Literature Review of Huntingtin Intrabodies/Nanobodies (2018/01/09)

Rationale

Preparation of the huntingtin protein for structural studies requires creating a stable and homogenous sample. The N-terminal exon 1 is disordered so finding protein binding partners which might specifically bind exon1 and stabilise its conformation would help generate a higher quality sample. Huntingtin-specific Ig domains (nanobodies/intrabodies), reviewed here, may be a good exon 1 binders for this reason. NB: iVHH4 already expressed and purified https://zenodo.org/record/1066182#.WlYdGrbMxE4

Methods

Pubmed search of ("huntingtin" of "huntington") + ("intrabodies" or "nanobodies")

General papers

Methods describing huntingtin intrabody production and screening can be found here (Khoshnan et al (2013) Methods Mol Biol Pubmed ID: 23754229 - Antibodies and intrabodies against huntingtin: production and screening of monoclonals and single-chain recombinant forms).

Huntingtin intrabodies mentioned in these review manuscripts: (Ali et al (2011) Neurobiology of HD https://www.ncbi.nlm.nih.gov/books/NBK55999/, Messer and Joshi (2013) Neurotherapeutics Pubmed ID: 23649691 - Intrabodies as Neuroprotective Therapeutics and Butler et al (2012) Prog Neurobiol Pubmed ID: 22120646 - Engineered antibody therapies to counteract mutant huntingtin and related toxic intracellular proteins).

C4 sFv

Type of Ig:
Single-chain Fv (sFv) antibody

Binding site:
N17 of exon 1

NCBI sequence ACA53373.1 anti-huntingtin intrabody single chain Fv antibody, partial [synthetic construct]
MAQVQLQESGGGLVQPGGSLRLSCAASGFTFSSYSMSWVRQAPGKGLEWVAVISYDGSNKYYADSVKGRFTISRDNSKNTLY
LQMNSLRAEDTAVYYCARDRYFDLWGRGTLVTVSSGGGGSGGGGSGGGGSQSALTQPASVSGSPGQSITISCTGTSSDIGAY
NYVSWYQQYPGKAPKLLIYDVSNRPSGISNRFSGSKSGDTASLTISGLQAEDEADYYCSSFANSGPLFGGGTKVTVLG

C4 sFv was shown to reduce aggregation of huntingtin in cellular HD models (Lecerf et al (2001) PNAS Pubmed ID: 11296304 - Human single-chain Fv intrabodies counteract in situ huntingtin aggregation in cellular models of Huntington's disease) and slice culture HD models (Murphy and Messer (2004) Molecular Brain Research Pubmed ID: 14969746 - A single-chain Fv intrabody provides functional protection against the effects of mutant protein in an organotypic slice culture model of Huntington's disease).

C4 sFv was shown to bind N-terminal huntingtin fragments 2D culture HD models (Miller et al (2005) Neurobiology of Disease Pubmed ID: 15837560 - A human single-chain Fv intrabody preferentially targets amino-terminal Huntingtin's fragments in striatal models of Huntington's disease).

