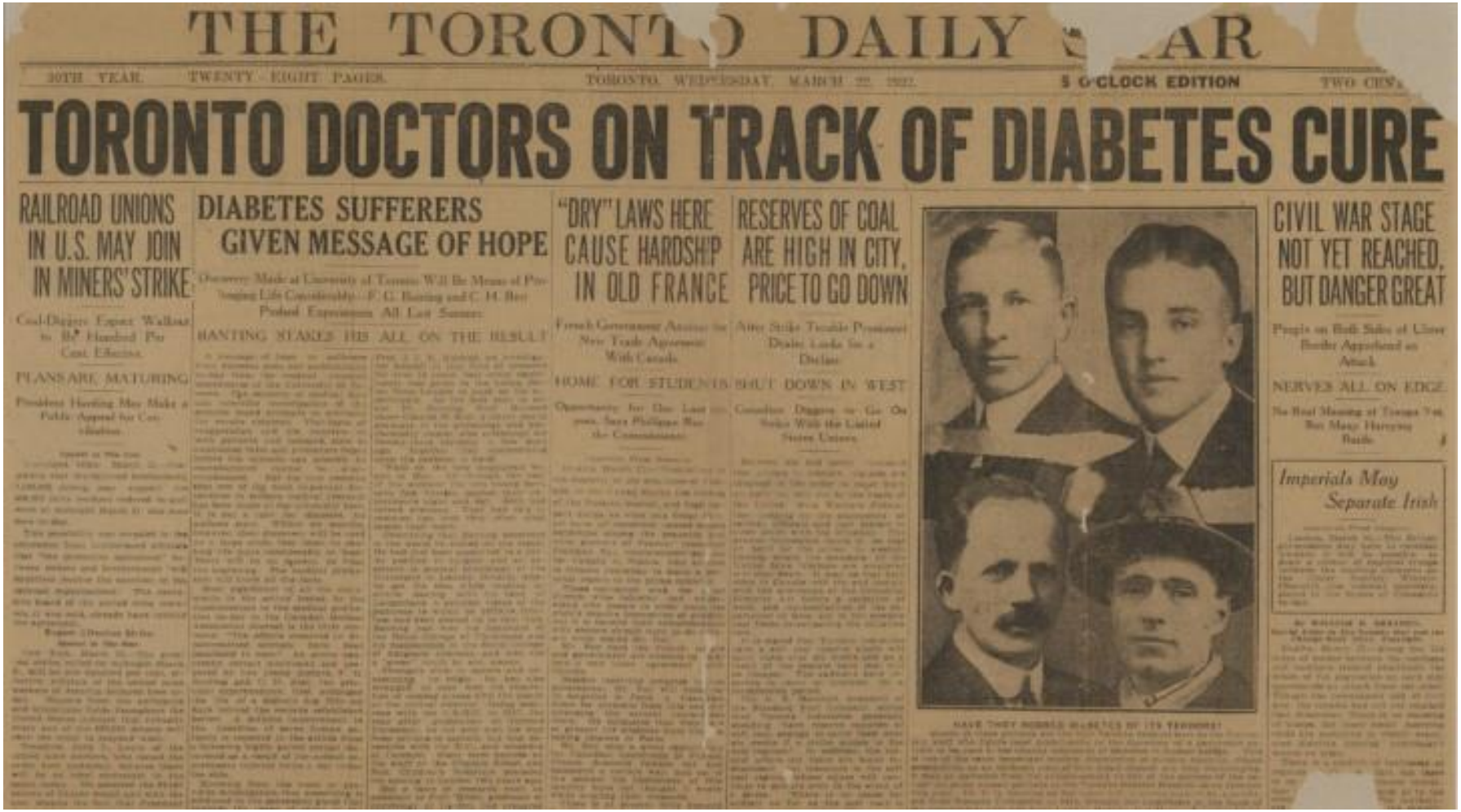
Use of Computational modeling and Continuous-Flow Solid-Phase Peptide Synthesis for the design and synthesis of peptide ligands targeting HLA and Hsp90

Ezequiel Silva Nigenda[1], Olena Tykhoniuk[1], Usman Mirza[1], Sarfraz Ahmad[1] and John F. Trant[1,*]

[1] Department of Chemistry and Biochemistry, University of Windsor. Windsor, On, Canada.

* Corresponding author: j.trant@uwindsor.ca

Therapeutic peptides: Where are we standing?



THE TORONTO DAILY STAR

30TH YEAR. TWENTY EIGHT PAGES. TORONTO, WEDNESDAY, MARCH 22, 1922. 5 O'CLOCK EDITION TWO CENTS

TORONTO DOCTORS ON TRACK OF DIABETES CURE

RAILROAD UNIONS IN U.S. MAY JOIN IN MINERS' STRIKE

Coal-Diggers Expect Walkout to Be Hundred Per Cent. Effective.

PLANS ARE MATURING

President Harding May Make a Public Appeal for Coal Abstinence

DIABETES SUFFERERS GIVEN MESSAGE OF HOPE

Discovery Made at University of Toronto Will Be Means of Prolonging Life Considerably—F. G. Banting and C. H. Best Probed Experiments All Last Summer.

BANTING STAKES HIS ALL ON THE RESULT

"DRY" LAWS HERE CAUSE HARDSHIP IN OLD FRANCE

French Government Anxious for New Trade Agreement With Canada.

HOME FOR STUDENTS SHUT DOWN IN WEST

Opportunity for One Last Year, Says Philippe Roy the Commissioner.

RESERVES OF COAL ARE HIGH IN CITY, PRICE TO GO DOWN

After Strike Trouble Promoters Dealer Looks for a Decline.

Canadian Diggers to Go On Strike With the United States Miners.

HAVE THEY ROBBED DIABETES OF ITS TERRORS?

CIVIL WAR STAGE NOT YET REACHED, BUT DANGER GREAT

People on Both Sides of Ulster Border Apprehend an Attack.

NERVES ALL ON EDGE

No Real Meaning of Troops Yet, But Many Harrowing Battle

Imperials May Separate Irish

Wang, L., Wang, N., Zhang, W., Cheng, X., Yan, Z., Shao, G., Wang, X., Wang, R., & Fu, C. (2022). Therapeutic peptides: current applications and future directions. In *Signal Transduction and Targeted Therapy* (Vol. 7, Issue 1). Springer Nature. https://doi.org/10.1038/s41392-022-00904-4

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The Nobel Prize in Chemistry 2024

Rosetta Commons

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Demis Hassabis

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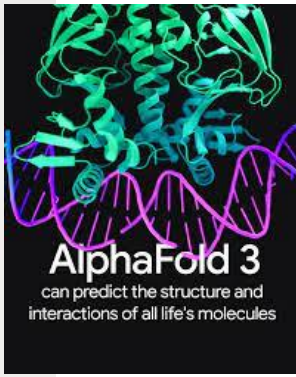
John Jumper



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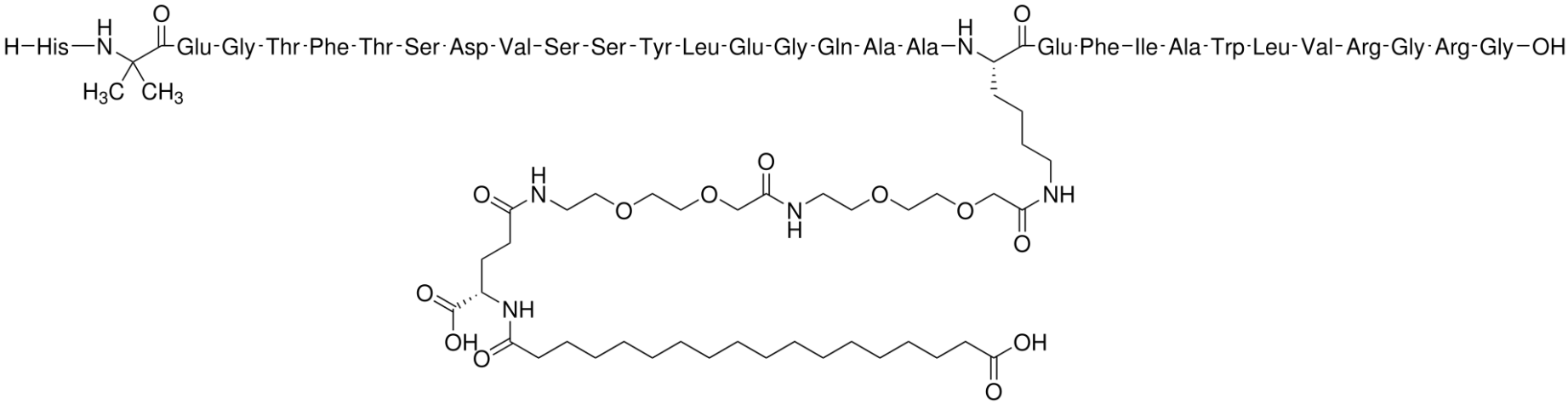
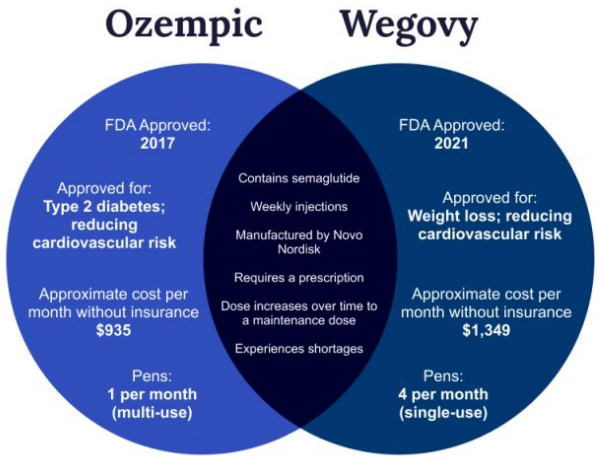
Target name	Peptide name	First approval	Approved indication(s)
GLP-1 receptor	Exenatide[462]	2005	Indicated for Type 2 Diabetes Mellitus
	Liraglutide[463]	2009	
	Lixisenatide[464]	2013	
	Albiglutide[465]	2014	
	Dulaglutide[466]	2014	
	Semaglutide[467]	2017	





