DEVELOPMENT OF A NOVEL ANTIBODY-BASED ORAL FACTOR VIIIa-MIMETIC DRUG CANDIDATE - Inno8

CONTROLLED RELEASE SOCIETY – ANNUAL MEETING

PHILADELPHIA, USA 14 – 18 JULY 2025

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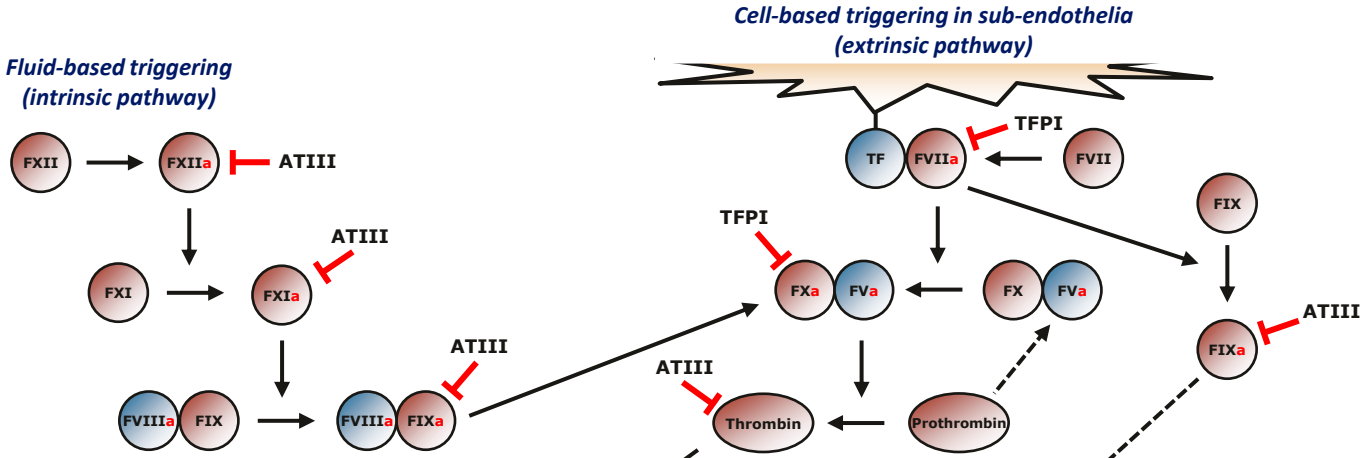
Oral Factor VIIIa mimetic project

Inno8

The **project aspiration** is to develop an orally administrated next-generation Factor VIII mimetic drug for convenient prophylactic treatment of Haemophilia A

Coagulation Cascade and Hemophilia

Initiation phase



Propagation phase

Hemophilia

Approx. 1:5,000 male births (X-linked)

Types

- Haemophilia A: lack FVIII activity
- Haemophilia B: lack FIX activity
- Other (FXI, FXIII, FVII..)

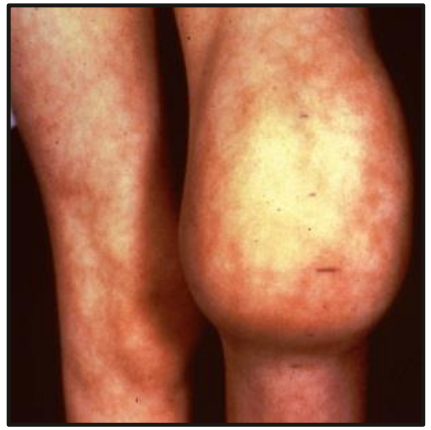
Severity

- Mild (5-50% activity)
- Moderate (1-5% activity)
- Severe (less than 1% activity)

Current treatments

- Plasma-derived factor products
- Recombinant factor products
- Bispecific antibody - Emicizumab

Typical joint bleeding in hemophilia patient



Cell-based coagulation model developed by Prof. Ulla Hedner and colleagues,
He S, Blombäck M, Jacobsson-Ekman G, Hedner U. (2003) J Thromb Haemost. 6:1215-9.

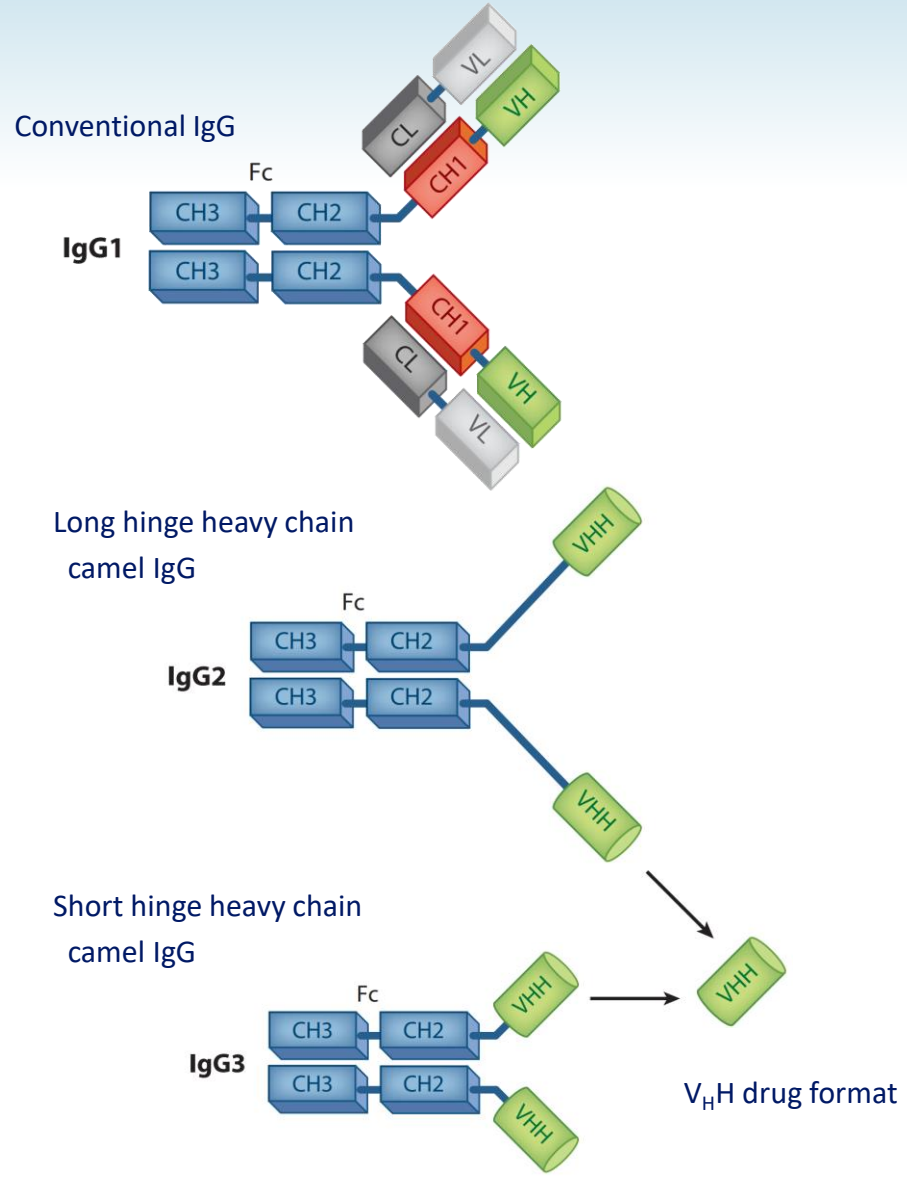
V_HHs

Camelid antibodies

- Camelids generate 3 types of antibodies
- Conventional IgG1
- Long hinge camel IgG2
- Short hinge camel IgG3

The V_HH format

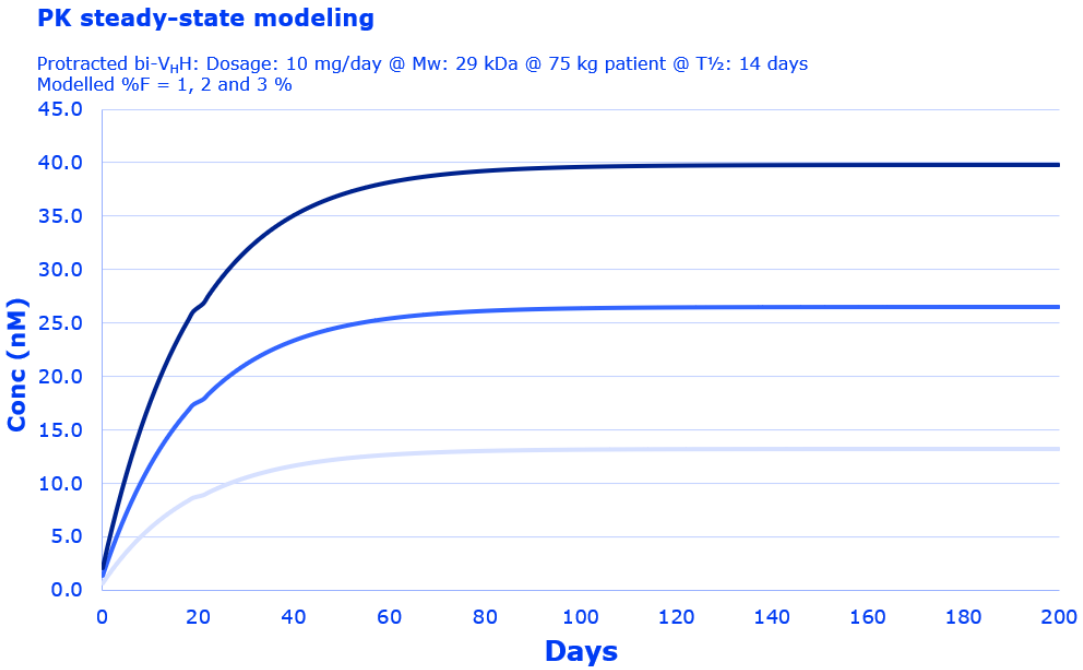
- V_HH: variable heavy domain of Heavy Chain antibody
- V_HH is small format (12-14 kDa) and can be expressed as a single domain with retained antigen binding
- Sequence similarity to human IgG is high

