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# EXPLORING THE ROLE OF BIOLOGICAL PROBES IN DNA REPAIR ENZYME MUTYH

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## Abstract

In this project pharmaceutical drugs were screened to find those that interacted with the DNA repair enzyme known as MUTYH. MUTYH prevents mutations and is thereby linked to preventing cancer. The ability of FDA-approved drugs to dock with the MUTYH enzyme was tested with a virtual screen. The findings suggest that these drugs are able to interact with MUTYH. However, a biochemical assay is needed to further study how the drugs are affecting enzyme activity. Studying the molecular interactions of the screened drugs may reveal new treatments for common diseases like cancer. Moreover, understanding the effect of these interactions on other pathways will prevent unintended consequences, such as increased mutation rate and inflammation.

## Introduction

Medicines come from the natural environment and through chemical design. These molecules have an intended purpose provided by evolution but opportunities for these molecules to interact with other targets still arise, a situation we will refer to as opportunistic molecular interactions. The interconnectivity of physiological systems implies that molecules interact with and impact more than one pathway. The willow tree illustrates this concept of an opportunistic molecular interaction. This tree produces salicylic acid, a plant hormone that serves as a defense mechanism to deter insects (Dempsey and Klessig, 2017). However, the willow tree also provides health benefits for humans. The earliest evidence of medicinal uses of the willow tree dates to 1500 BC, although use of this tree is suspected to be many years before that. In the 1920s, researchers extracted salicin and found that irritant properties were reduced by adding an acetyl group to the compound, now called acetylsalicylic acid, or Aspirin for short (Connelly, 2014). Over the past 100 years Aspirin has become a common name in treating aches and fevers. Aspirin operates by inhibiting the COX enzyme, an enzyme that is responsible for inflammation and pain (Vrane, 2003). Recent findings suggest that taking Aspirin daily also reduces the risk of colorectal cancer (Rothwell et al. 2010, Garcia-Albeniz et al. 2011). This multifaceted use of Aspirin demonstrates how a molecule designed by evolution for hormone signaling in plants can be repurposed to target other enzymes in insects as a deterrent and in humans as a medicine (Figure 1).

Many medications, like Aspirin, intend to target a specific enzyme but still have opportunities to target other pathways as well. “Off-label use” medications are medications not approved for the disease being treated but approved for other diseases. Off-label uses of drugs are widespread in cancer treatments especially since drug labelling is specific in the type of tumor the drug is intended to treat (Leveque, 2008). Despite the prevalence of off-label use, uncertainties surrounding the benefits versus the toxicities remain high (Saiyed et al, 2017). Given the potential for off-target effects, we questioned if some drugs are interacting with other physiological processes in the body, such as DNA repair pathways. DNA repair, and specifically the GO DNA repair pathway, have been validated as a target for cancer treatment (Curtin 2012, Hosoya and Miyagawa 2014) (Figure 2). We sought to discover opportunistic molecular interactions involving drugs and enzymes in the GO DNA repair pathway.

Researchers have been studying inhibitors that could block the activity of several enzymes in the GO DNA repair pathway. The inhibitors appeared to be part of the hydrazine and hydrazone classes and target the OGG1 enzyme, a DNA glycosylase (Donley et al. 2015, Edwards et al. 2015, Qin et al. 2020). While previous screens focused on the human OGG1 enzyme, much of the GO DNA repair pathway remains to be a mystery. Another key enzyme that functions in this pathway is MutY, as known in bacteria, or MUTYH, the homolog found in mammals. MUTYH is responsible for identifying and cutting out areas of specific DNA damage. By removing an adenine base that is incorrectly paired to an 8-oxo-guanine base, MUTYH prevents future mutations (Russelburg et. al., 2020) (Figure 3). Given the important role MUTYH plays in DNA repair, the search for cancer treatments warrants studying the effects drugs have on this enzyme. Probes that inhibit or activate MUTYH need to be identified to study the lesser known roles of the enzyme. Discovering opportunistic molecular interactions involving MUTYH may revolutionize cancer treatments and illuminate the impact on mutational burden that could cause inflammation.

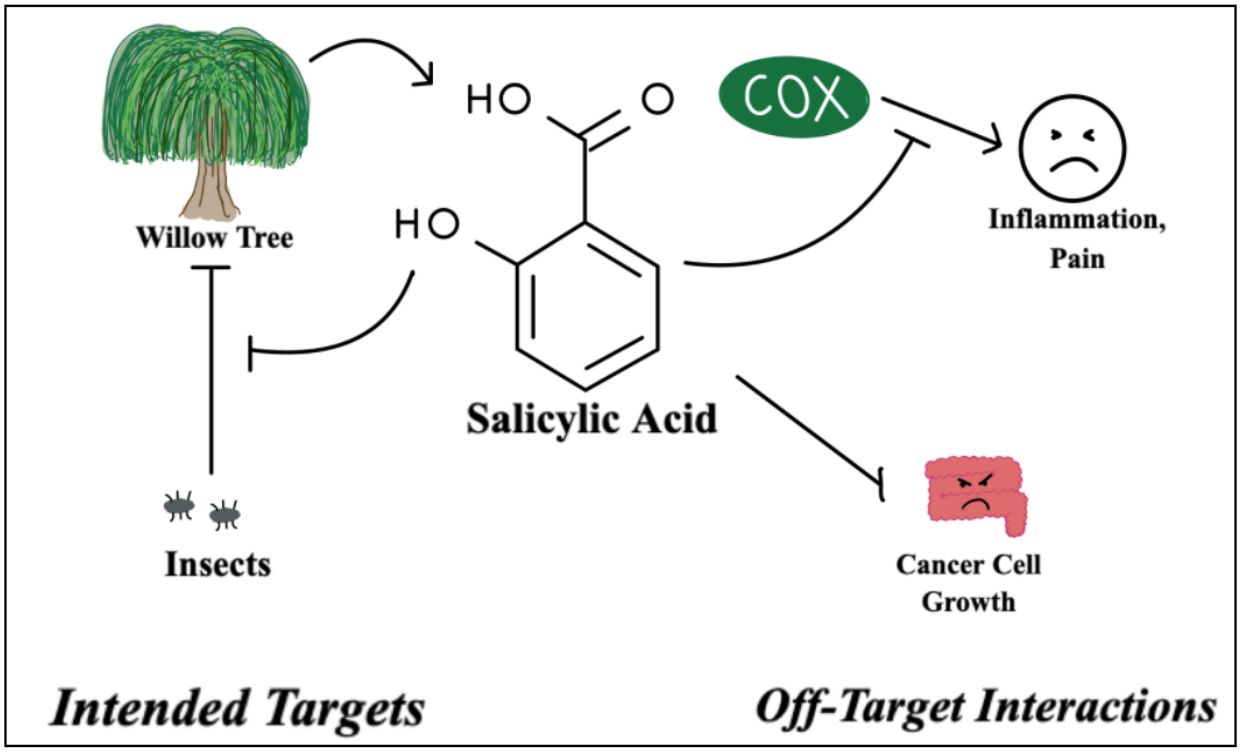



**Figure 1. Opportunistic molecular interactions of salicylic acid.** The willow tree produces salicylic acid to deter insects. However, salicylic acid also has off-target interactions. It inhibits the COX enzyme, thereby preventing inflammation and pain. It also prevents growth of cancer cells.

