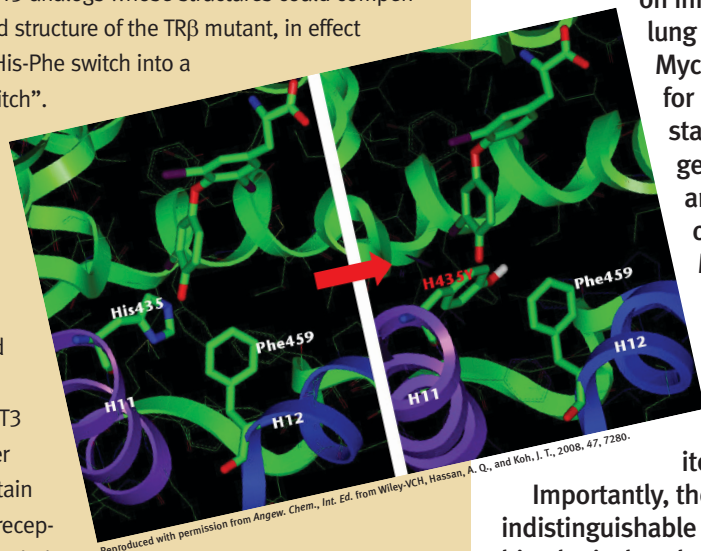
Spotlight

Switching Up the His-Phe Switch

Thyroid hormone receptors (TRs) are ligand-dependent transcriptional regulators that play key roles in development and metabolism. Loss of function mutations in these receptors are associated with an inheritable genetic disease, resistance to thyroid hormone, and several types of cancer. TRβ possesses a "His-Phe switch" motif that mediates interaction with its ligand, triiodothyronine (T3), and mutations to this region can dramatically impair receptor function in response to T3. Hassan and Koh (*Angew. Chem., Int. Ed.* 2008, *47*, 7280–7283) use a novel chemical strategy to rescue function in the naturally occurring TRβ mutant His435 → Tyr, which has been identified in both hormone resistance and pituitary cancer.

By examining the crystal structure of TRβ bound to T3, the authors designed T3 analogs whose structures could compensate for the altered structure of the TRβ mutant, in effect transforming the His-Phe switch into a novel "Tyr-Phe switch". This task was especially challenging because of the fact that the His435 → Tyr mutation results in a smaller ligand binding pocket, requiring that the T3 analogs be smaller than T3 yet still retain other key ligand–receptor interactions needed for a high-potency interaction. Clever tweaking of hydrogen bonding groups within the T3 structure led to analogs containing a pyridine moiety in place of the outer phenyl ring of T3. Indeed, these compounds were potent activators of TRβ(H435Y). Moreover, they showed a binding preference for the mutant TRβ over wild-type. By restoring function to otherwise damaged receptors, this innovative chemical rescue approach could lead to development of new drugs targeting cancers and inheritable genetic diseases linked to mutations in TRβ. **Eva J. Gordon, Ph.D.**



Making the Most of Myc

Myc is a transcription factor that is deregulated and overexpressed in most cancer cells. However, its critical role in the growth of all normal cells has raised concerns about its suitability as a cancer target, because severe side effects could result from inhibiting its function. To address whether inhibiting Myc could be an effective strategy for treating cancer without causing unacceptable side effects, Soucek *et al.* (*Nature* advance online publication Aug 17, 2008; DOI: 10.1038/nature07260) explore the effects of Myc inhibition in a mouse model of lung cancer.

By creating transgenic mice in which Myc-dependent transcriptional activation can be systemically yet reversibly blocked, the effect of Myc inhibition on initiation, early, and late phases of lung cancer was assessed. Remarkably, Myc function was found to be required for proliferation of lung tumors at all stages, confirming its role in tumorigenesis and validating its potential as an anticancer target. However, it was crucial to also address the effect that Myc inhibition had on proliferation of normal cells, especially those in continuously proliferating tissues, such as skin, the gastrointestinal tract, and bone marrow. Thus, Myc function was next inhibited in cancer-free transgenic mice.

