Systems Biology footprint

On the nutraceuticals and cosmetics arena

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SUMMARY

Systems Biology, a new and validated integrative technology, finds many uses in the fields of cosmetics and nutraceuticals, accelerating the design of new products, and providing scientific support for health claims. Three practical situations are presented to illustrate how systems biology can be applied in the nutraceuticals and cosmetics area.

1 Positive relation between ketogenic diets and diabetes
2 Cosmetics and skin aging
3 Enhancing wellbeing effect.

INTRODUCTION

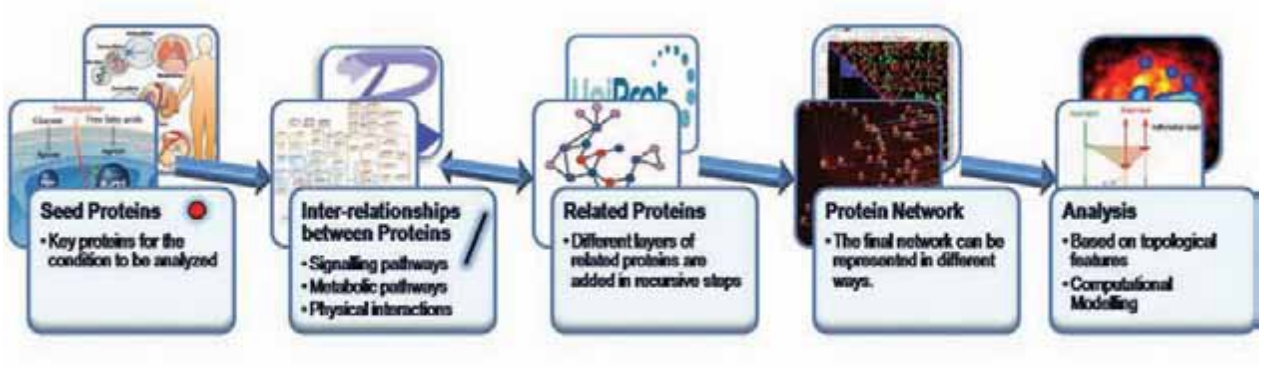
Systems Biology

Systems Biology emerged in recent years as 'the integrated approach' to studying biological systems by measuring and integrating genetic, proteomic and metabolic data. Nature mechanisms are blurry, redundant, complex, hyperconnected, variable ... but do work. Systems Biology offers a new vision of the complex biochemical and molecular interactions in the human body that lead to 'disease' and 'health' status (**1,2**). Systems Biology takes profit of the enormous amount of information generated by *Omics sciences* (Genomics, Proteomics, Transcriptomics, Metabolomics), and combines it with the extraordinary computing potency and cutting-edge mathematical analysis strategies, to offer from scientific explanations of disease processes, to more practical approaches like identification of new targets for drug discovery or drug safety prediction, optimization of cosmetics compounds or to explain the mechanism of action and new properties of functional foods (**3,4**).

Systems Biology approaches are based on the construction of complex interactions or cell networks that contain all the interactions between proteins in the human body. The analysis of this maps or interaction networks can explain the pathogenesis of diseases, or the mechanisms of action of drugs. The Systems Biology concept is increasingly being applied within life sciences for the development of drugs, but has found yet little attention in the nutraceuticals and cosmetics sectors. There is possibly a lack of understanding on how to integrate Systems Biology capabilities into innovation in those sectors. In order to bring an insight on how to integrate Systems Biology in those areas, we are presenting three practical situations where we have applied Systems Biology methods in the nutraceutics and cosmetics area.

