Application of label-free technologies in a core facility research

Ewa Folta-Stogniew
Yale University

Biophysics Resource of Keck Laboratory: Yale School of Medicine

Mission: quantitative characterization of interactions between biomolecules using in solution biophysical methods

Common questions:

- how tight is the binding ? (binding affinity: K_d, K_a)
- how many of each molecule are in the complex (stoichiometry)
- how fast does the complex form? (kinetics)
- is the binding event enthalpy or entropy-driven? (thermodynamics)

List of technologies:

- Size Exclusion Chromatography coupled with Light Scattering (SEC/LS)
- Dynamic Light Scattering (DLS)
- Isothermal MicroCalorimeter (ITC)
- CD-Spectrophotometer
- Stopped-Flow Spectrofluorometer
- Surface Plasmon Resonance (SPR) Sensor [BiaCore Biosensor; T100]
- Composition Gradient Static Light Scattering (CGSLS)
- Asymmetric flow Field-Flow Fractionation (AFFF)

http://info.med.yale.edu/wmkeck/biophysics/

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Application of label-free technologies in a core facility research

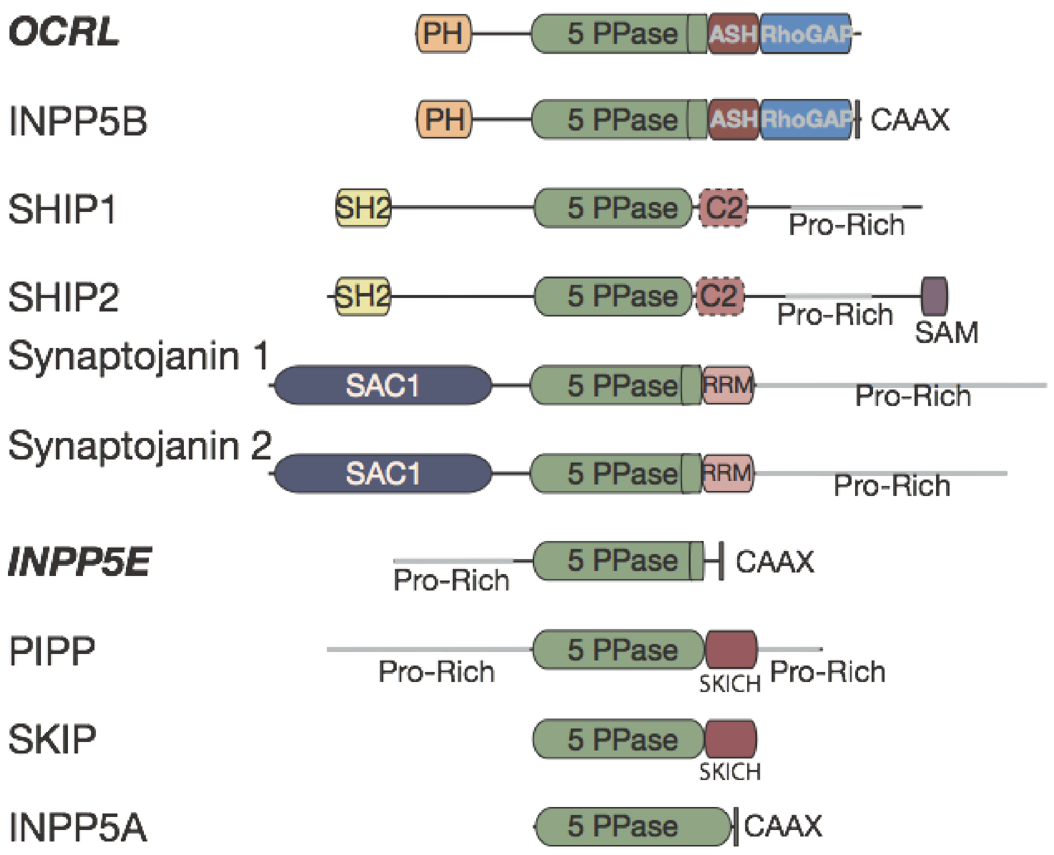
Recognition of the F&H motif by the Lowe syndrome protein OCRL

Michelle Pirruccello, Laura Swan, Ewa Folta-Stogniew, and Pietro DeCamilli

Yale University

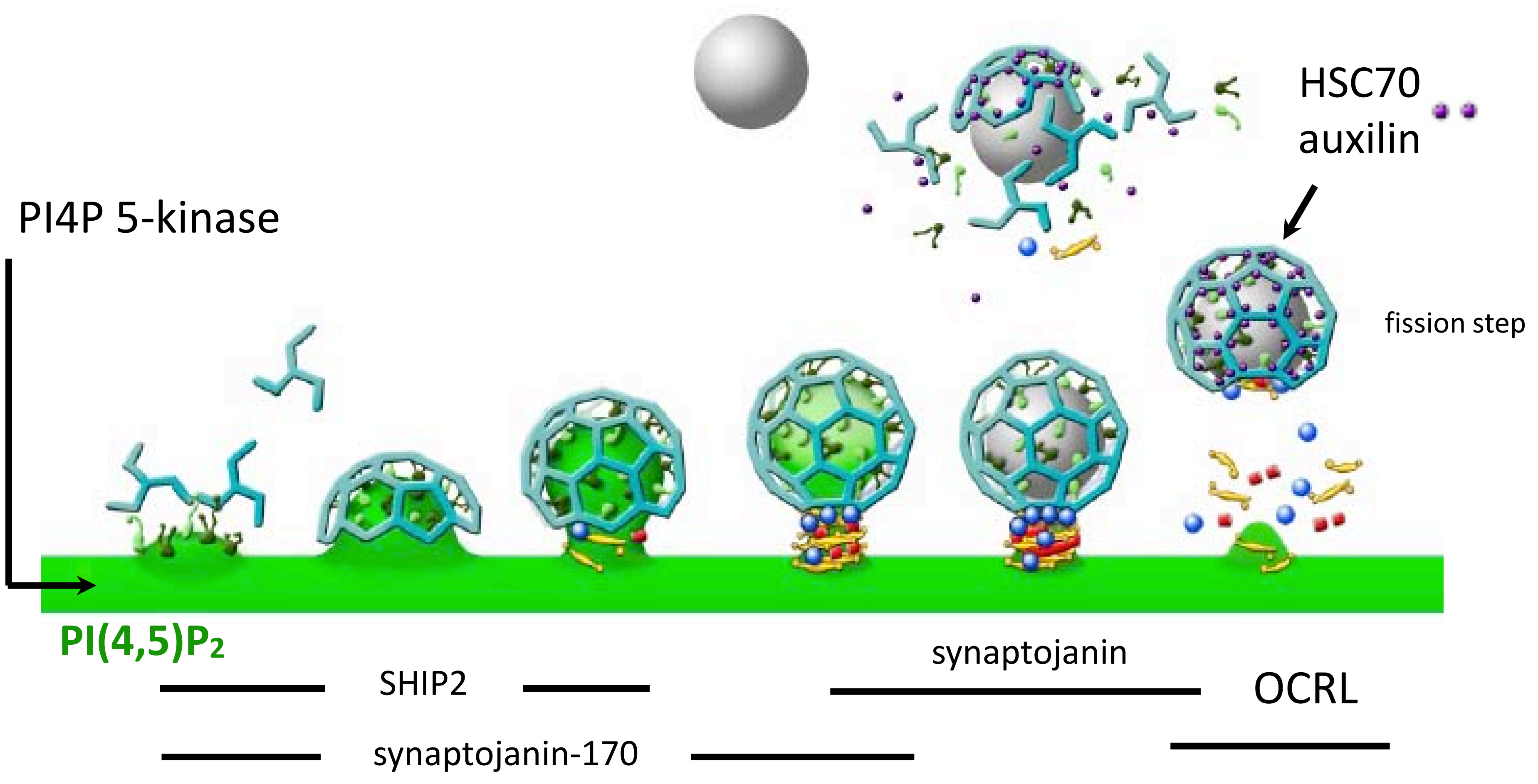
Pirruccello M, Swan L. E., Folta-Stogniew E., and De Camilli P. (2011) *Nat Struct.Mol.Biol* **18**; 789-795

Phosphoinositide 5-Phosphatases



Common phosphatase domain flanked by regions which direct the enzymes to the correct membrane target

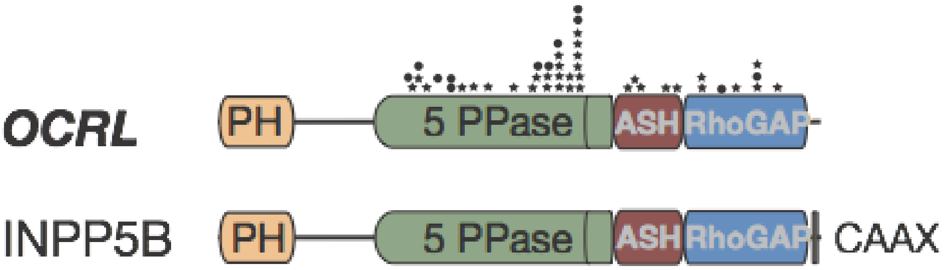
$PI(4,5)P_2$ in Clathrin-mediated endocytosis

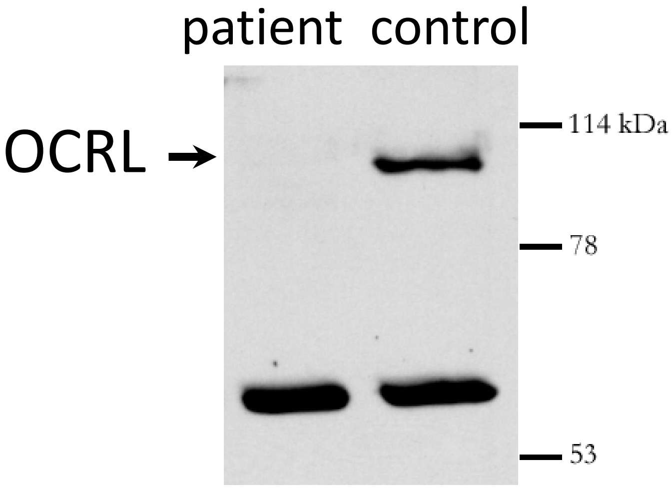


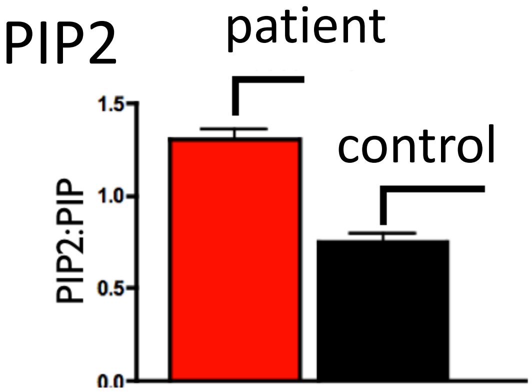
Loss of function of OCRL: **O**culo**c**erebro**r**enal syndrome of **L**owe and Dent's disease (OCRL phosphatase)

Lowe's syndrome
Kidney Reabsorbtive Defects
Congenital Cataracts
Cognitive Impairment

Dent 2 Disease
Mainly caused by mutations in ClC-5.
Some patients identified with OCRL mutations.
Kidney Reabsorbtive Defects

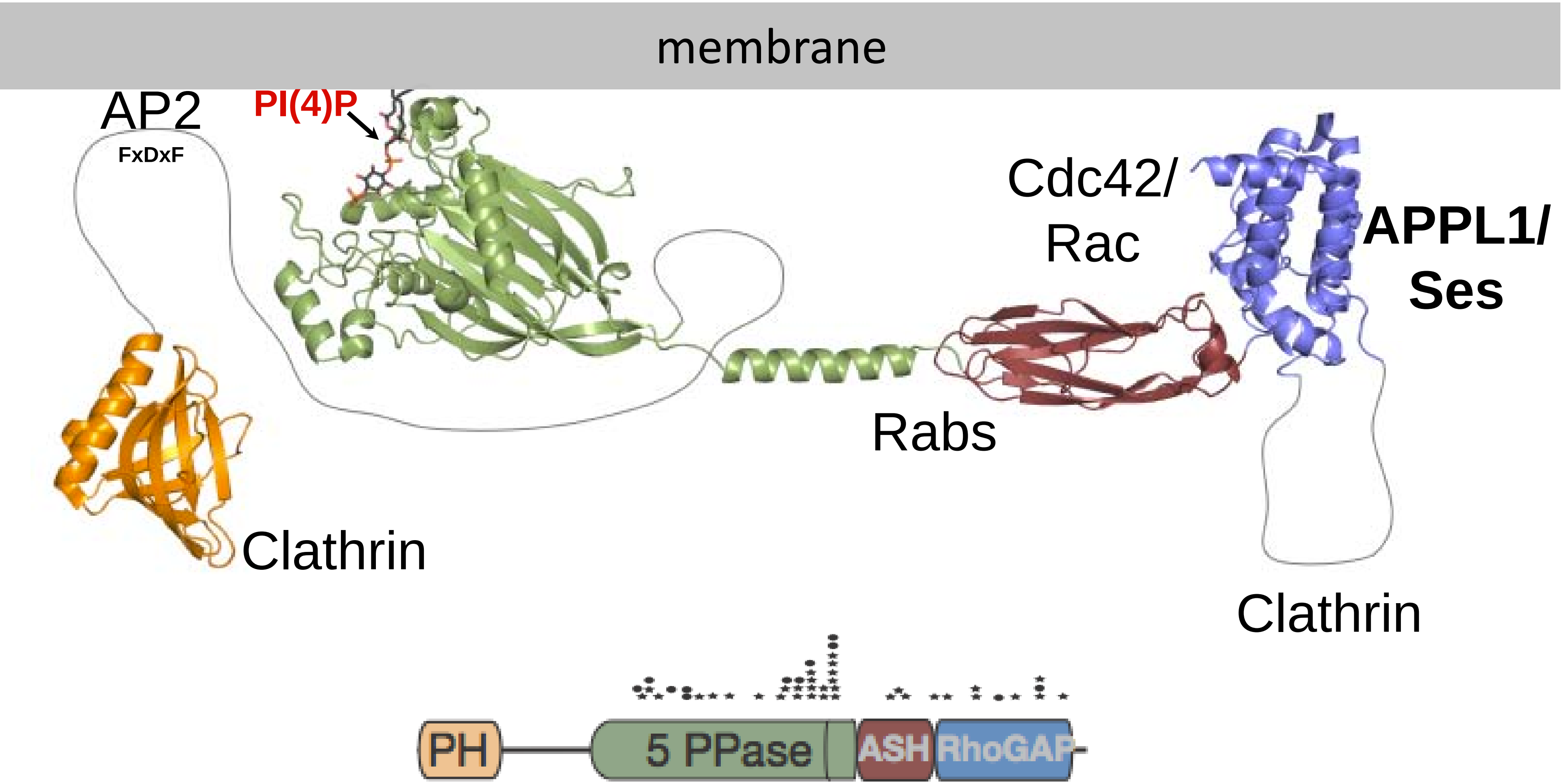






Disease causing mutations in C-terminus (non-catalytic domain)
Increased PIP2 level even for mutations in non-catalytic domains

