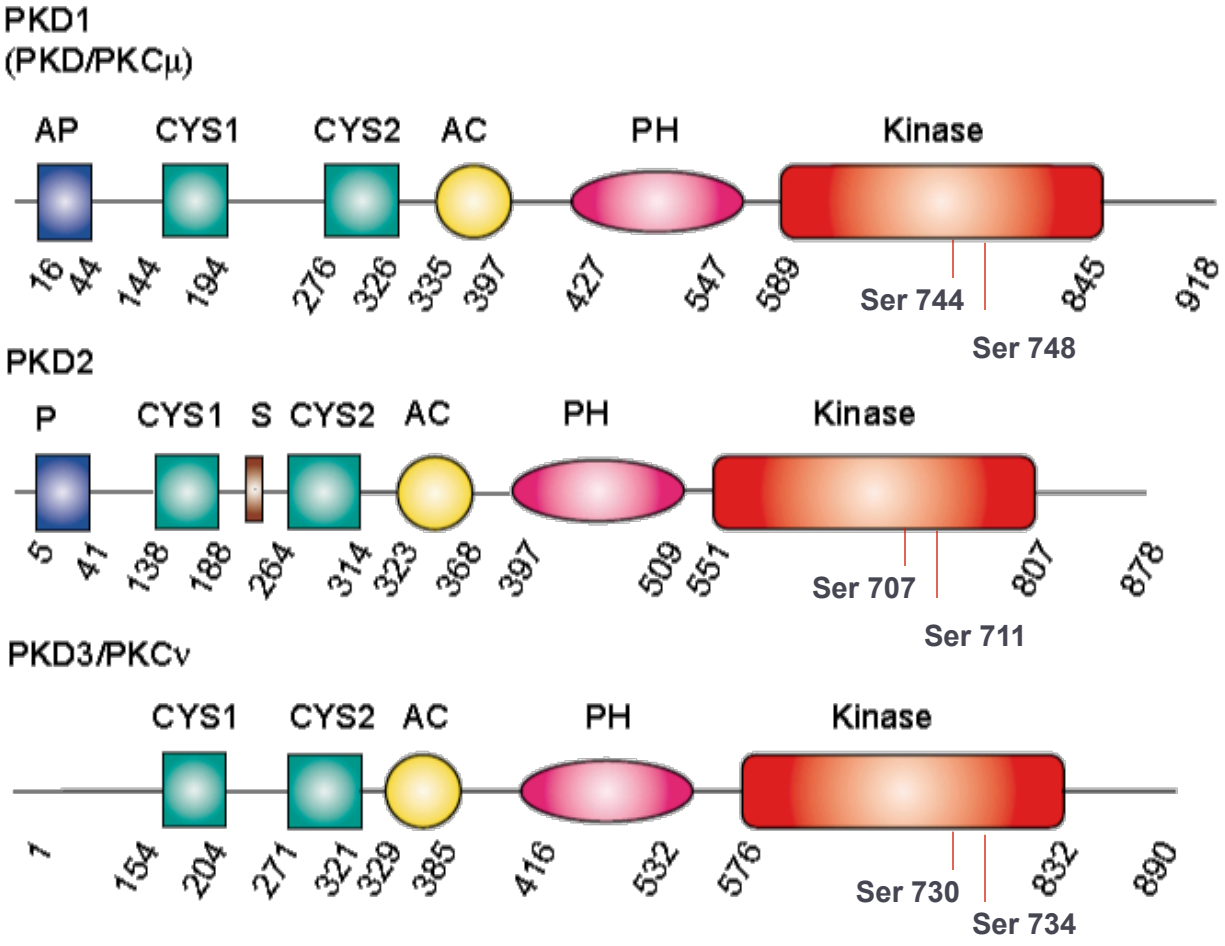
# Synthesis and Biological Evaluation of Protein Kinase D Inhibitors

Celeste Alverez
Topic Seminar
October 26, 2013

# Protein Kinase D (PKD)

- A novel family of serine/threonine kinases and diacylglycerol (DAG) receptors belonging to the $Ca^{2+}$/ calmodulin-dependent kinase (CaMK) superfamily
- Composed of 3 isoforms:
  - PKD1 (PKC $\mu$ )
  - PKD2
  - PKD3 (PKC $\nu$ )

# PKD Structure






van Lint, J., *TRENDS Cell Biol.*, **2002**, *12*, 193.
Fu, Y. *EMBO Reports*, **2011**, *12*, 785.

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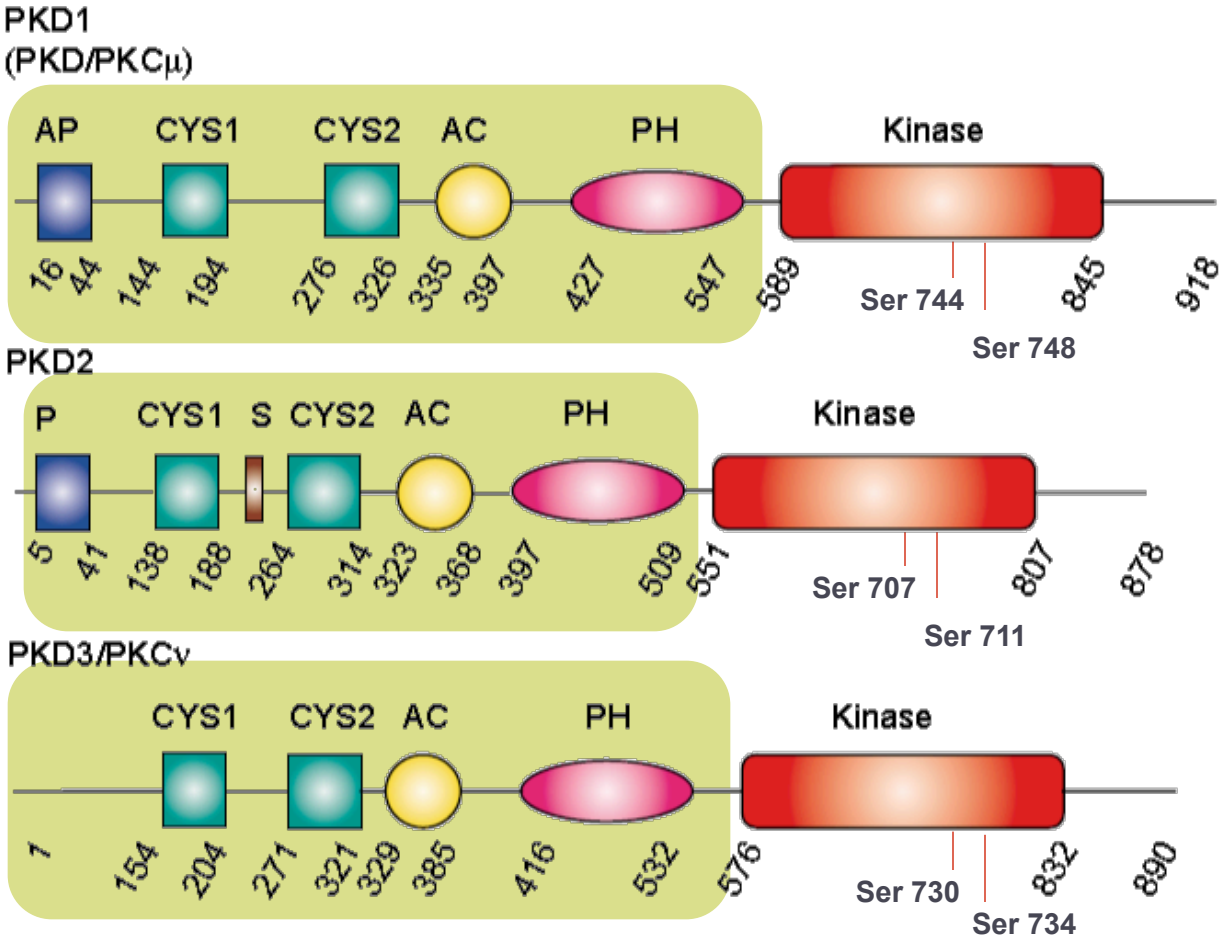




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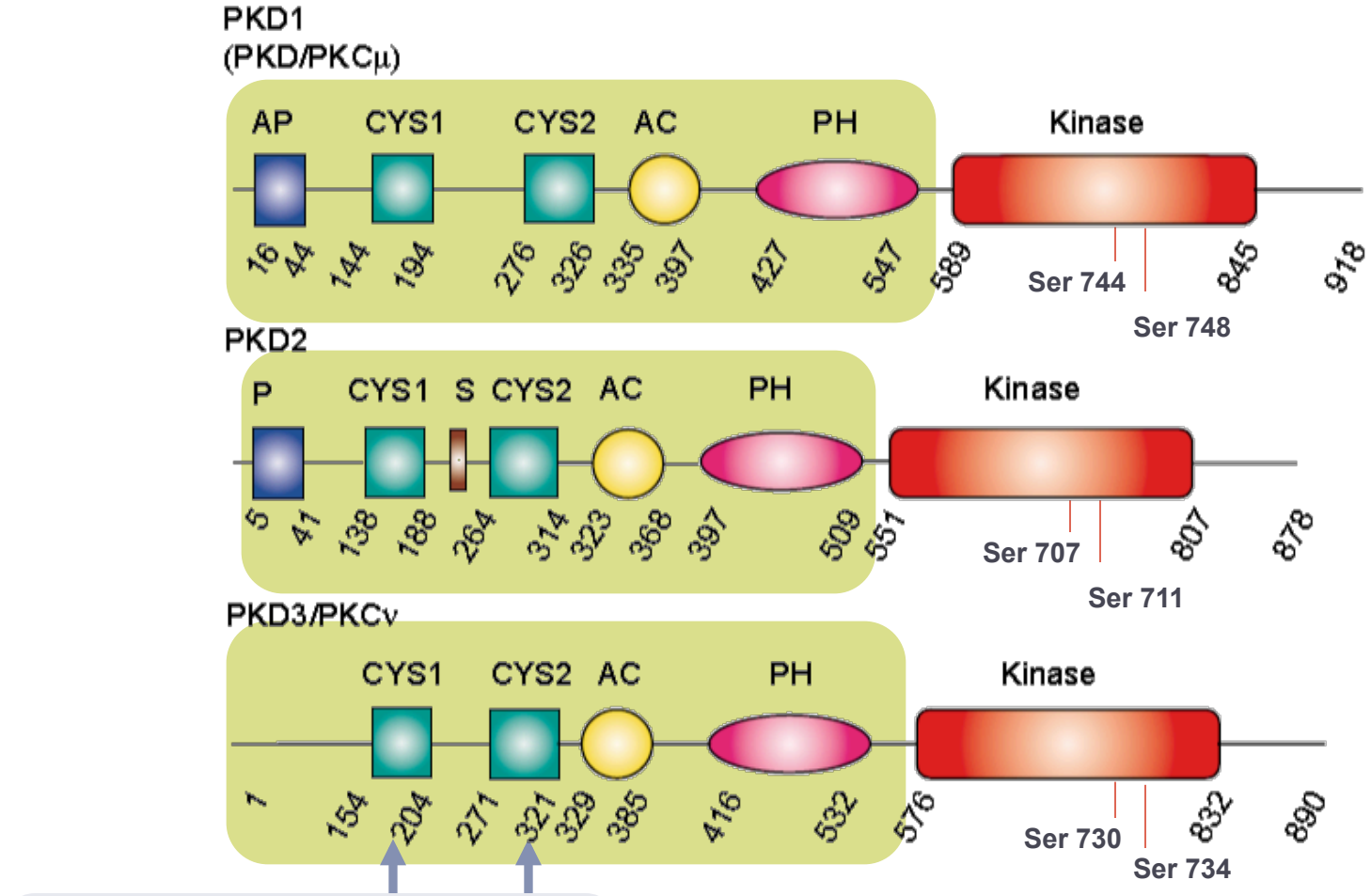
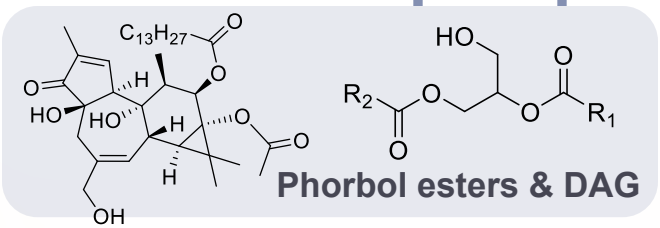
# PKD Structure



