CLINICAL PROTEOMIC TECHNOLOGIES FOR CANCER

National Cancer Institute Clinical Proteomic Technologies Initiative for Cancer (CPTI)

Adam Michael Clark, Ph.D.

http://proteomics.cancer.gov

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Challenges and Opportunities in Clinical Proteomics

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Clinical Cancer Proteomics

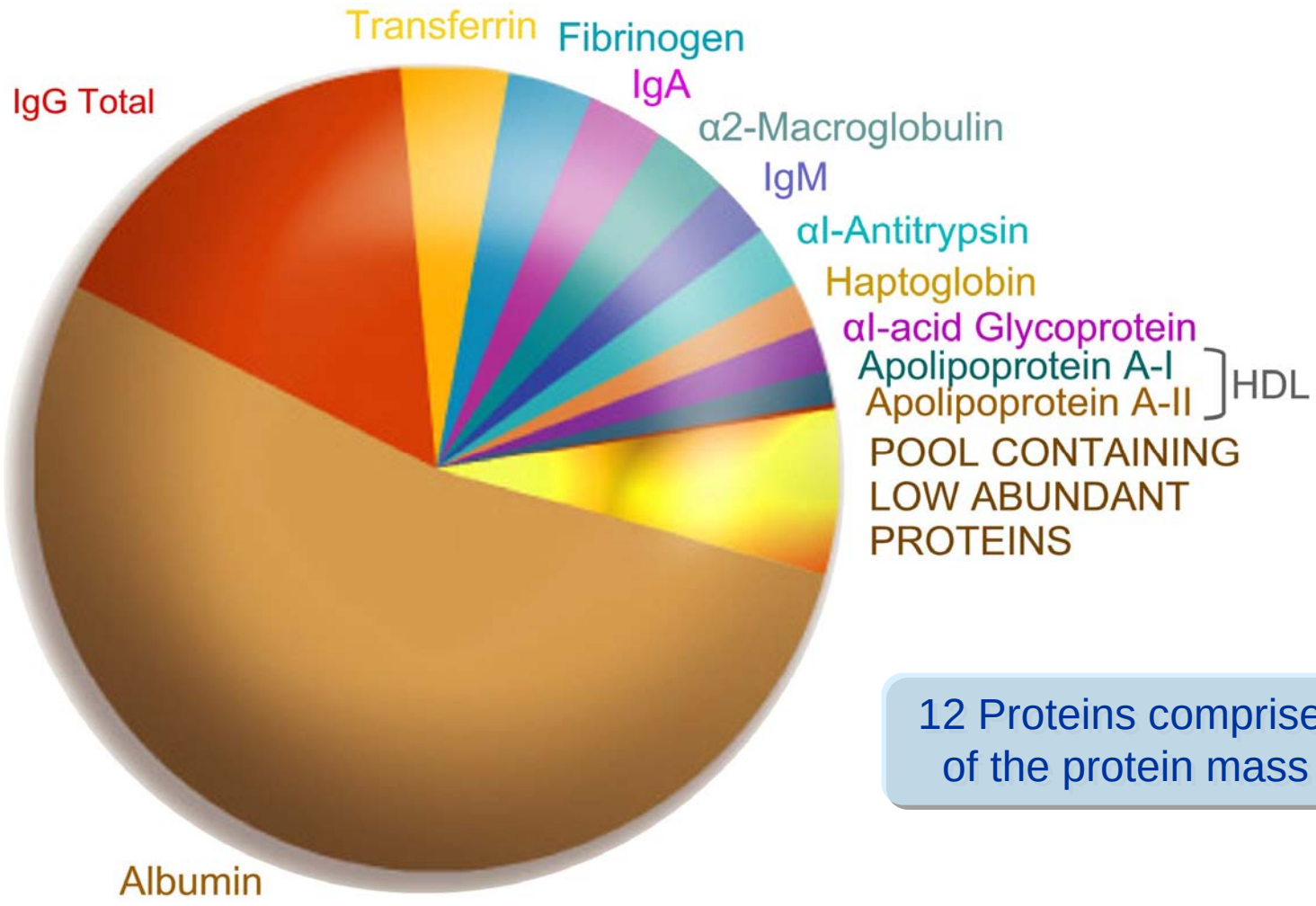
- **Problem:**
  - Cancer metastasizes before it can be detected
  - Tumors are difficult to control
- **Potential Solution:**
  - Protein-based detection and monitoring of cancer processes
- **Challenges**
  - Detection of low abundance proteins
  - High sensitivity and specificity
  - Label-free detection
  - High-throughput platform analysis
  - Clinical application

Community Input and Consensus

- **On the basis of discussions with a wide range of clinicians, cancer researchers, and technologists, the NCI recognizes that there are immense opportunities for using proteomics technologies to solve mission-critical problems in cancer research.**

- **Premises:**
  - Cancer-related proteins exist in readily-accessible body fluids
  - Panels of such proteins will be required to achieve high specificity and sensitivity
  - Current technology is capable of discovering these panels
  - Current application of this technology can be improved

