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Ingestion-Controlling Network: What's Language Got To Do With It?

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SYNOPSIS

The prevalence of obesity has increased dramatically worldwide whereas the types of treatment and their efficacy have not substantially changed over the last two decades. Additionally, drugs used to control weight gain could occasionally create untoward effects in cardiovascular functions, as well as behaviors, memory, sleep, and emotions since molecular machinery responsible for ingestion control is interconnected with or shared by the above domains. How each group of drugs preserves the privacy of its message in the mutual network is not fully understood. In the present essay, the graph theory approach was used to explore some aspects of molecular signaling as though they were a 'language'. Its emphasis is on 'molecular polysemy', a term that refers to the ability of biomolecules to be used like words in natural languages more than one-way. This has physiological and clinical implications, in particular when planning drug designs with "specially engineered shotgun loads" that target a combination of biomolecules that assure a better therapeutic outcome without causing deficits in connected but pathophysiologically irrelevant bystanders.

Keywords

networks; polysemy; molecular promiscuity; motifs; orexigenic and anorexigenic peptides

INTRODUCTION

The group of peptides implicated in food intake and weight regulation is organized into several layers of complicated networks. Some of them have an advanced system of receptors and thus an individual pathway of signaling. Despite their labels as orexigenic (feeding-generating) and anorexigenic (feeding-inhibiting) factors, both subsets of molecules are also implicated in a range of highly specialized processes that can be conceived of as modules / 1/, such as reproduction, immunity, cognition and emotion, circadian functions and sleep, plasticity, learning, and affiliative behaviors /2,3/. The number of such modules and the degree of their partitioning is unknown, but expectedly, they must create a system of intra-modular and inter-modular communication, akin to language. This has physiological and clinical implications since medicinal chemists tend to emulate such signaling by searching for molecular identity between particular structures of interest that assures their binding in three-dimensional space and triggers predictable biological responses. Otherwise, engaging the members of the 'ingestion module' pharmacologically may unwittingly create untoward

effects in diverse domains unless the messages are individualized and all innocent bystanders are shielded. This is a safe assumption since most drugs interact with multiple targets. According to a recent computation, /4/ the average number of target proteins per drug is 1.8.

A comparison with language, however tempting, has an important proviso: Languages with small-size vocabularies could be prone to confusion when they handle complex messages delivered in contexts other than expected. Even rich lexica do not protect against ambiguity when the same word is used with different connotation. In linguistics, such economy of words is defined as ‘polysemy’/5,6/. Although a pervasive phenomenon in natural languages (multiple meanings in human language), polysemy can be encountered in other codes. Even acoustic structures of rodents’ vocalizations may relate to multiple biological functions (on semiotic level) reminiscent of polysemy /7/. Based on his analysis of acoustic parameters in rat calls, Brudzynski maintained that “it is likely that most rodent calls are polysemic” (/7/ and references therein). He further indicated that rat communication seems probabilistic rather than deterministic. In this regard, it might be compared with betting on the outcome and thereby admitting irrationality in decision-making /8/.

Therefore, at first glance the metaphor of polysemy may seem somewhat strained when applied to molecular signaling since the latter cannot be irrational’ without convicting its ‘hearers’ to irregular functions. However, as Condit and Railsback /9/ observed, the blossoming of biology has shifted an emphasis “from identity to similarity as a basis for classification and generalization...” For example, biologists focus on pleiotropy, such as ‘hormonal pleiotropy’/10/. Ingestion peptides have the roles similar to those of hormones in the sense that they exert multiple phenotypic effects by signaling to proximal and distant tissue targets throughout the organism, which functions still await detailing. Therefore, polysemy is a useful metaphor for metabolic codes precisely because of its ambiguity and because these signals may communicate different things in different contexts.

