# SAC review 'Omic' technologies: genomics, transcriptomics, proteomics and metabolomics

Authors Richard P Horgan / Louise C Kenny

## Key content:

- 'Omic' technologies are primarily aimed at the universal detection of genes (genomics), mRNA (transcriptomics), proteins (proteomics) and metabolites (metabolomics) in a specific biological sample.
- Omic technologies have a broad range of applications.
- Genomic and transcriptomic research has progressed due to advances in microarray technology.
- Mass spectrometry is the most common method used for the detection of analytes in proteomic and metabolomic research.
- Data analysis is complex as a huge amount of data is generated and statistician and bioinformatician involvement in the process is essential.
- Much of the omic research in obstetrics and gynaecology has concentrated on using the technology to develop screening tests for gynaecological cancers and obstetric complications.

## Learning objectives:

- To learn about the omic disciplines and to be clear about the terminology in use.
- To appreciate that the omic experiment is a complex process requiring thorough study design and sample preparation, involving a number of technologies and requiring extensive data analysis.
- To gain a brief overview of the application of these approaches to obstetrics and gynaecology.

## Ethical issues:

- The use of genetic testing, particularly in the area of predisposition genes.
- Ethical issues surrounding the storage and use of samples in biobanks and the associated clinical data.
- The limited access to the technology involved in these techniques.

Keywords genomics / metabolomics / proteomics / technology / systems biology / transcriptomics

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Author details

**Richard P Horgan** MRCOG MRCPI
**Clinical Research Fellow and Specialist Registrar in Obstetrics and Gynaecology**
The Anu Research Centre, Department of Obstetrics and Gynaecology, University College Cork, Cork University Maternity Hospital, Cork, Republic of Ireland
Email: Richard.horgan@ucc.ie
(corresponding author)

**Louise C Kenny** MRCOG PhD
**Consultant Obstetrician Gynaecologist and Professor of Obstetrics**
The Anu Research Centre, Department of Obstetrics and Gynaecology, University College Cork, Cork, Republic of Ireland

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## Introduction

'Omic' technologies adopt a holistic view of the molecules that make up a cell, tissue or organism. They are aimed primarily at the universal detection of genes (genomics), mRNA (transcriptomics), proteins (proteomics) and metabolites (metabolomics) in a specific biological sample in a non-targeted and non-biased manner. This can also be referred to as high-dimensional biology; the integration of these techniques is called systems biology (**Figure 1**) (see **Box 1** for a list of definitions).[1,2] The basic aspect of these approaches is that a complex system can be understood more thoroughly if considered as a whole. Systems biology and omics experiments differ from traditional studies, which are largely hypothesis-driven or reductionist. By contrast, systems biology experiments are hypothesis-generating, using holistic approaches where no hypothesis is known or prescribed but all data are acquired and analysed to define a hypothesis that can be further tested.[3]

These strategies have many applications and much potential. Omic technology can be applied not only for the greater understanding of normal physiological processes but also in disease processes where they play a role in screening, diagnosis and prognosis as well as aiding our understanding of the aetiology of diseases. Omic strategies lend themselves to biomarker discovery as they investigate multiple molecules simultaneously. Omic investigation is increasingly being used in drug discovery and assessment of their toxicity and efficacy.[4,5] Pharmacogenomics — the intersection of genomics and pharmacology — is the study of the role of inheritance in individual variation in drug response which can potentially be used to individualise and optimise drug therapy.[6] Pharmacogenomics is especially important for oncology, as severe systemic toxicity and unpredictable efficacy are hallmarks of cancer therapies.[7] Systems approaches to conditions such as cancer, cardiovascular disease and obesity give the opportunity to facilitate greatly the success of selecting novel targets for treatments and drug development. In the future, systems biology may enable us to develop new approaches that will be predictive, preventive and personalised.

Research in obstetrics and gynaecology is currently taking advantage of these possibilities. The aim of this review is to provide an overview of the omic experiment and technologies and their potential application to women's health research.