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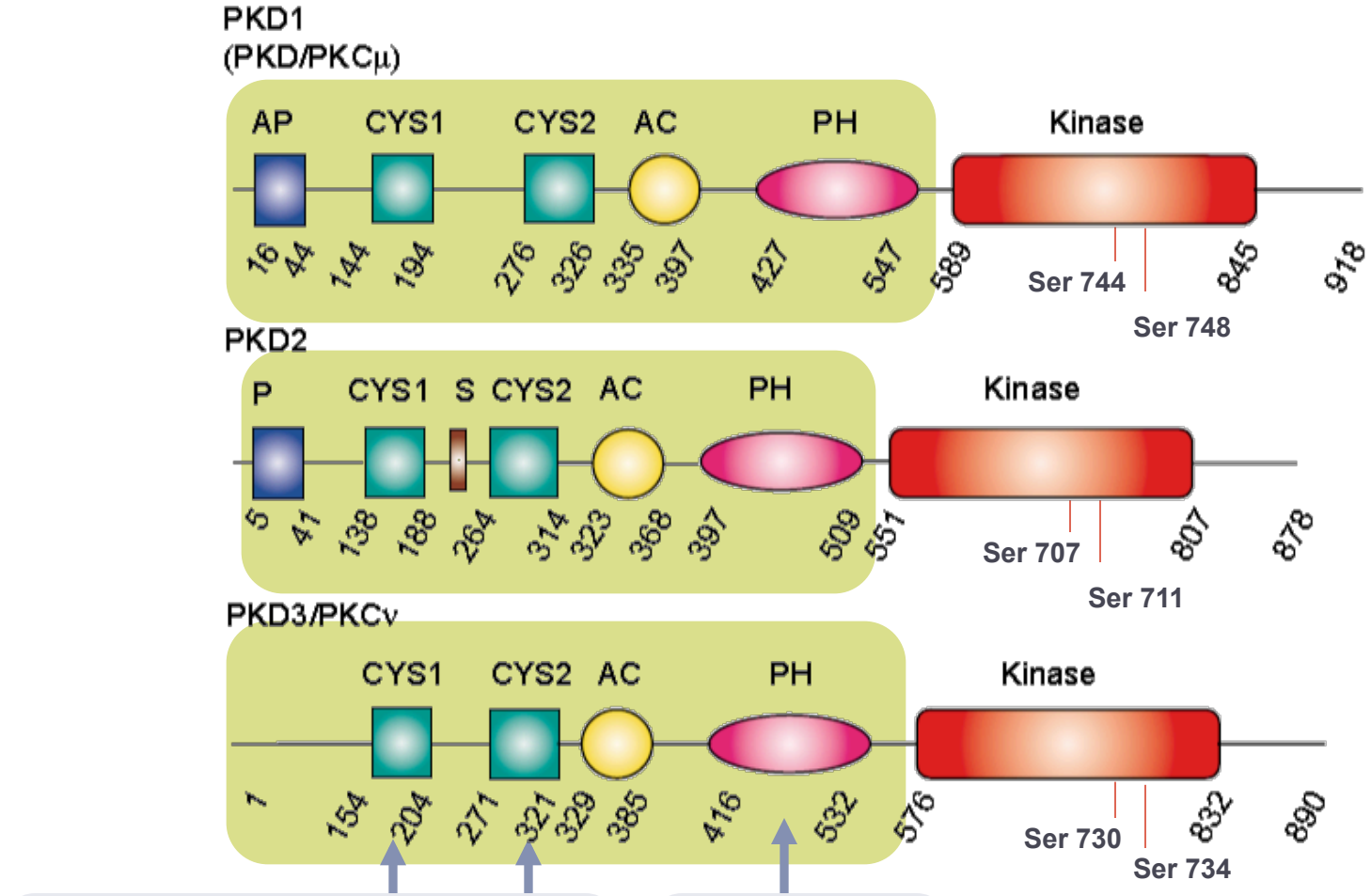
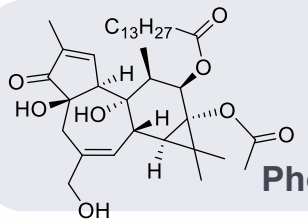
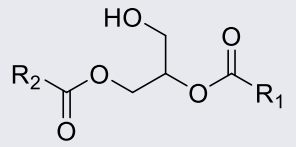
# PKD Structure




Phorbol esters & DAG

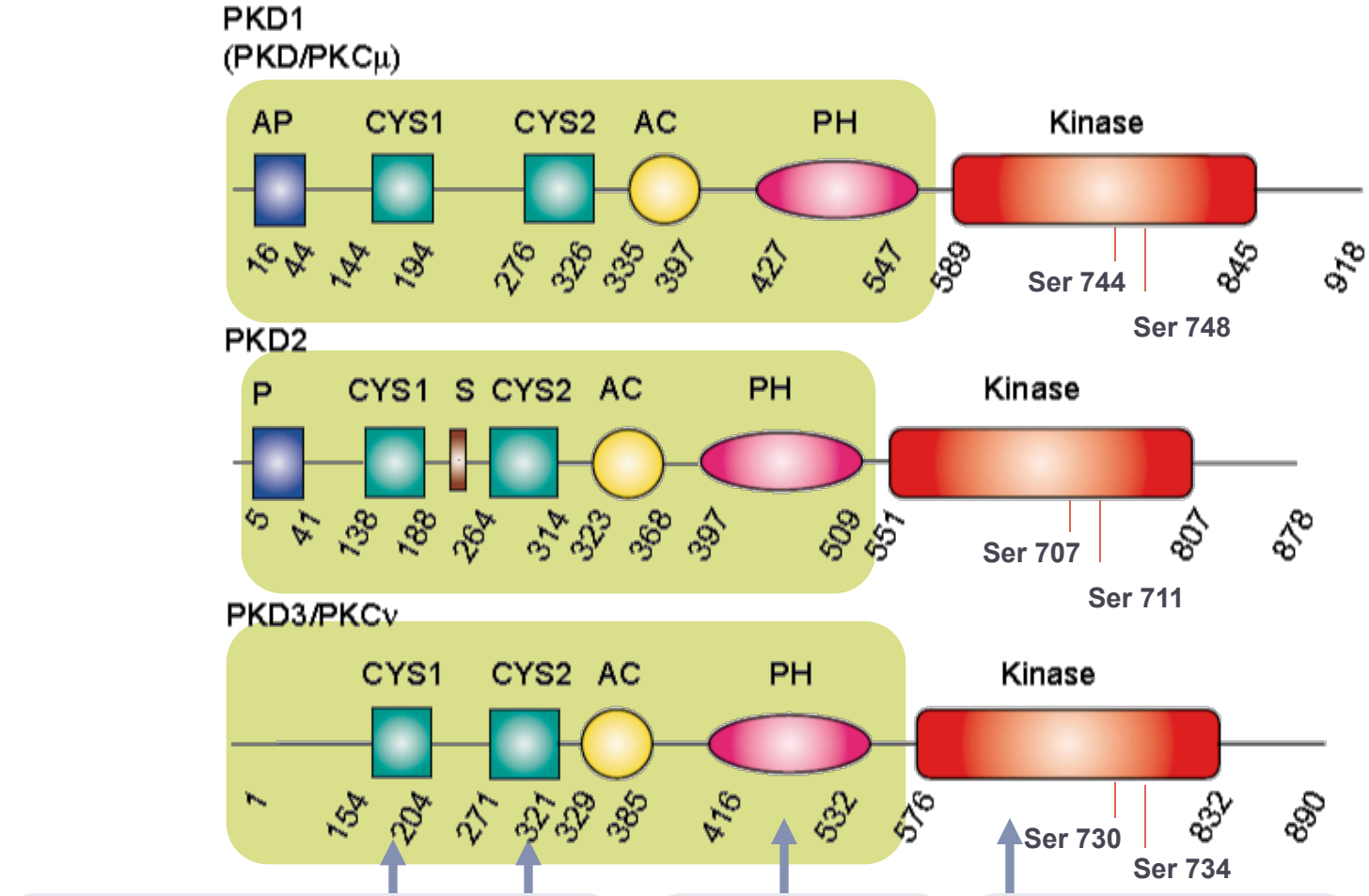
**Protein-Protein Interactions**

**Autoinhibition**



van Lint, J., *TRENDS Cell Biol.*, **2002**, *12*, 193.
Fu, Y. *EMBO Reports*, **2011**, *12*, 785.

# PKD Structure

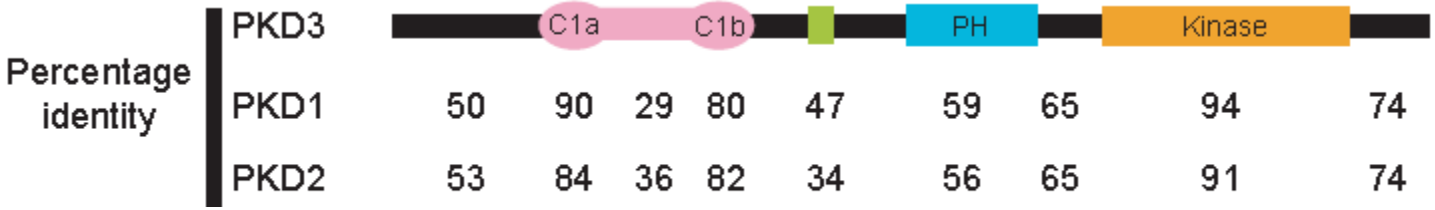





van Lint, J., *TRENDS Cell Biol.*, **2002**, *12*, 193.
Fu, Y. *EMBO Reports*, **2011**, *12*, 785.

