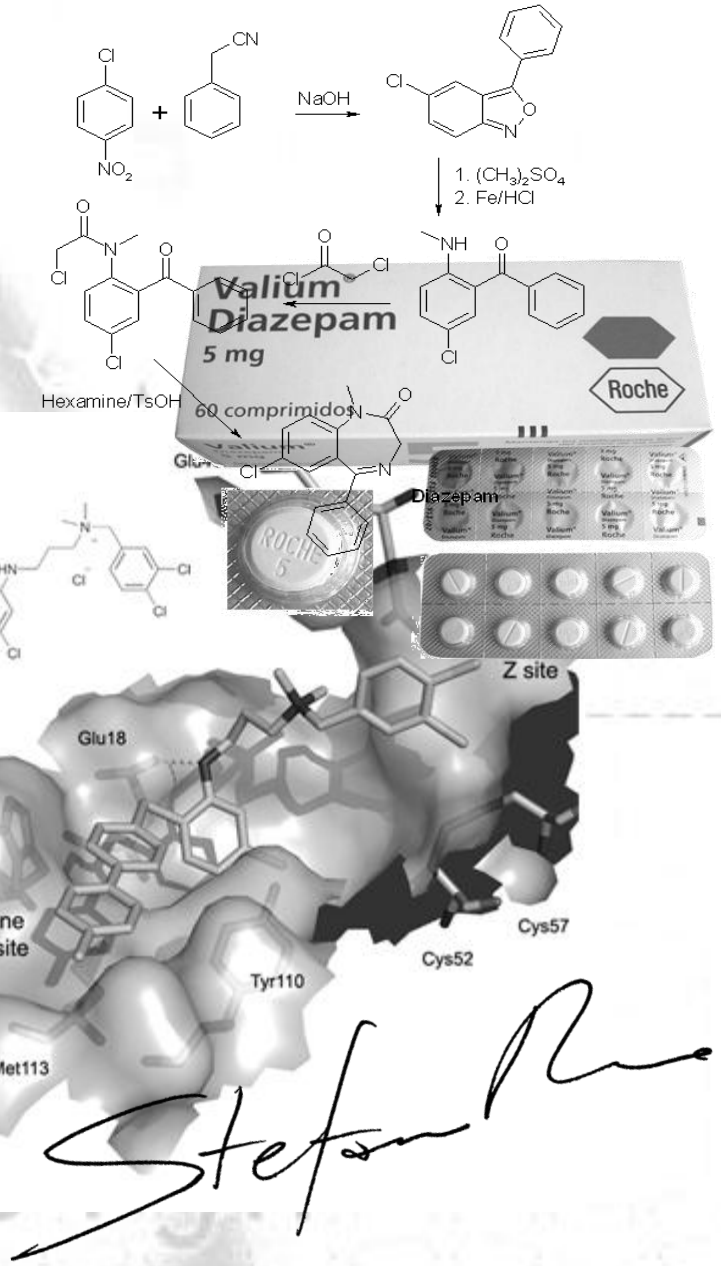
Chimica Farmaceutica



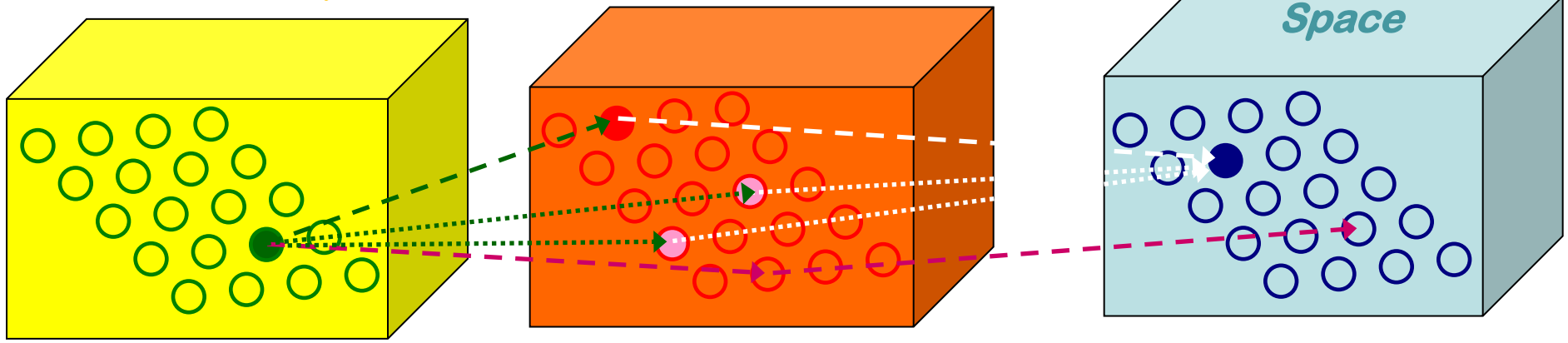
… here we are, some case studies.

1. Inflammation and Non-Steroidal Anti-Inflammatory Drug (NSAID);

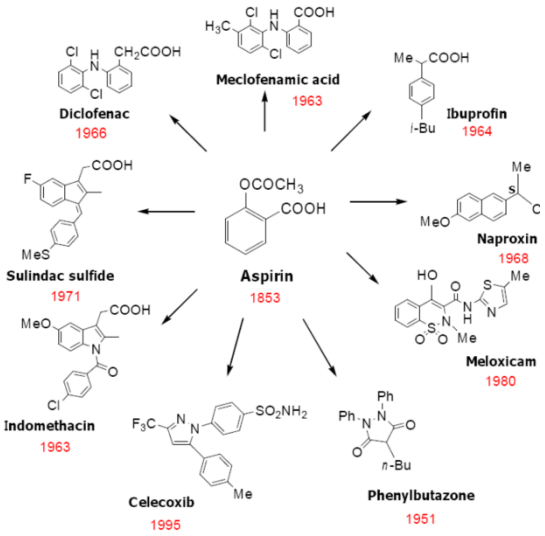
Chemical Space

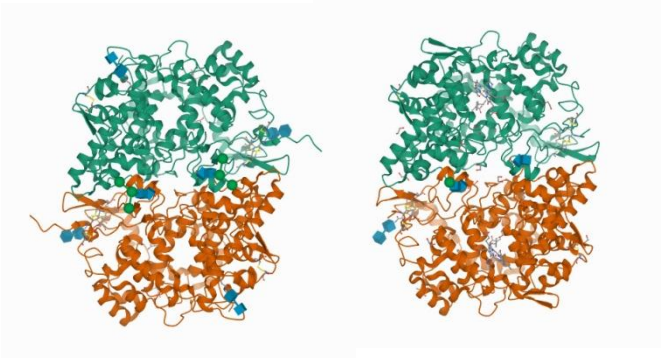
Biological Space

Physio-Pathological Space



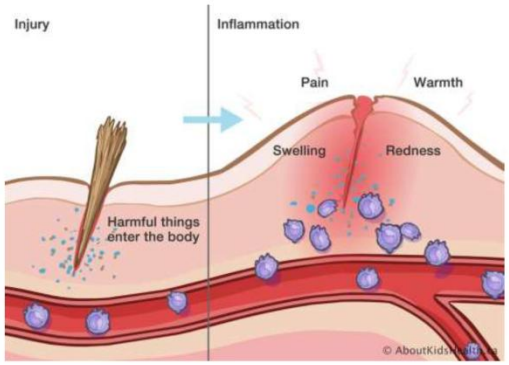
Non-Steroidal Anti-Inflammatory Drug (NSAID)*





COX-1 and COX-2

Inflammation



* Farmaci Antiinfiammatori Non Stereoidei (FANS)

… a short journey through inflammation.

Causes of inflammation

- Inflammation is defined as the local response of living mammalian tissues to injury due to any agent.
- **Causes of inflammation**
  - **Infective agents** like bacteria, viruses and their toxins, fungi, parasites.
  - **Immunological agents** like cell-mediated and antigen-antibody reactions.
  - **Physical agents** like heat, cold, radiation, mechanical trauma.
  - **Chemical agents** like organic and inorganic poisons.
  - **Inert materials** such as foreign bodies.

… a short journey through inflammation.

Signs of inflammation

- **Signs of inflammation**

Five cardinal signs of inflammation as

- ***rubor* (redness)**
- ***tumor* (swelling)**
- ***calor* (heat)**
- ***dolor* (pain)**
- **functio *laesa* (loss of function)**

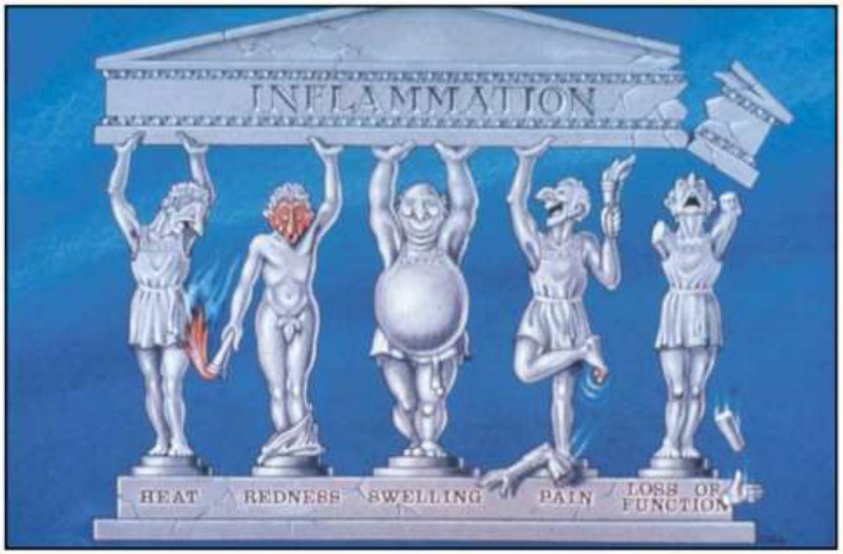
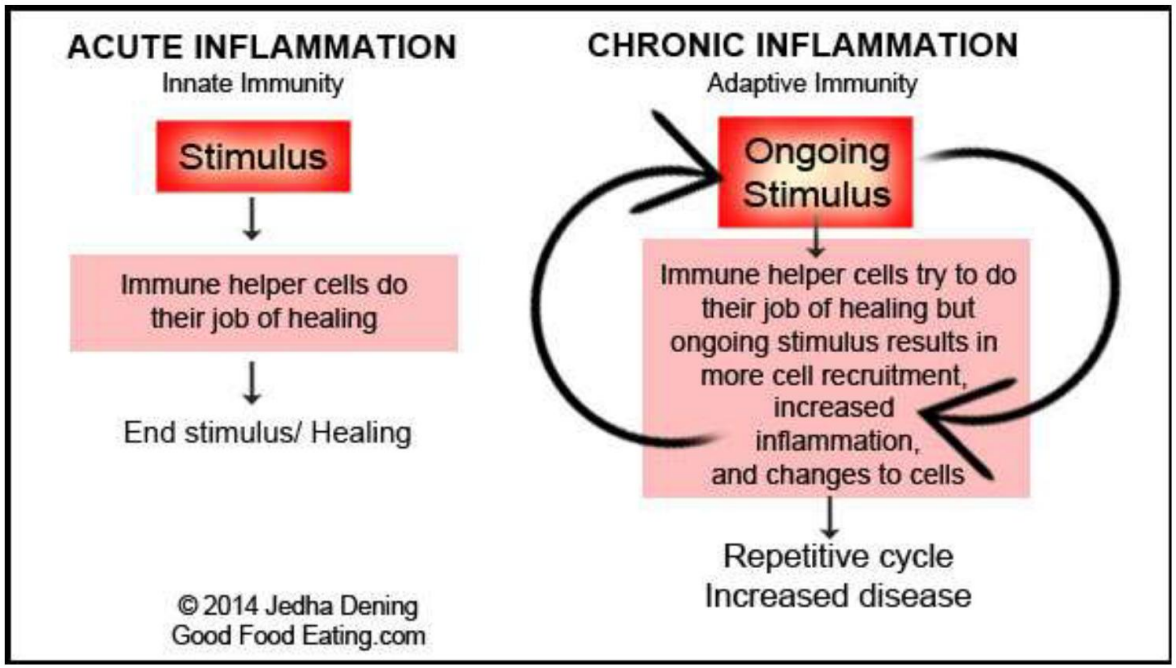


Figure 1 | **Cardinal signs of inflammation.** This cartoon depicts five Greeks representing the cardinal signs of inflammation — heat, redness, swelling, pain and loss of function — which are as appropriate today as they were when first described by Celsus more than 2000 years ago. This figure was commissioned by D.A.W. and drawn by P. Cull for the Medical Illustration Department at St Bartholomew's Medical College. LAWRENCE ET AL. NATURE REVIEWS IMMUNOLOGY 2002

… a short journey through inflammation.

Types of inflammation

- Depending upon the defense capacity of the host and duration of response, inflammation can be classified as
  - acute
  - chronic



… a short journey through inflammation.

Acute inflammation

- **Causes of acute inflammation:** Infection, trauma, physical and chemical agents, necrosis, foreign bodies, and immune reactions.
- **Stages of acute inflammation:**
  - **Vasodilation**
  - **Increased vascular permeability**
  - **Movement of white blood cells from blood vessels into soft tissue at the site of inflammation**

… a short journey through inflammation.

Acute inflammation

- **Stages of acute inflammation:**
  - **Vasodilation:** Vasodilation occurs through release of mediators [include **histamine, prostacyclin (PGI2), and nitric oxide (NO)**] from cells. Vasodilation increases the hydrostatic pressure by causing slowing (sludging) of blood flow. Sludging of blood also causes margination of leukocytes along the wall of the blood vessel
  - **Increased vascular permeability:** Increased vascular permeability occurs through release of mediators [**histamine**, **bradykinin, TNF, IL-1, leukotrienes C4, D4, and E4**] from cells.
  - **Movement of white blood cells from blood vessels into soft tissue at the site of inflammation:** The steps required are **rolling**, **pavementing**, and **transmigration**.

… a short journey through inflammation.

Chronic inflammation

- Prolonged inflammation consisting of active inflammation and tissue destruction and repair, all occurring simultaneously.
- **Causes of Chronic inflammation:**
  - Chronic inflammation following acute inflammation
  - Recurrent attacks of acute inflammation
  - Chronic inflammation starting *de novo*

… a short journey through inflammation.

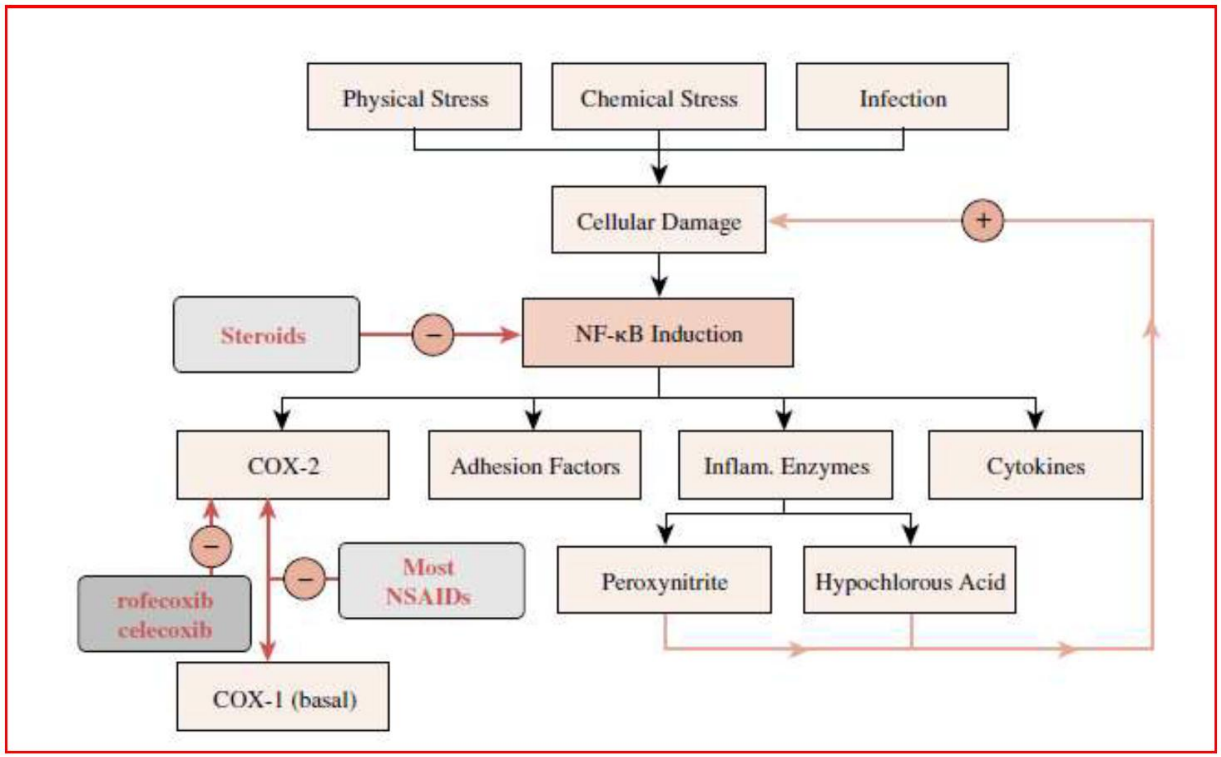
Cells involved in chronic inflammation

- **Macrophages:** Activated macrophages produce proteases, IL-1, TNF, arachidonic acid metabolites, NO, angiogenesis and **growth factors such as platelet-derived growth factor (PDGF) or fibroblast growth factor (FGF)**.
- **Lymphocytes :** Activated lymphocytes produce
  - FGF stimulates fibroblasts to produce collagen, which results in scarring.
  - PDGF and transforming growth factor-β (TGF-β).
  - Interferon-γ (activates macrophages).

