Field evaluation of novel livestock vaccines
EuFMD-IVVN workshop, 2018

Design of a multi-antigenic, multi-stage and multi-epitope vaccine candidate against onchocerciasis and related filarial diseases

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Supervisor:

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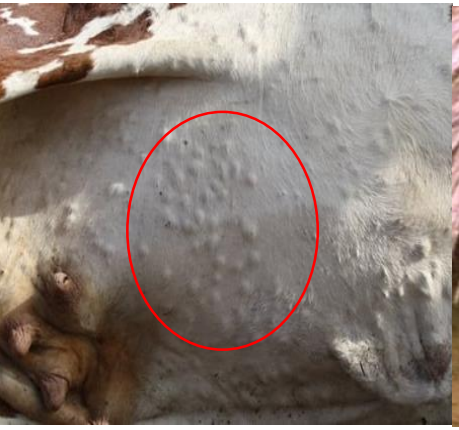
Onchocerciasis, what is it?

- **Causative agent:**
  - ✓ ***Onchocerca ochengi***
  - ✓ ***Onchocerca volvulus***

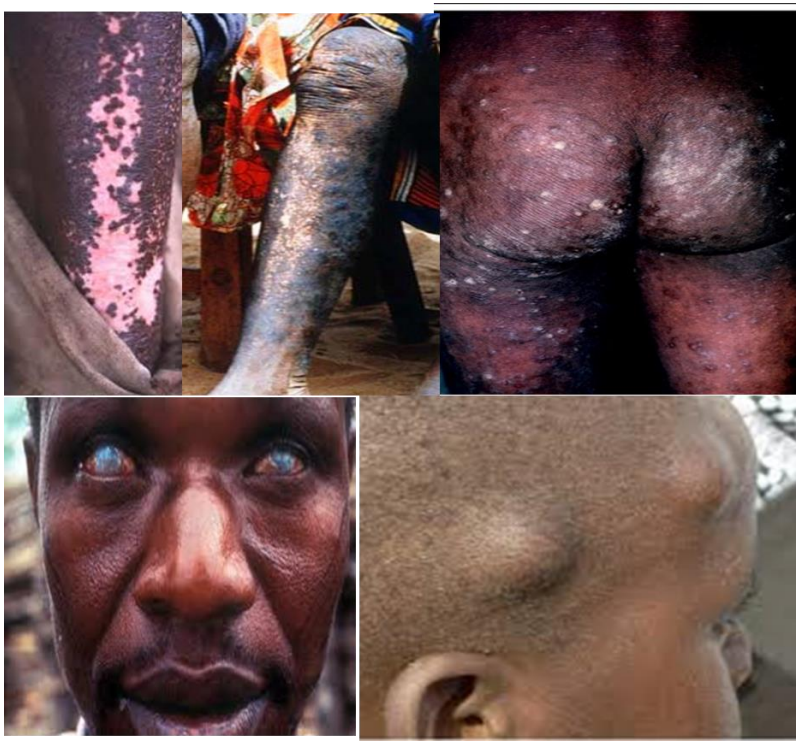


Bovine

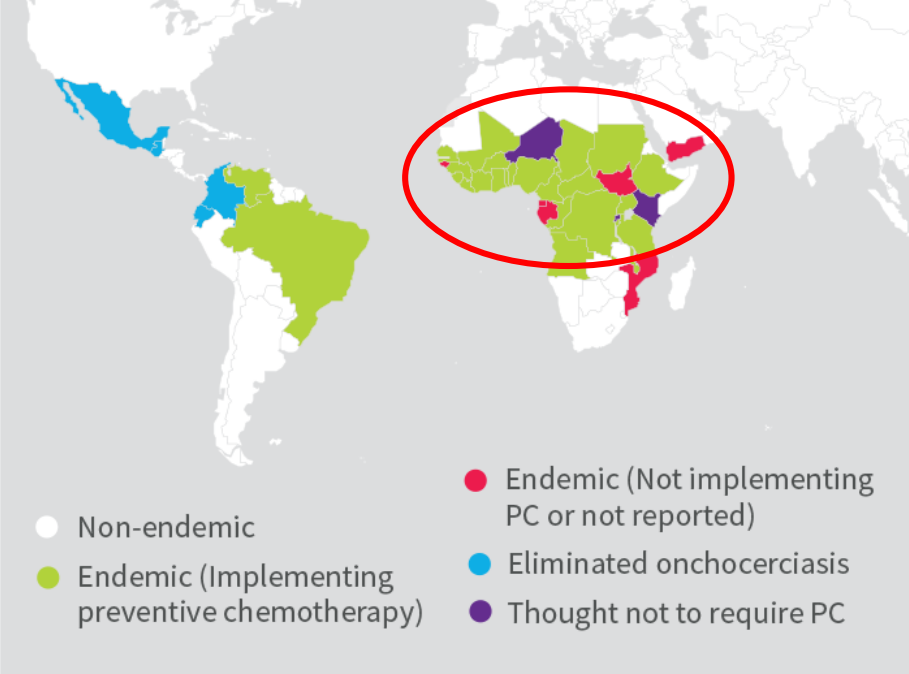
Human

- **Common Vector: Blackfly**

- **Pathology**: associated mainly with microfilariae



Onchocerciasis – the burden



- **15.5 million people infected**
- **10% visually impaired, 1% blind**
- **About 172 million people at risk**
- **High epilepsy and mortality reported**

Lustigman *et al.,* 2017

Control strategies & challenges

Vector

Insecticide Sprays

- Difficult to spray forest areas
- Black flies reinvade area

Control of onchocerciasis

Parasite

Chemotherapy