# Isoform Similarity

- PKD1 and PKD2 have 69% overall identity
  - 91% identity at the kinase domain
- PKD1 and PKD3 have 70% overall identity
  - 94% identity at the kinase domain
- PKD2 and PKD3 have 68% overall identity
  - 91% identity at the kinase domain



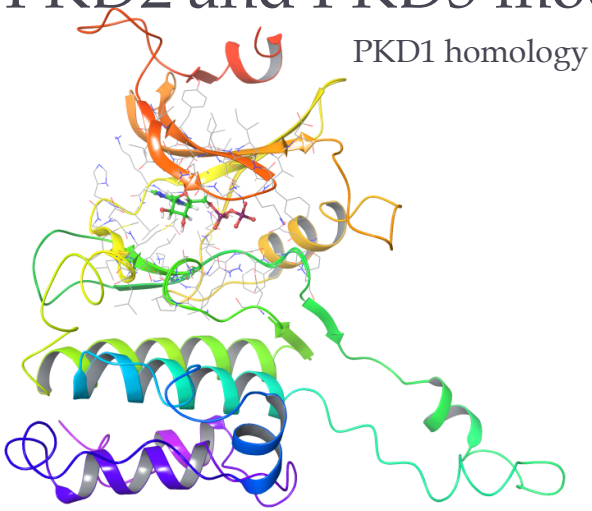



Wang, Q. J. *TRENDS Pharmacol. Sci.*, **2006**, *27*, 317.
Altschul S.F., *J. Mol. Biol.*, **1990**, *215*, 403.

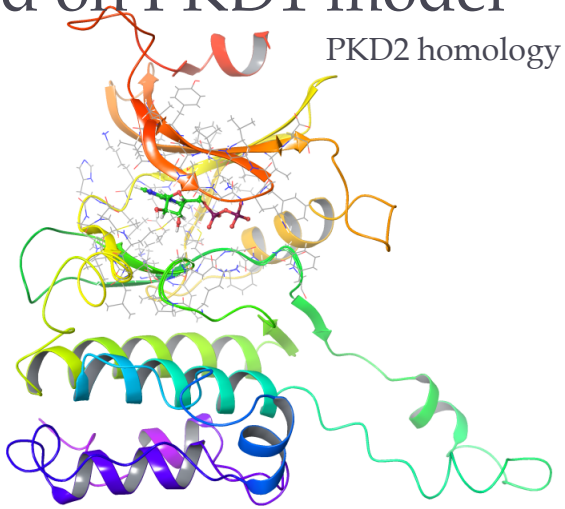
# PKD Homology modeling

- Necessary due to lack of crystal structure
- Kinase domain homology model was generated for PKD1, PKD2, and PKD3
  - PKD1 and PKD2 focused on
- PKD1 model based on crystal structures of CHK2
  - 39% homology
- PKD2 and PKD3 models based on PKD1 model

PKD1 homology model



PKD2 homology model

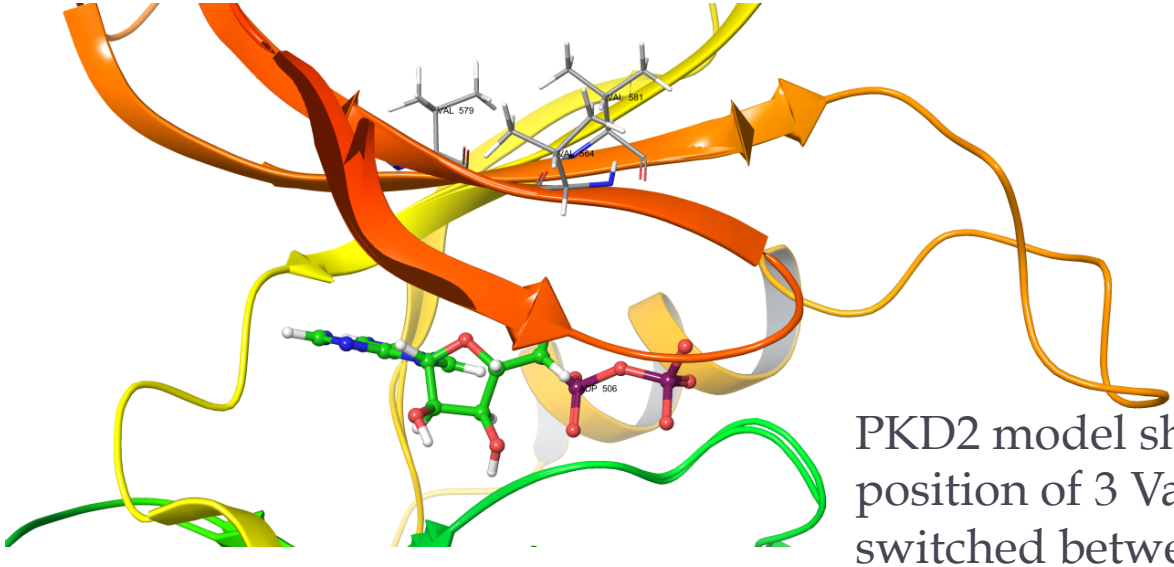




Kim Haas unpublished work.

# PKD Homology modeling

- Key difference between PKD1 and PKD2 in the ATP binding pocket, near the Gly rich loop there are 3 Ile to Val residues switched
- Key difference between PKD2 to PKD3 in the ATP binding pocket, there is only one of the Ile to Val residues switched

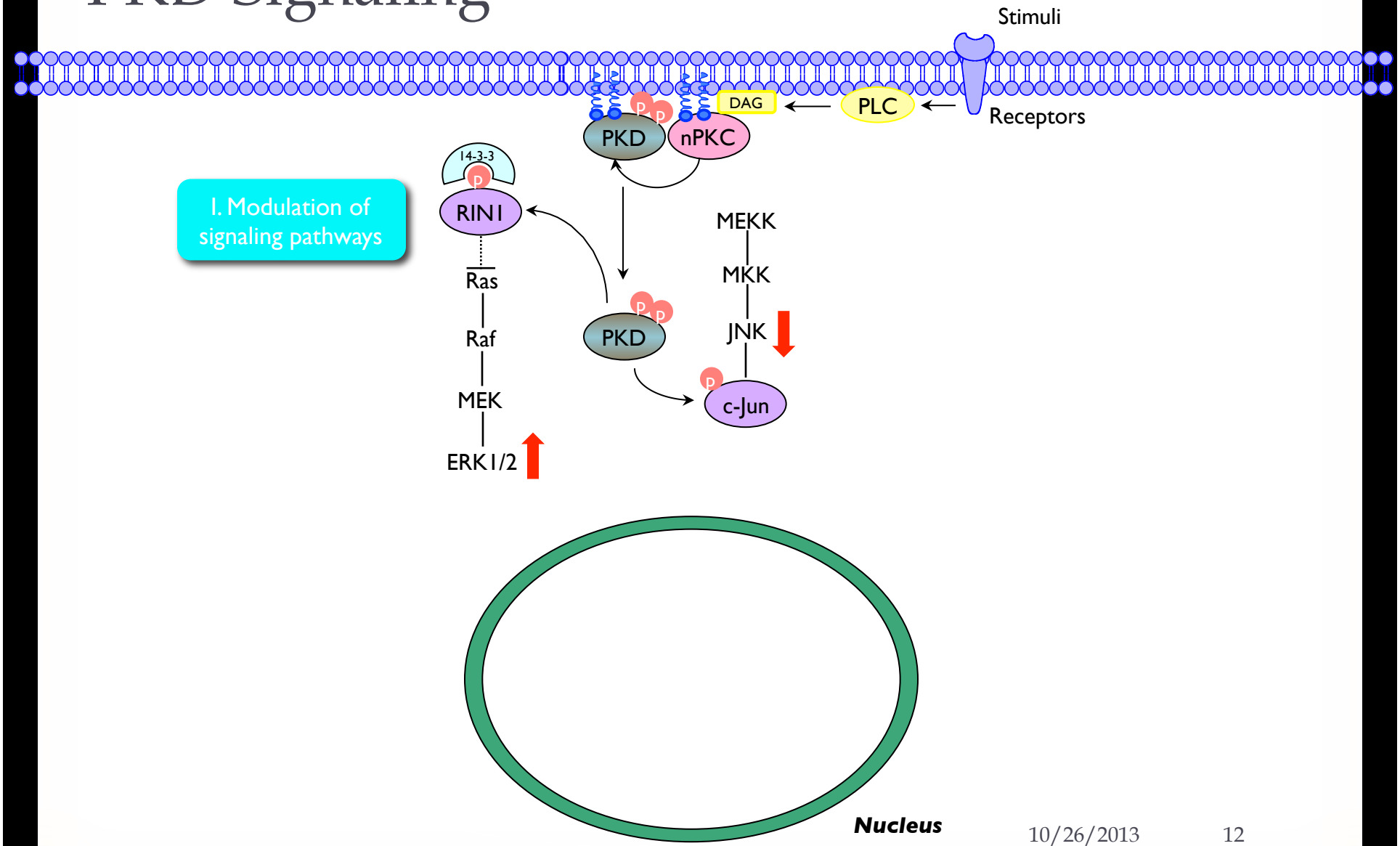



PKD2 model showing position of 3 Val residues switched between PKD1 and PKD2

# Roles of PKD

- Under normal physiological conditions
  - Apoptosis
  - Proliferation
  - Survival
  - Cell motility
  - Gene transcription
  - Cell signaling
  - Secretion/cellular trafficking
  - Immune response
- Abnormal regulation
  - Implicated in various cancers
    - Prostate, pancreatic, head and neck, colon, breast, ect.
  - Over activity promotes angiogenesis and metastasis in tumors
  - Cardiac hypertrophy

