Electronic Supplementary Material (ESI) for MedChemComm.

Electronic Supplementary Information

De Novo Design of Helical Peptides to Inhibit Tumor Necrosis Factor-α by Disrupting Its Trimer Formation

Qi Shen,[†a] Changsheng Zhang,[†b] Hongbo Liu,[b] Yuting Liu,[c] Junyue Cao,[d] Xiaolin Zhang,[a] Yuan Liang,[c] Meiping Zhao[c] and Luhua Lai[*abc]

[a] Center for Quantitative Biology, Peking University, Beijing 100871, China;
[b] BNLMS, State Key Laboratory for Structural Chemistry of Unstable and Unstable species, and Peking-Tsinghua Center for Life Sciences, Peking University, Beijing 100871, China;
[c] BNLMS, College of Chemistry and Molecular Engineering, Peking University, Beijing 100871, China;
[d] School of Life Sciences, Peking University, Beijing 100871, China;
[†] These authors contributed equally to this manuscript.

Correspondence should be addressed to Luhua Lai (lhlai@pku.edu.cn)

Content

Part I. Supplementary computational methods and results

Section 1: Target (TNFα dimer) structures

For treating structural flexibility, dimer chains in PDB 1TNF and 1A8M were all used as target structures, including 1TNF-AB, 1TNF-BC, 1TNF-CA, 1A8M-AB, 1A8M-BC, and 1A8M-CA. Residues on TNFα dimer-monomer interface (for example 1TNF AB-C interface) are listed in Table S1).

Table S1. Dimer-monomer interface residues in TNFα trimer structure.

Chain A	11, 13-15, 33-36, 57, 59, 61, 63, 66, 68, 69, 98, 100, 101, 103, 104, 112-117, 119, 143, 146-149, 151, 154-157
Chain B	53-55, 57, 72, 73, 75, 82, 91-99, 101-104, 116, 118-127, 157

Section 2: Computational methods and results for finding the best capsule-binding positions on the target surfaces

Similar to our previous methods (reference 1),[1] the target binding surfaces were systematically searched for finding the best capsule-binding positions. In this work, to get common binding positions, the six target structures (TNFα dimer) were superposed together and the scoring functions (Eq. S1 and S2) work on all of them together. The initial binding position was created based on the alignment to the targeting residues list in Table S1 (see details in reference 1). Then, the positions were sample by rotating and translating the capsule from the initial. The capsule was rotated to 2000 quasi-uniform directions, in which the maximum rotation angle was 45°. The translational step length in each of the three dimensions is 0.2 Å and the maximum translation distance is 10 Å. Every sampled position were evaluated from geometrical matching between the capsule and the crevice, and the hydrophobicity of attached target surface (achieved by INTERACT_score, Eq. S1). The surface area for each surface atom of TNF dimer ($surf\_area_i$ Å) was calculated first and used in INTERACT_score (Eq. S1). 6 different capsule radii, D_0 = 6.25, D_1 = 7.3, D_2 =8.3, D_3 =9.3, D_4 =10.0, and D_5 =11.0 Å, were used to probe the target surface. If the distance

between the atom and the capsule axis was lower than the tolerance value, a negative value would be added to INTERACT_score; otherwise, the area value was added with a weight, which was given based on the atom type and as Eq. S1 shows. Hydrophobic atom definitions are the same as those in reference 1.

$$\text{INTERACT\_score} = \left(\sum_{\text{structure } s=1}^{6} \sum_{\text{surface atom } i} \text{atom\_interaction}_i \right) \Big/ 6 \quad ,$$

$$\text{atom\_interaction}_i = \begin{cases} -300.0 & 4.25 < d_i < D_0 \\ -50.0 & d_i \le D_0 \\ \text{surf\_area}_i \times 2 & (D_1 < d_i \le D_2 \text{ or } D_3 < d_i \le D_4) \text{ and hydrophobic} \\ \text{surf\_area}_i & (D_1 < d_i \le D_2 \text{ or } D_3 < d_i \le D_4) \text{and non-hydrophobic} \\ \text{surf\_area}_i \times 4 & D_2 < d_i \le D_3 \text{ and hydrophobic} \\ \text{surf\_area}_i \times 2 & D_2 < d_i \le D_3 \text{ and non-hydrophobic} \\ \text{surf\_area}_i / 2 & D_4 < d_i \le D_5 \end{cases}$$

(**Eq. S1**)

Capsule positions with INTERACT_score > 90 ×residue_number (2700 for 30-residue and 2250 for 25 residue) were clustered with a detector-detector distance of 2.4 Å using the same clustering method in reference 1. The clusters were ranked by the highest score in each cluster, and the top 10 clusters were shown in Figure S1.

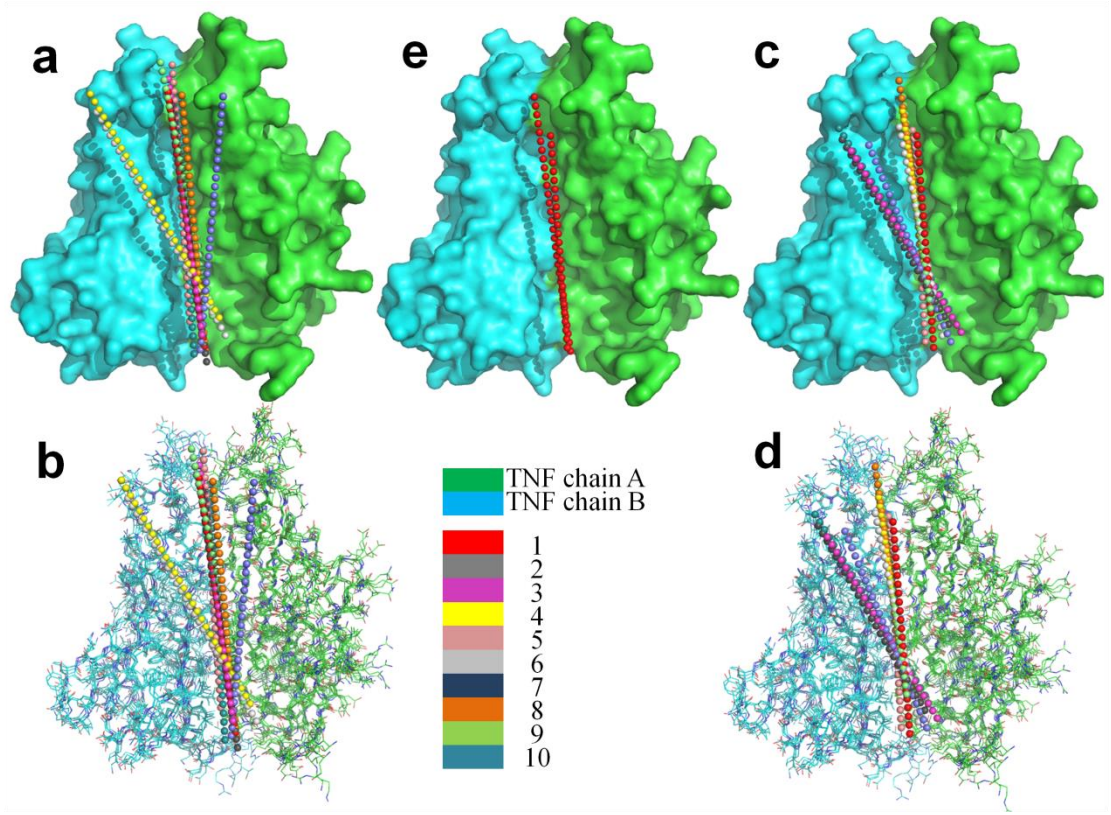


Figure S1. Best capsule-binding positions (position of the axis) on TNF dimer surface for 30-residue and 25-residue helix. (a) and (b) Top ten positions for 30-residue; (c) and (d) top ten positions for 25-residue. Positions are indicated by dotted lines. The colors in the color bar (middle lower) from top to bottom indicate the first detector to the tenth. Six TNFα dimer structures were superposed together in (b) and (d) as in the searching process. (e) shows the best binding position for 30-residue and 25-residue helix respectively. The best binding positions were used for generating the initial helix-binding structures.

