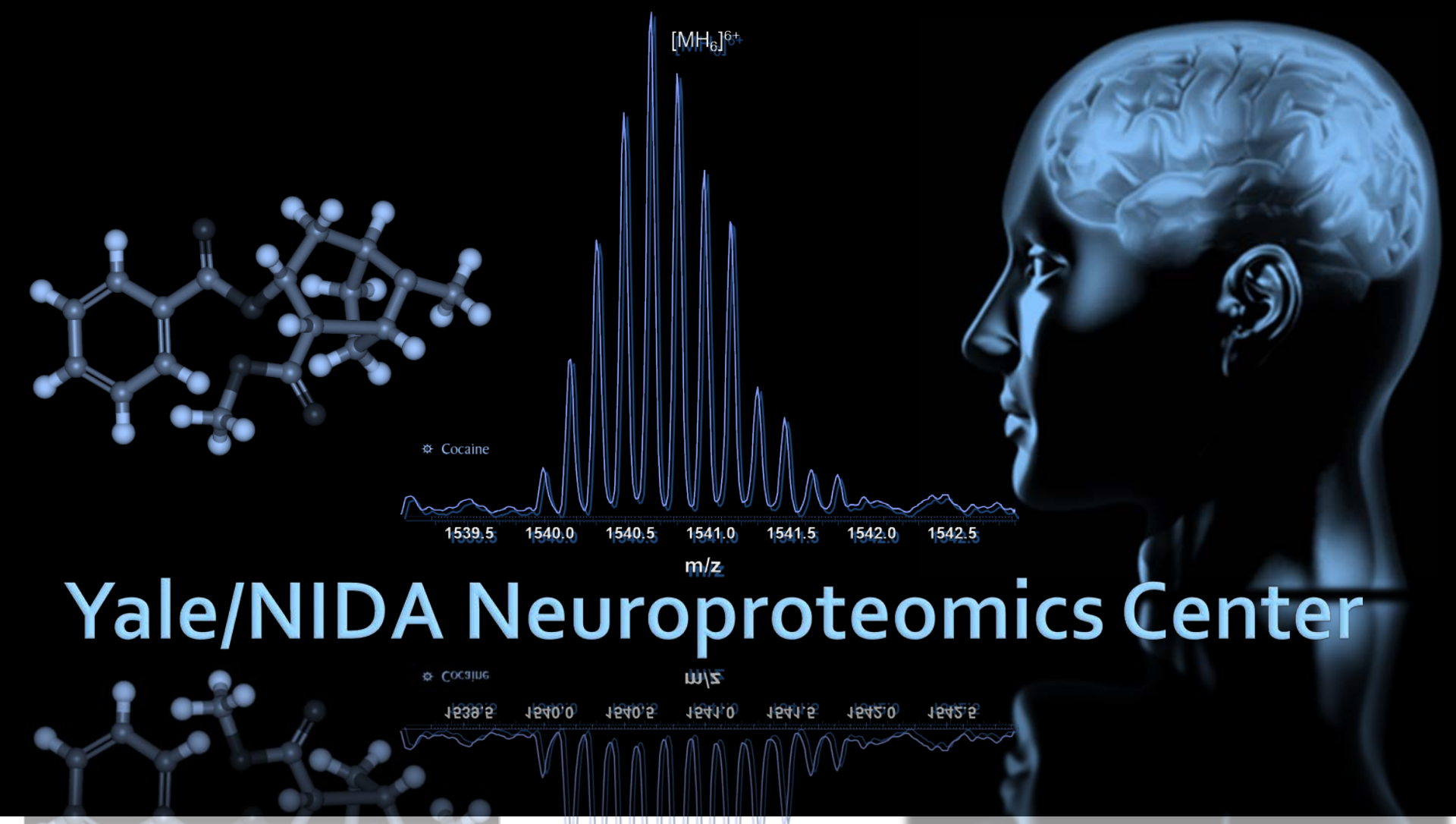
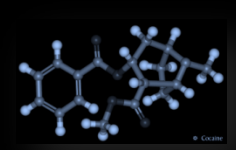


Yale/NIDA Neuroproteomics Center

Biophysics Core

Ewa Folta-Stogniew, Ph.D., M.S.

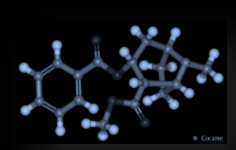


Biophysics Core

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

Expands the proteomic analyses beyond the identification of proteins' networks

Allows quantitative characterization of interactions between candidates identified through mass spectrometry approaches



Biophysics Core

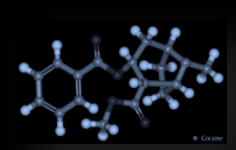
Mission: quantitative characterization of interactions between biomolecules using in solution biophysical methods

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)



Biophysics Core

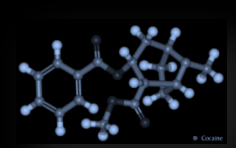
Mission: quantitative characterization of interactions between biomolecules using in solution biophysical methods

Common questions:

- how tight is the binding ? (light scattering: static LS and dynamic LS)
- how many of each molecule are in the complex ? (ITC, SPR, LS, stopped-flow)
- how fast does the complex form? (SPR and stopped-flow)
- is the binding event enthalpy or entropy-driven? (ITC)

List of technologies:

- Size Exclusion Chromatography coupled with Light Scattering (SEC/MALLS)
- 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)



Biophysics Core

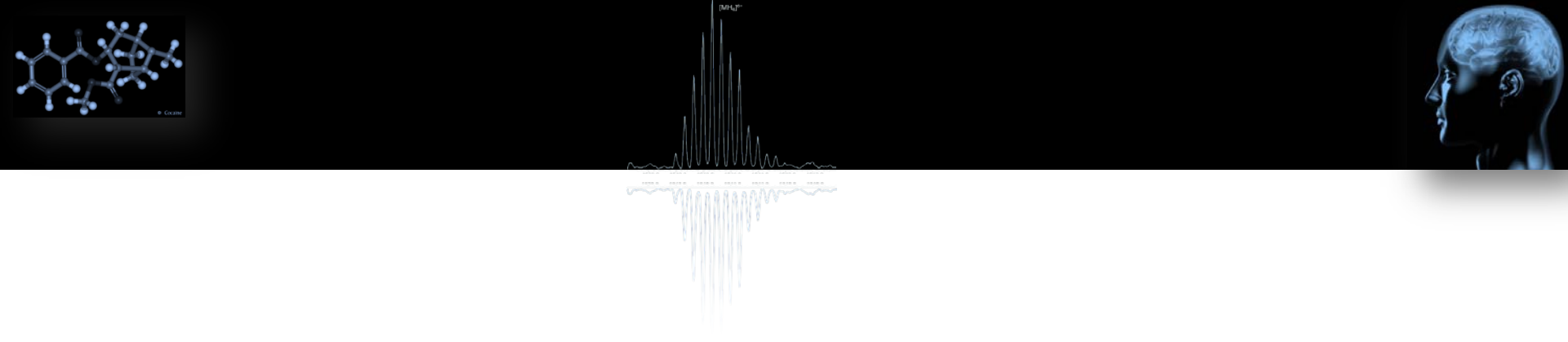
Mission: quantitative characterization of interactions between biomolecules using in solution biophysical methods

Common questions:

- how tight is the binding ? (light scattering: static LS and dynamic LS)
- how many of each molecule are in the complex ? (ITC, SPR, LS, stopped-flow)
- how fast does the complex form? (SPR and stopped-flow)
- is the binding event enthalpy or entropy-driven? (ITC)

List of technologies:

- **Size Exclusion Chromatography coupled with Light Scattering (SEC/MALLS)**
- **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)**



Application of label free technologies to study binding of the F&H motif to Phosphoinositide 5-Phosphatase: OCRL protein

