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Bacteriophage Capsid Modification by Genetic and Chemical Methods

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Abstract

Bacteriophages are viruses whose ubiquity in nature and remarkable specificity to their host bacteria enable an impressive and growing field of tunable biotechnologies in agriculture and public health. Bacteriophage capsids, which house and protect their nucleic acids, have been modified with a range of functionalities (e.g. fluorophores, nanoparticles, antigens, drugs) to suit their final application. Functional groups naturally present on bacteriophage capsids can be used for electrostatic adsorption or bioconjugation but their impermanence and poor specificity can lead to inconsistencies in coverage and function. To overcome these limitations, researchers have explored both genetic and chemical modifications to enable strong, specific bonds between phage capsids and their target conjugates. Genetic modification methods involve introducing genes for alternative amino acids, peptides, or protein sequences into either the bacteriophage genomes or capsid genes on host plasmids to facilitate recombinant phage generation. Chemical modification methods rely on reacting functional groups present on the capsid with activated conjugates under the appropriate solution pH and salt conditions. This review surveys the current state-of-the-art in both genetic and chemical bacteriophage capsid modification methodologies, identifies major strengths and weaknesses of methods, and discusses areas of research needed to propel bacteriophage technology in development of biosensors, vaccines, therapeutics, and nanocarriers.

Keywords

Bacteriophage; Genetic Engineering; Virus Bioconjugation; Capsid; Synthetic Biology

1. INTRODUCTION

Bacteriophages (phages) have played an instrumental role in biotechnology since their discovery in the late 1890's, including being utilized for antibacterial therapy, vaccines, and gene delivery vehicles.[1,2] Phages are viruses that infect bacteria within a typically narrow host range, and are non-infectious towards non-bacterial cells. Following recognition of their host and injection of their genetic material, phages take over the host bacterium's cellular machinery to propagate tens to thousands of more phages, lysing the host cell to release newly synthesized phage progeny into the environment. Due to this remarkable orders-of-

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magnitude replication cycle, phages are highly abundant (in fact, are reported to be the most abundant organism on Earth)[3] and have adapted to survive in a variety of environments across the globe including marine waters,[4–6] human bodies,[7–9] and diverse soils.[10–12] Phages can be propagated with standard lab glassware and equipment or in bioreactors for large-scale production.[13–15]

Wild type phages are employed as therapeutics, bioreceptors, and biocontrol agents. Several recent cases have demonstrated that patients infected with multi-drug resistant bacteria including *Staphylococcus aureus*,[16–18] *Acinetobacter baumannii*,[19–21] and *Pseudomonas aeruginosa*[22–24] have successfully recovered after phage administration. Commercial phage preparations can be applied to food products and contact surfaces to help control contamination by foodborne pathogens.[25–27] Biosensors relying on phages as bacteria biorecognition elements have allowed for the rapid detection of pathogens such as *Salmonella* Typhimurium and *Staphylococcus aureus*.[28,29]

Although phages offer several benefits, biological limitations of phage structural features prevent permanent evasion from mammalian immune systems, bacterial phage resistance, and non-specific immobilization. Advances in bioinformatics,[30–32] microscopy imaging,[33–35] and genetic engineering[36–38] have created new opportunities for modifying phage structural features to overcome some limitations of current phage-based biotechnologies. Capsids are highly ordered proteinaceous structures utilized by phages to protect their genomes. Phages with the most frequently identified capsid shapes, filamentous and icosahedral,[39] have been modified using genetic and chemical methods to functionalize phages with an array of organic and inorganic materials. For phage therapy applications, capsid modifications with polyethylene glycol have increased phages' bloodstream half-life *in vivo*.[40] To generate phage activated materials, site-specific capsid modifications have facilitated binding interactions with silica, cellulose, and magnetic particles to improve immobilized phages' orientation, stability, and density.[41–43] The potential of phage-based biotechnologies have expanded through the use of non-native proteins displayed on capsids to include applications of cell differentiation scaffolds,[44–48] targeted imaging platforms,[49–51] vaccines,[52–54] and nanocarriers.[55–57] This review adds to the excellent reports of Büning[58] and Xu[59] which largely focus on adenovirus capsid modification, where here advances in bacteriophage capsid engineering are highlighted. These genetic and chemical methods for modifying phage capsids can be applied to create a variety of phage-based biotechnologies including low-cost diagnostics, gene therapies, cell differentiation scaffolds, or vaccines.

2. CAPSIDS OVERVIEW

Capsids encapsulate the tightly packed nucleic acids of the phage. Icosahedral capsid geometries having 20 triangular faces are the most frequently observed phage capsid structures.[39] These capsids are typically composed of high numbers of repeating structures of a relatively few protein types. The triangulation number of the icosahedral capsid's central body and two caps can be used to further classify the capsid as the uniform isometric shape or the elongated prolate form.[60] These capsids vary in complexity and stability depending on the capsid subunits' composition (pentameric or hexameric), copy number, and organization.[61–63] Most icosahedral phages assemble utilizing scaffolding domains or

proteins to aid in proper formation of a stable procapsid shell intermediate which are subsequently removed from the mature capsid before or during genome packaging.[64–66] During the capsid maturation process, some phage capsids expand to reveal binding sites for decoration proteins[67] that provide a fitness advantage such as increased capsid stability.[68–71] Mature capsids range in size from 43 to 160 nm in diameter.[72,73] The spherical-like shape of these capsids has been utilized for protected delivery of therapeutic and imaging agents through internal modification of empty capsids.[55,74–78]

Filamentous phages from the *Inoviridae* family have a rod like capsid shape that can be 800-2,000 nm long and 6.5-7.5nm in diameter.[79] Filamentous capsids have a helical array which can be further identified as class I or class II based on having five-fold or one-fold rotational symmetry.[80,81] These capsids are constructed by anchoring all five structural proteins to the inner membrane of the bacteria host and assembling the capsid around the genome as it is translocated into the inner membrane.[82] The length of the capsid is dictated by the genome size and can be changed by adjusting the genome length.[83,84] High density display of foreign molecules has been achieved by modifying the major capsid protein of filamentous phages that is present in thousands of copies in the capsid.[85–87]

Some phages utilize lipid envelopes derived from the phospholipids of the bacteria host to aid in protecting their genome.[88–90] One such phage family which surround their capsids with an external lipid envelope is the *Cystoviridae*.[91] *Corticoviridae, Sphaerolipoviridae*, and *Tectiviridae* phages contain a lipid membrane surrounding the genome on the inside of their capsid.[92–94] To date, the *Plasmaviridae* phage MVL2 is the only phage to have a lipid membrane as the sole protection surrounding its genome.[95] The structural characteristics of lipid-containing phages have enabled them to be used as surrogates for enveloped pathogenic human viruses including Ebola virus, severe acute respiratory syndrome (SARS) coronavirus, and human immunodeficiency virus (HIV).[96–100]

Phages of all capsid types can be noncovalently immobilized to charged materials via electrostatic adsorption. Important to note is that solution conditions can affect bioconjugation by simple charge-charge interactions, with pH and salt concentration affecting the charge state of capsid protein amino acid residues.[101] Charged amino acid residues on phage capsids can be utilized to direct the orientation of phages during bioconjugation, with reported electrostatic adsorption onto modified silica,[102,103] cellulose,[104–106] and gold[103,107] to create bioactive materials. The charge of M13 phages has been exploited to create highly ordered phage monolayer films via electrostatic layer by layer technique.[108] To enhance phage adsorption capabilities, commonly used materials can be modified with charged functional groups including amines, carboxylic acids, and glycols to facilitate adhesion.[102,105,109,110] This immobilization method is simple, but the nonspecific nature can result in structural features crucial for infection to be interfered with during immobilization. An electric field can be incorporated into the process to properly orient phages during immobilization. This strategy has previously been used to increase the effect of charge during immobilization to direct bioconjugation of T4 phages by their negatively charged capsids during phage-based biosensor construction.[107,111]

3. GENETIC MODIFICATIONS

There are numerous genetic engineering approaches that can be used to modify phage capsid genes. Some methods rely on incorporation of the foreign DNA sequence into the phage genome by flanking it with DNA sequences identical to the desired insertion site in the genome. This “Donor DNA” construct creates a template that can seamlessly incorporate the foreign sequence into the phage genome via homologous recombination (Fig 1A). Recombination rates of phages with plasmid vectors containing a Donor DNA construct occur at very low frequencies ranging from 10^{-10} to 10^{-4}, [112–114] but increasing the length of the flanking DNA sequences identical to the insertion site can be used to increase recombination frequency.[115] Bacteria hosts used for genetically modified phage synthesis can be altered to increase recombinant phage generation. [116] The *in vivo* recombineering method utilizes an *Escherichia coli* strain containing an extra set of temperature inducible recombination genes to help facilitate homologous recombination.[117,118] This *E. coli* strain is infected with a wild type phage to deliver the phage genome into the cell, heated to induce recombination gene expression, and introduced with a single or double stranded Donor DNA construct that is delivered into the cell via electroporation, typically yielding 0.5-2% phage recombinants.[118] Bacteriophage recombineering of electroporated DNA (BRED) method utilizes a bacteria strain containing a plasmid encoding recombination genes that promote high levels of recombination.[116] A wild type phage genome and a double stranded Donor DNA construct are simultaneously delivered into the bacteria cell via electroporation which typically results in recombinant phage generation at a frequency of 10-15%.[116] Bacteria can be equipped with clustered regularly interspaced palindromic repeats (CRISPR) and CRISPR-associated proteins (Cas) to form a CRISPR/Cas system within the cell, which were originally derived from a bacteria defense mechanism able to identify and cleave foreign nucleic acids that invade the bacteria cell.[119] This system has been adapted into a genetic engineering tool that can be programmed to cleave a targeted gene at a specific location.[120] The genes required for the CRISPR/Cas system to function can be incorporated directly into the bacteria’s genome or encoded on a plasmid that is maintained in the cell. CRISPR/Cas systems have been programmed to cleave wild type phage genes resulting in a selection of recombinant phages from a mixed pool.[121] CIRSPR/Cas systems can also be used in combination with a plasmid containing a Donor DNA construct to improve recombination rates with the cleaved gene, due to DNA repair mechanisms activated when there is a double stranded DNA break. This method has resulted in a rate of recombination of 99% for some T4 phage genes.[36]

Genetic engineering approaches relying on a bacterial host can be limited by the destruction rate of the host during the lytic phage infection cycle that results in bacteria cell lysis. To overcome the limited rection time, phage genome recombination can be performed in a yeast cell intermediate, *Saccharomyces cerevisiae*, before insertion of the phage genome into the bacteria host via electroporation.[122] DNA sequences overlapping with a shuttle plasmid compatible in yeast and bacteria are incorporated into the ends of the phage genome to allow for genome transport between organisms using the shuttle plasmid[123] This method has been successful in engineering multiple phages from the T7-family.[122] *In vitro* phage genome assembly is another recombination method that is performed independent of the bacteria

host. DNA fragments are synthesized with overlapping ends via polymerase chain reactions (PCR) that can hybridize to form the desired recombinant genome sequence with the aid of enzymes.[124,125] The recombinant genome is then introduced into the bacteria host via electroporation to allow for generation of a functional phage This approach has been used to assemble full genomes of a ϕX174 phage and a T7 reporter phage.[126,127] However this method has a higher chance of introducing mutations through PCR errors and can be challenging when transforming large phage genomes into the bacterial host.[126] Gram positive bacteria's thick cell wall make it challenging to deliver DNA into the cell via electroporation, limiting *in vitro* genome assembly applications for phages with gram positive bacteria hosts. To overcome this limitation, cell wall deficient (L-form) bacteria cells can be used to uptake large molecules of DNA.[128] This approach has been used with L-form *Listeria monocytogenes* cells to uptake genomes of *Listeria*, *Bacillus*, and *Staphylococcus* phages via PEG mediated transfection.[129]

3.1. PHAGE DISPLAY

The well-established "Phage Display" method is used in combination with a genetic engineering method to construct a modified phage capsid.[130–132] Phage display involves genetically fusing a DNA sequence encoding for a foreign amino acid, peptide, or protein to a phage capsid gene that results in a phage displaying the recombinant capsid protein (Figure 1B). Modified capsid genes can be incorporated into the phage genome or exogenously expressed from a plasmid vector. Synthesis of genetically modified capsid proteins occurs in one production step within the bacteria cell. A mixture of modified and unmodified capsid proteins can be synthesized by incorporating a wild type and a modified capsid gene into the system to improve modified capsid stability.[133–135] Inducible promoters can be used to alter the percent of modified proteins displayed in mixed display systems.[136,137]. This method can be used on multiple capsid genes to create a multifunctional phage.[86,138] Phage display is time intensive upfront for proper design and development of the initial modified plasmids and genomes, but once established aliquots can be readily propagated and maintained for use in subsequent production batches.

Phage display libraries composed of a multitude of uniquely modified phage capsids can be subjected to bio-panning for identification of modified phages that best suit the desired application. Phage genotype and phenotype are linked, enabling traceback to reproduce the high preforming strains. Libraries can be randomly generated or specifically altered to elicit specific modification characteristics on the capsid. For example, cyclic peptide modified capsid libraries can be generated by intentionally incorporating cysteine residues to facilitate disulfide bond formation in all strains.[139,140] Bicyclic peptide libraries can be generated by genetically incorporating three cystines into the capsid gene followed reaction with a reagent containing three thiol-reactive groups after phage capsid assembly.[141–145] Forming peptides with nonreducible bonds has been achieved in libraries by utilizing enzymes to catalyze peptide crosslinking[146] or non-canonical amino acids that can undergo specific covalent reactions.[147,148] *In vitro* bio-panning assays have been used to identify modified phages with affinity to a variety of biological and inorganic substances including single-crystal semiconductors,[149] silica,[150] streptavidin,[151] cellulose,[152] and cell surface receptors.[153,154] *In vivo* bio-panning assays have been developed to identify modified phages that hone

specific tissues in mouse[155–157] and human[158] models. Co-display systems of enzymes and substrates have been developed to identity catalytically active modified capsids during bio-panning.[159–165]

3.2. AMINO ACIDS

Single amino acids can be added or substituted in capsids to modify the number of functional groups present in side chains capable of reacting in downstream chemical modification steps (Figure 2A).[166–168] By substituting a single amino acid for lysine in the major coat protein of M13 phage, Tridgett *et al.* were able to conjugate an additional 520 exogenous molecules to the phage via amine conjugation compared to the wild type.[169] In another study, a M13 phage displayed protein was modified to leave only one reactive cystine to increase site specificity of the subsequent thiol conjugation.[170] A similar method can be used to incorporate single unnatural amino acids that contain functional groups uncommon in nature to expand reaction capabilities.[171–175] Rare, nonsense, quadruplet, or reassigned amino acid codons can be added to a gene's sequence to create a site for unnatural amino acid incorporation by the corresponding unnatural amino acid specific t-RNA and t-RNA synthetase.[176] Unnatural amino acid modification reduces the potential for reacting unintended parts of the capsid but requires a more complex synthesis process that typically results in significantly lower yields compared to wild type protein synthesis.[177,178] This method was used in a phage-based biosensor to modify capsids of T4 phages for immobilization to ensure tail fibers crucial for infection were not altered.[171]

3.3. PEPTIDE MOTIFS

Peptide motifs are short structural regions conserved among different proteins that can often be linked to a particular biological function.[179] Peptide motifs that can recognized by enzymes can be displayed on the capsid to create a specific site for enzymatic modification after phage capsid assembly (Figure 2B). Protease cleavage sites have been added to T4 capsid gene constructs to allow for an affinity peptide to be displayed for chromatography purification of phage preparations that can be subsequently removed.[180] M13 and P22 phage nanocarriers containing protease cleavage sites have been used to facilitate enzyme driven release of contents.[49,181] Biotin ligase enzymes can utilize ATP to conjugate biotin to biotin carboxyl carrier protein (BCCP) via an amide linkage to one of BCCP's lysine residues. The components of this system can be used to biotinylate BCCP displaying phages *in vitro*.[182] Biotinylating can also occur *in vivo* during normal phage propagation, if a biotin ligase enzyme gene is present in the bacteria host or genetically incorporated into the phage or bacteria host.[183,184] BCCPs have been incorporated into T4, M13, and T7 phages to allow for site specific biotin addition.[57,185–188] Several commercially available biotin and streptavidin functionalized materials offer a diverse array of applications for BCCP tagged phages.

