



HAL
open science

Designing a Peptide-dendrimer for Use as a Synthetic Vaccine Against *Plasmodium falciparum*

Raúl Isea, Johan Hoebeke, Rafael Mayo-García

► **To cite this version:**

Raúl Isea, Johan Hoebeke, Rafael Mayo-García. Designing a Peptide-dendrimer for Use as a Synthetic Vaccine Against *Plasmodium falciparum*. *American Journal of Bioinformatics and Computational Biology*, 2013, 1, pp.1-8. 10.7726/ajbcb.2013.1001 . hal-03521081

HAL Id: hal-03521081

<https://hal.science/hal-03521081>

Submitted on 11 Jan 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Designing a Peptide-dendrimer for Use as a Synthetic Vaccine against *Plasmodium Falciparum*

Raúl Isea^{1*}, Johan Hoebeke¹, and Rafael Mayo-García²

Received 13 July 2012; Published online 20 October 2012

© The author(s) 2012. Published with open access at uscip.org

Abstract

This work presents a new computational analysis using post-genomic data in order to design a new vaccine candidate against *Plasmodium falciparum*, a parasite responsible for malaria. It is based on a poly(amidoamine) (PAMAM) eight-branched dendrimeric structure integrating B cell epitopes. To do so, all available experimental epitopes were identified, either stored in databases or bibliographic references, and analysed by means of bioinformatic tools for obtaining consensus epitopes up to six amino acids in length. An eight-branched tertiary structure integrating the consensus epitopes is proposed using a molecular mechanics tool. This computational technique could also be applied to other infectious agents as well as tumour antigens.

Keywords: Plasmodium falciparum, PAMAM, malaria, epitope, dendrimers.

1. Introduction

Dendrimers, also known as arborols or cascades, with cauliflower shapes, were first synthesized as 'ordered trees' by Vögtle's group in 1978 (Buhleier et al., 1978). Dendrimers are polymers that emerge from a centre or nucleus and are usually highly symmetric spherical compounds, with several branches in a great variety of patterns. As an example, Figure 1 shows a core of a second generation poly(amidoamine) (PAMAM) dendrimer. At the same time, it is well known that they can cross biological barriers such as cellular membranes or gut walls and can be designed to synthetically target specific proteins (Najlah and D'Emanuel, 2006).

Dendrimers are nontoxic agents, and are currently being used as drug carriers or as effective immunogens due to their compact nanometric structures. In fact, they are opening new strategies for drug delivery or vaccines to more conventional ones (Dan, 2006).

*Corresponding e-mail: raul.isea@gmail.com

1 IDEA. Fundación Instituto de Estudios Avanzados IDEA, Valle de Sartenejas, Hoyo de la Puerta, Baruta, 1 Venezuela.

2 CIEMAT - Avda. Complutense 22, 28040 Madrid - Spain

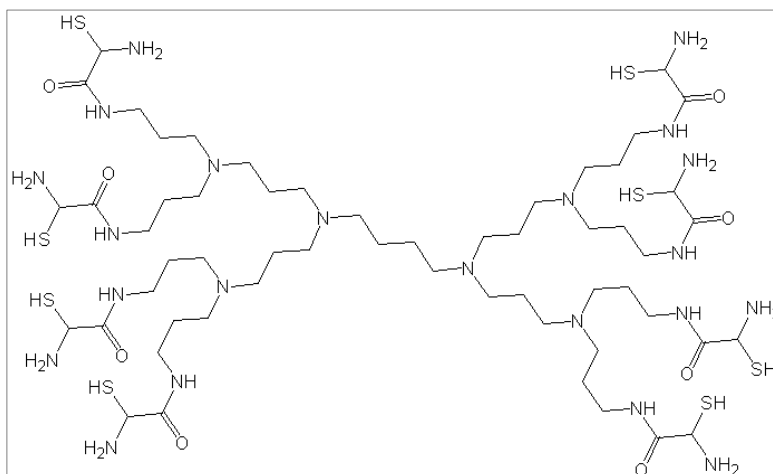


Fig 1. The core-shell architecture of a poly(amidoamine) (PAMAM) dendrimer with eight branches indicated with NH₂ terminals.

In recently published work by Hayder *et al.*, a dendrimer was administered intravenously to animals to cure arthritic inflammation (Hayder *et al.*, 2011); they have also been used as vaccines against Hepatitis B (Dutta *et al.*, 2008). Dendrimers are also being used as chemical ‘sensors’ (Tokuhisa *et al.*, 1997) and diagnostic agents (Majoros *et al.*, 2008); they are moreover commercialised as for example in VivaGel® to prevent HIV transmission (Rupp *et al.*, 2007) that has as an active component a dendrimeric polylysine that has been tested in animals. Recent reviews have been written on the subject (Heegaard *et al.*, 2010; Sadler and Tam, 2002).