The term “code”, is itself a polysemous word. When it is uttered, the listener needs to decipher whether it refers to a register of rules, signifies a specialized instruction, such as genetic code or code of computer communications, or when it is used as a set of intentionally cryptic symbols relevant only on the receiving (‘hearing’) side. The comparison of molecular code with languages, suggested the possibility of their compliance with the ‘principle of least effort’ due to an asymmetry in speaker and hearer needs. In turn, formally, a quantitative measure of ‘linguistic behavior’ of ingestion-controlling molecules could be tested by computing their agreement with Zipf law /11/. The law, so named after a linguist George Kingsley Zipf who showed early in the last century that the frequency of word occurrences in natural languages is proportional to the negative power of the rank order of the word. The law is formally expressed as $W \approx R^{-\alpha}$, where W is the frequency of some occurring object and R stands for the rank of this object. Zipf law is one of a few phenomenal findings that had acquired the status of law outside of linguistics. The existence of Zipfian rank-size distribution has been tested and corroborated in diverse research fields, as wide apart as theoretical physics and bioengineering where the exponent α value universally approaches 1 as in ‘corpora’ of linguistic data /12/. Mantegna et al. /13/ conducted ‘linguistic tests’ on DNA sequences and later, a relationship akin to Zipf law was shown to hold for a variety of biological objects /14–17/.

Here we ask what gives the frugal ‘ingestion vocabulary’ the capacity to reach beyond the requirements of food intake and endows its network with the pliability considered necessary for the multiplicity of non-nutritional functions without creating communication problems. In the following, Section 1 briefly illustrates the viability of the small nutritional vocabulary by taking advantage of a new paradigm offered by the network building capacity of

Ingenuity Pathway Analysis (IPA) (www.ingenuity.com). Mining data using IPA is not a new approach. This platform from high-quality systematic literature curation allows to characterize the degree of multi-functionality of diverse systems by providing the facilities for building networks and functional analyses [18]. In Section 2, the generality of Zipf's law is probed in the province of peptides' signaling. Section 3 examines the assumption of the interdependence of the multi-factorial molecules using Venn diagrams, whereas Section 4 explains the limitations of the categorical approach in network analysis. The final subsection (5) is driven by the idea that motif exploration would help in confronting the problem of drug discovery and drug targeting.

1. MULTI-FUNCTIONALITY OF INGESTION CONTROL NETWORK

The Ingenuity software makes the trial of multi-functionality reasonably uncomplicated. It opens with compiling a core 'vocabulary' derived from data in the experimental literature on the hypothetical nutritional signals. This was limited to sixteen well-known interacting biomolecules. There is little doubt that more molecules implicated in nutrition control and genes affecting obesity phenotypes will be discovered in the near future. Yet, the latter group is commonly selected for experimental testing and was explored elsewhere [19]. They were introduced into the IPA search engine, edited for accuracy, and used as 'molecular seeds' for core analysis. After running the analysis, the results were available for overview in several domains, such as 'Molecular and Cellular Functions', 'Physiological System Development and Function' and 'Diseases and Disorders'. Each could be explored in a variety of subcategories along with a population of associated molecules. IPA displays the top 500 functions that meet a p-value cutoff of 0.05.

A brief list of functions relevant for the present issue was selected for a histogram in Fig. 1. Note that the histogram is dominated by a 'behavior' bar, and ends with miniature bars representing tissue development and tissue morphology. IPA analysis arranges the findings from statistically more significant to the less significant. The top to bottom order of significance masks the presumed sequence of the events depicted in the figure in the sense that tissue development would come before an organism exhibits behaviors or psychological disorders. This logic suggests that the basic properties and roles of each group of molecules is probably determined during early development and guided by the specific temporal structural and metabolic needs of the embryo.

An important issue of the analysis is that although molecules supporting the selected subcategory within Ingenuity's knowledge base appear as a group, for obvious research reasons its dataset is a summary when one or two molecules are isolated or manipulated at a time in pharmacological or knockout studies. Scrutinizing the lists of molecules for each function in Fig. 1, one thus might be puzzled by noticing that a small number of biomolecules is repeatedly recruited for such an amazing array of roles.

Figure 2 provides a compelling illustration of the functional overlap by showing three arbitrarily selected groups of disconnected molecules implicated by the IPA analysis in 'Cellular Growth and Proliferation', 'Feeding' and 'Behavior'. All three groups were statistically significant under IPA criteria (p-values based on Fisher's exact test). IPA provides an overlay of the neurobiological functions associated with each of the molecular group sampled in the window. Among numerous options in the drop menu, we selected only 'vascular development', 'immune response', 'memory', and 'affiliative behavior'. The overlap is rather robust. A close-up that selected three molecules, CRH, LEP, and NPY further emphasized that all three appear in vascular development and immune response; as well, they are implicated in memory and affiliative behavior. Such a range of functions suggests their availability in early ontogeny and their role in cognition and behavior.