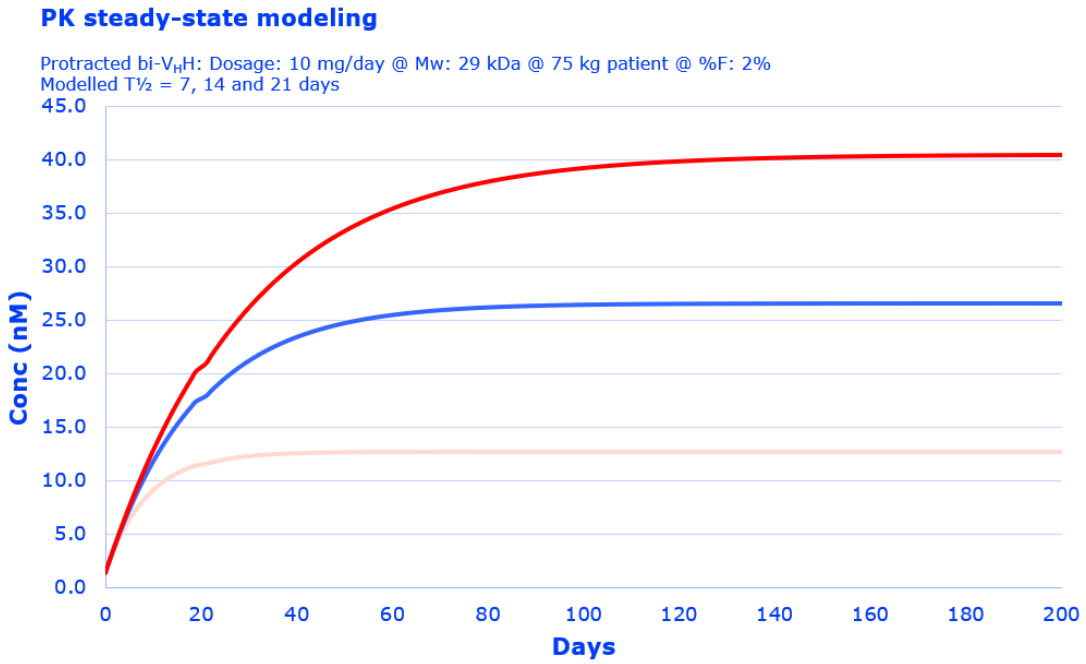


Muyldermans S. (2013) Nanobodies: natural single-domain antibodies. Annu Rev Biochem. 82: 775-97.

Hamers-Casterman C, Atarhouch T, Muyldermans S, Robinson G, Hamers C, Songa EB, Bendahman N, Hamers R. (1993) Naturally occurring antibodies devoid of light chains. Nature. 363: 446

V_HH as oral drug format





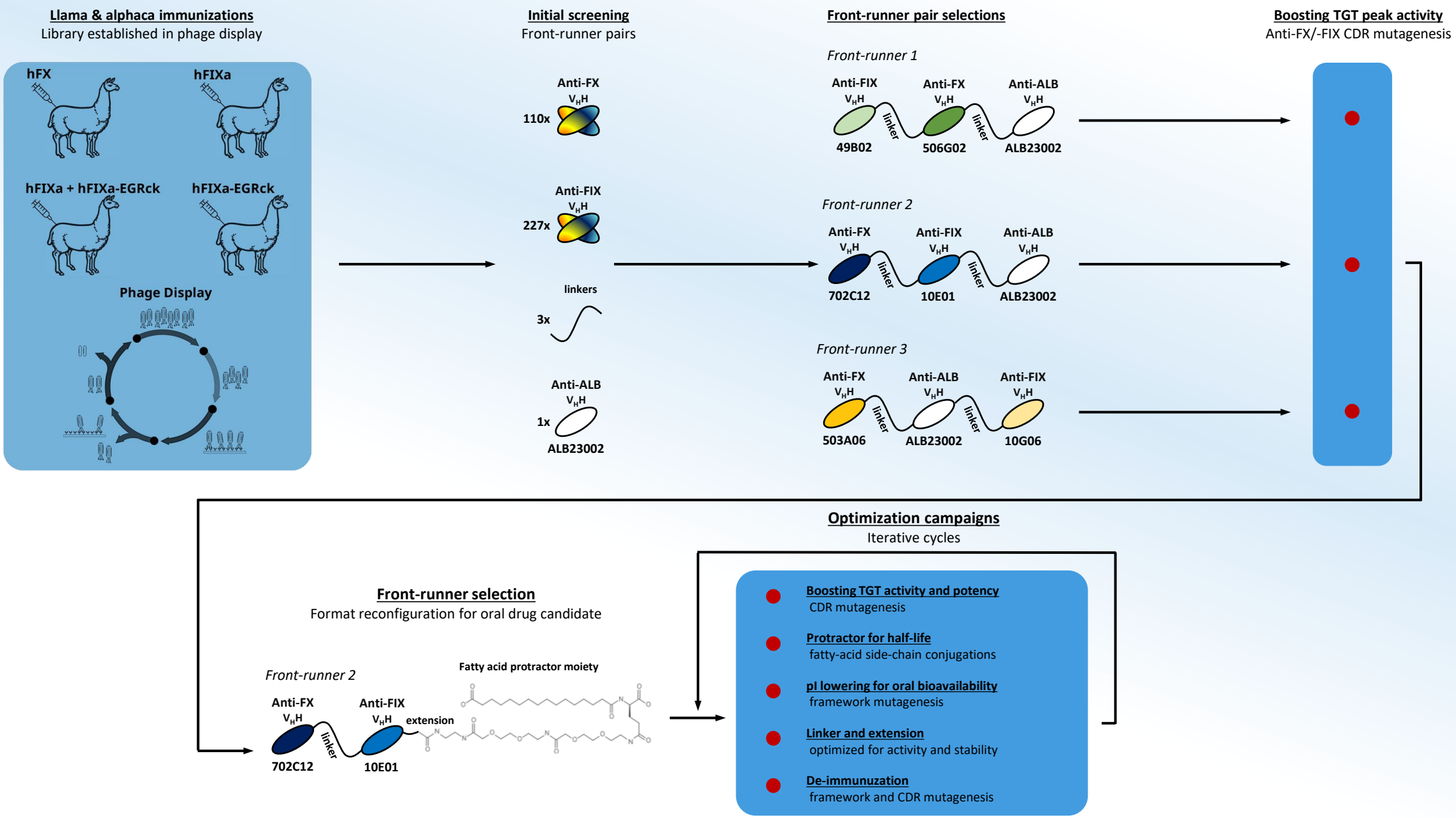
Basic principle for oral drug format

DOSAGE x POTENCY x HALF-LIFE x BIOAVAILABILITY

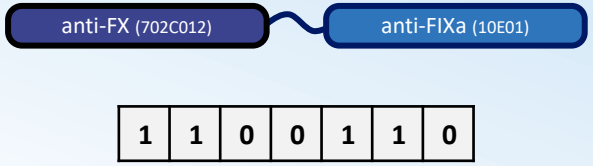
gives clinically relevant C_{ss}

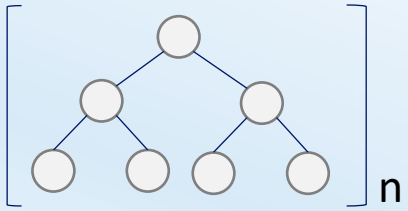
novo nordisk®

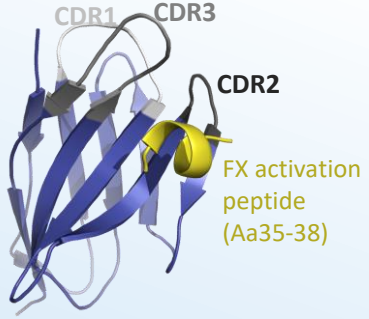
Inno8 | Screening for lead based on library from Ablynx

