



UNIVERSITY of  
**BRADFORD**

Institute of Cancer  
Therapeutics

# Liquid Biopsy Preparation

Chris Sutton

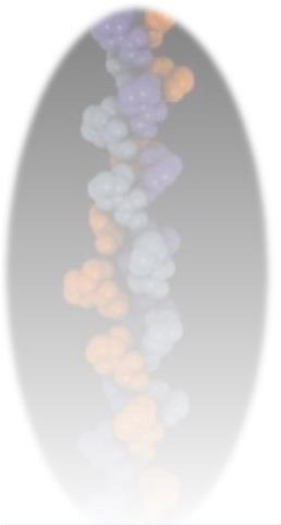
Institute of Cancer Therapeutics

25th September 2019



# Objectives

- P4 medicine
- Diagnostic biomarker roadmap
- Explore the different types of liquid biopsies - their application and diagnostic value



# Background

- Cancer
- Early detection
- Classical proteomics approach
- Diagnostics at the point of care
- Premise - early detection of cancer increases the chance of survival
- and reduces the therapeutic intervention

# P4 Medicine

- Predictive - genomic information, family history
- Preventive - avoid those lifestyle factors to which we are susceptible, e.g. smoking
- Personalised - understand individual variation
- Participatory - encourage individuals to take responsibility for their own health

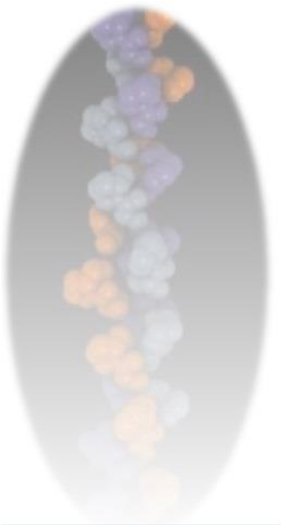
Hood and Friend, Nat Rev Clin Oncol. 8, 184-7 (2011)

Tian et al, J. Intern Med. 271, 111-121 (2012)

Hood and Tian, Genomics Proteomics Bioinformatics 10, 181-185 (2012)

# P4 Medicine

- Active surveillance/wellness profiling
- Healthy ageing
- Early intervention for disease reversibility - reduce impact of medical treatment
- Precision medicine
- Reduce healthcare costs



