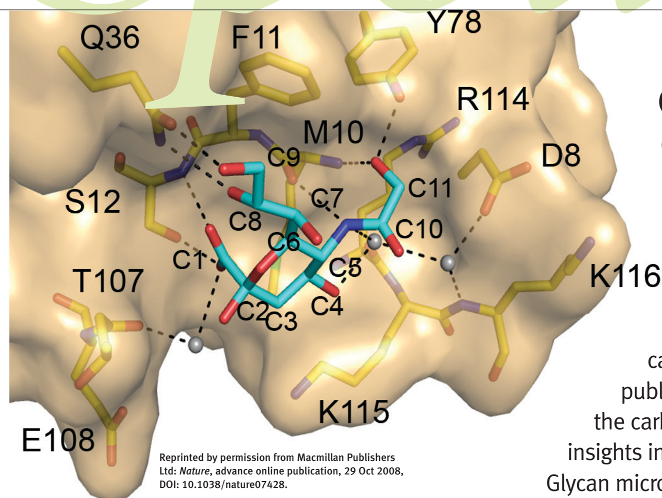
Spotlight



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Glycan Receptors: They Are What You Eat

Certain pathogenic bacteria secrete toxins of the type AB_5, in which the pentameric B-subunits bind to glycan-containing receptors on the surface of target cells. The AB_5 toxin subtilase cytotoxin (SubAB) is produced by some Shiga toxigenic *Escherichia coli* (STEC), the bacteria responsible for causing certain severe gastrointestinal disorders, but the carbohydrate-based elements recognized by SubB have not been elucidated. Using an impressive suite of biochemical, cell biological, and structural techniques, Byres *et al.* (*Nature*, published online Oct 29, 2008; DOI: 10.1038/nature07428) now report the carbohydrate structure recognized by SubB and present intriguing insights into how the toxin targets human cells.

Glycan microarrays, surface plasmon resonance, and X-ray crystallography were all used to determine unambiguously that SubB binds to glycans terminating with α2-3-linked residues of the sialic acid *N*-glycolylneuraminic acid (Neu5Gc). In addition, fluorescence microscopy experiments with SubB mutants provided further insight into the specific residues important for binding, and experiments with mouse tissues and human cells displaying varying levels of cell surface Neu5Gc confirmed the specificity of SubAB for the glycan. This is the first example of a bacterial toxin that specifically recognizes Neu5Gc, and it is especially fascinating in light of the fact that humans, unlike other mammals, are not actually capable of synthesizing Neu5Gc. However, small quantities of Neu5Gc have been found in certain human tissues such as colon and kidney, very likely as a result of metabolic incorporation from dietary sources such as red meats and milk. Ironically, red meats and milk are also the very foods that are most often contaminated with SubAB-producing STEC, thus making those who consume such products sensitized to infection. This unusual finding illuminates a novel method for the generation of a receptor for a bacterial toxin and may point to new approaches for treating infections caused by such bacteria. **Eva J. Gordon, Ph.D.**

PASTA Feeds Dormant Bacteria

When conditions are rough, some bacteria can enter into a dormant state where they temporarily halt metabolic activity. While in this dormant state, however, they monitor the local environment, and when it has improved sufficiently, a process called germination enables them to resume metabolism. Germination is known to be triggered by certain nutrients, but now Shah *et al.* (*Cell* 2008, *135*, 486–496) demonstrate that peptidoglycan fragments, which are components of the bacterial cell wall, can also function as germinants.

The authors hypothesized that the growth of other bacteria might signal dormant bacteria that conditions are favorable for exiting dormancy. During growth, bacteria release peptidoglycan fragments called muropeptides into the extracellular milieu. Indeed, incubation of dormant *Bacillus subtilis* spores with supernatants from certain bacterial cultures or purified muropeptides triggered germination, suggesting that dormant bacteria can sense when other bacteria are growing nearby. Subsequent structural and mechanistic studies provided valuable additional insight into this process. First, it was determined that a disaccharide tripeptide fragment of the muropeptide is sufficient to germinate spores and that the

