Getting to the point (mutations) in re-engineering biofuel-producing bacterial enzymes

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Converting fibrous plant waste, like corn stalks and wood shavings, into fermentable simple sugars for the production of biofuel is no simple process. Bacteria must break down tough leaves, stems and other cellulosic matter resistant to degradation to turn them into usable energy.

Helping bacteria become more efficient in this process could result in more affordable biofuels for our gas tanks and sustainable products such as bioplastics. One way to achieve this goal is to re-engineer the bacterial enzyme complexes, called cellulosomes, which serve as catalysts in the degradation process.

In an effort to produce these so-called designer cellulosomes, the international research consortium CellulosomePlus is developing methods to enhance the efficiency of this complex engineering process to make it economically feasible and effective. Consortium researchers from Spain, Poland and Ireland reported their findings for one method recently in *The Journal of Chemical Physics*.

The researchers focused on the *Clostridium thermocellum* (*C. thermocellum*) bacterium. Capable of directly converting cellulose into ethanol, especially at elevated temperatures, the bacterium has garnered much interest as an optimal biofuel catalyst.

Notably, *C. thermocellum* has a large cellulosome that degrades cellulose

through simultaneous action of many enzymes, mostly cellulases. The enzymes are parts of molecules called dockerins, which form non-covalent complexes with various protein domains called cohesin domains. These domains are connected segments of a scaffoldin, a large protein that serves as the cellulosome backbone. Cellulosome function requires the cohesins to be mechanically strong and the cellulases to be effective enzymes in transforming plant waste into sugar.

"One way to design a better cellulosome is to improve the mechanical stability of type-I cohesins and re-engineer the cellulase units," said Marek Cieplak, co-author of the paper who directs the Laboratory of Biological Physics at the Institute of Physics, Polish Academy of Sciences.

The researchers targeted the c7A cohesin of *C. thermocellum* because it appears to be subjected to more intense mechanical stress than other cohesins and is exceptionally mechanically stable.

A computational method was developed to identify which point mutations, single amino acid replacements, would lead to stronger mechanical stability as well as higher thermodynamic stability. Using all-atom computations, the researchers identified the mutations by systematically replacing all amino acids with either alanine or phenylalanine.

"One interesting result is that the mutations have a non-obvious impact on the internal structure of the protein and thus on the stabilities," said Mateusz Chwastyk, also one of the publication's authors and Cieplak's former student.

Specifically, the changes in the contact map (the list of amino acid pairs that affect conformational dynamics) can be non-local. The best choices were tested experimentally. The researchers also found that adding

disulfide bonds, which form between different amino acids in a protein chain, made the protein extremely resistant to stretching.

"Our theoretical method seems to be a valid approximation for screening the effects of mutations in the mechanical and thermal stabilities of proteins," Cieplak said.

The proposed method is universal, can be applied to multiple mutations, and is currently used to explain properties of bacteria that live in extreme environments.

More information: Mateusz Chwastyk et al, Non-local effects of point mutations on the stability of a protein module, *The Journal of Chemical Physics* (2017). DOI: 10.1063/1.4999703

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