CURRICULUM VITAE: Nicholas Grammatikakis, Ph.D.

Work Address:

Cell Signaling and Molecular Chaperones Laboratory, Director

Institute of Biology, National Centre for Scientific Research "Demokritos"

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Position Title: Research Director
Place of Birth: Lassithi, Crete

Citizenship: Greece/European Union (E.U.)

Current research activities

My general research expertise is in Cellular and Molecular Biology Research and, overall my graduate studies, training and work have all focused in the Signal Transduction in Cancer and in response to Stress. During the first part of my career (1982-1991), my laboratory investigations have focused experience in the areas of genomics and transcriptional gene regulation. Since 1992, working essentially as an independent investigator, I have focused on studying the basic mechanisms regulating cell signaling, cell cycle progression and cell transformation.

A) Cell Signaling

- Mechanisms of mammalian kinase regulation during normal differentiation and disease

- Chemotherapeutical inhibition of oncogenic kinase activity

B) Cellular Responses to Stress

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Regulation of Chaperone Protein Activity

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Identification of Signaling Mediators (including kinases and transcriptional factors) which are modulated by the Chaperone Machinery in transformed cells and in response to Stress

C) Cell Cycle Regulation

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The Chaperone Machinery as an effector of cellular Stress in cell cycle progression

D) Novel Molecular Chaperones

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Identification of novel Molecular Chaperones and study of their potential role as mediators of the assembly and activity of mammalian kinases in cell proliferation and cell cycle progression. Our study extends to learning how the activity of these novel signal modulators is regulated by

Growth conditions and Stress and in malignancy.

I have a long tracking record in research areas outlined above. For the last 16 years I have worked independently and I have been primarily responsible for designing and conducting experiments using various experimental models including cultured cells and animal models. During that time I have supervised and coordinated the efforts of laboratory staff (technical and students) in the field of Signaling Chaperones. In parallel, I have been involved in composing grants and editing all the manuscripts where I am the first or corresponding author (8 manuscripts in total).

Doctoral Dissertation:

In Molecular Biology: “Cloning and Functional Analysis of the Transferrin Gene”, Department of Cell Biology and Human Genetics, the University of Texas Medical Branch at Galveston, Texas, 1989.

Previous Professional Experience:

--**2001-2004:** Research Assistant Professor, Center for the Molecular Stress Response, Boston University School of Medicine; Harvard University/ Beth Israel Deaconess Medical Center Boston, MA (Instructor). **Research topics**: Regulation of heat shock factor intracellular trafficking and activity and identification of signaling pathways involved; Regulation of the ErbB2 and NF-kappaB pathways by the chaperone machinery; HSF (Heat Shock Factor) isoforms and activity; Cdc37-mediated IKK assembly and activity.

--2000-2001: Research Associate, Department of Microbiology and Immunology, Queen's University, Kingston, ON. The role of molecular chaperones in LPS and TNFa-mediated signaling in macrophages; Study of a specialized group of chaperones and chaperone cofactors serving as partners and potential regulators of viral oncoproteins (SV40 Large-T, Hepatitis B reverse transcriptase, Tax and Tat).

--10/2001-4/2001: Research Associate, Lankenau Institute

--1995-2000: Research Associate, Dept. of Physiology, Tufts Univ., Boston, MA (laboratory of Dr B. Cochran). Regulation of Raf-1 by the Cdc37/Hsp90 chaperone system; Identification and study of components of native mammalian kinase complexes; Characterization of a kinase-specific chaperone pathway in the production of a Cdk9/Cyclin T1 heterodimer during P-TEFb-mediated Tat stimulation and HIV-1 transcription.
--1991-1995: Postdoctoral Fellow, Dept. of Anatomy and Cell Biology, Tufts Univ., Boston, MA (laboratory of Dr Bryan Toole). Hyaluronan-receptors in malignancy; Isolation and study (structure-function) of the Cdc37 family proteins in human, mouse and chicken; Regulatory roles of Cdc37, Hsp90 and other members of the chaperone machinery in signal transduction; Regulation of Cdk4/Cyclin D assembly and activity by the chaperone machinery.
--1982-1989: Ph.D. Candidate, University of Texas Medical Branch and NCI/FCRF (laboratories of Drs L. Chan and J. Papaconstantinou). Isolation and study of the regulation of the transferrin and the transferrin receptor genes; Transcriptional regulation of viral and cellular genes by viral oncoproteins of the HTLV family.

