

Retrophin[®]

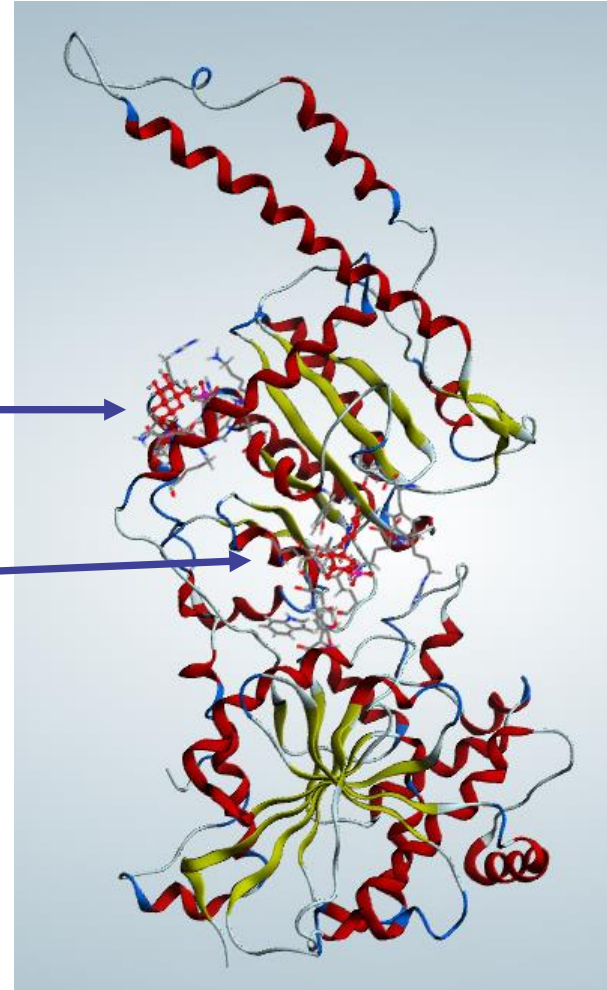
WaterSwap to Assess Target Druggability

E. Adam Kallel Ph. D.

Ligands directly Taken from 4KQ1

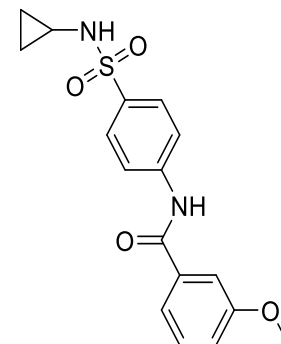
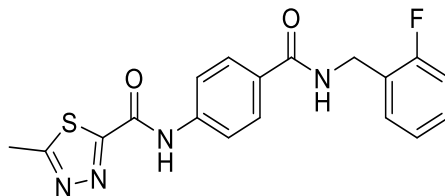
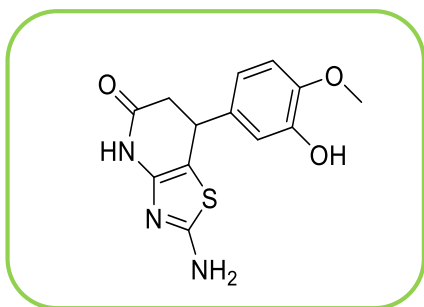
Glucose Monophosphate
In allosteric pocket
UDP Glucose in Catalytic
Pocket

Allosteric
Pocket →
Catalytic
Pocket →



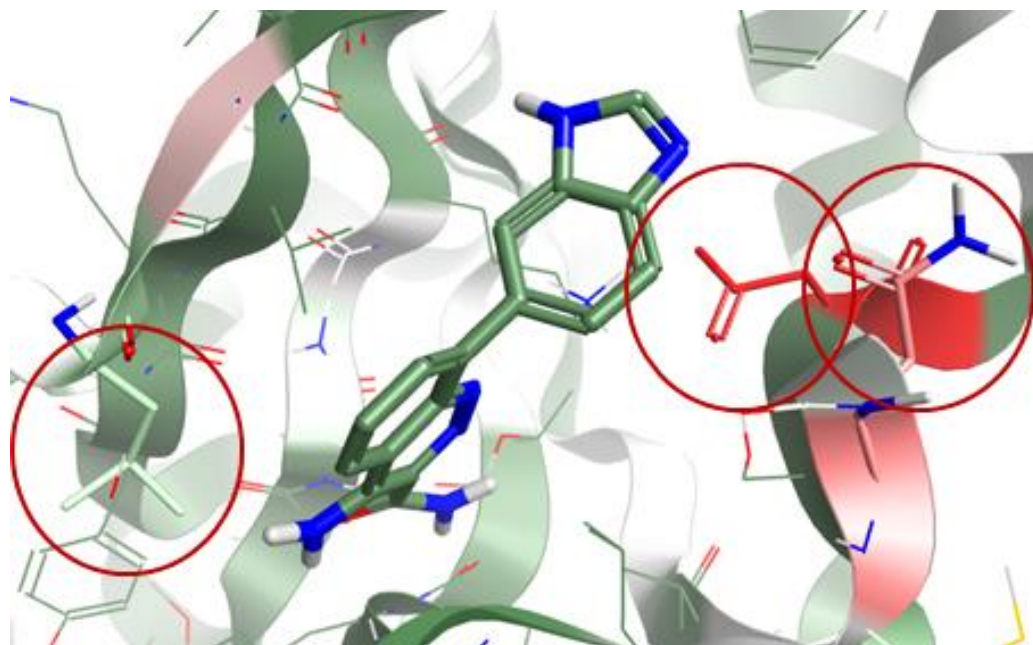
- **eMolecules has 13.5 compounds available at any given time**
 - What percentage of these do you think are worth screening?
- **250<MW<450, -3<cLogP<4.5, 30<TPSA>130**
- **REOS**
- **PAINS**

- **985,000 compounds left were docked**
- **3,998 compounds ordered and screened**
- **43 Confirmed hits in 3 classes 1% hit rate (high) (retest with .1% triton X)**



- The flat SAR led to the my performing the activity cliff analysis
- I was finally able to convince the biologists to run assays with triton X
- Triton X killed the activity indicating the entire project was derived from aggregation a lesson relearned.
- Could this have been stopped even earlier?

- Detailed energetic analysis of ligand-protein binding
- Find hotspots within the protein active site
- Understand the energetics of your ligand
- See the 'perfect' interaction pattern for hydrophilicity within the protein active site

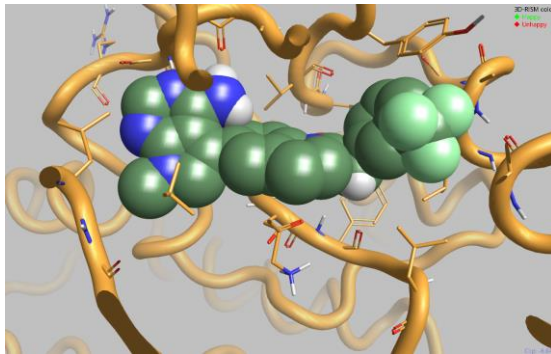


Woods, J Chem Phys 134, p054114, 2011
Woods et al. Faraday Discussions 169, p477, 2014

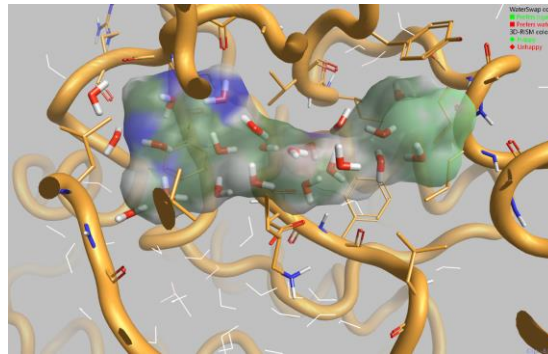
- **Thermodynamic Integration method to study energy of ligand-protein binding**
- **Details energetically favourable and unfavourable interactions within the protein active site**
- **Possible to predict binding energy for some systems**
- **Extensions to study selectivity in development**

Swap Uses λ to Fade Between the Two States

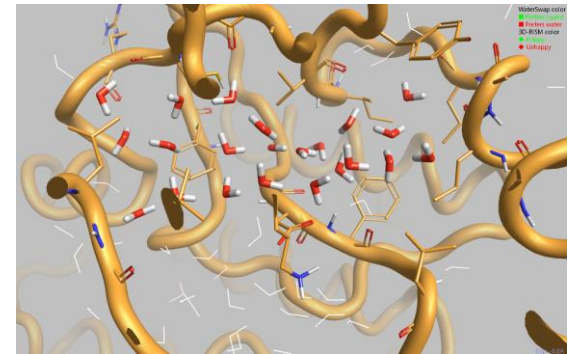
Retrophin[®]



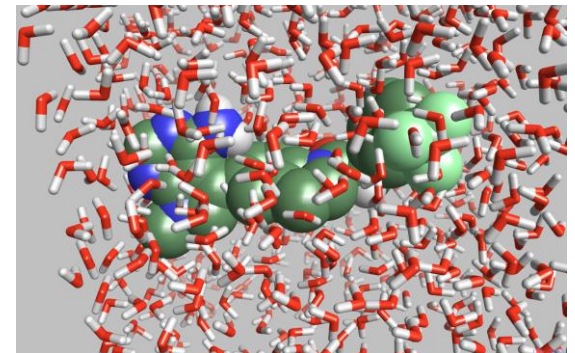
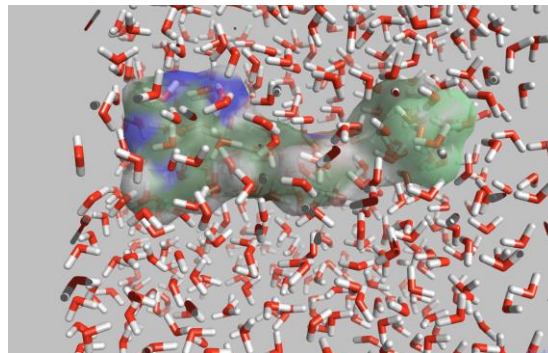
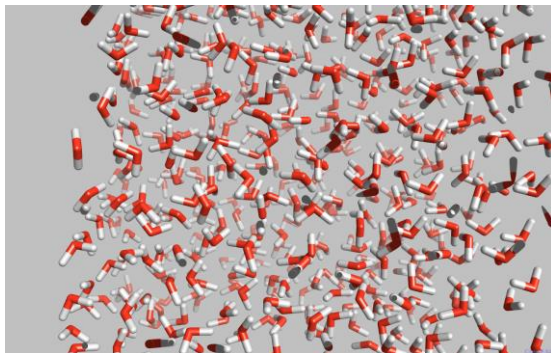
$\lambda = 0$



