Electronic Supplementary Material (ESI) for Chemical Science.

Supporting Information:

De novo Design of Peptides that Bind Specific Conformers of Amyloids; Methods and Application to α-Synuclein

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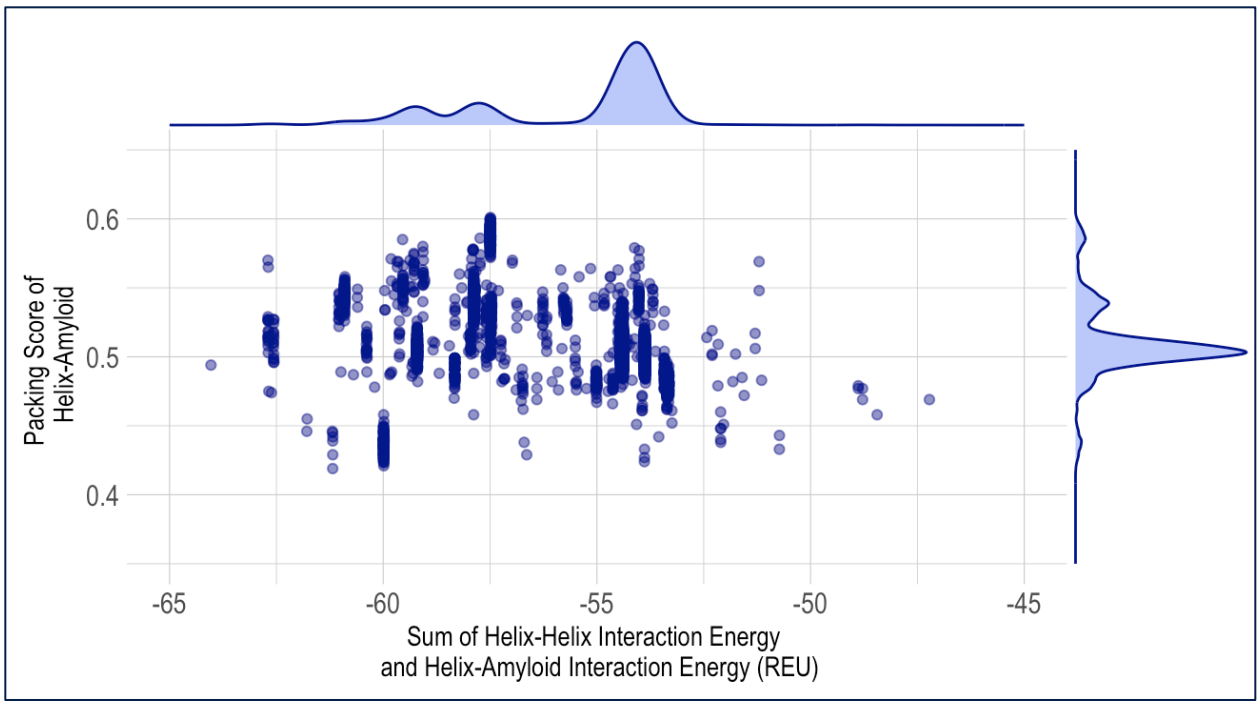

Fig S1. Interaction energies vs. packing scores of designed peptides to amyloid. Each point represents a different design model with plotted distributions on the x and y-axis. Each design model's interaction energy is plotted against the packing score.

$$\frac{[PL]}{[P]_T} = \frac{\left(K_D + \frac{[P]_T}{n} + [L]_T \pm \sqrt{\left(K_D + \frac{[P]_T}{n} + [L]_T\right)^2 - \frac{4[P]_T[L]_T]}{n}}\right)}{\frac{2[P]_T}{n}}$$

Fig S2. Quadratic binding formula to best fit ligand depletion sedimentation assay.

