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Designing Insulin Analogues with Lower Binding Affinity to Insulin Receptor Than that of Insulin Icodec

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Abstract: Insulin therapy is a cornerstone in the management of diabetes, yet the pursuit of optimizing its pharmacokinetic profile remains a focal point in diabetes research. For instance, insulin icodec of Novo Nordisk is a novel long-acting insulin analogue that exhibits an extended duration of action, providing a promising once-weekly treatment option for diabetic patients. However, designing insulin analogues with lower receptor affinity than that of insulin icodec could still produce a further extended duration of action than that of insulin icodec through the suppression of receptor-mediated internalization of insulin analogues. In this study, therefore, I present the design of a novel series of insulin analogues engineered towards lower binding affinity to the insulin receptor compared to that of insulin icodec. Through computational structural biophysics-based rational design strategies, this article aims to further extend the duration of action while maintaining therapeutic efficacy of insulin analogues. Utilizing homology molecular modeling and structural biophysics-based binding affinity calculations, this article puts forward a set of insulin analogues with lower binding affinity to insulin receptor than that of insulin icodec from a structural and biophysical point of view. Overall, this article calls for subsequent in vitro and in vivo evaluations of the efficacy and prolonged action of the engineered insulin analogues to pharmacokinetically test whether these analogues acutally surpass that of insulin icodec as hopeful candidates for next-generation insulin analogue therapies with improved duration of action and enhanced control of blood glucose levels for diabetic patients in future.

Keywords: insulin icodec; binding affinity; site-specific mutation; receptor-mediated internalization

1. Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia, which remains a global health challenge with significant morbidity and mortality [1]. Insulin therapy stands as a cornerstone in the management of diabetes, yet the quest for optimized insulin analogues keeps driving continued innovation in therapeutic development for diabetic patients [2]. The evolution from conventional insulin formulations to engineered analogues has been propelled by the pursuit of achieving enhanced pharmacokinetic profiles, notably extended duration of action coupled with reduced risk of hypoglycemia [3]. For instance, insulin icodec of Novo Nordisk is a long-acting insulin analogue for better management of blood sugar levels in people with diabetes [4–6]. Insulin icodec is designed to provide a steady release of insulin throughout the day, mimicking the natural insulin production in the body [7–9]. Insulin icodec is typically administered through injection once a week, which helps lower blood sugar levels by allowing glucose to enter the body's cells, where it is subsequently used for energy production [10,11]. Moreover, insulin icodec has a distinct pharmacokinetic profile compared to other long-acting insulin analogs. It exhibits a long duration of action, with a half-life of approximately 196 hours, leading to improved glycemic control and reduced hypoglycemia risk [12–14].

Interestingly, Icosema (of Novo Nordisk, too) [15–18] represents a combination medication that consists of insulin icodec and semaglutide, which belongs to a class of medications called glucagon-like peptide-1 (GLP-1) receptor agonists and helps regulate blood sugar levels by stimulating the release of insulin, reducing the production of glucagon (a hormone that increases blood sugar levels), and slowing down the digestion process for weight reduction [19–22].

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In light of the two-dimensional structures of native human insulin and insulin icodec as shown in Figure 1, below is a list of structural modifications of insulin icodec compared to native human insulin [27–29]:

1. addition of a fatty acid (K50B_C20, Figures 1 and 2);
2. deletion of threonine at position B30 (T51B_del, Figures 1 and 2);
3. a site-specific missense mutation, Y14A_E (Figures 1 and 2);
4. a site-specific missense mutation, Y37B_H (Figures 1 and 2);
5. a site-specific missense mutation, F46B_H (Figures 1 and 2).

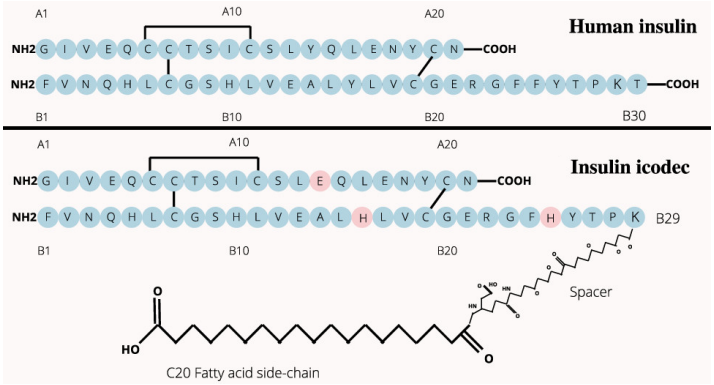

Figure 1. A brief illustration of the two-dimensional structures of native human insulin and insulin icodec [3,23,24]. In this figure, the amino acid residues with pink backgrounds represents the positions of the three site-specific mutations [25,26] (Y14A_E, Y37B_H, F46B_H) of insulin icodec.

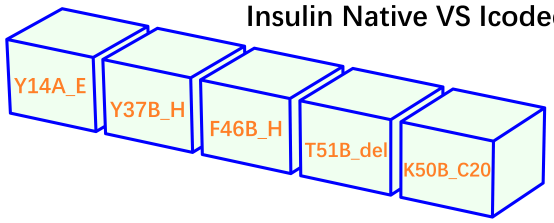

Figure 2. A summary of the structural modification of insulin icodec in comparison to native human insulin. In this figure, Y14A_E (i.e., replacement of Tyr14 (Y14) at position A14 (position 14 of chain A) by a histidine), Y37B_H (i.e., replacement of Tyr16 (Y16) at position B16 by a histidine) and F46B_H (i.e., replacement of Phe25 (F25) at position B25 by a histidine) represent three site-specific missense mutations of insulin icodec, T51B_del represents deletion of Thr30 (T30) at position B30, while K50B_C20 represents the addition of a 20-carbon fatty acid to the lysine amino acid (K50B) at position B29 [30–33].

Overall, these structural modifications (Figure 2) in insulin icodec result in a more stable and longer-acting insulin analogue compared to regular insulin, providing a more consistent and sustained blood glucose-lowering effect:

1. insulin icodec is able to form aggregates or clusters at the subcutaneous injection site, gradually releasing into the bloodstream over an extended period.
2. insulin icodec undergoes structural modifications that increases its stability and solubility and preventing enzyme-mediated degradation and rapid clearance [34].
3. insulin icodec is conjugated with a fatty acid at position B30 (K50B_C20, Figure 2). After injection, the fatty acid chain in insulin icodec interacts with albumin in the subcutaneous tissue, forming reversible albumin-insulin complexes. These complexes act as a reservoir, gradually releasing insulin icodec into the bloodstream, increasing its fat solubility and allowing it to bind to fatty acid-binding proteins, forming a depot of the insulin icodec reversibly bound to albumin.

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4. the incorporation of fatty acid chains facilitate the formation of stable hexameric structures, thereby delaying insulin absorption and promoting sustained release.
5. with respect to the addition of a fatty acid (K50B_C20, Figure 2), it is conceivable that the deletion of threonine at position B30 (T51B_del, Figure 2) is for K50B_C20 to take place easier and more efficiently than without the deletion of threonine at position B30.
6. two missense mutations of insulin icodec (Y37B_H and F46B_H) contributed to its prolonged duration of action through the induction of a modest decrease in the binding affinity of insulin icodec and its receptor (IR) [14]. Specifically, it is entirely due to the site-directed mutation Y37B_H that the salt bridge at the binding interface between insulin icodec and IR becomes weaker (from 3.204 Å to 3.669 Å), but is still not disrupted by the site-directed mutation Y37B_H, such that the binding affinity of ligand-receptor is lowered but not eliminated by the site-directed mutation Y37B_H [14], and thereby ensuring downstream signal transduction for the prolonged blood glucose-lowering effect of insulin icodec.

2. Motivation

As reported for the first time in [14], two missense mutations of insulin icodec (Y37B_H and F46B_H) contributed to its prolonged duration of action of insulin icodec through the induction of a modest decrease in the binding affinity of insulin icodec and its receptor [14], offering improved glycemic control and reduce treatment burden, thus enhancing patient adherence and overall outcomes in diabetes management [35,36]. The motivation behind this study stems from the need to exhaustively explore the entire insulin-IR molecular space [37–40] and to keep pushing forward the biophysical limit(s) of the binding affinity between insulin analogues and its receptor to finally reach a balance between the pharmacokinetic and therapeutic limits of structurally conceivable insulin analogues for improved duration of action and enhanced control of blood glucose levels for diabetic patients in future.

3. Materials and Methods

As listed in Table 1, as of April 29, 2024, there are a variety of experimental complex structures of insulin (analogues) bound to its receptor (IR), such as PDB entries 7PG3 (insulin receptor bound to 3 insulins), 7PG4 (insulin receptor bound to 2 insulins), 6SOF (insulin receptor bound to 4 insulins).

Table 1. Experimentally determined IR-related structures (released newest from oldest) in the Protein Data Bank (PDB [41–43]) as of April 29, 2024, QUERY code: UniProt Molecule Name = "Insulin receptor".

PDB ID	Structure Title (release date from newest to oldest)
8DWN	Crystal structure of bis-phosphorylated insulin receptor kinase domain
7YQ3	human insulin receptor bound with A43 DNA aptamer and insulin
7YQ4	human insulin receptor bound with A62 DNA aptamer and insulin - locally refined
7YQ5	human insulin receptor bound with A62 DNA aptamer and insulin
7YQ6	human insulin receptor bound with A62 DNA aptamer
8EYX	Cryo-EM structure of 4 insulins bound full-length mouse IR mutant with physically decoupled alpha CTs (C684S/C685S/C687S; denoted as IR-3CS) Asymmetric conformation 1
8EYY	Cryo-EM structure of 4 insulins bound full-length mouse IR mutant with physically decoupled alpha CTs (C684S/C685S/C687S, denoted as IR-3CS) Asymmetric conformation 2
8EZ0	Cryo-EM structure of 4 insulins bound full-length mouse IR mutant with physically decoupled alpha CTs (C684S/C685S/C687S; denoted as IR-3CS) Symmetric conformation
8GUY	human insulin receptor bound with two insulin molecules
7U6D	Head region of insulin receptor ectodomain (A-isoform) bound to the non-insulin agonist IM459
7U6E	Head region of insulin receptor ectodomain (A-isoform) bound to the non-insulin agonist IM462
7PHT	Structure of Insulin receptor's transmembrane domain
8DTL	Cryo-EM structure of insulin receptor (IR) bound with S597 peptide
8DTM	Cryo-EM structure of insulin receptor (IR) bound with S597 component 2

