Improved Preparation and Analysis of Biothreat Agents and Infectious Microbial Samples Using Pressure Cycling Technology (PCT)

Bradford Powell, Ph.D.

Cernomics Solutions
Representing

Pressure BioSciences Inc.

Related Publications

Bradford Powell and Robert Cybulski. “Inactivation and extraction of bacterial spores for systems biological analysis” p. 941-977. In, A. Ivanov, A. Lazarev (ed), **Sample Preparation in Biological Mass Spectrometry**, Springer, New York, 2011.

Powell BS, Lazarev AV, Carlson G, Ivanov AR, Rozak DA. “Pressure Cycling Technology in Systems Biology” in **Microbial Systems Biology: Methods and Protocols**, ed. Navid A, New York: Humana Press (in press).

Disclaimer

Opinions, interpretations, conclusions, and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

Funding Acknowledgement

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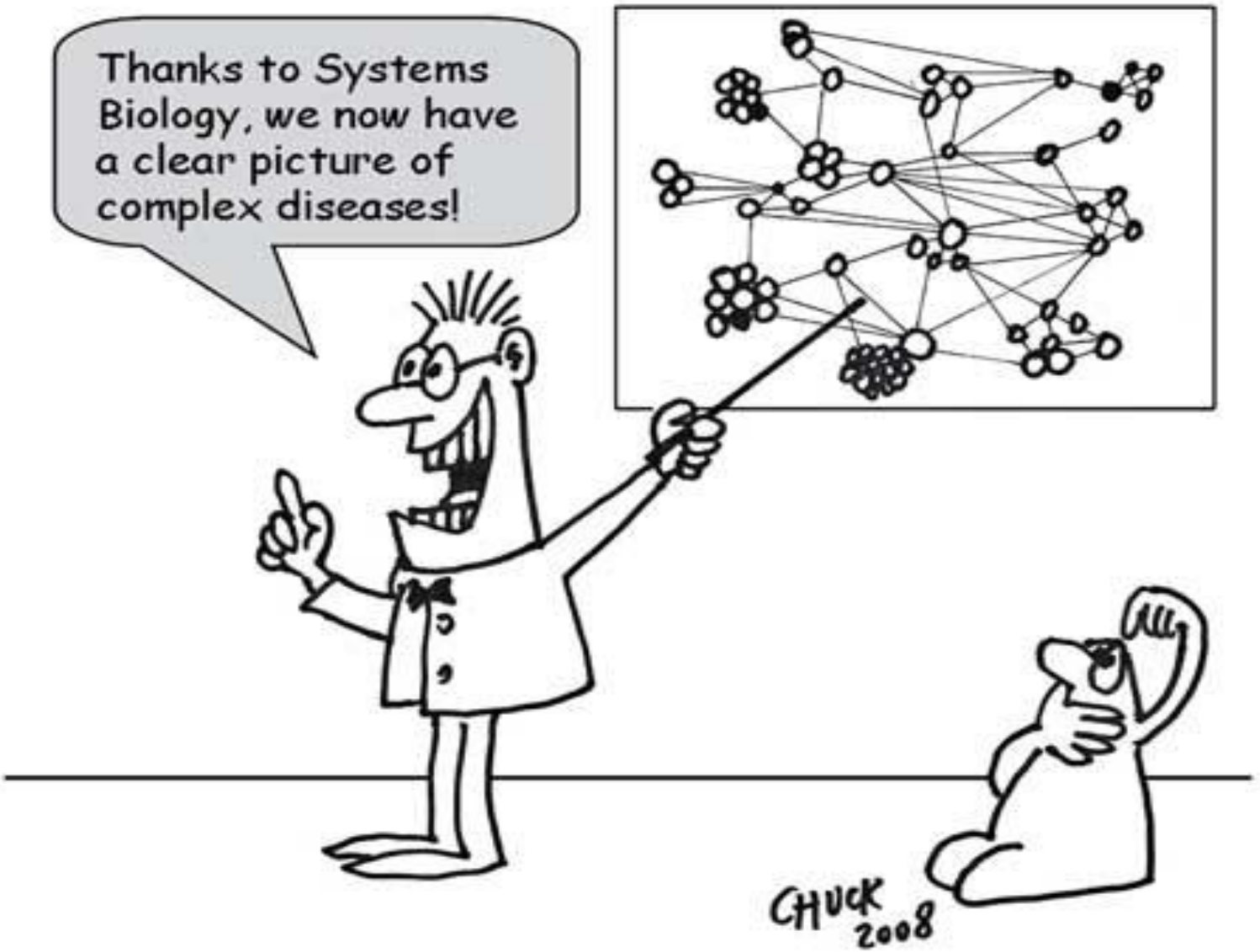
Sample Prep