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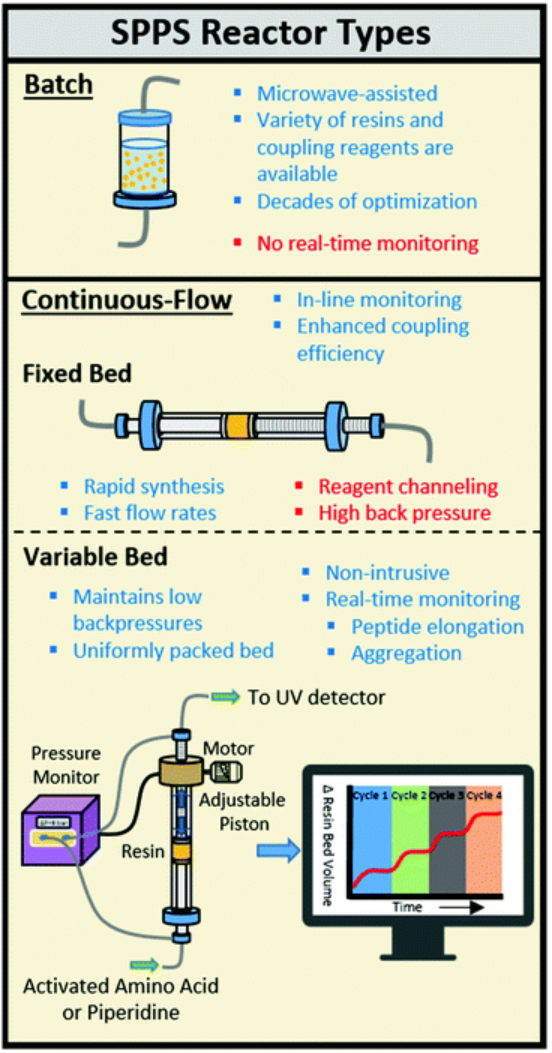
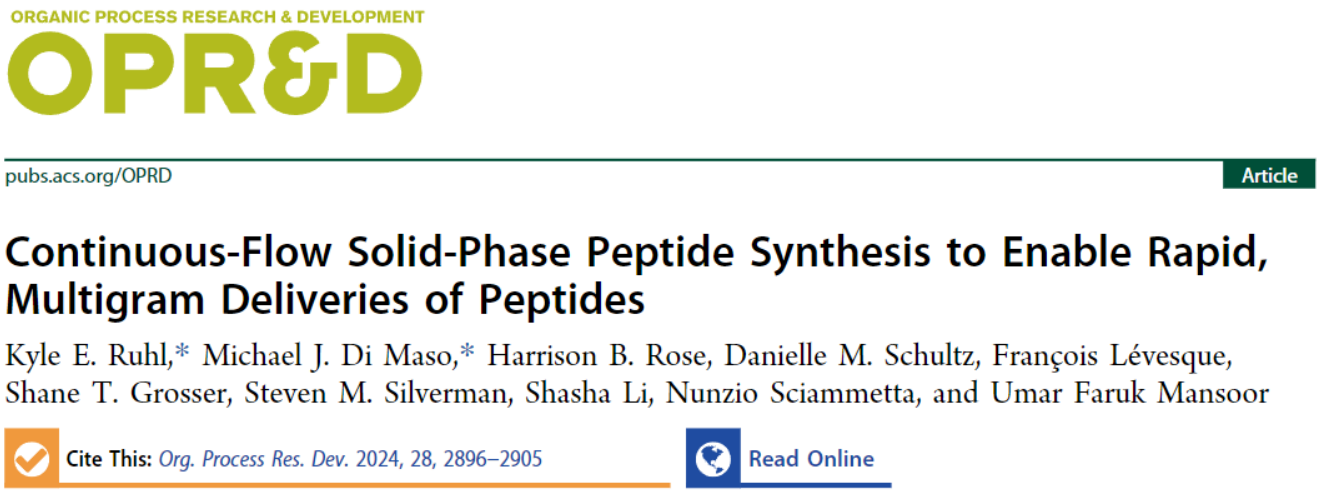
Batch vs Continuous Flow SPPS



Problems with conventional batch mode SPPS:

- Atom economy (Use of huge excess of starting materials to avoid inefficient couplings)
- Use of DMF or DCM as a solvents with high waste production.
- Aggregation when peptide chain gets over 70 a.a.



Sletten, E. T.; Nuño, M.; Guthrie, D.; Seeberger, P. H. Real-Time Monitoring of Solid-Phase Peptide Synthesis Using a Variable Bed Flow Reactor. *Chem. Commun.* **2019**, *55* (97), 14598–14601. / Ruhl, K. E.; Schultz, D. M.; Lévesque, F.; Grosser, S. T.; Mansoor, U. F. Continuous-Flow Solid-Phase Peptide Synthesis to Enable Rapid, Multigram Deliveries of Peptides. *Org. Process Res. Dev.* **2024**.

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In Silico peptide-drug design

Contents lists available at ScienceDirect

Journal of Pharmaceutical Analysis

journal homepage: www.elsevier.com/locate/jpa

Original article

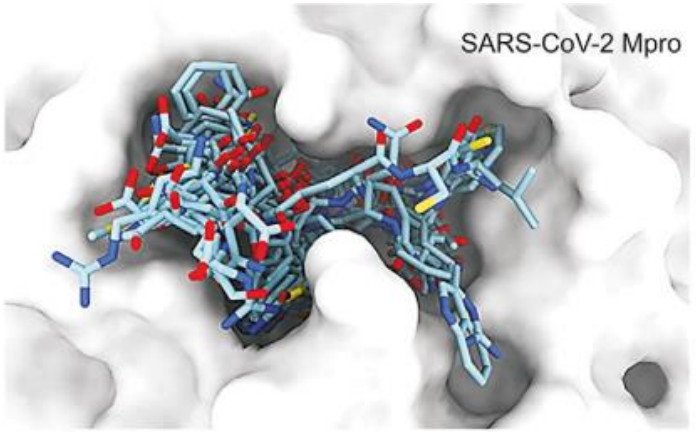
Dock-able linear and homodetic di, tri, tetra and pentapeptide library from canonical amino acids: SARS-CoV-2 Mpro as a case study

Sarfraz Ahmad [a, b], Muhammad Usman Mirza [a, b, *], John F. Trant [a, b, **]

[a] Department of Chemistry and Biochemistry, University of Windsor, Windsor N9B 3P4, Ontario, Canada

[b] Binary Star Research Services, LaSalle N9J 3X8, Ontario, Canada

Form	Dipeptide	Tripeptide	Tetrapeptide	Pentapeptide	Total
Linear	400	8,000	160,000	3,200,000	3,368,400
Linear filtered	(400)	(8,000)	(160,000)	(1,169,013)	(1,337,413)
Cyclic	210	2,680	40,110	640,016	683,016
Total	610	10,680	200,110	3,840,016 (1,809,029 filtered)	4,051,416 (2,020,429 filtered)



Ahmad, S., Mirza, M. U., & Trant, J. F. Dock-able linear and homodetic di, tri, tetra and pentapeptide library from canonical amino acids: SARS-CoV-2 Mpro as a case study. *Journal of Pharmaceutical Analysis*,. **2023,** *13*(5), 523–534.

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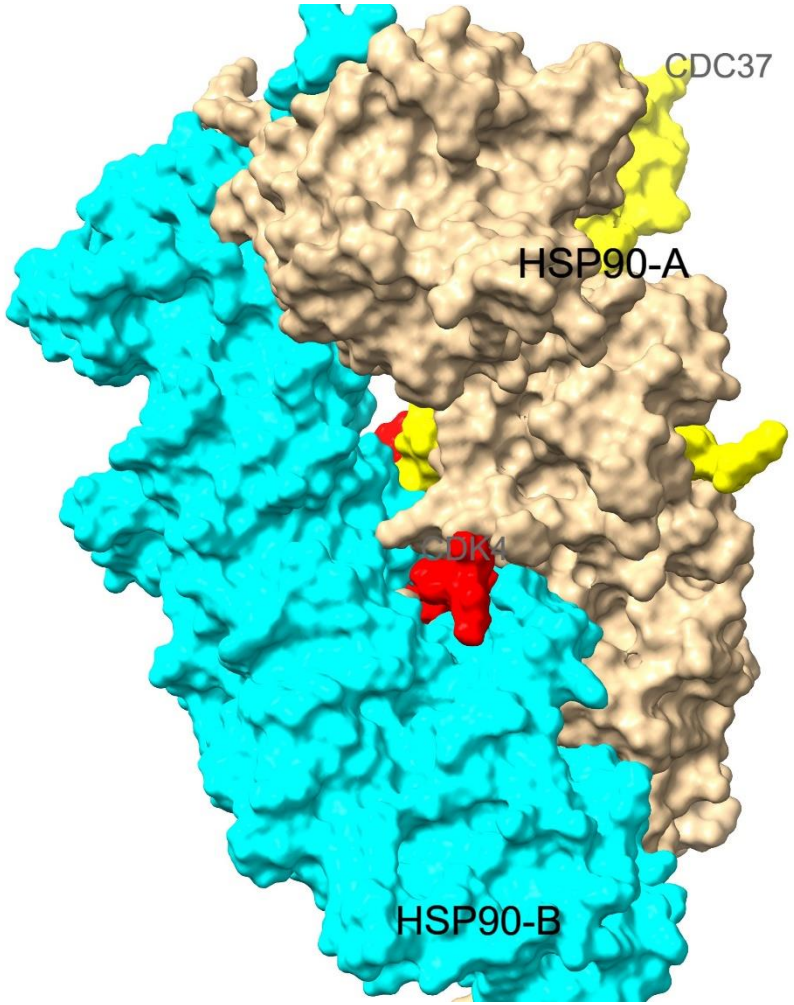
Hsp90

Heat Shock Proteins are a set of conserved proteins classified depending upon their molecular weights (Hsp100, Hsp90, Hsp70, Hsp60 and small Hsps).



- Most Hsp are molecular chaperons that are in charge of ***proper folding*** of polypeptides or ***misfolded*** proteins, ***degrade mutant proteins*** and ***repair DNA***.
- The most abundant Hsp is Hsp90, which interacts with more than 400 substrate proteins, half of which are believed to be involved in ***cancer***.

Jackson, S.E. Hsp90: Structure and Function. In: Jackson, S. (eds) Molecular Chaperones. Topics in Current Chemistry, vol 328. **2012.** Springer, Berlin, Heidelberg.

HLA-DR4

The Human Leukocyte Antigen DR4 was first associated with a higher risk of developing Rheumatoid Arthritis in the late 70´s.

