NANO HIGHLIGHT
Inteins as Nanoswitches for Biotechnology: Linking Molecular Modeling with Physical and Genetic Methods[1]

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PIs: Shekhar Garde (Co-PI)[1], Saroj Nayak (Co-PI)[2], and Georges Belfort[1] (PI)
[1]Department of Chemical Engineering and [2]Department of Physics
Rensselaer Polytechnic Institute, Troy NY 12180-3590
and
Patrick Van Roey, Marlene Belfort (Co-PI) and Victoria Derbyshire (Co-PI)
Wadsworth Center, New York State Department of Health, and
School of Public Health, State University of New York at Albany, Albany, NY 12201-2002

In this four-year cross-disciplinary, multi-investigator, multi-institutional research project we are planning to adapt an autocatalytic self-processing protein called an intein, as a nanoswitch. The goals of this research project are to determine the underlying principles of the splicing and cleavage (joining and cutting) reactions that occur during protein processing and to use this understanding to design a molecular nanoswitch that exhibits desirable properties for use in functional genomics and proteomics. This work will culminate in the use of the nanoswitch (mini-intein) to perform protein separation on a fluidics chip platform. The research approach involves combining molecular modeling with biophysical and genetic methods.

During the past three months, the following research highlights are noteworthy:

1. Using ***homology mapping***, we have constructed a proposed tertiary structure for our super-fast cleaving/splicing mini-intein[2], which we now plan to verify with the results from our collaborator's (Dr. Patrick Van Roey's) x-ray crystallographic structure analysis (#3. below).
2. To measure the ***conformational dynamics of cleavage*** with Fluorescence Resonance Energy Transfer (FRET), we have designed and ordered the clones for a tripart-fusion containing flanking exteins of a donor (cyan fluorescent protein, CFP) and an acceptor (yellow fluorescent protein) fluorophore[3].
3. The mini-intein has been ***crystallized*** (Dr. Patrick Van Roey) and X-ray diffraction patterns collected at the synchrotron at Brookhaven National Laboratories are being analyzed for structure determination and comparison with that obtained via homology mapping.
4. Using a ***silicon-etched micro-fluidics channel*** (27 □l; 1.5x0.6x30 mm) containing chitin affinity beads, both splicing and cleavage of the intein fused to a toxic model protein (ITEV-1) was demonstrated, to yield active products[4].

References

[1]. For further information about this project link to < http://www.rpi.edu/dept/chem-eng/WWW/faculty/belfort/> or email <belfog@rpi.edu>.
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