

## Supplementary file 1

# Design and computational analysis of an effective multi-epitope vaccine candidate using subunit B of cholera toxin as a build-in adjuvant against urinary tract infections

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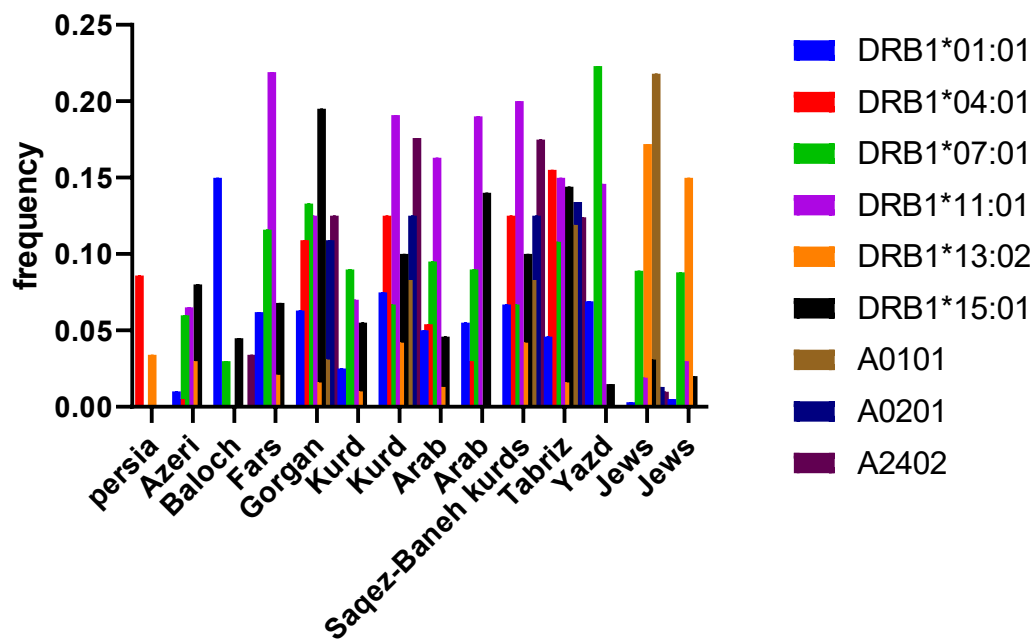
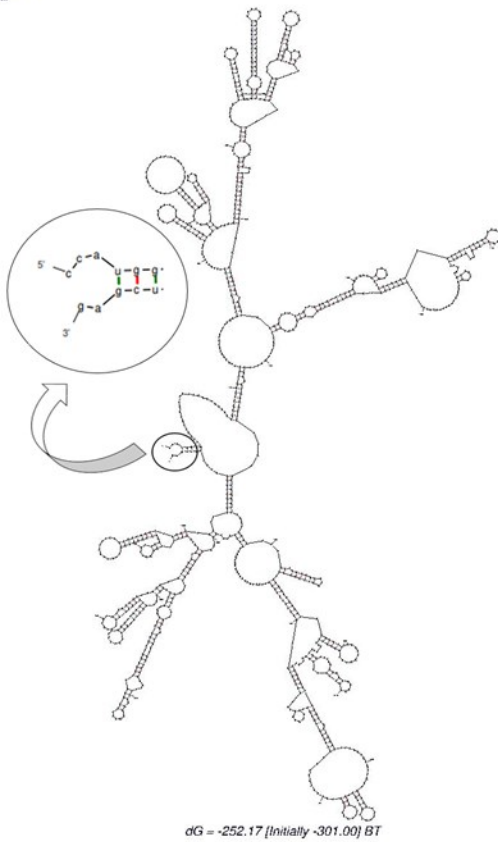