Sortase enzymes (those which modify surface proteins) can be incorporated into phage display systems to catalyze covalent bond formation between the N terminus of a substrate peptide or peptide conjugate to the C terminus of a cleaved sortase recognition motif.[189] Commonly used sortase A transpeptidation systems rely on a five amino acid long recognition motif and two-five amino acid long substrates.[190–192] This method has been

utilized to conjugate green fluorescent proteins and influenza antigens to P22 capsids.[189] Sortase motifs derived from different organisms have been used in the same system on different M13 capsid proteins to create multifunctional modified phage capsids.[193,194] After reacting for 3 hours, 56-74% of the M13 minor capsid proteins were attached with green fluorescent protein or biotin via sortase ligation.[193] Sortase catalyzed modification is advantageous for facilitating attachment of large complex proteins that may disrupt stable capsid assembly if fused directly to the phage capsid gene. This system is limited by the inefficiency of ligation by the sortase enzymes used which can be compensated for by using longer reaction times.[195]

3.4. DECORATION PROTEINS

Decoration proteins typically incorporated into icosahedral capsids after procapsid shell formation can assemble *in vitro* to mature capsids of strains where the gene for the decoration protein has been knocked out (Figure 2C).[196] This method allows for more purification and characterization of the modified proteins before incorporating them into the capsid to ensure they are in the correct form. It also allows for the displaying complex proteins that typically cannot be efficiently synthesized in the phage's bacteria host due to the protein's complexity or toxicity. Antigen constructs over twice the size of the decoration protein it is fused to have been successfully displayed on all capsid binding sites of T4 phage using this method.[197] Multiple antigen fusions to the same T4 decoration protein have been synthesized separately then mixed in equal molar ratios before *in vitro* binding to allow for mixed display of antigens on a single phage capsid.[198] *In vitro* assembly has been demonstrated in T4 phage with displayed antigens of swine fever virus,[54] human immunodeficiency virus,[53] foot and mouth disease virus,[52] *Bacillus anthracis*,[197] and *Neisseria meningitidis*[199] for initial vaccine development. Decoration protein homologs with conserved capsid binding domains have been bound to closely related phages to offer improved solubility or quantification capability compared to the native decoration proteins.[200–202]

4. CHEMICAL MODIFICATIONS

Another method for manipulating phages' structure and function is through chemical modification. Amino acids in the proteinaceou6s phage capsids offer a variety of reactive functional groups available for bioconjugation including carboxylic acids, amines, phenols, and thiols (Figure 3). The maximum degree of modification is determined by the number of reactive groups present that are sterically accessible for modification, their pKa, and solution conditions. The plethora of nucleophilic functional groups present creates potential for several amino acids to participate in chemical reactions. However, this can result in mixed reaction products from undesired side reactions even when conditions are optimized to favor a particular group.[203,204] Unintentional modification of residues that are crucial for proper structural feature function can decrease phage infectivity.[40] Low abundance amino acids such as cysteine or unnatural amino acids can be targeted for improved control over the site of modification.[205] Phospholipids in lipid enveloped phages provide another target for modification. Human enveloped viruses have been modified through propagating in a host engineered to metabolically incorporate chemically functionalized components into their cell

membranes.[206–208] This hybrid method provides a potential pathway for enveloped phage modification in the future.

4.1. AMINE GROUPS

Amine groups present at the N-terminus of all proteins and on lysine side chains are common targets for chemical modification. Reaction pH can be used to drive the reaction towards the α-amino group at the N-terminus (pKa ~8) or ε-amino group of lysine (pKa ~10) but still usually results in mixed modification.[209,210] Models have been created to help predict the molar ratio of NHS (N-hydroxysuccinimide) ester reagent to target protein to achieve the desired degree of modification.[211,212] NHS ester reagents are frequently used to form stable amide linkages with amines in one-step reactions. Several NHS ester conjugates are commercially available and stable under dry conditions. NHS esters in homo- and hetero-bifunctional reagents have been used to crosslink T4 phages to gold sensors and MS2 capsids to cell penetrating peptides.[168,213] NHS esters modified with a negatively charged sulfonate group can be used to increase the reagents solubility in water and reduce the need for organic solvents in reactions.[214] NHS[215–220] and Sulfo-NHS[168,221,222] esters have been used to modify phages from a variety of families including T7, M13, T4, A511, Felix-O1, SJ2, and MS2. Nonionic polyethylene glycol (PEG) linker arms have also been incorporated into NHS ester conjugates used for M13, fd, and G1 phage modifications to improve solubility and biocompatibilty.[223–225] Basic conditions required for amine deprotonation can be problematic with these reactions, because NHS esters are highly susceptible to base hydrolysis.[226–228] Tetrafluorophenyl (TFP) esters are another water soluble reagent able to form amide bonds with amines but are more stable in basic pH conditions and more hydrophobic.[229] Isocyanates and isothiocyanates can react with amines to form ureas and thioureas. Isothiocyanates are used more frequently for bioconjugations than isocyanates, because they are more stable in storage.[230,231] TFP esters[232,233] and isothiocyanates[216,234] have been used to modify T7, MS2, and M13 phages with fluorophores. For increased specificity, a two-step reaction can be used to modify only α-amino groups at the N-terminus of proteins under mild conditions. First the phage is incubated with pyridoxal 5'-phosphate (PLP) to undergo a transamination reaction that swaps the N-terminal amine for a ketone.[235] This ketone can undergo an oxime reaction with an aminooxy-functionalized substituent, which has been used for generation of fd phage biosensors.[236,237] N-terminal alanine residues with proximal lysine residues have shown to increase the efficiency of this bioconjugation reaction.[238] This method has been used for high density attachment of imaging agents and polymers to fd phage capsids.[51]

4.2. CARBOXYLATE GROUPS

Carboxylate groups are found at the C-terminus of proteins and on aspartate and glutamate side chains. Carboxylates have a low reactivity in water so they are commonly activated with a carbodiimide crosslinker like the water soluble 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) or water insoluble Dicyclohexylcarbodiimide (DCC) to form a reactive *O*-acylisourea intermediate.[239] Activated carboxylates can then be reacted with primary amines in mildly acidic pH conditions to form stable amide bonds that leave no trace of the carbodiimide used to facilitate the bond formation.[240] EDC coupling reactions have been used to conjugate

reactive moieties and chloramphenicol to filamentous phages as shown in Table 1.[241–243] NHS esters can be added to these reactions to generate a more stable NHS ester intermediate that can undergo reaction with primary amines under physiological pH.[244] This method has been used to attach fluorphores[222], magnetic beads,[225] folic acid,[245] and drugs[49,246] to M13 phage capsids. Glutaric anhydride has been conjugated to primary amines on M13 phages to increase the number of carboxylate groups accessible for polymer immobilization in downstream reactions.[247]

4.3. THIOL GROUPS

Thiol groups in cysteine side chains are the most reactive nucleophiles present in proteins.[248] Under oxidizing conditions, thiols from two cystines can form a disulfide bond. Thiols in cystine disulfide bonds can be liberated using reducing agents to increase the number of thiols accessible for modification, but this can cause protein destabilization or reversion back to disulfides.[249,250] Maleimides react with thiols under mild pH and temperature conditions to form stable thioether bonds.[251] Maleimides can also react with amines but their reaction with sulfhydryl groups occurs 1000-fold faster at pH 7 therefore is highly favored.[252] Maleimide conjugates functionalized for fluorescence,[167,241,253] water solubility,[166] neomycin[242] and improved stability[168,170] have been used to modify P22, MS2, M13, and fd phages. Thiol groups can also readily form dipolar bonds with metal ions and materials.[254–256] These bonds are not as strong as traditional covalent bonds, but allow even disulfide bonds to form bonds with metals.[252] Based on this principle, cysteine residues have been incorporated into fd phage capsids to improve binding to gold materials.[257] N-succinimidyl-S-acetylthiopropionate (SATP) has been chemically conjugated to M13 phages to increase the number of thiol groups capable of bonding with gold nanorods.[220]

4.4. PHENOL GROUPS

Phenol groups present on tyrosine and histidine residues can be modified using diazonium compounds to form diazo linked conjugates. Adjusting the reaction pH to 7 favors reaction with the histidine imidazole group, where higher pH's favor reaction with the tyrosine phenol group.[258] This strategy has been utilized to facilitate conjugation of imaging agents to M13 and MS2 phage capsids via tyrosine residues[74,222]

4.5. ALDEHYDE CROSS LINKERS

Glutaraldehyde is a dialdehyde that is frequently used as a crosslinking agent to form chemically and thermally stable crosslinks of biological materials.[259,260] Glutaraldehyde is present in many forms in solution leading to the precise mechanism and primary reactive species not being fully understood or agreed upon in the literature.[261] Glutaraldehyde can react with several nucleophilic functional groups present in proteins including amines, thiols, phenols, and imidazole, but the ε-amino group of lysine was found to be the most reactive.[262,263] This method has been used to facilitate crosslinking phages to amine functionalized magnetic microspheres and gold surfaces.[110,264] N-terminal serine and threonine residues contain a β-amino alcohol motif that can specifically undergo oxidative cleavage by sodium periodate to generate an aldehyde handle.[265] This handle can be subjected to a second reaction to add the desired conjugate. Oxime reactions have been used in aldehyde displaying phages to attach aminooxy conjugates. Oxime reactions can take

several hours at neutral pH but can be accelerated using acidic conditions (pH 4.5) and an aniline catalyst.[266] This two-step method was used in a one-pot reaction to add an aminooxy-functionalized glycan to M13 phages through an oxime condensation reaction in less than 1.5 hours.[267] In another study, an aldehyde conjugated to a NHS ester was used to attach aldehydes to amines on the exterior of MS2 phage capsids, followed by an 8 hour oxime condensation reaction at pH 6.5 to attach the desired contrast agent.[74] In a similar reaction, phage displayed aldehydes with 2-amino benzamidoxime derivatives have been used to conjugate materials to M13 phages in one hour resulting in bonds more resilient to hydrolysis than oximes.[268]

4.6. UNNATURAL AMINO ACIDS

Amino acids that are not encoded for in the natural genetic code of organisms can be incorporated into phage capsid protein constructs to display unique functional groups to generate sites on the capsid for selective chemical modification. Selenocysteine is an unnatural amino acid cysteine analog containing selenium in place of sulfur that has been incorporated into M13 phages for biotin conjugation.[269,270] This strong nucleophile reacts fast and at a low pH which can help prevent undesired side reactions that occur at a higher pH.[271] Multiple uncommon or unnatural amino acids have been synthesized to contain azide or alkyne groups capable of undergoing a copper catalyzed azide-alkyne cycloaddition click chemistry reaction.[272–274] This fast, highly specific reaction occurs among functional groups that are not common in biological substances, making it a good candidate for site specific modification with limited potential for unwanted side reactions.[275] Azide containing unnatural amino acid displaying M13 phages have facilitated conjugation to alkyne functionalized fluorophores and gold particles.[172,175] A tyrosine unnatural amino derivative, p-aminophenylalanine, can undergo sodium periodate mediated oxidative coupling for phage capsid bioconjugation. This method has been used to modify MS2 phage like particles and shown selective even in the presence of tyrosine.[76,276,277]

5. OPPORTUNITIES AND CHALLENGES

The modification approaches described in this review highlight advances made to functionalize phages for a diverse array of applications. These strategies can be used alone or in combination depending on the phage's characteristics and desired end modification. Adsorption to materials through electrostatic interactions offers the simplest method for functionalizing phages but lacks specificity and permanence. Genetic engineering can be used to fuse DNA sequences encoding for amino acids, peptides, or proteins to phage capsid genes through the well-established "Phage Display" method. These recombinant capsid proteins create additional sites for capsid modification by enzymes or chemicals. To accommodate large complex foreign protein fusions, recombinant capsid decoration proteins can be assembled *in vitro* to phages with accessible binding sites. Chemical modifications can also be used to target functional groups on amino acids of phage capsids for modification. Chemical reactions have potential for undesired side reactions to occur, but pH and temperature conditions can be adjusted to drive the reaction towards a particular functional group to mitigate side reactions. Protective groups can also be utilized during multistep reactions to improve selectivity of the reaction.

The plethora of genetic and chemical phage modification strategies have expanded the possibilities of phage-based biotechnologies. Hybrid methods utilizing genetic engineering to incorporate a low abundance or unnatural amino acid residue that can undergo a specific chemical reaction downstream appear to be the most promising for specific capsid modification. Current site-specific chemical methods are time-intensive and require multiple steps leaving much room for improvement to streamline the phage engineering process. To date, icosahedral and filamentous phage capsids have been the most frequently modified with no reported lipid modification of enveloped phages. Future studies adapting human enveloped virus modification methods to enveloped phages could tap into an underutilized capsid feature. Decoration proteins have been substituted from closely related phages to improve recombinant phage solubility. Investigating substitutions of other closely related phage capsid proteins could be a promising approach to alter phage capsid properties. Methods described in this review for capsid protein modification can be adapted to modify other phage proteins. For example, phage tail fibers that act as receptors for binding to bacteria hosts can be modified to alter a phage's bacteria host range. Overall, increased characterization of different phage capsids at the genomic and structural level will help expand the already promising opportunities of phage capsid engineering highlighted in this review.

