

As much as 80% of the LHC antenna is mobile in *Chlamydomonas* during state transitions²⁵, whereas in land plants the mobile fraction of LHCII is only 15–20%³. In spite of this relatively low value, our study shows that in land plants the STN7 kinase, and probably state transitions, are important for adaptation and that in their absence growth is significantly impaired under conditions in which light quality and quantity change frequently. This points to the importance of state transitions in a natural environment where plants are often subjected to light fluctuations of this sort. □

Methods

Plant material

Arabidopsis thaliana (L.) ecotype Columbia (Col-0) was used for all experiments. Plants were grown under controlled conditions of light (50 or 160 $\mu\text{mol m}^{-2} \text{s}^{-1}$; 8 h or 12 h photoperiods, 23/20 °C day/night, and relative air humidity of 50–70%).

All physiological and biochemical analyses were performed with rosette leaves harvested before flowering. We obtained the T-DNA insertion lines in the Columbia background for Atlg68830 (SALK 073254) and At5g01920 (SALK 060869 and SALK 064913) from the Salk Institute (see Supplementary Information for the characterization of these lines and for the DNA, RNA, protein and chlorophyll analyses).

State transitions and NPQ

State transitions and NPQ were measured as described^{11,26} (see Supplementary Information for details).

Photosynthetic measurements

Photosynthetic gas exchange and chlorophyll fluorescence measurements were simultaneously performed on detached leaves using a LI-6400 portable photosynthesis system equipped with a 6400-40 fluorometer (LI-COR Biosciences) (for details see Supplementary Information). PSII light was obtained with cool white fluorescent lamps (Osram L18W/20) with orange 105 Lee filters and PSI light was obtained with red fluorescent lamps (Osram L18W/60) with red 027 Lee filters. Chlorophyll fluorescence emission spectra of thylakoid membrane suspensions were recorded in liquid nitrogen (77 K) as described^{27,28} (see Supplementary Information).

In vivo and in vitro phosphorylation of the LHCII antennae

Leaves from dark-acclimated plants, floating on water, were exposed to low light (80 $\mu\text{mol m}^{-2} \text{s}^{-1}$) or kept in the dark for 30 min²⁹. Thylakoid membranes were isolated from the dark-incubated and illuminated leaves as described²⁷ in the presence of 10 mM NaF to inhibit phospho-LHCII phosphatase activity. Thylakoids were re-suspended in assay buffer consisting of 50 mM HEPES-KOH pH 7.5, 100 mM sucrose, 5 mM NaCl, 10 mM MgCl_2 and 10 mM NaF at a final chlorophyll concentration of 0.4 mg ml^{-1} .

After dark adaptation, thylakoids were isolated from plants according to ref. 30, and re-suspended in storage buffer (100 mM sorbitol, 5 mM MgCl_2 , 5 mM NaCl and 50 mM HEPES/KOH pH 7.5). They were used as substrate for the kinase assay. Thylakoid membrane proteins equivalent to 8 μg of chlorophyll were subjected to a 20 min light induction (80 $\mu\text{mol m}^{-2} \text{s}^{-1}$) at 25 °C in the presence of 10 μCi [γ -³²P]ATP (Amersham 3,000 Ci mmol^{-1}), 0.4 mM ATP and 10 mM NaF in 100 μl of storage buffer²⁹. Reactions were terminated by centrifugation, washing twice in storage buffer and addition of denaturing sample buffer, and were electrophoresed on 12% polyacrylamide-SDS gels, and finally analysed with a phosphorimager.

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Functional cartography of complex metabolic networks

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High-throughput techniques are leading to an explosive growth in the size of biological databases and creating the opportunity to revolutionize our understanding of life and disease. Interpretation of these data remains, however, a major scientific challenge. Here, we propose a methodology that enables us to extract

and display information contained in complex networks^{1–3}. Specifically, we demonstrate that we can find functional modules^{4,5} in complex networks, and classify nodes into universal roles according to their pattern of intra- and inter-module connections. The method thus yields a ‘cartographic representation’ of complex networks. Metabolic networks^{6–8} are among the most challenging biological networks and, arguably, the ones with most potential for immediate applicability⁹. We use our method to analyse the metabolic networks of twelve organisms from three different superkingdoms. We find that, typically, 80% of the nodes are only connected to other nodes within their respective modules, and that nodes with different roles are affected by different evolutionary constraints and pressures. Remarkably, we find that metabolites that participate in only a few reactions but that connect different modules are more conserved than hubs whose links are mostly within a single module.

If we are to extract the significant information from the topology of a large, complex network, knowledge of the role of each node is of crucial importance. A cartographic analogy is helpful to illustrate this point. Consider the network formed by all cities and towns in a country (the nodes) and all the roads that connect them (the links). It is clear that a map in which each city and town is represented by a circle of fixed size and each road is represented by a line of fixed width is hardly useful. Rather, real maps emphasize capitals and important communication lines so that we can obtain scale-specific information at a glance. Similarly, it is difficult, if not impossible, to obtain information from a network with hundreds or thousands of nodes and links, unless the information about nodes and links is conveniently summarized. This is particularly true for biological networks.

Here, we propose a methodology, which is based on the connectivity of the nodes, that yields a cartographic representation of a complex network. The first step in our method is to identify the functional modules^{4,5} in the network. In the cartographic picture, modules are analogous to countries or regions, and enable a coarse-grained, and thus simplified, description of the network. Then we classify the nodes in the network into a small number of system-independent ‘universal roles’.

It is common that social networks have communities of highly interconnected nodes that are less connected to nodes in other communities. Such modular structures have been reported not only in social networks^{5,10–12}, but also in food webs¹³ and biochemical networks^{4,14–16}. It is widely believed that the modular structure of complex networks plays a critical role in their functionality^{4,14,16}. There is therefore a clear need to develop algorithms to identify modules accurately^{5,11,17–20}.

We identify modules by maximizing the network’s modularity^{11,18,21} using simulated annealing²² (see Methods). Simulated annealing enables us to perform an exhaustive search and to minimize the problem of finding sub-optimal partitions. It is noteworthy that, in our method, we do not need to specify a priori the number of modules; rather, this number is an outcome of the algorithm. Our algorithm is able to reliably identify modules in a network whose nodes have as many as 50% of their connections outside their own module (Fig. 1).

When considering modular networks, it is plausible to surmise that the nodes in a network are connected according to the role they fulfil. This fact has been long recognized in the analysis of social networks²³. For example, in a classical hierarchical organization, the chief executive is not directly connected to plant employees but is connected to the members of the board of directors. Such a

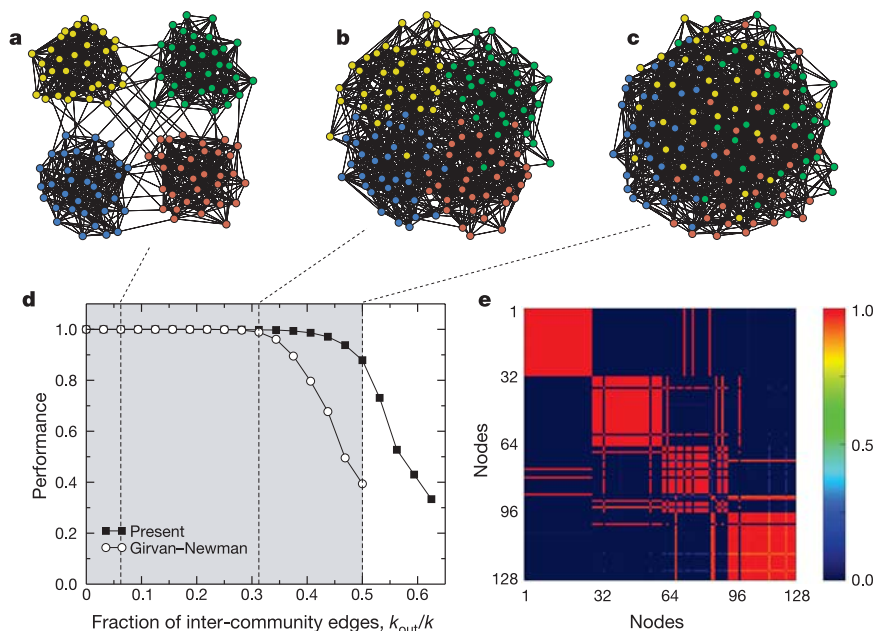


Figure 1 Performance of module identification methods. To test the performance of the method, we build ‘random networks’ with known module structure. Each test network comprises 128 nodes divided into 4 modules of 32 nodes. Each node is connected to the other nodes in its module with probability p_i , and to nodes in other modules with probability $p_o < p_i$. On average, thus, each node is connected to $k_{out} = 96 p_o$ nodes in other modules and to $k_{in} = 31 p_i$ in the same module. Additionally, p_i and p_o are selected so that the average degree of the nodes is $k = 16$. We display networks with: **a**, $k_{in} = 15$ and $k_{out} = 1$; **b**, $k_{in} = 11$ and $k_{out} = 5$; and **c**, $k_{in} = k_{out} = 8$. **d**, The performance of a module identification algorithm is typically defined as the fraction of correctly classified

nodes. We compare our algorithm to the Girvan–Newman algorithm^{5,18}, which is the reference algorithm for module identification^{11,18,19}. Note that our method is 90% accurate even when half of a node’s links are to nodes in outside modules. **e**, Our module-identification algorithm is stochastic, so different runs yield, in principle, different partitions. To test the robustness of the algorithm, we obtain 100 partitions of the network depicted in **c** and plot, for each pair of nodes in the network, the fraction of times that they are classified in the same module. As shown in the figure, most pairs of nodes are either always classified in the same module (red) or never classified in the same module (dark blue), which indicates that the solution is robust.

statement holds for virtually any organization; that is, the role of chief executive is defined irrespective of the particular organization considered.

We propose a new method to determine the role of a node in a complex network. Our approach is based on the idea that nodes with the same role should have similar topological properties²⁴ (see Supplementary Information for a discussion on how our approach relates to previous work). We predict that the role of a node can be determined, to a great extent, by its within-module degree and its participation coefficient, which define how the node is positioned in its own module and with respect to other modules^{25,26} (see Methods). These two properties are easily computed once the modules of a network are known.

