

Modelizations in 3D of the Anti-HRP Sea Star (or Starfish) Primitive Antibody (IPA): Their Relations with Mus Musculus Fab and In A General Way Mammal Antibodies

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ABSTRACT

Presentations of sea star primitive antibody 3D modelizations, according AlphaFold and Swiss Model predictions were performed in EMBL Grenoble A superposition of the 2 predictions lead us to envisage a true link with a « part » of Mus musculus IG antibody: the Fab one of Heavy Chain. But, at every time, in such presentations, it could be contested by those who don't believe in the existence of the IPA (Invertebrate Primitive Antibody) for various reasons we don't want enumerate.

Keywords: Invertebrates, CHO Protocol, Elisa, CDR1, CDR2, IPA.

INTRODUCTION

Recently, (2024) we have discovered CDR1, CDR2, CDR3? In sea star Immune system [1] with the help of Dr Sofia Kossida (IMGT Montpellier) [2,3]. We try in the present study to give modelizations of the sea star IGKappa protein [4] which recognizes specifically the antigen HRP. In the first part we recall the different data and characteristics of sea star IGKappa: i-e sequence and different alignments (aa) when compared to mammal sequences in aa. At last we attempt to find again, in the 3D modelization, the « regions » which may be compared to CDR1, CDR2, and CDR3.

MATERIALS AND METHODS

Alphafold and Swiss Model entities, which predict a protein's 3D structure based on its primary amino acid sequence, were used. Recalling of IGKappa gene sequence and comparison of aa alignments between this last one and mammal ones are first given:

Inputs: IMGT/DomainGapAlign version: 4.10.3 (2021-12-06)

Sequence:

```
MRGNMASLWMFFVVGITLQRSLAIYTFREQSDTSALQGS
TVVLHCSVEQYINTTAIVWWSRDSVISHNKDLKLSLNTDQ
LQRYISGDASRGEFNLKIVNFTATDAASYRCQMFA
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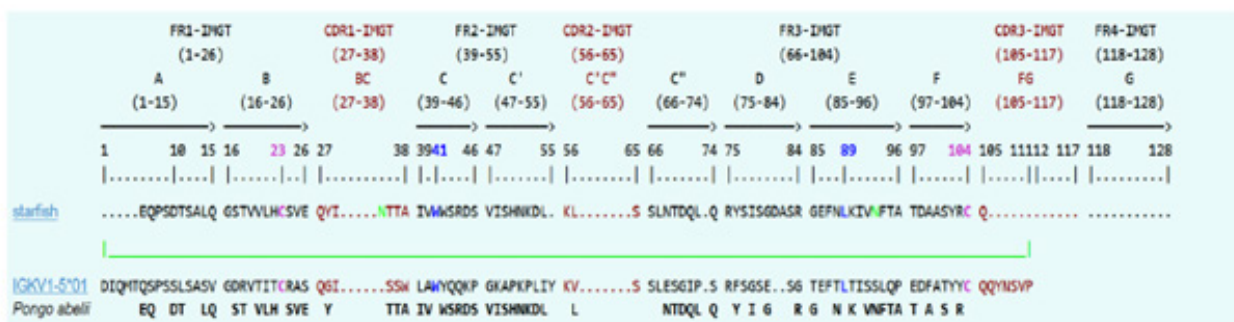
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Closest reference gene and allele(s) from the IMGT V domain directory: (All species)

Species	Gene and allele	Domain	Domain label	Smith-Waterman score	% identity	Overlap	Show alignment
<i>Pongo abelii</i>	IGKV1-5*01	1	V-KAPPA	121	33.3	90	
<i>Pongo pygmaeus</i>	IGKV1-8*01	1	V-KAPPA	121	33.3	90	
<i>Homo sapiens</i>	IGKV1-5*03	1	V-KAPPA	119	33.3	90	
<i>Homo sapiens</i>	IGKV1-5*04	1	V-KAPPA	119	33.3	90	
