1. Primary and Secondary Structure Analysis

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Introduction

Protein's primary structure is sequence of aminod acid. We can find some conservation of same protein which vary on different organs and different species. So it is possible to find out domain from these conservative sequence. Comparing amino acid sequence and certain algorithm, we can print the phylogenetic tree.

The information of amino acid sequence is not enough to build up protein with certain biological activity, but the secondary structure and higher dimemnsion structure do. Based on the features of known experiment result between primary structure and secondary structure, we can predict the helix, strand and turn structure in unknown protein.

Methods

In the first part, we selected 10 homology sequences of human UNC5b, including both orthologues and paralogues from uniprot.org. There are

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UNC5B_HUMAN,
K7DDTY1_PANTR,K7B3D8_PANTR,K7BVI5_PANTR,H2NAM6_PONAB,G7N281_MACMU,
F6UDU4_MACMU,F6UDV7_MACMU,F7A1Z5_CALJA,U3F339_CALJA
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Then we aligned and generated a phylogenetic tree sequence with the Clustal Omega program in uniprot.org.

In the second part, we used JPred to predicte secondary structure of the cytoplasmic domain of human UNC5b. Then we compared the predicted result with X-ray structure(PDB:3G5B) to get error.

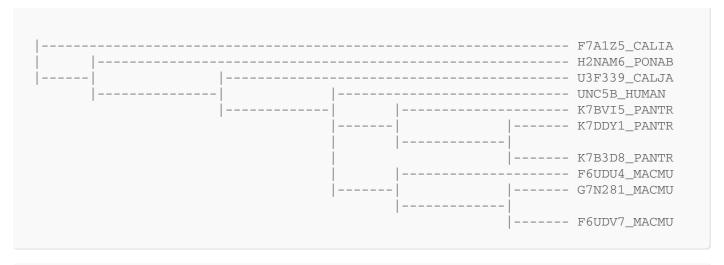
Last part, we design a truncation construct with minimal size, where retaining the overall fold. We keep all the E and H domain sequence and binding site for residue PO4A.

