PNAS Plus Significance Statements

Chaperone activation by unfolding

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For proteins, function is generally associated with order. Some proteins, however, are at least partially disordered. Because proteins tend to evolve into disorder in the absence of selection, it has been difficult to establish any significance of disorder for protein function. Here (pp. E1254–E1262), we isolate a constitutively active variant of the normally acid-activated, conditionally disordered chaperone HdeA. We find this mutant to be largely destabilized, partially unstructured, and monomeric at a concentration at which it prevents the aggregation of a client protein. Our data therefore provide experimental evidence for the significance of partial disorder in protein function.

Activity-enhancing mutations in an E3 ubiquitin ligase identified by high-throughput mutagenesis

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Ubiquitin is a 76 residue protein that is attached to target proteins as a posttranslational modification. This modification is dependent on the successive activity of three enzymes, designated E1, E2, and E3. We developed a high-throughput mutagenesis strategy to probe the mechanism of E3-catalyzed transfer of ubiquitin from the E2 to the target protein. By scoring the effect of nearly 100,000 mutations in an E3, we identified mutations that affect direct and allosteric interactions between the E3 and the E2. These results (pp. E1263–E1272) highlight the general utility of high-throughput mutagenesis in delineating the molecular basis of enzyme activity.

Transient protonation changes in channelrhodopsin-2 and their relevance to channel gating

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It was always a dream to control cells and living animals by light. Discovery of channelrhodopsin turned the dream into reality because this light-activated cation channel is able to elicit action potentials with unprecedented spatial and temporal resolution. To unravel the underlying molecular mechanism, we have applied time-resolved IR spectroscopy, and we suggest how the observed proton transfer and the protein conformational changes lead to opening of the cation channel. Our results (pp. E1273–E1281) will not only contribute to the rational design of channelrhodopsin variants with improved properties, but also help to decipher the temporal sequence in the gating of ion channels.

Targeting CXCL12/CXCR4 signaling with oncolytic virotherapy disrupts tumor vasculature and inhibits breast cancer metastases

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Novel advances in viral oncotherapy require effective direct oncolysis and manipulation of the tumor microenvironment, which has proven to be an important target in cancer treatment. The CXCR4 receptor for the CXCL12 chemokine is one of the key stimuli involved in signaling interactions between tumor cells and their microenvironment, suggesting that inhibition of this pathway by oncolytic viruses expressing the CXCR4 antagonist should increase efficacy over that mediated by oncolysis alone. We are unique in demonstrating (pp. E1291–E1300) that targeting CXCR4 signaling through an oncolytic vaccinia virus yields a significant therapeutic impact against primary and metastatic breast cancer.

Met synergizes with p53 loss to induce mammary tumors that possess features of claudin-low breast cancer

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Triple-negative breast cancers lack targeted therapies and are subdivided into molecular subtypes, including basal and claudin-low. Preclinical models representing these subtypes are limited. We have developed a murine model in which mammary gland expression of a receptor tyrosine kinase (MET) and loss of tumor suppressor gene p53 (*Trp53*), synergize to promote tumors with pathological and molecular features of claudin-low breast cancer. These tumors require MET signaling for proliferation, as well as mesenchymal characteristics, which are key features of claudin-low biology. This work (pp. E1301–E1310) associates MET expression and p53 loss with claudin-low breast cancers and highly proliferative breast cancers of poor outcome.