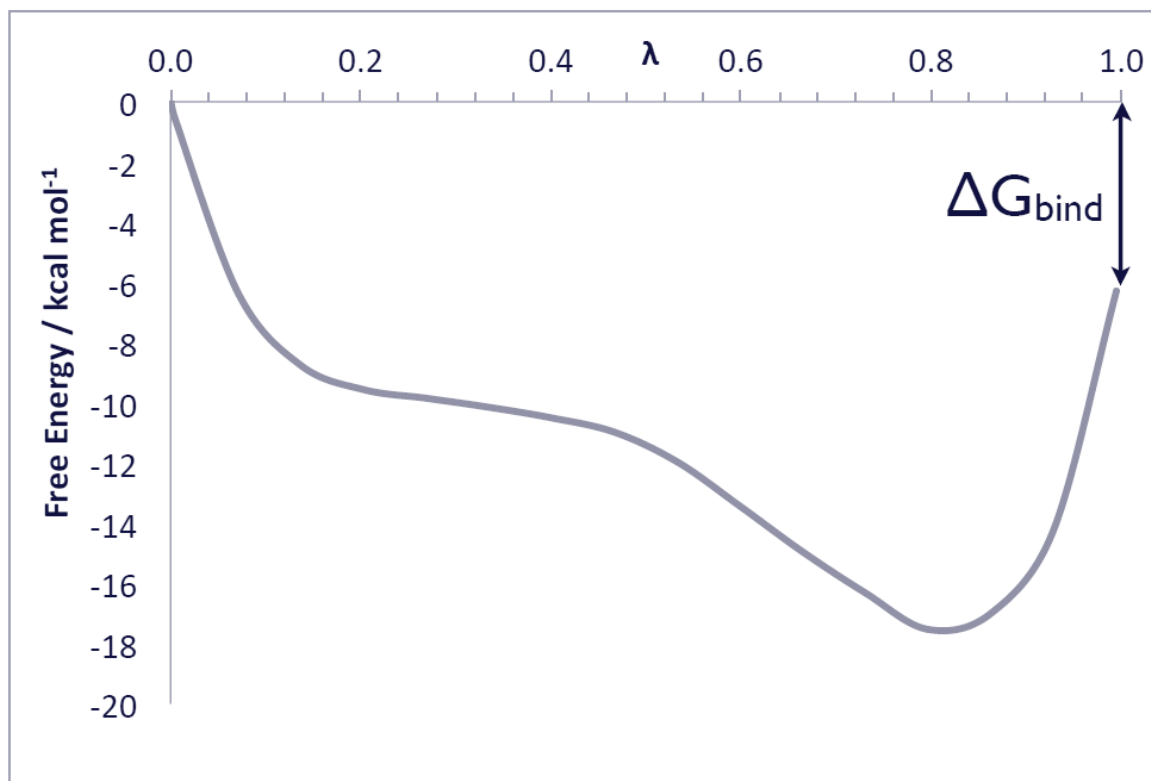
$\lambda = 0.5$

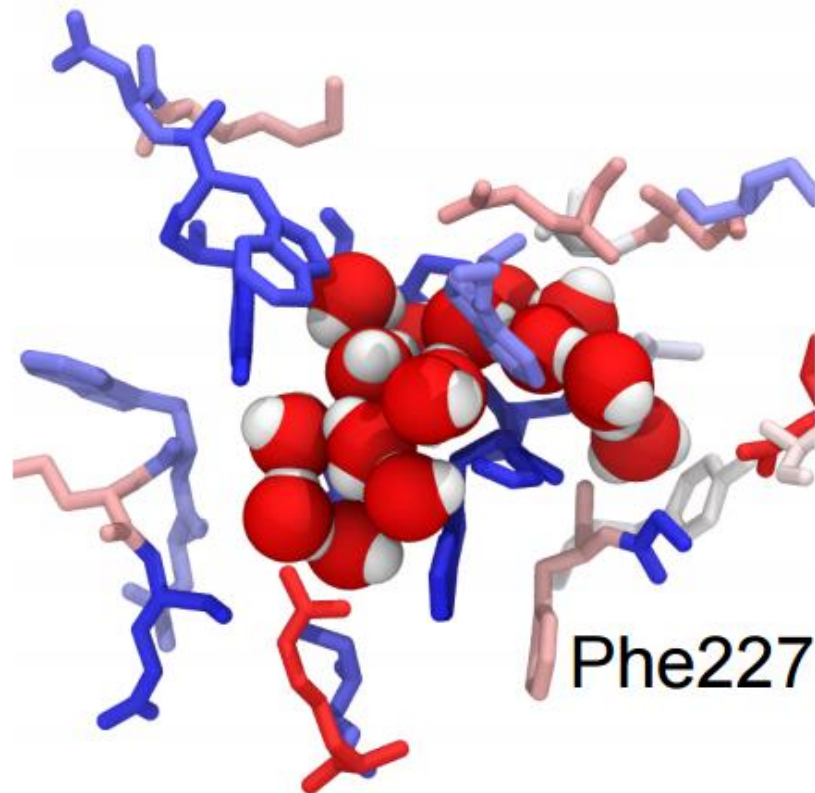
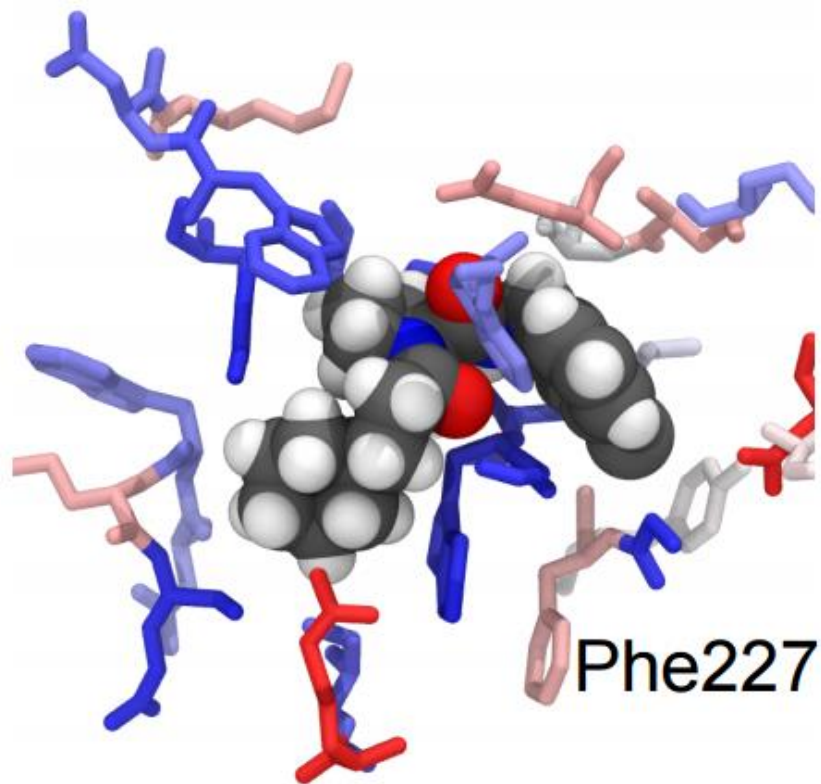


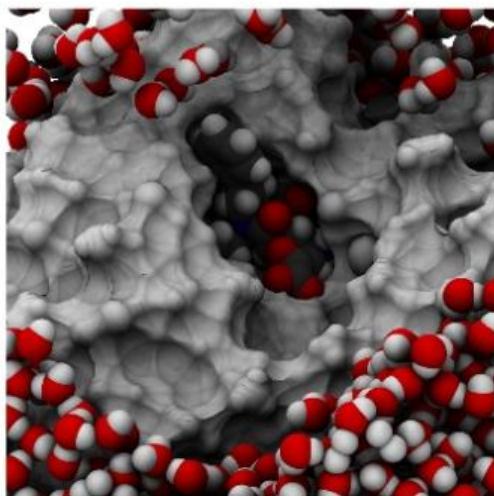
$\lambda = 1$



Perform Thermodynamic Integration (TI) along the Waterswap λ coordinate. This results, directly, in the absolute binding free energy



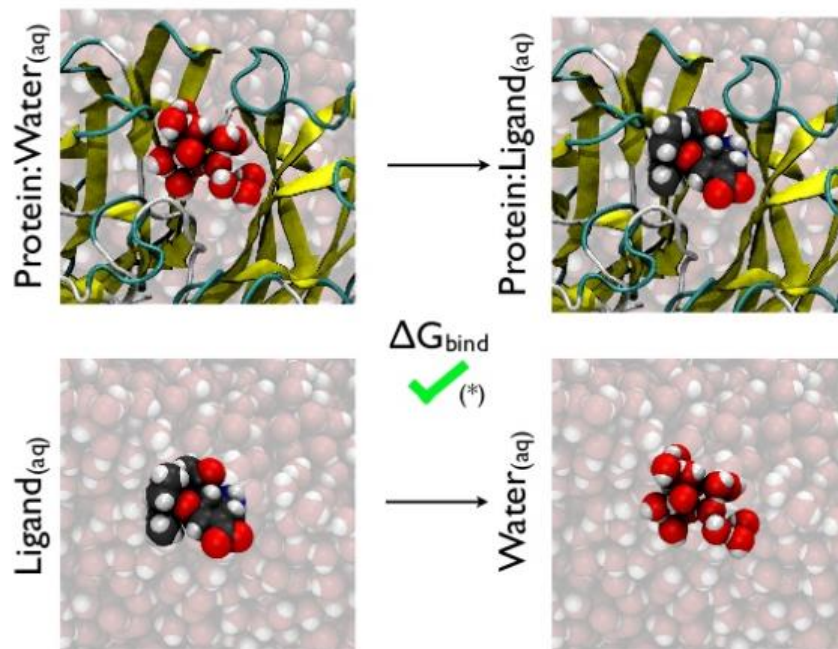




Waterswap Method

Uses the fact that protein-ligand binding is really a competition between the ligand and water for binding to the protein

- Woods, J Chem Phys, Vol 134, p054114, 2011
- <http://dx.doi.org/10.1063/1.3519057>



WaterSwap allows decomposition of ΔG by a per-residue basis. This permits interpretation of which residues prefer binding to water or ligand.