NIDA Investigator: Pietro DeCamilli

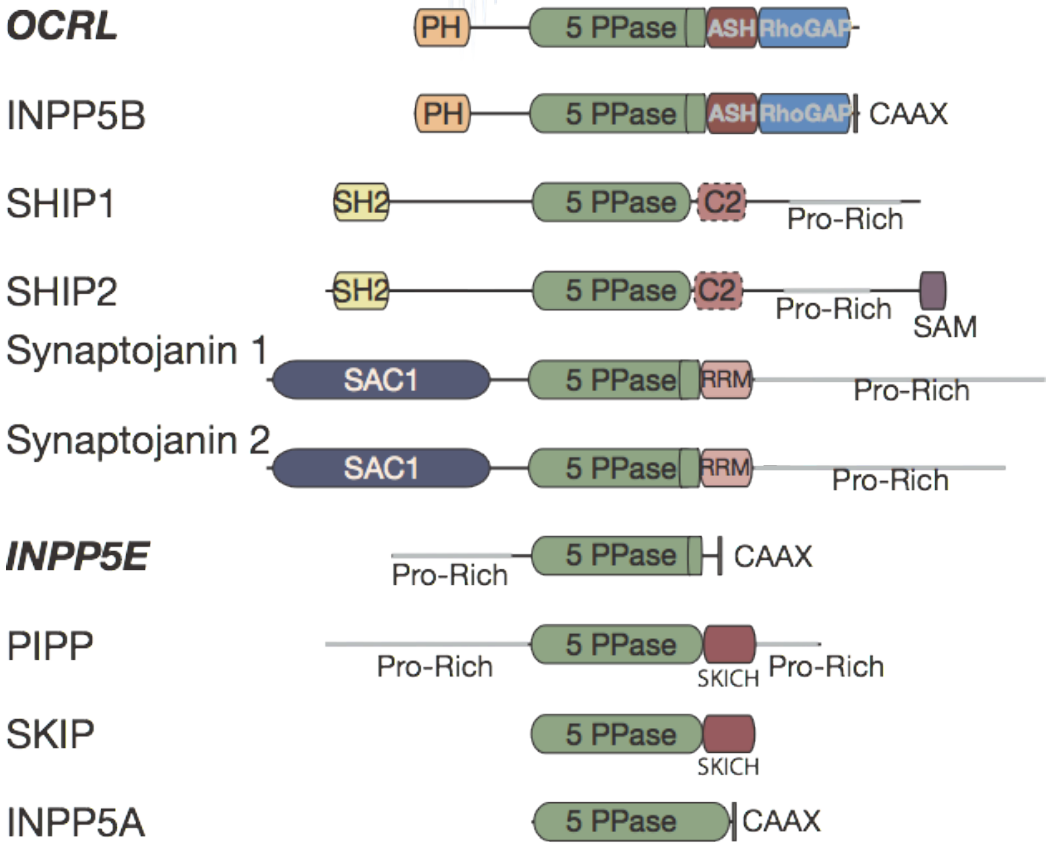
Technologies employed:

SEC/MALLS
ITC
SPR

Swan L E, Tomasini L., Pirruccello M., Lunardi J. l., and De Camilli P. (2010) *PNAS* **107**; 3511-3516

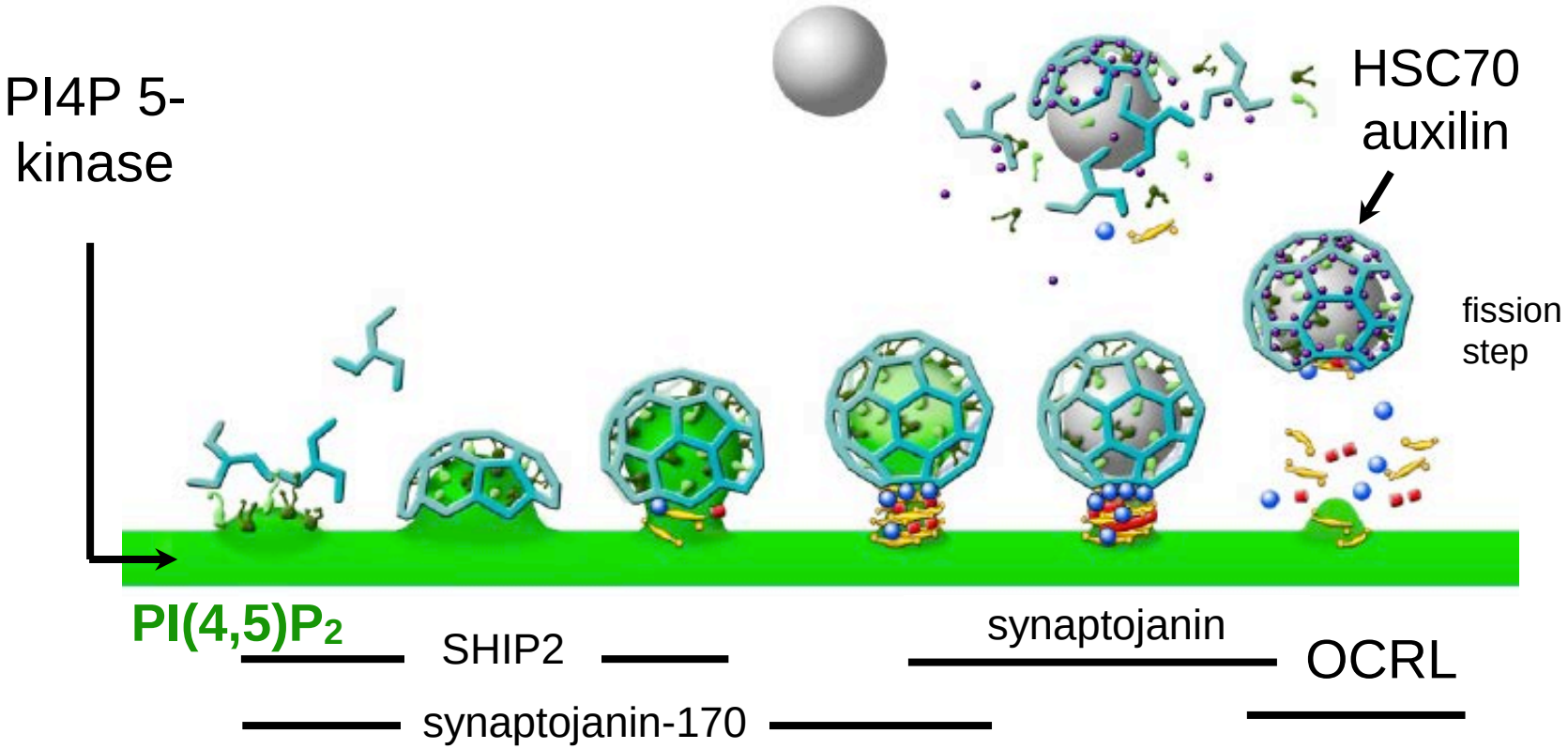
Pirruccello M, Swan L. E., Folta-Stogniew E., and De Camilli P. (2011) *Nat Struct.Mol.Biol* **18**; 789-795

Phosphoinositide 5-Phosphatases



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

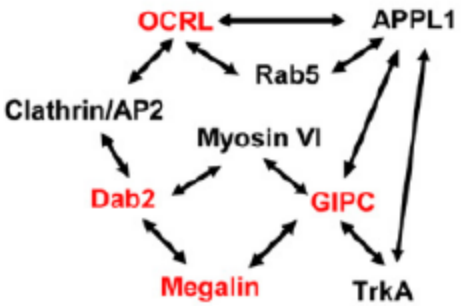
$PI(4,5)P_2$ in Clathrin-mediated endocytosis



Identification of Protein Network Implicated in Receptor Trafficking and Signaling

The pull-down assays used to analyze the clathrin binding of OCRL showed that the COOH-terminal regions of OCRL bound a 90 kDa band that was identified by mass spectrometry, and then western blot, as APPL1

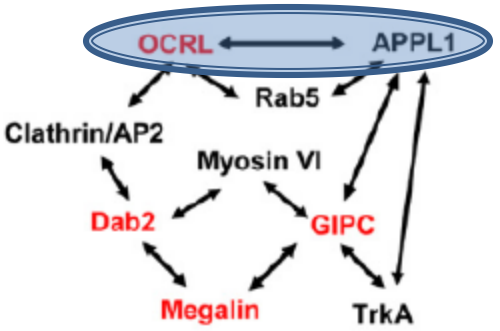
OCRL and APPL1 are components of a protein network implicated in receptor trafficking and signaling



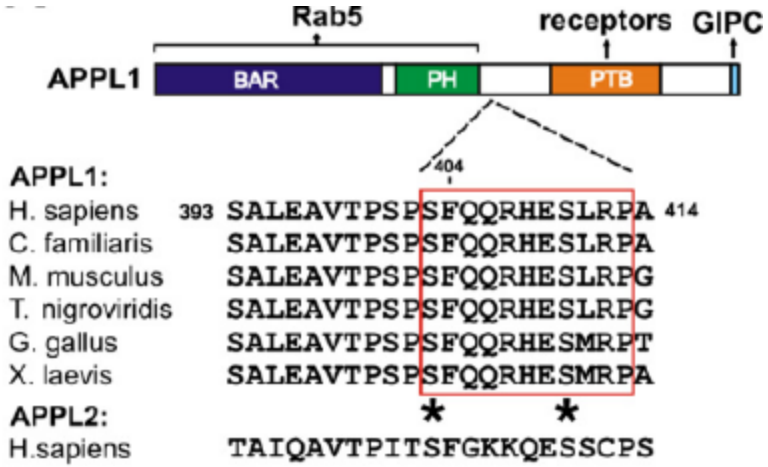
Erdmann, K.S., Mao, Y., McCrea, H.J., Zoncu, R., Lee, S., Paradise, S., Modregger, J., Biemesderfer, D., Toomre, D. and De Camilli, P. (2007) A role of the Lowe syndrome protein OCRL in early steps of the endocytic pathway. Dev. Cell 13, 377-390. (supported by NIDA Center)

