

Program Announcement To DOE National Laboratories
LAB 01-20
Microbial Cell Project

The Offices of Biological and Environmental Research (OBER), Basic Energy Sciences (BES), and Advanced Scientific Computing Research (ASCR) of the Office of Science (SC), U.S. Department of Energy, hereby announce their interest in receiving proposals for research in support of the Microbial Cell Project (MCP), an effort to build on information from completely sequenced microbial genomes to achieve a more comprehensive understanding of the functioning of a prokaryotic microbial cell. This announcement encourages proposals from interdisciplinary scientific partnerships or teams that include such disciplines as microbiology, molecular biology, applied mathematics, biochemistry, structural and computational biology, as well as physics, chemistry, engineering and computer science. The MCP is focused on fundamental research to understand those reactions, pathways, and regulatory networks that are involved in environmental processes of relevance to the DOE, specifically the bioremediation of metals and radionuclides, cellulose degradation, carbon sequestration, and the production, conversion, or conservation of energy (e.g. fuels, chemicals, and chemical feedstocks). Research areas of particular interest that should be integrated into an interdisciplinary approach can include studies of: 1) functional analysis of the microbial proteome; 2) biochemical and physiological characterization; 3) intracellular localization; and 4) cell modeling. This announcement represents a planned first step in an ambitious effort to understand the functions of all the macromolecular components in a microbial cell, to understand all their interactions as they form pathways and processes that are related to DOE-relevant activities, and to eventually build predictive models for microbial activities that address DOE mission needs.

SUPPLEMENTARY INFORMATION: The Microbial Cell Project (MCP) supports key DOE missions by building on the successful DOE Microbial Genome Program that has furnished microbial DNA sequence information on microbes relevant to environmental remediation, global carbon sequestration (e.g. CO₂ fixation), complex polymer degradation (e.g. cellulose and lignins), and energy production (fuels, chemicals, and chemical feedstocks). These microbial genome sequences provide a finite set of "working parts" for a cell; the challenge now is to understand how these parts are assembled into functional pathways and networks to accomplish activities of interest to the DOE (specifically those identified in the preceding sentence.) The traditional reductionist experimental approach has defined specific steps or stages within many physiological processes; however, the availability of whole genomes affords the opportunity to integrate these individual pathways into

a larger physiological or whole organism framework. The MCP seeks to integrate available information about individual processes and regulatory complexes to understand the intracellular environment in which these pathways and networks exist and function. The DOE Microbial Cell Project is part of a coordinated Federal effort called the Microbe Project involving elements from several other Federal agencies.

This announcement strongly encourages interdisciplinary teams that assemble a range of expertise into an integrated approach to characterizing the structure and function of a prokaryotic cell. The purpose of encouraging interdisciplinary teams is to combine diverse scientific talents into a coordinated program and thus it is very important that a coordination plan describing how the whole exceeds the sum of the parts be included in the proposal. In addition, the MCP seeks to promote research on the internal organization and complex control systems that allow microbial cells to respond to their environment, to make unique products, and to carry out specialized functions relevant to DOE missions in the bioremediation of metals and radionuclides, cellulose degradation, carbon sequestration, and the production, conversion, or conservation of energy. This effort will exploit a range of approaches, among them: 1) functional analyses of proteins and protein interactions; 2) metabolic and flux measurements; 3) intracellular imaging technologies for the localization and quantitation of proteins and other cellular constituents; and 4) computational modeling to represent the activities of a cell in ways that permit testable predictions of microbial cell functions.

Preference will be given to those proposals selecting prokaryotic microbes that satisfy all of the following criteria: a) the chosen microbe is of DOE mission-relevance, i.e., can bioremediate metals and radionuclides, sequesters environmental carbon, e.g., can fix CO₂, degrades significant biopolymers such as celluloses and lignins, or generates energy sources, fuels, chemicals, and chemical feedstocks. Strict pathogens or parasites will not be considered; b) complete or near-complete genomic sequencing information from the chosen microbe exists in the public domain; c) the chosen microbe grows sufficiently in culture to enable experimental work; d) the chosen microbe can easily be genetically transformed; and e) expression vectors are available. Of particular importance will be a clear description of a coherent plan for making efficient use of the available sequence information. (See <http://www.ornl.gov/microbialgenomes/organisms.html> for a current list of microbes that have been and are being sequenced.) If a group proposes to carry out work under this announcement on a specific microbe, it should be prepared to justify the merits of the chosen target organism to the peer review process. It is expected that each project supported by the MCP will be focused on an energy-related or environmentally relevant microbe (or group of microbes) for which extensive sequence information is known, although proposers may take advantage of relevant information derived from