<u>Section 3</u>: Methods for generating helical peptide sequences and binding conformations using Rosetta program (version 2.3.0)

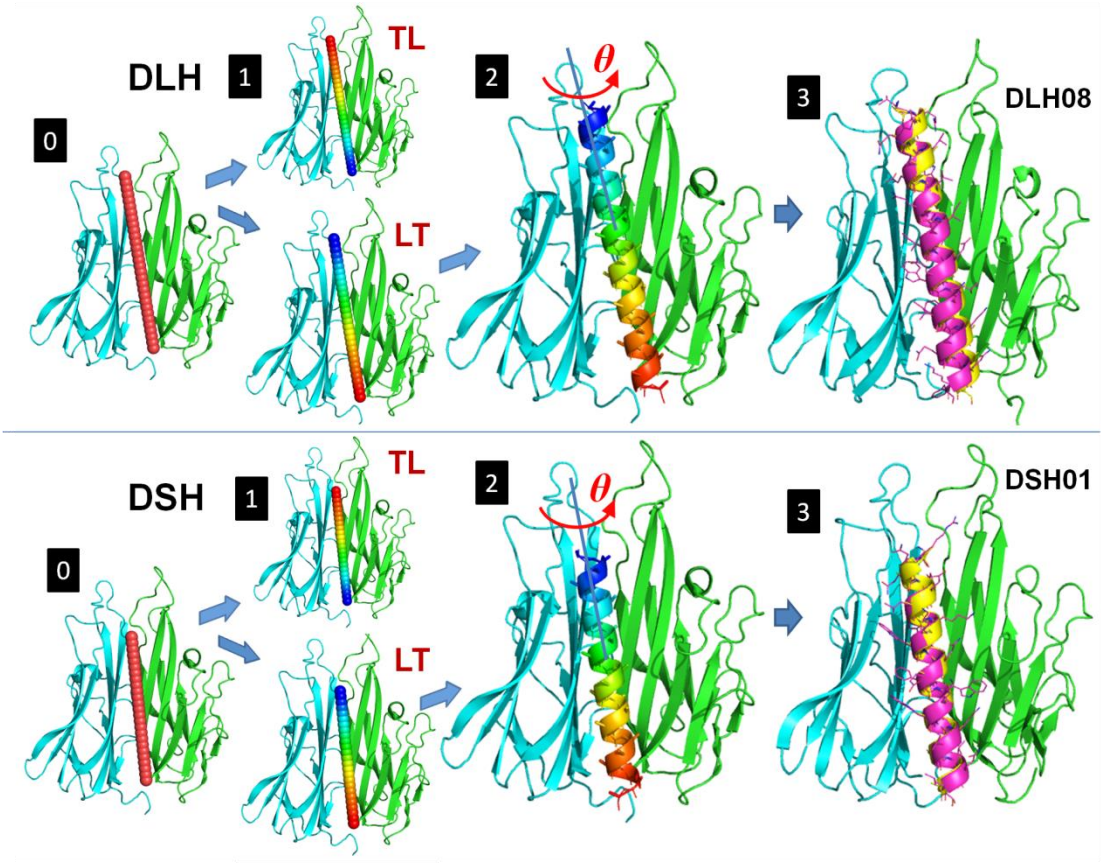


Figure S2. Design process, from initial binding position to helical peptide sequences and binding structures. 30-residue helices (upper line) and 25-residue helices (lower line) were name as DLH and DSH respectively. From the best binding positions ( ), both of the two opposite directions (named LT and TL) with alternative N- and C-termini were adopted for the initial helical polyalanine. ( ). Initial binding conformations of the helices, with main chain dihedral angles φ = -60.0 ° and ψ = -42.0 ° were generated by rotating the helix around its axis per θ=60 ° ( ). The diverse sequences weregenerated using Rosetta. Each initial structure with 100 different Rosetta trajectories "evolved" to 100 different binding structures with different sequences. For either 30-residue or 25-residue helices, 6 (target surfaces) × 2 (LT and TL directions) × 6 (rotations) × 100 = 7,200 sequences and corresponding binding structure models were obtained. DLH08 and DSH01 (magentas) were shown as an example for DLHs and DSHs respectively ( ) . The responding initial conformation was shown in yellow.

Rosetta program (version 2.3.0) was used to generate sequences and binding conformations from initial TNF dimer-peptide structure, in which the backbone of TNFα was fixed while the side chain conformations at the interface were allowed to be optimized (see details about the structure preparation and Rosetta command line in SI). The initial peptides were polyalanine, whose conformations were generated by rotating on the helical axis located on the best positions found in step 1. Both of the two reversed directions with alternate N- and C-termini were included in the initial helical structure (the initial structures were illustrated in Figure S2). Each initial structure with different Rosetta trajectories could "evolved" to different binding structures with different sequences. One hundred sequences were obtained from each initial structure for each TNFα dimer structure. For either the 25-residue or 30-residue peptide, a total of 7,200 sequences (Figure S2) and corresponding binding structure models were obtained.

As described in our previous work (reference 1), the loop design module in Rosetta 2.3.0 version was used for the helical peptide binding design. The command line was:

Rosetta –design –loops –design_loops dock –resfile resfile –s start.pdb

The start structure file, start.pdb, was prepared by adding the initial helix structure to the end of the target TNF dimer structure file. In the "resfile" file. All residues of TNF were set to native (NATAA), and the residues of the helix were set to "can be mutated to any type of residues" (ALLAA) or "can be mutated to polar residues" (POLAR). With these settings the helical peptide was treated like a loop of the target protein. and the loop design module in Rosetta was used to search for best binding conformations and best sequences by a Monte Carlo sampling strategy. The "dock" option means the side-chain conformation of target protein can be rearrangement during the helical peptide design. Interface design strategy of Rosetta was also used to optimize the binding interface.

Section 4: Computational methods and results for peptide sequence selection

Rosetta score, geometry score, and sequence score (including terms estimating folding probability, aggregation probability, and probability of folding to helix) were calculated using the same methods as our previous work (see details in reference 1).

Folding probability was estimated by the FoldIndex method, the probability of aggregation was estimated using AGGRESCAN, and the tendency of forming a helix was estimated by calculating the average residue propensity for α-helix. The stability of the entire system and the binding affinity was estimated using Rosetta energy, and the characteristics of the interface was examined in terms of the contact size and hydrophobic packing. Screening method is also similar, and the screening conditions were listed in Table S2. A hybrid scoring function (Eq. S2) was used to sort results, in which the weights of the three terms are estimated to balance their contributions:

$$\text{sorting\_score} = \text{RosettaScore} \times 5 + \text{GeometryScore} \times 2 + \text{SequenceScore}, \quad \textbf{(Eq. S2)}$$

where, SequenceScore = FoldIndex + Helix-Tendency ×3+ AggreScan/10

The top 50 sequences for DLH and DSH were listed in Table S3. Three of the 50 sequences were selected for experimental validation: DLH01, DLH08, and DLH16 for 30-residue, and DSH01, DSH03, and DSH 47 for 25-residue.

Table S2. Screen conditions in screening process.

