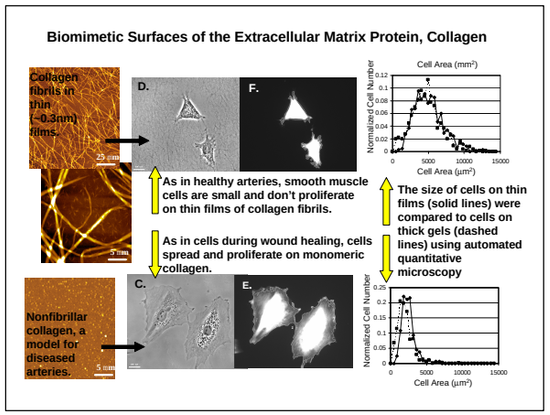

with surface plasmon resonance spectroscopy. Cell fragments containing the transmembrane proteins are immobilized to a solid support where cells containing the G-protein CCR5 bind to a surface. An antibody against CCR5 subsequently binds to these membranes, but not to a control surface. These results suggest that the technique may be useful for detecting the binding of small ligands to these receptors. The emphasis of the **mitochondrial proteomics** project will be to address needs of the mitochondrial and proteomics communities as outlined in a September 2002 workshop and to develop general protocols for handling and characterizing membrane associated proteins.

The Research Collaboratory for Structural Bioinformatics - Protein Data Bank:
http://www.rcsb.org/
http://rcsb.nist.gov/

The Biological Macromolecule Crystallization Database:
http://wwwbmcd.nist.gov:8080/bmcd/bmcd.html

The Short Tandem Repeat DNA Internet Database:
http://www.cstl.nist.gov/biotech/strbase/

Thermodynamics of Enzyme-Catalyzed Reactions:
http://wwwbmcd.nist.gov:8080/enzyme/enzyme.html

HIV Protease Database:
http://srdata.nist.gov/hivdb/

BIOTECHNOLOGY

http://www.cstl.nist.gov/biotech/
http://www.cstl.nist.gov/
http://www.nist.gov/

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United States Department of Commerce
Technology Administration
National Institute of Standards and Technology
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Division Overview

The Biotechnology Division is the focus of the NIST effort addressing critical measurement and data needs for the rapidly developing biotechnology industry.

MISSION:

The mission of the Division is to provide measurement infrastructure necessary to advance the comercialization and application of biotechnology. This is achieved by developing a scientific and engineering technical base along with reliable measurement techniques and data to enable U.S. industry to produce biochemical products, and to enable the government to apply advances in biotechnology to the benefit of societal needs. The Division has established a variety of long-range research projects to maintain critical expertise needed for the development of Standard Reference Materials, Standard Reference Databases, and advanced measurement methods. The Division fosters collaboration among NIST scientists conducting biology-related research, and raises the visibility of the NIST and Chemcial Science and Technology Laboratory Strategic Focus Areas (SFAs) that have strong biological focus.

Division scientists participate in scientific meetings, topical workshops, and numerous national and international organizations such as: Biotechnology Industry Organization (BIO), IUPAC Commission on Biophysical Chemistry, ASTM Committee E-48 on Biotechnology, the International Measurement Standards Consultative Committee for the Amount of Substance (CCQM), Bioanalytical Working Group. Division members were also active as reviewers for the NIST Advanced Technology Program (ATP), for several NSF and DOE programs, NIH study section panels, and for the Office of Science and Technology Policy on issues related to bioterrorism defense, and with the Department of Justice on issues related to forensics and human identification.

STANDARD REFERENCE MATERIALS
- Human Identification and Forensics
  - –DNA Profiling
  - –Mitochondrial DNA
- DNA Diagnostics
- DNA Damage & Repair
- Fluorescence
- Peptides

DATA ACTIVITIES
- Research Collaboratory for Structural Bioinformatics – Protein Data Bank
- Biological Macromolecule Crystallization Database (Standard Reference Database 21)
- Short Tandem Repeat DNA Database
- Thermodynamics of Enzyme-Catalyzed Reaction (Standard Reference Database 74)

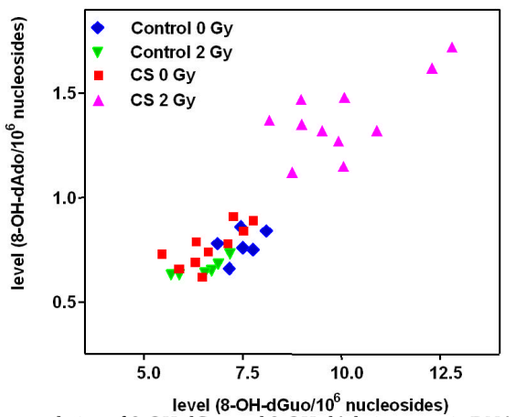
The staff of the Biotechnology Division consists of 50 NIST employees and a comparable number of contract researchers, guest scientists, and postdoctoral fellows. The Division is organized into four groups: (1) **DNA Technologies**; (2) **Bioprocess Engineering**; (3) **Structural Biology**; and (4) **Biomolecular Materials**. Brief descriptions of technical highlights from each Group are given below.

