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

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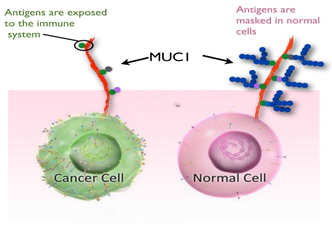
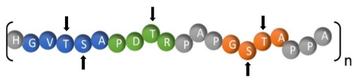
Mucin-1; a promising cancer antigen



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



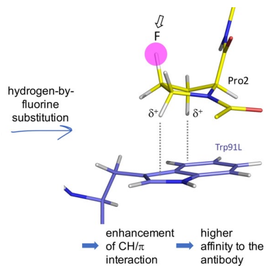
MUC1 tandem repeat domain, including the APDTRP and GSTAP epitopes recognised by the SM3 and 5E5 antibodies, respectively. The arrows indicate the possible glycosylation positions.

Mucin-1 is…

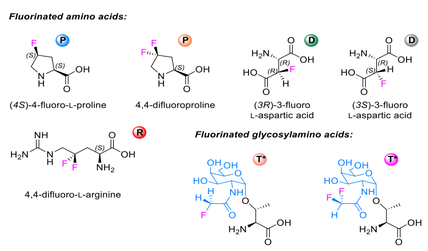
- ✓ Abdunant in the majority of the cancers[1,2]
- ✓ Different post-translational modifications in healthy and cancer cells[1]
- X Low Immunogenicity
- X Low Stability

Incorporation of unnatural amino acids offers:

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

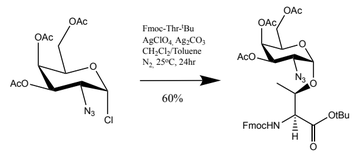


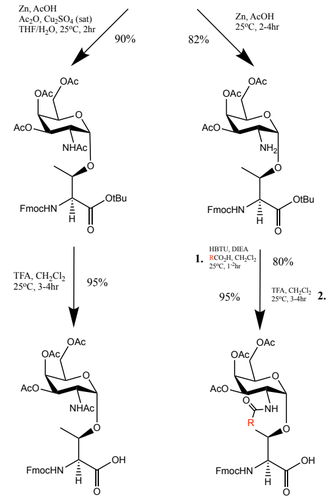
Hydrogen-by-fluorine *substitution to enhance the CH/π interactions, and overall antibody binding affinity.*[3]



Promising unnatural fluorinated amino acis

Unnatural amino acids featuring sugar modifications

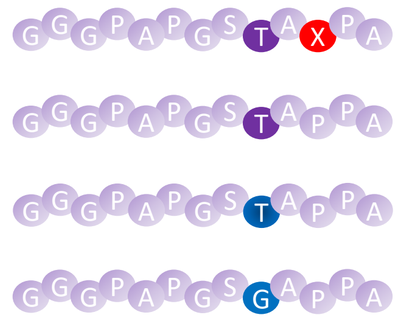




Based on previous studies, ***certain sugar modifications*** *can:*

- Enhance antibody binding affinity
- Increase stability significantly
- Maintain peptide original structure & natural folding

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



Positive Control

Negative Control

T Indicates unnatural amino acid. X Indicates fluorinated unnatural amino acid.

T Indicates the Tn-Threonine.

Mucin-1 Oligomer - Secondment Activity

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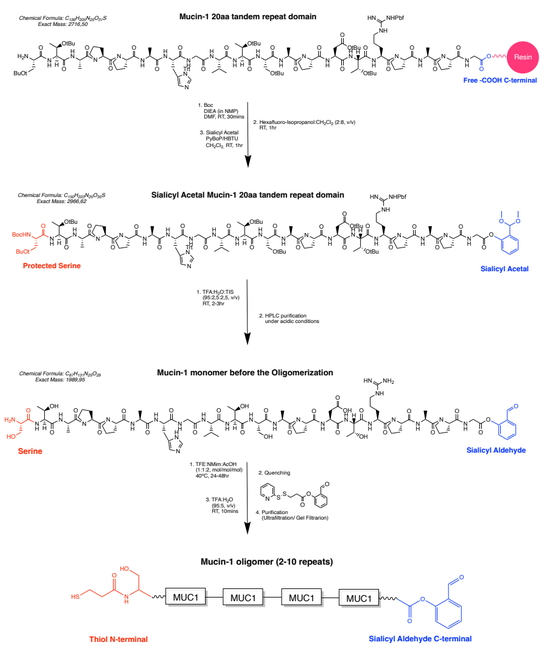
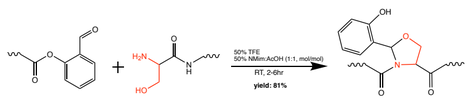
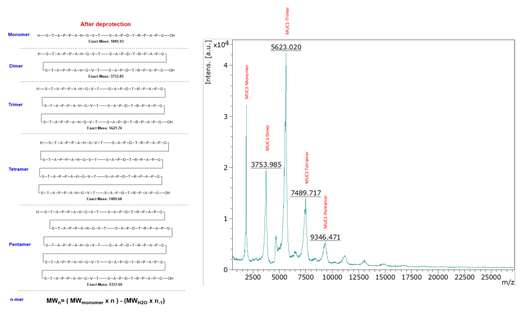


