This has been a rather successful grant, leading to several major discoveries that not only have fundamentally altered our understanding of metabolic networks, but have also lead to several highly cited publications on the subject. We started by addressing the existence of modules in the metabolism. An elegant proposal addressing the cell's functional architecture is offered by the concept of modularity. This concept assumes the possibility of seamlessly partitioning the cell into a collection of modules, in which each module, a discrete entity of several elementary components, performs an identifiable task, separable from the functions of other modules. Simultaneously, it is now widely recognized that the components of a living cell are dynamically connected to one another, such that the cell’s functional properties are ultimately encoded into a complex intracellular web of molecular interactions. This is perhaps most evident when inspecting the cellular metabolism, for which the probability that a substrate can react with \( k \) other substrates decays as a power law \( P(k) \sim k^{-\gamma} \) with \( \gamma \simeq 2.2 \) suggesting that metabolic networks have a scale-free topology. A distinguishing feature of such scale-free networks is the existence of a few highly connected metabolites which participate in a very large number of metabolic reactions. With a large number of links, these hubs integrate all substrates into a single, integrated web in which the existence of fully separated modules is prohibited by definition.

Yet, the dilemma of a modular- vs. a highly integrated module-free metabolic network organization remains. The most important result was our ability to show that the metabolic networks of 43 distinct organisms are discernibly organized into many small, highly connected topologic modules that combine in a hierarchical manner into larger, less cohesive units, their number and degree of clustering following a power law. Within \textit{Escherichia coli} the uncovered hierarchical modularity closely overlaps with known metabolic functions. The paper describing these findings was published by \textit{Science}. [Ravasz et al. Hierarchical organization of modularity in metabolic networks, \textit{Science} 297, 1551-5 (2002)].

We have also completed a second major project, addressing the issue of how frequently a given metabolic pathway is utilized in setting up metabolic states of \textit{E. coli}. Various biochemical reactions
are known to have widely different roles and impact, some being highly active under most growth conditions, while others are switched on only under rare environmental circumstances. Recent advances in metabolic flux analysis have significantly improved our ability to generate quantitative predictions on the relative importance of various reactions. Following flux-balance analysis we first established a solution space using constraints imposed by the conservation of mass and the stochiometry of the reaction system for the reconstructed \textit{E. coli} MG1655 metabolic network. Assuming that cellular metabolism is in a steady state and optimized for the maximal growth rate, we calculated the flux for each reaction, providing a measure of each reaction’s relative activity. A striking feature of the obtained flux distribution is its overall inhomogeneity; i.e., reactions with orders of magnitude different fluxes coexist under the same conditions. We found that the distribution is best fitted with a power law with a small k cutoff. The observed local flux inhomogeneity indicates that for each metabolite \(i\) we can identify a single reaction that dominates its production (consumption). We find that this approach uncovers a distinct structure of disjoint pathways, which, representing the high flux backbone of the metabolism, largely overlap with the traditional, biochemistry-based partitioning of cellular metabolism. The paper describing these was published by \textit{Nature}, and featured in the journal’s cover [E. Almaas et al., Global organization of metabolic fluxes in the bacterium Escherichia coli, \textit{Nature} 427, 839-843 (2004)].

Finally, we used flux-balance analysis to thoroughly assess the activity of \textit{Escherichia coli}, \textit{Helicobacter pylori}, and \textit{Saccharomyces cerevisiae} metabolism in 30,000 diverse simulated environments. This work allowed us to identify a set of metabolic reactions forming a connected metabolic core that carry non-zero fluxes under all growth conditions, and whose flux variations are highly correlated. Furthermore, we found that the enzymes catalyzing the core reactions display a considerably higher fraction of phenotypic essentiality and evolutionary conservation than those catalyzing noncore reactions. Cellular metabolism is characterized by a large number of species-specific conditionally active reactions organized around an evolutionary conserved, but always active, metabolic core. Finally, we found that most current antibiotics interfering with bacterial metabolism target the core enzymes, indicating that our findings may have important implications for antimicrobial drug-target discovery. The paper describing these was published by \textit{PLOS Computational Biology} [E. Almaas et al., \textit{PLoS Computational Biology} 1, 0557-0563 (2005)].
MEMORANDUM

TO: William Kokal, H. A. King  
Department of Energy  
Chicago Operations Office  
Office of Acquisition and Assistance  
9800 S. Cass Ave.  
Argonne, IL 60439

FROM:  
Department of Research & Sponsored Programs Accounting  
University of Notre Dame

DATE: April 5, 2006

RE: Contract No.: DE-FG02-01ER25495  
ND No. 200203

Please find enclosed the original and two copies of Financial Status Report for the subject contract covering the period September 1, 2001 through August 31, 2005.

