Mapping the G-Quadruplex Binding Determinants of a Vimentin-Derived Peptide by Alanine Scanning

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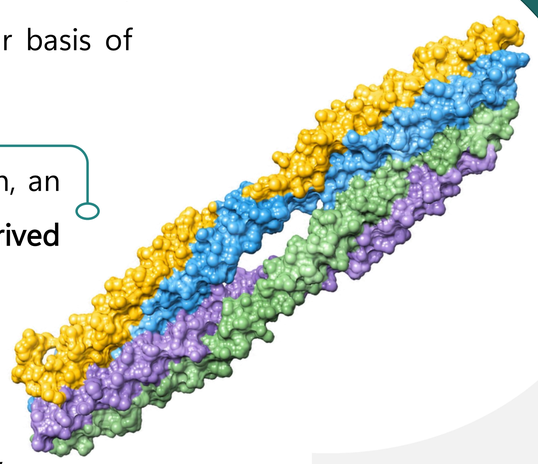
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VIMENTIN AND G4-RECOGNITION

Vimentin is a type III intermediate filament protein involved in the epithelial-mesenchymal transition (EMT) process and cancer progression. Soluble tetrameric Vimentin **selectively recognises G-quadruplex (G4) DNA structures**, suggesting a potential in gene regulation, although the molecular basis of this interaction remains unclear.

In this study, to investigate the residues involved in G4 recognition, an **alanine-scanning approach was applied to the Vimentin-derived peptide 11-30.**



CONVERGENT SYNTHESIS AND ALA-SCAN

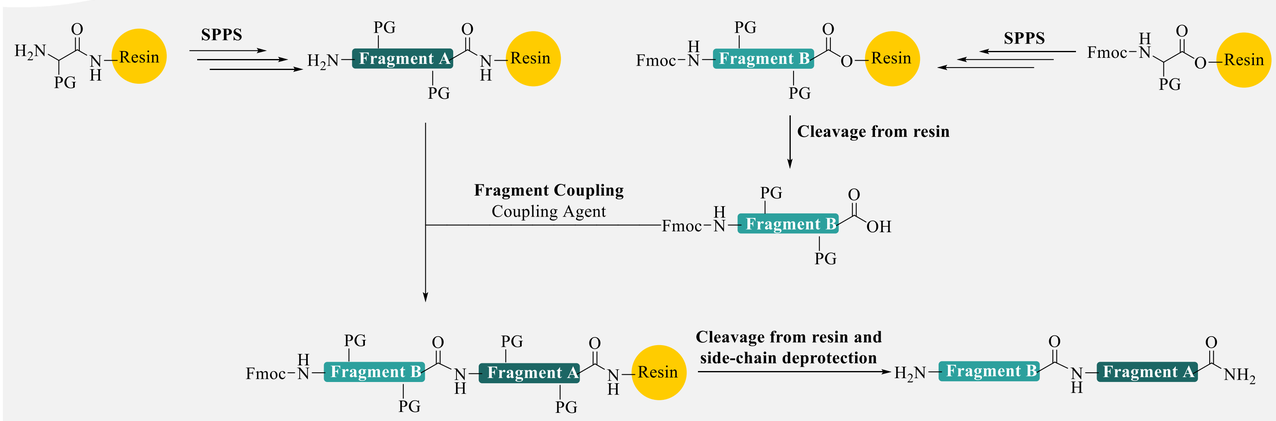
The Vimentin-derived peptide and its analogue were prepared by Fmoc-based solid-phase peptide synthesis using a **convergent synthetic strategy**.

Independent peptide fragments were assembled separately and subsequently combined, reducing the number of sequential coupling steps and improving the overall efficiency of the synthesis. This approach proved particularly advantageous for the preparation of a relatively long sequence such as Vimentin 11-30 fragment.

EXPERIMENTAL APPROACH

1. **Peptide Synthesis.** The reference peptide and a series of alanine-substituted analogues were synthesized by fragment-based and convergent peptide synthesis.
2. **Biophysical Characterization.** Peptide-G4 interactions were evaluated through fluorescence melting assays (FM) and surface plasmon resonance (SPR).
3. **Structure-activity Relationship Analysis.** Assessment of the contribution of individual residues to G4 recognition.

Schematic representation of fragment based and convergent peptide synthesis



PEPTIDES SYNTHESISED

Reference sequence:

^{11}Y R R M F G G P G^{20}T A S R P S S S R S^{30}Y

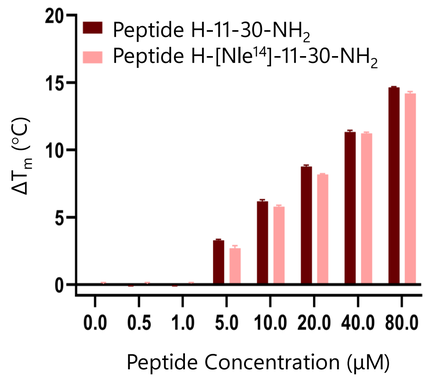
Met^{14} → Nle^{14}

Targeted alanine substitutions:

Aromatic residues Basic residues

A focused library of single-point mutants was generated through systematic **alanine substitution**. This approach preserves the peptide backbone while enabling the evaluation of the contribution of individual side chains to peptide activity.

