

From Systems to Structure: Bridging Networks and Mechanism

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There is a wide gap between the generation of large-scale biological data sets and more-detailed, structural and mechanistic studies. However, recent studies that explicitly combine data from systems and structural biological approaches are having a profound effect on our ability to predict how mutations and small molecules affect atomic-level mechanisms, disrupt systems-level networks, and ultimately lead to changes in organismal fitness. In fact, we argue that a shared framework for analysis of nonadditive genetic and thermodynamic responses to perturbations will accelerate the integration of reductionist and global approaches. A stronger bridge between these two areas will allow for a deeper and more-complete understanding of complex biological phenomenon and ultimately provide needed breakthroughs in biomedical research.

As sequencing efforts reveal unprecedented levels of genetic diversity in populations, key challenges remain in linking heritable variation to organismal fitness (Lander, 2011). The functional effect of sequence changes are commonly predicted starting from the top down (using a network from systems biology) or the bottom up (using an atomic structure from structural biology). However, biologists now consciously (and unconsciously) straddle the line between global, systems and reductionist, structural approaches. Here, we review how the growing synergies between these two tactics are advancing our understanding of the mechanisms of phenotypic change. A theme emerging from both systems and structural biology is that multiple perturbations can function nonadditively. We anticipate that further parallels between nonadditive effects on both network and macromolecular dynamics will emerge as scientists working at the systems-to-structure interface probe complex biological phenomena. Learning how mutations and small molecules affect both atomic interactions and network organizations will advance our ability to predict the phenotypic responses to perturbations and to design new therapies that restore homeostasis.

The Interface between Systems and Structure: Conservation Is King

A major goal of systems biology is to determine the abundance of each protein, nucleic acid, or metabolite component and all the interactions that exist between them. In protein-protein interaction networks, an edge represents more than just a construct of graph theory—a connection between nodes implies a direct atomic contact between proteins. Aside from using a cocrystal structure to provide the ultimate “true positive” confirmation of an interaction, what can structural biology contribute to our knowledge of systems-level organization?

The number of interactions discovered by proteomics experiments (Havugimana et al., 2012) can dwarf the total number of

protein structures available in the Protein Data Bank (Rose et al., 2011). To bridge this gap, one can use homology models to approximate the interfaces of many interactions (Pieper et al., 2011; Zhang et al., 2012a). Using these principles, Kim and Gerstein developed a systems-level structural interaction network in yeast (Kim et al., 2006). The atomic-level details afforded by this structural network representation allowed them to distinguish interactions that can simultaneously assemble on a single receptor from those that are mutually exclusive (Figure 1A). For example, the GTPase Ras interacts with multiple proteins that compete for an overlapping interaction surface (Figure 1B). Only one of these proteins can therefore bind Ras at any given time (Wittinghofer and Vetter, 2011). In contrast, macromolecular machines often assemble many interaction partners simultaneously. Indeed, the SCF ubiquitin ligase complex must assemble Rbx1, Skp1, and a member of the F-box protein family, along a Cullin scaffold, to properly ubiquitinate target proteins (Zheng et al., 2002). Systematic characterization of overlapping and simultaneous binding within a protein-protein interaction network has resolved long-standing ambiguities about the relationship between a protein's evolutionary rate and its degree (i.e., number of interaction partners) by establishing a central role for the amount of accessible surface area buried upon protein-protein association (Kim et al., 2006). This approach can also be inverted: structural information can be leveraged to predict new protein-protein interactions. Remote structural alignments that consider geometric relationships between interacting proteins have recently been used to predict protein-protein interactions on a proteomic scale (Zhang et al., 2012a).

Similarly, structural insights can be used to assess the evolution and functional role of posttranslational modifications, such as phosphorylation, ubiquitination, and acetylation (Beltrao et al., 2012). The increased sensitivity of mass spectrometry to