Challenges in Blood-based Proteomics



12 Proteins comprise up to 96% of the protein mass in plasma

The Human Plasma Proteome:
History, Character, and Diagnostic Prospects

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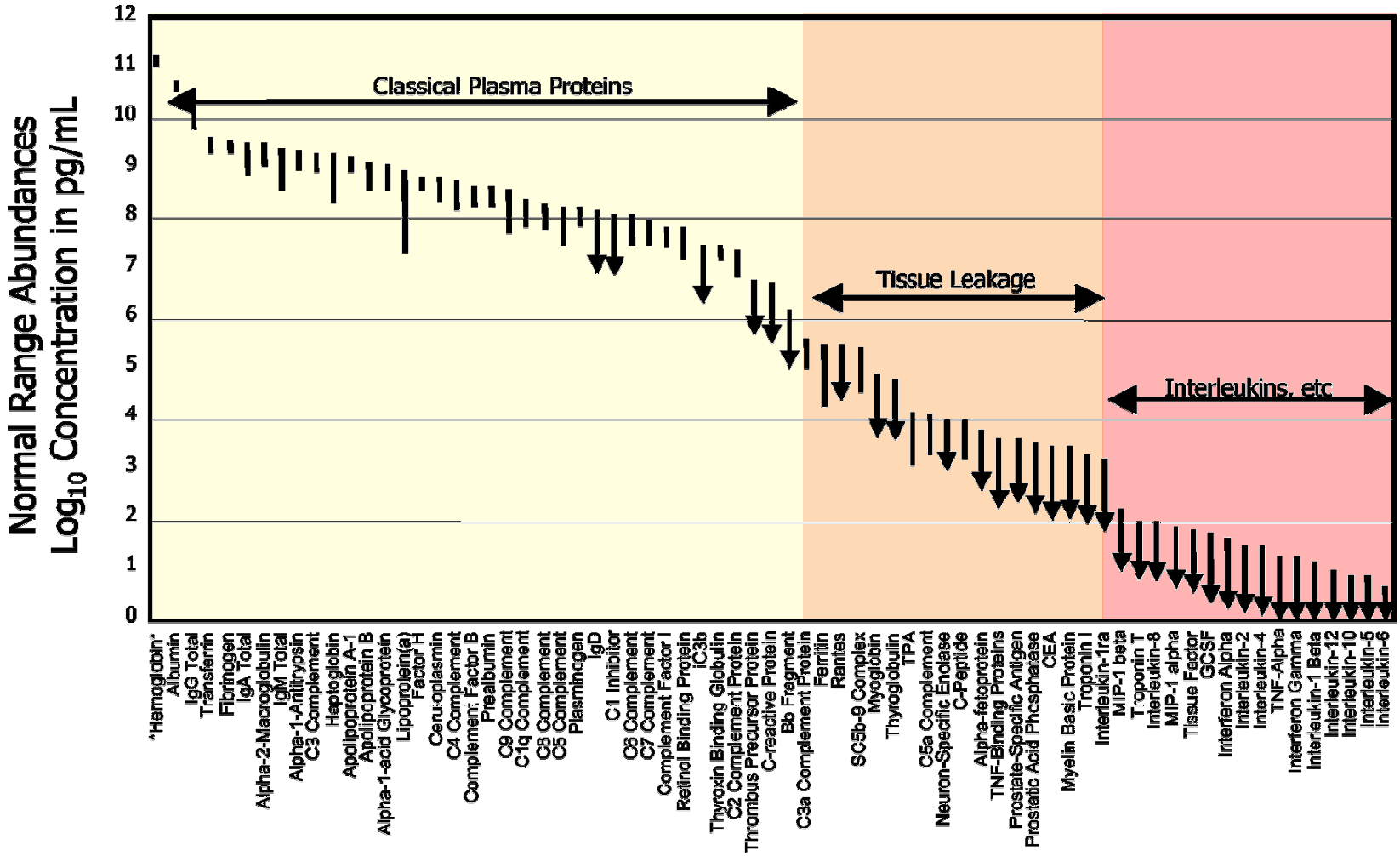
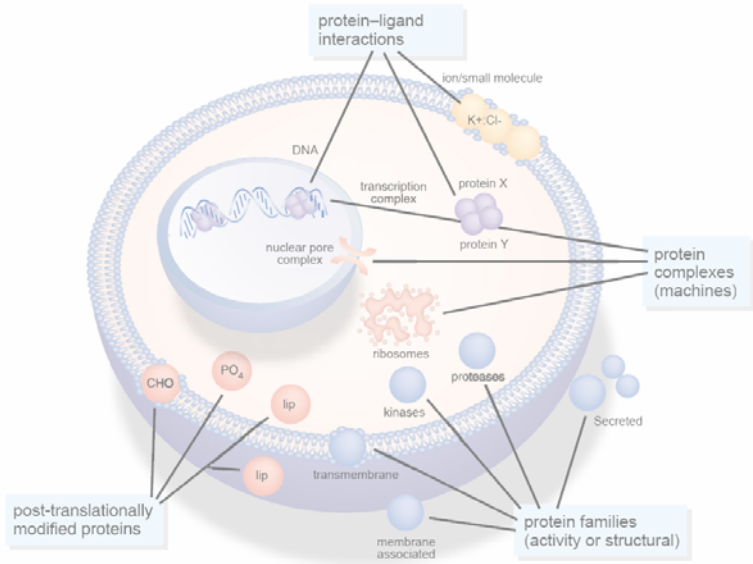
Reference intervals for 70 protein analytes in plasma



Anderson, N. L., and Anderson, N. G. (2002) The Human Plasma Proteome: History, Character, and Diagnostic Prospects. *Molecular & Cellular Proteomics*, 1, 845-867.