Analysis

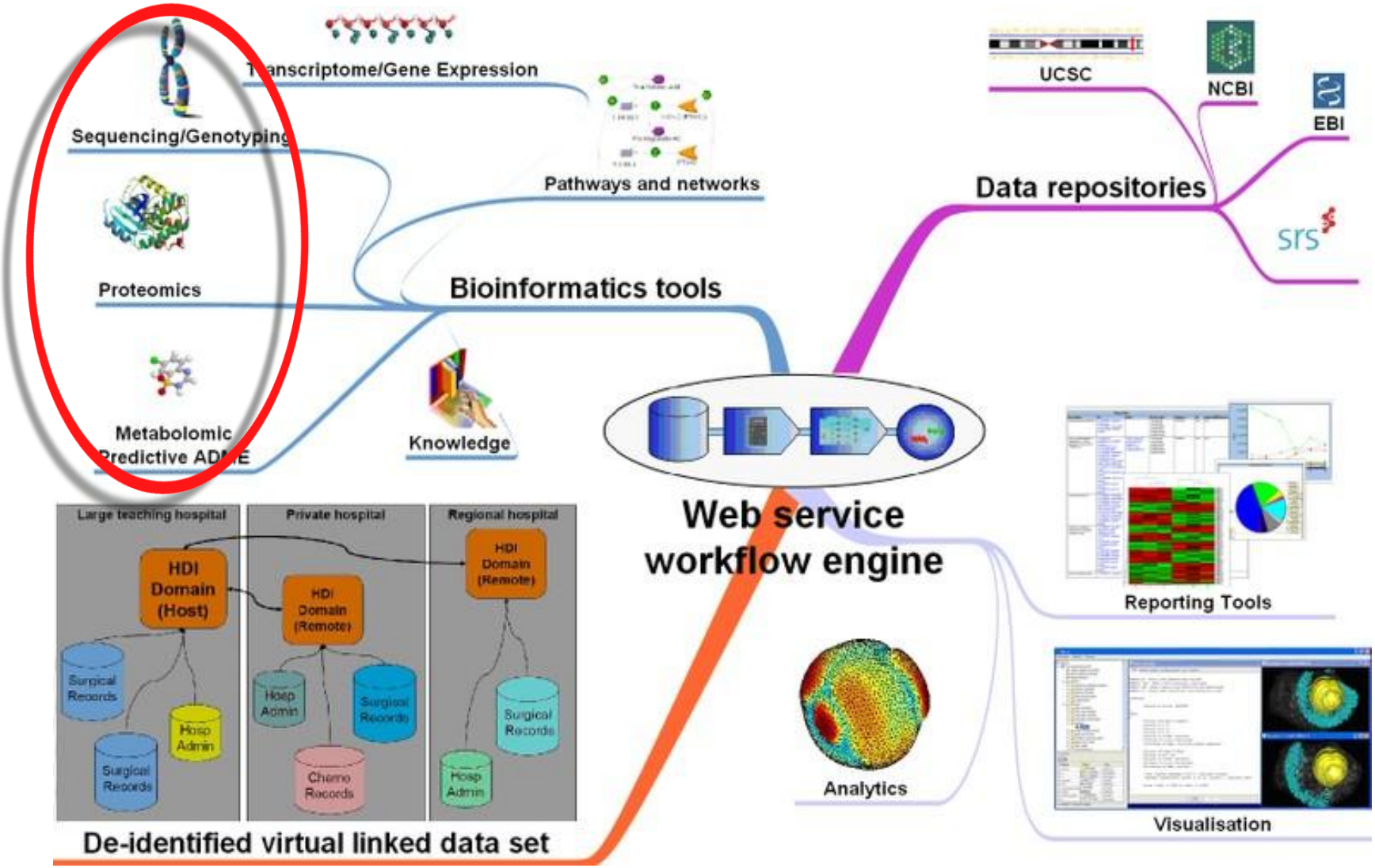
R & D

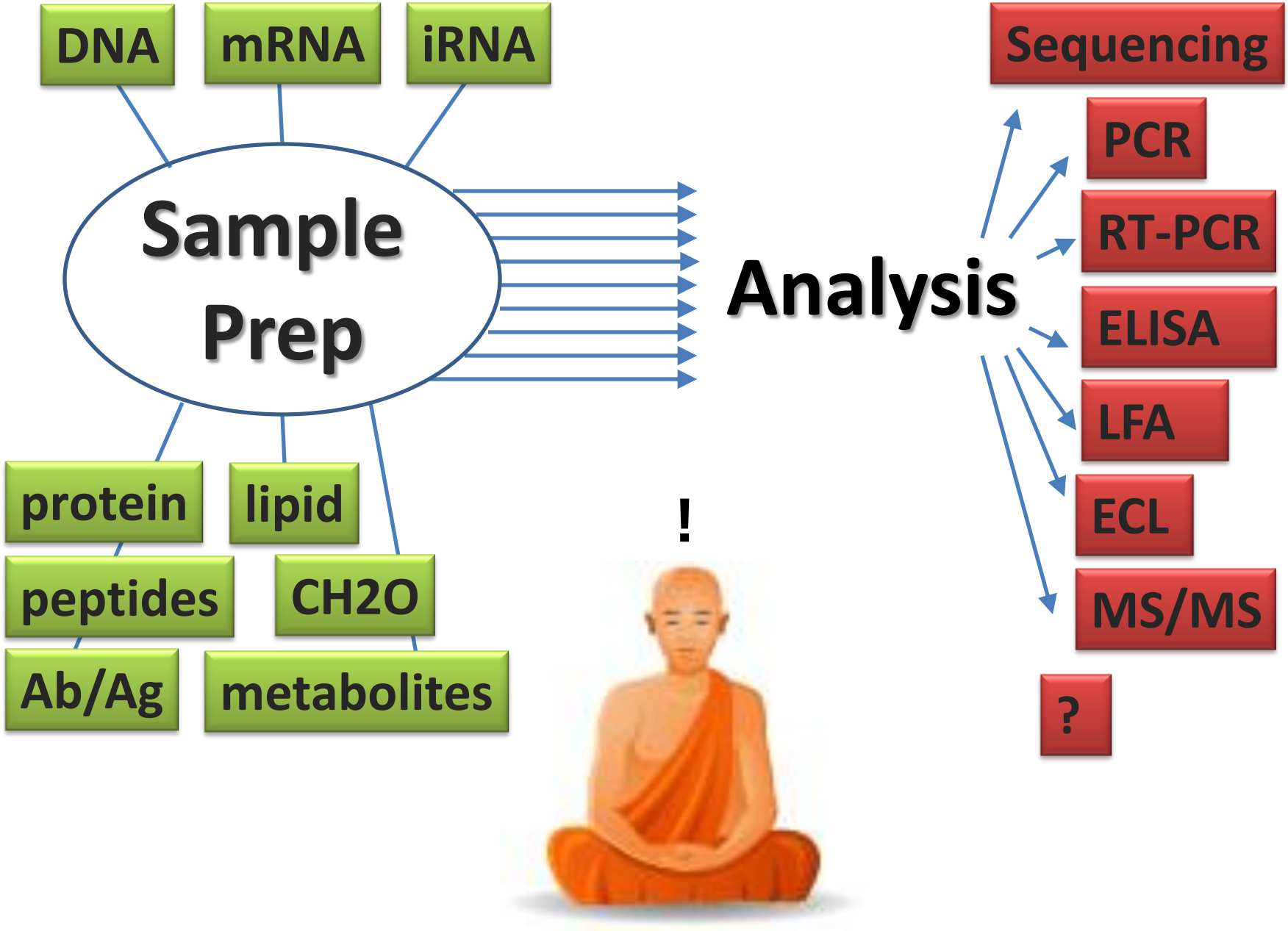
Dx

PCT

