ParaFishControl

Innovative solutions to improve fish health in aquaculture: from diagnostics to vaccines

ParaFishControl Final Conference

"Innovative Strategies to Control Parasites in Aquaculture Farms"

Brussels, 11th March 2020

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This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 634429. This output reflects the views only of the author(s), and the European Union cannot be held responsible for any use which may be made of the information contained therein.

Outline

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1. **Overview of VAL technologies**
2. **VAL antibody technology**
   - **Solution 1:** develop antibodies to commercial species
3. **Development of AI software**
   - Applications of AI software
   - **Solution 2:** Diagnostic design
   - **Solution 3:** Vaccine design
4. **VAL Recombinant Technology**
   - Recombinants applications
   - **Solution 4:** Develop recombinant proteins
   - **Solution 5:** 1st , 2nd generations of adjuvants & immunostimulants

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Overview of VAL technologies

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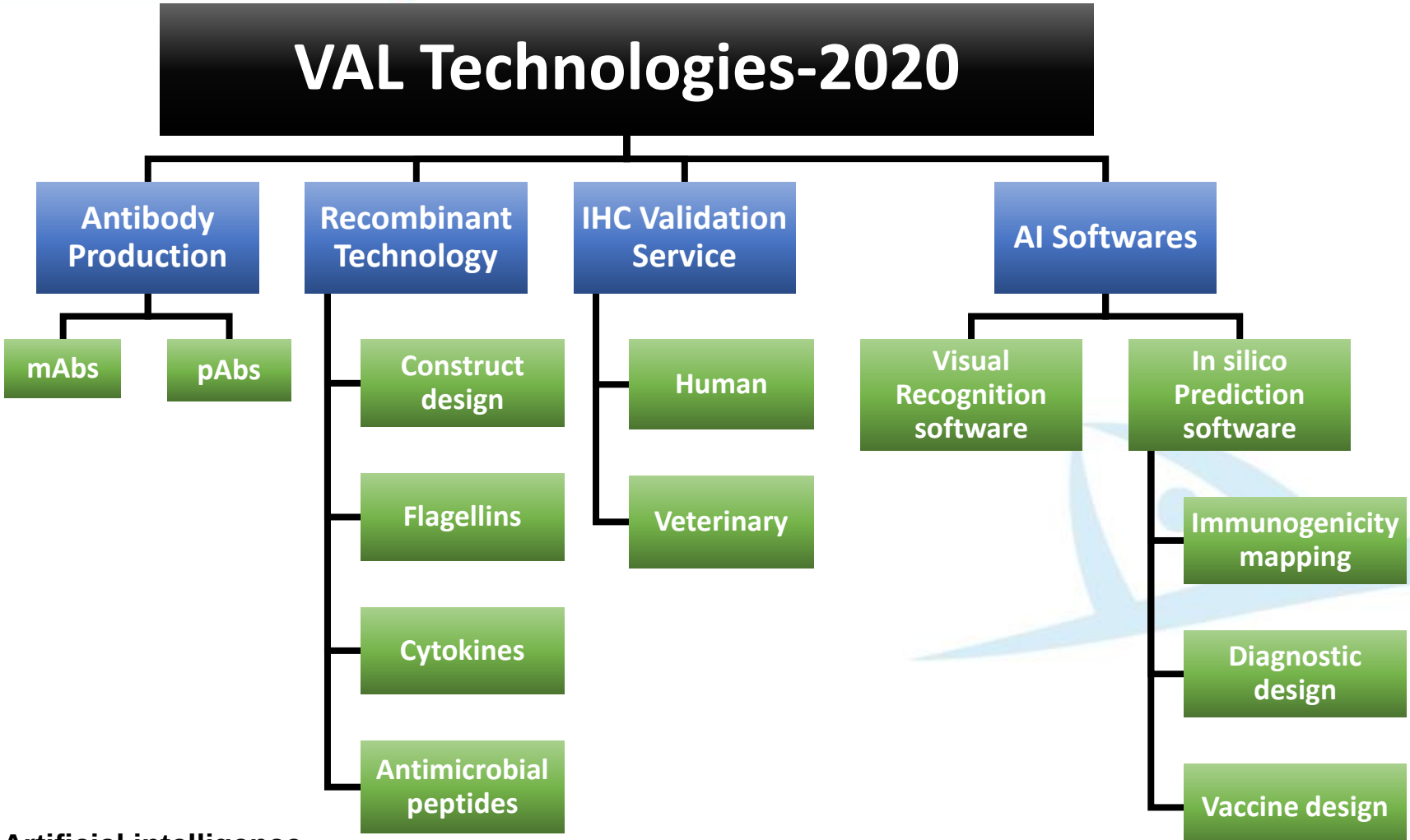

