# THE DOSSIER

The Digest on CCR Staff Scientists and Staff Clinicians: Information, Employment and Research

September 2015 Issue 21

## From the Editor

**Welcome to the September issue of The Dossier, a newsletter dedicated to the Staff Scientists and Staff Clinicians (SSSC) of the CCR!**

This issue contains important messages from the Director's Office and a special article by Giorgio Trinchieri, M.D. New resources to assist displaced SSSC are presented by Christophe Marchand, Ph.D., and in our SSSC Corner, we feature Francois Van Laethem, Ph.D. The published work of Alberto Bartesaghi, Ph.D., is highlighted in our Author's Corner, while Diana A. Starvreva, Ph.D., and Gianluca Pegoraro, Ph.D., describe their collaborative efforts at the High-Throughput Imaging Facility.

We hope to continue to provide pertinent information to aid in the success of SSSCs. Please send your contributions, suggestions, and comments to budhua@mail.nih.gov.

***Anuradha Budhu, Ph.D. (SS)***
***Editor-in-Chief***
*Laboratory of Human Carcinogenesis*

## In This Issue



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# From the Office of the Director

## Partnership with Walter Reed National Military Medical Center

The CCR has a long and fruitful history of collaborations with the Walter Reed National Military Medical Center (WRNMMC), formerly known as the Bethesda Naval Hospital, which is a hub of national military medicine. At one time, NCI had a branch located on the military campus and scientific accomplishments over the partnership's rich history include:

- The first clinical randomized trial demonstrating that standard dose therapy was as effective as double therapy dose in small cell lung cancer.
- Established a common staging system for mycosis fungoides.
- Established the cell line that led to the initial isolation of the first human leukemia retrovirus (in acute T-cell leukemia/lymphoma).
- Established the world's largest cell line bank in lung cancers, which has led to seminal molecular insights into lung cancer.

After a hiatus, CCR has reengaged with the new, combined military organization, WRNMMC, that formed from the Base Realignment and Consolidation (BRAC) process and relocation of the Walter Reed Medical Center to the National Naval Medical Center. The key leaders of the oversight group are Drs. Lee Helman, Acting CCR Director and Scientific Director for Clinical Research, Bill Dahut, Clinical Director, CCR, and Craig D. Shriver, COL, Director, Murtha Cancer Center (MCC). Brigadier General Jeffrey B. Clark, Director, WRNMMC, and Acting NCI Director, Doug Lowy, have already met several times to discuss ways to continue to deepen the partnership and leverage the considerable resources of each organization.

These initiatives and activities include the following:

- Approval was recently granted to allow CCR patients to be cared for at Walter Reed. The WRNMMC MCC has already provided care in a variety of specialties not available at the NIH Clinical Center (CC).
- Approval has been granted for WRNMMC Institutional Review Boards (IRB) to accept a master protocol for large NCI studies.
- Approval was granted for a reliance agreement to allow clinical studies to be conducted based on using NCI's IRB approval.
- Efforts are under way to establish overarching agreements for tissue, reagent, and data sharing between the two organizations.
- Many Walter Reed physicians and surgeons now are credentialed at the CC and CCR physicians and surgeons are credentialed at Walter Reed enabling them to join research efforts at both institutions through Clinical Collaborator appointments.
- The MCC and CCR jointly supported projects through Activation Funds in FY14, and FY15 project proposals are under review. In FY14, two projects were funded: "Genomic Characterization of Breast Cancer in High Risk Subsets of Breast Cancer" (PIs: Drs. Stanley Lipkowitz, Hai Hu, Patricia Steeg, and Jeremy Perkins) and "Breast Cancer Specific Immunome" (PIs: Drs. Jan Davidson-Moncada, Giorgio Trinchieri, Jeffrey Hooke, Christopher Gallagher, Hai Hu, and Albert Kovatich).

