A better understanding of the von Willebrand Factor's A2 domain

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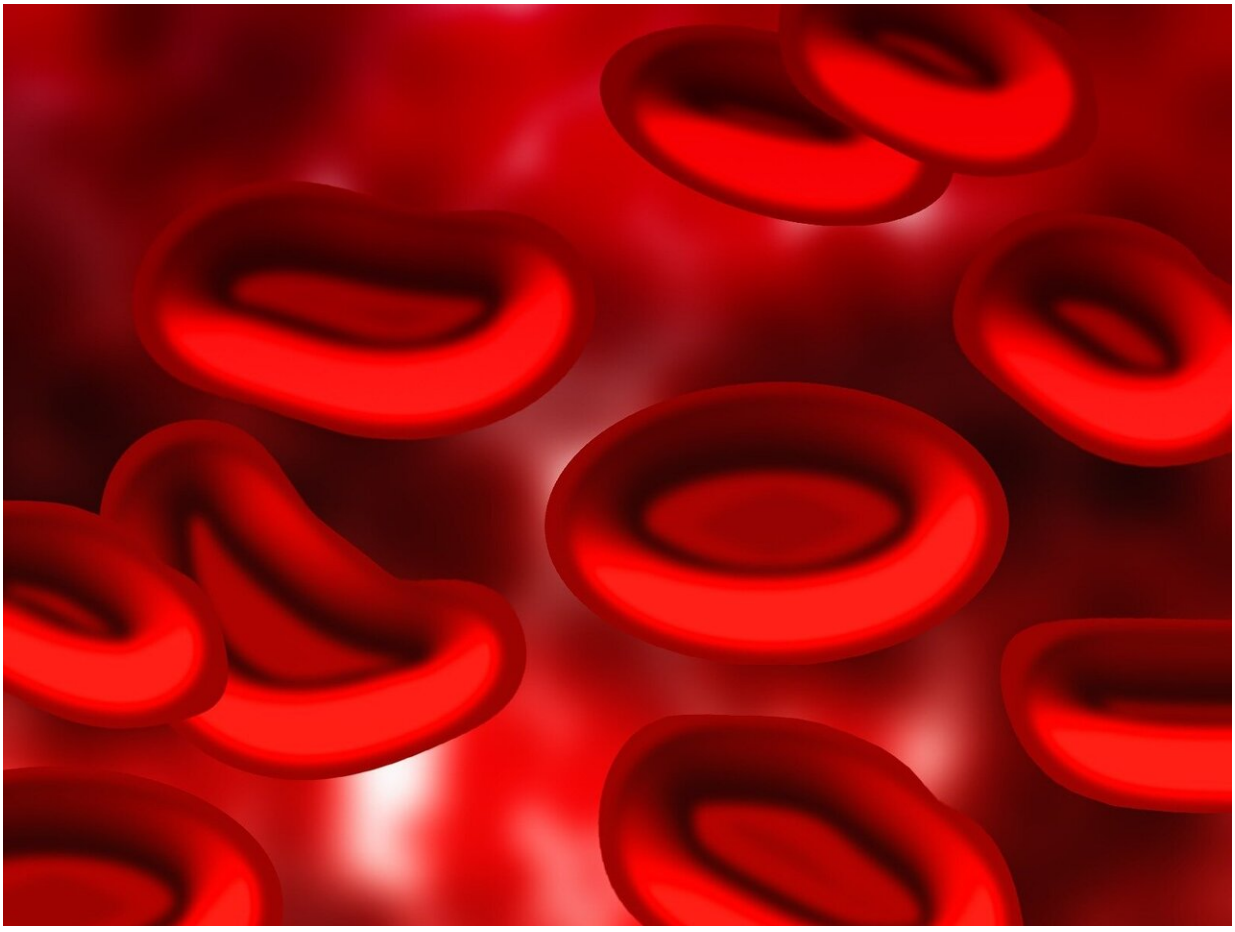

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Under normal, healthy circulatory conditions, the von Willebrand Factor

(vWF) keeps to itself. The large and mysterious multimeric glycoprotein moves through the blood, balled up tightly, its reaction sites unexposed. But when significant bleeding occurs, it springs into action, initiating the clotting process.

When it works properly, vWF helps stop bleeding and saves lives. However, about one to two percent of the world's population is affected by vWF mutations that result in bleeding disorders. For those with more rare, severe forms, a very expensive treatment in the form of blood plasma replacement may be required.

On the other hand, if vWF activates where it isn't needed, it can trigger a stroke or heart attack.

A better understanding of how vWF functions could result in drugs that replace it in those who lack it. It could also lead to the development of new drugs or drug carriers that mimic the protein's behavior for more effective drug delivery.

With that in mind, a team of Lehigh University researchers is working to characterize this mysterious protein. In a recent paper published in *Biophysical Journal*, they advance experimental data for the shear-induced extensional response of vWF, using a microfluidic device and fluorescence microscopy. Further, they use the results from tandem Brownian dynamics simulations of an experimentally parameterized coarse-grained VWF model to help explain some of their central observations from experiment. This work elucidates further details of the flow-induced biomechanical response behaviors of tethered VWF and demonstrates the power and capabilities of increasingly complex coarse-grained models employed in tandem with experiment.

