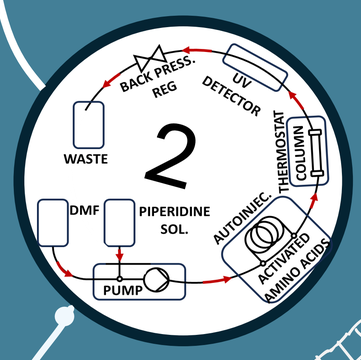
Methionine Oxidation Effect on GLP-2 Synthesis and Amyloid Kinetics

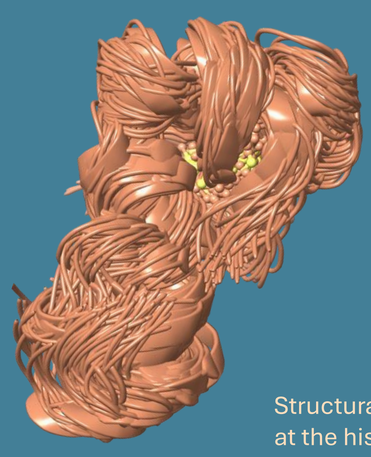
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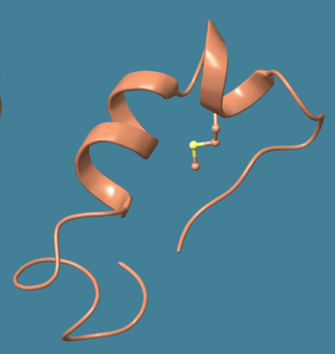
Glucagon-like peptide-2 (GLP-2) might just be the "second most important" peptide after GLP-1
It exerts key physiological functions, including stimulating intestinal epithelial growth, enhancing nutrient absorption, and reducing intestinal inflammation. However, it's one of those "difficult peptides," which makes solid-phase peptide synthesis (SPPS) challenging.
To overcome this, we set out to improve the synthesis by testing different amino acid protecting groups and structural tricks, including pseudoproline derivatives and Asp(Bno). Ultimately, the best effect came from using methionine sulfoxide.
Beyond improving yield, we were also curious to see what happens if we leave the sulfoxide derivatives in after synthesis to observe how they influence the peptide's behavior.
Below are the results of our synthesis trials, along with CD spectra, ThT tests, molecular dynamics (MD) simulations, and AFM images.

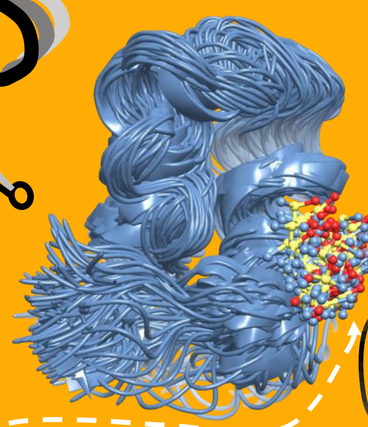


FLOW PEPTIDE SYNTHESIS



Structural alignment of GLP-2 variants protonated at the histidine side chain (162 structures)





Structural alignment of protonated oxidized variant GLP-2-M(O^R) (157 structures) and its representative.

5 CONCLUSION

Synthetic Handle: Reversible Fmoc-Met(O)-OH incorporation overcomes SPPS aggregation bottlenecks to deliver high crude GLP-2 purity, with post-purification reduction restoring the authentic native peptide.

Pathway Selector: Rather than acting as a simple aggregation inhibitor, methionine sulfoxidation functions as a pH-dependent pathway selector—bypassing off-pathway amorphous collapse near the pI (pH 4) to promote ordered fibril assembly (like a zipper).

Molecular Mechanism: Molecular dynamics simulations reveal that sulfoxidation disrupts the buried, hydrophobic "methionine cage" around Met10, exposing the polar sidechain to act cooperatively with His1 protonation to preserve N-terminal helicity.

