Expanding the chemical diversity of M13 bacteriophage

Grace L. Allen¹, Ashley K. Grahn¹, Katerina Kourentzi², Richard C. Willson², Sean Waldrop³, Jiantao Guo³ and Brian K. Kay¹*

¹Tango Biosciences, Inc., Chicago, IL, United States, ²Department of Chemical and Biomolecular Engineering, University of Houston, Houston, TX, United States, ³Department of Chemistry, University of Nebraska at Lincoln, Lincoln, NE, United States

Bacteriophage M13 virions are very stable nanoparticles that can be modified by chemical and genetic methods. The capsid proteins can be functionalized in a variety of chemical reactions without loss of particle integrity. In addition, Genetic Code Expansion (GCE) permits the introduction of non-canonical amino acids (ncAAs) into displayed peptides and proteins. The incorporation of ncAAs into phage libraries has led to the discovery of high-affinity binders with low nanomolar dissociation constant ($K_D$) values that can potentially serve as inhibitors. This article reviews how bioconjugation and the incorporation of ncAAs during translation have expanded the chemistry of peptides and proteins displayed by M13 virions for a variety of purposes.

KEYWORDS
bioconjugation, phage-display, stop codon suppression, peptide, cyclization, cross-linking, combinatorial peptide libraries, antibody fragments

Introduction

Since the seminal work of Professor George Smith (Smith, 1985) in displaying a protein fragment on the surface of M13 bacteriophage, a large number of peptides and proteins have been displayed for antibody discovery, protein engineering, and mapping protein-protein interactions. While phage-display is a prize-worthy technique (Smith, 2019), it has generally been limited in chemistry to the canonical set of 20 amino acids of L-chirality. Both chemical modification and genetic methods have been used to expand the types of functional groups displayed on the surface of virions.

Chemically modified virions have been used in a variety of applications (Mohan and Weiss, 2016), such as lateral flow assays (Hagström et al., 2015; Kim et al., 2015, 2017), biosensors (Moon et al., 2019), nanomaterials (Petrenko, 2018), nanomedicine (Ulfo et al., 2022), and batteries (Lee et al., 2009). In M13 bacteriophage (Figure 1), there are ~2,700 copies of the major capsid protein, pVIII, which makes it a desirable target for bulk chemical modification of virions (Keohoe and Kay, 2005). The major capsid protein is 50 amino acids long and it contains a number of residues with functional
groups suitable for bioconjugation, such as amines, carboxylic acids, and phenols (Carmody et al., 2021). The reactivity of these functional groups is dependent on steric accessibility, ionization state, and solvent conditions. For example, the ε-amino group of the lysine at position 8 can be derivatized with glutaraldehyde or N-hydroxysuccinimide esters (NHS-esters) to attach fluorescent dyes, biotin, drugs, DNA, enzymes, or gold nanoparticles (Carmody et al., 2021), although the N-terminus of pVIII is preferentially targeted because of its higher solvent accessibility and lower pKa value (Li et al., 2010). While pVIII lacks cysteines, thiolation of primary amines with 2-iminothiolane (Traut's Reagent) generates sulfhydryl groups that can be coupled to maleimide-activated antibodies or enzymes. Moreover, ε-amino groups are reactive toward aldehydes that are easily generated by mild periodate-mediated oxidation of the sugars on antibodies or horseradish peroxidase (HRP), enabling favorable conjugation directed away from binding sites and active sites of antibodies or HRP, respectively (Adhikari et al., 2013, 2015). In another scheme, using 4-formyl succinimidyl benzoate, amine groups are converted to aromatic aldehydes (2013, 2015). In another scheme, using 4-formyl succinimidyl benzoate, amine groups are converted to aromatic aldehydes that readily react with hydrazide derivatized DNA under mild conditions (Domaille et al., 2013). Carboxylate groups at the C-terminus and within aspartic (D) and glutamic (E) acids of virion coat proteins can be activated with a carbodiimide crosslinker [e.g., EDC (1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride)]. It is also possible to incorporate a methionine analog, L-azidohomoalanine (Urquhart et al., 2016), in strains of Escherichia coli that are auxotrophic for methionine and produce virions displaying hundreds of azide groups for downstream chemical conjugation.

Peptides and proteins that have been displayed on the surface of virions have also been the target of chemical or enzymatic modification. One can engineer a free cysteine (Junutula et al., 2008) for bioconjugation or, alternatively, disulfides in a displayed peptide, which can be reduced and then reacted with a cross-bridging molecule (i.e., linchpin), thereby creating macroyclic or bicyclic peptides for the purpose of discovering novel peptide ligands of target proteins (Ng and Derda, 2016; Doyle et al., 2017; Chen et al., 2021; Ekanayake et al., 2021). Tyrosine (Y) in pVIII can be selectively activated by laccase to produce a free radical species that can be conjugated to acrylates (Vignali et al., 2018). Virions that have been engineered to display the AviTag (Scholle et al., 2004) or sortase tag (Hess et al., 2012) can be biotinylated by BirA or ligated to a variety of labeled peptides with sortase, respectively. Promising directions in the future will be to exploit the toolbox of enzymes capable of protein ligation (Nuijens et al., 2019; Weeks and Wells, 2020) and the SpyTag/SpyCatcher system (Keeble and Howarth, 2020) to build novel virion structures.

