

Supporting Materials

Focused Design of Novel Cyclic Peptides Endowed with GABARAP Inhibiting Activity

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Table S1. Summary table of the Microscale Thermophoresis (MST) experiments performed on both Monolith NT.115 and Monolith NT.115Pico instruments. At least two independent experiments were performed to compute the K_d value.

| Monolith NT.115 | | | | | | | |
|---------------------|--------|-----------|------------|-------|-------------------------|------|------------------|
| Protein | Ligand | MST Power | Exc. Power | Temp. | [Ligand] Range | SNR* | K_d (μ M) |
| GABARAP 25 nM | K1 | 40% | 100% | 25°C | 312.5 μ M - 19.1 nM | 20.7 | 2.6 \pm 0.4 |
| GABARAP 200 nM | WC8 | 40% | 85% | 25°C | 1.25 mM - 38.1 nM | 12.3 | 22.0 \pm 5.9 |
| GABARAP 25 nM | WC10 | 40% | 80% | 25°C | 25 μ M - 0.8 nM | 11.7 | 4.2 \pm 1.8 |
| Monolith NT.115Pico | | | | | | | |
| Protein | Ligand | MST Power | Exc. Power | Temp. | [Ligand] Range | SNR* | K_d (μ M) |
| GABARAP 10 nM | K1 | 40% | 20% | 25°C | 312.5 μ M - 38.1 nM | 13.3 | 3.5 \pm 0.9 |
| GABARAP 10 nM | WC8 | 40% | 20% | 25°C | 625 μ M - 38.1 nM | 27.6 | 20.8 \pm 2.7 |
| GABARAP 10 nM | WC10 | 40% | 20% | 25°C | 39.1 μ M - 9.5 nM | 10.8 | 3.8 \pm 1.5 |

