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Computational Biology and High Performance Computing

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recycled paper

Computational Biology and High Performance Computing

Presenters:

Manfred Zorn – Co-Head, Center of Bioinformatics and Computational Genomics, NERSC

Teresa Head-Gordon – Scientist, Physical Biosciences Division, LBNL

Adam Arkin – Scientist, Physical Biosciences Division, LBNL

Brian Shoichet, Northwestern University

Organizer: Horst D. Simon – NERSC Director

November 15, 1999

Abstract

- **The pace of extraordinary advances in molecular biology has accelerated in the past decade due in large part to discoveries coming from genome projects on human and model organisms. The advances in the genome project so far, happening well ahead of schedule and under budget, have exceeded any dreams by its protagonists, let alone formal expectations. Biologists expect the next phase of the genome project to be even more startling in terms of dramatic breakthroughs in our understanding of human biology, the biology of health and of disease. Only today can biologists begin to envision the necessary experimental, computational and theoretical steps necessary to exploit genome sequence information for its medical impact, its contribution to biotechnology and economic competitiveness, and its ultimate contribution to environmental quality.**

Abstract (cont.)

- High performance computing has become one of the critical enabling technologies, which will help to translate this vision of future advances in biology into reality. Biologists are increasingly becoming aware of the potential of high performance computing. The goal of this tutorial is to introduce the exciting new developments in computational biology and genomics to the high performance computing community.

Tutorial Outline

- **1:30 - 2:00 p.m.** **Overview of Computational Biology --Teresa Head-Gordon**
- **2:00 - 3:00 p.m.** **Bioinformatics -- Manfred Zorn**
- **3:00 - 3:30 p.m.** **Break**
- **3:30 - 4:00 p.m.** **Protein Structure Prediction and Folding --Teresa Head-Gordon**
- **4:00 - 4:30 p.m.** **Docking/Molecular Recognition -- Brian Shoichet**
- **4:30 - 5:00 p.m.** **Cellular Networks -- Adam Arkin**

Computational Challenges in Structural and Functional Genomics

Teresa Head-Gordon
Physical Biosciences and Life Sciences Divisions
Lawrence Berkeley National Laboratory

November 15, 1999

(1) Why computational biology?

(2) Community effort to define problems with genuine computational complexity

- Genome analysis, gene modeling, sequence-based annotation
- Low resolution fold prediction: Single Molecule
- High resolution structure prediction and protein folding: Single Molecule
- Molecular recognition or Docking: Multi-molecule complexes
- Cellular Decision modeling

(3) Putting it all together:

- Deinococcus radiodurans
- Center for Integrative Physiome Analysis (CIPhA)

Revolutionary Experimental Efforts in Biology

Sequence → Structure → Function

Sequence

Genome projects
Microbial organisms
C elegans
Human

Structure

Structural Genomics Initiative
High throughput effort underway
NIH, new beamlines
LBNL: ALS

Function

Functional Annotation Initiatives
Gene deletion projects
Yeast two-hybrid screening
Gene expression micro-arrays
In vivo GFP protein (kinetics)

Computational Biology White Paper

http://cbcg.lbl.gov/ssi-csb

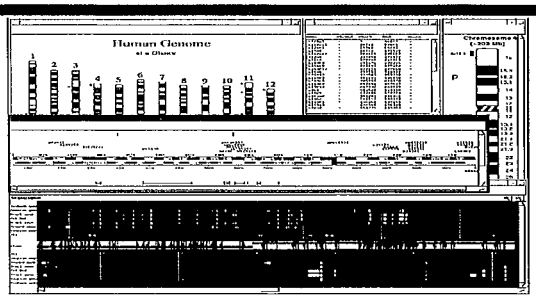
A technical document to define areas of biology exhibiting computational problems of scale

Organization:
- Introduction to biological complexity and needs for advanced computing (1)
- Scientific areas (2-6)
- Computing hardware, software, CSET issues (7)
- Appendices

For each scientific chapter:
- illustrate with state of the art application (current generation hpc platform)
- define algorithmic kernals
- deficiencies of methodologies
- define what can be accomplished with 100 teraflop computing

- ➢Community document
- ➢More organized CB community in government labs, universities
- ➢Support for CB by the broader biological community

High-Throughput Genome Sequence Assembly, Modeling, and Annotation

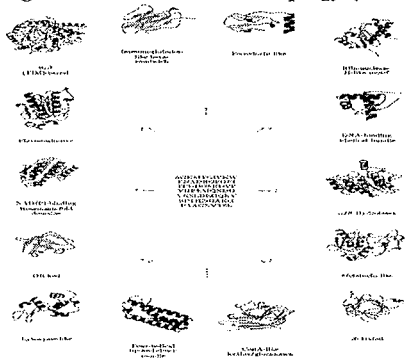


The Genome Channel Browser to access and visualize current data flow, analysis and modeling. (Manfred Zorn, NERSC)

- Genome sequencing and annotation ⟶ Bioinformatics
  - 100,000 human genes; genes from other organism
  - Structure/functional annotation at the sequence level
- Computation to determine regions of a genome that might yield new folds
  - Experimental Structural Genomics Initiative
  - Functional annotation at the structure level by experiment

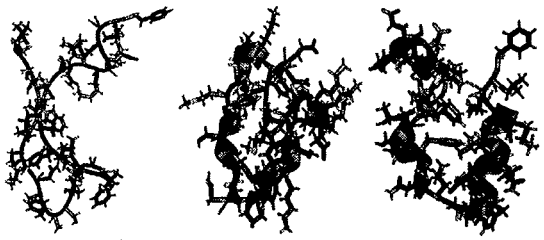
Characterize the Link Between Protein Sequence and Fold Topology

Sequence Assignments to Protein Fold Toplogy (David Eisenberg, UCLA)

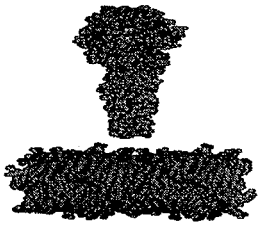


- Experimental Structural Genomics Initiative
  - Define basis set of folds: ~10^3 structures to be determined
- Predict Fold Topology from Computation (~10^5 folds)
  - Functional annotation at the structural level by computation

Low Resolution Fold Topologies to High Resolution Structure



One microsecond simulation of a fragment of the protein, Villin.
Duan & Kollman, Science 1998



Influenza virus poised above a model of a lipid membrane will involve a 100,000 atom MD simulation over long timescales to understand this step in the mechanism of viral infection. (Tobias, UCI)

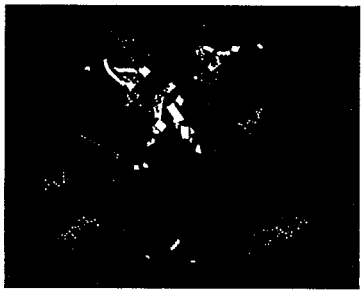
Low Resolution Structures from Predicted Fold Topology

Fold class gives some idea of biological function, but....

Higher Resolution Structures with Biochemical Relevance

Drug design, bioremediation, diseases of new pathogen

Simulating Molecular Recognition/Docking



Changes in the structure of DNA that can be induced by proteins. Through such mechanisms proteins regulate genes, repair DNA, and carry out other cellular functions.

Improvements in Methodology and Algorithms of Higher Resolution Structure

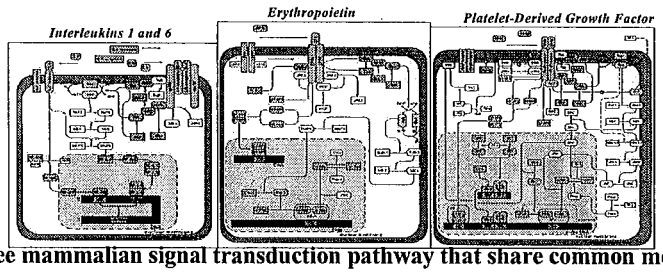
Breaking down size, time, lengthscale bottlenecks (IT^2, algorithms, teraflop computing)

Protein, DNA recognition, binding affinity, mechanism with which drugs bind to proteins

Simulating two-hybrid yeast experiments

Protein-protein and Protein-nucleic acid docking

Modeling the Cellular Program



Three mammalian signal transduction pathway that share common molecular elements (i.e. they cross-talk). From the Signaling PAthway Database (SPAD) (http://www.grt.kyushu-u.ac.jp/spad/)

- Integrating Computational/Experimental Data at all levels
  - Sequence, structural functional annotation (Virtually all biological initiatives)
- Simulating biochemical/genetic networks to mode cellular decisions
  - Modeling of network connectivity (sets of reactions: proteins, small molecules, DNA)
  - Functional analysis of that network (kinetics of the interactions)

Implicit Collaborations Across the DOE Mission Sciences

Computer Hardware & Portability
- Applications described running on various platforms
  - T3D, T3E, IBM SP's, ASCI Red, Blue

Information Technologies and Database Management
- Integrating biological databases; CORBA and java
- Data Warehousing
- ultra-high-speed networks

Ensuring Scalability on Parallel Architectures
- implicit algorithmic scaling
- paradigm/software library support tools for effective parallelization
- strategies: 100 teraflop

Meta Problem Solving Environments
- geographically distributed software paradigm: "plug and play" paradigm

Visualization
- Querying data which is "information dense"

Feedback from Biotech Industry Meeting

LBNL 2/25/99

Jim Cavalcoli, Ph.D. **Bioinformatics Manager, PDLMG** **Parke-Davis, Warner-Lambert**	**Patrick O'Hara** **VP, BioMolecular Informatics** **ZymoGenetics, Inc** **Seattle WA**	**Herve Recipon** **Asst. dir. bioinformatics** **diaDexus (Incyte)**
Pete Smietana, Ph.D. **Senior Staff Software Engineer,** **Bioinformatics** **Ciphergen**	**Peter Karp, Ph.D.** **Scientific Fellow** **Pangea Systems**	**Rick Bott** **X-ray crystallographer** **Genencor**
Julie Rice **Computational Chemist** **IBM-Almaden**	**Eric Martin** **Sr. Scientist Small Molecule Discovery** **Chiron**	

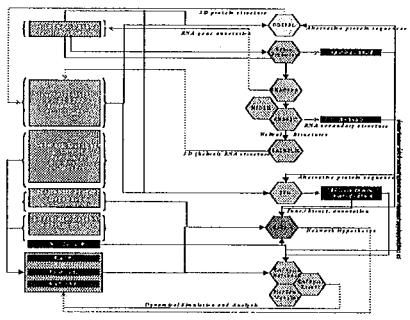
LBNL: Gilbert, Head-Gordon, Holbrook, Mian, Rokhsar, Simon, Spengler, Zorn

We want to listen to Biotech industry perspective on Computational Biology white paper

- **Is there strong objection to any of the content?**
  - **NO, very supportive**
- **Are there other areas to be included, stronger emphasis placed?**
  - **Will be a new chapter on databases: integrating, querying, visualization**
- **Technical input: contribute a "vignette" on important Comp. Bio. application**
  - **Parke-Davis, Chiron, Zymogenetics, Pangea**

Center for Integrative Physiome Analysis (CIPhA)

NCRR submitted 2/1/99 **P.I.: Adam Arkin**



Cell cycle, asymmetric division and differentiation in Caulobacter crescentus

Analysis of developmental pathways in C. Elegans

Analysis of databases of two-hybrid interactions

The role of cytomechanical and nuclear structure in mammary gland transformation

Interrelationships among the various tools and databases used and developed by the Center. Blue rectangles are databases built by the Center (with the exception of *Interact 1.0* which is provided courtesy of Roger Brent, Molecular Sciences Institute). Green boxes are off-site database. Hexagons are tools to be developed by this Center.

Adam Arkin, Mina Bissell, Roger Brent, Silvia Crivelli, Tarek Elaydi, Teresa Head-Gordon, Stephen Holbrook, Stuart Kim,
Casimir Kulikowski, Harley McAdams, Saira Mian, Ilya Muchnik, Lucy Shapiro, NERSC

Deinococcus Radiodurans (DR: Strange Berry That Withstands Radiation)

Bacteria isolated from tins of spoiled meat given "sterilizing" doses of γ radiation.
- $3x10^6$ base pairs, or ~3000 protein products
- fully sequenced by TIGR under DOE/OBER sponsorship

Three components to DR's successful DNA repair strategy
- specifics of the DNA repair mechanism
- the fact that it is multi-genomic
- coupling of repair, replication, export of damaged DNA from intracellular medium.

Propose to construct molecular models of key components of the DNA repair system:
- Damaged DNA
- Multigenomic repair intermediates such as Holliday junctions
- Proteins known are yet to be discovered to be involved in DNA repair
- Protein-protein or protein-nucleic acids that couple repair, replication, transport.

Developing better fold recognition, comparative modeling, and ab initio prediction methods, and docking methods to describe macromolecular complexes.
- Application of methodologies will be to fully and completely annotate the DR genome
- Learn underlying components of highly-honed strategies for DNA repair in DR.

