Scaffolds within scaffolds: generating ion channel blocking antibodies by fusing knottin to peripheral CDR loops

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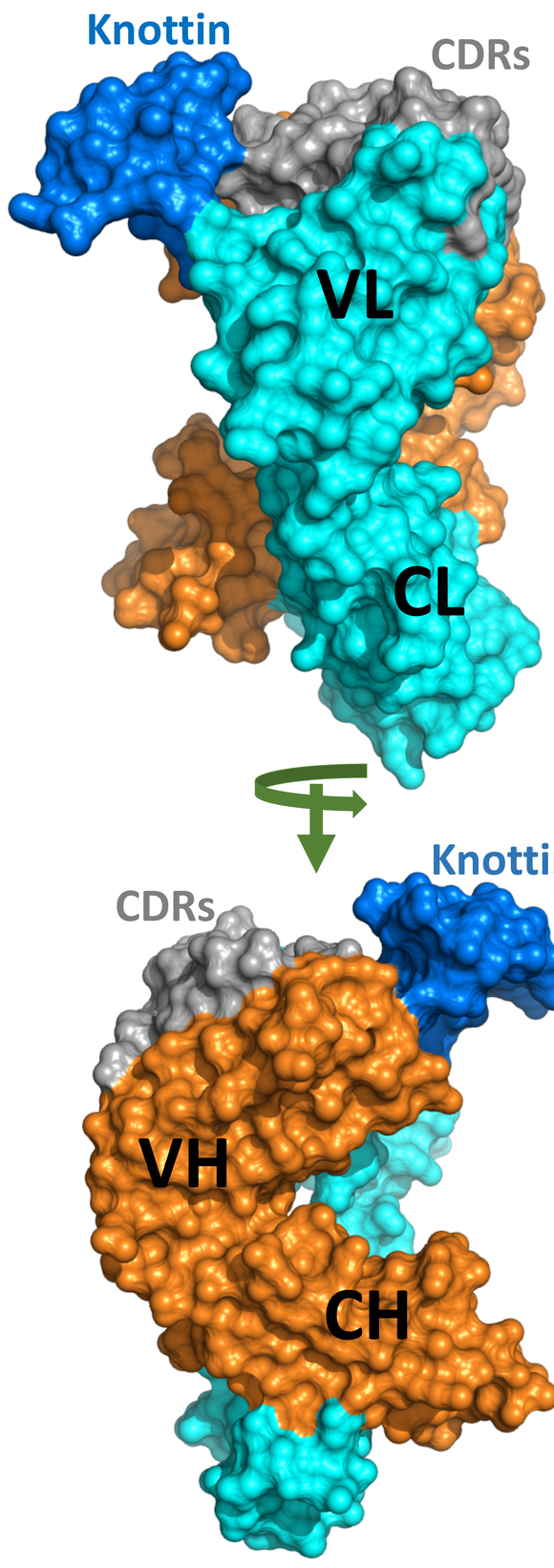
Background

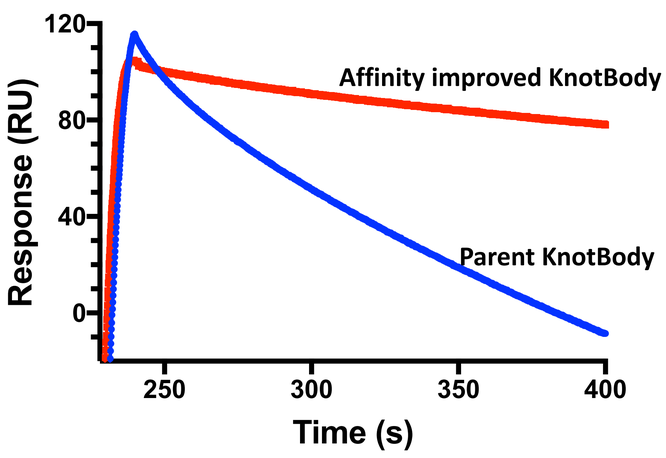
Cysteine-knot miniproteins (knottins) have potential as therapeutic agents to block ion channels involved in cancer, autoimmunity and pain but suffer from manufacturing difficulties, short half-lives and a lack of specificity. IONTAS have invented a novel molecular format wherein a peripheral CDR loop (e.g. VL CDR2) of an antibody has been removed and replaced by a naturally occurring knottin. In this novel format (termed a KnotBody™), the knottin enjoys the extended half-life of an antibody molecule and the peripheral CDRs gain additional diversity within a scaffold which is pre-disposed to blockade of ion channels. This example of successful fusion of one structural domain within another was initially achieved by inserting a trypsin binding knottin (EETI-II) flanked by diverse repertoire of short linker sequences into the CDR2 position of naïve antibody light chain sequences. Functional KnotBodies were selected from this library using phage display technology on the basis of retained trypsin binding and the correct folding of both domains were confirmed using X-ray crystallography. To further demonstrate the merits of this novel format, the modular nature of the KnotBody binding surface was exploited to: (i) improve existing knottin binding by introducing additional VH contacts; (ii) create a bispecific molecule by introducing a VH chain that binds to a different target; (iii) engineer novel binding specificity on the knottin scaffold by loop diversification; (iv) substitute the selected (EETI-II trypsin binding) knottin with ion channel blocking knottins.