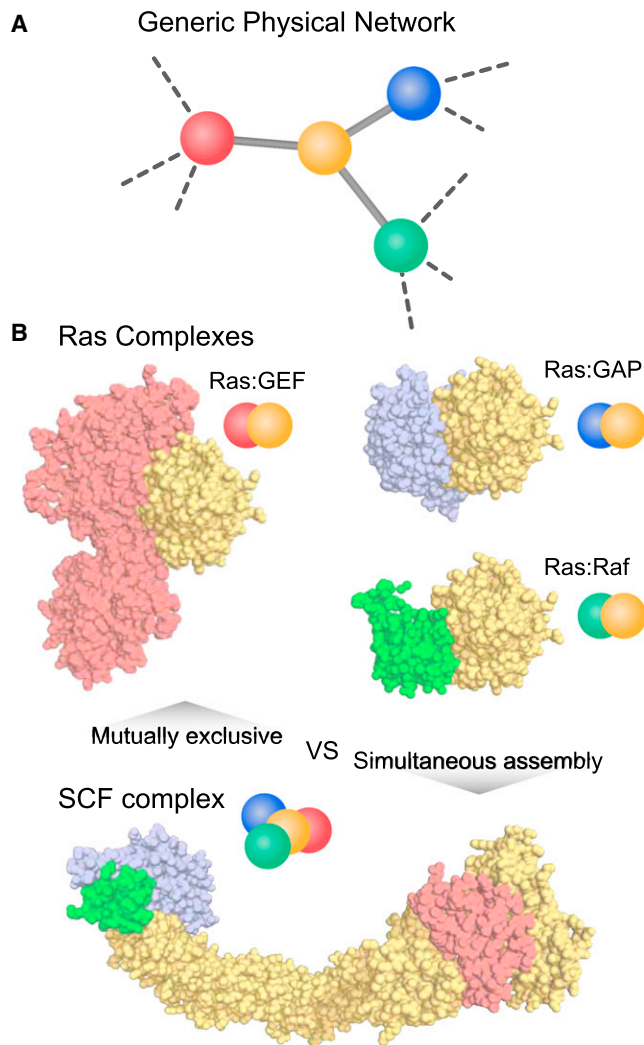


Figure 1. Interpretation of Physical Networks

(A) A generic physical interaction network that could be derived from various systematic protein-protein interaction platforms. (B) The interaction map in (A) could represent a set of mutually exclusive protein-protein interactions involving one common target (e.g., Ras complexes: Ras:GEF [PDB accession number 1BKD], Ras:GAP [1WQ1], or Ras:Raf [3KUD]) or a simultaneous assembly of proteins into a complex (e.g., SCF complex [1LDK]).

identify posttranslational modifications in proteomic samples has been a blessing and a curse: while new hypotheses can be generated based on the identification of modified sites, the functional significance of the vast majority of these modifications remains unknown. By examining posttranslational modifications in a structural context, Beltrao et al. found that phosphosites located at protein-protein interaction interfaces are more highly conserved (Beltrao et al., 2012). Additionally, this study proposed and tested the hypothesis that posttranslational hotspots for controlling protein function are structurally conserved across family members. However, these data suggest that most post-translational modifications are likely to have no biological role. They proposed that the near neutrality of modifications probably

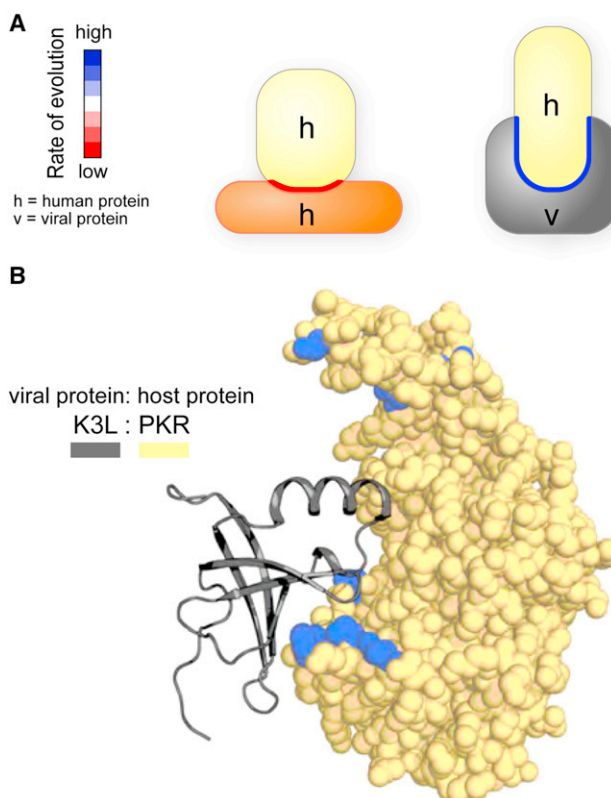


Figure 2. Evolutionarily Analysis of Interfaces Involved in Mediating Protein-Protein Interactions

(A) Regions of host proteins that are directly hijacked by viral factors are less conserved than interfaces that mediate interactions between host proteins. (B) The human kinase PKR interacts with eIF2 α and the viral protein K3L via a common surface. Residues colored in blue line the interface region and display evidence for positive selection (elevated rates of amino acid substitutions). K3L (PDB accession number 1LUZ) was docked to PKR based on a superposition of the PKR:eIF2 α cocrystal structure (2A1A).

increases the evolvability of interaction networks. Structural principles, therefore, can be applied to proteomic studies to pinpoint only those modifications that are highly conserved and likely to be functional.

