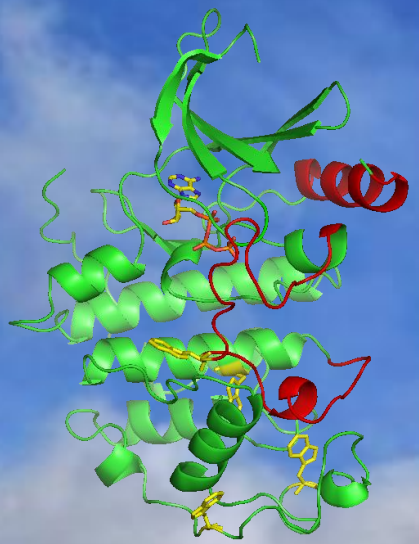
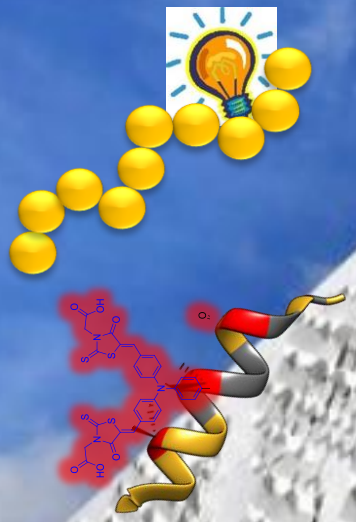
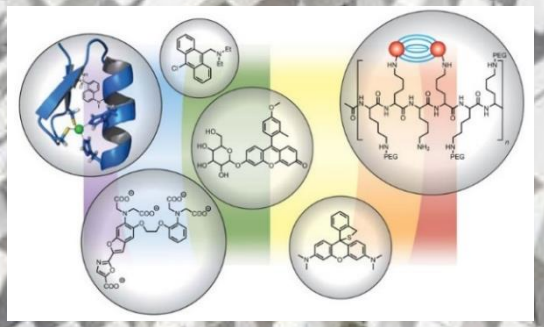
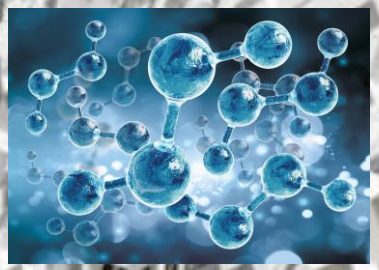
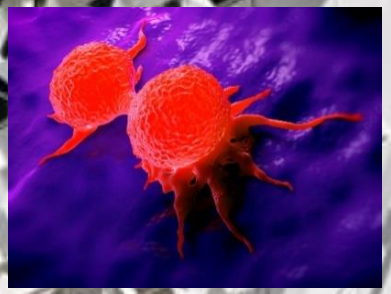
BIOSENSEURS CONFORMATIONNELS POUR LE CRIBLAGE D'INHIBITEURS ALLOSTERIQUES DES KINASES CYCLINE-DEPENDENTES











May C. Morris

Institut des Biomolécules Max Mousseron, Pôle Chimie Balard Recherche

CNRS UMR5247, Montpellier, France

IBMM Institut des Biomolécules Max Mousseron

POLE chimie Balard

ensCM CHIMIE Montpellier

cnrs

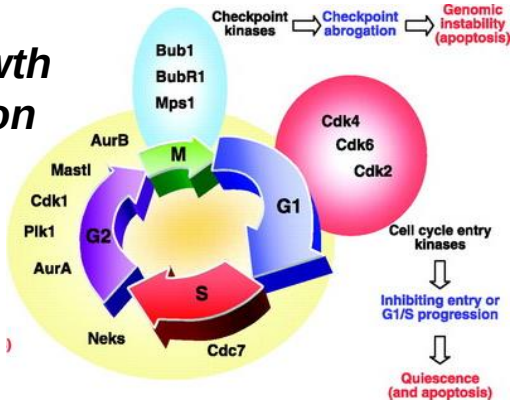
UNIVERSITE MONTPELLIER

MUSE MONTPELLIER UNIVERSITÉ D'EXCELLENCE

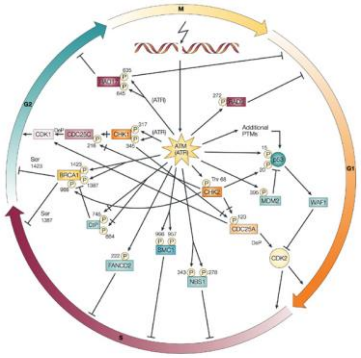
La Région Occitanie Pyrénées · Méditerranée

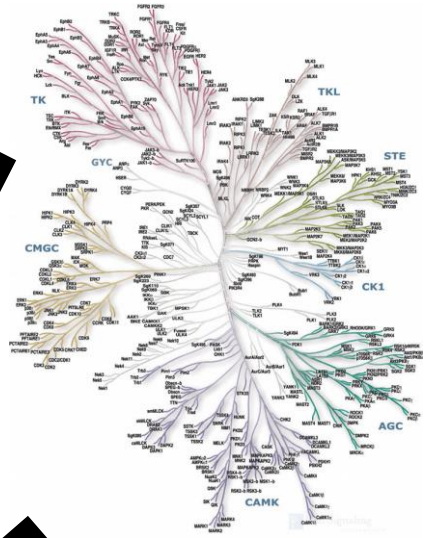
PROTEIN KINASES IN HEALTH & DISEASE

Cell Growth & Division

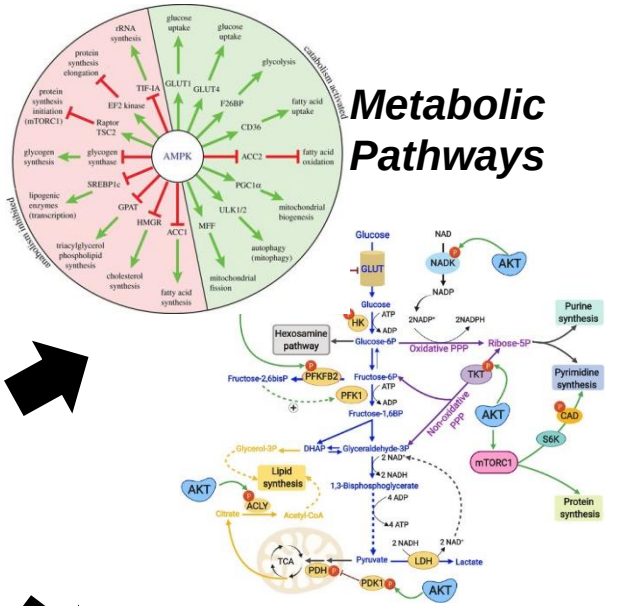


Checkpoint Signalling

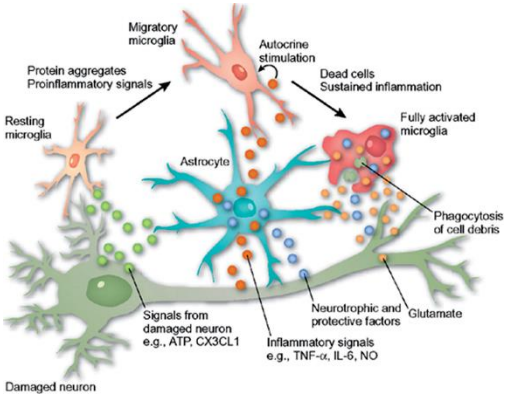




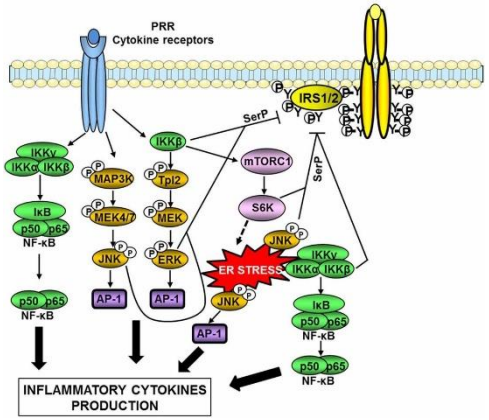
Metabolic Pathways



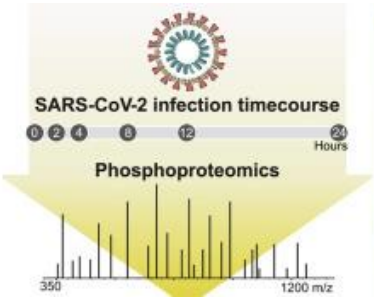
Neuronal Functions

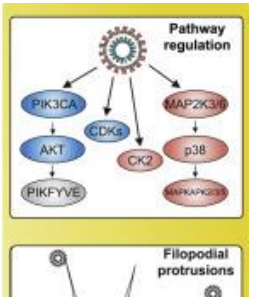


Inflammation & Immunity



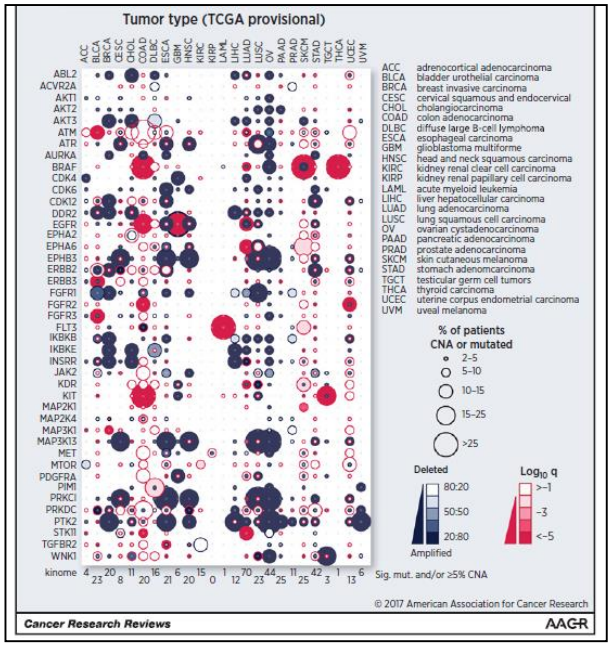
Viral & Bacterial Infection



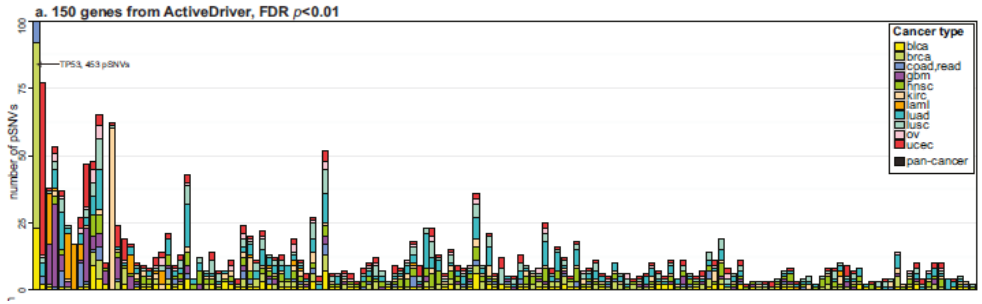


PROTEIN KINASES : CANCER BIOMARKERS

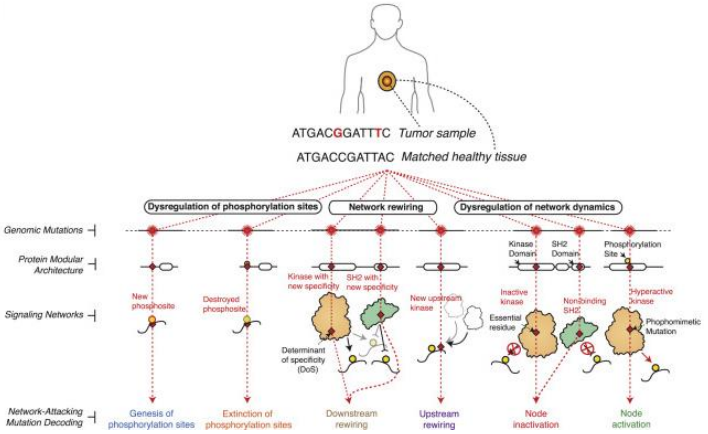
Genetic, transcriptomic & proteomic evidence for protein kinase dysregulation in cancer



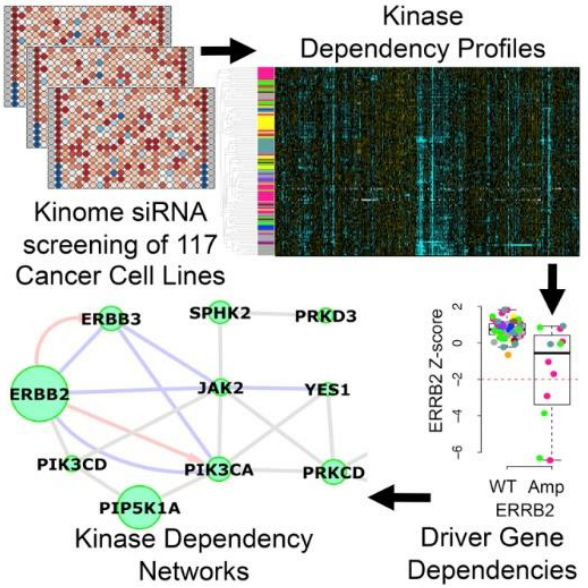
New Perspectives, Opportunities, and Challenges in Exploring the Human Protein Kinome
Wilson et al. Cancer Res. 2018



The mutational landscape of phosphorylation signaling in cancer
Reimand et al. Sci.Rep. 2013



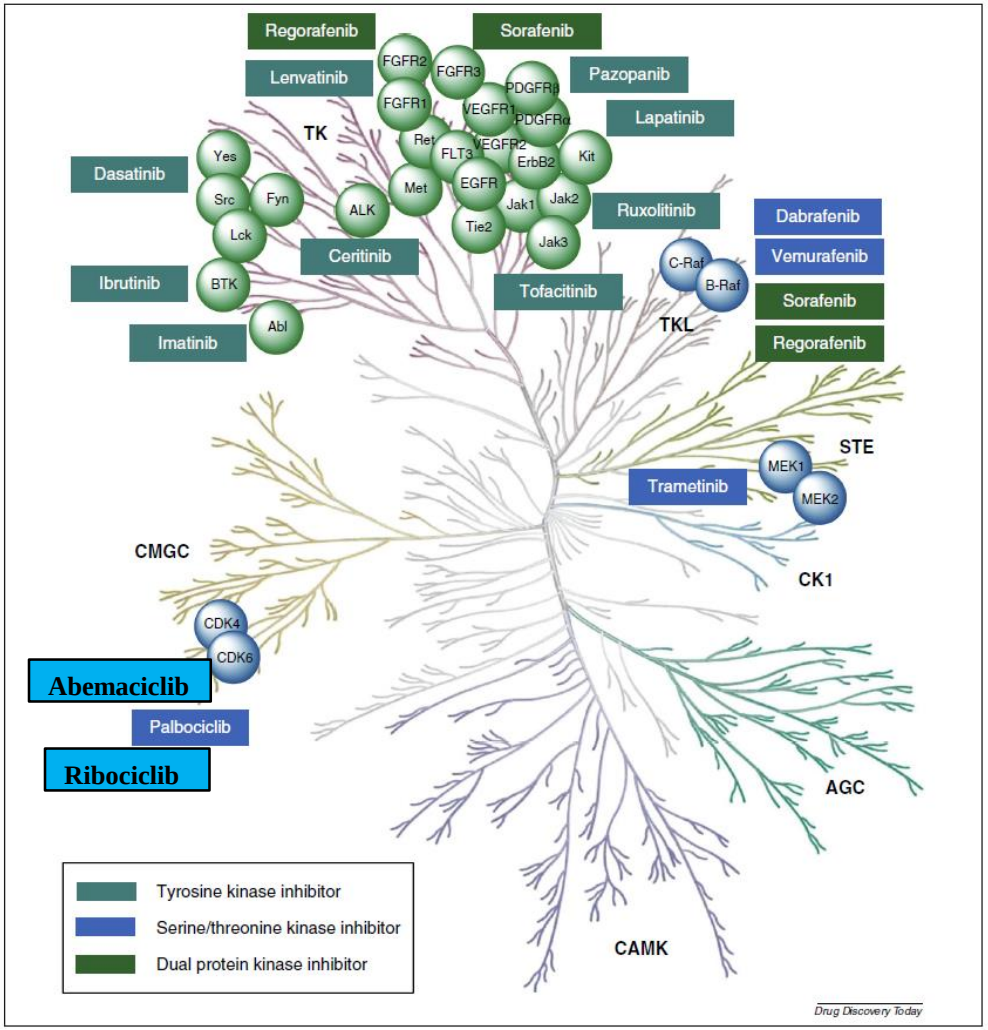
Kinase Network Rewiring in Cancer
Creixell et al. 2015



Kinase Dependencies in Cancer
Campbell et al. 2016

THERAPEUTIC TARGETING of PROTEIN KINASES

> 75 FDA-approved drugs: most Kinase Inhibitors are for oncological indications



Wu et al. Small-molecule kinase inhibitors: an analysis of FDA-approved drugs, Drug Discovery Today 2016

Cancer, Hypertension, Parkinson's Disease, Inflammatory & Autoimmune diseases

Table 1. Small Molecule Inhibitors of Protein Kinases Approved for Clinical Use or in Advanced Clinical Trials

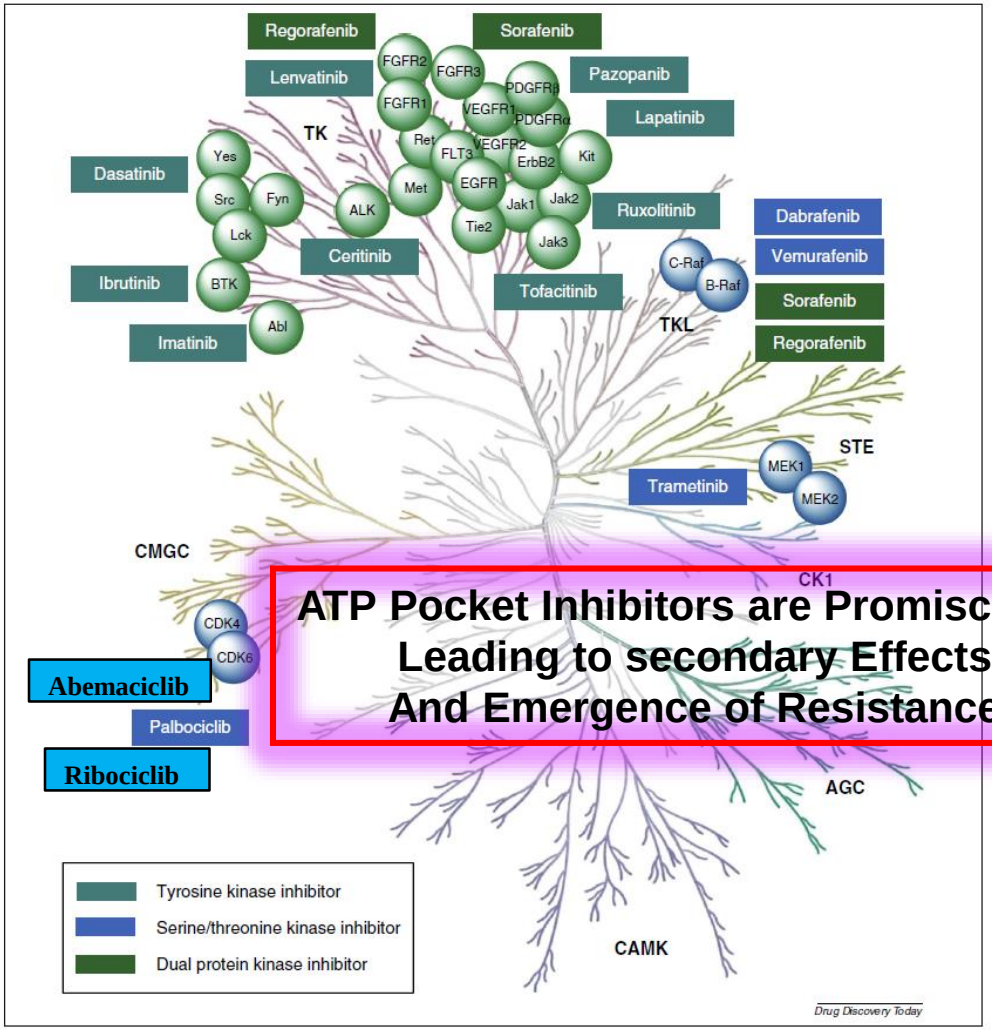
Name	Structure	Reported target	Company	Approved for clinical use
Eril		ROCK	Eisai	1995 cerebral vasospasm (Japan)
Rapamune		mTOR	Wyeth Pfizer	2000 kidney transplantation
Temsirolimus		mTOR	Wyeth Pfizer	2007 advanced renal cell carcinoma
Everolimus		mTOR	Novartis	2009 several cancers
Imatinib		Bcr-Abl c-KIT PDGFR	Novartis	2001 chronic myelogenous leukaemia
Gefitinib		EGFR	Astra Zeneca	2005 lung cancer
Erlotinib		ErbB1	Genetech Roche	2005 lung,pancreatic and others cancers
Sorafenib		Multiple Tyrosine kinases targeted	Onyx Bayer	2005 renal cancer
Dasatinib		Multiple Tyrosine-kinases targeted	Bristol Myers Squibb	2006 chronic myelogenous leukaemia, All
Sunitinib		Multiple Tyrosine-kinases targeted	SUGEN Pfizer	2006 renal cancer and GIST
Nilotinib		Bcr-Abl	Novartis	2007 chronic myelogenous leukaemia
Lapatinib		Her2 EGFR	GlaxoSmith Kline	2009 renal cancer
Pazopanib		VEGFR2 PDGFR c-KIT	GlaxoSmith Kline	2009 renal cancer
Ruxolitinib		JAKs	Incyte	2011 myelofibrosis
Crizotinib		ALK/Met	Pfizer	2011 NSCLC with Alk mutation
Vemurafenib		BRAF	Roche	2011 melanoma
Vadetinib		Multiple Tyrosine kinases targeted	Caprelsa IPR Pharms	2012 thyroid cancer
Axitinib		VEGFRs PDGFRB c-KIT	Pfizer	2012 renal cell carcinoma
Bosutinib		Bcr/Abl SRC	Pfizer	2012 chronic myelogenous leukemia
Tivozanib		VGFRs	AVEO Pharms	2012 kidney cancer
Tofacitinib		JAKs	Pfizer	2012 rheumatoid arthritis
Regorafenib		Multiple Tyrosine kinases targeted	Stivarga Bayer	2012 thyroid cancer
Lenvatinib		VEGFR2/ VEGFR2	Eisai	2012 thyroid cancer (Japan)
Toceranib		Multiple Tyrosine kinases targeted	Pfizer	2009 canine mastocytoma
Masivet Kinavet		c-KIT PDGFR	AB Science	2010 canine mastocytoma
Cabozantinib		VEGFRs KIT / Axl	Cometriq Exelixis	2012 canine thyroid cancer
Afatinib		Her2 EGFR	Boehringer Ingelheim	Not yet NSCLC
Dabrafenib		BRaf	GlaxoSmith Kline	Not yet metastatic melanoma
Trametinib		MEK1/2	GlaxoSmith Kline	Not yet metastatic melanoma

