Modeling General Proteostasis: Proteome Balance in Health and Disease

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Abstract

Protein function is generated and maintained by the proteostasis network (PN) (Balch et al. (2008) Science, 319:916). The PN is a modular, yet integrated system unique to each cell type that is sensitive to signaling pathways that direct development and aging, and respond to folding stress. Mismanagement of protein folding and function triggered by genetic, epigenetic, and environmental causes poses a major challenge to human health and lifespan. Herein, we address the impact of proteostasis defined by the FoldFx model on our understanding of protein folding and function in biology. FoldFx describes how general proteostasis control (GPC) enables the polypeptide chain sequence to achieve functional balance in the context of the cellular proteome. By linking together the chemical and energetic properties of the protein fold with the composition of the PN we discuss the principle of the proteostasis boundary (PB) as a key component of GPC. The curved surface of the PB observed in 3-dimensional space suggests that the polypeptide chain sequence and the PN operate as an evolutionarily conserved functional unit to generate and sustain protein dynamics required for biology. Modeling general proteostasis provides a rational basis for tackling some of the most challenging diseases facing mankind in the 21st century.

Newly synthesized proteins must fold into a unique three-dimensional (3D) structure to become functionally active. We now appreciate that all proteins likely require the assistance of the "proteostasis network" (PN) to generate and maintain function. The PN comprises not less than a 1000 factors that regulate protein synthesis, folding, function, and degradation [1–3] (FIG. 1). These form the Yin and Yang environment that promotes what we have referred to recently as proteome balance in health [4]. Importantly, the composition of the PN is dynamically regulated by a variety of signaling pathways [5,6], and in response to developmental cues, genetic changes, epigenetic marks, environmental stress and aging; challenges that all cells encounter during their lifespan to maintain normal organismal physiology [3,7,8]. Of importance, is that a very large number of inherited diseases are caused by mutations in the sequence of a polypeptide chain, leading to loss of protein stability, misfolding and disease. While genetic changes often severely challenge the

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dynamics of proteostasis to retain proteome balance, the response of the PN to mutation can significantly contribute to organismal evolution [9]. Given the multiplicity of cellular PN stress responses, it is not surprising that the PN has evolved to be highly versatile in its capacity to maintain proteome balance. Herein, we discuss the role of protein energetics and kinetics in generating and maintaining proteome balance through the activity of the PN. We explore how modeling of proteostasis opens new avenues to the management of human health and disease.

Principles guiding the function of the Proteostasis network

While small, single domain proteins can fold efficiently in the test tube, we now appreciate that these and multi-domain proteins generated in the crowded environment of the cell often fail to do so. This is because there are energy barriers in the landscape model (Fig. 2a, peaks and troughs) [10,11], that dictate the kinetics and thermodynamics of folding intermediates in the path(s) required to achieve the native folded state (Fig. 2a, red circles and 'N' in figure). The native state here is defined as the state with the lowest energy- which may or may not be the biologically important state [4]. To avoid off-pathway misfolding, degradation and/or aggregation that can occur during progress through intermediate folding steps in biology (Fig. 2a, red circles and white arrows), PN components are thought to interact with the polypeptide chain to generate and protect biological function (Fig. 2a, black and gray arrows) [1,2].

The PN is an integrated system consisting of chaperones, folding enzymes, degradation components, and regulatory pathways that control the composition and concentration of the general proteostasis system [5,12] (Fig. 1). PN components include the molecular chaperones/co-chaperones belonging to the Hsc/Hsp (Hsc/p) 70 and 90 families [13], the GroEL/TCP1-ring complex (TRiC)/chaperonin family of folding machines [14], tetratricopeptide repeat (TRP)-domain containing proteins, proteins that modulate oxidative folding (e.g., protein disulfide isomerases [15]), and degradation components comprising both the cytosolic ubiquitin-proteasome and membrane-linked autophagy-lysosome systems [6,16] (Fig. 1). Some PN components are highly abundant (e.g., ribosome, Hsc/p70-Hsp90, proteasome/lysosome) and provide a cellular 'buffer' for synthesis, folding and/or degradation [12]. Most others function as specialists, either alone or together with the Hsc/p70 and Hsp90 systems, to synthesize and/or maintain specific folds for the highly evolved dynamic functions dictating extant organismal physiology. Regulation of the composition of the PN occurs through a number of signaling pathways including the unfolded protein response (UPR) [17], the heat shock response (HSR) [7,18,19], oxidative stress pathways [20], and growth factor and diet sensitive pathways [1–3], among others. A simplistic view of the PN is that components directing synthesis, (un)folding, and degradation could be considered as a triad of modules (Fig. 1, dark black lines). Triad modules recognize the chemical properties of polypeptide folding intermediates (Fig. 2), yet are integrated by the overall composition of the PN to maintain normal biological function.