Results

Target Sequence and Aligment results

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08IZJ1 UNC5B HUMAN
K7DDY1 K7DDYI PANT
                                                TLSDPNSHLLEASGDAALYAGLVVAIFVVVAILMAVGVVVYRRNCRDFDTDITDSSAALT
                                                                                                                                                 418
         K7DDYI PANTR
K7B3D8_PANTR
                                       359
                                                TLSDPNSHLLEASGDAALYAGLVVAVFVVVAILMAVGVVVYRRNCRDFDTDITDSSAALT
                                                                                                                                                 418
                                        359
                                                TLSDPNSHLLEASGDAALYAGLVVAVFVVVAILMAVGVVVYRRNCRDFDTDITDSSAALT
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K7BV15 K7BV15 PANTR
K7BV15 K7BV15 PANTR
H2NAM6 H2NAM6 PONAB
G7N281 G7N281 MACMU
F6UDU4 F6UDU4 MACMU
                                       359
73
                                                TLSDPNSHLLEASGDAALYAGLVVAVFVVVAILMAVGVVVYRHNCRDFDTDITDSSAALT
                                                                                                                                                418
                                                TLSDPNSHLLEASGDAALYAGLVVAIFVVVAILMAVGVVVYRRNCRDFDTDITDSSAALT
                                       359
                                                TLSDPNSHLLEASGDAALYAGLVVAVFVVVAILMAVGVVVYRRNCRDFDTDITDSSAALT
                                        356
                                                          -MLEASGDAALYAGLVVAVFVVVAILMAVGVVVYRRNCRDFDTDITDSSAALT
F6UDV7 F6UDV7 MACMU
F7A1Z5 F7A1Z5 CALJA
                                       359
                                                TLSDPNSHLLEASGDAALYAGLVVAVFVVVAILMAVGVVVYRRNCRDFDTDITDSSAALT
                                                                                                                                                 418
F7A1Z5 F7A1Z5 CALJA
U3F339 U3F339 CALJA
                                                TLSDPNSHLLEASGDAALYAGLVVAVFVVVAVLMAVGVVVYRRNCHDFDTDITDSSAALT
                                                TLSDPNSHLLEASGDAALYAGLVVAVFVVVAVLMAVGVVVYRRNCHDFDTDITDSSAALT
Q8IZJ1 UNC5B HUMAN
                                                GGFHPVNFKTARPSNPQLLHPSVPPDLTASAGIYRGPVYALQDSTDKIPMTNSPLLDPLP
                                                                                                                                                478
                                       419
K7DDY1 K7DDY1 PANTR
K7B3D8 K7B3D8 PANTR
K7BV15 K7BV15 PANTR
