Supporting information

for

# Insulin–Insulin-like Growth Factors Hybrids as Molecular Probes of Hormone:Receptor Binding Specificity

Květoslava Křížková[1,2], Martina Chrudinová[1,2], Anna Povalová[1,2], Irena Selicharová[1], Michaela Collinsová[1], Václav Vaněk[1], Andrzej M. Brzozowski[3], Jiří Jiráček[1], Lenka Žáková[1,*]

[1]Institute of Organic Chemistry and Biochemistry, Academy of Science of the Czech Republic v.v.i., Flemingovo nám. 2, 166 10, Praha 6, Czech Republic, [2]Charles University in Prague, Faculty of Science, Department of Biochemistry, Hlavova 8, 128 43, Praha 2, Czech Republic and [3]York Structural Biology Laboratory, Department of Chemistry, University of York, Heslington, York YO10 5DD, United Kingdom

* Correspondence e-mail: zakova@uochb.cas.cz

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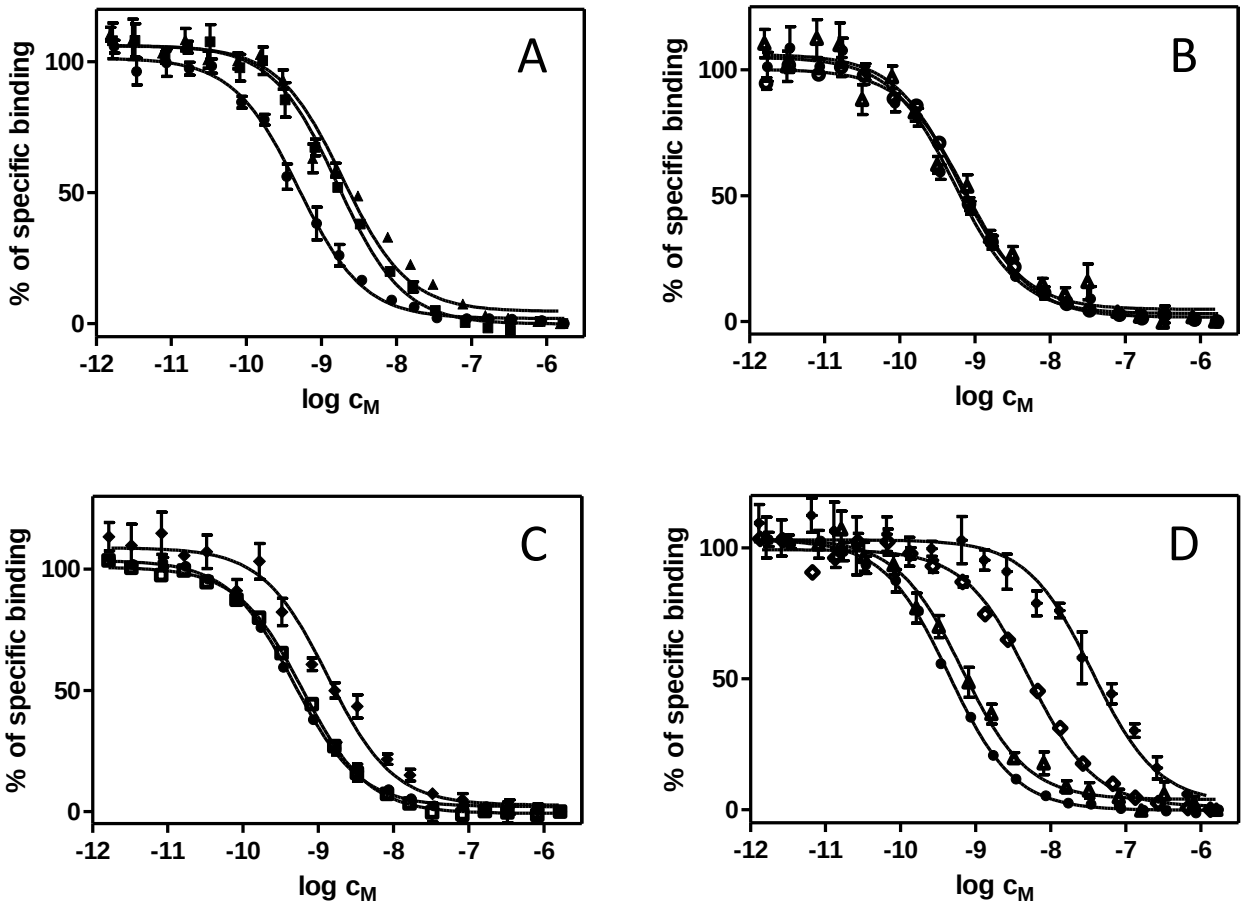