<i>Homo sapiens</i>	IGKV1-5*05	1	V-KAPPA	119	33.3	90	

Species	Gene and allele	Domain	Domain label	Smith-Waterman score	% identity	Overlap
<i>Pongo pygmaeus</i>	IGKJ4*01	1		7	100.0	1

Alignment with the closest gene and allele from the IMGT V domain directory: (All species)



RESULTS

1) Comparisons with Fab Light chain(crystal): Swiss Model (<https://swissmodel.expasy.org/>)

Summary Templates **50** Models Project Data ▾

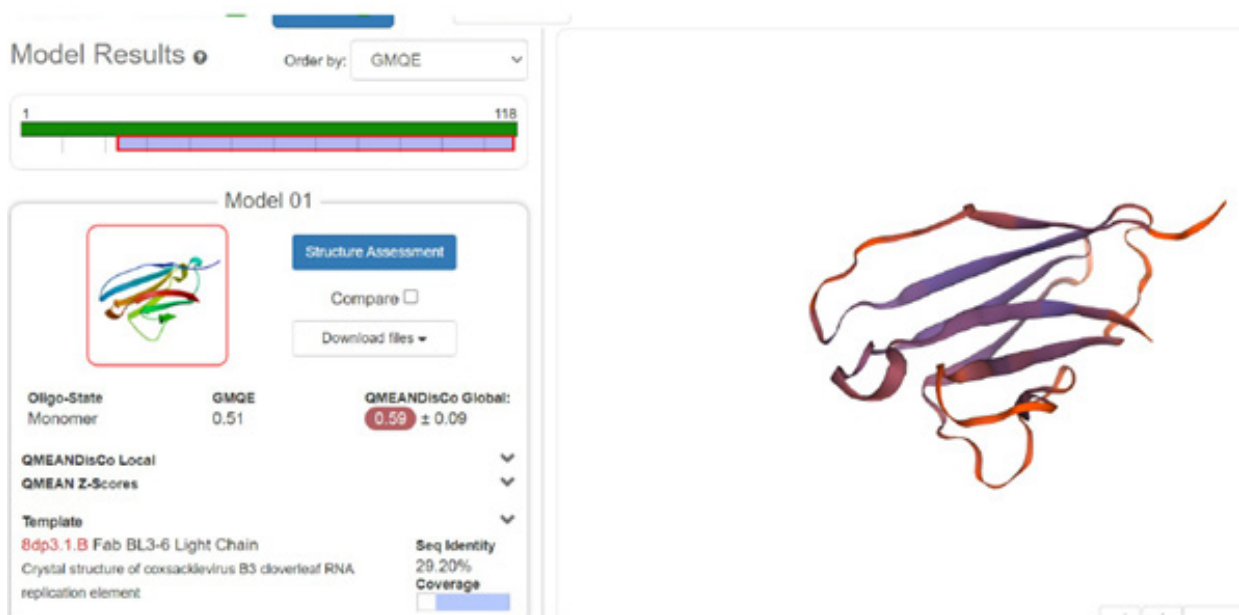
Template Results **0**

Templates Quaternary Structure Sequence Similarity Alignment More ▾

Sort Coverage GMQE QSQE Identity Method Oligo State Ligands

- 8xp3.1.B** Fab BL3-6 Light Chain
Crystal structure of coxsackievirus B3 cloverleaf RNA replication element
0.57 - 29.20 X-ray, 1.9A hetero-dimer Δ None
- 6xjy.1.C** Fab HAVx Light Chain
Crystal structure of a self-alkylating ribozyme - short time incubation with the epoxide substrate
0.57 - 28.32 X-ray, 2.2A hetero-dimer Δ None
- 6xpz.2.C** Fab HAVx Light Chain
Crystal structure of a self-alkylating ribozyme - apo form
0.56 - 28.32 X-ray, 2.5A hetero-dimer Δ None
- 6xjq.2.C** Fab HAVx Light Chain
