Insulin Signaling and Pharmacology in Corals

Whitney Vizgaudis[1], Lokender Kumar[1], Monsurat Olaosebikan[2], Liza Roger[3], Nathanael Brenner[1], Samuel Sledzieski[4], Jinkyu Yang[5], Nastassja Lewinski[3], Rohit Singh[4], Noah Daniels[6], Lenore Cowen[2], and Judith Klein-Seetharaman[1]

[1]Colorado School of Mines
[2]Tufts University
[3]Virginia Commonwealth University
[4]MIT
[5]University of Washington
[6]University of Rhode Island

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Abstract

Once thought to be a unique capability of the Langerhans Islands in the pancreas of mammals, insulin production is now recognized as an evolutionarily ancient function going back to prokaryotes, ubiquitously present in unicellular eukaryotes, fungi, worm, Drosophila and of course human. While the functionality of the signaling pathway has been experimentally demonstrated in some of these organisms, it has not yet been exploited for pharmacological applications. To enable such applications, we need to understand the extent to which the structure and function of the insulin-insulin receptor system is conserved. To this end, we analyzed the insulin signaling pathway in corals through remote homology detection and modeling. By docking known insulin receptor ligands to a coral homology structure, we locate ligand binding pockets and demonstrate their conservation suggesting that it may be possible to exploit the structural conservation for pharmacological applications in non-model organisms. We also identified the coral homologues of the over 100 signaling proteins involved in insulin and its related signaling pathways, demonstrating their wide-spread conservation. Notable exceptions are glucagon and somatostatin. It is tempting to speculate that under high light conditions, when the algae synthetize excess sugars, the cnidarian host may experience insulin resistance, and that the cnidarian microbiome may be involved in manipulating the insulin signaling system.

Introduction

Corals are colonies of marine invertebrates (cnidarians) that depend on a symbiotic relationship with algae in the family Symbiodiniaceae (LaJeunesse et al., 2018). The algae harvest light and synthesize nutrients in exchange for shelter and nitrogen sources (Putnam et al., 2017). Coral reefs cover only 0.1% of the ocean floor, but are home to the largest density of animals on earth, rivaling rain forest habitats in species diversity (Hoegh-Guldberg et al., 2017). The symbiosis, which was originally thought to be restricted to algae, is now known to extend to a much more complex community than anticipated with thousands of bacteria, bacteriophages, viruses and fungi, in addition to algae. The entirety of the organism community in a coral is referred to as a holobiont. Individual cnidarian host animals are called polyps.

Symbiosis characterizes the healthy host-microbial coral community. It is essentially unknown what molecules are responsible for the complex communication mechanisms that allow symbiosis to occur (Gates et al., 1995). This is a particularly severe gap in our knowledge, since it is at the heart of the worldwide phenomenon of coral reef bleaching, in which the algae are leaving the cnidarian host as a result of temperature stress, including that brought about by global warming. A recent study assessed 100 worldwide locations and found

that the annual risk of coral bleaching has increased from an expected 8% of locations in the early 1980s to 31% in 2016 (Gates et al., 1995; Hughes et al., 2018). Human impacts on coral reef ecosystems threaten fishing and tourism industries that are valued at hundreds of billion of dollars annually (Putnam et al., 2017). Finding potential solutions to assist the corals in the survival of human impact is an urgent task.

The symbiotic algae are believed to provide as much as 90% of the energy the corals consume by light harvesting and photosynthesis. Thus, it is likely that corals can measure and regulate nutrient balance. Support for this hypothesis comes from transcriptomic studies (Yuyama et al., 2018). A comparison between the expression of insulin signaling related genes in the presence and absence of the symbiotic algae strongly suggests that insulin signaling is induced at the transcriptomic level in response to population of the corals by the algae. A likely interpretation of this finding is that the coral needs to respond to the sugars that are produced by the algae and perhaps too much sugar could have detrimental effects on corals, similar to the diabetic response through aberrant insulin signaling in humans. It is also possible that the mechanism for bleaching (loss of symbiotic algae from the holobiont) involves an imbalance in nutrient regulation and possible involvement of the insulin signaling pathway. Could corals have diabetes?

The first step in addressing such questions is to establish the extent to which insulin signaling in corals is analogous to human insulin signaling. The animal host in corals are cnidarians which have evolved before the split into deuterostomia such as humans and protostomia like the model organisms *Caenorhabditis elegans* and *Drosophila melanogaster* 700 Million years ago. During this time, mutations accumulated, so we expect many homologues between humans and corals to be in the gray zone of 20-30% sequence identity, usually referred to as remote homology. Therefore, identifying similarity between human and coral genes requires remote homology detection and analysis. The coral we have chosen for this project is Pocillopora damicornis (pdam), a stony coral that makes its own calcium carbonate skeleton and houses colonies of individual animals, the polyps, just barely visible by eye. First, we identified the most likely homologue of human insulin and insulin receptor (IR) as well as downstream pdam homologues in the insulin related signaling pathways involving over 100 proteins using a Hidden Markov Modeling approach suitable for the large divergence between sequences, hhblits (Remmert et al., 2011). Next, we investigated in detail through computational structural modeling the conservation of amino acids crucial for function, especially ligand binding. Finally, we compared the conservation of the interface of the IR with its natural ligand insulin to those of small molecule pharmacological agents developed originally for targeting the human IR. This included small molecule agonists and sensitizers as well as inhibitors, which have been studied in humans for their potential clinical applications in treatment of diabetes and cancer, respectively. Our study opens the door to a new field in coral biology, that of coral pharmacology.

Methods

Sequence sources. The FASTA sequence files for human and coral proteins used in this study were acquired from the UniProt database website at *www.uniprot.org*.

Sequence analysis. In-depth sequence analysis of coral was performed as (Ye et al., 2017). The 3D reconstruction was made to provide detailed, domain wise information of the coral insulin receptor. Full coral receptor model (left, right, top, and bottom view) was represented as surface representation with color coded description of individual domain.

Hhblits alignment and selection . Protein sequences were imported to the online hhblits coral protein remote homology search tool at*www.hhblits.cs.tufts.edu*based on (Remmert et al., 2011). The sequences were provided as a FASTA sequence file format and the results of the remote homology detection were received via email. Coral protein sequence pdam_00006633-RA showed homology with human insulin protein and pdam_00013976-RA protein sequence with human insulin receptor.

Structural mapping of conservation. The ConSurf2016 server at*www.consurf.tau.ac.il* was used to identify sequence conservation and plot it onto the structure of insulin and insulin receptors. ConSurf is a bioinformatics application for estimating the evolutionary conservation of amino acid position in a protein molecule between homologous sequences based on the phylogenetic relations.

Insulin signaling information: To uncover the coral insulin signaling we use the literature information from KEGG: Kyoto Encyclopedia of Genes and Genomes at*www.genome.jp/kegg*. KEGG database consists of high-level functions of molecular information from large-scale datasets of organisms generated by high-throughput sequencing techniques.