Proteomics Today

- **No** single technology platform that can satisfy all of the desired proteomic measurements
- **No** mature, “true” proteomic technology
- **No** performance criteria
  - Poor confidence in protein measurement results
  - Difficulty in assessing agreement of different experiments
  - Conflicting reports in the literature
  - Lost opportunities



Scott D. Patterson & Ruedi H. Aebersold, Proteomics: the first decade and beyond, *Nature Genetics* 33, 311-323 (2003)

If proteomics technologies are to successfully make their way into clinical diagnostics, universally accepted metrics will be needed to help clarify protocols and experimental results to make them comparable.

Troponin I
… a marker for the occurrence and damage from heart attack

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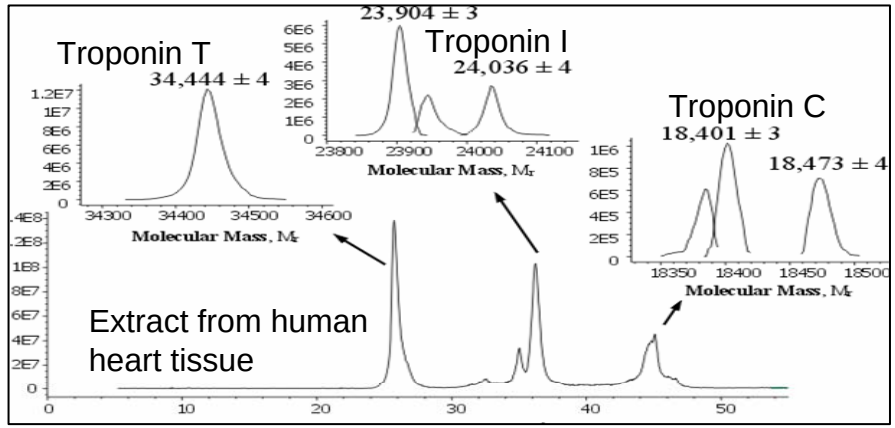
Assay	Conc.	# Labs
Manufacturer	ng/mL	
A	19.9	115
B	6.7	489
C	0.85	27

From G. S. Bodor, Denver Health and Hospitals -- personal communication 1997



PROBLEM:

Troponin I is a complex, heterogeneous protein that may be free or may be complexed with Troponin C and/or Troponin T. Different assay antibodies do not recognize the same form.

NIST RESPONSE:

Developed SRM 2921, a Troponin - I, C, T - complex from human heart tissue. Concentrations of Troponin I and Troponin-T value assigned by NIST. **Proper use of this SRM by the industry has been shown to reduce data variability by a factor of 10.**

Informatics and Data Analysis Challenges in Clinical Proteomics

- **Data validation and reproducibility**
  - Instrument calibration and comparison
  - Peptide/Protein identification
  - Quantification
  - Data normalization
  - Annotation and ontologies
- **Statistical considerations and planning**
  - Chance versus reproducibility
  - Bias
  - “One-hit wonders”
  - Non-disease and/or co-morbid proteomic evaluation references

Proteomics Data Analysis

- **Instruments**
  - Which instruments used for protein mixture studies?
  - Any special (not widely used) features?
  - Are instrument parameter files accessible, readable?
- **Data Files**
  - What form of ‘raw data’ files are available?
  - Can they be converted to a standard text format?
  - Where data will be held at an individual lab? At a national repository?
- **Analysis Description**
  - Is proposed data analysis workflow understood and acceptable?
- **Results**
  - Are confidence levels and reporting of IDs understood?
  - Is the data represented in a format that is understandable across broad disciplines?