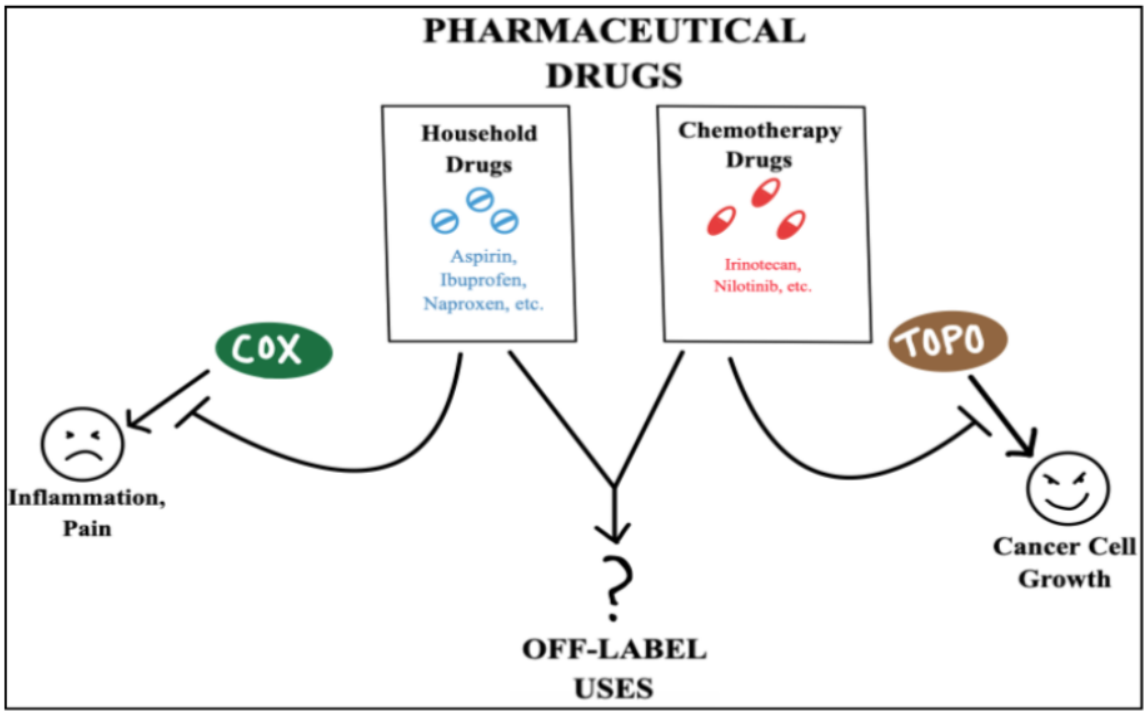



**Figure 2. Off-label use concept map.** Common household drugs, like Aspirin, Ibuprofen, and Naproxen are known to treat pain and inflammation. Aspirin achieves this by inhibiting the COX enzyme. Chemotherapy drugs like Irinotecan inhibit the Topoisomerase-1 enzyme to induce apoptosis in cancer cells. This map suggests there may be other off-label uses of these drugs. Looking for such molecular interactions was the subject of this study.

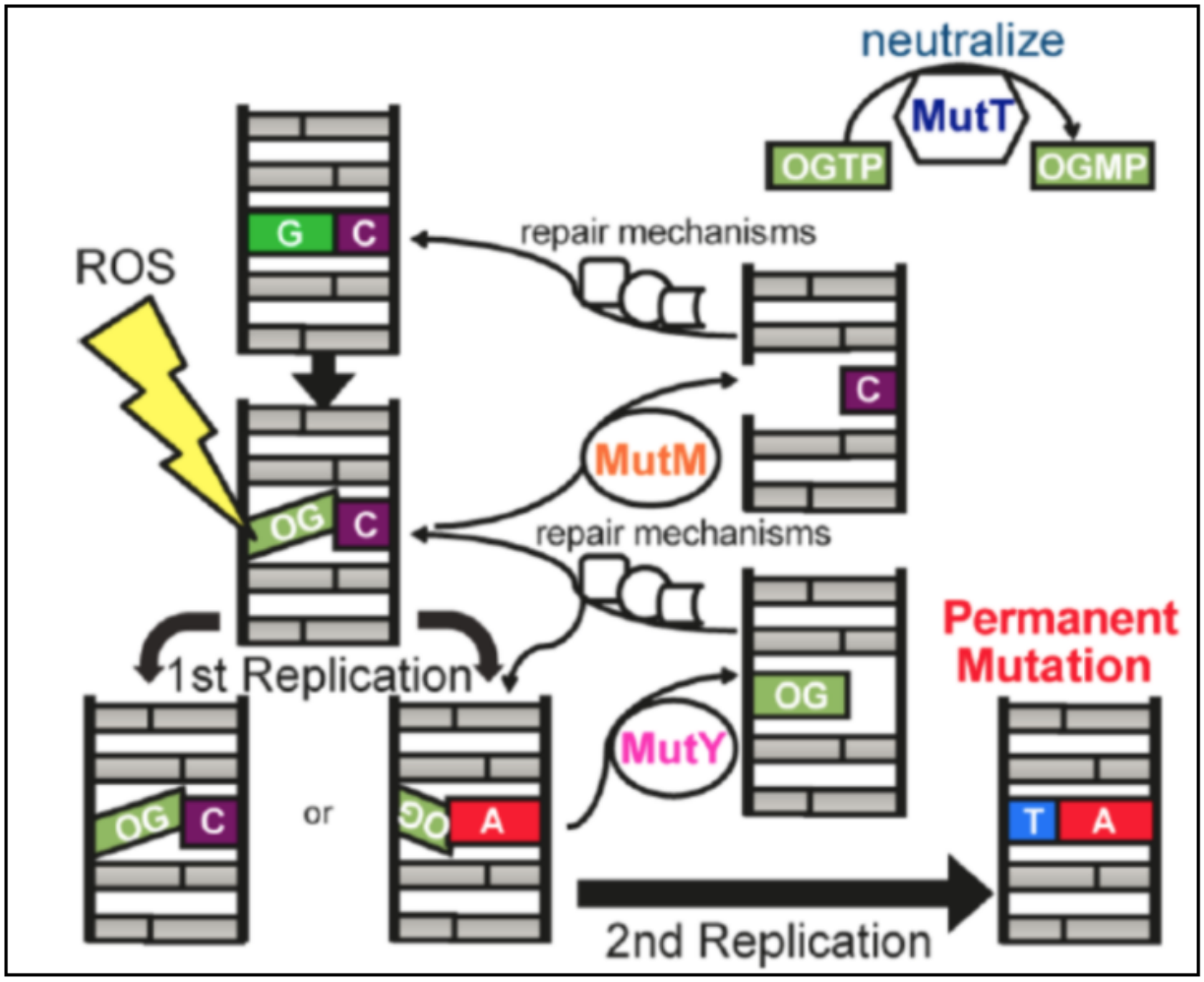



**Figure 3. GO DNA Repair Pathway (Base Excision Repair).** Several enzymes ensure that oxidization of G to form 8-OG (aka GO) does not result in a permanent mutation. One key enzyme is MutY. MutY removes an incorrectly paired adenine base from an OG:A pairing, thereby preventing permanent mutations after future rounds of replication. *Figure from Russelburg et. al., 2020.*

This project explored the potential for opportunistic molecular interactions by testing if drugs interact with MUTYH. Studying the opportunistic molecular interactions of the drugs is significant because it may present new approaches to cancer treatment. Additionally, opportunistic molecular interactions may also increase mutation rates in the microbes that live in our body. To test if common medicines like Aspirin and other FDA-approved drugs could interact with MUTYH, a docking simulation identified probes that could dock to the enzyme with favorable binding energies. While previous studies used a high throughput screen of thousands of molecules in the chemical catalog, this project narrowed the screen down to drugs that were already FDA approved and available for purchase. The findings suggest that both common household drugs and FDA-approved drugs are able to interact with MUTYH, indicating a potential for opportunistic molecular interactions. These verified drugs may expose cheaper alternatives to expensive treatments. Moreover, these drugs can serve as probes to modulate MUTYH activity to reveal its unknown role in other pathways. For example, inhibited MUTYH may increase the mutational burden in cells and lead to inflammation. The results call for an *in vitro* activity assay to determine how the drugs are impacting MUTYH.

