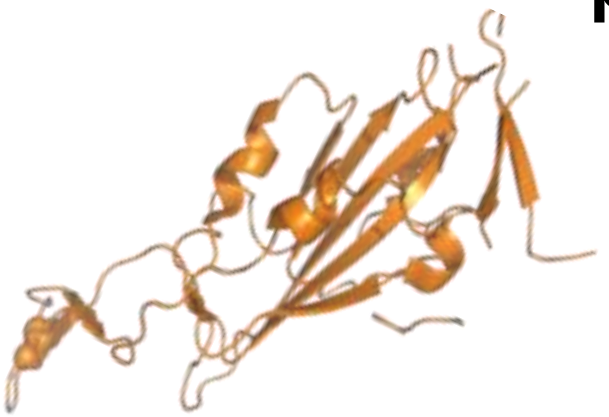
Soberana vaccines against SARS-CoV2: coping with complexity in a versatile biotechnological process



CIM Centro de Inmunología Molecular

A chapter in the story of anti-COVID-19 Cuban vaccines

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Cuba

Area: 109,884 km^2

Over 11 million inhabitants

Capital city: La Habana

A brief overview of Cuban Biotechnology:

before COVID-19

✓ How did Cuban Biotech start?

Several Research-Production biotech institutes were created in the 80s-90s (early stages of world Biotech)

Center of Genetic Engineering and Biotechnology

Immunoassay Center

Finlay Institute of Vaccines

BioCEN

Center of Molecular Immunology

A brief overview of Cuban Biotechnology: before COVID-19

✓ How did Cuban Biotech evolve?

BioCubaFarma (2012) is a group which coordinates the activities of more than 30 biotechnological and pharmaceutical enterprises, including joint ventures in Cuba and abroad.

The Protein Engineering lab at the Center of Molecular Immunology

where new proteins are born

- **Use of large molecular libraries to discover and optimize immune-related molecules to perform the desired functions**
- ✓ **Anti-cancer antibodies**
- ✓ **Immune modulators that mediate cell-to-cell communication (cytokines)**
- **High throughput production of multiple proteins at laboratory scale to study their functions**
- **Generation of cell lines stably producing therapeutic proteins (protein factories) suitable for industrial scale**

What happened after COVID-19 emergence?

- **2020: Cuban scientists started to prepare the scenario for treating and preventing COVID-19 (at public health and biotechnology institutions).**
- **March 2020: First cases of COVID-19 were detected in Cuba.**
- **March 2020: Protein engineering lab at CIM incorporated to the vaccine development project.**
- **May 2020: The Cuban president organized a meeting with scientists from the biotechnology field to confirm the decision of making our own vaccines.**
- **July 2021: Cuban regulatory authorities (CECMED) authorized the use of emergency of the first anti-COVID vaccine in Latin America (Abdala)**
- **August 2021: Soberana vaccines also received the authorization for emergency use in Cuba**

What is a vaccine made of?

- **Non-specific components that enhance immune responses against the vaccine or even global immune responsiveness of the individual: multiple substances of different chemical nature (biological, organic, inorganic) that can be included in diverse vaccines…**
- **Specific components or vaccine antigens: Particular pathogens, pathogen parts, or compounds that resemble pathogens. The response against them attacks the target pathogen, protecting vaccinated individuals from the disease.**

Examples of specific components in the case of anti-viral vaccines:

- ✓ **whole viruses (attenuated or inactivated to make them harmless)**
- ✓ **modified non-pathogenic viruses that contain molecules that simulate the original target virus**
- ✓ **synthetic compounds resembling virus parts**
- ✓ **viral proteins or their fagments obtained through genetic engineering**
- ✓ **nucleic acids coding for the synthesis of viral proteins once in the body**

Cuban strategy to produce anti-COVID vaccines

1. **involved several institutions and multiple production facilities (redundancy as a way to robustness).**

