State-of-Art Infrastructure for Biomedical Research: The potential for Regional and National Collaborations – *the NYSBC experience*

- Scientific accomplishments
  - Infrastructural components
- Organization of NYSBC
  - Governance
    - Program
    - Capital
    - Revenue
    - Compliance
- Model for regional collaborations
  - Pluses and minuses
- Improving the quality
  - The “Wealth of Science”
  - changing the social structure
    - Do we want to?
    - How?

AAMC

NEW YORK STRUCTURAL BIOLOGY CENTER

Scientific goals

- Structure determination

  proteins, nucleic acids, macromolecular assemblies
- Structure/function relationships
- Therapeutics discovery

Methodologies

- X-ray Crystallography

  Beam line X-4A, X-4C Brookhaven synchrotron
- Cryo-Electron Microscopy
- NMR

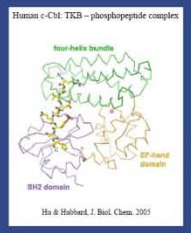
AAMC

Some NYSBC core foci

- Structure of Membrane Proteins
- Structures of Protein Complexes
- Dynamics of proteins and their interactions
- Mapping of small molecule interactions on protein complexes

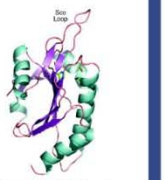
AAMC

NYSBC X4 Beamline at NSLS 2003-2005



Human c-Cbl: TKB – phosphopeptide complex

Hu & Hubbard, J. Biol. Chem. 2005


Human SCO1 "assembly" protein

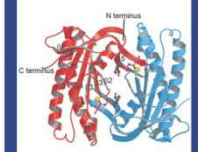
Williams ... Schon, J. Biol. Chem. 2005

hOGG1 repair protein – DNA complex

Banerjee ... Verdine, Nature 2005

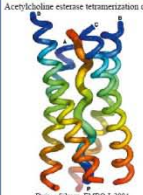

human FGF10 – FGFR2b complex

Ibrahimi ... Mohammadi, Mol. Cell. Biol. 2005



Soluble adenylyl cyclase

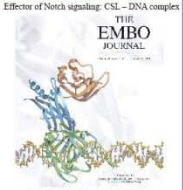
Steegborn ... Wu, Nat. Struct. Mol. Biol. 2005


Acetylcholine esterase tetramerization complex

Dvir ... Silman, EMBO J. 2004

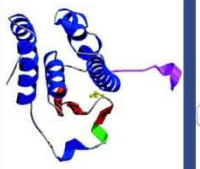
Human fatty acid synthase: thioesterase domain

Chakravarty ... Quiocho, PNAS 2004



Effector of Notch signaling: CSL – DNA complex

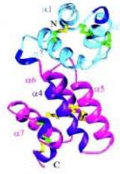
Kovall & Hendrickson, EMBO J. 2004


SufE sulfur-acceptor protein

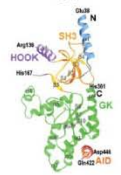
Goldsmith-Fischman ... Hunt, J. Mol. Biol. 2004


Human Senp2 domain – SUMO complex

Reverter & Lima, Structure 2004

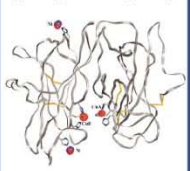

HER-1 sexual differentiation factor

Hamaoka ... Leahy, PNAS 2004

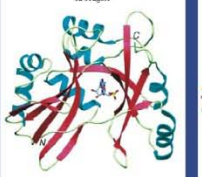


α1-β interactions of voltage-gated Ca2+ channels

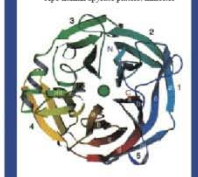
Chen ... Yang, Nature 2004


Copper – dioxygen PHM monooxygenase complex

Prigge ... Amzel, Science 2004


RNA ligase

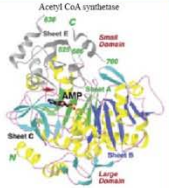
Ho ... Shuman, Structure 2004


Apo human apyrase platelet inhibitor

Dai ... Lu, Cell 2004

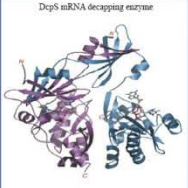

Resistin

Patel ... Shapiro, Science 2004

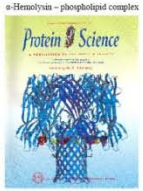


Acetyl CoA synthetase

Jogl & Tong, Biochemistry 2004

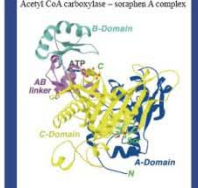

DcpS mRNA decapping enzyme

Gu ... Lima, Mol. Cell 2004



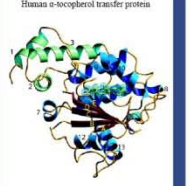
α-Hemolysin – phospholipid complex

Galdiero & Gouaux, Protein Science 2004

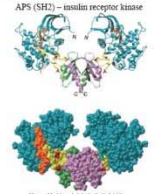


Acetyl CoA carboxylase – soraphen A complex

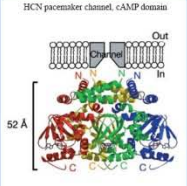
Shen ... Tong, Mol. Cell 2004


Human α-tocopherol transfer protein

Min, Kovall & Hendrickson, PNAS 2003

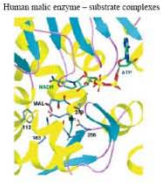

APS (SH2) – insulin receptor kinase

Hu ... Hubbard, Mol. Cell 2003

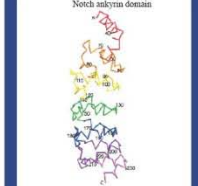


HCN pacemaker channel, cAMP domain

Zagotta ... Gouaux, Nature 2003

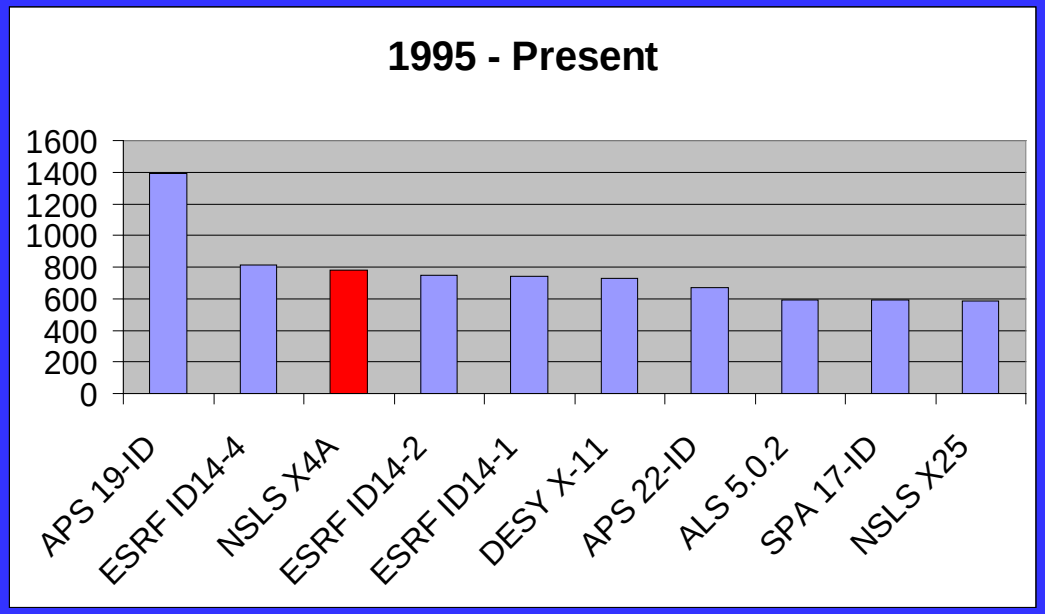

Human malic enzyme – substrate complexes

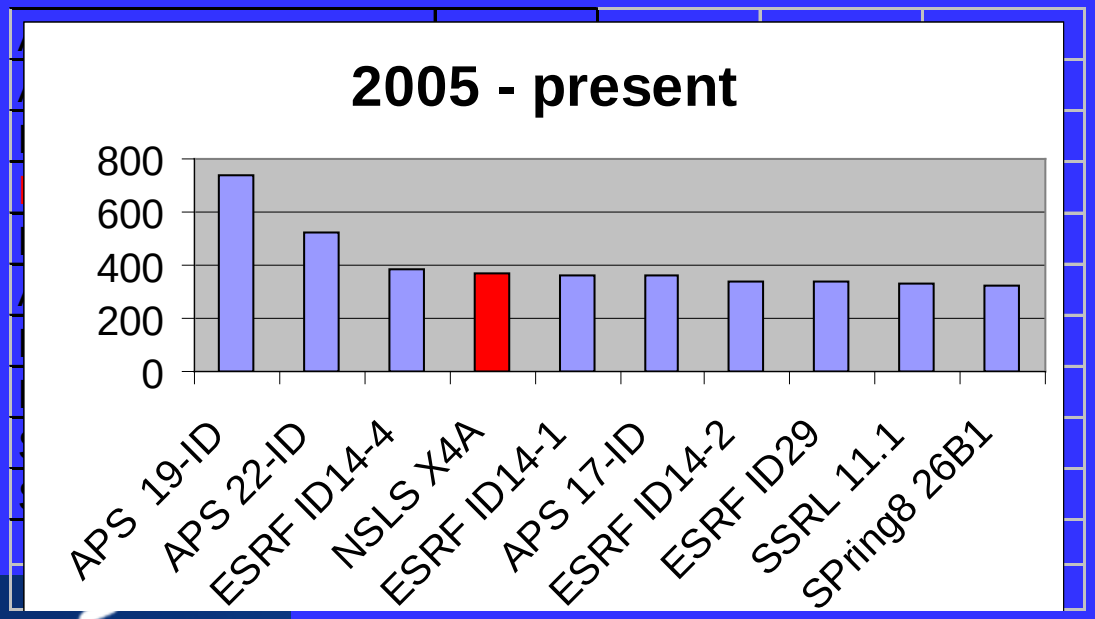
Tao, Yang & Tong, Structure 2003


Notch ankyrin domain

Zweifel ... Barrick, Protein Science 2003

AAMC



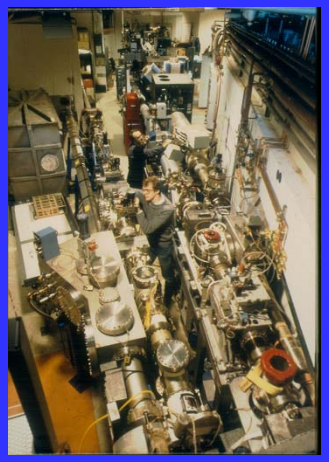


Historical and continuing high productivity at Beamlines X-4A & C at BNL. (PDB deposits)