The recent construction of a human-viral structural interaction network revealed an exception to the principle that highly conserved surfaces are the most interesting regions (Franzosa and Xia, 2011). Consistent with the earlier work, these studies confirmed that interfaces involved in host protein-protein interactions evolve more slowly. However, interfaces exploited by pathogens tend to evolve much faster (Figure 2A). This result provides structural rationale for an evolutionary arms race between host and pathogen: there is selective pressure on host interfaces to evolve incompatibilities with their pathogen interaction partners. For example, the human kinase PKR has evolved under intense positive selection to avoid interactions with viral proteins such as poxvirus K3L, while maintaining interactions with endogenous interaction partners such as eIF2 α (Elde et al., 2009) (Figure 2B). In the case of PKR, coevolving mutations on multiple structural elements are likely to be required to decrease the affinity of binding the pathogenic protein

while maintaining the proper connections to the host protein-protein interaction network.

The idea that coevolution of interface residues across interacting proteins plays an important role in the evolution of network structure has also been tested in model systems. Using the yeast proliferating cell nuclear antigen (PCNA) DNA clamp as a model for studying protein-protein interaction network evolution, Aharoni and colleagues discovered that disruption of the delicate balance of affinities between different interaction partners can yield more severe phenotypes than gene deletions (Fridman et al., 2010). These studies suggest that large-scale genetic interaction studies, using deletion libraries or RNA interference (RNAi) screens, should be supplemented with targeted mutations on specific components to tease apart the roles of multifunctional proteins. Because these mutations can, in principle, affect only one edge of a protein-protein interaction network, they have been termed “edgetic” mutations, to contrast from gene deletions, which remove all edges associated with a given node (Amberg et al., 1995; Charlotiaux et al., 2011). Similarly, structurally guided mutants could be used to dissect the roles of multifunctional macromolecular machines, such as RNA polymerase II (N.J.K., unpublished data), which interact with different protein partners during complex functional cycles.

Mutations that tune the affinity of specific interactions or introduce new, unwanted interactions may have more severe phenotypic consequences than can be accessed with traditional gene knockouts. Mapping of human disease-associated mutations onto structures has provided a structural rationale for pleiotropic genes, in which distinct mutations are implicated in different diseases (Wang et al., 2012a). Mutations on a single interface are more commonly associated with a single disease, whereas mutations at different interfaces within the same protein tend to be associated with distinct diseases. Furthermore, mutations at interfaces with known disease implications provide a guilt-by-association indication for new genes, which may be more therapeutically tractable. For proteins with multiple domains or protein-protein interaction interfaces, these analyses can greatly narrow the scope of candidate genes to those most likely to be functionally relevant for the disease of interest. As both structural coverage of the human proteome and disease associated mutations discovered by exome sequencing studies increase, application of structural insights will likely increase the leverage of network-level observations to understand the genetic basis for disease (Aloy and Russell, 2003; Wang and Moul, 2001).

Making the Complex Whole: Systems Organize Structure

As structural biology shifts increasingly to characterizing complex, heterogeneous macromolecular assemblies, data sets generated from unbiased, global systems biology studies are playing a prominent role. The influence of systems biology has already begun changing the normal course for identifying members of protein complexes from a “fishing trip” to a “mining expedition.” Because identifying all members of a protein complex is often a necessary prerequisite for structural characterization, a common fishing approach is to use a yeast two-hybrid screen with single defined bait against a large, proteomic-scale prey library. An early example of how this approach can facilitate

a deeper structural and mechanistic understanding of an important biological problem involves BRCA2, a DNA binding protein with important roles in breast cancer (Zheng et al., 2000). When structural studies of BRCA2 were initiated, baculovirus expression of BRCA2 in the absence of any interaction partners yielded insoluble protein (Yang et al., 2002). A two-hybrid screen based on complementary DNA derived from HeLa cells and mammary tissues identified DSS1 as a new interaction partner for BRCA2 (Marston et al., 1999). Coexpression of DSS1 and BRCA2 eventually yielded a cocrystal structure and the structure of a ternary complex with DNA, demonstrating the importance of fishing out new interaction partners to enable structural studies (Yang et al., 2002) (Figure 3A). Interestingly, DSS1 has been shown to be part of multiple complexes, including an evolutionarily conserved component of the 26S proteasome (Wilmes et al., 2008).