Development of MUC1 oligomers (2-10 repeats) of the 20aa tandem repeat domain

- *Idea based on the MUC1 100mer peptide vaccine*[4]
- *Protocol based on, and adapted from a previous research work*[5]
- *Specific conjugation through the functionalised Thiol N-terminal*
- *Enzymatic post-synthetic glycosylation*
- ***Cyclic monomer-peptide was observed!***



Oligomerization reaction through ***Serine/Threonine ligation.***[6]



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

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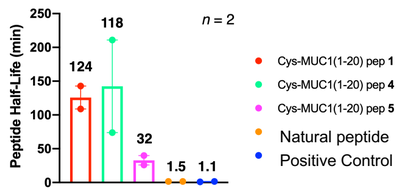
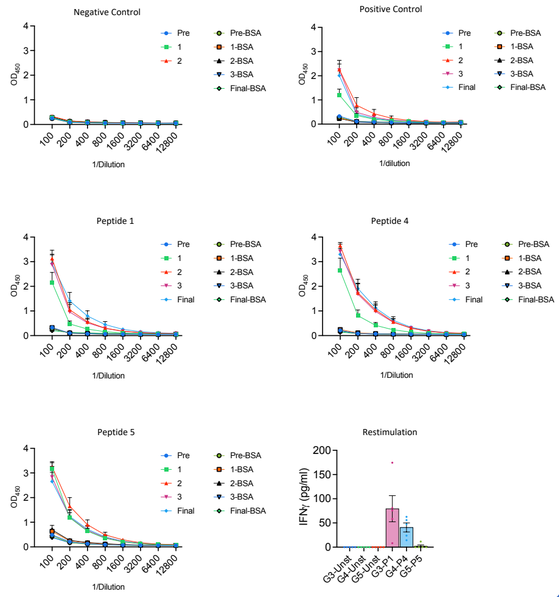
Affinity for the antibody SM3 (SPR assay)

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





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

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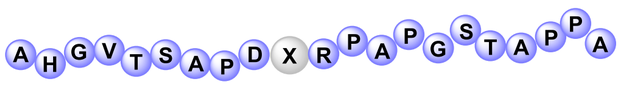
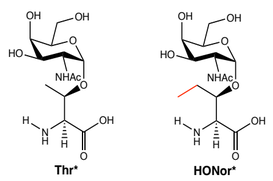
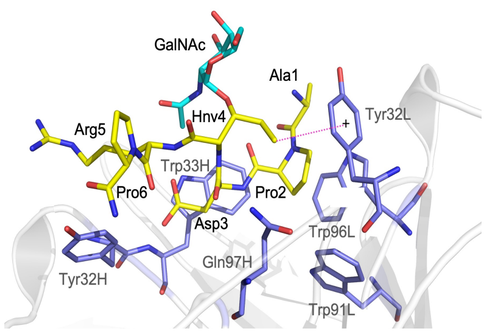
Nano-vaccine candidate utilizing an unnatural threonine derivative; ***hydroxy-norvaline***



X indicates the modified amino acid

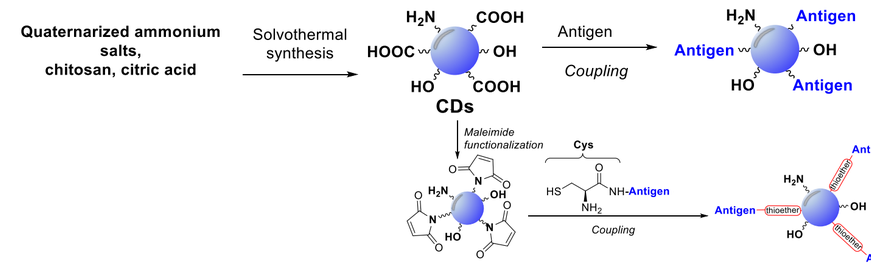


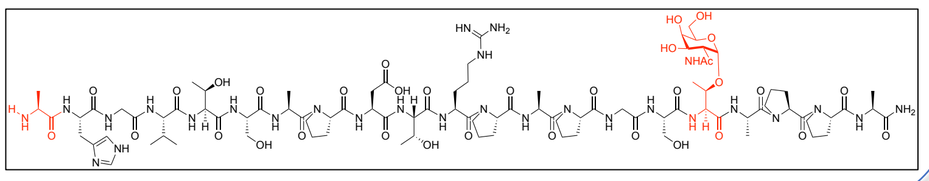
Structures of the ***Tn-Thr*** *and* ***HONor*** *amino acids*



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

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





For more information, check ***Ander Eguskiza's (ESR9)*** *poster*

Acknowledgements

References

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2. Hollingsworth, M., Swanson, B. *Nat. Rev. Cancer.* **2004,** 4, 45–60.
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4. Kimura *et al. Cancer Prev. Res.* **2013**, 6(1), 18-26.
5. Xu et al. *Chem. Commun.*, **2013**, 49, 6200.
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