# Delving Deep into the Structural Aspects of the BPro28-BLys29 Exchange in Insulin *Lispro*: A Structural Biophysical Lesson

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# 1 Supplementary Materials and Methods

## 1.1 Experimental insulin-insulin receptor complex and dimeric insulin structures in the Protein Data Bank: a brief update

| PDB ID | Resolution | Structure Title |
|---|---|---|
| 6PXW | 3.10 Å | Cryo-EM structure of full-length insulin receptor bound to 4 insulin. 3D refinement was focused on the top part of the receptor complex |
| 6PXV | 3.20 Å | Cryo-EM structure of full-length insulin receptor bound to 4 insulin. 3D refinement was focused on the extracellular region |
| 6CE7 | 7.40 Å | Insulin receptor ectodomain in complex with one insulin molecule |
| 6CEB | 4.70 Å | Insulin receptor ectodomain in complex with two insulin molecules - C1 symmetry |
| 6SOF | 4.30 Å | Human insulin receptor ectodomain bound by 4 insulin |
| 6CE9 | 4.30 Å | Insulin receptor ectodomain in complex with two insulin molecules |

Table 1: Insulin-insulin receptor (IR) complex structures inside Protein Data Bank (PDB [1]) as of July 12, 2020. All the structures were retrieved from the PDB website (https://www.rcsb.org/) directly. In this table, the resolution of **PDB ID: 1MHI** is not applicable (NA) because it was determined by liquid-state NMR spectroscopy.

| PDB ID | 6CE7 |
|---|---|
| Insulin receptor | A, B, P |
| Insulin chain A | N |
| Insulin chain B | O |
| PDB ID | 6CE9 |
| Insulin receptor | A, B, M, P |
| Insulin chain A | K, N |
| Insulin chain B | L, O |
| PDB ID | 6CEB |
| Insulin receptor | A, B, M, P |
| Insulin chain A | K, N |
| Insulin chain B | L, O |
| PDB ID | 6PXV |
| Insulin receptor | A, C |
| Insulin | D, E, F, G |
| PDB ID | 6PXW |
| Insulin receptor | A, B |
| Insulin | C, D, E, F |
| PDB ID | 6SOF |
| Insulin receptor | A, B, C, D |
| Insulin chain A | E, G, I, K |
| Insulin chain B | F, H, J, L |

Table 2: Chains and their respective PDB identifiers for the six experimental insulin-IR complex structures listed in Table 1. In this table, the chains and their respective PDB identifiers are to be used in the structural analysis of interfacial electrostatic interactions between insulin receptor and insulin or its fast acting analogues, and also in the interpretation of the result of the structural analysis of interfacial electrostatic interactions, where the residue naming scheme is **Chain ID_residue name_residue number** for all tables in this supplementary document (**supps.pdf**).

| PDB ID | 6PXV |
|---|---|
| Chains F, G | both P28 and K29 are missing |
| Chains D, E | K29 is missing |
| PDB ID | 6PXW |
| Chains E, F | both P28 and K29 are missing |
| Chains C, D | K29 is missing |
| PDB ID | 6SOF |
| Chains F,H,J,L | both P28 and K29 are present |

Table 3: Experimentally charted and uncharted territories (EUTs) [2] at positions 28 and 29 of the six experimental insulin-IR complex structures listed in Table 1.

Since the atomic coordinates of both P28 and K29 are present in the experimental complex structure (PDB ID: 6SOF) of insulin and IR, this structure was chosen to represent the regular (i.e., native) insulin-IR complex structure for subsequent illustrations of their interfacial electrostatic interactions in comparison with the fast-acting insulin analogue, i.e., insulin lispro [3, 4, 5].

## 1.2 Construction and structural electrostatic analysis of a homology structural model of insulin lispro in complex with IR

To begin with, the amino acid sequences of insulin lispro and IR are listed in italics in fasta format as below,

>IR

*HLYPGEVCPGMDIRNNLTRLHELENCSVIEGHLQILLMFKTRPEDFRDLSF*
*PKLIMITDYLLLFRVYGLESLKDLFPNLTVIRGSRLFFNYALVIFEMVHLKELG*
*LYNLMNITRGSVRIEKNNELCYLATIDWSRILDSVEDNYIVLNKDDNEECGDI*
*CPGTAKGKTNCPATVINGQFVERCWTHSHCQKVCPTICKSHGCTAEGLCCH*
*SECLGNCSQPDDPTKCVACRNFYLDGRCVETCPPPYYHFQDWRCVNFSFCQ*
*DLHHKCKNSRRQGCHQYVIHNNKCIPECPSGYTMNSSNLLCTPCLGPCPKVC*
*HLLEGEKTIDSVTSAQELRGCTVINGSLIINIRGGNNLAAELEANLGLIEEISGY*
*LKIRRSYALVSLSFFRKLRLIRGETLEIGNYSFYALDNQNLRQLWDWSKHNLT*
*ITQGKLFFHYNPKLCLSEIHKMEEVSGTKGRQERNDIALKTNGDQASCENEL*
*LKFSYIRTSFDKILLRWEPYWPPDFRDLLGFMLFYKEAPYQNVTEFDGQDAC*
*GSNSWTVVDIDPPLRSNDPKSQNHPGWLMRGLKPWTQYAIFVKTLVTFSDE*
*RRTYGAKSDIIYVQTDATNPSVPLDPISVSNSSSQIILKWKPPSDPNGNITHYL*
*VFWERQAEDSELFELDYCLKGLKLPSRTWSPPFESEDSQKHNQSEYEDSAGE*
*CCSCPKTDSQILKELEESSFRKTFEDYLHNVVFVPRPSRKRRSLGDVGNVTVA*
*VPTVAAFPNTSSTSVPTSPEEHRPFEKVVNKESLVISGLRHFTGYRIELQACN*
*QDTPEERCSVAAYVSARTMPEAKADDIVGPVTHEIFENNVVHLMWQEPKEP*
*NGLIVLYEVSYRRYGDEELHLCVSRKHFALERGCRLRGLSPGNYSVRIRATSL*
*AGNGSWTEPTYFYVTDYLDVPSNIAKIIIGPLIFVFLFSVVIGSIYLFLRKRQPD*
*GPLGPLYASSNPEFLTASDVFPCSVYVPDEWEVSREKITLLRELGQGSFGMV*

*YEGNARDIIKGEAETRVAVKTVNESASLRERIEFLNEASVMKGFTCHHVVRL*
*LGVVSKGQPTLVVMELMAHGDLKSYLRSLRPEAENNPGRPPPTLQEMIQMA*
*AEIADGMAYLNAKKFVHRNLAARNCMVAHDFTVKIGDFGMTRDIYETDYY*
*RKGGKGLLPVRWMAPESLKDGVFTTSSDMWSFGVVLWEITSLAEQPYQGLS*
*NEQVLKFVMDGGYLDQPDNCPERVTDLMRMCWQFNPKMRPTFLEIVNLLK*
*DDLHPSFPEVSFFHSEENKAPESEELEMEFEDMENVPLDRSSHCQREEAGGR*
*DGGSSLGFKRSYEEHIPYTHMNGGKKNGAAATAPRSNPSLESSGLEVLFQ*

>InsulinLispro

*FVNQHLCGSHLVEALYLVCGERGFFYTKPTRREAEDLQGSLQPLALEGSL*
*QKRGIVEQCCTSICSLYQLENYCN*

Afterwards, the two sequences above were plugged into the SWISS-MODEL homology modeling [6] server in search of an experimental structural model, which led to a Cryo-EM structure (PDB ID: 6PXV) with 99.86% sequence identity, representing a hetero-2-4-meric structure of full-length insulin receptor (IR) bound to 4 insulin [7]. After homology structural modeling [6], the UCSF Chimera software [8] was employed to add hydrogen atoms to the homology structural model (supplementary file **lispro.pdb**), which was subsequently subject to a comprehensive set of electrostatic interaction analysis as described previously in [9]. Take **6PXV.pdb** as an example again, after the addition of hydrogen atoms by Chimera [8], the PDB file was renamed to be **H6PXV.pdb**.