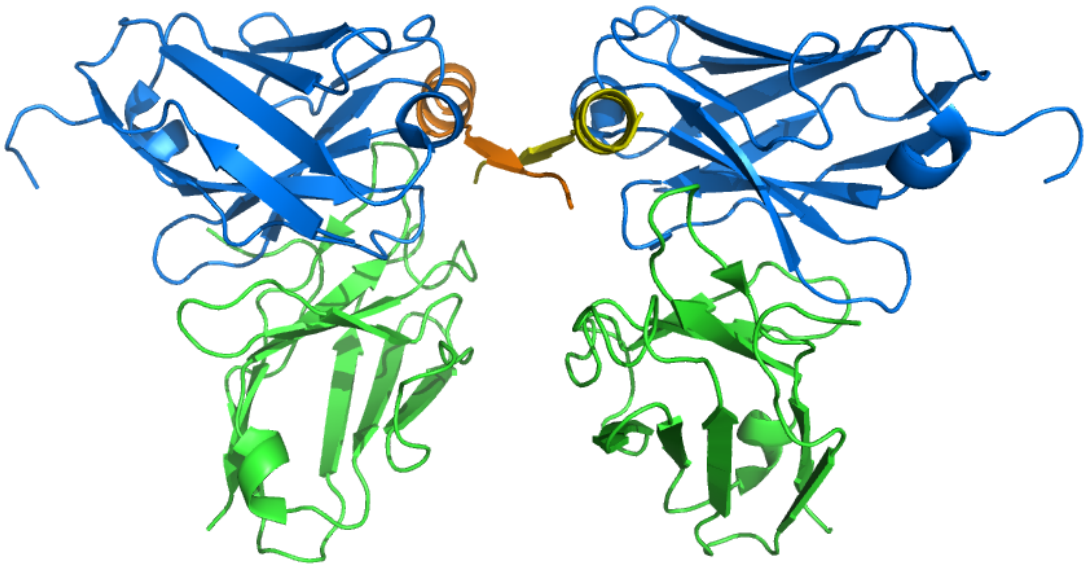
In fly HD models, C4 sFv can ameliorate HD phenotypes (Wolfgang et al (2005) PNAS Pubmed ID: 16061794 - Suppression of Huntington's disease pathology in Drosophila by human single-chain Fv antibodies). Similar effects are seen in combinatorial treatment regimens which enhance HSP70 levels (McLear et al (2008) FASEB J. Pubmed ID: 18199697 - Combinational approach of intrabody with enhanced Hsp70 expression addresses multiple pathologies in a fly model of Huntington's disease) and co-treat with cystamine (Bortvedt et al (2010) Neurobiol Dis Pubmed ID: 20399860 - Cystamine and intrabody co-treatment confers additional benefits in a fly model of Huntington's disease). In HD R6/1 mice, treatment with intrabody gene therapy reduces HD pathology (Snyder-Keller et al (2010) J. Neuropath Pubmed ID: 20838238 - Early or late-stage anti-N-terminal Huntingtin intrabody gene therapy reduces pathological features in B6.HDR6/1 mice).

When C4 sFv is tagged with a PEST motif to target C4 sFv complexes for UPS degradation, HTT exon1 fragments are significantly reduced in 2D striatal culture (Butler and Messer (2011) PLoS One Pubmed ID: 22216210 - Bifunctional anti-huntingtin proteasome-directed intrabodies mediate efficient degradation of mutant huntingtin exon 1 protein fragments in HDR6/1 mice).

Intracranial delivery to R6/1 mouse brains, cytoplasm localised C4 sFv appears to correct the mutant phenotype (Butler (2014) Protein Eng Des Sel Pubmed ID: 25301961 - Differential nuclear localization of complexes may underlie in vivo intrabody efficacy in Huntington's disease).

Sequence in crystal structure (4RAV): NB: sequence highlighted in red is not resolved in cocrystal structure
Full sequence expressed but linker missing in both copies of the molecule

VH – MAQVQLQESGGGLVQPGGSLRLSCAASGFTFSSYSMSWVRQAPGKGLEWVAVISYDGSNKYYADSVKGRFTISRDNSK
NTLYLQMNSLRAEDTAVYYCARDRYFDLWGRGTLVTVSSGGGSGGGSGGG
VL – SQSALTQPASVSGSPGQSITISCTGTSSDIGAYNYVSWYQQYPGKAPKLLIYDVSNRPSGISNRFSGSKSGDTASLTISGLQA
EDEADYYCSSFANSGPLFGGGTKVTVLGHHHHHH
HTT – MATLEKLMKAFESLKSF



VH (chains A and C) VL (chains B and D) HTT peptide (chain E) HTT peptide (chain F)

References and literature discussing this nanobody:

The structure of C4 sFv was solved in complex with HTT N-terminal N17 fragment (De Genst (2015) JMB Pubmed ID: 25861763 - Structure of a single-chain Fv bound to the 17 N-terminal residues of huntingtin

provides insights into pathogenic amyloid formation and suppression). The dimer is not observed in vitro so likely a crystallographic artefact. Potentially indicative of domain swapping – aa. 3-14 interact with C4 sFv whilst aa. 16-17 interact with the adjacent C4 sFv, perhaps they fold back to interact with the same molecule of C4 sFv in solution.

