SUPPLEMENTARY INFORMATION

**Development of superior antibodies against the S-protein of SARS-Cov-2 using macrocyclic epitopes**

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(Supplementary Information)

**HPLC Analyses**

The mobile phase consisted of 0.1%Trifluoroacetic in 100%, Acetonitrile and 0.1%Trifluoroacetic in 100% Water with a detection wavelength of 220 nm, Flow rate: 1.0 ml/min and volume: 20 µl.

The gradient mobile phase was set as follows for the different peptides:

**Table S 1**: Grade mobile phase used in the HPLC Analyses for the different peptides

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| ***Peptide*** | ***0.1%Trifluoroacetic in 100% Acetonitrile*** | | | ***0.1%Trifluoroacetic in 100% Water*** | | |
| ***0.01 min*** | ***25 min*** | ***25.01 min*** | ***0.01 min*** | ***25 min*** | ***25.01 min*** |
| ***(1)*** | *10.0 %* | *70.0 %* | *100.0 %* | *90.0 %* | *30.0 %* | *0.0 %* |
| ***(2)*** | *15 %* | *75.0 %* | *100.0 %* | *85.0 %* | *25.0 %* | *0.0 %* |
| ***(3)*** | *20 %* | *80.0 %* | *100.0 %* | *80.0 %* | *20.0 %* | *0.0 %* |

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**Figure S 1**: : Characterization of peptide **(1)** by HPLC

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**Figure S 2**: Characterization of peptide **(2)** by HPLC

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**Figure S 3**: Characterization of peptide **(3)** by HPLC

**LC-MS Analyses**

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**Figure S 4**: Characterization of peptide **(1)** by LC-MS

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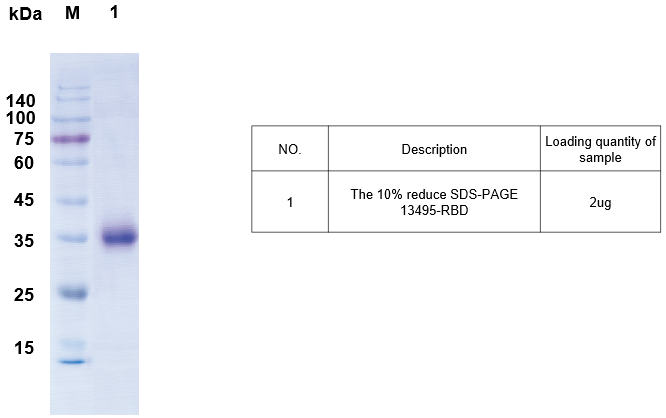
**Figure S 5**: Characterization of peptide **(2)** by LC-MS

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**Figure S 6:** Characterization of peptide **(3)** by LC-MS

**Recombinant COVID-19 RBD protein**

The purity of the RBD that will be used for Western Blot and ELISA studies has been verified by SDS-PAGE in reducing conditions as shown in Figure S 7.



**Figure S 7**: SDS-PAGE for the RBD protein. The sequence of the used RBD is the following: RVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLHAPATVCGPKKSTNLVKNKCVNFSGHHHHHHHH

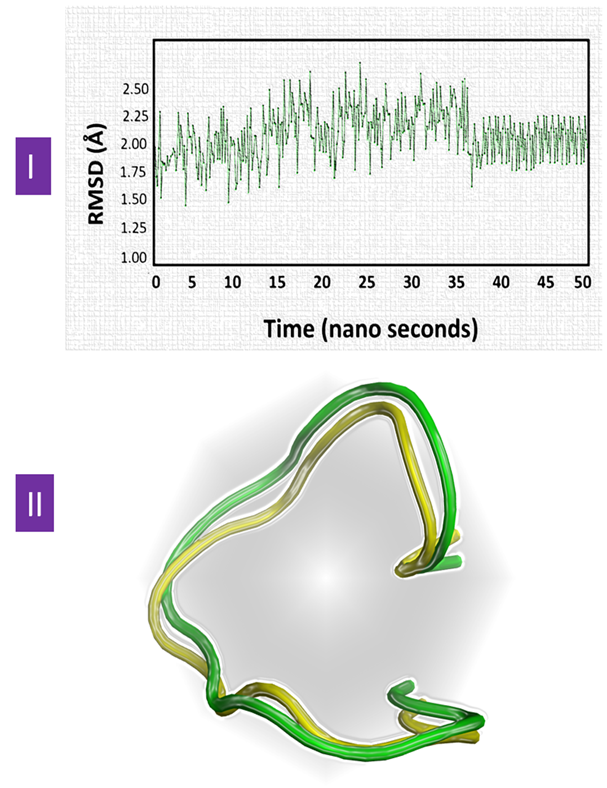
**Phsyico-chemical properties of the Designed peptides**