The CCR and WRNMMC also plan to continue the prostate cancer collaborative research initiatives that were launched under the NCI and the MCC Center of Excellence to foster and synergize research projects between NCI and the Department of Defense prostate cancer researchers at Walter Reed and the Uniformed Services University. Among the initiatives is a multidisciplinary advanced prostate cancer clinic established in 2003 in association with medical oncologists from our Medical Oncology Service that has provided valuable service to more than 1,500 patients. In the clinic, patients are offered conventional treatments as well as an opportunity to participate in state-of-the-art clinical trials and other innovative therapies. This clinic also provides an avenue to collect follow-up data on prostate cancer for the Center for Prostate Disease Research Multicenter National Database.

The WRNMMC prostate cancer research collaborations also include projects to develop new therapeutic

# From the Office of the Director Con't

strategies, to evaluate new prostate cancer markers in circulating tumor cells, and to evaluate the infiltrating immune cells within prostate specimens, which have been shown prognostic significance in colon and prostate cancer.

As Staff Scientists and Staff Clinicians, working together with your Lab/Branch Chief, you have already contributed to the success of our research collaborations by being directly involved in these initiatives or by contributing to the foundations on which they were built. I encourage you to discuss with your Lab/Branch Chief about becoming involved in this partnership.


***Lee Helman, M.D.***
Acting Director, Center for Cancer Research
Scientific Director for Clinical Research

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# The SSSC Corner

**Section Editor: Takashi Furukawa, Ph.D. (*SS*)**

I first heard about the NIH from my Ph.D. mentor, Oberdan Leo, Ph.D., who did his post-doctoral fellowship at the NCI. After hearing great things about the networking opportunities and the quality of research happening in Bethesda, I decided to start my own post-doctoral fellowship at the NCI in the Laboratory of Alfred Singer, M.D. In the Lymphocyte Development Section of the Experimental Immunology Branch of the NCI, our work is primarily focused on understanding how T lymphocytes develop and acquire their functional helper or cytotoxic potential. During my post-doctoral work, I was interested in understanding how T lymphocytes become MHC (Major-Histocompatibility Complex) -restricted. This obsession of T cells for MHC has been a central immunological dogma for many years and the molecular mechanisms underlying this concept had not been well characterized. Our work unraveled an essential role for the T-cell specific tyrosine kinase Lck during T-cell development. We indeed found that the association of Lck with the cytoplasmic tails of both CD4 and CD8 coreceptors ensures that the mature T cell repertoire only contains T cells that recognize antigens that are presented by MHC molecules and are therefore MHC-restricted. Being a Staff Scientist at the NIH has allowed me to develop complex and long-term projects. I have to admit that the resources available here at the NIH have been absolutely exceptional and the genetic manipulations required for our studies (for example quadruple knock-out mice) would have been impossible to generate anywhere else in the world. The relative independence given by my mentor also allowed me to supervise the research of a number of younger investigators (Ph.D. student, post-bacs and post-doctoral fellows) and this has been extremely rewarding on a both personal and scientific level. Allowing to closely monitor other people's work has given me and the group many new opportunities and opened quickly new areas of research. Like my Ph.D. mentor a few years back, working at the NIH has given me vast opportunities to meet an amazing number of immunologists on campus and to start collaboration with a few of them. The number of experts that are part of the NIH community is really impressive and it's very easy to find someone to help solve any theoretical or technical issue.