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ABBREVIATIONS

BCCP	biotin carboxyl carrier protein
BRED	bacteriophage recombineering of electroporated DNA
CRISPR	clustered regularly interspaced palindromic repeats
Cas	CRISPR-associated proteins
DCC	Dicyclohexylcarbodiimide
EDC	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride
HIV	human immunodeficiency virus
NHS	N-hydroxysuccinimide
PCR	polymerase chain reaction
PEG	polyethylene glycol
PLP	pyridoxal 5'-phosphate
SARS	severe acute respiratory syndrome
SATP	N-succinimidyl-S-acetylthiopropionate

TFP	tetrafluorophenyl

REFERENCES

(1). Gordillo Altamirano FL; Barr JJ Phage Therapy in the Postantibiotic Era. Clinical Microbiology Reviews. 2019. 10.1128/CMR.00066-18.

(2). Ul Haq I; Chaudhry WN; Akhtar MN; Andleeb S; Qadri I Bacteriophages and Their Implications on Future Biotechnology: A Review. Virology Journal. 2012. 10.1186/1743-422X-9-9.

(3). Keen EC A Century of Phage Research: Bacteriophages and the Shaping of Modern Biology. BioEssays 2015. 10.1002/bies.201400152.

(4). Wigington CH; Sonderegger D; Brussaard CPD; Buchan A; Finke JF; Fuhrman JA; Lennon JT; Middelboe M; Suttle CA; Stock C et al. Re-Examination of the Relationship between Marine Virus and Microbial Cell Abundances. Nat. Microbiol. 2016. 10.1038/nmicrobiol.2015.24.

(5). Brum JR; Schenck RO; Sullivan MB Global Morphological Analysis of Marine Viruses Shows Minimal Regional Variation and Dominance of Non-Tailed Viruses. ISME J. 2013. 10.1038/ismej.2013.67.

(6). Martinez-Hernandez F; Fornas O; Lluesma Gomez M; Bolduc B; De La Cruz Peña MJ; Martínez JM; Anton J; Gasol JM; Rosselli R; Rodriguez-Valera F et al. Single-Virus Genomics Reveals Hidden Cosmopolitan and Abundant Viruses. Nat. Commun. 2017. 10.1038/ncommsl5892.

(7). Manrique P; Bolduc B; Walk ST; Van Oost J. Der; De Vos WM; Young MJ Healthy Human Gut Phageome. Proc. Natl. Acad. Sci. U. S. A 2016. 10.1073/pnas.1601060113.

(8). Barr JJ; Auro R; Furlan M; Whiteson KL; Erb ML; Pogliano J; Stotland A; Wolkowicz R; Cutting AS; Doran KS et al. Bacteriophage Adhering to Mucus Provide a Non-Host-Derived Immunity. Proc. Natl. Acad. Sci. U. S. A 2013. 10.1073/pnas.1305923110.

(9). Minot S; Bryson A; Chehoud C; Wu GD; Lewis JD; Bushman FD Rapid Evolution of the Human Gut Virome. Proc. Natl. Acad. Sci. U. S. A 2013. 10.1073/pnas.1300833110.

(10). Williamson KE; Radosevich M; Wommack KE Abundance and Diversity of Viruses in Six Delaware Soils. Appl. Environ. Microbiol. 2005. 10.1128/AEM.7L6.3119-3125.2005.

(11). Chen L; Xun W; Sun L; Zhang N; Shen Q; Zhang R Effect of Different Long-Term Fertilization Regimes on the Viral Community in an Agricultural Soil of Southern China. Eur. J. Soil Biol. 2014. 10.1016/j.ejsobi.2014.03.006.

(12). Williamson KE; Radosevich M; Smith DW; Wommack KE Incidence of Lysogeny within Temperate and Extreme Soil Environments. Environ. Microbiol. 2007. 10.llll/j.1462-2920.2007.01374.x.

(13). Luong T; Salabarria AC; Edwards RA; Roach DR Standardized Bacteriophage Purification for Personalized Phage Therapy. Nat. Protoc. 2020. 10.1038/s41596-020-0346-0.

(14). Mancuso F; Shi J; Malik DJ High Throughput Manufacturing of Bacteriophages Using Continuous Stirred Tank Bioreactors Connected in Series to Ensure Optimum Host Bacteria Physiology for Phage Production. Viruses 2018. 10.3390/vl0100537.

(15). Bonilla N; Barr JJ Phage on Tap: A Quick and Efficient Protocol for the Preparation of Bacteriophage Laboratory Stocks. In Methods in Molecular Biology, 2018. 10.1007/978-l-4939-8682-8_4.

(16). Leszczyński P; Weber-Dąbrowska B; Kohutnicka M; Łuczak M; Górecki A; Górski A Successful Eradication of Methicillin-Resistant Staphylococcus Aureus (MRSA) Intestinal Carrier Status in a Healthcare Worker - Case Report. Folia Microbiol. (Praha). 2006. 10.1007/BF02932128.

(17). Fish R; Kutter E; Bryan D; Wheat G; Kuhl S Resolving Digital Staphylococcal Osteomyelitis Using Bacteriophage—A Case Report. Antibiotics 2018. 10.3390/antibiotics7040087.

(18). Zhvania P; Hoyle NS; Nadareishvili L; Nizharadze D; Kutateladze M Phage Therapy in a 16-Year-Old Boy with Netherton Syndrome. Front. Med. 2017. 10.3389/fmed.2017.00094.

(19). LaVergne S; Hamilton T; Biswas B; Kumaraswamy M; Schooley RT; Wooten D Phage Therapy for a Multidrug-Resistant Acinetobacter Baumannii Craniectomy Site Infection. Open Forum Infect. Dis. 2018. 10.1093/ofid/oly064.

(20). Schooley RT; Biswas B; Gill JJ; Hernandez-Morales A; Lancaster J; Lessor L; Barr JJ; Reed SL; Rohwer F; Benler S et al. Development and Use of Personalized Bacteriophage-Based Therapeutic Cocktails to Treat a Patient with a Disseminated Resistant Acinetobacter Baumannii Infection. Antimicrob. Agents Chemother. 2017. 10.1128/AAC.00954-17.

(21). Nir-Paz R; Gelman D; Khouri A; Sisson BM; Fackler J; Alkalay-Oren S; Khalifa L; Rimon A; Yerushalmy O; Bader R et al. Successful Treatment of Antibiotic-Resistant, Poly-Microbial Bone Infection with Bacteriophages and Antibiotics Combination. Clin. Infect. Dis. 2019. 10.1093/cid/ciz222.

(22). Marza JAS; Soothill JS; Boydell P; Collyns TA Multiplication of Therapeutically Administered Bacteriophages in Pseudomonas Aeruginosa Infected Patients. Burns 2006. 10.1016/j.bums.2006.02.012.

(23). Khawaldeh A; Morales S; Dillon B; Alavidze Z; Ginn AN; Thomas L; Chapman SJ; Dublanchet A; Smithyman A; Iredell JR Bacteriophage Therapy for Refractory Pseudomonas Aeruginosa Urinary Tract Infection. J. Med. Microbiol. 2011. 10.1099/jmm.0.029744-0.

(24). Aslam S; Yung G; Dan J; Reed S; LeFebvre M; Logan C; Taplitz R; Law N; Golts E; Afshar K et al. Bacteriophage Treatment in a Lung Transplant Recipient. J. Hear. Lung Transplant. 2018. 10.1016/j.healun.2018.01.376.

(25). Vikram A; Tokman JI; Woolston J; Sulakvelidze A Phage Biocontrol Improves Food Safety by Significantly Reducing the Level and Prevalence of Escherichia Coli O157:H7 in Various Foods. J. Food Prot. 2020. 10.4315/0362-028X.JFP-19-433.

(26). Gutiérrez D; Rodríguez-Rubio L; Fernández L; Martínez B; Rodríguez A; García P Applicability of Commercial Phage-Based Products against Listeria Monocytogenes for Improvement of Food Safety in Spanish Dry-Cured Ham and Food Contact Surfaces. Food Control 2017. 10.1016/j.foodcont.2016.ll.007.

(27). Magnone JP; Marek PJ; Sulakvelidze A; Senecal AG Additive Approach for Inactivation of Escherichia Coli O157:H7, Salmonella, and Shigella Spp. on Contaminated Fresh Fruits and Vegetables Using Bacteriophage Cocktail and Produce Wash. J. Food Prot. 2013. 10.4315/0362-028X.JFP-12-517.

(28). Lakshmanan RS; Guntupalli R; Hu J; Kim DJ; Petrenko VA; Barbaree JM; Chin BA Phage Immobilized Magnetoelastic Sensor for the Detection of Salmonella Typhimurium. J. Microbiol. Methods 2007. 10.1016/j.mimet.2007.07.012.

(29). Balasubramanian S; Sorokulova IB; Vodyanoy VJ; Simonian AL Lytic Phage as a Specific and Selective Probe for Detection of Staphylococcus Aureus-A Surface Plasmon Resonance Spectroscopic Study. Biosens. Bioelectron. 2007. 10.1016/j.bios.2006.04.003.

(30). Juds C; Schmidt J; Weller MG; Lange T; Beck U; Conrad T; Börner HG Combining Phage Display and Next-Generation Sequencing for Materials Sciences: A Case Study on Probing Polypropylene Surfaces. J. Am. Chem. Soc. 2020. 10.1021/jacs.0c03482.

(31). Garneau JR; Depardieu F; Fortier LC; Bikard D; Monot M PhageTerm: A Tool for Fast and Accurate Determination of Phage Termini and Packaging Mechanism Using next-Generation Sequencing Data. Sci. Rep. 2017. 10.1038/s41598-017-07910-5.

(32). Meng C; Zhang J; Ye X; Guo F; Zou Q Review and Comparative Analysis of Machine Learning-Based Phage Virion Protein Identification Methods. Biochimica et Biophysica Acta - Proteins and Proteomics. 2020. 10.1016/j.bbapap.2020.140406.

(33). Callaway E Revolutionary Cryo-EM Is Taking over Structural Biology. Nature. 2020. 10.1038/d41586-020-00341-9.

(34). Almeida GM; Leppänen M; Maasilta IJ; Sundberg LR Bacteriophage Imaging: Past, Present and Future. Res. Microbiol. 2018. 10.1016/j.resmic.2018.05.006.

(35). Coulibaly F Polyhedra, Spindles, Phage Nucleus and Pyramids: Structural Biology of Viral Superstructures. In Advances in Virus Research; 2019. 10.1016/bs.aivir.2019.08.001.

(36). Duong MM, Carmody CM, Qinqin M, Peters JE, Nugen SR Optimization of T4 Phage Engineering via CRISPR/Cas9. Sci. Rep 2020, 10.1038/s41598-020-75426-6.

(37). Pires DP; Cleto S; Sillankorva S; Azeredo J; Lu TK Genetically Engineered Phages: A Review of Advances over the Last Decade. Microbiol. Mol. Biol. Rev. 2016. 10.1128/mmbr.00069-15.

(38). Chen Y; Batra H; Dong J; Chen C; Rao VB; Tao P Genetic Engineering of Bacteriophages against Infectious Diseases. Frontiers in Microbiology. 2019. 10.3389/fmicb.2019.00954.

(39). Ackermann HW 5500 Phages Examined in the Electron Microscope. Archives of Virology. 2007. 10.1007/s00705-006-0849-1.

(40). Kim KP; Cha JD; Jang EH; Klumpp J; Hagens S; Hardt WD; Lee KY; Loessner MJ PEGylation of Bacteriophages Increases Blood Circulation Time and Reduces T-Helper Type 1 Immune Response. Microb. Biotechnol 2008. 10.1111/j.1751-7915.2008.00028.x.

(41). Bone S; Alum A; Markovski J; Hristovski K; Bar-Zeev E; Kaufman Y; Abbaszadegan M; Perreault F Physisorption and Chemisorption of T4 Bacteriophages on Amino Functionalized Silica Particles. J. Colloid Interface Sci. 2018. 10.1016/jjcis.2018.07.107.

(42). Bhardwaj N Phage Immobilized Antibacterial Silica Nanoplatform: Application against Bacterial Infections. Adv. Anim. Vet. Sci. 2015. 10.14737/journal.aavs/2015/3.1s.1.9.

(43). Tolba M; Minikh O; Brovko LY; Evoy S; Griffiths MW Oriented Immobilization of Bacteriophages for Biosensor Applications. Appl. Environ. Microbiol. 2010. 10.1128/AEM.02294-09.

(44). Merzlyak A; Indrakanti S; Lee S-W Genetically Engineered Nanofiber-Like Viruses For Tissue Regenerating Materials. Nano Lett. 2009. 10.1021/nl8036728.

(45). Yoo SY; Kobayashi M; Lee PP; Lee SW Early Osteogenic Differentiation of Mouse Preosteoblasts Induced by Collagen-Derived DGEA-Peptide on Nanofibrous Phage Tissue Matrices. Biomacromolecules 2011. 10.1021/bm1013475.

(46). Chung WJ; Merzlyak A; Yoo SY; Lee SW Genetically Engineered Liquid-Crystalline Viral Films for Directing Neural Cell Growth. Langmuir 2010. 10.1021/la100226u.

(47). Lee HS; Kang JI; Chung WJ; Lee DH; Lee BY; Lee SW; Yoo SY Engineered Phage Matrix Stiffness-Modulating Osteogenic Differentiation. ACS Appl. Mater. Interfaces 2018. 10.1021/acsami.7b17871.

(48). Yoo SY; Shrestha KR; Jeong SN; Kang JI; Lee SW Engineered Phage Nanofibers Induce Angiogenesis. Nanoscale 2017. 10.1039/c7nr03332j.

(49). Ghosh D; Kohli AG; Moser F; Endy D; Belcher AM Refactored M13 Bacteriophage as a Platform for Tumor Cell Imaging and Drug Delivery. ACS Synth. Biol. 2012. 10.1021/sb300052u.

(50). Newton JR; Kelly KA; Mahmood U; Weissleder R; Deutscher SL *In Vivo* Selection of Phage for the Optical Imaging of PC-3 Human Prostate Carcinoma in Mice. Neoplasia 2006. 10.1593/neo.06331.

(51). Carrico ZM; Farkas ME; Zhou Y; Hsiao SC; Marks JD; Chokhawala H; Clark DS; Francis MB N-Terminal Labeling of Filamentous Phage to Create Cancer Marker Imaging Agents. ACS Nano 2012. 10.1021/nn301134z.

(52). Ren ZJ; Tian CJ; Zhu QS; Zhao MY; Xin AG; Nie WX; Ling SR; Zhu MW; Wu JY; Lan HY et al. Orally Delivered Foot-and-Mouth Disease Virus Capsid Protomer Vaccine Displayed on T4 Bacteriophage Surface: 100% Protection from Potency Challenge in Mice. Vaccine 2008. 10.1016/j.vaccine.2007.12.053.

(53). Sathaliyawala T; Rao M; Maclean DM; Birx DL; Alving CR; Rao VB Assembly of Human Immunodeficiency Virus (HIV) Antigens on Bacteriophage T4: A Novel In Vitro Approach To Construct Multicomponent HIV Vaccines. J. Virol. 2006. 10.1128/jvi.00235-06.