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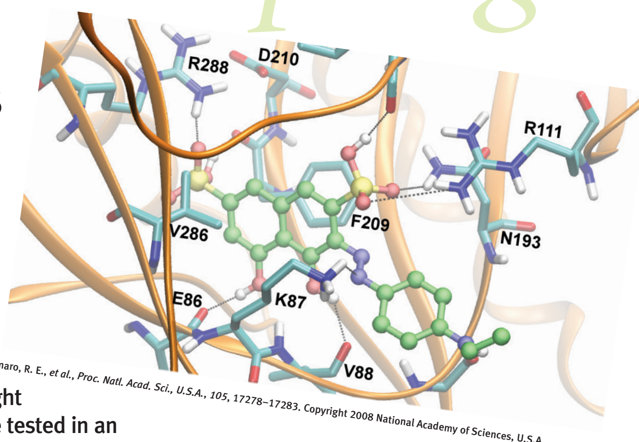
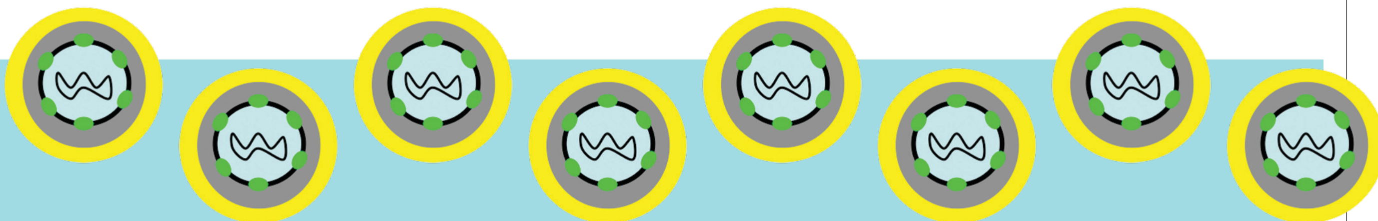
Editing Out Trypanosomal Diseases



Amaro, R. E., *et al.*, *Proc. Natl. Acad. Sci., U.S.A.*, *105*, 17278–17283. Copyright 2008 National Academy of Sciences, U.S.A.

Despite recent advances in our understanding of the biology of trypanosomes, the parasites responsible for causing debilitating and often fatal tropical diseases such as human African trypanosomiasis, Chagas' disease, and leishmaniasis, treatments against these elusive pathogens are frighteningly scarce. Interestingly, the mitochondrial messenger RNAs of trypanosomes undergo a unique and essential editing process facilitated in part by the enzyme RNA-editing ligase 1 (REL1). Because of its key role in trypanosomal survival and the lack of known human homologs, REL1 has emerged as a potential therapeutic target for trypanosomal diseases. Now, Amaro *et al.* (*Proc. Natl. Acad. Sci. U.S.A.* 2008, *105*, 17278–17283) report their use of computational methods to help identify small-molecule inhibitors of REL1 as potential new drug leads for these devastating conditions.

Using the REL1 crystal structure and an innovative combination of computational methods, the authors screened ~1800 compounds from the National Cancer Institute Diversity Set *in silico* for their predicted binding affinity to REL1. The top eight compounds identified were tested in an *in vitro* assay for their ability to inhibit the first step of the ligase reaction, and of these, two exhibited encouraging inhibitory activity. Based on the structure of the most potent inhibitor, a second, larger computational screen was conducted to identify structurally related compounds with potential activity. Of six new compounds identified, two displayed potent activity against REL1 in the biochemical assay. Notably, the top inhibitors, which are predicted to bind in the ATP binding cleft of REL1, are azo dyes with structural similarities to the anti-trypanosome drug suramin, which are promising in terms of their pharmacological properties and the possibility of therapeutic development. The computational approach taken here illustrates the power of combining computational and experimental methods to accelerate the drug discovery process and has provided promising new small-molecule leads for anti-trypanosome drug development. **Eva J. Gordon, Ph.D.**



Reprinted from *Cell*, *135*, Shah, I. M., *et al.*, A eukaryotic-like Ser/Thr kinase signals bacteria to exit dormancy in response to peptidoglycan fragments, 486–496, Copyright 2008, with permission from Elsevier.