It is important to recognize that the PN is unique for each type of cell and the numerous subcellular compartments within a eukaryotic cell. These environments change differentially during development, aging and in response to physiological stress [2,21]. Moreover, the PN is constantly challenged by changes in the composition of the amino acid pool, metabolites/co-factors, ion balance, genetic-epigenetic-environmental triggers, and viral/bacterial pathogens. These factors not only affect the inherited capacity of the proteostasis program, but are readily sensed by the above regulatory signaling pathways that attempt to rebalance the proteome to preserve healthspan [1,3,4]. Thus, the PN is dynamically tuned to cellular function as prescribed by cell autonomous processes and cell non-autonomous signals that optimize folding for function in complex organismal environments [7,19].

Role of ATP in maintaining proteome balance

The capacity of the PN to maintain proteome balance in the cytosol and exocytic/endocytic trafficking compartments, that is, the Yin-Yang relationship between generating and maintaining a functional fold or targeting a protein for degradation [4], is referred here as general proteostasis control (GPC). GPC emphasizes that function of a polypeptide chain is tightly linked to the local composition of the PN triad- the environment being the ultimate arbitrator of biological folding for function. What is a wild-type protein fold in one PN environment becomes a 'mutant' in another and can be removed and/or challenge the health status of the cell. The former is evident in the cyclical stability of proteins during cell cycle, or the transient stability observed in developmental programs. The latter is observed in, for example, numerous sporadic aggregation diseases, type II diabetes and cancer [2].

Both subtle and global challenges to protein folding energetics directly challenge the dynamics of the kinetics of protein folding and its thermodynamic stability. Thus, there is a close link between PN folding for function (Fig. 2a) and ATP-based proteostasis machines that manipulate the energy landscape dictated by the unique chemistries of amino acid sequence of each polypeptide chain (Fig. 2b). For example, during protein biogenesis, newly synthesized polypeptides are generated by the ribosome at a very high energy cost and in response to protein specific translational control programs. They emerge from the ribosome with exposed hydrophobic residues that are recognized by the folding module of the PN to prevent protein aggregation. This first step of GPC faced by nascent proteins is often regulated by members of the Hsc/p70 family [12] and/or the TRiC/chaperonin ATPase machines [22]. While TRiC ATPases appear to be dedicated to folding, the Hsc/p70 ATPases function to either promote folding/assembly of newly synthesized proteins or direct 'non-native' polypeptides to degradation [23], serving as a key linker between the various PN modules (Fig. 1). Thus, the Hsc/p70 system plays a critical role in proteome balance in response to energetics of the polypeptide chain [4].

Hsc/p70 family of chaperones, utilize ATP-dependent cycles of client binding and release in response to a plethora of accessory proteins, called co-chaperones. In the case of Hsc/p70 these include nucleotide exchange factors (NEFs) composed by the Bag (BCL2 associated athanogene) family of proteins, which facilitate ADP release and ATP binding to promote Hsc/p70 client substrate release, and a large Hsp40/DnaJ family of co-chaperones that stimulate the ATPase activity of Hsc/p70 and stabilize protein client-chaperone interactions [24]. Thus, Hsc/p70 co-chaperones will not only fine-tune Hsc/p70 client substrate specificity but dictate the cellular fate of the protein client [13,25,26].