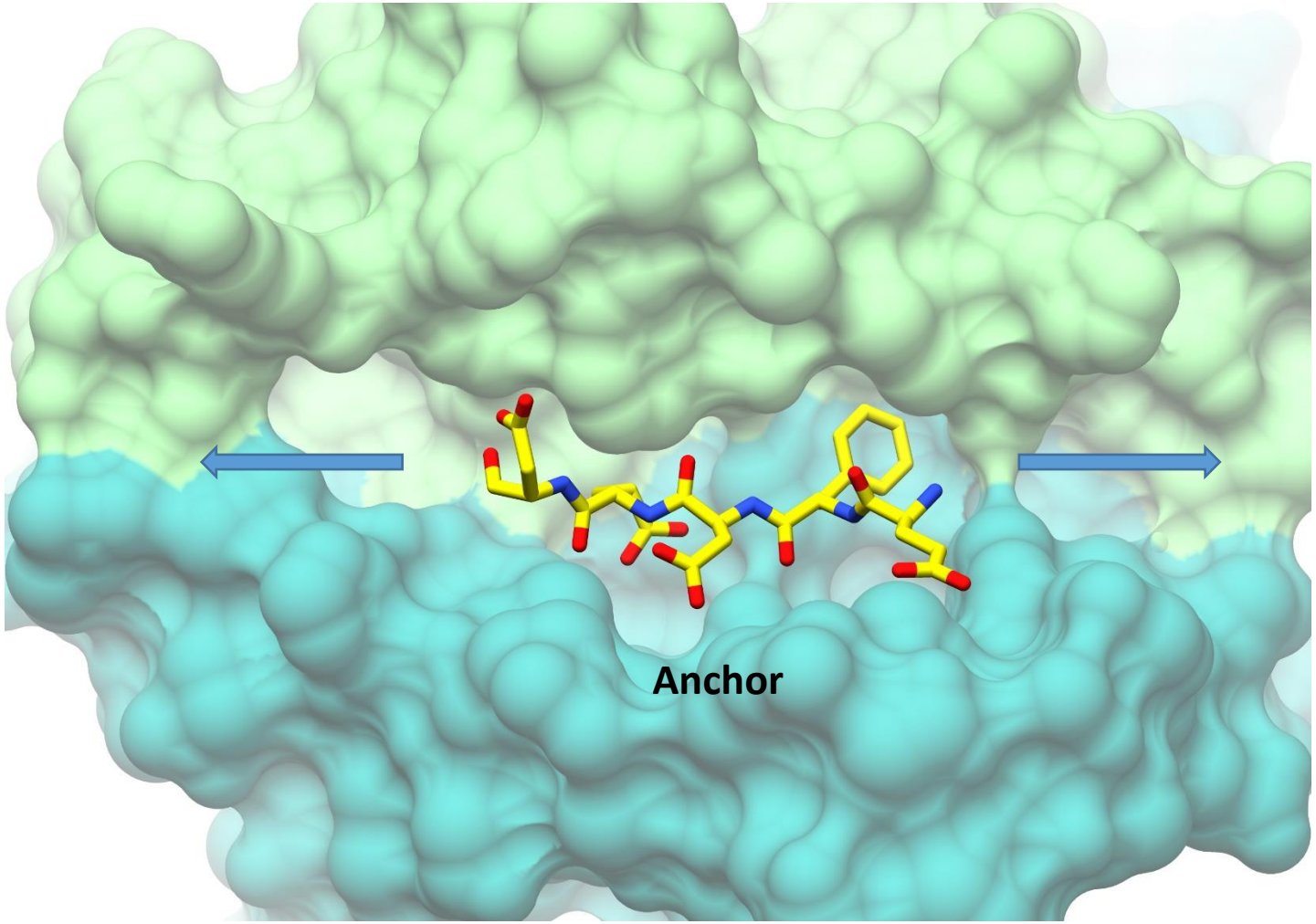
This type of Major Histocompatibility II complexes work as surface receptors whose function is to bound to pathogen antigens and present them as recognizable structures to T-cells, thus initiating the immune response to a potential infection.

From time to time, upregulation caused by extra coding information, can lead to the recognition of self peptides (like the ones present in the connective tissue of the joints) which can lead to an “auto” attack from the immune system.

Stastny, P.; Ball, E. J.; Khan, M. A.; Olsen, N. J.; Pincus, T.; Gao, X. Hla-Dr4 and Other Genetic Markers in Rheumatoid Arthritis. *Rheumatol.* **1988**, *27* (suppl II), 132–138. Fugger, L.; Svejgaard, A. Association of MHC and Rheumatoid Arthritis HLA-DR4

Rheumatoid Arthritis: Studies in Mice and Men. *Arthritis Res.* **2000**, *2* (3), 208–211.

HLA blocker design:



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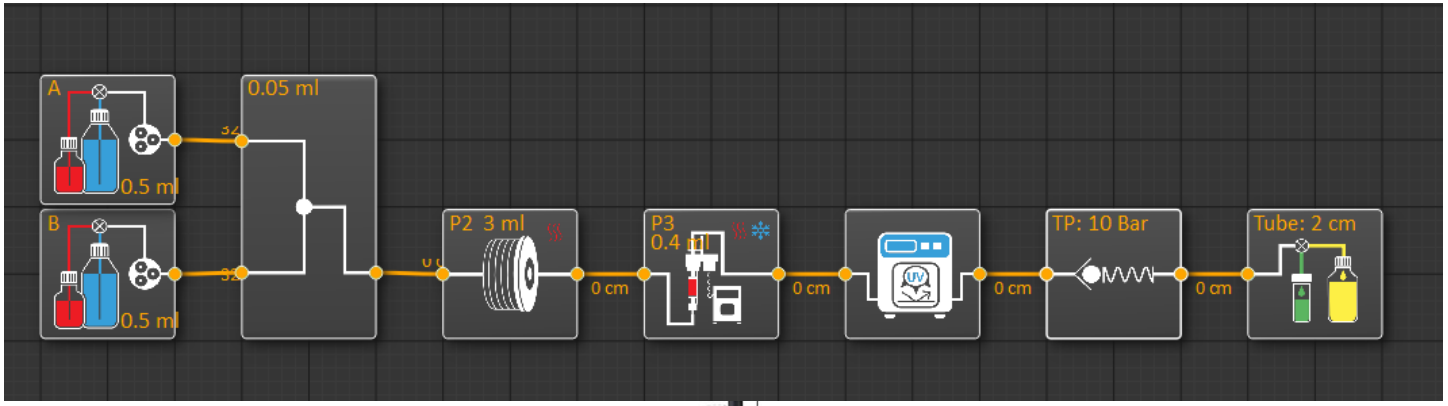
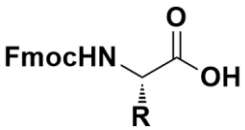
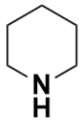
VBFR CF-SPPS

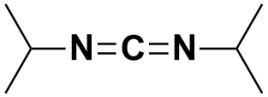




Fmoc-a.a-COOH



Piperidine



Dilsopropyl Carbodiimide

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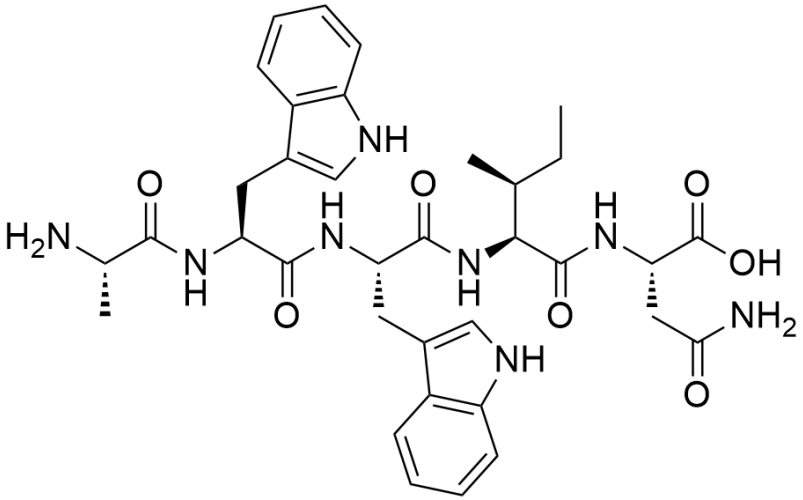
Hsp90 ligands:

TrantTeam_ID	Sequence	M.W.	LC 214nm	LC 250nm	LR-MS
TTSA-0001	AWWIN	688.7840	83.1%	82.3%	687.8
TTSA-0002	WQWWE	833.9014	100.0%	100.0%	646.9
TTSA-0003	WWWQY	867.9619	81.7%	75.4%	867.1
TTSA-0004	WYWCY	819.9386	81.9%	73.9%	819.0
TTSA-0005	SWRYY	773.8463	88.2%	84.4%	1550
TTSA-0006	WYWWS	826.9093	91.5%	91.6%	826.1
TTSA-0007	QWMPY	723.8506	55.4%	56.7%	723.1
TTSA-0008	CWWDN	722.7791	75.9%	82.0%	722.0
TTSA-0009	FTWWG	695.7755	67.5%	76.1%	696.0
TTSA-0010	WRWRY	865.9930	69.6%	81.2%	865.3
TTSA-0011	WFYGW	757.8464	84.7%	80.8%	758.0
TTSA-0012	NTWWW	791.8641	71.5%	75.8%	792.4
TTSA-0013	WWNWI	803.9185	72.9%	70.0%	804.5
TTSA-0014	AWWYW	810.9099	76.2%	71.8%	811.4
TTSA-0015	FWCWN	754.8671	85.4%	87.3%	755.2
TTSA-0016	QWYWQ	809.8793	42.1/57.9%	44.4/55.6%	
TTSA-0017	WWQWW	890.9992	77.8%	71.9%	891.6
TTSA-0018	IYWWE	795.8928	75.9%	72.7%	796.4
TTSA-0019	WSWQD	720.7394	84.10%	84.98%	721.1
TTSA-0020	WMWWTG	866.0108	43.7%	37.4%	867.5

