tant BCL-X_L protein that has reduced affinity for gossypol, consistent with the previous demonstration of BH3-dependent association of BCL-G(S) with BCL-X_L. Cytotoxic activity of apogossypol is also demonstrated against chronic lymphocytic leukemia (CLL) cells derived from untreated and treatment-refractory patients. As CLL is most commonly associated with high expression levels of BCL-2, it is probable that apogossypol inhibits both BCL-X_L and BCL-2. The current generation of BCL-2 inhibitors appear to have similar binding affinities for BCL-2 and BCL-X_L, suggesting that conserved features of the binding pockets are being sampled. An important test of the static and dynamic features of the binding models will be the development of inhibitors with greater specificity for individual survival proteins.

With several BCL-X_L/BCL-2 antagonists now available [14], important questions for the functions of these proteins can be better addressed. Are proapoptotic members required for the lethal effects of BCL-2 inhibition? What are their effects on isolated mitochondria? Is there more than one class of chemical inhibitors? Different pharmacophores? Is chemical inhibition different from acute loss of expression (using antisense or RNAi methods)? The answers to these and other questions are eagerly awaited.

David Hockenbery
University of Washington
Box 358080
Fred Hutchinson Cancer Research Center
1100 Fairview Avenue North, C3-155
Seattle, Washington 98104

Selected Reading

1. Beccatini, B., Kitada, S., Leone, M., Monosov, E., Chandler, S., Zhai, D., Kipps, T.J., Reed, J.C., and Pellecchi, M. (2004). Chem. Biol. *11*, 389–395.
2. Amundson, S.A., Myers, T.G., Scudiero, D., Kitada, S., Reed, J.C., and Fornace, A.J., Jr. (2000). Cancer Res. *60*, 6101–6110.
3. Toogood, P.L. (2002). J. Med. Chem. *45*, 1543–1548.
4. Sattler, M., Liang, H., Nettesheim, D., Meadows, R.P., Harlan, J.E., Eberstadt, M., Yoon, H.S., Shuker, S.B., Chang, B.S., Minn, A.J., et al. (1997). Science *275*, 983–986.
5. Cheng, E.H., Wei, M.C., Weiler, S., Flavell, R.A., Mak, T.W., Lindsten, T., and Korsmeyer, S.J. (2001). Mol. Cell *8*, 705–711.
6. Wang, J.L., Liu, D., Zhang, Z.J., Shan, S., Han, X., Srinivasula, S.M., Croce, C.M., Alnemri, E.S., and Huang, Z. (2000). Proc. Natl. Acad. Sci. USA *97*, 7124–7129.
7. Degterev, A., Lugovskoy, A., Cardone, M., Mulley, B., Wagner, G., Mitchison, T., and Yuan, J. (2001). Nat. Cell Biol. *3*, 173–182.
8. Tzung, S.P., Kim, K.M., Basanez, G., Giedt, C.D., Simon, J., Zimmerberg, J., Zhang, K.Y., and Hockenbery, D.M. (2001). Nat. Cell Biol. *3*, 183–191.
9. Hsu, Y.T., and Youle, R.J. (1997). J. Biol. Chem. *272*, 13829–13834.
10. Vander Heiden, M.G., and Thompson, C.B. (1999). Nat. Cell Biol. *1*, E209–E216.
11. Manion, M.K., O'Neill, J.W., Giedt, C.D., Kim, K.M., Zhang, K.Y., and Hockenbery, D.M. (2004). J. Biol. Chem. *279*, 2159–2165.
12. Kitada, S., Leone, M., Sareth, S., Zhai, D., Reed, J.C., and Pellecchia, M. (2003). J. Med. Chem. *46*, 4259–4264.
13. Lugovskoy, A.A., Degterev, A.I., Fahmy, A.F., Zhou, P., Gross, J.D., Yuan, J., and Wagner, G. (2002). J. Am. Chem. Soc. *124*, 1234–1240.
14. O'Neill, J.W., and Hockenbery, D.M. (2003). Curr. Med. Chem. *10*, 1553–1562.

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Nano-Tailoring: Stitching Alterations on Viral Coats

Growing interest in utilizing protein assemblies for nanomaterials applications has spawned efforts to customize these scaffolds. Viral capsids have been modified with new chemical functionalities, typically at lysine or cysteine residues. Two innovative studies describe approaches to introduce modifications at virion tyrosine residues [1, 2].