- Ivermectin has been the mainstay to control

- **IVM is only microfilaricidal**
- **Resistance to IVM**
- **SAE in cases of co-endemicity with loiasis**

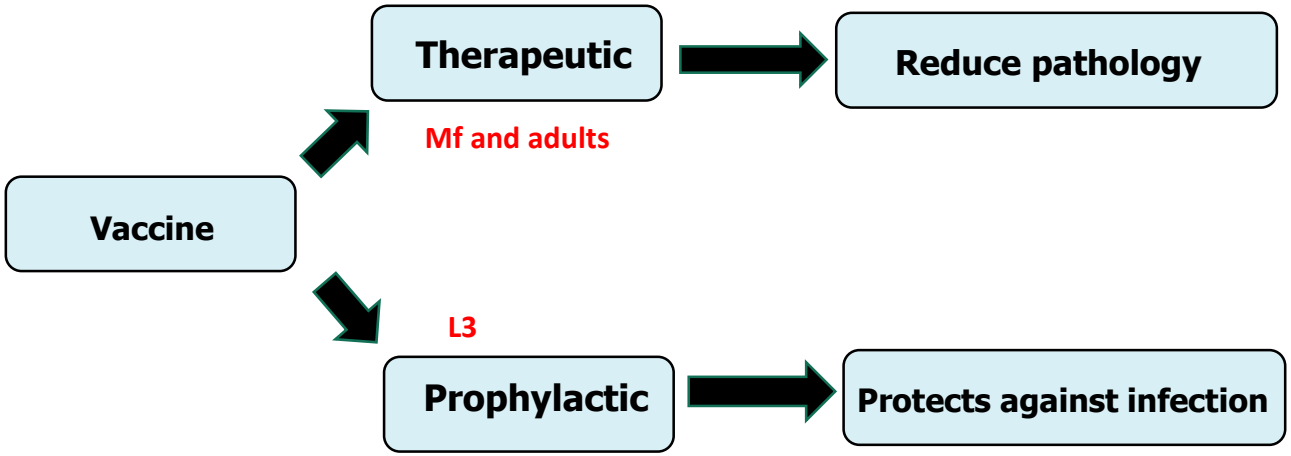
Onchocerciasis: NOT eradicable in Africa with current tools

Vaccines could accelerate towards elimination goal

Vaccine strategies in onchocerciasis

The Onchocerciasis Vaccine for Africa (TOVA) Initiative launched in 2012

- **Protective immunity exists**
  - ✓ 1-5% are putative immunes (PI)
  - ✓ Zooprophylaxis
  - ✓ Concomitant immunity
  - ✓ *In-vitro and in vivo* studies in animal models



- **Ov103, Ov-RAL-2, Ov-ASP-1, Ov-ALT-1 and Ov-ALT-2 planned for phase 1 trials**

Five Lead vaccine candidates selected for vaccine construct (Ov-DKR-2)

Single antigens are limited

- **Genetic responses from host**
- **Presence of tolerigenic or autoreactive epitopes**
- **Shifts in antigenic profiles**
- **Elicit insufficient responses**
  - ✓ **Multiple vaccine target hypothesis:**

$$C_v = 2n$$

    - ➢ **C_v = required targets**
    - ➢ **n = major parasite stages**
    - ➢ **C_v = 6**

