

Self-Assembled Synthetic Viral Capsids from a 24-mer Viral Peptide Fragment**

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Natural plant viruses are rodlike or spherical nanoassemblies with discrete size and morphology, in which genome nucleic acids are encapsulated by self-assembled coat proteins (capsids). Most capsids in spherical viruses have an icosahedral symmetry and the number and arrangement of subunits are related to the triangulation number (T number), which is derived from quasi-equivalence theory.^[1] For example, tomato bushy stunt virus (TBSV, $T=3$) consists of 180 quasi-equivalent protein subunits that comprise 388 amino acids each (diameter of capsid ca. 33 nm).^[2]

Recently, the application of bacteriophages such as M13 phage^[3] and plant viruses^[4] such as tobacco mosaic virus (TMV),^[5] cowpea mosaic virus (CPMV),^[6] and cowpea chlorotic mottle virus (CCMV)^[7] in nanotechnology have attracted much attention because of their fascinating nanostructures with a discrete nanospace. Virus nanotechnologies depend on the structure of “ready-made” capsids, however, the chemical strategy of *de novo* designed “tailor-made” viruslike nanocapsules is still in its infancy. The development of designed capsid molecules for the reconstruction of viral architectures would enhance the potential of viruslike nanocapsules and notably contribute to advance nanobioscience. To date, virus-inspired nanocapsules with a size of about 1–5 nm have been self-assembled by hydrogen bonds^[8] and

coordination bonds.^[9] However, the size of these supramolecular nanocapsules is evidently smaller than that of natural viruses, and consequently their applications have been limited to the inclusion of small guest molecules. Yeates and co-workers have developed a general strategy for the construction of protein architectures, such as cages and filaments, by the use of fusion proteins.^[10] We have demonstrated that virus-inspired C_3 -symmetric β -sheet-forming peptide conjugates self-assemble into nanocapsules^[11a,c] and nanofibers.^[11b,c] Recently, we have also reported that C_3 -symmetric glutathione conjugates self-assemble into nanospheres.^[11d,e]

Herein we show a first example of a synthetic viral capsid self-assembled from a 24-mer β -annulus peptide (1: INHVGGTGGAIMAPVAVTRQLVGS) in water (Figure 1). The β -annulus peptide motif (Ile69–Ser92) in the TBSV capsid participates in the formation of a dodecahedral internal skeleton,^[2b] thus we expected that the peptide 1

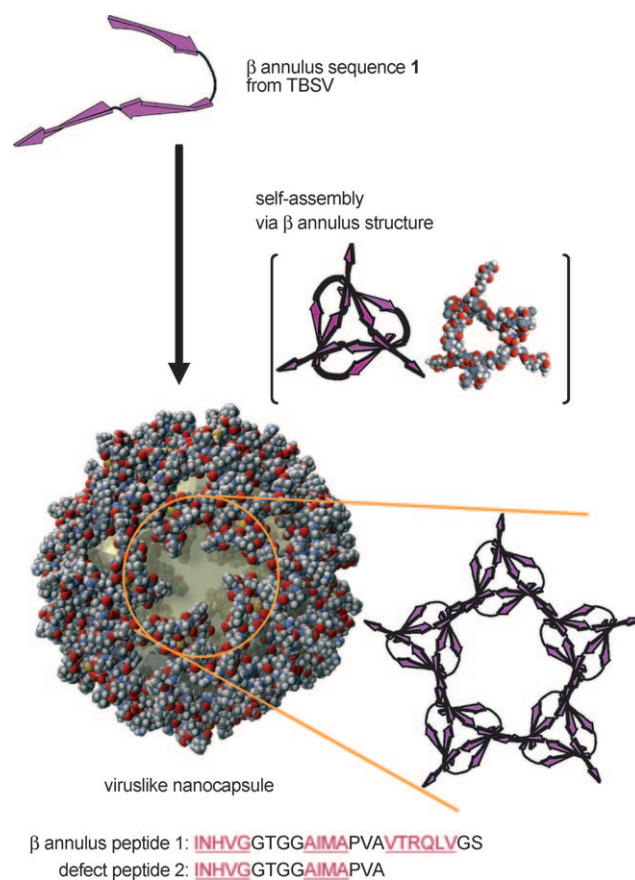


Figure 1. Illustration of the hypothesized formation of viruslike nanocapsules by self-assembly of 24-mer β -annulus peptide 1.