AI: Artificial intelligence

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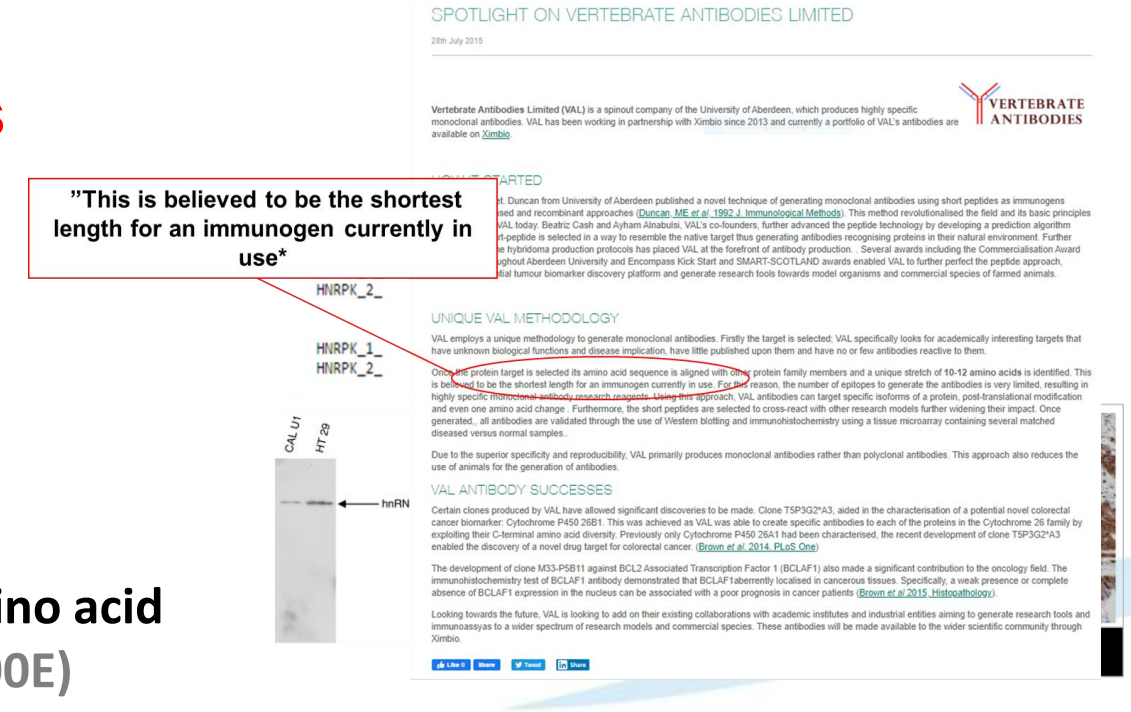
VAL antibody technology

VAL utilises short synthetic peptides technology



- **Advantages:**
  - **Highest specificity**
  - **Can target one amino acid change -(BRAF V600E)**
  - **Time, cost effective & high throughput**

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Solution 1– develop antibodies to commercial species

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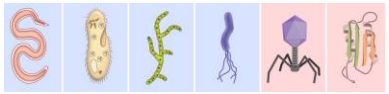
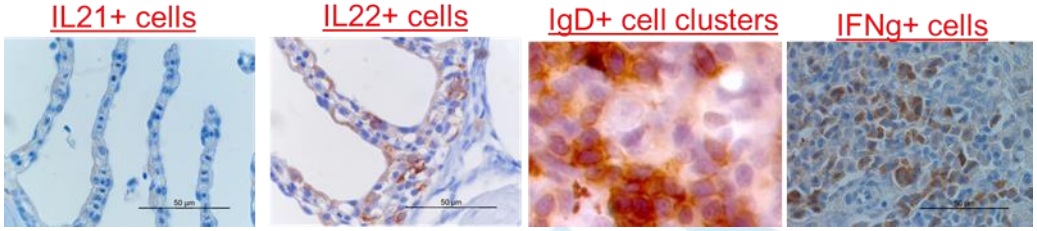


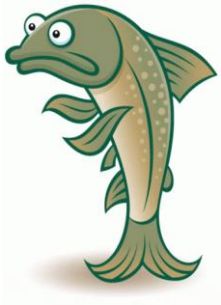
- **Pathogen Detection:**

Tilapia Lake virus
Iridovirus,
Extra small virus-like particles (XSV)
Infectious pancreatic necrosis virus
Betanodavirus

