A Novel Peptide Targeting PD-1: Implications for Protein-Protein Interaction Studies and Immunotherapy

Satya Prakash Arya[a], Hari Timilsina[a], Siddhartha Kalpa Samadhi Thennakoon[a], Rick Mason Postema[a], Raunak Jahan[a], Andrew Michael Reynolds[a], Xiaohong Tan*[a]

Department of Chemistry and Centre for Photochemical Sciences, Bowling Green State University

*To whom correspondence should be addressed: tanx@bgsu.edu

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Materials and methods-:

General reagents for phage display and peptide synthesis. Recombinant PD-1 Protein was purchased from Sino Biological (RBD proteins, Cat # 10377-H08H). Ph.D.-12 phage display peptide library, and the screening kit were purchased from New England Biolabs, Ipswich, MA (Cat# E8110S). The HRP-conjugated M13 antibody (Cat# 11973-MM05T-H) was purchased from Sino Biological. For peptide synthesis, Rink amide resin, Fmoc-amino acids, (1-Cyano-2-ethoxy-2-oxoethylidenaminooxy) dimethylamino-morpholino-carbenium hexafluorophosphate (COMU), N, N-diisopropylethylamine (DIPEA) were purchased from chem-impex international. Piperidine was purchased from Bean Town Chemical (BTC). Triisopropyl silane (TIPS) was purchased from TCI (Tokyo Chemical Industry, USA). Acetonitrile (HPLC grade), Diethyl ether, Trifluoroacetic acid (TFA), Dichloromethane (DCM), and Dimethylformamide (DMF) were purchased from Sigma Aldrich.

Phage-displayed peptide library screening. Recombinant PD-1 Protein was utilized for screening the Ph.D.-12 Phage Display Peptide Library (E8110S, New England Biolabs, MA) across si rounds with progressively increased stringency in each subsequent round. In the initial round of screening, we coated the recombinant PD-1 protein at a concentration of 1µg and used a tween concentration of 0.1%. The protein amount remained constant for rounds two and three, but the Tween concentration was increased to 0.2%, with a counter-selection time of 25 minutes. Maintaining the same protein amount in the later rounds, we further intensified the selection pressure by raising the tween concentration to 0.5% in round six. In brief, in the first round, 1 µg of PD-1 protein in 100 µL of 0.1 M $NaHCO_3$, pH 8.6, was coated on a Nunc Maxisorp flat-bottom plate (Cat# 44-2404-1, Thermofischer) at 4°C overnight. After removing the supernatant and blocking with 5 mg/mL bovine serum albumin (BSA), the plate was washed six times with TBST (TBS+ 0.1% Tween-20). One hundred microliters of diluted phage library (3×10^{11}) were pipetted into the well and incubated at room temperature for one hour. The unbound phages were removed and washed 10 times with 0.1% TBST. The bound phages were then eluted with 100 µL of 0.2 M glycine-HCl (Ph 2.2), 1 mg/ml BSA for 10 min and neutralized with 1M of Tris-HCl, pH 9.1.

Round	Conditions	Counter-selection	Input Library	Output Library	Enrichment Factor
1st	Target protein: 1 µg/50 µL PD1 Washing: 0.1% Tween-20 TBST, 10 times Elution: 0.2 M Glycine-HCl (pH 2.2)+ BSA Neutralization: 1M Tris-HCl, pH 9.1	0 min	3×10^{11}	1.3×10^{4}	2.3×10^{7}
2nd	Target protein: 1 µg/50 µL PD1 Washing: 0.1% Tween-20 TBST, 10 times Elution: 0.2 M Glycine-HCl (pH 2.2)+ BSA Neutralization: 1M Tris-HCl, pH 9.1	25 min	3×10^{11}	4.3×10^{4}	6.97×10^{6}
3rd	Target protein: 1 µg/50 µL PD1 Washing: 0.2% Tween-20 TBST, 10 times Elution: 0.2 M Glycine-HCl (pH 2.2)+ BSA Neutralization: 1M Tris-HCl, pH 9.1	25 min	3×10^{11}	8.6×10^{4}	3.48×10^{6}
4th	Target protein: 1 µg/50 µL PD1 Washing: 0.25% Tween-20 TBST, 10 times Elution: 0.2 M Glycine-HCl (pH 2.2)+ BSA Neutralization: 1M Tris-HCl, pH 9.1	25 min	3×10^{11}	1.8×10^{5}	1.66×10^{6}
5th	Target protein: 1 µg/50 µL PD1 Washing: 0.25% Tween-20 TBST, 10 times Elution: 0.2 M Glycine-HCl (pH 2.2)+ BSA Neutralization: 1M Tris-HCl, pH 9.1	30 min	3×10^{11}	2.6×10^{6}	1.16×10^{5}
6th	Target protein: 1 µg/50 µL PD1 Washing: 0.5% Tween-20 TBST, 10 times Elution: 0.2 M Glycine-HCl (pH 2.2)+ BSA Neutralization: 1M Tris-HCl, pH 9.1	30 min	3×10^{11}	3×10^{6}	1×10^{5}

Table S1-: Phage enrichment summary table for all six panning rounds. The enrichment factor is calculated as the ratio of the input phage (phage-forming unit) to the output phage. The enrichment factors were monitored over 6 panning and serve as relative indicators that PD-1 binding phages are being selected.

Amplification of the phage sub libraries. The eluted phage library was amplified in 20 mL of ER2738 *E. coli* (OD600 at 0.01-0.05) with shaking vigorously at 37 °C for 4.5 h. The culture was then centrifuged at 12,000*g* for 10 min at 4 °C, after which the supernatant was transferred into a new tube with the addition of one-sixth volume of 20% polyethylene glycol (PEG)/2.5 M NaCl and incubated at 4 °C overnight. The PEG-precipitated phages were centrifuged at 12,000*g* for 15 min at 4 °C. The phage pellet was resuspended in 500 µL of TBS, the first PD-1-specific sublibraries. These sub-libraries were subjected to phage titering and used for the next round of screening. The conditions for subsequent rounds of biopanning are described in supporting information table S1.

Titering of the Phage Libraries. Titering of the phage sublibraries was performed using the NEB Ph.D-12 protocol. In brief, 10 µL of serially diluted (100-fold) phage sublibraries was mixed with 200 µL of the mid-log phase of E. coli ER2738 cells, and the mixture was then added to 1 mL of prewarmed top agar and covered on a prewarmed LB/IPTG/X-gal plate. After cooling the plates at 4 °C for 5 min, the plates were inverted and incubated at 37 °C overnight. The phage plaques were counted, and the titers were calculated according to the plaque numbers and the corresponding dilution.

