ACTIVE INGREDIENT STUDY

1 - FOREWORD
2 - SAMPLE PREPARATION
3 - LC-MS/MS ANALYSIS
4 - DATA PROCESSING
5 - CLAIMING POTENTIAL
6 - CONCLUSION

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1-FOREWORD

Principle

A quantitative comparison between ingredient treated samples vs placebo treated samples

Why a new approach based on proteins:

Proteins are the molecules directly in charge of cell functions and structures, nucleic acid are not.

Why an untargeted method:

You can discover what happens and not only what you think could happen, including toxic effects. So you can screen an ingredient and see what effect(s) it has.

Why nanoLC-MS/MS:

High resolution LC-MS/MS allows to identify and quantify thousands of proteins. These last evolutions of Mass spectrometry allow to treat directly extracted proteins from samples and very few protein quantit are necessary (50µg).

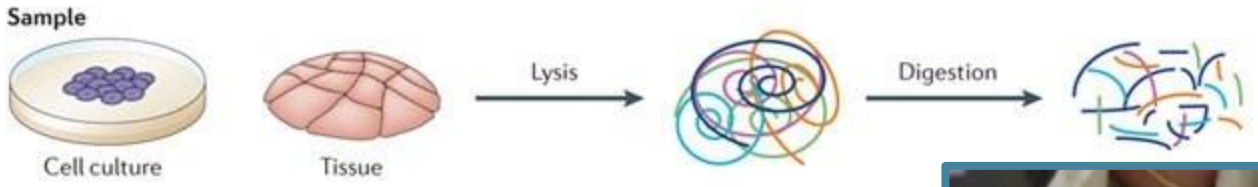
Why it is cost-effective:

You get a large view at a time for a cost of 2 to 3 € by protein

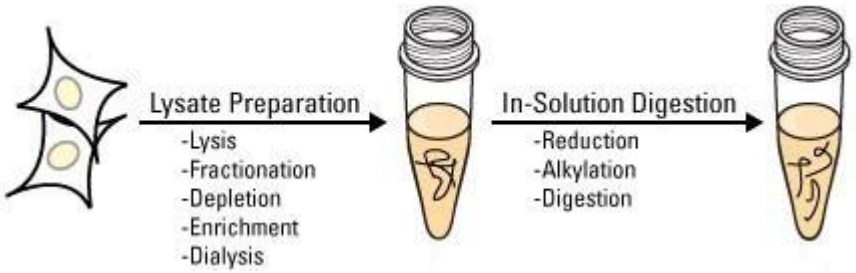
Why CORAVALID:

Huge amounts of data are generated by high resolution LC-MS/MS and each protein has to be included in the analysis in order to know its impact in a potential effect.

2-SAMPLE PREPARATION



… fibroblasts, keratinocytes, skin explants, rebuilt skin, D-Squame, swabs, extra cellular matrix, sub-proteomes….