Genetic methods have been separately applied to expanding the chemical space of virions. They are largely based on the pioneering work of Professor Peter Schultz’s research group (Liu et al., 1997; Xiao and Schultz, 2016; Young and Schultz, 2018) on recoding the amber codon (TAG) so that it is recognized by a mutant, suppressor tRNA molecule that is charged with an non-canonical amino acid (ncAA). Figure 2 illustrates the basics of tRNA suppression. One first uses molecular biology techniques to engineer a TAG mutation at a site in the coding region of a protein where the ncAA is desired. When the gene is transcribed, the UAG codon is recognized as a stop codon by the ribosome, thereby terminating translation and yielding a truncated protein; however, if a tRNA has an anti-codon (CUA) that can base pair with the UAG codon in an mRNA, the amino acid attached to the 3’ end of the tRNA will form a peptide bond with the nascent peptide in the ribosome, allowing translation to continue and yield a full-length protein. The introduced ncAA can be any that is compatible with charging by an aminoacyl-tRNA synthetase (aaRS). The amino acid pocket of the pyrrolysyl-tRNA synthetase has been shown to be remarkably malleable to engineering recognition and charging of its cognate tRNA with diverse ncAAs (Wan et al., 2014; Tharp et al., 2018). Stop codon suppression has also been achieved with opal (UGA) (Anderson and Schultz, 2003) and ochre (UAA) (Italia et al., 2019) codons. In addition to suppression of stop codons, an analogous method with quadruplet codons (Magliery et al., 2001; Anderson and Schultz, 2003; Anderson et al., 2004; Neumann et al., 2010; Niu et al., 2013; Wang et al., 2014) has been developed (Figure 2). Mutations in both the aaRS and tRNA, as well as the use of engineered bacterial hosts (Chatterjee et al., 2014), have enhanced suppression efficiency of a variety of quadruplet codons (Guo and Niu, 2022). To date, >200 different ncAAs have been introduced into different proteins by this technique (Xiao and Schultz, 2016), which has been termed Genetic Code Expansion (GCE). Some of GCE’s applications have been to probe the structure and function of proteins, alter their redox potential, introduce fluorophores, infrared, and spin label probes, encode post-translational modifications, and create sites for site-specific bioconjugation. Several recent reviews of how GCE has been used to modify proteins expressed in bacteria, yeast, and mammalian cells can be found elsewhere (Young and Schultz, 2018; Chung et al., 2020; Nikić-Spiegel, 2020; Manandhar et al., 2021; Ros et al., 2021; Shandell et al., 2021; Sanders et al., 2022).

GCE has been applied to phage-display. Figure 3 shows an E. coli cell containing three circular genomes: a plasmid encoding the orthogonal tRNA and cognate aaRS, a phagemid that carries a truncated form of capsid protein III (pIII), which is fused to the coding region of the displayed peptide or protein containing the suppressible amber codon, and an M13 helper virus. The helper virus encodes 10 proteins necessary for viral replication and assembly; it also contains a mutation that leads to preferential packaging of the phagemid genome over the helper virus genome. Secreter virions will display the recombinant peptide or protein and ncAA, only if suppression is successful. The culture medium is supplemented with the ncAA, where it enters the cell and is used by the engineered aaRS to
FIGURE 1
Cartoon of an M13 bacteriophage virion, three types of chemically reactive groups, and primary structure of the major capsid protein, pVIII. Each virus particle (A) is 900 nanometers long and 7 nanometers wide and contains one single-stranded, circular DNA molecule, and five copies of pIX (yellow), pVII (white), pIII (purple), and pVI (pink), and ∼2,700 copies of pVIII (blue). Chemical groups that are available for conjugation include the \( \alpha \)-amino groups on the N-terminus and \( \varepsilon \)-amino groups of lysines (K) of pVIII, the carboxylate groups at the C-terminus and aspartic (D) and glutamic (E) acids, and the phenol groups of tyrosine (Y) are shown in violet, orange, and fushia, respectively. The primary structure for mature pVIII (B) is shown with the N-terminus, C-terminus, and residues theoretically capable of being chemically modified highlighted in color.

It should be noted that the lysines in the anchoring region (KLFKKFTSKAS) of pVIII may not be sterically accessible for chemical modification.

