Supplementary Information

Design and Self Assembly of Tri-Terpene Peptide Conjugates and their Interactions with EGFR and EGFR Mutant Receptors -An In Silico and In Vitro Study

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Table S1a. Predicted binding interactions as determined by PLIP for Wild Type EGFR with VPWXE and its conjugates

VPWXE									
Hydrogen Bonds	Dis H-A (Å)	Hydrophobic Interactions	Dis (Å)	π-Stacking	Dis (Å)	π-Cation Interactions	Dis (Å)	Salt Bridges	Dis (Å)
LYS 721 GLU 738 ARG 817 ASN 818 ASN 818 ASP 831 ASP 831 GLY 883	3.28 2.47 3.06 3.00 2.86 2.60 2.71 3.57	ALA 698 ALA 731 ILE 735 LEU 834	3.85 3.65 3.63 3.75					LYS 721	4.87
Hydroxybetulinate-VPWXE									
Hydrogen Bonds	Dis H-A (Å)	Hydrophobic Interactions	Dis (Å)	π-Stacking	Dis (Å)	π-Cation Interactions	Dis (Å)	Salt Bridges	Dis (Å)
LEU 694 GLY 700 LYS 721 ARG 817 ASN 818 ASN 818 LYS 851	2.19 2.95 2.57 2.28 3.18 3.43 2.37	LEU 694 LEU 694 ALA 698 PHE 699 LYS 721 ILE 735 ARG 817 LEU 820 LEU 820 ASP 831 LEU 834	3.71 3.57 3.41 3.44 3.83 3.61 3.96 3.06 3.92 3.78 3.77						
Oleanolate-VPWXE									
Hydrogen Bonds	Dis H-A (Å)	Hydrophobic Interactions	Dis (Å)	π-Stacking	Dis (Å)	π-Cation Interactions	Dis (Å)	Salt Bridges	Dis (Å)
LYS 721 GLU 734 ASP 737 ASP 813 ARG 817 ARG 817 THR 830 LYS 851	2.55 3.35 3.06 2.64 1.92 2.40 2.38 2.43	ALA 698 PHE 699 VAL 702 GLU 734 LEU 820 THR 830 ASP 831 LEU 834 LEU 834	3.61 3.54 3.54 3.46 3.42 3.94 3.60 3.51 3.52					LYS 721	3.97

		PRO 853	3.80						
Ursolate-VPWXE									
Hydrogen Bonds	Dis H-A (Å)	Hydrophobic Interactions	Dis (Å)	π-Stacking	Dis (Å)	π-Cation Interactions	Dis (Å)	Salt Bridges	Dis (Å)
GLU 738 THR 766 ASP 831 ASP 831	3.17 3.20 3.03 4.06	LEU 694 ALA 698 VAL 702 ALA 719 MET 769 LEU 775 ARG 817 LEU 820 LEU 820 ASP 831	3.58 3.72 3.80 3.68 3.86 3.59 3.58 3.74 3.64 3.93						
Perillate-VPWXE									
Hydrogen Bonds	Dis H-A (Å)	Hydrophobic Interactions	Dis (Å)	π-Stacking	Dis (Å)	π-Cation Interactions	Dis (Å)	Salt Bridges	Dis (Å)
SER 696 GLY 700 THR 766 ASP 831	2.90 2.09 2.17 2.67	LEU 694 LEU 694 ALA 698 VAL 702 VAL 702 ALA 719 THR 766 LEU 768 LEU 820 LEU 820 THR 830	3.58 3.60 3.42 3.91 3.32 3.65 3.82 3.89 3.75 3.62 3.28					LYS 721 LYS 721	4.04 3.82

Table S1b. Predicted binding interactions as determined by PLIP for T90M/L858R EGFR with VPWXE and its conjugates

VPWXE									
Hydrogen Bonds	Dis H-A (Å)	Hydrophobic Interactions	Dis (Å)	π-Stacking	Dis (Å)	π-Cation Interactions	Dis (Å)	Salt Bridges	Dis (Å)
LYS 745 CYS 797 ASP 800 ARG 841 ASN 842	3.18 2.82 2.48 3.25 2.17	LEU 718 LEU 718 LEU 718 ALA 743 LYS 745 LEU 792 MET 793 LEU 844 PHE 997 PHE 997	3.95 3.50 3.93 3.54 3.59 3.79 3.81 3.69 3.63 3.69					LYS 745	4.58
Hydroxybetulinate-VPWXE									
Hydrogen Bonds	Dis H-A (Å)	Hydrophobic Interactions	Dis (Å)	π-Stacking	Dis (Å)	π-Cation Interactions	Dis (Å)	Salt Bridges	Dis (Å)
ALA 722 PHE 723 ARG 841 ASN 842 ASP 855 ASP 855 ASP 855 ILE 878 LYS 879	2.31 2.82 2.91 2.67 2.92 2.34 2.53 2.90 2.51	LEU 718 VAL 726 VAL 726 LEU 799 ASP 837 ARG 841 LYS 879	3.50 3.84 3.73 3.76 3,63 3.53 3.35					LYS 745 HIS 835	3.22 5.32