presence of a *meso*-diaminopimelic acid residue in the third position of the peptide plays a key role in peptidoglycan recognition. Next, after determination that none of the known nutrient germination receptors were involved in the peptidoglycan germination response, proteins thought to interact with peptidoglycan were examined as potential modulators of the response. The eukaryotic-like serine/threonine kinase from *B. subtilis*, $PrkC_{Bs}$, which contains several PASTA (penicillin and Ser/Thr kinase associated) repeats thought to interact with peptidoglycan, was shown to be required for germination by peptidoglycan. Moreover, germination induced by peptidoglycan resulted in $PrkC_{Bs}$ activation, and small-molecule modulators of $PrkC_{Bs}$ were also capable of regulating germination. These findings provide intriguing new insight into the diverse mechanisms devised by bacteria for regulating their growth and survival. **Eva J. Gordon, Ph.D.**

Spotlight

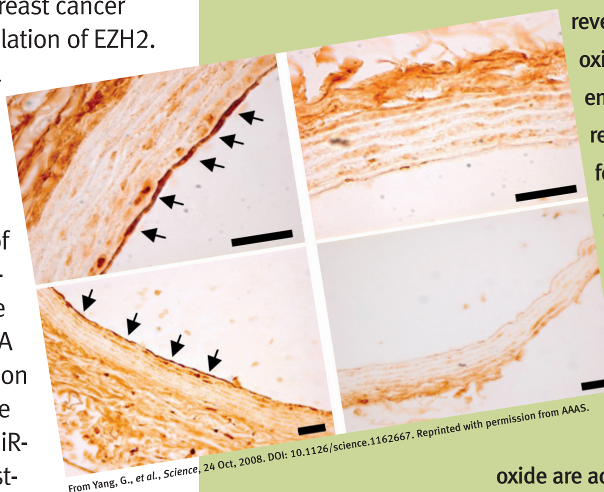
Little RNA Keeps Cancer in Check

Understanding the cell's potential to differentiate and divide is a task that extends beyond the genome and into what researchers term the epigenome. This is tied to the modification state of DNA itself and the histone proteins that package DNA. One modification of key interest, the trimethylation of Lys27 on Histone H3, leads to epigenetic silencing when H3 is bound to gene promoters. The enzyme that carries out this methylation, EZH2, has recently been tied to stem cell pluripotency, cancer, and the mysterious effects of long noncoding RNAs. Now, another family of noncoding RNAs enters the mix. Varambally *et al.* (*Science* 2008, published online Nov 13, 2008; DOI: 10.1126/science.1165395) used four computational approaches to look for microRNAs (miRNAs) that might regulate EZH2 gene expression in their cancer models. All of the methods pointed toward two miRNAs, miR-101 and miR-217, so the researchers focused on what effect these might have on EZH2 levels. Previous work showed that EZH2 levels are elevated in many aggressive forms of cancer, but could this change in protein abundance be mediated by one of these miRNAs? Of the two candidates, miR-101 emerged as a potent regulator of EZH2 expression. By forcing the overexpression of miR-101 in a human breast cancer cell line, the authors saw a strong down-regulation of EZH2. Further, these cells dosed with extra miR-101 displayed lower proliferation, a less invasive phenotype, and a reduced propensity for tumor growth. Diving into finer detail, the study looked at several loci for histone methylation marks and found that addition of extra miR-101 caused a decrease in methylation, consistent with a down-regulation of the methyl transferase EZH2. So could this miRNA be a player during an actual cancer progression event? To look at this, normal human prostate and various stage tumors were profiled for miR-101 and EZH2 messenger RNA levels. Interestingly, progression of prostate tumors was marked by loss of miR-101 expression and concurrent increase in EZH2. Further, many of the tumors showed chromosomal lesions causing loss of one or both of the two copies of miR-101 in the human genome. Taken together, this study displays how a single miRNA sequence can cause a global dysregulation of gene expression by loss of critical epigenetic marks. **Jason G. Underwood Ph.D.**

H_2S: A Not-So-Rotten Blood Pressure Regulator

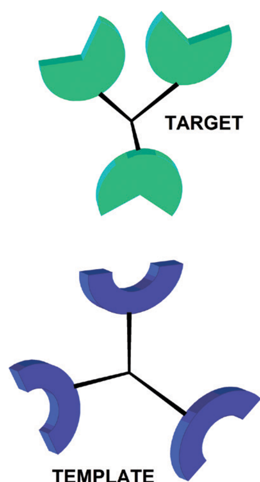
Though infamous as the source of the foul odor that emanates from rotten eggs, hydrogen sulfide has recently found new celebrity as a molecule with important physiological functions. Like the gaseous messenger molecule nitric oxide, evidence suggests that H_2S may be a blood pressure regulator, but its precise role in this arena remains unclear. By creating mice lacking an enzyme that produces H_2S, cystathionine γ-lyase (CSE), Yang *et al.* (*Science* 2008, *322*, 587–590) now provide direct evidence for H_2S as a vasorelaxant.

