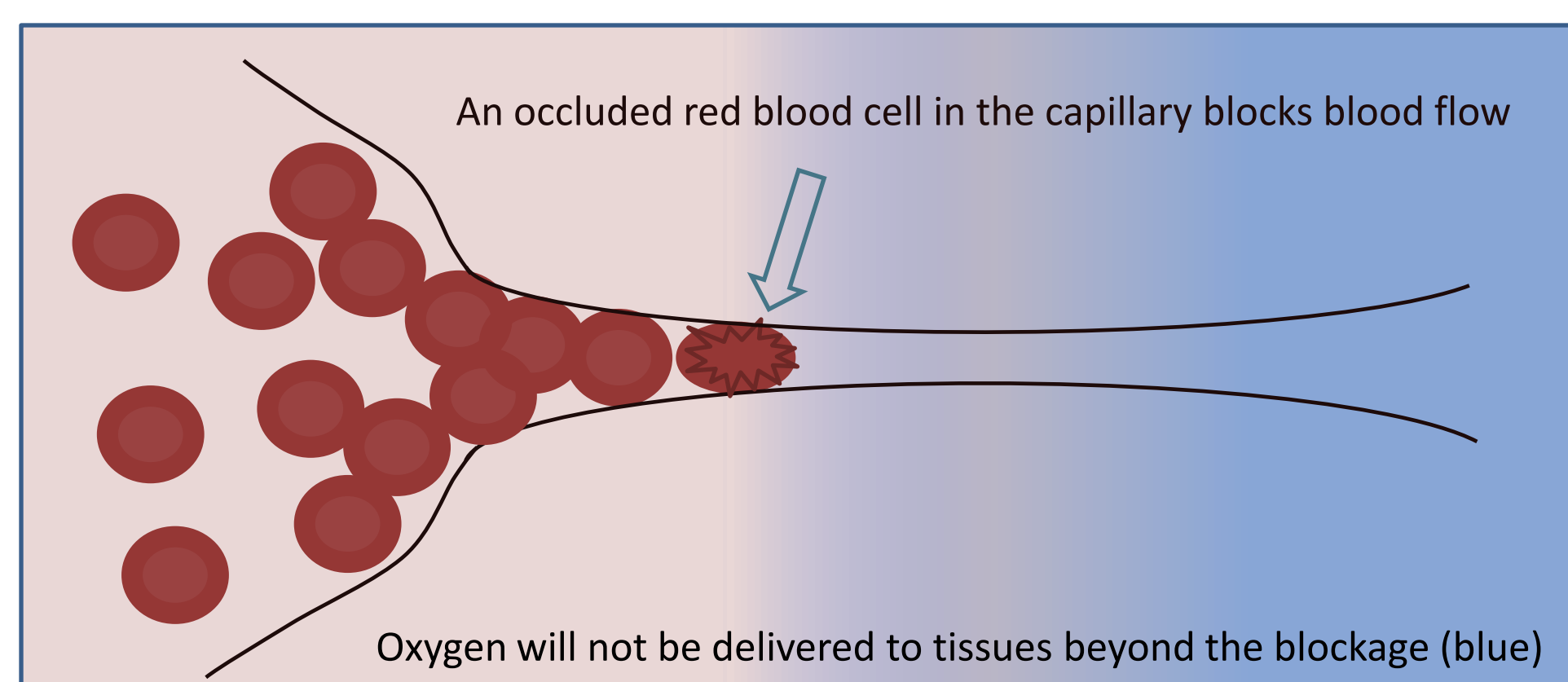


Do Different Ligands Produce Different Effects in Sickle Hemoglobin Polymer Growth?