(54). Wu J; Tu C; Yu X; Zhang M; Zhang N; Zhao M; Nie W; Ren Z Bacteriophage T4 Nanoparticle Capsid Surface SOC and HOC Bipartite Display with Enhanced Classical Swine Fever Virus Immunogenicity: A Powerful Immunological Approach. J. Virol. Methods 2007. 10.1016/jjviromet.2006.09.017.

(55). Liu CM; Jin Q; Sutton A; Chen L A Novel Fluorescent Probe: Europium Complex Hybridized T7 Phage. Bioconjug. Chem. 2005. 10.1021/bc050108+.

(56). Farkas ME; Aanei IL; Behrens CR; Tong GJ; Murphy ST; O'Neil JP; Francis MB PET Imaging and Biodistribution of Chemically Modified Bacteriophage MS2. Mol. Pharm. 2013. 10.1021/mp3003754.

(57). DePorter SM; McNaughton BR Engineered M13 Bacteriophage Nanocarriers for Intracellular Delivery of Exogenous Proteins to Human Prostate Cancer Cells. Bioconjug. Chem. 2014. 10.1021/bc500339k.

(58). Büning H; Srivastava A Capsid Modifications for Targeting and Improving the Efficacy of AAV Vectors. Molecular Therapy - Methods and Clinical Development. 2019. 10.1016/j.omtm.2019.01.008.

(59). Xu Z; Zhou X; Shi W; Qian Q Capsid Modification of Adeno-Associated Virus and Tumor Targeting Gene Therapy. Chinese Sci. Bull. 2008. 10.1007/s11434-008-0487-y.

(60). Prasad BVV; Schmid MF Principles of Virus Structural Organization. Adv. Exp. Med Biol. 2012. 10.1007/978-l-4614-0980-93.

(61). Zandi R; Reguera D Mechanical Properties of Viral Capsids. Phys. Rev. E - Stat. Nonlinear, Soft Matter Phys. 2005. 10.1103/PhysRevE.72.021917.

(62). Tama F; Brooks CL Diversity and Identity of Mechanical Properties of Icosahedral Viral Capsids Studied with Elastic Network Normal Mode Analysis. J. Mol. Biol. 2005. 10.1016/jjmb.2004.10.054.

(63). May ER; Feng J; Brooks CL Exploring the Symmetry and Mechanism of Virus Capsid Maturation via an Ensemble of Pathways. Biophys. J. 2012. 10.1016/j.bpj.2011.12.016.

(64). Casjens S; Hendrix R Control Mechanisms in DsDNA Bacteriophage Assembly. In The Bacteriophages; 1988. 10.1007/978-1-4684-5424-62.

(65). Homa FL; Brown JC Capsid Assembly and DNA Packaging in Herpes Simplex Virus. Reviews in Medical Virology. 1997. 10.1002/(SICI)1099-1654(199707)7:2<107::AID-RMV191>3.0.CO;2-M.

(66). Aksyuk AA; Rossmann MG Bacteriophage Assembly. Viruses 2011. 10.3390/v3030172.

(67). Prevelige PE Send for Reinforcements! Conserved Binding of Capsid Decoration Proteins. Structure. 2008. 10.1016/j.str.2008.08.003.

(68). Qin L; Fokine A; O'Donnell E; Rao VB; Rossmann MG Structure of the Small Outer Capsid Protein, Soc: A Clamp for Stabilizing Capsids of T4-like Phages. J. Mol. Biol. 2010. 10.1016/jjmb.2009.10.007.

(69). Sternberg N; Weisberg R Packaging of Coliphage Lambda DNA. II. The Role of the Gene D Protein. J. Mol. Biol. 1977. 10.1016/0022-2836(77)90067-5.

(70). Tang L; Gilcrease EB; Casjens SR; Johnson JE Highly Discriminatory Binding of Capsid-Cementing Proteins in Bacteriophage L. Structure 2006. 10.1016/j.str.2006.03.010.

(71). Banerjee R; Nath S; Ranjan A; Khamrui S; Pani B; Sen R; Sen U The First Structure of Polarity Suppression Protein, Psu from Enterobacteria Phage P4, Reveals a Novel Fold and a Knotted Dimer. J. Biol. Chem. 2012. 10.1074/jbc.M112.423202.

(72). Petrovski S; Dyson ZA; Seviour RJ; Tillett D Small but Sufficient: The Rhodococcus Phage RRH1 Has the Smallest Known Siphoviridae Genome at 14.2 Kilobases. J. Virol. 2012. 10.1128/jvi.05460-ll.

(73). Donelli G; Dore E; Frontali C; Grandolfo ME Structure and Physico-Chemical Properties of Bacteriophage G. III. A Homogeneous DNA of Molecular Weight 5 × 108. J. Mol. Biol. 1975. 10.1016/0022-2836(75)90321-6.

(74). Hooker JM; Datta A; Botta M; Raymond KN; Francis MB Magnetic Resonance Contrast Agents from Viral Capsid Shells: A Comparison of Exterior and Interior Cargo Strategies. Nano Lett. 2007. 10.1021/nl070512c.

(75). Wu W; Hsiao SC; Carrico ZM; Francis MB Genome-Free Viral Capsids as Multivalent Carriers for Taxol Delivery. Angew. Chemie - Int. Ed. 2009. 10.1002/anie.200902426.

(76). Tong GJ; Hsiao SC; Carrico ZM; Francis MB Viral Capsid DNA Aptamer Conjugates as Multivalent Cell-Targeting Vehicles. J. Am. Chem. Soc. 2009. 10.1021/ja903857f.

(77). Qazi S; Miettinen HM; Wilkinson RA; McCoy K; Douglas T; Wiedenheft B Programmed Self-Assembly of an Active P22-Cas9 Nanocarrier System. Mol. Pharm. 2016. 10.1021/acs.molpharmaceut.5b00822.

(78). Wu M; Sherwin T; Brown WL; Stockley PG Delivery of Antisense Oligonucleotides to Leukemia Cells by RNA Bacteriophage Capsids. Nanomedicine Nanotechnology, Biol. Med. 2005. 10.1016/j.nano.2004.ll.011.

(79). Xu J; Dayan N; Goldbourt A; Xiang Y Cryo-Electron Microscopy Structure of the Filamentous Bacteriophage IKe. Proc. Natl. Acad. Sci. U. S. A 2019. 10.1073/pnas.1811929116.

(80). Marzec CJ; Day LA A Theory of the Symmetries of Filamentous Bacteriophages. Biophys. J. 1988. 10.1016/S0006-3495(88)83119-9.

(81). Caspar DLD; Makowski L The Symmetries of Filamentous Phage Particles. J. Mol. Biol. 1981. 10.1016/0022-2836(81)90549-0.

(82). Feng JN; Russel M; Model P A Permeabilized Cell System That Assembles Filamentous Bacteriophage. Proc. Natl. Acad. Sci. U. S. A 1997. 10.1073/pnas.94.8.4068.

(83). Yoo SY; Jin HE; Choi DS; Kobayashi M; Farouz Y; Wang S; Lee SW M13 Bacteriophage and Adeno-Associated Virus Hybrid for Novel Tissue Engineering Material with Gene Delivery Functions. Adv. Healthc. Mater. 2016. 10.1002/adhm.201500179.

(84). Hunter GJ; Rowitch DH; Perham RN Interactions between DNA and Coat Protein in the Structure and Assembly of Filamentous Bacteriophage Fd. Nature 1987. 10.1038/327252a0.

(85). Sidhu SS; Weiss GA; Wells JA High Copy Display of Large Proteins on Phage for Functional Selections. J. Mol. Biol. 2000. 10.1006/jmbi.1999.3465.

(86). Fatemi F; Amini SM; Kharrazi S; Rasaee MJ; Mazlomi MA; Asadi-Ghalehni M; Rajabibazl M; Sadroddiny E Construction of Genetically Engineered M13K07 Helper Phage for Simultaneous Phage Display of Gold Binding Peptide 1 and Nuclear Matrix Protein 22 ScFv Antibody. Colloids Surfaces B Biointerfaces 2017. 10.1016/j.colsurfb.2017.08.034.

(87). Nam KT; Kim DW; Yoo PJ; Chiang CY; Meethong N; Hammond PT; Chiang YM; Belcher AM Virus-Enabled Synthesis and Assembly of Nanowires for Lithium Ion Battery Electrodes. Science (80-,). 2006. 10.1126/science.1122716.

(88). Laurinavičius S; Käkelä R; Bamford DH; Somerharju P The Origin of Phospholipids of the Enveloped Bacteriophage Phi6. Virology 2004. 10.1016/j.virol.2004.05.021.

(89). Laurinavičius S; Käkelä R; Somerharju P; Bamford DH Phospholipid Molecular Species Profiles of Tectiviruses Infecting Gram-Negative and Gram-Positive Hosts. Virology 2004. 10.1016/j.virol.2004.02.009.

(90). Hinnen R; Chassin R; SCHÄFER R; Franklin RM; Hitz H; SCHÄFER D Structure and Synthesis of a Lipid-Containing Bacteriophage: Purification, Chemical Composition, and Partial Sequences of the Structural Proteins. Eur. J. Biochem. 1976. 10.llll/j.1432-1033.1976.tbl0772.x.

(91). Mäntynen S; Sundberg LR; Poranen MM Recognition of Six Additional Cystoviruses: Pseudomonas Virus Phi6 Is No Longer the Sole Species of the Family Cystoviridae. Arch. Virol. 2018. 10.1007/s00705-017-3679-4.

(92). Pawlowski A; Rissanen I; Bamford JKH; Krupovic M; Jalasvuori M Gammasphaerolipovirus, a Newly Proposed Bacteriophage Genus, Unifies Viruses of Halophilic Archaea and Thermophilic Bacteria within the Novel Family Sphaerolipoviridae. Arch. Virol. 2014. 10.1007/s00705-013-1970-6.

(93). Abrescia NGA; Cockburn JJB; Grimes JM; Sutton GC; Diprose JM; Butcher SJ; Fuller SD; San Martín C; Burnett RM; Stuart DI et al. Insights into Assembly from Structural Analysis of Bacteriophage PRD1. Nature. 2004. 10.1038/nature03056.

(94). Kivelä HM; Kalkkinen N; Bamford DH Bacteriophage PM2 Has a Protein Capsid Surrounding a Spherical Proteinaceous Lipid Core. J. Virol. 2002. 10.1128/jvi.76.16.8169-8178.2002.

(95). Liss A; Heiland RA Characterization of the Enveloped Plasmavirus MVL2 after Propagation on Three Acholeplasma Laidlawii Hosts. Arch. Virol. 1983. 10.1007/BF01314132.

(96). Whitworth C; Mu Y; Houston H.; Martinez-Smith M; Noble-Wang J; Coulliette-Salmond A; Rose L Persistence of Bacteriophage Phi 6 on Porous and Nonporous Surfaces and the Potential for Its Use as an Ebola Virus or Coronavirus Surrogate. Appl. Environ. Microbiol. 2020. 10.1128/AEM.01482-20.

(97). Casanova LM; Waka B Survival of a Surrogate Virus on N95 Respirator Material. Infect. Control Hosp. Epidemiol. 2013. 10.1086/673994.

(98). Wood JP; Richter W; Sunderman M; Calfee MW; Serre S; Mickelsen L Evaluating the Environmental Persistence and Inactivation of MS2 Bacteriophage and the Presumed Ebola Virus Surrogate Phi6 Using Low Concentration Hydrogen Peroxide Vapor. Environ. Sci. Technol. 2020. 10.1021/acs.est.9b06034.

(99). Aquino De Carvalho N; Stachler EN; Cimabue N; Bibby K Evaluation of Phi6 Persistence and Suitability as an Enveloped Virus Surrogate. Environ. Sci. Technol. 2017. 10.1021/acs.est.7b01296.

(100). Lytle CD; Tondreau SC; Truscott W; Budacz AP; Kuester RK; Venegas L; Schmukler RE; Cyr WH Filtration Sizes of Human Immunodeficiency Virus Type 1 and Surrogate Viruses Used to Test Barrier Materials. Applied and Environmental Microbiology. 1992. 10.1128/aem.58.2.747-749.1992.

(101). Nap RJ; Božič AL; Szleifer I; Podgornik R The Role of Solution Conditions in the Bacteriophage Pp7 Capsid Charge Regulation. Biophys. J. 2014. 10.1016/j.bpj.2014.08.032.

(102). Cademartiri R; Anany H; Gross I; Bhayani R; Griffiths M; Brook MA Immobilization of Bacteriophages on Modified Silica Particles. Biomaterials 2010. 10.1016/j.biomaterials.2009.ll.029.

(103). Leppänen M; Maasilta IJ; Sundberg LR Antibacterial Efficiency of Surface-Immobilized Flavobacterium-Infecting Bacteriophage. ACS Appl. Bio Mater. 2019. 10.1021/acsabm.9b00242.

(104). Anany H.; Brovko L; El Dougdoug NK; Sohar J; Fenn H; Alasiri N; Jabrane T; Mangin P; Monsur Ali M; Kannan B et al. Print to Detect: A Rapid and Ultrasensitive Phage-Based Dipstick Assay for Foodborne Pathogens. Anal. Bioanal. Chem. 2018. 10.1007/s00216-017-0597-y.

(105). Farooq U; Ullah MW; Yang Q; Aziz A; Xu J; Zhou L; Wang S High-Density Phage Particles Immobilization in Surface-Modified Bacterial Cellulose for Ultra-Sensitive and Selective Electrochemical Detection of Staphylococcus Aureus. Biosens. Bioelectron. 2020. 10.1016/j.bios.2020.112163.

(106). Anany H.; Chen W; Pelton R; Griffiths MW Biocontrol of Listeria Monocytogenes and Escherichia Coli O157:H7 in Meat by Using Phages Immobilized on Modified Cellulose Membranes. Appl. Environ. Microbiol. 2011. 10.1128/AEM.05493-ll.

(107). Richter Ł; Bielec K; Leśniewski A; Łoś M; Paczesny J; Hołyst R Dense Layer of Bacteriophages Ordered in Alternating Electric Field and Immobilized by Surface Chemical Modification as Sensing Element for Bacteria Detection. ACS Appl. Mater. Interfaces 2017. 10.1021/acsami.7b03497.

(108). Yoo PJ; Nam KT; Qi J; Lee SK; Park J; Belcher AM; Elammond PT Spontaneous Assembly of Viruses on Multilayered Polymer Surfaces. Nat. Mater. 2006. 10.1038/nmatl596.

(109). Vonasek E; Lu P; Hsieh Y. Lo; Nitin N Bacteriophages Immobilized on Electrospun Cellulose Microfibers by Non-Specific Adsorption, Protein–Ligand Binding, and Electrostatic Interactions. Cellulose 2017. 10.1007/sl0570-017-1442-3.

(110). Singh A; Glass N; Tolba M; Brovko L; Griffiths M; Evoy S Immobilization of Bacteriophages on Gold Surfaces for the Specific Capture of Pathogens. Biosens. Bioelectron. 2009. 10.1016/j.bios.2009.05.028.