Crystal structure of a self-alkylating ribozyme - alkylated form with biotinylated epoxide substrate
0.56 - 28.32 X-ray, 1.7A hetero-dimer Δ None
- 5w23.1.E** 5C4 Fab light chain
Crystal Structure of RSV F in complex with 5C4 Fab
0.55 - 24.78 X-ray, 3.4A hetero-8-mer Δ 7 x ZN¹⁷

2) First Modelization of anti-HRP protein



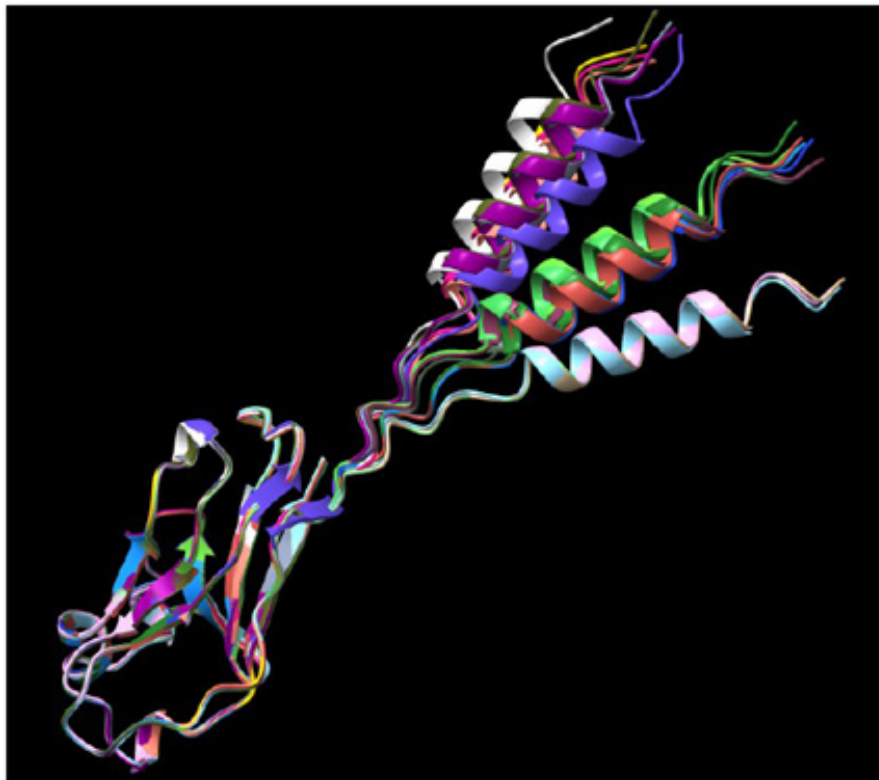
3) AlphaFOLD

5 models predicted and for each 4 seeds are tested → 20 predictions

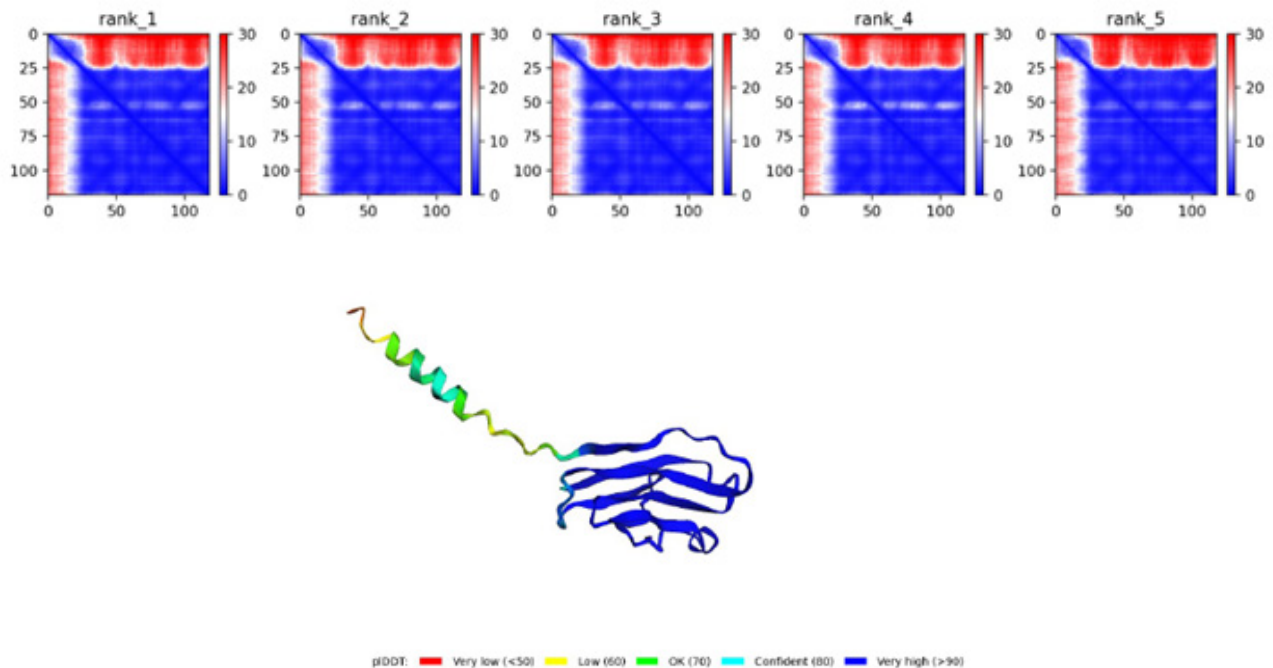
Superimposition done from residue 26

The RMSD is on average from 0.6 to 0.3 Å

The only variability is at the level of α -helix



4) Parameters of the alphafold prediction



Predicted alignment error plot (PAE). The absolute error in A of the relative position between residues

Local confidence of the model:

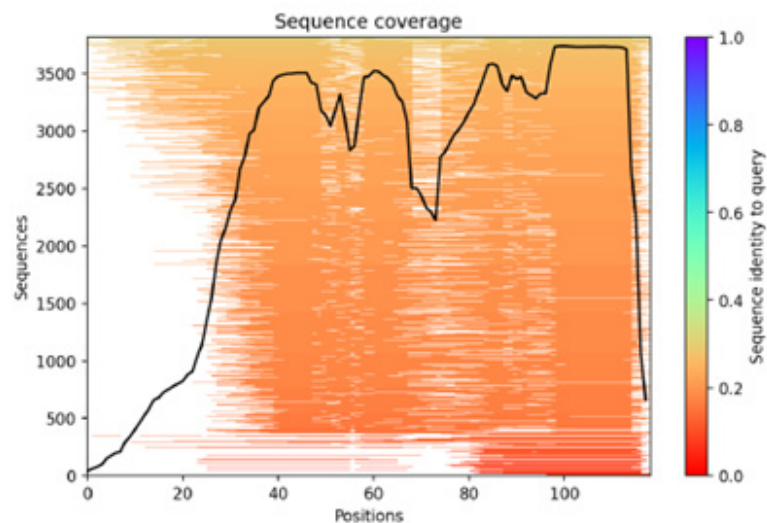
- Dark blue: large confidence
- Red: low confidence

0-25: red → low confidence (up to 30 A of error)

25-118: blue with some regions in white → more confident

5) Multiple-Sequence Alignment (MSA)

The sequences used in the alignment ordered from largest identity (top) to the lowest identity (bottom). White parts are not covered. The black lines indicate the coverage of the sequence.