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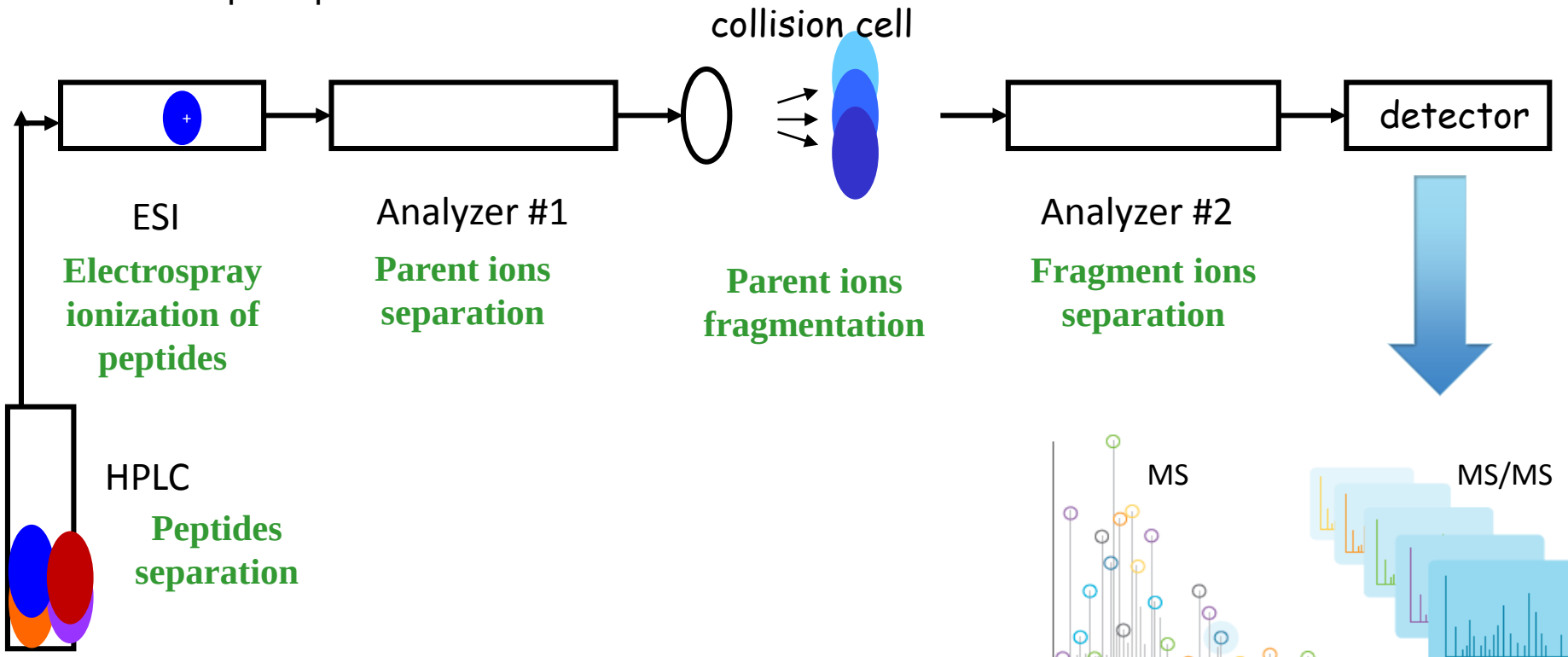
3-LC-MS/MS ANALYSIS