Swiss modeling (Coral IR model construction): The coral insulin receptor homolog model was constructed using Swiss Model at*www.swissmodel.expasy.org*. Swiss-Model is an integrated web-based service dedicated to homology modelling of proteins. We used the target-template alignment function of swiss model and provided the full structure of the human insulin receptor (6pxv) as a template to model pdam_00013976-RA. From the same structure we used insulin structure to model pdam_00006633-RA as coral insulin. The model was downloaded and analyzed using PyMOL software (Version-2.3.4, Schrodinger, LLC).

ClusPro protein-protein docking: Protein-protein docking studies were performed using Clus-Pro 2.0 protein-protein docking tool at *www.cluspro.bu.edu*. We performed the docking of coral insulin protein models with coral IR model (dimer). The docked poses were downloaded and analyzed using PyMOL software. We have analyzed the insulin binding site-1 and site-2 in detail and provided the information of interacting residues between coral insulin and coral IR. The information was extracted using PyMOL.

Autodock Vina (protein-ligand docking): Molecular docking of selected ligands with coral Insulin receptor EC and TK domain was performed using Autodock Vina (Trott and Olson, 2009). We constructed a grid box covering all desired residues using Autodock vina/Autodock tools 1.5.6 at*vina.scripps.edu*(Trott and Olson, 2009). The gridbox parameters for full protein include: center_x= -11.318, center_y= -15.45, center_z= 18.207, size_x=126, size_y=126, and size_z=126. We also used the full TK structure for identification of putative allosteric ligands. We used the latest inhibitor-bound structure (5HHW) for standardizing our docking experiments. Results are expressed as binding affinity for the top pose with polar contacts for the same.

Coral Insulin Receptor Kinase domain inhibitor binding site analysis: The most recent structures of human kinase domains with their inhibitors were analyzed and essential residues forming polar contacts with inhibitors were identified as the inhibitor pocket (see details in text). The identified residues were also mapped in the coral TK domain model and represented in the coral TK domain using PyMOL.

Results and Discussion

Remote homology detection of insulin and IR

Popular sequence alignment methods such as Blast are not suitable for the detection of sequence conservation between coral and human proteins because of the low level of conservation in the 20-30% sequence identity range. The method of choice for retrieval of coral homologs of human proteins was Hhblits. This is a so-called Hidden Markov Model based alignment approach developed by Johannes Soeding in 2005 (Remmert et al., 2011). Unlike traditional profile HMM's, both query and template are HMM's. The query HMM is generated by using amino acid distributions. Thus, the search for homologues is through an HMM-HMM alignment which makes this method extremely sensitive. This has been shown in many instances where hhblits has been able to successfully outperform the identification and alignment of remote homologues, as compared to the traditional profile HMM approach, such as HMMER3 (Remmert et al., 2011). Because of the 700 million years of evolution between corals and humans, this exquisite sensitivity of hhblits has been instrumental for the present study. The results of the hhblits search in the pdam genome for homologues of human insulin (uniprot ID **P01308**) and human IR (uniprot ID **P06213**) are shown in the **Supplementary**materials with filenames matching the uniprot ids.

Shown in **Figure 1** is the sequence alignment of human insulin with pdam protein pdam_00013976. Similarly, IR was aligned with pdam_00006633 with high confidence (data not shown). In both cases, the alignments cover a large fraction of the sequence, 1164 out of 1382 amino acids in the case of IR and 101 out of 110 in the case of insulin. Manual inspection of the sequence alignment also shows a clear matching of similar sequences despite the low overall sequence identity. This was not possible with regular profile HMM sequence

alignments (data not shown). To validate the alignment, we extracted known insulin residues involved in binding and you can see that these map to regions of high confidence alignment. Shown in different colors in **Figure 1** are various residue motifs identified to be important for receptor binding by cryoelectron microscopy (Uchikawa et al., 2019). There is a good overlap between these functionally important motifs and the regions of high confidence alignment (9=highest, 0=lowest). We did the same for the insulin receptor and again found overlap with the ligand binding residues and high confidence alignment (data not shown). We also found conservation of the human disulfide bond between Cys647-Cys860 connecting FnII-2a and FnIII-3 in the coral sequence. We had identified this disulfide bond to be important for receptor activation as a signaling bridge (Ye et al., 2017), a hypothesis that was validated by the recent structural analysis (Uchikawa et al., 2019).

3-Dimensional reconstruction of coral insulin and IR using hhblits alignments

The high conservation of known interfaces between insulin and IR gave us high confidence that there really are insulin and IR homologues in pdam and so we created coral homology models using Swiss modeler. There has been an incredible amount of structural data that has come out recently on the IR (McKern et al., 2006; Menting et al., 2013) (Gutmann et al., 2018) (Weis et al., 2018) especially due to the advances in cryoelectron microscopy (Uchikawa et al., 2019). This has allowed us to use the alignments to reconstruct three-dimensional structural homology models for the coral insulin and IR molecules using Swiss-Modeler (see Methods). This is a relatively complex task for IR, given the large size and dimeric configuration of the receptor. The human IR is composed of extracellular (EC or ecto) domain, transmembrane, and cytoplasmic (CP) domains. The IR EC domain consists of a full-length alpha chain and 194 residues of beta-chain including leucine-rich repeat domains (L-L2), a cysteine rich region (CR) and fibronectin type-III domains (FnIII-1-3). The transmembrane (TM) and juxtamembrane (JM) domains are present in the beta-chain and start after the c-terminal of FnIII. The cytoplasmic domain includes tyrosine kinase domain and betaCT domain. Based on our sequence analysis described in the accompanying paper (ref), we identified 6pxv as the structure that showed the highest sequence coverage. This structure was therefore selected for homology modeling, shown in **Figure 2** . The amino acid sequences of the corresponding domains in human and in coral are listed in **Table 1** .

Remote homology detection of proteins involved in insulin signaling

Next, we looked at the human proteins and their pdam homologues in three receptor activated signaling pathways related to insulin signaling. We extracted from the literature all the proteins involved in the signaling pathways activated by binding of insulin to the IR. In addition, we also extracted proteins from related pathways, glucagon and somatostatin, which are known to down-regulate insulin signaling. In **Figure 3** , all of the proteins shown in black have a predicted pdam homologue, while those shown in red, do not. Crosstalk between these pathways is mediated by a number of proteins that are common to two or even all three pathways.

For three proteins, we were not able to identify suitable pdam homologues, somatostatin, glucagon and BAD, as judged by their poor e-values of 0.52, 15 and 5.6 and small percent alignments 16% of 116 amino acids, 7% of 180 and 11% of 168 amino acids, respectively. Thus, in the insulin receptor signaling pathway, only one protein, BAD, is missing, at the effector end of the pathway, indicating that the pathway is mostly functional. Importantly, there is a clear homologue of both insulin and the insulin receptor present. In contrast, we were not able to find pdam homologues for either the pathway initiating ligands glucagon or somatostatin. These are both pathways that suppress insulin signaling.