* Signal to Noise Ratio

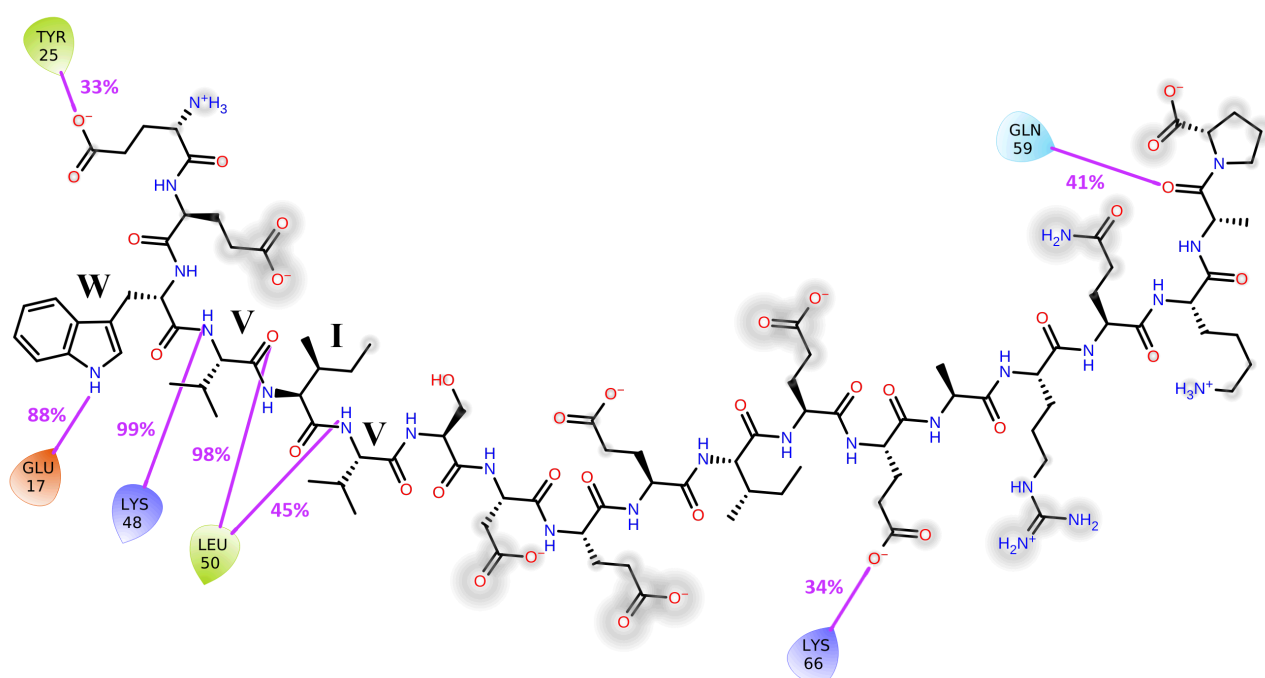


Figure S1. GABARAP H-bond interactions with AnkB-LIR ligand monitored throughout the simulation. The key residues of AnkB-LIR (WVIV) interacting with GABARAP protein are highlighted. Only the interactions that occur more than 30% of the simulation time are shown.

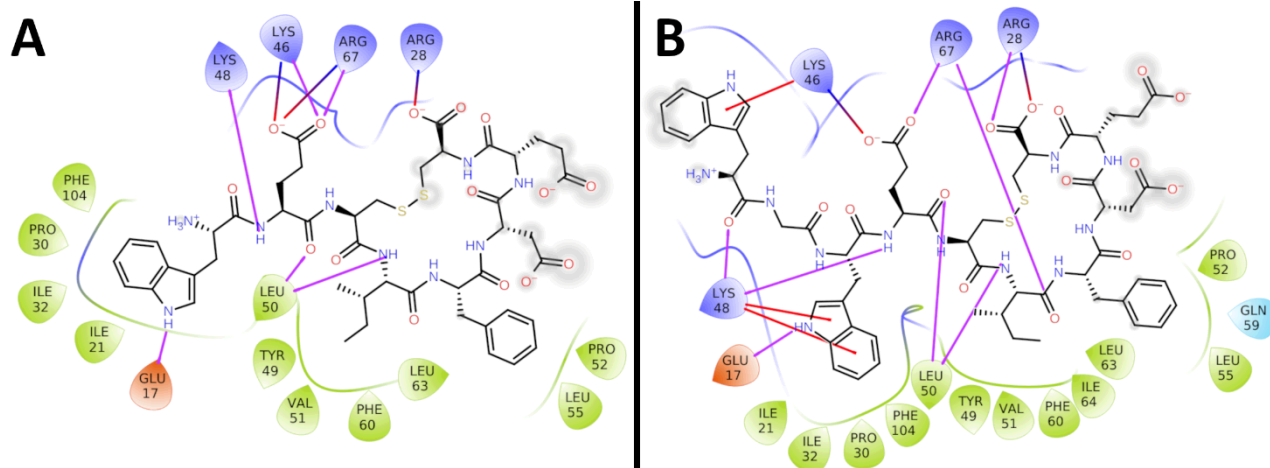


Figure S2. Ligand interaction diagram of WC8 (A) and WC10 (B) peptides with GABARAP protein. H-bond, salt bridges and π -cation interactions are highlighted in purple, blue/red and red lines, respectively. Residues involved in hydrophobic interactions are showed in green.