Proteins that interact with the Hsc/p70 arm of GPC are in many cases subject to a second level of maintenance by the Hsp90 system [27]. The Hsp90 ATPase machinery appears to recognize dynamic facets of more folded substrates to modulate their activity(s) (Fig. 2a, blue lines), [27,28] (www.picard.ch/downloads/Hsp90interactors.pdf). As is seen for the Hsc/p70 system, a unique collection of co-chaperones also regulate Hsp90 ATPase activity. Hsp90 co-chaperones include the ATPase activator Aha1 and the ATPase inhibitor p23, as well as Cdc37, HOP, protein-disulfide isomerases (PPIases), and a large family of TRP-domain containing proteins. Depending on the local activity/composition of the co-chaperone environment, Hsp90 can promote either protein stability or degradation of folding intermediates- dynamically altering the proteome balance [4] (Fig. 2b).

Like the folding GPC module (Fig. 1), the degradation module involving the proteasome and the autophagy-lysosome pathways are intensely ATP-dependent [29]. While many of the regulatory factors that control function of these degradative machines remain to be determined, client targeting to multiple degradative pathways is generally regulated by the

ubiquitin/sumoylation system. Targeting for degradation utilizes a highly diverse (>300) set of client specific ligases that utilize the energy of ATP to prime polypeptide targets for destruction [23]. Thus, ATP-dependent cycling of synthesis, folding, and degradation modules provides an energetic link between the functional and degradation prone states of a target protein in biology.

While GPC has a universal high level of energy demand, it is important to realize that folding/function is highly compartmentalized. For example, the cytosol is a reducing environment maintaining folded proteins in the absence of disulfide bonding. In contrast, the endoplasmic reticulum (ER), the first step in the secretory pathway, is an oxidative environment where protein folding is driven by an evolutionarily related, but distinct set of luminal PN folding components. The folding module in the ER is tightly coupled by membrane translocation pathways to the reducing cytosolic proteasomal degradation module [23,30,31]. Recent studies [32] have shown that cytosolic GPC components important for generation of newly synthesized transmembrane polypeptides in the ER also modulate protein stability at the cell surface. Likewise, the lysosome not only handles internalized cargoes from the cell surface, but is a critical partner of the autophagosome pathway that engulfs a wide range of misfolded cytosolic proteins and dysfunctional organelles [29,33]. These results suggest the importance of as yet unknown cellular proteostasis sensors that unify and balance folding throughout the cell.

In summary, it is now clear that GPC may define energetic standards for each cell type that is linked in as yet to be determined ways by the activity of PN-linked ATPase machines. By coupling the chemistry of the polypeptide chain sequence and its associated folding energetics with the ATPase activity of PN modules (Fig. 2b), a biologically dynamic GPC standard generates the proper balance between the triad of synthesis, (un)folding, and degradation modules (Fig. 1). This standard defines the proteome balance in a healthy cell and its response to stress, disease, injury and aging programs.

Modeling proteostasis

An understanding of the rules guiding GPC to achieve protein function involves integrating the chemistry of the polypeptide sequence with the activity of PN components (Fig. 2). For this purpose, we applied Michaelis-Menten formalism in the *FoldEx* model to describe how the inherent chemistry and energetics of the polypeptide chain can be read and manipulated by the PN for proteins trafficking through the exocytic pathway [34]. The concepts stemming from the FoldEx model were extended to describe a more encompassing model of how folding energetics and the PN work together. We refer to this new model as *FoldFx* [2]. FoldFx is applicable to folding of proteins in all compartments of the cell and the extracellular space in response to the composition of the local PN (Fig. 1). In FoldFx, the operational goal of the triad of protein synthesis, (un)folding, and degradation modules through GPC is to achieve 'function'.

