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Reaching the Target: Small Molecules Aim to Probe Barrier Quality

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The field of chemical genetics is based on the premise that small molecules can be used to perturb macromolecular function and, hence, to give insight into complex biological mechanisms.[1,2] For the systematic exploration of biology, this approach is usually considered to require a small-molecule partner for each protein target; indeed, one goal for chemical genomics is the identification of a small-molecule probe for each function of every protein.[1] Nonetheless, some of the most exciting strategic applications of chemical probes in biology challenge this paradigm.

The combination of small-molecule probes with classical genetic approaches, for example, can offer particularly valuable insights into protein function. For families of proteins with highly conserved active sites, the discovery of chemical probes that are selective for a specific protein is extremely challenging. However, by engineering the ATP-binding site, it is possible to design ligands that essentially target only the (mutant) protein kinase of interest.[3] The combination of the chemical genetic approach with reverse classical genetics has, therefore, given remarkable insight into the biological mechanisms controlled by protein kinases; representative examples include the molecular mechanisms that underpin cell-cycle regulation,[3] endocytosis,[4] neurotrophin signalling[5] and trafficking between cellular compartments.[6]

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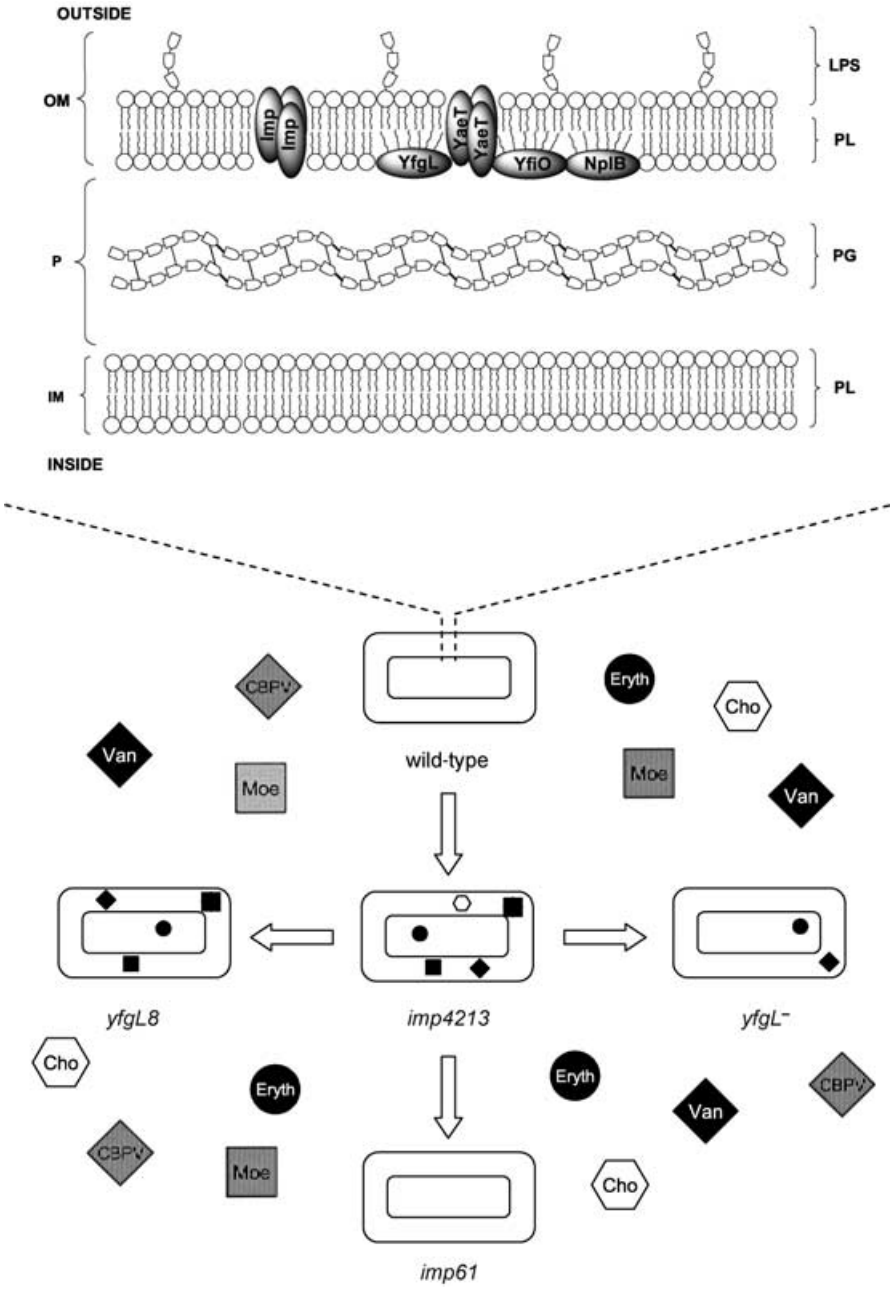


Figure 1. Schematic representation of the membrane permeability of various *E. coli* strains to small-molecule bactericides. The OM of the wild-type bacterium is impermeable to all of the probes studied. The OM of the *imp4213* strain is permeable to all the probes, although it can be mutated to restore resistance. OM, outer membrane; P, periplasm; IM, inner membrane; LPS, lipopolysaccharide; LP, lipoprotein; PG, peptidoglycan; PL, phospholipids; Eryth, erythromycin; Van, vancomycin; CBPV, chlorobiphenylvancomycin; Moe, moenomycin A; Cho, cholic acid.