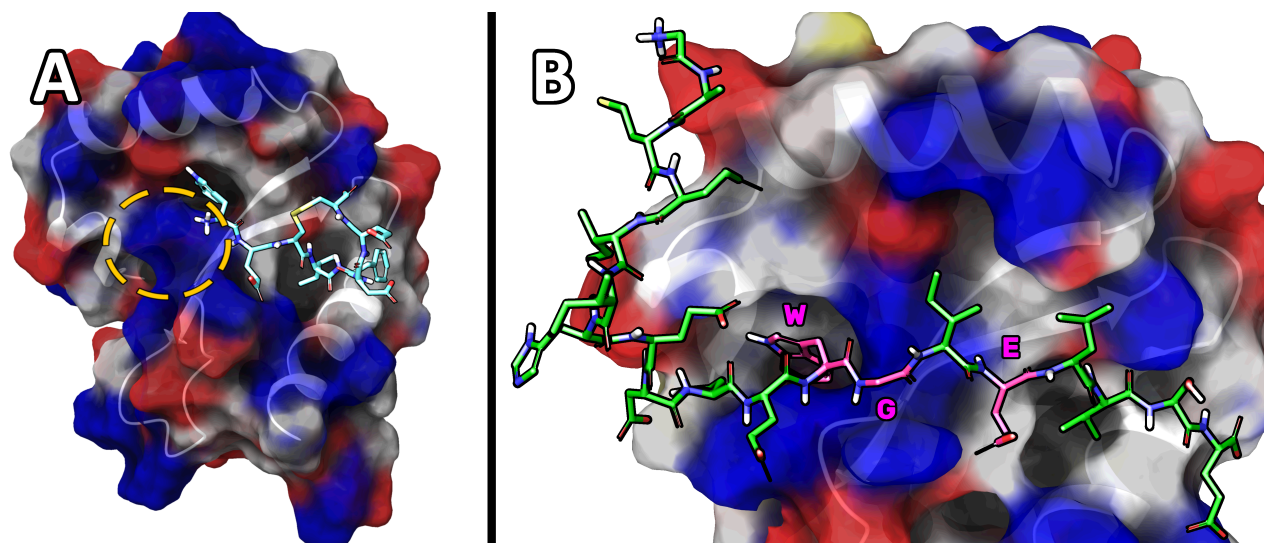


Figure S3. (A) GABARAP/WC8 most representative structure. The yellow circle highlights the protein area in which the two N-terminal residues added on WC8 could be inserted to improve theoretical binding affinity. (B) GABARAPL2/UBA5 NMR structure (PDB accession code 6H8C). In pink sticks are highlighted the residues of UBA5 (GAMEIIHEDNEWGIELVSE) in complex with GABARAP and GABARAPL2 whose interactions are reproduced by peptide WC10 (WGWECIFDEC). The GABARAP solvent-accessible surface is showed accordingly by residue charges: blue for positive and red for negative residues.

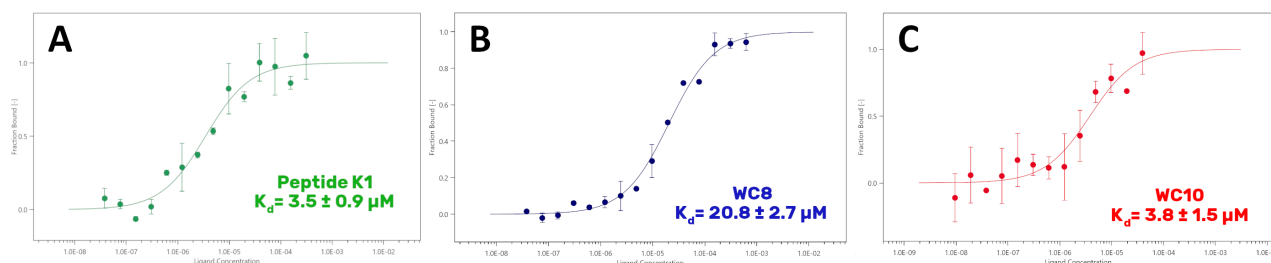


Figure S4. MST curves acquired by GABARAP recombinant protein incubated with different concentrations of K1 (A), WC8 (B) and WC10 (C) peptides using the Monolith NT.115Pico instrument.