LYS 879	2.47								
Oleanolate-VPWXE									
Hydrogen Bonds	Dis H-A (Å)	Hydrophobic Interactions	Dis (Å)	π-Stacking	Dis (Å)	π-Cation Interactions	Dis (Å)	Salt Bridges	Dis (Å)
LEU 718 ASP 837 ASN 842 ASN 842	2.66 2.19 1.99 2.00	LEU 718 LEU 718 VAL 726 VAL 726 ALA 743 LEU 792 LEU 799 LEU 844 THR 854 TRP 880 PHE 997	3.81 3.45 3.69 3.98 3.71 3.70 3.82 3.71 3.55 3.88 3.88						
Ursolate-VPWXE									
Hydrogen Bonds	Dis H-A (Å)	Hydrophobic Interactions	Dis (Å)	π-Stacking	Dis (Å)	π-Cation Interactions	Dis (Å)	Salt Bridges	Dis (Å)
MET 793 CYS 797 LYS 870	2.31 2.61 2.84	LEU 718 ALA 722 VAL 726 VAL 726 ARG 841 LEU 844 THR 854 LYS 879 LYS 879 TRP 880 PHE 997	3.54 3.70 3.71 3.74 3.53 3.68 3.83 3.59 3.66 3.39 3.85						
Perillate-VPWXE									
Hydrogen Bonds	Dis H-A (Å)	Hydrophobic Interactions	Dis (Å)	π-Stacking	Dis (Å)	π-Cation Interactions	Dis (Å)	Salt Bridges	Dis (Å)
GLY 796 THR 854	3.57 3.03	LEU 718 LEU 718 LEU 718 VAL 726 VAL 726 ARG 841 ARG 841 LEU 844 PHE 997 PHE 997 ALA 1000	3.90 3.94 3.39 3.54 3.63 3.98 3.96 3.65 3.92 3.45 3.76						

Table S2a. Predicted binding interactions as determined by PLIP for Wild Type EGFR with MEGPSKCCFSLALSH (MFSL) and its conjugates

MFSL									
Hydrogen Bonds	Dis H-A (Å)	Hydrophobic Interactions	Dis (Å)	π-Stacking	Dis (Å)	π-Cation Interactions	Dis (Å)	Salt Bridges	Dis (Å)
SER 696 ALA 698 PHE 699 GLY 700 GLY 700 LYS 721 ARG 779	2.52 2.64 2.44 2.05 2.42 2.86 2.10	ALA 698 TRP 856	3.78 3.71					ARG 779	5.27

ARG 779	3.10								
ARG 779	2.64								
ARG 817	2.85								
ARG 817	2.31								
ARG 817	3.35								
ARG 817	2.18								
ASP 831	2.99								
ASP 831	2.54								
ASP 831	2.26								
ASP 831	2.73								
GLY 833	3.14								
LYS 851	2.38								
(Hydroxy betulinate)$_2$-MFSL									
Hydrogen Bonds	Dis H-A (Å)	Hydrophobic Interactions	Dis (Å)	π-Stacking	Dis (Å)	π-Cation Interactions	Dis (Å)	Salt Bridges	Dis (Å)
LYS 692	2.21	LEU 649	3.89					ARG 817A	4.19
VAL 693	1.99	ALA 698	3.58						
LYS 704	2.27	VAL 702	3.47						
CYS 773	3.00	LYS 704	3.75						
ASP 776	1.92	LYS 721	3.55						
ASP 776	2.52	PRO 770	3.66						
LYS 822	2.07	LYS 855	3.45						
ASP 831	2.54	TRP 856	3.96						
ASP 831	2.68	ASP 988	3.98						
LYS 855	2.85								
(Oleanolate)$_2$-MFSL									
Hydrogen Bonds	Dis H-A (Å)	Hydrophobic Interactions	Dis (Å)	π-Stacking	Dis (Å)	π-Cation Interactions	Dis (Å)	Salt Bridges	Dis (Å)
SER 696	2.28	VAL 702	3.58						
LYS 721	2.69	PHE 771	3.68						
ARG 779	2.67	LEU 834	3.98						
ARG 779	2.02	LYS 851	3.61						
ARG 817	2.08	PRO 853	3.65						
ARG 817	3.26								
ASN 818	2.93								
ASN 818	3.24								
(Perillate)$_2$-MFSL									
Hydrogen Bonds	Dis H-A (Å)	Hydrophobic Interactions	Dis (Å)	π-Stacking	Dis (Å)	π-Cation Interactions	Dis (Å)	Salt Bridges	Dis (Å)
SER 696	2.59	LEU 775	3.93					LYS 721	3.96
LYS 721	2.12	ARG 817	3.93						
THR 830	3.57	ARG 817	3.69						
VAL 852	2.94	ASP 831	3.77						
LYS 855	3.14	LEU 834	3.91						
		PRO 853	3.65						
		LYS 855	3.73						
		TRP 856	3.45						
		LYS 889	3.65						
(Ursolate)$_2$-MFSL									
Hydrogen Bonds	Dis H-A (Å)	Hydrophobic Interactions	Dis (Å)	π-Stacking	Dis (Å)	π-Cation Interactions	Dis (Å)	Salt Bridges	Dis (Å)
LYS 692	2.51	PHE 771	3.57						
GLU 734	3.42	TYR 777	3.73						
CYS 773	2.21	LEU 820	3.48						
ASP 813	2.79	LEU 834	3.55						
ARG 817	1.85	LEU 834	3.79						
ARG 817	3.29	ALA 847	3.90						
ASN 818	2.88	LYS 855	3.96						
		TRP 856	3.79						
		TRP 856	3.25						
		LYS 889	3.03						

