

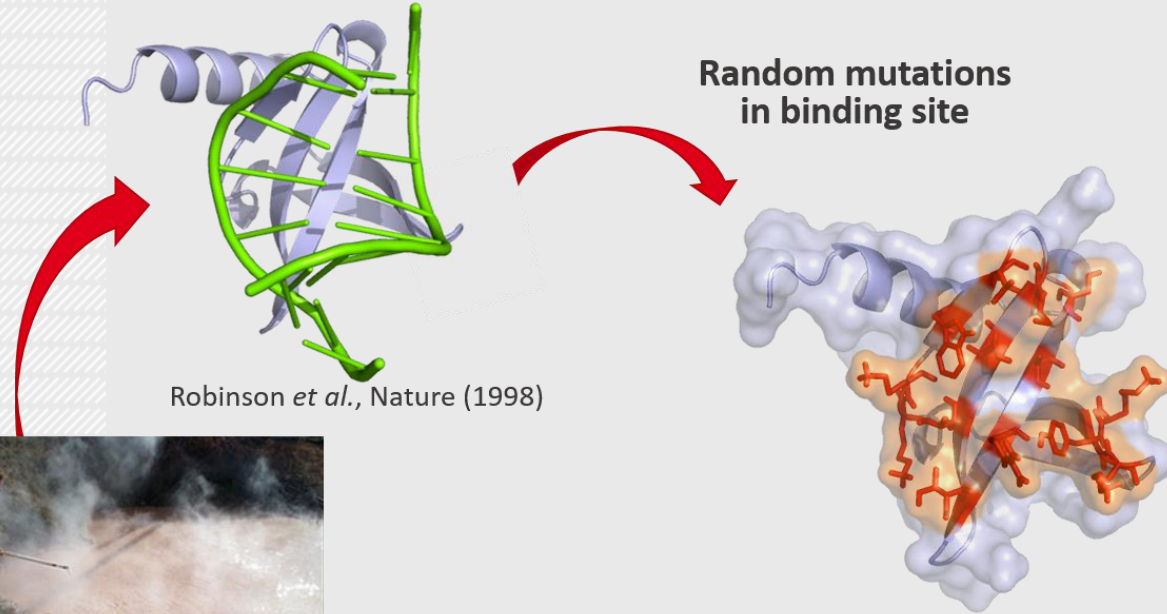
SADEL Project:

Scaffolds for Alternative Delivery

Magali Zeisser Labouèbe¹, Mathieu Cinier², **Christel Rousseaux³**,
Rute Castro⁴, António Cunha⁴, Nghiep Truong Tan³, **Olivier Kitten²**,
Robert Gurny¹, Leonardo Scapozza¹,

1. School of Pharmaceutical Sciences, University of Geneva, Geneva, Switzerland
2. Affilogic, Nantes, France
3. Intestinal Biotech Development, Lille, France
4. iBET, Oeiras, Portugal

Nanofitins : small antibody mimetics



Robinson *et al.*, Nature (1998)

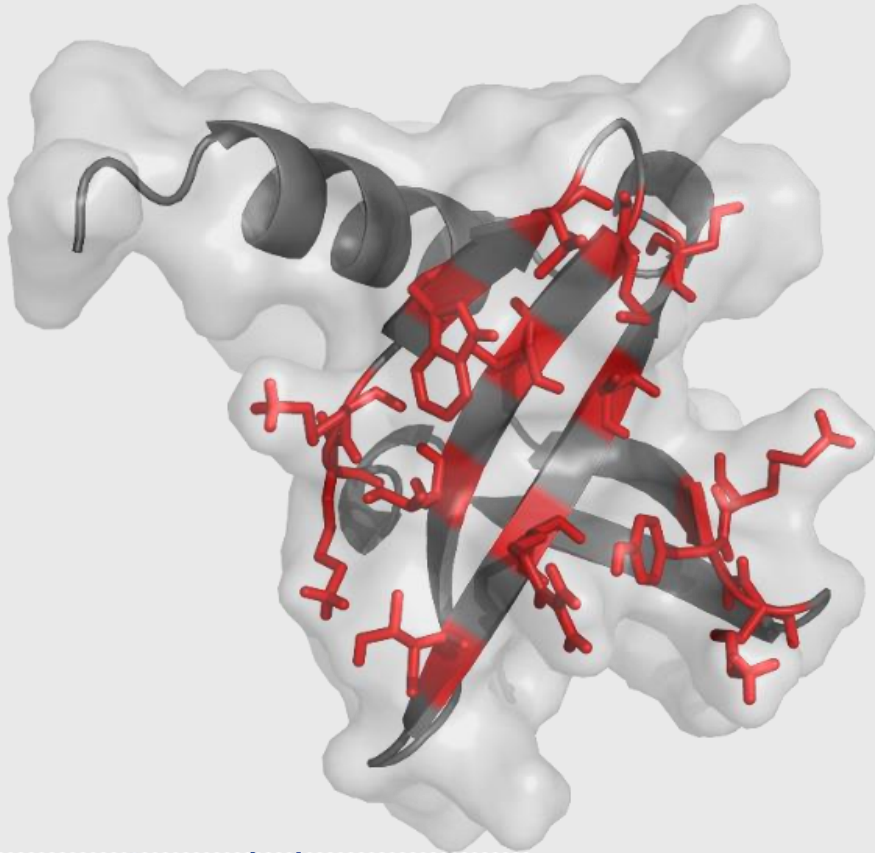


Discovered in Yellowstone geysers
Extremely stable to heat and acid
(85°C and pH 2).