Protein Network Implicated in Receptor Trafficking and Signaling

Protein network implicated in receptor trafficking and signaling



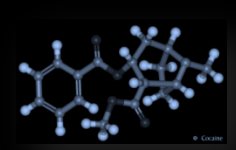
A short peptide in APPL1 mediates binding to OCRL and INPP5B



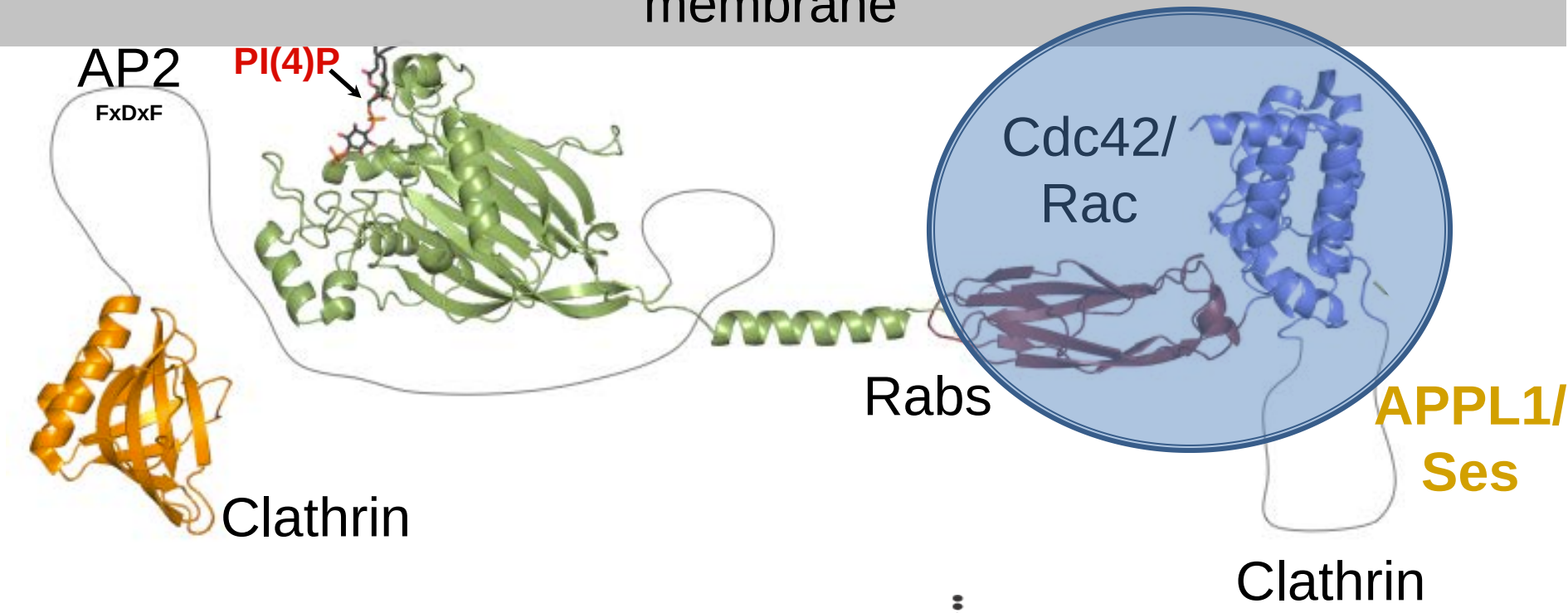
direct binding confirmed by ITC

Erdmann, K.S., Mao, Y., McCrea, H.J., Zoncu, R., Lee, S., Paradise, S., Modregger, J., Biemesderfer, D., Toomre, D. and De Camilli, P. (2007) A role of the Lowe syndrome protein OCRL in early steps of the endocytic pathway. Dev. Cell 13, 377-390. (supported by NIDA Center)

