

Enzyme E is a homodimer that can be inactivated at 60°C. A semilog plot of remaining activity versus time was found to be linear, indicating that the inactivation of Enzyme E is a first order process.

The inactive Enzyme E can be dialyzed at 4°C to reactivate it, leading to an increase in enzyme activity versus time. The time course of reactivation at two different initial enzyme concentrations is shown below. Note that the $[E]_0$ values shown represent the total concentration of subunit active sites (i.e., the total monomer concentrations).

1. Analyze the data using appropriate plots to determine the kinetic nature of the reactivation process (zero, first or second order?).

This will be best done by determining the concentration of inactive Enzyme E present during the time course of dialysis (this is the substrate that decreases with time).

The plot that is closest to linear and also provides the correct relationship between inactive $[E]_0$ and the half life should give you the answer.

2. Provide a likely reaction model of the inactivation/reactivation process.

Dialysis at 5°C (min)	Activity (Units x mg protein ⁻¹)	
	$[E]_0 = 10$ nM	$[E]_0 = 30$ nM
0	0	0
2	2.3	3.87
3	2.72	4.91
4	3.05	6.17
5	3.58	6.9
6	4.01	7.6
7	4.34	8.32
8	4.87	9.03
9	5.3	9.53
10	5.63	9.93
11	5.95	10.25
12	6.3	10.9
13	6.7	11
14	6.81	11.5
15	7.13	11.72
Overnight incubation	17.9	17.9