In contrast to the BRCA2-focused yeast-two hybrid study that identified DSS1, most systems biology studies are performed with limited bias, systematically focusing on functionally related sets of proteins (Babu et al., 2012; Behrends et al., 2010; Krogan et al., 2004; Sowa et al., 2009) or even entire the entire proteome within an organism (Gavin et al., 2006; Havugimana et al., 2012; Hu et al., 2009; Krogan et al., 2006). These comprehensive approaches facilitate “mining expeditions” through a large database of interactions to identify multiprotein complexes that can be reconstituted biochemically and structurally (Figure 3B) (Aloy et al., 2004; Brooks et al., 2010). For example, in a recent global survey of the HIV proteome, Jäger et al. identified interactions between human proteins and all 18 HIV-1 proteins and polyproteins (Jäger et al., 2012a). The resulting 497 high-confidence interactions provide many new opportunities to observe the atomic details of how a pathogen utilizes and subverts the normal function of host proteins. Examination of the interaction maps across two different cell lines revealed a surprising association of the transcription cofactor CBF- β , which heterodimerizes with Runx to regulate transcription in T cells (Wong et al., 2011), with the HIV protein Vif. Although Vif was previously known to hijack an endogenous CUL-5 ubiquitin ligase complex to target for ubiquitination and degradation the host restriction factor APBCEC3G (Yu et al., 2003), the architecture of this complex had remained elusive. Similar to DSS1 with BRCA2, coexpression of CBF- β provided the missing puzzle piece and enabled biochemical reconstitution and structural characterization of the Vif-Cul-5 complex (Jäger et al., 2012b; Zhang et al., 2012b). Interestingly, Vif may be facilitating a dual hijack, one that affects a cullin-containing ubiquitination pathway as well as the rewiring of the transcriptional landscape regulated by the CBF- β -RUNX1 complex (Kim et al., 2013). Consistent with these results, a knock-down of CBF- β adversely affected HIV infectivity (Jäger et al., 2012b; Zhang et al., 2012b). Future studies will investigate whether and how both functional hijackings are required for efficient HIV infection. As further atomic-level details of this interaction emerge, it will likely serve as a paradigmatic example of how unexpected interaction partners can emerge from unbiased global studies to enable mechanistic studies.

Hybrid strategies that build on “-omics” techniques normally associated with systems biology are being used to uncover new binding partners and to accelerate biophysical studies for