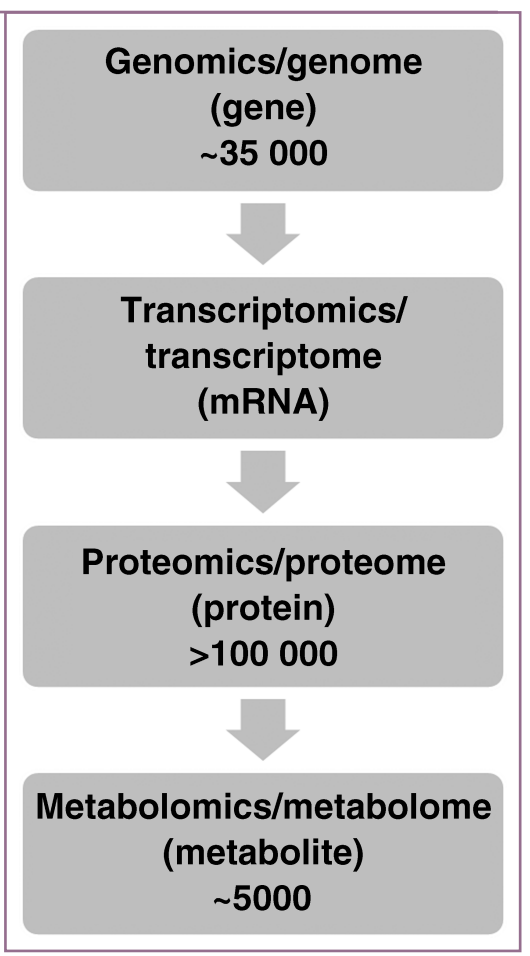


**Figure 1**
**Omic sciences and their interaction. The flow of biological information is bidirectional. The numbers are the approximate quantity at each functional level**

## High-dimensional biology

Genomics is the systematic study of an organism's genome. The genome is the total DNA of a cell or organism. The human genome contains 3.2 billion bases[8] and an estimated 30 000–40 000 protein-coding genes. Traditionally, genes have been analysed individually but microarray technology has advanced substantially in recent years. DNA microarrays measure differences in DNA sequence between individuals and the expression of thousands of genes can be analysed simultaneously. They can reveal abnormalities such as chromosomal insertions and deletions or abnormal chromosomal numbers in a process called comparative genomic hybridisation. The most common variations in DNA sequences between people are single nucleotide polymorphisms (SNPs), in which one nucleotide is substituted for another; this may have functional significance if the change results in a codon for a different amino acid. They are of particular interest when linked with diseases with a genetic determination. Single nucleotide polymorphism profiling also has a role in pharmacogenomics in exploring individual patient responses to drugs.

The transcriptome is the total mRNA in a cell or organism and the template for protein synthesis in a process called translation. The transcriptome reflects the genes that are actively expressed at any given moment. Gene expression microarrays measure packaged mRNA (mRNA with the introns spliced out) as a summary of gene activity.

While advances in microarray technology have resulted in progress in genomics and transcriptomics (and the resultant literature), it is important to

highlight some limitations. Specifically, gene expression microarrays measure changes in mRNA abundance, not protein, and thus there is a lack of consensus around the interpretation of microarray data.

The proteome is defined as the set of all expressed proteins in a cell, tissue or organism.[9] Proteomics aims to characterise information flow within the cell and the organism, through protein pathways and networks,[10] with the eventual aim of understanding the functional relevance of proteins.[11] While we can gain much information from proteomic investigation, it is complicated by its domain size (>100 000 proteins) and the inability to detect accurately low-abundance proteins. The proteome is a dynamic reflection of both genes and the environment and is thought to hold special promise for biomarker discovery because proteins are most likely to be ubiquitously affected in disease and disease response.[12] This is reflected in the many protein disease biomarkers already available (e.g. CA125 and alpha-fetoprotein).

Metabolomics can generally be defined as the study of global metabolite profiles in a system (cell, tissue or organism) under a given set of conditions.[13] Metabolomics has a number of theoretical advantages over the other omic approaches. The metabolome is the final downstream product of gene transcription and, therefore, changes in the metabolome are amplified relative to changes in the transcriptome and the proteome.[14] Additionally, as the downstream product, the metabolome is closest to the phenotype of the biological system studied. Although the metabolome contains the smallest domain (~5000 metabolites), it is more diverse, containing many different biological molecules, making it more physically and chemically complex than the other 'omes'.