Table S2b. Predicted binding interactions as determined by PLIP for T790M/L858R EGFR with MEGPSKCCFSLALSH (MFSL) and its conjugates

MFSL									
Hydrogen Bonds	Dis H-A (Å)	Hydrophobic Interactions	Dis (Å)	π-Stacking	Dis (Å)	π-Cation Interactions	Dis (Å)	Salt Bridges	Dis (Å)
SER 720	3.58	LEU 718	3.35	PHE 856A	5.16				
LYS 745	2.58	ALA 722	3.6						
LYS 745	3.09	VAL 726	3.15						
ARG 803	2.29	LYS 745	3.91						
ARG 841	2.09	LEU 792	3.6						
ARG 841	2.49	LEU 844	3.63						
ARG 841	2.48	LEU 844	3.87						
ARG 841	2.83	TRP 880	3.58						
THR 854	3.24	PHE 997	3.67						
THR 854	2.1								
THR 854	1.78								
ASP 855	1.91								
ASP 855	2.89								
PHE 856	3.17								
VAL 876	2.08								
ILE 878	3.09								
(Hydroxy betulinate)$_2$-MFSL									
Hydrogen Bonds	Dis H-A (Å)	Hydrophobic Interactions	Dis (Å)	π-Stacking	Dis (Å)	π-Cation Interactions	Dis (Å)	Salt Bridges	Dis (Å)
ALA 722	2.40	LEU 718	3.58					LYS 745	4.71
ASP 800	2.68	ALA 722	3.32						
ASP 800	2.88	PHE 723	3.90						
ARG 803	2.49	ALA 743	3.66						
ARG 841	2.91	ARG 803	3.40						
ARG 841	2.62	GLU 804	3.88						
ASN 842	3.05	VAL 876	3.37						
THR 854	2.19	ILE 878	3.43						
THR 854	2.31	LYS 879	3.60						
ASP 855	2.48	TYR 891	3.83						
ASP 855	3.37	ALA 920	3.62						
ASN 996	1.76	PHE 997	3.55						
(Oleanolate)$_2$-MFSL									
Hydrogen Bonds	Dis H-A (Å)	Hydrophobic Interactions	Dis (Å)	π-Stacking	Dis (Å)	π-Cation Interactions	Dis (Å)	Salt Bridges	Dis (Å)
LEU 718	2.16	VAL 717	3.93			ARG 803	5.09		
ARG 803	1.92	VAL 717	3.68						
ARG 803	2.36	LEU 718	3.50						
ARG 841	2.96	ASN 996	3.05						
ARG 841	2.94	PHE 997	3.49						
LYS 879	2.67	PHE 997	3.67						
LYS 879	3.12								
GLU 906	3.54								
(Perillate)$_2$-MFSL									
Hydrogen Bonds	Dis H-A (Å)	Hydrophobic Interactions	Dis (Å)	π-Stacking	Dis (Å)	π-Cation Interactions	Dis (Å)	Salt Bridges	Dis (Å)
LEU 718	2.75	ALA 722	3.54						
ALA 722	2.21	PHE 723	3.94						
MET 793	3.15	LEU 799	3.63						
MET 793	2.83	ARG 836	3.72						
ARG 803	2.28	ASP 837	3.90						
ARG 803	2.90	ARG 841	3.45						
ASP 837	2.44	LEU 844	3.60						
ASP 837	2.16	LYS 879	3.84						
ARG 841	3.19	TRP 880	3.25						
ASN 842	2.10	TYR 891	3.67						
ASN 842	3.17	TYR 891	3.82						
THR 854	3.22	LYS 913	3.37						
VAL 876	3.11	PRO 914	3.87						

		ALA 920	3.41						
(Ursolate)$_2$-MFSL									
Hydrogen Bonds	Dis H-A (Å)	Hydrophobic Interactions	Dis (Å)	π-Stacking	Dis (Å)	π-Cation Interactions	Dis (Å)	Salt Bridges	Dis (Å)
SER 720	2.21	VAL 717	3.42						
SER 720	2.52	LEU 718	3.97						
GLY 721	2.14	VAL 726	3.66						
MET 793	2.28	ALA 743	3.50						
ARG 841	2.20	ARG 748	3.92						
ARG 841	2.85	LEU 799	3.65						
ARG 841	2.76	PRO 877	3.60						
THR 854	2.01	PHE 997	3.94						
ASP 855	2.41	PHE 997	3.76						
VAL 876	2.44								
VAL 876	1.87								

Table S3a. Predicted binding interactions as determined by PLIP for Wild Type EGFR with neat, unconjugated terpenes.