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would self-assemble into virus-sized nanocapsules by adopting a C_3 -symmetric β -annulus structure. Although it is known that the deletion mutants of spherical viral capsids affect the morphology of the capsids,^[11] there have been no reports on the construction of nanocapsules by the self-assembly of viral peptide fragments.

The 24-mer β -annulus peptide (**1**) and a 16-mer defect peptide (**2**) were synthesized by solid-phase fluorenylmethyloxycarbonyl (Fmoc) chemistry. The VTRQLVGS sequence of peptide **1**, which acts as a sticky end in the self-assembly, is not comprised in peptide **2**. Peptide **1** was purified by reverse-phase HPLC (RP-HPLC), and its primary structure was confirmed by MALDI-TOF mass spectrometry (m/z 1464.7 $[M+H]^+$) and MS/MS analysis (see Figure S1 in the Supporting Information). A lyophilized powder of peptide **1** was readily dissolved in deionized water without sonication or heating, and the resulting solution showed a pH value of 4.3. The CD spectrum of the aqueous solution of peptide **1** (Figure 2a) showed a negative peak at 197 nm and a negative shoulder at 218 nm, thus indicating the coexistence of random-coil and β structures, which is consistent with the β -annulus structure of TBSV. TEM images of the aqueous

solution of peptide **1** show that spherical assemblies with diameters of 30–50 nm are abundant (Figure 2b, the size distribution is shown in Figure S2 in the Supporting Information). The black centers of the structures observed in the TEM images might be caused by an accumulation of uranyl acetate in the interior of the spherical assemblies.^[7c] The average hydrodynamic diameter of the assemblies as determined by dynamic light scattering (DLS) was (48 ± 7) nm (hydrodynamic radius: $R_h = (24 \pm 3.5)$ nm, Figure 2c), which is comparable to the size of the spherical assemblies observed by TEM ((44 ± 8) nm, see Figure S2 in the Supporting Information). In contrast, for an aqueous solution of the 16-mer defect peptide **2**, an average diameter of 1.5 nm was determined by DLS (Figure 2d), thus indicating the absence of self-assembled structures. These results suggest that peptide **1** spontaneously forms spherical assemblies with a diameter of 30–50 nm by interactions between sticky ends of β -annulus structures. The spherical structures were stable at room temperature for more than six months. It is noteworthy that peptide **1** selectively self-assembled into nanospheres, without the formation of nanofibers or unimolecular folding structures.

The scattering intensity (DLS count rate) observed for the aqueous solutions of peptide **1** (pH 4.3, 25 °C) was approximately constant below a concentration of 25 μ M, and increased at concentrations above 25 μ M (Figure 3a). This result indicates that the critical aggregation concentration (CAC) of peptide **1** in water at 25 °C is 25 μ M. At concentrations below 25 μ M, DLS autocorrelation data suitable for analysis could not be obtained (see Figure S3a in the Supporting Information), hence indicating the absence of assemblies. In contrast, at concentrations well above 25 μ M,