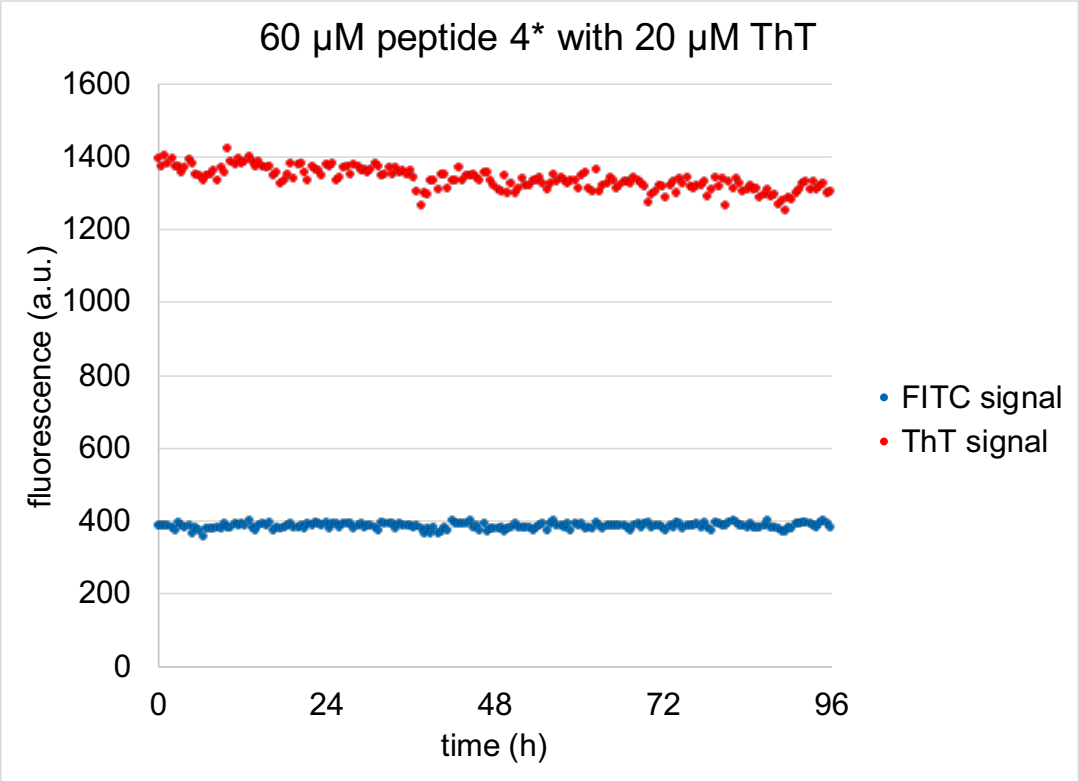

Fig S3. ThT fluorescence kinetics assay with peptide 4* (60 µM) and ThT (20 µM). ThT fluorescence was measured at ex/em of 440 nm and 490 nm and FITC fluorescence at ex/em of 490 nm and 525 nm over 96 h.

Materials and Methods

Preparation of α-Syn monomers.

α-Synuclein (α-Syn) fibrils were generated following established protocols to ensure consistent preparations.[1,2] In a concise description, the pET28a vector carrying the human α-synuclein gene was introduced into the BL21(DE3) *E. coli* strain, facilitating the overexpression of α-synuclein protein through IPTG induction. Subsequently, the cells were pelleted and suspended in an osmotic shock buffer (20 mM Tris-HCl, 40% sucrose, 2 mM EDTA at pH 7.2) and subjected to centrifugation. The resulting pellet was resuspended in cold water containing $MgCl_2$, followed by another round of centrifugation. The supernatant obtained was collected and subjected to lyophilization. E46K α-Syn was prepared the same way.

Preparation of fibrils.

α-Synuclein: The lyophilized α-synuclein monomers were reconstituted in a fibrillization solution containing 15 mM tetrabutylphosphonium bromide, resulting in a final concentration of 300 μM α-synuclein. Over a period of seven days, fibrils were grown through continuous shaking at room temperature in non-stick Eppendorf tubes.

Aβ40, 0N3R tau, α-Synuclein (RIBBON), α-Synuclein (FIBRIL): Previously reported procedures were followed.[3,4,5]

Solid-phase peptide synthesis.

The designed peptides were synthesized using Fmoc solid-phase peptide synthesis. An automated peptide synthesizer (Biotage Initiator+ Alstra peptide synthesizer) was employed, utilizing TentageIS RAM resin at a 0.05 mmol scale (0.10 g with 0.3 mmol/g loading size). Each amino acid was coupled in five equiv Fmoc-protected amino acid with five equiv of HCTU and ten equiv of *N,N*-diisopropylethylamine (DIPEA) in DMF relative to the peptide functional sites, at 75 ºC for 5 min. Deprotection was achieved by treating the resin-bound peptides with 4.5 mL of 20% 4-methylpiperidine in DMF at 70 ºC for 5 min.

For FITC-tagged peptides, Fmoc-beta-alanine-OH was coupled and Fmoc-deprotected, then the N-terminus was conjugated with 2.5 equiv FITC along with 5 equiv of DIPEA relative to the peptide functional site for 5 h at room temperature on resin. The resin was washed with three rinses of DMF and four rinses of CH_2Cl_2 before being dried for 1 h.

Peptide cleavage was performed following a standard protocol in a solution of 95% trifluoroacetic acid (TFA), 2.5% H_2O, 2.5% triisopropylsilane (TIPS), and 20 mg/mL dithiothreitol (DTT) for 1 h at room temperature. The crude peptide was then precipitated in cold diethyl ether and subsequently lyophilized. The resulting crude peptide was dissolved in H_2O + 0.1% TFA and purified by preparative reverse-phase HPLC using a Vydac C4 column (22 mm × 250 mm, 10 μm particle size) and a linear gradient of 5-100% MeCN + 0.1% TFA (with the remaining fraction H_2O + 0.1% TFA) over 25 min at a flow rate of 10 mL/min. The peptide eluted at approximately 40% MeCN. Fractions containing the peptide were combined and peptide purity was assessed by MALDI mass spectrometry and analytical HPLC. The combined fractions were frozen in liquid nitrogen followed by lyophilization. Approximately 20 mg of purified peptide was obtained as a white powder from the 0.05 mmol synthesis scale, corresponding to an overall yield of approximately 5%.

Confocal microscopy imaging.

Fluorophore-conjugated peptides were prepared in 1x PBS varying concentrations (128, 64, 32, 16, 8, 4, 2, 1, 0.1, 0.01 nM). For the experimental setup, 50-µL aliquots of each peptide solution were transferred into each wells of a 384-well plate (Corning™ BioCoat™ 384-Well, Collagen Type I-Treated, Flat-Bottom Microplate). Subsequently, 1-µL fibril solution was added to each well, achieving a final fibril concentration of 600 nM. Thorough mixing of the fibril and peptide solutions was achieved by pipetting up and down. The plate was centrifuged at 50 x g to pellet the fibrils.

Imaging of the fibril-peptide complexes was performed using a Leica SP8 confocal microscope equipped with a 40× water immersion lens (1.1 NA), white light and 405 nm lasers, and a HyD detector at 512 x 512 with a 1x zoom factor and a LightGate (0.5 to 18 ns). Excitation and emission detection wavelengths were collected at 490/525 nm.