FIGURE 2
Insertion of ncAAs into peptides or proteins by GCE. (A) An mRNA containing an engineered quadruplet or stop codon for insertion of an ncAA at a designated location in a protein. During translation, the ribosome (purple) decodes the engineered stop codon (B) or quadruplet codon (C) with a tRNA charged with the ncAA (blue). The tRNA anticodon is shown in bold and the growing polypeptide chain is shown in green. (D) Peptide or protein with an ncAA at the desired location.
FIGURE 3
Incorporation of ncAAs into phage-displayed constructs. (A) E. coli cell showing three genomes: phagemid with suppressible mutation (TAG, blue) in the coding region (green) of a protein fused to a truncated form of capsid protein pIII (purple), a plasmid encoding an orthogonal aaRS/tRNA$^{CUA}$ pair (orange/red), and an M13 helper virus (i.e., K07) encoding full length pIII (black). Each of these genomes carry a different antibiotic resistance marker to allow for selective growth of bacterial cells containing all three. (B) Virions secreted from such cells contain both wild type (black) and recombinant (purple) pIII capsid proteins, the latter of which display the protein of interest (green) incorporating the desired ncAA (blue).

charge the suppressor tRNA. Table 1 lists some examples of publications describing the incorporation of ncAAs into virions, which are also summarized in more detail below.

The earliest experiments of engineered replacement of amino acids with M13 bacteriophage took advantage of its lifecycle properties. Scientists at New England Biolabs inserted the opal (TGA) stop codon and a downstream selenocysteine insertion sequence (SECIS) upstream of the signal sequence in pIII (Sandman and Noren, 2000). Viral secretion was dependent on the addition of selenium to the culture medium and the incorporation of selenocysteine was confirmed by chemical reactivity. The efficiency of incorporation was influenced by the choice of nucleotide downstream of the opal codon, although the frequency of TGG revertants, encoding tryptophan, was high. Later, the authors (Beech et al., 2015) utilized the selenocysteine-displaying virions to chemically attach five different adenosine receptor ligands and successfully demonstrated that the pentavalently decorated virions could activate the adenosine A1 receptor, a G protein-coupled receptor (GPCR), of cultured cells. An early example of utilizing stop codon suppression to control the replacement of an amino acid was that of the Schultz group (Pastrnak and Schultz, 2001). They introduced an amber codon in lieu of the asparagine (N) in a 10-mer peptide sequence (PASTTNKDKL) at the N-terminus of pIII of a phage genome. Virions were propagated in an E. coli strain that carried the supE mutation, which encodes a suppressor tRNA that is charged with asparagine. The virions were then used to infect a second bacterial strain that carried a plasmid encoding a yeast tRNA and its cognate aaRS that inserted N into the 10-mer peptide. Only virions secreted by this strain bound well to a monoclonal antibody that had been generated against the 10-mer peptide, demonstrating successful suppression of the stop codon. This proof-of-concept experiment set the stage for engineering aaRSs that could charge their cognate tRNA with ncAAs and suppress amber codons inserted into pIII of virions. Phage-assisted continuous evolution (PACE), phage-assisted non-continuous evolution (PANCE), and phage- and robotic-assisted near-continuous evolution (PRANCE) have enabled rapid laboratory evolution of orthogonal aaRSs over hundreds of generations of mutation, selection, and replication (Eswell et al., 2011; Bryson et al., 2017; Miller et al., 2020; DeBenedictis et al., 2021, 2022; Fischer et al., 2022). As coat protein pIII is essential for phage, this selection scheme directly links the production and titer of virions with the efficiency of stop codon suppression and incorporation of the desired ncAA in a stop codon inserted into gene III.

The Schultz group was among the first to incorporate ncAAs into displayed peptides and proteins. A variety of ncAAs have been incorporated into peptides permitting bioconjugation (Tian et al., 2004), binding to metal ions (Day et al., 2013), and replacing Zn(II) with Fe(II) for DNA binding domains (Kang et al., 2014). Non-canonical amino acids have also been incorporated into human single-chain variable fragments (scFvs) displayed on virions. In fact, a sulfitotyrosine has been incorporated into an scFv that contributed to binding to HIV glycoprotein 120 (Liu et al., 2008). The electrophilic
TABLE 1 Examples of GCE involving M13 virions in the literature.

