SNAP25 knockout embryonic mouse chromaffin cells. We demonstrated that all parameters of exocytosis in SNAP25 knockout cells are completely rescued by SCORE2 (SNARE Complex REporter2) and unchanged compared to wild type cells. Combining electrochemical imaging of individual release events using electrochemical detector arrays with total internal reflection fluorescence resonance energy transfer (TIR-FRET) imaging of SCORE2 conformational changes we detected a rapid FRET increase specifically associated with individual fusion events. This conformational change precedes fusion by ~60 ms. Calibrated by the SCORE2 overexpression factor, we estimate that in wt cells ~7 copies of SNAP25 change conformation preceding a fusion event. This number increased by ~20% in L-Dopa-loaded vesicles which exhibited 1.67 fold larger quantal size and presumably 21% larger diameter. The number of SNAP25 copies undergoing a conformational change increases with vesicle size in parallel to the estimated area of the vesicle-plasma membrane contact zone. The results suggest that the FRET change of SCORE2 reports the docking of the vesicle, which is associated with a conformational of all SNAP25 copies in the contact zone.
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Platform: Computational Methods and Machine Learning, Artificial Intelligence, and Bioinformatics

2243-Plat
Key interactions convert amino acid side chains into strong acids and bases in the active sites of enzymes
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Enzymes catalyze reactions under mild conditions that might otherwise require extreme conditions such as high temperature or strong acid or base. To achieve this, amino acid side chains that are weak Brønsted acids or bases in the free amino acid become strong acids and bases in the active sites of many enzymes. Here we report on a set of 30 enzymes that represent all six major EC classes and a variety of different folds for which experimental studies of the mechanistic roles of the catalytic residues have been reported in the literature. Using macromolecular electrostatics techniques, it is shown that the catalytic aspartate and glutamate residues are strongly coupled to at least one other aspartate or glutamate residue, and often to multiple other carboxylate residues, with intrinsic pK_a differences less than 1 pH unit. Sometimes these catalytic acidic residues are also coupled to a histidine residue, such that the intrinsic pK_a of the acidic residue is higher than that of the histidine. Most catalytic lysine residues studied here are strongly coupled to tyrosine or cysteine residues, wherein the intrinsic pK_a of the anion-forming residue is higher than that of the lysine. Some catalytic lysines are also coupled to other lysines with intrinsic pK_a differences within 1 pH unit. Some general principles about the design of enzyme active sites are presented. These principles have significant implications for the engineering of novel enzymes. Supported by NSF CHE-1905214 (MJO).

2244-Plat
Harnessing natural evolution and computation towards systems enzymology
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Rationally reprogramming enzyme catalysis requires systems-level knowledge of various enzyme mutations, which is extremely challenging. Learning from nature is inescapable to overcome this barrier. We recently distilled the evolutionary information from natural homologous sequences using a maximum-entropy model and established a connection between enzyme evolution and enzyme catalysis. The finding also provides a rational enzyme engineering approach, and about half of the predicted mutations improve enzyme catalytic power in experiment. Furthermore, we utilized natural evolution to systematically rationalize laboratory evolution of designer enzymes; we identified the sequence determinants for the selectivity of kinase covalent inhibitors and confirmed the insight using enzyme modeling. Overall, our studies show the availability of vast protein sequences from nature is promising to advance enzymology to a systems-level, an emerging field termed 'systems enzymology.'

2245-Plat
Bayesian structure determination of multiple conformational structures from single molecule X-ray scattering images
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Single molecule X-ray diffraction experiments are a promising new method for the structure determination of biomolecules. The refinement of the structure from these experiments is quite challenging: The scattering images are sparse, each containing only 10-50 photons on average, the signal to noise ratio is very low, and the orientations of the molecules at the time of scattering are unknown.
Despite these challenges, using rigorous Bayesian approaches, we have recently demonstrated that near-atomistic resolution can be achieved. However, many biomolecules show structural heterogeneity and conformational dynamics between different distinct structures; to extract these from single molecule scattering data has so far been elusive. The main bottleneck here is that not only the orientation, but also the current conformer for each scattering image is unknown.
Generalizing our Bayesian approach, we demonstrate that it is possible to determine not only a single structure, but the molecule's entire structural ensemble from these experiments. Using synthetic scattering images generated from molecular dynamics trajectories, we demonstrate that the method is able to determine the conformational structures of alanine dipeptide at 2-3Å resolution using 10 images, and of the unfolded ensemble of the synthetic protein chignolin at 5-6Å resolution using 10 images.

2246-Plat
Ot2Rec: A semi-automatic, extensible, multi-software tomographic reconstruction workflow
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Electron cryo-tomography (cryo-ET) is an imaging technique that produces relatively high-resolution three-dimensional images. With the advancement in microscopic and computational technologies, data collection and processing on both the hardware and software facets have become ever faster and more automated. Nowadays, tens of tilt series (up to several terabytes in total) can be collected per day, in turn shifting the experimental bottleneck to data processing. Here we present Ot2Rec, a novel workflow for performing tomographic image processing, providing access to several state-of-the-art tools within a single ''wrapper'' user interface. The functionality of Ot2Rec covers most tasks within a common image processing pipeline, from the motion-correction of raw multi-frame micrographs to the estimation of the true defoci from which the contrast transfer function (CTF) of images is calculated, and from the alignment of tilted image stacks to the full reconstruction of the final 3D tomogram. Moreover, Ot2Rec can carry out several novel post-processing operations such as simulation of stack point-spread functions and CTF corrections by means of deconvolution. Ot2Rec also provides automated reporting of processing parameters and quality metrics for optimisation and performance evaluation of processing workflows. Ot2Rec has been developed with the central philosophy of metadata unification. We have unified the metadata structure across the suite, so that the components within the suite can be used in a user/developer defined order (for instance, MotionCor2 -> CTFFind4 -> IMOD) with data flowing seamlessly between any pair of components and with consideration of required prerequisites. Lastly, Ot2Rec is an open-source program suite (Apache License V2). The raw code and documentation can be found at https://github.com/rosalindfranklininstitute/Ot2Rec.

2247-Plat
A deep learning method to separate fluorophores based on their fluorescence lifetime
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Fluorescence lifetime in biological samples is a useful feature to demultiplex the fluorescence signal of spectrally overlapping fluorophores. A problem that frequently occurs when multiple fluorophores are in use is the possible spectral overlap. Therefore, selected fluorophores should be chosen to avoid this issue, consequently constraining the number and the type of fluorophores that can be used at the same time on one sample. Moreover the ability to discriminate fluorophores from temporal information does not come from their own emission spectra, but from the (fluorescent-life) time information. A well-established method for the analysis of lifetime images is the phasor approach. The phasor