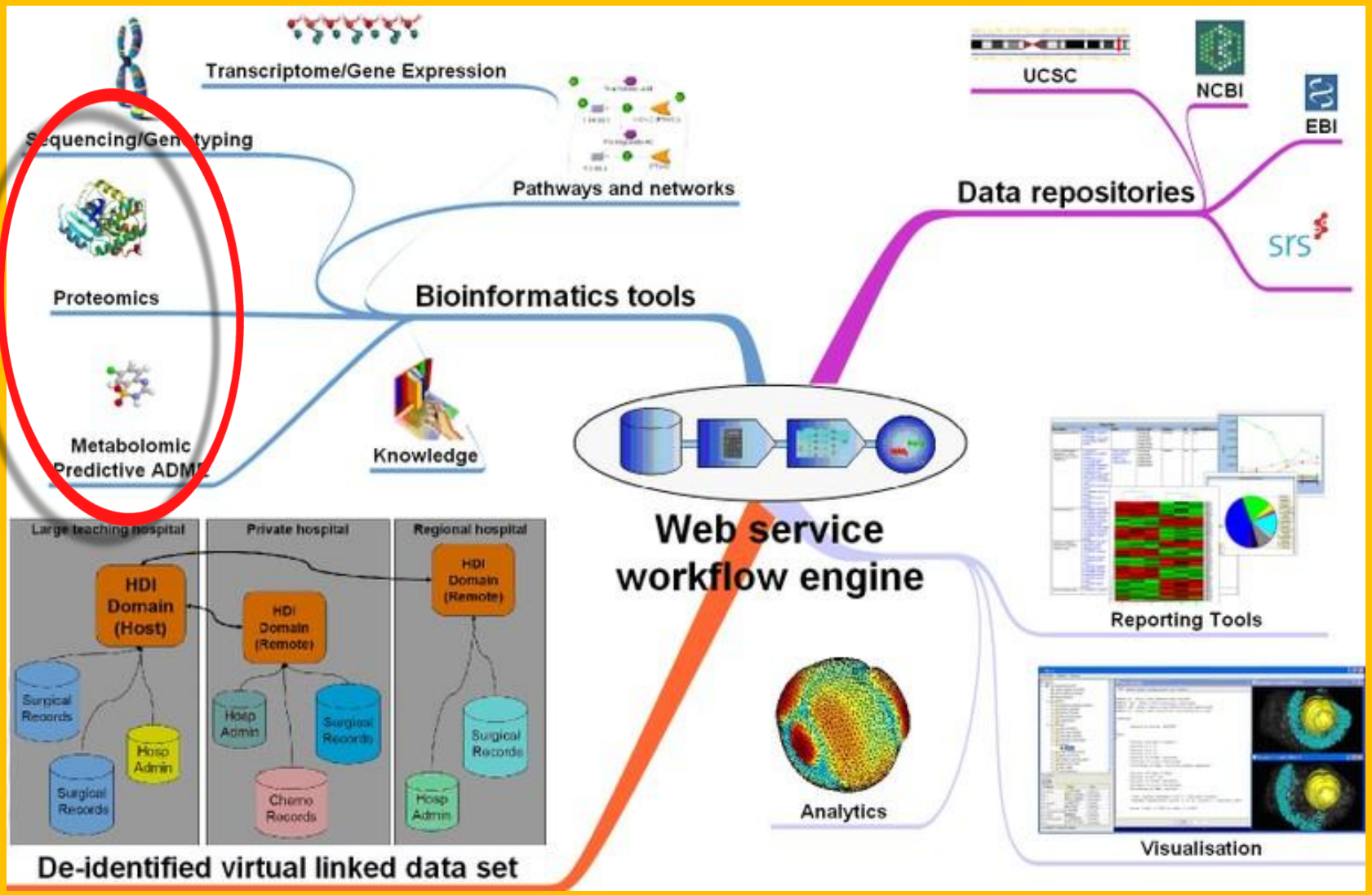

Thanks to Systems Biology, we now have a clear picture of complex diseases!
CHUCK 2008

