Supplemental Information

The Rational Design of Therapeutic Peptides for Aminopeptidase N using a Substrate-Based Approach

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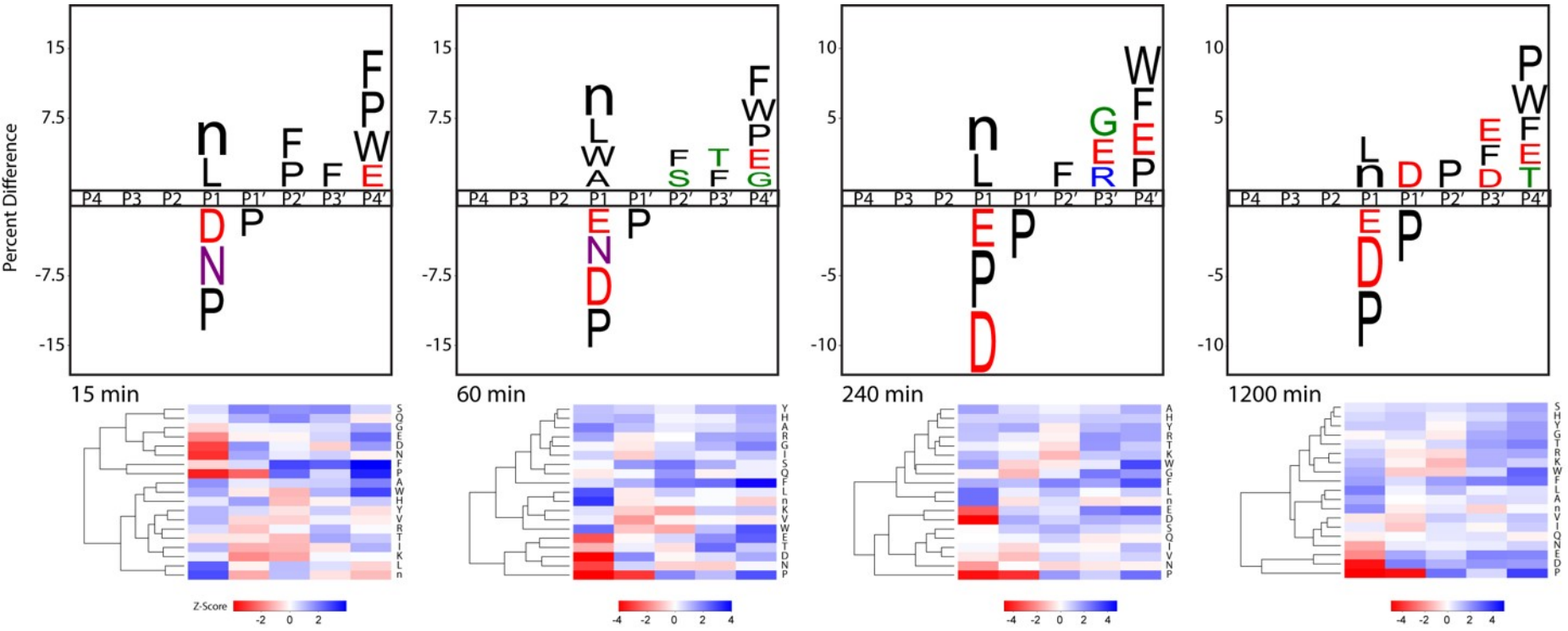
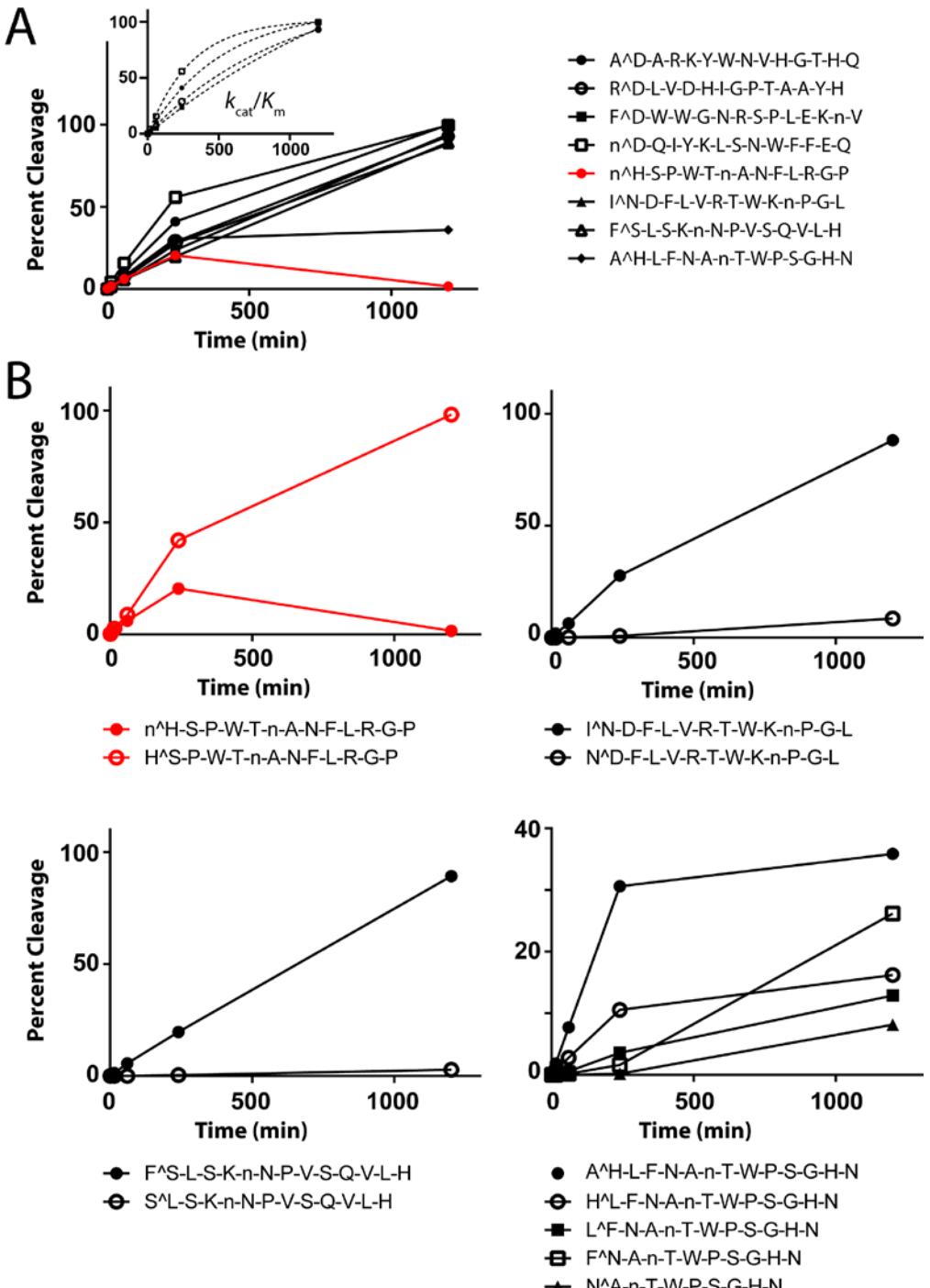
Supplemental Figure 1