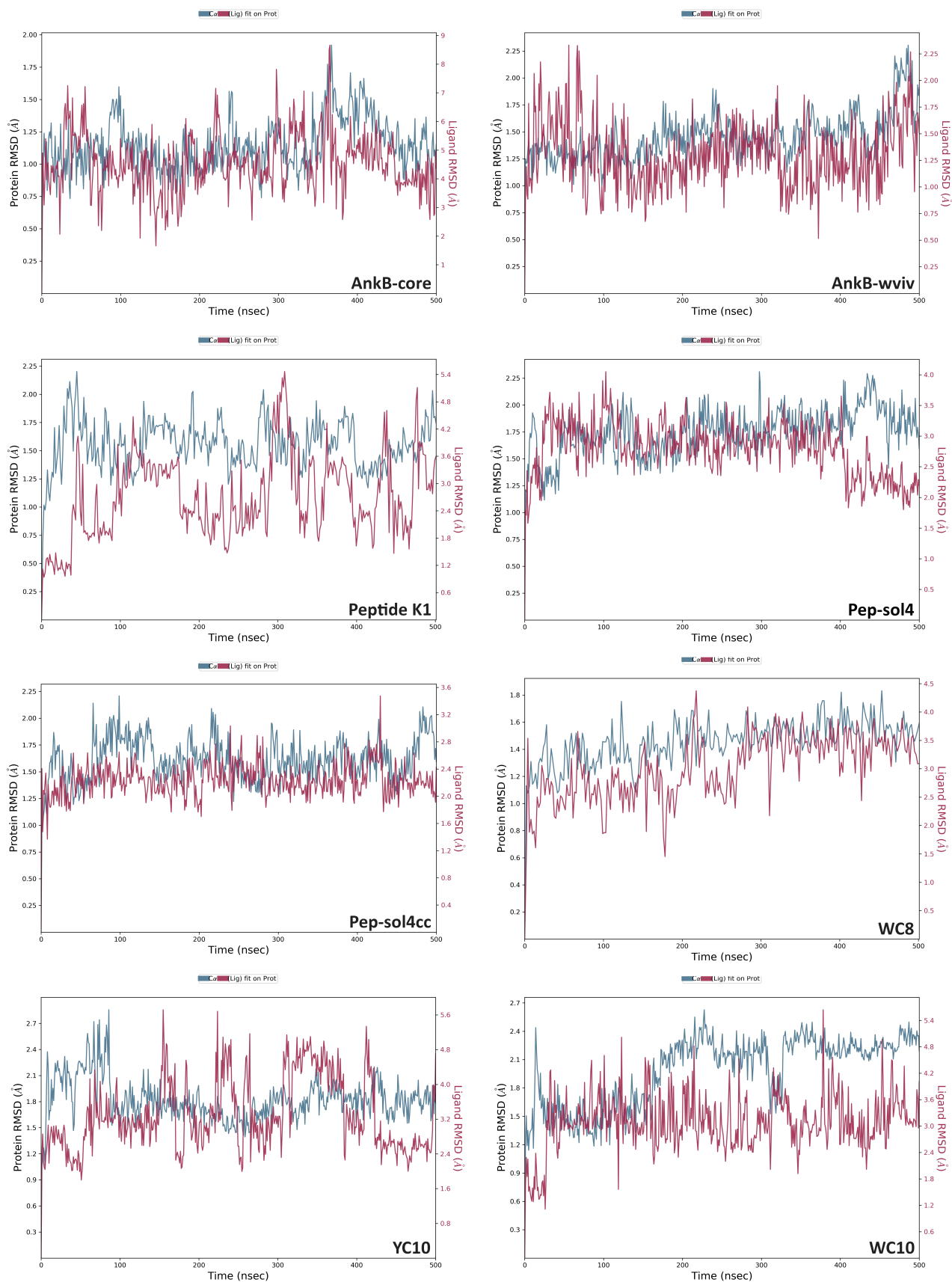
