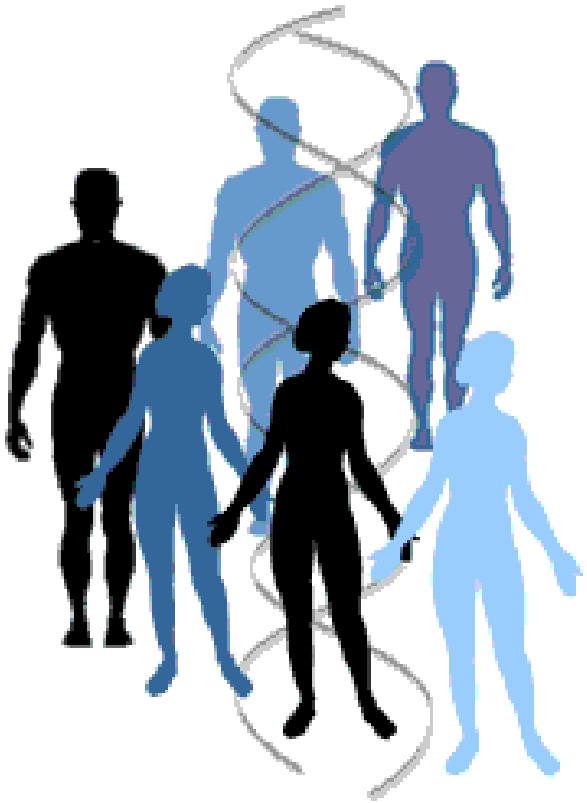
Molecular and Cell Biology methods for validation of (imaging) biomarkers

Summer school
Development and Pre-clinical Evaluation of Radiopharmaceuticals

Filipa Mendes
Center for Nuclear Sciences and Technologies
IST, Universidade de Lisboa

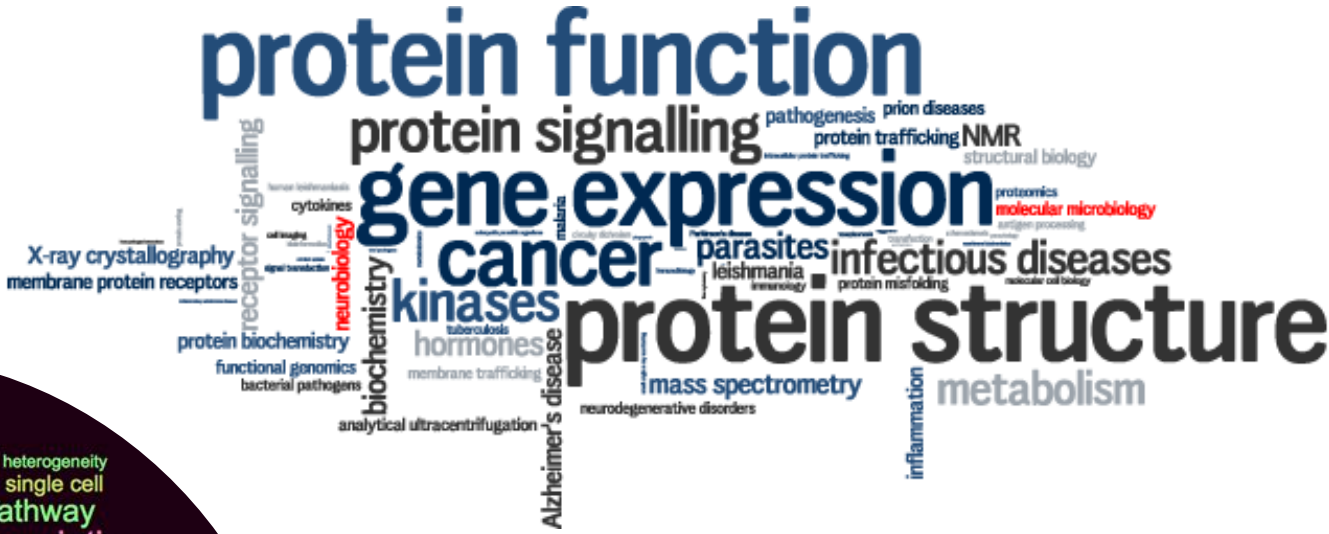
- Molecular and Cell Biology
- Biomarkers vs targets – imaging and therapy
- Transcriptomics and Proteomics
- Antibody fragments

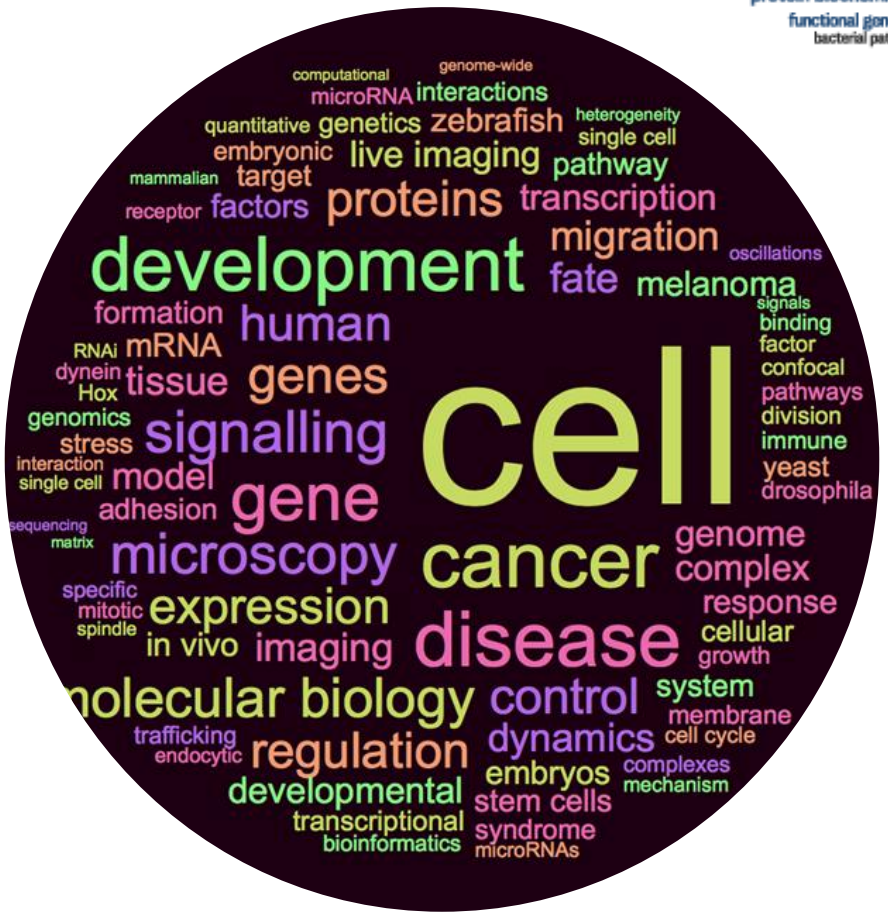


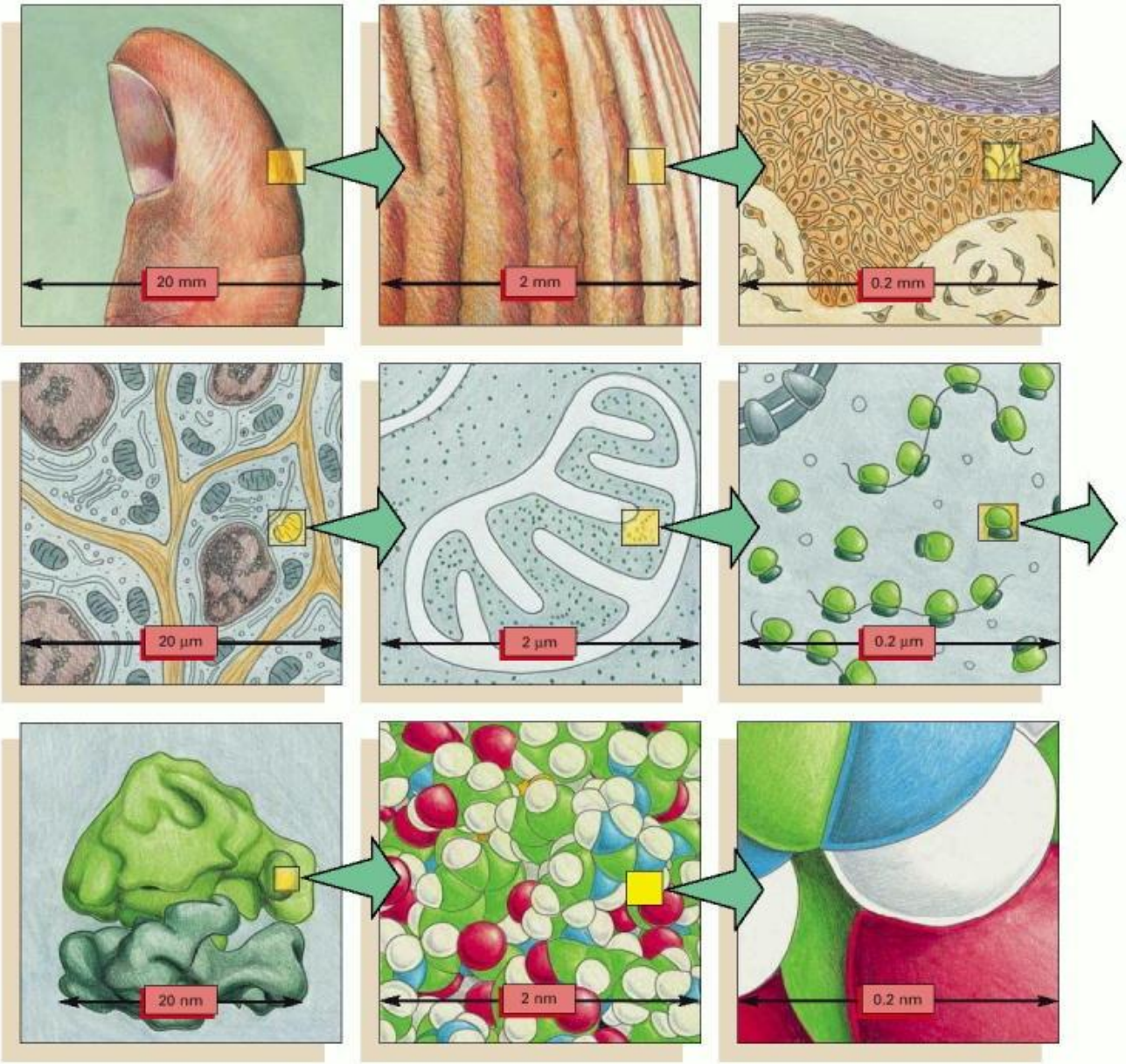
Molecular Biology is the field of biology that studies the composition, structure and interactions of macromolecules – e.g. nucleic acids and proteins – that carry out the biological processes essential for the cell's functions and maintenance.

Cell Biology is the study of structure and function of cells, which are the fundamental units of life.

Focusing on the cell allows a detailed understanding of the tissues and organisms that cells compose.

