OBITUARY
887 Roger Y. Tsien
Amy E Palmer & Jin Zhang

RESEARCH HIGHLIGHTS
889 Our choices from the recent literature

NEWS AND VIEWS
890 Protein dynamics: Conformational footprints
Buyong Ma & Ruth Nussinov  ▶ Article p944
891 Antibiotics: New recipe for targeting resistance
Balázs Papp & Viktória Lázár  ▶ Brief Communication p902
892 Enzyme mechanisms: Sugary shears
Ethan D Goddard-Borger  ▶ Brief Communication p899
894 Metabolic engineering: Biosensors get the green light
Sarah K Hammer & José L Avalos  ▶ Article p951

BRIEF COMMUNICATIONS
896 Small-molecule WNK inhibition regulates cardiovascular and renal function
A selective inhibitor of the With-No-Lysine (K) (WNK) kinase family reduces blood pressure and increases electrolyte excretion in hypertensive rats.

ON THE COVER
SINGLE-MOLECULE BIOPHYSICS
Insert at random
Article, p911

PROTEIN EVOLUTION
Enzyme history lesson
Article, p944

BIOSYNTHESIS
SAM closes the loop
Brief Communication, p905

COVER IMAGE
A new study enables CRISPR-Cas9-mediated genome modification in a temporally regulated manner. The cover features a modified Cas9 enzyme containing four copies of the hormone-binding domain of the estrogen receptor (lower panel with red and brown squares) that is inactive (faded outline) in the absence of the estrogen-receptor ligand 4-hydroxytamoxifen (4-HT). The addition of 4-HT stimulates Cas9 activity (upper panel with green squares), enabling genome editing to occur.
Cover design by Erin Dewalt, based on an image created by Muhammad Nadzim Bin Ramli.
Article, p980

WNK463 in complex with rat WNK1

Pan-WNK-kinase inhibitor
• Decreases blood pressure
• Increases electrolyte excretion
899 How the glycosyltransferase OGT catalyzes amide bond cleavage
J Janetzko, S A Trauger, M B Lazarus & S Walker

The glycosyltransferase OGT cleaves a substrate, HCF-1, via a glutamyl-sugar intermediate, defining a reaction mechanism that requires UDP-GlcNAc and involves the formation of an internal pyroglutamate that undergoes spontaneous backbone hydrolysis.

▶ N&V p892

902 Compounds that select against the tetracycline-resistance efflux pump
L K Stone, M Baym, T D Lieberman, R Chait, J Clardy & R Kishony

A high-throughput screen against the E. coli tetracycline-resistance efflux pump, TetA, identifies two ‘selection-inverting’ compounds that swap tetracycline resistance for resistance to another antibiotic, paving the way for two-phase antibiotic treatment protocols.

▶ N&V p891

905 Carbon extension in peptidyl nucleoside biosynthesis by radical SAM enzymes
E A Lilla & K Yokoyama

Nikkomycins and polyoxins are peptidyl nucleosides with antifungal activity. The biosynthetic routes to these natural products share a bicyclic intermediate formed by a carbon radical–centered ring closure catalyzed by the radical SAM enzymes NikJ or PolH.

908 Thermal profiling reveals phenylalanine hydroxylase as an off-target of panobinostat
I Becher, T Werner, C Doce, E A Zaal, I Tögel, C A Khan, A Rueger, M Muelbaier, E Salzer, C R Berkers, P F Fitzpatrick, M Bantscheff & M M Savitski

A chemoproteomics approach utilizing the thermal shift assay and quantitative MS resulted in the identification of phenylalanine hydroxylase as an off-target of the histone deacetylase inhibitor panobinostat.