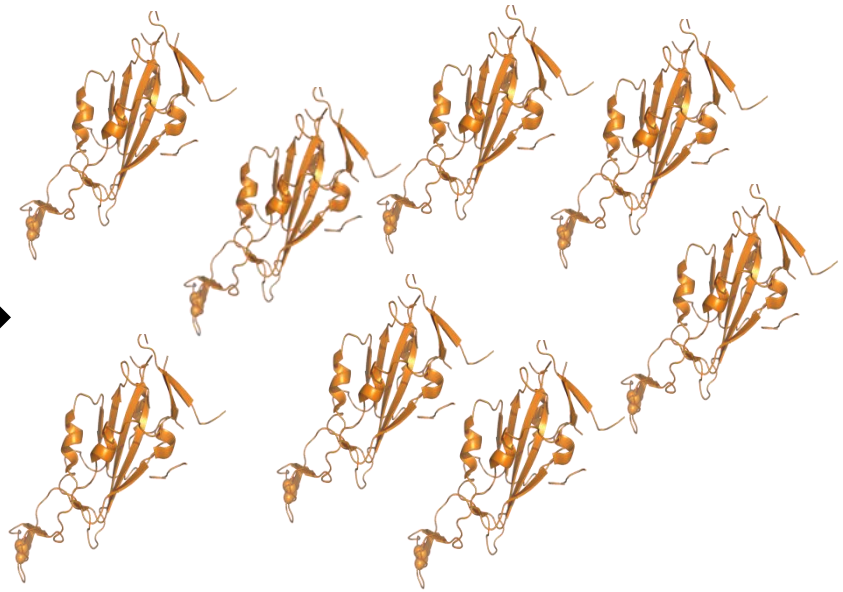
2. **included the development of several vaccine candidates, which together could cover the whole Cuban population and eventually could be combined to achieve better results.**

3. **took advantage of already established technological platforms to accelerate development.**