Binding specificity redirected to
the target of choice

**Specific, high affinity binders
with conserved original features**

Why would we need mimetics over antibodies ?



features in 66 amino acids

✓
ity



manufacturing

- Blockade -> Inflammation
- Non-i.v. -> oral
- Tissue penetration -> gut
- Affordable CoG



Orally administered
targeted therapies
in IBD

Anti-TNF α therapy in Inflammatory Bowel Diseases

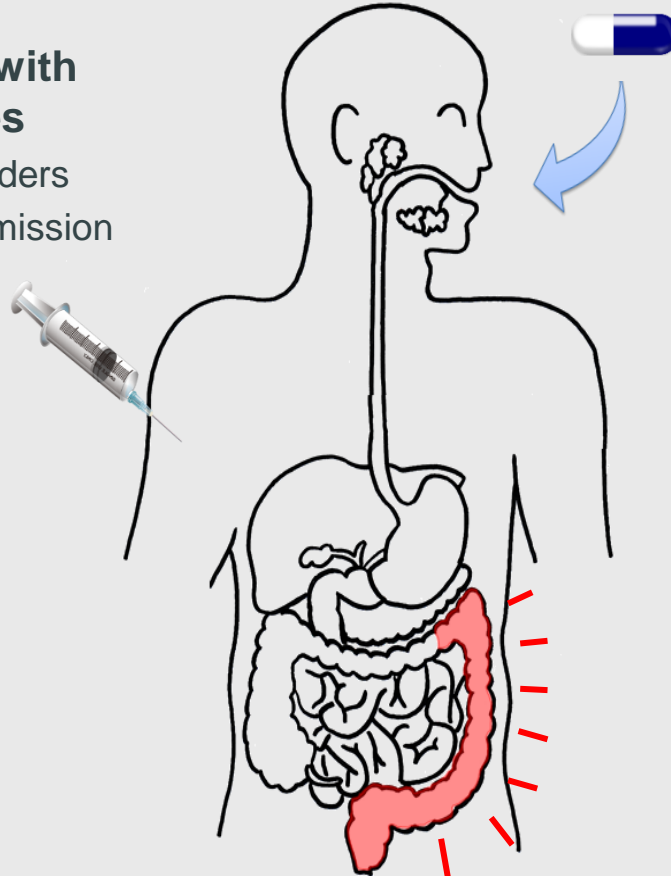
Oral delivery challenge

Targeted therapies with anti-TNF α antibodies

- High efficacy in responders
- Corticosteroids free remission

but

- **Only injectable drugs**
- Injection reaction
- Systemic side effects
- Immunogenicity
- Expensive therapies



Patients and Clinicians associations such as ECCO highlight the need for oral therapeutics.

Unmet medical need

Treating patients with Crohn's disease (CD) and Ulcerative colitis (UC) with a **safe orally administered therapy**.

Nanofitins, a novel family of proteins

Alternative to antibodies

Affordable biologics

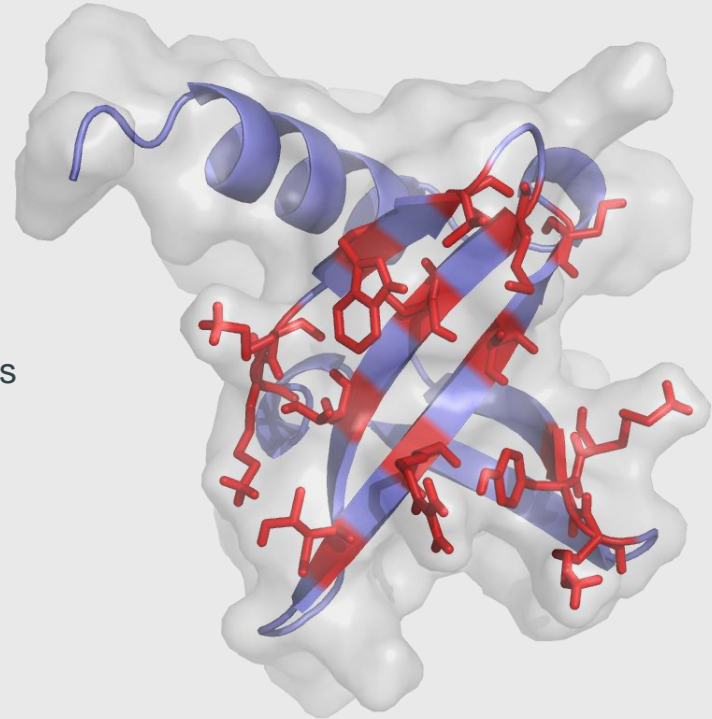
- Simple and cost-effective manufacturing by *E.coli* fermentation

Fit for oral administration

- Stable to T° and pH
- Highly resistant to human intestinal fluids
- Long half-life in digestive track