**Figure S1. Inhibition of binding of human [$^{125}$I]monoiodotyrosylA14-insulin to IR-A isoform in membranes of human IM-9 lymphocytes by human insulin, insulin analogues, IGF-1 and IGF-2.** (A) • - human insulin; ■ - $A^{A21}PLK^{A24}$-insulin (**1**); ▲ - $A^{A21}PLKPAKSA^{A29}$-insulin (**2**). (B) • - human insulin; Δ - $A^{A21}TPAKSE^{A27}$-insulin (**3**); ○ - $S^{B31}$-insulin (**4**). (C) • - human insulin; ♦ - $S^{B31}K^{B32}$-insulin (**5**); □ – $S^{B31}KV^{B33}$-insulin (**6**). (D) • - human insulin; ▼ - $S^{B31}KVS^{B34}$-insulin; ∗ - IGF-1; ◊ - IGF-2.

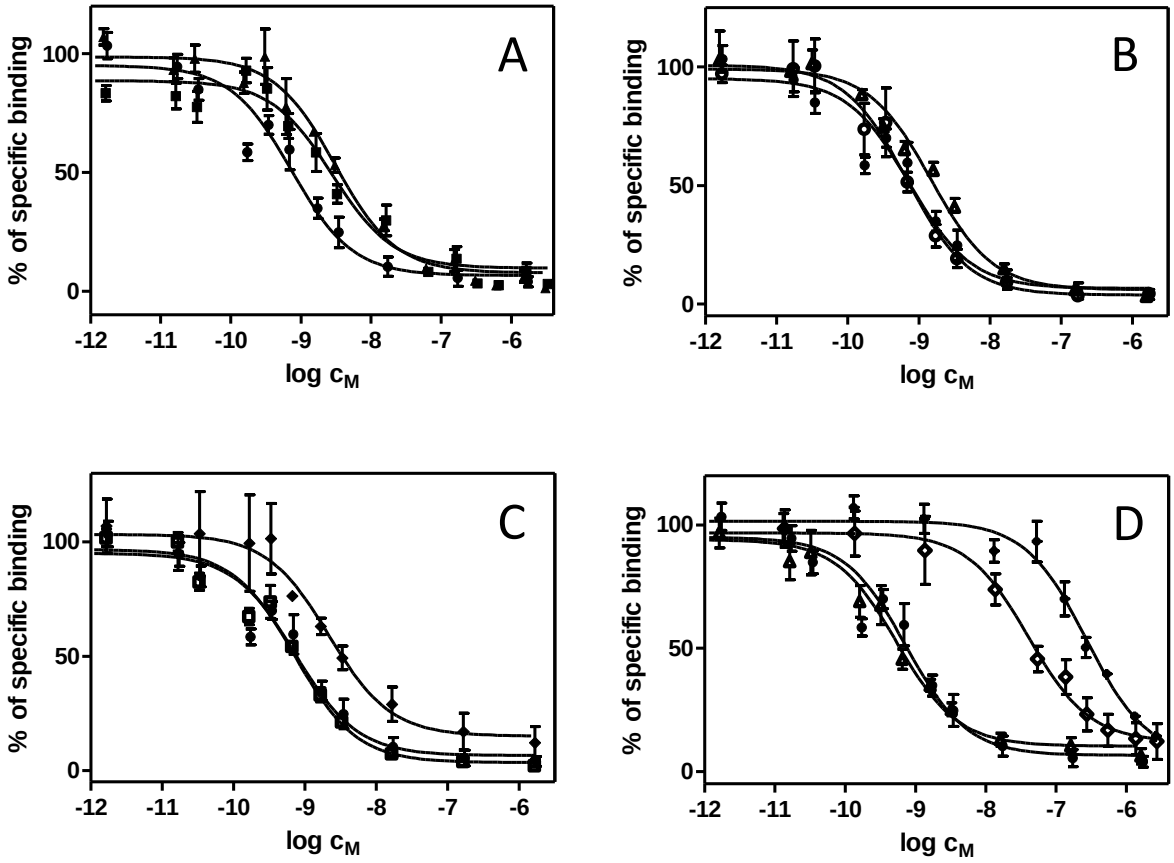


**Figure S2. Inhibition of binding of human [$^{125}$I]monoiodotyrosylA14-insulin to IR-B isoform in membranes of mouse fibroblasts by human insulin, insulin analogues, IGF-1 and IGF-2.** (A) • - human insulin; ■ - A$^{A21}$PLK$^{A24}$-insulin (**1**); ▲ - A$^{A21}$PLKPAKSA$^{A29}$-insulin (**2**). (B) • - human insulin; Δ - A$^{A21}$TPAKSE$^{A27}$-insulin (**3**); ○ - S$^{B31}$-insulin (**4**). (C) • - human insulin, ♦ - S$^{B31}$K$^{B32}$-insulin (**5**); □ – S$^{B31}$KV$^{B33}$-insulin (**6**). (D) • - human insulin; ▼ - S$^{B31}$KVS$^{B34}$-insulin; ∗ - IGF-1; ◊ - IGF-2.