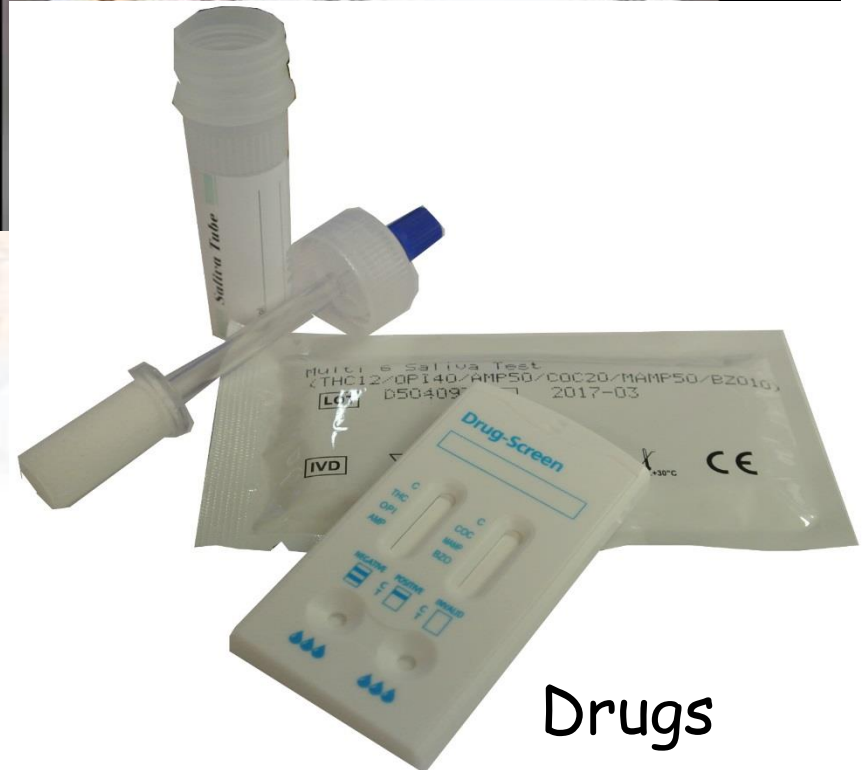
Pregnancy



Colorectal cancer



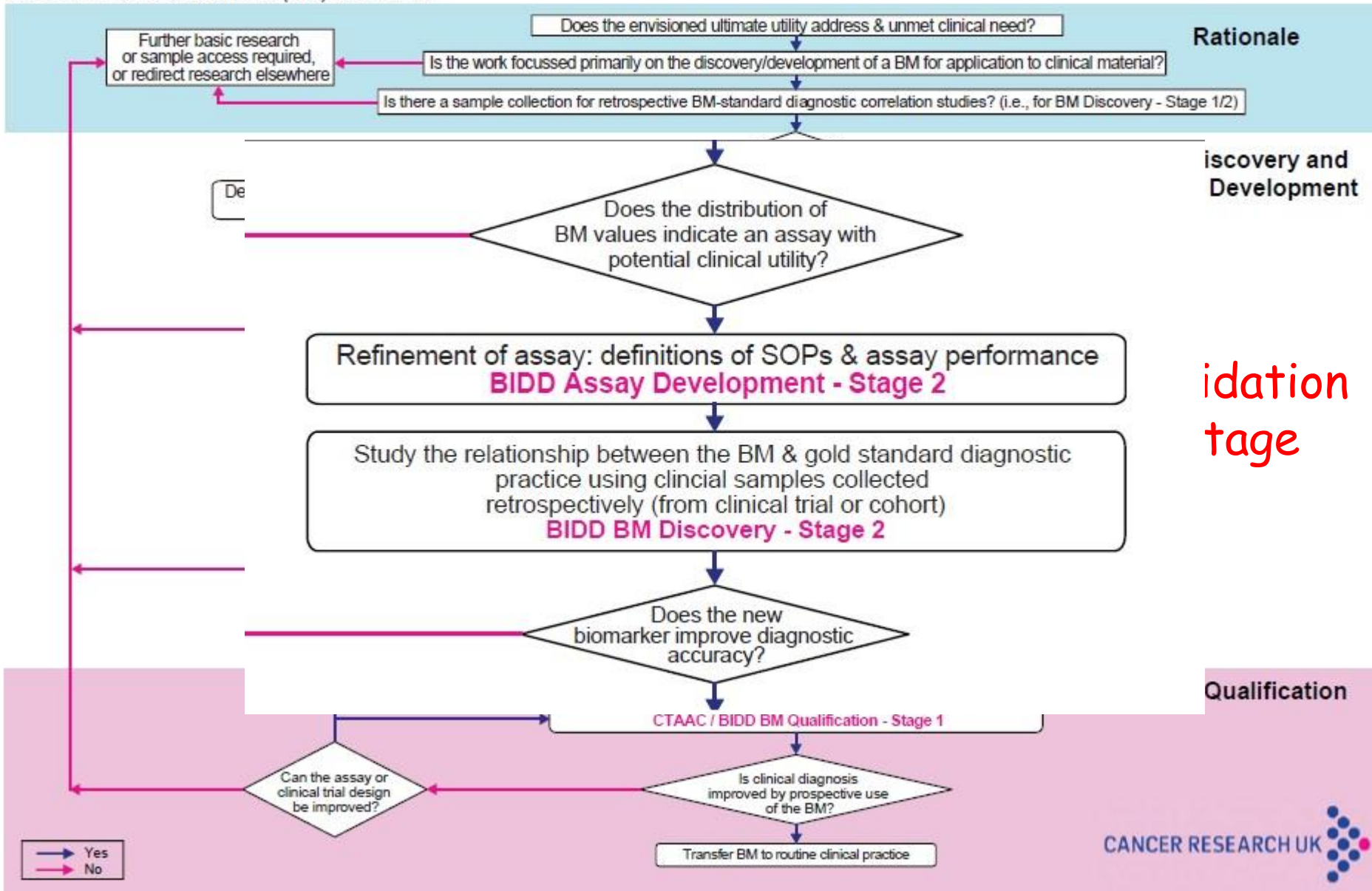
Metabolites - Dip.io



Drugs



## DIAGNOSTIC BIOMARKER (BM) ROADMAP



# Biological liquids

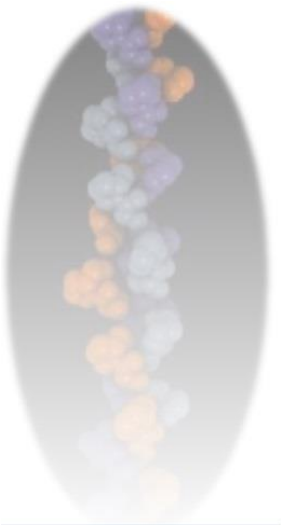
- Definition of a biofluid

biological fluid, can be excreted, secreted, obtained with a needle, or developed as a result of a pathological process

- Definition of a liquid biopsy

the sampling and analysis of non-solid biological tissue, primarily blood..... mainly used as a diagnostic and monitoring tool for diseases

- Composition - endogenous biomolecules, cells, exosomes, exogenous compounds (environmental, medicines, metabolites)

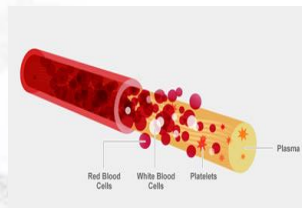
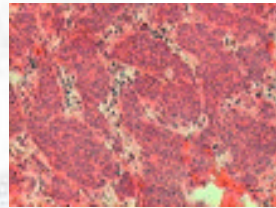




# Tissue vs biofluid (blood)

tissue

blood



	tissue	blood
disease proximity	+	-
biomarker concentration	+	-
biological complexity	-	-
sample accessibility	-	+
sample supply	-	+
sample processing	-	+
control	+	-