Cohen & Alessi, Kinase Drug Discovery – What's next in the field, ACS Chem Biol. 2013

- only a small number of protein and lipid kinase targets (about 80) out of the 500+ protein kinases in the human kinome have been successfully targeted
- most of the kinase inhibitor drugs are used for oncological indications
- many kinase inhibitor drugs are used to target the same indication (mainly due to the generation of resistance)

THERAPEUTIC TARGETING of PROTEIN KINASES

> 75 FDA-approved drugs: most Kinase Inhibitors are for oncological indications



ATP Pocket Inhibitors are Promiscuous
Leading to secondary Effects
And Emergence of Resistance

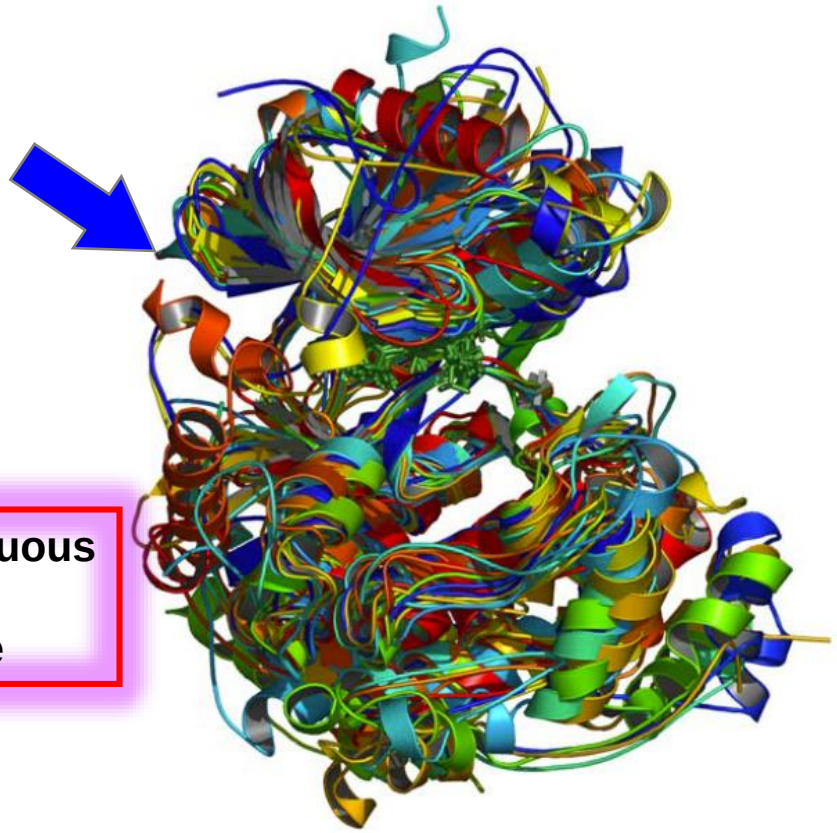
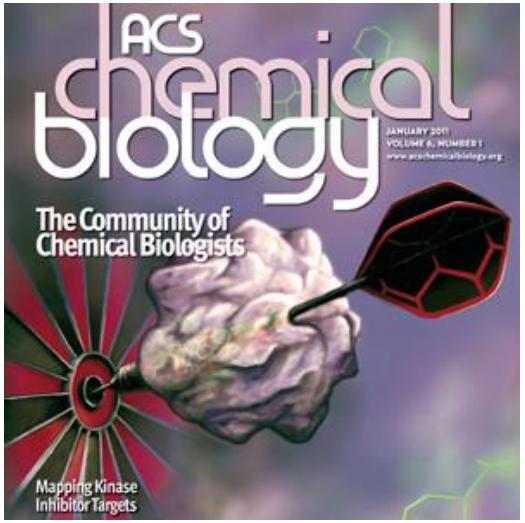


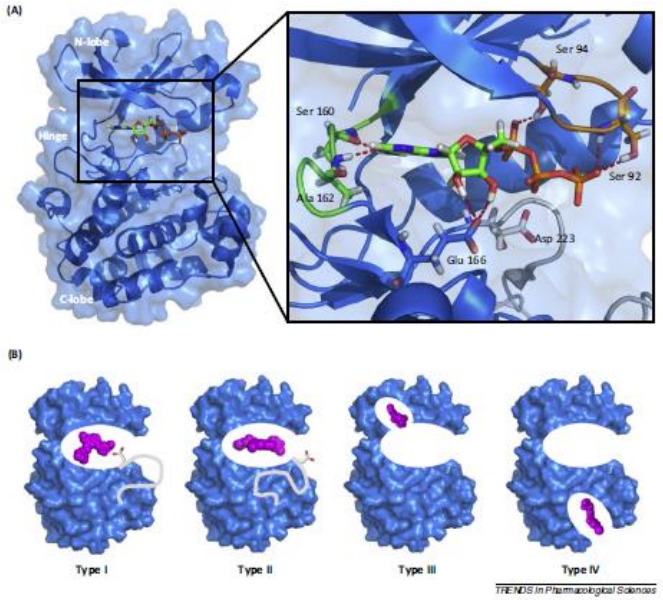
Figure 2. Multiple kinase alignment. The fifteen active-conformation kinase structures listed in Table 1 were aligned using our modified Procrustes approach. Shown in green sticks is the ATP or ATP analog molecule of each structure. Each kinase is colored uniquely.
doi:10.1371/journal.pone.0000982.g002

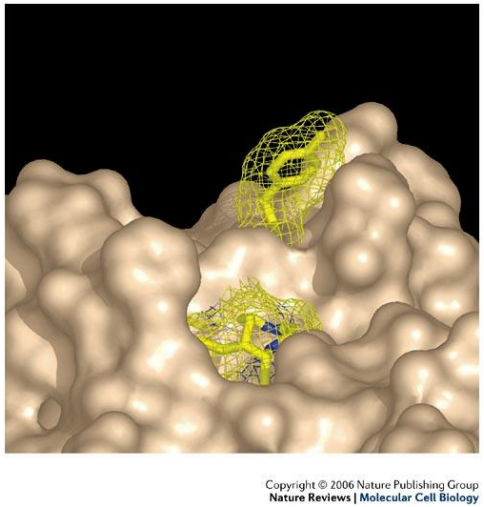
Wu et al. Small-molecule kinase inhibitors: an analysis of FDA-approved drugs, Drug Discovery Today 2016

Knight et al. PLOS ONE 2007

KINASE INHIBITORS : IN SEARCH OF SELECTIVITY







Wu et al. Trends Pharmacological Sciences 2015

New strategies are required to develop inhibitors that do not target the ATP-binding pocket : targeting essential protein / protein interfaces or conformational transitions

ATP Pocket Inhibitors
Promiscuous
Secondary Effects
Resistance

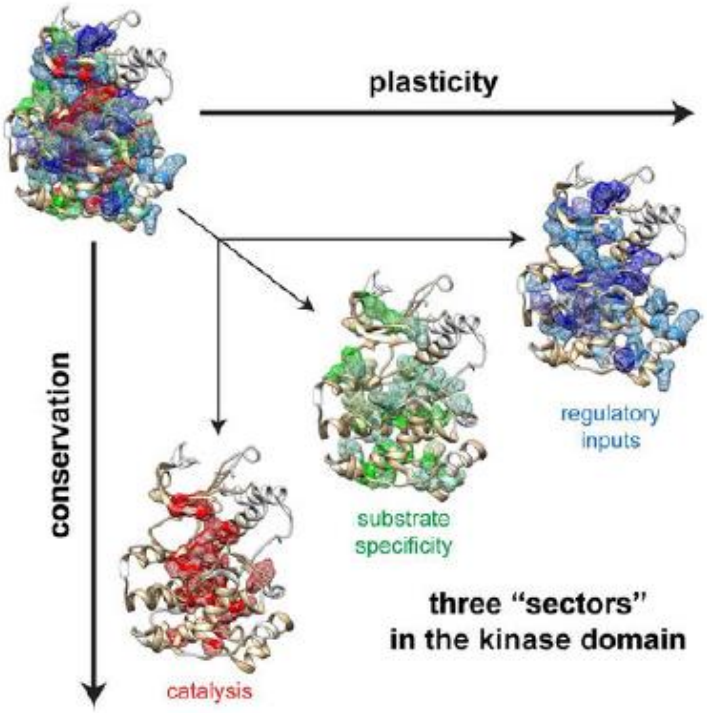
- **Targeting « non-catalytic » sites**
- **Targeting surface hotspots**
- **Targeting essential PPIs**
- **Preventing Kinase Activation**
- **Trapping Inactive Conformation**

Gain of Selectivity
Less Adverse Effects

PROTEIN KINASES

PLASTICITY & NON-CATALYTIC FUNCTIONS

Structural Plasticity



Creixell P. et al. Cell Syst. 2018

Hierarchical organization endows the kinase domain with regulatory plasticity

Non Catalytic Functions of PKs

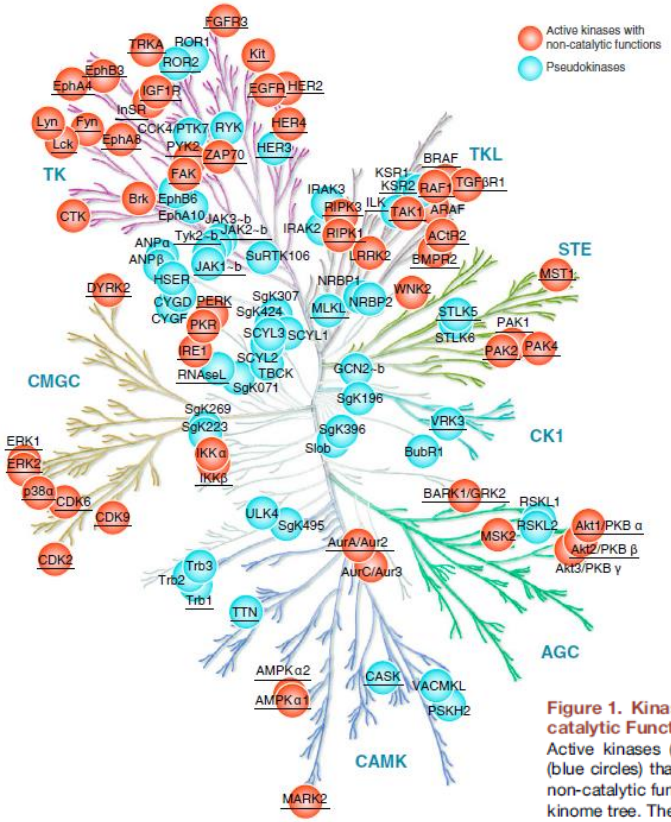


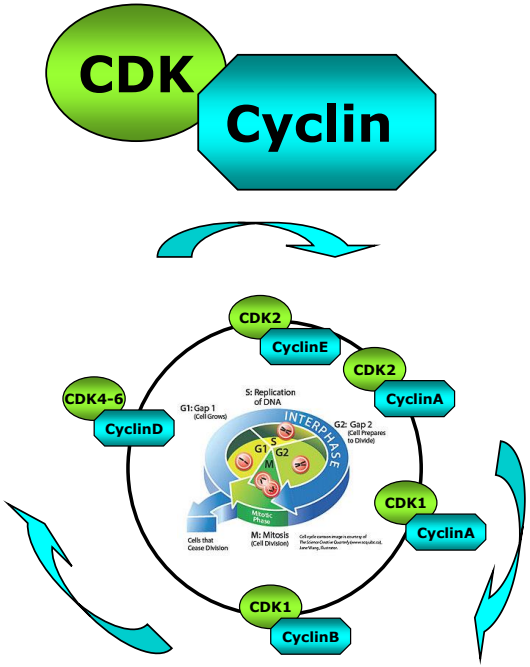
Figure 1. Kinases Reported to Have Non-catalytic Functions
Active kinases (red circles) and pseudokinases (blue circles) that have been reported to perform non-catalytic functions are marked on the human kinome tree. The names of kinases whose crystal structures are available are underlined. Illustration reproduced courtesy of Cell Signaling Technology, Inc. (www.cellsignal.com).

Kung & Jura 2015

Structural basis for the non-catalytic functions of protein kinases

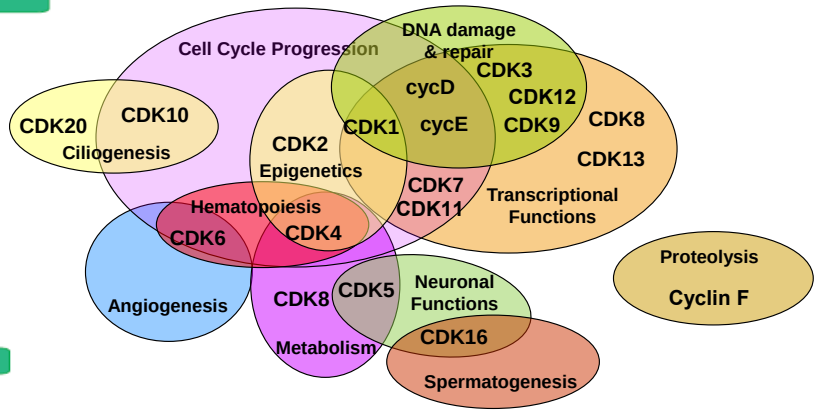
CYCLIN-DEPENDENT KINASES

Master Kinases coordinate cell cycle progression and ....



CDK1	Cyclins A, B, D, E, J, O
CDK2	Cyclins A, B, D, E, O, Cables 1
CDK3	Cyclin A, E, C, Cables 1
CDK4	Cyclins D
CDK5	Cyclin D, G, I, CDK5R1/2, Cables 1
CDK6	Cyclins D
CDK7	Cyclin H
CDK8	Cyclin C
CDK9	Cyclin K, T
CDK10	Cyclin M
CDK11	Cyclins D, L
CDK12	Cyclin K, L
CDK13	Cyclin K, L
CDK14	Cyclins D, Y
CDK15	
CDK16	Cyclin Y, CDK5R1/2, Cables 1
CDK17	Cables 1
CDK18	Cyclin K
CDK19	Cyclin C
CDK20	Cyclins H, D, T

Malumbres M. & Barbacid M.
TIBS 2005

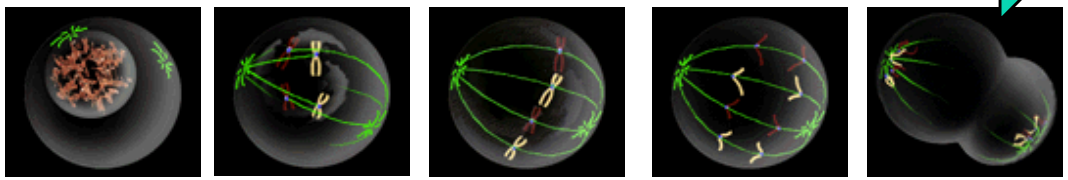


Prével C. & Morris M.C.
Eur J.Med. Chem. 2014

Leland Hartwell, Tim Hunt and Paul Nurse

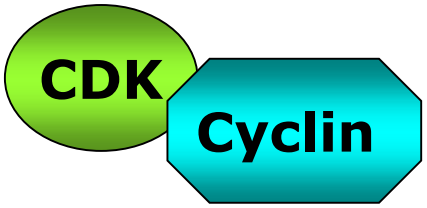
The Nobel Prize in Physiology or Medicine 2001
"for their discoveries of key regulators of the cell cycle"

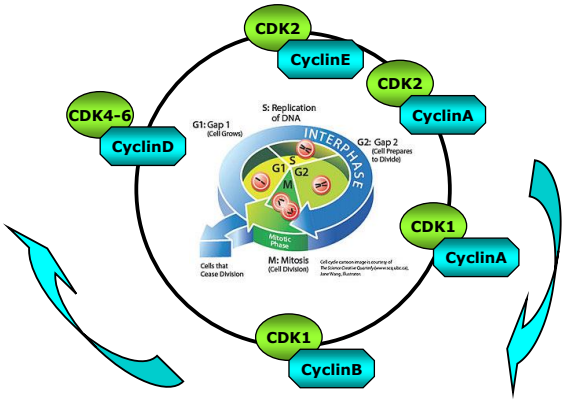
Regulation of Cell growth and Division

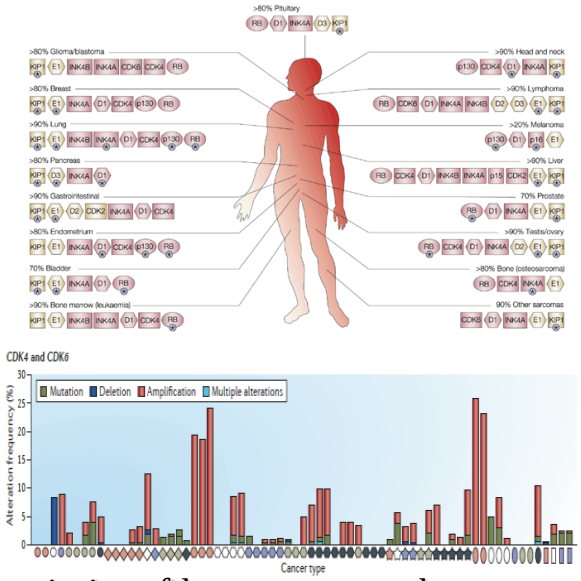


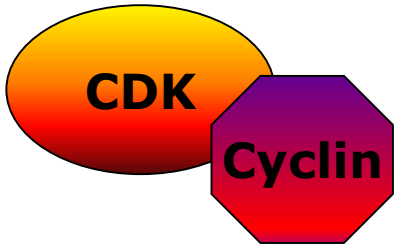
CYCLIN-DEPENDENT KINASES

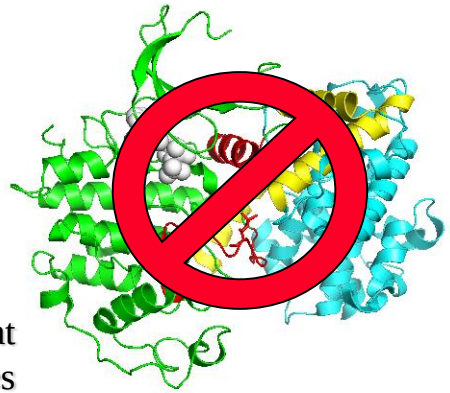
Hyperactivated Cancer Biomarkers and Targets







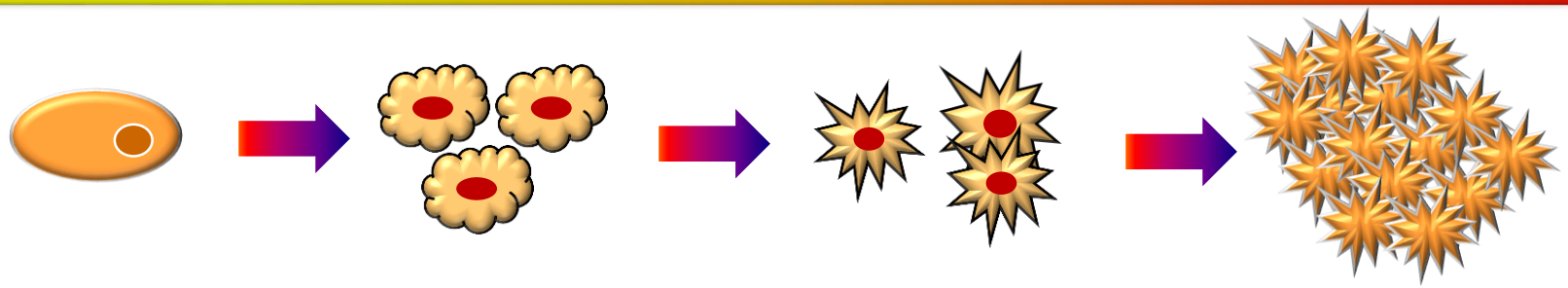




The majority of human cancers bear mutations that lead to hyperactivation of cyclin-dependent kinases

Malumbres & Barbacid, Nat. Rev. Cancer 2001

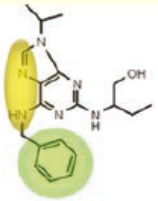
Contribute and sustain hyperproliferation of cancer cells