http://biosync.rcsb.org/BiosyncStat.html

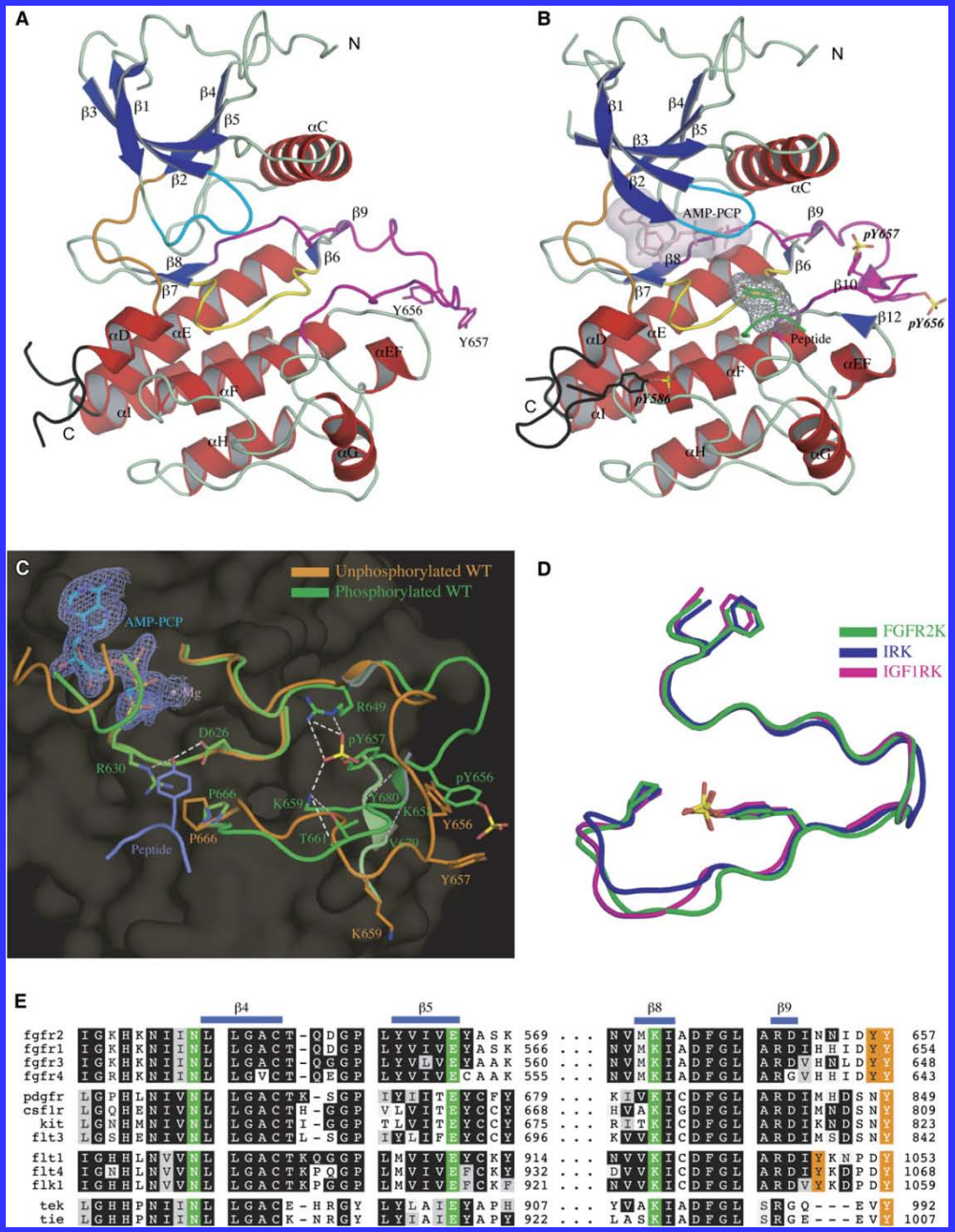
105 active beam lines worldwide

4 April 2008



AAMC

Beam line access permits detailed comparisons of multiple isoforms



A Molecular Brake in the Kinase Hinge Region Regulates the Activity of Receptor Tyrosine Kinases

Huaibin Chen, Jinghong Ma Wanqing Li, Anna V. Eliseenkova Chongfeng Xu, Thomas A. Neubert, W. Todd Miller, and Moosa Mohammadi Mol. Cell 27, 717–730,, 2007

AAMC

Cryoelectron microscopy at NYSBC

Goals

- Novel protein structures
- Structural mechanisms of macromolecular machines
- Bridge gap between atomic structures and cell physiology

Methods

- Electron crystallography
- Single particle analysis
- Electron tomography

Machines

- 300kV FEG liquid He with energy filtration
- 200kV FEG liquid N_2
- 200kV LaB_6 liquid N_2

AAMC

Two examples of using and expanding the interactome knowledge and bridging gaps -- EM

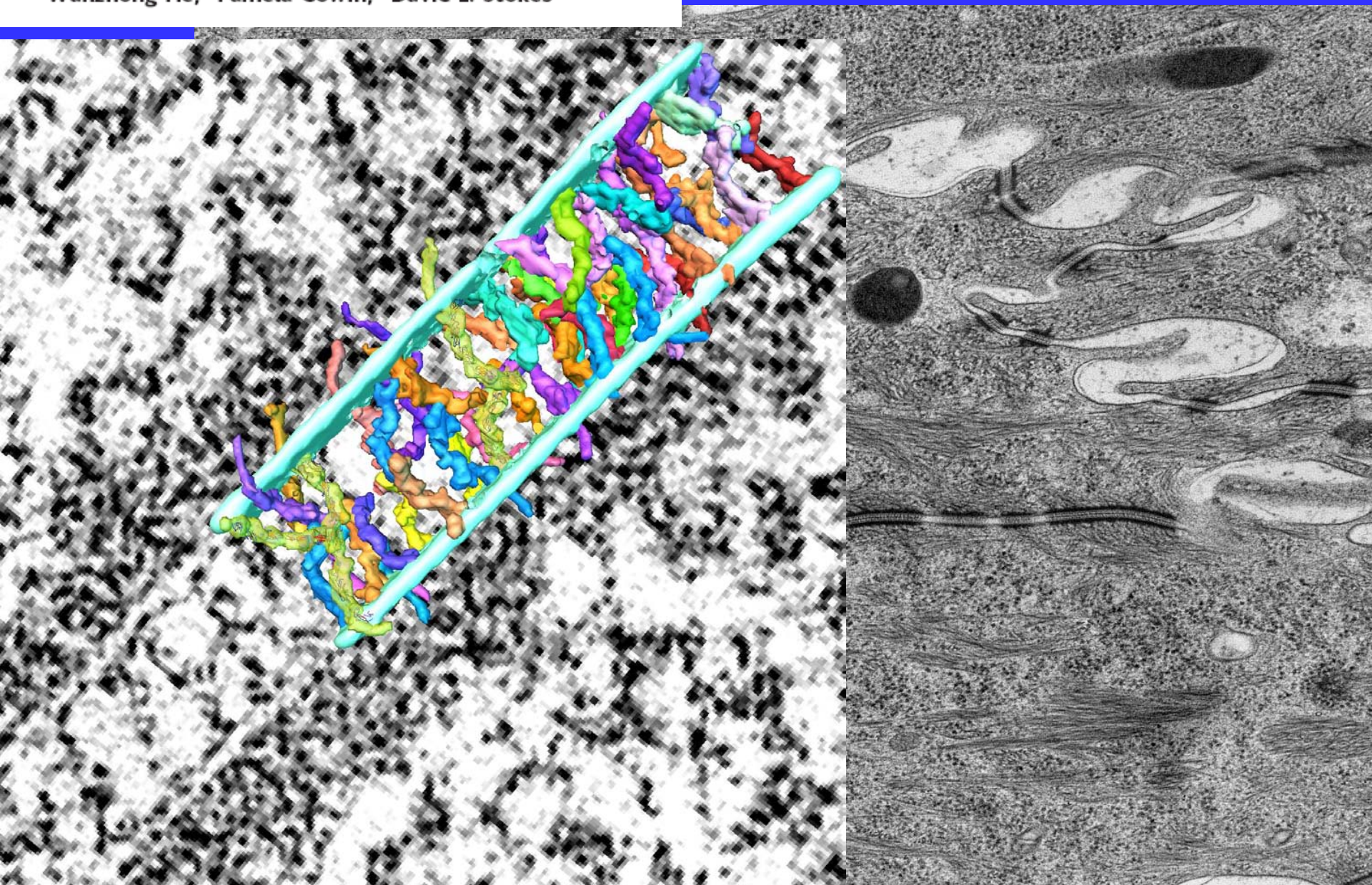
- Role of cadherins in desmosomal knots – Stokes et al.
- Interaction of endonuclease with DNA – Aggarwal et al