+

+

-

-

-

-

+

-

-

-

+

+

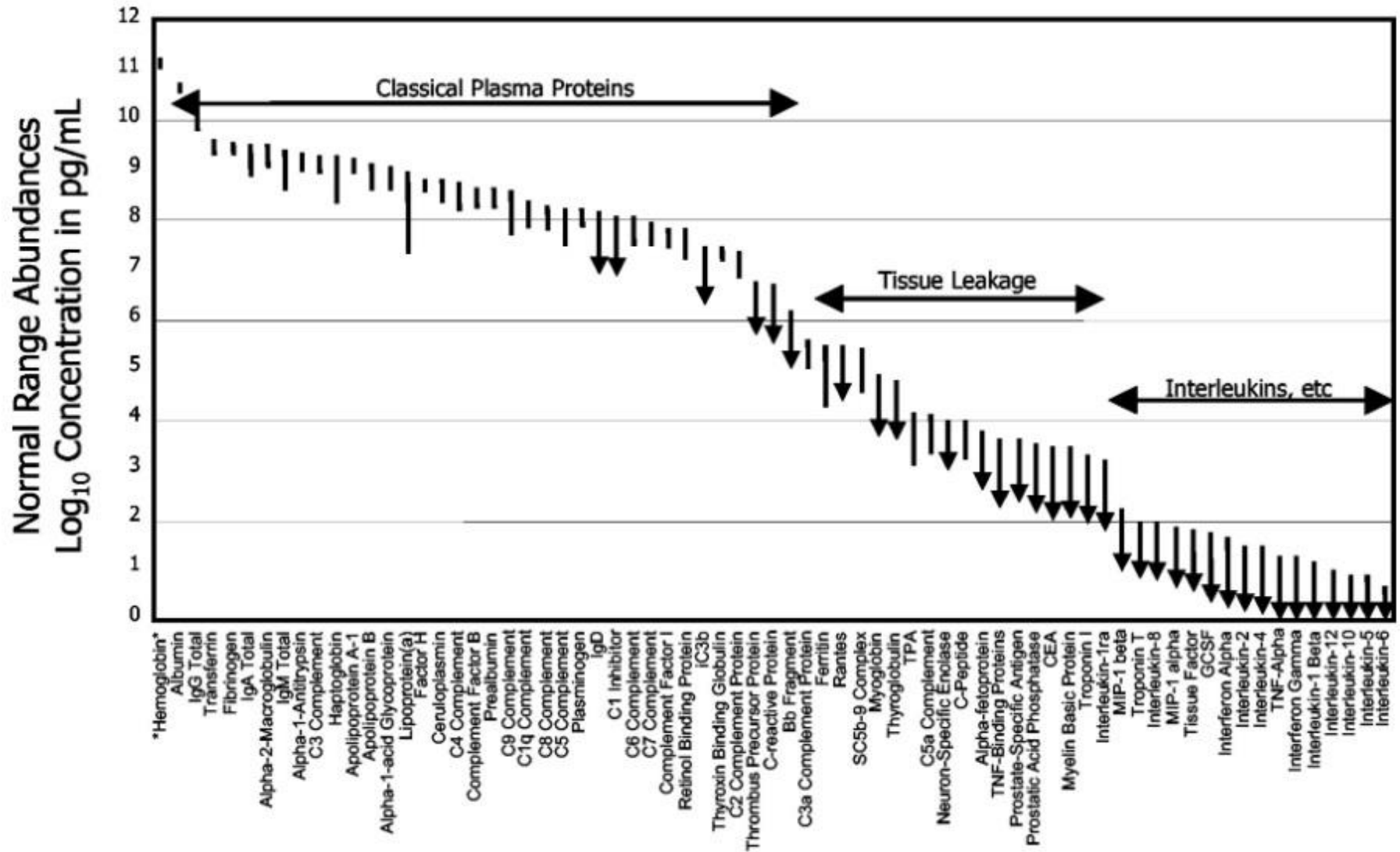
+

-

+ = good

- = poor

# Plasma Protein Abundance

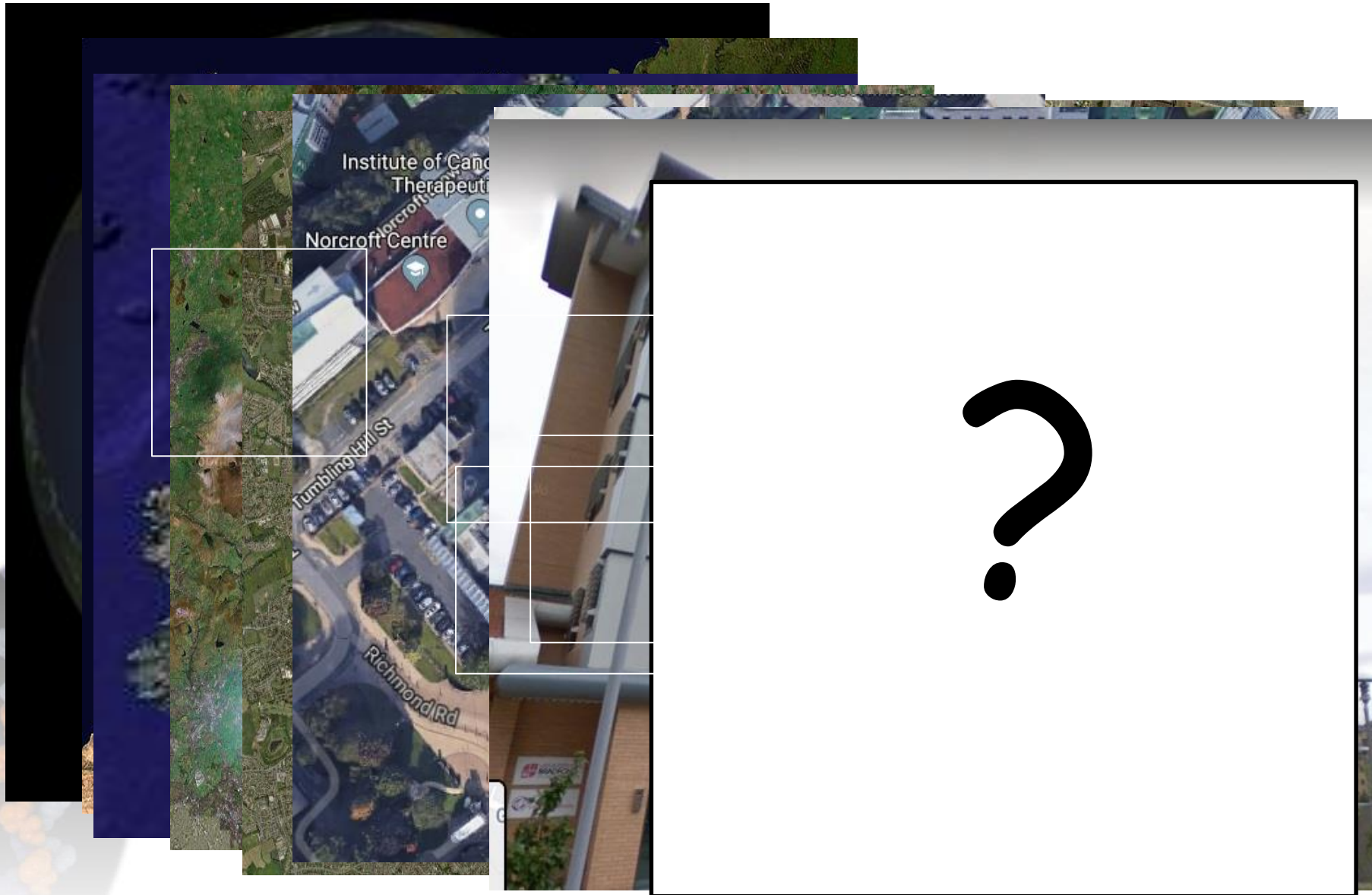


> 9000 proteins identified

<http://www.plasmaproteomedatabase.org/>

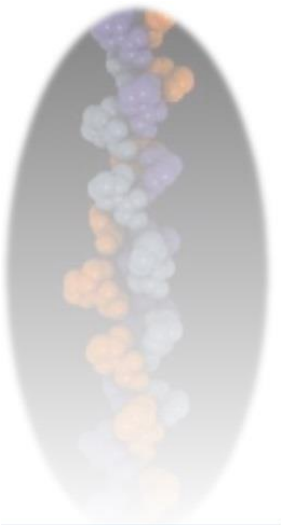
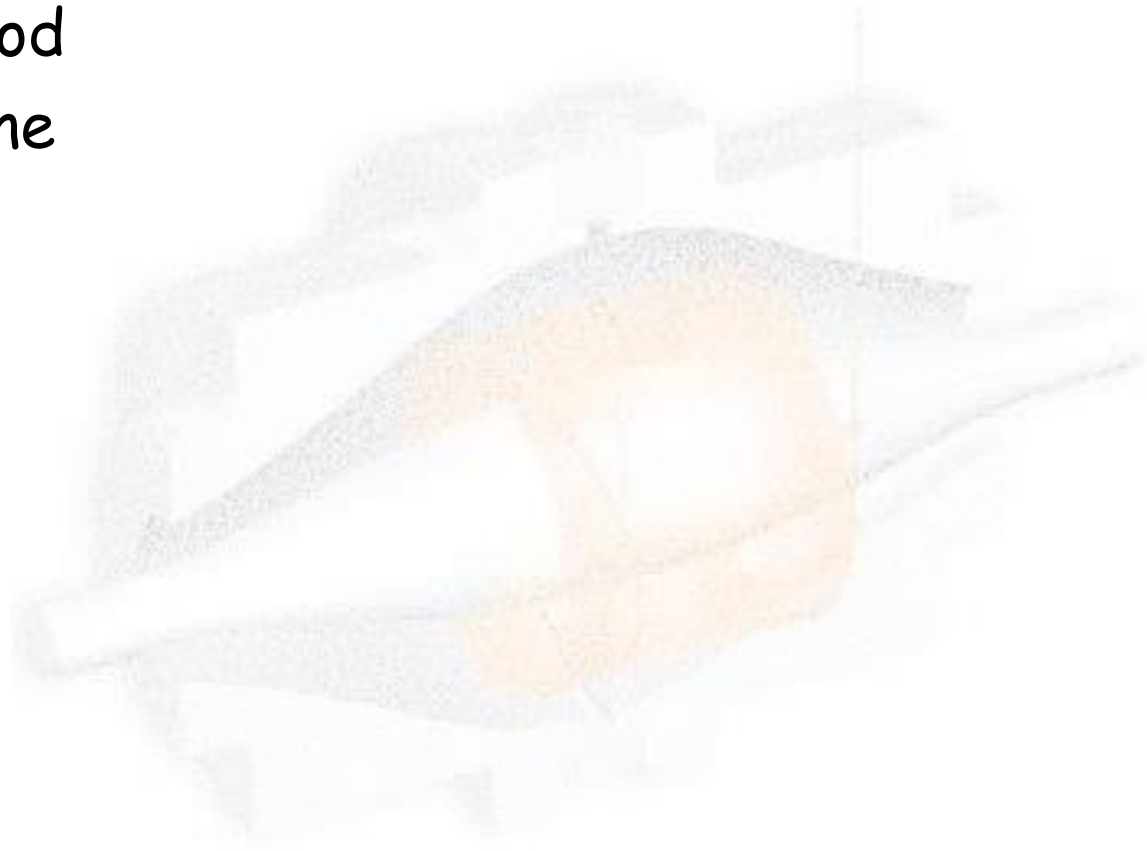
Anderson, N. L. *et al*, *Mol. & Cell. Proteomics* 3, 311-326 (2004)

