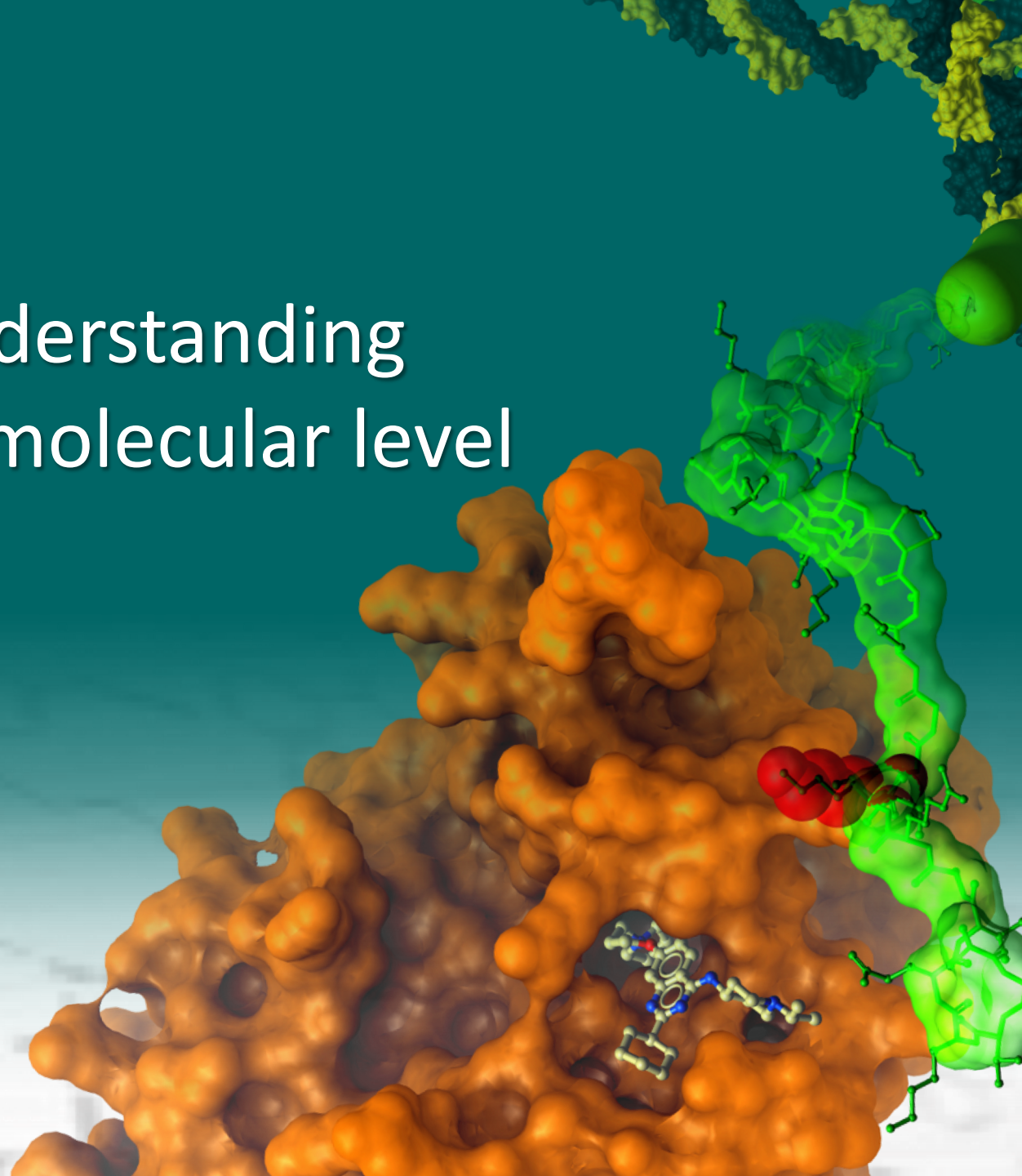
SGC

From proteins to people: understanding Huntington's disease at the molecular level

Dr Rachel J. Harding

HDSA Convention, Boston MA

28th June 2019

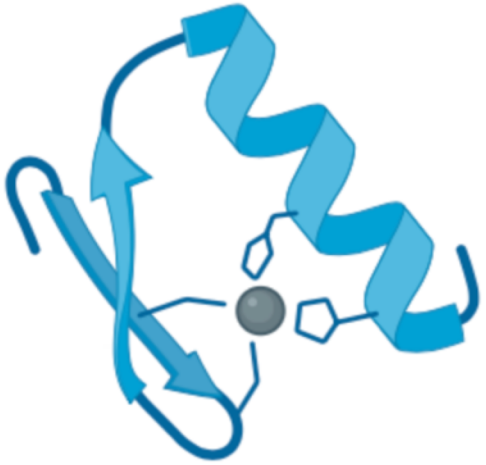


Understanding biology at the molecular level

2 metres
2 m

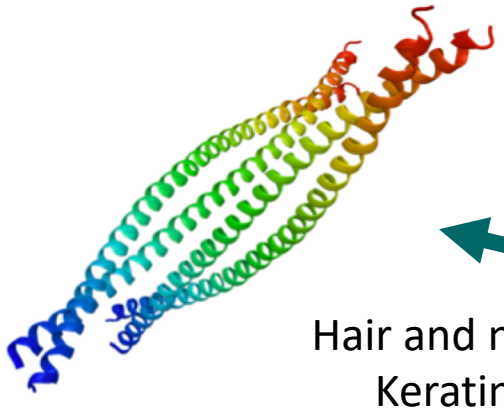
15 centimetres
0.15 m

20 micrometres
0.00002 m

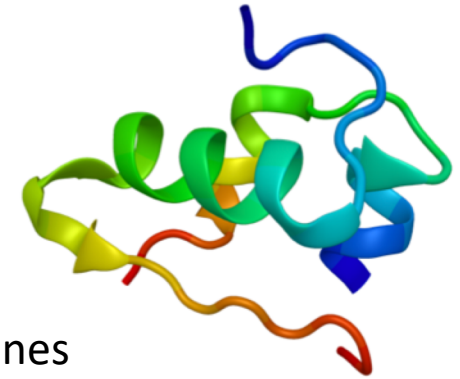


5 nanometres
0.000000005 m

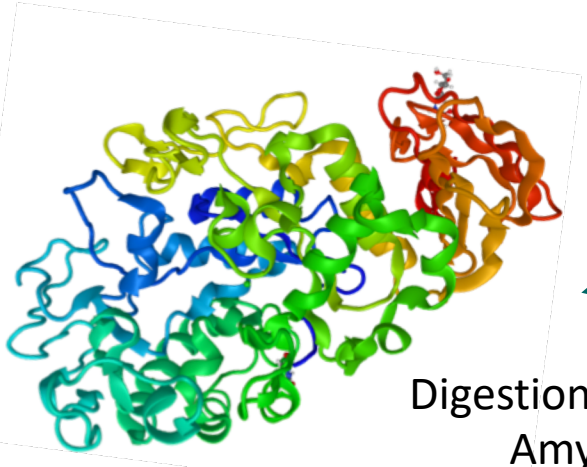
Proteins are the molecules which do the work in cells of our bodies



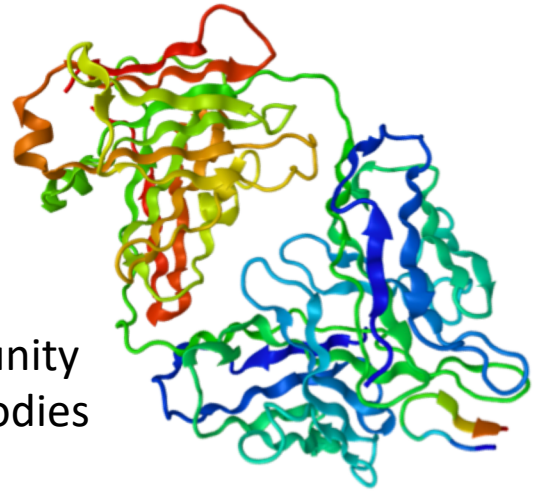
Hair and nails
Keratin



Hormones
Insulin



Digestion enzymes
Amylase



Immunity
Antibodies

Understanding protein molecules: the devil is in the details

?