WaterSwap Analysis of Glycogen Binding Pocket with UDP Glucose

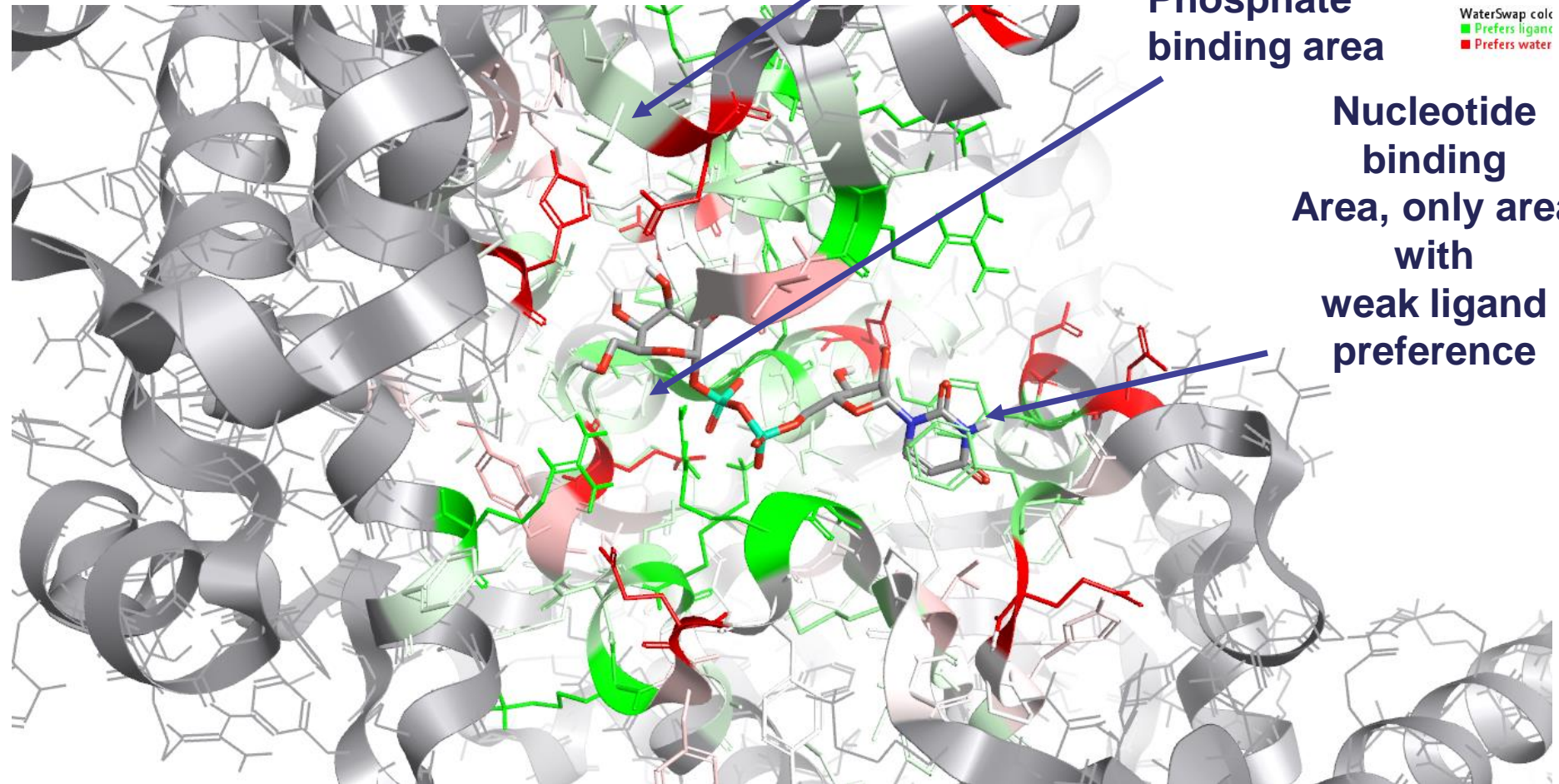
Retrophin®

Ligand binding area;
more preference for water

Phosphate
binding area

WaterSwap color
■ Prefers ligand
■ Prefers water

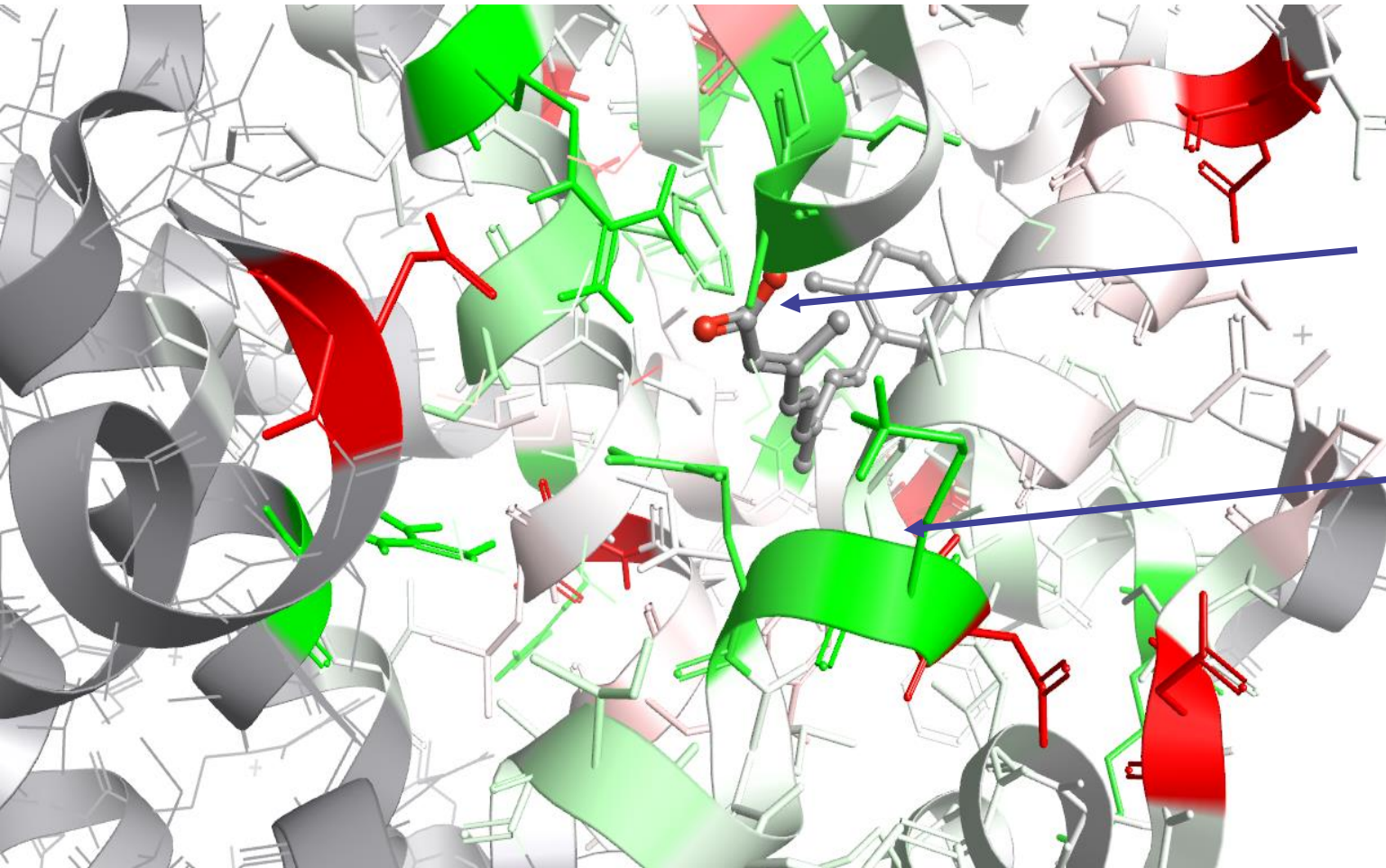
Nucleotide
binding
Area, only area
with
weak ligand
preference



- **Since the part of the binding domain that had strong ligand preference was the pyrophosphate binding area, this was predisposed to highly polar compounds**
- **The binding pocket did not evince features that would have bound drug like molecules**
- **Coupled with the shape of the binding pocket, this target is not druggable**
- **We should not have started here**

- Only 2% of human proteins interact with currently approved drugs
- 10-15% of human proteins are disease-modifying
- 10-15% are druggable
- 5% are both disease-modifying and druggable
- What can we do *a priori* to identify druggable proteins?
- Identifying cavities or pockets on the structure
- Calculating physicochemical and geometric properties of the pocket
- Assessing how these properties fit a training set of known druggable targets, typically using machine learning algorithms