Docking

MOE: Rigid-body protein-protein docking was performed by MOE software 2022.02 (Chemical Computing Group ULC, Montreal, Quebec, Canada). The receptor structure was obtained from alpha-syn fibril structure (PDB ID: 6CU7) and the ligand structure was imported from the designed trimeric helical bundle. Refinement was performed using rigid body and the top 100 poses were visually examined.

HDOCK: Molecular docking simulations were conducted using HDOCK.[6] The simulations were run on default parameters for protein-protein docking and the top 100 predictions were generated. The poses corresponding to the lowest energy scores is shown in the figure.

Molecular dynamics simulations.

Four copies of each designed peptide were simulated in complex with ten fibrils (except peptide 5, which was simulated with 9 fibrils because the peptide is much shorter in length and does not cross over multiple fibrils). The *N*- and *C*-termini of the amyloid regions were capped with *N*-methyl and acetyl group, respectively. The residues of protein and peptide were assigned their standard protonation states at pH 7.

The MD system was prepared using tleap.[7,8] The simulation box was built by solvating each complex with OPC model waters[9] with 8 Å padding from the complex, and sodium and chloride ions were added to reach a charge-neutral 100 mM concentration. All simulations were performed in Amber18 with the ff19SB forcefield.[10] Simulations began with 1,000 restrained steepest-descent minimization steps before switching to a maximum of 7,000 steps in conjugate gradient minimization. The system was then heated up from 150K to 300 K over 50 ps in the NVT ensemble with Langevin thermostat control of temperature, using a 1 fs integration timestep. The system was then switched to the NPT ensemble, and pressure was maintained at 1 atm using the Monte Carlo barostat. Peptide residues were restrained with a 10 kcal/(mol·Å2) force constant initially and very slowly ramped down to 0 kcal/(mol·Å2) over 6 equilibration steps, comprising 3 ns in total. Amyloid C-alpha atoms were restrained with a 0.5 kcal/(mol·Å2) force constant throughout the whole simulation to maintain their positional integrity.

Following minimization and equilibration, the simulation was carried out for a 500 ns production run under periodic boundary conditions with 2 fs timesteps. The SHAKE algorithm[11] was used to restrain hydrogens, the Particle Mesh Ewald method[12,13,14,15] was used to calculate long-range electrostatics, and non-bonded interactions were cut off at 10 Å. Three independent simulations were performed for each design.

Ligand depletion sedimentation assay.

The WT αSyn fibril was diluted 2-fold with a PBS solution containing 1 μM peptide 4*, resulting in concentrations of 100, 50, 25, 12.5, 6.25, 3.125, 1.5625, and 0.78 μM. After incubating the mixture at room temperature for 30 minutes, it was centrifuged at 14,000 rpm for 20 minutes to pellet the fibrils and fibril-bound peptide 4*. The supernatant, containing the fibril unbound peptides, was carefully collected. The fluorescence signal associated with the FITC-labeled peptide in the supernatant was quantified by measuring the absorbance of FITC at 490 nm. Each experiment was conducted in duplicates, and the same procedure was followed for the scramble* peptide.

ThT fluorescence assay.

α-Synuclein fibrillization was monitored using the FLUOstar OMEGA microplate reader. A 50 μL of 300 μM α-synuclein monomers containing 15 μM Thioflavin T (ThT) was transferred to a 384-well plate. The ThT fluorescence resulting from 440 nm excitation and 490 nm emission was recorded at every 10-min interval over the course of 6000 min period. The plate was agitated for 5 min between each data collections.

Supplementary Text

RosettaScript (design_hbnet.xml)

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# with ALL AA (amino acids) except cysteine (XC) -- to avoid disulfide linkages


# the following lines are commented out, but this is what the rest of the
# resfile would look like if you wanted to design specific amino acids on to
# specific resdiue locations