The within-module degree z_i measures how ‘well-connected’ node i is to other nodes in the module. High values of z_i indicate high within-module degrees and vice versa. The participation coefficient P_i measures how ‘well-distributed’ the links of node i are among different modules. The participation coefficient P_i is close to 1 if its links are uniformly distributed among all the modules, and 0 if all its links are within its own module.

We define heuristically seven different universal roles, each defined by a different region in the z - P parameter space (Fig. 2). According to the within-module degree, we classify nodes with $z \geq 2.5$ as module hubs and nodes with $z < 2.5$ as non-hubs. Both hub and non-hub nodes are then more finely characterized by using the values of the participation coefficient (see Supplementary Information for a detailed justification of this classification scheme, and for a discussion on possible alternatives).

We find that non-hub nodes can be naturally divided into four different roles: (R1) ultra-peripheral nodes; that is, nodes with all their links within their module ($P \leq 0.05$); (R2) peripheral nodes; that is, nodes with most links within their module ($0.05 < P \leq 0.62$); (R3) non-hub connector nodes; that is, nodes with many links to other modules ($0.62 < P \leq 0.80$); and (R4) non-hub kinless nodes; that is, nodes with links homogeneously distributed among all modules ($P > 0.80$). We find that hub nodes can be naturally divided into three different roles: (R5) provincial hubs; that is, hub nodes with the vast majority of links within their module ($P \leq 0.30$); (R6) connector hubs; that is, hubs with many links to most of the other modules ($0.30 < P \leq 0.75$); and (R7) kinless hubs; that is, hubs with links homogeneously distributed among all modules ($P > 0.75$).

To test the applicability of our approach to complex biological networks, we consider the cartographic representation of the metabolic networks^{6–9,14} of twelve organisms: four bacteria (*Escherichia coli*, *Bacillus subtilis*, *Lactococcus lactis* and *Thermosynechococcus elongatus*), four eukaryotes (*Saccharomyces cerevisiae*, *Caenorhabditis elegans*, *Plasmodium falciparum* and *Homo sapiens*), and four archaea (*Pyrococcus furiosus*, *Aeropyrum pernix*, *Archaeoglobus fulgidus* and *Sulfolobus solfataricus*). In metabolic networks, nodes represent metabolites and two nodes i and j are connected by a link if there is a chemical reaction in which i is a substrate and j a product, or vice versa. In our analysis, we use the database developed by Ma and Zeng⁸ (MZ) from the Kyoto Encyclopedia of Genes and Genomes²⁷ (KEGG). The results we report are not altered if we consider the complete KEGG database instead (Figs 2c and 4b, and Supplementary Information).

First, we identify the functional modules in the different metabolic networks (Fig. 3). Finding modules in metabolic networks purely on the basis of topological properties is an extremely important task. For example, Schuster *et al.* have reported on the impossibility of obtaining elementary flux modes²⁸ from complete metabolic networks due to the combinatorial explosion of the number of such modes²⁹. Our algorithm identifies an average of 15 different modules in each metabolic network—with a maximum of 19 for *E. coli* and *H. sapiens*, and a minimum of 11 for *A. fulgidus*. As expected, the density of links within each of the modules is

significantly larger than between modules—typically 100–1,000 times larger (see Supplementary Information).

To assess how each of the modules is related to the pathways traditionally defined in biology, we use the classification scheme proposed in KEGG, which includes nine major pathways: carbohydrate metabolism, energy metabolism, lipid metabolism, nucleotide metabolism, amino-acid metabolism, glycan biosynthesis and metabolism, metabolism of cofactors and vitamins, biosynthesis of secondary metabolites and biodegradation of xenobiotics. Each metabolite in the KEGG database is assigned to at least one pathway; thus, we can determine to which pathways the metabolites in a given

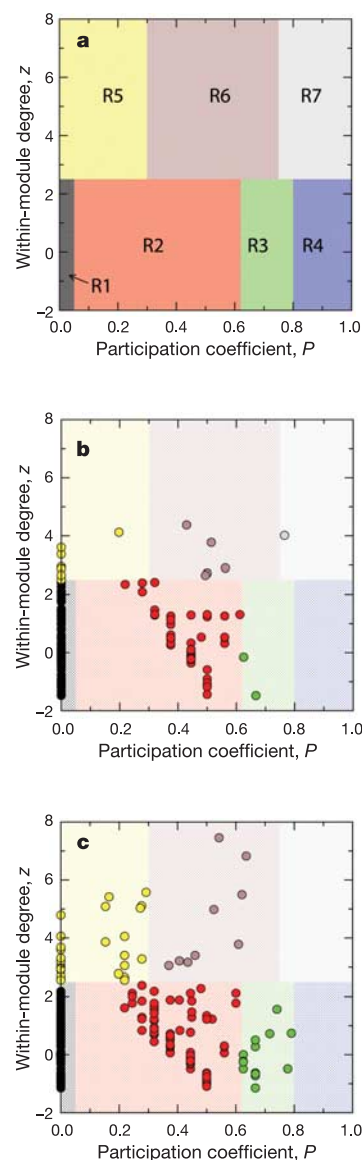


Figure 2 Roles and regions in the z - P parameter space. **a**, Each node in a network can be characterized by its within-module degree and its participation coefficient (see Methods for definitions). We classify nodes with $z \geq 2.5$ as module hubs and nodes with $z < 2.5$ as non-hubs. We find that non-hub nodes can be naturally assigned into four different roles: (R1) ultra-peripheral nodes; (R2) peripheral nodes; (R3) non-hub connector nodes; and (R4) non-hub kinless nodes. We find that hub nodes can be naturally assigned into three different roles: (R5) provincial hubs; (R6) connector hubs; and (R7) kinless hubs (see text and Supplementary Information for details). **b**, Metabolite role determination for the metabolic network of *E. coli*, as obtained from the MZ database. Each metabolite is represented as a point in the z - P parameter space, and is coloured according to its role. **c**, Same as **b** but for the complete KEGG database.

module belong. We find that most modules contain metabolites mostly from one major pathway. For example, in 17 of the 19 modules identified for *E. coli*, more than one-third of the metabolites belong to a single pathway. Interestingly, some other modules—two in the case of *E. coli*—cannot be trivially associated with a single traditional pathway. These modules are typically central in the metabolism and contain, mostly, metabolites that are classified in KEGG as belonging to carbohydrate and amino-acid metabolism.

Next we identify the role of each metabolite. In Fig. 2b we show the roles identified in the metabolic network of *E. coli*. Other organisms show a similar distribution of the nodes in the different roles, even though they correspond to organisms that are very distant from an evolutionary standpoint (see Supplementary Information). Role R1, which contains ultra-peripheral metabolites with small degree and no between-module links, comprises 76–86% of all the metabolites in the networks. This considerably simplifies the coarse-grained representation of the network as these nodes do not need to be identified separately. Note that this finding alone represents an important step towards the goal of extracting scale-specific information from complex networks.

The information about modules and roles enables us to build a cartographic representation of the metabolic network of, for example, *E. coli* (Fig. 3). This representation enables us to recover relevant biological information. For instance, we find that the metabolism is mostly organized around the module containing pyruvate, which in turn is strongly connected to the module whose hub is acetyl-coenzyme A (CoA). These two molecules are key to connecting the metabolism of carbohydrates, amino acids and lipids to the tricarboxylic acid (TCA) cycle from which ATP is obtained.

These two modules are connected to more peripheral ones by key metabolites such as D-glyceraldehyde 3-phosphate and D-fructose 6-phosphate (which connect to the glucose and galactose metabolisms), D-ribose 5-phosphate (which connects to the metabolism of certain nucleotides), and glycerone phosphate (which connects to the metabolism of certain lipids).

Our analysis also uncovers nodes with key connector roles that take part in only a small but fundamental set of reactions. For example, *N*-carbamoyl-L-aspartate takes part in only three reactions but is vital because it connects the pyrimidine metabolism, whose hub is uracil, to the core of the metabolism through the alanine and aspartate metabolism. The potential importance of such non-hub connectors points to another consideration. It is a plausible hypothesis that nodes with different roles are under different evolutionary constraints and pressures. In particular, we expect that nodes with structurally relevant roles are more necessary and therefore more conserved across species.

To quantify the relation between roles and conservation, we define the loss rate $p_{\text{lost}}(R)$ (see Methods). Structurally relevant roles are expected to have low values of $p_{\text{lost}}(R)$ and vice versa. We find that the different roles have different loss rates (Fig. 4). As expected, ultra-peripheral nodes (role R1) have the highest loss rate whereas connector hubs (role R6) are the most conserved across all species considered.