The objective of this work is to computationally obtain an eight-branching unit of the (PAMAM) dendrimer that would be formed by combinations of consensus epitopes obtained from the whole sequenced genome of *P. falciparum*. Such a dendrimer could be used as a multiantigenic vaccine candidate against malaria, since nowadays there is no efficient vaccine for this species (Sauerwein *et al.*, 2011).

The importance of such a vaccine cannot be stressed enough, since the disease causes almost 1 to 1.5 million deaths and an estimated 243 million clinical cases annually, explaining the big research efforts that have been made for many years now (Herrington *et al.*, 1987; Bojang *et al.*, 2001). Moreover, work with T and B epitopes for obtaining chemically defined synthetic vaccines against malaria remain an open issue (Tam *et al.*, 1990; Nardin *et al.*, 2001) and studies based on tetrabranching peptide dendrimers as vaccines against *Plasmodium falciparum* have already been shown to be possible (Chaves *et al.*, 2001).

2. Materials and methods

The methodology is twofold. First, we searched for consensus epitopes on the whole genome of *Plasmodium falciparum* and secondly, we determined the tertiary peptide-dendrimer using molecular dynamics.

2.1 Search for consensus epitopes

Plasmodium falciparum data are available through the open source PlasmoDB database at <http://plasmodb.org>. We selected antigenic sequences with special attention to proteins that could interfere with parasite metabolism without interfering with the metabolism of the host. This last condition is required for non-toxic effects on the host even when no experimental demonstration is provided.

Every epitope derived from such proteins was collected, eliminating protein repetitions in order to produce a uniform distribution of the information and prevent bias in the consensus determination leading to a wrong epitope combination. To do so, the Expert Protein Analysis System (ExPASy) server was used to select consensus epitopes having at least 50% sequence similarity between them. The resulting epitopes were grouped in a cluster according to the results provided by the Nomad server (<http://expasy.org/tools/nomad.html>). This tool performs a local multi-alignment that does not allow any gap between the amino acids forming those epitopes and identifies blocks composed of six amino acids with a higher likelihood value among all of them. This action is carried out by means of iterative evaluation of relative entropy as described in (Hernández et al., 2006). This procedure enabled us to identify consensus epitopes that could be used as key factors in the development of vaccines against malaria.

2.2 Determination of the tertiary peptide-dendrimer

The next action was to design the eight branched (PAMAM) dendrimer, using pairwise combinations of epitopes but avoiding the use of identical epitopes in the same branch in order to restrict enhanced immune responses due to repetitive epitopes. In order to find the most favourable branch, molecular mechanics calculations were used to select the eight energetically most favourable structures. To do so, we used the following methodology (all calculations were run on the Linux operating system):

1. The tertiary structure of any possible branch was obtained using PerlMol program (www.perlmol.org) for each linear combination of the pair consensus epitopes. These branches are calculated this way:
 - a. The initial tertiary structure was visualised with VMD software (Humphrey et al., 1996).
 - b. Solvate plugin available in VMD was used in order to distribute a solvent medium only formed by water molecules surrounding the protein, such that when measured from the outer atoms of this molecule, it had a 5 Å thickness.
 - c. A second plugin called autoionize was executed for neutralizing the charge of the system by the addition of Cl⁻ or Na⁺ ions.
 - d. The necessary parameters for the molecular mechanics calculation were taken from the field force CHARMM version 27 (Mackerell et al., 1998). The terms for non-bond energy and van der Waals force were performed with a cut distance of 12 Å. All calculations were done in periodical contours and the hydrogen atoms were aggregated according to the ShakeH algorithm (Andersen, 1983).
 - e. NAMD (NAnoscale Molecular Dynamics) version 2.8 was used as the molecular mechanics and molecular dynamics program (Phillips et al., 2005) used. It works

such that the energy minimization (*i.e.*, adjust the three dimension structure with force field, solve the system, and relax the systems near of equilibrium position), heating (*i.e.*, adjust gradual adjust of temperature of system in lineal form with increased of 0.001 K), equilibration (*i.e.*, equilibration kinetic and potential energy at temperature constant) and, finally, molecular dynamics itself are determined. In the minimization process carried out at 50 ps, water molecules were free to move while the protein configuration remained unchanged.