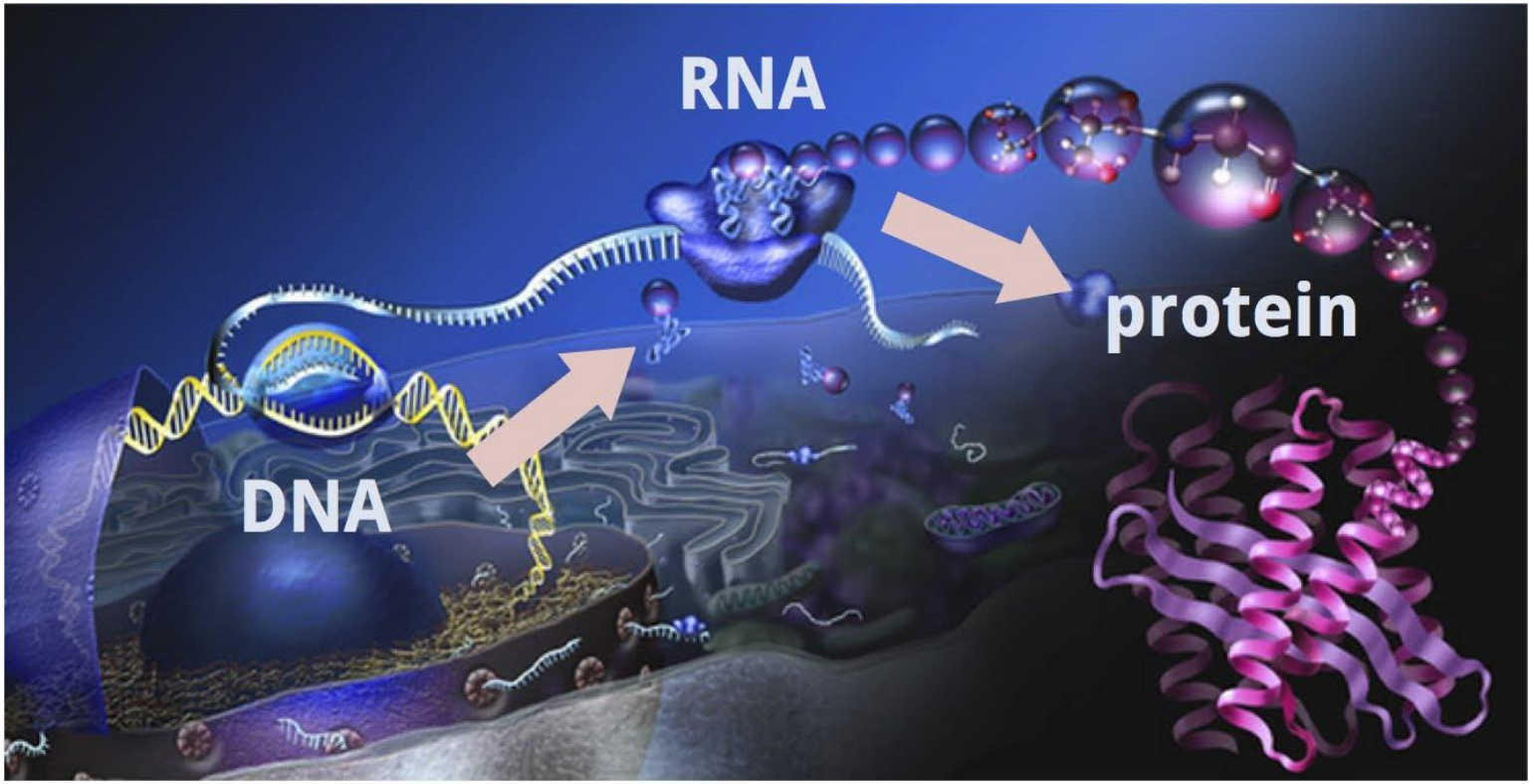

protein function
protein signalling
pathogenesis
prion diseases
protein trafficking
NMR
structural biology
gene expression
proteomics
molecular microbiology
cytokines
receptor signalling
X-ray crystallography
membrane protein receptors
neurobiology
cancer
parasites
leishmania
infectious diseases
protein misfolding
biochemistry
kinases
protein structure
protein biochemistry
hormones
functional genomics
bacterial pathogens
membrane trafficking
mass spectrometry
metabolism
analytical ultracentrifugation
neurodegenerative disorders
Alzheimer's disease
inflammation


computational
genome-wide
microRNA
interactions
quantitative
genetics
zebrafish
heterogeneity
single cell
embryonic
live imaging
pathway
mammalian
target
receptor
factors
proteins
transcription
migration
oscillations
development
fate
melanoma
formation
human
signals
binding
factor
RNAi
mRNA
dynein
tissue
genes
confocal
Hox
pathways
genomics
signalling
cell
division
stress
immune
interaction
single cell
model
yeast
gene
drosophila
adhesion
sequencing
matrix
microscopy
cancer
genome
complex
specific
response
mitotic
expression
cellular
spindle
in vivo
imaging
disease
growth
molecular biology
control
system
membrane
trafficking
endocytic
regulation
dynamics
cell cycle
embryos
complexes
developmental
stem cells
mechanism
transcriptional
syndrome
bioinformatics
microRNAs

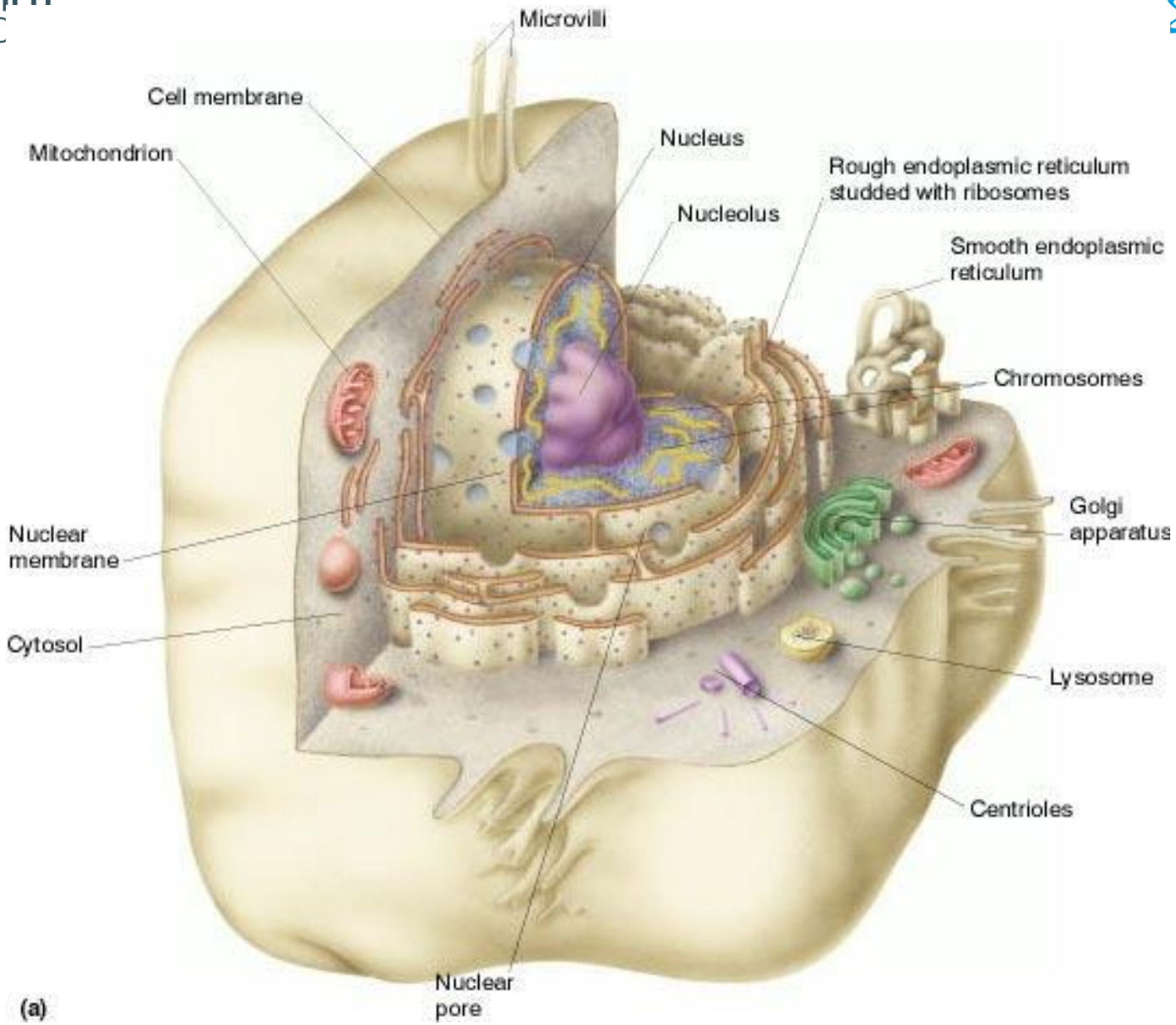


Molecular Biology of the Cell. 4th edition. Alberts B, Johnson A, Lewis J, et al., New York: Garland Science; 2002

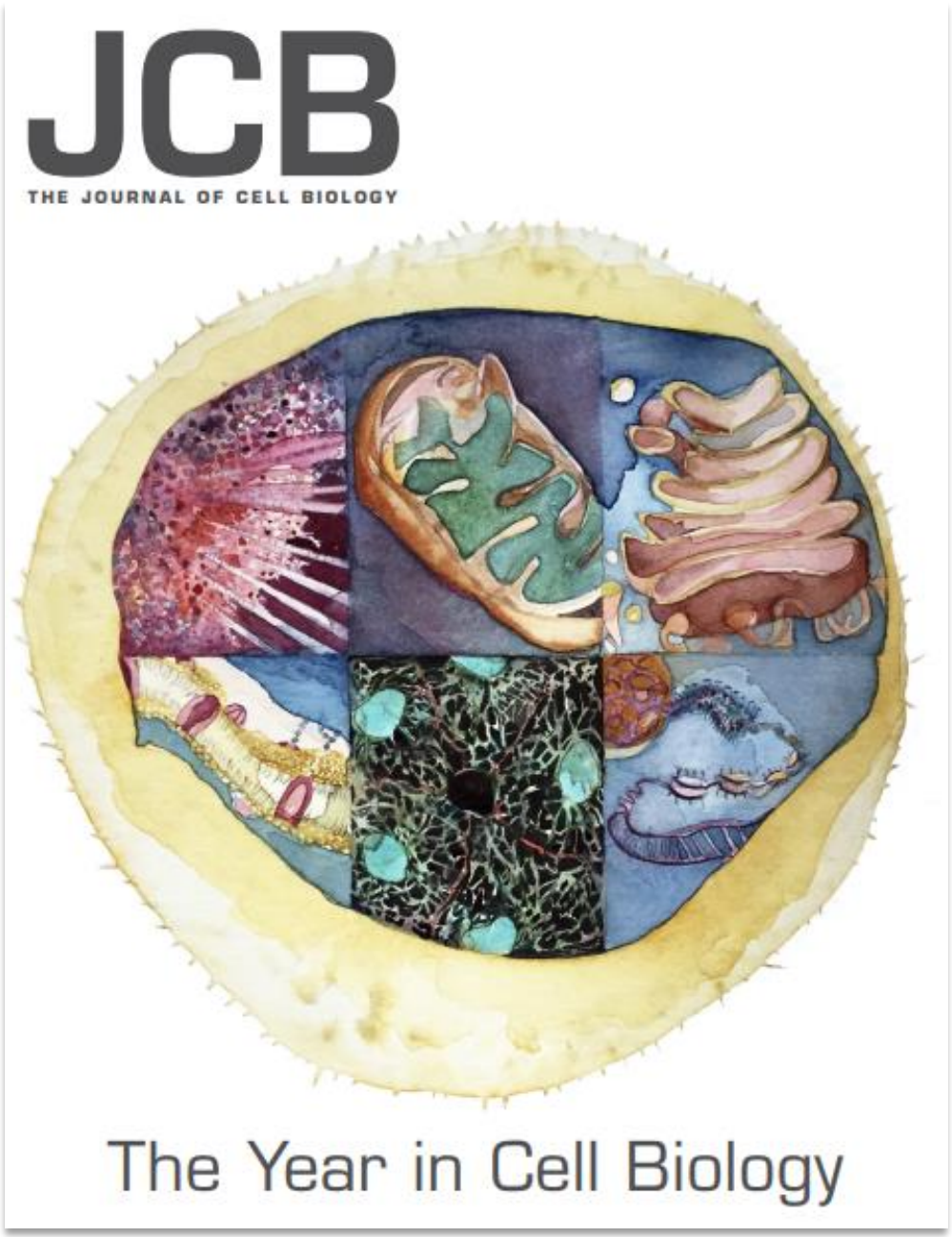
Central dogma of molecular **biology**



the two-step process, transcription and translation, by which the information in genes flows into proteins: DNA → RNA → protein



