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## Engineering high pH-resistant inteins by phage display

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Inteins are self-splicing proteins found in all three domains of life. Inteins have interesting properties that are useful in biotechnology and medicine. Inteins reside within host proteins and inhibit the protein activities. Protein splicing is required for activation of host protein function. Intein-mediated splicing happens rapidly under physiological conditions in uncontrollable fashion. It can be a very useful molecular tool if we could control splicing events by simply changing environment such as shifting the pH. Previously our lab developed an Escherichia coli-based selection system and we isolated several smaller inteins, a splicing improved intein, and a pH-controllable cleavage intein. However, these in vivo selection systems cannot be used to derive inteins that work under non-physiological conditions, because of the difficulty in modulating in vivo selection conditions. To select inteins that are active at high pH, we used a phage display. A randomly mutated mini-intein library inserted into a chitin-binding domain (ChBD) was displayed on the surface of T7 phage. The phages displaying ChBD disrupted by an inactive intein had low affinity for chitin, while the phages displaying an active intein had high affinity, since ChBD was reconstructed by splicing. We tested intein mutants in a splicing context by using green fluorescent protein (GFP) as an indicator of protein splicing. We thus isolated and characterized novel intein mutants with enhanced splicing activity at high pH. We also test intein mutants for cleavage activity using GFP as a reporter, in a different fusion context.