A key feature of the FoldFx model which rigorously defines the activity of GPC is the concept of the 'proteostasis boundary' or PB [2] (Fig. 3a). The PB can be used to define the minimal energetic properties that a protein must have to achieve normal function in response to the local PN. The PB is best illustrated in a 3-dimentsional (3D) space diagram as a curved surface. The position of a protein in 3D is determined by its inherent folding kinetics, misfolding kinetics, and thermodynamics (Fig. 3a). The curved shape of the PB is dictated by the variable concentration of proteostasis components. These are, in turn, defined by the genetic, epigenetic, and intrinsic and extrinsic factors that regulate PN pathways and thereby tune the PN for specific client functionality. Beneath the boundary is a normal biological network, defined by nodes (the proteins) and edges (their links to other proteins within the

network) (Fig. 3a). Each node is positioned according to its folding energetics (its unique folding and misfolding rate and stability). In a healthy cell, each node and its link (the edges in Fig. 3a) are embraced by the curved space of the PB, indicating that the PN is sufficient to maintain normal function (Fig. 3a). In disease, a node falls outside the embrace of the PB, resulting in misfolding, aggregation and/or degradation (Fig. 3b).

Current evidence suggests that in healthy cells the PB is protective but does not have excess of proteostasis capacity [2,5,7]. To compensate for this limit threshold (Fig. 3), the PB is highly responsive to numerous signaling pathways (Fig. 1). Thus, the GPC operates as a rheostat to control the shape of the PB in time and space- it can be turned up or down to adjust the folding capacity in response to the environment to achieve biology.

GPC modeling in health and disease

Given the FoldFx model and the impact of GPC on protein function, when is a protein folded and functional from a biological perspective? Put in another way- what is disease? This remains a challenging question, as up to 30% of proteins are now thought to have some level of intrinsic 'disorder'-that is, unfolded. One possibility suggested by FoldFx is that folding kinetics and energetics defined by the energy landscape (Fig. 2) are continuously defined and redefined by the Yin and Yang of the GPC triad in a fashion that considerably extends the chemistry imparted by the polypeptide sequence to achieve normal cellular healthspan (Fig. 4a-GPC1). Thus, we would suggest that the the polypeptide sequence per se can be of limited utility to the cell [10,11]. Rather, what it does provide is a dynamic template for the GPC to sustain and evolve biology [9]. In contrast to a healthy proteome, in inherited folding disorders, during aging and in environmentally triggered pathologies (including physical and pathological insults), the Yin-Yang of the proteome imbalance challenges GPC biology [4], exceeding the capacity of the GPC to manage the polypeptide template folding to achieve function (Fig. 4b-GPC2).

Of particular contemporary interest is the aging-related decline in the functionality of the GPC triad, triggering systemic and neurodegenerative disease [3,35,36]. Increased protein 'wear and tear', transcriptional and translational dysfunction, and altered signaling pathways associated with aging can significantly challenge the PB, reducing the effective capacity of GPC triad and thereby triggering proteome imbalance (Fig. 3b,Fig. 4b). It is now recognized that aging related decay of the PN can be remediated by IGF1-R (FOXO and HSF-1 transcription factors), diet, and oxidative stress pathways. For example, the levels of expression of the Hsc/p70 chaperone system decrease in senescent fibroblasts and in the human brain during aging [3,35]. Likewise, the DNA binding activity of the HSF-1 transcription factor is diminished with aging in rat hepatocytes, causing an imbalance in the expression of the genes encoding chaperones during the stress response [37]. Studies have demonstrated that delaying the aging process by improving the activity of the Hsc/p70 system in both Huntington disease (HD) and Alzheimer disease (AD) models prevent disease onset [38]. During aging, protein degradation by autophagy is enhanced and Bag3 (an Hsc/p70 ATPase regulator), has been implicated as the main player linking the Hsc/p70 chaperone module and misfolded clients to the autophagy system [39]. Interestingly, senescent cells switch expression from the Bag1 to Bag3 co-chaperone [25], possibly as a compensatory mechanism to clear all the misfolded proteins that increasingly accumulate in aged cells. Similar to genetic and aging related changes in the GPC triad, environmental triggered pathologies are more cumulative and hugely impact the capacity of the PN to maintain proteome balance during aging as illustrated by both systemic (type II diabetes) and neurodegenerative (amyloid) disease.

In summary, it is becoming increasingly evident that we will need to further develop the FoldFx model in the context of genetic, epigenetic, and environmental factors that directly affect the status of the GPC triad during aging and its effect on proteome balance (Fig. 4a) to improve human healthspan.

