Supplemental Information

for

Enhancing the Activity of Insulin at the Receptor Edge:

Crystal Structure and Photo-Cross-Linking of A8 Analogues

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Purpose of Supplement

The purpose of the Supplement is to provide two figures and five tables. The figures illustrate the Zn-binding sites of the $T_3R_3^f$ coordination geometry model (Fig. S1**)** and the environment of the phenol molecule bound in the R_3^f trimer (Fig. S2). These features are essentially identical to those observed in nature phenol-stabilized Zn hexamer. The site of mutation ($Thr^{A8} \rightarrow$ His) is on the surface of the hexamer distant from the two Zn-binding sites and three phenol-binding pockets.

Table S1 and S2 provide RMSD values obtained on alignments of the present crystal structure with respect to previous crystal structures of insulin hexamers,. Protomer-specific alignments are provided for the T state (S1) and R state (S2). As controls for the variability observed away native structures, Tables S3 and S4 provide RMSD values on alignment of the present structure with native $T_3R^f_3$ hexamers (S3, T-state; and S4, R-state). Comparison of these RMSD values indicates that His^{A8}–insulin falls within the range of native structure,
Table S5 provides analogous RMSD values for alignment of the present crystal structure with the solution structure of [4E+A8H]-insulin.

Figure S1. *2Fo-Fc* electron density map contoured at a level of 1.0 σ, showing a phenol molecule bound in the R_3^f trimer of $T_3R_3^f$ His^{A8}- insulin.

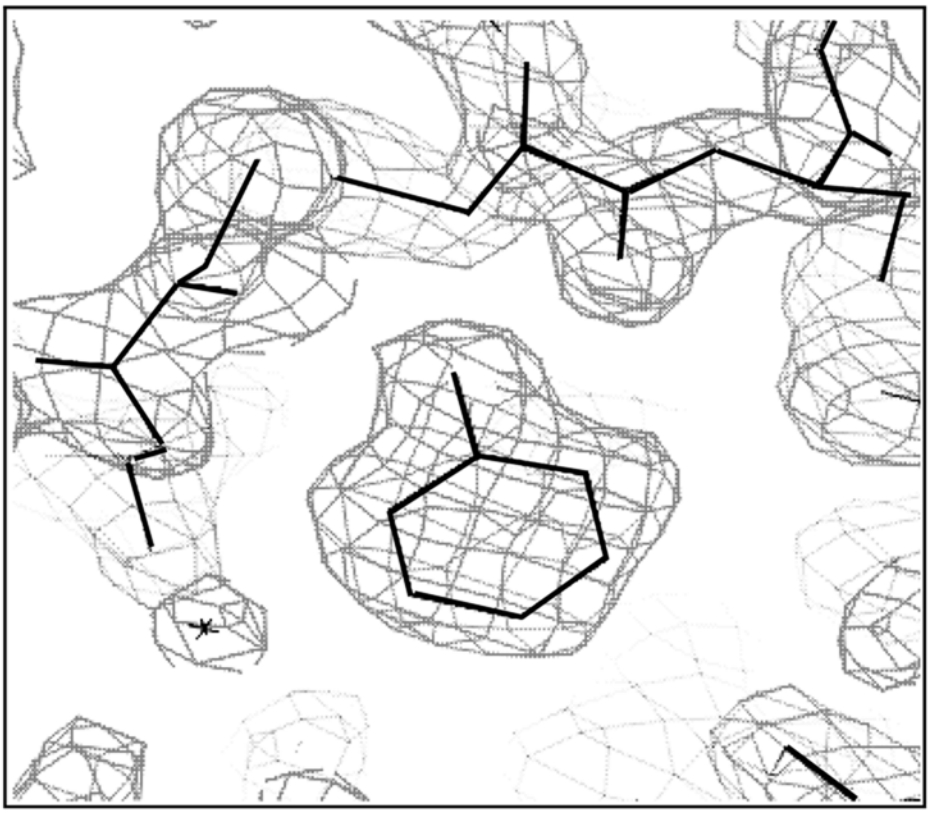

Figure S1

Figure 2. 2Fo-Fc electron density map contoured at a level of 1.0 σ, showing the T3 trimer the coordination geometry is octahedral (A), in the R3f trimer the coordination geometry is tetrahedral (B).