TTSA-0001



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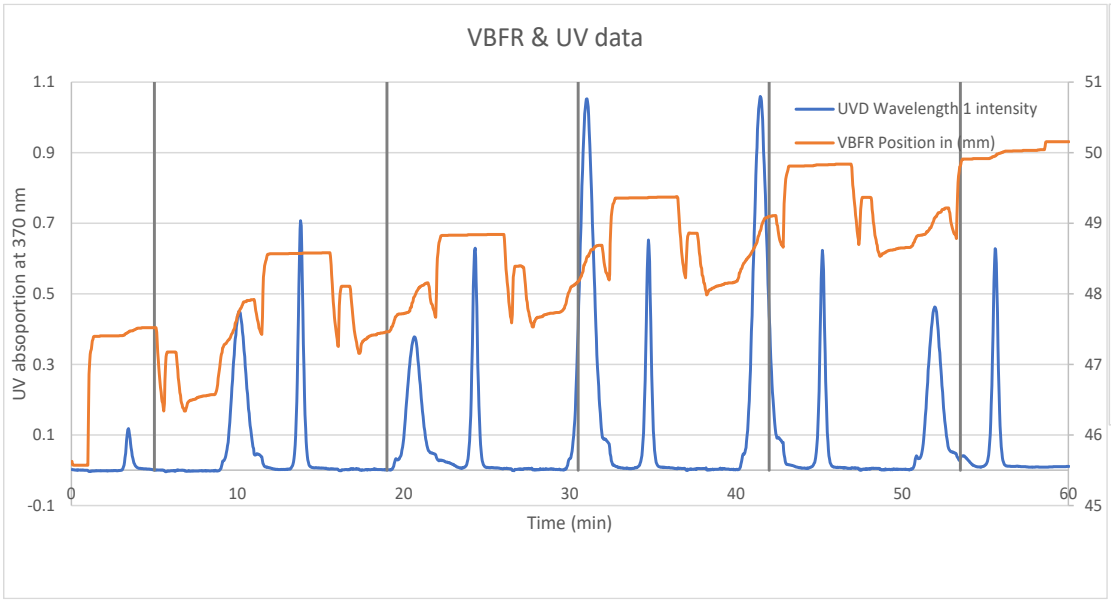

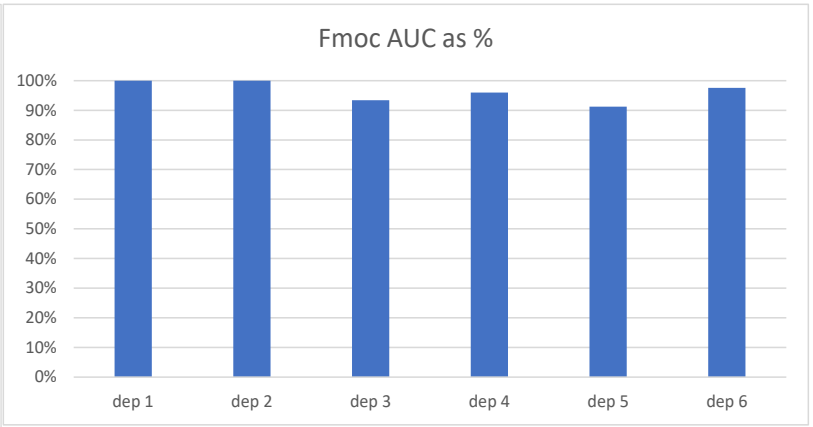

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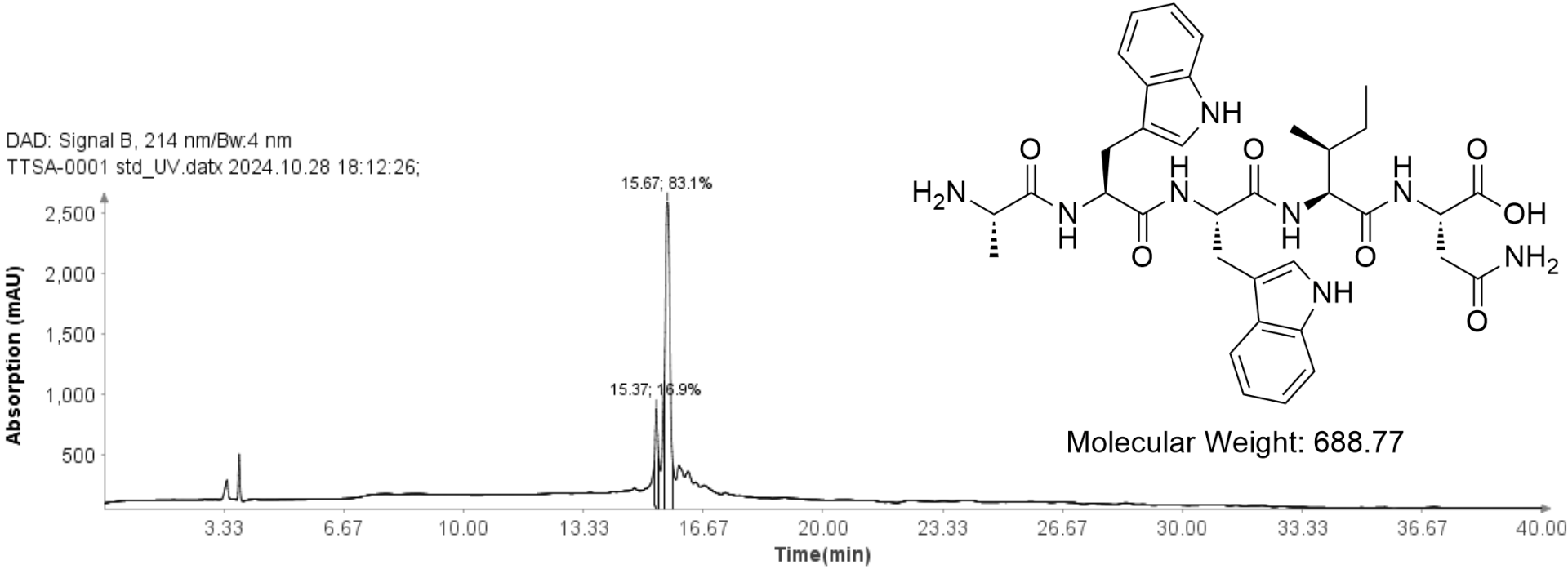

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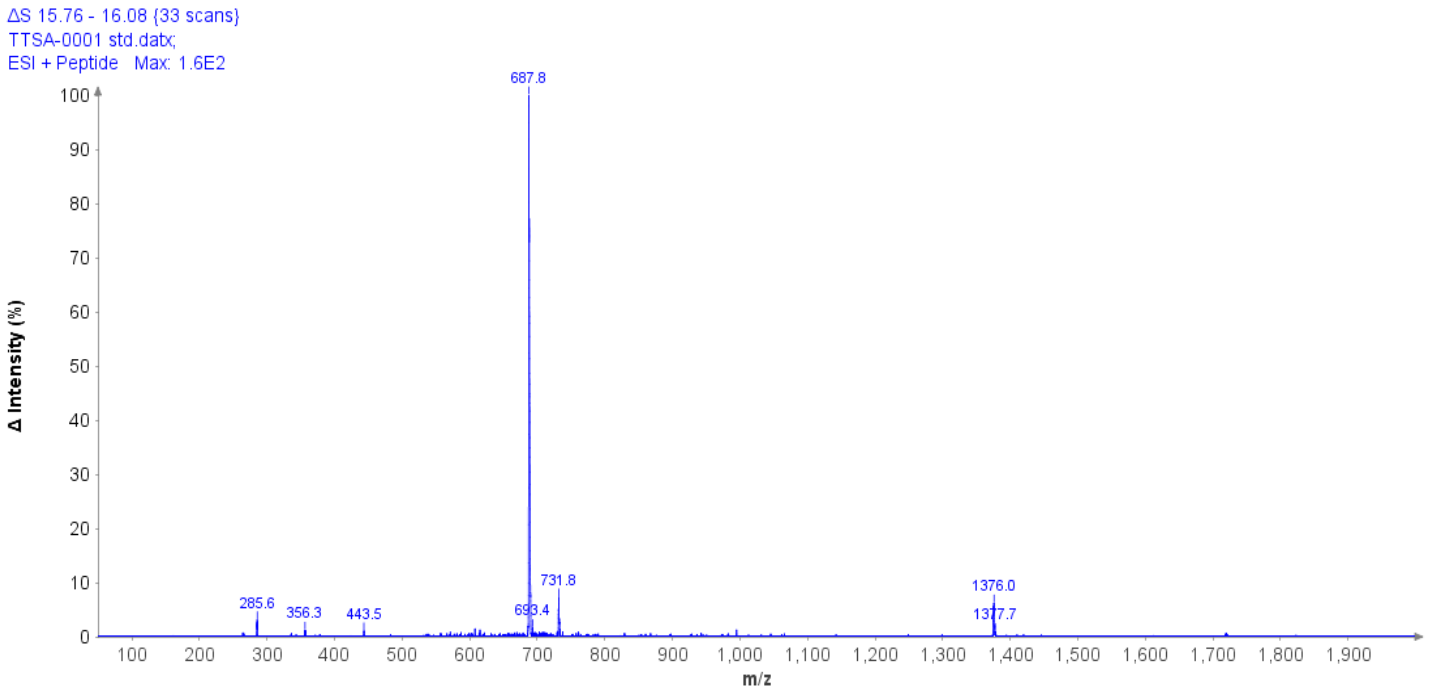

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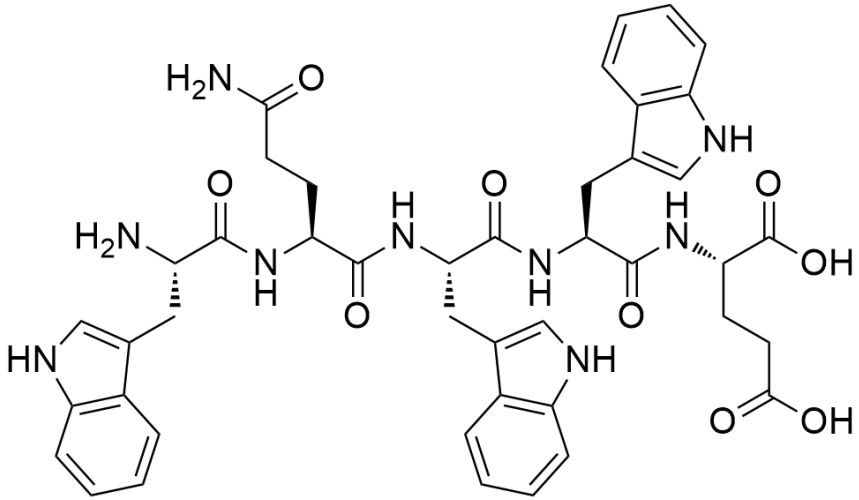
TTSA-0002