The results for the comparison of $p_{\text{lost}}(R)$ for ultra-peripheral nodes and connector hubs is illustrative, but hardly surprising. The comparison of $p_{\text{lost}}(R)$ for non-hub connectors (role R3) and provincial hubs (role R5), however, yields a surprising finding. The metabolites in the provincial hubs class have many within-module

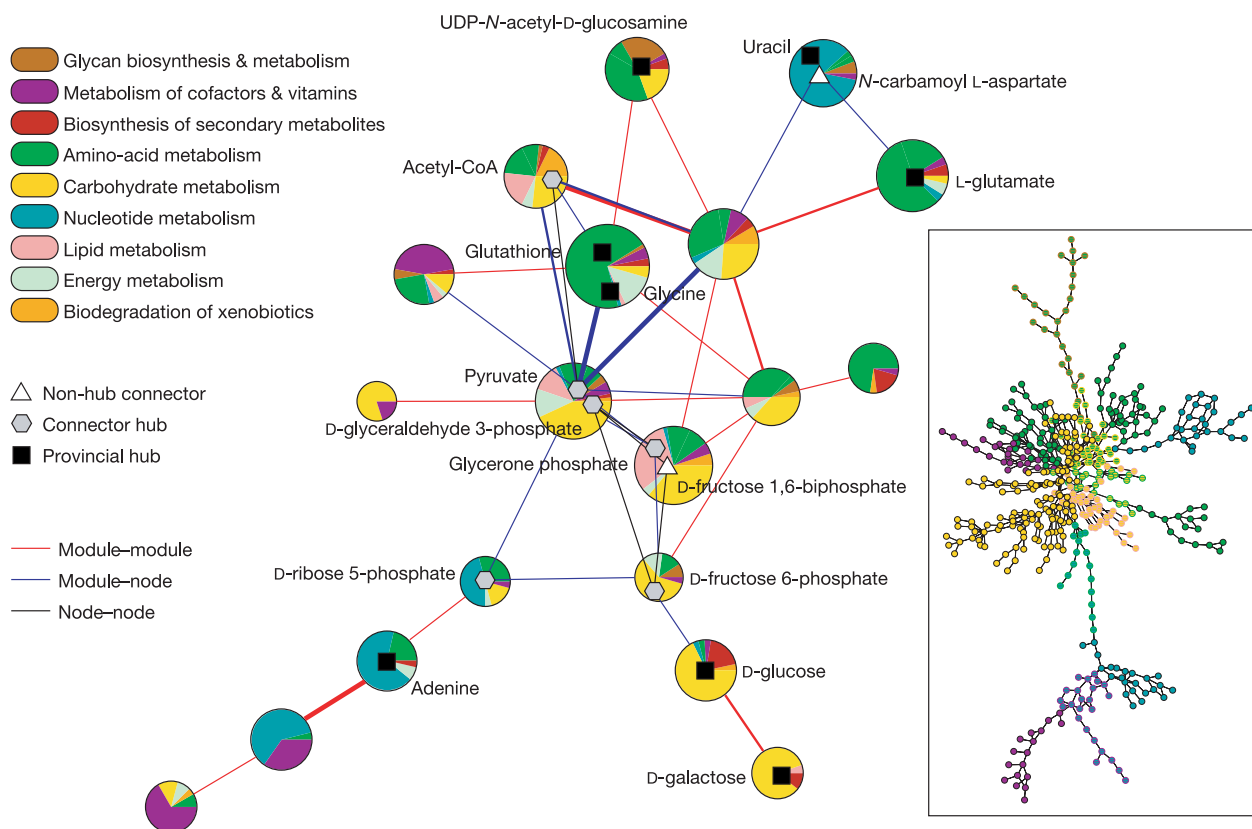


Figure 3 Cartographic representation of the metabolic network of *E. coli*. Each circle represents a module and is coloured according to the KEGG pathway classification of the metabolites it contains. Certain important nodes are depicted as triangles (non-hub connectors), hexagons (connector hubs) and squares (provincial hubs). Interactions between modules and nodes are depicted using lines, with thickness proportional to the

number of actual links. Inset: metabolic network of *E. coli*, which contains 473 metabolites and 574 links. This representation was obtained using the program Pajek. Each node is coloured according to the 'main' colour of its module, as obtained from the cartographic representation.

connections, sometimes as many as five standard deviations more connections than the average node in the module. Conversely, non-hub connector metabolites have few links relative to other nodes in their modules—and fewer total connections than the metabolites in role R5 (see Supplementary Fig. S12b, c). The links of non-hub connectors, however, are distributed among several different

modules, whereas the links of provincial hubs are mainly within their modules. We find that non-hub connectors are systematically and significantly more conserved than provincial hub metabolites (Fig. 4).

A possible explanation for the high degree of conservation of non-hub connectors is as follows. Connector nodes are responsible for inter-module fluxes. These modules are otherwise poorly connected or not connected at all to each other, so the elimination of connector metabolites will probably have a large impact on the global structure of fluxes in the network. On the contrary, the pathways in which provincial hubs are involved may be backed up within the module in such a way that elimination of these metabolites may have a comparatively smaller impact, which in addition would probably be confined to the module containing the provincial hub.

Our results therefore point to the need to consider each complex biological network as a whole, instead of focusing on local properties. In protein networks, for example, it has been reported that hubs are more essential than non-hubs³⁰. Notwithstanding the relevance of such a finding, our results suggest that the global role of nodes in the network might be a better indicator of their importance than degree²⁶.

Our ‘cartography’ provides a scale-specific method to process the information contained in the structure of complex networks, and to extract knowledge about the function performed by the network and its constituents. An open question is how to adapt current module-detection algorithms to networks with a hierarchical structure.

For metabolic networks—a comparatively well studied and well understood case—our method allows us to recover firmly established biological facts, and to uncover important new results, such as the significant conservation of non-hub connector metabolites. Similar results can be expected when our method is applied to other complex networks that are not as well studied as metabolic networks. Among those, protein interaction and gene regulation networks may be the most significant. □

Methods

Modularity

For a given partition of the nodes of a network into modules, the modularity M of this partition is^{11,18,21}:

$$M = \sum_{s=1}^{N_M} \left[\frac{l_s}{L} - \left(\frac{d_s}{2L} \right)^2 \right] \quad (1)$$

where N_M is the number of modules, L is the number of links in the network, l_s is the number of links between nodes in module s , and d_s is the sum of the degrees of the nodes in module s . The rationale for this definition of modularity is the following. A good partition of a network into modules must comprise many within-module links and as few as possible between-module links. However, if we just try to minimize the number of between-module links (or, equivalently, maximize the number of within-module links) the optimal partition consists of a single module and no between-module links. Equation (1) addresses this difficulty by imposing that $M = 0$ if nodes are placed at random into modules or if all nodes are in the same cluster^{11,18,21}.

The objective of a module identification algorithm is to find the partition with largest modularity, and several methods have been proposed to attain such a goal. Most of them rely on heuristic procedures and use M , or a similar measure, only to assess their performance. In contrast, we use simulated annealing²² to find the partition with the largest modularity.

Simulated annealing for module identification

Simulated annealing²² is a stochastic optimization technique that enables you to find ‘low-cost’ configuration without getting trapped in ‘high-cost’ local minima. This is achieved by introducing a computational temperature T . When T is high, the system can explore configurations of high cost whereas at low T the system only explores low-cost regions. By starting at high T and slowly decreasing T , the system descends gradually towards deep minima, eventually overcoming small cost barriers.

When identifying modules, the objective is to maximize the modularity, and thus the cost is $C = -M$, where M is the modularity as defined in equation (1). At each temperature, we perform a number of random updates and accept them with probability:

$$P = \begin{cases} 1 & \text{if } C_f \leq C_i \\ \exp\left(-\frac{C_f - C_i}{T}\right) & \text{if } C_f > C_i \end{cases} \quad (2)$$

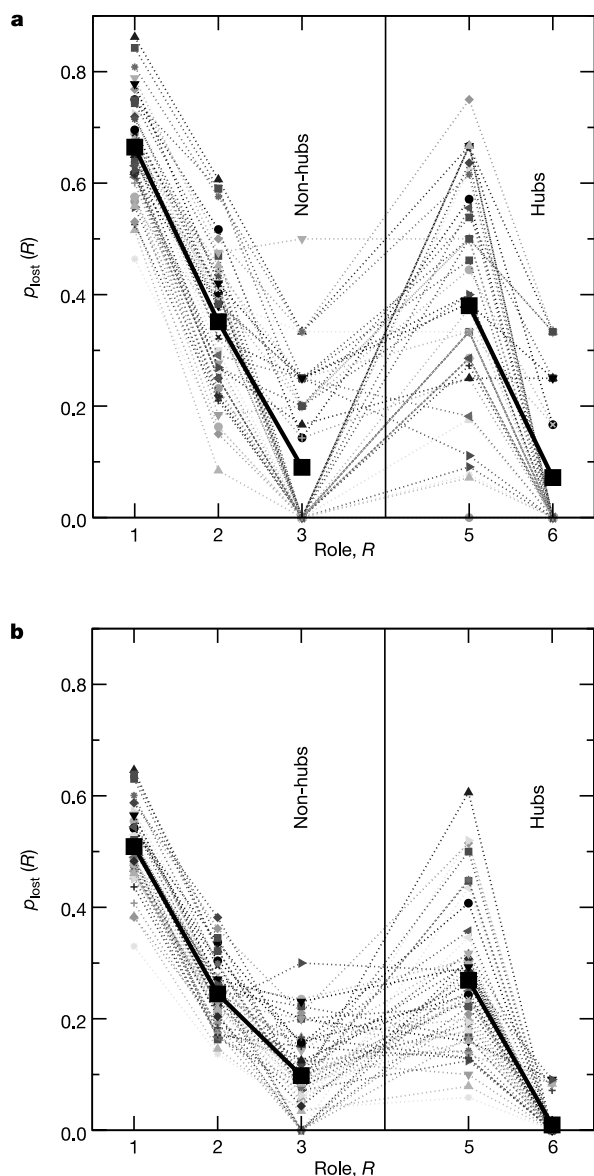


Figure 4 Roles of metabolites and inter-species conservation. To quantify the relation between roles and conservation, we calculate the loss rate $p_{\text{lost}}(R)$ of each metabolite (see Methods). Each thin line in the graph corresponds to a comparison between two species. Because we are interested in metabolites that are present in some species but missing in others, metabolic networks of species within the same superkingdom—bacteria, eukaryotes and archaea—are usually too similar to provide statistically sound information, especially for roles containing only a few metabolites. Therefore, we consider in our analysis only pairs of species that belong to different superkingdoms. The thick line is the average over all pairs of species. The loss rate $p_{\text{lost}}(R)$ is maximum for ultra-peripheral (R1) nodes and minimum for connector hubs (R6). Provincial hubs (R5) have a significantly and consistently higher $p_{\text{lost}}(R)$ than non-hub connectors (R3), even though the within-module degree and the total degree of provincial hubs is larger. Note that, out of the total 48 pair comparisons, only in two cases is $p_{\text{lost}}(R)$ lower for provincial hubs than for non-hub connectors, whereas the opposite is true in 44 cases. **a, b**, Results obtained for the MZ database (**a**) and the complete KEGG database (**b**).

where C_f is the cost after the update and C_i is the cost before the update.

Specifically, at each T we propose $n_i = fS^2$ individual node movements from one module to another, where S is the number of nodes in the network. We also propose $n_c = fS$ collective movements, which involve either merging two modules or splitting a module. For f we typically choose $f = 1$. After the movements are evaluated at a certain T , the system is cooled down to $T' = cT$, with $c = 0.995$.