With demonstrated efficacy

- Preclinical efficacy
- Preventive and curative modes

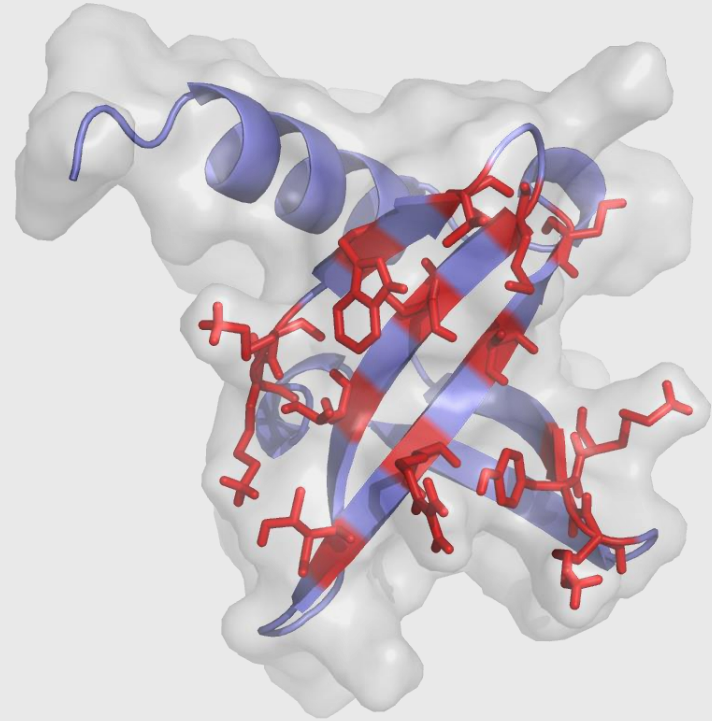


Nanofitins, a novel family of proteins

Alternative to antibodies

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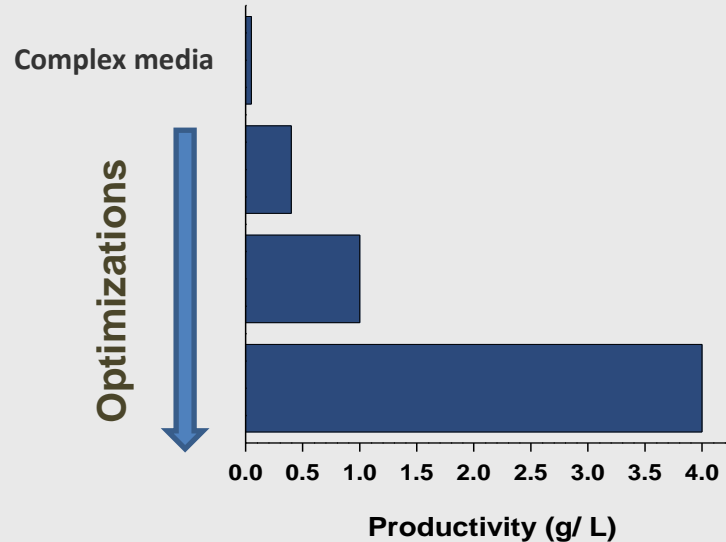
- Simple and cost-effective manufacturing by *E.coli* fermentation



Profile of Nanofitins: Affordable biologics

Manufacturing: High Yield Bacterial Production

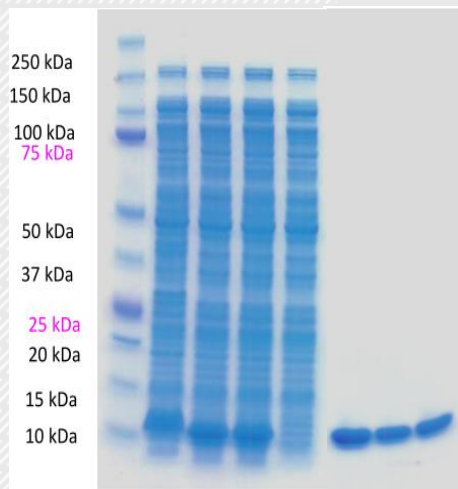
- Expression in *E. coli* (30L bioreactor)



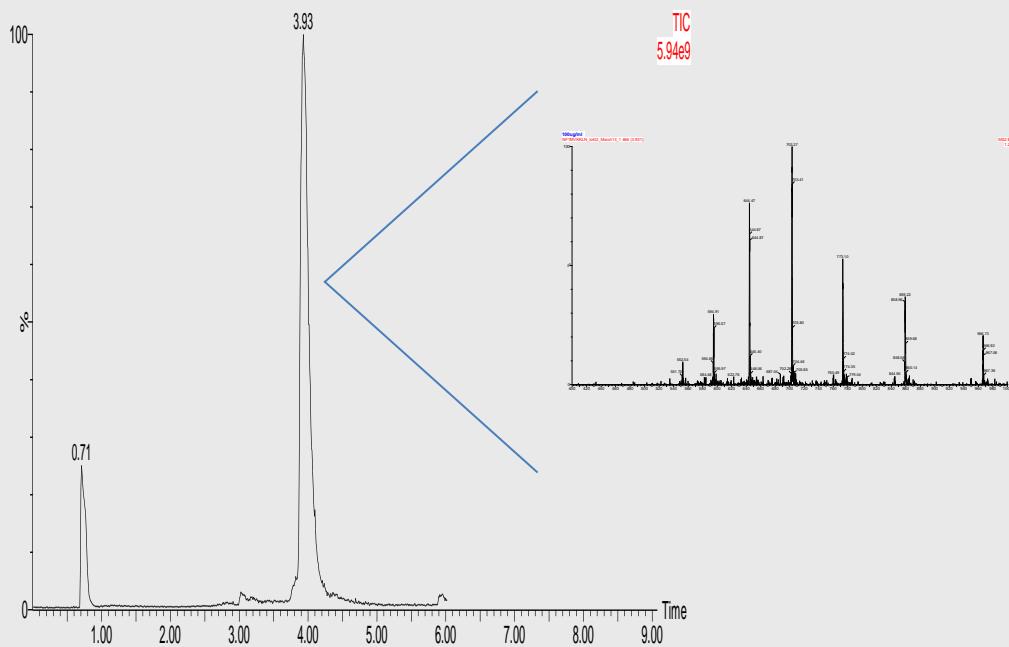
- Optimization of the productivity up to 4 g/L