This result suggests that molecules that control ingestion also provide scores of roles that are dissimilar and even remote from nutrition unless the term is used metaphorically. If the systems ‘controlling a function’ are understood as particular sets of units with their unique connections, developmental history, boundary maintenance, and hierarchical order of activation, the kind of vocabulary used for the ingestion system certainly defies this understanding. To paraphrase Jacob /20/, evolution has a frugal way in offering a patchwork of ancestral sets pieced together when and where opportunities arise to service a new process. Presumably, in molecular communications as in language, developmental (evolutionary) needs outpace the growth of vocabulary thereby recycling available labels without increasing the size of the lexicon /21/.

2. A GLITCH OF THE ZIPF DISTRIBUTION

Combing through the lists generated in the course of functional analyses it was easy to notice that several biomolecules (LEP, NPY, CRH, POMC, and INS; see Fig. 3, for abbreviations) were most consistently chosen to team up in different constellations, thereby suggesting that they were hardly drawn blindly out of the evolutionary pool for coding a message. A familiar example is that of LEP inaugurated as a gene that encodes a protein with a major role in the regulation of body weight, but was also discovered to act as a multifunctional hormone implicated in control of reproduction /22/, memory /23/, cognition /24/, and cardiovascular regulation /25/. The components of hypothalamic circuitry that integrate aspects of feeding behavior and energy homeostasis, such as NPY and CRH also regulate a variety of other functions implicated in wakefulness, sleep and circadian rhythms, memory, and cognition (/26/ and references therein).

In order to test the applicability of Zipf’s Law in the domain of ingestion-controlling molecules we used a series of lists collected in functional analyses discussed elsewhere /1/. In order to validate their selection, the list of molecules (as their standard abbreviations from several networks compiled from obesity analysis and clozapine effects in the process of IPA functional analysis) was compared for their frequency and rank. This was done with commercial software *Hermetic Word Frequency Counter* (<http://www.hermetic.ch/wfca/wfca.htm>), as if they were words of a text. Following traditional practice, the *Counter* was programmed to compute the Zipf data, which are logarithms of the rank and frequency values of such words. At first glance, by ranking in its advanced version 850 molecular names, the fitness to the Zipf law was found to be adequate ($Y = -1.1885x + 4.5779$; $R^2 = 0.9516$) (Fig. 2).

However, Zipf distribution shown in Fig. 3 revealed a group of terms with the same frequency and different rank. These top ranking molecules of the same multi-functional category pull the left tail of the curve away from the regression line on the plot. The fact that Zipf law did not hold exactly as would be expected in linguistic corpora was nothing new /27/, so that in itself it does not contest the universality of the exponent of Zipf law in the realm of ingestion-controlling peptides. What might be the ‘wrinkle’ of the Zipf exponent in the list of molecular terms?

One finding /28/ invited the possibility that all words with the same length have the same frequency of occurrence. In the present context, all molecules were mostly three-four letter terms. Assuming that ‘particular length’ might refer to the number of amino acids each peptide comprises, LEP that is composed of 167 amino acids and adiponectin, a 244-amino-acid-long polypeptide would be of the same rank since technically, both are proteins. However, LEP and NPY that is a 36-amino-acid peptide are neighbors whereas adiponectin is lost in the middle of distribution together with somatostatin (14-amino acids), neurotensin

(13-amino acids), and oxytocin (9-amino acid peptide). Therefore, the 'length' of peptides is unlikely to account for the rate of their delivery into the reaction space.

A better explanation of the result is that in the English language, words such as articles, prepositions, special adverbs, and conjunctions (e.g., 'the', 'and', 'of', 'up', 'who', or 'but') are frequently encountered and are useful for language comprehension. Consequently, they dependably lift the left tail of the curve in word-frequency analyses/27/, but have no inherent reading. The morphologically impoverished lexicons, such as pidgins at their simplest mainly consist of nouns, verbs, and adjectives with no similar dependence on articles, prepositions, conjunctions, or auxiliary verbs /29/. Their phonemic messaging may be less comprehensible outside the context of statements of posture, gesture, facial expression or lip movements. In humans, language and mouth motor command system involved in ingestion activities shares neurophysiological resources with gesture /30/. At first blush, the corpus of 'language of ingestion' may behave similar to the pidgins by exhibiting a power-law distribution with an exponent deviating from -1 since molecular lists do not have a similar number of elements corresponding to such useful items with no inherent meaning.