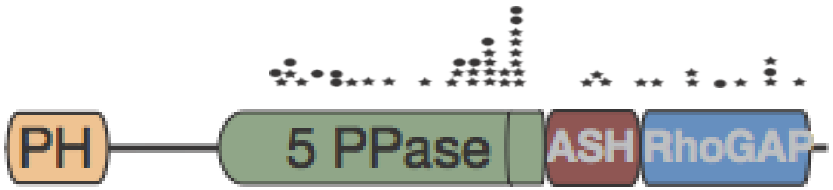
OCRL membrane recruitment



membrane

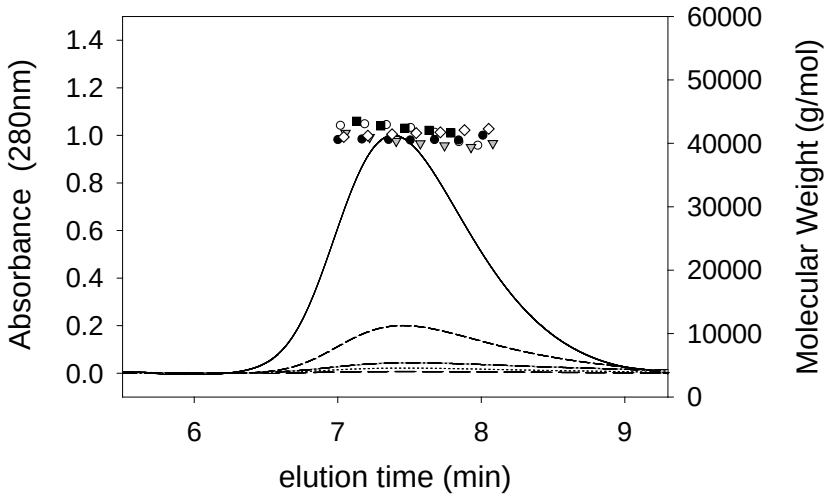


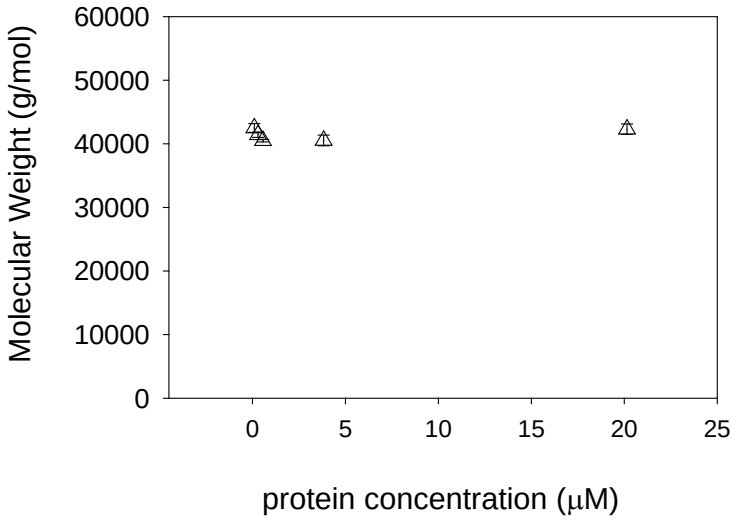
OCRL



Oligomeric state of OCRL ASh- RhoGAP

SEC/MALLS used for determination of oligomeric state

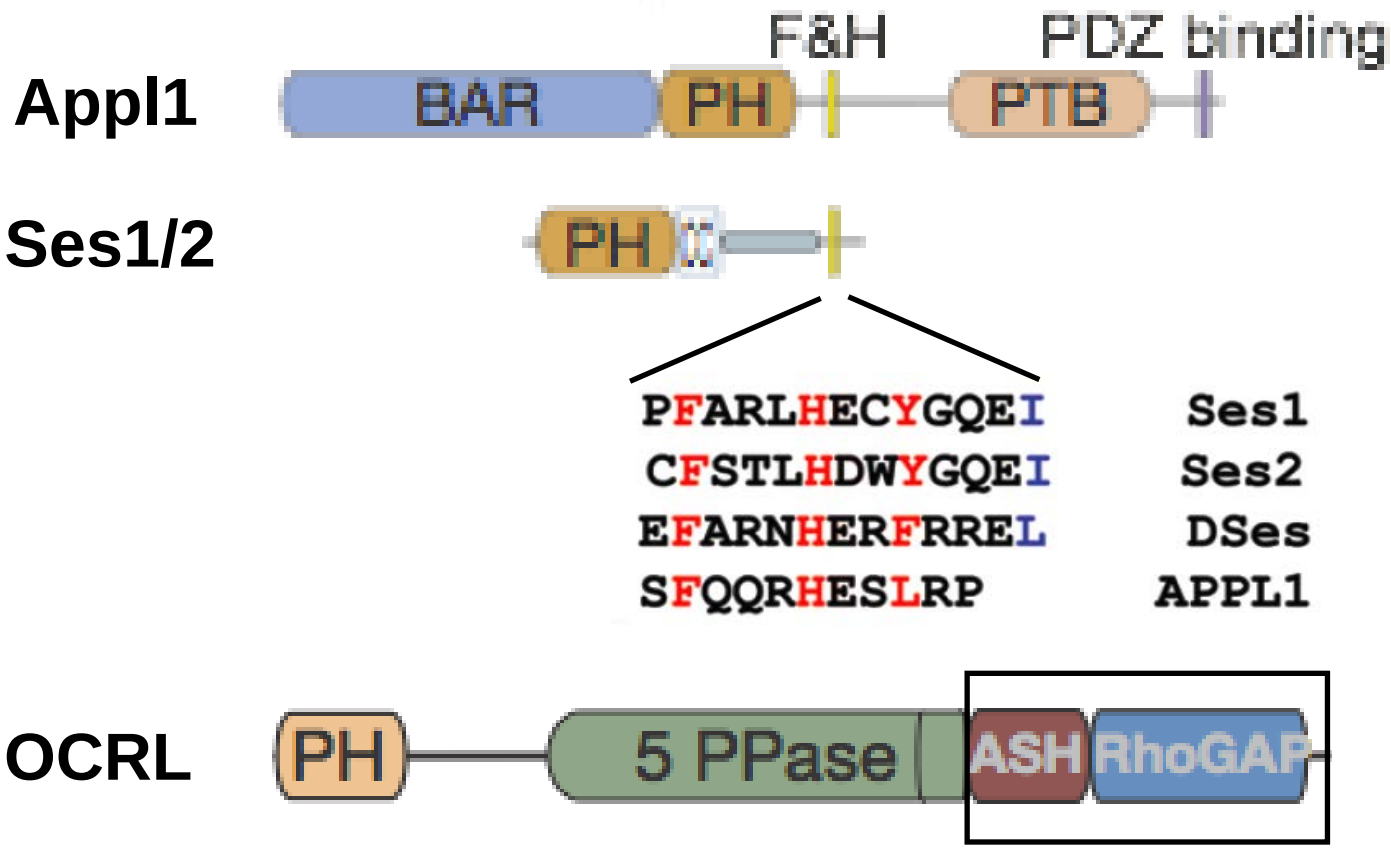




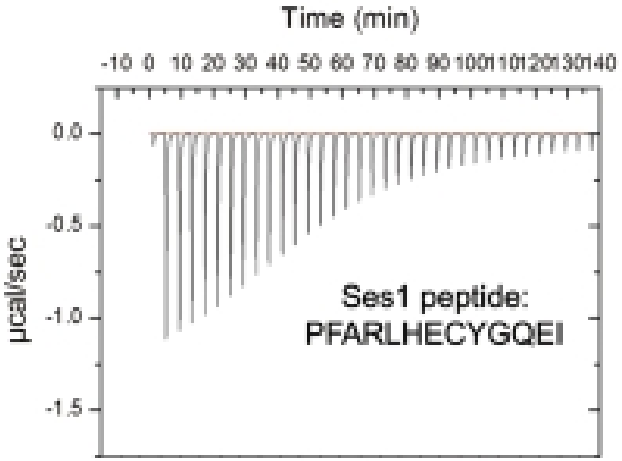
0.1 uM to 20 uM (200 fold change in concentration) protein remains monomeric

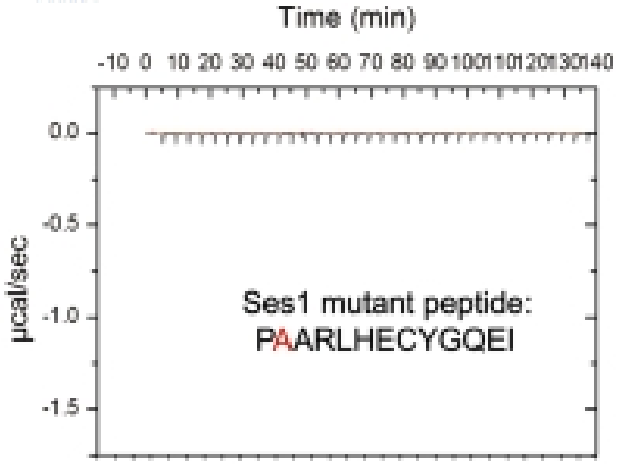
The ASH domain has an immunoglobulin-like fold similar to major sperm protein (MSP), which exists as a dimer

Definition of the consensus F&H motif

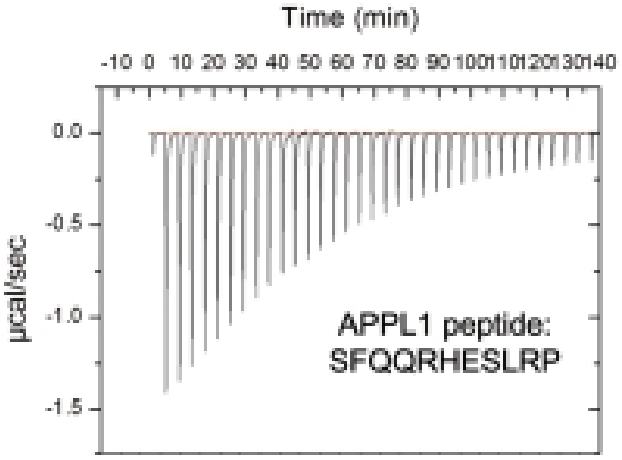


Direct binding of the minimal consensus peptides to the OCRL ASH-RhoGAP–like domain





F to A mutant



Kd (µM) ITC			
Proteins	F&H Peptides		
	APPL	Ses1	Ses1 (F to A)
WT OCRL ASH-RhoGAP	12 ± 2	0.70 ± 0.08	NB

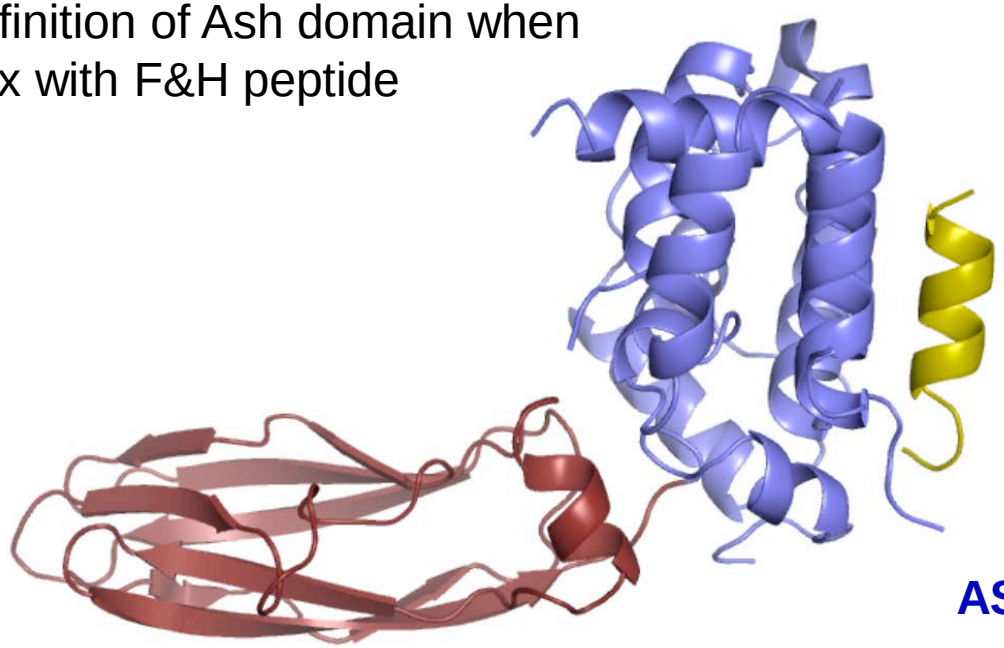
Swan L E, Tomasini L., Pirruccello M., Lunardi J. l., and De Camilli P. (2010) . *Proceedings of the National Academy of Sciences* **107**; 3511-3516