Production of recombinant viral proteins by genetic engineering

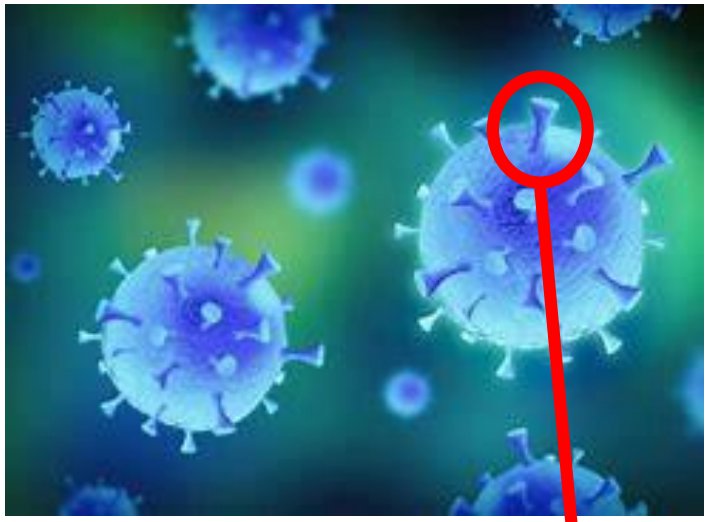
DNA

living host cells

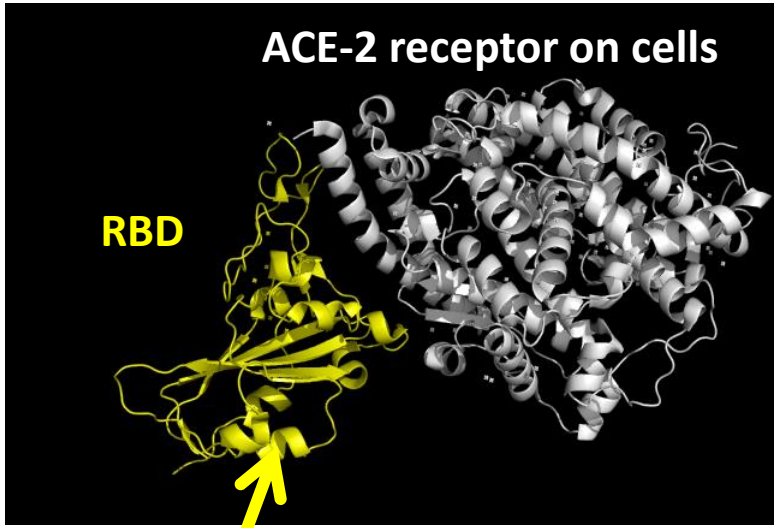


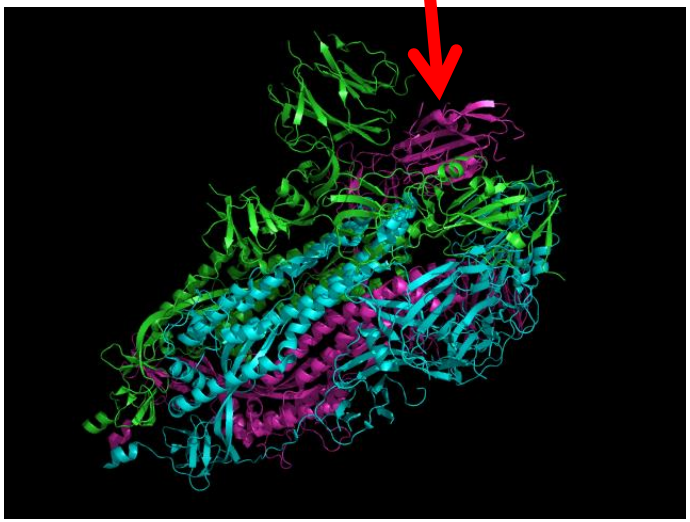
High level secretion of protein coded by the introduced DNA

RBD is the specific component of Cuban anti-COVID-19 vaccines



SARS-CoV2 representation





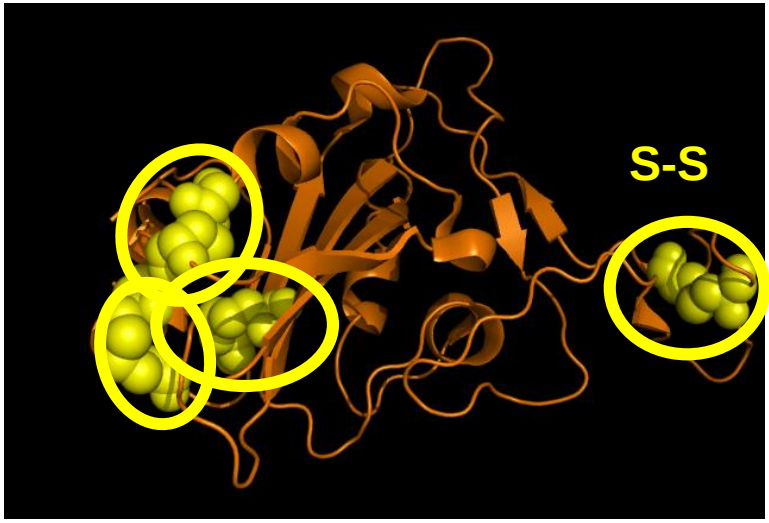
trimer of spike proteins

receptor binding domain (RBD)

RBD is a complex protein

RFPNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNV
YADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKP
FERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLHAPATVCGPKKST
NL

- ✓ Each letter represents a protein building block (amino acid).
- ✓ C means Cysteine, a particular amino acid with a highly reactive sulfhydryl group (SH).
- ✓ Two cysteines form disulfide bonds (S-S), which connect distant building blocks and are essential to shape the tridimensional structure (8 cysteines and four S-S bonds in RBD)



Both yeast and mammalian cells were able to poduce properly folded RBD resembling the protein fragment in the virus

- **Using yeast as host cells to produce viral RBD: Abdala and Mambisa vaccine candidates produced by the Center of Genetic Engineering and Biotechnology**

- **Using mammalian cells to produce viral RBD: Soberana vaccine candidates produced by Finlay Vaccine Institute (Department of Protein Engineering, Center of Molecular Immunology)**

"Soberana" means sovereign

IFV
INSTITUTO FINLAY
DE VACUNAS

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Why was it so challenging to obtain the vaccine antigens for Soberana vaccines?

