Lit Lunch
January 9, 2013
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1. Science. 2012 Nov 9;338(6108):810-4. doi: 10.1126/science.1226854.

Optical control of protein activity by fluorescent protein domains.
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Fluorescent proteins (FPs) are widely used as optical sensors, whereas other light-absorbing domains have been used for optical control of protein localization or activity. Here, we describe light-dependent dissociation and association in a mutant of the photochromic FP Dronpa, and we used it to control protein activities with light. We created a fluorescent light-inducible protein design in which Dronpa domains are fused to both termini of an enzyme domain. In the dark, the Dronpa domains associate and cage the protein, but light induces Dronpa dissociation and activates the protein. This method enabled optical control over guanine nucleotide exchange factor and protease domains without extensive screening. Our findings extend the applications of FPs from exclusively sensing functions to also encompass optogenetic control.

2. Science. 2012 Nov 9;338(6108):783-5. doi: 10.1126/science.1227032.

Discovery of an organic trefoil knot.
Ponnuswamy N, Cougnon FB, Clough JM, Pantoş GD, Sanders JK.

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Comment in
Science. 2012 Nov 9;338(6108):752-3.

Molecular knots remain difficult to produce using the current synthetic methods of chemistry because of their topological complexity. We report here the near-quantitative self-assembly of a trefoil knot from a naphthalenediimide-based aqueous disulfide dynamic combinatorial library. The formation of the knot appears to be driven by the hydrophobic effect and leads to a structure in which the aromatic components are buried while the hydrophilic carboxylate groups remain exposed to the solvent. Moreover, the building block chirality constrains the topological conformation of the knot and results in its stereoselective synthesis. This work demonstrates that the hydrophobic effect provides a powerful strategy to direct the synthesis of entwined architectures.

3. Science. 2012 Nov 9;338(6108):822-4. doi: 10.1126/science.1225720.

Mitochondrial network size scaling in budding yeast.

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Mitochondria must grow with the growing cell to ensure proper cellular physiology and inheritance upon division. We measured the physical size of mitochondrial networks in budding yeast and found that mitochondrial network size increased with increasing cell size and that this scaling relation occurred primarily in the bud. The mitochondria-to-cell size ratio continually decreased in aging mothers over successive generations. However, regardless of the mother's age or mitochondrial content, all buds attained the same average ratio. Thus, yeast populations achieve a stable scaling relation between mitochondrial content and cell size despite asymmetry in inheritance.

4. Science. 2012 Nov 16;338(6109):949-53. doi: 10.1126/science.1227157.

Pathological α-synuclein transmission initiates Parkinson-like neurodegeneration in nontransgenic mice.

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Parkinson's disease is characterized by abundant α-synuclein (α-Syn) neuronal inclusions, known as Lewy bodies and Lewy neurites, and the massive loss of midbrain dopamine neurons. However, a cause-and-effect relationship between Lewy inclusion formation and neurodegeneration remains unclear. Here, we found that in wild-type nontransgenic mice, a single intrastriatal inoculation of synthetic α-Syn fibrils led to the cell-to-cell transmission of pathologic α-Syn and Parkinson's-like Lewy pathology in anatomically interconnected regions. Lewy pathology accumulation resulted in progressive loss of dopamine neurons in the substantia nigra pars compacta, but not in the adjacent ventral tegmental area, and was accompanied by reduced dopamine levels culminating in motor deficits.This recapitulation of a neurodegenerative cascade thus establishes a mechanistic link between transmission of pathologic α-Syn and the cardinal features of Parkinson's disease.

5. Science. 2012 Nov 23;338(6110):1065-9. doi: 10.1126/science.1227833. Epub 2012 Oct 11.

Flows of research manuscripts among scientific journals reveal hidden submission patterns.

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Comment in
Science. 2012 Nov 23;338(6110):1029.

The study of science-making is a growing discipline that builds largely on online publication and citation databases, while prepublication processes remain hidden. Here, we report on results from a large-scale survey

of the submission process, covering 923 scientific journals from the biological sciences in years 2006 to 2008. Manuscript flows among journals revealed a modular submission network, with high-impact journals preferentially attracting submissions. However, about 75% of published articles were submitted first to the journal that would publish them, and high-impact journals published proportionally more articles that had been resubmitted from another journal. Submission history affected post-publication impact: Resubmissions from other journals received significantly more citations than first-intent submissions, and resubmissions between different journal communities received significantly fewer citations.

6. Science. 2012 Nov 29. [Epub ahead of print]

Natively Inhibited Trypanosoma brucei Cathepsin B Structure Determined by Using an X-ray Laser.