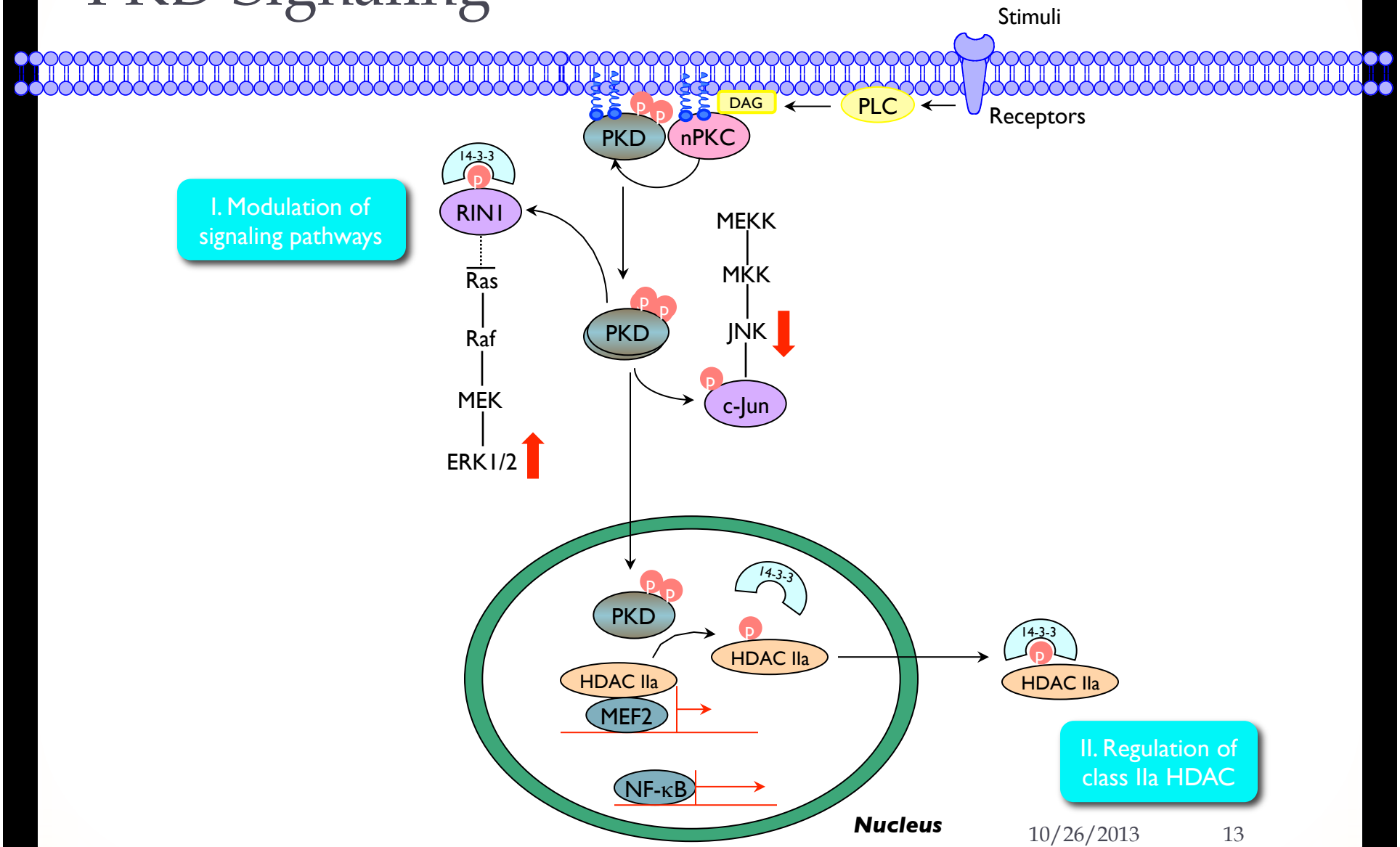
# PKD Signaling






Adapted from Kara George-Rosenker by Wang, Q. J. *TRENDS Pharmacol. Sci.*, **2006**, *27*, 317.

# PKD Signaling



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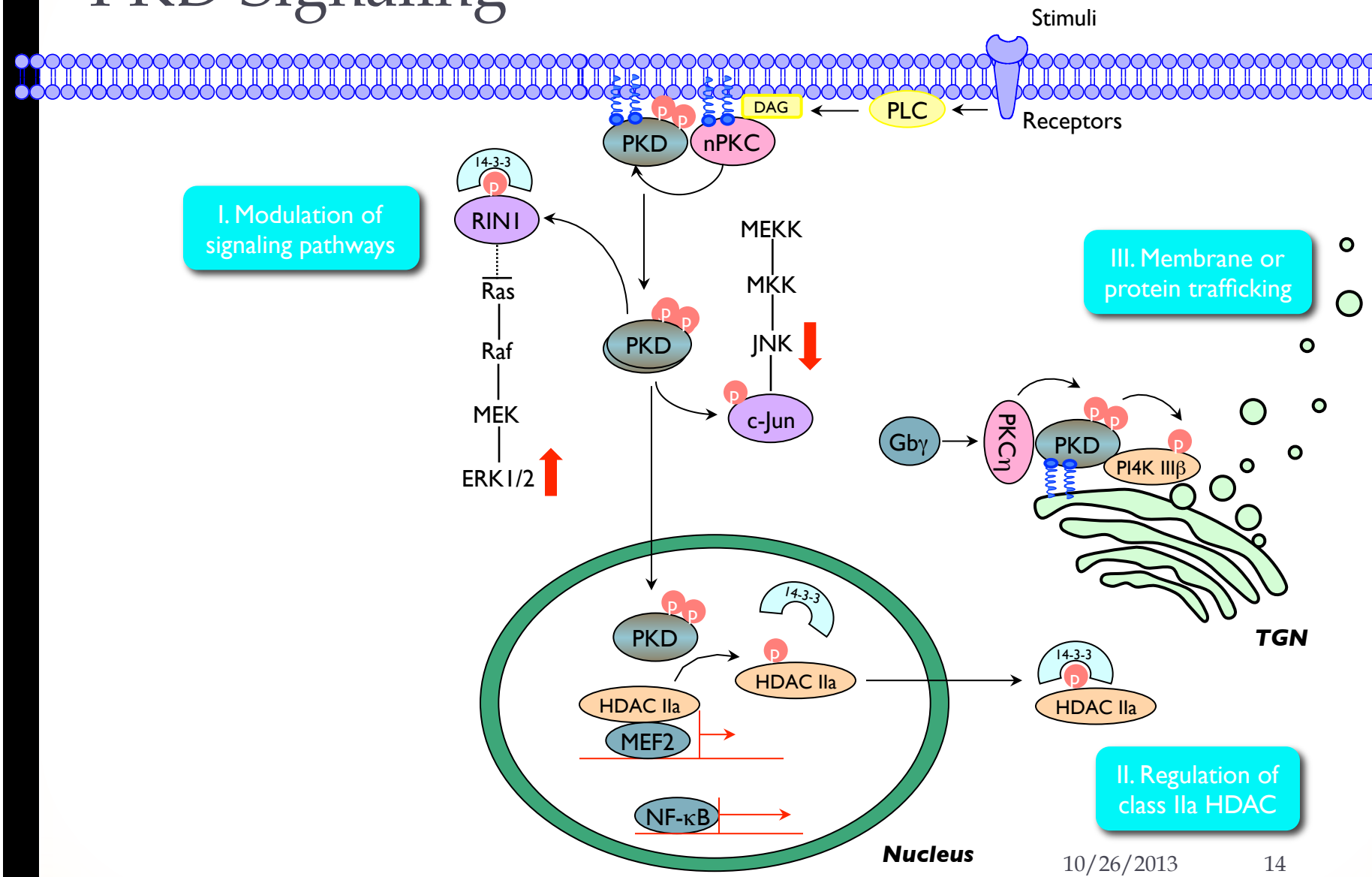




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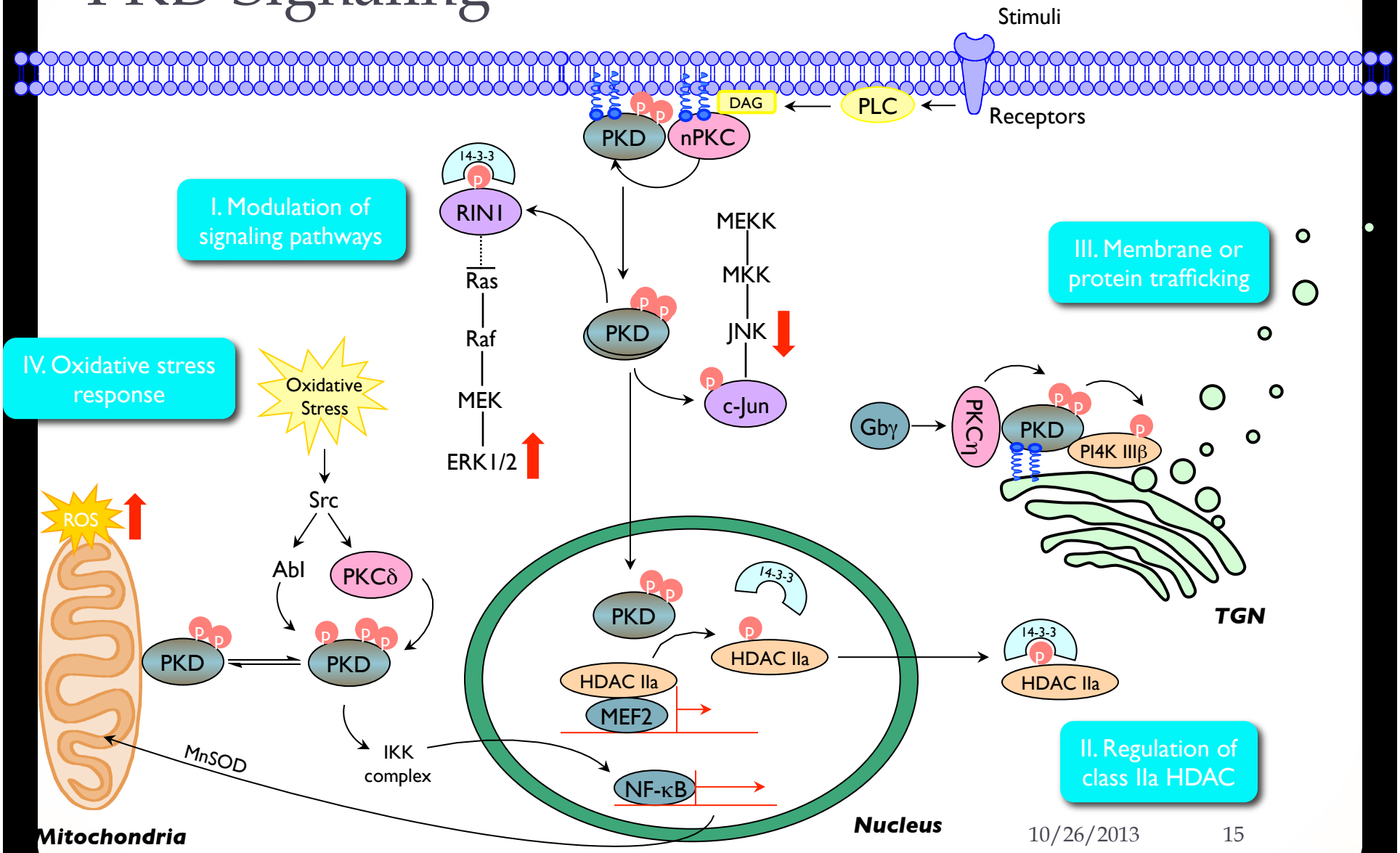
# PKD Signaling