Methods describing huntingtin intrabody production and screening can be found here (Khoshnan et al (2013) Methods Mol Biol Pubmed ID: 23754229 - Antibodies and intrabodies against huntingtin: production and screening of monoclonals and single-chain recombinant forms).

Huntingtin intrabodies mentioned in these 2 review manuscripts (Ali et al (2011) Neurobiology of HD https://www.ncbi.nlm.nih.gov/books/NBK55999/ and Butler et al (2012) Prog Neurobiol Pubmed ID: 22120646 - Engineered antibody therapies to counteract mutant huntingtin and related toxic intracellular proteins).

Happ1

Type of Ig:
Single domain intrabody

Binding site:
Proline rich domain of exon 1 aa. 41-81 (Ref Mauiri et al 2016), P-rich epitope located between the two polyP epitopes aa. 52-68 (Ref Southwell et al 2008).

Sequence (from Addgene deposit https://www.addgene.org/98582/):
MQSVLTQPPSASGTPGQRVTISCSGSSSNIGSNYVYWYQQLPGTAPKLLIYRNNQRPSGVPDRFSGSKSGTSASLAISGLRPEDE
ADYYCAAWDDSLCVALVFGGGTNGGGGVDGTAG

References and literature discussing this nanobody:

Happ1 binds the P-rich epitope located between the two polyP epitopes (Southwell et al (2008) J Neurosci Pubmed ID: 18768695 - Intrabodies binding the proline-rich domains of mutant huntingtin increase its turnover and reduce neurotoxicity). Binding reduces mHTT exon1 aggregation and toxicity due to increased exon 1 turnover.

When administered via adeno-associated virus in live brain of a number HD mouse models (R6/2, N171-82Q, YAC128, and BACHD), Happ1 treatment conferred significant beneficial effects in a variety of assays of motor and cognitive deficits (Southwell et al (2009) J Neurosci Pubmed ID: 19864571 - Intrabody gene therapy ameliorates motor, cognitive, and neuropathological symptoms in multiple mouse models of Huntington's disease).

Happ1 clearance of HTT exon1 is independent of UPS and macroautophagy pathways. However, inhibition of calpain or lysosomal pH block reduces Happ1-mediated HTT exon 1 clearance suggesting that the complex is cleared via this process. Calpain cleaves HTT at aa. 15 – blocked when bound to VL12.3 which binds N17 (Southwell et al (2011) PLoS One Pubmed ID: 21304966 - Perturbation with intrabodies reveals that calpain cleavage is required for degradation of huntingtin exon 1).

Referred to as HCB1 (huntingtin chromobody 1), used to visualise endogenous huntingtin in living cells (Maiuri et al (2016) HMG Pubmed ID: 28017939 - Huntingtin is a scaffolding protein in the ATM oxidative DNA damage response complex).

Happ3

Type of Ig:
Single domain intrabody

Binding site:
P-rich epitope located between the two polyP epitopes aa. 52-68

Sequence (from Addgene deposit https://www.addgene.org/98582/):
MQSVLTQPPSASGTPGQRVTISCSGSSSNIGSNYVYWYQQLPGTAPKLLIYRNNQRPSGVPDRFSGSKSGTSASLAISGLRPEDE
ADYYCAAWDDSLCVALVFGGGTNGGGGVDGTAG

References and literature discussing this nanobody:

Happ3 binds the P-rich epitope located between the two polyP epitopes (Southwell et al (2008) J Neurosci Pubmed ID: 18768695 - Intrabodies binding the proline-rich domains of mutant huntingtin increase its turnover and reduce neurotoxicity). Binding reduces mHTT exon1 aggregation and toxicity due to increased exon 1 turnover.