OCRL membrane recruitment



membrane

AP2

FxDxF

PI(4)P

Cdc42/
Rac

**APPL1/
Ses**

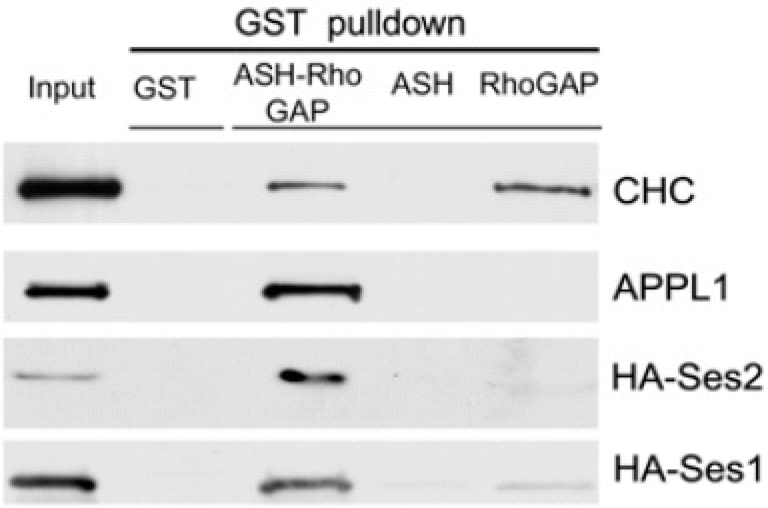
Rabs

Clathrin

Clathrin

Mao et. al. 2009
3MTC.pdb (Structural Genomics Consortium)
Pirruccello et. al. 2011

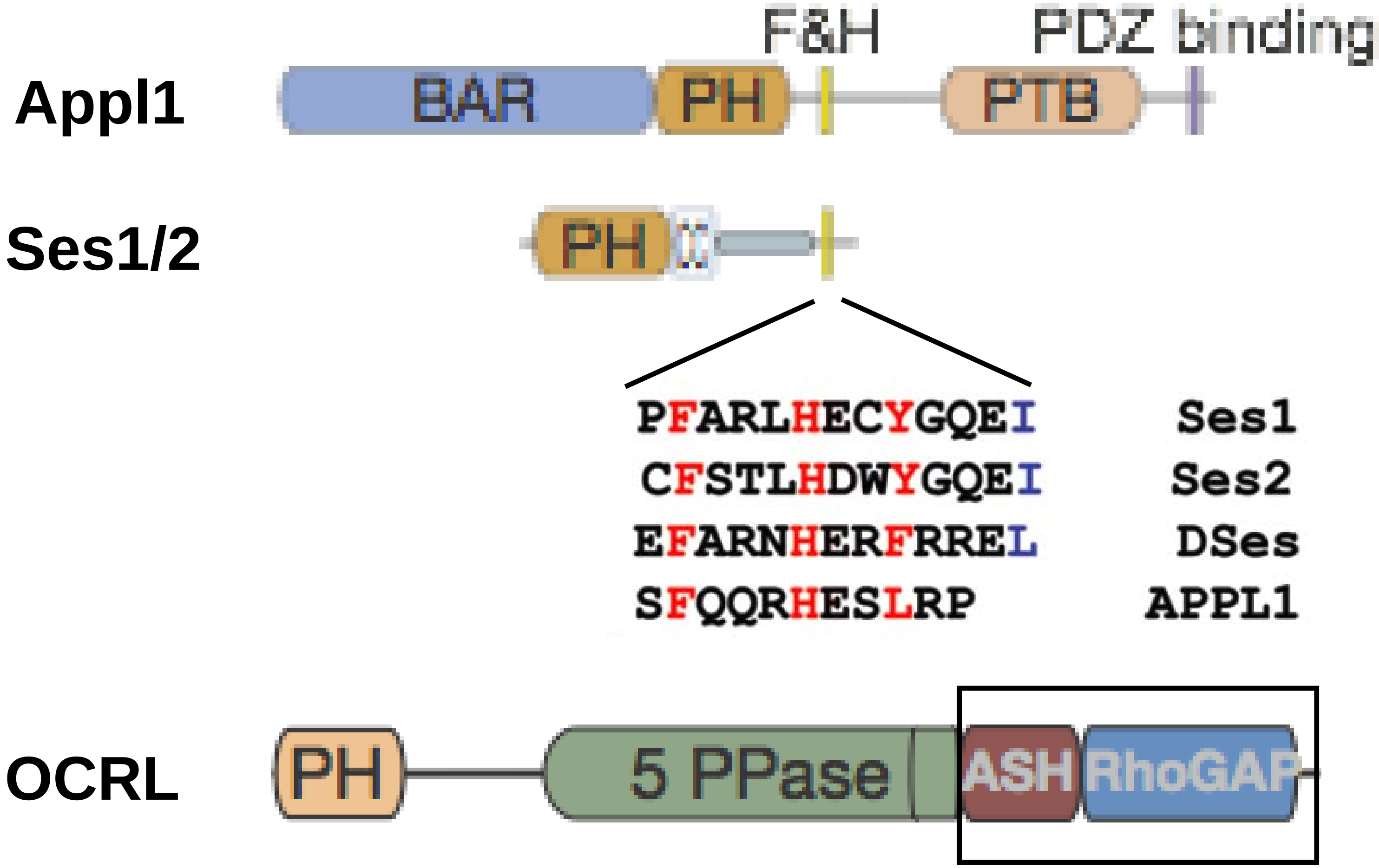
A new class of endocytic adaptors containing an F&H motif



Clathrin Heavy Chain

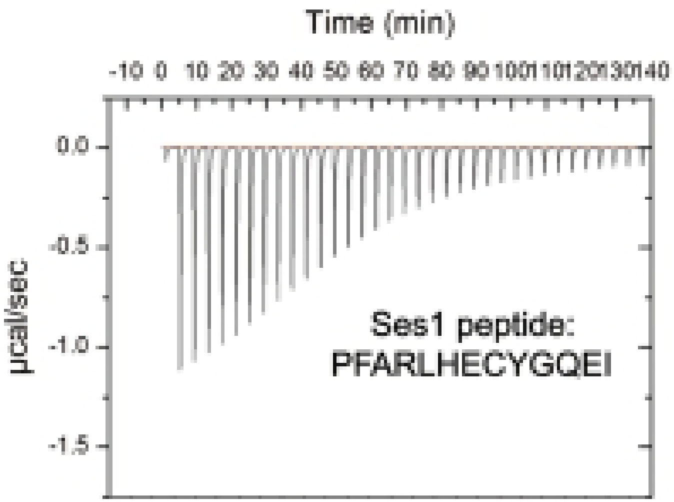
Binding of F&H motif requires the presence of both: the ASH and RhoGAP domains

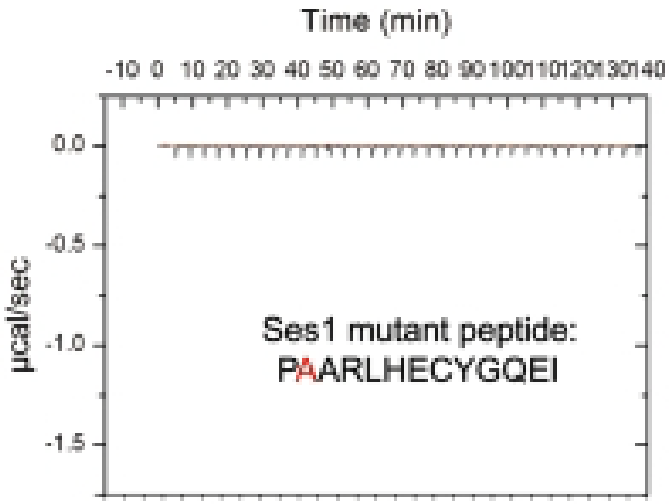
Definition of the consensus F&H motif



PFARLHECYGQEI	Ses1
CFSTLHDWYGQEI	Ses2
EFARNHERFRREL	DSes
SFQQRHESLRP	APPL1

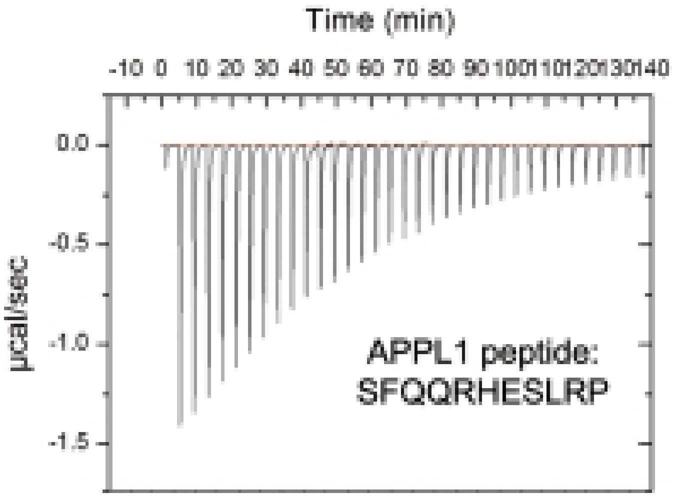
Direct binding of the minimal consensus peptides to the OCRL ASH-RhoGAP–like domain





F to A mutant

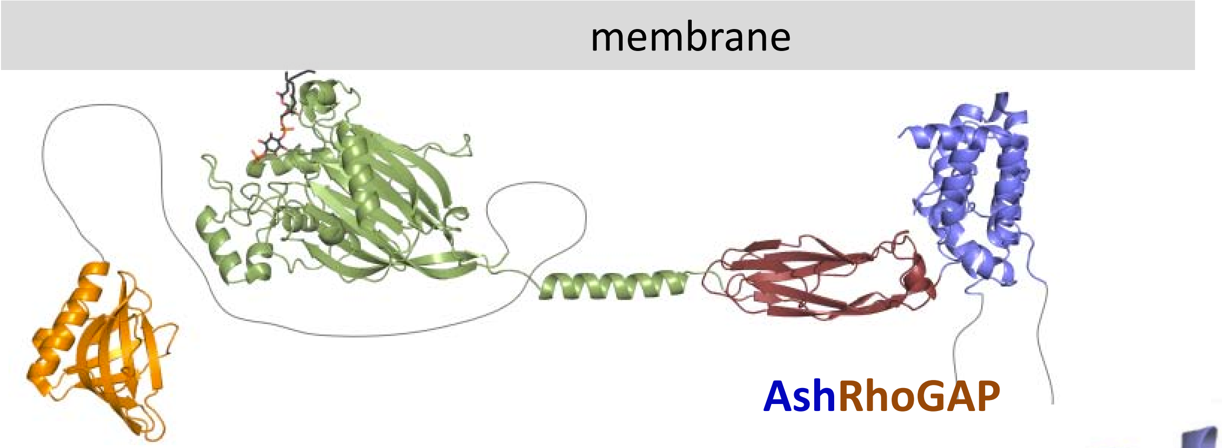
30 μM OCRL
2 mM peptide



Kd (μM) **ITC**			
Proteins	**F&H Peptides**		
	APPL	**Ses1**	**Ses1 (F to A)**
Wt OCRL ASH-RhoGAP	12 ± 2	0.7 ± 0.08	NB

Swan L E, Tomasini L., Pirruccello M., Lunardi J. l., and De Camilli P. (2010) Two closely related endocytic proteins that share a common OCRL-binding motif with APPL1. *Proceedings of the National Academy of Sciences* **107**; 3511-3516

