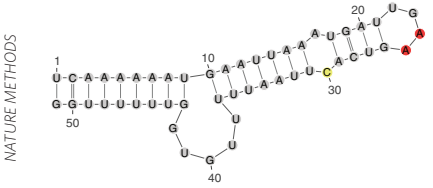
RNA STRUCTURE

Widening the probe

Nat. Methods doi:10.1038/nmeth.4057 (2016)



One method for genome-wide probing of RNA structures in cells combines the use of dimethyl sulfate (DMS), which reacts with unpaired adenine and cytosine residues, with deep sequencing. However, this approach is unable to detect RNA species with low expression or to reliably distinguish heterogeneous RNA populations. To address this limitation, Zubradt *et al.* devised a modified approach called DMS-MaPseq, which uses DMS-mediated RNA modification with RT-PCR performed by a high-fidelity thermostable group II intron reverse transcriptase (TGIRT) combined with next-generation sequencing. DMS-MaPseq reduced the signal-to-noise ratio and produced reproducible results between samples. This method enabled the authors to probe the RNA structure of *oskar* and *gurken* in *Drosophila* ovaries and to observe low-abundance mRNA species such as human *FXR2*. It also allowed discernment of distinct RNA structures in a heterogeneous population, such as a single-nucleotide polymorphism in *MRPS21* that produced a local RNA structural shift. Finally, this method enabled the comparison of the premature and mature mRNA structures of the yeast ribosomal protein RPL14A. Overall, the DMS-MaPseq approach offers an improved method that expands the analysis of diverse RNA secondary structures with the potential to provide more insight into RNA function. *GM*

BIOCATALYSIS

Custom carbon cycling

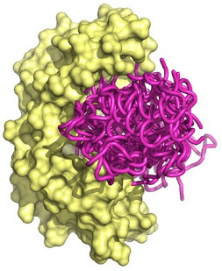
Science **354**, 900–904 (2016)

In addition to the widespread and well-known Calvin–Benson–Bassham (CBB) cycle, five other carbon-fixation pathways exist in nature. Schwander *et al.* now add a designer *in vitro* option to that portfolio. Beginning with a collection of enoyl–CoA carboxylases/reductases (ECRs), the authors composed several theoretical CO_2 fixation cycles, then calculated their Gibbs free energy profiles and estimated their consumption of ATP and NADPH per molecule of CO_2 converted. Guided by this analysis, they chose a crotonyl–CoA/ethylmalonyl–CoA/hydroxybutyryl–CoA (CETCH) cycle and selected enzymes to experimentally catalyze each step in the pathway. Further optimization of the CETCH cycle involved rational structure-guided engineering of three enzymes to exhibit desirable activities, the addition of enzymes for ATP and NADPH regeneration, protection against oxidative damage from H_2O_2 production, and incorporation of metabolic proofreading enzymes to correct for unwanted side reactions. In total, the optimized CETCH cycle comprised 17 enzymes originating from nine different organisms across all three domains of life (including bacteria, archaea, plants, and humans) and had CO_2-fixation efficiency comparable with the best estimates for the CBB cycle. A reliance on ECR activity for autotrophic CO_2 fixation is unprecedented in nature, and realization of the CETCH cycle demonstrates the potential not only for improving carbon fixation but also for the rational design of custom metabolic pathways. *CD*

PROTEIN FOLDING

Minimizing frustration

Sci. Adv. **2**, e1601625 (2016)



Chaperones have key roles in promoting protein folding events. In the energy landscape theory of protein folding, chaperones mitigate the 'frustration' and increased dynamics that arise from the drive to minimize interaction free energy within the context of a protein sequence. To better understand the mechanistic principles involved in client interaction as well as a chaperone's influence on client dynamics and native structure, He *et al.* used NMR spectroscopy to examine the atomic-level interactions between the ATP-dependent bacterial chaperone Spy and its client Im7 in a partially folded as well as in an unfolded state. Their data are consistent with a model in which Spy acts as a passive interaction surface, exhibiting minimal structural changes while binding to regions of Im7 with increased local flexibility and high local frustration levels, but leaving the Im7 backbone essentially intact. Binding of the frustrated regions by Spy is associated with increases in dynamics and internal motion within Im7. Studies of two additional chaperones gave similar results, suggesting that the recognition of frustrated segments that leads to increased dynamics and the opportunity this creates for the client protein to search available conformations are fundamental features of chaperone function. *MB*

NEURODEGENERATION

Untangling tau

eLife **5**, e18691 (2016)

Alzheimer's disease (AD) is characterized by amyloid-β (Aβ) plaques in the brain and neurofibrillary tangles of hyperphosphorylated tau, a microtubule-binding protein. The neurotoxicity of Aβ *in vitro* requires tau, but the mechanism linking tau and Aβ and its relevance to neurodegeneration and memory loss in AD are unclear. Using neuronal cell cultures and mouse models of AD, Kam *et al.* showed that tau hyperphosphorylation and memory impairments induced by Aβ oligomers are dependent on Fc gamma receptor IIb (FcgRIIb) a receptor that mediates Ab neurotoxicity. Moreover, the effects of Aβ treatment—on tau and on neuronal toxicity—required phosphorylation of FcgRIIb at Tyr273 and its recruitment of the inositol phosphatase SHIP2. Metabolism of phosphoinositides—phosphorylated derivatives of phosphatidylinositol—has been reported to be dysregulated in AD. When the authors exposed neuronal cells to Aβ, they found altered levels of PIP2 and PIP3, dependent on expression of FcgRIIb and SHIP2. Direct treatment of cells with PIP2 increased activation of GSK3β, a tau kinase, as well as tau hyperphosphorylation. Using a SHIP2 inhibitor, the authors showed that interrupting this pathway can reduce memory impairment in a mouse model of AD, suggesting a new candidate target for the treatment of AD. *AF*

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