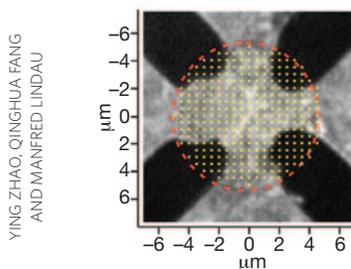


IMAGING

Conformational SNARE

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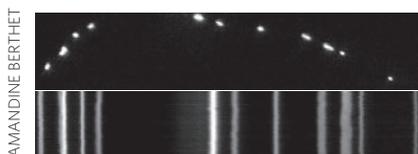
Compounds that mediate signaling across cellular synapses are released by exocytosis when the secretory vesicles that contain them form a fusion pore with the plasma membrane. Three proteins, including synaptosomal-associated protein 25 (SNAP25), make up the SNARE complex, which drives the formation of the fusion pore during exocytosis. The fluorescence resonance energy transfer (FRET)-based SNARE complex reporter (SCORE), a fusion protein containing SNAP25, CFP and Venus, was previously developed to assess whether conformational changes in SNARE are linked to fusion pore formation, but the time resolution afforded by the application of this tool exceeded the timing of the fusion event. Zhao *et al.* now develop event correlation microscopy (ECOM) that improves time resolution to ~1 ms. In the authors' system, time and location of single fusion events in cells expressing SCORE were monitored in tandem. They recorded images from total internal reflection FRET

microscopy and overlaid the images with maps of catecholamine release, which marks fusion events, derived from data collected via a four-electrode electrochemical detector. The authors collected data from over 900 fusion events, revealing a consistent ~90-ms delay between FRET changes in SCORE and the amperometric spike associated with catecholamine release. Collectively, the data are consistent with a rapid conformational change in the SNARE complex preceding the opening of the fusion pore by milliseconds. **AD**

NEURODEGENERATION

Tickled PINK1

Cell **154**, 737–747 (2013)



AMANDINE BERTHET

Parkinson's disease (PD) is a progressive neurological disorder in which the substantia nigra region of the brain is depleted in dopaminergic neurons. Mitochondrial defects have long been associated with PD. For example, mutations in PTEN-induced kinase 1 (PINK1), which is involved in mitochondrial maintenance in neurons, have been linked to an early-onset form of PD, prompting suggestions that enhancers of PINK1 activity could be neuroprotective. Hertz *et al.* now identify a nucleotide triphosphate 'neo-substrate' that elevates PINK1 activity in neurons and provide cellular data in support of this

therapeutic model. Examination of PINK1 sequences, particularly of the G309D mutant associated with PD, led the authors to hypothesize that PINK1 enzymes may be able to accommodate larger ATP analogs as substrates. A screen of a small panel of N6-alkyl-ATP analogs revealed that PINK1 and its G309D mutant use the neo-substrate kinetin triphosphate (KTP, N6-furfuryl ATP) more efficiently than ATP itself. Cells treated with kinetin, the nucleoside precursor of KTP, readily accumulate KTP at amounts that restore nearly wild-type activity of PINK1. Finally, the authors showed that neurons treated with kinetin display several molecular phenotypes consistent with PINK1 activation. In addition to validating a potential therapeutic avenue for early-onset PD, the study points toward the possibility that neo-substrate activation could be a general approach for modulating disease-related kinase activity. **TLS**

DRUG DISCOVERY

Look within

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doi:10.1038/nm.3262

Killing *Mycobacterium tuberculosis* (*Mtb*) inside of host cells is critical for effective treatment of tuberculosis. To find new potential drugs that are capable of doing this, Pethe *et al.* used an automated microscopy platform to screen a library of 121,156 compounds for those that could interfere with replication of *Mtb* within macrophages. Two compounds with an imidazopyridine amide scaffold were isolated, and they had low micromolar potencies against a reference *Mtb* strain as well as against multidrug-resistant (MDR) clinical isolates. Lead optimization led the authors to Q203, a compound with an MIC50 of 0.28 nM within macrophages that is capable of killing MDR and also extensively drug-resistant (XDR) strains. Q203 was not toxic to macrophages and had other properties that make it an attractive drug lead, including metabolic stability and an absence of drug-drug interactions, and there was a low rate of spontaneous mutation with the potential to lead to resistance in treated *Mtb* cells. Q203 was effective in mouse models of acute and established tuberculosis. Sequencing the genomes of Q203-resistant *Mtb* mutants and an assay monitoring ATP synthesis suggested that the QcrB subunit of the cytochrome *bc₁* complex is the direct efficacy target. These studies demonstrate that Q203 has desirable features for clinical treatment of MDR and XDR tuberculosis. **MB**

PROTEIN DYNAMICS

Shake it up, baby

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doi:10.1038/nmeth.2592

The amino acids in proteins are highly dynamic, with their side chains sampling many different conformations every second. Although X-ray crystal structures of proteins are usually presented as a single, static structure, the diffraction data may contain additional information about transient, alternative conformations of the protein. van den Bedem *et al.* now report a new algorithm, CONTACT, that can be used to identify networks of dynamic amino acids in a protein from high-resolution X-ray crystallography data. In a proof-of-principle study involving dihydrofolate reductase, the algorithm predicted that the NADP cofactor might mediate long-range interactions between the FG loop and the adenosine-binding domain; NMR spectroscopic measurements were shown to support this hypothesis. The authors then solved a high-resolution, room-temperature X-ray crystal structure of a variant of dihydrofolate reductase in which the Met20 loop was destabilized by making two mutations. CONTACT indicated that there was a large increase in the number of dynamic contacts between amino acids in the double mutant, and its active site was significantly more heterogeneous than that of the wild-type protein. This heterogeneity may explain why the rate of hydride transfer for the double mutant is much lower than that of the wild-type enzyme. Algorithms like CONTACT will make it easier to identify and study dynamic regions of proteins, which will help structural biologists relate protein motions to function. **JMF**