other microbes that are not considered DOE targets, e.g. *E. coli* or yeast. While integrated and multidisciplinary consortia are strongly encouraged, exceptional proposals from individual investigators focused on more confined aspects or areas may be considered.

This program announcement encourages research proposals that integrate the following highly interrelated thrusts, using a single, sequenced, DOE-relevant microbe as the unifying cornerstone. For the purposes of this announcement, the interests of DOE are the bioremediation of metals and radionuclides, cellulose degradation, carbon sequestration, and energy production, conversion, or conservation. Integrated proposals should include a careful description of how the project's proposed interdisciplinary research team will integrate all or most of the following components into a single research project. These components are:

1) Functional Analysis of the Microbial Proteome. It is presently difficult, and in many instances impossible, to predict biological function from microbial genomic sequence data, even when the entire genome has been sequenced and is available for inspection. Proposals should discuss better ways to exploit sequence data from novel open reading frames, and even whole genomes, to characterize the pathways and networks that mediate microbial physiology and function, and how they are regulated under different environmental conditions. This effort can take place at different levels of resolution: a medium-resolution (less detailed) analysis of novel or unannotated genes and open reading frames across an entire sequenced microbial genome or a higher-resolution (more comprehensive) analysis of novel or unannotated genes and open reading frames that participate in one or a few processes supporting the stated interests of DOE. The research emphasis should be on whole genome approaches to functional prediction, functional regulation, functional categorization (at medium resolution), or on specific systems, e.g., redox enzymes, metal reductases, or hydrogen or methane production components (at high resolution). Proposals may include the use of new high-throughput technologies/tools to better understand expression patterns and protein profiles, as well as the exploitation of functional manipulations to better understand pathways relevant to the DOE. Identification of domains in gene sequences that mediate protein-protein interactions that are part of these kinds of pathways are also of great interest. An explicit intention of this announcement is to promote research on DOE mission relevant protein complexes, pathways, and processes and their biochemistry, physiology and regulation as a basis for understanding function. Studies on individual proteins are not encouraged.

2) Biochemical and Physiological Characterization. The MCP seeks to go beyond identifying discrete genes and proteins that participate in a few isolated

enzymatic reactions; the interest is in defining the global interactions among multiple cellular components. How do these proteins, metabolites, or cellular biomolecules interact with each other to form functional networks or linkages between the constituents of traditionally described modular pathways? There is an acute need to know more about the quantitative intracellular physiology and biochemistry of a microbial cell's constituents, e.g., assembly dynamics, kinetics, and fluxes of relevant proteins and cytoplasmic components under *in vivo* conditions. Proposals may include the use of new high-throughput technologies/tools to better quantify protein biochemistry inside a cell in response to different conditions and to better understand regulatory molecules and noncoding regulatory sequences that affect pathways relevant to the DOE. Of particular interest, are explorations of the physical mechanisms of intracellular communication and information exchange that underlie the DOE mission relevant processes listed earlier in this announcement. This announcement does not encourage research proposals directed toward microarray or "gene-chip" development or construction; however, such arrays or chips may be used to address the aims of this announcement.

3) Intracellular Localization. A microbial cell is not a simple "bag of dilute saline" in which proteins freely diffuse and interact in ways solely governed by simple diffusion. Although this assumption (of simplicity) has proven useful in studying protein biochemistry and reaction kinetics at the level of single enzymes, it does not represent the internal reality of even a simple microbial cell. This announcement encourages research on the intracellular physico-chemical environment, including the intracellular distribution, localization, movement, temporal variations, and topological or mechanical constraints on physiological function of microbial proteins involved in reaction pathways and networks that are of interest to DOE. Technologies for imaging microbial cell constituents in real time are also of interest.