Profile of Nanofitins: Affordable biologics

Manufacturing: High Purity



SDS page



Analysis by UPLC-MS

Nanofitins, a novel family of proteins

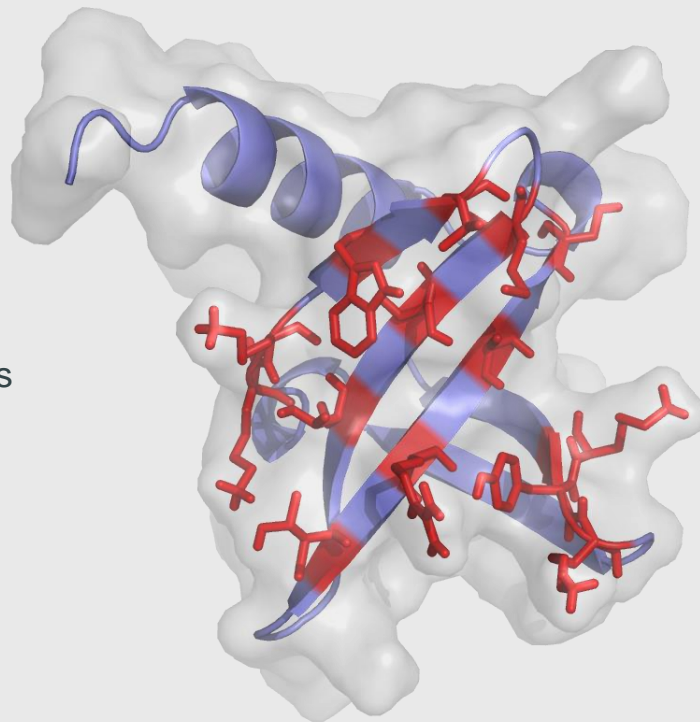
Alternative to antibodies

Affordable biologics

- Simple and cost-effective manufacturing by *E.coli* fermentation

Fit for oral administration

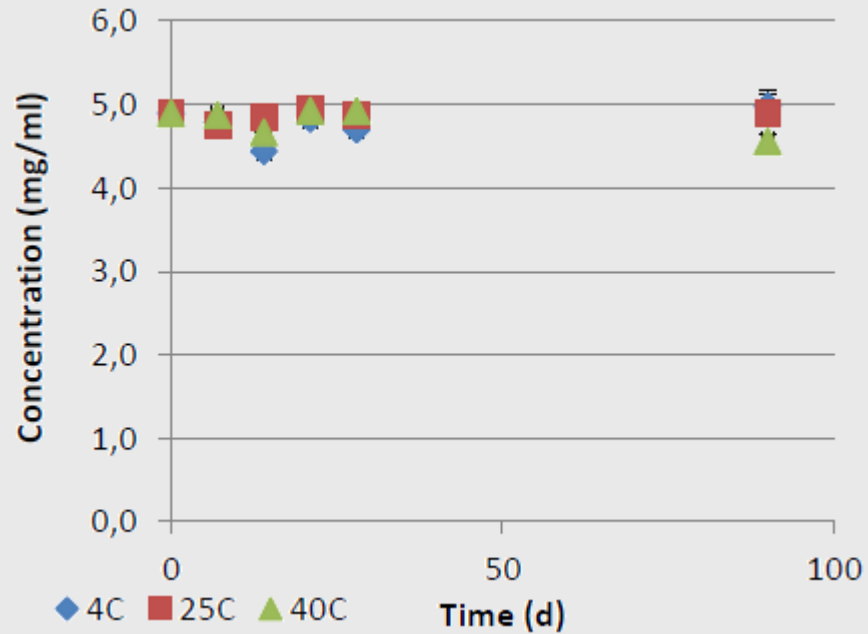
- Stable to T° and pH
- Highly resistant to human intestinal fluids
- Long half-life in digestive track



Profile of Nanofitins: Fit for formulation

Long-term stability of PBS formulation

- Nanofitin (5 mg/ml) PBS
pH7.4, filter-sterilized
- Storage @ 4, 25 and 40°C
- **Read-out** : Analysis by
UPLC-UV/MS

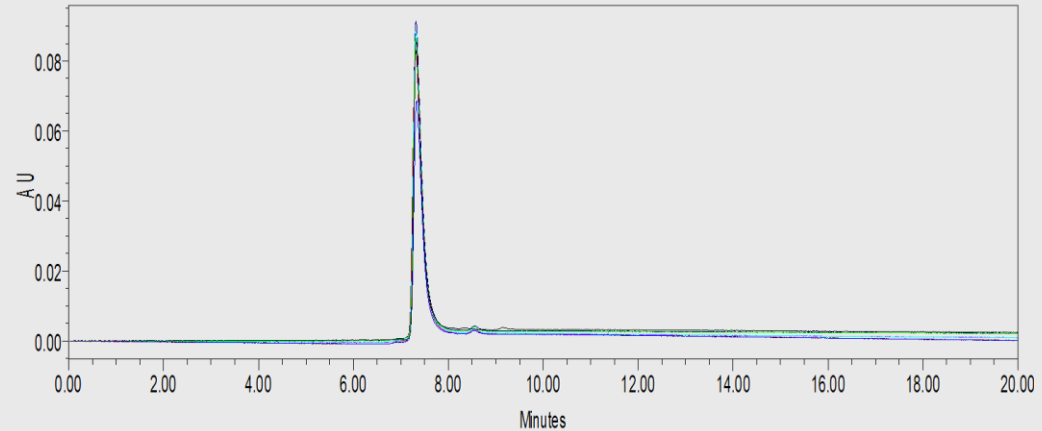


- Nanofitins are stable up to 40°C for three months.

Profile of Nanofitins: Fit for formulation

Stability to stress

- Nanofitin (5 mg/ml) PBS pH7.4, filter-sterilized
- Storage @ 40°C with shaking or shearing up to 72h
- **Read-out** : Analysis by SEC

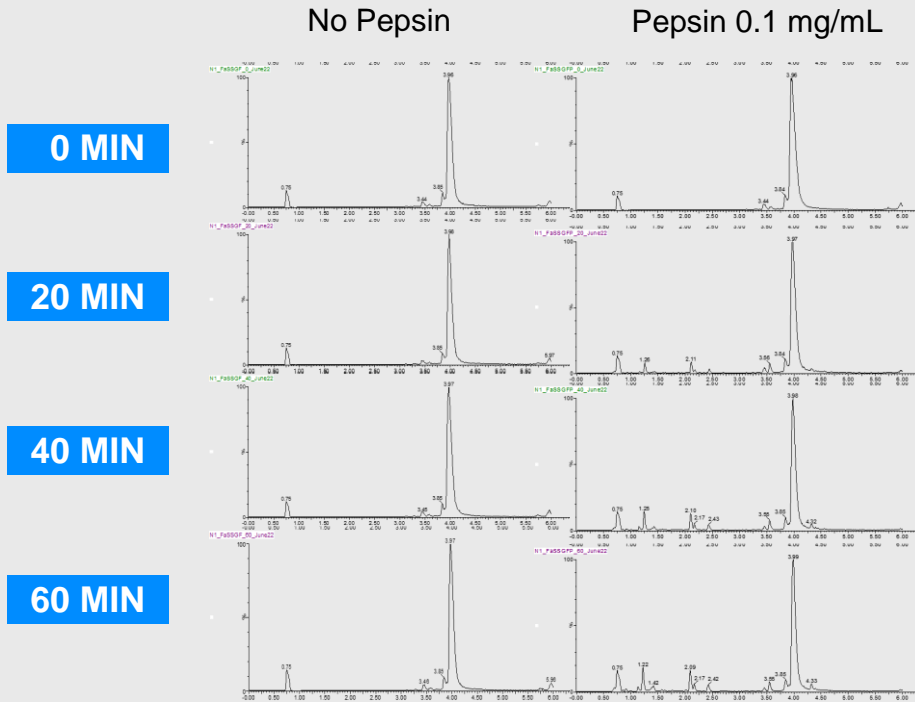


- **Clear solution, no visible precipitation**
- **No soluble aggregates visible by SEC**