… a short journey through inflammation.

Overview of inflammatory processes

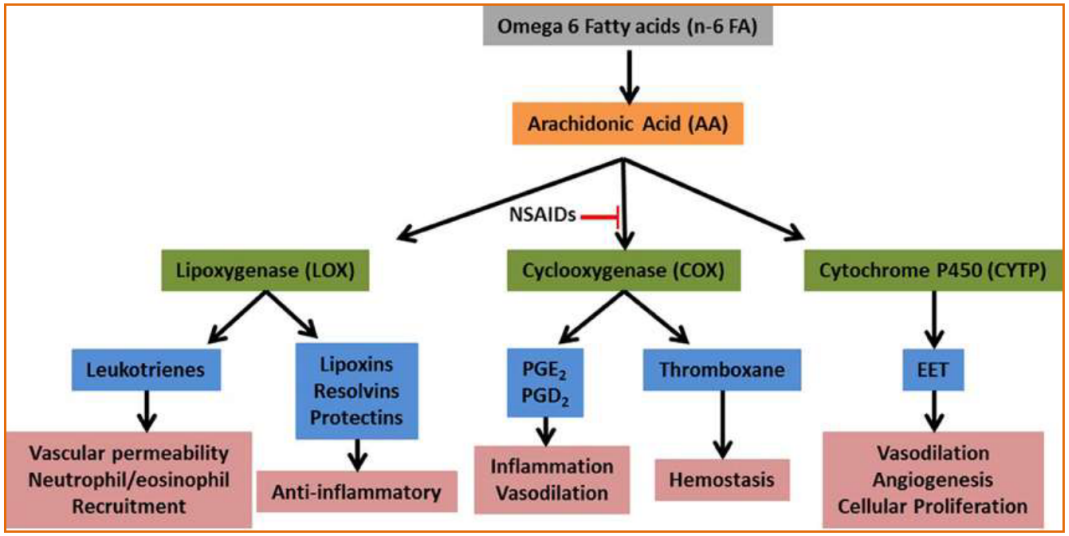
- Inflammation begins when a stimulus, such as infection, physical stress, or chemical stress, produces cellular damage.



… a short journey through inflammation.

Overview of inflammatory processes

- The more important inflammatory mediators are the **eicosanoids, biological oxidants, cytokines**, adhesion factors, and digestive enzymes (proteases, hyaluronidase, collagenase, and elastase).
- ***EICOSANOIDS:*** The pathway initiated by cyclooxygenase (COX) produces prostaglandins; the lipoxygenase pathway generates leukotrienes.



… a short journey through inflammation.

Overview of inflammatory processes

- ***Biological Oxidants:*** The biologically derived oxidants are potent bacterial killers but are also a major contributing factor in tissue injury that results from the inflammatory response. These oxidants include the superoxide anion ($^{\cdot}O_2$-), hydrogen peroxide (H_2O_2), nitric oxide ($^{\cdot}NO$), peroxynitrite ($^{\cdot}OONO^{\cdot}$), hypochlorous acid (HOCl), peroxidase-generated oxidants of undefined character, probably the hydroxyl radical ($^{\cdot}OH$), and possibly singlet oxygen ($^1O_2$).
- ***Cytokines:*** *Tumor necrosis factor-α* (TNF- *α*) and *interleukin 1*(IL-1) are produced primarily by cells of the monocyte–macrophage lineage.

… a short journey through inflammation.

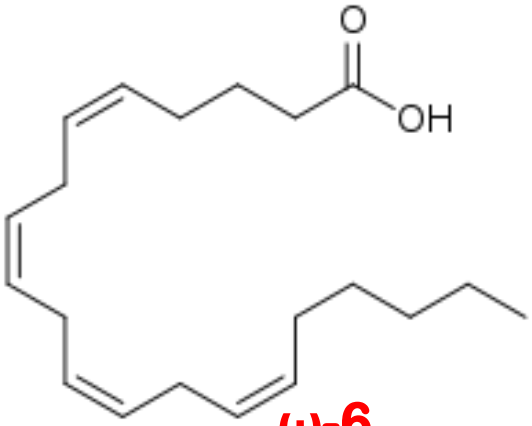
Overview of inflammatory processes

- Biological effects of eicosanoids

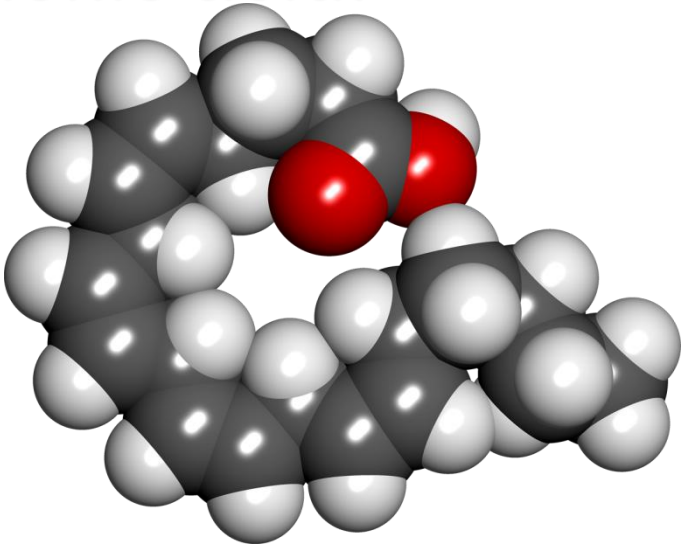
Eicosanoid	Primary Biological Effects
PGE_1	Vasodilation, decreased gastric acid secretion, bronchodilation
PGE_2	Vasodilation, decreased gastric acid secretion, pain sensitization, uterine contraction, cervical ripening, maintenance of patent ductus arteriosus, bronchodilation, fever
$PGF_{2\alpha}$	Bronchoconstriction, uterine contraction, increases drainage from aqueous humor
PGI_2	Vasodilation, maintenance of patent ductus arteriosus, inhibition of platelet aggregation, pain sensitization, gastric cytoprotection
TXA_2	Platelet aggregation, bronchoconstriction
LTC_4, D_4, E_4	Bronchoconstriction
LTB_4	Chemoattraction and activation of polymorphonuclear leukocytes

cytoplasmic membrane
PLA2
arachidonic acid
5-LOX
12/15-LOX
COX-1
COX-2
LTA4 hydrolase
LTB4
LTA4
12S-H(P)ETE
15S-H(P)ETE
LTC4S
12/15-LOX
5-LOX
LTC4
LXA4
γ-glutamyl transpeptidase
LTD4
dipeptidase
LTE4
PGG2
COX-1
COX-2
PGH2
PGIS
PGDS
PGFS
PGES
TXAS
PGI2
PGD2
PGF2a
PGE2
TXA2

… the magic arachidonic acid.



ω-6



Arachidonic acid **(AA, sometimes ARA) is a polyunsaturated omega-6 fatty acid 20:4(ω-6), or 20:4(5,8,11,14).**

Chemical formula	$C_{20}H_{32}O_2$
Molar mass	304.474 g·mol^{-1}
log *P*	6.99
Acidity (pK_a)	4.75

cytoplasmic membrane

PLA2

arachidonic acid

COX-1
COX-2

5-LOX

12/15-LOX

LTA4 hydrolase

LTB4

LTA4

12S-H(P)ETE
15S-H(P)ETE

LTC4S

12/15-LOX

5-LOX

LTC4

LXA4

γ-glutamyl transpeptidase

LTD4

dipeptidase

LTE4

PGG2

COX-1
COX-2

PGH2

PGIS

PGDS

PGFS

PGES

TXAS

PGI2

PGD2

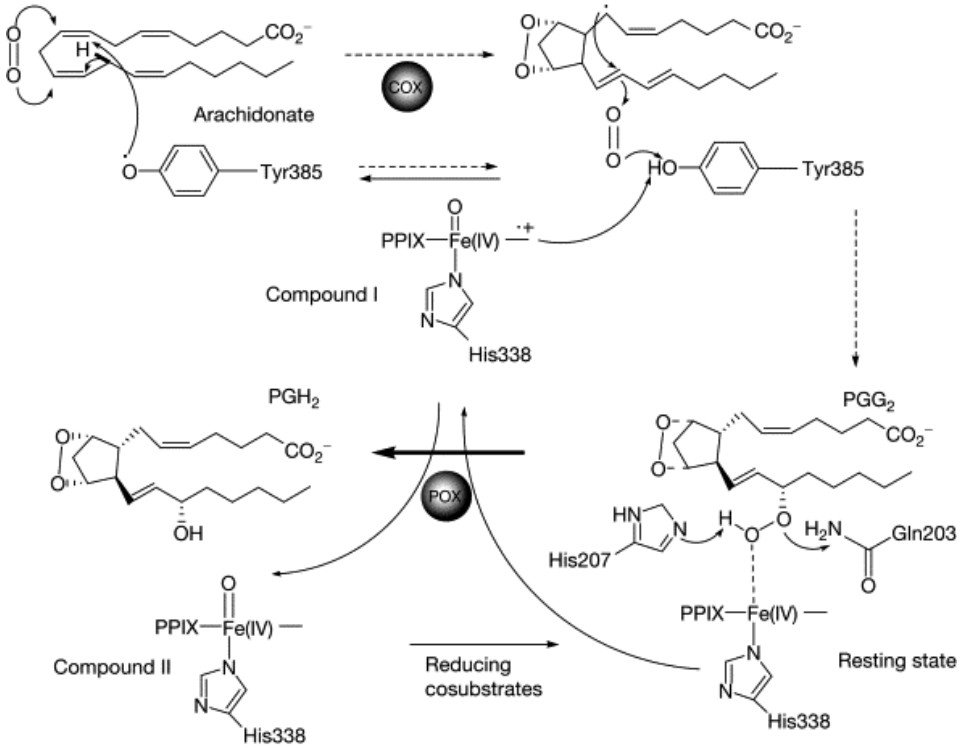
PGF2a

PGE2

TXA2

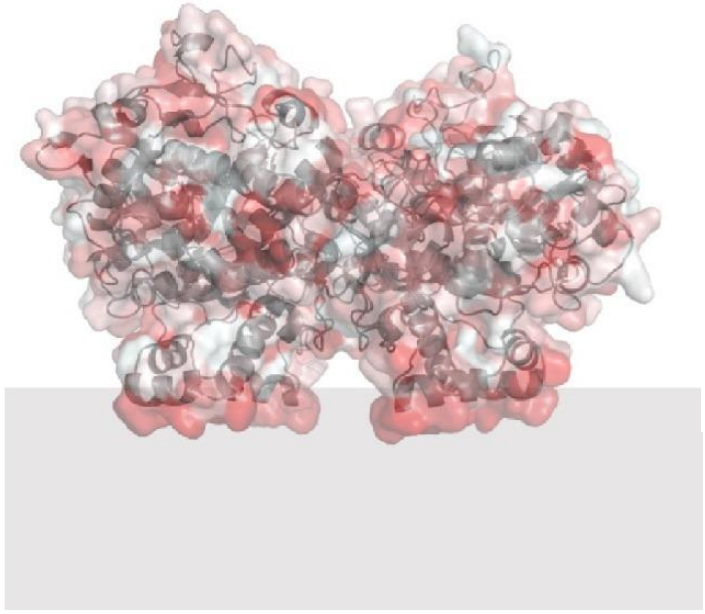
… and the two magic enzymes COX-1 and COX-2.

Cyclooxygenase (COX), officially known as prostaglandin-endoperoxide synthase (PTGS), is an enzyme that is responsible for formation of *prostanoids*, including *thromboxane* and prostaglandins such as *prostacyclin*, from arachidonic acid. The specific reaction catalyzed is the conversion from arachidonic acid to *Prostaglandin H2*, via a short-living *Prostaglandin G2* intermediate.



… and the two magic enzymes COX-1 and COX-2.

In terms of their molecular biology, COX-1 and COX-2 are of similar molecular weight, approximately 70 and 72 kDa, respectively, and having 65% amino acid sequence similarity and near-identical catalytic sites. Both proteins have three domains: an N-terminal EGF-like domain, a small 4-helical membrane anchor, and a core heme-peroxidase catalytic domain. Both form dimers. The membrane anchor fixes the proteins into the endoplasmic reticulum (ER) and microsome membrane.



COX-2
PBD ID: 1CQE
~9 Å depth
7.6% embedded

… and the two magic enzymes COX-1 and COX-2.

There are two isozymes of COX encoded by distinct gene products: a *constitutive COX-1* and an *inducible COX-2*, which differ in their regulation of expression and tissue distribution. The expression of these two transcripts is differentially regulated by relevant cytokines and growth factors. This gene encodes COX-1, which regulates angiogenesis in endothelial cells. COX-1 is also involved in cell signaling and maintaining tissue homeostasis. A splice variant of COX-1 termed COX-3 was identified in the CNS of dogs, but does not result in a functional protein in humans.

COX-1 promotes the production of the natural mucus lining that protects the inner stomach and contributes to reduced acid secretion and reduced pepsin content. COX-1 is normally present in a variety of areas of the body, including not only the stomach but any site of inflammation.

… and the two magic enzymes COX-1 and COX-2.

