

Structures of Ribosome’s Two Subunits Revealed at NSLS, Published in Two Back-to-Back Articles in August 26 *Nature*

Detailed models for the structures of the two subunits making up the ribosome — the essential organelle that synthesizes proteins within living cells — have, for the first time, been developed, following two studies performed at BNL’s National Synchrotron Light Source (NSLS).

In addition, in revealing the sur-

face contours of both subunits, this research has also resulted in the first model of how the two subunits fit together to form a whole, functional ribosome.

These results were published in back-to-back articles in the August 26 issue of the journal *Nature*. Both *Nature* reports were based on high-reso-

lution diffraction data from studies of crystals using high-intensity x-rays at NSLS beam lines operated by BNL’s Biology Department and the NSLS. From those data, the structures were determined to the resolution of five angstroms, about one ten-thousandth the thickness of a human hair.

To date, the ribosome is the largest and most complex cellular component to have its structure successfully determined using x-ray crystallography. Composed of protein and ribonucleic acid (RNA), each ribosome consists of two subunits, one approximately twice as large as the other.

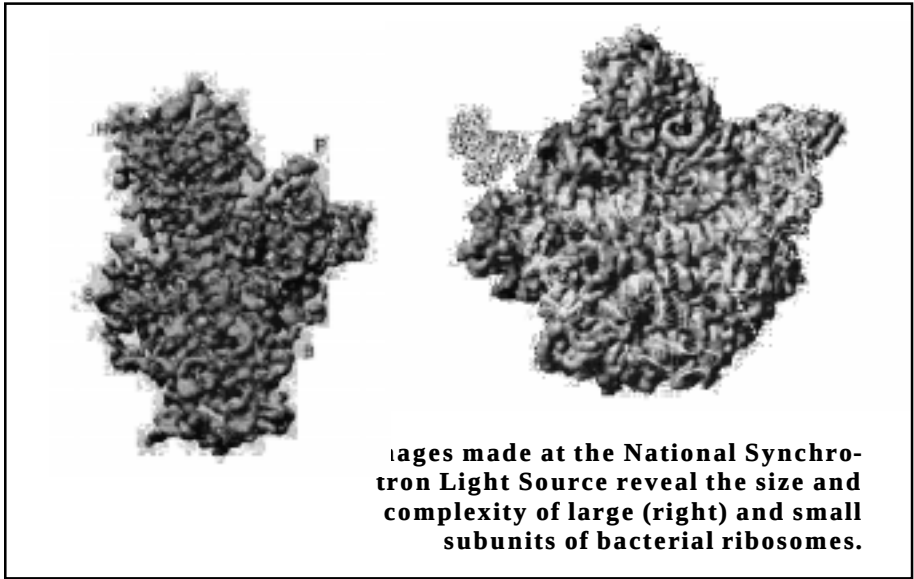
A collaboration led Venki Ramakrishnan of the Medical Research Council in England published a structure of the smaller of the two ribosome pieces, what is called the 30S subunit, which was extracted from the bacterium *Thermus thermophilus*. A former BNL employee, Ramakrishnan had performed this research while on the staff of the University of Utah.

A structure for the larger piece of the ribosome, the 50S subunit, of the bacterium *Haloarcula marismortui* is

described in *Nature* by a collaboration led by Yale University researchers.

“These two independent research efforts are each a tour de force,” says Biophysicist Malcolm Capel of the Biology Department, who is a coauthor of both *Nature* articles. “And they point the way toward the future emphasis of biological crystallography: to the study of other large, multi-subunit, functional complexes made up of proteins and nuclei acids.”

As Capel explains, this research is (continued on page 2)



Images made at the National Synchrotron Light Source reveal the size and complexity of large (right) and small subunits of bacterial ribosomes.

Employee Reminder: Meet With Marburger Next Thursday, Sept. 9

Next Thursday, September 9, the Lab community is invited to Berkner Hall at 11 a.m., to hear Laboratory Director John Marburger discuss the state of the Lab, the latest news on budget for the fiscal year 1999, and more.

Brookhaven-Patented Inventions Transferred to Industry

Right now, thanks to Lab inventors and the Office of Economic Development & Technology Transfer Office (OTT), five departments and divisions have ten products being marketed by industry under patent licenses from BSA. While these products differ widely — they range from red-blood-cell-labeling kits to mirror surface-profiling interferometers to asbestos remediation — they are alike in their practical value to the world and the respect for BNL’s accomplishments that they engender.

