# Indirect $k_{II}$: Degradation of Phenol and Propranolol by B* TAML with Safranin-O and Orange II Dyes

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**Abstract**

High Performance Liquid Chromatography (HPLC) is a scientific instrument designed to separate a liquid mixture in order to identify and quantify the components inside. The HPLC is a very accurate technique that few instruments can compare with. However, it take hours to read and run a sample, and large mixtures of the solution are required for the instrument. Furthermore, the machine itself is quite costly to use and maintain.

Thus, Professor Terrence Collins and his research group at Carnegie Mellon University are turning to the UV-Visible spectroscopy to analyze the degradation of micropollutants by TAML catalysts. The UV-vis is an ideal instrument to use, since it only requires small volumes of solution and analyzes solutions by light detection within seconds. Hence, the Collins group is determined to find a way to use the UV-vis to analyze invisible, or colorless, substrates, instead of relying on the time-consuming and expensive HPLC to run experiments. A new method, the indirect $k_{II}$, is being developed and tested, which utilizes competition between an invisible substrate and a known visible dye against the B* TAML catalyst. Through this project and previous works done, it becomes clear that the indirect $k_{II}$method has potential, but will require more time and research to confirm its reliability. Furthermore, this project has shown more evidence of the presence of substrate-catalyst interactions, and the importance of considering those interactions for the accuracy of calculations.

**Introduction**

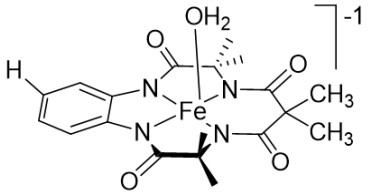
As more countries are developing, harmful chemicals and compounds are being thrown into bodies of water without the consideration of the effects on human health and the environment. The majority of these pollutants come from common pharmaceuticals, fertilizers, and pesticides. Because of this, micropollutants, extremely small particles that are toxic at tiny concentrations, are common in our water sources, harming all living things (Reference 6). Current technologies, such as ozone and powdered activated carbon, are expensive procedures and often release more toxic by-products during the process of decomposing micropollutants, making them not commercial-friendly methods (Reference 2).

With this in mind, Professor Collins and his research group looked to a new solution, tetra-amido macrocyclic ligand (TAML) catalysts, mimic enzymes that decompose any micropollutants into its basic harmless compounds, and are cost-effective, small, and efficient. The experiments on the rate of decomposition of micropollutants substrates are measured by the UV-vis or the HPLC. The UV-vis is able to measure a sample in just a few seconds, whereas the HPLC can take up to an hour to separate and analyze sample solutions. However, if a colorless substrate is placed into

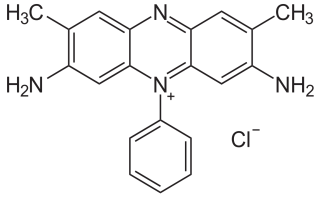
the UV-vis, its absorbance curve becomes indistinguishable from the absorbances of the catalyst and the oxidant. Although we can measure rate constants ($k_{II}$) of invisible substrates using HPLC, the amount of time and money required makes it a hard instrument to work with. Without data on the decomposition of colorless micropollutants, we cannot check the effectiveness of the TAML catalysts. Therefore, a new method is being tested in order to use the time and cost efficiency of the UV-vis to measure the invisible substrate rate constant using a known visible substrate.

This new method will determine the most efficient way to identify and measure reaction of non-colored micropollutants with TAMLs using the UV-vis by using dyes. Since absorbances of visible substrates (dyes) are easily distinguishable by the UV-vis, this new method will react both a visible substrate and an invisible substrate with the TAMLs catalyst. This will create competitive reaction between the two substrates, causing the concentration of the visible substrate to decrease at a slower rate since the catalyst will be used up faster. With the change in rate of visible substrate concentration decrease, it will be possible to calculate the rate constant of decomposition of the invisible substrate. Comparing this result with the $k_{II}$ obtained from using the HPLC will determine the accuracy of this method.

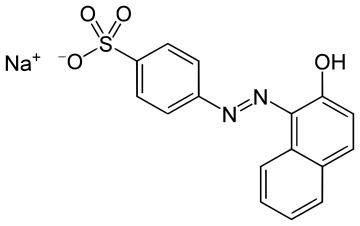
This report highlights the effectiveness of the indirect $k_{II}$ method for determining the rate constants of the invisible substrates propranolol and phenol against B* TAML catalysts. Both invisible substrates were tested with Orange II and Safranin-O as the visible dyes.