AAMC

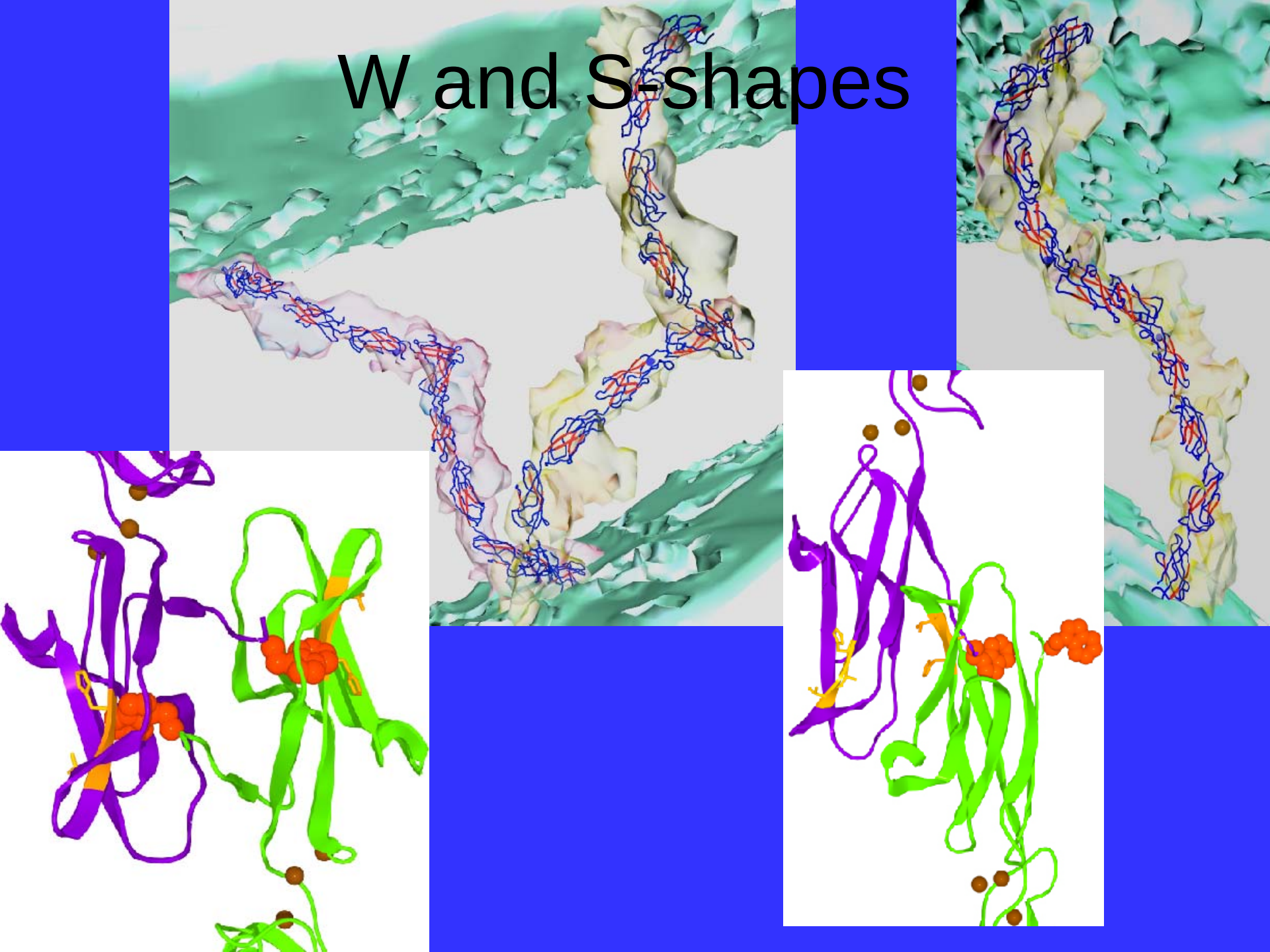
Untangling Desmosomal Knots with Electron Tomography

Wanzhong He,[1] Pamela Cowin,[2] David L. Stokes[1,2,3]*

SCIENCE VOL 302 3 OCTOBER 2003



W and S-shapes



doi:10.1016/j.jmb.2007.04.066 J. Mol. Biol. (2007) 370, 207–212

JMB

Available online at www.sciencedirect.com

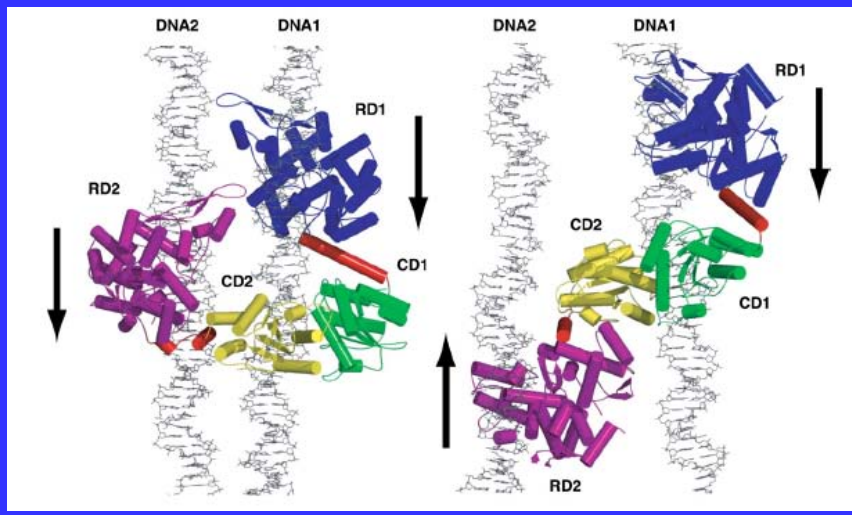
ScienceDirect

ELSEVIER

COMMUNICATION

An EM View of the FokI Synaptic Complex by Single Particle Analysis

Éva Scheuring Vanamee[1]*, John Berriman[2] and Aneel K. Aggarwal[1]



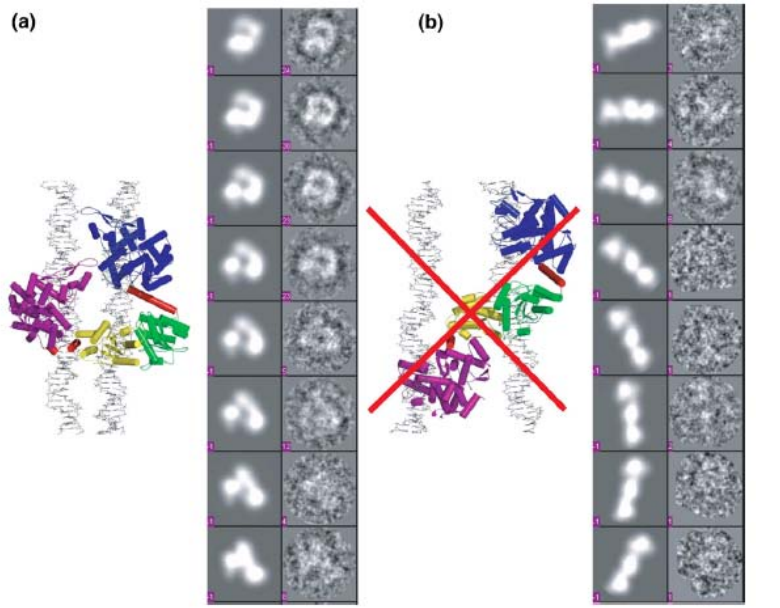


Figure 3. 2D projection matches generated (a) from model 1 and (b) from model 2. The left panels show representative projections generated from the models at 9° intervals and the right panels show the matched classified particles. The numbers in the right panels indicate the number of particles in each projection. The atomic models used to generate the 2D

AAMC

Some NMR – structural biology areas at NYSBC

- Signal transduction
- Chromatin remodeling
- RNAi and related protein interactions
- Small Transporters
- Ubiquitin – proteosome
- Bacterial sigma factors
- Apoptosis
- Viral Assembly
- Intrinsically unstructured proteins

