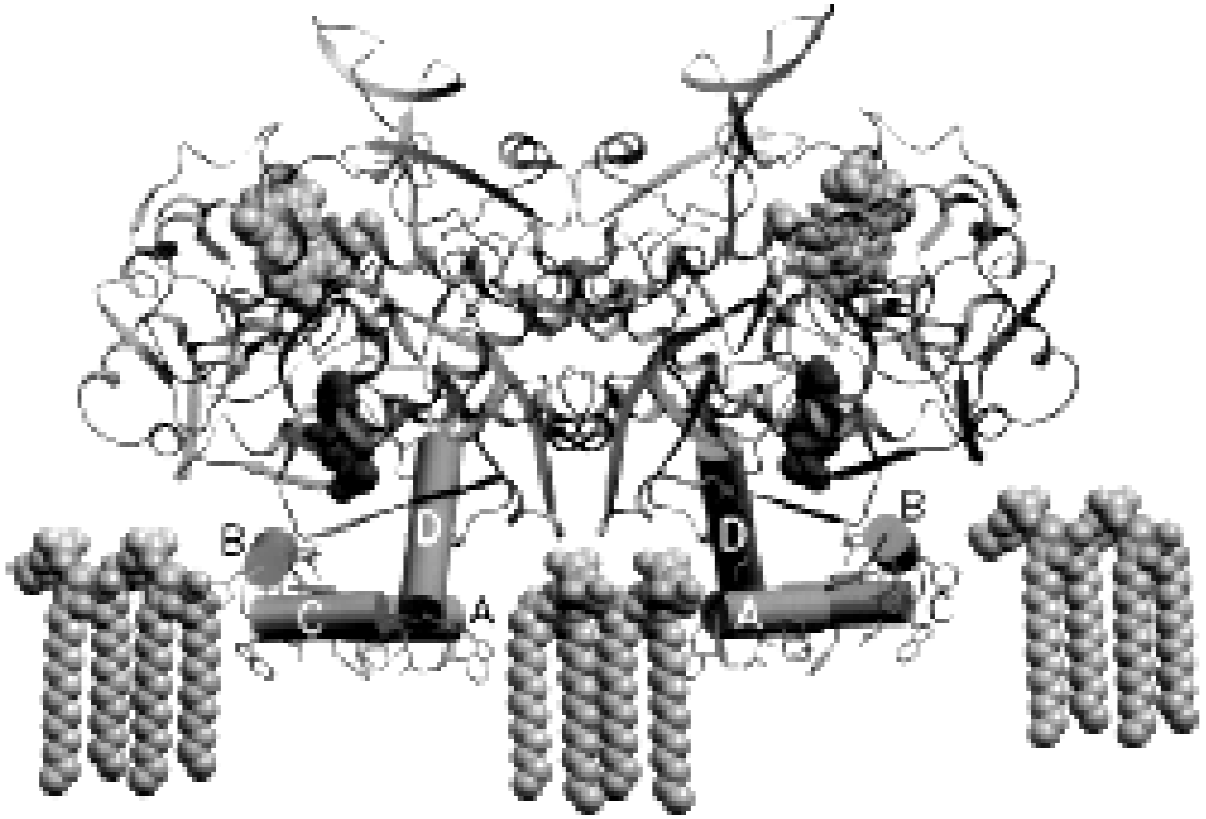
	COX I	COX II
Gene	**22 Kb, cromosoma 9 mRNA 2.8 Kb**	**8 Kb, cromosoma 1 mRNA 4.3 Kb**
Enzima	**70 Kd proteina di membrana**	**70 Kd proteina di membrana**
Substrato	**Acido arachidonico**	**Acido arachidonico, altri acidi grassi simili**
Km (µmol/L) (Acido Arachidonico)	**5.4**	**5.6**

Differenze biologiche fra le due isoforme

	COX I	COX II
Espressione	**Costitutiva**	**Inducibile**
Funzioni	• **Controllo delle funzioni cellulari** • **Piastrine** • **Stomaco** • **Rene**	• **Processi infiammatori** • **Macrofagi** • **Leucociti** • **Fibroblasti**
Inibizione	**Aspirina, Farmaci antinfiammatori non steroidei (FANS)**	**Aspirina, FANS coxibici**

La struttura e la catalisi

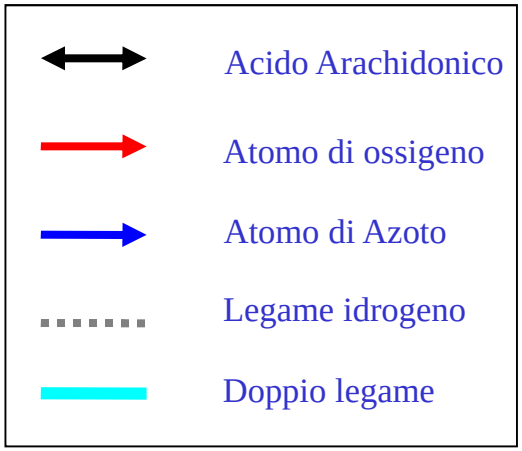
Dominio “membrane-binding”

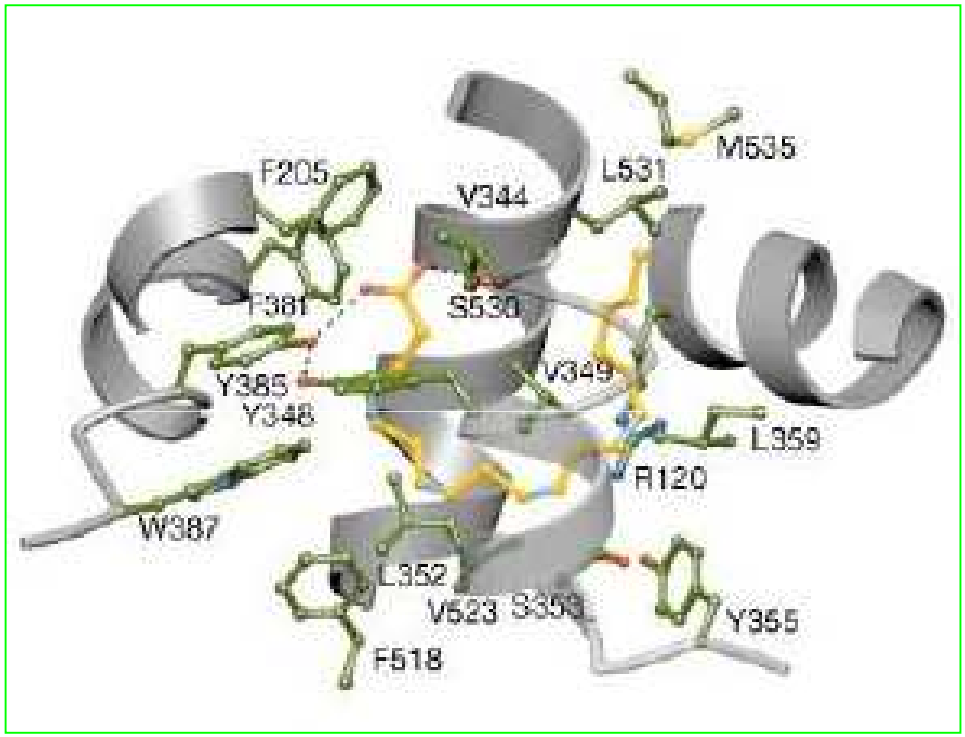


Ogni subunità ha un dominio per il legame con le membrana

Questo dominio è composto da 4 a-eliche anfipatiche che si legano ad una precisa e ottimale profondità ad uno solo dei due strati della membrana cellulare

