# Peptide Based Nanoparticles as a Platform for Vaccine Design

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## ABSTRACT

Artificial particulate systems such as polymeric beads and liposomes have many applications in drug delivery, drug targeting, antigen display, vaccination, and other technologies. We have recently designed a novel type of nanoparticle with regular icosahedral symmetry and a diameter of about 16 nm, which self-assembles from single polypeptide chains [1].

The geometry of the nanoparticle and its resemblance to small viruses will trigger a strong immune response based on repetitive antigen display - a concept that now is increasingly exploited for producing novel vaccines that yield high titers of specific antibodies by using virus-like-particles as repetitive antigen display systems. Such *de novo* designed nanoparticles eliminates the need for virus-based designs, and allow for high flexibility in vaccine design. Epitopes of any pathogen can readily be engineered onto the surface of the nanoparticle, thus allowing for the easy generation of a whole variety of different vaccines.

To validate our system as a novel platform for vaccine design, we have designed nanoparticles that display antigenic sequences at their surface. As a test model we have inserted the pilin sequence from *Pseudomonas* at the C-terminus of the constructs. Immunization experiments using such nanoparticles for repetitive antigen display are described and presented.

***Keywords***: repetitive antigen display, peptide nanoparticle, synthetic vaccines, pseudomonas, virus-like particle

## 1 INTRODUCTION

Modern vaccine strategies can roughly be classified as live attenuated, whole inactivated, subunit protein, live vector DNA, and combinatorial (for reviews see [2, 3]. Most licensed vaccines used today are either live attenuated or whole inactivated vaccines and are not devoid of risk factors, especially in the case of HIV. On the other hand subunit vaccines based on recombinant proteins can be considered safe but they can suffer from poor immunogenicity owing to incorrect folding of the target protein or poor presentation to the immune system. Virus-like particles (VLPs) represent a specific class of subunit vaccine composed of the structural envelope proteins of authentic virus particles and are recognized readily by the immune system [4]. They present viral antigens in a more authentic conformation than other subunit vaccines if derived from the original pathogen. Furthermore, VLPs can be used as carrier and presentation systems for B-cell epitopes of foreign pathogens and have shown dramatic effectiveness as candidate vaccines [5-7]. Epitopes of a pathogen can be displayed and therapeutic vaccines can possibly be engineered at will. The best known and most frequently used carrier system is the hepatitis C core capsid to which either an immunogen is chemically attached or where the immunodominant loop of the capsid protein is replaced by the respective B-cell epitope. This has been used e.g. for the design of a malaria vaccine [8].

Therefore the concept of presenting antigens in the form of repetitive arrays - i.e. by using surface-customized virus capsids or bacterial envelopes - is being increasingly employed for producing novel vaccines that yield high titers of specific antibodies [7, 9-11]. Engineering antigens as repetitive supramolecular arrays is now leading the way to novel vaccine strategies including strategies for therapeutic vaccines for chronic diseases [12].

So far such repetitive antigen display systems as B-cell epitope carriers have largely involved viral capsid structures. As an alternative, we therefore propose to use our recently designed nanoparticles as a novel tool for repetitive antigen display. For vaccination purposes such a peptidic nanoparticle will have the same properties as a VLP, but it can more easily be handled. The protein can be cloned and expressed in an *E. coli* system. The antigenic epitope can be customized at either end of the sequence as both the N-terminal and the C-terminal ends of the peptide sequence extend from the surface of the nanoparticle (Figures 1 & 3). Also, the size of the nanoparticle can easily be varied to obtain optimal immune responses (Figure 3). Hence, an antigenic peptide sequence at the C-terminus of the construct will be repetitively displayed at the surface of the nanoparticle.

## 2 RESULTS

In collaboration with Dr. Hodges from the University of Colorado Health Sciences Center we are developing a

series of peptidic nanoparticles displaying the epitope from the *Pseudomonas* fimbrial protein precursor (Pilin) (Strain PAO) residues 124 - 149 on thier surface. *Pseudomonas aeruginosa* and *Pseudomonas maltophilia* account for 80% of opportunistic infections by *Pseudomonas*. *Pseudomonas aeruginosa* is an opportunistic pathogen that causes urinary tract infections, respiratory system infections, dermatitis, soft tissue infections, bacteremia, and a variety of systemic infections, particularly in patients with severe burns, and in cancer and AIDS patients who are immunosuppressed. *Pseudomonas aeruginosa* is notable for its resistance to antibiotics, and is therefore a particularly dangerous pathogen. Only a few antibiotics are effective against *Pseudomonas*, including fluoroquinolones, gentamicin, and imipenem, and even these antibiotics are not effective against all strains. The difficulty in treating *Pseudomonas* infections with antibiotics is most dramatically illustrated in cystic fibrosis patients, virtually all of whom eventually become infected with a strain that is so resistant that it cannot be treated. Since antibiotic therapy has proved so ineffective as a treatment, we embarked on a research program to investigate the development of a synthetic peptide consensus sequence vaccine for this pathogen [13].

