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Robert H Singer, PhD

Harold and Muriel Block Chair
Anatomy & Structural Biology
Professor of Cell Biology
Professor of Neuroscience
Co-Director of the Gruss Lipper Biophotonics Center

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March 3, 2015

Richard Conroy, Ph.D.
Director, Division of Applied Sciences and Technology
National Institute of Biomedical Imaging and Bioengineering (NIBIB)
National Institutes of Health
Democracy Plaza II, Suite 200
Bethesda, MD 20892-5469

Re: 1U01EB021236-01

Dear Dr. Conroy,

I would like to add these post-submission materials to our grant application for the 4D Nucleome as provided by NOT-OD-13-030.

The purpose is to clarify the role of the Janelia Research Campus of the HHMI in this proposal. There was not a mechanism to include them in the application process, since no funds are being requested for their participation. Therefore, I am providing information on the personnel involved in this project through the Transcription Imaging Consortium of the Janelia Research Campus including the lead investigator Timothee Lionnet and a letter from the Senior Director of Scientific Services, Reed George describing the acceptable avenue for their participation as described by their policy.

Since this is the first formal NIH-Janelia scientific cooperation, it is important to set appropriate legal boundaries for these interactions.

I hope this helps to clarify the relationship.

Yours,



Robert H. Singer

cc: Maria DeBenardi
Scientific Review Administrator
Center for Scientific Review

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Richard Conroy, Ph.D.
Director, Division of Applied Science and Technology
National Institute of Biomedical Imaging and Bioengineering (NIBIB)
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Re: Application 1U01EB021236-01

Dear Richard,

Referring to the grant proposal submitted by Robert H. Singer, Ph.D., of Albert Einstein College of Medicine for RFA-RM-14-009, entitled 4D Nucleome Imaging Tools (U01), I am writing to clarify institutional contact information for Janelia Research Campus (Janelia), and the role of Janelia and our employee, Timothee Lionnet, Ph.D. in the work proposed.

As Janelia policy precludes us from accepting federal funding, we cannot be considered as a participating institution according to the NIH definition. As a Janelia employee, Dr. Lionnet cannot serve as a Co-Investigator according to the NIH definition, per the same Janelia policy. The policy is posted at <http://www.hhmi.org/sites/default/files/About/Policies/sc900.pdf> should you wish to review it.

As the letter of support from Drs. Tjian and Wu indicates, Janelia's Transcription Imaging Consortium (TIC) is pleased to collaborate with Drs. Singer, Darzacq, and Coleman and their groups, with Janelia bearing its own costs in that pursuit.

While Janelia's budget for efforts including the TIC and the Advanced Imaging Center are proposed and approved on an annual basis, these programs are progressing well, and our intention is for these efforts to continue and for them to be an ideal place for collaboration with groups at other institutions, such as that proposed in the grant application.

For collaboration that occurs at the Janelia campus, Timothee Lionnet, Project Scientist for the TIC, will plan, lead, and participate in the execution of the research described. Therefore, while he cannot serve as a Co-Investigator, we recognize Timothee's leadership role in the collaboration.

Please consider me as your institutional contact at Janelia, full contact information below.

Richard Conroy, PhD
March 3, 2015
Page 2

I hope that these clarifications are useful in your consideration of the proposal.

Best Regards,



Reed A. George
Senior Director of Scientific Services

cc: Maria DeBenardi
Scientific Review Administrator
Center for Scientific Review

Janelia Research Campus of the HHMI Transcription Imaging Consortium (TIC)

TIC Personnel

Timothée Lionnet, TIC Project Scientist and Lead Investigator for the U01 proposal: Timothée leads the TIC core team at Janelia and coordinates the exchange of tools between the different labs participating to the TIC (Berkeley, New York, Janelia and Institut Curie, Paris). He has a strong quantitative background (see biosketch) and oversees the development of the novel microscopes (Multifocus and others), as well as innovative experimental approaches and data analysis methods by the Janelia Team.