4) Cell Modeling. It is not presently possible to model every single interaction in a cell, much less represent its overlapping but distinct networks and pathways in sufficient detail to capture most its complexity. This announcement encourages research proposals to develop and explore computational models of those networks and pathways of interest to the DOE. Computational models are sought to simulate the intracellular environment at different levels of resolution: a) at medium resolution, i.e., modeling most of a cell's proteome, to generate a rough or approximate predictive understanding of the "minimal metabolic scaffold" for processes such as methanogenesis, photosynthesis, or metal reduction, or b) at higher resolution: i.e. for a detailed quantitative representation of a relevant physiological process to optimize or

manipulate a particular reaction, and to accurately predict responses to environmental perturbations. It is important that any proposed software development activities be based on modular design, which enables upgrades and expansions to the predictive modeling capability as more quantitative data about protein biochemistry, physiology, and intracellular topology becomes available. Of particular importance is that modeling efforts not be conducted in isolation from the biological "reality" derived from experimental research. Of special interest will be computational models that would effectively utilize investments made by the Office of Science in massively parallel, high-performance computing hardware and software libraries. It is expected that computational tools developed under these awards will be widely distributed to the scientific community (e.g. via a WWW site) and that some level of user support will be available. Applicants with an interest in this thrust area are strongly encouraged to explore the companion Program Announcement LAB 01-21, *Advanced Modeling and Simulation of Biological Systems*, which encourages the submission of research proposals that emphasize the applied mathematics and computer science advances needed to provide the computational modeling foundation upon which this announcement is focused.

DATES: Preproposals referencing Program Announcement LAB 01-20 should be received by February 21, 2001. Earlier submissions will be gladly accepted. A response to timely preproposals will be communicated to the applicant by March 9, 2001.

Formal proposals in response to this announcement should be received by 4:30 p.m., E.D.T., April 24, 2001, to be accepted for merit review and funding in FY 2001.

ADDRESSES: Preproposals referencing Program Announcement LAB 01-20 should be sent to Dr. Daniel W. Drell, Office of Biological and Environmental Research, SC-72, Office of Science, U.S. Department of Energy, 19901 Germantown Road, Germantown, MD 20874-1290; e-mail is encouraged (but not required) for submitting preproposals using the following address: joanne.corcoran@science.doe.gov.

Formal proposals referencing Program Announcement LAB 01-20, should be forwarded to: U.S. Department of Energy, Office of Science, Grants and Contracts Division, SC-64, 19901 Germantown Road, Germantown, MD 20874-1290, ATTN: Program Announcement LAB 01-20. This address must be used when submitting proposals by U.S. Postal Service Express Mail or any commercial mail delivery service, or when hand-carried by the applicant.

FOR FURTHER INFORMATION CONTACT:

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Preproposals

Potential applicants are strongly encouraged to submit a brief preproposal that consists of two to three pages of narrative describing the research objectives, the technical approach(s), and the proposed team members and their expertise. The intent in requesting a preproposal is to save the time and effort of applicants in preparing and submitting a formal project proposal that may be inappropriate for the program. Preproposals will be reviewed relative to the scope and research needs of the Microbial Cell Project, as outlined in the summary paragraph and in the SUPPLEMENTARY INFORMATION. The preproposal should identify, on the cover sheet, the title of the project, the institution, principal investigator name, telephone, fax, and e-mail address. No budget information or biographical data need be included, nor is an institutional endorsement necessary. A response to timely preproposals will be communicated to the Principal Investigator by March 9, 2001.

Program Funding

It is anticipated that up to \$6 million will be available for all MCP awards in Fiscal Year 2001. It is anticipated that at least 4 awards will be made to interdisciplinary scientific teams, contingent on satisfactory peer review, the availability of funds, and the size of the awards. Multiple year funding is expected, also contingent on availability of funds and progress of the research; pending the availability of future funding, it is anticipated that this initiative will reflect a long term commitment to understanding the workings of a microbial cell. Awards to interdisciplinary teams are expected to range from \$0.5 million to \$1.5 million per year, total costs, with terms of one to three years. (A number of awards in the \$100 – 200 thousand range, total annual costs, may be made to exceptional individual investigator proposals). The DOE is under no obligation to pay for any costs associated with the preparation or submission of an proposal. DOE reserves the right to fund, in whole or in part, any, all, or none of the proposals submitted in response to this announcement. Proposals received by the Office of Science under its normal competitive proposal mechanisms

may also be deemed appropriate for consideration under this announcement and may be funded under this program.