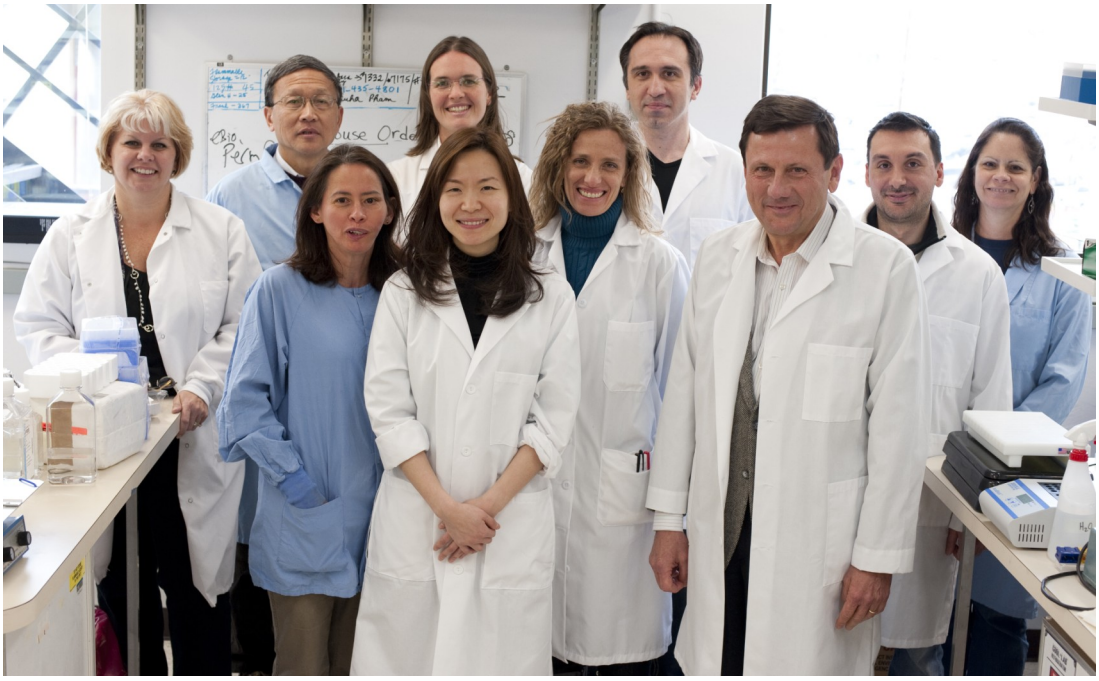
Francois is pictured above riding his grey gelding, Little Prince, in Poolesville, MD.

Outside of the lab, I love listening to music, in particular classical music and opera. I also have two major sport passions. I play competitive tennis on a weekly basis in Potomac with some great friends and competitive horseback riding is my second big passion. It's great to spend time outdoors with my lovely horse called Little Prince (or Petri for short). Getting him and myself fit and ready for jumping shows does require, like in science, a lot of work and dedication.

**Francois Van Laethem, Ph.D. *(SS)***
*Lymphocyte Development Section*
*Experimental Immunology Branch*

# The PI Corner

**Section Editor: Lakshmi Balagopalan, Ph.D. (*SS*)**



The Trinchieri lab is pictured. **Giorgio Trinchieri**, M.D., Director, Cancer and Inflammation Program (third from right). At his right, SS **Amiran Dzutsev**, Ph.D., and next to him is former SS and presently Earl Stadtman Tenure-Track iIvestigator **Romina Goldszmid**, Ph.D. SS **Rosalba Salcedo**, Ph.D., is third from the left.

The landscape of scientific research in the United States and in other countries is rapidly evolving. New technologies and analytical methods have fostered the generation of new results and scientific knowledge at an unprecedented pace. This is contributing to great advances in medicine and many technological fields such as food production, energy, and electronics, that are positively affecting quality of life across the globe. Particularly in the United States, it has been recognized for a long time that basic science analyzing the fundamental mechanisms of biology, chemistry, and physics contributes to the scientific knowledge that fosters technological and analytical advances and allows the progress of translational and applied research. Regrettably, these scientific advances, both at basic and translational levels, have not coincided with a needed sustained support for scientific research, but have taken place in an environment of stagnant or contracting funding for science in most countries. This is creating a major challenge for continuous scientific progress and, more immediately, for the scientific careers of new Ph.D. and young investigators that are confronted with shrinking possibilities for independent academic careers. Conversely, the PIs in academic or government institutions find it difficult to maintain in their groups the knowledge needed to conduct innovative research utilizing the sophisticated technological and analytical tools available and essential for modern science by relying solely on graduate students or short-term postdoctoral fellows. Thus, the Staff Scientist position offers a good opportunity for young investigators that are enthusiastic in their passion for science, yet unwilling or unable to go through the uncertainty as well as the personal and family challenges that a career as an independent scientist would impose on them. At the same time, their scientific expertise and technical and analytical prowess provide to their scientific group stability, the possibility of outstanding training for new students and fellows, and the knowledge needed to keep the technology and analytical effort in the group at a state-of-the-art level. This is particularly important in the collaborative team effort required by modern science and, thus, the Staff Scientist may provide an important contribution to different groups, facilitating team integration.