Systems Biology Workflow



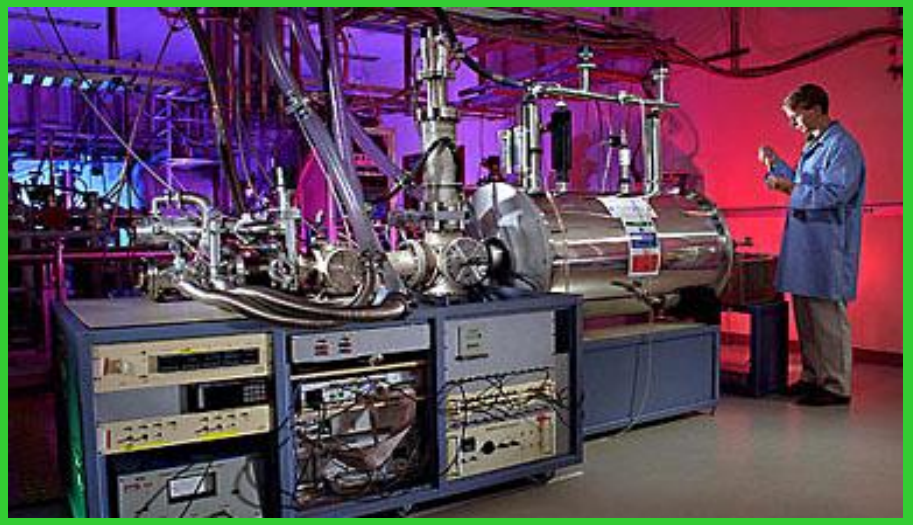

DNA
mRNA
iRNA
Sample Prep
protein
lipid
peptides
CH2O
Ab/Ag
metabolites
!
Analysis
Sequencing
PCR
RT-PCR
ELISA
LFA
ECL
MS/MS
?

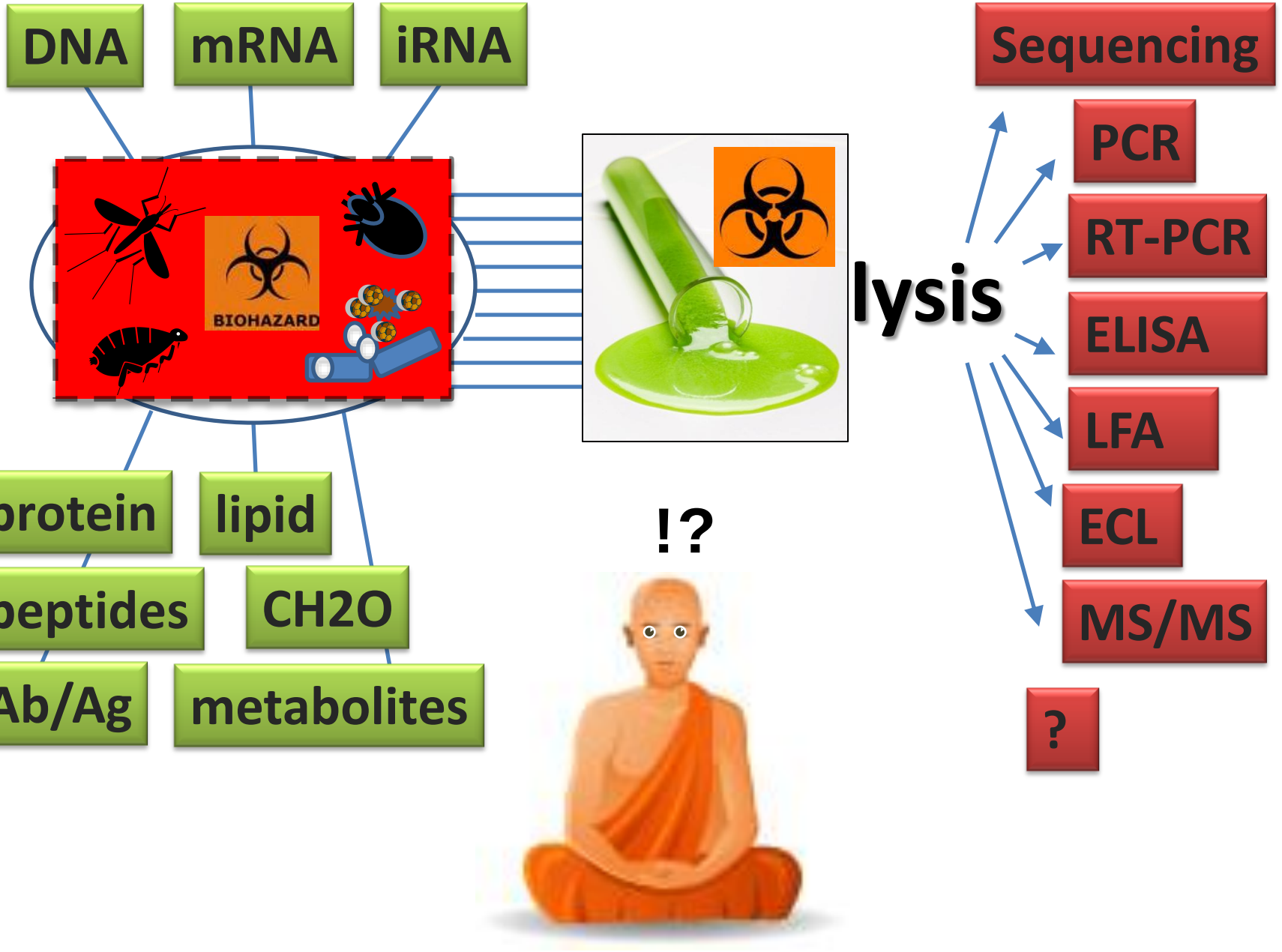
Systems Biology Workflow



BSL-3/-4

BSL-2




DNA
mRNA
iRNA
BIOHAZARD
protein
lipid
peptides
CH2O
Ab/Ag
metabolites
lysis
!?
Sequencing
PCR
RT-PCR
ELISA
LFA
ECL
MS/MS
?