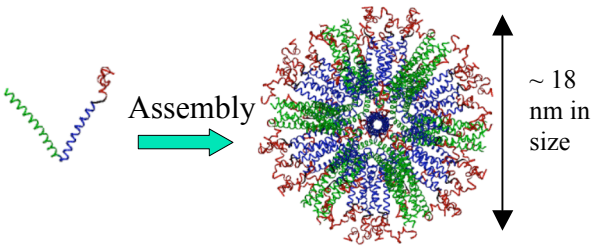


Figure 1: Concept of peptidic nanoparticle as vaccination tool. The pentameric coiled coil (green) attached to the trimeric coiled coil (blue) by a short linker, which forms the basic building blocks. We extended the C-terminus of trimeric coiled coil by antigenic sequence (red). Assembly of such building blocks yields a repetitive antigen display system.

## 2.1 Cloning, Expression and Purification

For the recombinant protein expression of the constructs BL21 (DE3) pLysS cells were used as the over-expression system according to the following procedure: LB meduim containing Ampicillin (200µg/ml) and Chloramphenicol (30µg/ml) was inoculated with an overnight preculture (28°C) of bacteria expressing the protein. The bacteria were grown at 37°C. Protein expression was induced using 1 mM IPTG. After 3-4 hours of expression the cells were harvested and centrifuged for 30 min at 5000rpm. The pellet was washed using Tris buffer pH 7.5 and kept at -80°C.

The purification was done under denaturing conditions according to the following procedure: The pellet was resuspended in a Lysis buffer at pH 8.0 containing 8M urea, 100mM $NaH_2PO_4$, 10mM Tris, 10mM β-Mercaptoethanol. After sonication, cell membranes were removed by centrifugation (40 min at 17 000rpm). The supernatant was then incubated with Nickel beads (Qiagen) for 2 hours. Then the protein contaminants were washed from the column using a pH gradient Washing buffer I pH 6.3 (8M urea, 100mM $NaH_2PO_4$, 10mM Tris, 10mM β-Mercaptoethanol), Washing buffer II pH 5.9, Washing buffer III pH 5.0. Finally, the protein was eluted from the resin using the elution buffer (8M urea, 100mM $NaH_2PO_4$, 10mM Tris, 10 mM β-Mercaptoethanol) pH 8.0 containing 1M Imidazole.

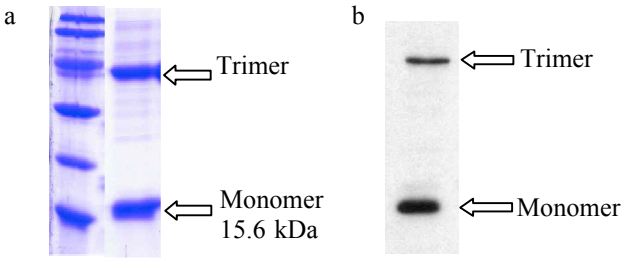


Figure 2. (a) Picture of an SDS-PAGE gel of purified nanoparticle constructs. Bands corresponding to monomeric and trimeric constructs are visible even in 8M urea. (b) Western blot confirms that both bands correspond to the same construct.

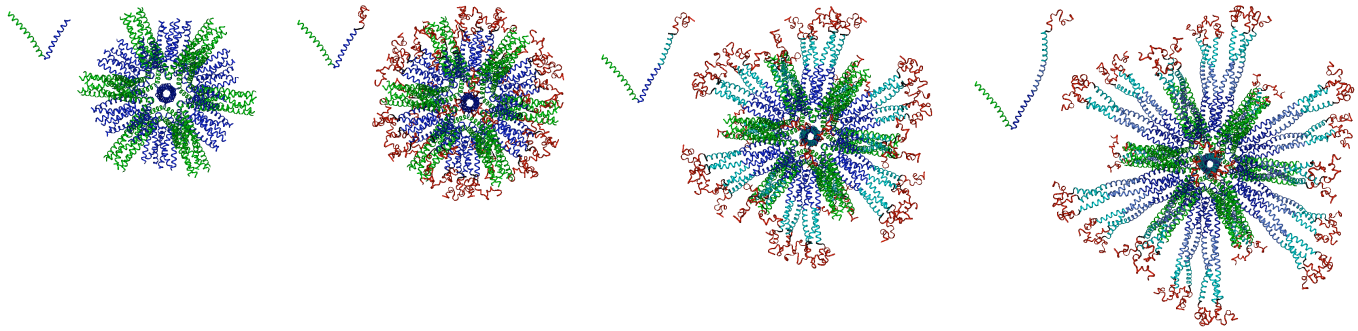

Figure 3: Computational visualization of the different sized monomers and their corresponding icosahedral nanoparticles. The size of the nanoparticles increases from 14.5 nm up to 29.0 nm and the antigenic sequence (at the C-terminus of the sequence and hence at the end of the trimeric coiled-coil helix) is displayed repetitively on the surface of the nanoparticle.