Il Sito Ciclossigenasico





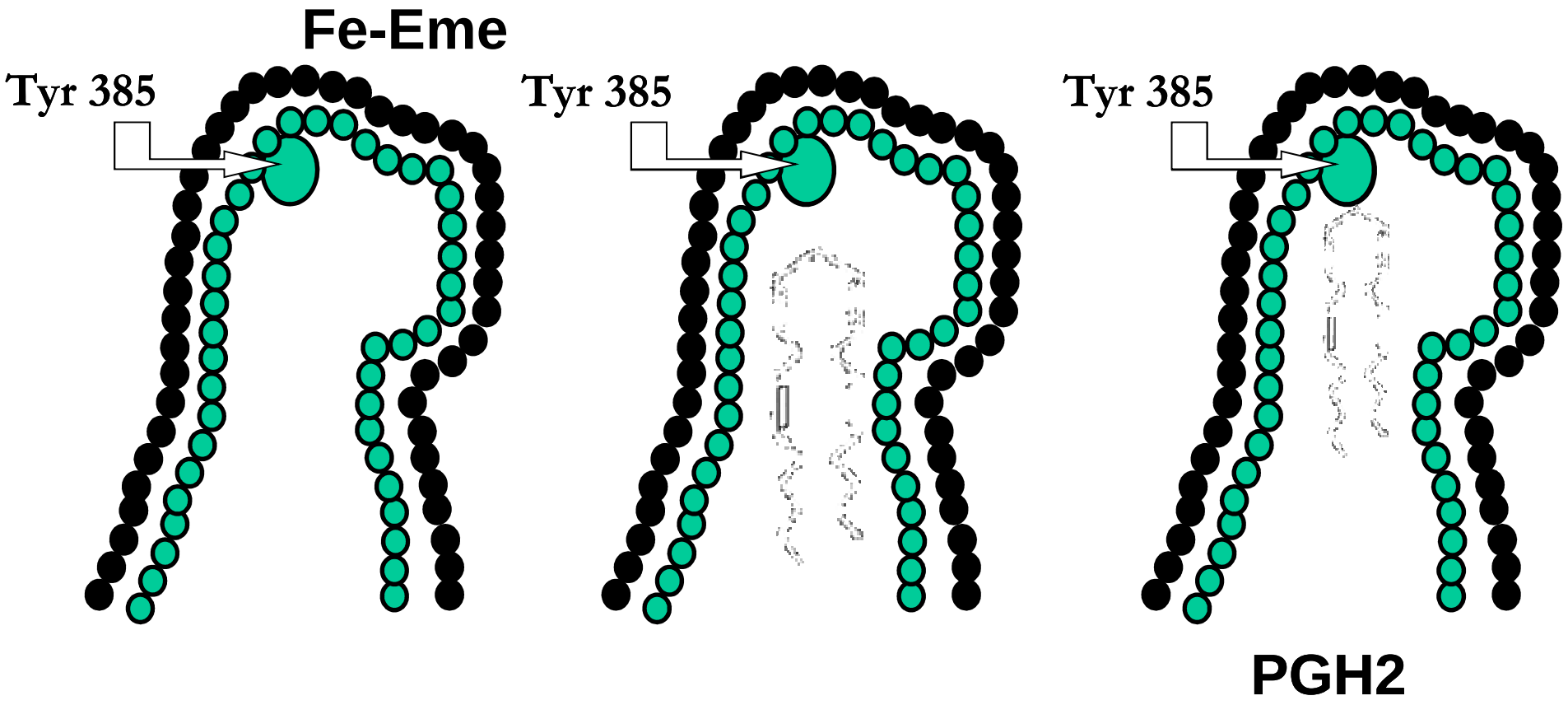
L'Acido Arachidonico (AA) viene legato in questa tasca formata da 4 α eliche e tappezzata da amminoacidi idrofobici W F L V.

La testa polare dell'acido è tenuta in posizione dai legami idrogeno formati con la serina 530 e la tirosina 385 e 348.

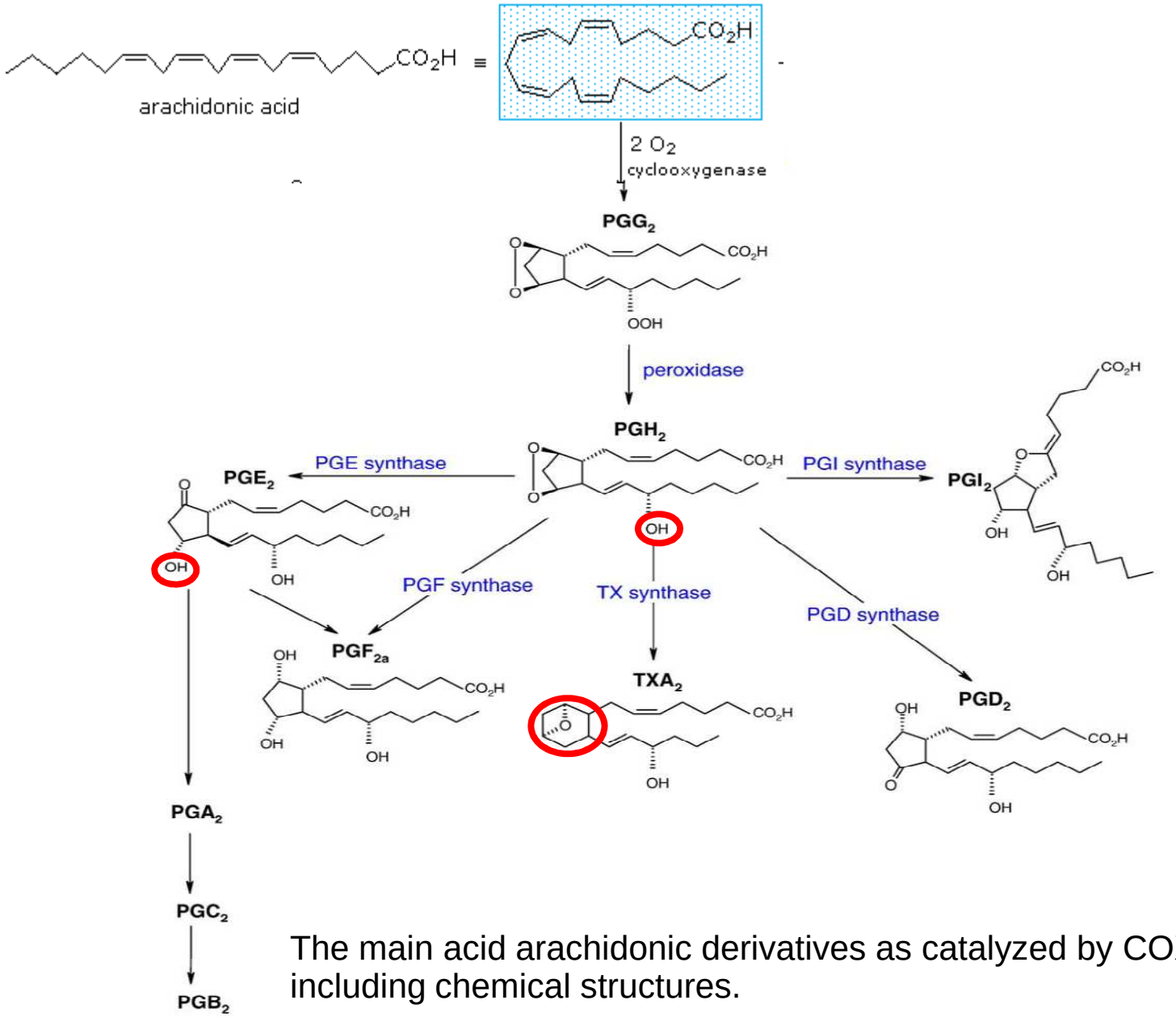
In posizioni vicine ai carboni 13-15 dell'AA, l'idrofobicità della catena è interrotta da residui di serina, tirosina e arginina, importani per la catalisi.