Within-module degree and participation coefficient

Each module can be organized in very different ways, ranging from totally centralized—with one or a few nodes connected to all the others—to totally decentralized, with all nodes having similar connectivities. Nodes with similar roles are expected to have similar relative within-module connectivity. If κ_i is the number of links of node i to other nodes in its module s_i , $\bar{\kappa}_{s_i}$ is the average of κ over all the nodes in s_i , and $\sigma_{\kappa_{s_i}}$ is the standard deviation of κ in s_i , then:

$$z_i = \frac{\kappa_i - \bar{\kappa}_{s_i}}{\sigma_{\kappa_{s_i}}} \quad (3)$$

is the so-called z -score. The within-module degree z -score measures how well-connected node i is to other nodes in the module.

Different roles can also arise because of the connections of a node to modules other than its own. For example, two nodes with the same z -score will play different roles if one of them is connected to several nodes in other modules while the other is not. We define the participation coefficient P_i of node i as:

$$P_i = 1 - \sum_{s=1}^{N_M} \left(\frac{\kappa_{is}}{k_i} \right)^2 \quad (4)$$

where κ_{is} is the number of links of node i to nodes in module s , and k_i is the total degree of node i . The participation coefficient of a node is therefore close to 1 if its links are uniformly distributed among all the modules and 0 if all its links are within its own module.

Loss rate

To quantify the relation between roles and conservation, we calculate to what extent metabolites are conserved in the different species depending on the role they play. Specifically, for a pair of species, A and B , we define the loss rate as the probability $p(R_A = 0 | R_B = R) = p_{\text{lost}}(R)$ that a metabolite is not present in one of the species ($R_A = 0$) given that it plays role R in the other species ($R_B = R$). Structurally relevant roles are expected to have low values of $p_{\text{lost}}(R)$ and vice versa.

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SUPPLEMENTARY DISCUSSION

Functional cartography of complex metabolic networks

Roger Guimerà and Luís A. Nunes Amaral

1 Roles and blocks

Already in 1957, Nadel argued that “roles” are the central elements in the analysis of social systems^{1,2}, and in the 1970s White and coworkers introduced the concepts of *structural equivalence* and *blockmodel* to address this issue from a network perspective^{3,4,5,2}. Two nodes are structurally equivalent if they are connected to the same nodes^{3,5}. Therefore, any network can be divided into blocks of structurally-equivalent nodes in such a way that the structure of the network is *summarized* in a blockmodel by stating the relations between the blocks. Usually, structural equivalence is too strong a requirement for a large complex network; it is very unlikely that two nodes are connected to the exact same set of other nodes. Regular structural equivalence^{6,5} relaxes this requirement by requiring that regularly equivalent nodes have identical links with other *equivalent* nodes (Fig. 1). Formally, if nodes i and j are regularly equivalent and i has a link to/from some node k , then node j must have a link to/from some node l , and nodes k and l must be, also, regularly equivalent⁵.

Real networks are likely to have both modular structure and block structure. This fact raises serious concerns about the conceptual relationship between blocks and roles. Although blocks certainly give interesting information about the overall structure of the network, simple examples, such as the one shown in Fig. 1c, demonstrate that, in general, *blocks cannot be interpreted as roles*.

Motivated by this handicap of the block-scheme, we propose a new method to determine

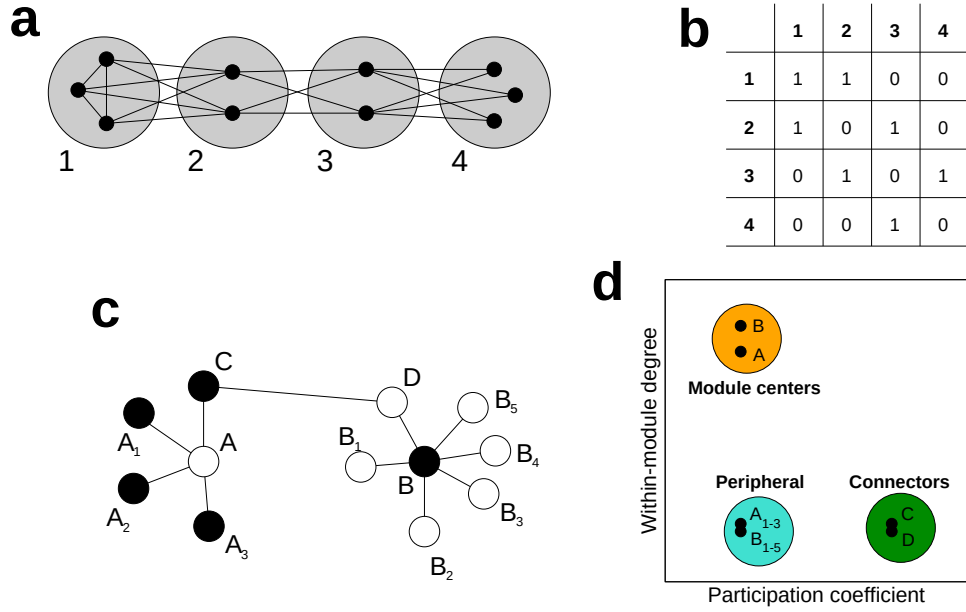


Figure 1: Weaknesses of the block approach to the identification of roles in modular networks. **a**, Structural equivalence and blocks. The network depicted can be divided into four blocks of structurally equivalent nodes. All nodes in block 1 are connected to each other and to all nodes in block 2. All nodes in block 2 are connected to all nodes in blocks 1 and 3, and so forth. **b**, The structure of the network can be conveniently summarized using a blockmodel matrix. **c**, To illustrate the weaknesses of the blockmodel approach to the identification of roles in modular networks, consider the network shown. Black nodes are connected to white nodes only, and white nodes are connected to black nodes only. Therefore, black nodes are regularly structurally equivalent to each other and white nodes are regularly structurally equivalent to each other. An ideal block detection algorithm may thus partition the nodes into two blocks, black and white. Significantly, this partition fails to capture the truly significant roles of the nodes in the network. Namely, nodes A and B are the “centers” of their modules, nodes C and D are module connectors, and all the other nodes are peripheral. **d**, Identification of roles based on the “within-module degree” and the “participation coefficient” (see Methods for definitions.)

the role of a node in a complex network. Our approach is not based on the idea of blocks but on the general idea that nodes with the same role should have similar topological properties (Fig. 1c,d).

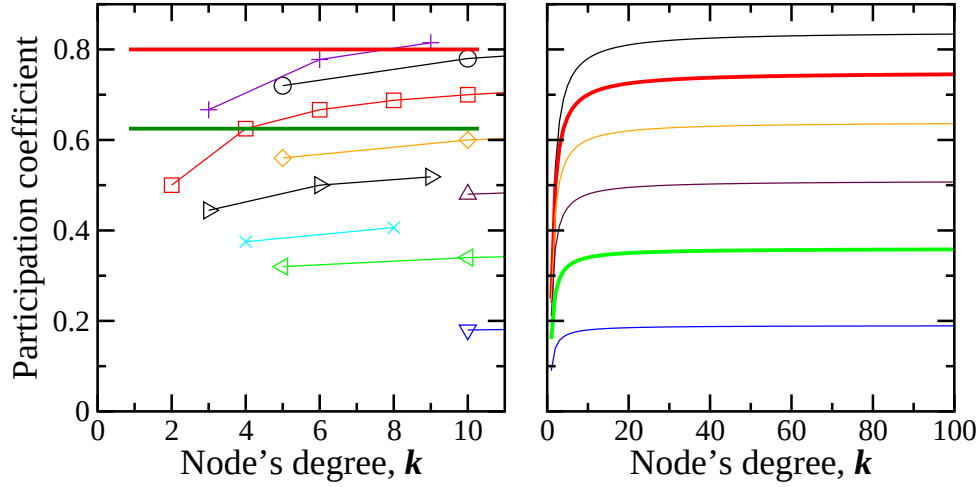


Figure 2: Dependence of value of participation coefficient on total degree and fraction of within-module links. **a**, P for, from top to bottom, 1/3, 0.4, 1/2, 0.6, 0.66, 0.7, 0.75, 0.8, and 0.9 of within-module links. The red horizontal line corresponds to $P = 0.8$ and the dark green to $P = 0.625$. These results suggest that $P > 0.8$ occurs only for cases in which the assignment of a node to a role is mostly a matter of chance. **b**, P for, from top to bottom, 0.4, 1/2, 0.6, 0.7, 0.8, and 0.9 of within-module links. The red curve, which correspond to half of the links within-module, converges to $P = 0.75$. The green curve, which correspond to 80% of the links within-module, converges to $P = 0.35$.

2 Heuristic determination of a set of discrete roles

We surmise that the role of a node is defined mainly by its within-community degree and its participation coefficient. Our definition of the roles is firstly determined by the within-module degree. We classify nodes with $z \geq 2.5$ as module hubs and nodes $z < 2.5$ as non-hubs. Both hub and non-hub nodes are then more finely characterized by using the values of the participation coefficient. Simple calculations suggest that non-hub nodes can be naturally assigned into four roles.

- *Ultra-peripheral nodes* (Role R1).

If a node has all its links within its module ($P \approx 0$).

- *Peripheral nodes* (Role R2).

If a node has at least 60% its links within-module, then for $k < 4$ it follows that $P < 0.625$

(Fig. S2a).

- *Non-hub connectors* (Role R3).

If a node with $k < 4$ has half of its links (or at least two links, whichever is larger) within-module, then it follows that $P < 0.8$ (Fig. S2a). Thus, a plausible region for non-hub connectors is $0.62 < P < 0.8$.

- *Non-hub kinless nodes* (Role R4).

If a node has fewer than 35% of its links within-module, it implies that $P > 0.8$. We surmise that such nodes cannot be clearly assigned to a single module. We thus classify them as kinless nodes. We will demonstrate later that non-hub kinless nodes are found in most network growth models, but not in real-world networks.

Similarly, hubs can be naturally assigned into three different roles:

- *Provincial hubs* (Role R5).