Profile of Nanofitins: Fit for oral treatment

Stability in biorelevant simulated fluids

Fasted Simulated
Gastric Fluid
(FaSSGF) pH 1.6

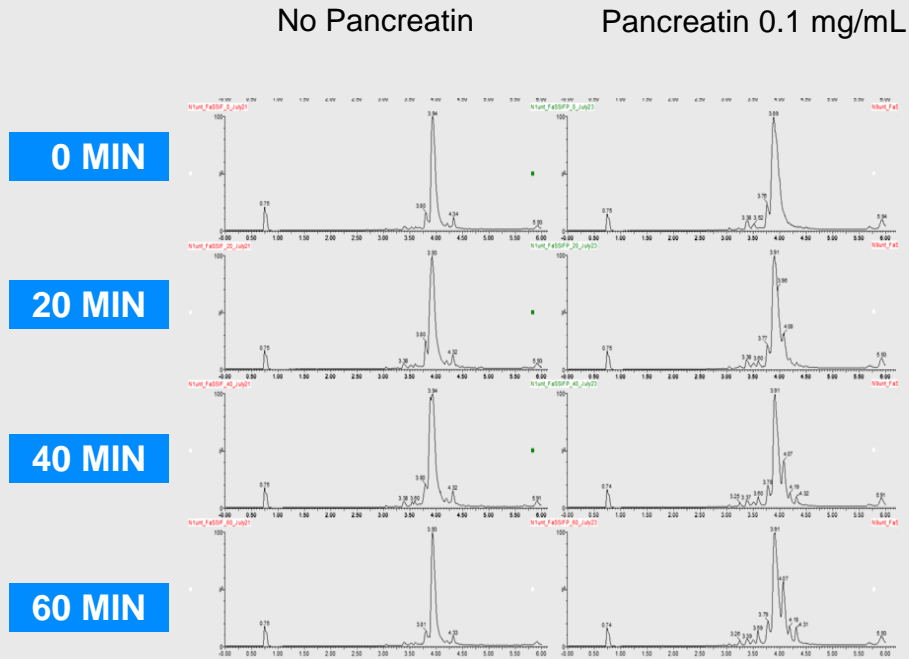


➤ Nanofitins are highly stable to very acidic pH and protease degradation.

Profile of Nanofitins: Fit for oral treatment

Stability in biorelevant simulated fluids

Fasted simulated
Intestinal Fluid
(FaSSIF) pH 6.5

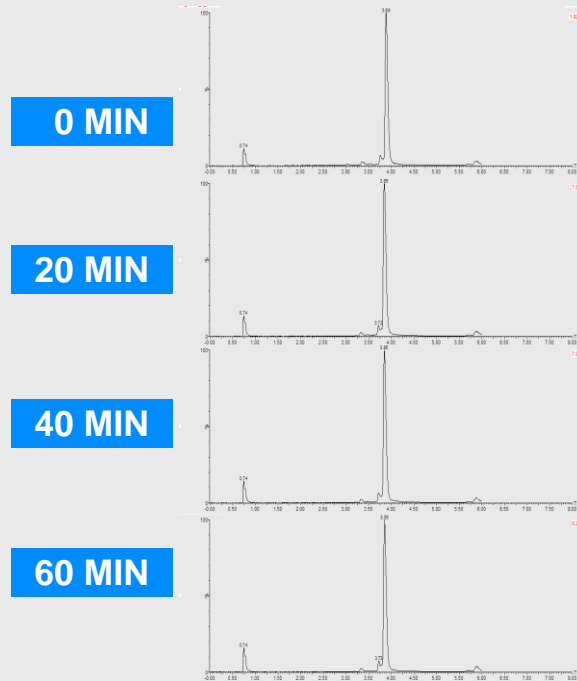


➤ Nanofitins show a high stability profile in simulated intestinal fluids

Profile of Nanofitins: Fit for oral treatment

Stability in biorelevant simulated fluids

Simulated Colon Fluid
(SCoF) pH 5.8



➤ Nanofitins show a high stability profile in colon fluids.

Nanofitins, a novel family of proteins

Alternative to antibodies

Affordable biologics

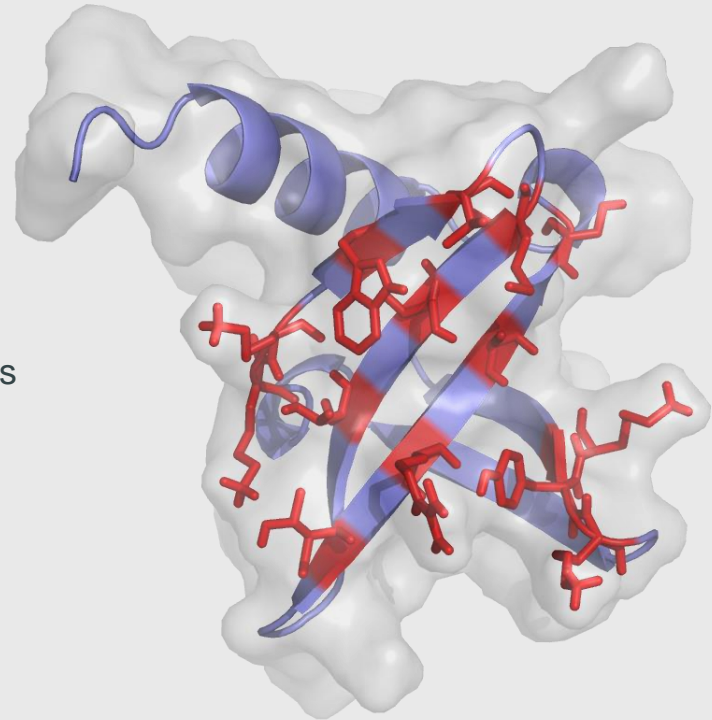
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Fit for oral administration

- Stable to T° and pH
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- Long half-life in digestive track