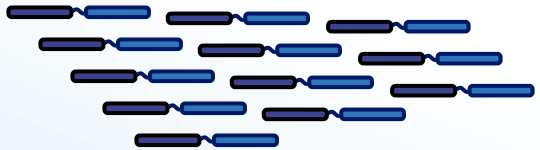


Inno8 | Boosting FVIIIa-mimetic activity via machine learning

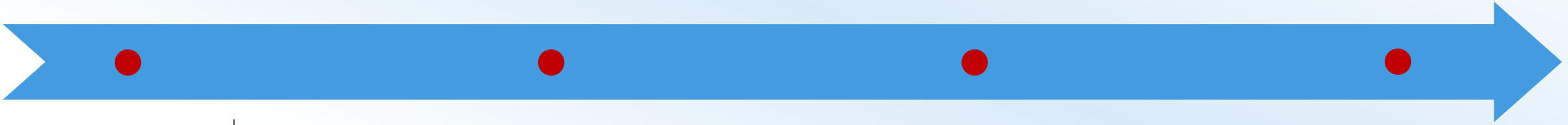


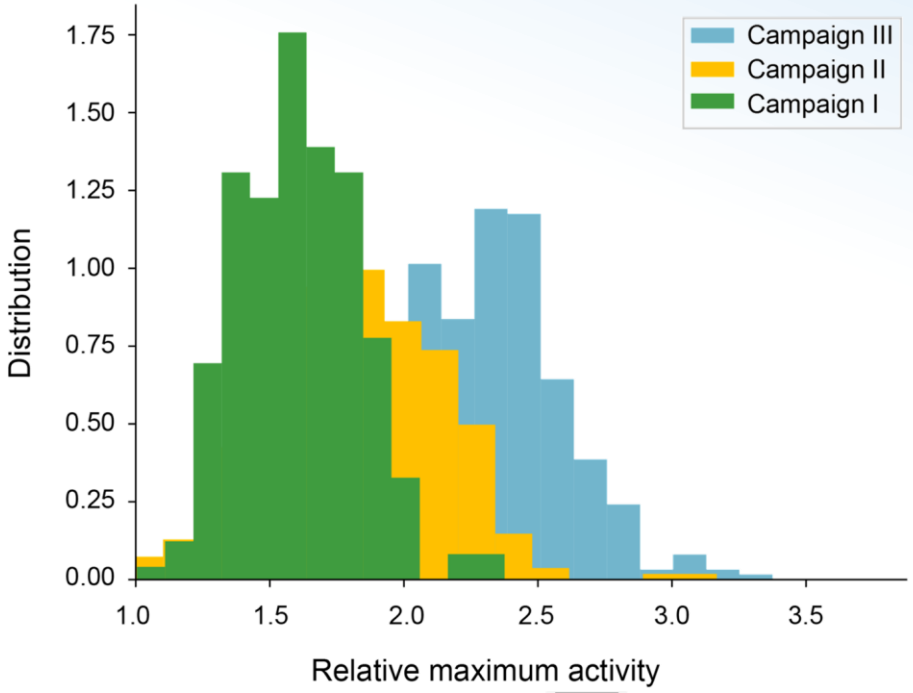


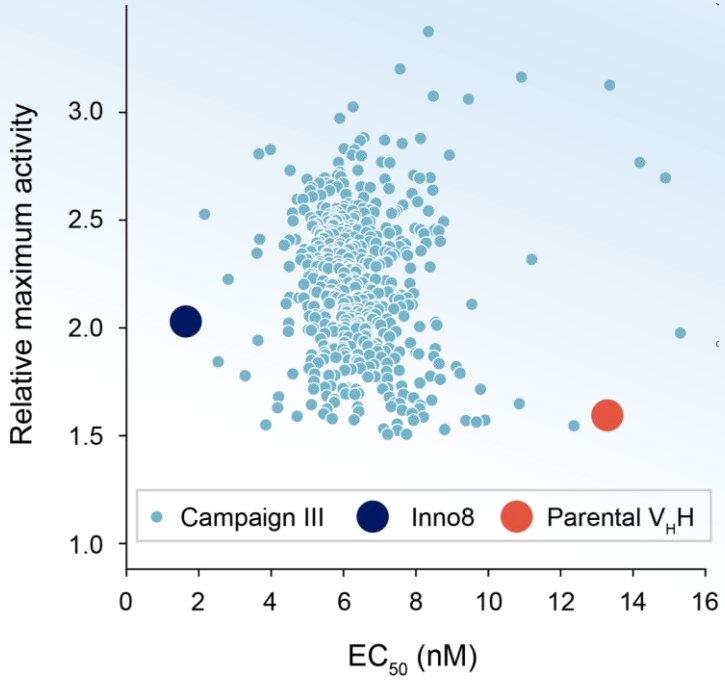




sequence encoding | random forest algorithm | interpret data on structure | new variant designs



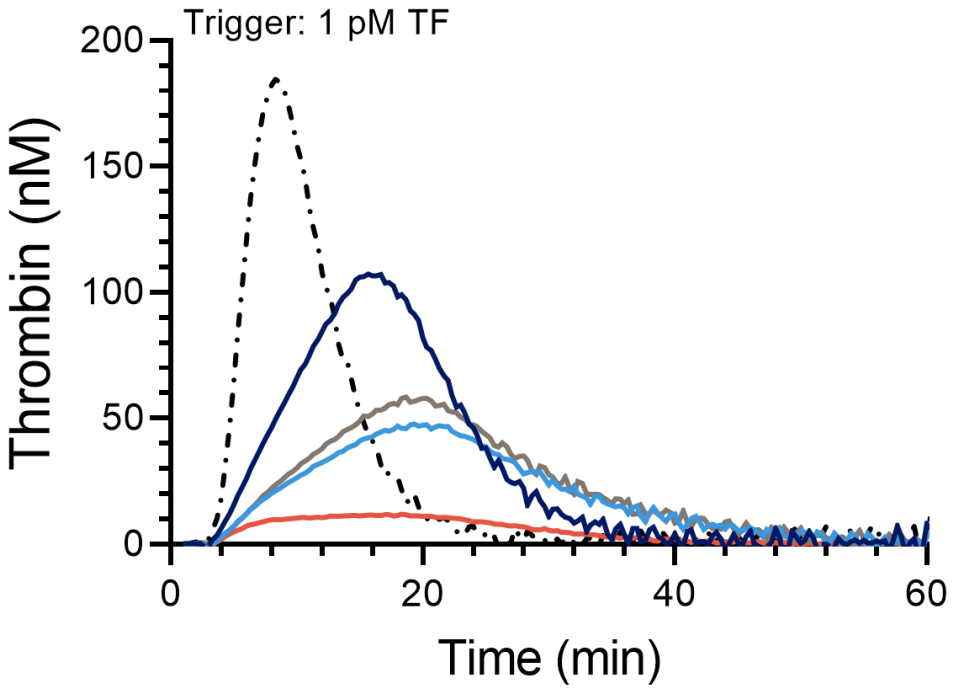


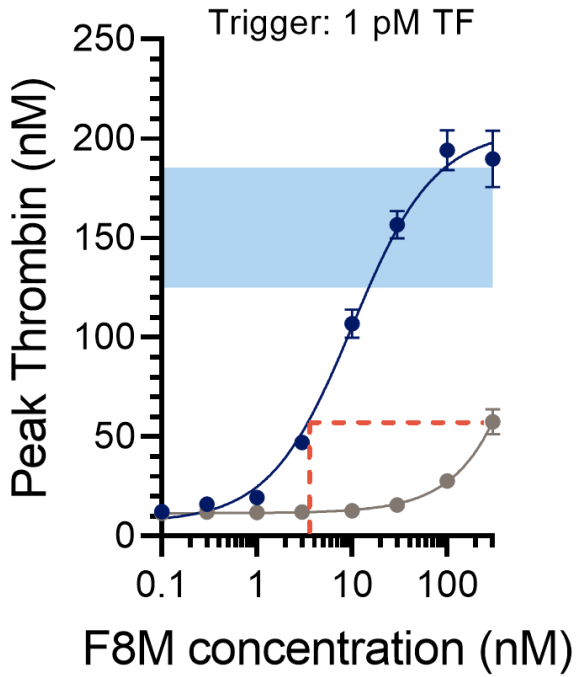


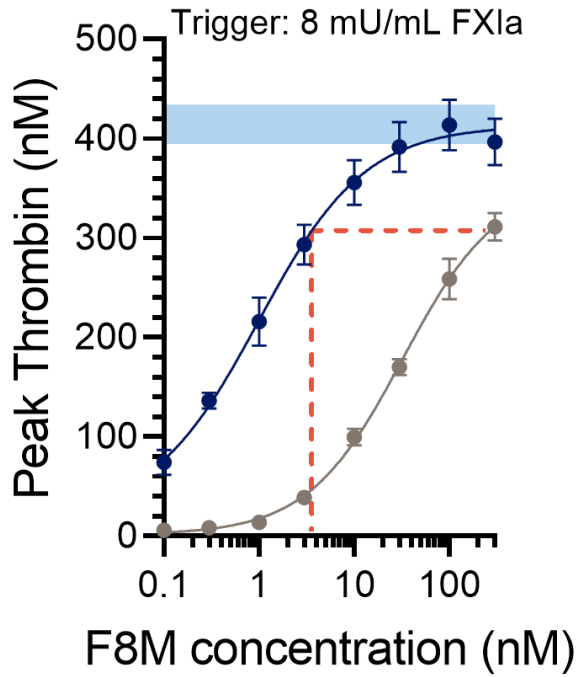
Inno8 | A very potent Factor VIIIa-mimetic molecule

Thrombin generation *in vitro* assay testing of Inno8

Tested with coagulation triggers tissue factor (TF) and factor XIa (FXIa)







Inno8 10 nM
Inno8 3 nM
HA control
Emicizumab SIA 300 nM
HA + 100 IU/dL FVIII

Inno8
Emicizumab SIA
Normal level

- Inno8 shows similar *in vitro* effect as Emicizumab SIA at ~90-folds lower molar concentrations.