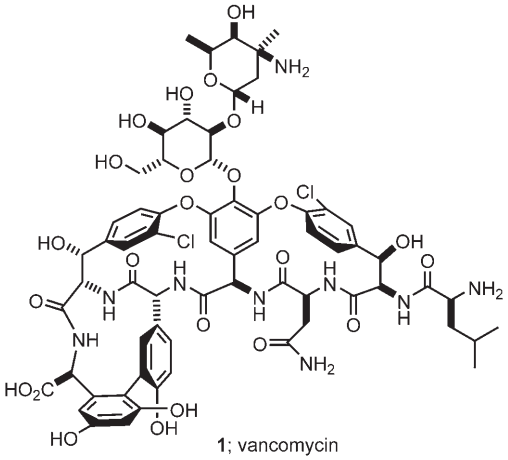
Recently, another variation on the usual chemical genetic approach has emerged.[7] A range of toxic compounds with diverse physicochemical properties has been used to investigate the mechanisms that underpin the biogenesis of the outer membrane (OM) of *Escherichia coli*. The approach was unusual for two reasons. First, small molecules were used to unravel the biology of proteins other than their direct molecular targets and, second, the chemical-genetic approach was used in combination with forward classical genetics. Ultimately, this approach revealed some roles for a bacterial OM–protein complex comprising a number of proteins of previously unknown function.[8]

The bactericidal compounds **1**–**5** were exploited as specific chemical probes of barrier quality in *E. coli*. The OM of *E. coli*, which surrounds the peptidoglycan layer, is usually impermeable to all of the chemical probes **1**–**5** used in the investigation. It is a highly asymmetric bilayer, in which the outer leaflet is largely composed of lipopolysaccharides and the inner leaflet is composed of phospholipids (Figure 1).[9] The mutant strain *imp4213* displays *i*ncreased outer-*m*embrane *p*ermeability; this "leaky" phenotype stems from a mutation in the *imp* gene that encodes the Imp protein and is essential for OM assembly. Consequently, the mutant strain is sensitive to probes **1**–**5**, since they are now able to penetrate the OM and reach their molecular targets.

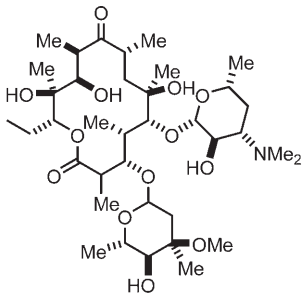
The investigators used a forward genetic approach, in which they selected for spontaneous mutations that restored resistance to each of the chemical probes **1**–**5**.[7] Only specific mutations in the *imp* gene were able to restore resistance to vancomycin and erythromycin: by restoring the functions of the essential Imp protein, the OM was correctly assembled once more. With the permeability of the OM re-established, the cells were not only resistant to vancomycin (**1**) and erythromycin (**2**), but also to the other chemical probes (**3**–**5**).

The analogous experiments with chlorobiphenyl vancomycin (**3**; CBPV) and moenomycin A (**4**) were more revealing. Some mutations in the *yfgL* gene were able to restore resistance to these toxic compounds. Hence, in this investigation, small-molecule probes were found to give insight into the biology of proteins other than their direct targets, in this case, proteins involved in the biogenesis of the OM. The *yfgL* gene encodes a protein, YfgL, that was also shown to be involved in OM biogenesis.

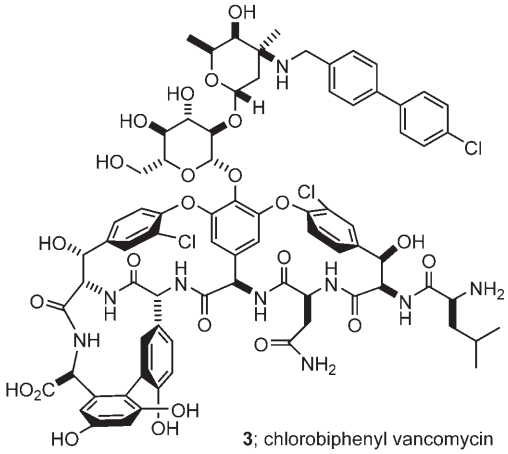
Ultimately, a hierarchical relationship between chemical probes of barrier quality was uncovered (see Figure 2). Only specific mutations in the *imp* gene were able to restore the resistance of the *imp4213* strain to vancomycin and erythromycin, and these mutations resulted in resistance to all of the chemical



