Supplementary Materials: Understanding Insulin Endocrinology in Decapod *Crustacea*: Molecular Modelling Characterization of an Insulin-Binding Protein and Insulin-Like Peptides in the Eastern Spiny Lobster, *Sagmariasus verreauxi*

Figure S1: Modelling criteria for (a) Sv-IGFBP; (b) Cq-IGFBP_N'; (c) Sv-IAG; (d) Sv-ILP1; (e) Sv-ILP2. In each case the trimmed modelled sequence is given (subscript numbering in brackets indicating the native amino acid position) with the following associated details listed: (i) model templates; (ii) input sequence alignments; (iii) secondary structure predictions and resulting additional restraints (implemented due to deviation from the chosen template), also shown in sequence in red bold; and (iv) defined bonds and any additional refinement. Key as shown, colouration as previously described.

Alpha helix	**(Hh)**
3_{10} helix	**(Gg)**
Pi helix	**(Ii)**
Beta bridge	**(Bb)**
Extended strand	**(Ee)**
Beta turn	**(Tt)**
Bend region	**(Ss)**
Random coil	**(Cc)**
Removed in refinement	**(~~strike~~)**

(a) Sv-IGFBP

$_{(21)1}$~~QDVVT~~TECGECDRSNCPEVKTCLGGKVQDACGCCEVCALGLGQRCDSQDSGDSTDYGSCGEYLVCRTRTDTG
GTDEATCVCENPDPVCGSDGITYSTLCHLLQETTEKPDVFVAVRGPCKGVPVIKSKPEDKIRPLGSILVLDCEAA
GYPVPEITWELNRPDGSTMKLPGDDSSFAVQVRGGPEDHMVTGWVQIMRITKKSLGIYTCVATNTEGETRVSATV
ALKEHGEKEDSMNKL$_{236(256)}$

(i) Model template selections

Insulin-binding + kazal domains: HTRA1 (3TJQ_A)

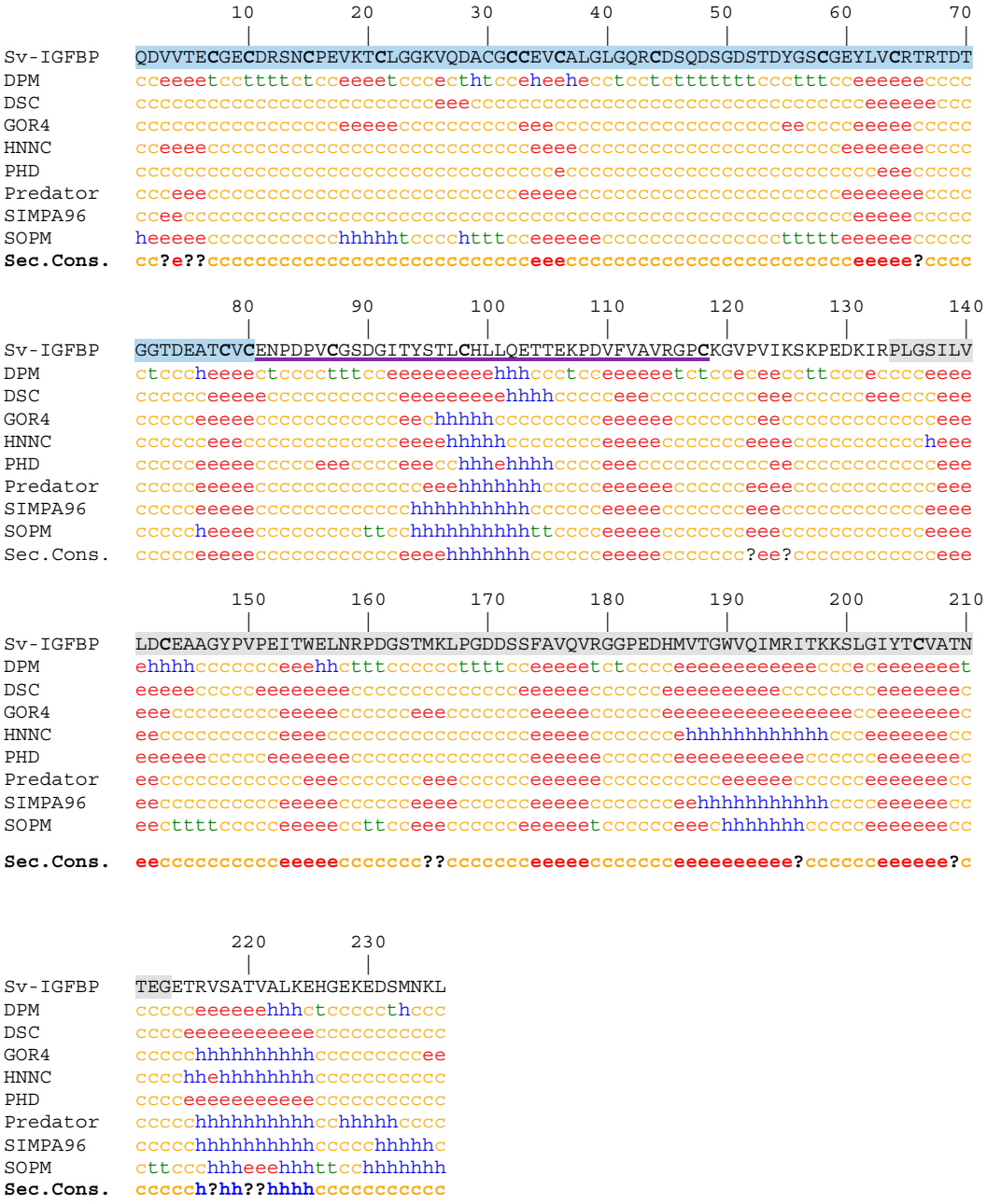
Immunoglobulin domain: Myosin light chain kinase (2CQV_A)