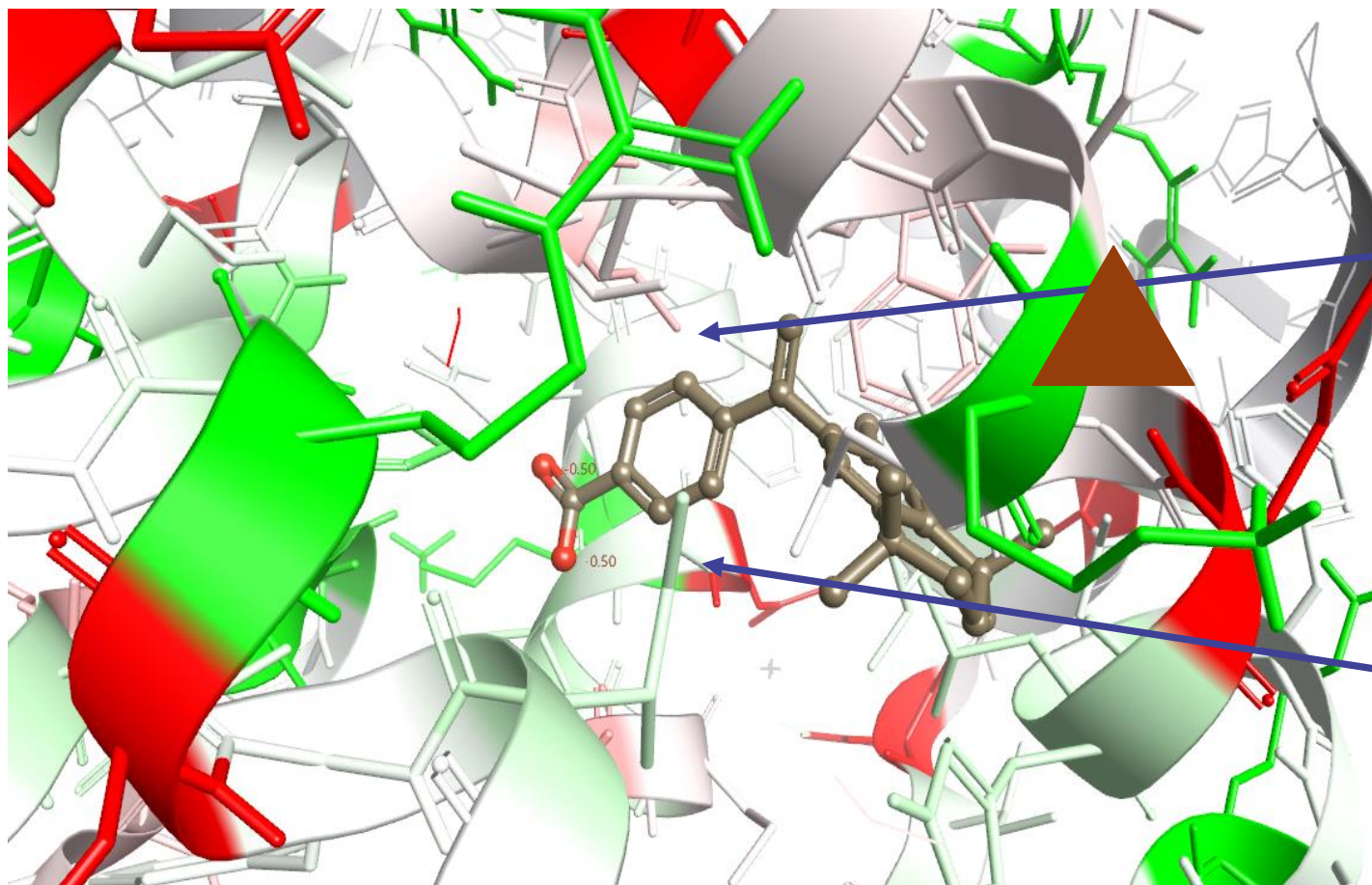
- The retinoid X receptor (RXR) is a type of nuclear receptor that is activated by 9-cis retinoic acid
- There are three retinoic X receptors (RXR): RXR-alpha, RXR-beta, and RXR-gamma, encoded by the RXRA, RXRB, RXRG genes, respectively.
- RXR heterodimerizes with subfamily 1 nuclear receptors including CAR, FXR, LXR, PPAR, PXR, RAR, TR, and VDR.
- As with other type II nuclear receptors, the RXR heterodimer in the absence of ligand is bound to hormone response elements complexed with corepressor protein
- Binding of agonist ligands to RXR results in dissociation of corepressor and recruitment of coactivator protein, which, in turn, promotes transcription of the downstream target gene into mRNA and eventually protein.



WaterSwap color
■ Prefers ligand
■ Prefers water

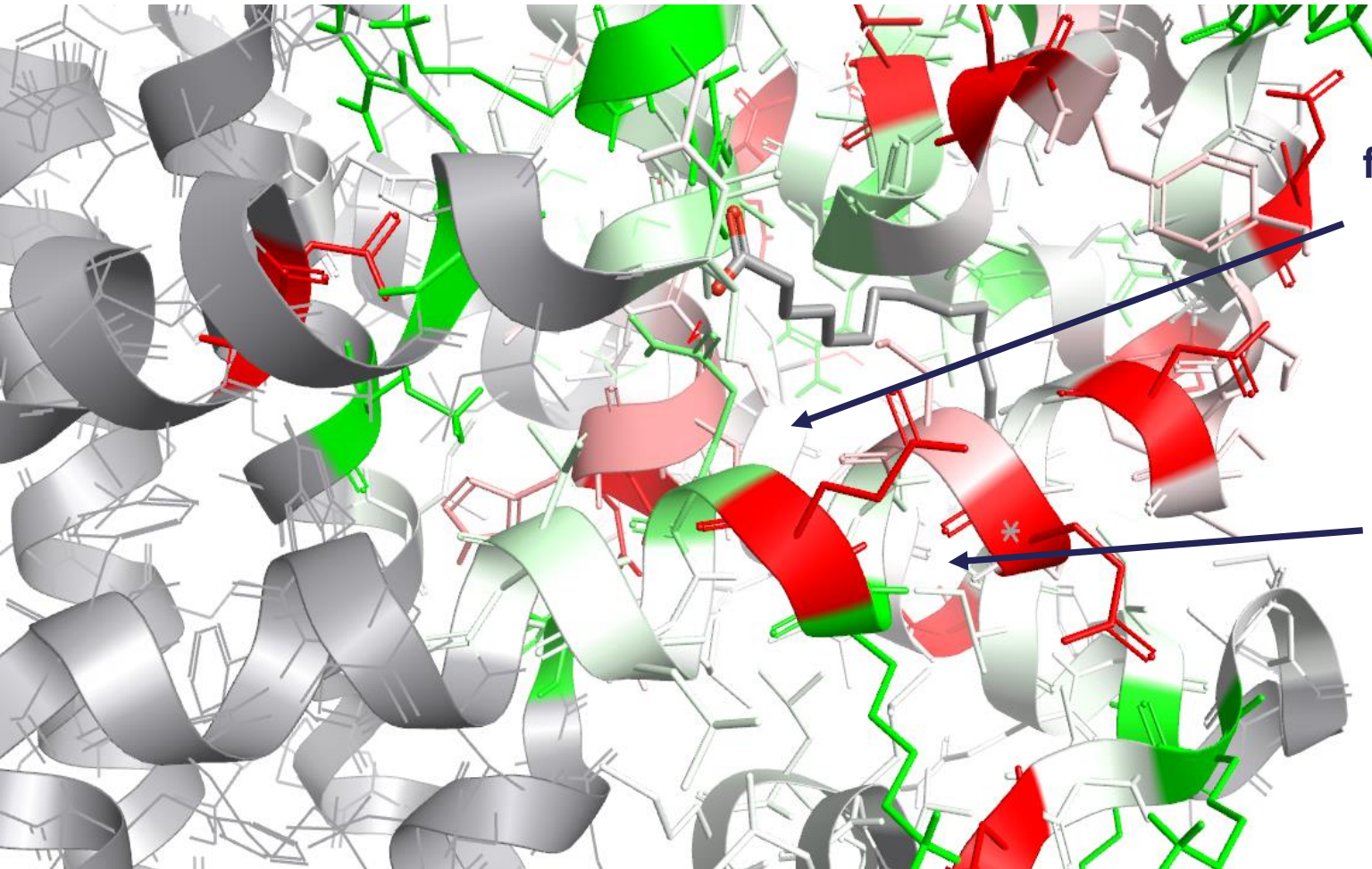
The retinoid binding domain has a clear strong preference for ligand

WaterSwap
■ Prefers H
■ Prefers V



The benzoic acid in Bexarotene is buried in a deep pocket with clear preference for ligand

- **HNF4 (Hepatocyte Nuclear Factor 4) is a nuclear receptor protein mostly expressed in the liver, gut, kidney, and pancreatic beta cells that is critical for liver development. In humans, there are two isoforms of HNF4, HNF4 α and HNF4 γ , encoded by two separate genes HNF4A and HNF4G respectively.**
- **HNF4 was originally classified as an orphan receptor that exhibits constitutive transactivation activity apparently by being continuously bound to a variety of fatty acids. The existence of a ligand for HNF4 has been somewhat controversial, but linoleic acid (LA) has been identified as the endogenous ligand of native HNF4 expressed in mouse liver; the binding of LA to HNF4 is reversible.**
- **The ligand binding domain of HNF4, as with other nuclear receptors, adopts a canonical alpha helical sandwich fold and interacts with co-activator proteins.**



water
Prefers
Prefers

Ligand favorable areas oriented away from ligand binding domain

Area of ligand binding domain more favorable to water

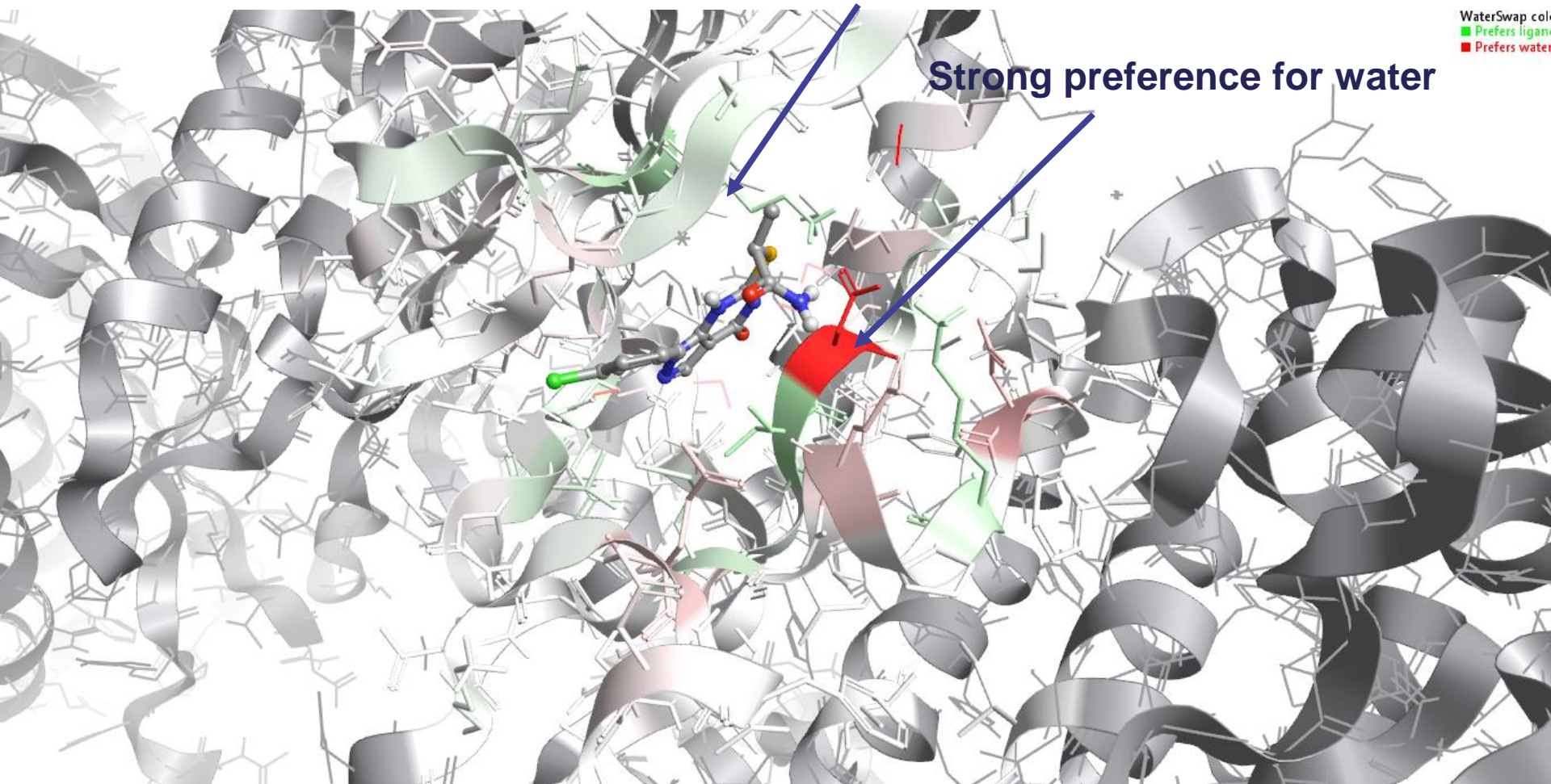
Human Zipper-Interacting Protein Kinase aka DAPK: Undrugged

