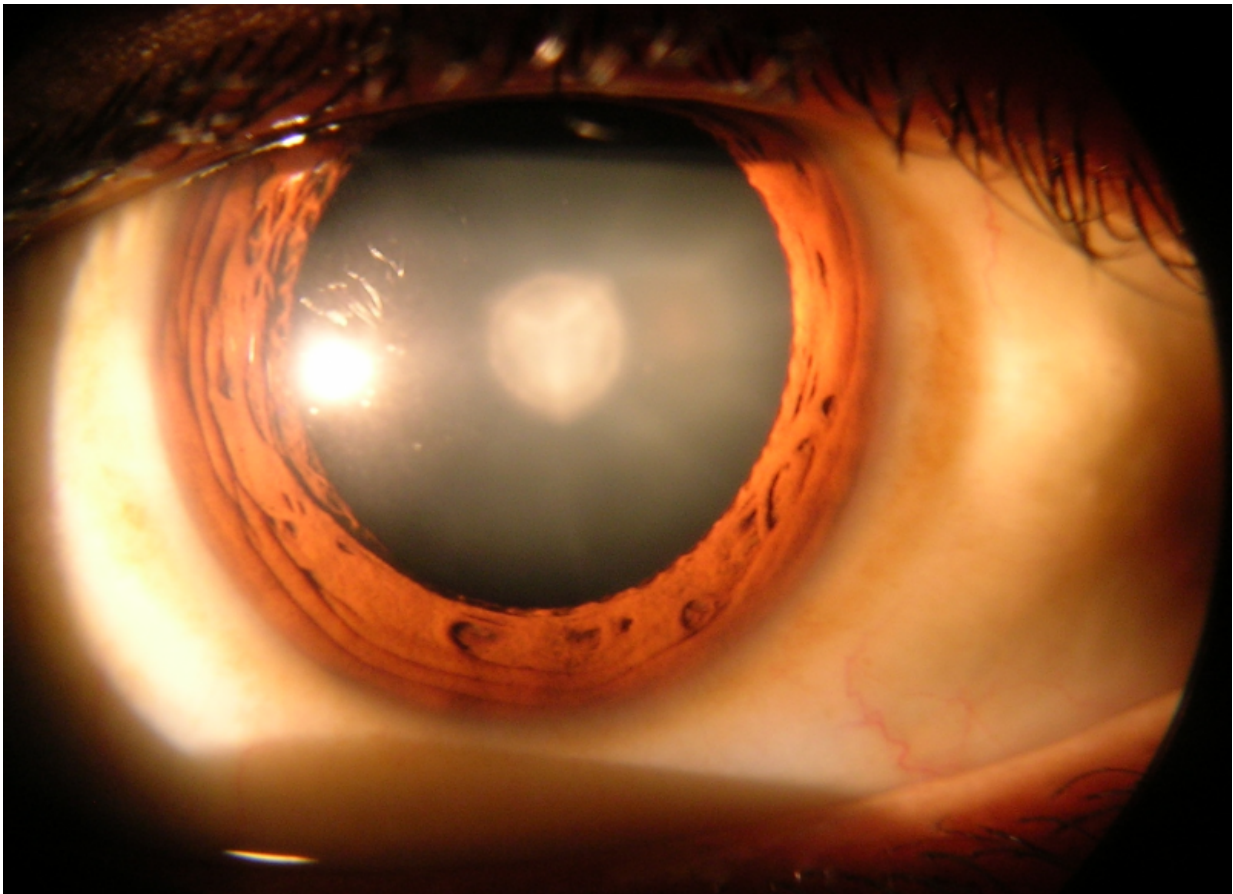


# How changing labs revealed a chemical reaction key to cataract formation

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Cataract in human eye. Credit: Wikipedia.

Researchers working to understand the biochemistry of cataract formation have made a surprising finding: A protein that was long

believed to be inert actually has an important chemical function that protects the lens of the eye from cataract formation.

The lens is made up of cells packed with structural proteins called crystallins. Crystallins within each lens cell form a protein-dense gel, and the gel's optical properties—like its transparency and the way it refracts light—help focus light onto the retina.

But when crystallin proteins clump together, they are no longer so transparent. If enough of the proteins go from their usual water-soluble, densely packed organization to clumpy aggregates, they begin to scatter incoming light, forming cloudy deposits known as cataracts.