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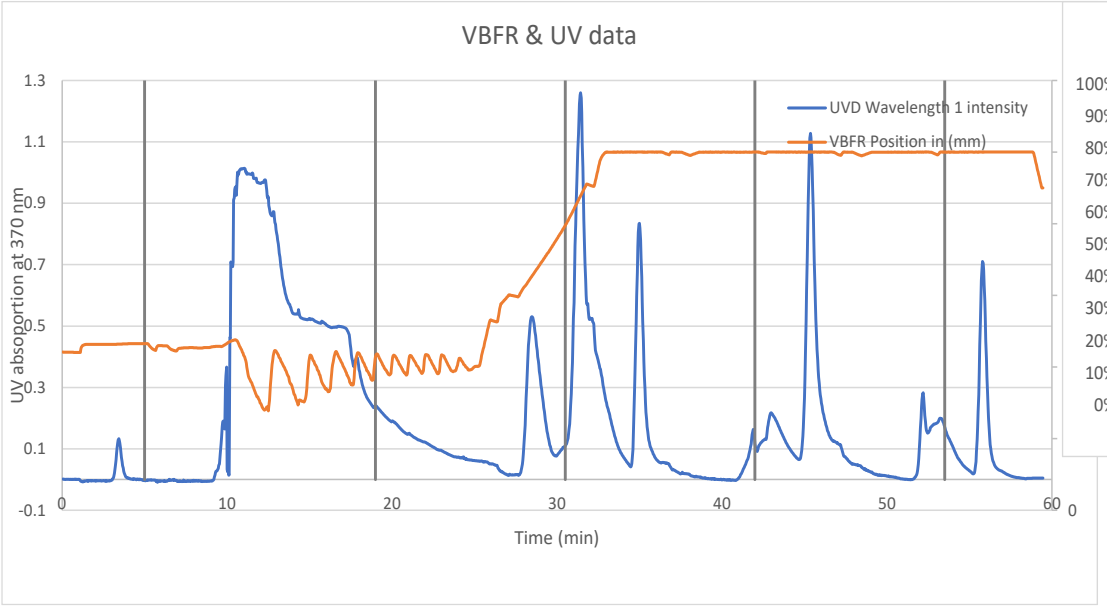

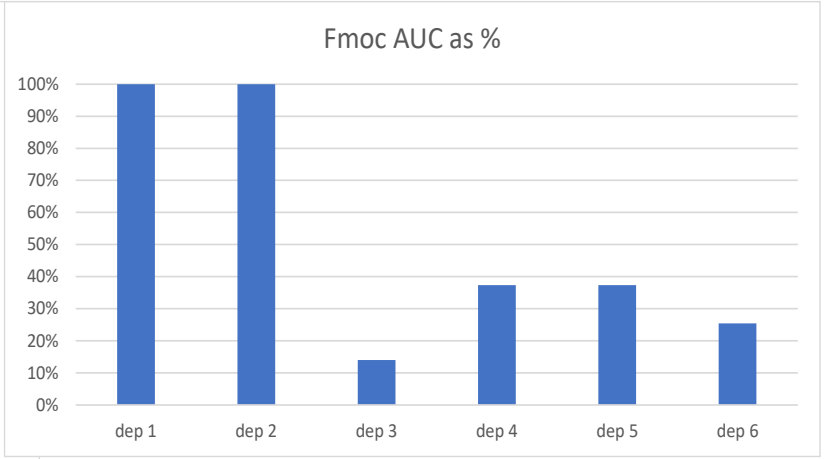

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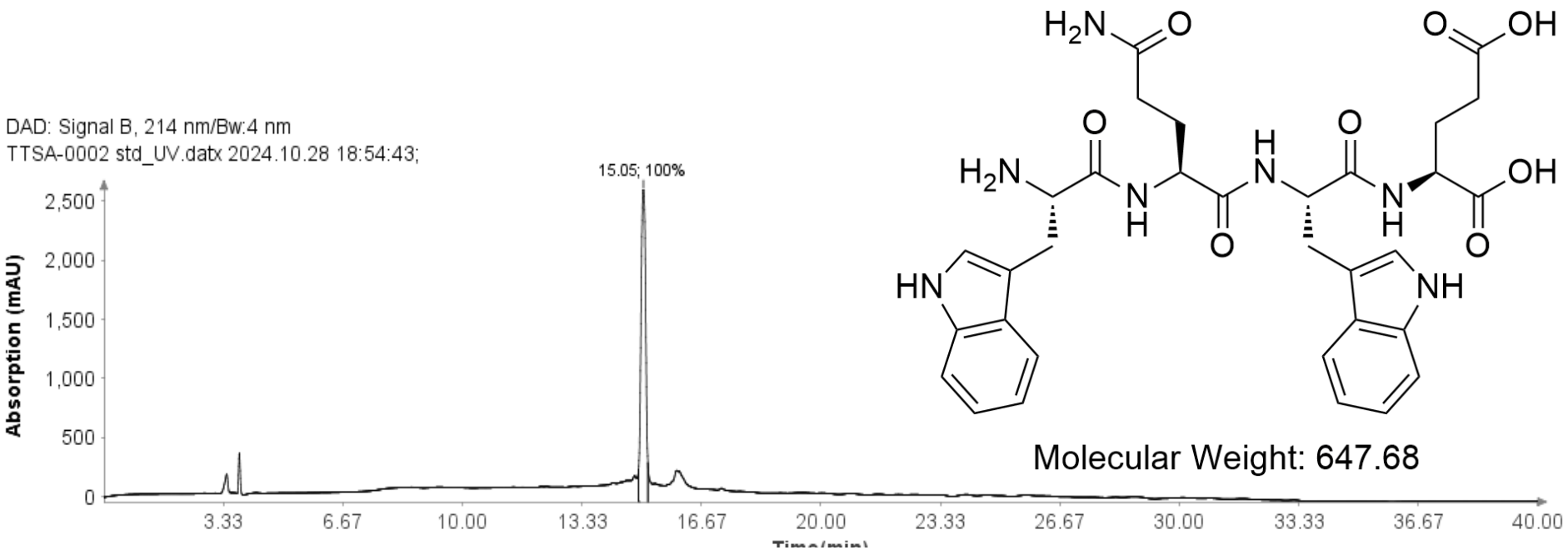
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Absorption (mAU)
2,500
2,000
1,500
1,000
500
0
3.33
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10.00
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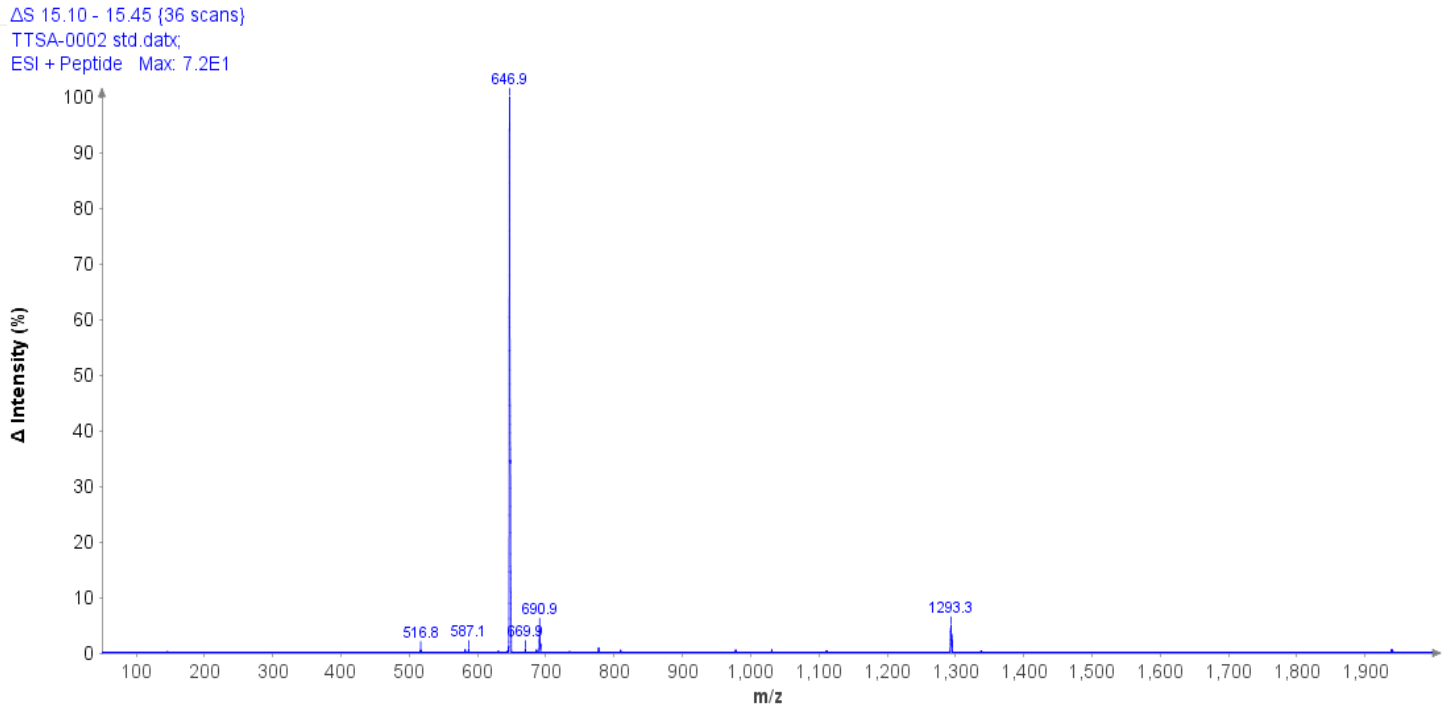
16.67
20.00
23.33
26.67
30.00
33.33
36.67
40.00
Molecular Weight: 647.68


ΔS 15.10 - 15.45 {36 scans}
TTSA-0002 std.datx;
ESI + Peptide Max: 7.2E1
Δ Intensity (%)
646.9
690.9
516.8
587.1
669.9
1293.3
m/z

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HLA blockers:

TrantTeam_IDs	Sequence	M.W.	LC 214nm	LC 250nm	LR-MS
TTM006	WIAEWDEELRG	1403.5143	64.5%	71.3%	1404.3
TTM017	YWVEWDYEFRG	1549.6622	54.1%	53.2%	1550.1
TTM041	YHVEWDEDWWG	1521.5651	32.7%	39.7%	1522.1
TTM069	WFVDWDEDHQG	1433.4562	84.7%	100.0%	1434.0
TTM089	YHVEFDEDFKG	1385.4525	63.3%	68.7%	1386.2
TTM108	WYVEYDEDWRG	1517.5742	53.2%	63.2%	1518.5
TTM175	WYVEFDEDFKG	1434.5247	64.2%	66.5%	1435.8
TTM314	YFVEFDEDFQG	1395.4445	100.0%	100.0%	1397.7
TTM405	YFVDWDEDHQG	1410.4189	100.0%	100.0%	1413.1
TTM667	YIAEWDYELRG	1414.5374	51.6%	59.3%	1416.2

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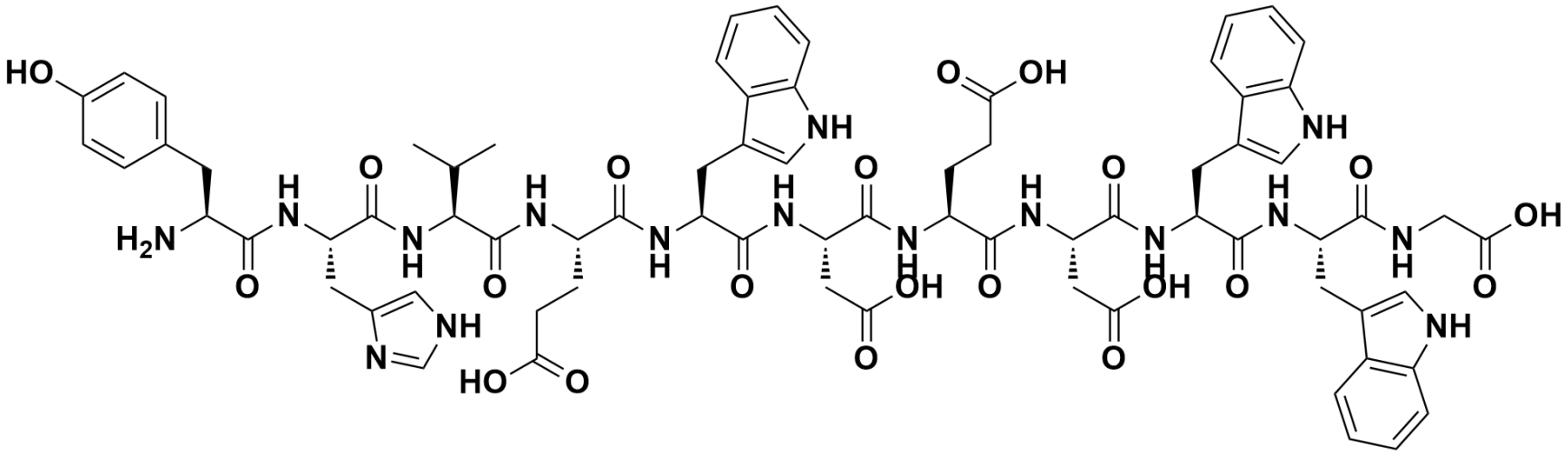
TTM-0041



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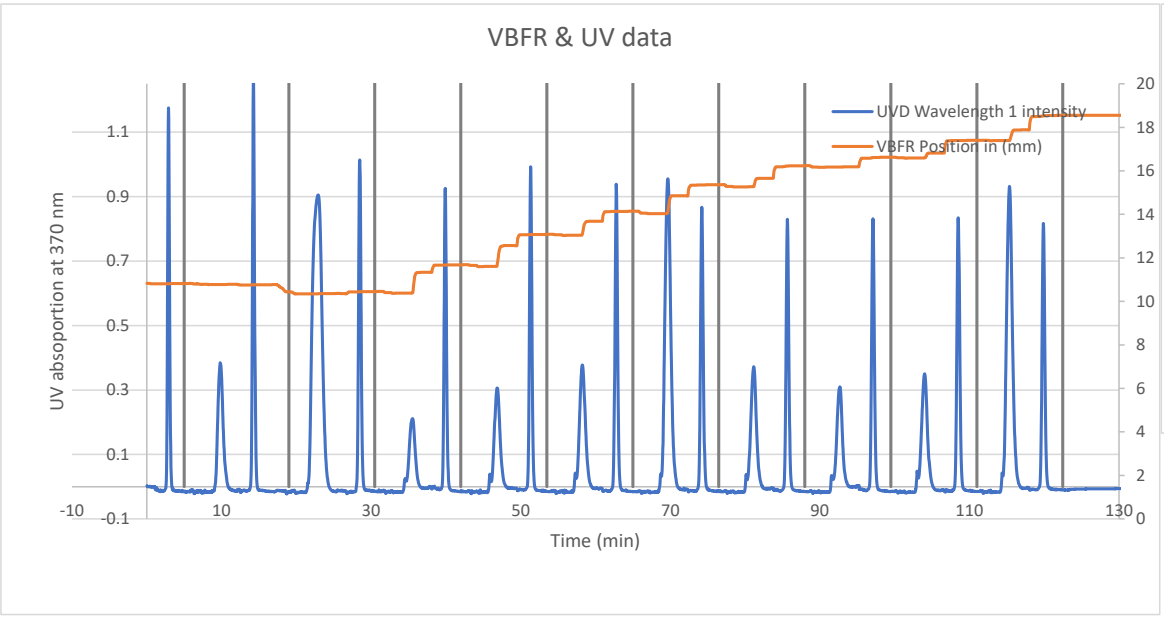

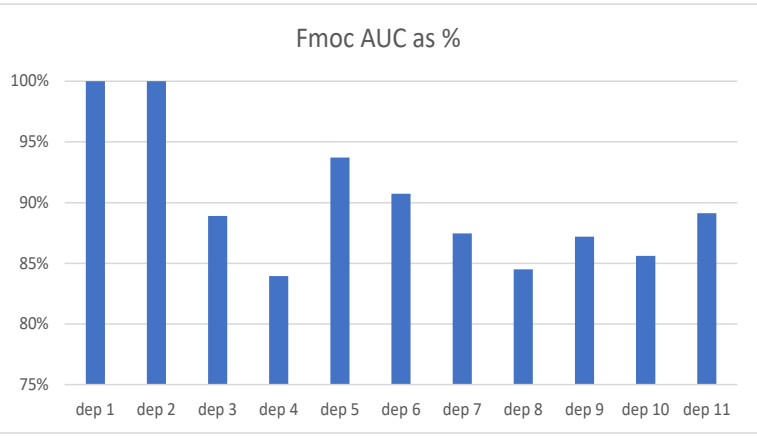

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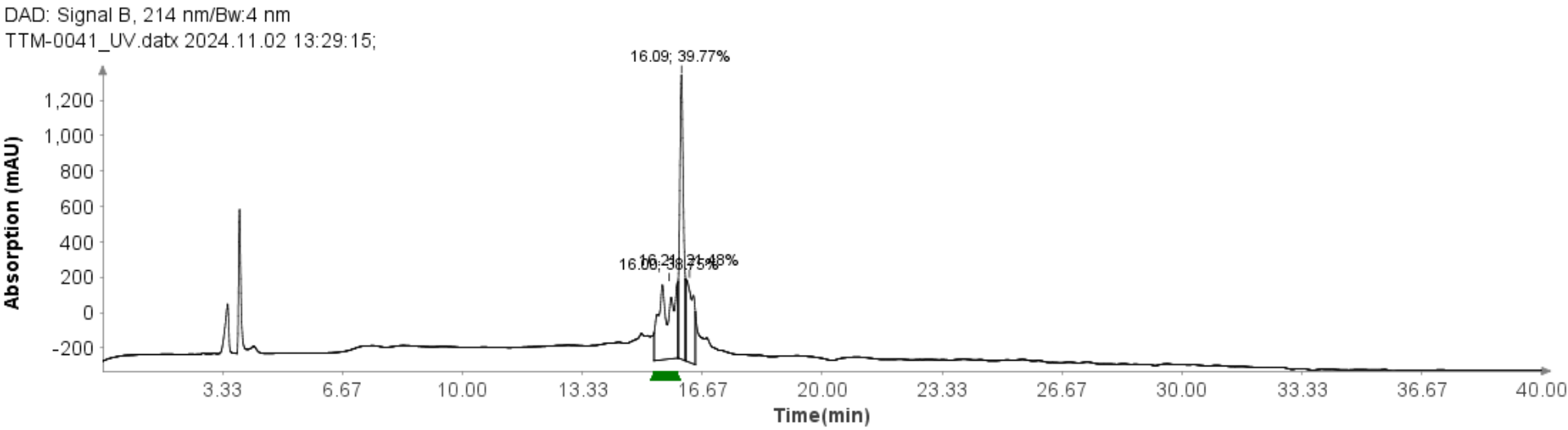
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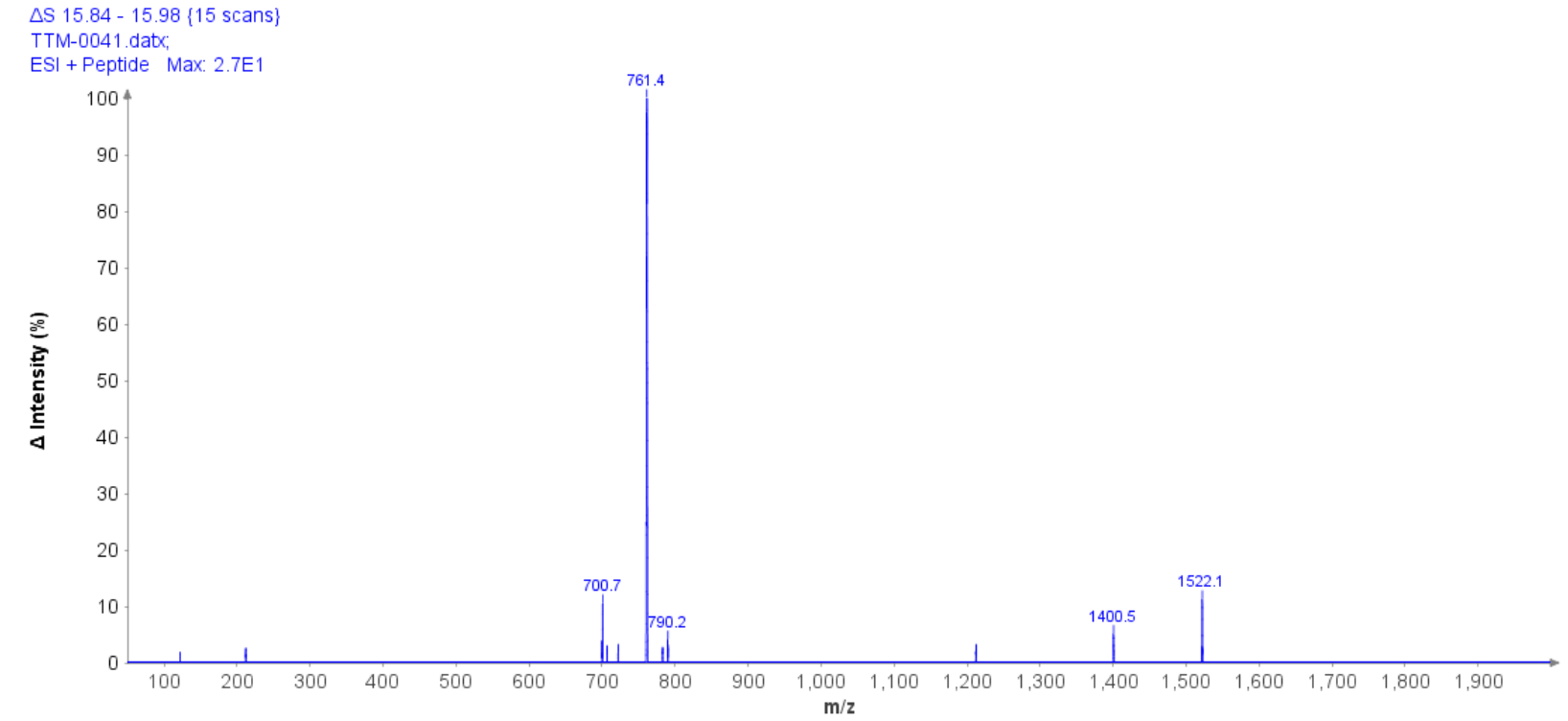
16.09; 39.77%
16.00; 38.75%
Absorption (mAU)
1,200
1,000
800
600
400
200
0
-200
3.33
6.67
10.00
13.33
16.67
20.00
23.33
26.67
30.00
33.33
36.67
40.00
Time(min)