# ICT, University of Bradford



# Types of biofluids

- blood
- urine

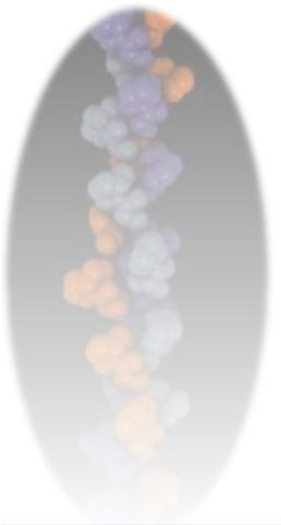


# Types of biofluids

- Blood
- Lymph
- Dermal Interstitial Fluid
- Urine
- Stool
- Cerebral Spinal Fluid
- Cervicovaginal Fluid
- Saliva
- Seminal Fluid
- Sweat
- Milk
- Gastric reflux
- Bone marrow
- Tears
- Breast Cyst Fluid
- Nipple Aspirate Fluid
- Ductal Lavage
- Gastric acid
- Synovial Fluid
- Sputum
- Bile
- Cerumen (earwax)
- Aqueous Humour
- Vitreous Humour
- Amniotic fluid

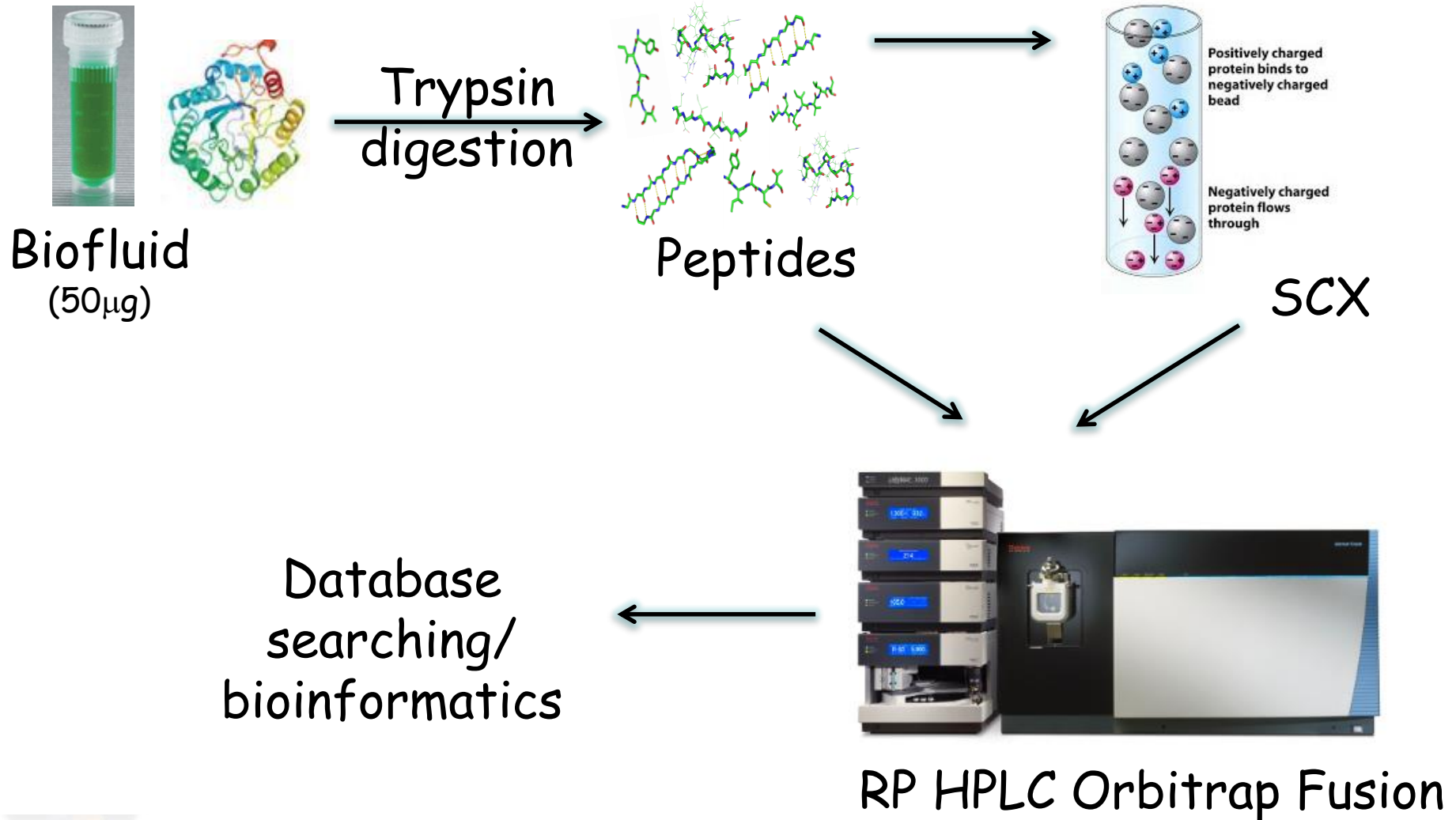
# Sample collection - standard operating procedures

- Time of the day, week, month
- Frequency of collection
- Diet/fasting
- Storage
- Volume
- Physical properties
- Patient/volunteer health status
- Accurate records (GCP)
- Reference/standards