Submission Information

The Project Description must be 25 pages or less, exclusive of attachments. It must contain an abstract or project summary on a separate page with the name of the proposer, mailing address, phone FAX and E-mail listed. The proposal must include letters of intent from collaborators (briefly describing the intended contribution of each to the research), and short curriculum vitae, consistent with NIH guidelines, for the proposer and any co-PIs.

DOE policy requires that potential proposers adhere to 10 CFR Part 745 "Protection of Human Subjects" (if applicable), or such later revision of those guidelines as may be published in the Federal Register.

Any recipient of an award from the Office of Science, performing research involving recombinant DNA molecules and/or organisms and viruses containing recombinant DNA molecules shall comply with the National Institutes of Health "Guidelines for Research Involving Recombinant DNA Molecules," which is available via the World Wide Web at: <http://www.niehs.nih.gov/odhsb/biosafe/nih/rdna-apr98.pdf>, (59 FR 34496, July 5, 1994), or such later revision of those guidelines as may be published in the Federal Register.

Other useful web sites include:

MCP Home Page – <http://microbialcellproject.org>

Microbial Genome Program Home Page -
<http://www.er.doe.gov/production/ober/microbial.html>

DOE Joint Genome Institute Microbial Web Page -
http://www.jgi.doe.gov/JGI_microbial/html/

GenBank Home Page - <http://www.ncbi.nlm.nih.gov/>

Human Genome Home Page - <http://www.ornl.gov/hgmis>

The instructions and format described below should be followed. Reference Program Announcement LAB 00-20 on all submissions and inquiries about this program.

OFFICE OF SCIENCE
GUIDE FOR PREPARATION OF SCIENTIFIC/TECHNICAL PROPOSALS
TO BE SUBMITTED BY NATIONAL LABORATORIES

Proposals from National Laboratories submitted to the Office of Science (SC) as a result of this program announcement will follow the Department of Energy Field Work Proposal process with additional information requested to allow for scientific/technical merit review. The following guidelines for content and format are intended to facilitate an understanding of the requirements necessary for SC to conduct a merit review of a proposal. Please follow the guidelines carefully, as deviations could be cause for declination of a proposal without merit review.

1. Evaluation Criteria

Proposals will be subjected to formal merit review (peer review) and will be evaluated against the following criteria which are listed in descending order of importance:

1. Scientific and/or technical merit of the project
2. Appropriateness of the proposed method or approach
3. Competency of the personnel and adequacy of the proposed resources
4. Reasonableness and appropriateness of the proposed budget

In addition to the above evaluation criteria, proposals will also be evaluated on the following:

5. The robustness of the organizational framework and its coordination plan if a consortium is proposed.

The evaluation will include program policy factors such as the relevance of the proposed research to the terms of the announcement, the uniqueness of the proposer's capabilities, and demonstrated usefulness of the research for proposals in other DOE Program Offices as evidenced by a history of programmatic support directly related to the proposed work.

2. Summary of Proposal Contents

Field Work Proposal (FWP) Format (Reference DOE Order 5700.7C) (DOE ONLY)
Proposal Cover Page
Table of Contents
Abstract

Narrative
Literature Cited
Budget and Budget Explanation
Other support of investigators
Biographical Sketches
Description of facilities and resources
Appendix

2.1 Number of Copies to Submit

An original and seven copies of the formal proposal/FWP must be submitted.

3. Detailed Contents of the Proposal

Proposals must be readily legible, when photocopied, and must conform to the following three requirements: the height of the letters must be no smaller than 10 point with at least 2 points of spacing between lines (leading); the type density must average no more than 17 characters per inch; the margins must be at least one-half inch on all sides. Figures, charts, tables, figure legends, etc., may include type smaller than these requirements so long as they are still fully legible.

3.1 Field Work Proposal Format (Reference DOE Order 5700.7C) (DOE ONLY)

The Field Work Proposal (FWP) is to be prepared and submitted consistent with policies of the investigator's laboratory and the local DOE Operations Office. Additional information is also requested to allow for scientific/technical merit review.