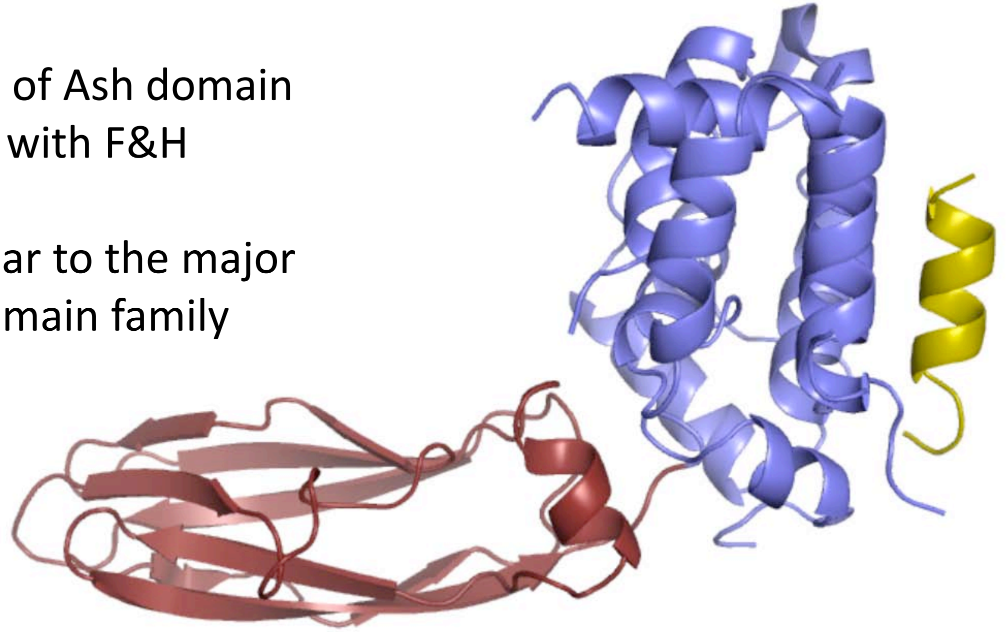
Crystallographic Analysis of the F&H/OCRL interaction



F&H peptides

PFARLHECYGQEI	Ses1
CFSTLHDWYGQEI	Ses2
EFARNHERFRREL	DSes
SFQQRHESLRP	APPL1

- better definition of Ash domain when in complex with F&H peptide
- structurally similar to the major sperm protein domain family



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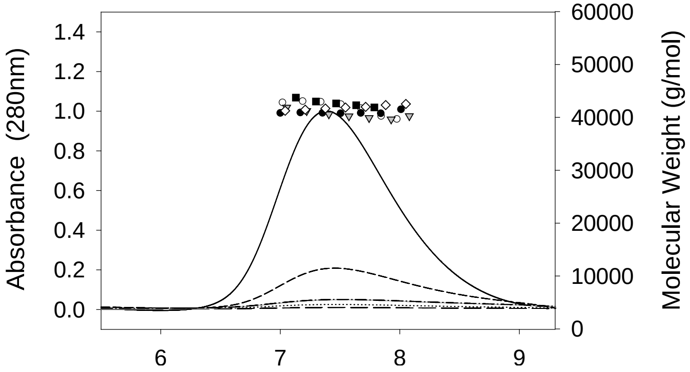

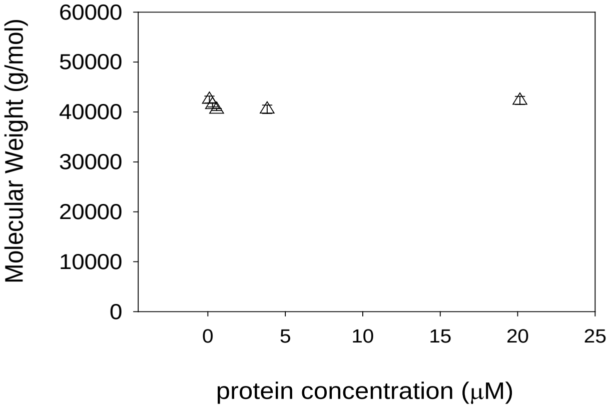

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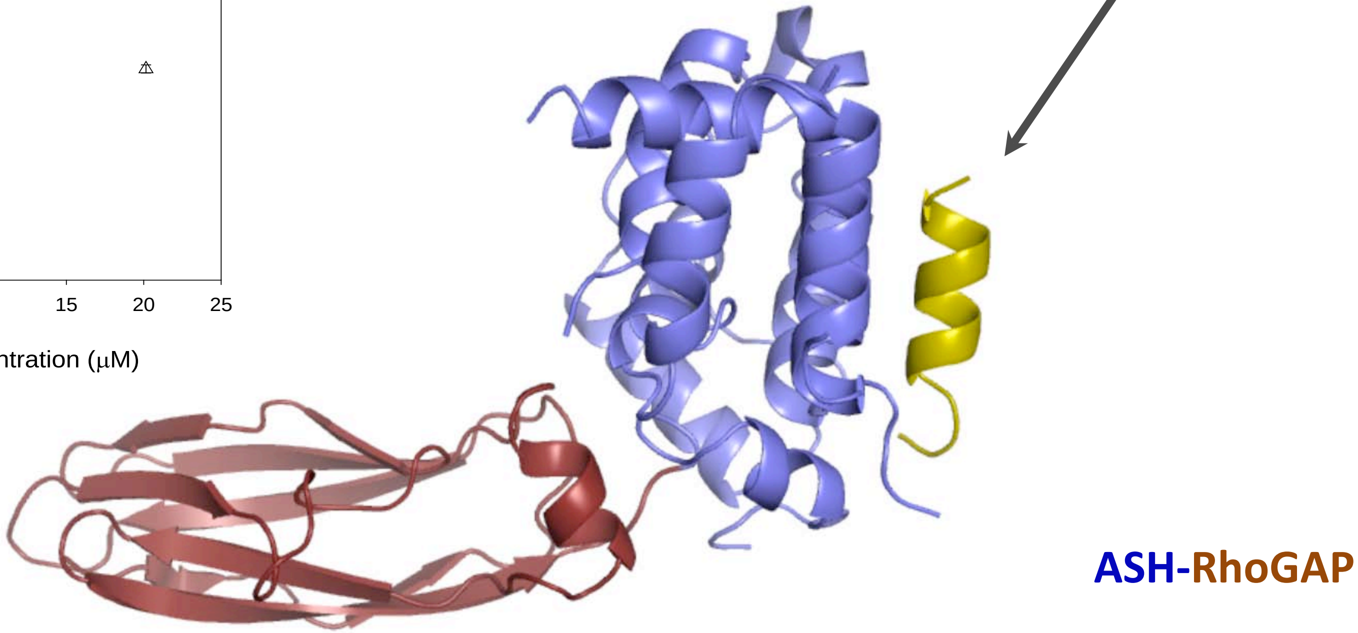

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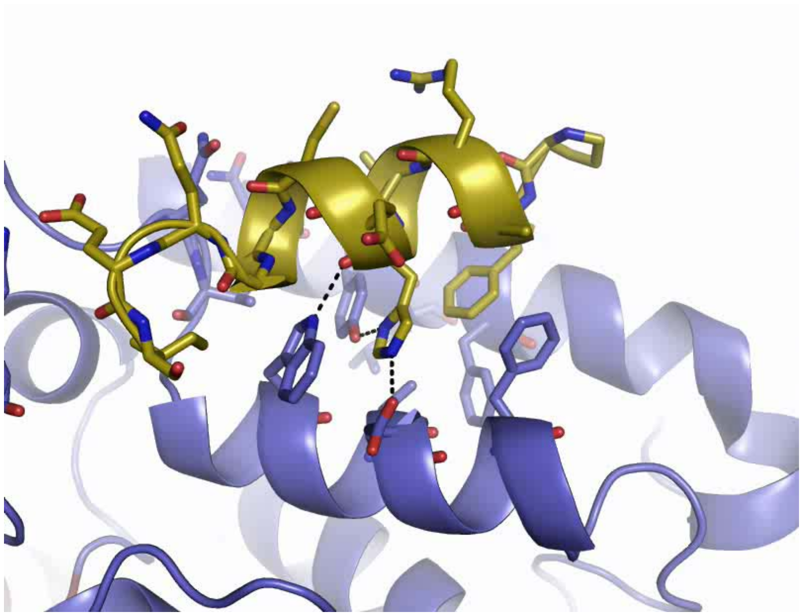

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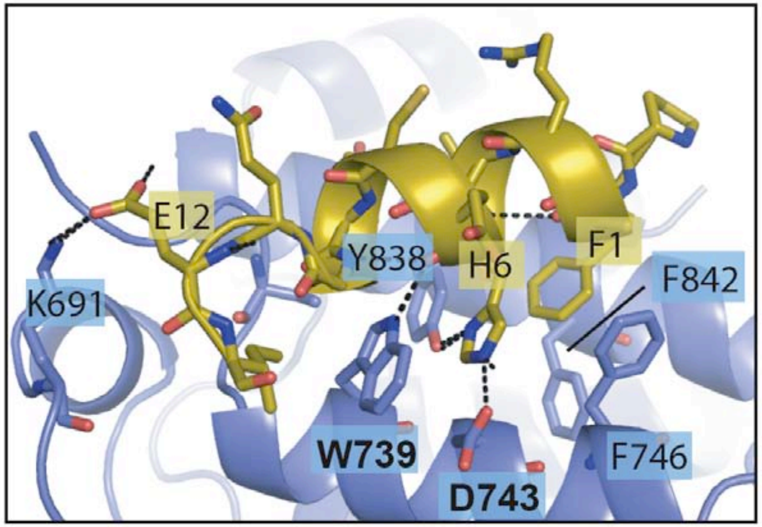

F&H peptide	**OCRL**
Phenylalanine (F&H motif)	hydrophobic pocket Phe842 and Phe746
His (F&H motif)	Side chain forms hydrogen bond with Asp743 Main chain carbonyl H- bond with Indole nitrogen of Trp739

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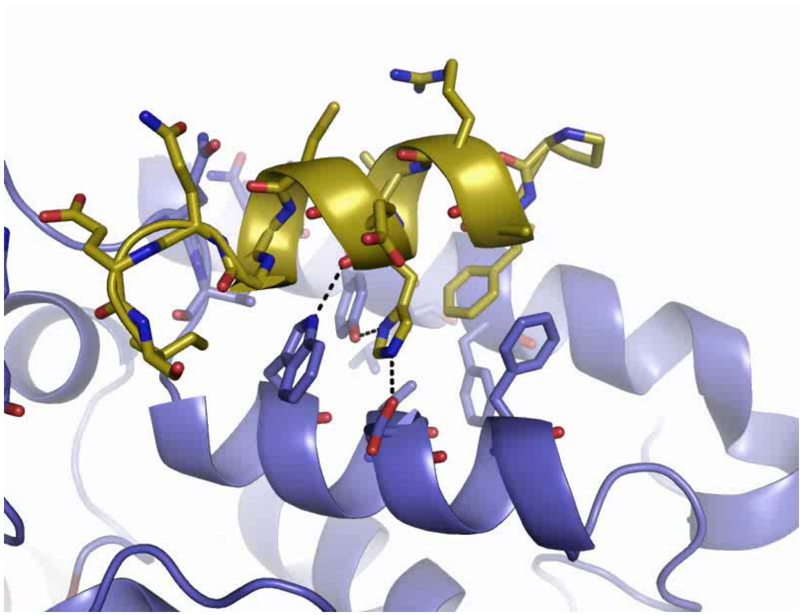

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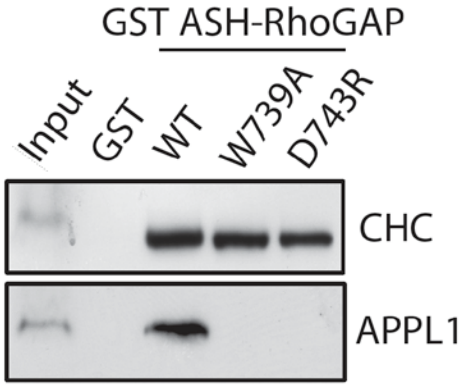
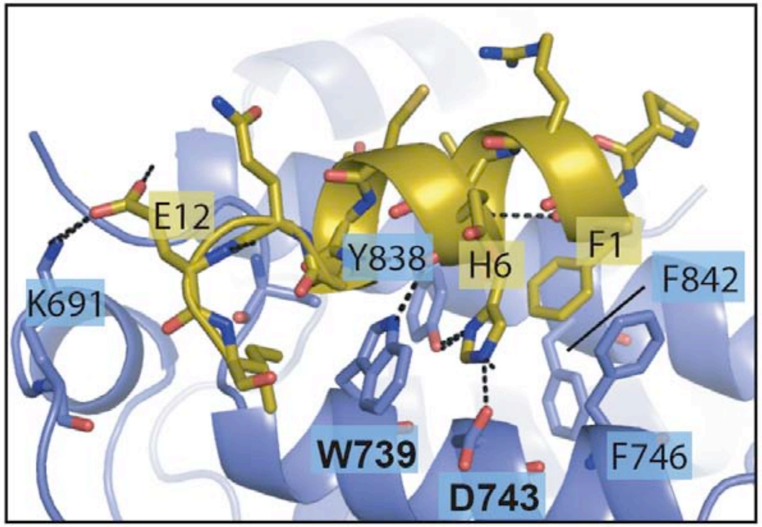