Hydroxybetulinic acid									
Hydrogen Bonds	Dis H-A (Å)	Hydrophobic Interactions	Dis (Å)	π-Stacking	Dis (Å)	π-Cation Interactions	Dis (Å)	Salt Bridges	Dis (Å)
ASP 831	2.26	LEU 694	3.87					LYS 721	3.18
		LEU 694	3.98						
		VAL 702	3.39						
		VAL 702	3.89						
		LYS 721	3.80						
		LYS 721	3.72						
		LEU 820	3.39						
Oleanolic acid									
Hydrogen Bonds	Dis H-A (Å)	Hydrophobic Interactions	Dis (Å)	π-Stacking	Dis (Å)	π-Cation Interactions	Dis (Å)	Salt Bridges	Dis (Å)
MET 769	2.10	LEU 694	3.70						
		LEU 694	3.46						
		VAL 702	3.29						
		LEU 820	3.76						
Ursolic acid									
Hydrogen Bonds	Dis H-A (Å)	Hydrophobic Interactions	Dis (Å)	π-Stacking	Dis (Å)	π-Cation Interactions	Dis (Å)	Salt Bridges	Dis (Å)
LYS 721	3.17	LEU 820	3.22						
GLU 738	3.78								
Perillic acid									
Hydrogen Bonds	Dis H-A (Å)	Hydrophobic Interactions	Dis (Å)	π-Stacking	Dis (Å)	π-Cation Interactions	Dis (Å)	Salt Bridges	Dis (Å)
		ALA 731	3.39						
		ILE 735	3.53						
		ILE 735	3.64						

Table S3b. Predicted binding interactions as determined by PLIP for EGFR (T790M/L858R) with neat, unconjugated terpenes.

Hydroxybetulinic acid									
Hydrogen Bonds	Dis H-A (Å)	Hydrophobic Interactions	Dis (Å)	π-Stacking	Dis (Å)	π-Cation Interactions	Dis (Å)	Salt Bridges	Dis (Å)
ILE 878	2.14	PRO 877	3.61						
LYS 879	2.20	LYS 879	3.20						
		TRP 880	3.60						
Oleanolic acid									
Hydrogen Bonds	Dis H-A (Å)	Hydrophobic Interactions	Dis (Å)	π-Stacking	Dis (Å)	π-Cation Interactions	Dis (Å)	Salt Bridges	Dis (Å)
		LEU 718	3.55						
		LEU 718	3.35						
		VAL 726	3.40						
		ALA 743	3.09						
		ARG 841	3.53						
		THR 854	3.67						
Ursolic acid									

Hydrogen Bonds	Dis H-A (Å)	Hydrophobic Interactions	Dis (Å)	π-Stacking	Dis (Å)	π-Cation Interactions	Dis (Å)	Salt Bridges	Dis (Å)
		LEU 718	3.84						
		LEU 718	3.49						
		VAL 726	3.48						
		VAL 726	3.56						
		ALA 743	3.89						
		ARG 841	3.50						
		LEU 844	3.47						
		LEU 844	3.68						
		THR 854	3.69						
Perillic acid									
Hydrogen Bonds	Dis H-A (Å)	Hydrophobic Interactions	Dis (Å)	π-Stacking	Dis (Å)	π-Cation Interactions	Dis (Å)	Salt Bridges	Dis (Å)
GLY 857	2.25	PHE 723	3.81						
ALA 859	3.16	PHE 723	3.78						
		PHE 723	3.53						
		LEU 747	3.57						
		LEU 747	3.80						
		ILE 759	3.98						
		GLU 762	3.74						
		ARG 858	3.56						