If a node with a large degree, $k \gg 1$, has at least 80% of its links within-module, then it follows that $P = 1 - (0.8)^2 - (k/5)(1/k^2) = 0.36 - 1/(5k)$.

- *Connector hubs* (Role R6).

If a node with a large degree has at least half of its links within module, then it follows that $P = 1 - 1/4 - (k/2) * (1/k^2) = 0.75 - 1/(2k)$. Since $k \gg 1$, $P < 0.75$ for such nodes.

- *kinless hubs* (Role R7).

If a hub has fewer than half its links within-module, i.e., $P > 0.75$, then we surmise that it may not be clearly associated with a single module. We then classify it as a kinless hub.

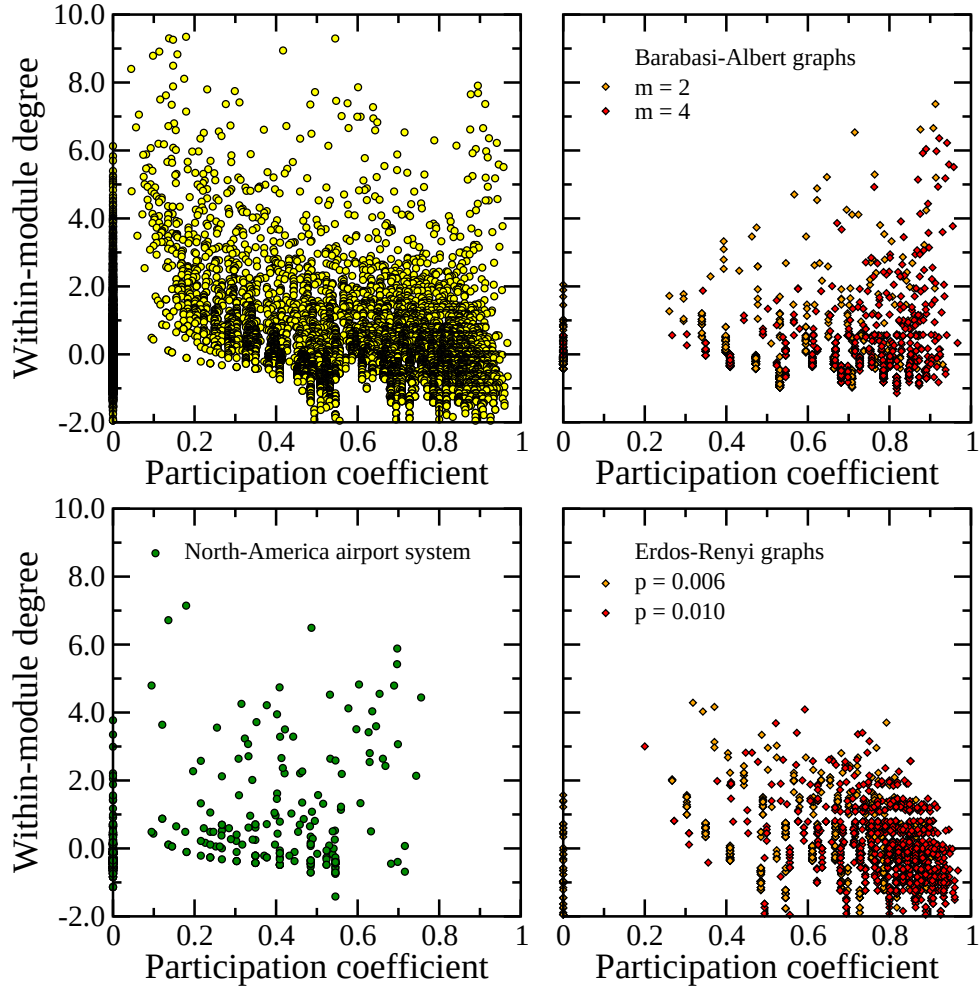


Figure 3: **a**, Values of z and P for 26771 nodes from 16 networks, including the metabolic networks of three organisms, the proteome of *C. elegans*, the North-American airport network, the collaboration networks of chemical engineers obtained from two journals (Chemical Engineering Science and AIChE Journal), the Internet at the autonomous system level, four Erdős-Rényi graphs with $p = 0.004, 0.006, 0.008$ and 0.010 , and four Barabási-Albert graphs with $m = 1, 2, 3$ and 4 . **b**, Values of z and P for two Barabási-Albert graphs with 1000 nodes each. **c**, Values of z and P for 940 nodes in the largest fully-connected component of the North-American airport network. **d**, Values of z and P for two Erdős-Rényi graphs with 1000 nodes each.

We will demonstrate later that hubs in most network growth models are actually kinless hubs.

2.1 Uncertainty in node position in parameters-space

In our analysis, we estimate the z value of the intra-module degree of each node and its participation coefficient. Since, we have access to these networks at a single moment in time, it is plausible to assume that the values we measure for z and P for a given node are not error-free. To take this uncertainty into consideration, we assign to each node a Gaussian peak with specific widths σ_z and σ_P . Figure S4 show the spread of the Gaussian peaks for two values of these parameters.

In order to obtain as complete as possible a picture of how the nodes in a given network might populate the zP parameters-space, we calculate z and P values for all the nodes in a large number of networks. Specifically, we obtain these values for (i) the metabolic networks of three organisms, (ii) the proteome of *C. elegans*, (iii) the North-American airport network, (iv) the collaboration networks of chemical engineers as defined by publications in two different journals, (v) the Internet at the autonomous-system level. Additionally, we obtain these values for nodes in model networks generated by the Barabási-Albert network growth model and the Erdős-Rényi model. In all, we consider in our analysis 26,771 nodes. We plot the density

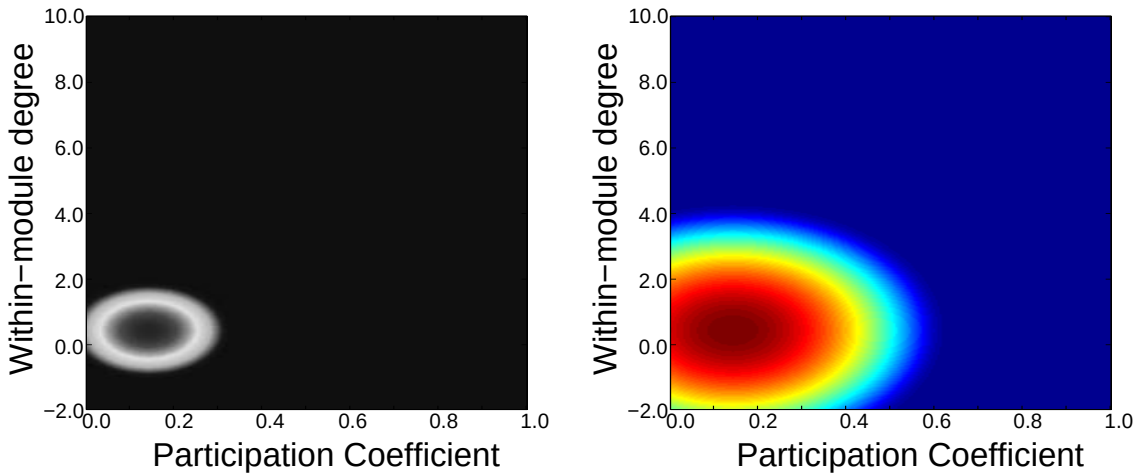


Figure 4: Gaussian peak decay for a single node as a function of the peak width. Note that the scale is logarithmic. Node density for **a**, $\sigma_P = 0.03$, and **b**, $\sigma_P = 0.08$. In both cases, $\sigma_z = 10\sigma_P$.

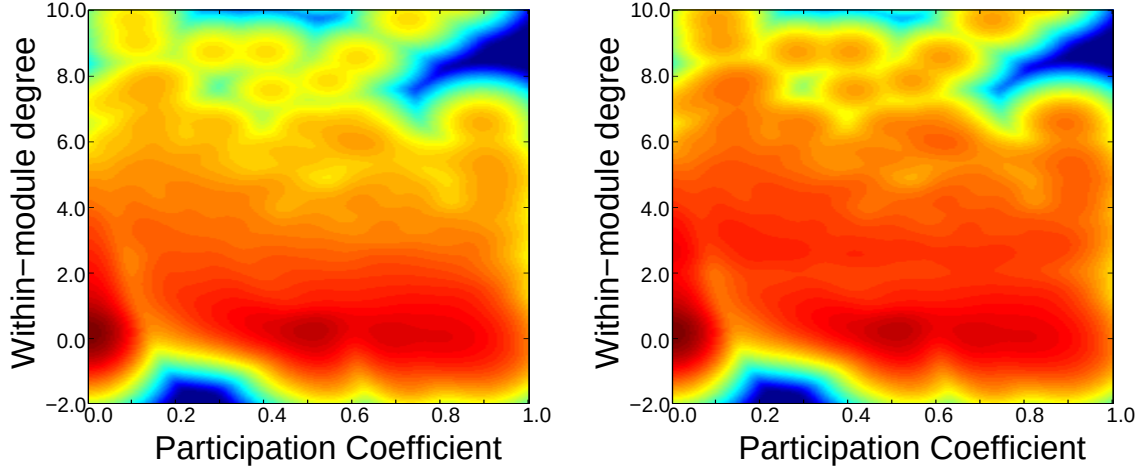


Figure 5: Density landscape for the nodes belonging to 8 real-world networks and 8 model networks. Due to the fact that more than 98% of the nodes have $z < 2.5$, one finds that the density landscape for $z > 2.5$ is quite “washed” down by the background of the non-hub region. For this reason, we obtain the density landscape under two distinct conditions: **a**, In the first, we weigh each hub with a weight of one. **b**, In the second, we weigh each hub with a weight of five.

landscape obtained for these nodes with $\sigma_P = 0.035$ in Fig. S5.

2.2 “Basins of attraction” for non-hub nodes

One can see the probability of finding a node with given values of z and P as a density landscape, with high probability regions as valleys and low probability regions as peaks. Then, at (almost) every point of the landscape, one can “follow” the gradient to reach a local minimum. The region of the space that “flows” toward a certain minimum is what we call a “basin of attraction.”