Importante per il funzionamento della proteina è anche il Triptofano 387 anche se non se ne conosce la motivazione

Sintesi delle prostaglandine a partire dall'acido arachidonico



Acido arachidonico



The main acid arachidonic derivatives as catalyzed by COX and its isomerases including chemical structures.

I prodotti: I PROSTANOIDI

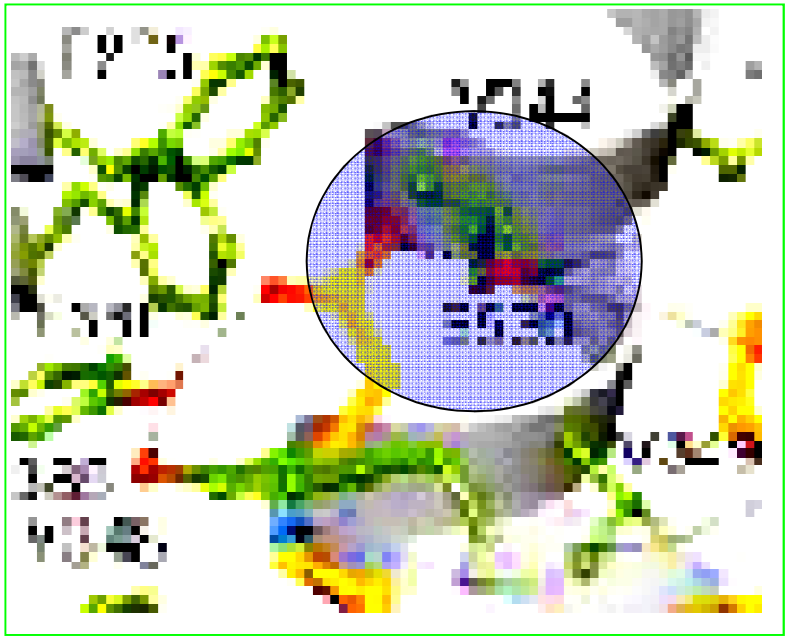
PGI_2	derivazione vasale	vasodilatazione inibiz. aggregazione piastrinica
PGE_2	deriva dai macrofagi	mediatore dell' infiammazione mediatore della febbre
PGD_2	deriva dai mastociti	vasodilatazione inibiz. aggregazione piastrinica
TXA_2	deriva dalle piastrine	vasocostrizione aggregazione piastrinica

Gli inibitori

I farmaci si dividono in due classi: inibitori aspecifici e inibitori della cox-2.

Della prima Famiglia il più conosciuto è l'acido acetil salicilico, la sua azione è semplice quanto efficace perché acetilando il residuo Ser530 altera il sito di legame, non solo stericamente, ma anche riducendo la polarità di quella zona e alterando l'ordine del legami idrogeno.

I farmaci di nuova generazione invece sfruttano la maggiore ampiezza del canale idrofobico nella Cox-2 e competono per il sito di legame dell'AA



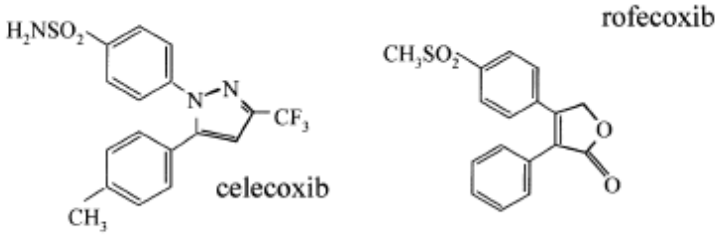
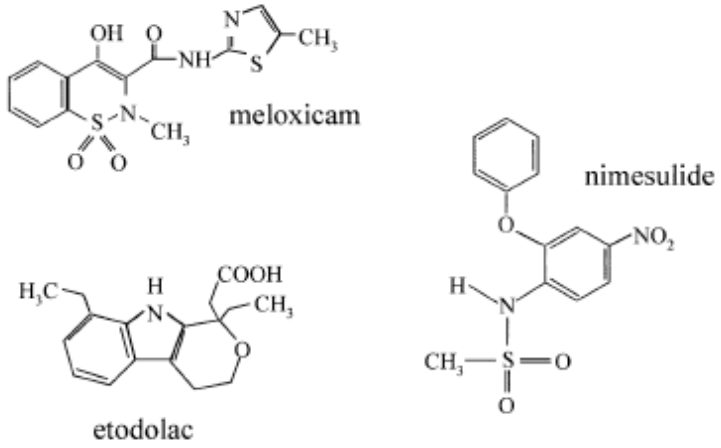
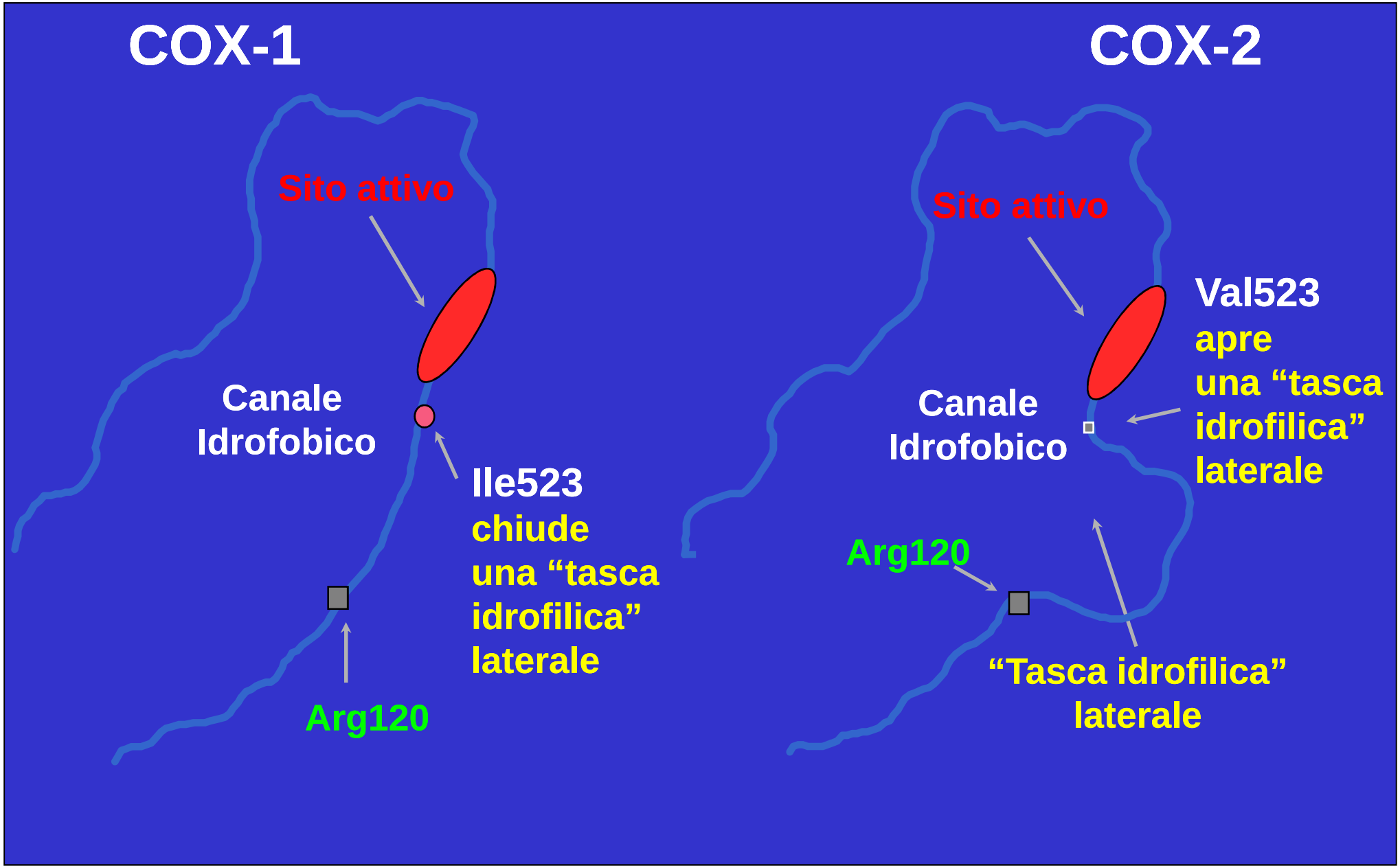