# 2 Supplementary Results

## 2.1 Interfacial electrostatic interactions between IR and regular insulin: a brief update

| PDB file name | Residue A | Atom A | Residue B | Atom B | Distance (Å) |
|---|---|---|---|---|---|
| H6CE7 | A_ARG_65 | NH1 | O_GLU_13 | OE2 | 2.835 |
| H6PXV | A_ARG_479 | NH2 | G_GLU_21 | OE1 | 3.630 |
| H6PXV | A_ARG_479 | NH2 | G_GLU_21 | OE2 | 3.051 |
| H6PXV | C_ARG_479 | NH2 | F_GLU_21 | OE1 | 3.657 |
| H6PXV | C_ARG_479 | NH2 | F_GLU_21 | OE2 | 3.064 |
| H6PXW | A_ARG_479 | NH2 | F_GLU_21 | OE1 | 3.630 |
| H6PXW | A_ARG_479 | NH2 | F_GLU_21 | OE2 | 3.052 |
| H6PXW | B_ARG_479 | NH2 | E_GLU_21 | OE1 | 3.656 |
| H6PXW | B_ARG_479 | NH2 | E_GLU_21 | OE2 | 3.065 |
| H6SOF | C_LYS_40 | NZ | H_GLU_21 | OE1 | 3.890 |
| H6SOF | C_LYS_40 | NZ | H_GLU_21 | OE2 | 3.086 |
| H6SOF | C_LYS_484 | NZ | L_GLU_13 | OE1 | 3.321 |
| H6SOF | L_HIS_10 | NE2 | C_GLU_674 | OE1 | 2.709 |
| H6SOF | L_HIS_10 | NE2 | C_GLU_674 | OE2 | 3.653 |
| H6SOF | J_HIS_10 | NE2 | A_ASP_483 | OD2 | 3.765 |

Table 4: Salt bridging networks [9] within the six experimental insulin-IR structures (Table 1). In this table, the residue naming scheme is **Chain ID_residue name_residue number**, and '.pdb' is not included in the **PDB file name**.

| PDB file name | Acceptor (A) | Donor (D) | Hydrogen (H) | D-A (Å) | H-A (Å) | $\angle ADH$(°) |
|---|---|---|---|---|---|---|
| H6PXV | O, E_CYS_75 | NE, A_ARG_717 | HE, A_ARG_717 | 2.59 | 1.65 | 16.71 |
| H6PXV | O, D_CYS_75 | NE, C_ARG_717 | HE, C_ARG_717 | 2.64 | 1.69 | 15.81 |
| H6PXV | ND2, A_ASN_15 | N, D_PHE_24 | H, D_PHE_24 | 3.13 | 2.17 | 14.93 |
| H6PXV | OD1, C_ASN_711 | N, D_VAL_58 | H, D_VAL_58 | 3.46 | 2.45 | 4.00 |
| H6PXV | ND2, C_ASN_15 | N, E_PHE_24 | H, E_PHE_24 | 3.17 | 2.21 | 15.07 |
| H6PXV | OD1, A_ASN_711 | N, E_VAL_58 | H, E_VAL_58 | 3.47 | 2.47 | 4.10 |
| H6PXW | O, D_CYS_75 | NE, A_ARG_717 | HE, A_ARG_717 | 2.59 | 1.65 | 16.71 |
| H6PXW | O, C_CYS_75 | NE, B_ARG_717 | HE, B_ARG_717 | 2.64 | 1.69 | 15.82 |
| H6PXW | ND2, A_ASN_15 | N, C_PHE_24 | H, C_PHE_24 | 3.13 | 2.17 | 14.95 |
| H6PXW | OD1, B_ASN_711 | N, C_VAL_58 | H, C_VAL_58 | 3.46 | 2.45 | 4.05 |
| H6PXW | ND2, B_ASN_15 | N, D_PHE_24 | H, D_PHE_24 | 3.17 | 2.21 | 15.06 |
| H6PXW | OD1, A_ASN_711 | N, D_VAL_58 | H, D_VAL_58 | 3.47 | 2.47 | 4.04 |

Table 5: Side chain and main chain hydrogen bonding networks [9] of the six experimental insulin-IR structures (Table 1), except that the distance $(D - A)$ cutoff was set to be 4 Å. In this table, the residue naming scheme is **Chain ID_residue name_residue number**, $\angle ADH$ represents the angle formed by acceptor (A), donor (D) and hydrogen (H) ($\angle ADH$), and '.pdb' is not included in the **PDB file name**.