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Table 1. *Cont.*

PDB ID	Structure Title (release date from newest to oldest)
7S0Q	Head region of a complex of IGF-I with the ectodomain of a hybrid insulin receptor / type 1 insulin-like growth factor receptor
7S8V	Leg region of a complex of IGF-I with the ectodomain of a hybrid insulin receptor / type 1 insulin-like growth factor receptor
7SL1	Full-length insulin receptor bound with site 1 binding deficient mutant insulin (A-V3E)
7SL2	Full-length insulin receptor bound with site 2 binding deficient mutant insulin (A-L13R) – asymmetric conformation
7SL3	Full-length insulin receptor bound with site 2 binding deficient mutant insulin (A-L13R) – symmetric conformation
7SL4	Full-length insulin receptor bound with site 2 binding deficient mutant insulin (B-L17R) – asymmetric conformation
7SL6	Full-length insulin receptor bound with site 2 binding deficient mutant insulin (B-L17R) – symmetric conformation
7SL7	Full-length insulin receptor bound with both site 1 binding deficient mutant insulin (A-V3E) and site 2 binding deficient mutant insulin (A-L13R)
7STH	Full-length insulin receptor bound with unsaturated insulin WT (2 insulin bound) symmetric conformation
7STI	Full-length insulin receptor bound with unsaturated insulin WT (1 insulin bound) asymmetric conformation
7STJ	Full-length insulin receptor bound with unsaturated insulin WT (2 insulins bound) asymmetric conformation (Conformation 1)
7STK	Full-length insulin receptor bound with unsaturated insulin WT (2 insulins bound) asymmetric conformation (Conformation 2)
7MQO	The insulin receptor ectodomain in complex with a venom hybrid insulin analogue - "head" region
7MQR	The insulin receptor ectodomain in complex with four venom hybrid insulins - symmetric conformation
7MQS	The insulin receptor ectodomain in complex with three venom hybrid insulin molecules - asymmetric conformation
7MD4	Insulin receptor ectodomain dimer complexed with two IRPA-3 partial agonists
7MD5	Insulin receptor ectodomain dimer complexed with two IRPA-9 partial agonists
7PG0	Low resolution Cryo-EM structure of full-length insulin receptor bound to 3 insulin with visible ddm micelle, conf 1
7PG2	Low resolution Cryo-EM structure of full-length insulin receptor bound to 3 insulin, conf 1
7PG3	Low resolution Cryo-EM structure of the full-length insulin receptor bound to 3 insulin, conf 2
7PG4	Low resolution Cryo-EM structure of the full-length insulin receptor bound to 2 insulin, conf 3
7QID	tentative model of the human insulin receptor ectodomain bound by three insulin
7KD6	Insulin Receptor L1-CR plus alphaCT fragment in co-complex with Fv 83-7 and single-chain insulin SCI-b
7BW7	Cryo-EM Structure for the Ectodomain of the Full-length Human Insulin Receptor in Complex with 1 Insulin.
7BW8	Cryo-EM Structure for the Insulin Binding Region in the Ectodomain of the Full-length Human Insulin Receptor in Complex with 1 Insulin
7BWA	Cryo-EM Structure for the Ectodomain of the Full-length Human Insulin Receptor in Complex with 2 Insulin
6VEP	Human insulin in complex with the human insulin microreceptor in turn in complex with Fv 83-7
6VEQ	Con-Ins G1 in complex with the human insulin microreceptor in turn in complex with Fv 83-7
6SOF	human insulin receptor ectodomain bound by 4 insulin
6PXV	Cryo-EM structure of full-length insulin receptor bound to 4 insulin. 3D refinement was focused on the extracellular region.
6PXW	Cryo-EM structure of full-length insulin receptor bound to 4 insulin. 3D refinement was focused on the top part of the receptor complex.
6HN4	Leucine-zippered human insulin receptor ectodomain with single bound insulin - "lower" membrane-proximal part
6HN5	Leucine-zippered human insulin receptor ectodomain with single bound insulin - "upper" membrane-distal part
6CE7	Insulin Receptor ectodomain in complex with one insulin molecule

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Table 1. *Cont.*

PDB ID	Structure Title (release date from newest to oldest)
6CE9	Insulin Receptor ectodomain in complex with two insulin molecules
6CEB	Insulin Receptor ectodomain in complex with two insulin molecules - C1 symmetry
5U1M	Structure of the IRS-1 PTB Domain Bound to the Juxtamembrane Region of the Insulin Receptor
5KQV	Insulin receptor ectodomain construct comprising domains L1,CR,L2, FnIII-1 and alphaCT peptide in complex with bovine insulin and FAB 83-14 (REVISED STRUCTURE)
5TQ1	Phospholipase C gamma-1 C-terminal SH2 domain bound to a phosphopeptide derived from the insulin receptor
5J3H	Human insulin receptor domains L1-CR in complex with peptide S519C16 and 83-7 Fv
5HHW	Crystal structure of insulin receptor kinase domain in complex with cis-(R)-7-(3-(azetidin-1-ylmethyl)cyclobutyl)-5-(3-((tetrahydro-2H-pyran-2-yl)methoxy)phenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine
4ZXB	Structure of the human insulin receptor ectodomain, IRDeltabeta construct, in complex with four Fab molecules
5E1S	The Crystal structure of INSR Tyrosine Kinase in complex with the Inhibitor BI 885578
4XSS	Insulin-like growth factor I in complex with site 1 of a hybrid insulin receptor / Type 1 insulin-like growth factor receptor
4XST	Structure of the endoglycosidase-H treated L1-CR domains of the human insulin receptor in complex with residues 697-719 of the human insulin receptor (A-isoform)
4XLV	Crystal structure of the activated insulin receptor tyrosine kinase dimer
4OGA	Insulin in complex with Site 1 of the human insulin receptor
2MFR	Solution structure of the transmembrane domain of the insulin receptor in micelles
4IBM	Crystal structure of insulin receptor kinase domain in complex with an inhibitor Irfin-1
3W11	Insulin receptor ectodomain construct comprising domains L1-CR in complex with human insulin, Alpha-CT peptide(704-719) and FAB 83-7
3W12	Insulin receptor ectodomain construct comprising domains L1-CR in complex with high-affinity insulin analogue [D-PRO-B26]-DTI-NH2, alpha-CT peptide(704-719) and FAB 83-7
3W13	Insulin receptor ectodomain construct comprising domains L1-CR in complex with high-affinity insulin analogue [D-PRO-B26]-DTI-NH2, alphact peptide(693-719) and FAB 83-7
3ETA	Kinase domain of insulin receptor complexed with a pyrrolo pyridine inhibitor
3EKN	Insulin receptor kinase complexed with an inhibitor
3EKK	Insulin receptor kinase complexed with an inhibitor
2Z8C	Phosphorylated insulin receptor tyrosine kinase in complex with (4-[5-carbamoyl-4-(3-methylanilino)pyrimidin-2-yl]aminophenyl)acetic acid
3BU3	Crystal structure of the insulin receptor kinase in complex with IRS2 KRLB peptide
3BU5	Crystal structure of the insulin receptor kinase in complex with IRS2 KRLB peptide and ATP
3BU6	Crystal structure of the insulin receptor kinase in complex with IRS2 KRLB phosphopeptide
2HR7	Insulin receptor (domains 1-3)
2B4S	Crystal structure of a complex between PTP1B and the insulin receptor tyrosine kinase
2AUH	Crystal structure of the Grb14 BPS region in complex with the insulin receptor tyrosine kinase
1RQQ	Crystal Structure of the Insulin Receptor Kinase in Complex with the SH2 Domain of APS
1LK2	1.35A crystal structure of H-2Kb complexed with the GNYSFYAL peptide
1P14	Crystal structure of a catalytic-loop mutant of the insulin receptor tyrosine kinase
1I44	CRYSTALLOGRAPHIC STUDIES OF AN ACTIVATION LOOP MUTANT OF THE INSULIN RECEPTOR TYROSINE KINASE
1GAG	CRYSTAL STRUCTURE OF THE INSULIN RECEPTOR KINASE IN COMPLEX WITH A BISUBSTRATE INHIBITOR
1IR3	PHOSPHORYLATED INSULIN RECEPTOR TYROSINE KINASE IN COMPLEX WITH PEPTIDE SUBSTRATE AND ATP ANALOG
1IRK	CRYSTAL STRUCTURE OF THE TYROSINE KINASE DOMAIN OF THE HUMAN INSULIN RECEPTOR

Among the insulin receptor-related structures listed in Table 1, PDB entry 6SOF (Figure 3) [44] is the only experimental complex structure of insulin bound to IR, where all four distinct binding sites of the IR dimer are saturated by four insulin molecules. Therefore, PDB entry 6SOF [44] is chosen here as the structural template for subsequent structural modelling of insulin icodec bound to IR.

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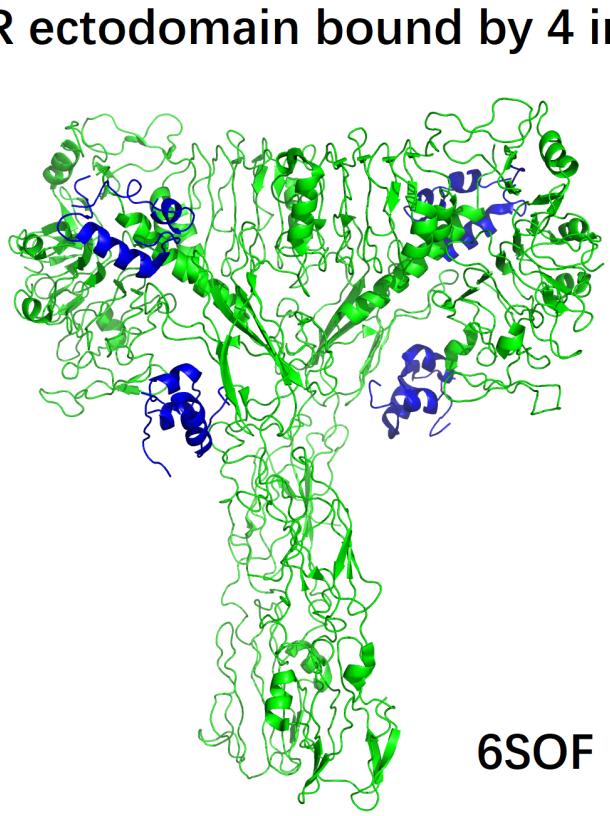

Figure 3. CryEM structure of human insulin receptor ectodomain bound by 4 insulin [30–33]. This figure is prepared with PyMol [45].