The effect of FVIIIa mimetics on *in vitro* thrombin *generation does* not necessarily translate directly to clinical efficacy.

SIA = Sequence identical analogue, SD = standard deviation

Inno8 | Screening for fatty acid conjugation site

NB bi-V_HH model sequence, not Inno8

$V_HH_{anti\text{-}FX}$

CDRs: A...A

```
1         10        20        30        40        50        60        70        80        90        100       110       120
DVQLVESGGGVVQPGGSLRLSCAASAAAAAAAAAAWFRQAPGKEREFVAAAAAAAAAAAYADSVKGRFTISRDNSKNTVYLQMNSLRPEDTALYYAAAAAAAAAAAAAAWGQGTLVTVSS
```

```
↑↑↑↑↑↑ ↑↑↑   ↑↑↑↑↑↑↑↑↑   ↑↑↑↑↑↑   ↑ ↑ ↑↑↑↑↑
CCCCCC CCC   CCCCCCCCC   CCCCCC   C C CCCCC
 ↓↓↓   ↓↓     ↓↓↓   ↓↓     ↓ ↓  ↓    ↓ ↓ ↓↓↓↓ ↓
 EEE   EE     EEE   EE     E E  E    E E EEE E
  ↓↓           ↓↓   ↓           ↓          ↓
  FF           FF   F            F          F
```

$V_HH_{anti\text{-}FIX}$

```
1         10        20        30        40        50        60        70        80        90        100       110       120
EVQLVESGGGVVQPGGSLRLSCAASAAAAAAAAAWFRQAPGKEREFVAAAAAAAAAAAYADSVKGRFTISRDNSKNTVYLQMNSLRPEDTALYYAAAAAAAAAAAAAAAAAAWGQGTLVTVSS
```

```
↑↑↑↑↑↑↑↑↑↑   ↑↑ ↑↑ ↑↑   ↑↑↑↑↑↑↑↑↑↑   ↑↑ ↑↑↑↑ ↑
CCCCCCCCCC   CC CC CC   CCCCCCCCCC   CC CCCC C
 ↓↓↓↓↓↓↓     ↓↓ ↓↓ ↓     ↓↓↓↓↓ ↓↓↓     ↓ ↓
 EEEEEEE     EE EE E     EEEEE EEE     E E
  ↓↓↓↓↓↓      ↓ ↓↓ ↓      ↓↓↓↓          ↓
  FFFFFF      F FF F      FFFF          F
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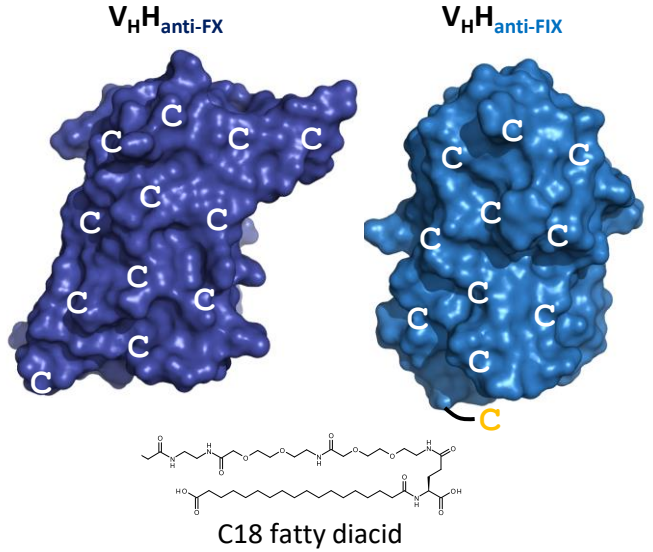


C18 fatty diacid

C-term seq. extension