An Introduction to Genetic Analysis. 7th edition. Griffiths AJF, Miller JH, Suzuki DT, et al. New York: W. H. Freeman; 2000

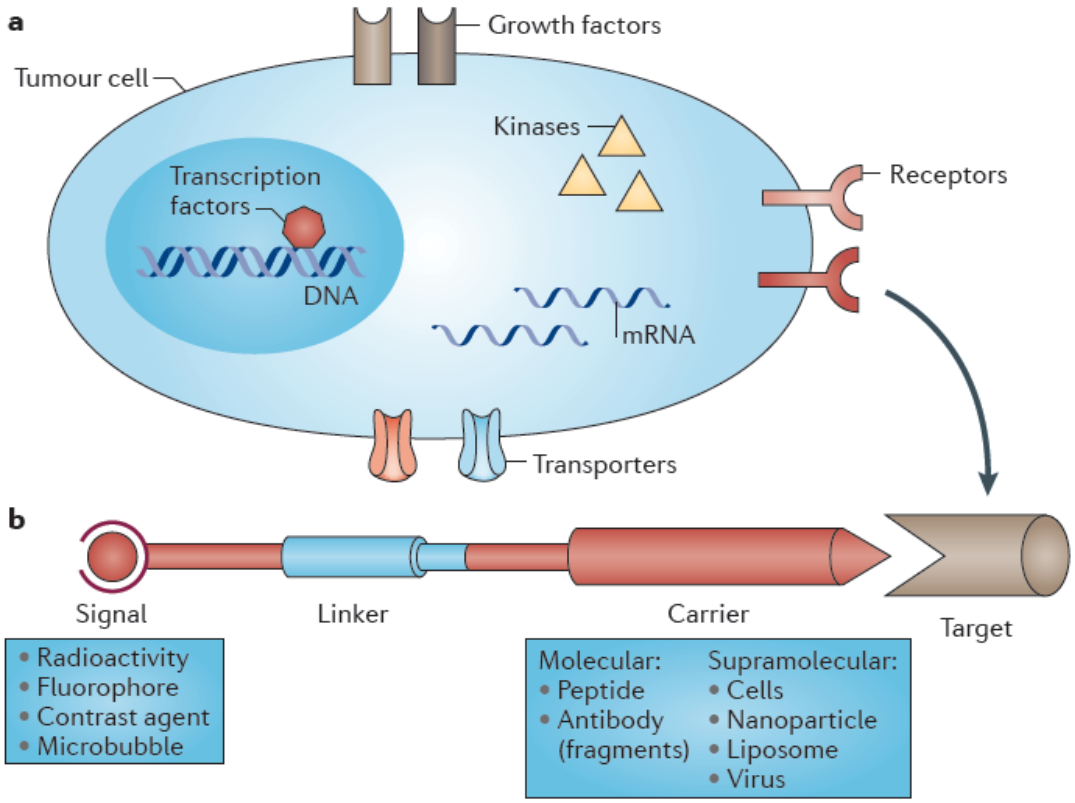

JCB
THE JOURNAL OF CELL BIOLOGY
The Year in Cell Biology

Molecular Imaging

In vivo **characterization and measurement of biological/biochemical processes at the cellular/molecular level, allowing the noninvasive visualization of a target macromolecule *in vivo* by virtue of its interaction with a specific imaging probe.**

Since certain molecular markers (e.g. cell surface receptors and enzymes) anticipate macromolecular manifestations of disease, namely aberrant anatomy and organ dysfunction, their visualization by imaging modalities may allow early disease detection.

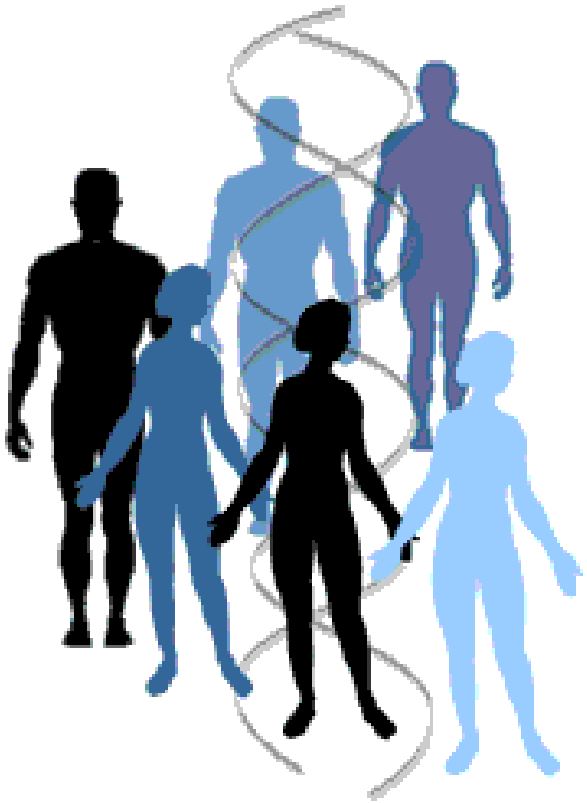
Molecular imaging targets in (tumour) cells and structure of imaging probes



Changing times in medicine - Paradigm shift in disease detection and treatment from a focus on morphology, function, and pathophysiology to **genetic and molecular events**

n this way, molecular nuclear medicine will play an increasing role in disease definition, preventive medicine, and treatment planning and monitoring – Personalized medicine

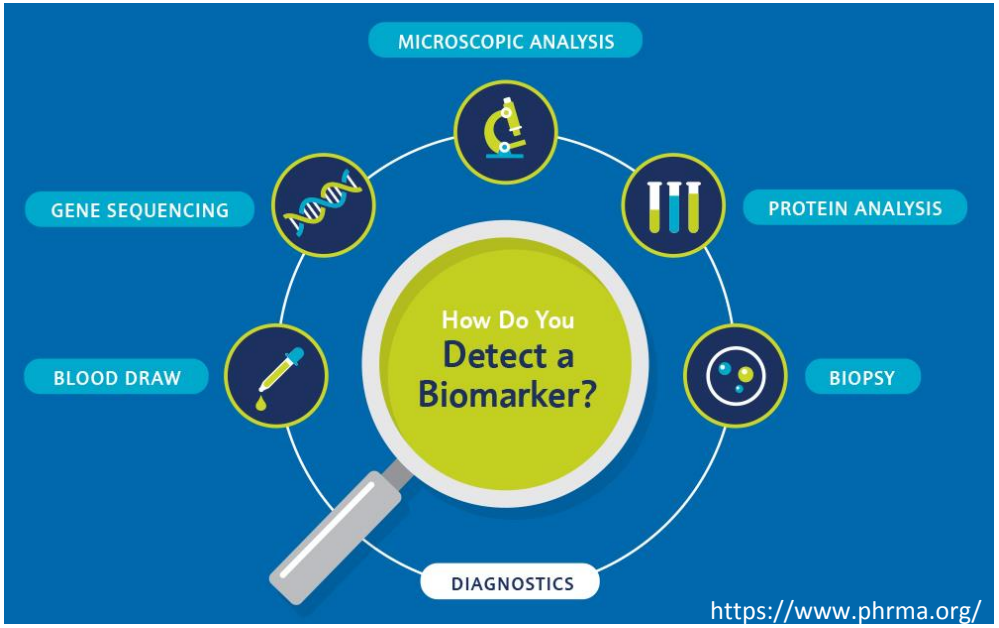
- Molecular and Cell Biology
- **Biomarkers vs targets – imaging and therapy**
- Transcriptomics and Proteomics
- Antibody fragments



Biomarkers

NIH Biomarkers Definition Working Group

Cellular, biochemical, and molecular alterations by which a normal, abnormal, or simply biologic process can be recognized or monitored and are used to objectively **measure and evaluate** normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention



~30 cancer biomarkers approved by the FDA

Cancer biomarkers have typically relied on assays of blood or tissue; however, **molecular imaging (MI)** has a promising and complementary role as a cancer biomarker

Why?
Non-invasive (serial assay)
Entire disease burden (whole-body)
Avoidance of sampling errors (tumor heterogeneity)

Disadvantages:
Sample only 1 or 2 processes simultaneously
One subject at a time

Biomarkers

- Detect cancer
- Direct cancer therapy

Types of biomarkers

- <u>Predictive and prognostic</u>
  Indication of aggressiveness, course of disease, likelihood of response to treatment
  e.g. ERexpression in breast cancer - ^{18}F-estradiol

- <u>Early-response</u>
  PhD markers – indication of biological response to treatment
  e.g. Ki 67 tumor proliferation - ^{18}F-FDG

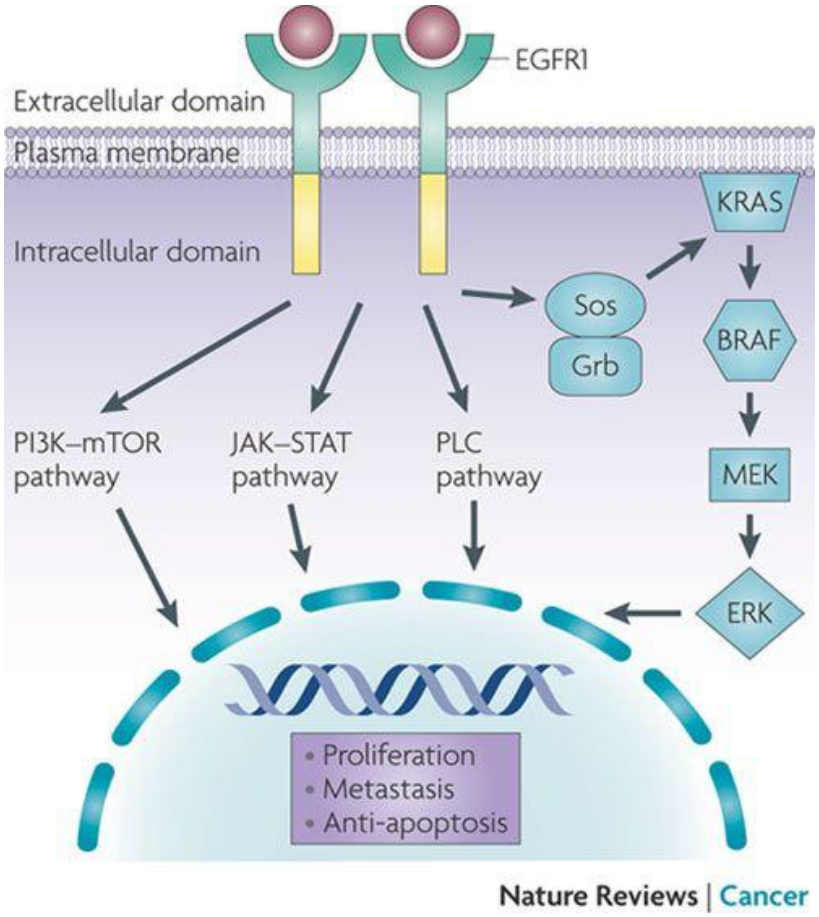
- <u>Surrogate endpoints</u>
  Indication of therapeutic success associated with patient outcome
  e.g. tumor regression (histology) – ^{18}F-FDG

