

PKC-alpha Phosphorylates Cytoskeleton Protein *in vitro*

Liang @ Schaller's Lab

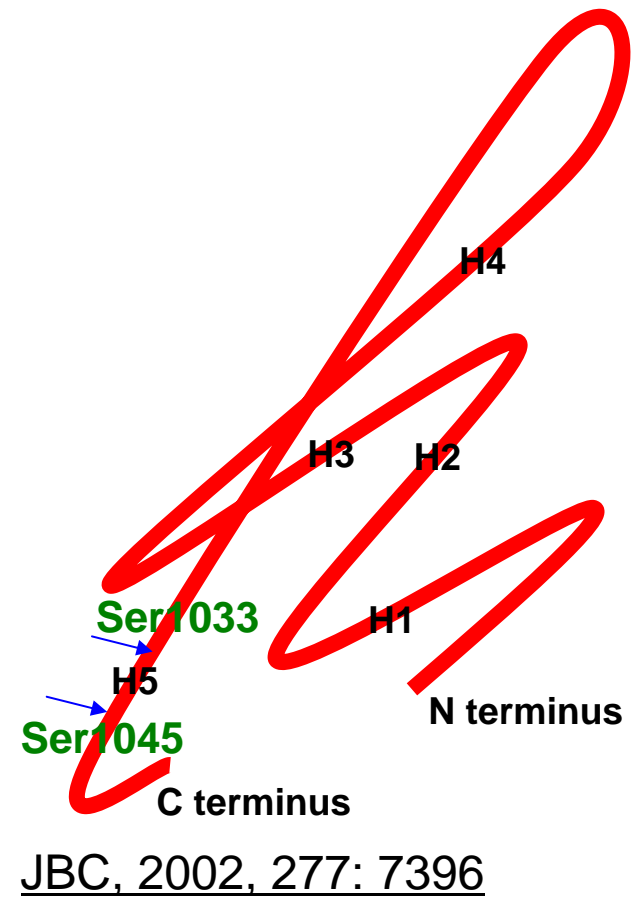
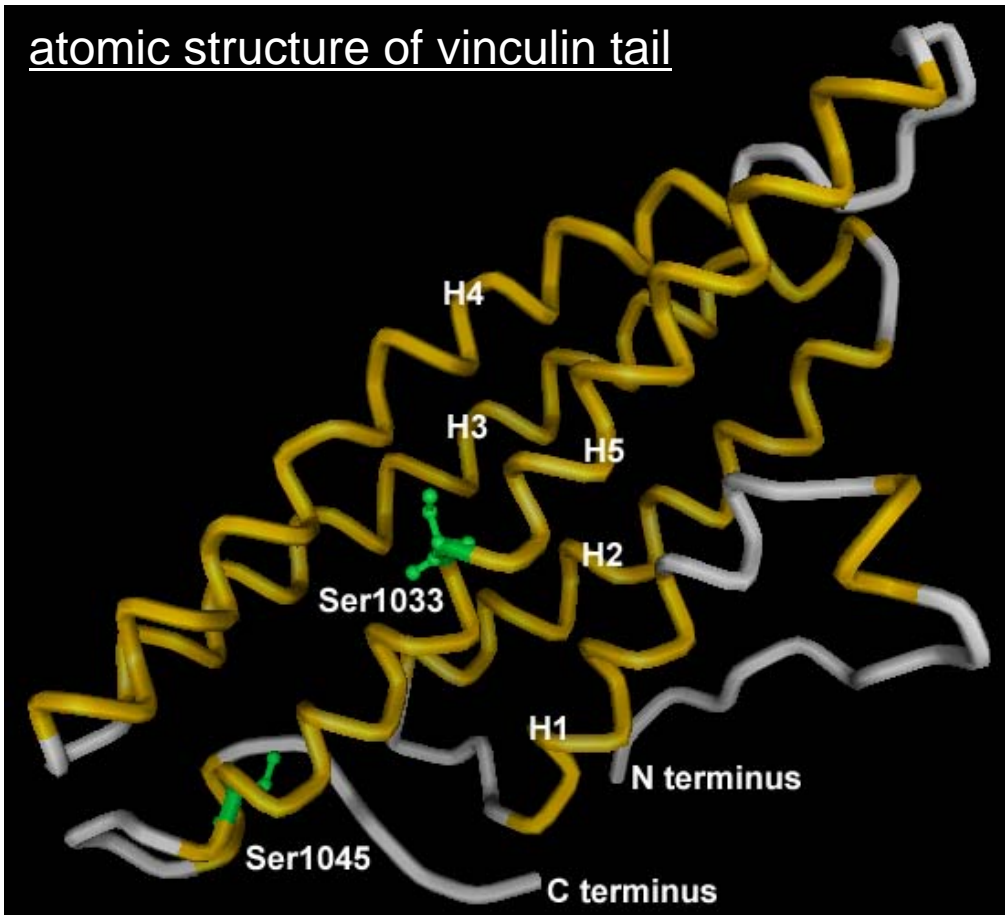
Feb ~ Apr, 2004

