# Smart peptide nanoparticles for efficient and safe gene therapy

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Co-PI: Yaakov Benenson, Department of Biosystems Science and Engineering, ETH Zürich

**1. Current state of research in the field**: Gene therapy constitutes a promising novel modality for treatment of genetic diseases as well as cancer; recently, the first gene therapy agent for an inherited disorder LPLD (Glybera)[1] and an anti-melanoma agent (Oncovex[2]) have been approved by the regulators. However, widespread clinical adoption of gene therapies is still in its infancy not least due to various issues associated with using viral vectors to deliver transgenes to target tissues or tumors[3] in patients. These problems include, among others, innate or acquired immune response against the viral capsid. This limits the delivery regimens to single injections and excludes large cohorts of patients with preexisting immunity from accessing the treatment in the first place. Therefore, non-viral delivery methods for DNA and possibly RNA could have advantages in terms of immunogenicity, toxicity, and more[4]. In particular, peptide-based self-assembled nanostructures developed in the field of nanomedicine are inherently biocompatible and biodegradable[5]. However, there are only a few peptidic systems described to date capable of co-delivery of nucleic acids and small molecules[6,7]. In addition, they still lag behind viral vectors when it comes to delivery efficiency *in vivo*[4]. **The aim of this project is to develop the next generation of non-viral DNA delivery tools using smart peptide nanoparticles specifically designed to carry theranostic agents comprising DNA and fluorescent molecules (as reporters), and to test them both *in vitro* and *in vivo*.**

**2. Research Proposal:** In this project we plan to design amphiphilic peptides we reported previously for entrapment of short sequence DNA[7] by implementing extended nucleotide condensing sites of oligo histidines, and decreasing the size of the self-assembled system below 50 nm (Figure). Smaller particle size is beneficial for deep tissue penetrance, and an effort will be made to generate particles with a uniform size distribution between 20-40 nm, close to the size of an Adeno-Associated Virus (AAV), 20 nm. The project will be divided into three milestones. **Importantly, this study takes advantage of established expertise and experimental capabilities in both collaborating labs.**

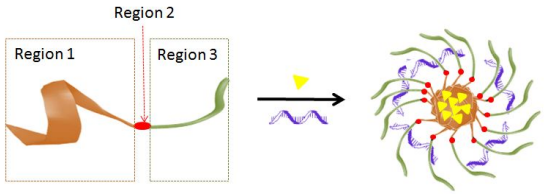


**Figure.** Schematic representation of the design of smart amphiphilic peptides based nanoparticles for theranostic strategies that combine simultaneous fluorescent detection of fluorescent dyes (yellow triangle) and delivery of therapeutic DNA.

The **first milestone** will encompass the design and development, in a bottom-up approach, of amphiphilic peptides to support a theranostic strategy. Peptides will be designed combining three different regions (Figure): i) a hydrophobic L-tryptophan-D-leucine repeating unit derived from a truncated sequence of gramicidin A (gT)[8] - *region 1*, ii) a hydrophilic moiety of histidines to provide electrostatic affinity to nucleotides - *region 3*, and iii) an artificial amino acid (*region 2*) bearing a disulfide functional group between the hydrophobic and hydrophilic regions[7]. The disulfide cleavage site will serve to induce responsiveness to physiological concentrations of reducing agent, allowing release of the incorporated molecules upon up-take by cells. We will modulate the length and charge of the hydrophilic region to enhance the electrostatic interactions with DNA in order to allow delivery of dsDNA or ssDNA of up to 6,000 base pairs or bases. Moreover, using oligo histidine as hydrophilic part will preserve secondary aggregation to nanoparticles due to a pKa of 6.0. The experience of Palivan group with tuning conditions for the self-assembly of amphiphilic macromolecules will be invaluable[7-9], as will be the experience in designing stimuli-responsive systems[9].The self-assembled peptide nanoparticles will be characterized by light scattering techniques and electron microscopy (TEM, SEM), whilst the charge will be assessed by zeta-potential measurements. Both DNA and the fluorescent dyes will be entrapped during the self-assembly process of nanoparticles formation, and characterized *in situ* by fluorescence correlation spectroscopy.

