Generating potent and selective inhibitors of Kv1.3 ion channels by fusing venom derived mini proteins into peripheral CDR loops of antibodies

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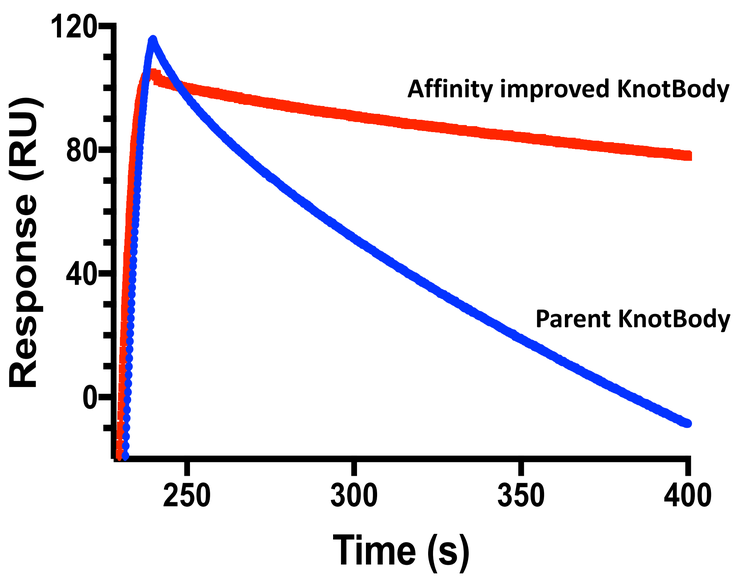
Background

Pathogenic T cell effector memory (TEM) cells drive many autoimmune disorders and are uniquely dependent on the Kv1.3 channel. A number of venom derived knottin (cysteine-rich mini-protein) inhibitors of Kv1.3 are being developed as potential drug candidates, but can suffer from manufacturing difficulties, short half-lives and a lack of specificity. We have developed a novel molecular format wherein a peripheral CDR loop of an antibody has been replaced by a knottin. In this novel KnotBody™ format, the knottin benefits from the improved therapeutic functionality of an antibody and the antibody gains additional diversity by the addition of a scaffold which is pre-disposed to blockade of ion channels.

A proof-of-concept fusion protein of one structural domain within another was initially achieved by inserting a trypsin inhibiting knottin (EETI-II) flanked by diverse repertoire of short linker sequences into the CDR2 position of naïve antibody light chain sequences. Functional KnotBody™ molecules were selected from this library using phage display technology on the basis of retained trypsin binding, with the correct folding of both domains confirmed using X-ray crystallography. To further demonstrate the benefits of this novel format, the modular nature of the KnotBody™ binding surface was exploited to: (i) improve existing knottin binding by introducing additional V_H contacts; (ii) create a bispecific molecule by introducing a V_H chain that binds to a different target; (iii) substitute the proof-of-concept knottin (EETI-II, a trypsin inhibitor) with ShK, a Kv1.3 ion channel blocking toxin; (iv) develop a panel of low-nM Kv1.3 inhibitors with selectivity exceeding 3000-fold over the Kv1.1 channel, a closely related Kv family member.

