NANO HIGHLIGHT
Inteins as Nanoswitches for Biotechnology: Linking Molecular Modeling with Physical and Genetic Methods

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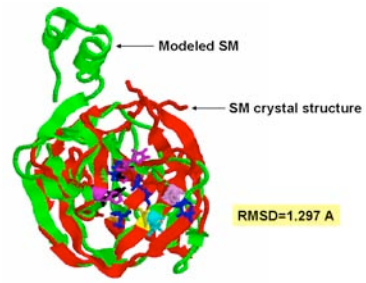
In this work, "inteins", a particularly unique class of enzymes, are being harnessed to develop highly efficient single-step affinity separation in bioprocessing and micro-fluidic devices and to design sensitive nanoswitches that could be used in sensors and devices for treatment of diseases [1]. The goals of this four-year cross-disciplinary, multi-investigator, multi-institutional research project are to determine the underlying principles of the cutting and joining reactions that occur during intein processing and to use this understanding to design a molecular nanoswitch that exhibits desirable properties for use in functional genomics and proteomics. In the first year, we have focused on the mechanism of cleavage and splicing of autocatalytic self-processing mini-inteins (mutants SM and CM from the *Mtu RecA* intein molecule) and orchestrated a visit of talented high-school seniors as part of an **Educational Outreach Initiative**. Three complementary synergistic approaches are being pursued: (i) A novel two-prong theoretical approach involving classical molecular dynamics and quantum *ab initio* calculations is used to unravel the mechanism of intein cleavage, (ii) genetic approaches to search for improved characteristics such as reduced size, faster cleavage rate and different triggers for cleavage, and (iii) modern biophysical methods to follow the global and secondary structural changes occurring within the intein during cleavage and splicing. Three new exciting research results have been obtained during the first year. **(i) Obtained the crystal structures of our two mini-inteins (for splicing, SM and for cleaving, CN).** P. Van Roey, a collaborator, has obtained the crystal structures of the mini-inteins. By comparing our predicted (homology mapping) structure with the crystal structure, we find that the locations of active site residues (which are also conserved in many canonical inteins) as well as the overall horseshoe-like structure of the protein are well reproduced in our predictions (**Fig. 1**). **(ii) Generated a number of smaller stable pH-controllable mini-inteins.** Using targeted mutagenesis, in combination with genetic screeening schemes, we have derived active, stable, pH-controllable mini-inteins from a 168-amino acid parent. These range in size from 135-139 amino acids. **(iii) Scaled-down single-step intein-based affinity purification in a fluidic device.** Proteins from *E.coli* lysates with pH-controllable CM and SM intein linkers and a chitin binding domain (CBD) were used to recover three different proteins in a rapid single-step affinity purification.

Fig. 1. Comparison of the molecular model and crystal structure of Mtu RecA intein (SM) (From P. Van Roey).



Active site residues are shown in sticks, and the root mean square distance (RMSD) is that of those residues.

References: [1] For further information about this project email Georges Belfort <belfog@rpi.edu>, Marlene Belfort <Marlene.Belfort@wadsworth.org>, Victoria Derbyshire <vicky.derbyshire@wadsworth.org>, Shekhar Garde <gardes@rpi.edu>, and Saroj Nayak <nayaks@rpi.edu>.