Involves significant portions of community white paper on high end computing needs

The Need for Advanced Computing for Computational Biology

Computational Complexity arises from inherent factors:
- 100,000 gene products just from human; genes from many other organisms
- Experimental data is accumulating rapidly
- N^2, N^3, N^4, etc. interactions between gene products
- Combinatorial libraries of potential drugs/ligands
- New materials that elaborate on native gene products from many organisms

Algorithmic Issues to make it tractable
- Objective Functions
- Optimization
- Treatment of Long-ranged Interactions
- Overcoming Size and Time scale bottlenecks
- Statistics

Acknowledgements for Community White Paper in Computational Biology

The First Step Beyond the Genome Project: High-Throughput Genome Assembly, Modeling, and Annotation
P. LaCascio, R. Mural, J, Snoddy, E. Uberbacher, ORNL
S. Mian, F. Olken, S. Spengler, M. Zorn: LBNL
David States, Washington University

From Genome Annotation to Protein Folds: Comparative Modeling and Fold Assignment
D. Eisenberg, UCLA
A. Lapedes, LANL
A. Sali, Rockefeller University
B. Honig, Columbia University

Low Resolution Folds to High Resolution Protein Structure and Dynamics
C. Brooks, Scripps Research Institute
P. Kollman &Y. Duan, UCSF
A. McCammon & V. Helms, UCSD
G. Martyna, Indiana University
D.Tobias, UCI
T. Head-Gordon, LBNL

Biotechnology Advances from Computational Structural Genomics: In Silico Drug Design and Mechanistic Enzymology
R. Abagyan, NYU, Skirball Institute
P. Bash, ANL
J. Blaney, Metaphorics, Inc.
F. Cohen, UCSF
M. Colvin, LLNL
I. Kuntz, UCSF

Linking Structural Genomics to Systems Modeling: Modeling the Cellular Program
A. Arkin & D. Wolf, LBNL
P. Karp, PangeaS. Subramaniam, U Illinois Urbana

Implicit Collaborations Across the DOE Mission Sciences
M. Colvin & C. Musick, LLNL
T. Gaasterland, ANL (now Rockefeller)
S. Crivelli & T. Head-Gordon, LBNL
G. Martyna, Indiana University

Bioinformatics

Manfred D. Zorn
November 15, 1999

Overview

- 30 seconds of Biology
- DNA Sequencing: View from 10,000 feet
- Genome Analysis
  - Genome Projects
  - Identify a possible gene
  - Characterize a gene
- Large-scale Genome Annotation
- What's supercomputing got to do with it?
- Challenges

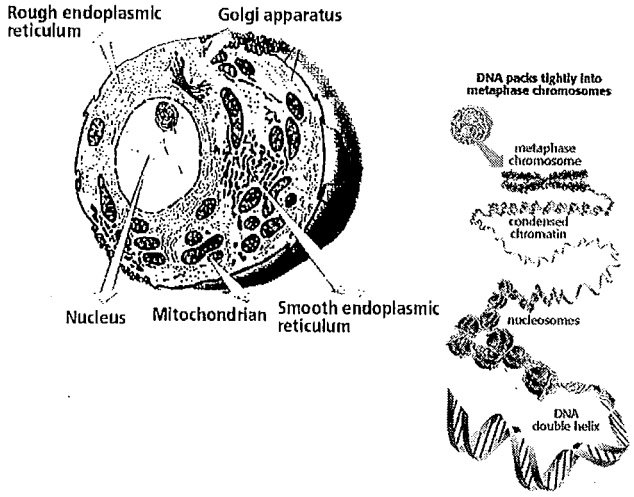
Biology is Special

Life is characterized by

- *Individuality*
- *Historicity*
- *Contingency*
- *high (digital) information content*

No law of large numbers, since every living thing is genuinely unique

Basic Biology



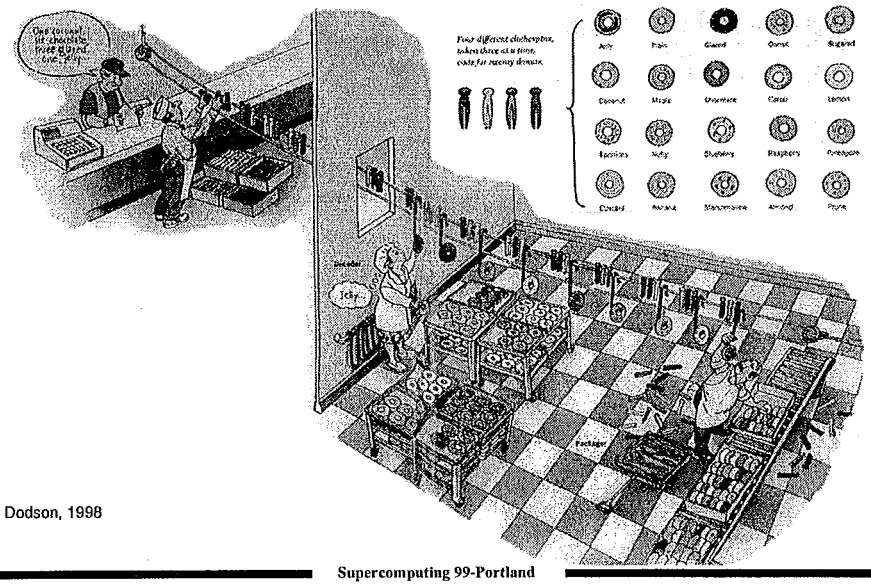
Fundamental Dogma

The fundamental dogma of molecular biology is that genes act to create phenotypes through a flow of information form DNA to RNA to proteins to interactions among proteins (regulatory circuits and metabolic pathways) and ultimately to phenotypes.

Collections of individual phenotypes constitute a population

DNA
↓
RNA
↓
Proteins
↓
Circuits
↓
Phenotypes
↓
Populations

DNA Codes



Dodson, 1998

DNA Sequencing

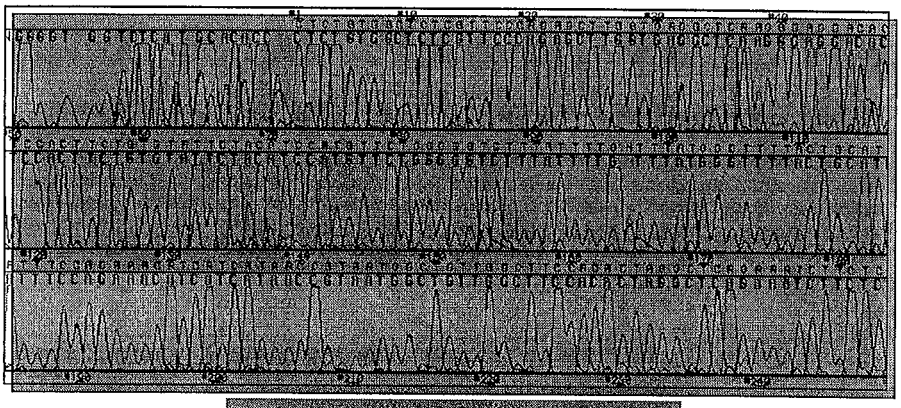
Read base code from storage medium!

- **Read length: About 600 bases at once**
- **Reader capacity**
  - ✓ **100 lanes in parallel in about 2-5 hours**

Sequencing: "bird's eye view"

- Prepare DNA
  - about a trillion DNA molecules
- Do the sequencing reactions
  - synthesize a new strand with terminators
- Separate fragments
  - by time, length = constant
- Sequence determination
  - automatic reading with laser detection systems

Sequence Traces



Good quality sequence needs about 10X Coverage

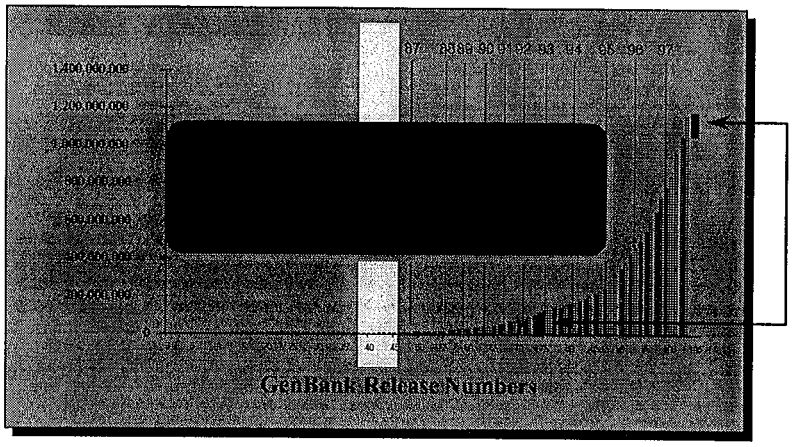
Human Genome Project - Goals

- Construction of a high-resolution genetic map
- Production of a variety of physical maps of all human chromosomes and of selected model organisms
- Determination of the complete sequence of human DNA and DNA of selected model organisms
- Development of capabilities for collecting, storing, distributing, and analyzing the data produced
- Creation of appropriate technologies necessary to achieve these objectives

Genome Projects

- Model organisms sequenced
  - E. coli — 4.5 Mb
  - S. cerevisiae
  - C. elegans — 100 Mb
  - Dozens of bacteria — 1 - 6 Mb
  - D. melanogaster — 140 Mb
- Human
  - 408 Mb
  - ~14% of the genome

Base Pairs in GenBank

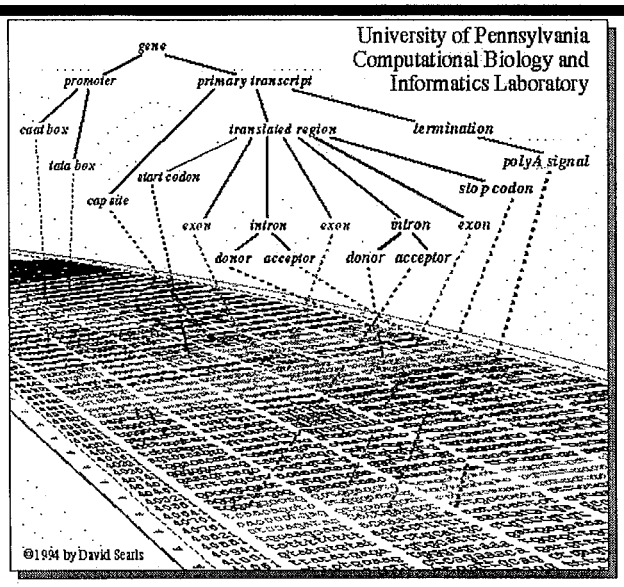


DNA Analysis

Disassemble the base code!

- **Find the genes**
  - **Heuristic signals**
  - **Inherent features**
  - **Intelligent methods**
- **Characterize each gene**
  - **Compare with other genes**
  - **Find functional components**
  - **Predict features**

What is a Gene?

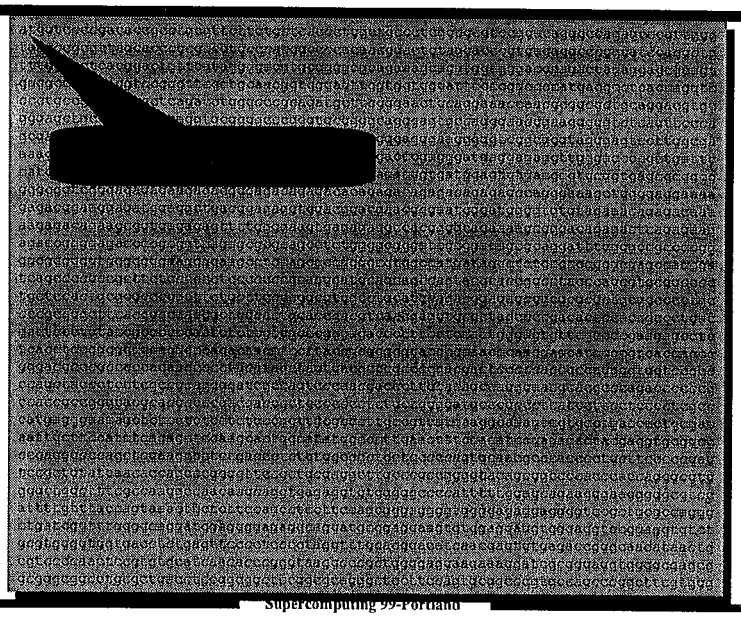


Heuristic Signals

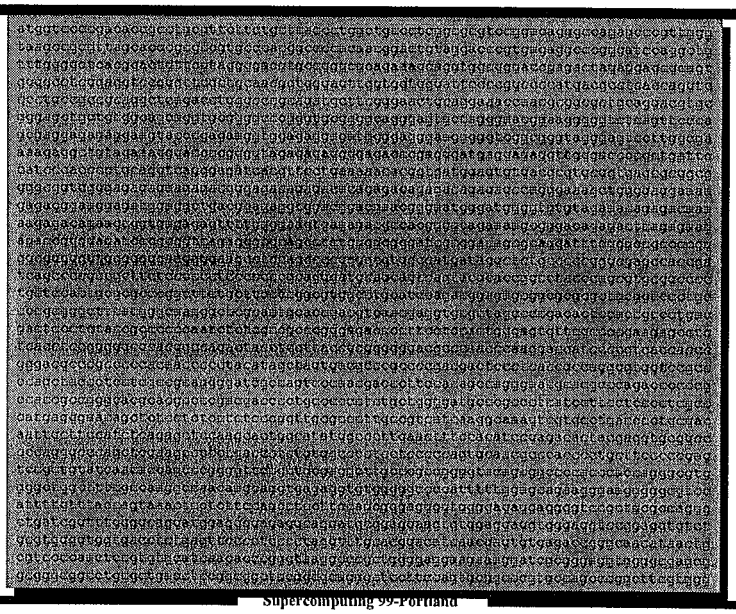
DNA contains various recognition sites for internal machinery