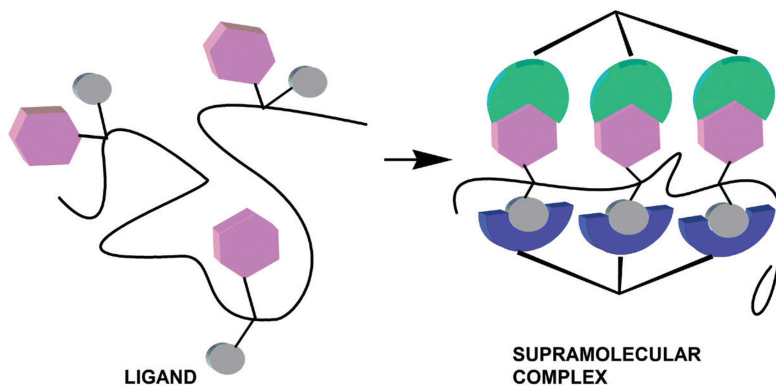
Mice with a targeted deletion of the gene encoding CSE, though viable, fertile, and displaying similar growth patterns as wild-type animals, exhibited decreased levels of H_2S in aorta, heart, and serum. Most notable, however, was the observation that these mice developed age-dependent high blood pressure. In addition, when exogenous H_2S was supplied to mice lacking the CSE gene, a decrease in blood pressure was observed. Investigation into the mechanisms behind the hypertension observed in mice without CSE revealed that, like nitric oxide, H_2S acts as an endothelial cell-derived relaxing factor, as it is formed in endothelium and is involved in neurotransmitter-induced blood vessel relaxation. Furthermore, just as the enzymes responsible for generation of nitric oxide are activated by calcium-calmodulin, generation of H_2S by CSE is also regulated by calcium-calmodulin. These studies establish that CSE does in fact produce physiologically relevant amounts of H_2S in vascular tissues and that H_2S plays an important role in regulating blood pressure, comparable to that of nitric oxide. The findings further suggest modulation of H_2S levels as a new avenue for treatment of high blood pressure. **Eva J. Gordon, Ph.D.**


From Yang, G., *et al.*, *Science*, 24 Oct, 2008. DOI: 10.1126/science.1162667. Reprinted with permission from AAAS.

Shiga Toxin Takes the BAIT

Receptors that engage in multivalent interactions with their ligands, which include many carbohydrate-binding proteins, can be especially tricky to inhibit because of the increased complexity of the geometry and dynamics of the interactions. Shiga toxins (Stx), which are produced by pathogenic *Escherichia coli* bacteria responsible for causing serious digestive system disorders, contain radially symmetric, pentameric B-subunits that bind to a trisaccharide moiety, called the P^k-trisaccharide, that is present on cell-surface glycolipids. Though multivalent P^k-derived Stx inhibitors have been generated with some success, design improvements are needed for enhanced activity and efficacy, especially in an *in vivo* setting. Kitov *et al.* (*Proc. Natl. Acad. Sci. U.S.A.* 2008, *105*, 16837–16842) now report the creation of a novel polymer displaying preorganized, P^k-containing heterobifunctional pairs as a highly potent Stx antagonist both *in vitro* and *in vivo*.





Kitov, P. I., *et al.*, *Proc. Natl. Acad. Sci., U.S.A.*, *105*, 16837–16842. Copyright 2008 National Academy of Sciences, U.S.A.