Importantly, the general health of these mice was indistinguishable from that of control mice, as was the histological evaluation of organs with relatively slow growth profiles. However, tissues that exhibit more rapid proliferation were indeed adversely affected. Cleverly, the reversibility of the system was brought into play by investigating the recovery of this tissue attrition when Myc function was no longer blocked. Notably, the negative effects on the rapidly proliferating tissues were completely reversed within a week of restoring Myc function. These encouraging results suggest that Myc may in fact be a viable cancer target and that accompanying side effects may be well-tolerated and temporary. **Eva J. Gordon, Ph.D.**

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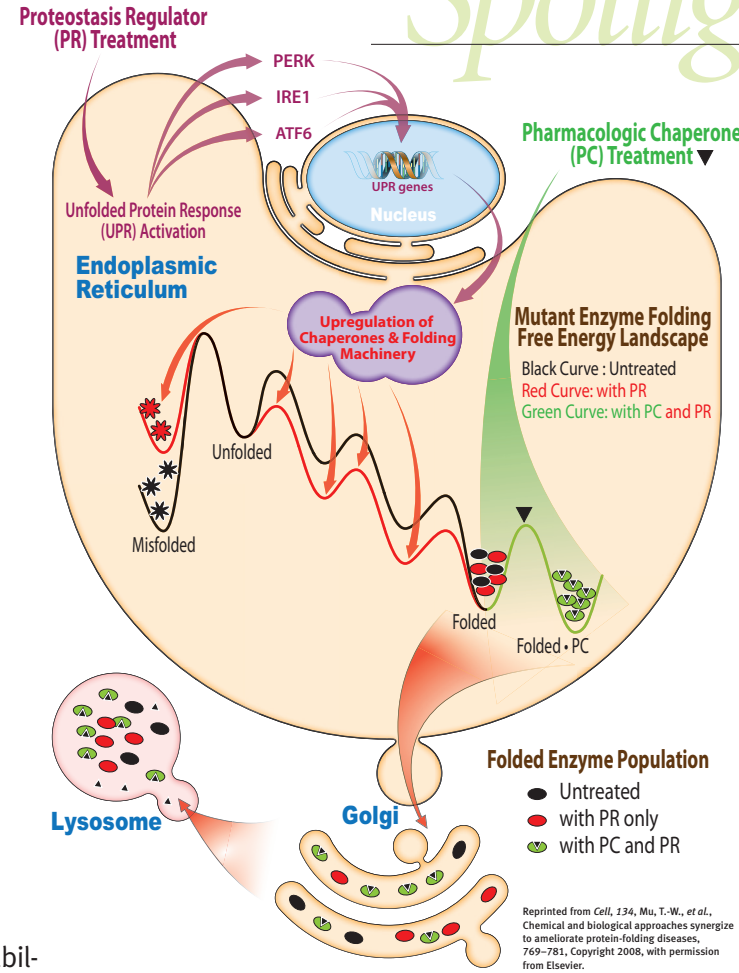
Promoting Proteostasis

Proteostasis, the homeostasis of cellular proteins, relies on the proper folding, localization, activity, and degradation of each of the proteins in the proteome of a given cell. Many diseases, including lysosomal storage diseases (LSDs) such as Gaucher disease and Tay–Sachs disease, result from improper folding and degradation of a mutated protein, a process that challenges proteostasis. Mu *et al.* (*Cell* 2008, *134*, 769–781) report the discovery of two small molecules that can function as "proteostasis regulators" and restore function to mutant enzymes associated with LSDs by enhancing the biology of protein folding.

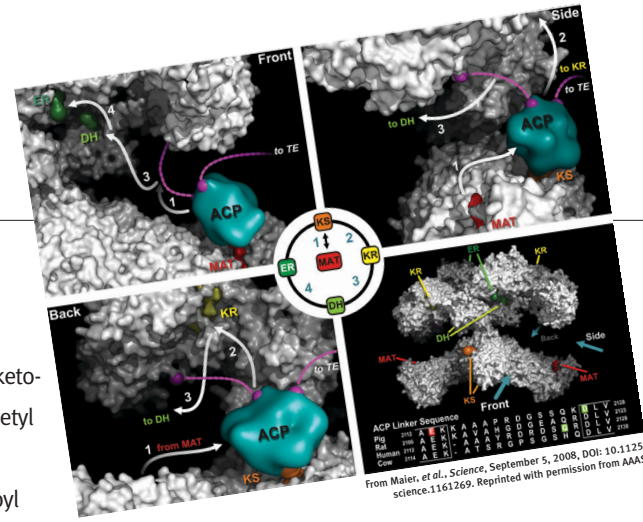


Reprinted from *Cell*, *134*, Mu, T.-W., *et al.*, Chemical and biological approaches synergize to ameliorate protein-folding diseases, 769–781, Copyright 2008, with permission from Elsevier.