- **Promoter signals**
- **Transcription start signals**
- **Start Codon**
- **Exon, Intron boundaries**
- **Transcription termination signals**

Heuristic Signals



Heuristic Signals

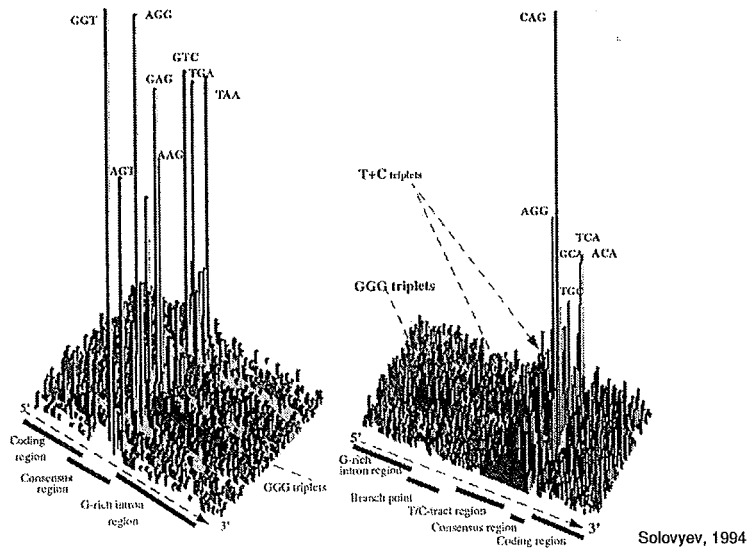


Inherent Features

DNA exhibits certain biases that can be exploited to locate coding regions

- Uneven distribution of bases
- Codon bias
- CpG islands
- In-phase words
- Encoded amino acid sequence
- Imperfect periodicity
- Other global patterns

Inherent Features



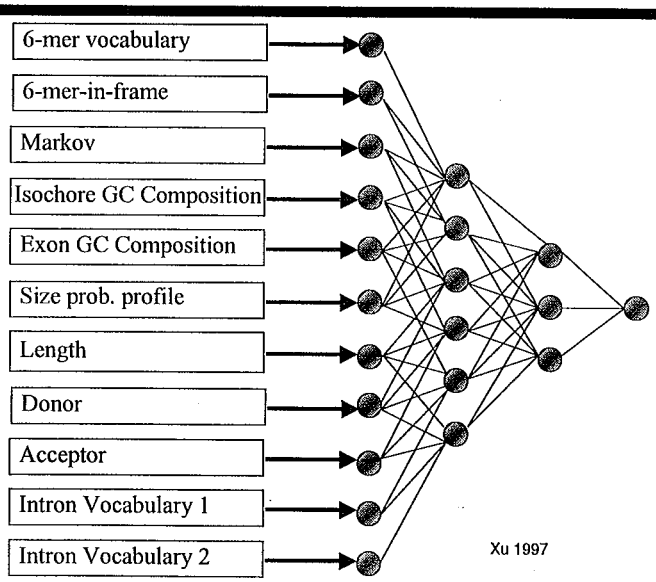
Solovyev, 1994

Intelligent Methods

Pattern recognition methods weigh inputs and predict gene location

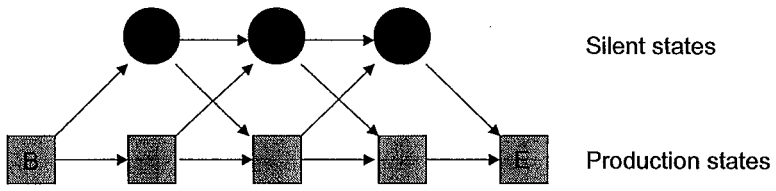
- **Neural Networks**
- **Hidden Markov Models**
- **Stochastic Context-Free Grammer**

Neural networks



Xu 1997

Hidden Markov Models



Characterize a Gene

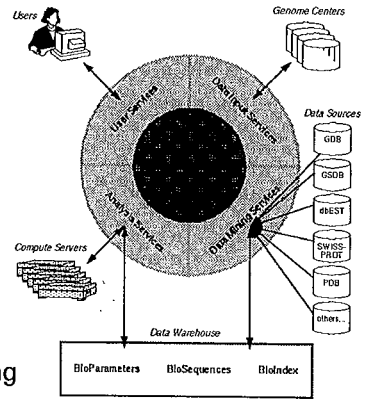
Collect clues for potential function

- **Comparison with other known genes, proteins**
- **Predict secondary structure**
- **Fold classification**

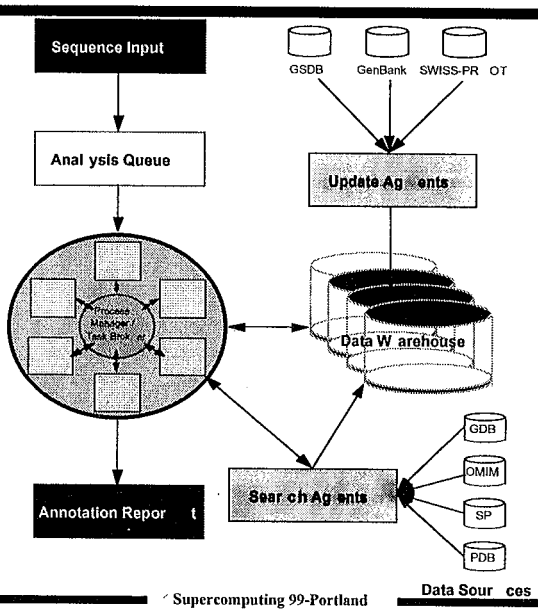
- **Gene Expression**
- **Gene Regulatory Networks**
- **Phylogenetic comparisons**
- **Metabolic pathways**

Large-scale Genome Annotation

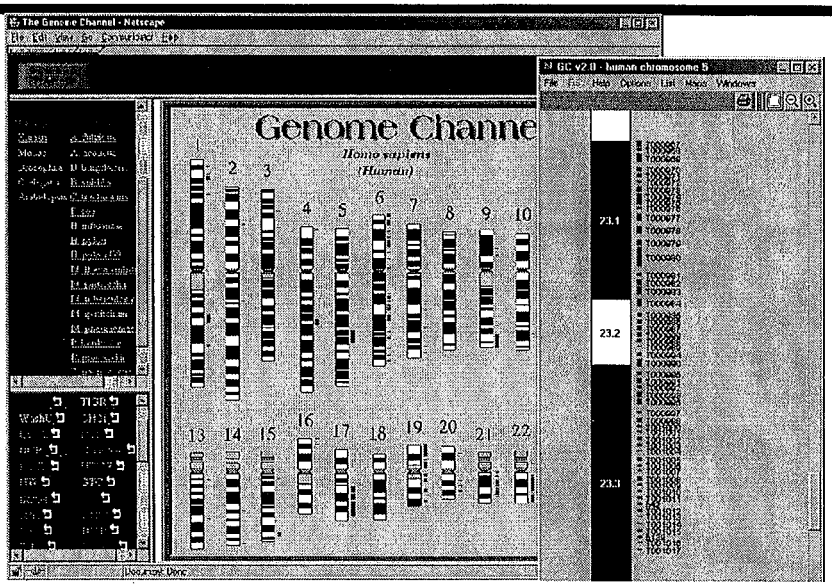
- Multi-laboratory Project
- Standard Annotation of Genomes
  - Genome Channel
  - Genome Catalog
- Comprehensive integration of
  - Analysis tools
  - Data management systems
  - Data mining
  - User services
- Extensible Framework
  - High-performance computing
  - Data integration technology
  - Artificial intelligence



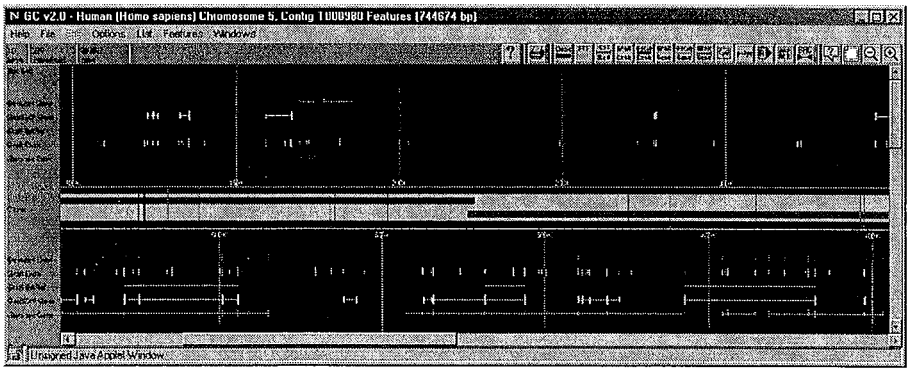
Annotation Pipeline



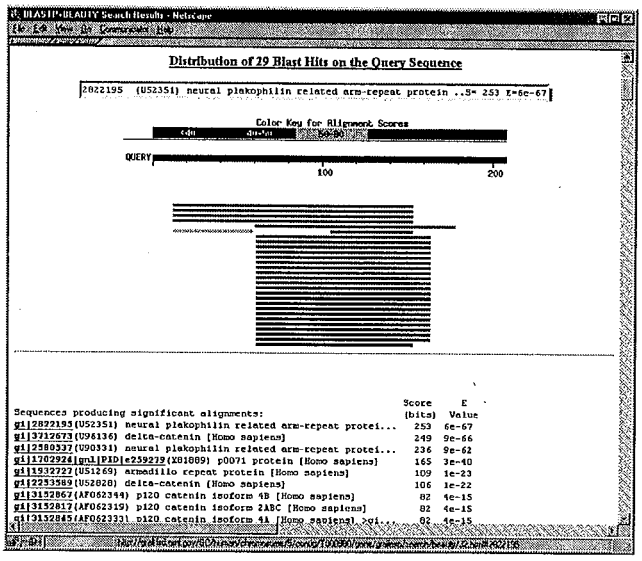
Genome Channel



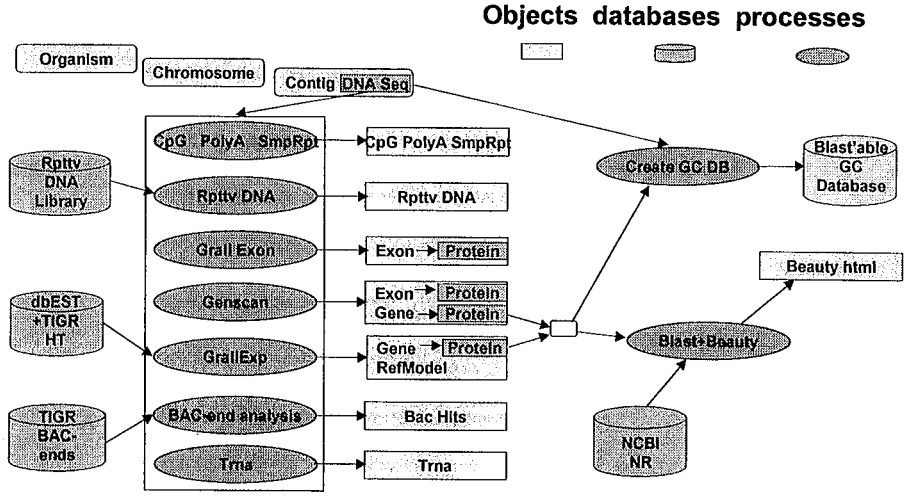
Feature Display



Gene Search - BEAUTY Results



Highlights - Data Analysis



What's supercomputing got to do with it?

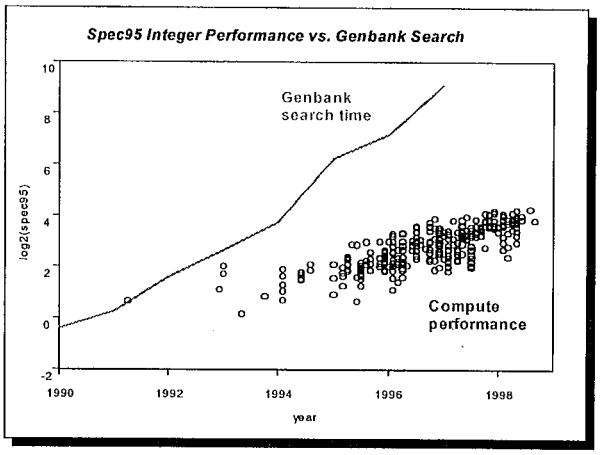
- Complexity of the information
- Amount of data
- Most applications are trivially parallel

Layers of Information

The same base sequence contains many layered instructions!