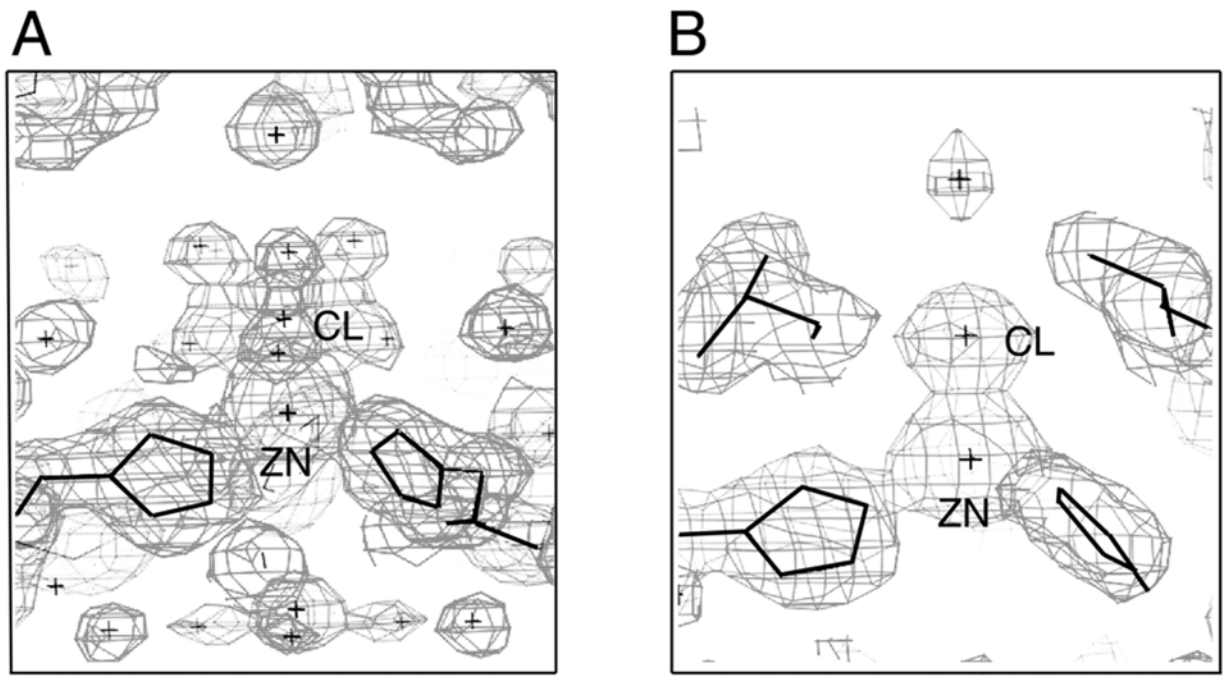

Figure S2

Table S1: RMSD of His^{A8}-insulin T-state Relative to Previous T-state Structures[a]

structure	main-chain	side-chain
1TRZ T (T_3R_3)	0.333 Å	0.676 Å
1TYL T (T_3R_3)	0.272	0.766
1TYM T (T_3R_3)	0.230	0.686
1ZNI T (T_3R_3)	0.172	0.884
1LPH T (KP-T_3R_3)	0.387	1.110
1G7A T1 (rhom-T_3R_3)	0.314	0.815
T2	0.440	0.950
6INS T(mini-proinsulin)	0.438	1.053
4INS T1(2ZN-T_6)	0.652	1.282
T2	1.020	1.860
2INS T1(desB1-T_6)	0.958	1.873
T2	1.098	1.339
1APH (cubic)	0.632	1.451
1BPH (cubic)	0.648	1.380
1CPH (cubic)	0.651	1.511
1DPH (cubic)	0.671	1.477
average	0.557 Å	1.315 Å

[a] The structures were aligned according to the main-chain atoms of residue on B3-B28 and A1-A21. "KP" designates variant insulin containing substitutions Pro^{B28}→Lys and Lys^{B29}→Pro. "rhom" indicates Rhombohedral crystal form."Mini-proinsulin" designate B29-A1 single-chain analog. "desB1" indicates removal of Phe^{B1}.

