 doi:10.20944/preprints202007.0552.v1

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

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July 13, 2020

Abstract

Insulin lispro was the first fast acting insulin analogue to obtain regulatory approval for therapeutic use. This article puts forward a novel biophysical mechanism where the net impact of the simple B28Pro-B29Lys exchange from regular insulin to insulin lispro is the establishment of a novel set of interfacial electrostatic interactions between Lys28 of insulin lispro and Asp12 of insulin receptor (IR). In addition, a set of structural analysis was presented in this article to further strengthen the binding of insulin lispro to IR, where two polar amino acid residues (Gln51 and Asn74 of insulin lispro) were put forward as two potential targets for site-directed mutagenesis of insulin lispro at its binding interface with IR.

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1 Introduction

It is almost a century since the discovery of insulin in 1921, a medical triumph which led to it being one of the most intensely studied molecules [1, 2, 3, 4, 5, 6]. While new methods were developed and used to change the structure of regular human insulin to make it more suitable for subcutaneous administration, various insulin analogues were developed with pharmacodynamic and pharmacokinetic features different from that of regular human insulin [7, 8, 9, 10, 11], among which insulin lispro was the first fast acting insulin analogue to obtain regulatory approval for therapeutic use in 1996 [12, 13, 14, 5, 15].

Genetically, insulin lispro is engineered in such a way that it has a similar amino acid sequence as the regular insulin but has an exchange of proline (Pro)-lysine (Lys) sequence at positions 28 and 29 in its B chain, leading to the creation of a fast acting B28Lys-B29Pro insulin mutant. Pharmacologically, due to its significantly reduced self-association, insulin lispro offers faster subcutaneous absorption, and begins to exert its effects earlier than its wild-type counterpart [16, 17, 18, 19, 20, 21, 22, 23].

2 Motivation

To date, insulin treatment still imposes a challenging regimen and provides sub-optimal outcomes for the majority of patients [24, 25, 15]. Moreover, despite continued advancements in the R&D of fast acting insulin analogues and continued depositions of biomolecular experimental structures in PDB [26], the biophysics underlying still remains not clear from a biomolecular structural point of view. Thus, this article aims to answer: what is the biophysical basis of the BPro28-BLys29 exchange from regular insulin to insulin lispro? what lesson can be learned from it?

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3 Materials and Methods

Of this article, all materials and methods are included the *Supplementary Materials and Methods* section in supplementary file **supps.pdf**.

4 Results

4.1 Biophysical basis of the simple BPro28-BLys29 exchange from regular insulin to insulin lispro

Here, a set of structural analysis (Tables 5-9, Figure 2, supplementary file **supps.pdf**) led to a direct structural observation (Figures 1 and 2) that the net impact of the simple B28Pro-B29Lys exchange from regular insulin to insulin lispro is the establishment of a novel set of interfacial electrostatic interactions between Lys28 of insulin lispro and Asp12 of insulin receptor (IR).

Specifically,

1. there is no interfacial salt bridge or hydrogen bond for Asp12 (of IR) or Pro28 (of regular insulin) or Lys29 (of regular insulin).

2. there is no interfacial salt bridge or hydrogen bond between Asp12 (of IR) and Pro29 (of insulin lispro).

3. there is one interfacial salt bridge (induced by the simple B28Pro-B29Lys exchange) between Asp12 (of IR) and Lys28 (of insulin lispro) (Table 1).

4. there is one interfacial side chain hydrogen bond (induced by the simple B28Pro-B29Lys exchange) between Asp12 (of IR) and Lys28 (of insulin lispro) (Table 2).

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Of further biophysical interest, the side chain nitrogen atom (carrying one unit of net positive electric charge) of C_LYS_28 is only 4.7 Å away from another side chain oxygen atom (carrying one unit of net negative electric charge) of A_ASP_12 (Table 1), which is rather close to the cutoff distance (4 Å) for salt bridge screening as used previously in [27].

PDB file	**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 1: Interfacial salt bridges at **vertex B** (Figures 3 and 4) 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**.