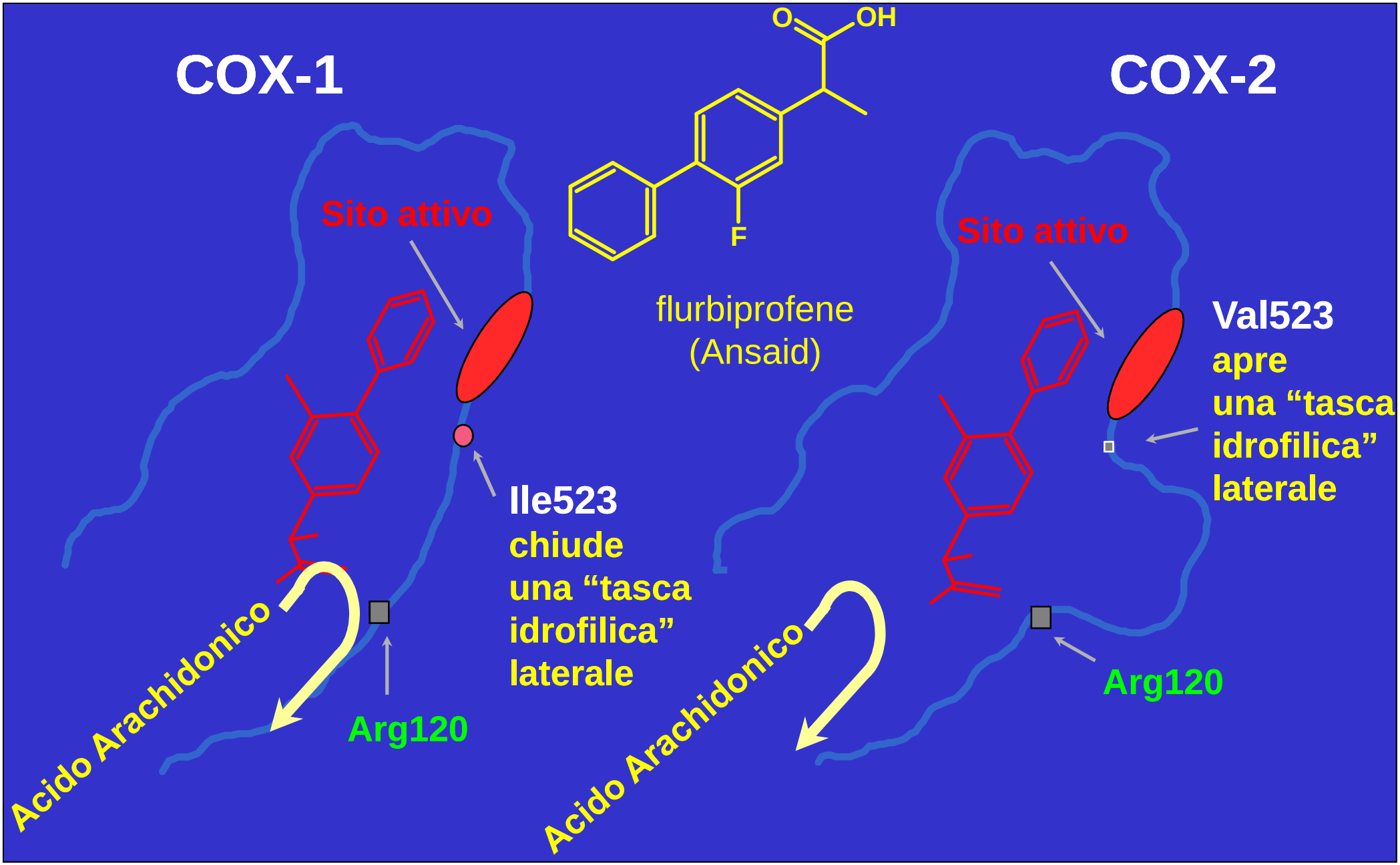
Figure 3 Chemical structures of celecoxib and rofecoxib.