Briefly, the amino acid sequences of the ten chains of native human insulin and IR (according to PDB entry 6SOF [44] are listed in italics in fasta format as below,

>6SOF_1 | Chains A | Insulin receptor | Homo sapiens (9606)

HLYPGEVCPGMDIRNNLTRLHELENCSVIEGHLQILLMFKTRPEDFRDLSFPKLIMITDYLLLFRV YGLESLKDLFPNLTVIRGSRLFFNYALVIFEMVHLKELGLYNLMNITRGSVRIEKNNELCYLATIDWSRI LDSVEDNYIVLNKDDNEECGDICPGTAKGKTNCPATVINGQFVERCWTHSHCQKVCPTICKSHGCT AEGLCCHSECLGNCSQPDDPTKCVACRNFYLDGRCVETCPPPYYHFQDWRCVNFSFCQDLHHKCK NSRRQGCHQYVIHNNKCIPECPSGYTMNSSNLLCTPCLGPCPKVCHLLEGEKTIDSVTSAQELRGCTV INGSLIINIRGGNNLAAELEANLGLIEEISGYLKIRRSYALVSLSFFRKLRLIRGETLEIGNYSFYALDNQN LRQLWDWSKHNLTITQGKLFFHYNPKLCLSEIHKMEEVSGTKGRQERNDIALKTNGDQASCENELLK FSYIRTSFDKILLRWEPYWPPDFRDLLGFMLFYKEAPYQNVTEFDGQDACGSNSWTVVDIDPPLRSN DPKSQNHPGWLMRGLKPWTQYAIFVKTLVTFSDERRTYGAKSDIIYVQTDATNPSVPLDPISVSNSSS QIILKWKPPSDPNGNITHYLVFWERQAEDSELFELDYCLKGLKLPSRTWSPPFESEDSQKHNQSEYED SAGECCSCPKTDSQILKELEESSFRKTFEDYLHNVVFVPRPS

>6SOF_2 | Chains B | Insulin receptor | Homo sapiens (9606)

HRPFEKVVNKESLVISGLRHFTGYRIELQACNQDTPEERCSVAAYVSARTMPEAKADDIVGPVT HEIFENNVVHLMWQEPKEPNGLIVLYEVSYRRYGDEELHLCVSRKHFALERGCRLRGLSPGNYSVRI RATSLAGNGSWTEPTYFYVTDYLDVPSNIAK

>6SOF_1 | Chains C | Insulin receptor | Homo sapiens (9606)

HLYPGEVCPGMDIRNNLTRLHELENCSVIEGHLQILLMFKTRPEDFRDLSFPKLIMITDYLLLFRV YGLESLKDLFPNLTVIRGSRLFFNYALVIFEMVHLKELGLYNLMNITRGSVRIEKNNELCYLATIDWSRI LDSVEDNYIVLNKDDNEECGDICPGTAKGKTNCPATVINGQFVERCWTHSHCQKVCPTICKSHGCT AEGLCCHSECLGNCSQPDDPTKCVACRNFYLDGRCVETCPPPYYHFQDWRCVNFSFCQDLHHKCK NSRRQGCHQYVIHNNKCIPECPSGYTMNSSNLLCTPCLGPCPKVCHLLEGEKTIDSVTSAQELRGCTV

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INGSLIINIRGGNNLAAELEANLGLIEEISGYLKIRRSYALVSLSFFRKLRLIRGETLEIGNYSFYALDNQN
LRQLWDWSKHNLTITQGKLFFHYNPKLCLSEIHKMEEVSGTKGRQERNDIALKTNGDQASCENELLK
FSYIRTSFDKILLRWEPYWPPDFRDLLGFMLFYKEAPYQNVTEFDGQDACGSNSWTVVDIDPPLRSN
DPKSQNHPGWLMRGLKPWTQYAIFVKTLVTFSDERRTYGAKSDIIYVQTDATNPSVPLDPISVSNSSS
QIILKWKPPSDPNGNITHYLVFWERQAEDSELFELDYCLKGLKLPSRTWSPPFESEDSQKHNQSEYED
SAGECCSCPKTDSQILKELEESSFRKTFEDYLHNVVFVPRPS

>6SOF_2 | Chains D | Insulin receptor | Homo sapiens (9606)

HRPFEKVVNKESLVISGLRHFTGYRIELQACNQDTPEERCSVAAYVSARTMPEAKADDIVGPVT
HEIFENNVVHLMWQEPKEPNGLIVLYEVSYRRYGDEELHLCVSRKHFALERGCRLRGLSPGNYSVRI
RATSLAGNGSWTEPTYFYVTDYLDVPSNIAK

>6SOF_3 | Chains E | Insulin | Homo sapiens (9606)

GIVEQCCTSICSLYQLENYCN

>6SOF_4 | Chains F | Insulin | Homo sapiens (9606)

FVNQHLCGSHLVEALYLVCGERGFFYTPKT

>6SOF_3 | Chains G | Insulin | Homo sapiens (9606)

GIVEQCCTSICSLYQLENYCN

>6SOF_4 | Chains H | Insulin | Homo sapiens (9606)

FVNQHLCGSHLVEALYLVCGERGFFYTPKT

>6SOF_3 | Chains I | Insulin | Homo sapiens (9606)

GIVEQCCTSICSLYQLENYCN

>6SOF_4 | Chains J | Insulin | Homo sapiens (9606)

FVNQHLCGSHLVEALYLVCGERGFFYTPKT

>6SOF_3 | Chains K | Insulin | Homo sapiens (9606)

GIVEQCCTSICSLYQLENYCN

>6SOF_4 | Chains L | Insulin | Homo sapiens (9606)

FVNQHLCGSHLVEALYLVCGERGFFYTPKT

In the only experimental complex structure of four insulins bound to IR (PDB entry 6SOF (Figure 3) [44]), the first IR molecule is defined as >6SOF_1 | Chains A and >6SOF_2 | Chains B, the second IR molecule is defined as >6SOF_1 | Chains C and >6SOF_2 | Chains D, the first insulin molecule is defined as >6SOF_3 | Chains E and >6SOF_4 | Chains F, the second insulin molecule is defined as >6SOF_3 | Chains G and >6SOF_4 | Chains H, the third insulin molecule is defined as >6SOF_3 | Chains I and >6SOF_4 | Chains J and the fourth insulin molecule is defined as >6SOF_3 | Chains K and >6SOF_4 | Chains L, respectively.

Subsequently, a huge ($s = g(51,3) = \frac{51!}{3!(51-3)!} \times 20^k$ [38]) set of insulin analogues were generated with in-house Python script with three site-specific missense mutations introduced into native insulin sequences. Afterwards, homology structural modeling was carried out using Modeller [46] with PDB entry 6SOF [44] as the structural template. Finally, the binding affinity between insulin analogues and IR was calculated using Prodigy [47,48] for native insulin (10000 times), insulin icodec (10000 times) and also for $s = g(51,3) = \frac{51!}{3!(51-3)!} \times 20^k$ [38] insulin analogues (1 time).

4. Results

As experimentally determined in human insulin receptor ectodomain bound by 4 insulin (PDB entry 6SOF [44,49]) using a combined approach of cryo-EM and atomistic molecular dynamics simulation, the structure of the entire dimeric insulin receptor ectodomain is saturated with four insulin molecules, i.e., the first IR molecule (abbreviated as *A* in Tables 2–5), the second IR molecule (abbreviated as *C* in Tables 2–5), the first insulin molecule (abbreviated as *E* in Tables 2–5) consisting of >6SOF_3 | Chains E and >6SOF_4 | Chains F, the second insulin molecule (abbreviated as *G* in Tables 2–5) consisting of >6SOF_3 | Chains G and >6SOF_4 | Chains H, the third insulin molecule (abbreviated as *I* in Tables 2–5) consisting of >6SOF_3 | Chains I and >6SOF_4 | Chains J and the fourth insulin molecule (abbreviated as *K* in Tables 2–5) consisting of >6SOF_3 | Chains K and >6SOF_4 | Chains L, respectively. Therefore,

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for each insulin analogue with one set of missense mutations, there are a total of eight values of intermolecular binding affinity (K_d) to be calculated by Prodigy [47,48], as listed in Tables 2–5.

Table 2. Prodigy-calculated K_d (37 °C) values for native insulin (10000 times) and insulin icodec (10000 times), respectively, as benchlines for the comparison of K_d values against $s = g(51,3) = \frac{51!}{3!(51-3)!} \times 20^k$ [38] insulin analogues.

No.	Muta	AE	AG	AI	AK	CE	CG	CI	CK
1	Y14A_E Y16B_H F25B_H	2.404e-7	9.736e-6	5.827e-4	4.849e-6	7.038e-6	2.042e-8	7.222e-8	2.815e-4
1	native insulin molecule	3.279e-7	8.736e-6	5.946e-4	3.897e-6	7.431e-6	2.439e-8	6.379e-8	2.747e-4

Table 3. Potential long acting insulin analogue candidates with significantly lower affinity to insulin receptor than native insulin with Prodigy-calculated K_d (37 °C) values.

No.	Muta	AE	AG	AI	AK	CE	CG	CI	CK
1	Y14A_T E34B_S H31B_S	1.6e-6	2.2e-5	1.1e-3	1.4e-5	1.2e-5	5.9e-8	3.8e-7	3.0e-4
1	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
2	H31B_Q Y14A_A H26B_Q	4.4e-7	1.1e-5	4.3e-3	6.1e-6	9.7e-6	5.2e-8	5.0e-7	3.4e-4
2	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
5	N18A_A Y47B_G H31B_S	2.3e-6	1.7e-5	6.5e-4	9.2e-6	1.0e-5	9.2e-8	1.0e-7	3.6e-4
5	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
6	E34B_A H26B_Q H31B_S	5.4e-7	1.2e-5	1.4e-3	1.2e-5	9.6e-6	4.3e-8	5.3e-7	2.9e-4
6	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
7	Y37B_P Y14A_A H31B_G	5.7e-7	1.3e-5	8.9e-4	1.0e-5	1.1e-5	1.4e-7	1.6e-7	4.0e-4
7	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
15	V23B_A H31B_G E34B_S	9.1e-7	1.2e-5	7.1e-4	1.4e-5	1.2e-5	3.6e-8	2.2e-7	3.2e-4
15	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
17	Y47B_G E34B_F E42B_T	6.6e-7	2.2e-5	8.2e-4	5.6e-6	2.3e-5	5.8e-8	1.1e-7	3.2e-4
17	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
23	N18A_A Y37B_P Y14A_M	1.8e-6	1.4e-5	1.0e-3	7.1e-6	1.3e-5	3.7e-8	1.1e-7	2.9e-4
23	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
29	Y14A_A K50B_A H31B_G	4.2e-7	1.0e-5	1.1e-3	6.4e-6	8.0e-6	9.7e-8	2.7e-7	4.1e-4
29	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
30	E42B_Q H26B_Q E34B_S	4.5e-7	1.2e-5	1.0e-3	8.2e-6	1.8e-5	3.3e-8	3.4e-7	2.8e-4
30	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
31	Y47B_G Y14A_A H31B_G	4.1e-7	2.5e-5	7.4e-4	1.0e-5	1.3e-5	3.3e-8	2.6e-7	2.9e-4
31	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
33	H31B_G E34B_S L32B_H	6.3e-7	9.9e-6	8.7e-4	8.0e-6	1.1e-5	1.4e-7	1.1e-7	3.3e-4
33	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
35	N18A_A Y37B_P Y14A_A	6.6e-7	1.3e-5	8.8e-4	1.3e-5	9.2e-6	3.1e-8	2.4e-7	3.4e-4
35	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
38	Y47B_G H31B_G Y14A_T	3.6e-7	1.2e-5	6.6e-4	2.2e-5	8.5e-6	4.4e-8	2.7e-7	3.5e-4
38	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
41	N18A_A E34B_K H31B_G	1.5e-6	1.1e-5	6.8e-4	1.8e-5	1.0e-5	3.0e-8	1.0e-7	3.6e-4
41	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
45	H31B_Q E4A_K E34B_A	4.5e-7	2.1e-5	6.1e-4	7.8e-6	1.1e-5	6.3e-8	2.0e-7	3.2e-4
45	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
51	K50B_N Y14A_T E34B_S	6.2e-7	1.6e-5	1.1e-3	1.0e-5	1.1e-5	5.5e-8	9.6e-8	3.0e-4
51	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
54	Y47B_G H26B_Q E34B_S	5.1e-7	1.4e-5	1.0e-3	7.7e-6	1.4e-5	2.6e-8	2.9e-7	3.2e-4
54	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
59	E42B_Q E34B_A H31B_G	1.1e-6	1.8e-5	8.8e-4	5.1e-6	1.5e-5	2.8e-8	1.4e-7	3.4e-4
59	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
64	H31B_Q Y37B_P E42B_Q	9.4e-7	1.2e-5	8.5e-4	4.7e-6	2.1e-5	3.0e-8	1.8e-7	3.3e-4
64	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4