“The scientists and engineers at Brookhaven are wonderfully creative and inventive,” said Robert Bari, Interim Associate Laboratory Director for Applied Science & Technology. “Our challenge is to identify and nurture those concepts that may lead to patents and commercial products which will benefit our economy

and the national well-being.”

To this end, Bari and OTT Manager and Laboratory Patent Counsel Margaret Bogosian strongly encourage BNLers to report their inventions to OTT so that, if commercial potential exists, then the inventions may be patented and licensed to industry.

“We want to expand the Lab’s intellectual property portfolio to foster commercial development of Lab technologies,” explained Bogosian. “By increasing the number of patent and license actions and equity business deals, not only does BNL benefit, but also BNL contributes to local and national economic development.”

The Lab and the individual inventors are both beneficiaries of income received (continued on page 2)

Patent No. 5,926,772

Encapsulating Hazardous Wastes

To contain wastes more effectively and inexpensively, Paul Kalb, Department of Advanced Technology (DAT), and DAT retiree Peter Colombo have improved on their previous patent,

which was the second received for what is called polyethylene microencapsulation. Now, the third patent for this process has been awarded.

Wastes are classified as: radioactive waste; hazardous waste, which includes toxic substances; or mixed waste, a combination of radioactive and hazardous waste. For safe handling, storage, and final disposal, wastes require treatment. Some of these (cont’d, page 2)



Roger Stoutenburgh

Paul Kalb, DAT, holds jars of recyclable plastic, which can be used in the polyethylene microencapsulation technique that he invented with DAT retiree Peter Colombo.



Roger Stoutenburgh

(From left) Richard Ferrieri, David Schlyer, and Conrad Koehler, Chemistry Department, are seen with their accelerator target, developed for use with low-energy, high-current particle beams.

U.S. Patent No. 5,917,874

New Target Reduces Health Costs

David Schlyer, Richard Ferrieri, and Conrad Koehler, all of the Chemistry Department, have invented a new accelerator target that is aimed at bringing down the cost of producing radioisotopes. Specifically, they have developed a target in which to produce radioisotopes that are essential in the

widely used medical-imaging technique called positron emission tomography (PET).

Radioisotopes may be made by putting a sample of a material in a suitable holder, or target, and irradiating the sample with a high-energy beam (continued on page 2)

Ribosomal Subunits (cont'd)

the culmination of years of work on the ribosome at BNL, much of which was performed at the Lab's facilities best suited for structural biology: the High Flux Beam Reactor, the Scanning Electron Transmission Microscope, and, of course, the NSLS (see story, below).

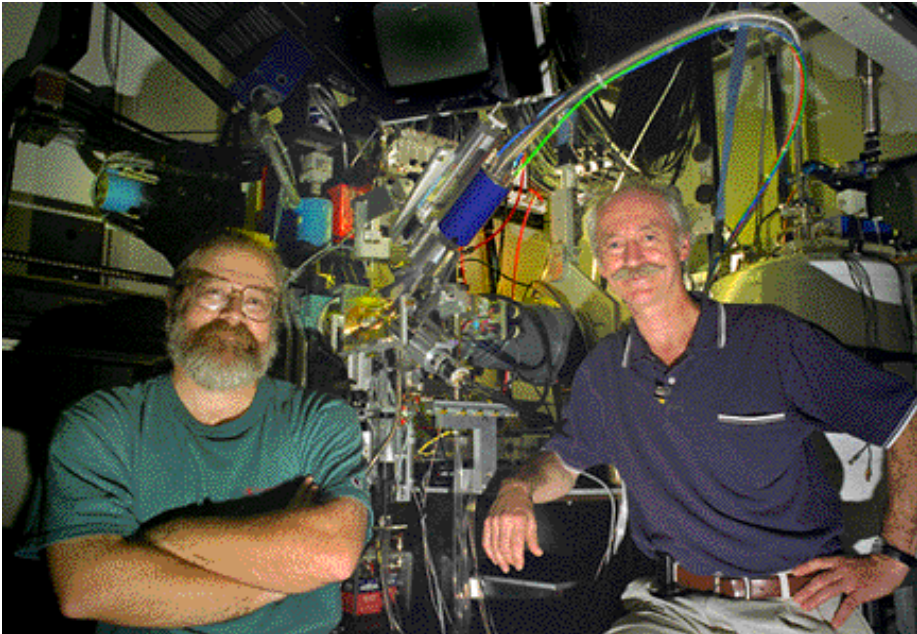
According to Capel, the recent findings are a giant step on the road to understanding how living organisms make proteins. This research can be applied, for instance, to the development of new antibiotics that work by inhibiting the production of ribosomes in bacteria.