The paper, called "Shear-Induced Extensional Response Behaviors of Tethered von Willebrand Factor," is authored by Xuanhong Cheng,

associate professor of materials science and engineering; Alparslan Oztekin, professor of mechanical engineering and mechanics; Edmund Webb III , associate professor of mechanical engineering and mechanics; and Frank Zhang, associate professor of bioengineering and mechanical engineering and mechanics; as well as doctoral students Michael Morabito and Yi Wang.

vWF at Work

At the location of a minor wound, platelets adhere to the collagen-exposed sites near the hole in the blood vessel wall on their own and act as a plug, effectively stopping the bleeding. Rapid blood flow, however, makes it difficult for platelets to do this. Fortunately, the von Willebrand Factor recognizes this rapid blood flow and activates: "It's a flow-mechanics-activated event, if you will," explains Webb.

The globular molecule unfolds like a Slinky, stretching to 10 times its original size and exposing its reaction sites. It clings to the broken blood vessel wall, where exposed collagen—the structural protein of the blood vessel wall—attracts platelets. vWF then captures platelets from blood as they flow by, acting like a bridge between the collagen and the platelets.

Although the biological function of vWF has long been recognized by scientists, not much is known about the specifics of how vWF functions, particularly under flow conditions.

"Most proteins in blood functions are executed through biochemical reactions," says Cheng. "This protein [vWF] also requires some biochemical reaction for its function, so it needs to grab onto platelets, grab onto collagen—those are biochemical reactions. At the same time, vWF relies on mechanical stimulation to execute the biochemical function, and that part is not very well known. That's what we're trying to study."

Adds Webb: "Some of the data that's coming out of our group but also from other groups indicates that those biochemical reactions are somehow abetted by there being some sort of a tension, a pulling force. So even the biochemical reactions appear to be somewhat mechanically mediated. Again, it was understood that there was this change from a compact, almost ball-like shape, if you will, to this long, stringy thing. But very recently people have been indicating it's not just that. For this chemical site to be active, you have to be pulling it, you have to be in a bit of tension, locally. So it's a really fascinating system."

Unraveling A2

The von Willebrand Factor is a particularly large protein made up of many monomers, or molecules that can be bonded to other identical molecules to form a polymer. Within each monomer of vWF are different domains: A, C and D. Each domain and each of its respective subdomains has its own role, and many of these roles are yet unknown. The A1 domain, for example, binds vWF to platelets. A3 binds vWF to collagen. The A2 domain unfolds to expose the protein's reaction sites, and, when fully opened, exposes a site that permits scission of the vWF molecule down to size. Members of the team have focused on the A2 domain, in particular.

"Understanding that domain and how it interacts with the flow, I think, is the best contribution from our group," says Oztekin.

Each member of the team plays a particular role. Cheng, Zhang and their graduate students work on the experimental side of the project; Oztekin, Webb and their graduate students focus on simulation. Each team's results inform the work of the other.

Zhang, who has been studying vWF for years and brought the project to Lehigh, specializes in single-molecule force spectroscopy and

mechanosensing, or how cells respond to mechanical stimuli. He uses a specialized tool called optical tweezers, which utilizes a focused laser beam to apply force to objects as small as a single atom.

"Optical tweezers can grab tiny objects," Zhang explains. "We can grab the vWF and at the same time we apply force to see how the protein changes shape, to see how the proteins are activated when there's a mechanical perturbation or a mechanical force."

Cheng develops microfluidic devices, which have a small diameter and can be used to analyze live bioparticles. She and her team make very small channels similar to the geometry of blood vessels—on the order of 10 micron in height, a few millimeters in length and width—so they can mimic the flow condition that vWF encounters in the body. They tag the vWF molecule fluorescently and use a confocal microscope to capture video and still images of the molecule as it flows through the channel at different rates.

"When we talk about this protein under normal flow, it's one conformation, and then when it's exposed to certain abnormal flow patterns, you'll have a different conformation," Cheng explains. "So we're trying to characterize or replicate that process in an in vitro system, trying to observe how this protein changes conformation under different flow patterns. And then, if we have mutants versus normal protein, how would they behave differently?"

Doctoral student Yi Wang works with Cheng on the microfluidics channel in which they can observe the vWF molecule unraveling and folding back again in real time under a microscope. To do so, they must create an environment that mimics the shear rate, or change in blood flow velocity, found in the body.

"Because we are using a pretty high shear rate to be comparable to the

physiological environment, and because of the limited moving speed of a microscope lens that images the molecule, it's actually pretty challenging to capture the movement of a molecule if it's moving," says Wang.

To solve that problem, the team binds one side of the molecule to the surface of the channel to immobilize it as they apply shear force. They have successfully captured the unfolding phenomenon on video.

"If it [the molecule] is bound too tight, it will just stay there [and not unfold]," says Wang. "If it is too loose, everything will be flushed away. So I was very excited when we got the sweet spot of binding it right there on the surface and so it can unfold and fold back."

More information: Yi Wang et al, Shear-Induced Extensional Response Behaviors of Tethered von Willebrand Factor, *Biophysical Journal* (2019). DOI: 10.1016/j.bpj.2019.04.025

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