harboring a different library member. They screened these droplets for variants able to hydrolyze sulfate monoesters or phosphate triesters (**Fig. 1**, bottom right). In total, 20 million droplets could be assayed by fluorescence measurements and sorted within 2 hours. After further verification, the authors characterized 14 final hits biochemically, resulting in the identification of a set of completely novel hydrolytic enzymes. These enzymes have the potential to degrade xenobiotics, provide unprecedented promiscuity and span three enzyme superfamilies. This exploration of sequence space also provided detailed insights into the routes of evolution. A further charm of the method is that the substrate scope of most of the novel enzymes could have not been predicted by bioinformatic tools from their sequences. Overall, the microfluidic system was sensitive enough to identify enzymes with very low activities and low natural abundance as compared to common hydrolases.

The discovery of novel enzymes and the use of protein engineering represent a vibrant area of research, especially for biocatalysis. Enzymes catalyzing novel chemistry, as well as improved ones with optimal performance under process conditions, are highly desired, with an impressive number of success stories being reported[5]. Since the early 1990s, when directed evolution "in the test tube" started to become the standard tool in protein engineering, scientists have struggled to deal with the large sizes of mutant libraries that are generated. To address this, various concepts for ultra-high-throughput screening (uHTS) have been developed, such as *in vitro* compartmentalization[6], fluorescence-activated cell sorting for screening or selection[7] and, more recently, microfluidic systems[8]. A common limitation of these approaches is that the conditions under which these assays yielding fluorescent products are run are usually far from reality. Thus, the putative hits from uHTS may not show the expected performance when exposed to (physiologically or chemically) relevant substrates and conditions ("You get what you screen for"[9]). Both Chen *et al.* and Colin *et al.* now offer advantages over established uHTS methods in determining the binding, single-turnover and real-time kinetics of an enzyme to be measured, despite the necessity of using fluorescence readouts. However, confirmation of these hits—commonly accomplished through microtiter-plate assays followed by classical biochemical characterization of purified protein—is still required and dictates the overall time scale for protein discovery and engineering projects. Thus a potential approach to deal with massive uHTS using the recently described microfluidic tools is to reduce the library size and at the same time increase the number of well-performing initial hits. This can be achieved through the use of bioinformatic tools to analyze and filter enzyme superfamilies by, for example, correlated mutation analysis for better predictions of function and key mutations. Overall, the microfluidic systems described offer the potential to increase the success rate in protein discovery and engineering even further. ■

Uwe T. Bornscheuer is at the Institute of Biochemistry, Department of Biotechnology and Enzyme Catalysis, Greifswald University, Greifswald, Germany.
e-mail: uwe.bornscheuer@uni-greifswald.de

Published online 7 December 2015
doi:10.1038/nchembio.1989

References

1. Brown, C.T. *et al. Nature* **523**, 208–211 (2015).
2. Chen, B. *et al. Nat. Chem. Biol.* doi:10.1038/nchembio.1978 (7 December 2015).
3. Colin, P.Y. *et al. Nat. Commun.* doi:10.1038/ncomms10008 (7 December 2015).
4. Kazlauskas, R.J. & Bornscheuer, U.T. *Nat. Chem. Biol.* **5**, 526–529 (2009).
5. Bornscheuer, U.T. *et al. Nature* **485**, 185–194 (2012).
6. Griffiths, A.D. & Tawfik, D.S. *Nat. Biotechnol.* **16**, 652–656 (1998).
7. Fernández-Álvaro, E. *et al. Angew. Chem. Int. Edn. Engl.* **50**, 8584–8587 (2011).
8. Colin, P.Y., Zinchenko, A. & Hollfelder, F. *Curr. Opin. Struct. Biol.* **33**, 42–51 (2015).
9. Schmidt-Dannert, C. & Arnold, F.H. *Trends Biotechnol.* **17**, 135–136 (1999).

Competing financial interests

The author declares no competing financial interests.

