Peptides Conjugated to Sugars as Therapeutics for Ebola & Cancer disease

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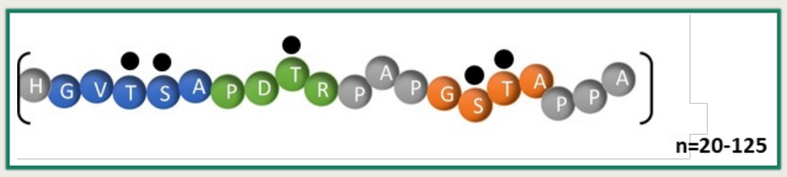
The use of synthetic biomolecules emulating biological systems has emerged as a promising frontier in therapy and medicine. Research presented here explores diverse strategies to target both Cancer and Ebola disease.

MUC1 Glycopeptide as Cancer Vaccine

Mucin 1 or (MUC1) is a membrane-associated protein highly *O*-glycosylated through Ser/Thr residues. This protein is highly glycosylated in healthy cells, whereas in tumour cells it carries simple and truncated carbohydrates and exposes fragments, such as APDTRP or Tn antigen [Thr/Ser(α-GalNAc)], which can trigger the production of anti-MUC1 antibodies. Vaccines incorporating these structures are a promising field of research (Asín *et al. Curr Med Chem*, **2022**).