Retrophin[®]

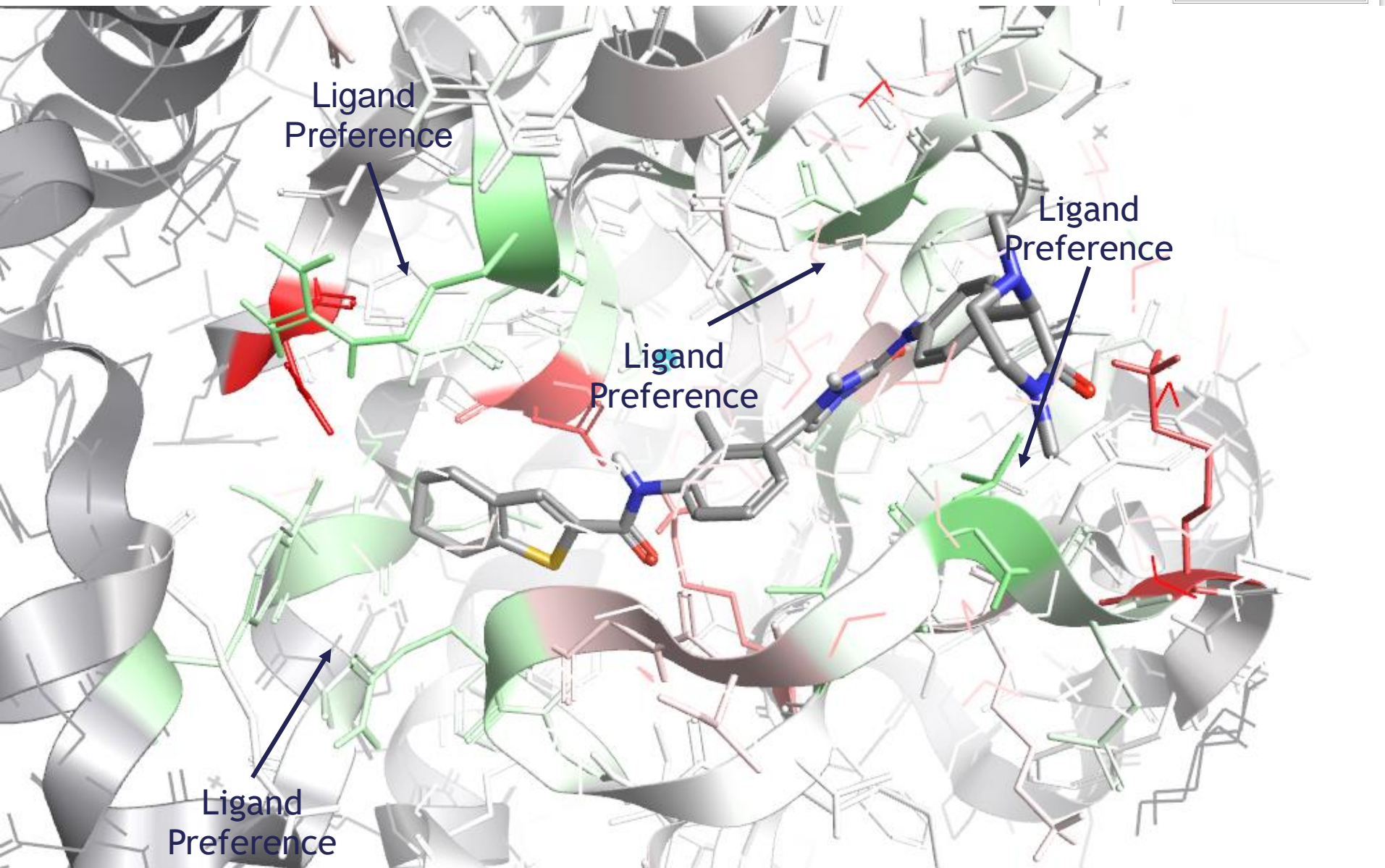
- **Serine/threonine kinase which is involved in the regulation of apoptosis, autophagy, transcription, translation and actin cytoskeleton reorganization**
- **Involved in the regulation of smooth muscle contraction**
- **Regulates both type I (caspase-dependent) apoptotic and type II (caspase-independent) autophagic cell deaths signal, depending on the cellular setting**
- **Involved in regulation of starvation-induced autophagy. Regulates myosin phosphorylation in both smooth muscle and non-muscle cells**
- **In smooth muscle, regulates myosin either directly by phosphorylating MYL12B and MYL9 or through inhibition of smooth muscle myosin phosphatase (SMPP1M) via phosphorylation of PPP1R12A**
- **As yet undrugged published ligands**

Weak preference for ligand

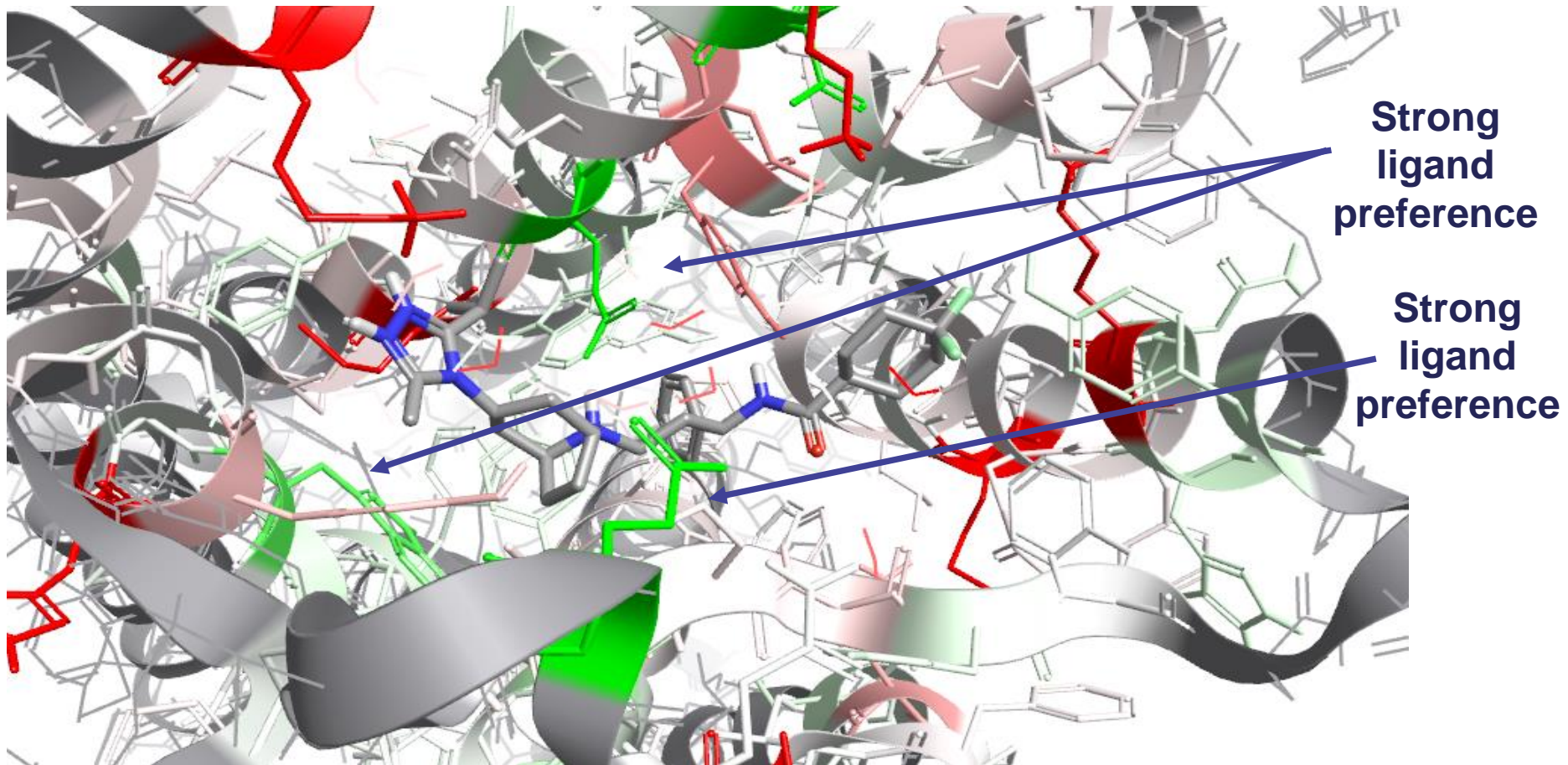
Strong preference for water



- **Bruton's tyrosine kinase also known as tyrosine-protein kinase BTK is an enzyme that in humans is encoded by the BTK gene. BTK is a kinase that plays a crucial role in B-cell development.**
- **Mutations in the BTK gene are implicated in the primary immunodeficiency disease X-linked agammaglobulinemia (Bruton's agammaglobulinemia); sometimes abbreviated to XLA. Patients with XLA have normal pre-B cell populations in their bone marrow but these cells fail to mature and enter the circulation. The Btk gene is located on the X chromosome. At least 400 mutations of the BTK gene have been identified.**