**Table S 2**: Physicochemical properties of the designed peptides

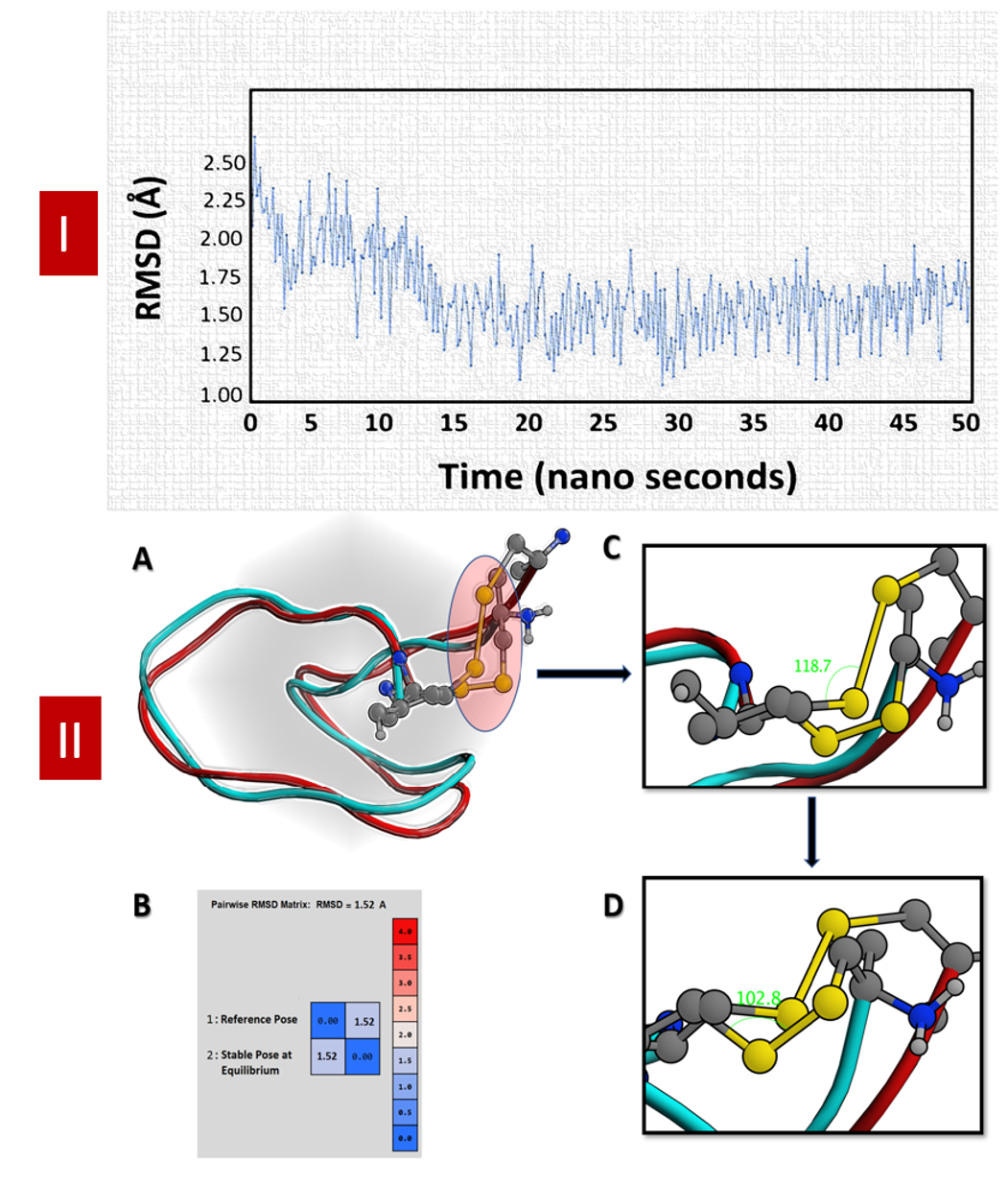
determined using PepCal (https://pepcalc.com/)

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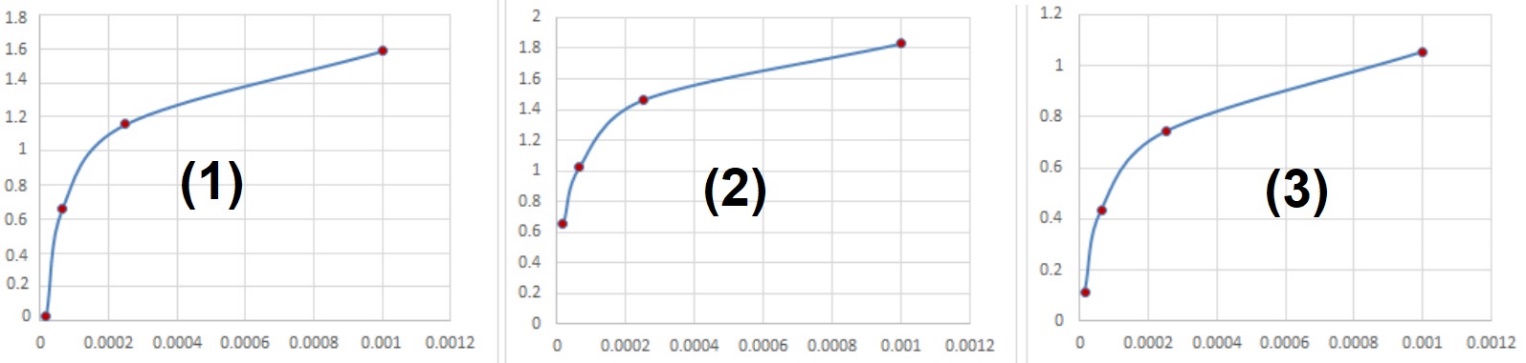
**Molecular Dynamics**



**Figure S 8**: I) MD of peptide **(1)** over 50 ns showing the least RMSD = 2.00 Å. II) Superimposition of the reference conformation of **(1)** (yellow) and the stable one at equilibrium (green).



**Figure S 9**: I) MD of peptide **(3)** over 50 ns showing the least RMSD = 0.61 Å. II) A) Superimposition of the reference conformation of **(3)** (red) and the stable one at equilibrium (blue), B) RMSD matrix , C) Measured angle before MD, D) Measured angle after MD.



**Figure S 10**: ELISA data conducted on the sera of rabbits immunized with epitopes **(1)**, **(2)** and **(3)**