ΔS 15.84 - 15.98 (15 scans)
TTM-0041.datx;
ESI + Peptide Max: 2.7E1
761.4
700.7
790.2
1400.5
1522.1
Δ Intensity (%)
100
90
80
70
60
50
40
30
20
10
0
100
200
300
400
500
600
700
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900
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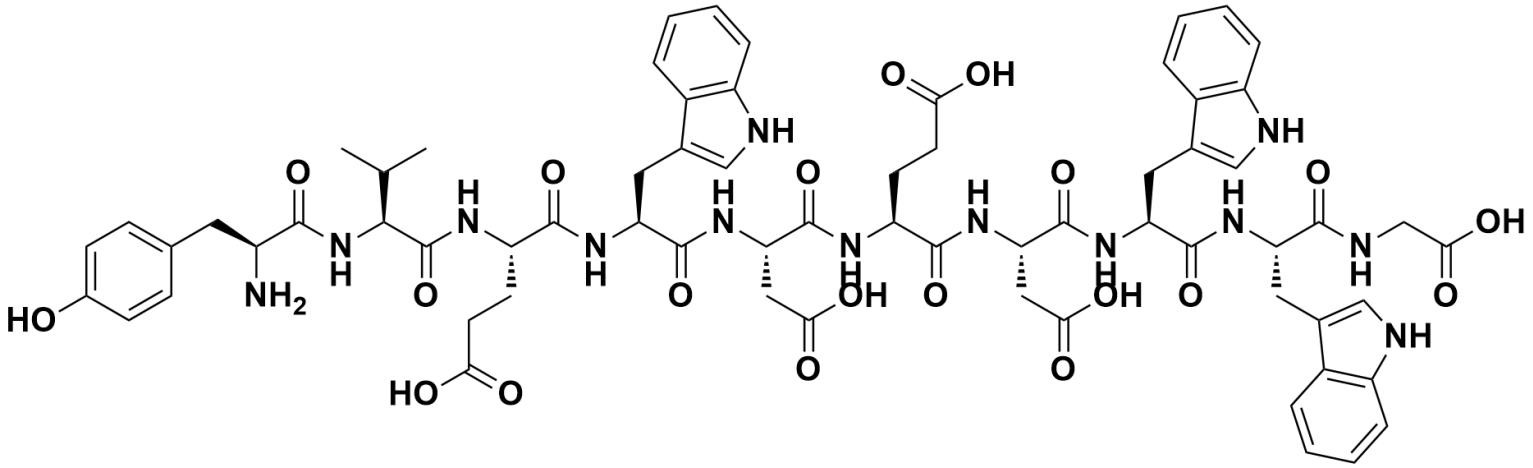
1,500
1,600
1,700
1,800
1,900
m/z

CPPC 2024



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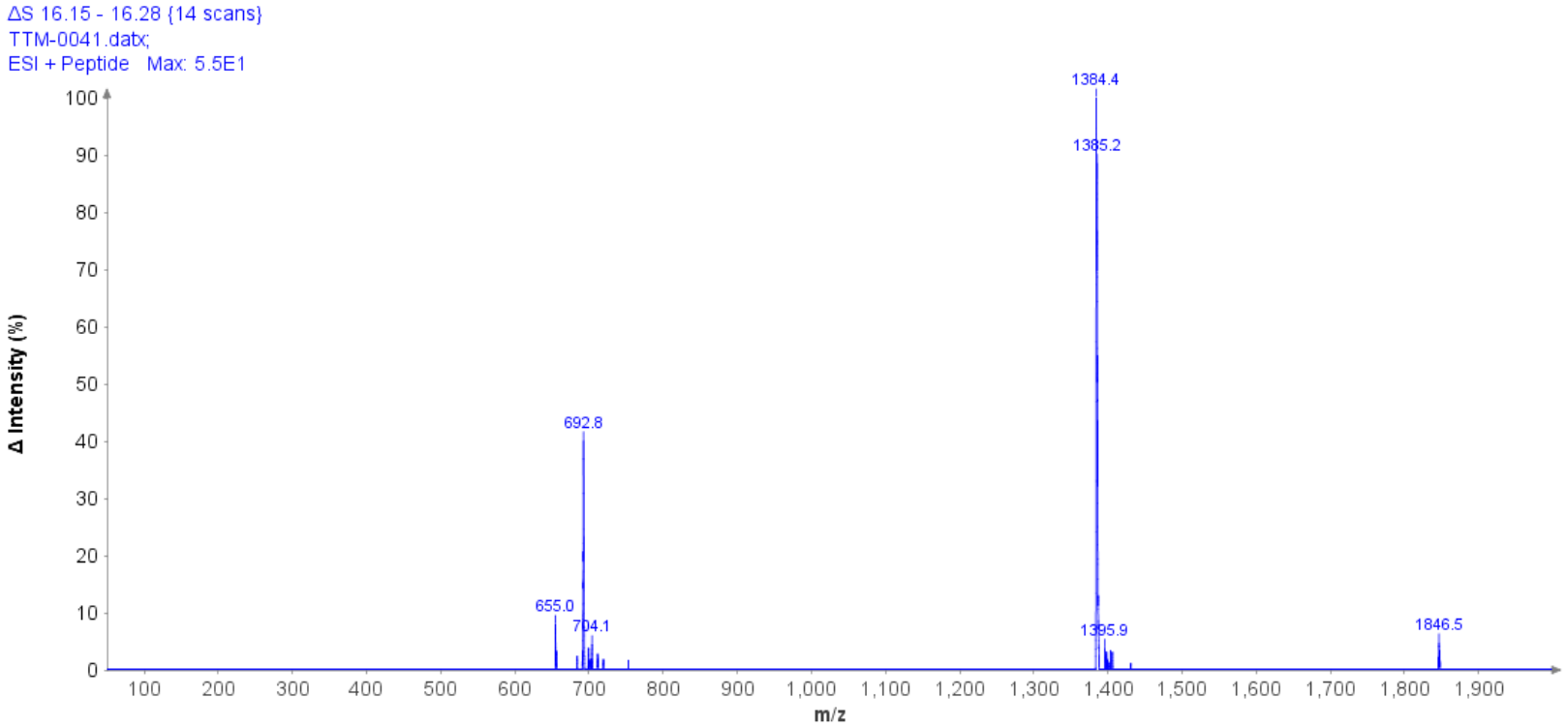
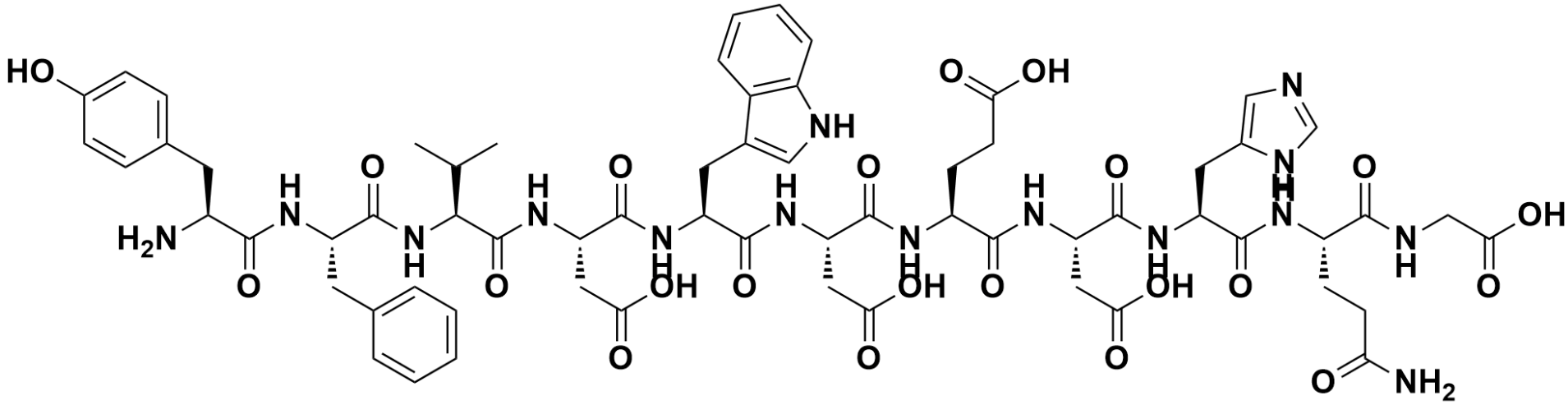
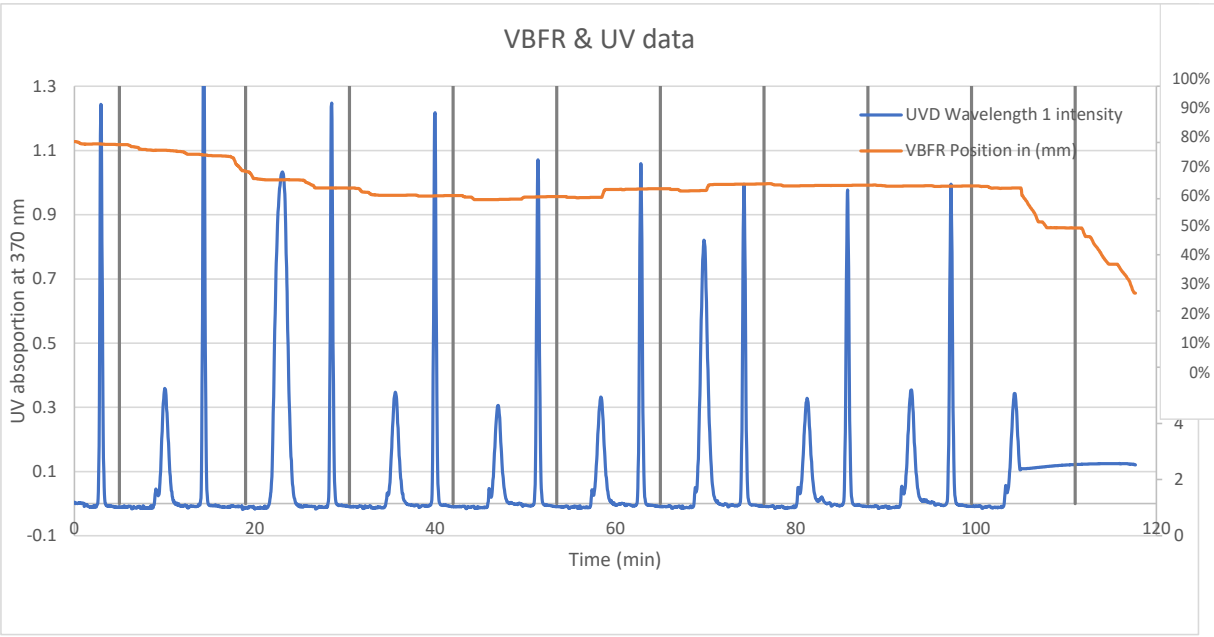