The bases of the methology applied in the three cases presented is the same. *Figure 1* depicts the basic steps of the procedure. The first step in the construction of the cell network, or map, representing the condition in study, consists in the identification of all those proteins known to be important for that particular condition (i.e. seed proteins). The map generation and extension process is conducted in recursive steps through the incorporation of all known relationships of the seed proteins derived from public data bases (KEGG, REACTOME, INTACT, BIOGRID) (**5**). As a result a complex map of protein-to-protein interactions is obtained. Those maps usually contain thousands of nodes (proteins) that are connected through edges that illustrate their inter-relationships. Additional information is mapped onto the interaction network. Nodes that are known drug targets are identified. Anaxomics data base ax_AES-DataTool™ is used to identify proteins related to different physiological conditions (syndrome, disease or pathological condition).
It consists of a hand-curated data base that contains more than 200 characterized conditions and their complete molecular mechanisms of action (or sub-networks). The resulting map can be analyzed using different algorithms that provide organizational information about the system from a top-down view.

Figure 1 *General outline of the Systems Biology strategy used in the different case studies*



The initial step consist in the identification of the key proteins for the condition to be analyzed (i.e. seed proteins). In a second step all the relations of the seed proteins retrieved from the literature are incorporated leading to a second layer of proteins. In recursive steps more layers of proteins are incorporated. The final map, interaction network, can be visualized in different ways, like a matrix of relations, or as graphs of connected nodes where proteins are represented by nodes (spheres) and they are connected through edges that illustrate their inter-relationships. Green triangles identify proteins which are known drug targets, seed proteins are coloured in dark green. On the map obtained different type of analysis can be performed: i) based on topological features like distance, centrality and clustering; ii) computational modelling which provides insights in the spatiotemporal dynamics.

Static, topological information is only a first step toward an understanding of the cell (**6**). Computational models provide into insights the intricate spatiotemporal dynamics that shape cell decisions. Model driven discovery is appealing because computation is far less expensive and time consuming than wet-lab experimentation (**7**).

1. Positive relation between ketogenic diets and diabetes

Certain food diets which are rich in proteins and that produce a metabolic ketosis status (ketogenic diets) apart from the concomitant weight loss, have shown beneficial effects on certain conditions like Alzheimer's disease or type 2 diabetes (**8**). Clinical trials provide evidence of the benefits of ketogenic diets in terms of clinical outcomes on patients (**9,10**). However, the molecular events responsible for these improvements still remain unclear.

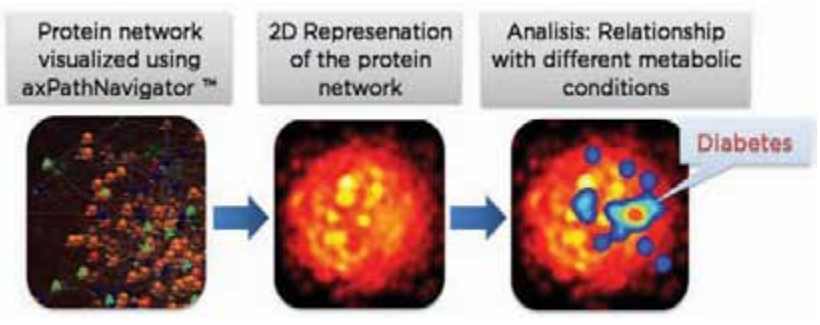
Our approach offers a new global vision of the interrelationship of those conditions, finding solid links between them, and explaining the main biological processes by which ketogenic diets are beneficial for Alzheimer's disease or diabetes in a way not previously described.

Our Systems Biology approach is based on the construction and analyses of the cell networks associated with the metabolic state observed in a very-low-calorie ketogenic diet. As outlined in the previous section the first step in the construction of the cell network, or map, representing the metabolic state of ketosis consists in the identification of all those proteins described to be important in this particular metabolic state (i.e. seed proteins). The ketosis map was built using 447 seed proteins derived from KEGG pathways corresponding to ketosis, fatty acid metabolism and other physiological pathways related to a protein-sparing modified fast (PSMF), a type of very-low-carbohydrate ketogenic diet. The final map includes 3,669 proteins and 142,531 known relationships.