## 2.2 Interfacial electrostatic interactions between IR and insulin lispro: a brief update

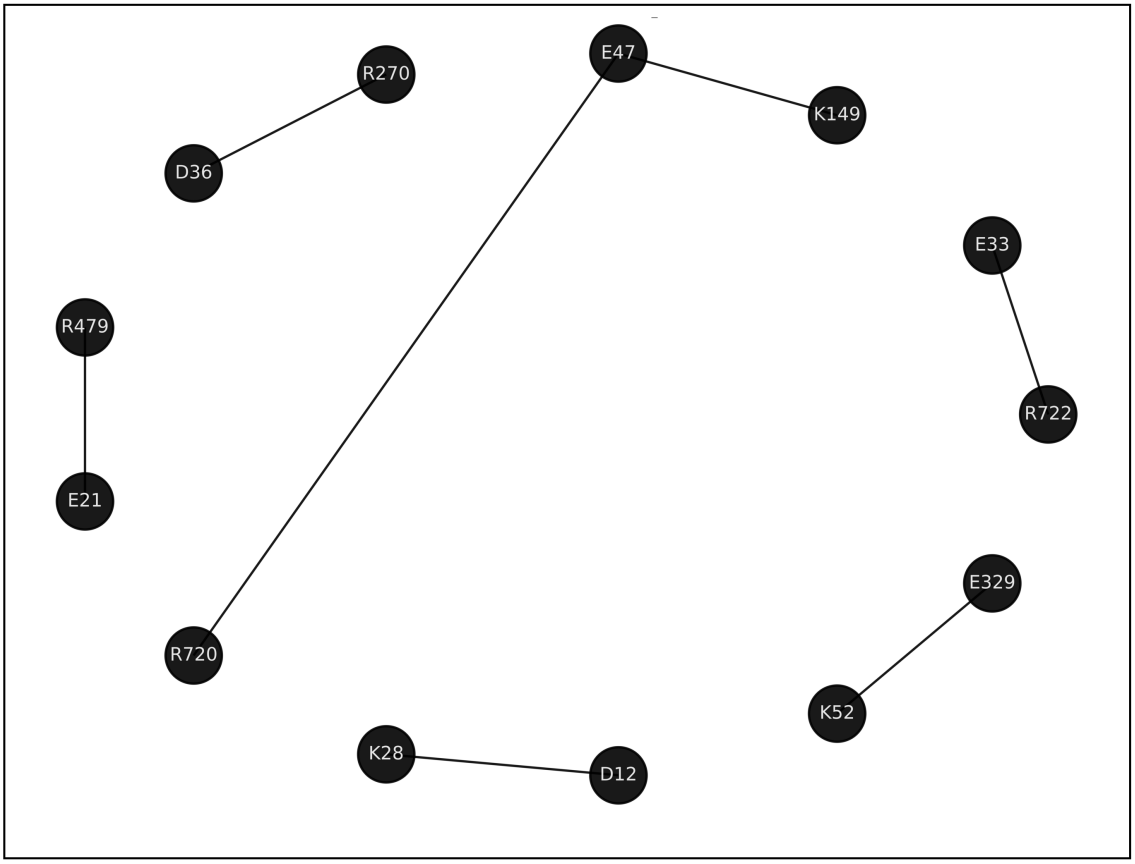


Figure 1: A computer-generated two-dimensional graph of the interfacial salt bridging network of insulin lispro. In this figure, the residue naming scheme is **one letter residue code_residue number**.

| PDB file name | Residue A | Atom A | Residue B | Atom B | Distance (Å) |
|---|---|---|---|---|---|
| lispro | A_ARG_14 | NH2 | A_ASP_12 | OD1 | 3.438 |
| lispro | A_ARG_19 | NH2 | A_ASP_12 | OD2 | 3.756 |
| lispro | A_ARG_42 | NH2 | A_GLU_44 | OE2 | 2.874 |
| lispro | A_ARG_65 | NH2 | A_GLU_97 | OE1 | 2.651 |
| lispro | A_LYS_73 | NZ | A_ASP_74 | OD1 | 2.765 |
| lispro | A_LYS_73 | NZ | A_ASP_74 | OD2 | 2.677 |
| lispro | A_ARG_86 | NH1 | A_ASP_59 | OD1 | 3.418 |
| lispro | A_ARG_86 | NH1 | A_ASP_59 | OD2 | 3.106 |
| lispro | A_LYS_102 | NZ | A_GLU_70 | OE1 | 2.609 |
| lispro | A_LYS_102 | NZ | A_GLU_70 | OE2 | 3.264 |
| lispro | A_ARG_114 | NH2 | A_GLU_353 | OE1 | 2.897 |
| lispro | A_ARG_114 | NH2 | A_GLU_353 | OE2 | 2.741 |
| lispro | A_ARG_118 | NH2 | A_GLU_120 | OE1 | 2.642 |
| lispro | A_LYS_121 | NZ | A_GLU_97 | OE2 | 2.667 |
| lispro | A_LYS_121 | NZ | B_GLU_706 | OE2 | 2.574 |
| lispro | A_LYS_149 | NZ | A_GLU_124 | OE1 | 2.552 |
| lispro | A_LYS_149 | NZ | A_GLU_124 | OE2 | 3.982 |
| lispro | A_LYS_149 | NZ | A_GLU_153 | OE1 | 3.996 |
| lispro | A_HIS_185 | ND1 | A_GLU_103 | OE1 | 3.891 |
| lispro | A_LYS_224 | NZ | A_ASP_221 | OD2 | 2.671 |
| lispro | A_ARG_252 | NH2 | A_GLU_30 | OE1 | 2.634 |
| lispro | A_ARG_252 | NH2 | A_GLU_30 | OE2 | 2.928 |
| lispro | A_LYS_310 | NZ | A_GLU_287 | OE1 | 2.612 |
| lispro | A_LYS_310 | NZ | A_GLU_287 | OE2 | 3.122 |
| lispro | A_LYS_319 | NZ | A_GLU_329 | OE1 | 2.537 |

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|---|---|---|---|---|---|
| lispro | A_ARG_345 | NH1 | B_GLU_697 | OE1 | 2.570 |
| lispro | A_LYS_369 | NZ | A_GLU_394 | OE1 | 2.629 |
| lispro | A_LYS_369 | NZ | A_GLU_394 | OE2 | 2.793 |
| lispro | A_ARG_383 | NH2 | A_GLU_355 | OE1 | 2.769 |
| lispro | A_ARG_383 | NH2 | A_GLU_355 | OE2 | 2.840 |
| lispro | A_ARG_386 | NH1 | A_GLU_363 | OE1 | 2.758 |
| lispro | A_ARG_386 | NH1 | A_GLU_363 | OE2 | 2.880 |
| lispro | A_ARG_386 | NH2 | A_GLU_363 | OE2 | 2.760 |
| lispro | A_ARG_389 | NH1 | A_GLU_391 | OE2 | 3.858 |
| lispro | A_LYS_425 | NZ | A_GLU_453 | OE1 | 2.615 |
| lispro | A_LYS_425 | NZ | A_GLU_453 | OE2 | 3.481 |
| lispro | A_LYS_425 | NZ | A_ASP_456 | OD1 | 2.780 |
| lispro | A_LYS_425 | NZ | A_ASP_456 | OD2 | 2.849 |
| lispro | A_HIS_440 | NE2 | A_GLU_443 | OE2 | 3.763 |
| lispro | A_LYS_441 | NZ | A_GLU_438 | OE1 | 3.793 |
| lispro | A_LYS_441 | NZ | A_GLU_438 | OE2 | 2.695 |
| lispro | A_LYS_449 | NZ | A_GLU_444 | OE1 | 2.533 |
| lispro | A_LYS_449 | NZ | A_GLU_444 | OE2 | 3.673 |
| lispro | A_LYS_460 | NZ | B_ASP_404 | OD1 | 2.979 |
| lispro | A_LYS_460 | NZ | B_ASP_404 | OD2 | 2.714 |
| lispro | A_LYS_474 | NZ | A_GLU_490 | OE1 | 2.686 |
| lispro | A_LYS_474 | NZ | A_GLU_490 | OE2 | 2.645 |
| lispro | A_LYS_484 | NZ | A_ASP_483 | OD2 | 2.629 |
| lispro | A_ARG_498 | NH1 | A_GLU_706 | OE1 | 2.702 |
| lispro | A_ARG_498 | NH1 | A_ASP_707 | OD1 | 2.673 |
| lispro | A_ARG_498 | NH1 | A_ASP_707 | OD2 | 3.685 |