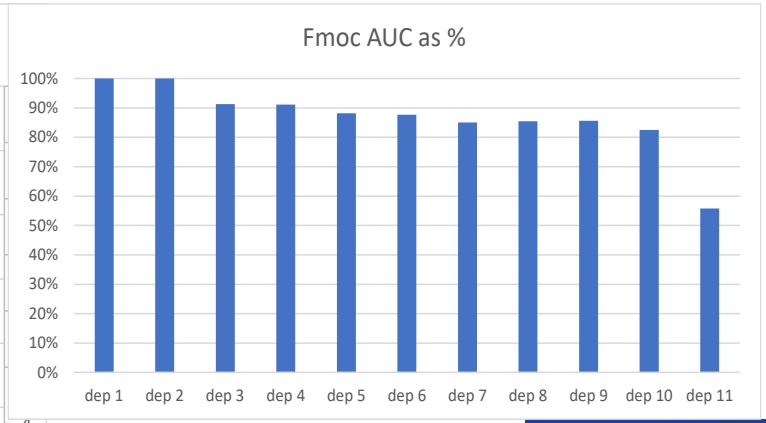
CPPC 2024

TTM-0405



Molecular Weight: 1410.40





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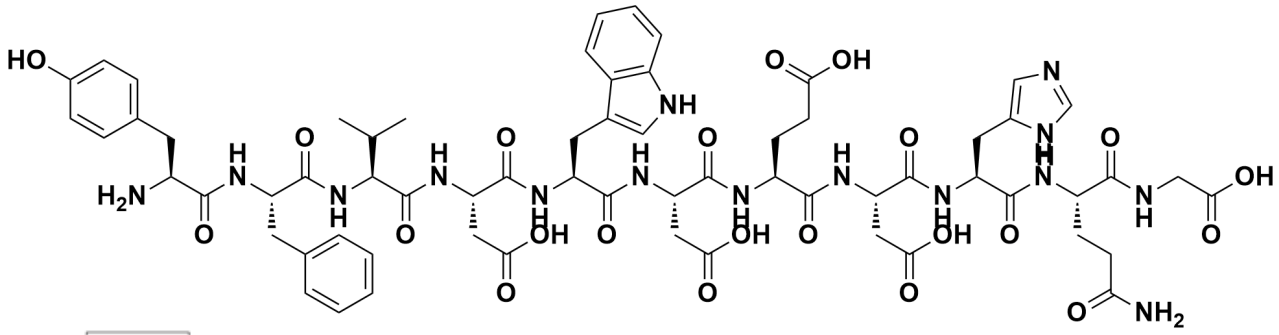

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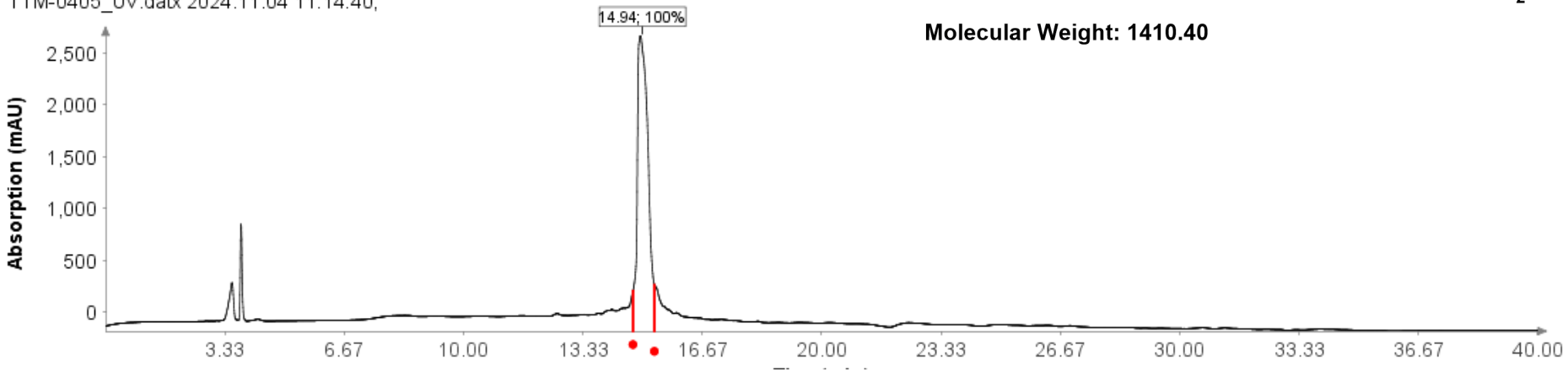
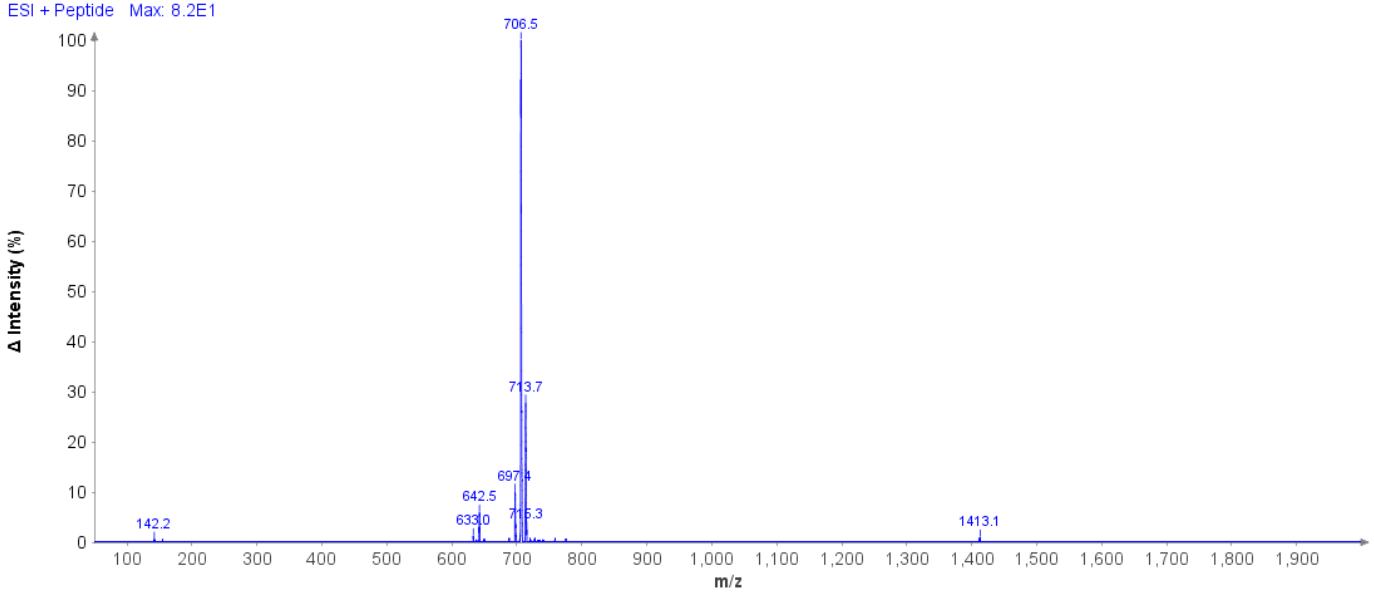
DAD: Signal B, 214 nm/Bw:4 nm
TTM-0405_UV.datx 2024.11.04 11:14:40;



ΔS 14.99 - 15.28 {30 scans}
TTM-0405.datx;
ESI + Peptide Max: 8.2E1



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Conclusions:

- We have created a library of di, tri, tetra and pentapeptides that is ready to use for computational purposes (docking)

- This library was used to create two different lists of candidates to target HLA-DR4 and Hsp90.

- 30 of these candidates were synthesized in a robust, efficient and quick fashion using CF-SPPS

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