Education:
1982-1989: University of Texas Medical Branch, Galveston, TX (Ph.D. in Molecular Biology)
1977-1982: School of Biology, University of Thessaloniki, Greece (B.Sc. in Biology)
1976-1977: Agricultural University of Thessaloniki, Greece.

Awards and Fellowships:

10/2005: Research Program was evaluated by the General Secretariat for Research and Technology (Ministry of Development, Greece) as one of the three most promising at the Institute of Biology (National Centre for Scientific Research "Demokritos"/ see APPENDIX)

4/2002: American Cancer Society Award for Beginning Investigators. Title of the study: “The IKK regulator p50 ^{cdc37} as a potential LMP1 effector and target in cancer chemotherapy”.
4/2001: Queen’s Postdoctoral Fellowship.
1997-2000: Research supported by the US/DOD Breast Cancer Research Program.
1996-1997: Supported by L.P. Markey Award (Tufts University).
1987-1989: Research supported by National Cancer Institute Fellowship (NCI/FCRF).
1982-1987: Graduate studies supported by full State Fellowship.

1982: I was awarded one of the two Scholarships given by of the Hellenic Scholarship Foundation for Graduate studies in England (declined).
1976-1982: Government Scholarship, awarded to top 10 out of 150 candidates on admission, and subsequently awarded each year until graduation for being among the top ten in each year

Teaching and Supervising Experience:
--1980-1982: As a 3rd and 4th year undergraduate student (University of Thessaloniki) I worked closely with Dr Nitska Beis in establishing a complete series of undergraduate labs for the Biology students. During that time, I also assisted Professor C. Arsenis in editing a three-volume textbook on Developmental Biology.

As a graduate student I taught labs in Cell Biology to the 2nd year medical students (University of Texas).

--1992-1995: I taught “Molecular Biology Techniques” to graduate students of the Sackler School of Graduate Studies (Tufts University).

In addition, I have supervised and directed the work of a great number of colleagues, including: Elizabeth Gerhardt, Jeanette Banks, Mark Eckley (1982-1989), Masahiko Yoneda, Aliki Siganou, Alfredo Sabbah, Santra Panse, Conrado Jones, Michael Alexandrakis, Lei Huang, Qin Yu, Majieh Kotecki, and Jay Lee (1991-2000), Shen Gan, Melik Okzoz, Adina Vultur and Hideobu Takahashi (2001-2004). At the NCSR Demokritos, I have supervised a research technician (Aliki Siganou) and the work of two Ph.D. candidates (Sofia Aliberti and Avraam El Hamidie).

Patents granted:
"Vertebrate Cdc37"
Country: WO World Intellectual Property Organization (WIPO)
World-Wide Patent Number: WO9801463A1
Application Number: WO1997US0011737
Date Issued: Jan.15, 1998

Inventors: Grammatikakis N, *et. al.,*

"Nucleic acids encoding vertebrate Cdc37"
Country: USA
USA Patent Number: US6066723
Application Number: US1996000675885
Date Issued: May 23, 2000
Inventors: Grammatikakis N, *et. al.,*

Scientific Articles:

1. Chan LN, **Grammatikakis N,** Banks JM, Gerhardt EM. Transferrin receptor gene: conservation of 3 noncoding sequences and expression in erythroid cells. Nucleic Acids Res 1988, 25; 17(10): 3763-71.
2. **Grammatikakis N**. Cloning and functional analysis of the murine transferrin gene. (Doctoral Dissertation, The University of Texas Medical Branch at Galveston, Texas, 1989).
3. Pavlakis G, Felber B, Kaplin G, Paskalis H, **Grammatikakis N,** and Rosenberg M 1989. Regulation of expression of the HTLV family of retroviruses. In The Control of Human Retrovirus Gene Expression (Franza BR, Jr, Cullen BR, and Wong-Staal F, eds.), CSH Press, Cold Spring Harbor, NY, pp. 281-289.
4. **Grammatikakis N**, Grammatikakis A, Yoneda M, Yu Q, Banerjee SD, Toole BP. A novel glycosaminoglycan binding protein is the vertebrate homologue of the cell cycle control protein, Cdc37. **J Biol Chem** 1995, Jul 7; 270(27): 16198-205.
5. **Grammatikakis N**, Toole BP. Functional domain mapping using M13 deletions. Biochemistry and **Mol Biol Int** 1995; 36(4): 771-9.

6. **Grammatikakis N**, Siganou-Grammatikakis A, Yoneda M, Yu Q, Banerjee SD, Toole BP. Cell cycle under control of glycosaminoglycans. Trends in Glycoscience and Glycotechnology 1995, Nov 5; 7(38): 525-536.

7. Yu Q, **Grammatikakis N,** Toole BP. Expression of multiple CD44 isoforms in the apical ectodermal ridge of the embryonic mouse limb. **Developmental Dyn** 1996; 207(2):

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8. Huang L, **Grammatikakis N,** Toole BP. Organization of the chick CDC37 gene. **J Biol Chem** 1998, 6; 273(6): 3598-603.
9. Silverstein AM, **Grammatikakis N**, Cochran BH, Chinkers M, Pratt WB. p50(cdc37) binds directly to the catalytic domain of Raf, as well as to a site on Hsp90 that is topologically adjacent to the tetratricopeptide repeat (TPR) binding site. **J Biol Chem**
1998; 273(32): 20090-5.
10. **Grammatikakis N** (Corresponding Author), Lin JH, Tsihlis P., Cochran BH. p50(cdc37) acting in concert with Hsp90 is required for Raf-1 function. **Mol. Cell. Biol.** 1999;
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11. Ramana CV, **Grammatikakis N**, Chernov M, Nguyen H, Goh K, Williams B, Stark GR. Regulation of c-myc expression by IFNg through Stat1 dependent and independent pathways. **EMBO J**
2000; 19(2):263-72.
12. Huang L, **Grammatikakis N**, Yoneda M, Banerjee SD, Toole BP. Molecular characterization of a novel intracellular hyaluronan-binding protein.
J Biol Chem.
2000 Sep 22; 275(38):29829-39.
13. Shao Y, **Grammatikakis N**, Scroggins B, Uma S, Huang W, Hartson SD and Matts RL, 2000. p50Cdc37 and Hsp90 -mediated Biogenesis of the Active Conformation of the Heme-regulated eIF2a Kinase. **J Biol Chem**. 2001 Jan 5;276(1):206-214.
14. Kumar R, **Grammatikakis N**, Chinkers M, 2001. Regulation of the ANP receptor by Heat Shock Protein 90 complexes. **J Biol Chem.** 2001 Apr 6;276(14):11371-11375.
15. **Grammatikakis N,** Jaronczyk K, Grammatikakis-Siganou A, Vultur A, Brownell HL, Benzaquen M, Rausch C, Lapointe R, Gjoerup O, Roberts TM, RaptisL.Simian virus 40 large tumor antigen modulates the Raf signaling pathway. **J Biol Chem** 2001 Jul 27;276(30):27840-27845.
16. Broustas C, **Grammatikakis N,** Eto, M, Dent, P., Braudigan, D., Kasid U. Phosphorylation of myosin-binding subunit of myosin phosphatase by Raf-1 and inhibition of phosphatase .
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, 2002 Jan 25;277(4):3053-9.
17. **Grammatikakis N** (Corresponding Author), Vultur, A., Ramana, C.V., Siganou, A., Schweinfest, C.W., Watson, D. and Raptis, L.The role of Hsp90N, a novel member of the Hsp90 family, in signal transduction and neoplastic transformation. **J Biol Chem.,** 2002
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18. Grinberg M, Sarig R, Zaltsman Y, Frumkin D, **Grammatikakis N**, Reuveny E, Gross A. tBID Homooligomerizes in the mitochondrial membrane to induce apoptosis.
J Biol Chem.
2002 April 5; 277(14):12237-45.
19. Vultur V, Keiski C-L, Maynard M, **Grammatikakis N** and Raptis L. Increased efficiency of in vivo isotopic labelling of adherent cells.