# Proteomic approach



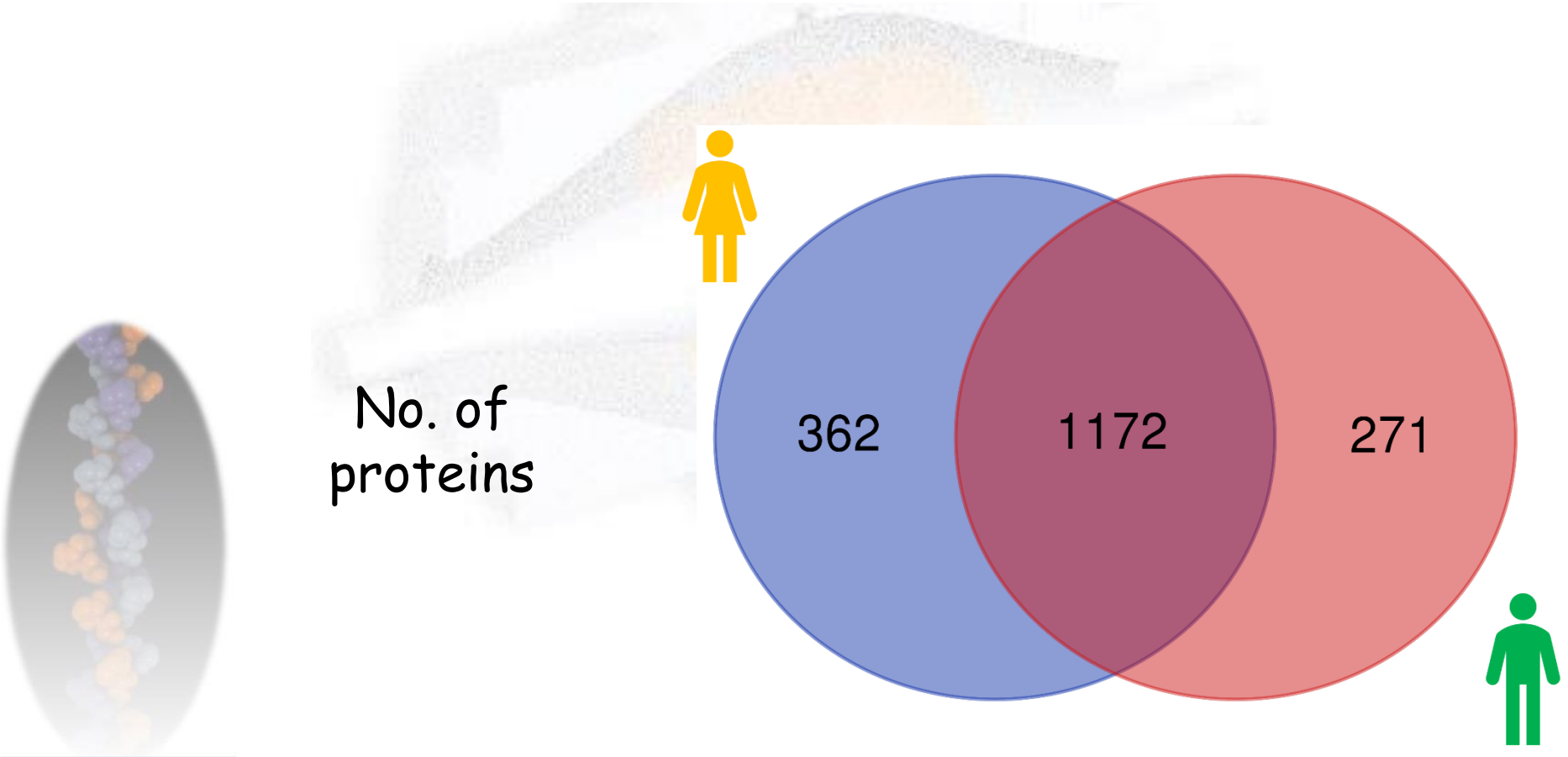
# saliva proteome

- Readily accessible, protein-rich , fasting
- Disease indicators - head and neck cancers
- Collection time important
- High protein concentration
- Minimum preparation
- Avoid centrifugation
- Viscous - high mucin composition



# saliva proteome

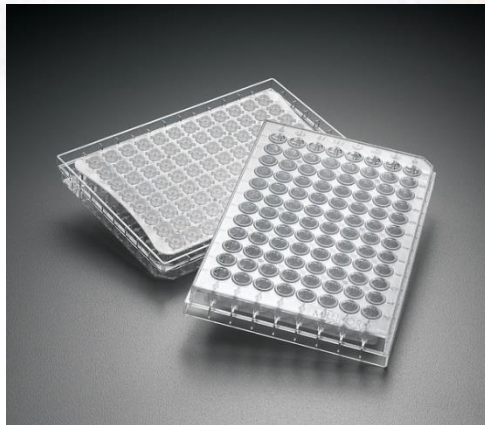
- 1400 to 1600 proteins identified
- MMPs 8 and 9, CYP2S1, CD44, vimentin, CA15-3, PIP



# urine proteome

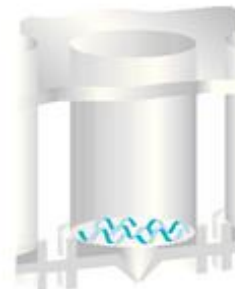
- Readily accessible, large volume, fasting
- Cancers - bladder, renal, prostate
- Dilute protein samples

## MultiScreen - concentrate proteins



1.

apply



2.