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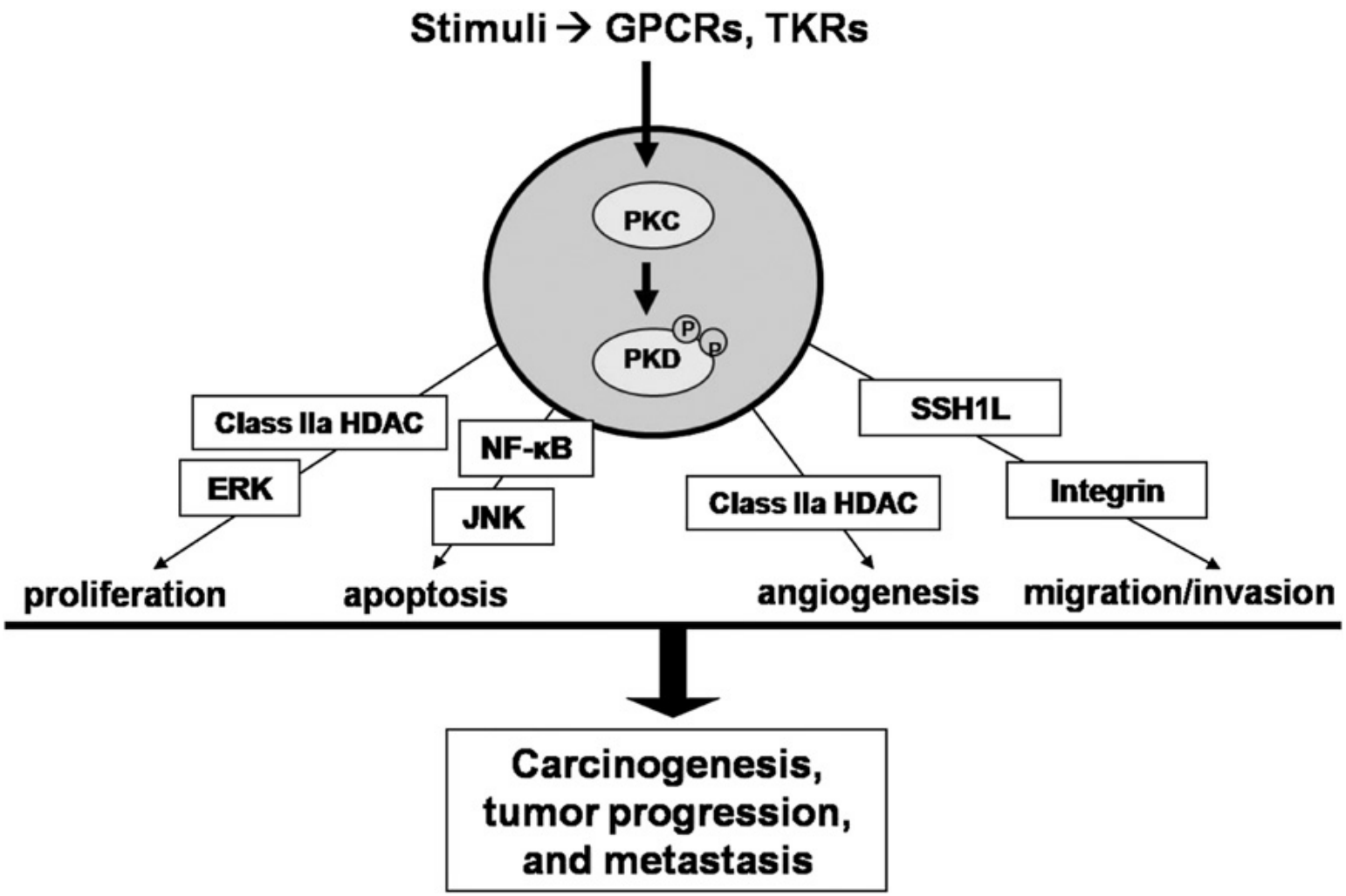
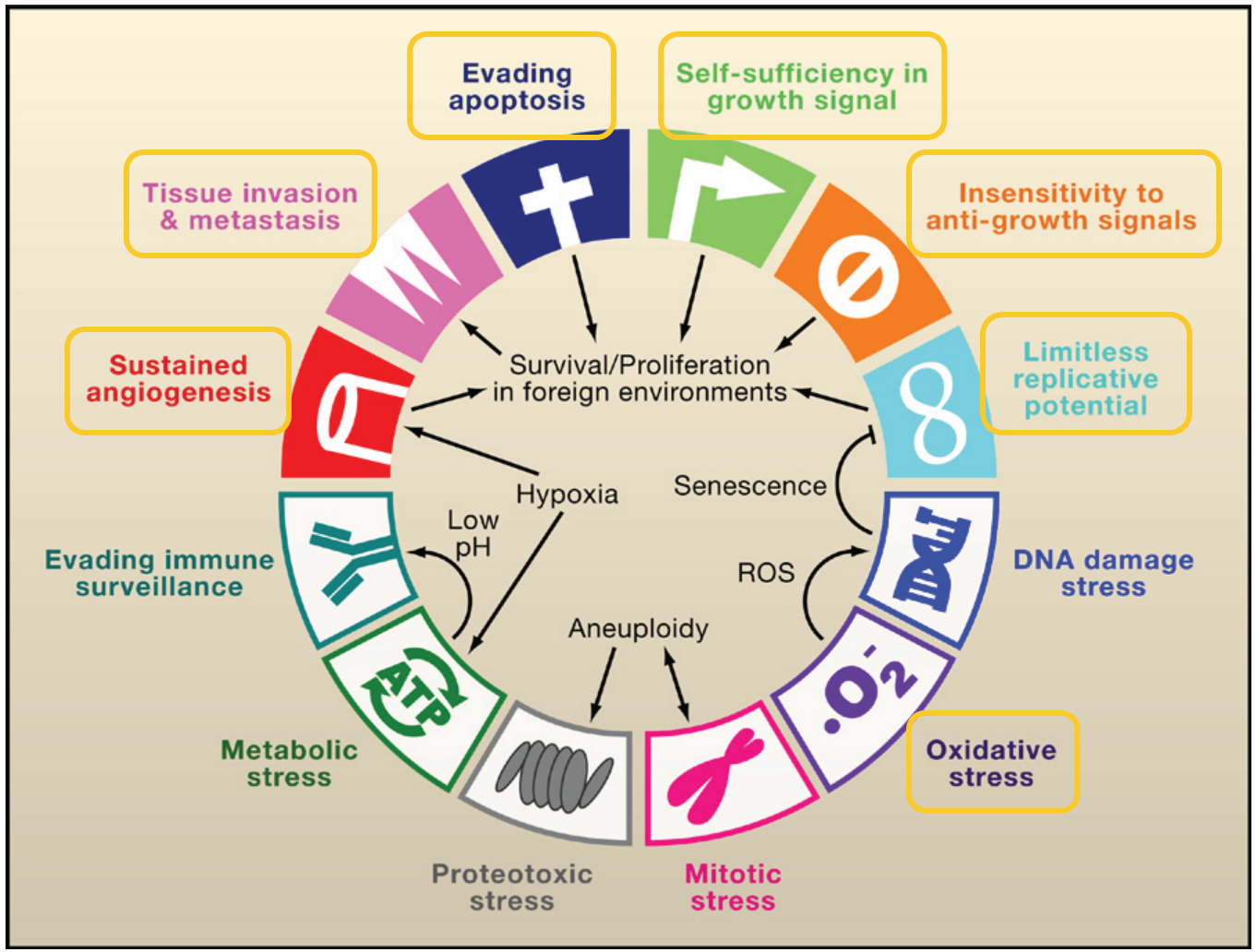
# PKD Role in Cancer






LaValle, C.R., *Biochim. Biophys. Acta,* **2010**. *1806* 183.

# PKD Role






Luo, J., *Cell*, **2009**, *136*, 823.

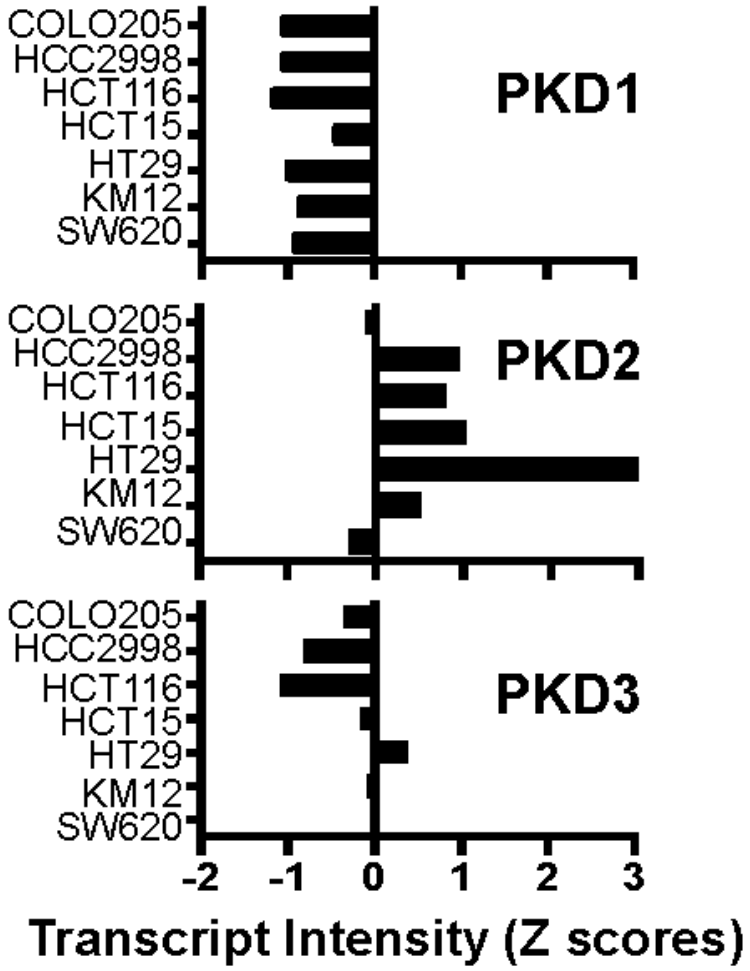
# Altered Expression of PKD in Cancer

| PKD Isoform | Cancer Type | Expression | Effect |
|---|---|---|---|
| PKD1 | Breast | Decreased | Correleated to more invasive tumors |
| | Basal Cell Carcinoma | Increased | Linked to increased proliferation |
| | Gastric | Decreased | |
| | Leukemia | Decreased | |
| | Pancreatic | Increased | |
| | Prostate | Increased/Decreased | Decreased in androgen-independent tumors |
| PKD2 | Colon | Increased | |
| | Gastric | Increased | |
| | Glioblastoma | Increased | Level of expression correlated to tumor grade |
| | Lymphoma | Increased/Decreased | |
| PKD3 | Prostate | Increased | Level of expression correlated to tumor grade |



LaValle, C.R., *Biochim. Biophys. Acta*, **2010**, *1806*, 183.
Sundram, V., *Mol. Cancer Res.*, **2011**, *9*, 985.
Azoitei, N., *Neuro Oncol.*, **2011**, *12*, 710.
Mihailovic, T., *Cancer Res.*, **2004**, *64*, 8939.
Shabelnik, M. Y., *Exp. Oncol.*, **2011**, 33, 206.