| Peptide or protein displayed on M13 virions | ncAA incorporated through GCE | Outcome | Reference |
|-------------------------------------------|-------------------------------|---------|-----------|
| Combinatorial peptide library             | Selenocysteine                | Proof-of-principle experiment | Sandman and Noren, 2000 |
| 10-mer peptide epitope                    | Gln with Asp                  | Directed evolution of aminocyl-tRNA synthetase | Pastinka and Schultz, 2001 |
| Short peptide                             | O-methyl-tyrosine             | Proof-of-principle experiments demonstrated fluorescence labeling | Tian et al., 2004 |
| scFv (TAG codon at position 111 of V\_H) CDR3 | Sulfotyrosine                 | Affinity selection of a sulfotyrosine-containing antibodies that can bind to gp120 | Liu et al., 2008 |
| scFv with six random NNK codons in V\_H CDR3 | p-boronophenylanline          | Affinity selection of an scFv capable of forming a covalent bond to a sugar | Liu et al., 2009 |
| CX\_6 Z, where X = NNK                    | Bipyridylalanine              | Affinity selection of cyclic Ni\^{2+} and Zn\^{2+} binding peptides | Day et al., 2013 |
| Five residues in the N-terminal finger of zf268 were randomized to include both canonical amino acids and p-Bpy-Ala | (2,2'-bipyridin-5-yl)alanine (Bpy-Ala) | DNA and Fe(III) binding domain | Kang et al., 2014 |
| CX\_6 Z                                  | N\(^{+}\) acryloyl-lysine      | Affinity selection of cyclic peptide ligands and inhibitor for TEV protease | Wang et al., 2019 |
| CX\_6 Z                                  | N\(^{+}\)-[(2-methylcyclopent-2-ene-1-
| | yl)methyl]carbonyl]-1-lysine       | Dual labeling of scFv on virions | Offer-Salvia and Chin, 2019 |
| CX\_6 Z                                  | four phenylalanine derivatives | Affinity selection of cyclic peptide ligands and inhibitors for Sirtuin 2 and TEV protease | Tharp et al., 2020 |
| ZxeC                                     | O-(2-bromoethyl)-tyrosine      | Affinity selection of cyclic peptide ligands to streptavidin, Kelch-like ECH-associated protein 1 (Keap1), and Sonic Hedgehog (Shh) | Owens et al., 2020 |

C = cysteine.  
X = NNK codons, where N is A, C, G, or T and K is G or T.  
Z = ncAA.

Recently, GCE has also been used to incorporate ncAAs for the purpose of cyclizing phage-displayed peptides. This effort represents an alternative route for cyclizing phage-displayed peptides to generate macrocycles with novel structures and the potential to inhibit protein-protein interactions in therapeutic applications (Deyle et al., 2017). In the original approach (Heinis et al., 2009), a library of peptides with three fixed cysteines was reduced and then reacted with a trifunctional compound, termed a “linchpin,” thereby creating bicyclic peptides. GCE has been used for the same purpose: researchers have built libraries of virions displaying CX\_6 Z (Wang et al., 2019; Tharp et al., 2020) and ZxeC (Owens et al., 2020), where X represents an amino acid encoded by NNK codons and Z is an ncAA encoded by the TAG codon that is capable of reacting with the adjacent cysteine residue to form a macrocycle (Figure 4B). These libraries were screened by affinity selection and yielded nanomolar inhibitors of Tobacco Etch Virus (TEV) protease (Wang et al., 2019), Sirt2 (Tharp et al., 2020), and Kelch-like ECH-associated protein 1 (Keap1) and Sonic Hedgehog (Shh) (Owens et al., 2020).
cylcined peptides all showed stronger affinity to their targets than their linear counterparts. It will be exciting to see how this approach unfolds over time. Perhaps covalent inhibitors can be discovered by incorporating ncAAs capable of cross-linking with a target as reported elsewhere (Chen et al., 2021).

GCE has been used to site-specifically dual-fluorophore-label proteins displayed on the surface of M13 virions (Figure 4C). Suppression of amber and quadruplet codons facilitated the insertion of Nε-[(2-methylcycloprop-2-en-1-yl)methoxy]carbonyl]-l-lysine (CypK) and p-propargyloxy-l-phenyl-alanine (PrpF) into separate sites of a phage-displayed anti-Her2 scFv (Oller-Salvia and Chin, 2019). The orthogonal Methanococcus janaschii (Mj) tyrosyl-tRNA synthetase (MjTyrRs)/tRNA_{CUA} pair was used to incorporate PrpF, which was labeled with an azide-fluorophore. The pyrrolysyl-tRNA synthetase (PylRS)/tRNA orthogonal pair was used to incorporate CypK, which was labeled with a tetrazine-fluorophore. By exploring a variety of variables, the authors were able to achieve near wild-type display levels of the scFv for dual labeled, phage-displayed proteins. This system allows for mutually orthogonal and site-specific, dual-labeling of a protein in a one-pot reaction.
Two other recent publications of note describe the incorporation of an ncAA into the target of an affinity selection experiment (Figure 5A). Human interleukin-1β (IL-1β) and complement 5a (C5a) proteins were prepared in bacterial cells with p-benzoyl-L-phenylalanine utilizing GCE and used to affinity select a phage-display scFv library (Chen et al., 2020). By cross-linking virions to the targets with ultraviolet (UV) irradiation and washing away non-covalently bound virions with pH 2.0 glycine, the bound virions were selectively recovered by trypsin digestion. Over one-third of the recovered scFvs could bind to the targets without cross-linking and their affinities could be improved by mutagenesis. Thus, GCE can be used to steer production of antibodies to epitopes of interest. In a related approach, the 3-nitrotyrosine (nitroTyr) modified form (nY133) of the 14-3-3 signaling protein was used to immunize alpacas and two nanobodies that recognize this post-translational modification at position 133 were recovered by phage-display (Van Fossen et al., 2022).