Body of Work

Comprised of the 30S small subunit and the 50S large subunit, a ribosome is itself made up of many different proteins and three RNA molecules.

In the case of bacteria, the 30S ribosomal subunit contains 21 different proteins and one RNA molecule, which is about 1540 structural units, or nucleotides, in length. The 50S subunit contains 33 different proteins and the remaining two RNA molecules, which are, respectively, 120 and 2900 nucleotides in length.

To synthesize proteins, ribosomes "translate" coded instructions, contained by what is called a messenger RNA, into chains of amino acids that



Roger Stoughton

At NSLS beam line X12B, where much of the data on the ribosomal subunits' structures were collected, are Malcolm Capel (left) and Dieter Schneider of BNL's Biology Department. Not pictured is Bob Sweet, also of the Biology Department.

make up proteins. During this process, the 30S subunit has the job of ensuring that the translation is accurate, that is, decoded into the specified amino acid in the proper sequence. The 50S subunit works with what is called transfer RNA to couple amino acids to the growing polypeptide chain.

Since the regularly repeating arrangements of atoms and molecules

within a crystal are most easy to use for structural studies, the researchers first crystallized purified solutions of ribosomal subunits.

To help map the structures, the scientists then modified the crystals by adding organic clusters of heavy metals, which function as a kind of road marker. Specifically, clusters containing tungsten and osmium were used,

and the latter, since they are not available commercially, were made for these studies by Bruce Brunschwig and Mei Chou of BNL's Chemistry Department.

To protect the crystallized ribosomes from radiation damage in the course of data collection, they were flash frozen to 100 Kelvin, which is -173°C. The scientists next took their crystals to the NSLS x-ray ring's beam lines: X12B and X12C, which are operated by the Biology Department and overseen, respectively, by Malcolm Capel and Robert Sweet; and X25, which is run by the NSLS under the jurisdiction of Lonny Berman.

Directing the NSLS's intense x-rays at their crystals to study the ribosomal subunits, the researchers employed the phenomena called diffraction, which is the spacial redistribution of the intensity of light waves due to the presence of their samples in the path of the x-rays. The results of the diffraction experiments were tens of thousands of spots recorded by a computerized imaging detector.

After measuring the position and intensity of each spot, the collaborators then calculated what is called the electron density of the two subunits. It was from the electron density maps that molecular models of the two ribosomal subunits emerged. — Marsha Belford & Diane Greenberg

BNL's Long-Term Investment in Structural Biology Pays Off

The proposed structures of the bacterial ribosome that were published in last week's *Nature* (see story, starting on page 1) are a culmination of years of work at BNL on the ribosome. For much of this research, the Lab's big machines best suited for structural biology and the complementary data that they generate were crucial.

In the 1970s and 80s, two of the present studies' authors, Malcolm Capel and Venki Ramakrishnan, worked at the High Flux Beam Reactor (HFBR) with collaborators from Yale, using neutron diffraction to determine the three-dimensional configuration of the 21 proteins of the 30s subunit of the ribosome within the bacterium *Escherichia coli*. This was done with the ribosomes in solution, at HFBR beam line H9B, which was operated by BNL's Biology Department and under the supervision of Dieter Schneider.

According to Capel, the spacial information within the 30S neutron map was key in interpreting Ramakrishnan's current x-ray diffraction data and, thus, in producing the just-published detailed, high-resolution model of the 30S ribosomal subunit.

To study how a ribosome itself is synthesized and assembled from its components, RNA and protein, Joe Wall in BNL's Biology Department and collaborators such as the late Miroslav Buoblik of the Roche Institute used the Lab's Scanning Transmission Electron Microscope (STEM). Their observations have been used to understand how proteins interact with ribosomal RNA in assembling a complete ribosome.

Using x-ray crystallography at the National Synchrotron Light Source in the early to mid-90s, Ramakrishnan and former BNL employee Stephen White determined the molecular struc-

tures of a number of proteins within the small and large subunits of the ribosome that they had isolated and crystallized.

With the structure of the bacterial ribosome revealed, what is left to do, says Capel, is: locate the remaining ribosomal proteins not currently in the x-ray crystallography map, determine how these proteins are oriented and folded within the ribosome, and ascertain the fold of the entire 16S ribosomal RNA.