As discussed above, we define non-hub nodes as those with $z < 2.5$. We then calculate the node density plot for different choices of the values of σ_Z and σ_P and identify the basins of attraction for the different node density plots (Fig. S6).

Based on the results of Figs. S2–S6, we partition the zP parameters space for $z < 2.5$ into four regions with boundaries at $P = 0.05, 0.62$ and 0.8 .

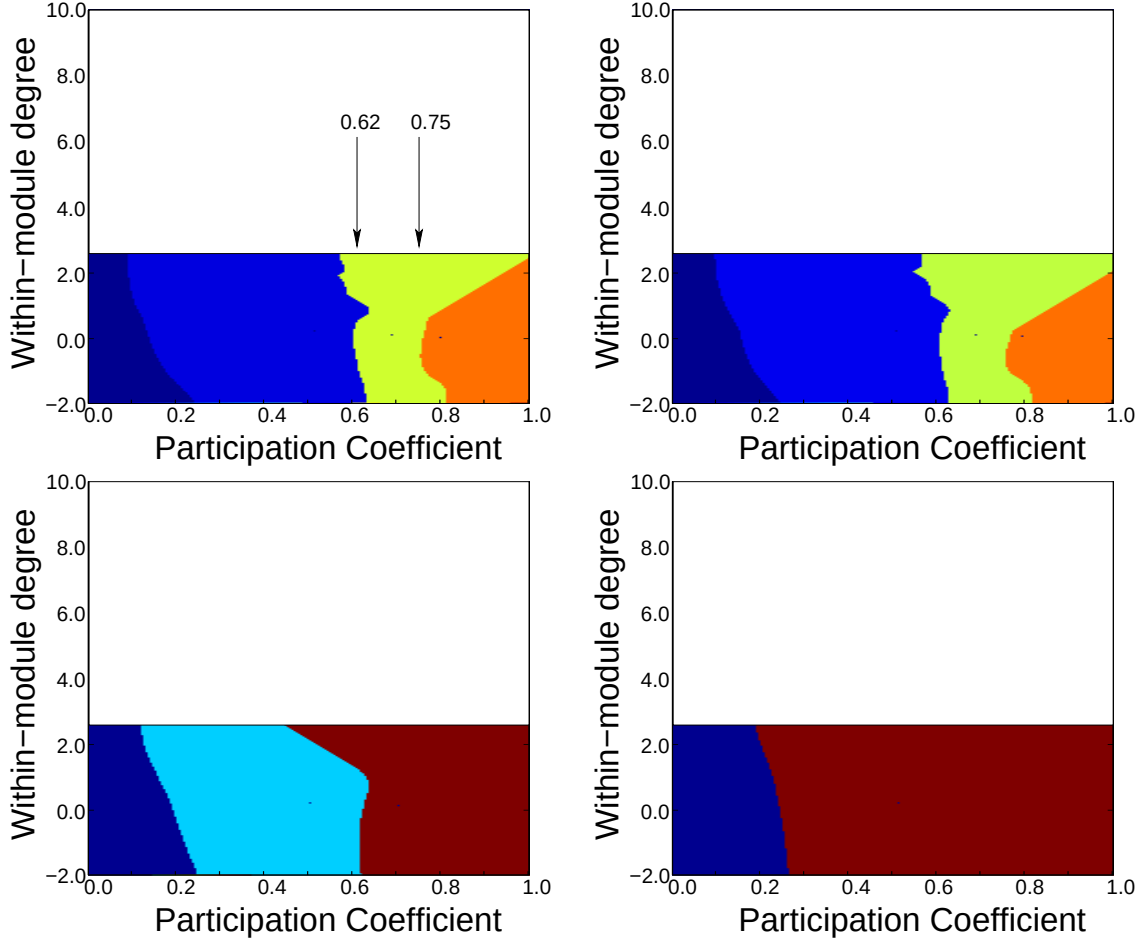


Figure 6: Basin of attraction identification for density landscapes obtained with **a**, $\sigma_P = 0.03$, **b**, $\sigma_P = 0.035$, **c**, $\sigma_P = 0.05$, and **d**, $\sigma_P = 0.08$. Note how the values of P identified in our simple analysis provide a good match to the boundaries of the basins of attraction in the node density landscapes.

2.3 “Basins of attraction” for hubs

We define non-hub nodes as those with $z > 2.5$. We then calculate the node density plot for different choices of the values of σ_z and σ_P and identify the basins of attraction for the different node density plots (Fig. S7) .

In this case the results are not as clear as for the non-hub region because of the scarcity of data points. However, the density plot are compatible with a selection of three regions corresponding to distinct roles. The boundary of these regions are at $P = 0.30$ and 0.75 .

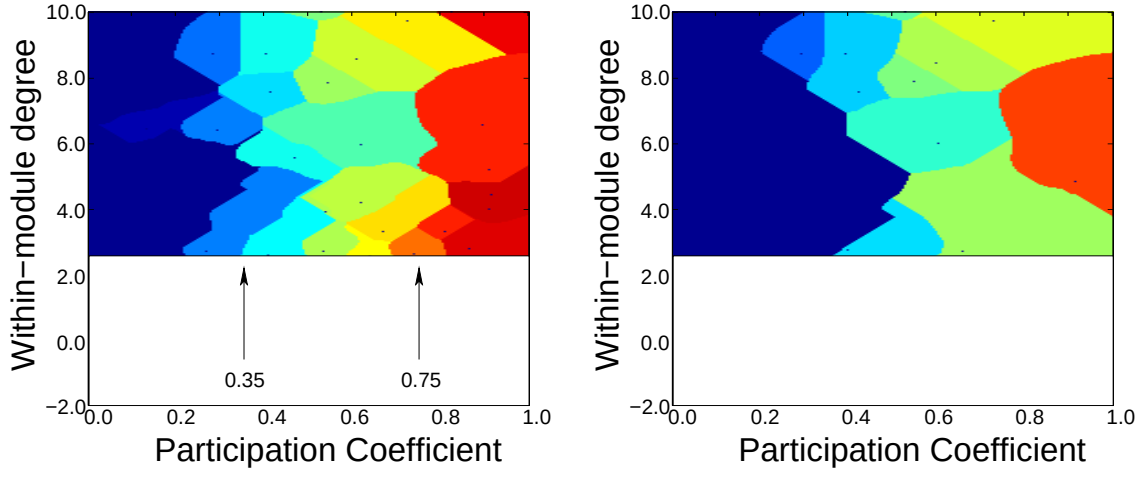


Figure 7: Basin of attraction identification for density landscapes obtained with **a**, $\sigma_P = 0.03$, and **b**, $\sigma_P = 0.05$.

The seven roles we identify and the corresponding regions in the zP parameters-space are displayed in Fig. S8.

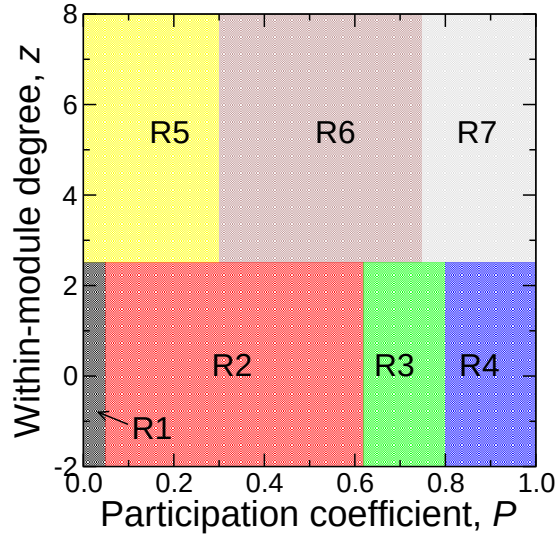


Figure 8: Role-specific regions in the zP parameters-space.

3 Metabolic networks

3.1 Modules in metabolic networks

Two issues related to the accuracy of the identification of modules in metabolic networks are worth analyzing in detail. First, nodes are expected to be more densely connected to nodes in the same module than to nodes in other modules. To quantify to which extent the algorithm accomplishes this task, we depict the within- and between-module connectivity density, that is, the ratio between the actual number of links and the maximum number of links within a module and between a pair of modules (Fig. S9a). As expected, the within-module density is much larger than the between-module density, typically 100-1000 times larger.

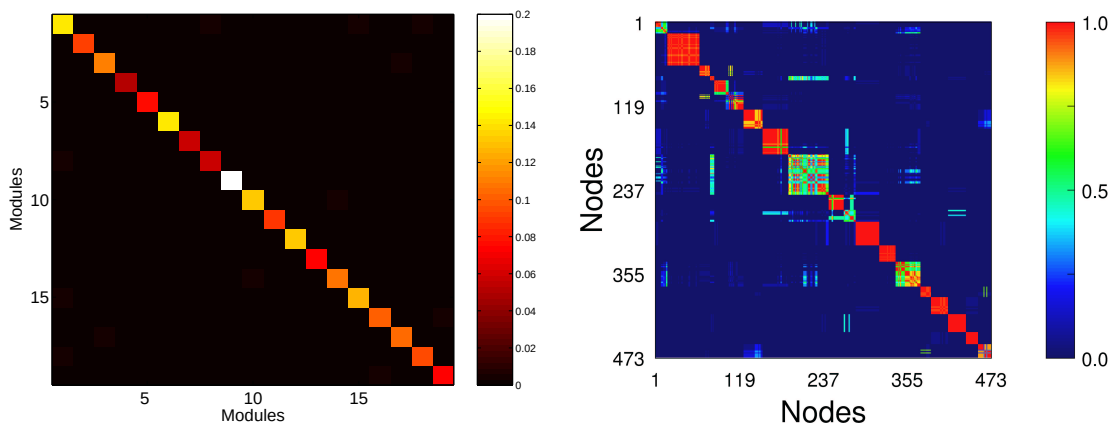


Figure 9: Accuracy of the module identification algorithm. **a**, Connection density between and within modules. The connection density is defined as the ratio between the actual number of links and the maximum possible number of links. **b**, To test the robustness of the algorithm when applied to metabolic networks, we obtain 25 partitions of the metabolic network of *E. coli* and plot, for each pair of nodes in the network, the fraction of times that they are classified in the same module.