Score item	Conditions for remaining
FoldIndex	>-0.60
AggreScan	<7.0
Helix-Tendency	>1.05
RosettaScore	>2.0
Hydrophobic core	<0.5
Geometry score	>0.0

Table S3. Top 50 sequences of DLH. Total score, Rosetta score, sequence score, geometry score, sequences, and corresponding targeting patch and initial binding conformation are listed. (1 for TNF, 2 for 1A8M in 'Initial' column)

No.	Score	Rosetta	Seq	Geo	Sequence	Initial
1	**16.717**	**2.441**	**3.280**	**0.615**	**LREQRLKEHLQEIQIFEQWLQIFQEQFQKF**	**1_BC_LT5**
2	16.679	2.483	3.174	0.546	QFEQAVKESLQEKQYFEQILQLLQEIFKKL	2_AB_LT5
3	16.583	2.420	3.501	0.490	LLELRFKEHQQEIEFFEKLLQIIEEVFKKI	1_BC_LT5
4	16.489	2.451	3.065	0.585	NRFLQRVQEELQEAQQHQQAMKLAMLMALQ	1_BC_LT3
5	16.287	2.398	2.940	0.679	DRRKQRIEEDLKEAQFHQKMMEIAVKQALK	1_BC_LT3
6	16.238	2.518	2.378	0.635	DNEDELKRNQQERQEFQQILELFFKLFQLD	2_CA_LT5
7	16.212	2.406	3.023	0.580	NEEDRFKEAQEEIQYFQQILQIFQQLWQLD	2_BC_LT5
8	**16.199**	**2.474**	**2.510**	**0.660**	**DAEDELKRNWQEIQEFQQILQMFFEWWQKD**	**2_CA_LT5**
9	16.173	2.380	2.896	0.689	YEEQLAKQIWQLMLQAIEQELKAVEMWLRW	1_BC_TL0
10	16.147	2.462	2.513	0.662	DQEDELKRNLQEIQEFQQILELFFKLFQLD	2_CA_LT5
11	16.139	2.348	3.309	0.544	LTDLRFIEHRQEEEFFEKLREIIEKVFKKD	1_BC_LT5
12	16.134	2.438	2.674	0.635	NLEDRFKEAWQEIQQFQQILKIWWQLWQLD	2_BC_LT5
13	16.112	2.477	2.823	0.451	LTEQRFKEELQEIEYFEKLLQIIIEEFLKD	1_BC_LT5
14	16.109	2.400	2.902	0.603	NEEEKRWQEELERAQKYQQWTQQILEQVFK	2_BC_LT3
15	16.098	2.408	2.865	0.597	NAREQRIQQELQAAQQYYQFALEALLLALS	2_CA_LT3
16	**16.092**	**2.382**	**2.820**	**0.681**	**NTDEEFAKKMLQYQEEFQKGDQIEQQFLQE**	**2_BC_TL5**
17	16.090	2.362	3.159	0.560	DLFEKRVKEELQEAQQHKQAMEQARKQALQ	1_BC_LT3
18	16.069	2.287	3.241	0.696	DEQQLAQQIKQLMEQAIEQELRAVEEKLKR	1_BC_TL0
19	16.068	2.338	3.170	0.605	TTEEWAKKIAELIERWRKMEQLERELREKE	2_BC_TL0
20	16.064	2.340	3.214	0.576	QFEQEVKRALQEEQEFRQILQQIQEWFKKL	2_AB_LT5
21	16.063	2.330	3.034	0.691	NLEQEWAQKMLQYQQQFRQGAEIAQKFRQE	2_BC_TL5
22	16.063	2.417	2.789	0.595	LTELRFKEHQQEIQYFEKLLQIFLQFIQQQ	1_BC_LT5
23	16.054	2.371	3.033	0.583	DRREQRIKQDLQEAQQHQQAAQIALEMALR	1_BC_LT3
24	16.053	2.366	3.067	0.579	NEQNKFQENQQEIQYFQQILQLLQQIFQQL	2_BC_LT5
25	16.037	2.468	2.473	0.613	NLENRFQEAQQEEQYFQKILKIWWELWQKD	2_BC_LT5
26	16.034	2.404	2.626	0.694	NTDDLFALWILQYQQEFQIAAQWHQQFGKD	2_BC_TL5
27	16.010	2.380	3.238	0.435	NLRDLRILRELQQAQQYQQALQIALEQALK	1_AB_LT3
28	15.971	2.366	2.817	0.663	NTDQIFAQWLLQYQEEFRKGLDIAEKFMKE	2_BC_TL5
29	15.957	2.304	3.203	0.617	TEQQLAQQIAQLMQQAIQQELQAVEMWLKW	1_BC_TL0
30	15.935	2.423	2.927	0.446	NLEDKFKEAWQEIEYFQKILEIIQKLLQLD	2_BC_LT5
31	15.925	2.306	3.140	0.626	QLEQEFKRHLQEKQYFEQLLQILQEIFKKL	2_AB_LT5
32	15.913	2.345	2.914	0.638	NMREQHIKQELQDAQKYQQFTQLALQQAKN	2_CA_LT3
33	15.893	2.343	2.877	0.651	NTDQLFAQWLLQYQEEFRKGLDIAEKFLKD	2_BC_TL5
34	15.892	2.309	3.095	0.627	NLDNEWAQKMLQFQQQFRQGAEMAEKFRAE	2_BC_TL5
35	15.891	2.383	3.043	0.466	LEQQWAQQIAELMEKWRKMEFLADIFKILE	2_BC_TL0
36	15.874	2.400	2.688	0.594	DKEQALKEEQQELQEFQKILQLILELFQKD	1_CA_LT5
37	15.861	2.449	2.470	0.574	DEEDELKRNWQEIQEFQQILQMFFQLWQLD	2_CA_LT5
38	15.853	2.315	3.106	0.586	NAREQKIKQELQAAQLYEQFAKLALLQALS	2_CA_LT3
39	15.849	2.369	2.927	0.538	LTELRILEELQEIQYFEQILQILLEFIQKV	1_BC_LT5

40	15.831	2.366	2.911	0.546	NLEDKFKEALQEIQYFQQILKLLFELWKKI	2_BC_LT5
41	15.809	2.421	2.555	0.575	DDEDELKRNLQEIQEFQQILQMFFELWKKD	2_CA_LT5
42	15.782	2.296	3.194	0.554	NRRLQKIQEDLQEAQWLQEMMEKALKQALK	1_BC_LT3
43	15.764	2.319	2.998	0.584	NRRLQKIQQDLQEAQWLQQQTEQALKQALK	1_BC_LT3
44	15.759	2.379	2.756	0.554	LTEQRIKEELQEIQQFEQLLKIFLQFIQQQ	1_BC_LT5
45	15.758	2.356	2.703	0.639	NLEDRFKEAWEEIQRFQQILQIWWQLLQLF	2_BC_LT5
46	15.747	2.351	3.073	0.459	NFEQKMKENWQEIEEFQKILQIFQELFQKD	2_BC_LT5
47	15.743	2.292	2.999	0.643	NAREQRIKQDLEDAQKYEQFAKEALEEAKK	2_CA_LT3
48	15.729	2.397	2.681	0.530	SAEDELKRNLQEIQEFQQILQMFFELWQKD	2_CA_LT5
49	15.726	2.389	2.624	0.578	DKQQALQEEQQELQEFQQILELIFKLFQLS	1_CA_LT5
50	15.720	2.330	2.896	0.587	LRELELKRHLQEIEEFEKLLKIFLEFIQKM	1_BC_LT5

Table S4. Top 50 sequences of DSH. Total score, Rosetta score, sequence score, geometry score, sequences, and corresponding targeting patch and initial binding conformation are listed.