Crystallographic Analysis of the F&H/OCRL interaction

F&H peptides

PFARLHECYGQEI	Ses1
CFSTLHDWYGQEI	Ses2
ARNHERFRREL	DSes
QQRHESLRP	APPL1

•better definition of Ash domain when in complex with F&H peptide

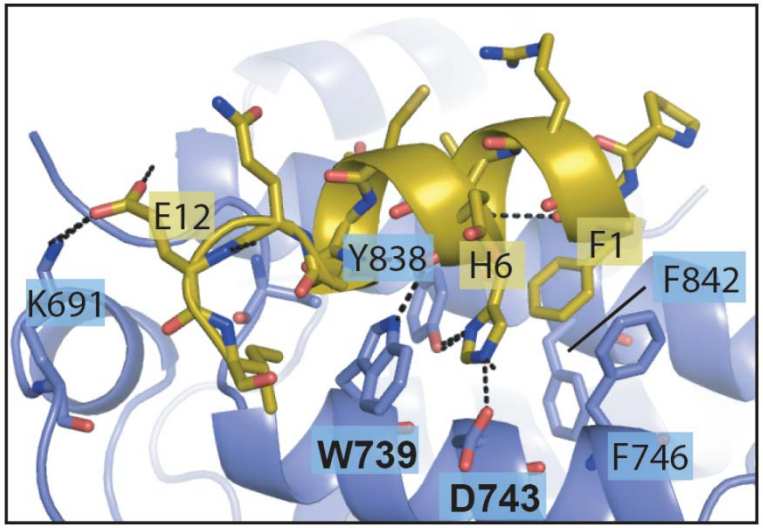


ASH-RhoGAP

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

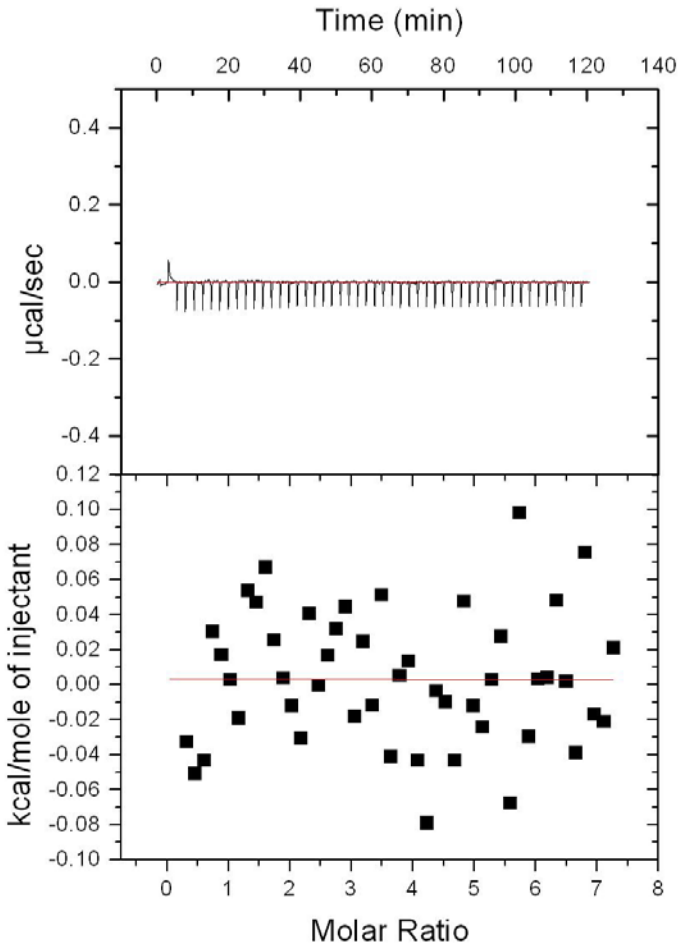
Crystallographic Analysis of the F&H/OCRL interaction

PFARLHECYGQEI	Ses1
CFSTLHDWYGQEI	Ses2
EFARNHERFRREL	DSes
SFQQRHESLRP	APPL1



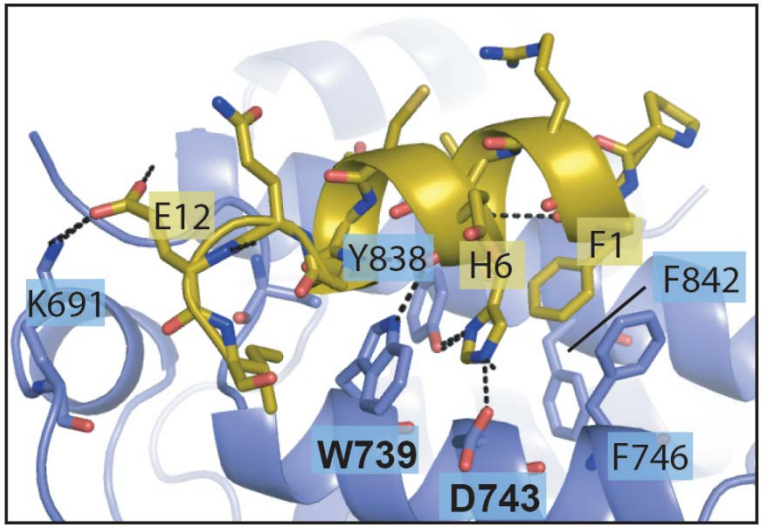
F&H peptide	**OCRL**
Phenylalanine (F&H motif)	hydrophobic pocket Phe842 and Phe746
His (F&H motif)	Side chain forms hydrogen bond with Asp743 Main chain carbonyl H- bond with Indole nitrogen of Trp739

Crystallographic Analysis of the F&H/OCRL interaction



Ses2 peptide
W739A ASH-RhoGAP

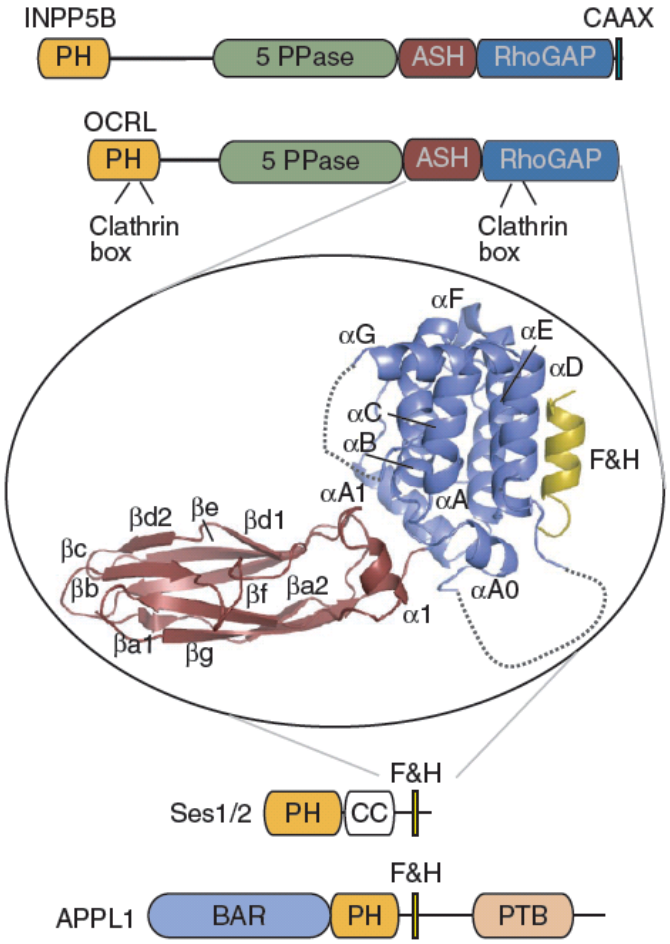
PFARLHECYGQEI	Ses1
CFSTLHDWYGQEI	Ses2
EFARNHERFRREL	DSes
SFQQRHESLRP	APPL1



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?