- **The inclusion of an additional (unpaired) cysteine at position 538 of Spike protein was required.**
- **The presence of nine (1+) highly reactive sulfhydryl groups complicated the folding process to obtain the correct protein structure.**
- **Simultaneous production of two different molecules derived from RBD in a single biotechnological process was requested by Finlay Inst collegues.**

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RVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTK

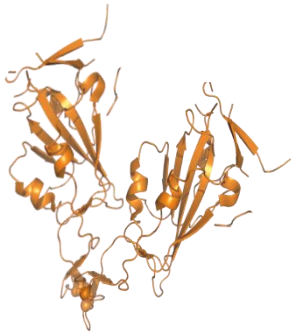
LNDLCFTNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYR

LFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLHAPA

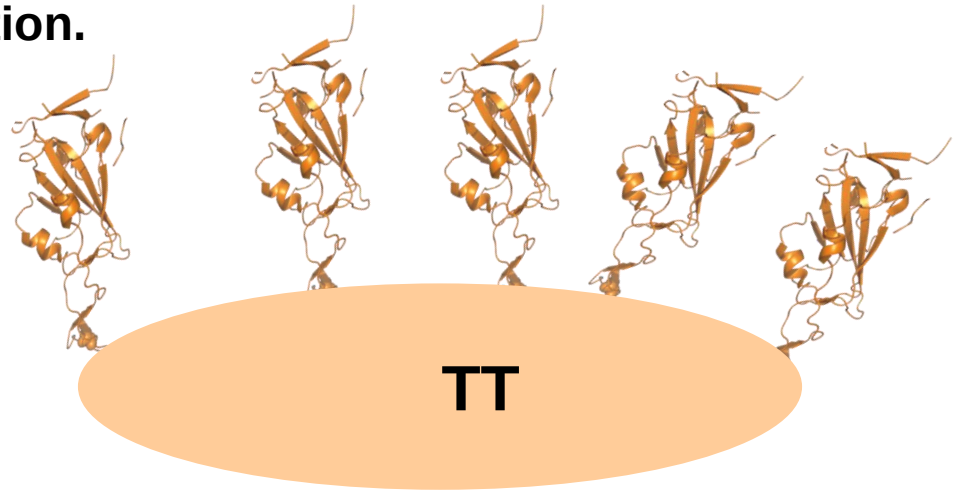
TVCGPKKSTNLVKNKCVNF

What to do with the highly reactive SH group during the biosynthesis process in mammalian cells?

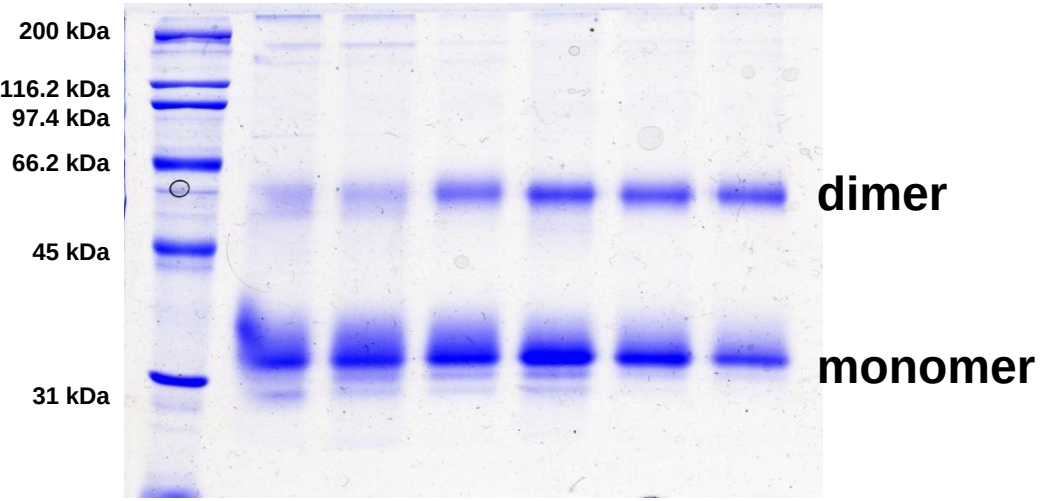
1. **Pairing of Cys 538 from two molecules was required to obtain a dimeric version of RBD antigen.**