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Table 3. *Cont.*

No.	Muta	AE	AG	AI	AK	CE	CG	CI	CK
65	Y37B_P L38B_D H31B_G	5.1e-7	1.6e-5	9.2e-4	9.5e-6	1.0e-5	2.8e-8	2.3e-7	3.6e-4
65	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
67	Y47B_G G44B_E H31B_S	9.5e-7	2.1e-5	6.3e-4	4.4e-6	1.2e-5	6.6e-8	1.1e-7	3.4e-4
67	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
70	N18A_A E4A_K H26B_Q	7.8e-7	1.4e-5	2.4e-3	5.5e-6	8.8e-6	4.2e-8	8.2e-8	3.6e-4
70	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
72	F46B_Q L38B_D E34B_S	3.3e-7	2.4e-5	7.4e-4	1.1e-5	1.1e-5	2.8e-8	2.8e-7	2.8e-4
72	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
79	L38B_D E34B_K H31B_G	5.6e-7	1.5e-5	6.0e-4	1.2e-5	9.1e-6	5.8e-8	1.4e-7	3.2e-4
79	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
85	E34B_A H26B_Q T8A_K	3.6e-7	1.1e-5	2.3e-3	6.2e-6	8.4e-6	4.7e-8	1.8e-7	3.5e-4
85	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
93	K50B_N Y14A_M E34B_S	6.4e-7	1.1e-5	1.1e-3	1.1e-5	1.3e-5	3.3e-8	1.2e-7	3.1e-4
93	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
99	H31B_Q Y47B_G L38B_D	7.9e-7	2.5e-5	6.1e-4	7.5e-6	1.1e-5	4.4e-8	8.8e-8	3.3e-4
99	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
107	E42B_Q H31B_G T8A_K	4.0e-7	1.3e-5	6.0e-4	4.8e-6	1.7e-5	8.0e-8	1.9e-7	3.2e-4
107	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
112	H31B_Q I2A_D Y14A_T	8.6e-7	1.2e-5	6.4e-4	1.1e-5	1.1e-5	2.7e-8	1.6e-7	3.4e-4
112	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
115	F22B_D E34B_A Y14A_T	3.9e-7	9.4e-6	7.8e-4	1.1e-5	1.0e-5	4.7e-8	2.6e-7	3.0e-4
115	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
117	V23B_A L38B_D H31B_G	4.9e-7	1.3e-5	7.3e-4	1.4e-5	9.3e-6	3.2e-8	1.8e-7	3.3e-4
117	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
118	E34B_A H31B_S T8A_K	5.8e-7	1.7e-5	6.2e-4	9.4e-6	7.6e-6	3.8e-8	2.1e-7	3.3e-4
118	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
127	Y47B_G Y14A_A H31B_S	5.8e-7	1.4e-5	8.5e-4	7.2e-6	1.3e-5	2.7e-8	1.9e-7	3.3e-4
127	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
128	Y37B_P Y14A_A E42B_T	3.5e-7	2.4e-5	6.2e-4	5.3e-6	1.3e-5	7.1e-8	1.3e-7	3.3e-4
128	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
132	V23B_A Y14A_A E34B_A	4.6e-7	1.3e-5	6.2e-4	1.8e-5	9.7e-6	3.0e-8	1.6e-7	3.5e-4
132	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
148	N18A_A E4A_K E42B_T	1.3e-6	1.0e-5	6.4e-4	4.4e-6	1.8e-5	4.6e-8	9.8e-8	3.4e-4
148	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
154	Y47B_G E34B_A H31B_S	3.5e-7	3.7e-5	6.7e-4	8.6e-6	1.0e-5	2.8e-8	1.3e-7	3.6e-4
154	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
162	Y14A_M K50B_A H31B_G	5.2e-7	2.4e-5	9.5e-4	9.4e-6	8.5e-6	3.0e-8	8.9e-8	3.7e-4
162	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
200	N18A_A K50B_N H31B_G	9.5e-7	1.4e-5	1.1e-3	5.6e-6	9.6e-6	3.2e-8	8.6e-8	3.6e-4
200	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
219	V23B_A Y37B_P E34B_S	7.2e-7	1.8e-5	7.8e-4	5.6e-6	1.2e-5	2.9e-8	1.2e-7	3.0e-4
219	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
222	H31B_Q E42B_T E34B_K	6.1e-7	1.1e-5	7.7e-4	9.2e-6	1.4e-5	3.6e-8	9.8e-8	3.0e-4
222	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
230	Y37B_P Y47B_G H31B_S	1.1e-6	1.7e-5	8.6e-4	5.0e-6	9.5e-6	2.8e-8	9.8e-8	3.3e-4
230	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
257	H31B_Q Y37B_P E4A_K	9.1e-7	1.4e-5	6.0e-4	5.9e-6	1.1e-5	3.1e-8	1.3e-7	3.2e-4
257	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
277	E34B_F H31B_G Y14A_T	3.8e-7	9.4e-6	9.6e-4	8.0e-6	7.6e-6	3.8e-8	2.0e-7	3.7e-4
277	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
337	Y37B_P E34B_F H31B_S	5.9e-7	1.4e-5	7.0e-4	4.5e-6	1.2e-5	2.9e-8	1.7e-7	3.2e-4
337	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
344	Y47B_G L38B_D K50B_A	5.8e-7	1.2e-5	8.6e-4	5.6e-6	7.6e-6	5.2e-8	1.1e-7	3.3e-4
344	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4

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Table 3. *Cont.*

No.	Muta	AE	AG	AI	AK	CE	CG	CI	CK
354	E34B_F H31B_S T8A_K	4.2e-7	1.4e-5	6.3e-4	5.6e-6	9.4e-6	4.7e-8	1.5e-7	3.4e-4
354	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
383	Y37B_P L27B_K E34B_S	4.9e-7	1.2e-5	1.0e-3	9.9e-6	1.3e-5	2.6e-8	7.9e-8	2.8e-4
383	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
398	V23B_A E42B_T H31B_S	3.7e-7	1.1e-5	1.1e-3	5.4e-6	1.4e-5	3.0e-8	1.4e-7	3.0e-4
398	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
423	E34B_F G44B_E L27B_K	4.6e-7	1.2e-5	6.0e-4	8.2e-6	9.6e-6	4.4e-8	1.1e-7	3.2e-4
423	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
429	V23B_A E4A_K E34B_K	4.2e-7	1.3e-5	8.4e-4	9.8e-6	7.8e-6	4.9e-8	8.3e-8	2.8e-4
429	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
433	H31B_Q E4A_K Y14A_M	3.7e-7	1.0e-5	7.2e-4	5.4e-6	7.8e-6	3.7e-8	3.0e-7	3.2e-4
433	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
461	Y47B_G K50B_A E34B_S	3.9e-7	1.9e-5	6.1e-4	4.9e-6	1.4e-5	2.7e-8	1.4e-7	3.2e-4
461	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
479	N18A_A F46B_Q Y14A_M	4.7e-7	1.7e-5	6.9e-4	5.0e-6	1.2e-5	2.7e-8	1.3e-7	3.1e-4
479	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
530	E4A_K H31B_G Y14A_T	3.6e-7	9.4e-6	9.1e-4	1.2e-5	8.5e-6	3.4e-8	8.5e-8	3.5e-4
530	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
604	N18A_A L38B_D H31B_G	5.7e-7	1.1e-5	6.5e-4	6.7e-6	8.3e-6	3.0e-8	1.1e-7	3.5e-4
604	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
613	V23B_A H31B_Q E42B_Q	4.8e-7	1.3e-5	7.4e-4	5.9e-6	9.0e-6	3.3e-8	1.1e-7	2.9e-4
613	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
616	H31B_Q Y37B_P F22B_D	5.6e-7	9.9e-6	6.0e-4	4.3e-6	9.1e-6	2.8e-8	2.2e-7	3.2e-4
616	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
667	H31B_Q E4A_K E34B_K	4.0e-7	1.1e-5	6.3e-4	8.0e-6	8.0e-6	4.7e-8	1.0e-7	2.8e-4
667	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
680	E34B_K I2A_D H31B_S	6.0e-7	1.1e-5	8.4e-4	4.0e-6	7.9e-6	4.6e-8	8.8e-8	3.2e-4
680	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
745	H31B_Q Y37B_P K50B_N	3.7e-7	1.2e-5	8.3e-4	4.6e-6	8.8e-6	3.9e-8	1.1e-7	3.2e-4
745	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
965	E34B_K H31B_G L32B_H	3.4e-7	1.0e-5	8.4e-4	7.0e-6	9.2e-6	3.4e-8	7.5e-8	3.2e-4
965	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
1306	E4A_K E34B_K T8A_K	3.3e-7	1.1e-5	6.4e-4	7.5e-6	8.2e-6	2.9e-8	6.9e-8	3.4e-4
1306	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
1432	E34B_K H31B_G T8A_K	3.3e-7	1.3e-5	6.7e-4	4.5e-6	1.0e-5	2.7e-8	6.9e-8	3.5e-4
1432	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4

Table 4. Potential long acting insulin analogue candidates with significantly lower affinity to insulin receptor than insulin icodec with Prodigy-calculated K_d (37 °C) values.

No.	Muta	AE	AG	AI	AK	CE	CG	CI	CK
1	Y14A_T E34B_S H31B_S	1.6e-6	2.2e-5	1.1e-3	1.4e-5	1.2e-5	5.9e-8	3.8e-7	3.0e-4
1	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
2	H31B_Q Y14A_A H26B_Q	4.4e-7	1.1e-5	4.3e-3	6.1e-6	9.7e-6	5.2e-8	5.0e-7	3.4e-4
2	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
5	N18A_A Y47B_G H31B_S	2.3e-6	1.7e-5	6.5e-4	9.2e-6	1.0e-5	9.2e-8	1.0e-7	3.6e-4
5	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
6	E34B_A H26B_Q H31B_S	5.4e-7	1.2e-5	1.4e-3	1.2e-5	9.6e-6	4.3e-8	5.3e-7	2.9e-4
6	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
7	Y37B_P Y14A_A H31B_G	5.7e-7	1.3e-5	8.9e-4	1.0e-5	1.1e-5	1.4e-7	1.6e-7	4.0e-4
7	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
15	V23B_A H31B_G E34B_S	9.1e-7	1.2e-5	7.1e-4	1.4e-5	1.2e-5	3.6e-8	2.2e-7	3.2e-4
15	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4