The important and essential role of the Staff Scientists is being increasingly recognized at NIH and in academic institutions, particularly in the large, best organized, and productive lab groups. At NIH, all Staff Scientists are regularly evaluated on their own scientific productivity and/or contribution to the scientific community through the Quad Review process, particularly for those managing different facilities. Although the Staff Scientist position at NIH is not a per-

-anent position, when the position occupied by a Staff Scientist disappears, due to reduction of PI resources, career choice, or retirement, efforts are made to help find a new position. Staff Scientists who find themselves in this position, and indeed all Staff Scientists, should be encouraged to network extensively and work closely with their Principal Investigator, Lab/Branch Chief and NIH leadership to identify new career opportunities if needed.

In the extramural academic community, recommendations have been made to identify a defined career path for investigators who are not in an independent position as head of a laboratory, and to create new definitions of research positions at different levels of seniority (2012 Report of the Biomedical Research Workforce, a Working Group of the Advisory Committee to the NIH Director). For example, in other countries, such as France, semi-independent investigator positions are available, in addition to the head of the research group: these investigators are evaluated for their individual contribution and ability and, when needed for scientific or organizational reasons, they are allowed the possibility to change the scientific group that they are supporting. It has also been recommended that NIH should consider the presence of Staff Scientists in a research group as a positive factor in making grant funding decisions and that the use of institutional funds should be increased compared to soft money to support Staff Scientists in order to increase their job stability. In this way, Staff Scientists will be personally and scientifically motivated and confident that their high-quality work and dedication will be recognized and, as a result, excellent fellows will feel that the Staff Scientist position is an attractive and real career choice.

In my ten years at NIH, I have been blessed with the excellent contribution of several Staff Scientists that not only have contributed to the success of my group even when I was distracted by administrative responsibilities, but most importantly have contributed to opening new areas of research and interest; an example is our move into the microbiome field as related to oncogenesis and cancer therapy. Among the most recent ones, Rosalba Salcedo, Ph.D. has been with me for all this time, organizing and supervising the experimental mouse work and the study of the role of the inflammation in carcinogenesis; Romina Goldszmid, Ph.D., recently appointed as an Earl Stadtman Tenure-Track Investigator in the Cancer and Inflammation Program, has brought our work on myeloid cells in infection and inflammation to a new level of sophistication and innovation; Amiran Dzutsev, M.D., Ph.D., has brought the microbiome methods and analyses to the laboratory and has convinced me, more than anybody else, of the requirement for an analytical and systems biology approach in any modern laboratory; and finally Jonathan Badger, Ph.D. has recently joined the Genetics and Microbiome Facility that I am organizing and will help the facility manager, Colm O'Huigin, Ph.D., to coordinate the bioinformatics effort and service provided by the facility. I owe our successes to them, as without their effort, none of our groups scientific progress and contributions would have been possible.

**Giorgio Trinchieri, M.D.**
Director, Cancer and Inflammation Program

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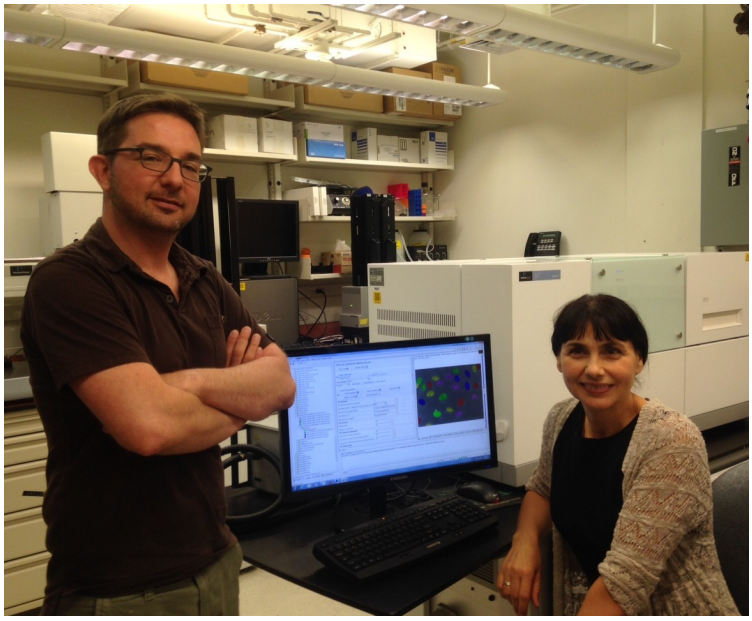
# Development and Validation of a High Throughput Microscopy-Based Assay for Detection of Chemicals with Endocrine Disrupting Properties