Vaccines, Adjuvants & Immunostimulants:

IgM, IgT, IFNg, IL4, IL22, IL17, IL21





Overall Health:

Serum amyloid A
C-reactive protein
Tumour Necrosis Factor alpha
Alanine aminotransferase
Ghrelin, Orexin, leptin, Irisin

Basic Research:

Mx, CD3, TIMP2a, MMP9, RYK, CCT2

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Peptide Technology

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Peptide	K & R	D & E	PI	Net charge	Hydropho kcal/mol	Molecular Weight	Immunogencity Score
KRPFPSILRF	3	0	12.49	3	9.27	1259.74	7
EDSQMVEMDR	1	4	3.53	-3	23.68	1238.48	0
KDPTSRTPVNQ	2	1	9.83	1	18.55	1241.63	0
GETVTLPRSK	2	1	9.8	1	16.68	1086.6	0
NEFKEVYQRA	2	2	6.91	0	19.01	1282.62	0
QYGTSDNNI	0	1	3.04	-1	14.04	1010.42	0
PIERKAIPQR	3	1	11.35	2	17.26	1206.71	0
KASNPEQTA	1	1	6.79	0	17.8	944.45	0
KAQLIDRGYP	2	1	10.1	1	15.63	1159.63	7
KEIPDDEQSD	1	5	3.4	-4	29.13	1174.49	7
KLIPRSHKEK	4	1	10.84	3	22.3	1234.74	7
TLEYKDENTNEY	1	4	3.57	-3	24.76	1517.64	7
TFLKDFCVHA	1	1	7.17	0	12.27	1179.57	7
LPYSILGTPY	0	0	5.47	0	5	1122.59	7
AQAIYSRPHP	1	0	10.35	1	12.72	1138.58	7

Generate a software to predict immunogenicity profile

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AI software- learning capabilities

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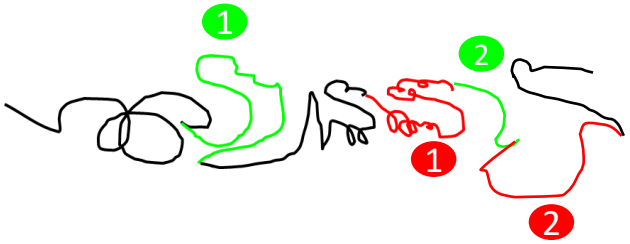
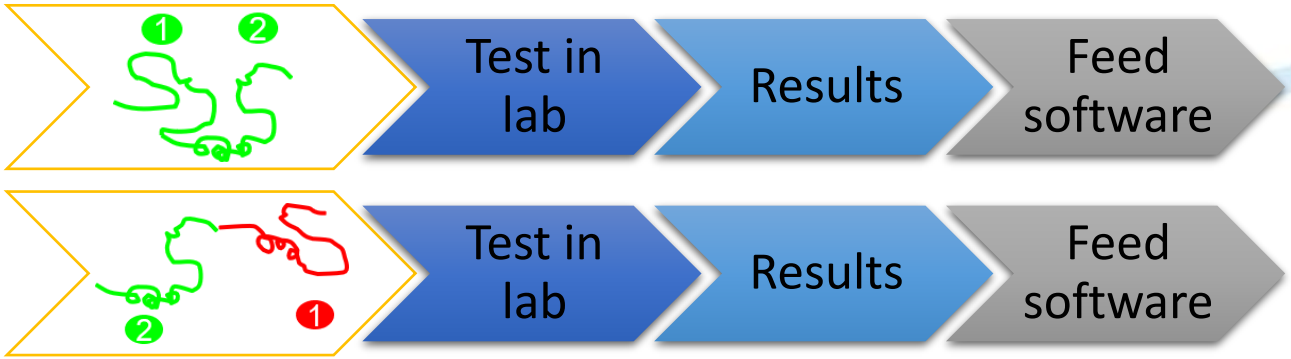


Green line is antigenic- able to elicit an immune response.
Red line is not antigenic- will not elicit immune response.
Black line is of medium antigenicity.