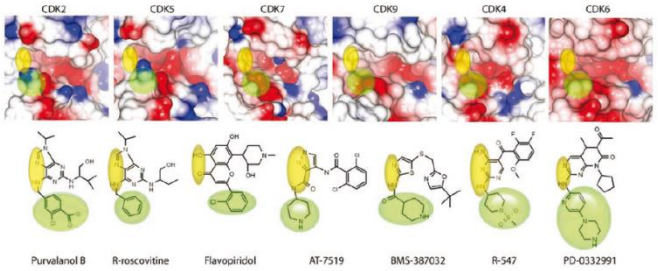
TARGETING CYCLIN-DEPENDENT KINASES

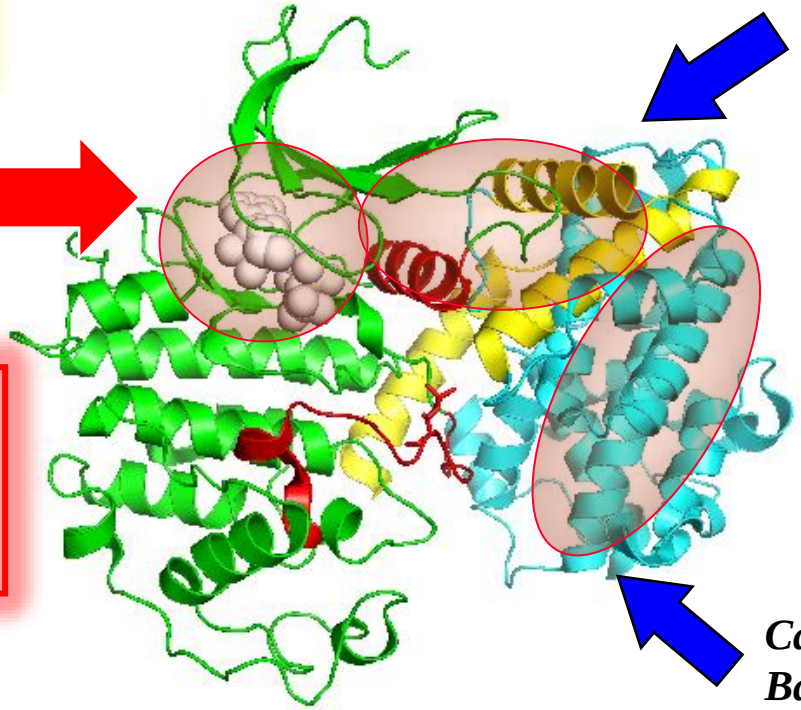
ATP-COMPETITIVE INHIBITORS

Promiscuous
Secondary Effects
Emergence Resistance



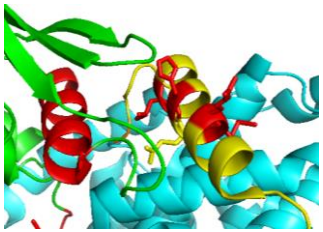
3 FDA-approved Type I ATP-competitive drugs : Abemaciclib, Palbociclib, Ribociclib





PSTAIRE / alpha 5 interface eg the C4 peptide

Gondeau et al. JBC 2005



Cyclin surface or pocket inhibitors

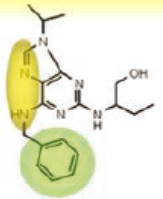
Canela et al. JBC 2006
Bagella et al. Cell Cycle 2007
Corbel et al. Chem. & Biol. 2015

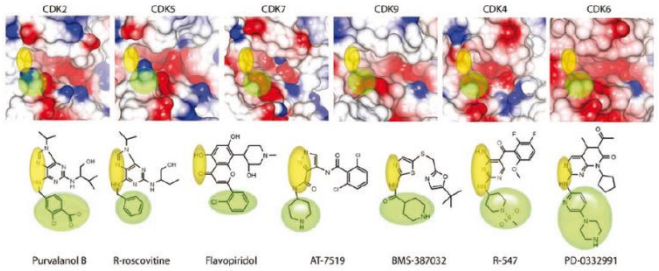
Asghar et al. Nat. Rev. Drug Disc. 2015 ; Bruyere & Meijer Curr.Opin.Cell Biol.2013 ; Lapenna and Giordano, Nat. Rev. Drug Disc., 2009

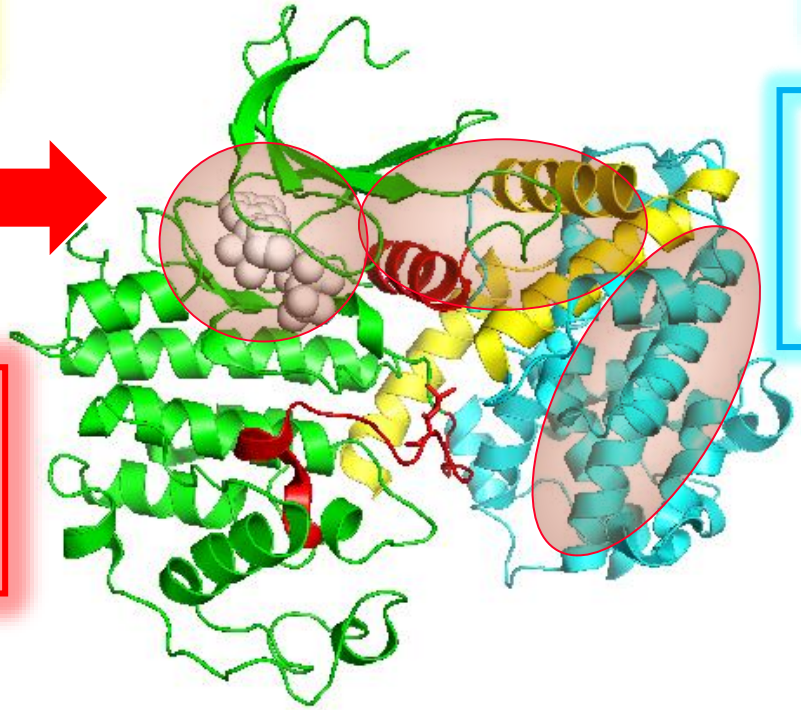
TARGETING CYCLIN-DEPENDENT KINASES

ATP-COMPETITIVE INHIBITORS

Promiscuous
Secondary Effects
Emergence Resistance







NO ALLOSTERIC INHIBITORS

ALTERNATIVE STRATEGIES
Targeting essential PPIs or conformational transitions
(with Type IV allosteric inhibitors)

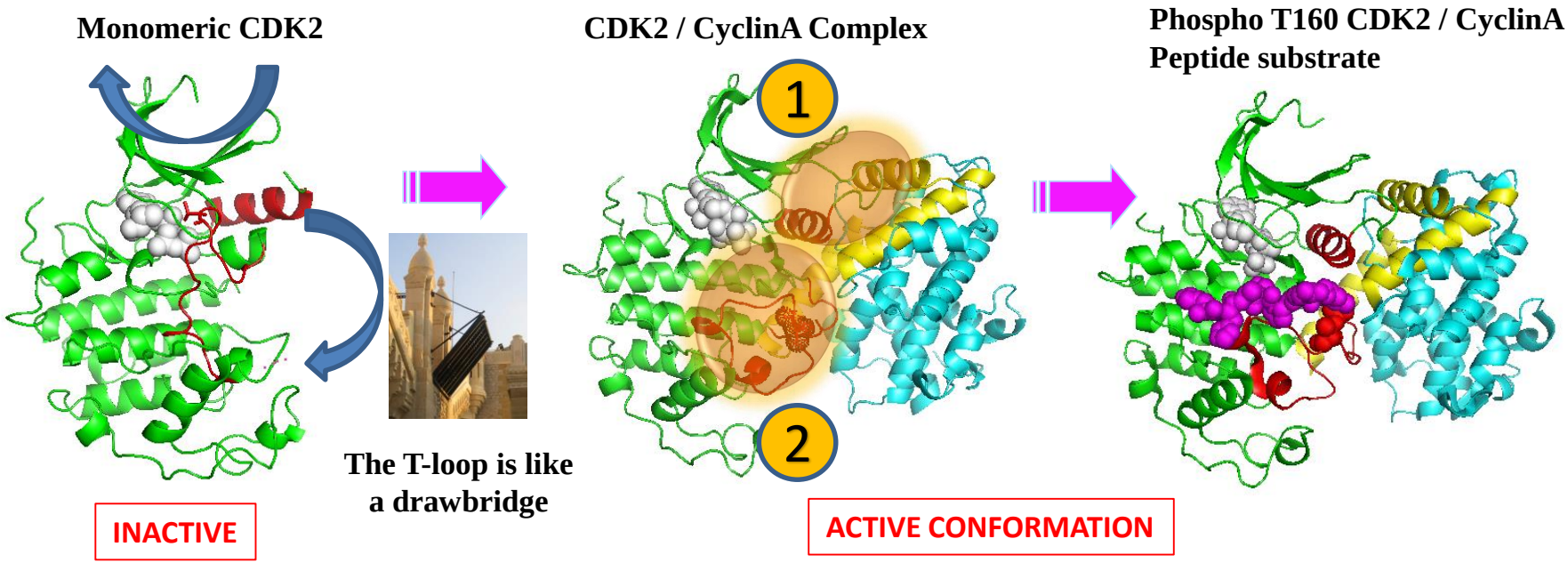
3 FDA-approved Type I ATP-competitive drugs : Abemaciclib, Palbociclib, Ribociclib

Different MOA
Gain of Selectivity
Less Adverse Effects

Asghar et al. Nat. Rev. Drug Disc. 2015 ; Bruyere & Meijer Curr.Opin.Cell Biol.2013 ; Lapenna and Giordano, Nat. Rev. Drug Disc., 2009

CDK/CYCLIN ASSEMBLY & ACTIVATION

A 2-STEP MECHANISM INVOLVING CONFORMATIONAL REORGANIZATION



The T-loop of the CDK (red) is a very dynamic, flexible unstructured loop, that behaves like a drawbridge, providing access to the substrate when the CDK binds to its cyclin partner.

STEP 1: rapid PPI and rotation of N-lobe leading to alignment of ATP pocket with catalytic site
STEP 2 : slow isomerization of C-lobe

⇒ **The primary interface between a CDK and a cyclin constitutes a first target : PPI inhibitor**

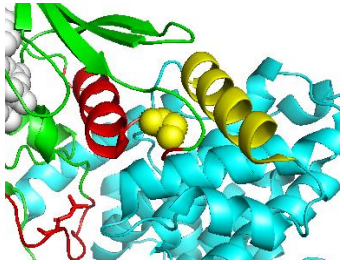
⇒ **The conformational transition constitutes a second target : allosteric inhibitor**

Morris, M. C. et al. J. Biol. Chem. (2002) Kinetic Mechanism of Activation of the Cdk2/Cyclin A Complex

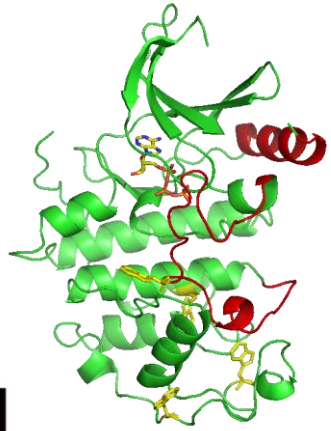
TARGETING CYCLIN-DEPENDENT KINASES
Novel Strategies for Cancer Therapeutics

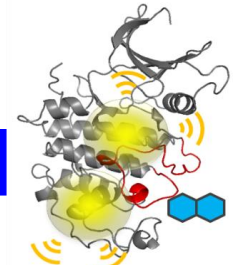
RATIONAL DESIGN
Targeting critical PPIs
Peptide & peptidomimetic therapeutics

BIOSENSOR DESIGN & HTS
Targeting conformational changes
Small molecule allosteric inhibitors

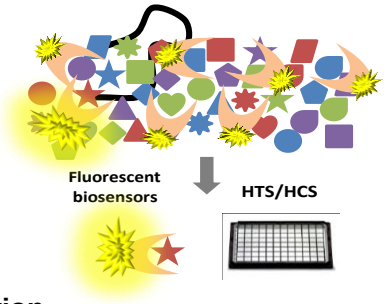


PPI Targeting

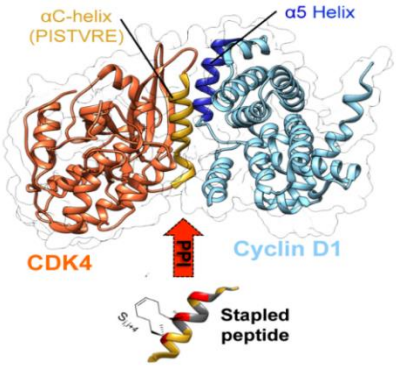


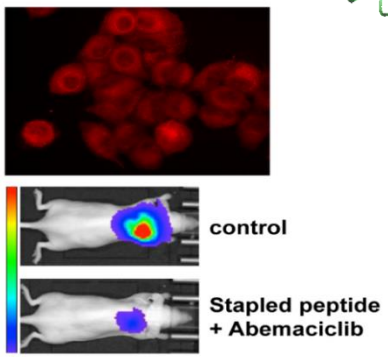


Biosensor Design & Validation

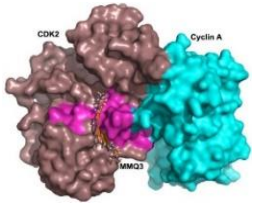


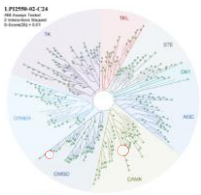
HTS Screening

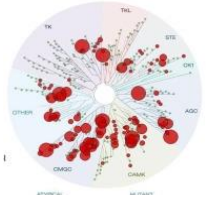




Bouclier et al. Theranostics (2020)







Pellerano et al. Biotechnology J. (2017)
Peyressatre et al. Frontiers Chemistry (2020)
Laure et al. ACS Pharmacol. Transl Sci. (2024)
Patent "New CDK4 Modulators" (2019)

INSTITUT NATIONAL DU CANCER

FOLDLUNGK4 INCA Project
Collab. M.Amblard, IBMM,
Collab. A.Hurbin, IAB, Grenoble
Collab. S.Lantuejoul CHU Grenoble

MATWIN
MATURATION & ACCELERATING TRANSLATION WITH INDUSTRY

MUSE
MONTPELLIER UNIVERSITÉ D'EXCELLENCE

CDK4PPI Project, Collab. F.Bihel, Strasbourg
CDK5 Project MUSE, Collab. N.Masurier, Montpellier
CDK5ALLOCANCER Project, PCSI INSERM
Collab. M.Tramier, Rennes & F. Bihel

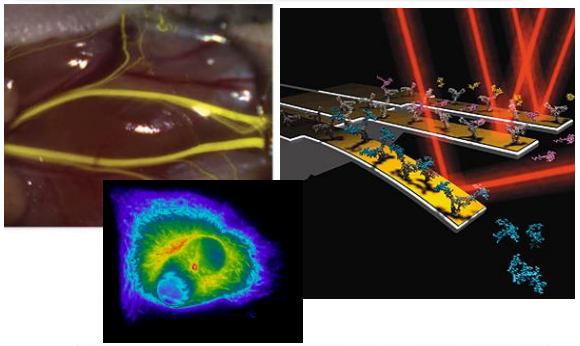
DID YOU SAY BIOSENSOR ?

Point-of-care Devices

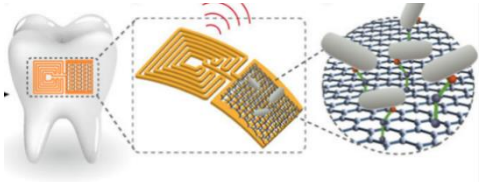
Health Monitoring

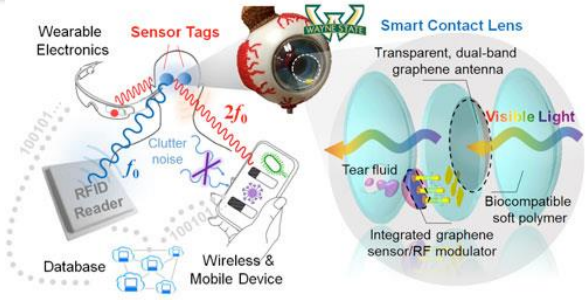
Forensics

Imaging - Optical Biosensing

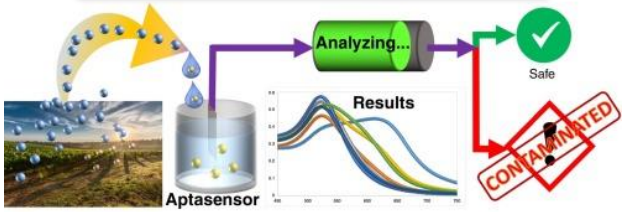


Wearable Biosensors

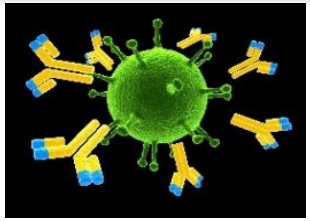




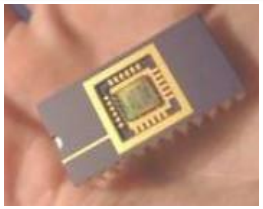
Environmental Biosensors



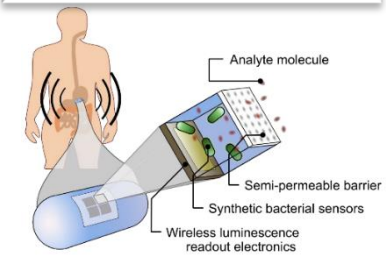
Immuno-biosensors



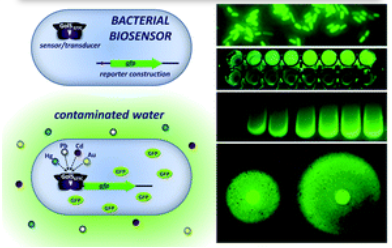
Nanobiosensor



Ingestible Biosensors



Bacterial Biosensors



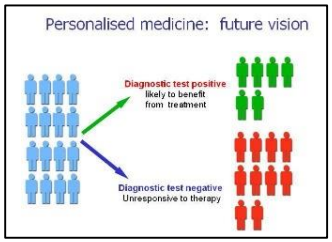
Water Biosensors

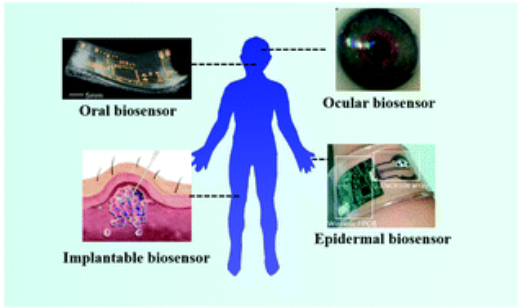
Biosensors for Health & Disease

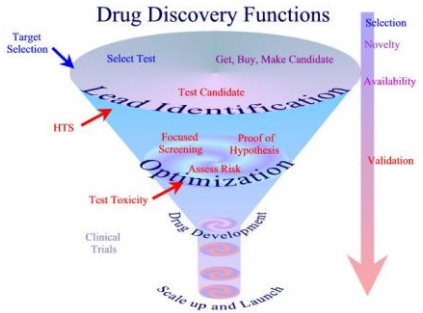
« Health Monitoring » : biosensors to follow physiological parameters

« Disease Monitoring » : biomarker detection & targeting

- Diagnostics for detection of relevant pathological biomarkers
- Patient / pathology stratification
- Point-of-care devices & Companion assays
- Personalized medicine - Theranostics
- Lab-on-a-chip – multiparametric; multiplexing
- Drug Discovery : HTS/HCS screening of new drugs





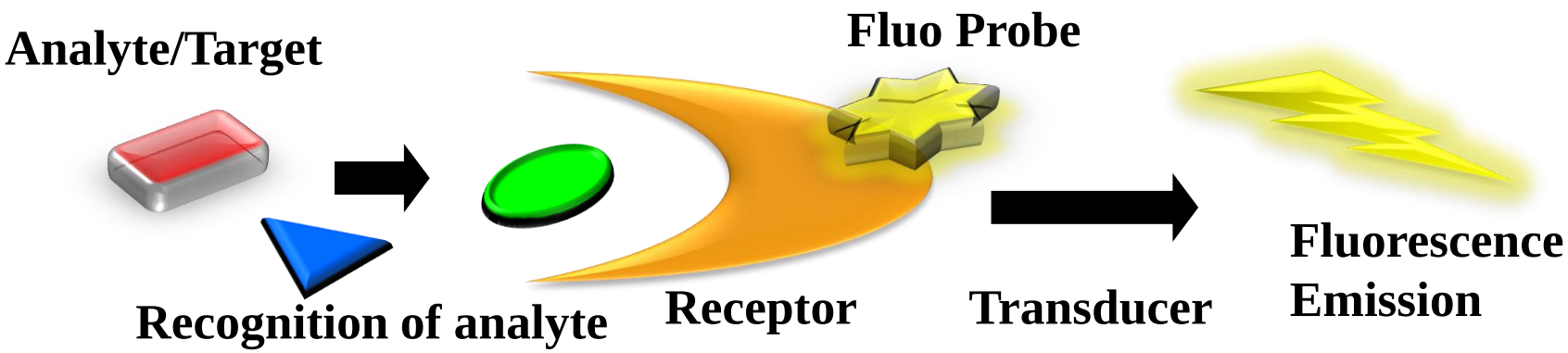


FLUORESCENT BIOSENSORS

Tools for Visualizing Biomolecules

Ions, metabolites
Enzymes & Nucleic Acids
Biomarkers & Targets

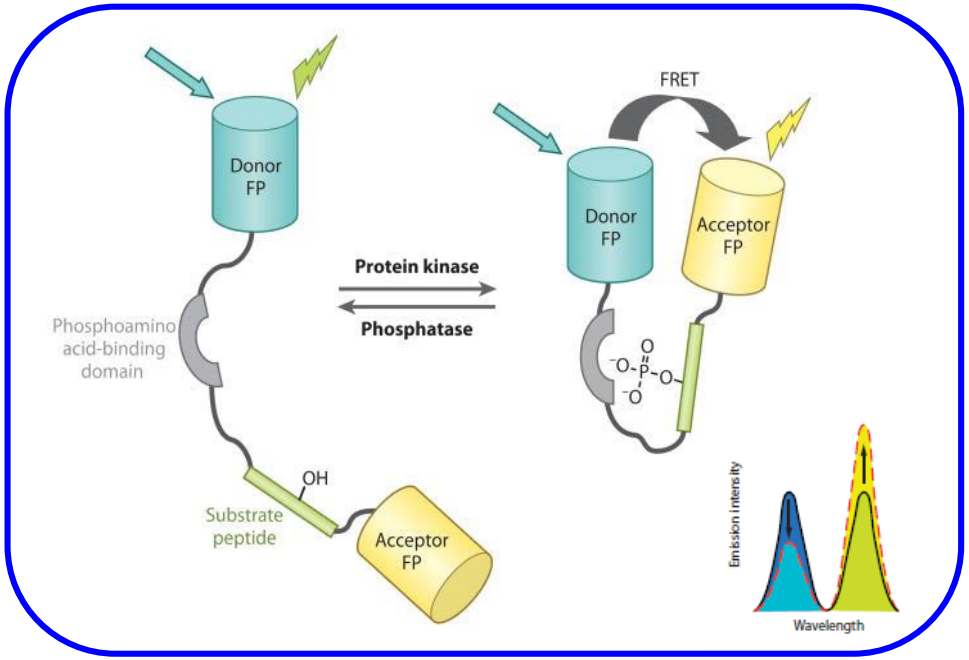
Abundance, Activity
Conformational Status
Subcellular Localization