Measurements Challenges for Clinical Proteomics

- **Pervasive problems with research design, data analysis, reproducibility, and comparability of research results**
  - Lack of common reagents and highly qualified public data sets
  - Inability to manage, interpret, and represent large quantities of processed data and associated metadata
  - Ineffective and inefficient transfer of platform technologies to clinical application
- **Private sector is unable and unlikely to address all these challenges**
- **A multidisciplinary, team-based approach that includes public-private partnerships and collaborations among academic institutions can better identify and address the multitude of factors better than any individual research team**

The Clinical proteomic Technologies Initiative for Cancer: Opportunities in Proteomic Cancer Research

CPTI teams will support the development of standards and resources for a clinical proteomics platform for cancer research

- Multidisciplinary Efforts in Clinical Cancer Proteomics
  - Cancer biology
  - Clinical research
  - Experimental design, statistical analysis, and metrology
  - Technology development
  - Bioinformatics and computational sciences
- Areas of Interest
  - Peptide/Protein identification and quantification
  - Detection of post-translational modifications, splice variants, and mutations
  - Improved statistical measures of confidence
  - Data normalization
  - QA/QC procedures
  - Standard reagents, resources, and protocols

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CPTI: Strategy and Programs

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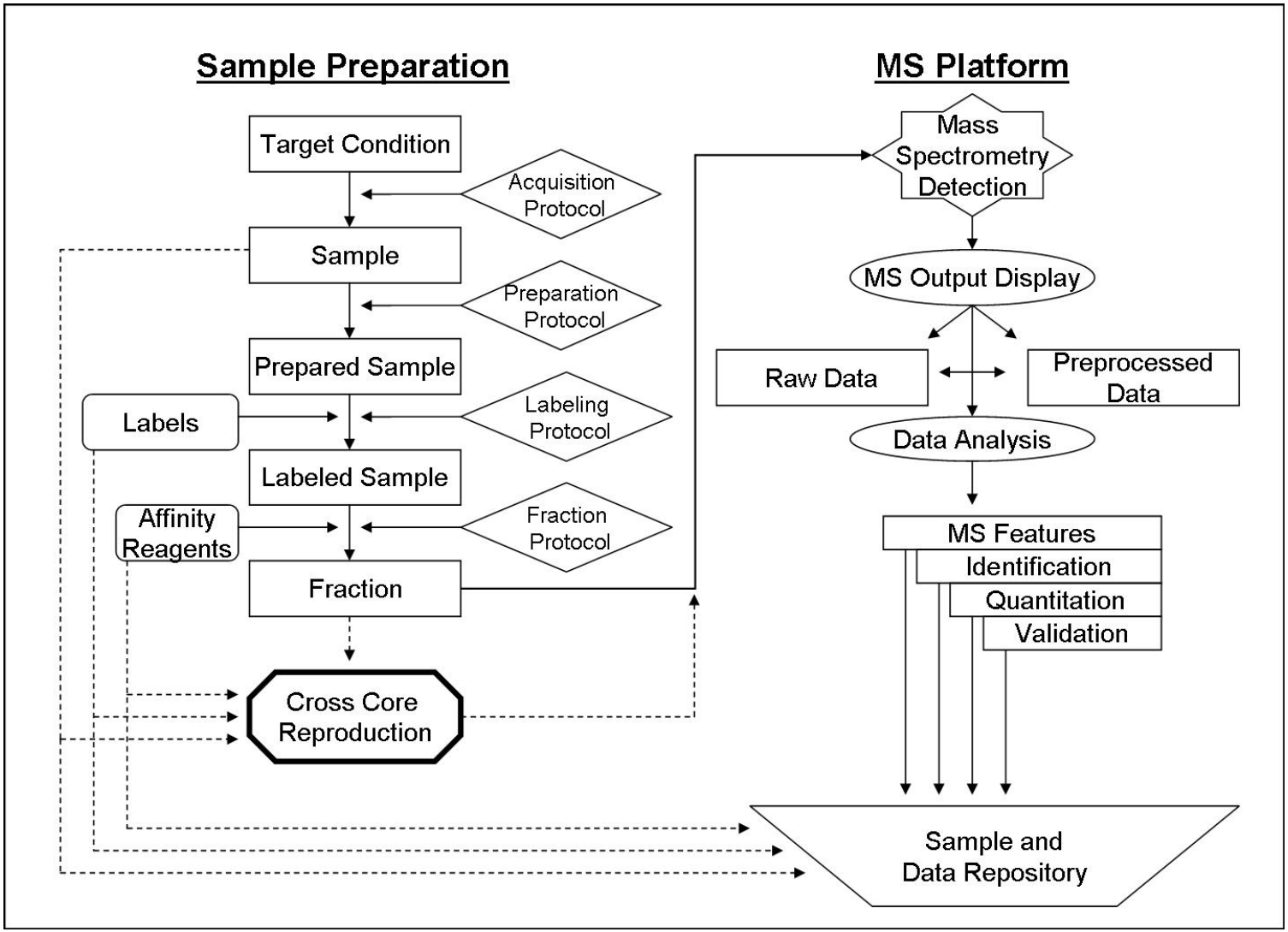
Clinical Proteomics: Technology Development, Assessment, and Standardization



Proteomics Experimental Design: Standards, Metrics, and Variability

- **Statistical and epidemiological consultants to advise on experimental design, processes, and reporting**
- **Program management and coordination**
  - Assessment of objectives versus study design and measurements
  - Certified standards and samples for calibration, bias, and uncertainty measures
    - Matrix
    - Peptides, proteins
  - Experimental design and comparison
  - Data standardization for sharing information (caBIG)
- **Control of systematic sources of variation**
  - Sample characteristics (e.g., age, gender, diet)
  - Sample handling and storage conditions
  - Instrument conditions and characteristics
- **Control of random variation**
  - Adequate sample sizes to control between sample variation
  - Replication of measurements on a given sample
- **Calibration of platform performance for laboratory comparison studies**