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Table 4. *Cont.*

No.	Muta	AE	AG	AI	AK	CE	CG	CI	CK
17	Y47B_G E34B_F E42B_T	6.6e-7	2.2e-5	8.2e-4	5.6e-6	2.3e-5	5.8e-8	1.1e-7	3.2e-4
17	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
21	H26B_Q Y14A_T H31B_S	6.9e-7	1.4e-5	1.4e-3	7.7e-6	7.3e-6	3.1e-8	4.1e-7	3.0e-4
21	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
23	N18A_A Y37B_P Y14A_M	1.8e-6	1.4e-5	1.0e-3	7.1e-6	1.3e-5	3.7e-8	1.1e-7	2.9e-4
23	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
24	Y14A_A E42B_T E34B_A	4.2e-7	2.0e-5	6.1e-4	2.4e-5	1.5e-5	2.3e-8	1.9e-7	3.3e-4
24	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
29	Y14A_A K50B_A H31B_G	4.2e-7	1.0e-5	1.1e-3	6.4e-6	8.0e-6	9.7e-8	2.7e-7	4.1e-4
29	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
31	Y47B_G Y14A_A H31B_G	4.1e-7	2.5e-5	7.4e-4	1.0e-5	1.3e-5	3.3e-8	2.6e-7	2.9e-4
31	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
32	N18A_A L38B_D E34B_S	1.1e-6	1.1e-5	5.9e-4	1.6e-5	1.0e-5	2.3e-8	3.0e-7	3.1e-4
32	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
33	H31B_G E34B_S L32B_H	6.3e-7	9.9e-6	8.7e-4	8.0e-6	1.1e-5	1.4e-7	1.1e-7	3.3e-4
33	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
35	N18A_A Y37B_P Y14A_A	6.6e-7	1.3e-5	8.8e-4	1.3e-5	9.2e-6	3.1e-8	2.4e-7	3.4e-4
35	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
38	Y47B_G H31B_G Y14A_T	3.6e-7	1.2e-5	6.6e-4	2.2e-5	8.5e-6	4.4e-8	2.7e-7	3.5e-4
38	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
41	N18A_A E34B_K H31B_G	1.5e-6	1.1e-5	6.8e-4	1.8e-5	1.0e-5	3.0e-8	1.0e-7	3.6e-4
41	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
45	H31B_Q E4A_K E34B_A	4.5e-7	2.1e-5	6.1e-4	7.8e-6	1.1e-5	6.3e-8	2.0e-7	3.2e-4
45	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
46	E34B_A H26B_Q H31B_G	3.0e-7	1.2e-5	8.3e-4	5.0e-6	9.4e-6	1.1e-7	3.9e-7	3.3e-4
46	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
51	K50B_N Y14A_T E34B_S	6.2e-7	1.6e-5	1.1e-3	1.0e-5	1.1e-5	5.5e-8	9.6e-8	3.0e-4
51	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
54	Y47B_G H26B_Q E34B_S	5.1e-7	1.4e-5	1.0e-3	7.7e-6	1.4e-5	2.6e-8	2.9e-7	3.2e-4
54	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
59	E42B_Q E34B_A H31B_G	1.1e-6	1.8e-5	8.8e-4	5.1e-6	1.5e-5	2.8e-8	1.4e-7	3.4e-4
59	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
61	E42B_T H31B_G Y14A_T	5.9e-7	1.3e-5	9.2e-4	1.5e-5	1.2e-5	2.1e-8	1.9e-7	3.5e-4
61	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
65	Y37B_P L38B_D H31B_G	5.1e-7	1.6e-5	9.2e-4	9.5e-6	1.0e-5	2.8e-8	2.3e-7	3.6e-4
65	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
70	N18A_A E4A_K H26B_Q	7.8e-7	1.4e-5	2.4e-3	5.5e-6	8.8e-6	4.2e-8	8.2e-8	3.6e-4
70	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
79	L38B_D E34B_K H31B_G	5.6e-7	1.5e-5	6.0e-4	1.2e-5	9.1e-6	5.8e-8	1.4e-7	3.2e-4
79	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
83	E34B_F H26B_Q L32B_H	3.2e-7	1.8e-5	9.9e-4	6.0e-6	8.1e-6	3.0e-8	5.0e-7	3.4e-4
83	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
85	E34B_A H26B_Q T8A_K	3.6e-7	1.1e-5	2.3e-3	6.2e-6	8.4e-6	4.7e-8	1.8e-7	3.5e-4
85	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
93	K50B_N Y14A_M E34B_S	6.4e-7	1.1e-5	1.1e-3	1.1e-5	1.3e-5	3.3e-8	1.2e-7	3.1e-4
93	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
94	H31B_Q Y47B_G E34B_A	5.5e-7	1.0e-5	5.9e-4	9.3e-6	7.5e-6	6.4e-8	2.8e-7	3.3e-4
94	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
99	H31B_Q Y47B_G L38B_D	7.9e-7	2.5e-5	6.1e-4	7.5e-6	1.1e-5	4.4e-8	8.8e-8	3.3e-4
99	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
112	H31B_Q I2A_D Y14A_T	8.6e-7	1.2e-5	6.4e-4	1.1e-5	1.1e-5	2.7e-8	1.6e-7	3.4e-4
112	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
117	V23B_A L38B_D H31B_G	4.9e-7	1.3e-5	7.3e-4	1.4e-5	9.3e-6	3.2e-8	1.8e-7	3.3e-4
117	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
118	E34B_A H31B_S T8A_K	5.8e-7	1.7e-5	6.2e-4	9.4e-6	7.6e-6	3.8e-8	2.1e-7	3.3e-4
118	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4

 doi:10.20944/preprints202404.1922.v1

Table 4. *Cont.*

No.	Muta	AE	AG	AI	AK	CE	CG	CI	CK
127	Y47B_G Y14A_A H31B_S	5.8e-7	1.4e-5	8.5e-4	7.2e-6	1.3e-5	2.7e-8	1.9e-7	3.3e-4
127	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
128	Y37B_P Y14A_A E42B_T	3.5e-7	2.4e-5	6.2e-4	5.3e-6	1.3e-5	7.1e-8	1.3e-7	3.3e-4
128	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
132	V23B_A Y14A_A E34B_A	4.6e-7	1.3e-5	6.2e-4	1.8e-5	9.7e-6	3.0e-8	1.6e-7	3.5e-4
132	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
134	Y14A_M I2A_D E34B_S	6.9e-7	3.3e-5	5.9e-4	9.5e-6	8.9e-6	3.3e-8	9.3e-8	3.1e-4
134	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
154	Y47B_G E34B_A H31B_S	3.5e-7	3.7e-5	6.7e-4	8.6e-6	1.0e-5	2.8e-8	1.3e-7	3.6e-4
154	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
160	Y14A_A G44B_E H31B_G	2.7e-7	2.2e-5	6.6e-4	5.5e-6	1.2e-5	5.2e-8	2.0e-7	3.5e-4
160	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
162	Y14A_M K50B_A H31B_G	5.2e-7	2.4e-5	9.5e-4	9.4e-6	8.5e-6	3.0e-8	8.9e-8	3.7e-4
162	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
168	N18A_A L38B_D I2A_D	1.0e-6	1.2e-5	8.8e-4	9.3e-6	8.2e-6	2.2e-8	1.5e-7	3.4e-4
168	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
200	N18A_A K50B_N H31B_G	9.5e-7	1.4e-5	1.1e-3	5.6e-6	9.6e-6	3.2e-8	8.6e-8	3.6e-4
200	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
204	Y14A_A E34B_A H31B_G	2.9e-7	2.6e-5	6.5e-4	7.1e-6	9.7e-6	7.0e-8	9.6e-8	3.4e-4
204	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
219	V23B_A Y37B_P E34B_S	7.2e-7	1.8e-5	7.8e-4	5.6e-6	1.2e-5	2.9e-8	1.2e-7	3.0e-4
219	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
222	H31B_Q E42B_T E34B_K	6.1e-7	1.1e-5	7.7e-4	9.2e-6	1.4e-5	3.6e-8	9.8e-8	3.0e-4
222	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
228	H31B_Q L38B_D K50B_A	5.1e-7	2.0e-5	7.9e-4	9.2e-6	7.1e-6	4.4e-8	9.7e-8	3.1e-4
228	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
230	Y37B_P Y47B_G H31B_S	1.1e-6	1.7e-5	8.6e-4	5.0e-6	9.5e-6	2.8e-8	9.8e-8	3.3e-4
230	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
235	H31B_Q Y37B_P Y14A_T	2.9e-7	1.1e-5	9.0e-4	8.9e-6	1.2e-5	3.2e-8	2.0e-7	3.5e-4
235	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
249	H31B_Q Y14A_A E42B_T	2.5e-7	1.4e-5	8.0e-4	1.4e-5	1.2e-5	3.0e-8	1.5e-7	3.1e-4
249	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
253	Y47B_G E42B_Q K50B_A	4.1e-7	2.2e-5	5.9e-4	5.6e-6	1.6e-5	4.0e-8	1.1e-7	3.1e-4
253	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
254	E42B_T H26B_Q E34B_A	4.2e-7	1.6e-5	1.5e-3	5.5e-6	1.0e-5	2.1e-8	1.8e-7	3.1e-4
254	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
257	H31B_Q Y37B_P E4A_K	9.1e-7	1.4e-5	6.0e-4	5.9e-6	1.1e-5	3.1e-8	1.3e-7	3.2e-4
257	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
294	K50B_N Y14A_A E34B_K	2.5e-7	1.5e-5	1.2e-3	6.5e-6	1.0e-5	6.0e-8	9.5e-8	3.3e-4
294	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
321	Y47B_G E34B_F Y14A_A	2.7e-7	1.6e-5	6.7e-4	1.6e-5	1.1e-5	2.1e-8	1.3e-7	3.7e-4
321	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
322	Y14A_M E34B_A T8A_K	7.4e-7	1.5e-5	6.4e-4	7.2e-6	9.6e-6	2.2e-8	1.4e-7	3.4e-4
322	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
344	Y47B_G L38B_D K50B_A	5.8e-7	1.2e-5	8.6e-4	5.6e-6	7.6e-6	5.2e-8	1.1e-7	3.3e-4
344	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
351	H31B_Q E34B_F Y14A_A	2.9e-7	1.7e-5	8.2e-4	5.0e-6	1.0e-5	2.6e-8	2.8e-7	3.2e-4
351	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
354	E34B_F H31B_S T8A_K	4.2e-7	1.4e-5	6.3e-4	5.6e-6	9.4e-6	4.7e-8	1.5e-7	3.4e-4
354	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
365	Y14A_M H31B_G R43B_F	6.9e-7	1.8e-5	6.3e-4	5.0e-6	7.7e-6	2.4e-8	1.9e-7	3.3e-4
365	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
368	Y47B_G E4A_K L38B_D	4.4e-7	3.4e-5	6.1e-4	8.9e-6	1.1e-5	2.1e-8	7.5e-8	3.2e-4
368	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4