- Predict immunogenic/antigenic regions
- Software developed using experimental data
- The software has deep learning capabilities
  - A **genetic Algorithm** **is incorporated into the software based on Darwin's theory of natural evolution and hybrid and crossover in order to improve prediction accuracy**



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AI software applications

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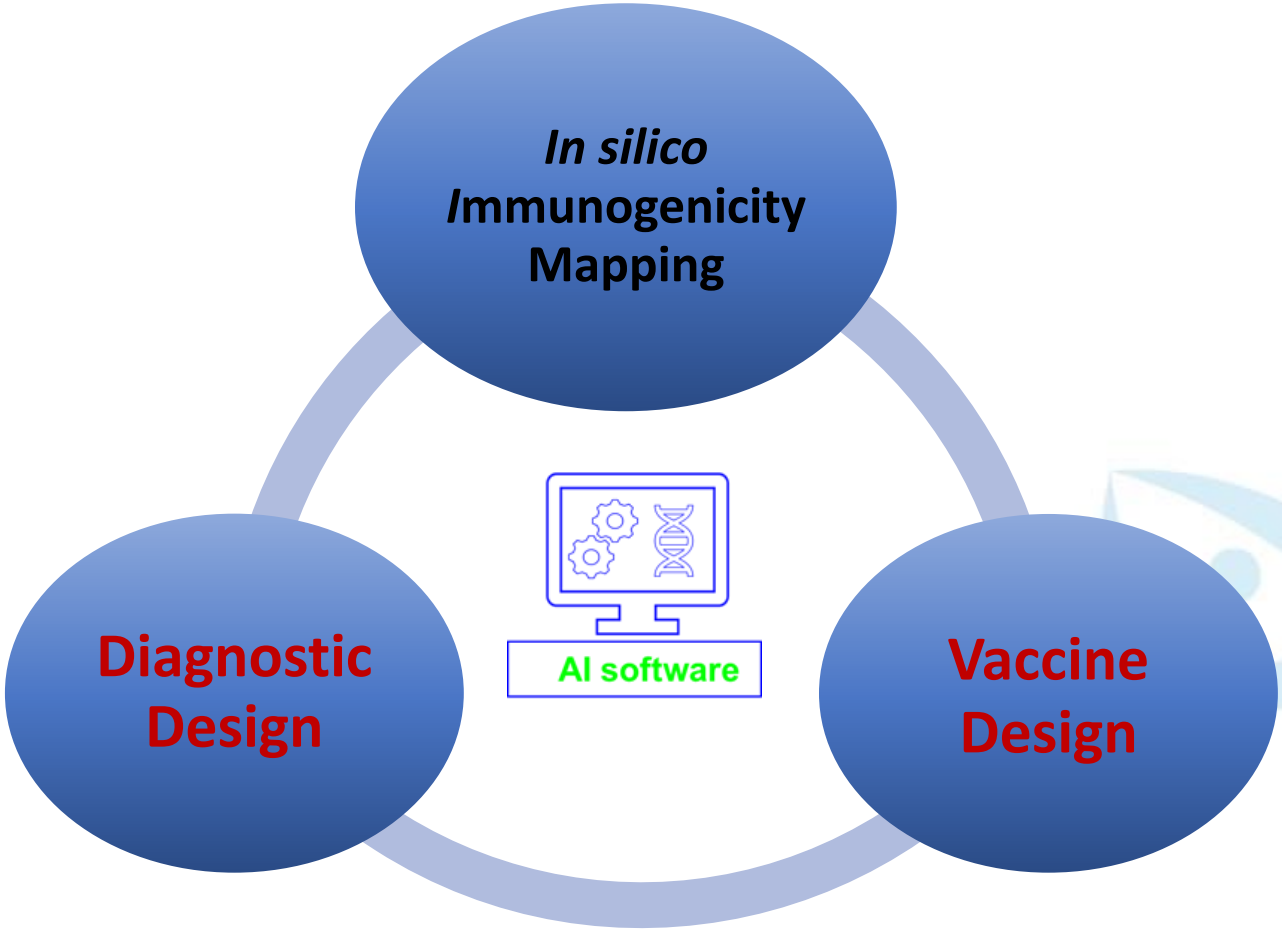

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Solution 2: Diagnostic design

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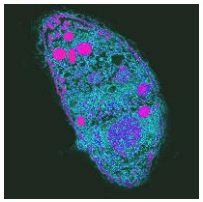
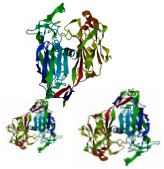
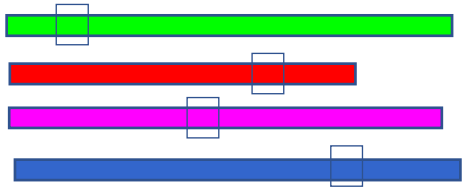




Identify most antigenic protein/s

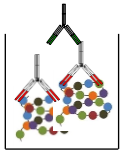


Identify most antigenic regions/epitopes

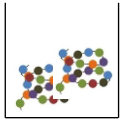
Epitope amplification

Promoter

Anti- IgM, IgG specific to studied species



Infected species sera



Coat plat

Expression of composite protein

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Solution 2: Diagnostic design