(i) Improving existing knottin binding

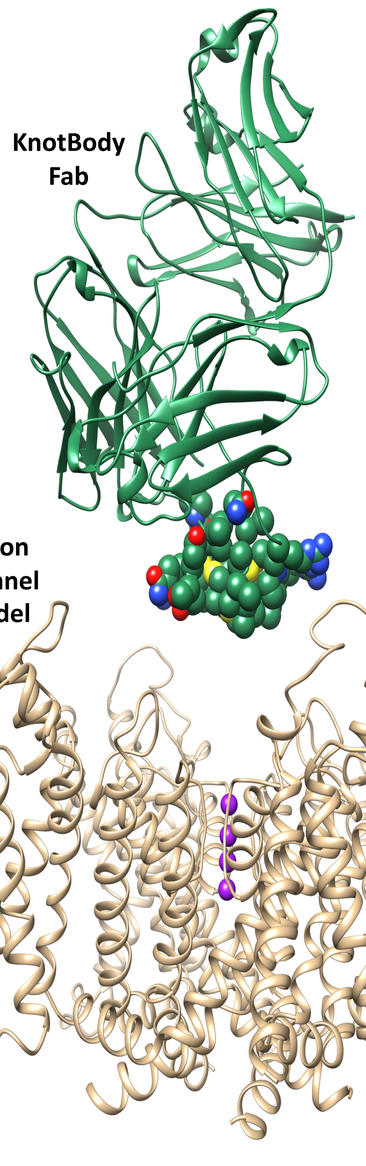


Using the trypsin inhibitor knottin EETI-II fused into the CDR2 position on an antibody, a trypsin binding KnotBody™ was made (blue). The affinity of this parental KnotBody™ to trypsin was improved by selecting a V_H that makes additional contacts (red). Improvement in "off-rate" was analysed using SPR.

Crystal structures of KnotBody™ Fab (1.9Å) and Kv ion channel

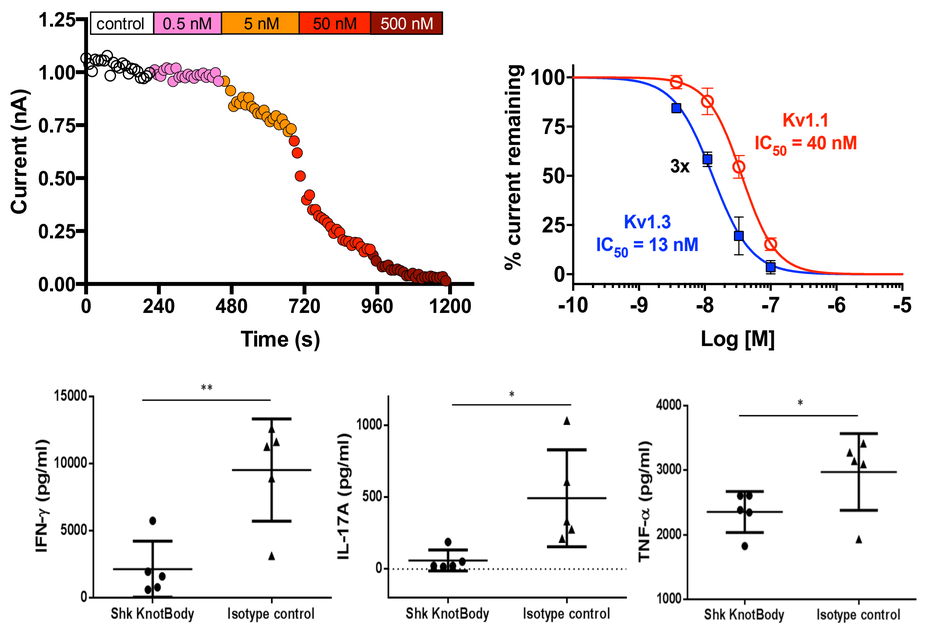
Adapted from: Wulff *et al.*, Nat. Rev. Drug Discovery, 2019.

Upper structure: KnotBody Fab composed of V_H and V_L as a ribbon structure (green) with fused knottin in space filling atoms.



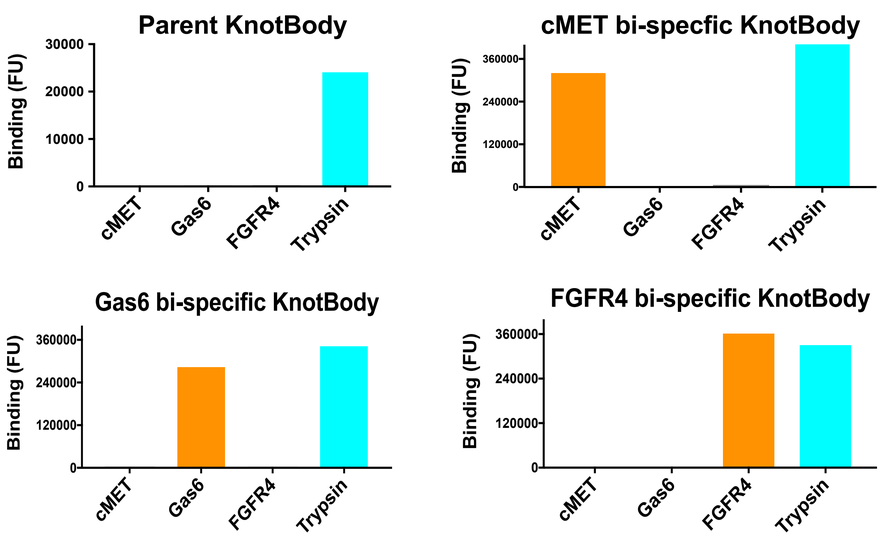
Lower structure: Kv ion channel ribbon structure (gold) with K^+ ions in selectivity filter (purple). Atomic coordinates from the Kv1.2/2.1 chimaera crystal structure (Long *et al.*, Nature, 2007).