Also enclosed is Financial Assistance Property Closeout Certification.

If there are any questions, please contact Ann P. Strasser, Director, Research & Sponsored Programs Accounting at (574) 631-5437.
**FINANCIAL STATUS REPORT**

*(Short Form)*

(Follow instructions on back)

---

1. Federal Agency and Organizational Element to Which Report is Submitted
   - **U.S. Department of Energy**

2. Federal Grant or Other Identifying Number
   - DE-FG02-01ER25495
   - OMB Approval No.: 0348-0039

3. Recipient Organization (Name and complete address, including ZIP code)
   - University of Notre Dame
   - 801 Grace Hall, Research & Sponsored Programs
   - Accounting
   - Notre Dame, Indiana 46556

4. Employer Identification Number
   - 35-0868188

5. Recipient Account Number or Identification Number
   - 200203

6. Final Report
   - X Yes
   - No

7. Basis
   - X Cash
   - Accrual

8. Funding/Grant Period (See instructions)
   - From: (Month, Day, Year)
     - 09/01/01
   - To: (Month, Day, Year)
     - 08/31/05

9. Period Covered by this Report
   - From: (Month, Day, Year)
     - 09/01/01
   - To: (Month, Day, Year)
     - 08/31/05

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**Transactions:**

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11. Indirect Expense
   - a. Type of Rate (Place "X" in appropriate box)
     - Provisional
     - Predetermined
     - Final
     - Fixed

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12. Remarks: Attach any explanations deemed necessary or information required by Federal sponsoring agency in compliance with governing legislation.

13. Certification: I certify to the best of my knowledge and belief that this report is correct and complete and that all outlays and unliquidated obligations are for the purposes set forth in the award documents.

**Typed or Printed Name and Title**

John H. Engel, RSPA Systems Analyst/Senior Accountant

**Telephone (Area code, number & extension)**

(574)631-7070

**Signature of Authorized Certifying Official**

**Date Report Submitted**

5-Apr-06

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Standard Form: 269A (Rev A-06)
The purpose of this report is to facilitate the closeout of the Award. Based on the records maintained by the Recipient in accordance with the Property Management standards set forth in the Award, the following reflects the Recipient’s closeout inventory of real and personal property that was provided by the Department of Energy (DOE) or partially or wholly acquired with project funds.

I. EQUIPMENT

A. Federally-Owned; (Government Furnished Equipment): (10 CFR 600.133(a), 600.232, 600.322, or Federal Demonstration Partnership (FDP) General Terms and Conditions No. 33, as applicable): ☒ No ☐ Yes  
   (If yes, attach property inventory list that includes item description, manufacturer, model, serial number, original acquisition date, original acquisition cost per the Federal Management Regulation 102-36.240)

B. Equipment Acquired with Award Funds where Title Vests in the Recipient with further obligations to DOE: (10 CFR 600.133, 600.134, 600.232, or 600.321, as applicable)
   ☐ No ☒ Yes  
   If yes, does the equipment have a per unit fair market value of $5,000 or more? ☒ No ☐ Yes  
   (If yes, attach a property inventory list that includes item description, manufacturer, model, serial number, original acquisition date, original acquisition cost, disposal condition code per the Federal Management Regulation 102-36-240 and one of the disposition codes listed below)

1. The property will continue to be used for the purposes authorized in the Award.  
2. The property is no longer needed for the purposes of the Award, and will be used on another Federally sponsored activity (List Activity and Federal Agency):  
3. The Recipient wishes to retain the property and compensate DOE for its share of the current per unit fair market value. (Identify the fair market value on the attached property inventory list and describe how the value was determined).  
4. The property is no longer needed for the purposes of the Award or other Federally sponsored activities and the Recipient requests DOE disposition instructions.