(ii) Input sequence alignments

```
Sv-IGFBP      1 QDVVTECG-ECDRSNCPEV-KTCLGGKVQDACGCCEVCALGLGQRCDSQDSGDSTDYGSCGEYLVCRTRT  68
3TJQ_A       13 FQGSAGCPDRCEPARCPPQPEHCEGGRARDACGCCEVCGAPEGAACGLQ-------EGPCGEGLQCVVPF  75
3TJQ SS Pred    ccCCCCCCCCCChhhCCCCCCCCCcccCCCCCcCccccccCCCCCCC-------CCCCCCCCeeCCC

Sv-IGFBP     69 D-------TGGTDEATCVCENPDPVCGSDGITYSTLCHLLQET-TE----KPDVFVAVRGPCKGV       121
3TJQ_A       76 GVPASATVRRRAQAGLCVCASSEPVCGSDANTYANLCQLRAASRRSERLHRPPVIVLQRGACGNS       140
3TJQ SS Pred    CCCccHHHHHccCCeEeecccCCeeecCCcEECCHHHHHHHHHHcccCCCCeEEeeecCCCCC

Sv-IGFBP    122 PVIKSKPEDKIRPLGSILVLDCEAAGYPVPEITWELNRPDGSTMKLPGDDSSFAVQVRGGPEDHMVTGWV  191
2CQV_A        8 PQIIQFPEDQKVRAGESVELFGKVTGTQPITCTWMKFRKQIQ------ESEHMKVENSEN------GSKL  65
2CQV SS Pred    CeEeeCCCceEEeCCCcEEEEEEEecCCCEEEEEeCCeecc------ccccEEEEEcCC------ceEE

Sv-IGFBP    192 QIMRITKKSLGIYTCVATNTEGETRVSATVALKEHGEKEDSMNKL                        256
2CQV_A       66 TILAARQEHCGCYTLLVENKLGSRQAQVNLTVVDKPDPPAGTPSG                        110
2CQV SS Pred    EEecchhhCEEEEEEEEecCcccEEEEEEEEecCCCCCCCCCCC
```

(iii) Additional secondary structure restraints

Secondary structure predictions fit model templates, no restraints applied.



(iv) Defined bonds and refinement

Insulin-binding domain: C_7-C_{30}; C_{10}-C_{32}; C_{15}-C_{33}; C_{21}-C_{36}; C_{58}-C_{78}; C_{44}-C_{64}; C_{98}-C_{80}

Kazal and immunoglobulin domain: C_{118}-C_{87}

Immunoglobulin domain: C_{143}-C_{206}; Cis-peptide bond P_{149}

$_{1}$QDVVT$_{5}$ removed during refinement due to steric clashes.

(b) Cq-IGFBP_N′

(25)1 TCGECDRSKCAVTASCPGGLVLDTCGCCEVCARGLGQLCENTTTEDTPTTTHYGVCGQYLVCRTRTDTGGT GEATCECEDSGAVCGSDGVTYTTLCHLLMETAERPDLSVAVRGPCKTAPVIKSKPEDKIR 131 (155)

(i) Model template selections

Insulin-binding + kazal domains: HTRA1 (3TJQ_A)

(ii) Input sequence alignments

```
Cq-IGFBP_N’    1 TCG-ECDRSKCAVT-ASCPGGLVLDTCGCCEVCARGLGQLCENTTTEDTPTTTHYGVCGQYLVCRTRTD-  67
3TJQ_A        18 GCPDRCEPARCPPQPEHCEGGRARDACGCCEVCGAPEGAACGLQ----------EGPCGEGLQCVVPFGV  77
3TJQ SS Pred     CCCCCCChhhCCCCCCCCCCcccCCCCCcCcccccccCCCCCCC----------CCCCCCCCceeCCCCC

Cq-IGFBP      68 ------TGGTGEATCECEDSGAVCGSDGVTYTTLCHLLMETA-E----RPDLSVAVRGPCKTA        119
3TJQ_A        78 PASATVRRRAQAGLCVCASSEPVCGSDANTYANLCQLRAASRRSERLHRPPVIVLQRGACGNS        140
3TJQ SS Pred     CcchhhhhhccCCeEeeccccCCeeecCCcEECCHHHHHHHHHhccccCCCCeEEeeecCCCCC
```

(iii) Additional secondary structure restraints

Secondary structure predictions fit model template, no restraints applied.

```
                       10        20        30        40        50        60        70
                        |         |         |         |         |         |         |
Cq-IGFBP_N’TCGECDRSKCAVTASCPGGLVLDTCGCCEVCARGLGQLCENTTTEDTPTTTHYGVCGQYLVCRTRTDTGG
DPM        ctccttttceheeccttcceeeececceeheehccccchtcccccccccccccceteeteeeeeeeeccccctt
DSC        cccccccccccccccccccccccccccccccccccccccccccccccccccccccccceeeeccccccc
GOR1       eettttttheeeeeeetteeeeettttteeeehtttceeeeeccсttcttееееееееееееееееeeettttct
GOR3       cccccccccceeccccccccceeccccccccccccccceeeeccccccccceeeeceeceeeeeeeeecccc
HNNC       ccccccccceeeeecccccceeeecccccceechccccccccccccccccccceeeeeceeeeeeeecccccc
MLRC       cccccccccccccccccccceeeccccccccccccccccccccccccccccccccccceeeeecccccc
PHD        cccccccccccccccccccceeccccccccceeeccccccceccccccccccccccccccccccceeeecccccc
Predator   cccccccccceeeecccccceeeccccceeecccccccccccccccccccccceeeccccceeeeeeecccccc
SOPM       ccccccccceeeeeccttceeeeccccchhhhhhhhhhcccccccccccceeeeeeeeeeeeccccccc
Sec.Cons.  ccccccccceeeecccccceeeccccccceeccccccccccccccccccccccceeceeceeeeeeeecccccc

                       80        90       100       110       120       130
                        |         |         |         |         |         |
Cq-IGFBP_N’TGEATCECEDSGAVCGSDGVTYTTLCHLLMETAERPDLSVAVRGPCKTAPVIKSKPEDKIR
DPM        ccchchhtttccсetttcceeeeeeeeeeehhhhhcccceeeeeetcccccceeccttccccc
DSC        cccceeeeeccccccccccccccceeeehhhhhhccceeeeccccccccceeecccccccc
GOR1       ccceeehhhhhhheeeetteeeeeeehhhhhhhhhhhheeeeeetteeeeeeeehhhhhhh
GOR3       ccceeeccccccccceeeccccceeehhhhhhcchccccccceeeccccccccccccccccchhh
HNNC       ccccccccccccccccccccceeeeeehhehcccccccceeeeeccccccccceeccccccccc
MLRC       ccceeeeecccccecccccccchhhhhhhhhhhhcccceeeeeccccccccceeecccccccc
PHD        ccceeeeeccccceeeccccccceehhhheeeeeccccceeeeeecccccccccceeecccccccc
Predator   cccceeeccccccccccccccchhhhhhhhhhhcccccceeeeeccccccccccccccccccccc
SOPM       ccccceehhccttteeeccttceeehhhhhhhhhttcccceeeecccccccceeeccccccce
Sec.Cons.  ccc?eee?cccccce?cccccccee?hhhhhhhhhcccc?eeeeccccccccceeecccccccc
```