PDB file	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 2: Interfacial side chain hydrogen bond at **vertex B** (Figures 3 and 4) 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**.

With a close inspection of the structural model of IR in complex with insulin lispro (supplementary file **lispro.pdb**), an electrostatic interaction triangle was found to sit at the binding interface of IR and insulin lispro, which constitutes an even more favourable contribution towards insulin-IR interfacial structural stabilization, as illustrated in Figures 3 and 4.

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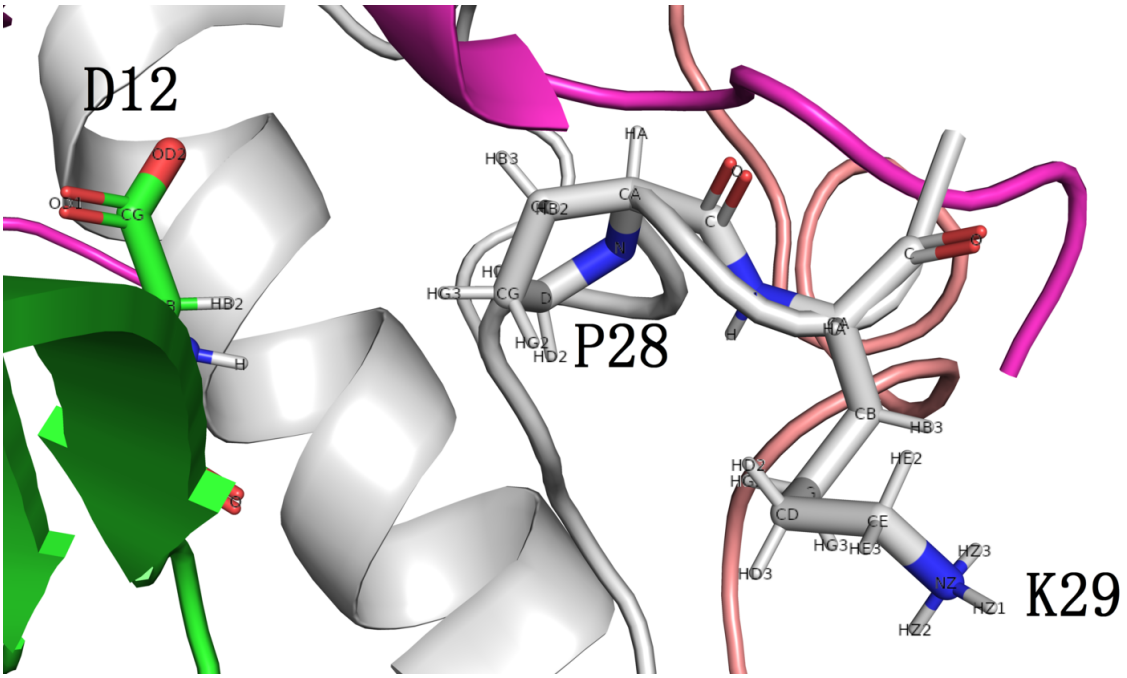

Figure 1: The spatial locations of Asp12 (of IR, green cartoon), Pro28 (of regular insulin, gray cartoon) and Lys29 (of regular insulin, gray cartoon) in the complex structure of regular insulin bound to IR (PDB ID: 6SOD). In this figure, all atoms of the three residues are labelled with their respective names, and the side chain nitrogen atom of Lys29 is as far as 13.9, 10.9, and 14.3 Å away from the two side chain oxygen atoms and the backbone oxygen atom, while the side chain carbon atoms of Pro28 are as far as 6 and 6.8 Å away from the the two side chain oxygens and backbone oxygen atom of Asp12.