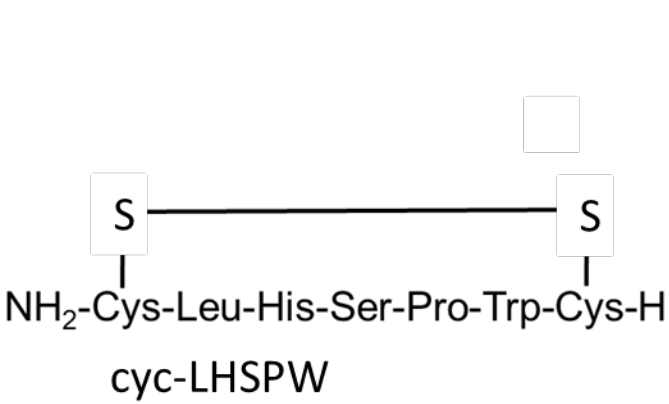
Supplemental Figure 1: Global identification of hAPN substrate specificity with the MSP-MS assay. (Top) iceLogo representation of P1-P4' specificity at the 15, 60, 240, and 1200 min assay time points ($P \leq 0.05$ for all residues shown; "n" is norleucine). (Bottom) Heat map representations of hAPN P1-P4' specificity at the 15, 60, 240, and 1200 min assay time points calculated using Z-scores at each position. Favored residues are colored blue (Z-score > 0) and disfavored residues are colored red (Z-score < 0).