Second, since our module-identification algorithm is stochastic, different runs yield, in general, different partitions of the nodes into modules. To test the robustness of the algorithm when applied to metabolic networks, we obtain 25 partitions of the metabolic network of *E. coli* and plot, for each pair of nodes in the network, the fraction of times that they are classified in the same module. As shown in Fig. S9b, modules are robustly and consistently identified.

3.2 Role assignment for the metabolites in 12 organisms

We analyze the metabolic networks of 12 organisms: four archaea—*P. furiosus*, *A. pernix*, *A. fulgidus*, and *S. solfataricus*—, four prokaryotes—*E. coli*, *B. subtilis*, *L. lactis*, and *T. elongatus*—, and four eukaryotes—*S. cerevisiae*, *C. elegans*, *P. falciparum*, and *H. sapiens*.

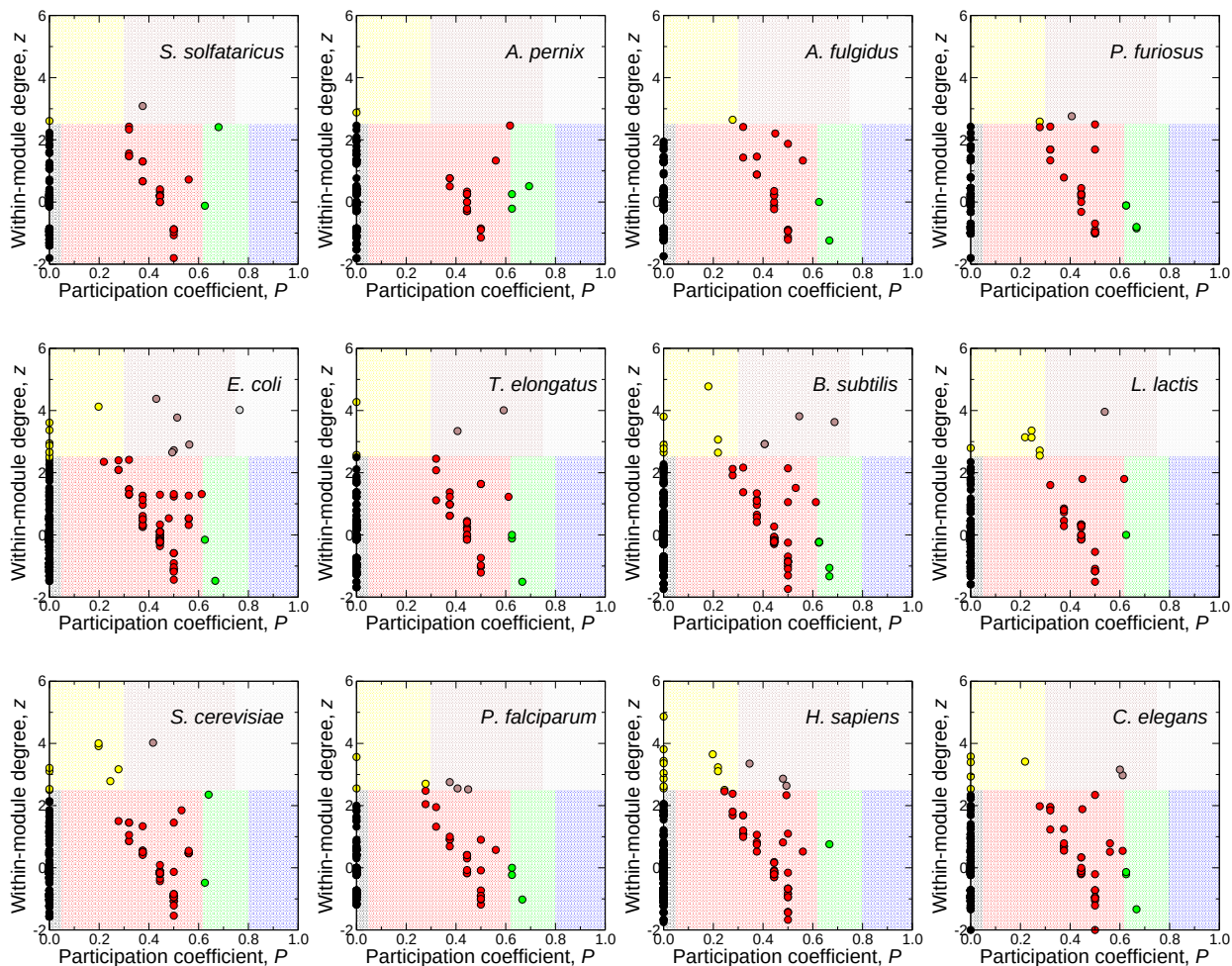


Figure 10: Metabolite role determination for the 12 organisms. Each metabolite is represented as a point in the zP phase-space, and is colored according to its role.

An issue of potential concern is the accuracy of the metabolic network database. The metabolic networks used in the paper were originally compiled by Ma and Zeng⁷ (MZ). These authors carefully considered the reactions contained in the KEGG database and manually cor-

rected inconsistencies and errors, removed current metabolites from reactions, and excluded polymerization reactions as well as reactions with macromolecule participation. Although this database is probably the most reliable to date⁸, it may be argued that it is very restrictive and that our results may be contingent on its use.

To assess this possibility, we analyze the complete, unfiltered, KEGG database. To build the metabolic networks, we use the data compiled in the LIGAND section of the KEGG database, publicly available at <ftp://ftp.genome.ad.jp/pub/kegg/ligand/>. In particular, we consider all bio-

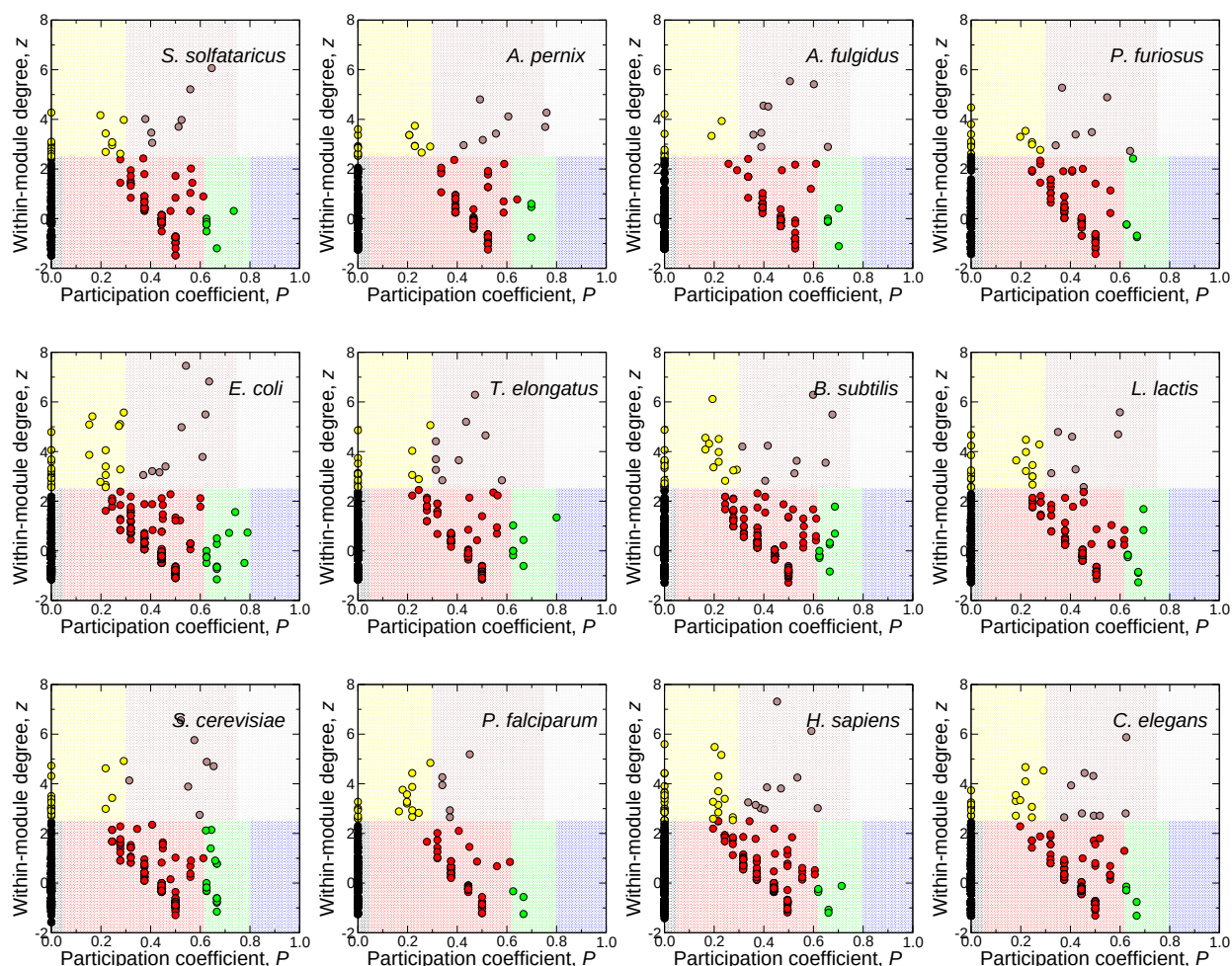


Figure 11: Metabolite role determination for the 12 organisms using unfiltered data from KEGG. Each metabolite is represented as a point in the zP phase-space, and is colored according to its role.

chemical reactions included in the *reaction_main.lst* file, which includes the main metabolites for each reaction. Then, for each organism, we only take into account reactions that are catalyzed by an enzyme that the organism is able to synthesize. We obtain the enzymes necessary for each reaction from the *reaction* file, and the enzymes synthesized by each organism from the organism database. For example, for *E. coli* the database is available at <ftp://ftp.genome.ad.jp/pub/kegg/genomes/genes/E.coli.ent>.

Since the KEGG metabolic networks are not subject to manual filtering, they contain many more metabolites and reactions than the networks in the MZ database. For *E. coli*, for example, the MZ network contains 473 nodes while the unfiltered network contains 1200 metabolites. This difference is due to two factors. First, some metabolites, such as macromolecules, are removed from the MZ database. Second, some reactions are also discarded, so the connectivity of the MZ networks is smaller and the giant component of the network contains a smaller fraction of nodes.