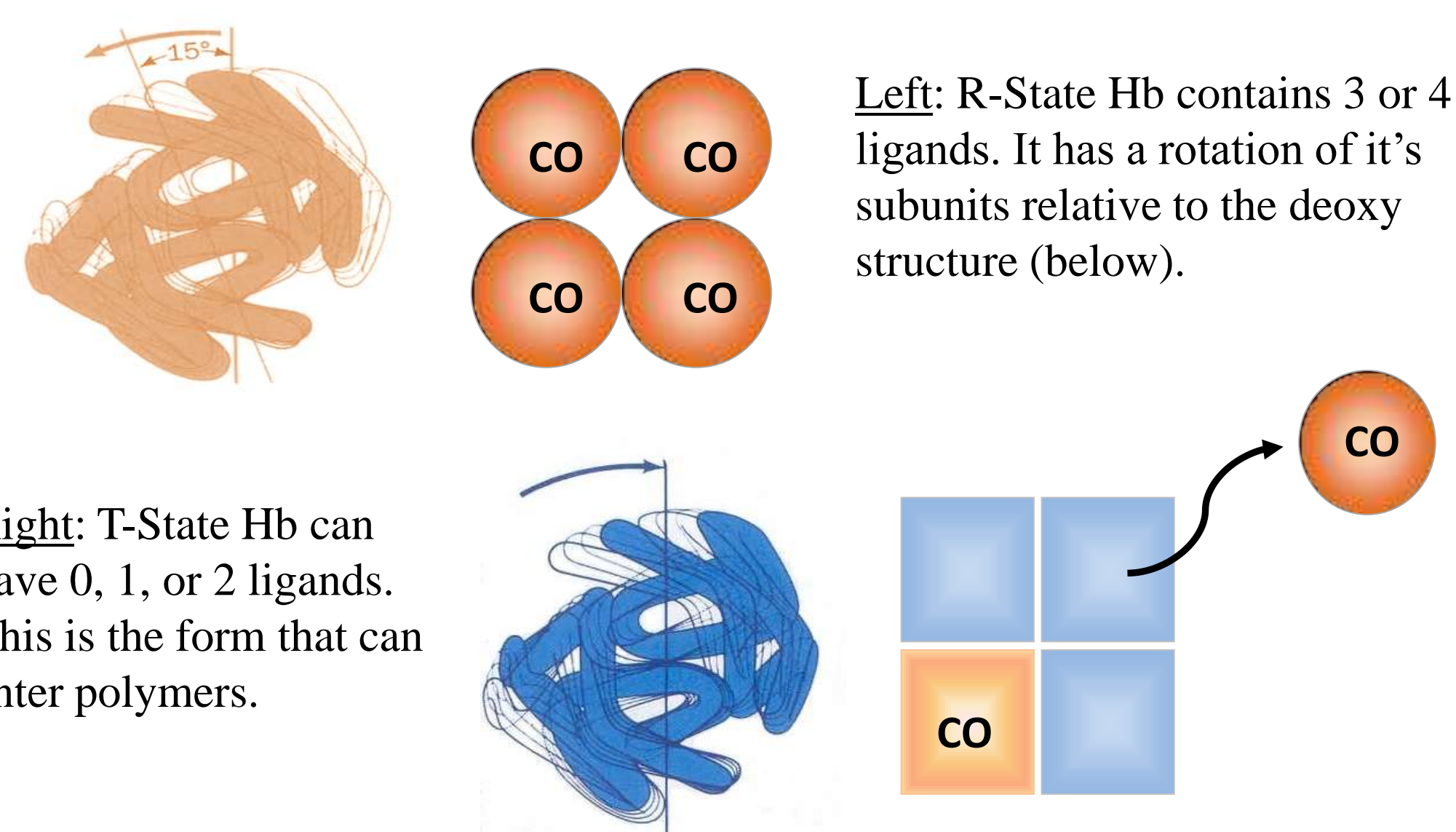
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Introduction