ARTICLES

911 YidC assists the stepwise and stochastic folding of membrane proteins

An AFM-based single-molecule approach shows how the chaperone and insertase YidC stabilizes E. coli LacY in the unfolded state and assists LacY to insert and fold transmembrane structural segments in random order until folding of the native state is complete.
Simultaneous analysis of enzyme structure and activity by kinetic capillary electrophoresis–MS
G G Mironov, C M Clouthier, A Akbar, J W Keillor & M V Berezovski

Combining the kinetic separation capability of capillary electrophoresis with the structural elucidation capacity of ion-mobility mass spectrometry, a coupled CE-UV-IM-MS system demonstrates utility in examining transglutaminase conformers and their enzymatic activity.

Overcoming resistance to HER2 inhibitors through state-specific kinase binding
C J Novotny, S Pollari, J H Park, M A Lemmon, W Shen & K M Shokat

High-throughput screening identified a small-molecule compound that targets the active conformation of HER2 and is effective against growth-factor-mediated drug resistance.

Inhibition of Mcl-1 through covalent modification of a noncatalytic lysine side chain
G Akçay, M A Belmonte, B Aquila, C Chuaqui, A W Hird, M L Lamb, P B Rawlins, N Su, S Tentarelli, N P Grimster & Q Su

The use of an aryl boronic acid carbonyl warhead to target a noncatalytic lysine side chain enables the development of covalent inhibitors against the anti-apoptotic protein myeloid cell leukemia 1 (Mcl-1).

Lactate metabolism is associated with mammalian mitochondria

The application of high-resolution metabolomics integrated with isotope labeling revealed that lactate is imported into the mitochondria and is metabolized by mitochondrial LDH into pyruvate.

The role of protein dynamics in the evolution of new enzyme function

Analysis of the structures and dynamics of intermediates and engineered mutants from directed protein evolution experiments reveals how dynamic conformational changes are harnessed across evolutionary trajectories to generate new catalytic functions.
Engineering prokaryotic transcriptional activators as metabolite biosensors in yeast

Transplantation of the prokaryotic LysR-type transcriptional regulator into yeast combined with \textit{in vivo} screening identifies yeast mutants that produce metabolic products with bacterial small molecule inducers.

\textgreater{} N&V p894

Ultra-deep tyrosine phosphoproteomics enabled by a phosphotyrosine superbinder
Y Bian, L Li, M Dong, X Liu, T Kaneko, K Cheng, H Liu, C Voss, X Cao, Y Wang, D Litchfield, M Ye, S-C Li & H Zou

A SH2-domain-derived superbinder that exhibits strong affinity for phosphotyrosine (pTyr) was used in conjugation with mass spectroscopy approaches to enrich and enable identification of pTyr sites in different cancer cell lines.

Amino-group carrier-protein-mediated secondary metabolite biosynthesis in \textit{Streptomyces}
F Hasebe, K Matsuda, T Shiraishi, Y Futamura, T Nakano, T Tomita, K Ishigami, H Taka, R Mineki, T Fujimura, H Osada, T Kuzuyama & M Nishiyama

Vzb22, an amino-group carrier protein from \textit{Streptomyces}, is required for biosynthesis of the noncanonical amino acid DADH, a biosynthetic precursor of vazabitide A and related azabicyclohexane natural products.

Structural basis for precursor protein-directed ribosomal peptide macrocyclization
K Li, H L Condroso, G Li, Y Ding & S D Bruner

Biosynthesis of the protease inhibitor microviridin J includes peptide macrocyclization catalyzed by two enzymes of the ATP-grasp family. Structures of these macrocyclases, MdnB and MdnC, reveal how they recognize their precursor-peptide substrates.

A chemical-inducible CRISPR-Cas9 system for rapid control of genome editing
K I Liu, M N Bin Ramli, C W A Woo, Y Wang, T Zhao, X Zhang, G R D Yim, B Y Chong, A Gowher, M Z H Chua, J Jung, J H J Lee & M H Tan

A modified version of Cas9 with a fusion of the hormone-binding domain of the estrogen receptor allows reversible control of Cas9 activity with high efficiency at multiple loci with 4-hydroxytamoxifen treatment.