## Results

To investigate whether molecules have the potential to interact with MUTYH, we applied molecular modeling with Autodock VINA, which is a computer program that tests possible combinations of molecule interactions between ligands resembling small druglike molecules and receptors of large proteins. The ligands were assigned charges and atom types based on a physiological pH. The structure of the receptors, bacterial MutY protein and human MUTYH protein, were retrieved from the Protein Data Bank.

*Household Drugs*

We initially started molecular modeling with common household drugs and the simpler MutY from a thermotolerant bacteria called *Geobacillus stearothermophilus*. The green box shows the search area explored by Autodock Vina. The search area focused on the entire MutY protein (Figure 4). Autodock VINA docked Naproxen (Figure 5a) and Ibuprofen (Figure 5b) with MutY. The docking simulation also tested 8-oxo-guanine (8-OG) to provide a basis of comparison (Figure 5c). 8-OG is the normal target MutY must find in order to carry out its biological function of preventing mutations.

Autodock VINA arrives at docking outcomes by optimizing the binding energy of the ligand-receptor interaction. When 8-OG docked to the receptor MutY, Autodock VINA calculated a large negative free energy of -7.8 kcal/mol, indicating favorable molecular interactions as expected (Table 1). Naproxen and Ibuprofen had binding energies of -7.1 kcal/mol and -6.6 kcal/mol respectively when docked with MutY. These household drugs have binding energies similar to the binding energy observed for 8-OG, the normal target of MutY. The relative binding affinity, compared to 8-OG, was calculated for each ligand. If the relative binding affinity value is greater than 1, then the ligand is expected to bind more tightly to the receptor than 8-OG. These docking experiments indicate Ibuprofen and Naproxen make favorable interactions and achieve relative binding affinities close to 1, but 8-OG is still predicted to bind more tightly.

*FDA-Approved Drug Screening*

Since molecular modeling of the household drugs was encouraging, we broadened the search to test if other medicines may also be interacting with MUTYH. Autodock VINA screened 1424 ligands which were obtained from a list of FDA-approved drugs. The receptor was a MUTYH chimera construct consisting of the N-terminal domain of MUTYH and the C-terminal domain of bacterial MutY (Figure 6). This strategy to dock molecules to a chimera protein rather than the entire MUTYH receptor was necessary because there is currently no complete structure that includes the C-terminal domain of MUTYH. The green box indicates the search area, which focused on the active site of MUTYH. We also docked adenine to MUTYH to obtain a basis of comparison. Adenine, like 8-OG, is a normal target for MutY and MUTYH.

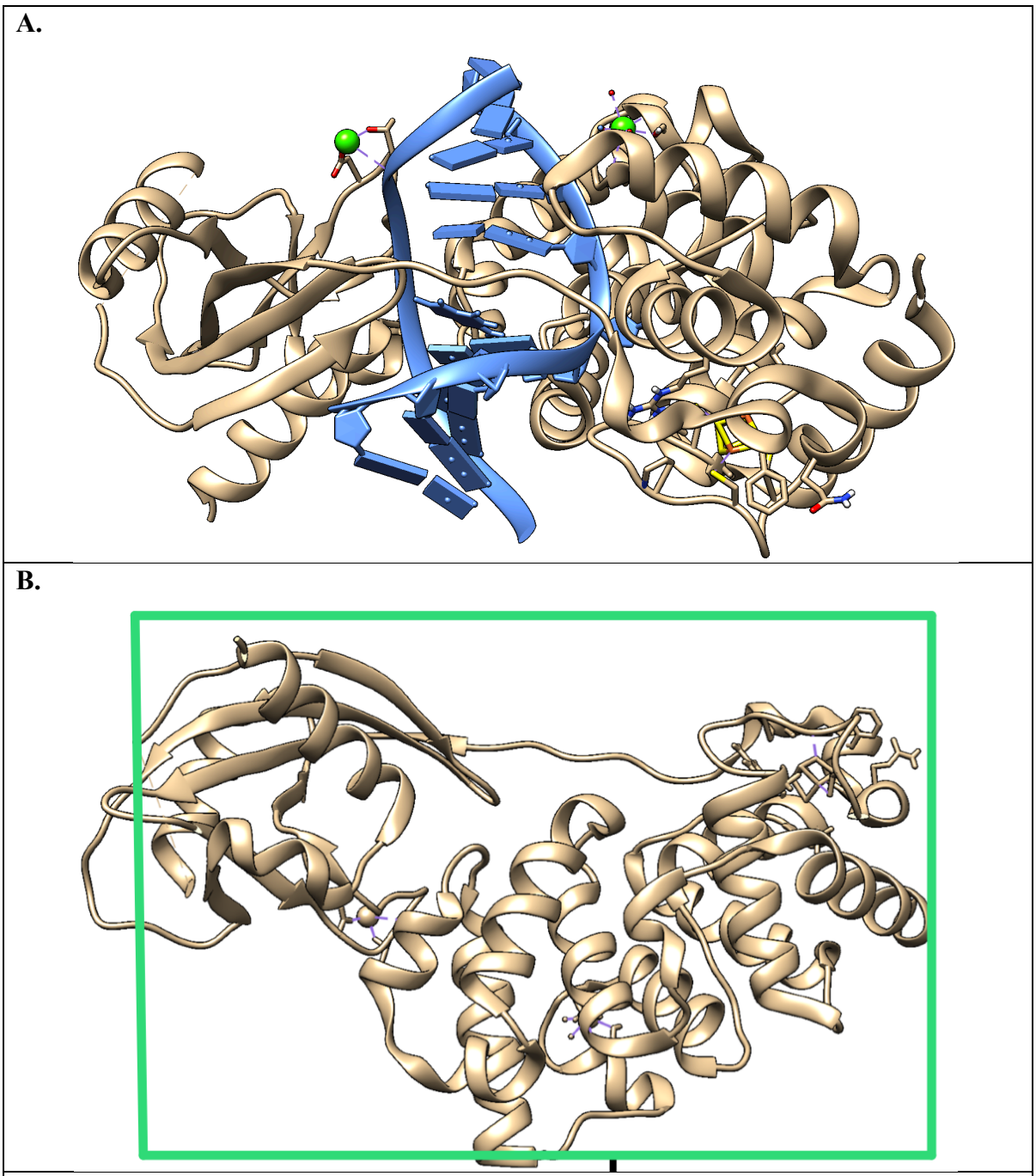



**Figure 4. Receptor MutY for docking of household drugs.** The MutY protein was obtained from *Geobacillus stearothermophilus*. Panel A shows MutY (tan) in complex with DNA (blue). Panel B shows MutY as prepared for docking trials with the DNA removed. The green box outlines the search area in Autodock Vina, which focused on the entire protein. Removing the DNA allowed access to the active site for ligand binding.

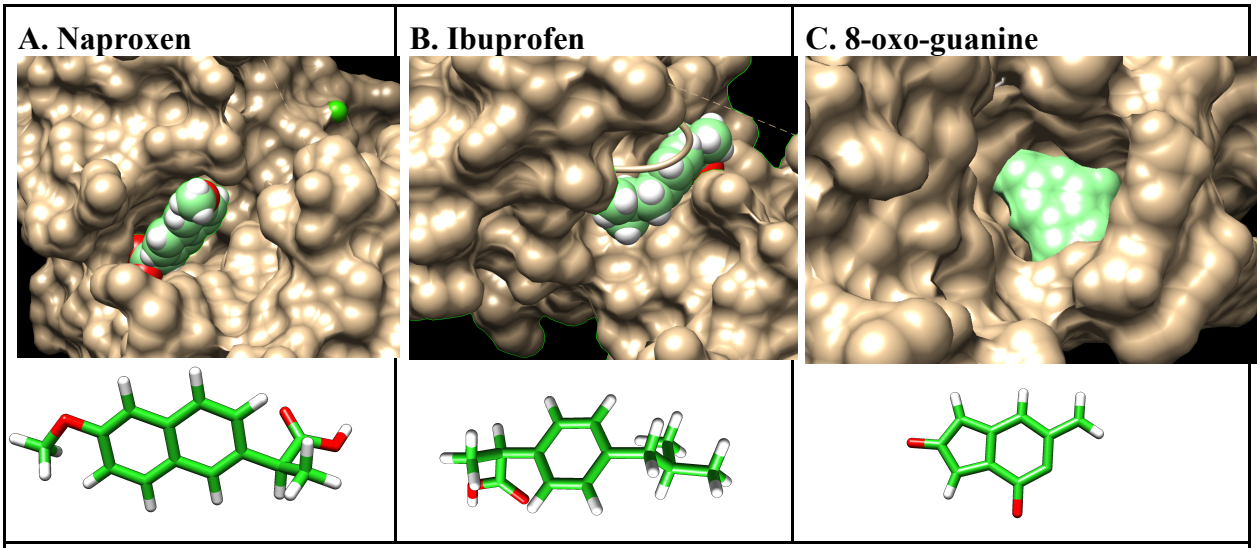



**Figure 5. Ligands docked with bacterial MutY.** The docking outcomes and the chemical structures of common household drugs (green) are shown in complex with MutY (tan). Each ligand docked with favorable binding energies and fit comfortably in the active site of MutY.