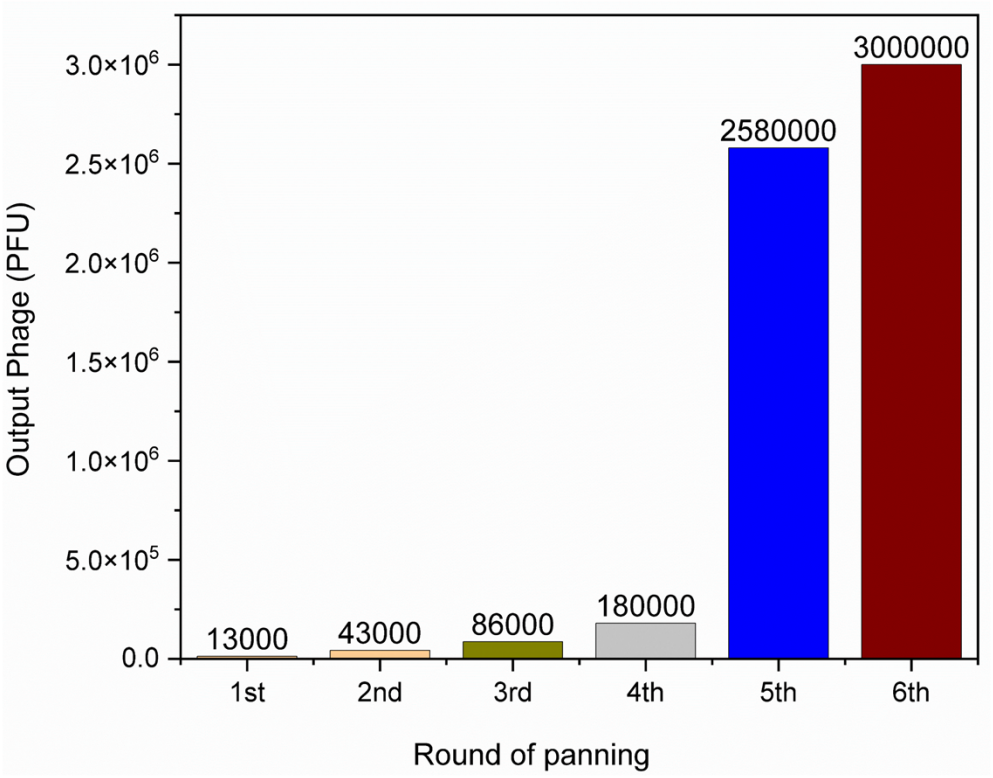


Figure S1-: Enrichment of phages from Ph.D-12 library by bio-panning against PD-1 protein.

Phage ELISA. Three different rounds of phage sublibraries were subjected to phage ELISA to determine the binding specificity and affinity toward the PD-1 protein. For this purpose, 0.5 µg of PD-1 as a target protein and BSA protein as a control protein in 0.1 M $NaHCO_3$, pH 8.6, was coated on a Nunc Maxisorp 96-well plate overnight at 4 °C. After blocking with 5 mg/mL BSA for 1 hr, the plate was washed six times with 0.1% TBST, and 5 x 10^9 phages from each round were applied to plates and incubated at 37 °C for 1h. After washing ten times with 0.5% TBST, the HRP-conjugated M13 antibody (1:2000 in 0.5% TBST) was incubated in the plate for 30 min. The plate was washed six times to remove the nonspecific antibody, followed by color development with the addition of 3,3',5,5'-tetramethylbenzidine (TMB) substrate. The OD values at 450 nm were recorded in a Clariostar microplate reader.

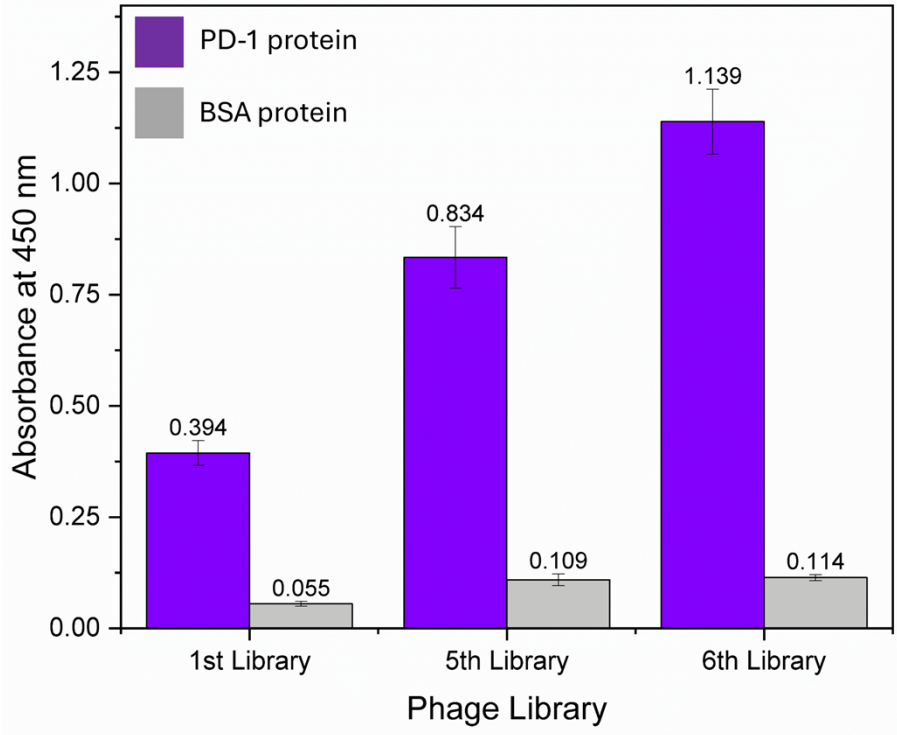


Figure S2-: Three rounds of phage libraries compared with phage ELISA.

Phage DNA Sequencing. After the last round of screening, 23 positive phages were randomly picked from the sixth round of the bio-panning cycle by blue and white assay (IPTG/X-Gal) and amplified in 2 mL of the culture. The individual phage DNA was extracted, purified, and sent for sequencing at the CRC-DNA Sequencing Facility, University of Chicago.