```
1         10        1
GGGGSGGGSCHHHHHH    GQACPC
```

```
↓                   ↓ ↓
E                   E E
↓                   ↓ ↓
F                   F F
```

Symbols

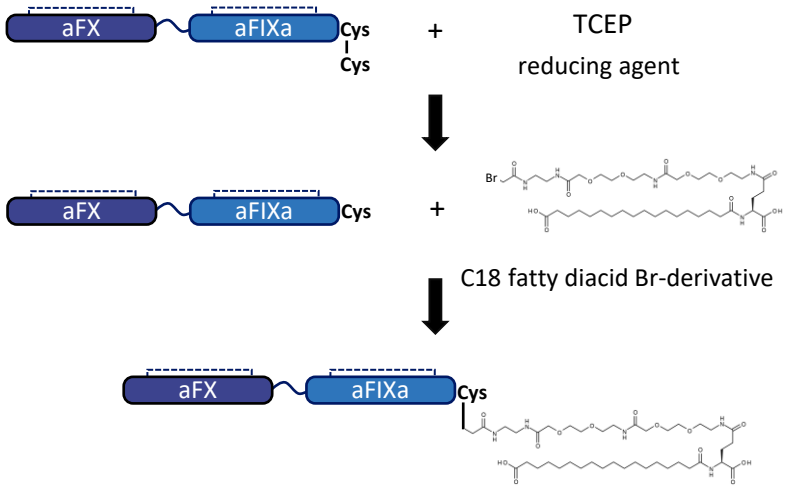
C: Site for introduced unpaired Cys

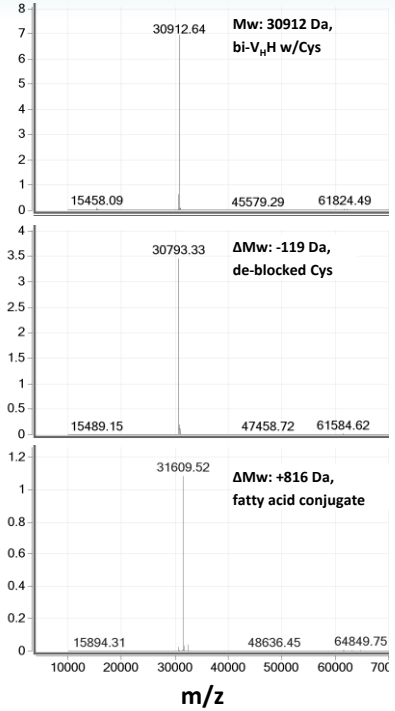
E: Expressable / purifiable bi-V_HH variant with free Cys

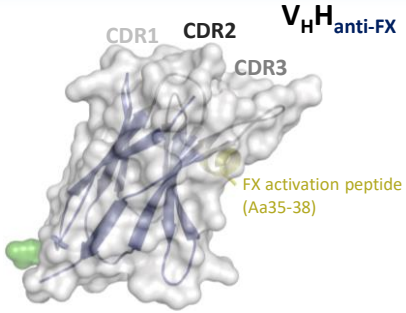
F: Conjugatable bi-V_HH variant with C18 fatty diacid

Inno8 | Screening for fatty acid conjugation site

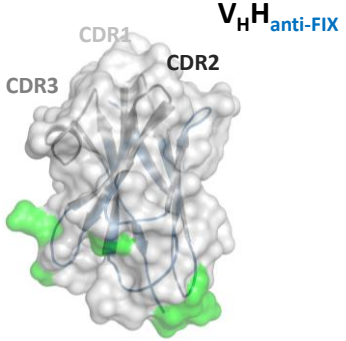
Conjugation





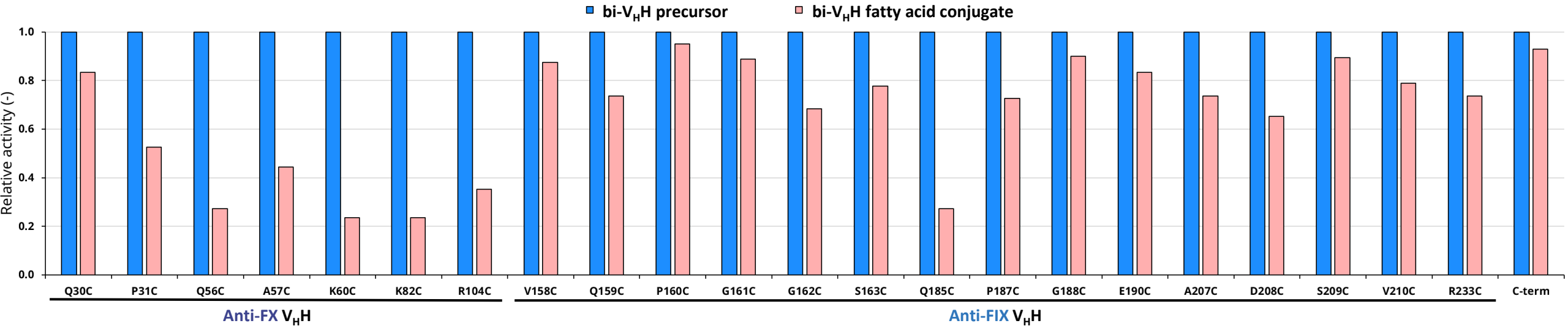


Θ: allowable sites

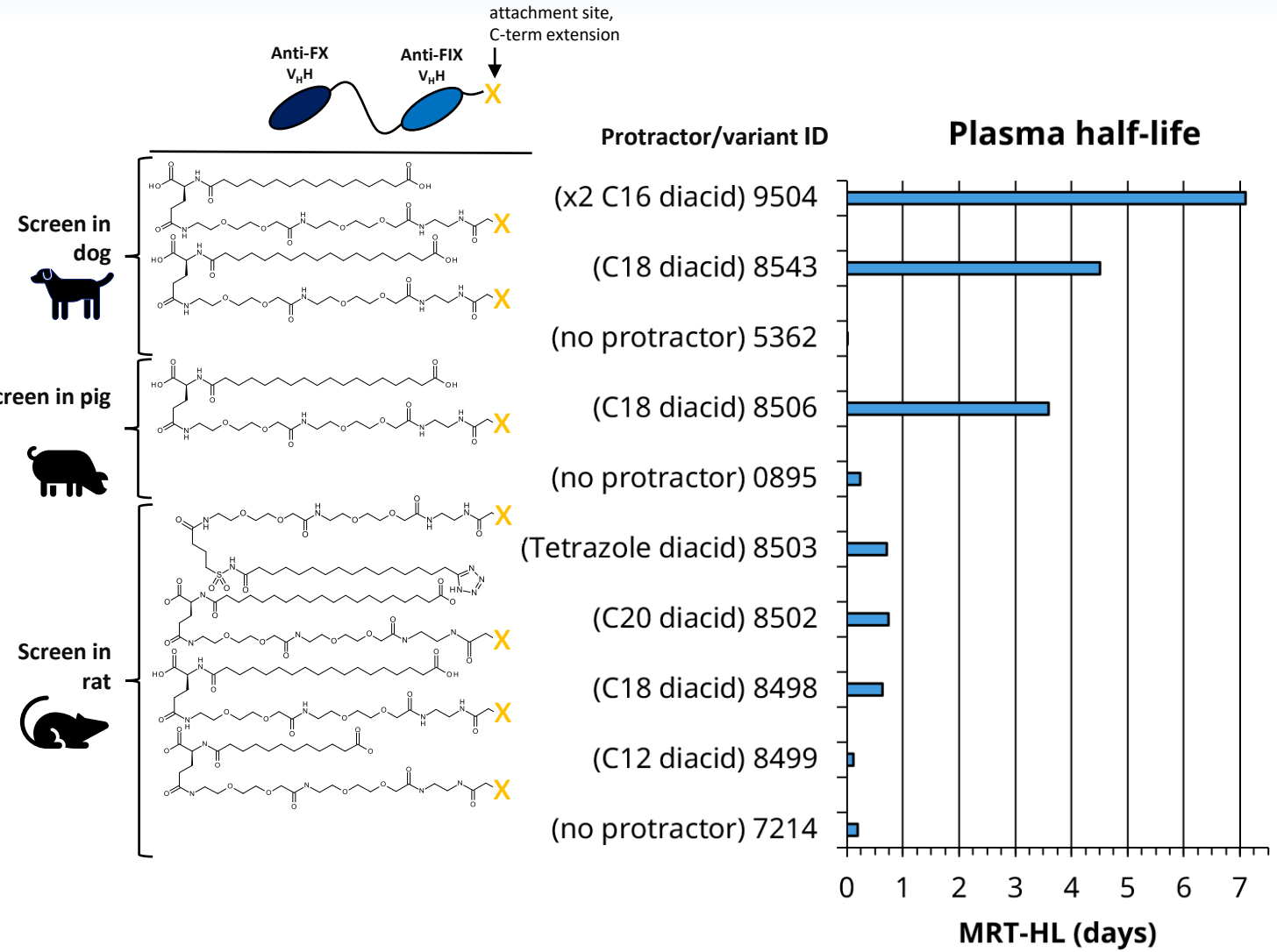


- Bi-V_HH screening for fatty acid conjugation sites identified expressable variants and conjugatable surface exposed sites.
- Penalty on activity of most fatty acid conjugation sites on anti-FX V_HH, while several allowable conjugation sites identified on anti-FIX V_HH.
- C-term extension of anti-FIX V_HH part chosen as fatty acid conjugation site for Inno8.

TGT activity assay

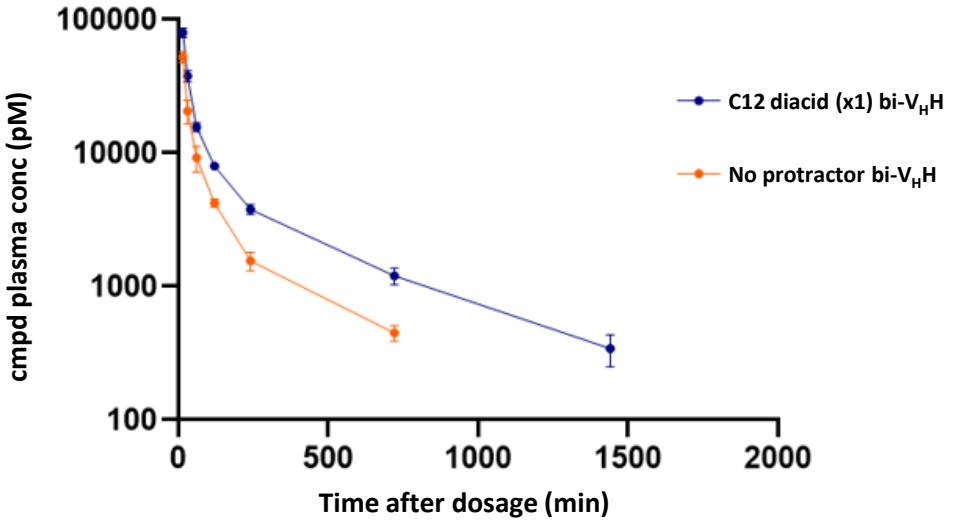


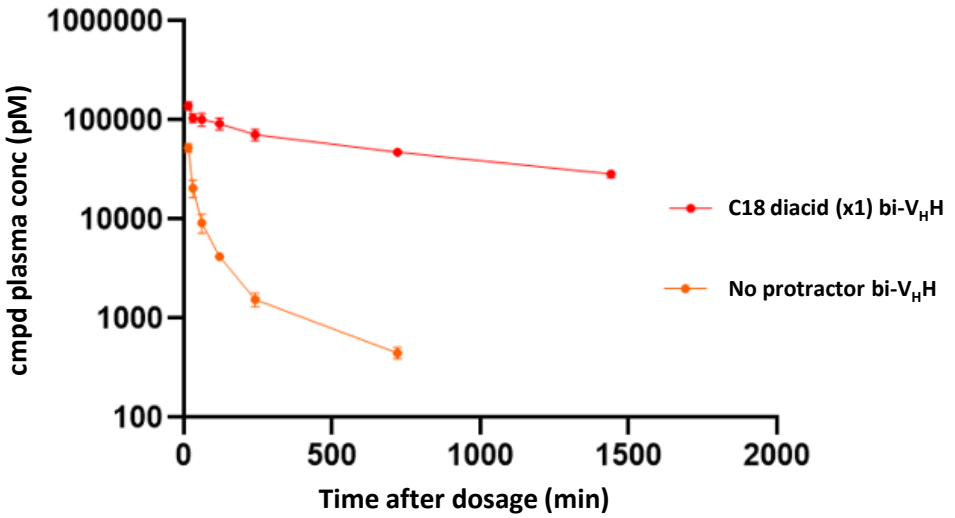
Inno8 | Fatty acid bi-V_HH conjugates screening *in vivo*



Example screening fatty diacid V_HH conjugates in IV rat

