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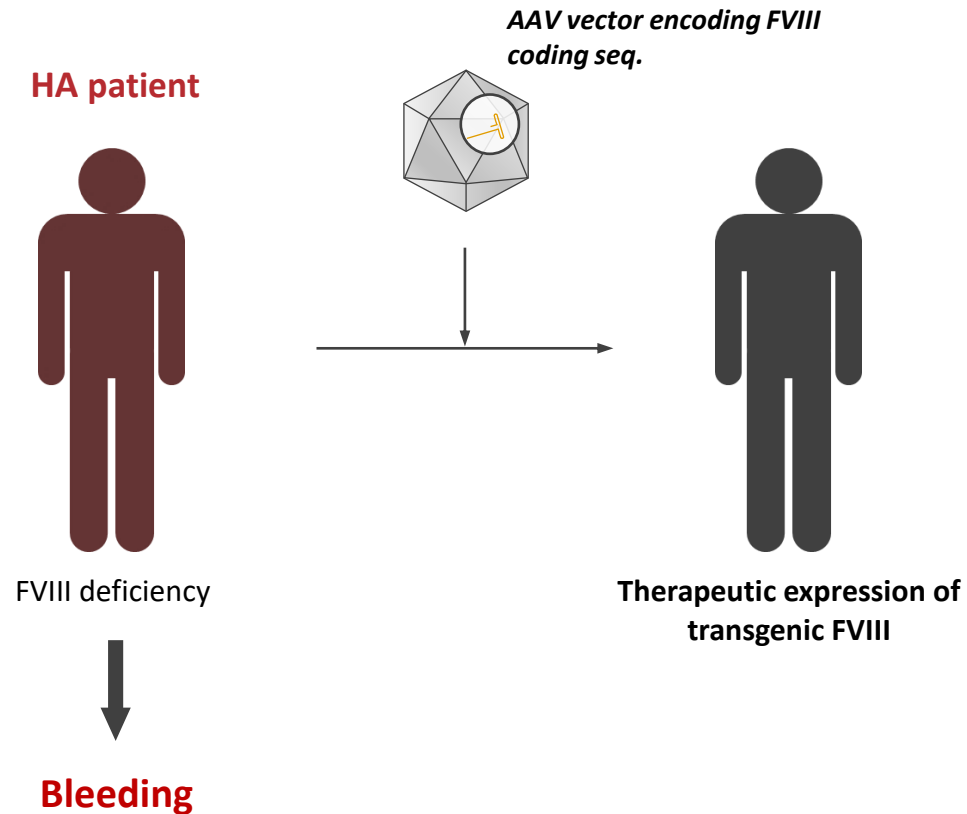
Single chain bispecific antibodies for gene therapy applications in haemophilia A

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AAV-mediated gene therapy for haemophilia A (HA)

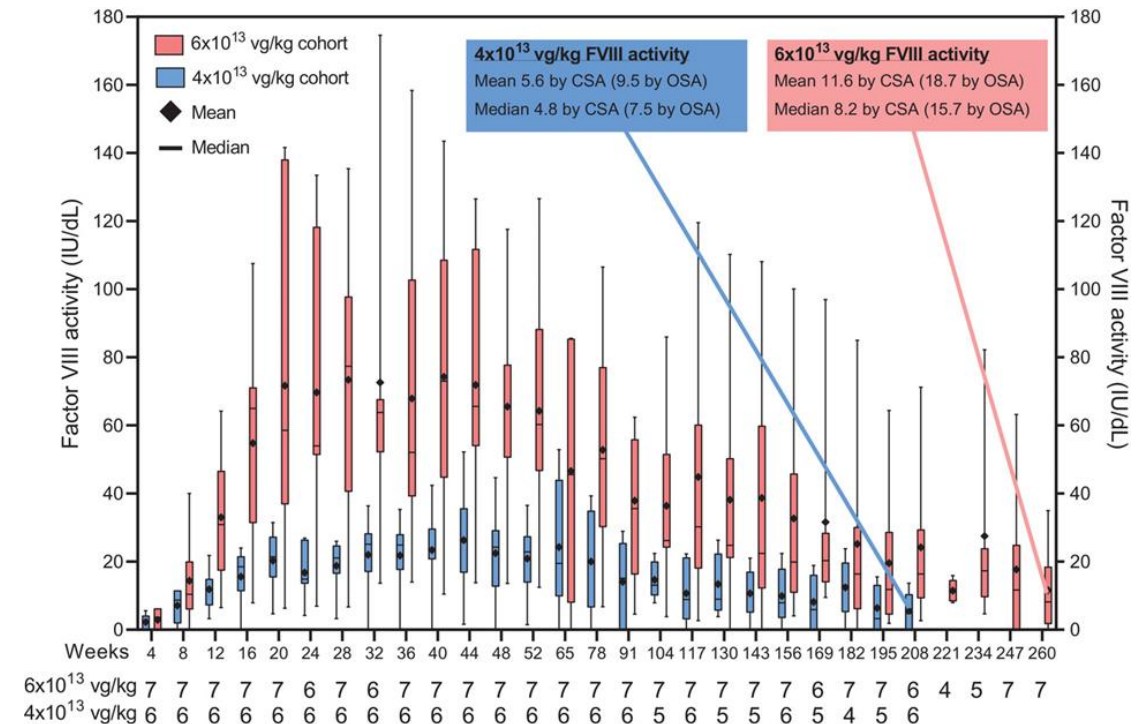
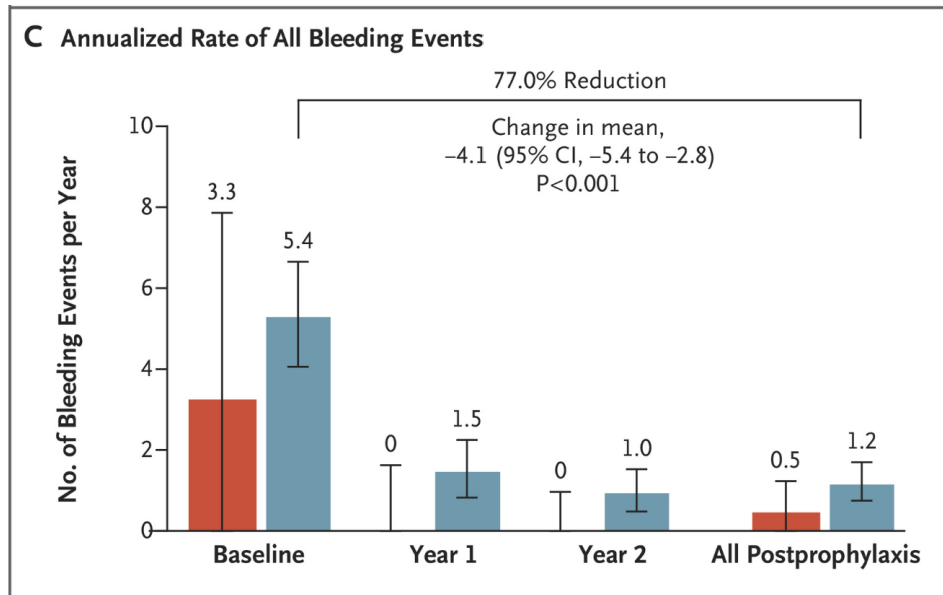
Haemophilia A (HA) is a congenital bleeding disorder caused by a **defect in coagulation FVIII** and affecting 1/5000 male birth