Therapeutics in modeling of the GPC triad

Multiple lines of evidence suggest that protection to misfolding disease and aging can be boasted through multiple pathways that regulate the expression of PN components (e.g., HSR, IGF1-R signaling, diet restriction and pathways that protect against oxidative stress mentioned above) [1,3] (Fig. 1,Fig. 4c -GPC3). Modulation of components of the PN biologically by targeting individual PN components with siRNA implicated in these pathways can dramatically affect the outcome of disease. For example, depletion of Aha1 (an Hsp90 ATPase regulator) or E3ligase RMA1/CHIP, partially restores functionality in cystic fibrosis (CF) models [40,41]. These represent changes in distinct arms of the Yin-Yang balancing act, involving both cytosolic and exocytic/endocytic membrane trafficking pathways managed by the GPC triad (Fig. 4c). Moreover, overexpression of Hsc/p70 and its co-chaperones has been shown to reduce aggregation and toxicity in models of neurodegenerative/misfolding diseases, such as AD [42], prion disease [43], and HD [24]. Overexpression of Hsp40 reduces polyQ inclusion formation and toxicity [24] while the co-chaperone CHIP suppresses the toxicity of α-synuclein and polyQ proteins [44], and reduces accumulation of tau and Aβ [45], possibly through removal of aggregated misfolded proteins via the proteasome. The cofactor Bag1 also has been shown to reduce toxicity caused by polyQ Huntington aggregates [46]. Indeed, the FoldFx model predicts that bolstering the operation of the PN along specific axis's is likely to not only improve healthspan, but also simultaneously improve longevity- the ultimate test of a therapeutic approach [1,2,7,8,47].

An increasing number of small molecules are now recognized to impact these pathways and provide protective function to human disease [4,48,49] (Fig. 4c). For example, the inhibition of the proteasome arrests myeloma disease [50], kinetic stabilizers arrest onset of TTR [51], histone deacetylases function to correct CF [52], Friedreich Ataxia [53], HD [54] and poly-glutamine (polyQ) disease [26] and have a strong like to GPC [18].

It is now clear that the ultimate goal for FoldFx modeling will be to utilize it as framework for further understanding of human biology and for development of small molecule therapeutics that manipulate GPC triad to maintain and restore human health.

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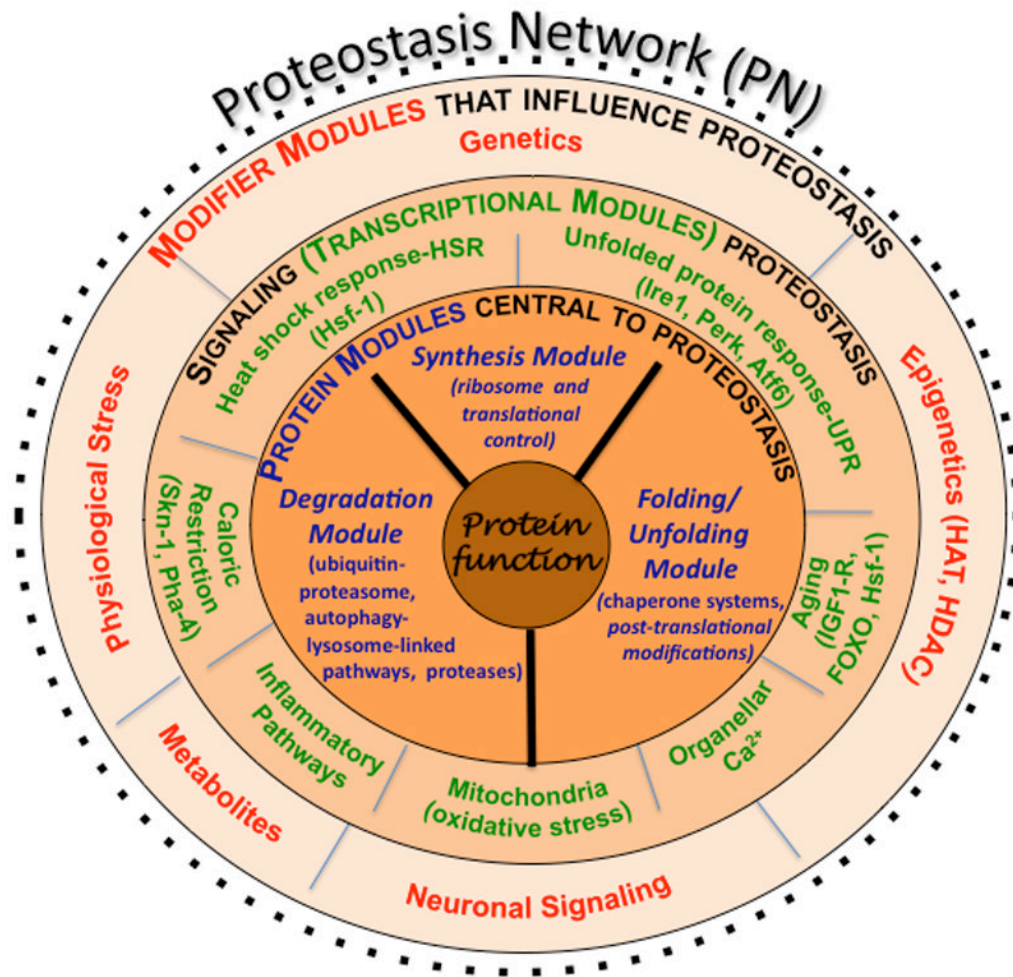
Figure 1. The PN

