Identification of TARP Phosphorylation Sites and Their Significance in the Brain

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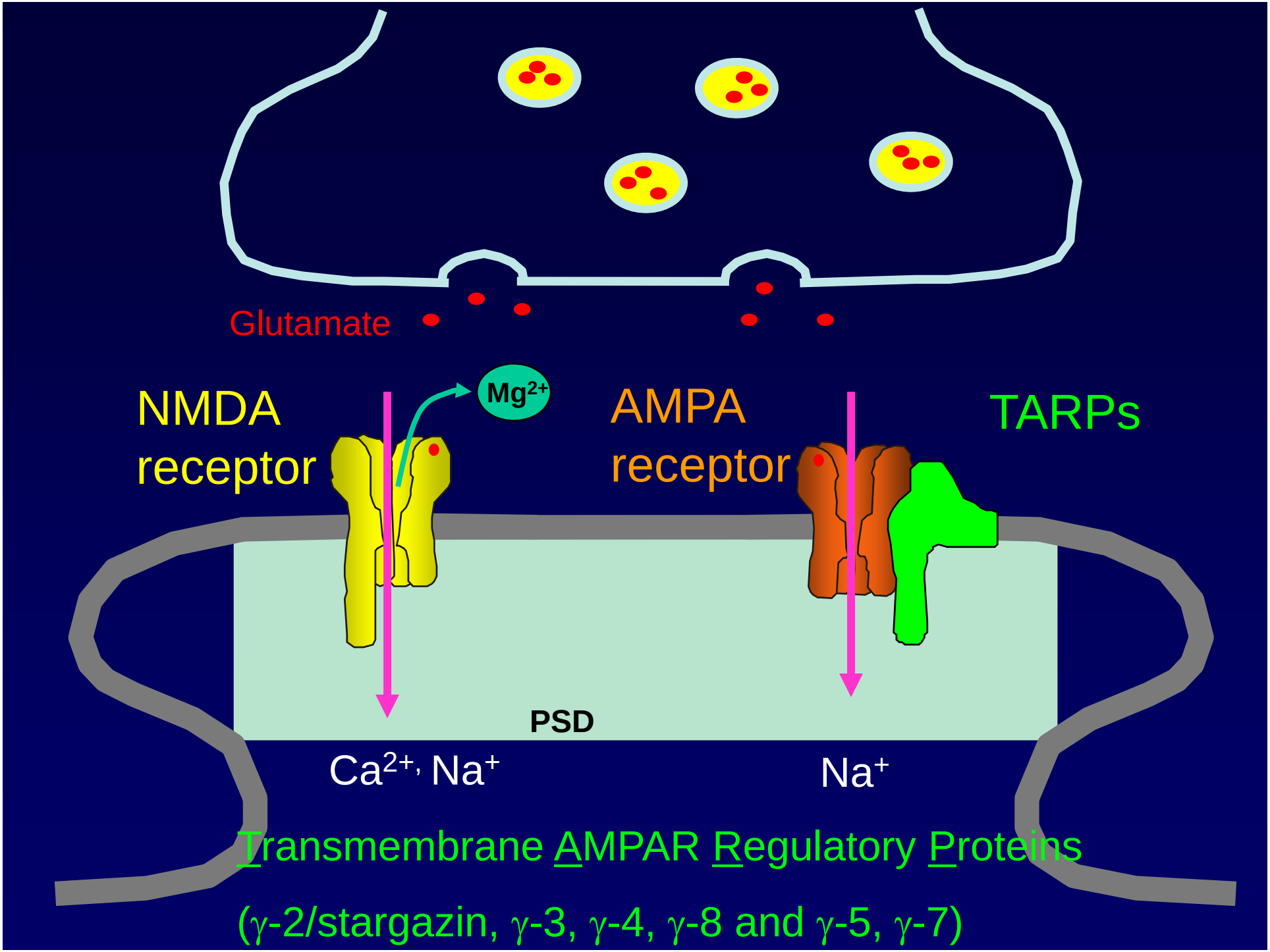
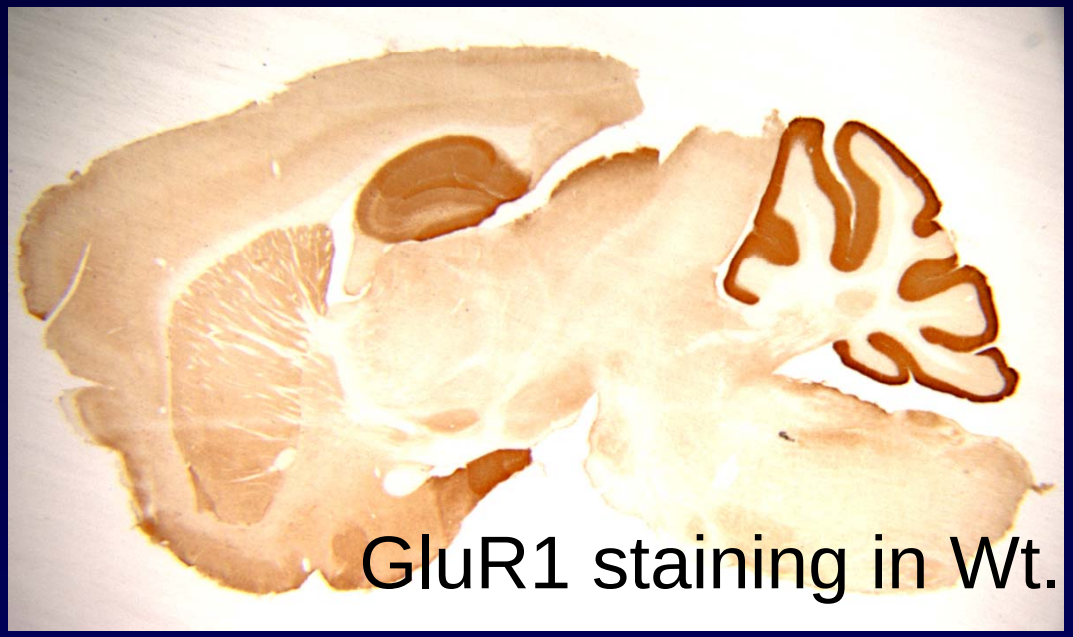
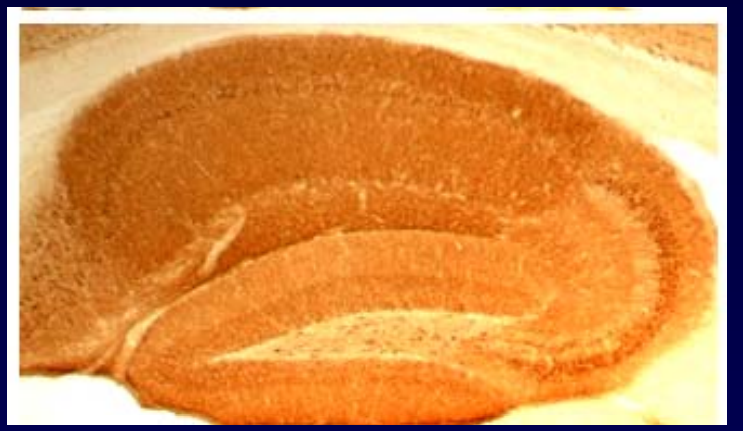
NIDA Neuroproteomics Center , December 13, 2008


Glutamate
NMDA receptor
Mg2+
AMPA receptor
TARPs
PSD
Ca2+, Na+
Na+
Transmembrane AMPAR Regulatory Proteins
(γ-2/stargazin, γ-3, γ-4, γ-8 and γ-5, γ-7)

Loss of AMPAR in hippocampus of TARP/γ-8 KOs



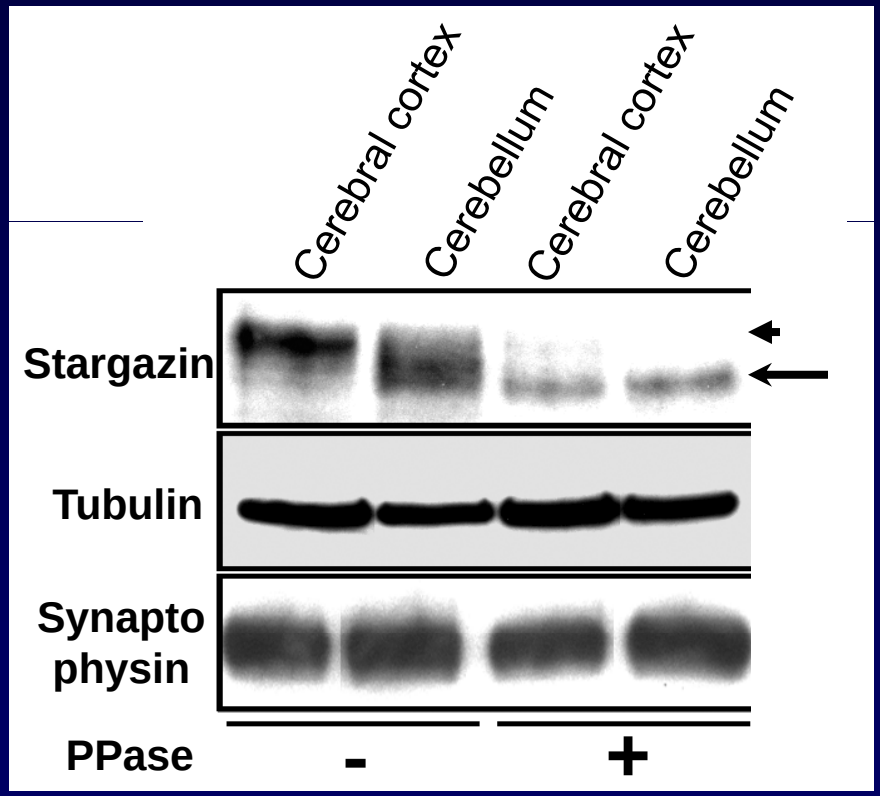
GluR1 staining in Wt.