The quest to create new nanometer-sized chemical architectures continues to accelerate, primarily along three broad trajectories: (1) top-down designs, using microlithographic and other techniques to embed increasingly smaller features into macroscopic materials; (2) bottom-up designs, using the techniques of supramolecular chemistry to control the self-assembly of multiple constituent molecules into well-defined complexes; and (3), nanoscale redesigns, utilizing existent biogenic assemblies of the proper dimension and altering their chemical composition to attain a desired structure and/or function.

Viruses are intrinsically attractive scaffolds for nanoscale constructions because they are predisposed for self-assembly to form highly stable symmetrical structures with dimensions in the tens of nanometers. The detailed three-dimensional structures are often known from X-ray crystallography, and it is possible to introduce specific positions of chemical reactivity on viral proteins through standard site-directed mutagenesis protocols. Virions can even be produced cost effectively on the gram scale. Self-assembly does not end at the level of the individual capsid; in many cases, the viral particles can be readily crystallized, even after modification, leading to organization of nanoblock arrays on the millimeter scale. A number of research groups are rapidly expanding the repertoire for nanochemistry on biological scaffolds [3]. Substantial effort has been devoted to utilizing biopolymers as templates for organized formation of inorganic materials [4, 5]. Recently, progress

Table 1.

Protein Assemblies Used for Nanometer-Scaled Scaffolds	Substituents Coupled to Protein-Based Scaffolds
cowpea mosaic virus (CPMV)	fluorescent dyes
M13 bacteriophage	biotin
tobacco mosaic virus (TMV)	carbohydrates (e.g., mannose)
cowpea chlorotic mottle virus (CCMV)	antigenic groups (e.g., stilbene)
bacteriophage MS2	gold nanoparticles
filamentous bacteriophage (Ff and Fd)	semiconductor materials (e.g., GaAs, ZnS, and CdS)
hepatitis B virus (HBV)	magnetic materials (e.g., FePt, CoPt)
immunoglobulins	peptide libraries
C_4-symmetric tetrameric aldolase (RhuA)	polyethylene glycol
leucine zipper proteins	
chaperonin	
ferritin	

has been made in the use of protein assemblies as covalent reagents, including the conjugation of an increasing variety of pendant groups, efforts to introduce new synthetic routes for addressing alternative chemical functionalities, and the development of new biological scaffolds to embellish.

Discoveries in the Finn group have featured covalent modifications to the cowpea mosaic virus (CPMV), an icosahedral assembly comprised of 60 copies of a large and small protein subunit. Standard maleimide or bromoacetamide reagents have been used to introduce fluorescent dyes at cysteine residues, while N-hydroxysuccinimide esters and isothiocyanate reagents have been conjugated at lysine residues [6–8]. The absence of cysteine residues on the outside surface combined with the somewhat porous nature of the virion structure allowed for a small thiol-specific reagent to modify all 60 copies of a specific cysteine residue on the interior surface of the capsid exclusively. Development of a mutant CPMV with an additional solvent-exposed cysteine residue allowed the attachment of fluorophores on the exterior surface [8].

A variety of different moieties have now been displayed by conjugation to viral particles (Table 1). These include antigenic groups (stilbene) [6], biotin [7, 8], gold clusters [8], carbohydrates [9], and polyethylene glycol [10]. Viruses have been used to synthesize and assemble inorganic materials for the formation of nanowires, nanotubes, and liquid crystals [4, 5]. Alternatively, engineered viruses displaying binding peptides or reactive thiols can be immobilized upon patterned inorganic substrates [11].

In the long term, the usefulness of virus-based scaffolds as building blocks for nanoscale assemblies may depend on two criteria: (1) the stability of the overall assembly, and (2) the access to orthogonal chemistries that allow introduction of specific modifications at distinct sites on the viral particles. Recent advancements from the Finn group and the Francis group show that it is also possible to selectively utilize tyrosine residues to attach olefin and disulfide substrates onto these protein assemblies (see Figure 1).

Francis and coworkers describe an efficient strategy based on a hetero Diels-Alder reaction for the attachment of olefin substrates to modified tyrosine residues on bacteriophage MS2 [2]. A three-step synthetic sequence was used to convert tyrosine residues on the phage particle first to *o*-amino tyrosine and then to *o*-imino-quinone, which served as the diene platform for the subsequent Diels-Alder reaction.