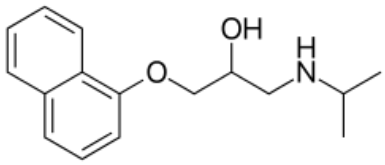

1. B* TAML



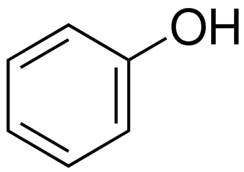

2. Safranin-O




3. Orange II




4. Propranolol




5. Phenol

**Materials**

All experiments were conducted with a Agilent 8453 UV-Vis spectroscopy, and pH of the buffer solution was measured using a Fisher Scientific Accumet pH meter.

HPLC grade water, hydrogen peroxide, and KOH pellets were obtained from Fisher Scientific. Safranin-O dye was purchased from Acros, and Orange II, propranolol hydrochloride, phenol, and KH2PO4 were purchased from Sigma Aldrich. The catalyst, B*, was synthesized by the Institute of Green Science.

All solutions were prepared in HPLC water, and experiments were conducted at 25℃ in 0.01M pH 7 KH2PO4 buffer.

Propranolol:

The propranolol experiments used 3 mM Hydrogen Peroxide, 500 nM B*, and 20 μM Orange II, with variation in propranolol concentration. All reactions were done with triplicates.

Phenol:

For phenol and Safranin-O, 3 mM Hydrogen Peroxide, 500 nM B*, and 30 μM Safranin-O were used. For phenol and Orange II, 2.5 mM Hydrogen Peroxide, 500 nM B*, and 50 μM Orange II were used. Both had variation on phenol concentration

All reactions were done with triplicates.

**Procedure**

UV-vis:

First, 2 mL of buffer was pipetted into a cuvette, which was blanked in the UV-vis. Then, the appropriate volumes of B*, visible dye, and invisible substrate were added into separate 2 mL cuvettes. KH2PO4 buffer was added to bring the total solution to 2 mL. The wavelength on the UV-vis was set to 520 nm for Safranin-O and 486 nm for Orange II, and the experiment was set to run for 20 min, taking a measurement every 30 seconds. After at least one measurement of the inactivated catalyst solution, $H_2O_2$ was added into the cuvettes to activate the catalyst.

Propranolol Variation:

The concentrations of propranolol used were 0 M, 60 μM, 200 μM, 400 μM, 600 μM, 1 mM, 3 mM, 5 mM, 8 mM, 10 mM, 15 mM, 20 mM. Triplicate initial rates were found for Orange II at each concentration of Propranolol.

Phenol Variation:
[Safranin-O] The concentrations of phenol used were in the range from 0 M to 0.15 mM. Triplicate initial rates were found for Safranin-O at each concentration of phenol.

[Orange II] The concentrations of phenol used were in the range from 0 M to 3 mM. Triplicate initial rates were found for Orange II at each concentration of phenol.

Calculations:
Given the extinction coefficient of the visible substrate (Safranin-O: 34000, Orange II: 21000) and the absorbance measured from the UV-vis, Eq (1), below, could be used to calculate the change in concentration of the visible substrate over time, thus obtaining the initial rates.

$$\text{Eq (1) } Concentration = \frac{Absorbance}{Extinction\ Coefficient}$$

The relationship between the visible and invisible substrates can be described by the following equation:

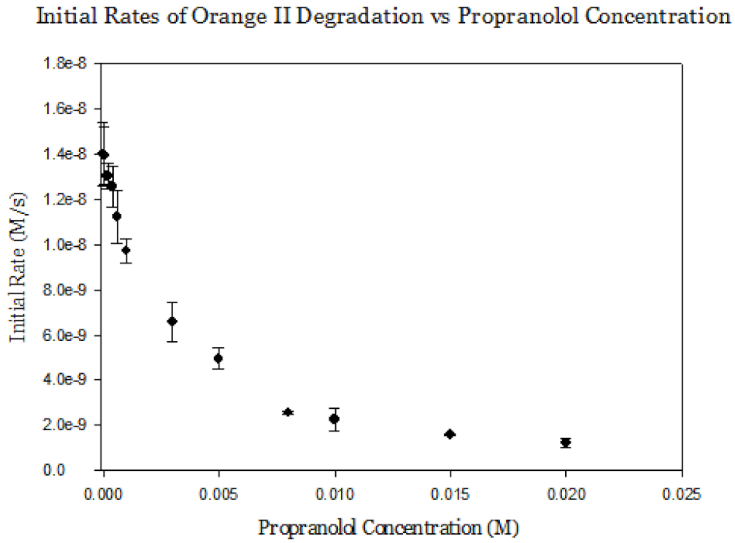
$$\text{Eq (2) } \frac{k_I k_{II-V}[Fe][H_2O_2][VS]dt}{d[VS]} = k_{II-I}[IS] + k_I[H_2O_2] + k_{II-V}[VS]$$

