

Untangling the Structure of Lyme Disease

by Michaela Mann

The Department of Energy's National Synchrotron Light Source at Brookhaven National Laboratory helped researchers discover new information about the bacterium that causes Lyme disease. Their work may lead to an effective vaccine and new treatment protocols.

"It's the perfect stealth bacteria," says one frustrated physician. He's talking about *Borrelia burgdorferi*, the bacterium that causes Lyme disease. This illness, which is often mistaken for diseases ranging from multiple sclerosis to Lupus, can inflict excruciating headaches and muscle pain, affect the brain and nervous system, attack major organs, and inflame joints. Although there have been more than 100,000 reports of the tick-borne Lyme disease in the U.S. since 1982, researchers are still struggling to create vaccines and treatments that are effective against *B. burgdorferi*.

New findings may explain vaccine failure, suggest treatment approaches. Investigators are particularly pleased with two recent discoveries made using the Department of Energy's National Synchronous Light Source (NSLS) at Brookhaven National Laboratory. The uniquely refined images they were able to create demonstrated the bacterium changes its outer surface protein according to its host, and that different strains of the bacterium have different electrical charges, which may determine their ability to cause disease.

Outer surface, or peripheral, proteins do not penetrate the cell wall and are easily shed, but they are significant in determining a cell's capabilities—for example, how it attaches to other cells or survives in specific environments.

"These findings make it clear what direction the research should take," says **John Dunn**, a biologist and principal investigator at Brookhaven National Laboratory. "There's a lot of work left to do, but now we have a much better sense of where we should be looking."

Researchers from Brookhaven, Stony Brook University's School of Medicine, the University of Rochester medical Center, and Rutgers University reported their findings on the **OspC** structure in the March 1, 2001 edition of the *EMBO Journal*.

Altered surface proteins

As *B. burgdorferi* moves by tick bite from the gut of a tick to the bloodstream of a mammal, it suppresses one outer surface protein (an "Osp"), called **OspA**, and switches on another, called **OspC**. The switch is regulated, at least partly, by temperature. **OspA** is expressed at temperatures below 32 degrees C, and is synthesized in the 24 degrees C environment of the tick gut. Between 32 to 37 degrees C, the range for mammalian blood, **OspA** is suppressed and **OspC** is synthesized. The genes for producing these proteins appear to be controlled by mRNA, and the process suggests that the bacterium has developed mechanisms that permit sustained survival in two very different hosts.

This switch may explain some of the problems encountered with the original Lyme disease vaccine, which was developed to counteract **OspA**. Vaccines that confer active immunity, such as the **OspA** vaccine, are very specific and stimulate the immune system to attack invading cells that exhibit a particular protein. Because *B. burgdorferi* does not exhibit **OspA** in the human body (or exhibits it weakly), the immune system of the vaccinated person doesn't "recognize" the bacterium.

Invasive bacterium may pack a negative charge

ticks

Ixodes scapularis (deer ticks) are the most common vector for Lyme disease. Larval and nymphal ticks are no bigger than the eye of a common sewing needle. Adult ticks are about the size of a small apple seed.

Osp-A structure

*Computer-generated image of the **OspA** structure found on the *B. burgdorferi* bacterium. **OspA** is suppressed when the bacterium moves from the tick gut into mammalian blood streams.*

The Brookhaven researchers also found that gene sequences within different groups of **OspC** itself are highly variable. To date, 19 major groups (A-S) have been identified; they differ from each other at the dimer interface on the surface of the cell (a dimer is molecule in the protein chain that is made up of two identical, simpler, molecules). Apparently only four **OspC** groups (A, B, I, and K) are "invasive"—that is, are responsible for systemic human disease.

Further, the invasive bacteria appear to share a common trait: they all have a strong negative charge in the area of the **OspC** dimer. The researchers postulate that this negative charge may help the bacterium attach to cell tissue, which carries a more positive charge.

"Understanding the correlation between surface charge and invasiveness may be useful not only in developing an effective vaccine, but also in predicting whether other **OspC** bacterium are likely to cause disease," said Subramanyam Swaminathan, another member of the Brookhaven research team.

Understanding the structure is the key

The new understanding of the structure was made possible by the protein fixation and imaging techniques at NSLS. The NSLS permits researchers to focus and control light beams such that images can be seen at resolutions as fine as 2 Å—near atomic resolution.

It is no easy matter to concoct fragile organic matter, such as protein chains, into crystals that can withstand the powerful radiation bombardment of the NSLS and yet retain their original structure. To do this, the Brookhaven team drew upon available nuclear magnetic resonance (NMR) information to identify the least stable areas of the **OspC** protein—the C and N termini. They truncated the protein to remove these termini and improve their chances of crystallizing portions of the protein into a stable, viewable form. They then expressed and purified the protein to ensure homogeneity, and grew them as crystals.

These crystals were frozen to liquid nitrogen temperature and then illuminated with the NSLS beams. By varying the wavelength of the light beams and by using a technique called multiple wavelength anomalous **diffraction** (MAD), the researchers generated more than 120,000 different "reflections" (**diffraction** patterns).

Using computer-assisted analysis and visual imaging techniques, researchers resolved the **diffraction** patterns into vivid 3-D views of both the shape and surface characteristics (such as the charge) of that portion of the **OspC** protein. Once the basic shape of the protein—how it "folds"—was determined for two of the invasive **OspC** groups, the researchers used computer modeling techniques to infer the structure of the remaining 17 groups, considerably speeding the investigative process. The technique and findings are discussed by Swaminathan and fellow researchers in the March 2001 edition of *Acta Crystallographica*, D57. This information about structure and the techniques used to derive it are expected to prove significant in understanding the behavior of other disease-causing bacteria.

The next step, developing an **OspC** vaccine, is not a simple task. However, says **Dunn**, having the structure of the **OspC** protein is a major step forward.

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OspC structure

*Computer-generated image of the **OspC**-HB19 dimer structure found on the *B. burgdorferi* bacterium. Four **OspC** groups are implicated in Lyme disease in humans.*

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Related web links from DOE's Virtual Resource Library:

"[Crystal structure of outer surface protein C \(OspC\) from the Lyme disease spirochete, *Borrelia burgdorferi*](#)," D. Kumaran, S. Eswaramoorthy, B.J. Luft, S. Koide, J.J. [Dunn](#), C.L. Lawson¹, and S. Swaminathan¹, *The EMBO Journal*, Vol. 20, No. 5 pp. 971-978, 2001 (European Molecular Biology Organization)

"*Borrelia burgdorferi* outer surface protein C (OspC)," Kumaran, D., Eswaramoorthy, S., [Dunn](#), J. J. & Swaminathan, S. (2001). Crystallization and preliminary **X-ray** analysis of *Acta Cryst.* D57, 298-300. (requires subscription - [Foundations of Crystallography Online](#))

[National Synchrotron Light Source website](#)

[Lyme Disease Network](#)

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[Protein Crystallization](#) (steps involved in protein production, purification, and crystallization)

[Protein Crystallography](#)

[Interactive tutorial about diffraction](#)

[X-ray Anomalous Scattering](#) (for crystallographers considering MAD (multiple-wavelength anomalous **diffraction**))

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Laboratory: [Brookhaven National Laboratory](#) creates and operates major facilities available to university, industrial and government personnel for basic and applied research in the physical, biomedical and environmental sciences, and in selected energy technologies. The Laboratory is operated by Brookhaven Science Associates, a not-for-profit research management company, under contract with the U.S. Department of Energy.

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