Several small molecules known to affect proteostasis were tested for their ability to increase the activity of mutant proteins linked to LSDs. The heat-shock transcription factor 1 activator celastrol and the proteasome inhibitor MG-132 were each found to partially restore the folding, localization, and activity of the mutated enzymes glucocerebrosidase and β-hexosaminidase A in cells derived from patients with Gaucher disease and Tay–Sachs disease, respectively. Exploration of the mechanism of action of these small molecules revealed that although they induce both the heat-shock response influencing cytoplasmic proteostasis and the unfolded protein response (UPR) affecting secretory pathway homeostasis, it is the activation of the UPR that facilitates their role as proteostasis regulators. Induction of the UPR affects the coordinated expression of chaperones and folding enzymes, such that folding efficiency of the mutant enzymes is enhanced. Moreover, when proteostasis regulators were co-administered with pharmacologic chaperones, small molecules that bind to mutant enzymes and stabilize them in the endoplasmic reticulum, a synergistic enhancement in enzyme activity was observed. Identification of such proteostasis regulators offers a promising jumping-off point for the discovery of novel therapeutic agents where one small molecule enhances the folding of several misfolding-prone proteins associated with distinct maladies of similar etiology. **Eva J. Gordon, Ph.D.**

Spotlight

Trimming the Fat on Fatty Acid Synthase



From Maier, *et al.*, *Science*, September 5, 2008, DOI: 10.1125/science.1161269. Reprinted with permission from AAAS.

Fatty acids serve a wide range of critical functions in biology, including being fundamental components of biological membranes, operating as energy storage compounds, acting as signaling molecules, and directing the localization of proteins. In humans, mammalian fatty acid synthase (mFAS) is responsible for fatty acid synthesis, but overexpression of this large multienzyme has been correlated with cancer. Toward gaining a better understanding of fatty acid biosynthesis and developing effective new anticancer agents targeting mFAS, Maier *et al.* (*Science* 2008, *321*, 1315–1322) present the crystal structure of mFAS alone and in complex with its cofactor $NADP^+$.

mFAS is made up of a β-ketoacyl synthase, a malonyl-acetyl transferase, a β-ketoreductase, a dehydratase, an enoyl reductase, an acyl carrier protein (ACP), and a thioesterase. With five of these domains resolved to 3.2-Å resolution, the structure reveals the intricate architecture through which the domains are connected, provides key features of many of the active sites, and highlights how the complex is organized into a homodimer composed of a lower condensing portion and an upper portion responsible for modification of the substrate β-carbon. The structure also offers compelling evidence for the evolutionary relationship between mFAS and bacterial and fungal polyketide synthases (PKSs), such as the presence of a nonfunctional ME-like domain in mFAS that is highly suggestive of a catalytically active domain found in several PKSs. Finally, the structure gives clues into how the substrate is shuttled between domains; flexible tethering of the ACP domain and limited contact between the upper and lower portions of the complex facilitate this complex process. Solving the structure of this important catalytic complex provides an exciting new framework for the design of novel FAS inhibitors. **Eva J. Gordon, Ph.D.**

When Inclusion Equals Stranding

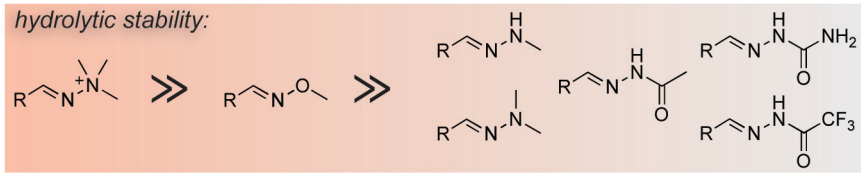
The aggregation of misfolded proteins into large self-associated bodies is thought to underlie the pathology of diseases such as Alzheimer's and type II diabetes. In the last few years, much progress has been made in studying the interaction of the proteins within Alzheimer's amyloid fibers. High-resolution structures of the fibers implicate β-strand contacts between neighboring proteins, an ordered interaction rather than a random adhesion of protein molecules. These aggregates have long eluded the structural biologist's eye because of the difficulty of working with proteins that tend to form large oligomers. In reality, obtaining a homogenous population of pure protein from a bacterial overexpression platform is often a rate-limiting step for structural studies of any protein. In bacteria, many proteins form insoluble aggregates, termed inclusion bodies, where proteins are thought to clump together randomly as a result of misfolding. Recently, a pair of groups (Wang *et al.*, 2008, *PLoS Biol. 6*, 1791–1801) collaborated to take a look at those pesky inclusion bodies from bacteria and found an impressive link with the pathologically relevant amyloid fibers.

Because the native fold of a protein could influence the type of aggregates that were formed, the

Oxime Examination

The chemical reactivity and biological prevalence of carbon-nitrogen double bonds make them intriguing and important functional groups. Indeed, studies of these compounds, which include imines, hydrazones, and oximes, date back to the 1930s. While it is well-established that among this trio, imines are the least stable to hydrolysis, few studies have addressed the relative stability of hydrazones and oximes. Now, Kalia and Raines (*Angew. Chem., Int. Ed.* 2008, *47*, 7523–7526) directly compare the hydrolytic stability of hydrazones and oximes.