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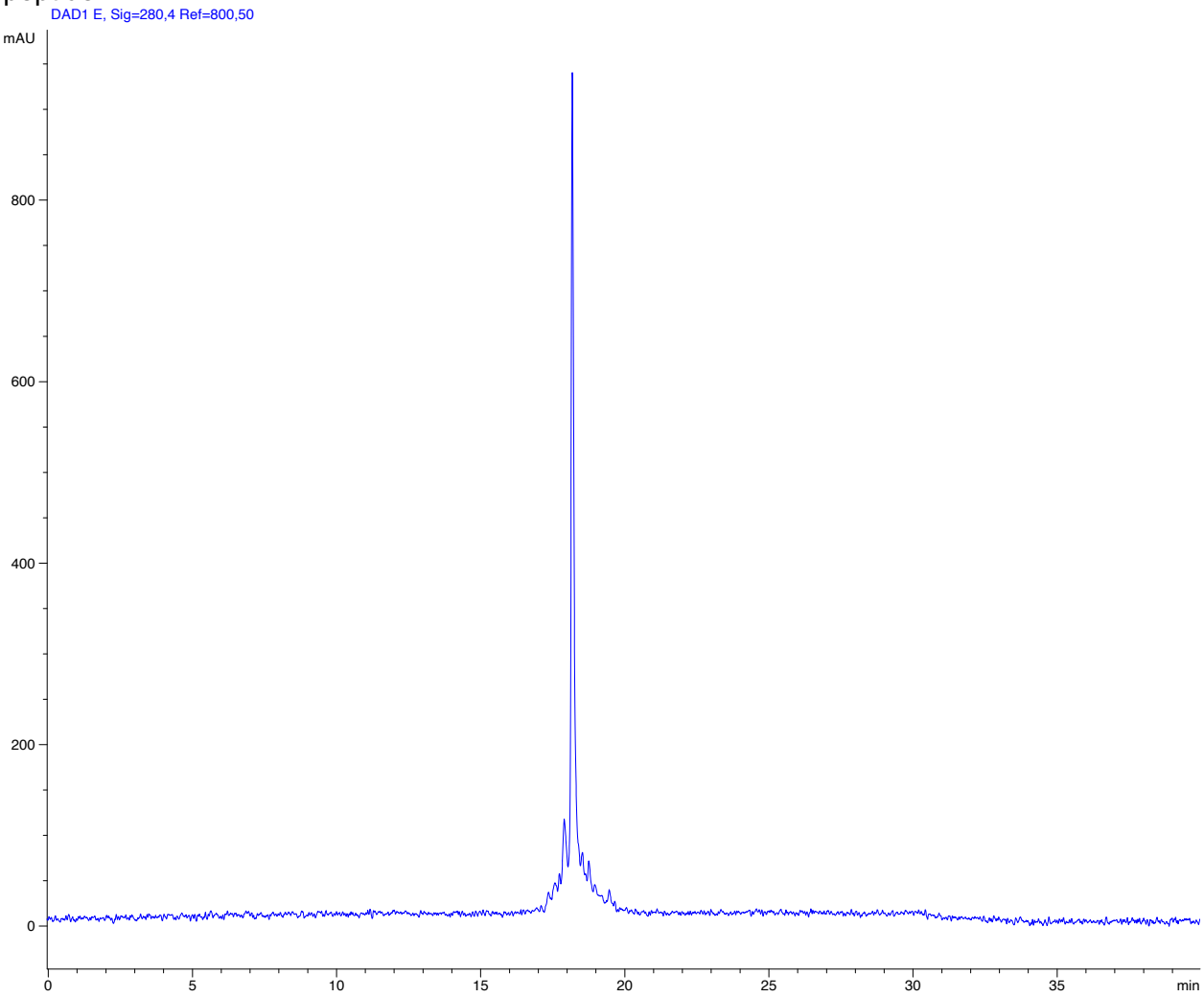
HPLC trace and mass spectrum of peptides

peptide 4



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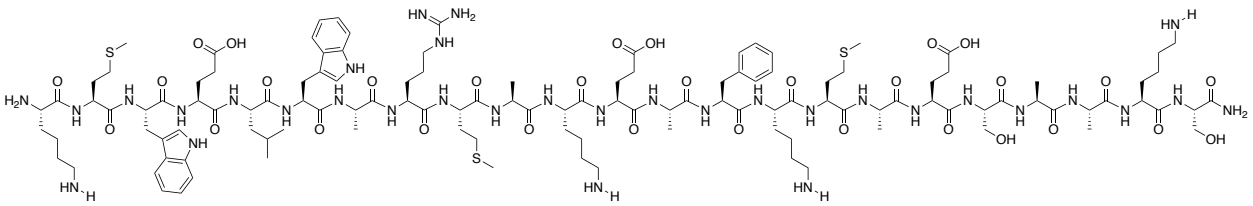