No.	Score	Rosetta	Seq	Geo	Sequence	Initial
1	**16.866**	**2.525**	**2.793**	**0.725**	**RQQEELYKKWLQEFWKWFQIALQLD**	**1_CA_LT2**
2	16.347	2.530	2.962	0.366	YDEQLLKLYEEWVLKFIIKELLRVL	2_CA_TL0
3	**16.256**	**2.390**	**2.982**	**0.663**	**QEKWFAEQYARMVLEWIEKELKESL**	**1_CA_TL0**
4	16.247	2.430	2.858	0.619	LEQEWKYKQLLQQFWQWIQIALQLE	1_CA_LT2
5	16.204	2.371	3.017	0.665	REQEQKYKELLQKFFQRRLEVFQKL	1_CA_LT2
6	16.131	2.330	3.404	0.537	NWEWQMEIEMAEKAKKALEIAKKAA	1_CA_LT3
7	16.105	2.383	2.907	0.641	NLDQLFAQWMLEFWKRWVELEEKTE	2_BC_TL5
8	16.100	2.394	3.002	0.565	NQEWQEELEMAERAKESLQKAEQAS	1_CA_LT3
9	16.081	2.414	2.693	0.660	REQEQKYKQLLQKFWQWIQIALQIE	1_CA_LT2
10	16.041	2.408	2.734	0.634	NRRLEEIQRAQQLWWWTIVAMQIAD	1_AB_LT4
11	16.002	2.289	3.000	0.778	NERQKAMQHYQQVRQWTEIAQKIEF	1_BC_LT4
12	15.998	2.317	3.213	0.600	NELQMAQQYAQMVQQFIEQELKRSQ	1_CA_TL0
13	15.965	2.216	3.603	0.642	NSTIEHILRMLQAWQSAMLARELAE	2_BC_LT4
14	15.919	2.298	3.143	0.644	QQQRTQEEQQIAQFFHEELKKLFQL	2_CA_LT1
15	15.862	2.269	3.439	0.539	NLDLQFAQQLLEHLLRMLQAWQQNE	2_BC_TL5
16	15.853	2.423	2.775	0.482	GDLQRLELYWKWVLQFLQQELQRVQ	2_CA_TL0
17	15.852	2.314	2.908	0.686	NEFLLGLIVAQLAQQFIQQEFQIAQ	1_BC_TL0
18	15.845	2.280	3.171	0.636	NEFQQALEVAQRALEFIQKEFQNTQ	2_AB_TL0
19	15.822	2.318	3.105	0.562	NLDELFAKWLLQAEKQMRIAWDLKE	2_BC_TL5
20	15.806	2.376	2.756	0.586	TELLLALLYAQWVEEWIKKELQRDQ	1_CA_TL0
21	15.806	2.319	3.099	0.556	NTEQELAQKMRQFTWQMLQDMLKDI	1_BC_TL5
22	15.795	2.359	2.836	0.583	NELQLAQWYAELVLKWIEIELKKSQ	1_CA_TL0
23	15.787	2.351	2.809	0.611	LEQEQKYKELLQKFFQMILEALQKE	1_CA_LT2
24	15.771	2.370	2.514	0.705	DEQLMAFLWVQWVQEWIQKEFQQDQ	1_AB_TL0
25	15.759	2.260	3.169	0.645	NQTIEYVKKVMEQMLKAEQQEEQVQ	1_CA_TL4

26	15.726	2.236	3.164	0.691	DDEQRGFEWAQRALEFIQQAFQISS	2_BC_TL0
27	15.717	2.350	2.924	0.521	NREQFNKQLQQEAQRLLQALLQWLL	2_BC_LT5
28	15.706	2.276	3.064	0.631	TELLWAFQIAQQIQQWIQREFKISS	2_BC_TL0
29	15.704	2.351	3.026	0.461	NLKWLWEIQMAQQAKESLQKAEQAS	1_CA_LT3
30	15.691	2.277	3.031	0.637	FQEEMQTKQLWQEFYQWKLEALQKD	2_CA_LT2
31	15.690	2.299	2.832	0.681	RQDDQQYKQLLQQFMQRWQEAFQEE	1_CA_LT2
32	15.688	2.288	3.154	0.546	NLEIQWEQQMAEQAKQSLQKAKQAA	1_CA_LT3
33	15.685	2.318	3.087	0.505	NEEWQRDLQMAQQAKQSLQLAKQAS	1_CA_LT3
34	15.674	2.364	2.651	0.600	REQDQKVKELWQKFFQWILEALQKE	1_CA_LT2
35	15.641	2.285	3.207	0.505	LRLIFNQWLQQEAQRLLQALEELLK	2_BC_LT5
36	15.634	2.376	2.874	0.439	GELQQLEQYWKMVLEFIEKELKRVQ	2_CA_TL0
37	15.617	2.282	2.992	0.607	REQEQKYKELLQKFEIRIRIALQLD	1_CA_LT2
38	15.616	2.351	2.921	0.470	LQQQFQVKELWEKFWKIIMEAFQKE	1_CA_LT2
39	15.612	2.186	3.435	0.622	NLREIEMKIAQQVRQWTEIAAKLEF	1_BC_LT4
40	15.609	2.321	2.720	0.642	REQEQKYKELLQKFFQMILEAIQKE	1_CA_LT2
41	15.598	2.334	2.656	0.637	PEQIMAFIVAQWIEQWIKQEFQQDQ	1_AB_TL0
42	15.596	2.302	2.937	0.574	TLEIIWMQIMAQQAKQSLQIAKQAA	1_CA_LT3
43	15.593	2.364	2.791	0.491	NLDQQVAQMILIFQQLWQIMEDLTE	2_BC_TL5
44	15.582	2.303	2.903	0.582	LELQQAEQYAKIVLEWIQKELQRAQ	1_CA_TL0
45	15.576	2.325	2.966	0.493	NELFQKMAQYILQALQQMDIWDDLK	1_BC_TL3
46	15.576	2.346	2.789	0.528	NLDDLFAKWMLLYQQWLEILEDLTE	2_BC_TL5
47	**15.570**	**2.340**	**2.608**	**0.632**	**NEQLLAFIIAQWIEQWIRQEFQEDQ**	**1_AB_TL0**
48	15.562	2.271	2.840	0.683	NELQMAFEYAQRVIEWILKELQESQ	1_CA_TL0
49	15.544	2.294	3.069	0.502	NREQFNKILQQIAQILLEALIKELL	2_BC_LT5
50	15.543	2.257	3.143	0.558	NQDRQYVEQVMKMAQISFQLELQVR	2_BC_TL4