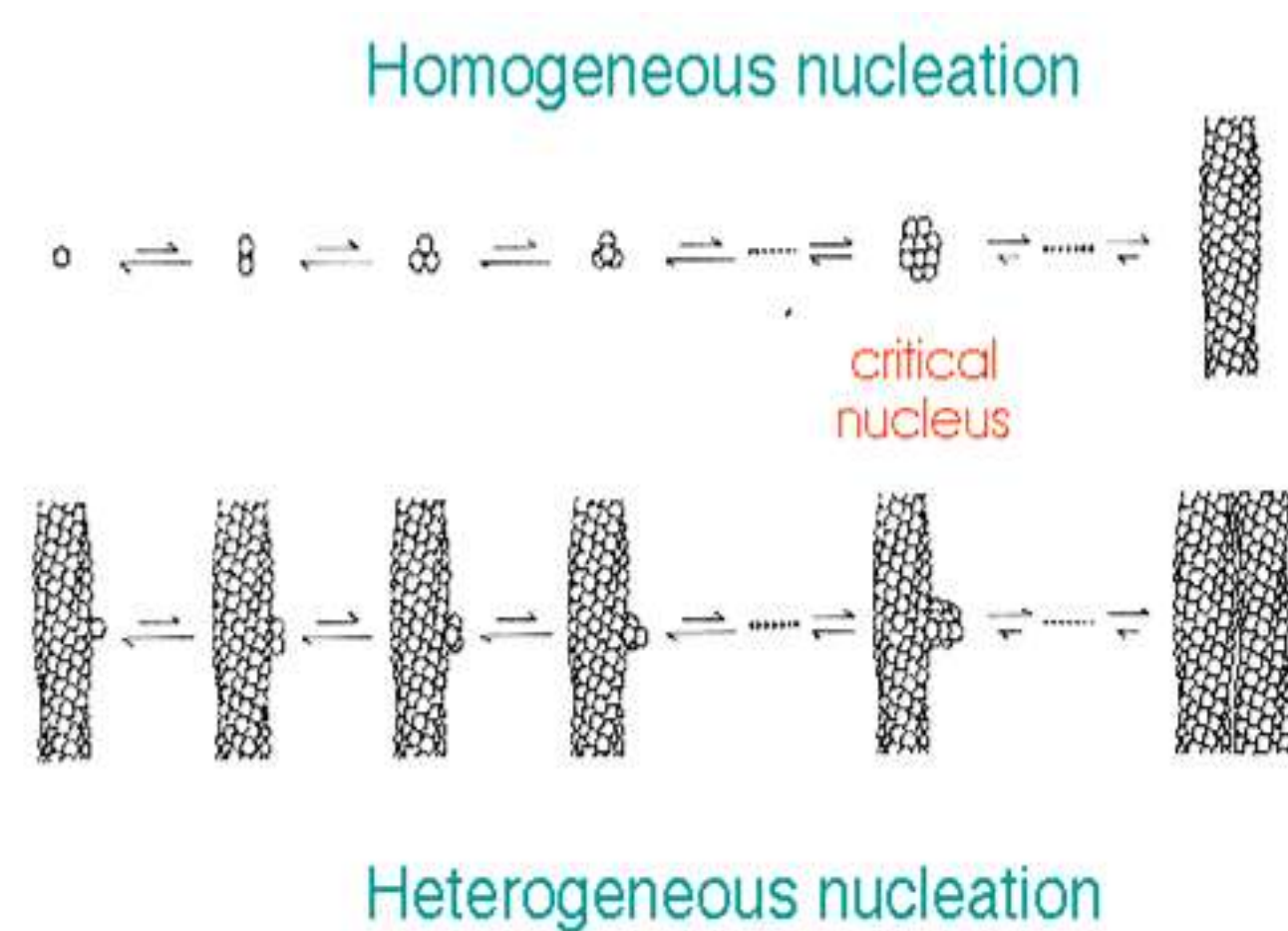
Hemoglobin (Hb) is the gas transporting protein found in high concentrations in human red blood cells (RBCs). Sickle Hemoglobin (HbS) is a mutated variant of Hb with a Glu replaced by Val on the two beta subunits of the molecule. This mutation causes HbS polymer masses to grow inside RBCs, making them too rigid to deform to pass through thin capillaries, causing vaso-occlusion, stroke and other complications for sufferers of Sickle Cell Disease.



Hb has four heme groups that bind one gas molecule each for a total of four ligands. When Hb is fully saturated, it has four ligands and is found in its relaxed R-state. However, as it releases gas ligands into the human body it will favor a T-state configuration with increasing probability. Only T-state structures contribute to polymerization, while R-states crowd the solution.



There are two pathways of polymerization: homogeneous and heterogeneous nucleation (figure below). Homogeneous nucleation is the formation of a nucleus which will become the first polymer, this is an event limited step. Heterogeneous nucleation is the formation of a nucleus for other polymers on the surface of pre-existing polymers. This contribution causes the growth of HbS fibers to be exponential.