Background about Vinculin

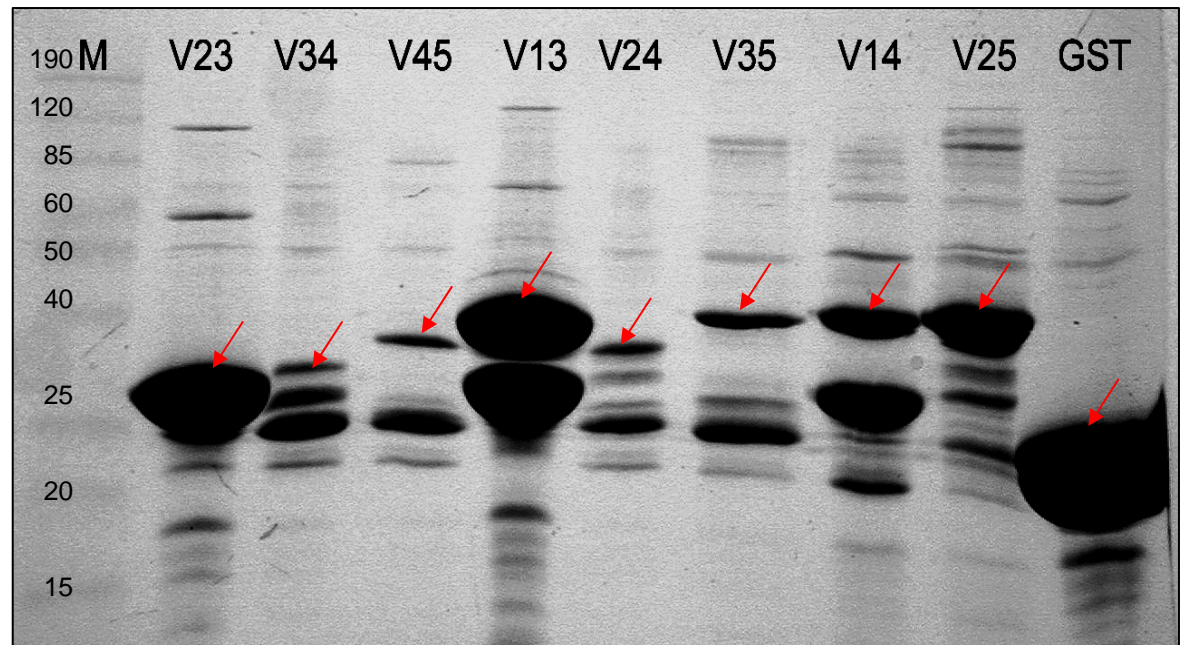
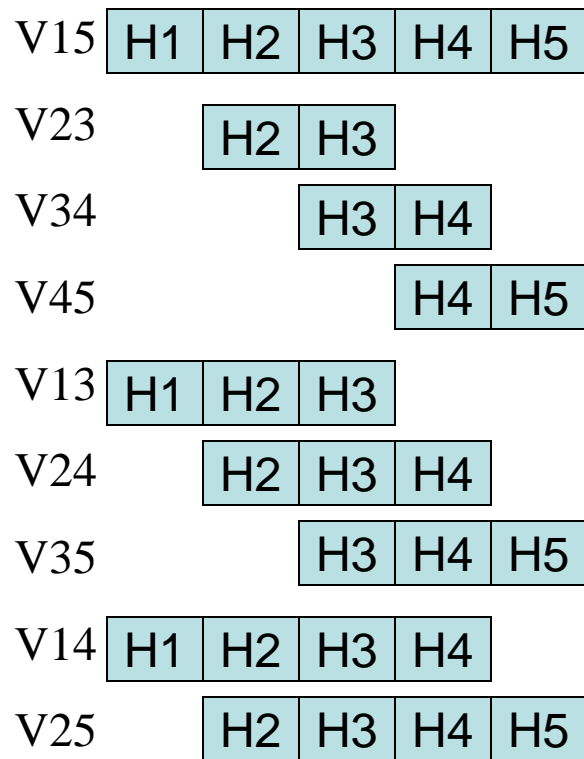
- Vinculin is an early and essential component of nascent cell adhesions.
- Vinculin is a tadpole-like molecule.
 - Globular head of vinculin binds to talin and alpha-actinin.
 - Rod-like tail of vinculin binds to F-actin and paxillin.
- A lipid-regulated docking site was found on the 5th helix of the vinculin tail.