Analysis and comparison of insulin-IR interactions

An important approach to confirm if the pdam receptor we identified with hhblits really does what the corresponding human receptor does, is to check for conservation of amino acids crucial for function, here ligand (insulin) binding. Similar to the human insulin-IR complex, we expect two distinct insulin binding sites per IR monomer (4 insulin molecules that can be bound in total). To verify if these binding sites are conserved in our coral homology model, we used ClusPro to dock coral insulin to coral IR (see Methods).

For comparison, we also docked human insulin to coral IR and *vice versa* (**Figure 4**). Our protein-protein docking clearly showed the binding of coral insulin protein to the coral insulin receptor at two sites (similar to human insulin-IR complex). In addition, we found some insulin molecules docked to additional sites in the lower parts of the EC structure. Because these are expected to be located close to the membrane, these structures may not be fully reliable and these putative insulin binding sites (labeled 5 and 6 in **Figure 4**) have been excluded from further analysis.

To systematically compare the interactions between insulin and IR at the two binding sites, we identified all amino acids within 5 Angstrom distance of either protein (insulin or IR). We find that site-1 of coral insulin binding to the IR consists of Arg532, Gly533, Ser531, Val530, Asp479, Asn529, Val528, Asp480, Tyr481, Arg482, Gln103, Leu71, Asp692, Gln691, Tyr695, Met43, Arg45, Lys46, Lys696, Phe699, Leu698, Thr697, Asn20, Ser19, Lys701, Thr700, Lys17, and Thr70 (**Figure 5, top**). Site-1 was found to be interacting with chain-A and chain-B residues of insulin. Site-2 was found at a similar position to the human insulin site-2 (**Figure 5, bottom** , and also see **Figure 4**). The site-2 residues in coral IR consist of Cys463, Asn464, Pro465, Arg461, Lys547, Pro460, Leu459, Lys546, Cys467, Thr458, Ile457, Thr544, Lys469, Glu456, Arg543, Val470, Glu542, Glu471, Thr522, Ile541, Val523, Lys540, Arg539, Pro524, Pro525, Asn159, Asn160, and Asp161.

Within these close-by residues, we identified polar contacts and hydrogen bonds in the human insulin-IR co-structure (**Figure 6**) and the coral insulin-IR homology models (**Figure 7**). We can see that there is overlap between the residues in both binding pockets, although the human interface is more extensive than the coral interface for both binding sites. This suggests that the human insulin-IR pair is likely a higher affinity complex than the coral one. Presumably, evolution has gradually improved and expanded the network of interactions between insulin and its receptor over the 700 million years in between human and pdam species.

Small molecule docking to the coral IR homology model

Encouraged by the clear conservation of the functional insulin binding pockets between human IR and coral IR, we investigated the potential of targeting the coral IR through pharmacology using existing human IR ligands. To this end, we systematically docked small molecule ligands to the coral receptor analogous to the human controls described in the accompanying paper (Vizgaudis et al, 2021). We created a table of ligands (**Table 2**) that directly interact with IR. After having found out where those ligands bind in human IR, we here investigated if they also bind to those locations in coral IR. Table 2 shows overall very similar results for docking of the ligands to the coral homology model, as compared to the human results. However, overall the predicted affinities tend to be slightly smaller for the coral-ligand complexes than the human-ligand complexes. This is in line with the observation of the insulin-IR interfaces and may indicate that while not *all*stabilizing interactions in human will extend to the coral system, a large extent of them do. This is illustrated for the CP ligands in**Figure 8** . There is almost complete conservation of the amino acids in contact with the CP inhibitors described in the accompanying paper (Vizgaudis et al, 2021). The only exception is Glu1074 which is a glycine (Gly1030) in human. **Figure 9** shows the top ranked poses for the three TLK inhibitors and DDN. Although the entire protein was included in the grid box for docking, all four inhibitors docked to the main ligand binding pocket in between the two lobes of the kinase domain described in the accompanying paper, characteristic for orthosteric tyrosine kinase inhibitors.

Discussion

Here, we describe for the first time the concept of "coral pharmacology" where we aim to develop pharmacological approaches towards potentially treating corals who have been harmed by human pollution. Using the insulin-IR system as a proof of concept, we developed a pipeline for establishing the functional similarities between human and coral membrane receptor signaling systems. Insulin is responsible for regulation of nutrient concentration in human, and will likely carry out a similar function in corals. This is the premise that we investigated in depth in this paper.

Structural biology has made major strides in understanding the mechanisms of insulin binding to the IR,

the conformational changes induced and subsequent modulation of downstream signal transduction. We have now investigated the evolution of the insulin signaling system through sequence and structure analysis. Evolutionary early species such as cnidarian animal hosts in corals use the system despite their simple organization. Transcriptomic analysis had revealed that insulin signaling plays a major role in the establishment of symbiosis between cnidarian host animals and algal symbionts (Yuyama et al., 2018). Given that one of the major benefits of symbiosis is the delivery of sugars obtained through photosynthesis of the algae to the host, we can expect that the role of insulin signaling is analogous in corals to that in humans, despite their evolutionary distance. It is tempting to speculate that under high light conditions, when the algae synthetize excess sugars, that the cnidarian host may experience insulin resistance, a hypothesis that remains to be validated experimentally.

We found that there is strong conservation at the structural level at the functionally important ligand interfaces, despite the large divergence in sequence, supporting that the insulin and IR homologues function similarly to their human counterparts. To further find support for this hypothesis, we extracted from the literature all the proteins involved in the signaling pathways activated by binding of insulin to the IR. In addition, we also extracted proteins from related pathways, glucagon and somatostatin, which are known to down-regulate insulin signaling. Interestingly, these two hormones are missing homologues in corals. It is tempting to conclude that while Corals can regulate nutrient uptake/release via insulin signaling, regulation of insulin signaling by other pathways may not function. We speculate that perhaps these ligands may be provided by the microbiome. Perhaps it is bacteria or algae which generate ligands for negative regulation of insulin signaling. This would make sense since this may save nutrients extracellularly for their consumption rather than allowing uptake into the coral cells.

The finding of conservation of the insulin system across 700 millions years linking human and coral proteins is not surprising and add a new system to a growing list of organisms: Insulin-like molecules have been identified in prokaryotes, microbial eukaryotes, insects, invertebrates, plants (LeRoith et al., 1981) (Le Roith et al., 1980) (Baig and Khaleeq, 2020). Antibodies raised against human insulin recognizes insulin like material from unicellular eukaryotes such as Tetrahymena pyriformis, a ciliated protozoan, and Neurospora crassa (Kole et al., 1991; Muthukumar and Lenard, 1991) and Aspergillus fumigatus, both fungi, and even prokaryotes (LeRoith et al., 1981). The fact that both prokaryotes and eukaryotes synthesize insulin suggests that it may play a role in co-evolution, potentially supporting our above hypothesis of the insulin system playing a role in the communication across the coral microbiome.