Wulan Deng, Research Specialist: Wulan joined the team after her graduate work with Gerd Blobel. She is an expert in cis-regulation of transcription by distal enhancers. Her project with the Janelia Team consists in developing tools to precisely map enhancer-promoter interactions in live cells. She is also developing a fixed cell alternative to DNA-FISH based on the CRISPR system (CASFISH) that will allow imaging DNA loci without the harsh treatments typically used in FISH, thereby preserving the chromatin structure.

Brian Mehl, Postdoctoral Associate: Brian has a spectroscopy background and is developing the next generation Multifocus microscope in collaboration with NIST and the original MFM developer, Sara Abrahamsson (now at Rockefeller). He oversees duplicating the MFM setup onsite (he assembled the system now used at the Janelia AIC) as well as offsite (Berkeley). He is also involved in other microscope development projects trying to improve the efficiency of live cell PALM/STORM experiments for longer experiments at decreased light doses.

Hervé Rouault, Postdoctoral Associate: Hervé is a theoretical physicist who has developed innovative dense single-molecule detection algorithms based on statistical physics computational methods (belief propagation). His work allows imaging more individual molecules per frame, increasing the throughput of PALM/STORM experiments. He is also developing new analysis tools that extract detailed spatial architectural features from chromatin superresolution maps.

Xi (Salina) Long, Postdoctoral Associate: Salina is working on single-molecule RNA FISH, trying to improve the technology for increased throughput using barcoded probes, and combining it with advanced clearing and imaging techniques for tissue imaging. She is also designing nascent RNA probes for superresolved *in situ* determination of genomic loci position as a complement to our live cell approaches.

Anand Ranjan, Research Specialist: Anand joined the team after a postdoctoral training with Carl Wu at NCI. Building on his biochemical expertise on the

remodeling of histone variants H2A.Z by the complex Swr1 in yeast, he is imaging the dynamics of this reaction at the single molecule level in live cells.

Vu Nguyen, Postdoctoral Associate: Vu has a structural biology background in EM microscopy of chromatin remodeling complexes. Using yeast as a system, he will develop strains where components of the transcription machinery are replaced by fluorescent fusions in order to correlate transcription dynamics with local histone properties.

Wu lab

Jan Wisniewski, Senior Scientist: Jan is developing superresolution techniques to image chromatin architecture. Using 3D superresolution, he has been able to resolve the structure of centromeric histones in yeast, and is now building tools to image the spatial dynamics of arbitrary chromatin loci in relationship with their transcriptional activity. He is also involved in testing the novel MFM gratings jointly with Brian Mehl.

Manabu (Gaku) Mizuguchi, Senior Scientist: Gaku is developing biochemical methods to analyze the genome-wide nascent chromatin landscape in the wake of DNA replication in budding yeast. He is also exploring imaging modalities to reveal the dynamics of transcription factors and chromatin regulators during chromosome replication.

Tjian Lab

Zhengjian Zhang, Senior Scientist: Zhengjian is a biochemist expert in purifying the components of the transcriptional machinery. He investigates the dynamics of reconstituted eukaryotic transcription systems in single-molecule *in vitro* experiments. These experiments provide an important complement to the TIC live cell work, as they allow extracting single molecule mechanisms in well-controlled system, and testing hypotheses elaborated from live cell work.

Singer Lab

Brian English, Research Scientist: Brian English is expert in single molecule tracking in live cells. He received his PhD with Sunney Xie at Harvard. He has built the three-color microscope and will provide advice and expertise transferring it to the Einstein site. Using his unique setup, he is developing novel ways to investigate transcription mechanisms in live cells at the single molecule level using multiple labels (e.g. TF, PolII and nascent mRNA).

Albert Tsai, Postdoctoral Associate: Albert Tsai has a background in single molecule *in vitro* imaging at Stanford where he received his PhD with Jody

Puglisi. He is investigating single molecule mechanisms of gene expression in live cells.

Zhe Liu, Junior Fellow: Zhe is expert in live cell imaging of transcription factors at the single molecule level. He did his PhD with Robert Tjian. His studies of the dynamics of the Master regulators of pluripotency in ES cells provide a foundation upon which our proposal will expand.