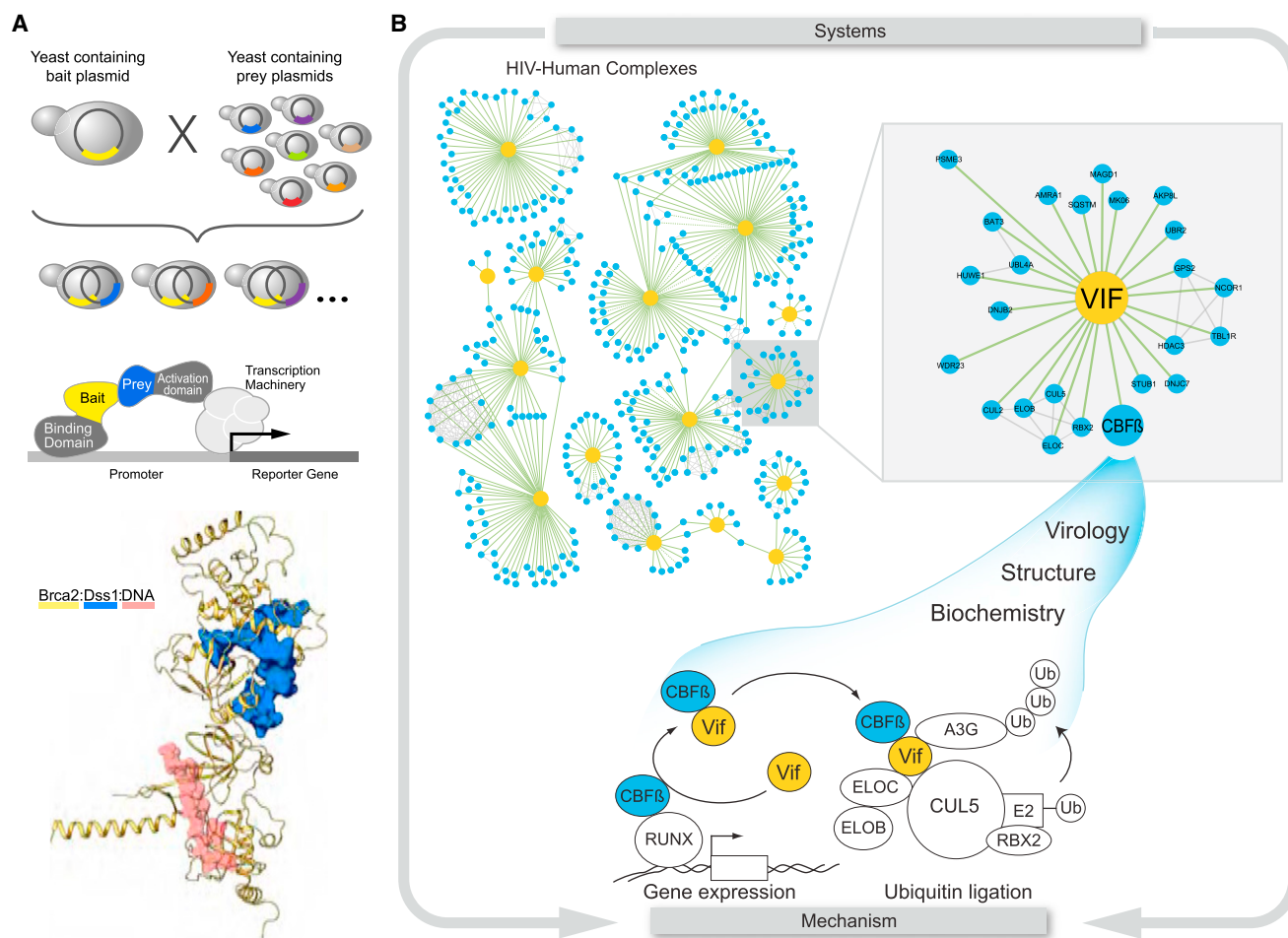


Figure 3. Using Unbiased Systems Approaches to Solve Important Biochemically Intractable Problems

(A) Use of a targeted two-hybrid approach for BRCA2 identified DSS1, which allowed for the structural elucidation (PDB accession number 1MJJE) of this important factor involved in breast cancer progression.

(B) An unbiased affinity tag-purification mass spectrometry approach on all HIV proteins revealed a connection between the accessory protein Vif and the human transcription factor CBF- β . This host protein, which is required for efficient HIV infection, allowed for the reconstitution and structural characterization of an active Vif-Cul-5 ubiquitination complex, which is required for the degradation of the host restriction factor APOBEC3G. The unbiased systems approach allowed for the discovery that by hijacking CBF- β , Vif is manipulating the ubiquitination machinery and adversely effecting host transcriptional regulation.

intrinsically disordered proteins (Dyson, 2011), macromolecular machines (Alber et al., 2007b), and transient complexes (Herzog et al., 2012). A particular challenge faced by these studies is how to integrate and weight diverse data types into a self-consistent structural model (Alber et al., 2007a). For example, when performed on purified proteins, chemical crosslinking coupled with mass spectrometry provides distance restraints (Rapsilber, 2011). Crosslinking restraints can be incorporated into structure determination with well-established methods similar to those used routinely in nuclear magnetic resonance (NMR) (Havel et al., 1983); however, additional complications are encountered as crosslinking studies are extended to even more heterogeneous samples. In these studies, network context inferred from previous systems-level studies is essential for separation of signals resulting from sterically incompatible complexes, allosteric distortion of individual subunits, and entirely

novel assemblies. The complexity of deconvoluting signals emerging from proteomic studies is exemplified by the protein phosphatase 2A (PP2A) holoenzymes, which consist of regulatory, catalytic, and scaffold subunits that provide combinatorial specificity to substrate proteins (Herzog et al., 2012). By purifying endogenous PP2A, Aebersold and colleagues revealed new players in the PP2A network but also provided insights into the structural basis for how specific PP2A holoenzymes achieve interactions with a wide array of cellular processes. Proteomic-based crosslinking methods are a valuable source of structural data that can be obtained directly from heterogeneous mixtures and complementary methods such as X-ray crystallography, NMR, and electron microscopy that rely on purified samples.