Binding APPL from rat brain homogenate



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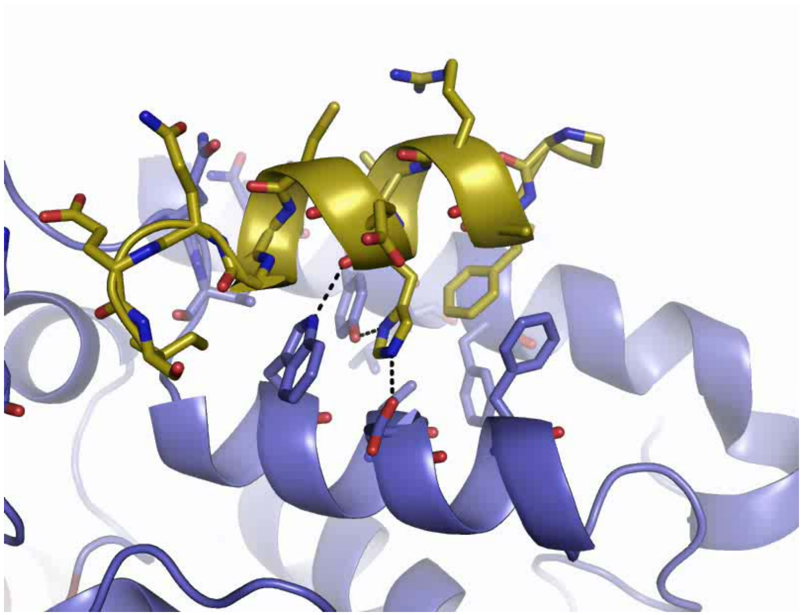


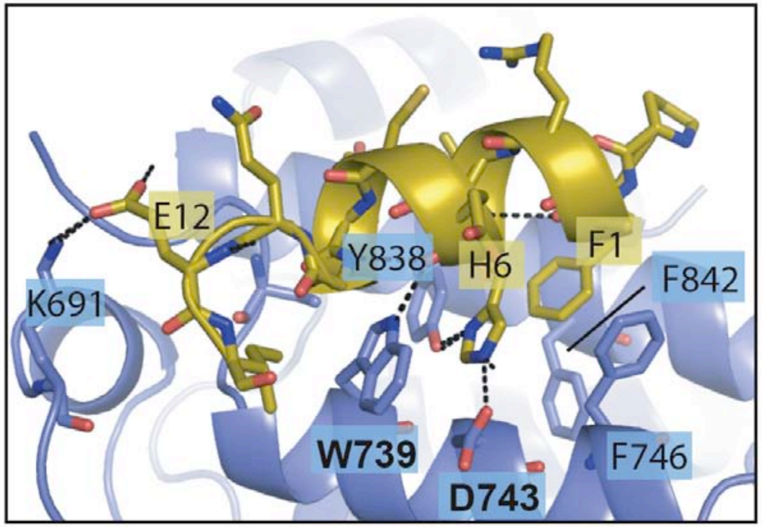
Ses1

Glu12 H-bond
Lys691

APPL

Terminal Proline 11
In the minimal APPL1 F&H peptide

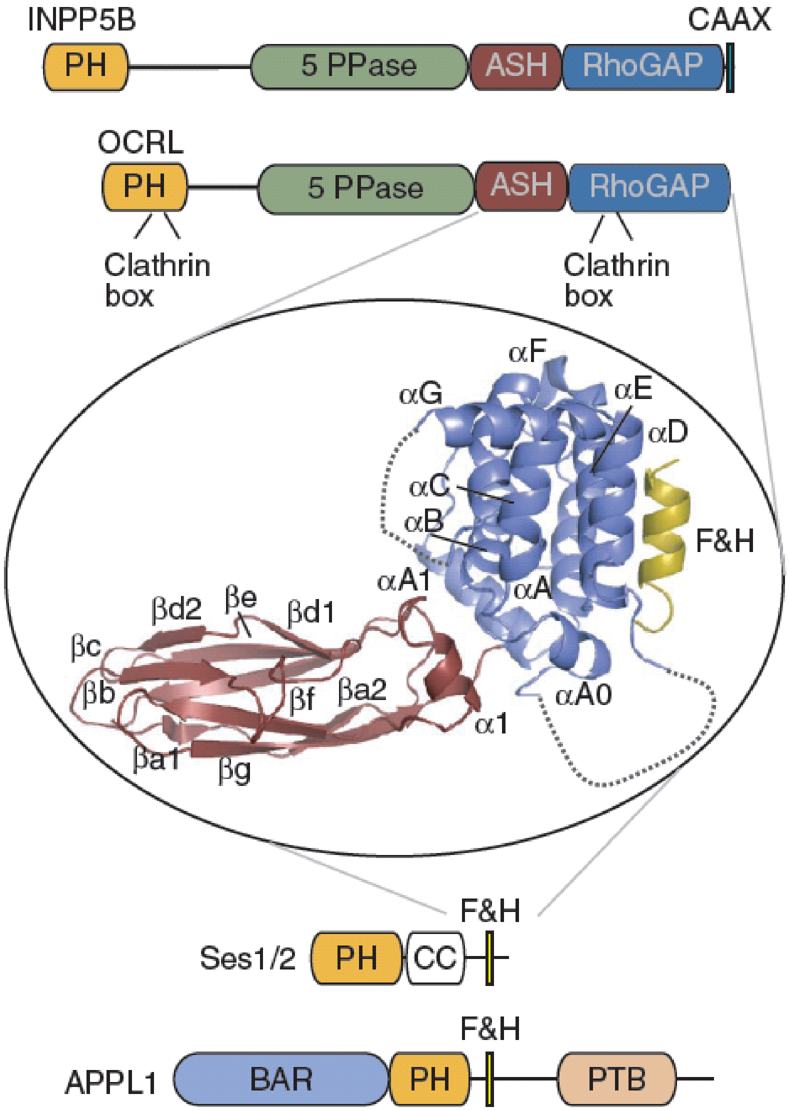
PFARLHECYGQEI Ses1
CFSTLHDWYGQEI Ses2
EFARNHERFRREL DSes
SFQQRHESLRP APPL1



F&H binding site on ASH-RhoGAP domain of OCRL is highly conserved throughout evolution.

This interface is conserved in lower organisms that encode an OCRL and INPP5B homolog but neither APPL1 nor Ses1/2.

What are the interacting partners in these organisms?



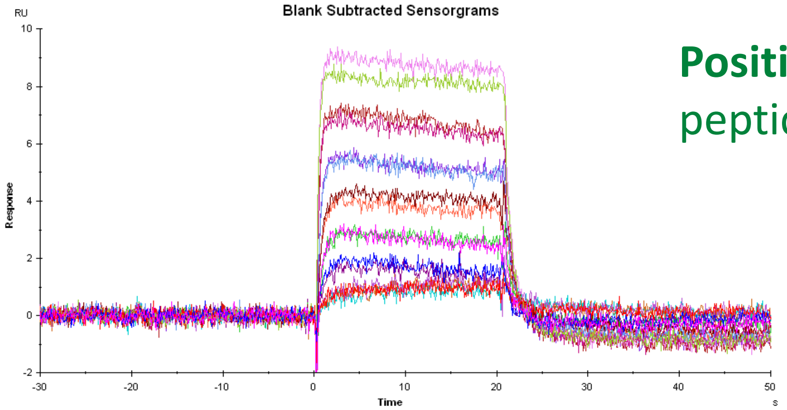
Utilize SPR and ITC to test for binding of F&H peptides to ASH-RhoGAP OCRL and rank their affinities

F&H peptides candidates

SF**ARL**HE**CYGQEI**	**Superclamp; positive control** (engineered F&H peptide)	
SFQQRHESLYRP	APPL1	Endocytic proteins
PFARLHECYGQEI	Ses1	
CFSTLHDWYGQEI	Ses2	

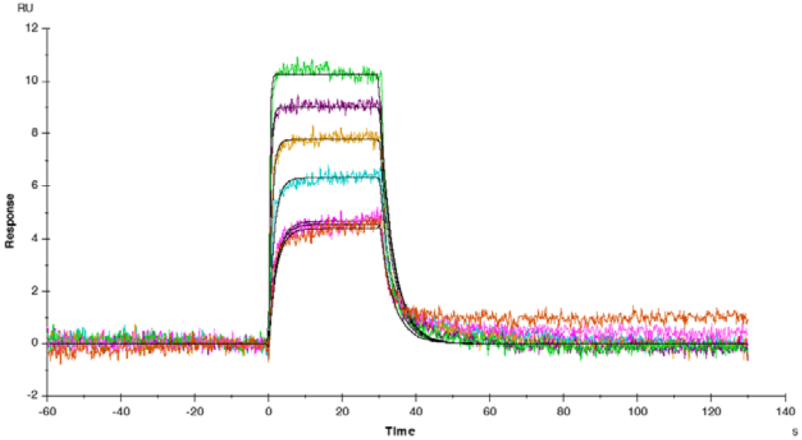
KFRRQHEQLRAVI	**Dynein Heavy Chain**	F&H peptide candidates Selected through bioinformatics
S**F**YVR**H**SCLREAL	**zFyve26** (Spastizin)	
S**F**STV**H**EKFNKSL	**WDR36**	
I**F**GLH**H**IGMQMRI	**CFTR**, cystic fibrosis	
S**F**ETQ**H**HHLLHCL	**kv4.2**	
E**F**CRN**H**FLVGLLL	**Dock9**	
A**F**IER**H**RIIEEP	**Fly Weeble**	

Utilize SPR and ITC to test for binding of F&H peptides to ASH-RhoGAP OCRL and rank their affinities



Blank Subtracted Sensorgrams

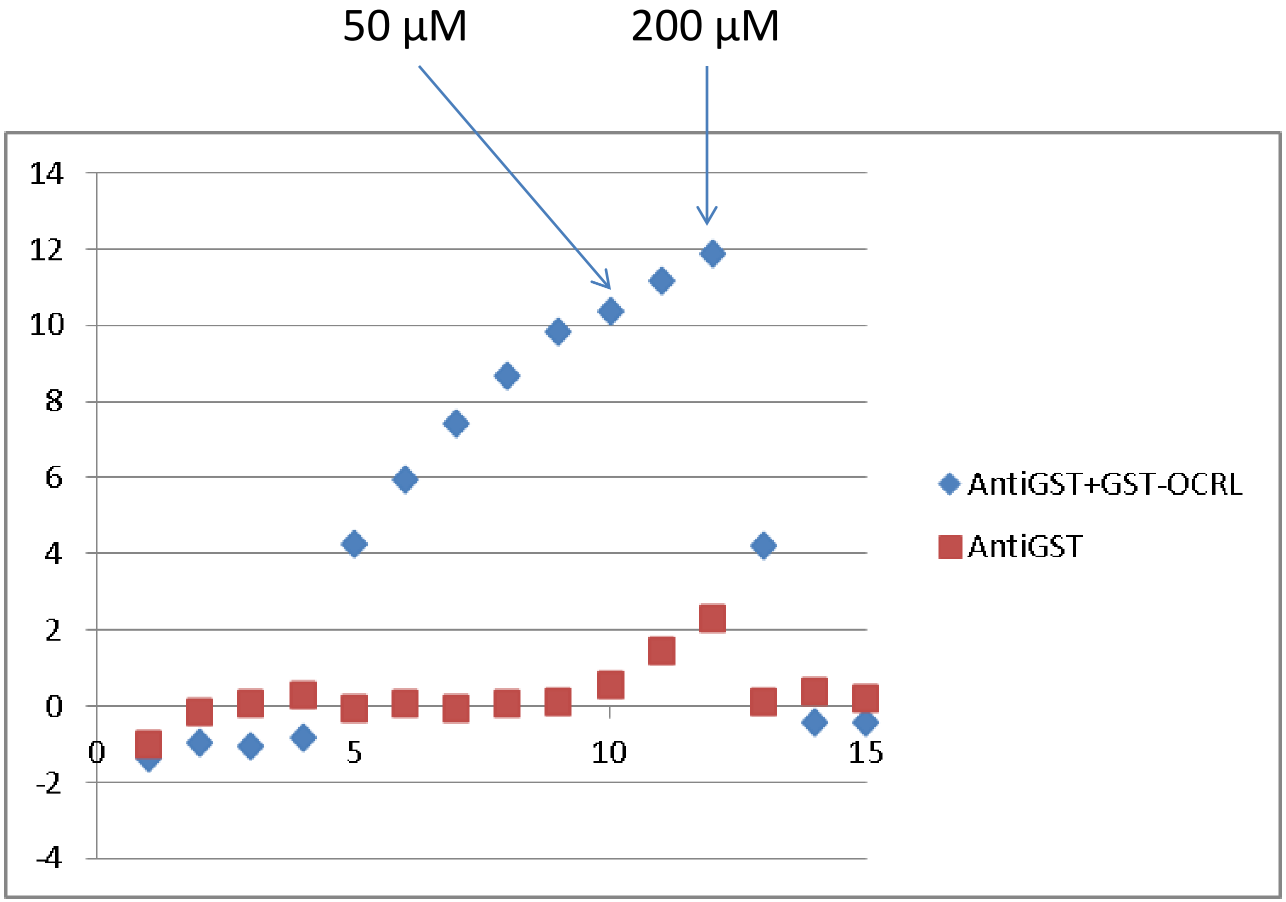
Positive Control: Superclamp (engineered F&H peptide)