- Chromosome structure and function
  - Telomers, centromers
- Gene Regulatory information
  - Enancers, promoters
- Instructions for gene structure
- Instructions for protein
- Instructions for protein post-processing and localization

Moore's Law and Genomics



States 1998

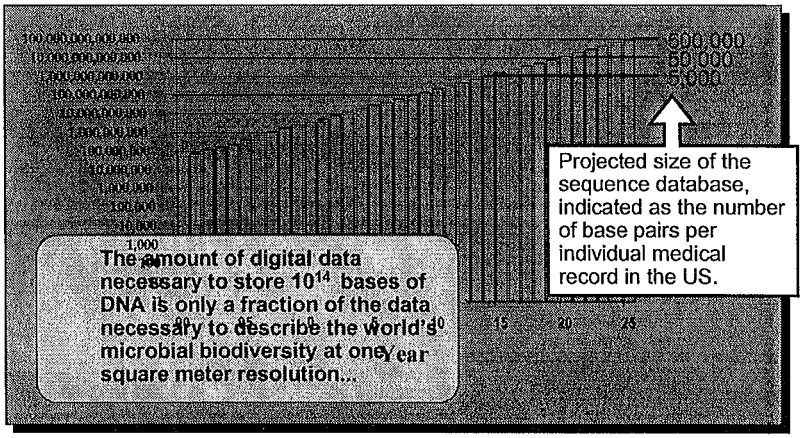
The Shape of the Wave

- 1999
  - ✓ JGI releases 150 Mbases draft
  - ✓ Celera releases the sequence of Drosophila (140 Mb)
  - ✓ Public "draft" effort reaches halfway point (1,500 Mb)
  - ✓ 20 more Microbial genomes completed (80 Mb but 60,000 genes)
  - ✓ First release of Celera "shotgun" (9,000 Mb)
- 2000
  - ✓ JGI releases 150 Mbases draft
  - ✓ Public "draft" completed (1,500 Mb)
  - ✓ Mouse "draft" begins (500 Mb - comparisons with human)
  - ✓ Two more Celera shotgun releases (18,000 Mb)
  - ✓ 40 more Microbial genomes sequenced (160 Mb -120,000 genes)

CPU Requirements

- Current annotation
  - 250 Mbases DNA yield ~125 Gbytes of data
  - It takes ~ 7.5 days on 20 workstations ~3,600nhr
- Celera Data
  - 9 Gbases (36x) in small pieces every 3 months ~2,000 hr.
  - Analysis time approx. quadratic (1300x)
  - 1,300 x 3,600nhr / 2,000 hr. = 2,340 nodes
- Celera Sequencing
  - Assembly of 1.7 Million reads in 25 hrs
  - Annotation 8-10 Mbases per months with 6 FTE
  - Assembly of Human Genome: expected ~ 3 months

Projected Base Pairs



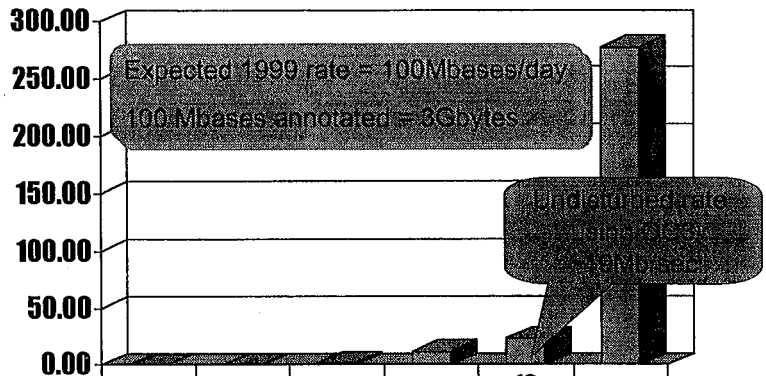
Sequence Assembly

- Complexity
  - Adding a day's read of 100 Mb to a billion base pairs of contig would require 100 Pops operations
  - A 1 Tops machine would take about one day to process 100 Mbases

Assembly / Integration / Modeling

- **BAC end integration**
  - ✓ **JGI draft (1st half) = 300 Pops**
  - ✓ **first Celera release requires = 3,000 Pops**
- **Draft and whole genome shotgun integration**
  - ✓ **JGI draft (1st half) + Celera first release = 1,300 Pops**
- **Gene modeling**
  - ✓ **Celera first release (9Gbases) - 1 day of Paragon time**
- **Placing STSs**
  - ✓ **JGI draft requires = 9 Pops**
  - ✓ **Celera first release = 90 Pops**

Data Transfer



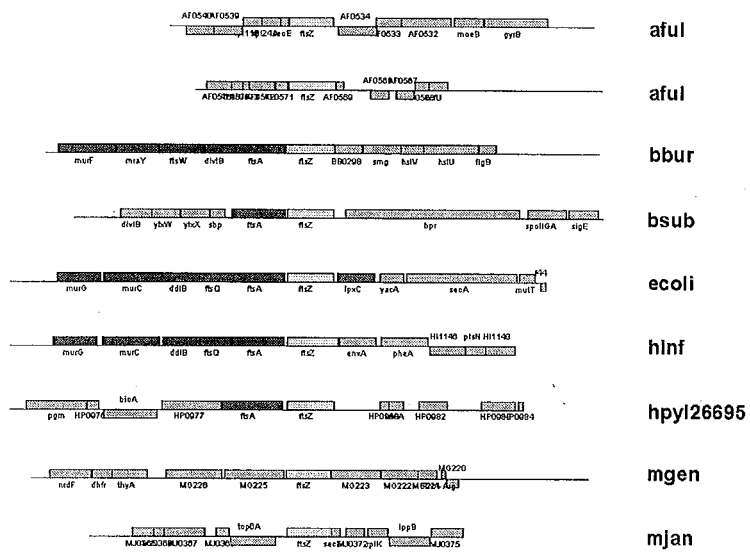
	year	month	week	day	12 hours	1 hour
Mbytes/sec	0.03	0.39	1.65	11.60	23.10	277.70

Challenges

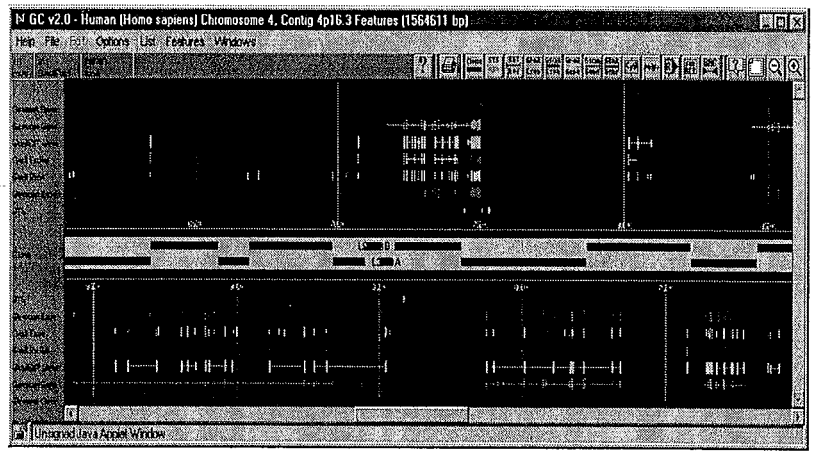
- Discovering new biology

- Lack of software integration
- Beginning to build high-performance applications
- Shortage of personnel

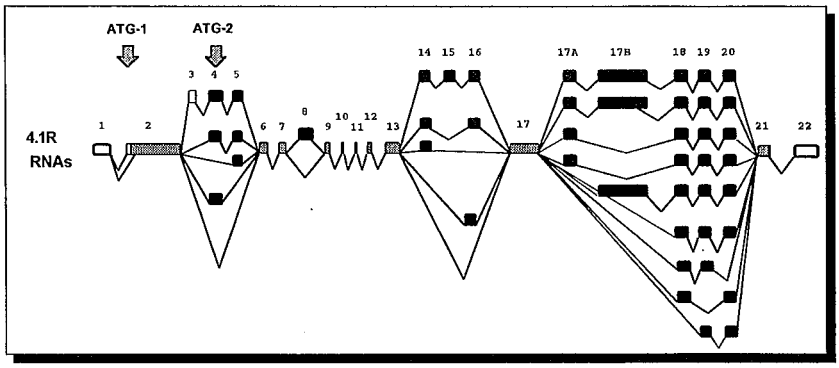
Comparative Genome Analysis



Alternatively Spliced ?



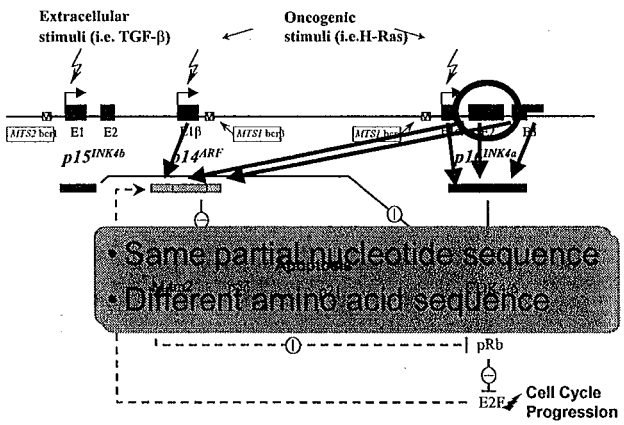
One Gene - Many Proteins



Conboy 1998

As many as 30% of human genes, in particular structural genes, may be alternatively spliced

9p21 Gene Cluster is a Nexus of the Rb and p53 Pathways



- Same partial nucleotide sequence
- Different amino acid sequence

Credits

- NERSC / LBNL
  - John Conboy
  - Donn Davy
  - Inna Dubchak
  - Sylvia Spengler
  - Denise Wolf
  - Eric P. Xing
  - Manfred Zorn
- ORNL
  - Ed Uberbacher
  - Richard Mural
  - Phil LoCascio
  - Sergey Petrov
  - Manesh Shah
  - Morey Parang

Protein Fold Recognition, Structure Prediction, and Folding

Teresa Head-Gordon
Physical Biosciences and Life Sciences Divisions
Lawrence Berkeley National Laboratory

November 15, 1999

Protein Fold Recognition, Structure Prediction, and Folding

(1) Drawing analogies with known protein structures
- Sequence homology, Structural Homology
- Inverse Folding, Threading

(2) Ab initio folding: the ability to follow kinetics, mechanism
- robust objective function
- severe time-scale problem
- proper treatment of long-ranged interactions

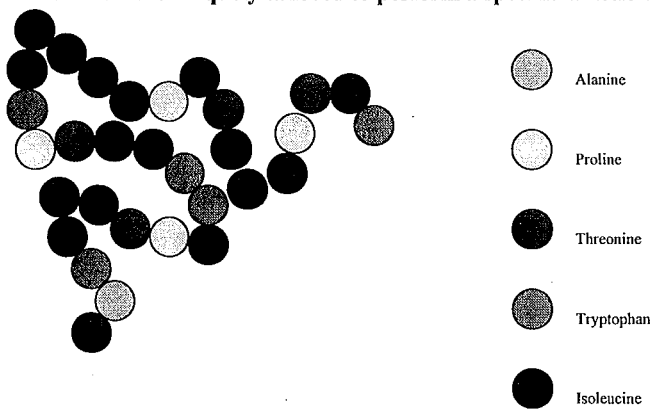
(3) Ab initio prediction: the ability to extrapolate to unknown folds
- multiple minima problem
- robust objective function
- Stochastic Perturbation and Soft Constraints

(4) Simplified Models that Capture the Essence of Real Proteins
- Lattice and Off-Lattice Simulations
- Off-Lattice Model that Connect to Experiments: Whole Genomes?

What is a protein?

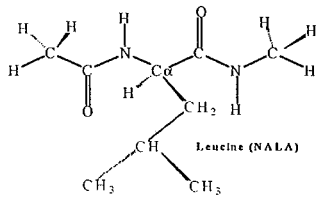
A biopolymer which is distinct from a heteropolymer in one very important way

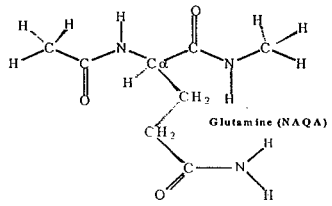
It's 3-D structure is uniquely tailored to perform a specific function



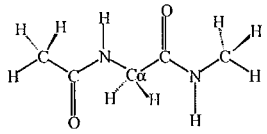
NMR, X-ray and electron crystallography solve structures slowly (1/2-3 yrs.)