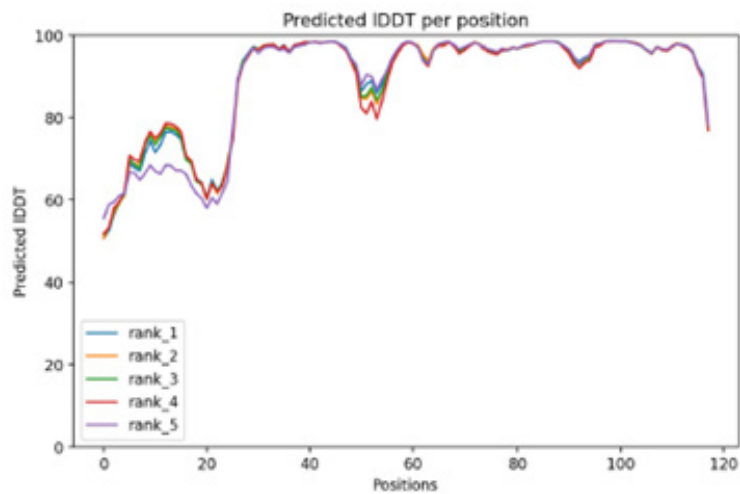


0-24 residues: not well covered by the sequence alignment → α -helix predicted

24- 118 residues: identity found in the range of 0.2-0.3, the pic is for the last 30 residues

6) IDDT: Local Distance Difference Test

It evaluates local distance differences of all atoms in a model including validation of stereochemical plausibility.



pLDDT > 90 modelled to high accuracy

pLDDT between 70 to 90 modelled well

pLDDT between 50 and 70 low confidence

0-20 residues have a pLDDT between 80 and 50

→ this region is majoritary modelled with low confidence, with a pick at 80 which indicated that a portion is well modelled
 20-118 residues have a IDDT higher than 90 however some regions (around position 50 and after 110) have a score between 90 and 70 → this regions is model with high accuracy with two regions well modelled.

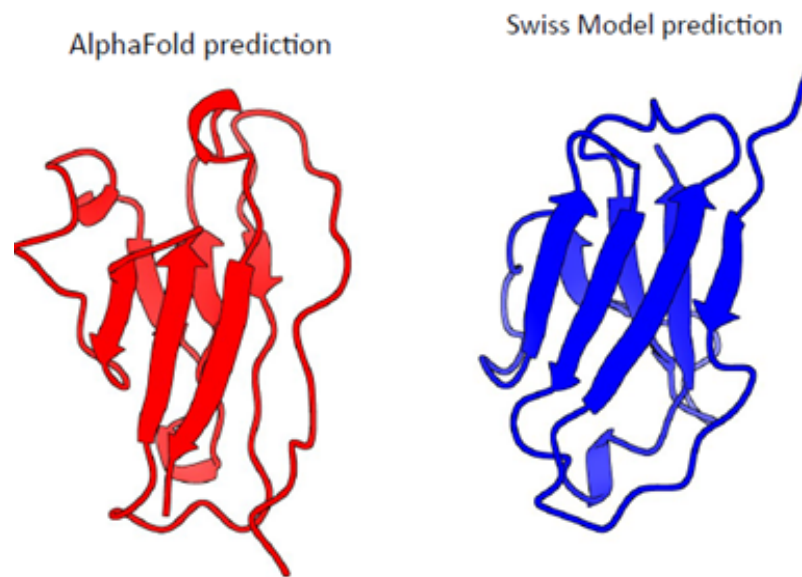
7) SCALOP: Sequence-based antibody Canonical LooP structure annotation

(<https://opig.stats.ox.ac.uk/webapps/sabdab-sabpred/sabpred/scalop>)

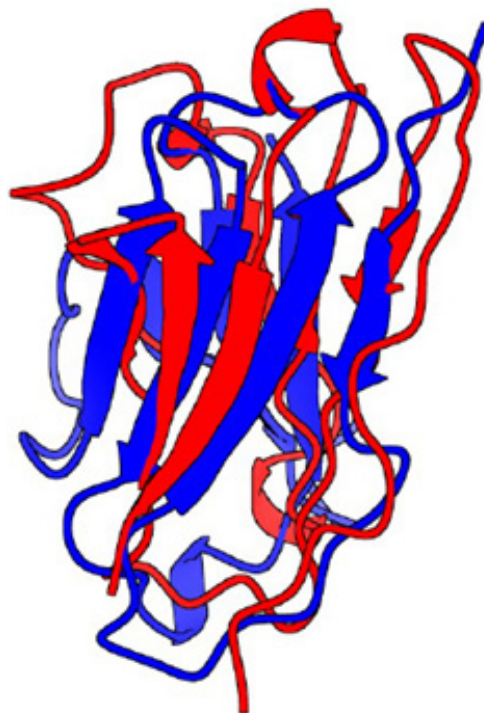
SCALOP is a sequence-based canonical form predictor for five of the six complementarity determining regions (H1, H2, L1, L2 and L3) on an antibody.