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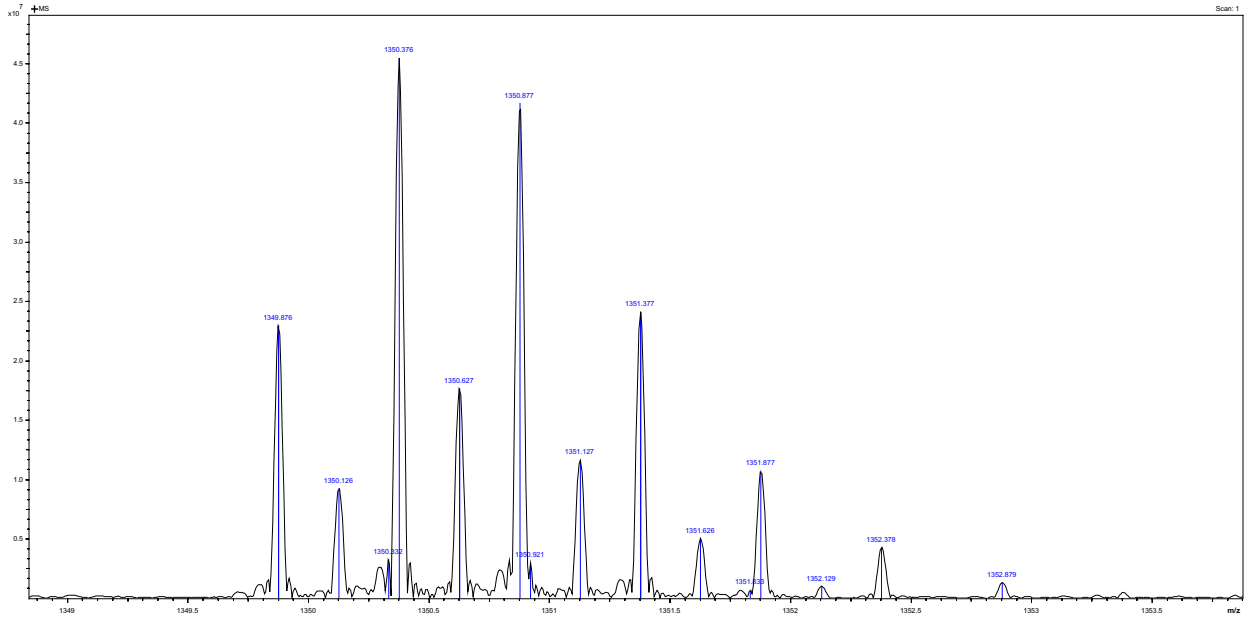
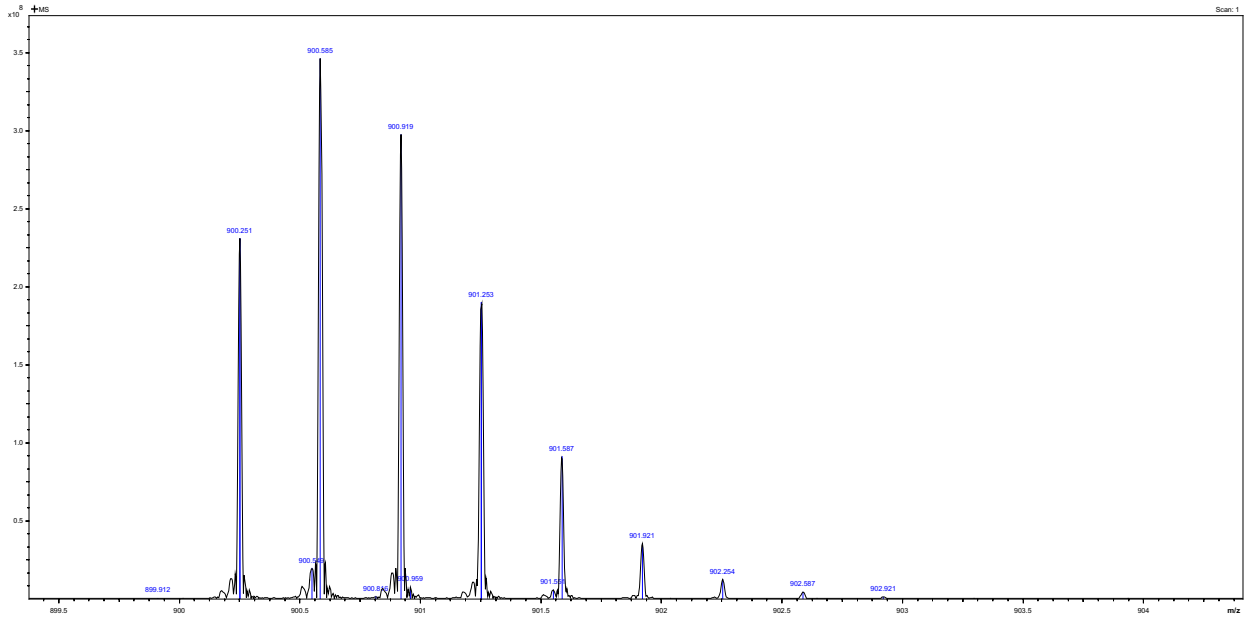
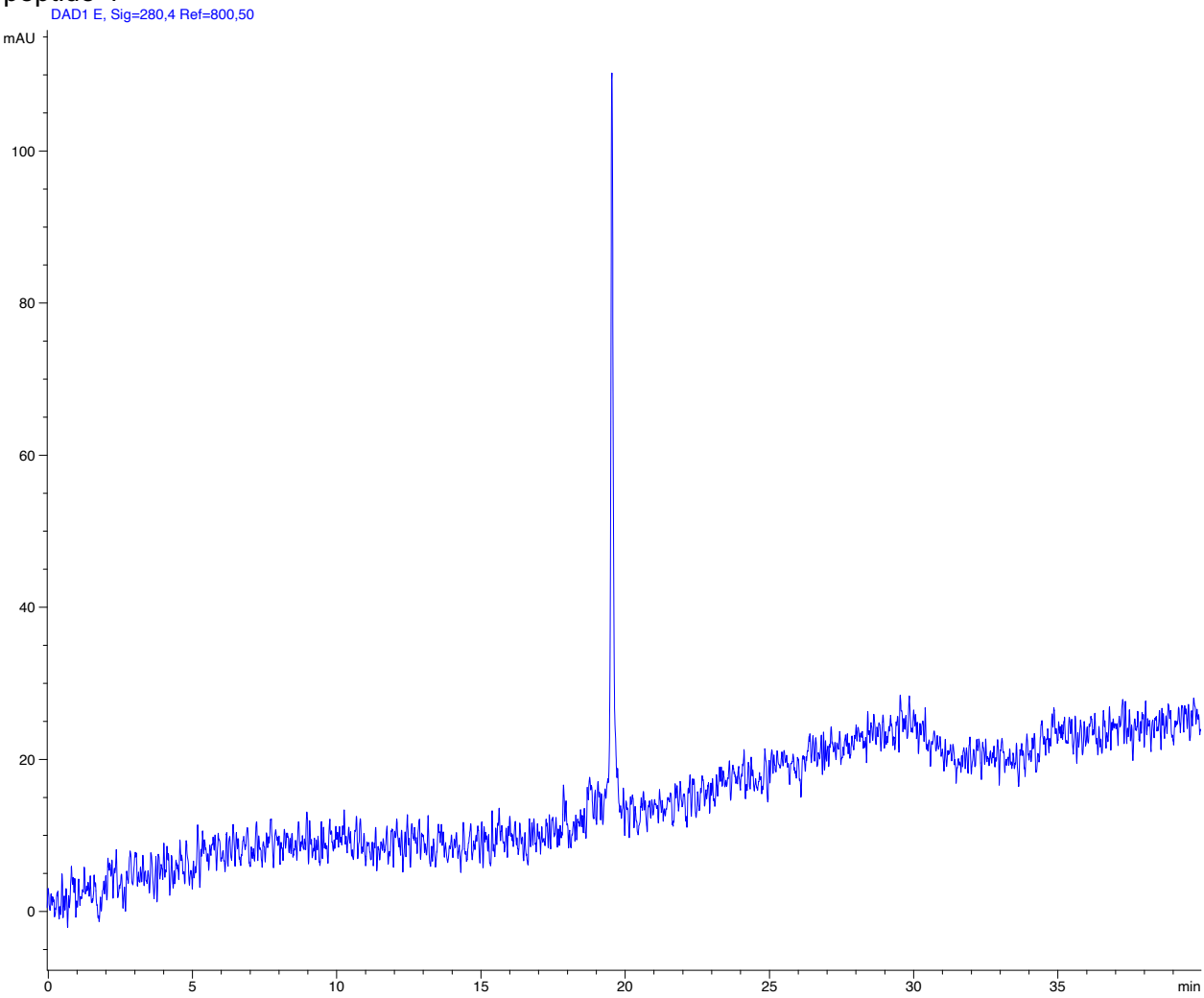