SYNTHETIC BIOLOGY

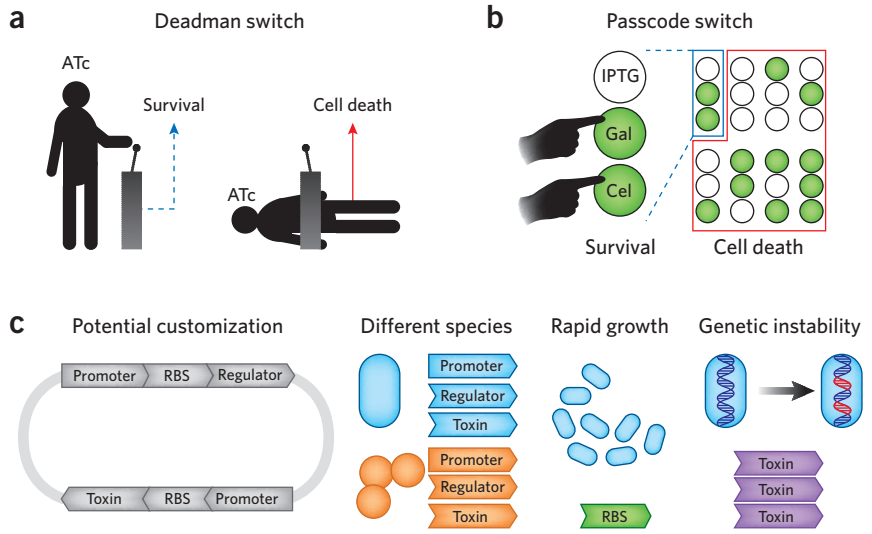
Building genetic containment

Since the 1980s, scientists have worked on designing genetic codes to reinforce containment and control of genetically engineered microbes. New mechanistic studies of "deadman" and "passcode" gene circuits provide a flexible platform to build new safety switches.

Karmella A Haynes

Currently, it is unclear whether the unmonitored release of every genetically engineered microbe (GEM) into the environment is absolutely harmful or absolutely safe. Decades of scientific research and changes in government policies may be needed to determine and define the danger or safety of every type of GEM, as current information is sparse[1]. Many GEMs provide valuable immediate benefits to human society, but these benefits could be delayed unnecessarily by drawn-out vetting processes. Today, GEMs are being used in closed industrial environments to generate scents, flavors, fragrances, solid materials, fuels and pharmaceuticals on demand[2]. Biocontainment, the prevention of unintended environmental release, is an approach that allows the use of beneficial GEMs while avoiding uncertain risks. Physical containment within flasks and bioreactors and regulated disposal of decontaminated GEM cultures constitute the standard approach in industry and research lab settings. If the use of GEMs continues to scale up and these organisms are used in open settings in the future, external physical containment measures will no longer be sufficient. We will need genetically programmed controls that operate within the GEMs themselves. Chan *et al.* report the construction and validation of DNA-based genetic safeguards[3] that prevent escaped microbes from proliferating unchecked. Two customizable designs are composed of 'nuts and bolts' sub-parts that potentially allow scientists to tune the function of the gene circuits to suit different microbial strains and conditions.

An ideal genetic safeguard, sometimes referred to as a 'kill switch', simultaneously harbors a neutral state that allows billions of microbes to happily thrive and an activatable state that swiftly kills the entire population. As a second requirement, the engineered



Kim Caesar/Nature Publishing Group

Figure 1 | Genetic safety switches built from interchangeable parts. (**a**) The "deadman" switch, named for a mechanism that is triggered if a human operator becomes incapacitated, allows cells to live as long as an operator compound (anhydrotetracycline, ATc) is present. (**b**) The "passcode" switch in this example requires the presence of galactose (Gal) and cellobiose (Cel), and the absence of IPTG, for cell survival. (**c**) Both systems are built from interchangeable DNA parts such as promoters, ribosome-binding sites (RBSs) and protein-coding regions (regulators and toxins), as roughly illustrated here. Potentially, a scientist could swap in species-specific parts, boost protein expression with stronger RBSs in rapidly growing cells, or add extra toxin genes to resist damage caused by genetic instability.

DNA encoding the safeguard should be blocked from transfer into non-GEM organisms. Several strategies have been pursued to meet these design needs. For example, kill switches use a lethal gene to actively kill the GEM either after a compound is taken away or when an inducer is added. Unintended transfer of the engineered DNA into non-GEM hosts can be prevented by embedding a toxic gene in the synthetic DNA plasmid and using an immune GEM host[4]. An irksome caveat for lethal genes is the occurrence of transposon insertions that can disrupt the toxin-expressing gene and break the safety switch. As an alternative, engineered auxotrophs are unable to synthesize an essential natural compound required for their survival, and starve when the compound is no longer supplied[4,5]. However, the microbe might survive long enough to find a new supply in the environment. Recent achievements in genome-wide engineering have addressed this caveat by rendering *Escherichia coli* dependent upon unnatural amino acids for survival[6,7]. However, other useful microbes such as the pollutant-eating *Pseudomonas putida*, the dairy bacterium *Lactococcus lactis*, the soil and gut microbe *Bacillus subtilis*, and yeast (a eukaryote with 16 chromosomes) may be less tractable to whole-genome recoding[6,7]. Bioengineers still need simple, portable systems that can easily be modified and introduced into different host cells.