AAMC

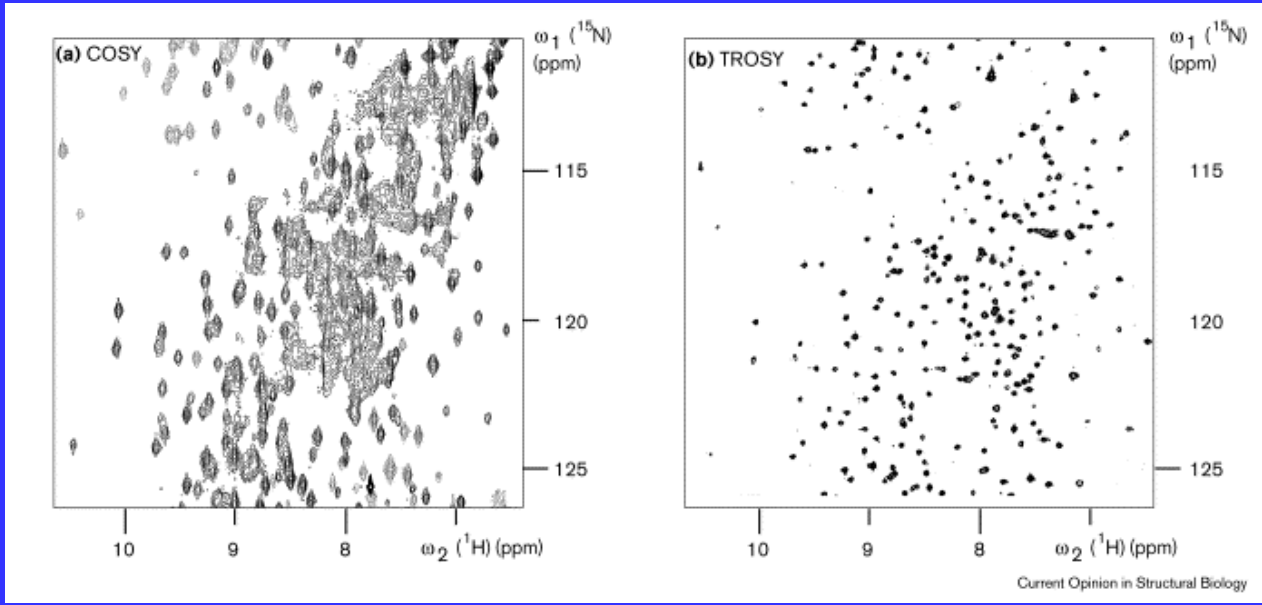
NYSBC NMR Performance

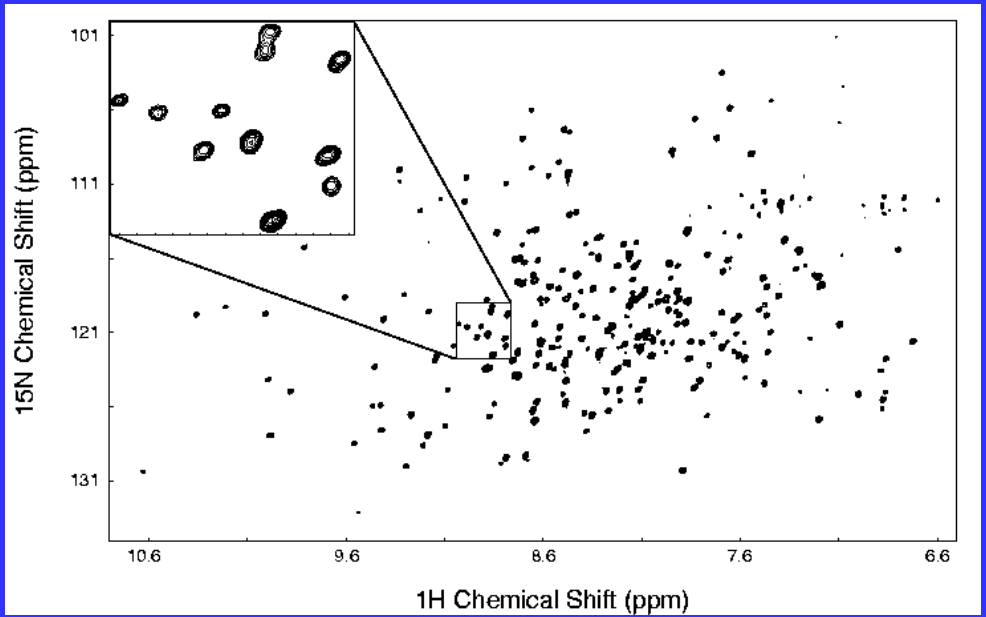
- The TROSY effect
  Magnets > 750 MHz
  Proteins > 30 kD

63 kD protein at 900 MHz,

- NYSBC

Courtesy Prof. Mark Girvin





Albert Einstein College of Medicine OF YESHIVA UNIVERSITY

AAMC

Understanding multiple weak interactions

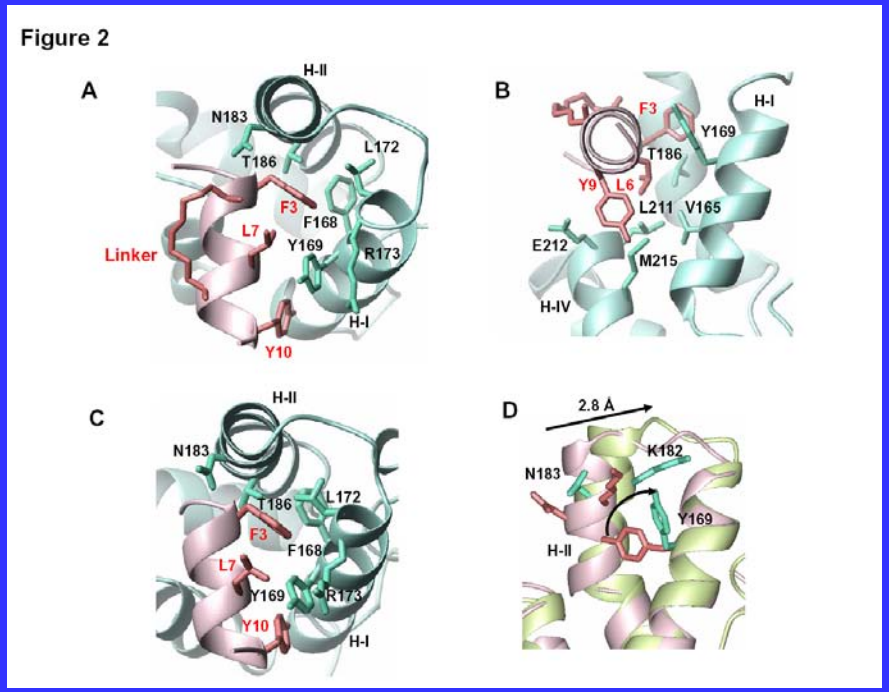
Structural Basis of Site-Specific Histone Recognition by the Bromodomains of Human Coactivators PCAF and CBP/p300

Lei Zeng,1 Qiang Zhang,1 Guillermo Gerona-Navarro,1 Natalia Moshkina,1 and Ming-Ming Zhou1,*

Structure 16, 643–652, April 2008

AAMC

Compact structure NYAD-13 / CTD CA mt

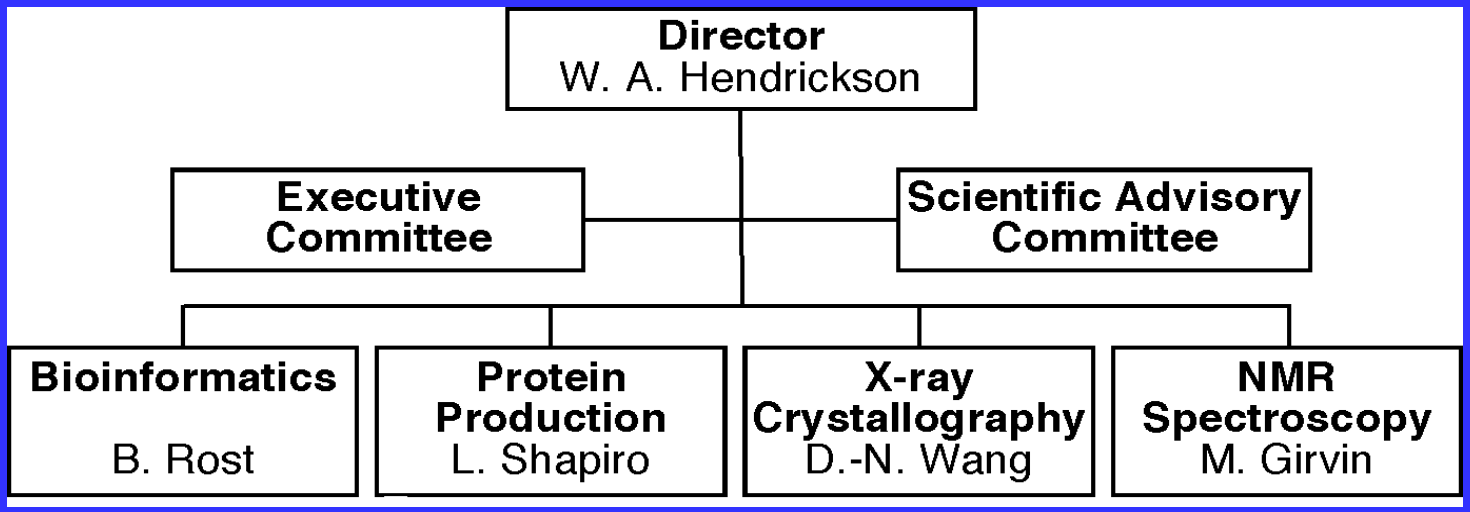


SOLUTION STRUCTURE OF A HYDROCARBON STAPLED PEPTIDE INHIBITOR IN COMPLEX WITH MONOMERIC C-TERMINAL DOMAIN OF HIV-1 CAPSID*.
Shibani Bhattacharya1, Hongtao Zhang2, Asim K Debnath2 & David Cowburn, J Biol Chem *in press*

AAMC

Cooperative ventures

AAMC



NYSBC	Wayne Hendrickson	James Love
Albert Einstein	Mark Girvin	
Columbia	Wayne Hendrickson	Barry Honig
	John Hunt	Ann McDermott
		Filippo Mancia
Burkhard Rost		
	Larry Shapiro	Ming Zhou
NYU	Da-Neng Wang	
Rockefeller	Rod MacKinnon	
Rutgers	Guy Montelione	
SUNY Buffalo	Thomas Szyperski	
Vollum	Eric Gouaux	
UMDNJ	Masayori Inouye	Nancy Woychik