Over the years our research group (Hormone Action and Ontogenesis, PI: Gordon Hager, Ph.D.) has developed a number of cell lines expressing fluorescently tagged nuclear receptors, which allow us to study receptor biology and transcription regulation in real time using sophisticated imaging methods. Since its establishment in 2009, the NCI High-Throughput Imaging Facility, previously headed by Ty Voss, Ph.D and now by Gianluca Pegoraro, Ph.D., has streamlined our research. The unique capacity of the Perkin Elmer Opera microscopes to image thousands of samples in a single day at high resolution generated a remarkable amount of high-quality datasets leading to a number of groundbreaking studies (http://www.nature.com/nature/journal/v484/n7392/full/nature10909.html; http://www.sciencedirect.com/science/article/pii/S0092867411007616; http://www.sciencemag.org/content/341/6146/660.long). It also provides scientist with a unique opportunity to expand their research programs in new and uncharted territories. One such example is the high throughput assay for detection of endocrine disrupting chemicals (EDCs), which we developed and validated in collaboration with HiTIF. As their name suggests, EDCs interfere with the endocrine (hormonal) system that governs the development and function of all tissues and organs. Because of the increased concern about contamination of the environment with EDCs, as well as the complexity of their biological effects, significant attention and resources have been devoted to their detection.

The method we developed is based on the fact that, in the absence of the hormone, some nuclear receptors like glucocorticoid or androgen receptors (GR and AR, respectively) reside in the cytoplasm. Upon binding hormone or chemicals with hormonal properties, these receptors translocate to the cell nucleus where they interact with DNA regulatory elements to elicit hormone-specific transcription regulation. The degree of translocation of the fluorescently-tagged receptors from the cytoplasm to the nucleus provides a proxy readout for the hormonal activities present in the cellular milieu. Using this principle we generated a number of mammalian cell lines that allow us to screen for contaminants with androgen, glucocorticoid, thyroid, estrogenic activities, etc. The major advantages of the imaging-based translocation assay over previously described methods are that it is rapid, quantitative, inexpensive and compatible with high-throughput screening. Receptor translocation is completed in 20-30 minutes, and multiple cell lines or multiple genetically tagged fluorescent receptors in the same cell line can be utilized simultaneously. In contrast to chemical methods used to detect EDCs, this assay does not reveal the chemical structure of the active contaminants.

Instead, it detects biologically active EDCs capable of interacting with a specific nuclear receptor (http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3515810/). Considering that nuclear receptors are well established drug targets in various endocrine disorders and cancers, this technology could also be used for discovery of novel therapeutic molecules interacting with target receptors. The development of this technology could not have been possible without the close collaboration between our group and the dedicated and knowledgeable staff of the High-Throughput Imaging Facility!

# The Core Corner Con't

**Section Editor: Anne Gegonne, Ph.D. (*SS*)**

**The High-Throughput Imaging Facility**

High-Content Imaging (HCI) can be employed to probe libraries of perturbing agents, such as siRNA or chemical compounds, in drug discovery, chemical genetics, or functional genomics screens. In addition, HCI is also becoming increasingly popular to systematically quantify biological phenotypes and molecular pathways in hypothesis-driven projects, both in heterogeneous cell populations and at the single cell level. The High-Throughput Imaging Facility (HiTIF) at NCI provides all the instrumentation, software, and expertise to set-up and implement HCI assays in collaboration with CCR investigators. For additional information regarding initiating possible collaborations with HiTIF, please contact the Facility Head: gianluca.pegoraro@nih.gov.