(multi-pathogen detection)

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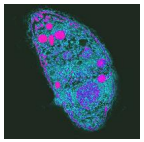

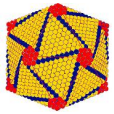

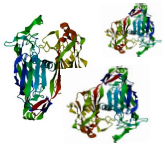

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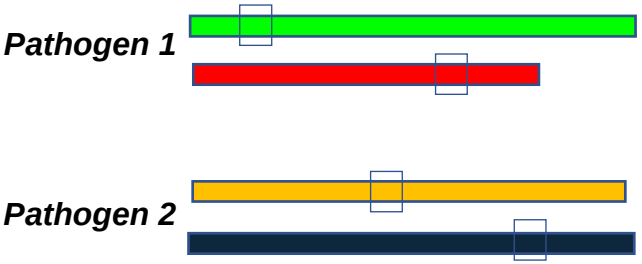
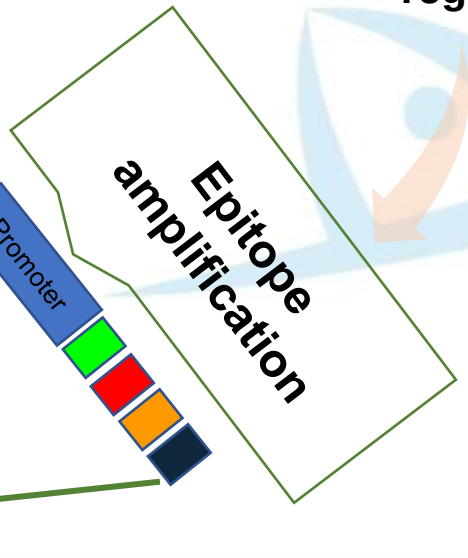
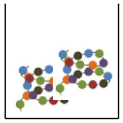


Identify most antigenic regions/epitopes

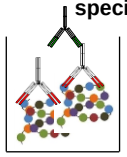


Expression of composite antigen

Coat plat



Infected species sera



Anti- IgM, IgG specific to studied species

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Solution 3: Subunit vaccine Design

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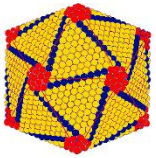
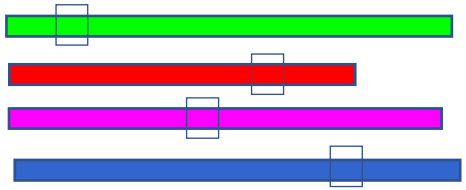
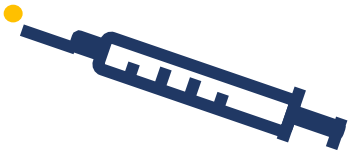


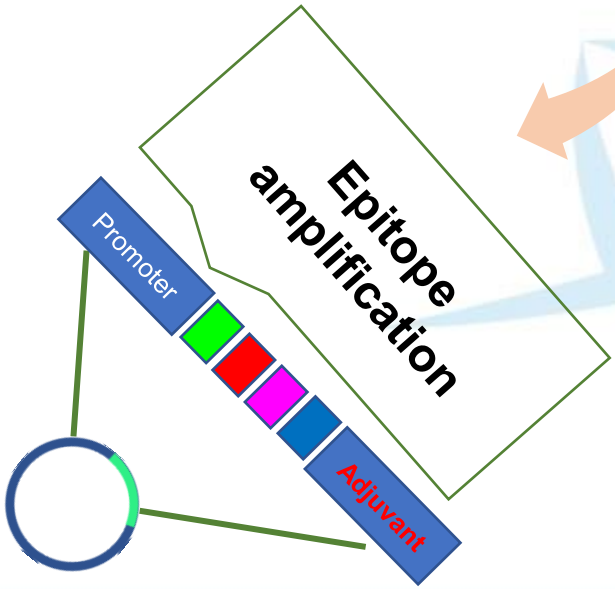
Identify most antigenic protein/s



Identify most antigenic regions/epitopes



Vaccine



Expression of composite antigen

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Solution 3: Multivalent vaccine