J. Virol. Methods
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21. Wang X, **Grammatikakis N**, Hu J. Role of p50Cdc37in hepadnavirus assembly and replication. **J Biol Chem.** 2002 Jul 5;277(27):24361-7.

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23. Wang A., **Grammatikakis N,** Siganou A, Calderwood SK. Regulation of Molecular Chaperone Gene Transcription Involves the Serine Phosphorylation, 14-3-3 epsilon Binding, and Cytoplasmic Sequestration of Heat Shock Factor 1. **Mol Cell Biol**. 2003 Sep; 23(17):6013-26.

24. Peng XY, Guo XX, Abdul KM, **Grammatikakis N**, Bharti A, Calderwood S, Sawyer DB, 2003. HSP90 regulates ErbB2 stability and expression in cardiac myocytes, **Circulation**
108 (17): 965

25. (Underlined authors had equal first author contribution) He H., Soncin F., **Grammatikakis N**, Siganou A., Yie Y., Calderwood, SK. Elevated expression of heat shock factor 2a stimulates HSF1-induced transcription during stress. **J Biol Chem**. 2003 Sep12;278(37): 35465-75.

26. Zhang H, Wu W, Du Y, Santos SJ, Conrad SE, Watson JT, **Grammatikakis N**, Gallo KA. Hsp90/p50cdc37 is required for mixed-lineage kinase (MLK) 3 signaling. J Biol Chem. 2004 May 7; 279(19): 19457-63.

27. (Underlined authors had equal first author contribution) Wang X, **Grammatikakis N**, Siganou A, Stevenson MA, Calderwood SK. Interactions between extracellular signal-regulated protein kinase 1, 14-3-3epsilon, and heat shock factor 1 during stress.
J Biol Chem
. 2004 Nov 19;279(47): 49460-9.

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J Cell Sci.
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Trends in Cell & Molecular Biology
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30. El Hamidieh A, **Grammatikakis N**. and Patsavoudi E. Cell surface Cdc37 participates in extracellular Hsp90 mediated cancer cell invasion. PLoS One. 2012; 7(8):e42722.

-- Zhang N., and **Grammatikakis N**. Cdk4 regulation by the Cdc37/Hsp90 foldosome (work presented at The Cell Cycle meeting, Cold Spring Harbor, NY and draft in preparation).

--El Hamidie A. and **Grammatikakis N**. Cdc37 is represented by multiple isoforms in human cells which have dinstinct functions in cell signaling (in preparation).

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Paper No.10 (**Grammatikakis N** (Corresponding Author), Lin JH, Tsihlis P., Cochran BH. p50(cdc37) acting in concert with Hsp90 is required for Raf-1 function.
Mol. Cell. Biol.
1999; 19(3): 1661-72) has been characterized as a “ground breaking” in the field; and has attracted close to 200 citations (self-references not included).