CDR	CDR sequence	Canonical Form	Median Structure
Not an antibody	None	None	None

8) Primitive invertebrate antibody - final predictions



9) Superpose of the predictions



Red: Alpha Fold prediction

Blue: Swiss Model prediction

10) 4HDI : 3E5 IgG3 FAB from mus musculus



Green: light chain

Grey: heavy chain

Light blue: CDRs

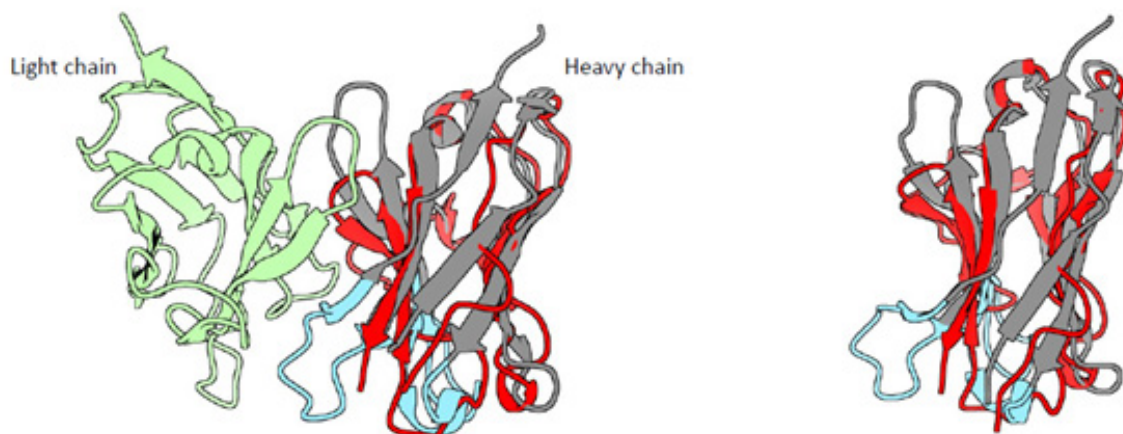
CDR1: 26-35

CDR2: 50-58

CDR3: 99-109

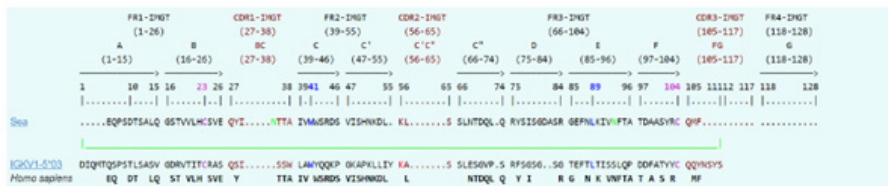
<https://www.rcsb.org/structure/4HDI>

11) Superpose of AlphaFold prediction and X-ray structure of a Fab (4HDI)



The SSM superpose in Coot aligns the prediction with the heavy chain

12) IMGT/DomainGapAlign



P01602 · KV105_HUMAN

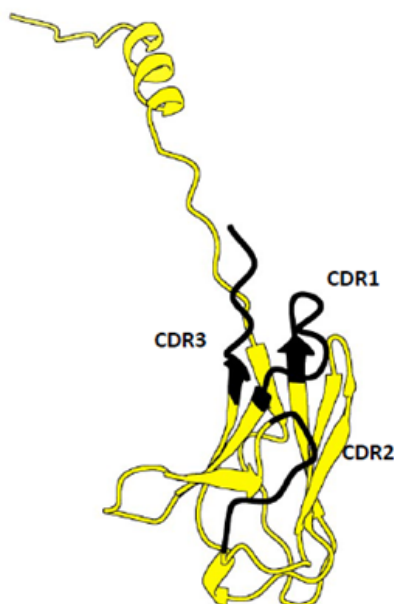
Protein: Immunoglobulin kappa variable 1-5
 Gene: IGKV1-5
 Status: UniProtKB reviewed (Swiss-Prot)
 Organism: Homo sapiens (Human)

Amino acids: 117 (to sequence)
 Protein existence: Evidence at protein level
 Annotation score: 5.5

Entry Variant viewer Feature viewer Genomic coordinates Publications External links History

BLAST Download Add Add a publication Entry feedback

13) AlphaFold prediction of IGKV1-5*03 Homo sapiens



Family & Domains

Features
 Showing features for region, domain.

1 25 50 75 100 117

MDHAYPQQLGLGLLVEPBAKCDIQHTQSPFTEPRASGDRVTITCRASGSSISHLAWYQKQPKKPKLLEIKASGLEAEVPRPFEGRGGSTIETETISLQLPQDFATYKCCQYNEYE

TYPE	ID	POSITION(S)	DESCRIPTION	BLAST	Add
Region		23-45	Framework-1 [1] Publication	BLAST	Add
			Ig-like [PROSITE-ProRule:PRU00114]		
Domain		24-117	Manual assertion according to rules UniRule PROSITE-ProRule:PRU00114 [3]	BLAST	Add

CDR1: 46-55 residues
 CDR2: 72-78 residues
 CDR3: 111-117 residues

14) AlphaFold predictions superposed

