The two reports in this issue establish a third route: modulating protein stability using a small molecule. Initially developed for mammalian cells, this method is based on fusion of the target protein to a mutant version of the human rapamycin binding protein FKBP12 (ref. 9). Addition of this destabilization domain (ddFKBP; **Fig. 1**) results in degradation of the target protein. The protein can be spared from this fate by addition of a cell-permeable small molecule ligand of ddFKBP dubbed Shield-1.

This system produces robust regulation of a variety of proteins in both parasites[2,3]. Depending on the configuration, this occurs with remarkable speed: in *T. gondii* a fusion of the destabilization domain and yellow fluorescent protein (ddFKBP-YFP) is upregulated to full level within 90 min upon addition of Shield-1 (ref. 3). Experiments using endogenous parasite proteins suggest that the approach is well suited for the construction of conditional knockout as well as dominant negative mutants. The ddFKBP model does not depend on a specific promoter element (as does the tetracycline system[10]) thus avoiding complications resulting from inappropriate promoter strength or timing.

Another strength of this system is the ability to precisely dose the level of target protein by varying the concentration of Shield-1. On the downside, it requires fusion of the target protein to the 12-kDa ddFKBP. One of the proteins tested here[3], myosinA, serves as an example where such fusion results in loss of function. Also destruction of the protein likely depends on the cytoplasmic proteoasome, which could imply that target proteins have to be cytoplasmic for optimal regulation. Additional work is needed to evaluate the regulation of proteins that are targeted to other cellular locales.

However, Armstrong and Goldberg already demonstrate regulation of falcipain-2 (ref. 2). This protease is used by the malaria parasite to digest red cell hemoglobin and is localized to the food vacuole, a compartment equivalent to the lysosome. This finding suggests that (at least some) parasite secretory proteins can be manipulated using this system. This is important because secretory proteins constitute a large portion of the parasite's host cell invasion and manipulation machinery.

What could be the broader uses for ddFKBP beyond the obvious gene-by-gene approach? Two areas come to mind. ddFKBP could enable work toward a comprehensive catalog of conditional mutants canvassing essentially the entire genome, similar to efforts in other model organisms. Combining ddFKBP with approaches already available[11] might yield conditional insertional mutants and open the door to forward genetic analysis of the rich and unique biology of Apicomplexan parasites.

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Illuminating aggregate heterogeneity in neurodegenerative disease

Walker S Jackson & Susan Lindquist

Luminescent conjugated polymers (LCPs) bind to prion aggregates and emit different fluorescent spectra depending on their binding conformation. As such, they are promising tools for investigating the biophysical basis of prion strains.

The transmissible spongiform encephalopathies, prion diseases, are perhaps the most bizarre and perplexing diseases known. Prions cause the notorious 'mad cow' disease in cattle, scrapie in sheep and chronic wasting disease in deer and elk. In humans they produce a spectrum of disorders known as fatal familial insomnia, Creutzfeldt-Jakob disease and Gerstmann-Sträussler-Scheinker syndrome. Despite years of study, many questions remain concerning how the infectious agent replicates in the brain, how it kills and how it is transmitted within and between species. In this issue of *Nature Methods*, Sigurdson and colleagues from the Aguzzi laboratory bring a powerful new histological imaging probe to bear on the problem[1].

The prion diseases are caused by misfolding of a protein known as PrP, which is the main component of the infectious agent. PrP is present in normal brains in an alpha helix–rich state. But it is capable of adopting an infectious conformation rich in beta sheet structure, which somehow entices the normal protein to adopt the same conformation. This protein-misfolding chain reaction generates new infections material and, in a manner still quite mysterious, wrecks havoc in the brain.

The process can start in several ways. In most cases, it appears to happen spontaneously, thankfully in only about one in a million individuals per year. It occurs with high likelihood in people harboring mutations in PrP that make the protein prone to misfolding. Less commonly (but certainly more newsworthy), it can be initiated by environmental exposure to the proteinaceous infectious agent via ingestion, infected surgical equipment or even blood transfusion. A strong species barrier greatly reduces cross-species transmission, which appears to have protected most, but not all, people who came into contact with infectious material during the epidemic of mad cow disease in Great Britain.

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PrP can adopt a variety of infectious misfolded states that confer unique disease properties including (i) the time required to cause terminal disease, (ii) the degree and distribution of neuropathology (including neuronal damage and PrP deposits) and (iii) the sequelae of infection, such as loss of muscle control, hyperactivity and excessive scratching. The different misfolded states are known as prion 'strains' because their properties are faithfully replicated upon serial transmission into animals expressing the same PrP sequence. However, prion strain properties can change when a different host is infected with a slightly different PrP sequence. To date the mechanism remains confounding, in part because the tools we have available to study it are crude: for example, different strains of prions show differences in the sizes of protein fragments detected by western blot analysis following partial digestion with proteinase K (the differences in digestion products are thought to be the result of differences in structure and therefore accessibility of the aggregate to be digested by proteinase K).