VL12.3

Type of Ig:
Single domain intrabody

Binding site:
N17 – structure shows epitope is aa. 5-18

Sequence:
MGSQPVLTQSPSVSAAPRQRVTISVSGSNSNIGSNTVNWIQQLPGRAPELLMYDDDLLAPGVSDRFSGSR
SGTSASLTISGLQSEDEADYYAATWDDSLNGWVFGGGTKVTVLSGHHHHHH

NB: retrieved sequence from https://www.ncbi.nlm.nih.gov/protein/AAV87178.1
>AAV87178.1 immunoglobulin variable light chain domain VL12.3
MGSQPVLTQSPSVSAAPRQRVTISVSGSNSNIGSNTVNWIQQLPGRAPELLMYDDDLLAPGVSDRFSGSR
SGTSASLTISGLQSEDEADYYAATWDDSLNGWVFGGGTKVTVLSGHHHHHH

References and literature discussing this nanobody:

Clone 2.4.3 for HTT N-terminal 20 amino acids developed by yeast surface display (Colby et al (2004) JMB Pubmed ID: 15342245 - Development of a human light chain variable domain (V(L)) intracellular antibody specific for the amino terminus of huntingtin via yeast surface display).

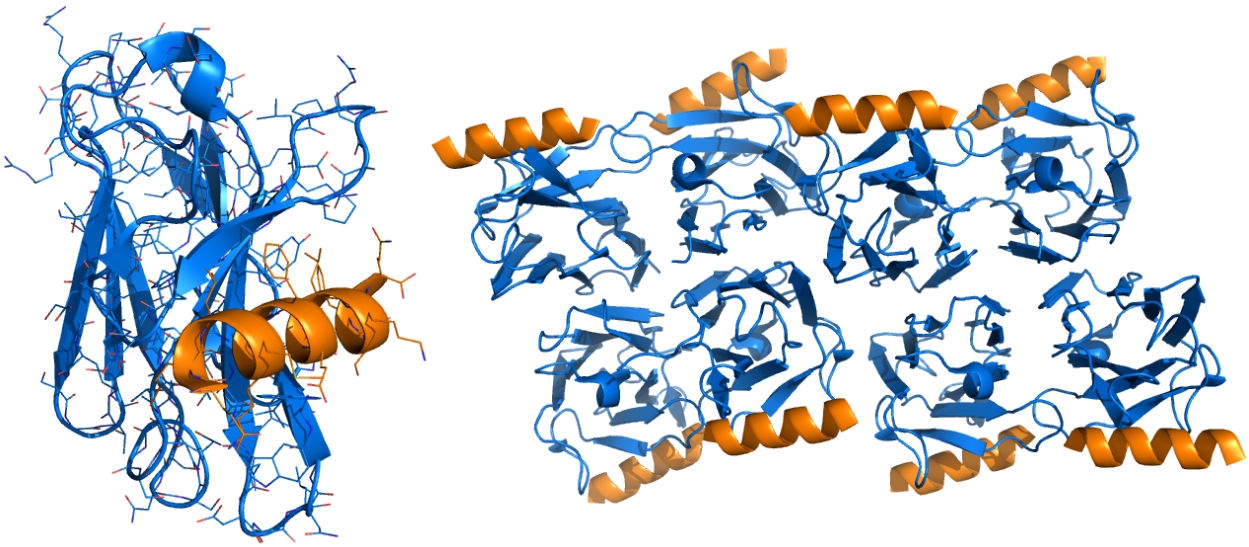
VL12.3 is a variable light chain disulphide free intrabody which binds HTT, reducing its aggregation and toxicity in a yeast model of HD (Colby et al (2004) PNAS Pubmed ID: 15598740 - Potent inhibition of huntingtin aggregation and cytotoxicity by a disulfide bond-free single-domain intracellular antibody).

VL12.3 binds the N17 of HTT (Southwell et al (2008) J Neurosci Pubmed ID: 18768695 - Intrabodies binding the proline-rich domains of mutant huntingtin increase its turnover and reduce neurotoxicity). Binding reduces mHTT exon1 aggregation and toxicity due to increased nuclear localisation and proposed reduction in toxic conformations of HTT exon 1.