In a related approach, the Finn group utilized mild oxidation chemistry to prepare tyrosyl radicals that se-

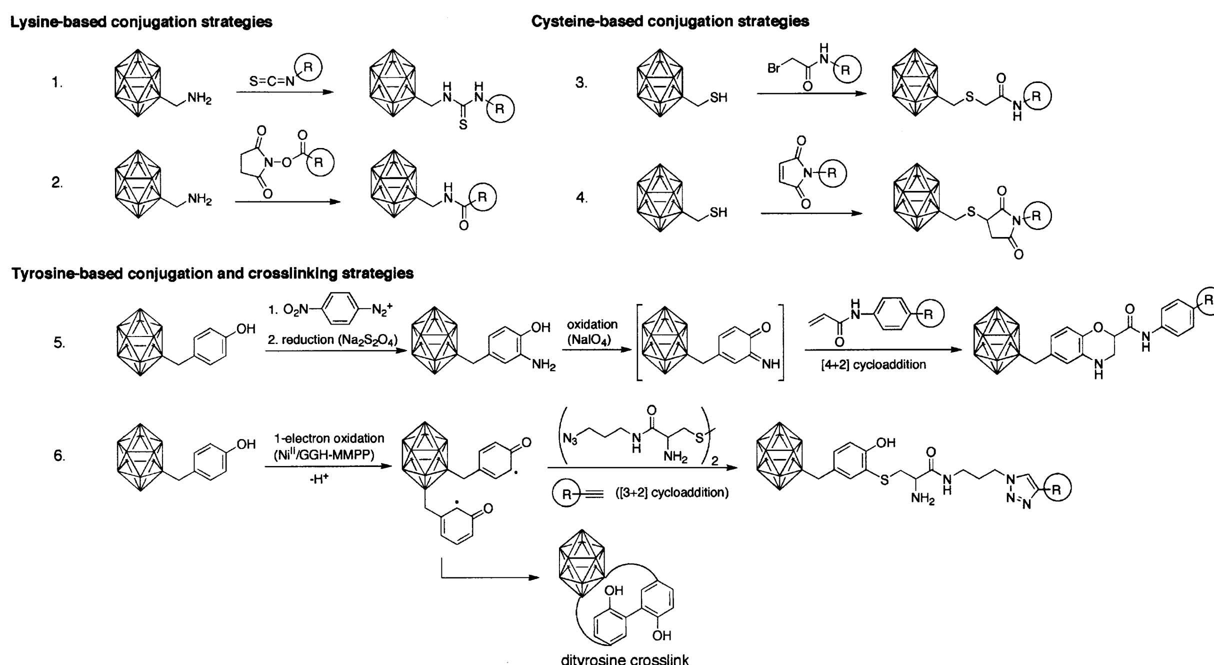

Figure 1. Strategies for Specific Chemical Modification of Viral Particles to Generate Virus-Based Scaffolds for Nanomaterials

lectively react with fuctionalized disulfides [1]. This method was used to append azide groups on CPMV. The azide groups were then utilized in a 1,3 dipolar cycloaddition reaction [12] with modified alkynes to affix the desired functionality on the virus. Importantly, in this study Finn and coworkers also discovered that it is possible to stitch a virus particle together at adjacent subunits through the reaction of a neighboring pair of tyrosine residues to form dityrosine crosslinks. It is puzzling that despite extensive "darning" of the viral coat with such intersubunit crosslinks, these particles were not observed to be more stable than the unmodified virions. Given the fairly rugged nature of CPMV, this finding is probably more of a curiosity than a concern and can probably be rectified by directing crosslink formation at more labile positions on the capsid. Overall, the studies by the Finn and Francis groups significantly increase the diversity of available reactions that can be utilized to decorate virus particles.

Potential applications of viruses as chemical reagents include the formation of nanometer-scale circuitry upon the appropriate immobilization of metal particles [5, 13], the ability to encapsulate drugs or inorganic species in viral cages [14], the possibility of creating isolated catalytic chambers inside viral capsids, and the use of virions as scaffolds for the polyvalent display of bioactive ligands [15, 16].

What lies beyond modification of virions at lysine, cysteine, aspartic acid, glutamic acid, and now tyrosine? The palette for decorating reactive residues on viral proteins has become quite robust. Further developments may await techniques for the introduction of nonnatural amino acids into viral proteins, which could potentially enable a new suite of synthetic strategies [17]. In addition, there is a wide diversity of nanometer-scaled protein scaffolds other than viruses, such as chaperonins. Now some of these are also being modified [18, 19], along with self-assembled DNA arrays [20].