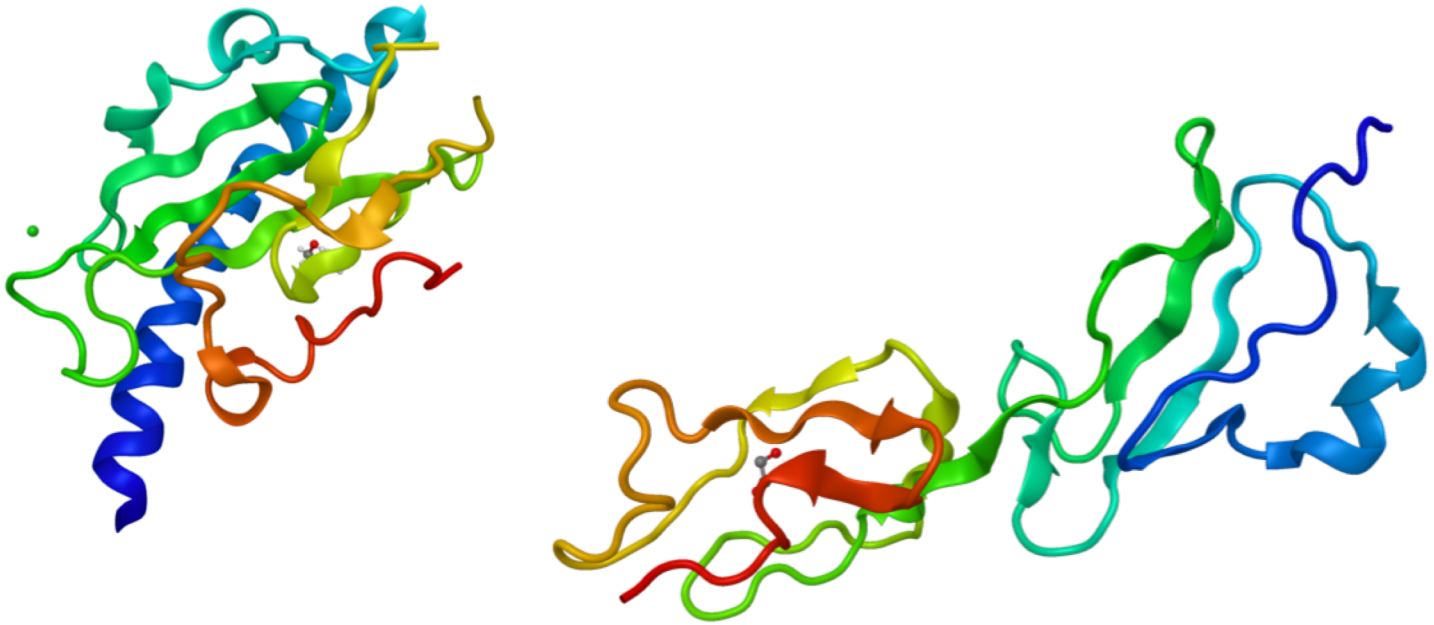
Understanding protein molecules: the devil is in the details

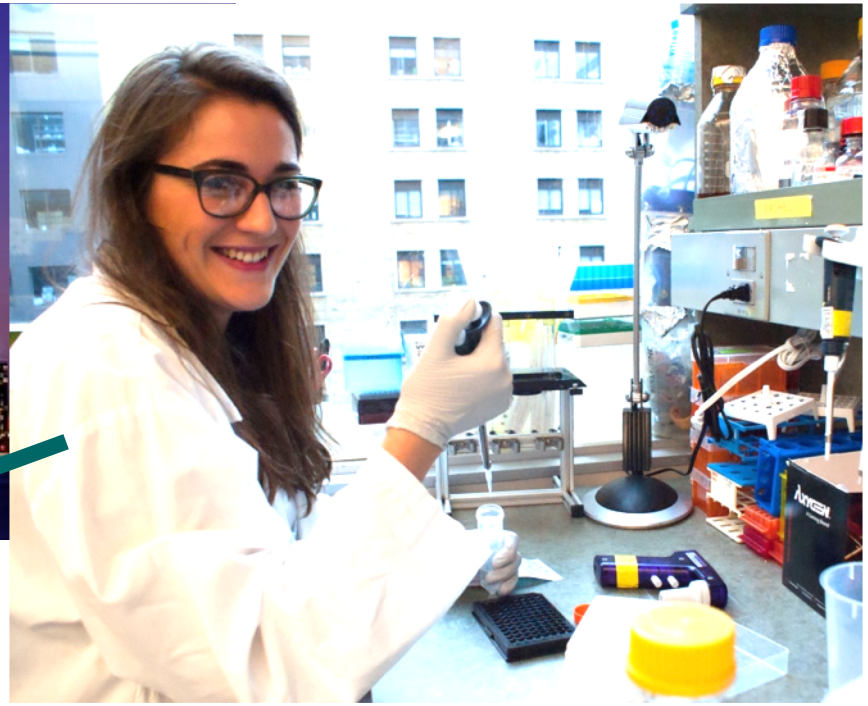
UNIVERSITY OF OXFORD

Professor Susan Lea

Thesis

Interactions of Neisseria meningitidis with the human immune system





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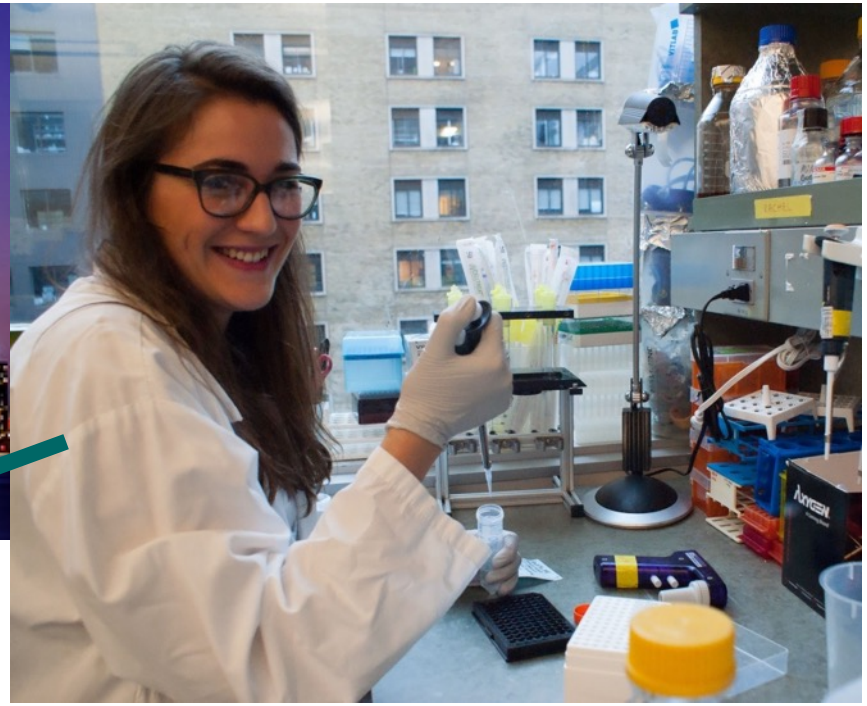
MaRS