(111). Xu J; Zhao C; Chau Y; Lee YK The Synergy of Chemical Immobilization and Electrical Orientation of T4 Bacteriophage on a Micro Electrochemical Sensor for Low-Level Viable Bacteria Detection via Differential Pulse Voltammetry. Biosens. Bioelectron. 2020. 10.1016/j.bios.2019.111914.

(112). Mahichi F; Synnott AJ; Yamamichi K; Osada T; Tanji Y Site-Specific Recombination of T2 Phage Using IP008 Long Tail Fiber Genes Provides a Targeted Method for Expanding Host Range While Retaining Lytic Activity. FEMS Microbiol. Lett. 2009. 10.llll/j.1574-6968.2009.01588.x.

(113). Le S; He X; Tan Y; Huang G; Zhang L; Lux R; Shi W; Hu F Mapping the Tail Fiber as the Receptor Binding Protein Responsible for Differential Host Specificity of Pseudomonas Aeruginosa Bacteriophages PaP1 and JG004. PLoS One 2013. 10.1371/joumal.pone.0068562.

(114). Loessner MJ; Rees CED; Stewart GSAB; Scherer S Construction of Luciferase Reporter Bacteriophage A511::LuxAB for Rapid and Sensitive Detection of Viable Listeria Cells. Appl. Environ. Microbiol. 1996. 10.1128/aem.62.4.1133-1140.1996.

(115). Tao P; Wu X; Tang WC; Zhu J; Rao V Engineering of Bacteriophage T4 Genome Using CRISPR-Cas9. ACS Synth. Biol. 2017. 10.1021/acssynbio.7b00179.

(116). Marinelli LJ; Piuri M; Swigoňová Z; Balachandran A; Oldfield LM; van Kessel JC; Hatfull GF BRED: A Simple and Powerful Tool for Constructing Mutant and Recombinant Bacteriophage Genomes. PLoS One 2008. 10.1371/joumal.pone.0003957.

(117). Court DL; Oppenheim AB; Adhya SL A New Look at Bacteriophage λ Genetic Networks. Journal of Bacteriology. 2007. 10.1128/JB.01215-06.

(118). Oppenheim AB; Rattray AJ; Bubunenko M; Thomason LC; Court DL *In Vivo* Recombineering of Bacteriophage λ by PCR Fragments and Single-Strand Oligonucleotides. Virology 2004. 10.1016/j.virol.2003.ll.007.

(119). Barrangou R; Fremaux C; Deveau H; Richards M; Boyaval P; Moineau S; Romero DA; Horvath P CRISPR Provides Acquired Resistance against Viruses in Prokaryotes. Science (80-,). 2007. 10.1126/science.1138140.

(120). Jin M; Garreau De Loubresse N; Kim Y; Kim J; Yin P Programmable CRISPR-Cas Repression, Activation, and Computation with Sequence-Independent Targets and Triggers. ACS Synth. Biol. 2019. 10.1021/acssynbio.9b00141.

(121). Kiro R; Shitrit D; Qimron U Efficient Engineering of a Bacteriophage Genome Using the Type I-E CRISPR-Cas System. RNA Biol. 2014. 10.4161/ma.27766.

(122). Ando H; Lemire S; Pires DP; Lu TK Engineering Modular Viral Scaffolds for Targeted Bacterial Population Editing. Cell Syst. 2015. 10.1016/j.cels.2015.08.013.

(123). Bradshaw MS; Bollekens JA; Ruddle FH A New Vector for Recombination-Based Cloning of Large DNA Fragments from Yeast Artificial Chromosomes. Nucleic Acids Res. 1995. 10.1093/nar/23.23.4850.

(124). Gibson DG; Young L; Chuang RY; Venter JC; Hutchison CA; Smith HO Enzymatic Assembly of DNA Molecules up to Several Hundred Kilobases. Nat. Methods 2009. 10.1038/nmeth.1318.

(125). TerMaat JR; Pienaar E; Whitney SE; Mamedov TG; Subramanian A Gene Synthesis by Integrated Polymerase Chain Assembly and PCR Amplification Using a High-Speed Thermocycler. J. Microbiol. Methods 2009. 10.1016/j.mimet.2009.09.015.

(126). Smith HO; Hutchison CA; Pfannkoch C; Venter JC Generating a Synthetic Genome by Whole Genome Assembly: ΦX174 Bacteriophage from Synthetic Oligonucleotides. Proc. Natl. Acad. Sci. U. S. A 2003. 10.1073/pnas.2237126100.

(127). Pulkkinen EM; Hinkley TC; Nugen SR Utilizing in Vitro DNA Assembly to Engineer a Synthetic T7 Nanoluc Reporter Phage for Escherichia Coli Detection. Integr. Biol 2019. 10.1093/intbio/zyz005.

(128). White TB; Doyle RJ; Streips UN Transformation of a Bacillus Subtilis L-Form with Bacteriophage Deoxyribonucleic Acid. J. Bacteriol. 1981. 10.1128/jb.145.2.878-883.1981.

(129). Kilcher S; Studer P; Muessner C; Klumpp J; Loessner MJ; Adhya S Cross-Genus Rebooting of Custom-Made, Synthetic Bacteriophage Genomes in L-Form Bacteria. Proc. Natl. Acad. Sci. U. S. A 2018. 10.1073/pnas.1714658115.

(130). Smith GP; Petrenko VA Phage Display. Chem. Rev. 1997. 10.1021/cr960065d.

(131). Bazan J; Calkosiński I; Gamian A Phage Display—A Powerful Technique for Immunotherapy. Hum. Vaccin. Immunother. 2012. 10.4161/hv.21703.

(132). Tan Y; Tian T; Liu W; Zhu Z; J. Yang C Advance in Phage Display Technology for Bioanalysis. Biotechnology Journal. 2016. 10.1002/biot.201500458.

(133). Malik P; Terry TD; Gowda LR; Langara A; Petukhov SA; Symmons MF; Welsh LC; Marvin DA; Perham RN Role of Capsid Structure and Membrane Protein Processing in Determining the Size and Copy Number of Peptides Displayed on the Major Coat Protein of Filamentous Bacteriophage. J. Mol. Biol. 1996. 10.1006/jmbi.1996.0378.

(134). Markland W; Roberts BL; Saxena MJ; Guterman SK; Ladner RC Design, Construction and Function of a Multicopy Display Vector Using Fusions to the Major Coat Protein of Bacteriophage M13. Gene 1991. 10.1016/0378-1119(91)90583-W.

(135). Chasteen L; Ayriss J; Pavlik P; Bradbury ARM Eliminating Helper Phage from Phage Display. Nucleic Acids Res. 2006. 10.1093/nar/gkl772.

(136). Zahn G; Skerra A; Höhne W Investigation of a Tetracycline-Regulated Phage Display System. Protein Eng. 1999. 10.1093/protein/12.12.1031.

(137). Huang W; McKevitt M; Palzkill T Use of the Arabinose p(Bad) Promoter for Tightly Regulated Display of Proteins on Bacteriophage. Gene 2000. 10.1016/S0378-1119(00)00210-9.

(138). Guo Y; Liang X; Zhou Y; Zhang Z; Wei H; Men D; Luo M; Zhang XE Construction of Bifunctional Phage Display for Biological Analysis and Immunoassay. Anal. Biochem. 2010. 10.1016/j.ab.2009.08.026.

(139). Bonnycastle LLC; Mehroke JS; Rashed M; Gong X; Scott JK Probing the Basis of Antibody Reactivity with a Panel of Constrained Peptide Libraries Displayed by Filamentous Phage. J. Mol. Biol. 1996. 10.1006/jmbi.1996.0284.

(140). McLafferty MA; Kent RB; Ladner RC; Markland W M13 Bacteriophage Displaying Disulfide-Constrained Microproteins. Gene 1993. 10.1016/0378-1119(93)90149-W.

(141). Angelini A; Cendron L; Chen S; Touati J; Winter G; Zanotti G; Heinis C Bicyclic Peptide Inhibitor Reveals Large Contact Interface with a Protease Target. ACS Chem. Biol. 2012. 10.1021/cb200478t.

(142). Heinis C; Rutherford T; Freund S; Winter G Phage-Encoded Combinatorial Chemical Libraries Based on Bicyclic Peptides. Nat. Chem. Biol. 2009. 10.1038/nchembio.184.

(143). Chen S; Morales-Sanfrutos J; Angelini A; Cutting B; Heinis C Structurally Diverse Cyclisation Linkers Impose Different Backbone Conformations in Bicyclic Peptides. ChemBioChem 2012. 10.1002/cbic.201200049.

(144). van de Langemheen H; Korotkovs V; Bijl J; Wilson C; Kale SS; Heinis C; Liskamp RMJ Polar Hinges as Functionalized Conformational Constraints in (Bi)Cyclic Peptides. ChemBioChem 2017. 10.1002/cbic.201600612.

(145). Rebollo IR; Angelini A; Heinis C Phage Display Libraries of Differently Sized Bicyclic Peptides. Medchemcomm 2013. 10.1039/c2md20171b.

(146). Hetrick KJ; Walker MC; Van Der Donk WA Development and Application of Yeast and Phage Display of Diverse Lanthipeptides. ACS Cent. Sci. 2018. 10.1021/acscentsci.7b00581.

(147). Simonetti L; Ivarsson Y Genetically Encoded Cyclic Peptide Phage Display Libraries. ACS Cent. Sci. 2020. 10.1021/acscentsci.0c00087.

(148). Wang XS; Chen PHC; Hampton JT; Tharp JM; Reed CA; Das SK; Wang DS; Hayatshahi HS; Shen Y; Liu J et al. A Genetically Encoded, Phage-Displayed Cyclic-Peptide Library. Angew. Chemie - Int. Ed. 2019. 10.1002/anie.201908713.

(149). Whaley SR; English DS; Hu EL; Barbara PF; Belcher AM Selection of Peptides with Semiconductor Binding Specificity for Directed Nanocrystal Assembly. Nature 2000. 10.1038/35015043.

(150). Oliver D; Michaelis M; Heinz H; Volkov VV; Perry CC From Phage Display to Structure: An Interplay of Enthalpy and Entropy in the Binding of the LDHSLHS Polypeptide to Silica. Phys. Chem. Chem. Phys. 2019. 10.1039/c8cp07011c.

(151). Caparon MH; De Ciechi PA; Devine CS; Olins PO; Lee SC Analysis of Novel Streptavidin-Binding Peptides, Identified Using a Phage Display Library, Shows That Amino Acids External to a Perfectly Conserved Consensus Sequence and to the Presented Peptides Contribute to Binding. Mol. Divers. 1996. 10.1007/BF01715528.

(152). Guo J; Catchmark JM; Mohamed MNA; Benesi AJ; Tien M; Kao TH; Watts HD; Kubicki JD Identification and Characterization of a Cellulose Binding Heptapeptide Revealed by Phage Display. Biomacromolecules 2013. 10.1021/bm4001876.

(153). Marks JD; Ouwehand WH; Bye JM; Finnern R; Gorick BD; Voak D; Thorpe SJ; Hughes-Jones NC; Winter G Human Antibody Fragments Specific for Human Blood Group Antigens from a Phage Display Library. Bio/Technology 1993. 10.1038/nbtl093-1145.

(154). Heitner T; Moor A; Garrison JL; Marks C; Hasan T; Marks JD Selection of Cell Binding and Internalizing Epidermal Growth Factor Receptor Antibodies from a Phage Display Library. J. Immunol. Methods 2001. 10.1016/S0022-1759(00)00340-9.

(155). Pasqualini R; Ruoslahti E Organ Targeting in Vivo Using Phage Display Peptide Libraries. Nature 1996. 10.1038/380364a0.

(156). Rajotte D; Arap W; Hagedorn M; Koivunen E; Pasqualini R; Ruoslahti E Molecular Heterogeneity of the Vascular Endothelium Revealed by in Vivo Phage Display. J. Clin. Invest. 1998. 10.1172/JCI3008.

(157). Robert R; Jacobin-Valat MJ; Daret D; Miraux S; Nurden AT; Franconi JM; Clofent-Sanchez G Identification of Human ScFvs Targeting Atherosclerotic Lesions: Selection by Single Round in Vivo Phage Display. J. Biol. Chem. 2006. 10.1074/jbc.M609344200.

(158). Krag DN; Shukla GS; Shen GP; Pero S; Ashikaga T; Fuller S; Weaver DL; Burdette-Radoux S; Thomas C Selection of Tumor-Binding Ligands in Cancer Patients with Phage Display Libraries. Cancer Res. 2006. 10.1158/0008-5472.CAN-05-4441.

(159). Delespaul W; Peeters Y; Herdewijn P; Robben J A Novel Helper Phage for HaloTag-Mediated Co-Display of Enzyme and Substrate on Phage. Biochem. Biophys. Res. Commun. 2015. 10.1016/j.bbrc.2015.03.019.

(160). Pedersen H; Hölder S; Sutherlin DP; Schwitter U; King DS; Schultz PG A Method for Directed Evolution and Functional Cloning of Enzymes. Proc. Natl. Acad. Sci. U. S. A 1998. 10.1073/pnas.95.18.10523.

(161). Demartis S; Huber A; Viti F; Lozzi L; Giovannoni L; Neri P; Winter G; Neri D A Strategy for the Isolation of Catalytic Activities from Repertoires of Enzymes Displayed on Phage. J. Mol. Biol. 1999. 10.1006/jmbi.1998.2476.

(162). Love KR; Swoboda JG; Noren CJ; Walker S Enabling Glycosyltransferase Evolution: A Facile Substrate-Attachment Strategy for Phage-Display Enzyme Evolution. ChemBioChem 2006. 10.1002/cbic.200600018.

(163). Sunbul M; Emerson N; Yin J Enzyme-Catalyzed Substrate Attachment to Phage Surfaces for the Selection of Catalytic Activities. ChemBioChem 2011. 10.1002/cbic.201000475.

(164). Sunbul M; Marshall NJ; Zou Y; Zhang K; Yin J Catalytic Turnover-Based Phage Selection for Engineering the Substrate Specificity of Sfp Phosphopantetheinyl Transferase. J. Mol. Biol. 2009. 10.1016/jjmb.2009.02.010.

(165). Zou Y; Yin J Cu-Free Cycloaddition for Identifying Catalytic Active Adenylation Domains of Nonribosomal Peptide Synthetases by Phage Display. Bioorganic Med. Chem. Lett. 2008. 10.1016/j.bmcl.2008.08.085.

(166). Kang S; Uchida M; Oneil A; Li R; Prevelige PE; Douglas T Implementation of P22 Viral Capsids as Nanoplatforms. Biomacromolecules 2010. 10.1021/bml00877q.

(167). Parent KN; Deedas CT; Egelman EH; Casjens SR; Baker TS; Teschke CM Stepwise Molecular Display Utilizing Icosahedral and Helical Complexes of Phage Coat and Decoration Proteins in the Development of Robust Nanoscale Display Vehicles. Biomaterials 2012. 10.1016/j.biomaterials.2012.04.026.