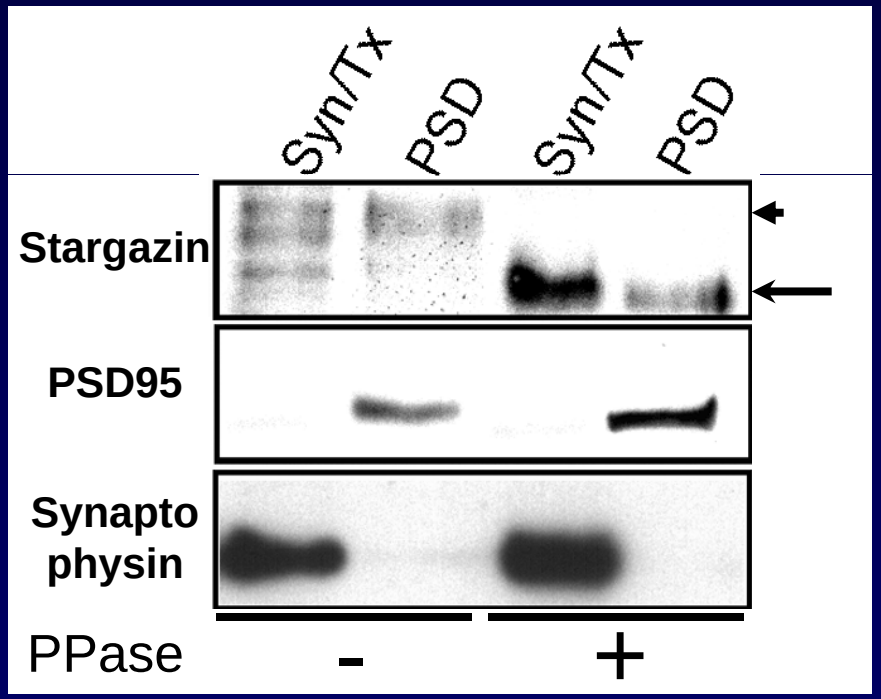


GluR1staining in γ8 KO

Rouach N. *et al.* (2005) *Nat. Neurosci.*

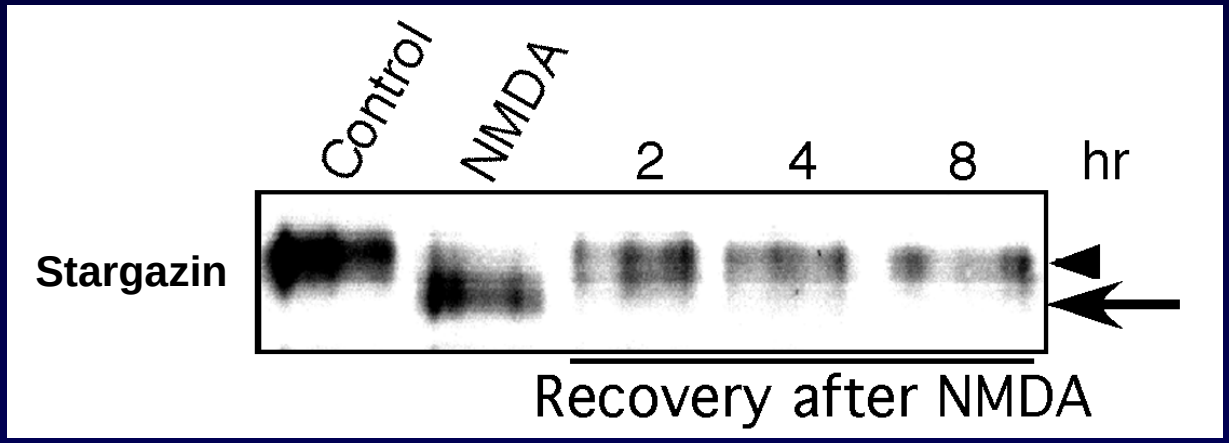
TARP/Stargazin is phosphorylated in brain

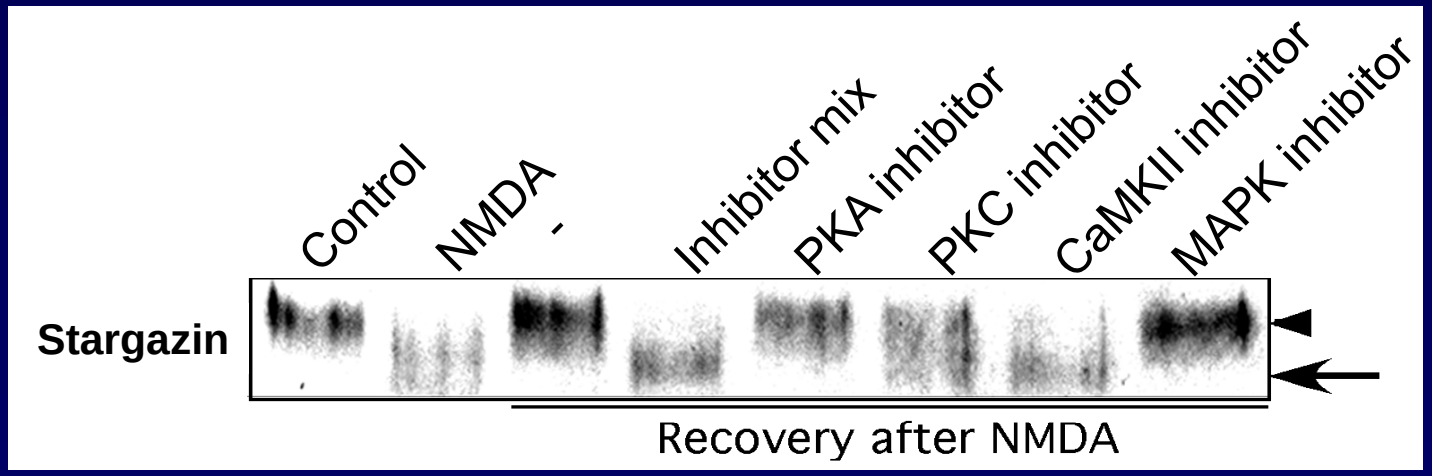




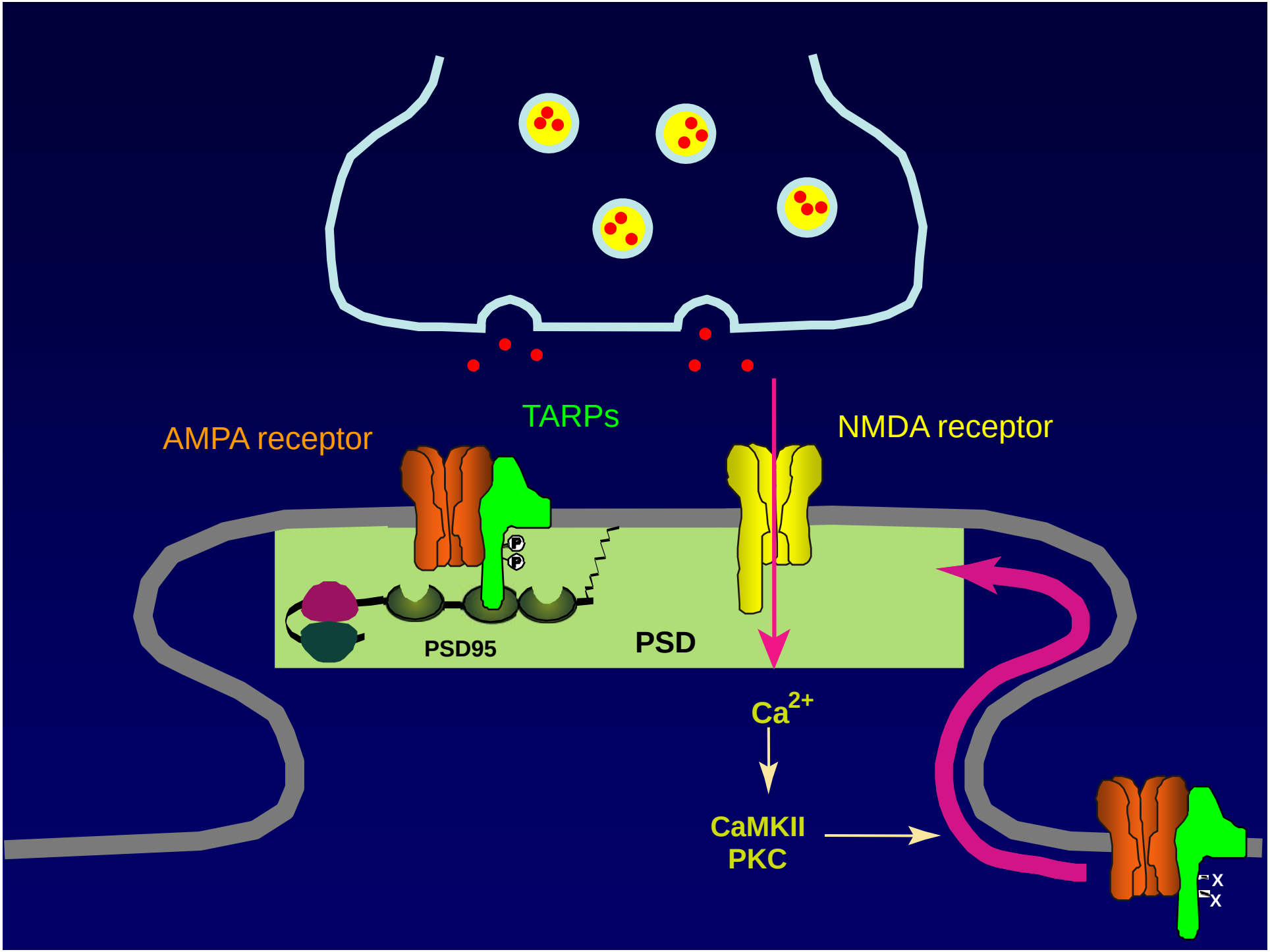
Tomita et al (2005) *Neuron*

CaMKII and PKC are involved in stargazin phosphorylation in neurons





Tomita et al (2005) *Neuron*


AMPA receptor
TARPs
NMDA receptor
P
P
PSD95
PSD
Ca2+
CaMKII
PKC

Amino acid sequence of stargazin cytoplasmic domain

DRHKQLRATA RATDYLQASA ITRIPSYRYR
YQRRSRSSSR STEPSHSRDA SPVGVKGFNT
LPSTEISMYT LSRDPLKAAT TPTATYNSDR
DNSFLQVHNC IQKDSKDSLH ADTANRRTTPV