(iv) Defined bonds and refinement

Insulin-binding domain: C_2-C_{25}; C_5-C_{27}; C_{10}-C_{28}; C_{16}-C_{31}; C_{39}-C_{62}; C_{56}-C_{76}; C_{78}-C_{96}

Kazal domain: C_{85}-C_{116}

(c) Sv-IAG

```
(23)1YNVSGLSEDFECGDFENVLGRICAETQSNIVRDTRSVSTVAVADSTHGGTDPSRRPYHHPRAIQVVLRHAA
NPPATQGAGAEEGVRVTSEAAFSLVKSRSIRDTRETNLQDECCPFPLVHCDKEEILHYCFLTEG135(157)
```

(i) Model template selections

Insulin: (2KQP_A)

ii) Input sequence alignments

```
Sv-IAG          7 SEDFECG-DFENVLGRICAETQSNIVRDTRSVSTVAVADSTHGGTDPSRRPYHHPRAIQVVLRHAANPPA  69
2KQP_A          9 VNQHLCGSDLVEALYLVCGERGFFYTKP------------------TRREAEDLQVGQVE---------  50
2KQP SS Pred      CCccccchhHHHhHHHHhcCcCeecCCC-----------------Ccccchhhhccccc---------

Sv-IAG         70 TQGAGAEEGVRVTSEAAFSLVKSRSIRDTRETNLQDECCPFPLVHCDKEEILHYCF              125
2KQP_A            -LGGGPGAGSL----QPLAL----EGSLQKRGIVEQCC---TSICSLYQLENYCN               86
2KQP SS Pred      -cCCCCCCccc----hhHHh----hccccCCchHHhc---CCCCCHHHHHHhcc
```

(iii) Additional secondary structure restraints

Additional restraints applied based on secondary structure predictions

C-peptide (later removed): A_{62}-R_{68}; G_{80}-A_{92}: helix restraint

```
                  10        20        30        40        50        60        70
                   |         |         |         |         |         |         |
Sv-IAG      YNVSGLSEDFECGDFENVLGRICAETQSNIVRDTRSVSTVAVADSTHGGTDPSRRPYHHPRAIQVVLRHA
DPM         ctecccteehhtcchcceechehhhhhhcceeeceeeeeeeeehctctctctttccccccchheeeeehhh
DSC         ccccccccccccccccccccccccccccccccccccceeeeeeecccccccccccccccccchheehhc
GOR1        eeecccchhhhhhhhhhhhhheeehhtteeeeeeeeeeeeeeeeeettttccctteettcccthheeeeeec
GOR3        eecccccccccccchcceeeeehccccceeeeeeeceeeeeeeecccccccccccccccchhheehhhc
HNNC        ccccccccccccchhhhheeeecccccceeecccceeeeeeecccccccccccccccchhhhhhhhhc
MLRC        ccccceecccccchhhhhhhhhhcccccccccccccceeeeecccccccccccccccchheehhhc
PHD         cceeeeeecccccchhhhhhhhhcccccccccccccccceeecccccccccccccccceeeeeecc
Predator    ccccccccccccccchhhhhhhcccccccccccccceeeeeecccccccccccccccchhhhhhhhhc
SOPM        echhcchhcttchhhhhhhhhhhcchheecttceeeeeeeccccccccccccccchhheeeeecc
Sec.Cons.   ccccccccccccchhhhhh?hhccccceecccccceeeeeecccccccccccccccc?hhheeehhc

                  80        90       100       110       120       130
                   |         |         |         |         |         |
Sv-IAG      ANPPATQGAGAEEGVRVTSEAAFSLVKSRSIRDTRETNLQDECCPFPLVHCDKEEILHYCFLTEG
DPM         htccccctcchhhceeeehhhhhhheethеehcchhcchhthccccceehhhhhhhhheeeeecc
DSC         ccccccccccccceeeehhhhhhhhhhhhhhhcccccccccccccccccchhhhhhheecccc
GOR1        ccccceeecchhhhhhhhhhhhhhhhhceeeeectttttttttetehhhhhhhhhhhhhhhhhht
GOR3        ccccchhcccceeeeeeehhhhhhheehcececccccccccccccccccccchhhhehhecccc
HNNC        cccccccccccchhhhhhhhhhhhhhhhcccccccccccccccccccccchhheeeeeeeccc
MLRC        ccccccccccchchchhhhhhhhhhhhhcccccccccccccccccccccchhhhhhhhhccc
PHD         ccccccccccceceeeechhhhhhhhhcccccchhhcccccccccccccccchhhhhhhecеecc
Predator    ccccccccccccccchhhhhhhhhhcccccchhhhhhcccccccccccccccceeeeeeccc
SOPM        cccccccccccchttceeehhhhhhhhhcccccccccccccccccceeecctthhhhhhhhhct
Sec.Cons.   cccccccccccchc?eee?hhhhhhhhh?ccccccccccccccccccccccchhhhhhheeeccc
```