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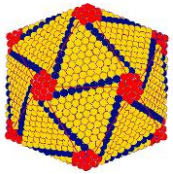
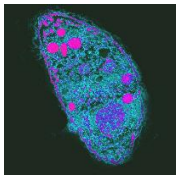
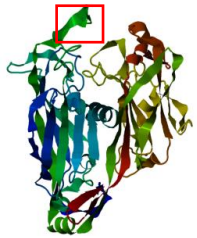
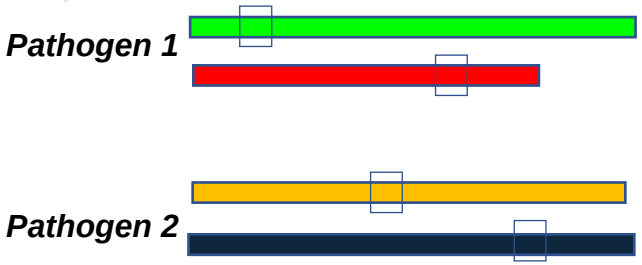




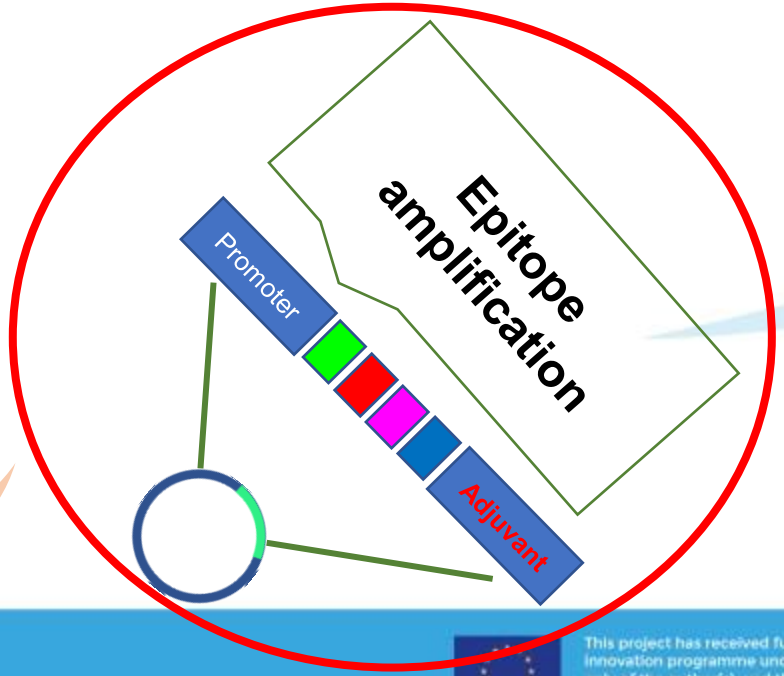


Identify most antigenic protein/s



Identify most antigenic regions/epitopes

Vaccine



Expression of Composite antigen

Recombinant protein technology

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VAL recombinant technology

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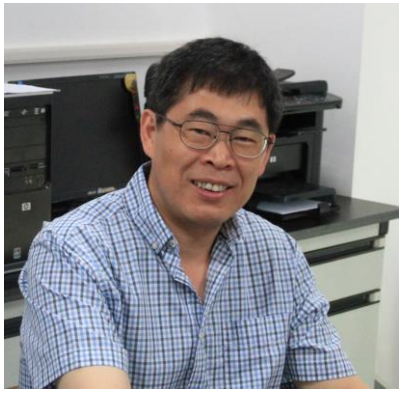
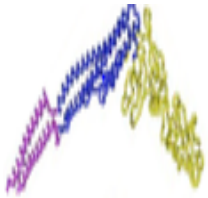


Tiehui Wang

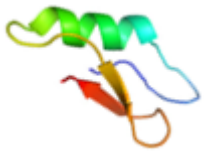
Options for *E. coli* and eukaryotic expression systems.
Codon-optimisation for high-level expression.
Ligation-free rapid cloning system
Streamlined production and purification
Bio-modelling and bioengineering

Flagellins

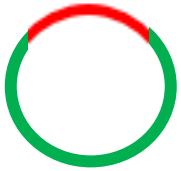


Cytokines

AMPs



DNA construct



Disease markers

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Solution 4: develop recombinant proteins

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Recombinant proteins produced

- **30 recombinant flagellins from broad arrange of bacterial species:**

Piscirickettsia salmonis	***Edwardsiella tarda***	***Edwardsiella ictalurid***
Listeria monocytogenes	***Pseudomonas aeruginosa***	***Salmonella typhimurium***
Vibrio anguillarum	***Pseudomonas putida***	***Aliivibrio salmonicida***
Aeromonas hydrophila	***Yersinia rucker***	***Pseudomonas syringae pv. pisi***
Pseudomonas putida	***Bordetella pertussis***	***Legionella pneumophila***
Bacillus cereus		

- **Bioactive recombinant cytokines from broad Atlantic salmon, tilapia and zebrafish.**
  Type I and II interferons, IL-1β, IFNγ, IL-2, IL-6, TNFα etc.