**Milestone 2** comprises the testing of the nanoparticles from Milestone 1 in live cultured mammalian cells. The nanoparticles will be evaluated for their DNA delivery efficiency judged by the expression of a fluorescent reporter protein as well as encapsulated fluorescent dyes, allowing separate assessment of delivery per se on one hand, and the delivery of functional DNA, on the other. We will build empirical correlations between the physico-chemical properties of the particles and their delivery efficiency, and iterate until satisfactory efficiency is reached. Based on the cellular uptake and DNA release, nanoparticles will be optimised in Palivan group by taking into account various molecular parameters: length of the hydrophilic sequence, hydrophilic to hydrophobic ratio, and stability of nanopaticles in various media. Co-entrapment efficiency will be modulated by increasing the amount of DNA and dye, whilst preserving the architecture of the nanoparticles; the release will be controlled by systematic modification of the molecular parameters.

**Milestone 3** is dedicated to testing the best-performing particles from Milestone 2 in laboratory mice. The Benenson lab is fully equipped to perform *in vivo* tests using systemic delivery, having already successfully done so for a variety of viral vectors (manuscript in preparation). The lab has established in-house a mouse model of metastatic liver cancer in immunocompromised mice using human cancer cell xenografts; it can be readily adapted to immunocompetent animals when using mouse cancer cell lines. We will use mice with pre-established liver tumors to assess delivery efficiency in healthy tissues and in the tumors simultaneously, reducing the number of experimental animals. The tests will be performed on immunocompromised as well as immunocompetent mice, to gauge the effects of the immune response. In this way, delivery efficiency can be assessed simultaneously in the tumor and in the healthy tissues using fluorescent protein expression and fluorescent dyes. The nanoparticles will be assessed against efficient AAV vectors such as AAV8 (liver delivery) and AAV6 (tumor delivery).

**3. Necessity of interdisciplinary collaboration**: In order to achieve the targets in this challenging project and support its medical application, it is necessary to combine expertise in nanoscience, translational genetic engineering, and physical chemistry. The project will benefit from the unique combination of expertise in Benenson and Palivan labs. The former is developing complex multi-gene systems ("gene circuits") for selective cancer therapies; they have established a mouse model of metastatic liver cancer and the necessary methods for detailed assessment of the delivery efficiency (imaging, tissue dissection and dissociation, flow cytometry, histology). Palivan group is currently involved in the design and characterisation of new hybrid systems at the nanoscale by combining self-assembling macromolecules (peptides, polymers, combinations of thereof) with active molecules (proteins, enzymes, mimics, DNA).

**4. Impact:** Our study will provide a novel solution for gene delivery due to the two-pronged strategy combining cutting-edge design of nanoscience based carriers with a suite of methods and assays that enable their detailed interrogation and understanding of their delivery mechanisms *in vitro* and *in vivo*. It will also form a basis for novel and expanded theranostic strategies due to co-entrapment of fluorescent molecules with large DNA payloads. This capability will, on one hand, enable the delivery of theranostic gene circuits[10,11]; on the other hand, the entrapment of fluorescent dyes will enable facile readout of particle delivery in time and space that does not rely on gene expression.

**5. Project management**: The PhD student, registered at the University of Basel with C.G.P, will be a full member of the both the Palivan and Benenson groups and will have access to state-of-the art facilities for all aspects of the project both at UniBasel and ETH (Basel and Zurich sites).

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8. T. B. Schuster, D. de Bruyn Ouboter, C.G. Palivan, W. Meier, *Langmuir* **2011**, 27, (8), 4578.
9. C.G. Palivan, R. Goers, A. Najer, X. Zhang, W. Meier, *Chem. Soc. Rev*, **2015**, 45, 377; T. Einfalt, R. Goers, A. I. Dinu, A. Najer, M. Spulber, O. Onaca-Fischer, C. G. Palivan, *Nano Letters*., **2015**, 15(11), 7596.
10. Z. Xie, L. Wroblewska, L. Prochazka, R. Weiss and Y. Benenson, *Science*, **2011**, 333, 1307.
11. N. Lapique, Y. Benenson. *Nat. Chem. Biol*. **2014**, 10, 1020.