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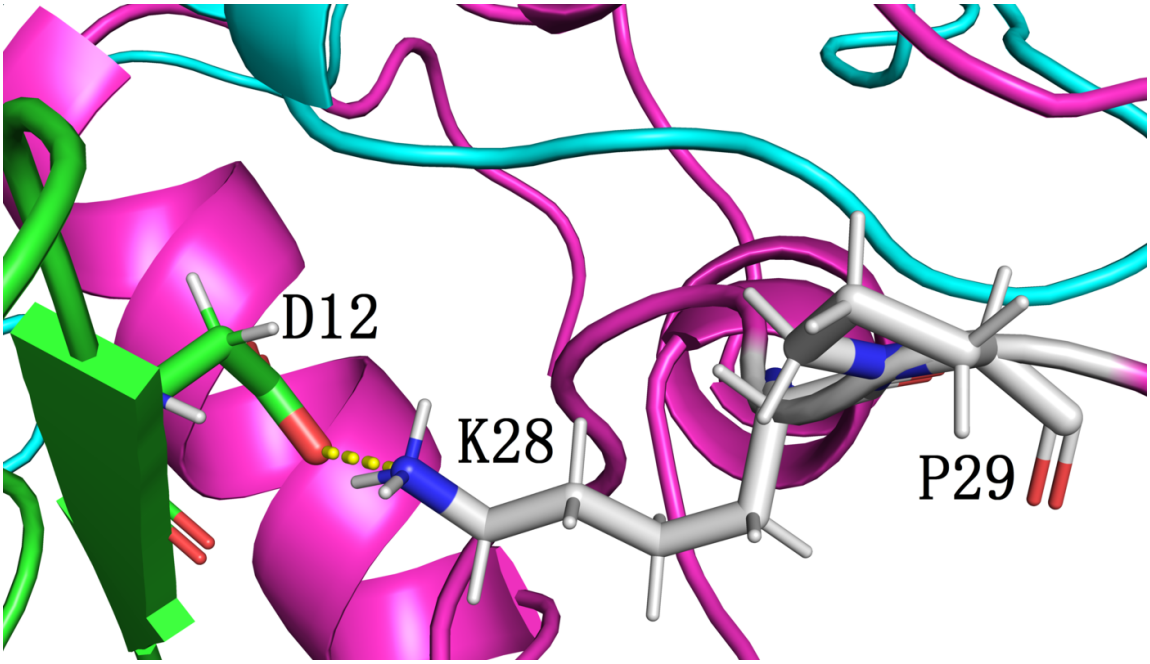

Figure 2: The spatial locations of Asp12 (of IR, green cartoon), Lys28 (of insulin lispro, purple cartoon) and Pro29 (of insulin lispro, purple cartoon) in the complex structure of insulin lispro bound to IR (supplementary file **lispro.pdb**). In this figure, the side chain nitrogen atom of Lys28 is only 2.7, 3.1 and 4.7 Å away from the two side chain oxygen atoms and the backbone oxygen atom of Asp12, allowing the establishment of a set of strong interfacial electrostatic interactions (salt bridging, dipole-dipole interaction and side chain hydrogen bonding) between insulin lispro and IR.

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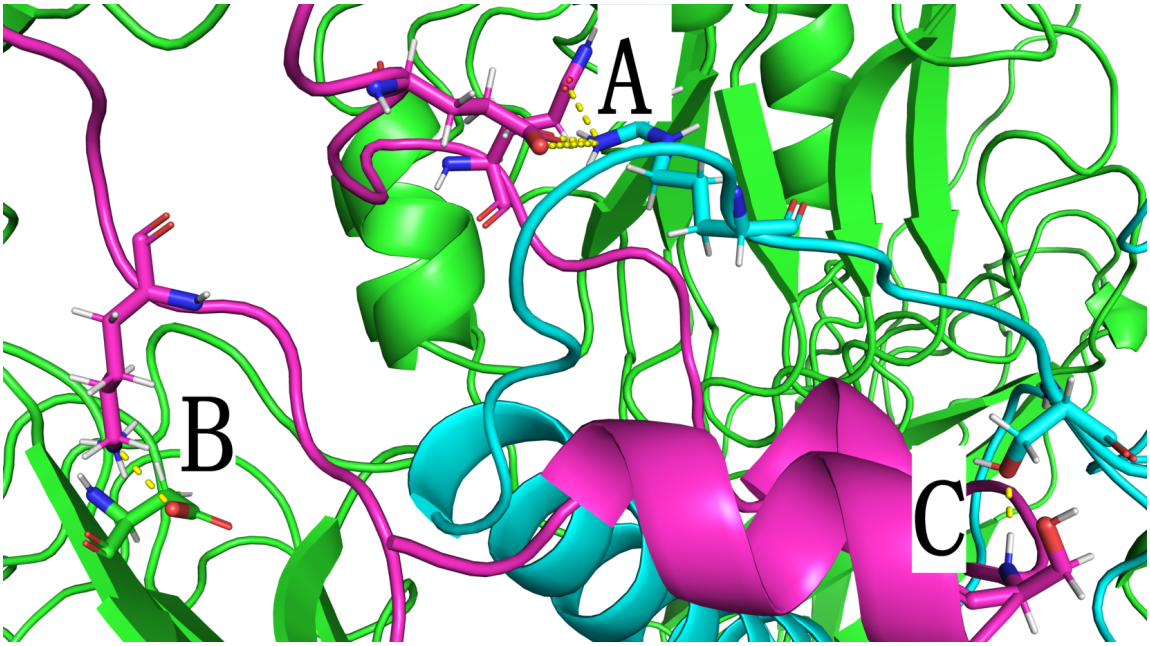