We are currently still improving the purification scheme. Our preliminary results show very strong binding of the constructs to our first purification matrix, nickel agarose. This is most likely due to a cooperativity of binding of the his-tag since the constructs remain trimeric even under drastic denaturing conditions such as 8 M urea (Figure 2). We could show two protein forms, monomeric and trimeric form on the SDS page confirmed by Western blot using a His-antibody. This proves the effectiveness of the rational design of trimeric coiled coils and might be of great help for purifying the protein as highly purified protein can be obtained after a single step. Moreover, such strong binding will also be very useful for the technical scale production where it will be necessary to switch to the less potent Zn- or Cu- matrices for protein purification.

## 1.2 Self Assembly of Nanoparticles

After purification the peptidic building blocks were self-assembled to nanoparticles in a stepwise dialysis from high (8M) to low (0M) urea concentration. This process was

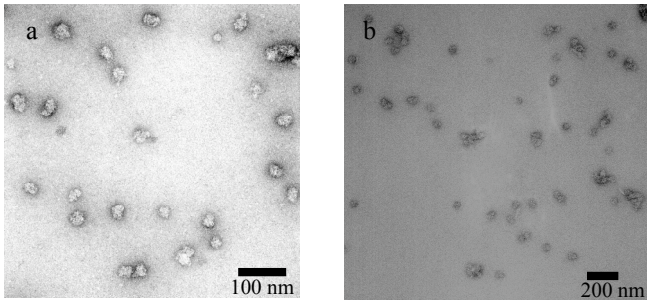


Figure 4. Electron micrographs of peptidic nanoparticles prepared by negative staining with 2% uranyl acetate at a concentration of 0.05 mg/ml. (a) Peptidic nanoparticle without epitope (100X magnification); (b) Peptidic nanoparticle with epitope (50X magnification)

performed under reducing conditions (2mM DTT) in a refolding buffer containing 20mM Tris pH 7.5, 150mM NaCl and 10% Glycerol. In a final step oxidizing conditions are established by removing DTT. Assembled nanoparticles were analyzed by transmission electron microscopy (TEM) to assess the size and shape of the particles (Figure 4). The electron micrographs reveal particles of the expected size ~ 16nm in case of peptidic nanoparticles without epitope and ~18nm in case of peptidic nanoparticles with epitope.

## 1.3 Preliminary immunization results

For our immunization experiments we have used rabbit as the animal model. Immunizations were performed by intermuscular (i.m.) injection of 15 μg of antigen (peptidic nanoparticle expressing pili antigen from *P. aeruginosa* - strain PAO) in TBS+10% Glycerol (tris buffered saline) together with Freund's complete/incomplete adjuvant. The animals were boosted at 4 week intervals with the same amount of antigen in adjuvant by the same route. A standard sandwich ELISA (enzyme-linked immunosorbent assay) was used to measure anti pilin antibodies. Briefly the ELISA plate is coated with pilin peptide conjugated to bovine serum albumin (BSA). This plate is then incubated with dilutions of rabbit sera and then incubated with an anti-rabbit IgG secondary antibody conjugated to horseradish peroxidase (HRP). Substrate is then added and product formation is measured by monitoring the absorbance at 450 nm.

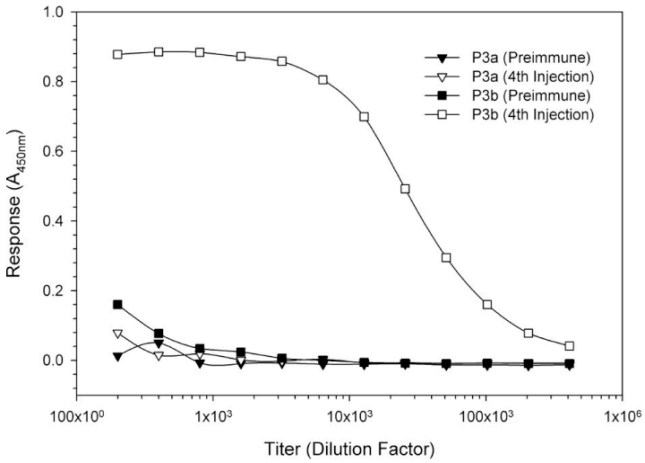


Figure 5: Titers of rabbits immunized with nanoparticles containing *Pseudomonas aeruginosa* (Strain PAO) epitope. Serum after 4 immunizations, ELISA plate coated with PAO-BSA conjugate.