The conservation at the sequence level is mirrored by conservation at the functional level (Abou-Sabe' and Reilly, 1978) (Le Roith et al., 1980). For example, effects of mammalian insulin on E. coli have been described. Similarly, insulin shows metabolic effects on Neurospora crassa cells such as enhanced glucose metabolism, enhanced growth, improved viability, accumulation of intracellular sodium. The insulin-like preparations from more primitive organisms have effects on rat cells. It has also been shown that these functional effects are likely achieved through a phosphorylation cascade as shown by the enhanced phosphorylation of specific proteins on serine/threonine and tyrosine residues (Kole et al., 1991).

Most recently, an insulin-IR pair has been described in detail for Acanthamoeba castellanii, an early mitochondria unicellular eukaryotic organism (Baig and Khaleeq, 2020). Not only did they show typical mammalian insulin-induced effects on Acanthamoeba cells, but they also investigated the anti-diabeteic drug, metformin, and conducted homology modeling of the putative Acanthamoeba insulin-IR pair. This study strongly supports the notion of a high degree of conservation of the insulin-IR pair across billions of years of evolution, and pioneers the use of a human antidiabetic drug (metformin) in the context of a primitive organism.

The findings described here for the insulin system may extrapolate also to other hormones. Insulin is not the only mammalian hormone that is found in many more primitive organisms. Prokaryotic and eukaryotic microbes contain calcitonin, corticotropin, gonadotropin, relaxin, somatostatin, thymosin and thyrotropin (Kole et al., 1991; Muthukumar and Lenard, 1991).

While the focus of this paper has been on a single ligand-receptor complex and its downstream signaling network, extending our detailed understanding from the well studied human organism to the new coral system (pdam, specifically), we would like to point out that such extensions from model to non-model organisms is general. The explosion in sequence data obtainable for non-model organisms makes approaches described here very desirable, as we can extrapolate to function from the sequence data via structural and systems biology.

Table 1 . Matching residues in human insulin receptor with corresponding residues in coral insulin receptor

Domains	Amino acid seq (Human IR) 6pxv	Amino acid (coral IR)
C1	HLYPGEVCPGMDIRNNLTRL	[illegible]
CR	IDWSRILDSVEDNYIVLNKD	[illegible]
L2	CVACRNFYLDGRCVETCPPP	[illegible] GNKSCLKCT-TEKCPRGIGTQLEENL-GQIEKVNGYIVIIESASLT-SLNFFKNLREIR-PRLIYNFLSRPPAMETDLYNERYALAIR
FN3-1	VSGTKGRQERNDIALKTNGD	[illegible] SDTTNGNAVACNVRKIN-VTVEEITLPRGCN-PVCVKVEWDDAIIND-DYRNVLFYTL-SYREAPNRQITEYTDVDACSSDSGDIWT VMTKESK
FN3-2	NPSVPLDPISVSNSSSQIIL	[illegible]
FN3-3	LVISGLRHFTGYRIELQACN	[illegible]
TM	RGCRLRGLSPGNYSVRIRAT	[illegible] SAQVRAITSSGNGSWS-NTVSFSYFIESQSTVPPIGE

*indicates the missing sequence in the structure

Table 2. Docking scores for different IR ligands to human IR and pdam IR homology model. All fields colored in gray are negative controls, i.e. known CP ligands docked to the EC domain, known EC ligands docked to the CP domain and ligands known to bind to proteins other than IR. While it cannot be excluded that these ligands may also bind to the IR and/or different domains within the IR, it is unlikely.

Drugs	Coral IR Extracellular Domain	Coral IR Extracellular Domain	Coral IR Extracellular Domain	Coral IR Cytoplasmic Domain	Coral IR Cytoplasmic Domain	Coral IR Cytoplasmic Domain
Ligands binding in the EC domain	Affinity coral (rank) Ligands binding in the EC domain	Notes (location etc) Ligands binding in the EC domain	Affinity human (rank) Ligands binding in the EC domain	Affinity coral (rank) Ligands binding in the EC domain	Notes (location etc) Ligands binding in the EC domain	Affinity human (rank) Ligands binding in the EC domain

Drugs	Coral IR Extracellular Domain	Coral IR Extracellular Domain	Coral IR Extracellular Domain	Coral IR Cytoplasmic Domain	Coral IR Cytoplasmic Domain	Coral IR Cytoplasmic Domain
1,2,3,4,6-penta-o-galloyl-D-glucopyranose	-9.9(4) -10.9(1)	Pose 4; Bonds with Pro308, Gly310, Lys324, Ile322, Ala289, Ile311, and Asn894. Many poses are near insulin binding pockets. Few are buried deep.	-11.4(1)	-8.8 (1)	Close to inhibitor binding site not buried deep in the pocket, No H-bond formation with TK domain	-9.2(1)
L-783,281	-9.4(1)	Pose 1; Bonds with Lys127 and Val154. All other poses are buried deep and not near insulin binding pockets	-9.2(1)	-7.5(1)	Bound away from the inhibitor binding site and not buried deep in the domain; H-bond with Gln1043	-8.4(1)
S597	-7.4(3) -7.0(9) -8.6(1)	Pose 3; Bonds with Arg482, Ala684, Ser683, Tyr481, Pro525, Glu527, Ile129, and Gln103 . Pose 9 is also near an insulin binding pocket. All other poses are buried deep and not near insulin binding pockets	-7.8(6) -8.3(1)	-7.4(1)	Bound away from the inhibitor binding site; H-bonds with Asn1001, Thr1148, Asp1150, Asn1131, Arg1130,	-8.3(1)
Thymolphthalein	-7.5(1)	All poses are buried deep and not near the insulin binding pocket	-7.7(6) -8.3(1)	-6.6(1)	Away from the inhibitor binding pocket, H-bond with Phe1138	-8.6(1)

Drugs	Coral IR Extracellular Domain	Coral IR Extracellular Domain	Coral IR Extracellular Domain	Coral IR Cytoplasmic Domain	Coral IR Cytoplasmic Domain	Coral IR Cytoplasmic Domain
4548g05	-9.0(1)	All poses are buried deep and not near the insulin binding pocket	-8.9(1)	-8.9(1)	Bound in the middle but away from inhibitor binding site, H-bonds with Asp1080, and Asn1083	-8.8(1)
Ligands binding to CP domain	**Ligands binding to CP domain**	**Ligands binding to CP domain**	**Ligands binding to CP domain**	**Ligands binding to CP domain**	**Ligands binding to CP domain**	**Ligands binding to CP domain**
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. (PDB: 5hhw)	NA	NA	NA	-7.7(1)	Bound to the inhibitor binding pocket; H-bond with Leu999.	-7.8 (1)
5,8-diacetyloxy-2,3-dichloro-1,4-naphthoquinone	-5.8(1)	All poses are buried deep and not near the insulin binding pockets	-4.9(9) -5.7(1)	-6.1(1)	Bound to the inhibitor binding pocket and buried deep; No H-bond formation	-5.4(1)