JBC, 2002, 277: 7396

atomic structure of vinculin tail

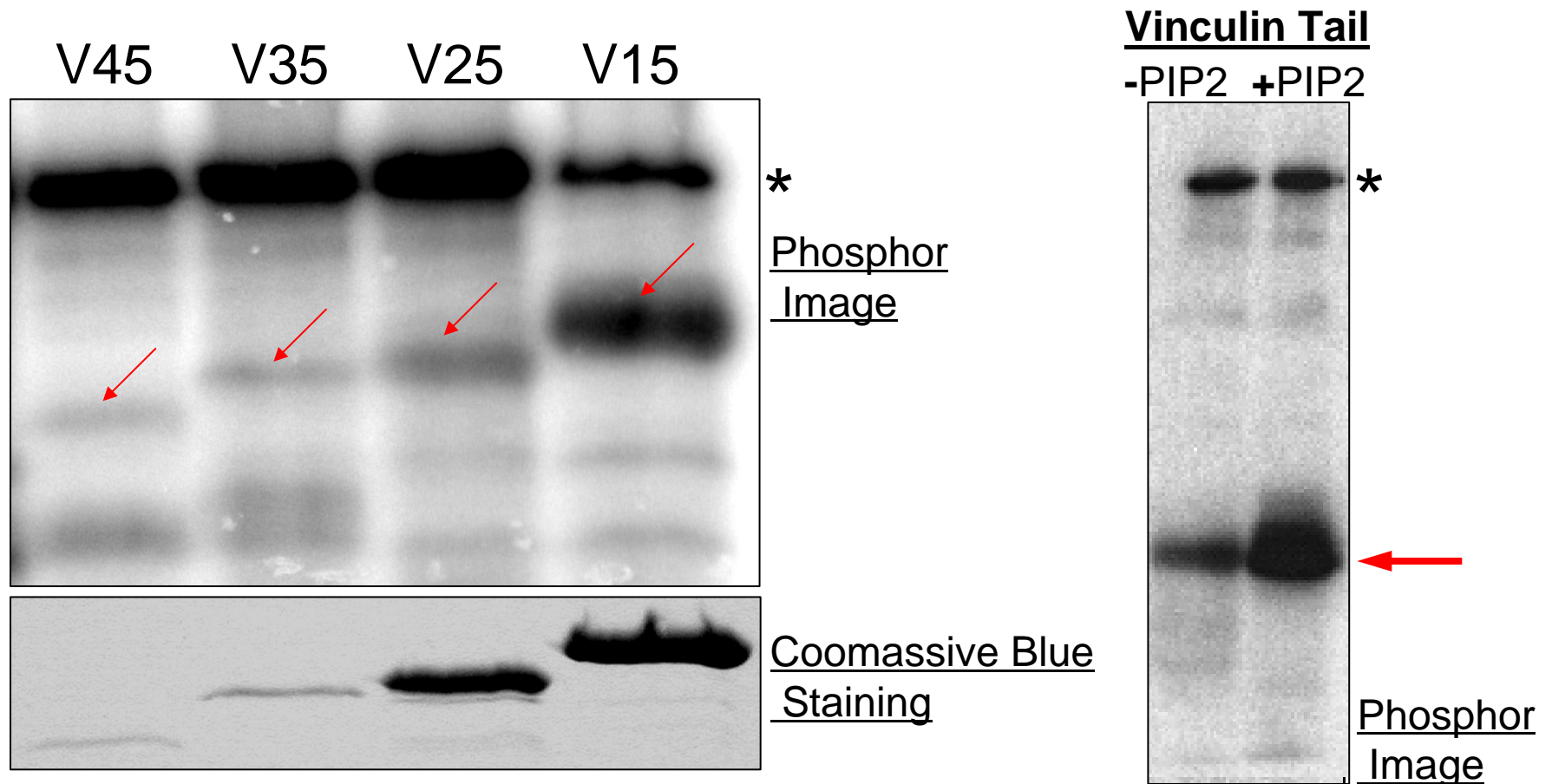


Purify Series of Vinculin Tail



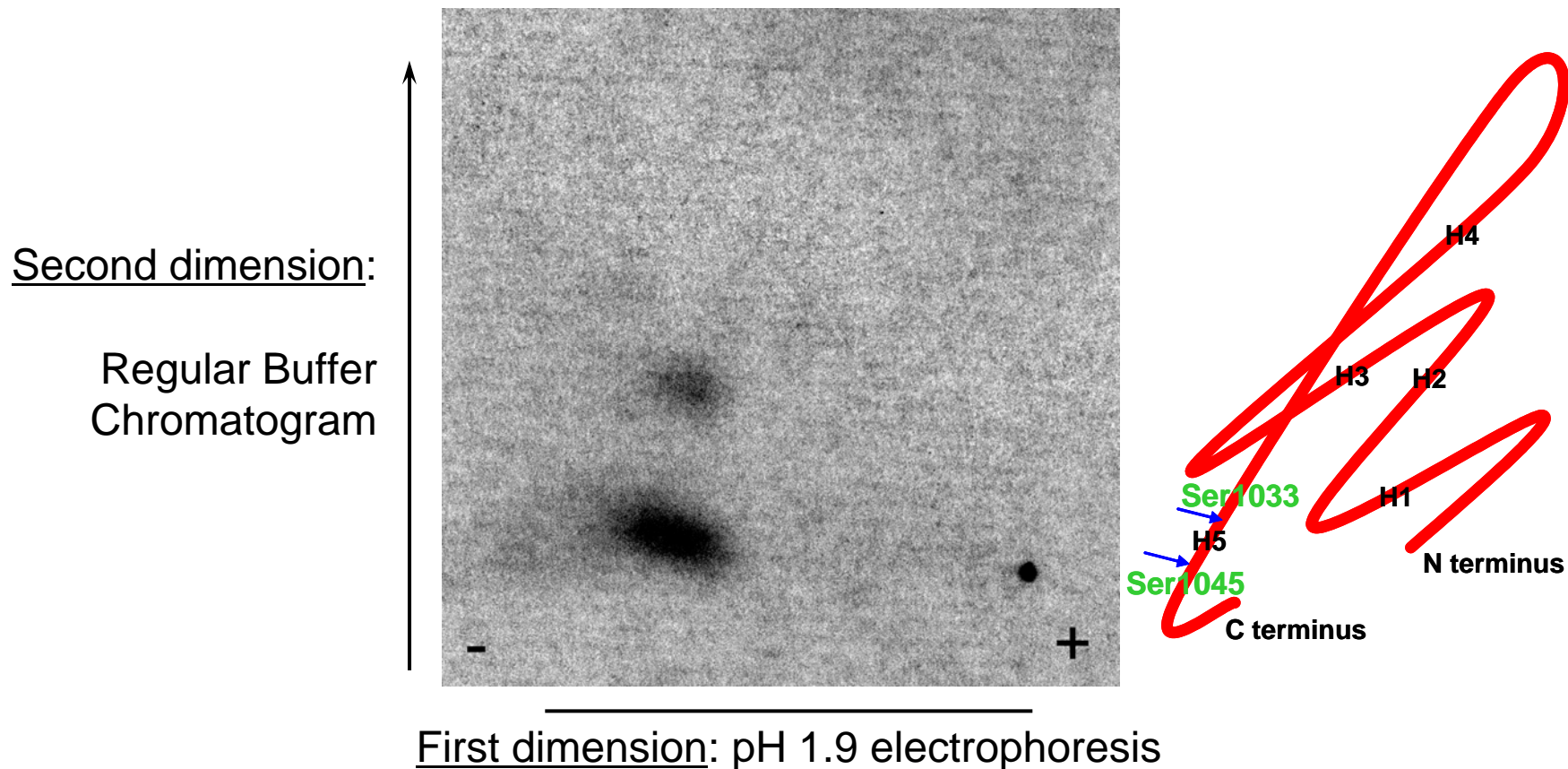
Coomassie Blue Staining

In Vitro Phosphorylation of Vinculin Tail



Tryptic Peptide Map of Vinculin Tail

Sample from: *in vitro* phosphorylation product of vinculin tail



Background about Coronin

- Coronin, an actin binding protein, is predicted to have diverse activities in nonmuscle cells.
- Coronin_{se}, isolated from rabbit parietal cells, is the downstream target of PKC. JBC, 1999, 274: 3017
- Protein sequence of Coronin_{se} has:
 - 89% identity with human coronin 1B,
 - 90% identity with mouse coronin 1B,
 - 88% identity with rat coronin 1B.

- 11 phosphorylated peptides may be generated by trypsin digestion.