In addition to the natural links between structural biology and proteomics, other “-omics” technologies are beginning to

generate interesting mechanistic problems. Synergies between genomics, metabolomics, and structural studies are exemplified by an interesting mutation (R132H) in the metabolic enzyme isocitrate dehydrogenase-1 (IDH1). This mutation was first uncovered in a large genomics study, where it was present in 12% of Glioblastoma multiforme brain cancer patients (Parsons et al., 2008). Initial studies suggested that the mutation led to a dominant negative effect on the catalytic formation of the metabolite α -ketoglutarate (Zhao et al., 2009). Surprisingly, subsequent metabolomics profiling revealed no significant changes in levels of α -ketoglutarate between cells expressing mutant or wild-type versions of the enzyme (Dang et al., 2009). However, levels of a different metabolite, R(-)-2-hydroxyglutarate, were significantly elevated. A crystal structure of the mutant enzyme revealed the key changes in active site geometry that enable this novel catalytic activity. The discovery of a new activity has motivated further systems-level studies into the role of R(-)-2-hydroxyglutarate in epigenetics (Sasaki et al., 2012), and the structural information provides additional hope that the mutant IDH1 enzyme represents a new target for therapeutic intervention in glioblastomas and acute myeloid leukemias.

A New Coupling of Thermodynamics and Genetic Interactions

The examples discussed above represent only the initial cross-pollination between reductionist and global approaches. By recognizing the importance of nonadditive interactions in genetics and biophysics, a common framework is being developed to more fully integrate systems and structural biology (Lehner, 2011). These interactions are commonly referred to as “epistatic” effects because the extent of the interaction depends on its genetic context (Phillips, 2008). In both genetics and biophysics, most perturbations act independently, yielding purely additive effects on fitness (Mani et al., 2008) or thermodynamic parameters (Wells, 1990). However, many functionally important perturbations may be benign in one context and deleterious in another (Cadwell et al., 2010). The nonadditive effects of mutations may underlie “missing” genetic heritability—the limitation in our ability to explain functional consequences of sequence changes (Zuk et al., 2012). Exciting new efforts are underway to correlate genetic interaction measurements used in systems biology (Mani et al., 2008) with thermodynamic coupling analyses used in structural biology (Horovitz, 1996) to establish general principles of nonadditivity and robustness that scale from systems to structure.

The most complete analyses of nonadditive effects at a systems-level are from genetic interaction screens in the budding yeast *Saccharomyces cerevisiae*. High-throughput measurements, such as synthetic genetic array (SGA) (Costanzo et al., 2010) or epistatic miniarray profile (E-MAP) experiments (Collins et al., 2010), compare quantitative measures of the fitness of individual gene knockouts to double knockouts (Figure 4). Positive genetic interactions occur when the fitness of the double mutant is higher than would be expected based on growth rates of the individual mutants. These alleviating interactions are enriched in gene products that physically interact or are part of a linear signaling pathway (Beltrao et al., 2010; Collins et al., 2007; Roguev et al., 2008). In contrast, cases where the double

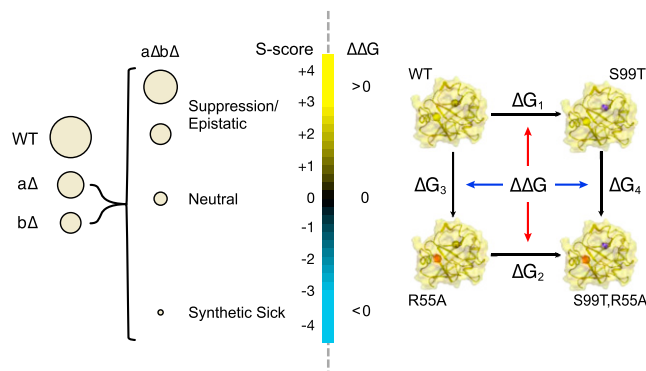


Figure 4. A Common Framework for Understanding Genetic and Thermodynamic Perturbations

Nonadditive effects are analyzed by similar methods and have similar distributions in a genetic (left) or biophysical context (right). For nonneutral genetic interactions and biophysical couplings, the effects of perturbations are context dependent. Quantitative genetic analysis distinguishes between negative [(aΔbΔ) < (aΔ)(bΔ)], positive [(aΔbΔ) > (aΔ)(bΔ)], and neutral [(aΔbΔ) = (aΔ)(bΔ)] interactions based on the fitness of the organism, represented here by colony size. Biophysical studies measure the free energy of a process, for example protein folding, across different variants to distinguish between negative ($\Delta G_3 > \Delta G_4$; $\Delta G_1 > \Delta G_2$), positive ($\Delta G_3 < \Delta G_4$; $\Delta G_1 < \Delta G_2$), or the absence ($\Delta G_3 = \Delta G_4$; $\Delta G_1 = \Delta G_2$) of thermodynamic coupling ($\Delta\Delta G$).