**Box 1**
**Omics-related definitions**

**Systems biology**

Biological research focusing on the systematic study of complex interactions in biological systems using integration models. The ultimate aim is to understand whole systems, e.g. complex cellular pathways, by studying the effect of altered external factors on the genome, transcriptome, proteome and metabolome simultaneously

**Genomics**

The study of the structure, function and expression of all the genes in an organism

**Genome**

The total DNA of a cell or organism

**Polymorphism**

Variations in DNA at a specific site

**Transcriptomics**

The study of the mRNA within a cell or organism

**Transcriptome**

The total mRNA in a cell or organism

**Proteomics**

The large-scale study of proteins, including their structure and function, within a cell/system/organism. A name coined as an analogy with the genome

**Proteome**

The set of all expressed proteins in a cell, tissue or organism

**Metabolomics**

The study of global metabolite profiles in a system (cell, tissue or organism) under a given set of conditions

**Metabolome**

The total quantitative collection of low molecular weight compounds (metabolites) present in a cell or organism that participate in metabolic reactions. It also includes those metabolites taken in from external environments or symbiotic relationships

**Metabonomics**

A measure of the fingerprint of biochemical perturbations caused by disease, drugs and toxins; some would say that metabonomics and metabolomics are the same and the terms are occasionally used interchangeably

**Mass spectrometry**

An analytical technique measuring the mass-to-charge (*m/z*) ratio of charged particles

## The omic experiment

### Experimental design

Areas that require careful thought include:

- the use of suitable biological samples: the choice of the sample type to investigate is dependent on the objectives of the experiment and the availability of the sample
- the technical/analytical variation: this is the relative standard deviation of a specific experimental technique and this needs to be validated
- the biological variation: in humans this can be very large, therefore it is important to collect meta-data and, in certain study designs, to match comparison groups strongly, to ensure that changes are not due to confounding factors.

A number of factors determine the sample size, but it has to be such that valid statistical conclusions can be made. Large numbers of biological specimens are required to generate the greatest predictive power, therefore, the collection of clinical data and biological samples in large biobanks is essential. The SCOPE (SCreening fOr Pregnancy Endpoints) study is an example of such a pregnancy-related biobank (see below and **Websites** section).

The purpose of the investigation governs what type of sample should be used. In terms of biomarker discovery, plasma is the obvious candidate, as the ultimate goal is usually a blood test.[15] However, biomarkers are likely to occur in low relative abundance and be massively diluted in the circulation.[15] Rigorous reproducible standard operating procedures are essential to ensure that samples are collected, stored and transported in an identical manner.

### Analytical techniques

Sample preparation for omic experiments is imperative and should be standardised and reproducible.

DNA microarrays have many modes of use, of which expression profiling is the dominant mode.