Redecke L, Nass K, Deponte DP, White TA, Rehders D, Barty A, Stellato F, Liang M, Barends TR, Boutet S, Williams GJ, Messerschmidt M, Seibert MM, Aquila A, Arnlund D, Bajt S, Barth T, Bogan MJ, Caleman C, Chao TC, Doak RB, Fleckenstein H, Frank M, Fromme R, Galli L, Grotjohann I, Hunter MS, Johansson LC, Kassemeyer S, Katona G, Kirian RA, Koopmann R, Kupitz C, Lomb L, Martin AV, Mogk S, Neutze R, Shoeman RL, Steinbrener J, Timneanu N, Wang D, Weierstall U, Zatsepin NA, Spence JC, Fromme P, Schlichting I, Duszenko M, Betzel C, Chapman HN.

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The Trypanosoma brucei cysteine protease cathepsin B (TbCatB), which is involved in host protein degradation, is a promising target to develop new treatments against sleeping sickness, a fatal disease caused by this protozoan parasite. The structure of the mature, active form of TbCatB has so far not provided sufficient information for the design of a safe and specific drug against T. brucei. By combining two recent innovations, in vivo crystallization and serial femtosecond crystallography, we obtain the room-temperature 2.1 Å resolution structure of the fully glycosylated precursor complex of TbCatB. The structure reveals the mechanism of native TbCatB inhibition and demonstrates that new biomolecular information can be obtained by the "diffraction before destruction" approach of x-ray free-electron lasers from hundreds of thousands of individual microcrystals.

Journal of Molecular Biology

Design Principles of a Universal Protein Degradation Machine

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The 26S proteasome is a 2.5-MDa, 32-subunit ATP-dependent protease that is responsible for the degradation of ubiquitinated protein targets in all eukaryotic cells. This proteolytic machine consists of

a barrel-shaped peptidase capped by a large regulatory particle, which contains a heterohexameric AAA + unfoldase as well as several structural modules of previously unknown function. Recent electron microscopy (EM) studies have allowed major breakthroughs in understanding the architecture of the regulatory particle, revealing that the additional modules provide a structural framework to position critical, ubiquitin-interacting subunits and thus allow the 26S proteasome to function as a universal degradation machine for a wide variety of protein substrates. The EM studies have also uncovered surprising asymmetries in the spatial arrangement of proteasome subunits, yet the functional significance of these architectural features remains unclear. This review will summarize the recent findings on 26S proteasome structure and discuss the mechanistic implications for substrate binding, deubiquitination, unfolding, and degradation.

Biophysical Characterization of Two Different Stable Misfolded Monomeric Polypeptides that Are Chaperone-Amenable Substrates

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Misfolded polypeptide monomers may be regarded as the initial species of many protein aggregation pathways, which could accordingly serve as primary targets for molecular chaperones. It is therefore of paramount importance to study the cellular mechanisms that can prevent misfolded monomers from entering the toxic aggregation pathway and moreover rehabilitate them into active proteins. Here, we produced two stable misfolded monomers of Luciferase and Rhodanese, which we found to be differently processed by the Hsp70 chaperone machinery and whose conformational properties were investigated by biophysical approaches. In spite of their monomeric nature, they displayed enhanced Thioflavin-T fluorescence, non-native β-sheets and tertiary structures with surface-accessible hydrophobic patches, but differed in their conformational stability and aggregation propensity. Interestingly, minor structural differences between the two misfolded species could account for their markedly different behavior in chaperone-mediated unfolding/refolding assays. Indeed, only a single DnaK molecule is sufficient to unfold by direct clamping a misfolded Luciferase monomer, while, by contrast, several DnaK molecules are necessary to unfold the more resistant misfolded Rhodanese monomer by a combination of direct clamping and cooperative entropic pulling.

Structural characterization of a eukaryotic chaperone—the ribosome-associated complex

Christoph Leidig, Gert Bange, Jürgen Kopp, Stefan Amlacher, Ajay Aravind, Stephan Wickles, Gregor Witte, Ed Hurt, Roland Beckmann & Irmgard Sinning

Ribosome-associated chaperones act in early folding events during protein synthesis. Structural information is available for prokaryotic chaperones (such as trigger factor), but structural understanding of these processes in eukaryotes lags far behind. Here we present structural analyses of the eukaryotic ribosome-associated complex (RAC) from *Saccharomyces cerevisiae* and *Chaetomium thermophilum*, consisting of heat-shock protein 70 (Hsp70) Ssz1 and the Hsp40 Zuo1. RAC is an elongated complex that crouches over the ribosomal tunnel exit and seems to be stabilized in a distinct conformation by expansion segment ES27. A unique α-helical domain in Zuo1 mediates ribosome interaction of RAC near the ribosomal proteins L22e and L31e and ribosomal RNA helix H59. The crystal structure of the Ssz1 ATPase domain bound to ATP-Mg^{2+} explains its catalytic inactivity and suggests that Ssz1 may act before the RAC-associated chaperone Ssb. Our study offers insights into the interplay between RAC, the ER membrane–integrated Hsp40-type protein ERj1 and the signal-recognition particle.