LC-MS/MS principle



MS and MS/MS data generation

Identification of thousands of protein specific fragments through databases,

Quantification of proteins through peptides signal intensity

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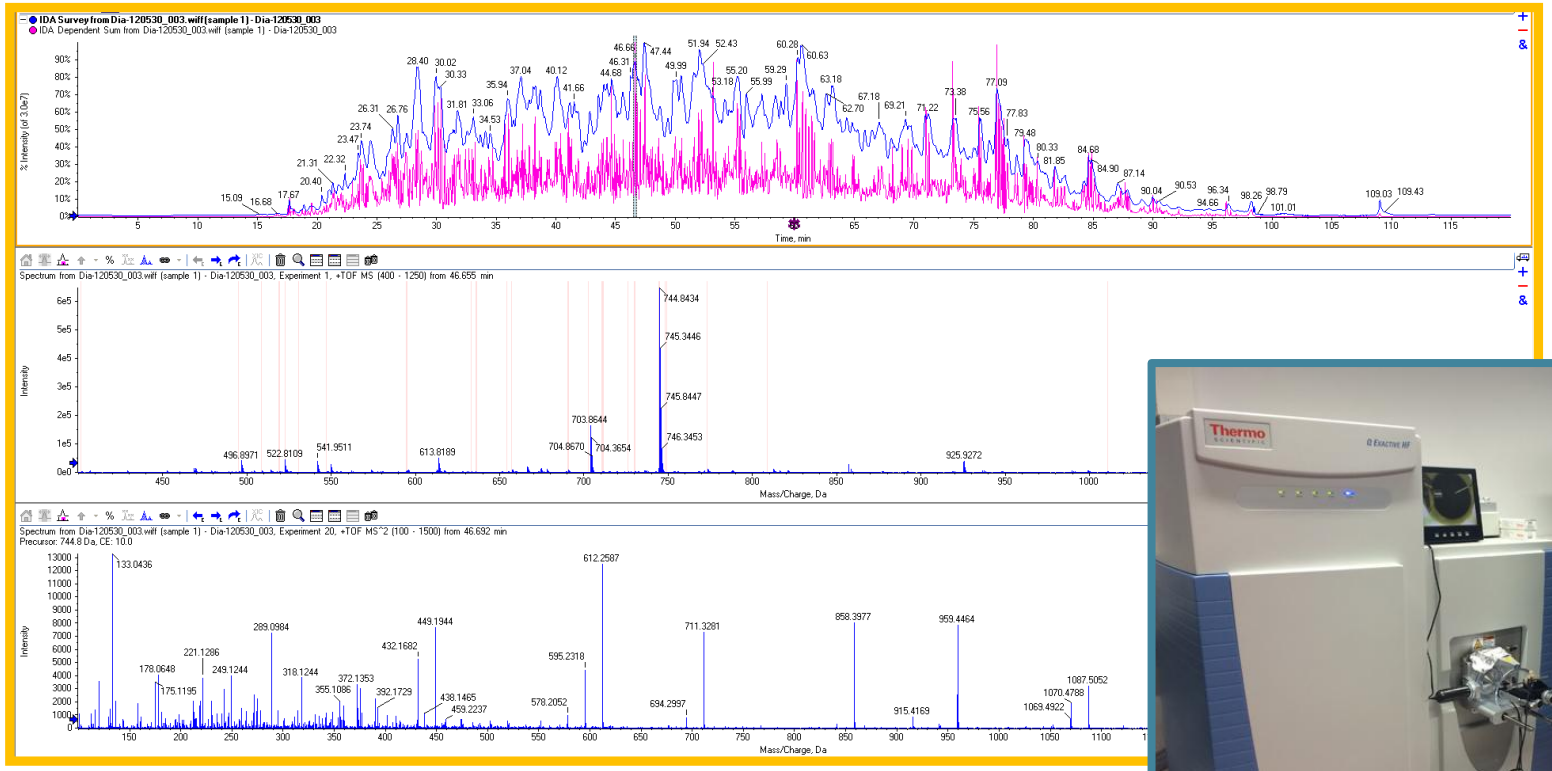
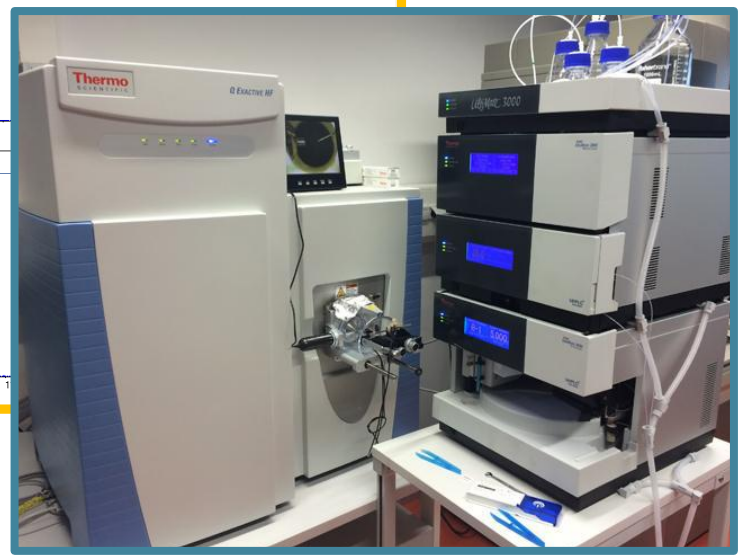
3-LC-MS/MS ANALYSIS





Untargeted highres nanoLC-MS/MS proteome label-free relative quantification

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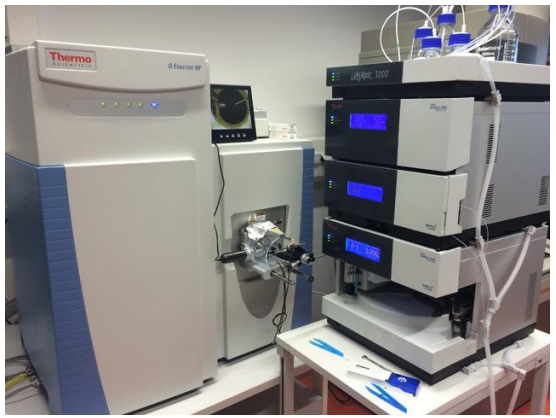
MS.PHYLOGENE equipments