Biomarkers are not (always) equivalent to targets

An important distinction should be made between biomarkers and targets

KRAS is an excellent biomarker in colorectal cancer- but it is not the target of therapy

mutations in KRAS render tumors less responsive to anti-**EGFR** therapies



https://media.nature.com/full/nature-assets/nrc/journal/v9/n7/images/nrc2645-f2.jpg

Imaging targets can be used as (imaging) biomarkers

tremendous variety of (potencial) biomarkers: proteins, nucleic acids, antibodies, and peptides

Imaging biomarkers

Angiogenesis
Proliferation
Infection
Apoptosis
Atherosclerosis (unstable plaques)
Neurodegenerative disorders
Inflammation
Renal function

Identification and validation of new targets

Validation

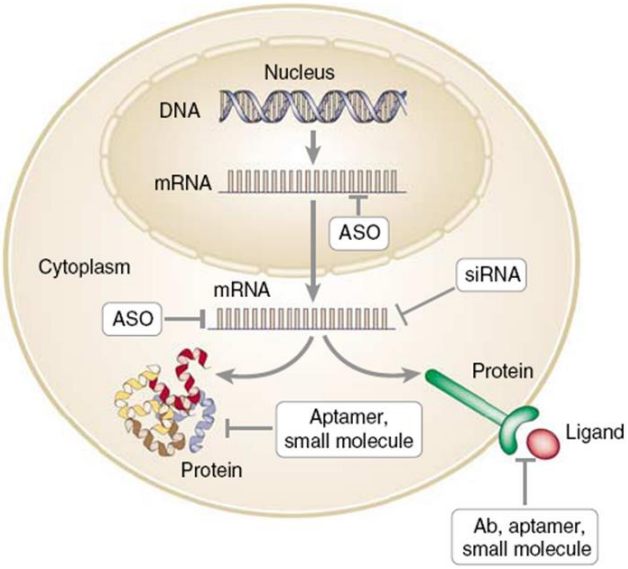
Process of determining the direct or indirect involvement of a specific protein in a disease and as a suitable target for new (radio)pharmaceuticals

Methods for the identification and validation of new targets - analysis of gene function in the different levels of expression

↓

DNA, RNA and protein

Emerging from investigations of the genomic and proteomic signatures of cancer cells, an increasing number of promising targets are being identified, including receptors, enzymes, transporters, micro RNA, and antigens (a biomarker can also be a collection of alterations, such as gene expression, proteomic, and metabolomic signatures)



Identification and validation of new targets

Potential markers can be identified through multiple approaches:

Classic approach - identify candidate biomarkers/targets based on the biology of the tumor and surrounding environment

"Discovery" approach – using techniques such as high-throughput sequencing, gene expression arrays, and mass spectroscopy to quickly identify individual or groups of targets that differ between cohorts

vast amount of data generated means that particular attention needs to be paid to the study design and the data analysis, in order to minimize the chance of identifying associations that are false positives

Key aspects- careful study design to avoid bias, comprehensive testing and validation, and accurate reporting

OPEN

Imaging biomarker roadmap for cancer studies

James P. B. O'Connor[1], Eric O. Aboagye[2], Judith E. Adams[3], Hugo J. W. L. Aerts[4], Sally F. Barrington[5], Ambros J. Beer[6], Ronald Boellaard[7], Sarah E. Bohndiek[8], Michael Brady[9], Gina Brown[10], David L. Buckley[11], Thomas L. Chenevert[12], [1]Laurence P. Clarke[13], Sandra Collette[14], Gary J. Cook[5], Nandita M. deSouza[15], John C. Dickson[16], Caroline Dive[17], Jeffrey L. Evelhoch[18], Corinne Faivre-Finn[19], Ferdia A. Gallagher[8], Fiona J. Gilbert[8], Robert J. Gillies[20], Vicky Goh[5], John R. Griffiths[8], Ashley M. Groves[16], Steve Halligan[16], Adrian L. Harris[9], David J. Hawkes[16], Otto S. Hoekstra[21], Erich P. Huang[22], Brian F. Hutton[16], Edward F. Jackson[23], Gordon C. Jayson[24], Andrew Jones[25], Dow-Mu Koh[15], Denis Lacombe[26], Philippe Lambin[27], Nathalie Lassau[28], Martin O. Leach[15], Ting-Yim Lee[29], Edward L. Leen[2], Jason S. Lewis[30], Yan Liu[26], Mark F. Lythgoe[31], Prakash Manoharan[1], Ross J. Maxwell[32], Kenneth A. Miles[16], Bruno Morgan[33], Steve Morris[34], Tony Ng[5], Anwar R. Padhani[35], Geoff J. M. Parker[1], Mike Partridge[9], Arvind P. Pathak[36], Andrew C. Peet[37], Shonit Punwani[16], Andrew R. Reynolds[38], Simon P. Robinson[15], Lalitha K. Shankar[13], Ricky A. Sharma[16], Dmitry Soloviev[8], Sigrid Stroobants[39], Daniel C. Sullivan[40], Stuart A. Taylor[16], Paul S. Tofts[41], Gillian M. Tozer[42], Marcel van Herk[19], Simon Walker-Samuel[31], James Wason[43], Kaye J. Williams[1], Paul Workman[44], Thomas E. Yankeelov[45], Kevin M. Brindle[8], Lisa M. McShane[22], Alan Jackson[1] and John C. Waterton[1]

Abstract | Imaging biomarkers (IBs) are integral to the routine management of patients with cancer. IBs used daily in oncology include clinical TNM stage, objective response and left ventricular ejection fraction. Other CT, MRI, PET and ultrasonography biomarkers are used extensively in cancer research and drug development. New IBs need to be established either as useful tools for testing research hypotheses in clinical trials and research studies, or as clinical decision-making tools for use in healthcare, by crossing 'translational gaps' through validation and qualification. Important differences exist between IBs and biospecimen-derived biomarkers and, therefore, the development of IBs requires a tailored 'roadmap'. Recognizing this need, Cancer Research UK (CRUK) and the European Organisation for Research and Treatment of Cancer (EORTC) assembled experts to review, debate and summarize the challenges of IB validation and qualification. This consensus group has produced 14 key recommendations for accelerating the clinical translation of IBs, which highlight the role of parallel (rather than sequential) tracks of technical (assay) validation, biological/clinical validation and assessment of cost-effectiveness; the need for IB standardization and accreditation systems; the need to continually revisit IB precision; an alternative framework for biological/clinical validation of IBs; and the essential requirements for multicentre studies to qualify IBs for clinical use.

Correspondence to J.P.B.O'C.
Cancer Research UK Manchester Institute, University of Manchester, Wilmslow Road, Withington, Manchester M20 4BX, UK.
james.o'connor@manchester.ac.uk

doi:10.1038/nrclinonc.2016.162
Published online 11 Oct 2016

A biomarker is a "defined characteristic that is measured as an indicator of normal biological processes, pathogenic processes or responses to an exposure or intervention, including therapeutic interventions" (REFS 1,2). The current FDA–NIH Biomarker Working Group definition — adopted in this consensus statement — states explicitly that "molecular, histologic, radiographic or physiologic characteristics are examples of biomarkers" (REF. 2). This approach seeks to clarify inconsistency in terminology, because some previous definitions have restricted