where $k_I$ is the rate constant of the activation of catalyst, $k_{II-V}$ is the rate of oxidation of visible substrate, $k_{II-I}$ is the rate of oxidation of invisible substrate (propranolol or phenol), and $[Fe]$, $[IS]$, $[VS]$, and $[H_2O_2]$ are the concentrations of catalyst (B*), invisible substrate, visible substrate, and hydrogen peroxide, respectively.

The independent variable is $[IS]$, the concentration of invisible substrate, and the dependent variable is the left-hand side of the equation. $k_I[H_2O_2] + k_{II-V}[VS]$ is the y-intercept, and $k_{II-I}$ is the slope of the graph.

**Results**

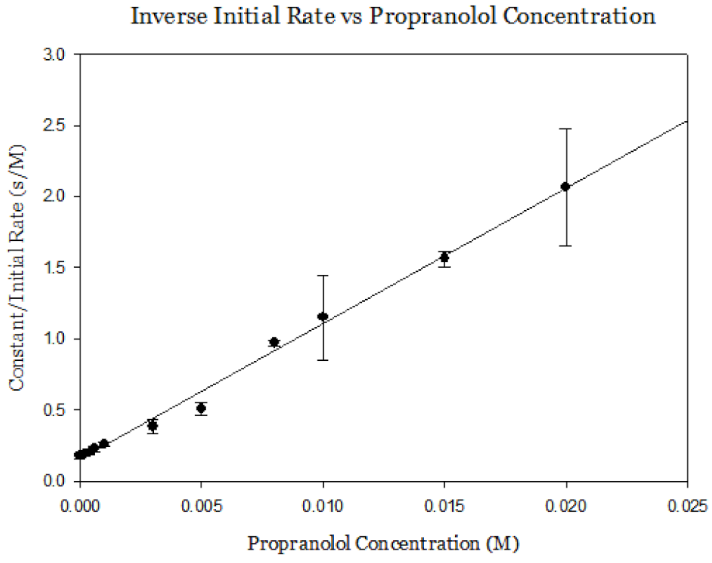
Propranolol:




*Graph 1:* The initial rates of Orange II degradation error bar plot at [500 x $10^{-9}$] M B*, [20 x $10^{-6}$] M Orange II, 0.01 M pH7 $KH_2PO_4$ buffer, 25℃

The above graph shows the exponential decay in the initial rate of Orange II degradation with B* as the concentration of Propranolol increases. This demonstrates the competitive inhibition occurring between the two substrates.

Using Eq (2) gives the following graph:

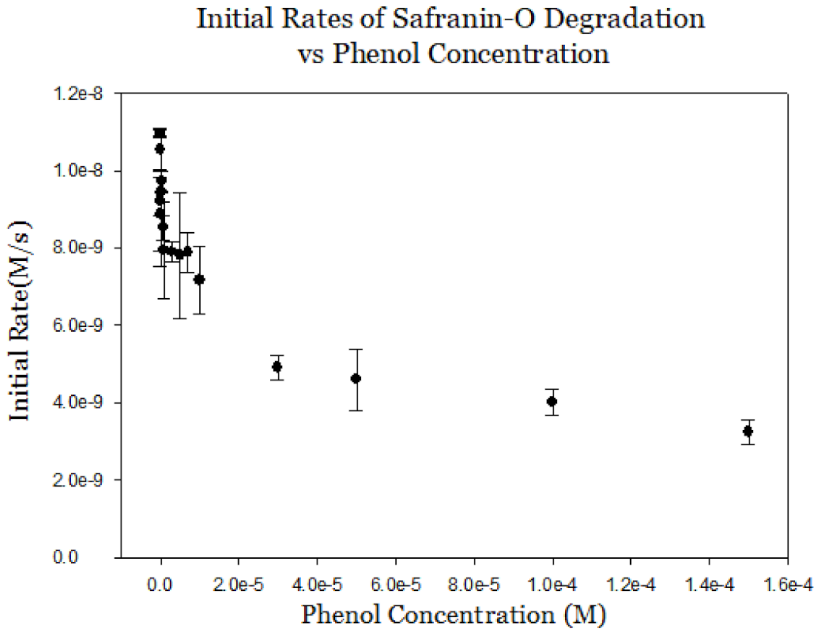


*Graph 2:* Propranolol variation error bar plot at [500 x $10^{-9}$] M B*, [20 x $10^{-6}$] M Orange II, 0.01 M pH7 KH2PO4 buffer, 25℃