Figure 5 shows the results of IgG measurements in serum as determined by ELISA using a pilin peptide conjugated to bovine serum albumin (BSA) coated to the solid phase. From the results it shows that none of the preimmune sera recognized the *P.aeruginosa* (strain PAO) epitope in the PAO-BSA conjugate. Also the immunized serum from the nanoparticle construct without the epitope showed no response to the PAO epitope as expected. The immunized serum against the nanoparticle construct with epitope, however, showed significant IgG titers (1:29600 dilution) against PAO-BSA conjugate (Table 1).

| Construct | Epitope | Serum | Titer |
|---|---|---|---|
| **P3a** | none | preimmune | No response |
| | | After 4th injection | No response |
| **P3b** | PAO | preimmune | No response |
| | | After 4th injection | 1:29600 |

Table 1: Preliminary immunization results of nanoparticles with *Pseudomonas* epitope.

# 3 OUTLOOK

This novel type of repetitive antigen display based on our peptide nanoparticles can be used to elicit high affinity

neutralizing antibodies against immunogenic epitopes from other pathogens. We are currently developing synthetic vaccine candidates against several major human pathogens. Immunogenic peptide sequences characteristic for these pathogens will be displayed on the surface of a peptidic nanoparticle. In addition to its ability to elicit high titer of serospecific neutralizing antibodies, the particulate structure will guide the nanoparticles to antigen presenting cells and induce CD4+ proliferative responses and cytotoxic T lymphocytes, thus inducing long-term immunologic memory.

Using recombinant protein expression, we are now also designing nanoparticles presenting the epitopes of any of the major *Plasmodium falciparum* protein antigens causing malaria (e.g. from the MSP-1, the AMA-1, the CS proteins, etc.) and HIV (e.g. from the surface proteins gp41, or gp120) repetitively and rigidly on their surface. Using the same approach, synthetic subunit vaccines can also easily be designed against any other pathogens. Especially enveloped viruses (e.g. influenza, Ebola, SARS, etc.) represent an ideal target as their surface proteins are characterized by trimeric coiled-coil proteins, which are also building blocks of our nanoparticles.

Since the peptidic nanoparticles will have the same immunological properties as VLPs and since this new technology allows for the design of novel types of vaccines, we expect that it will serve as a highly versatile platform for the design of synthetic subunit vaccines. The major advantages of this concept are **i)** its ease of handling in terms of protein expression (E. coli), protein purification and storage, hence leading to low production costs; **ii)** its flexibility allowing for a high throughput testing of different designs, which is especially important for HIV; **iii)** its suitability for the presentation of trimeric coiled-coil B-cell epitopes, which might prove to be an efficient vaccination strategy against enveloped viruses; **iv)** it does not need chemical coupling of the epitope to the particle as in some other approaches; and finally **v)** it is not associated with a remaining risk factor as it is not infectious.

## REFERENCES


[1] Burkhard, P. (2004). PCT patent application, WO 2004/071493.

[2] Zinkernagel, R.M. (2003). Annu Rev Immunol *21*, 515-546.

[3] Cho, M.W. (2003). Curr Mol Med *3*, 243-263.

[4] Noad, R., and Roy, P. (2003). Trends Microbiol *11*, 438-444.

[5] Bachmann, M.F., Rohrer, U.H., Kundig, T.M., Burki, K., Hengartner, H., and Zinkernagel, R.M. (1993). Science *262*, 1448-1451.

[6] Fehr, T., Skrastina, D., Pumpens, P., and Zinkernagel, R.M. (1998). Proc Natl Acad Sci U S A *95*, 9477-9481.

[7] Baschong, W., Hasler, L., Haner, M., Kistler, J., and Aebi, U. (2003). J Struct Biol *143*, 258-262.

[8] Birkett, A., Lyons, K., Schmidt, A., Boyd, D., Oliveira, G.A., Siddique, A., Nussenzweig, R., Calvo-Calle, J.M., and Nardin, E. (2002). Infect Immun *70*, 6860-6870.

[9] Zinkernagel, R.M. (1996). Science *271*, 173-178.

[10] Bachmann, M.F., and Zinkernagel, R.M. (1996). Immunol Today *17*, 553-558.

[11] Nieba, L., and Bachmann, M.F. (2000). Mod. Asp. Immunobiol. *1*, 56-39.

[12] Bachmann, M.F., and Dyer, M.R. (2004). Nat Rev Drug Discov *3*, 81-88.

[13] Cachia, P.J., and Hodges, R.S. (2003). Biopolymers *71*, 141-168.