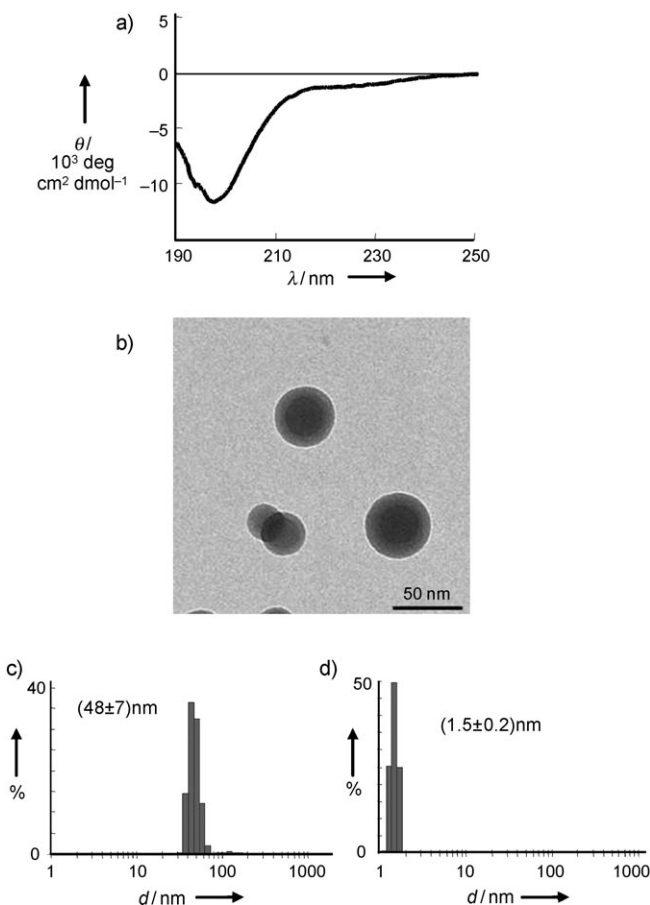


Figure 2. a) CD spectrum of an aqueous solution of β -annulus peptide **1**. b) TEM image of the assemblies obtained from an aqueous solution of **1**. The TEM sample was stained with uranyl acetate. c) Size distribution of an aqueous solution of **1** determined by DLS. d) Size distribution of an aqueous solution of defect peptide **2** determined by DLS. Concentration of peptides: 0.1 mM; pH 4.3; temperature: 25 °C.

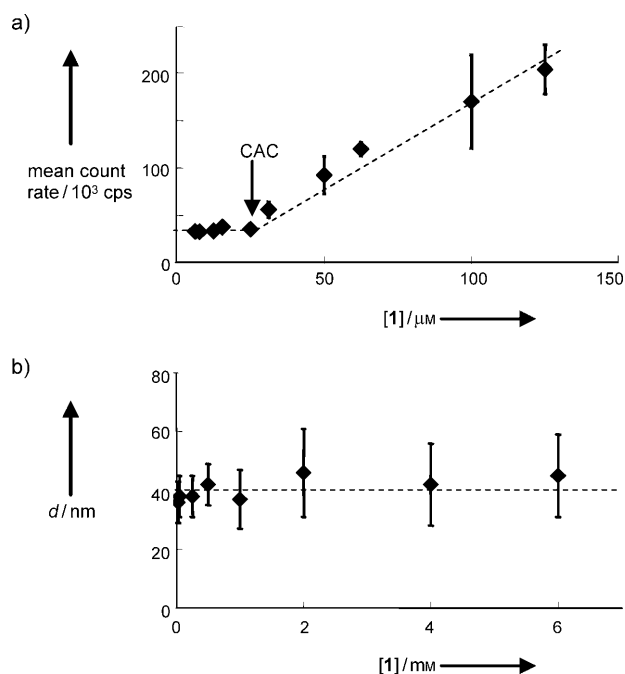


Figure 3. Effect of peptide concentration on a) scattering intensity and b) size distribution of an aqueous solution of **1** determined by DLS at 25 °C (pH 4.3).

DLS autocorrelation data suitable for analysis could be obtained (see Figure S3b in the Supporting Information). Similar assemblies with a size of about 30–50 nm were formed in the large concentration range of 0.025–6.0 mM (Figure 3b). The electron micrographs obtained at the different concentrations also showed the formation of spherical assemblies with a size of about 30–50 nm. These results indicate that the size and morphology of the assemblies are marginally affected by concentrations above CAC, and that the spherical assemblies with about 30–50 nm size are thermodynamically stable.