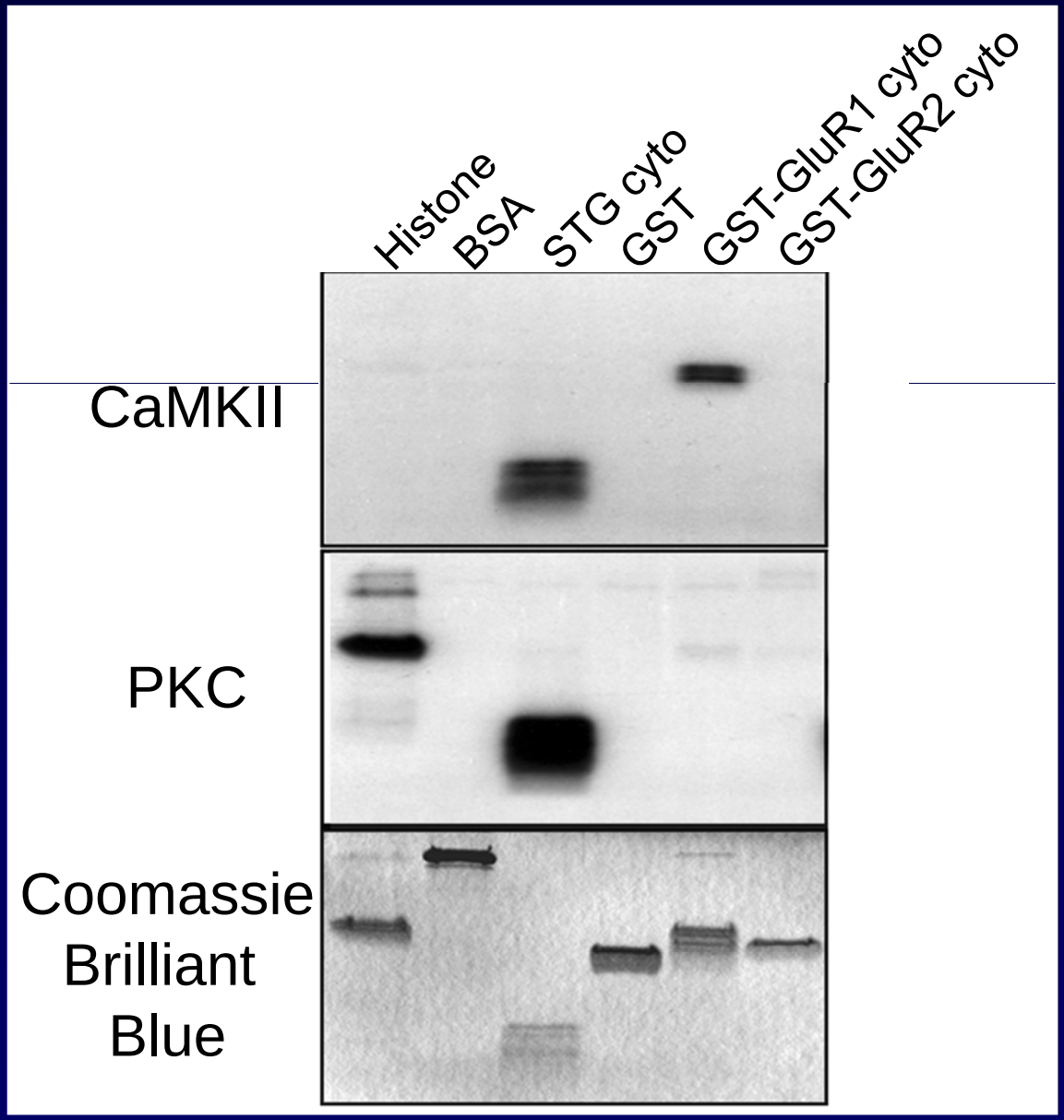
Phosphorylate recombinant proteins *in vitro*

Digest by trypsin

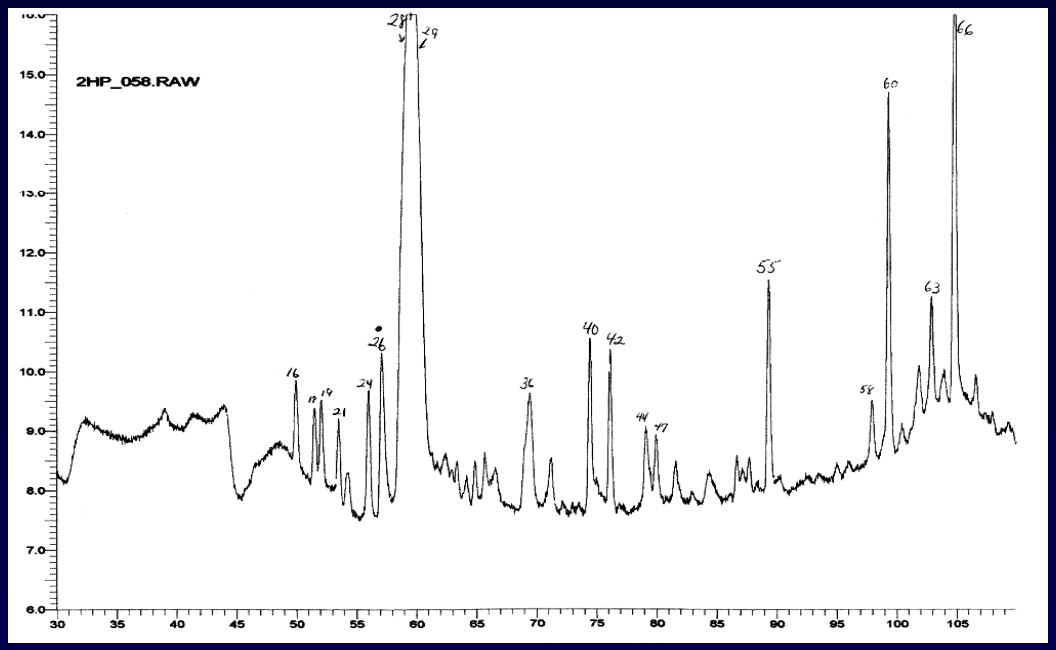
Fraction by HPLC

MASS-spec

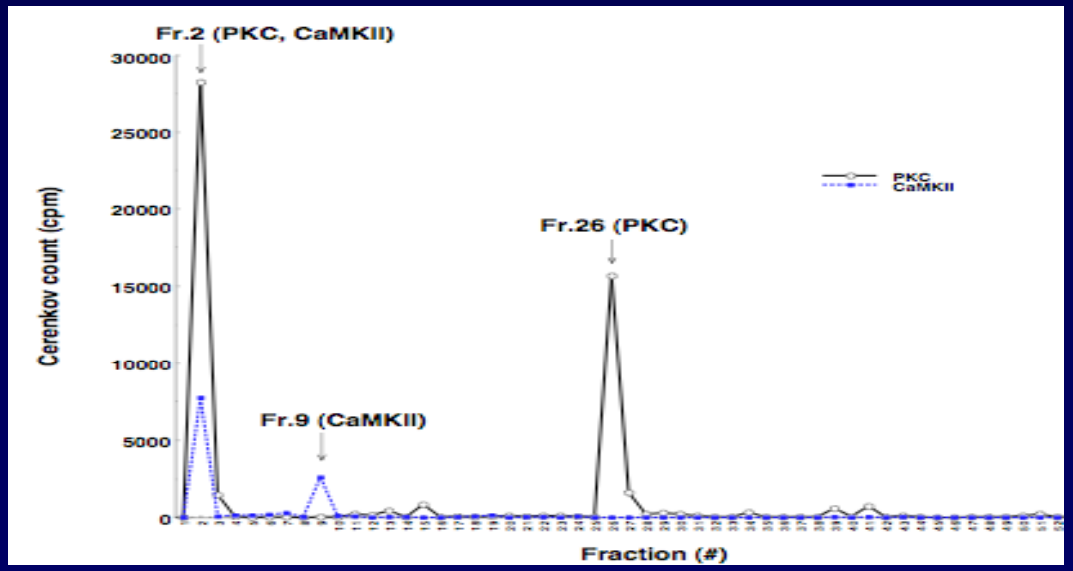
Both CaMKII and PKC directly phosphorylate stargazin cytoplasmic domain *in vitro*