- f. The eight combinations of pair of epitopes with the lowest energy were selected from the previous methodology applied in the previous step.
2. The tertiary structure of the (PAMAM) dendrimer was built with the xdrawchem program (available at <http://xdrawchem.sourceforge.net>). Later, it was linked with the eight branches obtained in the (f) step through NH₂ terminal.
3. Finally, the final tertiary structure of the peptide-dendrimer was derived by applying the computational methodology described in steps (b) to (f), while taking into account 50,000 ps of the molecular dynamics.

3. Results

The *Plasmodium falciparum* genomic 3D7 (taxonomic ID from NCBI: 36329) sequence was released in 2002 (Gardner et al., 2002). The genome is 23.26 Mb in size, with a karyotype of 14 linear chromosomes, with the size of chromosomes ranging from 0.75 Mb (154 genes in chromosome 1) up to 3.5 Mb (771 genes in chromosome 14). As can be seen, this database contains 5,373 genes, 10,999 proteins, 78 pseudo-genes and 73 RNA genes.

Table 1 shows some consensus merozoite epitopes obtained in this work. The consensus epitope was obtained according to their physicochemical properties and the local non-gap alignment. Finally, we only considered the most frequent consensus epitopes reported in the Table 1, *i.e.*, TSPRSD, NEGFCF, TDIHNS, YLKKIL, NKETKL, ALEKAV, LDNIKD, and KIFINN.

Table 1 Partial list of epitopes present in the *P. falciparum* genome debugged by means of the program available at ExPASy.

TSPRSD	NEGFCF	DIKCLT	TDIHNS	TSVLAG
YLKKIL	EEAHNL	NKETKL	KKDMLG	ALEKAV
NLIDTS	SYLEDY	NELDVL	AQAYDL	YTYNVE
SAQSGT	TGLEAD	DGYEEI	LDNIKD	KIFINN
DPTKSV	DVTPKS	SKKDYE	LKSHMD	EDYSLR
KSSENK	KFPSSP	EKIITD	PYYLIV	FDLLRA
HNVLQN	VPYPNG	NFPNTI	SVYNVQ	KYYNGE
FKGLTH				

Table 2 lists the first 15 combinations of pairs of epitopes sorted out for decreasing energy obtained from molecular mechanics calculations that could act as branches in the peptide-dendrimer structure. They were obtained by molecular mechanics and sorted for decreasing

energetic values. The pairs of epitopes most favourable were TLKKILGGNKETKL, TLKKILGGLDNIKD, TSPRSDGGNKETKL, TSPRSDGGNEGFCF, TSPRSDGGLDNIKD, TLKKILGGKIFINN, TDIHNSGGNKETKL, TSPRSDGGKIFINN. The results in Table 2 show that the same peptide, linked in different pairs, conserved the same total energy. This suggests that the calculations performed in this work reflect an intrinsic property of the peptide and, by extension of the peptide in the protein. This may be due to the interpolation of two glycines, avoiding new secondary structures in the peptide pair. It also comforts the hypothesis that the dendrimer can induce an immune answer against the targeted parasite proteins.

Table 2 List of the 15 lowest energetic structures obtained by molecular mechanics with the possible dendrimer branches. Branch identification and the energy values (Kcal/mol) are shown.

TLKKILGGNKETKL	-10132,69
TLKKILGGLDNIKD	-9506,94
TSPRSDGGNKETKL	-9452,45
TSPRSDGGNEGFCF	-9084,08
TSPRSDGGLDNIKD	-9081,45
TLKKILGGKIFINN	-8764,08
TDIHNSGGNKETKL	-8549,80
TSPRSDGGKIFINN	-8385,10
TDIHNSGGLDNIKD	-8347,68
TSPRSDGGALEKAV	-8272,81
ALEKAVGGLDNIKD	-8174,00
TDIHNSGGYLKKIL	-8072,47
TSPRSDGGTDIHNS	-8033,29
NEGFCGGNKETKL	-7862,67
LDNIKDGGKIFINN	-7662,31

Fig. 2 shows the final structure of the peptide-dendrimer obtained in the final step (3) using molecular dynamics methodology. This structure forms fibers around a condensed nucleus. However, it is important to indicate that the current work is mainly focused on describing a systematic methodology for developing new vaccines designed from experimental data rather than directly obtaining that experimental structure.