Perspectives

Several challenges and opportunities remain for optimizing GCE for phage-display. First, in order to incorporate a particular ncAA, the ncAA or its precursor must be supplied to the culture medium for it to cross the bacterial cell membrane where it can cross the cell membrane and be used by an aaRS to charge the suppressor tRNA. This challenge may be solved through the use of mutated strains, the expression of exogenous transporters, and the design of gene clusters capable of cellular synthesis of certain ncAAs. Second the use of bacterial strains that lack release factor 1 (RF-1) (Lajoie et al., 2013; Mukai et al., 2015; Fredens et al., 2019) will suppress TAG codons more efficiently.

One might ask, what are some possible experiments that can be accomplished by combining GCE and phage-display? An example that comes to mind is the production of phage-displayed combinatorial peptide libraries that carry phosphoserine (Park et al., 2011; Zhu et al., 2021),...
phosphothreonine (Zhang et al., 2017), or phosphotyrosine (Hoppmann et al., 2017; Loo et al., 2017). Such libraries could then be used to define the specificity of antibodies generated to phosphopeptides (Mandell, 2003). It might even be possible to use this general approach to generate recombinant proteins that bind other post-translational modifications. Another application might be to generate recombinant proteins that bear a phosphorylated amino acid at a position found in cellular proteins and then generate recombinant affinity reagents that recognize the folded, phosphorylated target (Figure 5B). Thus, it may even be possible to use this general approach to generate recombinant antibodies to many of the site-specific post-translational modifications of interest to cell biologists and biochemists. Finally, it is likely that chemically-and genetically-modified virions will prove beneficial in such emerging applications of phage in drug delivery (Karimi et al., 2016), immune- oncology (Foglizzo and Marchió, 2021), synthetic biology (Lemire et al., 2018), tissue regeneration (Cao et al., 2019), and vaccines (Henry et al., 2015; González-Mora et al., 2020).

Author contributions

AG and GA prepared the figures. All the authors contributed to the writing or editing the manuscript.

Funding

Funding was provided by grant 1 R43 GM146514-01 from the National Institutes of Health.

Conflict of interest

GA, AG, and BK were employed of a biotech start-up company (Tango Biosciences, Inc.).

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher’s note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

Adhikari, M., Dhamane, S., Hagstrom, A. E., Garvey, G., Chen, W. H., Kourrentzi, K., et al. (2013). Functionalized viral nanoparticles as ultrasensitive reporters in lateral-flow assays. Analyst 138, 5584–5587. doi: 10.1039/c3an0891f

Adhikari, M., Strych, U., Kim, J., Goux, H., Dhamane, S., Poongavanam, M. V., et al. (2015). Aptamer phage reporters for ultrasensitive lateral flow assays. Analy. Chem. 87, 11660–11665. doi: 10.1021/acs.analchem.5b00702

Anderson, J. C., and Schultz, P. G. (2003). Adaptation of an orthogonal archaean leucyl-tRNA and synthetase pair for four-base, amber, and opal suppression. Biochemistry 42, 9598–9608. doi: 10.1021/bi034550w

Anderson, J. C., Wu, N., Santoro, S. W., Lakshman, V., King, D. S., and Schultz, P. G. (2004). An expanded genetic code with a functional quadruplet codon. Proc. Natl. Acad. Sci. U S A 101, 7566–7571.

Beech, J., Saleh, L., Frentrel, J., Figler, H., Corrêa, I. R., Baker, B., et al. (2015). Multivalent site-specific phage modification enhances the binding affinity of receptor ligands. Bioconjug. Chem. 26, 529–536. doi: 10.1021/bc500811w

Bryson, D. I., Fan, C., Guo, L. T., Miller, C., Soll, D., and Liu, D. R. (2017). Continuous directed evolution of aminocyl-tRNA synthetases. Nat. Chem. Biol. 13, 1253–1260.

Cao, B., Li, Y., Yang, T., Bao, Q., Yang, M., and Mao, C. (2019). Bacteriophage-based biomaterials for tissue regeneration. Adv. Drug Del. Rev. 145, 73–95. doi: 10.1016/j.addr.2018.11.004

Carmody, C. M., Goddard, J. M., and Nugen, S. R. (2021). Bacteriophage Capsid Modification by Genetic and Chemical Methods. Bioconjug. Chem. 32, 486–481.

Chatterjee, A., Lajoie, M. J., Xiao, H., Church, G. M., and Schultz, P. G. (2014). A bacterial strain with a unique quadruplet codon specifying non-native amino acids. ChemBioChem 15, 1782–1786. doi: 10.1002/cbic.201402104

Chen, L., Zhu, C., Guo, H., Li, R., Zhang, L., Xing, Z., et al. (2020). Epitope-directed antibody selection by site-specific photocrosslinking. Sci. Adv. 6.eaaz7825. doi: 10.1126/sciadv.aaz7825

Chen, S., Lovell, S., Lee, S., Fellner, M., Mace, P. D., and Bogyn, M. (2021). Identification of highly selective covalent inhibitors by phage display. Nat. Biotechnol. 39, 490–498. doi: 10.1038/s41587-020-0733-7

Chung, C. Z., Amikura, K., and Soll, D. (2020). Using genetic code expansion for protein biochemical studies. Front. Bioeng. Biotechnol. 8:598577.