**Table 1. Binding energies for household drugs docked to MutY.**

| Ligand | Binding Energy (kcal/mol) | Relative Binding Affinity£ |
|---|---|---|
| Naproxen | -7.1 | 0.3 |
| Ibuprofen | -6.6 | 0.1 |
| 8-OG | -7.8 | 1 |

£ Relative binding affinity is the ratio of expected dissociation constants and was calculated as exp(ΔBE/RT), where ΔBE is the difference in binding energy comparing ligand to 8-OG, R = 0.00198 kcal mol-1 K-1 and T = 310 K. A value greater than 1 indicates greater affinity; a value less than 1 indicates lower affinity.

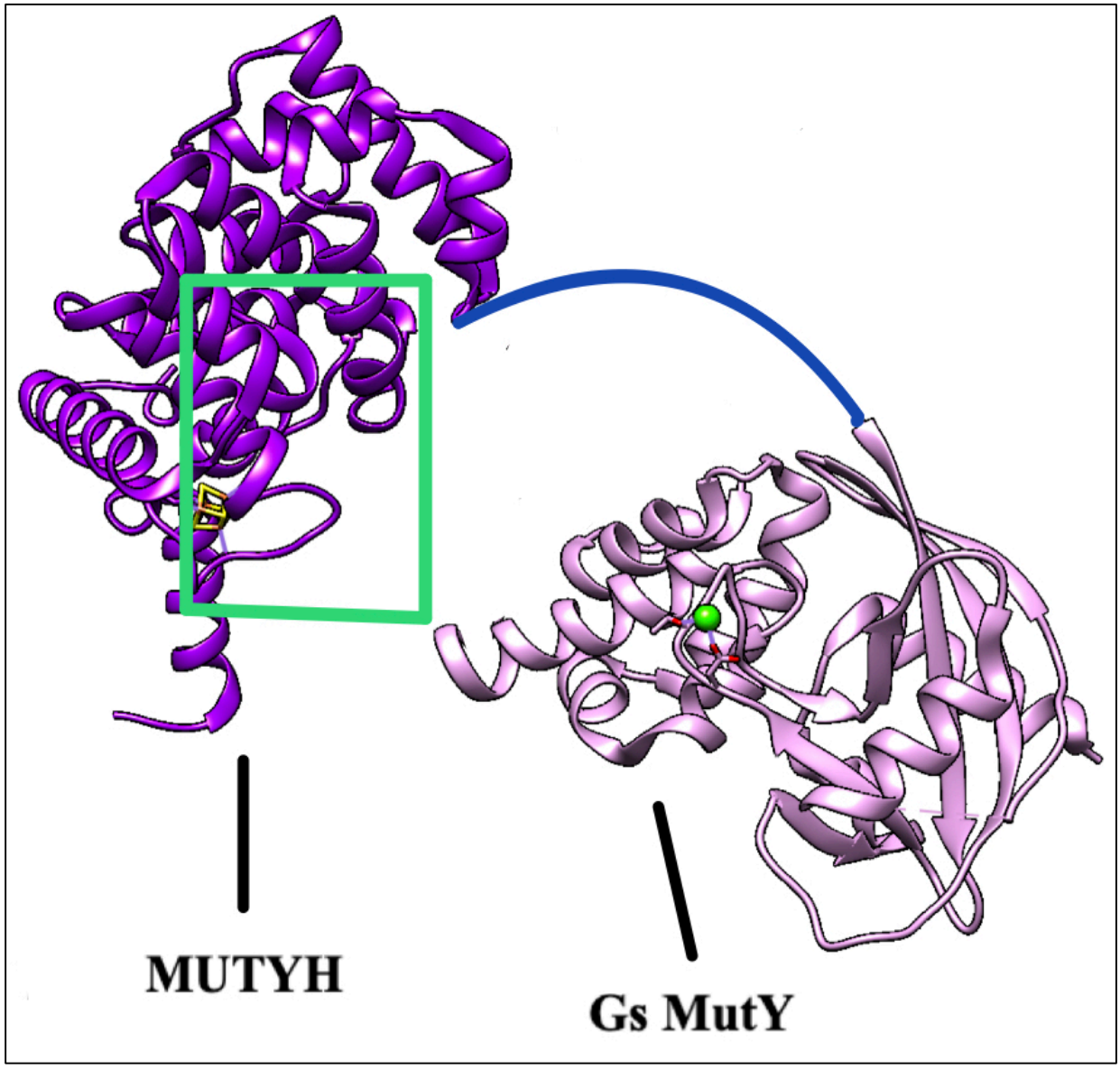



**Figure 6. MUTYH chimera protein.** The MUTYH chimera protein was constructed by joining the N-terminal domain of MUTYH from humans (purple) and the C-terminal domain of MutY from *Geobacillus stearothermophilus* (pink). The green box outlines the search area in Autodock Vina, which is focused on the active site in MUTYH.

The binding energies of the top ten "winners" ranged from -10.1 kcal/mol to -11.3 kcal/mol (Table 2). Interestingly, a couple of the winners (those with asterisks) are drugs already applied in chemotherapy for treatment of cancer. Figure 7 shows Irinotecan and Nilotinib in complex with the MUTYH chimera receptor. Docking of adenine to MUTYH resulted in a binding energy of -7.4 kcal/mol. The relative binding affinity was calculated for each ligand by comparing its binding energy to that obtained in docking adenine. All of the winners had a relative binding affinity greater than 1, indicating that the ligands are predicted to bind more tightly to MUTYH compared to adenine, MUTYH's natural target.

The high affinities measured for docking outcomes, while highly encouraging, require verification through a biochemical activity assay. The molecules may not interact as strongly as suggested by the virtual docking experiments described here or binding may not interfere with normal MutY function. We can rule out these possibilities by directly testing for drug-MUTYH interactions. The following section describes the first step towards developing this biochemical activity assay by engineering a MUTYH expression system.

*Engineered MUTYH Chimera*

As a first step in development of the activity assay, we applied protein engineering to create an easy to express and stable MUTYH protein that can be tested with potential inhibitors and activators. This engineered MUTYH chimera protein consists of a maltose-binding protein tag that helps with purification, the N-terminal sequence of MUTYH, and the C-terminal sequence of *Geobacillus stearothermophilus* MutY which is predicted to be more stable. We constructed a plasmid DNA encoding the MUTYH chimera through ligation-independent cloning. The MUTYH chimera was confirmed through Sanger sequencing. The NCBI Blast tool searched for other sequences in known databases that match the protein encoded by the MUTYH chimera plasmid DNA (Figure 8). NCBI Blast matched the first third of the MUTYH chimera with maltose-binding protein from *E. coli* (not shown). The second third of the MUTYH chimera matched with the MUTYH isoform in chimpanzees (*Pan troglodytes*) which is identical to the human sequence. The last third matched with MutY from *Geobacillus stearothermophilus*. Additional sequencing is needed to establish complete coverage of the coding DNA. The chimera protein will be further confirmed through an SDS PAGE gel analysis to compare the experimental band sizes with the expected size for the MBP tagged MUTYH chimera (83 kDa).