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Table 4. *Cont.*

No.	Muta	AE	AG	AI	AK	CE	CG	CI	CK
380	Y47B_G Y14A_A E42B_Q	3.1e-7	1.3e-5	7.8e-4	9.6e-6	1.8e-5	3.0e-8	7.7e-8	3.5e-4
380	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
381	L38B_D E34B_K I2A_D	2.6e-7	1.4e-5	8.0e-4	8.0e-6	1.0e-5	3.2e-8	1.9e-7	3.1e-4
381	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
397	N18A_A Y47B_G K50B_A	1.1e-6	1.2e-5	6.8e-4	5.8e-6	7.4e-6	3.1e-8	9.4e-8	3.8e-4
397	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
398	V23B_A E42B_T H31B_S	3.7e-7	1.1e-5	1.1e-3	5.4e-6	1.4e-5	3.0e-8	1.4e-7	3.0e-4
398	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
423	E34B_F G44B_E L27B_K	4.6e-7	1.2e-5	6.0e-4	8.2e-6	9.6e-6	4.4e-8	1.1e-7	3.2e-4
423	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
433	H31B_Q E4A_K Y14A_M	3.7e-7	1.0e-5	7.2e-4	5.4e-6	7.8e-6	3.7e-8	3.0e-7	3.2e-4
433	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
439	H31B_Q F46B_Q E34B_F	3.1e-7	1.5e-5	7.6e-4	8.2e-6	9.7e-6	3.0e-8	1.6e-7	2.9e-4
439	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
461	Y47B_G K50B_A E34B_S	3.9e-7	1.9e-5	6.1e-4	4.9e-6	1.4e-5	2.7e-8	1.4e-7	3.2e-4
461	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
473	Y14A_A E42B_T H31B_S	3.2e-7	1.3e-5	6.8e-4	9.3e-6	1.4e-5	3.0e-8	1.1e-7	3.0e-4
473	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
479	N18A_A F46B_Q Y14A_M	4.7e-7	1.7e-5	6.9e-4	5.0e-6	1.2e-5	2.7e-8	1.3e-7	3.1e-4
479	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
495	Y47B_G E34B_K H26B_Q	3.9e-7	1.3e-5	8.3e-4	8.7e-6	8.2e-6	2.4e-8	1.4e-7	3.4e-4
495	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
504	E42B_T Y14A_M E34B_K	2.7e-7	1.1e-5	6.8e-4	7.9e-6	1.4e-5	2.7e-8	1.8e-7	3.1e-4
504	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
586	H31B_Q Y47B_G T8A_K	2.6e-7	1.4e-5	7.0e-4	5.0e-6	1.1e-5	7.2e-8	8.5e-8	3.2e-4
586	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
604	N18A_A L38B_D H31B_G	5.7e-7	1.1e-5	6.5e-4	6.7e-6	8.3e-6	3.0e-8	1.1e-7	3.5e-4
604	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
613	V23B_A H31B_Q E42B_Q	4.8e-7	1.3e-5	7.4e-4	5.9e-6	9.0e-6	3.3e-8	1.1e-7	2.9e-4
613	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
614	S9A_D Y14A_K I2A_R	2.9e-7	2.0e-5	6.8e-4	9.7e-6	7.4e-6	3.2e-8	7.9e-8	3.6e-4
614	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
632	Y47B_G E34B_A H31B_G	4.1e-7	1.3e-5	6.4e-4	5.9e-6	1.9e-5	2.4e-8	8.1e-8	3.4e-4
632	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
679	Y37B_P E4A_K H26B_Q	2.7e-7	1.4e-5	1.5e-3	4.9e-6	1.5e-5	2.3e-8	7.4e-8	3.2e-4
679	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
735	N18A_A E42B_Q H26B_Q	4.7e-7	1.6e-5	9.2e-4	5.7e-6	8.1e-6	2.2e-8	9.9e-8	3.0e-4
735	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
784	Y47B_G E34B_K Y14A_T	4.0e-7	1.4e-5	5.9e-4	8.9e-6	1.0e-5	2.3e-8	9.1e-8	3.1e-4
784	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
821	L38B_D Y14A_M K50B_A	4.1e-7	9.9e-6	8.2e-4	1.1e-5	7.3e-6	2.4e-8	8.7e-8	3.2e-4
821	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
837	K50B_A H31B_G Y14A_T	2.9e-7	1.1e-5	6.5e-4	8.3e-6	7.9e-6	2.9e-8	1.3e-7	3.4e-4
837	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
860	H31B_Q E34B_A K50B_A	3.1e-7	1.1e-5	8.1e-4	5.4e-6	8.4e-6	2.9e-8	1.5e-7	3.1e-4
860	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
965	E34B_K H31B_G L32B_H	3.4e-7	1.0e-5	8.4e-4	7.0e-6	9.2e-6	3.4e-8	7.5e-8	3.2e-4
965	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
994	Y14A_A I2A_D E34B_A	3.0e-7	1.1e-5	8.8e-4	7.4e-6	9.5e-6	2.1e-8	1.0e-7	3.4e-4
994	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
1000	L38B_D I2A_D T8A_K	7.5e-7	1.0e-5	6.1e-4	7.6e-6	7.8e-6	2.2e-8	7.6e-8	3.2e-4
1000	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
1310	Y14A_A E34B_K K50B_A	3.1e-7	1.2e-5	6.2e-4	5.3e-6	7.5e-6	2.9e-8	1.1e-7	3.3e-4
1310	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4

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Table 5. Potential long acting insulin analogue candidates with significantly lower affinity to insulin receptor than insulin icodec and native insulin with Prodigy-calculated K_d (37 °C) values.

No.	Muta	AE	AG	AI	AK	CE	CG	CI	CK
1	Y14A_T E34B_S H31B_S	1.6e-6	2.2e-5	1.1e-3	1.4e-5	1.2e-5	5.9e-8	3.8e-7	3.0e-4
1	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
2	H31B_Q Y14A_A H26B_Q	4.4e-7	1.1e-5	4.3e-3	6.1e-6	9.7e-6	5.2e-8	5.0e-7	3.4e-4
2	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
5	N18A_A Y47B_G H31B_S	2.3e-6	1.7e-5	6.5e-4	9.2e-6	1.0e-5	9.2e-8	1.0e-7	3.6e-4
5	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
6	E34B_A H26B_Q H31B_S	5.4e-7	1.2e-5	1.4e-3	1.2e-5	9.6e-6	4.3e-8	5.3e-7	2.9e-4
6	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
7	Y37B_P Y14A_A H31B_G	5.7e-7	1.3e-5	8.9e-4	1.0e-5	1.1e-5	1.4e-7	1.6e-7	4.0e-4
7	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
15	V23B_A H31B_G E34B_S	9.1e-7	1.2e-5	7.1e-4	1.4e-5	1.2e-5	3.6e-8	2.2e-7	3.2e-4
15	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
17	Y47B_G E34B_F E42B_T	6.6e-7	2.2e-5	8.2e-4	5.6e-6	2.3e-5	5.8e-8	1.1e-7	3.2e-4
17	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
23	N18A_A Y37B_P Y14A_M	1.8e-6	1.4e-5	1.0e-3	7.1e-6	1.3e-5	3.7e-8	1.1e-7	2.9e-4
23	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
29	Y14A_A K50B_A H31B_G	4.2e-7	1.0e-5	1.1e-3	6.4e-6	8.0e-6	9.7e-8	2.7e-7	4.1e-4
29	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
31	Y47B_G Y14A_A H31B_G	4.1e-7	2.5e-5	7.4e-4	1.0e-5	1.3e-5	3.3e-8	2.6e-7	2.9e-4
31	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
33	H31B_G E34B_S L32B_H	6.3e-7	9.9e-6	8.7e-4	8.0e-6	1.1e-5	1.4e-7	1.1e-7	3.3e-4
33	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
35	N18A_A Y37B_P Y14A_A	6.6e-7	1.3e-5	8.8e-4	1.3e-5	9.2e-6	3.1e-8	2.4e-7	3.4e-4
35	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
38	Y47B_G H31B_G Y14A_T	3.6e-7	1.2e-5	6.6e-4	2.2e-5	8.5e-6	4.4e-8	2.7e-7	3.5e-4
38	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
41	N18A_A E34B_K H31B_G	1.5e-6	1.1e-5	6.8e-4	1.8e-5	1.0e-5	3.0e-8	1.0e-7	3.6e-4
41	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
45	H31B_Q E4A_K E34B_A	4.5e-7	2.1e-5	6.1e-4	7.8e-6	1.1e-5	6.3e-8	2.0e-7	3.2e-4
45	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
51	K50B_N Y14A_T E34B_S	6.2e-7	1.6e-5	1.1e-3	1.0e-5	1.1e-5	5.5e-8	9.6e-8	3.0e-4
51	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
54	Y47B_G H26B_Q E34B_S	5.1e-7	1.4e-5	1.0e-3	7.7e-6	1.4e-5	2.6e-8	2.9e-7	3.2e-4
54	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
59	E42B_Q E34B_A H31B_G	1.1e-6	1.8e-5	8.8e-4	5.1e-6	1.5e-5	2.8e-8	1.4e-7	3.4e-4
59	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
65	Y37B_P L38B_D H31B_G	5.1e-7	1.6e-5	9.2e-4	9.5e-6	1.0e-5	2.8e-8	2.3e-7	3.6e-4
65	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
70	N18A_A E4A_K H26B_Q	7.8e-7	1.4e-5	2.4e-3	5.5e-6	8.8e-6	4.2e-8	8.2e-8	3.6e-4
70	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
79	L38B_D E34B_K H31B_G	5.6e-7	1.5e-5	6.0e-4	1.2e-5	9.1e-6	5.8e-8	1.4e-7	3.2e-4
79	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
85	E34B_A H26B_Q T8A_K	3.6e-7	1.1e-5	2.3e-3	6.2e-6	8.4e-6	4.7e-8	1.8e-7	3.5e-4
85	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
93	K50B_N Y14A_M E34B_S	6.4e-7	1.1e-5	1.1e-3	1.1e-5	1.3e-5	3.3e-8	1.2e-7	3.1e-4
93	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
99	H31B_Q Y47B_G L38B_D	7.9e-7	2.5e-5	6.1e-4	7.5e-6	1.1e-5	4.4e-8	8.8e-8	3.3e-4
99	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
112	H31B_Q I2A_D Y14A_T	8.6e-7	1.2e-5	6.4e-4	1.1e-5	1.1e-5	2.7e-8	1.6e-7	3.4e-4
112	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
117	V23B_A L38B_D H31B_G	4.9e-7	1.3e-5	7.3e-4	1.4e-5	9.3e-6	3.2e-8	1.8e-7	3.3e-4
117	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
118	E34B_A H31B_S T8A_K	5.8e-7	1.7e-5	6.2e-4	9.4e-6	7.6e-6	3.8e-8	2.1e-7	3.3e-4
118	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4