In this case, the independent variable is[$IS$], the concentration of Propranolol, and the dependent variable is the left-hand side of the equation, with the inverse of the initial rate as the changing variable. $k_I[H_2O_2] + k_{II-V}[VS]$is the y-intercept, and $k_{II-I}$is the slope of the graph.

For this particular graph, $k_I$= 16.7 $M^{-1}s^{-1}$, which is the average between the $k_I$of Orange II (31.4) and the $k_I$of Propranolol (2) was used for calculation. The y-intercept was 0.1525, which is very close to the calculated y-intercept of 0.1491. The slope was found to be 95.19.

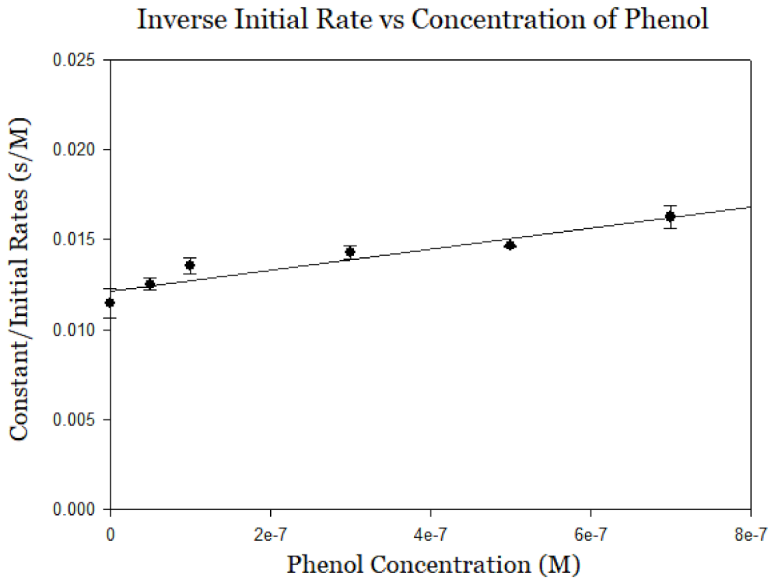
Phenol:
[Safranin-O]




*Graph 3*: The initial rates of Safranin-O degradation vs. phenol concentration error bar plot at [500 x $10^{-9}$] M B*, [30 x $10^{-6}$] M Safranin-O, [3 x $10^{-3}$] M $H_2O_2$, 0.01 M pH7 KH2PO4 buffer, 25℃

Graph 3, above, shows the exponential decay of the initial rate of Safranin-O degradation from B* as the concentration of phenol increases. Thus, there is evidence of competitive inhibition between the two substrates.

Eq (2) was used to make the following plot:

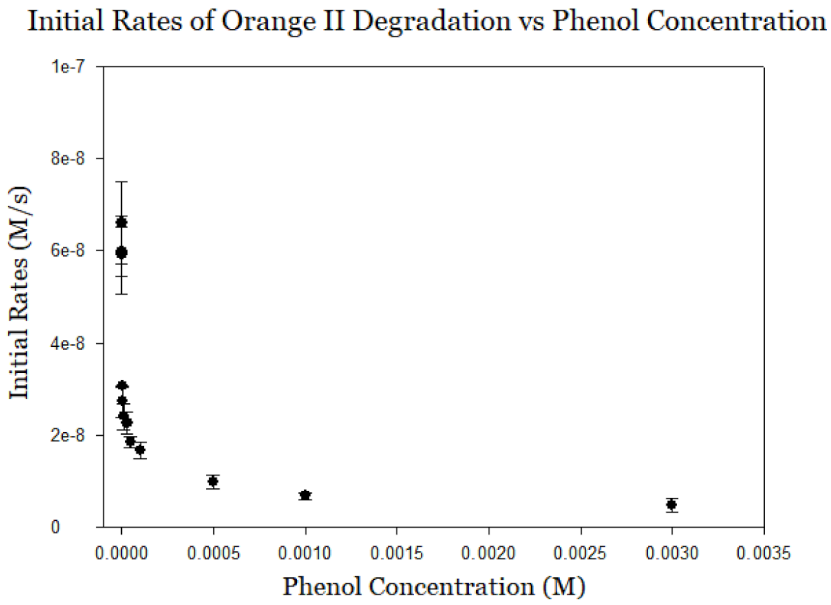



*Graph 4*: Phenol variation error bar plot at [500 x $10^{-9}$] M B*, [30 x $10^{-6}$] M Safranin-O, [3 x $10^{-3}$] M $H_2O_2$, 0.01 M pH7 KH2PO4 buffer, 25℃

A smaller phenol concentration range was used for Graph 4 because Equation (2) only works within a linear region. Thus, the range was narrowed down until a reasonable graph could be drawn. The rate constants used for this calculations were $k_I$= 4 $M^{-1}s^{-1}$, $k_{II-V}$= 770 $M^{-1}s^{-1}$.

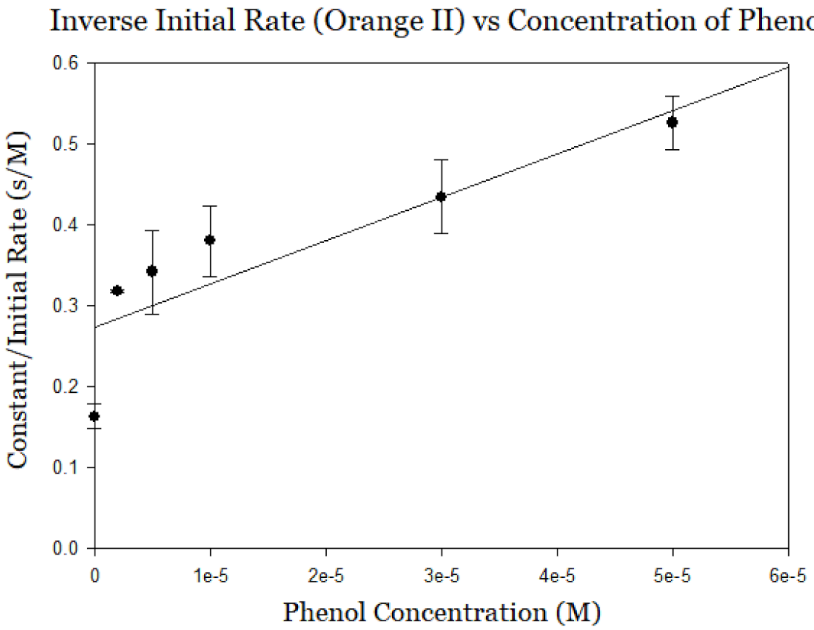
The y-intercept of the graph was 0.0121 ± 2.36 x$10^{-4}$s/M, compared to the calculated value of 0.0351 s/M. The slope was determined to be 5827 ± 616 $M^{-1}s^{-1}$, compared to the known kII of phenol of 7400 $M^{-1}s^{-1}$. Because the actual and expected y-intercepts were very closely related, Eq could be confirmed to be a good linear representation of the relationship between phenol and Safranin-O. In addition, the 5827 $M^{-1}s^{-1}$ was to the same magnitude as the expected value of 7400 $M^{-1}s^{-1}$, which also gave solid evidence of an effective indirect kII result.

[Orange II]




*Graph 5*: The initial rates of Orange II degradation error bar plot at [500 x $10^{-9}$] M B*, [50 x $10^{-6}$] M Orange II, [2.5 x $10^{-3}$] M $H_2O_2$, 0.01 M pH7 KH2PO4 buffer, 25℃

Similar to the past experiments, Graph 5 showed that there was evidence of competitive inhibition between Orange II and phenol. Using a $k_{II-V}$ value of 4950 $M^{-1}s^{-1}$ and $k_I$ value of 31.4 $M^{-1}s^{-1}$, a plot of Eq (2) resulted in Graph 6 shown below.



*Graph 6*: Phenol variation error bar plot at [500 x $10^{-9}$] M B*, [50 x $10^{-6}$] M Orange II, [2.5 x $10^{-3}$] M $H_2O_2$, 0.01 M pH7 KH2PO4 buffer, 25℃

The y-intercept of Graph 4 was found to be 0.274 ± 0.0210 s/M, as compared to the calculated value of 0.326 s/M. The slope was found to be 5359 ± 864 $M^{-1}s^{-1}$, as compared to 7400 $M^{-1}s^{-1}$, the known $k_{II}$ of phenol. Both the calculated y-intercept and slope depict a similar relationship to the expected values, further confirming the effectiveness of the indirect $k_{II}$ method for the determination of phenol rate constant.