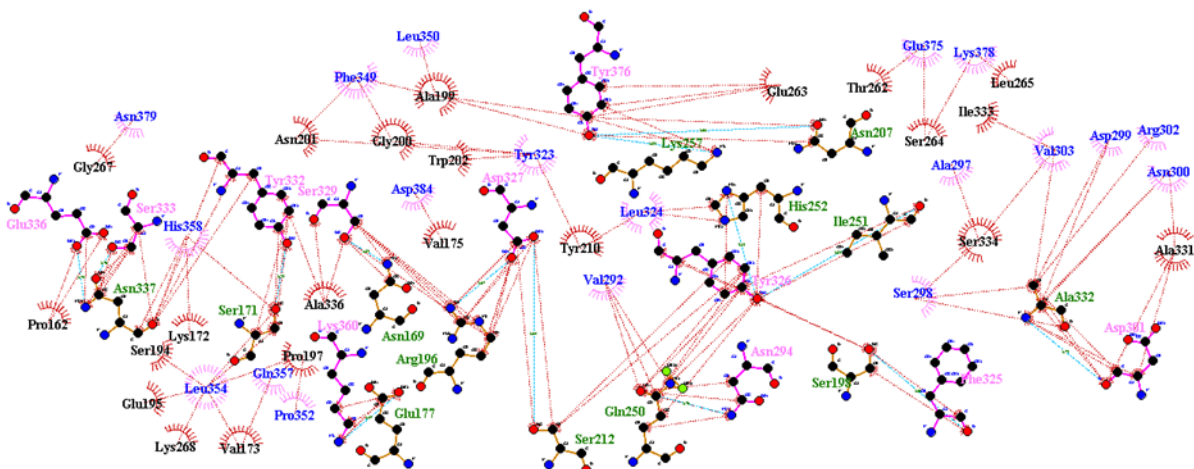
Figure S1: The frequency of MHC alleles used in our study is shown based on the data extracted from [www.allelefreqencies.net](http://www.allelefreqencies.net).



Structural element	$\delta G$	Information
External loop	- 0.60	5 ss bases & 1 closing helices.
Stack	- 1.40	External closing pair is u <sup>4</sup> -g <sup>1023</sup>
Stack	- 1.50	External closing pair is g <sup>5</sup> -c <sup>1022</sup>
Stack	- 2.10	External closing pair is g <sup>6</sup> -u <sup>1021</sup>
Stack	- 3.30	External closing pair is g <sup>7</sup> -c <sup>1020</sup>

**Table S1:** Free energy details related to 5' end of chimeric gene mRNA structure

**Figure S2:** The best predicted mRNA structure ( $\Delta G = -252.17$  kcal/mol).



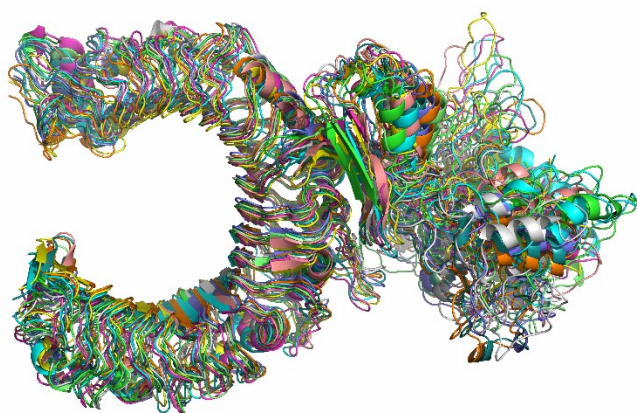
**Figure S3:** The two-dimensional diagram of the vaccine protein-TLR2 docked complex. The hydrogen bonds and hydrophobic interactions are shown as a Deep sky blue dashed line, and a red spline curve, respectively. The intermolecular bonds of the vaccine and TLRs are shown as orange and pink lines, respectively. The plots provided by the DIMPLOT tool of LigPlot+ v2.2 program.



**Figure S4:** The merge tertiary structure of the TLR2- vaccine protein complex at 75,95 and 99ns.

	Interacting amino acids of ligand	Interacting amino acids of receptor
<b>75 ns</b>	His247, Thr249, Gln250, His252, Leu254	Val292, Asn294, Leu324, Tyr323, Tyr326
<b>95ns</b>	Thr249, Gln250, His252, Leu254, Ala336	Val292, Asn294, Ala297, Tyr323, Leu324
<b>99ns</b>	Thr249, Gln250, Ile251, His252, Pro327, His328	Val292, Asn294, Asp301, Arg302, Leu324, Tyr326

**Table S2:** Interacting amino acids of vaccine protein and TLR2 at 75ns, 95ns and 99 ns by Ligplot + software.



**Figure S5.:** Conformational changes of the TLR2- vaccine protein complex at 10, 20, 31, 40, 50, 60, 70, 80, 90 and 95ns during simulation time after clustering analysis.