Hit	DNA Sequence	Peptide Sequence
1	ATTGATACGGGTCGGAAGGGGCCTGAGCGGGCGATT	IDTGRKGPERAI
2	ATTGATACGGGTCGGAAGGGGCCTGAGCGGGCGATT	IDTGRKGPERAI
3	ATTGATACGGGTCGGAAGGGGCCTGAGCGGGCGATT	IDTGRKGPERAI
4	ATTGATACGGGTCGGAAGGGGCCTGAGCGGGCGATT	IDTGRKGPERAI
5	ATTGATACGGGTCGGAAGGGGCCTGAGCGGGCGATT	IDTGRKGPERAI
6	ATTGATACGGGTCGGAAGGGGCCTGAGCGGGCGATT	IDTGRKGPERAI
7	ATTGATACGGGTCGGAAGGGGCCTGAGCGGGCGATT	IDTGRKGPERAI
8	ATTGATACGGGTCGGAAGGGGCCTGAGCGGGCGATT	IDTGRKGPERAI
9	ATTGATACGGGTCGGAAGGGGCCTGAGCGGGCGATT	IDTGRKGPERAI
10	ATTGATACGGGTCGGAAGGGGCCTGAGCGGGCGATT	IDTGRKGPERAI
11	ATTGATACGGGTCGGAAGGGGCCTGAGCGGGCGATT	IDTGRKGPERAI
12	ATTGATACGGGTCGGAAGGGGCCTGAGCGGGCGATT	IDTGRKGPERAI
13	ATTGATACGGGTCGGAAGGGGCCTGAGCGGGCGATT	IDTGRKGPERAI
14	ATTGATACGGGTCGGAAGGGGCCTGAGCGGGCGATT	IDTGRKGPERAI
15	ATTGATACGGGTCGGAAGGGGCCTGAGCGGGCGATT	IDTGRKGPERAI
16	ATTGATACGGGTCGGAAGGGGCCTGAGCGGGCGATT	IDTGRKGPERAI
17	ATTGATACGGGTCGGAAGGGGCCTGAGCGGGCGATT	IDTGRKGPERAI
18	ATTGATACGGGTCGGAAGGGGCCTGAGCGGGCGATT	IDTGRKGPERAI
19	ATTGATACGGGTCGGAAGGGGCCTGAGCGGGCGATT	IDTGRKGPERAI
20	ATTGATACGGGTCGGAAGGGGCCTGAGCGGGCGATT	IDTGRKGPERAI
21	CATGATAATCATGGGCCGAATGGGTTGGCGGTTGGG	HDNHGPNGLAVG
22	CATGATAATCATGGGCCGAATGGGTTGGCGGTTGGG	HDNHGPNGLAVG
23	ATTGATACGGGTCGGAAGGGGCCTGAGCGGGCGATT	IDTGRKGPERAI

Table S2-: Phage DNA Sequence from bio-panning against PD-1 protein from round-6.

Peptide Synthesis. The Solid Phase Peptide Synthesis (SPPS) technique was used to synthesize peptides, utilizing rink amide resin as the solid support. Initially, 50 µmoles (100 mg) of the resin were soaked in dry DMF for 1 hour to allow for swelling. The resin was then subjected to deprotection to remove the Fmoc group, which was accomplished using a 20% piperidine solution in dry DMF, applied in two successive 10-minute treatments. For the coupling of amino acids, a mixture of amino acids, DIEA, and COMU—in a ratio of 150 µmol: 300 µmol: 150 µmol—was used for each coupling step in the peptide sequence. Capping was performed after each coupling to prevent undesired reactions. The resin was washed with DMF twice, followed by DCM once, then DMF again twice, DCM once, and finally DMF twice. After the final coupling, the resin was washed with DMF twice, DCM once, DMF twice, and DCM once, and then with methanol six times before drying under a vacuum in the hood. For cleavage, 0.1-0.5 mL of the cleavage mixture, prepared in a ratio of 18:1:1 TFA: H2O: TIPS, was added and incubated for 3 hours. The peptide was precipitated using 10 mL of pre-cooled ether, and the resulting solution was centrifuged for 10 minutes in the hood. The pellet was then dried and purified by reverse-phase high-performance liquid chromatography (RP-HPLC). For FITC-ahx-II peptide synthesis, the Fmoc group of the N-terminal amino acid of II (IDTGRKGPERAI) peptide was removed using 20% piperidine in DMF. After deprotection, amino hexanoic acid (ahx) was attached as a linker by using the amino acid coupling method. FITC reagent was dissolved in DMF at least 3 molar excess along with the addition of DIPEA (6 molar), and mixture was added to resin for 3-4 hours in the dark at room temperature with agitation. The resin was washed with DMF and standard peptide cleavage method was performed. After precipitation, the pellet was dried and purified by reverse-phase high performance liquid chromatography (RP-HPLC).

Peptide ELISA to measure binding specificity of the II peptide to the PD-1 protein. The synthesized peptide (IDTGRKGPERAI) was utilized in the ELISA assay. It was coated on a maleic anhydride-activated plate (Thermo-Fisher, Carlsbad, CA) at 2 µg per well in 0.1 M NaHCO3 (pH 8.6) and incubated at 4 °C overnight. After coating, the plate was blocked with 5 mg/mL bovine serum albumin (BSA) for 2 hours. Following the blocking step, the plate was washed six times with TBST (0.1% Tween). It was then incubated with 1 µg per well of his-tagged PD-1 as the target protein and his-tagged PDL1 as the control protein for 2 hours. After incubation, unbound proteins were removed by washing the plate six times with 0.5% TBST. Next, a horseradish peroxidase (HRP) conjugated anti-his tag antibody (Cat# HRP-66005) was applied at a dilution of 1:1500, followed by a 1-hour incubation at room temperature. The plate was washed six times with 0.5% TBST again, and then TMB substrate was added and incubated for 10 minutes to visualize the color change.

Flow cytometry experiment to measure the binding specificity of II peptide to PD-1 protein. The 100 µl of 250 nM his-tagged PD1 protein was prepared in TBS buffer and incubated with 1 µL (2 x diluted) of Ni-NTA magnetic beads (G-biosciences, Ref. 062N-A). The bead/protein complex was washed twice with 200 µL of TBST (0.1% tween) buffer. Then, 500 picomoles of FITC-ahx- II peptide were prepared in 50 µl of TBS buffer and incubated with a complex of protein and Ni-NTA beads for 1h at RT with rotation. After incubation, the beads were washed two times with 200 µl of TBST (0.5% tween) and resuspended with 100 µl of TBS buffer. The fluorescence emission produced by the FITC II peptide bound on the complex of protein and Ni-NTA bead was analyzed by flow cytometer measurement (Guava easyCyte 5HT, Catalog# 0500-4005).