Titanji *et al.,* 2017

Immunoprotection is multifactorial

Protective Immunity to the Larval Stages of *Onchocerca volvulus* Is Dependent on Toll-Like Receptor 4

Roles for both CD4+ and CD8+ T cells in protective immunity against *Onchocerca lienalis* microfilariae in the mouse

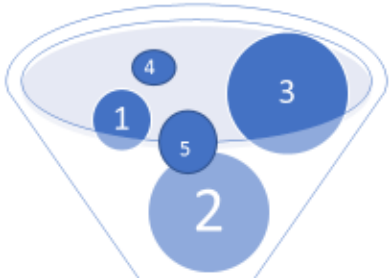
IgG1, IgG2 and IgE were higher, but IgG4 was lower in endemic controls compared with post-patent onchocerciasis patients

...support the possible involvement of anti-Ov-CPI-2 IgG1 and/or IgG3 cytophilic antibodies in the development of protective immunity

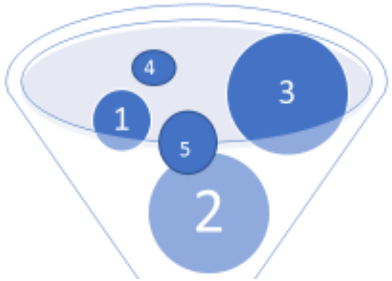
IgG3 levels have a significant negative correlation with the Mf load

Antigens contains B/T cell epitopes

Epitope type	**Linear-B**	**Helper-T**	**Cytotoxic-T**
Tool used	Bcpreds, ABCPred, BepiPred	NetMHC 2.3 server	NetCTL 1.2
Basis	Recurrence	Affinity	Cut-off score