The "Beads" are Chemically Complex Structures

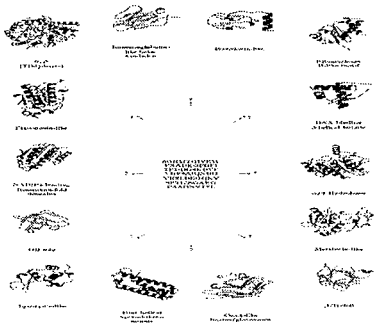




Glycine (NAGA)



Protein Fold Recognition: Threading



Sequence Assignments to Protein Fold Toplogy (David Eisenberg, UCLA)

Take a sequence with unknown structure and align onto structural template of a given fold

Score how compatible that sequence is based on empirical knowledge of protein structure

Right now 25-30% of new sequences can be assigned with high confidence to fold class

100,000's of sequences and 10,000's of structures (each of order 10^2-10^3amino acids long)

Protein Fold Recognition: Threading

Computational Approach:

Dynamic programming: capable of finding optimal alignments if

optimal alignments of subsequences can be extended to optimal alignments of whole

objective functions that are one-dimensional $E=\Sigma V_i + \Sigma V_{gap}$

Complexity: all to all comparison of sequence to structure scales as L^2

Whole human genome: 10^{13} flops

Improve Objective function:

Take into account structural environment

3D→1D: dynamic programming, L^2

Build pairwise or multi-body objective function

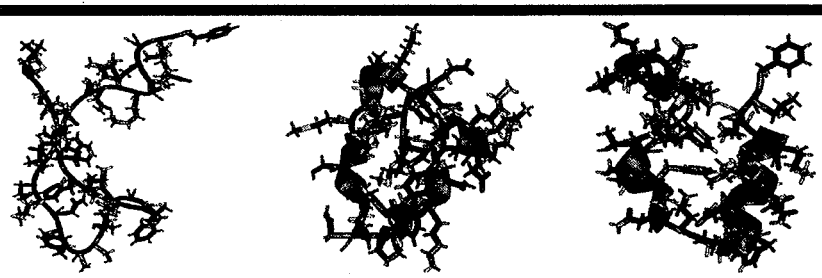
NP-hard if: variable-length gaps and model nonlocal effects such as distance dependence

Recursive dynamic programming, Hidden markov models, stochastic grammers

Complexity: all to all comparison of sequence to structure scales as L^3

Whole human genome: ~10^{16} flops

Computational Protein Folding



One microsecond simulation of a fragment of the protein, Villin. (Duan & Kollman, Science 1998)

(1) robust objective function✓

all atom simulation with molecular water present: some structure present

(2) severe time-scale problem✓

required 10^9 energy and force evaluations: parallelization (spatial decomposition)

(3) proper treatment of long-ranged interactions X

cut-off interactions at 8Å, poor by known simulation standards

(4) Statistics (1 trajectory is anecdotal) X

Many trajectories required to characterize kinetics and thermodynamics

Computational Protein Folding

(1) Size-scaling bottlenecks: Depends on complexity of energy function, V

Empirical (less accurate): cN^2; ab initio (more accurate): CN^3 or worse ; $c \ll C$

empirical force field used

"long-ranged interactions" truncated so cM^2 scaling; $M < N$

spatial decomposition, linked lists

(2) Time-Scale of motions bottlenecks (Δt)

$$r_i(t+\Delta t) = 2r_i(t) - r_i(t-\Delta t) + \frac{f_i(t)}{m_i}\frac{(\Delta t)^2}{2!} + O\left[(\Delta t)^4\right]; v_i(t) = \frac{r_i(t+\Delta t) - r_i(t-\Delta t)}{2\Delta t} + O\left[(\Delta t)^3\right]$$

$$f_i = m_i a_i = -\nabla_i V(r_1, r_2, \ldots r_N)$$

Use timestep commensurate with fastest timescale in your system

bond vibrations: 0.01Å amplitude: 10^{-15} seconds (1fs)

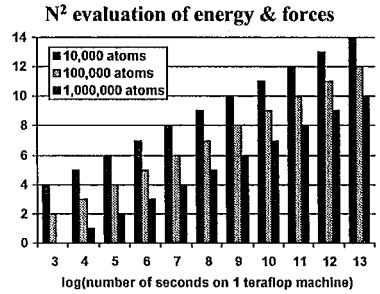
Shake/Rattle bonds (2fs)

Multiple timescale algorithms (~5fs) (not used here)

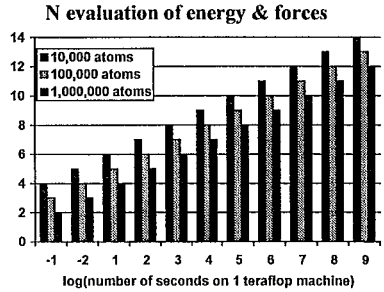
1 Microsecond simulation of Villin Headpiece in Water

Generate 10^9 steps; Assume 1 teraflop machine; 1000 Flops per energy/force evaluation

N^2 evaluation of energy & forces



N evaluation of energy & forces



Ewald Sums:

$$qq = \sum_{i>j}^{N}\left(\sum_{n=0}^{\infty} q_i q_j \frac{erfc\left(\kappa\left|r_{ij}+n\right|\right)}{\left|r_{ij}+n\right|}\right) + \frac{1}{\pi L^3}\sum_{k\neq 0} q_i q_j \frac{4\pi^2}{k^2} exp\left(-k^2/4\kappa^2\right) cos\left(k\cdot r_{ij}\right)\Bigg) + V_{self}$$

- Particle Mesh Ewald (N)
  Spatial Decomposition in r-space; Parallelization of FFT's in k-space
- Evaluate full Ewald sum in r-space using FMM techniques

Ab Initio Protein Structure Prediction

Primary Squence and an Energy function → Tertiary structure

Empirical energy functions:

(1) Detailed, Atomic description: leads to enormous difficulties!

$$V_{MM} = \sum_{i}^{\#\,Bonds} k_b\left(b_i - b_o\right)^2 + \sum_{i}^{\#\,Angles} k_\theta\left(\theta_i - \theta_o\right)^2 + \sum_{i}^{\#\,Impropers} k_\tau\left(\tau_i - \tau_o\right)^2 +$$

$$\sum_{i}^{\#\,dihedrals} k_\phi\left[1 + cos(n\phi + \delta)\right] + \sum_{i}^{\#\,atoms}\sum_{i<j}^{\#\,atoms}\left\{\frac{q_i q_j}{r_{ij}} + \varepsilon_{ij}\left[\left(\frac{\sigma_{ij}}{r_{ij}}\right)^{12} - \left(\frac{\sigma_{ij}}{r_{ij}}\right)^{6}\right]\right\} + \sum_{i}^{\#\,atoms} \Delta\sigma A$$

(1) Multiple minima problem is fierce

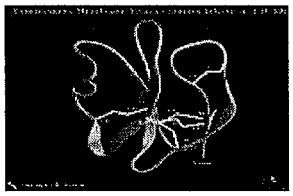
Find a way to effectively overcome the multiple minima problem

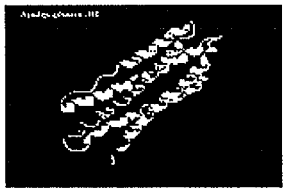
(2) Objective Functions: <u>Replaceable</u> algorithmic component?

Global energy minimum should be native structure, misfolds higher in energy

The Objective (Energy) Function

Empirical Protein Force Fields: AMBER, CHARMM, ECEPP
"gas phase"





CATH protein classification: http://pdb.pdb.bnl.gov/bsm/cath

α-helical sequence/ β-sheet structure β-sheet sequence/a-helical structure

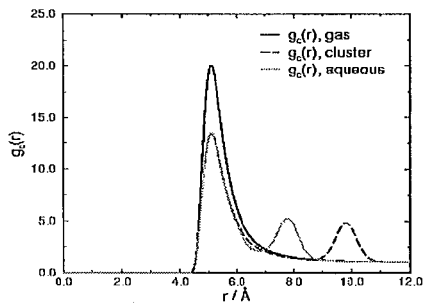
Energies the same! Makes energy minimization difficult!

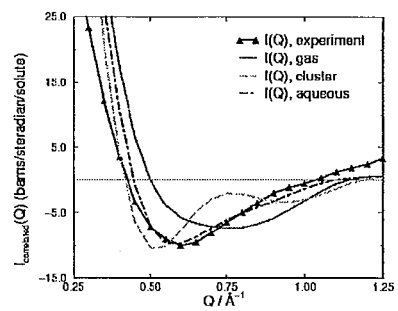
Add penalty for exposing hydrophobic surface: favors more compact structures

$E_{native\ folds} < E_{misfolds}$ for a few test cases

Solvent accessible surface area functions: Numerically difficult to use in optimization

Hydration Forces from Experiment/ Simulation and Optimization





Find model $g_c(r)$ that best reproduces excess experimental signal, $I_{c-c}(Q)$
W(r) is "potential of mean force" between two hydrophobic solutes
(Feature Article, J. Phys. Chem., 1999)

V= AMBER + (predicted helices fixed) + W(r) like that from experiment

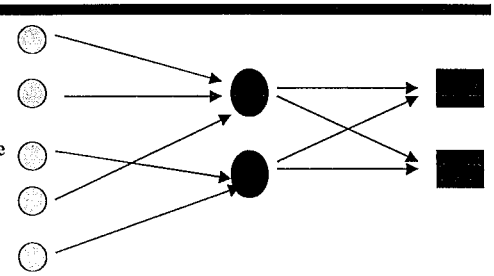
Global optimization can find no lower energy structures than crystal structures
1pou (72 aa), 3icb (77 aa), 2utg_A (70 aa), 3cln (145)

Neural Networks for 2° Structure Prediction

Input units represent amino acid sequence

Hidden units map sequence to structure

Output Units represent secondary structure class (helix, sheet, coil)



Weights are optimizable variables that are trained on database of proteins

Poorly designed networks result in overfitting, inadequate generalization to test set

Neural network design
- **input and output representation**
- **number of hidden neurons**
- **weight connection patterns that detect structural features**

Neural Network Results

No sequence homology through multiple alignments

Train			Test		
Total predicted correctly = 66%			Total predicted correctly = 62.5%		
Helix:	51%	C_a=0.42	Helix:	48%	C_a=0.38
Sheet:	38%	C_b=0.39	Sheet:	28%	C_b=0.31
Coil:	82%	C_c=0.36	Coil:	84%	C_c=0.35

Network with Design: Yu and Head-Gordon, Phys. Rev. E 1995

Train			Test		
Total predicted correctly = 67%			Total predicted correctly = 66.5%		
Helix:	66%	C_a=0.52	Helix:	64%	C_a=0.48
Sheet:	63%	C_b=0.46	Sheet:	53%	C_b=0.43
Coil:	69%	C_c=0.43	Coil:	73%	C_c=0.44

Combine networks of Yu and Head-Gordon with multiple alignments

Neural Network Predictions As Soft Constraints In Local Optimization

Make neural network prediction of 2° structure for each amino acid

Network Output: Helix (P_α, -1), Sheet (-1, P_β), Coil (-1, -1)

P_α= probability of being helix P_β= probability of being sheet

Optimize on following energy surface: $_{Bias} = V_{MM} + V_{\phi\psi} + V_{HB}$

$$_{\phi\psi} = k_\phi[1-\cos(\phi-\phi^o)] + k_\psi[1-\cos(\psi-\psi^o)] \quad ; \quad V_{HB} = q_i q_j / r_{i,j}$$

ϕ_0 and ψ_0 define perfect helix values predictions define k_ϕ, k_ψ, and q_i

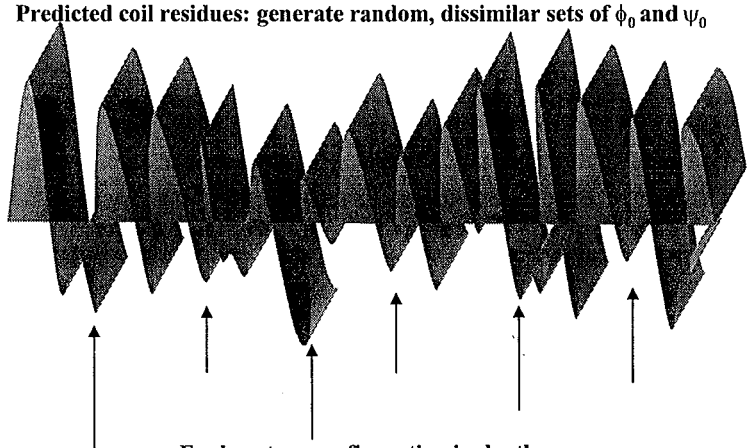
Using optimized structure from V_{bias}

optimize on V_{MM} (AMBER: unbiased objective function)

Neural Networks Used To Guide Global Optimization Methods

Generate expanded tree of configurations

Predicted coil residues: generate random, dissimilar sets of ϕ_0 and ψ_0



Explore tree configuration in depth:

Global Optimization in sub-space of coil residues: walk through barriers, move downhill

Neural Networks Used To Guide Stochastic Perturbation Algorithm

Stochastic/perturbation in sub-space of dihedral angles predicted to be coil

(1) Local minimization of a set of start points in sub-space

(2) Define a critical radius

$$r_k = \left[\left(\frac{1}{\pi} \right)^{n/2} \Gamma\left(1 + \frac{n}{2} \right) \frac{V \sigma \log \rho}{\rho} \right]^{1/n}$$

a measure of whether a point is within a basis of attraction

(3) Generate many sample points in sub-space volume, V

(4) Evaluate r.m.s. between new sample points and minimizers of (1)

If (r.m.s. < r_k) ignore this sample point

(5) Minimize sample points not in any critical distance and merge into (1)

Choose new set of dihedral angles and repeat

Probabilistic theoretical guarantees of global optimum in sub-spaces

Global optimization by solving a successive series of global optimum in sub-spaces?