Supplemental Figure 2



Supplemental Figure 2: Comparison of hAPN cleavage against a panel of 14-mer peptides from the MSP-MS library that underwent complete hydrolysis during the assay time course and that were selected for inhibitor development (Table I). A.) Cleavage time courses for hAPN removal of the primary N-terminal amino acid in the panel of substrates. Inset.) Determination of k_{cat}/K_m values for substrates that did not undergo secondary cleavage events. Notably, these substrates contain aspartic acid, which is a disfavorable P1 residue for hAPN, at the second position. Arrows (^) indicate the site(s) of cleavage. k_{cat}/K_m values were as follows: 1.67 (± 0.08) x 10^4, 9.03 (± 0.36) x 10^3, 4.46 (± 0.75) x 10^3, and 2.85 (± 0.15) x 10^4 $M^{-1}s^{-1}$ for A^D-A-R-K-Y-W-N-V-H-G-T-H-Q, R^D-L-V-D-H-I-G-P-T-A-A-Y-H, F^D-W-W-G-N-R-S-P-L-E-K-n-V, and n^D-Q-I-Y-K-L-S-N-W-F-F-E-Q, respectively. B.) Time courses showing secondary cleavage events for a subset of the panel. The five terminal residues (P1-P4') of the substrate n-H-S-P-W-T-n-A-N-F-L-R-G-P (red) was the most effective hAPN inhibitor in the absence of cyclization (Table I).

Supplemental Figure 3



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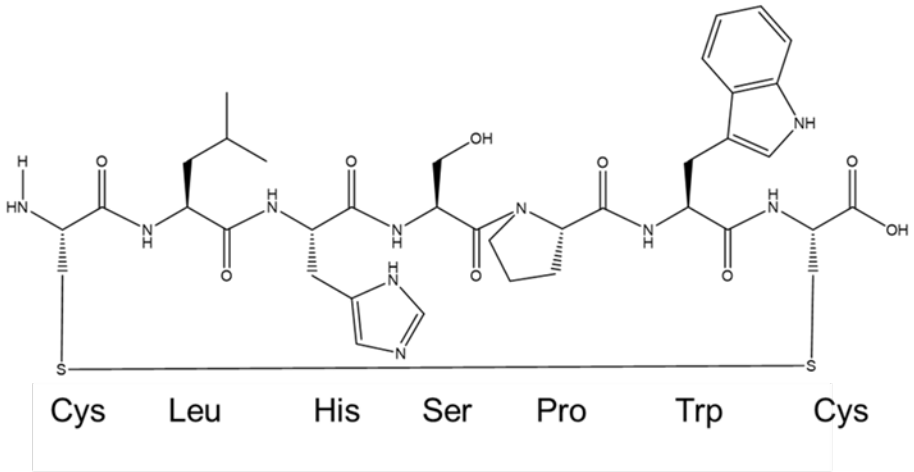