Shown are the interactions that comprise the PN, the composition of which is responsible for generating and maintaining the biological protein fold. Components comprising the PN outlined in the inner-most layer (in *blue font*) involve the synthesis module, the folding/ unfolding module, and the degradation module (the GPC triad). A second layer shows the signaling transcriptional pathways (in *green font*) that influence the level and activity of the triad found in the innermost layer. The third layer (in *red font*) includes modifiers that influence and/or integrate the activities defined by the second and first layers. Modifiers and signaling pathways from both cell autonomous and cell non-autonomous origin. Modified figure reproduced with permission from Elsevier Press [2].
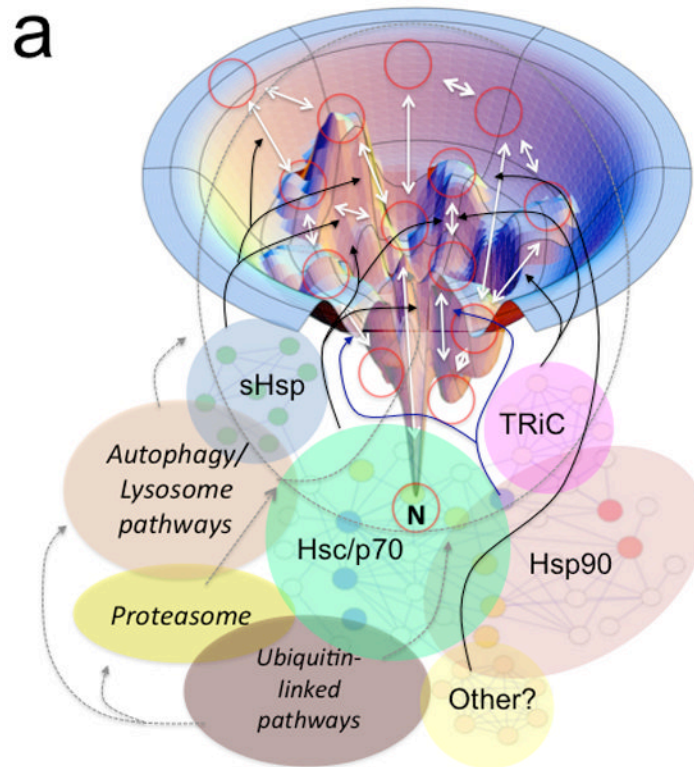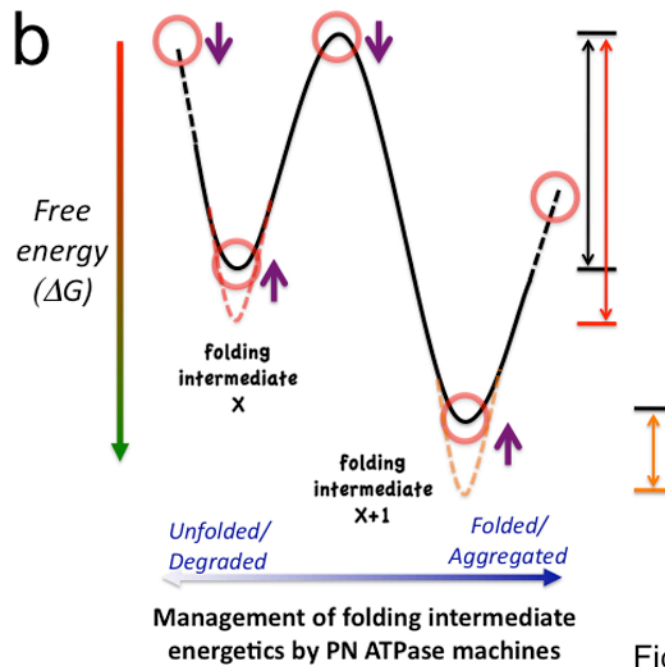
Figure 2. Coupling of the folding energy landscape with the PN
(**a**) Illustrated is a bumpy energy landscape funnel (http://www.dillgroup.ucsf.edu/) in which an unfolded protein proceeds along various intermediate steps (red circles) that can pose energetic barriers to achieve the native state (N) at the base of the funnel. The white arrows indicate potential pathways through various folding intermediates that the nascent protein may take to reach the native state. The solid black and blue (synthesis and folding modules)