(iv) Defined bonds and refinement

C_{12}-C_{114}; C_{23}-C_{130}; C_{113}-C_{121}

(d) Sv-ILP1

```
24LEPDLISQIESRTEKEWQELWTEERLTLCRSRLRHNLDAICGKDVYRRSSMLPPRTRHRRWSRAKRNTDIFLE
VHDTDTARGDSRKKEKRMKTMSVDLPTTRIEISPSVPDTGQHSTHTRSPFLSVHQANLFVTTWVGGHHRHRRQSP
SITSECCTTVGCTWEEYAEYCPTSSRLRPGVTLI205
```

(i) Model template selections

Insulin: (2KQP_A)

(ii) Input sequence alignments

```
Sv-ILP1       24 ERLTLCRSRLRHNLDAICGKDVYRRSSMLPPRTRHRRWSRAKRNTDIFLEVHDTDTARGDSRKKEKRMKT  93
2KQP_A         2 VNQHLCGSDLVEALYLVCGERGFFYTKP----TR-------REAEDLQVGQVEL----------------  44
2KQP SS Pred     CCccccchHHHHHHHHHHcCcCCCCCCC----Cc-------cccHHHHccccc----------------

Sv-ILP1       94 MSVDLPTTRIEISPSVPDTGQHSTHTRSPFLSVHQANLFVTTWVGGHHRHRRQSPSITSECCTTVGCTWE  163
2KQP_A        45 ----------------------GGGPGAGSLQPL------------ALEGSLQKRGIVEQCCT-SICSLY  79
2KQP SS Pred     ----------------------CCCCCCcccHHH------------HHhccccCCcHHHHcC-CCCCHH

Sv-ILP1      164 EYAEYCP                                                               170
2KQP_A        80 QLENYCN                                                               86
2KQP SS Pred     HHHHHcC
```

(iii) Additional secondary structure restraints

Additional restraints applied based on secondary structure predictions

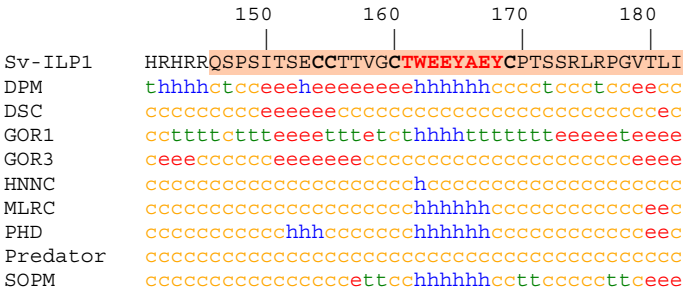
B-chain: P_3-C_{41}: helix restraint

A-chain: T_{162}-Y_{168}: helix restraint

C-peptide (later removed): R_{60}-R_{66}; S_{84}-M_{94}: helix restraint

NB: note the highly disordered nature of the A-chain, predicted as random coil

```
                    10        20        30        40        50        60        70
                    |         |         |         |         |         |         |
Sv-ILP1     LEPDLISQIESRTEKEWQELWTEERLTLCRSRLRHNLDAICGKDVYRRSSMLPPRTRHRRWSRAKRNTDI
DPM         ctcchheeeehhhhhhhhhhhhhhhhheeehhhhhhchhhectcceeeeccccccchhhhhhhhhhtcce
DSC         ccccceeeeeccccccchhhhhhccccccccchhhhheeeccccccccccccccccccchhhhhcccccce
GOR1        hhhhhhhhhhcccchhhhhhhhhhhhhhhhhhheettteeeeeeeeeeeeetcccttteeetttctttttccee
GOR3        ccccchhhhhhhhhhhhhhhhhhhhhhhhhhhhhcccccchhccccececccccccceeeeeehhcccchhh
HNNC        ccccchhhecccccchhhhhhhhhhhhhhhhhhhhhcccccheccccchhhcccccccccchhhhhhhhcccce
MLRC        ccchhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhcccchcccccceecccccccccccccchhhcccee
PHD         ccccceecccccchhhhhhhhhhcccchhhhhhhhhhhhhhhcccceeecccccccccccccccccccccce
Predator    ccccchhhhhhhhhhhhhhhhhhhhhhhhhhhhhchhhhhccccccccccccccccccccchhhhhhhhhhhe
SOPM        ccthhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhttcceeecccccccccccchhhhcccccee
Sec.Cons.   ccccchhhhhhhhhhhhhhhhhhhhhhhhhhhhhhh?hch?ccccceecccccccccccchhhhhh?cccce

                    80        90        100       110       120       130       140
                    |         |         |         |         |         |         |
Sv-ILP1     FLEVHDTDTARGDSRKKEKRMKTMSVDLPTTRIEISPSVPDTGQHSTHTRSPFLSVHQANLFVTTWVGGH
DPM         eehehccccttttcccchhhhheeeecccceeehheecccccctccccccccceeeehhhheeeeeeeccc
DSC         eeeeccccccccccchhhhheeeeeecccceeeeecccccccccccccccccceeecccceeeeeeeccc
GOR1        eeehhhhhhhhhhhhhhhhhheeeeecceeeeeeetccctctteeeeetcctteeeehhheeeeeeectcc
GOR3        ehheccccchhcccchhhhhhhhhhecccccccceeeecccccccceeceeceeeehhhhhhheeeeeeecc
HNNC        eeeecccccccccccchhhhccceeecccccceeeecccccccccccccccccceeecccceeeeeeeccc
MLRC        eeeeecccccccccchhhhhhhhhhhcccccccceccccccccccccccccceeeecccceeeeecccccc
PHD         eecccccccccccccccccccccccccccccccccccccccccccccccceeeecchhheeeeeccccc
Predator    eeeecccccccccchhhhhhhhhhcccccccceeeecccccccccceeecccccccccccccceeeecccc
SOPM        eeeeecccccttcchhhhhhhheeeecccccceecccccccccccccccceeeecttteeeeeeetcc
Sec.Cons.   eeeecccccccccchhhhhhhheeecccccceeeecccccccccccccccceeeec??ceeeeeeeccc
```