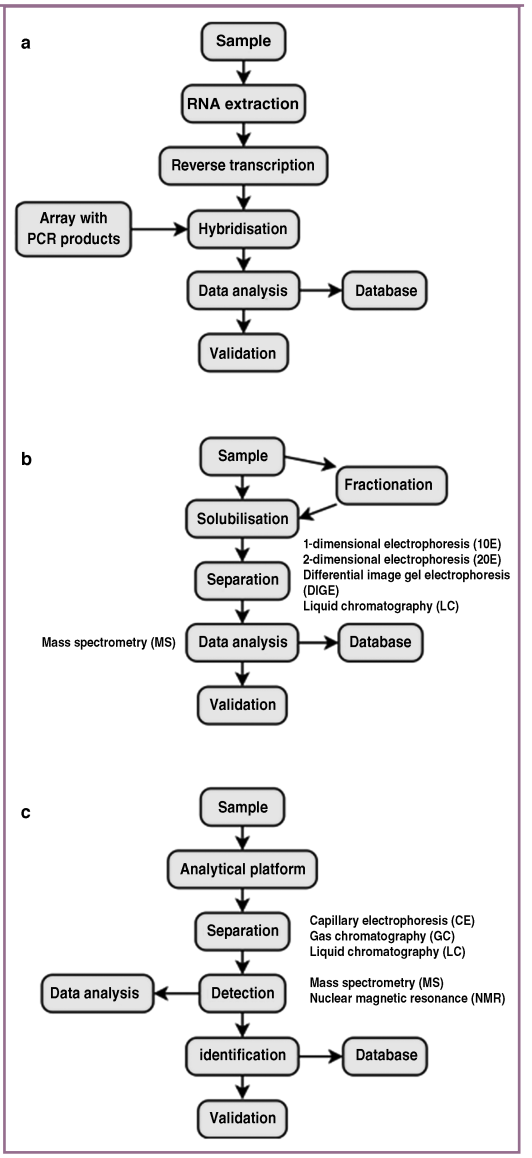


**Figure 2**
**Analytical techniques**
**(a) Process flow for gene expression microarray experiment**
**(b) Simplified schematic workflow for proteomics experiments**
**(c) Simplified schematic workflow for metabolomics experiments**
**PCR = polymerase chain reaction; RNA = ribonucleic acid**

In gene expression microarray, the probe used to assess the amount of mRNA can be either complementary DNA (cDNA) or an oligonucleotide. The probe is amplified by polymerase chain reaction (PCR) and spotted onto an array which is then immobilised on a solid support (glass slide).[16] In the experiment RNA is extracted from the samples and, by the process of reverse transcription and addition of fluorescent dyes, labelled cDNA is formed (both normal/control and diseased/case) which is then combined and hybridised with the microarray slide. These microarray glass slides are often called chips. The amount of the two dyes is representative of the gene expression in the different samples. An ultraviolet laser is used to scan the slide which detects the amount of fluorescent signal for each gene. This image is then analysed (Figure 2a).

The principal considerations in a proteomic experiment are protein concentration, sample purification and protein digestion, plus affinity capture and sample fractionation (using gel-based or chromatography techniques) to reduce the complexity of the target fluid. These steps require different emphasis, depending on the biological sample being used (Figure 2b). For example, urine provides different analytical challenges to plasma, including the need for preparation techniques such as ultrafiltration and precipitation, which are necessary to remove salts and concentrate urinary proteins. Different problems can arise for each fluid, such as inter-individual variation in urinary protein concentration in diseases such as pre-eclampsia.

The degree of sample preparation required for metabolomic experiments depends on the type of sample. Metabolomic samples also require fractionation (usually chromatography or electrophoresis) prior to analysis. These fractionation techniques use the different chemical/physical properties of molecules and enable the separation of proteins/peptides/metabolites in liquid or gas phase (Figure 2c).

Mass spectrometry is the most commonly used method for the investigation/identification of analytes in both proteomics and metabolomics. Ions are created from neutral proteins, peptides or metabolites, which are then separated according to their mass-to-charge ratio ($m/z$) and detected to create a mass spectrum, which is characteristic of the molecular mass and/or structure. Figure 3 shows a general flow chart for mass spectrometry. Each analytical technique offers different advantages and limitations in terms of instrument sensitivity, resolution, mass accuracy, dynamic range and throughput: a number of techniques are needed to analyse the entire proteome or metabolome. In proteomics, several ionisation techniques have improved the way proteins are characterised and sequenced, in particular, electrospray ionisation (ESI) and matrix-assisted laser desorption/

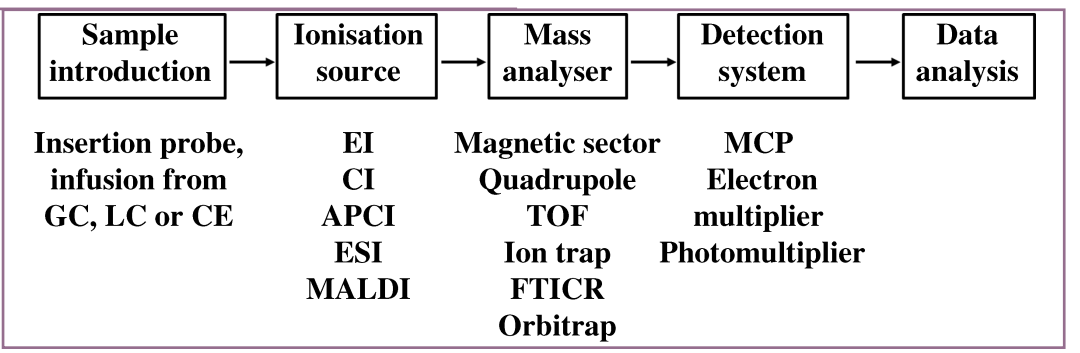


**Figure 3**
**General flow chart for mass spectrometry. APCI = atmospheric pressure chemical ionisation; CE = capillary electrophoresis; CI = chemical ionisation; EI = electron impact ionisation; ESI = electrospray ionisation; FTICR = Fourier transform ion cyclotron resonance; GC = gas chromatography; LC = liquid chromatography; MALDI = matrix-assisted laser desorption/ ionisation; MCP = microchannel plate; TOF = time of flight**