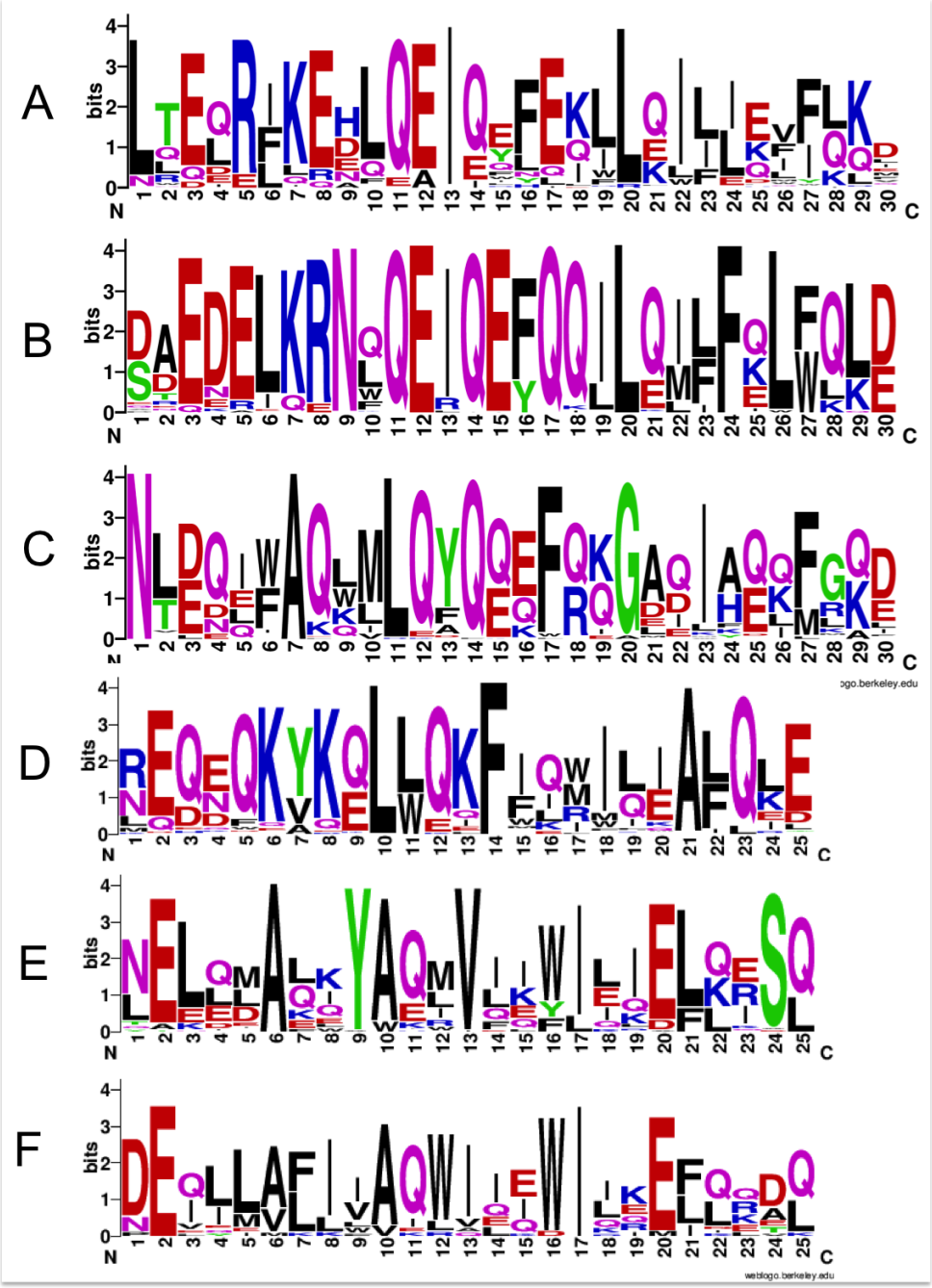


Figure S3. Sequence conservation of the six selected sequences. (A)-(F): DLH01, DLH08, DLH16, DSH01, DSH03, and DSH47. The conservation of a sequence was expressed by multi-sequence alignment of helices generated from one initial conformation and represented by WebLogo[2].

Part II. Experimental procedures and results

Section 5: Experimental materials and methods

Experimental Materials

The clone of hTNFα in pUC18 vector was a generous gift from Professor Beifen Shen (Academy of Military Medical Sciences, China). HEK293T cells were received as a gift from Professor Jincai Luo (Peking University, China). Designed peptides were synthesized by GL Biochem (Shanghai, China).

Cloning, Expression, and Purification of Human TNFα and GST-fusion Peptides

The details of experimental procedures for protein expression and purification are described in our previous publication.[1]

Circular Dichroism.

Peptide samples were dissolved to a final concentration of 0.2 mg/ml in Milli-Q water. CD spectra were recorded on a MOS 450 AF/CD (Biologic, France) at 25 ^{o}C, using 1 mm quartz cuvettes for the far-UV region (190-260 nm).

Luciferase Activity Assay

The cell assay was performed following our previously reported procedures.[1] Briefly, cells were co-transfected with transfection reagent (0.1 μl) and purified plasmids (100 ng pGL4.74[hRluc/TK] plasmid and 100 ng pGL4.32 [luc2P/NF-kB-RE/Hygro] plasmid) in 50 μl DMEM/10% FBS per well. Twenty-four hours later, cells were stimulated with 50 μl of pre-incubated TNFα and peptide mixture for 6 hrs and the luciferase assays were carried out using the Dual-Glo Luciferase Assay System. The final results were converted to percentage inhibition rates of the peptides and expressed as Mean ± SD from three independent experiments.

Binding Assays by Surface Plasmon Resonance

Binding interactions between TNFα and the peptides were examined using a BIAcore 3000 biosensor system (BIAcore). TNFα was immobilized on a CM-5 senor chip via an amine coupling reaction according to the manufacturer's instructions. All binding experiments were performed in PBS-EP buffer (10 mM NaH_2PO_4/Na_2HPO_4, 150 mM NaCl, 3.7 mM EDTA, 0.005% Surfactant P20, pH 7.4) at 25℃ at a flow rate of 50 μl/min. Preliminary screening of peptide candidates was performed using 1 μM GST-peptide fusions. GST protein was used as a control. In the kinetics study, synthetic peptides with no tags were used. Peptide samples at various concentrations (as indicated in Results) were injected over the chip. Using the BIAcore 3000 evaluation software, the response curves of peptides at different concentrations were globally fitted to the nonlinear 1:1 Langmuir model to achieve the K_D, k_{on} and k_{off} of each peptide.

Cross-linking Experiment [3]

Purified TNFα was buffer-exchanged into 10 mM HEPES buffer (pH 7.5) and pre-incubated with the peptides at 4 ^oC for 12 hrs. The final concentrations of TNFα and the peptides were 2.0μM, respectively. The crosslinker, disuccinimidyl suberate (DSS), was dissolved in DMSO at 5 mM for a 100-fold molar excess over the protein. For each 20 μl of the TNFα-peptide solution, 0.6 μl of the DSS solution was added. TNFα mixed with 2.0 μM TBHa31 was set as a control. TNFα with or without treatment of DSS were also used as controls. After incubation at room temperature for 15 min, ammonium bicarbonate was added to the system at a final concentration of 20 mM to terminate the reaction. Samples were then subjected to SDS-PAGE, visualized by silver-stain using Protein Silver Stain Kit (cwbio).

Mass Spectrometry Analysis of TNFα Oligomeric State [4]

Purified TNFα was buffer-exchanged into MilliQ water (pH7.0). Peptide samples, DLH01, DLH08, DSH01 or DSH03, were incubated with TNFα at 37 ^oC for 12 hrs. The final concentrations of TNFα and peptide were 1 μM and 5 μM, respectively.

Then, the analysis was performed on an Agilent (USA) 1200 HPLC coupled to a 6510 quadrupole-time of flight mass spectrometry (QTOF MS). The HPLC system was not equipped with any column and the samples were quickly flushed in to MS source after injection. The mobile phase was 0.01% aqueous formic acid at a flow rate of 0.25 ml/min. The main parameters were set as follows: in dual-spray souce (Vcap 3500V, fragmentor 200V), the drying gas temperature (350^{o}C), flow rate (12 L/min), nebulizing gas pressure (35 psig). Data are acquired from m/z 100-3000. All data were collected and processed using the Agilent Technologies MassHunter Workstation Software.

Cell Viability Assay

In order to evaluate the cytotoxicity of the selected peptides, cell viability assays were performed using Cell Counting Kit-8 (Dojindo). Cells were plated in 96-well plates at 1×10^4 cells per well and cultured in 100 μl of DMEM at 37 ^{o}C for 24 hrs. Then, to each well was added 50 μl of peptide or SPD304 solutions at various concentrations (as indicated in Results). In the control groups, 50 μl of DMEM was used. Wells containing only media without cells and additives were used as blanks. After 24 hrs, 15 μl of reduced WST-8 (2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt) was added to each well and the cells were incubated at 37 ^{o}C for 1 hr. The cell numbers in triplicate wells were measured by the absorbance at 450 nm, with that at 630 nm as a reference.

Section 6: Experimental materials and methods

Table S5. Summary of SPR-measured binding parameters for peptide-TNFα interactions

Peptide	k_a ($\times 10^4$ $M^{-1}s^{-1}$)	k_d ($\times 10^{-2}$ s^{-1})	K_D ($\times 10^{-7}$ M)
DLH01	8.39	4.90	5.84
DLH08	7.50	2.92	3.89

DSH01	1.56	1.06	6.78
DSH03	7.41	3.87	5.22

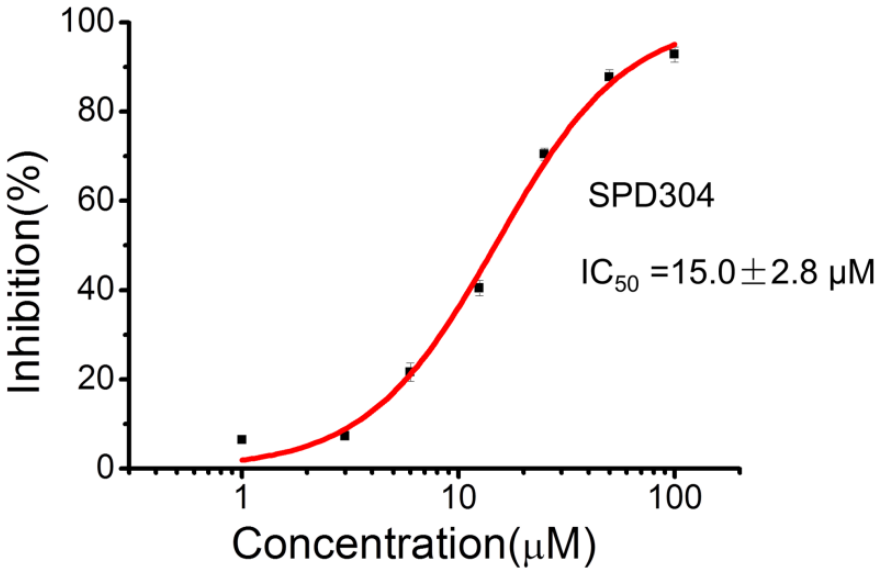


Figure S4. Dose-response curve of SPD304 in the cell luciferase assay. The data are reported as means ±errors from three independent experiments.