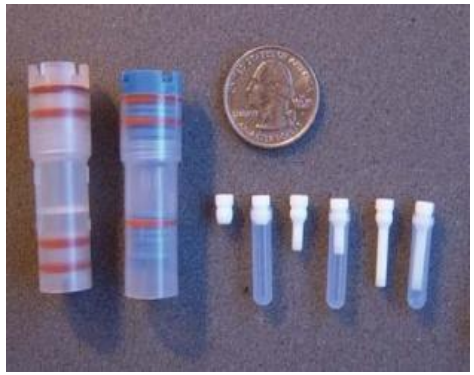
?

Analysis

Inactivation for Systems Biology

Inactivation Method	Molecular Analysis	Comments
Autoclave		PCR: Physical hazard, facilities, instrument & training requirements
γ-irradiation		sical hazard, facilities, instrument & training
Formaldehyde	NO	mical hazard, Facility & training requirements
UV-irradiation		ument & training requirements
Ethylene oxide		ies, instrument & training requirements
*TriZol™/$CHCl_3$	Partial	; chemical hazard
Bead Beater		vn breaches
Filtration		umented incidences

*TriZol extraction contains guanidinium thiocyanate, phenol and chloroform. Positive strand virus (e.g., VEE) requires additional care and treatment due to infectivity of RNA.



Pressure Cycling Technology (PCT)

vs

Focused Microwave Technology (FMT)

Approaches to safely inactivate BSAT

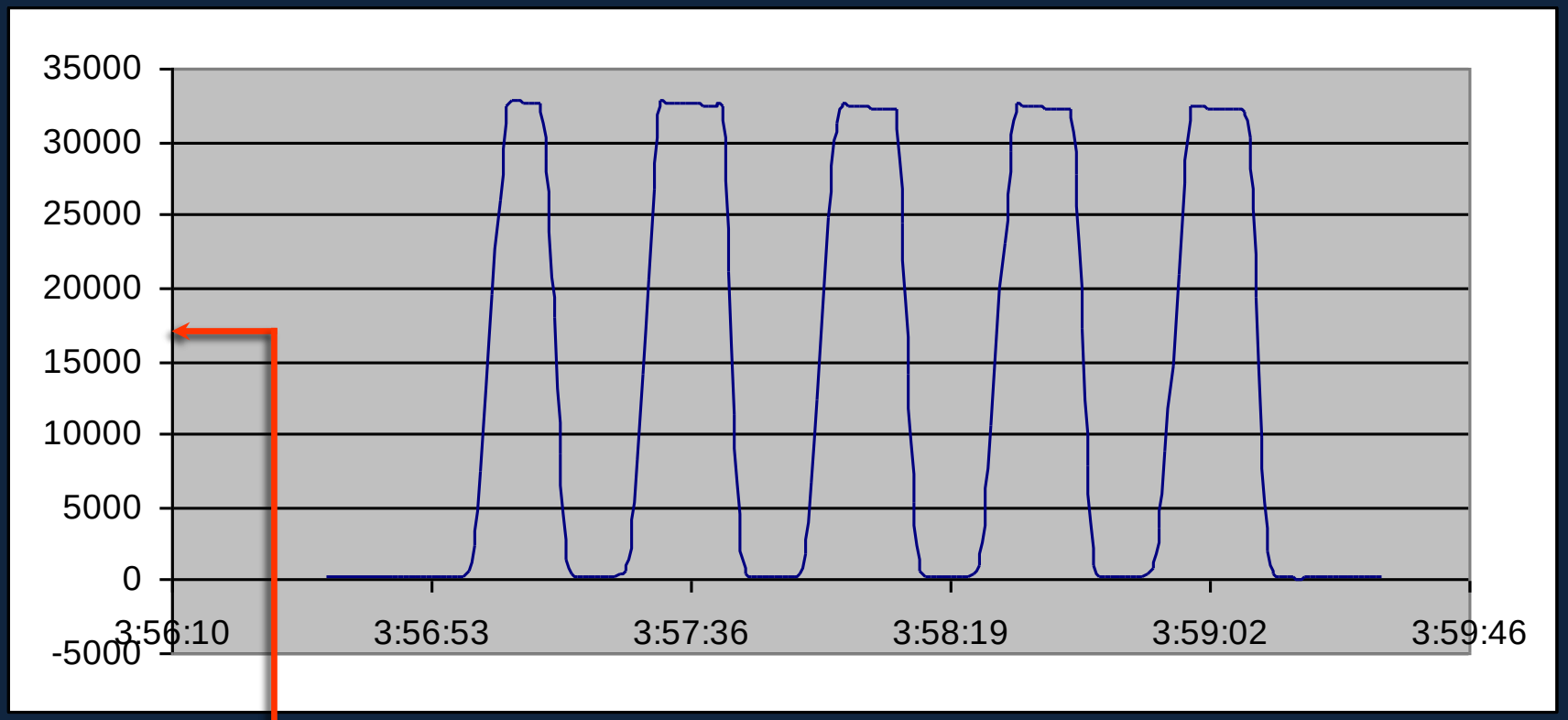
Approach	Inactivation		Analysis
	Spores [1]	Virus[2]	
PCT	7 log	4.4×10^6	PCR, LC-MS/MS
FMT	4 log	nt	nt