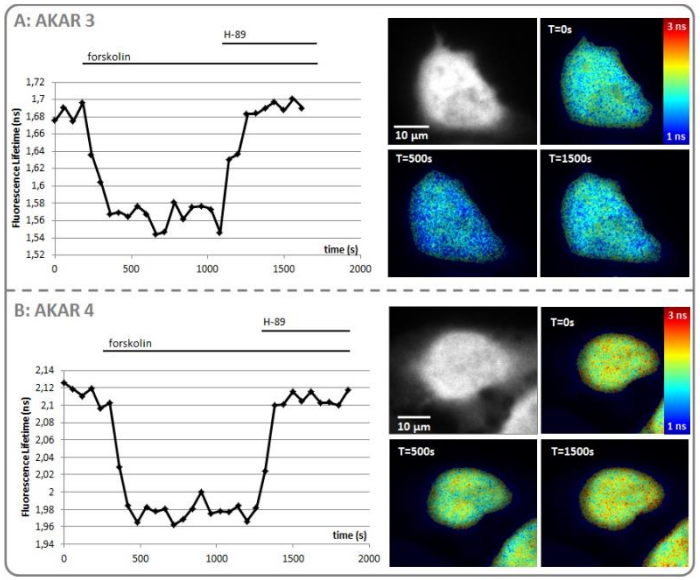


Sensitive & Non-Invasive
Dynamic & Reversible
High spatial and temporal resolution

Fluorescent biosensors are useful tools for a wide variety of applications in vitro, in cellulo and in vivo: fundamental studies, molecular diagnostics, drug discovery programmes, biotechnology, etc…

GENETICALLY-ENCODED BIOSENSORS
Single-chain FRET Biosensors





Courtesy F. Riquet & P.Vincent

Kinase Activity Reporters (KARs)

Phosphorylation of the substrate sequence favours its intramolecular interaction with the PAABD, bringing together the AFPs, and favouring FRET between donor and acceptor.

AKAR BIOSENSORS – Probing PKA

U20S cells transfected with AKAR3 or AKAR4

PKA activated with adenylate cyclase activator (forskolin) and then inhibited with H-89.

Lifetime measurements - frequency domain

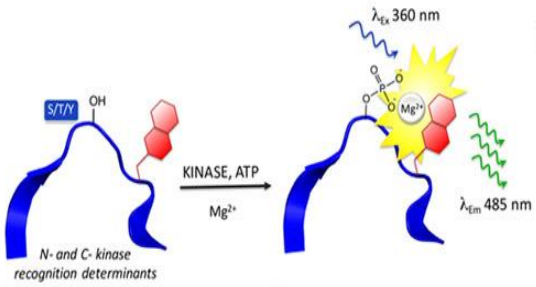
FRET between a donor and an acceptor promotes increase in **fluorescence intensity** of acceptor (decrease of donor) associated with decrease in **life-time** of the donor

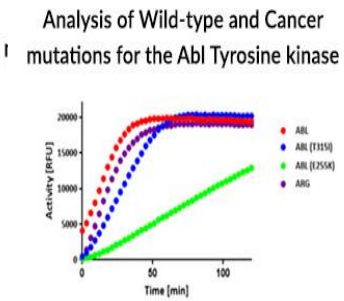
FLUORESCENT BIOSENSORS - APPLICATIONS

CHEF: Chelation Enhanced Fluorescence of Sox dye (Sulfanamido Oxine)

ASSAYQUANT TECHNOLOGY

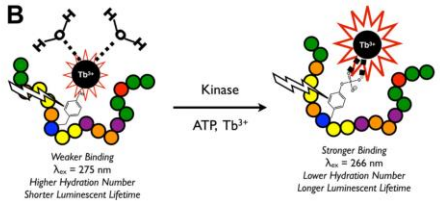
B. Imperiali Laboratory

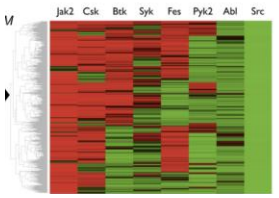




Lukovic et al. Angewandte Chemie 2009

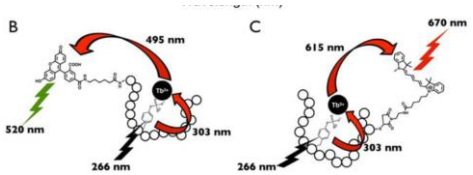
KINATEST-ID PIPELINE: Phosphorylation-dependent Terbium-sensitizing Kinase Assays

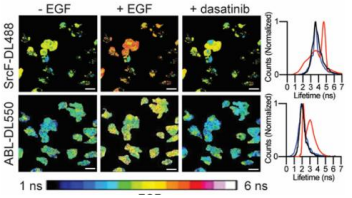




Laurie Parker Laboratory

Lipchik et al. JACS 2015

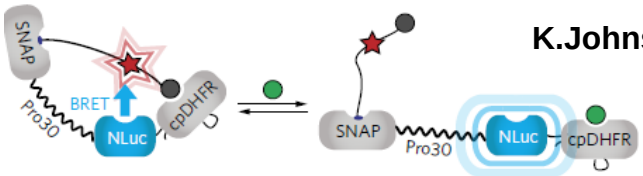


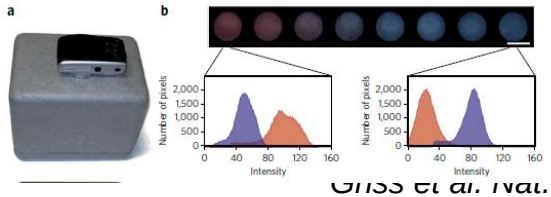


Sampreeti et al. ChemCommun 2020

LUCIDS: Luciferase Indicators of Drugs Bioluminescent sensor protein for point-of-care therapeutic drug monitoring

K.Johnsson Laboratory

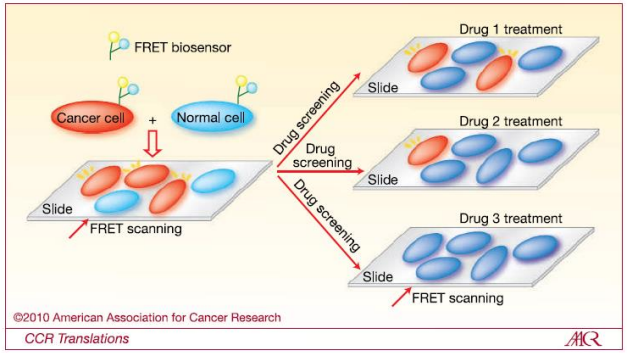




Gnss et al. Nat.Chem.Biol. 2014

A Novel FRET-Based Biosensor for the Measurement of BCR-ABL Activity and Its Response to Drugs in Living Cells

Tatsuaki Mizutani, Takeshi Kondo, Stephanie Darmanin, et al.



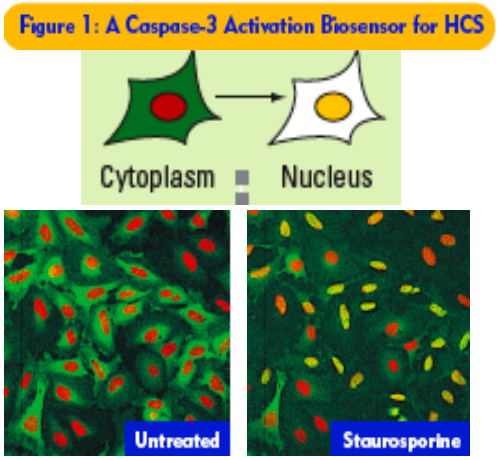
Lu & Wang, Clin. Cancer Res. 2010

FLUORESCENT BIOSENSORS FOR HTS/HCS

Drug discovery applications in vitro, in extracto and in cellulo

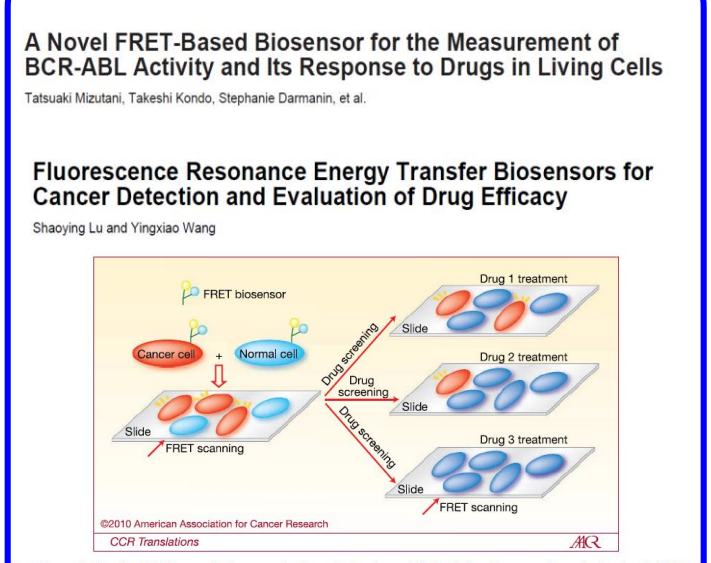
- Drug Discovery Programmes : HTS & HCS
- Positional, FRET, Intensity-based biosensors
- Multiparametric Screens
- Postscreen validation of hits
- Characterization and Optimization of hits to leads
- Preclinical Evaluation of Drugs (biodistribution, pharmacokinetics, response)

Positional Biosensors



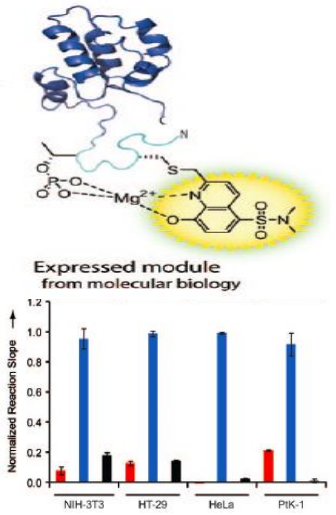
K.A. Giuliani et al.
Modern Drug Discovery 2003

FRET Biosensors



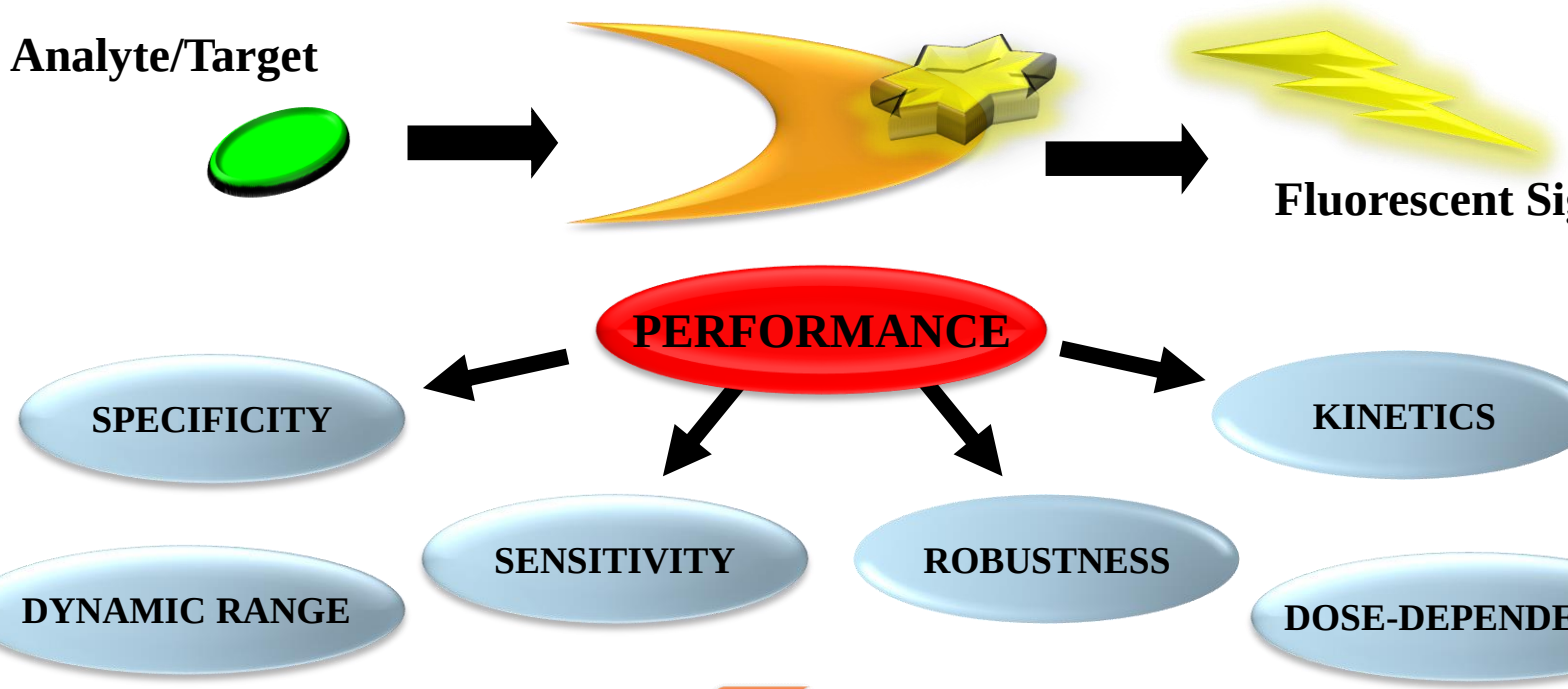
Lu & Wang, Clin. Cancer Res. 2010

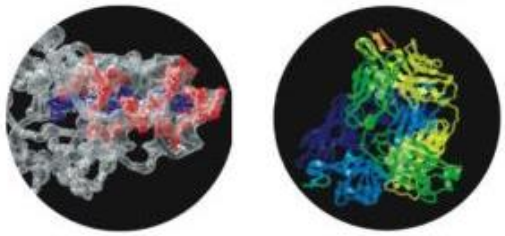
Environmentally-sensitive Biosensors

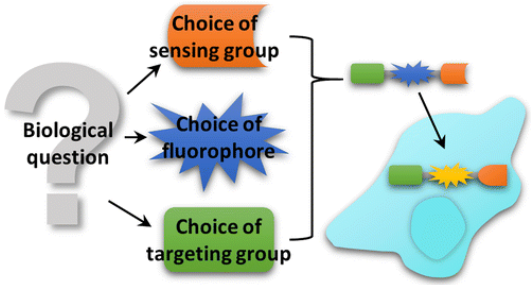


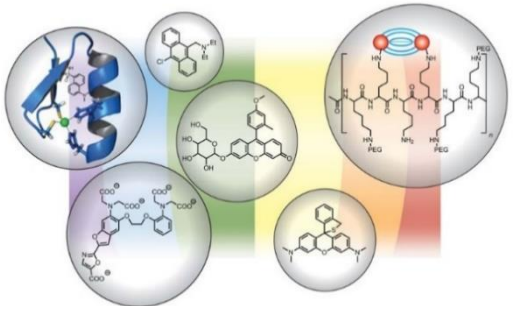
Lukovic et al.
Angewandte Chemie 2009

DESIGNING SELECTIVE & SENSITIVE BIOSENSORS









DESIGN & ENGINEERING SCAFFOLD

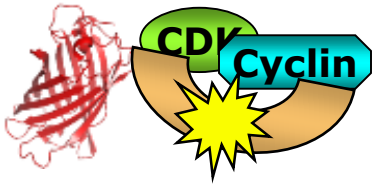
Selectivity – Stability - Robustness

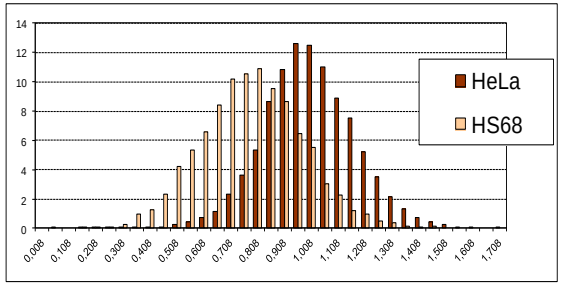
CHOICE OF FLUORESCENT PROBE

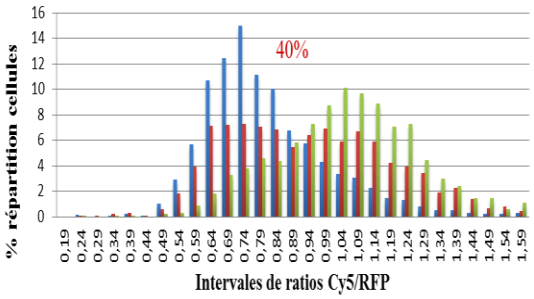
Sensitivity - Robustness

CDKSENS, CDKACT & CDKCONF BIOSENSORS

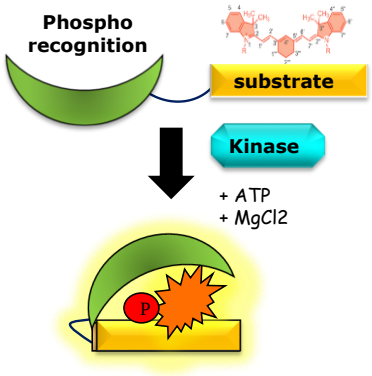
CDKSENS : probing relative abundance



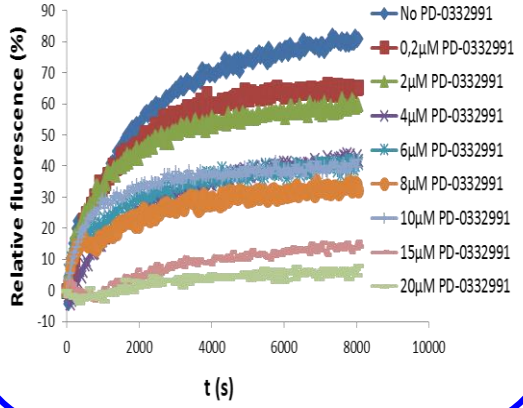




CDKACT : probing kinase activity

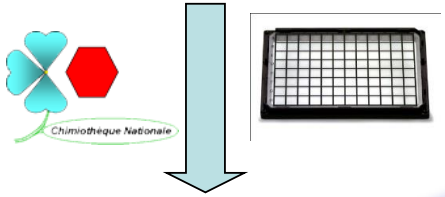


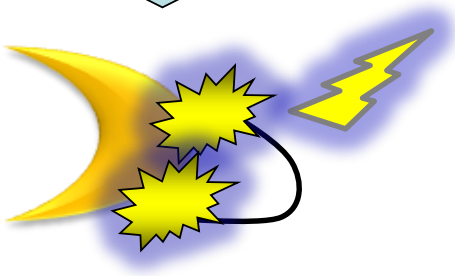
melanoma cell extracts (A375)



CDKCONF : probing conformational dynamics

« Molecular Hinges »



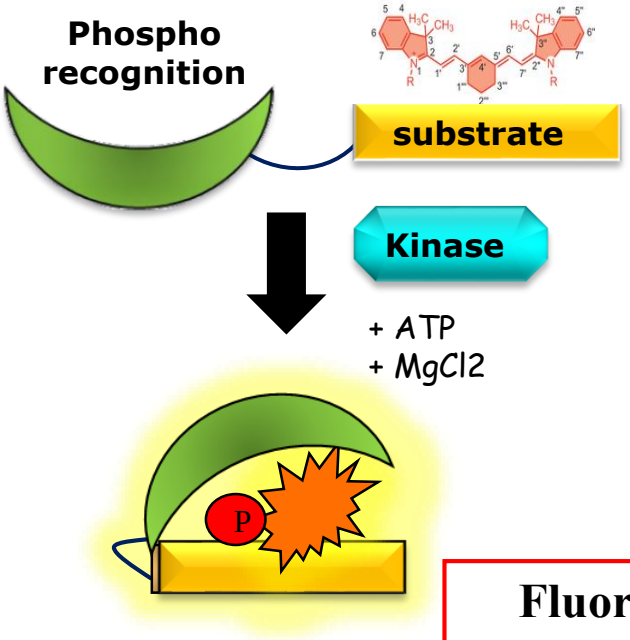


Multiparametric High Content Screen
Cellomics ArrayScan Robot

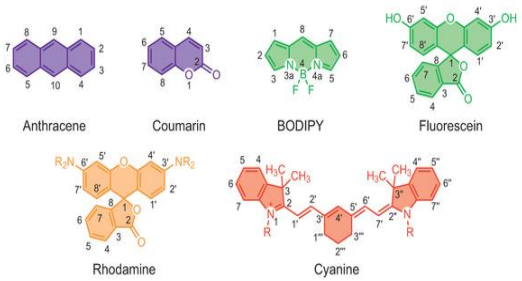
High Throughput Screen
Mammalian Cell Extracts