ionisation (MALDI), while questions have been raised regarding reproducibility, accuracy, mass range and dynamic range of another technique, surface-enhanced laser desorption/ionisation (SELDI). Quantitative analysis has been improved by several techniques, including differential image gel electrophoresis (DIGE), which uses fluorescent tags in gel-based techniques, and strategies of isotopically coded affinity tag (ICAT) labelling have been used along with tandem mass spectrometry. In metabolomics, other analytical platforms, including nuclear magnetic resonance (NMR) spectroscopy and infra-red spectroscopy, have also been used for metabolite identification but this is still an area that requires significant improvement.[17] Nuclear magnetic resonance spectroscopy is disadvantaged by its poor sensitivity and can only reliably detect and quantify metabolites present in relatively high concentrations. Spectroscopy also has limited sensitivity and poor ability to identify metabolites in complex samples.

It is important to realise that not all omic techniques can be interpreted equally and that each analytical technique offers different advantages and limitations. There often has to be a trade-off between the technique and the experimental objectives.

### Data analysis

Given the enormous amount of data generated in these studies, sophisticated bioinformatics and dedicated statisticians are fundamental.

In genomics and transcriptomics microarray data analysis can prove difficult. Huge numbers of variables (each gene) in microarray experiments complicate the statistics and increase the likelihood of false positives. Microarray changes should be validated using real-time PCR. In proteomics, the properties of many thousands of ions are recorded in a single experiment and complex algorithms are used to match these data to a theoretical database to enable protein identification and/or quantification. In metabolomics, raw data require transformation to a suitable format prior to processing. The methods available for analysis comprise various statistical techniques including univariate and multivariate analysis, supervised and unsupervised learning tools and system-based analyses. The aim of these strategies is to find data patterns that provide useful biological information which can be used to generate further hypotheses for testing.

Omic strategies generate huge amounts of data and multiple testing increases the likelihood of false positives. Data validation is essential to ensure that findings are not just random findings. *P*-values can be corrected for multiple testing (false discovery rate). Other methods of model validation include the use of a 'hold-out' or 'test' set.[18] The set used in producing the model is called the training set. Models built using the training data can then be independently validated using the hold-out set. An alternative method of independent model validation is to use permutation testing.[19] More robust methods include confirming the observations with a complementary technique and replicating the experiment in a different sample set.[20]

There are many publications, across all the biological sciences, pointing out the potential folly of using profiling techniques such as metabolomics, proteomics, transcriptomics and genomics in order to discover clinically significant biomarkers.[21–25]

These areas of experimental design, sample preparation, analytical techniques and data analysis are covered in greater detail in a number of review articles.[13,21,26–30]

## Omic research in obstetrics and gynaecology

While omic studies in the area of obstetrics and gynaecology are relatively small in number, with advancing technology, it is a rapidly expanding field of research. It is not possible in this article to give an in-depth discussion of all omic research in obstetrics and gynaecology. As may be anticipated, most of the omic research in gynaecology is centred on oncology and cancer screening and much of the work in obstetrics on identifying biomarkers for complications of pregnancy such as pre-eclampsia and preterm birth.

A wide range of biological samples has been used for omic research, including plasma/serum, urine, amniotic fluid, cultured trophoblasts and cervicovaginal and follicular fluid.