II. SUPPLIES (10 CFR 600.135, 600.233, 600.324, or FDP General Terms and Conditions No. 35, as applicable)

Does the residual inventory of unused supplies exceed $5,000 in total aggregate value? ☒ No ☐ Yes (If yes, check block below)

☐ The supplies will be used on another Federally sponsored activity (List Activity and Federal Agency).  
☐ The supplies will be sold or retained for use on non-Federally sponsored activities and the Recipient will compensate DOE for its share of the sales proceeds (or estimate of current fair market value). Attach a list of the supplies and complete the following Worksheet:

| Sale proceeds or estimate of current fair market value | $ _________ |
| Percentage of Federal participation | _________ % |
| Federal share | $ _________ |
| Selling and handling allowance | $ _________ |
| Amount to be remitted to DOE | $ _________ |
III. REAL PROPERTY: (Real Estate - 10 CFR 600.132, 600.231, 600.321, or FDP General Terms and Conditions No. 32 as applicable)  

A. Description of Real Property:

B. Complete Address of Real Property:

C. Period of Federal Interest in the Property: From _______ To _______. (Unless the award specifies otherwise, the Federal Interest in the property ends when the award project period ends.)

D. Disposition Preference Request. If the period of Federal Interest in the property exceeds the project period, check one of the following blocks to indicate your disposition preference:

☐ Transfer property to another Federal award.
☐ Sell and compensate DOE.
☐ Return to DOE.
☐ Retain title and compensate DOE for its share of the current fair market value of the property.

Certification: I certify to the best of my knowledge and belief that all information presented in this report is true, correct and complete, and constitutes a material representation of fact upon which the Federal government may rely.

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Page 2 of 3
U.S. DEPARTMENT OF ENERGY
FINANCIAL ASSISTANCE
PROPERTY CLOSEOUT CERTIFICATION

To be completed by the Department of Energy:

DOE PROPERTY DISPOSITION

☒ Negative Report

☐ Real Property:

☐ Equipment:

☐ Supplies:

Property Management Official Name  Signature  Date
PATENT CERTIFICATION

University of Notre Dame
Awardee
☐ Interim Certification

DE-FG02-01ER25495
DOE Prime and/or Subcontract Nos.
☒ Final Certification

Awardee hereby certifies unless indicated to the contrary, that:

1. All procedures for identifying and disclosing subject inventions as required by the patent clause of the contract have been followed throughout the reporting period.

2. There were no subcontracts or purchase orders involving research, development, and demonstration except as follows: (a separate certification must be provided to DOE for each subcontract or purchase order awarded.)

3. No inventions or discoveries were made or conceived in the course of or under this contract other than the following (Certification includes ☒, does not include ☐ all subcontracts):

   | TITLE | INVENTOR | DATE REPORTED | DOE “S” NO.*
   |-------|----------|---------------|-------------

4. The completion date of this contract is as follows: August 31, 2006

5. The following period is covered by this certification:

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   Signature
   Michael T. Edwards
   Director, Office of Research
   Title
   University of Notre Dame Contractor
   511 Main Building
   Notre Dame, Indiana 46556 Address
   March 16, 2006 Date of Certification

* Also include Subcontract No. if available.

NOTE: A positive certification for this Item 3 does not negate the requirement for furnishing to DOE a fully executed Patent Certification from each subawardee identified in Item 2.
PATENT CERTIFICATION

Zoltan Oltvai, M.D.
Northwestern University via Subcontract from Notre Dame
Awardee

☐ Interim Certification

DE-FG02-01ER25495
DOE Prime and/or Subcontract Nos.

☐ Final Certification

Awardee hereby certifies unless indicated to the contrary, that:

1. All procedures for identifying and disclosing subject inventions as required by the patent clause of the contract have been followed throughout the reporting period.

2. There were no subcontracts or purchase orders involving research, development, and demonstration except as follows: (a separate certification must be provided to DOE for each subcontract or purchase order awarded.)

3. No inventions or discoveries were made or conceived in the course of or under this contract other than the following (Certification includes ☑, does not include ☐ all subcontracts):

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4. The completion date of this contract is as follows: March 10, 2006

5. The following period is covered by this certification:

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Northwestern University Contractor

633 Clark Street
Address
Evanston, IL 60208

Signature: Bruce W. Elliott, Jr., Ph.D.
Director Office of Sponsored Research
Title Chicago Campus

Date of Certification 3/13/06

* Also include Subcontract No. if available.

NOTE: A positive certification for this Item 3 does not negate the requirement for furnishing to DOE a fully executed Patent Certification from each subawardee identified in Item 2.