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|---|---|---|---|---|---|
| lispro | A_ARG_498 | NH2 | A_ASP_496 | OD1 | 3.749 |
| lispro | A_ARG_498 | NH2 | A_ASP_496 | OD2 | 3.139 |
| lispro | A_ARG_498 | NH2 | A_ASP_707 | OD1 | 3.056 |
| lispro | A_ARG_498 | NH2 | A_ASP_707 | OD2 | 2.889 |
| lispro | A_LYS_557 | NZ | A_ASP_677 | OD2 | 2.638 |
| lispro | A_ARG_576 | NH1 | A_GLU_575 | OE1 | 2.699 |
| lispro | A_ARG_576 | NH1 | A_GLU_575 | OE2 | 3.381 |
| lispro | A_ARG_576 | NH2 | A_GLU_575 | OE1 | 3.563 |
| lispro | A_ARG_576 | NH2 | A_GLU_575 | OE2 | 2.645 |
| lispro | A_LYS_616 | NZ | A_ASP_600 | OD2 | 2.638 |
| lispro | A_HIS_627 | NE2 | A_GLU_793 | OE1 | 2.732 |
| lispro | A_ARG_656 | NH1 | A_ASP_638 | OD1 | 3.075 |
| lispro | A_ARG_656 | NH1 | A_ASP_638 | OD2 | 2.694 |
| lispro | A_ARG_656 | NH2 | A_GLU_637 | OE1 | 2.931 |
| lispro | A_ARG_656 | NH2 | A_ASP_638 | OD1 | 2.615 |
| lispro | A_ARG_656 | NH2 | A_ASP_638 | OD2 | 3.814 |
| lispro | A_ARG_702 | NH2 | B_GLU_120 | OE2 | 2.732 |
| lispro | A_LYS_703 | NZ | A_ASP_496 | OD2 | 2.679 |
| lispro | A_LYS_703 | NZ | A_ASP_499 | OD1 | 2.606 |
| lispro | A_LYS_703 | NZ | A_ASP_499 | OD2 | 2.969 |
| lispro | A_ARG_722 | NH1 | D_GLU_33 | OE2 | 3.008 |
| lispro | A_ARG_757 | NH1 | A_GLU_633 | OE2 | 3.205 |
| lispro | A_ARG_757 | NH1 | A_GLU_782 | OE1 | 2.714 |
| lispro | A_ARG_757 | NH2 | A_GLU_782 | OE1 | 2.741 |
| lispro | A_ARG_757 | NH2 | A_GLU_782 | OE2 | 3.551 |
| lispro | A_LYS_761 | NZ | A_ASP_789 | OD1 | 2.771 |

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|---|---|---|---|---|---|
| lispro | A_LYS_761 | NZ | A_ASP_789 | OD2 | 2.698 |
| lispro | A_LYS_761 | NZ | A_GLU_793 | OE1 | 3.131 |
| lispro | A_ARG_774 | NH2 | A_GLU_837 | OE1 | 2.593 |
| lispro | A_ARG_774 | NH2 | A_GLU_837 | OE2 | 3.875 |
| lispro | A_ARG_780 | NH1 | A_GLU_633 | OE1 | 3.041 |
| lispro | A_ARG_780 | NH1 | A_GLU_633 | OE2 | 2.733 |
| lispro | A_ARG_804 | NH1 | A_GLU_637 | OE1 | 3.114 |
| lispro | A_ARG_804 | NH1 | A_GLU_637 | OE2 | 2.708 |
| lispro | A_ARG_804 | NH2 | A_GLU_637 | OE1 | 2.742 |
| lispro | A_ARG_804 | NH2 | A_GLU_637 | OE2 | 2.987 |
| lispro | A_LYS_810 | NZ | A_ASP_813 | OD2 | 3.192 |
| lispro | A_HIS_829 | ND1 | A_GLU_821 | OE1 | 3.166 |
| lispro | A_LYS_836 | NZ | A_GLU_837 | OE2 | 2.622 |
| lispro | A_ARG_850 | NH1 | A_GLU_856 | OE1 | 2.565 |
| lispro | A_ARG_850 | NH2 | A_GLU_855 | OE2 | 2.632 |
| lispro | A_ARG_850 | NH2 | A_GLU_856 | OE1 | 3.332 |
| lispro | A_ARG_851 | NH2 | A_ASP_854 | OD1 | 2.731 |
| lispro | A_ARG_851 | NH2 | A_ASP_854 | OD2 | 2.992 |
| lispro | A_HIS_858 | NE2 | A_GLU_856 | OE2 | 3.716 |
| lispro | A_LYS_864 | NZ | A_GLU_643 | OE2 | 2.822 |
| lispro | A_HIS_865 | NE2 | A_GLU_869 | OE1 | 3.220 |
| lispro | A_HIS_865 | NE2 | A_GLU_869 | OE2 | 3.115 |
| lispro | A_ARG_870 | NH1 | A_GLU_821 | OE1 | 3.896 |
| lispro | A_ARG_870 | NH2 | A_GLU_821 | OE1 | 3.357 |
| lispro | A_ARG_875 | NH1 | A_GLU_869 | OE1 | 3.500 |
| lispro | A_ARG_875 | NH2 | A_GLU_869 | OE1 | 2.611 |

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|---|---|---|---|---|---|
| lispro | A_ARG_875 | NH2 | A_GLU_869 | OE2 | 3.409 |
| lispro | A_ARG_885 | NH1 | A_GLU_899 | OE1 | 2.804 |
| lispro | A_ARG_885 | NH1 | A_GLU_899 | OE2 | 3.609 |
| lispro | A_ARG_885 | NH2 | A_GLU_899 | OE1 | 3.312 |
| lispro | A_ARG_885 | NH2 | A_GLU_899 | OE2 | 2.610 |
| lispro | A_ARG_887 | NH2 | A_GLU_846 | OE1 | 3.093 |
| lispro | B_ARG_14 | NH2 | B_ASP_12 | OD1 | 3.254 |
| lispro | B_ARG_42 | NH2 | B_GLU_44 | OE2 | 2.875 |
| lispro | B_ARG_65 | NH2 | B_GLU_97 | OE1 | 2.651 |
| lispro | B_LYS_73 | NZ | B_ASP_74 | OD1 | 2.764 |
| lispro | B_LYS_73 | NZ | B_ASP_74 | OD2 | 2.676 |
| lispro | B_ARG_86 | NH1 | B_ASP_59 | OD1 | 3.417 |
| lispro | B_ARG_86 | NH1 | B_ASP_59 | OD2 | 3.110 |
| lispro | B_LYS_102 | NZ | B_GLU_70 | OE1 | 2.609 |
| lispro | B_LYS_102 | NZ | B_GLU_70 | OE2 | 3.288 |
| lispro | B_ARG_114 | NH2 | B_GLU_353 | OE1 | 2.895 |
| lispro | B_ARG_114 | NH2 | B_GLU_353 | OE2 | 2.742 |
| lispro | B_ARG_118 | NH2 | B_GLU_120 | OE1 | 2.642 |
| lispro | B_LYS_121 | NZ | A_GLU_706 | OE2 | 2.573 |
| lispro | B_LYS_121 | NZ | B_GLU_97 | OE2 | 2.667 |
| lispro | B_LYS_149 | NZ | B_GLU_124 | OE1 | 2.609 |
| lispro | B_LYS_149 | NZ | F_GLU_47 | OE1 | 2.562 |
| lispro | B_HIS_185 | ND1 | B_GLU_103 | OE1 | 3.891 |
| lispro | B_LYS_224 | NZ | B_ASP_221 | OD2 | 2.671 |
| lispro | B_ARG_252 | NH2 | B_GLU_30 | OE1 | 2.634 |
| lispro | B_ARG_252 | NH2 | B_GLU_30 | OE2 | 2.929 |

