DIRNANO
Directing the immune response through designed nanomaterials
H2020-MSCA-ITN-2020

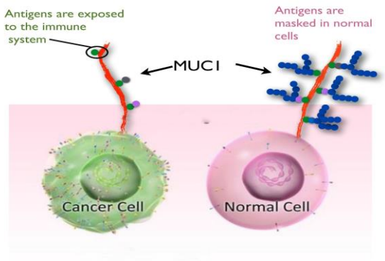
Impact of structure-guided cancer nano-vaccine candidates and their respective nano-formulations in preclinical cancer models & biosensor development

UNIVERSIDAD DE LA RIOJA

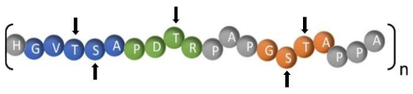
Foivos S. Lazaris[a], Mattia Ghirardello[a], and Francisco Corzana[a]

[a]Universidad de La Rioja, Centro de Investigación en Síntesis Química, Departamento de Química, Madre de Dios, 53, 26006, Logroño, La Rioja, España

Mucin-1; a promising cancer antigen



Mucins are large extracellular glycoproteins met with different post-translational modifications in healthy and cancer cells.[1,2]



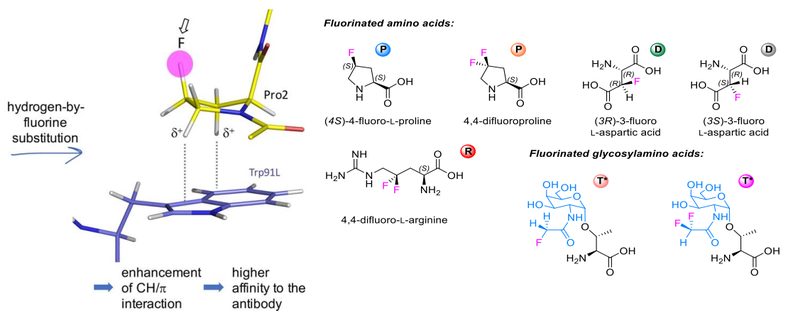
MUC1 tandem repeat domain, including the APDTRP and GSTAP epitopes recognised by the SM3 and 5E5 antibodies, respectively. The arrows indicate the possible glycosylation positions.

Mucin-1 is…

- ✓ Abdunant in the majority of the cancers[1,2]
- ✓ Different post-translational modifications in healthy and cancer cells[1]
- X Low Immunogenicity
- X Low Stability

Incorporation of unnatural amino acids offers:

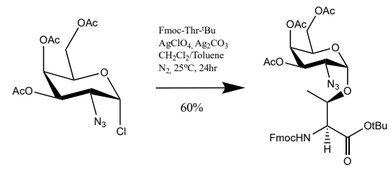
- *Increased stability*
- *Increased immunogenicity*
- *Enhanced binding affinity*



Hydrogen-by-fluorine *substitution to enhance the CH/π interactions, and overall antibody binding affinity.[3]*

Promising unnatural fluorinated amino acis

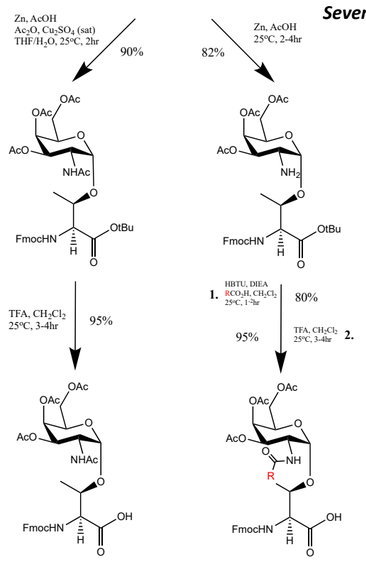
Unnatural amino acids featuring sugar modifications

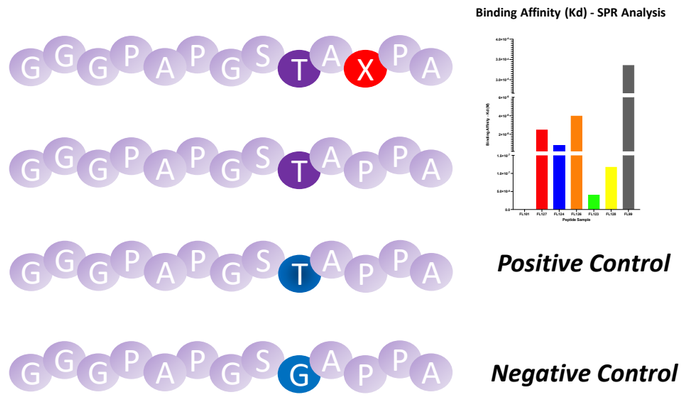


Surface Plasmon Resonance (SPR) *results:*

- Confirmed the enhanced binding affinity of certain biomimetics towards the 5E5 antibody
- Most promising peptide candidates have been functionalized with a DBCO linker and are mounted to an *N_3-functionalized coating* provided by ***SuSoS AG****
- Development of a more sensitive & specific cancer diagnostic assay
- In anticipation of preliminary results.