- Current **AAV-mediated** gene therapy enables expression of **transgenic FVIII**
- **First gene therapy** product EMA/FDA approved with valoctocogene roxaparvovec

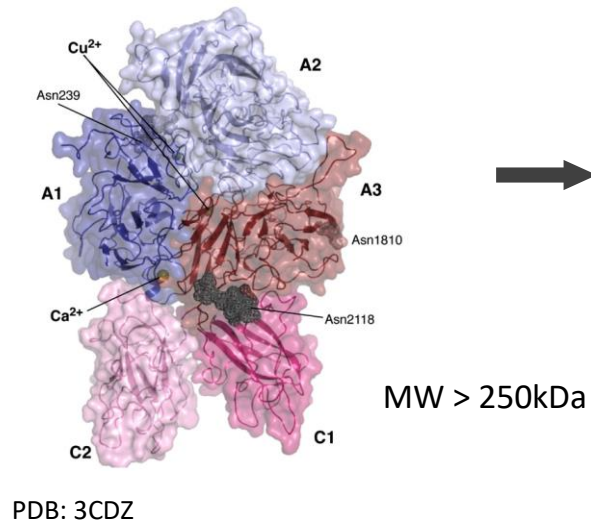
AAV-mediated gene therapy for haemophilia A (HA)

- valoctocogene roxaparvovec demonstrated clinical efficacy with significantly reduced bleeding rate in treated patients (Genr8-3)
- Progressive loss of transgenic expression overtime

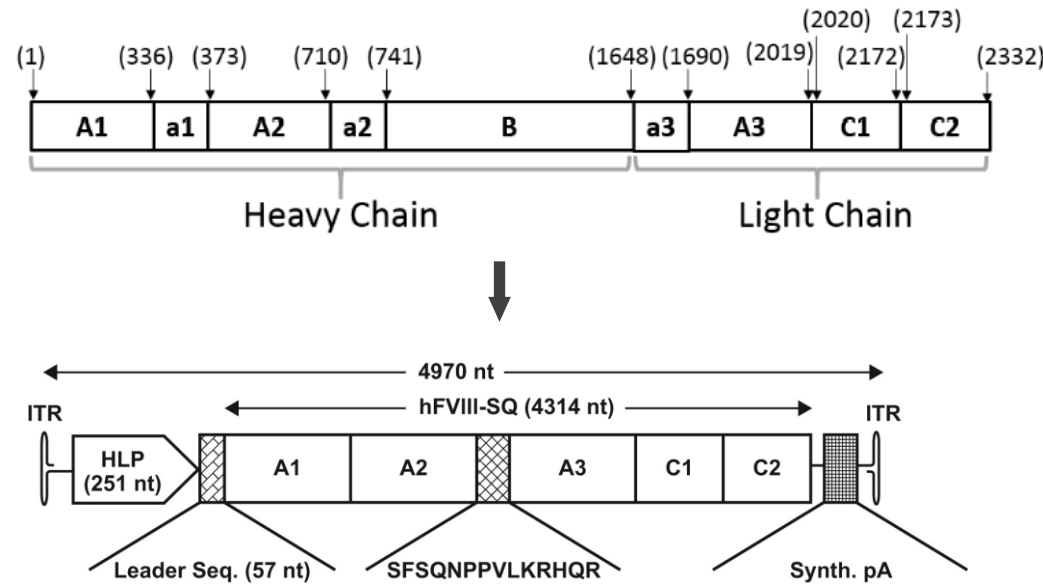


AAV-mediated gene therapy for haemophilia A (HA)

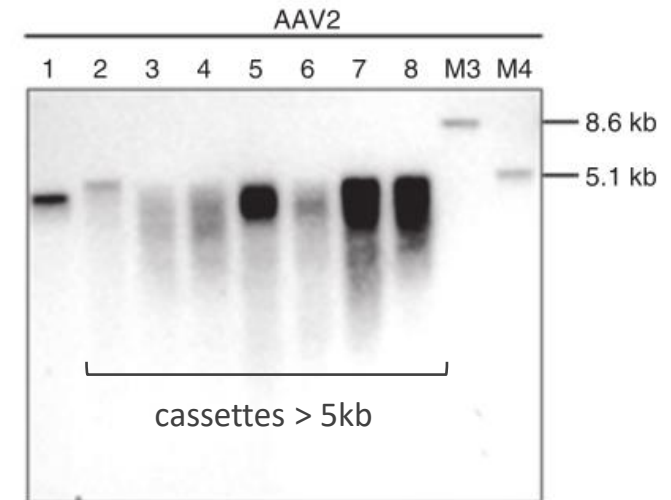
FVIII coding sequence **exceed the capacity** of transgene compatible with AAV capsid packaging capacity



MW > 250kDa



Coding seq. ~ 7kb

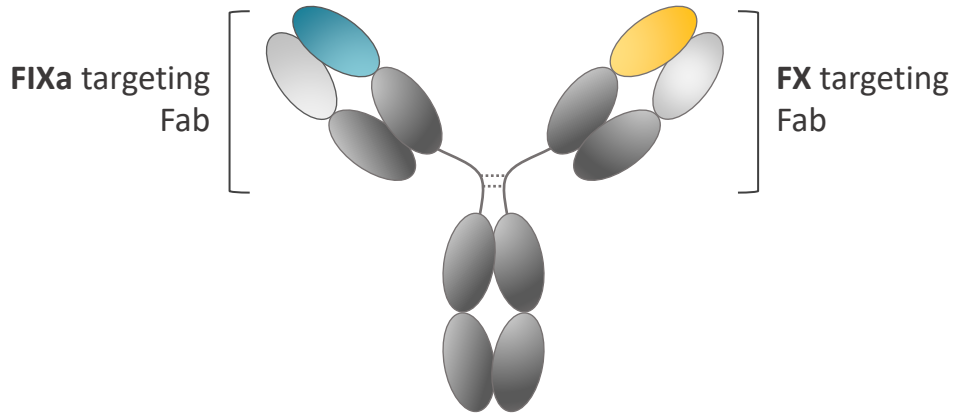


⇒ Requires the use of **oversized expression cassettes** above the packaging limit of AAV capsids