Hierarchical Parallel Implementation of Global Optimization Algorithm

Static vs. Dynamic Load Balancing of Tasks

Central Processor

GOPT1 → $W_{1,1} \rightarrow W_{1,11}$

GOPT2 → $W_{2,1} \rightarrow W_{2,11}$

GOPT3 → $W_{3,1} \rightarrow W_{3,11}$

GOPT4 → $W_{4,1} \rightarrow W_{4,11}$

GOPT5 → $W_{5,1} \rightarrow W_{5,11}$

Central Processor: Assigns starting coordinates to GOPT's

Task time is highly variable

GOPT's: Divide up sub-space into N regions for global search

Task time is variable

Workers: Generate sample points; find best minimizer in region

(Number of workers depends on sub-space)

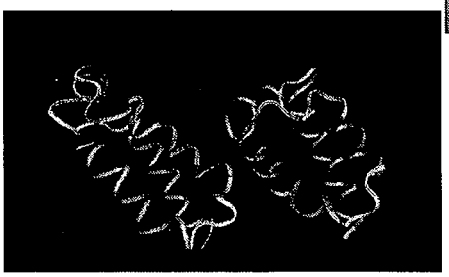
Dynamical load balancing of tasks: reassigning GOPT/workers to GOPT/workers

Gain in efficiency of a factor of 5-10

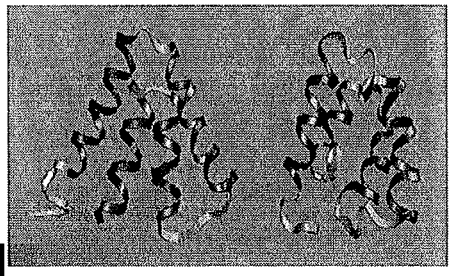
Global Optimization Predictions of α-Helical Proteins

Crystal (left), Prediction (right)
R.M.S. 7.0Å

1pou: 72 aa DNA binding protein



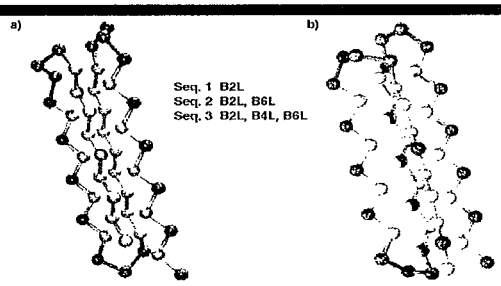
2utg_A: 70aa α-chain of uteroglobin:



Prediction (left) and crystal (right)
R.M.S. 6.3Å

Still have not reached crystal energy yet!

Simplified Models for Simulating Protein Folding



Simplifies the "real" energy surface topology sufficiently that you can do

(1) Statistics ✓

Can do many trajectories to converge kinetics and thermodynamics

(2) severe time-scale problem✓

characterize full folding pathway: mechanism, kinetics, thermodynamics

(3) proper treatment of long-ranged interactions ✓

all interactions are evaluated; no explicit electrostatics

(4) robust objective function?

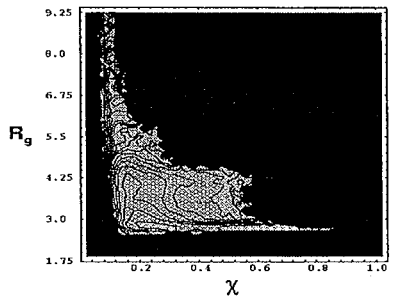
good comparison to experiments

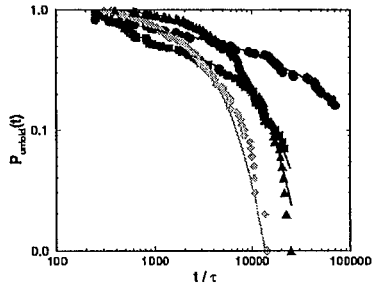
α/β Protein Model Resembling IgG-binding Proteins L and G

- Folding is highly cooperative, chain collapse accompanying folding.
- Two parallel folding pathways:
  - One pathway contains an intermediate–protein G
  - One pathway contains no intermediates–protein L.
- Sequence mutations affecting secondary structure propensities
  - Similar to mutational experiments on Protein G & L

Same Hamiltonian can model all-β (SH3) and all-α proteins (four helix bundles)

Computational Complexity of Simplified Models for Protein Folding





Thermodynamics of the folding process are characterized using
- multi-histogram method: complexity increases with multiple order parameters
- constant-temperature Langevin simulations

Folding kinetics are characterized by tabulating
- mean-first passage times, and temperature scans

One week using two Compaq/Dec EV10000 (~50 specfp95) per protein sequence
- 100,000 sequences for Human Genome; Ample mutational study data

Acknowledgements for THG Research in Computational Biology at LBNL

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Greg Hura, Graduate Group in Biophysics, UCB

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Robert M. Glaeser, Mol. & Cell Biology, UCB and Life Sciences Division, LBNL

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Structure-Based Drug Discovery

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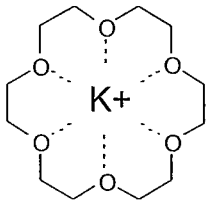
303 E. Chicago Ave, Chicago, IL 60611-3008
Nov 15, 1999

Problems in Structure-Based Inhibitor Discovery & Design

- Balance of forces in binding
  - Energies in condensed phases
    - ✓ interaction energies
    - ✓ desolvation
- Problem scales badly with degrees of freedom
  - Configuration
    - ✓ configs α (prot-features)^4 X (lig-features)^4
  - Conformation
    - ✓ Ligand & Protein, confs α 3^{lbonds} X 3^{pbonds}
- Sampling chemical space (scales *very* badly)
- Defining binding sites

The Pros & Cons of Proteins



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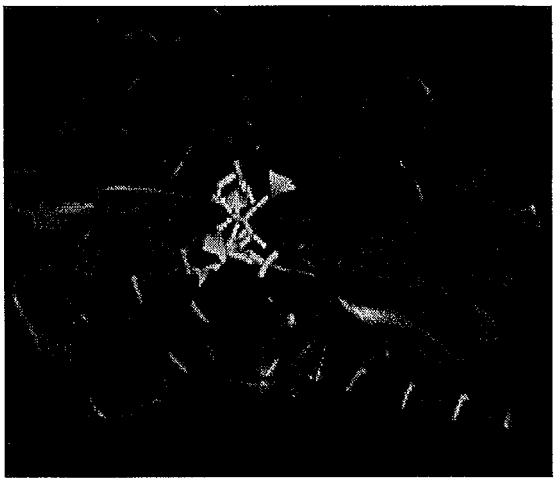
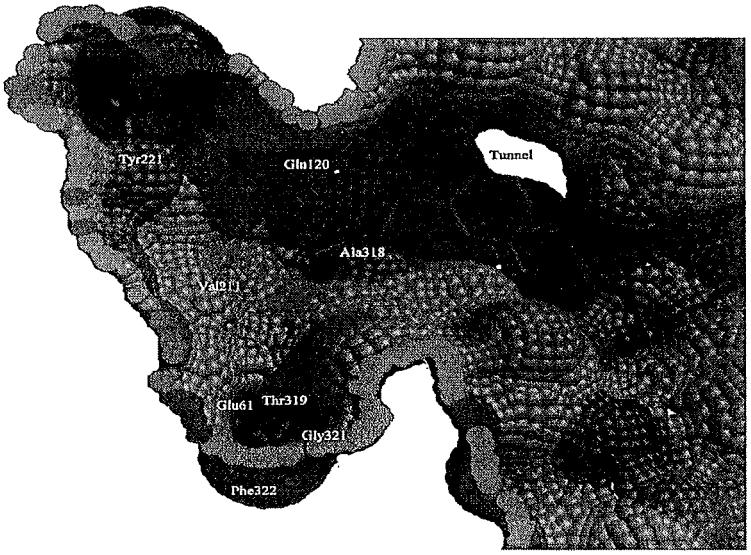


sulfate binding protein

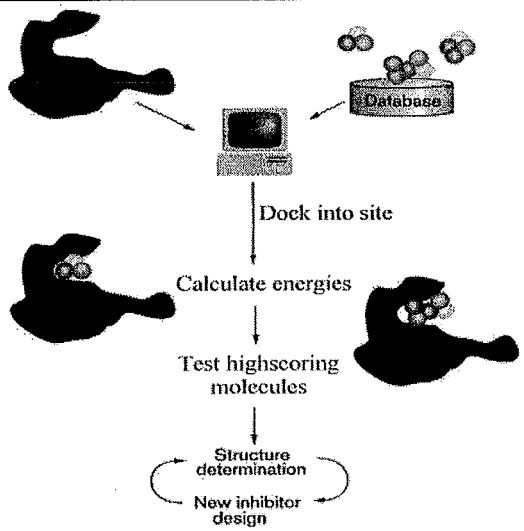
Conserved Residues, Ordered Structure, Function Unknown



Inhibitor Discovery or Design?

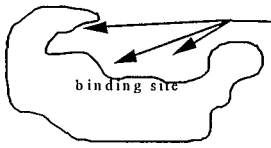
- **Design ligands**
  - Ludi (Bohm)
  - Grow (Moon & Howe)
  - Builder (Roe & Kuntz)
  - MCSS-Hook (Miranker & Karplus)
  - SMOG (DeWitte & Shaknovitch)
  - Others...
- **Discover Ligands**
  - DOCK (Kuntz, et al., Shoichet)
  - CAVEAT (Bartlett)
  - Monte Carlo (Hart & Read)
  - AutoDock (Goodsell & Olson)
  - SPECITOPE (Kuhn et al)
  - Others...

Screening Databases by Molecular Docking



© Chemistry & Biology, 1996

Database Screening Using DOCK

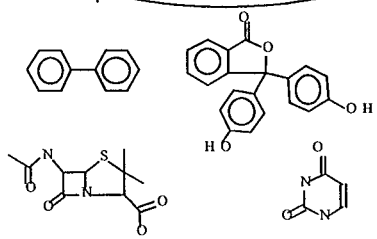


Database of commercially available small molecules

Each molecule is fit into the binding site in multiple orientations.
Multiple conformations of each ligand are considered.

Each orientation is evaluated for complementarity, using van der Waals and electrostatic interaction energies.

Solvation energies are subtracted.



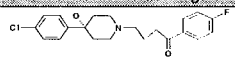
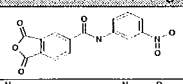
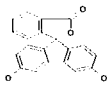
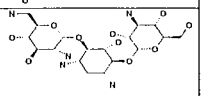
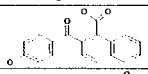
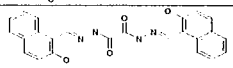
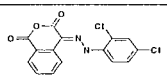
... ~200,000 compounds

The inhibition constants of the best fitting molecules are established in an enzyme assay

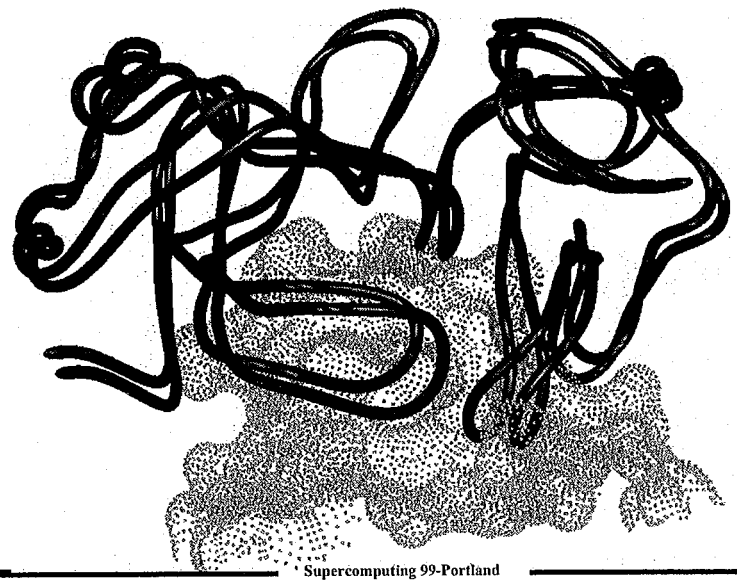
Inhibitor-receptor complex structures are determined.

New interactions with the enzyme are targeted.