[1] *Bacillus anthracis* Sterne spores, cfu/ml decrease

[2] Venezuelan equine encephalitis virus (VEE) TC83, PFU/ml limit

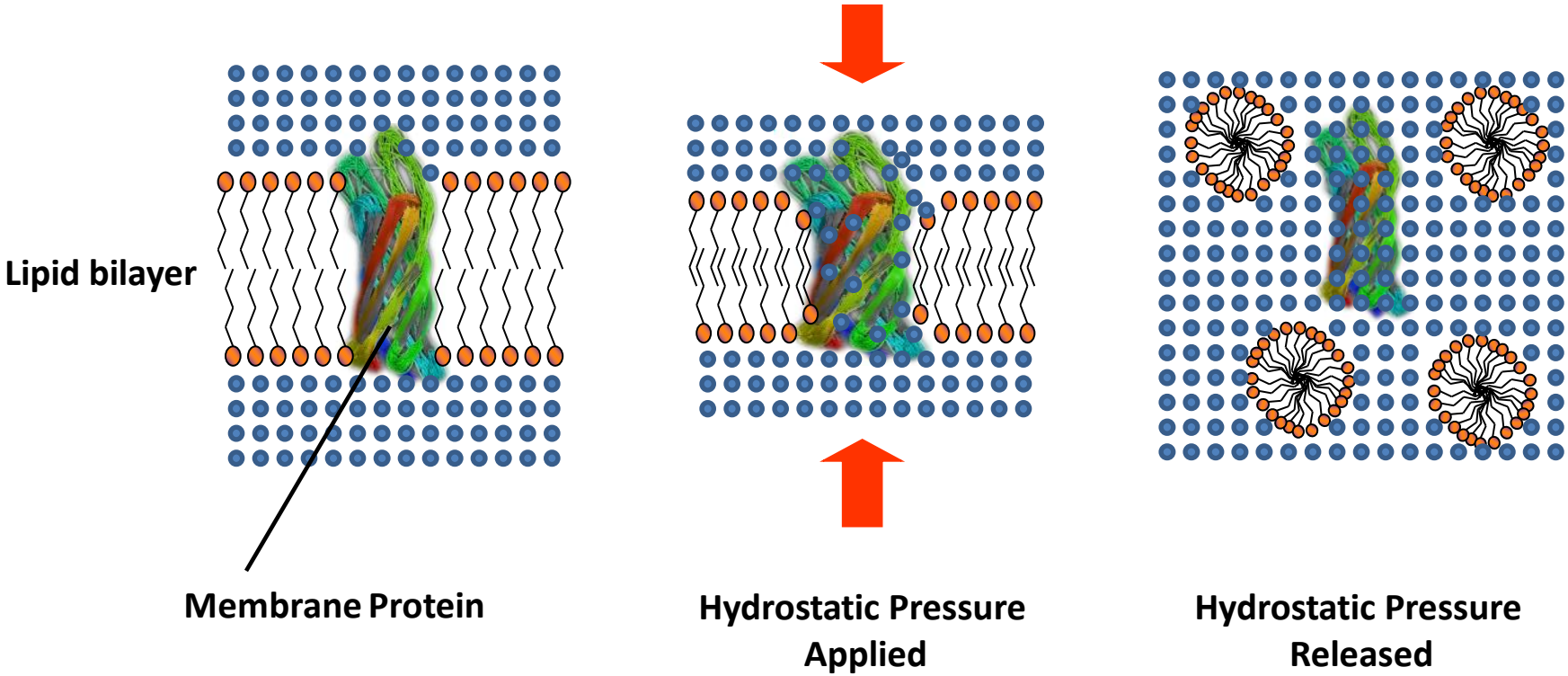
Powell and Cybulski. In, Sample Preparation in Biological Mass Spectrometry, Springer, New York, 2011.

Pressure Cycling Technology (PCT)