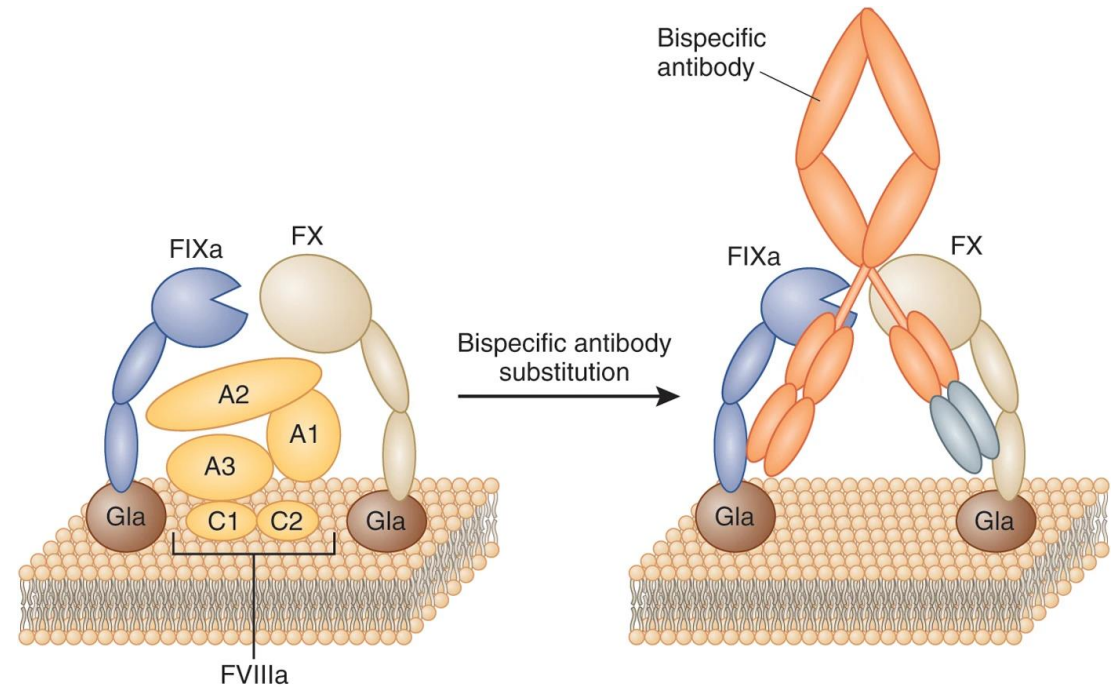
⇒ **Minimal space** for regulatory elements

Alternative gene therapy approach for HA

FVIII mimetic antibody

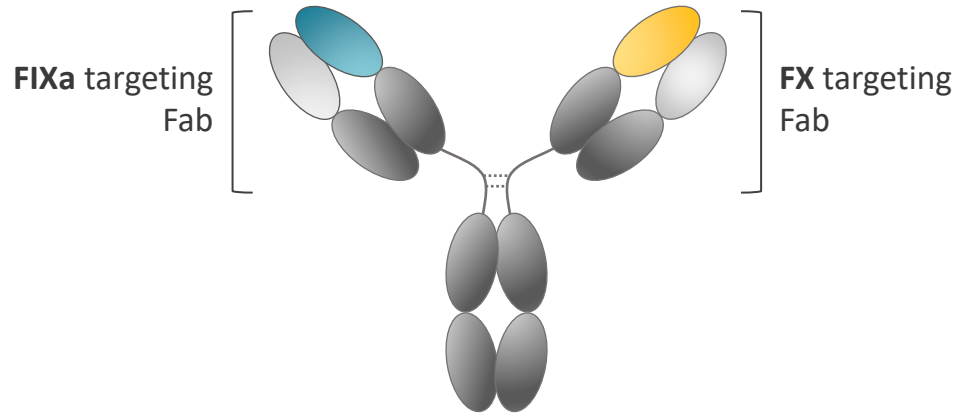


- Pioneered with the development of emicizumab
- Bypass FVIII deficiency to restore the coagulation cascade
- Efficient in Haemophilia A patient with and without FVIII neutralizing inhibitors

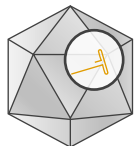


Alternative gene therapy approach for HA

FVIII mimetic antibody



AAV vectors



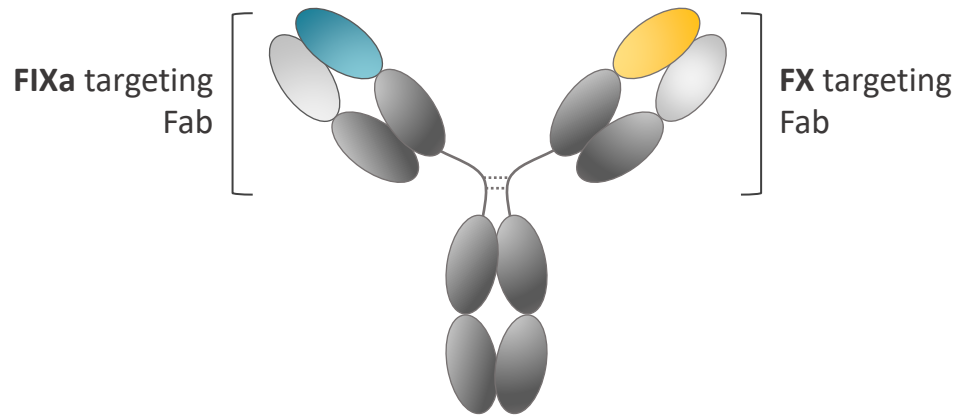
- Pioneered with the development of emicizumab
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⇒ Strong potential of antibody engineering

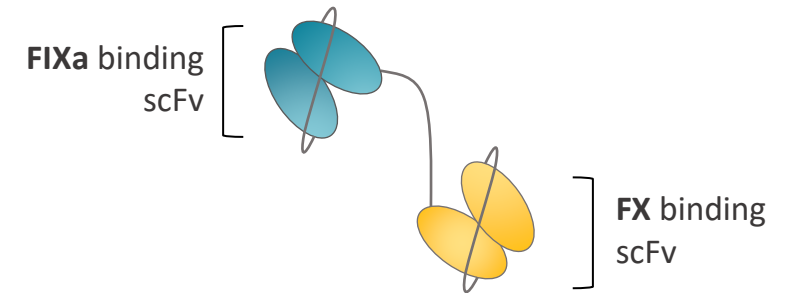
⇒ **Can we combine the therapeutic potential of FVIII mimetic antibodies with AAV-mediated gene therapy ?**