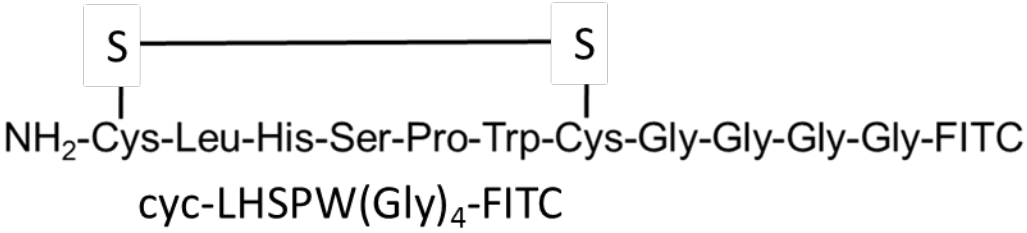

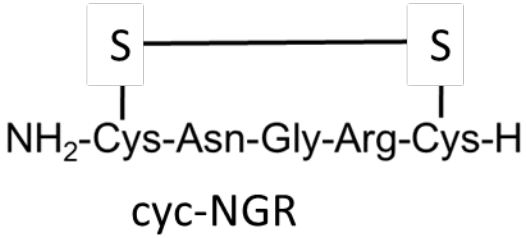

Supplemental Figure 3: Structures of the salient cyclic peptides used in this study.

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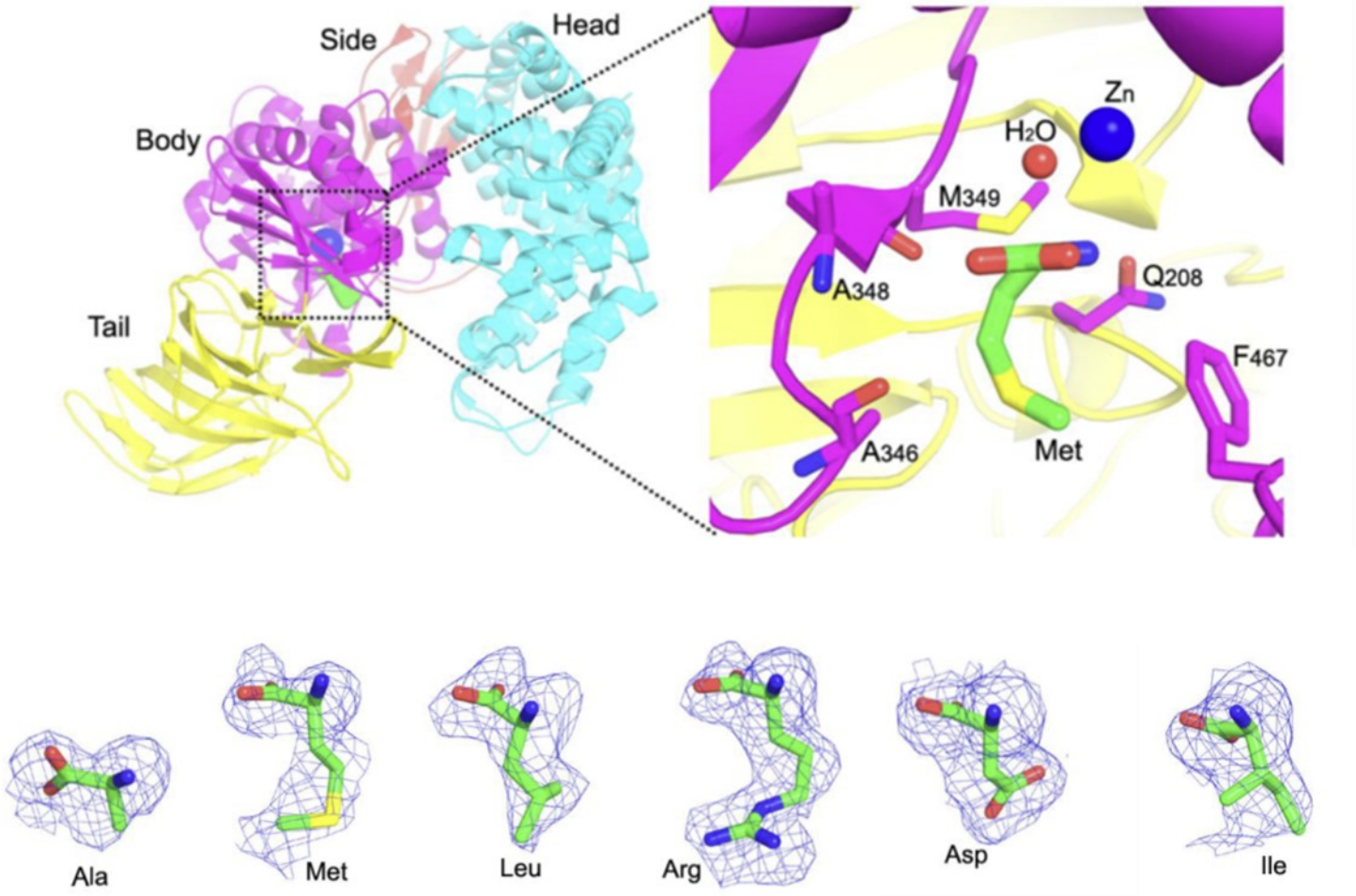
Supplemental Figure 4



Supplemental Figure 4: Electron density of the amino acid side chains.