Future Emphasis Areas
- Proteomics
- Tissue Engineering
- Microbial Forensics
- DNA Diagnostics
- Bioinformatics
- Structural and Functional Genomics
- Genetically Modified Organisms

Selected Program Highlights

DNA TECHNOLOGIES

The DNA Technologies Group has research projects that are included in the Strategic Focus Areas of Health and Medical Products, Forensics and Homeland Security, and Food and Nutritional Products. **Advanced mass spectrometry measurements of DNA damage** are used to describe the cellular accumulation of two major oxidative stress-induced DNA lesions in cells of Cockayne syndrome (CS) patients after exposure to

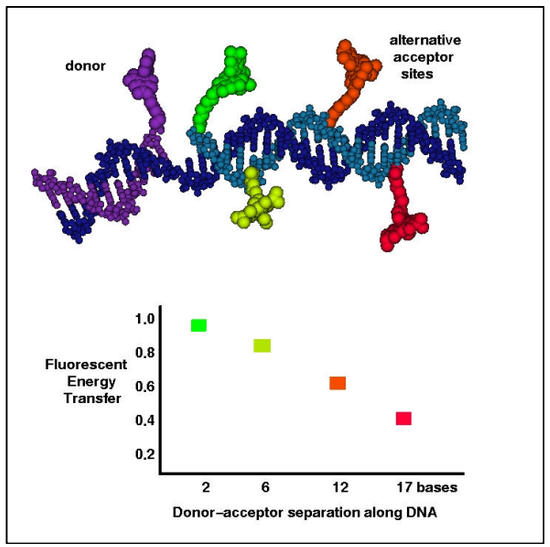


Accumulation of 8-OH-dGuo and 8-OH-dAdo in genomic DNA of Cockayne syndrome patients after exposure to γ-radiation. Cells that were deficient in DNA repair (▲CS 2 Gy) exhibit greater accumulation of the DNA lesions.

ionizing radiation. As a disease with implications for understanding the human aging process, these studies are undertaken as a collaborative effort with scientists at the National Institute of Aging. Projects in the area of **DNA diagonostics for the detection of human disease** include the NIST-National Cancer Institue Biomarkers Validation Laboratory (BVL), the NIST component of the Early Detection Research Network which serves to refine recently discovered cancer biomarkers, and to format new research tests for field trials in EDRN clinical laboratories. Another area is the study of cellular biomarkers that can be used for quality assurance of tissue-engineered medical products in terms of genetic damage. In the **human identity/ forensic science** project, the group focuses on new methods for DNA profiling, ranging from developing well-characterized DNA standards for restriction fragment length polymorphisms (RFLPs) to performing research for rapid determination of DNA profiles by polymerase chain reaction (PCR) amplification and automated detection of fragments. New methods were developed for identification of victims of the World Trade Center (WTC) disaster of September 11, 2001 where the high degree of DNA fragmentation due to the severe environmental conditions has meant that only about 50% of the specimens yielded results with standard DNA testing methods.

BIOPROCESS ENGINEERING

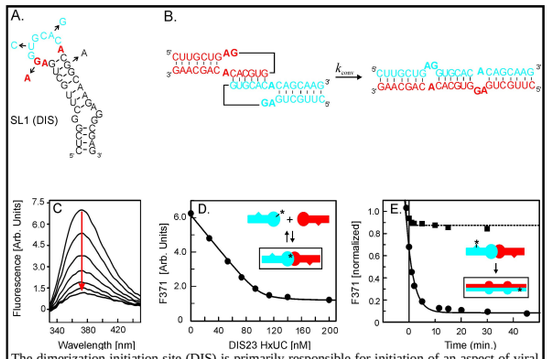
The Bioprocess Engineering Group (**http://www.cstl.nist.gov/biotech/bioprocess/**) is concerned with the development of measurement methods, databases, and generic technologies related to the use of biomolecules and biomaterials. The results are directed at the biomanufacturing and pharmaceutical industries and, most recently, to Homeland Defense, where there are needs for the detection and quantification of very small amounts of biological materials. The effort is organized into four project areas that are part of the Pharmaceuticals and Biomanufacturing, Food and Nutritional Products, and Forensics and Homeland Security SFAs. In the **biospectroscopy** project, one study is directed at investigation of the mechanism of fluorescence resonance energy transfer (FRET) when it is used to quantify the extent of a polymerase chain reaction (PCR). In the figure, FRET efficiency is seen to decline by five-fold as a function of fluorophore separation, counted as number of nucleic bases between the fluorophore labeling sites. In the **biocatalysis** project, enzyme characterization is being carried out to address industrially important biotransformation