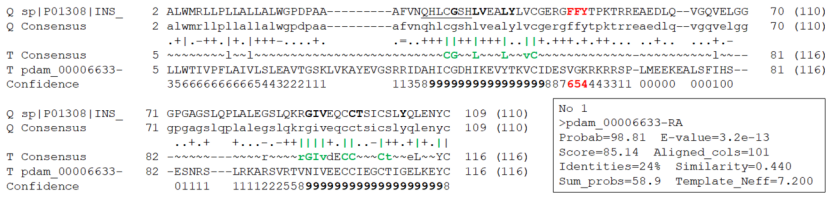
Drugs	Coral IR Extracellular Domain	Coral IR Extracellular Domain	Coral IR Extracellular Domain	Coral IR Cytoplasmic Domain	Coral IR Cytoplasmic Domain	Coral IR Cytoplasmic Domain
TLK 16998	-9.3(1) -9.1(2,3) -8.9(4,5)	Pose 1; Bonds with Gly310, Ile311, Arg309, Lys324, GLu323, Ile322, Asn326, Ser336, Ile349, and Cys287. Poses 1-5 are near insulin binding pockets. Poses 6-9 are buried deep and not near insulin binding pockets.	-10.3(1)	-9.2(1)	Close to the inhibitor binding pocket, not buried deep, H-bond with Asp1150.	-8.4(1)
TLK 19780	-9.2(1)	All poses are buried deep and not near the insulin binding pockets	-9.0(4) -10.3(1)	-9.1(1)	Close to the inhibitor binding site, not buried deep; H-bond with Arg997, and Asn1001	-11.7(1)
TLK 19781	-9.1(1)	All poses are buried deep and not near the insulin binding pockets	-8.7(3) -9.8(1)	-8.6(1)	Close to the inhibitor binding site, not buried deep in the pocket, H-bonds with Asn1083, and Asp1080	-7.7(1)
Ligands binding to other diabetes targets (negative controls)	**Ligands binding to other diabetes targets (negative controls)**	**Ligands binding to other diabetes targets (negative controls)**	**Ligands binding to other diabetes targets (negative controls)**	**Ligands binding to other diabetes targets (negative controls)**	**Ligands binding to other diabetes targets (negative controls)**	**Ligands binding to other diabetes targets (negative controls)**

Drugs	Coral IR Extracellular Domain	Coral IR Extracellular Domain	Coral IR Extracellular Domain	Coral IR Cytoplasmic Domain	Coral IR Cytoplasmic Domain	Coral IR Cytoplasmic Domain
Glimepiride	-8.3(1)	All poses are buried deep and not near the insulin binding pocket	-9.6(1)	-7.7(1)	Bound close to the inhibitor binding site, not buried deep in the pocket; H-bond with Leu999	-8.4(1)
Ursolic Acid	-8.7(1)	All poses are buried deep and not near the insulin binding pockets	-8.7(4) -8.9(1)	-8.0(1)	Bound away from the inhibitor binding pocket, not buried deep; no H-bond formation	-8.3(1)
Sitagliptin			-7.1(1,3)	-9.0(1)	Bound to the inhibitor binding site; H-bond with Gly1000	-7-2(1)
Diazoxide	-6.2(1)	All poses are buried deep and not near the insulin binding pockets	-6.0(1)	-6.0(1)	Bound to the inhibitor binding site; No-H bond formation	-6.0(1)
Metformin	-4.7(1)	All poses are buried deep and not near the insulin binding pockets	-4.2(1)	5.5(1)	Bound to the inhibitor binding pocket; H-bond formation with Thr1148, and Asp1144	-5.0(1)
Miglitol	-5.3(1)	All poses are buried deep and not near the insulin binding pockets	-5.0(1)	NA	Did not dock.	NA

Drugs	Coral IR Extracellular Domain	Coral IR Extracellular Domain	Coral IR Extracellular Domain	Coral IR Cytoplasmic Domain	Coral IR Cytoplasmic Domain	Coral IR Cytoplasmic Domain
Pioglitazone	-4.9(1)	All poses are buried deep and not near the insulin binding pockets	-5.7(7) -6.6(1)	-7.7(1)	Bound to the inhibitor binding site; H-bond with Thr1148	-6.5(1)
Repaglinide	-6.4(1)	All poses are buried deep and not near the insulin binding pockets	-7.2(1)	-6.3(1)	Away from the inhibitor binding site; H-bond formation Glu1044	-7.4 (1)

Figures

Figure 1. Remote sequence homology detection of insulin. Hhblits was used to align the human and pdam sequences. Shown in different colors are various residue motifs identified to be important for receptor binding by cryoelectron microscopy (Uchikawa et al., 2019).



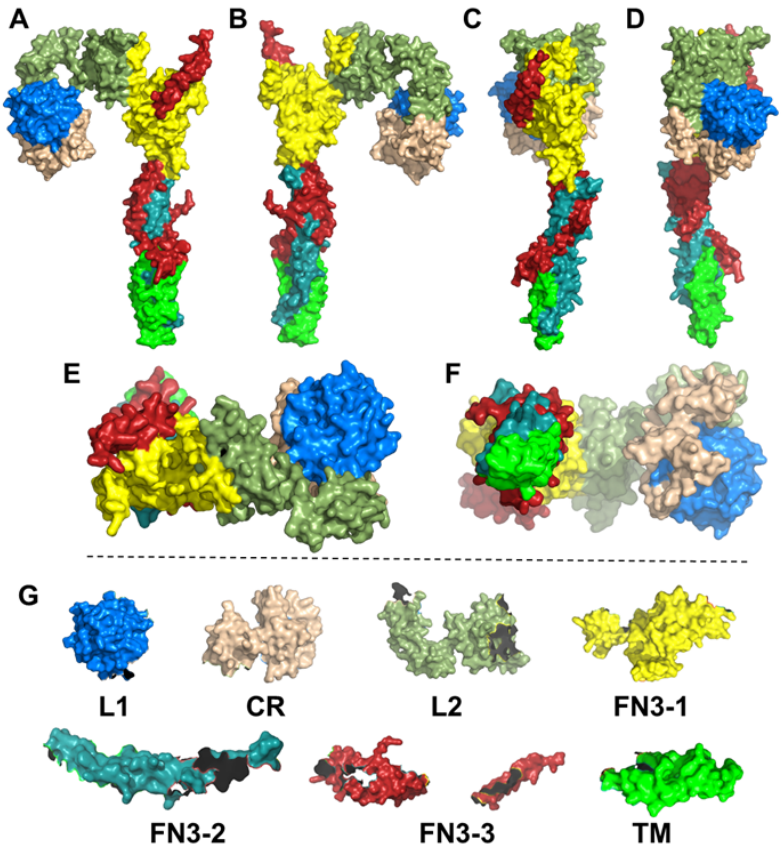


Fig.1. 3D reconstruction of Coral Insulin Receptor. (A) Side view-1 (B) Side view-2 (C) Side view-3 (D) Side view-4 (E) Top view (F) Bottom view (G) Individual domains of Coral IR

Figure 2. 3D reconstruction of Coral Insulin Receptor. (A) Side view-1 (B) Side view-2 (C) Side view-3 (D) Side view-4 (E) Top view (F) Bottom view (G) Individual domains of Coral IR

Figure 3. Conservation of insulin-related signaling pathways.