HPLC

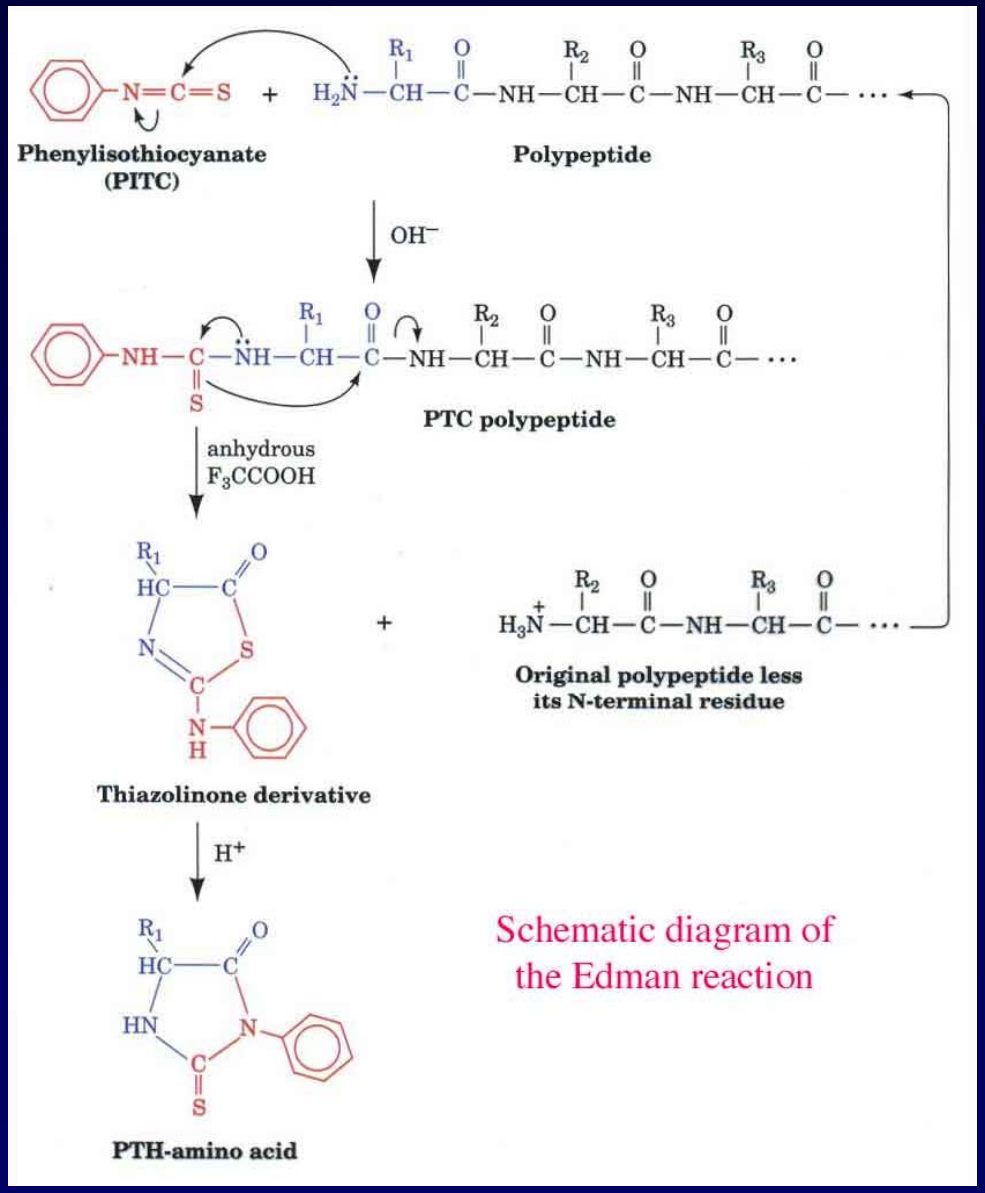


P32



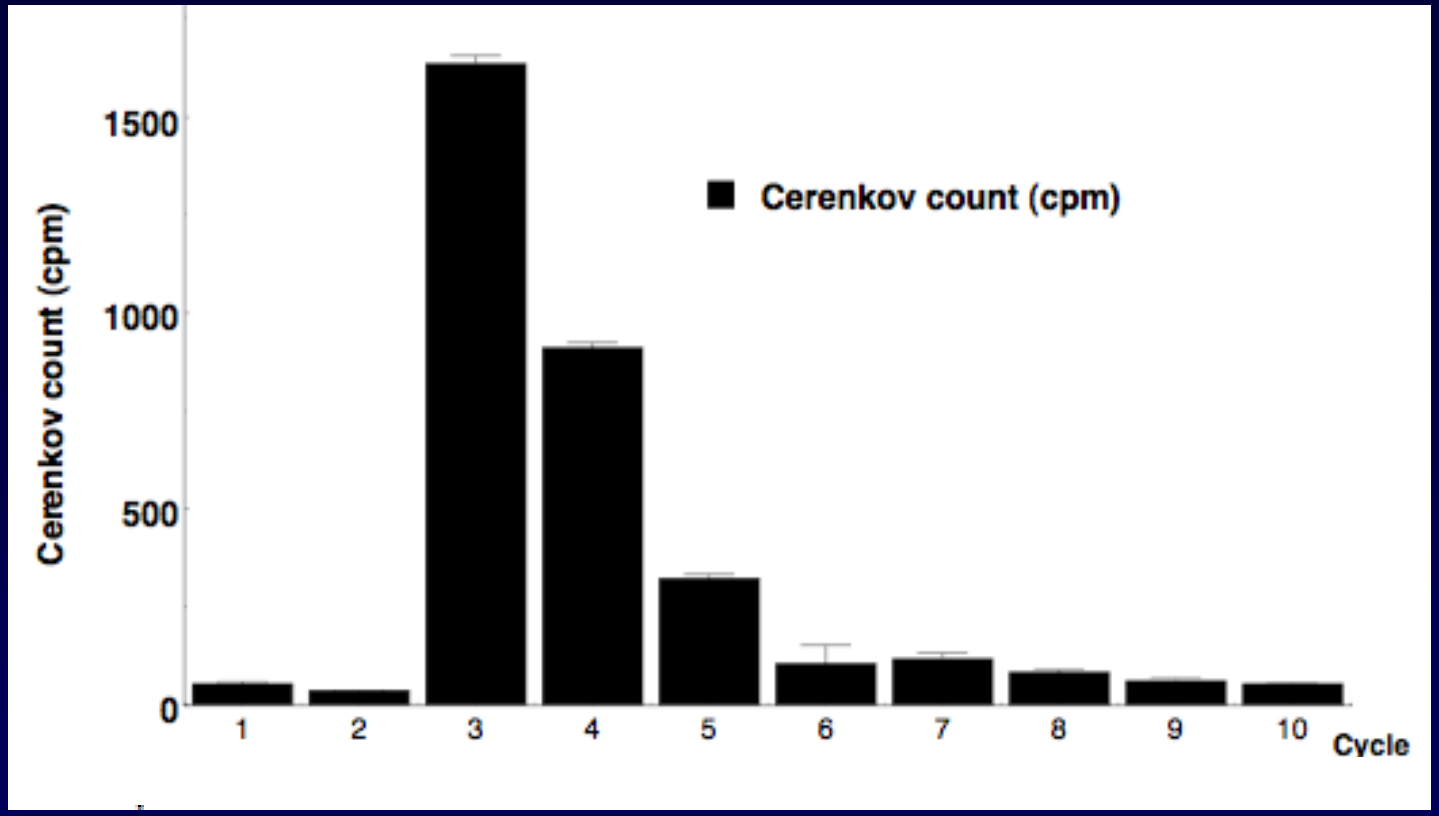
715.2638 Da, corresponding to residues 223-227 with one phosphorylation site (IPpSYR)

Radio Edman sequencing



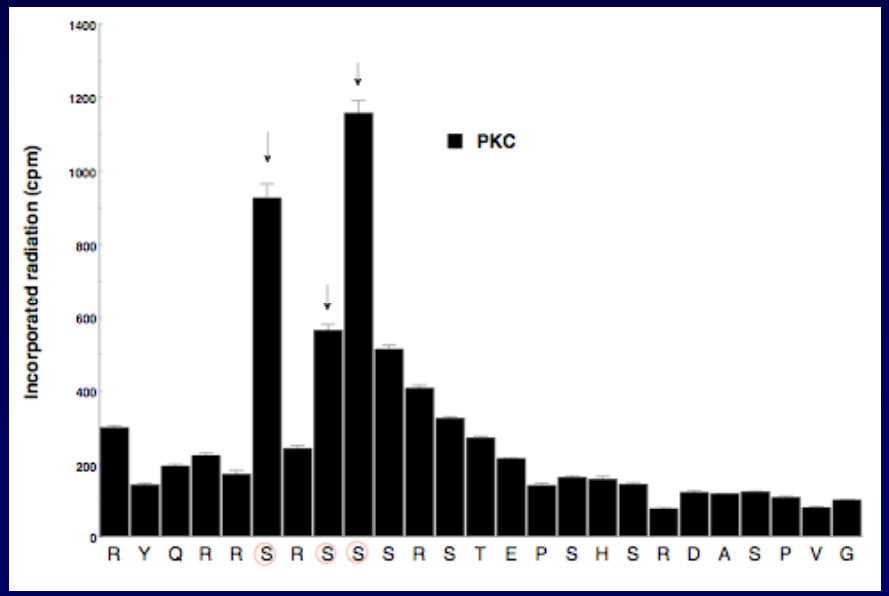
Schematic diagram of the Edman reaction

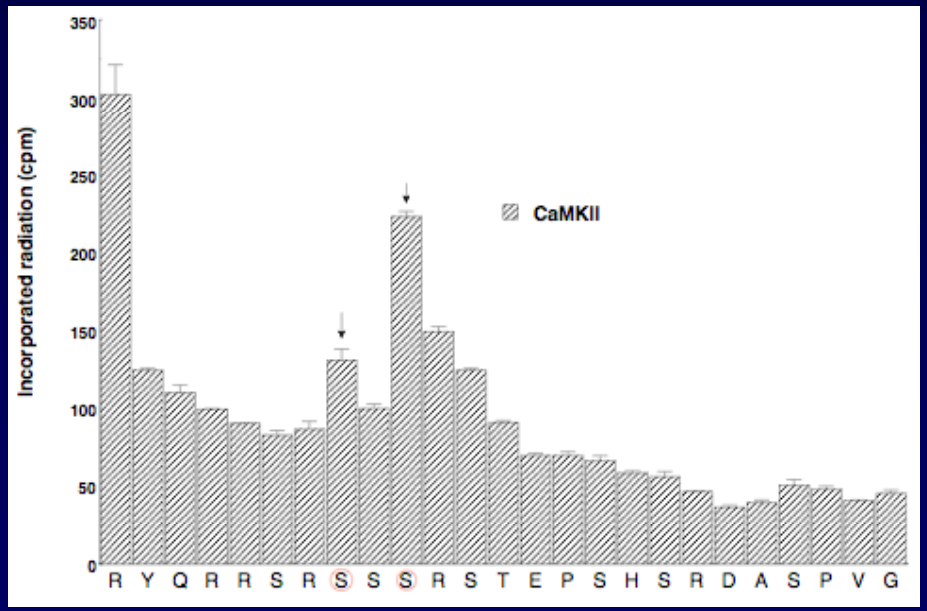
http://www2.bioch.ox.ac.uk/immunoch/PCFWebSite/Sequencing.



DRHKQLRATA RATDYLQASA ITRIPSYRYR
YQRRSRSSSR STEPSHSRDA SPVGVKGFNT
LPSTEISMYT LSRDPLKAAT TPTATYNSDR
DNSFLQVHNC IQKDSKDSLH ADTANRRTTPV

DRHKQLRATA RATDYLQASA ITRIPSYRYR
YQRRSRSSSR STEPSHSRDA SPVGVKGFNT
LPSTEISMYT LSRDPLKAAT TPTATYNSDR
DNSFLQVHNC IQKDSKDSLH ADTANRRTTPV