Figure 3: An electrostatic interaction triangle ($\triangle ABC$) stabilizes the insulin lispro-IR complex structure (supplementary file **lispro.pdb**). Of the $\triangle ABC$ in this figure, all details of the three insulin lispro-IR-stabilizing vertices are listed in Tables 10-14 in supplementary file **supps.pdf**, all interfacial electrostatic interactions are indicated with yellow dotted lines beside the three letters **A, B and C**. In this figure, IR is shown as green cartoon, chains A and B of insulin lispro in cyan and purple cartoon, respectively.

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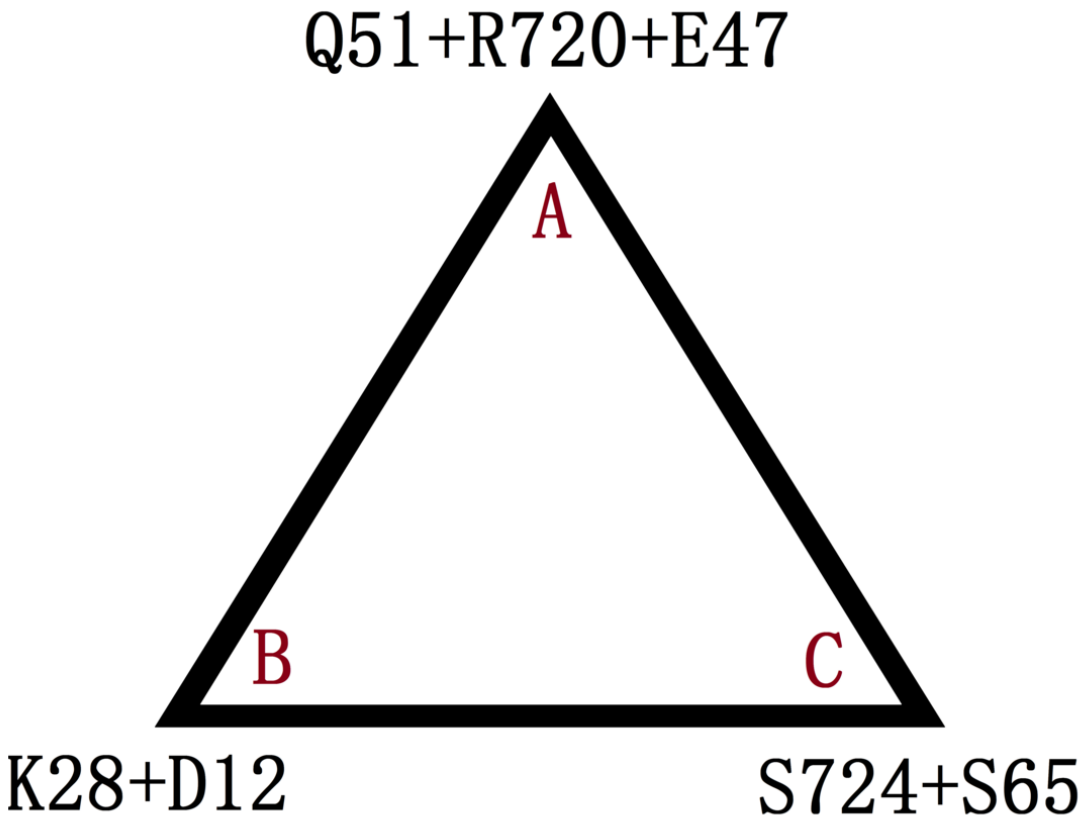