The relationship between two groups of proteins of interest (proteins related to ketosis and proteins related with type 2 diabetes) is assessed by means of standard measures in the field of biological networks, such as presence, centrality or clustering analysis (***Fig 2***). The map obtain is multidimensional an thus a limited number of analysis can be performed in this form. A conservative nonlinear projection method to visualize high-dimensional data into 2D map has been applied. The relationship between two groups of proteins of interest (proteins related to ketosis and proteins related with type 2 diabetes) was assessed by means of standard measures in the field of biological networks, such as presence, centrality or clustering analysis.

Our analysis suggests that the relationship between the ketosis status induced by PSMF and diabetes is closest to the insulin resistance pathway in respect to the other two identified pathways for diabetes (insufficient insulin production and destruction of pancreatic β-cells). Furthermore, the analysis at protein level highlights two complementary hypothesis of functional relationship between the molecules involved in both physiological processes (**11**). Clinical observations identify beneficial effects in the outcome of patients with type 2 diabetes following a ketogenic diet that cannot be attributed, only, to weight loss since the improvements in the glycemic state are detected before a significant reduction of weight (**12**). Our approach provides for the first time a molecular explanation of the additional beneficial effect seen in the

Figure 2 *The metabolic state of ketosis represented as a protein network*



The first panel shows the ketosis map visualized using the software axPathNavigator developed by ANAXOMICS. Proteins are depicted as spheres and the edges illustrate their inter-relationships.
The second panel shows the ketosis map in a 2D representation. The colour grading of the image reflects the protein density, from black (no protein) until yellow (high protein density).
The third panel shows the results of the clustering analysis, one of the different topological analyses performed. The underlying image shows the ketosis map in 2D (same as medium panel). The overlaying image depicts the location and density of the diabetes cluster on the ketogenic-diet map. Again, the colour grading of the image reflects the protein density: from blue (low protein density) to dark red (high protein density)

outcome of diabetic patients following a ketogenic diet. The analysis of the ketogenic-diet map from the diabetes perspective offers a unique insight on the mechanism of action that will be further validated with biochemical approaches.

2. Cosmetics & skin aging

The whole concept is based on products being efficient and safe. A number of cosmetic companies are exploring the use of growth factors like epidermal growth factor (EGF), fibroblast growth factor (FGF), and interleukins (IL), along with other active ingredients of different origin, in order to stimulate growth of the human skin cells for cosmetic uses. But there is a need to find optimal combinations of these compounds to minimize the quantity used, as they are expensive. Finding synergistic combinations that maximize the desired effect and/or substitute them with other cosmetic active compounds that might have the same effect but that might be more convenient from the cosmetics perspective are two possible ways to achieve a reduction (**3**).

Starting from the human skin protein interactions map, we have developed a network model that reproduces biochemical behaviour in the skin (metabolic, signalling and growing), and have developed a 'virtual mixer' that allows to simulate combinations of compounds to maximize desired effects. Those effects can be direct (like adding fibroblast growth factor (FGF) to stimulate cell growth and collagen production), but also we can find more convenient cosmetic ingredients that have the same desired effect in an indirect manner, i.e. by indirectly stimulating the same biological pathway as FGF.

The assembly of the human skin protein interaction map starts with the selection of seed proteins. For this purpose, the known biochemical processes associated with skin aging are characterized in detail. Skin aging is a complex biological process resulting from both intrinsic aging (or genetically programmed) that occurs with time and extrinsic aging caused by environmental factors acting simultaneously (**13,14**).