- **Recombinant proteins for health markers.**
  SAA5, CRP, Leptin,

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Solution 5: Flagellins are the most potent immune stimulants *in vitro* in fish cells

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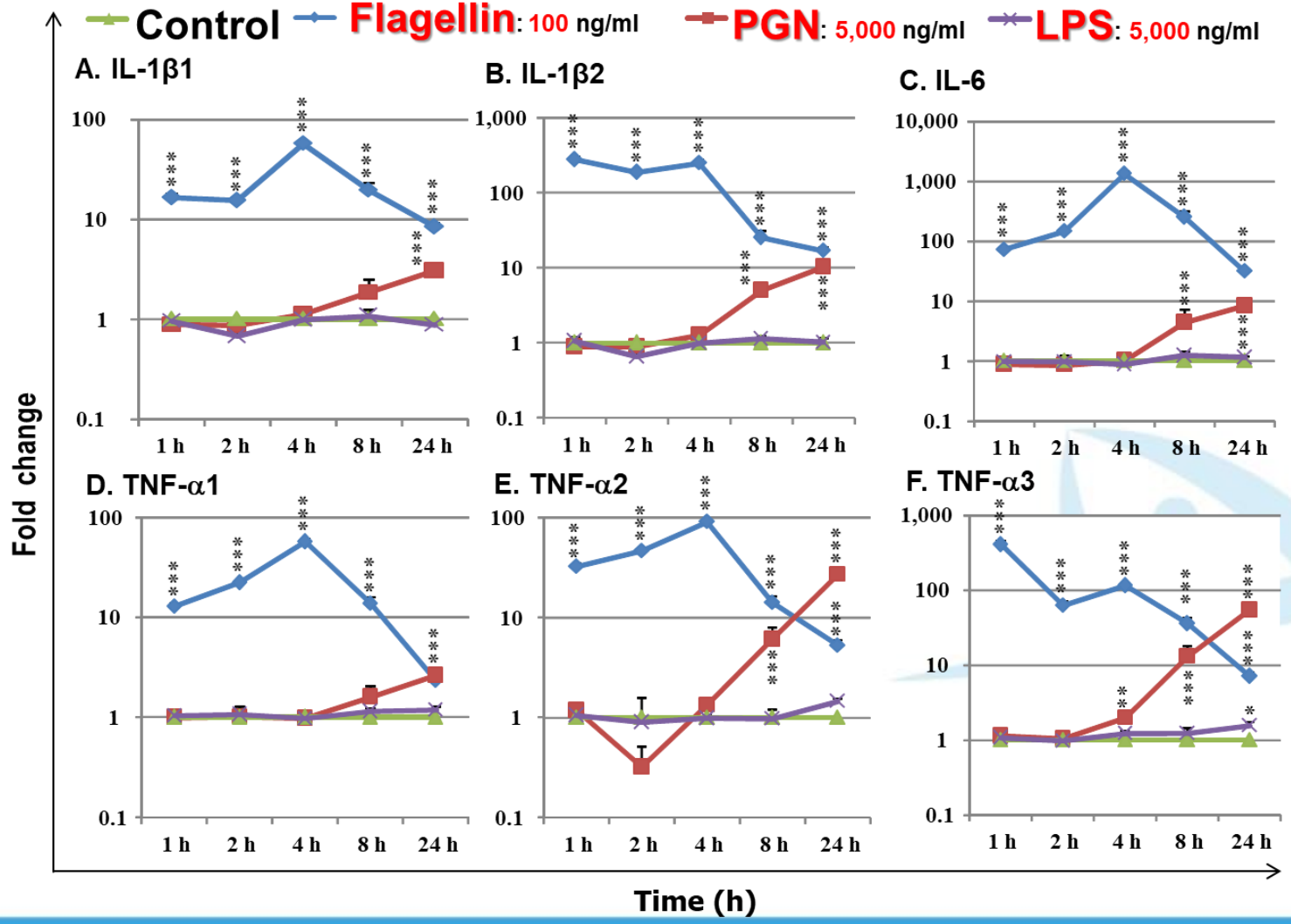

