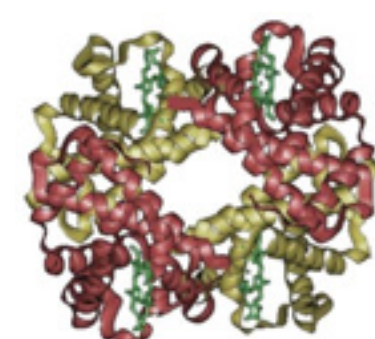


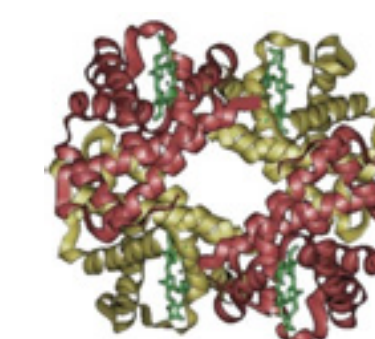
# ADVANCING REGULATORY SCIENCE

## Identification of oxidative reactions of hemoglobin (Hb) with membranes of sickled red blood cells that produce microparticles containing highly reactive Hb

The abnormal oxidative environment of red blood cells in sickle cell disease (SCD) produces highly reactive forms of SCD hemoglobin (HbS) that either create HbS-containing microparticles or are degraded in the proteasome. Interrupting these oxidative reactions might lessen the intensity of disease symptoms in patients with SCD.



### Hemoglobin oxidation-dependent reactions promote interactions with band 3 and oxidative changes in sickle cell-derived microparticles