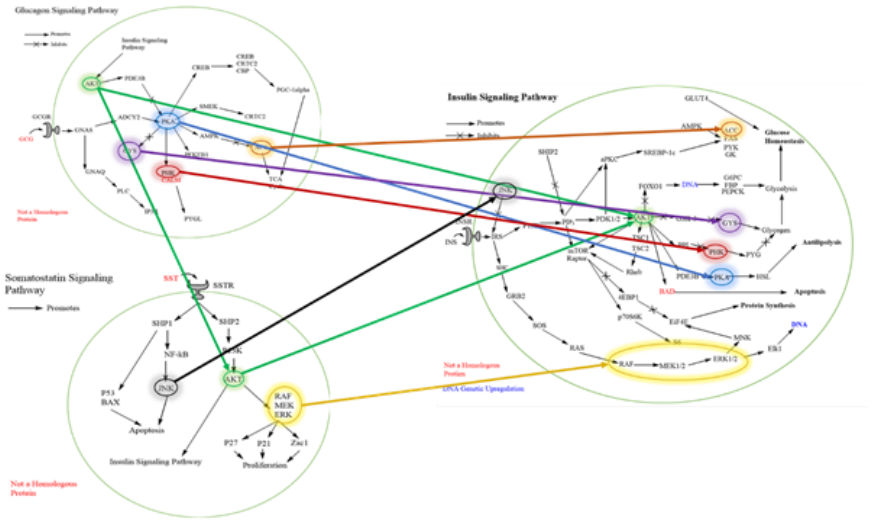
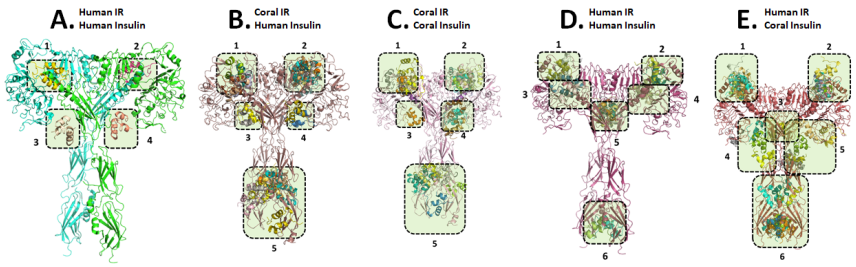
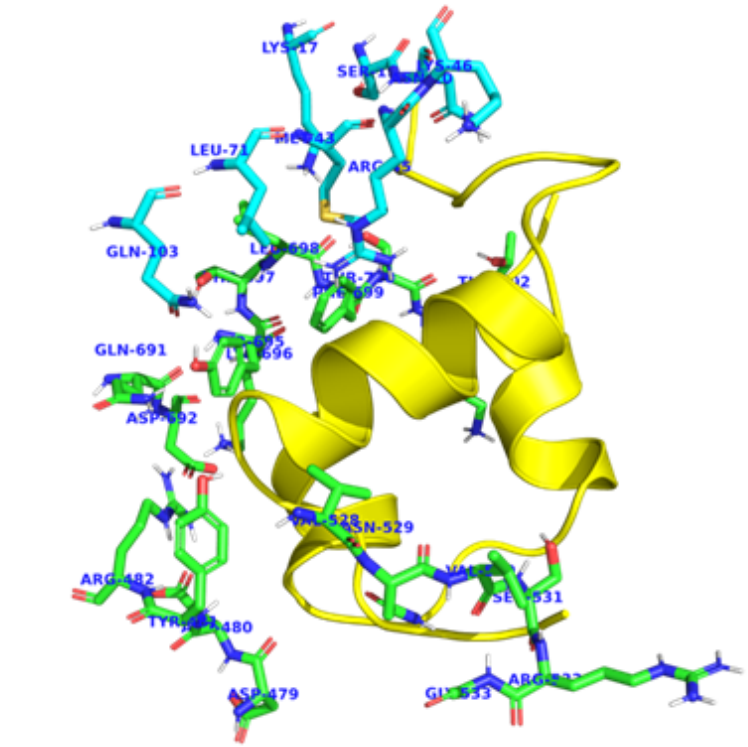


Figure 4. Swiss-homology model of human and pdam IR with locked ligand (human and coral insulin).



Coral Insulin binding site-1 (Top)



Coral Insulin binding site-2 (bottom)

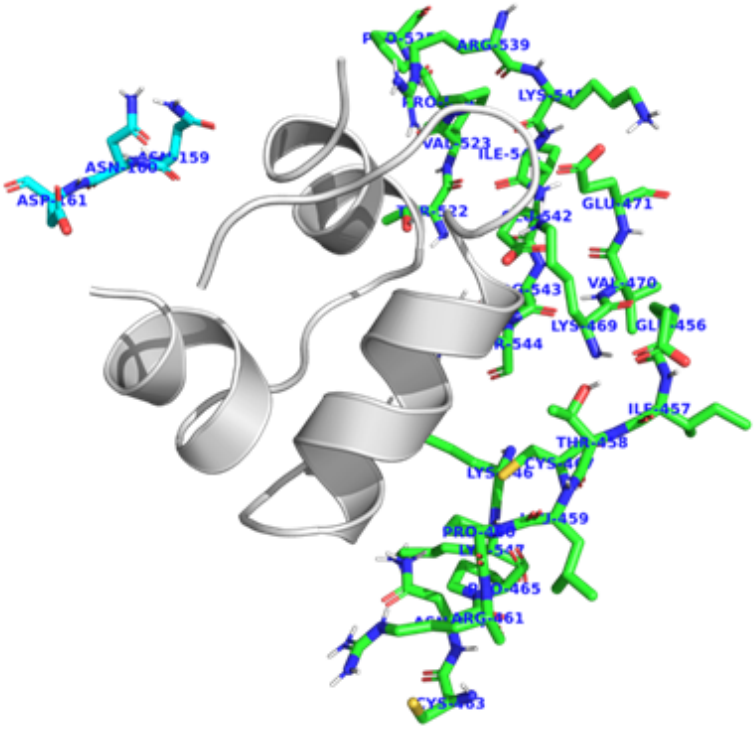
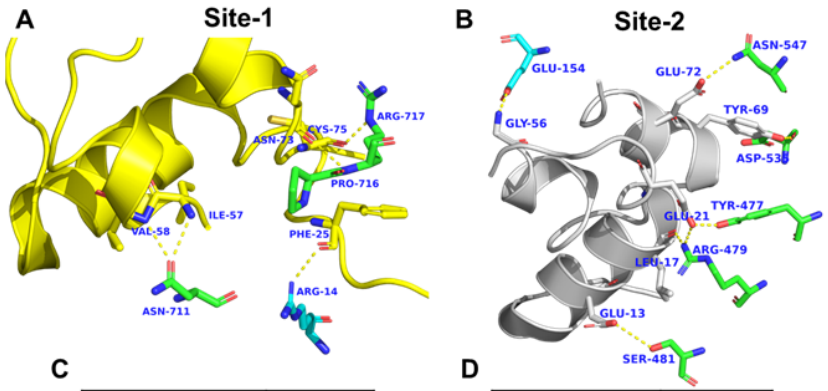