Folding of large multidomain proteins by partial encapsulation in the chaperonin TRiC/CCT

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The eukaryotic chaperonin, TRiC/CCT (TRiC, TCP-1 ring complex; CCT, chaperonin containing TCP-1), uses a built-in lid to mediate protein folding in an enclosed central cavity. Recent structural data suggest an effective size limit for the TRiC folding chamber of ~70 kDa, but numerous chaperonin substrates are substantially larger. Using artificial fusion constructs with actin, an obligate chaperonin substrate, we show that TRiC can mediate folding of large proteins by segmental or domain-wise encapsulation. Single or multiple protein domains up to ~70 kDa are stably enclosed by stabilizing the ATP-hydrolysis transition state of TRiC. Additional domains, connected by flexible linkers that pass through the central opening of the folding chamber, are excluded and remain accessible to externally added protease. Experiments with the physiological TRiC substrate hSnu114, a 109-kDa multidomain protein, suggest that TRiC has the ability to recognize domain boundaries in partially folded

intermediates. In the case of hSnu114, this allows the selective encapsulation of the C-terminal ~45-kDa domain and segments thereof, presumably reflecting a stepwise folding mechanism. The capacity of the eukaryotic chaperonin to overcome the size limitation of the folding chamber may have facilitated the explosive expansion of the multidomain proteome in eukaryotes.

J.BiolChem.

Dynamic Nucleotide-dependent Interactions of Cysteine- and Histidine-rich Domain (CHORD)-containing Hsp90 Cochaperones Chp-1 and Melusin with Cochaperones PP5 and Sgt1.

Hong TJ, Kim S, Wi AR, Lee P, Kang M, Jeong JH, Hahn JS.

Abstract
Mammals have two cysteine- and histidine-rich domain (CHORD)-containing Hsp90 cochaperones, Chp-1 and melusin, which are homologs of plant Rar1. It has been shown previously that Rar1 CHORD directly interacts with ADP bound to the nucleotide pocket of Hsp90. Here, we report that ADP and ATP can bind to Hsp90 cochaperones Chp-1 and PP5, inducing their conformational changes. Furthermore, we demonstrate that Chp-1 and melusin can interact with cochaperones PP5 and Sgt1 and with each other in an ATP-dependent manner. Based on the known structure of the Rar1-Hsp90 complex, His-186 has been identified as an important residue of Chp-1 for ADP/ATP binding. His-186 is necessary for the nucleotide-dependent interaction of Chp-1 not only with Hsp90 but also with Sgt1. In addition, Ca(2+), which is known to bind to melusin, enhances the interactions of melusin with Hsp90 and Sgt1. Furthermore, melusin acquires the ADP preference for Hsp90 binding in the presence of Ca(2+). Our newly discovered nucleotide-dependent interactions between cochaperones might provide additional complexity to the dynamics of the Hsp90 chaperone system, also suggesting potential Hsp90-independent roles for these cochaperones.

Heme oxygenase-1 is involved in nitric oxide- and cGMP-induced *α-Amy2/54* gene expression in GA-treated wheat aleurone layers

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Here, *α-Amy2/54* gene expression was used as a molecular probe to investigate the interrelationship among nitric oxide (NO), cyclic GMP (cGMP), and heme oxygenase-1 (HO-1) in GA-treated wheat aleurone layers. The inducible expressions of *α-Amy2/54* and *α*-amylase activity were respectively amplified by two NO-releasing compounds, sodium nitroprusside (SNP) and spermine NONOate, in a GA-dependent fashion. Similar responses were observed when an inducer of HO-1, hemin—or one of

its catalytic products, carbon monoxide (CO) in aqueous solution—was respectively added. The SNP-induced responses, mimicked by 8-bromoguanosine 3′,5′-cyclic monophosphate (8-Br-cGMP), a cGMP derivative, were NO-dependent. This conclusion was supported by the fact that endogenous NO overproduction was rapidly induced by SNP, and thereafter induction of *α-Amy2/54* gene expression and increased α-amylase activity were sensitive to the NO scavenger. We further observed that the above induction triggered by SNP and 8-Br-cGMP was partially prevented by zinc protoporphyrin IX (ZnPPIX), an inhibitor of HO-1. These blocking effects were clearly reversed by CO, confirming that the above response was HO-1-specific. Further analyses showed that both SNP and 8-Br-cGMP rapidly up-regulated *HO-1* gene expression and increased HO activity, and SNP responses were sensitive to cPTIO and the guanylate cyclase inhibitor 6-anilino-5,8-quinolinedione (LY83583). Molecular evidence confirmed that GA-induced *GAMYB* and ABA-triggered *PKABA1* transcripts were up-regulated or down-regulated by SNP, 8-Br-cGMP or CO cotreated with GA. Contrasting changes were observed when cPTIO, LY83583, or ZnPPIX was added. Together, our results suggested that HO-1 is involved in NO- and cGMP-induced *α-Amy2/54* gene expression in GA-treated aleurone layers.