Histological methods also have shortcomings. Immunohistochemical approaches require harsh treatment of tissue sections to expose antibody epitopes of aggregated PrP, potentially denaturing the aggregates, whereas Congo red staining only gives information about whether or not an aggregate is in an ordered, formal amyloid structure. To understand the structural and mechanistic basis for prion strains as well as a host of other questions surrounding the prion diseases, we urgently need tools to qualitatively distinguish the different types of aggregates in their cellular environments during the course of infection.

The problem of multiple folds and aggregated protein states is common to many neurodegenerative diseases, and aggregates come in a variety of shapes and sizes. In virtually all of these diseases, the precise roles of protein aggregates remain unclear. The hypotheses that they are helpful, harmful or neutral are all viable and quite contentious. A new series of conformation-specific protein dyes called luminescent conjugated polymers (LCPs) hold great promise for untangling this troubled web. LCPs probably bind to most natively structured proteins but provide a much stronger and uniform signal when bound to aggregates, especially ordered aggregates, emitting unique fluorescence profiles. *In vitro* experiments have suggested that these emission signatures are due to ultrastructural differences in aggregate conformation[2]. LCPs were recently shown to be effective for detecting differences between Alzheimer's disease–related aggregates[3].

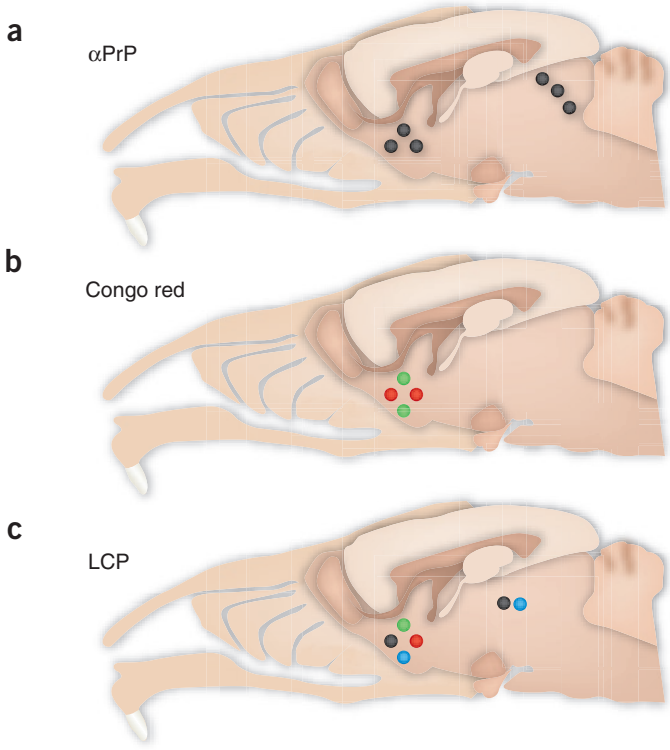

Figure 1 | LCPs provide a unique set of information about protein aggregates in brain tissue. All three diagrams depict serial brain slices from the same infected mouse. (**a**) A slice stained for PrP deposits using PrP antibodies and conventional immunohistochemical methods. Immunohistochemistry detects many aggregates (black dots) with good spatial resolution but provides no structural information. (**b**) A slice stained with Congo red, producing green birefringence with polarized light when bound to amyloid (green dots). Some aggregates bound by Congo red do not show birefringence (red dots), potentially meaning they are not formally amyloid. (**c**) A slice stained with an LCP. Different aggregates emit different spectra of light (different colored dots). Comparison of the three diagrams shows that some aggregates are not detected by all three methods. Therefore combinations of these methods will provide the richest datasets.

Sigurdson and colleagues determined that LCPs are useful for distinguishing different structures of PrP aggregates, which are thought to be the basis for differences between prion strains[1]. Indeed, they show that LCPs reveal a much greater diversity of PrP aggregate types than was previously appreciated (**Fig. 1**). The authors also show that as prions are passaged into new host species, LCPs illuminate changes in PrP aggregate structures; this has long been proposed but was difficult to test experimentally. Three different species of animals with unique prion diseases served as source infectious materials for these transmission experiments, indicating these probes work on a wide range of aggregate types. Notably, the new LCP signatures were stabilized after a few serial transmissions in the same host species, consistent with the prions having successfully crossed a species barrier.

In future experiments, LCPs should be useful for making correlations between which aggregates are toxic based on their proximity to damaged cells or circuits. Biochemists will find them a handy tool for purifying infectious aggregates from non-infectious aggregates, allowing for more detailed studies of their structures. LCPs could also have clinical and agricultural applications, for example, as tools for tracking the source of prion infections in humans or farm animals.

The development of conformation-dependent imaging agents will undoubtedly have an enormous impact on the protein misfolding field. As LCPs are useful for optical imaging, analogous probes would be useful for other imaging modalities such as magnetic resonance imaging or microultrasound.

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