Work reported by Chan *et al.* addresses this need. Their group used combinations of DNA fragments to optimize two types of safety switches—"deadman" and "passcode" for *E. coli*. Earlier studies have tinkered with lethal-gene copy numbers and activation kinetics[2]. Since then, the synthetic biology community has developed libraries of DNA fragments that exhibit ranges of function that enable more sophisticated fine-tuning. In the "deadman" circuit developed by Chan *et al.*, ribosome-binding sites were selected to generate an unbalanced toggle that, once triggered, ensures a strong, irreversible kill state. The cells are allowed to live as long as a single compound (anhydrotetracycline, ATc) is present in the medium (**Fig. 1a**). Removal of ATc stimulates toxic EcoRI endonuclease and anti-*murC* protease activity, which kill the cells.

The "passcode" circuit uses Boolean logic to detect a combination of compounds that allows cell survival (**Fig. 1b**). Chan *et al.* used two interchangeable modules from the hybrid *LacI-GalR* transcription regulator system to build three different passcode switches. These required the presence of two and the absence of one of the compounds galactose, cellobiose and IPTG. In one version of the passcode circuit, the presence of galactose and cellobiose blocks two negative transcription regulators, allowing the production of a third, protective regulator. This protective regulator stops the toxin genes from being activated, but only as long as IPTG is absent. Any other combination of compounds triggers the toxic EcoRI and the anti-*murC* protease activities, which kill the cells.

The study from Chan *et al.* harkens back to the 2000 toggle-switch study, a seminal synthetic biology report[8] that showed how well-characterized DNA fragments can be assembled to engineer cell behavior. Fans of composable parts–based design may be delighted to see the toggle switch reappear in a practical context. Are the new switches ready to be implemented in industrial settings? Unfortunately, DNA deletions and transposable elements reappear as a problem, as with previously reported lethal gene–based safeguards. Using a recombination- and transposon-lacking strain improved the stability of the switches. However, an ideal switch is inherently resistant to damage and does not require a compromised host cell. Furthermore, the system's flexibility will only be truly realized when scientists determine the specifications required for different contextual aspects such as host strain type, cell division rates and mutation rates (**Fig. 1c**).

Nonetheless, the authors' consideration of the customizable aspects of safety switches could generate impact by steering biocontainment regulations towards standards that are rooted in research data and engineering. Currently, the most specific recommendation for biocontainment from the US National Institutes of Health (NIH) is descriptive: an escape limit of less than 1 per 1×10^8 cells[9,10]. More technical recommendations, such as gene circuit architecture or the number of toxic genes needed to control certain host strains, could provide better practical guidance for effective GEM containment and stimulate more research in this important area of synthetic biology.

Karmella A. Haynes is in the Ira A. Fulton School of Biological and Health Systems Engineering, Arizona State University, Tempe, Arizona, USA.
e-mail: karmella.haynes@asu.edu

References

1. Kimman, T.G., Smit, E. & Klein, M.R. *Clin. Microbiol. Rev.* **21**, 403–425 (2008).
2. Moe-Behrens, G.H., Davis, R. & Haynes, K.A. *Front. Microbiol.* **25**, 4–5 (2013).
3. Chan, C.T.Y. *et al. Nat. Chem. Biol.* **12**, 82–86 (2016).
4. Wright, O. *et al. ACS Synth. Biol.* **4**, 307–316 (2015).
5. Steidler, L. *et al. Nat. Biotechnol.* **21**, 785–789 (2003).
6. Mandell, D.J. *et al. Nature* **18**, 55–60 (2015).
7. Rovner, A.J. *et al. Nature* **518**, 89–93 (2015).
8. Gardner, T.S., Cantor, C.R. & Collins, J.J. *Nature* **403**, 339–342 (2000).
9. Wilson, D.J. *Account. Res.* **3**, 177–185 (1993).
10. *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* http://osp.od.nih.gov/sites/default/files/NIH_Guidelines.html (National Institutes of Health, November 2013).

Competing financial interests

The author declares no competing financial interests.