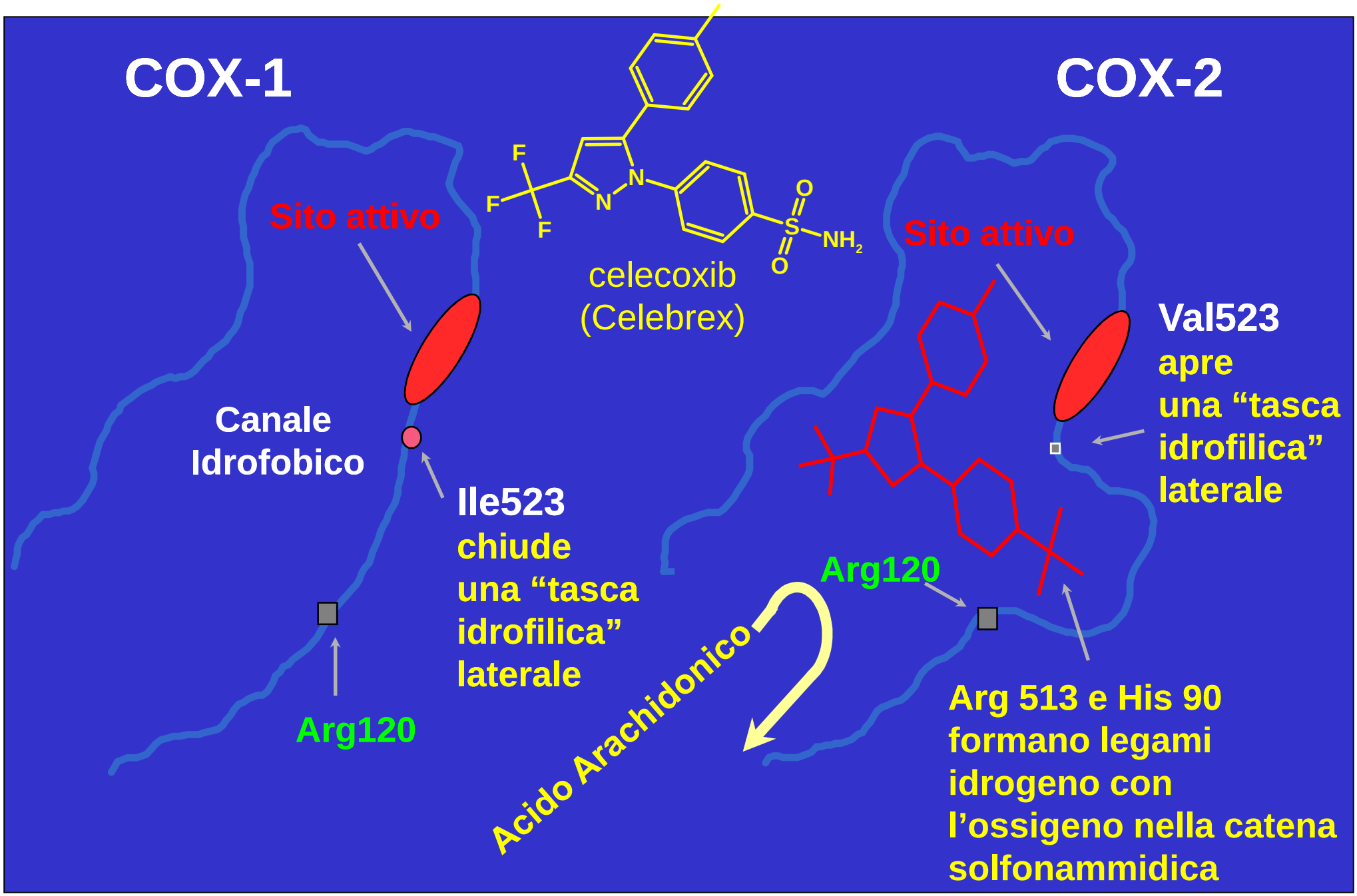
Le Due Isoforme della Ciclossigenasi



Le Due Isoforme della Ciclossigenasi



Le Due Isoforme della Ciclossigenasi



Selective inhibition of cyclooxygenase-2 enhances platelet adhesion in hamster arterioles in vivo.

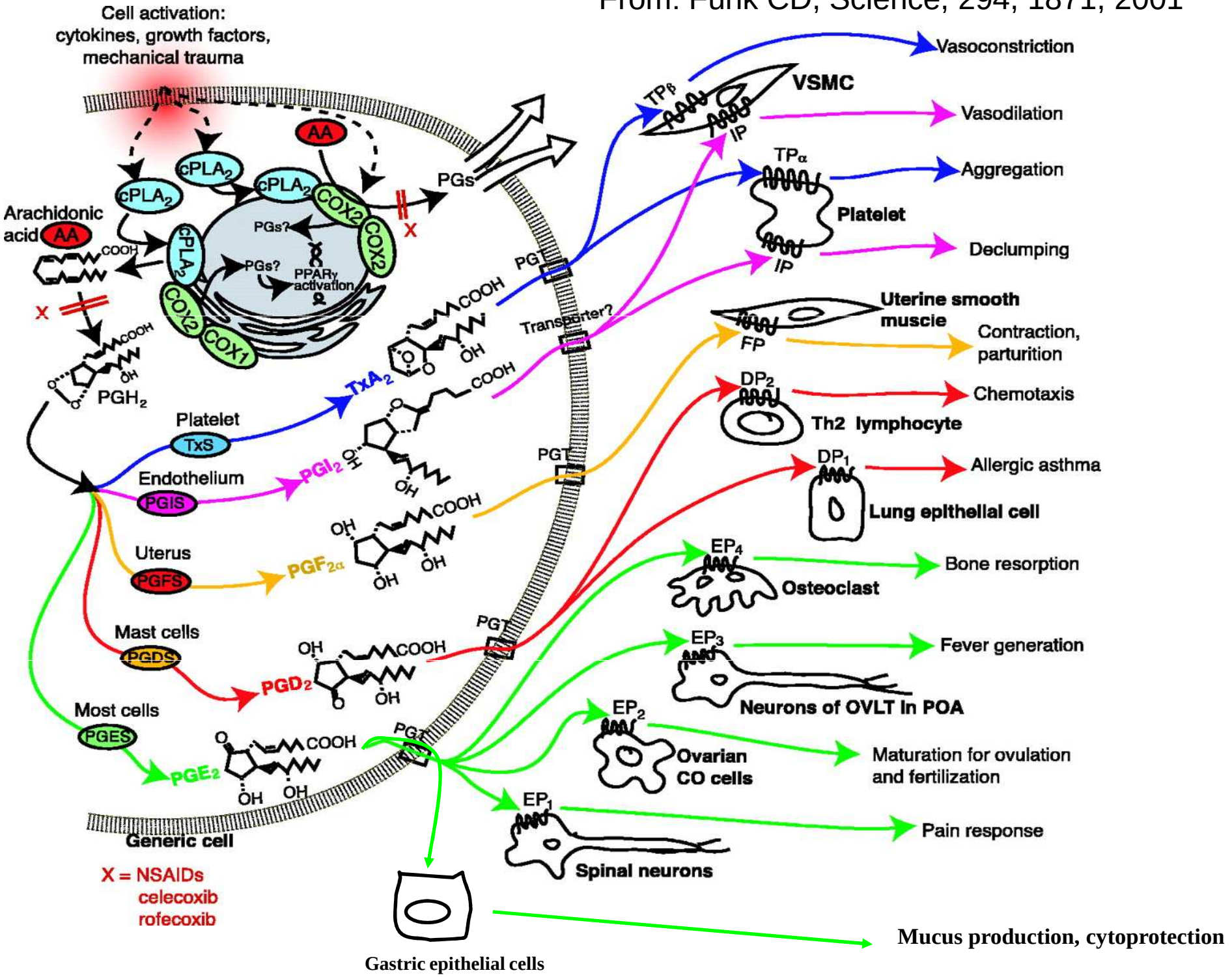
Martin A. Buerkle, Selim Lehrer, Hae-Young Sohn, Peter Conzen, Ulrich Pohl and Florian Krötz

"Our experiments demonstrate that selective inhibition of Cox-2 results in an increase in transient platelet interactions with the vessel wall in vivo, resulting in significant firm platelet adhesion that normally does not take place in these intact arterioles."