With demonstrated efficacy

- Preclinical efficacy
- Preventive and curative modes

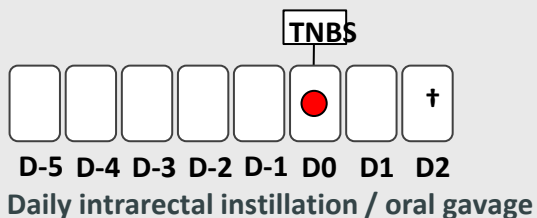


Screening 9 anti-TNF α Nanofitin candidates

Gold standard Model of colitis induced by TNBS



Preventive effect / Intrarectal > Oral

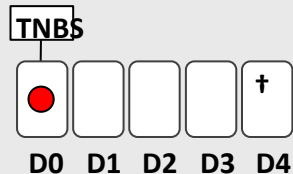


Nanofitins

5-ASA control

Curative effect / Oral

Daily oral gavage



Nanofitins

Prednisolone

- Mortality rate
- Macroscopic score
- Histologic score
- Collection of samples

Preventive set-up intrarectally

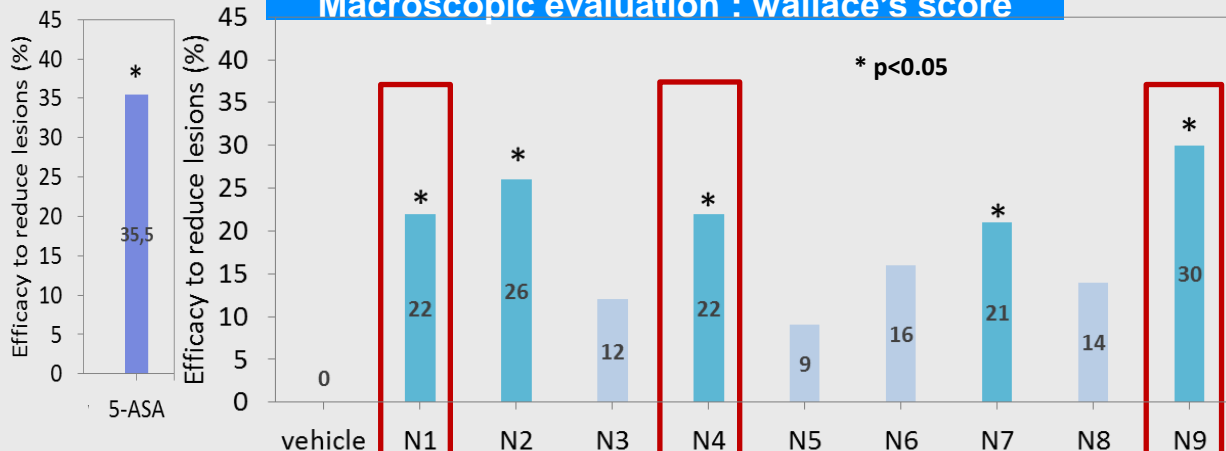
Nanofitins in PBS pH7.4
@ 10 mg/kg

C57bl6 mice
n = 20 mice/group

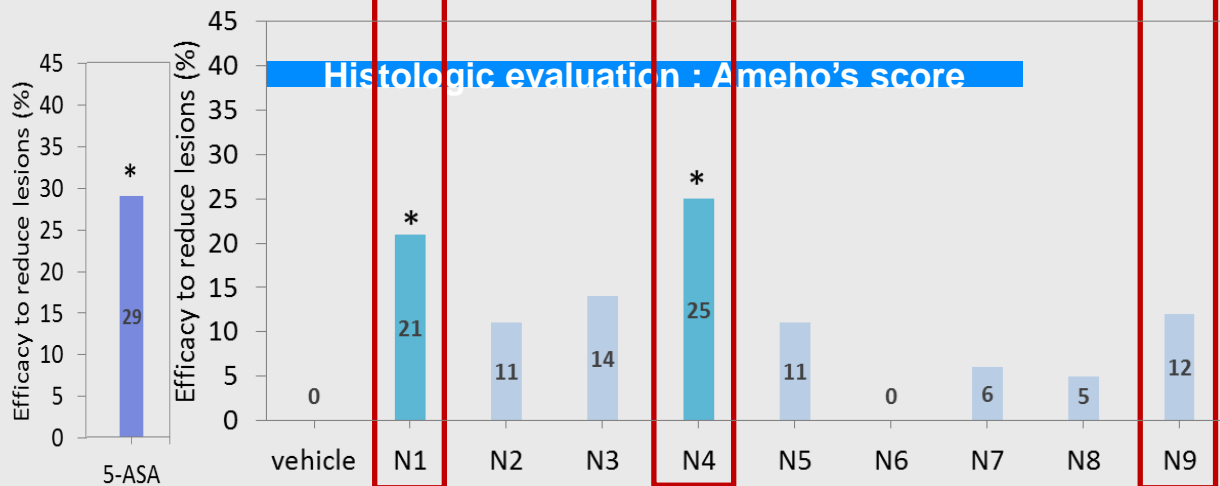


- Nanofitins locally reduce inflammation
- Efficacy confirmed in a DSS-induced model
- Good correlation between *in vivo* data and data obtained by Surface Plasmon Resonance (SPR)