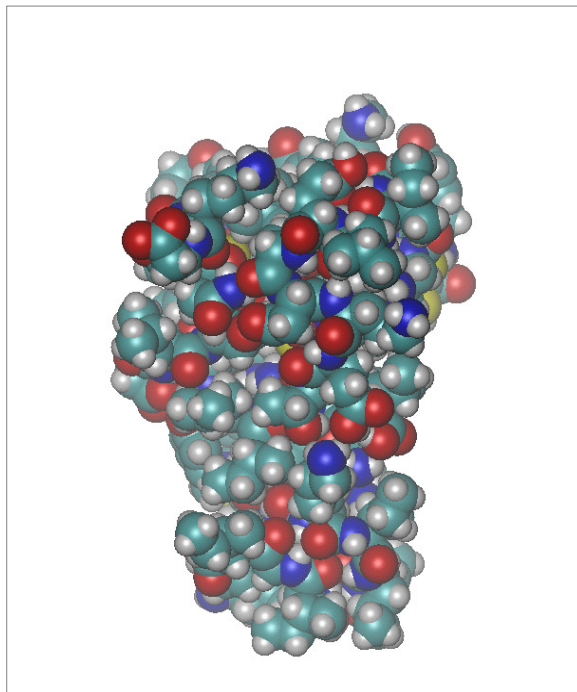


Fig 2. The final 3D structure of a peptide-dendrimer obtained by using molecular dynamics (see text for details).

4. Conclusion

A computational methodology that can be reasonably used for obtaining compact and non-toxic vaccine candidates on a nano-scale is presented. It makes use of experimental data that are available through databases and literature. The work was focused on malaria as a worldwide epidemic disease but the same methodology could be used for other infectious diseases or for anti-tumour vaccines using tumour-specific antigens.

The innovation in computational techniques that could drive the scientific community to better design such vaccines is of utmost importance since it can be seamlessly coupled to traditional techniques and procedures. The future work is the synthesis of the peptide-dendrimer for validating this computational methodology.

Acknowledgement

We thank the Ibero-American Network of the Nano-Bio-Info-Cogno Convergent Technologies (Ibero-NBIC)

References

- Andersen, H.C., 1983. Rattle: A velocity version of the Shake algorithm for molecular dynamics calculations. *J. Comp. Phys.* 52, 24–34.
[http://dx.doi.org/10.1016/0021-9991\(83\)90014-1](http://dx.doi.org/10.1016/0021-9991(83)90014-1)
- Bojang, K.A., Milligan, P.J., Pinder, M., Vigneron, L., Allouche, A., Kester, K.E., et al., 2001. Efficacy of RTS,S/AS02 malaria vaccine against Plasmodium falciparum infection in semi-immune adult men in The Gambia: a randomised trial. *Lancet.* 358, 1927-1934.
[http://dx.doi.org/10.1016/S0140-6736\(01\)06957-4](http://dx.doi.org/10.1016/S0140-6736(01)06957-4)
- Buhleier, E., Wehner, W., Vögtle, F., 1978. Cascade and Nonskid-chain-like Synthesis of Molecular Cavity Topologies. *Synthesis.* 2, 155–158.
<http://dx.doi.org/10.1055/s-1978-24702>
- Chaves, F., Calvo, J.C., Carvajal, C., Rivera, Z., Ramírez, L., Pinto, M., Trujillo, M., et al., 2001. Synthesis, isolation and characterization of Plasmodium falciparum antigenic tetrabranched peptide dendrimers obtained by thiazolidine linkages. *J. Pept. Res.* 58, 307–316.
<http://dx.doi.org/10.1034/j.1399-3011.2001.00921.x>
PMid:11606215
- Dan, H. B., 2006. Rational design of gene-based vaccines. *J. Pathol.* 208, 283–289.
<http://dx.doi.org/10.1002/path.1874>
PMid:16362986
- Dutta, T., Garga, M., Jaina, N.K., 2008. Poly(propyleneimine) dendrimer and dendrosome mediated genetic immunization against hepatitis B. *Vaccine*, 3389-3394.
<http://dx.doi.org/10.1016/j.vaccine.2008.04.058>
PMid:18511160
- Gardner, M.J., Hall, N., Fung, E., White, O., Berriman, M., Hyman, R.W., et al., 2002. Genome sequence of the human malaria parasite Plasmodium falciparum. *Nature.* 419, 498-511.
<http://dx.doi.org/10.1038/nature01097>
PMid:12368864
- Hayder, M., Poupot, M., Baron, M., Nigon, D., Turrin, C.O., Caminade, A. M., et al., 2011. A phosphorus-based dendrimer targets inflammation and osteoclastogenesis in experimental arthritis. *Sci. Transl. Med.* 81.
- Heegaard, P.M., Boas, U., Sorensen, N.S., 2010. Dendrimers for Vaccine and Immunostimulatory Uses. A Review. *Bioconjugate Chem.* 21, 405–418.
<http://dx.doi.org/10.1021/bc900290d>
PMid:19886668
- Hernández, D., Gras, R., Appel, R., 2006. Neighborhood functions and hill-climbing strategies dedicated to the generalized ungapped local multiple alignment. *European J. Operat. Res.* 185, 1276-1284.
<http://dx.doi.org/10.1016/j.ejor.2005.10.076>
- Herrington, D.A., Clyde, D.F., Losonsky, G., Cortesia, M., Murphy, J.R., Davis, J., et al., 1987. Safety and immunogenicity in man of a synthetic peptide malaria vaccine against Plasmodium falciparum sporozoites. *Nature.* 328, 257 – 259.
<http://dx.doi.org/10.1038/328257a0>
PMid:2439920
- Humphrey, W., Dalke, A., Schulten, K., 1996. VMD - Visual Molecular Dynamics. *J. Molec. Graphics.* 14, 33-38.
[http://dx.doi.org/10.1016/0263-7855\(96\)00018-5](http://dx.doi.org/10.1016/0263-7855(96)00018-5)
- Mackerell, A.D., Bashford, D., Bellot, M., Dunbrack, R.L., Evanseck, J.D., M. Field, J., et al., 1998. All-atom empirical potential for molecular modeling and dynamics studies of proteins. *J. Phys. Chem. B.* 102, 3586-3616.
<http://dx.doi.org/10.1021/jp973084f>
- Majoros, I.J., Williams, C.R., Baker, J.R., 2008. Current dendrimer applications in cancer diagnosis and therapy. *Curr Top Med Chem.* 8, 1165-1179.
<http://dx.doi.org/10.2174/156802608785849049>