The Stx antagonist, termed (S)-PolyBAIT, is composed of an acrylamide-based polymer scaffold displaying a P^k-derived moiety, which is recognized by Stx, integrated with a cyclic pyruvate ketal (CP), which binds to the circulating plasma protein human serum amyloid P component (HuSAP). Like the pentameric subunit of Stx, HuSAP is also a radially arranged pentamer. Thus, binding of CP to HuSAP has the potential to structurally arrange the neighboring P^k groups for efficient binding to Stx1, resulting in a face-to-face, nonfunctional HuSAP-Stx aggregate. Indeed, in *in vitro* binding-inhibition assays and cytoxicity-neutralization assays, (S)-PolyBAIT exhibited potent inhibitory activity that was strictly dependent on the presence of HuSAP. Moreover, in mice genetically modified to express HuSAP, exposure to lethal levels of Stx1 combined with administration of (S)-PolyBAIT resulted in protection from the toxic effects of Stx1. In addition to the promising therapeutic potential exhibited by these innovative Stx1 antagonists, the design strategy used here could be extended to create inhibitors of other multivalent interactions of therapeutic interest.

Eva J. Gordon, Ph.D.

Spotlight

Proteins with GPS Coordinates

In the past decade, many high-throughput methods have become commonplace for assaying mammalian gene expression, such as the chromatin immunoprecipitation to look at regulatory DNA on a genome-wide scale or the microarray to quantify mRNA expression. Ironically, the downstream products of most gene expression circuits, the proteins, have been lost in the mix, and the dynamic proteome remains more difficult to study on a large scale. Particularly mysterious is how proteins perish and what factors determine the half-lives of each polypeptide. The regulated degradation of proteins plays key roles in cell cycle control, response to stimuli, and feedback loops. So far, these events have been studied on a gene-by-gene basis, but a recent study by Yen *et al.* (*Science* 2008, *322*, 918–923) takes a bold new approach to the problem with impressive results. They used their method, termed global protein stability profiling, or GPS, to accomplish a previously unfeasible task: the stability figures for 8000 human proteins.

The GPS method used a bicistronic reporter that expressed both a red fluorescent protein and a green variant fusion to the protein of interest from the same mRNA. Using fluorescence-activated cell sorting (FACS), the authors quantified the intensity of the channels, and the ratio of these intensities reflects the stability of the green fluorescent protein–test protein fusion. After the calibrations with proteins of known half-lives gave promising results, they scaled the procedure to take on a human open reading frame library of 8000 genes. Because the FACS experiments were performed in a pooled fashion, a deconvolution procedure involving a DNA microarray was used to trace back which protein fusion was expressed each cell type. They placed the proteins into stability classes, and several unique findings emerged. Membrane and signal transduction proteins were enriched in the short half-life category, whereas cytoskeletal proteins tended to live longer. The large data set even allowed for identification of which amino acids tend to be enriched in stable *versus* labile proteins. Finally, in a proof of principle for how the GPS assay can be morphed into another discovery platform, the study used a proteasome inhibitor drug in a parallel run to uncover many new proteins that undergo this particular decay pathway. This study paves the way for many new modifications to the GPS procedure and a whole new level of high-throughput science stationed at the protein graveyard. **Jason G. Underwood, Ph.D.**

Painting the Cells Red

Labeling of whole cells with fluorescent proteins offers a unique and informative view of cellular processes. Whereas green fluorescent protein variants are commonly used to label whole cells, the inherent cytotoxicity of known red fluorescent protein (RFP) derivatives has limited their use in this capacity. Using protein engineering methods, Strack *et al.* (*Nat. Methods* 2008, *5*, 955–957) now present a new RFP with reduced cytotoxicity for whole-cell labeling.

Because of its relatively fast maturation and high photostability, the RFP DsRed-Express was chosen as a starting point for the engineering of an improved RFP. Because protein aggregation is thought to be a source of cytotoxicity, directed evolution techniques were used to generate a DsRed-Express protein surface less prone to aggregation, resulting in the DsRed-Express variant DsRed-Express2. Like DsRed-Express, DsRed-Express2 is not quite as bright as the original DsRed, so protein mutagenesis was used to generate a second variant, DsRed-Max, that is 30% brighter than DsRed-Express2 but also more photolabile. In both bacteria and mammalian cells, DsRed-Express2 and DsRed-Max were less toxic than other DsRed variants when produced using plasmid expression vectors. In addition, expression of DsRed-Express2 in various mammalian cell lines using retroviral transduction resulted in highly fluorescent cells exhibiting minimal signs of toxicity. DsRed-Express2 is excited by lasers commonly used in flow cytometry, is expressed more strongly than DsRed-Express, and exhibits low phototoxicity, making it especially well-suited for whole-cell labeling applications. Notably, the improved properties of DsRed-Express2 suggest its potential in the generation of fluorescent transgenic animals as well. These new and improved RFP derivatives are valuable additions to the toolkit of fluorescent proteins used for whole-cell labeling applications. **Eva J. Gordon, Ph.D.**