- **C-C chemokine receptor type 5, also known as CCR5 or CD195, is a protein on the surface of white blood cells that is involved in the immune system as it acts as a receptor for chemokines**
- **This is the process by which T cells are attracted to specific tissue and organ targets. Many forms of HIV, the virus that causes AIDS, initially use CCR5 to enter and infect host cells**
- **Certain individuals carry a mutation known as CCR5-Δ32 in the CCR5 gene, protecting them against these strains of HIV**
- **Several CCR5 antagonists advanced to late clinical trials with Maraviroc being approved in 2007**



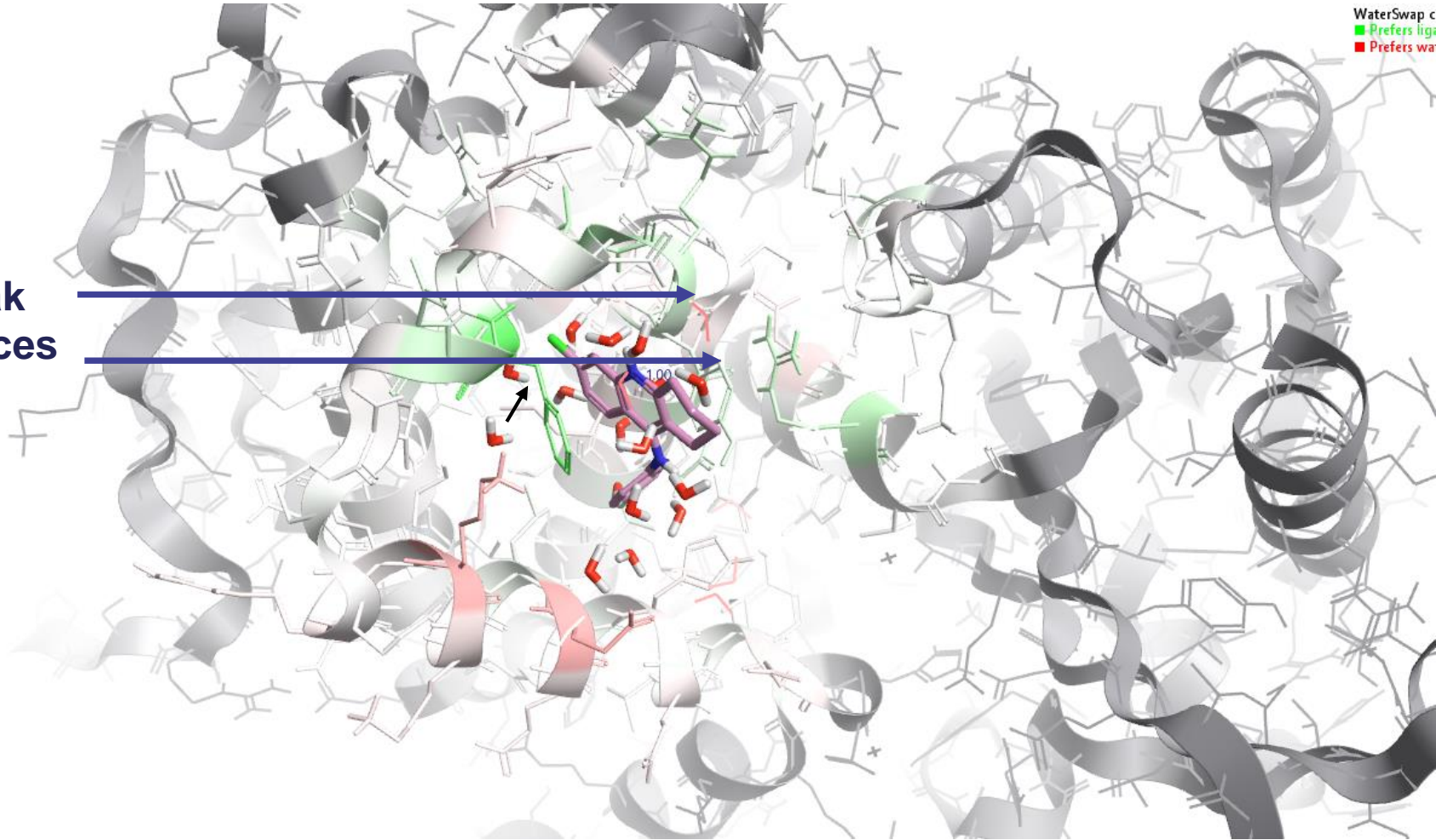
- The RAS subfamily of the RAS Superfamily has long been unsuccessfully looked at as a potential drug target
- KRAS (K-ras or Ki-ras) is a gene that acts as an on/off switch in cell signaling. When it functions normally, it controls cell proliferation. When it is mutated, negative signaling is disrupted. Thus, cells can continuously proliferate, and often develop into cancer
- In this case the K-RAS Son of Sevenless (SOS) complex was examined
- Activation of RAS is catalyzed by the guanine nucleotide exchange factor (GEF) and SOS, which is responsible for catalyzing the exchange of GDP for GTP on RAS