Ultimate 3000 + Q-Exactive Plus

a new configuration upgraded on:
- Enhanced dynamic range
- Higher resolution

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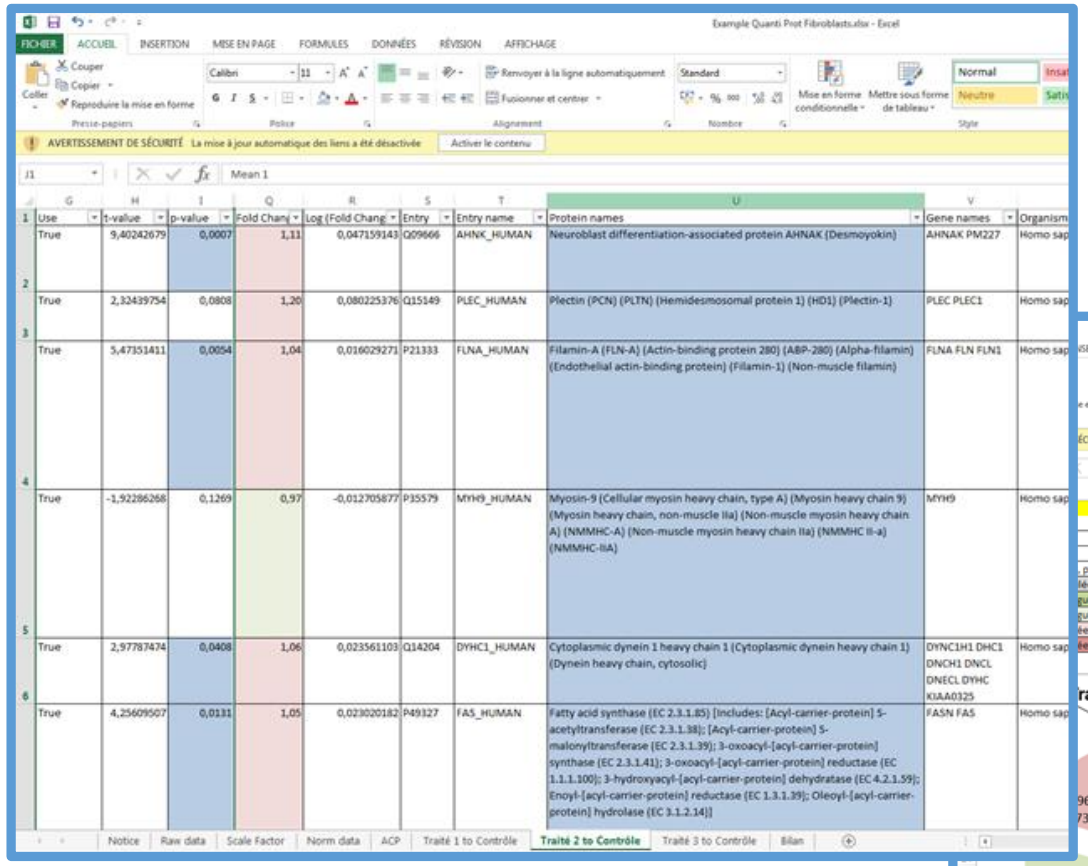
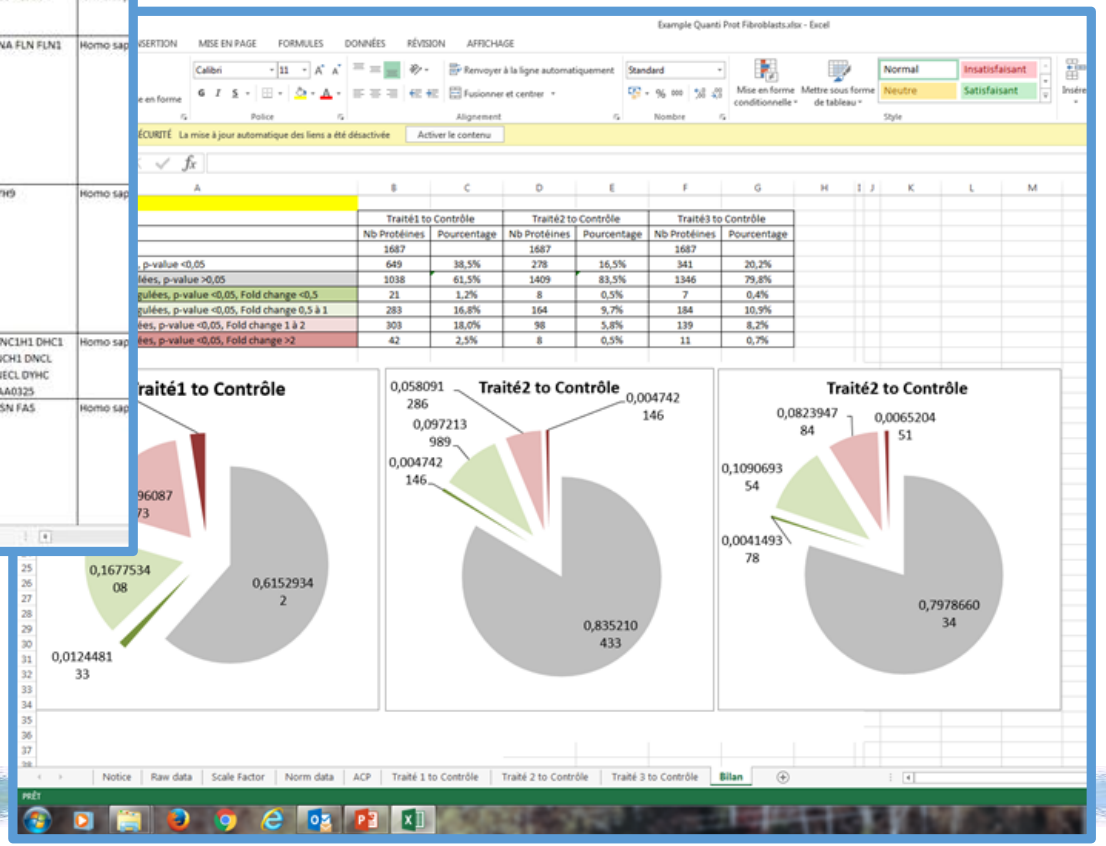
3-LC-MS/MS ANALYSIS