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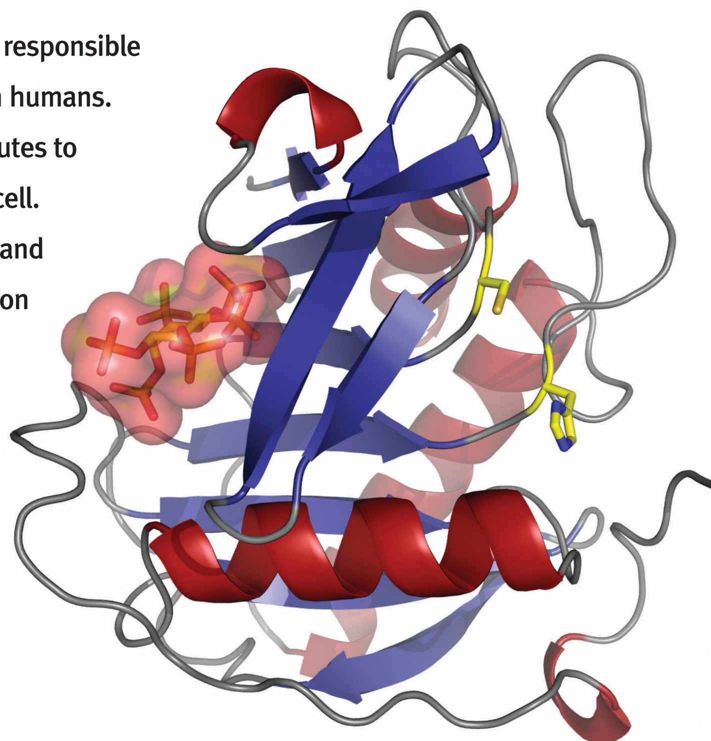
Cholera Bacteria Hijacks Host Small Molecules



From Lupardus, P. J., *et al.*, *Science* 10 Oct, 2008, DOI: 10.1126/science.1162403. Reprinted with permission from AAAS.

Vibrio cholerae is a Gram-negative bacteria that is responsible for causing the gastrointestinal disorder cholera in humans. RTX is a toxin produced by *V. cholerae* that contributes to its virulence by disrupting actin fibers in the host cell. RTX possesses a cysteine protease domain (CPD), and it has recently been discovered that upon interaction with the host small molecule inositol hexakisphosphate ($InsP_6$), the CPD autocatalyzes the release of the actin-disrupting effector domains in RTX. To gain insight into the mechanism behind this autocatalytic activation, Lupardus *et al.* (*Science* 2008, *322*, 265–268) now present the crystal structure of the RTX CPD in complex with $InsP_6$.

With an overall structure similar to those of the caspase and gingipain cysteine protease families, the RTX CPD is composed of a seven-stranded β-sheet and three helices, with a key cysteine–histidine catalytic dyad at the active site. A notable exception is the presence of a three-strand flap structure located a fair distance from the catalytic center, which forms a pocket ideally suited for the binding of a single $InsP_6$ molecule. The fact that the binding site for $InsP_6$ is physically separated from the active site and that $InsP_6$ binding does not appear to affect the binding or affinity of the substrate to the enzyme suggested that $InsP_6$ binding may instead serve as an allosteric regulator of substrate binding. Indeed, biochemical and protein mutagenesis experiments revealed that the presence of $InsP_6$ is required for the active site cysteine to react with enzyme substrates or inhibitors. The authors hypothesize that this process may in fact be a rather ingenious mechanism devised by the pathogen for protecting the CPD cysteine from reacting until the toxin has entered a eukaryotic cell. Once in the cell, the toxin cleverly hijacks $InsP_6$ present in the cytosol, which triggers autocatalysis and consequent actin disruption. **Eva J. Gordon, Ph.D.**