**Diana A. Stavreva, Ph.D. *(SS)***
Laboratory of Receptor Biology and Gene Expression

**Gianluca Pegoraro, Ph.D.**
Head, High-Throughput Imaging Facility,
Laboratory of Receptor Biology and Gene Expression

# The Professional Development Corner

Following a discussion initiated with CCR leadership on SSSC displacement at the 3rd SSSC Professional Development Day in September 2014, the Professional Development Committee has taken some initiatives that were presented at the 2015 SSSC Annual Retreat.

The first initiative is the creation of a CCR SSSC Alumni Database. Using CCR data, the Professional Development Committee has assembled a list of former SSSC who have left CCR since 2007. The Professional Development Committee has retrieved the current affiliation and current job title for most former SSSCs. The Alumni Database has since been uploaded on the SSSC website and is now accessible to all NCI employees. We believe this database represents an incredible tool for SSSCs who may become displaced and who want to explore alternative career paths previously experienced by former CCR SSSCs.

The second initiative undertaken by the Professional Development Committee has been the preparation of a Displacement Checklist for SSSCs. This document is focused on two areas: the first portion is about what can be done before becoming displaced and the second portion is about what can be done after receipt of a 12-month termination notice. The Displacement Checklist for SSSCs contains numerous tips and suggestions to improve SSSC visibility and readiness for professional transition.

Finally, this Displacement Checklist for SSSC has been incorporated in the SSSC New Hiring Handbook, which has also been updated for content and links. The new SSSC Handbook v2.0 will soon be available at the SSSC website after being validated by CCR.

***Christophe Marchand, Ph.D. (SS)***
Laboratory of Molecular Pharmacology

# The Author's Corner

**Section Editor: Cristina Bergamaschi, Ph.D. (*SS*)**

## 2.2 Å Resolution Cryo-EM Structure of β-galactosidase in Complex with a Cell-Permeant Inhibitor

A. Bartesaghi, A. Merk, S. Banerjee, D. Matthies, X. Wu, J.L.S. Milne and S. Subramaniam, "2.2 Å resolution cryo-EM structure of β-galactosidase in complex with a cell-permeant inhibitor," Science 348:1147-1151, (2015).

Cryo-electron microscopy (cryo-EM) is rapidly emerging powerful tool for protein structure determination at high resolution. In this study, we show that it is possible to use this technique to elucidate the architecture of a bacterial metabolic enzyme bound to a drug that blocks its activity, at a resolution high enough to reveal near-atomic detail. This advance provides a new path for solving molecular structures under near-native conditions at resolutions that rival those obtained using X-ray crystallography but without the need for crystallization.

Using cryo-EM, we imaged the small Escherichia coli enzyme β-galactosidase bound to the cell-permeant inhibitor phenylethyl β-D-thiogalactopyranoside (PETG) (Figure 1). Understanding the structure of an enzyme, both with and without a drug bound to it, can aid the design of new inhibitors that either block or enhance the activity of the enzyme.

Using about 40,000 molecular images corresponding to projections of the complex into different spatial orientations, we were able to compute a 2.2 Å resolution map of the structure of β-galactosidase bound to PETG. This map allowed us to determine the exact positioning of PETG within the binding pocket and also enabled us to resolve more than 800 water molecules, visualize density for magnesium and sodium ions, and resolve features of protein secondary structure at resolutions never achieved before by cryo-EM.

At these high resolutions, there is enough information in the structure to enable mapping of contacts between small molecules and their binding sites, information that is useful to reliably assist drug design and development. This process requires not only tracing the shape of the protein chain accurately, but also resolving the hydrogen bonds between the small molecules and the protein it interacts with.

One of the technological advances that helped us achieve these resolutions was the use of direct electron detector technology capable of detecting individual electron events and producing time-resolved movies of the specimen during the exposure to electrons. This process coupled with the use of advanced image processing tools developed in our lab, helped to significantly reduce the incidence of resolution limiting factors like microscope stage drift and beam induced specimen motion.