and the dashed gray (degradative module) arrows illustrate how different components of the PN may influence pathway choice. The dashed oval indicates that all intermediates are potential steps in which a protein can be targeted for degradation. Hsp90 is thought to principally facilitate late folding events (solid blue lines). (**b**) The energy landscape (a slice through the funnel illustrated in panel **a**) illustrates the central role of the ATPase cycle in synthesis, folding and degradative modules in managing the biology of the protein structure in the cell to achieve function. By coupling the energy of ATP hydrolysis (*X-axis*) by PN ATPase machines with the energetics imparted in the chemistry of the amino acid sequence of the polypeptide chain (*Y-axis*), PN ATPases maintain the protein fold in a dynamic state-essential for biology. Right side of panel illustrates energy barriers necessary to achieve a functional fold (black arrow) relative to the energetics associated with misfolding (red and orange arrows). The additional energetic demands challenging GPC through misfolding (red line) or aggregation (short orange line) are illustrated. Purple arrows indicate potential steps for GPC to alter folding kinetics and energetics to promote proteome balance and cell health. Abbreviations: sHsp (small heat shock proteins); TRiC (TCP1-ring complex).
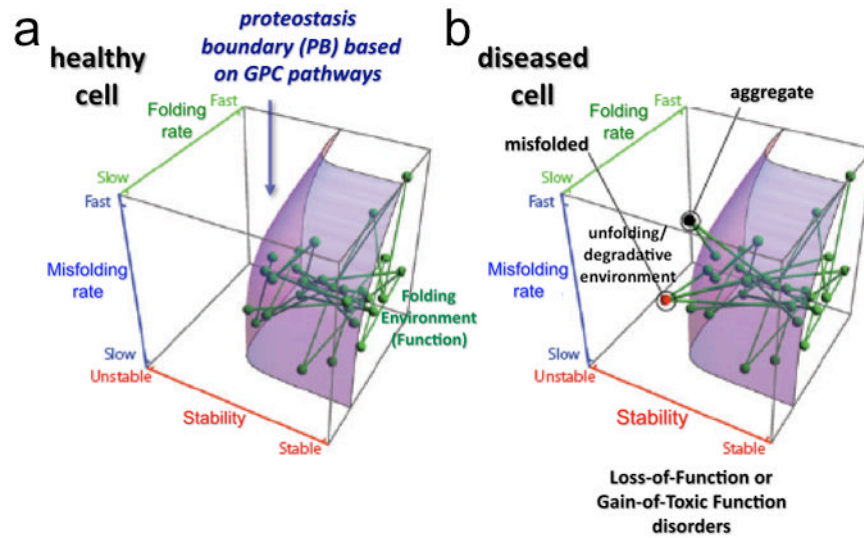
Figure 3. The proteostasis boundary (PB)
The position of each node (protein) relative to the PB (curved surface) responsible for biological function is defined by a protein's folding properties: folding kinetics (Z-axis), misfolding kinetics (Y-axis) and thermodynamic stability (X-axis). Each line defines a physical or functional interaction between two proteins in the system. The location of the PB in 3D space is established by the composition