H2NAM6 H2NAM6 PONAB
G7N281 G7N281 MACMU
F6UDU4 F6UDU4 MACMU
                                               GGFHPVNFKTARPSNPQLLHPSVPPDLTASAGIYRGPVYALQDSTDKIPMTNSPLLDPLP
GGFHPVNFKTARPSNPQLLHPSVPPDLTASAGIYRGPVYALQDSTDKIPMTNSPLLDPLP
GGFHPVNFKTARPSNPQLLHPSVPPDLTASAGIYRGPVYALQDSTDKIPMTNSPLLDPLP
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133
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                                                GGFHPVNFKTARPSNPQLLHPSVPPDLTASAGIYRGPVYALQDSTDKIPMTNSPLLDPLP
                                                                                                                                                192
                                               GGFHPVNFKTARPNNPQLLHPSVPPDLTASAGIYRGPVYALQDSTDKIPMTNSPLLDPLP
GGFHPVNFKTARPNNPQLLHPSVPPDLTASAGIYRGPVYALQDSTDKIPMTNSPLLDPLP
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408
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F6UDU4
F6UDV7 F6UDV7 MACMU
F7A1Z5 F7A1Z5 CALJA
U3F339 U3F339 CALJA
                                               GGFHPVNFKTARPNNPÖLLHPSVPPDLTASAGIYRGPVYALÖDSTDKIPMTNSPLLDPLP
GGFHPVNFKTARPSNPÖLLHPSVPPDLTASAGIYRGPMCALÖDSTDKILMTNSPLLDPLP
                                       419
                                                                                                                                                 478
                                                GGFHPVNFKTARPSNPQLLHPSVPPDLTASAGIYRGPMCALQDSTDKILMTNSPLLDPLP
   <u>IZJ1</u> UNC5B HUMAN
DDY1 K7DDYI PANTR
B3D8 K7B3D8 PANTR
                                                SLKVKVYSSSTTGSGPGLADGADLLGVLPPGTYPSDFARDTHFLHLRSASLGSQQLLGLP
                                                SLKVKVYSSSTTGSGPGLADGADLLGVLPPGTYPSDFARDTHFLHLRSASLGSQQLLGLF
                                                SLKVKVYSSSTTGSGPGLADGADLLGVLPPGTYPSDFARDTHFLHLRSASLGSQQLLGLP
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K7BVI5 K7BVI5 PANTR H2NAM6 H2NAM6 PONAB G7N281 G7N281 MACMU F6UDU4 F6UDV7 MACMU F7A1Z5 F7A1Z5 CALJA U3F339 U3F339 CALJA	479 193 479 468 479 455 479	SLKVKVYSSSTTGSGPGLADGADLLGVLPPGTYPSDFARDTHFLHLRSASLGSQQLLGLP SLKVKVYSSSTTGSGPGLADGADLLGVLPPGTYPSDFARDTHFLHLRSASLGSQQLLGLP SLKVKVYSSSTTGSGPGLADGADLLGVLPPGTYPSDFARDTHLLHLRSASLGSQQLLGLP SLKVKVYSSSTTGSGPGLADGADLLGVLPPGTYPSDFTRDTHLLHLRSASLGSQQLLGLP SLKVKVYSSSTTGSGPGLADGADLLGVLPPGTYPSDFTRDTHLLHLRSASLGSQQLLGLP SLKVKVYSSSTTGSGQGLTDGADLLGVLPPGTYPSDFTRDTHLLHLRSASLGSQQLLGLP SLKVKVYSSSTTGSGQGLTDGADLLGVLPPGTYPSDFTRDSHFLHLRSASLGSQQLLGLP SLKVKVYSSSTTGSGQGLTDGADLLGVLPPGTYPSDFTRDSYFLHLRSASLGSQQLLGLP **********************************	538 252 538 527 538 514 538