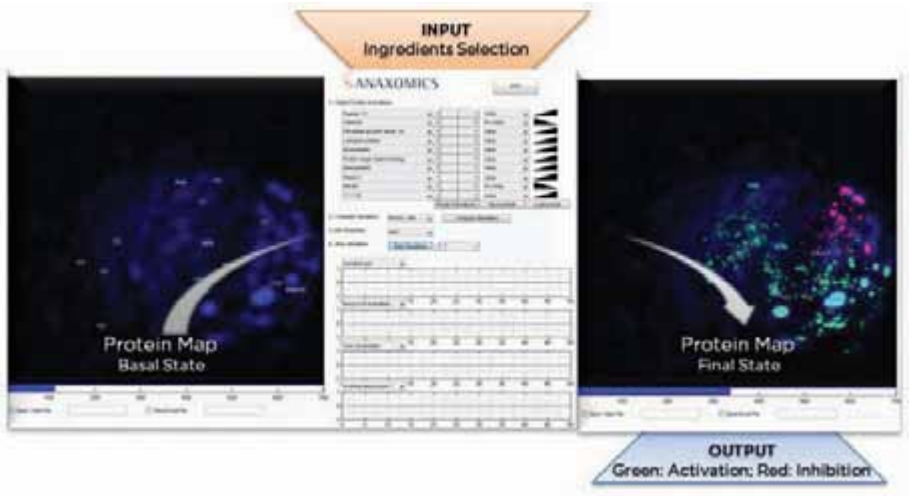
Only intrinsic factors can be traced to proteins. Intrinsic or sun-protected aging of skin is mediated by the 'biologic clock' that affects the skin in the same manner as it affects the internal organs, i.e., by slow, irreversible tissue degeneration. Telomere shortening combined with metabolic oxidative damage is believed to play a major role in the intrinsic aging process. ROS detoxifying activity is reduced among the years which contribute to a higher extracellular matrix degradation process (**15**). Collagen is the most abundant protein at the extracellular matrix. Therefore modifications of its structure, less synthesis rate or more degradation, are key points for matrix elasticity and structure (**16,17**). Moreover, skin acts as a barrier mediating solute flow-through and water maintenance (**18**). In our approach 3 different pathophysiological motives were established and the key proteins for each of them identified (***Table 1***)

Table 1 *Skin-aging pathophysiological motives as identified in ax_AES-DataTool™*

Increase of reactive oxygen species = Oxidative damage
• *Superoxyde dismutase*. The cytosolic Cu/Zndependent enzyme (SOD1), the mitochondrial Mn-dependent variant (SOD2), or an extracellular variant (SOD3).
• *Peroxiredoxin*. Prdx comprise a family of six enzymes that catalyze the reduction of a broad spectrum of peroxides.
•...
Collagen degradation and synthesis in skin = Elasticity
• *Nuclear factor NF-kappa-B*. NF-κB controls cell cycle exit and gene expression signature of aging.
• *Matrix metalloproteinases*. Degrade collagen.
•...
Hydrophobic and hydrophilic skin alteration = Skin dehydration
• *Aquaporins*. Several (AQPs) are expressed in mammalian skin, some are directly involved in water transport.
•...

The identified proteins for each of the motives serve as a seed protein. A protein interaction map, considering all types of interactions, is build around them.
The methodological approach has the same bases as the one already

Figure 3 *The Virtual Mixer for the selection of ingredients*



Snapshot of the software application, the Virtual Mixer. On the left panel, a protein network map is created following the steps described in the text. This map includes all the proteins related with the 3 main arguments under study, i.e., increase of reactive oxygen species, collagen degradation and synthesis and hydrophobic / hydrophilic skin alteration.
The analysis is conducted by producing perturbations (activation or inhibition) over a specific point or a group of points (medium panel). The input signal will be transported as a perturbation over the map and it will move its consequences to other regions of the Map. Each perturbation uses the rules given by the mathematical model (previously trained with known cases). The nodes affected by the perturbation can have effects over the individual; these will be the output signals (right panel).
This software allows assessing the effect of different active compounds for a cosmetic application on the key proteins involved in skin aging, identifying the active compound combinations that best provides the optimum effect while minimizing undesired side effect

described in Chapter 1. The resulting map describes the relations of the proteins involved in skin aging. By means of artificial intelligence techniques we have developed an algorithm able to accurately model skin aging processes, which will allow us to query the map. The new developed algorithm is the bases of our software application, the *Virtual Mixer* (***Fig 3***).
This software allows assessing the effect of different active compounds for a cosmetic application on the key proteins involved in skin aging. Identify the active compound combinations that best provides the optimum effect while minimizing any undesired side effect. The analysis of different active compound combinations has been used for the selection of the sets that best act on a certain skin aging motive, while minimizing any side effects. Some of the predicted combinations have been tested *in vitro* and are currently in clinical trials with positive preliminary results. Our approach provides a very cost-effective way of optimizing the composition of cosmetic products, suggesting the molecular bases for the proposed effect.