3. THE ASSUMPTION OF MOLECULAR INTERDEPENDENCE

The assumption of molecules multi-functionality can be further explored using Venn diagrams as the logical base for contrasting the relationship between objects implicated in dissimilar roles, such as suggested by Fig. 1 and 2 of IPA functional analysis. To this end, their IPA terms were submitted into a web software program that allows creating Venn diagrams (<http://www.cmbi.ru.nl/cdd/biovenn/index.php>). In order to increase their functional distance, the present lists were again selected such as to allude to the presumed chronological timetable of their roles, such as categorized by IPA as 'Behavior', 'Immune response', and 'Cellular Growth and Proliferation'. Figure 4 demonstrates that when samples from these data sets are submitted to the program it directly extracts the genes responsible for the overlap. The shared variance appeared to be substantial, such that 30% of molecules involved in 'behaviors' were also implicated in cellular development and immune functions.

Such power sharing of the regulatory molecules appears in a number of signaling pathways and networks signifying that the genetic programs regulating them are likely to be related. Using Venn diagram analysis, one study /31/ demonstrated that pollination/fertilization-related genes are also regulated by dehydration and injury. Venn diagrams do not explain why communications based on such shared vocabulary are so robust as to be conserved in evolution. That is, if polysemy is such a regular feature in molecular communication, it could impose a heavy processing cost on the regulated system (i.e., 'hearing side') of the inter-modular interactions. The other issue is the shared variance in Venn diagrams that implies the presence of complex functional code that unites all modules with the common pool of the molecular network. If these molecular signals are uttered as sentences, it is yet to be determined how they become legible or become "content-relevant" (in linguistic terms), and in what manner their specialized and shared signals are understood in different constellations. Consider the following example of the context utility captured by the title 'The Dangers of Love in Antony and Cleopatra'. A mere change of the sequences of words, say, into "The Love of Dangers..." translates the message from a Shakespeare play to events on Double-Black Diamond ski runs of the Rocky Mountains enjoyed by two teenagers.

A recent linguistic experiment helps to provide a plausible clue. In it, Selten and Warglien / 32/ had 10 pairs of subjects develop a common language in the course of interaction using a limited repertoire of letters by referring to items in varying lists of geometrical figures distinguished by up to three features. As the number of potential signals threatened

coordination failures, participants began to rely on the positional structure (combination) of messages. A similar situation exists in human languages when ‘phonic signs’ can be of less importance once other signs are available to communicate meaning /33/. In free constituent order languages such as Esperanto or Turkish, interlocutors select their actions by disambiguating the message in the pragmatic “cultural context.” In regular languages, ‘information’ is synonymous with ‘knowledge of order’ /34/.

Since free morphemes (the smallest important linguistic units) or even one-word utterances out of context have a sizeable amount of ambiguity, the smallest unit of linguistic description is not a word but the word in its syntagmatic context or sentences when combinatorics would be a natural way of molecular signals disambiguation in relatively small-size vocabulary /35/. This example suggests that in molecular communications, the usefulness of signals would originate from syntax rather than their presumed importance / 27/, so that the statistics of molecule frequencies (much like those of word frequencies) may tell us little of their relations with other molecules. In contrast to language, overlap of molecular functions cannot be validated by ‘functional semantics’. The only way to visualize molecular interactions is to marry Venn diagrams with network analysis. In the next step, we explored what happens when molecules of each of the compared groups were bonded into a single system.

4. NETWORK ANALYSIS OF SHARED VARIANCE

Networks are sets of nodes linked by connections, mathematically described as graphs that create order out of randomness /36/. Throughout the text, the terms graphs/networks and subgraphs/subnetworks have similar meaning. Nodes (vertices) of networks symbolize specific molecules, neurotransmitters or their receptors whereas edges or directed connections (denoted as arcs) represent in IPA graphs the interactions between them based on published data.

A satisfying representation of Venn’s overlaps would be created if the abbreviations of all molecules of interest or their full names (e.g., LEP/leptin) were entered into the IPA search engine in order to portray them as a group of isolated items in the processing window as shown in Fig. 2. They could be assembled into graphs using a ‘connect’ macro. The present graphs are termed *digraphs* since their connections programmed by IPA filters to show all recognized relationships between vertices, regardless of how they were established (e.g., in dissimilar tissues, paradigms, processes, and species). Such three digraphs were put together for connections based on nodes already demonstrated in Fig. 2. These are ‘Cellular Growth and Proliferation’, ‘Feeding Response’ and ‘Behavior’. They show parameter-free network architecture in the sense of coding the potential propagation of reactions rather than a timed model of information flow.