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Table 5. *Cont.*

No.	Muta	AE	AG	AI	AK	CE	CG	CI	CK
127	Y47B_G Y14A_A H31B_S	5.8e-7	1.4e-5	8.5e-4	7.2e-6	1.3e-5	2.7e-8	1.9e-7	3.3e-4
127	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
128	Y37B_P Y14A_A E42B_T	3.5e-7	2.4e-5	6.2e-4	5.3e-6	1.3e-5	7.1e-8	1.3e-7	3.3e-4
128	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
132	V23B_A Y14A_A E34B_A	4.6e-7	1.3e-5	6.2e-4	1.8e-5	9.7e-6	3.0e-8	1.6e-7	3.5e-4
132	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
154	Y47B_G E34B_A H31B_S	3.5e-7	3.7e-5	6.7e-4	8.6e-6	1.0e-5	2.8e-8	1.3e-7	3.6e-4
154	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
162	Y14A_M K50B_A H31B_G	5.2e-7	2.4e-5	9.5e-4	9.4e-6	8.5e-6	3.0e-8	8.9e-8	3.7e-4
162	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
200	N18A_A K50B_N H31B_G	9.5e-7	1.4e-5	1.1e-3	5.6e-6	9.6e-6	3.2e-8	8.6e-8	3.6e-4
200	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
219	V23B_A Y37B_P E34B_S	7.2e-7	1.8e-5	7.8e-4	5.6e-6	1.2e-5	2.9e-8	1.2e-7	3.0e-4
219	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
222	H31B_Q E42B_T E34B_K	6.1e-7	1.1e-5	7.7e-4	9.2e-6	1.4e-5	3.6e-8	9.8e-8	3.0e-4
222	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
230	Y37B_P Y47B_G H31B_S	1.1e-6	1.7e-5	8.6e-4	5.0e-6	9.5e-6	2.8e-8	9.8e-8	3.3e-4
230	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
257	H31B_Q Y37B_P E4A_K	9.1e-7	1.4e-5	6.0e-4	5.9e-6	1.1e-5	3.1e-8	1.3e-7	3.2e-4
257	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
344	Y47B_G L38B_D K50B_A	5.8e-7	1.2e-5	8.6e-4	5.6e-6	7.6e-6	5.2e-8	1.1e-7	3.3e-4
344	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
354	E34B_F H31B_S T8A_K	4.2e-7	1.4e-5	6.3e-4	5.6e-6	9.4e-6	4.7e-8	1.5e-7	3.4e-4
354	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
398	V23B_A E42B_T H31B_S	3.7e-7	1.1e-5	1.1e-3	5.4e-6	1.4e-5	3.0e-8	1.4e-7	3.0e-4
398	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
423	E34B_F G44B_E L27B_K	4.6e-7	1.2e-5	6.0e-4	8.2e-6	9.6e-6	4.4e-8	1.1e-7	3.2e-4
423	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
433	H31B_Q E4A_K Y14A_M	3.7e-7	1.0e-5	7.2e-4	5.4e-6	7.8e-6	3.7e-8	3.0e-7	3.2e-4
433	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
461	Y47B_G K50B_A E34B_S	3.9e-7	1.9e-5	6.1e-4	4.9e-6	1.4e-5	2.7e-8	1.4e-7	3.2e-4
461	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
479	N18A_A F46B_Q Y14A_M	4.7e-7	1.7e-5	6.9e-4	5.0e-6	1.2e-5	2.7e-8	1.3e-7	3.1e-4
479	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
604	N18A_A L38B_D H31B_G	5.7e-7	1.1e-5	6.5e-4	6.7e-6	8.3e-6	3.0e-8	1.1e-7	3.5e-4
604	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
613	V23B_A H31B_Q E42B_Q	4.8e-7	1.3e-5	7.4e-4	5.9e-6	9.0e-6	3.3e-8	1.1e-7	2.9e-4
613	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4
965	E34B_K H31B_G L32B_H	3.4e-7	1.0e-5	8.4e-4	7.0e-6	9.2e-6	3.4e-8	7.5e-8	3.2e-4
965	native insulin molecule	3.2e-7	8.7e-6	5.9e-4	3.8e-6	7.4e-6	2.4e-8	6.3e-8	2.7e-4

In Tables 3, 4 and 5, to increase the likelihood of the insulin analogues designed here possessing an extended duration of action than insulin icodec, all $s = g(51,3) = \frac{51!}{3!(51-3)!} \times 20^k$ [38] insulin analogues were computationally screened with a collection of criteria as below:

1. the Prodigy-calculated K_d of the insulin analogue to its receptor (**AE**) is larger than that of native insulin or insulin icodec or both, i.e., the binding affinity of the first engineered insulin analogue to its receptor is lower that that of native insulin or insulin icodec or both.
2. the Prodigy-calculated K_d of the insulin analogue to its receptor (**AG**) is larger than that of native insulin or insulin icodec or both, i.e., the binding affinity of the second engineered insulin analogue to its receptor is lower that that of native insulin or insulin icodec or both.
3. the Prodigy-calculated K_d of the insulin analogue to its receptor (**AI**) is larger than that of native insulin or insulin icodec or both, i.e., the binding affinity of the third engineered insulin analogue to its receptor is lower that that of native insulin or insulin icodec or both.

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4. the Prodigy-calculated K_d of the insulin analogue to its receptor (**AK**) is larger than that of native insulin or insulin icodec or both, i.e., the binding affinity of the fourth engineered insulin analogue to its receptor is lower that that of native insulin or insulin icodec or both.
5. the Prodigy-calculated K_d of the insulin analogue to its receptor (**CE**) is larger than that of native insulin or insulin icodec or both, i.e., the binding affinity of the first engineered insulin analogue to its receptor is lower that that of native insulin or insulin icodec or both.
6. the Prodigy-calculated K_d of the insulin analogue to its receptor (**CG**) is larger than that of native insulin or insulin icodec or both, i.e., the binding affinity of the second engineered insulin analogue to its receptor is lower that that of native insulin or insulin icodec or both.
7. the Prodigy-calculated K_d of the insulin analogue to its receptor (**CI**) is larger than that of native insulin or insulin icodec or both, i.e., the binding affinity of the third engineered insulin analogue to its receptor is lower that that of native insulin or insulin icodec or both.
8. the Prodigy-calculated K_d of the insulin analogue to its receptor (**CK**) is larger than that of native insulin or insulin icodec or both, i.e., the binding affinity of the fourth engineered insulin analogue to its receptor is lower that that of native insulin or insulin icodec or both.

5. Conclusion and Discussion

For the first time, through a comprehensive structural and biophysical analysis [50] of the insulin (both native and icodec) structures bound to its receptor, this article puts forward a a set of insulin analogues with lower binding affinity to insulin receptor than that of insulin icodec from a structural and biophysical point of view [51]. Overall, this article calls for subsequent in vitro and in vivo evaluations of the efficacy and prolonged action of the engineered insulin analogues to pharmacokinetically test whether these analogues acutally surpass that of insulin icodec as hopeful candidates for next-generation insulin analogue therapies with improved duration of action [14,52] and enhanced control of blood glucose levels for diabetic patients in future.

The development of insulin analogues with extended duration of action while maintaining therapeutic efficacy represents a significant advancement in diabetes management [14]. With computational modeling and structure-based sequence design, this study leveraged insights from protein engineering and molecular biophysics to guide the rational design of insulin analogues with potentially prolonged duration of action compared to insulin icodec, as evidenced by the structural biophysical analysis as listed in Tables 2–5.

Furthermore, while this study represents another step towards the development of next-generation insulin therapies with improved duration of action, the entire process of the design of insulin analogues with lower binding affinity to insulin receptor than that of insulin icodec, along with the structural biophysics-based strategy for the molecular design, is essentially also a process of the construction of a insulin-IR based mini general intermolecular binding affinity calculator [22,37,38] based on the experimentally determined human insulin receptor ectodomain bound by 4 insulin (PDB entry 6SOF).

6. Ethical Statement

No ethical approval is required.

7. Declaration of Generative AI and AI-Assisted Technologies in the Writing Process

During the preparation of this work, the author used OpenAI's ChatGPT in order to improve the readability of the manuscript, and to make it as concise and short as possible. After using this tool, the author reviewed and edited the content as needed and takes full responsibility for the content of the publication.

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References

1. Bajaj, H.S.; Bergenstal, R.M.; Christoffersen, A.; Davies, M.J.; Gowda, A.; Isendahl, J.; Lingvay, I.; Senior, P.A.; Silver, R.J.; Trevisan, R.; Rosenstock, J. Switching to Once-Weekly Insulin Icodec Versus Once-Daily Insulin Glargine U100 in Type 2 Diabetes Inadequately Controlled on Daily Basal Insulin: A Phase 2 Randomized Controlled Trial. *Diabetes Care* **2021**, *44*, 1586–1594.
2. Wick, J.Y. Insulin: Almost a Century of Lifesaving. *The Consultant Pharmacist* **2017**, *32*, 190–198.
3. Griffin, T.P.; Dinneen, S.F. In T2DM, weekly insulin icodec did not differ from daily glargine for reducing HbA1c or significant/severe hypoglycemia. *Annals of Internal Medicine* **2021**, *174*, JC34.
4. Philis-Tsimikas, A.; Bajaj, H.S.; Begtrup, K.; Cailleteau, R.; Gowda, A.; Lingvay, I.; Mathieu, C.; Russell-Jones, D.; Rosenstock, J. Rationale and design of the phase 3a development programme (ONWARDS 1–6 trials) investigating once-weekly insulin icodec in diabetes. *Diabetes, Obesity and Metabolism* **2022**, *25*, 331–341.
5. Philis-Tsimikas, A.; Asong, M.; Franek, E.; Jia, T.; Rosenstock, J.; Stachlewska, K.; Watada, H.; Kellerer, M. Switching to once-weekly insulin icodec versus once-daily insulin degludec in individuals with basal insulin-treated type 2 diabetes (ONWARDS 2): a phase 3a, randomised, open label, multicentre, treat-to-target trial. *The Lancet Diabetes & Endocrinology* **2023**, *11*, 414–425.
6. Mathieu, C.; Ásbjörnsdóttir, B.; Bajaj, H.S.; Lane, W.; Matos, A.L.S.A.; Murthy, S.; Stachlewska, K.; Rosenstock, J. Switching to once-weekly insulin icodec versus once-daily insulin glargine U100 in individuals with basal-bolus insulin-treated type 2 diabetes (ONWARDS 4): a phase 3a, randomised, open-label, multicentre, treat-to-target, non-inferiority trial. *The Lancet* **2023**, *401*, 1929–1940.
7. Lingvay, I.; Asong, M.; Desouza, C.; Gourdy, P.; Kar, S.; Vianna, A.; Vilsbøll, T.; Vinther, S.; Mu, Y. Once-Weekly Insulin Icodec vs Once-Daily Insulin Degludec in Adults With Insulin-Naive Type 2 Diabetes. *JAMA* **2023**, *330*, 228.
8. Lingvay, I.; Buse, J.B.; Franek, E.; Hansen, M.V.; Koefoed, M.M.; Mathieu, C.; Pettus, J.; Stachlewska, K.; Rosenstock, J. A Randomized, Open-Label Comparison of Once-Weekly Insulin Icodec Titration Strategies Versus Once-Daily Insulin Glargine U100. *Diabetes Care* **2021**, *44*, 1595–1603.
9. Kjeldsen, T.B.; Hubálek, F.; Hjørringgaard, C.U.; Tagmose, T.M.; Nishimura, E.; Stidsen, C.E.; Porsgaard, T.; Fledelius, C.; Refsgaard, H.H.F.; Gram-Nielsen, S.; Naver, H.; Pridal, L.; Hoeg-Jensen, T.; Jeppesen, C.B.; Manfè, V.; Ludvigsen, S.; Lautrup-Larsen, I.; Madsen, P. Molecular Engineering of Insulin Icodec, the First Acylated Insulin Analog for Once-Weekly Administration in Humans. *Journal of Medicinal Chemistry* **2021**, *64*, 8942–8950.
10. Rosenstock, J.; Bajaj, H.S.; Janež, A.; Silver, R.; Begtrup, K.; Hansen, M.V.; Jia, T.; Goldenberg, R. Once-Weekly Insulin for Type 2 Diabetes without Previous Insulin Treatment. *New England Journal of Medicine* **2020**, *383*, 2107–2116.
11. Bajaj, H.S.; Goldenberg, R.M.; and. Insulin Icodec Weekly: A Basal Insulin Analogue for Type 2 Diabetes. *European Endocrinology* **2023**, *19*, 4.
12. DiMarchi, R.D.; Mayer, J.P. Icodec Advances the Prospect of Once-Weekly Insulin Injection. *Journal of Medicinal Chemistry* **2021**, *64*, 8939–8941.
13. Rosenstock, J.; Prato, S.D. Basal weekly insulins: the way of the future! *Metabolism* **2022**, *126*, 154924.
14. Li, W. How Structural Modifications of Insulin Icodec Contributes to Its Prolonged Duration of Action: A Structural and Biophysical Perspective **2023**. doi:10.20944/preprints202311.1048.v1.
15. Kalra, S.; Bhattacharya, S.; Kapoor, N. Contemporary Classification of Glucagon-Like Peptide 1 Receptor Agonists (GLP1RAs). *Diabetes Therapy* **2021**, *12*, 2133–2147.
16. Pratley, R.; Amod, A.; Hoff, S.T.; Kadowaki, T.; Lingvay, I.; Nauck, M.; Pedersen, K.B.; Saugstrup, T.; Meier, J.J. Oral semaglutide versus subcutaneous liraglutide and placebo in type 2 diabetes (PIONEER 4): a randomised, double-blind, phase 3a trial. *The Lancet* **2019**, *394*, 39–50.
17. Anderson, S.L.; Beutel, T.R.; Trujillo, J.M. Oral semaglutide in type 2 diabetes. *Journal of Diabetes and its Complications* **2020**, *34*, 107520.