Figure 5. Mapping of coral insulin binding site in coral IR. Top: residues highlighted within 5Å of insulin at site-1 and at site-2 at the bottom.



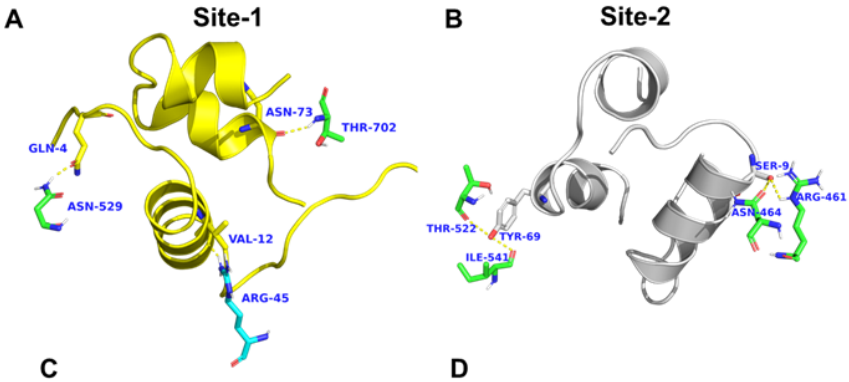
C

Human Insulin Site1	Human IR
Val58	Asn711
Ile57	Asn711
Phe25	Arg14
Asn73	Pro716
Cys75	Arg717

D

Human Insulin Site2	Human IR
Gly56	Glu154
Glu72	Asn547
Tyr69	Asp536
Glu21	Arg479
Leu17	Arg479
Glu13	Ser481

Figure 6. Mapping of H-bonds between human insulin and human insulin receptor (6pxv) (A) Polar contacts at site-1 (B) Polar contacts at site-2 (C) residues involved in H-bond formation at site-1 (D) residues involved in H-bond formation at site-2



C

Coral Insulin Site1	Coral IR
Gln4	Asn529
Val12	Arg45
Asn73	Thr702

D

Coral Insulin Site2	Coral IR
Tyr69	Thr522
Tyr69	Ile541
Ser9	Arg461
Ser9	Asn464

Figure 7. Mapping of H-bonds between coral insulin and coral insulin receptor (Model) (A)

Polar contacts at site-1 (B) Polar contacts at site-2 (C) residues involved in H-bond formation at site-1 (D) residues involved in H-bond formation at site-2.

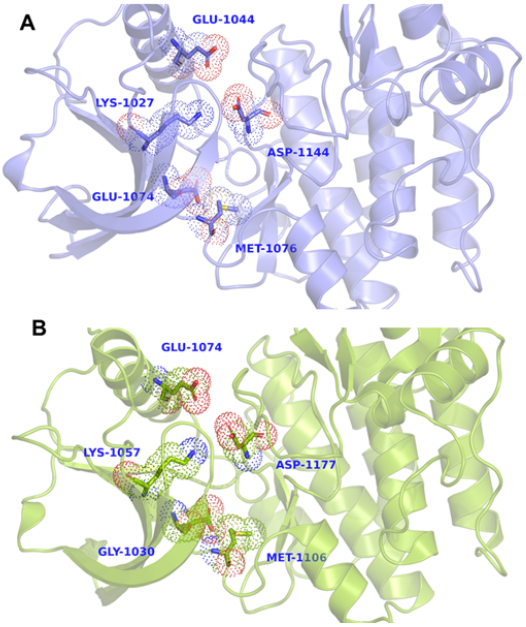


Figure 8 . Map of TK domain inhibitory binding pocket of coral IR and human IR (A) Coral inhibitory binding pocket (Model) (B) Human inhibitory binding pocket (5hhw)

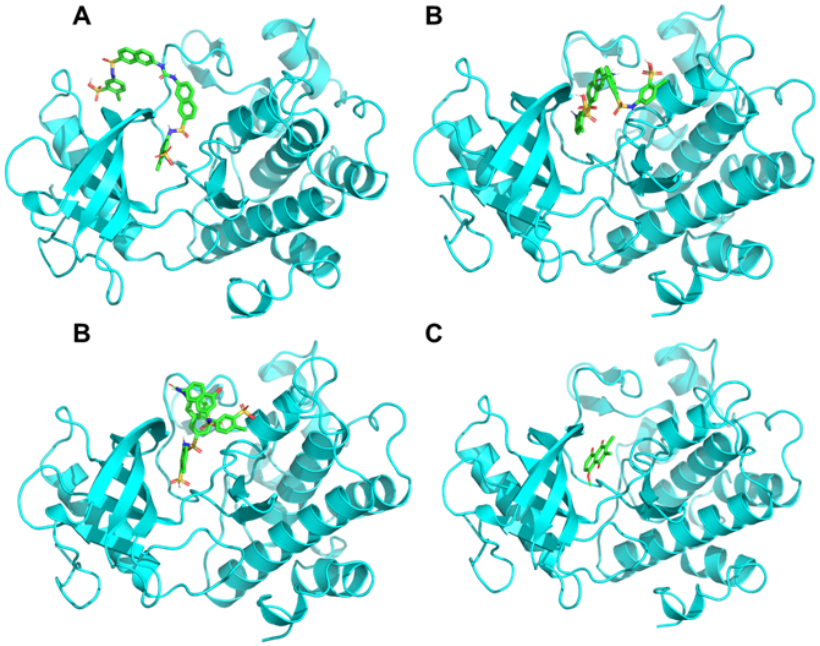


Figure 9. Binding conformations of drugs with coral tyrosine kinase domain with full protein selected as

the gridbox for docking. (a) TLK16998, (b) = TLK19780, (c) TLK19781, and (d) DDN.

References

Abou-Sabe', M., and Reilly, T. (1978). Insulin action on Escherichia coli. Regulation of the adenylate cyclase and phosphotransferase enzymes. Biochim. Biophys. Acta*542*: 442–455.