A group of seven compounds, composed of structurally similar hydrazones and an oxime, was synthesized and compared in this study. Hydrolytic stability was evaluated by using NMR spectroscopy to determine the half-lives for the hydrolysis of the compounds as well as their pK_a values. Deuterated buffers and an excess of deuterated formaldehyde, which helps drive the hydrolysis reaction to completion, were used in proton NMR studies. These studies revealed that the oxime exhibits a significantly longer half-life relative to that of the hydrazones, with the surprising exception of the trimethylhydrazonium ion, which has a half-life even longer than that of the oxime. pD-Titration experiments provided intriguing additional information about the protonation state of the compounds and suggested that the rate of hydrolysis is correlated with the susceptibility to protonation of the nitrogen atom that participates in the carbon-nitrogen double bond. Kalia and Raines could not form the "hyperstable" trimethylhydrazonium ion by a condensation reaction, leaving a worthwhile challenge for future investigators. Such insight into the characteristics of oximes and hydrazones will facilitate their expeditious use for chemical and biological applications. **Eva J. Gordon, Ph.D.**



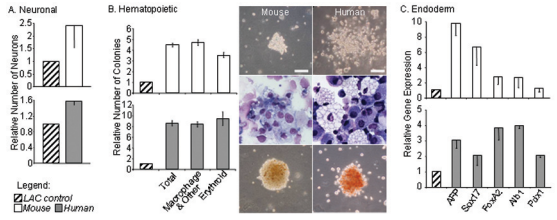
Reproduced with permission from *Angew. Chem., Int. Ed.* from Wiley-VCH, Kalia, J. and Raines, R., 2008, *47*, 7523.

researchers picked three proteins with known native 3D structures that displayed either helical, β-strand, or mixed structural features. All of the proteins formed inclusion bodies upon expression in bacteria, and despite their differences in native fold, they showed remarkable similarity in one key assay. By placing the proteins in heavy water, or D_2O, the authors could monitor the proton exchange of the proteins. Those protons that were protected from free solvation were visualized by NMR. All three proteins displayed a short region of exchange protection, indicating an ordered association event. As in amyloid strands, mutations in these regions to arginine broke the associations and even caused protein solubility in some cases. Further, all three proteins' inclusion bodies diffracted X-rays with the characteristic pattern of a cross-β-strand structure. Finally, electron microscopy showed that amyloid-like fibrils could actually grow out of bacterial inclusion bodies of the protein BMP2 upon incubation at 37 °C. This study shows a new side to the old problem of inclusion bodies, but more importantly, it provides new insights and even new model systems to watch how amyloid fibrils form and grow. **Jason G. Underwood, Ph.D.**

Spotlight

Embryoid Bodies Rise to the Surface

The tantalizing therapeutic promise of embryonic stem (ES) cells has fueled intense efforts to induce the differentiation of ES cells into specific cell types with clinical potential. Coaxing ES cells to form embryoid bodies (EBs), or 3D cell aggregates, is a key strategy used in the differentiation induction process. EBs can be generated using a suspension culture technique in which aggregation of ES cells is promoted through the use of special low-attachment cell culture plates, but the EBs



Valamehr, B., *et al.*, *Proc. Natl. Acad. Sci., U.S.A.*, DOI: 10.1073/pnas.0807235105. Copyright 2008 National Academy of Sciences, U.S.A.

generated using this method tend to vary significantly in size and morphology. In an effort to find optimal conditions for EB formation, Valamehr *et al.* (*Proc. Natl. Acad. Sci.*, published online Sept 12, 2008; DOI: 10.1073/pnas.0807235105) demonstrate that cells grown on hydrophobic surfaces form more uniform EBs and, as a result, exhibit enhanced differentiation yields.

