SUPPORTING INFORMATION

Inhibition of PCSK9^{D374Y}/LDLR Protein-Protein Interaction by Computationally Designed T9 Lupin Peptide Analogs

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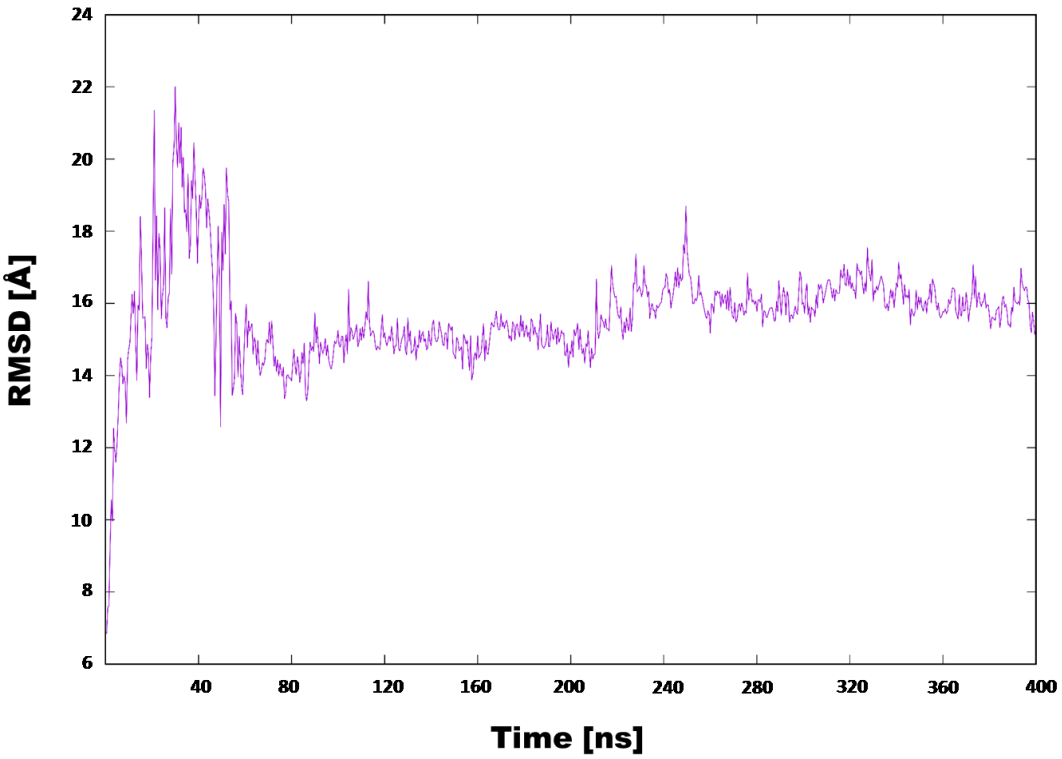

Figure S1. Distance between the T9 center of mass and the Cα atoms of F379-, R192- and Y374-PCSK9^{D374Y} residues.

Table S1. Contact analysis performed on the 400 ns-long MD simulations of $PCSK9^{D374Y}$/T9 complex. Only residues with a contact frequency ≥ 70 % (i.e. the ligand is in contact with the residue in more than the 70 of the analyzed frames) are reported. To run the analysis, the system was divided in two groups of atoms (enzyme and peptide) and the minimum contact distance was set to 0.4 nm. The analysis was carried out by *g_contacts* algorithm, written by Blau and Grubmuller.[1]

$PCSK9^{D374Y}$ residue	**T9 residue**	**Percentage of contact**
Y374	I12	100
V380	I12	96
Q382	R14	96
S372	I12	95
V380	I12	90
S381	R14	89
C378	I12	89
Y374	V11	88
F379	I12	87
C385	H6	84
Y374	H6	83
Y374	G10	81
Y374	Q7	81
C385	S5	81
Y374	V13	80
K222	Q7	79
S376	S5	78
Y374	E9	78
C385	Q7	75
S221	V13	73

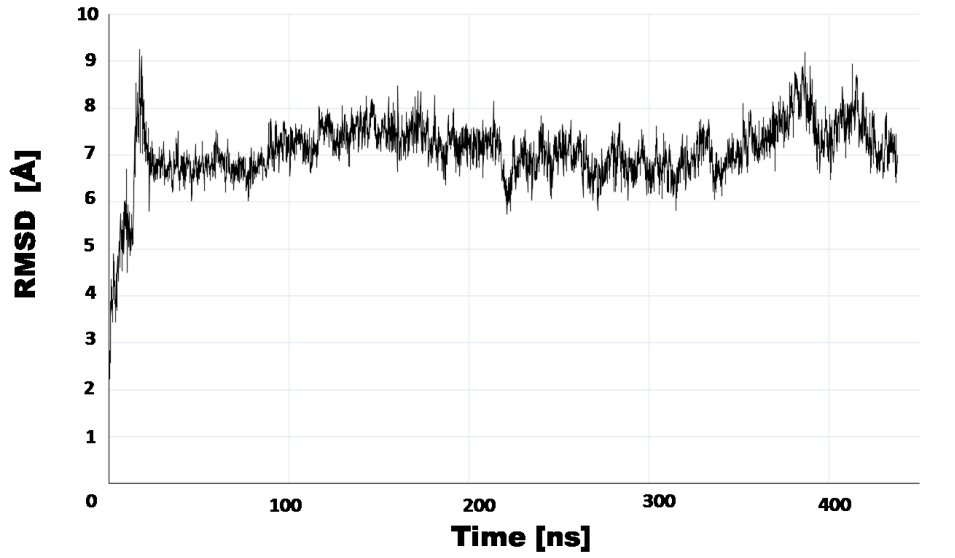

Figure S2. The RMSD of T9D8A_1 Cα atoms, over more than 400 ns of MD simulations, is shown with respect to the initial minimized structure of PCSK9^{D374Y}/T9D8A_1 complex.

ADDITIONAL DETAILS ON EXPERIMENTAL PROCEDURES

Chemicals. The CircuLex recombinant PCSK9^{D374Y} and the *in vitro* PCSK9^{D374Y}-LDLR binding Assay Kit were bought from CycLex Co. (Nagano, Japan). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), stable L-glutamine, penicillin/streptomycin, phosphate buffered saline (PBS), chemiluminescent reagent, and 96-well plates were purchased from Euroclone (Milan, Italy). The antibody against LDLR and the TMB substrate were bought from Pierce (Rockford, IL, USA). Janus Green B, bovine serum albumin (BSA), formaldehyde, HCl and H_2SO_4 were from Sigma-Aldrich (St. Louis, MO, USA). The LDL-DyLight^{TM} 550 was from Cayman Chemical (Ann Arbor, MI, USA). The synthetic peptides T9D8A_1 was synthesized by the company GeneScript (Piscataway, NJ, USA) at >95% purity.

PCSK9^{D374Y}-LDLR PPI evaluation. Peptides T9D8A and T9D8A_1 (0.1 μM - 1.0 mM) was investigated using the *in vitro* PCSK9-LDLR binding assay from CircuLex, following the manufacturer instructions after small modification of the conditions previously described.[19] Briefly, peptide T9D8A_1 was tested in presence of purified recombinant PCSK9^{D374Y}. For the *in vitro* screening of the PCSK9^{D374Y}-LDLR PPI inhibition, T9D8A_1 was added at different concentrations, ranging from 0.1 μM to 1.0 mM, to the His-tagged PCSK9^{D374Y} in the wells that had been coated with recombinant LDLR-AB domain. Then, the evaluation of inhibitory effects on PCSK9-LDLR PPI was carried out through the measurement of the amount of His-tagged PCSK9^{D374Y} on the wells which is correlated to the absorbance signals at 450 nm was read using the Synergy H1.

Fluorescent LDL uptake cell-based assay. A total of 3 x 104 HepG2 cells/well were seeded in black 96-well plates with clear bottom and kept in complete growth medium for 2 days before treatment. The following day after 2 h treatment with 4.0 μg/mL PCSK9D374Y or PCSK9 D374Y 10.0 μM T9D8A_1

in presence of PCSK9D374Y (4.0 μg/mL), and vehicle (H2O) at 37 °C under 5% CO2 atmosphere, the fluorescent LDL uptake cell-based assay was carried out using the procedure previously reported.5

References

1. Blau, C., and Grubmuller, H. (2013) g_contacts: Fast contact search in bio-molecular ensemble data. *Comp. Physics Commun.* **2013**, *184*, 2856-2859