When administered via adeno-associated virus in live brain of a number HD mouse models (R6/2 and YAC128,), VL12.3 treatment increased disease severity and mortality despite promising data from an HD lentiviral model (Southwell et al (2009) J Neurosci Pubmed ID: 19864571 - Intrabody gene therapy ameliorates motor, cognitive, and neuropathological symptoms in multiple mouse models of Huntington's disease).

Calpain cleaves HTT at aa. 15 – blocked when bound to VL12.3 which binds N17 (Southwell et al (2011) PLoS One Pubmed ID: 21304966 - Perturbation with intrabodies reveals that calpain cleavage is required for degradation of huntingtin exon 1).

Structure solved of VL 12.3 apo (PDB ID: 3LRG) and bound (PDB ID: 3LRH) to HTT sequence EKLMKAFESLKSFQ (Schiefner et al (2011) JMB Pubmed ID: 21968397 - A disulfide-free single-domain V(L) intrabody with blocking activity towards huntingtin reveals a novel mode of epitope recognition). HTT epitope is α-helical and is bound non-canonically at the base of the CDRs at the concave β-sheet at the interface between the paired VL and VH domain, while few interactions with CDR-L1 and 3 are formed.



VH (chains A, C, E, G, I, K, M and O) HTT peptide (chain B, D, F, H, J, L, N and P)

Intracranial delivery to R6/1 mouse brains, nuclear localised VL12.3 appears to accelerate the mutant phenotype (Butler (2014) Protein Eng Des Sel Pubmed ID: 25301961 - Differential nuclear localization of complexes may underlie in vivo intrabody efficacy in Huntington's disease).

INT41

Type of Ig:
Single domain intrabody

Binding site:
Proline rich domain of exon 1

Sequence:
Not provided

References and literature discussing this nanobody:

INT41 binds the proline rich domain of HTT exon 1 and expression in 2D culture reduces mHTT gene dysregulation (Amaro and Henderson (2016) Journal of Neurodegen Pubmed ID: 27595037 - An Intrabody Drug (rAAV6-INT41) Reduces the Binding of N-Terminal Huntingtin Fragment(s) to DNA to Basal Levels in PC12 Cells and Delays Cognitive Loss in the R6/2 Animal Model). Transduction with rAAV6-INT41 reduced DNA binding of mHTT in the nucleus and reduced nuclear translocation of mHTT fragments. rAAV6-INT41 delivery into the striatum in the R6/2 mouse model, reduced in HTT aggregates in the striatum and recovered HD phenotypes to WT levels.

iVHH4

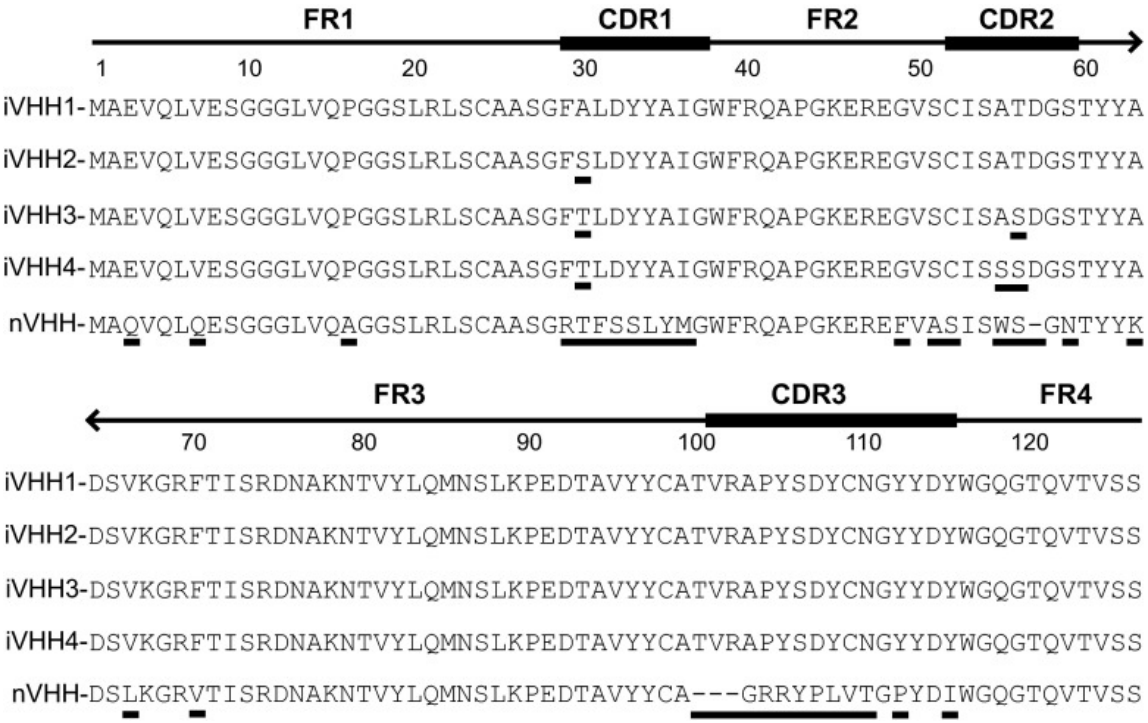
Type of Ig:
Single domain camelid intrabody

