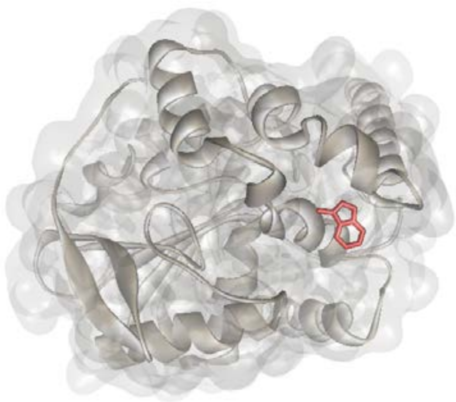
Task 4

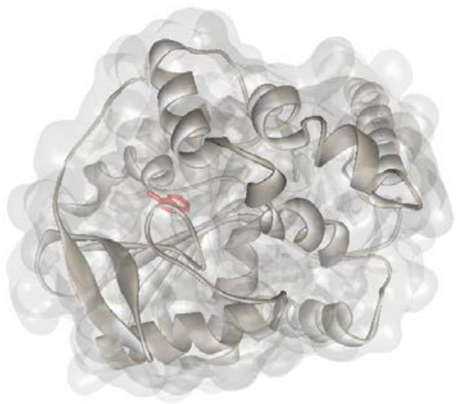
15 December 2020

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

Tthis 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 the videotutorial here.

		rezervoár	Koncentrace akrylamidu [M]					
			0	**0.1**	**0.2**	**0.3**	**0.4**	**0.5**
1	Dikunová Alžbeta	**A**	944	911	891	870	853	834
		B	944	794	697	621	560	510
2	Dzurov Matej	**A**	977	943	922	901	883	864
		B	977	822	722	643	580	528
3	Faturová Jana	**A**	940	908	887	867	850	831
		B	940	791	694	619	558	508
4	Gašparik Norbert	**A**	951	918	898	877	860	841
		B	951	800	703	626	565	514
5	Hesko Ondrej	**A**	960	927	907	886	868	849
		B	960	808	709	632	570	519
6	Jahodová Kateřina	**A**	986	952	931	910	891	872
		B	986	830	729	649	585	533
7	Kameniarová Michaela	**A**	938	906	886	865	848	829
		B	938	790	693	617	557	507
8	Konečná Kateřina	**A**	957	924	903	882	865	846
		B	957	805	707	630	568	517
9	Korytářová Anna	**A**	986	952	931	910	891	872
		B	986	830	729	649	585	533
10	Kozeleková Aneta	**A**	975	942	921	899	881	862
		B	975	821	720	642	579	527
11	Kubinyiová Lenka	**A**	981	947	926	905	886	867
		B	981	825	724	646	582	530
12	Kůřilová Eliška	**A**	957	924	903	882	865	846
		B	957	805	707	630	568	517
13	Lysáková Klára	**A**	961	928	908	887	869	850
		B	961	809	710	633	571	520
14	Mikšátková Barbora	**A**	855	834	813	794	776	758
		B	855	732	639	568	510	464
15	Nováková Barbora	**A**	943	911	891	870	853	834
		B	943	794	697	621	560	510
16	Prabhullachandran Unnikannan	**A**	978	943	922	901	883	864
		B	978	822	722	643	580	528
17	Procházková Markéta	**A**	941	908	887	867	850	831
		B	941	791	694	619	558	508
18	Šimek Jan	**A**	953	918	898	877	860	841
		B	953	800	703	626	565	514
19	Tužinčin Dávid	**A**	962	927	907	886	868	849
		B	962	808	709	632	570	519