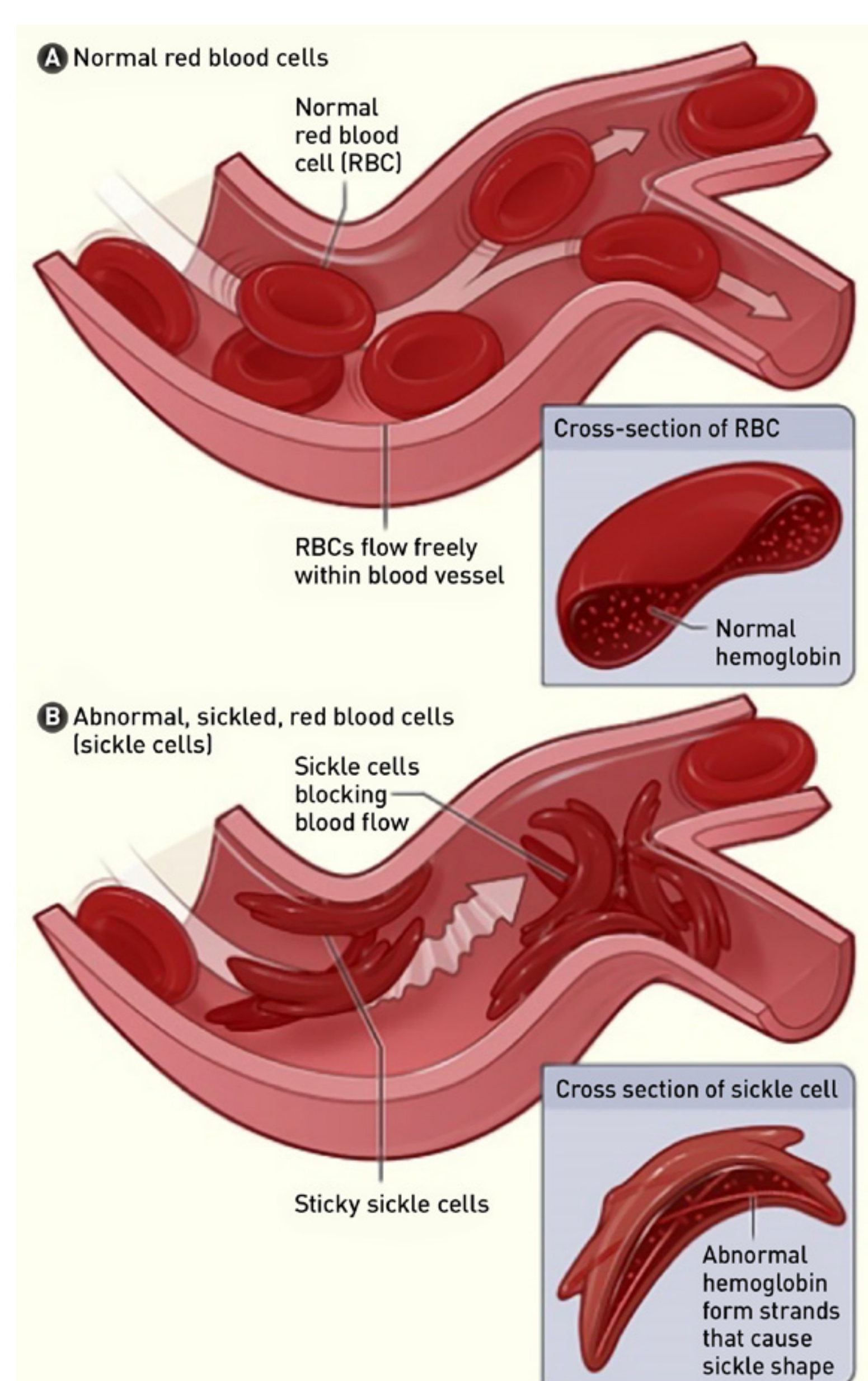


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Credit: National Heart, Lung, and Blood Institute

### HbS contributes to the symptoms of SCD

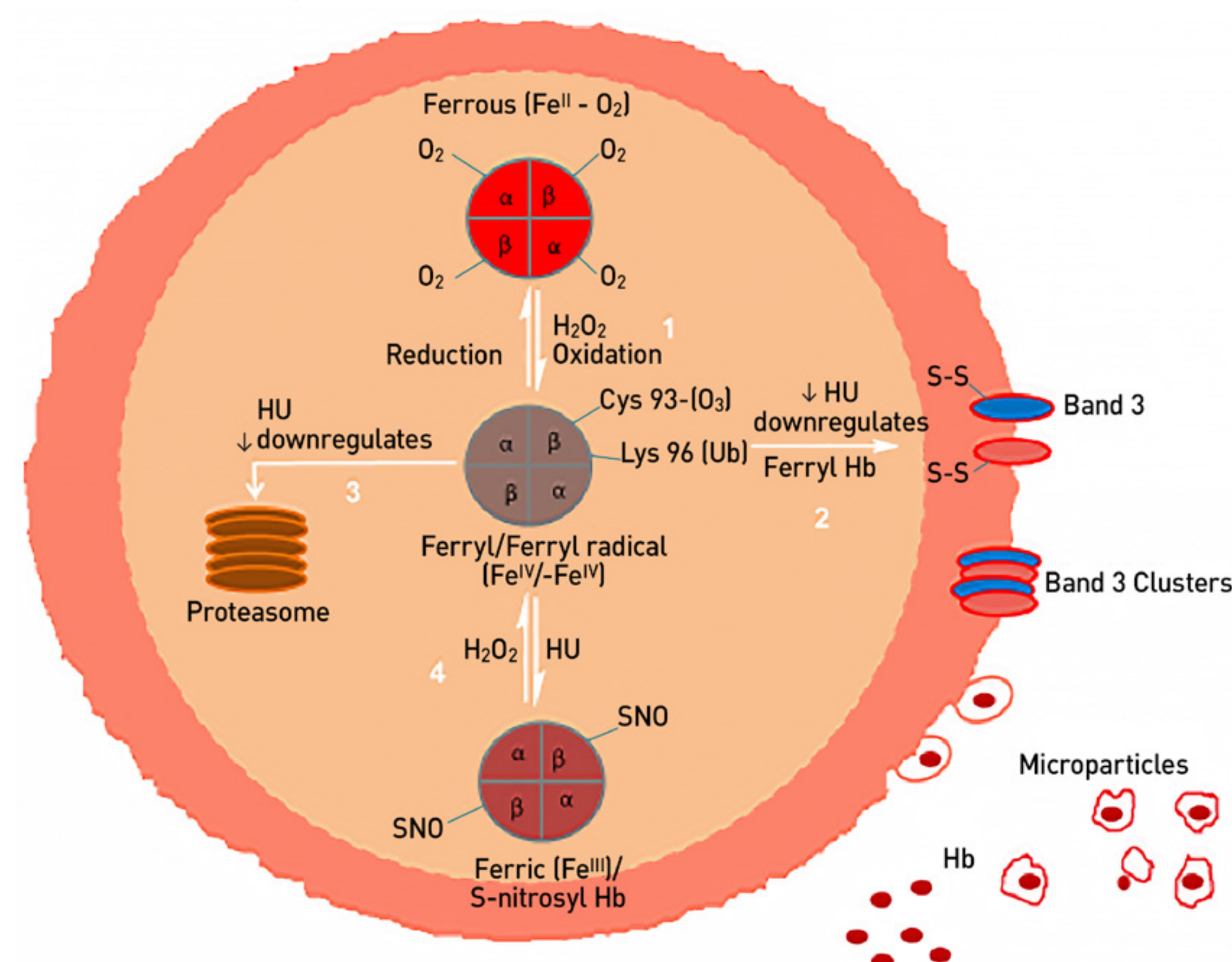
- The disruption of blood flow caused by sickled red blood cells reduces oxygen delivery and causes pain and damage to organs and tissues.
- Sickled cells eventually break apart, releasing highly reactive HbS, which damages blood vessels.