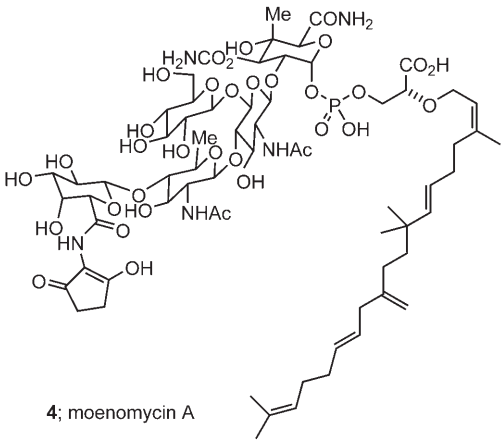
1; vancomycin



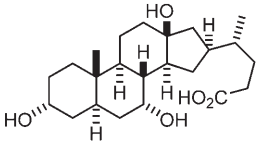
2; erythromycin



3; chlorobiphenyl vancomycin



4; moenomycin A



5; cholic acid

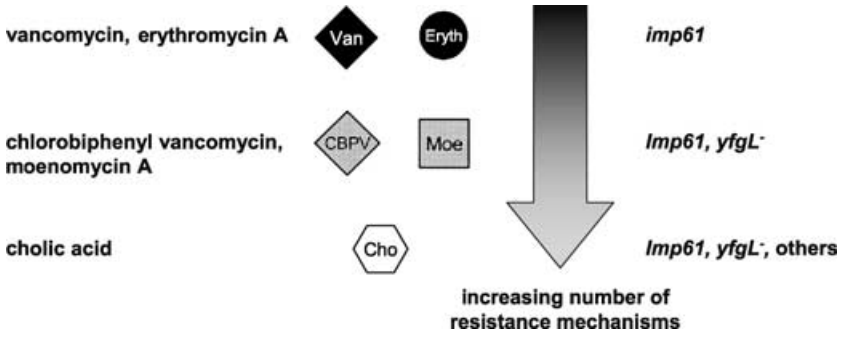


Figure 2. Hierarchical illustration of the relationship between bactericides and the number of mutations able to restore resistance in the *imp4213* strain. Molecules at the top are probes of high-quality OM barriers.

probes. Similarly, mutations in *yfgL* restored resistance to CBPV and moenomycin A, as well as to toxins lower in the hierarchy in Figure 2. In contrast, many mutations conferred resistance to cholic acid (**5**), none of which protected the bacterium from probes **1**–**4.** There was a clear correlation between the number of ways to suppress toxicity to a small molecule, and the quality of the barrier provided to that small molecule.

The study also revealed a link between the functions of YfgL and Imp.[7] The wild-type organism is resistant to both CBPV and moenomycin A. Furthermore, individually, certain mutations in either *yfgL* or *imp* can confer sensitivity to both of these compounds. However, the selection experiments described above demonstrated that, in combination, these same mutations restored the antibiotic-resistant phenotype, provided, it turns out, that cell growth is slow. The genetic link between *yfgL* and *imp* argues strongly for a cooperative role for YfgL and Imp in OM biogenesis, and a (direct or indirect) interaction between the proteins.

So what, then, is the link between YfgL and Imp? Other investigations[8] have shown that YfgL is a lipoprotein that is directed to the periplasmic face of the OM. A series of experiments[8] were conducted that aimed to detect a direct interaction between YfgL and Imp, but no such interaction was found. However, coimmunoprecipation experiments and genetic evidence suggested that YfgL forms a multiprotein complex with three other proteins (YaeT, YfiO and NlpB) that is implicated in the assembly of OM β-barrel proteins. Tight control of OM composition—in particular the highly asymmetric structure of the membrane—is required for impermeability. We have seen that certain YfgL mutants are able to confer CBPV and moenomycin A resistance to the *imp4213* strain. The authors suggest that the mutation of YgfL does not simply reduce the function of the multiprotein complex, but, rather, affects the homeostatic control of OM composition in a specific way.

The use of forward genetics in combination with small-molecule probes of barrier quality is an exciting alternative to conventional chemical genetic approaches. In particular, the use of toxic molecules with very different physicochemical properties allowed permeability defects in the OM to be probed in a highly specific manner. The field of chemical genetics is not restricted to the study of the actual targets of small-molecule probes. Small molecules need to reach their target to function, and can be used to investigate the mechanisms that control the organisation of biological systems. This highlight has outlined the power of small molecules in the context of biological research and the powerful interplay[10] that is possible between classical and chemical genetics. These approaches are united in their goals: the detailed dissection of the molecular basis of biological mechanisms.

Keywords: bacteria · chemical genetics · membranes · proteins · small-molecule probes

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