(168). Wei B; Wei Y; Zhang K; Wang J; Xu R; Zhan S; Lin G; Wang W; Liu M; Wang L; Zhang R; Li J Development of an Antisense RNA Delivery System Using Conjugates of the MS2 Bacteriophage Capsids and HIV-1 TAT Cell Penetrating Peptide. Biomed. Pharmacother. 2009. 10.1016/j.biopha.2008.07.086.

(169). Tridgett M; Lloyd JR; Kennefick J; Moore-Kelly C; Dafforn TR Mutation of M13 Bacteriophage Major Coat Protein for Increased Conjugation to Exogenous Compounds. Bioconjug. Chem. 2018. 10.1021/acs.bioconjchem.8b00307.

(170). Larocca D; Witte A; Johnson W; Pierce GF; Baird A Targeting Bacteriophage to Mammalian Cell Surface Receptors for Gene Delivery. Hum. Gene Ther. 1998. 10.1089/hum.1998.9.16-2393.

(171). S. Zurier H; M. Duong M; M. Goddard J; R. Nugen S Engineering Biorthogonal Phage-Based Nanobots for Ultrasensitive, In Situ Bacteria Detection. ACS Appl. Bio Mater. 2020, 0 (0). 10.1021/acsabm.0c00546.

(172). Tian F; Tsao ML; Schultz PG A Phage Display System with Unnatural Amino Acids. J. Am. Chem. Soc. 2004. 10.1021/ja045673m.

(173). Strable E; Prasuhn DE; Udit AK; Brown S; Link AJ; Ngo JT; Lander G; Quispe J; Potter CS; Carragher B et al. Unnatural Amino Acid Incorporation into Virus-like Particles. Bioconjug. Chem. 2008. 10.1021/bc700390r.

(174). Prasuhn DE; Singh P; Strable E; Brown S; Manchester M; Finn MG Plasma Clearance of Bacteriophage Qβ Particles as a Function of Surface Charge. J. Am. Chem. Soc. 2008. 10.1021/ja075937f.

(175). Urquhart T; Daub E; Honek JF Bioorthogonal Modification of the Major Sheath Protein of Bacteriophage M13: Extending the Versatility of Bionanomaterial Scaffolds. Bioconjug. Chem. 2016. 10.1021/acs.bioconjchem.6b00460.

(176). Xie J; Schultz PG Adding Amino Acids to the Genetic Repertoire. Current Opinion in Chemical Biology. 2005. 10.1016/j.cbpa.2005.10.011.

(177). Ryu Y; Schultz PG Efficient Incorporation of Unnatural Amino Acids into Proteins in Escherichia Coli. Nat. Methods 2006. 10.1038/nmeth864.

(178). Smolskaya S; Zhang ZJ; Alfonta L Enhanced Yield of Recombinant Proteins with Site-Specifically Incorporated Unnatural Amino Acids Using a Cell-Free Expression System. PLoS One 2013. 10.1371/joumal.pone.0068363.

(179). Tompa P; Davey NE; Gibson TJ; Babu MM A Million Peptide Motifs for the Molecular Biologist. Molecular Cell. 2014. 10.1016/j.molcel.2014.05.032.

(180). Oślizlo A; Miemikiewicz P; Piotrowicz A; Owczarek B; Kopciuch A; Figura G; Dabrowska K Purification of Phage Display-Modified Bacteriophage T4 by Affinity Chromatography. BMC Biotechnol. 2011. 10.1186/1472-6750-11-59.

(181). O'Neil A; Reichhardt C; Johnson B; Prevelige PE; Douglas T Genetically Programmed *in Vivo* Packaging of Protein Cargo and Its Controlled Release from Bacteriophage P22. Angew. Chemie - Int. Ed 2011. 10.1002/anie.201102036.

(182). Chen J; Duncan B; Wang Z; Wang LS; Rotello VM; Nugen SR Bacteriophage-Based Nanoprobes for Rapid Bacteria Separation. Nanoscale 2015. 10.1039/c5nr03779d.

(183). Duffy S; Tsao KL; Waugh DS Site-Specific, Enzymatic Biotinylation of Recombinant Proteins in Spodoptera Frugiperda Cells Using Biotin Acceptor Peptides. Anal. Biochem. 1998. 10.1006/abio.1998.2770.

(184). Yoda K; Yamasaki M; Kojima H *In Vivo* Biotinylation of Fusion Proteins Expressed in Escherichia Coli with a Sequence of Propionibacterium Freudenreichii Transcarboxylase 1.3S Biotin Subunit. Biosci. Biotechnol. Biochem. 1992. 10.1271/bbb.56.1017.

(185). Gervais L; Gel M; Allain B; Tolba M; Brovko L; Zourob M; Mandeville R; Griffiths M; Evoy S Immobilization of Biotinylated Bacteriophages on Biosensor Surfaces. Sensors Actuators, B Chem. 2007. 10.1016/j.snb.2007.03.007.

(186). Edgar R; McKinstry M; Hwang J; Oppenheim AB; Fekete RA; Giulian G; Merril C; Nagashima K; Adhya S High-Sensitivity Bacterial Detection Using Biotin-Tagged Phage and Quantum-Dot Nanocomplexes. Proc. Natl. Acad. Sci. U. S. A 2006. 10.1073/pnas.0601211103.

(187). Wang Z; Wang D; Chen J; Sela DA; Nugen SR Development of a Novel Bacteriophage Based Biomagnetic Separation Method as an Aid for Sensitive Detection of Viable Escherichia Coli. Analyst 2016. 10.1039/c5an01769f.

(188). Bardhan NM; Ghosh D; Belcher AM M13 Virus Based Detection of Bacterial Infections in Living Hosts. J. Biophotonics 2014. 10.1002/jbio.201300010.

(189). Patterson D; Schwarz B; Avera J; Western B; Hicks M; Krugler P; Terra M; Uchida M; McCoy K; Douglas T Sortase-Mediated Ligation as a Modular Approach for the Covalent Attachment of Proteins to the Exterior of the Bacteriophage P22 Virus-like Particle. Bioconjug. Chem 2017. 10.1021/acs.bioconjchem.7b00296.

(190). Ton-That H; Liu G; Mazmanian SK; Faull KF; Schneewind O Purification and Characterization of Sortase, the Transpeptidase That Cleaves Surface Proteins of Staphylococcus Aureus at the LPXTG Motif. Proc. Natl. Acad. Sci. U. S. A 1999. 10.1073/pnas.96.22.12424.

(191). Antos JM; Chew GL; Guimaraes CP; Yoder NC; Grotenbreg GM; Popp MWL; Ploegh HL Site-Specific N- and C-Terminal Labeling of a Single Polypeptide Using Sortases of Different Specificity. J. Am. Chem. Soc. 2009. 10.1021/ja902681k.

(192). Race PR; Bentley ML; Melvin JA; Crow A; Hughes RK; Smith WD; Sessions RB; Kehoe MA; McCafferty DG; Banfield MJ Crystal Structure of Streptococcus Pyogenes Sortase A: Implications for Sortase Mechanism. J. Biol. Chem. 2009. 10.1074/jbc.M805406200.

(193). Hess GT; Cragnolini JJ; Popp MW; Allen MA; Dougan SK; Spooner E; Ploegh HL; Belcher AM; Guimaraes CP M13 Bacteriophage Display Framework That Allows Sortase-Mediated Modification of Surface-Accessible Phage Proteins. Bioconjug. Chem. 2012. 10.1021/bc300130z.

(194). Hess GT; Guimaraes CP; Spooner E; Ploegh HL; Belcher AM Orthogonal Labeling of M13 Minor Capsid Proteins with DNA to Self-Assemble End-to-End Multiphage Structures. ACS Synth. Biol. 2013. 10.1021/sb400019s.

(195). Chen L; Cohen J; Song X; Zhao A; Ye Z; Feulner CJ; Doonan P; Somers W; Lin L; Chen PR Improved Variants of SrtA for Site-Specific Conjugation on Antibodies and Proteins with High Efficiency. Sci. Rep. 2016. 10.1038/srep31899.

(196). Tao P; Li Q; Shivachandra SB; Rao VB Bacteriophage T4 as a Nanoparticle Platform to Display and Deliver Pathogen Antigens: Construction of an Effective Anthrax Vaccine. In Methods in Molecular Biology, 2017. 10.1007/978-l-4939-6869-5_15.

(197). Shivachandra SB; Rao M; Janosi L; Sathaliyawala T; Matyas GR; Alving CR; Leppla SH; Rao VB *In Vitro* Binding of Anthrax Protective Antigen on Bacteriophage T4 Capsid Surface through Hoc-Capsid Interactions: A Strategy for Efficient Display of Large Full-Length Proteins. Virology 2006. 10.1016/j.virol.2005.10.037.

(198). Shivachandra SB; Li Q; Peachman KK; Matyas GR; Leppla SH; Alving CR; Rao M; Rao VB Multicomponent Anthrax Toxin Display and Delivery Using Bacteriophage T4. Vaccine 2007. 10.1016/j.vaccine.2006.10.010.

(199). Jiang J; Abu-Shilbayeh L; Rao VB Display of a PorA Peptide from Neisseria Meningitidis on the Bacteriophage T4 Capsid Surface. Infect. Immun. 1997. 10.1128/iai.65.ll.4770-4777.1997.

(200). Qin L; Fokine A; O'Donnell E; Rao VB; Rossmann MG Structure of the Small Outer Capsid Protein, Soc: A Clamp for Stabilizing Capsids of T4-like Phages. J. Mol. Biol. 2010. 10.1016/jjmb.2009.10.007.

(201). Sathaliyawala T; Islam MZ; Li Q; Fokine A; Rossmann MG; Rao VB Functional Analysis of the Highly Antigenic Outer Capsid Protein, Hoc, a Virus Decoration Protein from T4-like Bacteriophages. Mol. Microbiol. 2010. 10.llll/j.1365-2958.2010.07219.x.

(202). Forrer P; Chang C; Ott D; Wlodawer A; Plückthun A Kinetic Stability and Crystal Structure of the Viral Capsid Protein SHP. J. Mol. Biol. 2004. 10.1016/jjmb.2004.09.030.

(203). Abello N; Kerstjens HAM; Postma DS; Bischoff R Selective Acylation of Primary Amines in Peptides and Proteins. J. Proteome Res. 2007. 10.1021/pr070154e.

(204). Schmidt A; Kellermann J; Lottspeich F A Novel Strategy for Quantitative Proteomics Using Isotope-Coded Protein Labels. Proteomics 2005. 10.1002/pmic.200400873.

(205). Marino SM; Gladyshev VN Cysteine Function Governs Its Conservation and Degeneration and Restricts Its Utilization on Protein Surfaces. J. Mol. Biol. 2010. 10.1016/jjmb.2010.09.027.

(206). Huang L; Wu L; Li X; Liu K; Zhao D;Xie HY Labeling and Single-Particle-Tracking-Based Entry Mechanism Study of Vaccinia Virus from the Tiantan Strain. Anal. Chem. 2018. 10.1021/acs.analchem.7b05183.

(207). Zhao X; Cai L; Adogla EA; Guan H; Lin Y; Wang Q Labeling of Enveloped Virus via Metabolic Incorporation of Azido Sugars. Bioconjug. Chem. 2015. 10.1021/acs.bioconjchem.5b00310.

(208). Huang LL; Liu K; Zhang Q; Xu J; Zhao D; Zhu H; Xie HY Integrating Two Efficient and Specific Bioorthogonal Ligation Reactions with Natural Metabolic Incorporation in One Cell for Virus Dual Labeling. Anal. Chem. 2017. 10.1021/acs.analchem.7b03043.

(209). Grimsley GR; Scholtz JM; Pace CN A Summary of the Measured PK Values of the Ionizable Groups in Folded Proteins. Protein Sci. 2009. 10.1002/pro.19.

(210). Chan AOY; Ho CM; Chong HC; Leung YC; Huang JS; Wong MK; Che CM Modification of N-Terminal α-Amino Groups of Peptides and Proteins Using Ketenes. J. Am. Chem. Soc. 2012. 10.1021/ja208009r.

(211). Jin X; Newton JR; Montgomery-Smith S; Smith GP A Generalized Kinetic Model for Amine Modification of Proteins with Application to Phage Display. Biotechniques 2009. 10.2144/000113074.

(212). Smith GP Kinetics of Amine Modification of Proteins. Bioconjug. Chem. 2006. 10.1021/bc0503061.

(213). Arya SK; Singh A; Naidoo R; Wu P; McDermott MT; Evoy S Chemically Immobilized T4-Bacteriophage for Specific Escherichia Coli Detection Using Surface Plasmon Resonance. Analyst 2011. 10.1039/c0an00697a.

(214). Mujumdar RB; Ernst LA; Mujumdar SR; Lewis CJ Cyanine Dye Labeling Reagents: Sulfoindocyanine Succinimidyl Esters. Bioconjug. Chem. 1993. 10.1021/bc00020a001.

(215). World Bank Group. Drug-Resistant Infections: A Threat to Our Economic Future. World Bank Rep. 2017. 10.1007/sll947-009-0181-3.

(216). Kelly KA; Waterman P; Weissleder R *In Vivo* Imaging of Molecularly Targeted Phage. Neoplasia 2006. 10.1593/neo.06610.

(217). Nakamura M; Tsumoto K; Ishimura K; Kumagai I A Visualization Method of Filamentous Phage Infection and Phage-Derived Proteins in *Escherichia Coli* Using Biotinylated Phages. Biochem. Biophys. Res. Commun. 2001. 10.1006/bbrc.2001.5973.

(218). Zhu J; Tao P; Mahalingam M; Rao V Preparation of a Bacteriophage T4-Based Prokaryotic-Eukaryotic Hybrid Viral Vector for Delivery of Large Cargos of Genes and Proteins into Human Cells. BIO-PROTOCOL 2020. 10.21769/bioprotoc.3573.

(219). Wang Z; Wang D; Kinchla AJ; Sela DA; Nugen SR Rapid Screening of Waterborne Pathogens Using Phage-Mediated Separation Coupled with Real-Time PCR Detection. Anal. Bioanal. Chem. 2016. 10.1007/s00216-016-9511-2.

(220). Peng H; Borg RE; Dow LP; Pruitt BL; Chen IA Controlled Phage Therapy by Photothermal Ablation of Specific Bacterial Species Using Gold Nanorods Targeted by Chimeric Phages. Proc. Natl. Acad. Sci. U. S. A 2020. 10.1073/pnas.1913234117.

(221). Sun W; Brovko L; Griffiths M Use of Bioluminescent Salmonella for Assessing the Efficiency of Constructed Phage-Based Biosorbent. J. Ind. Microbiol. Biotechnol. 2001. 10.1038/sj.jim.7000198.

(222). Li K; Chen Y; Li S; Nguyen HG; Niu Z; You S; Mello CM; Lu X; Wang Q Chemical Modification of M13 Bacteriophage and Its Application in Cancer Cell Imaging. Bioconjug. Chem. 2010. 10.1021/bc900405q.

(223). Newton JR; Miao Y; Deutscher SL; Quinn TP Melanoma Imaging with Pretargeted Bivalent Bacteriophage. J. Nucl. Med. 2007.