AAMC

NY COMPS
NEW YORK CONSORTIUM ON MEMBRANE PROTEIN STRUCTURE

Production of prokaryotic membrane proteins in E. Coli hosts

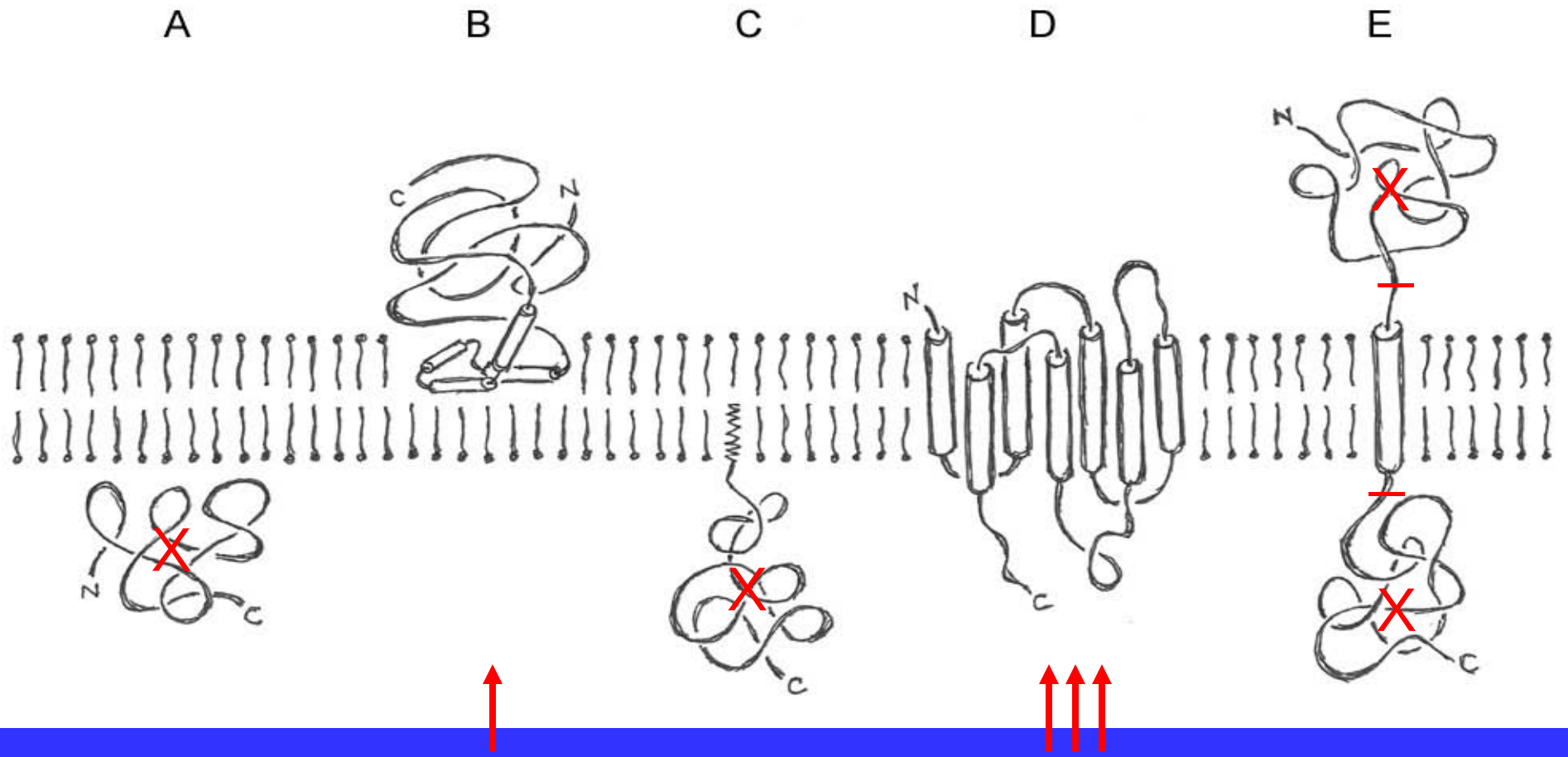
The application of HT techniques developed for soluble proteins to membrane proteins

AAMC

Membrane Proteins

25 - 30% of genomes < 1% Protein Data Bank (PDB)

_____Monotopic_____ _____Polytopic_____



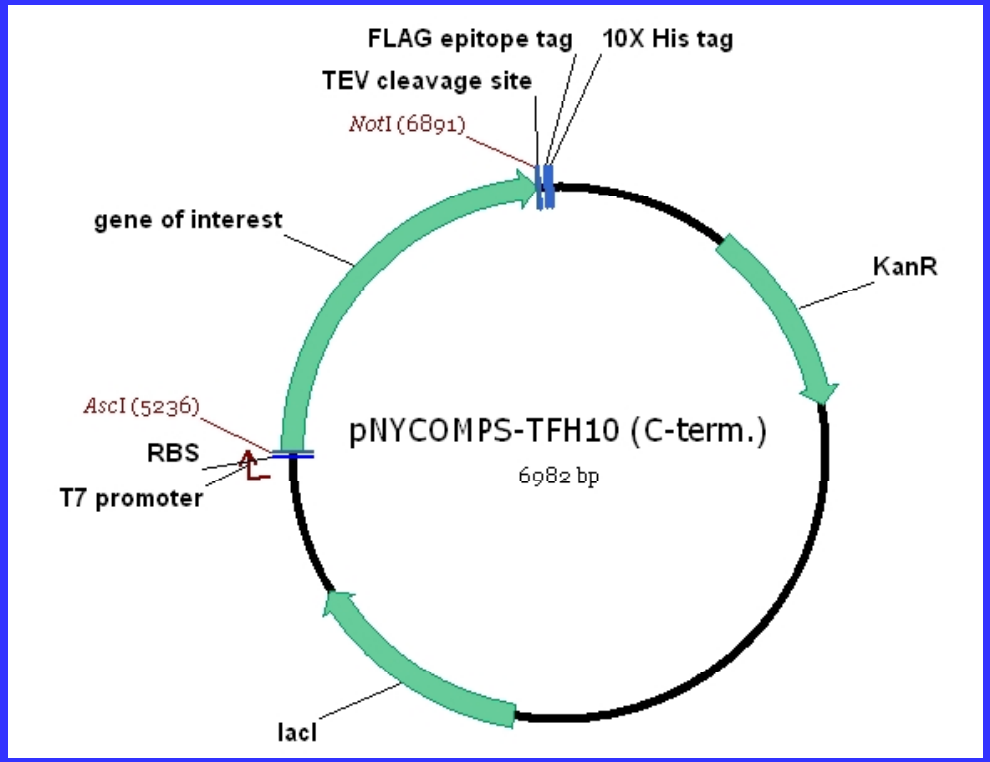
NYCOMPS Challenge: Integral Membrane Proteins having membrane domains

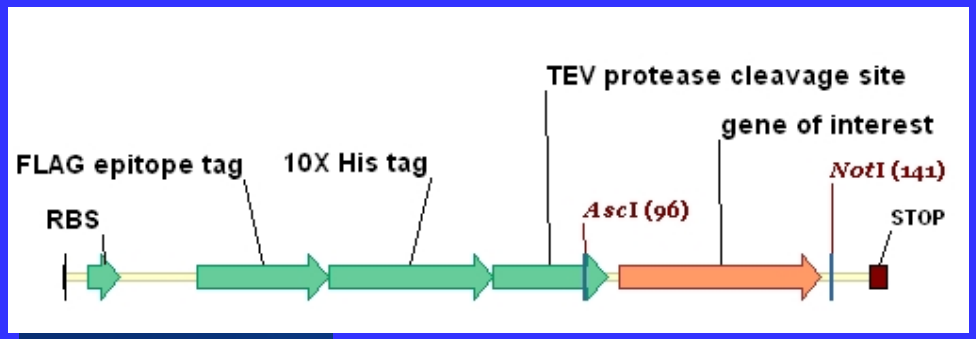
AAMC

New Robot Deck

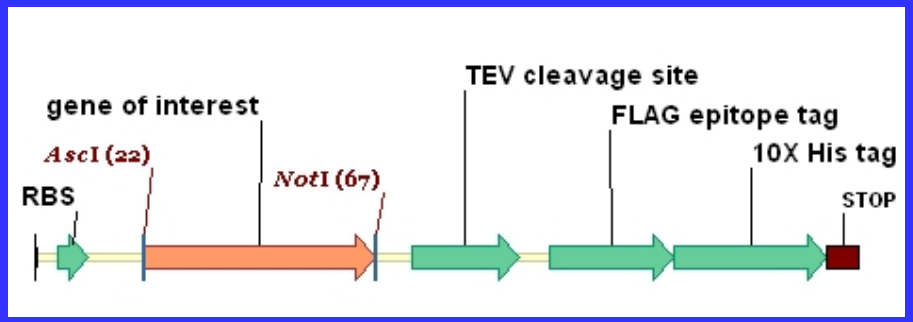
NY COMPS
NEW YORK CONSORTIUM ON MEMBRANE PROTEIN STRUCTURE

NYCOMPS vectors for protein expression in *E. coli*





N-terminal fusion



C-terminal fusion

AAMC

Ligation Independent Cloning (LIC)

