

# Judit Puskas

A polymer scientist explores and exploits the heterogeneity of natural biopolymers.

There is often no better rallying cry for scientific inquiry than the expectation that a thing can't be done. Judit Puskas learned this firsthand when she left her native Hungary and arrived at the University of Akron to study polymer synthesis as a postdoctoral associate with Joseph Kennedy. The lab was focused on rubber chemistry, in particular on developing a living carbocationic polymerization—marrying unstable carbocationic intermediates with the stable 'living' technique in which polymer termination does not occur. Puskas recalls, "At the time, people said that this was not possible. It was theoretically impossible." Yet she and her colleagues succeeded, and a connection forged at a subsequent conference led first to the demonstration that one of their newly created polymers had outstanding properties for biomaterials applications and then to a career devoted to biologically inspired and biologically derived polymers.

Synthetic polymers are often created in a controlled but random fashion, governed by rules of statistics, reaction mechanisms and experimental conditions. As a result, polymers typically have significant heterogeneity in their structure. However, even though the construction of synthetic polymers appears messy in comparison to the highly regulated biosynthesis of proteins and DNA, the properties of synthetic polymers can still be significantly dependent on variations in polymer length and composition and even on the identity of single monomers or functional groups within the chains. Thus, manipulating polymer structure through the development of new catalysts and strategies is a major goal in polymer science. Biopolymers such as polylysine, starch and polyisoprene (natural rubber) occupy some middle ground between synthetic polymers and informational polymers like DNA; for example, their architecture may be precisely controlled, but their length may be variable. However, many of the details of biopolymer synthesis, as well as the functional consequences of their heterogeneity, remain unclear. As a result, duplicating or altering their structures has proven difficult.

In her continued work on rubber chemistry at Bayer, Inc., Puskas was particularly intrigued by the discrepancy



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between the properties of natural rubber and synthetic polyisoprene. Earlier chemistry had determined the structure of natural rubber as nearly 100% *cis*-1,4-polyisoprene, and synthetic methods successfully recreated this structure, yet the two polymers did not behave the same. Puskas notes, "In spite of years of history, we still cannot make a synthetic equivalent of natural rubber." As there are some applications for which synthetic rubber simply cannot substitute for natural rubber, new insights are desperately needed. Thus, in launching an academic career at the University of Western Ontario, and now at the University of Akron, she and her colleagues have considered several differences between the natural and synthetic counterparts that may be important for function. For example, in natural rubber, though the bulk of the material is in a *cis* configuration, the first few units are in a *trans* configuration. In contrast, synthetic rubber is almost completely *cis*. Puskas wonders, "Does that matter? Does that do anything? We don't know." Although many scientists have pondered this and related issues, Puskas believes she has identified a new clue. In particular, rubber contains a gel that must be purified away to create a soluble polymer fraction. When Puskas and her colleagues injected the soluble material on a size-exclusion chromatography system, she remembers "the first thing I saw was that 70% of the sample was low-molecular weight—below 4,000 g mol<sup>-1</sup>—and the other 30% had a molecular weight of one million." Though no one had previously emphasized these small pieces, Puskas hopes they may prove to be the critical difference between natural and synthetic rubber.

One of the challenges of working on this topic is that the natural rubber enzyme, *cis*-prenyltransferase, is a membrane protein. Moreover, it has not been isolated from its associated rubber particle, has only limited sequence information available and, when purified, is only functional for a few hours at room temperature. Thus the next step in understanding the function and significance of this new low-molecular weight fraction is simply, as Puskas says, "to separate it and put it back with synthetic rubber and see what happens." This trial-and-error approach categorizes much of the enzyme chemistry going on in the lab as, for many of these systems, Puskas explains, "we still don't understand the mechanism. You know it works, but you don't know why." For these kinds of experiments, Puskas emphasizes that scientists must approach the data with no preconceptions. In the case of the low-molecular weight rubber, she cautions that "people just discarded the low-molecular weight fraction, perhaps because they made the judgment that the small-size fractions were not important."

One way Puskas is trying to bring some clarity to the field is through the recent founding of a new company, Biopolymers International, Inc., which provides synthetic samples of a range of biopolymers. This work is a natural extension of the projects in her lab, which have expanded from her early interests and now use enzymes in a variety of polymer applications, both to exploit their natural functions and inherent specificity and to gain insight into their inner workings. She is perhaps most enthusiastic about her efforts to explore the reaction pathway of *cis*-prenyltransferase, in which her studies of reaction rates, substrate and cosubstrate promiscuity and polymeric intermediates, as well as a careful analysis of available evidence, have led to the hypothesis that the enzyme is using a living carbocationic polymerization mechanism similar to the one she and her colleagues developed more than two decades ago. Perhaps it should be no surprise that something deemed impossible by so many had been working in nature all along. ■

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