Remarkably, the distribution of nodes in the different regions of the zP space is similar in networks obtained from the MZ and the unfiltered databases (Figs. S10 and S11). In particular, roles R4 and R7 are unpopulated, and there are usually only a few connectors—both hub and non-hub. These results are in stark contrast to those observed in some other networks (Fig. S3). The North American airport network, for example, contains many more connector hubs than metabolic networks (Fig. S3c), but fewer non-hub connectors. On the other hand, module-less model networks contain many nodes in role R4—Erdős-Rényi and Barabási-Albert networks—and in role R7—Barabasi-Albert networks—but few ultra-peripheral nodes (Fig. S3b and d).

3.3 Node degree and metabolite conservation

We find that non-hub connector metabolites are more conserved than provincial hubs, which have larger within-module degree z -score. This is a remarkable result and its soundness and

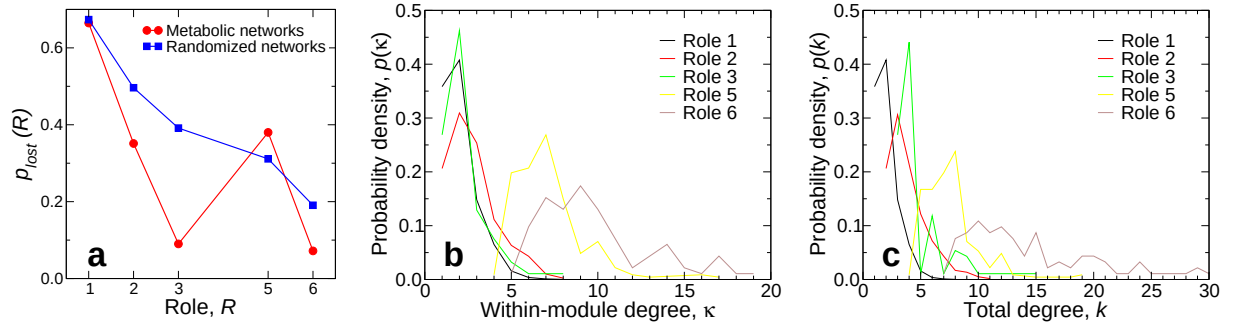


Figure 12: Node degree and role conservation results. **a**, Comparison of role conservation for real metabolic networks and for the corresponding randomized networks. **b**, Distribution $p(\kappa)$ of within-module degrees for nodes with different roles. **c**, Distribution $p(k)$ of total degrees for nodes with different roles.

robustness deserve to be considered carefully.

First, it is worth pointing out that studies of other complex biological networks have stressed the relevance of the degree of nodes. Jeong and coworkers showed that, in protein interaction networks, nodes with high degree are more essential than those with low degree⁹. In our analysis, then, one may expect that the total degree of a node is a key determinant of its inter-species conservation. Our claim is, however, that not only degree but also the position—role—of the node in the network is a crucial consideration regarding essentiality.

To determine to which extent degree can account for the reported results, we randomize the metabolic networks by keeping constant the degree of each node. By doing this, all connectivity correlations are “removed” from the network, and the total degree of a node is the only relevant property kept. In Fig. S12a, we show that the conservation pattern is significantly altered for the randomized networks. In this case, $p_{\text{lost}}(R3) > p_{\text{lost}}(R5)$ because nodes in R3 have lower degree than those in R5. It is also illustrative to compare the conservation of each role in the real and randomized networks. Roles with some connector function—R2, R3, and R6—are more conserved in the real case than in the random case, while provincial hubs—R5—are less conserved. Ultra-peripheral nodes are similarly conserved in the random and real networks.

The second point that needs to be checked is the following. It may be that, due to the use

of the z -score instead of the raw degree, some low-degree nodes in very sparse modules were classified as provincial hubs. Then, one may expect the loss rate to be large for provincial hubs just as an artifact, because of the influence of these nodes.

We have tested this possibility and found that this is not the case. In Fig. S12b, we show that the distribution $p(\kappa)$ of within-module degrees is essentially identical for roles 1-3, with approximately 95% of the nodes having $\kappa \in [1, 4]$. In contrast, less than 1% of the nodes with role 5 have $\kappa = 4$ and none have $\kappa < 4$. Similarly, if one considers the total degree k , 71% of the nodes in role 3 have $k < 5$, while less than 1% of the nodes in role 5 have such small degrees (Fig. S12c). The conclusion is therefore sound: connectors are more conserved than provincial hubs in spite of having smaller within-module degree and smaller total degree. We have added this discussion to the Supplementary Material.

3.4 Metabolite conservation results and role definition

In the first section of this Supplementary Information, we have analyzed the considerations that lead to the definition of seven system-independent roles. One could, however, divide the zP phase-space in other manners. Next, we show that the metabolite conservation results are independent of the particular definition of roles one uses, as long as the definition of roles, specially for non-hubs, takes into account certain fundamental considerations. This also serves as further evidence that our definition of roles is a parsimonious definition.

Let us start by considering an alternative partition of the zP space, in which nodes are divided, first, into three classes according to their within-module degree: anti-hubs ($z < 0$), non-hubs ($0 \leq z < 2.5$), and hubs ($z \geq 2.5$). Then, each one of these three classes is further subdivided into five groups according to the participation coefficient: $P < 0.2$, $0.2 \leq P < 0.4$, $0.4 \leq P < 0.6$, $0.6 \leq P < 0.8$, and $P \geq 0.8$. This is a finer definition of roles than the one we propose because it divides the zP phase-space into 15 regions instead of 7. In Fig. S13 we

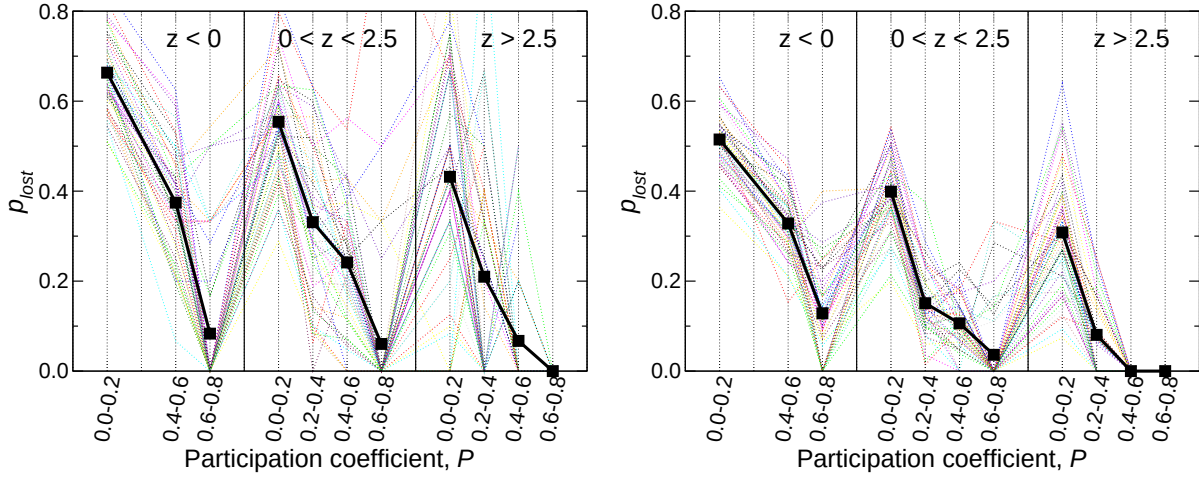


Figure 13: Robustness of the role conservation results. To test the robustness of our results for role-dependent conservation of metabolites, we investigate alternative definitions of the roles. Specifically, we partition the nodes into three grouping according to their degree: “anti-hubs” ($z < 0$), non-hubs ($0 < z < 2.5$) and hubs ($z > 2.5$). We then further subdivide the nodes according to the value of P into five equally-wide regions. Inter-species metabolite conservation as a function of the values of z and P for the **a**, MZ database and the **b**, unfiltered KEGG database. We find that: (i) the results are pretty insensitive to the database used, (ii) there is a very strong dependence of p_{lost} on the participation coefficient, and a somewhat weaker dependence on z , i.e., the degree, (iii) the seven roles described in the manuscript fully capture the results obtained with a finer definition of the roles.

show that such a definition of the roles does not alter the main conclusions drawn in the paper. Namely, connector nodes with low degree and high participation coefficient are systematically and consistently more conserved than hubs with low participation coefficient. Even anti-hubs with high P are considerably more conserved than hubs with low P . Thus, the results obtained from this finer partition of the zP phase-space emphasize, even more clearly, to which extent the participation coefficient is more relevant than the degree.

Now, a relevant methodological question is: would *any* partition of the zP phase-space yield *correct* results? The answer is that, specially for non-hubs, one must be careful. Non-hubs are nodes with low degree, and therefore integer constraints on the possible values of P become important. For example, as discussed in the first section of this Supporting Information and in Fig. S2, a node with degree $k = 2$ can only have $P = 1$ or $P = 0.5$.

To see how this fact potentially affects the definition of roles, consider a situation in which

one divides the the zP space into hubs ($z \leq 2.5$) and non-hubs ($z < 2.5$), and each of these two classes into five groups according to P as before: $P < 0.2$, $0.2 \leq P < 0.4$, $0.4 \leq P < 0.6$, $0.6 \leq P < 0.8$, and $P \geq 0.8$. Then, the region that includes $P = 0.5$ will contain many nodes with $k = 2$, while the region that includes $P = 0.3$ will include none. Since degree also plays a role in metabolite conservation, it may happen that nodes in the region containing $P = 0.5$ are less conserved than those in the region that contains $P = 0.3$, but this would be entirely due to a poor sampling of the degrees within each of the regions, and not to P itself.

In our definition of roles described in the first section, this problem is addressed by assigning a larger range of P to peripheral nodes (R2), in such a way that degrees are more uniformly sampled. Other partitions of the zP space into 7 or a similar number of roles must take this into consideration and, consequently, should be similar to our “universal roles.”

References and Notes

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