	10	20	30	40	50	60
	MSFRKVVVRQS	KFRHVFVGQPV	KNDQCYEDIR	VSRVTWDSTF	CAVNPCKFLAV	IVEASGGGAF
	70	80	90	100	110	120
	LVLPLSKTGR	IDKAYPTVCG	HTGPVLDIDW	CPHNDEVIAS	GSEDCTVMVW	QIPENGLTSP
	130	140	150	160	170	180
	LTEPVVVLEG	HTKRVGIIAW	HPTARNVLLS	AGCDNVVLIW	NVGTAEELYR	LDSLHPDLIY
	190	200	210	220	230	240
	NVSWNHNGSL	FCSACKDKSV	RIIDPRRGTL	VAEREKAHEG	ARPMRAIFLA	DGKVFTTGFS
	250	260	270	280	290	300
	RMSERQLALW	DPENLEEPMA	LQELDSSNGA	LLPFYDPDTS	VVYVCGKGDS	SIRYFEITEE
	310	320	330	340	350	360
	PPYIHFLNTF	TSKEPQRGMG	SMPKRGLEVS	KCEIARFYKL	HERKCEPIVM	TVPRKSDLFQ
	370	380	390	400	410	420
	DDLYPDTAGP	EAALEAEWVSGR	DADPILISLR	EAYVPSK	QRDLKISR	VLSDSRPAMA
	430	440	450	460	470	480
	PGSSHLGAPA	STTTAADATP	SGSLARAGEA	GKLEEVMQEL	RALRALVKEQ	GDRICRLEEQ
	489					
	LGRMENGDA					