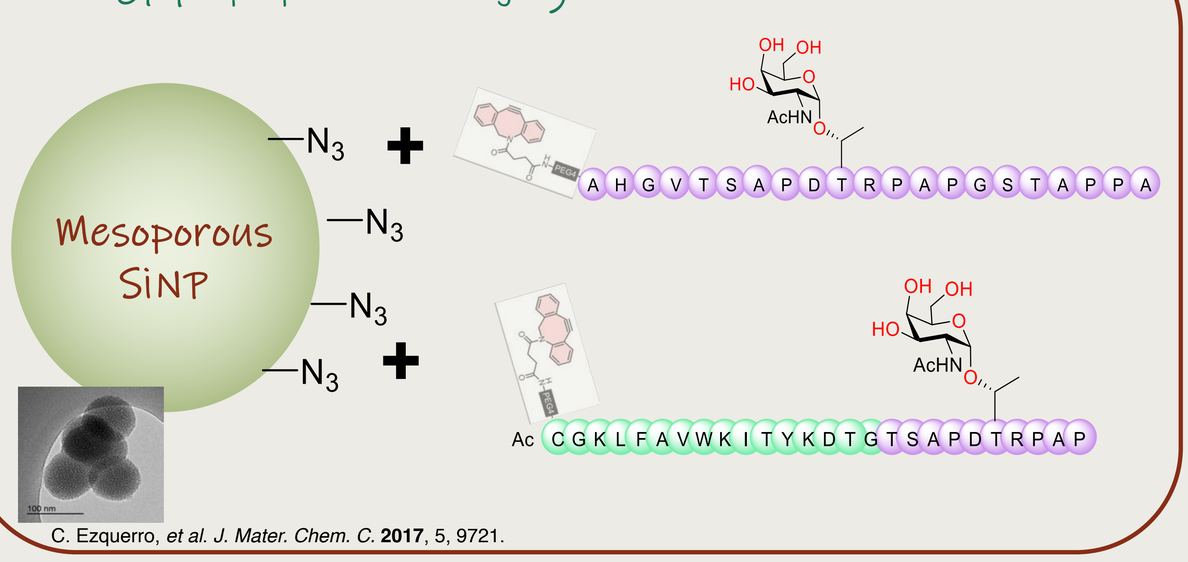
MUC1



Synthesis and Conjugation

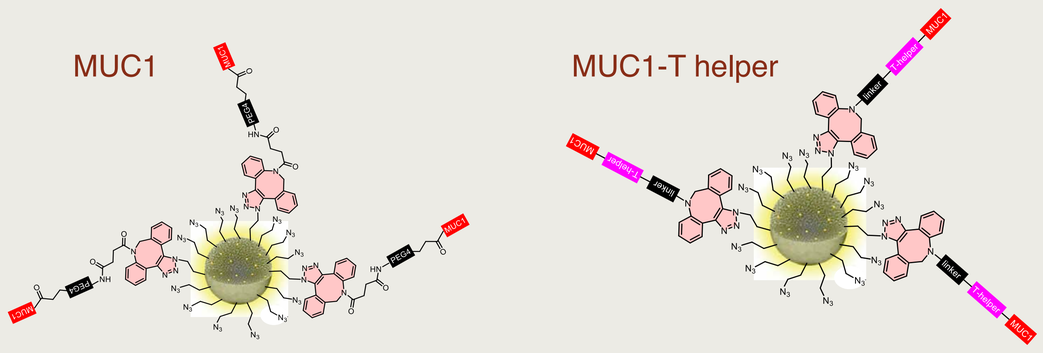
Using SPPS with microwave energy, we synthesized a multi-epitope with MUC1 epitope (PDT(GalNAc)RP) without and with a Thelper attached, and then conjugated to mesoporous silica nanoparticles (siNPs) by copper-free click chemistry.
The compounds were evaluated *in vivo* through biological assays, both alone and in combination with an aluminum adjuvant.

Glycopeptides Conjugation via Click reaction

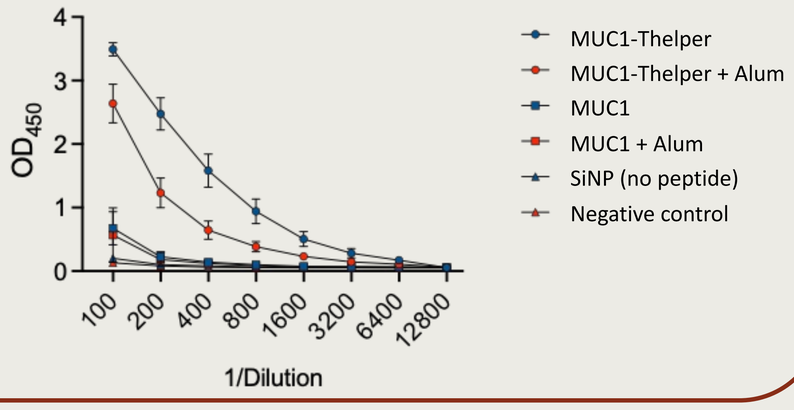


C. Ezquerro, *et al. J. Mater. Chem. C.* **2017**, 5, 9721.

Glycopeptides-SiNP compounds

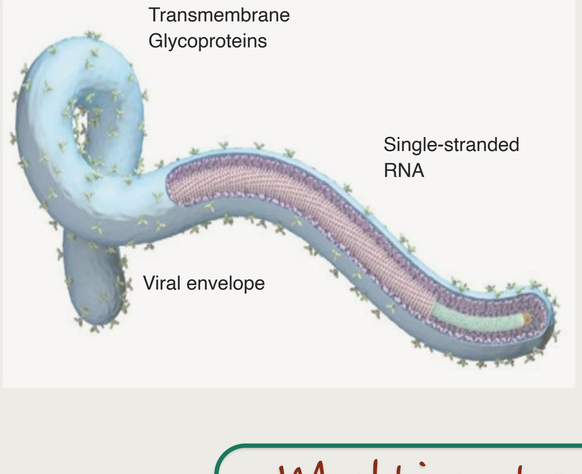


Vaccination & Biological Assays

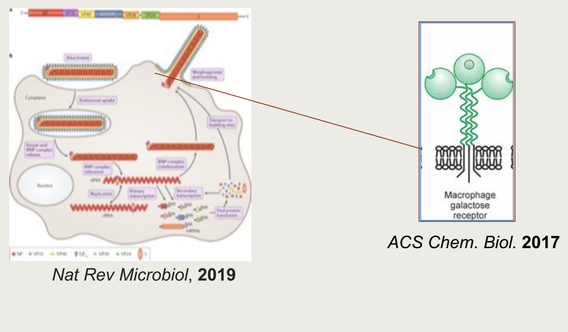


Peptides conjugated to sugars as Inhibitors of Ebola virus

Ebola virus utilizes glycoproteins of the extracellular capsid for host cellular entry, to attach to several receptors such as Macrophage Galactose Lectin (MGL).