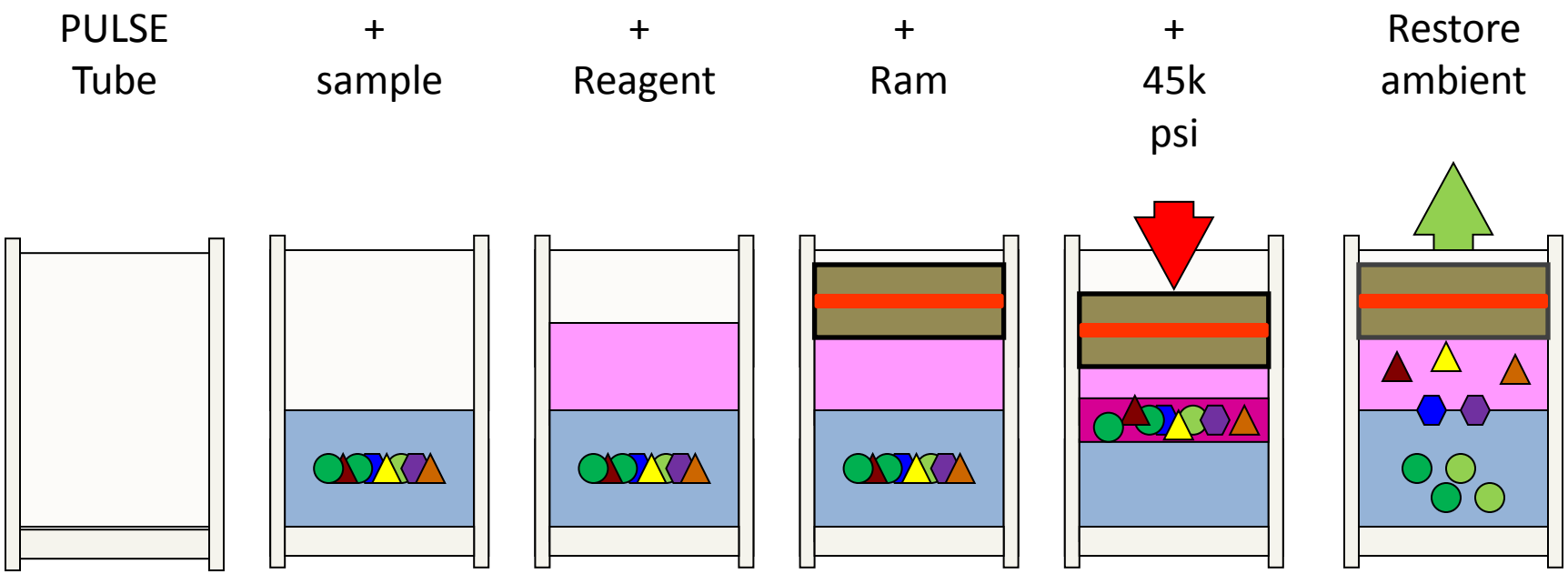
16,000 psi @ Marianas Trench

PCT Destabilizes Biological Membranes



1. Interdigitated bilayer
2. Hydrophobic hydration

PCT-mediated liquid-liquid extraction



Gross, *et al.*, JBT (2008) 19*:187–197*

ProteoSolve-SB Workflow

Detergent-Free Extraction of Proteins, DNA , RNA and lipids

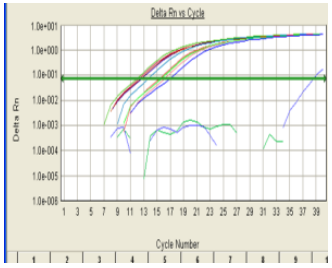
SAMPLE + REAGENTS

Gross et al, JBT, 2008 19:189–199

CENTRIFUGATION ~12,000g for 10 min.

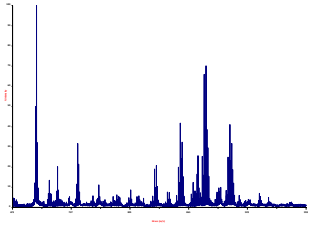
Pellet: Nucleic Acids

GENOMICS:
DNA Sequencing
SNPs, RNAi



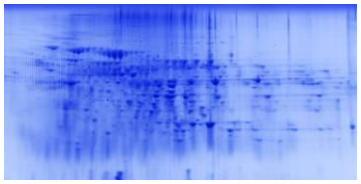
Top Phase: Lipids

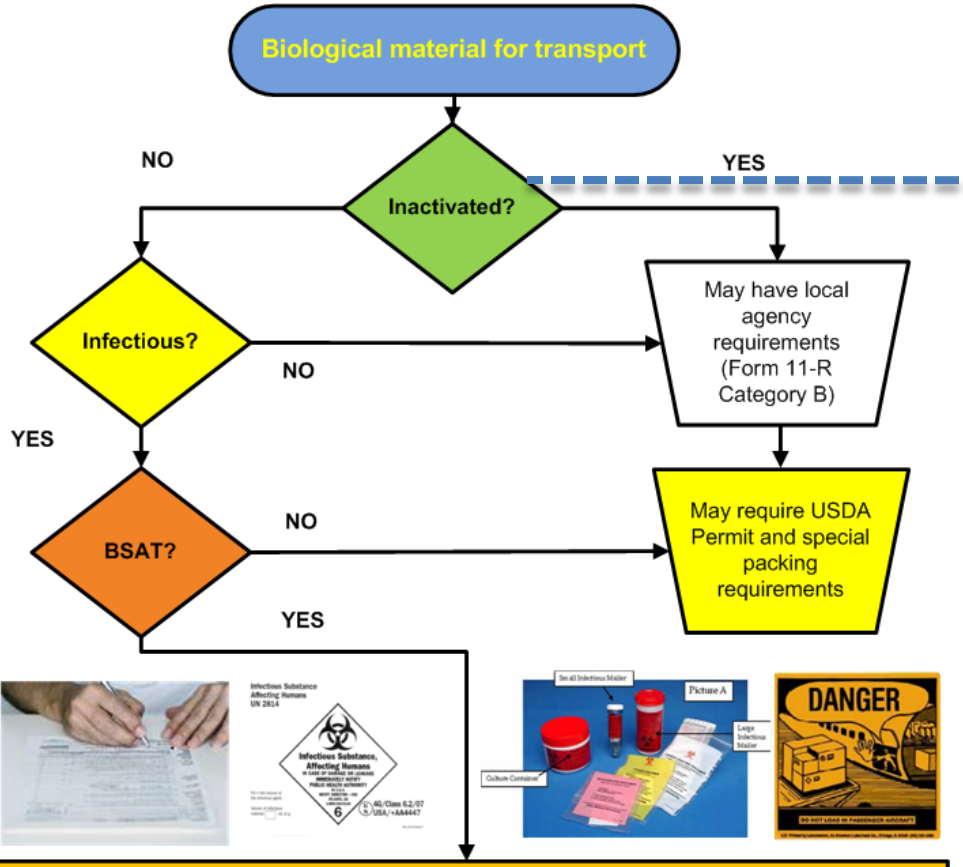
LIPIDOMICS:
GC-MS, LC-MS,
MALDI/TOF
TLC, NMR

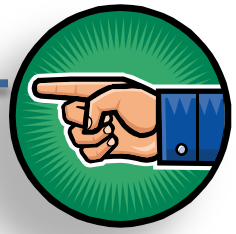


Bottom Phase: Proteins

PROTEOMICS
SDS-PAGE, 2D PAGE,
Western blotting,
LC-MS/MS,
MALDI-TOF, etc...