PKC targeting site

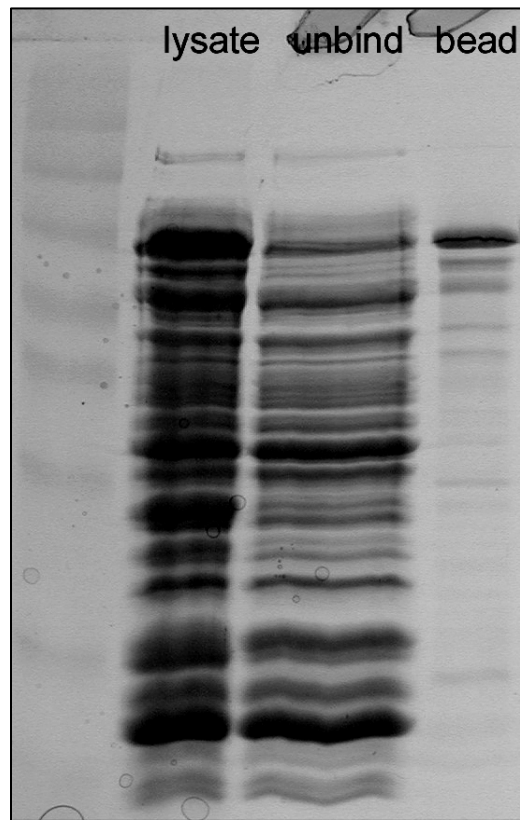
Thr containing peptide

Ser containing peptide

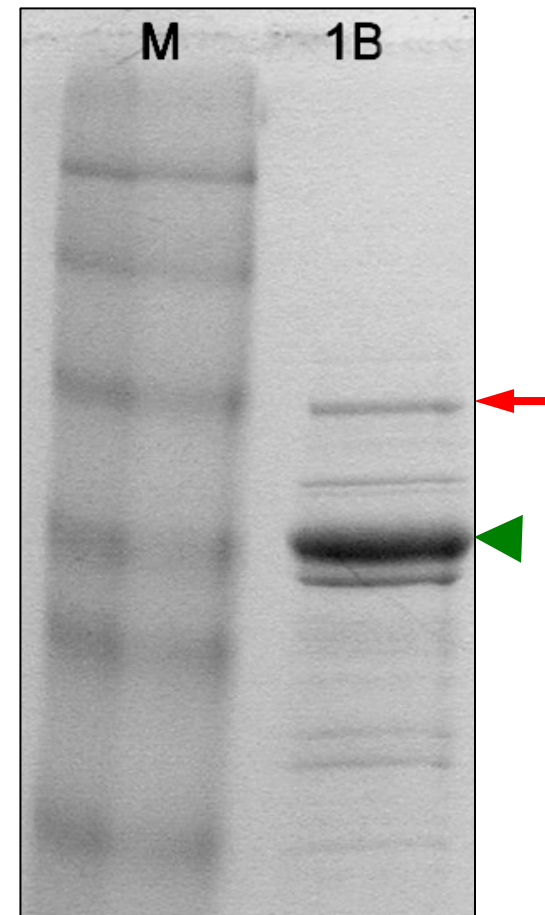
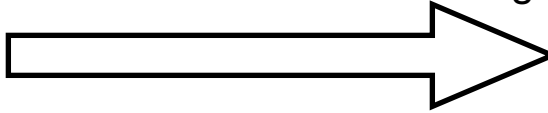
Predicted by ScanProsite & PeptideCutter