Table S2. RMSD of His^{A8}-Insulin R-state Relative to Previous R-state Structures[a]

structure	main-chain	side-chain
1TRZ R (T_3R_3)	0.837 Å	1.133 Å
6INS R(mini-proinsulin)	0.586	1.273
1ZNI R (T_3R_3)	0.739	1.259
1LPH R (KP-T_3R_3)	0.964	1.553
1EV3 R1(rhombohedral)	0.497	1.194
R2	0.444	0.948
1ZNJ R1(monoclinic)	0.532	1.260
R2	0.496	1.341
R3	0.551	1.248
R4	0.496	1.129
R5	0.414	1.015
R6	0.514	0.980
1EV6 R1 (monoclinic)	0.524	1.250
R2	0.465	1.217
R3	0.718	1.508
R4	0.507	1.155
R5	0.401	1.251
R6	0.579	1.108
1G7A R1 (rhom-T_3R_3)	0.672	1.285
R2	0.601	1.099
average	0.577 Å	1.210 Å

[a] The structures were aligned according to the main-chain atoms of residue on B3-B28 and A1-A21. "KP" designates variant insulin containing substitutions Pro^{B28}→Lys and Lys^{B29}→Pro.

Table S3: RMSD of Native 2-Zinc Insulin T-state with Collection of Previous T-state Structures[a]

Structure		main-chain	side-chain
4ZN	T2(2ZN-T_6)	0.684 Å	1.343 Å
2ZN	T1(desB1-T_6)	0.333	0.582
	T2	0.711	1.410
1APH	T(cubic)	0.734	1.540
1BPH	T(cubic)	0.735	1.536
1CPH	T(cubic)	0.766	1.688
1DPH	T(cubic)	0.775	1.625
1TRZ	T(T_3R_3)	0.602	1.185
1TYL	T(T_3R_3)	0.602	1.186
1TYM	T(T_3R_3)	0.636	1.194
1ZNI	T(T_3R_3)	0.612	1.261
1LPH	T(KP-T_3R_3)	0.627	1.253
1G7A	T1(rhom-T_3R_3)	0.687	1.355
	T2	0.714	1.384
average		0.658 Å	1.324 Å

[a] The structures were aligned according to the main-chain atoms of residue on B3-B28 and A1-A21. "KP" designates variant insulin containing substitutions Pro^{B28}→Lys and Lys^{B29}→Pro. "rhom" indicates Rhombohedral crystal form."desB1" indicates removal of Phe^{B1}.

Table S4. RMSD of Native Insulin R-state (1TRZ) Relative to Collection of Previous R-state Structures[a]

structure		main-chain	side-chain
1EV6	R1 (monoclinic)	0.851 Å	1.350 Å
	R2	0.731	1.167
	R3	0.994	1.567
	R4	0.759	1.199
	R5	0.748	1.410
	R6	0.728	1.243
1ZNJ	R1 (monoclinic)	0.779	1.225
	R2	0.694	1.422
	R3	0.783	1.389
	R4	0.713	1.032
	R5	0.760	1.228
	R6	0.747	1.193
1G7A	R1 (rhom-T_3R_3)	0.535	1.031
	R2	0.779	1.246
1LPH	R (KP-T_3R_3)	0.535	0.997
1ZNI	R (T_3R_3)	0.209	0.573
average		0.622 Å	1.205 Å

[a] The structures were aligned according to the main-chain atoms of residue on B3-B28 and A1-A21. "KP" designates variant insulin containing substitutions $Pro^{B28} \rightarrow Lys$ and $Lys^{B29} \rightarrow Pro$.

Table S5. RMSD of His^{A8}-Insulin with NMR Structure (4E+A8H) Alignment[a]

structure	main-chain	side-chain
NMR model-1	1.547 Å	2.438 Å
NMR model-2	2.060	2.870
NMR model-3	1.688	2.619
NMR model-4	1.952	2.681
NMR model-5	1.794	2.698
NMR model-6	2.067	2.864
NMR model-7	1.818	2.907
NMR model-8	1.638	2.607
NMR model-9	1.770	2.703
NMR model-10	1.511	2.358
NMR model-11	1.901	2.925
NMR model-12	2.027	2.788
NMR model-13	1.739	2.871
NMR model-14	1.815	2.722
NMR model-15	1.862	2.785
NMR model-16	1.648	2.475
NMR model-17	2.272	3.099
NMR model-18	1.779	2.833
NMR model-19	1.979	2.803
NMR model-20	1.865	2.724
NMR model-21	1.634	2.652
NMR model-22	1.679	2.518
NMR model-23	1.639	2.561
NMR model-24	1.499	2.599
NMR model-25	1.826	2.662
NMR model-26	2.097	2.490
average	1.812 Å	2.702 Å

[a] The structures were aligned according to the main-chain atoms of residue on B3-B28 and A1-A21.