K-RAS SOS K-RAS Complex

Retrophin®

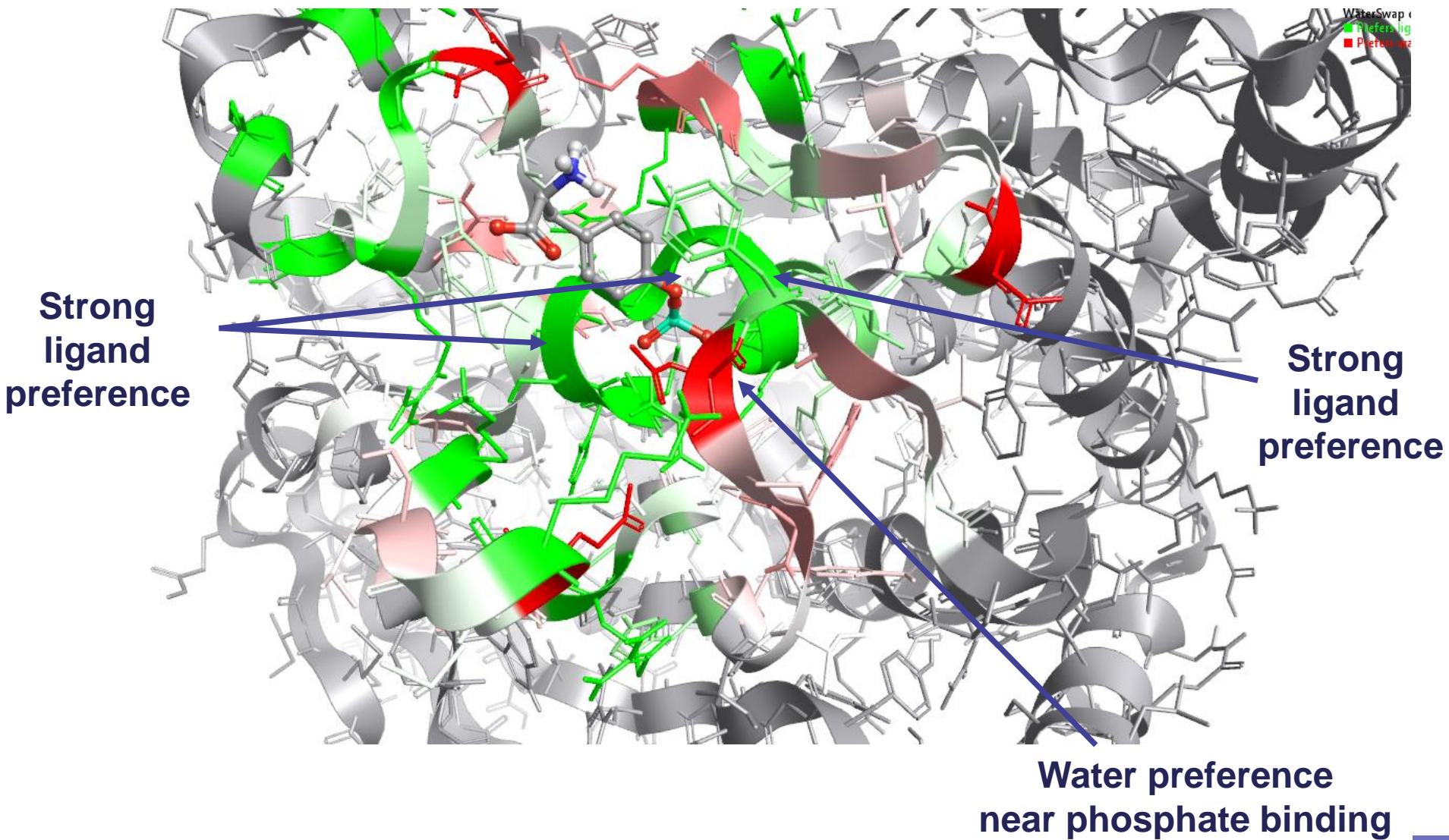
WaterSwap c
■ Prefers liga
■ Prefers wa

Very weak preferences



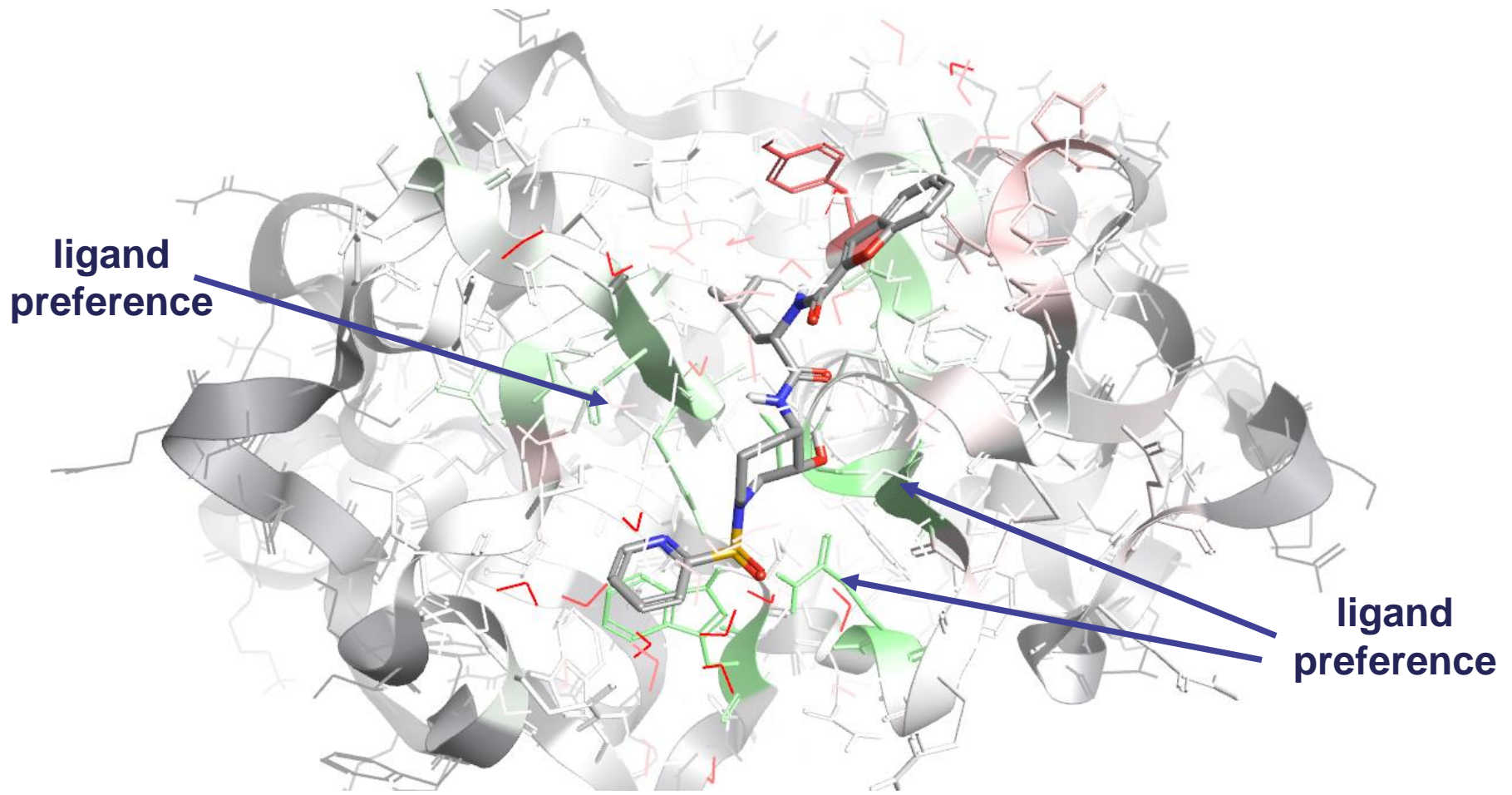
- Tyrosine-protein phosphatase non-receptor type 1 also known as protein-tyrosine phosphatase 1B (PTP1B) is an enzyme that is the founding member of the protein tyrosine phosphatase (PTP) family
- PTP1B is a negative regulator of the insulin signaling pathway and is considered a promising potential therapeutic target, in particular for treatment of type 2 diabetes. It has also been implicated in the development of breast cancer and has been explored as a potential therapeutic target in that avenue

- **PTP1B has clinical implications in the treatment of type 2 diabetes as well as cancer. Gene knockout studies conducted in murine models has provided substantial evidence for the role PTP1B plays in the regulation of insulin signaling and the development of obesity.**
- **PTPN1 knockout mice kept on high fat diets showed a resistance to obesity and an increased degree of insulin sensitivity as compared to their wild-type counterparts.**
- **As such, the design and development of PTP1B inhibitors is a growing field of research for the treatment of type 2 diabetes and obesity**

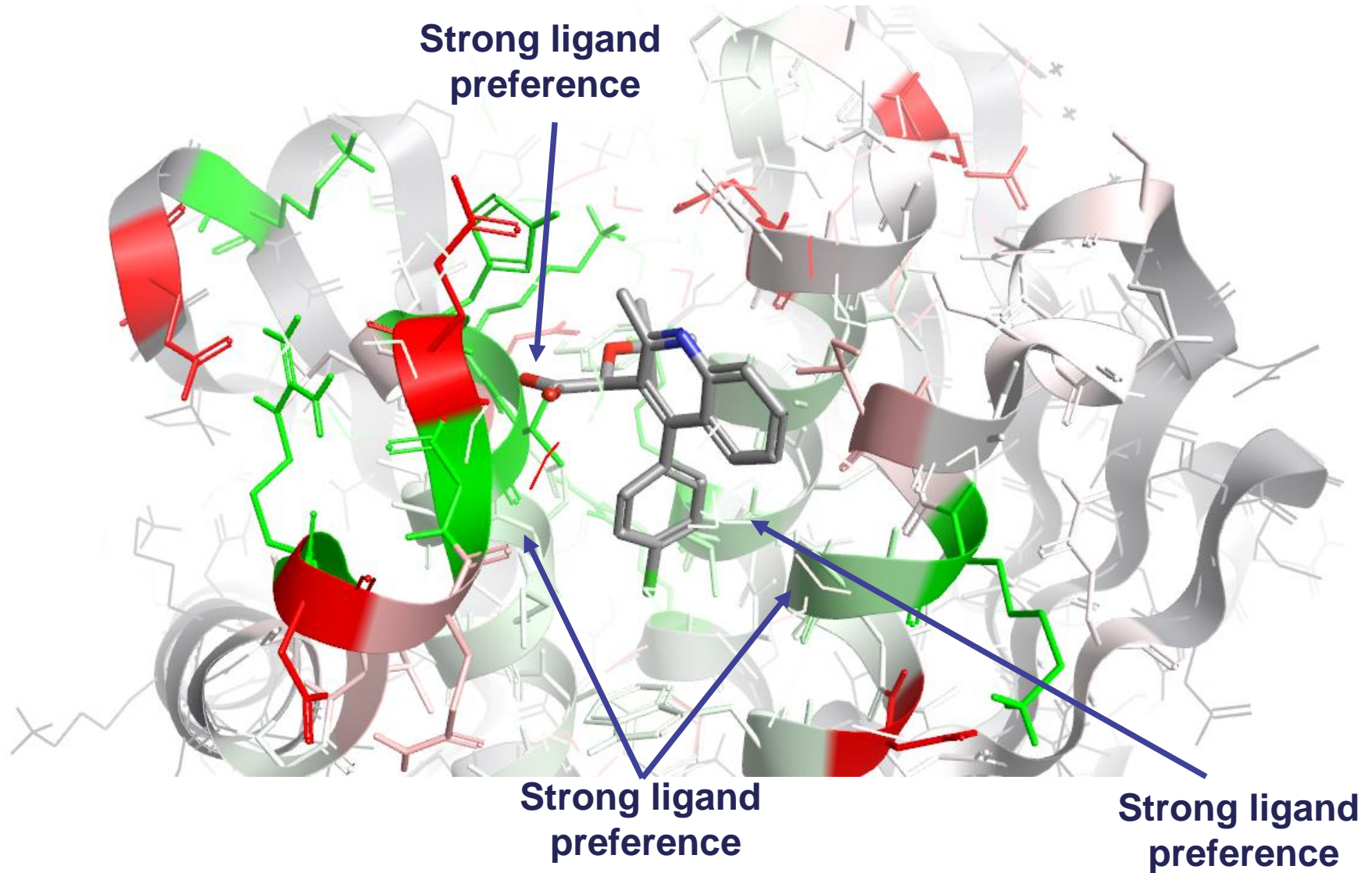


- **The protein encoded by this gene is a lysosomal cysteine protease involved in bone remodeling and resorption. This protein, which is a member of the peptidase C1 protein family, is expressed predominantly in osteoclasts.**
- **Cathepsin K is a protease, which is defined by its high specificity for kinins, that is involved in bone resorption. The enzyme's ability to catabolize elastin, collagen, and gelatin allows it to break down bone and cartilage. This catabolic activity is also partially responsible for the loss of lung elasticity and recoil in emphysema. Cathepsin K inhibitors show great potential in the treatment of osteoporosis. Cathepsin K is degraded by Cathepsin S, called Controlled Cathepsin Cannibalism.**