Several peptides have already been prepared, in anticipation of SPR results.





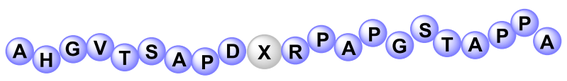
Nanovaccine candidate utilizing an unnatural threonine derivative & PEG-coated AuNPs

UNIVERSITÀ di VERONA

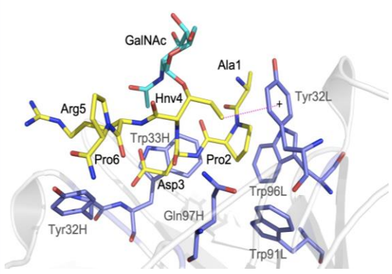
Instituto de Medicina Molecular | João Lobo Antunes

*Accepted manuscript; published in **JACS Au** on November 21, 2023[4]*

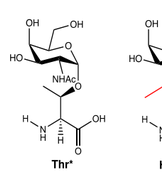
*Nano-vaccine candidate utilizing an unnatural threonine derivative; **hydroxy-norvaline***



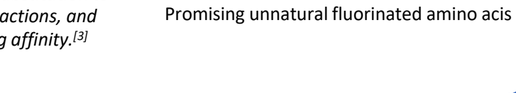
X indicates the modified amino acid



*Crystal structure of the complex **Ab-HONor-antigen***



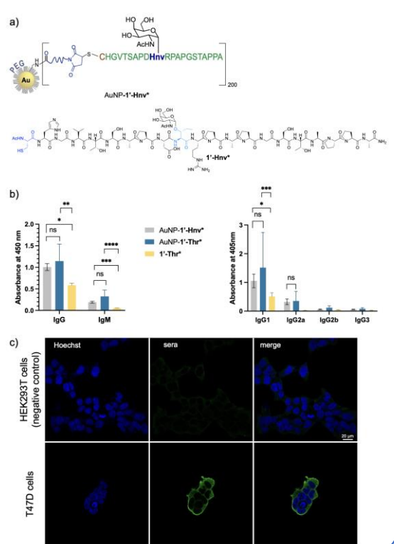
*Structures of the **Tn-Thr** and **HONor** amino acids*



a: The nanovaccine candidate comprising of ***MUC1 peptides*** conjugated onto a ***AuNP***

b: The results of the vaccination campaign; depicting ***similar antibody titers*** between the natural and the artificial antigen

c: Incubation of the sera with healthy and cancer cell lines; ***the abs recognize specifically the MUC1 cancer-expressing cells***



Nano-vaccine candidate utilizing unnatural amino acids & PEG-coated AuNPs

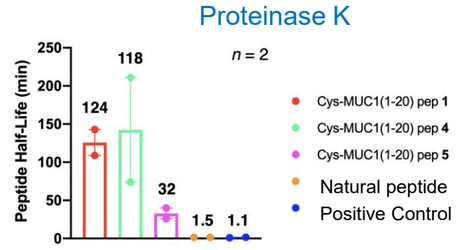
UNIVERSITÀ di VERONA

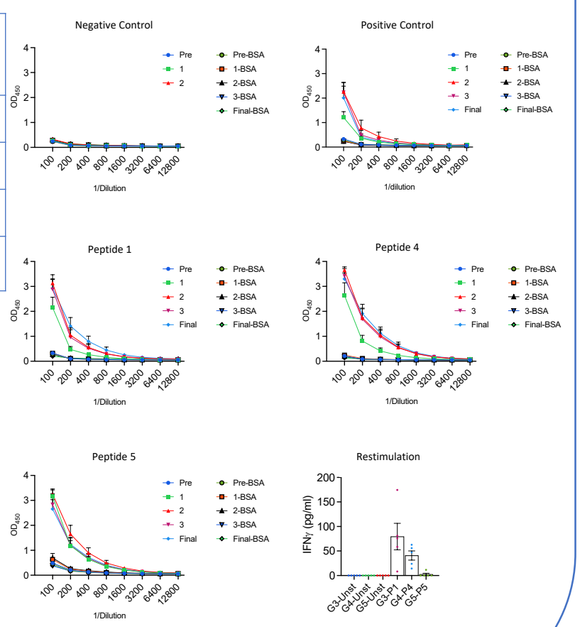
CIC bioGUNE
MEMBER OF BASQUE RESEARCH & TECHNOLOGY ALLIANCE

Affinity for the antibody SM3 (SPR assay)

Compound	Sequence (underlined aa = unnatural aa)	K_D (M)	K_D/K_D (natural)
Pep **1**	CAH**X**VT**X**APDT*RPAP**X**STA**X**P-NH2	1.6E-6	1.44
Pep **4**	CAH**X**VT**X**APDT*RPA**X**GSTA**X**P-NH2	3.29E-6	2.96
Pep **5**	CAH**X**VTS**X**PDT*RPA**X**GST**X**PP-NH2	1.67E-5	15.05
Positive Control	CAHGVTSAPDT*RPAPGSTAPP-NH2	1.11E-6	1