bind

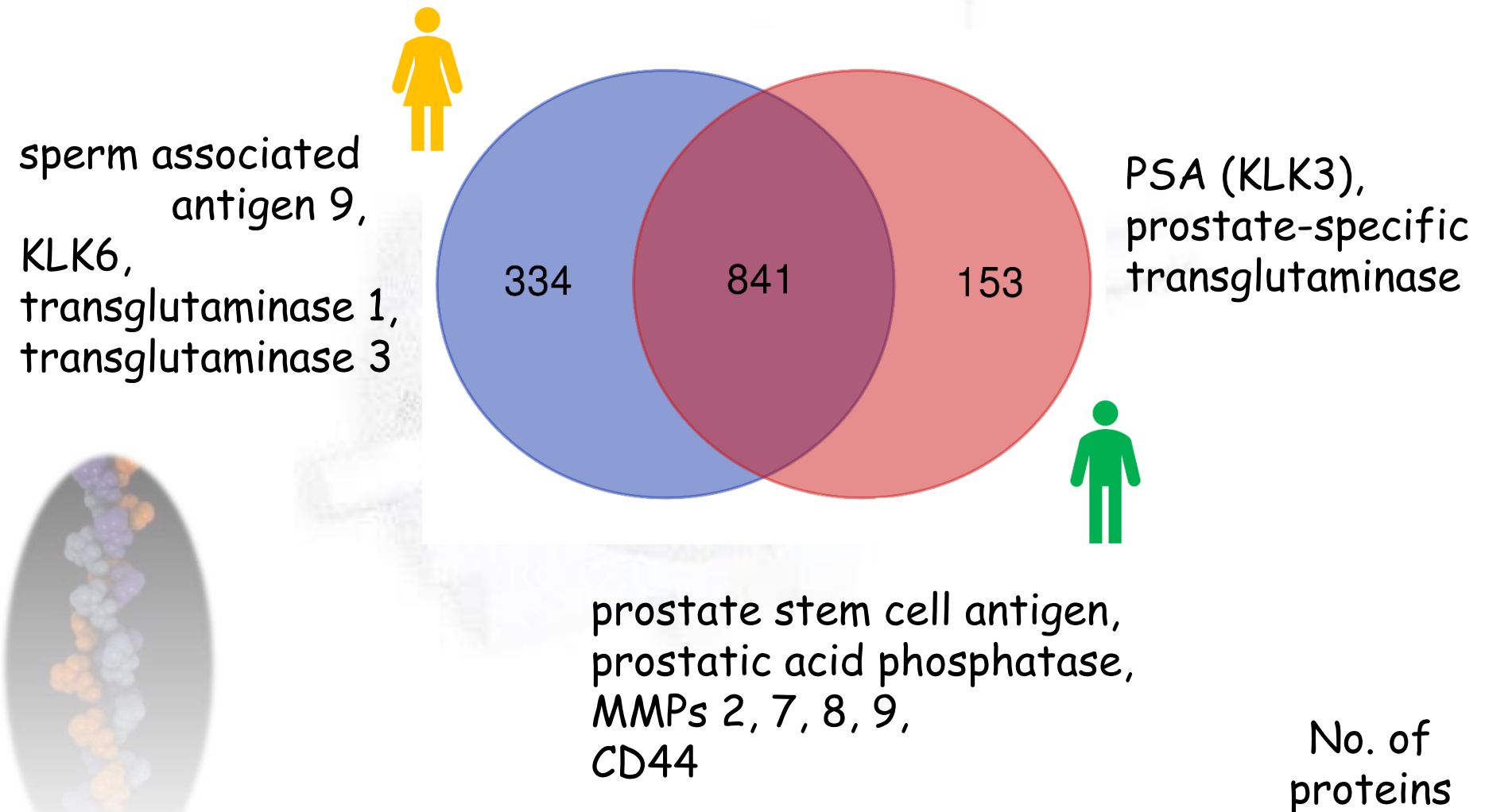


3.

digest

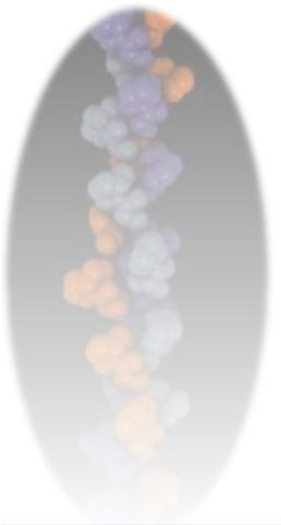
# urine proteome

- 600 to 800 proteins



# tear proteome

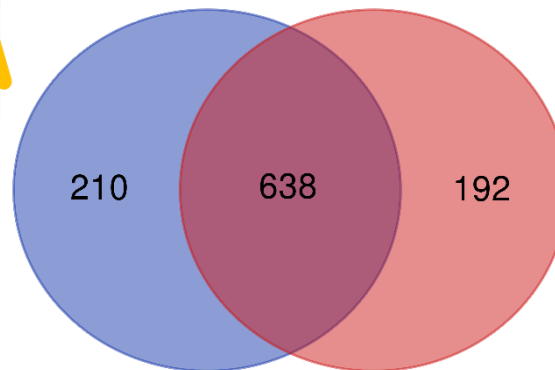
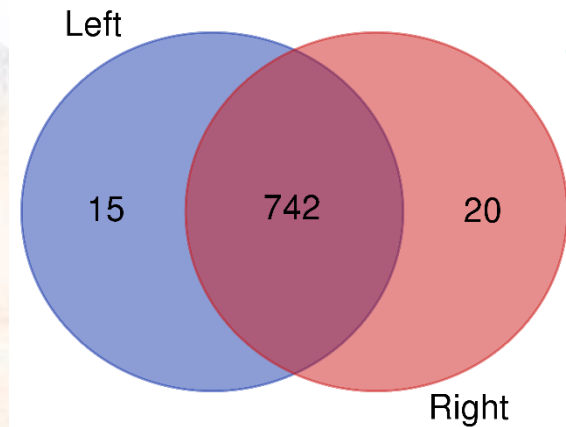
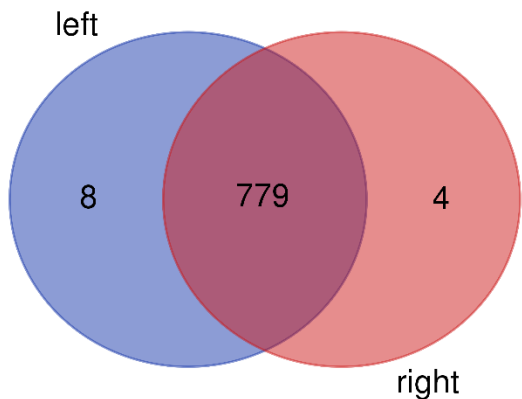
- Schirmer strips, *in-situ* trypsin digest, reference
- Disease indicators - breast cancer, colorectal cancer, eye-related cancers, non-cancer diseases





# tear proteome

- 700 to 800 proteins identified
- PIP, CA125, CA15-3

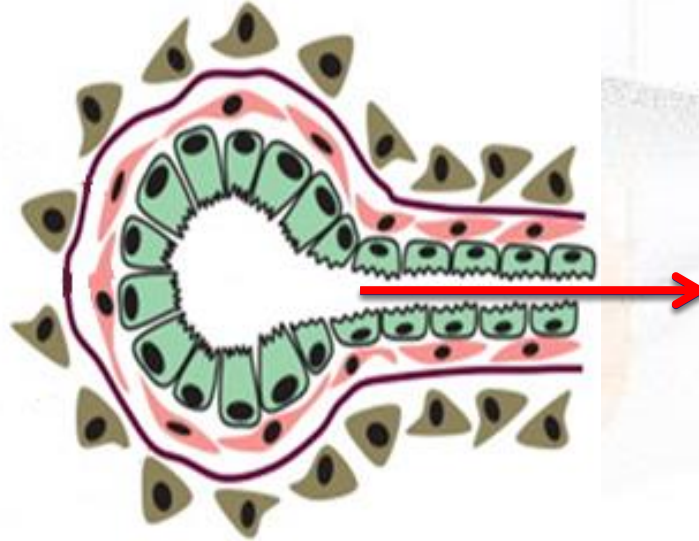


No. of  
proteins

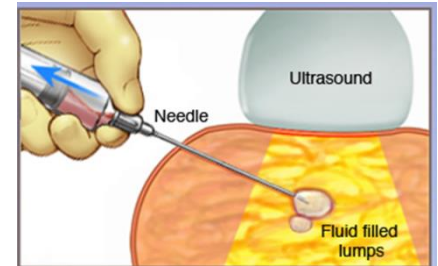
# Breast ductal fluids



Ductal Lavage



Nipple  
Aspirate Fluid  
(NAF)