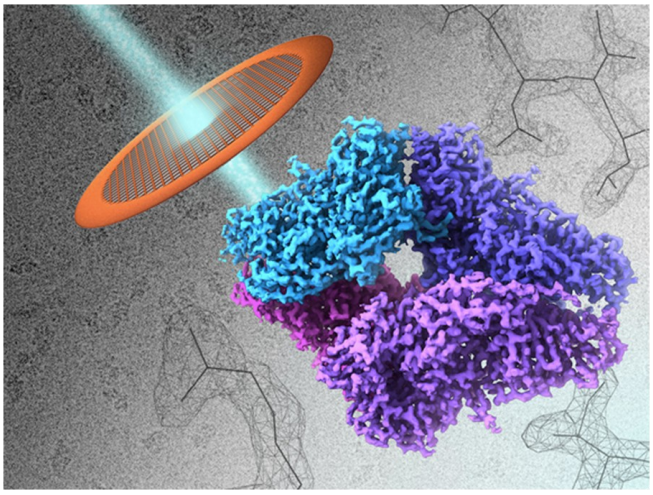


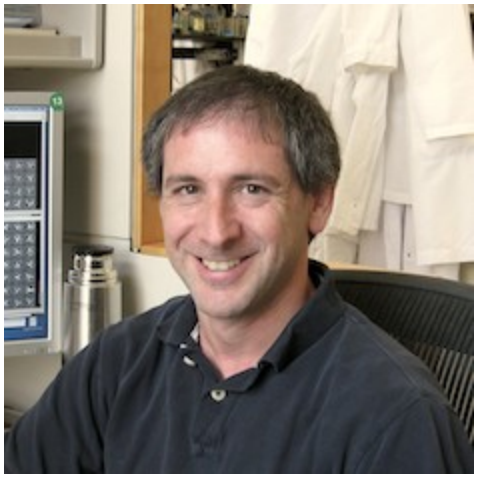
***Figure 1.*** Suspensions of cell-permeant inhibitor PETG bound to β-galactosidase are flash-frozen at liquid nitrogen temperatures onto holey carbon grids, imaged with electrons to obtain two-dimensional molecular images, and combined computationally in three-dimensions to elucidate protein structure at near atomic resolution. Illustration by Veronica Falconieri, CCR, NCI.

Although it is likely that continued technological advances may further enhance the resolutions achieved by cryo-EM, the findings in our work already demonstrate that cryo-EM is positioned to become a very useful tool in structural biology and drug design and development. As such, this work represents an important milestone in the cryo-EM field, serving both as proof that near-atomic resolution can be achieved using this technique and also to spur further technological development aimed at improving even more the resolutions that can achieved using cryo-EM.

# The Author's Corner Con't

**Section Editor: Cristina Bergamaschi, Ph.D. (*SS*)**

*Dr. Alberto Bartesaghi is an Associate Scientist at the Biophysics Section of the Laboratory of Cell Biology. Among his scientific interests, there is the development of novel image processing technologies for cryo-EM to enable determination of protein structure at the highest possible resolution. The tools and training he provides within the lab help support the work of graduate and post-doctoral fellows in the laboratory of Sriram Subramaniam, Ph.D., at the Center for Cancer Research, National Cancer Institute. Dr. Bartesaghi is also involved in various projects in cellular and molecular imaging and collaborates with several intramural and extramural academic partners as well industrial partners through Cooperative Research And Development Agreements (CRADAs).*



**Alberto Bartesaghi Ph.D.** ***(AS)***
*Biophysics Section*
*Laboratory of Cell Biology*

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# A Call for Content

**We need your input! Send your articles or suggestions with subject title "The Dossier" to budhua@mail.nih.gov.**

This newsletter is an avenue for you to express your ideas and thoughts on being a Staff Scientist or Staff Clinician at CCR and to make pertinent announcements.

Your contribution is very important to the success of The Dossier. Please send us your commentary, announcements, and suggestions for topics/subject matter and we will do our utmost to include your material in upcoming issues.

## *Join a SSSC Committee*

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Gabriella Andreotti, Ph.D.



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