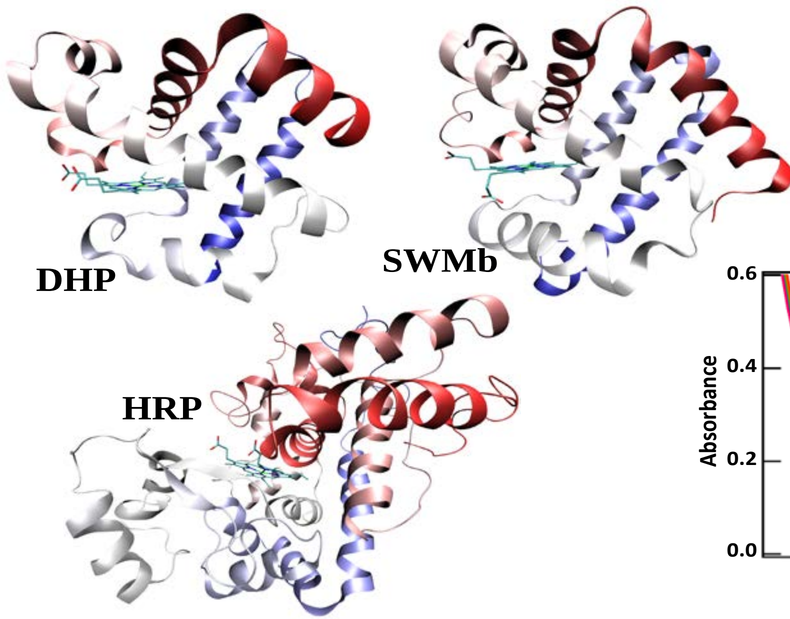
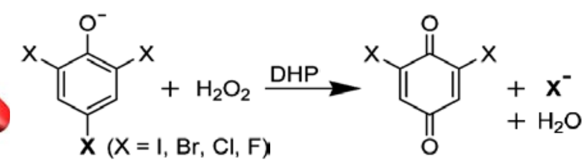
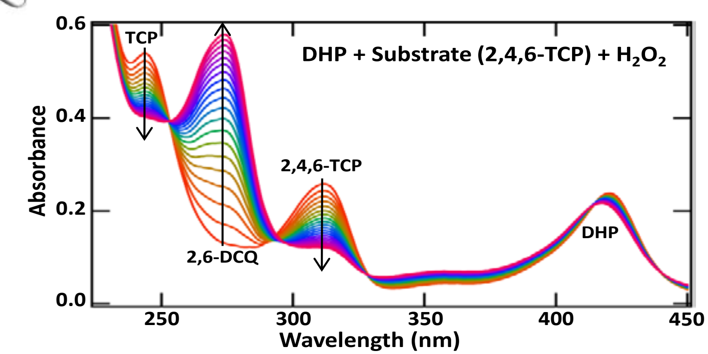
Michaelis-Menten Catalysis

The enzyme dehaloperoxidase has measured value of V_{max} = 1.5 micromolar/sec and K_m = 800 micromolar when the enzyme concentration is 3 micromolar. The association constant for the substrate, 2,4,6-trichlorophenol was measured separately and is K_a = 10,000 M^{-1}. Using these values, calculate the values of the intrinsic rate constants, k_{on}, k_{off} and k_{cat}.







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Solution: We can calculate the intrinsic k_{cat} using the fact that $V_{max} = k_{cat}[E]_0$.

$$k_{cat} = \frac{V_{max}}{[E]_0} = \frac{1.5 \times 10^{-6}\ Ms^{-1}}{3 \times 10^{-6}\ M} = 0.5\ s^{-1}$$

Then we can obtain k_{on} and k_{off} from two simultaneous equations given the association:

$$E + S \leftrightarrow ES$$

And the equilibrium constant:

$$K_a = \frac{k_{on}}{k_{off}} = \frac{[ES]}{[E][S]}$$

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We can solve for k_{off}

$$k_{off} = \frac{k_{on}}{K_a}$$

And then substitute that value into the Km equation

$$K_m = \frac{k_{cat} + \frac{k_{on}}{K_a}}{k_{on}}$$

Using algebra we find an expression for k_{on}

$$k_{on}\left(K_m - \frac{1}{K_a}\right) = k_{cat}$$

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We can solve for kon and insert the known values:

$$k_{on} = \frac{k_{cat}}{K_m - \frac{1}{K_a}} = \frac{0.5\ s^{-1}}{800 \times 10^{-6}\ M - \frac{1}{10000\ M^{-1}}}$$

To find

$$k_{on} = \frac{0.5\ s^{-1}}{7 \times 10^{-4}\ M} = 714\ M^{-1}s^{-1}$$

And therefore

$$k_{off} = \frac{714\ M^{-1}s^{-1}}{10000\ M^{-1}} = 0.071\ s^{-1}$$

Note that k_{on} is a second order rate constant while k_{off} is a first order rate constant.