Yellow: IGKV1-5*03 Homo sapiens

Red: Invertebrate Primitive Antibody from Starfish



Possible CDRs based on the superposition

CDR1: 48-58

CDR2: 69-73

CDR3: 115-118

DISCUSSION AND CONCLUSION

It seems clearly that our primitive antibody shares similarities with the Fab (4HDI) from the Heavy chain of *Mus musculus* in terms of amino-acids when we have a look to CDR1:26-35 residues. CDR2: 50-58 residues CDR3: 99-109 residues and in terms of morphology in 3D (Modelization), when compared always to the Fab '4HDI: 3E5 IGG3 Fab. No similarities were found with THE LIGHT CHAIN, except in the model 01, where it is shown slight similarities (Sequence identities of 29, 20 %) with Fab BL3-6 Light Chain: what is a weak percentage.

A comparison of our antibody with IGKV1-5 03 was also productive in terms of alignments for CDR1: 46-55 residues, CDR2:72-78 residues, CDR3: 111-117 residues. These last predictions were done with AlphaFold ones. This work presents the interest to name CDR3 in our research. It was not the case in our precedent work (1) our primitive antibody would present CDR1, CDR2, CDR3 (determining regions) as seen precedently and, more specially, similarities with Fab (4HDI) (by the superpose in Coot aligns the prediction with the Heavy Chain). Less with IGKV1-5 03 Homo sapiens. The main work is now to persuade that it consists in true observations+ and confirm the existence of the IPA (Invertebrate Primitive Antibody).

+We thanks greatly the Doctor J.A Marquez: EMBL (Grenoble) and his laboratory for their skillful and technical competences in this work in which, more and more people, dare to speak of Invertebrate Primitive Antibod.

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