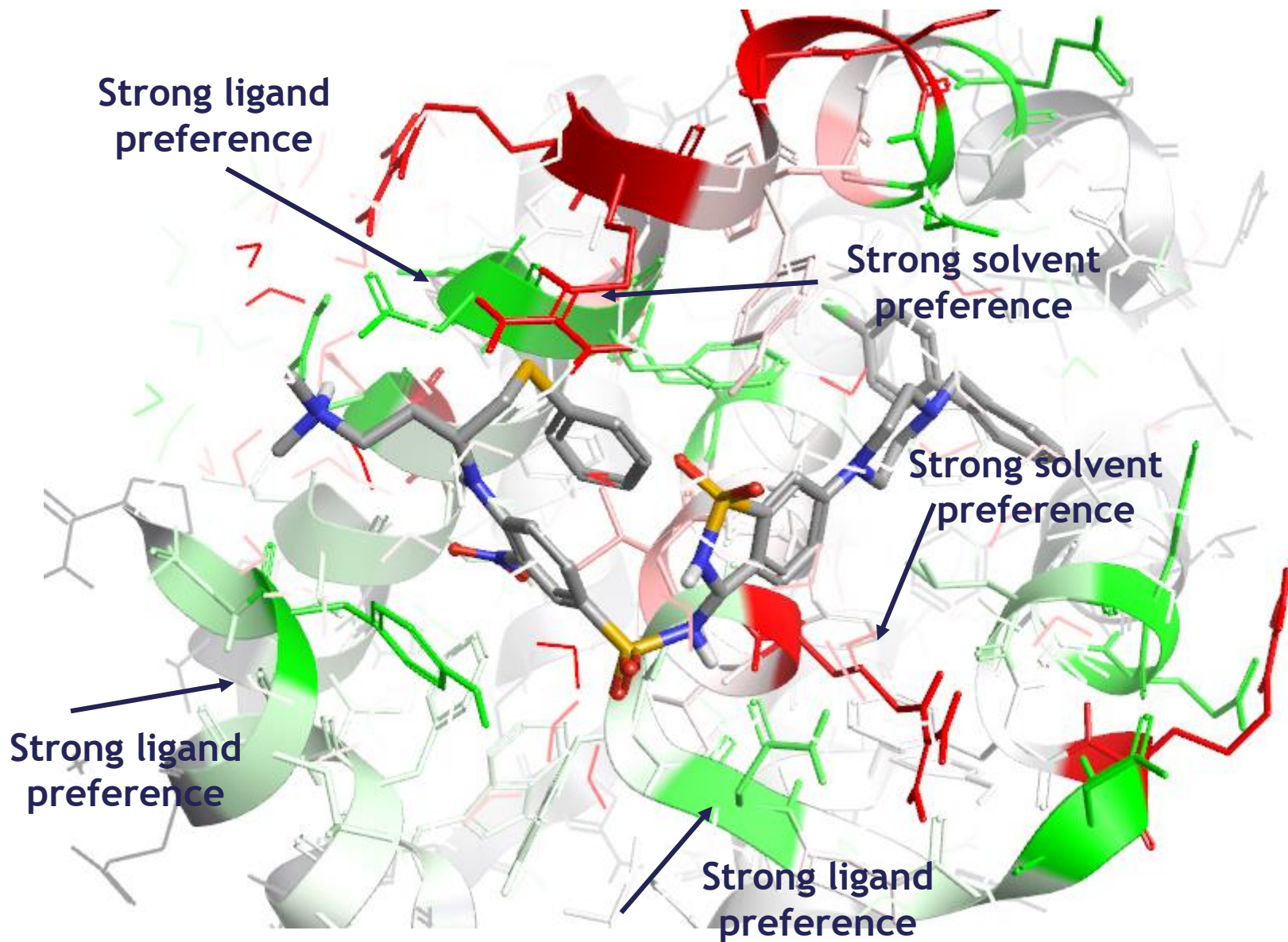
- **Cathepsin K is expressed in a significant fraction of human breast cancers, where it could contribute to tumor invasiveness. Mutations in this gene are the cause of pycnodysostosis, an autosomal recessive disease characterized by osteosclerosis and short stature. Cathepsin K has also been found to be over-expressed in glioblastoma**
- **Merck had a cathepsin K inhibitor, odanacatib, in Phase III clinical trials for osteoporosis. In September, 2016, Merck announced they were discontinuing development of odanacatib after their own assessment of adverse events and an independent assessment showed increased risk of stroke. Other cathepsin K inhibitors are in various stages of development. Medivir has a cathepsin K inhibitor, MIV-711 (L-006235), in Phase IIa clinical trial, as a disease modifying osteoarthritis drug, as of October 2017**



- **Retroviral integrase (IN) is an enzyme produced by a retrovirus (such as HIV) that enables its genetic material to be integrated into the DNA of the infected cell. Retroviral INs are not to be confused with phage integrases, such as λ phage integrase (Int) (see site-specific recombination)**
- **IN is a key component in the retroviral pre-integration complex (PIC). The complex of integrase bound to cognate viral DNA (vDNA) ends has been referred to as the intasome**
- **HIV integrase is a 32 kDa protein produced from the C-terminal portion of the Pol gene product, and is an attractive target for new anti-HIV drugs**
- **In November 2005, data from a phase 2 study of an investigational HIV integrase inhibitor, MK-0518, demonstrated that the compound has potent antiviral activity. On October 12, 2007, the Food and Drug Administration (U.S.) approved the integrase inhibitor Raltegravir (MK-0518, brand name Isentress). The second integrase inhibitor, elvitegravir, was approved in the U.S. in August 2012**



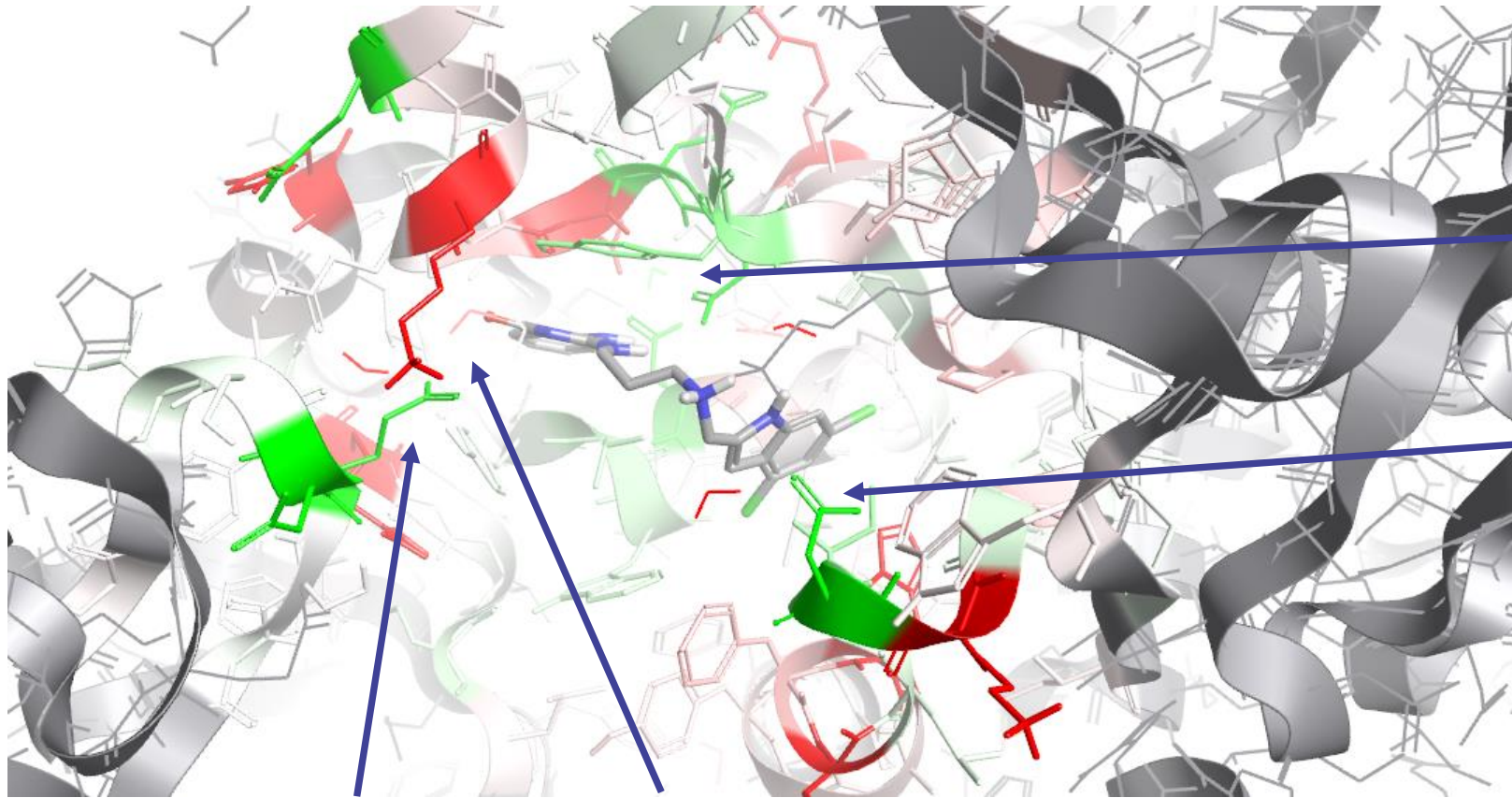
- **BCL-2 is localized to the outer membrane of mitochondria, where it plays an important role in promoting cellular survival and inhibiting the actions of pro-apoptotic proteins. The pro-apoptotic proteins in the BCL-2 family, including Bax and Bak, normally act on the mitochondrial membrane to promote permeabilization and release of cytochrome C and ROS, that are important signals in the apoptosis cascade. These pro-apoptotic proteins are in turn activated by BH3-only proteins, and are inhibited by the function of BCL-2 and its relative BCL-XI.**
- **There are additional non-canonical roles of BCL-2 that are being explored. BCL-2 is known to regulate mitochondrial dynamics, and is involved in the regulation of mitochondrial fusion and fission. Additionally, in pancreatic beta-cells, BCL-2 and BCL-XI are known to be involved in controlling metabolic activity and insulin secretion, with inhibition of BCL-2/XI showing increasing metabolic activity, but also additional ROS production; this suggests it has a protective metabolic effect in conditions of high demand.**



- Methionyl-tRNA synthetase of *Trypanosoma brucei* (TbMetRS) is an important target in the development of new antitrypanosomal drugs
- Generally, aaRS recognize a specific amino acid and charge it to its cognate tRNA through a two-step reaction: (1) recognition of the amino acid and ATP to form an aminoacyl-adenylate intermediate, and (2) recognition of the cognate tRNA to transfer the aminoacyl group to the 3'-terminal adenosine of the tRNA
- TbMetRS has been validated as a possible drug target through RNAi experiments [12]. In addition, a series of aminoquinolone-based inhibitors (ABIs) was shown to have potent antitrypanosomal activity in vitro
- Human African trypanosomiasis (HAT), also called sleeping sickness, is a disease caused by the protozoan parasite *Trypanosoma brucei*. Up to 60 million people in sub-Saharan Africa are estimated to be at risk for the infection [1]. The disease usually occurs in two stages. In the first, haemolymphatic, stage, the parasites multiply in blood and lymph. In the second, meningoencephalitic, stage, the parasites cross the blood-brain barrier (BBB) to invade the central nervous system. Most of the reported cases of HAT are caused by *T. brucei gambiense* which progresses slowly in months to years. In contrast, HAT caused by *T. brucei rhodesiense* progresses very rapidly in weeks. HAT is uniformly fatal if left untreated

Methionyl TRNA Synthase (TRYPANOSOMA BRUCEI)

Retrophin®



Ligand preference

Ligand preference

Ligand preference Water preference

- **Drugged**
 - Correctly identified
 - RXR, CCR5, HIV Integrase, BTK, BCL-2
- **Undrugged**
 - Predicted to be undruggable
 - HNF4, DAPK, ZLK
 - Predicted to be ambiguous
 - MTRNAS , Cathepsin K
 - Predicted to be druggable
 - Protein Tyrosine Phosphatase 1B

- **Unlike pocket finding methodologies, or other automated methods WaterSwap requires more interpretation**
- **It is useful to asses target druggability and provided important information about ligand binding and affinities to binding pockets**