F&H peptides candidates

SFARLHECYGQEI	**Superclamp; positive control** (engineered F&H peptide)
SFQQRHESLYRP	APPL1
PFARLHECYGQEI	Ses1
CFSTLHDWYGQEI	Ses2

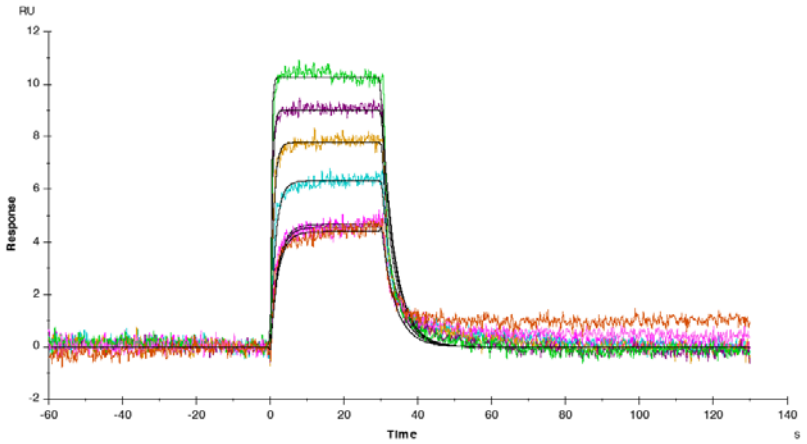
Endocytic proteins

KFRRQHEQLRAVI	**Dynein Heavy Chain**
SFYVRHSCLREAL	**zFyve26** (Spastizin)
SFSTVHEKFNKSL	**WDR36**
IFGLHHIGMQMRI	**CFTR**, cystic fibrosis
SFETQHHHLLHCL	**kv4.2**
EFCRNHFLVGLLL	**Dock9**
AFIERHRIIEEP	**Fly Weeble**

F&H peptide candidates Selected through bioinformatics

Test for binding of F&H peptides to ASH-RhoGAP OCRL and rank their affinities

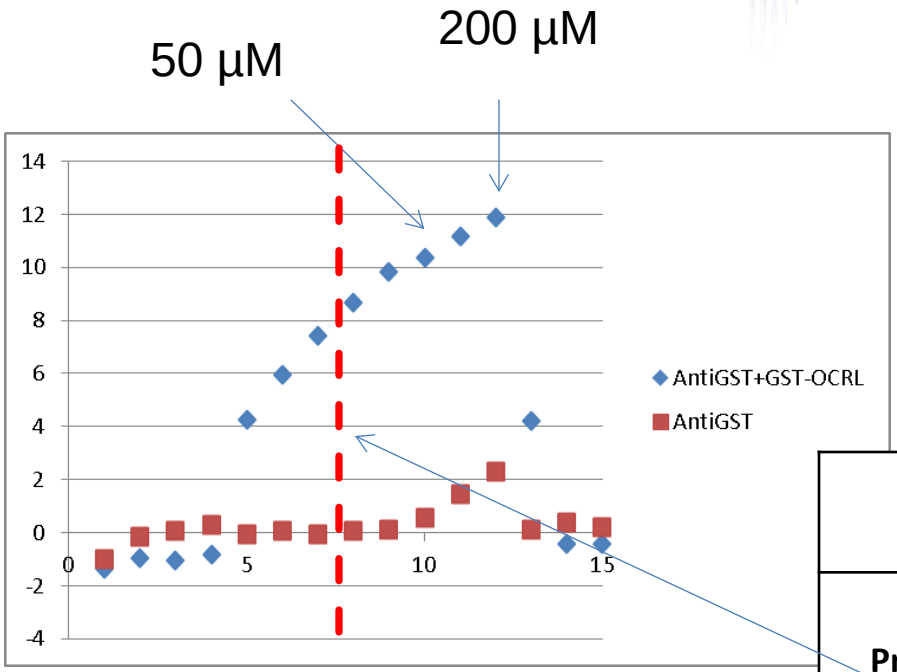
- **Positive Control:** Superclamp (engineered F&H peptide)



Peptides

GST-OCRL; captured on anti-GST Ab

Non-specific binding to antiGST Ab surface



Kd (µM) ITC			
Proteins	F&H Peptides		
	APPL	Ses1	Ses1 (F to A)
WT OCRL ASH-RhoGAP	12 ± 2	0.70 ± 0.08	NB

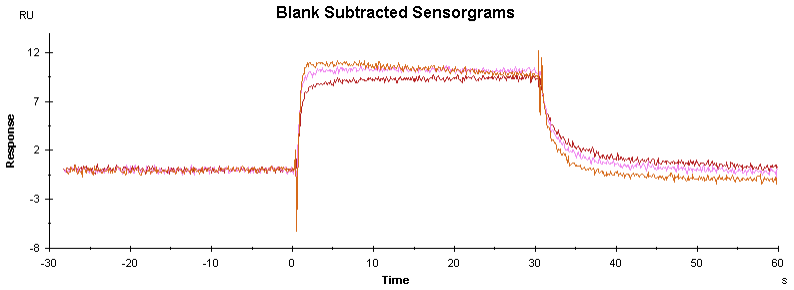
Testing binding of F&H peptide candidates to ASH-RhoGAP

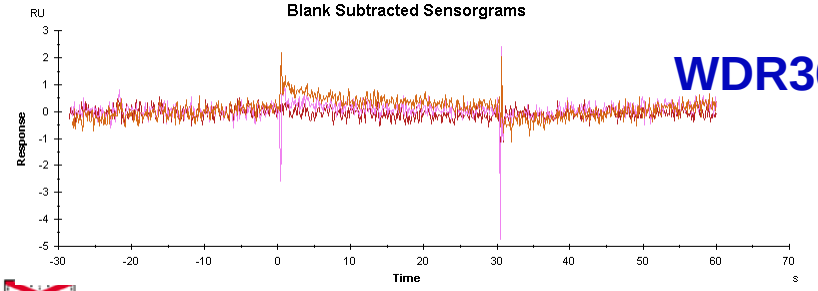
GST_OCRL (WT) captured on anti-GST surface

AntiGST
AntiGST+GST-OCRL

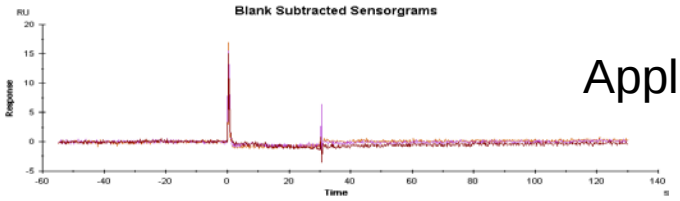
peptides at 50 μM,16,7, and 5.56 μM

positive control; Superclamp

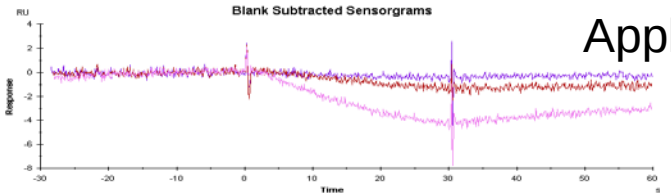




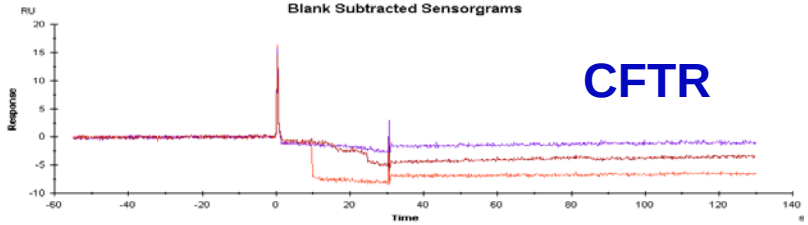
WDR36



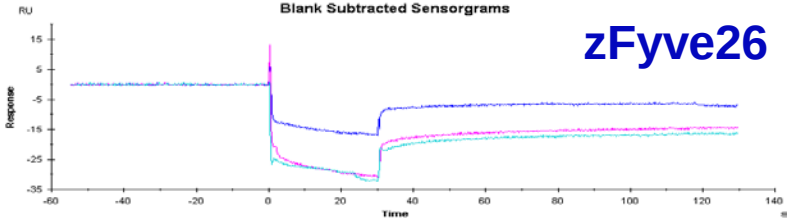
ApplP1



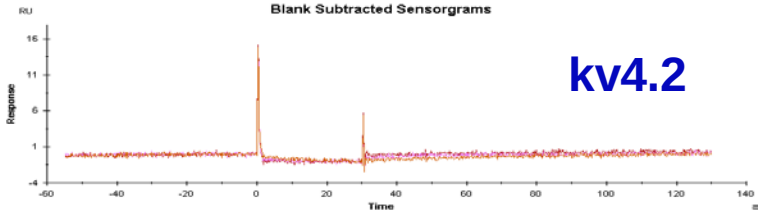
ApplP2



CFTR



zFyve26



kv4.2

Results from preliminary screen

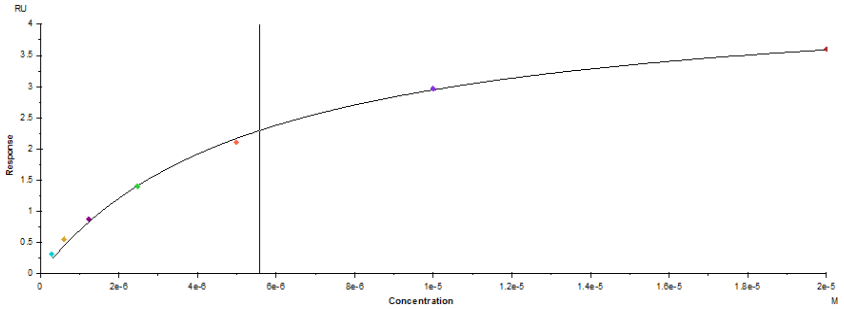
SFARLHECYGQEI	Superclamp; positive control
SFQQRHESLYRP	APPL1
PFARLHECYGQEI	Ses1
CFSTLHDWYGQEI	Ses2