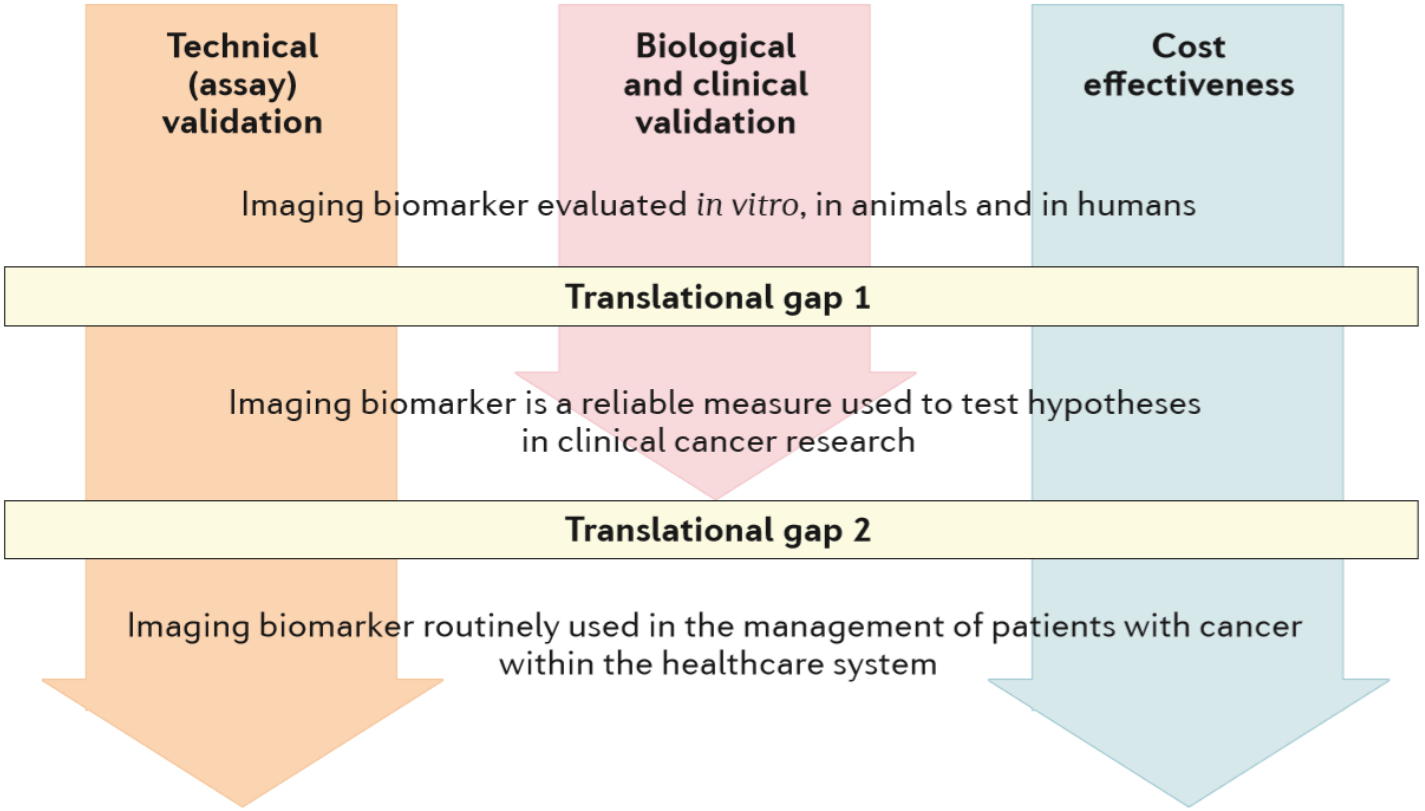
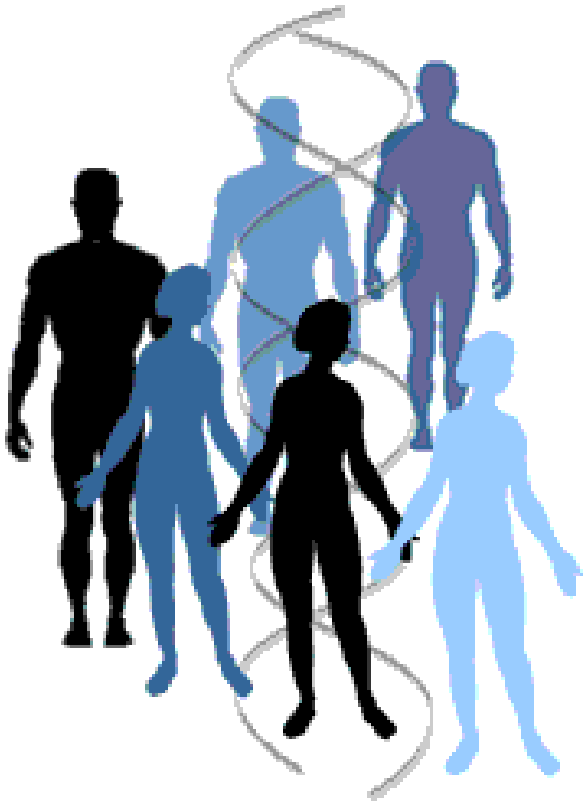
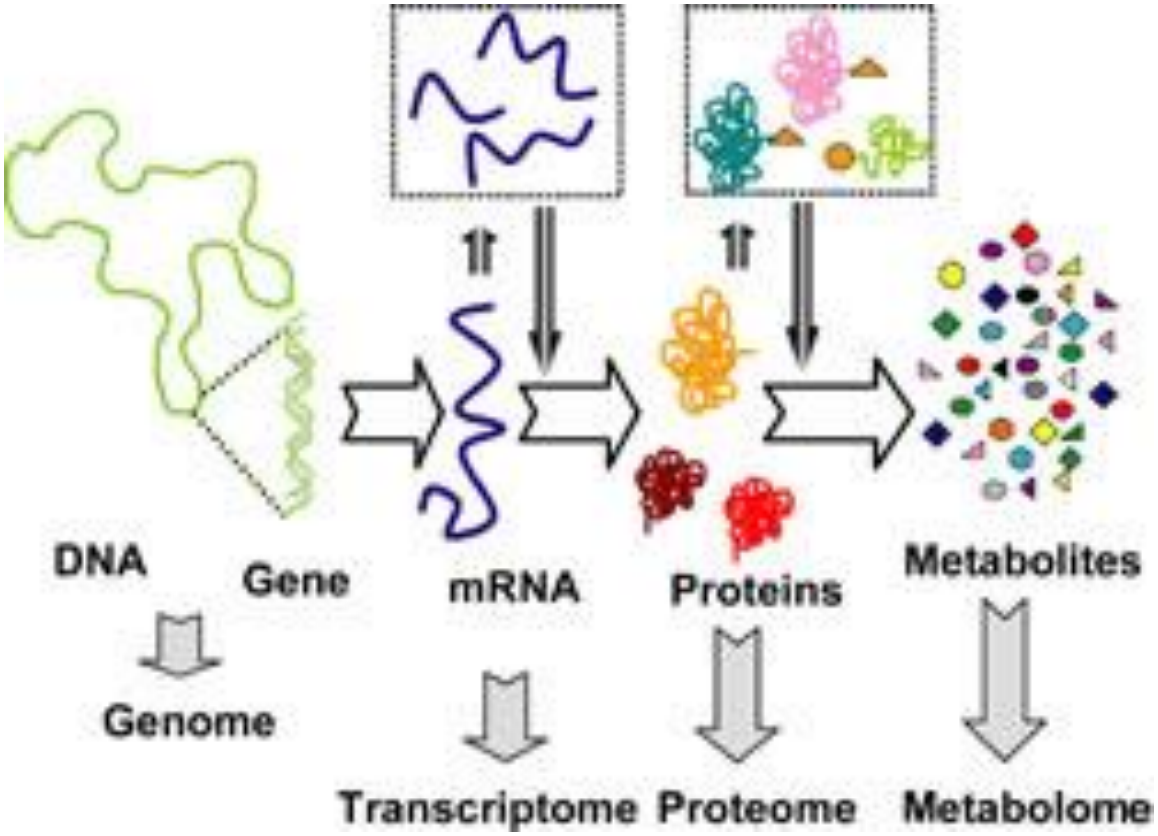


Figure 1 | **Overview of the imaging biomarker roadmap.** Imaging biomarkers must cross translational gap 1 to become robust medical research tools, and translational gap 2 to be integrated into routine patient care. This goal is achieved through three parallel tracks of technical (assay) validation, biological and clinical validation, and cost effectiveness.

- Molecular and Cell Biology
- Biomarkers vs targets – imaging and therapy
- **Transcriptomics and Proteomics**
- Antibody fragments



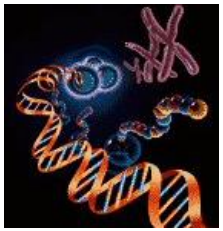
Omics: global (integrated) cell biology



The Era of Omics

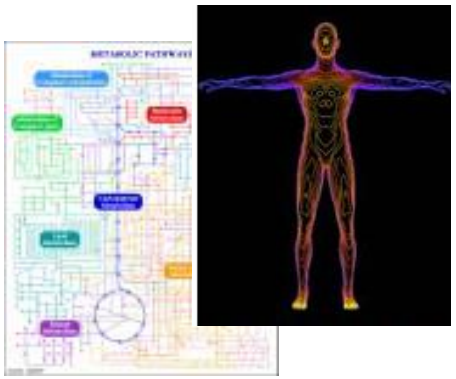
What might happen

Genomics



Genotype

Fenotype



What is happening (now)

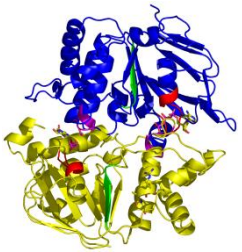
Metabolomics

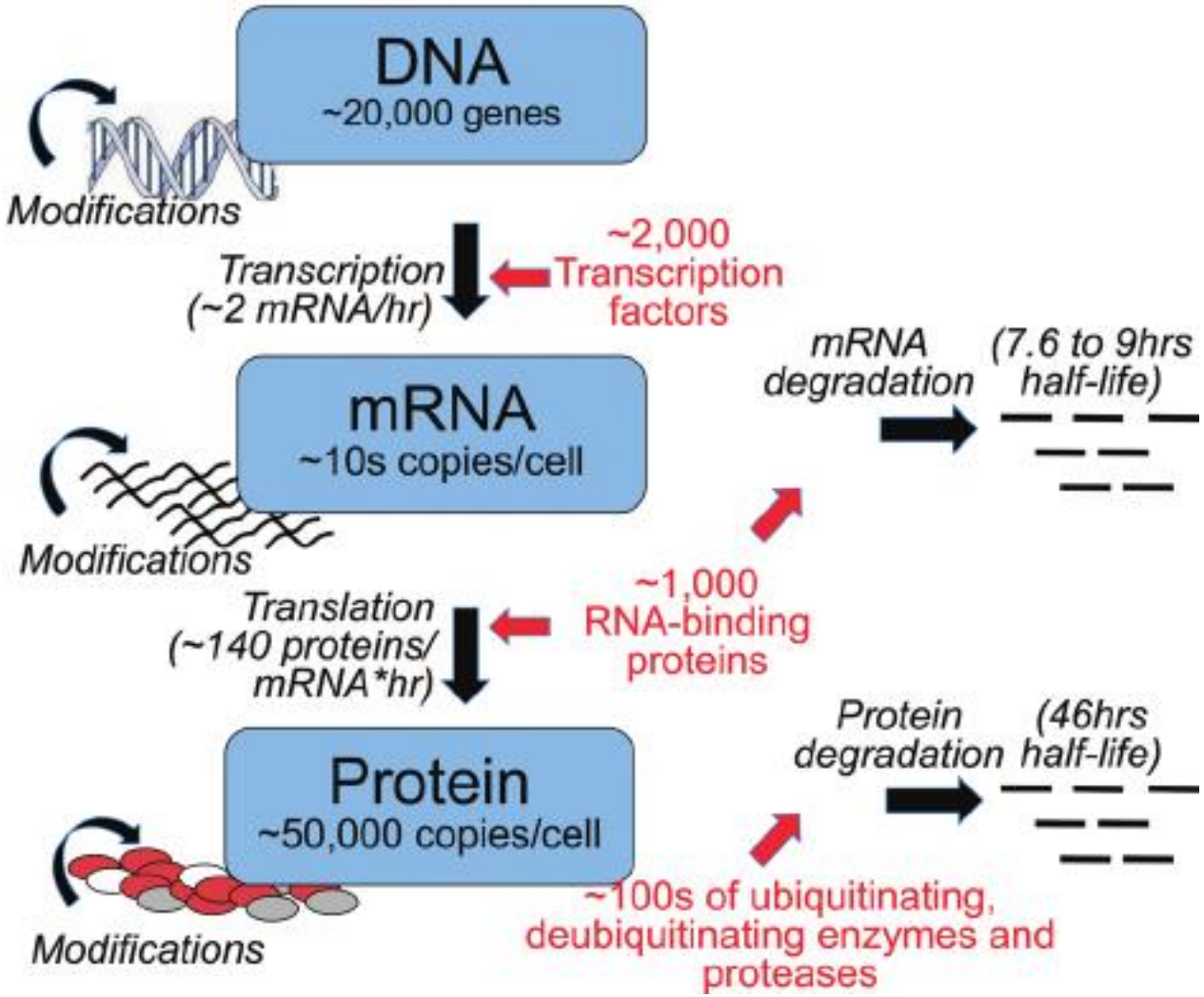
What seems to be happening

Transcriptomics

What makes it happen

Proteomics





> 68.000 protein variants

Next-generation analysis of gene expression regulation – comparing the roles of synthesis and degradation
July 2015, Molecular BioSystems 11(10)

Transcriptomics

DNA Arrays vs RNA sequencing

Dominant contemporary techniques: microarrays and RNA-Seq, were developed in the mid-1990s and 2000s

Microarrays that measure the abundances of a defined set of transcripts via their hybridisation to an array of complementary probes were first published in 1995.

