DRUG DISCOVERY

Know your chemical space

The susceptibility of organisms to chemical perturbation differs as a result of defenses that limit the permeation of small molecules. Screening for permeation, rather than bioactivity, to identify a priori organism-specific chemical space offers an intriguing approach to phenotypic assays and potentially addresses some fundamental challenges in drug discovery.

Andrew L Hopkins & G Richard Bickerton

Killing a bug is not easy. Even when applied in doses far in excess of those applied to mammalian cells, only 2% of small-molecule pharmaceuticals induce a strong phenotypic response in *Caenorhabditis elegans*[1]. Gene deletion and gene silencing techniques have been successful in enabling whole-genome analysis of gene function in many pathogens and model organisms. The accessibility of gene knockouts and knockdowns has fueled their use as tools in target validation in anti-infective drug discovery[2]. However, a fundamental problem in translating this target validation into drug leads is the lack of knowledge of what type of compounds are likely to even get into an organism to reach the site of desired drug action. If, despite genetic validation of a putative drug target, a compound that modulates that target *in vitro* turns out to be unlikely to permeate an organism *in vivo*, much effort will have been wasted. For example, *C. elegans* holds immense value as a model organism in genetics, but its potential in chemical biology and drug discovery has been limited by the relative difficulty of discovering small molecules that robustly perturb it. On page 549 of this issue, Burns *et al.* present an approach to screen directly for compound bioaccumulation rather than bioactivity in *C. elegans*, to map the chemical space that can permeate the worm's formidable defenses.

The worm has a four-layer cuticle, a rapidly pumping intestine and over 140 genes in its xenobiotic detoxification system. Using a high-throughput, high-performance liquid chromatography (HT-HPLC) method, Burns *et al.* conducted a survey to measure the accumulation and metabolism of over 1,000 diverse small molecules that were dosed to *C. elegans*[3]. Fewer than 10% of the molecules screened were found to accumulate in concentrations greater than 50% of the concentration in the worm's external environment. Using the chemical structure data of the accumulated compounds, Burns *et al.* developed and validated a Bayesian model to predict which compounds have a greater likelihood of bioavailability in *C. elegans*. The method and model that Burns *et al.* present could be valuable tools in selecting chemical libraries with a greater chance of showing bioactivity in *C. elegans*. Perhaps even more importantly, the innovation of screening chemical space a priori for bioavailability rather than bioactivity may have wider implications for drug discovery beyond *C. elegans* chemical biology.

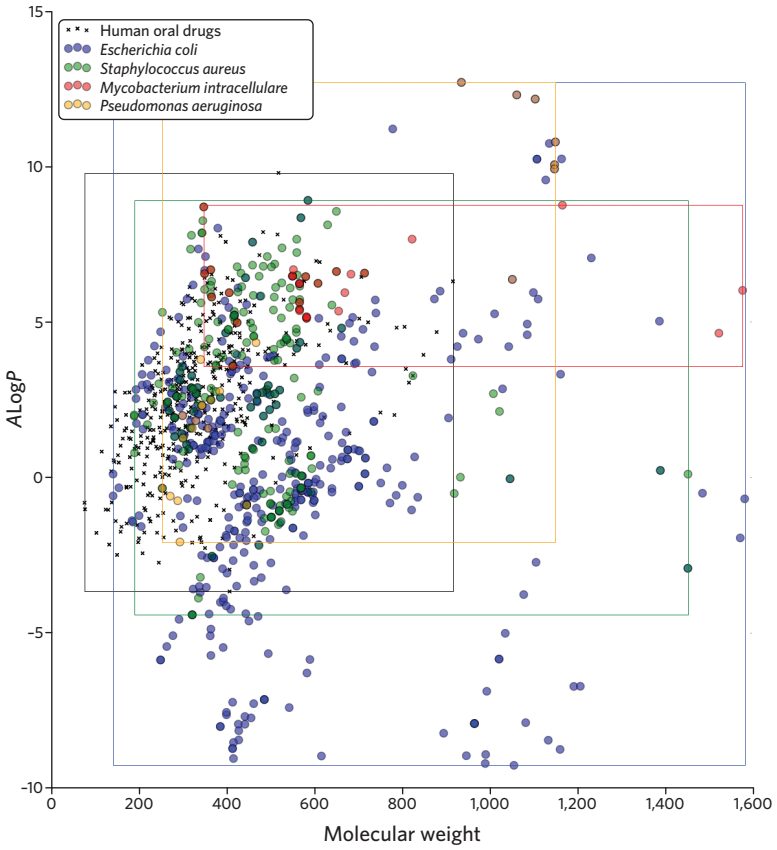

Figure 1 | Chemical space by species. Scatter plot of molecular weight against ALogP reflecting differing chemical spaces for compounds with *in vivo* activity against four pathogenic bacterial species and a set of oral drugs targeting human proteins. Antibacterial compounds were selected from the ChEMBL database using the criteria that they showed activity with 100 μm or less in an *in vivo* functional assay with an IC_{50} endpoint against the species of interest (*Escherichia coli,* 425 compounds; *Staphylococcus aureus,* 1,280 compounds; *Mycobacterium intracellulare,* 29 compounds; *Pseudomonas aeruginosa,* 23 compounds). The drug set (415 compounds) was selected as the active molecular component of an orally administered small-molecule therapeutic that is known to target one or more human proteins.