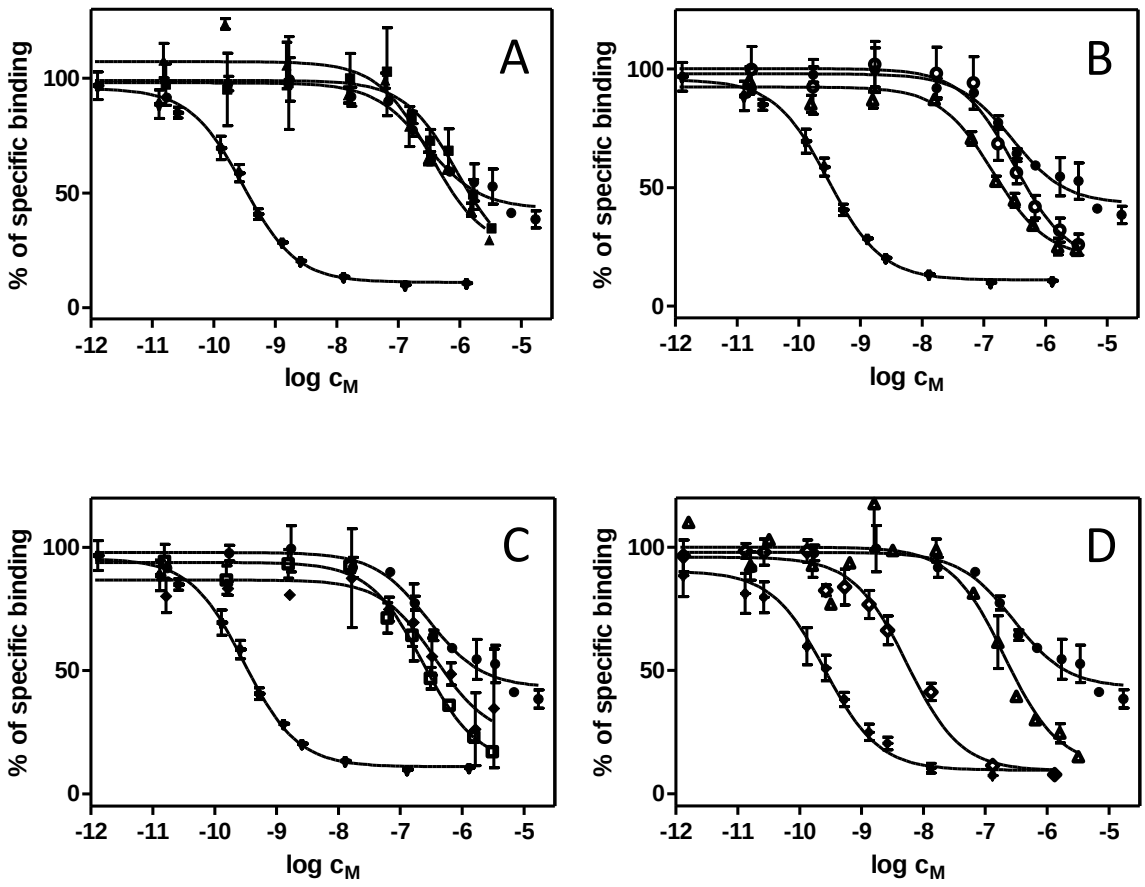


**Figure S3. Inhibition of binding of human [$^{125}$I]monoiodotyrosyl-IGF-1 to IGF-1R in membranes of mouse fibroblasts by human insulin, insulin analogues, IGF-1 and IGF-2.** (A) • - human insulin, ■ - A$^{A21}$PLK$^{A24}$-insulin (**1**); ▲ - A$^{A21}$PLKPAKSA$^{A29}$-insulin (**2**); ∗ - IGF-1. (B) • - human insulin; Δ - A$^{A21}$TPAKSE$^{A27}$-insulin (**3**); ○ - S$^{B31}$-insulin (**4**); ∗ - IGF-1. (C) • - human insulin; ♦ - S$^{B31}$K$^{B32}$-insulin (**5**); □ – S$^{B31}$KV$^{B33}$-insulin (**6**); ∗ - IGF-1. (D) • - human insulin; ▼ - S$^{B31}$KVS$^{B34}$-insulin; ∗ - IGF-1; ◊ - IGF-2.

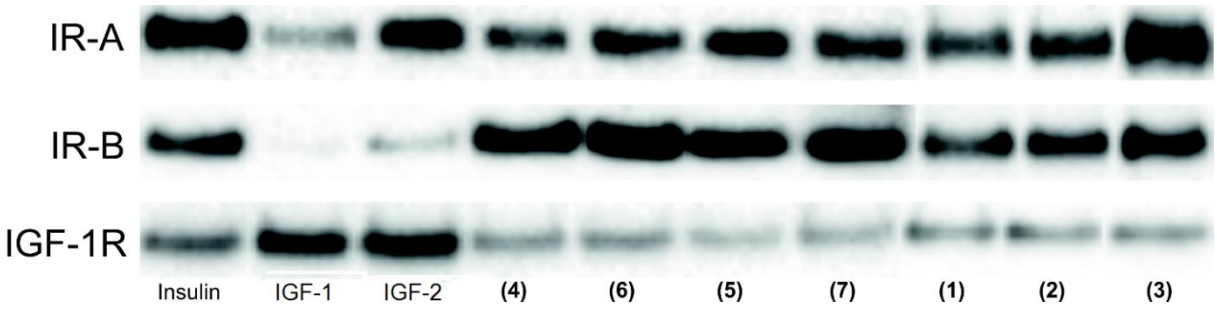


**Figure S4**. **Autophosphorylation of IR and IGF-1R.** Stimulation of phosphorylation of receptors in the IR-A, IR-B and IGF-1R cells by human insulin (HI), IGF-1, IGF-2 and insulin analogs containing sequences derived from D-domain of IGF-1 (**1** and **2**), IGF-2 (**3**) or from C-domain of IGF-2 (**4**-**7**). The cells were stimulated by 10 nM ligands for 10 min. Phosphorylated receptors were detected on western blots by anti-phospho-IGF-1R β-subunit (Tyr1131) /IRβ (Tyr1146) antibody. One representative blot is shown for each receptor (IR-A, IR-B and IGF-1R). Values including error bars shown in Figure 4 were calculated from four independent blots.