mutant is less fit than expected based on the individual mutations are termed negative genetic interactions. These aggravating interactions have been used to often identify proteins that function in parallel pathways of a given process (Tong et al., 2001). The extension of high-throughput genetic interaction methods to other organisms (Horn et al., 2011; Lin et al., 2012; Tischler et al., 2006; Typas et al., 2008) (N.J.K., unpublished data) has created great interest in mining these data sets to generate combination therapies for human diseases. In general, genetic interactions are most conserved between species within protein complexes, less conserved within biological processes, and least conserved across distinct biological processes. This suggests a hierarchical modularity that governs how nonadditive connections between proteins, complexes, and processes are functionally rewired and repurposed during evolution (Ryan et al., 2012). These genetic interaction approaches can extend across species to reveal dependencies between organisms in disease and symbiotic contexts (Fischbach and Krogan, 2010). A recent integration of insertional mutagenesis and depletion (iMAD) in the bacteria *Legionella* combined with RNAi in the host *Drosophila melanogaster* identified interesting cross-species synthetic lethal interactions that are relevant for pathogenesis (O'Connor et al., 2012). The fundamental principles of these genetic conflicts between species will likely be revealed by careful studies in classic host-pathogen systems such as bacteriophages and bacteria (Bondy-Denomy et al., 2012).

The hierarchical design principles revealed by high-throughput genetic interaction studies have many parallels in the principles revealed by biophysical studies of protein function. Analysis of nonadditive effects in biophysics studied in the context of protein folding (Horovitz and Fersht, 1990; Perry et al., 1989), enzyme mechanism (Carter et al., 1984), allostery

(Sadovsky and Yifrach, 2007), and protein-ligand interactions (Baum et al., 2010; Schreiber and Fersht, 1995). As with genetic interaction studies, biophysical double-mutant cycles reveal that most perturbations act independently (Wells, 1990) (Figure 4). However, both bioinformatics (Breen et al., 2012) and experimental (Bershtein et al., 2006; Tenaillon et al., 2012) studies suggest that nonadditive (epistatic) effects play a dominant role in shaping the evolution of proteins. In the context of protein structure, nonadditive effects of mutations often occur between residues that are in direct contact (Horovitz, 1987). For example, mutating two alanine residues to form a new lysine-aspartate salt bridge results is only beneficial when both mutations are made as neither the lysine-alanine nor aspartate-alanine pair is likely to be stabilizing. This “contact-based mechanism” of interactions parallels the positive genetic interactions observed between gene products that physically interact (Collins et al., 2007). A similar analogy of “parallel pathway mechanisms” exists between negative genetic interactions (Tong et al., 2001) and mutations that form new stabilizing intraprotein contacts or alternative intermolecular interaction binding modes. Promiscuous proteins with multiple binding modes may mask the negative effects of substitutions that only affect one interaction mechanism. Ancestral reconstruction of a nuclear hormone receptor revealed how two potential binding modes can be interconverted through conformational epistasis: where mutation at one site indirectly repositions the three-dimensional position of a remote second site and changes the functional effects of mutations at the second site (Ortlund et al., 2007). Similar to negative genetic interactions, conformational epistasis can lead to double mutations that affect both binding modes that are not apparent from the individual mutations.

However, despite general trends across atomic and network scales for positive and negative interactions, it remains difficult to predict the strength of non-additive interactions from network, structural, or evolutionary principles (Lehner, 2011). The simple contact-based or parallel pathway models can break down when long-range compensatory interactions occur (Lunzer et al., 2010), consistent with the fact that the correlation between direct protein-protein interactions and positive genetic interactions is not absolute, especially for complexes containing essential proteins (Costanzo et al., 2010). Mapping of the genotype-phenotype-fitness relationship (Lunzer et al., 2005) can be additionally complicated by the ability of molecular chaperones to act as buffers that may enhance nonadditive interactions as a protein nears its stability limit (Tokuriki and Tawfik, 2009) or a network is challenged by an external perturbation (Geller et al., 2007). For the significance of these trends to be addressed, comprehensive studies of protein mutations—on a scale similar to the genetic interaction studies—must be carried out. The advent of new sequencing methods now makes it possible to survey comprehensive sets of mutations under different selective pressures (Hietpas et al., 2011). One recent survey of all possible mutations in a PDZ domain revealed a strongly coevolving set of amino acids within which two mutations act nonadditively to change peptide binding specificity (McLaughlin et al., 2012). Studies on this newly accessible scale have the potential to reveal new principles of nonadditivity underlying protein stability and function (Arya et al., 2012).