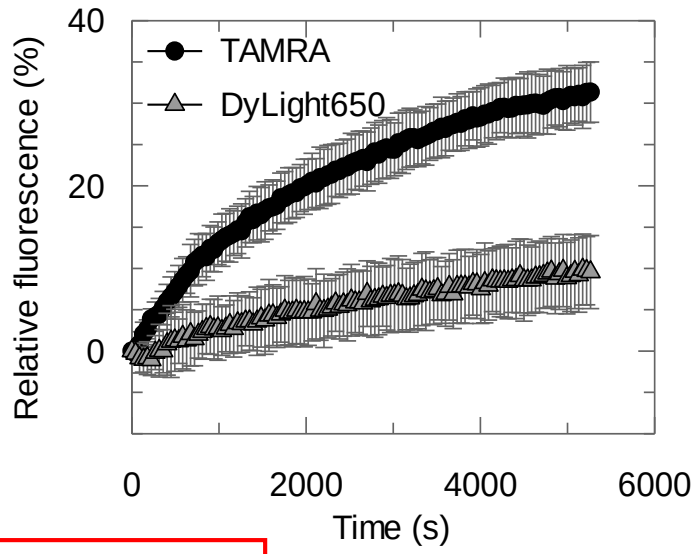
High Throughput Screen
In vitro, in solution

CDKACT BIOSENSOR TECHNOLOGY



Environmentally-sensitive Fluorescent Dye





Fluorescence enhancement in response to kinase activity and substrate phosphorylation

- Peptide / polypeptide conjugated to synthetic fluorescent probe
- Specific recognition (bioreceptor) : substrate or interface
- Reponse, sensitivity, dynamic range : choice of fluorescent probes
- Modularity & Versatility

- **Simple**
- **Sensitive**
- **Selective**
- **Reversible**
- **Dynamic**

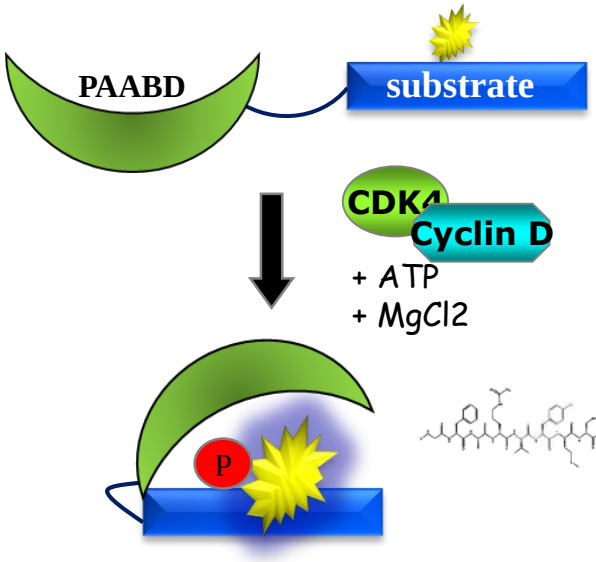
IBMM Institut des Biomolécules Max Mousseron

cnrs

Van TNN et al. ChemBioChem 2014

CDKACT Technology – Patent WO2014 / 057044

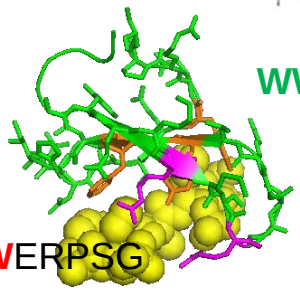
CDKACT4 BIOSENSOR TECHNOLOGY



Rb substrate sequence
YKFCSSPLRIPG

PAABD short:
GFARVYMSRSSGWERPSG

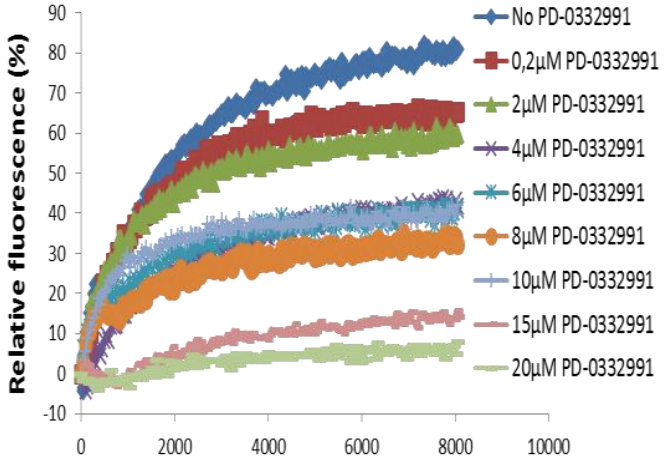
WW Pin 1:
GWEKRMSRSSGRVYYFNHITNASQWERPSG



WW Pin1

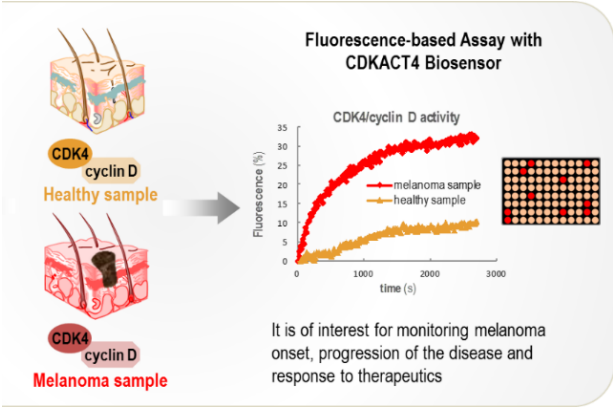
phSer peptide

melanoma cell extracts (A375)



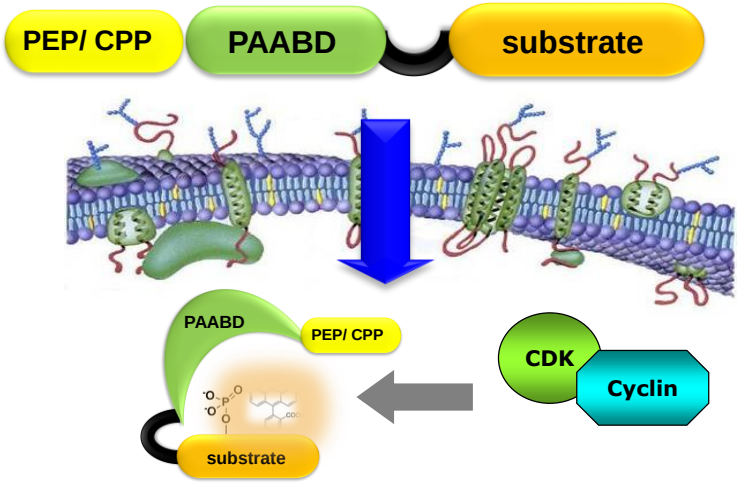
150nM CDKACT4-TAMRA

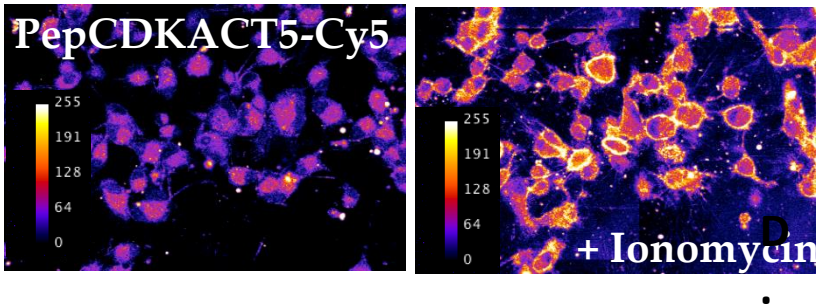
- Dose-dependent response to CDK4 kinase activity
- In cell extracts, mouse xenografts & biopsies
- Monitoring Response to Therapeutics
- Towards Diagnostic assays

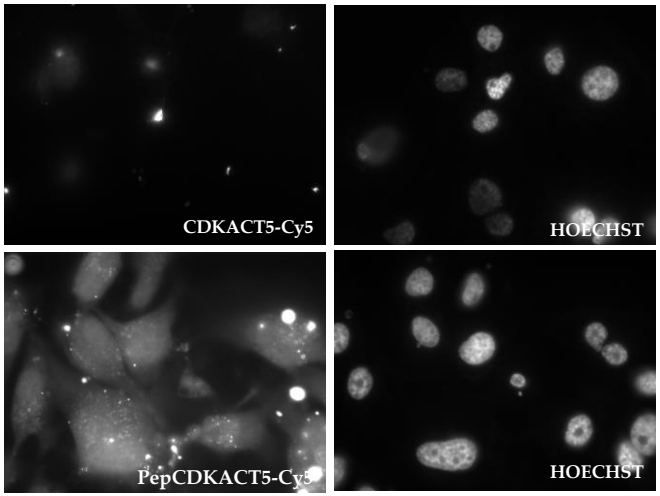


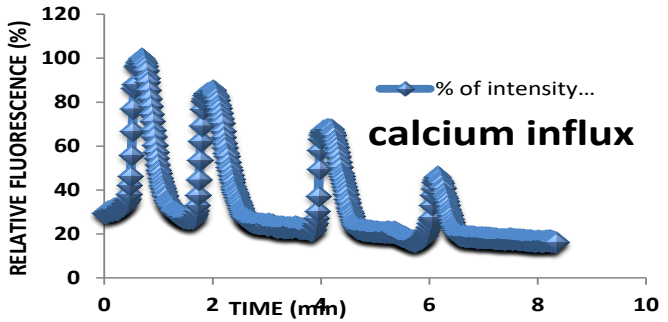
Prével C. et al. (2016) Fluorescent peptide biosensor for monitoring CDK4/cyclin D kinase activity in melanoma cell extracts, mouse xenografts and skin biopsies. *Biosens. Bioelectron*.

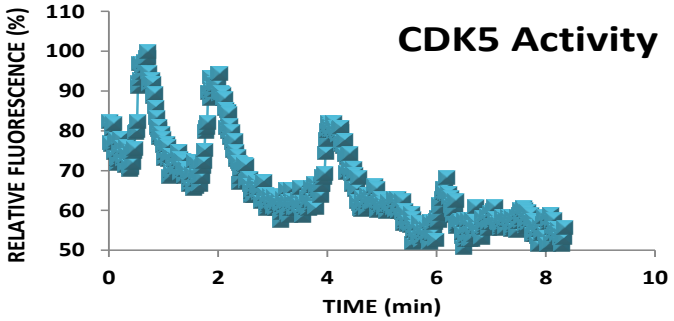
CELL-PENETRATING CDKACT5 TO IMAGE CDK5 ACTIVITY IN U87 CELLS







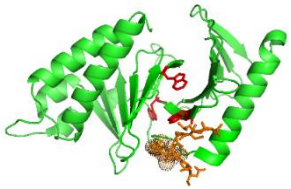


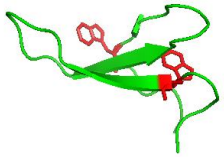


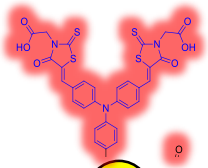
Peyressatre et al. Biotechnology J. 2020

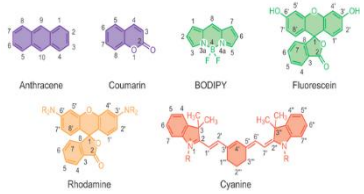
Fluorescent Peptide Biosensor Toolbox

Gaining Insight into the Landscape of Protein Kinase Activities ih cancer



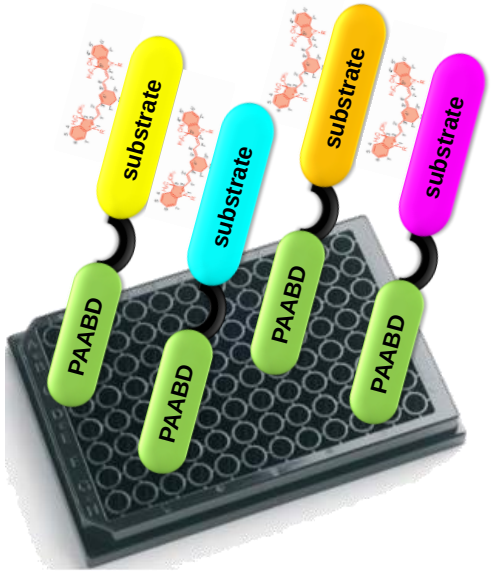






C -S/T-P

Phosphoamino acid binding domain | **linker** | **Substrate sequence**



- Multisensing platform
- Multiplex sensing
- Kinase activity profiling

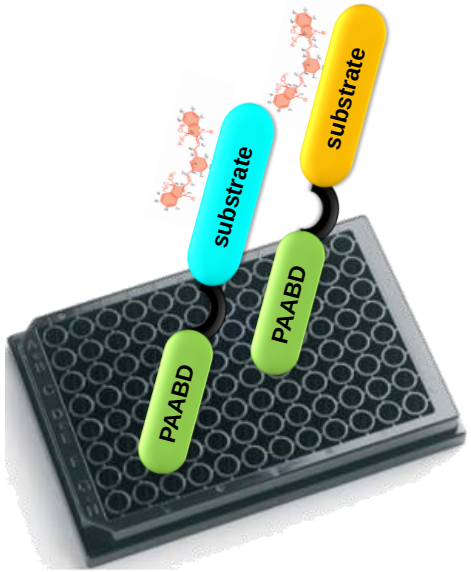
Substrate sequence

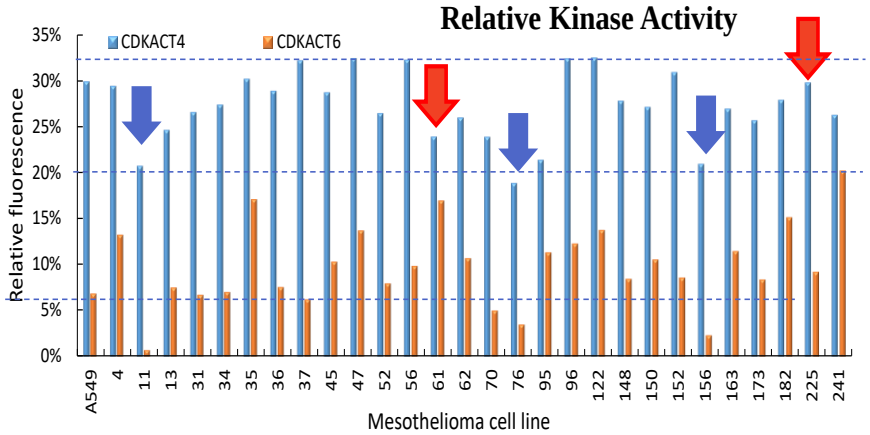
Substrate sequence

Substrate sequence

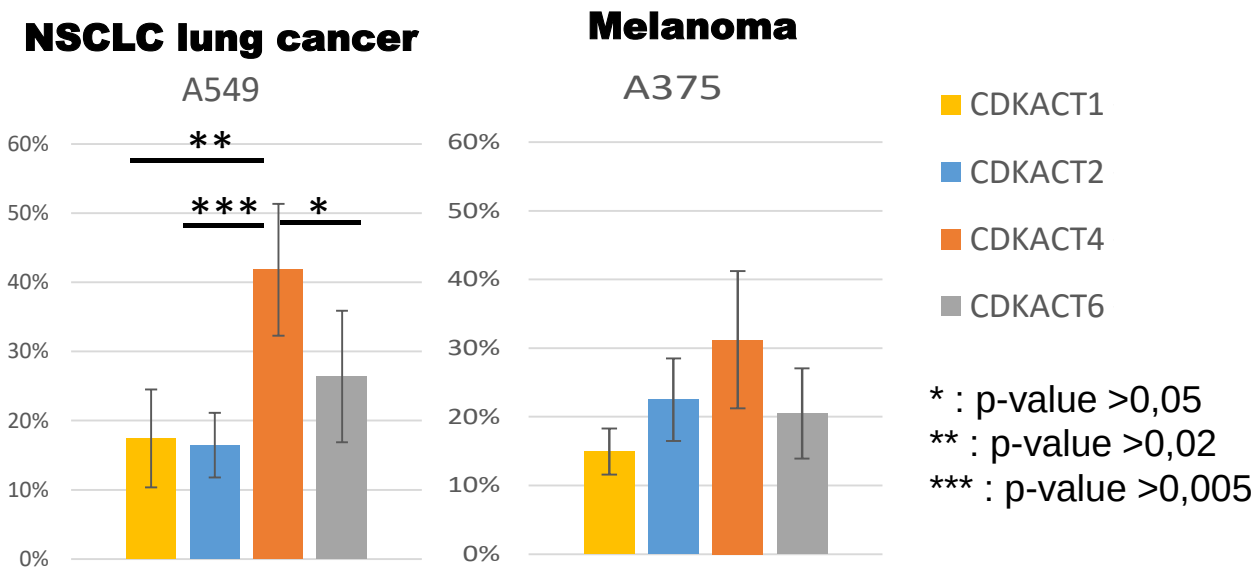
l'Europe s'engage en Occitanie
UNION EUROPÉENNE
La Région Occitanie Pyrénées · Méditerranée
GENEPEP PEPTIDE & PROTEIN SYNTHESIS
CONTRE LE CANCER LA LIGUE pour la vie COMITÉ DE L'HÉRAULT
PROJET COFINANCÉ PAR LE FONDS EUROPÉEN DE DÉVELOPPEMENT RÉGIONAL

Profiling CDK Activities in cell lines



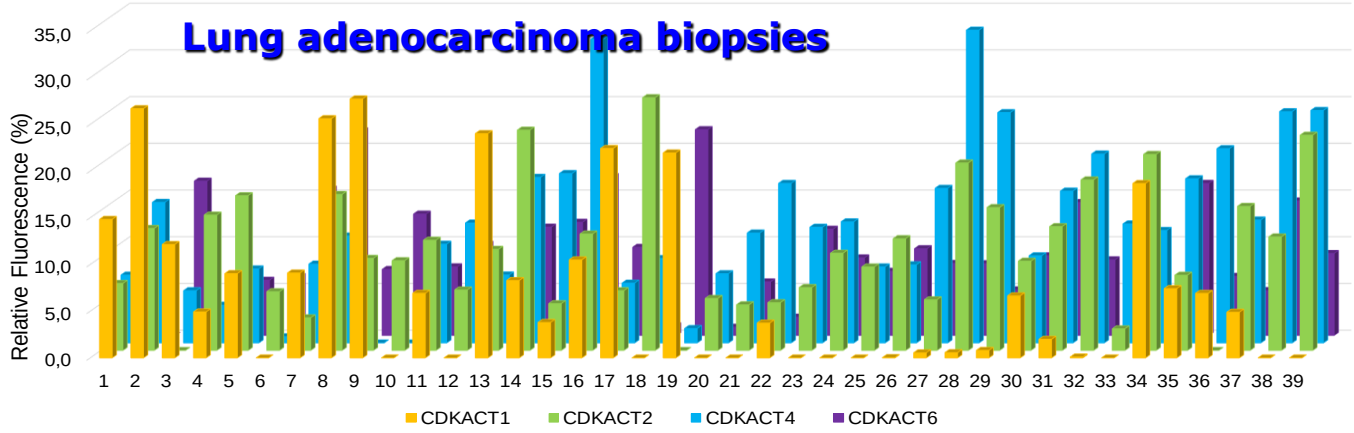


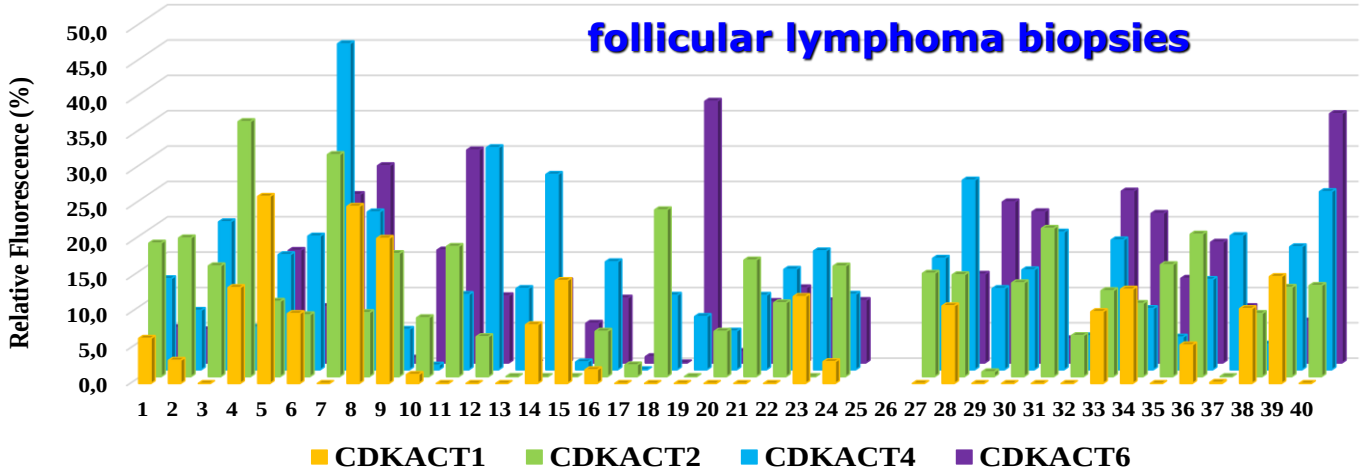
Collaboration Christophe Blanquart, Université Nantes, INSERM CRCINA, France