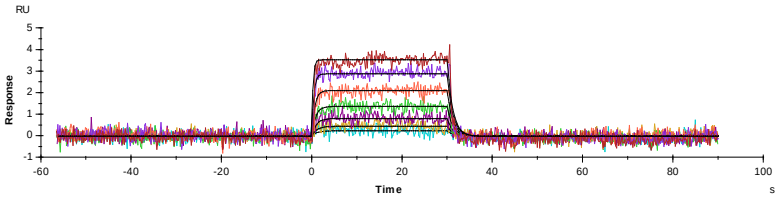
Candidates for SPR

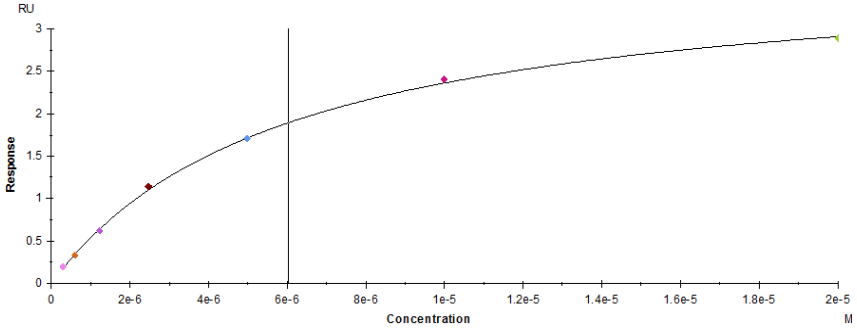
KFRRQHEQLRAVI	**Dynein Heavy Chain**	anti-GST binder
SFYVRHSCLREAL	**zFyve26** (Spastizin)	anti-GST binder
SFSTVHEKFNKSL	**WDR36**	no binding in SPR
IFGLHHIGMQMRI	**CFTR**, cystic fibrosis	no binding in SPR (?)
SFETQHHHLLHCL	**kv4.2**	no binding in SPR
EFCRNHFLVGLLL	**Dock9**	insoluble in SPR buffer
AFIERHRIIEEP	**Fly Weeble**	anti-GST binder

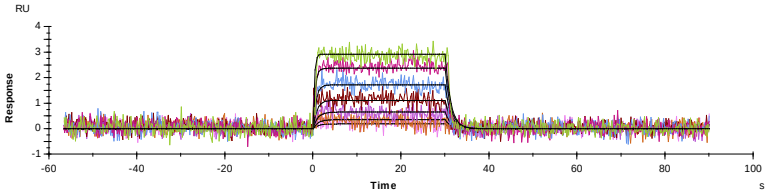
Kd measured from SPR experiments

Peptide #1







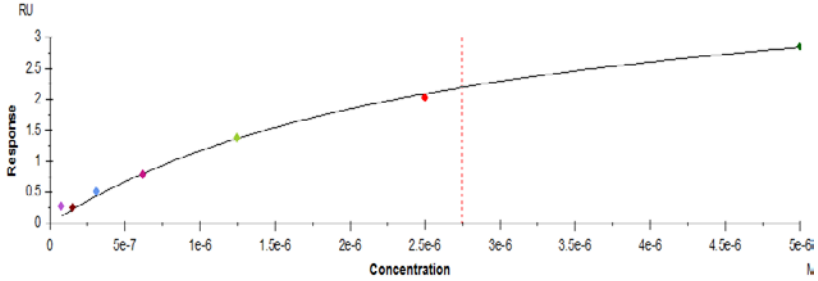


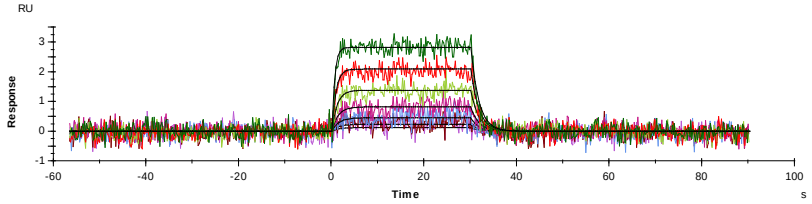
Ses #1

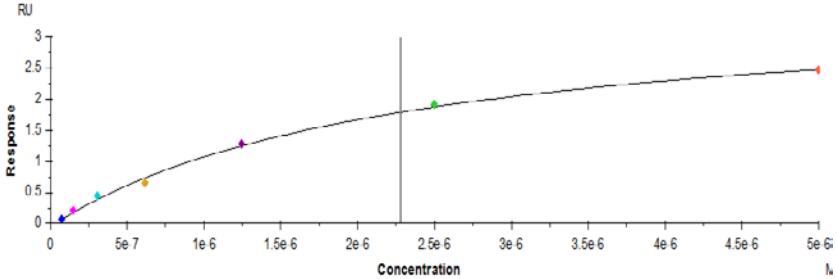
Kd 5.8 ± 0.3 uM

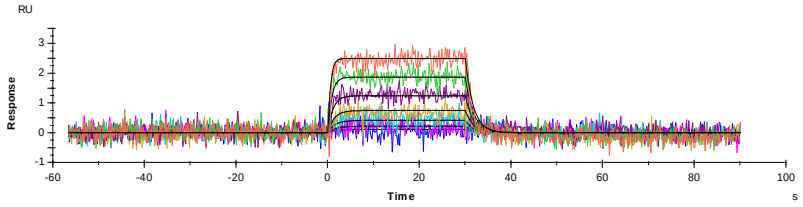
Kd measured from SPR experiments

Peptide #2







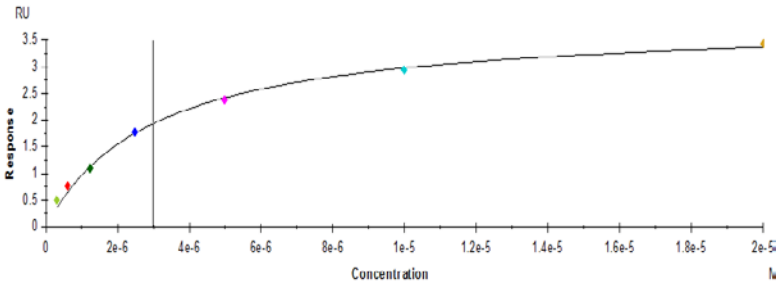


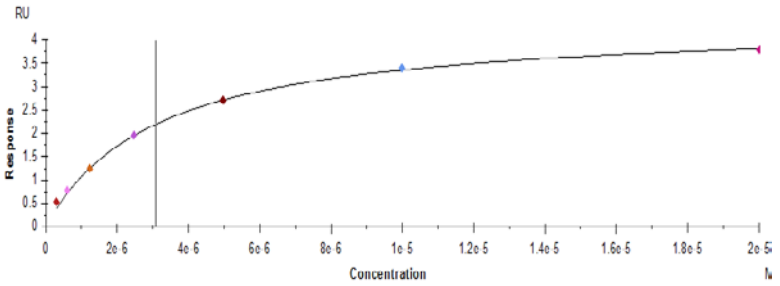
Ses2

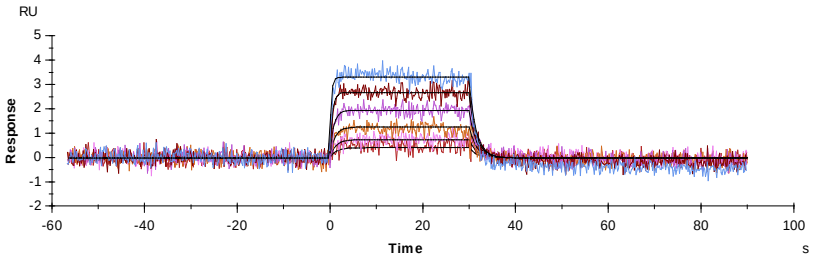
Kd 2.5 ± 0.4 uM

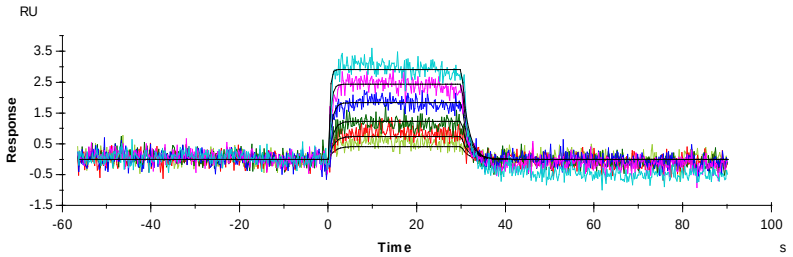
Kd measured from SPR experiment

Peptide #5









Superclamp
Kd 3.0 ± 0.2 uM

Kd measured from SPR experiment

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
SFYVRHSCLREAL	**zFyve26** (Spastizin)	anti-GST binder
SFSTVHEKFNKSL	**WDR36**	no binding in SPR
IFGLHHIGMQMRI	**CFTR**, cystic fibrosis	no binding in SPR
SFETQHHHLLHCL	**kv4.2**	no binding in SPR
EFCRNHFLVGLLL	**Dock9**	insoluble in SPR buffer
AFIERHRIIEEP	**Fly Weeble**	anti-GST binder