# PKD2 in Colon Cancer - RNA Expression






Wei, N.; Chu, E.; Wipf, P.; Schmitz, J., Manuscript Submitted.
John Schmitz unpublished data.

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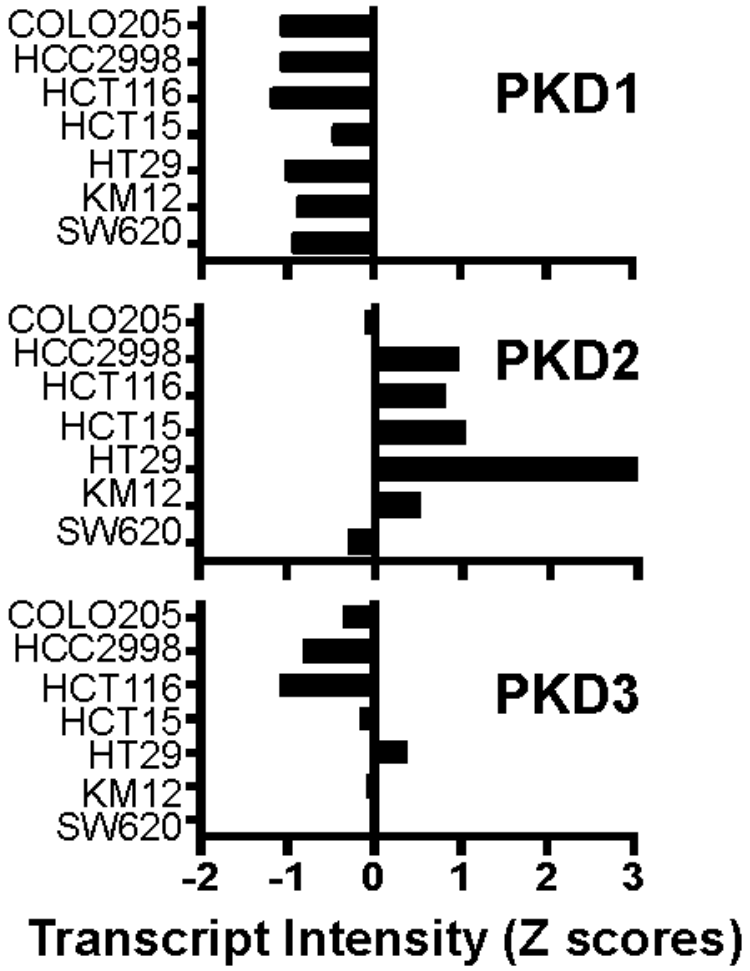


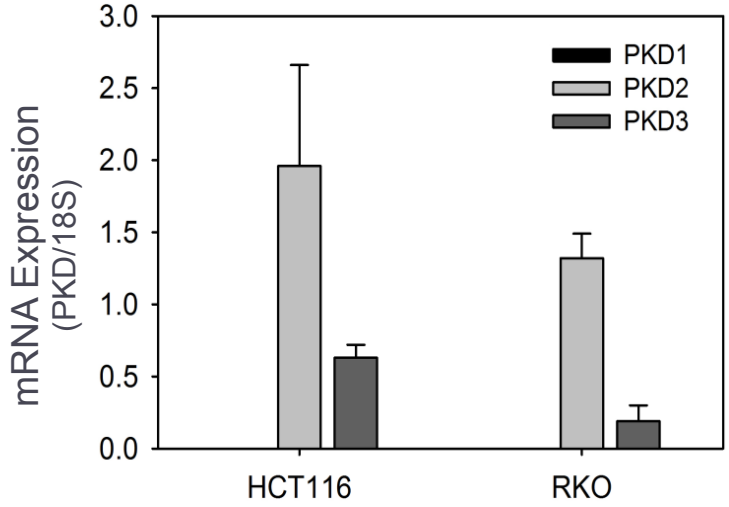




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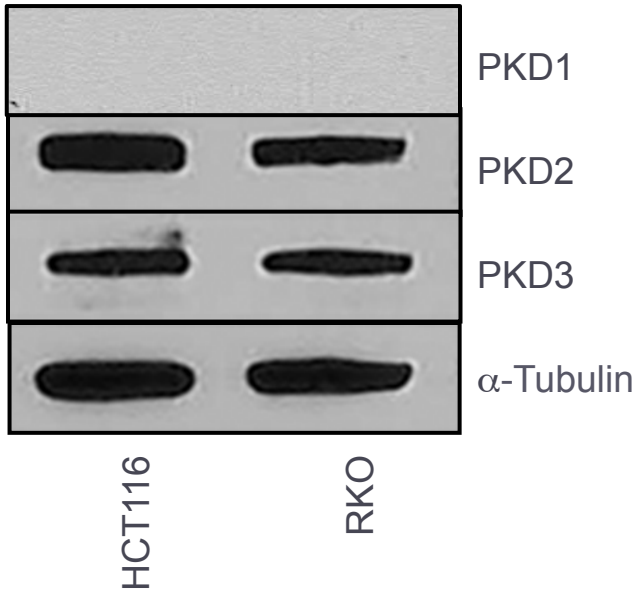
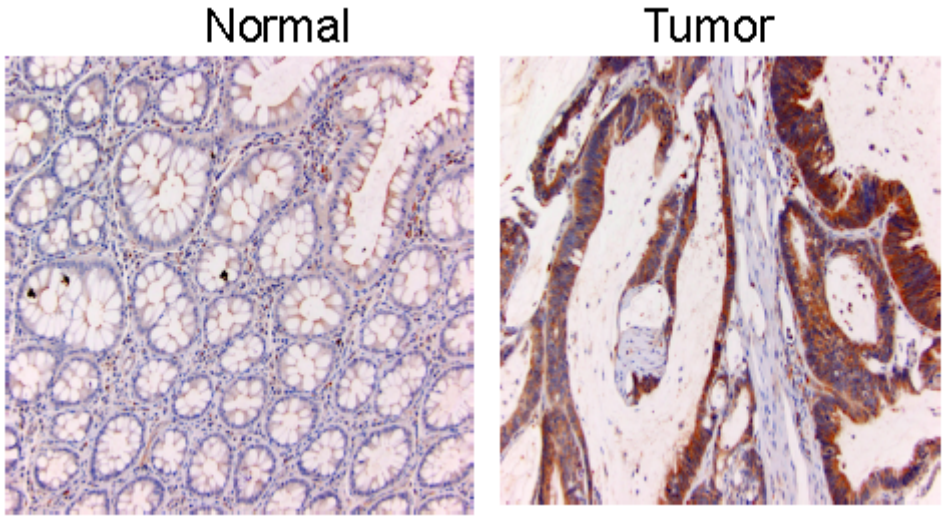
John Schmitz unpublished data.

# PKD in Colon Cancer – Protein Expression




Western blot showing PKD isoform expression in two colorectal cell lines

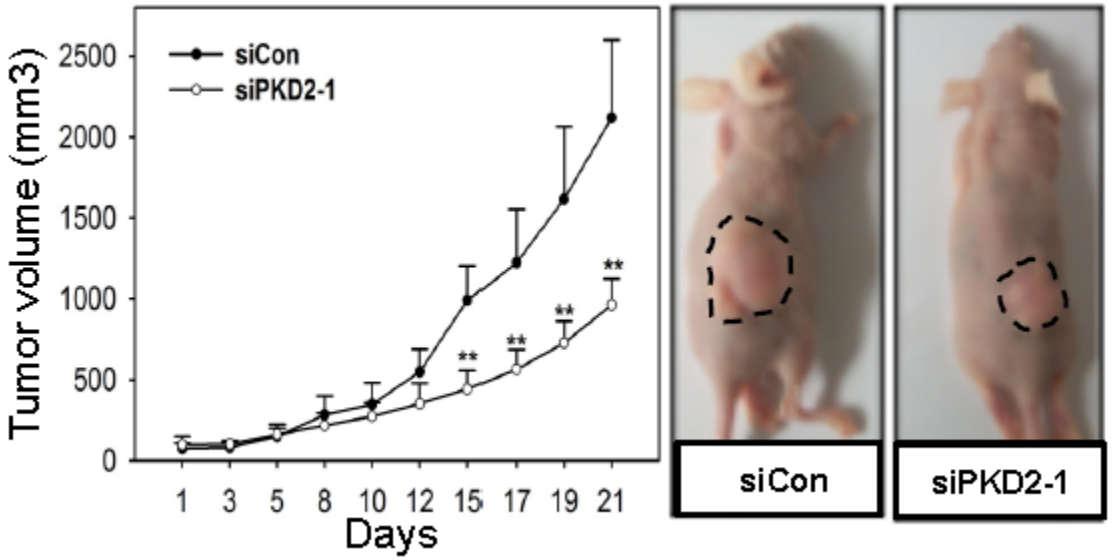



Immunohistochemical staining for PKD2 protein expression in human normal colon epithelium and colorectal tumor



Wei, N.; Chu, E.; Wipf, P.; Schmitz, J., Manuscript Submitted.
John Schmitz unpublished data.

# PKD2 in Colon Cancer



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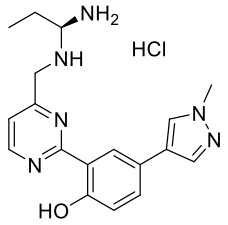