A comparison between the phenol concentration range used for the two visible substrates show that Safranin-O is affected by phenol at the nM range, but Orange II faces no significant change at such low concentrations. Given that Safranin-O has a lower $k_{II}$ than Orange II and phenol, it is a harder substrate to oxidize. Thus, it is reasonable to suggest that low concentrations of phenol can still be oxidized by the catalyst when in a system with Safranin-O, whereas the catalyst is much less likely to react with low concentrations of phenol in a system with Orange II. This assumption would explain the reasoning behind the observed differences of phenol concentration used for the two visible substrates. However, such assumptions cannot be confirmed until further experiments with invisible substrates have been conducted.

[Summary]

Table (1) below summarizes results found for the two invisible substrates

| **Invisible Substrate** | **Visible Substrate** | **$k_I$ ($M^{-1}s^{-1}$)** | **$k_{II}$ ($M^{-1}s^{-1}$) (indirect)** | **$k_{II}$ ($M^{-1}s^{-1}$) (HPLC)** |
|---|---|---|---|---|
| Propranolol | Orange II | 16.7 | 95 ± 4 | 70 ± 20 |
| | Safranin-O | 4 | 110 (Reference 4) | |
| Phenol | Orange II | 31.4 | 5359 ± 616 | 7400 ± 230 |
| | Safranin-O | 4 | 5827 ± 864 | |

*Table (1)*: Summary of propranolol/phenol $k_{II}$ from indirect $k_{II}$ method and comparison with HPLC data

Substrate-catalyst interactions were observed for the calculation of propranolol $k_{II}$ with Orange II. The $k_I$ of 16.7 $M^{-1}s^{-1}$ was the average of the $k_I$ for B* with Orange II

and $k_I$ for B* with propranolol. The table below demonstrates the variability of propranolol $k_{II}$ based on the $k_I$ used for calculations.

| | Orange II ($k_I$= 31.4) | Propranolol ($k_I$= 2) | Average ($k_I$= 16.7) |
|---|---|---|---|
| Propranolol $k_{II}$ ($M^{-1}s^{-1}$) | 179 ± 7 | 11.4 ± 0.42 | 95 ± 4 |

*Table (2)*: Summary of variability in propranolol $k_{II}$ based on $k_I$

The $k_{II}$ value for propranolol calculated from the average $k_I$ of 16.7 $M^{-1}s^{-1}$ was the most consistent with the HPLC $k_{II}$ value of 70 ± 20. This provides evidence of potential substrate-catalyst interactions within the two substrate system that may affect the $k_{II}$ calculations for invisible substrates.

## Conclusion

This project has further confirmed the prospect of using the UV-Vis spectroscopy as a method to measure the rate constant of invisible substrates using competitive inhibition. This can be proven by the clear exponential decay of the initial rates of propranolol and phenol with both Safranin-O and Orange II, and the linear relationship between the invisible and visible substrates.

The experiment with propranolol highlighted the differences in $k_{II}$ of propranolol depending on the $k_I$ used in the calculations. It is reasonable to attribute these differences to the substrate-catalyst interactions that may influence the activation of B* with hydrogen peroxide. Previous research within the Collins group (Reference 3) has shown that substrate-catalyst interactions do exist, and since this project involves two systems, it is very likely for the $k_I$ value to be dependent on both Orange II and Propranolol.

Furthermore, this could apply the experiment with phenol. Since the rate constant of phenol with B* was found to be lower than expected with both visible substrates, it is possible that this was simply due to the lack of consideration of substrate-catalyst interactions. The same method used for propranolol can be applied with the phenol project, which can help determine the existence of substrate-catalyst interactions regarding a system with phenol.

Since the indirect $k_{II}$ project involves a two-substrate system, it is reasonable to assume that using the $k_I$ of simply one of the substrates is not a good depiction of the interactions occurring with the catalyst. Thus, more research must be done to confirm

the presence of substrate-catalyst interactions, as well as the influence of a two-substrate system on the $k_I$ of the reaction. Doing so will also refine the indirect $k_{II}$ method and develop more accurate calculations for the rate constants of invisible substrates.

**References**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