Professor Cheryl Arrowsmith



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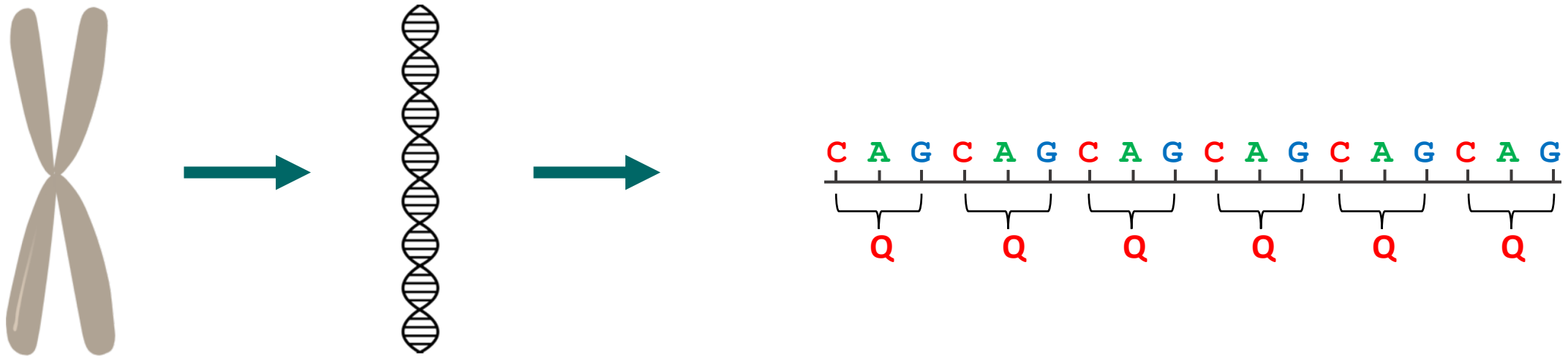
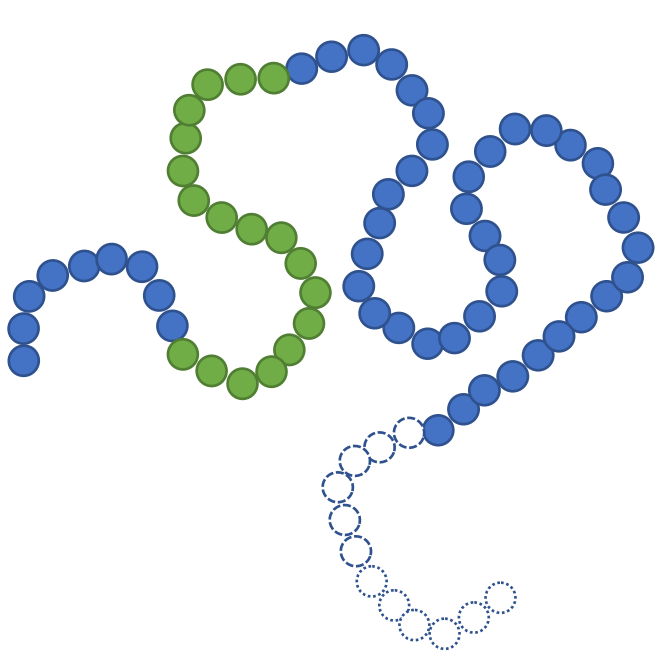
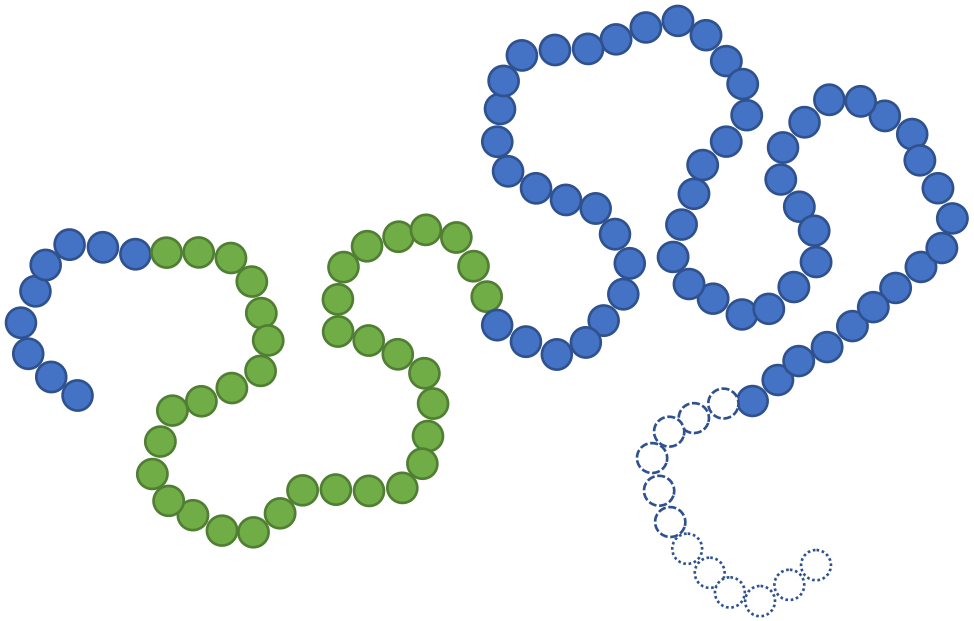
TORONTO RAPTORS

CAG repeats in the huntingtin gene encode a polyQ repeat in the huntingtin protein