The Vimentin 11-30 sequence (**YRRMFGGPGTASRPSSSRSY**) was used as the reference peptide. To improve oxidative stability, methionine was replaced by norleucine (**Nle^{14}**) without significantly affecting G4 recognition, as confirmed by fluorescence melting experiments. Aromatic (**Tyr, Phe**) and basic (**Arg**) residue were systematically replaced by alanine to assess their contribution to G4 binding.

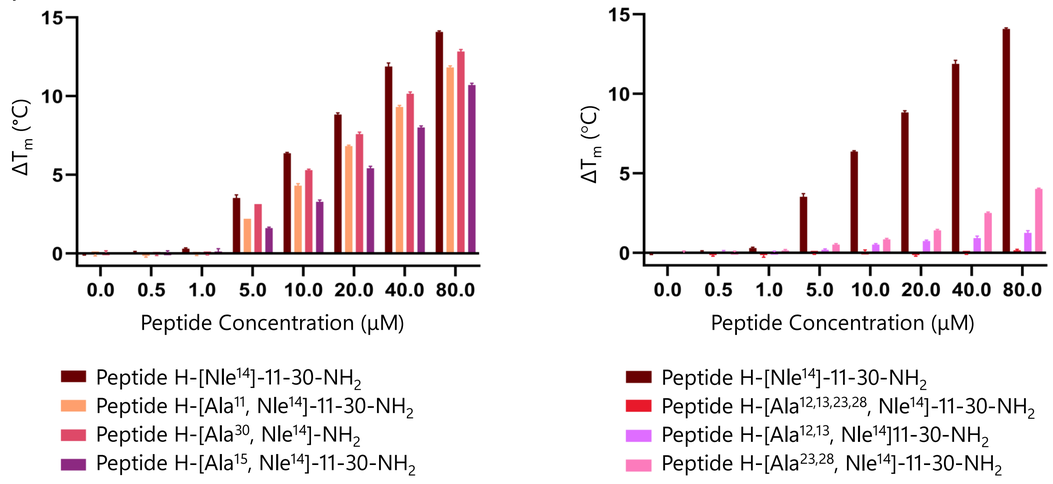


Fluorescence melting analysis of the interaction between H-11-30-NH_2 peptide and its Nle substituted analogue H-[Nle^{14}]-11-30-NH_2 peptide with the G4-forming sequence Tel23.

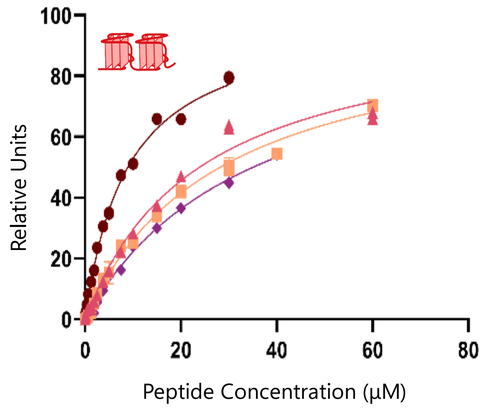
Peptide	Retention Time (min)	Purity (%)	MW_{calc} (g/mol)	$[M+H]^+$
H-11-30-NH_2	10.20	98	2219.1	2218.9
H-[Nle^{14}]-11-30-NH_2	10.10	99	2201.4	2201.0
H-[Ala^{11}, Nle^{14}]-11-30-NH_2	9.38	99	2108.1	2108.8
H-[Ala^{30}, Nle^{14}]-NH_2	10.22	98	2109.3	2108.9
H-[Ala^{15}, Nle^{14}]-11-30-NH_2	9.03	99	2125.2	2124.9
H-[Ala^{12,13,23,28}, Nle^{14}]-11-30-NH_2	11.94	99	1861.0	1860.3
H-[Ala^{12,13}, Nle^{14}]-11-30-NH_2	10.94	98	2031.2	2030.8
H-[Ala^{23,28}, Nle^{14}]-11-30-NH_2	10.94	99	2031.2	2030.8

BIOPHYSICAL CHARACTERIZATION

Peptide-G4 interactions were characterized by **fluorescence melting** and **surface plasmon resonance** experiments to evaluate G4 stabilization, binding affinity and kinetic parameters



Fluorescence melting analysis of the interaction between H-[Nle^{14}]-11-30-NH_2 peptide and its aromatic (left) and basic (right) substituted analogue peptides with G4 forming sequence Tel23.



Peptide	K_D
H-11-30-NH_2	8.9 ± 1.6μM
H-[Ala^{11}, Nle^{14}]-11-30-NH_2	28.1 ± 3.9μM
H-[Ala^{30}, Nle^{14}]-NH_2	23.8 ± 5.0μM
H-[Ala^{15}, Nle^{14}]-11-30-NH_2	35.0 ± 7.7μM

SPR analysis of the interaction between H-11-30-NH_2 peptide and its aromatic substituted analogue peptides with G4-repeat forming sequence.

CONCLUSION AND FUTURE PERSPECTIVES

The Vimentin-derived peptide 11-30 was identified as the minimal region responsible for selective G4 recognition with micromolar affinity. Alanine-scanning studies revealed that arginine residues are the main determinants of the binding and G4 stabilization, while aromatic residues provide an additional contribution to molecular recognition. The identified binding determinants will guide the design of optimized peptide ligands for investigating and modulating Vimentin–G4 interactions in cancer-related pathways.

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