Assessing Variability of Proteomic Technologies

- **Specimen handling and processing**
- **Platform evaluation**
  - Technical (resolution, accuracy, dynamic range, sensitivity, reproducibility)
  - Cross verification among platforms
- **Data acquisition/Bioinformatics**
- **Data analysis**
- **Publication uniformity**

Goal: Assurance that protein measurement results are due to changes in the sample and not changes or variability due to:

- Instrument
- Assay performance
- Reagents
- Operator
- Site

Overcoming Technical Barriers

- **Build a multidisciplinary team framework**
  - To permit large-scale, real-time exchange and application of existing and newly development protein measurement technologies, biological resources, and data dissemination
- **Refine and standardize technologies, and statistical and analytical methods**
  - To ensure reliable and reproducible separation, capture, identification, quantification, and validation of protein measurements
- **Develop and evaluate new technical approaches**
  - To separate and recognize proteins of clinical significance
- **Harness efforts and align strategies across the global scientific community**

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Funding Mechanisms and Process

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Components of the Initiative

http://proteomics.cancer.gov

Funding Mechanisms ($100+ million)

- **I. Clinical Proteomic Reagents Resource**
  - **RFP**
  - **DOE/ANL and NIST**
- **II. Advanced Proteomic Platforms and Computational Sciences**
  - Next generation technologies
  - R01, R21/R33
- **III. Clinical Proteomics Technology Assessment for Cancer (CPTAC)**
  - Evaluate existing technologies
  - U24

New SBIR Contract Topics

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Clinical Proteomic Technologies for Cancer

Development of Clinical Automated Multiplex Affinity Capture Technology for Detecting Low Abundance Cancer-related Proteins/Peptides

- Develop a quantitative automated high-throughput multiplex affinity/protein capture technology for detecting low abundance cancer related proteins/peptides from bodily fluids
- Proposed technologies should be highly specific, highly selective and have ultra-sensitive detection capabilities with limited sample preparation

Development of Alternative Affinity Capture Reagents for Cancer Proteomics Research

- Develop reproducible, highly qualified/characterized alternative protein capture reagents for the cancer research community
- Capture reagents are expected to effectively compete against ELISA-based antibody technologies in terms of protein recognition, binding affinity, and detection and should be reproducibly produced in a cost-effective and efficient manner