Finally, the biochemical mechanisms in each stage of protein synthesis remain to be correlated with detailed molecular models of the ribosome and its complexes — including transfer and messenger RNAs and elongation factors.

According to Capel, completing this work is going to be difficult, but will be made possible by using facilities such as BNL's NSLS. — Marsha Belford

Inventions Transferred

from patent licensing. After all expenses associated with patenting and licensing an invention are recovered, the individual inventors are given a share of the licensing income received by BSA after the invention is licensed to private industry.

After distribution to the inventors, the remaining net licensing income is distributed to the research departments and divisions, with 40 percent of the income shared among those that generated it. These funds are used to support cooperative research and development (R&D) projects with

U.S. industry, technology transfer activities, and new R&D initiatives (see list of products, right).

"So inventors benefit in two ways," said Bogosian. "They get a personal share, and they know that their efforts win licencing income for their departments or divisions to reinvest in more research."

Those researchers who want to explore filing an invention disclosure may contact Margaret Bogosian, Ext. 7338.

Two recent patents granted to BNLeers are featured starting on page 1. — Liz Seubert

Products Marketed Under BNL License

These commercialized technologies were invented by current and former BNL employees:

- **Apparatus and method for biological purification of wastes** - Eugene Premuzic and Mow Lin, Department of Applied Science (DAS).
- **Asbestos remediation** - Toshifumi Sugama and others, DAS.
- **Autogenes encoding RNA polymerases** - F. William Studier, Biology Department.
- **Cytoplasmic bacteriophage display system** - F. William Studier, Biology Department.
- **Fast repetition-rate fluorometers and method for measuring fluorescence and photosynthetic parameters** - Zbigniew Kolber and Paul Falkowski, DAS.
- **Polyethylene encapsulation of wastes** - Paul Kalb, Department of Advanced Technology (see story, page 1).
- **Recombinant plasmids for encoding restriction enzymes DpnI and DpnII of *Streptococcus pneumoniae*** - Sanford Lacks, Biology Department.
- **Red blood-cell labeling kit for selectively labeling whole blood with Tc-99m** - Suresh Srivastava and George Meinken, Medical Department.
- **Surface-profiling interferometers for accurately measuring irregularities in the surfaces of mirrors and lenses** - Peter Takacs and Shinan Qian, Instrumentation Division.
- **T7 gene expression system, vectors, and protein products produced with the T7 system** - F. William Studier and John Dunn, Biology Department.

Encapsulation

(cont'd)

wastes need to be solidified in a matrix to reduce the chance of contaminants dispersing or leaching from them over time. Conventional technologies, which use cement or thermosetting polymers, rely on a chemical reaction to solidify the wastes and are limited by chemical interactions with the wastes.

Developed within the Environmental & Waste Management Group in DAT's Environmental & Systems Engineering Division, the BNL process involves mixing a broad range of waste types together with virgin or recycled polyethylene to form a homogenous solid matrix. This form of matrix significantly reduces the potential for releasing the stored waste into the environment. Performance-enhancing additives and a clean, outer polymer layer are also used to help ensure the integrity of the waste.

In addition to improving performance, this BNL technology saves expense. The process is not susceptible to chemical interactions with the wastes, so more waste per unit volume can be encapsulated. About 50 to 70 percent of the total weight of each BNL-designed polyethylene-encapsulated container is waste, while conventional cement solidification containers hold only 15 to 30 percent waste.

Envirocare of Utah, the largest commercial mixed-waste treatment company in the U.S., has signed a license agreement with BNL and is currently using this new process (see Brookhaven Bulletin, June 20, 1997). — Liz Seubert

New Target

(cont'd)

of particles from an accelerator. Production costs are high, so the use of low-power accelerators which create low-energy particle beams is being explored. To retain the required beam density, however, the beam intensity must be increased to a relatively large current of about 100-150 microamps.

The increased intensity made the conventional targets inefficient. So Schlyer, Ferrieri and Koehler developed a target to use with low-energy, high-current particle beams, making it possible to produce lower cost radioisotopes at many more facilities. The target was developed under a Cooperative Research and Development Agreement (CRADA) with AccSys Technologies, Inc., a manufacturer of low-energy, high-current accelerators.