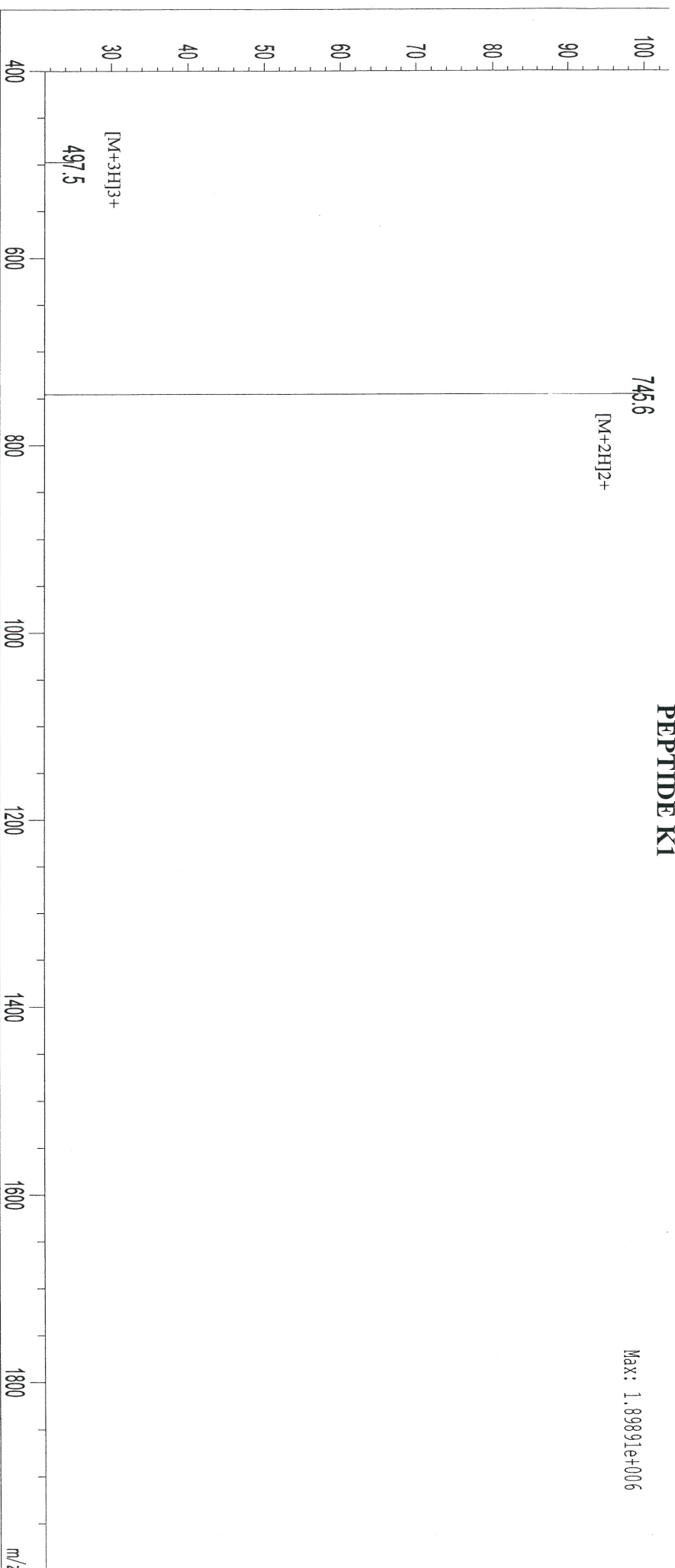