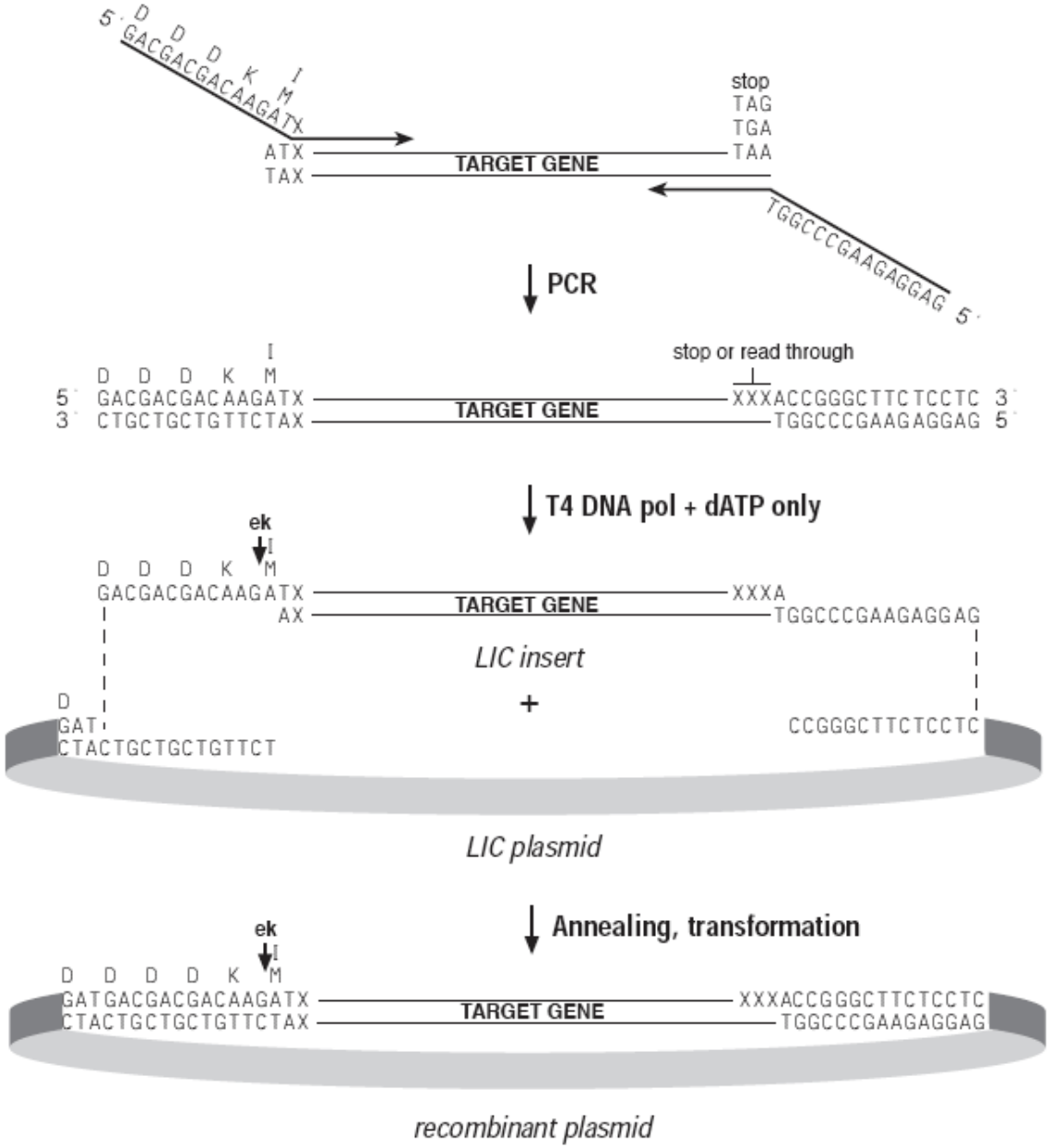
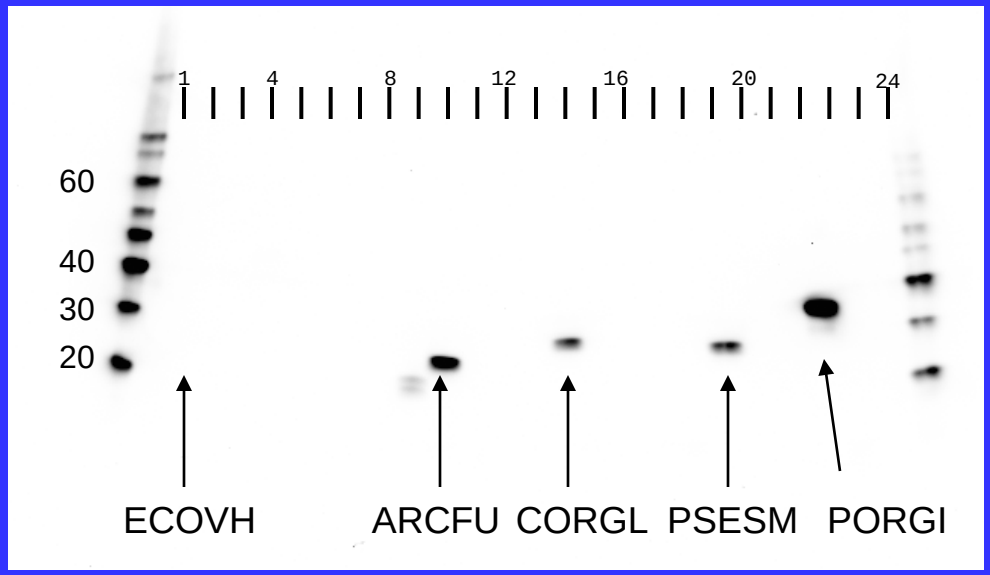


Figure from Novagen

Homologues are worth the effort

YnjF seed



0.5ml BL21 pLysS culture (96 well)
Lysis by sonication
DDM solubilisation
Metal affinity purification (96 well)
SDS PAGE of elutions
Western Blot (FLAG)
Chemiluminecent detection

E.Coli version does not express

Detergent selection by Gel Filtration

192 sample auto sampler
15 possible mobile phases
20 minutes per run, 5µl injections

2 operational systems

Statistics from distributed scale-up

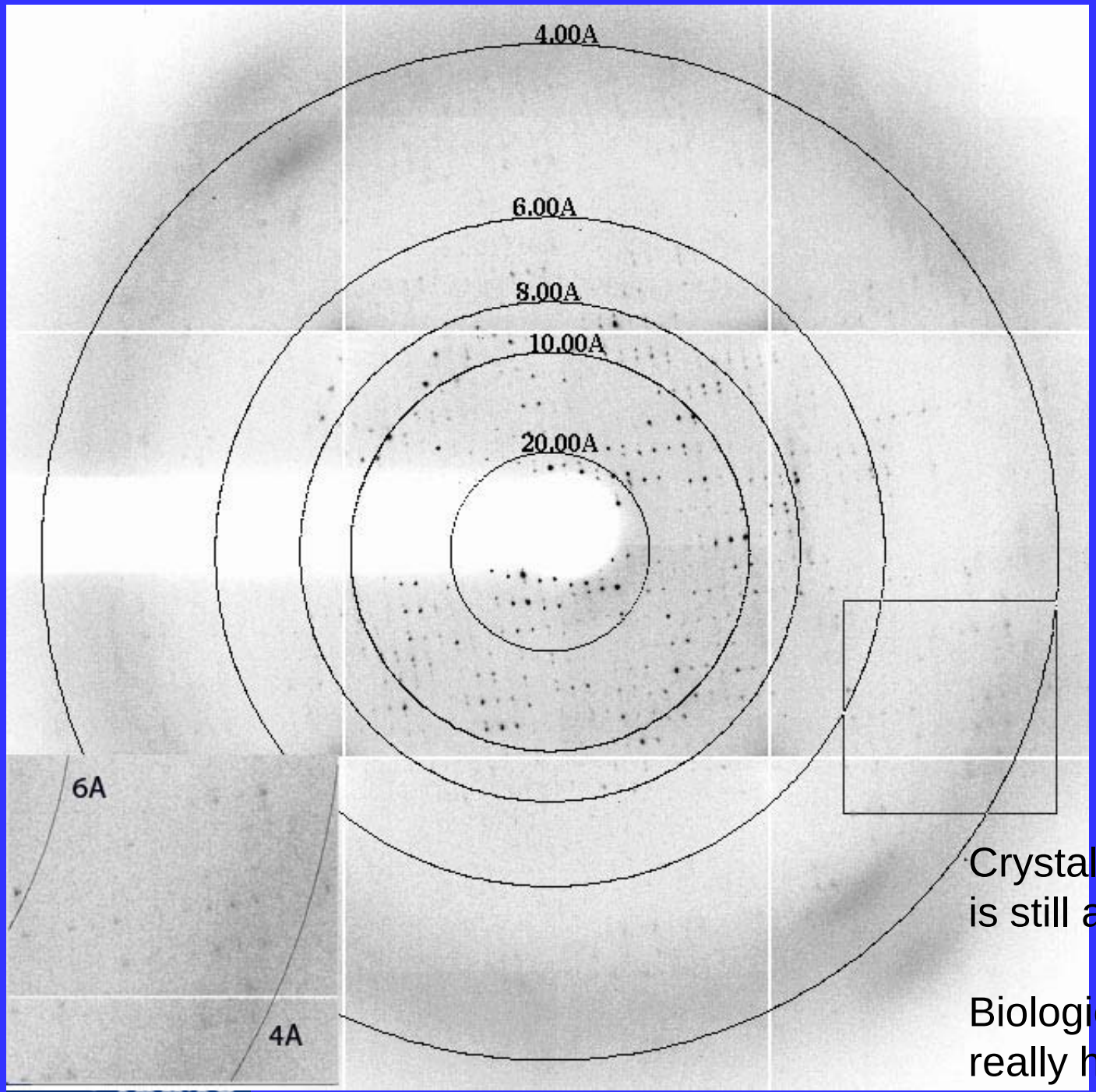
target clusters	28
clones received	432
processed at 1 liter scale	207 (47%)
good at 1 liter scale	58 (28%)
purified at large scale	40
purified to near-homogeneity	29 (73%)
with acceptable gel filtration	16 (55%)
set up for crystallization	10
yielding crystals	4 (40%)
acceptable diffraction limit	0