Bi8 – A single chain FVIII mimetic antibody

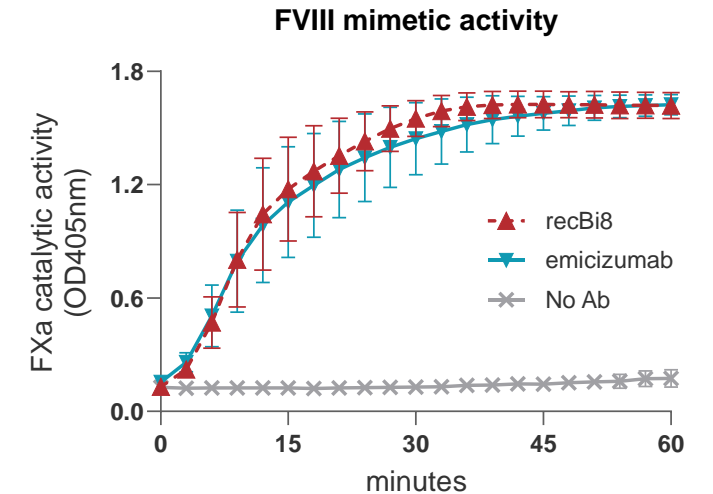
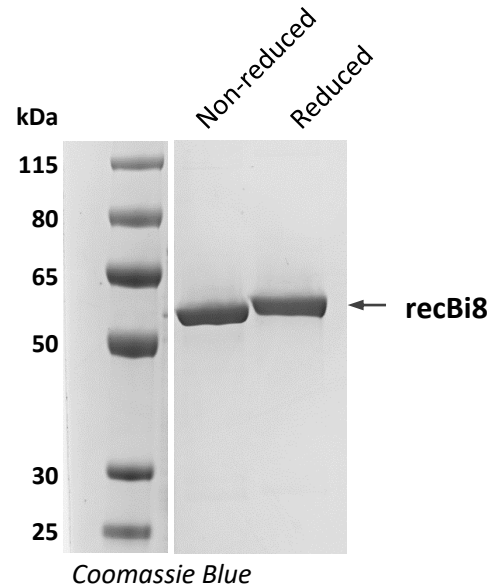
“Full-sized” FVIII mimetic antibody



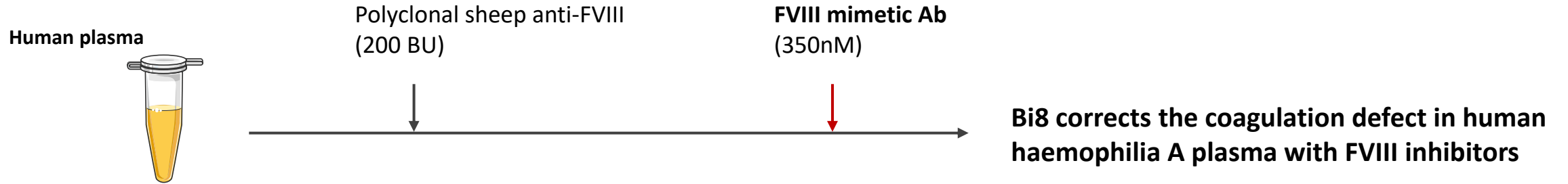
Bi8 – single chain FVIII mimetic antibody



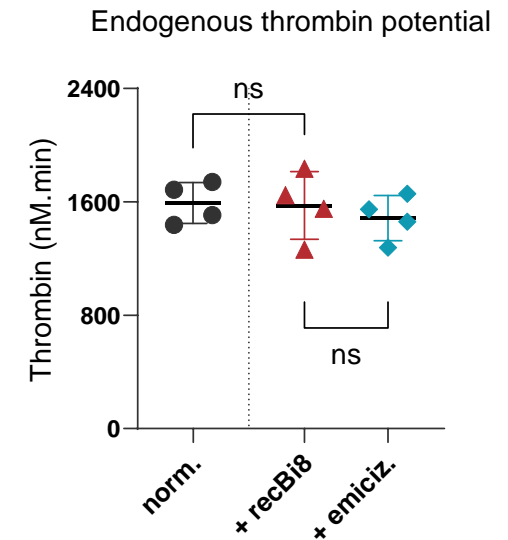
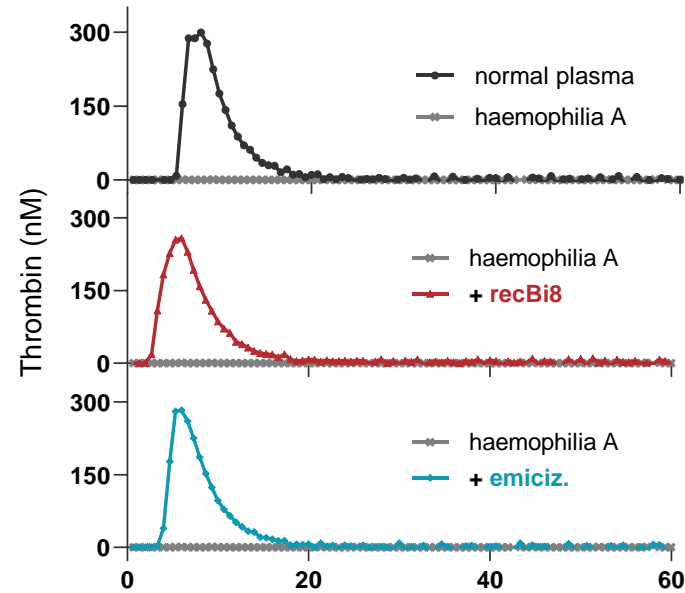
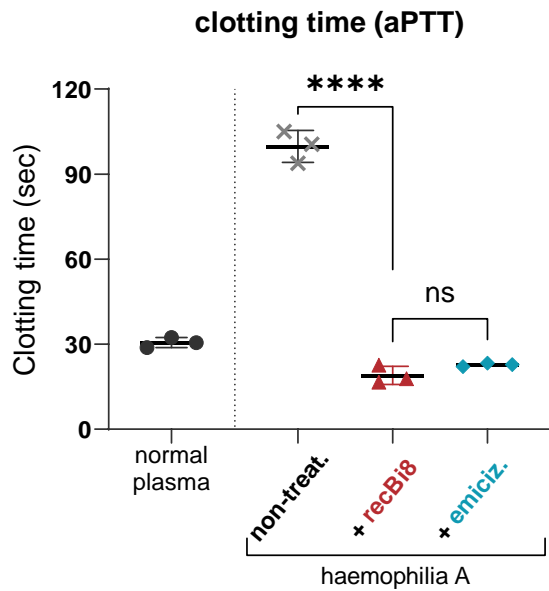
- Single chain FVIII mimetic antibody
- Discrete band at 54.5 kDa when produced in Expi293 mammalian cells
- Shows efficient FVIII mimetic activity