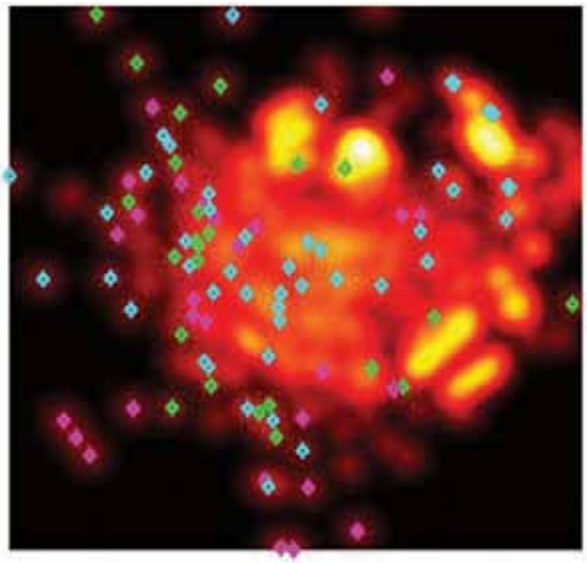
3. Enhancing wellbeing effect

Nowadays, there is increasing emphasis on health and wellness derived from food. Companies working in the area of functional foods make efforts to: a) justify mechanistically their health claims and b) find new properties for their products. One of the new ways to confer desired properties to foods is in the area of 'foods that induce wellbeing'.
The wellbeing concept is a complex one with many interplaying factors. Systems Biology approaches provide the way to analyze multifactor systems. The same bases as the other two approaches presented have been applied in this case.
In a first step, the concept is traced to its molecular bases.
The wellbeing sensation is being modulated by intrinsic factors (emotional, physical, cognitive...) and extrinsic factor (social, material,...) (**19**). The intrinsic factors related to wellbeing have been characterized at the protein level, like activation of certain metabolic and signalling routes (dopamine, serotonin, oxytocin, etc.) We have constructed a protein map built around the identified key proteins for the intrinsic factors of wellbeing.

Some food compounds can have effect over these routes and produce pleasant sensations by mechanisms of action not yet clearly understood. Our aim is to create a platform to allow *ad-hoc* design of food products with desired properties. We have analyzed the sensation of wellbeing produced by chocolate. There are many active ingredients in chocolate (**20**), some of the alkaloids contained in chocolate, like tetrahydro-β-carbolines and tetrahydroisoquinolines, have neuroactive properties that can explain part of the wellbeing sensation (**21**).

We have analyzed the action of those alkaloids in their target proteins and how these chocolate target proteins relate with wellbeing by means of standard measures in the field of biological networks, such as presence, centrality or clustering analysis. The initial multidimensional map obtained has been transformed into a 2D format using a conservative mathematical algorithm, MDS (**22**). The representation of Well-Being map in 2D is presented in *Figure 4*. The fist point of connection has been the identification of common proteins between wellbeing seed proteins and the target proteins of chocolate alkaloids. The different topological analyses performed describe, at a molecular level, the neuroactive properties attributed to chocolate. We are currently constructing a

Figure 4 *Protein map representing the wellbeing concept*



2D representation of the map of wellbeing. The color grading of the image reflects the protein density, from black (no protein) until yellow (high protein density). The localization of key proteins for wellbeing and protein targets for chocolate alkaloids are marked with a diamond in overlaying image. Cian: Wellbeing seeds; Green: chocolate targets; Magenta: proteins shared by the two groups.

model on the wellbeing map that will enable the optimization of the formulation of nutritional and phytoingredients in functional food, in the same way we have optimized cosmetic active ingredients.

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