Circulation, 2004

Regolazione infiammazione

From: Funk CD, Science, 294, 1871, 2001



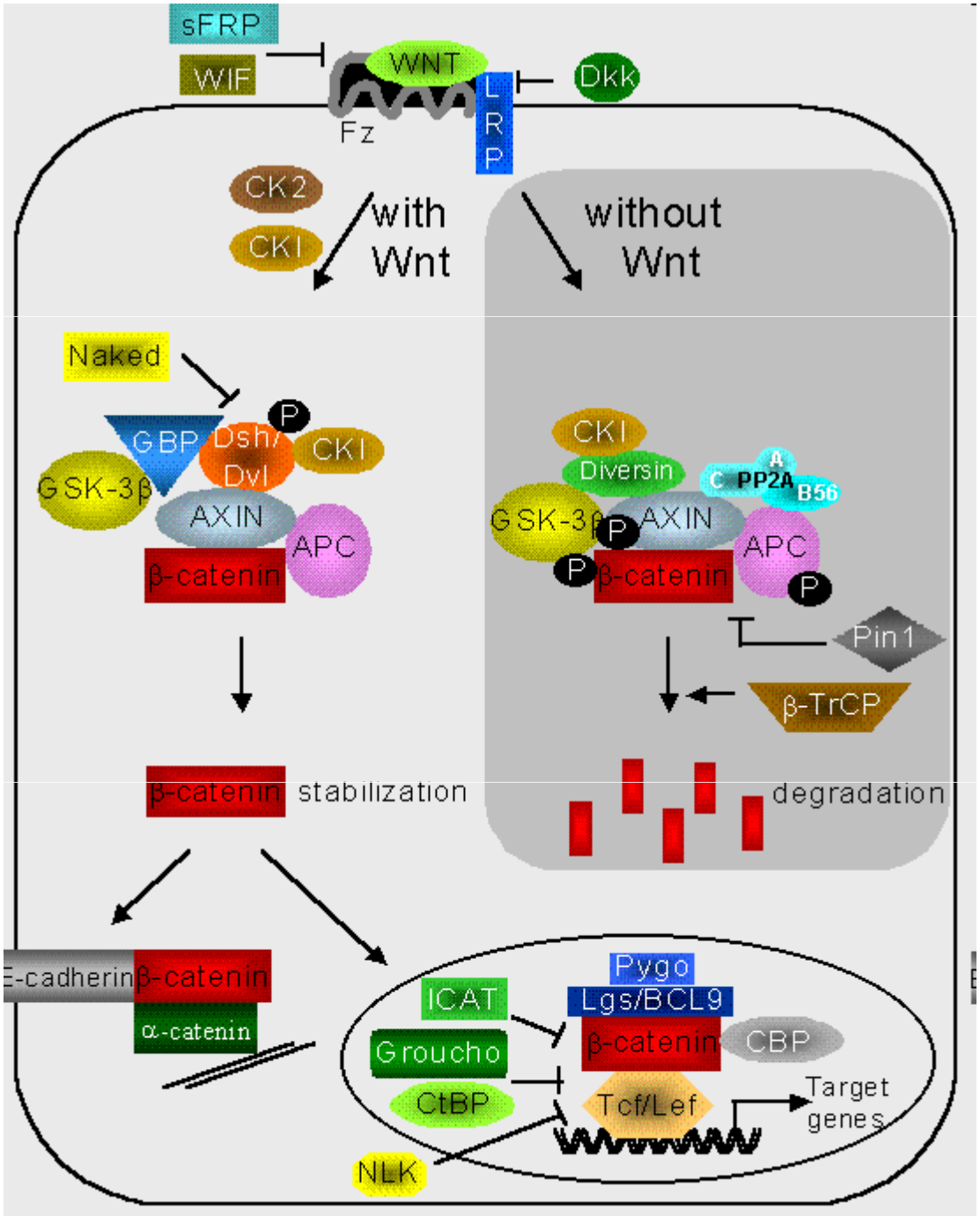
Wnt/b-Catenin Signaling Enhances Cyclooxygenase-2 (COX2) Transcriptional Activity in Gastric Cancer Cells

Felipe Nun˜ ez1, Soraya Bravo1, Fernando Cruzat1, Martı´n Montecino1,2, Giancarlo V. De Ferrari1,2*

Here we studied the transcriptional regulation of the COX2 gene in gastric cancer (GC) cell lines and assessed whether this phenomenon is modulated by Wnt/b-catenin signaling. Wnt3a significantly enhanced COX2 mRNA expression in a dose- and time-dependent manner. Serial deletion of a 1.6 Kbp COX2 promoter fragment and gain- or loss-of-function experiments allowed us to identify a minimal Wnt/b-catenin responsive region consisting of 0.8 Kbp of the COX2 promoter (pCOX2-0.8), which showed maximal response in genereporter assays. The activity of this pCOX2-0.8 promoter region was further confirmed by DNA-protein binding assays.

Plusone 2011

The Wnt signaling pathway



COX2 gene expression and nuclear localization of b-catenin in GC cells.

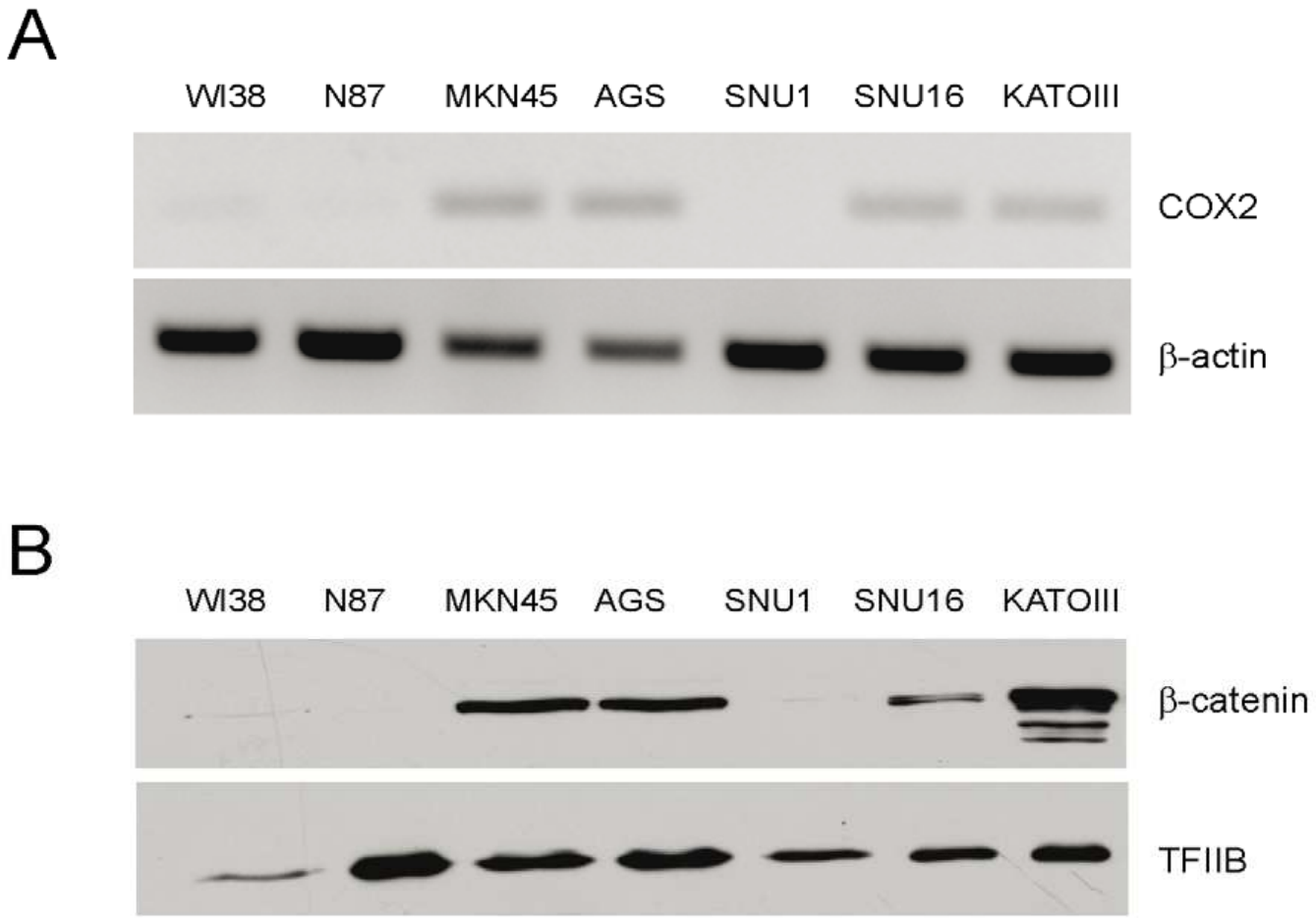


Figure 1. (A) COX2 mRNA expression in control (WI38) and GC cell lines (MKN45, AGS, SNU1, SNU16, KATOIII and N87). Total RNA was extracted from cultured cells and semiquantitative RT-PCR was used to determine COX2 and b-actin RNA levels as an internal control. Twenty-six cycles were chosen as an adequate PCR cycle. (B) Nuclear levels of b-catenin protein in the same cell-lines, as shown in (A), were examined through Western Blot analysis using nuclear extracts. The TFIIB general transcription factor was used as an internal control.