* : p-value >0,05
** : p-value >0,02
*** : p-value >0,005

Large-scale Profiling of CDK Activities in tumour biopsies from human patients



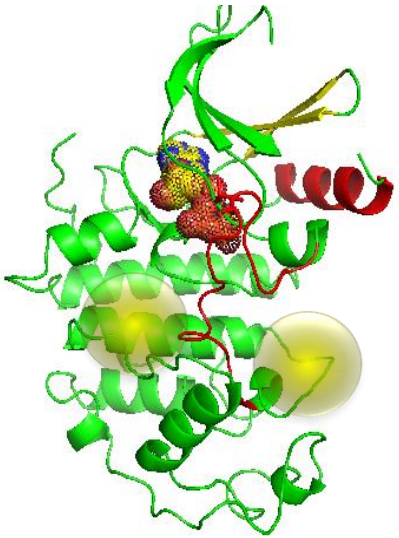


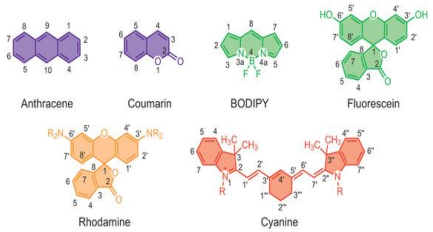
Profiling Protein Kinase Activities in Tumour Biopsies through multiplexed biosensing with fluorescent peptides
Royet C , Diot S, Lecki L, Lorcy F, Lacheretzszablewski V, Serre I and Morris M.C, *ACS Sensors 2024*

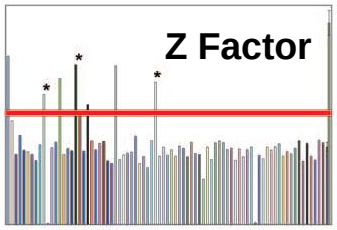
DESIGNING CONFORMATIONAL BIOSENSORS & SCREENING FOR ALLOSTERIC MODULATORS

Incorporation of fluorescent probes into the kinase scaffold

FLIK – Fluorescent Labels in Kinases

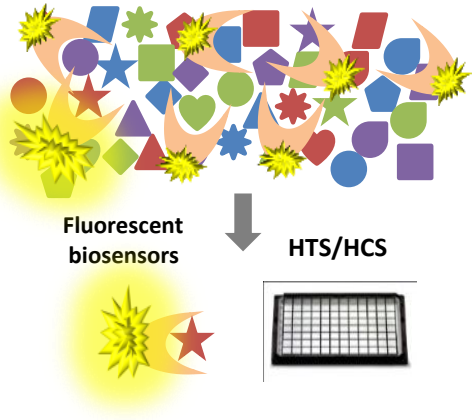






- Validation
- Optimization
- Miniaturization
- Performance
- Statistical value

Establishment of Screening Assay & HTS




Camille Prével


Morgan Pellerano


Marion Peyressatre


Juan Antonio Gonzalez Vera

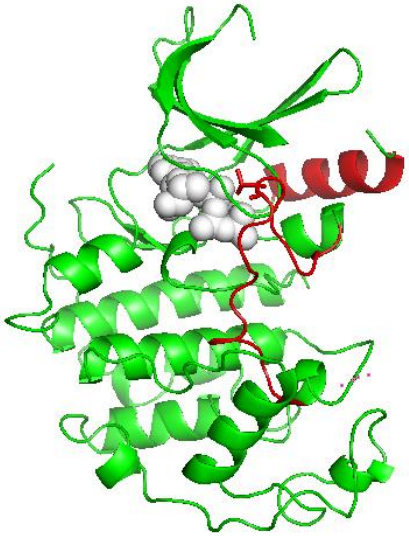

Sergey Tcherniuk

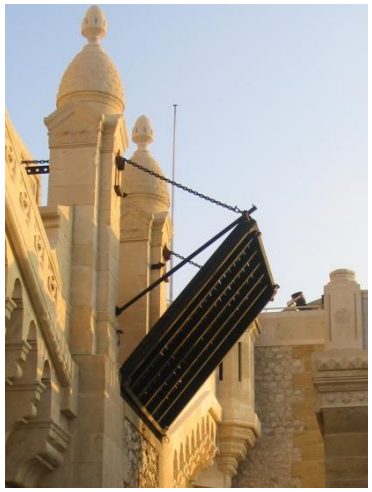
CDKCONF TECHNOLOGY PCT/EP2013/ 073747 - WO2014 / 076140

CDKCONF BIOSENSORS
Sensing Conformational Changes

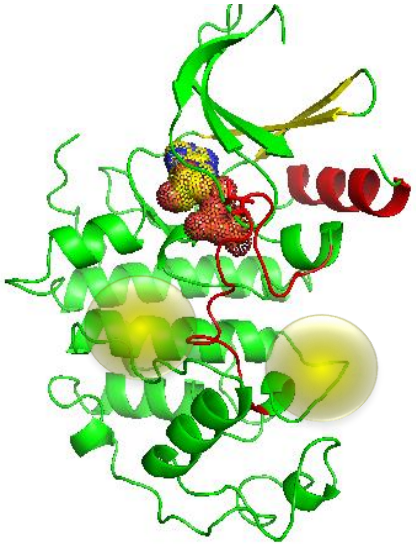
Incorporation of fluorescent probes into the kinase scaffold

Monomeric CDK2



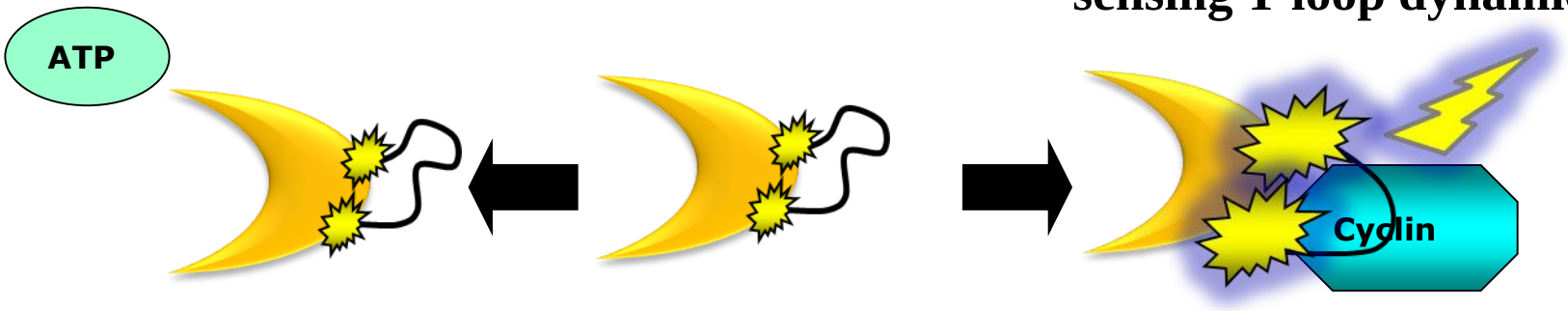


The T-loop is like a drawbridge



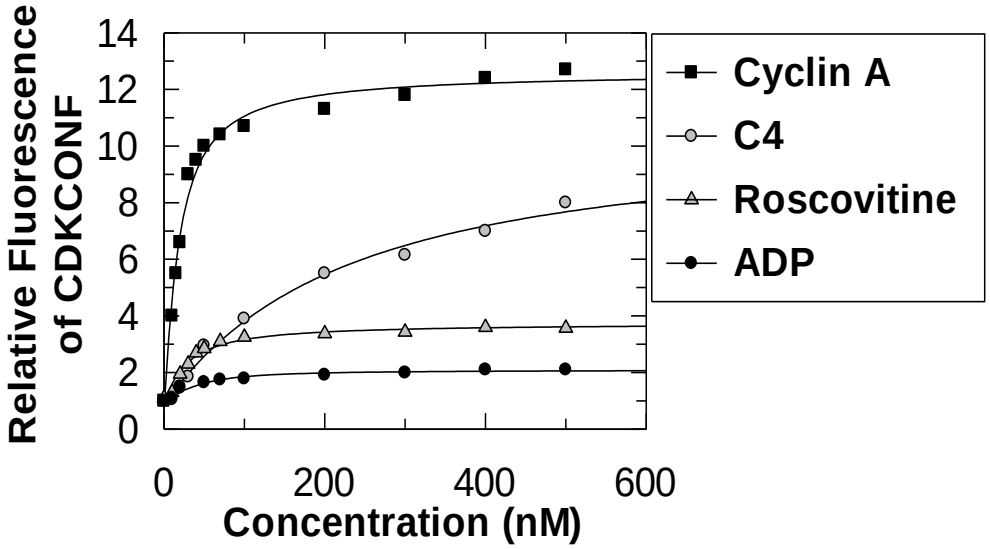
- **Insensitive to ATP pocket binders**
- **Responsive to allosteric modulators**

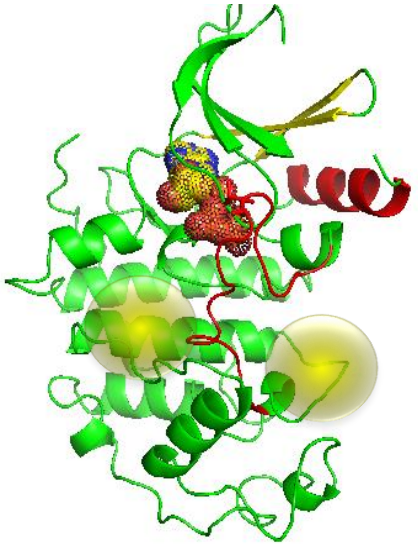
CDKCONF BIOSENSOR « Molecular hinges » for sensing T-loop dynamics



CDKCONF BIOSENSORS
Sensing Conformational Changes

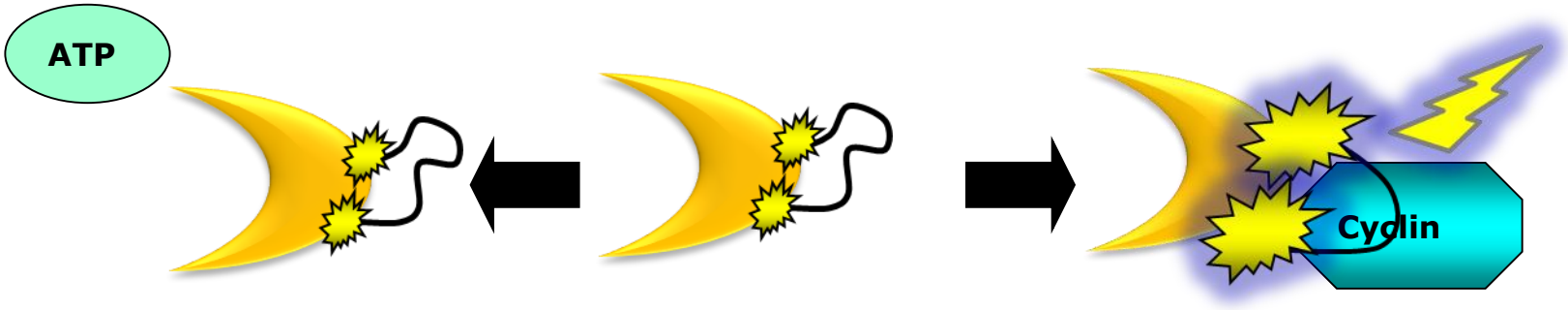
Incorporation of fluorescent probes into the kinase scaffold





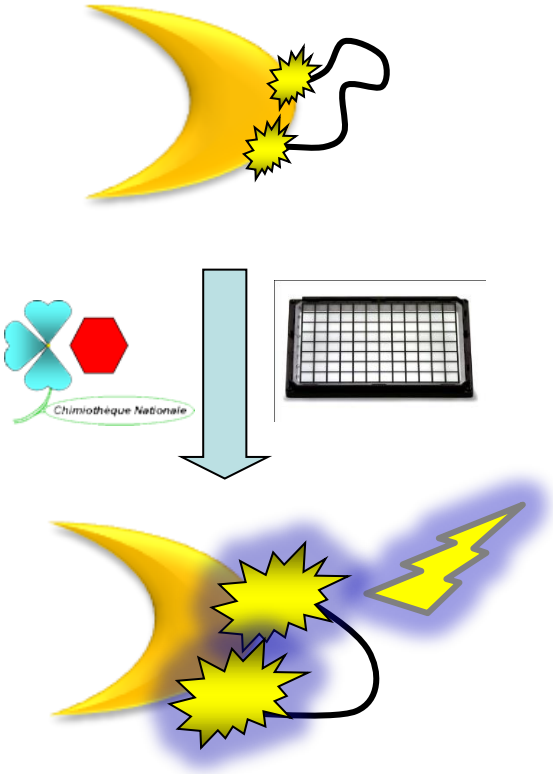
CDKCONF BIOSENSOR
« Molecular hinges » for sensing T-loop dynamics

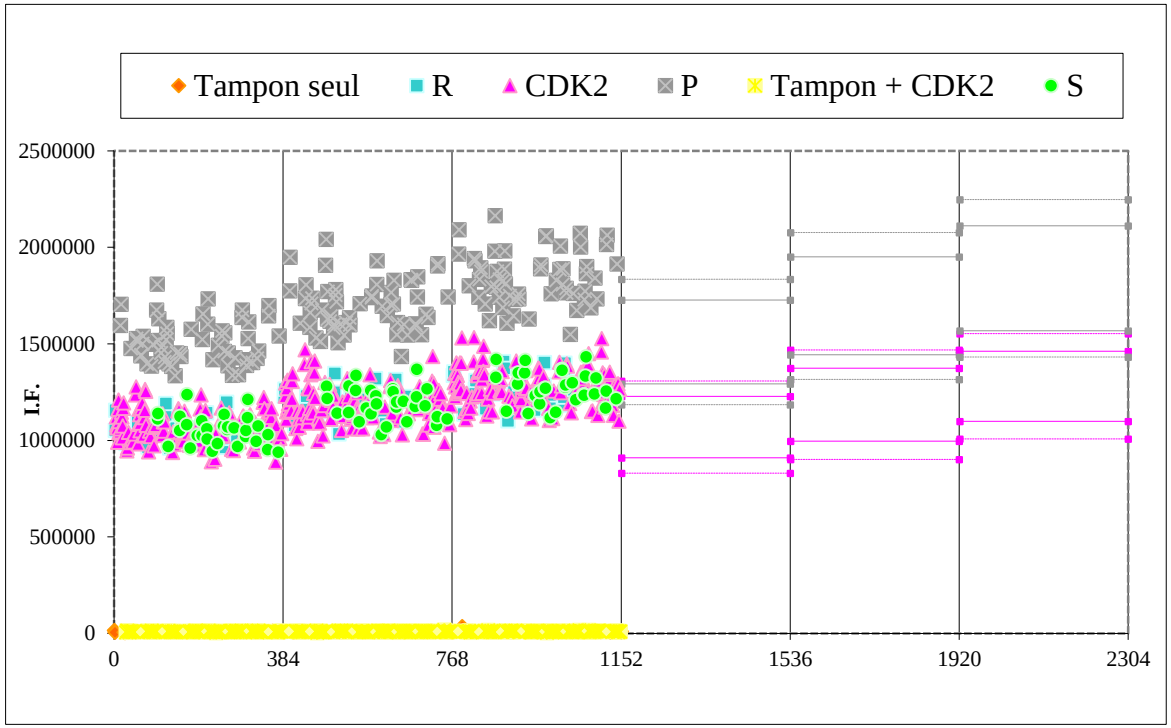
- **Insensitive to ATP pocket binders**
- **Responsive to allosteric modulators**



CDKCONF BIOSENSOR
Downscaling & Optimization of Screen

« Molecular Hinges »



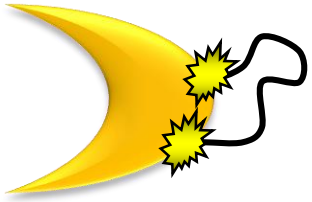


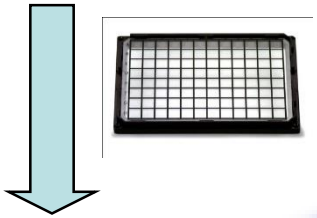
Orange squares : buffer
Yellow squares : buffer + CDK2
Pink triangles: CDKCONF 5nM
Green circles: CDKCONF 5nM+ Staurosporine 10µM
Blue squares: CDKCONF 5nM+ Roscovitine 10µM
Grey Squares CDKCONF 5nm +C4 Peptide at 5µM
lines: ± 2 SDE ; dotted lines :+/-3 SDE

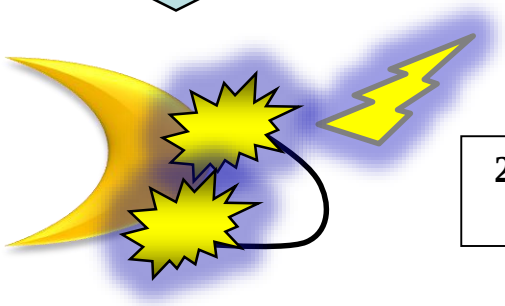
CDKCONF BIOSENSOR
In search of allosteric inhibitors of CDK2

High throughput Screen French National Library of Chemical Compounds : 18480 cpds

« Molecular Hinges »





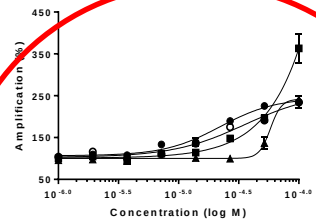


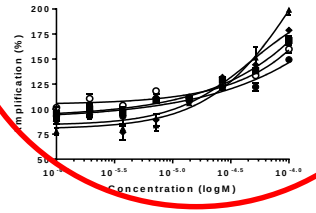
CDKCONF-Cy3 Screen
18480 chemical compounds

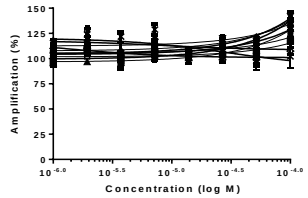
264 hits
amplification of fluorescence
equal to or greater to that of C4

201 autofluorescent compounds

47 hits reconfirmed manually







9 selected compounds

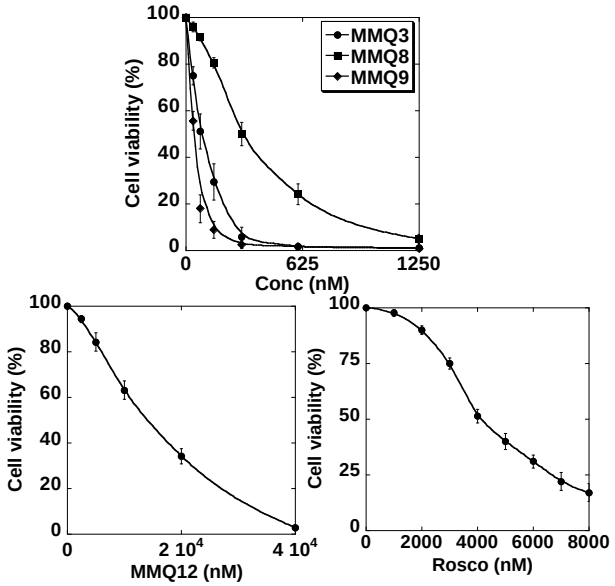
Collaboration C.Pérals, USR3388 CNRS-Pierre Fabre

NEW CLASS OF CDK2 INHIBITORS : MMQs

MMQs – Metaquinacridines
modulators of CDK2 conformation and Inhibitors of cell proliferation

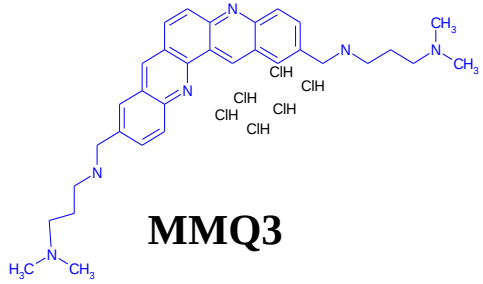
Coll. Florence Mahuteau-Betzer (Orsay)

Anti-proliferative effect in HeLa cells

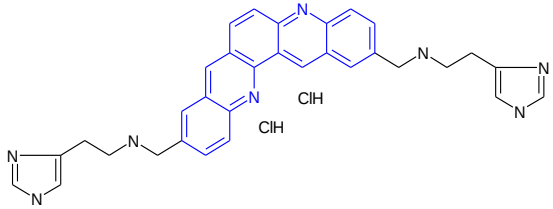


IC50 values (nM)

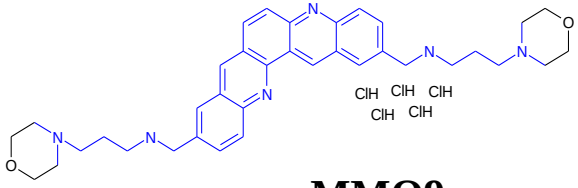
MMQ3	**70+/- 6**
MMQ8	**280 +/- 15**
MMQ9	**50 +/- 8**
MMQ12	**15000+/-500**
Roscov.	**4500 +/-100**