15-mers

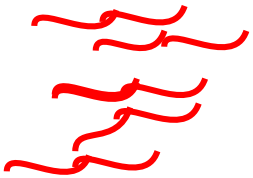


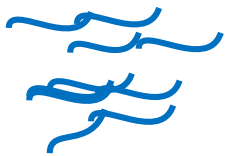
15-mers

9-mers

Antigenicity — **Vaxijen & ANTIGENPro**

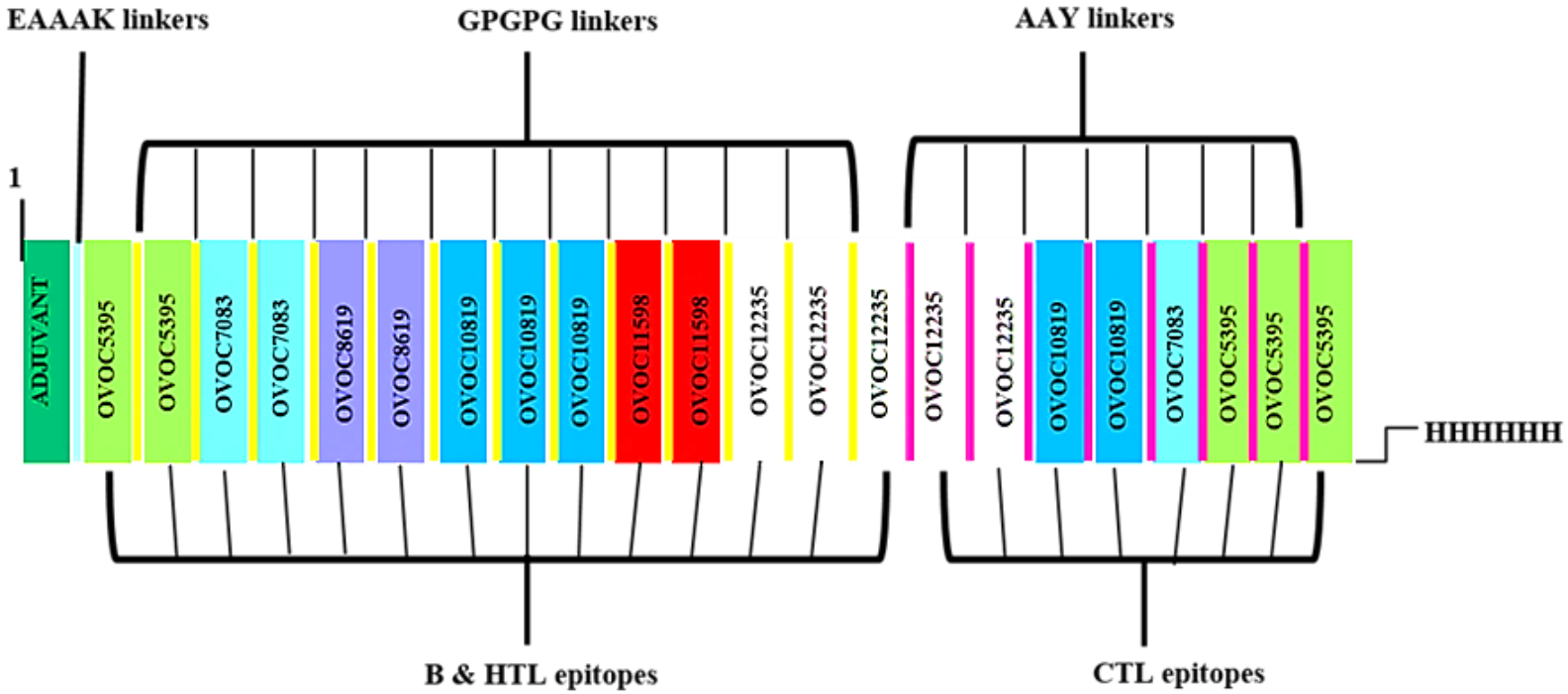
Epitopes selected





Epitope assembly and antigen design

599 aas, N-terminal adjuvant, 14 B-epitopes, 14-CTLs and 8 HTLs, flexible linkers and 6xHis tag



Adjuvant: TLR4 agonist *(Mycobacterium tuberculosis* 50S ribosomal protein L7/L12)

Constituent proteins are conserved

Protein	Percentage identity				
	O. ochengi	*O. flexuosa*	*L. loa*	*B. malayi*	*W. bancrofti*
Ov103	99.4	81.6	72.8	68.4	70.3
Ov-RAL-2	99.4	45.5	49.3	56.1	55.8
Ov-ASP-1	95.4	48.3	69.5	73.6	74.1
Ov-ALT-1	99.3	45.5	38.4	46.2	43.8
Ov-ALT-2	76.6	45.5	36.0	47.7	47.3

Epitopes, MSA: **36.0 - 99.4%**

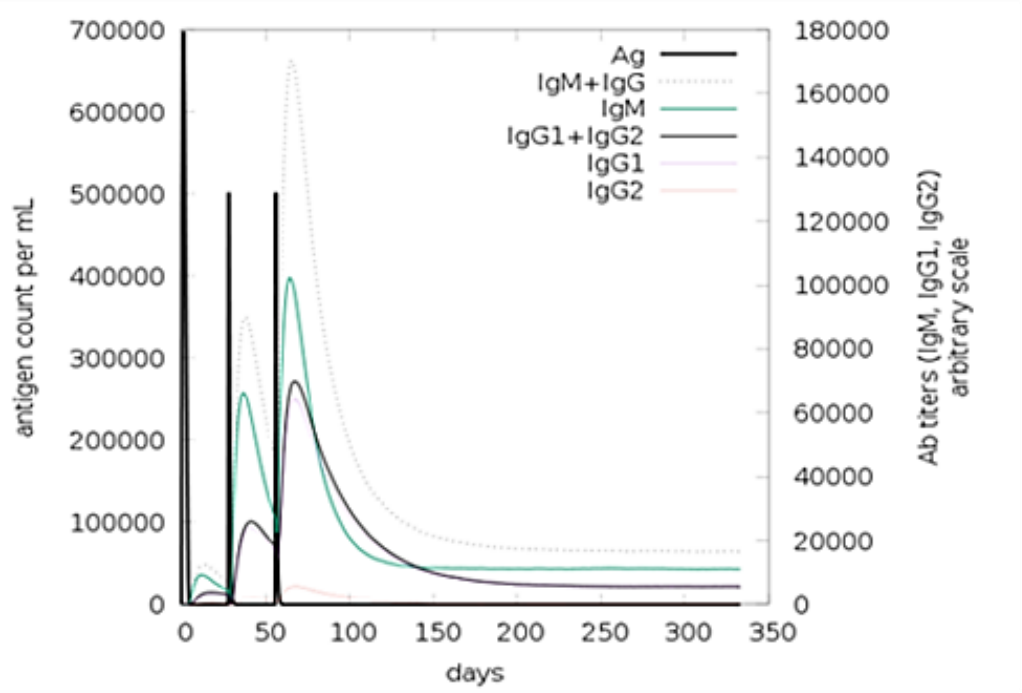
Chimera is antigenic and non-allergenic

Protein	Antigenicity (ANTIGENpro)	Antigenicity (VaxiJen 2.0)	Instability index	Allergenicity	
				AllerTOP	AllergenFP
Ov-DKR-2 (core)	0.931448	0.6202	41.92	NO	NO
Ov-DKR-2	0.953572	0.5472	32.19	NO	NO
Ov-103	0.594738	0.5230	37.44	NO	NO
Ov-RAL-2	0.887977	0.5141	67.79*	NO	NO
Ov-ASP-1	0.901496	0.5449	23.03	NO	NO
Ov-ALT-1	0.138690	1.4362	35.39	NO	NO
Ov-ALT-2	0.905444	0.4598	35.97	NO	NO