18. Li, W. Strengthening Semaglutide-GLP-1R Binding Affinity via a Val27-Arg28 Exchange in the Peptide Backbone of Semaglutide: A Computational Structural Approach. *Journal of Computational Biophysics and Chemistry* **2021**, *20*, 495–499.
19. Nadkarni, P.; Chepurny, O.G.; Holz, G.G. Regulation of Glucose Homeostasis by GLP-1. In *Progress in Molecular Biology and Translational Science*; Elsevier, 2014; pp. 23–65.
20. Lau, J.; Bloch, P.; Schäffer, L.; Pettersson, I.; Spetzler, J.; Kofoed, J.; Madsen, K.; Knudsen, L.B.; McGuire, J.; Steensgaard, D.B.; Strauss, H.M.; Gram, D.X.; Knudsen, S.M.; Nielsen, F.S.; Thygesen, P.; Reedtz-Runge, S.; Kruse, T. Discovery of the Once-Weekly Glucagon-Like Peptide-1 (GLP-1) Analogue Semaglutide. *Journal of Medicinal Chemistry* **2015**, *58*, 7370–7380.
21. Gabery, S.; Salinas, C.G.; Paulsen, S.J.; Ahnfelt-Rønne, J.; Alanentalo, T.; Baquero, A.F.; Buckley, S.T.; Farkas, E.; Fekete, C.; Frederiksen, K.S.; Helms, H.C.C.; Jeppesen, J.F.; John, L.M.; Pyke, C.; Nøhr, J.; Lu, T.T.; Polex-Wolf, J.; Prevot, V.; Raun, K.; Simonsen, L.; Sun, G.; Szilvásy-Szabó, A.; Willenbrock, H.; Secher, A.; Knudsen, L.B. Semaglutide lowers body weight in rodents via distributed neural pathways. *JCI Insight* **2020**, *5*.
22. Li, W. High-Throughput Extraction of Interfacial Electrostatic Features from GLP-1-GLP-1R Complex Structures: A GLP-1-GLP-1R-Based Mini GIBAC Perspective **2024**. doi:10.20944/preprints202402.1519.v1.
23. Pieber, T.R.; Arfelt, K.N.; Cailleteau, R.; Hart, M.; Kar, S.; Mursic, I.; Svehlikova, E.; Urschitz, M.; Haahr, H. Hypoglycaemia frequency and physiological response after double or triple doses of once-weekly insulin icodec vs once-daily insulin glargine U100 in type 2 diabetes: a randomised crossover trial. *Diabetologia* **2023**, *66*, 1413–1430.
24. Zerihun, K.; Mhanna, M.; Ayesh, H.; Ghazaleh, S.; Khader, Y.; Beran, A.; Aldhafeeri, A.; Sharma, S.; Iqbal, A.; Legesse, H.; Jaume, J. Efficacy and Safety of Insulin Icodec Versus Glargine U100: A Meta-Analysis of Randomized Controlled Trials. *American Journal of Therapeutics* **2022**, *30*, e480–e483.
25. Li, W. Structural and Functional Consequences of the SMA-Linked Missense Mutations of the Survival Motor Neuron Protein: A Brief Update. In *Novel Aspects on Motor Neuron Disease*; IntechOpen, 2019.
26. Li, W. How do SMA-linked mutations of *SMN1* lead to structural/functional deficiency of the SMA protein? *PLOS ONE* **2017**, *12*, e0178519.
27. Plum-Mörschel, L.; Andersen, L.R.; Hansen, S.; Hövelmann, U.; Krawietz, P.; Kristensen, N.R.; Lehrskov, L.L.; Haahr, H. Pharmacokinetic and Pharmacodynamic Characteristics of Insulin Icodec After Subcutaneous Administration in the Thigh, Abdomen or Upper Arm in Individuals with Type 2 Diabetes Mellitus. *Clinical Drug Investigation* **2023**, *43*, 119–127.
28. Belal, H.; Gandhi, G.Y. In uncontrolled T2DM treated with a basal-bolus insulin regimen, weekly icodec was noninferior to daily glargine for HbA1c at 26 wk. *Annals of Internal Medicine* **2023**, *176*, JC94.
29. Anderson, S.L.; Bassetti, M.; Mangoni, A.A. Drugs in Context Editorial: Review of 2020 and what lies ahead in therapeutic interventions. *Drugs in Context* **2021**, *10*, 1–5.
30. Bellary, S.; Barnett, A.H. Insulin icodec: evolution or revolution in diabetes therapy? *The Lancet Diabetes & Endocrinology* **2023**, *11*, 379–380.
31. e Silva, R.R.; de Miranda Gauza, M.; Guisso, M.E.S.; da Silva, J.O.N.; Kohara, S.K. Once-Weekly Insulin Icodec vs. Once-Daily Insulin Glargine U100 for type 2 diabetes: a systematic review and meta-analysis of phase 2 randomized controlled trials. *Archives of Endocrinology and Metabolism* **2023**, *67*.
32. Pieber, T.R.; Asong, M.; Fluhr, G.; Höller, V.; Kristensen, N.R.; Larsen, J.H.; Ribel-Madsen, R.; Svehlikova, E.; Vinther, S.; Voortman, M.; Haahr, H. Pharmacokinetic and pharmacodynamic properties of once-weekly insulin icodec in individuals with type 2 diabetes. *Diabetes, Obesity and Metabolism* **2023**.
33. Russell-Jones, D.; Babazono, T.; Cailleteau, R.; Engberg, S.; Irace, C.; Kjaersgaard, M.I.S.; Mathieu, C.; Rosenstock, J.; Woo, V.; Klonoff, D.C. Once-weekly insulin icodec versus once-daily insulin degludec as part of a basal-bolus regimen in individuals with type 1 diabetes (ONWARDS 6): a phase 3a, randomised, open-label, treat-to-target trial. *The Lancet* **2023**.
34. Feher, J. Digestion and Absorption of the Macronutrients. In *Quantitative Human Physiology*; Elsevier, 2017; pp. 821–833. doi:10.1016/b978-0-12-800883-6.00081-1.
35. Ericsson, Å.; Fridhammar, A. Cost-effectiveness of once-weekly semaglutide versus dulaglutide and lixisenatide in patients with type 2 diabetes with inadequate glycemic control in Sweden. *Journal of Medical Economics* **2019**, *22*, 997–1005.

 doi:10.20944/preprints202404.1922.v1

36. Han, J.; Fu, J.; Yang, Q.; Zhou, F.; Chen, X.; Li, C.; Yin, J. Rational design and biological evaluation of gemfibrozil modified Xenopus GLP-1 derivatives as long-acting hypoglycemic agents. *European Journal of Medicinal Chemistry* **2020**, *198*, 112389.
37. Li, W. Towards a General Intermolecular Binding Affinity Calculator **2022**.
38. Li, W.; Vottevor, G. Towards a Truly General Intermolecular Binding Affinity Calculator for Drug Discovery & Design **2023**. doi:10.20944/preprints202208.0213.v2.
39. Pharmaceuticals, R. Recursion Bridges the Protein and Chemical Space with Massive Protein-Ligand Interaction Predictions Spanning 36 Billion Compounds, 2023. Accessed: (September 1, 2023).
40. Reymond, J.L.; van Deursen, R.; Blum, L.C.; Ruddigkeit, L. Chemical space as a source for new drugs. *MedChemComm* **2010**, *1*, 30.
41. Berman, H.; Henrick, K.; Nakamura, H. Announcing the worldwide Protein Data Bank. *Nature Structural & Molecular Biology* **2003**, *10*, 980–980.
42. Li, W. Visualising the Experimentally Uncharted Territories of Membrane Protein Structures inside Protein Data Bank **2020**.
43. Li, W. Half-a-century Burial of ρ, θ and φ in PDB **2021**.
44. Gutmann, T.; Schäfer, I.B.; Poojari, C.; Brankatschk, B.; Vattulainen, I.; Strauss, M.; Ünal Coskun. Cryo-EM structure of the complete and ligand-saturated insulin receptor ectodomain. *Journal of Cell Biology* **2019**, *219*.
45. DeLano, W.L. Pymol: An open-source molecular graphics tool. *CCP4 Newsletter On Protein Crystallography* **2002**, *40*, 82–92.
46. Webb, B.; Sali, A. Protein Structure Modeling with MODELLER. In *Methods in Molecular Biology*; Springer US, 2020; pp. 239–255.
47. Vangone, A.; Bonvin, A.M. Contacts-based prediction of binding affinity in protein-protein complexes. *eLife* **2015**, *4*.
48. Xue, L.C.; Rodrigues, J.P.; Kastritis, P.L.; Bonvin, A.M.; Vangone, A. PRODIGY: a web server for predicting the binding affinity of protein-protein complexes. *Bioinformatics* **2016**, p. btw514.
49. Li, W. Gravity-driven pH adjustment for site-specific protein pKa measurement by solution-state NMR. *Measurement Science and Technology* **2017**, *28*, 127002.
50. Li, W. Calcium Channel Trafficking Blocker Gabapentin Bound to the α-2-δ-1 Subunit of Voltage-Gated Calcium Channel: A Computational Structural Investigation **2020**.
51. Li, W. Delving Deep into the Structural Aspects of the BPro28-BLys29 Exchange in Insulin Lispro: A Structural Biophysical Lesson **2020**.
52. Li, W. Extracting the Interfacial Electrostatic Features from Experimentally Determined Antigen and/or Antibody-Related Structures inside Protein Data Bank for Machine Learning-Based Antibody Design **2020**.