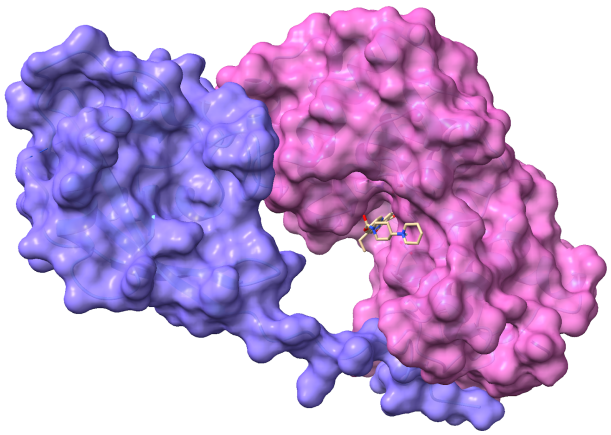
**Table 2. Top ten FDA-approved drugs docked to MUTYH chimera.**

| Ligand Docked | Binding Energy (kcal/mol) | Relative Binding Affinity£ |
|---|---|---|
| *Irinotecan (ZINC1612996) | -11.3 | 540 |
| Adapalene (ZINC3784182) | -11.1 | 390 |
| Dasabuvir (ZINC95616937) | -10.9 | 280 |
| *Nilotinib (ZINC6716957) | -10.4 | 130 |
| Lumacaftor (ZINC64033452) | -10.2 | 91 |
| Paliperidone (ZINC1481956) | -10.1 | 78 |
| Dutasteride (ZINC3932831) | -10.1 | 78 |
| Tadalafil (ZINC3993855) | -10.1 | 78 |
| Lifitegrast (ZINC84668739) | -10.1 | 78 |
| Grazoprevir (ZINC95551509) | -10.1 | 78 |
| Adenine | -7.4 | 1 |

* These two ligands are drugs currently used in chemotherapy treatments.

£ Relative binding affinity is the ratio of expected dissociation constants and was calculated as exp(ΔBE/RT), where ΔBE is the difference in binding energy comparing ligand to adenine, R = 0.00198 kcal mol-1 K-1 and T = 310 K. A value greater than 1 indicates greater affinity; a value less than 1 indicates lower affinity.

A



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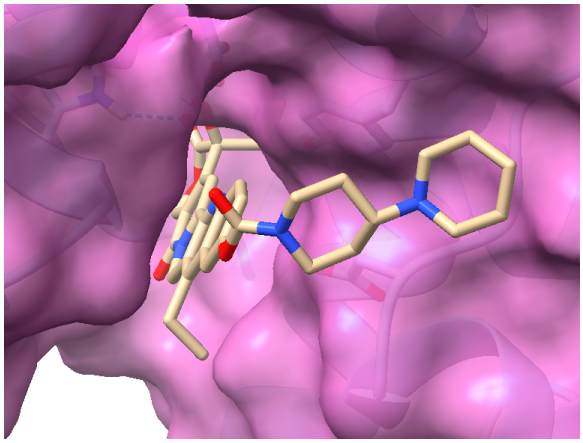

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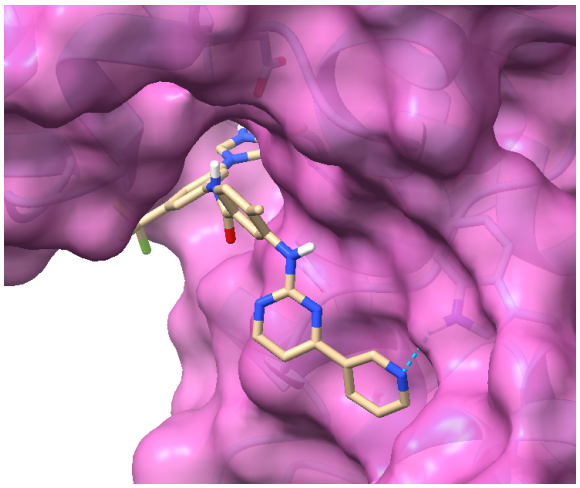

**Figure 7. Docking of Irinotecan and Nilotinib.** Panel A shows the MBP-MUTYH-GsMUTY chimera receptor (blue and pink protein structure) docked with one of the ligand winners, Irinotecan. Panel B shows a zoomed in view of the Irinotecan-MUTYH complex. Panel C shows a zoomed in view with another docking winner, Nilotinib. Despite being fairly large molecules, both of the drug winners fit snugly into the active site.

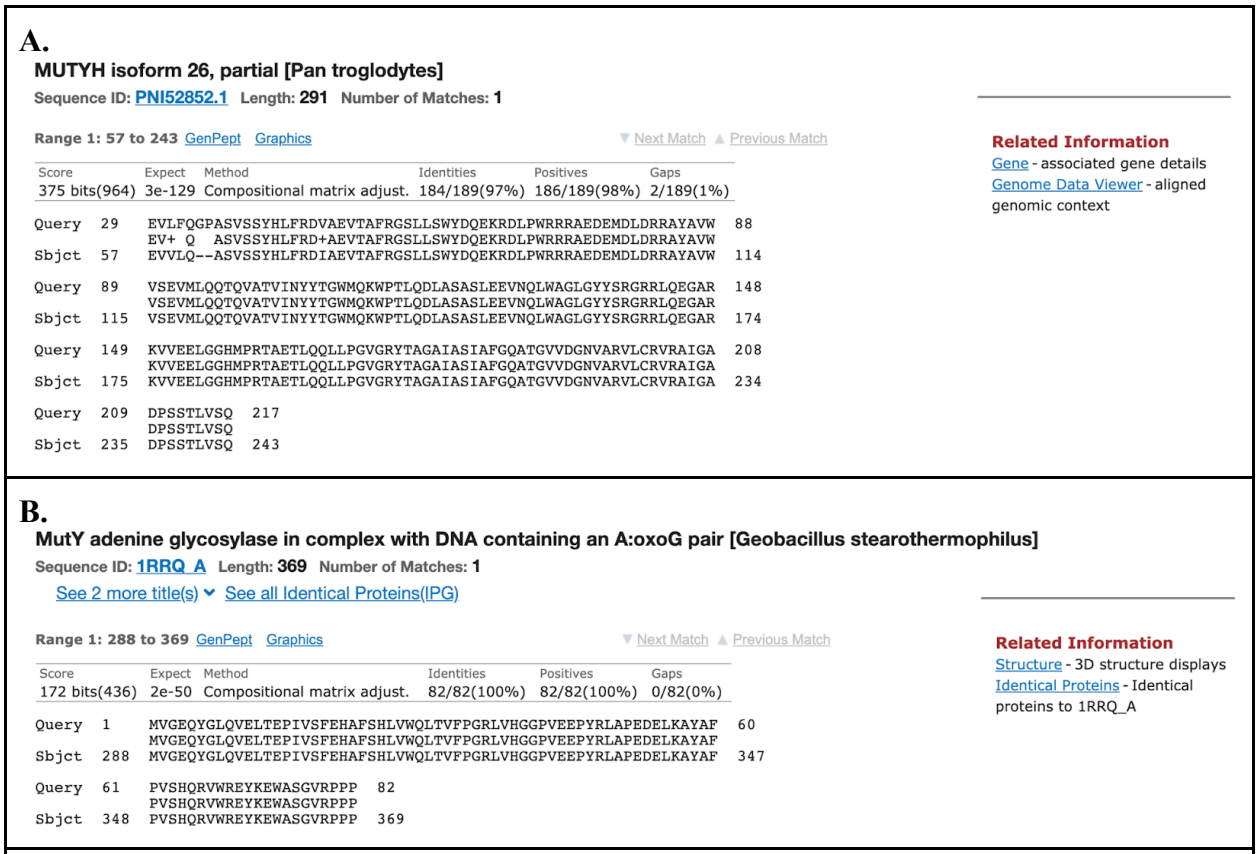


**Figure 8. Confirmation of MUTYH chimera protein.** To confirm the MUTYH chimera construct, we generated a conceptual translation of the DNA sequence obtained by Sanger sequencing and searched all databases for a good match. The second third of the sequence aligns with an isoform in chimpanzee (*Pan troglodytes*), a primate with MUTYH identical to human MUTYH (A). The final third of the sequence aligns with MutY from *Geobacillus stearothermophilus* (B). The MUTYH chimera protein aligned with the intended sequences, suggesting the cloning was successful.