### Microparticles (MPs) are an additional source of chemically reactive free HbS within the blood vessels

- MPs are small packets of highly chemically reactive HbS that bud off from the membrane of sickled red blood cells.
- MPs break up in the bloodstream, releasing HbS, which damages blood vessels and reduces the flow of blood to organs and tissues.

### FDA scientists discovered how the abnormal, strongly oxidative internal environment of sickled red blood cells gives rise to MPs and triggers degradation of HbS by the proteasome

Oxidative Changes to HbS in the Sickled Red Blood Cell



### In vitro studies of SCD mouse HbS clarify the oxidative

- Ferrous Hb (Fe<sup>II</sup>, top Hb in figure) spontaneously oxidizes to Ferric Hb (Fe<sup>III</sup>, bottom Hb).
- In the presence of H<sub>2</sub>O<sub>2</sub> and compromised antioxidative mechanisms, the initially spontaneous oxidation of Ferric Hb continues, forming Ferryl Hb (Fe<sup>IV</sup>) (middle Hb).
- Ferryl Hb undergoes oxidative changes, especially:
  - transformation to higher oxidative forms that was markedly increased in MPs generated from SCD mice in a pattern similar to their parent RBCs
  - irreversible oxidation of the Hb amino acid, βCys93
  - attachment of the protein ubiquitin to Hb lysine amino acids readied for degradation.
- Ferryl Hb with oxidized βCys93 and ubiquitinated lysines can undergo either of two subsequent reactions:
  - oxidatively target the portion of the cell membrane called band 3, which can lead to microparticle formation.
  - become denatured and be degraded in the proteasome
- Endothelial cells incubated with MPs underwent mitochondrial dysfunction and eventually apoptotic cell death, demonstrating the destructive effects of MPs.

### Therapeutic implication of FDA findings

- Hydroxyurea acts as an antioxidant by adding its nitric oxide group to a cysteine thiol of the Hb, forming SNO-Hb at βCys93 (S-nitrosylation, middle Hb). This minimizes the consequences of the oxidative processes, which suggests that it might reduce symptoms in patients with SCD.

The identification by FDA scientists of the oxidative reactions that HbS undergoes in sickled cells clarifies the chemical reactions that determine the two alternative fates of this molecule: 1) formation of microparticles; 2) designation for degradation by the ubiquitin-proteasome system. These findings suggest potential therapeutic targets aimed at reducing SCD symptoms caused by degradation of HbS and the release of

HbS into the bloodstream from microparticles. Hydroxyurea, an FDA-approved drug, is known to interfere with specific steps in these oxidative processes. These studies are also relevant to the ongoing evaluation of encapsulated hemoglobin as an oxygen therapeutics or blood substitute.