Macroscopic evaluation : wallace's score



Histologic evaluation : Ameho's score



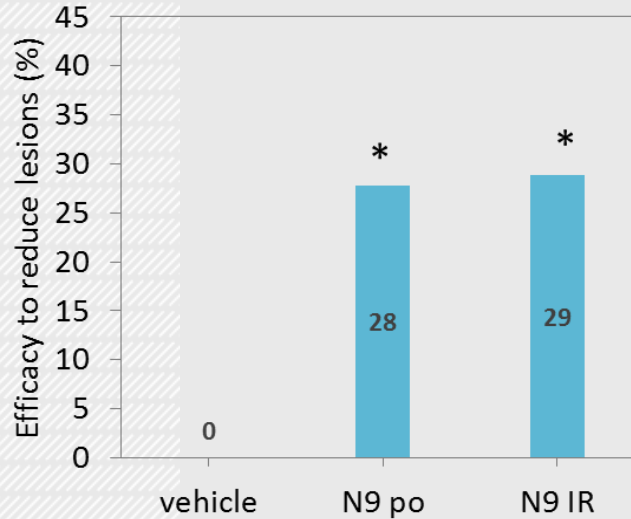
Comparison intrarectal vs. oral effect

C57bl6 mice
n = 20 mice/group



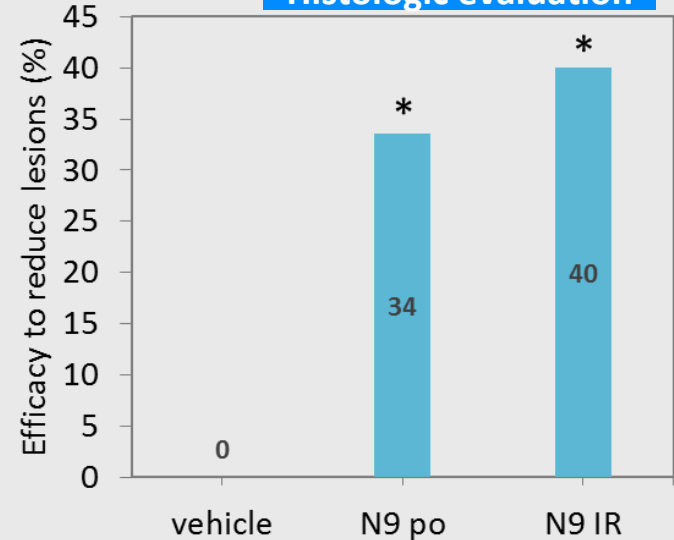
Nanofitin N9 in PBS pH7.4 @ 10 mg/kg

Macroscopic evaluation



Wallace's score

Histologic evaluation



Ameho's score

**Similar anti-inflammatory properties of N9
after intrarectal and oral administration**

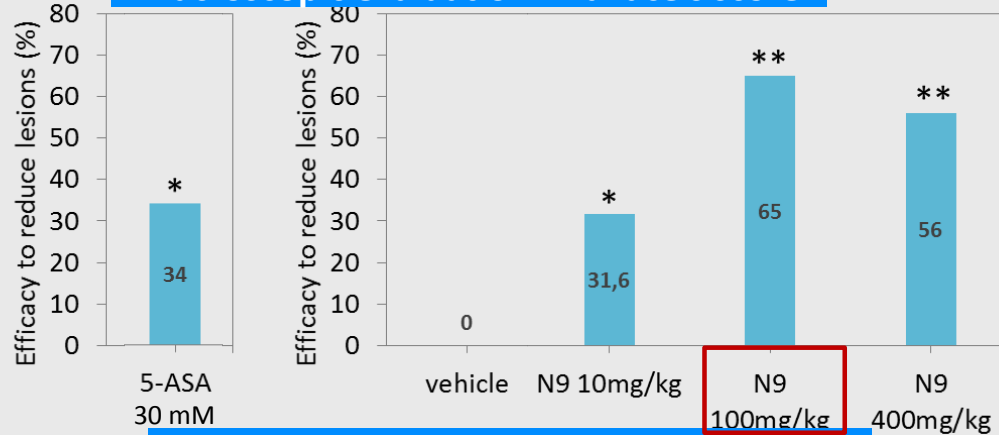
Preventive set-up orally: dose response

Nanofitin N9 in PBS
pH7.4
@ 10, 100 and 400 mg/kg
C57bl6 mice
n = 20 mice/group

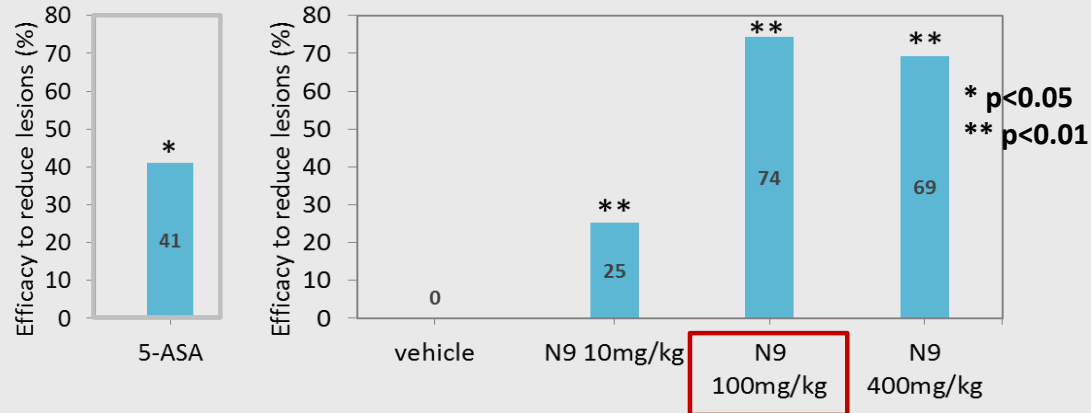


Higher preventive effect @100 mg/kg by oral administration

Macroscopic evaluation: Wallace's score



Histologic evaluation : Ameho's score



Curative set-up orally

Nanofitins in PBS pH7.4

@ 100 mg/kg

C57bl6 mice

n = 20 mice/group



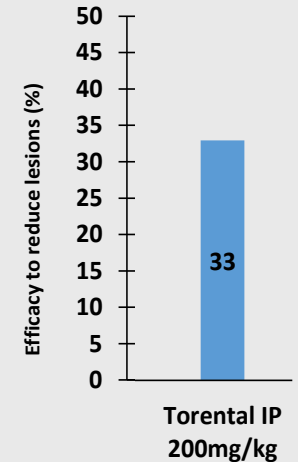
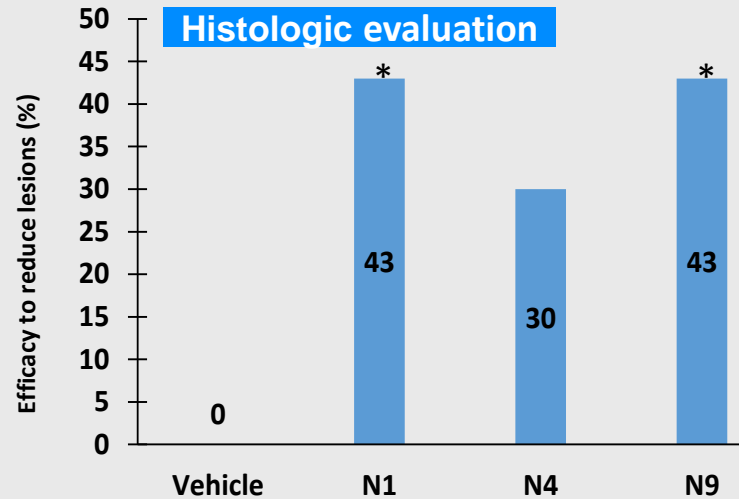
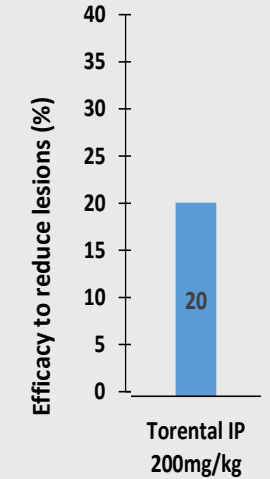
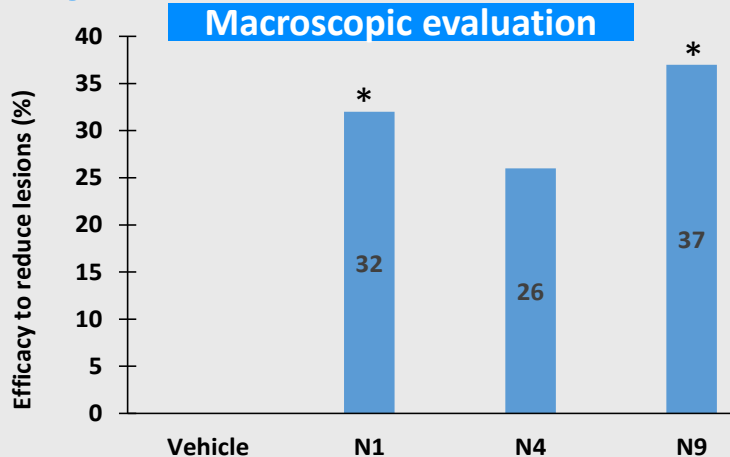
* p<0.05

