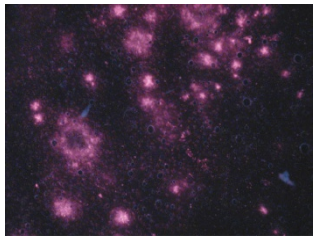
AR proteins good kinase inhibitors?

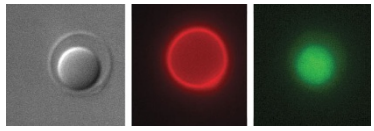
Eukaryotic protein kinases (EPKs) are central players in cellular signaling pathways. To understand the roles of specific kinases, recent studies have focused on selective EPK inhibition by small molecules. Achieving kinase inhibition specificity and *in vivo* efficacy has been challenging because of the conserved ATP-binding site shared by all EPKs. Non-natural peptide or protein ligands may offer an alternative approach for downregulation of kinase activity. However, the protein inhibitors must selectively bind the target EPK with high affinity, yet remain stable under the cell's reducing and proteolytic conditions. To address these challenges, Plückthun and coworkers have reported an *in vitro* selection and bacterial screen to identify small protein inhibitors of a bacterial protein phosphotransferase. The authors targeted aminoglycoside phosphotransferase IIIa (APH), a protein that confers bacterial drug resistance by phosphorylation of aminoglycoside antibiotics. The authors previously demonstrated that ankyrin repeat (AR) proteins offer an attractive scaffold for identifying protein inhibitors because they adopt a stable folded structure, even after amino acid substitutions. Plückthun and coworkers constructed randomized libraries of AR proteins and used *in vitro* ribosome display to identify AR modules that bound to APH. The resulting library hits were refined further to AR proteins that inhibited the phosphotransferase activity of APH and showed tight binding with dissociation constants in the nanomolar range. Several AR protein inhibitors elicited a full knockout phenotype in bacterial cells, and these also showed complete inhibition with the substrate amikacin. The authors point out that APH is an EPK model and that future work could improve the understanding of kinase biology or validate therapeutic targets. (*J. Biol. Chem.*, published online 25 April 2005, doi:10.1074/jbc.M501746200) *TLS*

Seeing amyloid in the IR

Experimental evidence for Alzheimer disease is typically obtained by histological analysis of brain tissue to detect the diagnostic amyloid plaques, which are formed by the accumulation of amyloid-β peptides (Aβ). Methods for the direct *in vivo* monitoring of plaque formation would be advantageous for diagnosis and for monitoring disease progression. Near-infrared fluorescence (NIRF) imaging offers a non-invasive and inexpensive approach for optical imaging in animals. To obtain sufficient sensitivity, however, it is necessary to have a NIRF ligand that increases the contrast between the plaques and surrounding tissue. Gremlich and coworkers have developed an oxazine derivative with fluorescence in the near-infrared region that binds selectively to Aβ deposits in brain slices from a mouse model of Alzheimer disease (APP23 mice). The authors found that the oxazine dye crossed the intact blood-brain barrier, which allowed *in vivo* imaging of amyloid plaques in APP23 mice. Compared to wild-type animals, the APP23 mice showed increased fluorescence at the site of plaque deposition. Although the authors speculate that the oxazine dye intercalates into the β-sheets of the plaques, the mechanism of dye-specific targeting remains to be determined. This technique offers promise for following disease progression over the lifetime of a single mouse and assessing the efficacy of potential Alzheimer disease treatments. (*Nat. Biotechnol.* **23**, 577–583, 2005) *JK*



A new phase in synthetic cells



Microcompartmentation is thought to play significant parts in cellular function. In living cells, local concentration differences in the cytoplasm are common, even in areas not separated by lipid membranes. Yet understanding the roles of microcompartmentation has been complicated by the complexity of living cells and the lack of an alternative experimental model. Long *et al.* report a new experimental model of cytoplasmic microcompartmentation that lacks membranous barriers. The synthetic cell encapsulates a polymeric aqueous two-phase system composed of polyethylene glycol (PEG) and dextran. At higher temperatures the two encapsulated polymers existed as a single phase, whereas at lower temperatures phase separation caused distinct dextran-rich microcompartments to form, which were observed using differential interface contrast microscopy and fluorescence-labeled dextran. Fluorescently labeled lectins concentrated in the dextran-rich phase, where raising the temperature caused the protein to diffuse equally throughout the cell as the two phases became miscible. This process was reversed by lowering the temperature. Treatment of the cells with a sucrose solution drove the dextran phase to the border of the outer membrane, a process thought to result from osmotic dehydration. Fluorescence-labeled DNA was found to concentrate preferentially in this outer dextran phase. The microcompartmentation of biological molecules in synthetic cells described here offers a simple cytoplasmic model to explore events such as protein folding or enzymatic reactions. (*Proc. Natl. Acad. Sci. USA* **102**, 5920–5925, 2005) *GW*

Probing outer membrane biogenesis

The outer membrane (OM) of Gram-negative bacteria is a complex, layered structure that acts as a barrier to harmful compounds such as antibiotics. The results of studies of OM biosynthesis using mutant strains have lacked clarity because OM-deficient mutants generally have defects in nonspecific permeability. In a recent issue of *Cell*, Ruiz *et al.* describe 'chemical conditionality' screens to identify mutant *E. coli* strains that confer enhanced resistance to antibiotics. Because the OM is generally impermeable to antibiotics, the authors started with an antibiotic-sensitive strain of *E. coli* (*imp4213*) that had a mutation affecting the Imp (increased membrane permeability) protein, causing an OM permeability defect. The authors screened *imp4213* cells in the presence of two antibiotics, moenomycin and chlorobiphenyl vancomycin, and selected for mutants with restored antibiotic resistance. The suppressor phenotypes were mapped to loss-of-function mutations in the *yfgL* gene. Further characterization showed that reduction or absence of YfgL led to altered membrane permeability and increased antibiotic resistance. In a follow-up paper, the authors identified three proteins that bind to YfgL and form a previously unidentified multiprotein complex that is required for OM assembly. Taken together, these studies demonstrated that chemical conditionality screens offer a unique approach to identifying components of complex molecular machines or organelles within cells. (*Cell* **121**, 235–245 and 307–317, 2005) *GW*

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