peptide 4*



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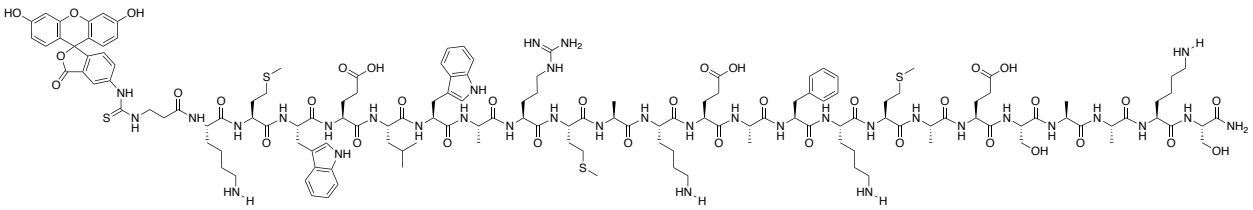

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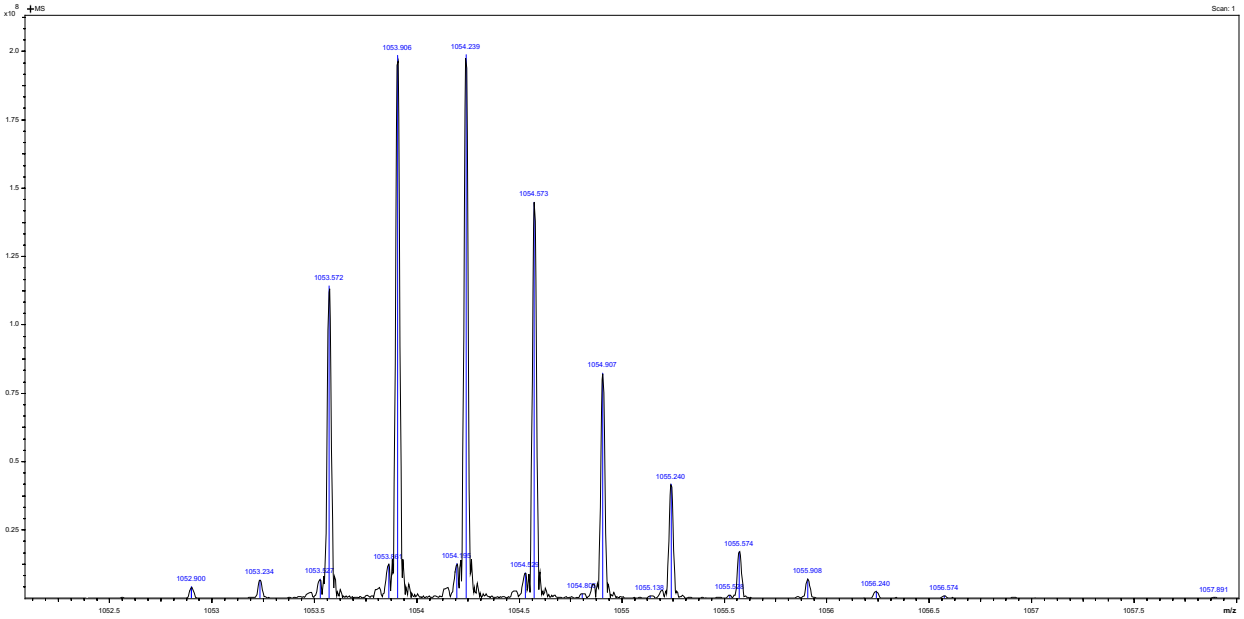