PMid:18855703

Najlah, M., and D'Emanuel, A., 2006. Crossing cellular barriers using dendrimer nanotechnologies. *Curr. Opin. Pharmacol.* 6, 522-527.

<http://dx.doi.org/10.1016/j.coph.2006.05.004>

PMid:16890022

Nardin, E.H., Calvo-Calle, J.M., Oliveira, G.A., Nussenzweig, R.S., Schneider, M., Tiercy, J.M., et al., 2001. A Totally Synthetic Polyoxime Malaria Vaccine Containing Plasmodium falciparum B Cell and Universal T Cell Epitopes Elicits Immune Responses in Volunteers of Diverse HLA Types1. *J Immunol.* 166, 481-489.

PMid:11123327

Phillips, J.C., Braun, R., Wang, W., Gumbart, J., Tajkhorshid, E., Villa, E., et al., 2005. Scalable molecular dynamics with NAMD. *J. Computat. Chemistry.* 26, 1781-1802.

<http://dx.doi.org/10.1002/jcc.20289>

PMid:16222654 PMCID:2486339

Prieto, J.H., Koncarevic, S., Park, S.K., Yates, J. III., Becker, K., 2008. Large-Scale Differential Proteome Analysis in Plasmodium falciparum under Drug Treatment. *PLoS ONE*, 3, e4098.

<http://dx.doi.org/10.1371/journal.pone.0004098>

PMid:19116658 PMCID:2605551

Rupp, R., Rosenthal, S.L., Stanberry, L.R., 2007. VivaGel™ (SPL7013 Gel): A candidate dendrimer – microbicide for the prevention of HIV and HSV infection. *Int J Nanomedicine.* 2, 561–566.

PMid:18203424 PMCID:2676817

Sadler, K. and Tam, J.P., 2002. Peptide dendrimers: applications and synthesis. *J. Biotechnol.* 90, 195-229.

PMid:12071226

Sauerwein, R.W., Roestenberg, M., Moorthy, V.S., 2011. Experimental human challenge infections can accelerate clinical malaria vaccine development. *Nat. Rev. Immunol.* 11, 57-64.

<http://dx.doi.org/10.1038/nri2902>

PMid:21179119

Tam, J.P., Clavijo, P., Lu, Y.A., Nussenzweig, V., Nussenzweig, R., Zavala, F., 1990. Incorporation of T and B epitopes of the circumsporozoite protein in a chemically defined synthetic vaccine against malaria. *J. Exp. Med.* 171, 299-306.

<http://dx.doi.org/10.1084/jem.171.1.299>

PMid:1688609

Tokuhisa, H., Crooks, R.M., Ricco, A.J., Osbourn, G.C., 1997. Chemical Sensors Based on Surface-Confined Dendrimers. *Proc. – Electrochem. Soc.* 19, 134-140.