Figure 4: An electrostatic interaction triangle ($\triangle ABC$, Figure 3) stabilizes the complex structure of insulin lispro and IR (supplementary file **lispro.pdb**). Of the $\triangle ABC$ in this figure, all details of the three insulin lispro-IR-stabilizing vertices (**A, B and C**, the same as those in Figure 3) are listed in Tables 10-14 in supplementary file **supps.pdf**.

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5 Conclusion

Incorporating currently available structural data, this article puts forward a biophysical mechanism where the net impact of the simple B28Pro-B29Lys exchange from regular insulin to insulin lispro is the establishment of a novel set of interfacial electrostatic interactions between Lys28 of insulin lispro and Asp12 of IR at their binding interface (Figures 1 and 2), which constitutes an energetically favourable contribution towards the binding of insulin lispro and IR and towards their interfacial structural stabilization, too.

In addition to the biophysical basis discussed above, this article also puts forward a set of structural analysis towards the design of novel insulin analogues to enhance the binding affinity between insulin and IR, which is to be discussed as below.

6 How insulin-IR binding can be improved using mutation-induced interfacial electrostatic perturbation: a structural biophysical lesson

Another insulin analogue, peglispro [28, 29, 30, 31] was derived by covalent attachment of a linear 20 kD polyethylene polymer to the LysB28 side chain in insulin lispro. With such modification, extended action of insulin peglispro was achieved through increased hydrodynamic size of the analogue owing to the PEG conjugate [15, 32]. This increase in size results in slower subcutaneous absorption, which contributes to the appreciable prolongation of the half-life of insulin peglispro [15, 32].

However, as mentioned above, insulin treatment to date is still a challenging field and provides sub-optimal outcomes for the majority of patients [24, 25, 15]. Here,

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with a set of electrostatic interaction analysis in supplementary file **supps.pdf**, this article puts forward two structural clues for the design of novel insulin analogues with higher binding affinity to IR than insulin lispro [33, 34]. For examples, two side chain hydrogen bonds already exist at the insulin-IR complex structural interface (Table 3). On top of the two hydrogen bonds (Table 3), site-directed mutagenesis [34] (Gln51Glu or Asn74Asp, Table 3) of insulin lispro can be generated to create stronger charge-charge interactions at the insulin-IR complex structural interface, where two sets of novel interfacial salt bridges can be created between Arg717 and Asp74, and between Arg720 and Glu51, on top of the two side chain hydrogen bonds which already exist at the insulin lispro-IR interface (Table 3).

PDB file	Acceptor (A)	Donor (D)	Hydrogen (H)	D-A (Å)	H-A (Å)	$\angle ADH(^\circ)$
lispro	OXT, D_ASN_74	NH1, A_ARG_717	HH12, A_ARG_717	2.67	1.77	21.20
lispro	OE1, C_GLN_51	NH2, B_ARG_720	HH22, B_ARG_720	2.71	1.86	26.51

Table 3: Two interfacial side chain hydrogen bonds for IR in complex with 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) ($\angle ADH$). With site-directed mutants (Gln51Glu or Asn74Asp).

Biophysically, these site-directed mutagenesis for Asn74 and Gln51 (Gln51Glu or Asn74Asp) are able to strengthen the binding affinity between IR and novel insulin lispro analogues, in a manner similar to the biophysical mechanism discussed above, where the simple B28Pro-B29Lys exchange from regular insulin to insulin lispro strengthens the binding of insulin lispro to IR.

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