Cycle of the viral infection



Nat Rev Microbiol, **2019**

ACS Chem. Biol. **2017**

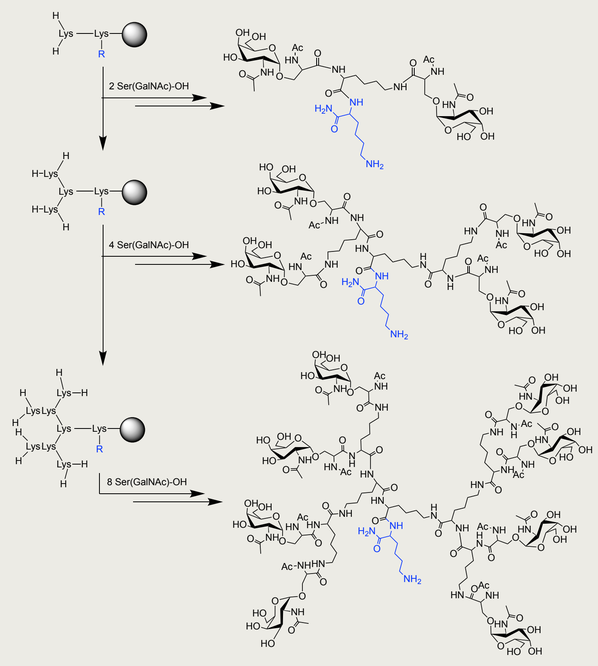
Multiantenna Ligands Design & Synthesis

To create entry inhibitors, we aim to mimic the virus using the Lys multiantigenic peptide system (MAP) (Tam *et al. J Immunol Meth*, **1989**).

Our compound of interest is the sugar *N*-acetylgalactosamine (GalNAc) linked to Ser, a ligand for one of the main viral entry receptors.

We synthesized a small library with varying Ser(GalNAc) repetitions to assess ligand density influence.

In characterization, NMR structure analyses revealed a symmetric dendritic shape for the multi-antenna platforms with the ligand.

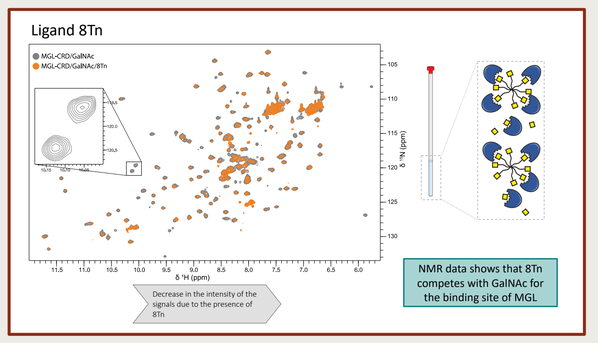


Affinity Assays

Affinity assays by calorimetry and NMR competition assays with the Ebola virus receptor, demonstrated higher ligand display correlating with increased affinity.

NMR Competition Assays

HSQC NMR using 15N-labeled MGL can be a powerful technique to study ligand binding and interactions in competition assays.



Calorimetry ITC assays

	GalNAc*	2Tn	4Tn	8Tn
N		0.4±0.01	0.2±0.02	0.1±0.01
ΔG (kcal/mol)		-8.2±0.1	-8.5±0.08	-9.0±0.1
ΔH (kcal/mol)	-14.7	-23±2	-40.1±0.4	-121±11
-TΔS (kcal/mol)		15±2	32.0±0.5	112±12
K_D (μM)	1400	1.1±0.2	0.6±0.1	0.3±0.04

* A Gabba *et al. J Am Chem Soc* , **2023**

CONCLUSIONS & FUTURE PROSPECTS

- We could obtain MUC1 derivatives and Ebola virus inhibitors in high purity.
- Biological assays of SiNP-MUC1 demonstrated a significant enhancement of the immune response following vaccination.
- Studies include binding affinity with MGL lectin by HSQC NMR & calorimetry assays.
- Best candidates will be proven as pathogen entry inhibitors.
- Peptides conjugated to sugars serve as highly effective probes for therapeutic applications.

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