Flow cytometry experiment to measure the binding affinity of the II peptide to PD-1 protein. The 50 µL of 250 nM His-tagged PD-1 protein was prepared in TBS buffer and incubated with 1 µL of Ni-NTA magnetic beads (2 x diluted, G-Biosciences, Ref # 062N-A), rotating for 1 h at RT. The protein/bead complex was then washed twice with 200 µL of TBST (0.1% tween). Then 10,30,100,300,3000,10000 nM solutions FITC-ahx -II peptide was prepared in 50 µL of TBS buffer and incubated with the bead/protein complex for 1h at RT with rotation. After incubation, the beads were washed twice with 200 µL of TBST (0.5% tween) buffer and finally resuspended with 100 µL of TBS buffer. The FITC-ahx-II peptide bound on protein/bead complex was then analyzed by flow cytometry (Guava easyCyte 5HT, Catalog # 0500-4005). Each experiment was performed for three trials, and flow events were integrated to calculate average fluorescence emission and binding affinity (K_d).

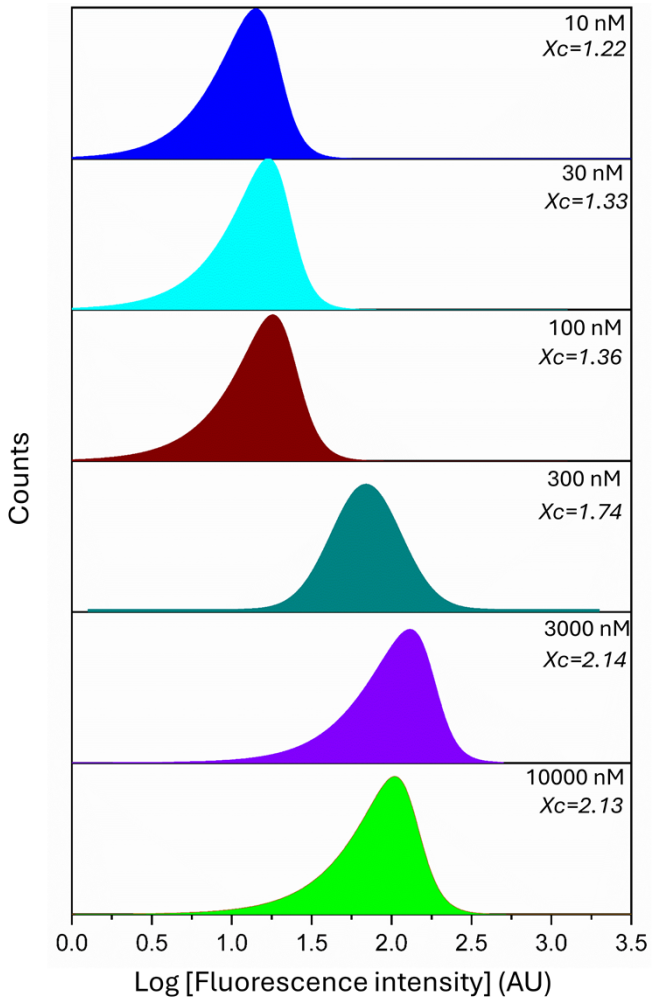


Figure S3-: The average green fluorescence emission intensity (Xc) of the Fitc-II peptide bound to a complex of Ni-NTA beads and His-tagged PD-1 protein was measured using flow cytometry.

Peptide inhibition assay:

0.5 µg of recombinant commercial human Fc-tagged PD-1 protein (Sino Bio, catalog# 10377-H02H) was coated per well on a 96-well plate overnight at 4℃. The plate was blocked with 5mg/mL BSA at room temperature for 2h. 10 µM of II (IDTGRKGPERAI) peptide was added to the well and incubated for two hours at room temperature. The commercial human His-tagged PD-L1 (0.5 µg, Sino Bio, catalog# 10084-H08H) was then added and incubated for 1 hour. HRP conjugated anti-his-tagged antibody, and TMB were added to each well, and the readings were taken after 10 min.