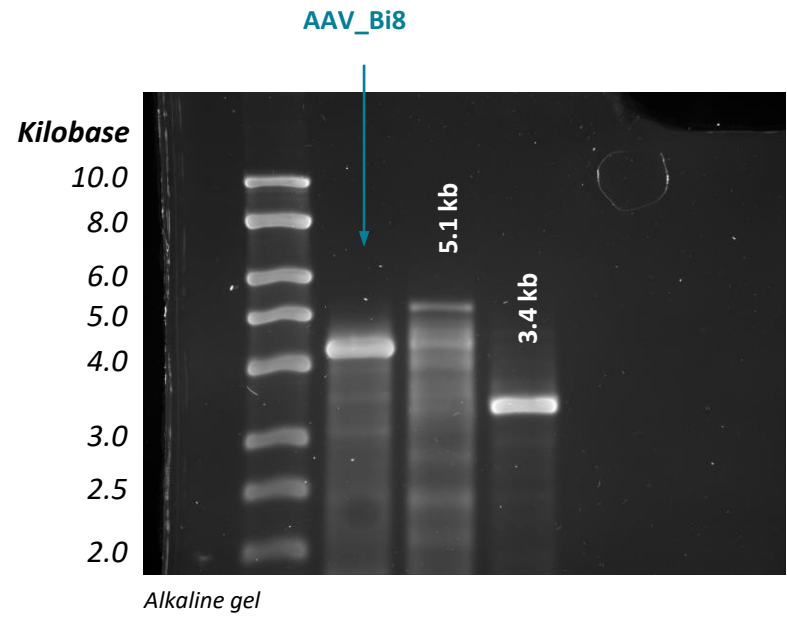
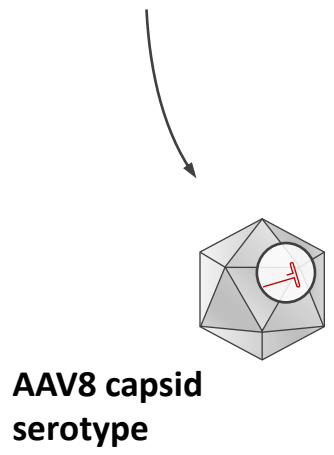
Haemostatic potential of Bi8 in haemophilia A plasma



Thrombin generation assay (*FXIa* triggered)

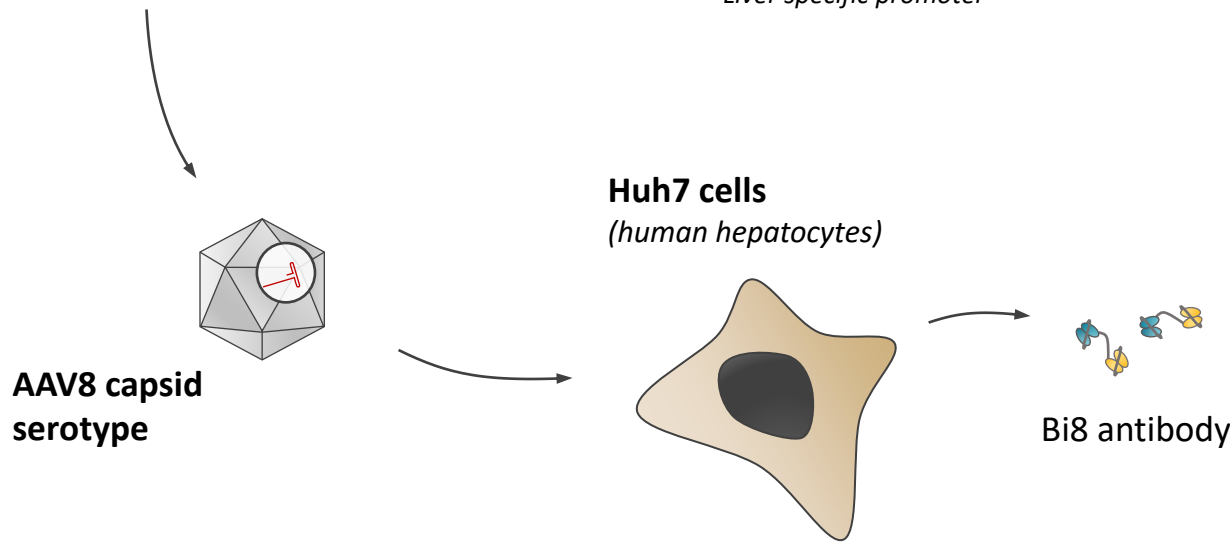


Viral expression cassette encoding Bi8

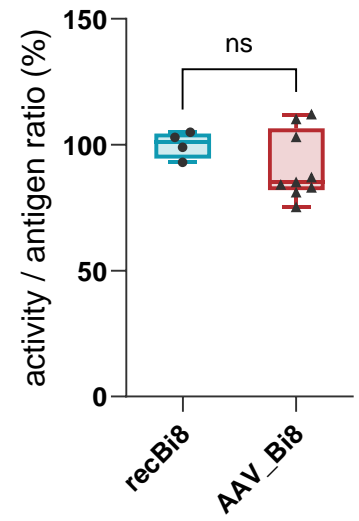
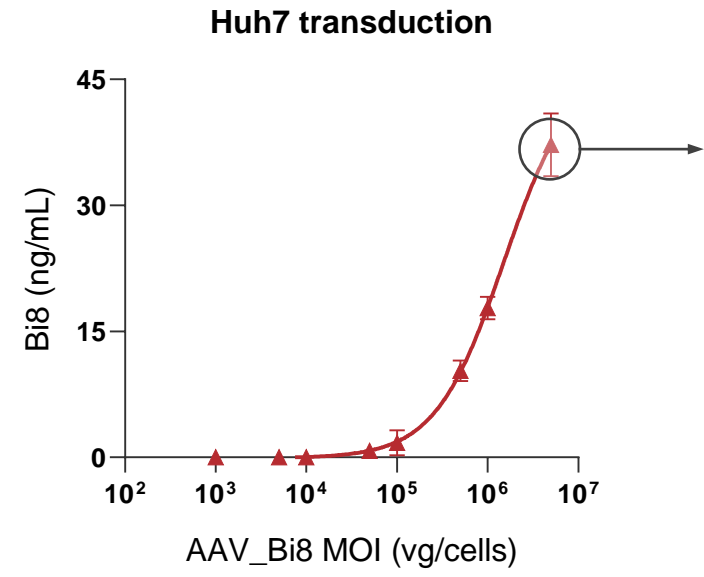