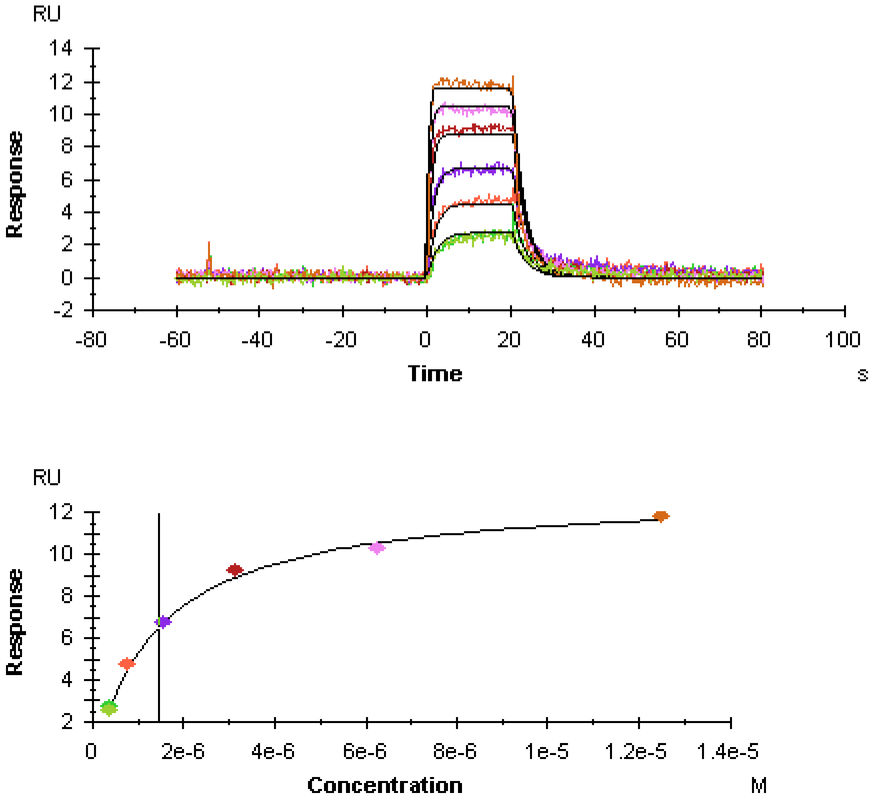
Peptides →

GST-OCRL; captured on anti-GST Ab

Non-specific binding to antiGST Ab surface



Kinetics of binding beyond SPR capabilities; determination of affinity from steady-state amplitudes



Peptides →

OCRL; captured on anti-GST Ab

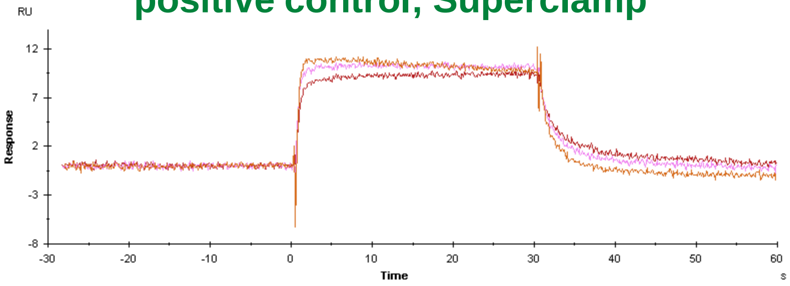
Testing binding of F&H peptide candidates to ASH-RhoGAP OCRL

GST_OCRL (WT) captured on anti-GST surface

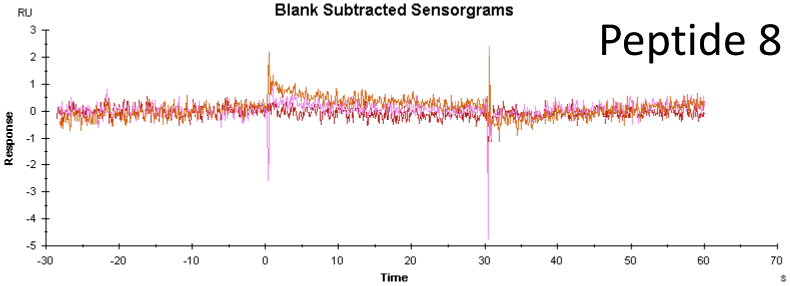
AntiGST
AntiGST+GST-OCRL

peptides at 50 μM,16,7, and 5.56 μM

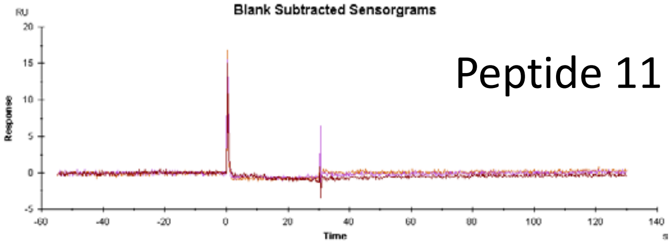
positive control; Superclamp



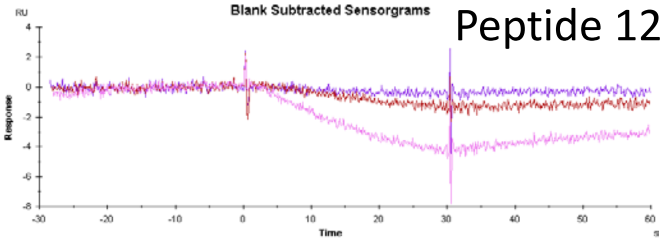
Peptide 8



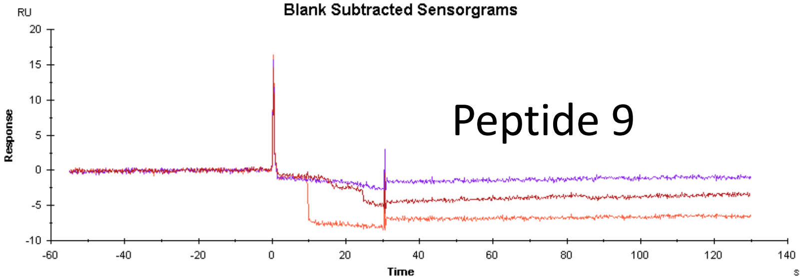
Peptide 11



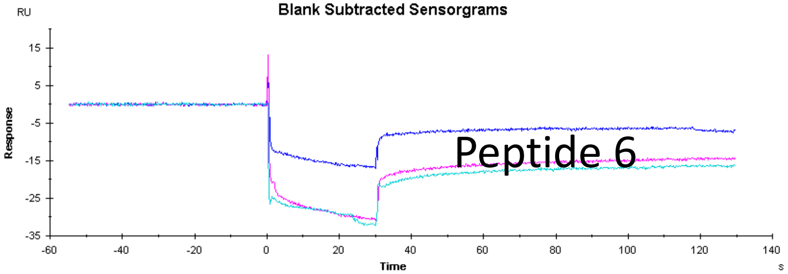
Peptide 12



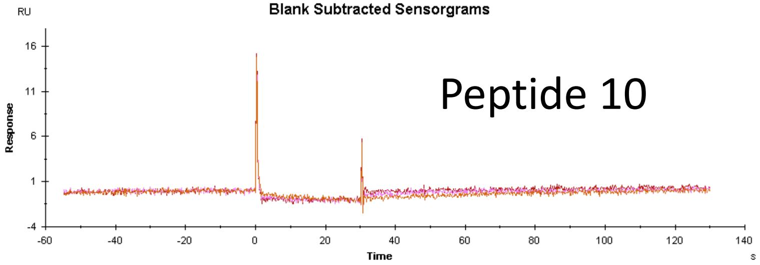
Peptide 9



Peptide 6



Peptide 10



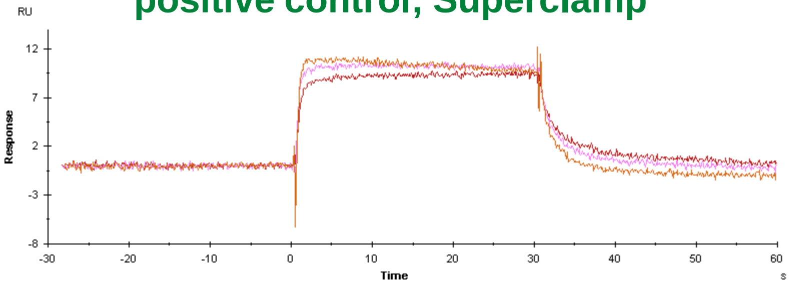
Testing binding of F&H peptide candidates to ASH-RhoGAP OCRL

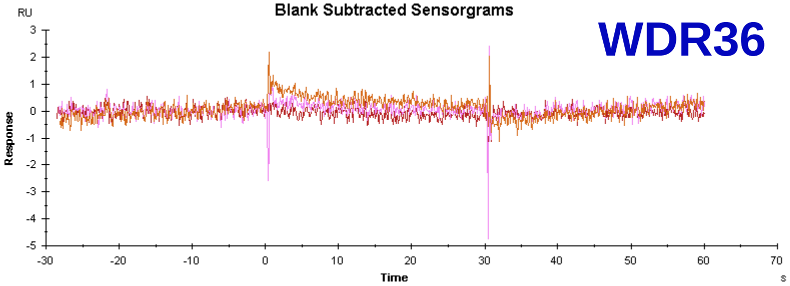
GST_OCRL (WT) captured on anti-GST surface

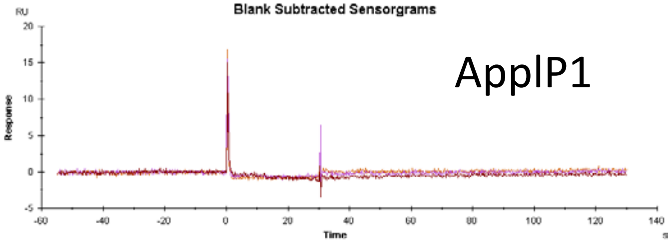
AntiGST
AntiGST+GST-OCRL

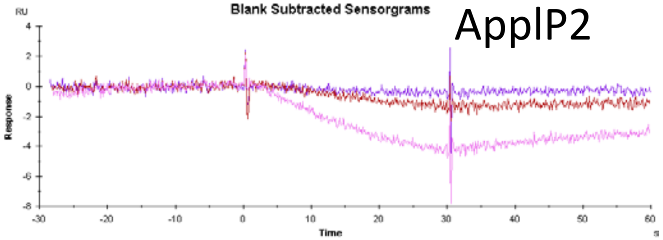
peptides at 50 μM,16,7, and 5.56 μM

positive control; Superclamp




WDR36


AppIP1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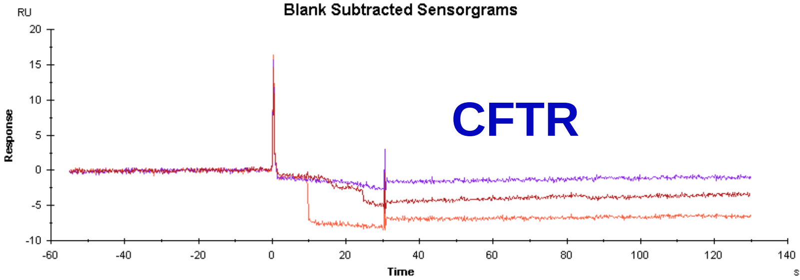
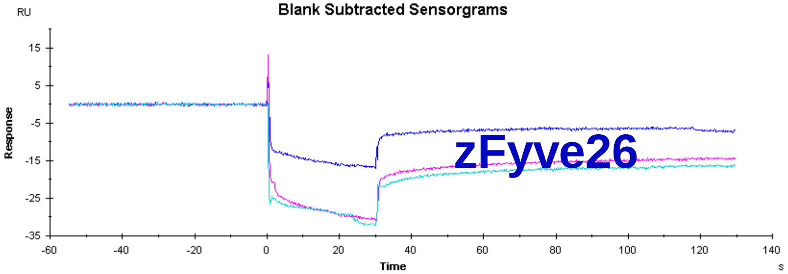
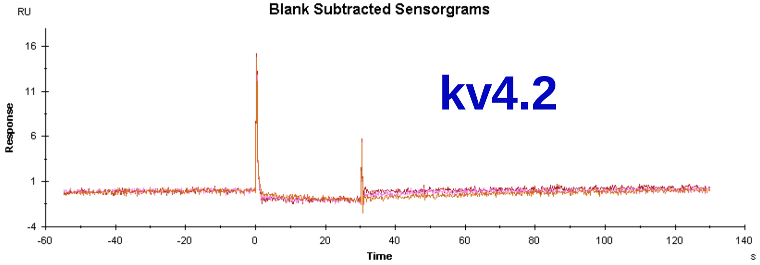

CFTR


zFyve26


kv4.2

Testing binding of F&H peptide candidates to ASH-RhoGAP OCRL by SPR

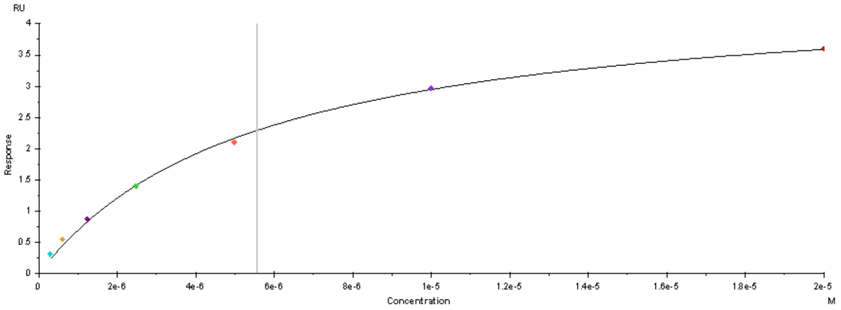
SFARLHECYGQEI	Superclamp; positive control
SFQQRHESLYRP	APPL1
PFARLHECYGQEI	Ses1
CFSTLHDWYGQEI	Ses2