(iv) Defined bonds and refinement

C_{29}-C_{155}; C_{41}-C_{169}; C_{154}-C_{160}

(e) Sv-ILP2

$_{21}$RPYEETRSYKI**C**TSRDVKVMANYV**C**NLHRRRRSVLSLDDARDNYGVPGLLLENRSRRLALPQHWRPEDDTDTG
NVSRRDPSFLQFTRIRRQVLLGEIRKQ**CC**VHGCTPRDFYGA**C**Q$_{136}$

(i) Model template selections

Insulin: (2KQP_A)

(ii) Input sequence alignments

```
Sv-ILP2        8 SYKICTSRDVKVMANYVCNLHRRRRSVLSLDDARDNYGVPGLLLENRSRRLALPQHWRPEDDTDTGNVSR   77
2KQP_A         3 NQHLCGSDLVEALY-LVC-------------------GERGFFYTKPTRR--------EAEDLQVGQVEL   44
2KQP SS Pred     CccccchHHHHHHHHHc-------------------CcCeecCCCCcc--------cchhhhccccccC

Sv-ILP2       78 R---DPSFLQFTRIRRQVLLGEIRKQCCVHGCTPRDFYGAC                                115
2KQP_A        45 GGGPGAGSLQPLALEGSLQKRGIVEQCCTSICSLYQLENYC                                85
2KQP SS Pred     CCCCCcccCCcHhhccccccCCchHHHcC-CCCCHHHHHHHc
```

(iii) Additional secondary structure restraints

Additional restraints applied based on secondary structure predictions

B-chain: V_{17}-R_{29}: helix restraint

A-chain: Q_{100}-V_{103}; P_{108}-F_{111}: helix restraint

C-peptide (later removed): N_{53}-A_{59}: helix restraint

NB: note the very short helix of the alpha chain won't make a full helix, rather an alpha turn

```
                  10        20        30        40        50        60        70
                   |         |         |         |         |         |         |
Sv-ILP2     RPYEETRSYKICTSRDVKVMANYVCNLHRRRRSVLSLDDARDNYGVPGLLLENRSRRLALPQHWRPEDDT
DPM         tcchhchceeeeeceeehehhheeechhhhhheeehhhthtttccccchhhthhhhhhhcchccctccc
DSC         ccccccccceeeccccceeeeehhcchhccccceeeccccccccccccceeehhhhhhhcccccccccccc
GOR1        cchhhhttteeeeeeheeeeehhheeeeeeeeeeeeeeeeeettteeeeeeehhhhhheeecttcccttttt
GOR3        ccccecceeeeeeccccceeeehhhecccccceeeeeecccccccccceeehhhhhhhhhcccccccccccc
HNNC        cccccccceeeeeecccchhhhhhhhhhhhcccceeeecccccccccccceeecccccecccccccccccc
MLRC        cccccccceeeecccchhhhhhhhhhhhhhhhcccceeeeecccccccccceeecccccccccccccccccc
PHD         cccccccceeeeeeeecehhhhhhhhhhcccceeeeeecccccccccceeeeecccccccccccccccccc
Predator    ccccccccccccccccceeeecccccccccccccccccccccccccccccchhhhhhhhcccccccccccc
SOPM        ccttcccceeeeccchhhhhhhhhhhhhhhhhtcceeeeehhhttttcccteeecccccccccccccccccc
Sec.Cons.   ccccccc?eeeeccccccehhhhhh?hhcccceeeeecccccccccccceeeh?hhhhhccccccccccccc

                  80        90       100       110
                   |         |         |         |
Sv-ILP2     DTGNVSRRDPSFLQFTRIRRQVLLGEIRKQCCVHGCTPRDFYGACQ
DPM         ccctettcccchhheehehhheehchhhhheeecccccccccchcc
DSC         ccccccccccchhhhhhhhhhhhccccceeeecccccccccccccc
GOR1        tttteeetectteeeeeeeeeeeeethhhteeeettteettttttteee
GOR3        ccccceccccchheehhhhhhheeccccceeeecccccccccccccee
HNNC        ccccccccccchhhhhhhhhhhhhhhhchceecccccccccccccc
MLRC        ccccccccccchhhhhhhhhhhhhhhhhhcccccccccccccccc
PHD         ccccccccccccchhhhhhhhhhhhhhhhhheeecccccccccccc
Predator    ccccccccccccchhhhhhhhhhhhhhhhhhcccccccccccccccc
SOPM        ccccccccccceeeehhhhhhhhhhhhhcttceettccccchechhh
Sec.Cons.   ccccccccccchhhhhhhhhhhhhhhhhh?eecccccccccccccc
```

(iv) Defined bonds and refinement

C_{12}-C_{102}; C_{25}-C_{115}; C_{101}-C_{106}

Figure S2. PDB predicted interactions of A and B-chains of IAG, ILP1 and ILP2. Interaction map describing residue specific interactions between A- and B-chains of each ligand. Standard amino acid abbreviations are used, with colours indicating physicochemical properties as follows: blue- positive, red- negative, green- neutral, grey- aliphatic, mauve- aromatic, orange- proline and glycine, yellow- cysteine. Number of interacting residues given in brackets.