Human pCOX2-1.6 promoter activity in response to Wnt/b-catenin signaling.

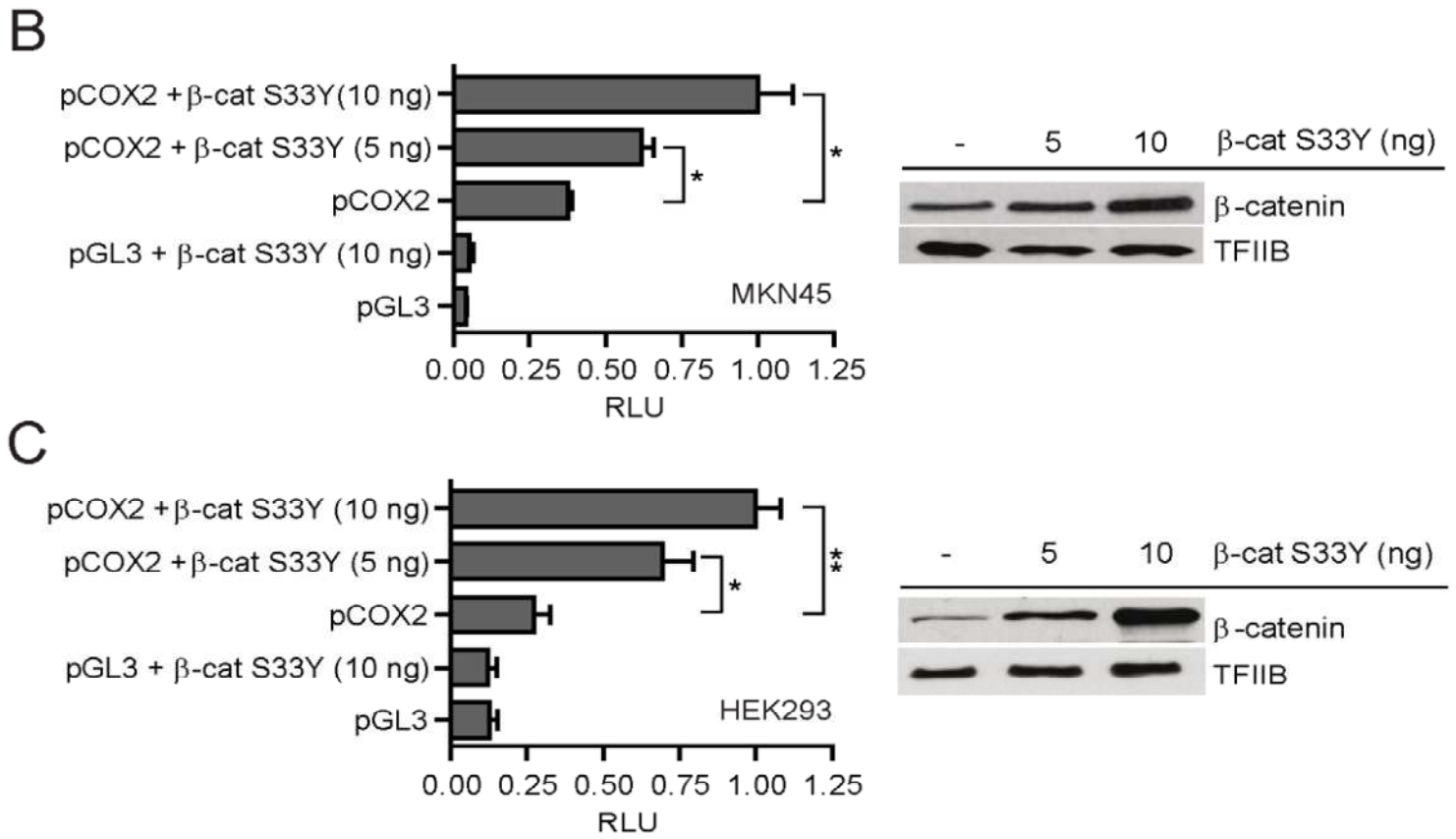


Figure 3 (B & C)Gene reporter assays in MKN45 (B) and HEK293 (C) cells co-transfected with 10 ng of pCOX2 and increasing concentrations of a constitutively active b-catenin (S33Y) protein (left panel). Cells were co-transfected with 1 ng of PRL-SV40 Renilla as an internal control. Promoter activity was normalized as the ratio between firefly luciferase and Renilla luciferase units. RLU: Relative Luciferase Units. Each figure corresponds to at least three independent experiments. Statistical significance was determined through ANOVA test (* p,0.05, ** p,0.01). Nuclear levels of b-catenin protein were examined in same cell lines through Western Blot analysis (right panel). The TFIIB general transcription factor was used as an internal control.

pCOX2-0.8 as a minimal COX2 promoter with maximum basal response in GC cells.