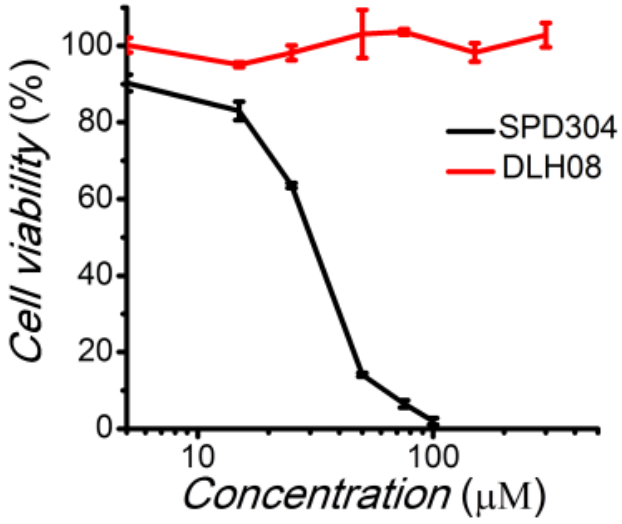


Figure S5. Cell cytotoxicity comparison of SPD304 and DLH08.

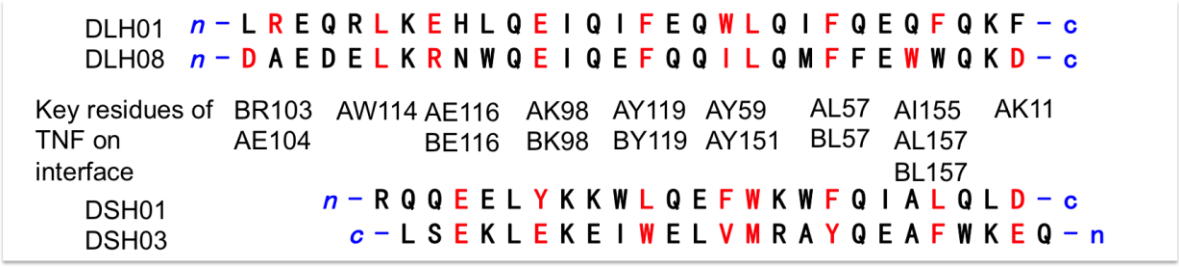
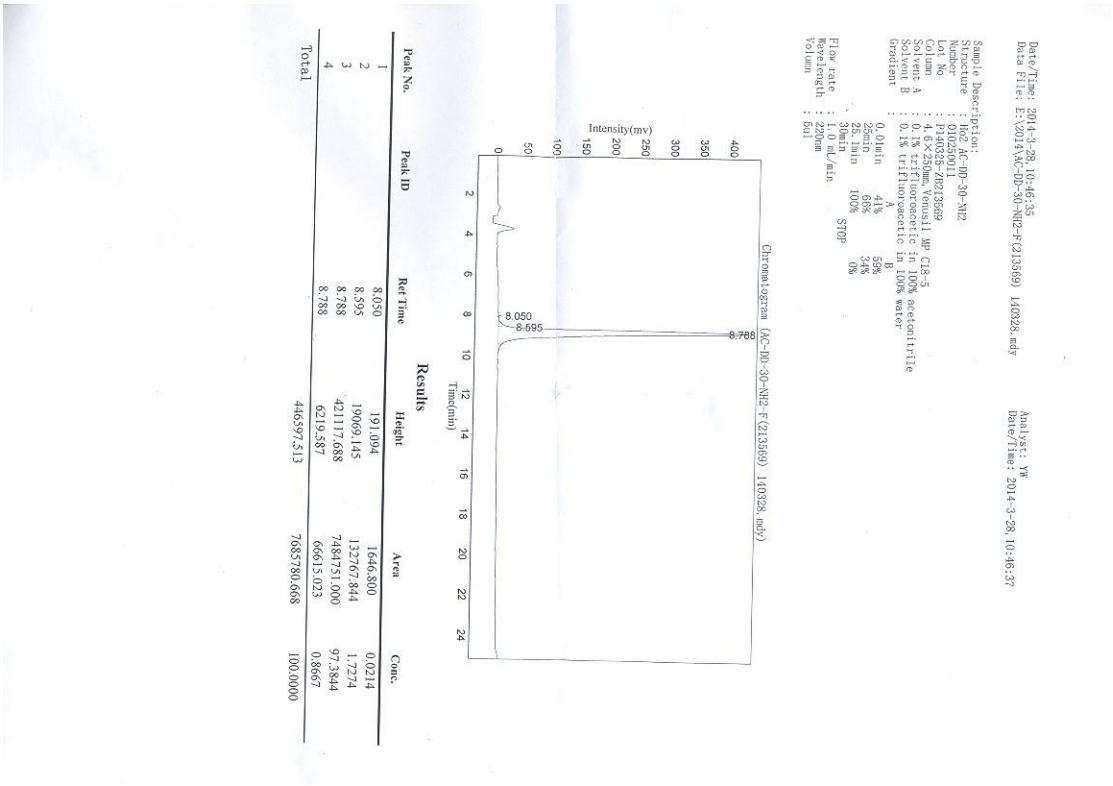
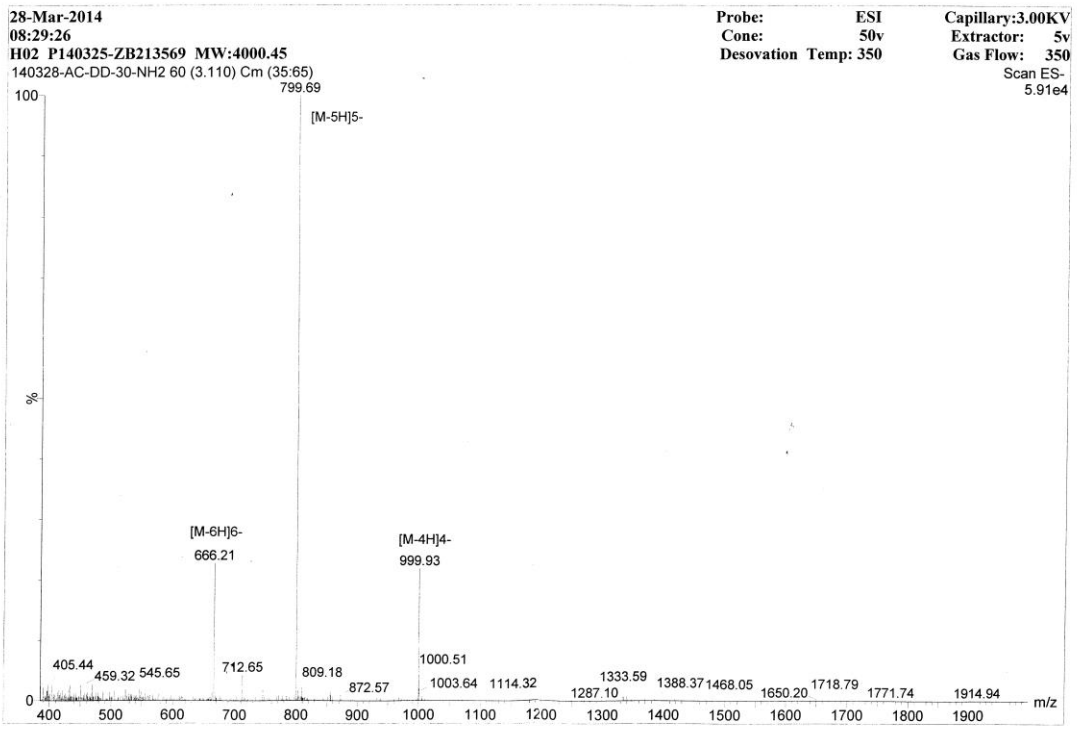


Figure S6. Interfacial key residues on designed helices and TNF dimer. Key residues of the peptides are labeled in red. The sequence of DSH03 is shown from C terminal to N terminal and the other three are shown from N terminal to C terminal.