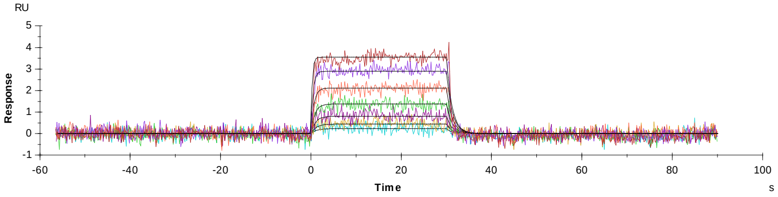
Candidates for SPR

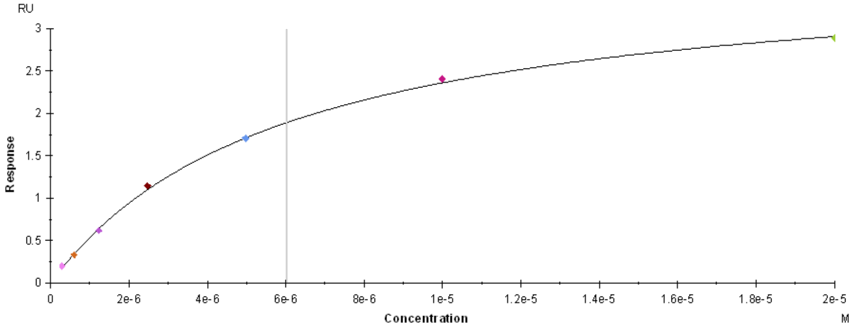
KFRRQHEQLRAVI	**Dynein Heavy Chain**	anti-GST binder
S**F**YVR**H**SCLREAL	**zFyve26** (Spastizin)	anti-GST binder
S**F**STV**H**EKFNKSL	**WDR36**	no binding in SPR
I**F**GLH**H**IGMQMRI	**CFTR**, cystic fibrosis	no binding in SPR (?)
S**F**ETQ**H**HHLLHCL	**kv4.2**	no binding in SPR
E**F**CRN**H**FLVGLLL	**Dock9**	insoluble in SPR buffer
A**F**IER**H**RIIEEP	**Fly Weeble**	anti-GST binder

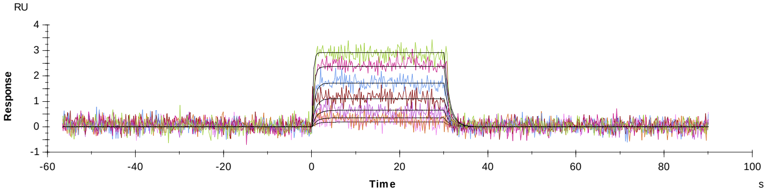
Affinity for binding of F&H peptides to ASH-RhoGAP OCRL

Peptide #1







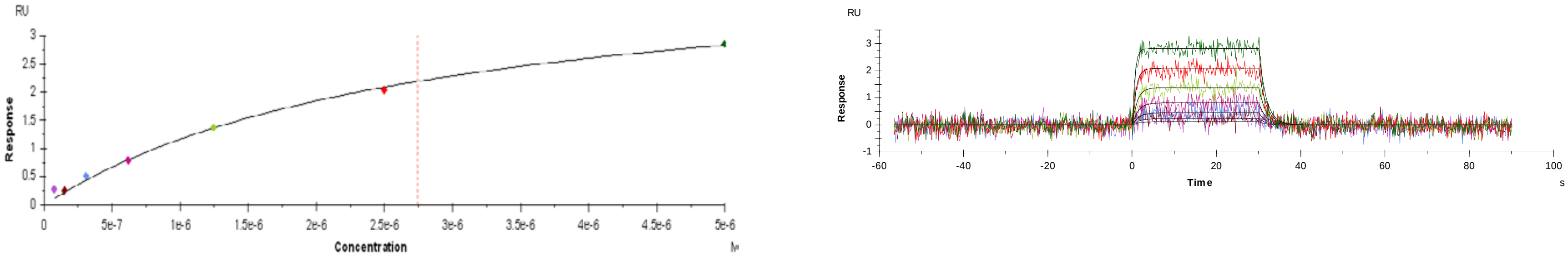


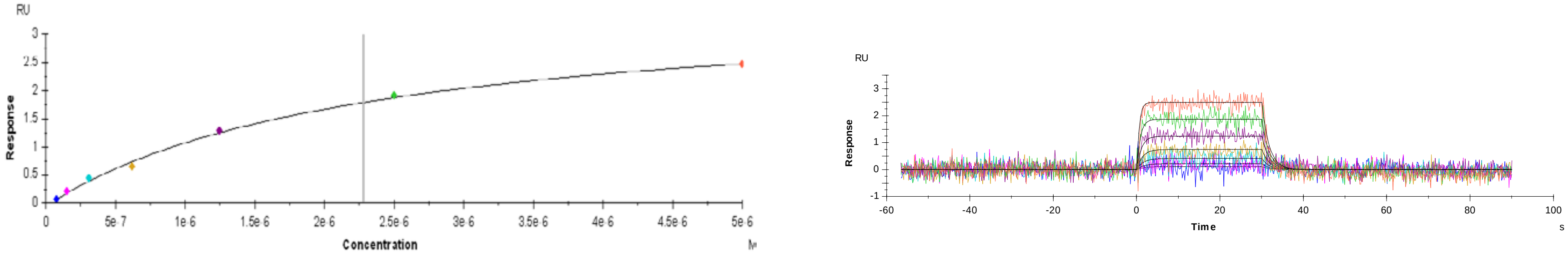
Ses #1

Kd 5.8 ± 0.3 uM

Affinity for binding of F&H peptides to ASH-RhoGAP OCRL

Peptide #2



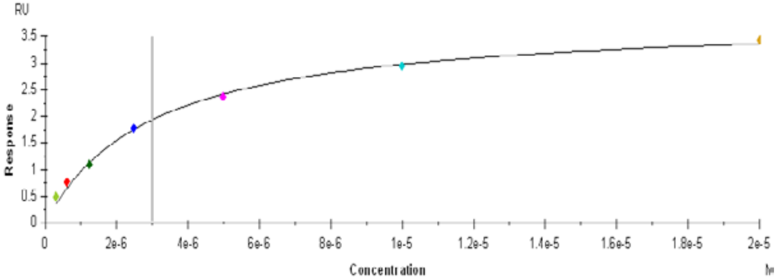


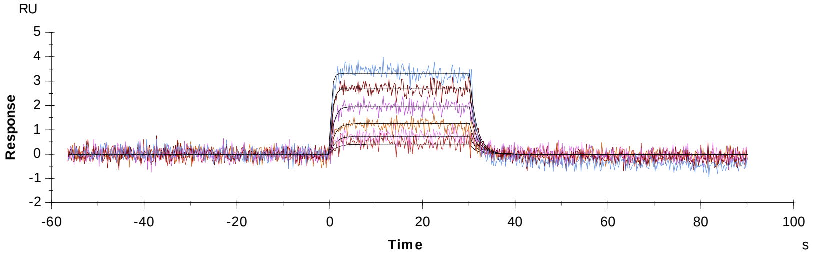
Ses2

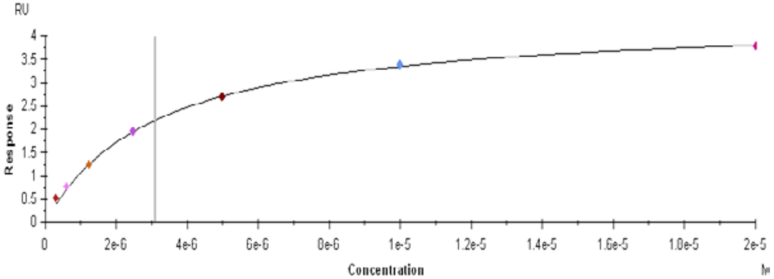
Kd 2.5 ± 0.4 uM

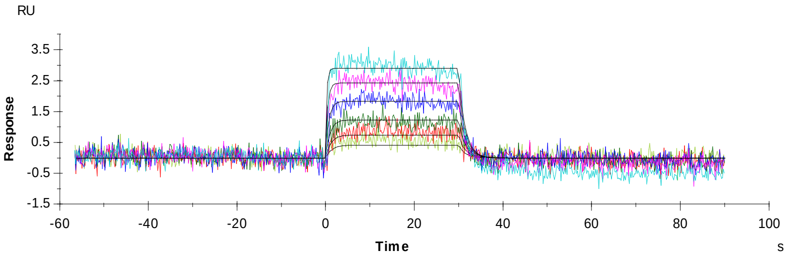
Affinity for binding of F&H peptides to ASH-RhoGAP OCRL

Peptide #5









Superclamp
Kd 3.0 ± 0.2 uM

Testing binding of F&H peptide candidates to ASH-RhoGAP OCRL by SPR

SFARLHECYGQEI	Superclamp; positive control
SFQQRHESLYRP	APPL1
PFARLHECYGQEI	Ses1
CFSTLHDWYGQEI	Ses2

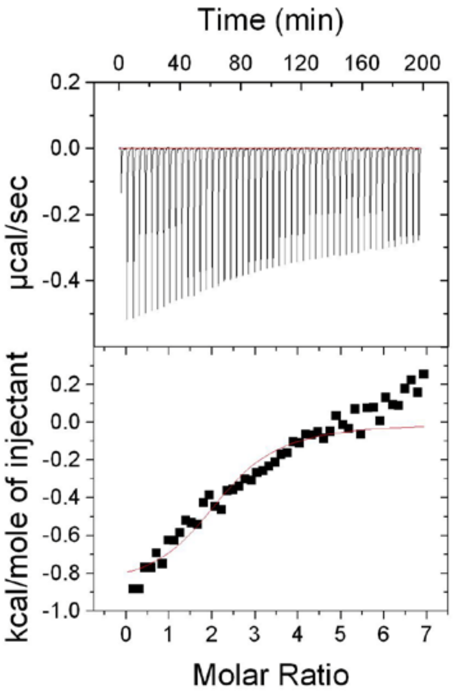
Kd measured by SPR

Candidates for ITC follow up

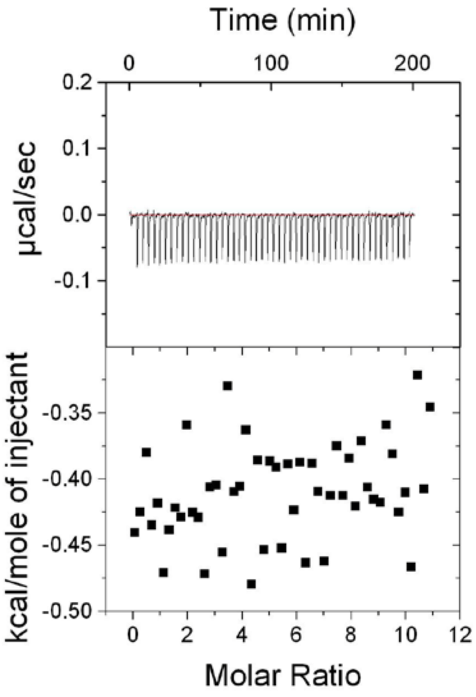
KFRRQHEQLRAVI	**Dynein Heavy Chain**	NON-SPECIFIC
S**F**YVR**H**SCLREAL	**zFyve26** (Spastizin)	anti-GST binder
S**F**STV**H**EKFNKSL	**WDR36**	no binding in SPR
I**F**GLH**H**IGMQMRI	**CFTR**, cystic fibrosis	no binding in SPR (?)
S**F**ETQ**H**HHLLHCL	**kv4.2**	no binding in SPR
E**F**CRN**H**FLVGLLL	**Dock9**	insoluble in SPR buffer
A**F**IER**H**RIIEEP	**Fly Weeble**	anti-GST binder

Testing binding of F&H peptide candidates to ASH-RhoGAP OCRL by ITC

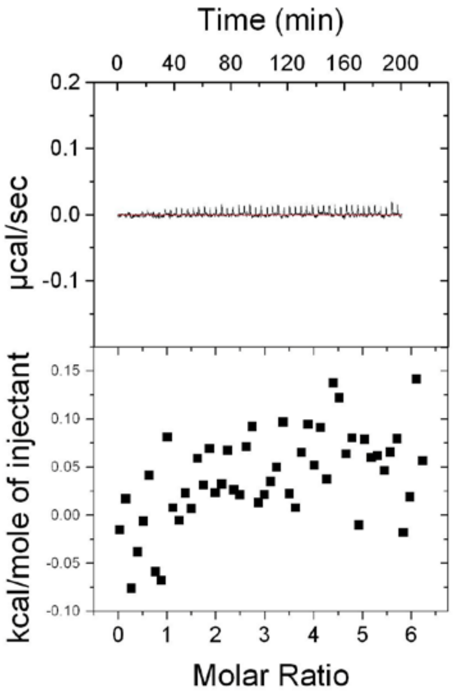
Zfyve-26



Fly Weeble

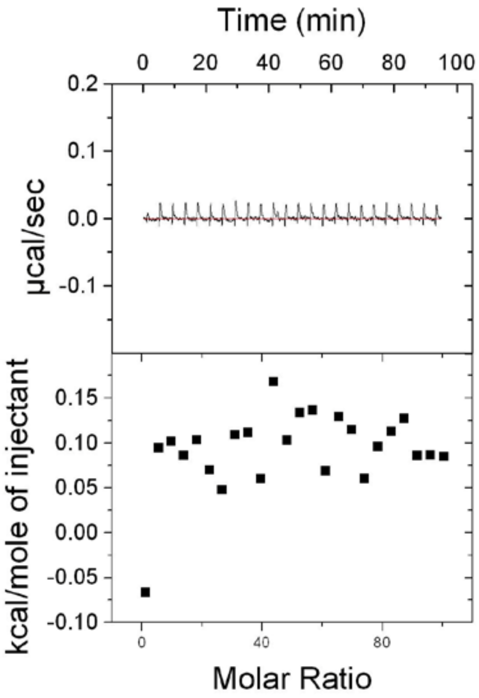


Kv 4.2

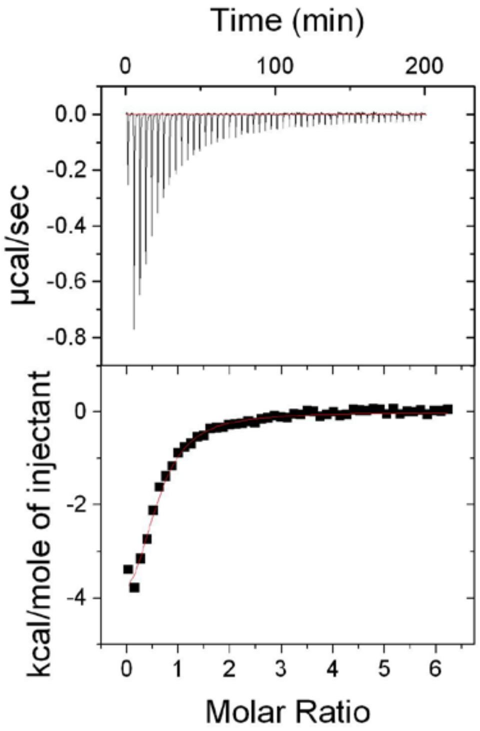


solubility artifact?