We can eagerly anticipate that chemists will continue to exploit virus particles for nanotechnology applications. After all the sustenance higher organisms have provided viruses, isn't it about time they did something for us?

Paramjit S. Arora and Kent Kirshenbaum
Department of Chemistry
New York University
100 Washington Sq. E., Room 1001
New York, New York 10003

Selected Reading

1. Meunier, S., Strable, E., and Finn, M.G. (2004). Chem. Biol. *11*, 319–326.
2. Hooker, J.M., Kovacs, E.W., and Francis, M.B. (2004). J. Am. Chem. Soc. *126*, 3718–3719.
3. Niemeyer, C.M. (2001). Angew. Chem. Int. Ed. Engl. *40*, 4128–4158.
4. Douglas, T., and Young, M. (1999). Adv. Mater. *11*, 679–681.
5. Flynn, C.E., Lee, S.-W., Peelle, B.R., and Belcher, A.M. (2003). Acta Mater. *51*, 5867–5880.
6. Wang, Q., Lin, T., Tang, L., Johnson, J.E., and Finn, M.G. (2002). Angew. Chem. Int. Ed. Engl. *41*, 459–462.
7. Wang, Q., Kaltgrad, E., Lin, T.W., Johnson, J.E., and Finn, M.G. (2002). Chem. Biol. *9*, 805–811.
8. Wang, Q., Lin, T.W., Johnson, J.E., and Finn, M.G. (2002). Chem. Biol. *9*, 813–819.
9. Raja, K.S., Wang, Q., and Finn, M.G. (2003). Chembiochem *4*, 1348–1351.
10. Raja, K.S., Wang, Q., Gonzalez, M.J., Manchester, M., Johnson, J.E., and Finn, M.G. (2003). Biomacromolecules *4*, 472–476.
11. Smith, J.C., Lee, K.-B., Wang, Q., Finn, M.G., Johnson, J.E., Mrksich, M., and Mirkin, C.A. (2003). Nano Lett. *3*, 883–886.
12. Wang, Q., Chan, T.R., Hilgraf, R., Fokin, V.V., Sharpless, K.B., and Finn, M.G. (2003). J. Am. Chem. Soc. *125*, 3192–3193.
13. Mao, C., Solis, D.J., Reiss, B.D., Kottmann, S.T., Sweeney, R.Y., Hayhurst, A., Georgiou, G., Iverson, B., and Belcher, A.M. (2004). Science *303*, 213–217.
14. Douglas, T., and Young, M. (1998). Nature *393*, 152–155.
15. Raja, K.S., Wang, Q., and Finn, M.G. (2003). Chembiochem *4*, 1348–1351.
16. Gillitzer, E., Willits, D., Young, M., and Douglas, T. (2002). Chem. Commun. 2390–2391.
17. Link, A.J., Mock, M.L., and Tirrell, D.A. (2003). Curr. Opin. Biotechnol. *14*, 603–609.
18. McMillan, R.A., Paavola, C.D., Howard, J., Chan, S.L., Zaluzec, N.J., and Trent, J.D. (2002). Nat. Mater. *1*, 247–252.
19. Flenniken, M.L., Willits, D.A., Brumfield, S., Young, M.J., and Douglas, T. (2003). Nano Lett. *3*, 1573–1576.
20. Yan, H., Park, S.H., Finkelstein, G., Reif, J.H., and LaBean, T.H. (2003). Science *301*, 1882–1884.

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Heparin-Induced Cancer Cell Death

Heparin uptake into cancer cells can be promoted by conjugation to poly (β-amino ester)s. Internalized heparin is cytotoxic, causing cancer cell death by interfering with transcription factor activity and inducing apoptosis, but only certain poly(β-amino ester)s promote this activity.

The heparin polysaccharide is among the most acidic of all natural products with a single molecule carrying from 75 to 100 negative charges [1]. As a result, heparin interacts with a large number of proteins and other basic molecules through ionic and hydrogen bonding interactions [2]. A widely used therapeutic anticoagulant, heparin is biosynthesized and stored intracellularly exclusively in mast cells. Mast-cell-rich animal tissues, such as porcine intestine and bovine lung, are used as commercial sources of heparin. Many of the biological activities ascribed to heparin (i.e., anticoagulation, regulation