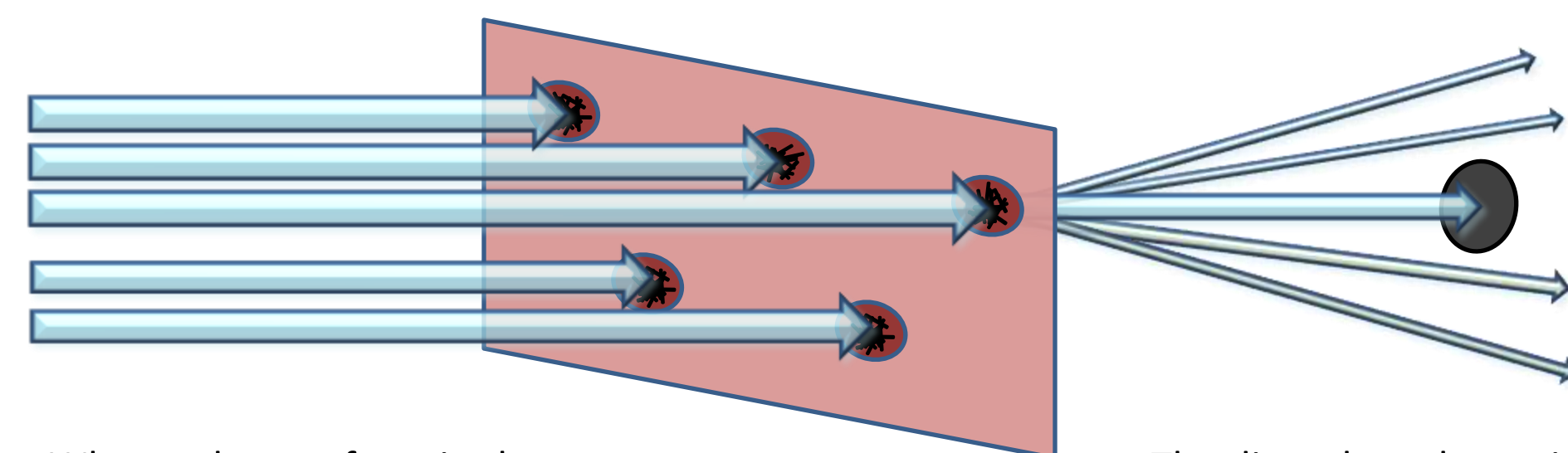
A study of the structural mechanisms that can affect HbS polymerization:

In vivo, RBCs rarely lose more than 50% of their O₂ saturation, therefore partially liganded T-states of HbS are usually present. Polymer growth has already been extensively studied for fully unliganded solutions. How do partial liganded T-state HbS influence polymer growth and nucleus formation? Does this influence differ based on the type ligand, such as NO, O₂, or CO? Can this be explained through differences in Hb protein crystal structures?

Experimental Procedure

Polymer growth is measured by the illumination of a solution of HbS by laser light. As pictured below:

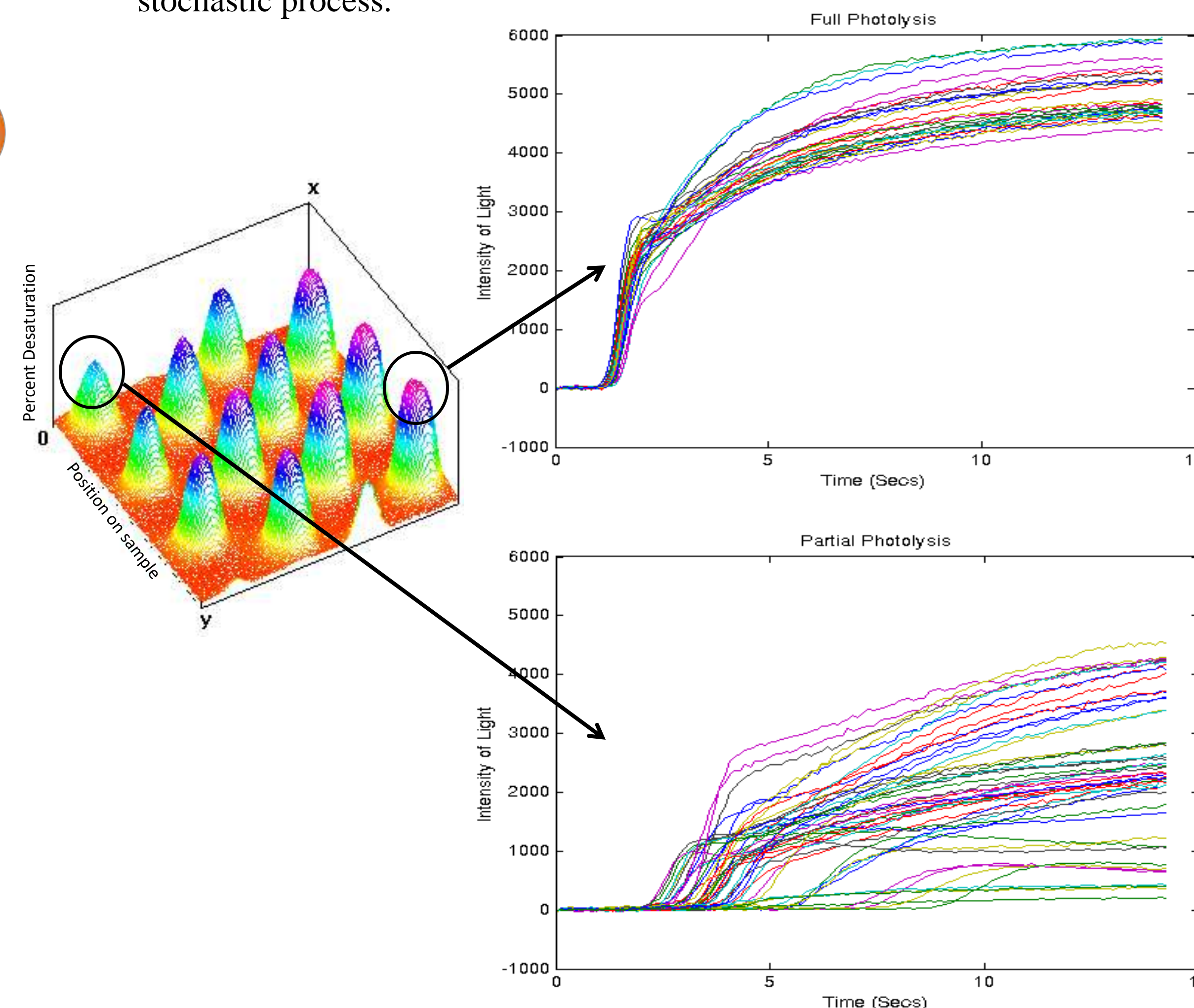
A laser is split into several beams that illuminate spots on the sample, creating deoxy areas, which form polymers.