(iii) ShK-KnotBody - Kv1.3 ion channel blocking



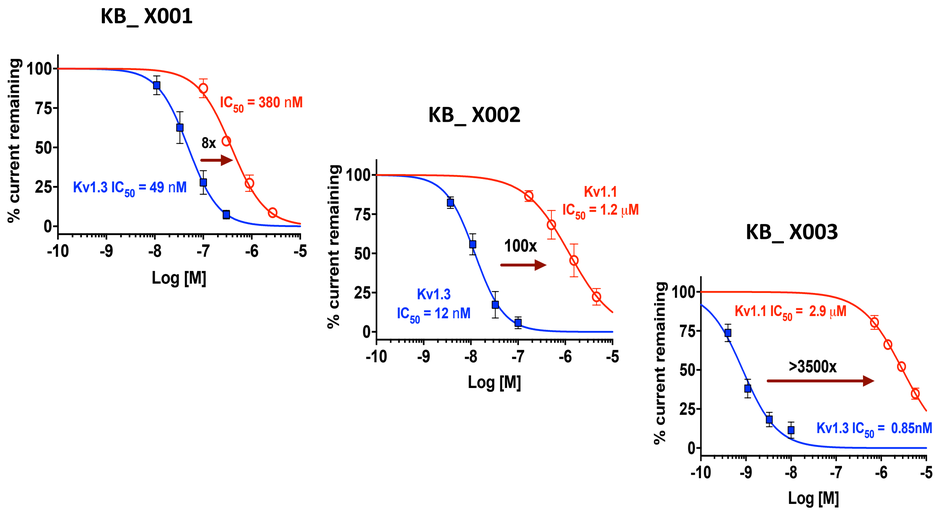
EETI-II (trypsin binding knottin) in the CDR2 position was substituted with ShK (Kv1.3 blocking toxin) to generate a Kv1.3 current blocking KnotBody™ (upper left). Concentration-dependent inhibition of Kv1.3 (blue) and the isoform Kv1.1 (red) shows similar IC50 potency (upper right). Activated PBMC cytokine secretion is reduced by ShK-KnotBody vs isotype control (lower panels).

(ii) Creating bi-specific binding in a novel format



Bi-specific KnotBody™ molecules were selected against cMET, Gas6 and FGFR4 from a phage display library created by shuffling the "Parent KnotBody" light chain (with trypsin binding knottin EETI-II at V_L CDR2 position) with a repertoire of naïve heavy chains.

(iv) Improved Kv1.3 selectivity and potency



By inserting alternative Kv1.3 blocking knottins and/or via modifications in the 'linker' residues a second generation of KnotBody™ molecules were generated. These KnotBody™ molecules showed improved Kv1.3 channel selective inhibition (blue) over Kv1.1 (red), a closely related isoform - the most improved KnotBody™ showed over 3500x selectivity for Kv1.3.

Summary

- IONTAS have invented a novel molecular format that encapsulates the benefits of antibodies and naturally occurring knottins:
  - Antibodies gain the functional diversity of the knottin, whilst the knottin gains the improved therapeutic functionality (e.g. longer half life) of an antibody.
  - Both knottin and antibody CDR loops can be further engineered using phage display technology to increase affinity and specificity.
- Due to the modular nature of the KnotBody™ binding surface, this format can be used to create bi-specifics.
- Functional ion channel blocking KnotBody™ molecules were generated by substituting trypsin binding knottin at V_L CDR2 position with Kv1.3 blocking knottins.
  - Nav1.7 (a chronic pain target) and ASIC1a (stroke) ion channel blocking KnotBody™ molecules have also been generated – data not shown.
- **This technology unlocks new possibilities for the blockade of ion channels using "engineerable" antibody based drugs.**