Day, J. W., Kim, C. H., Smider, V. V., and Schultz, P. G. (2013). Identification of metal ion binding peptides containing unnatural amino acids by phage display. Bioorg. Med. Chem. Lett. 23, 2598–2600. doi: 10.1016/j.bmcl.2013.02.106
Dellenedictis, E. A., Carver, G. D., Chung, C. Z., Soll, D., and Badran, A. H. (2021). Multiplex suppression of four quadruplet codons via rRNA directed evolution. Nat. Commun. 12, 5706. doi: 10.1038/s41467-021-25948-y

Dellenedictis, E. A., Chory, E. J., Gretton, D. W., Wang, B., Golas, S., and Esvelt, K. M. (2022). Systematic molecular evolution enables robust biomolecule discovery. Nat. Methods 19, 55–64. doi: 10.1038/s41592-021-01348-4

Deyle, K., Kong, X. D., and Heinis, C. (2017). Phage selection of cyclic peptides for application in research and drug development. Acc. Chem. Res. 50, 1866–1874.

Domaillé, D. W., Lee, J. H., and Cha, J. N. (2015). High density DNA loading on the M13 bacteriophage provides access to colorimetric and fluorescent protein microarray biosensors. Chem. Commun. 49, 1759–1761. doi: 10.1039/c3cc38871a

Ekanayake, A. I., Sobie, L., Kelich, P., Youk, J., Bennett, N. J., Mukherjee, R., et al. (2021). Genetically encoded fragment-based discovery from phage-displayed macrocyclic libraries with genetically encoded unnatural phosphorothioates. J. Am. Chem. Soc. 143, 5497–5507. doi: 10.1021/jacs.0c11866

Esvelt, K. M., Carlson, J. C., and Liu, D. R. (2011). A system for the continuous directed evolution of biomolecules. Nature 472, 499–503.

Fischer, J. T., Soll, D., and Tharp, J. M. (2022). Directed evolution of methanomethylophilus albus Pyruvyl-srRNA synthetase generates a hyperactive and highly selective variant. Front. Mol. Biol. 9:850613. doi: 10.3389/fmolb.2022.850613

Fogliasso, V., and Marchio, S. (2021). Bacteriophages as Therapeutic and Diagnostic Vehicles in Cancer. Pharmaceuticals 14:161.

Fredj, W., Wang, K., de la Torre, D., Funke, L. F. H., Robertson, W. E., Christova, V., et al. (2019). Total synthesis of Escherichia coli with a recorded genome. Nature 569, 514–518.

Gonzelez-Mora, A., Hernandez-Perez, J., Iqbal, H. M. N., Rito-Palmares, M., and Benavides, J. (2020). Bacteriophage-based vaccines: a potent approach for antigen delivery. Vaccines 8:504. doi: 10.3390/vaccines8030504

Guo, J., and Niu, W. (2022). Genetic code expansion through quadruplet codon decoding. J. Mol. Biol. 434, 175346.

Hagstrom, A. E., Garver, G., Paterson, A. S., Dhamane, S., Adhikari, M., Estes, M. K., et al. (2015). Sensitive detection of norovirus using phage nanoparticle reporters in lateral-flow assay. PLoS One 10:e0126571. doi: 10.1371/journal.pone.0126571

Heng, C., Rutherford, T., Freund, S., and Winter, G. (2009). Phage-encoded combinatorial chemical libraries based on bicyclic peptidic acids. Nat. Chem. Biol. 5, 502–507.

Henry, K. A., Arbab-Ghahroudi, M., and Scott, J. K. (2015). Beyond phage display: non-traditional applications of the filamentous bacteriophage as a vaccine carrier, therapeutic biologic, and bioconjugation scaffold. Front. Microbiol. 6:755. doi: 10.3389/fmicb.2015.00755

Hess, G. T., Cragnolini, J. J., Popp, M. W., Allen, M. A., Dougan, S. K., Spooner, E., et al. (2012). M13 bacteriophage display framework that allows sortase-mediated construction of surface-accessible phage proteins. Biocconjug. Chem. 23, 1478–1487. doi: 10.1021/bc300130z

Hoppmann, C., Wong, A., Yang, B., Li, S., Hunter, T., Shokat, K. M., et al. (2017). Power to the protein: enhancing and combining activities using the Spy toolbox. Nat. Chem. Biol. 13, 845–849. doi: 10.1038/nchembio.2405

Hagiwara, K., et al. (2015). Highly reproductive non-continuous evolution. Nat. Protoc. 10, 1613–1627.