*<u>Section 7</u>**:** HPLC and ESI-MS of the active peptides*

DLH08




28-Mar-2014
08:29:26
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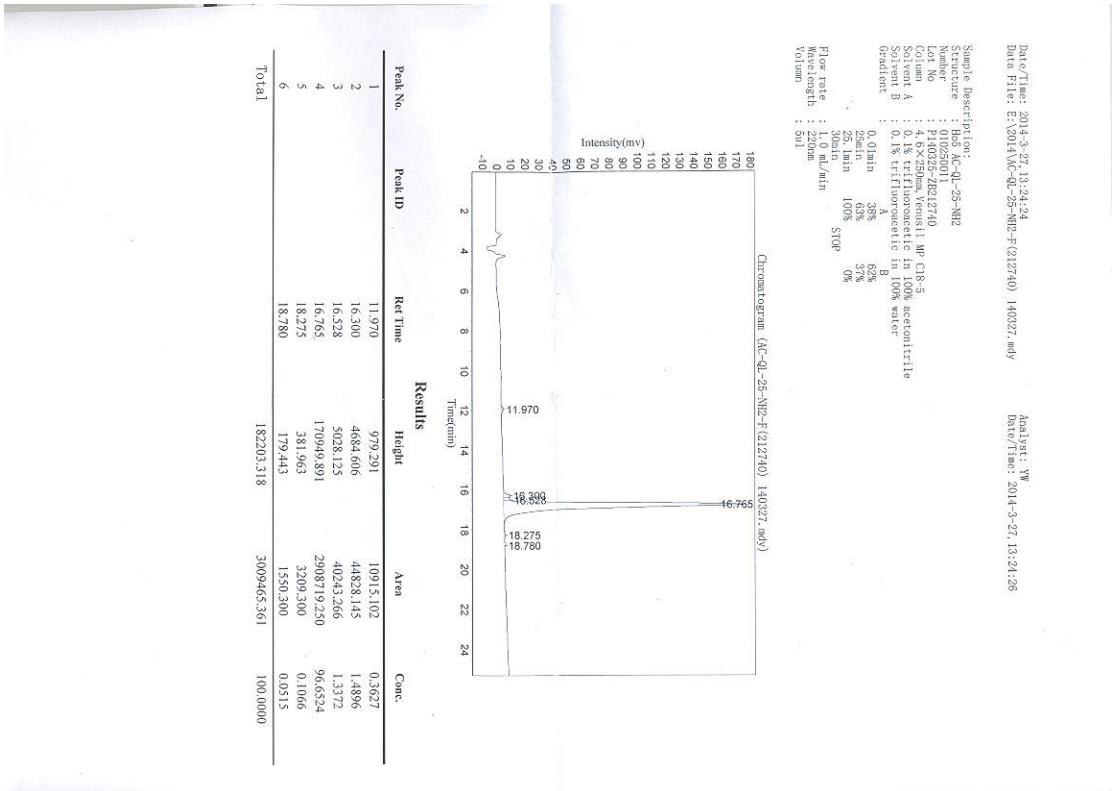
140328-AC-DD-30-NH2 60 (3.110) Cm (35:65)
Probe: ESI
Cone: 50v
Desovation Temp: 350
Capillary:3.00KV
Extractor: 5v
Gas Flow: 350
Scan ES-
5.91e4
799.69
[M-5H]5-
[M-6H]6-
666.21
[M-4H]4-
999.93
405.44
459.32
545.65
712.65
809.18
872.57
1000.51
1003.64
1114.32
1287.10
1333.59
1388.37
1468.05
1650.20
1718.79
1771.74
1914.94
%
m/z

DSH01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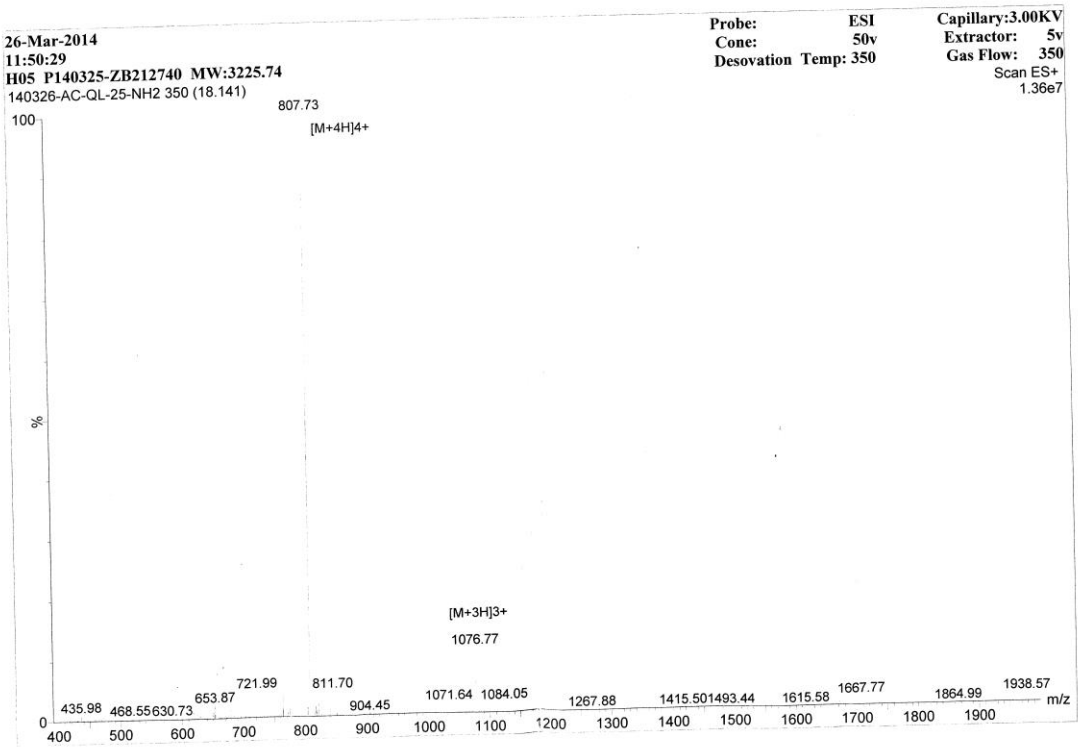
Data File: E:\2014\AC-QL-25-NH2-F(212740) 140327.mdy
Analyst: YW
Date/Time: 2014-3-27, 13:24:26
Sample Description:
Structure : Hc5-AC-QL-25-NH2
Number : 01025001
Lot No : P140325-ZB212740
Column : 4.6×250mm,Yemsil MP C18-5
Solvent A : 0.1% trifluoroacetic in 100% acetonitrile
Solvent B : 0.1% trifluoroacetic in 100% water
Gradient : 0.01min 38% B
25min 63% B
25.1min 100% B
30min STOP
Flow rate : 1.0 ml/min
Wavelength : 220nm
Volumn : 5ul
Intensity(mv)
Time(min)
Chromatogram (AC-QL-25-NH2-F(212740) 140327.mdy)
11.970
16.300
16.523
16.765
18.275
18.760
Results
Peak No. Peak ID Ret Time Height Area Conc.
1 11.970 979.291 10915.102 0.3627
2 16.300 4684.606 44828.145 1.4896
3 16.528 5028.125 40243.266 1.3372
4 16.765 170949.891 2908719.250 96.6524
5 18.275 381.963 3209.300 0.1066
6 18.780 179.443 1550.300 0.0515
Total 182203.318 3009465.361 100.0000