## Discussion

This project sought to determine if pharmaceutical drugs could modulate the activity of the DNA repair enzyme MUTYH. Previous research screened large chemical libraries to identify inhibitors to hOGG1 enzyme in the GO DNA repair pathway (Donley et al. 2015, Edwards et al. 2015, Qin et al. 2020). Studies have demonstrated a correlation between up-regulation of the hOGG1 enzyme and increased allergic immune reactions and suggest that modulation of the enzyme could provide clinical benefits (Bacsi et al., 2013). The hOGG1 structure possesses a domain homologous to MUTYH and both enzymes work cohesively in the GO DNA repair pathway (Arai et al., 1997). Given the collaboration between the enzymes, the full picture of DNA repair is lacking without probes identified for MUTYH. We chose to focus on MUTYH amidst the many studies centered on hOGG1. We narrowed down the screen to pharmaceutical drugs already FDA approved for certain treatments to expedite the drug development process by allowing researchers to skip validation protocols that other novel drugs must endure. We applied the Autodock VINA program to test whether these drugs could interact with the enzyme receptor. The search started with screening over-the-counter drugs like Ibuprofen and Naproxen. While these drugs are known to treat headaches and fevers, we wanted to examine if they interact with other enzymes, specifically the MUTYH enzyme. We found that these household drugs are favorably docking with MUTYH, suggesting that molecular interactions *in vivo* are plausible (Figure 9). Other docking experiments between Ibuprofen, Naproxen, and the COX enzyme indicate that the docking energies with bacterial MutY are comparable to the docking energies observed for the enzyme targets of these drugs (Martin Horvath, personal communication). The success of the household drugs test compelled us to apply a broader screening of medications, so we tested 1424 FDA-approved drugs that were available for purchase to see if the trends were similar. We again found that these drugs were favorably docking with the human homolog MUTYH, suggesting that these drugs are able to outcompete MUTYH's usual target.

The docking was executed with the 8-OG and adenine ligands individually without the context of DNA. As a result, the true enzyme-substrate interaction may be underestimated. If this is the case, *in vitro* binding of the drugs with MUTYH may produce different results. However, these findings were obtained through a virtual screen. Autodock VINA takes into account potential dockings of the proteins without other cellular factors regarded. These results serve as a starting point in persuading us that MUTYH is possibly able to interact with the drugs. It will be interesting to see how these results would compare when the drugs are tested *in vitro*. Additionally, what are these interactions doing to MUTYH? Will these drugs still bind to MUTYH in the presence of DNA? While chemotherapy drugs dock favorably with MUTYH, an assay is warranted to determine how these drugs affect the activity of MUTYH.

*Future Directions*

With the high-affinity binding winners identified, we now have a short list of molecules to test by biochemistry. We will test these drugs in the lab to see how the drugs affect the activity of the enzyme (Figure 10). In the biochemical assay, a fluorescent tag is associated with the DNA containing an OG:A lesion. When MUTYH excises adenine, DNA hydrolysis will occur and lead to strand breakage. The tagged fragment decreases in size indicating successful cleavage by MUTYH. By analyzing products generated by enzyme catalysis through FSEC, the intensity of the different peaks will be used as an indicator of MUTYH activity. A taller peak at an earlier time in the chromatograph will indicate inhibited MUTYH activity, while a taller peak at a later time

will indicate retained MUTYH activity. Modulation in MUTYH activity is significant in understanding how the drug probes are affecting other enzymes besides their intended targets. Being able to quantify this data is crucial in demonstrating the effect of the screened chemotherapy drugs on MUTYH activity. Moreover, future studies may focus on developing treatments that manipulate MUTYH to aid in efficient DNA repair. By using these drugs to turn MUTYH either on or off, we will be able to see the role it plays in DNA repair pathways and the connection to other diseases.

*Conclusion*

With pharmaceutical drugs shown to interact with an unintended target like MUTYH, the opportunistic molecular interactions of the tested drugs are validated and can be further developed. Studying the opportunistic molecular interactions of pharmaceutical drugs can present alternative uses for these medications that provide a more expedient path to clinical trials. The answers to prevalent diseases like respiratory distress and cancer could be amidst these already approved drugs, including the household drugs that are much cheaper than current chemotherapy treatments. As seen with Aspirin, daily consumption of this affordable household drug reduces the risk of colorectal cancer (Garcia-Albeniz et al. 2011). What other approved drugs have the same response? Ignoring the opportunistic molecular interactions of drugs hinders the discovery of valuable applications that may revolutionize cancer treatment and drug therapies for respiratory distress syndromes.

Moreover, understanding of how drugs are interacting with other physiological systems is necessary to prevent unintended consequences. Similar to hOGG1, MUTYH plays a role in various pathways, some of which may not yet be known. Since inhibited MUTYH leads to an increased mutation rate, studying the effects on microbes in the human microbiome is compelling. The gut microbiota in humans has been linked to the overall health of the immune system (Kelly et al., 2007). Inhibited MUTYH and MutY may increase the mutational burden in cells, thereby impacting the microbiome and inducing more diseases. Examining the interaction between these drugs and MUTYH prevents unexpected detrimental effects in humans and other species.