The IPA connected the submitted nodes using 162 edges. Unlike traditional Venn computations, it did not combine subnetworks by filing shared items to appear in the overlap region (the ‘intersection’). Rather it prompted to remove the duplicates in the lists that otherwise would be represented separately in Venn’s system as common to the relevant sets. Figure 5A shows a network thus created in an autolayout form that represented what would have been an overlapping pool of biomolecules in a traditional Venn’s computation. The logic of this algorithm is that it unveils numerous connections between the nodes, some of which appear as hubs. These connections mark the relevant functional and chemical points of interactions thereby illustrating why the natural universe for Venn’s circles is tenuous without documenting the presence of the rich molecular interconnections along with specific roles signified by edges that are implied by the overlapped circles.

Figure 5 shows that among numerous interconnected nodes, many form a recurrent three-node cliques, a sequence that overlaps the subgraphs of specific responses in all three groups of molecules. There may be other kinds of small circuits with dissimilar topological features that are commonly defined as network motifs. They are tacitly assumed following /37,38/ to be a group of interacting components performing elementary computational and regulatory roles. Implicit in this understanding is the notion of a ‘minimal functional unit’ capable of turning an input signal into a functional output signal. This concept of the motif is an acceptable one. However, its universality may be questioned when scrutinizing the triad of LEP-CRH-NPY because its nodes are united into a “maximal motif”, defined as a set that has more edges than are necessary to connect them. The prevalence of this motif requires a greater sample size.

Figure 5A indicates though that a sample of any size would not eliminate the need to cast the flow in network in decision-theoretical terms /8/. In order to show a computational puzzle this clique offers, it is cut off in Fig. 5B along with a few relevant molecules obscured in the clutter of the maternal graph. Its edges indicate that each node is a participant in more than one biochemical reaction (e.g., activation, inhibition, biochemical modification, protein-protein interaction, transcription, trans-location, and other) thereby potentially creating a requisite without action. In this directed clique, the orientation of the arcs cannot reflect the course of information flow, i.e., it cannot be disclaimed as ‘pseudodynamics’ /39/ representing propagation of reactions in chemical space. Granted, maximal motifs may not be more than a ‘stack’ of options available in the Ingenuity dataset, as maintained elsewhere /40/. That does not abolish the question of how a specific action is pulled from the stack at some nodes when called for.

As alluded to earlier, Venn diagrams do not take into account the surplus ‘terms’ conveyed by multifunctional arcs. Analytic investigations of these relationships are lacking. These terms may be likened to what Ziv et al. /41/ provocatively defined as edge modularity. However, this feature of vocabulary is recognized in semiotics /42/. Of central interest to the present undertaking is an attractive approach in the quantitative analysis of sense as codified by arcs that departs from the idea of *sème* (an elementary or minimal unit of meanings) taken by the French linguist Guiraud /43/. In his model, information is composed of a ‘signifier’ (i.e., a physical medium such as any coded signal) and a ‘signified’ or significance. If each signal coded in arcs or edges is proposed to act as a *sème*, the options for transmissions expand spectacularly. Arc information ought to be considered in drug designs as additional molecular interaction descriptors in order to generate functional alignments, somewhat similar to the property-based approach. A possible example of vocabulary extensions offered by arcs (acting as *sèmes*) is limited to at least ten interactions currently identified for LEP, NPY, and CRH triad (Fig. 5B).

How does this clique behave in terms of regulated physiological function? As we have seen above, small subnetworks are involved in numerous roles including the continuous monitoring of environmental inputs in order to repair damage, ensure growth, and contain stress. To answer this question, we first try to imagine the expression of CRH and its receptors in the brain of rats subjected to stress. CRH is a key mediator of endocrine, autonomic, behavioral, and immune responses to acute and chronic stress /45/, and a promising drug controlling CRH would be of interest. For example, LEP-type ligand might be a therapeutic agent of choice since its infusion reduces food intake and body weight, at least partially due to the enhancement of the anorectic effect via type 2 CRH receptor in the ventromedial hypothalamus /46/. However, LEP is a pluripotent regulatory protein with diverse non-feeding effects, such as modulation of immune function, memory, bone growth, reproduction, and neurogenesis among others. It was recently shown to blunt the stress-induced expression of type 1 CRH receptor /47/. Clinically, anorexia may not be a desirable

effect in the treatment of all stress-related illnesses, and so to be therapeutically useful our hypothetical 'LEP-type' CRH antagonist should be redesigned to allow better selectivity.