problems such as those found in hydroxylation and aromatic amino acid metabolic pathways. The methods used include site-directed mutagenesis, circular dichroism, ellipsometry, spectroelectro-chemistry, and X-ray diffraction to characterize several key steps along metabolic pathways. In the **biothermodynamics** project, chromatography and microcalorimetry are used with chemical equilibrium analysis of complex reacting systems to develop thermodynamic data for industrially important biotransformations that are included in the NIST Standard Reference Database "Thermodynamics of Enzyme-catalyzed Reactions." A new project, **bioterrorism research**, has recently started to develop standard methods, materials and data related to the national efforts to defend against threats of biological warfare.

STRUCTURAL BIOLOGY

The Structural Biology Group at the Center for Advanced Research in Biotechnology (CARB) is focused in key areas of industrial biotechnology, especially in the Pharmaceuticals and Biomanufacturing SFA. These areas are supported at CARB through a highly interactive group of scientists, from both the University of Maryland Biotechnology Institute (UMBI) and NIST. In the project **macromolecular structure determination by X-ray crystallography**, a new effort has been launched in structural genomics. The goal is to develop high-throughput approaches for elucidating the structures and functions of all the proteins encoded by entire genomes, with a focus on determination of the structures for 'hypothetical' proteins of microbial genomes that may be useful drug targets. The **molecular structure and dynamics** project includes a study of the dimerization of two homologous strands of genomic RNA, an essential reaction in the replication of retroviruses such as HIV-1 (see figure). Results from the **physical, molecular and cellular biochemistry** project on the structures and interactions of key recognition elements in G coupled protein receptors suggest new, quantitative models for signal transduction pathways in vision and viral infection. The energetics of enzyme catalyzed reactions are being studied by differential stopped flow microcalorimetry. The temperature dependence of the kinetics of the acylase hydrolysis reaction has recently been determined. The staff of the **computational biology** project has been investigating the reaction mechanisms of two enzymes, zinc lactamase and chorismate mutase, that are representative of their enzyme class. The **bioinformatics** project is striving to establish data uniformity and to develop the physical archive of the NSF/DOE/NIH supported Protein Data Bank (**http://pdb.nist.gov/**) within the Research Collaboratory for Structural Bioinformatics (RCSB) partnership that includes groups from Rutgers University, the University of California San Diego Supercomputer Center, and the University of Wisconsin.



The dimerization initiation site (DIS) is primarily responsible for initiation of an aspect of viral assembly. The loop contains an auto-complementary hexanucleotide sequence, highlighted in blue, and is flanked by highly conserved 5' and 3' purines (bold red). The structural rearrangement activated by the HIV-1 nucleocapsid protein (NCp7), believed to be associated with viral particle maturation, converts the stem-loop from an intermediate 'kissing' to an extended duplex isoform (Figure B). Fluorescence methods (the asterisk shows location of the 2-AP probe) were used to determine the kinetics of RNA structural isomerization and the role of NCp7.

BIOMOLECULAR MATERIALS

The Biomolecular Materials Group studies the behavior of biological molecules and adapts them for novel technological and scientific applications, and to emerging needs of bioterrorism research. Measurement methods including surface plasmon resonance, IR spectroscopy, ellipsometry, electrophysiology, impedance spectroscopy, chemical synthesis, atomic force microscopy, and confocal microscopy are combined with computer simulations to carry out projects in **nanobiotechnology, tissue engineering**, and **mitochondrial proteomics**. In the **nanobiotechnology** project, single nanometer-scale pores were employed to study biological transport processes, to read information within single biomolecules and to detect multiple analytes in solution. In the **tissue engineering** project, the need for biomarkers, physical standards and measurement technologies for tissue engineering are being addressed to assure quality control during manufacturing and storage of engineered medical products. A method for reproducibly and reliably fabricating films of collagen to provide surfaces on which cells can be grown is under development. Data shown in the figure illustrate that the films can induce morphological changes in vascular smooth muscle cells that mimic their response in healthy and diseased arteries. The ability to characterize these films with surface analytical techniques permits the evaluation of how changes in the collagen substrate influence cellular responses, potentially leading to reference materials. In another study, the ability of chemokines to interact with G-proteins, which are important target molecules of the pharmaceutical industry, is interrogated