Breast Cyst  
Fluid



Fine Needle  
Aspiration

# NAF proteome

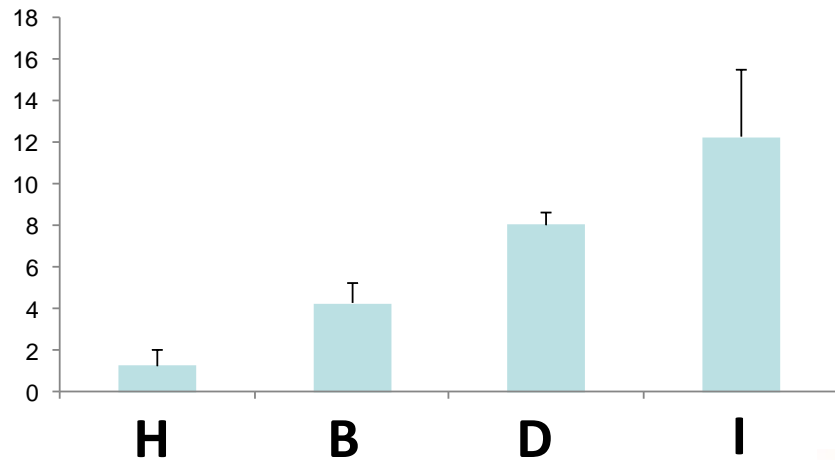
- rich in breast cancer associated proteins
- panel of related proteins differentiate non-cancer from cancer
- validation with a larger cohort
- BUT.....
- challenges of accessibility
- small volume

# NAF proteome

- >5000 proteins
- strong correlation between matched pairs
- variation between individuals
- rich in cell adhesion and stromal proteins, mitogenic factors and receptors
- unique proteins **not** detected in human plasma
- rich in Early Cancer Detection Network targets (<https://edrn.nci.nih.gov/>)

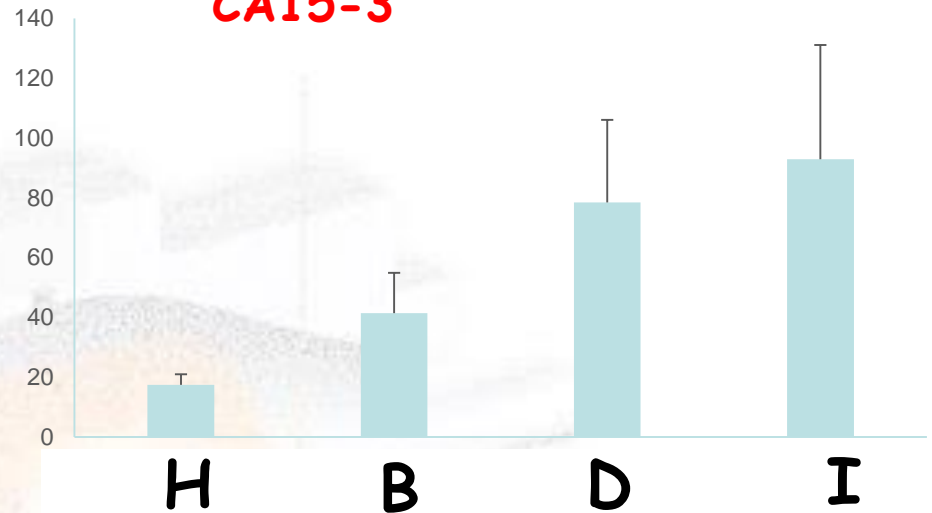
# Established markers

**CA125**



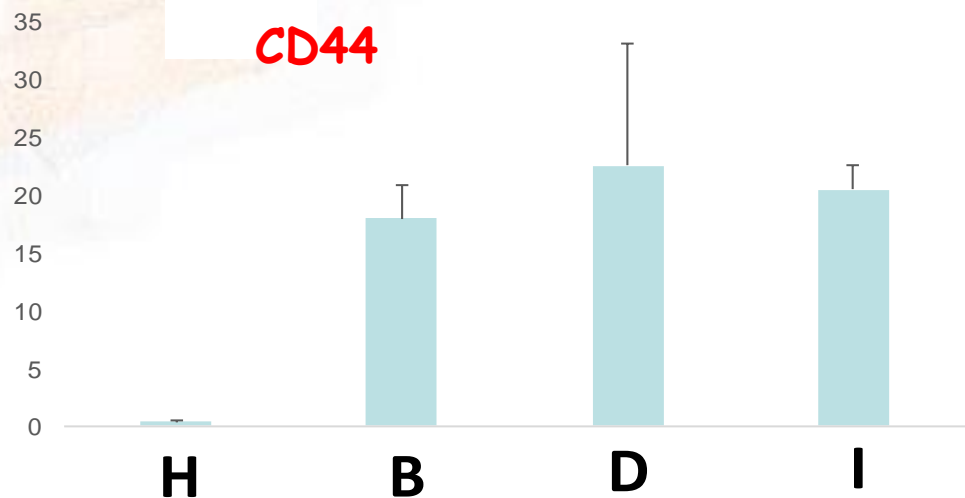
Mucin-16

**CA15-3**



Mucin 1

**CD44**



H = healthy

B = benign

D = DCIS

I = invasive

carcinoma

# Translation to a clinical diagnostic

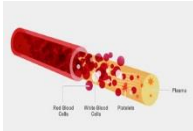




- Systemic vs proximal to the disease site
- Accessibility - invasive vs non-invasive
- Volume, marker concentration
- Sample collection
- Sample processing/preparation
- Reference sample



+ = low

+++ = high

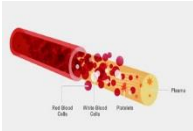




# Liquid biopsies

Liquid Biopsy	Blood	NAF	Urine	Tears	Saliva
					
Disease proximity	+				
Accessibility	Invasive				
Discomfort	+++				
Sample preparation	+++				
Reference Sample	No				
Biomarker concentration	+				
Patient-led collection	No				

+ = low

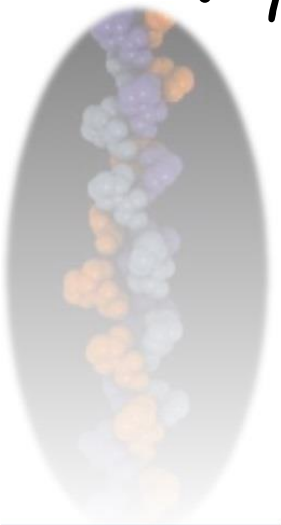
+++ = high

# Liquid biopsies

Liquid Biopsy	Blood	NAF	Urine	Tears	Saliva
					
Disease proximity	+	+++	++	+++	+++
Accessibility	Invasive	Non-invasive	Non-invasive	Non-invasive	Non-invasive
Discomfort	+++	++	+	+	+
Sample preparation	+++	+	+	+	+
Reference Sample	No	Yes	No	Yes	No
Biomarker concentration	+	+++	+	+++	+++
Patient-led collection	No	Yes	Yes	No	Yes