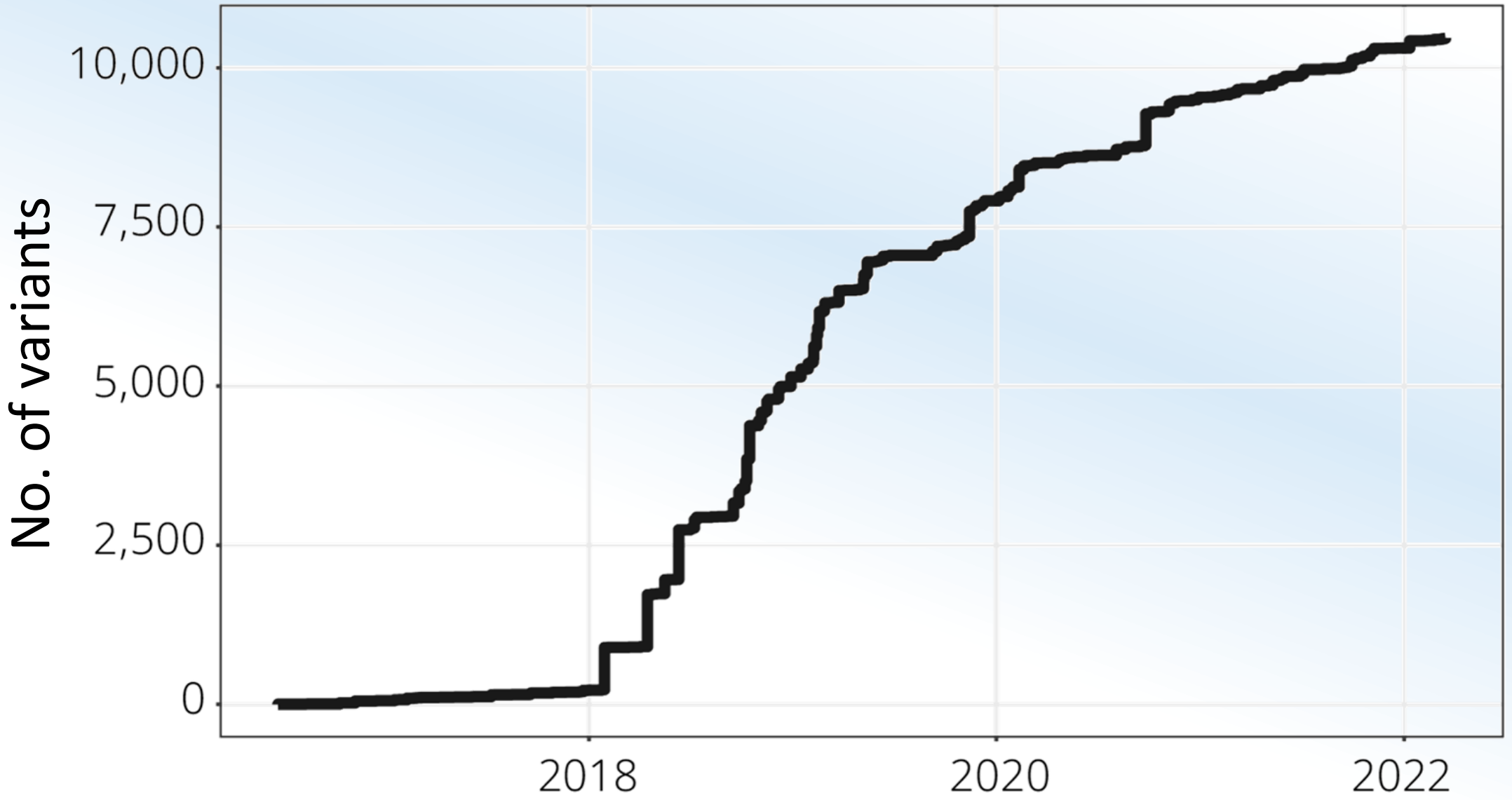
No protraction vs. C12 diacid vs. C18 diacid





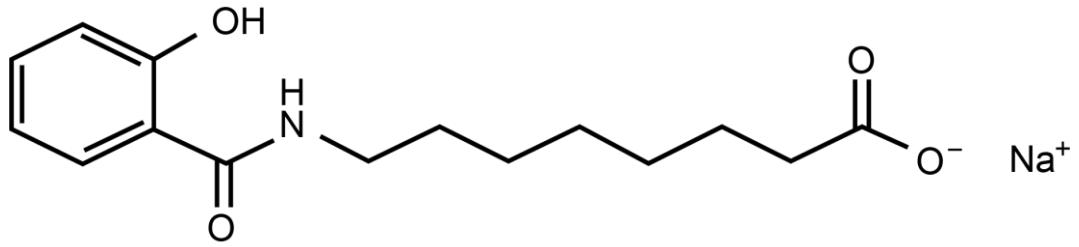
- Fatty diacid conjugates of bi-V_HHs identified as protractors that provide orders-of-magnitude enhanced half-life compared to non-protracted bi-V_HH.
- Double C16 fatty diacid chosen as protractor for Inno8 based on *in vivo* screenings.

Inno8 | Screened variants in numbers

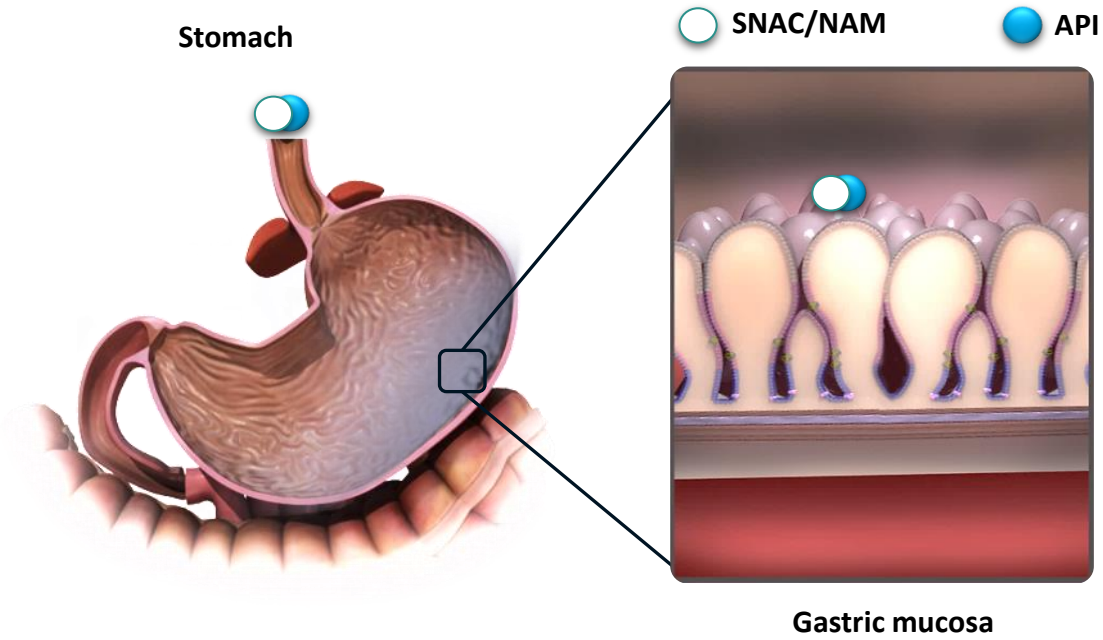


Inno8 | API tablets with absorption enhancer SNAC and NAM

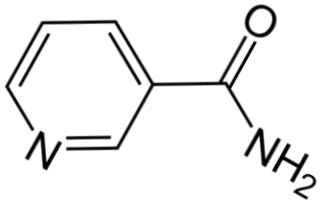
SNAC Sodium N-[8-(2-hydroxybenzoyl)amino]caprylate



- SNAC: Small fatty acid derivative API absorption enhancer
- Absorption takes place in the stomach in a local buffered environment
- The effect is time-dependent and occurs primarily via transcellular route
- SNAC have been used for development of oral GLP-1 (peptide) drug



NAM niacinamide



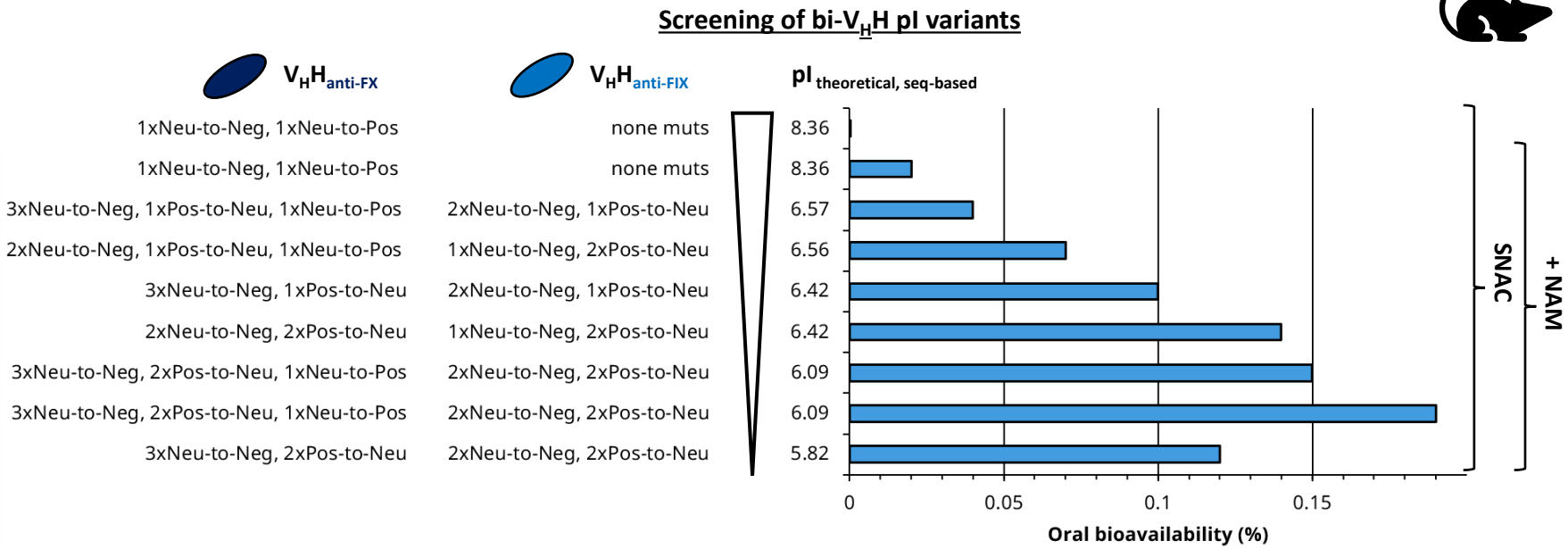
- NAM: B3 vitamin precursor and used as formulation excipient
- NAM shows efficient solubilizing effects, especially for Inno8 and derived bi-V_HHs
- SNAC/NAM co-formulation show enhanced effect on oral uptake of Inno8 and derived bi-V_HHs

Inno8 | Screening of pI variants for oral bioavailability

