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Title:	Tailored Polyproteins Using Sequential Staple and Cut
Authors:	Garg, Surbhi (/jspui/browse?type=author&value=Garg%2C+Surbhi) Singaraju, G.S. (/jspui/browse?type=author&value=Singaraju%2C+G.S.) Yengkhom, S. (/jspui/browse?type=author&value=Yengkhom%2C+S.) Rakshit, S. (/jspui/browse?type=author&value=Rakshit%2C+S.)
Keywords:	Peptides and proteins Genetics Polyproteins Fluorescence
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Publisher:	American Chemical Society
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Abstract:	Polyproteins, individual protein units joined covalently in tandem, have evolved as a promising tool for measuring the dynamic folding of biomacromolecules in single-molecule force spectroscopy. However, the synthetic routes to prepare polyproteins have been a bottleneck, and urge development of in vitro methods to knit individual protein units covalently into polyprotein. Employing two enzymes of orthogonal functionalities periodically in sequence, we synthesized monodispersed polyproteins on a solid surface. We used Sortase A (SrtA), the enzyme known for sequence specific transpeptidation, to staple protein units covalently through peptide bonds. Exploiting the sequence-specific peptide cleaving ability of TEV protease, we controlled the progress of the reaction to one attachment at a time. Finally, with unique design of the unit proteins we control the orientation of proteins in polyprotein. This simple conjugation has the potential to staple proteins with different functionalities and from different expression systems, in any number in the polyprotein and, above all, via irreversible peptide bonds. Multiple chimeric constructs can also be synthesized with interchangeable protein units.
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