Purify GST-hCoro1B

BL21 (DE3) +RP

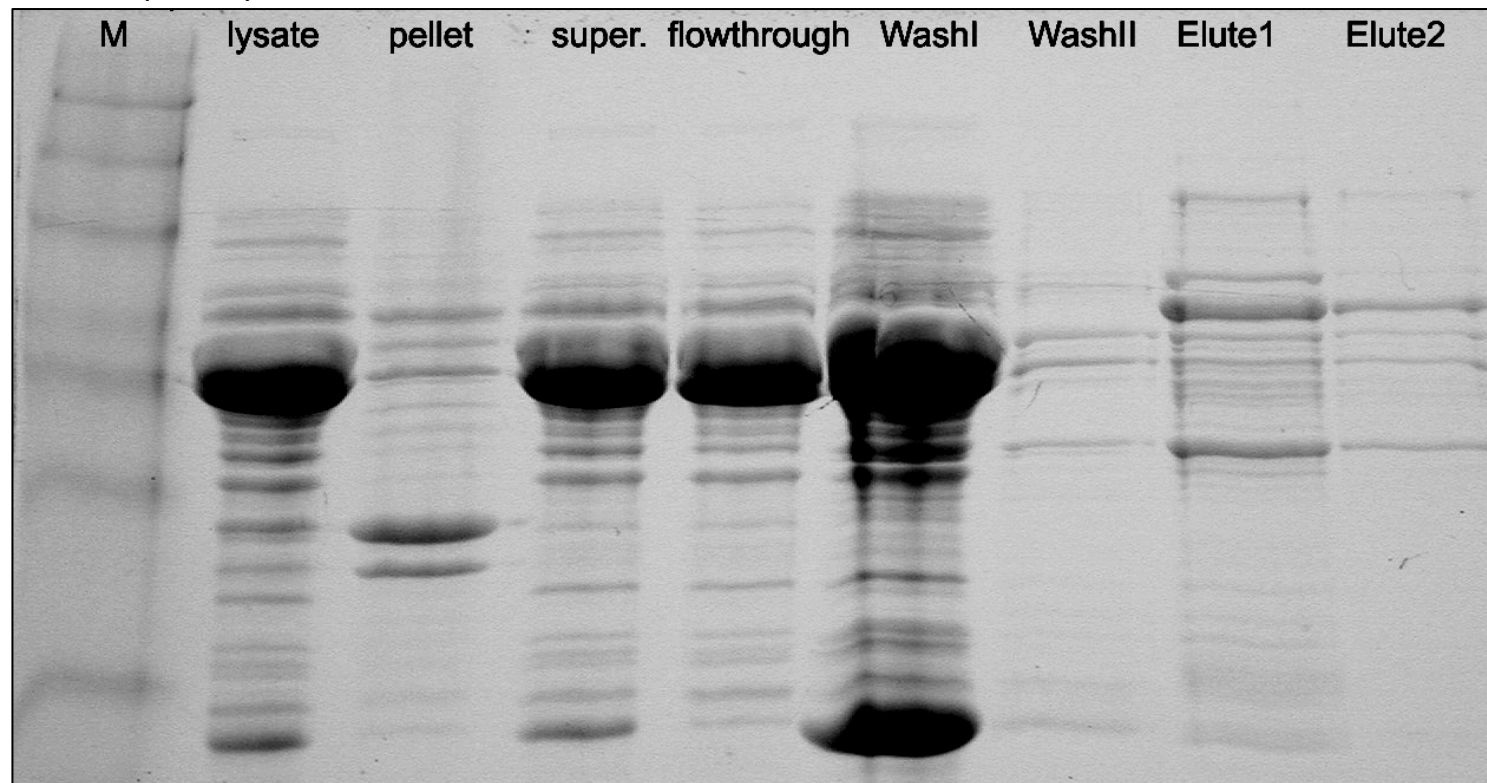


Proteolysis



Purify HIS-hCoro1B

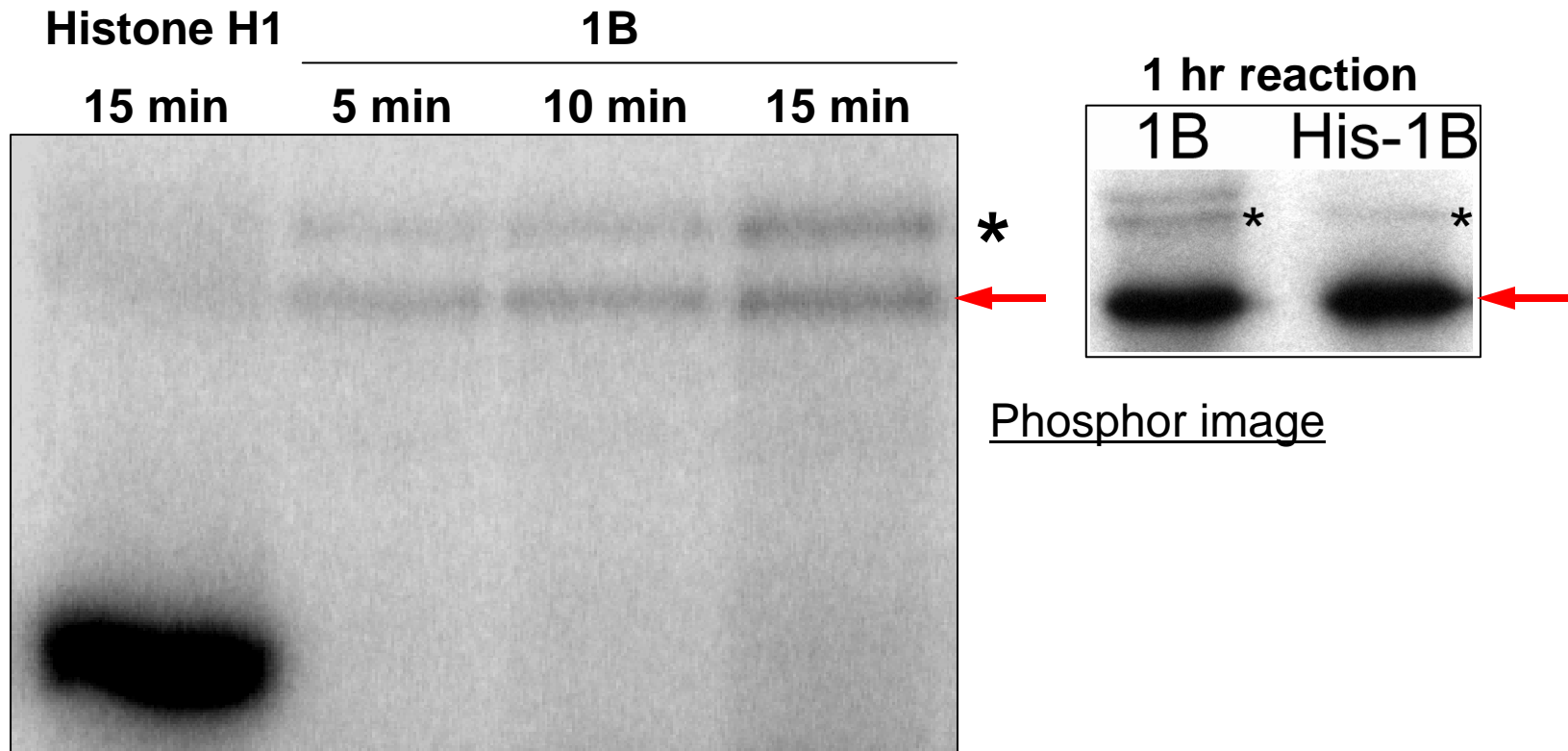
BL21 (DE3) + TF + GroEL + GroES



Coomassie Blue Staining

App.Env.Micro., 2000, 66: 884