# Curriculum vitae of Prof. Dr. Cornelia G. Palivan

**Date of birth:** April 6Th 1959.
**Civil status**: Married, 2 children.

**EDUCATION**

| | |
|---|---|
| 1995 | Doctor es Science, Summa cum laudae, University of Bucharest |
| 1983 | M.Sc. thesis, honoured University of Bucharest, physics. |

**HONORS**

Prize for excellence in research, Bucharest, *1987*; Royal Society Fellow SCRI, UK, *1992*; EC-COST Research Fellow UK, 1993; Visiting Fellow CNRS, France, *1995*; TEMPUS Fellow SCRI UK, *1998*; Visiting lecturer, University of Geneva, *1999*.

**EMPLOYMENT**

| | |
|---|---|
| Since 2013 | Titular Professor, Dept. of Chemistry, University of Basel |
| 2010 - 2013 | PD, Dept. of Chemistry, University of Basel |
| 2004 - 2010 | Senior Group Leader, Dept. of Chemistry, University of Basel |
| 2003 - 2004 | Visiting Researcher, ETH Zürich |
| 1999 - 2003 | Post-doctoral scientist University of Basel |
| 1997 - 2004 | Lecturer at the Faculty of Physics, University of Bucharest |
| 1995 –1997 | Post-doctoral scientist University of Bucharest |

**PUBLICATIONS**

ca. 115 publications in peer-reviewed journals, books and chapters of books; > 100 oral presentations. Examples of publications relevant for the proposal:

- T. B. Schuster, D. de Bruyn Ouboter, C. G. Palivan, W. Meier, ***Langmuir***, **2011**, *27* (8), 4578–4584.
- P. Baumann, V. Balasubramanian, O. Onaca, A. Sienkiewicz, C. G. Palivan ***Nanoscale***, **2013**, 5 (1), 217 - 224
- S.J. Sigg, V. Postupalenko, J. T. Duskey, C. G. Palivan, W. Meier ***Biomacromolecules***, **2016**, DOI:10.1021/acs.biomac.5b01614.
- X. Zhang, M. Lomora, T. Einfalt, W. Meier, N. Klein, D. Schneider, C. G. Palivan ***Biomaterials***, **2016**, 89, 79-88
- G. Gunkel-Grabode, S. Sigg, M. Lomora, S. Lörcher,C. G. Palivan, W. Meier, ***Biomater. Sci***., **2015**, 3, 25-40.
- C.G. Palivan, R. Goers, A. Najer, X. Zhang, W. Meier, ***Chem. Soc. Rev***, **2015**, 45, 377-411.

**MANAGEMENT EXPERIENCE**

**Project leader** in Drug research industry (ICPR Bucharest), Electron Paramagnetic Resonance-based projects (University of Bucharest), and Nanoscience (University of Basel)
**Funding record** (University of Basel): SNF (11 projects), Swiss Nanoscience Institute (2 projects), CIBA Spezialitatenchemie GmbH Germany (1 project).

**OTHER SCIENTIFIC/ACADEMIC ACTIVITIES:**

- Member in the Management Committee for: COST P15 Action (2007- 2010), COST IPROMEDAI Action (since 2014); Swiss Soft Days (since 2010).
- **Grant reviewer**: Council of Chemical Sciences of the Netherlands Organization for Scientific Research, and Technology Foundation NWO-Nano (NL); FWO – Flanders (B)**;** SNSF – Swiss National Science Foundation (CH)**;** ERA-Chemistry (EU), ERC (EU).
- **Scientific reviewer** for Elsevier (Polyhedron, J.Inorg.Biochem.), ACS (J. Am. Chem. Soc., J. Phys.Chem.B, J. Phys. Chem. C, Langmuir, ACS Nano), Wiley (Macromol. Biosci., Macromol. Rapid Communications, Small, Chem. Eur. J., J. Pol. Sci A.), Nature Communication.: currently 70+ manuscripts/year.