Appentix:

Η τελευταία αξιολόγηση του Ινστιτούτο Βιολογίας από την διεθνή επιτροπή κατά την αξιολόγηση των Ερευνητικώ ν Κέντρων της χώ ρας, Γενική Γραμματεία Έρευνας και Τεχνολογίας (ελήφθη από την ιστοσελίδα της GGET)

PHASE 2 EVALUATION REPORT

INSTITUTE OF BIOLOGY

NATIONAL CENTRE OF SCIENTIFIC RESEARCH "DEMOKRITOS"

October 11, 2005

General Comments

The Institute of Biology (IB) has a diverse research agenda. It is striving to be competitive in three general areas identified as Programs. The Programs are:

Program A: Regulation of Cellular Function/Age-Related Diseases

Program B: Model Biological Systems for the Study of Cellular Functions

Program C: Structural and Computational Biology

The Programs have two thrusts, biomedicine and biotechnology, which, in turn, are divided into the following topics.

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In vitro models of cancer, diabetes, senescence, and neurodegenerative diseases

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Molecular mechanisms of normal cellular signaling

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Changes occurring in diseased conditions

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Development of pharmaceuticals, diagnostics and therapeutics to interfere with these diseases or their progress and complications

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Genetic and biotechnological approaches for the development of insect cell and viral vectors for recombinant proteins of agricultural importance

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Proteins of medical relevance with the prospect of gene therapy

The IB is one of eight institutes in the National Center for Scientific Research “Demokritos” (NCSR). Significantly, it represents life sciences in a center that is dedicated primarily to nuclear physics and technology, materials science, informatics/telecommunications and radio-technological applications. As such, it appears to be an island unto itself despite the newly established collaboration with the Institutes of Physical Chemistry and Materials Science to develop and use new biomedical and nanoscience-related approaches for interfering with certain human diseases. Within the IB, there are four special laboratories listed.

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Human Tissue Bank

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Animal Facility

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Structural Unit

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Confocal Microscopy

There are 18 Researchers in the IB with the rank of Researcher A, B or C (2004 data). This number represents approximately 14% of the Researchers in the NCSR. Based on total income (total expenditure) from regular public budget funding, public investment funds and EU framework programs, the IB income represents 6.7% of the NCSR income (2004 data).

Assessment of the IB

Research in the IB is varied and broad ranging—a factor that has prevented the Institute from becoming recognized nationally and internationally. The only program that enjoys true

international recognition is Insect Molecular Genetics and Biotechnology led by Dr. K. Iatrou. It has great potential for biotechnological applications in agriculture, medicine and the environment. All areas can be leveraged scientifically and commercially. However, the highest payoff would be in agricultural and environmental applications. Dr. Iatrou is an accomplished scientist. He has vision and foresight. He is industrious, hard-working and creative. He appears to have good people skills and is highly organized.

All other research areas in the IB are diffuse with no obvious focus although there is an attempt to relate several research projects to age-related diseases such as diabetes, senescence and cancer, among others. From this particular effort, one unifying theme does emerge—cell signaling. The IB has a window of opportunity, albeit rapidly closing, to become premier in molecular signaling as it relates to oncogenesis and cell death. Research in this area would be useful for identifying potential drug targets. The development of radio-diagnostics also has potential but the efforts in this area are not well defined or coordinated. Dr. D. Kletsas is a bright and shining star who, with appropriate and adequate support, could bring prominence to a research program that emphasizes cellular signaling and cell-cell communication. **Dr. N. Grammatikakis is a valuable addition to the IB who also could help establish the Institute as an international leader in this important and exciting area of science. These two investigators should be able to formulate a research program in cellular signaling, with the help of other scientists in the Institute, which is second to none. Their presence in Greece provides a tremendous opportunity to promote basic science in the Country as well as to attract attention and financial support from the pharmaceutical industry.**

An additional program area that deserves considerable attention in terms of funding support is Structural and Computational Biology led by Drs. M. Vlassi and M. Pelecanou. This program area could be an integral part of the two aforementioned program areas as well as provide some service outside the Institute. Furthermore, this program area should be able to stand on its own by attracting a considerable amount of extramural funding from the government of Greece, the EU and private industry. Regretfully, the funding level for the entire program is insufficient. Dr. Vlassi has had moderate success in securing research monies whereas Dr. Pelecanou has not been very successful in this regard. Dr. Pelecanou has an excellent scientific publication record both in quantity and quality whereas Dr. Vlassi has been much less productive. The lack of research focus is a serious problem for this program area.