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CYCLOOXYGENASE

Help

in Additional Structure Keywords

Cyclooxygenase, COX, NSAID, OXIDOREDUCTASE

COX-2, **Cyclooxygenase**-2, Endocannabinoid, OXIDOREDUCTASE

Cyclooxygenase, COX-2, Flurbiprofen, FBP, OXIDOREDUCTASE

Protein, Monotopic, **Cyclooxygenase**, **Cyclooxygenase**-2, COX, PGHS, Salicylic

Cyclooxygenase, COX-2, celebrex, coxib, celecoxib, OXIDOREDUCTASE

COX-1, **CYCLOOXYGENASE**, PEROXIDASE, PROSTAGLANDIN, HEME, OXIDOREDUCTASE

COX-1, **CYCLOOXYGENASE**, PEROXIDASE, PROSTAGLANDIN, HEME, P6, OXIDOREDUCTASE

COX-2, **CYCLOOXYGENASE**, PROSTAGLANDIN, DICLOFENAC, ENDOPEROXIDE

cyclooxygenase, indomethacin, OXIDOREDUCTASE-OXIDOREDUCTASE INHIBITOR

COX-2, **CYCLOOXYGENASE**, PROSTAGLANDIN, ARACHIDONATE, ENDOPEROXIDE

in Structure Title

bound to the **cyclooxygenase** channel of **cyclooxygenase**-2

Structure of Apo Murine **Cyclooxygenase**-2

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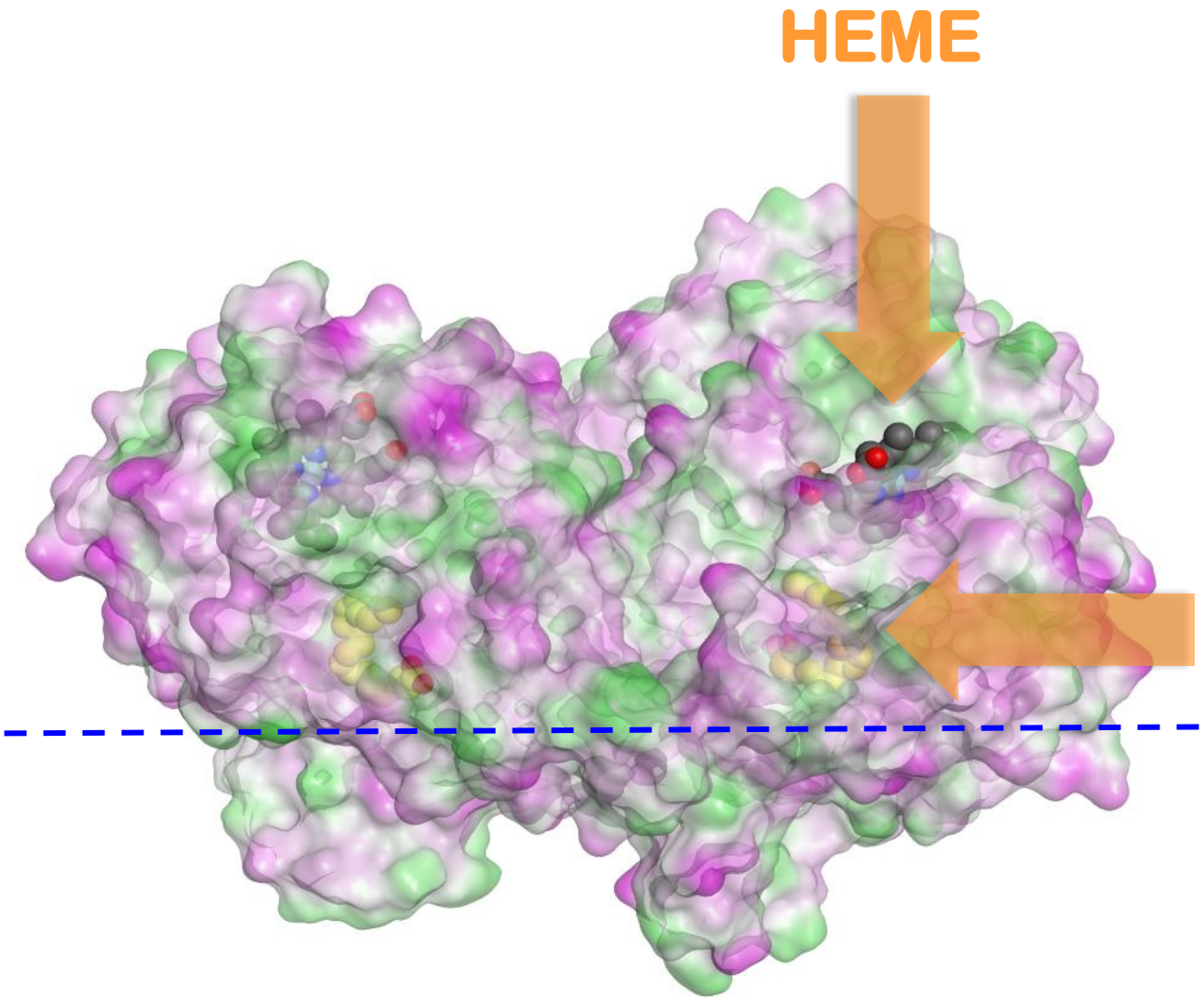
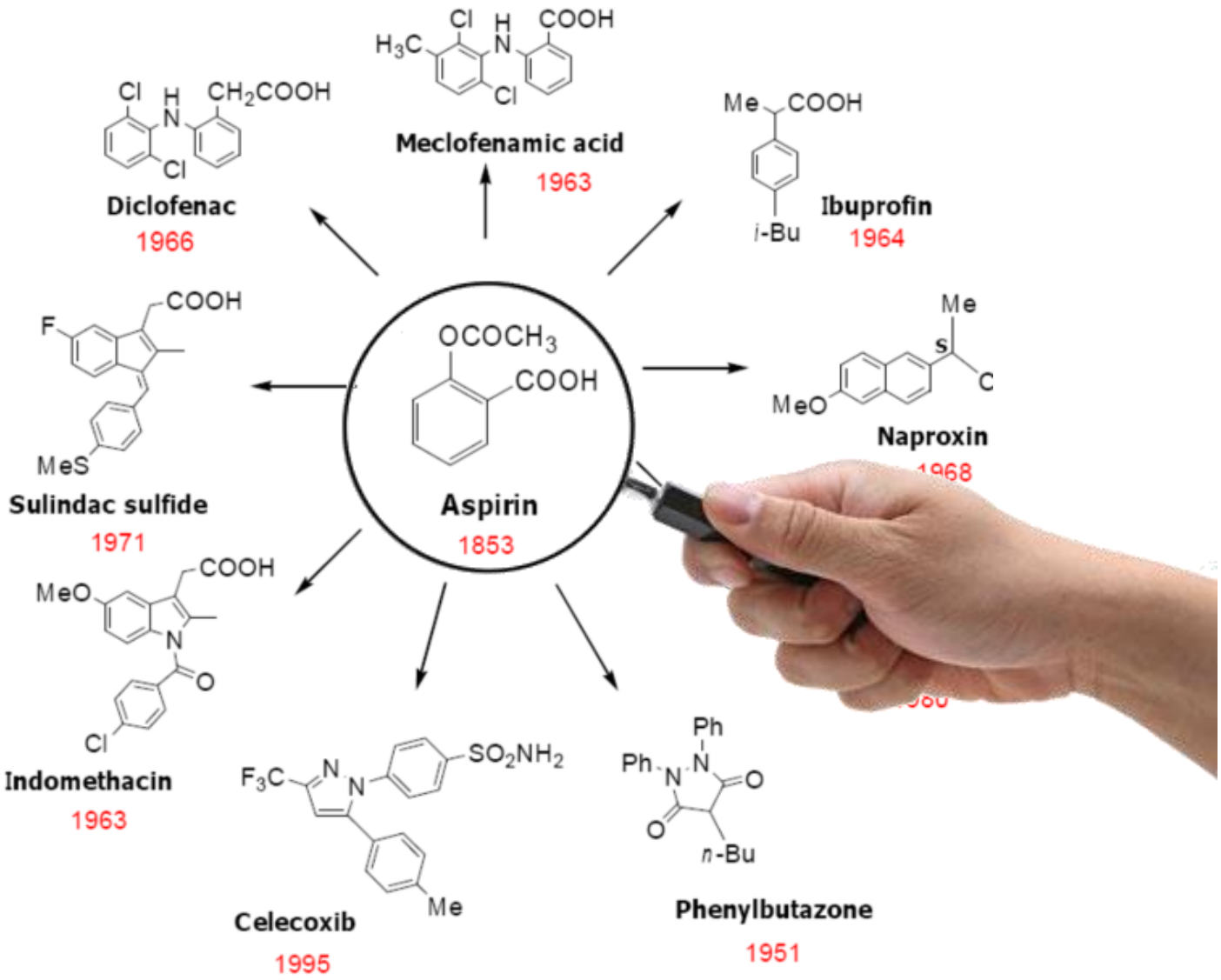
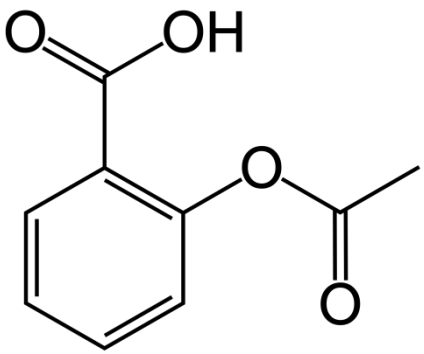
… and the two magic enzymes COX-1 and COX-2.

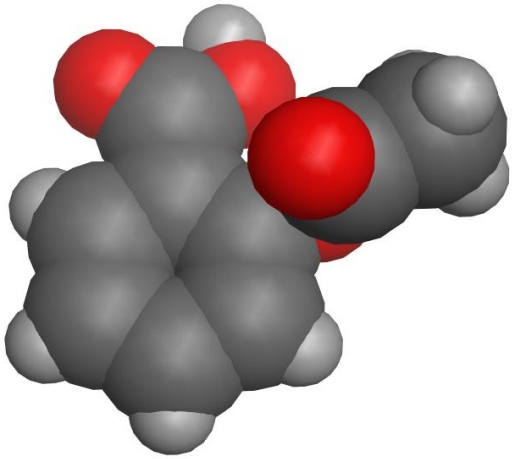


Non-Steroidal Anti-Inflammatory Drug (NSAID)



Acetylsalicylic acid (ASA)… better known as Aspirin

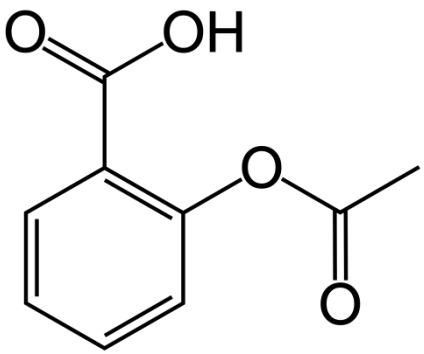


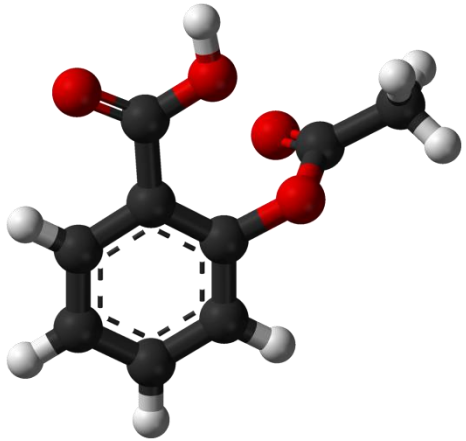


Also known as Aspirin, acetylsalicylic acid (ASA) is a commonly used drug for the treatment of pain and fever due to various causes. Acetylsalicylic acid has both anti-inflammatory and antipyretic effects. This drug also inhibits platelet aggregation and is used in the prevention of blood clots stroke, and myocardial infarction (MI)

Chemical formula	$C_9H_8O_4$
Molar mass	180.159 g·mol^{-1}
log *P*	1.18
Acidity (pK_a)	3.50

Acetylsalicylic acid (ASA)… better known as Aspirin





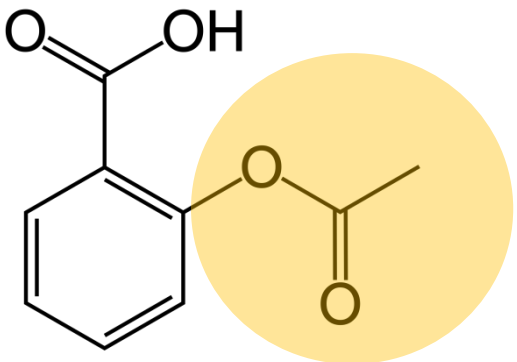
In 1971, British pharmacologist John Robert Vane, then employed by the Royal College of Surgeons in London, showed aspirin suppressed the production of prostaglandins and thromboxanes For this discovery he was awarded the 1982 Nobel Prize in Physiology or Medicine, jointly with Sune Bergström and Bengt Ingemar Samuelsson.

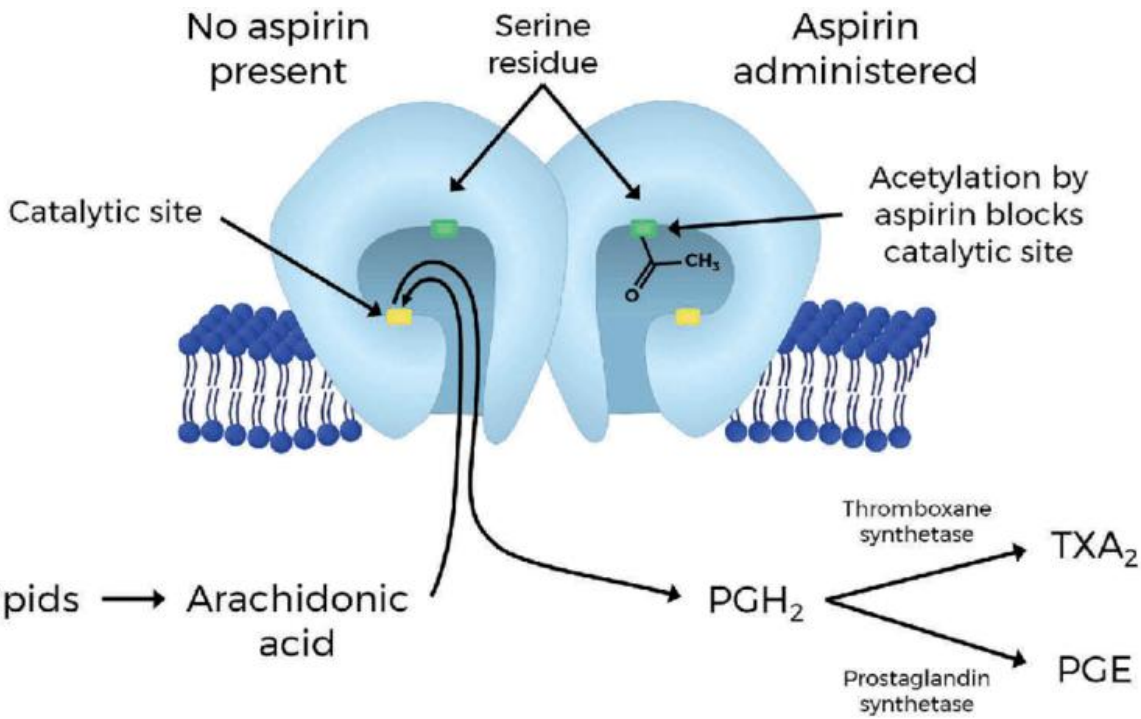
Aspirin is a medication used to reduce pain, fever, or inflammation. Specific inflammatory conditions which aspirin is used to treat include Kawasaki disease, pericarditis, and rheumatic fever.

Aspirin given shortly after a heart attack decreases the risk of death.] Aspirin is also used long-term to help prevent further heart attacks, ischaemic strokes, and blood clots in people at high risk.

Acetylsalicylic acid (ASA)… better known as Aspirin

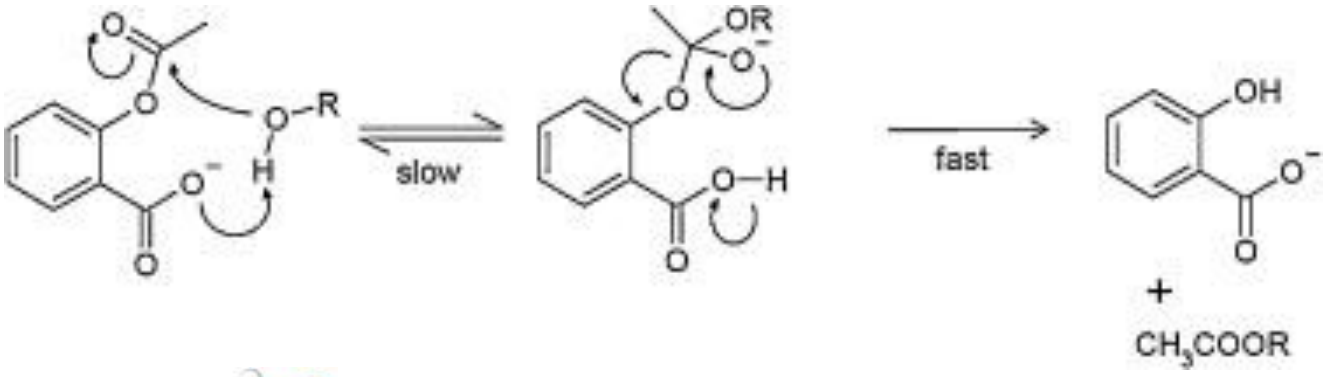
Aspirin acts as an *acetylating agent* where an acetyl group is covalently attached to a serine residue in the active site of the COX enzyme (suicide inhibition). This makes aspirin different from other NSAIDs (such as diclofenac and ibuprofen), which are reversible inhibitors.