The extension of these principles to the coevolution of protein-protein interactions will address the similarities in molecular mechanisms underlying epistatic interactions between and within individual gene products (Fridman et al., 2010; Skerker et al., 2008). Therefore, quantitative measures that intrinsically calibrate thermodynamic quantities to fitness measurements present new opportunities to discover unifying principles of buffering, nonadditivity, and adaptation across the atomic and network scales.

Looking Dynamically Forward

As these more comprehensive surveys of protein sequence and genetic interaction space continue to emerge, an additional challenge will be to adapt the common analytical models used in both systems and structural biology to include the fourth dimension: time. Much of the work we have highlighted here has focused on integrating multiple static representations, as exemplified by incorporating crystal structure information in a protein-protein interaction network diagram. Recently, structural interaction networks have been adapted to incorporate multiple protein conformations (Bhardwaj et al., 2011). Disease mutations that have only a minor effect on the dominant structure of a protein can exert a deleterious function by altering the ability to dynamically switch between near-native conformations (Shan et al., 2012). Indeed, mutations remote (Fraser et al., 2009; Wang et al., 2012b) and within (Bhabha et al., 2011) the active sites of enzymes can have large functional consequences without dramatically altering protein structure. Conservation of protein motions have been proposed to be a major constraint in protein evolution and may provide a mechanistic explanation for nonadditive effects between residues that are not in direct contact (Reynolds et al., 2011). Microsecond-millisecond protein conformational dynamics can be exploited in the development of new protein therapeutics (Levin et al., 2012) and in tuning the biophysical properties of small molecule inhibitors (Carroll et al., 2012).

At longer timescales, measurement of the dynamics of nonadditive responses in network contexts will also likely reveal new principles of modularity (Alexander et al., 2009). Indeed, profiling the changes in protein-protein interactions of the scaffold protein GRB2 after perturbations designed to activate different receptor tyrosine kinases revealed that the time-dependent rewiring and elaboration of new complexes centered around a conserved set of core interactions (Bisson et al., 2011). A comparison of genetic interactions in yeast in the presence and absence of DNA-damaging agents revealed that although relationships within protein complexes are largely conserved, there is substantial rewiring in the functional relationships between complexes (Bandyopadhyay et al., 2010). These results suggest that the concept of a network “module” may become more diffuse as a time dimension and additional perturbations are incorporated. Similar to genetic interactions, current efforts are underway to discover nonadditive effects in drug combinations (Yeh et al., 2009). Multiplex profiling of approved and clinically tested drugs uncovered new interactions that synergize to inhibit HIV replication (Tan et al., 2012). Variation of the timing and dynamics of different perturbations might reveal additional synergistic combinations. Methods, such as mass cytometry,

that can dynamically read out the response of the cell across many parameters simultaneously can be used to guide the optimization of perturbation dynamics (Bodenmiller et al., 2012). Indeed, new therapeutic regimens are currently being developed around nonadditive effects on the order and time course of drug combinations by exploiting network level principles (Lee et al., 2012). This study found that EGFR inhibition was ineffective when combined simultaneously with genotoxic drugs; however, staggered administration of the two perturbations dynamically rewires the cells and leading to a more efficient potential therapy. Collectively, these studies point to emerging parallels of the role of nonadditivity on the subsecond timescale in protein dynamics, on the hours-weeks timescale in the cellular response to perturbations, and on the million-year timescale in the adaptations that shape interaction networks (Ideker and Krogan, 2012).

In this review, we highlighted how the use of systems and structural biological approaches are truly synergistic when used in combination to understand complex biological phenomenon. For example, unbiased, systems approaches can identify key components that facilitate structural analysis, whereas structural information can provide insight into and even guide large-scale biological studies. Furthermore, we argue that there are striking similarities between the framework used to interpret quantitative genetic interactions and thermodynamic responses. Finally, detailed “small-scale” systems and synthetic biology efforts, which examine network motifs and quantitatively model signaling networks, are complementary to the large-scale studies since they can be used to define core interactions and network principles (see the review by Lim et al. (2013) in this issue of *Molecular Cell*). A challenge for future work will be to determine and integrate the quantitative relationships that exist at these different levels, including cell biology (Slack et al., 2008), pharmacology (Lounkine et al., 2012), and ecology (Hekstra and Leibler, 2012). In the near future, we expect methods that explicitly investigate these parallels from “systems to structure” will play an increasingly larger role in efforts to predict the phenotypic response to mutation, small molecules, pathogens, and other perturbations.

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