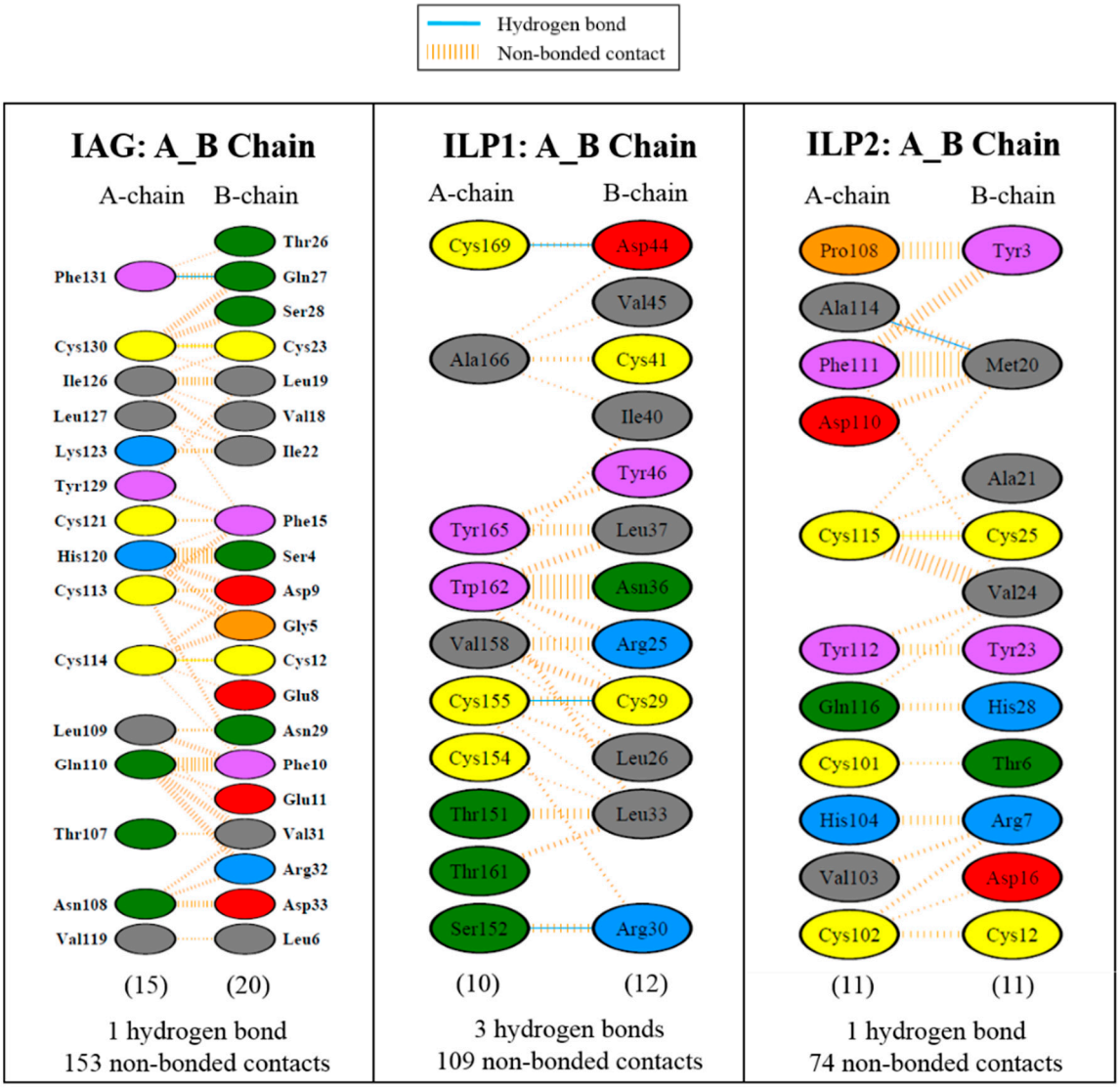


Figure S3. HADDOCK2.2 molecular docking as assessment of reliability of complex formation. Structural alignment of Sv complexes (darker shade) with the best-fit models generated by Haddock (lighter shade), shown in ribbon format. Good alignment suggests reliability of our generated complexes.

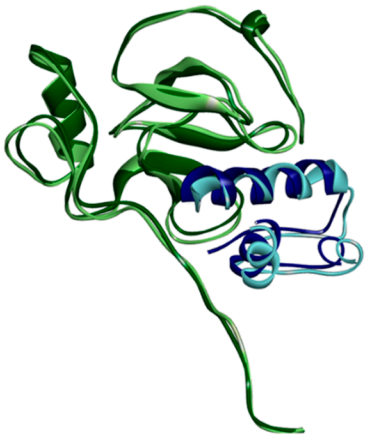
IGFBP_N' - IAG	IGFBP_N' - ILP1	IGFBP_N' - ILP2
		
Aligned with Haddock complex 138/174 from 3/6 ranked cluster	Aligned with Haddock complex 1/150 from 1/13 ranked cluster	Aligned with Haddock complex 22/171 from 3/12 ranked cluster

Figure S4: Electrostatic potential surface of human IGFII (PDB: 2L29). Binding orientation depicted, with the binding interface bracketed. Surface is coloured by potential on the solvent accessible surface on a scale of -kT/e (red) to +kT/e (blue), as indicated by the scale bar.