Figure 4 shows the synchrotron small-angle X-ray scattering (SAXS) profile of a 2.0 mM aqueous solution of peptide **1** (40B2 and 45XU beam lines of Spring-8, wavelength:

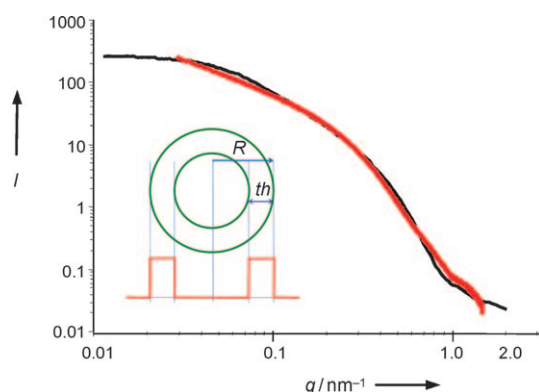


Figure 4. SAXS profile (red curve) of an aqueous solution of β -annulus peptide **1** (2 mM, pH 4.3). The black curve corresponds to the theoretical SAXS profile of a hollow capsule (see the Supporting Information).

0.15 nm, sample to detector distances: 1800 and 3500 mm). The SAXS profile shows slopes related to two different power laws: q^{-1} in the lower scattering-vector region ($q = 0.03$ – 0.1 nm^{-1}) and q^{-4} in the intermediate q region (0.3 – 1.0 nm^{-1}), and a second peak in the higher q region (1.0 – 1.5 nm^{-1}). The SAXS profile could be fitted well with a theoretical equation for a vesicle model^[13] with a radius of gyration (R_g) of $(25 \pm 11.2) \text{ nm}$ and a wall thickness (th) of $(7.0 \pm 1.4) \text{ nm}$ (see the Supporting Information). This result indicates the existence of a hollow interior of the particle. Attempts to fit the experimental data with a filled spherical model, such as a solid or core-shell sphere, were unsuccessful (see Figure S4 in the Supporting Information). In the case of filled spheres, it is known that the relationship between R_g and R_h from DLS corresponds to $\frac{R_g}{R_h} = \sqrt{\frac{3}{5}} \approx 0.77$.^[14] The R_g/R_h ratio of nano-assemblies of peptide **1** at 2 mM is $25/23 = 1.09$, which is evidently larger than $\sqrt{\frac{3}{5}}$. This finding supports that the self-assembled architecture has a hollow interior.

In conclusion, we have found that 24-mer β -annulus peptides of TBSV spontaneously self-assemble into hollow nanocapsules with a size of 30–50 nm. The nanocapsules possess a defined CAC (25 μM), and the size of the nanocapsules is almost unaffected by peptide concentrations above CAC. The hollow structure of the present β -annulus peptide

assemblies was clearly revealed by SAXS. It should be noted that viral capsidlike structures were spontaneously formed by the self-assembly of 24-mer β -annulus peptide fragments from TBSV capsids (in total 388 amino acids). The present findings provide a minimized design of artificial viral capsids that can be modified to extend the molecular design of functional nanocapsules. We envisage that these viruslike nanocapsules by proper surface modifications could be applied to DNA- and protein-carriers and platforms for artificial vaccines.