Use	t-value	p-value	Fold Chang	Log (Fold Chang	Entry	Entry name	Protein names	Gene names	Organism
True	9,40242679	0,0007	1,11	0,047159143	Q09666	AHNK_HUMAN	Neuroblast differentiation-associated protein AHNAK (Desmoyokin)	AHNAK PM227	Homo sap
True	2,32439754	0,0808	1,20	0,080225376	Q15149	PLEC_HUMAN	Plectin (PCN) (PLTN) (Hemidesmosomal protein 1) (HD1) (Plectin-1)	PLEC PLEC1	Homo sap
True	5,47351411	0,0054	1,04	0,016029271	P21333	FLNA_HUMAN	Filamin-A (FLN-A) (Actin-binding protein 280) (ABP-280) (Alpha-filamin) (Endothelial actin-binding protein) (Filamin-1) (Non-muscle filamin)	FLNA FLN FLN1	Homo sap
True	-1,92286268	0,1269	0,97	-0,012705877	P35579	MYH9_HUMAN	Myosin-9 (Cellular myosin heavy chain, type A) (Myosin heavy chain 9) (Myosin heavy chain, non-muscle IIa) (Non-muscle myosin heavy chain A) (NMMHC-A) (Non-muscle myosin heavy chain IIa) (NMMHC II-a) (NMMHC-IIA)	MYH9	Homo sap
True	2,97787474	0,0408	1,06	0,023561103	Q14204	DYHC1_HUMAN	Cytoplasmic dynein 1 heavy chain 1 (Cytoplasmic dynein heavy chain 1) (Dynein heavy chain, cytosolic)	DYNC1H1 DHC1 DNCH1 DNCL DNECL DYHC KIAA0325	Homo sap
True	4,25609507	0,0131	1,05	0,023020182	P49327	FAS_HUMAN	Fatty acid synthase (EC 2.3.1.85) [Includes: [Acyl-carrier-protein] S-acetyltransferase (EC 2.3.1.38); [Acyl-carrier-protein] S-malonyltransferase (EC 2.3.1.39); 3-oxoacyl-[acyl-carrier-protein] synthase (EC 2.3.1.41); 3-oxoacyl-[acyl-carrier-protein] reductase (EC 1.1.1.100); 3-hydroxyacyl-[acyl-carrier-protein] dehydratase (EC 4.2.1.59); Enoyl-[acyl-carrier-protein] reductase (EC 1.3.1.39); Oleoyl-[acyl-carrier-protein] hydrolase (EC 3.1.2.14)]	FASN FAS	Homo sap



