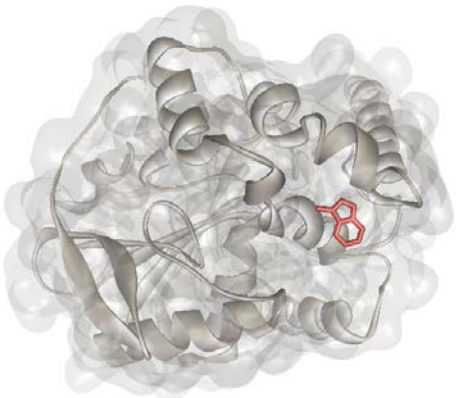
Task 4

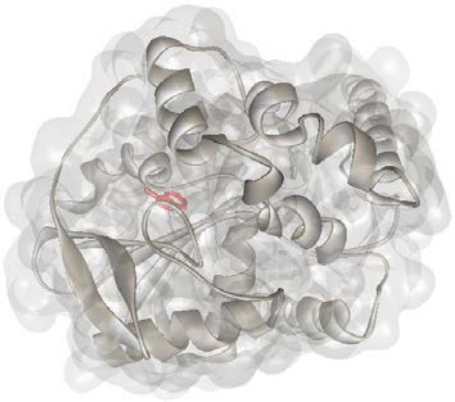
10 May 2017

Mutant identification

Your first real task in biotechnological laboratories BTP (Biotechnology for purification) is to distinguish between two mutant variants X1 and X2 of a protein which is used for the decomposition of toxic substances in drinking water. Accidentally, labels of reaction reservoirs with enzymes were confused. You have found out that both types of enzyme contain one tryptophan. Additionally, you know that tryptophan in case of mutant X1 is placed much closer to the surface. Therefore, X1 is in contact with surrounding solution more than X2.



X1



X2

The addressing of this task is important for the supply of drinking water to residents in an area affected by drought. You can use your knowledge of fluorescence quenching for the problem solution. You remember that the protein with a tryptophan located on the surface can be determined from the dependence of fluorescence intensity on the concentration of the quencher. For quenching fluorophore, the basic Stern-Volmer equation can be applied:

$$\frac{F_0}{F} = 1 + K_{SV}[Q]$$

where F_0 is fluorescence intensity in the absence of quencher, F is the fluorescence intensity in the presence of quencher, K_{SV} is Stern-Volmer constant and [Q] is the concentration of the quencher.

You have carried out measurements of fluorescence intensity of proteins taken from reservoirs A and B. The fluorescence intensity was measured in the absence of the quencher. Then you measured fluorescence decrease after gradual addition of quencher (acrylamide). The obtained values of fluorescence intensity are in the table below.

Plot the dependence of relative fluorescence intensity decrease on the acrylamide concentration in the form of Stern-Volmer graph and answer the following questions:

1. **Is acrylamide a dynamic or static quencher?**
2. **What are constant K_{SV} values corresponding to each mutant of the enzyme?**
3. **Determine in which reservoir X1 mutant is located.**

Please send me your answers together with Stern-Volmer plot for A and B via email.
Correct answer = 0.5 point

Note: Determine Ksv as a slope of linear regression according to a videotutorial here.
https://is.muni.cz/el/1431/jaro2017/S2006/um/Tasks/Regression_Linear_Excel.wmv

		reservoir	Acrylamide concentration [M]					
			0	0.1	0.2	0.3	0.4	0.5
1	Akhmetgalieva, Valentina	**A**	944	911	891	870	853	834
		B	944	794	697	621	560	510
2	Alispahic, Elma	**A**	977	943	922	901	883	864
		B	977	822	722	643	580	528
3	Atatri, Sura S. M.	**A**	940	908	887	867	850	831
		B	940	791	694	619	558	508
4	Janovič, Tomáš	**A**	951	918	898	877	860	841
		B	951	800	703	626	565	514
5	Lobello, Cosimo	**A**	960	927	907	886	868	849
		B	960	808	709	632	570	519