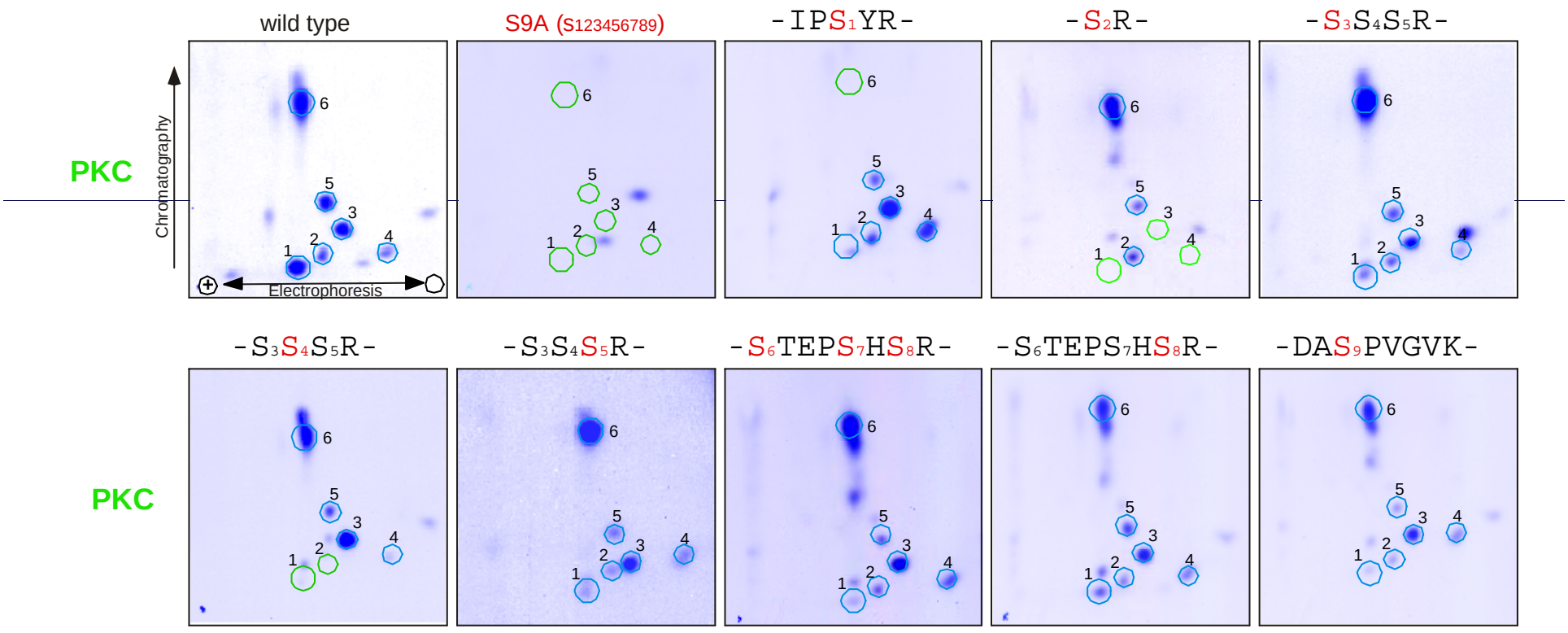
D R H K Q L R A T A R A T D Y L Q A S A I T R I P S_1 Y R Y R
Y Q R R S_2 R S_3 S_4 S_5 R S_6 T E P S_7 H S_8 R D A S_9 P V G V K G F N T
L P S T E I S M Y T L S R D P L K A A T T P T A T Y N S D R
D N S F L Q V H N C I Q K D S K D S L H A D T A N R R T T P V

Alternative approach with 2D gel

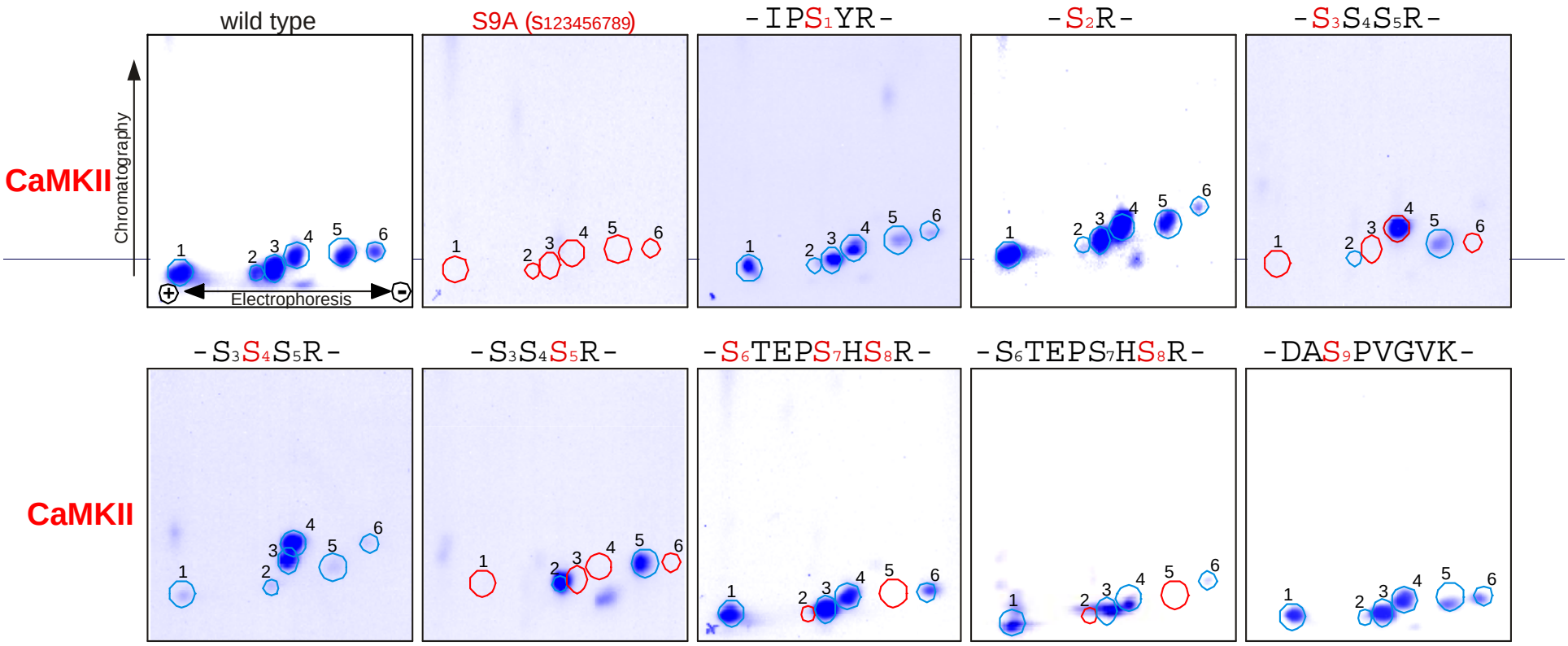
DRHKQLRATA RATDYLQASA ITRIPSYRYR
YQRRSRSSSR STEPSHSRDA SPVGVKGFNT
LPSTEISMYT LSRDPLKAAT TPTATYNSDR
DNSFLQVHNC IQKDSKDSLH ADTANRRTTPV

Purified recombinant STG-mut
In vitro phosphorylation by CaMKII and PKC
TPCK-Trypsin: K-X; R-X
2D gel (1st Electrophoresis, 2nd PLC)

PKC phosphorylation sites



CaMKII phosphorylation sites



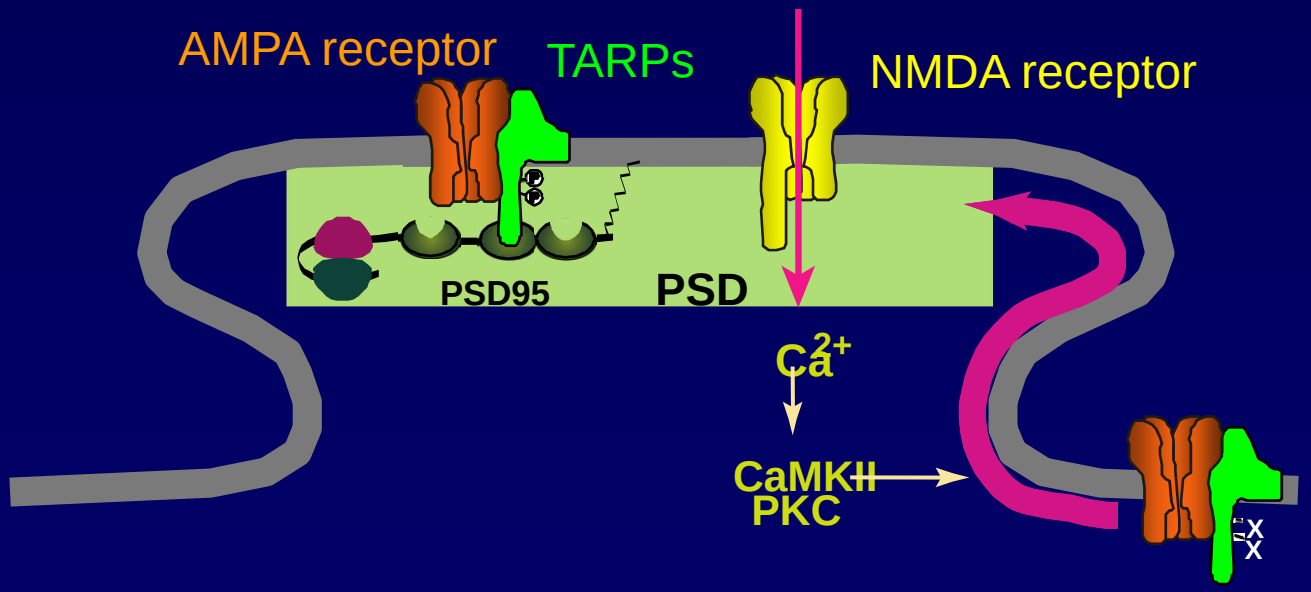
D R H K Q L R A T A	R A T D Y L Q A S A	I T R I P S_1 Y R Y R
Y Q R R S_2 R S_3 S_4 S_5 R	S_6 T E P S_7 H S_8 R D A	S_9 P V G V K G F N T
L P S T E I S M Y T	L S R D P L K A A T	T P T A T Y N S D R
D N S F L Q V H N C	I Q K D S K D S L H	A D T A N R R T T P V

Molecular Mechanisms for synaptic plasticity

Genetic mouse model

Electrophysiological analysis

Isolation of P-specific binding proteins



Acknowledgements

Tomita Lab.

