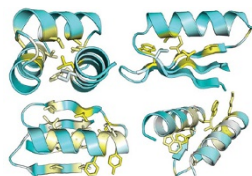


PROTEIN DESIGN

I like to fold it, fold it

Science 357, 168–175 (2017)

AAAS



Most proteins have evolved for function rather than stability, which makes it difficult to ascertain the connections between sequence and folding and, in turn, confounds *de novo* protein design. Combining the expression of synthetic DNA libraries with proteolysis resistance assays, Rocklin *et al.* have now systematically determined the stability of over 15,000 designed miniproteins in a high-throughput fashion. Following the screen, each sequence was assigned a ‘stability score’, and a subset was validated by structural analyses including circular dichroism and NMR. In-depth analysis of the features of successfully designed stable miniproteins revealed how multiple determinants (such as buried nonpolar surface area) are balanced, leading to refinements in the design and modeling procedures. With iterative rounds of design, synthesis, and stability testing, the number of stable designs multiplied from 200 in the first round to over 1,800 in the fourth round, spanning four topologies, only one of which is found in natural miniproteins. Comparison to naturally occurring miniproteins further revealed that 774 of the designed miniproteins identified in this study outperformed the most stable natural miniprotein in the Protein Data Bank. This collection of thousands of new

stable miniproteins with diverse topologies may themselves have applications in synthetic biology, and the insights gleaned from the way their global and sequence determinants contribute to folding will be useful for improving computational design strategies.

CD

BACTERIAL IMMUNITY

A virtuous CRISPR cycle

Nature doi:10.1038/nature23467 (2017)

Bacterial type II CRISPR–Cas systems are well suited for genome engineering because they target double-stranded DNA using a single effector nuclease, Cas9. In contrast, type III CRISPR–Cas systems have nuclease activities that target both DNA and RNA, mediated by a multiprotein interference complex. Type III CRISPR–Cas systems contain another ribonuclease, Csm6, that is not physically associated with the interference complex but can also degrade invader RNAs. Niewoehner *et al.* now show that the activities of the interference complex and Csm6 are linked by a cyclic oligoadenylate signaling molecule that is synthesized by Cas10 and allosterically activates Csm6. Structural data suggested that Csm6 might bind oligonucleotides, and the authors showed that synthetic oligoadenylates stimulated the RNase activity of Csm6 at a site in the enzyme’s CARF domain. They further demonstrate that the natural regulatory nucleotide cyclic 3’,5’-linked hexaadenylate (cA6) is produced by the Cas10-containing interference complex in a reaction that requires ATP, Mg<sup>2+</sup> and the Palm domain of Cas10. Studies in Csm6-knockout strains of *Staphylococcus epidermidis* provided evidence that the cyclase activity of Cas10 is required for regulating the RNA cleavage activity of Csm6

and bacterial immunity *in vivo*. By identifying this hidden activity of Cas10 and cA6 as a signaling molecule for type III systems, Niewoehner *et al.* highlight the diversity of bacterial CRISPR–Cas pathways and point to molecular regulators that may be adapted for biotechnological applications.

TLS

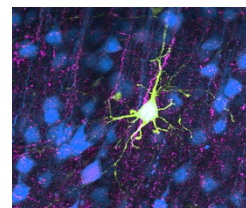
NEUROBIOLOGY

Lighting up neurons

Nat. Biotech. doi:10.1038/nbt.3902 (2017)

Nat. Biotech. doi:10.1038/nbt.3909 (2017)

GILLIAN MATTHEWS, CRAIG WILDES AND KAY TYE



Calcium-specific fluorescent indicators have been used to identify activated neurons, but these reporters are only passive tools that allow experimentalists to observe, but not to perturb, neuronal activity, and do not enable the alteration of gene expression to manipulate neuronal function and behavior. Lee *et al.* and Wang *et al.* developed two optogenetic systems, Cal-Light and FLARE, respectively, that rapidly label a neuronal population in the presence of both calcium signaling and light exposure. Both systems utilize a transcription factor that is tethered to a caged protease cleavage site adjacent to a light-sensitive LOV2 domain and a second protein that contains a protease linked to the calcium-sensing protein. An increase in calcium levels during neuronal activity promotes the dimerization of these two proteins, while blue-light exposure mediates a conformational change in the LOV2 domain to expose the cleavage site to processing by the neighboring protease. The released transcription factor then enters the nucleus to promote gene expression. Lee *et al.* infected mouse brains with a Cal-Light virus expressing halorhodopsin to silence neuronal activity with yellow-light exposure, which selectively suppressed lever-pressing motor behavior without perturbing other motor functions. Meanwhile, Wang *et al.* used their FLARE system to drive expression of a Chromson–mCherry fusion protein that promoted action potentials in specific neurons upon red-light exposure. Altogether, both Cal-Light and FLARE systems offer exciting opportunities to discover, image, and manipulate functional neuronal circuits.

GM

Written by Mirella Bucci, Caitlin Deane, Grant Miura & Terry L. Sheppard

UBIQUITIN-PROTEASOME SYSTEM

Rescuing EBV latency

PLoS Pathog. 13, e1006517 (2017)

Therapies against Epstein-Barr Virus (EBV) have focused on activating the virus from its latent state to enter a lytic cycle, where it becomes susceptible to treatment by antivirals. The small molecule C60 can induce the expression of markers of the lytic cycle, ZTA and EA-D, in EBV-infected cells. To determine its mechanism of action, Tikhmyanova *et al.* used an affinity selection approach in parallel with an shRNA screen to identify genes that restrict spontaneous lytic reactivation. Both approaches converged on CAND1, a component of the ubiquitin–proteasome system that acts as an exchange factor for complexes containing Cullin-type E3 ligases. Further mechanistic studies of C60 included mRNA expression profiling, which indicated regulation of a DNA damage checkpoint and of viral infection pathways. Immunoprecipitation experiments revealed that C60 destabilized the interaction between CAND1 and Cullin 1, increased the abundance of ubiquitylated proteins, and selectively stabilized ZTA and the tumor suppressor p53. C60 treatment showed distinct effects compared to histone deacetylase (HDAC) or proteasome inhibition and may provide a novel pathway for reactivation of EBV from latency. These results suggest that C60 targets the CAND1–Cullin pathway, ultimately preventing the degradation of such proteasome substrates as p53 and EBV lytic factor ZTA.

MB