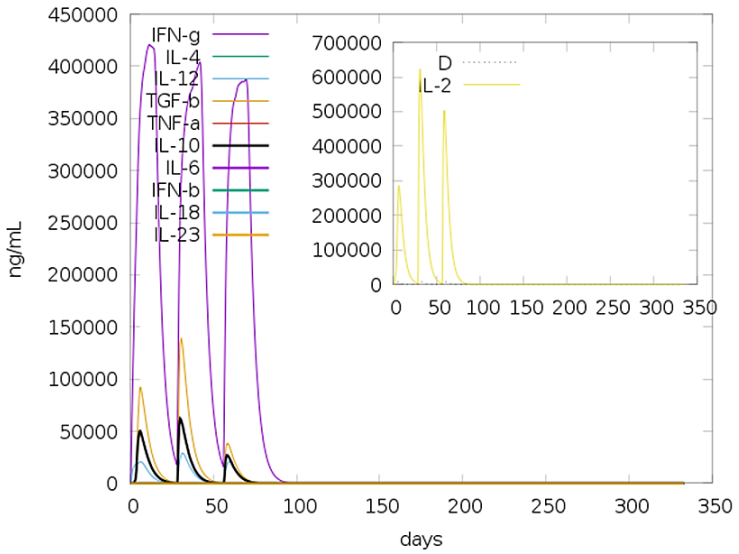
Cut-off: 40

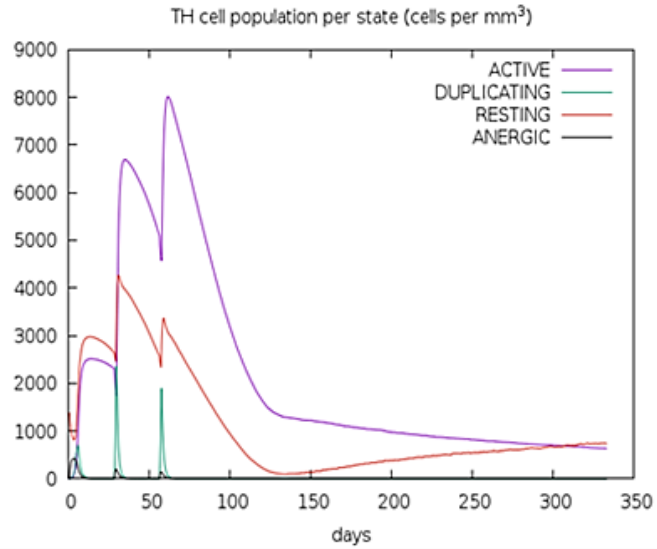
Chimera induces IgG, IgM and IFN-γ secretion

50 IFN-γ inducing epitopes



High titers of IgG1+ 2 and IFN-γ



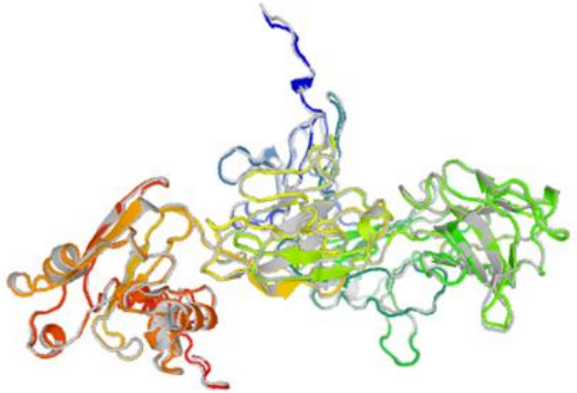


Large Th cell populations

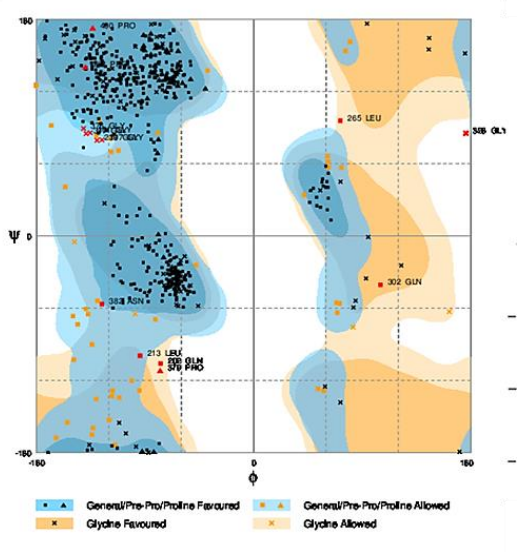
Structure predicted, refined and validated