Laboratories may submit proposals directly to the SC Program office listed above. A copy should also be provided to the appropriate DOE operations office.

3.2 Proposal Cover Page

The following proposal cover page information may be placed on plain paper. No form is required.

Title of proposed project
SC Program announcement title
Name of laboratory
Name of principal investigator (PI)
Position title of PI
Mailing address of PI
Telephone of PI

Fax number of PI
Electronic mail address of PI
Name of official signing for laboratory*
Title of official
Fax number of official
Telephone of official
Electronic mail address of official
Requested funding for each year; total request
Use of human subjects in proposed project:

If activities involving human subjects are not planned at any time during the proposed project period, state "No"; otherwise state "Yes", provide the IRB Approval date and Assurance of Compliance Number and include all necessary information with the proposal should human subjects be involved.

Use of vertebrate animals in proposed project:

If activities involving vertebrate animals are not planned at any time during this project, state "No"; otherwise state "Yes" and provide the IACUC Approval date and Animal Welfare Assurance number from NIH and include all necessary information with the proposal.

Signature of PI, date of signature

Signature of official, date of signature*

*The signature certifies that personnel and facilities are available as stated in the proposal, if the project is funded.

3.3 Table of Contents

Provide the initial page number for each of the sections of the proposal. Number pages consecutively at the bottom of each page throughout the proposal. Start each major section at the top of a new page. Do not use unnumbered pages and do not use suffices, such as 5a, 5b.

3.4 Abstract

Provide an abstract of no more than 250 words. Give the broad, long-term objectives and what the specific research proposed is intended to accomplish. State the hypotheses to be tested. Indicate how the proposed research addresses the SC scientific/technical area specifically described in this announcement.

3.5 Narrative

The narrative comprises the research plan for the project and is limited to 25 pages. It should contain the following subsections:

Background and Significance: Briefly sketch the background leading to the present proposal, critically evaluate existing knowledge, and specifically identify the gaps which the project is intended to fill. State concisely the importance of the research described in the proposal. Explain the relevance of the project to the research needs identified by the Office of Science. Include references to relevant published literature, both to work of the investigators and to work done by other researchers.

Preliminary Studies: Use this section to provide an account of any preliminary studies that may be pertinent to the proposal. Include any other information that will help to establish the experience and competence of the investigators to pursue the proposed project. References to appropriate publications and manuscripts submitted or accepted for publication may be included.

Research Design and Methods: Describe the research design and the procedures to be used to accomplish the specific aims of the project. Describe new techniques and methodologies and explain the advantages over existing techniques and methodologies. As part of this section, provide a tentative sequence or timetable for the project.

Subcontract or Consortium Arrangements: If any portion of the project described under "Research Design and Methods" is to be done in collaboration with another institution, provide information on the institution and why it is to do the specific component of the project. Further information on any such arrangements is to be given in the sections "Budget and Budget Explanation", "Biographical Sketches", and "Description of Facilities and Resources".

3.6 Literature Cited

List all references cited in the narrative. Limit citations to current literature relevant to the proposed research. Information about each reference should be sufficient for it to be located by a reviewer of the proposal.

3.7 Budget and Budget Explanation

A detailed budget is required for the entire project period, which normally will be three years, and for each fiscal year. It is preferred that DOE's budget page, Form 4620.1 be used for providing budget information*. Modifications of categories are permissible to comply with institutional practices, for example with regard to overhead costs.

A written justification of each budget item is to follow the budget pages. For personnel this should take the form of a one-sentence statement of the role of the person in the project. Provide a detailed justification of the need for each item of permanent equipment. Explain each of the other direct costs in sufficient detail for reviewers to be able to judge the appropriateness of the amount requested.

Further instructions regarding the budget are given in section 4 of this guide.

* Form 4620.1 is available at web site:

<http://www.sc.doe.gov/production/grants/forms.html>

3.8 Other Support of Investigators

Other support is defined as all financial resources, whether Federal, non-Federal, commercial or institutional, available in direct support of an individual's research endeavors. Information on active and pending other support is required for all senior personnel, including investigators at collaborating institutions to be funded by a subcontract. For each item of other support, give the organization or agency, inclusive dates of the project or proposed project, annual funding, and level of effort devoted to the project.