Thousands of proteins identification and relative quantification between treated sample and control sample (Id, fold Change, p-value)

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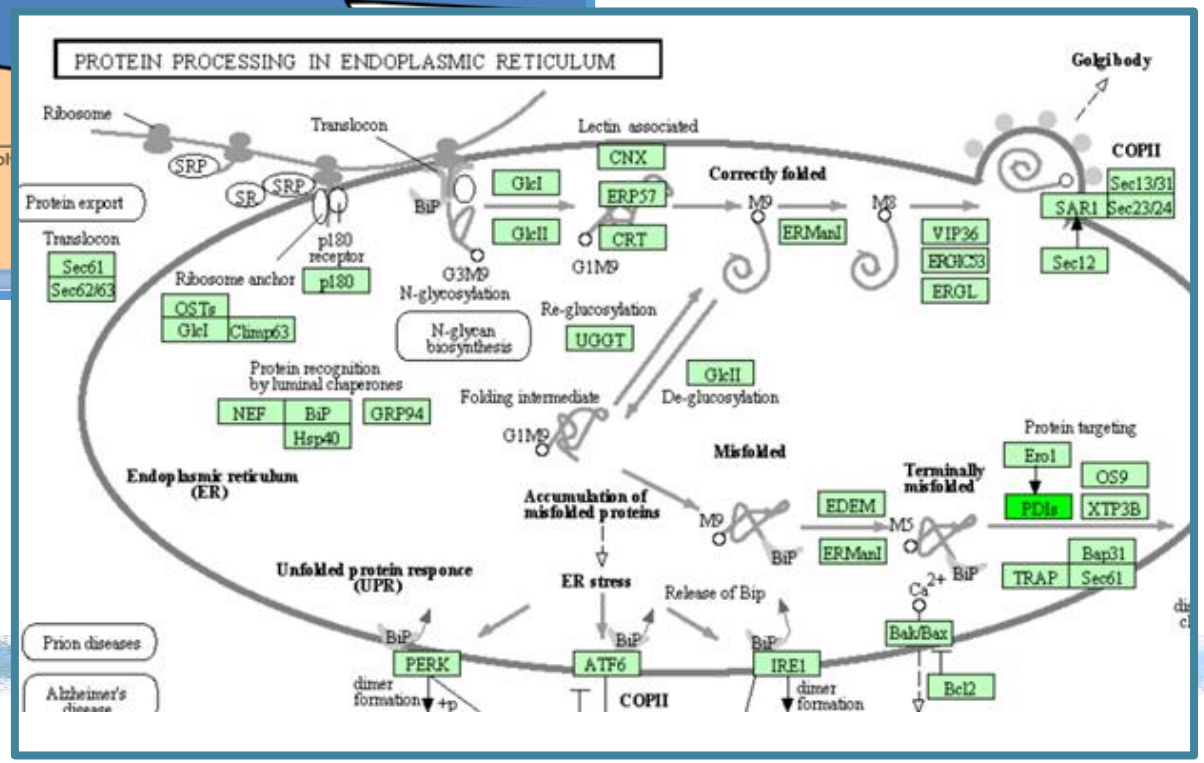
4-DATA PROCESSING WITH CORAVALID™