When polymers form in the spots, they will scatter the laser light

The direct laser beam is blocked, and the scattered light is recorded by a fast sensitive camera.

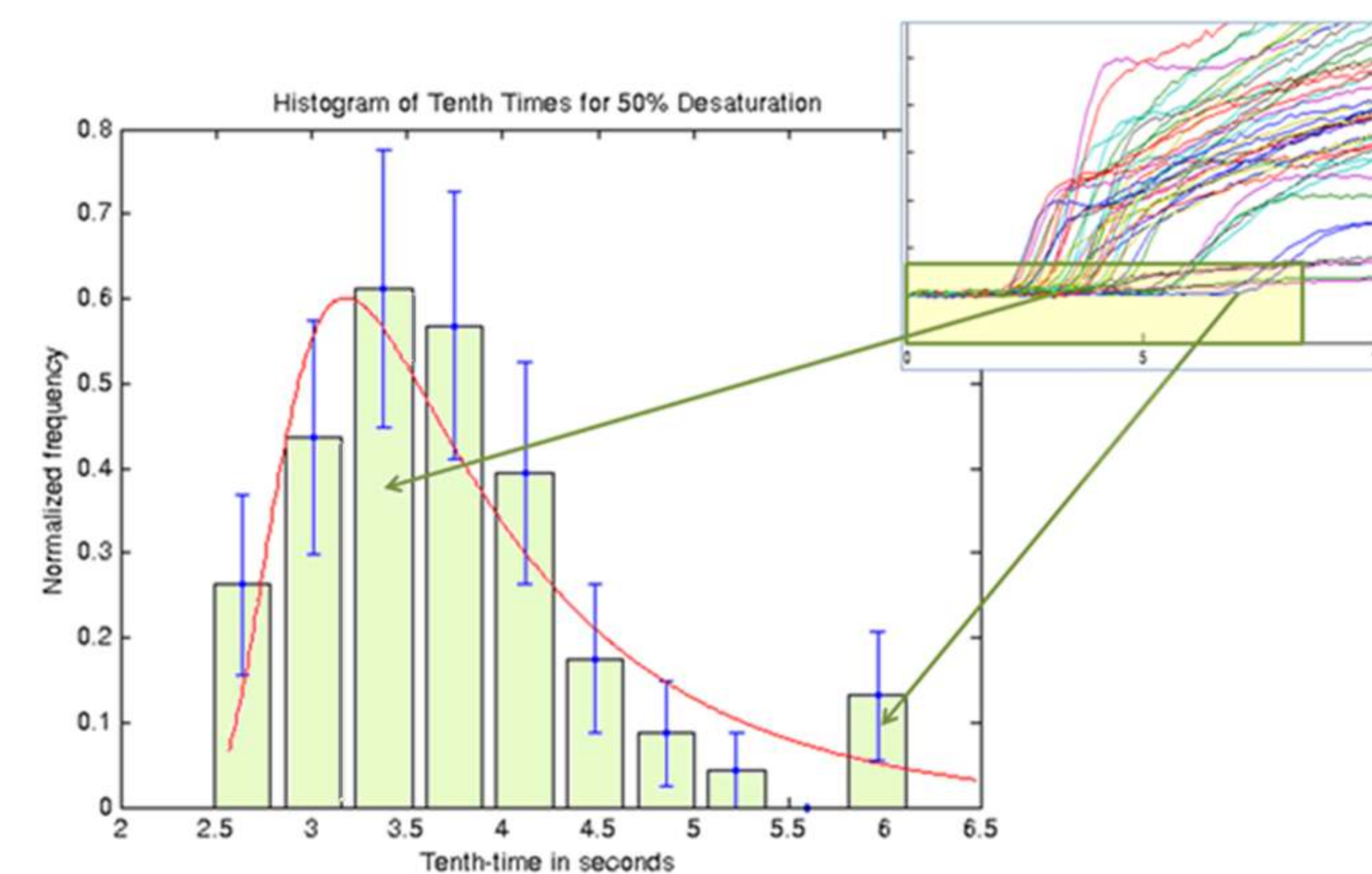
This process is done on as many as 30-50 spots on a single sample simultaneously and is done for several repetitions. The large amount of data is necessary because the formation of a polymer nucleus is a stochastic process.