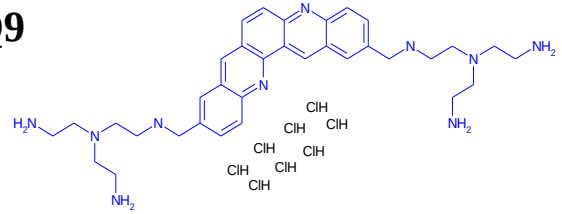
MMQ3



MMQ8

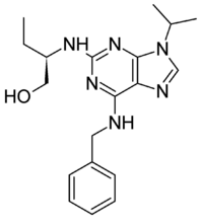


MMQ9



MMQ12

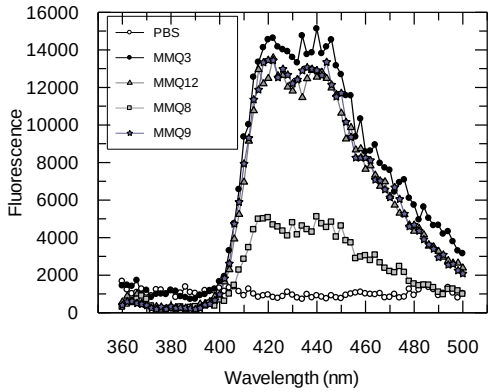
Roscovitine

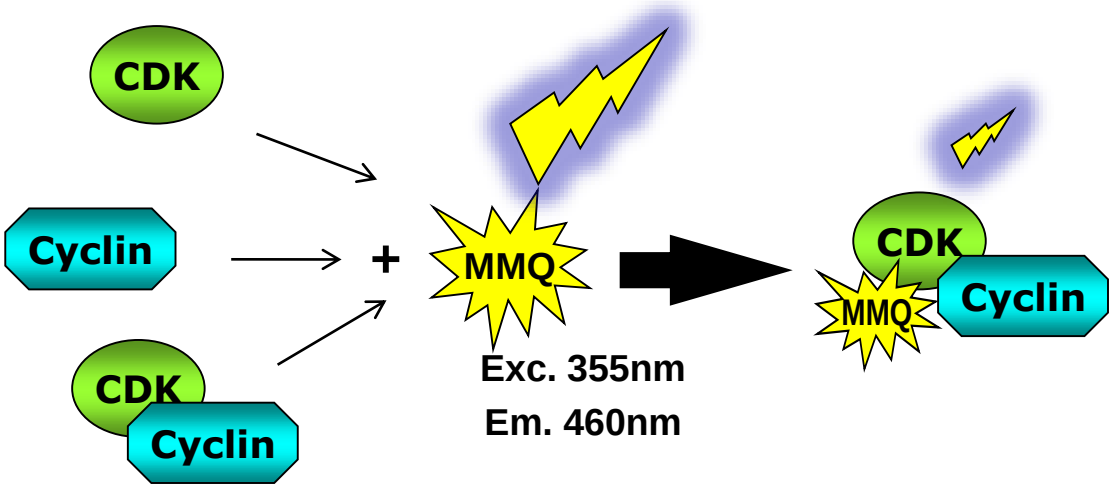


Telomerase inhibitors based on quadruplex ligands selected by a fluorescence assay

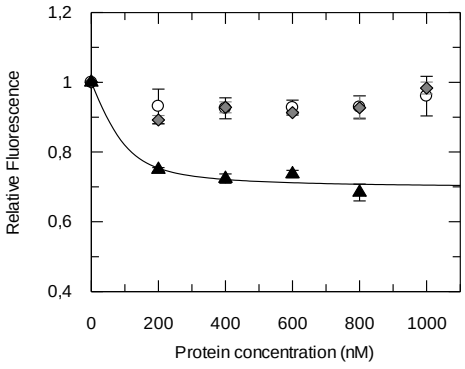
Jean-Louis Mergny*†, Laurent Lacroix*‡, Marie-Paule Teulade-Fichou§, Candide Hounsou§, Lionel Guittat*, Magali Hoarau*, Paola B. Arimondo*¶, Jean-Pierre Vigneron§, Jean-Marie Lehn§, Jean-François Riou‖**, Thérèse Garestier*, and Claude Hélène*

MMQs bind the CDK2/CyclinA complex



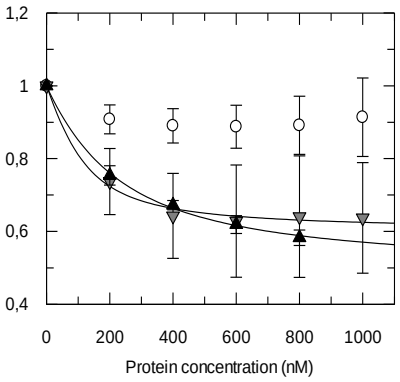


MMQ3



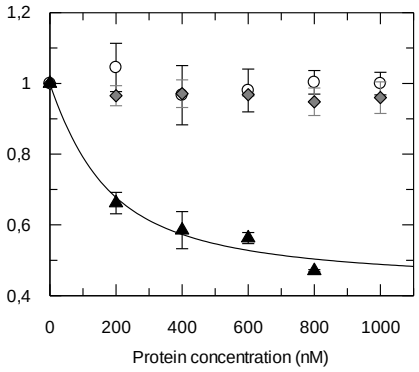
Kd (nM) 28 ± 26

MMQ12



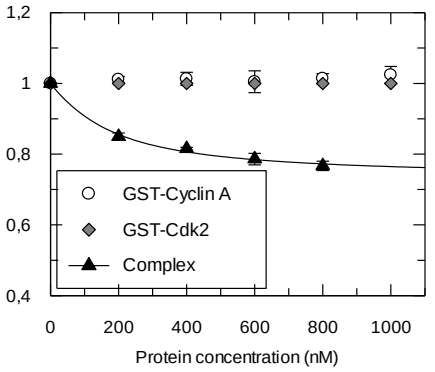
Kd (nM) 174 ± 26

MMQ8



Kd (nM) 114 ± 26

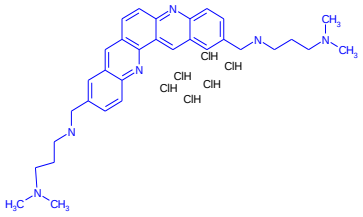
MMQ9



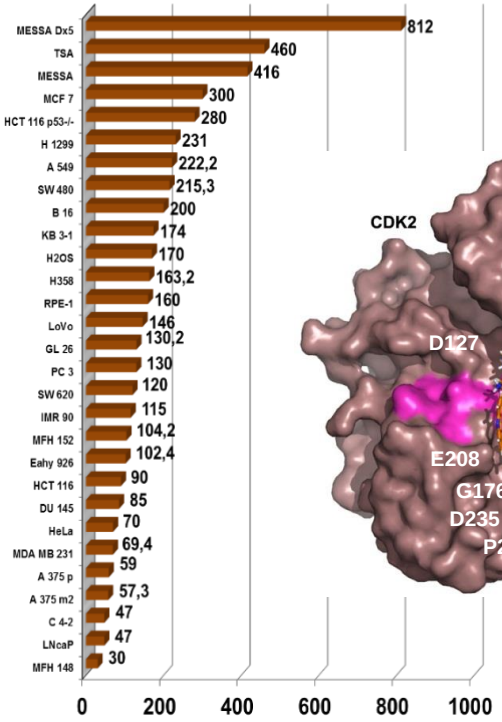
Kd (nM) 125 ± 35

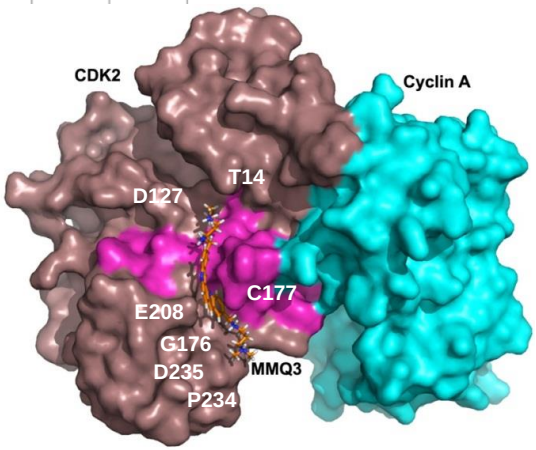
PROOF-OF-CONCEPT FOR SCREENING FOR ALLOSTERIC MODULATORS OF CDK2

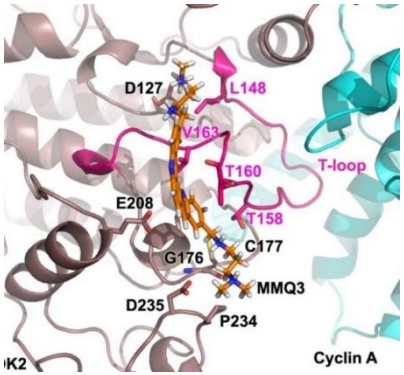
HTS -SCREEN
20'000 Compounds
(Chimiothèque Nationale)



Growth inhibition of different cell lines by MMQ3 – IC50 (nM).







MMQ3 staples onto the T-loop and binds several T-loop residues, as well as several hotspots adjacent to this activation segment, which are critical for CDK regulation

MMQs – Metaquinacridines modulators of CDK2 conformation and inhibitors of cell proliferation

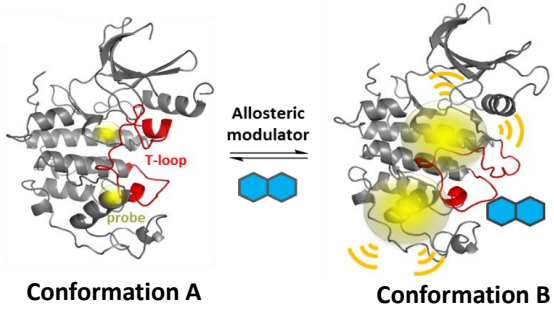
Coll. C.Pérals, USR3388 CNRS-Pierre Fabre

Coll. Florence Mahuteau-Betzer (Orsay) ; Coll. Elsa Garcin (Marseille)

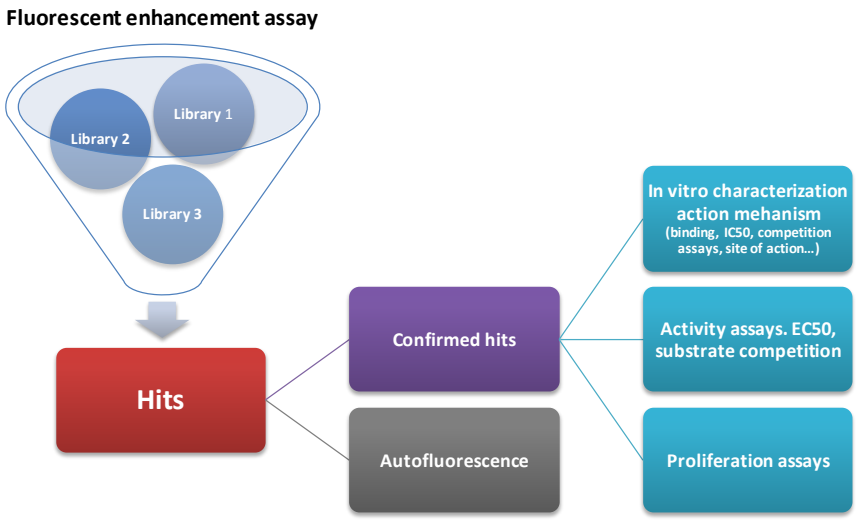
Pellerano et al. Biotechnology J. (2017) Targeting Conformational Activation of CDK2

DESIGNING CONFORMATIONAL BIOSENSORS & SCREENING FOR ALLOSTERIC MODULATORS

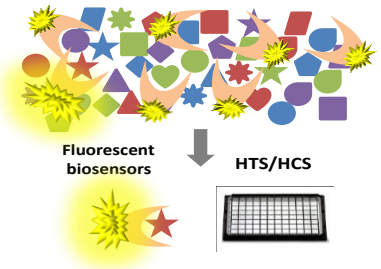
Biosensor Design & Validation



Characterization of Hits in vitro / in cellulo



Establishment of Screening Assay & HTS



Compounds were screened at 10µM with 10nM CDKCONF biosensors on a TECAN™ freedom EVO

Camille Prével

Morgan Pellerano

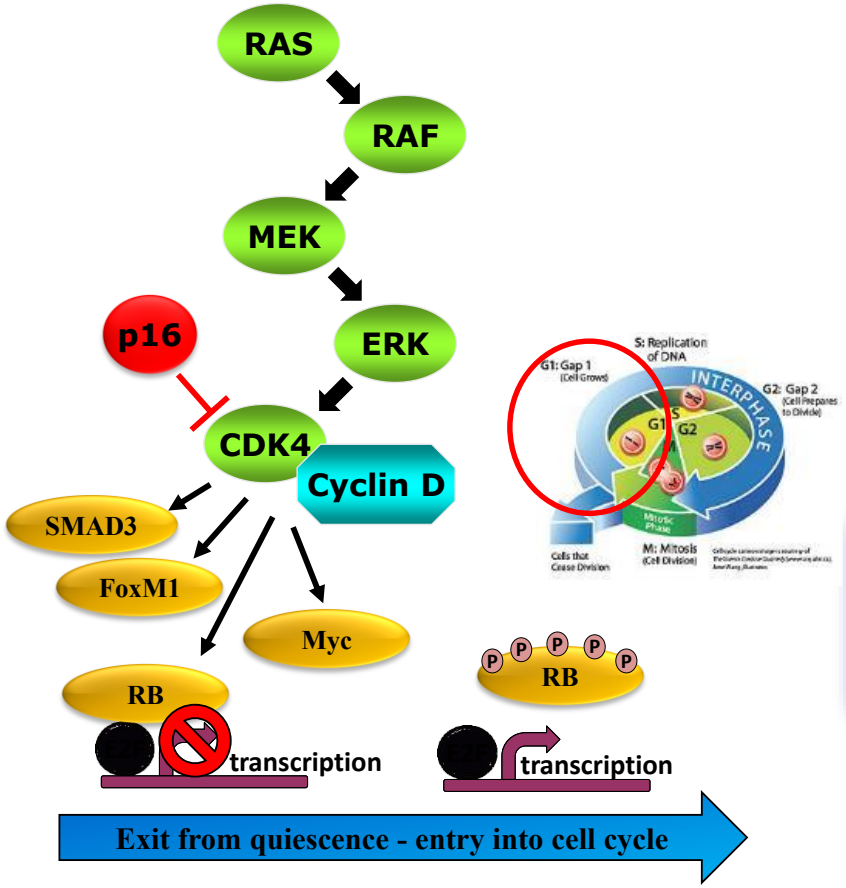
Marion Peyressatre

Juan Antonio Gonzalez Vera

Sergey Tcherniuk

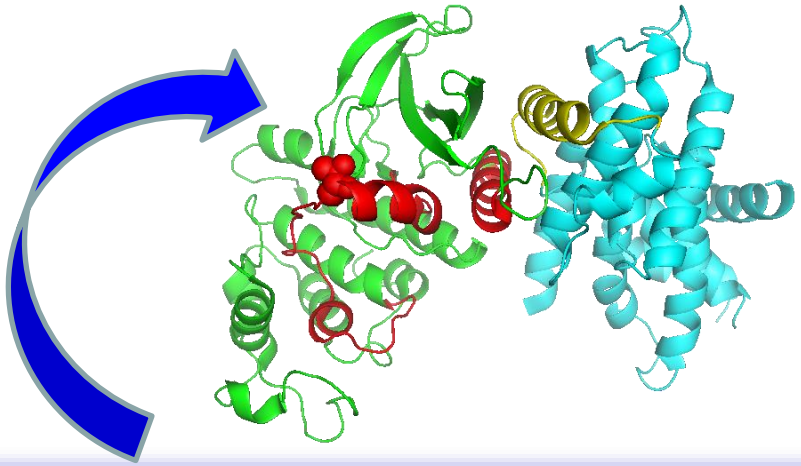
Frédéric BIHEL

TARGETING CDK4 KINASE IN CANCER



The Cell-Cycle Regulator CDK4: An Emerging Therapeutic Target in Melanoma *Clin Cancer Res* 2013
Karen E. Sheppard and Grant A. McArthur

A Synthetic Lethal Interaction between K-Ras Oncogenes and Cdk4 Unveils a Therapeutic Strategy for Non-small Cell Lung Carcinoma
Marta Puyol,[1,6,7] Alberto Martín,[1,6,8] Pierre Dubus,[4] Francisca Mulero,[2] Pilar Pizcueta,[3] Gulfaraz Khan,[5] Carmen Guerra,[1] David Santamaría,[1] and Mariano Barbacid[1,*]



PALBOCICLIB (IBRANCE)	RIBOCICLIB (KISQALI)	ABEMACICLIB (VERZENO)
PD-0332991 - Pfizer	LEE011 - Novartis	LY2835219 - Lilly

Mechanisms of CDK4 hyperactivation
CDK4 overexpression
Cyclin D amplification /overexpression
p16^{INK4A} *deletion*
CDK4 R24C mutation

Hamilton et Infante
Cancer Treatment Rev. 2016

Cyclin D overexpression in cancers	
Lung	18 – 76%
Melanoma	30 – 65%
Pancreatic	42 – 82%
Breast	50 – 70%
Colorectal	55%
Lymphoma	> 90%

Musgrove et al.
Nat Rev Cancer 2011

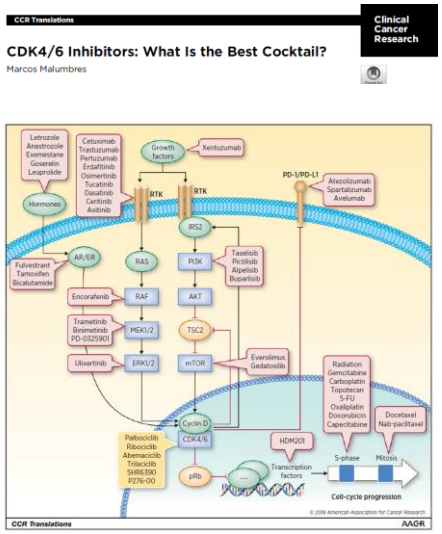
THERAPEUTIC LANDSCAPE OF CDK4/CDK6 INHIBITORS

		Palbociclib PD-0332991 - Pfizer	**Ribociclib** LEE011 - Novartis	**Abemaciclib** LY2835219 - Lilly
Marketed		Breast (IBRANCE)	Breast (KISQALI)	Breast (VERZENIO)
Clinical trials	***Phase III***	**NSC lung cancer**		**NSC lung cancer**
	Phase II	Head & Neck, Urogenital	Gastrointestinal, teratoma	Mantle cell lymphoma
	Phase I	Malignant melanoma, Colorectal, MC lymphoma, pancreatic, solid tumours	Glioma, liposarcoma, malignant melanoma, NSC lung, prostate, head & Neck, lymphoma, neuroblastoma, renal, solid tumours	
Sensitivity		**Loss of $p16^{INK4A}$**		**KRAS mutations in NSCLC**
Dose		125 mg/day for 4 weeks	900 mg/day for 4 weeks	200 mg twice/day
Combined Therapy		- with endocrine therapies (Letrozole, Fulvestrant) => Breast cancer - with PI3K/AKT/mTOR pathway inhibitors (buparlisib, alpelisib) => Breast, NSCLC, pancreatic - with RAS/RAF/MEK/ERK pathway inhibitor (binimetinib) => Melanoma - with chemotherapies (paclitaxel, gemcitabine, doxorubicin) => NSCLC, Breast, Neuroblastoma - with radiotherapy (POC in vitro)		
Adverse Secondary Effects		Neutropenia, anemia, fatigue, nausea, diarrhea, dyspnea, arthralgia	Neutropenia, leukopenia, lymphopenia, nausea, fatigue, thrombocytopenia, mucositis, pulmonary embolism, hyponatremia, QTC prolongation, increased creatinine	Neutropenia, diarrhea, nausea, fatigue, vomiting

ATP-competitive

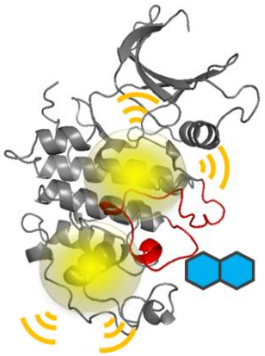
Type 1 Inhibitors

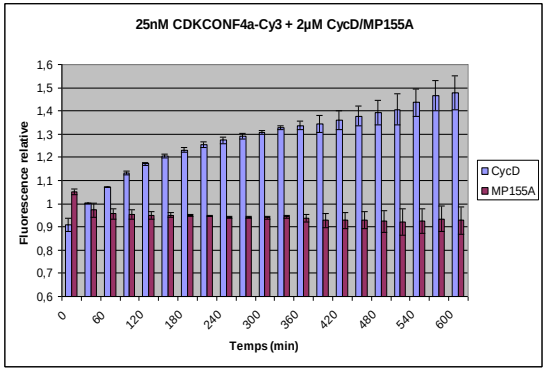
Limited efficacy in monotherapy

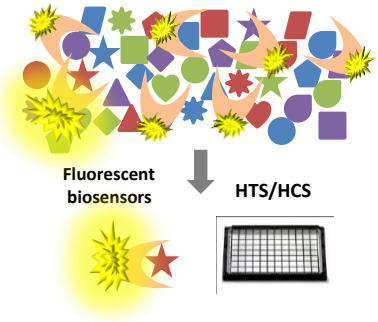


SCREENING WITH CDKCONF4 BIOSENSORS

Camille Prével







Morgan Pellerano

Screen 1 -480 cpds

15 & 9 hits (A&B) Fluorescence amplification

ZFactor : 0.89

Screen 2 -640 cpds

2 hits (the same) Fluorescence amplification

ZFactor : 0.68

Source of Compounds

Library 1 – essential French National Library of Chemical Compounds

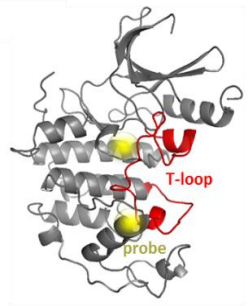
Library 2 - Faculté de Strasbourg/UMR 7175-LC1 CNRS-ULP/Illkirch

Compounds screened at 10µM with 10nM CDKCONF5 on a TECAN freedom EVO

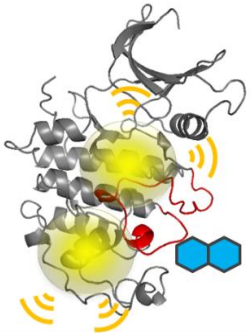
Automated HTS performed at Montpellier Imaging Facility (MRI)

ALLOSTERIC CDK4 MODULATORS OF CDK4

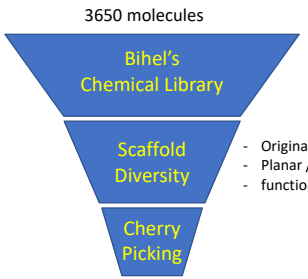
CDKCONF4 Conformational Biosensor discriminates against ATP-pocket binders