	L	M	N	O	P	Q
	Delt	p-value	Fold Change	Protein names	Log (Fold Chang	Entry
07	2,58E+07	0,0146	1,45	Collagen, type III, alpha 1	0,16	D2JYH5
06	1,50E+08	0,0001	2,25	Collagen, type I, alpha 1, isoform CRA_a	0,35	D3DTX7
07	2,66E+08	0,0000	2,17	Collagen alpha-1(I) chain (Alpha-1 type I collagen)	0,34	P02452
05	1,57E+06	0,0474	1,12	Gelsolin (AGEL) (Actin-depol		

1287 identified proteins, fold change, p-value



Events occurring on biological processes, molecular functions and cellular components

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5- CLAIMING POTENTIAL

WHAT WAS OBSERVED

-Decreased skin senescence

- *Extracellular matrix conservation* (COL1A4, TNSCX, SDC4)
- *Cornification decrease:*

  -Decrease of Keratins,

  -Decrease of Cornifin SPRR1B & HUTH, which are only expressed in corneocytes,

  -Decrease of focal adhesions, these characterizing the tight cellular mesh in *stratum corneum.*

- *Oxidative damage decrease:*

  -Induction of SODM & of glutathione regenerative mechanisms (IDH increased, hence probably NADPH production which reduced GSSG, while IDH decrease is associated to aging),

  -Decrease of DNA damage induced proteins (XRCC6, which repairs double strand DNA damages),

  -Decrease of apoptosis related proteins (HIST1C),

  -Decrease of protective mechanisms, supposedly less needed (ALDH3A1 decrease, has to be checked through biomarker metabolites).

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5- CLAIMING POTENTIAL

SKIN MACROSCOPIC FEATURES BENEFITS:

-Suppleness, Softness, Elasticity, Firmness, Glow:

- Decrease of keratinization,
- Reinforcement of extracellular matrix and cell-matrix interactions (collagen 1A4, tenascin, syndecan 4),
- Decrease of cornification/proliferation & terminal differentiation as senescent cells,
- Decrease of skin dryness, ichtyosis, shedding associated proteins (SPRR1B, ALDH3A1),
- *Potential melanogenesis decrease* (consistent with its UV damage induction as well as ALDH3A1 & IDH2 expression modifications, with possible tyrosine metabolism switching toward alternatives pathways; and potential effect on age spots/lentigo, possible disappearance of existing spots through induction of peroxisomal recycling?),
- *Possible effect on lipid storage or on lipid bilayers composition and related characteristics*:
  -effect on fatty acids metabolism and binding: FABP, TECR

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5-CLAIMING POTENTIAL

DERMATOLOGICAL CARE POTENTIAL

- Cicatrization improvement (keratinization regulation, potential activity on TNF-α),

- Hyperkeratosis improvement (symptoms & comfort)/healing (keratinization, cell proliferation),

- Skin dryness, ichtyosis, shedding phenomena improvement (symptoms & comfort)/healing (SPRR1B, ALDH3A1),
- Abnormal melanogenesis diseases improvement (at least UV related, but not necessarily as different pathway are involved in this potential),

- Skin inflammatory disease improvement (Potential activity on TNF-α & glutathione, activity on SOD2 allowing inflammatory damages restriction), particularly for **psoriasis** (Potential /TNF-α & PPAR, activity on fatty acids involved in disease physiopathology), and related diseases (**eczema**),

- Skin Allergic reaction & hypersensitivities improvement (idem)

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6-CONCLUSION

Knowledge depends on existing technos.
Since the years 1990, DNA sequencing techniques opened the door to untargeted approaches of genes.
For 15 years, mass spectrometry evolution allows to analyze large molecules.
For 7 years, **LC-MS/MS and databases** evolutions allows to **identify, quantify in an untargeted** mode

GENOMICS *DNA*	What can happen but what about EPIGENOMICS?	*Targeted*: PCR, DNA chip *Untargeted*: sequencing, NGS
TRANSCRIPTOMICS *mRNA*	What appears to be happening	*Targeted*: Rt-PCR, *Untargeted:* RNAseq
PROTEOMICS *proteins and PTMs*	What happens and makes it happen	*Targeted:* Immunotechniques *Untargeted:* LC-MS/MS and Bioinfo
METABOLOMICS metabolites	What has happened and is happening	*Targeted:* LC-MS/MS *Untargeted:* LC-MS/MS but with poor database

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A workflow particularly well adapted to effects discovery in cosmetics