http://sbir.cancer.gov

CPTAC Team Network:
Bringing Cutting-Edge Expertise

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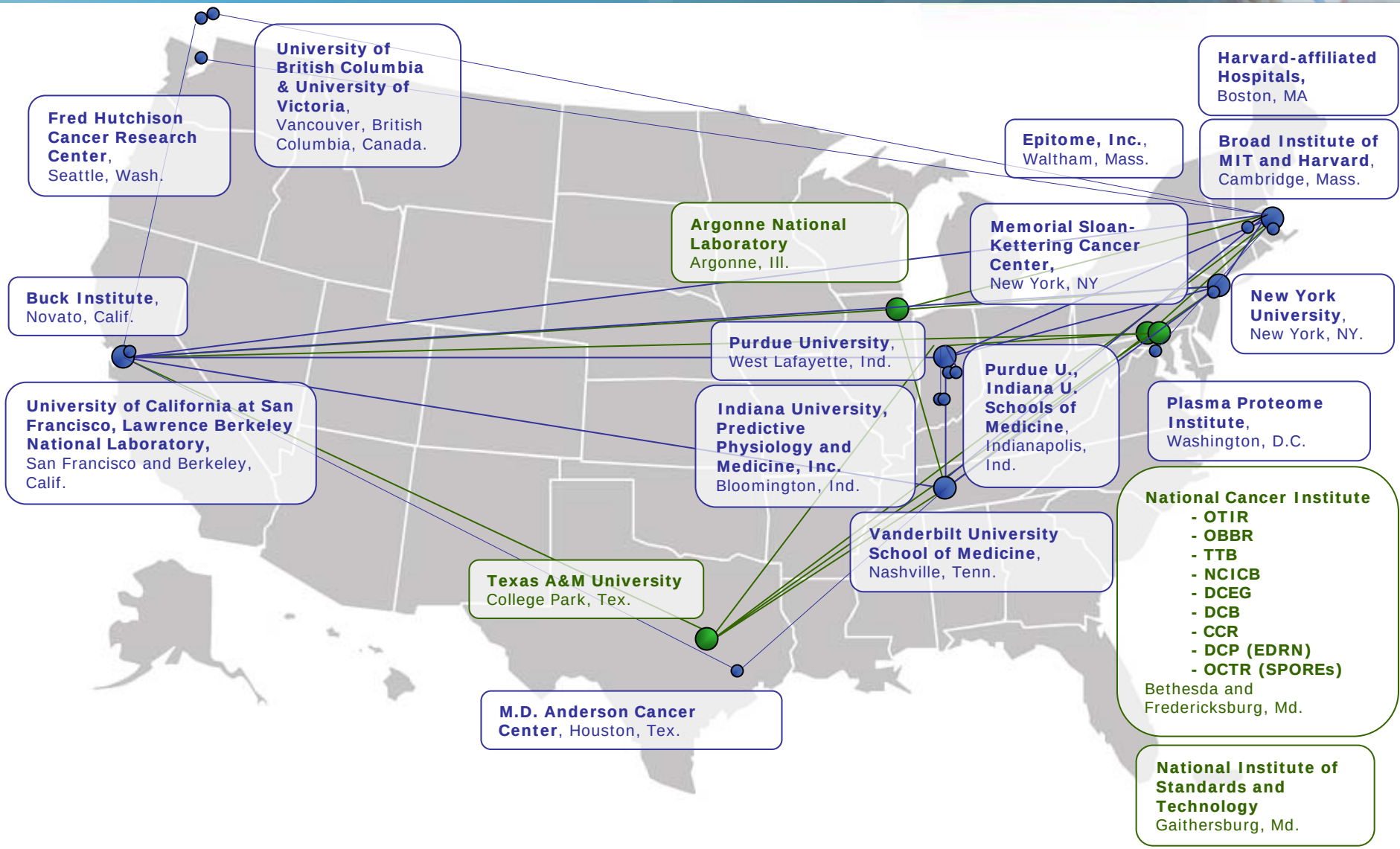
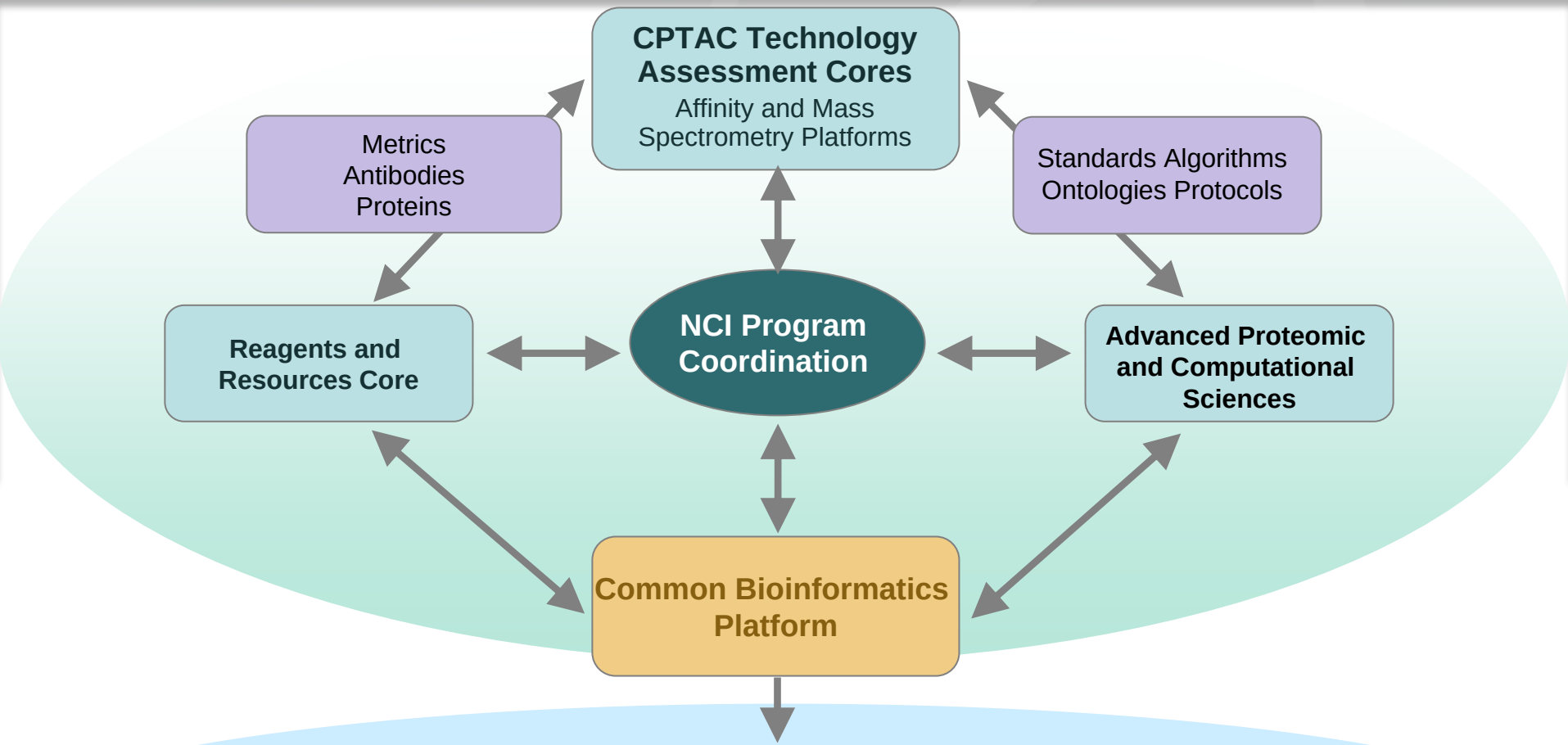