Huntingtin – 18 Q (glutamine) residues



Huntingtin – 36 Q (glutamine) residues

PolyQ lengths above 35 change the function of the huntingtin protein

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TCLRNVHKVTTC

PolyQ lengths above 35 change the function of the huntingtin protein

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MATLEKLMKAFESLKSFQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQPPPPPPPPPPQLPQPPPQAQPLLPQPQPPPPPPPPPPGPAVAEEPLHRPKKELSATKKDR
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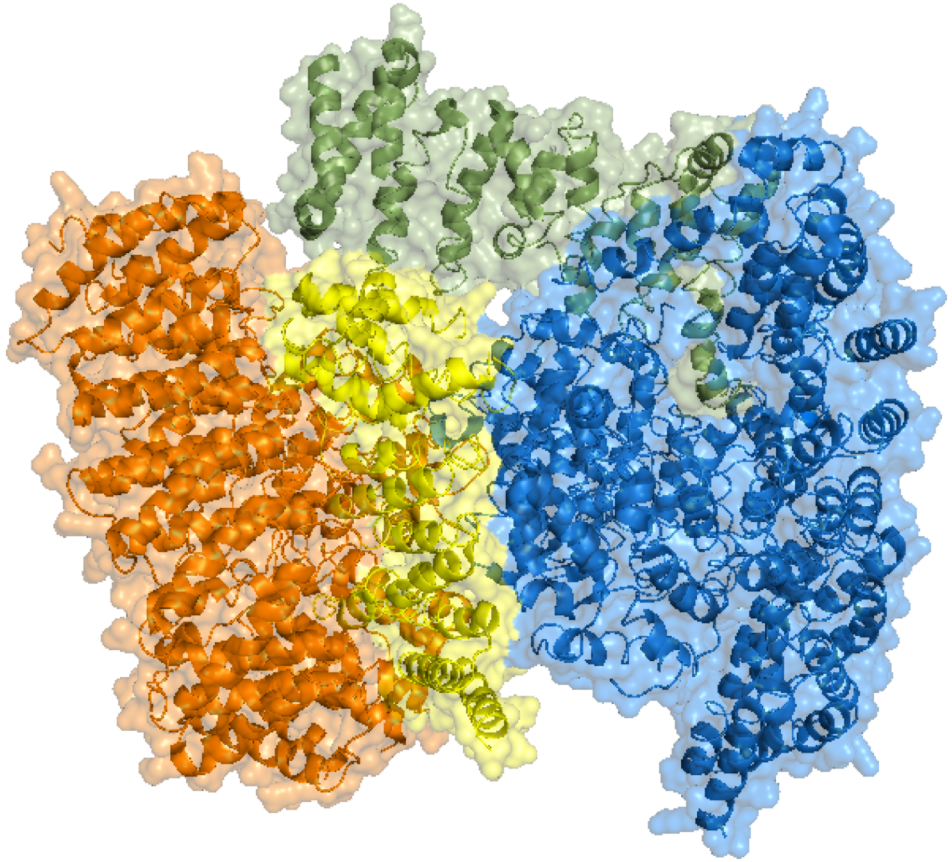
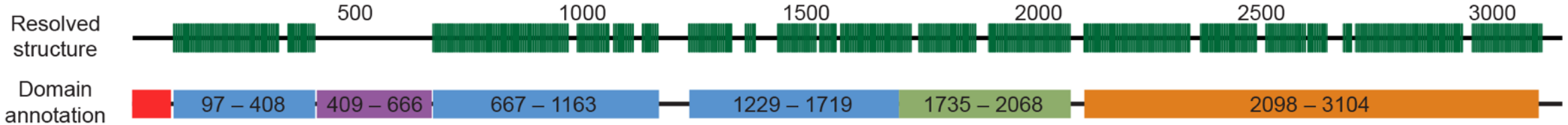
LEVVAAPGSPYHRLLTCLRNVHKVTTC

What does the huntingtin protein look like? What does this tell us about HD?



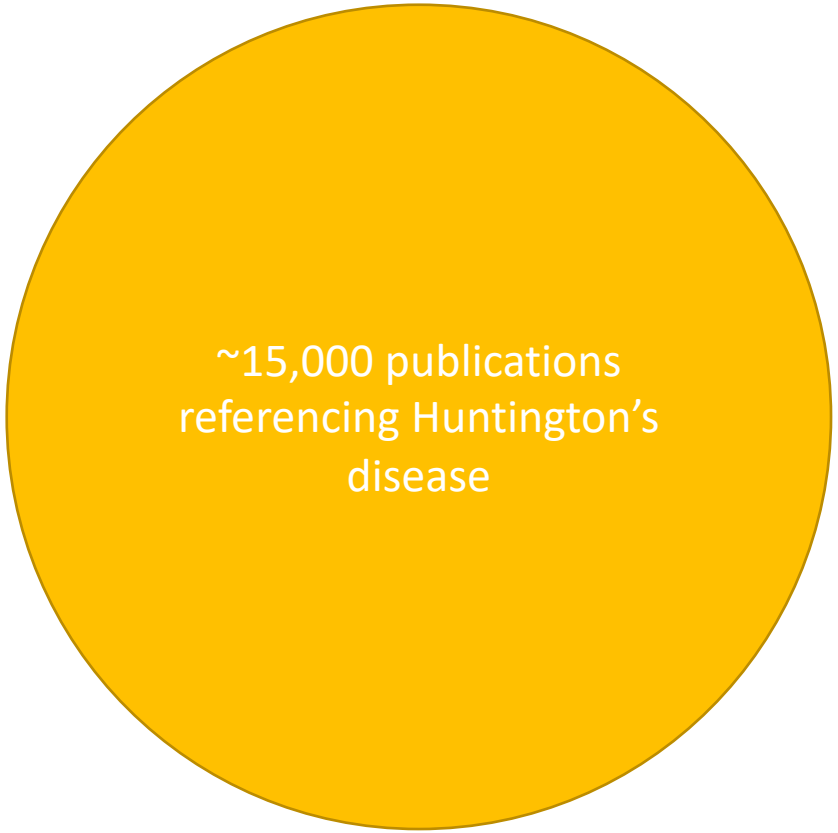
Exon 1
Intrinsically Disordered Region
N-terminal HEAT
Bridge
C-terminal HEAT
HAP40



Guo et al (2018)
Harding et al (2019)

A lack of available biochemical tools have limited investigation of the HTT protein

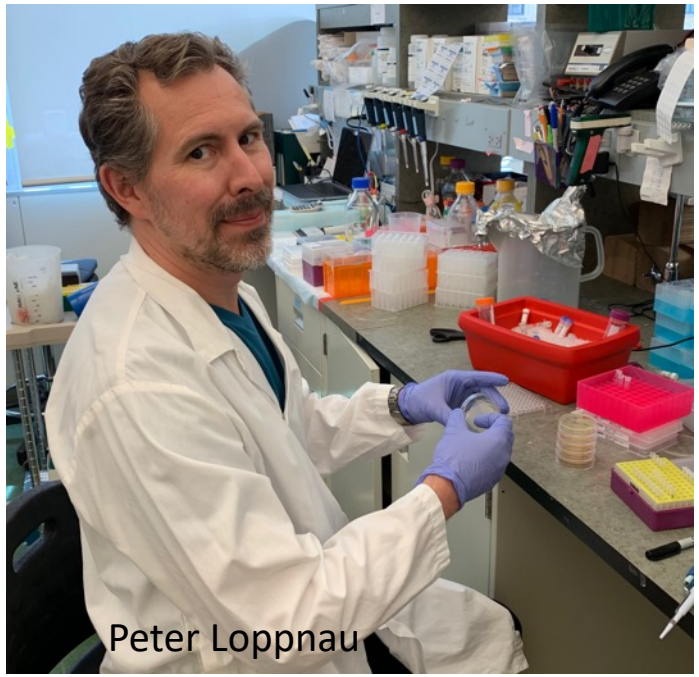
Data from PubMed search March 2019 for "Huntington's disease" & "purified huntingtin protein"