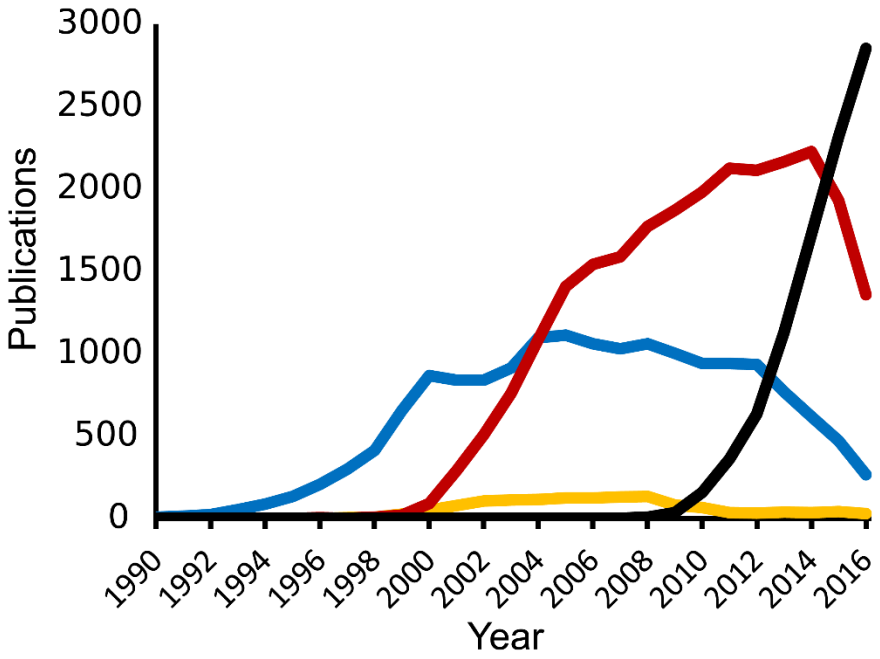
RNA-Seq refers to the sequencing of transcript cDNAs, in which abundance is derived from the number of counts from each transcript.

Method	RNA-Seq	Microarray
Throughput	High [10]	Higher [10]
Input RNA amount	Low ~ 1 ng total RNA [25]	High ~ 1 μg mRNA [26]
Labour intensity	High (sample preparation and data analysis) [10][23]	Low [10][23]
Prior knowledge	None required, though genome sequence useful [23]	Reference transcripts required for probes [23]
Quantitation accuracy	~90% (limited by sequence coverage) [27]	>90% (limited by fluorescence detection accuracy) [27]
Sequence resolution	Can detect SNPs and splice variants (limited by sequencing accuracy of ~99%) [27]	Dedicated arrays can detect splice variants (limited by probe design and cross-hybridisation) [27]
Sensitivity	10^{-6} (limited by sequence coverage) [27]	10^{-3} (limited by fluorescence detection) [27]
Dynamic range	$>10^5$ (limited by sequence coverage) [28]	10^3–10^4 (limited by fluorescence saturation) [28]
Technical reproducibility	>99% [29][30]	>99% [31][32]

RNA-Seq, RNA Sequencing

https://doi.org/10.1371/journal.pcbi.1005457.t001

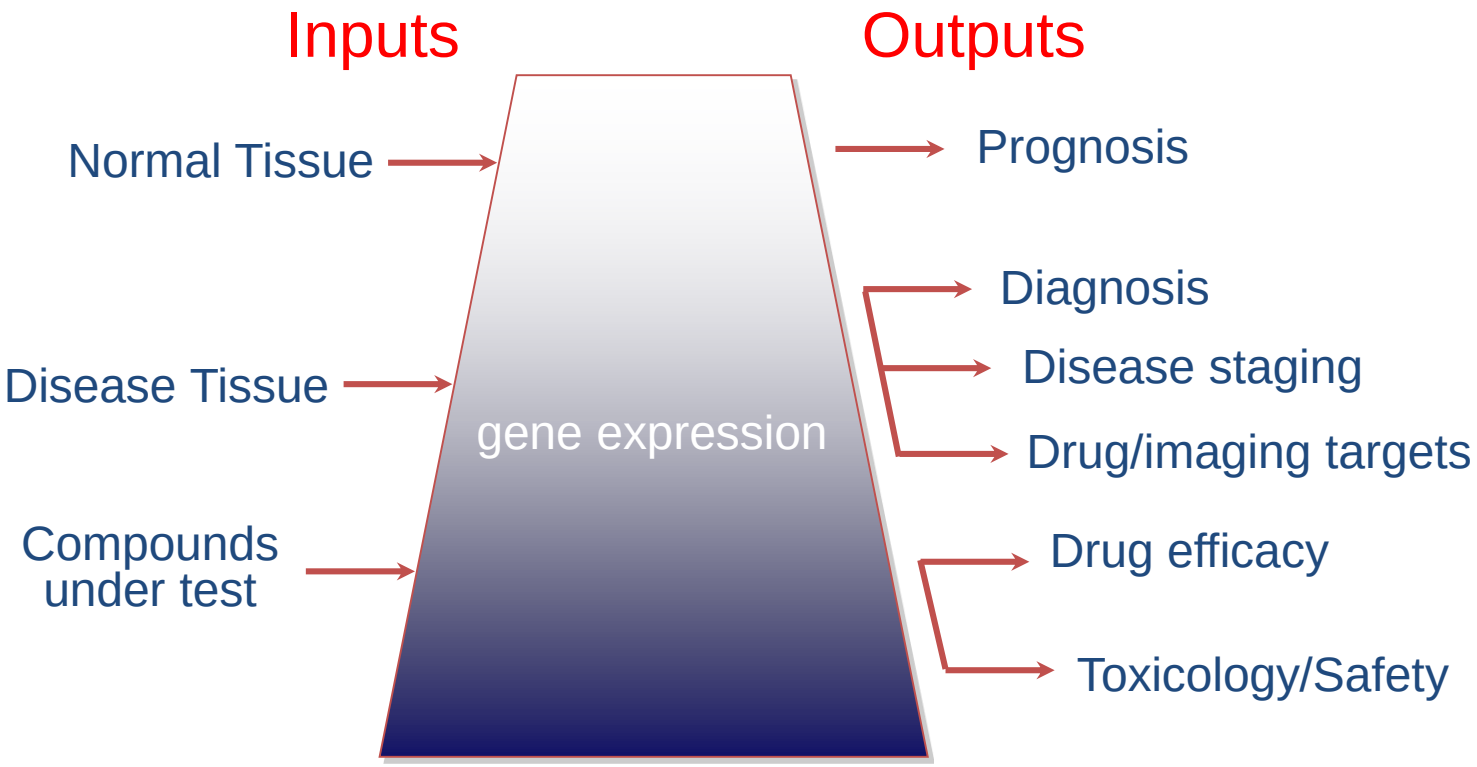
Transcriptomics methods use over time



Published papers since 1990, referring to RNA sequencing (black), RNA microarray (red), expressed sequence tag (blue), and serial/cap analysis of gene expression (yellow)

https://doi.org/10.1371/journal.pcbi.1005457

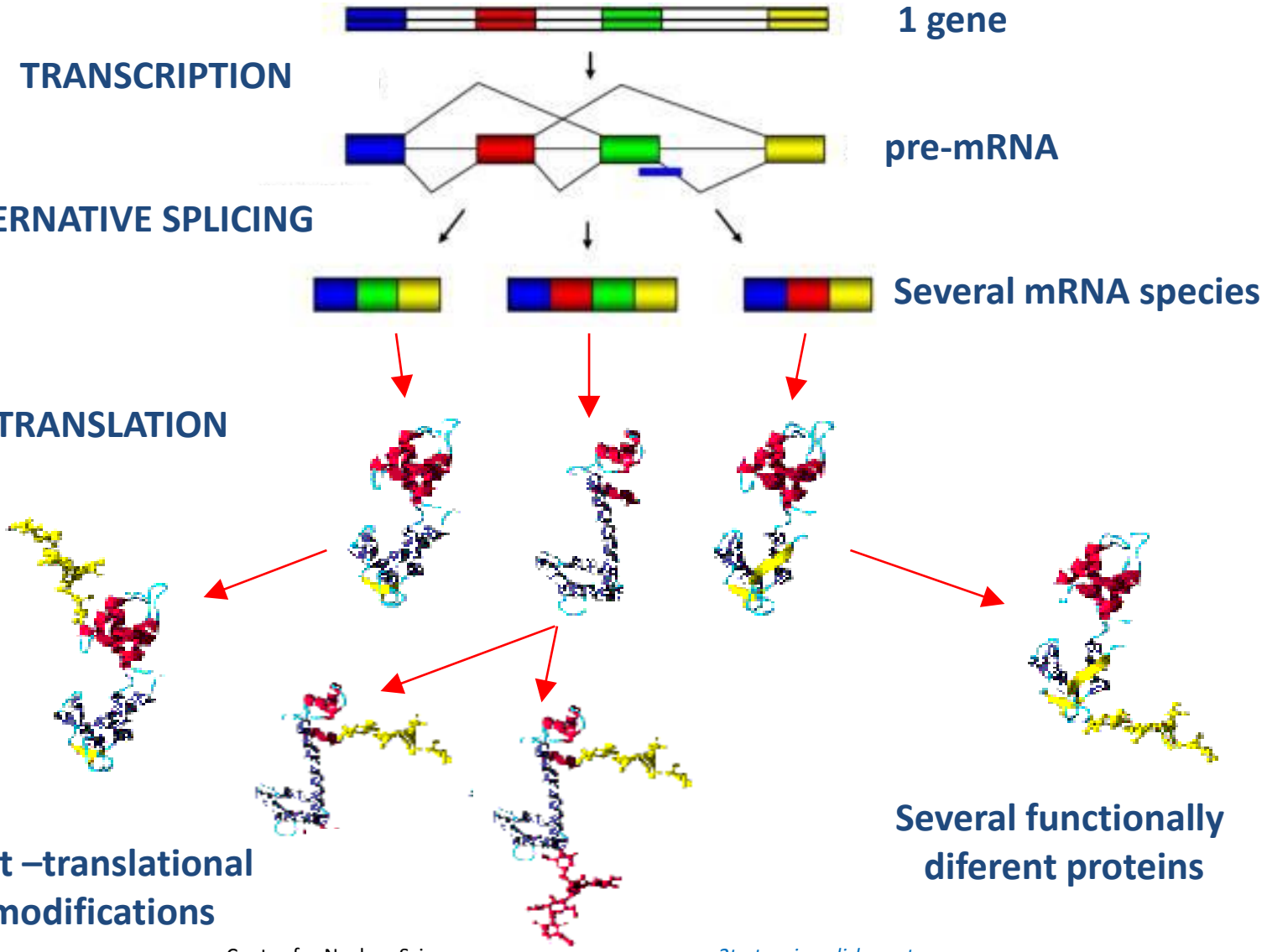
Transcriptomics - Aplications



Limitations of (genomics) and Transcriptomics

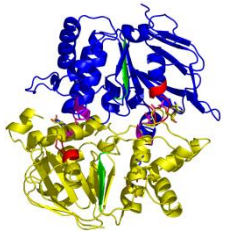
- ✓ The genome does not demonstrate the dynamic processes occurring at the protein and celular levels
- ✓ The sequence of a gene does not allow the identification of the post translational modifications (glicosilation, phosphorylation), which are essencial for protein funciton and activity
- ✓ The expression level of a gene does not correlate with the amount of active protein in the cell