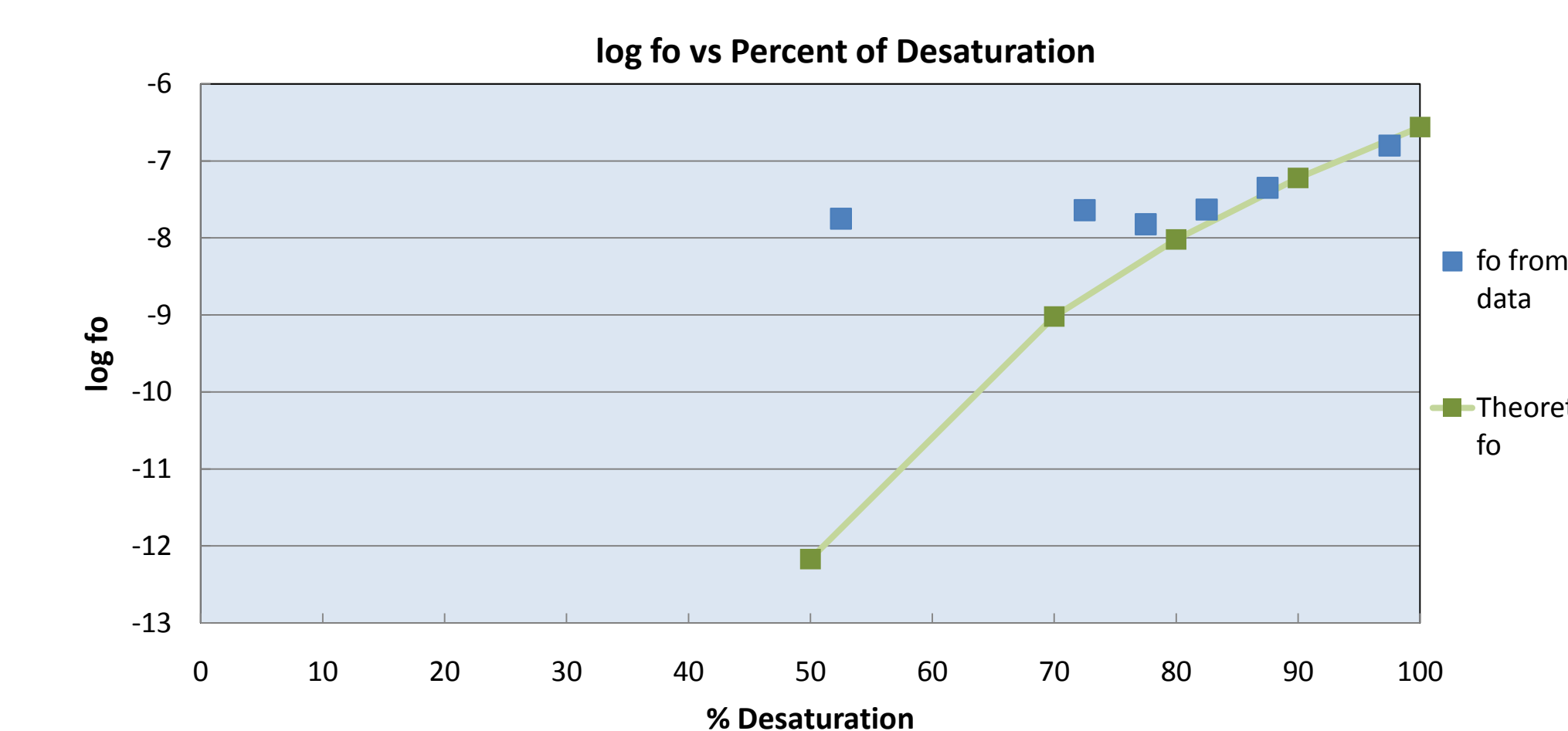
Each laser spot has a unique desaturation value and is analyzed independently.