I-TASSER – homology modelling

ModRefiner + GalaxyRefine



RAMPAGE

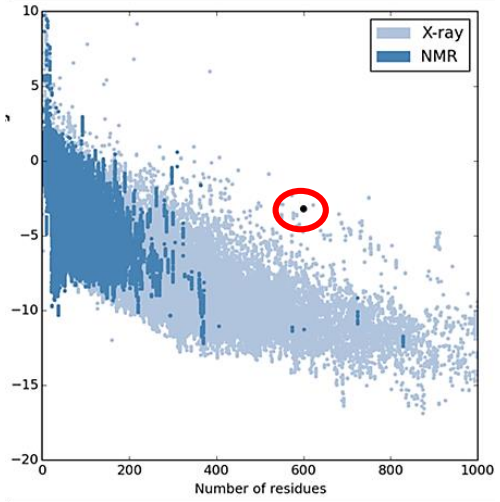


Favoured: 94.5%,

Allowed : 4.5% and

Outlier: 1.0 %

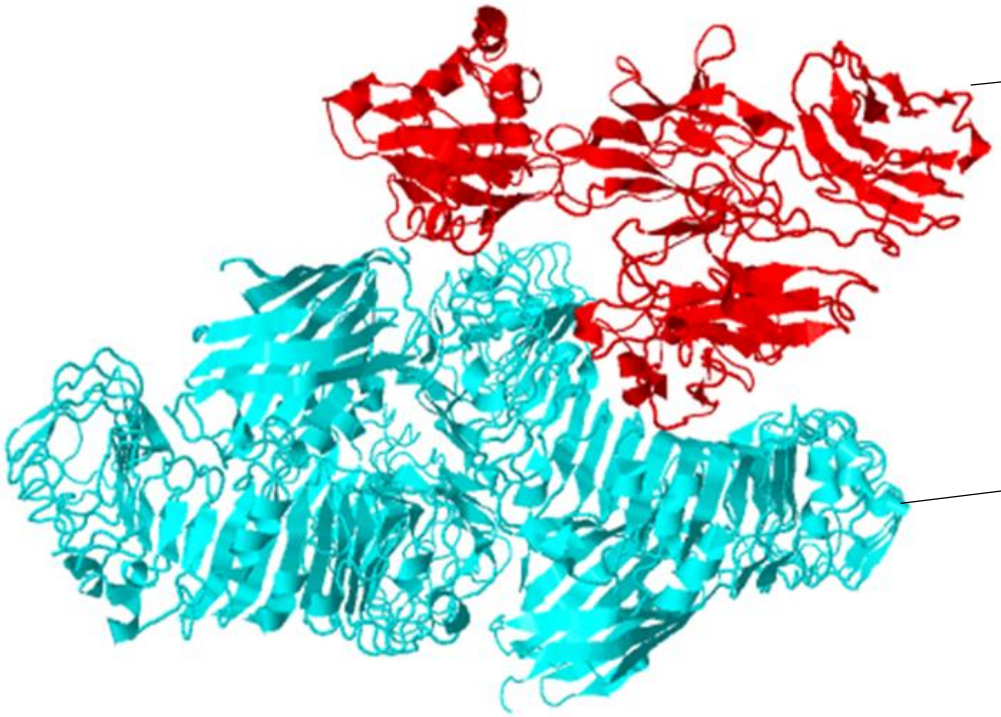
ProSA-Web



Z-score of −3.18.

3D structure interacts with TLR4

FRODOCK



Based on the calculated energies

Conclusions

- **Ov-DKR2 demonstrate superior antigenicity to the current individual lead vaccine candidates.**

- **Ov-DKR could act as a therapeutic and prophylactic vaccine.**

Recommendation

- **Ov-DKR2 should be cloned and expressed for *in-vitro/in-vivo* studies**

Acknowledgements

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Thanks for your kind attention