In the present study, we did not intend to trace the signals from their origin in network to a specific effect. In this regard, Fig. 5B emulates endgame positions in chess where motifs are also a basic element in determining how and when the piece moves. There is a difference from moving chess pieces since each node of LEP, NPY, and CRH triad stands for a high level of density of similar entities in local connectivity. Therefore, traffic traversing the LEP-CRH-NPY clique may require a particular profile for channeling a selective action. A subgraph in Fig. 5B is shown in a subcellular layout showing that most biomolecules reside in the extracellular space. As soon as CRH and arginine vasopressin (AVP) are secreted into the circulation, they act synergistically to increase pro-opiomelanocortin (POMC) transcription thereby complicating the prediction of traffic in the network by including LEP-melanocortin pathway. There are other choices in AVP-CRH synergism as shown next to the connecting arcs. Assuming that the order of interactions matters, a crude permutation estimated for one clique suggests that there can be 720 single-sème choices at a time. A key role played by these interactions is that of directing, calibrating, and timing particular actions. This indicates how difficult it is to compute the sème values in terms of the order of reaction initiations, their choice, direction, and the amount of time elapsed along the transmission trajectory. The presence of such embedded cliques with nodes co-regulated by more than one dissimilar enzymatic and biophysical option executed in uncertain successions defies at present the efforts to approximate signal propagations from ligand occupancy of receptors with a satisfying methodology similar to the currently available for portrayal of information-processing in brain networks /48/.

A geometric layout shown in Fig. 5 conveys a deceptive visual image as though the motifs are structural anatomical entities/49/ with the motionless cast of characters embedded in the lattice. In reality, the majority of biomolecules and other ligands that would be interacting with them presumably remain in a dynamic equilibrium of the self-assembling/controlled disassembling processes/50/ where in keeping with the repertory of interactions codified by the arcs, some connections may disappear almost as soon as they are formed. Many of these reactions do not require anatomical connections so that maximal motifs fall into the category of truly functional entities /49/. When targeting the NPY member of the motif in order to modulate its levels during stress situations /51/ another unexpected complication might arise that was not reflected in the graph. NPY receptors are widely distributed in the central nervous system and could be co-expressed, thereby resulting in heterodimerization /52/. NPY receptors are implicated in anxiolytic and orexigenic effects, as well as neuroproliferative /53/ and different pathological conditions such as epilepsy and neurodegenerative diseases/54/. In addition, NPY acts through Y1 and Y5 receptors to stimulate feeding, decrease thermogenesis, and increase weight /55/, so that predicting responses to NPY agonist and antagonist could be exceedingly complex for it requires taking into account an immediate prehistory of responses of each particular node. The detection of heteromers with different pharmacological and signaling properties showed that agonists and antagonists acting on the same receptor could induce different cellular response patterns. The designs for the multiple-action compounds, such as many receptor-heteromer-specific compounds /56, 57/ that could achieve completely opposite read-outs are on the agenda. This leads us to the final point.

5. CONCLUSIONS AND CAVEATS: BACK TO 'MAGIC SHOTGUNS'

The study began with the declaration that nutritional biomolecules form a small-vocabulary system that was conserved in evolution and utilized also outside of nutritional context. Inevitably then, the same bioactive peptides appear in different situations with different

meaning. This feature was likened to polysemy. What can we learn from this metaphor? For one, the notion of polysemy helped to organize the logic of presentation and exposed difficulties when trying to show why the same nodes must be biochemically shielded when interacting in network motifs for the gamut of non-nutritional functions. This metaphor cannot tell us how a molecule is selected from similar other molecules in the same environment. It is tempting to think of flow directions in networks as governed through learning. That is, each molecular pattern initially tested in transmission trials and later, the fine-tuning of prior knowledge, becomes 'stronger' through some form of molecular memory. Subsequently, a used path could be chosen as a preferred alternative under nutritional demands and rewards /58/. Likewise, the path of interaction may be facilitated by taking into account the patterns of physical and/or psychological stressors experienced in the past /59/.