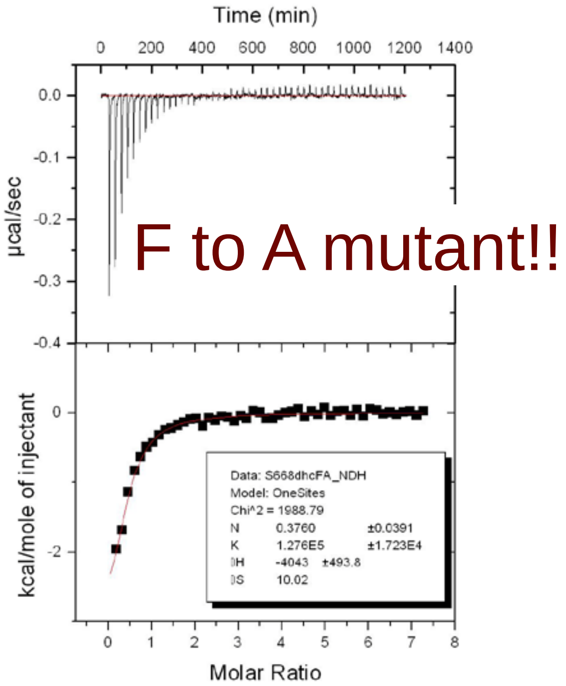
Identification of Dynein heavy chain as a potential interactor



Dynein Heavy Chain Into Buffer



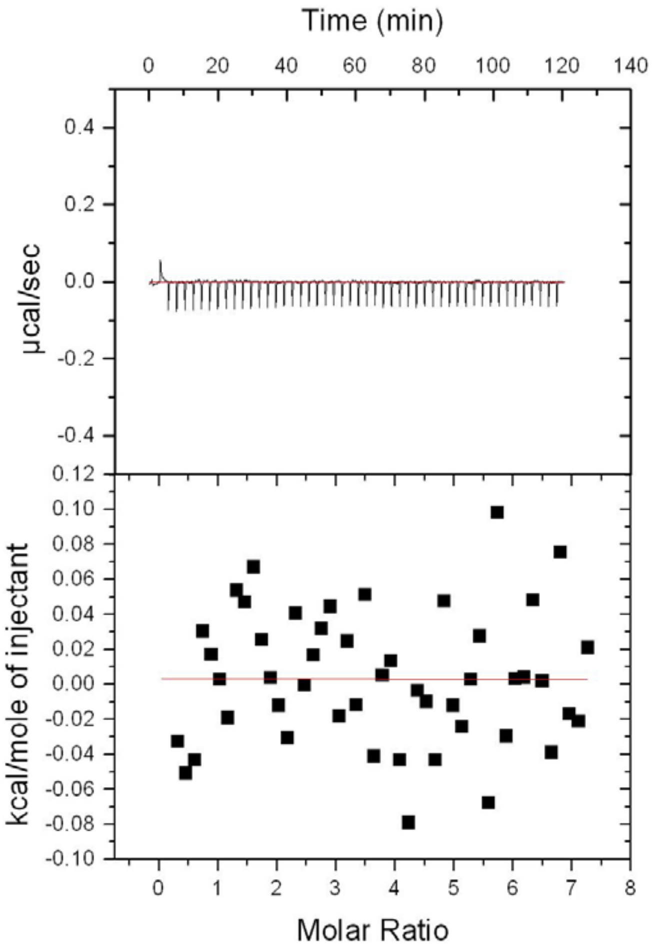
Dynein Heavy Chain Into OCRL



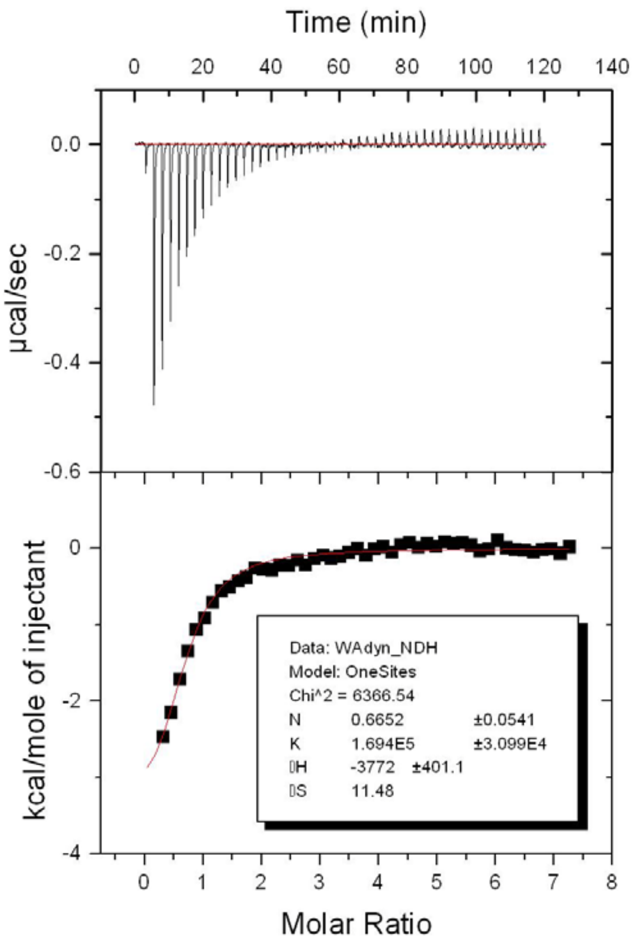
F to A mutant!!

Dynein F to A Mutant Into OCRL

Control ITC: OCRL W739A mutant

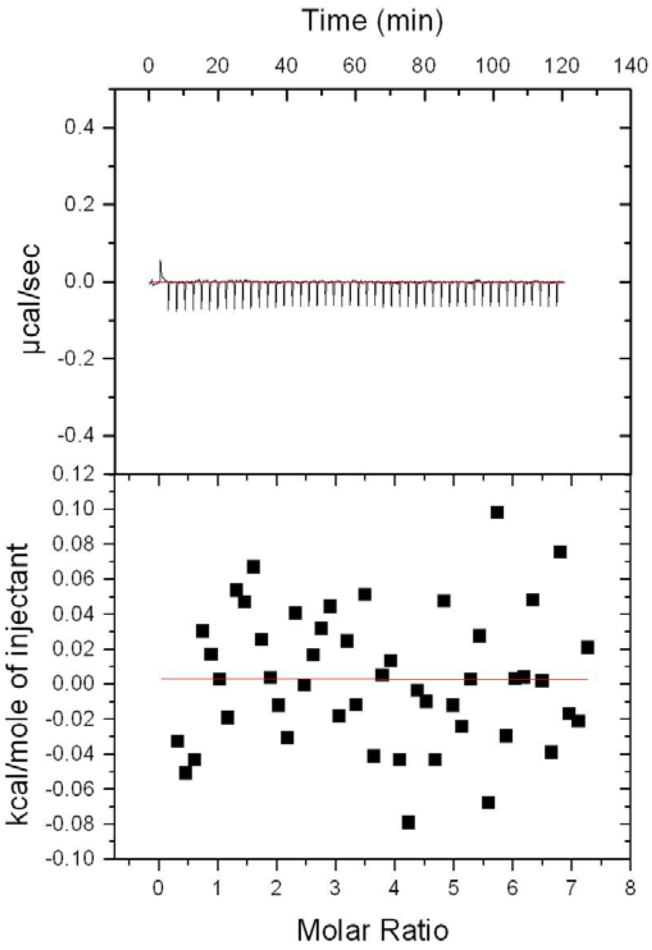


Ses2 peptide
W739A ASH-RhoGAP

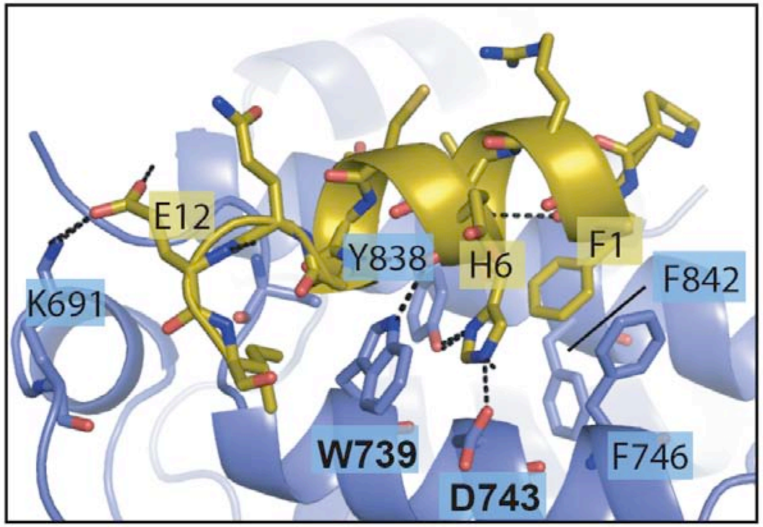


Dynein peptide
W739A ASH-RhoGAP

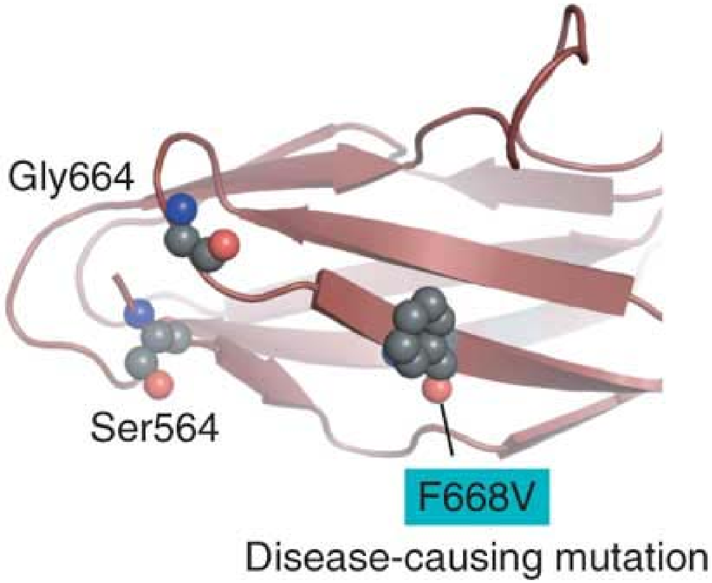
Control ITC: OCRL W739A mutant

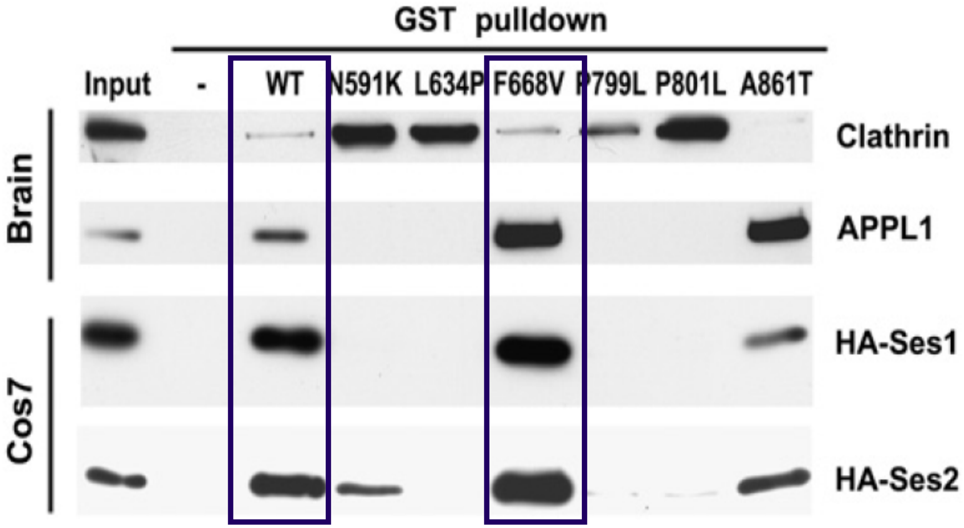


Ses2 peptide
W739A ASH-RhoGAP



Utilize SPR and ITC to determine affinity for binding of F&H peptides to ASH-RhoGAP OCRL (wt and disease-causing mutants)