Hagiwara, K., et al. (2015). Evolution of proteins with genetically encoded “chemical warheads”. J. Am. Chem. Soc. 137, 9616–9617. doi: 10.1021/jacs.5b04225

Liang, X., and Van Doren, S. R. (2008). Mechanistic insights into phosphoprotein-binding FHA domains. Acc. Chem. Res. 41, 991–999. doi: 10.1021/ar700148u

Luo, X., Fu, G., Wang, R. E., Zhu, X., Zambaldo, C., Liu, R., et al. (2017). Genetically encoding phosphorothioine and its nonhydrolyzable analog in bacteria. Nat. Chem. Biol. 13, 845–849. doi: 10.1038/nchembio.2405

Lemire, S., Yehl, K. M., and Lu, T. K. (2018). Phage-based applications in synthetic biology. Annu. Rev. Virol. 5, 453–476.

Lee, Y., Ch., Kim, W., Kang, K., Yun, D. S., Strano, M. S., et al. (2009). Fabricating genetically engineered high-power lithium-ion batteries using virus microarrays. Science 324, 1051–1055. doi: 10.1126/science.1171541

Mohan, K., and Weiss, G. A. (2016). Chemically modifying viruses for diverse applications. J. Antimicrob. Chemother. 71, 2224–2233.

Mohan, K., and Weiss, G. A. (2016). Chemically modifying viruses for diverse applications. J. Antimicrob. Chemother. 71, 2224–2233.

Ng, S., and Derda, R. (2016). Phage-displayed macrocyclic glycopeptide libraries. Bioconjug. Chem. 27, 553–567. doi: 10.1021/bc500838h

Niu, W., Schulz, P. G., and Guo, J. (2013). An expanded genetic code in mammalian cells with no specific assignment for stop codons. J. Mol. Biol. 425, 9616–9617. doi: 10.1016/j.jmb.2013.05.018

Niu, W., Schulz, P. G., and Guo, J. (2013). An expanded genetic code in mammalian cells with no specific assignment for stop codons. J. Mol. Biol. 425, 9616–9617. doi: 10.1016/j.jmb.2013.05.018

Oliveira-Salvia, B., and Chin, J. W. (2019). Efficient phage display with multiple distinct non-canonical amino acids using orthogonal ribosome-mediated genetic code expansion. Angew. Chem. Int. Ed. Engl. 58, 10844–10848. doi: 10.1002/anie.201902658

Kim, J., Vu, B., Kousrentzi, K., Willson, R. C., and Conrad, J. C. (2017). Increasing binding efficiency via reporter shape and flux in a viral nanoparticle lateral-flow assay. ACS Appl. Mater. Interfaces 9, 6879–6884. doi: 10.1021/acsami.6b05728

Lajoie, M. J., Roivner, A. J., Goodman, D. B., Aerni, H. R., Haimovich, A. D., Kuznetsov, G., et al. (2013). Genetically reprogrammed organisms expand biological function. Science 342, 357–360.

Lee, J. Y., H., Kim, W., Kang, K., Yun, D. S., Strano, M. S., et al. (2009). Fabricating genetically engineered high-power lithium-ion batteries using virus microarrays. Science 324, 1051–1055. doi: 10.1126/science.1171541
Owens, A. E., Iannuzzelli, J. A., Gu, Y., and Fasan, R. (2020). MOriPH-PhD: an integrated phage display platform for the discovery of functional genetically encoded peptide macrocycles. ACS Cent. Sci. 6, 368–381.

Park, H. S., Hohn, M. J., Umehara, T., Guo, L. T., Osborne, E. M., Benner, J., et al. (2011). Expanding the genetic code of Escherichia coli with phosphoserine. Science 333, 1151–1154.

Pastnak, M., and Schultz, P. G. (2001). Phage selection for site-specific incorporation of unnatural amino acids into proteins in vivo. Bioorg. Med. Chem. 9, 2377–2379. doi: 10.1016/s0968-0896(01)00157-2

Petrenko, V. A. (2018). Landscape phage: evolution from phage display to nanobiotechnology. Viruses 10:E311.

Qin, X., and Liu, T. (2022). Recent advances in genetic code expansion techniques for protein phosphorylation studies. J. Mol. Biol. 434:167406.

Ros, E., Torres, A. G., Ribas, and de Pouplana, L. (2021). Learning from nature to expand the genetic code. Trends Biotechnol. 39, 460–473.

Sanders, J., Hoffmann, S. A., Green, A. P., and Cai, Y. (2022). New opportunities for genetic code expansion in synthetic yeast. Curr. Opin. Biotechnol. 75:102691. doi: 10.1016/j.copbio.2022.102691

Sandman, K. E., and Noren, C. J. (2000). The efficiency of Escherichia coli selenocysteine insertion is influenced by the immediate downstream nucleotide. Nucleic Acids Res. 28, 755–761. doi: 10.1093/nar/28.3.755

Scholle, M. D., Collart, F. R., and Kay, B. K. (2004). In vivo biotinylated proteins as targets for phage-display selection experiments. Protein Expr. Purif. 37, 243–252.

