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RATIONAL DESIGN AND SYNTHESIS OF BIOACTIVE MIPEP205-DERIVED FRAGMENTS AS MODULATORS OF WNT/B-CATENIN SIGNALING IN TRIPLE-NEGATIVE BREAST CANCER

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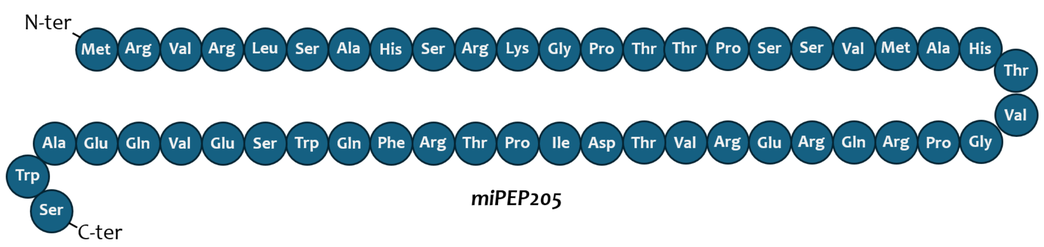
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ABSTRACT

Triple-Negative Breast Cancer (TNBC) is the most aggressive breast cancer subtype, accounting for 15 to 20% of cases and showing poor survival outcomes despite chemotherapy, largely due to tumor relapse and an immunosuppressive microenvironment [1]. These limitations highlight the necessity for new molecular targets. **miPEP205**, a 49-aminoacid micropeptide encoded by MIR205HG, acts as a negative regulator of the canonical **Wnt/β-catenin** pathway, a key driver of TNBC progression. By interacting with **GSK-3β,** miPEP205 promotes β-catenin phosphorylation and degradation, ultimately reducing tumor cell proliferation [2].

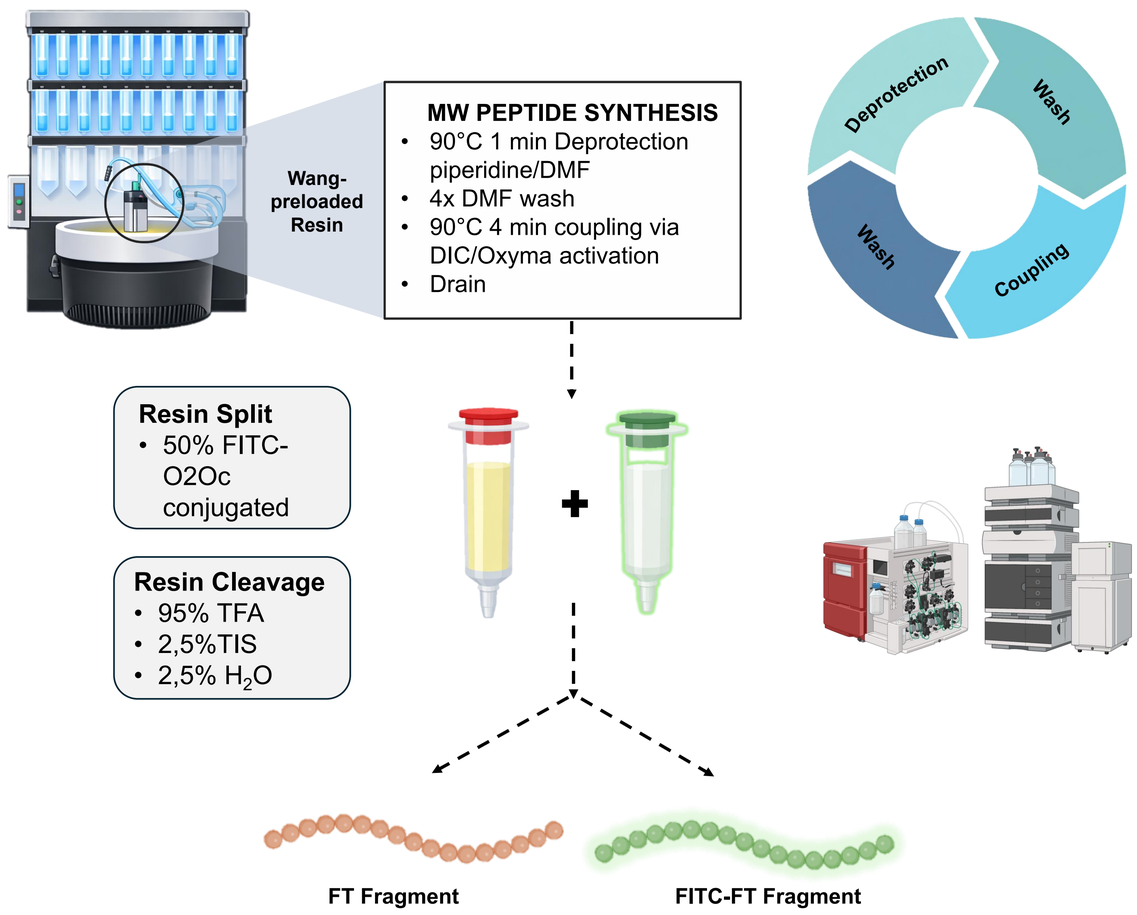
SCAN ME FOR 3D MODEL

RATIONALE & DESIGN



Representation of rationale design of FT-fragments deriving from miPEP205: **overlapping peptide mapping** consists in maintaining for each fragment a certain number of amminoacidic residues from the previous & the successive peptide fragment