In PET, a radioisotope is attached to a compound that is then administered to a patient. A PET machine can detect and image the gamma rays produced by positron particles emitted by these short-lived radioisotopes. So physicians and researchers can study the images to learn the distribution and location of the compound in the patient over time, "seeing" what is happening in the body or brain.

BNL research to study the brain using PET has resulted in discoveries on aging, schizophrenia, Alzheimer's and Parkinson's diseases, brain tumors, drug action, and substance abuse. — Liz Seubert

Coming Up

On Friday, September 24, at 8 p.m. in Brookhaven Center, physicist and motorcyclist Charles Falco, University of Arizona, Tucson, who helped curate “The Art of the Motorcycle” exhibition at the Guggenheim Museum last summer, will present a talk on “The Art and Science of the Motorcycle.” All are invited.

Holiday Notes

In observance of Labor Day, the Lab will be closed on Monday, September 6 and there will be no Bulletin on Friday, September 10.

In addition, the Upton Post Office and the on-site Teachers Federal Credit Union will be closed on 9/6. The automatic teller machine in the foyer of Berkner Hall will remain open. The Omega Leisure Travel Office in Berkner Hall will be closed on 9/6&7, reopening on Wednesday, 9/8.

The cafeteria will be open 7:30 a.m.-2 p.m., Saturday-Monday, 9/4-6. The Brookhaven Center will be closed on Sunday evening, 9/5; it will reopen on Monday, 9/6, 5-9 p.m. During September, the cafeteria will remain open on weekends for morning and lunch service.

The swimming pool will be closed Saturday-Monday, 9/4-6. Fall pool hours resume on Tuesday, 9/7. The gymnasium, which has been closed weekends throughout the summer, will reopen 10 a.m.-5 p.m. on Saturdays from 9/11.

To Your Health

The following activities are offered by the Health Promotion Program of the Occupational Medicine Clinic. To register or for more information, call Health Promotion Specialist Mary Wood, Ext. 5923.

A Better You

The “A Better You!” weight-loss program offers participants consultations with a dietitian or nutritionist to customized the program, healthy cooking techniques, dining-out strategies, food-shopping tips, exercise approaches, stress-reduction hints, and more. This ten-session program meets on Thursdays, starting September 9, and costs \$99 per person.

Cardio Kickboxing

By popular demand, cardio kickbox classes will be again offered on site, from noon to 1 p.m. on Mondays and Thursdays, starting Monday, September 13. Each class is \$5 per participant, payable monthly.

Water Aerobics

Water stretching, strengthening and aerobic exercise classes will again be offered at the Lab pool, Bldg. 478, from 5:20 to 6:20 p.m., on Tuesdays and Thursdays. The first classes will be on September 14 and 16, respectively. Water-aerobics classes are free, but participants must pay the pool fee of \$2 a session or show their season pool pass.

Weight Watchers at Work

The next session of the on-site Weight Watchers classes will have its first class on Wednesday, September 15, from noon to 1 p.m. in the Brookhaven Center.

Fall Into Fitness

Starting on September 20, travel to different cities around the world, beginning and ending in New York City — without leaving the area and while getting fit. Do this by joining the five-week Fall Into Fitness program. Similar to the annual March Into May activity program, Fall Into Fitness has participants track miles instead of points. Accordingly, 20 minutes of any exercise equals 600 miles of travel.

TFCU at Berkner 9/15

Representatives from Teachers Federal Credit Union and MEMBERS Financial Services, a financial-management program, will be in Berkner Hall lobby on Wednesday, September 15, 11 a.m.-2 p.m. Stop by for information on accounts and other services.

BERA News

For more information, stop at the BERA Sales Office in Berkner Hall, Tuesday-Friday, 9 a.m.-1:30 p.m., or call Andrea Dehler, Ext. 3347, or M. Kay Dellimore, Ext. 2873.

Walk for Healthier Hearts

Join the fight against heart disease and stroke by participating in the 1999 American Heart Walk on Sunday, October 3. Walkers may choose from two locations, Suffolk County Community College in Riverhead, or the State University of New York at Farmingdale.

Volunteers Wanted for Science, Environment Festival in October

On Saturday, October 16, the Lab will be holding an outdoor Festival of Science & the Environment, which will be open to the public. Similar to last year’s successful Environmental Fair, this year’s festival will feature more science exhibits and science-based activities for the kids.

To volunteer for this event, contact its coordinator, Janet Tempel, Community Relations Office, Ext. 4049 or jtempel@bnl.gov.