Baig, A.M., and Khaleeq, A. (2020). First Reports of Effects of Insulin, Human-like Insulin Receptors and Adapter Proteins in Acanthamoeba castellanii. Scientific Reports *10.*:

Gates, R.D., Hoegh-Guldberg, O., McFall-Ngai, M.J., Bil, K.Y., and Muscatine, L. (1995). Free amino acids exhibit anthozoan 'host factor' activity: they induce the release of photosynthate from symbiotic dinoflagellates in vitro. Proc. Natl. Acad. Sci. U. S. A. *92*: 7430–7434.

Gutmann, T., Kim, K.H., Grzybek, M., Walz, T., and Coskun, Ü. (2018). Visualization of ligand-induced transmembrane signaling in the full-length human insulin receptor. J. Cell Biol. *217*: 1643–1649.

Hoegh-Guldberg, O., Poloczanska, E.S., Skirving, W., and Dove, S. (2017). Coral Reef Ecosystems under Climate Change and Ocean Acidification. Frontiers in Marine Science *4.*:

Hughes, T.P., Anderson, K.D., Connolly, S.R., Heron, S.F., Kerry, J.T., Lough, J.M., et al. (2018). Spatial and temporal patterns of mass bleaching of corals in the Anthropocene. Science *359*: 80–83.

Kole, H.K., Muthukumar, G., and Lenard, J. (1991). Purification and properties of a membrane-bound insulin binding protein, a putative receptor, from Neurospora crassa. Biochemistry *30*: 682–688.

LaJeunesse, T.C., Parkinson, J.E., Gabrielson, P.W., Jeong, H.J., Reimer, J.D., Voolstra, C.R., et al. (2018). Systematic Revision of Symbiodiniaceae Highlights the Antiquity and Diversity of Coral Endosymbionts. Curr. Biol. *28*: 2570–2580.e6.

Le Roith, D., Shiloach, J., Roth, J., and Lesniak, M.A. (1980). Evolutionary origins of vertebrate hormones: substances similar to mammalian insulins are native to unicellular eukaryotes. Proc. Natl. Acad. Sci. U. S. A. *77*: 6184–6188.

LeRoith, D., Shiloach, J., Roth, J., and Lesniak, M.A. (1981). Insulin or a closely related molecule is native to Escherichia coli. Journal of Biological Chemistry*256*: 6533–6536.

McKern, N.M., Lawrence, M.C., Streltsov, V.A., Lou, M.-Z., Adams, T.E., Lovrecz, G.O., et al. (2006). Structure of the insulin receptor ectodomain reveals a folded-over conformation. Nature *443*: 218–221.

Menting, J.G., Whittaker, J., Margetts, M.B., Whittaker, L.J., Kong, G.K.-W., Smith, B.J., et al. (2013). How insulin engages its primary binding site on the insulin receptor. Nature *493*: 241–245.

Muthukumar, G., and Lenard, J. (1991). A preproinsulin-like pseudogene from Neurospora crassa. Mol. Cell. Endocrinol. *82*: 275–283.

Putnam, H.M., Barott, K.L., Ainsworth, T.D., and Gates, R.D. (2017). The Vulnerability and Resilience of Reef-Building Corals. Curr. Biol. *27*: R528–R540.

Remmert, M., Biegert, A., Hauser, A., and Söding, J. (2011). HHblits: lightning-fast iterative protein sequence searching by HMM-HMM alignment. Nat. Methods *9*: 173–175.

Trott, O., and Olson, A.J. (2009). AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. Journal of Computational Chemistry NA–NA.

Uchikawa, E., Choi, E., Shang, G., Yu, H., and Bai, X.-C. (2019). Activation mechanism of the insulin receptor revealed by cryo-EM structure of the fully liganded receptor-ligand complex. Elife *8.*:

Vizgaudis, W., Kumar, L., Olaosebikan, M., Roger, L., Brenner, N., Sledzieski, S., Yang, J.K., Lewinski, N., Singh, R., Daniels, N., Cowen, L. and Klein-Seetharaman, J. (2021) Insulin Signaling and Pharmacology in Corals. British Journal of Pharmacology, submitted.

Weis, F., Menting, J.G., Margetts, M.B., Chan, S.J., Xu, Y., Tennagels, N., et al. (2018). The signalling conformation of the insulin receptor ectodomain. Nat. Commun. *9*: 4420.

Ye, L., Maji, S., Sanghera, N., Gopalasingam, P., Gorbunov, E., Tarasov, S., et al. (2017). Structure and dynamics of the insulin receptor: implications for receptor activation and drug discovery. Drug Discov. Today *22*: 1092–1102.

Yuyama, I., Ishikawa, M., Nozawa, M., Yoshida, M.-A., and Ikeo, K. (2018). Transcriptomic changes with increasing algal symbiont reveal the detailed process underlying establishment of coral-algal symbiosis. Scientific Reports *8*.:

Gates, R.D., Hoegh-Guldberg, O., McFall-Ngai, M.J., Bil, K.Y., and Muscatine, L. (1995). Free amino acids exhibit anthozoan 'host factor' activity: they induce the release of photosynthate from symbiotic dinoflagellates in vitro. Proc. Natl. Acad. Sci. U. S. A. 92: 7430–7434.

Hoegh-Guldberg, O., Poloczanska, E.S., Skirving, W., and Dove, S. (2017). Coral Reef Ecosystems under Climate Change and Ocean Acidification. Frontiers in Marine Science 4.:

Hughes, T.P., Anderson, K.D., Connolly, S.R., Heron, S.F., Kerry, J.T., Lough, J.M., et al. (2018). Spatial and temporal patterns of mass bleaching of corals in the Anthropocene. Science 359: 80–83.

LaJeunesse, T.C., Parkinson, J.E., Gabrielson, P.W., Jeong, H.J., Reimer, J.D., Voolstra, C.R., et al. (2018). Systematic Revision of Symbiodiniaceae Highlights the Antiquity and Diversity of Coral Endosymbionts. Curr. Biol. 28: 2570–2580.e6.

Putnam, H.M., Barott, K.L., Ainsworth, T.D., and Gates, R.D. (2017). The Vulnerability and Resilience of Reef-Building Corals. Curr. Biol. 27: R528–R540.

Trott, O., and Olson, A.J. (2009). AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. Journal of Computational Chemistry NA–NA.

Ye, L., Maji, S., Sanghera, N., Gopalasingam, P., Gorbunov, E., Tarasov, S., et al. (2017). Structure and dynamics of the insulin receptor: implications for receptor activation and drug discovery. Drug Discov. Today 22: 1092–1102.

Yuyama, I., Ishikawa, M., Nozawa, M., Yoshida, M.-A., and Ikeo, K. (2018). Transcriptomic changes with increasing algal symbiont reveal the detailed process underlying establishment of coral-algal symbiosis. Scientific Reports 8.: