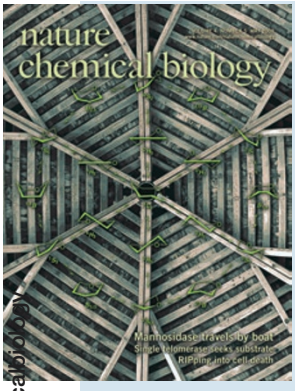


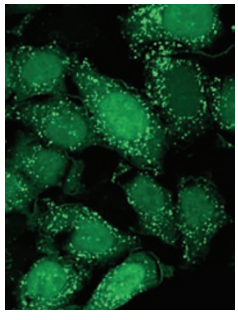
Mannoside hydrolysis: it's a boat!

Enzymatic hydrolysis of glycosidic bonds generally proceeds through a half-chair transition state. However, previous studies had suggested that retaining glycosidases hydrolyze α- or β-mannosides via a boat-like $B_{2,5}$ transition state. Tailford *et al.* now provide direct structural and biochemical evidence that β-mannosidase catalysis proceeds through this unusual pathway. Crystal structures of a β-mannosidase in complex with six tightly binding inhibitors revealed that all bound in a boat-like conformation. Importantly, there was a high correlation between log K_i and log K_m/k_{cat} for a series of active site mutants. This linear free energy relationship study confirms that these inhibitors are acting as transition state mimics. This strong evidence that retaining mannosidases use a boat-like transition state can be used to design more effective inhibitors as potential therapeutics. [**Articles, p. 306; News & Views, p. 269**] *JK*

Protease engineering makes the cut

The ability to alter protease specificity would open up new biotechnological and therapeutic applications for these enzymes. Although much work has been focused on redesigning proteases, there have been few successes in which engineered proteases exhibit both high activity and specificity. Varadarajan *et al.* have designed a selection-counterselection method for altering the specificity of the endopeptidase OmpT, which exhibits a natural preference for cleaving between two arginine residues. By introducing mutations in the S1 pocket, the authors have identified OmpT variants that preferentially cleave at altered P1 and P1′ positions, including between Glu-Arg, Thr-Arg and Glu-Ala bonds, with activity at least as high as that of the wild-type enzyme. Although these results suggest that screening for proteases with altered specificity will now be relatively straightforward, at least for OmpT, rationally choosing mutations to confer a desired specificity remains challenging. [Articles, p. 290; News & Views, p. 270] *JK*

Alternative path to autophagy



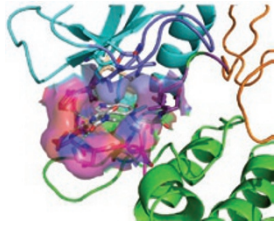
Autophagy is an important route for clearing proteins and organelles inside eukaryotic cells. As such, inducing autophagy represents a potential approach for treating neurodegenerative diseases that are caused by the aggregation of intracellular proteins. Rapamycin inhibition of the mammalian target of rapamycin (mTOR) is currently the only pharmacological strategy for inducing autophagy. To look for additional pathways, Williams *et al.* screened a small library of FDA-approved drugs for those that altered the rate of cellular clearance of mutant α-synuclein. By investigating the mechanism of action of seven hits, the authors identified a pathway in which elevated cAMP levels act via Epac (a guanine nucleotide exchange factor) and a phospholipase C (PLC-ε) to increase the activity of calpains (a family of cysteine proteases) and lead to reduced levels of autophagy. In animal models of Huntington's disease, hits from the screen had protective effects, supporting the therapeutic potential of targeting this pathway. [**Articles, p. 295**] *JK*

Written by Catherine Goodman, Joanne Kotz & Kenneth Sercy

Telomerase you can count on

Human telomerase is a ribonucleoprotein that repairs telomeres and is often overexpressed in cancer cells. The functional telomerase complex is known to comprise the protein hTERT, an RNA component and the DNA substrate; however, the absolute stoichiometry has remained unclear because of the difficulty of purifying sufficient quantities of the complex. Two-color coincidence detection (TCCD) is a single-molecule labeling technique that requires only femtomolar quantities of analyte and is viable even with large percentages of unbound components. Alves *et al.* have now used this method to examine dual-labeled active telomerase complexes in solution. They obtained 1:1:1 relative and absolute stoichiometries for the complex and determined an apparent dissociation constant for the telomerase–substrate complex. This study demonstrates the utility of TCCD for analyzing multicomponent biomolecular systems and provides a framework for understanding the mechanism of telomerase function. [Brief Communications, p. 287] *KS*

R.I.P., cell death



Apoptosis is an extensively studied pathway of regulated cell death. In contrast, though necroptosis and other types of regulated non-apoptotic cell death have been observed in animal models of human disease, including heart attack and stroke, their molecular mechanisms are poorly understood. Through biochemical analyses, molecular modeling and mutagenesis studies, Degterev *et al.* have shown that the known small-molecule necroptosis inhibitor nectrostatin-1 acts by directly and selectively inhibiting RIP1 kinase, an adaptor kinase known to be involved in regulated cell death. Necrostatin-3 and necrostatin-5 were also shown to inhibit RIP1 kinase activity, both through mechanisms differing from that of necrostatin-1. The study identifies RIP1 as a critical mediator of necroptosis and establishes the kinase as a potential therapeutic target in conditions involving pathologic tissue necrosis. It also establishes three potent small-molecule inhibitors of RIP1 kinase as promising therapeutic leads. [**Articles, p. 313**] *KS*

ROS under the microscope

The important role of reactive oxygen species (ROS) in cellular stress and signaling is becoming increasingly clear. However, the challenges of observing these small and transient species coupled with the complexity of developing suitable model systems has also resulted in a wealth of speculation about the abundance and biological reactivity of ROS. In this Review, Winterbourn outlines some common misconceptions in and considerations for ROS research and highlights specific biological systems that may prove promising for understanding ROS *in vivo*. [Review, p. 278] *CG*