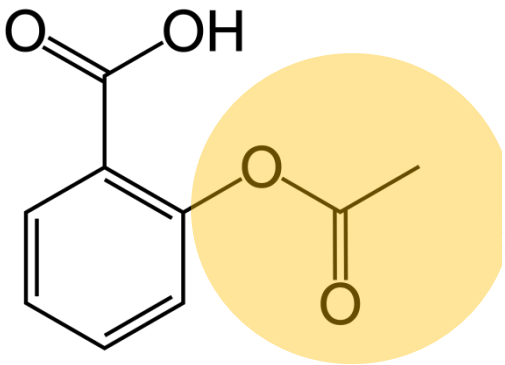


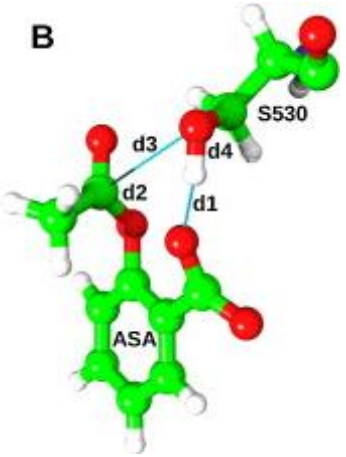


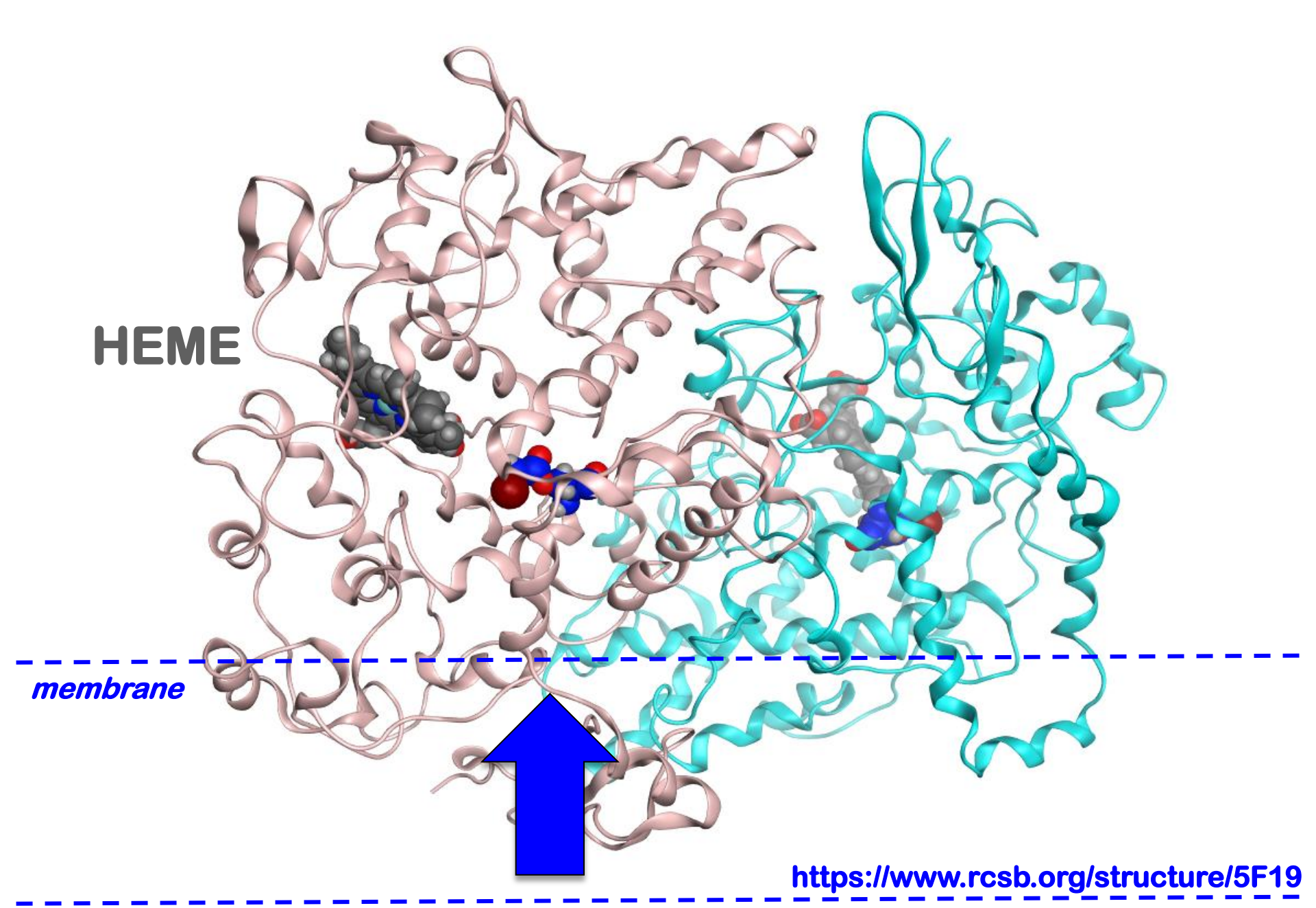
Acetylsalicylic acid (ASA)… better known as Aspirin

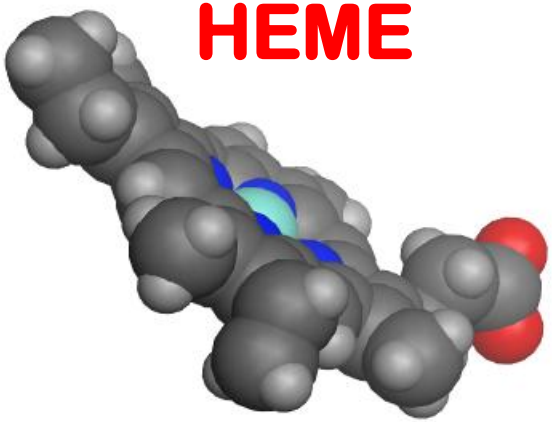
General scheme for the trans-esterification reaction (R = H, alkyl, enzyme)… here is, why ortho!

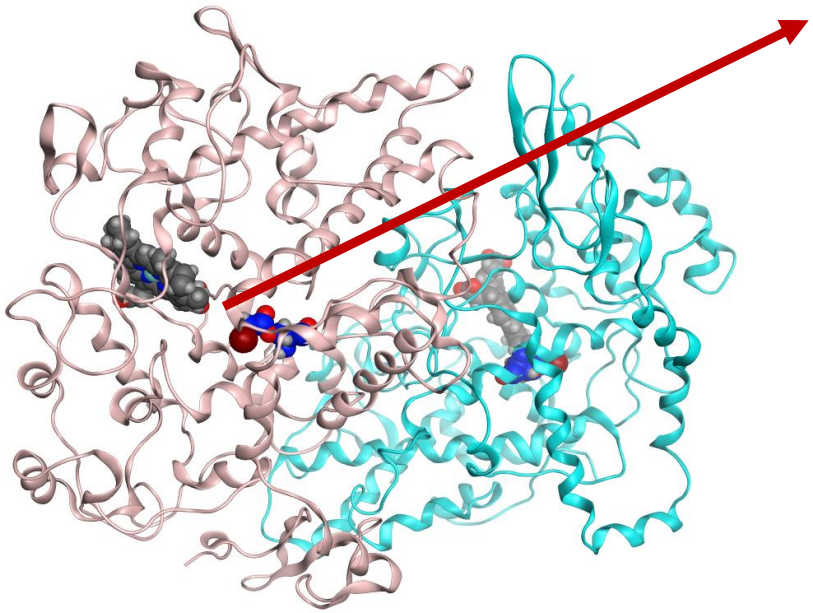


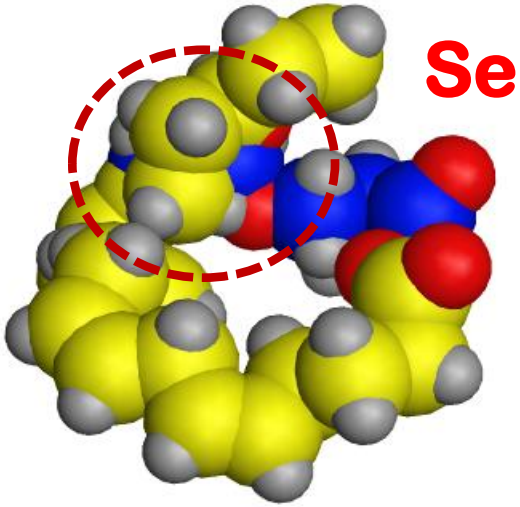




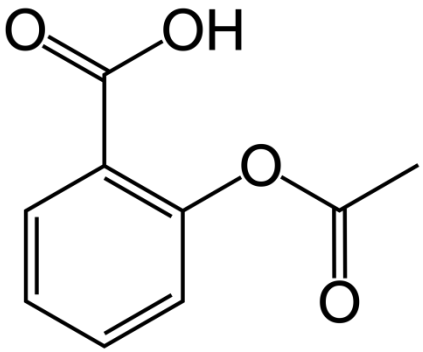



HEME

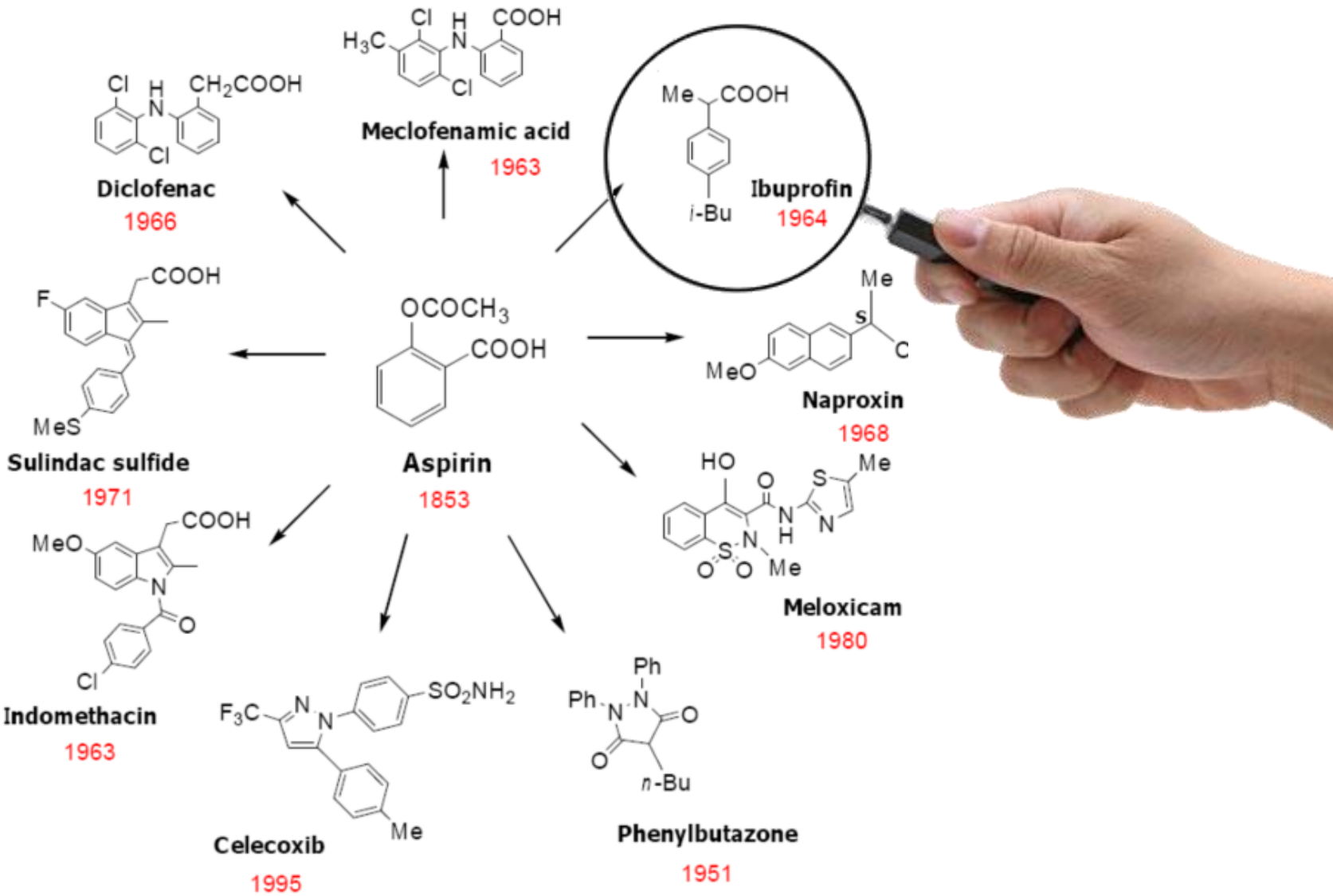



Ser530 (COX-2)

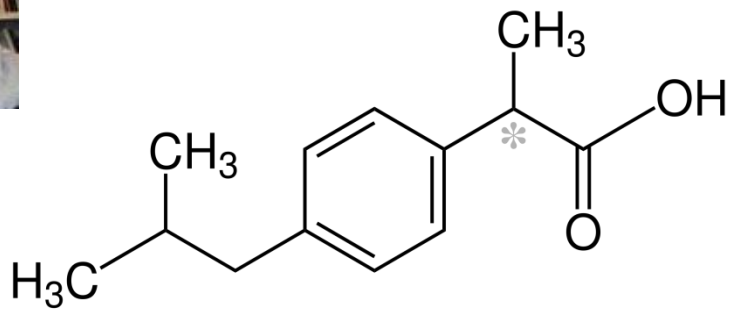
Just to remember…

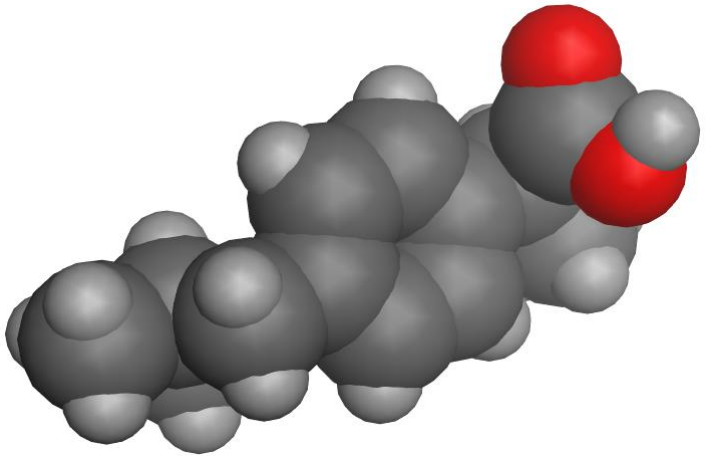


Non-Steroidal Anti-Inflammatory Drug (NSAID)



Ibuprofen… isobutyl-propionic acid-fenyl

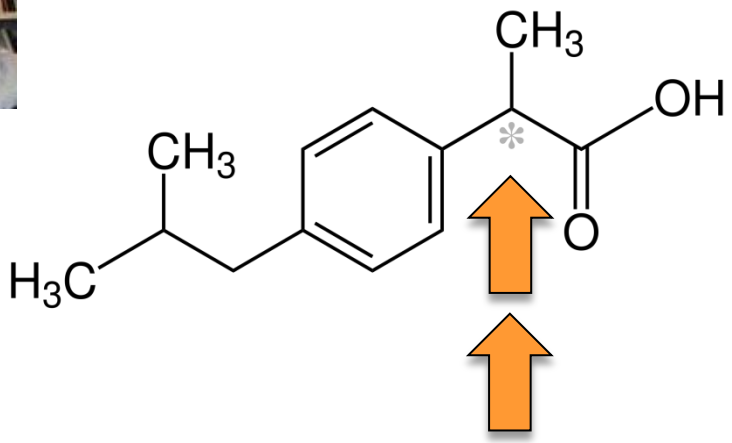


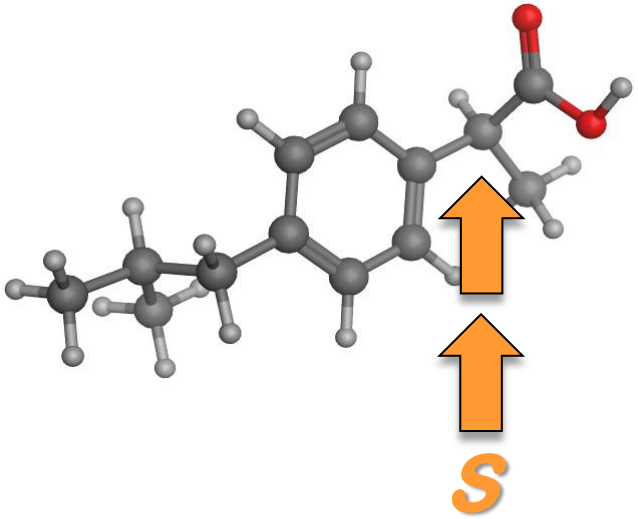


Ibuprofen is a non-steroidal anti-inflammatory drug (NSAID) derived from propionic acid and it is considered the first of the propionics. The formula of ibuprofen is 2-(4-isobutylphenyl) propionic acid and its initial development was in 1960 while researching for a safer alternative for aspirin. Ibuprofen was finally patented in 1961 and this drug was first launched against rheumatoid arthritis in the UK in 1969 and USA in 1974. It was the first available over-the-counter NSAID.