Proteolysis stability in the presence of Proteinase K

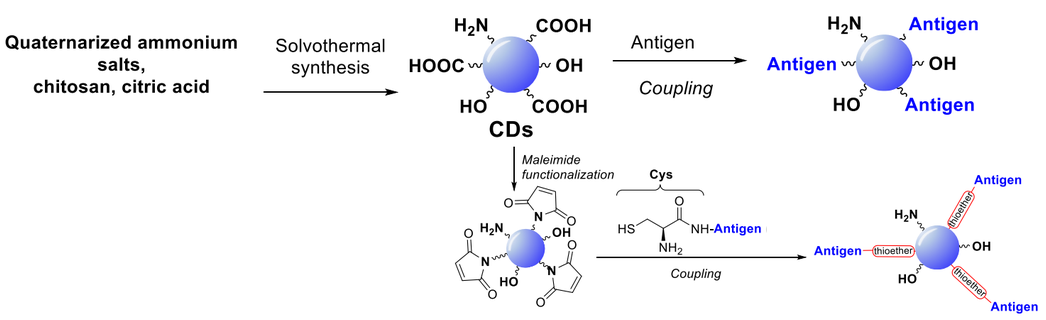


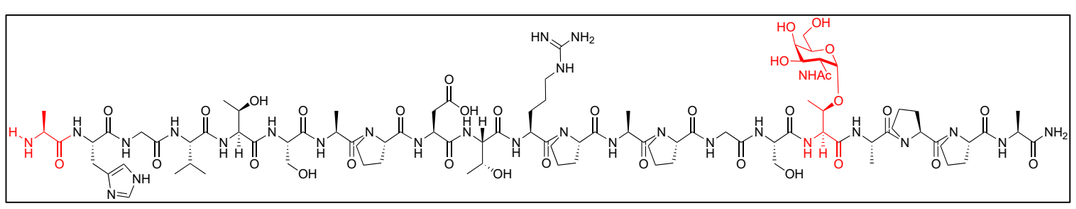


Other nano-vaccine candidates utilizing unnatural amino acids & different nanomaterials

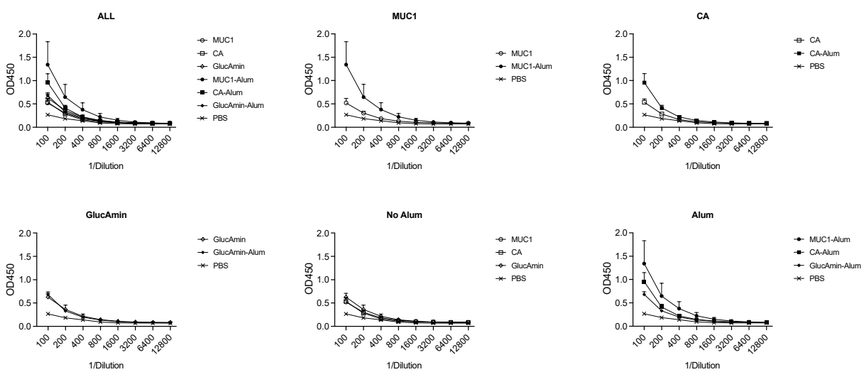
CIC bioGUNE
MEMBER OF BASQUE RESEARCH & TECHNOLOGY ALLIANCE

*Nano-vaccine candidate utilizing a **natural Mucin-1** and **carbon dots***



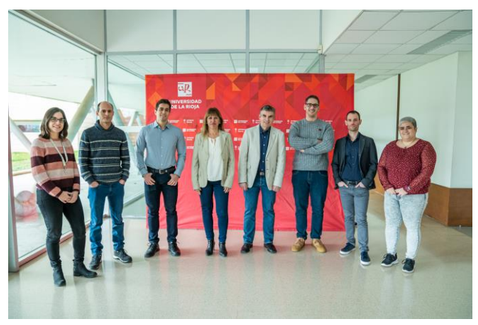


- *Carbon Dots (CD) **do not enhance the antigen's immunogenicity** even in combination with an adjuvant*
- *Similar antibody titers between MUC1+Alum & MUC1+Alum-CD observed*
- *Physicochemical characterization of the CD-MUC1 complexes still pending*
- *MUC1 attachment to the CD assessed by ^{1}H-NMR*



The vaccination campaign will be repeated but with the incorporation of a T-helper sequence in the N-terminal of the peptide. Following the results of our latest vaccination campaign.

Acknowledgements



References

1. Pihno and Reis *Nat. Rev. Cancer* **2015**, *15*, 540–555.
2. Hollingsworth, M., Swanson, B. *Nat. Rev. Cancer.* **2004,** 4, 45–60.
3. Corzana *et al. J. Am. Chem. Soc.* **2017**, *139*, 18255.
4. Bermejo *et al. J. Am. Chem. Soc. Au* **2023**, doi.org/10.1021/jacsau.3c00587.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 956544.