08IZJ1 UNC5B HUMAN K7DDY1 K7DDYT PANTR K7B3D8 K7B3D8 PANTR K7BVI5 K7BVI5 PANTR H2NAM6 H2NAM6 PONAB G7N281 G7N281 MACMU F6UDU4 F6UDU7 MACMU F6UDY7 F6UDV7 MACMU F7A1Z5 F7A1Z5 CALJA U3F339 U3F339_CALJA	539 539 539 253 539 528 539 515 539	RDPGSSVSGTFGCLGGRLSIPGTGVSLLVPNGAIPQGKFYEMYLLINKAESTLPLSEGTQ RDPGSSVSGTFGCLGGRLSIPGTGVSLLVPNGAIPQGKFYEMYLLINKAESTLPLSEGTQ RDPGSSVSGTFGCLGGRLSIPGTGVSLLVPNGAIPQGKFYEMYLLINKAESTLPLSEGTQ RDPGSSVSGTFGCLGGRLSIPGTGVSLLVPNGAIPQGKFYEMYLLINKAESTLPLSEGTQ RDPGSSVSGTFGCLGGRLSIPGTGVSLLVPNGAIPQGKFYEMYLLINKAESTLPLSEGTQ RDPGSSVSGTFGCLGGRLSIPGTGVSLLVPNGAIPQGKFYEMYLLINKAESTLPLSEGTQ RDPGSSVSGTFGCLGGRLSIPGTGVSLLVPNGAIPQGKFYEMYLLINKAESTLPLSEGTQ RDPGSSVSGTFGCLGGRLSIPGTGVSLLVPNGAIPQGKFYEMYLLINKAESTLPLSEGTQ RDPGSSVSGTFGCLGGRLSIPGTGVSLLVPNGAIPQGKFYEMYLLINKAESTLPLSEGTQ RDPGSSVSGTFGCLGGRLSIPGTGVSLLVPSGAIPQGKFYEMYLLINKAESTLPLSEGTQ RDPGSSVSGTFGCLGGRLSIPGTGVSLLVPSGAIPQGKFYEMYLLINKAESTLPLSEGTQ RDPGSSVSGTFGCLGGRLSIPGTGVSLLVPSGAIPQGKFYEMYLLINKAESTLPLSEGTQ RDPGSSVSGTFGCLGGRLSIPGTGVSLLVPSGAIPQGKFYEMYLLINKAESTLPLSEGTQ RDPGSSVSGTFGCLGGRLSIPGTGVSLLVPSGAIPQGKFYEMYLLINKAESTLPLSEGTQ ************************************	598 598 598 312 598 587 597 598
08IZJ1 UNC5B HUMAN K7DDY1 K7DDYT PANTR K7B3D8 K7B3D8 PANTR K7BVI5 K7BVI5 PANTR H2NAM6 H2NAM6 PONAB G7N281 G7N281 MACMU F6UDU4 F6UDU7 F6UDV7 MACMU F7AL75 F7AL75 CALJA U3F339 U3F339_CALJA	599 599 599 313 599 588 599 575 599	TVLSPSVTCGPTGLLLCRPVILTMPHCAEVSARDWIFQLKTQAHQGHWEEVVTLDEETLN TVLSPSVTCGPTGLLLCRPVILTMPHCAEVSARDWIFQLKTQAHQGHWEEVVTLDEETLN TVLSPSVTCGPTGLLLCHPVILTMPHCAEVSARDWIFQLKTQAHQGHWEEVVTLDEETLN TVLSPSVTCGPTGLLLCHPVILTMPHCAEVSARDWIFQLKTQAHQGHWEEVVTLDEETLN TVLSPSVTCGPTGLLLCRPVILTLPHCAEVSARDWIFQLKTQAHQGHWEEVVTLDEETLN TVLSPSVTCGPTGLLLCRPVILTVPHCAKVSVGDWIFQLKTQAHQGHWEEVVTLDEETLN TVLSPSVTCGPTGLLLCRPVILTVPHCAKVSVGDWIFQLKTQAHQGHWEEVVTLDEETLN TVLSPSVTCGPTGLLLCRPVILTVPHCAKVSVGDWIFQLKTQAHQGHWEEVVTLDEETLN TVLSPSVTCGPAGLLLCRPVILTVPHCAEVSAGDWIFQLKTQAHQGHWEEVVTLDEETLN TVLSPSVTCGPAGLLLCRPVILTVPHCAEVSAGDWIFQLKTQAHQGHWEEVVTLDEETLN TVLSPSVTCGPAGLLLCRPVILTVPHCAEVSAGDWIFQLKTQAHQGHWEEVVTLDEETLN ************************************	658 658 658 372 658 647 658 634 658
08IZJ1 UNC5B HUMAN K7DDY1 K7DDYT PANTR K7B3D8 K7B3D8 PANTR K7BVI5 K7BVI5 PANTR H2NAM6 H2NAM6 PONAB G7N281 G7N281 MACMU F6UDU4 F6UDU4 MACMU F6UDV7 F6UDV7 MACMU F7A1Z5 F7A1Z5 CALJA U3F339 U3F339_CALJA	659 659 659 373 659 648 659 635	TPCYCQLEPRACHILLDQLGTYVFTGESYSRSAVKRLQLAVFAPALCTSLEYSLRVYCLE TPCYCQLEPRACHILLDQLGTYVFTGESYSRSAVKRLQLAVFAPALCTSLEYSLRVYCLE TPCYCQLEPRACHILLDQLGTYVFTGESYSRSAVKRLQLAVFAPALCTSLEYSLRVYCLE TPCYCQLEPRACHILLDQLGTYVFTGESYSRSAVKRLQLAVFAPALCTSLEYSLRVYCLE TPCYCQLEPRACHILLDQLGTYVFTGESYSRSAVKRLQLAVFAPALCTSLEYSLRVYCLE TPCYCQLEPRACHILLDQLGTYVFTGESYSRSAVKRLQLAVFAPALCTSLEYSLRVYCLE TPCYCQLEPRACHILLDQLGTYVFTGESYSRSAVKRLQLAVFAPALCTSLEYSLRVYCLE TPCYCQLEPRACHILLDQLGTYVFTGESYSRSAVKRLQLAVFAPALCTSLEYSLRVYCLE TPCYCQLEPRACHILLDQLGTYVFTGESYSRSAVKRLQLAVFAPSLCTSLEYSLRVYCLE TPCYCQLEPRACHILLDQLGTYVFTGESYSRSAVKRLQLAVFAPSLCTSLEYSLRVYCLE TPCYCQLEPRACHILLDQLGTYVFTGESYSRSAVKRLQLAVFAPSLCTSLEYSLRVYCLE	718 718 718 718 432 718 707 718 694 718