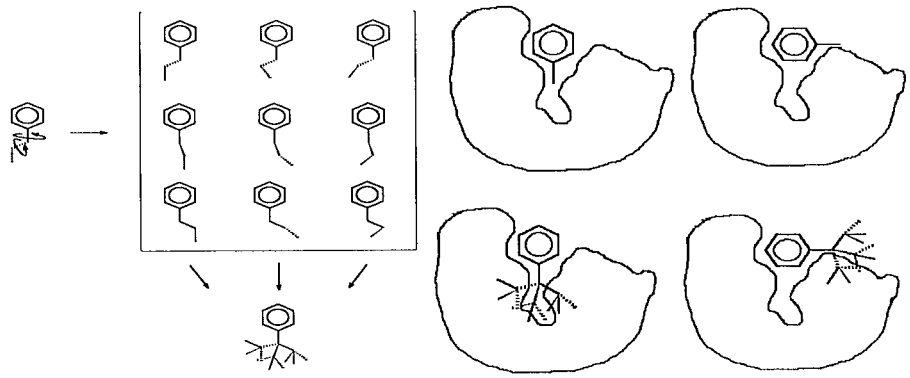
Novel Ligand Discovery Using Molecular Docking

Receptor	Lead from molecular docking	Receptor	Lead from molecular docking
HIV protease		HGXPRTase	
thymidylate synthase		RNA	
hemagglutinin		Zn β-lactamase	
cercarial elastase		Thrombin	
malarial protease		AmpC β-lactamase	
CD4-gp120	unpublished	thymidylate synthase	
		HGXPRTase	unpublished

de Novo Structure Prediction: blip/tem-1

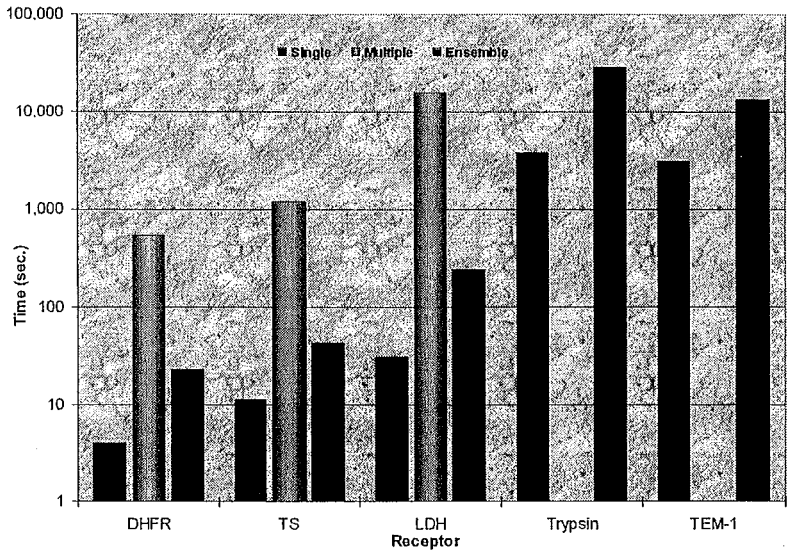


Ligand Flexibility: Conformational Ensembles



Generate an ensemble dock it into the site

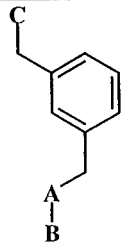
Conformational Ensembles vs. Brute Force



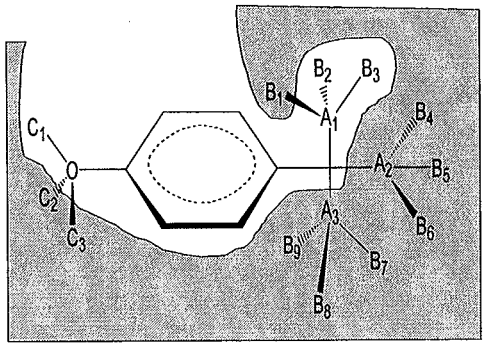
Database Docking

Enzyme	Number of Confs	Number of Comps	Time (hrs.)	Known ligand results: Score (kcal/mol)	RMS (Å)	Rank in Database
Single Conformation Database						
Complexed DHFR	**5,761**	5,761	**0.58**	----	----	----
Uncomplexed DHFR	**5,761**	5,761	**1.40**	91.9	8.32	**16.09%**
Complexed TS	**281**	281	**0.31**	----	----	----
Uncomplexed TS	**281**	281	**0.51**	-8.3	3.67	**97.15%**
Multi Conformation Database						
Complexed DHFR	**867,822**	5,656	**0.94**	-12.5	1.20	**99.33%**
Uncomplexed DHFR	**867,822**	5,656	**2.96**	-7.4	1.34	**98.83%**
Complexed TS	**88,487**	263	**0.27**	-89.2	0.77	**99.62%**
Uncomplexed TS	**88,487**	263	**0.18**	-31.5	2.71	**99.24%**
Full Multi Conformation Database						
Complexed DHFR	**33,717,639**	115,349	**26.50**	-12.5	1.20	**99.72%**
Complexed TS	**33,715,748**	117,240	**80.90**	-89.2	0.77	**99.93%**

Hierarchical Docking



Flexible docking:	Hierarchical docking:
27 confs	27 confs
x3 atoms	3C + 3A + 9B
81 atom positions	15 atom positions



Correcting for Ligand Solvation Energies

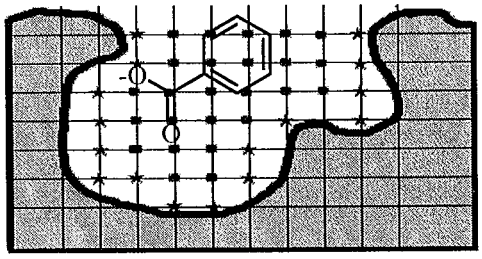
$$\Delta G_{bind} = \Delta G_{interact} - \Delta G_{solv,\,L} - \Delta G_{solv,\,R}$$

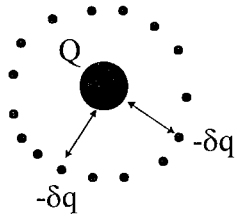
$$\Delta G_{interact} = \sum(q_i P_i + v_i P_v)$$

$$\Delta G_{elec,solv} = (q^2/2r)\,(1/D_0 - 1/D_w)$$

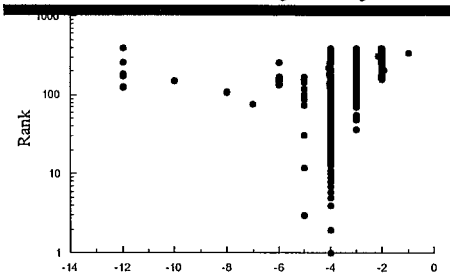
$$= (1/D_0 - 1/D_w)/2r \sum\sum Q_i \delta q_j$$

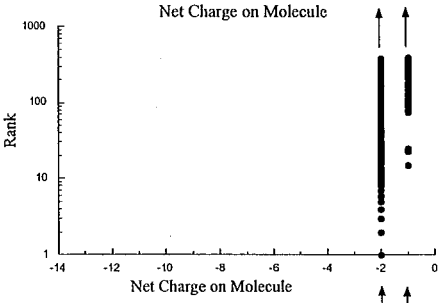
$$\ddot{A}H_{np} = -621.48 - 25.890 \times \text{area}$$

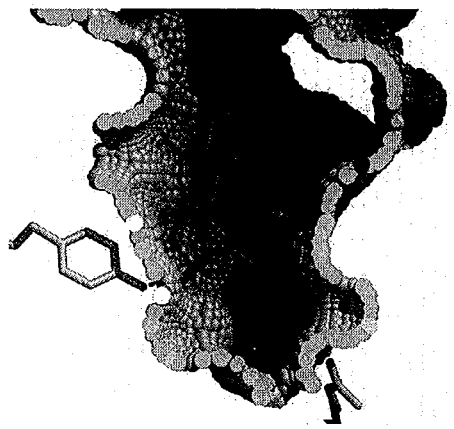




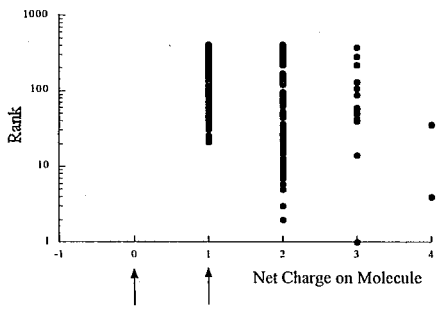
Solvation Corrections: Thymidylate Synthase Screen

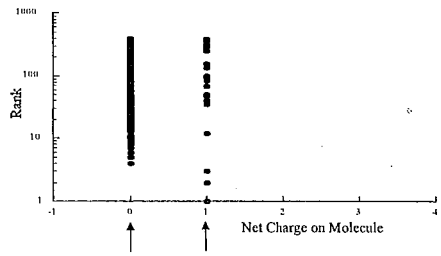


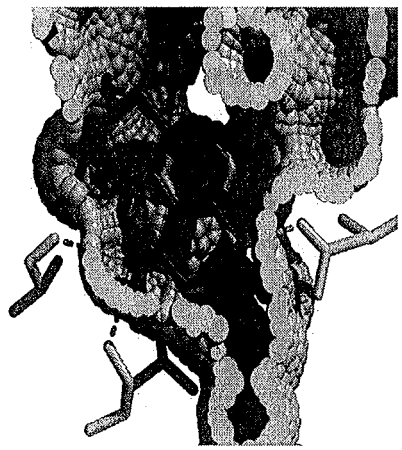




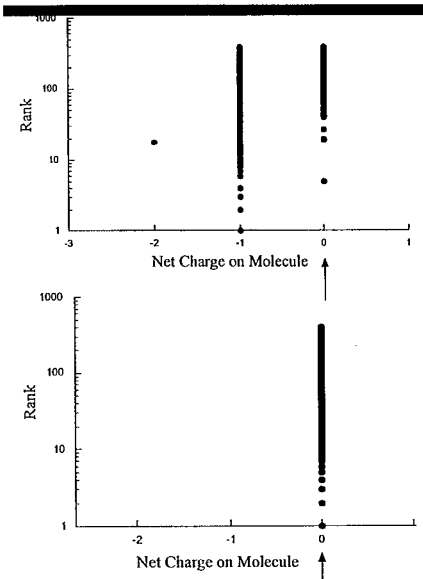
Solvation Corrections: DHFR Screen

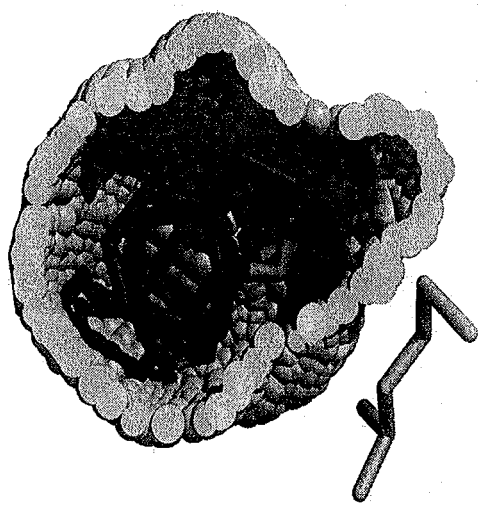






Solvation Corrections: Benzene Cavity Screen





Hit Rates

Enzyme	Hit Rate	IC50 for 'Hit'	Compounds Tested	Random Hit Rate
AmpC (E. coli)	50%	<10 μM	20	???
HXPRTase (T. cruzi)	60%	<12 μM	22	???
Corporate (homology modeled)	2%	???	895	0.04% (per 102,000)

Unmet Challenges

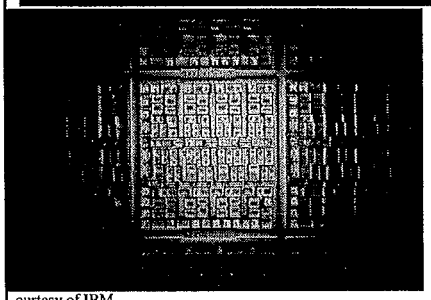
- Better Scoring
  - context dependent desolvation
  - receptor desolvation
  - better force-fields
- Receptor Flexibility
- Cominatorial Chemistry

- This work supported by the NIH, Genetics Institute, and Procter & Gamble

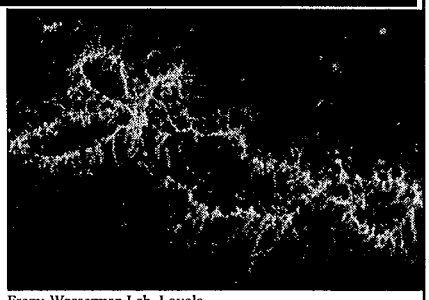
Cellular Network Analysis

Adam Arkin
Physical Biosciences
Lawrence Berkeley National Laboratory
Bioengineering and Chemistry
University of California, Berkeley
11/15/99

Engineering of Cellular Circuitry



ourtesy of IBM



From: Wasserman Lab, Loyola

Asynchronous Digital Telephone Switching Circuit

Full knowledge of parts list
Full knowledge of "device physics"
Full knowledge of interactions

No one fully understands how this circuit works!!
Its just too complicated.