Chemical formula	$C_{13}H_{18}O_2$
Molar mass	206.285 g·mol^{-1}
log *P*	3.97
Acidity (pK_a)	5.30

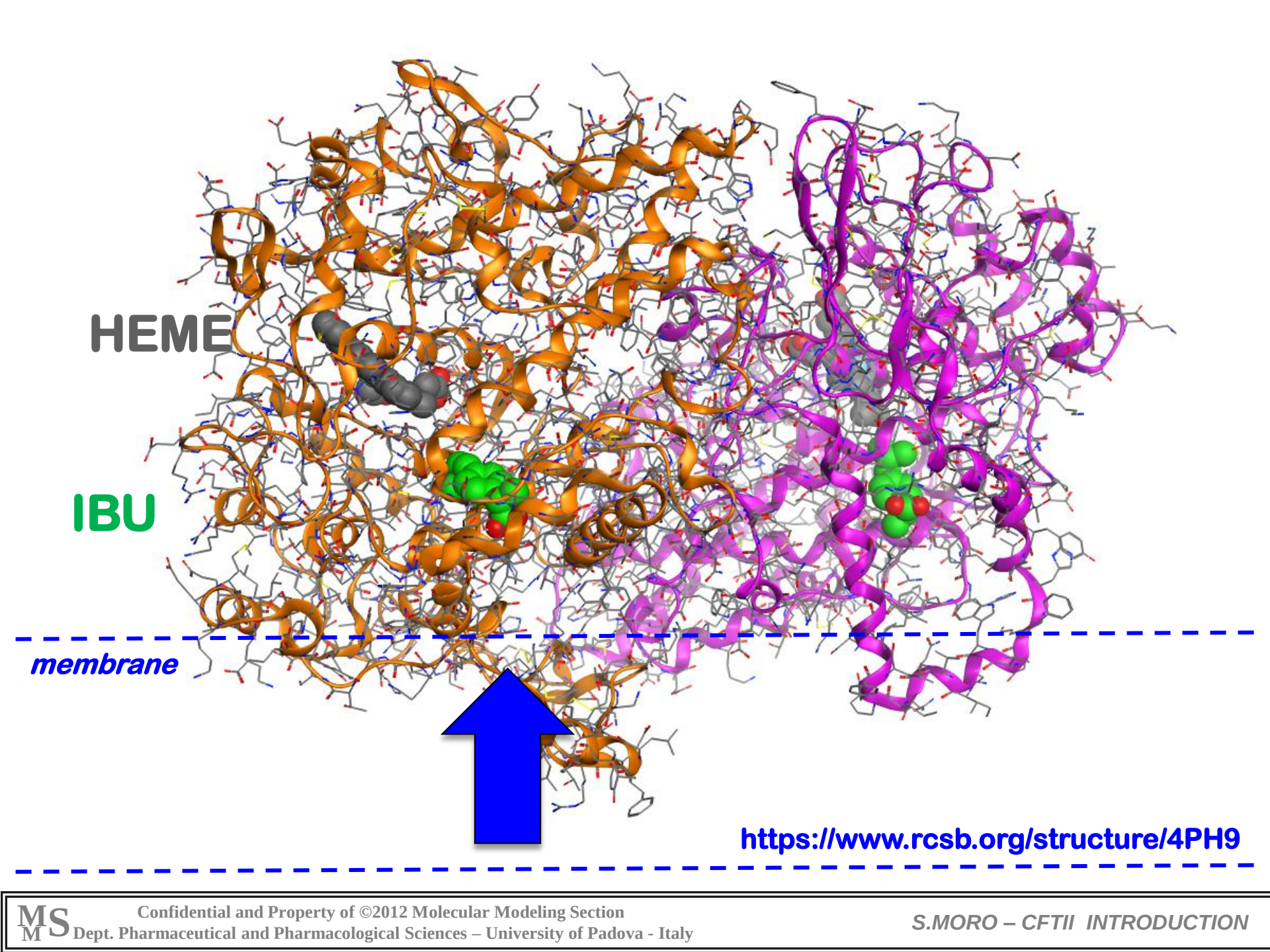
Ibuprofen… isobutyl-propionic acid-fenyl

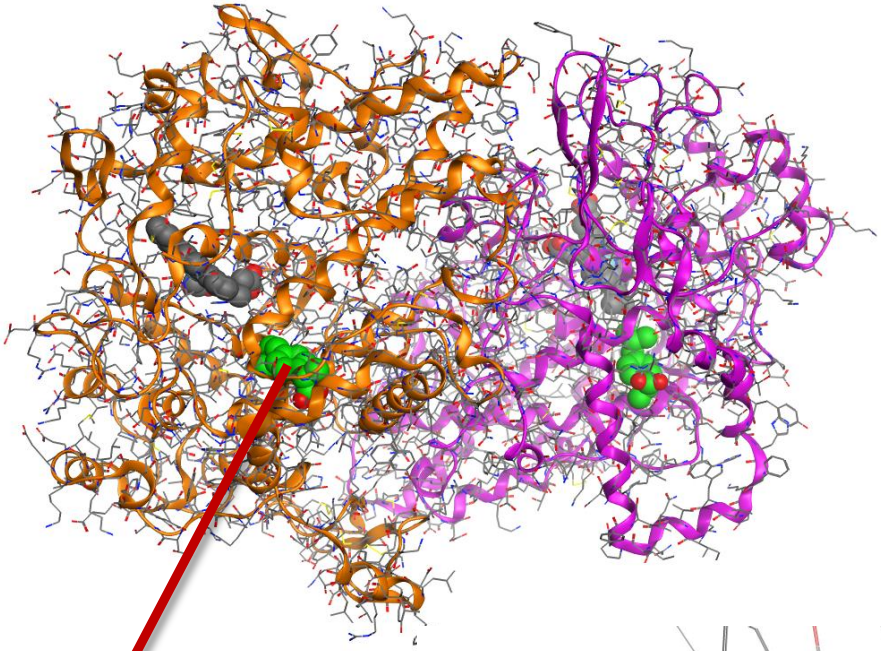


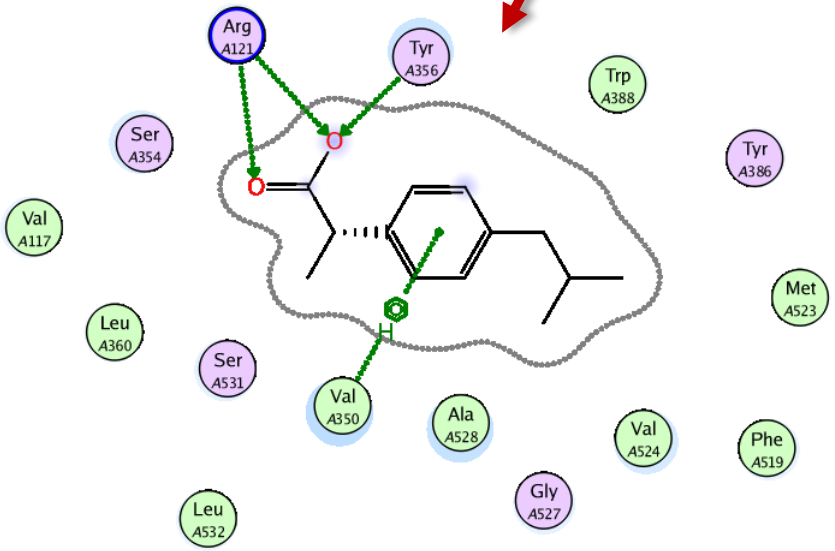


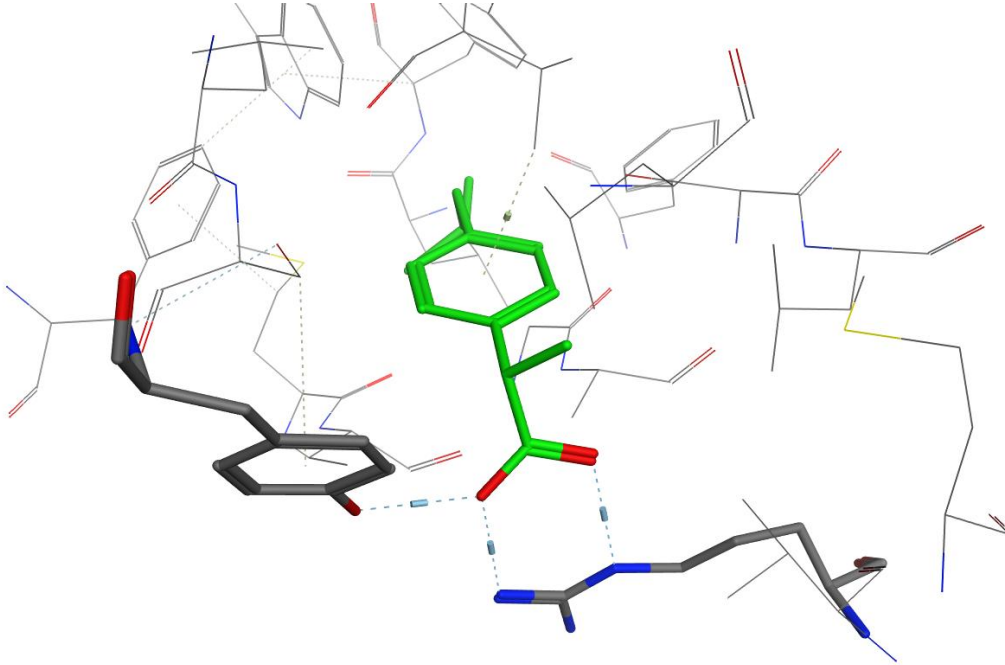
Ibuprofen, like other 2-arylpropionate derivatives such as ketoprofen, flurbiprofen and naproxen, contains a stereocenter in the α-position of the propionate moiety. The product sold in pharmacies is a racemic mixture of the *S* and *R*-isomers. The *S* (dextrorotatory) isomer is the more biologically active; this isomer has been isolated and used medically (see dexibuprofen for details).

The isomerase enzyme, alpha-methylacyl-CoA racemase, converts (*R*)-ibuprofen into the (*S*)-enantiomer

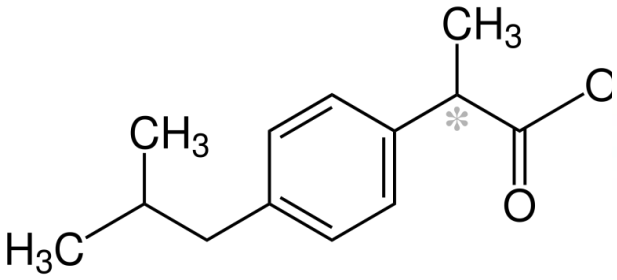




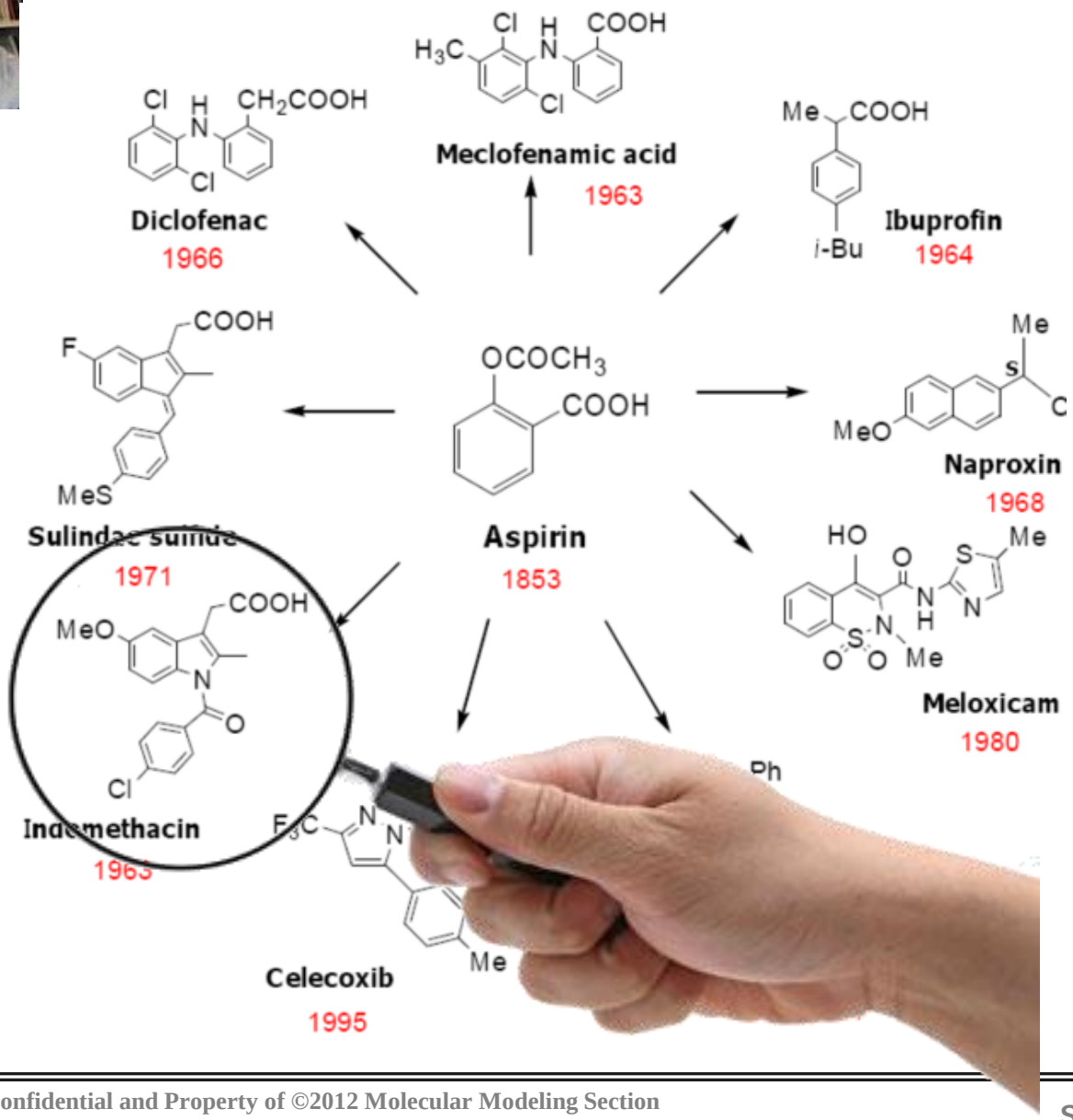

Arg A121
Tyr A356
Trp A388
Ser A354
Tyr A386
Val A117
Met A523
Leu A360
Ser A531
Val A350
Ala A528
Val A524
Phe A519
Gly A527
Leu A532