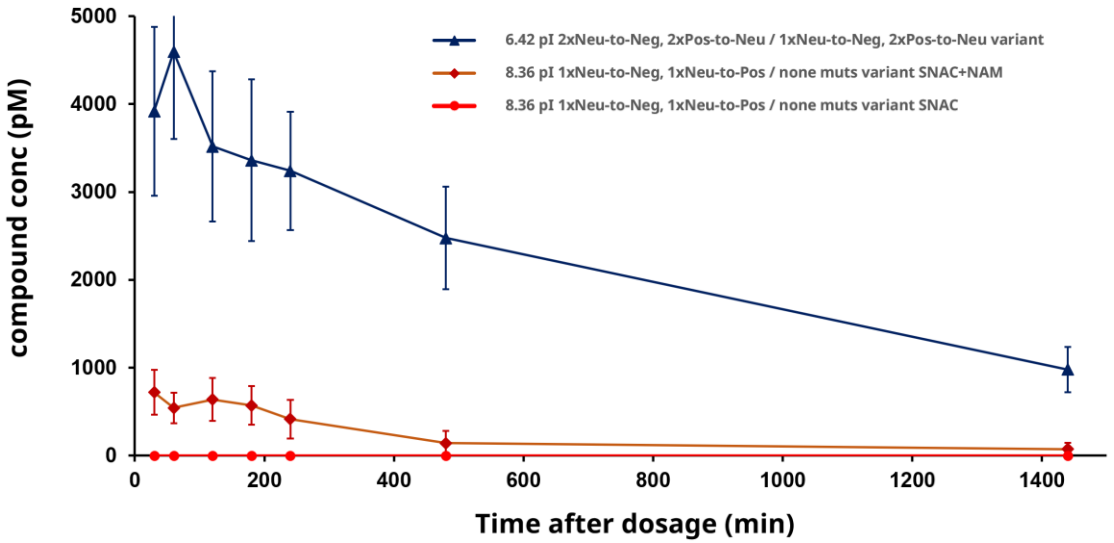
Variant screening *in vivo*

oral gavage rat model

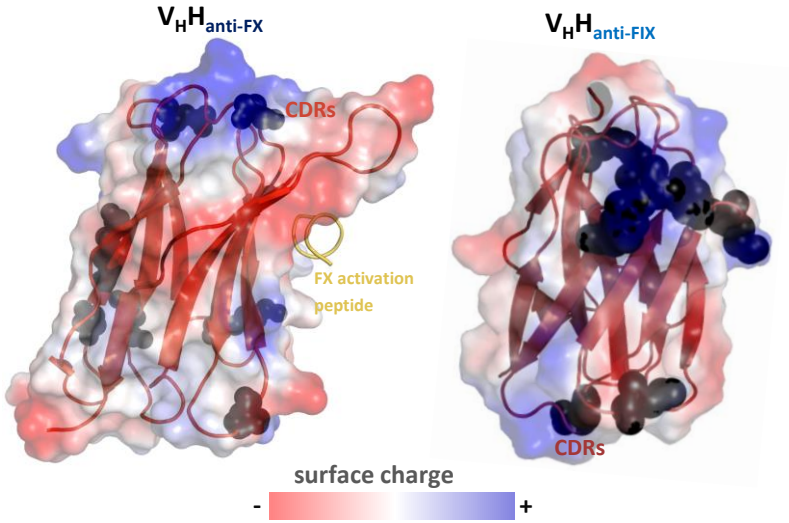
Screening of bi-V_HH pI variants



Example of oral gavage screening in rat



pI surface residues investigated for effect on oral uptake

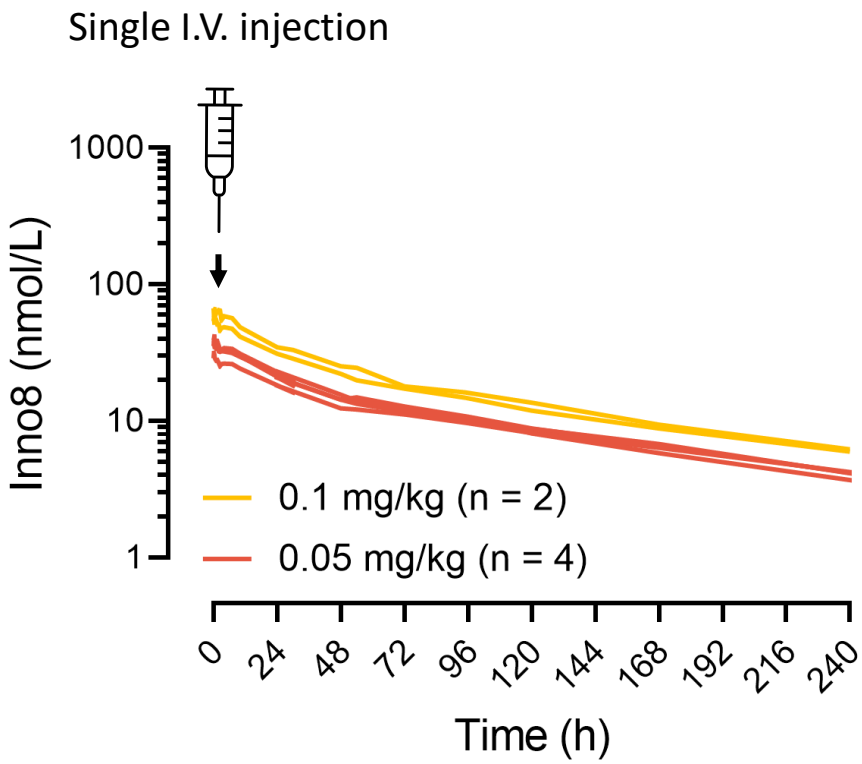


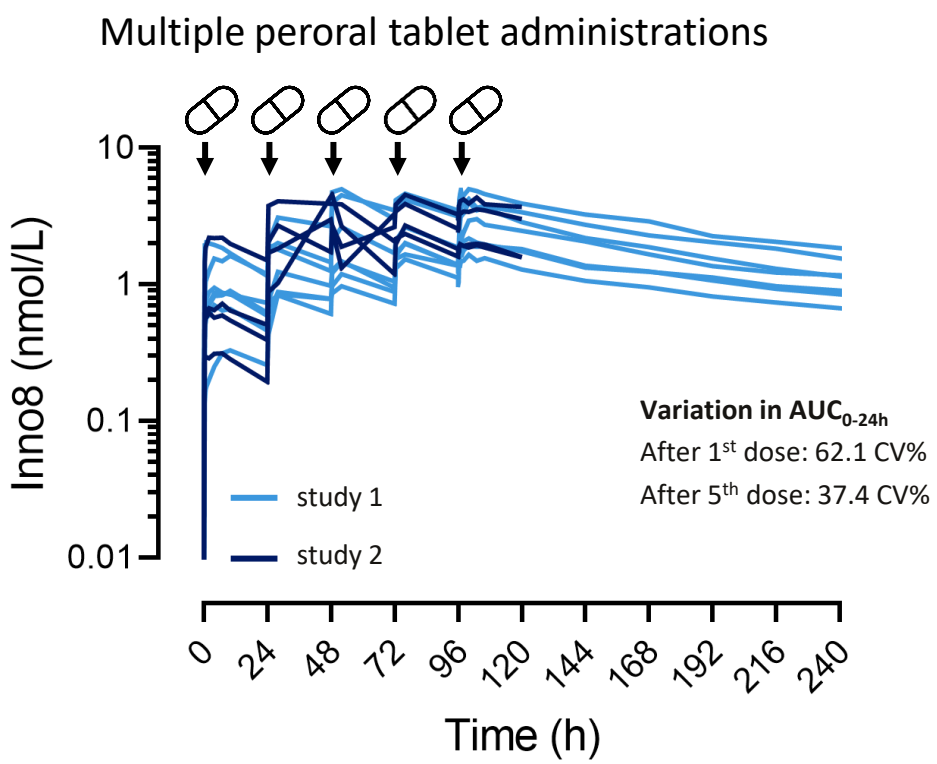
- Improved effect on oral uptake in oral rat model by lowering pI and introducing negative or neutralizing surface charges of bi-V_HHs.
- Selected mutations used in Inno8 to lower pI from 8.3 to 5.2.

Inno8 | Oral uptake of Inno8 tablet in dog

Tablet formulation testing *in vivo*

Trained beagle dogs for testing pharmacokinetics of Inno8 administered intravenously and orally in SNAC/NAM tablets

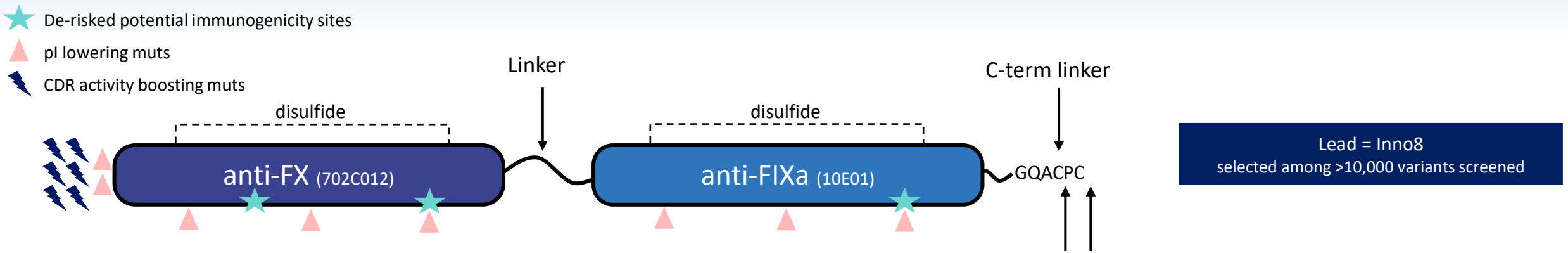




Pharmacokinetics

- Terminal half-life of Inno8 in beagle dog:
  115 hrs based on I.V. administration.
- 5-days repeated dosage of Inno8 tablets in beagle dog:
  Observed exposure concentrations between 1-5 nM w/reduced variability over dosage time.

Inno8 | Lead compound developed for oral treatment



Engineered bi-V_HH fatty diacid conjugate

- Mw 29.5 kDa (from tri- to **bi-V_HH**)
- **pI 5.2**, reduced from pI 8.3