Crystal structure of a KnotBody Fab (1.9Å)

VH in orange, VL in cyan, knottin in blue and CDR loops of the antibody in grey

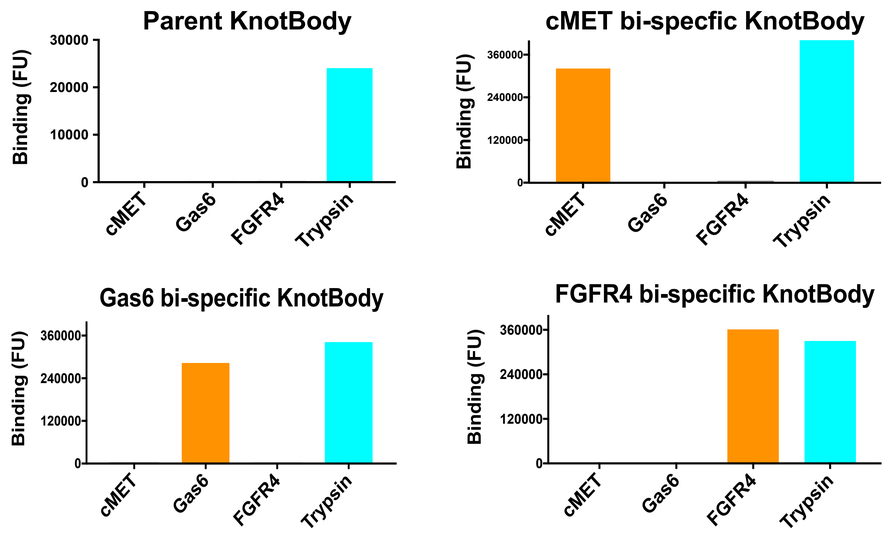


(i) Improving existing knottin binding



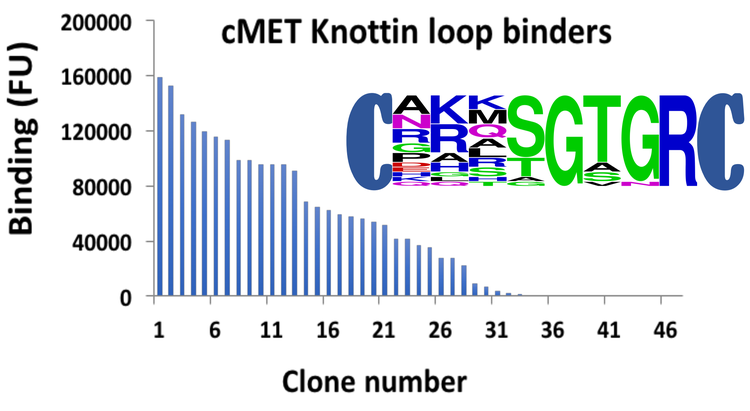
The affinity of the parent KnotBody to trypsin was improved by selecting a VH that makes additional contacts. Improvement in "off-rate" was analysed using SPR.

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



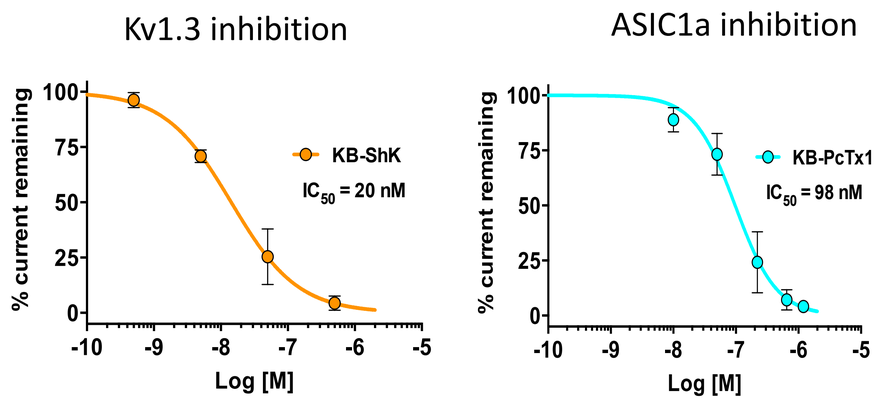
Bi-specific KnotBodies were selected against cMET, Gas6 and FGFR4 from a phage display library created by shuffling the "parent KnotBody" light chain (with trypsin binding knottin EETI-II at CDR2 position) with a repertoire of naïve heavy chains.

(iii) Generating novel binding specificities through knottin loop engineering



The trypsin binding loop of the EETI-II knottin was randomised and novel binders with diverse sequences were generated against cMET. The loop sequences of the binders are presented as a sequence logo with the flanking cysteine residues) highlighted in blue).

(iv) Generating ion channel modulators



The trypsin binding knottin at the VL CDR2 positon was substituted with ShK and PcTx1 (toxins) to generate blockers of Kv1.3 and ASIC1a ion channels respectively. Concentration-dependent inhibition of Kv1.3 and ASIC1a currents were measured using QPatch automated patch clamp. ASIC1a data were generated at Sophion.

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 long half life of an antibody molecule.
  - 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 KnotBodies were generated by substituting trypsin binding knottin at VL CDR2 position with Kv1.3 and ASIC1a blocking knottins.
- **This technology unlocks new possibilities for the blockade of ion channels using "engineerable" antibody based drugs**