SYNTHETIC PROCEDURES

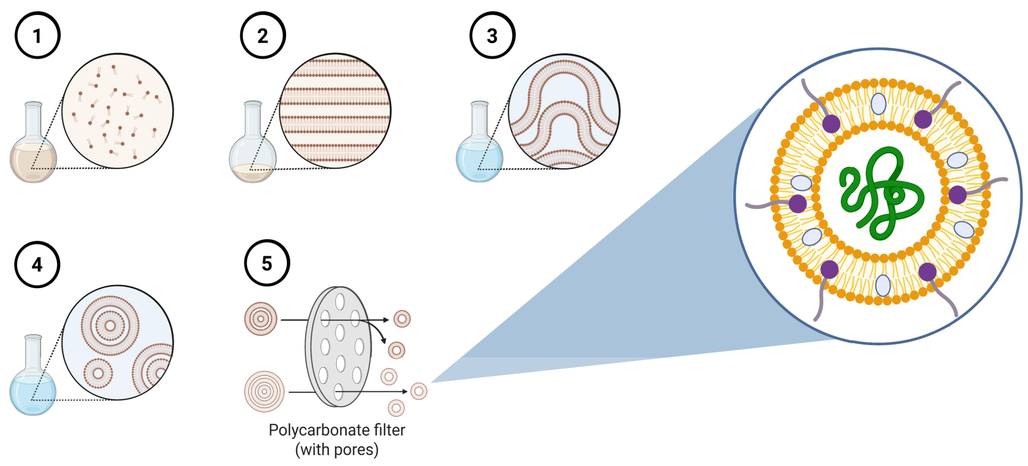


miPEP205-derived fragments were synthesized by automated microwave-assisted Fmoc-SPPS using **a Liberty Blue™ 2.0 peptide synthesizer**, followed by cleavage in acid condition, purification and analytical characterization via analytical & preparative HPLC.

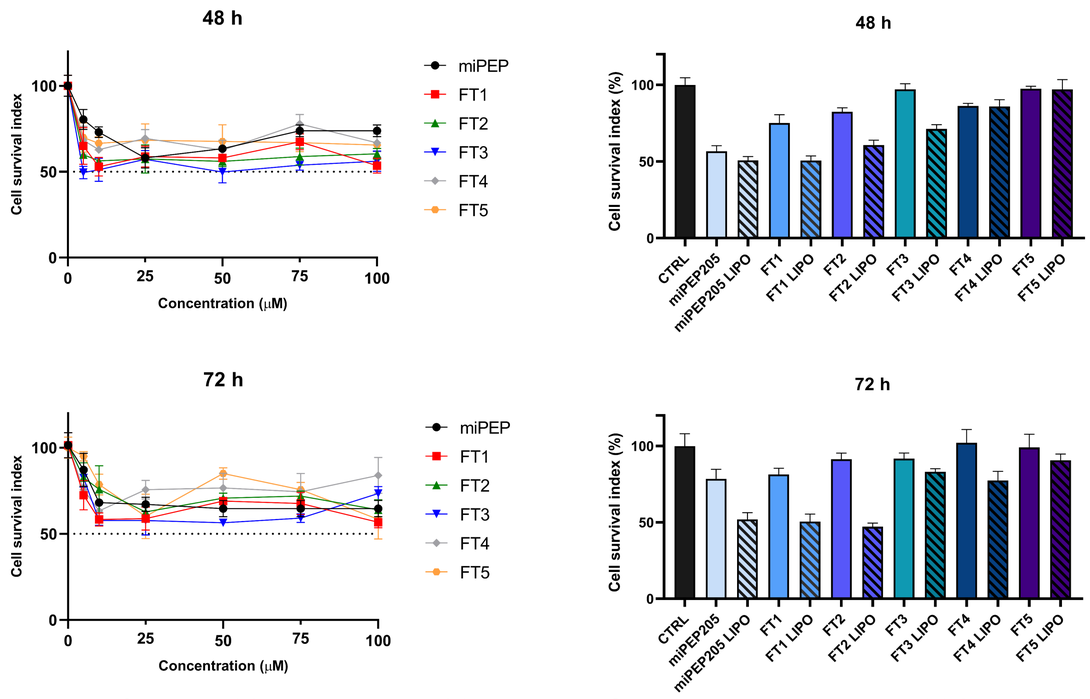
CONCLUSIONS

- Five overlapping fragments (FT1-5) were rationally designed from miPEP205 and synthesized
- In vitro testing on **MDA-MB-231** TNBC cells→ **FT1** and **FT2** most promising candidates
- Liposome-formulated FT1-FT2 cytotoxicity ≈ liposome miPEP205 citotoxicity
- Focus on FT1 & FT2 for future therapeutical optimizations

RESULTS



Liposome composition	Molar ratio (%)	Lipid concentration	Peptide concentration	Reference
DSPC/Cholesterol/DSPEPEG2000	32:60:8	1 mg/mL	0,08 mg/mL	[3]



Bioscreening of miPEP205 and miPEP205-derived fragments in **MDA-MB-231 cells**. **[A]** Cell survival index was assessed after treatment with miPEP205 and its fragments at concentrations ranging from 0 to 100 μM for 48 h and 72 h. **[B]** miPEP205 and the most active fragments, FT1, FT2, and FT3, were further evaluated at the selected concentration of 20 μM, either as free/naked peptides or in liposome formulation, after 48 h and 72 h of treatment.

	48H	72H
MIPEP205	56,6 %	78,5 %
MIPEP205-LIPO	50,7 %	51,9 %
FT1	75,1 %	81,3 %
FT1-LIPO	50,6 %	50,5 %
FT2	82,4 %	91,3 %
FT2-LIPO	60,7%	47,1 %
FT3	97,1 %	91,7 %
FT3-LIPO	71,2 %	83,1 %

Summary table showing cell survival index values in MDA-MB-231 cells, expressed as percentages relative to untreated control cells, after 48 h and 72 h of treatment with the selected free/naked and liposome-formulated peptides at 20 μM.

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