Experimental Section

Synthesis of β -annulus peptide **1:** The peptide H-Ile-Asn(Trt)-His(Trt)-Val-Gly-Gly-Thr(*t*Bu)-Ile-Met-Ala-Pro-Val-Ala-Val-Thr(*t*Bu)-Arg(Mtr)-Gln(Trt)-Leu-Val-Gly-Ser(*t*Bu)-Alko-PEG resin was synthesized with an ABI 433A synthesizer (Applied Biosystems) on Fmoc-Ser(*t*Bu)-Alko-PEG resin (Watanabe Chemical Ind. Ltd, 0.21 mmol g^{-1}) using standard Fmoc-based FastMoc coupling reactions (5 equiv Fmoc amino acids). Solutions of 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU, 0.5 M) and 1-hydroxybenzotriazole hydrate (HOBt·H₂O, 0.5 M) in *N,N*-dimethylformamide (DMF) were used as coupling reagents. Diisopropylamine (2.0 M) in *N*-methylpyrrolidone (NMP) and 20% piperidine in NMP were used for neutralization and for Fmoc deprotection, respectively. The peptidyl resin was washed with NMP and then dried under vacuum. The peptide was deprotected and cleaved from the resin by treatment with a mixture of trifluoroacetic acid (TFA)/thioanisole/phenol/water/1,2-ethanedithiol/triisopropylsilane = 8.15/0.5/0.5/0.5/0.25/0.1 at room temperature for 4 h. The reaction mixture was filtered to remove the resin and the filtrate was concentrated under vacuum. The peptide was precipitated by adding ice-cooled methyl *tert*-butyl ether (MTBE) to the residue and the supernatant was decanted. After repeating the washing with MTBE 5 times, the precipitated peptide was dried under vacuum. The crude product was purified by reverse-phase HPLC (RP-HPLC, Inertsil ODS-3) eluting with a linear gradient of CH₃CN/water containing 0.1% TFA (25/75 to 35/65 over 100 min). The fraction containing the desired peptide was lyophilized to give 157 mg of a flocculent solid (67% yield). MALDI-TOF MS (matrix: α -cyano-4-hydroxycinnamic acid, α -CHCA): m/z 2305.3 [$M+H$]⁺ (calcd 2304.2). The existence of the peptide sequence was confirmed by MS/MS analysis (see Figure S1 in the Supporting Information).

Defect peptide **2** (H-Ile-Asn-His-Val-Gly-Gly-Thr-Ile-Met-Ala-Pro-Val-Ala-OH) was prepared by a procedure similar to the one described above (yield: 66 mg (45%)). MALDI-TOF MS (matrix: α -CHCA): m/z 1464.7 [$M+H$]⁺ (calcd 1463.8).

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- [1] C. Branden, J. Tooze, *Introduction to Protein Structure*, 2nd ed., Garland Publishing, New York, 1999.
- [2] a) S. C. Harrison, A. J. Olson, C. E. Schutt, F. K. Winkler, *Nature* **1978**, 276, 368; b) A. J. Olson, G. Bricogne, S. C. Harrison, *J. Mol. Biol.* **1983**, 171, 61.
- [3] a) C. Mao, C. E. Flynn, A. Hayhurst, R. Sweeney, J. Qi, G. Georgiou, B. Iverson, A. M. Belcher, *Proc. Natl. Acad. Sci. USA* **2003**, 100, 6946; b) C. Mao, D. J. Solis, B. D. Reiss, S. T. Kottmann, R. Y. Sweeney, A. Hayhurst, G. Georgiou, B. Iverson, A. M. Belcher, *Science* **2004**, 303, 213.