Designed and prototyped on a computer (SPICE analysis)
Experimental implementation fault tested on computer

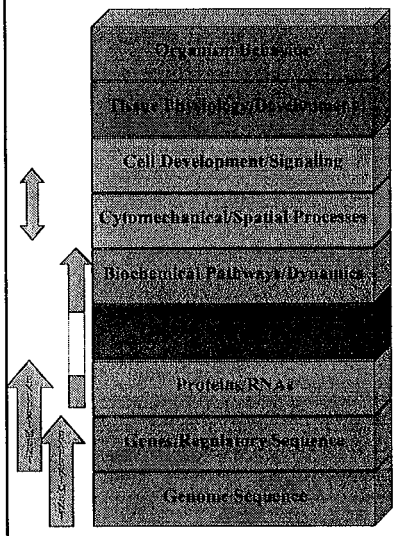
Asynchronous Analog Biological Switching Circuit

Partial knowledge of parts list
Partial knowledge of "device physics"
Partial knowledge of interactions

No one fully understands how this circuit works!!
Its just too complicated.

We *need* a SPICE-like analysis for biological systems

Analysis of Cell Function



The challenge is to integrate data from all levels to produce a description of cellular function.

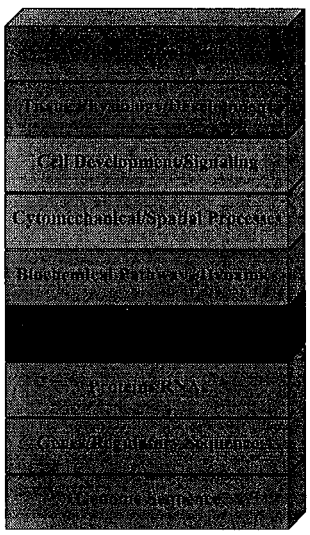
There are challenges in:

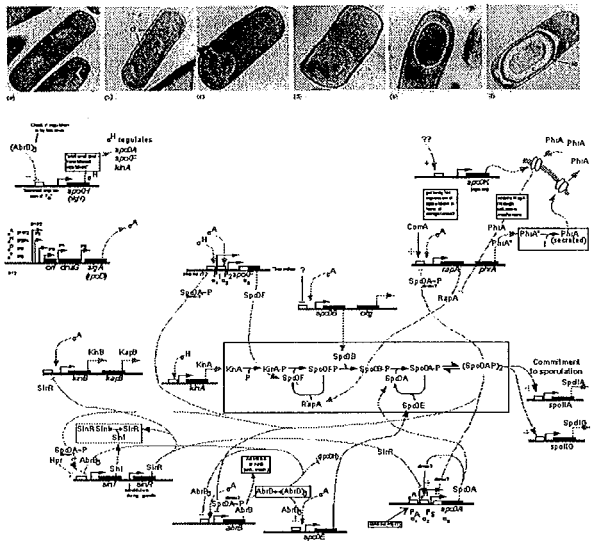
- Systematization and structuring of data
- Serving and query this data
- Representing the data
- Building multiscale, multiresolution models
- Dynamic and static analysis of these models

Pay-off in

- Industrial bioengineering
- Rational pharmaceutical design
- Basic biological understanding

Complexities of Cellular Function





Complexities of Cellular Function

Title:
/dak2/people/sparkin/fig2
Creator:

Preview:
This EPS picture was not saved with a preview included in it.
Comment:
This EPS picture will print to a PostScript printer, but not to other types of printers.

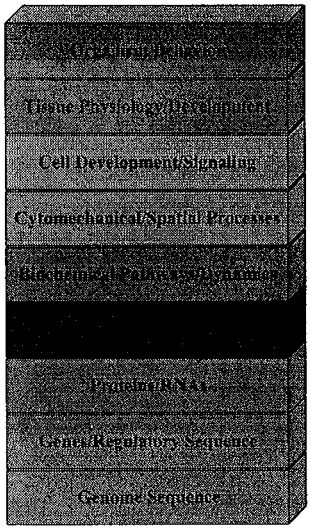
Spatiotemporally resolved pictures of developmental processes take up Gigs of storage.

Analyses takes days-weeks.

Models are in early days.

Each of those little bright spots contains networks vastly more complicated than those on the last slide!

Heterogeneity of Data

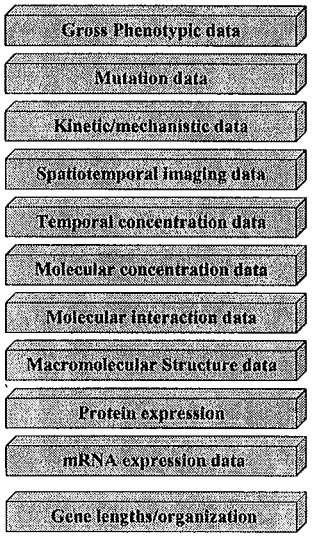


Data are:

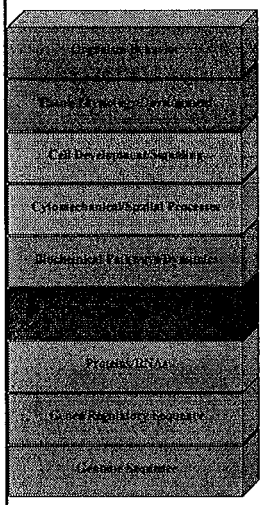
1) Qualitative-->Quantitative
2) Collected at many levels
3) Of heterogeneous structure
4) Of heterogeneous availability

Challenge:

Optimal use of available data to make predictions about cell function and failure.



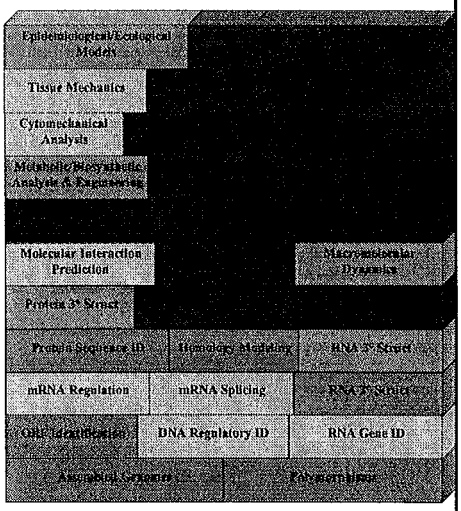
Tools for "multilevel" analysis



Cellular networks

Physical properties

Finding Parts



Why now?

•Genome projects are providing a large (but partial) list of parts

•New measurement technologies are helping to identify further components, their interactions, and timings

- Gene microarrays
- Two-Hybrid library screens
- High-throughput capillary electrophoresis arrays for DNA, proteins and metabolites
- Fluorescent confocal imaging of live biological specimens
- High-throughput protein structure determination

•Data is being compiled, systematized, and served at an unprecedented rate
- Growth of GenBank and PDB > polynomial
- Proliferation of databases of everything from sequence to confocal images to literature

•The tools for analyzing these various sorts of data are also multiplying at an astounding rate

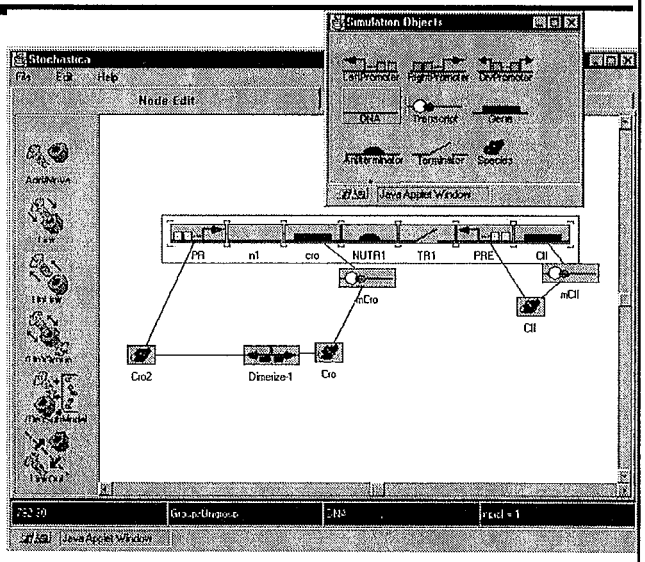
SPICE Tools for Biology?

Bio/Spice: A Web-Servable, Biologist-Friendly, database, analysis and simulation interface was developed into a true beta product.

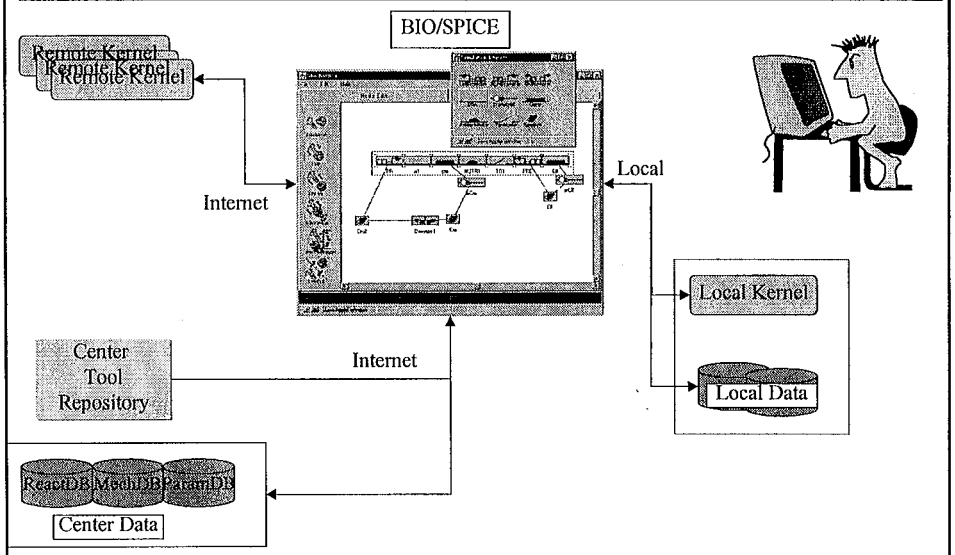
Interfaces to ReactDB, MechDB, and ParamDB.

With Kernel, performs basic:
flux-balance analysis,
stochastic and deterministic kinetics,
Scientific Visualization of results.

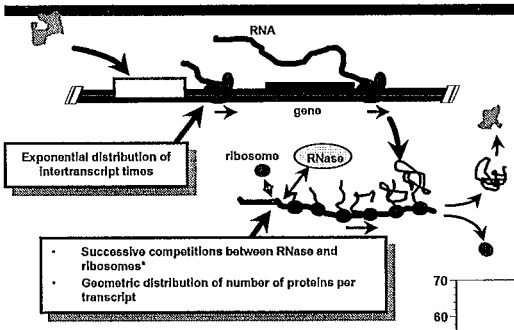
Notebook/Kernel design optimized for distributed computing.



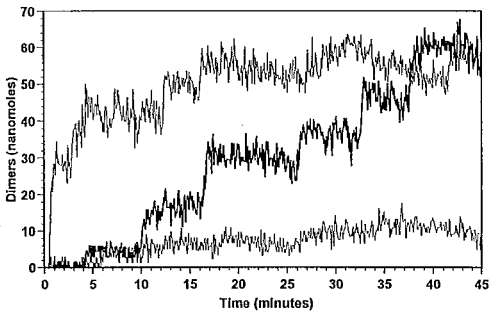
Components of Bio/Spice



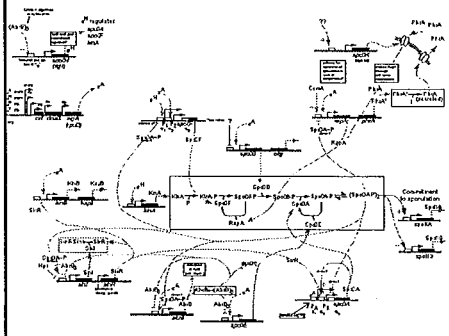
An Example of "Device Physics"



Simulation methodology for full-up simulation of chemical Markov-Process scales ***exponentially*** with number of reactions



Complexities of Cellular Function



This is approximately 1/3 of just the initiation of the sporulation program from *Bacillus subtilis*.

There are over 100 proteins, 40 genes, 300 reactions for which data is available.

The total data on just this process is a tens of Gigs and it is incomplete.
Microarray and microscope data are added 100 Megs per week.
Model builders need to query this data and arrange it for simulation.
Simulations must be run under many different condition and hypotheses.

The Need for Advanced Computing

Data Handling:

- The total data necessary for network analysis is huge.
- By nature it will be distributed and heterogeneous
- We need:
  - Database standard and new query types
  - Means of secure,fast transmission of information
  - Means of quality control on data input

Tool integration:

- Centralization of computational biology tools and standards
- Ability to use tools together to generate good network hypotheses
- Good quality ratings on Tool outputs

Advanced Simulation Tools:

- Fast, distributed algorithms for dynamical simulation
  - Mixed mode systems (differential, Markov, algebraic, logical)
  - Spatially distributed systems