Clinical Proteomic Technologies Initiative Strategy



Community Resources:

- Integrated Searchable Proteomic Database
- Highly Qualified Biospecimens
- Standardized Reagents
- Optimized Technology Platforms
- Proteomic Standards
- New Technologies

CPTI: Expected Results

- **Creation of public resources**
  - Reference sets
  - Reagents
  - Protocols
  - Algorithms and databases
- **Accelerated protein-related discovery research and applications**
- **Enhanced knowledge base to support discovery and translational research**
- **Well-characterized and documented candidate-based approaches for peptide/protein identification**
- **Technology Development and Assessment:**
  - Sample Preparation and Labeling Technologies
  - Sample Fractionation Technologies
  - Mass Spectrometry and Microarray/Protein Capture Technologies
  - Data Analysis
  - Microsimulation
  - Validation

Computational Proteomics Analysis System: Integrated Suite of Tools to Facilitate Proteomics Collaborations

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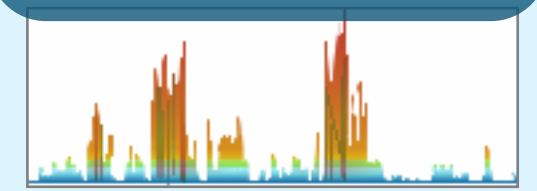
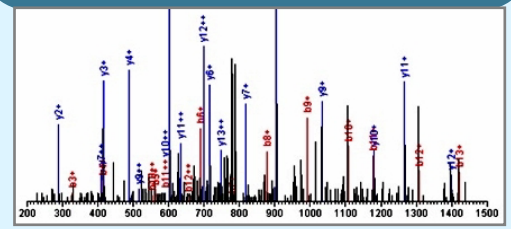
Proteomics data: multiple formats, multiple analytic methods



CPAS Suite



Proteomics Collaborations

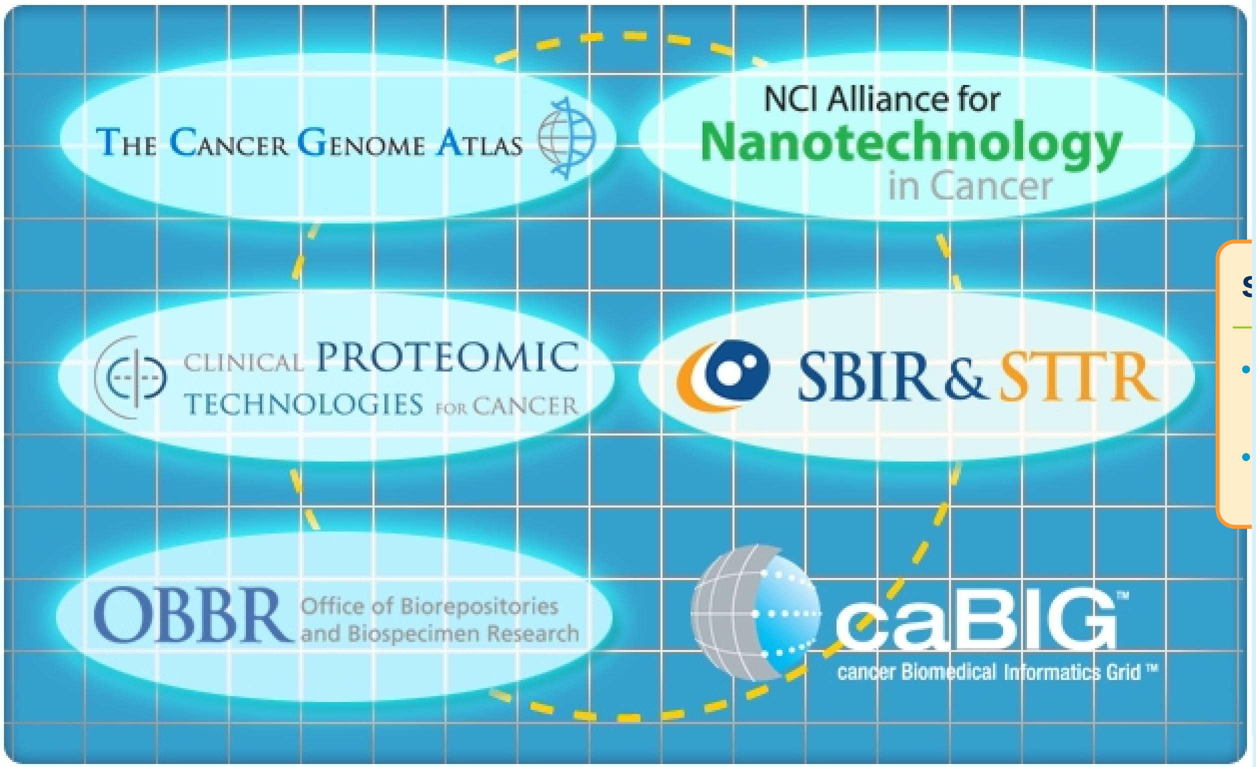
Laboratories can rapidly disseminate findings, algorithms and procedures that will enable replication and validation of discoveries

