

Microphotolysis Studies Illuminate Sickle Hemoglobin Polymerization
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Sickle cell disease arises from a genetic substitution on the surface of the normally highly-soluble hemoglobin molecule. The replacement of a negatively charged glutamic acid by a hydrophobic valine allows deoxygenated hemoglobin to form long, multi-stranded fibers that rigidify the cell. Because hemoglobin is so well studied, the investigation of this assembly process can act as a paradigm for many assembly diseases, such as Alzheimer's disease, mad cow disease, and Huntington's disease. The reaction can also be controlled by light, since carbon monoxide acts as a surrogate for oxygen to prevent assembly, but can be released efficiently by readily available laser sources. We illustrate here some of the results generated by use of this approach in a microscopic arrangement.

Nucleation rates can be determined by dividing the photolytic beam (provided by the 488 nm line of an Argon ion laser) into several hundred spots which illuminate areas small enough that only one nucleus forms in each. Small angle scattered laser light then acts as a diagnostic for the appearance of polymers. The distribution of times for first observing polymerization gives nucleation rates directly. Despite the high flux, the microscopic system dissipates heat effectively.

The enhancing effects of cell membranes on homogeneous nucleation of polymers can be determined by using the nucleation approach above on a solution which contains membrane fragments and free hemoglobin solution. The system permits a built in control experiment by use of absorption spectroscopy to identify areas of hemoglobin with and without membranes, so that the precision of the comparison is at least an order of magnitude improved over what was available previously, and has settled a long controversy over the role of the membrane.

The rigidity of fibers can be determined by "dragging" the photolytic region across the observed region at low levels of illumination and high magnification (100x objectives) thereby elongating fibers without further nucleation. A feedback system coupled to micrometer controls on the illuminating laser optics establishes a growth velocity. An abrupt turn in the dragging direction then determines how likely a polymer is to be bent in the particular direction.