3.9 Biographical Sketches

This information is required for senior personnel at the laboratory submitting the proposal and at all subcontracting institutions. The biographical sketch is limited to a maximum of two pages for each investigator.

3.10 Description of Facilities and Resources

Describe briefly the facilities to be used for the conduct of the proposed research. Indicate the performance sites and describe pertinent capabilities, including support facilities (such as machine shops) that will be used during the project. List the most important equipment items already available for the project and their pertinent capabilities. Include this information for each subcontracting institution, if any.

3.11 Appendix

Include collated sets of all appendix materials with each copy of the proposal. Do not use the appendix to circumvent the page limitations of the proposal. Information should be included that may not be easily accessible to a reviewer.

Reviewers are not required to consider information in the Appendix, only that in the body of the proposal. Reviewers may not have time to read extensive appendix materials with the same care as they will read the proposal proper.

The appendix may contain the following items: up to five publications, manuscripts (accepted for publication), abstracts, patents, or other printed materials directly relevant to this project, but not generally available to the scientific community; and letters from investigators at other institutions stating their agreement to participate in the project (do not include letters of endorsement of the project).

4. Detailed Instructions for the Budget

(DOE Form 4620.1 "Budget Page" may be used)

4.1 Salaries and Wages

List the names of the principal investigator and other key personnel and the estimated number of person-months for which DOE funding is requested. Proposers should list the number of postdoctoral associates and other professional positions included in the proposal and indicate the number of full-time-equivalent (FTE) person-months and rate of pay (hourly, monthly or annually). For graduate and undergraduate students and all other personnel categories such as secretarial, clerical, technical, etc., show the total number of people needed in each job title and total salaries needed. Salaries requested must be consistent with the institution's regular practices. The budget explanation should define concisely the role of each position in the overall project.

4.2 Equipment

DOE defines equipment as "an item of tangible personal property that has a useful life of more than two years and an acquisition cost of \$25,000 or more." Special purpose equipment means equipment which is used only for research, scientific or other technical activities. Items of needed equipment should be individually listed by description and estimated cost, including tax, and adequately justified. Allowable items ordinarily will be limited to scientific equipment that is not already available for the conduct of the work. General purpose office equipment normally will not be considered eligible for support.

4.3 Domestic Travel

The type and extent of travel and its relation to the research should be specified. Funds may be requested for attendance at meetings and conferences, other travel associated with the work and subsistence. In order to qualify for support, attendance at meetings or conferences must enhance the investigator's capability to perform the

research, plan extensions of it, or disseminate its results. Consultant's travel costs also may be requested.

4.4 Foreign Travel

Foreign travel is any travel outside Canada and the United States and its territories and possessions. Foreign travel may be approved only if it is directly related to project objectives.

4.5 Other Direct Costs

The budget should itemize other anticipated direct costs not included under the headings above, including materials and supplies, publication costs, computer services, and consultant services (which are discussed below). Other examples are: aircraft rental, space rental at research establishments away from the institution, minor building alterations, service charges, and fabrication of equipment or systems not available off-the-shelf. Reference books and periodicals may be charged to the project only if they are specifically related to the research.

a. Materials and Supplies

The budget should indicate in general terms the type of required expendable materials and supplies with their estimated costs. The breakdown should be more detailed when the cost is substantial.

b. Publication Costs/Page Charges

The budget may request funds for the costs of preparing and publishing the results of research, including costs of reports, reprints page charges, or other journal costs (except costs for prior or early publication), and necessary illustrations.

c. Consultant Services

Anticipated consultant services should be justified and information furnished on each individual's expertise, primary organizational affiliation, daily compensation rate and number of days expected service. Consultant's travel costs should be listed separately under travel in the budget.

d. Computer Services

The cost of computer services, including computer-based retrieval of scientific and technical information, may be requested. A justification based on the established computer service rates should be included.

e. Subcontracts

Subcontracts should be listed so that they can be properly evaluated. There should be an anticipated cost and an explanation of that cost for each subcontract. The total amount of each subcontract should also appear as a budget item.

4.6 Indirect Costs

Explain the basis for each overhead and indirect cost. Include the current rates.