Binding site:
Proline rich domain aa. 49-148

Sequence:
MAEVQLVESGGGLVQPGGSLRLSCAASGFTLDYYAIGWFRQAPGKEREGVSCISSSDGSTYYADSVKGRFTISRDNAKNTVYLQ
MNSLKPEDTAVYYCATVRAPYSDYCNGYYDYWGQGTQVTVSS

References and literature discussing this nanobody:

VHH produced are single domain nanobodies which can bind WT HTT and mHTT under native and denatured conditions (Schut et al (2015) Neuro Sci Pubmed ID: 25294428 - Selection and characterization of llama single domain antibodies against N-terminal huntingtin). All VHH bind N-terminal HTT aa. 49-148. iVHH4 binds weakest of the 4 iVHH nanobodies developed:



Referred to as HCB2 (huntingtin chromobody 2), used to visualise endogenous huntingtin in living cells (Maiuri et al (2016) HMG Pubmed ID: 28017939 - Huntingtin is a scaffolding protein in the ATM oxidative DNA damage response complex).

scFv-EM48

Type of Ig:
Single-chain Fv (sFv) antibody

Binding site:
Not mapped but likely downstream of proline rich domain at ~ aa. 82-83

Sequence:
Not provided

References and literature discussing this nanobody:

EM48 preferentially binds mHTT but not other polyQ proteins, but as developed against a HTT construct with no polyQ or PRD, likely downstream of here at aa. 82-83 (Wang et al (2009) JCB Pubmed ID: 18504298 - Suppression of neuropil aggregates and neurological symptoms by an intracellular antibody implicates the cytoplasmic toxicity of mutant huntingtin). The intrabody suppresses mHTT aggregates and cytotoxicity in R6/2 mouse brain. The intrabody also focusses the cellular distribution of mHTT and increases mHTT degradation. Motor deficits of treated R6/2 mice were alleviated.

MW series and 3B5H10 (mAbs – not intra/nanobodies)

8 mAbs (MW1-8) generated to bind HTT exon 1 epitopes polyQ (3B5H10), polyP (MW7) or the C terminal tract. Described in these references (Khoshnan et al (2002) PNAS Pubmed ID: 11792860 - Effects of intracellular expression of anti-huntingtin antibodies of various specificities on mutant huntingtin aggregation and toxicity), (Southwell et al (2008) J Neurosci Pubmed ID: 18768695 - Intrabodies binding the proline-rich domains of mutant huntingtin increase its turnover and reduce neurotoxicity) and (Legleiter et al (2009) JBC Pubmed ID: 19491400 - Monoclonal antibodies recognize distinct conformational epitopes formed by polyglutamine in a mutant huntingtin fragment).