of the PN and modulated by the GPC triad. **(a)** All of the nodes are within the PB boundary in a healthy cell. **(b)** Mutations or aberrant post-translational modifications can alter folding kinetics and energetics, making their corresponding nodes and edges fall outside (above the curved surface) of the PB. This space in the 3D plot does not support function of the energetically destabilized variant and can lead to either degradation (red node) or protein aggregation (black node). The loss of connectivity to proteins within the embrace of the PB can challenge the entire PN leading to cell, tissue, and organismal disease. Reproduced with permission from Elsevier Press [2].
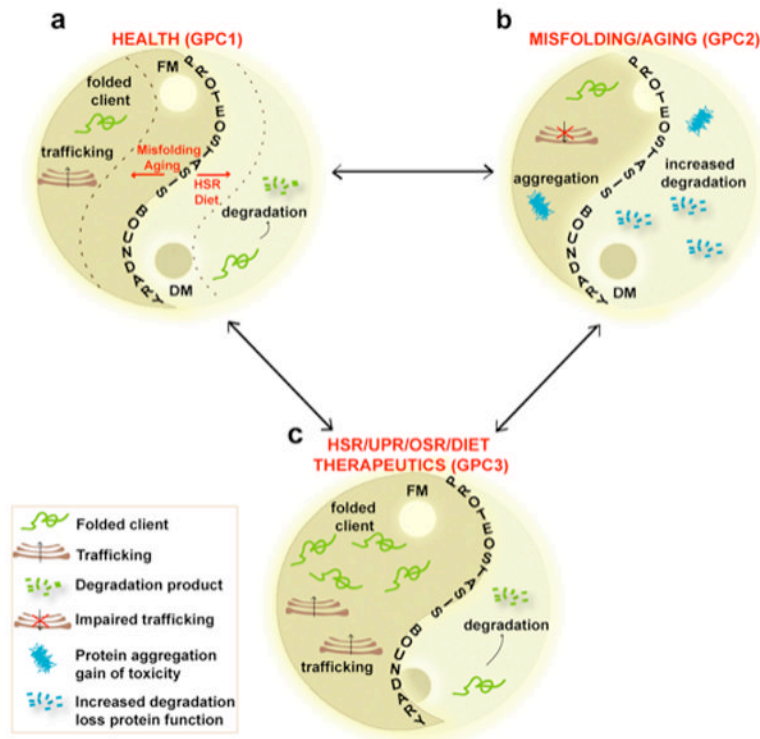
Figure 4. Modeling the Yin-Yang of proteome balance in health, disease, and in response to proteostasis therapeutics

(Panel **a**) Proteome balance [4] in a healthy cell is determined by the composition of the synthesis and folding modules (FM) (the Yang on the left side of diagram) and degradative module (DM) (the Yin on the right side of diagram). GPC1 determines the position of the PB (the S-shaped curve) and healthspan. The dashed lines illustrate that misfolding and aging can challenge the position of the PB. (Panel **b**) Aging and unfolding move the PB to the left resulting in compromised proteostasis function (GPC2) and an unhealthy cell by triggering increased degradation and/or accumulation of protein aggregates. (Panel **c**) Biological signaling pathways including the HSR (HSF1 and IGF1-R/FOXO pathways), UPR, oxidative stress response (OSR), diet, IGF1-R and/or proteostasis targeted therapeutics can move the Yin-Yang balance defined by the PB to the right generating GPC3. GPC3 provides an environment that protects the cell from physiological stress, misfolding and aging, allowing the cell to return to GPC1.