Hypothesis

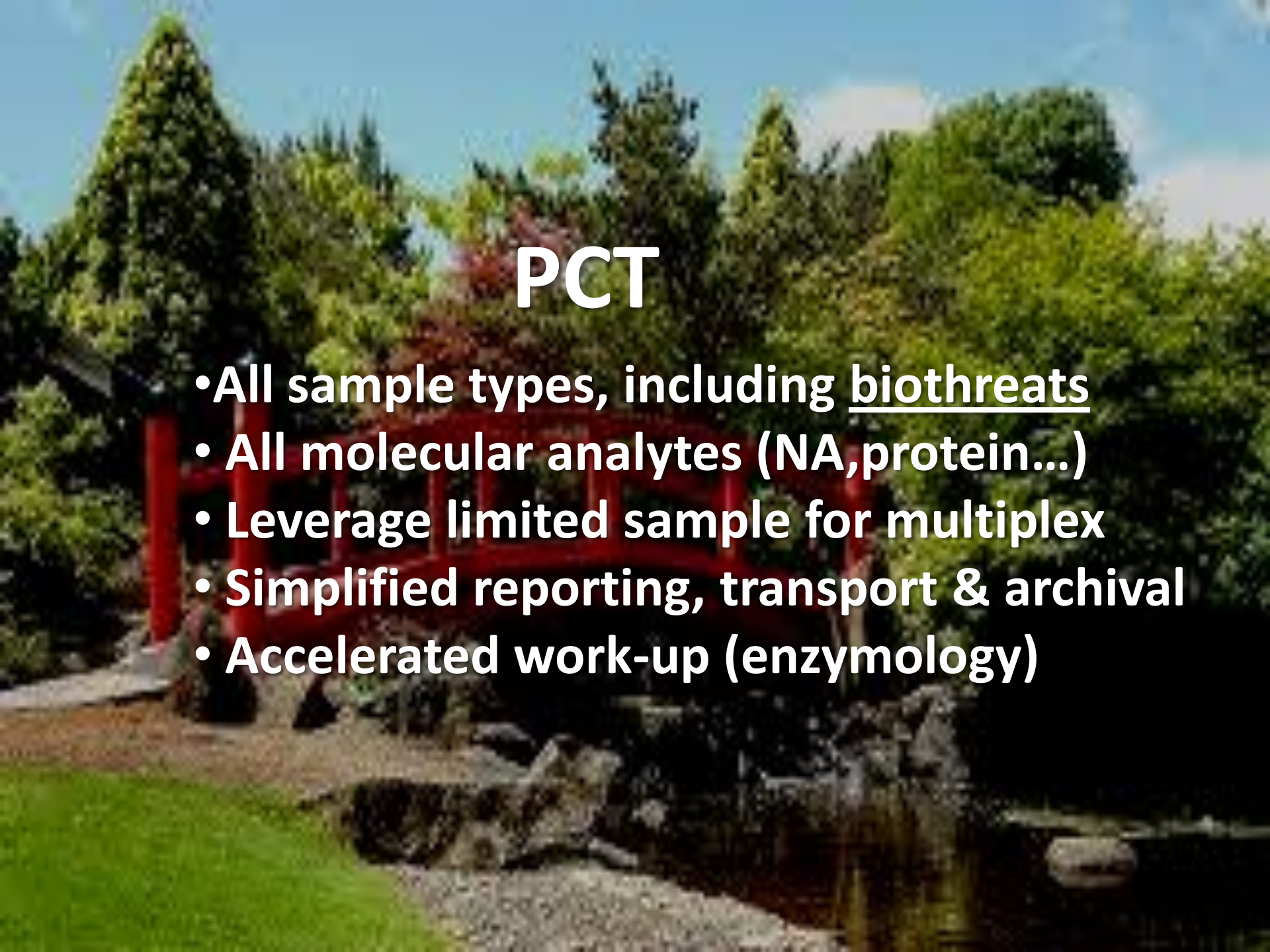
A new approach could expand research capability and save cost/time

- Recipient Institution/Agency must have CDC Registration for Agent
- Recipient PI must have CDC Registration for Agent
- Recipient requires CDC or USDA permit (AP
- If not federal agency or contractor, then app
- If recipient is a military installation or its con Personnel Reliability Program is mandatory
- Internal Chain of Custody forms for the BSA
- USDA Permit may be needed prior to shipp
- Special Packing requirements (needs certifi
- Special Shipping (FedEx White glove servi

http://www.selectagents.gov/TransferForm.html

A long list of sequential permitting is required before shipping infectious BSAT.

PCT


PCT
•All sample types, including biothreats
• All molecular analytes (NA,protein…)
• Leverage limited sample for multiplex
• Simplified reporting, transport & archival
• Accelerated work-up (enzymology)

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