*Advanced Computational Protocol for Atomistic Understanding and Modulation of Insulin Binding to Insulin Receptor*

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The binding of insulin to its receptor (insulin receptor, IR) is mediated by an extensive network of non-covalent interactions. Their quantitative characterization is difficult due to the huge size of the interface, its flexibility and thus also imprecise structural information. Major breakthroughs in the cryo-EM methodology yielded several structures of insulin bound to its receptor (IR) at sufficient resolution (3.2 Å) for atomistic modeling and structure-based drug design. Taking this into account, we have post-processed the recent cryo-EM structure of insulin/IR complex with a hierarchical computational protocol developed in this work. It entails molecular dynamics (MD), fragmentation and quantum chemical (QM) and faster molecular mechanics (MM) calculations to identify interaction “hotspots” in the IR primary site. The identified interaction hotspots in insulin Sites 1a and 1b (Ile A2, Glu A4, Tyr A19, Cys B7, Val B12, Glu B13, Tyr B16, Phe B24 and Phe B25) are in excellent agreement with the available experimental data. The energetic description of individual non-covalent interactions at the interface by the SQM and MM methods was checked on smaller fragments (amino acid dimers and trimers, up to 200 atoms) against DFT-D3/COSMO calculations. Both methods satisfactorily reproduce weaker contacts and strong NH…O hydrogen bonds but give a different ordering of individual residues. This proves a suboptimal description of the interactions using the MM method as compared to SQM. The SQM-based computational protocol developed in this work was validated against experimental and higher-level QM data and found applicable to very large and extremely flexible protein-protein interfaces.