Native GLP-2: Remains practically unchanged in overall helical content, as the hydrophobic "methionine cage" keeps Met buried **(SASA = 32.3 Å²).**

Met(O) Variants: Triggers a notable **increase in helicity** (rising to 30.5% for the *R*-epimer). **(SASA = 110.2 Å²)**

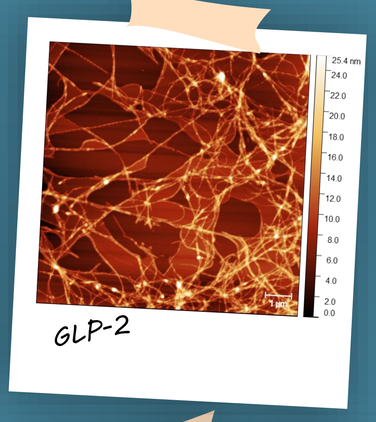
Mechanism: Because His1 interacts more heavily with nearby residues or hydrophobic regions in the oxidized states, its protonation destabilizes off-pathway contacts and exposes the polar Met(O) sidechain further to the solvent (SASA increases up to 110.2 AA^2). This stabilizes the helical fold and boosts resistance to aggregation.

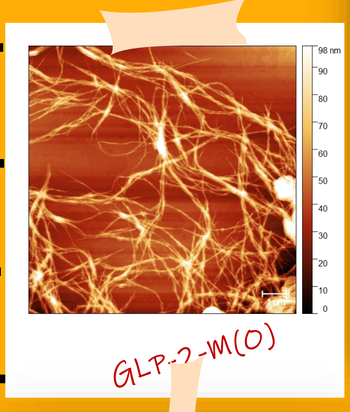
Protonating His1 (modeling pH < 6.5) affects the variants in the following ways:

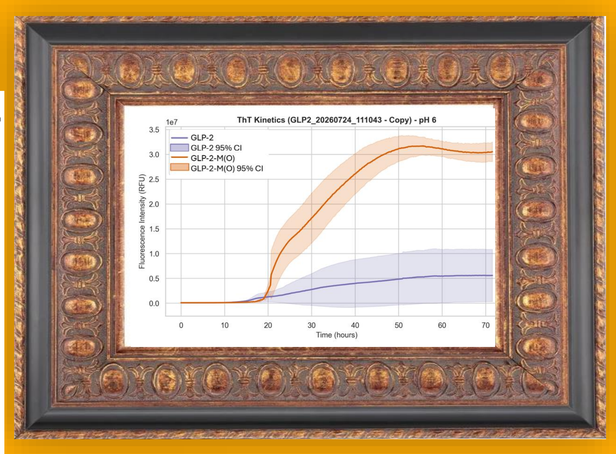
4 MOLECULAR DYNAMICS SIMULATIONS

pH 6

At pH 6 (~50% His protonation), GLP-2-Met(O) accelerates elongation ($k_{app} = 0.04\ h^{-1}$) and exhibits a ~4-fold higher ThT peak ($F_{max} = 3.19 \times 10^7$ vs. 8.32×10^6 RFU) than native GLP-2.
While native GLP-2 retains higher total β-sheet content (43% vs. 32%), AFM confirms that GLP-2-Met(O) uniquely assembles into thick, multi-filament hierarchical cables (10–28 nm).