⇒ **Bi8 transgenic cassette is well within the packaging capacity of AAV vectors**

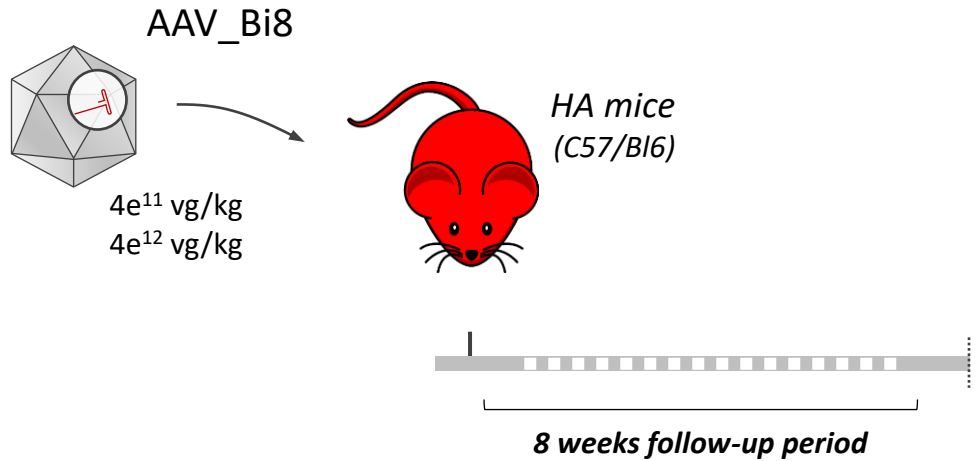
Viral expression cassette encoding Bi8



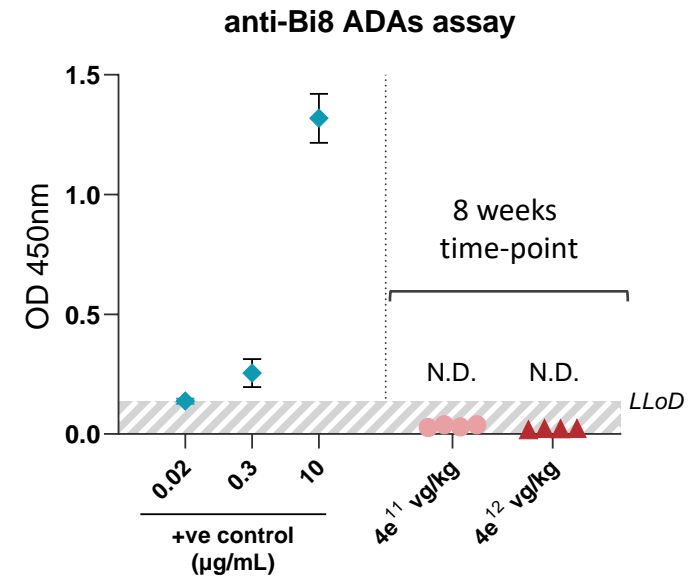
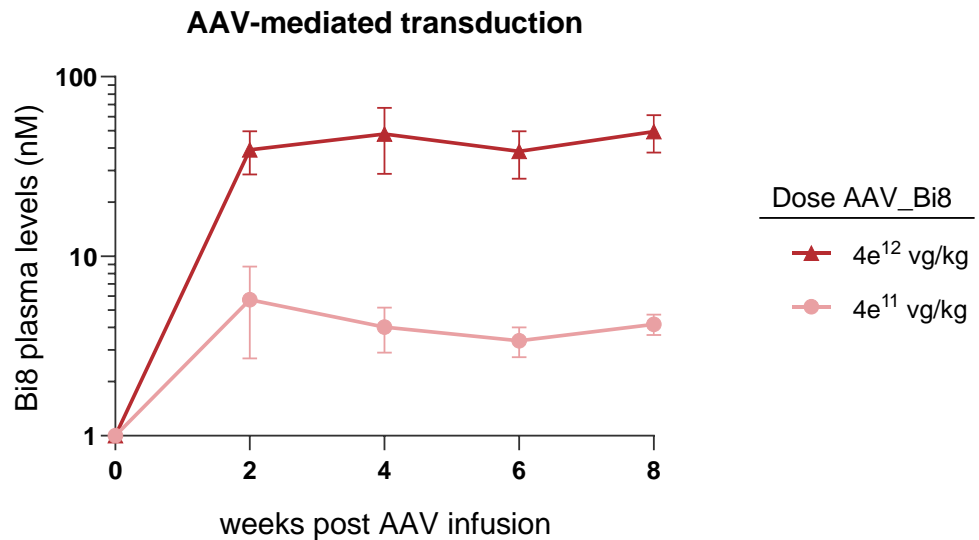
⇒ AAV derived Bi8 showed identical activity to the purified recombinant antibody