# Types of biofluids

- Lymph
- Stool
- Cerebral Spinal Fluid
- Cervicovaginal Fluid
- Seminal Fluid
- Sweat
- Milk
- Gastric acid
- Synovial Fluid
- Sputum
- Bile
- Cerumen (earwax)
- Aqueous & Vitreous Humour
- Amniotic fluid

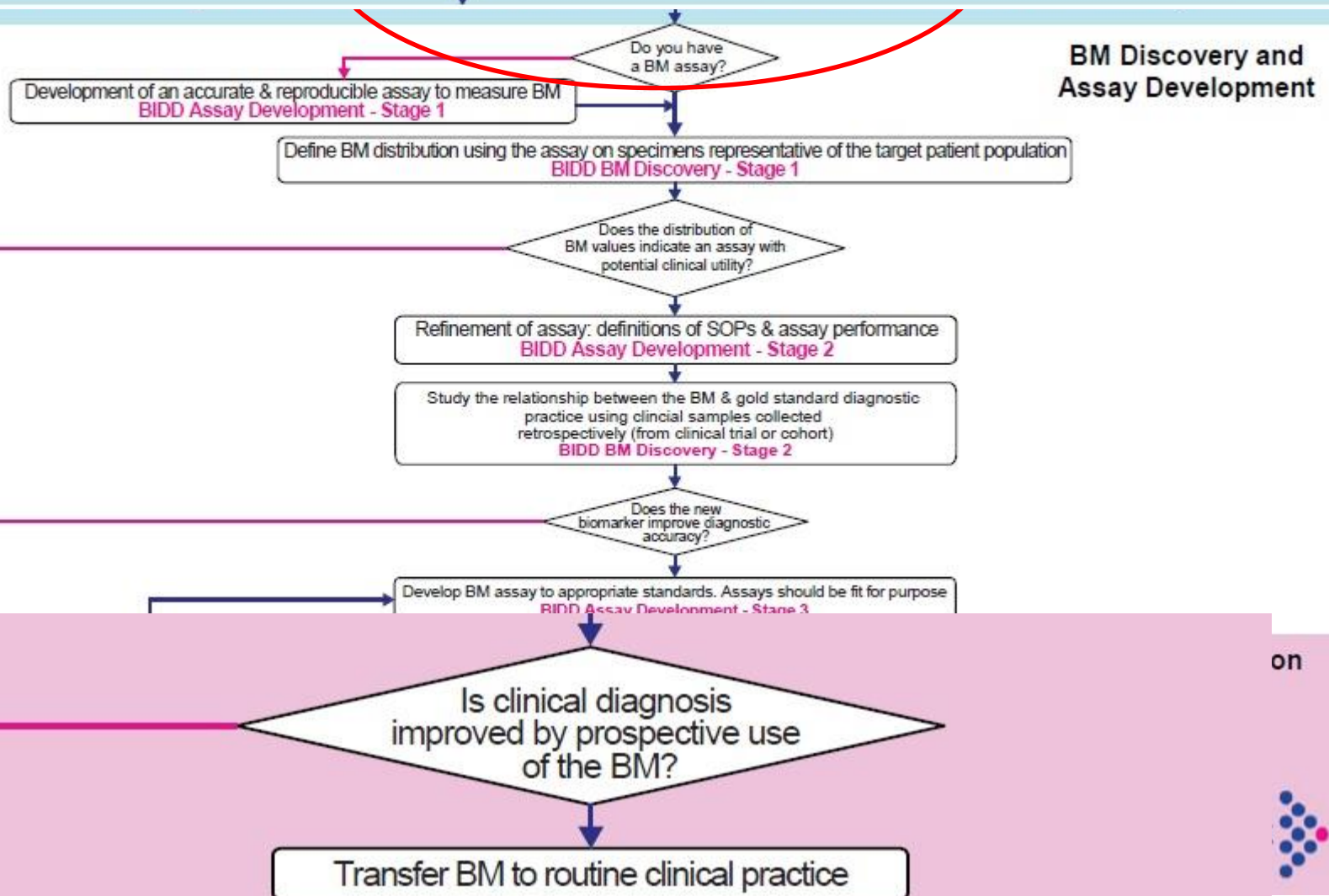


Does the envisioned ultimate utility address & unmet clinical need?

## Rationale

Is the work focussed primarily on the discovery/development of a BM for application to clinical material?

Is there a sample collection for retrospective BM-standard diagnostic correlation studies? (i.e., for BM Discovery - Stage 1/2)



# Purpose

- What is the objective of the test?
  - Diagnosis, prognosis, prediction, prevention, early detection
- Where is the sample collected?
  - Home, community centre, medical centre, mobile units, clinical centre
- Where will the sample be tested?
  - Home, community centre, medical centre, mobile units, analytical centre - single or multiple
- Frequency of collection and testing?
- Meaning of the result?
  - Harm
- It is important to have a good idea how the test or product for testing will be implemented before extensive research is undertaken

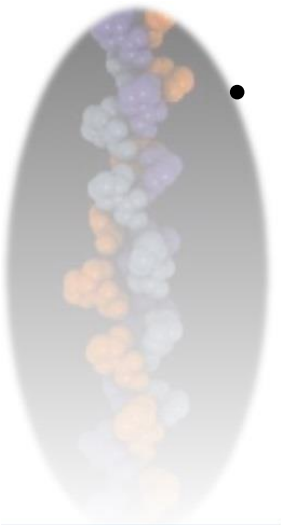
# The validation team

- Clinicians/research nurses
- Patient advocate groups/stakeholders
- Patients and healthy volunteers
- Biobankers
- Researchers
- Biostatisticians
- Psychologists
- **Regulatory authorities**



# Conclusions

- Understanding normal variation
  - strict SOPs
- Personalised profiles
- Longitudinal studies
- Correlate with disease



# Biological mass spectrometry

Nobel Prize in Chemistry in 2002



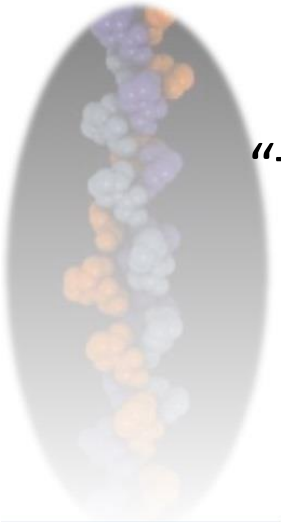
John Fenn – 1917 - 2010

Koichi Tanaka – b. 1959

“for the development of methods for identification and structure analysis of biological macromolecules”

ESI MS 1984

MALDI MS 1987



# Acknowledgements

## Institute of Cancer Therapeutics

Sadr ul-Shaheed  
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## Bradford Royal Infirmary

Rick Linforth  
Mohammed Salhab  
Catherine Tait  
Kathryn Rigby  
Sue Hignett

## Patients and volunteers



*Dedicated to Yorkshire for ninety years*



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