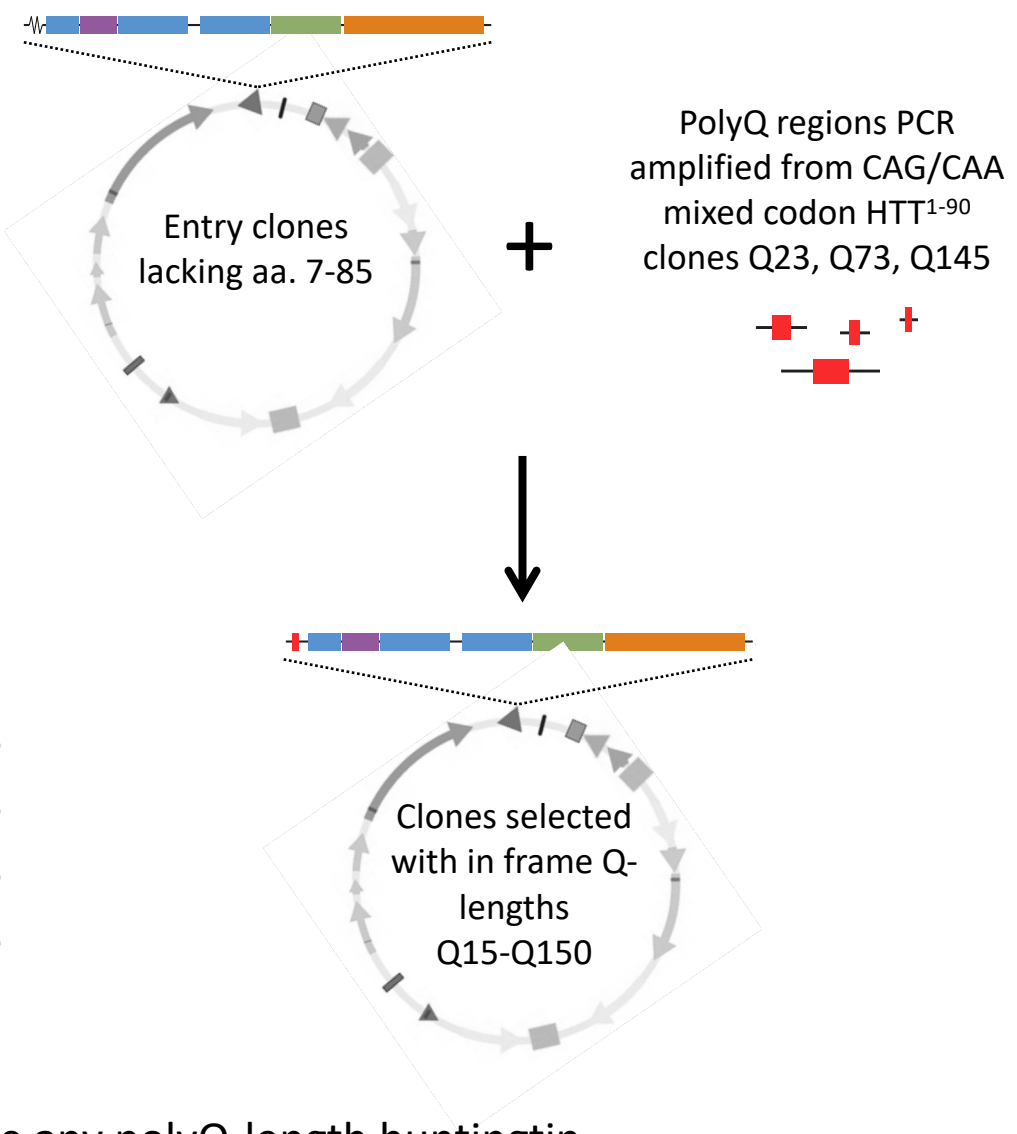


<50 publications referencing purified full-length huntingtin protein samples

Aim: Design and characterise an open toolkit of Huntington's disease research resources for biochemical investigation of full-length HTT

pBacMam full-length huntingtin constructs cloned to freely share with HD community

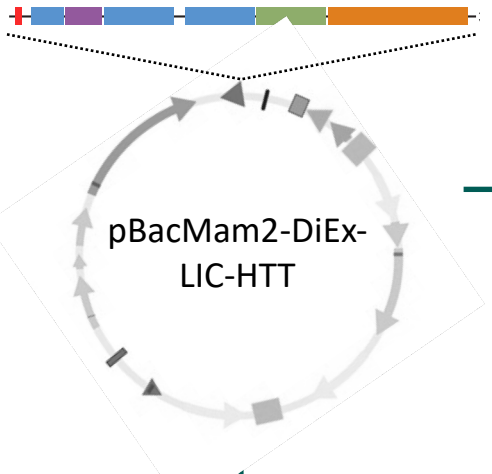




	N-terminal FLAG-tag	C-terminal FLAG-tag
General population	15, 19, 23	19, 23, 24, 25, 30
HD patients		36, 42, 48
Juvenile HD patients	51, 66, 73, 78, 79, 85	52, 54, 60, 66, 73, 79
Extreme expansions	139, 142, 145	109, 145

Our series of huntingtin constructs allow you to make any polyQ-length huntingtin

Huntingtin expression in insect and human cells using pBacMam2-DiEx-LIC constructs