- Protractor: **2 x C16 fatty diacid** C-term Cys-conjugated

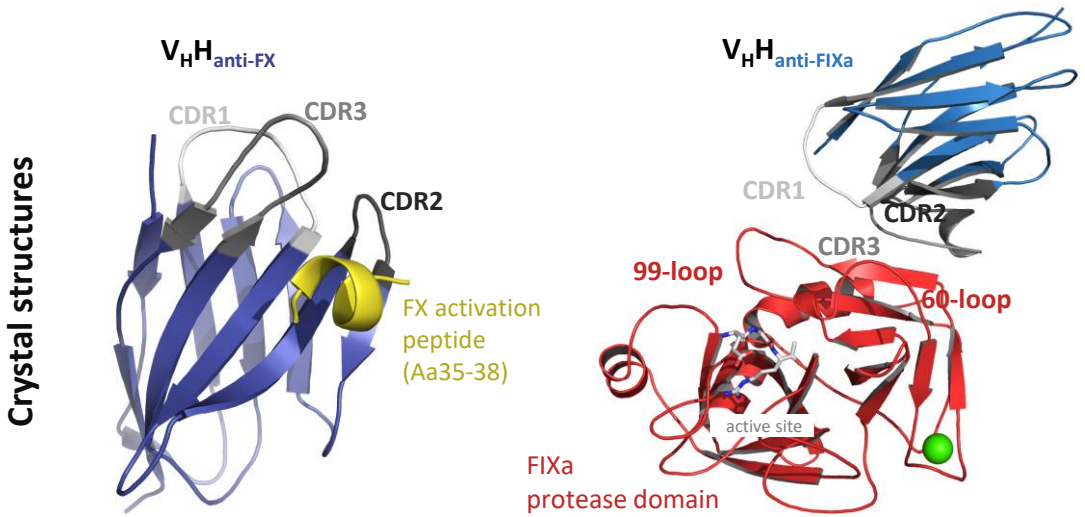
Optimized for oral PK

Anti-FX domain (lineage, 702C012)

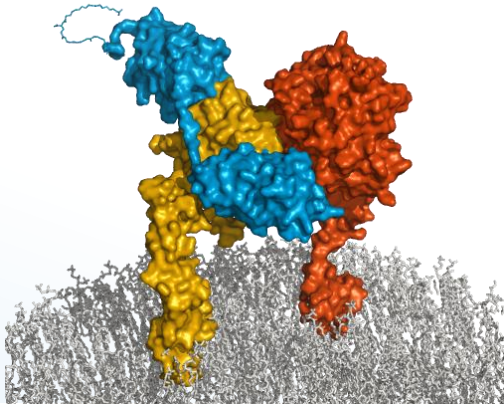
- Binds the Aa35-38 segment of the activation peptide in FX
- 6 activity boosting muts
- 2 hallmark/humanization muts
- 3 (+2) pI reducing muts
- 1 (+1) immunogenicity de-risking muts

Anti-FIXa domain (lineage, 10E01)

- Binds the protease domain of FIX(a)
- 9 hallmark/humanization muts
- 3 pI reducing muts
- (+1) immunogenicity de-risking mut



Conclusion



Inno8 was developed from comprehensive engineering of V_HHs obtained from llama & alphaca

Extensive optimization campaigns lead to **Inno8** demonstrating

- high potency *in vitro*
- long half-life *in vivo* using C16 fatty diacid protractor
- oral bioavailability *in vivo* using SNAC enhancer and NAM
- de-risked immunogenicity profile *in vitro*

Thus, **Inno8** may have the potential to be the first-in-class for oral treatment of Haemophilia A

Acknowledgments

Project team at Novo Nordisk

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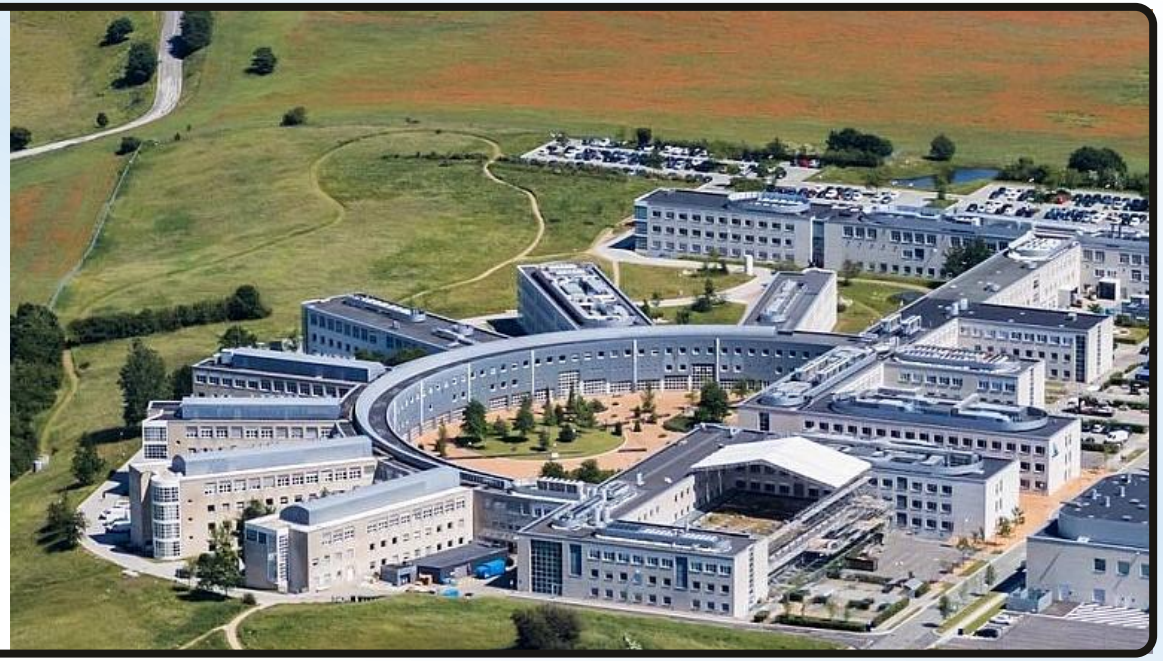
Collaborator

Ablynx



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