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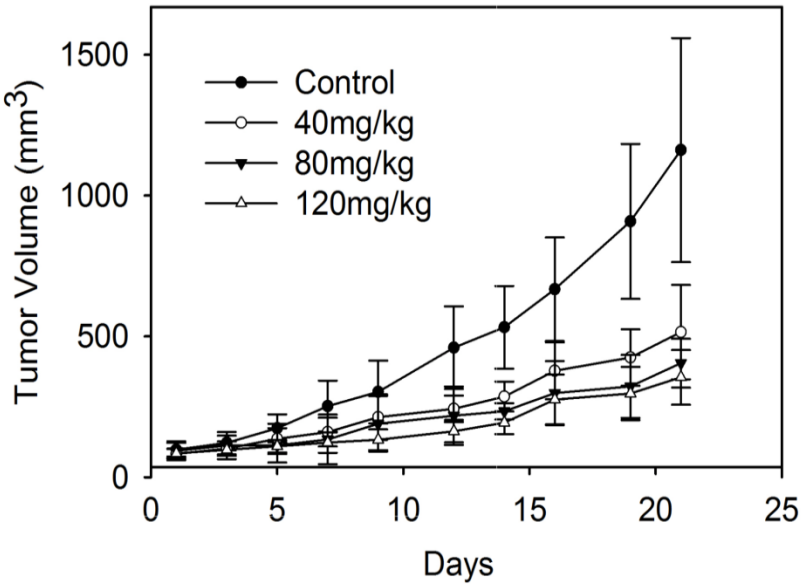
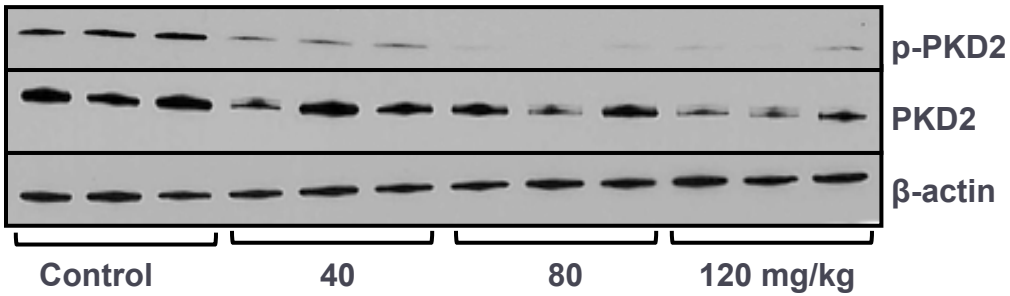
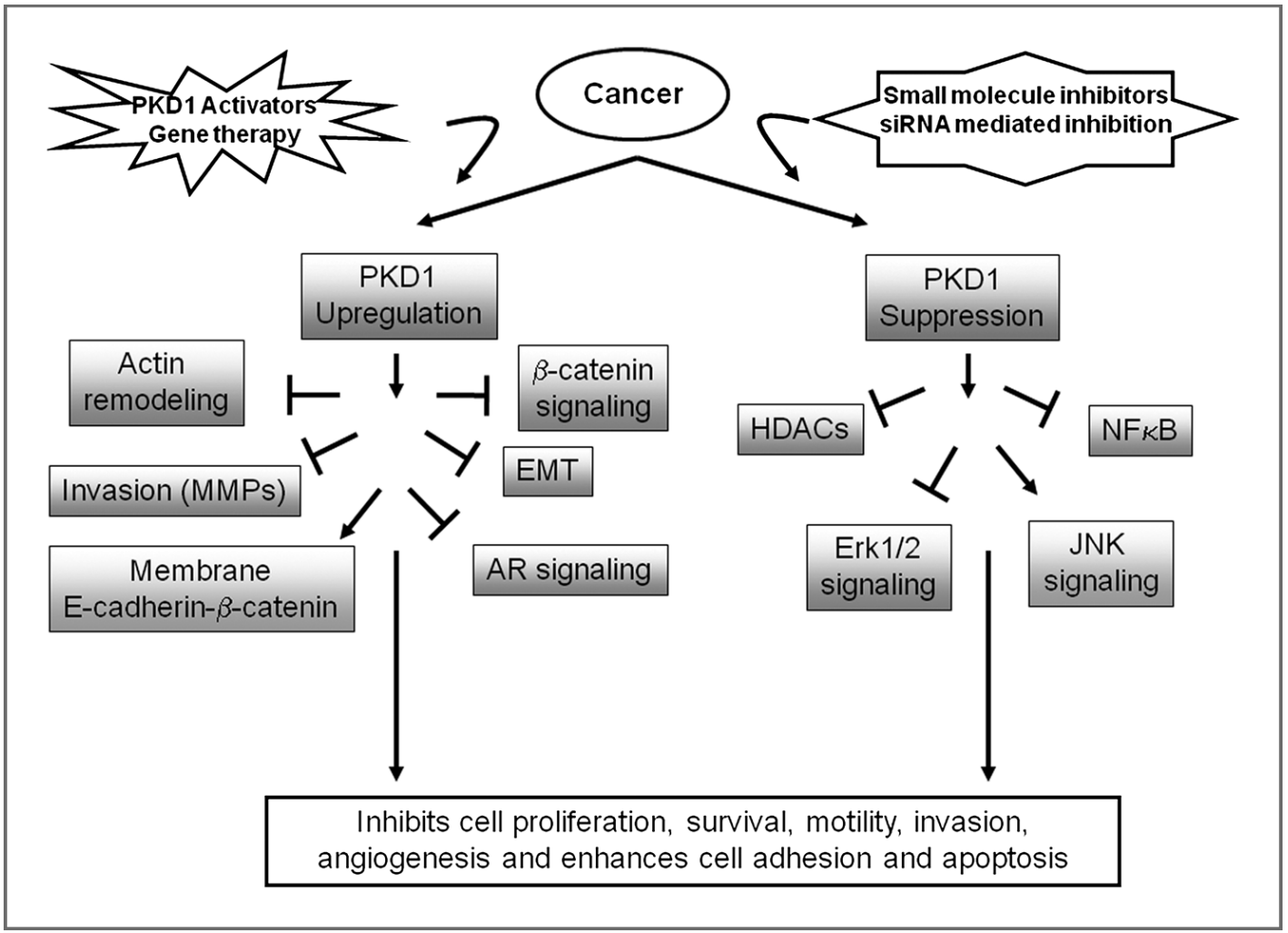


# PKD as a Therapeutic Target



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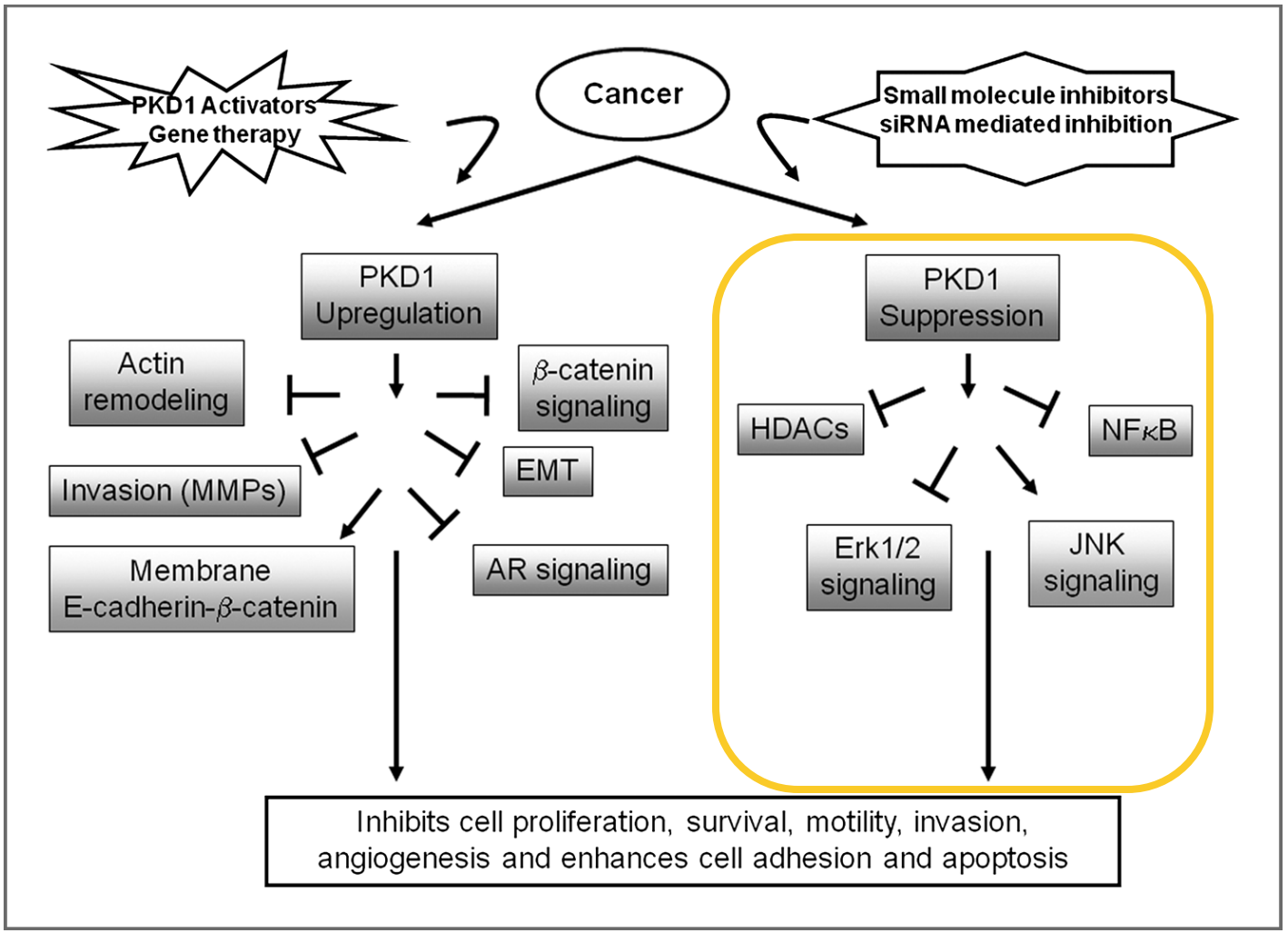
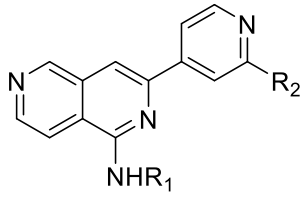





Sundram, V., *Mol. Cancer Res.*, **2011**, **9**, 985.

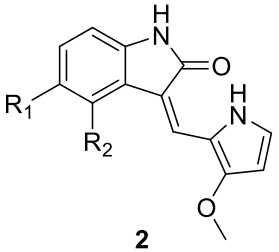
# PKD Inhibitors

- Upon review of the literature 11 PKD inhibitor chemotypes were identified:



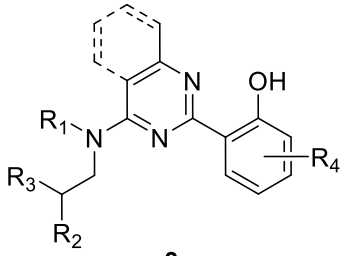
**1**

Most potent inhibitor:
PKD1 $IC_{50}$ = 0.6 nM
Cellular $IC_{50}$ = 32 nM
*Pan-PKD activity



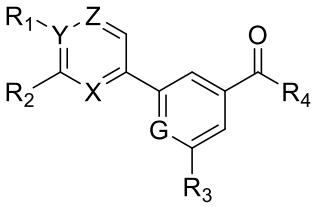
**2**

Most potent inhibitor:
97% PKD1 inhibition
at 1 $\mu$M
*known CDK2 inhibitor



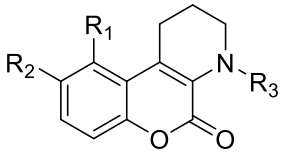
**3**

Most potent inhibitor:
PKD1 $IC_{50}$ = 1 nM
Cellular $IC_{50}$ (PANC-1) = 1 nM
*Pan-PKD activity



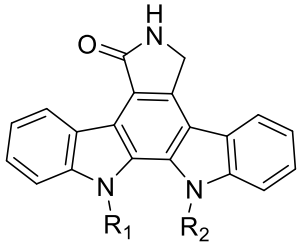
**4**

Most potent inhibitor:
PKD1 $IC_{50}$ = 4 nM
*Moderate PKD2 activity



**5**

Most potent inhibitor:
PKD1 $IC_{50}$ = 2.1 $\mu$M
Cellular $IC_{50}$ (LNCaP) =
>50 $\mu$M

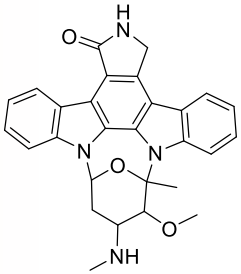


**6**

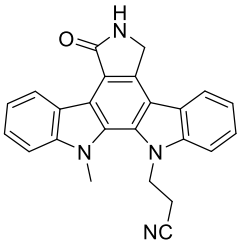
Most potent inhibitor:
PKD1 $IC_{50}$ = 7 nM
Cellular $IC_{50}$ (sf 158) = 7 nM
*Pan-PKD activity
*known PKC inhibitor


1. Meredith, E. L., *J. Med. Chem.*, **2010**, *53*, 5400.
2. Tandon, M., *PLoS ONE*, **2012**, *7*, e44653.
3. Harikumar, K. B., *Mol. Cancer Ther.*, **2010**, *9*, 1136.
4. Evans, J. M., *Biochem. J.*, **2010**, *429*, 565.
5. Sharlow, E. R., *J. Biol Chem.*, **2008**, *283*, 33516.
George, K. M., *Pharmaceutics*, **2011**, *3*, 186.
6. Gschwendt, M., *FEBS Lett.*, **1996**, *392*, 77.