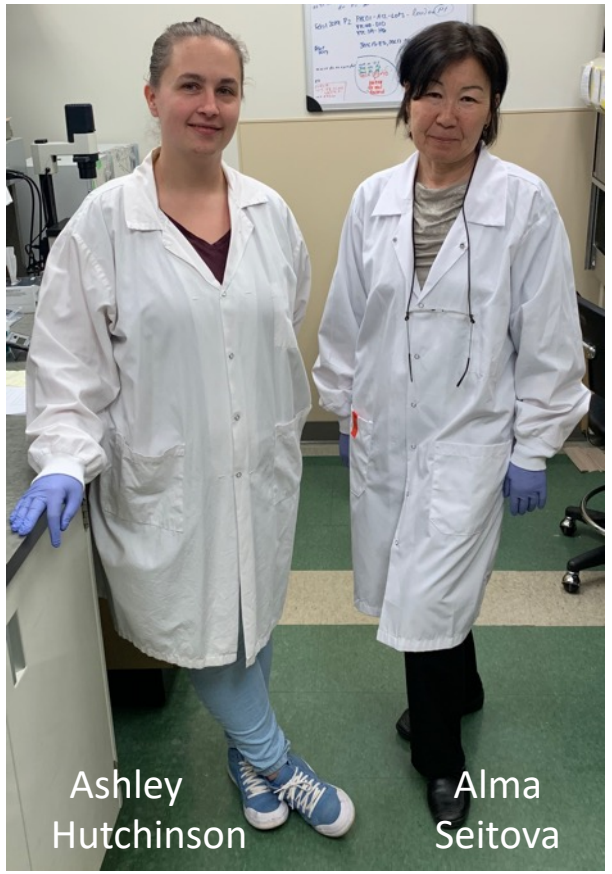


Bacmid DNA → Sf9 cell Transfection → Baculovirus recovery and amplification

1. Baculovirus expression in sf9 cells

2. Baculoviral transduction in EXPI293F

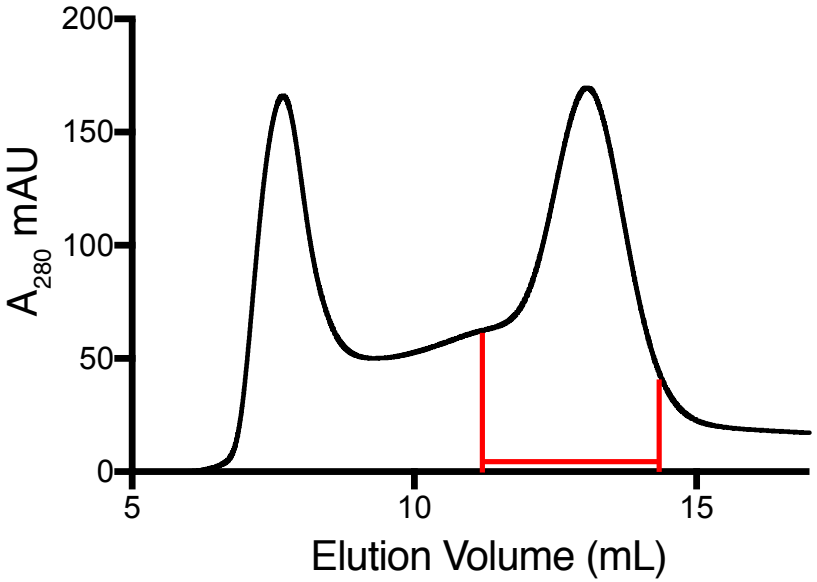
3. Transient transfection in EXPI293F



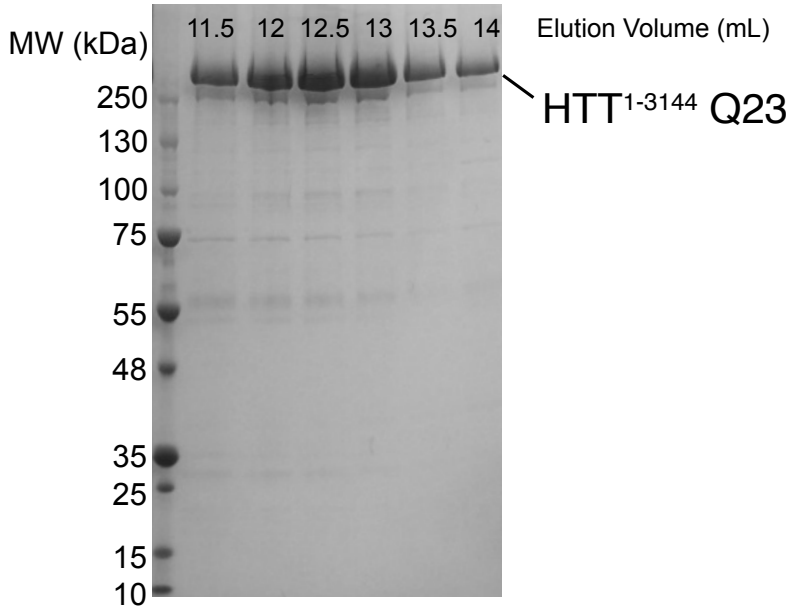
Suspension culture allows scalable production so you can make lots of huntingtin!

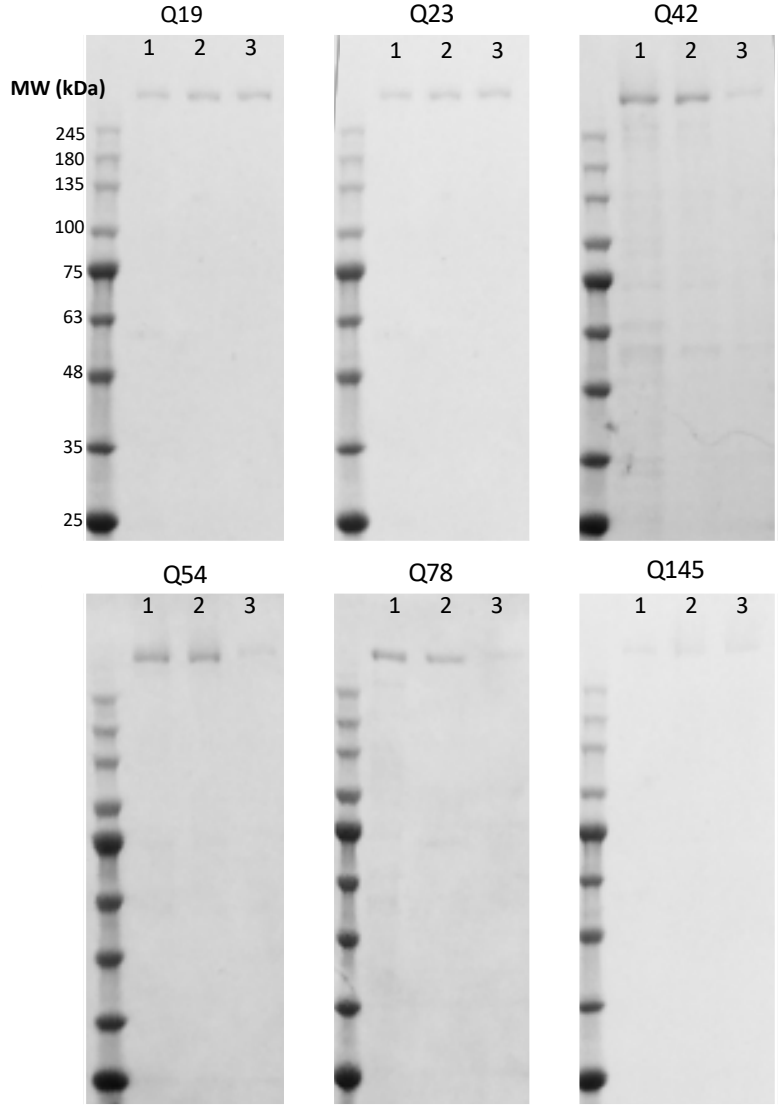
Full-length huntingtin of different Q-lengths can be trivially purified

Gel Filtration of insect HTT$^{1-3144}$ Q23



4-20% SDS-PAGE of monomer peak fractions





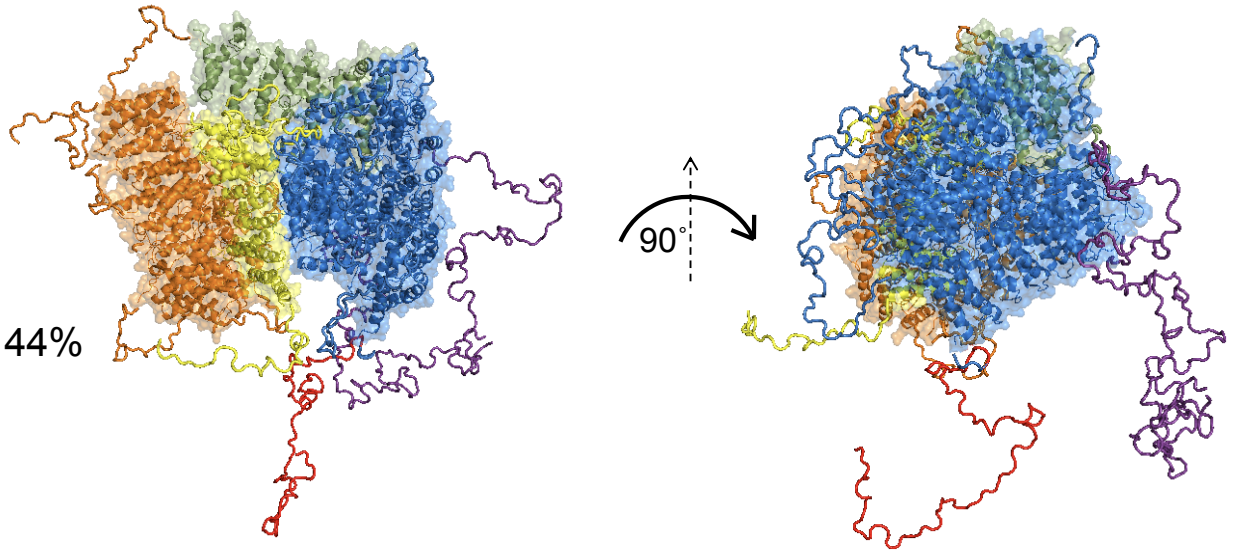
Huntingtin proteins can be extracted to high purity and high yield