### Pre-eclampsia

Studies have found gene expression to differ between pregnancies with pre-eclampsia and uncomplicated pregnancies in peripheral blood,[31] first-trimester placentas[32] and placentas at delivery.[33]

Much of the proteomic research in pregnancy has been in the area of pre-eclampsia. Studies have shown differences in women with pre-eclampsia compared with women with uncomplicated pregnancies, including differing serum levels of clusterin[34] and ficolin[35] but these were time-of-disease samples. One study[36] showed differences in five proteins at 26 weeks of gestation but these proteins could not be identified. Recently the plasma proteome at 20 weeks in women who subsequently developed pre-eclampsia ($n = 39$) was compared with that in normal healthy controls ($n = 57$): 39 proteins were identified and two protein clusters identified as fibrinogen gamma-chain and alpha-1-antichymotrypsin accurately classified women at risk of developing pre-eclampsia.[37]

Most metabolomic research has focused on pre-eclampsia and a number of researchers have found differences between women with pre-eclampsia and women with uncomplicated pregnancies using a number of different analytical techniques,[38–42] but these used time-of-disease blood samples or placentas at delivery and further work needs to concentrate on early pregnancy if predictive markers are to be found.

Not only are omic strategies of value in potential biomarker discovery, they also help to elucidate the molecular mechanisms involved in both normal and diseased states. Hypoxia plays a role in the pathophysiology of pre-eclampsia and fetal growth restriction and Hoang *et al.*[43] have detected distinct changes in the protein expression of first-trimester cytotrophoblasts under hypoxic conditions. Metabolomic differences have also been demonstrated on placental explants under different oxygen conditions[42] and from women with pre-eclampsia.[44] This demonstrates the systems biology approach to experimental models.

### Preterm birth

Romero *et al.*[45] have extensively reviewed the application of high-dimensional biology to the preterm parturition syndrome incorporating spontaneous preterm birth and preterm prelabour rupture of membranes. Numerous genetic polymorphisms have been reported that confer increased risk of preterm birth or preterm prelabour rupture of membranes, in particular those coding for matrix metalloproteinases (MMPs) and interleukins.

In preterm birth, differential expression of proteins has been found in placental membranes,[46] cervicovaginal fluid,[47,48] amniotic fluid[49] and maternal serum.[50]

The metabolic profiling of amniotic fluid has been reported to identify women at risk of preterm delivery and intra-amniotic infection with a precision of 96%.[51]

### Oncology

Profiling of gynaecological cancers has provided insight into the origin of these tumours as well as attempting to find diagnostic markers and potential therapies and to predict response to treatment. Predictive markers or early diagnostic markers may be of great benefit, particularly in ovarian cancers, as most cases are detected at an advanced stage and 5-year survival is poor. The literature in this area is vast; for example, various genes have been implicated in epithelial ovarian cancer, such as *p53*, *BCL-2*, *BAX*, *EGFR* and *c-erB2*. Numerous studies have reported multigene signatures of prognosis and response to chemotherapy in epithelial ovarian cancer. Gene expression profiling is also used for the early detection of epithelial ovarian cancer and one study using three markers (osteopontin, kallikrein 10 or MMP-7) in combination with CA125 had sensitivity and specificity values approaching 100%.[52]

In gynaecological oncology, proteomic profiling was initially applied to human serum to identify ovarian cancer by Petricoin *et al.*[53] Gadducci *et al.*[54] give a good overview of the markers associated with ovarian, endometrial and cervical cancer and the role proteomic profiling has to play. CA125 is the most reliable marker in ovarian cancer. Recently the autoantibody against the S100A7 protein has been found to be elevated in early and late-stage ovarian cancer but the clinical usefulness has yet to be investigated.[55]

Metabolic profiling using gas chromatography–mass spectrometry has also shown differences between malignant and borderline ovarian tumours using fresh-frozen tumour samples.[56]

## Conclusion

Omic strategies still provide many challenges: the technology and the software are still evolving and mapping the human proteome and metabolome is still ongoing. Pregnancy is a unique physiological state and pregnancy conditions can be extremely heterogeneous. Carefully designed experiments, accompanied by appropriate analytical techniques and statistical analyses, will assist in tackling many of these challenges, with the potential to generate reliable validated data to answer important biological questions.

The SCOPE study (see **Websites**) is actively recruiting. This is a collaboration of many international leading obstetricians and scientists seeking to develop novel, effective ways for the early prediction of nulliparous women at high risk of the three major complications of late pregnancy: pre-eclampsia, spontaneous preterm birth and fetal growth restriction. An extensive clinical database combined with genomic/proteomic/metabolomic data may provide the means for predictive screening tests to be developed.

### Websites

The SCOPE (SCreening fOr Pregnancy Endpoints) study [www.scopestudy.net/]

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