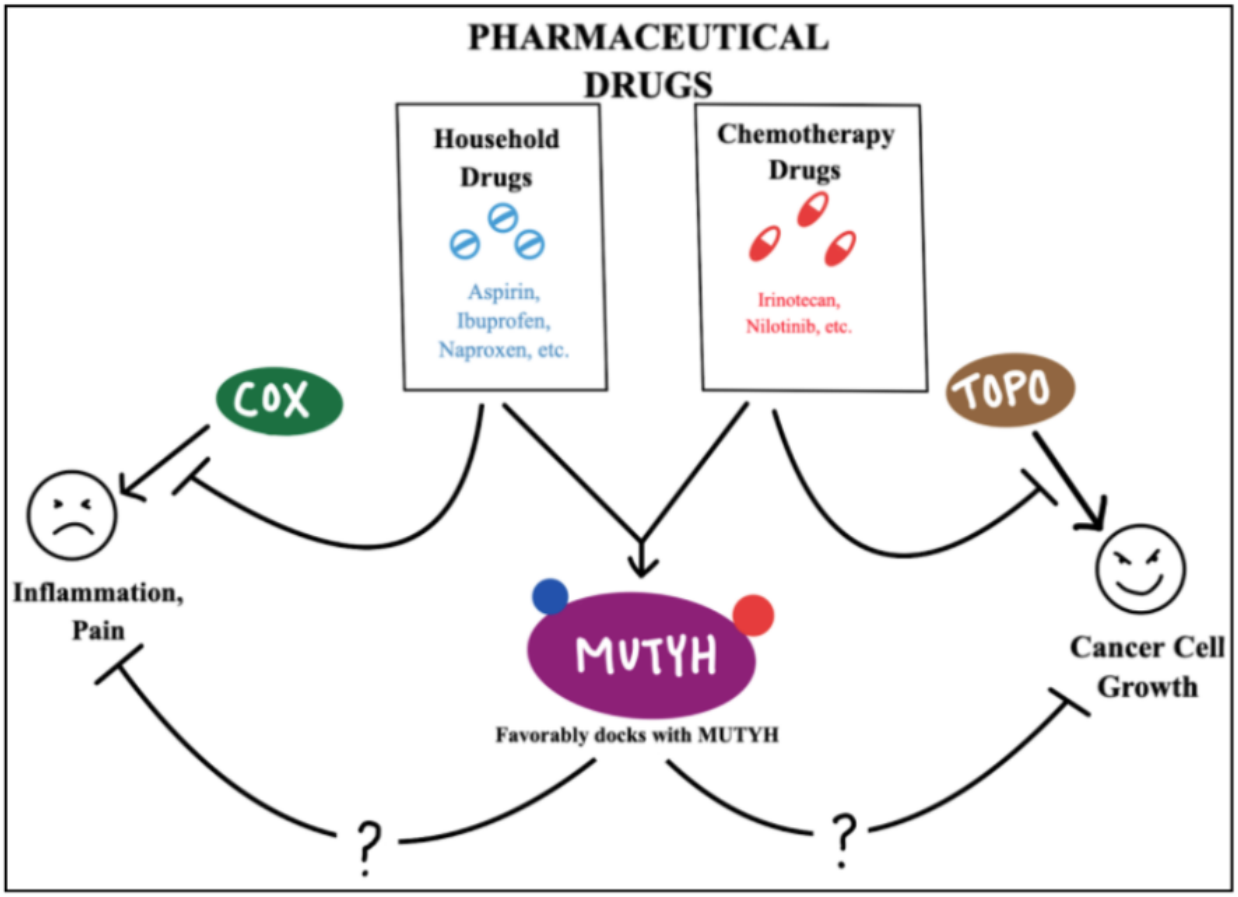


**Figure 9. Off-label use of drugs with MUTYH.** The outcomes from the virtual docking indicate that the pharmaceutical drugs are able to favorably bind to MUTYH. However, more questions arise surrounding how the enzyme's activity is impacted. The impact on cancer growth and inflammation may be better understood with modulation of MUTYH.

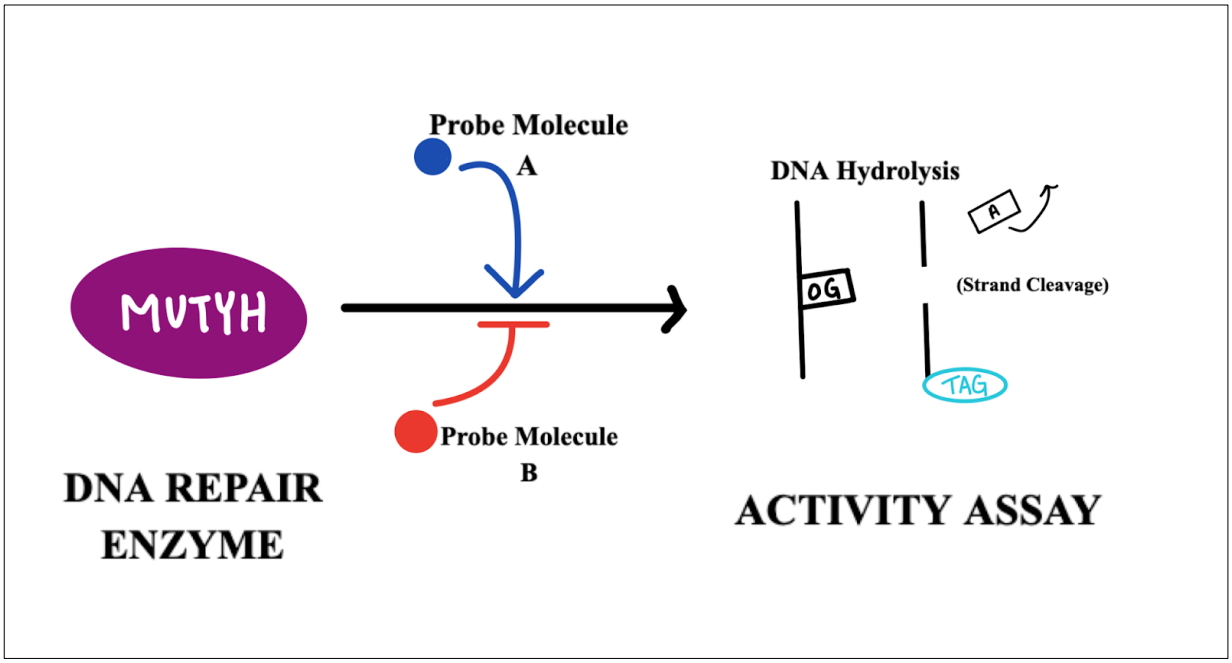


**Figure 10. Biochemical assay.** This assay analyzes how the docked drug winners (Probe Molecule A and Probe Molecule B) will affect the activity of the MUTYH enzyme. Fluorescent Size Exclusion Chromatography will be used to measure the hydrolysis of the DNA strand containing the OG:A lesion. Increased MUTYH activity will result in an increase in the smaller strand product. Modulating MUTYH activity is crucial in order to understand how the enzyme functions in various pathways.

## Methods

*Finding the Docking Winners: Autodock VINA and UCSF Chimera*

A list of potential biological ligands was gathered from the ZINC15 library (Sterling and Irwin, 2015). These ligands were categorized under the 'For Sale' and 'FDA Approved' search filter. The MUTYH-GsMutY chimera receptor was prepared for docking with Autodock Tools. Non-polar hydrogens were merged, leaving only polar hydrogens, and charges were assigned to each atom by the Gasteiger calculation. This receptor structure was saved as a PDBQT file. A python script that automatically docks each of the ligands with the receptor was written by Vincent Mays, a graduate student in the Horvath Lab (SBS, University of Utah). The docking 'winners' were ranked based on the best binding affinity. Results were visualized through the UCSF Chimera program.

Python script for docking:

```
import subprocess
import os
import csv


path_for_ligand = 'mol2s_to_pdbqts1201_1425/'
path_for_docked_pdbqt = '3n5n_6u7t_active_site_docked/'
path_for_logs = '3n5n_6u7t_active_site_logs/'
configuration = 'conf2.txt'
receptor_protein = '3n5n_merged_6u7t_model1+2'

files = os.listdir(path_for_ligand)


docking_count = 0
for file in files:
    x = file.index('.')
    name = file[slice(0, x)]
    subprocess.call(['vina',
        '--ligand', path_for_ligand + file,
        '--config', configuration,
        '--out', path_for_docked_pdbqt + name + '_' +
receptor_protein + '.pdbqt',
        '--log', path_for_logs + name + '_' + receptor_protein
+ '_log' + '.txt'])
    docking_count += 1
    print('Docked ' + str(docking_count) + ' ligands so far.')

print('Finished. Docked ' + str(docking_count) + ' total
ligands!')
```

*Creating the MUTYH Chimera*

Primers were designed to clone the MBP-MUTYH_NTD-GsMutY_CTD chimera. This chimera was clone with PCR, gel purified, and then transformed with DH5α cells on LB-Amp plates. The colonies were grown up, miniprepped, and then sequenced through Sanger sequencing.