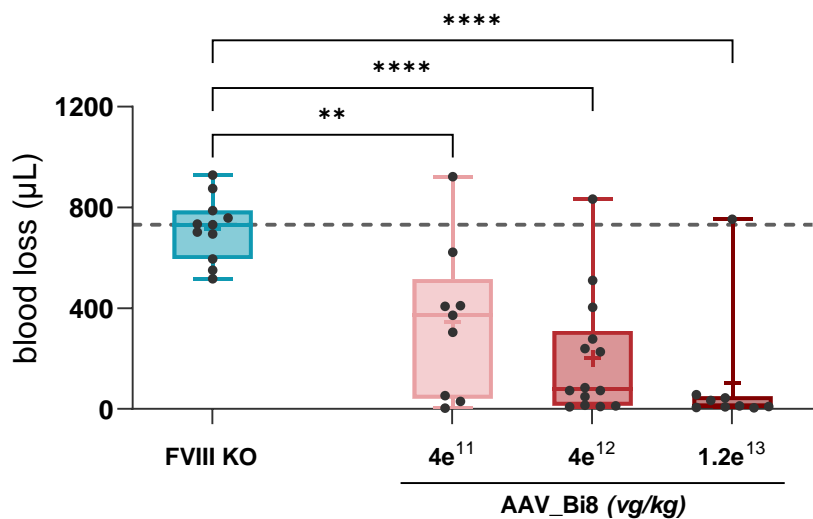
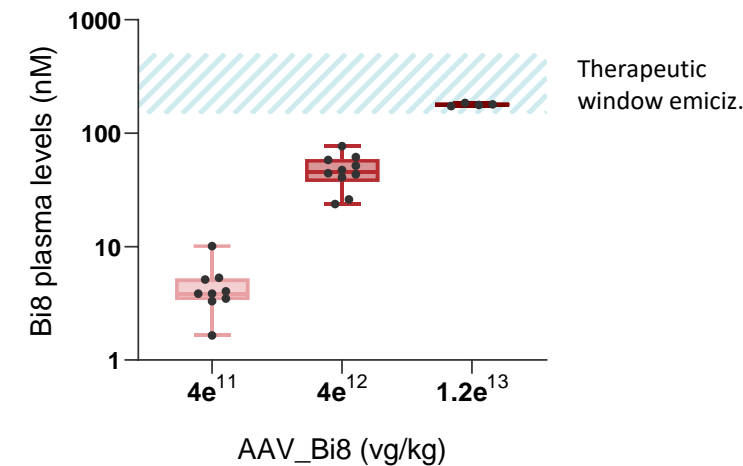
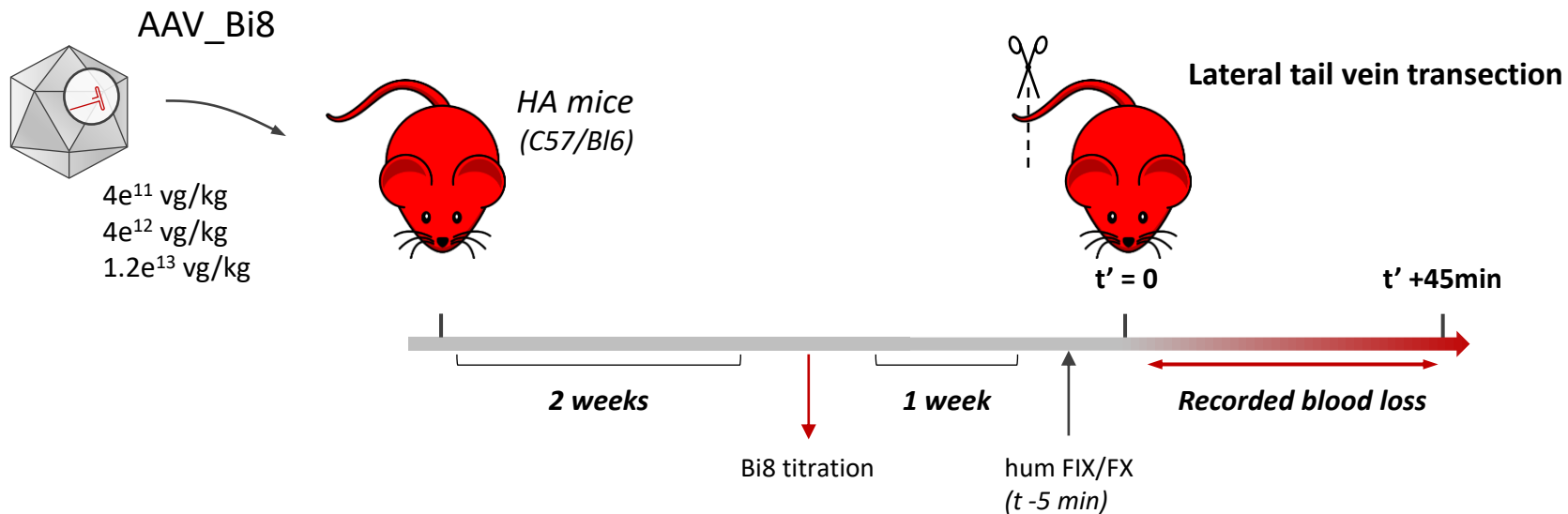
AAV-mediated expression of Bi8 in haemophilia A mice



- Dose-dependent expression of Bi8 following AAV-mediated gene transfer
- No evidence of anti-drug antibodies 8-weeks post gene transfer

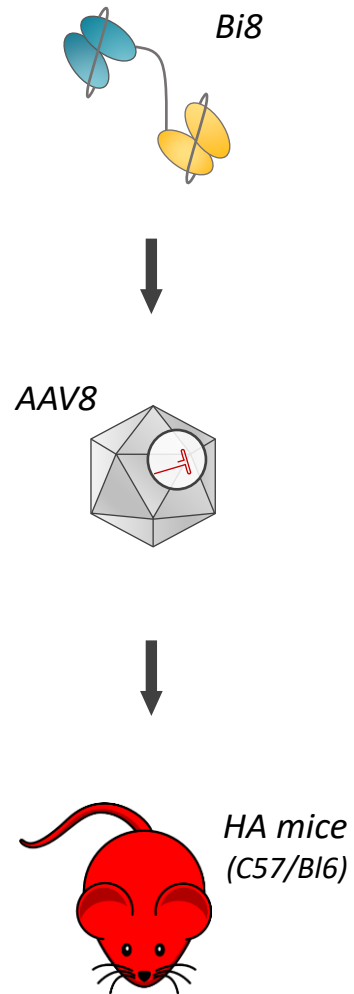


AAV_Bi8 corrects the bleeding tendency in HA mice



- Expression of single chain Bi8 up to the therapeutic range of the marketed emicizumab antibody
- Significant reduction of blood loss is observed for all tested doses of AAV_Bi8