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Potency of flagellins

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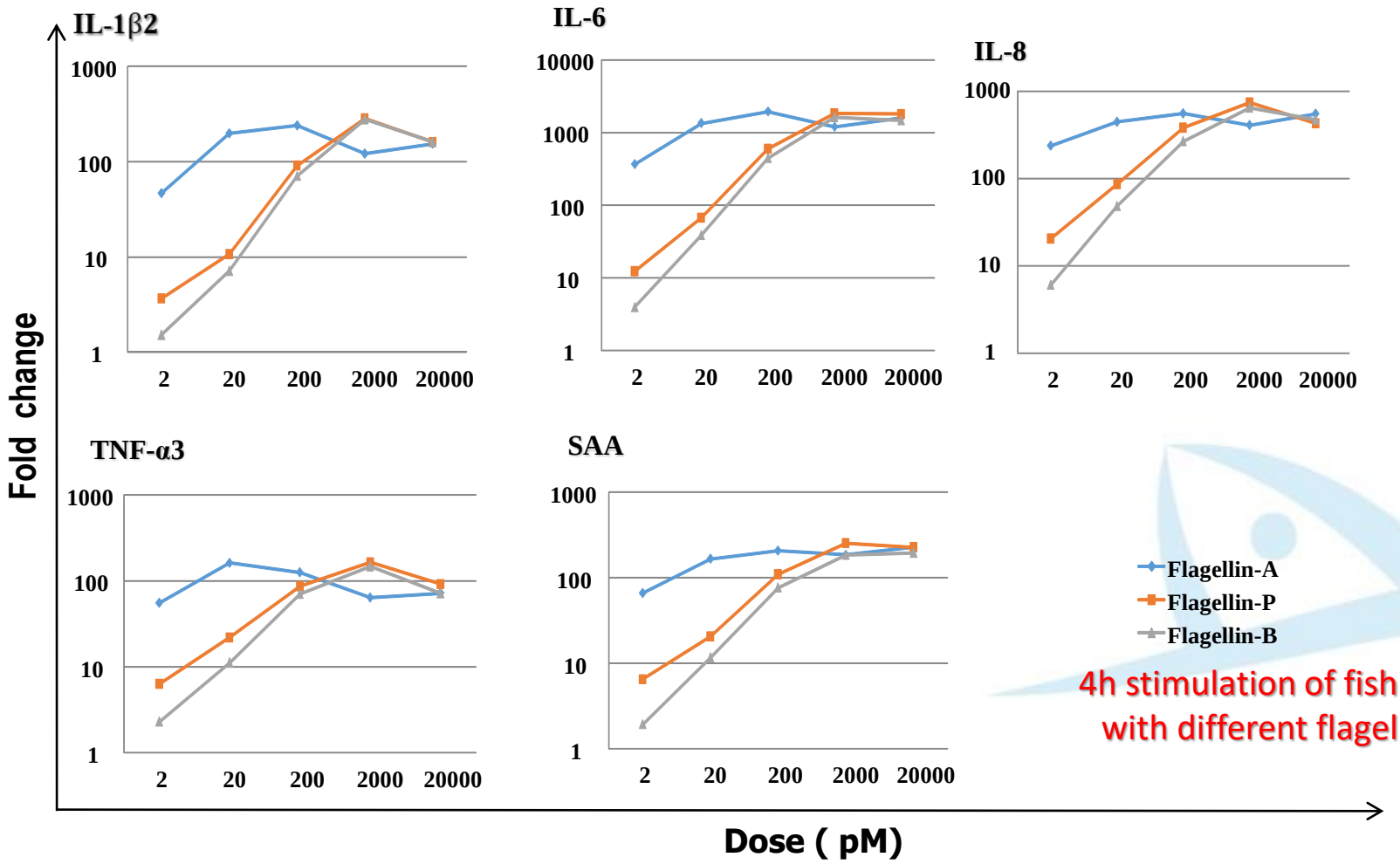

4h stimulation of fish cells with different flagellins

Different flagellins affect fish cells differently

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Summary

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- Develop urgent antibodies & recombinants- available for research
- Provide custom antibody & recombinant production services
- Provide IHC validation service
- AI software
  - Visual scanning (i.e. gill's health, skeletal muscle deformities)
  - Vaccine and diagnostic design
- Generation-1 of bioactive molecules
  - *in vitro* & *in vivo* research
- Generation-2 of Bioengineered molecules
  - vaccine adjuvants
  - immunostimulants

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Thank You

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Dr. Ariadna Sitja-Bobadilla
Dr. Carolina Tafalla
Prof James Bron
Prof. Niels Lorenzen
Dr. Panos Christofilogiannis
Dr. Kurt Buchmann
Dr. Geert F. Wiegertjes
Dr Ema Bello Gomez
Prof. Chris Secombes
Dr Jason Holland

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