26-Mar-2014
11:50:29
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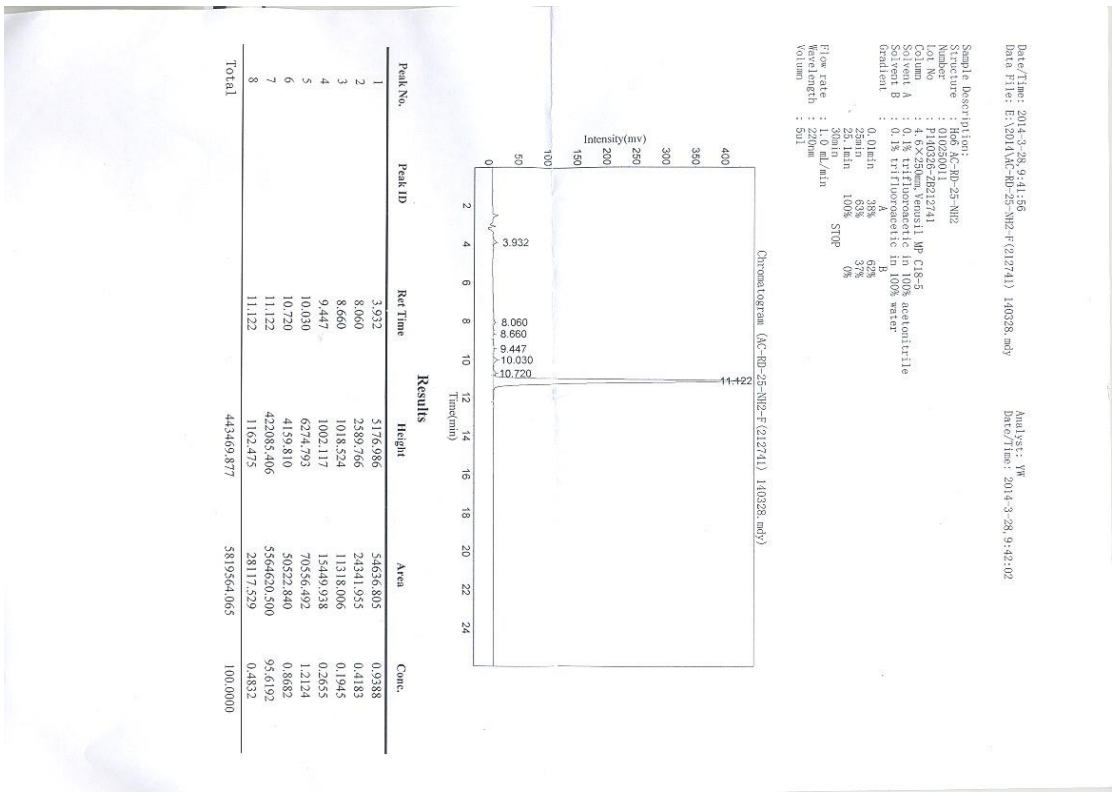
140326-AC-QL-25-NH2 350 (18.141)
Probe: ESI
Cone: 50v
Desovation Temp: 350
Capillary:3.00KV
Extractor: 5v
Gas Flow: 350
Scan ES+
1.36e7
807.73
[M+4H]4+
[M+3H]3+
1076.77
%
m/z

DSH03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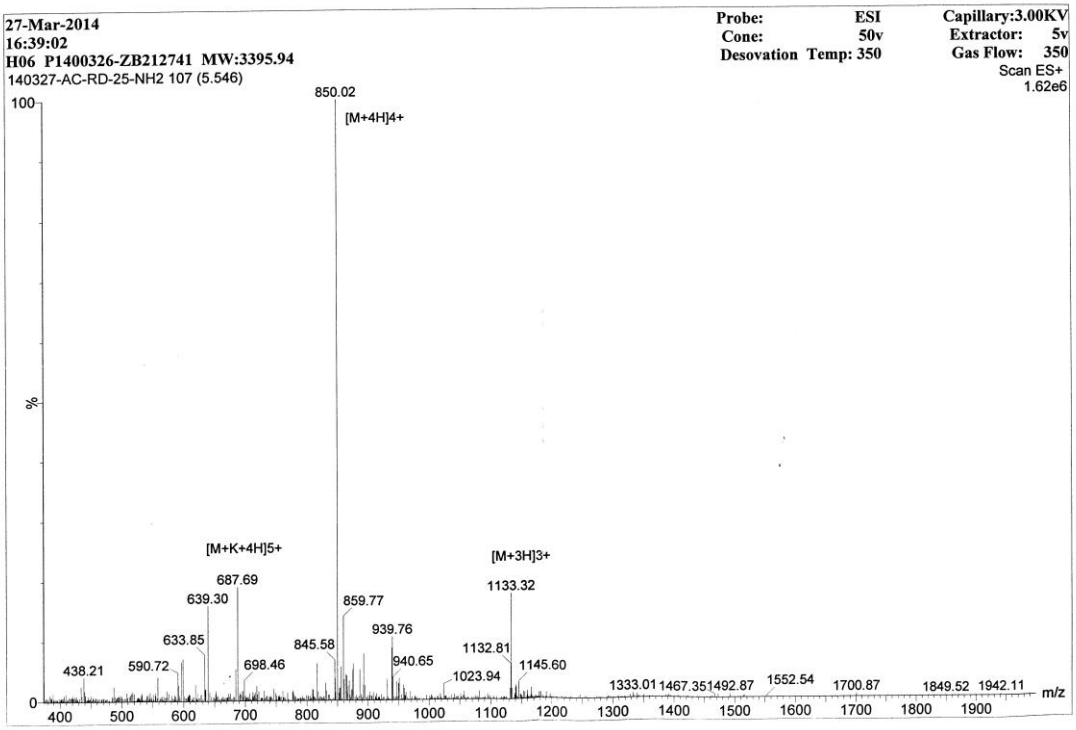
Data File: E:\2014\AC-HD-25-NH2-f(21274) 140328.mdy
Analyst: YW
Date/Time: 2014-3-28, 9:42:02
Sample Description
Structure : HD-AC-HD-25-NH2
Number : 01025001
Lot No. : P140326-ZB212741
Column : 4.6×250mm,Venusil MP C18-5
Solvent A : 0.1% trifluoroacetic in 100% acetonitrile
Solvent B : 0.1% trifluoroacetic in 100% water
Gradient : 0.01min 38% B 62%
25min 63% 37%
25.1min 100% 0%
30min STOP
Flow rate : 1.0 mL/min
Wavelength : 220nm
Volume : 5ul
Chromatogram (AC-HD-25-NH2-F(21274) 140328.mdy)
Intensity(mv)
Time(min)
3.932
8.060
8.660
9.447
10.030
10.720
11.122
Results
Peak No. Peak ID Ret Time Height Area Conc.
1 3.932 5176.986 54636.805 0.9388
2 8.060 2589.766 24341.955 0.4183
3 8.660 1018.524 11318.006 0.1945
4 9.447 1002.117 15449.938 0.2655
5 10.030 6274.793 70556.492 1.2124
6 10.720 4159.810 50522.840 0.8682
7 11.122 422085.406 5564620.500 95.6192
8 11.122 1162.475 28117.529 0.4832
Total 443469.877 5819564.065 100.0000



Supplementary references

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2. G. E. Crooks, G. Hon, J. M. Chandonia and S. E. Brenner, *Genome Res.*, 2004, **14**, 1188.

3. O. Yoshie, K. Tada and N. Ishida, *J. Biochem.*, 1986, **100**, 531.

4. M. M. He, A. S. Smith, J. D. Oslob, W. M. Flanagan, A. C. Braisted, A. Whitty, M. T. Cancilla, J. Wang, A. A. Lugovskoy, J. C. Yoburn, A. D. Fung, G. Farrington, J. K. Eldredge, E. S. Day, L. A. Cruz, T. G. Cachero, S. K. Miller, J. E. Friedman, I. C. Choong and B. C. Cunningham, *Science*, 2005, **310**, 1022.