Another problem in this program area is the lack of support personnel and insufficient funds for repair and maintenance of the equipment. The x-ray crystallographic system is less than state-of-the-art despite the statement to the contrary in the IB Business Plan. The NMR spectrophotometers are adequate for a variety of research applications although there should be serious consideration of acquiring higher resolution NMR capability. Overall, the current instrumentation available to this program area is not of the caliber suitable to support world-class research in structural biology. If this program area is to flourish, considerable effort and money need to be expended to bring it up to internationally competitive standards.

In spite of the weaknesses and problems mentioned above, this particular research area holds much promise. The program needs immediate direction. It needs to be led by one person either by Dr. Vlassi, Dr. Pelecanou or an individual recruited from outside the Institute. Dr. Pelecanou obviously is an aggressive and productive scientist. Given adequate support and consideration, she should be able to turn the program around and point it in the right direction. Otherwise, a new person should be recruited immediately. The program needs an infusion of new money (at least 1.5M€) from within Greece to purchase new instrumentation and hire support personnel.

The productivity of each individual scientist in the IB is given in Table 1. The data indicate the amount of competitive funds received by each individual serving as a principal investigator (Coordinator). It does not take into account monies received from collaborative grants. The number of publications is a quantitative index of the productivity of each researcher. It does not reflect the “quality” of science accomplished nor does it indicate the contribution made by each person to each paper published or to science in general. However, it does reflect the wide variation in research funding and productivity among the scientists.

Table 1. Productivity of individual scientists

Name	Total Amount of Funding (€)*	Number of Publications in Refereed Journals
1. Y. Almrantis	0	7
1. Z. Georgoussi	164,814	2
1.		

N. Grammatikakis

71,740

16

1.

K. Iatrou

1,307,500

13

1.

P. Kitsiou

90,000

4

1.

D. Kletsas

97,720

13

1.

V. Labropoulou

0

0

1.

M. Pelecanou

23,500

17

1.

A. Prombona

0

3

1.

K. Sekeri-Pataryas

24,460

12

1.

E. Sideris

0

9

1.

V. Sophianopoulou

161,800

8

1.

T. Sourlingas

32,608

13

1.

K. Stamatakis

0

1

1.

L. Swevers

0

7

1.

E. Tsilibary

187,466

6

1.

A. Tzinia

50,000

4

1.

M. Vlassi

207,646

4

*Competitive funds received by individuals listed as Coordinator in the Business Plan (2004 data)

Recommendations

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Reorganize the IB into two new institutes, one that encompasses insect molecular genetics and biotechnology and another that involves cellular signaling and human disease.

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Appoint Dr. Iatrou as Institute Director for Insect Molecular Genetics and Biotechnology.

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Appoint Dr. Kletsas as Institute Director for Cellular Signaling and Human Disease.

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Assign the most productive scientists in the IB to either of the two institutes as appropriate.

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Relocate both institutes to other research centers.

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If the above recommendations are not possible, the following actions should be taken:

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1.

Reorganize the IB into two program areas: (i) insect molecular genetics and biotechnology and (ii) cellular signaling and human disease with Dr. Iatrou and Dr. Kletsas serving as program leaders of the respective units.

2.

Upgrade the structural biology unit so that it can stand alone as a single program area and appoint a highly qualified individual to lead the program.

3.

Integrate the three program areas so that true synergy can be realized.

4.

Recruit a new director of the IB from outside the Institute. A national/international search is necessary.