

# CLASSE Seminar

## Low-resolution shape determination of large multi-subunit complexes of the restriction-modification system EcoR124I

September 14, 2009, MONDAY

1:00 p.m.

3rd Fl Wilson Commons

Refreshments served

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NOTE: SPECIAL  
DATE AND TIME



**Abstract:** Type I restriction-modification (R-M) systems are comprised of three genes HsdS, HsdM and HsdR. The R-M system EcoR124I, is composed of two multi-subunit complexes, the 162 kDa methyltransferase (MTase), responsible for methylation of DNA and the 400 kDa restriction endonuclease (ENase), responsible for DNA cleavage. I will describe our use of small-angle neutron scattering complemented by other low-resolution techniques such as analytical ultracentrifugation and high-resolution techniques such X-ray crystallography, to describe the subunit assembly and structural changes that occur when the methyltransferase is switched to an endonuclease through the addition of two R subunits. Small-angle neutron scattering data was collected at ILL, Grenoble. The resulting scattering profiles and distance distribution functions of the genetically engineered MTase were similar to those both for the wild-type MTase and related methyltransferase, M.AhdI<sup>1</sup>. Next, the restriction endonuclease R.EcoR124I, was reconstituted both as a R<sub>1</sub> and R<sub>2</sub> complex (having either one or two copies of the R subunit) and neutron scattering data was collected in the absence and presence of DNA. *Ab initio* methods have been used to determine the shape of R, which was used as a constraint for determining a high resolution model<sup>2</sup>. Furthermore, the *ab initio* model of R has assisted the determination of the position of each R subunit when bound to the MTase, to form the ENase. This has been achieved by using contrast variation, whereby, scattering measurements of ENase (composed of deuterated R and protonated MTase) were made in a range of D<sub>2</sub>O/H<sub>2</sub>O ratios. Finally, rigid-body modelling has been used to construct a high-resolution model of the location of each R subunit. This has revealed for the first time, how the endonuclease domains of each R subunit could cause single-stranded DNA breaks on each strand of foreign DNA from bacteriophage and viruses, thus protecting the host cell.

This abstract has been truncated, the full abstract can be seen at <http://jclub.chess.cornell.edu>

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Cornell Laboratory for Accelerator-based Sciences and Education is primarily supported by the National Science Foundation.