Janelia Environment

Janelia provides extensive support to the onsite members, from instrument design and fabrication, to molecular, cell biology and animal care. In addition, the Janelia team benefits from constant interactions with other group leaders at the forefront of the chemistry (Luke Lavis) and imaging (Eric Betzig, Harald Hess, Philipp Keller).

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Timothée Lionnet

eRA COMMONS USER NAME (credential, e.g., agency login): TLIONNET

POSITION TITLE: Project Scientist

EDUCATION/TRAINING (*Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.*)

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
Ecole Normale Supérieure, Paris, France.	BS	2001	Physics
Université Pierre & Marie Curie, Paris VI, France	MS	2002	Condensed Matter Physics
Ecole Normale Supérieure, Paris, France	Ph.D.	2006	Biophysics

A. Personal Statement

I am a biophysicist who develops novel imaging technologies to understand the fundamental principles of gene expression.

During my PhD in the group of Vincent Croquette (Ecole Normale Supérieure, Paris), I designed and built a highly sensitive single-molecule magnetic tweezers microscope. This system allowed me to discover that the DNA double helix stretches when overwound, a property bearing profound consequences for DNA sequence recognition. I also uncovered new principles driving the mechanochemistry of DNA helicases. I then joined the laboratory of Robert H. Singer (A. Einstein College of Medicine, New York) in order to continue developing single molecule technologies, this time inside cells and animals. These include a mouse line in which individual mRNA molecules of an endogenous gene can be detected by fluorescence, as well as a biosensor reporting on the translational state of single mRNAs in live cells and animals. Using these technologies, I was able to discover fundamental principles in various biological systems, from the “clueless gene” model in yeast, to the spatiotemporal regulation of the influenza virus genome assembly.

I am now the Project Scientist for the Transcription Imaging Consortium (TIC) at the H.H.M.I. Janelia Research Campus. In this position, I lead a Janelia-based research group that develops innovative fluorescence technologies to understand the rules that govern transcription at the single molecule level. We engineer tools that range from novel microscopes, fluorescent reagents and labeling techniques, to image analysis and visualization software. With assistance from other Steering Committee members, I also drive the global effort of the Consortium. Our mission is to accelerate our understanding of transcription through collaborative work and free flow of tools, technologies, people and ideas between participating labs. The Consortium currently consists of ten core laboratories worldwide, plus ten partner labs.

B. Positions and Honors**Positions and Employment**

2002-2006 Doctoral student, Ecole Normale Supérieure, Paris.
2006-2007 Postdoctoral fellow, Ecole Normale Supérieure, Paris.
2007-2012 Research Associate, Albert Einstein College of Medicine, Bronx, NY
2012-2013 Instructor, Albert Einstein College of Medicine, Bronx, NY
2013-present Project Scientist, H.H.M.I. Janelia Research Campus, Ashburn VA

Other Experience

2004-2006 Graduate teaching assistant, Biophysics, Université Pierre & Marie Curie, Paris.
2003-2005 Undergraduate teaching assistant, Physics, Université Pierre & Marie Curie, Paris.
2001 Undergraduate research assistant, supervisor: Carlos Bustamante, U.C. Berkeley.

Honors

2011 Albert Einstein College of Medicine Dennis Shields Postdoctoral award
2008-2011 Human Frontier Science Program long term fellowship
2003-2006 Graduate fellowship, French Ministry of National Education.
1999-2002 Undergraduate fellowship, Ecole Normale Supérieure, Paris.

B. Contribution to Science

1. During my PhD, I designed and built a magnetic tweezers microscope to manipulate individual DNA molecules with nanometer precision. I first studied how helicases use chemical energy (ATP) to unzip DNA. I discovered that when isolated from the replisome, replicative helicases only have the ability to passively unwind DNA, functioning as brownian ratchets that lock transient openings of the fork. In a separate project, I discovered a novel, counterintuitive property of DNA: the double helix stretches when overwound. This effect strongly depends on the nature of the basepairs, suggesting a sequence recognition mechanism through mechanical rather than biochemical means. This work resulted in 6 publications and 289 citations.