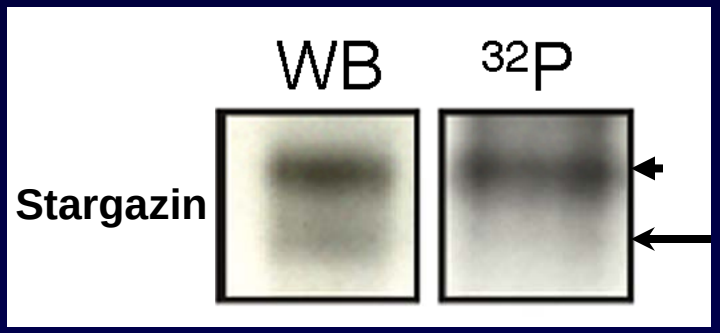
Kwang Kim

NIDA Neuroproteomics Center / KECK facility

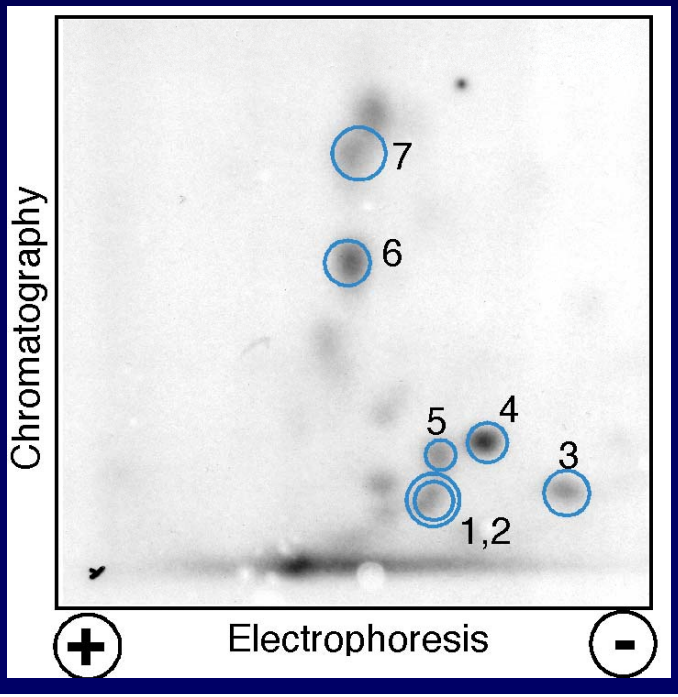
Kathryn Stone
Mary Lpresti
J. Myron Crawford

Stargazin phosphorylation in cerebro-cortical neurons

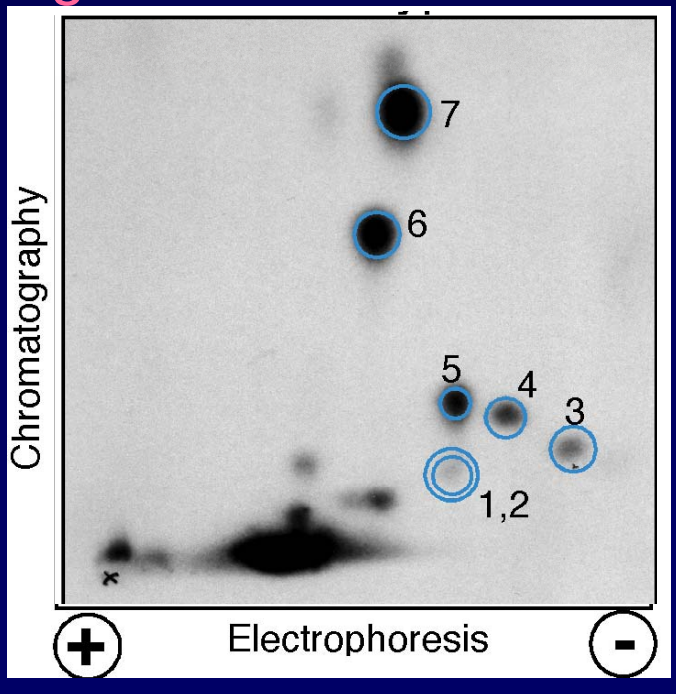
Phospho amino acid analysis



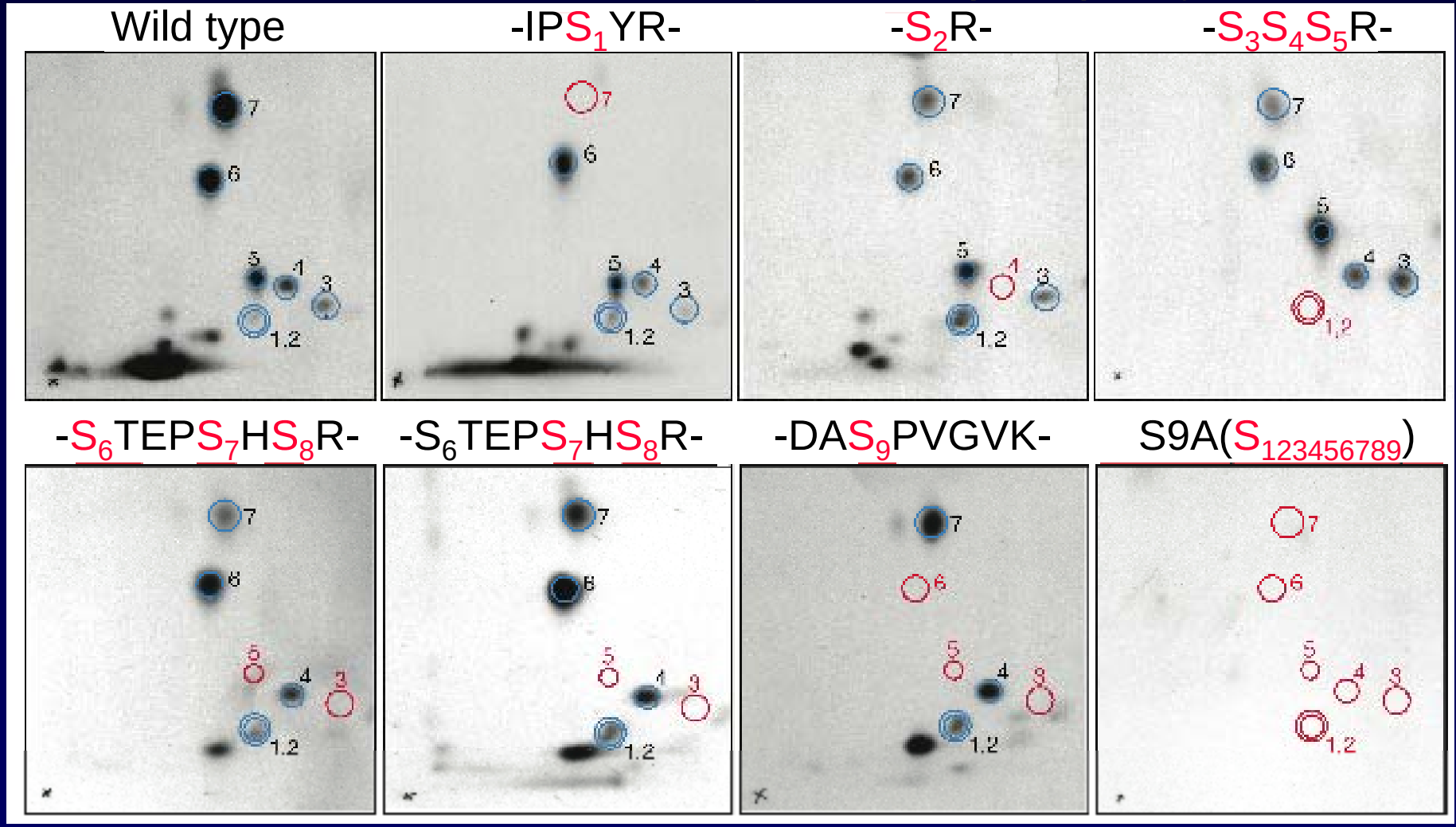
Cerebro-cortical neuron

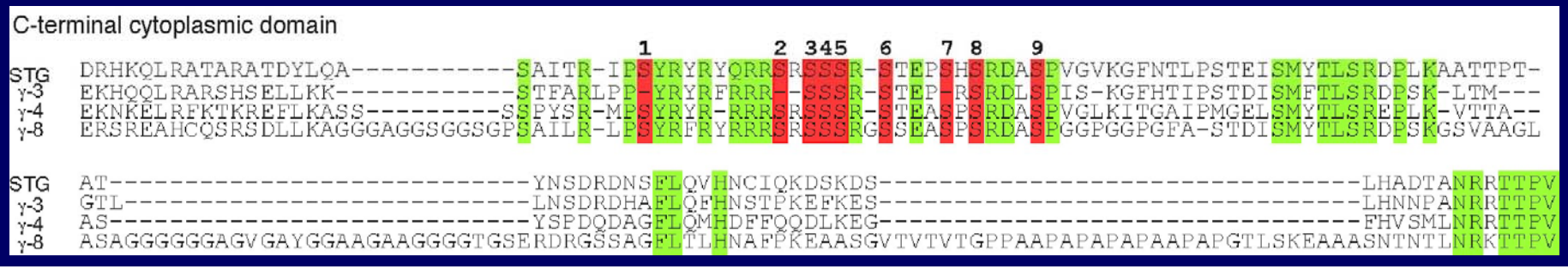


Stargazin transfected CHO cells

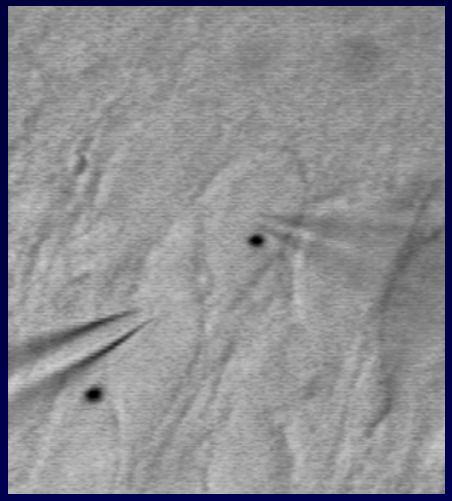


Nine serine residues in stargazin are phosphorylated.