Kd measured from SPR experiment

Bioinformatics approach did not yield new F&H candidates that interact with F&H motif binding surface of OCRL

No binding was detected – tested by SPR and ITC

K**F**RRQ**HE**QLRAVI	**Dynein Heavy Chain**
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**

Summary

Kd (μM) SPR/*ITC*							
Proteins	**Peptides**						
	APPL	**Ses1**	**Ses2**	**SesFA**	**Superclamp**	**APPLP1**	**APPLP2**
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*
W739A (engineered)	ND	ND	ND *ND*	ND	ND	ND	ND

- The affinities for binding of F&H peptides to ASH-RhoGAP OCRL were determined using SPR and ITC
- No binding was observed for phosphorylated forms of APPL1
- The peptides were rank Ses2≥Ses1>>APPL1

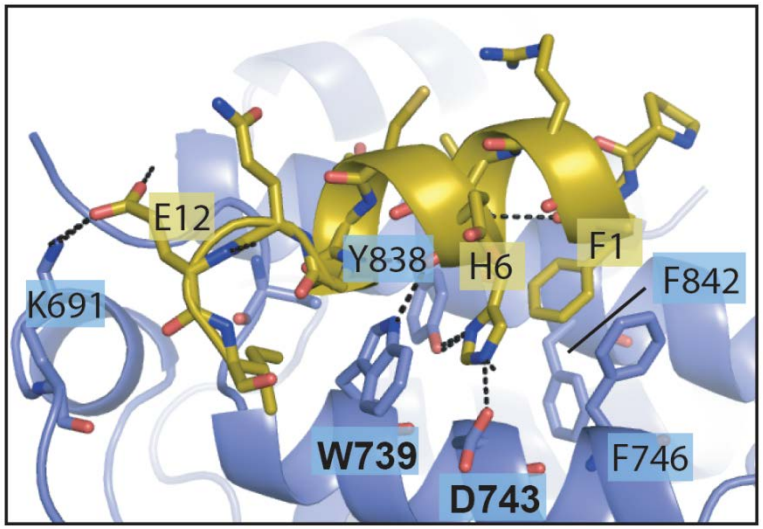
Summary

Ses1 **APPL**

Terminal Proline 11
In the minimal APPL1 F&H peptide

Glu12 H-bond
Lys691

PFARLHECYGQEI	Ses1
CFSTLHDWYGQEI	Ses2
EFARNHERFRREL	DSes
SFQQRHESLRP	APPL1



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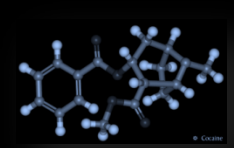


Summary

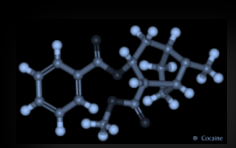
ITC

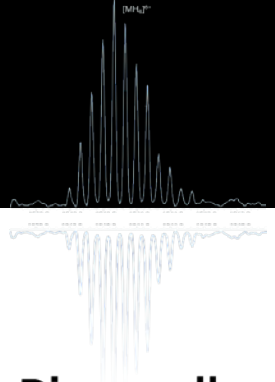
Peptide	Kd (uM)	ΔG (kcal/mol)
Ses1	0.7	-8.4
APPL1	12	-6.7
Delta(ΔG)		-1.7

SPR

Peptide	Kd (uM)	ΔG (kcal/mol)
Ses1	2.5	-7.6
APPL1	43	-5.9
Delta(ΔG)		-1.7

- The peptides were rank Ses1>>APPL1 by both technologies: SPR and ITC





Michelle Pirruccello and Laura Swan

Pietro DeCamilli

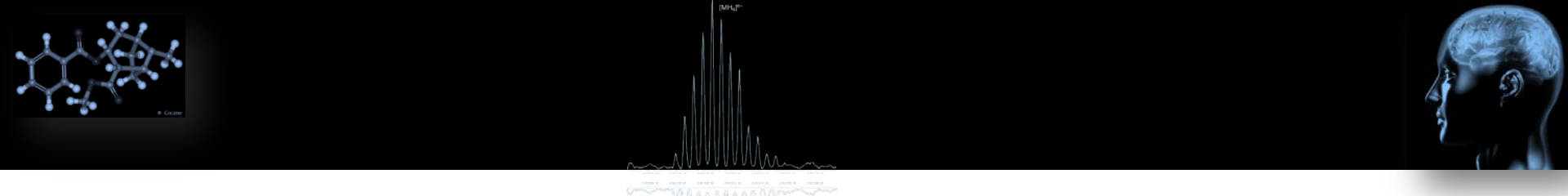
Department of Cell Biology Yale University and HHMI

Swan L E, Tomasini L., Pirruccello M., Lunardi J. l., and De Camilli P. (2010) *PNAS* **107**; 3511-3516

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

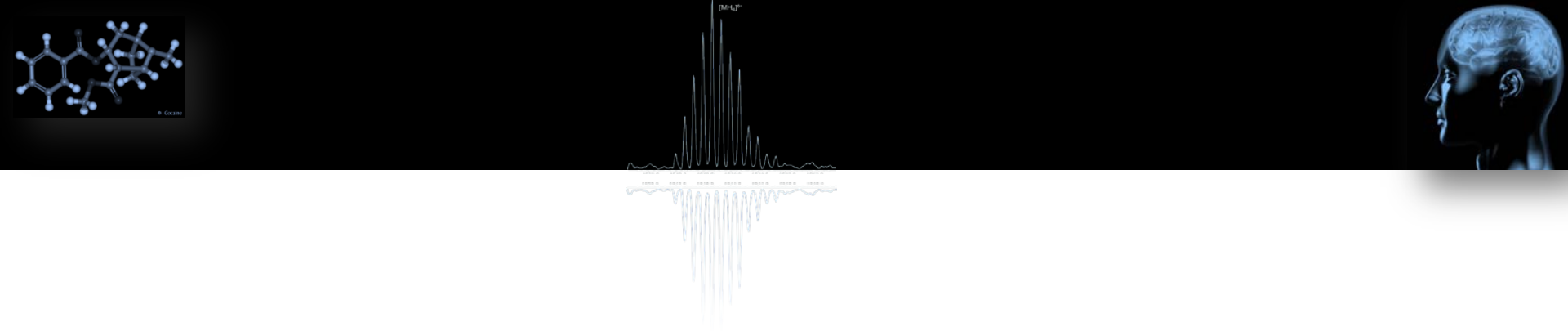
NIH
Yale/NIDA Center support
SEC/MALLS system; SIG 2007
Biacore T100; SIG 2009

HHMI (VP-ITC purchase)



Additional applications of Biophysics Core are presented in the poster.

- Use of SPR for screening small molecules candidates as possible drug candidates (DeCamilli; Strittmatter)
- Use of SEC/MALLS for characterization of oligomers of Cysteine string protein alpha, CSPα (Chandra)
- Use of SEC/MALLS for detection of phosphorylation-dependent dimerization of ΔFosB (Nestler)



- Use of SPR for screening small molecules candidates as possible drug candidates (DeCamilli; Strittmatter)

"To Affinity and Beyond"
From Screened Compounds To Optimized Leads With Label Free

Speakers: Olof Karlsson[1]and Paul Belcher[2]
[1]GE Healthcare Bio-Sciences AB, Uppsala, Sweden, *[2]GE Healthcare, Piscataway, NJ, USA*,