- a. Dessinges MN, **Lionnet T**, Xi XG, Bensimon D, Croquette V (2004). Single-molecule assay reveals strand switching and enhanced processivity of UvrD. *Proc. Natl. Acad. Sci.*, 101 (17): 6439-44
- b. **Lionnet T**, Spiering MM, Benkovic SJ, Bensimon D, Croquette V (2007). Real-time observation of bacteriophage T4 gp41 helicase reveals unwinding mechanism. *Proc. Natl. Acad. Sci.* 104 (50): 19790
- c. **Lionnet T**, Joubaud S, Lavery R, Bensimon D, Croquette V (2006). Wringing out DNA. *Phys. Rev. Lett.*, 96 (17): 178102

2. As part of my postdoctoral work, I developed multiple technologies to detect single mRNAs in live cells and animals. These include a reporter mouse we used to characterize the regulation of actin transcription; a biosensor that identifies which mRNA molecules have undergone translation. Combining quantitative imaging with theoretical modeling, I was able to uncover fundamental regulatory rules: for example, yeast cells do not coordinate the transcription of even functionally related genes, leading to a “clueless gene” model of transcription. I was also able to identify a novel regulatory step in the assembly of the RNA segments that form the influenza genome, a result important enough to become part of the latest Influenza textbook. This work resulted in 13 papers cited 330 times.

- a. Halstead, JM*, **Lionnet T***, Wilbertz JH*, Wippich F*, Ephrussi A, Singer RH, Chao JA (2015) An RNA biosensor for imaging the first round of translation in single cells and living animals. *Science* 347 (6228) 1367 (*: **equal contributions**)
- b. **Lionnet T**, Czaplinski K, Darzacq X, Shav-Tal Y, Wells AL, Chao JA, Park HY, de Turris V, Lopez-Jones M, Singer RH. (2011) A transgenic mouse for in vivo detection of endogenous labeled mRNA. *Nature Methods* 8 (2) 165-70.
- c. Chou YY, Heaton NS, Gao Q, Palese P, Singer R, **Lionnet T**. (2013) Colocalization of different influenza viral RNA segments in the cytoplasm before viral budding as shown by single-molecule sensitivity FISH analysis. *PLoS Pathogens*, 9 (5) e1003358
- d. Gandhi SJ, Zenklusen D, **Lionnet T**, Singer RH (2011). Transcription of functionally related genes is not coordinated. *Nature Structural & Molecular Biology*, 18 (1) 27-34

3. In my current work at Janelia, I develop technologies for single molecule imaging of various transcription regulators. Collaborating with the various groups composing the Transcription Imaging Consortium, we have characterized new fluorescent reagents and dissected the target search mechanism of Transcription Factors. We now are trying to uncover the rules leading to robust long-lived gene expression profiles from transient, noisy interactions between the various players in the transcription process.

- a. Grimm JB, English BP, Chen J, Slaughter JP, Zhang Z, Revyakin A, Patel R, Macklin JJ, Normanno D, Singer RH, **Lionnet T***, Lavis L*. (2015) A general method to improve fluorophores for live-cell and single-molecule microscopy. *Nature Methods* 12 (3) 244-50 (*: **corresponding authors**)

- b. Chen J, Zhang Z, Li L, Chen BC, Revyakin A, Hajj B, Legant W, Dahan M, **Lionnet T**, Betzig E, Tjian R, Liu Z. (2014). Single-molecule dynamics of enhanceosome assembly in embryonic stem cells. *Cell* 156 (6) 1274-85

Complete List of Published Work in MyBibliography:

<http://www.ncbi.nlm.nih.gov/sites/myncbi/1hmfASIRN8nAH/bibliography/47318382/public/?sort=date&direction=ascending>

D. Research Support

The research of my group at Janelia is funded internally. As an HHMI employee of the Janelia Research Campus, I am not allowed to request external funding.

Completed Research Support:

French Ministry of education/Paris VII University Graduate Fellowship Lionnet Timothee	2003-2006
LT00331/2008-L Human Frontier Science Foundation Long Term Fellowship Lionnet Timothee	2008-2011