Nov 07

Now 7, as Feb 08

AAMC

NYCOMPS
NEW YORK CONSORTIUM ON MEMBRANE PROTEIN STRUCTURE

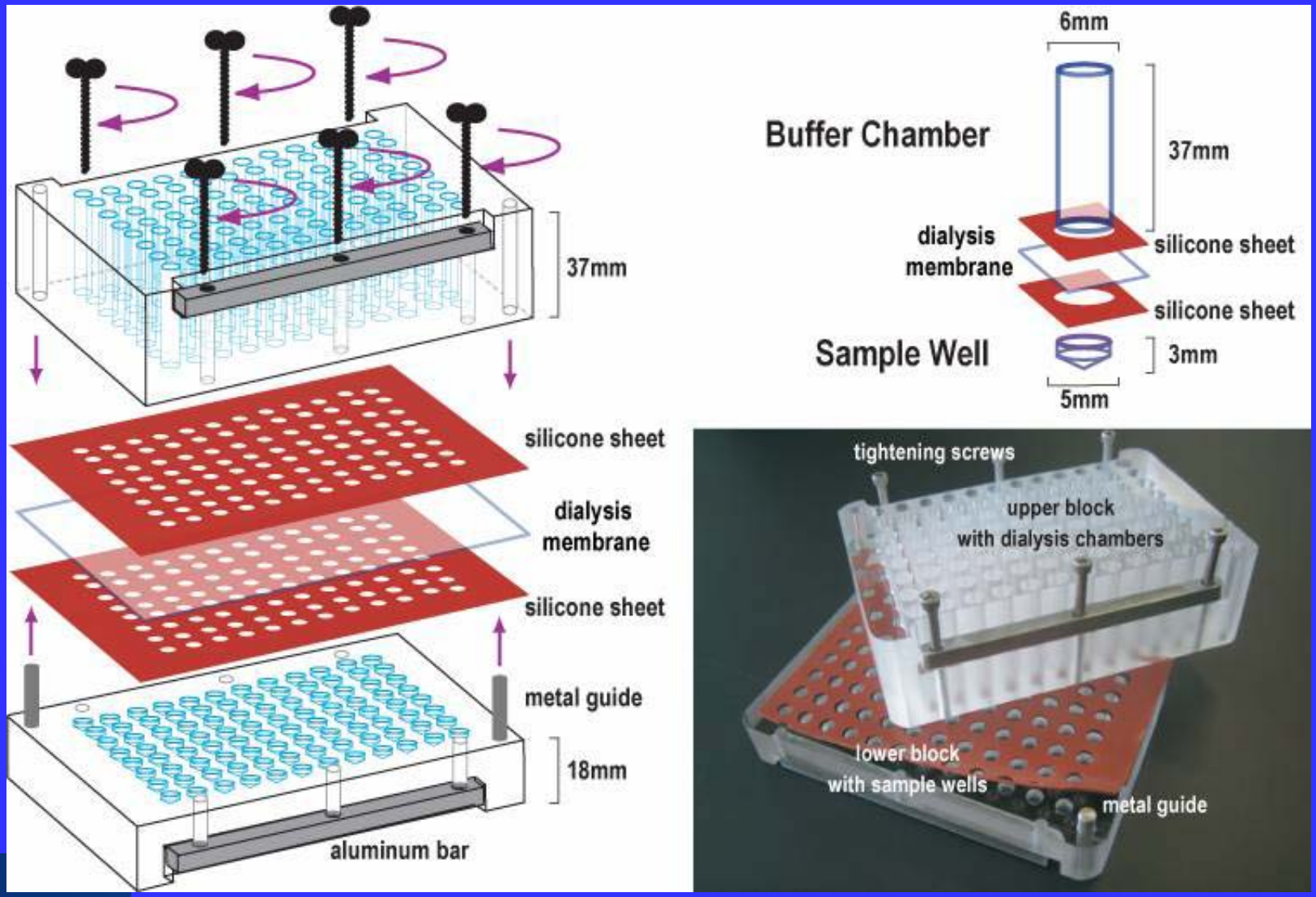


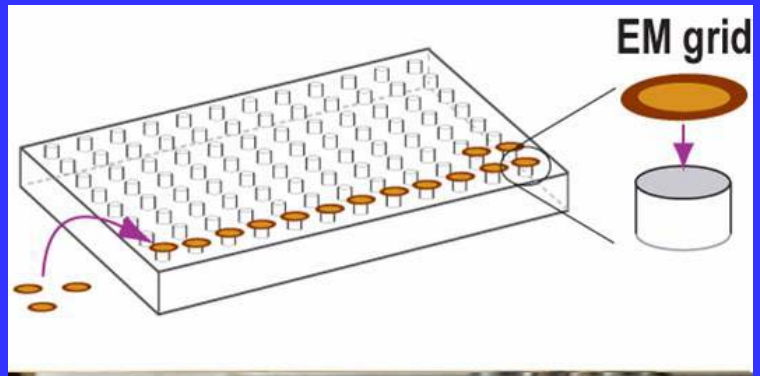
Sugar transport protein

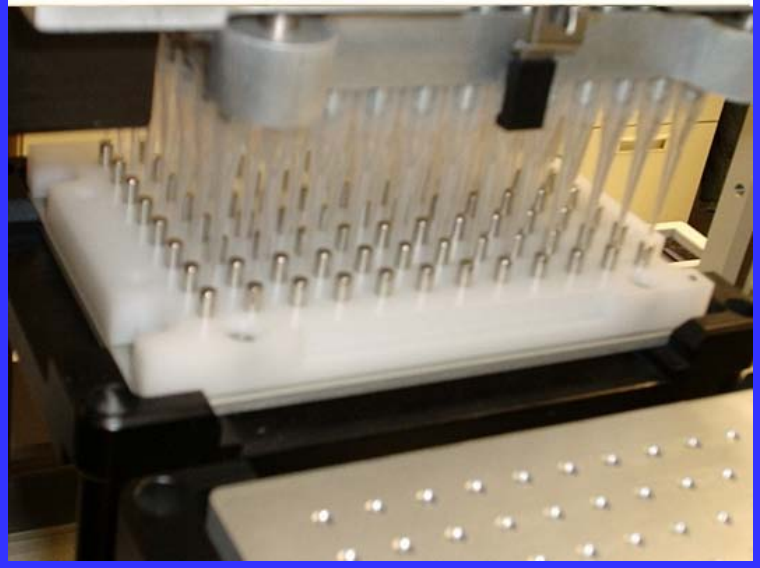
Crystal optimization is still a major bottleneck

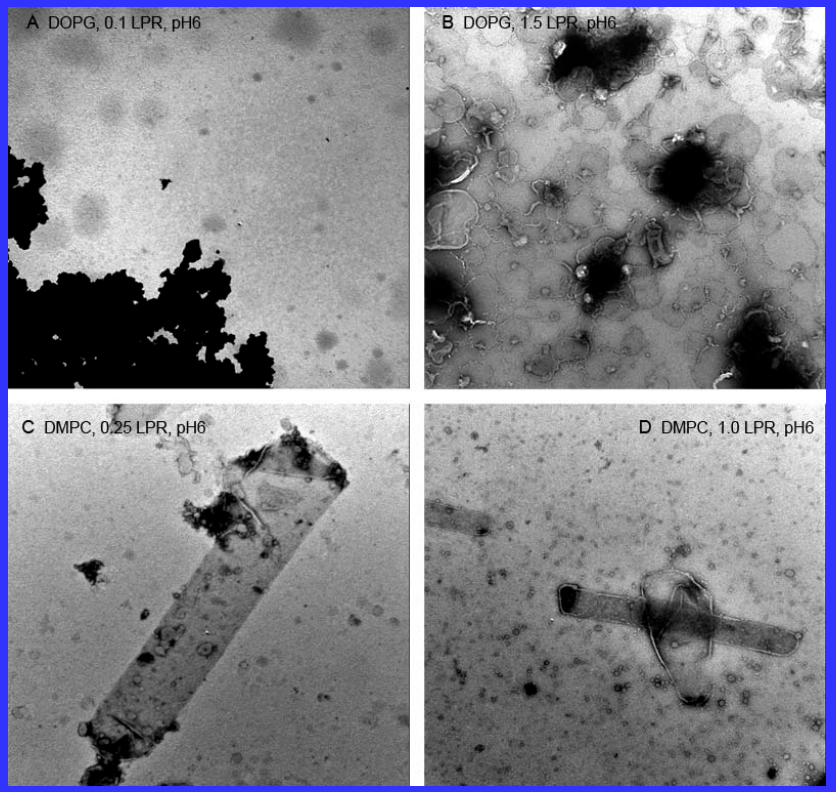
Biological information really helps (ligands etc)

HT 2D EM


37mm
silicone sheet
dialysis membrane
silicone sheet
metal guide
18mm
aluminum bar
6mm
Buffer Chamber
37mm
dialysis membrane
silicone sheet
silicone sheet
Sample Well
3mm
5mm
tightening screws
upper block with dialysis chambers
lower block with sample wells
metal guide


EM grid




A DOPG, 0.1 LPR, pH6
B DOPG, 1.5 LPR, pH6
C DMPC, 0.25 LPR, pH6
D DMPC, 1.0 LPR, pH6

Synergistic efforts

- Membrane protein structures – HT expression, crystallography, solution and solid state NMR (NYCOMPS), 2D crystallography (EM)
- Large ensembles – NMR screening *in cell,* single particle reconstruction (CEM), specific isotope labeling methods (NMR), crystallography
- Irregular ensembles – inter cellular contacts e.g. desmosomes by CEM, crystallography/NMR of multi-domain systems

AAMC

More than 250 publications benefited from NYSBC 7/02 -- present

- What was done…
  - Incorporation, 12/99
  - Site and architect selection 06/00
  - Phase I construction ended 7/02
  - Phase II construction ended 9/03
  - Beam line management initiated 9/03
  - Phase III construction ended 12/06

AAMC

New York Structural Biology Center

Formed as a 501 (c) (3) corporation with Board representation of ten Institutional Members

Albert Einstein College of Medicine
City University of New York
Columbia University
Memorial Sloan Kettering Cancer Center
Mount Sinai School of Medicine
New York University
Rockefeller University
State University of New York
Wadsworth Center, State Health Dept.
Weill Medical College of Cornell University

AAMC

NEW YORK STRUCTURAL BIOLOGY CENTER

New York Structural Biology

Phase I – 3x 800 MHz
750 MHz (solid state) 2100 m²
600 MHz
500MHz
200kV cryo-EM (to be moved)

Phase II – 900 MHz x 2
700 MHz 1200 m²

Phase III – 300kV cryo-EM
200kV cryo-EM 1100 m²
120 kV screening cEM

Educational Activities

- NYSBC currently offers the following semester-long, graduate level course, which are fully accredited. The courses are held at NYSBC.
- "NMR Spectroscopy of Macromolecules" (accredited Columbia University) is taught by Profs. A. G. Palmer, and A. McDermott. The course offers a detailed survey of the principles of NMR as applied to macromolecules.
- "NMR Structure Determination" (accredited CUNY) is taught by a team of staff and affiliate faculty members lead by Prof. Ranajeet Ghose. The course offers training in protein structure determination.
- "Cryoelectron Microscopy of Macromolecular Assemblies" (accredited NYU) is taught by a team of staff and affiliate faculty members lead by Prof. David Stokes. The course covers the theory and practice of solving structures by CEM.
- NYSBC offers non-credit short courses for instrument training in CEM and NMR.
- A bi-weekly seminar series "Metagroup" offers presentations by scientists from member institutions, from staff, and from visitors.