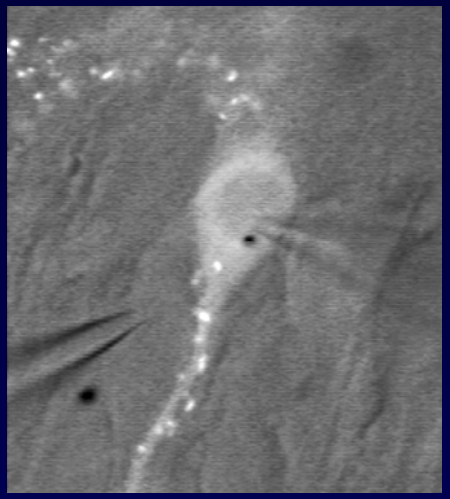


Phospho-mimic stargazin (S9D) enhances the AMPA EPSCs

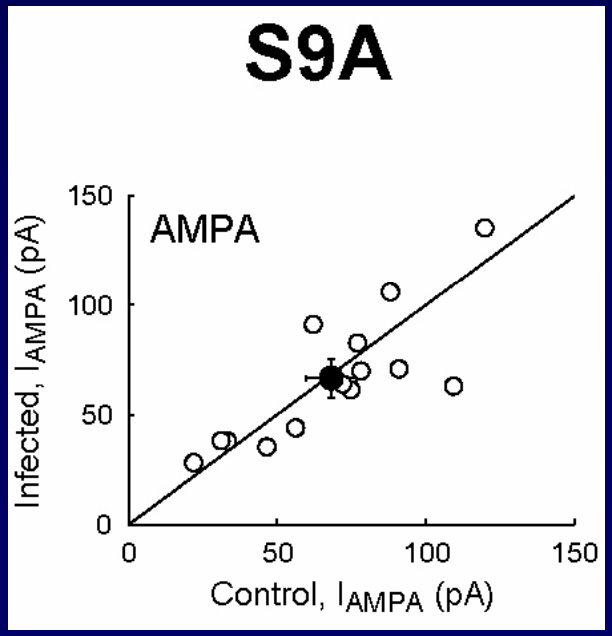


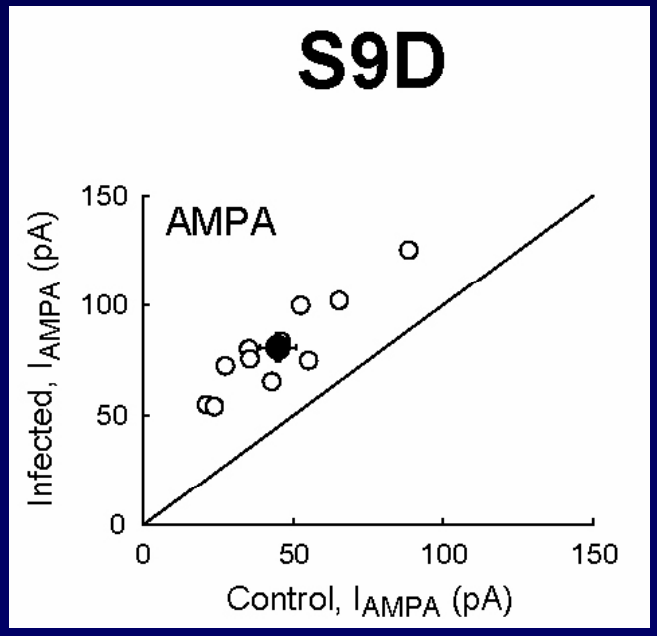
DIC

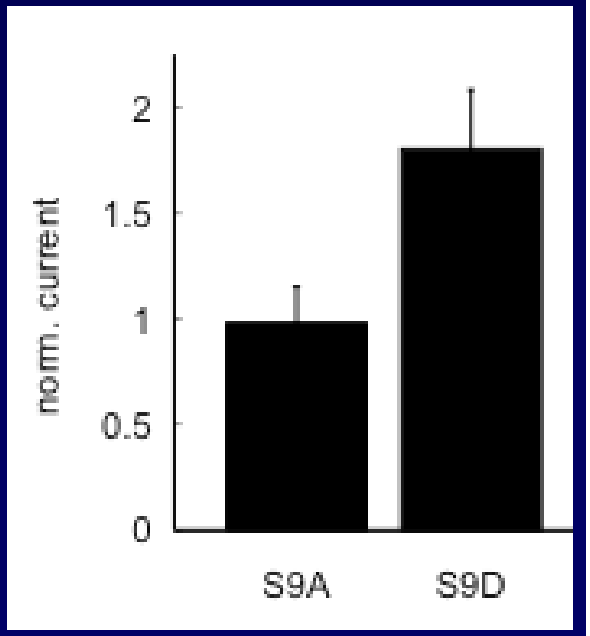
GFP-fluorescence



Overlay

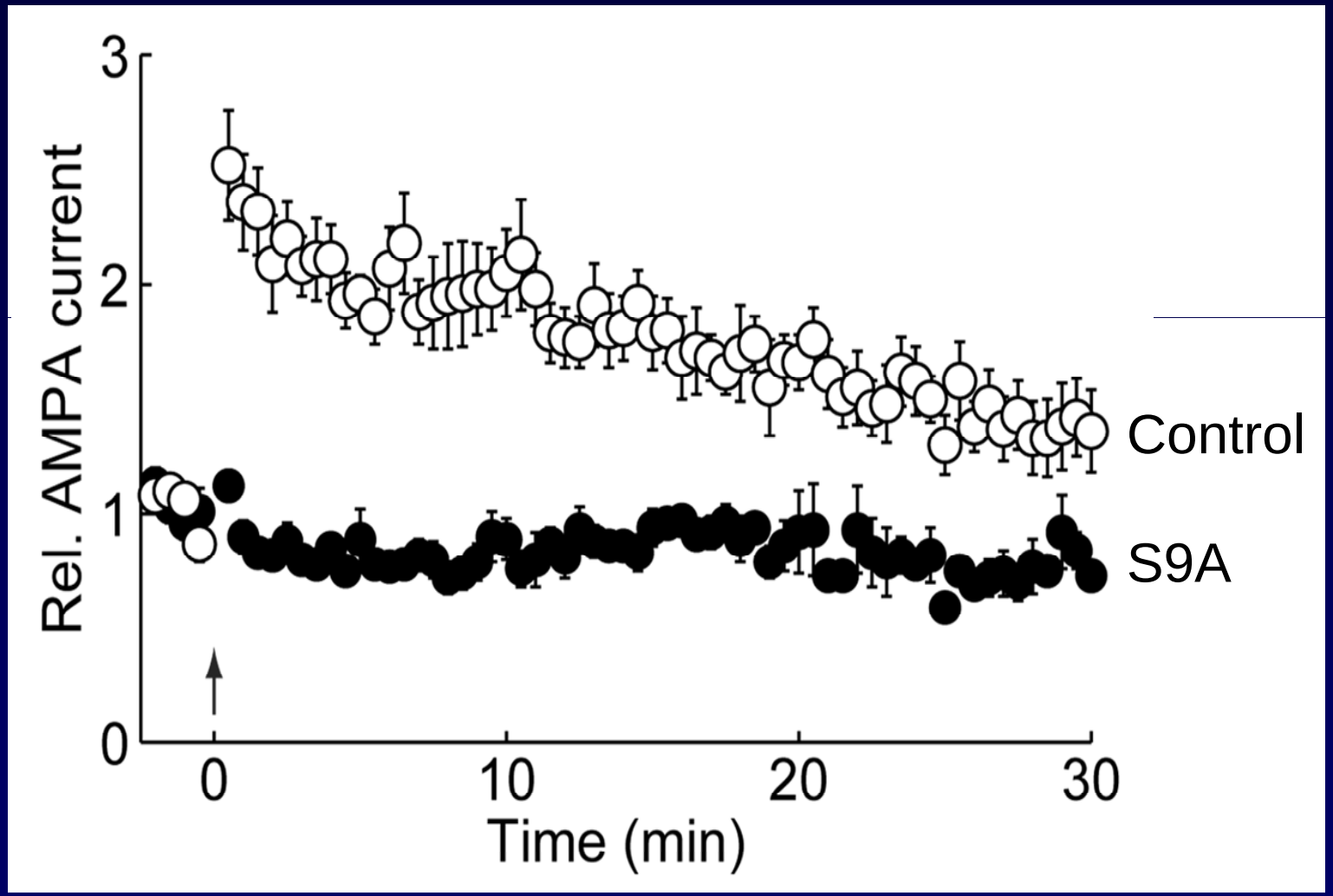






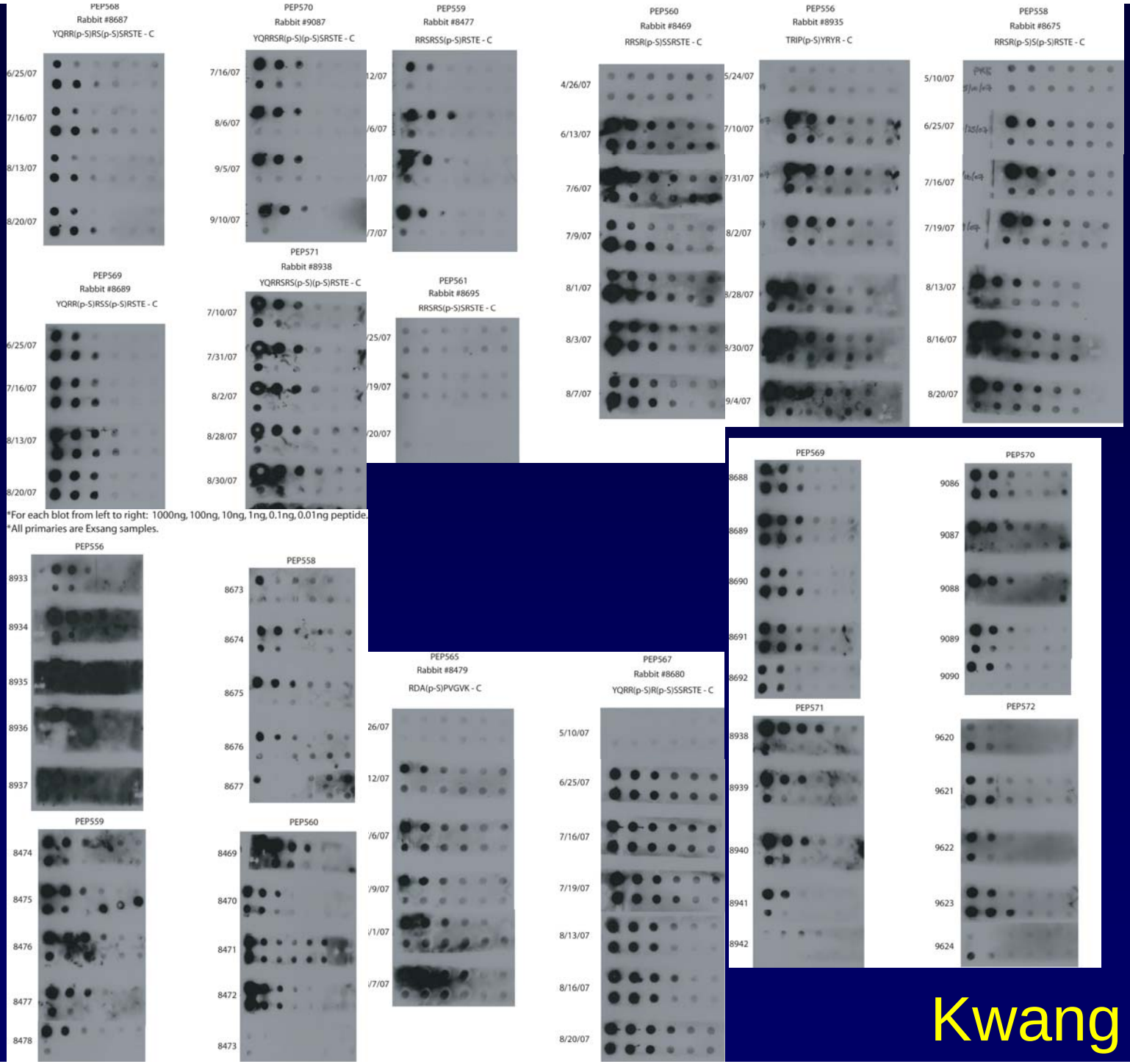
Tomita et al (2005)