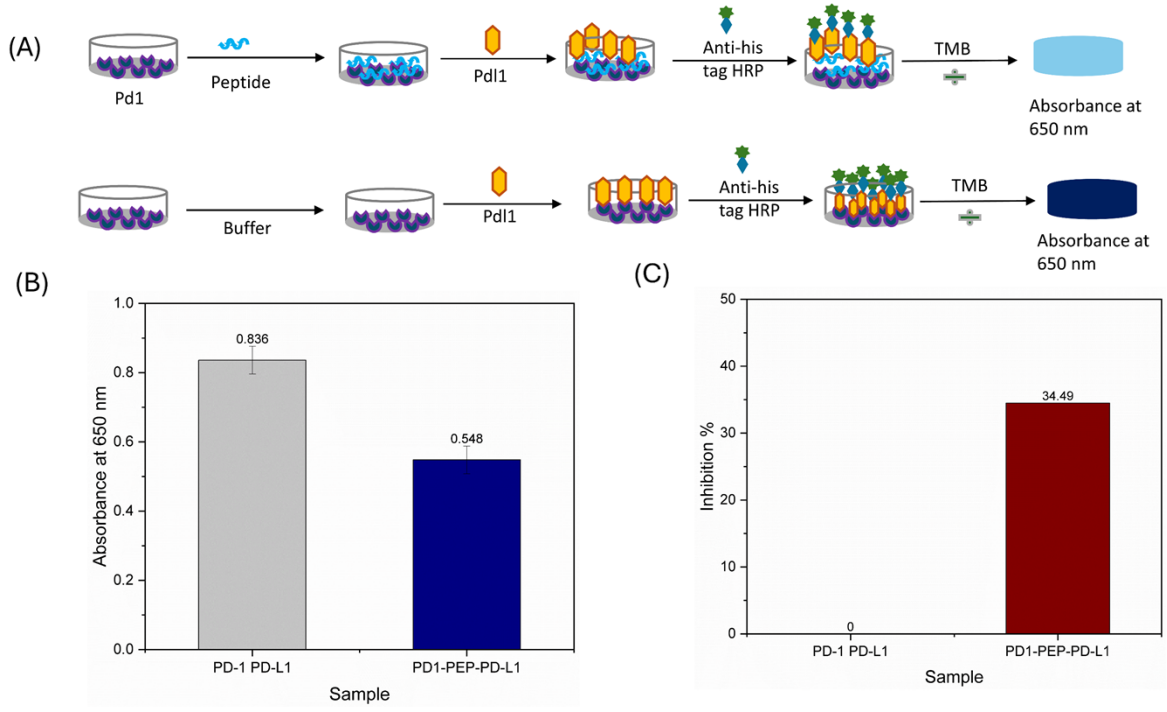


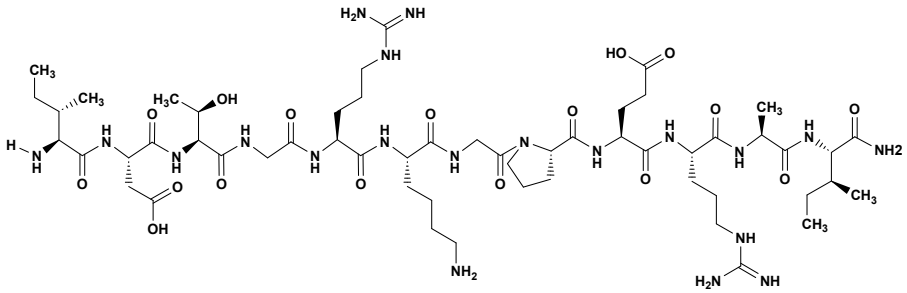
Figure S4-:The determination of inhibition efficacy of II peptide against PD-1/PD-L1 interaction. (A) The schematic representation of the ELISA competition assay to measure the inhibition efficacy of the ligand (not to scale). (B) The inhibition efficacy of the ligand was determined as an opposite function of absorbance caused by colored products. The blue color is formed due to HRP-mediated oxidation of TMB. The absorbance is measured by employing a Clariostar microplate reader at 650 nm. The error bar indicates the standard deviation of the absorbance from their mean observed trial. (C) Inhibition efficacies (%) of II peptides against PD-1/PD-L1.

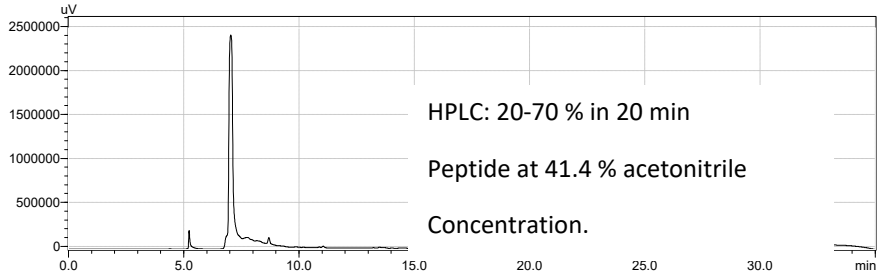
RP-HPLC analysis and mass spectrum of II (IDTGRKGPERAI) peptide.

IDTGRKGPERAI

Calculated molecular weight =1310.48 g/mol.

Observed molecular weight = 1310.51 g/mol.





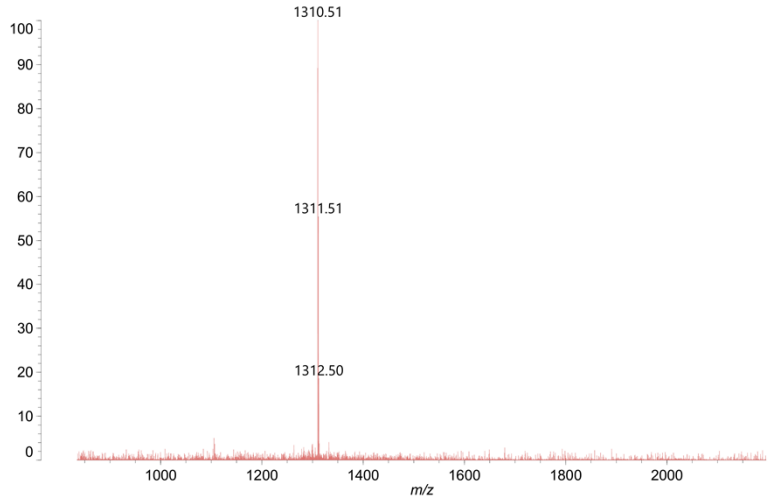


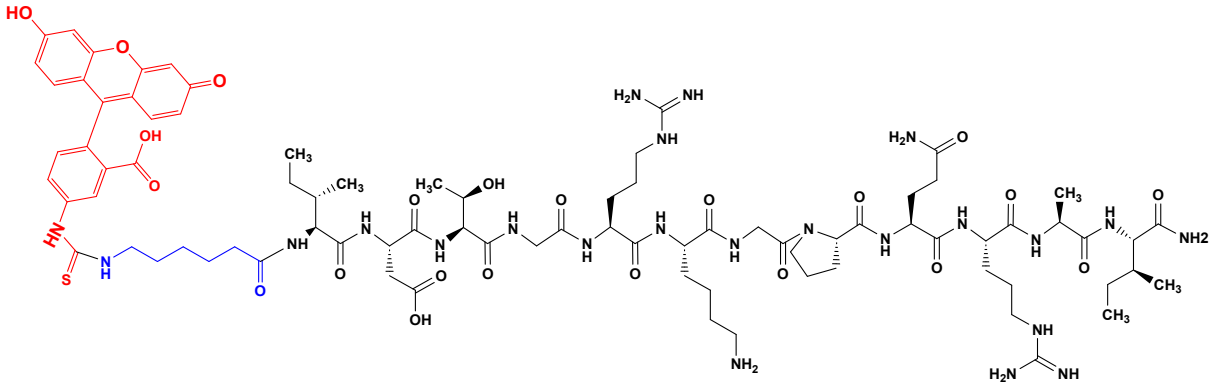
Figure S5-: RP-HPLC analysis and mass spectrum of II peptide.

RP-HPLC analysis and mass spectrum of FITC-ahx-II peptide.

FITC-ahx-IDTGRKGPERAI

Calculated molecular weight-: 1814 g/mol

Observed molecular weight-: 1815.8 g/mol.



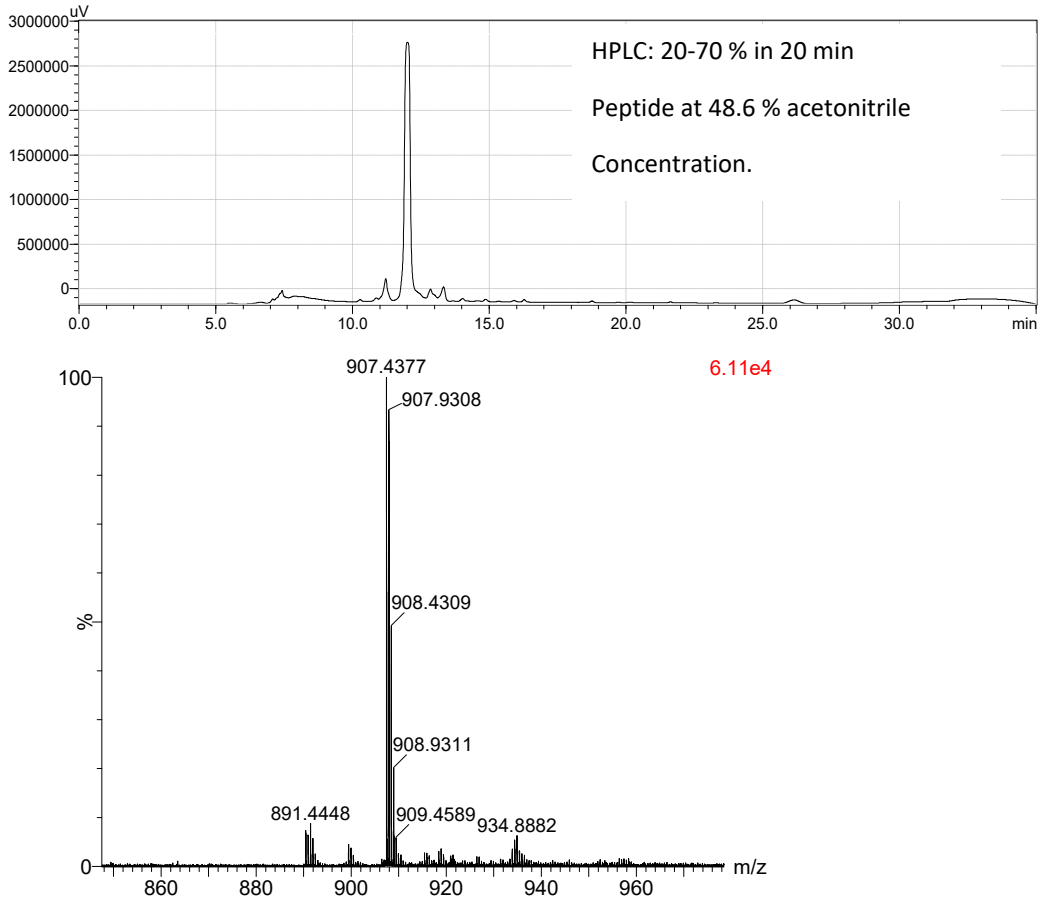


Figure S6-: RP-HPLC analysis and mass spectrum of FITC-ahx- II peptide.

Docking method and results analysis

CABS-dock method. The docking of peptide and protein was performed using the CABS-dock web server for flexible protein-peptide docking (freely available at http://biocomp.chem.uw.edu.pl/CABSdock) without prior knowledge about the binding site. This method enables full flexibility of the peptide structure and large-scale flexibility of protein fragments during a blind search of binding sites. This server procedure generates 10,000 model structures of the protein-peptide complex. These resulting complexes are further grouped in clusters of similar complexes and ranked according to cluster size from the largest to the 10th largest. The clustering is based on the RMSD of the entire protein-peptide complex. Kurcinski, Mateusz, et al. have provided detailed descriptions of this server and its benchmark tests.[1]

Input data. The complex structure of the PD-1/PD-L1 interaction is available in the Protein Data Bank (PDB) under the code 4ZQK.[2] This PDB ID was utilized as the input receptor structure. The input peptide data consists of a sequence containing 12 amino acid residues: IDTGRKGPERAI (note that no information regarding the peptide's secondary structure was used). In CABS-dock, we employed the default settings, ensuring that the peptide's structure remained fully flexible while the receptor structure was maintained close to its input conformation. Further, details about the II peptide and 4ZQK complex can be found on the CABS-DOCK server, Project name Satya Arya 4ZQK. The docking project was submitted to the server on Jan 18th, 2025, at 16:53:40 p.m.

(a)

Cluster name	Cluster density	Average cluster RMSD (A^0)	Maximum RMSD within the cluster (A^0)	Number of cluster element
Cluster 1	67.63	1.84	17.88	125
Cluster 2	28.60	4.68	24.18	134
Cluster 3	28.11	3.48	14.82	98
Cluster 4	22.28	7.35	23.77	164
Cluster 5	11.44	6.55	26.96	75
Cluster 6	8.92	12.09	30.79	108
Cluster 7	7.55	13.36	35.03	101
Cluster 8	7.19	13.47	34.71	97
Cluster 9	5.50	11.26	34.60	62
Cluster 10	2.63	13.68	40.82	36

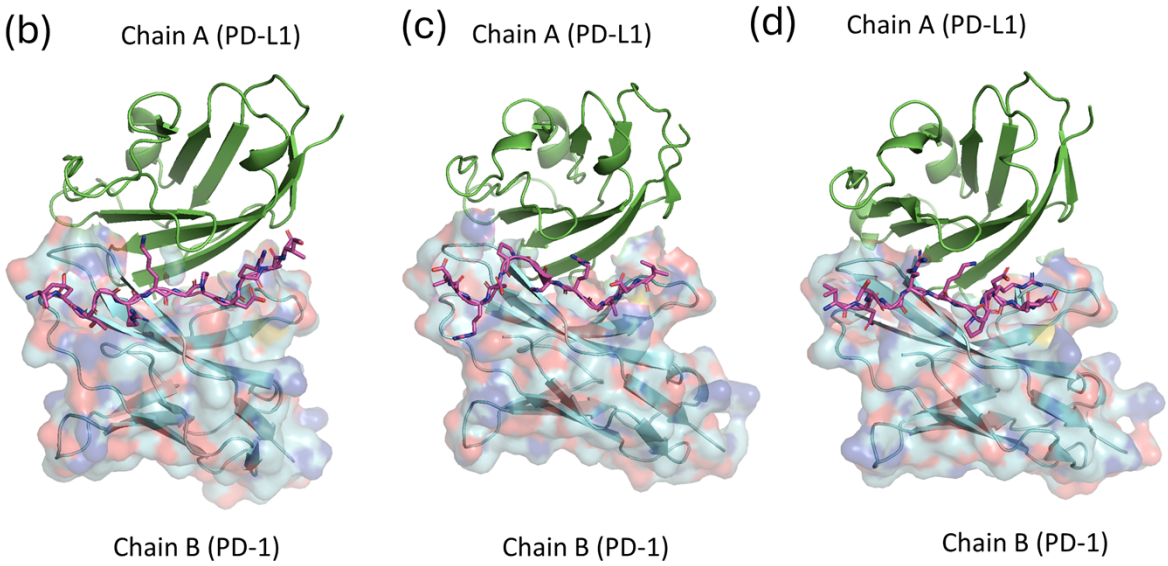


Figure S7-: Cluster analysis and molecular docking posing poses for 2nd, 3rd and 4th best models. (a) The table presents the details of the top 10 structural clusters displayed on the CABS-dock server, ranked according to their cluster density. (b-d) Molecular docking poses for the 2nd, 3rd, and 4th best models of the II peptide in complex with PD-1 and PD-L1 (PDB ID: 4ZQK). The receptor protein consists of two chains: Chain A (PD-L1) is represented in green as a cartoon, while Chain B (PD-1) is shown as a surface model in magenta with 60% transparency. The peptide is depicted in purple. The RMSDs for peptide II in the models are as follows: (b) RMSD = 4.68 Å, (c) RMSD = 3.48 Å, and (d) RMSD = 7.35 Å.

Cluster analysis and molecular docking poses for the 2nd,3rd, and 4th best models.

Amino acid interaction with chain-A (PD-L1) closer than 4.5 A^0	Peptide amino acid residue	Amino acid interaction with chain-B (PD-1) closer than 4.5 A^0
0	**I (Ile)**	Asn 74, Met 70
0	**D (Asp)**	Thr 76, Asn 74
Tyr 123	**T (Thr)**	Glu 136, Thr 76, Leu 122, Tyr 66
Tyr 123, Arg 113	**G (Gly)**	Ile 134, Glu 136, Thr 76
Glu 60, Arg 113, Asp 61, Glu 58	**R (Arg)**	Ile 134, Glu 136
Asp 61, Glu 58	**K (Lys)**	Gln 133, Lys 135, Ile 134
0	**G (Gly)**	Lys 135, Pro 34
0	**P (Pro)**	Gln 133
0	**E (Glu)**	Asn 33, Gln 133, Asn 58, Lys 135
His 78, Asn 63, Val 10, Lys 75	**R (Arg)**	Gln 133, Lys 131
0	**A (Ala)**	Lys 131
0	**I (Ile)**	Ser 127, Glu 61, Asn 58, Lys 135, Lys 131, Pro 130, Ser 60, Gln 133, Ala 129, Thr 59.

Table S3-: Showing interactions of amino acid residue from peptide interacting with Chain A (PD-1) and Chain B (PD-L1) amino acid closer than 4.5 A^0 with frequency of contact from 0.1 to 1.0.

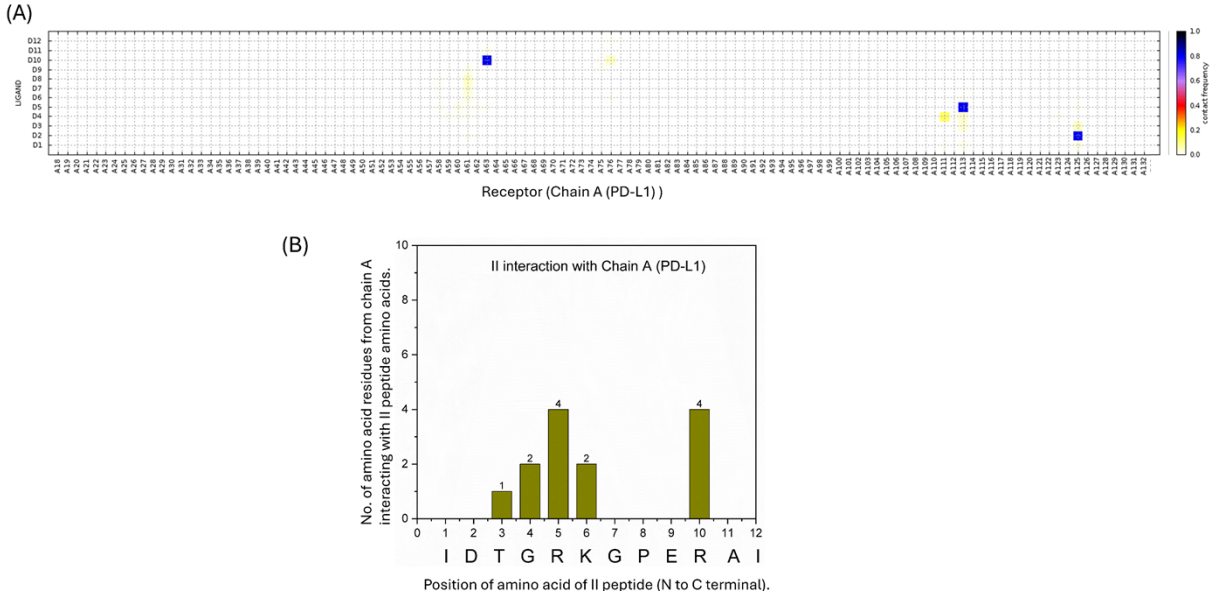


Figure S8-: The contact between II peptide amino acid residues and PD-L1 protein amino acid residues. (A) The contact frequency map illustrates the interactions between residues, with Chain A representing PD-L1 from the PD-1/PD-L1 complex and the II peptide residue. The x-axis shows the amino acid residues of the receptor protein, while the y-axis displays the amino acid residues of the peptide. The contact frequency scale ranges from 0.0 (indicated by white) to 1.0 (represented by intense blue).(B) Shows the number of contacts between each amino acid of the peptide and the amino acid residues of Chain A (PD-L1). In total, there are 13 contacts (out of 50) between the II peptide residue and Chain A (PD-L1). Among these, **only 3** residues from the receptor exhibit a better contact frequency with the peptide residue, which are highlighted in blue in Figure A.

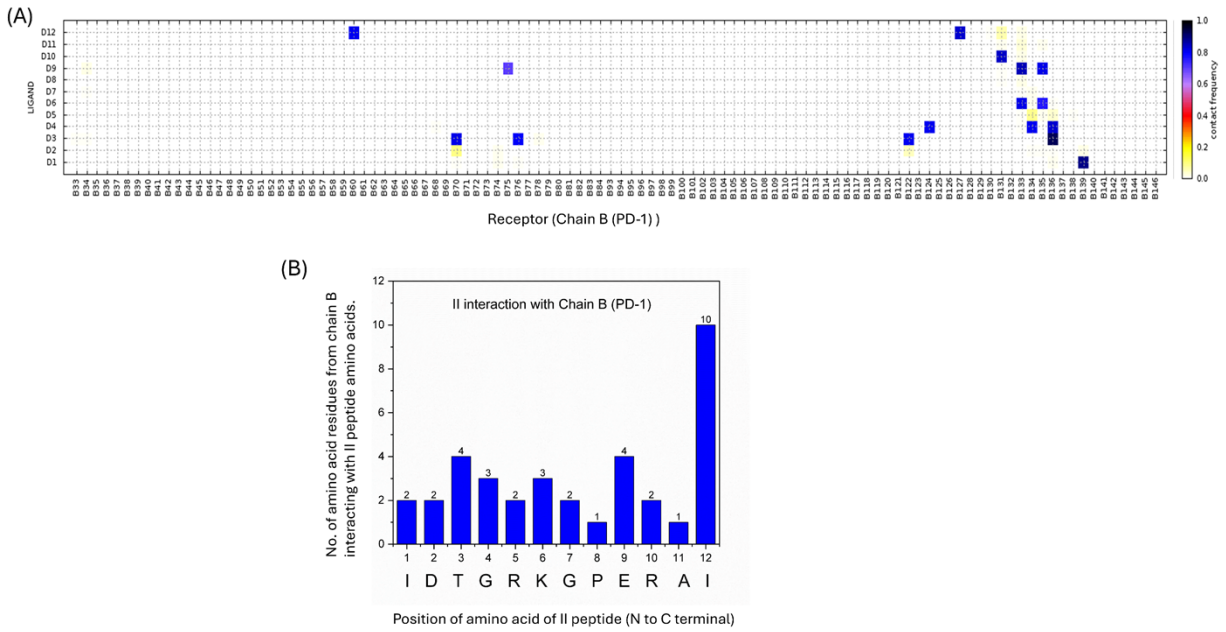


Figure S9-: The contact between II peptide amino acid residues and PD-1 protein amino acid residues. (A) The contact frequency map illustrates the interactions between residues, with Chain B representing PD-1 from the PD-1/PD-L1 complex and the II peptide residue. The x-axis shows the amino acid residues of the receptor protein, while the y-axis displays the amino acid residues of the peptide. The contact frequency scale ranges from 0.0 (indicated by white) to 1.0 (represented by intense blue). (B) Shows the number of contacts between each amino acid of the peptide and the amino acid residues of Chain A (PD-L1). There are 37 contacts (out of 50) between the II peptide residue and Chain A (PD-L1). Among these, **16 residues** from the receptor exhibit a better contact frequency with the peptide residue, which are highlighted in blue in Figure A.

Amino acid interaction with chain-A (PD-L1) with best frequency contact	Peptide amino acid residue	Amino acid interaction with chain-B (PD-1) with best frequency contact
0	I (Ile)	0
0	D (Asp)	0
0	T (Thr)	Thr 76, Leu 122
0	G (Gly)	Glu 136
Glu 58	R (Arg)	
Glu 58	K (Lys)	Gln 133, Ile 134
0	G (Gly)	0
0	P (Pro)	0
0	E (Glu)	Gln 133
0	R (Arg)	Gln 133
0	A (Ala)	0
0	I (Ile)	0

Table S4-: Showing interactions of amino acid residue from peptide interacting with Chain A (PD-1) and Chain B (PD-L1) amino acid closer than 3A^{o}.

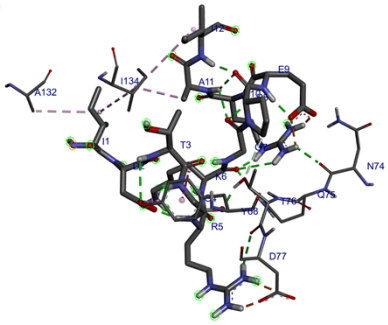
HADDOCK and M-DOCK docking of II peptide to PD-1 protein and PD-1/PD-L1 complex protein.

To further clarify the docking results in support of our experimental results, we conducted additional docking experiments with the II peptide (IDTGRKGPERAI) using different software. Specifically, we employed the peptide sequence to dock against the PD-1 protein using HADDOCK.[3, 4] Our analysis revealed that 100 out of 176 structures in cluster 1 exhibited successful docking with the peptide ligand, indicating potential contact at the interface of the PD-1/PD-L1 interaction. The common interacting residues A132, I134, and T76, identified from both docking analyses, further strengthen the contact residue at the interface of the PD-1/PD-L1 interaction by the II peptide as mentioned in (B) and (C).To further validate the docking, we use the MDOCK[5] server to locate the position of the II peptide in the complex of the PD-1/PD-L1 protein. We got the structure as mentioned in (D), which further validates the contact region of the II peptide toward the interface of PD-1/PD-L1.

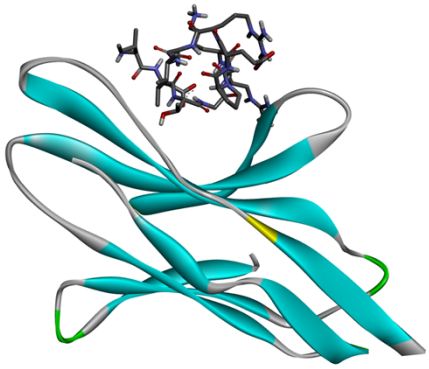
(A)

Cluster	1	2	3	4
HADDOCK score	-49.7+/-0.4	-43.3+/-4.7	-33.2 +/- 4.7	-32.4
Cluster size	100	18	15	11
RMSD from overall lowest energy structure	2.9+/-0.2	1.0 +/- 0.7	2.8 +/- 0.3	2.9 +/- 0.2
Van der waals energy	-31.6+/- 5.2	-18.3 +/- 4.7	-30.9 +/- 5.7	-24.9 +/- 1.8
Electrostatics energy	-138.9+/-27.1	-173.5 +/-27.6	-74 +/- 16.1	-88.4 +/- 17.4
Desolvation energy	7.7 +/-1.1	5.7 +/- 1.9	5.9 +/- 5.2	6.8 +/- 0.4
Restraint violation energy	19.8+/-7.0	39.4 +/-16.0	65.7 +/- 33.7	34.6 +/- 19.2
Buried surface area	1014.2 +/-88.7	854.8 +/- 55.9	879.1 +/- 88.5	817.9 +/- 14.1
Z-score	-1.9	-1.3	-0.2	-0.2

(C)



(B)



(D)

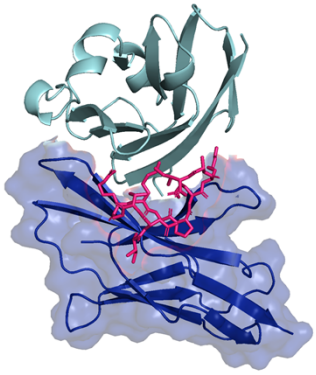


Figure S10-: (A) Table showing data for the top four models with their different sets of characteristic values. (B) Cluster 1 represents the docking of the II (IDTGRKGPERAI) peptide to the PD-1 protein. Out of 176 structures, 100 structures represent cluster 1. (C) Contact map between PD-1 protein and II peptide for cluster 1. The numbers 1 to 12 represent the amino acid residue from the peptide, whereas the numbers higher than 12 are from the PD-1 protein amino acid residues. D) The docking of the II peptide with the PD-1/PD-L1 complex. The light green represents the PD-L1 protein chain, blue represents PD-1, and hot pink represents the II peptide chain.

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