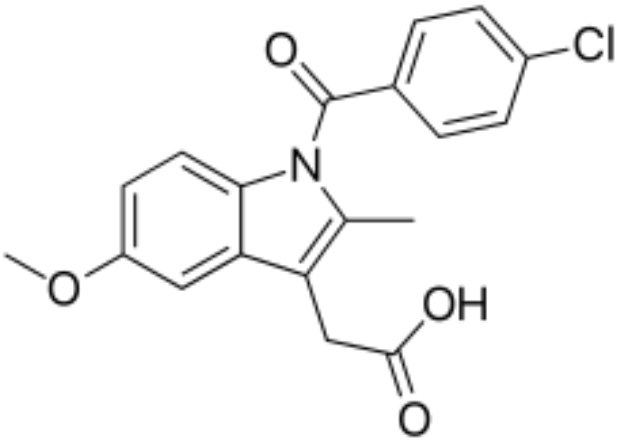
Just to remember…

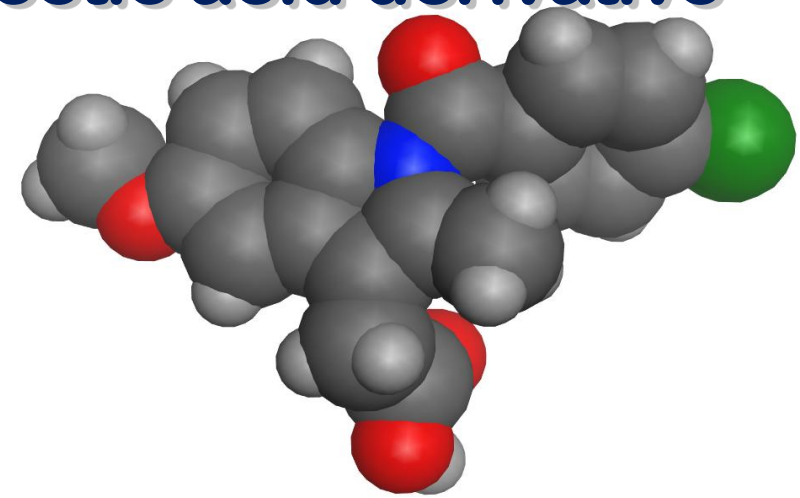


Non-Steroidal Anti-Inflammatory Drug (NSAID)



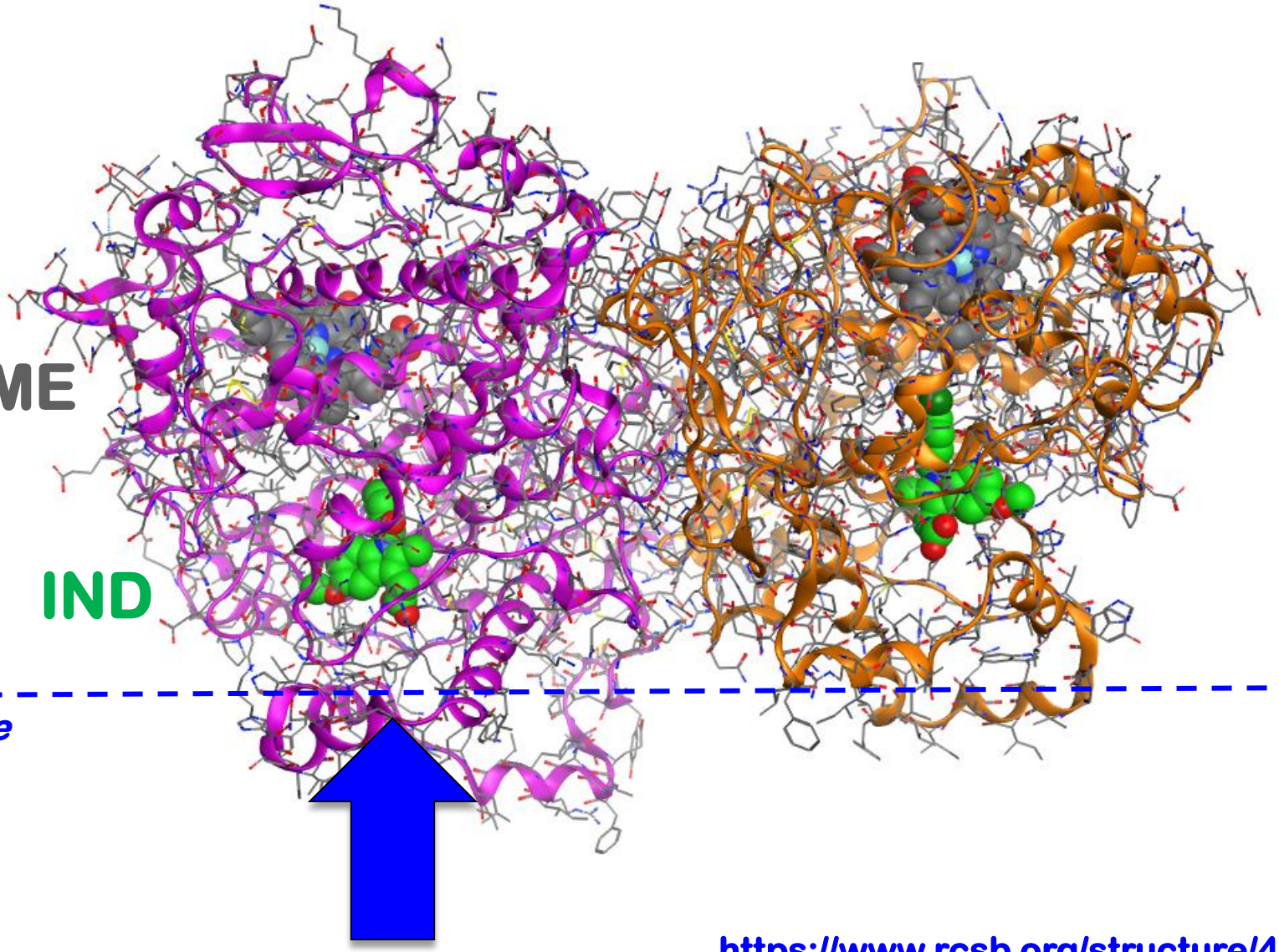
Indometacin... an acetic acid derivative



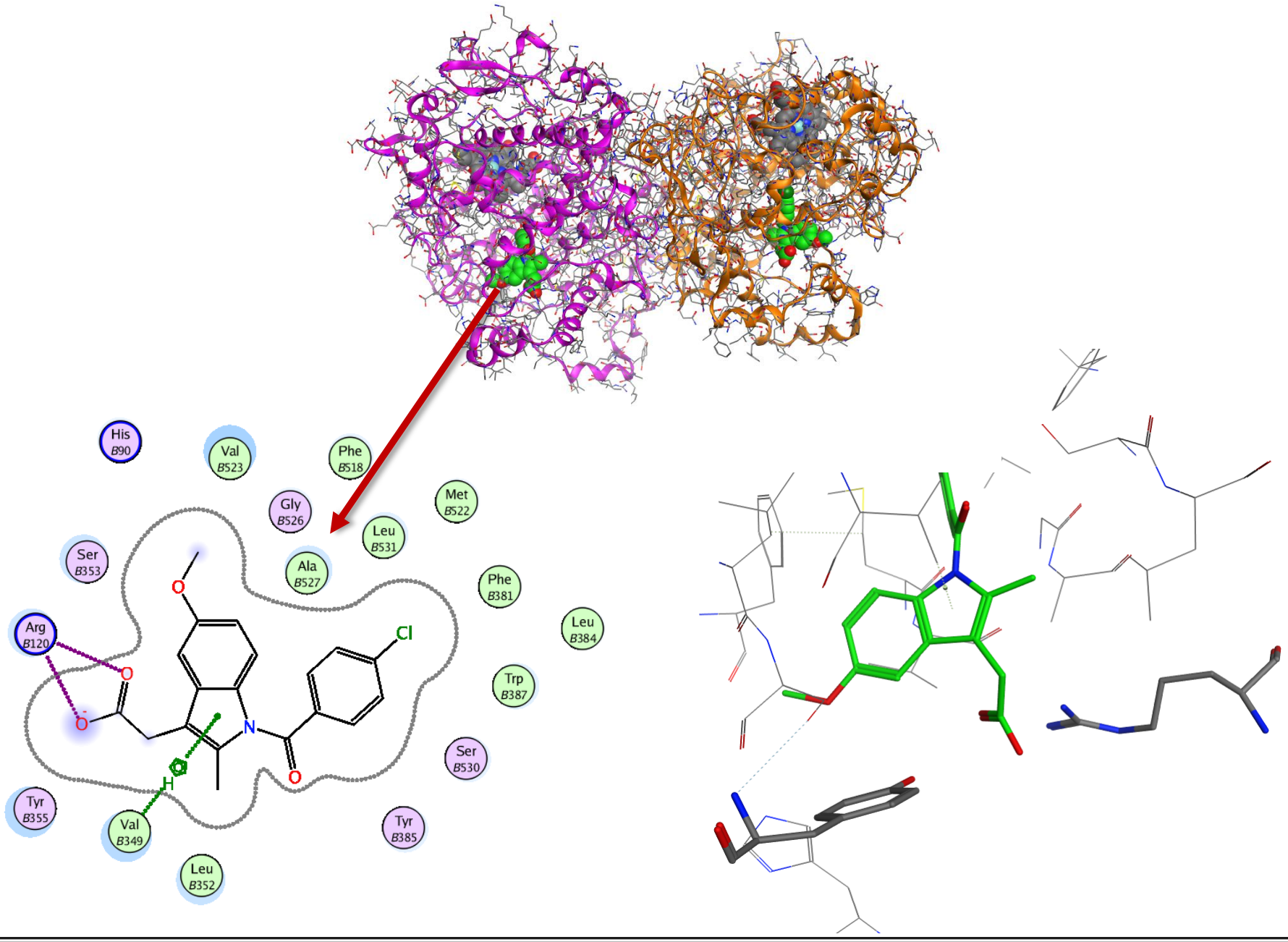


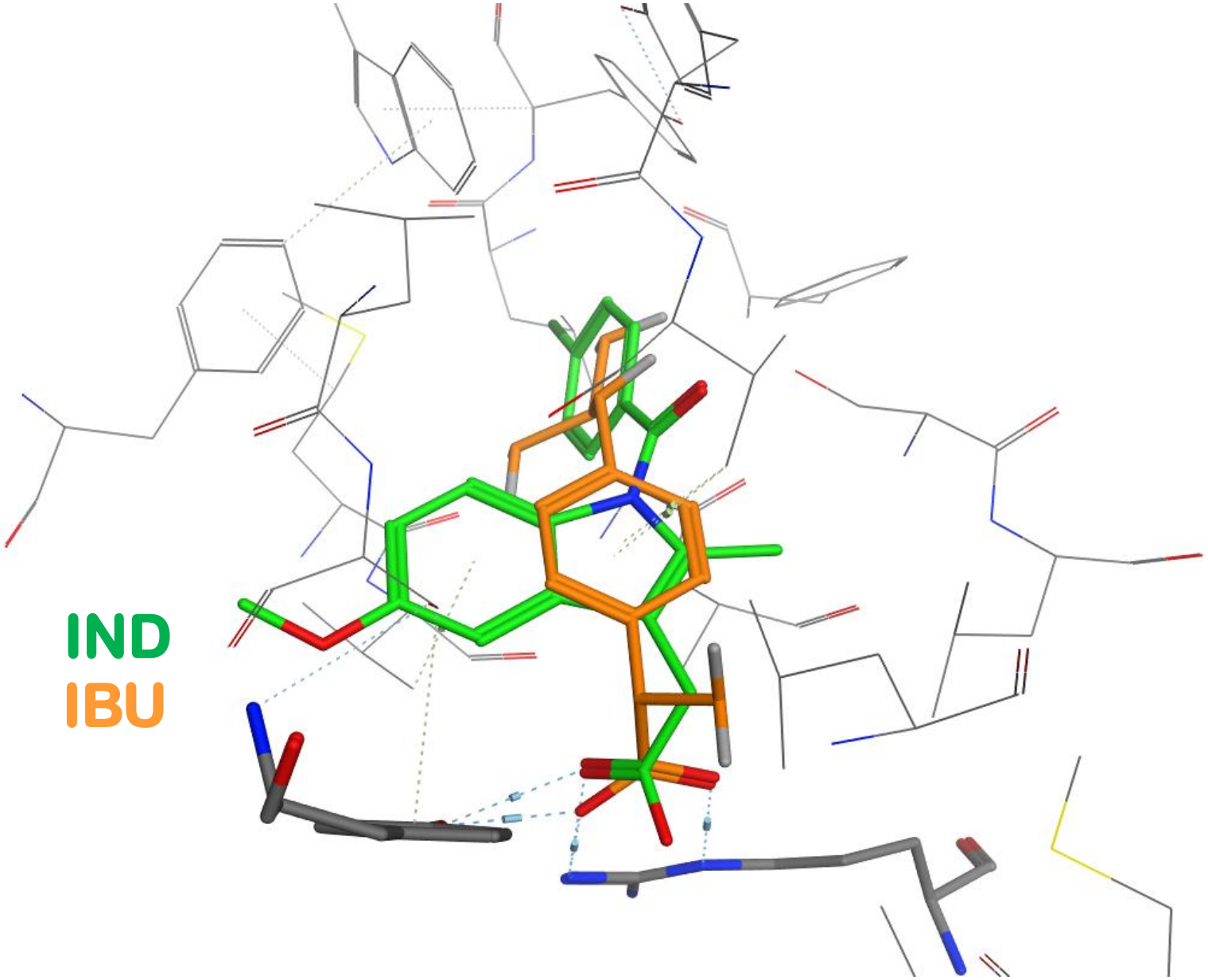
Indometacin, or indomethacin, is a non-steroidal anti-inflammatory drug (NSAID) with anti-inflammatory, analgesic, and antipyretic properties. NSAIDs consist of agents that are structurally unrelated; the NSAID chemical classification of indometacin is an indole-acetic acid derivative with the chemical name 1- (p-chlorobenzoyl)25-methoxy-2-methylindole-3-acetic acid. The pharmacological effect of indometacin is not fully understood, however, it is thought to be mediated through potent and nonselective inhibition of the enzyme cyclooxygenase (COX)

Chemical formula	$C_{19}H_{16}ClNO_4$
Molar mass	357.787 g·mol^{-1}
log *P*	4.27
Acidity (pK_a)	4.5