1 - baculovirus expression in sf9 insect cells
2 - transient transfection in mammalian EXPI293F cells
3 - transduction in mammalian EXPI293F cells

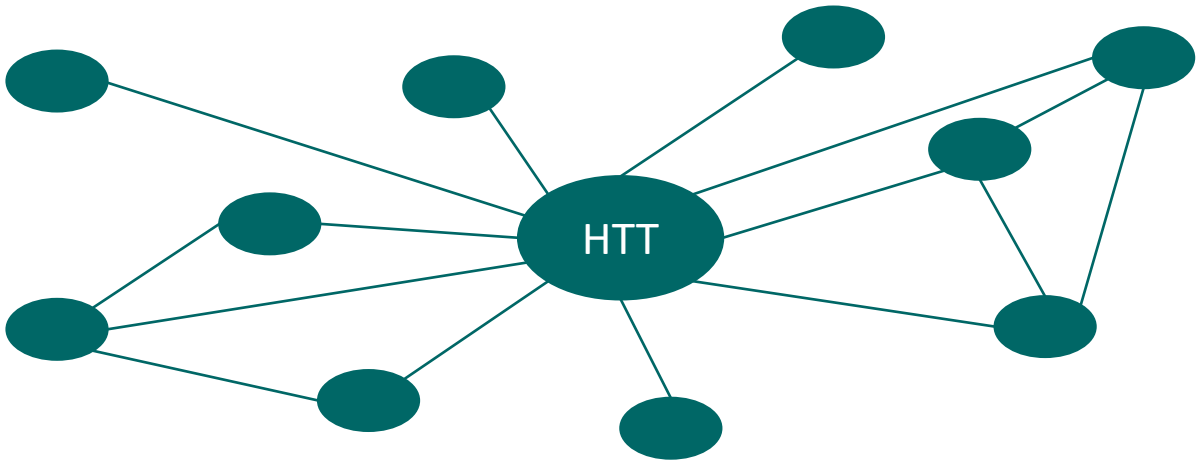
Trying to get a look at the polyQ region of the huntingtin protein molecule

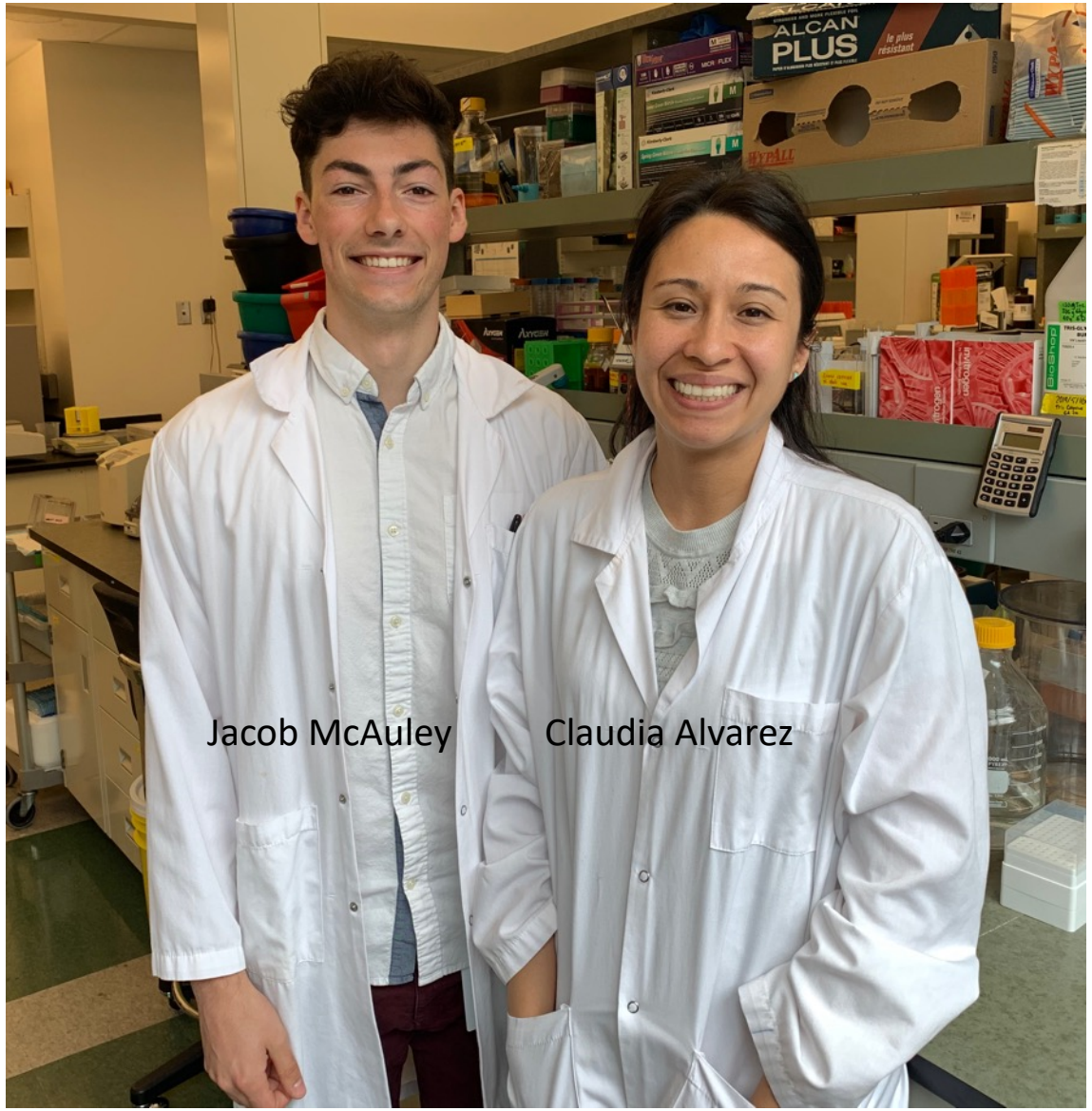
SGC

Computational approaches:

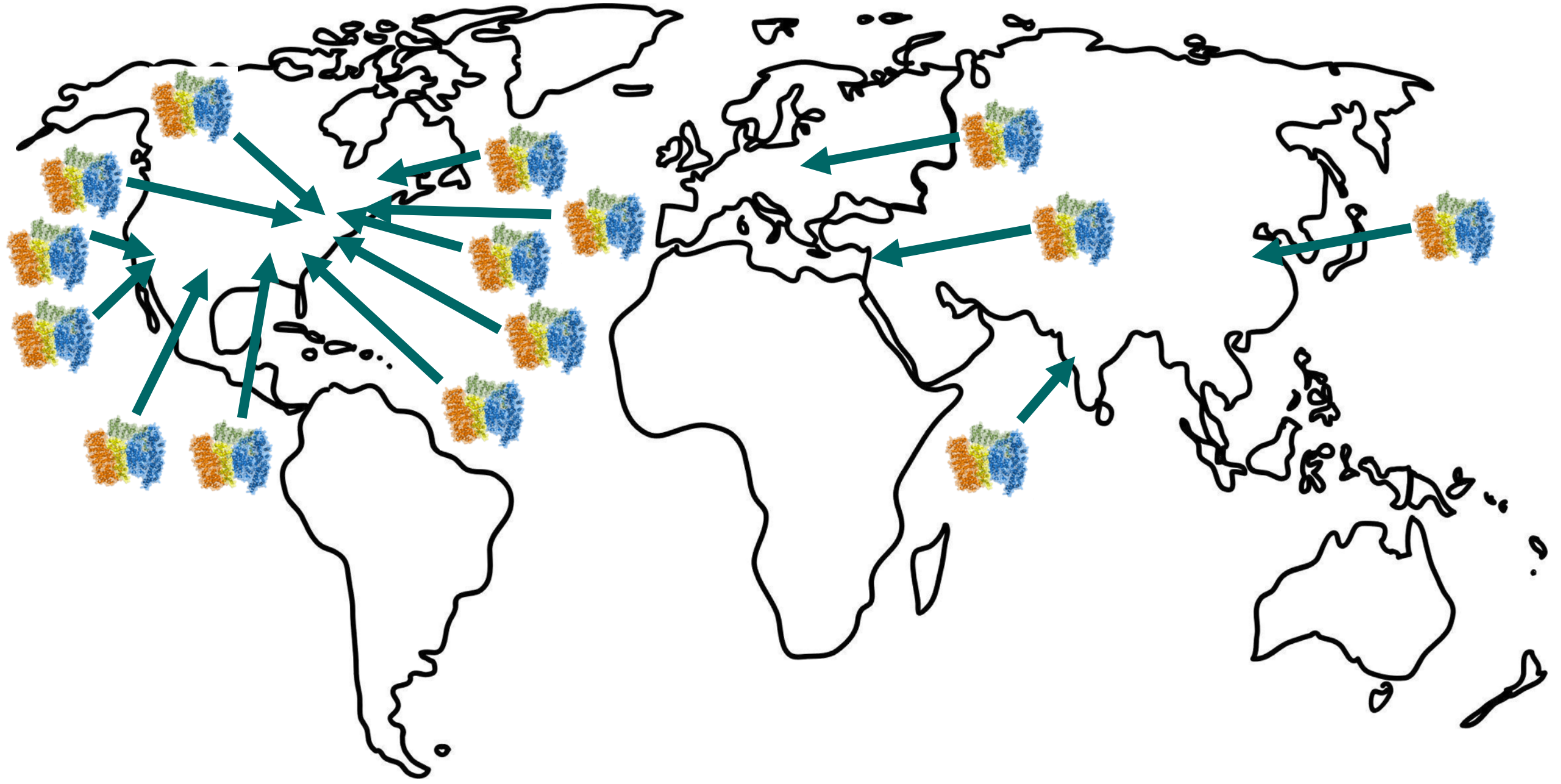


Finding other proteins which bind the huntingtin molecule:





pBacMam full-length huntingtin constructs freely shared with HD community



pBacMam full-length huntingtin constructs freely shared with HD community

All huntingtin plasmids and entry clones from this study are available through the Addgene repository

addgene
A better way to share plasmids

31	12	15
Total no. samples requested since December 2018	No. research groups requesting samples since December 2018	No. different constructs (Q-lengths) requested since December 2018

custom™ BIOLOGICS

We help provide scientists with huntingtin protein of any Q-length either via collaboration or CRO partnership

12	2
No. research groups huntingtin protein samples shared through collaboration since October 2017	CRO requests for purified HTT protein since January 2019

Full-length huntingtin protein interaction investigating collaborative consortium

Rachel Harding and Cheryl Arrowsmith
SGC, University of Toronto

Stefan Kochanek
Ulm University

Erich Wanker and Philipp Trepte
Max-Delbrück-Center for Molecular Medicine

Christopher Ross and Tamara Ratovitski
John Hopkins University

Publication of this work through my open lab notebook LabScribbles.com

1. Experiments completed in lab

2. Materials, methods, data and analysis uploaded to https://zenodo.org in open notebook community

3. Lay summary of experiment including discussion of context, aims and next steps plus links to Zenodo data upload

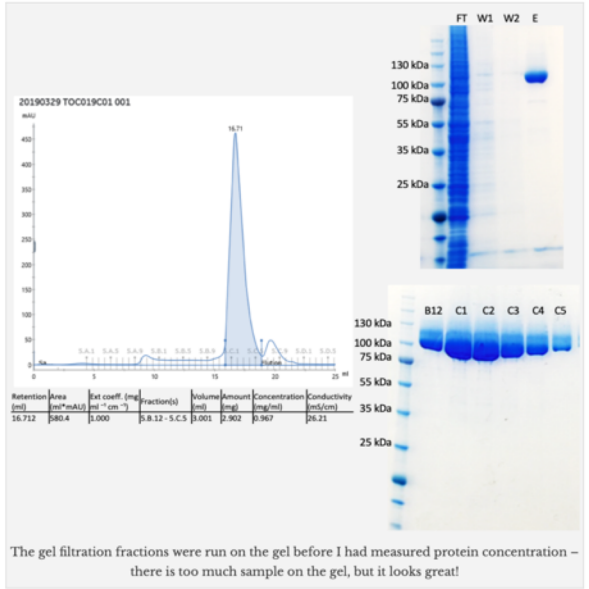
4. Share open notebook post via Twitter

Successful generation of fragments of the HTT protein and improving the purification procedure for the HTT-HAP40 complex

April 3, 2019 racheljaneharding Leave a comment Edit

It has now been almost 2 years since I set out to try and make fragments of the huntingtin protein which might be amenable to structural analysis with X-ray crystallography. X-ray crystallography is a fantastic method and allows us to see the molecules in very fine atomic detail which is important if we are to understand the intricacies of the elusive huntingtin protein molecule. It has been a long hard road with almost none of our extensive cloning efforts producing any expression constructs which made sufficient yields of protein. X-ray crystallography is a protein-expensive method so we need milligram (lots of protein). But finally, I have purified milligrams of different huntingtin fragment protein samples!!!

It should be made very clear that this has been a big team effort with cloner extraordinaire, Peter Loppnau, the eukaryotic production, Ashley Hutchinson and Alma Seitova as well as Linda Lin doing a lot of the heavy lifting on our cloning and eukaryotic production pipeline so I am very grateful for all of their hard work. Turns out that my construct design was fine but the expression vector we used made a huge difference (this is the piece of DNA which we insert different parts of the huntingtin gene). Switching from pFBOH-MHL to pBMDEL gave us great yields! Here is the C-terminal HEAT domain protein I purified – so much pure protein! All of the other data can be found on Zenodo.



The gel filtration fractions were run on the gel before I had measured protein concentration – there is too much sample on the gel, but it looks great!

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SGC

LAB SCRIBBLES
DR. RACHEL HARDING

labscribbles.com

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