Shandell, M. A., Tan, Z., and Cornish, V. W. (2021). Genetic code expansion: a brief history and perspective. Biochemistry 60, 3455–3469. doi: 10.1021/acs.biochem.1c00286

Smith, G. P. (1985). Filamentous fusion phage: novel expression vectors that display cloned antigens on the virion surface. Science 228, 1315–1317. doi: 10.1126/science.4001944

Smith, G. P. (2019). Phage display: simple evolution in a petri dish (noble Lecture). Angew. Chem. Int. Ed. Engl. 58, 14428–14437. doi: 10.1002/anie.201903808

Tharp, J. M., Ehnborn, A., and Liu, W. R. (2018). tRNA.SS. Structure, function, and applications. RNA Biol. 15, 441–452. doi: 10.1080/15476286.2017.1356561

Tharp, J. M., Hampton, J. T., Reed, C. A., Ehnborn, A., Chen, P. C., Morse, J. S., et al. (2020). An amber obligate active site-directed ligand evolution technique for phage display. Nat. Commun. 11:1392. doi: 10.1038/s41467-020-15057-7

Tian, F., Tao, M. L., and Schultz, P. G. (2004). A phage display system with unnatural amino acids. J. Am. Chem. Soc. 126, 15962–15963

Ullo, L., Cantelli, A., Petrosino, A., Costantini, P. E., Negro, M., Starmieri, F., et al. (2022). Orthogonal nanoarchiteconics of M13 phage for receptor targeted anticancer photodynamic therapy. Nanoscale 14, 632–641. doi: 10.1039/d1nr06053h

Urquhart, T., Daub, E., and Honek, J. F. (2016). Bioorthogonal modification of the major sheath protein of bacteriophage M13: extending the versatility of biomolecular scaffolds. Bioconjug. Chem. 27, 2276–2280. doi: 10.1021/acs.bioconjchem.6b00460

Van Fossen, E. M., Grutzius, S., Ruby, C. E., Mourich, D. V., Cebra, C., Bracha, S., et al. (2022). Creating a Selective Nanobody Against 3-Nitrotyrosine Containing Proteins. Front. Chem. 10:835229. doi: 10.3389/fchem.2022.835229

Vignali, V., Miranda, S., Lodoso-Torrecilla, L., van Nisselroy, C. A. J., Hoogenberg, B. J., Dantuma, S., et al. (2018). Biocatalytically induced surface modification of the tobacco mosaic virus and the bacteriophage M13. Chem. Commun. 55, 51–54. doi: 10.1039/c8cc08042a

Wan, W., Tharp, J. M., and Liu, W. R. (2014). Pyrrolyl-tRNA synthetase: an ordinary enzyme but an outstanding genetic code expansion tool. Biochim. Biophys. Acta 1844, 1059–1070. doi: 10.1016/j.bbapap.2014.03.002

Wang, K., Sachddra, A., Cox, D. J., Wilf, N. M., Lang, K., Wallace, S., et al. (2014). Optimized orthogonal translation of unnatural amino acids enables spontaneous protein double-labelling and FRET. Nat. Chem. 6, 393–403. doi: 10.1038/nchem.1919

Wang, X. S., Chen, P. C., Hampton, J. T., Tharp, J. M., Reed, C. A., Das, S. K., et al. (2019). A genetically encoded, phage-displayed cyclic peptide library. Angew. Chem. Int. Ed. Engl. 58, 15904–15909.

Weeks, A. M., and Wells, J. A. (2020). Subtiligase-catalyzed peptide ligation. Chem. Rev. 120, 3127–3160.

Xiao, H., and Schultz, P. G. (2016). At the interface of chemical and biological synthesis: an expanded genetic code. Cold Spring Harb. Perspect. Biol. 8:a023945.

Yaffe, M. B., and Elia, A. E. (2001). Phosphoserine/threonine-binding domains. Adv. Enzyme Regul. 39, 301–346.

Young, D. D., and Schultz, P. G. (2018). Playing with the molecules of Life. ACS Chem. Biol. 13, 854–870.

Zhang, M. S., Brunner, S. F., Huguenin-Desot, N., Liang, A. D., Schmied, W. H., Rogerson, D. T. et al. (2017). Biosynthesis and genetic encoding of phosphothreonine through parallel selection and deep sequencing. Nat. Methods 14, 729–736. doi: 10.1038/nmeth.4302

Zhu, P., Franklin, R., Vogel, A., Stanishevski, S., Reardon, P., Sluchanko, N. N., et al. (2021). PermaPhos: a selective PhoP-ChIP chromatin-remodelling system for the visualization of phosphothreonine-containing proteins. bioRxiv 2021:465468. doi: 10.1101/2021.10.22.65468