(224). Newton-Northup JR; Figueroa SD; Quinn TP; Deutscher SL Bifunctional Phage-Based Pretargeted Imaging of Human Prostate Carcinoma. Nucl. Med. Biol. 2009. 10.1016/j.nucmedbio.2009.04.010.

(225). Muzard J; Platt M; Lee GU M13 Bacteriophage-Activated Superparamagnetic Beads for Affinity Separation. Small 2012. 10.1002/smll.201200099.

(226). Dordi B; Schönherr H; Vancso GJ Reactivity in the Confinement of Self-Assembled Monolayers: Chain Length Effects on the Hydrolysis of N-Hydroxysuccinimide Ester Disulfides on Gold. Langmuir 2003. 10.1021/la0343066.

(227). Tournier EJM; Wallach J; Blond P Sulfosuccinimidyl 4-(N-Maleimidomethyl)-1-Cyclohexane Carboxylate as a Bifunctional Immobilization Agent. Optimization of the Coupling Conditions. Anal. Chim. Acta 1998. 10.1016/S0003-2670(98)00008-7.

(228). Grumbach IM; Veh RW Sulpho-N-Hydroxysuccinimide Activated Long Chain Biotin. A New Microtitre Plate Assay for the Determination of Its Stability at Different PH Values and Its Reaction Rate with Protein Bound Amino Groups. J. Immunol. Methods 1991. 10.1016/0022-1759(91)90372-M.

(229). Lockett MR; Phillips MF; Jarecki JL; Peelen D; Smith LM A Tetrafluorophenyl Activated Ester Self-Assembled Monolayer for the Immobilization of Amine-Modified Oligonucleotides. Langmuir 2008. 10.1021/la702493u.

(230). Coons AH; Creech HJ; Norman JR; Berliner E The Demonstration of Pneumococcal Antigen in Tissues by the Use of Fluorescent Antibody. J. Immunol. 1942.

(231). Tsao R; Yu Q; Friesen I; Potter J; Chiba M Factors Affecting the Dissolution and Degradation of Oriental Mustard- Derived Sinigrin and Allyl Isothiocyanate in Aqueous Media. J. Agric. Food Chem. 2000. 10.1021/jf9906578.

(232). Jayanna PK; Bedi D; Deinnocentes P; Bird RC; Petrenko VA Landscape Phage Ligands for PC3 Prostate Carcinoma Cells. Protein Eng. Des. Sel. 2010. 10.1093/protein/gzq011.

(233). Slootweg EJ; Keller HJHG; Hink MA; Borst JW; Bakker J; Schots A Fluorescent T7 Display Phages Obtained by Translational Frameshift. Nucleic Acids Res. 2006. 10.1093/nar/gkl600.

(234). Anderson EA; Isaacman S; Peabody DS; Wang EY; Canary JW; Kirshenbaum K Viral Nanoparticles Donning a Paramagnetic Coat: Conjugation of MRI Contrast Agents to the MS2 Capsid. Nano Lett. 2006. 10.1021/nl060378g.

(235). Gilmore JM; Scheck RA; Esser-Kahn AP; Joshi NS; Francis MB N-Terminal Protein Modification through a Biomimetic Transamination Reaction. Angew. Chemie - Int. Ed. 2006. 10.1002/anie.200600368.

(236). Jencks WP Studies on the Mechanism of Oxime and Semicarbazone Formation. J. Am. Chem. Soc. 1959. 10.1021/ja01511a053.

(237). Palaniappan KK; Ramirez RM; Bajaj VS; Wemmer DE; Pines A; Francis MB Molecular Imaging of Cancer Cells Using a Bacteriophage-Based 129Xe NMR Biosensor. Angew. Chemie - Int. Ed 2013. 10.1002/anie.201300170.

(238). Witus LS; Moore T; Thuronyi BW; Esser-Kahn AP; Scheck RA; Iavarone AT; Francis MB Identification of Highly Reactive Sequences for PLP-Mediated Bioconjugation Using a Combinatorial Peptide Library. J. Am. Chem. Soc. 2010. 10.1021/ja105429n.

(239). Cammarata CR; Hughes ME; Ofner CM Carbodiimide Induced Cross-Linking, Ligand Addition, and Degradation in Gelatin. Mol. Pharm. 2015. 10.1021/mp5006118.

(240). Yamada H; Imoto T; Fujita K; Okazaki K; Motomura M Selective Modification of Aspartic Acid-101 in Lysozyme by Carbodiimide Reaction. Biochemistry 1981. 10.1021/bi00520a005.

(241). Lee JH; Cha JN Amplified Protein Detection through Visible Plasmon Shifts in Gold Nanocrystal Solutions from Bacteriophage Platforms. Anal. Chem. 2011. 10.1021/ac200222d.

(242). Yacoby I; Bar H; Benhar I Targeted Drug-Carrying Bacteriophages as Antibacterial Nanomedicines. Antimicrob. Agents Chemother. 2007. 10.1128/AAC.00163-07.

(243). Vaks L; Benhar I In Vivo Characteristics of Targeted Drug-Carrying Filamentous Bacteriophage Nanomedicines. J. Nanobiotechnology 2011. 10.1186/1477-3155-9-58.

(244). Staros JV; Wright RW; Swingle DM Enhancement by N-Hydroxysulfosuccinimide of Water-Soluble Carbodiimide-Mediated Coupling Reactions. Anal. Biochem. 1986. 10.1016/0003-2697(86)90176-4.

(245). Suthiwangcharoen N; Li T; Li K; Thompson P; You S; Wang Q M13 Bacteriophage-Polymer Nanoassemblies as Drug Delivery Vehicles. Nano Res. 2011. 10.1007/s12274-011-0104-2.

(246). Yacoby I; Shamis M; Bar H; Shabat D; Benhar I Targeting Antibacterial Agents by Using Drug-Carrying Filamentous Bacteriophages. Antimicrob. Agents Chemother. 2006. 10.1128/AAC.00169-06.

(247). Niu Z; Bruckman MA; Harp B; Mello CM; Wang Q Bacteriophage M13 as a Scaffold for Preparing Conductive Polymeric Composite Fibers. Nano Res. 2008. 10.1007/s12274-008-8027-2.

(248). Brotzel F; Mayr H Nucleophilicities of Amino Acids and Peptides. Org. Biomol. Chem. 2007. 10.1039/b713778h.

(249). Getz EB; Xiao M; Chakrabarty T; Cooke R; Selvin PR A Comparison between the Sulfhydryl Reductants Tris(2-Carboxyethyl)Phosphine and Dithiothreitol for Use in Protein Biochemistry. Anal. Biochem. 1999. 10.1006/abio.1999.4203.

(250). Braakman I; Helenius J; Helenius A Manipulating Disulfide Bond Formation and Protein Folding in the Endoplasmic Reticulum. EMBO J. 1992. 10.1002/j.1460-2075.1992.tb05223.x.

(251). Martínez-Jothar L; Doulkeridou S; Schiffelers RM; Sastre Torano J; Oliveira S; van Nostrum CF; Hennink WE Insights into Maleimide-Thiol Conjugation Chemistry: Conditions for Efficient Surface Functionalization of Nanoparticles for Receptor Targeting. J. Control. Release 2018. 10.1016/jjconrel.2018.03.002.

(252). Hermanson G Bioconjugate Techniques, 2nd ed.; Academic Press, 2008.

(253). Peabody DS A Viral Platform for Chemical Modification and Multivalent Display. J. Nanobiotechnology 2003. 10.1186/1477-3155-1-5.

(254). Di Felice R; Selloni A; Molinari E DFT Study of Cysteine Adsorption on Au(111). J. Phys. Chem. B 2003. 10.1021/jp0272421.

(255). Vallee A; Humblot V; Pradier CM Peptide Interactions with Metal and Oxide Surfaces. Acc. Chem. Res. 2010. 10.1021/ar100017n.

(256). Xue Y; Li X; Li H; Zhang W Quantifying Thiol-Gold Interactions towards the Efficient Strength Control. Nat. Commun. 2014. 10.1038/ncomms5348.

(257). Korkmaz N Recombinant Bacteriophages as Gold Binding Bio-Templates. Colloids Surfaces B Biointerfaces 2013. 10.1016/j.colsurfb.2013.07.063.

(258). Leier S; Richter S; Bergmann R; Wuest M; Wuest F Radiometal-Containing Aryl Diazonium Salts for Chemoselective Bioconjugation of Tyrosine Residues. ACS Omega 2019. 10.1021/acsomega.9b03248.

(259). Nimni ME; Cheung D; Strates B; Kodama M; Sheikh K Chemically Modified Collagen: A Natural Biomaterial for Tissue Replacement. J. Biomed. Mater. Res. 1987. 10.1002/jbm.820210606.

(260). Walt DR; Agayn VI The Chemistry of Enzyme and Protein Immobilization with Glutaraldehyde. TrAC - Trends in Analytical Chemistry. 1994. 10.1016/0165-9936(94)85023-2.

(261). Migneault I; Dartiguenave C; Bertrand MJ; Waldron KC Glutaraldehyde: Behavior in Aqueous Solution, Reaction with Proteins, and Application to Enzyme Crosslinking. BioTechniques. 2004. 10.2144/04375rv01.

(262). Hopwood D; Allen CR; McCabe M The Reactions between Glutaraldehyde and Various Proteins. An Investigation of Their Kinetics. Histochem. J. 1970. 10.1007/BF01003541.

(263). Avrameas S; Ternynck T The Cross-Linking of Proteins with Glutaraldehyde and Its Use for the Preaparation of Immunoadsorbernts. Immunochemistry 1969. 10.1016/0019-2791(69)90178-5.

(264). Zhu G; Zhao P; Deng N; Tao D; Sun L; Liang Z; zhang L; Zhang Y Single Chain Variable Fragment Displaying M13 Phage Library Functionalized Magnetic Microsphere-Based Protein Equalizer for Human Serum Protein Analysis. Anal. Chem. 2012. 10.1021/ac3017746.

(265). Geoghegan KF; Stroh JG Site-Directed Conjugation of Nonpeptide Groups to Peptides and Proteins Via Periodate Oxidation of a 2-Amino Alcohol. Application to Modification at N-Terminal Serine. Bioconjug. Chem 1992. 10.1021/bc00014a008.

(266). Dirksen A; Hackeng TM; Dawson PE Nucleophilic Catalysis of Oxime Ligation. Angew. Chemie - Int. Ed. 2006. 10.1002/anie.200602877.

(267). Ng S; Jafari MR; Matochko WL; Derda R Quantitative Synthesis of Genetically Encoded Glycopeptide Libraries Displayed on M13 Phage. ACS Chem. Biol. 2012. 10.1021/cb300187t.

(268). Kitov PI; Vinals DF; Ng S; Tjhung KF; Derda R Rapid, Hydrolytically Stable Modification of Aldehyde-Terminated Proteins and Phage Libraries. J. Am. Chem. Soc. 2014. 10.1021/ja5023909.

(269). Saleh L; Noren CJ Chapter 9. Site-Directed Chemical Modification of Phage Particles; 2011. 10.1039/9781847559920-00202.

(270). Sandman KE; Banner JS; Noren CJ Phage Display of Selenopeptides [6]. Journal of the American Chemical Society. 2000. 10.1021/ja992462m.

(271). Hondal RJ; Nilsson BL; Raines RT Selenocysteine in Native Chemical Ligation and Expressed Protein Ligation. Journal of the American Chemical Society. 2001. 10.1021/ja005885t.

(272). Tookmanian EM; Fenlon EE; Brewer SH Synthesis and Protein Incorporation of Azido-Modified Unnatural Amino Acids. RSC Adv. 2015. 10.1039/c4ra14244f.

(273). Lang K Installing Terminal-Alkyne Reactivity into Proteins in Engineered Bacteria. Biochemistry. 2019. 10.1021/acs.biochem.9b00392.

(274). Kiick KL; Tirrell DA Protein Engineering by *in Vivo* Incorporation of Non-Natural Amino Acids: Control of Incorporation of Methionine Analogues by Methionyl-TRNA Synthetase. Tetrahedron 2000. 10.1016/S0040-4020(00)00833-4.

(275). Kolb HC; Finn MG; Sharpless KB Click Chemistry: Diverse Chemical Function from a Few Good Reactions. Angewandte Chemie - International Edition. 2001. 10.1002/1521-3773(20010601)40:11<2004::AID-ANIE2004>3.0.C0;2-5.

(276). Carrico ZM; Romanini DW; Mehl RA; Francis MB Oxidative Coupling of Peptides to a Virus Capsid Containing Unnatural Amino Acids. Chem. Commun. 2008. 10.1039/b717826c.

(277). Aanei IL; Elsohly AM; Farkas ME; Netirojjanakul C; Regan M; Taylor Murphy S; O'Neil JP; Seo Y; Francis MB Biodistribution of Antibody-MS2 Viral Capsid Conjugates in Breast Cancer Models. Mol. Pharm. 2016. 10.1021/acs.molpharmaceut.6b00566.

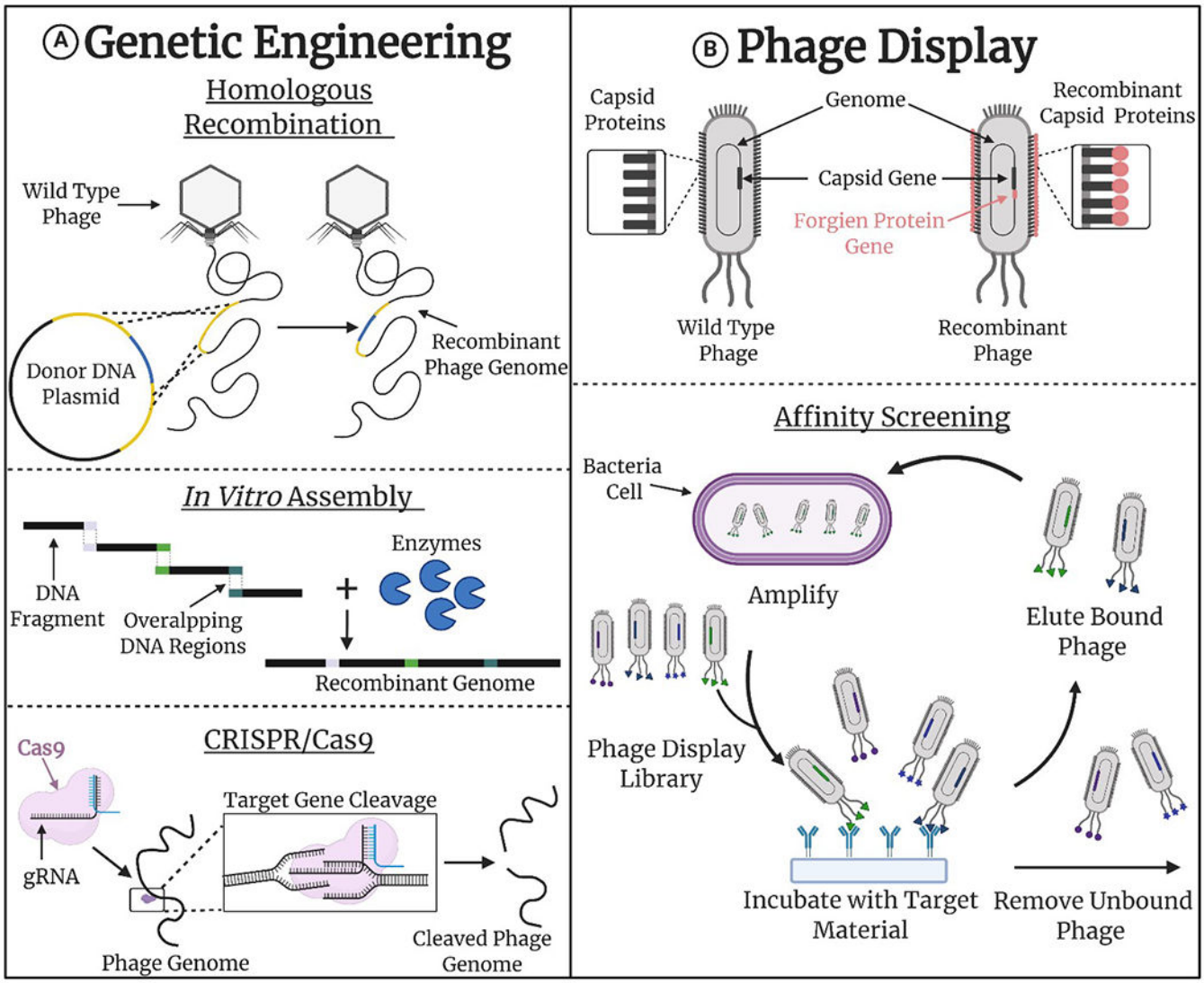

Figure 1.