Analyses of apoptosis markers, cell cycle phase, and expression of genes associated with differentiation were used to evaluate cell viability, cellular proliferation, and differentiation potential of EBs produced in suspension culture. Using these parameters, the authors observed that EBs of intermediate size are the most proliferative, viable, and differentiation-competent compared with both smaller- and larger-sized populations. The authors then went in search of alternative surface conditions that could enrich the generation of the intermediate-sized EBs. Of several biocompatible surfaces explored, the hydrophobic surface polydimethylsiloxane (PDMS) was found to promote formation of the most homogeneous population of intermediate-sized EBs. Further exploration revealed that EBs formed on PDMS also exhibited increased cell viability, proliferation, and differentiation potential compared with those formed on the low-attachment culture plates routinely used for EB formation. To investigate the mechanism behind uniform EB formation, the authors utilized self-assembled monolayers of alkanethiolates on gold-coated substrates and demonstrated that hydrophobic surfaces play a key role in preventing cell-surface attachment while exhibiting beneficial effects on EB development. **Eva J. Gordon, Ph.D.**

Fishing in a Nanoliter Pond

Cell membranes harbor pharmacologically interesting families of proteins, including ion channels, transporters, and neurotransmitter receptors. In the case of viral infections, transmembrane proteins can act as chaperones to help shuttle the virus into or out of the cell. Unfortunately, the very property that anchors these proteins into membranes, highly hydrophobic polypeptide stretches, makes biochemical tricks such as recombinant protein expression and structure determination notoriously difficult. To get around this issue and take a closer look at the hepatitis C transmembrane protein, NS4B, a new study by Einav *et al.* (*Nat. Biotechnol.* 2008 *26*, 1019–1027) adapts a novel approach previously used for DNA binding proteins.

The technique, mechanical trapping of molecular interactions (MITOMI), used microfluidics to mix together a tagged and fluorescently labeled NS4B protein and putative target RNA sequences from HCV's genome in a tiny nanoliter reaction volume. The protein was synthesized by *in vitro* translation in the presence of microsomal membranes, a key condition that helps membrane proteins fold into their native confirmation. Using a microarray scanner, the authors quantified immobilized NS4B protein and the labeled RNA, and using a dilution series of the RNA, they generated legitimate binding constants. Although NS4B was suspected to be in a complex with the RNA genome, this study represents the first *bona fide* demonstration of the protein binding to the negative strand of the viral RNA, with an impressive K_d of 3.4 nM. Next, the authors went on to show that arginine-rich motifs on the cytoplasmic side of the protein were critical to the high-affinity interaction. Finally, with an RNA–protein interaction assay in hand, the authors added a library of 1280 small molecules into the mix to look for a molecule that could interfere with the interaction and thus represent a candidate antiviral drug. Those that stood out in this test were then assayed for their effect on HCV replication *in vitro*. One drug, clemizole, emerged as a potent inhibitor of replication without cell toxicity, and the study focused on this drug because it had already seen years of use in humans. Interestingly, this drug, though now obsolete, was once used as an antihistamine to fight itching. This study represents an impressive step forward in studying hard-to-manage membrane proteins, nucleic acid binding, and drug screening, all in just a nanoliter reaction volume. **Jason G. Underwood, Ph.D.**

Getting the 411 on the CYP74s

Jasmonates, hormones involved in plant development, and green leaf volatiles (GLVs), compounds released in response to environmental stresses, are members of the oxylipin family of bioactive lipids. Two enzymes involved in oxylipin biosynthesis, allene oxide synthase (AOS) and hydroperoxide lyase (HPL), are members of the cytochrome P450 (CYP) superfamily. While most CYP enzymes function as monooxygenases, AOS and HPL, which belong to the CYP74 family, instead convert hydroperoxide-containing fatty acids to allene oxide (an essential intermediate in jasmonate biosynthesis) and GLVs, respectively. Although crystal structures have been solved for close to 30 bacterial and human CYP enzymes, no structural information exists for plant CYPs. Now, Lee *et al.* (*Nature* advance online publication Aug 20, 2008; DOI: 10.1038/nature07307) report the crystal structure of AOS from *Arabidopsis thaliana* alone and in complex with substrate and intermediate analogs.

The crystal structure of AOS alone revealed that it adopts the characteristic fold of CYPs; however, three significant differences were found that distinguish AOS from all other known CYP structures. These structural changes offered an explanation for the lack of monooxygenase activity in AOS and provided the framework for examining the structural basis of AOS substrate recognition and mechanism of action. Indeed, structures of AOS with a substrate analog and reaction intermediate analog revealed a critical role for Phe137 in converting substrate to allene oxide. Strikingly, mutating Phe137 in AOS to a conserved residue observed in HPL renders AOS incapable of generating allene oxide yet bestows on it the ability to generate products normally generated by HPL. Bioinformatic, biochemical, and phylogenetic analysis revealed the existence of a previously unknown HPL in plant-growth-promoting bacteria and an AOS in coral, indicating that the CYP74 enzymes were present in the last common ancestor of plants and animals. These contributions to uncovering the structures, mechanisms, biology, and evolutionary histories of the CYP74 family have broad applications in both agriculture and medicine, including modulating plant responses to stress and altering flavors and aromas. **Eva J. Gordon, Ph.D.**