Determine whether the F668V mutant binds more tightly to F&H peptides

Swan L E, Tomasini L., Pirruccello M., Lunardi J. l., and De Camilli P. (2010) Two closely related endocytic proteins that share a common OCRL-binding motif with APPL1. *Proceedings of the National Academy of Sciences* **107**; 3511-3516

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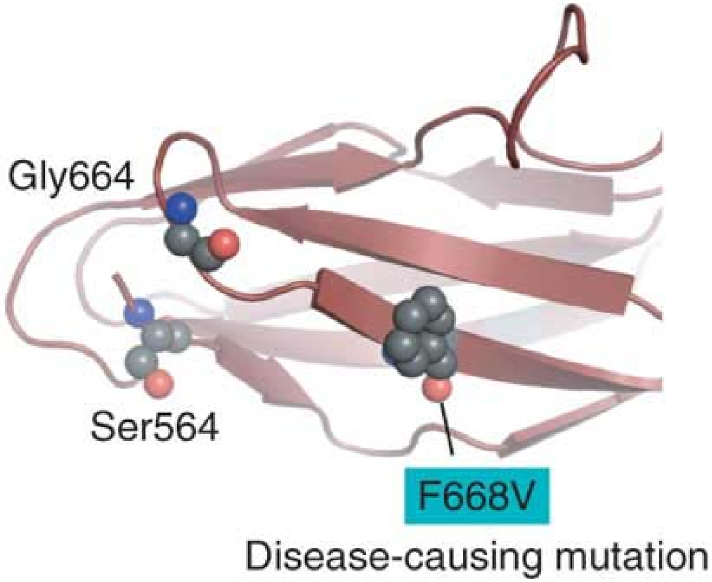

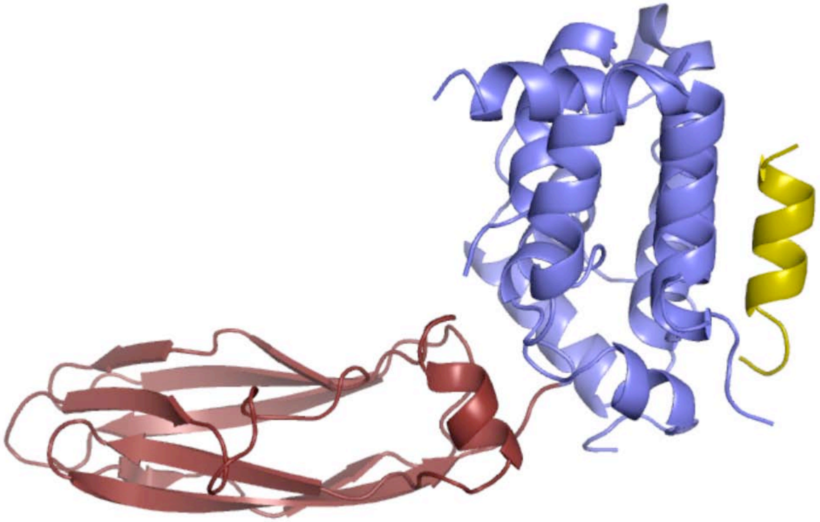

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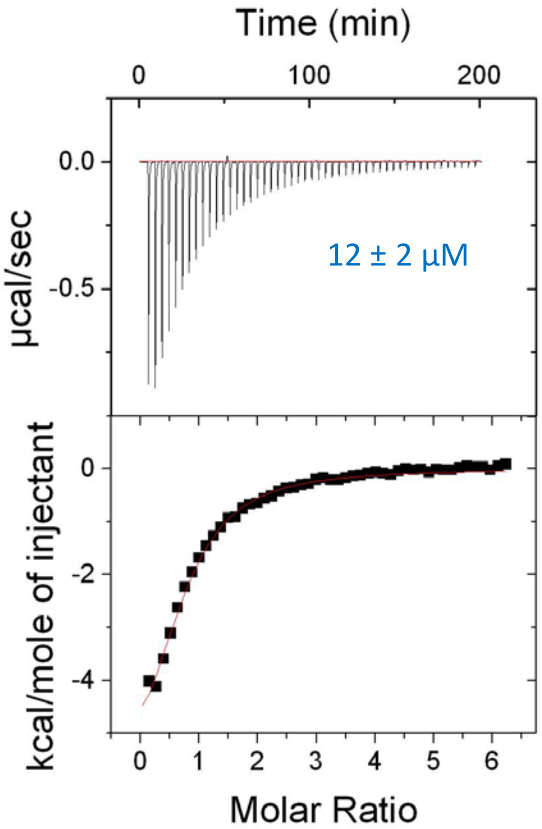
Utilize SPR and ITC to determine affinity for binding of F&H peptides to ASH-RhoGAP OCRL (wt and disease-causing mutants)

Kd (µM) **SPR/*ITC***		
Proteins	**Peptides**	
	APPL	**Superclamp**
Wt OCRL	*12 ±2*	3.0 ± 0.2
F668V	*12 ±2*	2 ± 1

F668V mutant has identical binding affinities to the F&H proteins as the wt protein



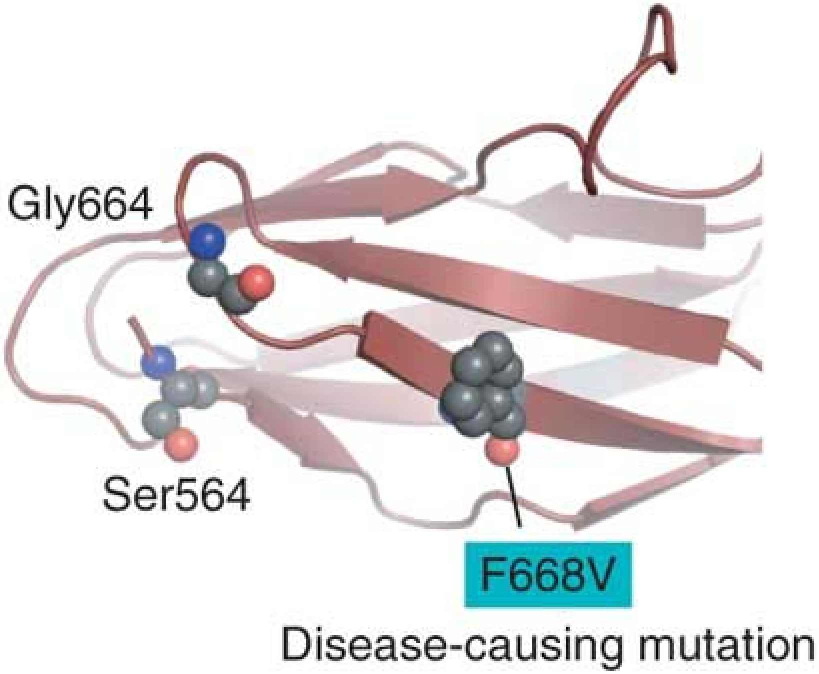
APPL binding to F668V OCRL

Summary

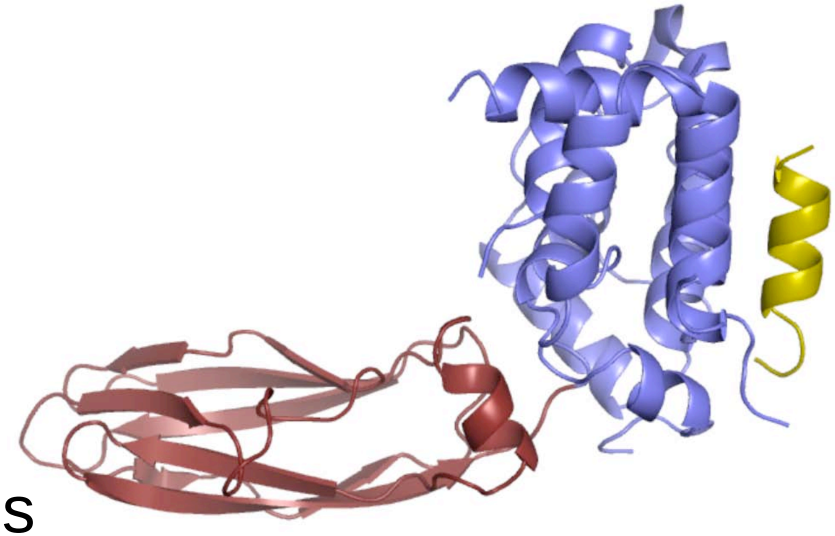
Kd (μM) **SPR/*ITC***								
Proteins	**Peptides**							
	APPL	**Ses1**	**Ses2**	**SesFA**	**Superclamp**	**APPLP1**	**APPLP2**	**Dynein Heavy Chain**
Wt OCRL	43.1 ± 0.4 *12 ±2*	5.8 ± 0.3 *0.70 ± 0.08*	2.5 ± 0.4	ND *ND*	3.0 ± 0.2	ND *ND*	ND *ND*	*92 ± 4*
W739A (engineered)	ND	ND	ND	ND	ND	ND	ND	*8±1*
F668V (disease causing)	*12 ±2*				2 ± 1			

- **F668V** and wt **AshRhoGAP** bind to F&H peptides with similar affinities
- No binding of F&H peptides was observed for **W739A** mutant
- Bioinformatics approach did not yield new F&H candidates that interact with F&H motif binding surface of OCRL

Future Direction



Rabs



Characterize the **Rab binding surface** of a full length OCRL

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Pietro DeCamilli

Department of Cell Biology Yale University and HHMI

Ken Williams

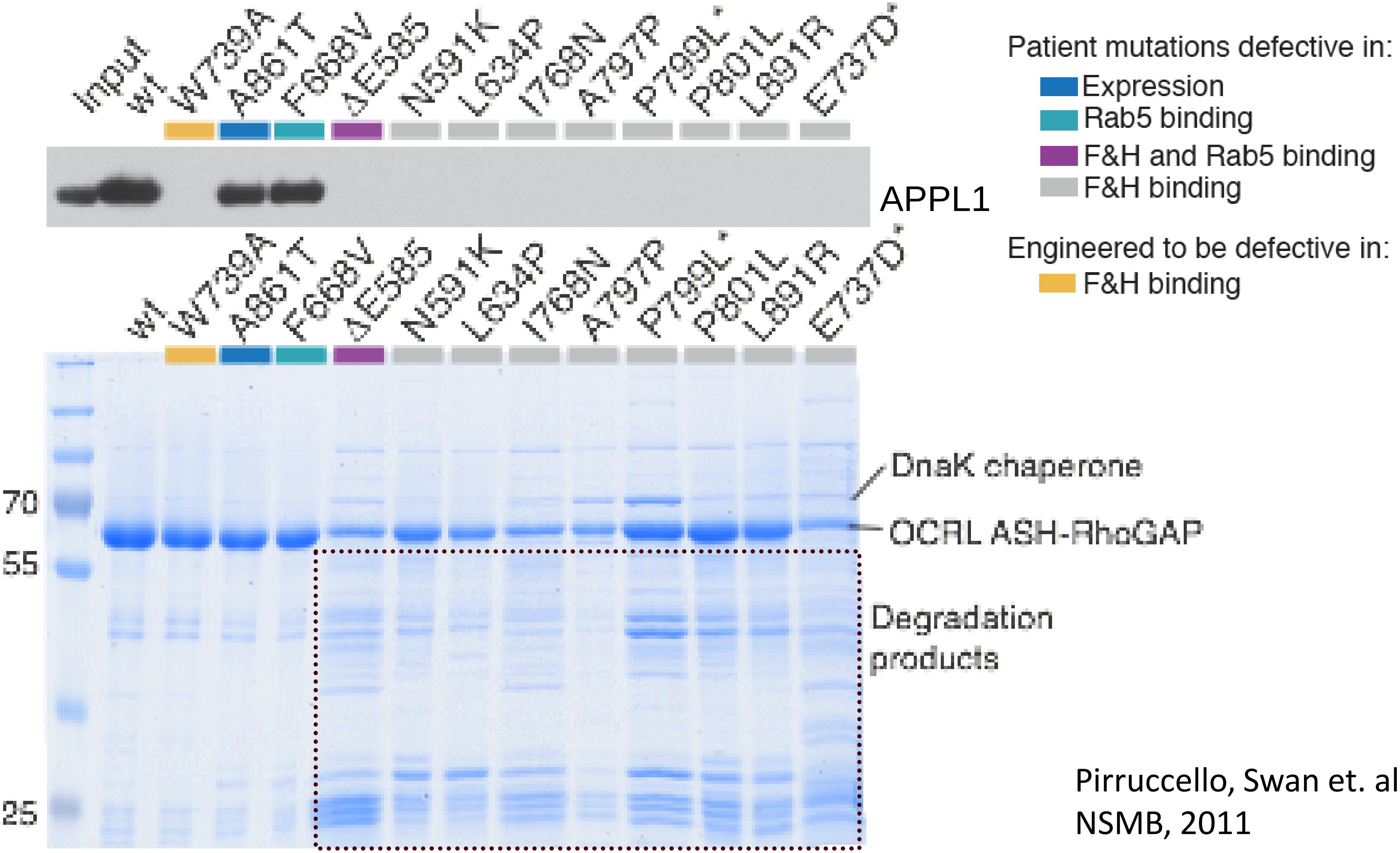
Yale/NIDA Neuroproteomics Center
W.M. Keck Biotechnology Resource Laboratory at Yale University School of Medicine

NIH (Biacore T100 purchase, Yale/NIDA Center support)
HHMI (VP-ITC purchase)

http://info.med.yale.edu/wmkeck/biophysics

Ewa.Folta-Stogniew@yale.edu

Patient mutations affecting F&H binding in the ASH-RhoGAP domain of OCRL destabilize the protein



Pirruccello, Swan et. al.
NSMB, 2011