

Towards a tunable architecture of protein-only artificial viruses

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Arginine-rich peptides are well known in molecular medicine by their abilities to condense DNA, to cross cell membranes and as efficient nuclear localization signals [1-4]. Altogether, these properties make them very useful functional elements of artificial viruses [5] for gene therapy and drug delivery. Very recently, we have shown that the homogeneous peptide R9 acts, in addition, as an unexpected architectonic agent at the nanoscale, promoting the self-assembling of a multifunctional protein that contains it, as protein-only, planar nanoparticles of 20 nm in diameter [6]. These particles, that show a strong nuclear avidity and accumulate in the cell nucleus a few minutes after exposition [7], are able to bind, condense and deliver expressible DNA [6].

As the self-organizing properties of R9 seemed to result in much more regular nanoparticles than those offered by conventional self-assembling amyloidogenic peptides, that render either fibers [8] or amorphous aggregates [9], we explored if cationic peptides other than R9 could also promote the self-assembling of holding building blocks. A series of unrelated peptides with diverse amino acid sequences and structures were tested as architectonic tags by using an EGFP as convenient building block. Interestingly, all these peptides were able, at different extents, to promote the spontaneous formation of protein nanoparticles of different sizes, ranging from 20 to 100 nm, in a process in which the arginine residues are critical for the final geometry of the resulting particles. On the contrary, Lysine-rich peptides, although very useful as DNA condensers, do not show any architectonic ability when incorporated to artificial viruses [10].

The use of arginine-rich peptides as structural agents of protein-only nanoparticles opens intriguing possibilities to the tailoring particle geometry through conventional protein engineering, a possibility so far unapproachable in bionanomedicine [11].

References

- [1] E. Vazquez, N. Ferrer-Miralles, R. Mangues, J. L. Corchero, Schwartz S Jr, and A. Villaverde, Modular protein engineering in emerging cancer therapies, *Curr. Pharm. Des.*, **15** (2009) 893-916.
- [2] P. Saccardo, A. Villaverde, and N. Gonzalez-Montalban, Peptide-mediated DNA condensation for non-viral gene therapy, *Biotechnol. Adv.*, **27** (2009) 432-438.
- [3] E. Vazquez, N. Ferrer-Miralles, and A. Villaverde, Peptide-assisted traffic engineering for nonviral gene therapy, *Drug Discov. Today*, **13** (2008) 1067-1074.
- [4] N. Ferrer-Miralles, E. Vazquez, and A. Villaverde, Membrane-active peptides for non-viral gene therapy: making the safest easier, *Trends Biotechnol.*, **26** (2008) 267-275.
- [5] E. Mastrobattista, M. A. van der Aa, W. E. Hennink, and D. J. Crommelin, Artificial viruses: a nanotechnological approach to gene delivery, *Nat. Rev. Drug Discov.*, **5** (2006) 115-121.
- [6] Vazquez, E., Roldán, M, Diez-Gil, C, Unzueta, U., Domingo-Espin, J., Cedano, J, Conchillo-Sole, O., Ratera, I, Veciana, J, Ferrer-Miralles, N, and Villaverde, A. Protein nanodisk assembling and intracellular trafficking powered by an arginine-rich (R9) peptide. *Nanomedicine* **5** (2010).259-268.
- [7] E. Vazquez, R. Cubarsi, U. Unzueta, M. Roldan, J. Domingo-Espin, N. Ferrer-Miralles, and A. Villaverde, Internalization and kinetics of nuclear migration of protein-only, arginine-rich nanoparticles, *Biomaterials*, **31** (2010) 9333-9339.
- [8] Y. Zhao, M. Tanaka, T. Kinoshita, M. Higuchi, and T. Tan, Self-assembling peptide nanofiber scaffolds for controlled release governed by gelator design and guest size, *J. Control Release*, **147** (2010) 392-399.
- [9] W. Wu, L. Xing, B. Zhou, and Z. Lin, Active protein aggregates induced by terminally attached self-assembling peptide ELK16 in *Escherichia coli*, *Microb. Cell Fact.*, **10** (2011) 9.
- [10] J. Domingo-Espin, E. Vazquez, J. Ganz, O. Conchillo-Sole, E. García-Fruitós, J. Cedano, U. Unzueta, V. Petegnief, N. Gonzalez-Montalban, A. M. Planas, X. Daura, H. Peluffo, N. Ferrer-Miralles, and A. Villaverde, The nanoparticulate architecture of protein-based artificial viruses is supported by protein-DNA interactions, *Nanomedicine*, **In press**, DOI 10.2217/NNM.11.28 (2011).
- [11] E. Vazquez and A. Villaverde. Engineering building blocks for self-assembling protein nanoparticles, *Microb. Cell Fact.*, **9** (2010) 101.