DNA *versus* Protein



Proteome

DNA Information → Proteins Activity



GENOME — PROTEOME

One genome

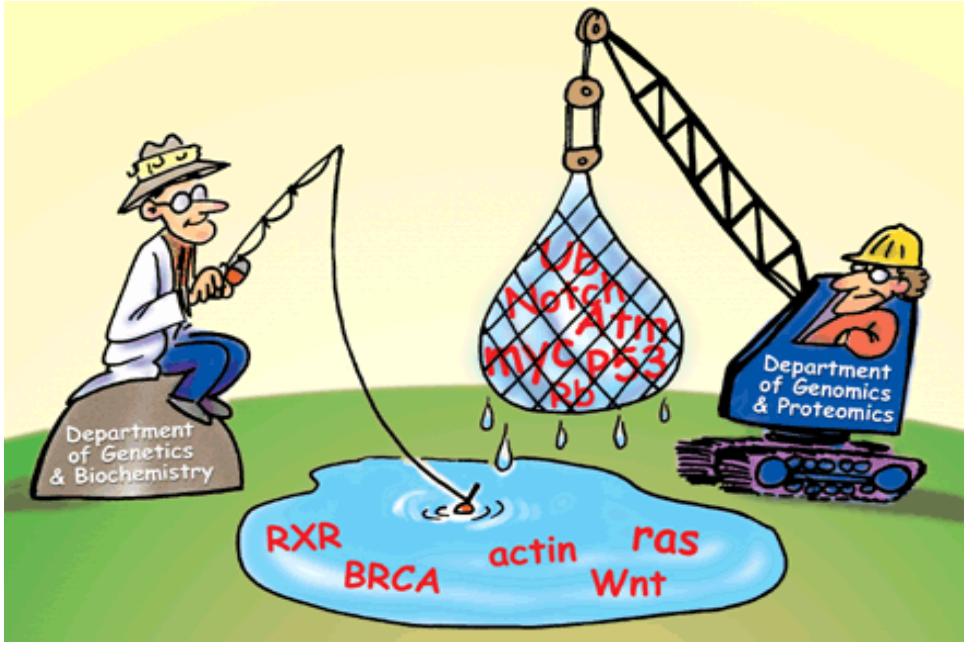
Two proteomes

It is the set of all proteins expressed by a determined type of cells, organisms, etc, in a determined time, under determined conditions, by a genome.

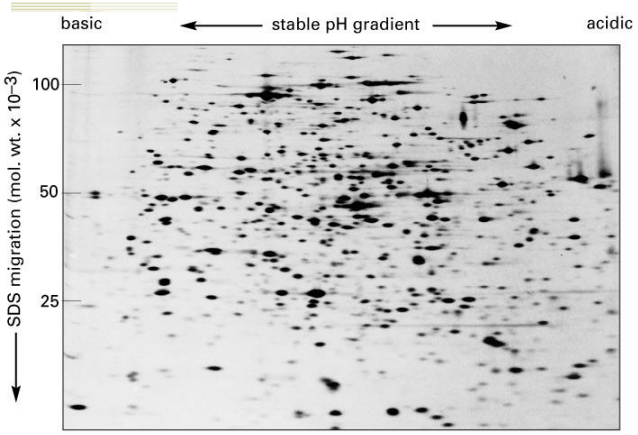
Marc R. Wikins, 1994

Proteomics

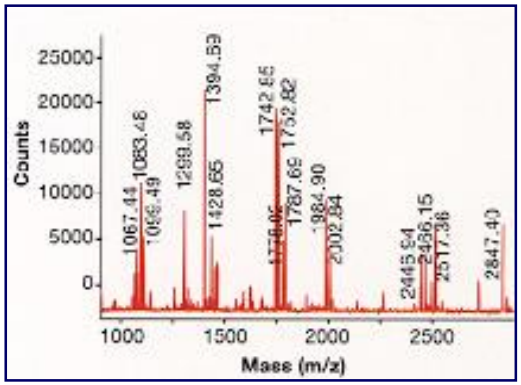
Methodologies to quantitatively determine the pattern of protein expression of a genome



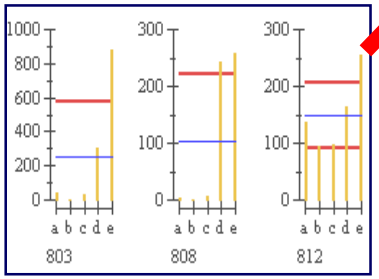
Proteomics classical approach



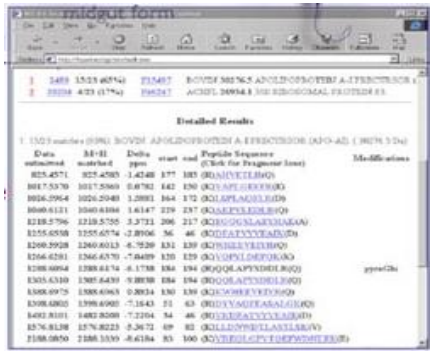
1. 2-DE separation



3. Mass spectroscopy ID



2. Bioinformatic analysis



4. Databases

Proteomics- alternatives to 2DE

- ✓ HPLC
- ✓ ICAT (Isotope Coded Affinity Tags)
- ✓ Protein Chip
- ✓ High Performance mass spectroscopy - iTRAQ, label-free LFQ…

Review Article

The Discovery of Novel Genomic, Transcriptomic, and Proteomic Biomarkers in Cardiovascular and Peripheral Vascular Disease: The State of the Art

Stefano de Franciscis,[1,2] Laurent Metzinger,[3] and Raffaele Serra[1,2]

Guest *et al. Genome Medicine* 2013, 5:17
http://genomemedicine.com/content/5/2/17

Genome Medicine

EDITORIAL

Proteomics: improving biomarker translation to modern medicine?

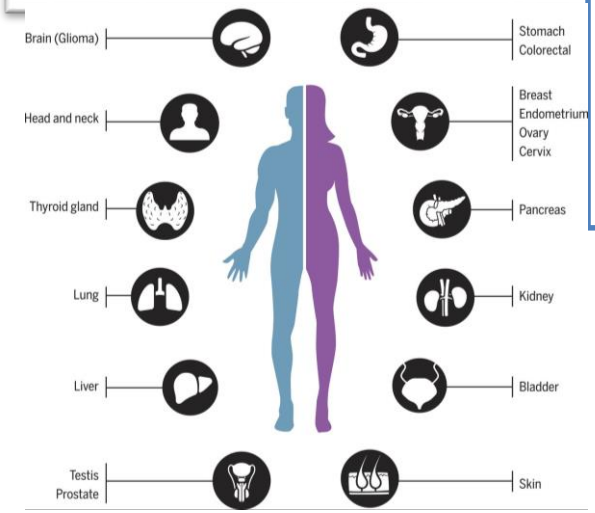
Paul C Guest[1*], Michael G Gottschalk[1] and Sabine Bahn[1,2]

RESEARCH ARTICLE

A pathology atlas of the human cancer transcriptome

Mathias Uhlen[1,2,3,*], Cheng Zhang[1], Sunjae Lee[1], Evelina Sjöstedt[1,4], Linn Fagerberg[1], Gholamreza Bidkhori[1], Rui Benfeitas[1],...
+ See all authors and affiliations

Science 18 Aug 2017:
Vol. 357, Issue 6352, eaan2507
DOI: 10.1126/science.aan2507



transcriptome of 17 major cancer types, based on a genome-wide transcriptomics analysis of ~8000 individual patients

Contents lists available at ScienceDirect

Translational Proteomics

journal homepage: http://www.elsevier.com/locate/trprot

Plasma proteomics to identify biomarkers – application to cardiovascular diseases

Hans Christian Beck[a,b,*], Martin Overgaard[a,b], Lars Melholt Rasmussen[a,b,c]

[a] Department of Biochemistry and Pharmacology, Odense University Hospital, University of Southern Denmark, Odense, Denmark
[b] Centre for Clinical Proteomics (CCP), Odense University Hospital, Odense, Denmark
[c] Center for Individualized Medicine in Arterial Diseases (CIMA), Odense University Hospital, Denmark

Ivyspring International Publisher

Theranostics

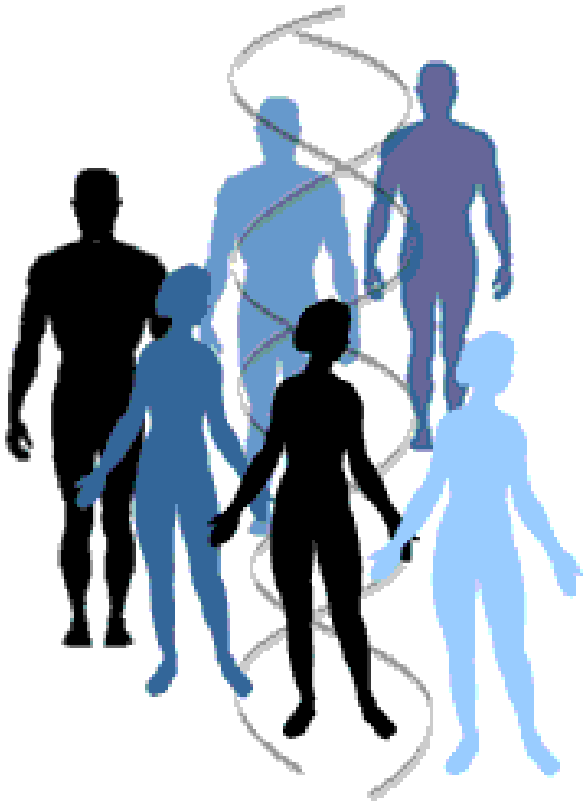
2017; 7(14): 3559-3572. doi: 10.7150/thno.20797

Review

Mass spectrometry-assisted gel-based proteomics in cancer biomarker discovery: approaches and application

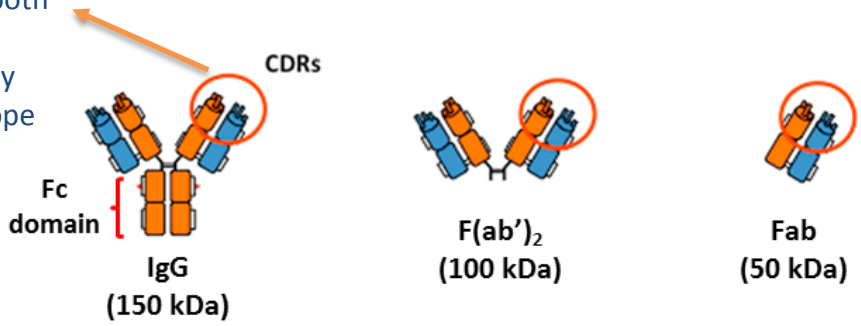
Rongrong Huang[1], Zhongsi Chen[1], Lei He[1], Nongyue He[1,4✉], Zhijiang Xi[2], Zhiyang Li[1,3], Yan Deng[1,4✉], Xin Zeng[5✉]

- Molecular and Cell Biology
- Biomarkers vs targets – imaging and therapy
- Transcriptomics and Proteomics
- **Antibody fragments**