In different linguistic environments, polysemy demands a considerable cross-cultural acumen needed by the hearer to disambiguate the messages. In Jones' reflections on Grice, /60/ the hearer must be prepared to make out what is said, what is conventionally implicated, and what is conversationally implicated. Using individual molecules in predictable sequences may conceivably overcome the ambiguity of polysemy. Organized in motifs, the same molecule could appear in different physiological and developmental contexts, thereby conforming to the requirement of clarity on the receiving end. One might object to using this example by pointing out that molecular targets are less likely to require verifying arriving signals since they exist in the medium of chemical and/or electrical parameters. If for consistency sake, we are to insist on the utility of linguistic metaphors, such identically written signals would be defined as 'homographs' /61/. Yet homographs, too, may have dissimilar connotation in different contexts and thus may not eliminate semantic uncertainty. Consider, the word 'mean' that could *mean* (imply, among others things) being cruel, as well as an average. Would then molecular homographs secure against errors in the course of nutritional 'texting'? The answer is in the negative. That is why.

It was assumed in the past that all ligands that occupy the same receptor elicit responses that differ only quantitatively. There is no empirical or theoretical basis to expect that the shape of the ligand assure the fidelity of messages in the crowded extracellular space filled with 'water'. The now classical studies of David Koshland alert to the presence of a degree of binding promiscuity that violates an antiquated lock-and-key view of ligand-receptor interaction /62,63/. Even rigid binding sites may accept a variety of partners /64/. In addition, allosteric proteins are capable of propagating conformational charges over significant distances. That indicates that substrates at active sites would generate analogous changes of sufficient amplitude such as to be read by a number of unintended recipients /65/. Although functional promiscuity may seem as an undesirable 'noise' in the system, gradually, it was recognized as an essential instrument of evolution /63/, possibly adopted ever since bacteria were the dominant species of the planet. For example, *E. coli* has only five chemoreceptor proteins to activate their chemotaxis to obtain food. At the same time, some chemoreceptors recognize multiple compounds such that bacteria are directed toward greater than 30 compounds /66/. The utility of these functions is still in the realm of developmental and evolutionary biology examining "how cells are able to reign in biochemical noise" /67/. Besides the fascination of the challenges posed by the puzzle, a practical question that occupies medicinal chemists is how they could make the most of it (or at least, cope with it), and by what means.

Pharmacotherapy of recent years was driven by the view that therapeutic agents must fit receptor monomers for a very narrow therapeutic profile (the "magic bullets"). Such designs were kept in circulation by the concepts created on the model of infections attributing numerous disorders to aberrations within a pathogenesis-specific single-neurotransmitter

system or some unique single-target diseases. The majority of diseases, however, including those associated with obesity are now recognized to require handling numerous therapeutic targets at once. This would entail a change of the paradigm since the amount of money a typical company is expected to spend on drug designed the 'classical way' and then approved for marketing is staggering /68/. For neurological disorders alone, an estimated total R&D cost is over US\$ 1,000 million in 2000 dollars /69/ that is likely to exceed that figure today. The rate of failure of presumably successful preclinical candidates is hardly expected to diminish in the near future. In a change of paradigm, Roth et al. /70/ propose designing multitargeted agents dubbed as "selectively non-selective drugs" or "specially engineered shotgun loads"/71/ that by interacting with several molecular targets should lead to new and more effective medications.

What seems to count in polypharmacology is not just the maximal specificity of particular agents, but also their ability to generate effects by engaging numerous 'primary' binding sites on the predictable time-scale. The present study adds one more group of targets, which entails multiple types of interactions themselves (edge modularity /41/). As represented by the arcs, the latter act as scaffolds of networks by determining the kind of structure networks assume when their nodes undergo repeated uncoupling and binding. There are a number of elegant pharmacophore models /44,72–75/ that use the point-based alignment algorithms searching for "chemical functionalities" for engaging each target of interest. However, locating an efficacious fit of ligands to their respective biological target structures is unlikely to resolve the messages when they are 'texted' by molecules with identical chemical features, but on different paths in networks, 3D position or distinctive features of interaction within the functional group. In this regard, scaffoldings become targets and also affect the order of encounters of extrinsic agents with nodes, and establish the readiness of nodes for interactions. The future drug-like pharmaceutical for multiple targets need to handle many network members collectively. Notwithstanding the difficulties, the functionality of multitargeted therapy and the strategies of drug discovery have become a topic of ongoing discussions (/76–80/ and references therein).