Supplemental Figure 5

Data	Arg/pAPN complex	Asp/pAPN complex	Ala/pAPN complex	Ile/pAPN complex	Leu/pAPN complex	Met/pAPN complex
PDB code	4IYV	4IYW	4IYY	4IYZ	4IZ1	4IZ2
Data collection						
Space group	C2	C2	C2	C2	C2	C2
Cell parameter						
a(Å)	260.7	259.8	260.6	260.4	259.6	260.7
b(Å)	62.9	62.7	62.7	62.9	63.0	62.9
c (Å)	81.7	81.7	81.6	81.7	81.6	81.6
α(°)	90.0	90.0	90.0	90.0	90.0	90.0
β(°)	100.6	100.4	100.3	100.5	100.4	100.4
γ (°)	90.0	90.0	90.0	90.0	90.0	90.0
Wavelength (Å)	1.00003	0.97918	0.97918	1.00003	1.00003	1.00003
Resolution (Å)	50 - 1.86	50 - 2.00	50 - 2.10	50 - 2.15	50 - 2.20	50 - 2.03
No. reflections	107699	85779	75984	69735	65231	84218
% completeness	98.2	97.8	99.9	99.0	99.1	98.9
(last shell)	(84.4)	(97.2)	(99.8)	(92.6)	(95.7)	(90.0)
Rmerge	0.075	0.062	0.061	0.116	0.092	0.085
(last shell)	(0.484)	(0.574)	(0.464)	(0.572)	(0.601)	(0.425)
I/ σ	18.6	19.6	20.1	16.3	22.8	21.4
(last shell)	(1.9)	(2.4)	(3.1)	(2.3)	(2.6)	(3.6)
Redundancy	4.3	3.7	3.7	6.5	7.1	7.4
(last shell)	(2.4)	(3.7)	(3.8)	(4.4)	(4.8)	(5.5)
Refinement						
Resolution (Å)	50 - 1.86	50 - 2.00	50 - 2.10	50 - 2.15	50 - 2.20	50 - 2.03
Rwork / Rfree	0.154/0.229	0.140/0.223	0.135/0.213	0.143/0.231	0.142/0.245	0.141/0.216
No. Atom						
Protein	7241	7241	7241	7241	7241	7241
Carbohydrate	322	322	322	322	322	322
Ligand	12	9	5	9	9	9
Ion	1	1	1	1	1	1
Solvent	1533	1633	1537	1497	1566	1457
RMS Deviations						
Bond length (Å)	0.006	0.005	0.005	0.006	0.006	0.006
Bond angle (°)	0.995	0.964	0.973	1.062	1.076	0.990
B factor	30.5	31.0	29.1	36.8	34.1	31.3
Ramachandran plot:						
core,	96.4%,	96.4%,	96.9%,	96.3%,	95.3%,	96%,
allow,	2.9%,	2.9%,	2.4%,	2.9%,	3.9%,	3.2%,
disallow	0.7%	0.7%	0.7%	0.8%	0.8%	0.8%

Data were collected at APS beamline 24-ID and ALS beamline 4.2.2.

$R_{merge} = \Sigma_{i,h} | I_{i,h} - \langle I_h \rangle | / \Sigma_{i,h} | I_{i,h} |$ where $\langle I_h \rangle$ is the mean of the i observations of the reflection h.

$R_{work} = \Sigma||F_o| - |F_c|| / \Sigma|F_o|$. R_{free} is the same statistic, but calculated from a subset of the data (5%) that has not been used in refinement.

Supplemental Figure 5: Statistics of the X-ray crystallographic data.