Genetic approaches for modified phage capsid engineering, display, and screening. (A) The main approaches for engineering phage genes are highlighted. In homologous recombination, a plasmid containing a donor DNA insert flanked by regions of homology to the desired insert site can be used to facilitate donor DNA insertion into a wild type phage genome to generate a recombinant phage. For *in vitro* assembly, phage genome fragments synthesized with overlapping ends can be stitched together with the aid of enzymes to construct a recombinant phage genome outside of the bacteria cell. In CRISPR/Cas9 systems, an enzyme-RNA complex can be used to specifically cleave a target sequence in the phage genome to increase the rate of recombination with donor DNA or select out wild type phages. (B) In phage display, genetic engineering is used to fuse an amino acid, peptide, or protein sequence to phage capsid gene resulting in display of the foreign gene product on the phage capsid. In affinity screening, repeated rounds of selection can be used to identify recombinant capsid sequences with strong affinity to the desired target from phage display libraries.

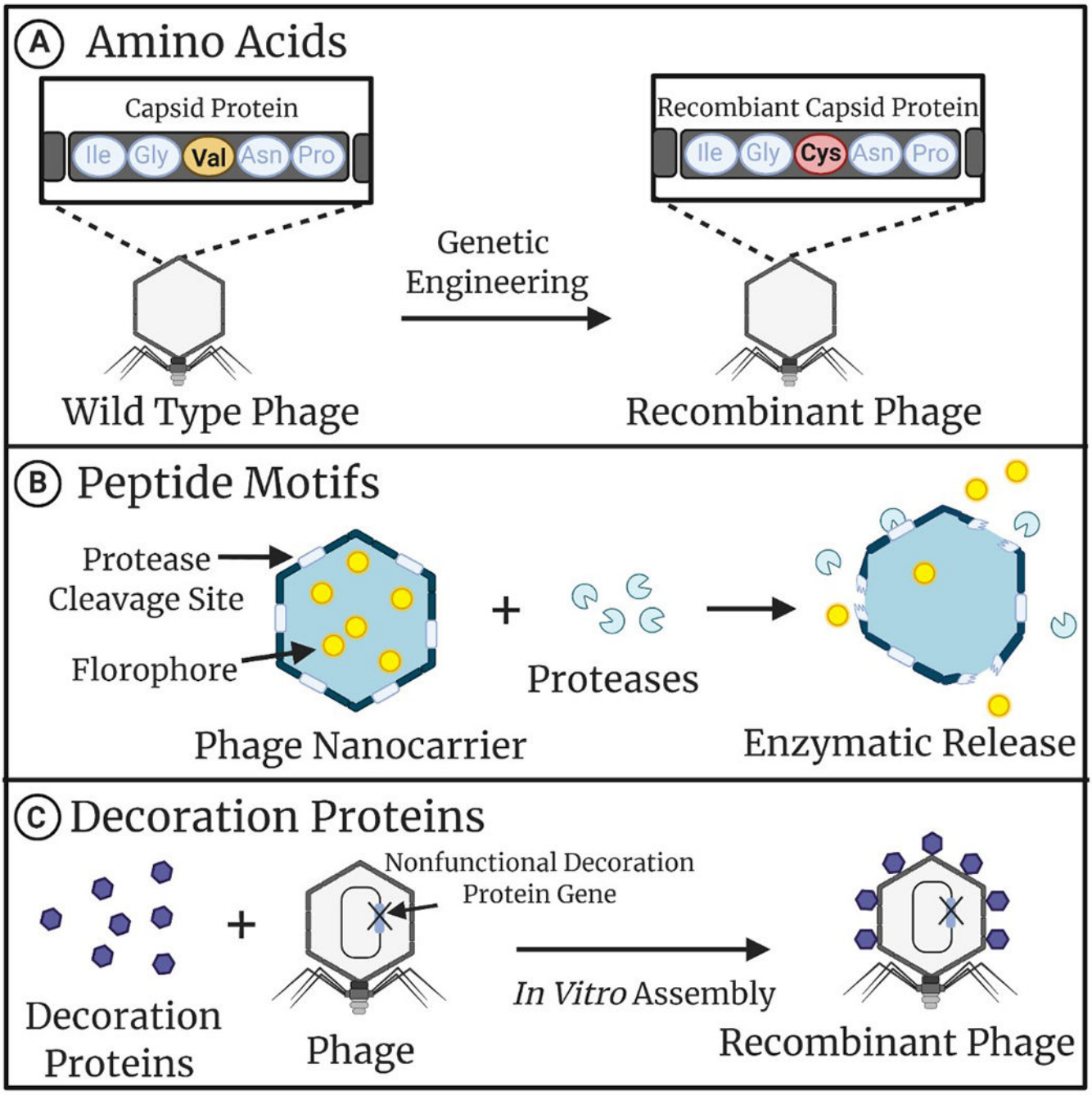

Figure 2.

Genetic modifications to phage capsids. (A) Single amino acids in phage capsids can be substituted to alter the number and type of functional groups accessible for downstream chemical modification. (B) Peptide motifs recognized by specific enzymes can be incorporated into phage capsids for downstream enzymatic modification or controlled release of contents. (C) Recombinant capsid decoration proteins can be synthesized separately from the phage and assembled to the capsid *in vitro*, allowing for large complex proteins to be displayed.

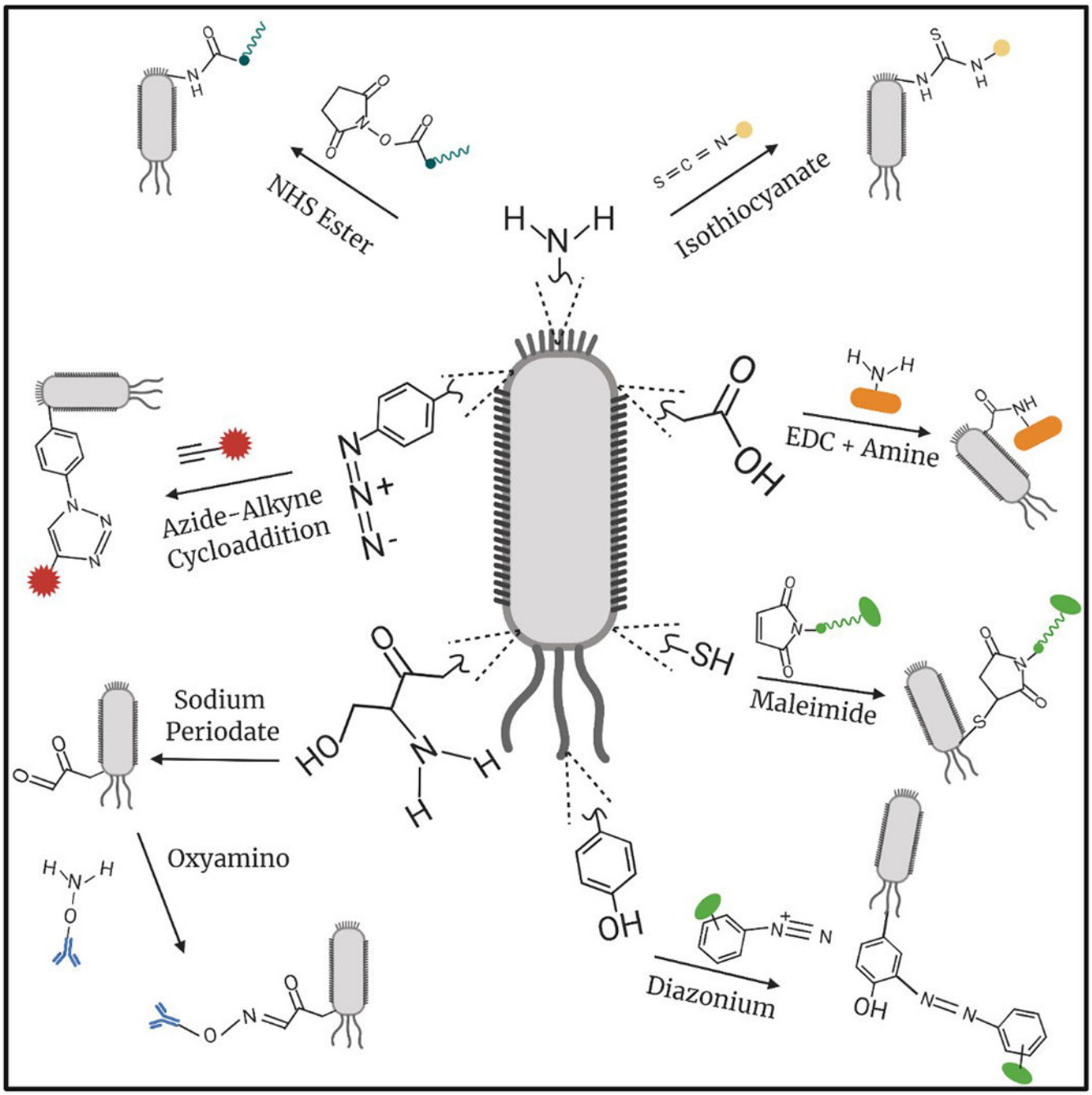

Figure 3.

Chemical modifications of filamentous phage capsids. Functional groups present on amino acids or unnatural amino acids can be utilized to add desired conjugates to phage capsids in a semi-selective manner. Though depicted on a filamentous phage capsid, these modification chemistries can be applied to other phage capsid architectures.

Table 1.

Modified Phage Capsids Summary

GENETIC	
Protease Cleavage Site Addition	AcTEV protease mediated affinity tag removal[180] Cathepsin B mediated release of chemotherapeutic[49] Thrombin mediated release of fluorescent protein[181]
Biotin Carboxyl Carrier Protein Addition	Horseradish peroxidase conjugation[57] Immobilization on gold surfaces[185] Antibody conjugation[188] Quantum dot conjugation[186] Immobilization on magnetic beads[187]
Sortase Recognition Motif Addition	Green fluorescent protein conjugation[189,193] Influenza antigen conjugation[189] Biotin conjugation[193]
In Vitro Display	Anthrax protective antigen conjugation[197] Anthrax protective antigen, lethal factor, and edema factor conjugation[198] *Neisseria meningitidis* PorA peptide conjugation[199] Foot and mouth disease virus capsid precursor polyprotein or proteinase peptide conjugation[52] Human immunodeficiency virus antigens gp24, Nef, and gp41 conjugation[53] Classical swine fever virus primary antigen and major antigenic determinant cluster conjugation[54]
CHEMICAL	
NHS Ester	PEG conjugation[40] Fluorescent dye conjugation[199,201] Biotin or PEG-Biotin conjugation[200,203,206,207]
NHS Ester; Oxime Formation	Magnetic resonance contrast agent conjugation[74]
NHS Ester; Metal Binding	Gold nanorod conjugation[220]
Sulfo-NHS Ester	Biotin conjugation[221]
TFP Ester	Fluorescent dye conjugation[232,233]
Isothiocyanate	Fluorescent dye conjugation[216,234]
EDC + NHS Ester Coupling	Immobilization to superparamagnetic particles[225]
DCC + NHS Ester Coupling	Chloramphenicol conjugation[246]
EDC + Sulfo-NHS Ester Coupling	Doxorubicin conjugation[49] Fluorophore conjugation[222] Folic acid conjugation[245]
EDC Coupling	Cysteamine conjugation[241] Chloramphenicol conjugation[242,243]
Diazonium; Copper Catalyzed Azide-Alkyne Cycloaddition	Biotin or folate conjugation[222]
Diazonium; Oxime Formation	Magnetic resonance contrast agent conjugation[74]
PLP Transamination; Oxime Formation	PEG conjugation[51] Fluorescent dye conjugation[51] Xe binding molecule conjugation[237]
Glutaric Anhydride Addition	Polymer conjugation[247]
Metal Bonding	Gold nanoparticle conjugation[241,257]
Glutaraldehyde Crosslinking	Immobilization on amino acid functionalized gold surfaces[110] Immobilization on amine functionalized magnetic microspheres[264]

Sodium Periodate Oxidation; Oxime Formation	Biotin conjugation[267] Mannose conjugation[267]
2-Amino Benzamidoxime	Biotin conjugation[268]
HYBRID *	
Amino Acid Substitution; Maleimide	Biotin or PEG-Biotin conjugation[166,170] Fluorescent dye conjugation[76,167,169,253]
Amino Acid Substitution; Sulfo-NHS Ester; Maleimide	Crosslinking cell penetrating peptides to phage capsids[168]
Amino Acid Substitution; Isothiocyanate	Fluorescent dye conjugation[169]
Sortase Recognition Motif Addition; Maleimide	Fluorescent dye conjugation[194] DNA conjugation[194]
Unnatural Amino Acid Addition; Selenide-Sulfide Crosslinking	Biotin conjugation[269,270]
Unnatural Amino Acid Addition; Copper Catalyzed Azide- Alkyne Cycloaddition	Gold nanoparticle conjugation[175] Fluorescent dye conjugation[172] Magnetic bead conjugation[171]
Unnatural Amino Acid Addition; Sodium Periodate Oxidation	Cell penetrating peptide conjugation[276] Antibody conjugation[277]

Left column is the modification method used. Right column is the application.

* Hybrid includes genetic and chemical modification methods.