Figure S5. α atoms RMSD plots of protein and ligand of the simulated systems over MD simulation time (500 ns).

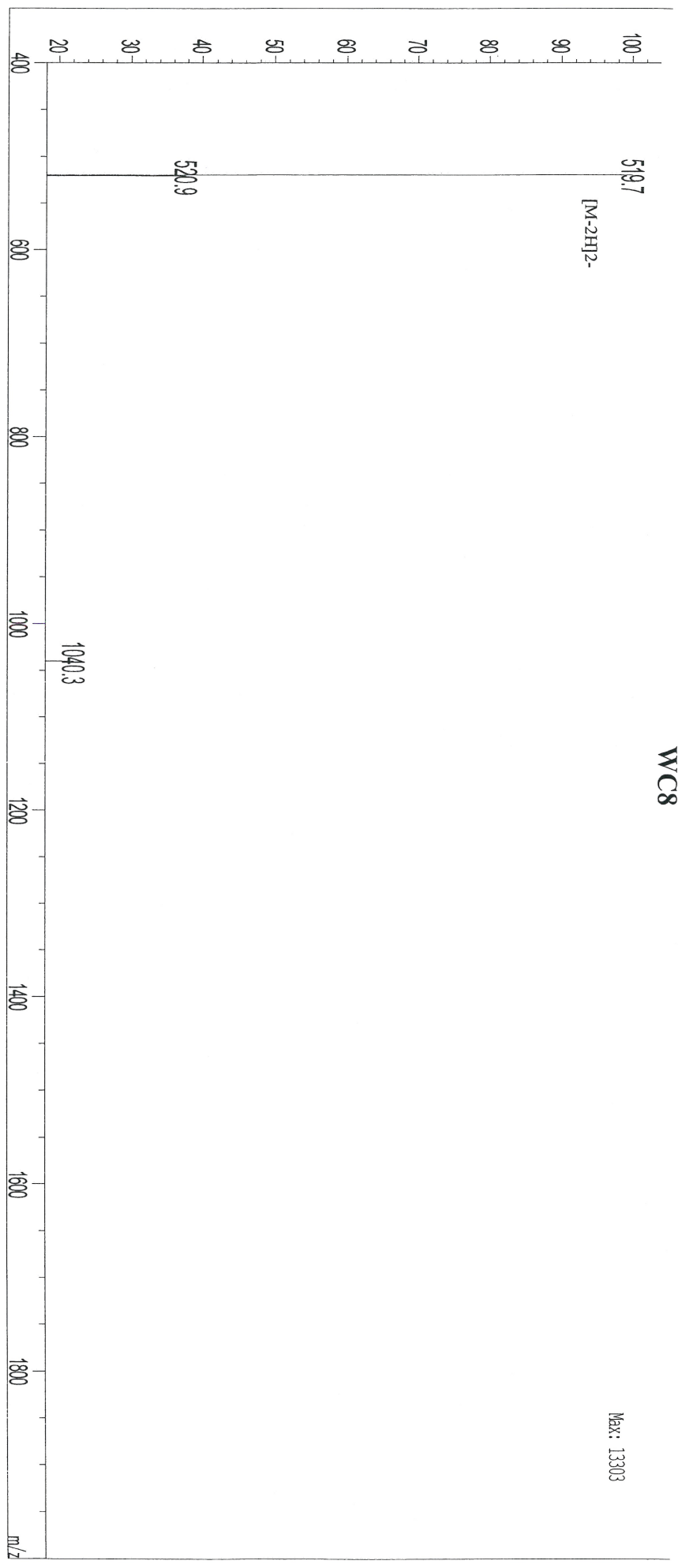
MASS SPECTROMETRY REPORT

PEPTIDE K1



| | | | | |
|----------------------------|---------------------|----------|-------------|---------------|
| Sample Information | Probe: | ESI | Probe Bias: | +4.5kv |
| Injection Volume : 1.00 µl | Nebulizer Gas Flow: | 1.5L/min | Detector: | 1.5kv |
| Sample: DP-12 | CDL: | -20.0v | T. Flow: | 0.2ml/min |
| M.W.: 1489.58 | CDL Temp.: | 250 °C | B. Conc.: | 50%H2O/50%ACN |
| Lot. No.: P201104-HS843557 | Block Temp.: | 200 °C | | |