- [4] For reviews: a) C. M. Niemeyer, *Angew. Chem.* **2001**, *113*, 4254; *Angew. Chem. Int. Ed.* **2001**, *40*, 4128; b) N. F. Steinmetz, D. J. Evans, *Org. Biomol. Chem.* **2007**, *5*, 2891; c) D. Papapostolou, S. Howorka, *Mol. Biosyst.* **2009**, *5*, 723.
- [5] a) M. Endo, H. Wang, M. Fujitsuka, T. Majima, *Chem. Eur. J.* **2006**, *12*, 3735; b) R. A. Miller, A. D. Presley, M. B. Francis, *J. Am. Chem. Soc.* **2007**, *129*, 3104; c) R. A. Miller, N. Stephanopoulos, J. M. McFarland, A. S. Rosko, P. L. Geissler, M. B. Francis, *J. Am. Chem. Soc.* **2010**, *132*, 6068.
- [6] a) Q. Wang, T. Lin, L. Tang, J. E. Johnson, M. G. Finn, *Angew. Chem.* **2002**, *114*, 477; *Angew. Chem. Int. Ed.* **2002**, *41*, 459; b) E. Strable, J. E. Johnson, M. G. Finn, *Nano Lett.* **2004**, *4*, 1385; c) C. Li Cheung, S.-W. Chung, A. Chatterji, T. Lin, J. E. Johnson, S. Hok, J. Perkins, J. J. De Yoreo, *J. Am. Chem. Soc.* **2006**, *128*, 10801.
- [7] a) T. Douglas, E. Strable, D. Willits, A. Aitouchen, M. Libera, M. Young, *Adv. Mater.* **2002**, *14*, 415; b) M. Comellas-Aragonès, H. Engelkamp, V. I. Claessen, N. A. J. M. Sommerdijk, A. E. Rowan, P. C. M. Christianen, J. C. Maan, B. J. M. Verduin, J. J. L. M. Cornelissen, R. J. M. Nolte, *Nat. Nanotechnol.* **2007**, *2*, 635; c) I. J. Minten, L. J. A. Hendriks, R. J. M. Nolte, J. J. L. M. Cornelissen, *J. Am. Chem. Soc.* **2009**, *131*, 17771.
- [8] M. M. Conn, J. Rebek, Jr., *Chem. Rev.* **1997**, *97*, 1647.
- [9] a) M. Tominaga, K. Suzuki, M. Kawano, T. Kusakawa, T. Ozeki, S. Sakamoto, K. Yamaguchi, M. Fujita, *Angew. Chem.* **2004**, *116*, 5739; *Angew. Chem. Int. Ed.* **2004**, *43*, 5621; b) M. Tominaga, K. Suzuki, T. Murase, M. Fujita, *J. Am. Chem. Soc.* **2005**, *127*, 11950; c) Q.-F. Sun, J. Iwasa, D. Ogawa, Y. Ishido, S. Sato, T. Ozeki, Y. Sei, K. Yamaguchi, M. Fujita, *Science* **2010**, *328*, 1144.
- [10] J. E. Padilla, C. Colovos, T. O. Yeates, *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 2217.
- [11] a) K. Matsuura, K. Murasato, N. Kimizuka, *J. Am. Chem. Soc.* **2005**, *127*, 10148; b) K. Murasato, K. Matsuura, N. Kimizuka, *Biomacromolecules* **2008**, *9*, 913; c) K. Matsuura, H. Hayashi, K. Murasato, N. Kimizuka, *Chem. Commun.* **2010**, DOI: 10.1039/C0CC01324B; d) K. Matsuura, H. Matsuyama, T. Fukuda, T. Teramoto, K. Watanabe, K. Murasato, N. Kimizuka, *Soft Matter* **2009**, *5*, 2463; e) K. Matsuura, K. Fujino, T. Teramoto, K. Murasato, N. Kimizuka, *Bull. Chem. Soc. Jpn.* **2010**, *83*, 880.
- [12] a) C. Hsu, P. Singh, W. Ochoa, D. J. Manayani, M. Manchester, A. Schneemann, V. S. Reddy, *Virology* **2006**, *349*, 222; b) A. Pappachan, C. Subashchandrabose, P. S. Satheshkumar, H. S. Savithri, M. R. N. Murthy, *Virology* **2008**, *375*, 190.
- [13] M. Hirai, H. Iwase, T. Hayakawa, M. Koizumi, H. Takahashi, *Biophys. J.* **2003**, *85*, 1600.
- [14] I. Teraoka, *Polymer Solutions*, Wiley-Interscience, New York, **2002**, chap. 3, p. 187.