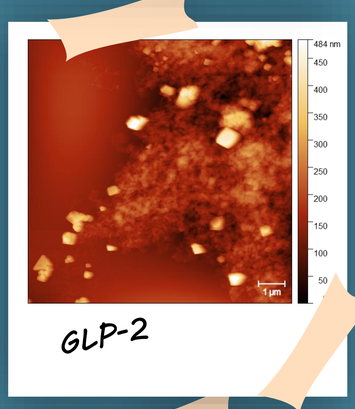


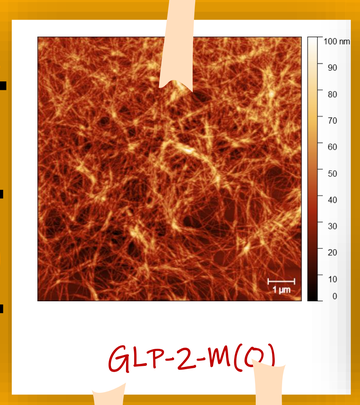


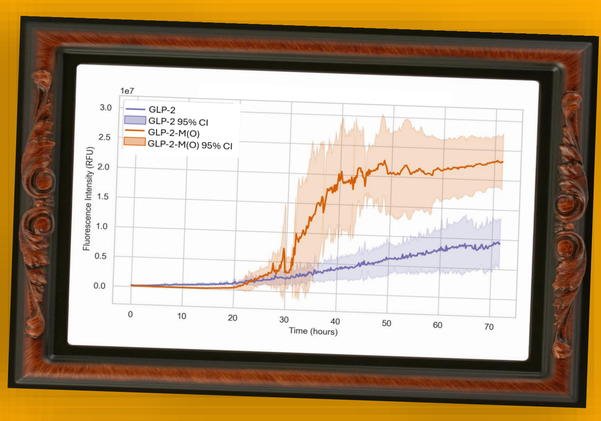
pH 4

At pH 4 (near pI = 3.9), native **GLP-2** undergoes immediate amorphous collapse into static, high β-sheet character (64% at $t = 0$ h → 68% at 72 h), trapping it in unorganized aggregates (~2 nm).

In stark contrast, GLP-2-Met(O) avoids kinetic trapping, navigating a progressive structural evolution from 52% to 76% β-sheet.



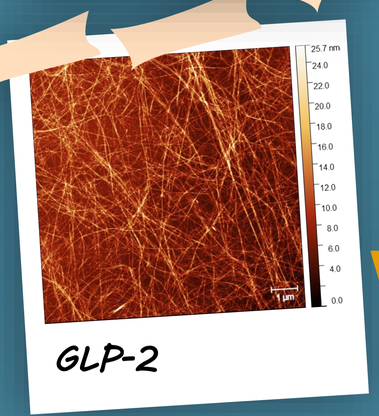


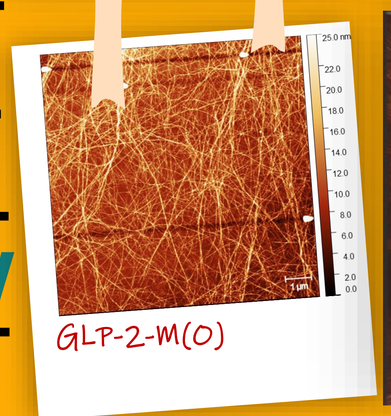


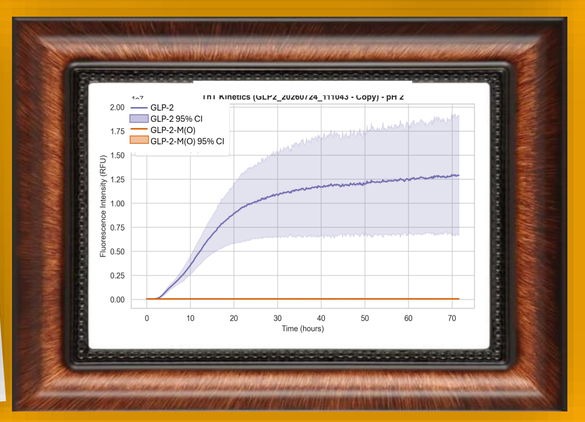
Data for pH 3, 5, and 7 are not presented on the poster.

pH 2

At pH 2, both variants assemble rapidly ($t_{lag} = 0$ h) into β-sheet-rich mature fibrillar networks over 72 h (60% β-sheet, 2.5–10 nm for GLP-2; 52% β-sheet, 3–6 nm for GLP-2-Met(O)). Notably, GLP-2-Met(O) exhibits a ~200-fold quenching in peak ThT fluorescence relative to native GLP-2.