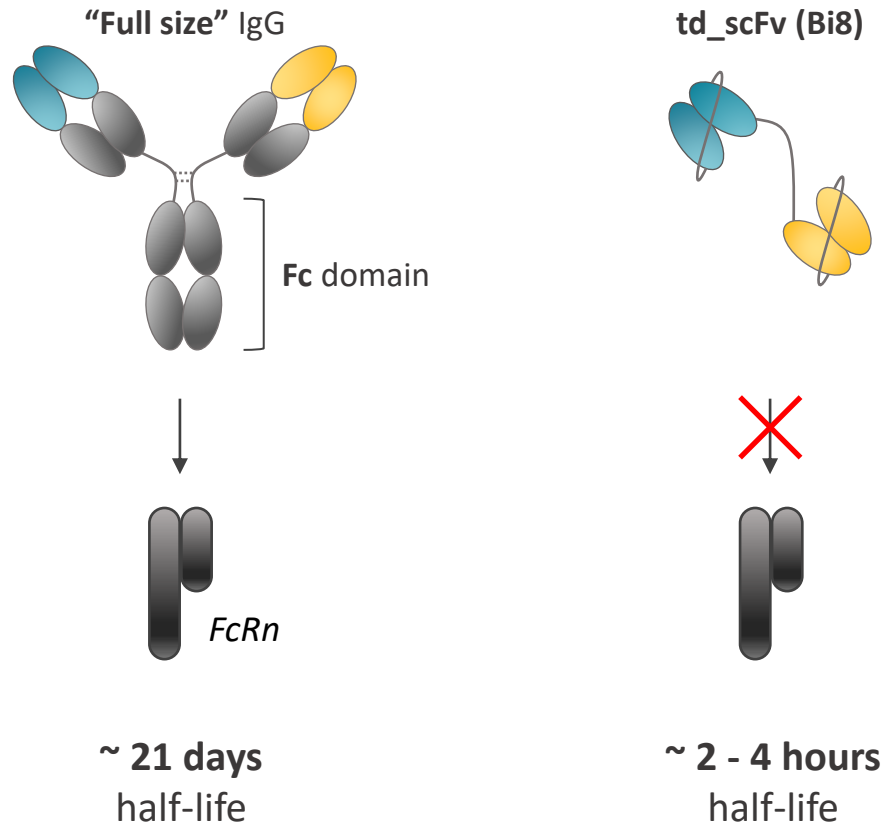
Overview



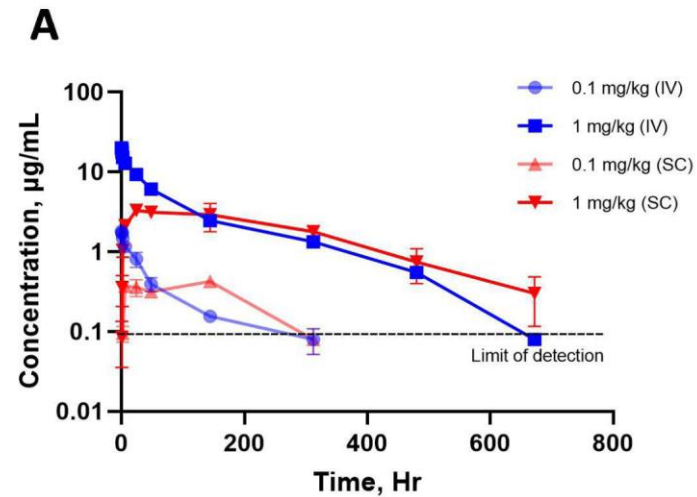
- Bi8 is designed small-sized single chain bispecific antibody with FVIII mimetic activity
- Bi8 enabled the development of a 4.4kb transgenic expression cassette, within the packaging limits of AAV capsids
- Infusion with AAV_Bi8 induces expression of the single chain FVIII mimetic antibody and successfully corrects the bleeding tendency in HA mice
- AAV_Bi8 provides an alternative gene therapy approach for HA which may be applicable to patients with and without FVIII neutralising antibodies

Limitations of gene therapies – the dose of AAV particles

Half-life of a transgene is a limiting factor to maintain high steady-state levels of a protein of interest

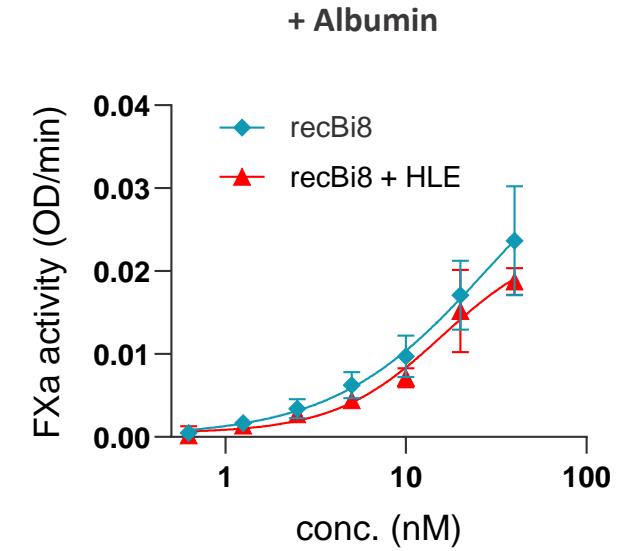
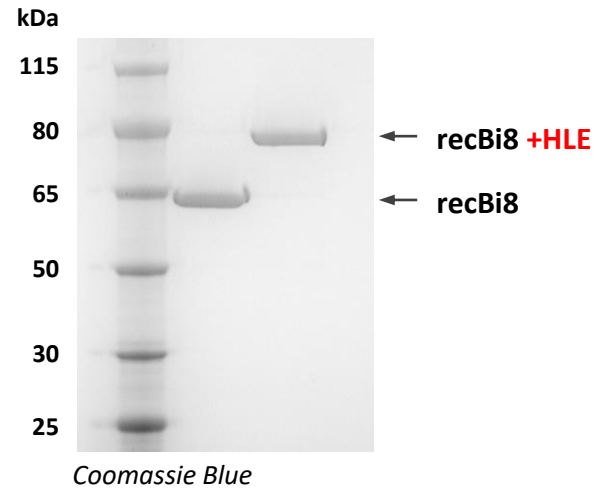
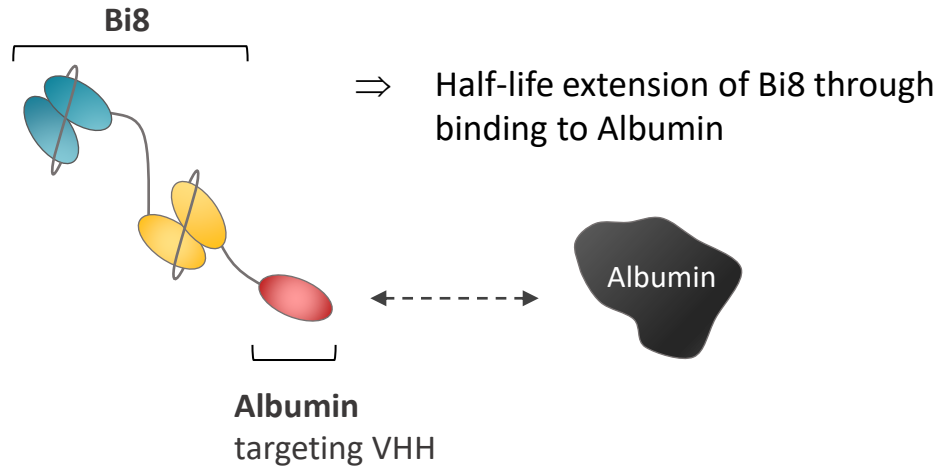


- ⇒ The half-life of albumin is also driven by binding to FcRn
- ⇒ **Binding to Albumin can extend the half-life of a protein of interest**



~ 5 - 7 days half-life

One step further : Half-life extended Bi8



⇒ Activity is preserved in presence of albumin



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