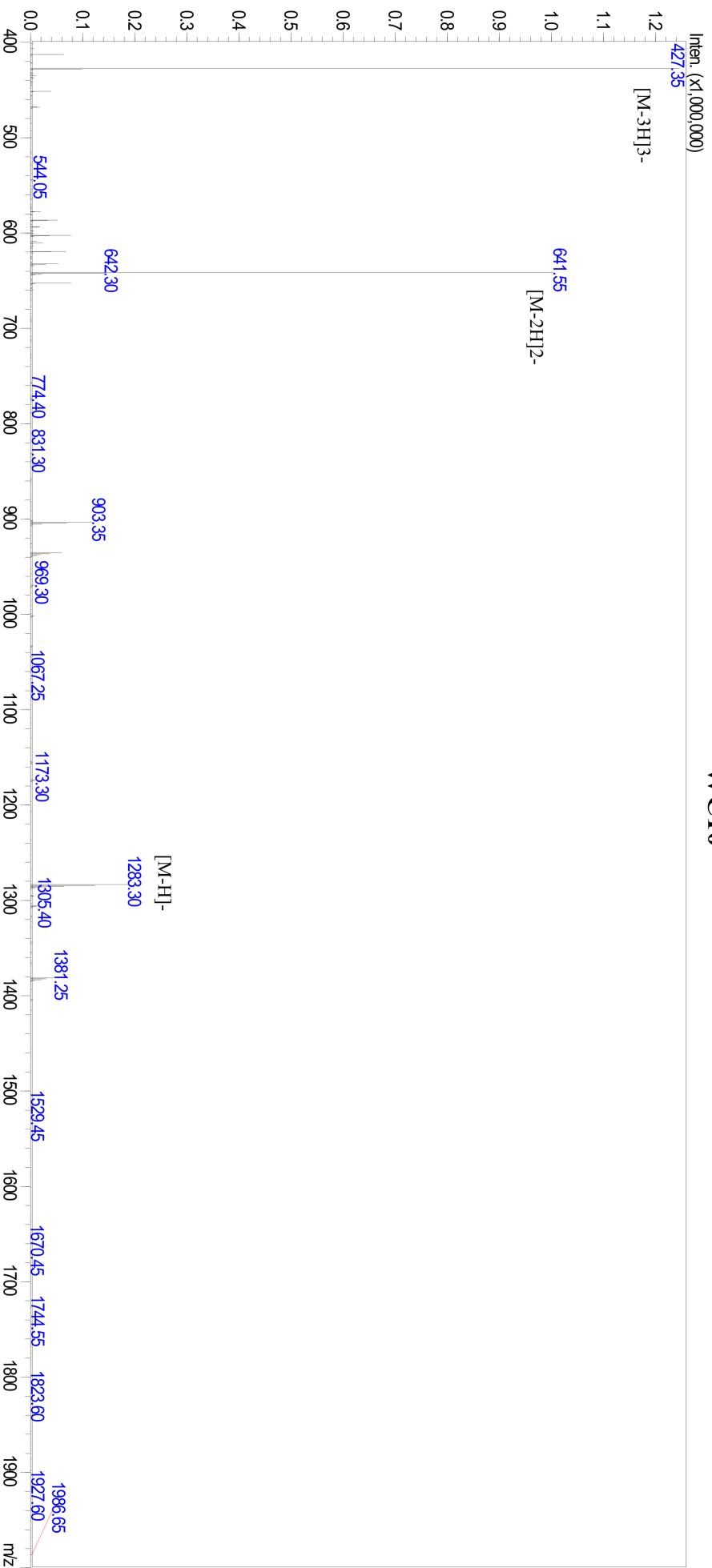
MASS SPECTROMETRY REPORT
WC8



| | |
|----------------------------|------------------------------|
| Sample Information | |
| Injection Volume : 1.00 µl | Probe: ESI |
| Sample: Sol4f-cc WC-8(O) | Nebulizer Gas Flow: 1.5L/min |
| M.W.: 1042.14 | CDL: -20.0v |
| Lot. No.: P200318-SY788987 | CDL Temp.: 250 °C |
| | Block Temp.: 200 °C |
| | Probe Bias: +4.5kv |
| | Detector: 1.5kv |
| | T. Flow: 0.2ml/min |
| | B. Conc.: 50%H2O/50%ACN |

MASS SPECTROMETRY REPORT

WC10



| | | | |
|----------------------------|--|---------------------|---------------|
| Sample Information | | | |
| Injection Volume : 1.00 µl | | Probe: | ESI |
| Sample: WC10 WC-10 | | Nebulizer Gas Flow: | 1.5L/min |
| M.W.: 1285.40 | | CDL: | -20.0v |
| Lot. No.: P210305-CQ874405 | | CDL Temp.: | 250 °C |
| | | Block Temp.: | 400 °C |
| | | Probe Bias: | +4.5kv |
| | | Detector: | 1.2kv |
| | | T. Flow: | 0.2ml/min |
| | | B. Conc.: | 50%H2O/50%ACN |