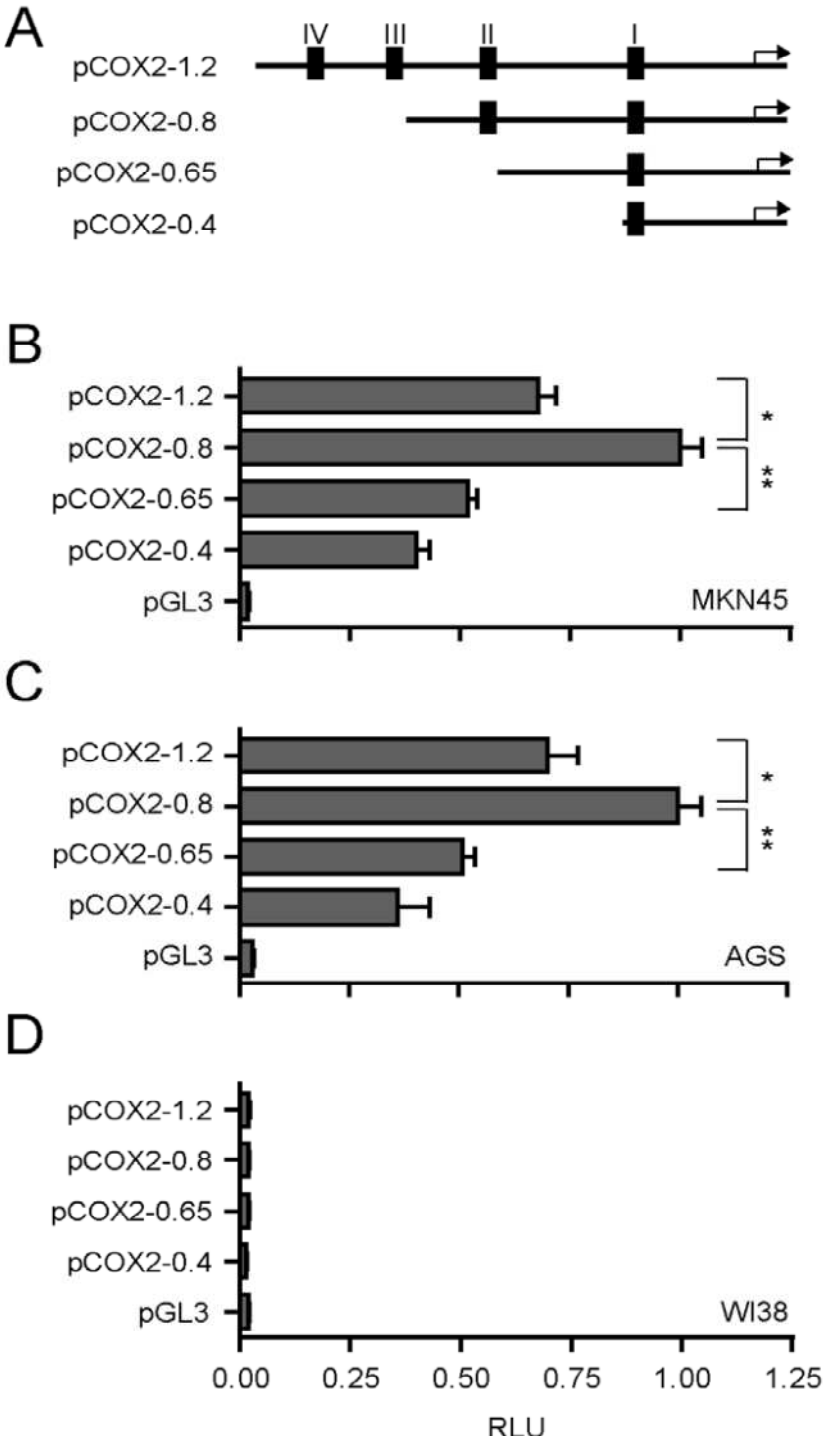


Figure 4. (A) Schematic representation of pCOX2 deletions. (B–D) Gene reporter assay in MKN45 (B), AGS (C) and WI38 (D) cell lines transiently transfected with 50 ng pCOX deletions (pCOX2-1.2; pCOX2-0.8; pCOX2-0.65 and pCOX2-0.4) and 50 ng of empty vector. In all experiments cells were transfected with 1 ng of PRL-SV40 Renilla as an internal control. Promoter activity was normalized as the ratio between firefly luciferase and Renilla luciferase units. RLU: Relative Luciferase Units. Each figure corresponds to at least three independent experiments. Statistical significance was determined through ANOVA test (* p,0.05, ** p,0.01).

Wnt/b-catenin signaling modulates pCOX2-0.8 activity in MKN45 cells.

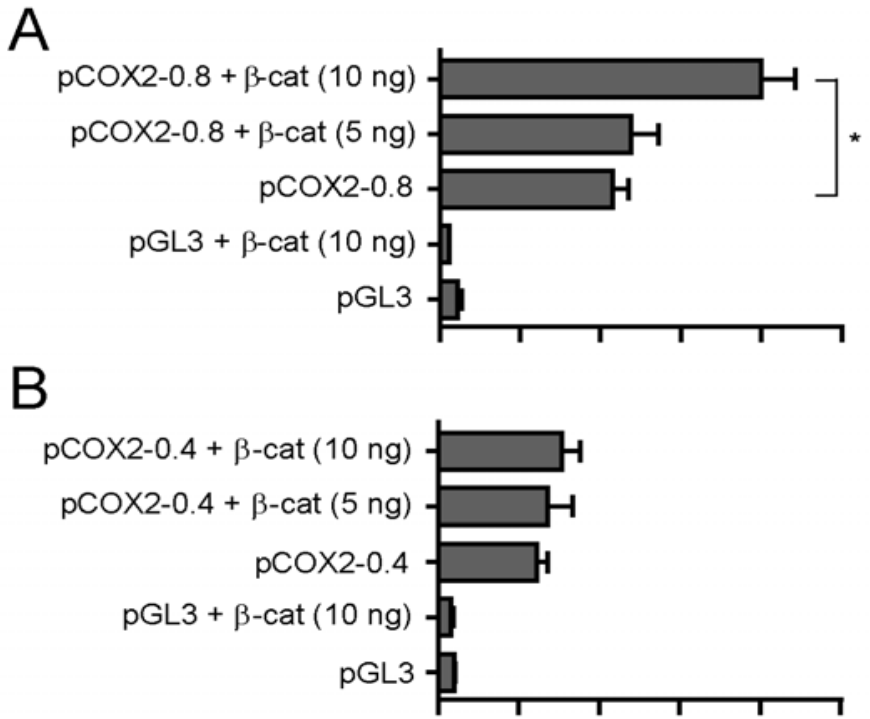


Figure 5. Gene reporter assays in MKN45 cells transiently transfected with either 10 ng pCOX2-0.8 (A) or pCOX2-0.4 (B), plus 5–10 ng of b-catenin S33Y and 10 ng of empty vector as control. Cells were transfected with increasing concentrations of pCOX2-0.8, MpCOX-08, or equal amounts of empty vector as a control. Promoter activity was normalized as the ratio between firefly luciferase and Renilla luciferase units. RLU: Relative Luciferase Units. Each figure corresponds to at least three independent experiments. Statistical significance was determined through ANOVA test (* p,0.05, ** p,0.01).

Binding of b-catenin to the TBE Site II (2689/2684) in the COX2 promoter

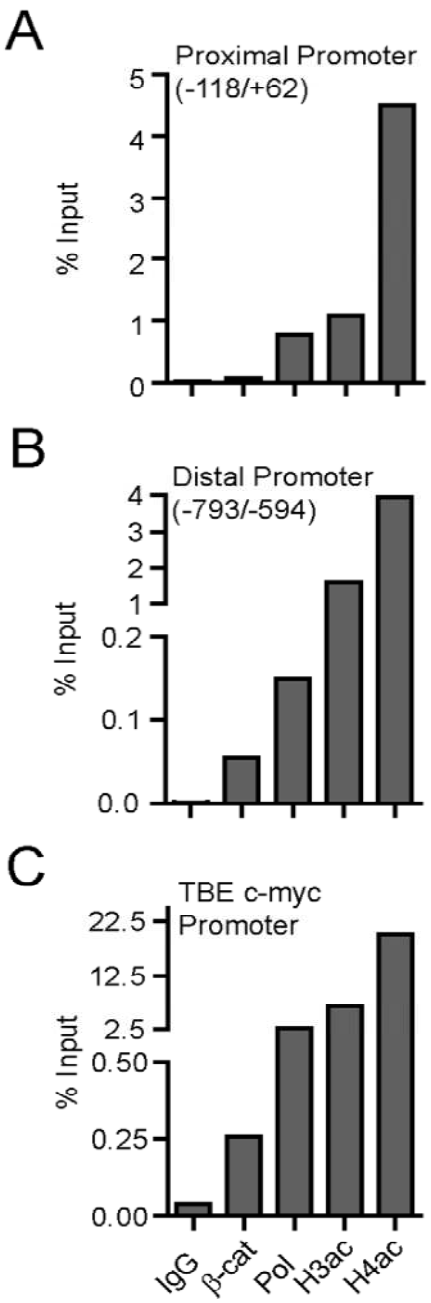


Figure 6.. (A–C) ChIP assays in MKN45 cells using specific antibodies for b-catenin (b-cat), polymerase II (Pol), H3 and H4 acetylated histones (H3ac; H4ac) and immunoglobulin G (IgG). Quantification was done by real time PCR using specific primers for the proximal promoter (PP) region (A), the TBE Site II (2689/2684) in the human COX2 promoter (B) and a TBE site within the c-myc promoter, as a positive control (C).