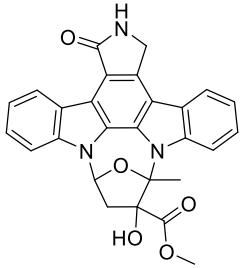
# PKD Inhibitors




Staurosporine




Gö6976




K252a

- ATP competititve
- Non PKD specific
- Gö6976 is more selective for PKC
- Staurosporine and K252a inhibit many kinases

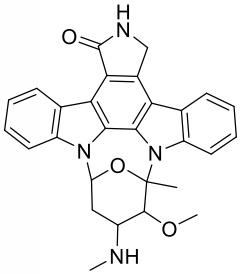
*In vitro* inhibition of PKD1 by known inhibitors

| **Compounds** | **$IC_{50}$ (nM)** |
|---|---|
| Staurosporine | 40 |
| Gö6976 | 20 |
| K252a | 7 |
| BPDKi | 1 |
| CRT0066101 | 1 |
| CID755673 | 182 |

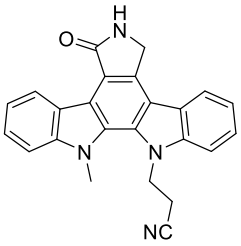


LaValle, C. R. et al *Biochim. Biophys. Acta*, **2010**, *1806*, 183.

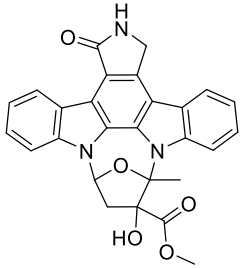
# PKD Inhibitors



Staurosporine



Gö6976



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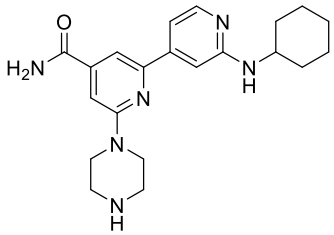

BPKDi

- ATP competitive
- Selective for PKDs
- 100% inhibition at 1 $\mu$ M

*In vitro* inhibition of PKD1 by known inhibitors

| **Compounds** | **$IC_{50}$ (nM)** |
|---|---|
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LaValle, C. R. et al *Biochim. Biophys. Acta*, **2010**, *1806*, 183.

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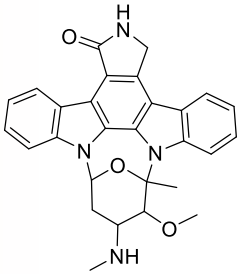
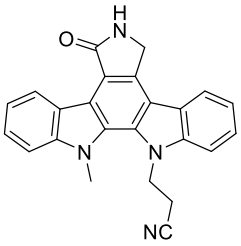
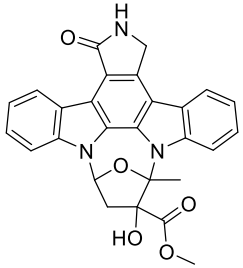


Staurosporine

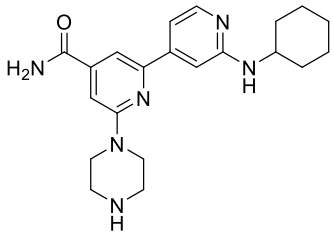


Gö6976



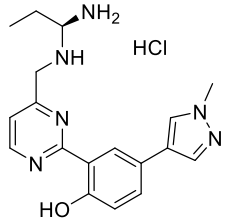
K252a

- ATP competititve
- Non PKD specific
- Gö6976 is more selective for PKC
- Staurosporine and K252a inhibit many kinases



BPKDi

- ATP competitive
- Selective for PKDs
- 100% inhibition at 1 $\mu$ M



CRT0066101

- ATP competitive
- Selective for PKDs
- Cellular $IC_{50}$ of 0.5 $\mu$ M in PANC-1 cells
- Orally bioavailable
- Effective *in vivo* against pancreatic cancer

*In vitro* inhibition of PKD1 by known inhibitors

| **Compounds** | **$IC_{50}$ (nM)** |
|---|---|
| Staurosporine | 40 |
| Gö6976 | 20 |
| K252a | 7 |
| BPDKi | 1 |
| CRT0066101 | 1 |
| CID755673 | 182 |



LaValle, C. R. et al *Biochim. Biophys. Acta*, **2010**, *1806*, 183.

# Lead Selection: HTS

**Identification of Primary Hits**

- Screen **196,173** compounds in IMAP HTS Assay
- Identify **109** compounds to validate

**Secondary Hit Confirmation**

- Hit validation
- Determination of $IC_{50}$
- Radiometric PKD kinase assay to confirm $IC_{50}$ values
- Screen for specificity
- Identify **14** compounds to screen in cells

**Tertiary Screen**

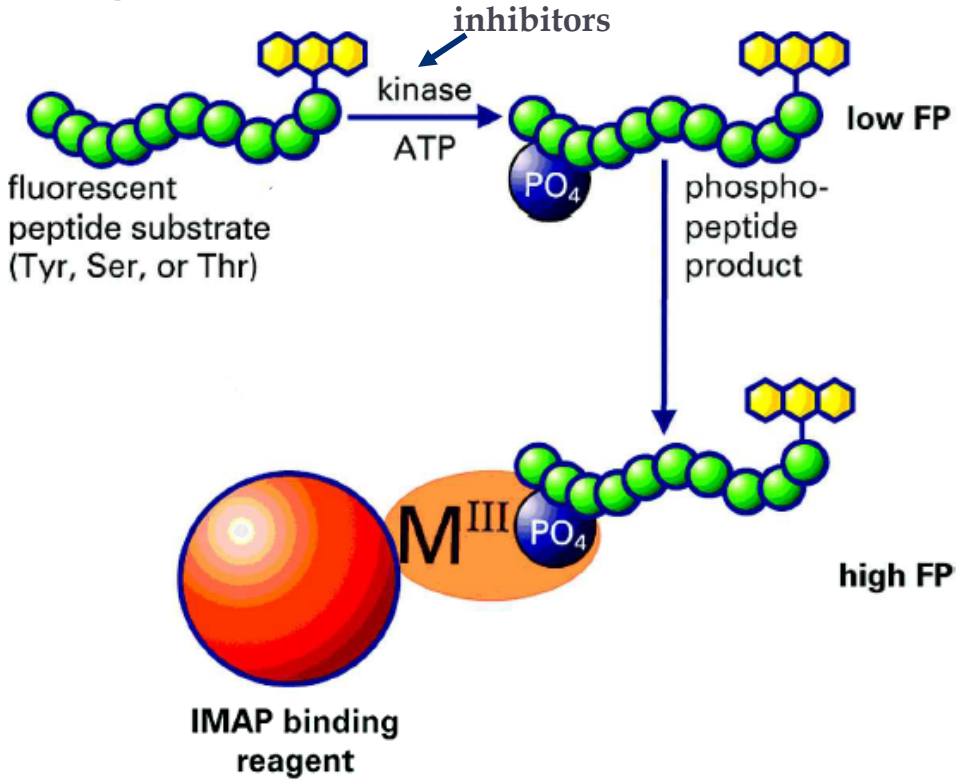
- Cell based assays
- Identify **8** compounds to select from for synthesis, SAR, and biological evaluation



Sharlow, E. R. *PLoS ONE*, **2011**, *6*, e25134.

# IMAP - Immobilized metal ion affinity-based fluorescence polarization detection

- Used for kinases and phosphatases
- Homogeneous
- High throughput (>40,000 compounds/day)






Sportsman, J. R., *Assay Drug Dev. Technol.*, **2004**, *2*, 205.

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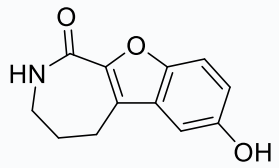
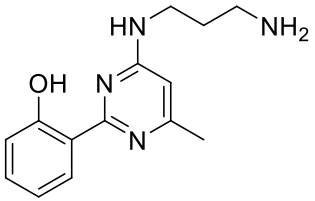
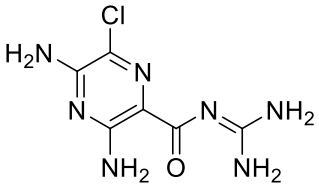
# HTS Hits



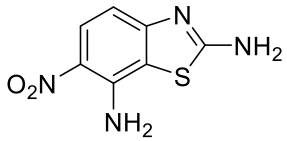
CID755673
$IC_{50}$ = 0.2 uM



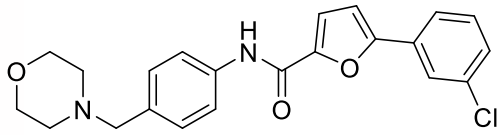
CID5389142
$IC_{50}$ = 1.1 uM



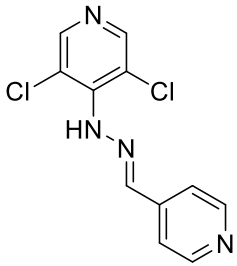
SID16230
$IC_{50}$ = 0.9 uM



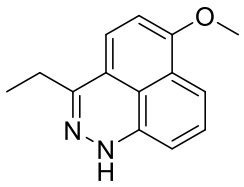
CID5086667
$IC_{50}$ = 1.5 uM



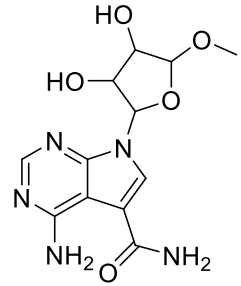
CID2011756
$IC_{50}$ = 3.3 uM



CID4438738
$IC_{50}$ = 0.6 uM



CID2876479
$IC_{50}$ = 2.2 uM



SID9549170
$IC_{50}$ = 0.5 uM



Sharlow, E. R. *PLoS ONE*, **2011**, *6*, e25134.

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