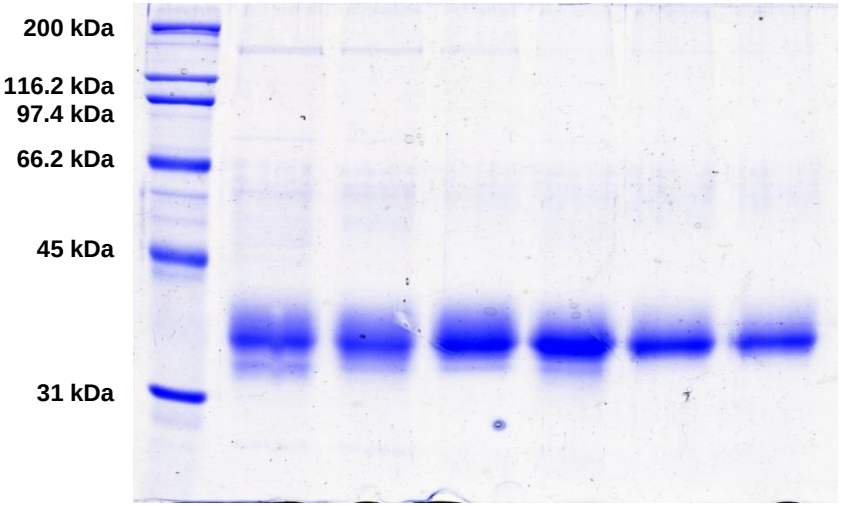
2. **Preserving the SH group to be conjugated the antigen to a second highly immunogenic carrier protein (tetanus toxoid) was also required for another vaccine formulation.**