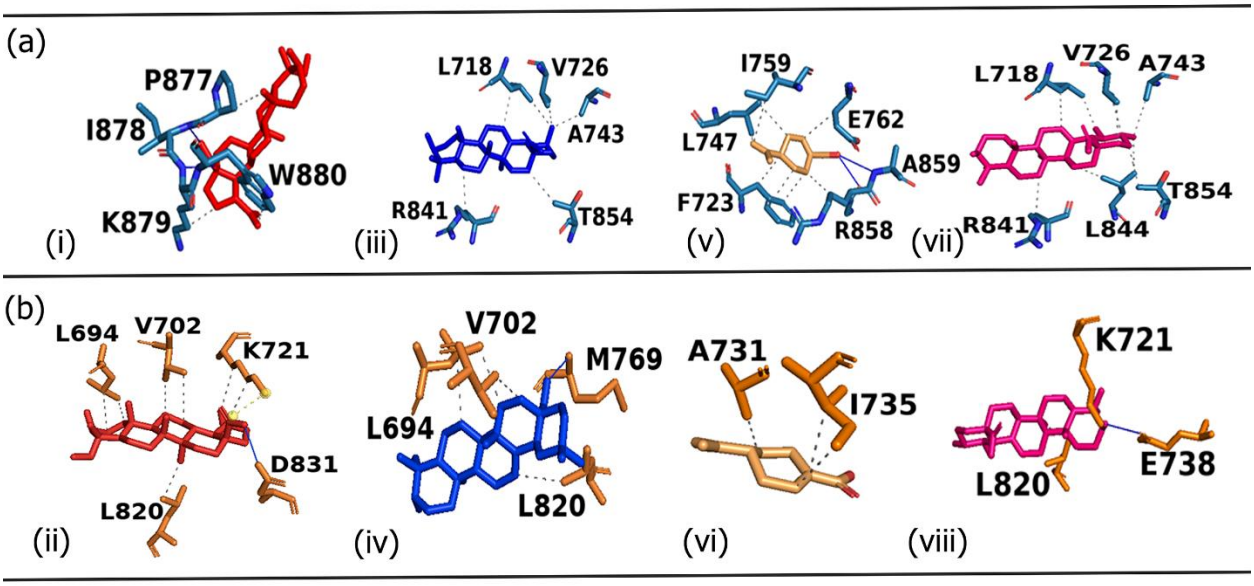

Figure S1. Binding interactions with neat terpenes. (a) with T790M/L858R; (b) with wild type EGFR. (i) and (ii) Hydroxybetulinic acid; (iii) and (iv) oleanolic acid; (v) and (vi) perillic acid; (vii) and (viii) ursolic acid .

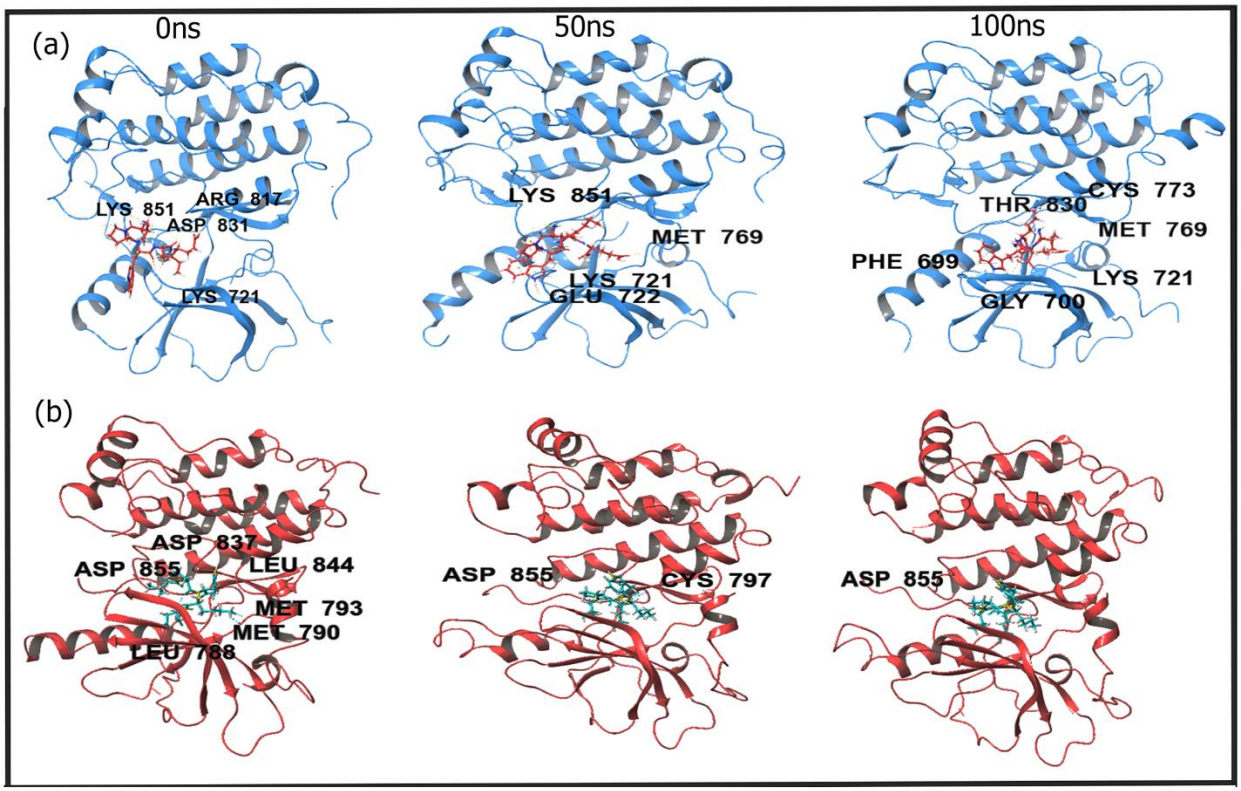

Figure S2. Trajectory snapshots of 100 ns MD simulation of VPWXE peptide with (a) EGFR (wild type); (b) EGFR T790M/L858R. Left to right – 0ns, 50ns and 100ns.