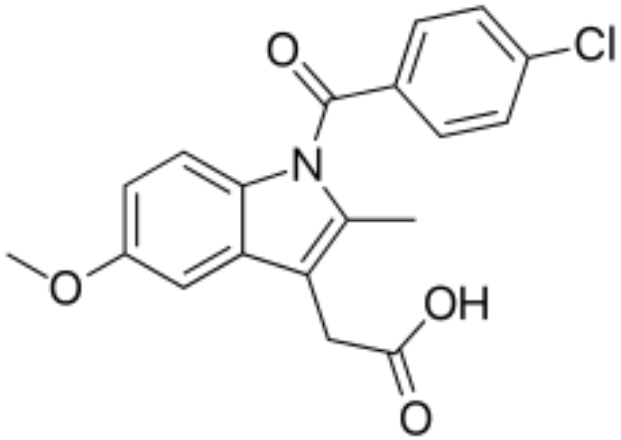


https://www.rcsb.org/structure/4COX

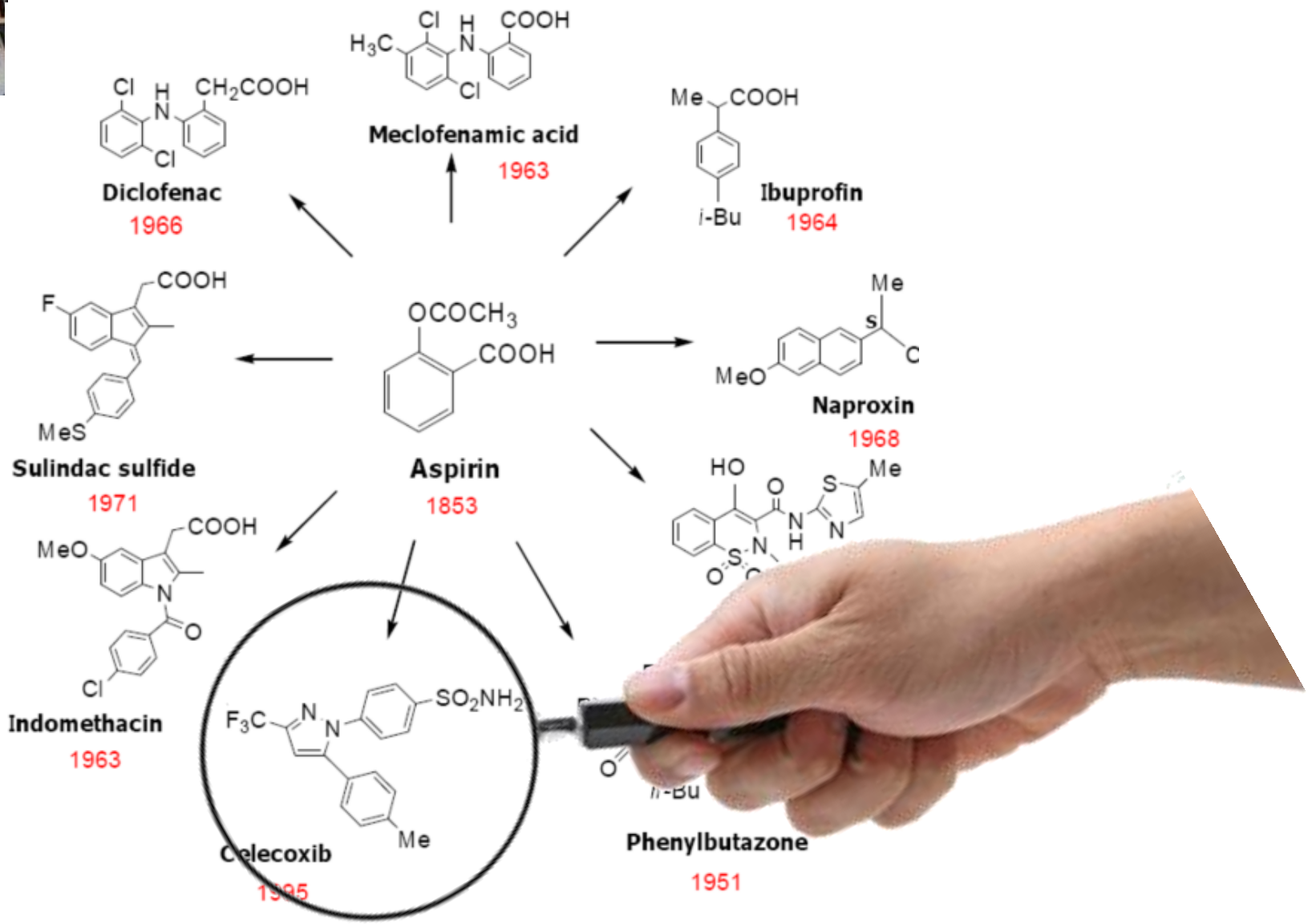

His B90
Val B523
Phe B518
Gly B526
Met B522
Leu B531
Ser B353
Ala B527
Phe B381
Arg B120
Leu B384
Cl
Trp B387
Ser B530
Tyr B355
Val B349
Tyr B385
Leu B352
O
N
H



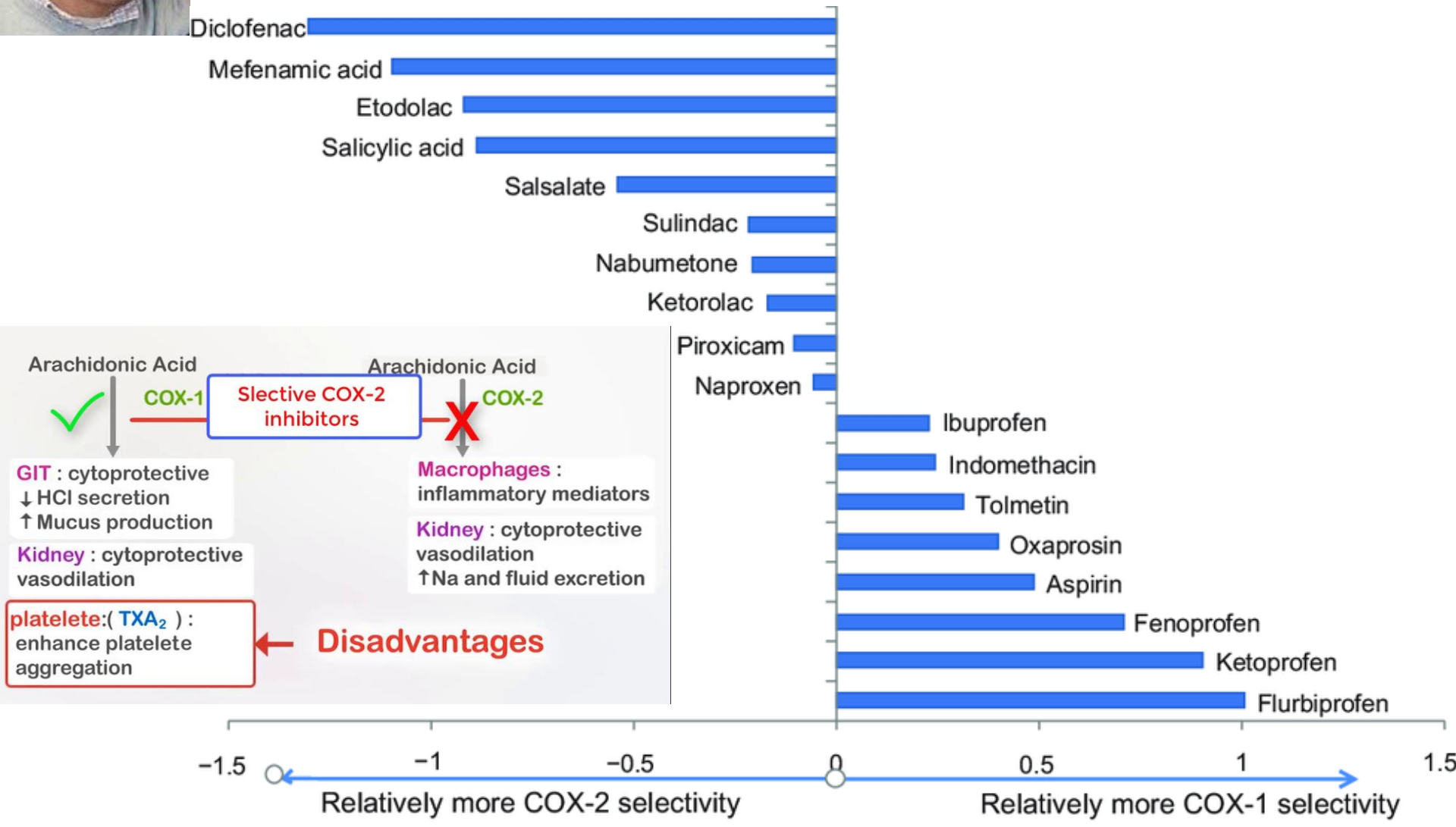
Just to remember…



Non-Steroidal Anti-Inflammatory Drug (NSAID)

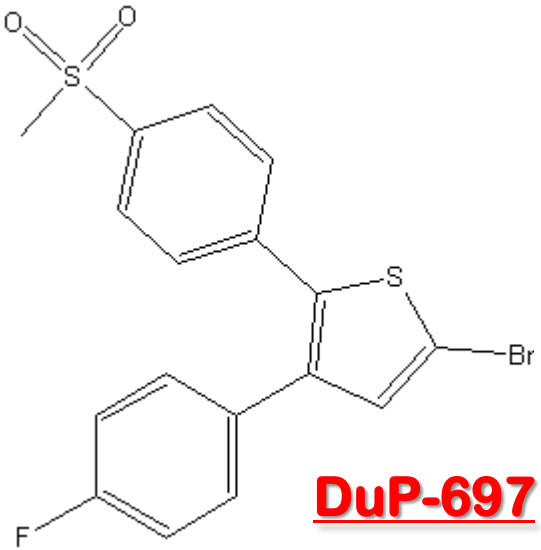


COX-1 versus COX-2 selectivity…



COX-1 versus COX-2 selectivity: the rise for development of selective COX-2 inhibitors

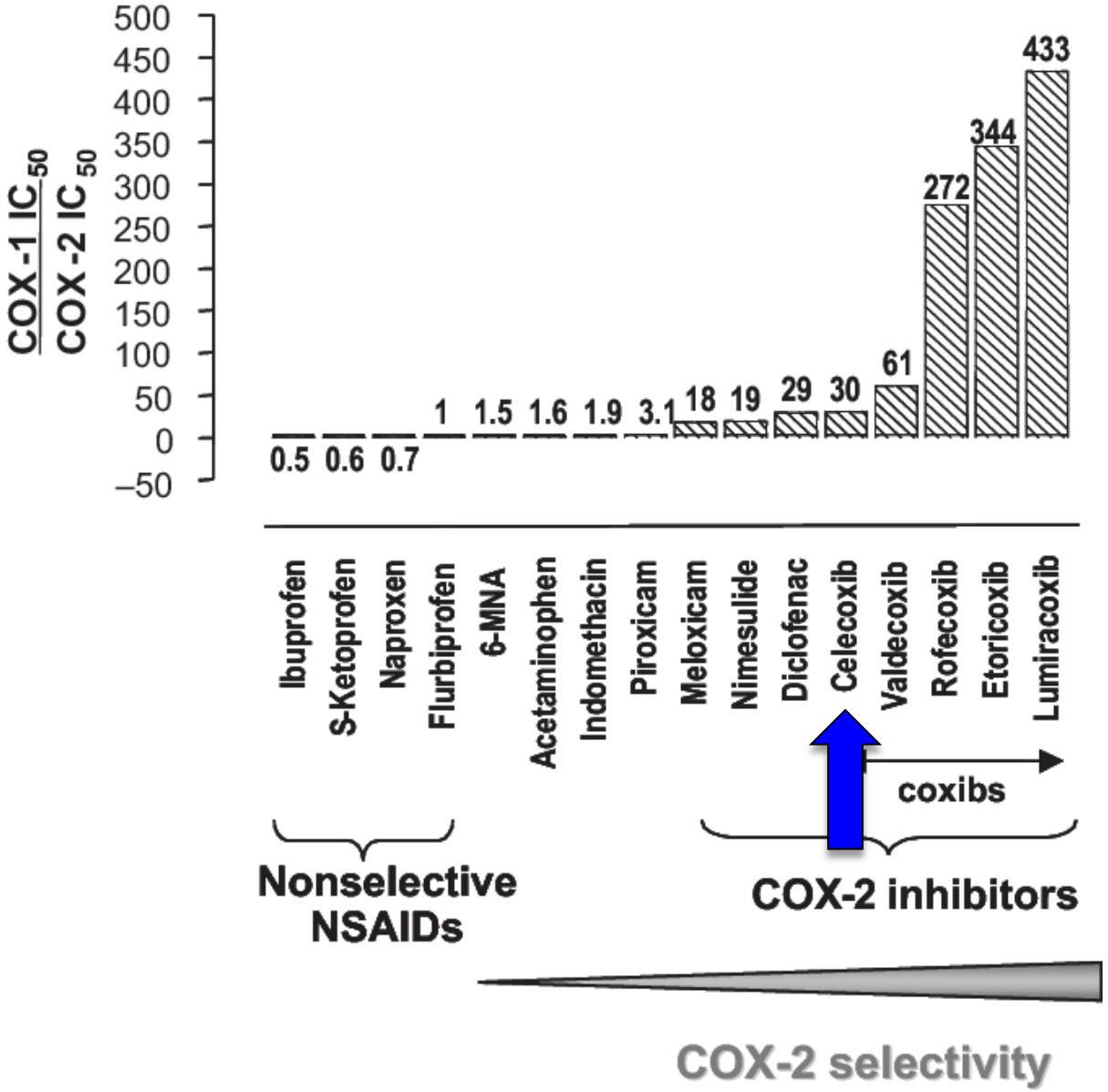
The impetus for development of selective COX-2 inhibitors was the adverse gastrointestinal side-effects of NSAIDs. Soon after the discovery of the mechanism of action of NSAIDs, strong indications emerged for alternative forms of COX, but little supporting evidence was found. COX enzyme proved to be difficult to purify and was not sequenced until 1988. In 1991 the existence of the COX-2 enzyme was confirmed by being cloned by Dr. Dan Simmons at Brigham Young University.



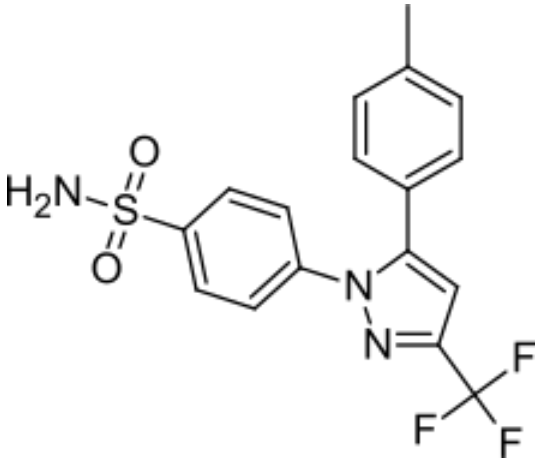
DuP-697

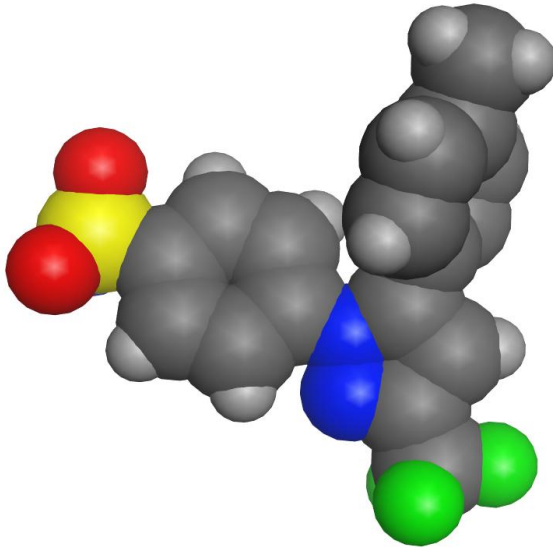
Before the confirmation of COX-2 existence, the Dupont company had developed a compound, DuP-697, that was potent in many anti-inflammatory assays but did not have the ulcerogenic effects of NSAIDs. Once the COX-2 enzyme was identified, Dup-697 became the building-block for synthesis of COX-2 inhibitors. Celecoxib and rofecoxib, the first COX-2 inhibitors to reach market, were based on DuP-697. It took less than eight years to develop and market the first COX-2 inhibitor, with Celebrex (celecoxib) launched in December 1998 and Vioxx (rofecoxib) launched in May 1999. Celecoxib and other COX-2 selective inhibitors, valdecoxib, parecoxib, and mavacoxib, were discovered by a team at the Searle division of Monsanto led by John Talley.

COX-1 versus COX-2 selectivity: …coxib!!!