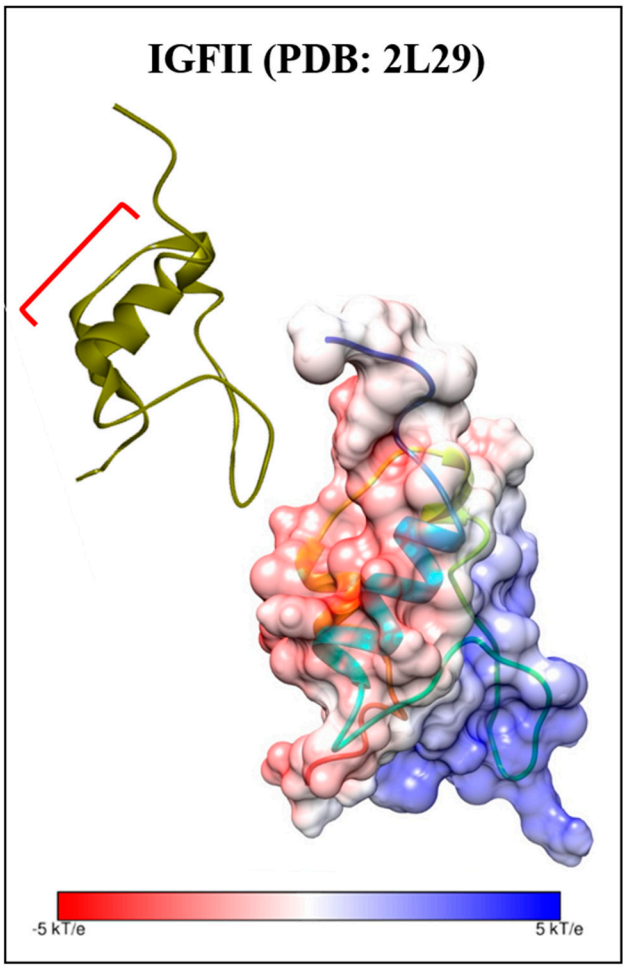


Table S1: PRODIGY predicted interactions of Sv-IGFBP_N' and all ligands, complementary to PDBsum predictions. a) All residue contacts of IGFBP_N' (I chain) and ligand (A, orange and B, blue chains) listed with text in the corresponding colours. Text box colouration is used to represent the physicochemical properties (red- negative, green- neutral, grey- aliphatic, orange- proline and glycine, yellow- cysteine) of the consistently interacting contacts of Sv-IGFBP_N'. The number of predicted contacts of these residues is given, with the average as indication of the weighted importance of the residue. b) Comparison of interaction contacts predicted by PRODIGY and PDBsum and the PRODIGY description of the physicochemical nature of these contacts. c) Summary of the consistently interacting contacts of the IGFBP_N' interface, predicted by both PRODIGY and PDBsum. 'All' indicates a conserved prediction of an interaction with all three ligands by both PRODIGY and PDBsum, otherwise the ligand(s) with which PDBsum predicts an interaction is shown.

(a)