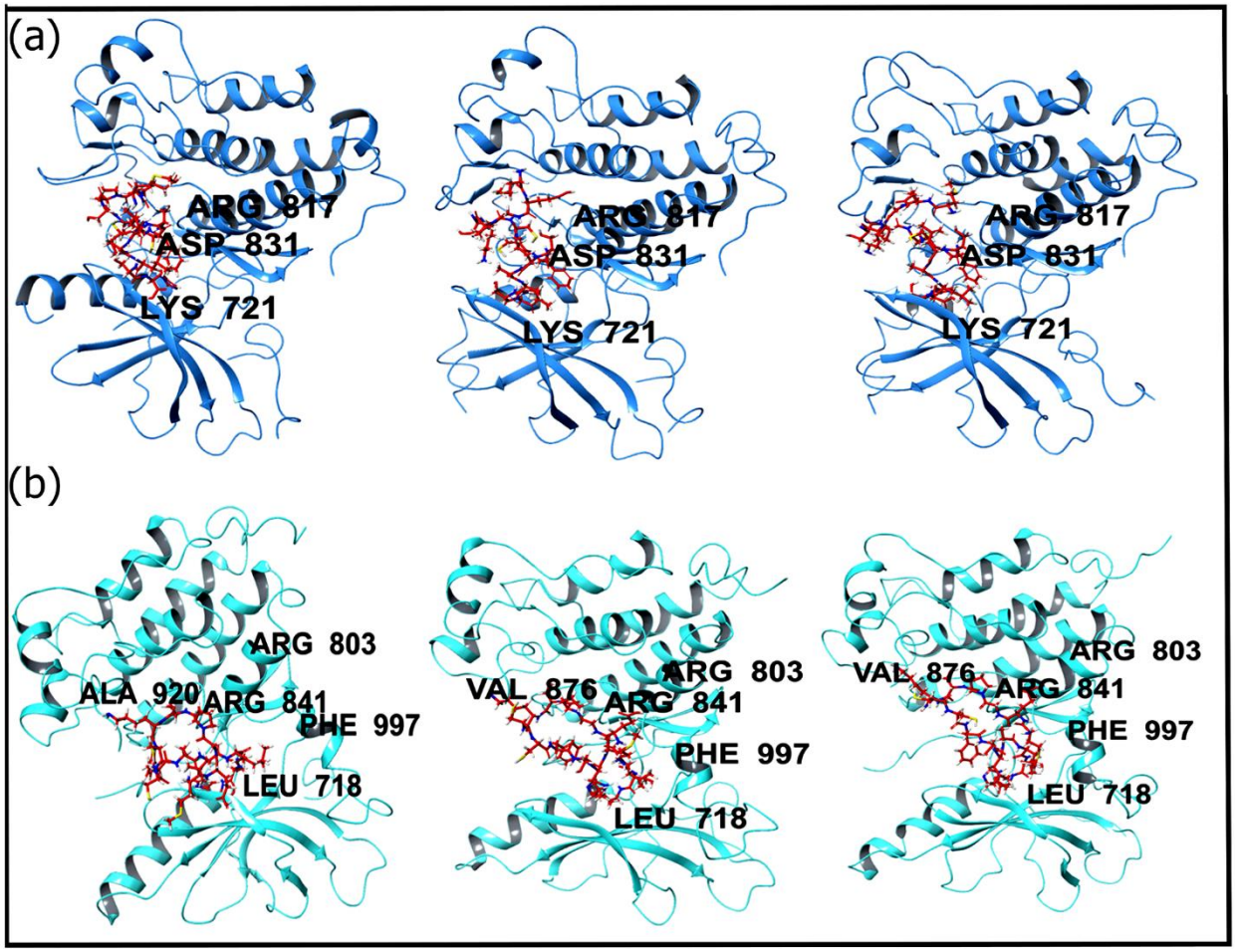

Figure S3. Trajectory snapshots of 100 ns MD simulation of neat MEGPSKCCFSLALSH (MFSL) peptide with (a) EGFR (wild type); (b) EGFR T790M/L858R. Left to right – 0ns, 50ns and 100ns.

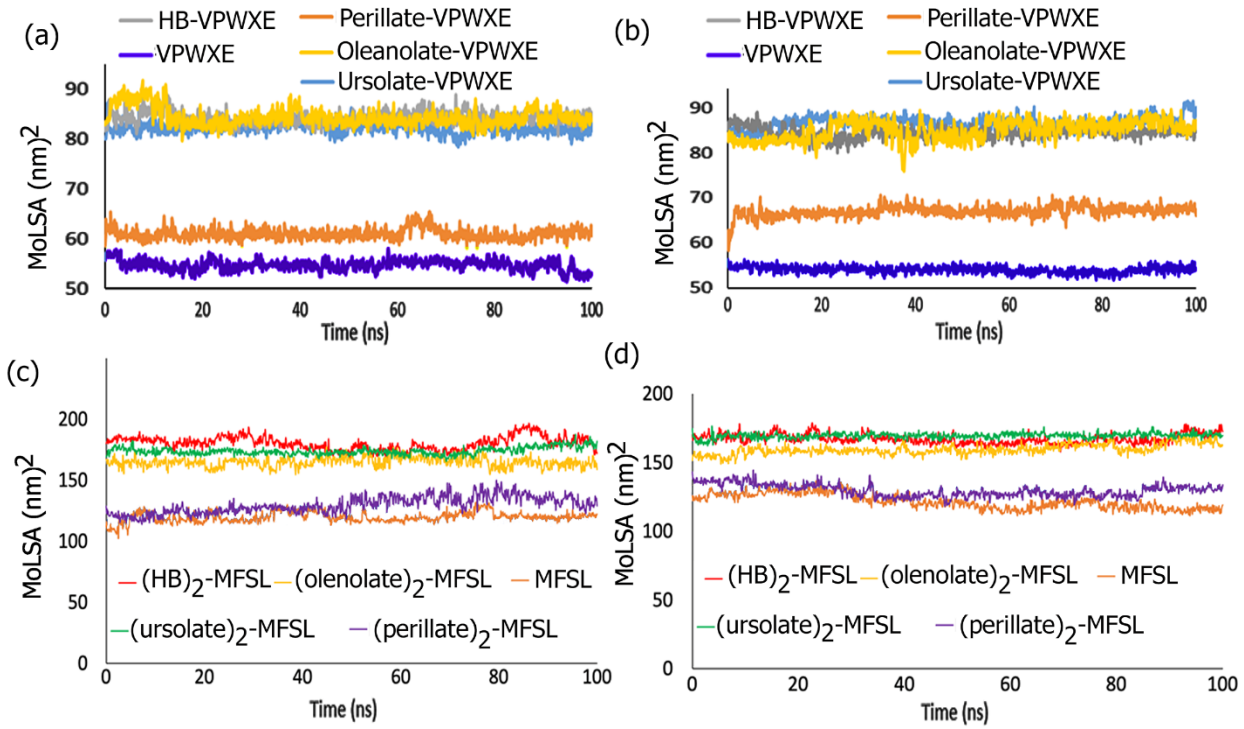

Figure S4. Comparison of MoLSA for (a) and (b) VPWXE and its conjugates with wild type and T790M/L858R EGFR receptors respectively. (c) and (d) MFSL and its conjugates with wild type and T790M/L858R EGFR receptors respectively. All simulations were carried out for 100 ns.

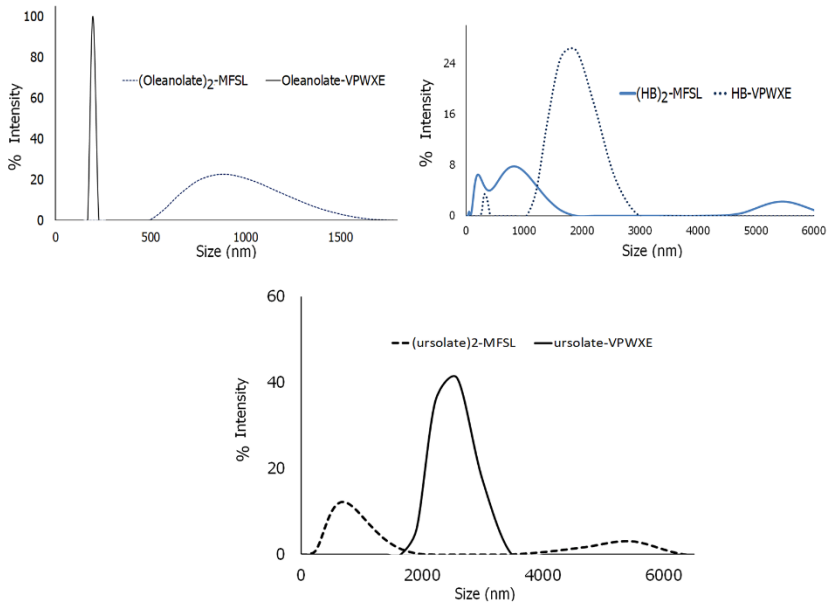

Figure S5. Comparison of Dynamic Light Scattering Analysis results to determine average size and distribution of nanoassemblies of peptide conjugates with tri-terpenes. Results shown are after two weeks of growth.

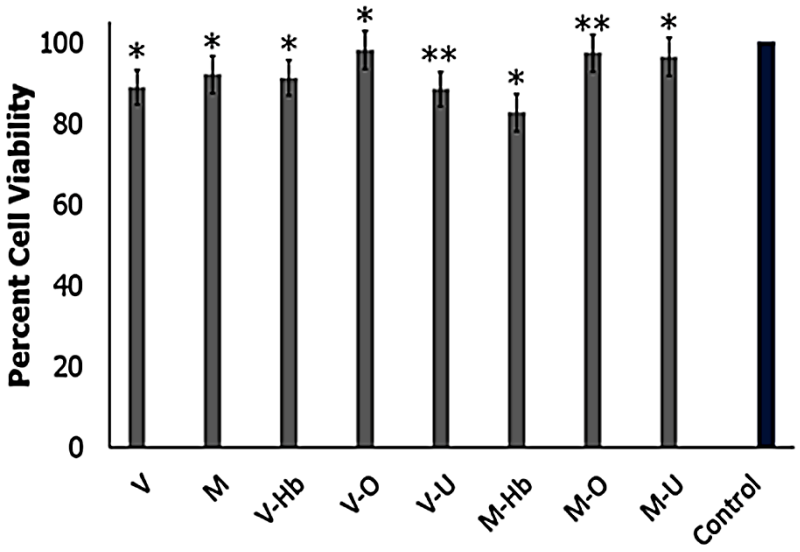

Figure S6. Fibroblast cell viability upon treatment for 24 hours with various nanoassemblies at a concentration of 3 μM. (V-Hb) = HB-VPWXE; V-O = oleanoalate-VPWXE; V-U = Ursolate-VPWXE; M-HB = $(HB)_2$-MFSL; M-O = $(oleanolate)_2$-MFSL; M-U = $(ursolate)_2$-MFSL. V= VPWXE; M = MFSL peptide * represents p value<0.05,

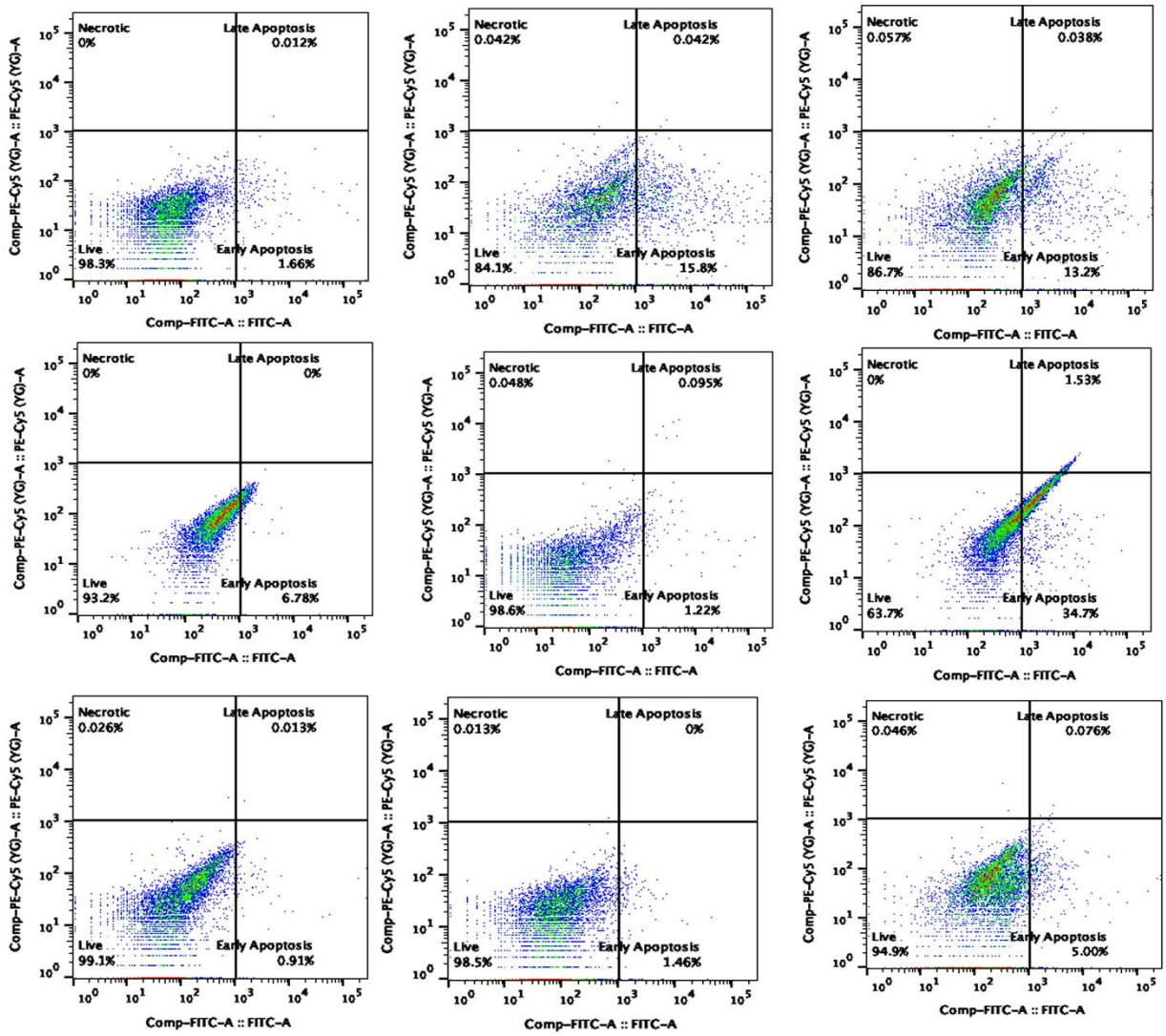

Figure S7. Apoptosis assay using FACS analysis for fibroblast cells upon treatment with nanoassemblies and peptides for 24 hours. Top row left to right: Control cells; cells treated with MFSL peptide; cells treated with VPWXE peptide; Middle row left to right: $(oleanolate)_2$-MFSL treated cells; $(ursolate)_2$-MFSL treated cells; $(HB)_2$-MFSL treated cells; Bottom Row: Left to right: oleanolate-VPWXE treated cells; ursolate-VPWXE treated cells; HB-VPWXE treated cells.

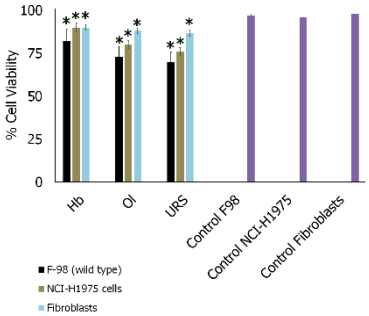

Figure S8. Viability studies with the three cell lines with unconjugated terpenes after 24-hour incubation. The concentration utilized was 3μM.