21Th May, 2016 Cornelia G. Palivan

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**Education**
2005: PhD, Departments of Computer Science & Applied Mathematics and Biological Chemistry, Weizmann Institute of Science, Israel
1999: MSc, Master of Science, Department of Chemistry, Technion, Israel
1996: BA (summa cum laude), Department of Chemistry, Technion, Israel

**Academic Appointments**
2015-now: Associate Professor with tenure, Department of Biosystems Science and Engineering (D-BSSE), ETH Zürich
2010-2015: Assistant Professor (tenure track), Department of Biosystems Science and Engineering (D-BSSE), ETH Zürich
2005-2010: Bauer Fellow & Principal Investigator, FAS Center for Systems Biology, Harvard University

**Selected Honors and Scholarships**
2011 ERC Starting Grant, European Research Council
2005 Kennedy Prize for achievements in PhD research, Feinberg Graduate School

**Service**
Scientific Advisory Board member, National Center for Biotechnology (CNB), Spain
Editorial Board member, ACS Synthetic Biology and OUP Synthetic Biology
Grant Reviewer for ERC, NSF, FP7, ISF, *etc*.
Project Reviewer for European Commission
Manuscript reviewer for Science, Nature Biotechnology, Nature Nanotechnology, Nature Materials, Nature Methods, Nature Communications, PNAS, Molecular Cell, *etc*.
Institutional duties: Departmental representative for Scientific Integrity
Departmental committees: Doctoral committee, ETH Silver Medal committee, Awards committee

**Selected Publications**
1. Haefliger, B., Prochazka, L., Angelici, B & Benenson, Y. Precision multidimensional assay for high-throughput microRNA drug discovery. ***Nature Communications*** **7**:10709 (2016).
2. Hansen, J., Mailand, E., Swaminathan, K. K., Schreiber, J., Angelici, B., Benenson, Y. Transplantation of prokaryotic two-component signaling pathways into mammalian cells. ***PNAS*** **111**, 15705–15710 (2014).
3. Lapique, N & Benenson, Y. Digital switching in a biosensor circuit via programmable timing of gene availability. ***Nature Chemical Biology*** **10**, 1020–1027 (2014).
4. Prochazka, L., Angelici, A., Haefliger, B and Benenson, Y. Highly modular bow-tie gene circuits with programmable dynamic behavior. ***Nature Communications*** **5**:4729 (2014).
5. Xie, Z., Wroblewska, L., Prochazka, L., Weiss, R., Benenson, Y. Multi-input RNAi-based logic circuit for identification of specific cancer cells. ***Science*** **333**, 1307-1311 (2011).
6. Bleris, L., Xie, Z., Glass, D., Adadey, A., Sontag, E., Benenson, Y. Synthetic incoherent feedforward circuits show adaptation to the amount of their genetic template. ***Molecular Systems Biology*** **7**:519 (2011).
7. Leisner, M., Bleris, L., Lohmueller, J., Xie, Z. & Benenson, Y. Rationally designed logic integration of regulatory signals in mammalian cells. ***Nature Nanotechnology*** **5**, 666-670 (2010).
8. Rinaudo, K., Bleris, L., Maddamsetti, R., Subramanian, S., Weiss, R. & Benenson, Y. A universal RNAi-based logic evaluator that operates in mammalian cells. ***Nature Biotechnology*** **25**, 795-801 (2007).
9. Benenson, Y., Gil, B., Ben-Dor, U., Adar, R. & Shapiro, E. An autonomous molecular computer for logical control of gene expression. ***Nature*** **429**, 423-429 (2004).
10. Benenson, Y., Paz-Elizur, T., Adar, R., Keinan, E., Livneh, Z. & Shapiro, E. Programmable and autonomous computing machine made of biomolecules. ***Nature*** **414**, 430-434 (2001).