The origin of 'magic' moniker was rooted in an auspicious scenario. As the German folklore has it, the protagonist of Carl Maria von Weber's opera "Der Freischütz" (*The Marksman*) accepted seven magic bullets in a misguided contract with the Devil. Unbeknown to him, the price of winning in the shooting contest must have been associated with a deadly 'side effect' for his bride, but fortuitously was not. By contrast, the avant-garde version of the legend, in the show "The Black Rider", all bullets offered by Satan were also able to hit magically any target as promised. Yet, in a less forgiving but medically a more realistic scenario, the final bullet strikes the beloved of the 'marksmen'. Amazingly, some drugs that were contemptuously branded as pharmaceutical shotgun remedies of the turn of 19th century appeared to be more efficacious than meticulously crafted 'clean' drugs /70/. Since then, time has moved on. In the past decades, the 'magic-bullets' approach produced no efficacious drugs for treating eating disorders. The development of compounds binding to a combination of docking sites seems to yield a better chance to assure therapeutic outcome without causing untoward effects in connected but pathophysiologically irrelevant bystanders. Whether this hope actually bears fruit remains to be seen.

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Fig. 1.

A bar chart of functions generated with IPA (Ingenuity R Systems, www.ingenuity.com) functional analysis that implicate ingestions-control peptides. Taller bars are more significant than shorter bars. The probability that each biological function assigned to the set of molecules is due to chance alone was determined using Fischer's exact test. Y-axis represents the significance score (negative log of p value; B-H: Benjamini-Hochberg method was used for multiple testing corrections). The vertical line denotes the cutoff for significance (p -value of 0.05).

Fig. 2. Molecules isolated during functional analyses as playing statistically significant roles in functions, such as ‘Cellular Growth and Proliferation’, ‘Feeding’ and ‘Behavior’ (see legend on the right). Here, as well as in Fig. 1, functions that exceed the random expectation of their probability are shown in p -values based on Fisher’s exact test. A sample of functions shown in IPA overlay indicates the same molecules a number of identical biomolecules recruited for Vascular development, Memory, Immune response, and Affiliative behavior. These roles were arbitrarily selected from a wider display of available in the overlay macro in order to emphasize the fact they evolved at dissimilar time in individual development.

Fig. 3.

A plot testing Zipf distribution using *Hermetic Word Frequency Counter*¹ based on ranking 850 terms of 91,322 scanned items from IPA-generated studies pooled together. Points are the experimental data and straight line with a negative slope is the best fit to Zipf's law. Note a group of terms (shown by arrows) with the same frequency and different rank. Despite an adequate agreement with Zipf's law first five top-ranking datapoints, (LEP, NPY, CRH, POMC, and INC) pull the left tail of the curve down.

Abbreviations: CRH - corticotropin releasing hormone; INS – insulin; LEP – leptin; NPY – neuropeptide Y; POMC – proopiomelanocortin.

¹(<http://www.hermetic.ch/wfca/wfca.htm>)

Fig. 4.

Venn diagram with three sets that represent in the various distinct areas of overlapping circles all possible combinations of the relevant molecules (in %) playing statistically significant roles in corresponding functions ('Behavior', 'Immune response', and 'Cellular Growth and Proliferation').

Fig. 5.

(A) Biomolecules playing statistically significant roles in ‘Cellular Growth and Proliferation’, ‘Feeding’ and ‘Behavior’; (see Fig. 2 and legend) connected in a single graph (in autolayout). All duplicate molecules (Venn’s “overlap”) were eliminated when submitted to ‘connect’ macro. (B) Maximal triad CRH, LEP, and NPY oversized for visibility along with AVP and POMC in a toggle subcellular layout. Note that in distinction from the classical Venn computations, network rendering emphasized the role of interconnections that includes all relevant information such as the layout constraints for specific functions and type of reactions involved. Arrowheads signify direct interactions between nodes. Lines with a terminal bar indicate negative regulation. In order to reduce the clutter, self-interaction arcs (loops) were removed. Edge labels: A - Activation; B - Binding; - expression (includes metabolism); I - inhibition; LO - localization; P - Phosphorylation/Dephosphorylation; PP - protein-protein binding; T - transcription