Wallace's score

Efficacy confirmed orally
in a curative model

-> Very good stability of
Nanofitins without any
formulation optimization

Ameho's score



Second generation Nanofitins

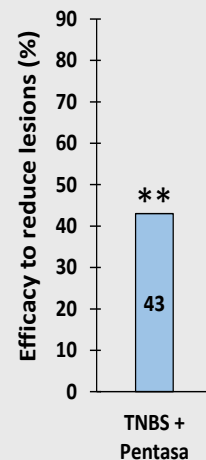
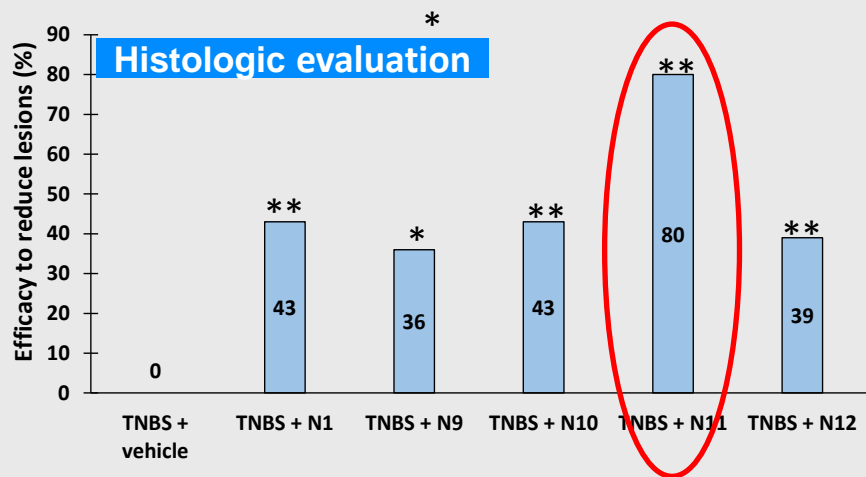
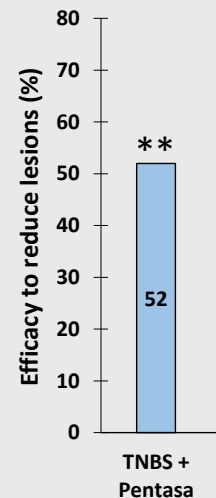
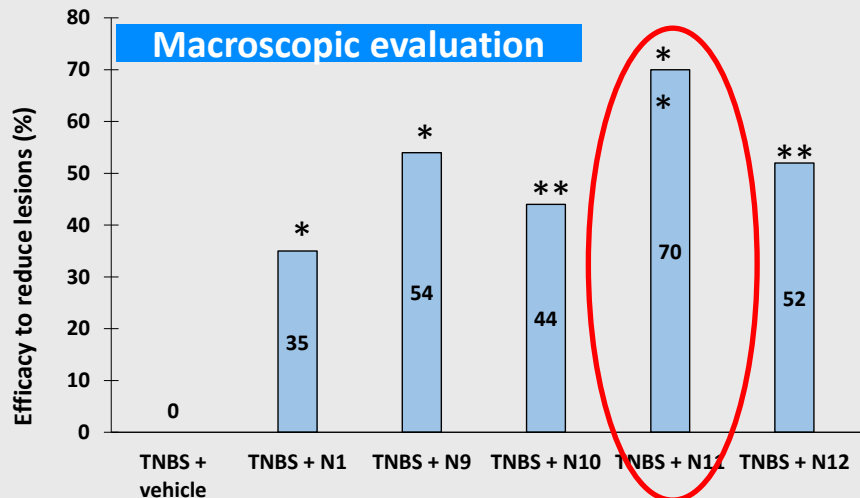
Nanofitins in PBS pH7.4
@ 10 mg/kg
C57bl6 mice
n = 20 mice/group



* p<0.05
** p<0.01

Wallace's score

Comparison of efficacy in
preventive mode



Ameho's score

The SADEL talent chain



Mathieu Cinier
Justine Picot
Simon Huet
Olivier Kitten



Magali Zeisser Labouèbe
Robert Gurny
Leonardo Scapozza



Ilaria Bonetti



Georges Kalias
Piyi Papadaki
Manolis Roulis



Joao V. Rodrigues
Antonio Cunha
Manuel Carrondo



Julia Gabriel
Johannes Meier



Christel Rousseaux
Nghiep Truong Tan



Andrew Sadler
Thomas Jorgensen
Daniel Plaksin



Raquel Fortunato
Teresa Alves

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Next steps – SADEL and Beyond

Document MoA/Tox

- Move towards the clinic

After Oral administration

- Bioavailability ?
- Towards oral antibody-mimetics

Beyond TNF

- Other IBD-related cytokines
- Oncology targets
- Microbiome modulation