08IZJ1 UNC5B HUMAN K7DDY1 K7DDYI PANTR K7B3D8 K7B3D8 PANTR K7BVI5 K7BVI5 PANTR H2NAM6 H2NAM6 PONAB G7N281 G7N281 MACMU F6UDU4 F6UDU4 MACMU F6UDV7 MACMU F7A1Z5 F7A1Z5 CALJA U3F339 U3F339 CALJA	719 719 719 719 433 719 708 719 695 719	DTPVALKEVLELERTLGGYLVEEPKPLMFKDSYHNLRLSLHDLPHAHWRSKLLAKYQEIP DTPVALKEVLELERTLGGYLVEEPKPLMFKDSYHNLRLSLHDLPHAHWRSKLLAKYQEIP DTPVALKEVLELERTLGGYLVEEPKPLMFKDSYHNLRLSLHDLPHAHWRSKLLAKYQEIP DTPVALKEVLELERTLGGYLVEEPKPLMFKDSYHNLRLSLHDLPHAHWRSKLLAKYQEIP DTPVALKEVLELERTLGGYLVEEPKPLMFKDSYHNLRLSLHDLPHAHWR-KLLAKYQEIP DTPVALKEVLDLERTLGGYLVEEPKPLMFKDSYHNLRLSLHDLPHAHWRSKLLAKYQEIP DTPVALKEVLDLERTLGGYLVEEPKPLMFKDSYHNLRLSLHDLPHAHWRSKLLAKYQEIP DTPVALKEVLDLERTLGGYLVEEPKPLMFKDSYHNLRLSLHDLPHAHWRSKLLAKYQEIP DTPVALKEVLELERTLGGYLVEEPKPLVFKDSYHNLRLSLHDLPHAHWRSKLLAKYQEIP DTPVALKEVLELERTLGGYLVEEPKPLVFKDSYHNLRLSLHDLPHAHWRSKLLAKYQEIP DTPVALKEVLELERTLGGYLVEEPKPLVFKDSYHNLRLSLHDLPHAHWRSKLLAKYQEIP DTPVALKEVLELERTLGGYLVEEPKPLVFKDSYHNLRLSLHDLPHAHWRSKLLAKYQEIP DTPVALKEVLELERTLGGYLVEEPKPLVFKDSYHNLRLSLHDLPHAHWRSKLLAKYQEIP DTPVALKEVLELERTLGGYLVEEPKPLVFKDSYHNLRLSLHDLPHAHWRSKLLAKYQEIP	778 778 778 778 491 778 767 778 754 778
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08IZJ1 UNC5B HUMAN K7DDY1 K7DDYT PANTR K7B3D8 K7B3D8 PANTR K7BV15 K7BV15 PANTR H2NAM6 H2NAM6 PONAB G7N281 G7N281 MACMU F6UDU4 F6UDU4 MACMU F6UDV7 F6UDV7 FAURACMU F7A1Z5 F7A1Z5 CALJA U3F339 U3F339 CALJA	894 894 894 894 607 894 888 892 870 894	NYFATKASPTGVILDLWEALQQDDGDLNSLASALEEMGKSEMLVAVATDGDC	945 945 945 945 658 945 939 943 921 945



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Sequence total length: 339 Error ratio= (mismatch of predition compare eith correct)/(total sequence length)=60/339=17%

Minisize sequence:

The minisize sequence are marked as *

Seq : YRRNCRDFDTDITD; Known : Design :	SSAALTGGFHPVNFK	TARPSNPQLLHP	SVPPDLTASAC	GIYRGPVYALQDS	TDKIPMTNSPLLI	PLP		
SLKVKVYSSSTTGS	GPGLADGADLLGVLP	PGTYPSDFARDT:	HFLHLRSASL(GSQQLLGLPRDPG	SSVSGTFGCLGGF	LSI		
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PGTGVSLLVPNGAT	POGKFYEMYLLINKA	ESTLDLSEGTOT	VI SPSVTCGP	TGT.T.T.CR PV/TT.TM	PHCAEWSARDWTE	'OI.K		
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Conclusions

Here are conservation of protein in different organs and speices, which suggested the evolution of protein. Using cetain technique, secondary structure can be predicted in acceptable range. But we think that the different of our choose target is small and not enough to show the essential domain. And we risks to keep beta strand and alpha helix.