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|---|---|---|---|---|---|
| lispro | B_ARG_270 | NH1 | D_ASP_36 | OD1 | 3.818 |
| lispro | B_LYS_310 | NZ | B_GLU_287 | OE1 | 2.613 |
| lispro | B_LYS_310 | NZ | B_GLU_287 | OE2 | 3.115 |
| lispro | B_LYS_319 | NZ | B_GLU_329 | OE1 | 2.533 |
| lispro | B_ARG_345 | NH1 | A_GLU_697 | OE1 | 2.571 |
| lispro | B_LYS_369 | NZ | B_GLU_394 | OE1 | 2.628 |
| lispro | B_LYS_369 | NZ | B_GLU_394 | OE2 | 2.791 |
| lispro | B_ARG_383 | NH2 | B_GLU_355 | OE1 | 2.770 |
| lispro | B_ARG_383 | NH2 | B_GLU_355 | OE2 | 2.840 |
| lispro | B_ARG_386 | NH1 | B_GLU_363 | OE1 | 2.758 |
| lispro | B_ARG_386 | NH1 | B_GLU_363 | OE2 | 2.880 |
| lispro | B_ARG_386 | NH2 | B_GLU_363 | OE2 | 2.760 |
| lispro | B_ARG_389 | NH1 | B_GLU_391 | OE2 | 3.855 |
| lispro | B_LYS_425 | NZ | B_GLU_453 | OE1 | 2.616 |
| lispro | B_LYS_425 | NZ | B_GLU_453 | OE2 | 3.486 |
| lispro | B_LYS_425 | NZ | B_ASP_456 | OD1 | 2.779 |
| lispro | B_LYS_425 | NZ | B_ASP_456 | OD2 | 2.849 |
| lispro | B_HIS_440 | NE2 | B_GLU_443 | OE2 | 3.764 |
| lispro | B_LYS_441 | NZ | B_GLU_438 | OE1 | 3.794 |
| lispro | B_LYS_441 | NZ | B_GLU_438 | OE2 | 2.695 |
| lispro | B_LYS_449 | NZ | B_GLU_444 | OE1 | 2.533 |
| lispro | B_LYS_449 | NZ | B_GLU_444 | OE2 | 3.672 |
| lispro | B_LYS_460 | NZ | A_ASP_404 | OD1 | 2.974 |
| lispro | B_LYS_460 | NZ | A_ASP_404 | OD2 | 2.716 |
| lispro | B_LYS_474 | NZ | B_GLU_490 | OE1 | 2.772 |
| lispro | B_LYS_474 | NZ | B_GLU_490 | OE2 | 2.666 |

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|---|---|---|---|---|---|
| lispro | B_ARG_479 | NH2 | E_GLU_21 | OE1 | 3.575 |
| lispro | B_ARG_479 | NH2 | E_GLU_21 | OE2 | 2.685 |
| lispro | B_LYS_484 | NZ | B_ASP_483 | OD2 | 2.575 |
| lispro | B_ARG_498 | NH1 | B_GLU_706 | OE1 | 2.703 |
| lispro | B_ARG_498 | NH1 | B_ASP_707 | OD1 | 2.673 |
| lispro | B_ARG_498 | NH1 | B_ASP_707 | OD2 | 3.675 |
| lispro | B_ARG_498 | NH2 | B_ASP_496 | OD1 | 3.750 |
| lispro | B_ARG_498 | NH2 | B_ASP_496 | OD2 | 3.131 |
| lispro | B_ARG_498 | NH2 | B_ASP_707 | OD1 | 3.065 |
| lispro | B_ARG_498 | NH2 | B_ASP_707 | OD2 | 2.878 |
| lispro | B_ARG_576 | NH1 | B_GLU_575 | OE1 | 2.692 |
| lispro | B_ARG_576 | NH1 | B_GLU_575 | OE2 | 3.408 |
| lispro | B_ARG_576 | NH2 | B_GLU_575 | OE1 | 3.527 |
| lispro | B_ARG_576 | NH2 | B_GLU_575 | OE2 | 2.630 |
| lispro | B_LYS_616 | NZ | B_ASP_600 | OD2 | 2.620 |
| lispro | B_HIS_627 | NE2 | B_GLU_793 | OE1 | 2.748 |
| lispro | B_ARG_656 | NH1 | B_ASP_638 | OD1 | 3.607 |
| lispro | B_ARG_656 | NH1 | B_ASP_638 | OD2 | 2.645 |
| lispro | B_ARG_656 | NH2 | B_GLU_637 | OE1 | 3.891 |
| lispro | B_ARG_656 | NH2 | B_ASP_638 | OD1 | 2.686 |
| lispro | B_ARG_656 | NH2 | B_ASP_638 | OD2 | 3.300 |
| lispro | B_ARG_702 | NH2 | A_GLU_120 | OE2 | 2.731 |
| lispro | B_LYS_703 | NZ | B_ASP_496 | OD2 | 2.674 |
| lispro | B_LYS_703 | NZ | B_ASP_499 | OD1 | 2.606 |
| lispro | B_LYS_703 | NZ | B_ASP_499 | OD2 | 2.965 |
| lispro | B_ARG_720 | NH2 | C_GLU_47 | OE1 | 2.958 |

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|---|---|---|---|---|---|
| lispro | B_ARG_757 | NH1 | B_GLU_633 | OE2 | 3.229 |
| lispro | B_ARG_757 | NH1 | B_GLU_782 | OE1 | 2.716 |
| lispro | B_ARG_757 | NH2 | B_GLU_782 | OE1 | 2.749 |
| lispro | B_ARG_757 | NH2 | B_GLU_782 | OE2 | 3.672 |
| lispro | B_LYS_761 | NZ | B_ASP_789 | OD1 | 2.767 |
| lispro | B_LYS_761 | NZ | B_ASP_789 | OD2 | 2.695 |
| lispro | B_LYS_761 | NZ | B_GLU_793 | OE1 | 3.181 |
| lispro | B_ARG_774 | NH2 | B_GLU_837 | OE1 | 2.594 |
| lispro | B_ARG_774 | NH2 | B_GLU_837 | OE2 | 3.876 |
| lispro | B_ARG_780 | NH1 | B_GLU_633 | OE1 | 3.071 |
| lispro | B_ARG_780 | NH1 | B_GLU_633 | OE2 | 2.729 |
| lispro | B_ARG_804 | NH1 | B_GLU_637 | OE1 | 3.255 |
| lispro | B_ARG_804 | NH1 | B_GLU_637 | OE2 | 2.641 |
| lispro | B_ARG_804 | NH2 | B_GLU_637 | OE1 | 2.632 |
| lispro | B_ARG_804 | NH2 | B_GLU_637 | OE2 | 3.252 |
| lispro | B_LYS_810 | NZ | B_ASP_813 | OD2 | 3.191 |
| lispro | B_HIS_829 | ND1 | B_GLU_821 | OE1 | 3.166 |
| lispro | B_LYS_836 | NZ | B_GLU_837 | OE2 | 2.620 |
| lispro | B_ARG_850 | NH1 | B_GLU_856 | OE1 | 2.564 |
| lispro | B_ARG_850 | NH2 | B_GLU_855 | OE2 | 2.633 |
| lispro | B_ARG_850 | NH2 | B_GLU_856 | OE1 | 3.331 |
| lispro | B_ARG_851 | NH2 | B_ASP_854 | OD1 | 2.731 |
| lispro | B_ARG_851 | NH2 | B_ASP_854 | OD2 | 2.991 |
| lispro | B_HIS_858 | NE2 | B_GLU_856 | OE2 | 3.716 |
| lispro | B_LYS_864 | NZ | B_GLU_643 | OE2 | 2.829 |
| lispro | B_HIS_865 | NE2 | B_GLU_869 | OE1 | 3.220 |