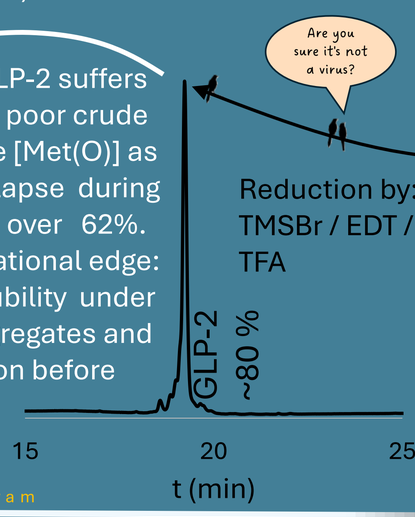


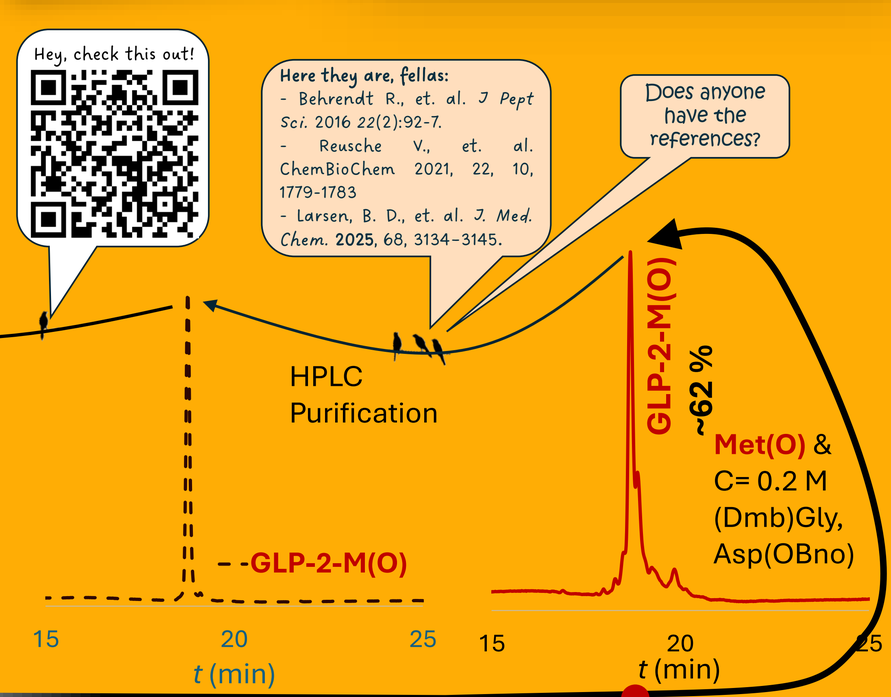


3

To compare native GLP-2 and its **methionine sulfoxide** variant [**GLP-2-Met(O)**], we integrated a multi-technique biophysical platform combining Far-UV circular dichroism (CD), Thioflavin T (ThT) fluorescence kinetics, and atomic force microscopy (AFM) across a broad pH gradient (pH 2–7):

Automated solid-phase peptide synthesis of GLP-2 suffers from severe interchain aggregation, resulting in poor crude purity (~19%). Introducing methionine sulfoxide [Met(O)] as a reversible handle disrupts hydrophobic collapse during chain elongation, boosting crude purity to over 62%. Beyond purity, Met(O) provides a decisive operational edge: it dramatically improves crude peptide solubility under identical conditions, eliminating insoluble aggregates and enabling seamless preparative HPLC purification before reduction back to native GLP-2.





methionine sulfoxide improves crude purity, as seen in the red chromatogram

HAARDFINWILLIQTKITD