IAG - IGFBP_N'	n contact	ILP1 - IGFBP_N'	n contact	ILP2 - IGFBP_N'	n contact	Consistent	Average n contacts
ALA 49 I ALA 24 B		ALA 49 I ASP 38 B		ALA 49 I ASN 22 B			
ALA 49 I ASN 17 B		ALA 49 I GLY 42 B		ALA 49 I ASN 26 B			
ALA 49 I GLU 25 B	3	ALA 49 I HIS 35 B	3	ALA 49 I CYS 25 B			
ASP 65 I ARG 21 B	1	ASP 68 I ARG 32 B	1	ALA 49 I LYS 18 B	4	**ALA 49**	3.3
ASP 68 I ASP 14 B	1	ASP 71 I ALA 39 B		ARG 87 I ARG 1 B	1		
ASP 71 I ARG 21 B		ASP 71 I ARG 32 B		ASP 48 I ASN 26 B	1		
ASP 71 I ASN 17 B		ASP 71 I ARG 34 B		ASP 65 I ARG 15 B			
ASP 71 I ASP 9 B		ASP 71 I ASN 36 B		ASP 65 I ASN 22 B	2		
ASP 71 I ASP 14 B		ASP 71 I ASP 38 B		ASP 68 I ARG 15 B	1	**ASP 68**	1
ASP 71 I GLU 16 B		ASP 71 I HIS 35 B		ASP 71 I ARG 15 B			
ASP 71 I GLY 20 B		ASP 71 I LEU 33 B		ASP 71 I ASN 22 B			
ASP 71 I LEU 19 B		ASP 71 I SER 31 B		ASP 71 I ASP 16 B			
ASP 71 I PHE 15 B		ASP 71 I TRP 162 A	9	ASP 71 I LYS 18 B			
ASP 71 I VAL 18 B	9	ASP 89 I TRP 21 B	1	ASP 71 I SER 14 B			
ASP 94 I ARG 21 B		ASP 94 I ASN 36 B		ASP 71 I TYR 23 B			
ASP 94 I ILE 22 B		ASP 94 I ILE 40 B		ASP 71 I VAL 17 B			
ASP 94 I LYS 123 A		ASP 94 I TRP 162 A	3	ASP 71 I VAL 19 B	8	**ASP71**	8.7
ASP 94 I VAL 18 B	4	CYS 27 I ARG 34 B		ASP 89 I ARG 1 B			
CYS 27 I ASN 17 B	1	CYS 27 I ASP 38 B		ASP 89 I PRO 2 B	2		
CYS 84 I LYS 123 A		CYS 27 I TYR 46 B	3	ASP 94 I TYR 3 B			
GLN 47 I GLU 25 B	1	CYS 50 I ARG 34 B		ASP 94 I TYR 23 B			
GLN 67 I ASN 17 B	1	CYS 50 I ASP 38 B	2	ASP 94 I VAL 19 B	3	**ASP 94**	3.3
GLU 26 I GLU 16 B	1	GLN 47 I LYS 43 B	1	CYS 27 I LYS 18 B	1	**CYS 27**	1.7
GLY 70 I ASN 17 B		GLN 67 I ARG 32 B		CYS 78 I ASN 26 B	1		
GLY 70 I ASP 14 B		GLN 67 I HIS 35 B	2	CYS 84 I TYR 23 B	1		
GLY 70 I GLU 16 B		GLU 26 I ARG 34 B		GLN 47 I ASN 26 B			
GLY 70 I VAL 18 B	4	GLU 26 I ASP 38 B		GLN 47 I CYS 25 B	2	**GLN 47**	1.3
GLY 91 I ASP 122 A		GLU 26 I HIS 35 B		GLN 67 I ARG 32 B			
GLY 91 I CYS 121 A		GLU 26 I TYR 46 B	4	GLN 67 I LYS 18 B	2	**GLN 67**	1.7
GLY 91 I HIS 120 A		GLY 70 I ARG 32 B		GLU 26 I ALA 21 B			
GLY 91 I LYS 123 A		GLY 70 I ARG 34 B		GLU 26 I LYS 18 B	2	**GLU 26**	2.3
GLY 91 I SER 4 B	5	GLY 70 I ASN 36 B		GLY 70 I ARG 15 B			
GLY 92 I ARG 21 B		GLY 70 I HIS 35 B		GLY 70 I LYS 18 B			
GLY 92 I ASP 9 B		GLY 70 I LEU 33 B		GLY 70 I SER 14 B			
GLY 92 I ASP 122 A		GLY 70 I SER 31 B	6	GLY 70 I THR 13 B			
GLY 92 I CYS 121 A		GLY 91 I ARG 25 B		GLY 70 I VAL 17 B			
GLY 92 I HIS 120 A		GLY 91 I TRP 21 B	2	GLY 70 I VAL 19 B	6	**GLY 70**	5.3
GLY 92 I SER 4 B		GLY 92 I ARG 25 B		GLY 91 I ARG 1 B			
GLY 92 I VAL 18 B	7	GLY 92 I TRP 21 B		GLY 91 I GLU 4 B			
SER 66 I ARG 21 B	1	GLY 92 I TRP 162 A		GLY 91 I PRO 2 B			
SER 69 I ASN 17 B		GLY 92 I VAL 158 A	4	GLY 91 I TYR 3 B	4	**GLY 91**	3.7
SER 69 I ASP 14 B	2	LEU 82 I LYS 43 B	1	GLY 92 I GLU 4 B			
SER 72 I ARG 21 B		LYS 145 I ALA 166 A		GLY 92 I GLU 5 B			
SER 72 I ASN 17 B		LYS 145 I ASP 44 B		GLY 92 I PRO 2 B			
SER 72 I GLY 20 B		LYS 145 I GLU 167 A		GLY 92 I TYR 3 B			
SER 72 I VAL 18 B	4	LYS 145 I TYR 168 A	4	GLY 92 I VAL 19 B	5	**GLY 92**	5.3
THR 73 I ARG 21 B	1	SER 66 I ALA 39 B		LEU 82 I ASN 26 B	1		
THR 90 I ASP 122 A		SER 66 I ARG 32 B		LYS 145 I GLN 116 A	1		
THR 90 I LYS 123 A	2	SER 66 I ASN 36 B	3	SER 66 I ARG 15 B			
THR 93 I ARG 21 B		SER 69 I ARG 32 B		SER 66 I ASN 22 B			
THR 93 I ASP 9 B		SER 69 I HIS 35 B		SER 66 I VAL 19 B	3	**SER 66**	2.3
THR 93 I GLU 8 B		SER 69 I SER 31 B	3	SER 69 I LYS 18 B			
THR 93 I HIS 120 A		SER 72 I ALA 39 B		SER 69 I SER 14 B	2	**SER 69**	2.3
THR 93 I SER 4 B		SER 72 I ARG 34 B		SER 72 I ASN 22 B			
THR 93 I VAL 18 B	6	SER 72 I ASN 36 B		SER 72 I LYS 18 B			
VAL 83 I ILE 22 B		SER 72 I ASP 38 B		SER 72 I VAL 19 B	3	**SER 72**	4
VAL 83 I LYS 123 A	2	SER 72 I HIS 35 B	5	SER 77 I ASN 26 B	1		
VAL 143 I PHE 131 A	1	THR 73 I ALA 39 B	1	THR 73 I ASN 22 B	1	**THR 73**	1
		THR 90 I TRP 21 B	1	THR 88 I ARG 1 B	1		
		THR 93 I ARG 25 B		THR 90 I ARG 1 B			
		THR 93 I ARG 32 B		THR 90 I GLU 4 B			
		THR 93 I ASN 36 B		THR 90 I PRO 2 B			
		THR 93 I LEU 28 B		THR 90 I TYR 3 B	4	**THR 90**	2.3
		THR 93 I TRP 162 A	5	THR 93 I ARG 15 B			
				THR 93 I GLU 4 B			
				THR 93 I TYR 3 B			
				THR 93 I VAL 19 B	4	**THR 93**	5
				VAL 83 I LEU 27 B			
				VAL 83 I TYR 23 B			

(b)

	IAG - IGFBP_N'	ILP1 - IGFBP_N'	ILP2 - IGFBP_N'
Number of interacting residues PRODIGY/ PDBsum			
IGFBP_N'	21/12	21/17	28/14
A Chain	4/4	3/2	1/1
B Chain	15/7	17/11	18/10
Contact properties PRODIGY			
charged - charged	9	10	8
charged - polar	12	14	15
charged - apolar	18	21	16
polar - polar	4	4	6
polar - apolar	8	8	13
apolar - apolar	7	7	11

(c)

Consistent IGFBP_N' contacts	
PRODIGY (17)	**PDBsum (8)**
ALA 49	ILP1
ASP 68	ILP1
ASP 71	**All**
ASP 94	**All**
CYS 27	ILP1
GLN 47	None
GLN 67	**All**
GLU 26	ILP1
GLY 70	**All**
GLY 91	**All**
GLY 92	**All**
SER 66	IAG and ILP2
SER 69	ILP1 and ILP2
SER 72	**All**
THR 73	None
THR 90	IAG and ILP2
THR 93	**All**