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|---|---|---|---|---|---|
| lispro | B_HIS_865 | NE2 | B_GLU_869 | OE2 | 3.116 |
| lispro | B_ARG_870 | NH1 | B_GLU_821 | OE1 | 3.898 |
| lispro | B_ARG_870 | NH2 | B_GLU_821 | OE1 | 3.358 |
| lispro | B_ARG_875 | NH1 | B_GLU_869 | OE1 | 3.500 |
| lispro | B_ARG_875 | NH2 | B_GLU_869 | OE1 | 2.610 |
| lispro | B_ARG_875 | NH2 | B_GLU_869 | OE2 | 3.407 |
| lispro | B_ARG_885 | NH1 | B_GLU_899 | OE1 | 2.805 |
| lispro | B_ARG_885 | NH1 | B_GLU_899 | OE2 | 3.610 |
| lispro | B_ARG_885 | NH2 | B_GLU_899 | OE1 | 3.312 |
| lispro | B_ARG_885 | NH2 | B_GLU_899 | OE2 | 2.610 |
| lispro | B_ARG_887 | NH2 | B_GLU_846 | OE1 | 3.092 |
| lispro | C_LYS_28 | NZ | A_ASP_12 | OD2 | 2.702 |
| lispro | C_ARG_31 | NH2 | C_GLU_33 | OE1 | 3.676 |
| lispro | C_ARG_31 | NH2 | C_GLU_33 | OE2 | 3.242 |
| lispro | C_ARG_32 | NH1 | C_GLU_35 | OE1 | 2.889 |
| lispro | C_ARG_32 | NH1 | C_GLU_35 | OE2 | 2.825 |
| lispro | D_ARG_22 | NH2 | D_GLU_70 | OE2 | 3.935 |
| lispro | D_LYS_52 | NZ | B_GLU_329 | OE2 | 3.665 |
| lispro | D_ARG_53 | NH1 | D_GLU_47 | OE1 | 2.776 |
| lispro | D_ARG_53 | NH2 | D_GLU_47 | OE1 | 2.757 |
| lispro | E_ARG_32 | NH1 | E_GLU_33 | OE1 | 2.677 |
| lispro | E_ARG_32 | NH2 | E_GLU_33 | OE1 | 2.746 |
| lispro | F_ARG_31 | NH1 | F_GLU_33 | OE2 | 2.647 |
| lispro | F_ARG_31 | NH2 | F_GLU_33 | OE2 | 2.692 |
| lispro | F_ARG_32 | NH2 | F_ASP_36 | OD1 | 2.564 |
| lispro | F_ARG_32 | NH2 | F_ASP_36 | OD2 | 3.516 |

Table 6: Salt bridging [9] network of insulin lispro. In this table, the residue naming scheme is **Chain ID_residue name_residue number**.

| PDB file name | Acceptor (A) | Donor (D) | Hydrogen (H) | D-A (Å) | H-A (Å) | $\angle ADH(°)$ |
|---|---|---|---|---|---|---|
| lispro | OE2, F_GLU_13 | OG, A_SER_481 | HG, A_SER_481 | 2.66 | 1.74 | 14.06 |
| lispro | ND1, F_HIS_10 | NZ, A_LYS_484 | HZ2, A_LYS_484 | 3.68 | 2.81 | 26.35 |
| lispro | OE1, F_GLU_70 | ND2, A_ASN_547 | HD21, A_ASN_547 | 3.60 | 2.65 | 17.10 |
| lispro | OE2, F_GLU_70 | ND2, A_ASN_547 | HD21, A_ASN_547 | 3.21 | 2.37 | 27.99 |
| lispro | O, F_CYS_64 | NH1, A_ARG_554 | HH12, A_ARG_554 | 3.11 | 2.21 | 22.25 |
| lispro | OE2, D_GLU_57 | ND2, A_ASN_711 | HD22, A_ASN_711 | 3.22 | 2.39 | 29.20 |
| lispro | OXT, D_ASN_74 | NH1, A_ARG_717 | HH12, A_ARG_717 | 2.67 | 1.77 | 21.20 |
| lispro | OE2, D_GLU_33 | NH1, A_ARG_722 | HH11, A_ARG_722 | 3.01 | 2.06 | 16.41 |
| lispro | OE2, D_GLU_70 | N, A_SER_724 | H, A_SER_724 | 3.03 | 2.05 | 11.22 |
| lispro | O, F_TYR_16 | N, A_ASN_743 | H, A_ASN_743 | 3.92 | 2.93 | 11.33 |
| lispro | OE1, F_GLU_13 | N, A_SER_745 | H, A_SER_745 | 3.89 | 2.89 | 6.62 |
| lispro | OE1, F_GLU_47 | NZ, B_LYS_149 | HZ3, B_LYS_149 | 2.56 | 1.65 | 19.89 |
| lispro | O, D_ALA_34 | NH1, B_ARG_270 | HH12, B_ARG_270 | 2.70 | 1.77 | 18.02 |
| lispro | OE1, D_GLN_38 | NH2, B_ARG_271 | HH21, B_ARG_271 | 3.63 | 2.64 | 10.85 |
| lispro | OE2, E_GLU_21 | NH2, B_ARG_479 | HH21, B_ARG_479 | 2.69 | 1.80 | 23.08 |
| lispro | OE2, E_GLU_13 | OG, B_SER_481 | HG, B_SER_481 | 2.65 | 1.82 | 24.30 |
| lispro | ND1, E_HIS_10 | NZ, B_LYS_484 | HZ3, B_LYS_484 | 3.68 | 2.81 | 26.81 |
| lispro | OE1, E_GLU_70 | ND2, B_ASN_547 | HD21, B_ASN_547 | 3.92 | 2.99 | 19.43 |
| lispro | OE2, E_GLU_70 | ND2, B_ASN_547 | HD21, B_ASN_547 | 3.21 | 2.39 | 29.52 |
| lispro | O, E_CYS_64 | NH1, B_ARG_554 | HH12, B_ARG_554 | 3.03 | 2.15 | 24.41 |
| lispro | O, C_ASN_71 | N, B_ARG_717 | H, B_ARG_717 | 2.82 | 1.84 | 11.43 |
| lispro | NE2, C_GLN_51 | NH1, B_ARG_720 | HH12, B_ARG_720 | 3.76 | 2.77 | 9.52 |
| lispro | OE1, C_GLN_51 | NH2, B_ARG_720 | HH22, B_ARG_720 | 2.71 | 1.86 | 26.51 |
| lispro | ND2, A_ASN_15 | N, C_PHE_24 | H, C_PHE_24 | 3.36 | 2.35 | 1.97 |
| lispro | OD2, A_ASP_12 | NZ, C_LYS_28 | HZ2, C_LYS_28 | 2.70 | 1.81 | 22.61 |
| lispro | NH2, A_ARG_270 | N, C_THR_30 | H, C_THR_30 | 3.97 | 3.06 | 22.71 |
| lispro | O, B_VAL_712 | NZ, C_LYS_52 | HZ2, C_LYS_52 | 2.70 | 1.71 | 8.73 |
| lispro | OD1, B_ASN_711 | N, C_ILE_55 | H, C_ILE_55 | 2.86 | 1.96 | 22.14 |
| lispro | OD1, B_ASN_711 | N, C_VAL_56 | H, C_VAL_56 | 3.28 | 2.28 | 4.88 |
| lispro | OG, B_SER_724 | N, C_SER_65 | H, C_SER_65 | 3.02 | 2.04 | 10.93 |
| lispro | O, A_ASN_730 | N, D_VAL_2 | H1, D_VAL_2 | 2.70 | 1.88 | 29.20 |
| lispro | ND2, B_ASN_15 | N, D_PHE_24 | H, D_PHE_24 | 3.40 | 2.39 | 3.40 |
| lispro | OD1, A_ASN_711 | N, D_ILE_55 | H, D_ILE_55 | 2.87 | 1.93 | 17.59 |
| lispro | OD1, A_ASN_711 | N, D_VAL_56 | H, D_VAL_56 | 3.28 | 2.27 | 6.08 |
| lispro | O, B_ARG_554 | NE2, E_GLN_4 | HE22, E_GLN_4 | 3.45 | 2.55 | 22.80 |
| lispro | OE2, A_GLU_154 | N, E_GLY_54 | H, E_GLY_54 | 2.69 | 1.72 | 13.17 |

| | | | | | | |
|---|---|---|---|---|---|---|
| lispro | O, B_ASP_535 | OH, E_TYR_67 | HH, E_TYR_67 | 2.71 | 1.89 | 26.05 |
| lispro | O, A_ARG_554 | NE2, F_GLN_4 | HE22, F_GLN_4 | 3.62 | 2.77 | 27.69 |
| lispro | OD1, A_ASN_743 | OH, F_TYR_16 | HH, F_TYR_16 | 3.48 | 2.67 | 27.69 |
| lispro | O, A_ASP_535 | OH, F_TYR_67 | HH, F_TYR_67 | 2.66 | 1.87 | 28.17 |

Table 7: The side chain and main chain hydrogen bonding analysis [9] of IR in complex with insulin lispro, except that the distance ($D-A$) cutoff for hydrogen bond screening was set to be 4 Å. In this table, the residue naming scheme is **Chain ID_residue name_residue number**, $\angle ADH$ represents the angle formed by acceptor (A), donor (D) and hydrogen (H) ($\angle ADH$).

## 2.3 A comparison of interfacial electrostatic interactions between IR-insulin and IR-insulin lispro

| PDB file name | Residue A | Atom A | Residue B | Atom B | Distance (Å) |
|---|---|---|---|---|---|
| H6PXV | A_ARG_479 | NH2 | G_GLU_21 | OE1 | 3.630 |
| H6PXV | A_ARG_479 | NH2 | G_GLU_21 | OE2 | 3.051 |
| H6PXV | C_ARG_479 | NH2 | F_GLU_21 | OE1 | 3.657 |
| H6PXV | C_ARG_479 | NH2 | F_GLU_21 | OE2 | 3.064 |
| H6PXW | A_ARG_479 | NH2 | F_GLU_21 | OE1 | 3.630 |
| H6PXW | A_ARG_479 | NH2 | F_GLU_21 | OE2 | 3.052 |
| H6PXW | B_ARG_479 | NH2 | E_GLU_21 | OE1 | 3.656 |
| H6PXW | B_ARG_479 | NH2 | E_GLU_21 | OE2 | 3.065 |
| H6SOF | C_LYS_40 | NZ | H_GLU_21 | OE2 | 3.086 |
| H6SOF | C_LYS_484 | NZ | L_GLU_13 | OE1 | 3.321 |
| H6SOF | L_HIS_10 | NE2 | C_GLU_674 | OE1 | 2.709 |
| H6SOF | L_HIS_10 | NE2 | C_GLU_674 | OE2 | 3.653 |
| H6SOF | J_HIS_10 | NE2 | A_ASP_483 | OD2 | 3.765 |

Table 8: Salt bridges formed at the binding interface of insulin and IR for three experimental insulin-IR structures (PDB IDs: 6PXV, 6PXW and 6SOF, Table 1). In this table, the residue naming scheme is **Chain ID_residue name_residue number**, and '.pdb' is not included in the **PDB file name**.

| PDB file name | Residue A | Atom A | Residue B | Atom B | Distance (Å) | Note |
|---|---|---|---|---|---|---|
| lispro | A_ARG_722 | NH1 | D_GLU_33 | OE2 | 3.008 | E33 missing |
| lispro | B_LYS_149 | NZ | F_GLU_47 | OE1 | 2.562 | E47 missing |
| lispro | B_ARG_270 | NH1 | D_ASP_36 | OD1 | 3.818 | E36 missing |
| lispro | B_ARG_479 | NH2 | E_GLU_21 | OE1 | 3.575 | same |
| lispro | B_ARG_479 | NH2 | E_GLU_21 | OE2 | 2.685 | same |
| lispro | B_ARG_720 | NH2 | C_GLU_47 | OE1 | 2.958 | E47 missing |
| lispro | C_LYS_28 | NZ | A_ASP_12 | OD2 | 2.702 | novel |
| lispro | D_LYS_52 | NZ | B_GLU_329 | OE2 | 3.665 | K52 missing |

Table 9: Salt bridges formed at the binding interface of insulin lispro and IR for supplementary file **lispro.pdb**. In this table, the residue naming scheme is **Chain ID_residue name_residue number**, and '.pdb' is not included in the **PDB file name**.

In Table 9, the last column accounts for the differences in the interfacial salt bridging networks of the structures of IR in complexes with both regular insulin and insulin lispro. For instance,

1. **E33 missing** represents that while an interfacial salt bridge was structurally identified between A_ARG_722 and D_GLU_33 for insulin lispro in complex with IR, no interfacial salt bridge was structurally identified for regular insulin in complex with IR because the atomic coordinates of residue Glu33 (E33) are missing in the original PDB file (PDB IDs: 6PXV and 6PXW, Table 1).

2. **same** means that the interfacial salt bridges between ARG_479 and GLU_21 were structurally identified for IR structures in complexes with both regular insulin and insulin lispro.

3. **novel** means that an entirely new interfacial salt bridge was formed between C_LYS_28 and A_ASP_12 at the binding interface between IR and insulin lispro

due to the simple Pro28-Lys29 exchange in the B chain of regular human insulin. Of further biophysical interest, the side chain nitrogen atom (carrying positive electric charge) of C_LYS_28 is only 4.7 Å away (Table 12) from another side chain oxygen atom (carrying negative electric charge) of A_ASP_12, listed in Table 12, which is rather close to the cutoff distance for salt bridge screening as described previously in [9].

