An evolutionary method for reprogramming proteases

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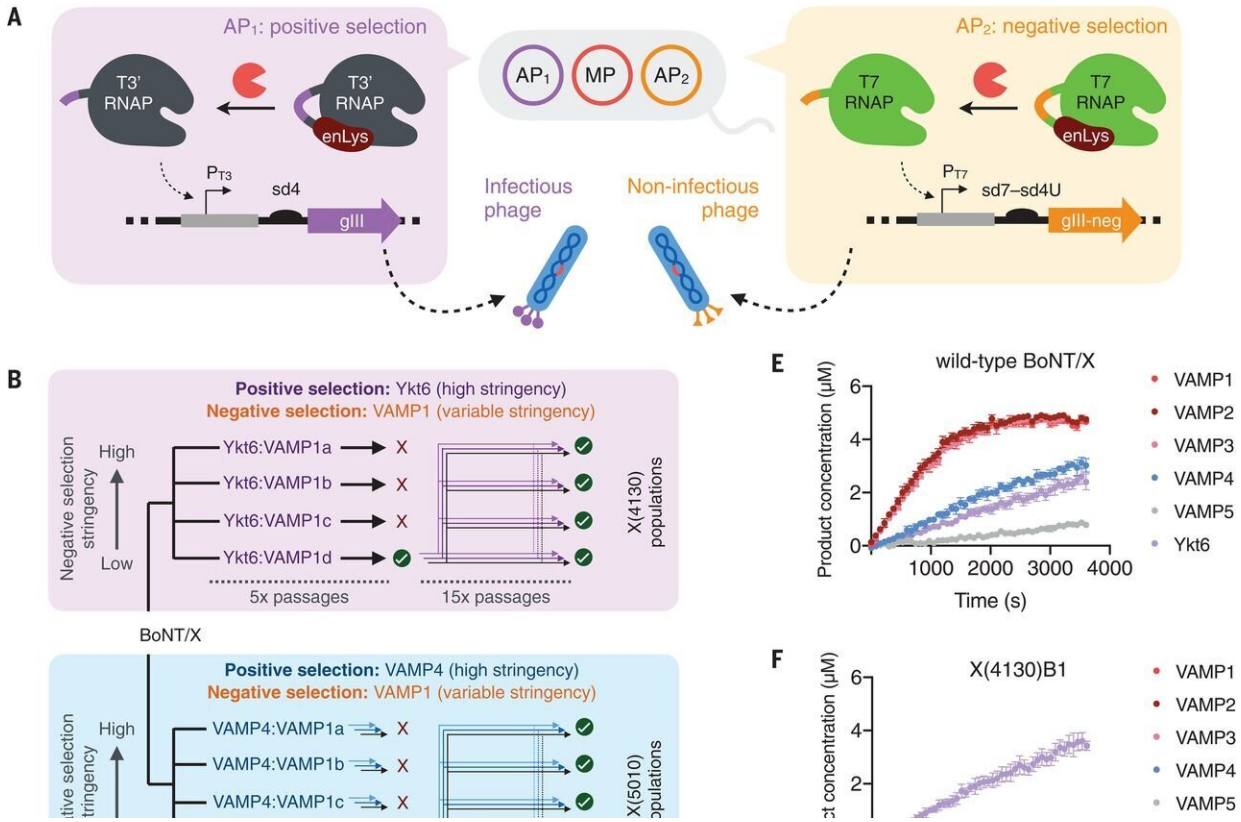

Phage-assisted protease positive and negative selection systems and their application to BoNT/X LC proteases. Credit: *Science* 19 Feb 2021: Vol. 371, Issue 6531, pp. 803-810, DOI: 10.1126/science.abf5972

A team of researchers affiliated with multiple institutions in the U.S. has developed an evolutionary method for reprogramming proteases. In their

paper published in the journal *Science*, the group describes how their technique works and how well it performed when tested. Pål Stenmark, with Stockholm University has published a Perspectives piece in the same journal issue outlining efforts to re-engineer Botox and how the work by the researchers in this new effort could apply to such research.

Proteases are types of enzymes that break down proteins and peptides. In many cases, they cut proteins at certain spots as parts of natural bodily processes. For several years, scientists have been looking at ways to program proteases to cut enzymes in desired ways. It is believed that doing so would allow for the development of a host of new therapies and biotechnological applications. In this new effort, the researchers have developed a means for doing just that using an evolutionary approach.

The work involved creating a variant of a phage-assisted, evolution-based process that allowed for both simultaneous positive and negative selection of certain protease activities over time. It relied on putting together the properties of a given protease domain with the infectivity aspects of a bacteriophage and then letting the protease evolve over several generations until desired characteristics appeared.

The effort involved working with botulinum neurotoxins, which are widely known to be toxic under some circumstances and therapeutic under others. They can present as botulism, for example, a well-known kind of food poisoning—but they are also used in another form to make Botox, which is used to treat migraines and as cosmetic therapies. The method they developed allowed them to first recognize desired substrates and then to select for them while selecting against others as an evolutionary process unfolded.

The researchers used their technique to reprogram a protease to make it cut one of the substrates of botulinum neurotoxin E in a way that they desired. They also showed that their technique could be used to

reprogram a protease to create entirely new substrates.

Stenmark notes that the work done by the team could have a major impact in some areas of medical research—reprogramming proteases on demand could lead to cleaving proteins involved in pain sensation, for example.

More information: Phage-assisted evolution of botulinum neurotoxin proteases with reprogrammed specificity, *Science* 19 Feb 2021: Vol. 371, Issue 6531, pp. 803-810, DOI: 10.1126/science.abf5972 , science.sciencemag.org/content/371/6531/803