In Vitro Phosphorylation of Coronin 1B

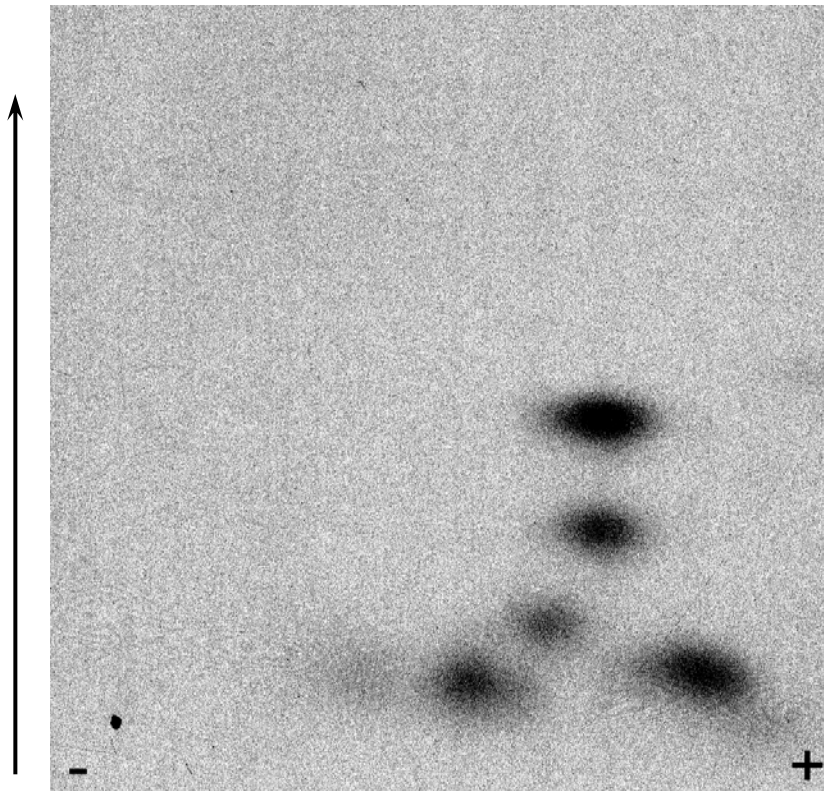


Tryptic Peptide Map of Coronin 1B

Sample from: *in vitro* phosphorylation product of Coronin 1B

Second dimension:

Regular Buffer
Chromatogram



First dimension: pH 8.9 electrophoresis

Special thanks to

- **Schaller's Lab**

- Martin Playford
- Michelle Brantley
- Danielle Scheswohl

- **Bear's Lab**

- Nick Holoweckyj
- Tom Marshall

- **Weiss's Lab**

- Thierry Horner
- Peng Liu
- Shoji Osawa

• Suggestion from Dr. Cyr; Water bath in Dr. Brennwald's Lab.