The idea that the bioavailability of a compound is probabilistically related to its chemical structure rose to prominence with the publication of Lipinski's seminal work on the 'Rule of Five', which describes how the likelihood of oral bioavailability of drugs in humans is correlated within defined

physicochemical parameters[4]. However, Lipinski's Rule of Five does not apply to many antibacterial drugs. Indeed, drugs for Gram-negative and Gram-positive bacterial strains appear to occupy distinct areas of chemical space[2,5] (**Fig. 1**). Screening rules to describe the distinct chemical space occupied by fungicides, herbicides and insecticides have also recently been proposed[6]. Central nervous system drugs that pass the human blood-brain barrier have an even greater restriction on their chemical property space than the Rule of Five permits[7].

Ensuring that a drug reaches its site of action, whether in specific human tissue types or in a pathogenic organism, is an important task of medicinal chemistry. Good bioavailability is composed of a mixture of high passive permeability through the appropriate cell physical barriers, low transporter efflux and an acceptable rate of xenobiotic degradation. Historically, our knowledge of specific bioavailable chemical spaces has been derived from the chemical structures and physicochemical properties of compounds that are bioactive *in vivo*[8]. What makes the Burns *et al.* paper[3] interesting is the idea of screening for bioavailability as a goal in its own right to define the chemical space for bioactive discovery, a priori. Given the challenges of translating *in vitro* hits for genetically validated targets into *in vivo* leads in many areas of drug discovery, particularly anti-infectives[2], surveying the bioavailable chemical space for a specific organism or tissue type could be a wise investment.

The relatively low throughput of the HPLC method Burns *et al.*[3] use to measure bioaccumulation, compared to conventional high-throughput screening for bioactivity, suggests there may be a role for organizing pre-competitive projects to screen larger, more diverse and more representative chemical libraries across a group of collaborators, both academic and industrial. Alternatively, pre-competitive chemical space screening to deliberately build structure and property-based predictive models for bioavailability may be a valuable undertaking for publicly funded screening initiatives, such as the US National Institutes of Health Molecular Libraries Screening Center Network. As each drug target has a propensity to bind ligands in a certain region of chemical space, the mapping of target chemical space with the specific bioavailable chemical space of an organism may enable the prioritization of targets likely to bind bioavailable ligands[8,9].

The use of bioavailability screening as a means to map the permeable chemical space of a pathogen organism is an oblique strategy that could benefit both drug discoverers and chemical biologists.

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Competing financial interests

The authors declare no competing financial interests.

PROTEIN LOCALIZATION

Can too much lipid glue stop Ras?

The kinetics of the acylation, deacylation and reacylation cycle are important for localization and function of Ras as well as other key signaling proteins. A new small-molecule inhibitor may put the brakes on Ras by inhibiting the deacylation enzyme APT1.

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Controlling the subcellular localization of cell signaling components aids in precise regulation of signaling, a task well suited to highly dynamic posttranslational modifications such as palmitoylation, the only reversible and dynamically regulated lipid modification of proteins[1]. Acylation by *S*-palmitoylation, the covalent thioester linkage of 16-carbon long-chain fatty acids to cysteine residues in proteins, is critical for proper localization and activity of peripheral membrane proteins such as Ras GTPases and heterotrimeric Gα subunits, both of which are important targets for drug discovery. Yet the identification of compounds that are both capable of interfering in this process and suitable for drug development has been hampered by the lack of consensus about the molecular machinery and mechanisms necessary for protein palmitoylation and depalmitoylation. These have been the subject of vigorous debate but remain poorly understood[2]. Two recent publications from the groups of Bastiaens and Waldmann address how the molecular acylation machinery controls spatial distribution of Ras[3] and identify a new small-molecule inhibitor of protein depalmitoylation that alters which pools of Ras become activated in response to growth factor signaling[4].

Many small GTPases and other signaling proteins exist in multiple isoforms that differ primarily in the membrane targeting sequences in their tails, regions that are post-translationally modified to dictate their localization and functions. Among the Ras proteins, the isoforms H-Ras, N-Ras and K-Ras4A are palmitoylated whereas K-Ras4B is not. Although it had long been thought that Ras signaling emanated only from the plasma membrane, Chiu *et al.* demonstrated in 2002 that palmitoylated H-Ras is resident on both plasma membrane and Golgi and that it signals from both platforms but with different outcomes and different kinetics[5]. Two groups later used live cell imaging after fluorescence-activated photobleaching (FRAP) of fluorescently tagged wild-type[6] or semisynthetic[7] H-Ras and N-Ras proteins to identify the kinetics of an acylation, deacylation and reacylation cycle important for Ras membrane targeting and activity. The cycle was surprisingly rapid, and the deacylation phase was as critical as the acylation phase in determining when and where Ras proteins localized. Yet the rules that governed reversible palmitoylation and the trafficking of palmitoylated versus depalmitoylated Ras remained unclear. The depalmitoylating enzyme APT1, an acyl