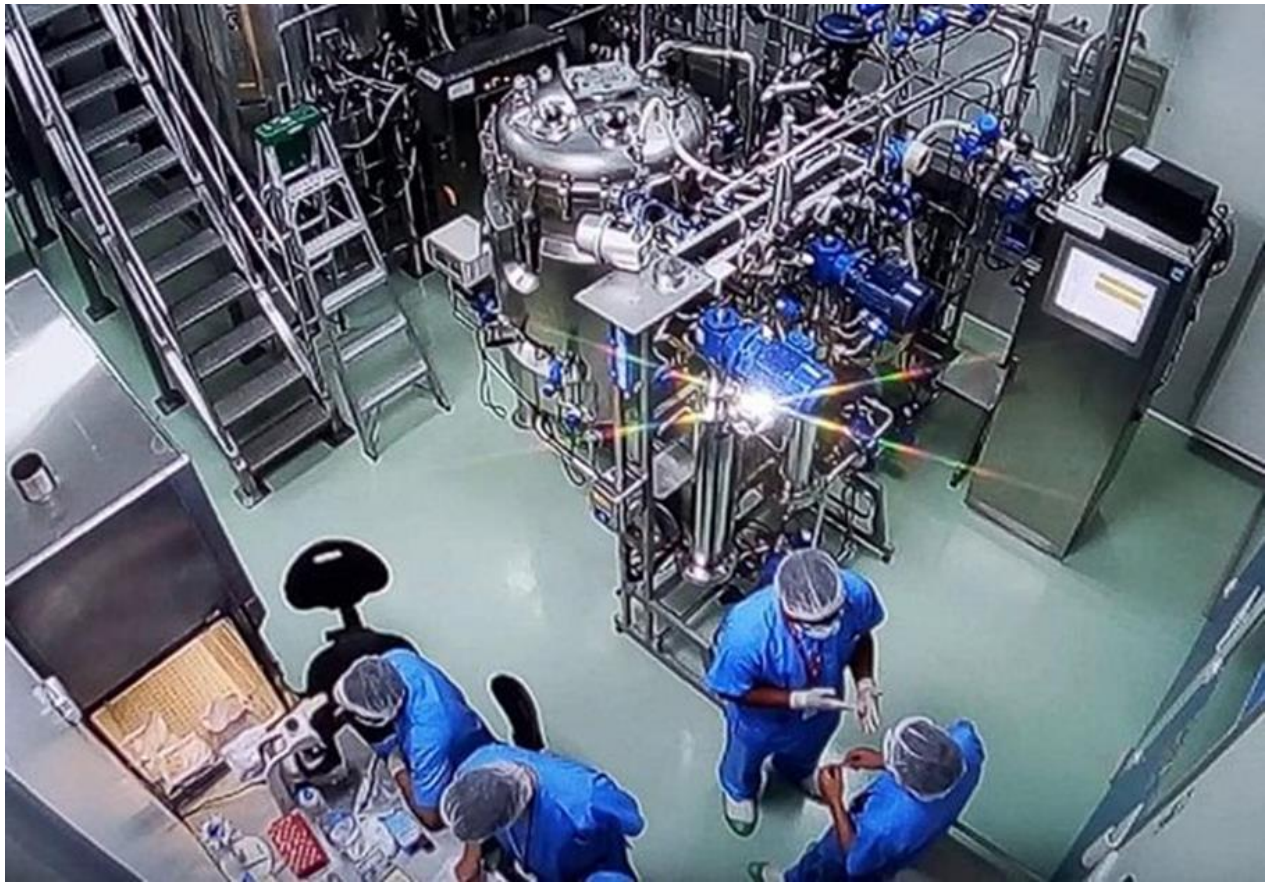
Dozens of cell lines producing a mixture of monomeric and dimeric RBD were obtained at the lab (cells of hamster ovary/CHO)



After breaking S-S bonds:



The best producer cell line was transferred to industrial bioreactors (up to 2000 L) and is used to produce both vaccine antigens



All Soberana formulations have been useful

- **Two doses of Soberana-02 (RBD+TT) were used to induce immunity in children and adults, followed by a third dose to recall the immune response with Soberana-Plus (dimeric RBD)**

- **A single dose of Soberana-Plus has been very effective in boosting the response of convalescent people**

- **Soberana-01 (dimeric RBD + membrane vesicles of *Neisseria meningitidis*) is being studied for booster doses due to its capacity to mobilize the innate immune system (first anti-pathogen defense line)**

Cuban vaccination in numbers (April 2022)

- ✓ **10 651 028 persons have received at least one dose**
- ✓ **9 399 049 have received two doses**
- ✓ **9 092 982 have received three doses**

- ✓ **9 939 645 individuals have completed the immunization schedule (89.7% of Cuban population)**

- ✓ **6 505 387 persons have received additional boosters after the initial immunization schedule**

Source: official site of MINSAP (Ministry of Public Health, Cuba)

What did we learn from the Cuban battle against COVID-19?

1. **It was possible, although quite difficult, to control the disease despite the negative impact of economical problems and external pressures.**

2. **Preventive and therapeutic agents we used were mainly products developed at Cuban labs, and produced by national industry, which were possible because scientists and facilities were ready.**

3. **The organization of the public and universal healthcare system guaranteed access to these products, including a very fast vaccination campaign.**

4. **The strategy of communication was effective to promote people understanding and support of vaccination and other sanitary measures.**

5. **Soveraignity did not mean isolation: help from foreign scientists, from other goverments, and from solidarity groups, was greatly appreciated and made a real contribution.**

Ackowledgements

All the teams involved in Cuban vaccines´ development

Finlay Inst, CIGB, CIM, Biocen, publich health system…

Thanks to the organizers of these lectures

Thank you!