AAMC

Current usage

~ 250 scientific affiliates
~ 37 NMR focused PI's
~ 32 EM focused PI's
~ 40 crystallographic focused PI's

~ 95 Direct use PI's
> 21 indirect collaborators etc

Governance

- Board represents the stake holding institutions
- Resources are distributed in proportion to capital stake, accommodating grants and contracts.
- Resource management per institution is by the institutions
- A single annual subscription is charged.
- IP belongs to the institutions.
- Well crafted by laws

AAMC

Governance - Program

- Program areas prioritized by
  - Wide interest by scientists at Member Institutions
  - High capital/ operating cost ratio
  - Likely long term scientific impact

AAMC

Governance – Capital

- Opportunistic
- Past success with State, Federal, City agencies
- Original member institution investment more than 400% exceeded by new external capital
- Exceptional work by Executive Vice President/ CEO Dr. Willa Appel

AAMC

Governance -- Revenue

- A natural cap on the ability to pay subscription by member institutions
- Difficulty of control of flow of F&A for small scale
- Changes of policy by NIH (e.g. NCRR BRTP P41's) problematic
- Level of new recruiting by member institutions below initial expectations

AAMC

Governance -- Compliance

- Highly burdensome on small independent institution.
- Significant legal and other consultant expenses
- Unavoidable complexity and restriction associated with a high density urban environment

AAMC

A model for regional cooperation?

- Pluses
  - It can be done! (even in NY)
  - It provides disconnection of resource management from research careers
  - Provides scale and backup of critical resources
  - Synergizes other potential interactions of benefit
- Negatives
  - Someone has to leave their ego(s) at the door
  - Is there an exit strategy?
  - Cost control, best to be hoped for is a “natural tension” between management and Board
  - Maybe the multiplicity and close packing of institutions in NY limit generic applicability

AAMC

Effect on quality of science

- A natural desire for justification beyond “faster, broader, cheaper”
- Generally accepted ideas for quality improvement
  - Personnel selection
    - No input from NYSBC, other than for staff
  - Selection of focal programs
    - Locked in by the program area choices
  - Minimization of non-research activities
    - NYSBC helps
  - Emphasis on impact and timeliness of projects
    - For the supported program areas, can be of significance

AAMC

The “Wealth of Science”

- Liberal (19th Cent. definition) policies for increasing the “wealth”…
  - Emphasis of individual creativity
  - Specialization of labor
  - Reduction of barriers to execution of ideas

AAMC

Individual creativity

- New ideas more readily tested with larger scale and scope of resources
- Successes of others provide competitive stimulus and resource availability minimizes “helplessness” that resources are unavailable
- Reduced political role in resource allocation

AAMC

Specialization of Labor

- Example 1 – technical NMR specialist can setup experiments for structure determination in 30’ which might take a trainee student weeks to do, (maybe months successfully)
- Example 2 – training of a large number of EM users identifies common problems and opportunities

AAMC

Reduction of barriers

- For structural biology, emphasis should be on the biology not necessarily the technique
- Applications in chemical biology, molecular & cellular biology, medicinal chemistry, …. should be available without the need for intercession with a nominal expert in structural biology
- An expert/specialist role reduces barriers by identifying best practices, removable bottle necks, and previously unrecognized opportunities

AAMC

Changing the social structure

- ? Is it needed –
  - Extraordinary scale of new information from genomics etc
  - Perception of lack of impact of basic science advances on human health
    - Drop in new chemical entities registered
    - Lag of impact of human genomics on individual health
  - Scale and scope of institutional size for biomedical research objectives not naturally the same as for the educational/professional role

AAMC

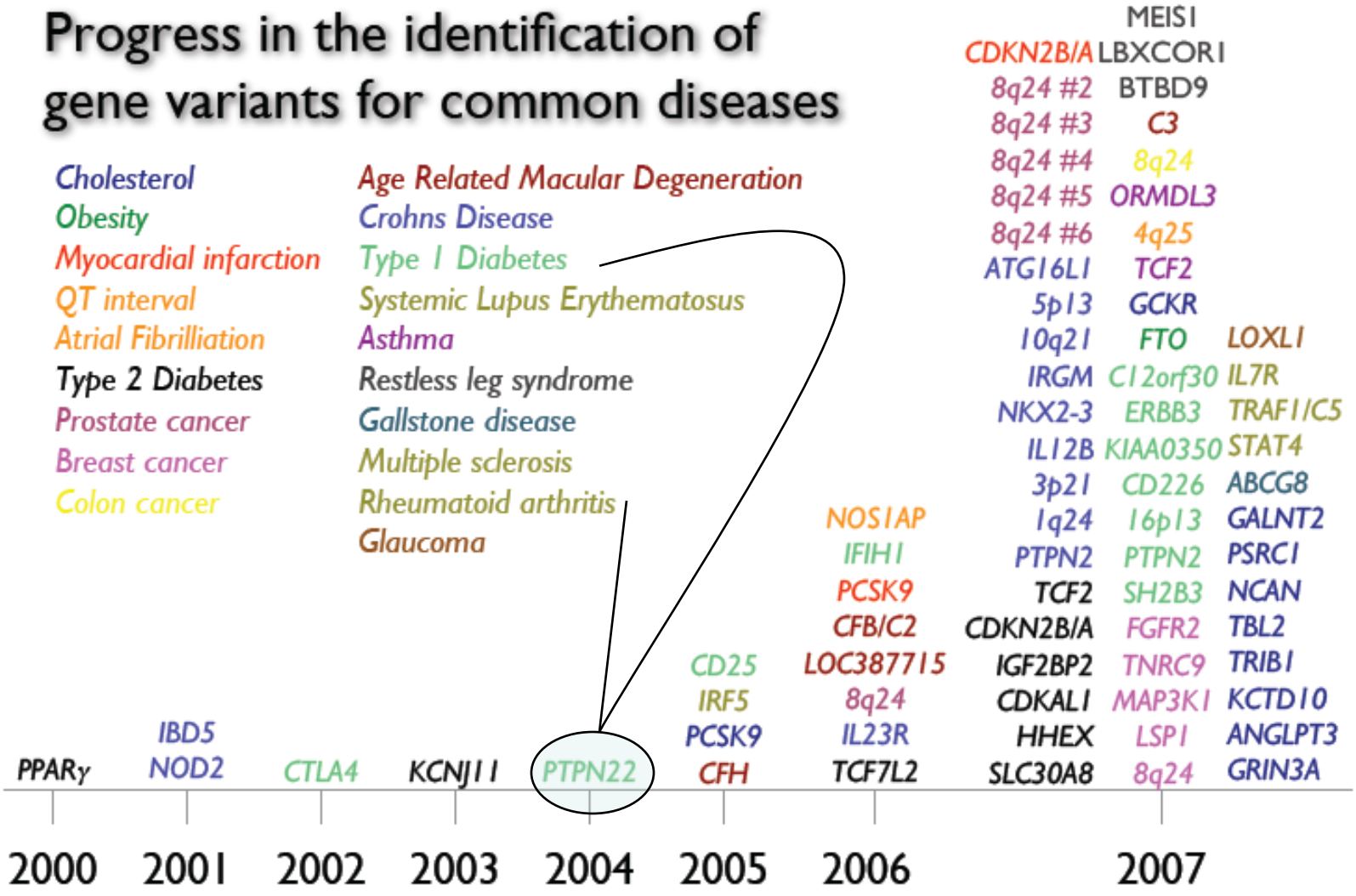
For the first time, rapid progress identifying the inherited basis of common human diseases

Long-term benefit will come from biological understanding and proven beneficial interventions

Typing of new genes variants (and the hyping of their potential value) will start immediately

AAMC

Progress in the identification of gene variants for common diseases



Scope and the importance of the knowledge of the commons

- The success of big pharma was significantly linked to the larger SCOPE of programs, and not to overall scale of operations
- The effect of Scope stems from capturing knowledge spillovers between programs
- Significant components of the common knowledge from academic research

Henderson R, Cockburn I, Rand J. Economics, **27**, 32-59, 1996

AAMC

Some uncomfortable questions

- Should every institution try to compete in all areas of resources (or indeed in scientific areas) ?
- What are the best practices for cross capture of basic biology and application to human disease (“translational research”?)
- Should there be regional/national centers to improve the “Wealth of Science” in defined areas?

AAMC

In conclusion, for NYSBC

- The long term benefits to the “Wealth of Science” are still yet to be established
- The short term scientific impact and success indicate that for this specific genotype and secular factors, the phenotype is currently healthy

AAMC


•THANK YOU
Like skiing, research should be exhilarating, and best enjoyed in fine surroundings and excellent company. Like skiing, there may be hidden hazards and changes in the weather.