Data Analysis

From a histogram of the time it took for the growth to reach one tenth of its maximum value (tenth time) the homogeneous nucleation rate f_0 can be determined, specifically from the exponential tail of the distribution.



Hundreds of growth curves are gathered and sorted according to the amount of ligand saturation, and the data is combined to obtain a single value for f_0 . This is done for HbS solutions with a constant total concentration, but varying CO ligand saturation. Below is a graph comparing collected data to a theoretical model. The model predicts what f_0 values should be obtained for an HbS solution that does not exhibit inhibition of partial ligands. Values are calculated based on the HbS concentration able to participate in polymer growth (T-state), and what percent of HbS (R-state) just "crowds" the solution:

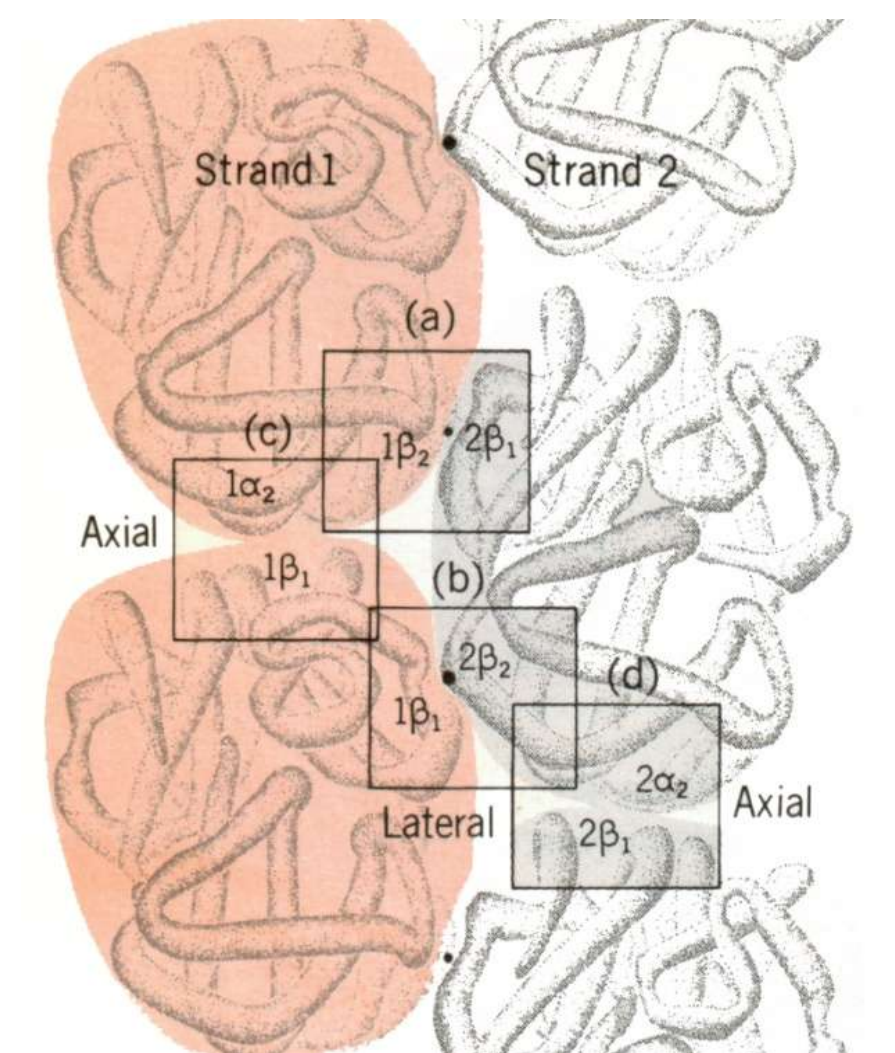


We previously collected data from samples partially saturated with NO. From this data, a sample with just 6.5% NO has growth similar to a sample that has lost 12.3% of its concentration to crowding of the sample with non participating molecules. This shows that Hb partially liganded with NO has a different effect on nucleation and growth:

Total Concentration of the sample (mM)	%NO on Hemes	% of Sample Not Participating in Kinetics	Polymerizing Concentration (mM)
4.76	6.5%	12.3%	4.18
4.48	12.1%	21.0%	3.54
4.61	12.3%	22.0%	3.60
4.92	16.7%	26.1%	3.63
4.92	17.3%	26.1%	3.63
4.90	21.0%	39.4%	2.97
4.77	27.4%	37.6%	2.98

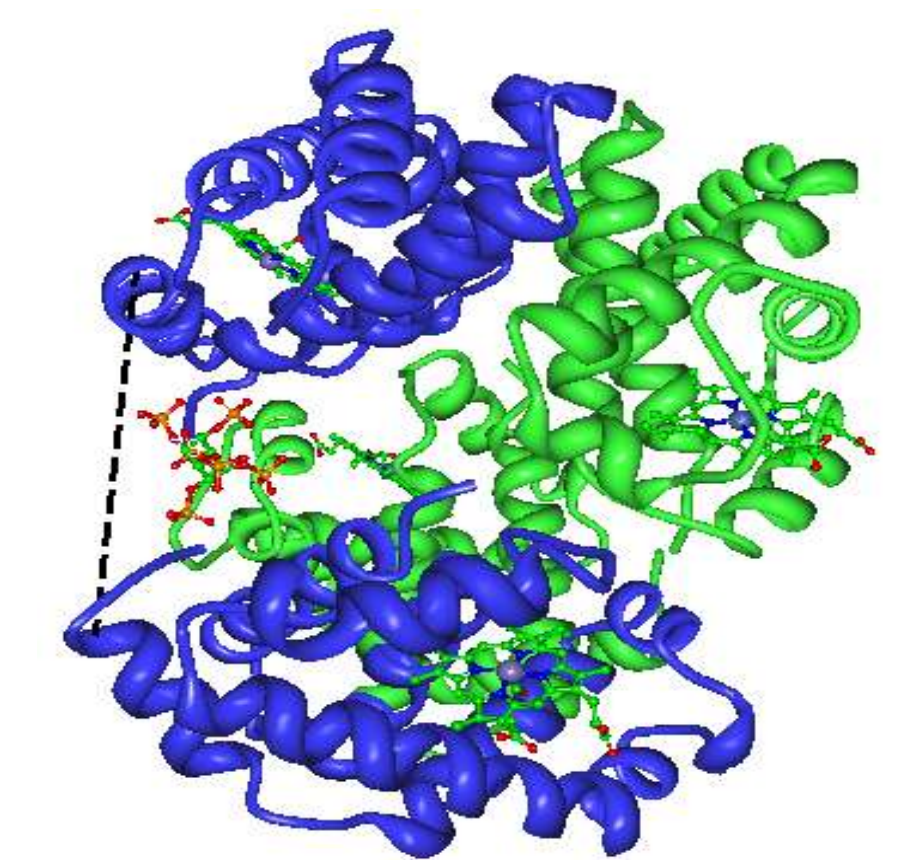
Conclusion: Relation to Crystal Structures

The data collected for partially liganded COHbS samples is similar to theoretical predictions for a solution that has a decreased concentration due to a crowder in the solution (i.e. R-state COHbS). Below 80%, we suspect our measurements are being affected by unmelted polymer pieces. Nitric Oxide HbS shows a partially liganded effect. A sample containing 6.5% NO, for example, will behave as though 12.3% of the concentration cannot polymerize. A sample with 12.5% CO, will behave as though 11-12% does not participate in polymer growth. This shows a dramatic decrease in how Hb partially liganded with CO affect the system.



How does this relate to structures? In order to form a polymer, the mutated amino acid on the beta subunits of HbS need to form a donor-acceptor site with another HbS molecule:

The amino acids involved in this (in order of significance), are the mutation β6, and corresponding β84, β85 and β88 on the other Beta subunit. Below is an image from the Protein Data Base of a T-State deoxy Hb. Beta subunits are blue, the dotted line shows the distance between the β6 and β84 alpha carbons:



The distance between the alpha carbons of β6-β84, β6-β85, β6-β88 were compared for four different Hb states: R-state HbCO (does not enter polymer), HbNO T-state, deoxy T-state Hb and deoxy T-state Hb with two CO ligands bound on the Beta units. There appears to be a stronger similarity between T-state deoxy Hb and HbCO T-state, than HbNO T-state and Ddeoxy T-state. This is a simple comparison, yet it still shows a structural relation. To further explore these structures it would be necessary to build a simulation that would track the alpha carbons positions while the protein was allowed to move in solution.