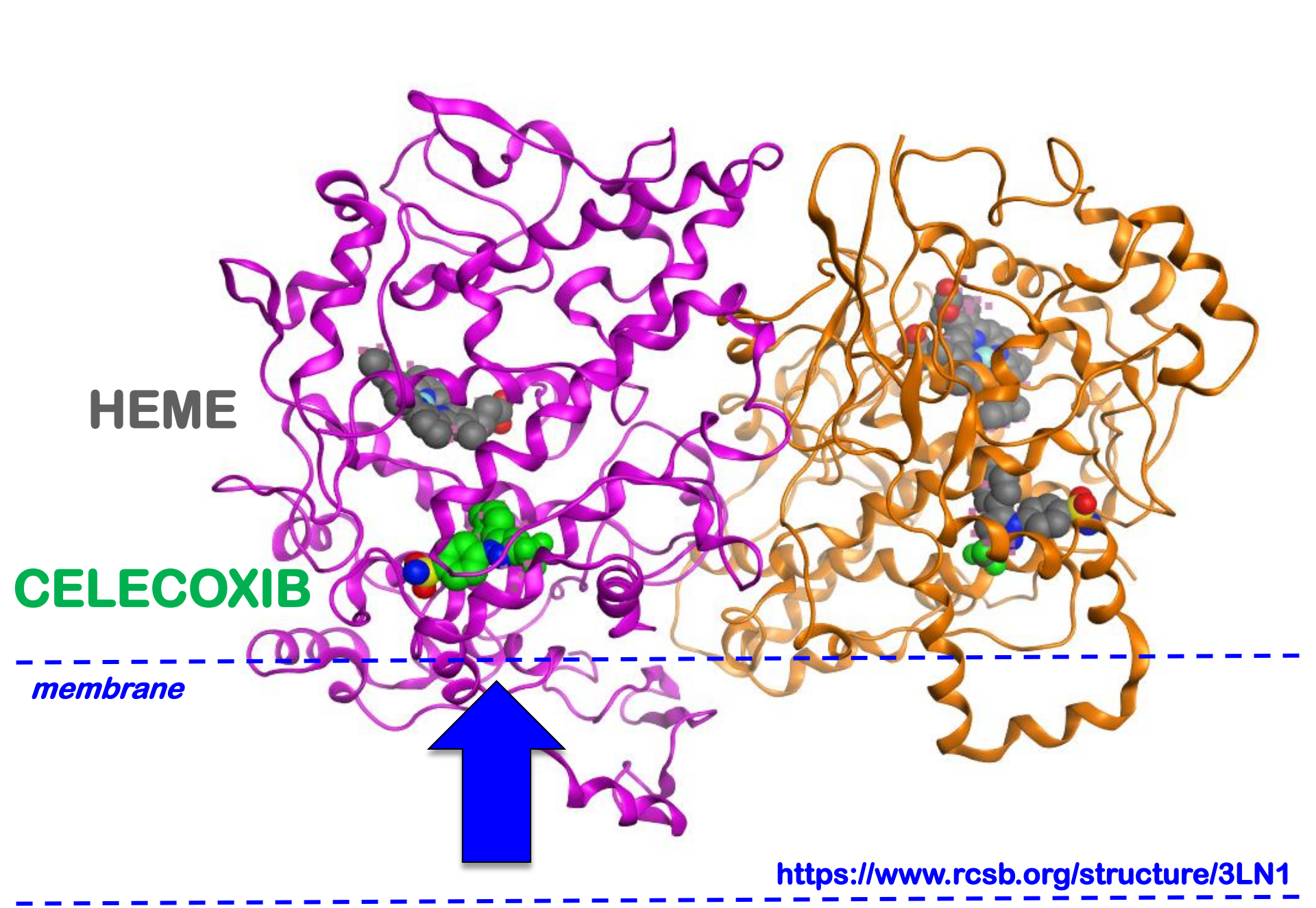
Celecoxib…

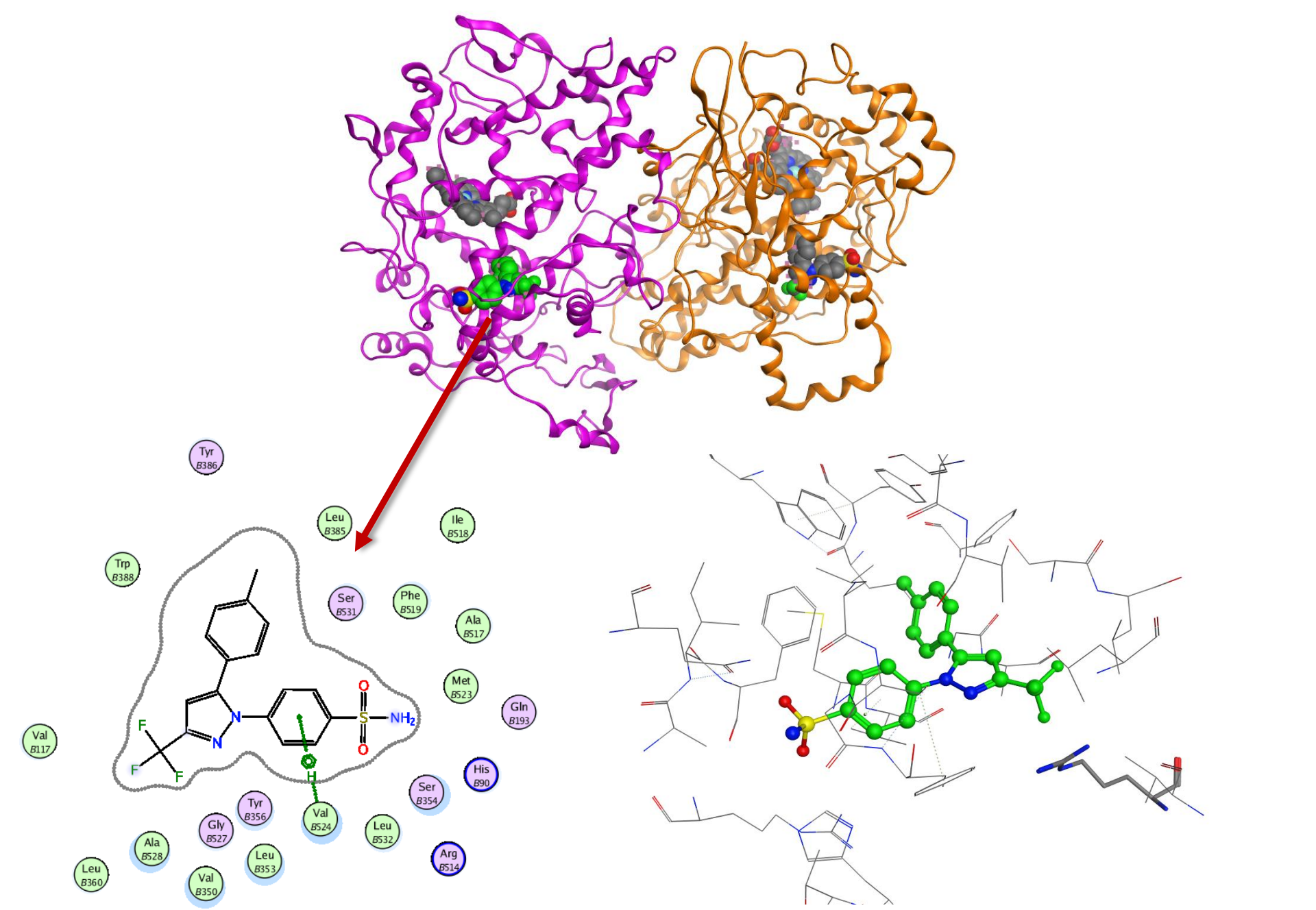




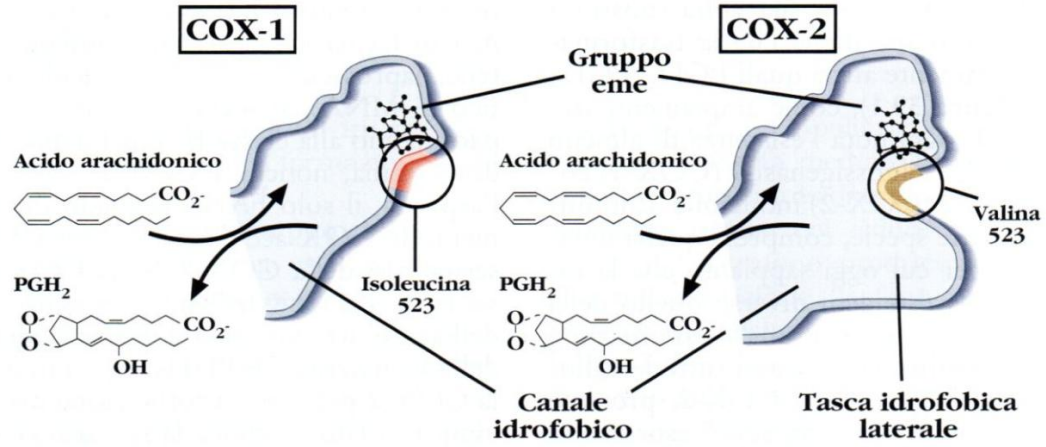
Celecoxib, a selective cyclooxygenase-2 (COX-2) inhibitor, is a nonsteroidal anti-inflammatory drug (NSAID) which is known for its decreased risk of causing gastrointestinal bleeding compared to other NSAIDS. It is used to manage symptoms of various types of arthritis pain and in familial adenomatous polyposis (FAP) to reduce precancerous polyps in the colon. It is marketed by Pfizer under the brand name Celebrex, and was initially granted FDA approval in 1998.

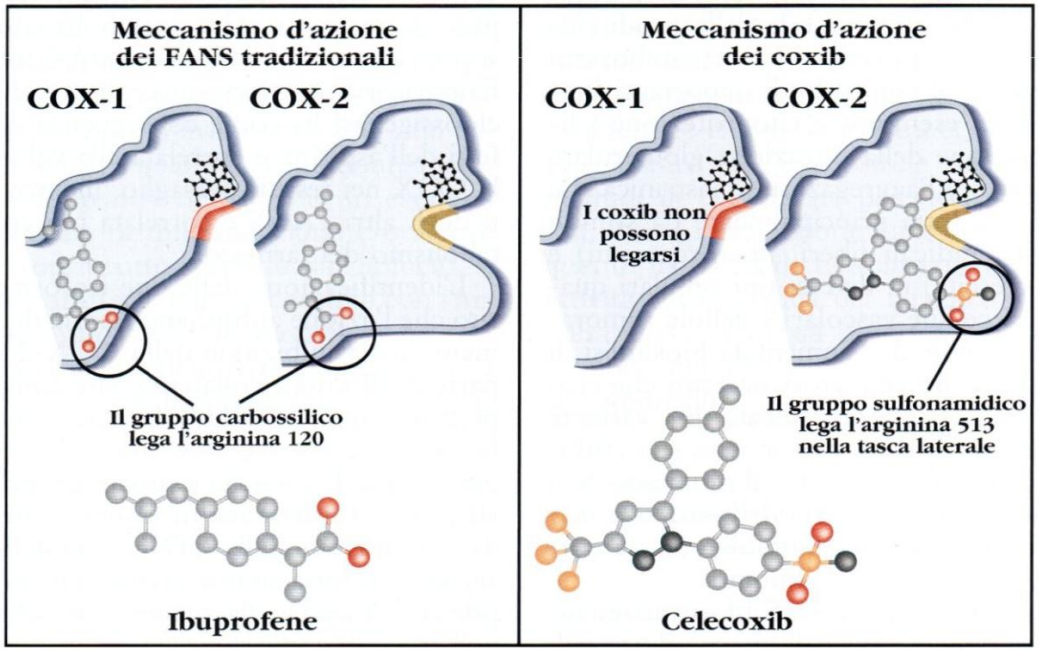
Chemical formula	$C_{17}H_{14}F_3N_3O_2S$
Molar mass	381.87 g·mol^{-1}
log *P*	3.53
Acidity (pK_a)	11.1



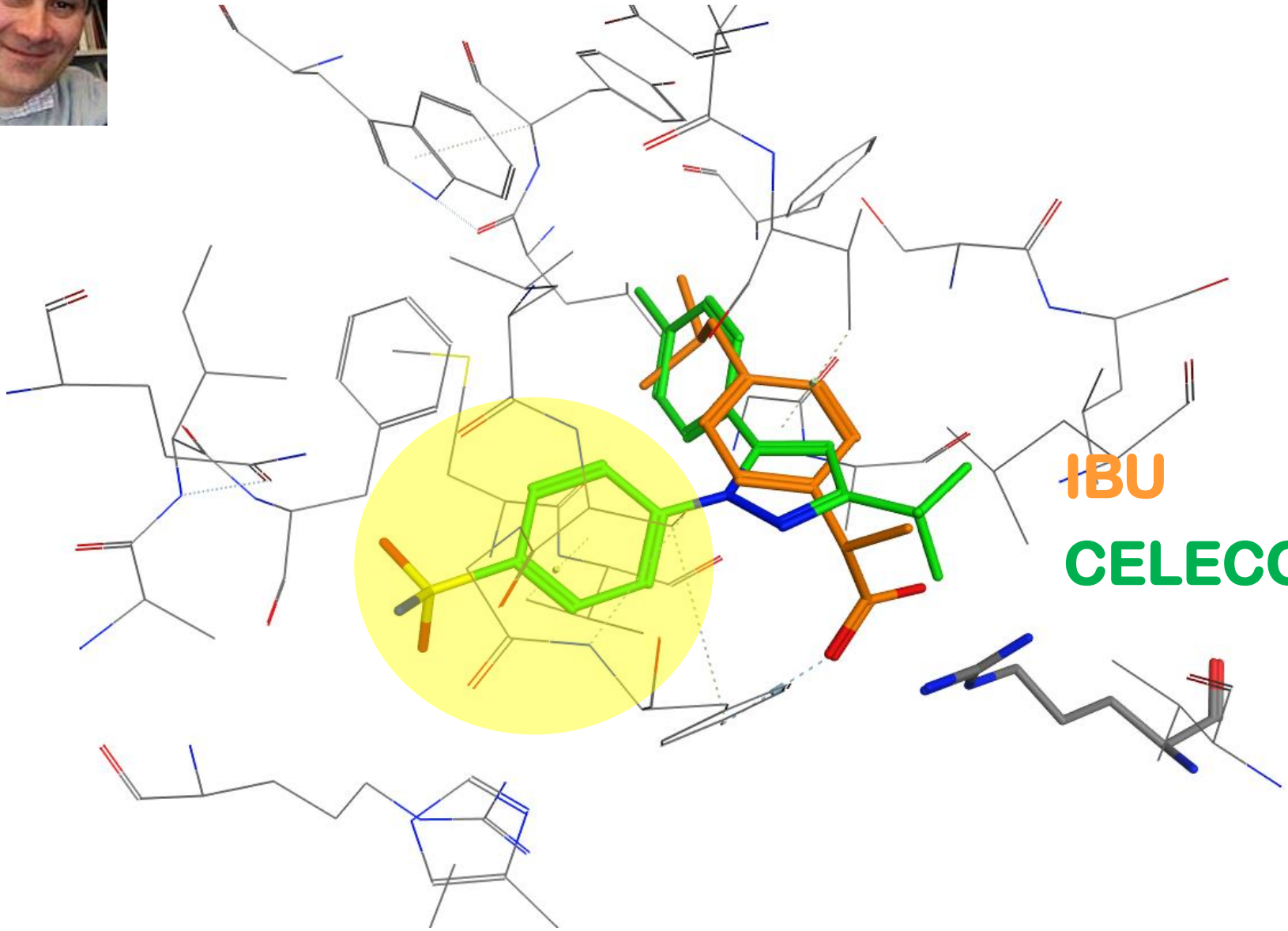


COX-1 versus COX-2 selectivity: …coxib!!!

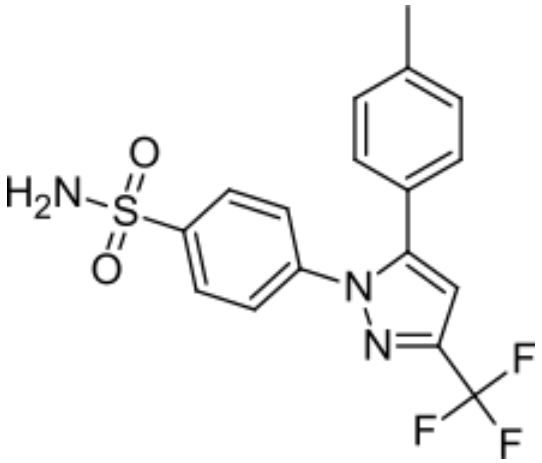




COX-1 versus COX-2 selectivity: …coxib!!!



Just to remember…



GRAZIE
PER LA PAZIENZA