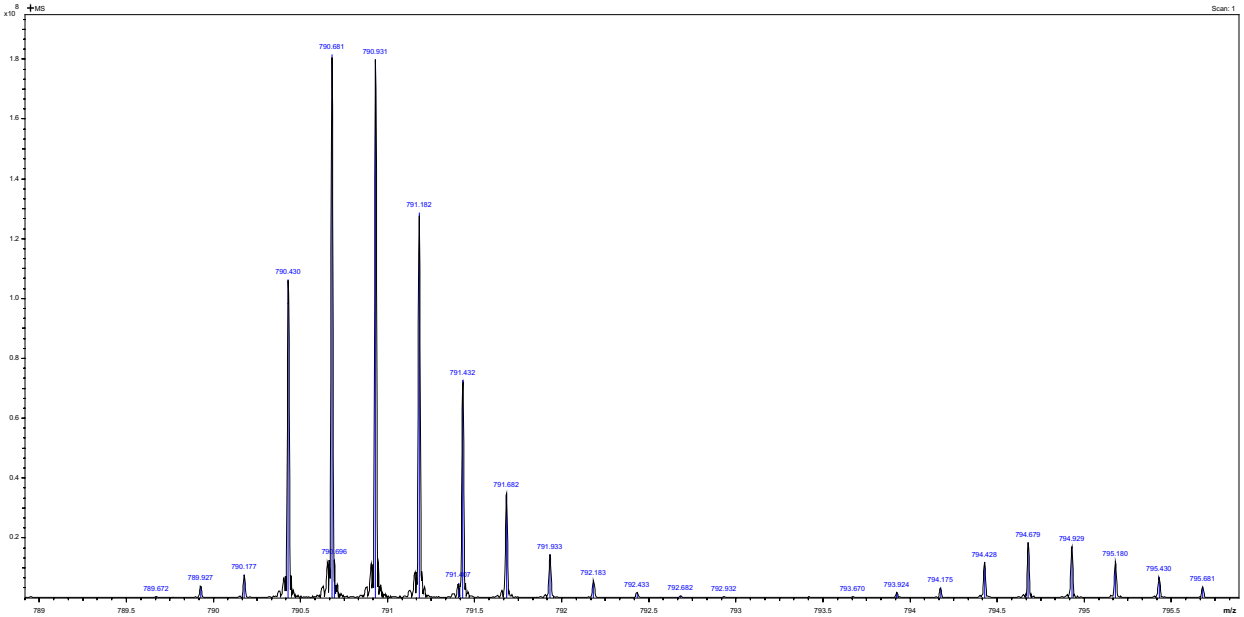

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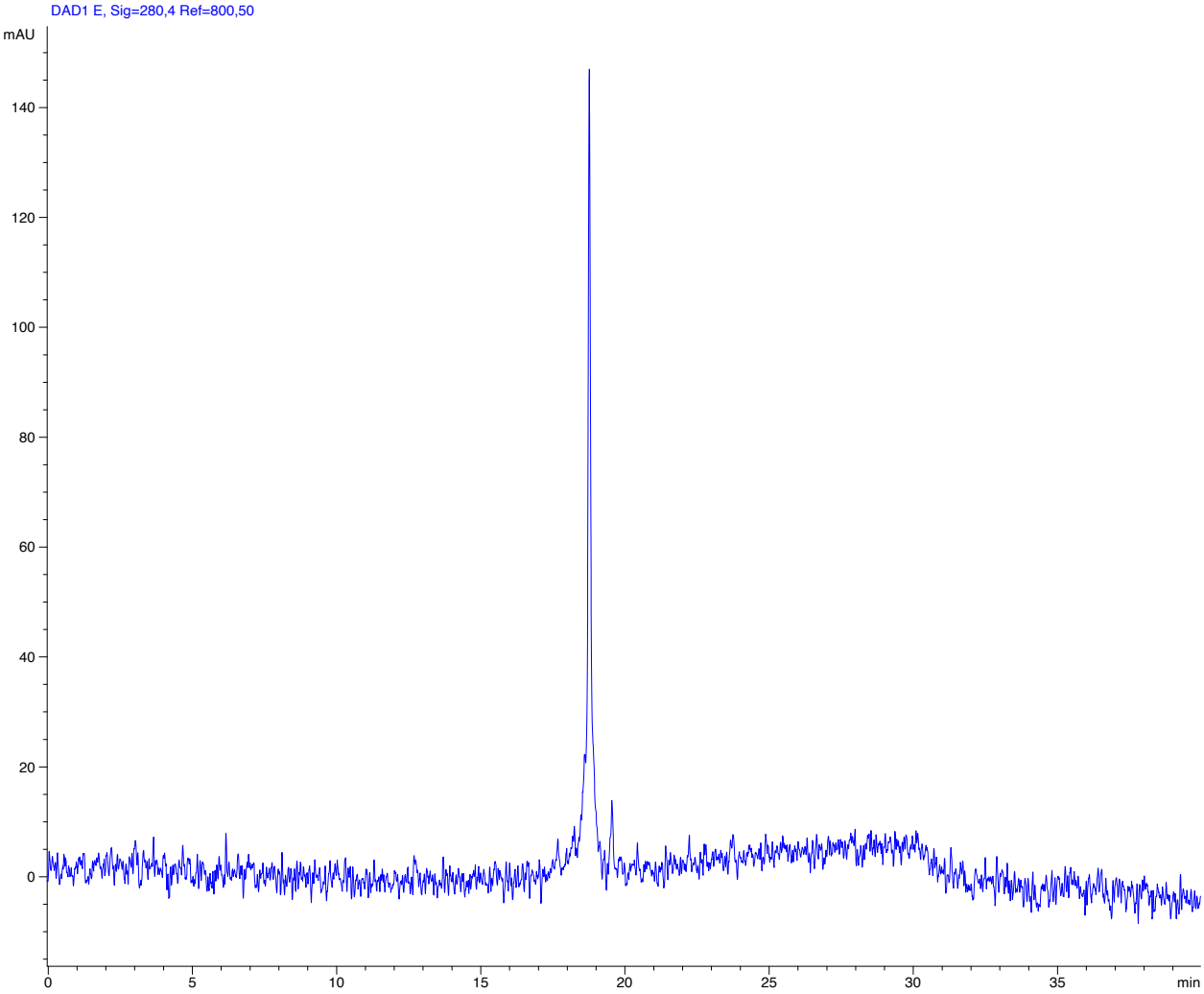

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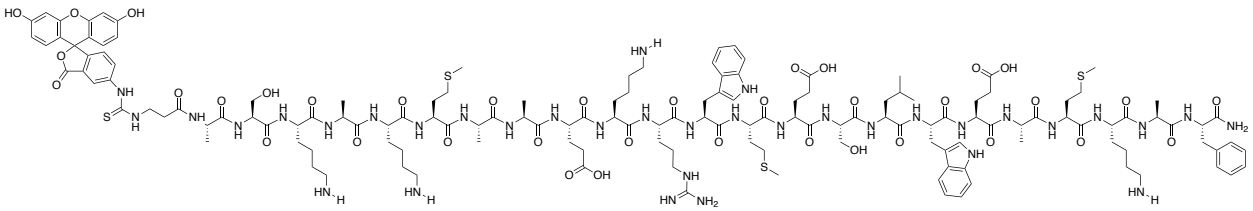