According to Harvard postdoctoral fellow Eugene Serebryany, lead author on a recent study in the *Journal of Biological Chemistry*, for a long time researchers believed that crystallin proteins were chemically inert. That is, except for aggregating as an individual ages, the proteins were not believed to interact much with fellow proteins. Serebryany said, "This was the model: (crystallin's) real function is to remain monomeric and transparent and avoid aggregating for as long as possible."

Back when he was a graduate student at MIT, Serebryany used a mutant form of the lens protein gamma-crystallin to mimic UV damage to the protein. While studying how that mutation leads crystallin to aggregate into clumps, Serebryany found something surprising: The mutant was more likely to aggregate if wild-type, or undamaged, protein was also present.

Harvard professor Eugene Shakhnovich, who collaborated with Serebryany and his graduate adviser, Jonathan King, on the earlier studies, described the finding as "a fairly striking phenomenon" and explained: "If you had these damaged proteins in a test tube, they would not aggregate for a while. If you had the wild-type protein, it would not

aggregate forever. But then, when you mix the two, you see rapid and precipitous aggregation."

In other words, the healthy version of a protein everyone had thought was inert was somehow causing a slightly damaged version to get much worse—and fast.

When Serebryany graduated, Shakhnovich hired him to continue working to understand how a supposedly inactive protein could cause this effect. Serebryany said, "The first thing I had to do was basically try to get the experiments from my Ph.D. lab to work in this (new) lab."

"They're just two stops apart on the subway!" Shakhnovich joked.

But, for some reason, Serebryany had trouble replicating the results. "It's a different place, it's a different set of instruments, a slightly different set of procedures. You see where this is going," he said. "All of a sudden, experiments that were highly reproducible before were giving a lot of variability."

Indeed, in the Harvard lab sometimes the wild-type crystallin caused mutant crystallin to aggregate, and sometimes it didn't. The scientists were mystified.

Serebryany said, "Obviously, if there is suddenly variability, there is a hidden variable that we didn't see before." He set up a series of experiments trying to pinpoint that variable.

A close comparison of the molecular weights of the wild-type protein that caused the mutant to clump and the protein that didn't revealed a difference equivalent to the weight of two hydrogen atoms. This gave the researchers a hint that the redox state—whether two sulfur atoms within a protein molecule were bound to one another instead of to

hydrogen atoms—might make a difference.

"By carrying out isotopically resolved mass spectrometry experiments, we got more than we bargained for," Serebryany explained. "Not only did the aggregation-prone mutant acquire one internal disulfide bond per molecule during the aggregation reaction, but the aggregation-promoting wild-type protein lost its disulfide at the same time."

By mutating the sulfur-containing cysteine [amino acid residues](#) one by one to non-sulfur-containing residues, Serebryany found that two cysteine amino acids close together on the surface of gamma-d-crystallin acted as a kind of switch. When the two bound, making a structure called a disulfide bond, crystallin seemed to be able to push damaged fellow molecules toward aggregation. When the two cysteines were not bound, each instead took on a hydrogen atom, explaining the protein's tiny change in mass. Under that condition, wild-type crystallin was inert.

But how could one bond between amino acids on the surface of this protein make it drive other proteins to aggregate?

Using biophysical and biochemical techniques, the team found that although the disulfide bond forms easily, it also introduces strain into the protein's structure. This made each protein molecule likely to pass along the disulfide bond to a nearby molecule of the protein, receiving two protons in return. In this way the disulfide bond could be constantly passed around among crystallin protein molecules. The authors compared the process to passing a hot potato.

Given a whole population of healthy, undamaged crystallin proteins, this process could go on indefinitely. But if one protein was already a little damaged, the authors showed, it caught the hot potato with a different set of cysteines, which were less able to pass it on. This drove the damaged protein to clump up. The authors' previous work revealed that

mutations mimicking damage caused by UV changed the stability of the [protein](#), making it more floppy, and therefore more likely to acquire the conformation that exposes new cysteines that could catch the hot potato.

This helps us understand cataract formation. According to Shakhnovich, the team is working on peptide treatments that might keep the "hot potato" from reaching damaged proteins. Serebryany hopes such peptides "could actually soak up some of those disulfides and delay the time that it takes to form the more aggregation-prone species." That could lead to slower cataract formation for patients.

**More information:** Eugene Serebryany et al, Dynamic disulfide exchange in a crystallin protein in the human eye lens promotes cataract-associated aggregation, *Journal of Biological Chemistry* (2018). [DOI: 10.1074/jbc.RA118.004551](#)

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