Researchers develop technique for measuring stressed molecules in cells

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Biophysicists at the University of Pennsylvania have helped develop a new technique for studying how proteins respond to physical stress and have applied it to better understand the stability-granting structures in normal and mutated red blood cells.

The research was conducted by Dennis Discher and Christine Krieger in the Molecular and Cell Biophysics Lab in Penn's School of Engineering and Applied Science, along with researchers from the New York Blood Center and the Wistar Institute.

Discher's research was published online in the journal Proceedings of the National Academy of Sciences.

In stark contrast with much of the architecture people interact with every day, the internal architecture of the human body is predominantly soft. Other than bones, all of the organs, tissues and structures in the body are pliable and flexible and need to be that way in order to work.

The Discher lab's research aims to understand what keeps these flexible structures stable, especially when they are under constant physical stress. Discher selected red blood cells as a model for this stress, as they make a complete lap of the turbulent circulatory system every few minutes but survive for months.

"Red blood cells are disks, and they have proteins right below the membrane that give it resilience, like a car tire," Discher said. "The cells

are filled with hemoglobin like the tires are filled with air, but where the rubber meets the road is the exterior."

To measure stress in that membrane on an atomic level, the Discher team needed a way to track changes to the shape of those supporting proteins. They found an ideal proxy for that stress in the amino acid cysteine.

Proteins are long chain of amino acids that are tightly folded in on themselves. The order and chemical properties of the acids determine the locations of the folds, which in turn determine the function of the protein. Cysteine is "hydrophobic"; it interacts poorly with water and so it is usually on the inside of a protein. And because stress changes the shape of these folded proteins, Discher reasoned that measuring the degree to which cysteine is exposed would in effect measure how stressed the protein and cells containing it are.

Discher's team simulated the shear forces originating from the beating heart, which forcefully pumps blood and ultimately pulls apart the folds that keep cysteine on the inside of proteins at the red blood cell membrane, allowing it to bind with a fluorescent marker dye. The team could visually confirm that more stressed cells were more fluorescent under the microscope but actually tested the levels of marked cysteine using mass spectrometry.

"Just like a polymer engineer designing a tire, we're looking at the relationship between the chemical makeup and the physical stability of the structure and how it performs," Discher said. "We can use this technique to look at the relationship between structure, flexibility and function."

Investigating the structural elements of blood cells could pave the way to breakthroughs for human health.

"How long can blood be stored? Why are there no good blood substitutes? There are a lot of things we don't understand about the forces cells can sustain before fragmenting and falling apart, especially when we consider age and mutations," he said.

The Discher team studied the mutated blood cells that result in disorders known as elliptocytosis; cells are elliptical, rather than round, and therefore have shorter functional lifespans. These elliptical cells are often missing a chemical "rivet" that anchors the support proteins to the outer membrane, which means that stress causes them to "disassociate," or disconnect, rather than unfold.

That kind of structural change is crippling to the function of anatomical structures like blood cells. The flexibility provided by unfolding is therefore key to their overall stability.

"At least for this cell, the first mechanism of response is to unfold proteins and keep the interactions between proteins the same," Discher said. "That constant back and forth with unfolding within these cells as the cells flow and distort while in the blood stream, allows their architecture to be maintained."

Discher and his colleagues plan to use their cysteine-mass-spectometry technique to investigate the role of softness and flexibility in responding to stress in other biological systems, particularly stem cells, and to better understand why those traits are intrinsic to life on this planet.

Provided by University of Pennsylvania

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