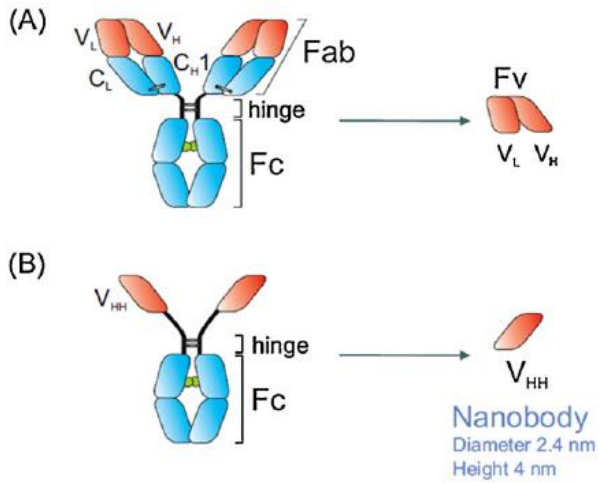
Antibody fragments

Complementarity-determining regions (CDR) are hypervariable domains in both light and heavy chains that determine specific antibody binding to an antigen epitope



Monovalent and monospecific recombinant fragments

Multivalent and monospecific recombinant fragments



Smallest available intact antigen binding fragments, only 15 kDa,

Alvarenga *et al.*, 2014

Production of antibody and antibody fragments

Full (natural) antibodies

Immunization + antibody isolation (hybridoma technology)

Antibody fragments

Libraries + antibody-fragment isolation & sequencing (biopanning technology)

Production of Ab fragments

→ **ANTIBODY FRAGMENT LIBRARY:**

- **naive library**
- **immune library**

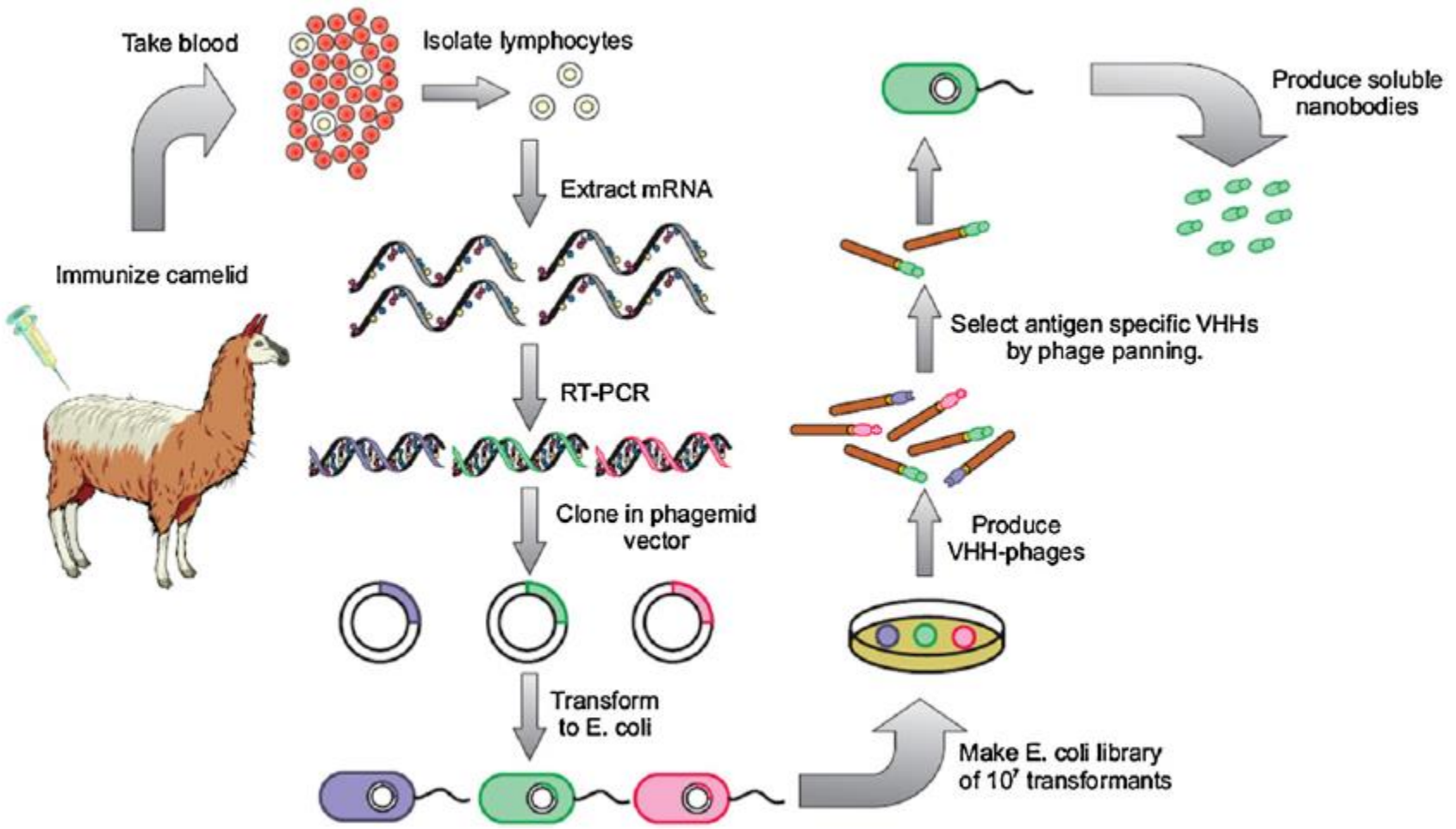
} **natural**

- **synthetic library**

- antibody libraries based solely on natural repertoires typically yield panels of hits with good levels of functionality but with limited diversity

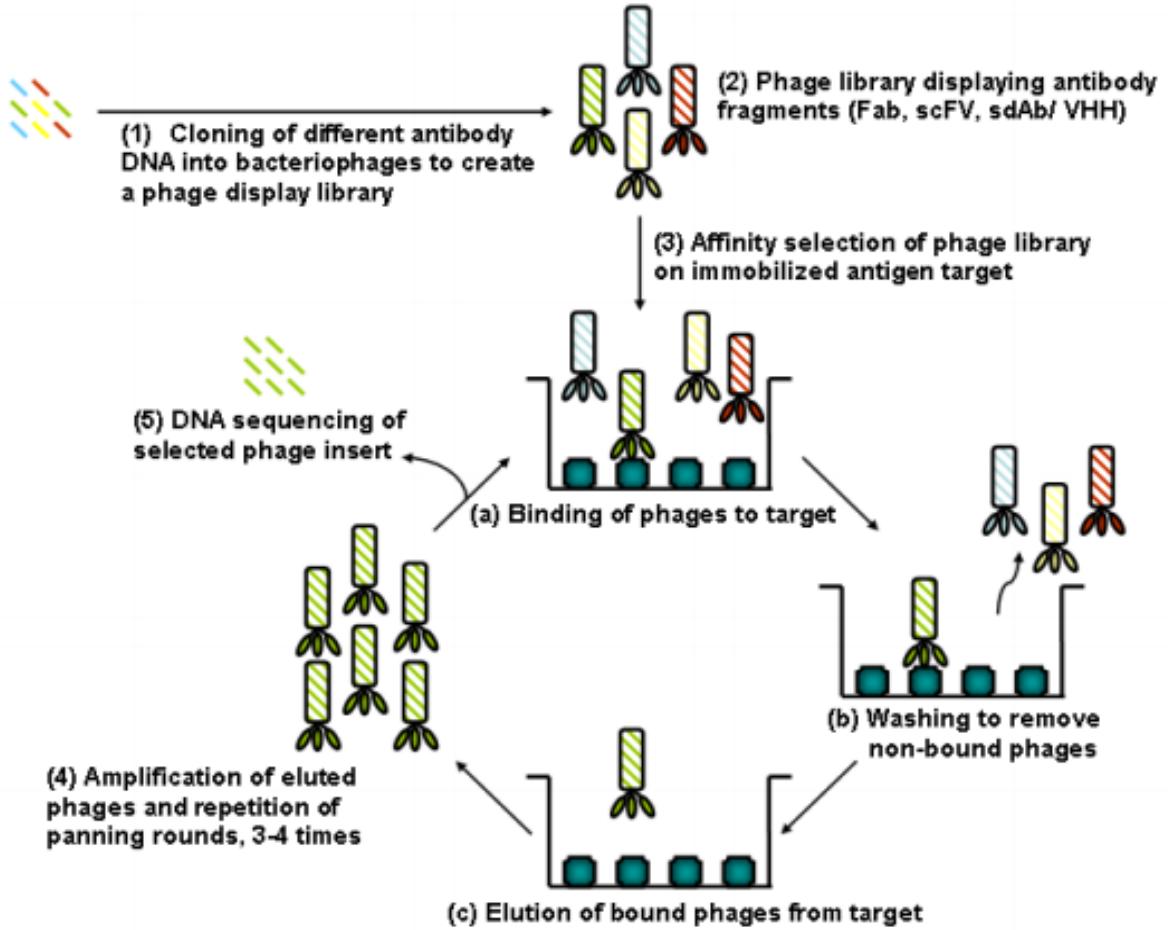
- synthetic libraries will yield far higher encoded diversity but will generally suffer from a high representation of antibodies with poor functionality.

Nanobody Generation



After immunization of a camelid, cDNA is generated from the animal's lymphocytes. This cDNA is introduced in a phage display system which is consequently used for panning on the antigen of interest. Positive clones are easily expressed or recloned into any desired format.

Selection of target-specific antibodies from an antibody phage display library



(1) DNA encoding different antibodies is cloned into the genome of a filamentous bacteriophage linked to one of the phage coat protein genes; (2) Each DNA variant is packed into a separate phage particle, and the antibody displayed on the phage coat protein; (3) Phage displaying an antibody that bind to the target analyte is selected using biopanning cycles of (a) binding, (b) washing, and (c) elution; (4) Eluted phages are reinfected into *E. coli* cells and amplified for further rounds of affinity selection; (5) Clones from the enriched library are characterized for binding properties using appropriate techniques (from Yun *et al.*, 2009).

To finish ….

May 2018 – 50th anniversary

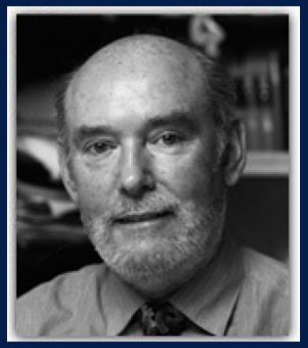
nature
International journal of science

NEWS AND VIEWS · 29 MAY 2018

Fifty years since DNA repair was linked to cancer

In 1968, a defect in DNA repair was found to underlie a disorder that makes people extremely sensitive to sunlight. This finding continues to influence research into the origins, diagnosis and treatment of cancer.

J Cleaver, "Defective Repair Replication of DNA in Xeroderma Pigmentosum" *Nature*, **218**, pp 652–656 (18 May 1968)



James E. Cleaver, PhD

Professor Emeritus, Departments of Dermatology and Pharmaceutical Chemistry, UCSF

Some people are born with exceptional sensitivity to sunlight. Fifty years ago, JCleaver reported a study of one such condition, and concluded that a failure of DNA repair was related to the extreme susceptibility of affected individuals to skin cancer. This was the first description of defective DNA repair in a genetically inherited disorder that makes people prone to cancer.


Thank you!
Any questions?
Filipa Mendes
fmendes@ctn.tecnico.ulisboa.pt