Phosphorylation of stargazin is necessary for LTP



Generate P-specific antibodies

E	SEQUENCE	PROJECT NAME	PROJECT#
	TRIP(P-S)YRYR - C	PHOSPHO STARGAZIN (S22 8) PKC SITE	AB1543 5
	TRIPSYRYR - C	STARGAZIN (S22 8) PKC SITE CONTROL	AB1543 5
	RRSR(P-S)S(P-S)RSTE - C	PHOSPHO STARGAZIN (S239/241)CAMKII	AB15436-1
	RRSRSS(P-S)RSTE - C	PHOSPHO STARGAZIN (S241) CAMKII	AB15436-3
	RRSR(P-S)SSRSTE - C	PHOSPHO STARGAZIN (S239) CAMKII	AB15436-2
	RRSRS(P-S)SRSTE - C	PHOSPHO STARGAZIN (S240)PKC/CAMKII	AB15438-8
	RRSRSSSRSTE - C	STARGAZIN (S239/240/241) CAMKII-CONTROL	AB15436
	YQRRSRSSSRSTE - C	STARGAZIN (S237/239/240/241) PKC/CAMKII - UNMODIFIED CONTROL	AB15438
	RDA(P-S)PVGVK - C	PHOSPHO STARGAZIN (S253)OTHER KINASE	AB15437
	RDAS PVGVK - C	PHOSPOSTARGAZIN (S253) OTHER KINASE-CONTROL	AB15437
	YQRR(PS)R(PS)SSRSTE - C	STARGAZIN (S237/239) PKC/CAMKII	AB15438-2
	YQRR(PS)RS(PS)SRSTE - C	STARGAZIN (S237/240) PKC/CAMKII	AB15438-3
	YQRR(PS)RSS(PS)RSTE - C	STARGAZIN (S237/241) PKC/CAMKII	AB15438-4
	YQRRSR(PS)(PS)SRSTE - C	STARGAZIN (S239/240) PKC/CAMKII	AB15438-5
	YQRRSRS(PS)(PS)RSTE - C	STARGAZIN (S240/241) PKC/CAMKII	AB15438-6
	YQRR(PS)RSSSRSTE - C	STARGAZIN (S237) PKC/CAMKII	AB15438-7


PEP568
Rabbit #8687
YQRR(p-S)RS(p-S)SRSTE - C
6/25/07
7/16/07
8/13/07
8/20/07
PEP570
Rabbit #9087
YQRRSR(p-S)(p-S)SRSTE - C
7/16/07
8/6/07
9/5/07
9/10/07
PEP559
Rabbit #8477
RRSRSS(p-S)RSTE - C
PEP560
Rabbit #8469
RRSR(p-S)SSRSTE - C
4/26/07
6/13/07
7/6/07
7/9/07
8/1/07
8/3/07
8/7/07
PEP556
Rabbit #8935
TRIP(p-S)YRYR - C
5/24/07
7/10/07
7/31/07
8/2/07
8/28/07
8/30/07
9/4/07
PEP558
Rabbit #8675
RRSR(p-S)S(p-S)RSTE - C
5/10/07
6/25/07
7/16/07
7/19/07
8/13/07
8/16/07
8/20/07
PEP569
Rabbit #8689
YQRR(p-S)RSS(p-S)RSTE - C
6/25/07
7/16/07
8/13/07
8/20/07
PEP571
Rabbit #8938
YQRRSRS(p-S)(p-S)RSTE - C
7/10/07
7/31/07
8/2/07
8/28/07
8/30/07
PEP561
Rabbit #8695
RRSRS(p-S)SRSTE - C
*For each blot from left to right: 1000ng, 100ng, 10ng, 1ng, 0.1ng, 0.01ng peptide.
*All primaries are Exsang samples.
PEP556
8933
8934
8935
8936
8937
PEP558
8673
8674
8675
8676
8677
PEP559
8474
8475
8476
8477
8478
PEP560
8469
8470
8471
8472
8473
PEP565
Rabbit #8479
RDA(p-S)PVGVK - C
PEP567
Rabbit #8680
YQRR(p-S)R(p-S)SSRSTE - C
5/10/07
6/25/07
7/16/07
7/19/07
8/13/07
8/16/07
8/20/07
PEP569
8688
8689
8690
8691
8692
PEP570
9086
9087
9088
9089
9090
PEP571
8938
8939
8940
8941
8942
PEP572
9620
9621
9622
9623
9624

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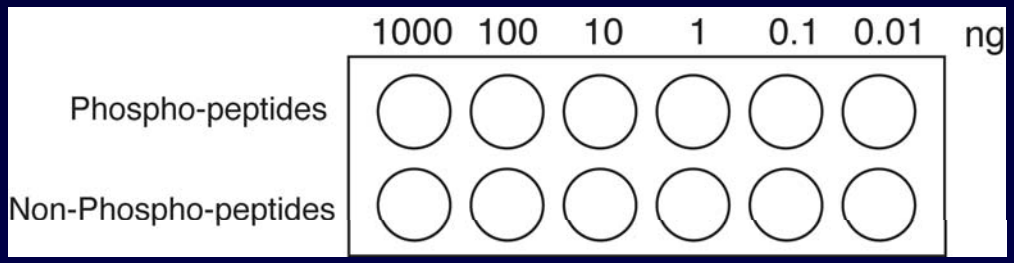
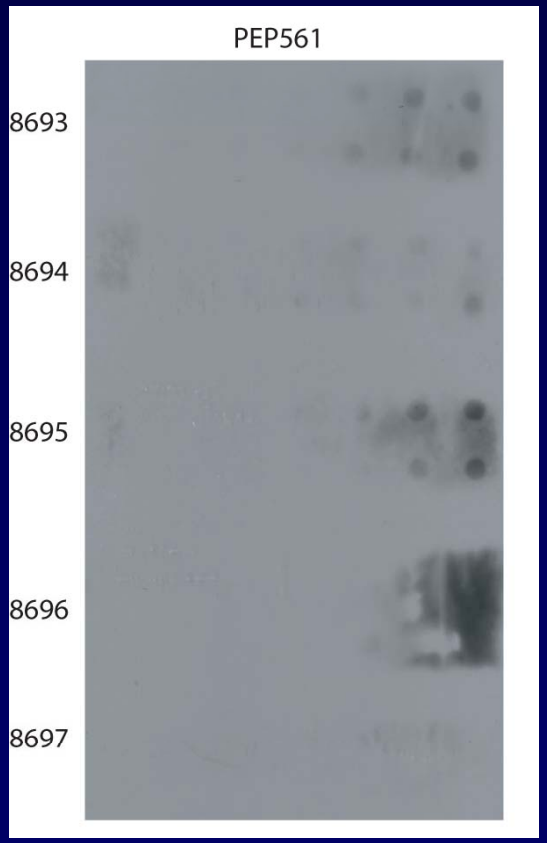
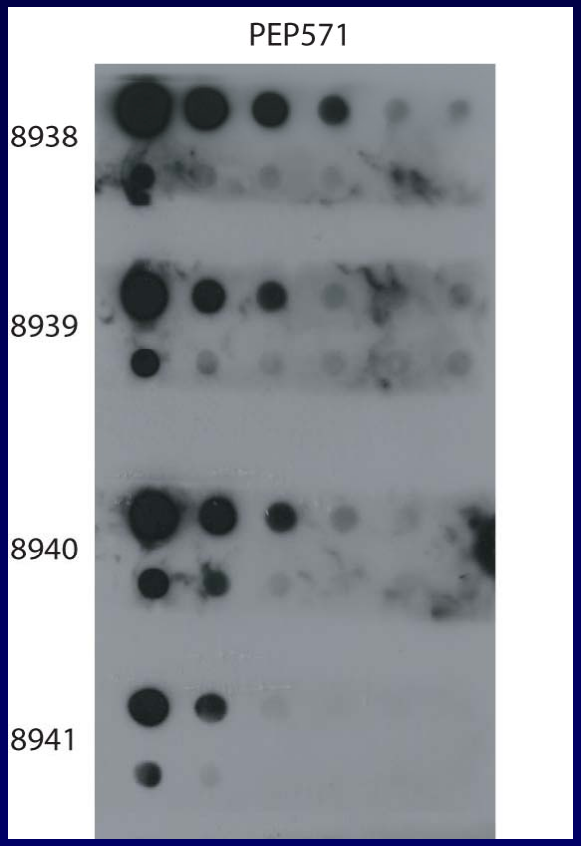
Dot blotting with P- & non-P peptides







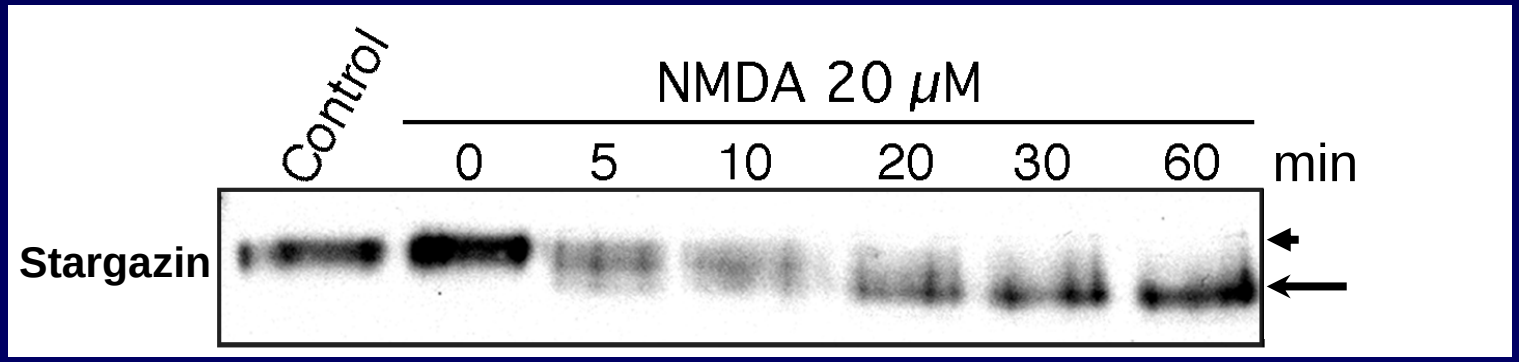
Kwang Kim

9 sites x (A or D) = 2^9 = 512 ……

Categorize each sites by kinases.

2 classes (total 9 sites) x (A or D) = 4 !

NMDA induces dephosphorylation of stargazin



Tomita et al (2005)