**Table 3. Primer Sequences for PCR Reactions.**

| | |
|---|---|
| Gene Block (Optimized MUTYH Sequence) | CTCGAAgttctcttccagggcccagCCTCAGTTTCTAGCTATCATCTTTTTCGCG<br>ACGTGGCTGAGGTGACTGCGTTTAGAGGGAGCTTATTAAGTTGGTACGATCAGGA<br>GAAGCGTGACTTACCCTGGCGCCGCCGCGCTGAGGACGAGATGGACTTAGATCGC<br>CGTGCCTACGCCGTATGGGTATCGGAAGTGATGCTTCAACAAACACAAGTAGCAA<br>CCGTCATCAATTATTATACTGGATGGATGCAGAAGTGGCCGACTCTGCAAGACCT<br>GGCCAGTGCATCATTGGAAGAAGTTAATCAGCTTTGGGCTGGCCTGGGTTACTAC<br>AGCCGCGGACGTCGCCTTCAGGAAGGTGCCCGTAAAGTTGTTGAAGAGTTAGGCG<br>GTCATATGCCACGTACTGCTGAAACGCTTCAGCAGTTGTTGCCGGGTGTTGGACG<br>TTACACCGCTGGTGCAATCGCAAGTATTGCTTTCGGGCAAGCAACAGGGGTTGTT<br>GATGGTAATGTAGCCCGCGTGCTTTGCCGTGTGCGCGCGATCGGAGCCGACCCGA<br>GTTCTACGCTGGTTAGCCAGCAGCTTTGGGGTTTGGCACAACAACTTGTAGATCC<br>AGCACGTCCTGGCGACTTTAATCAGGCCGCAATGGAATTGGGAGCTACCGTCTGT<br>ACGCCCCAACGCCCCTTGTGCTCACAGTGCCCTGTCGAATCCCTATGTCGCGCGC<br>GCCAACGTGAGGGAGTAGCGGAGGAATTGCCAGTGAAGATGAAGaaaacaGCGGT<br>CAAACAAGTGC |
| MBP-MutYHNTD-AB-Crk | CTCGAAgttctcttccagggcccagCCTCAGTTTCTAGC |
| MBP-MutYHNTD-AB-Wat | GCTAGAAACTGAGGctgggccctggaagagaacTTCGAG |
| MutYHNTD-GsCTD-CD-Crk | GTGAAGATGAAGaaaacaGCGGTCAAACAAGTGC |
| MutYHNTD-GsCTD-CD-Wat | GCACTTGTTTGACCGCtgttttCTTCATCTTCAC |

PCR Protocol

Master Mix (1x Reaction)

- 17.9 uL H2O
- 3 uL 10x Pfu II Buffer
- 1.2 uL DMSO
- 0.6 uL dNTPs 10mM
- 0.4 uL Pfu Ultra II Polymerase

Add 23uL of Master Mix to each tube. Each tube will contain its own specific set of primers.

Thermocycler Settings

- 96℃ 10 seconds
- 96℃ 2 minutes
  - 96℃ 20 seconds
  - 60℃ 40 seconds
  - 72℃ 4 minutes
  - *Repeat for 30 cycles
- 72℃ 10 seconds
- Hold at 4℃

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## References


Arai, K., Morishita, K., Shinmura, K., Kohno, T., Kim, S. R., Nohmi, T., ... & Yokota, J. (1997). Cloning of a human homolog of the yeast OGG1 gene that is involved in the repair of oxidative DNA damage. *Oncogene*, *14*(23), 2857-2861. https://doi.org/10.1038/sj.onc.1201139

Bacsi, A., Aguilera-Aguirre, L., Szczesny, B., Radak, Z., Hazra, T. K., Sur, S., ... & Boldogh, I. (2013). Down-regulation of 8-oxoguanine DNA glycosylase 1 expression in the airway epithelium ameliorates allergic lung inflammation. *DNA repair*, *12*(1), 18-26. https://doi.org/10.1016/j.dnarep.2012.10.002

Connelly, D. (2014, September 26). A history of aspirin. The Pharmaceutical Journal. https://pharmaceutical-journal.com/article/infographics/a-history-of-aspirin.

Curtin N. J. (2012). DNA repair dysregulation from cancer driver to therapeutic target. Nature reviews. Cancer, 12(12), 801–817. https://doi.org/10.1038/nrc3399

Dempsey, D. A., & Klessig, D. F. (2017). How does the multifaceted plant hormone salicylic acid combat disease in plants and are similar mechanisms utilized in humans?. *BMC biology*, *15*(1), 23. https://doi.org/10.1186/s12915-017-0364-8

Donley, N., Jaruga, P., Coskun, E., Dizdaroglu, M., McCullough, A. K., & Lloyd, R. S. (2015). Small molecule inhibitors of 8-oxoguanine DNA glycosylase-1 (OGG1). ACS chemical biology, 10(10), 2334-2343. 10.1021/acschembio.5b00452

Edwards, S. K., Ono, T., Wang, S., Jiang, W., Franzini, R. M., Jung, J. W., Chan, K. M., & Kool, E. T. (2015). In Vitro Fluorogenic Real-Time Assay of the Repair of Oxidative DNA Damage. Chembiochem : a European journal of chemical biology, 16(11), 1637–1646. https://doi.org/10.1002/cbic.201500184

Garcia-Albeniz, X., & Chan, A. T. (2011). Aspirin for the prevention of colorectal cancer. Best practice & research. Clinical gastroenterology, 25(4-5), 461–472. https://doi.org/10.1016/j.bpg.2011.10.015

Hosoya, N., & Miyagawa, K. (2014). Targeting DNA damage response in cancer therapy. Cancer science, 105(4), 370–388. https://doi.org/10.1111/cas.12366

Kelly, D., King, T., & Aminov, R. (2007, April 6). Importance of microbial colonization of the gut in early life to the development of immunity. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis. https://doi.org/10.1016/j.mrfmmm.2007.03.011.

Leveque, D. (2008). Off-label use of anticancer drugs. The lancet oncology, 9(11), 1102-1107. https://doi.org/10.1016/S1470-2045(08)70280-8

Orlando, B. J., Lucido, M. J., & Malkowski, M. G. (2015). The structure of ibuprofen bound to cyclooxygenase-2. Journal of structural biology, 189(1), 62-66. https://doi.org/10.1016/j.jsb.2014.11.005

Qin, S., Lin, P., Wu, Q., Pu, Q., Zhou, C., Wang, B., Gao, P., Wang, Z., Gao, A., Overby, M., Yang, J., Jiang, J., Wilson, D. L., Tahara, Y. K., Kool, E. T., Xia, Z., & Wu, M. (2020). Small-Molecule Inhibitor of 8-Oxoguanine DNA Glycosylase 1 Regulates Inflammatory Responses during Pseudomonas aeruginosa Infection. Journal of immunology (Baltimore, Md. : 1950), 205(8), 2231–2242. https://doi.org/10.4049/jimmunol.1901533

Rothwell, P. M., Fowkes, F. G., Belch, J. F., Ogawa, H., Warlow, C. P., & Meade, T. W. (2011). Effect of daily aspirin on long-term risk of death due to cancer: analysis

of individual patient data from randomised trials. Lancet (London, England), 377(9759), 31–41. https://doi.org/10.1016/S0140-6736(10)62110-1

Russelburg, L. P., O'Shea Murray, V. L., Demir, M., Knutsen, K. R., Sehgal, S. L., Cao, S., David, S. S., & Horvath, M. P. (2019). Structural Basis for Finding OG Lesions and Avoiding Undamaged G by the DNA Glycosylase MutY. ACS Chemical Biology, 15(1), 93–102. https://doi.org/10.1021/acschembio.9b00639

Saiyed, M. M., Ong, P. S., & Chew, L. (2017). Off-label drug use in oncology: a systematic review of literature. Journal of clinical pharmacy and therapeutics, 42(3), 251-258. https://doi.org/10.1111/jcpt.12507

Sterling, T., & Irwin, J. J. (2015). ZINC 15–ligand discovery for everyone. *Journal of chemical information and modeling*, *55*(11), 2324-2337. https://doi.org/10.1021/acs.jcim.5b00559

Vane, J. R., & Botting, R. M. (2003). The mechanism of action of aspirin. Thrombosis research, 110(5-6), 255-258. 10.1016/s0049-3848(03)00379-7

Xu, Y., & Villalona-Calero, M. A. (2002). Irinotecan: mechanisms of tumor resistance and novel strategies for modulating its activity. Annals of oncology, 13(12), 1841-1851. https://doi.org/10.1093/annonc/mdf337