Advanced Technology Initiatives

NCI Initiatives to Enable 21st Century Cancer Care

Alliance for Nanotechnology in Cancer

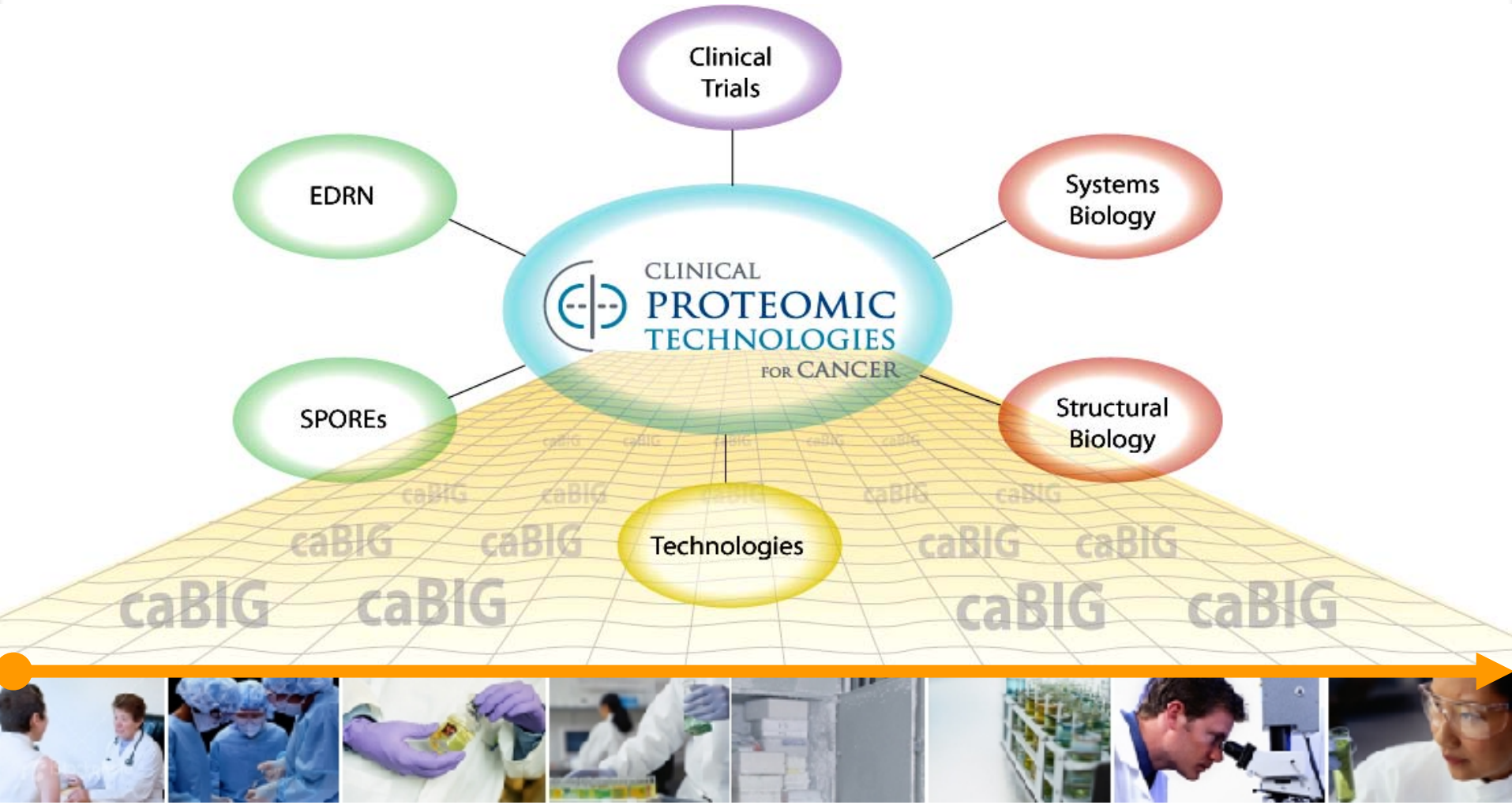
The Cancer



biorepositories

- OBBR: **Resources for molecular profiling and therapeutic optimization**
- CPTI: **Standardized measurement platforms for biomarker discovery**
- TCGA: **Molecular sub-groups for stratification and therapeutic targets**
- Nano: **Sentinels, trackers, reporters, carriers for detection, targeting and destroying cancer cells in real time**
- SBIR & STTR: **Funding of novel technologies, commercialization**
- caBIG™: **Data collection, analysis, dissemination and CONNECTIVITY**

NCI Programmatic Integration



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