## 2.4 Three sets of interfacial electrostatic interactions that stabilize structure of insulin lispro in complex with IR

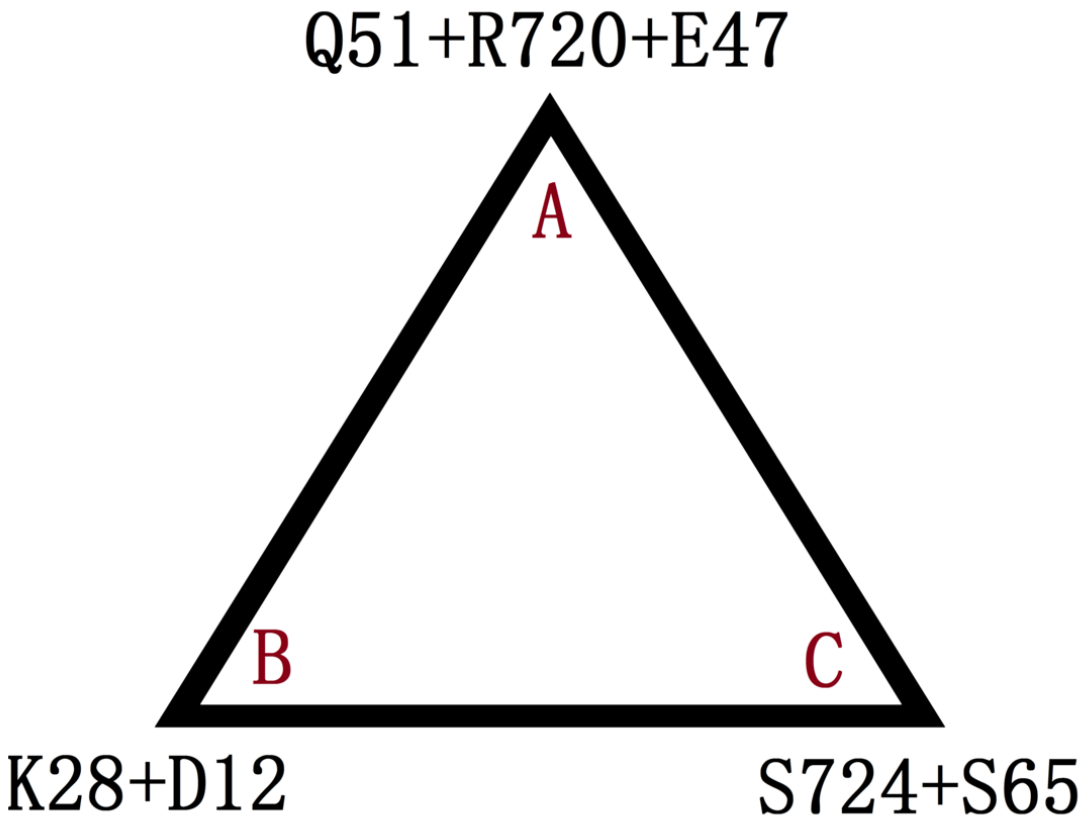


Figure 2: An electrostatic interaction triangle ($\triangle ABC$) that stabilizes the complex structure of insulin lispro and IR. Of $\triangle ABC$ in this figure, all details of the three vertices are listed in Tables 10, 11, 12, 13 and 14 as below.

### 2.4.1 Vertex A of an electrostatic interaction triangle

| **PDB file name** | **Residue A** | **Atom A** | **Residue B** | **Atom B** | **Distance (Å)** |
|---|---|---|---|---|---|
| lispro | B_ARG_720 | NH1 | C_GLU_47 | OE1 | 4.751 |
| lispro | B_ARG_720 | NH2 | C_GLU_47 | OE1 | 2.958 |

Table 10: Interfacial salt bridges at **vertex A** of the electrostatic triangle in the complex structure of IR and insulin lispro. In this table, the residue naming scheme is **Chain ID_residue name_residue number**, and '.pdb' is not included in the **PDB file name**.

| PDB file name | Acceptor (A) | Donor (D) | Hydrogen (H) | D-A (Å) | H-A (Å) | $\angle ADH(°)$ |
|---|---|---|---|---|---|---|
| lispro | NE2, C_GLN_51 | NH1, B_ARG_720 | HH12, B_ARG_720 | 3.76 | 2.77 | 9.52 |
| lispro | OE1, C_GLN_51 | NH2, B_ARG_720 | HH22, B_ARG_720 | 2.71 | 1.86 | 26.51 |

Table 11: Interfacial side chain hydrogen bonds at **vertex A** of the electrostatic triangle in the complex structure of IR and insulin lispro. In this table, the residue naming scheme is **Chain ID_residue name_residue number**, $\angle ADH$ represents the angle formed by acceptor (A), donor (D) and hydrogen (H) ($\angle ADH$), and '.pdb' is not included in the **PDB file name**.

### 2.4.2 Vertex B of an electrostatic interaction triangle

| PDB file name | Residue A | Atom A | Residue B | Atom B | Distance (Å) |
|---|---|---|---|---|---|
| lispro | C_LYS_28 | NZ | A_ASP_12 | OD1 | 4.687 |
| lispro | C_LYS_28 | NZ | A_ASP_12 | OD2 | 2.702 |

Table 12: Interfacial salt bridges at **vertex B** of the electrostatic triangle in the complex structure of IR and insulin lispro. In this table, the residue naming scheme is **Chain ID_residue name_residue number**, and '.pdb' is not included in the **PDB file name**.

| PDB file name | Acceptor (A) | Donor (D) | Hydrogen (H) | D-A (Å) | H-A (Å) | $\angle ADH(°)$ |
|---|---|---|---|---|---|---|
| lispro | OD2, A_ASP_12 | NZ, C_LYS_28 | HZ2, C_LYS_28 | 2.70 | 1.81 | 22.61 |

Table 13: Interfacial side chain hydrogen bond at **vertex B** of the electrostatic triangle in the complex structure of IR and insulin lispro. In this table, the residue naming scheme is **Chain ID_residue name_residue number**, $\angle ADH$ represents the angle formed by acceptor (A), donor (D) and hydrogen (H) ($\angle ADH$), and '.pdb' is not included in the **PDB file name**.

### 2.4.3 Vertex C of an electrostatic interaction triangle

| PDB file name | Acceptor (A) | Donor (D) | Hydrogen (H) | D-A (Å) | H-A (Å) | $\angle ADH$(°) |
|---|---|---|---|---|---|---|
| lispro | OG, B_SER_724 | N, C_SER_65 | H, C_SER_65 | 3.02 | 2.04 | 10.93 |

Table 14: Interfacial hydrogen bond at **vertex C** of the electrostatic triangle in the complex structure of IR and insulin lispro. In this table, the residue naming scheme is **Chain ID_residue name_residue number**, $\angle ADH$ represents the angle formed by acceptor (A), donor (D) and hydrogen (H) ($\angle ADH$), and '.pdb' is not included in the **PDB file name**.

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