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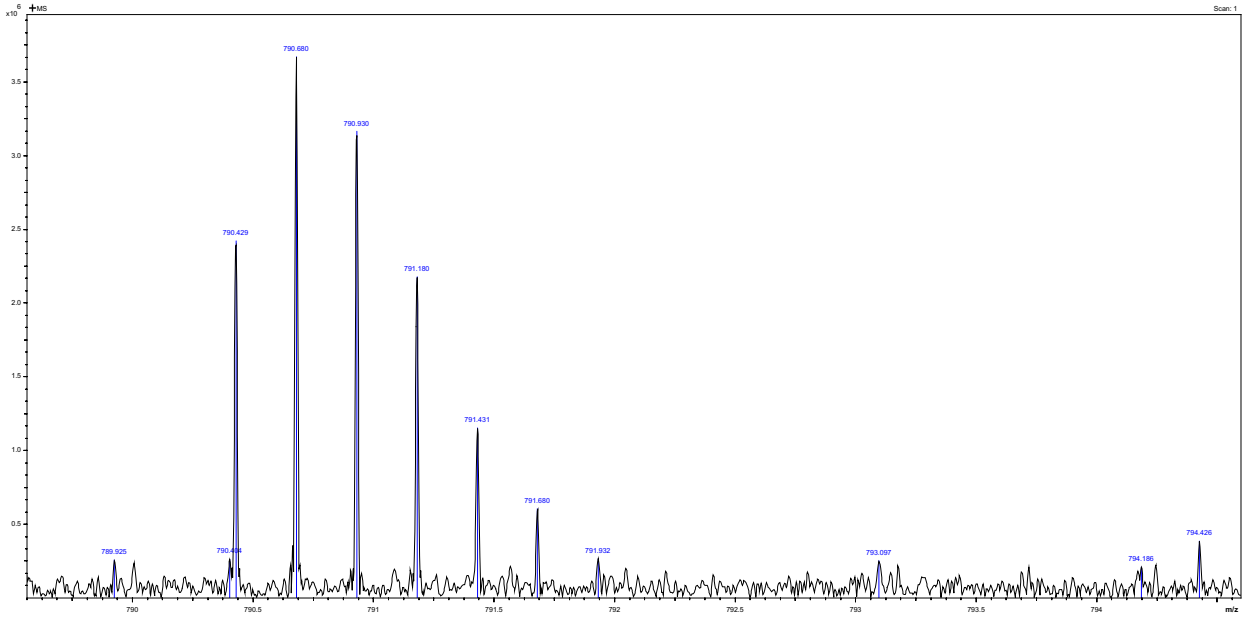

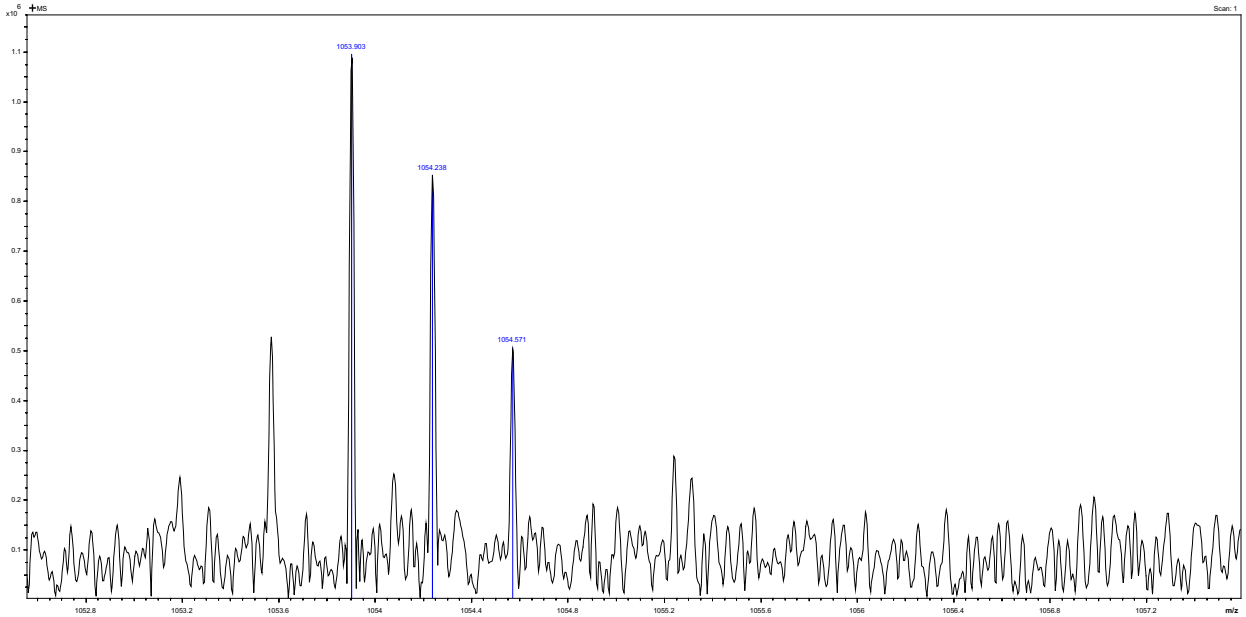

References

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