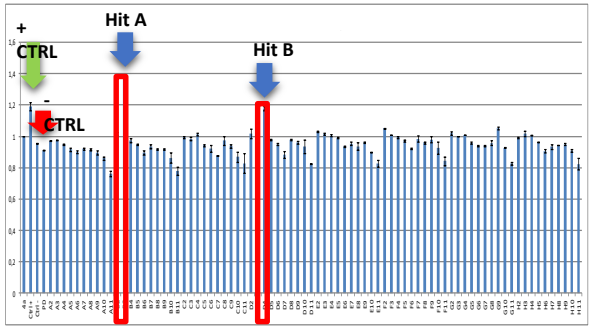
Allosteric modulator



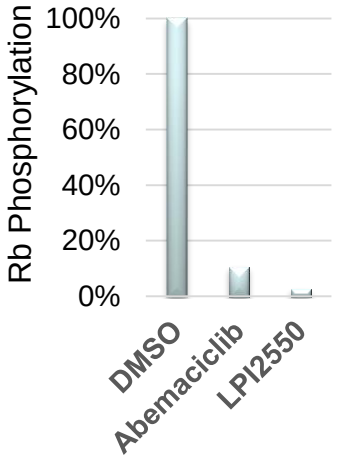
Conformation A **Conformation B**



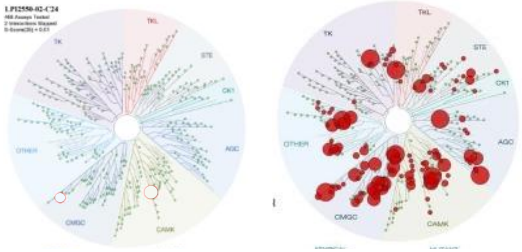
Fluorescence Based Screen: Response of Biosensor to small molecules



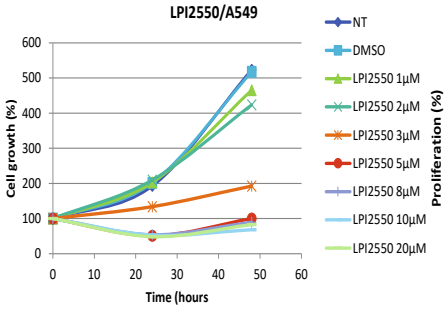
Source of Compounds – F. Bihel's Library
Faculté de Strasbourg/UMR 7175-LC1 CNRS-ULP/Illkirch



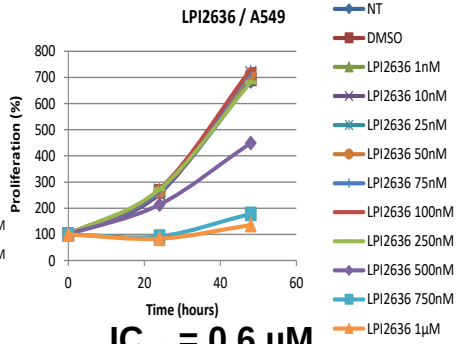
LPI2550 Abemaciclib



KinomeScan DiscoverX (456 kinases)



IC_{50} = 1.9 µM



IC_{50} = 0.6 µM

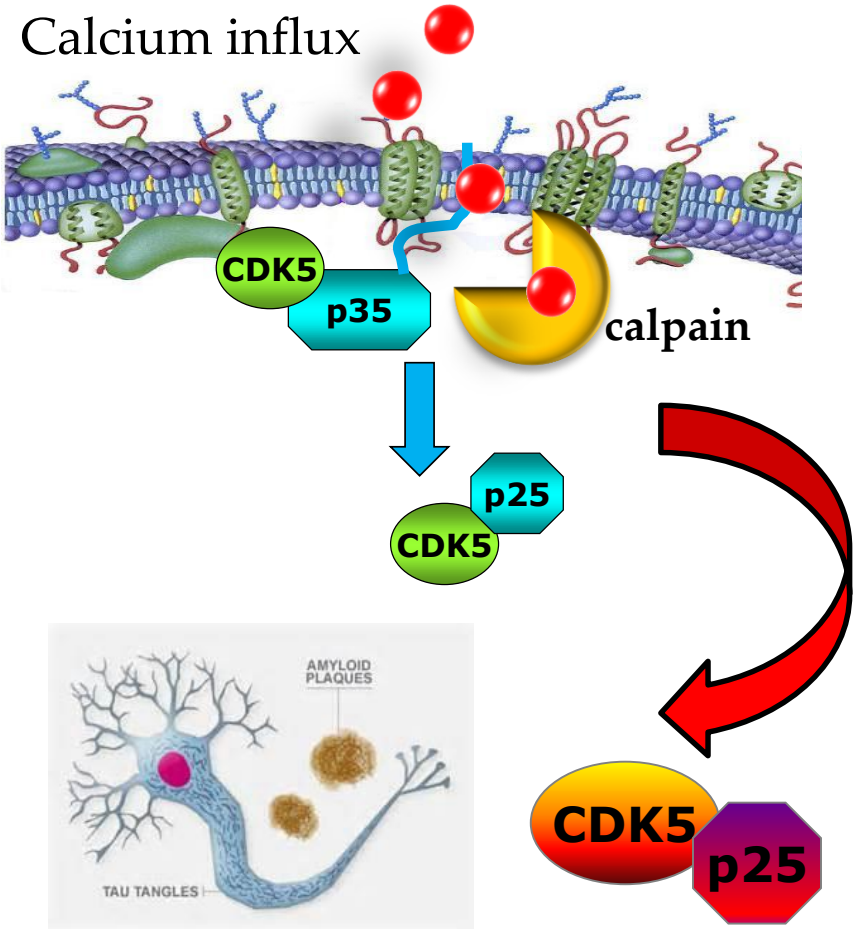
LPI2550 IC50 2.5 uM – optimization to LPI2636 IC50 0.3 uM

Collab. Frederic Bihel, Strasbourg - CDK4PPI project

MATWIN
MATURATION & ACCELERATING TRANSLATION WITH INDUSTRY

CDK5/p25 – an atypical CDK kinase

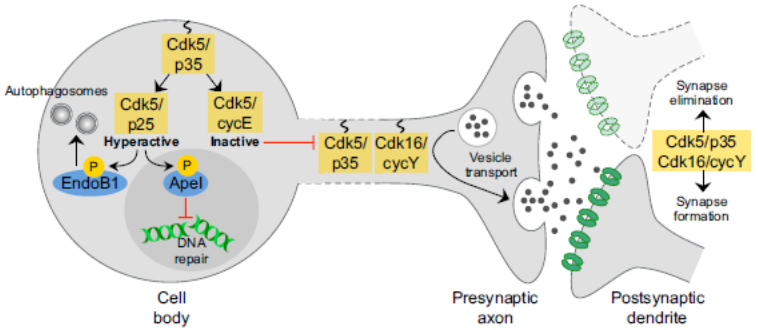
coordinates neuronal functions - hyperactivated in cancer



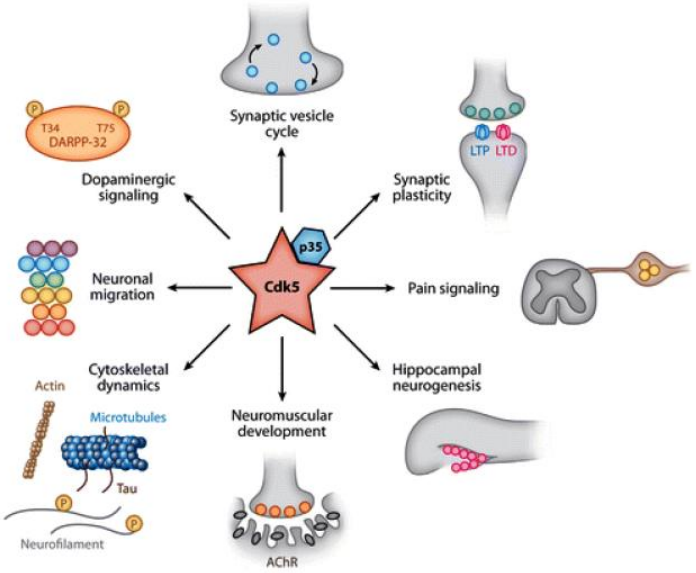
Cdk5: a multifaceted kinase in neurodegenerative diseases

Zelda H. Cheung and Nancy Y. Ip

Trends in Cell Biology March 2012, Vol. 22, No. 3

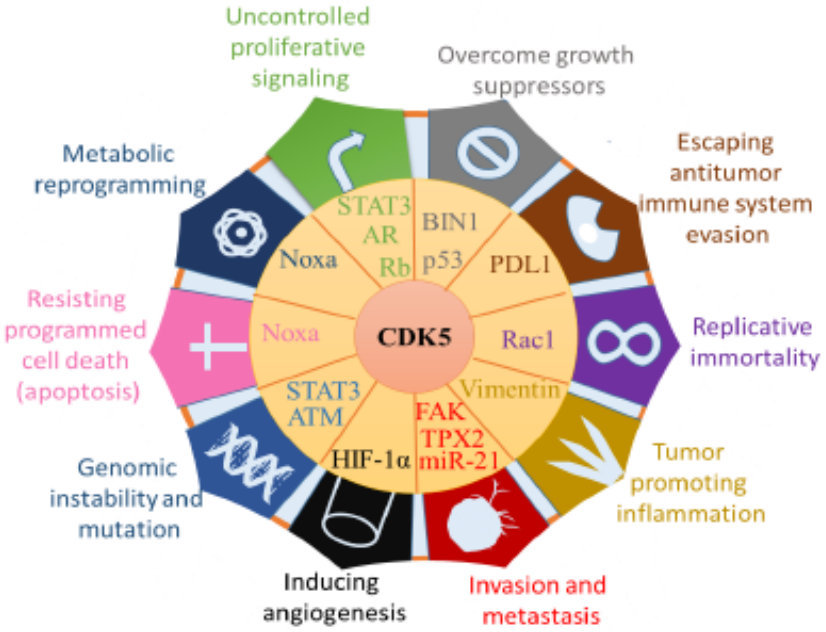


Lim & Kaldis, Development, 2013



Su & Tsai, Ann. Rev. Cell.Dev. Biol. 2011

CDK5 - RELEVANT CANCER BIOMARKER & TARGET

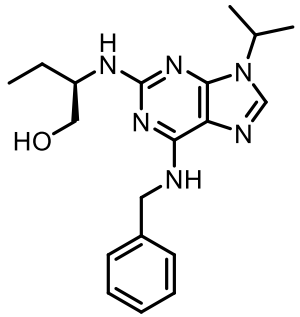


- Promotes proliferation and growth of cancer cells (including stem cells)
- Induces Rb-mediated senescence
- Stimulates angiogenesis
- Regulates motility, migration and metastasis of cancer cells
- Involves in cellular adaptations to hypoxic stress
- Favors the emergence of resistance to chemotherapies
- Up-regulates the formation of PD-L1, a checkpoint protein on the surface of tumor cells that reduces their recognition by the immune system

Lenjisa et al. *Future Med. Chem.* (**2017**) 9, 16, 1939-62

THERAPEUTIC LANDSCAPE OF CDK5

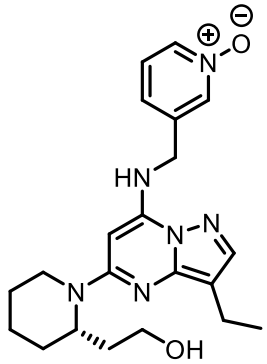
- **Orthosteric Inhibitors**
- **Two pan-inhibitors of the ATP binding site pushed into clinical trials**
- **One new selective inhibitor**



CDK1 : 650 nM
CDK2 : 700 nM
CDK5 : 160 nM
CDK7 : 460 nM
CDK9 : 600 nM

Seliciclib
(alias **(R)-Roscovitine**)
Pan-inhibitor of the ATP-binding site

Clinical trials phase 2
Against many cancers
=> Partial response – tumor stabilization



CDK1 : 3 nM
CDK2 : 1 nM
CDK5 : 1 nM
CDK9 : 4 nM

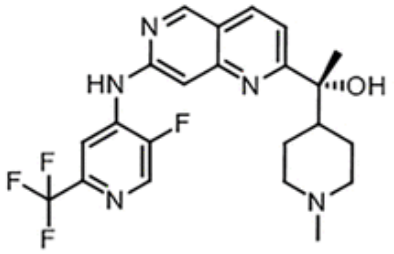
Dinaciclib
Pan-inhibitor of the ATP-binding site

Clinical trials phase 3
Chronic Lymphocytic Leukemia

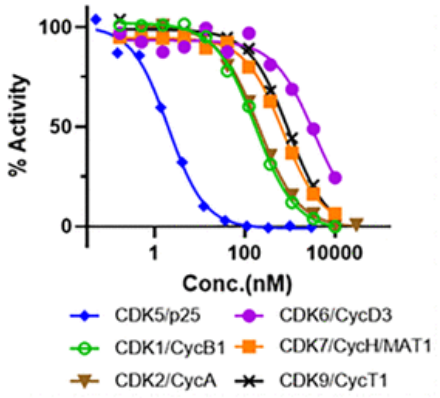
Journal of Medicinal Chemistry
pubs.acs.org/jmc Article

Discovery and Optimization of Highly Selective Inhibitors of CDK5

Matthew H. Daniels,* Goran Malojcic, Susan L. Clugston,* Brett Williams, Marie Coeffet-Le Gal, Xin-Ru Pan-Zhou, Srinivasan Venkatachalan, Jean-Christophe Harmange, and Mark Ledeboer

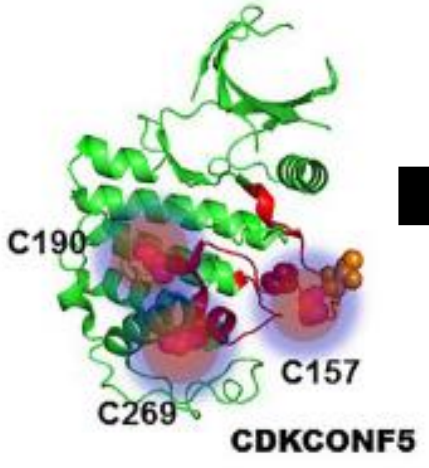


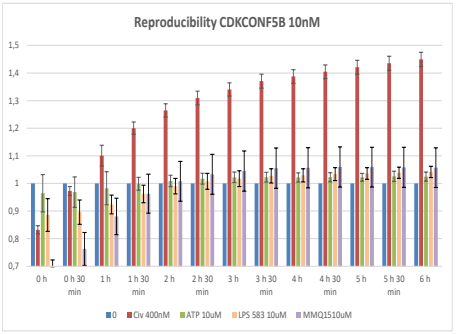
GFB-12811
CDK5 IC_{50} = 2.3 nM
Selective over CDK1/2/6/7/9
Orally bioavailable

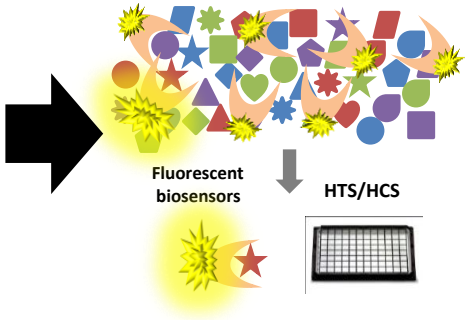


SCREENING FOR ALLOSTERIC INHIBITORS OF CDK5

Marion Peyressatre







Juan Antonio Gonzalez Vera

Morgan Pellerano

Screen 1 -640 cpds → **5 hits (enhancers) (1/5 autofluorescent) 9 quenchers**

Screen 2 -640 cpds → **17 hits (enhancers) 8 quenchers**

Screen 3 -221 cpds → **Hits Only quenchers**

Source of Compounds

Library 1 – essential French National Library of Chemical Compounds
Library 2 - Faculté de Strasbourg/UMR 7175-LC1 CNRS-ULP/Illkirch
Library 3 – Institut des Biomolécules Max Mousseron, Montpellier

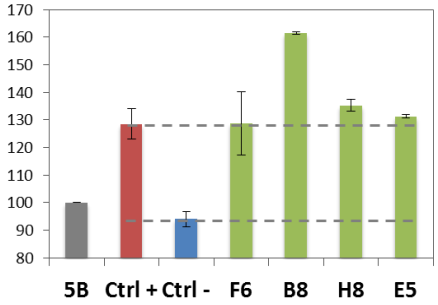
Compounds screened at 10µM with 10nM CDKCONF5 on a TECANTM freedom EVO

Automated HTS performed at Montpellier Imaging Facility (MRI)

CHARACTERIZATION OF HITS

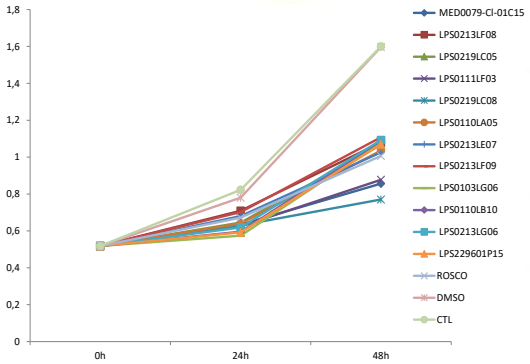
HITS

Hits: potential allosteric inhibitors

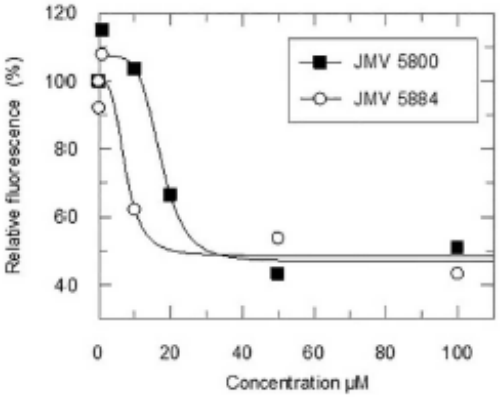


Fluorescence-based identification of allosteric modulators (NB Ctrl- is an ATP inhibitor)

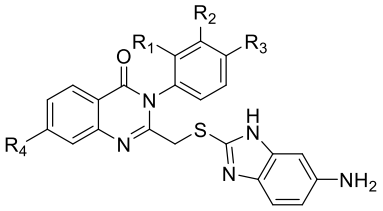
PROLIFERATION ASSAYS & KINASE ACTIVITY ASSAYS



Relative Inhibition of neuronal cell proliferation by allosteric modulators of CDK5



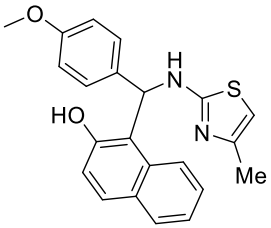
1) QUINAZOLINONES



M. Peyressatre et al.

Frontiers in Chemistry 2020

2) AMINO-THIAZOLES

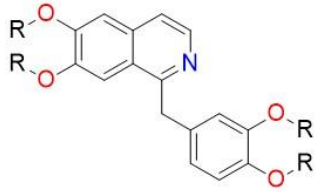


Ameni Hadj Mohamed

Nicolas Masurier

MUSE MONTPELLIER UNIVERSITÉ D'EXCELLENCE

3) ETHAVERINE PAPAVERINE



Papaverine, R = Me
Ethaverine, R = Et

Arthur Laure

Laure et al. ACS Pharmacol Trans Sci 2024

4) CONFIDENTIAL

Chloé Royet

Yacine Djellal

Collab. F.Bihel, Strasbourg

Collab. M.Tramier, Rennes

aviesan alliance nationale pour les sciences de la vie et de la santé ITMO Cancer

ALLOSTERIC INHIBITORS OF CDK5

Identification of Quinazolinone Analogs Targeting CDK5 Kinase Activity and Glioblastoma Cell Proliferation

*Marion Peyressatre, Dominique Patomo Arama, Arthur Laure, Juan A. González-Vera†, Morgan Pellerano, Nicolas Masurier, Vincent Lisowski and May C. Morris**

Frontiers in Chemistry 2020

Source of compounds

IBMM IN-HOUSE LIBRARY

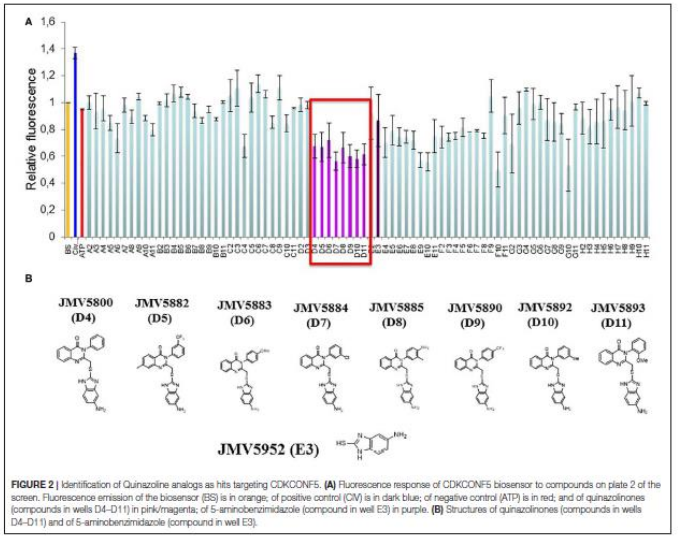


FIGURE 2 | Identification of Quinazoline analogs as hits targeting CDKCONF5. **(A)** Fluorescence response of CDKCONF5 biosensor to compounds on plate 2 of the screen. Fluorescence emission of the biosensor (BS) is in orange; of positive control (CM) is in dark blue; of negative control (ATP) is in red; and of quinazolinones (compounds in wells D4–D11) in pink/magenta; of 5-aminobenzimidazole (compound in well E3) in purple. **(B)** Structures of quinazolinones (compounds in wells D4–D11) and of 5-aminobenzimidazole (compound in well E3).

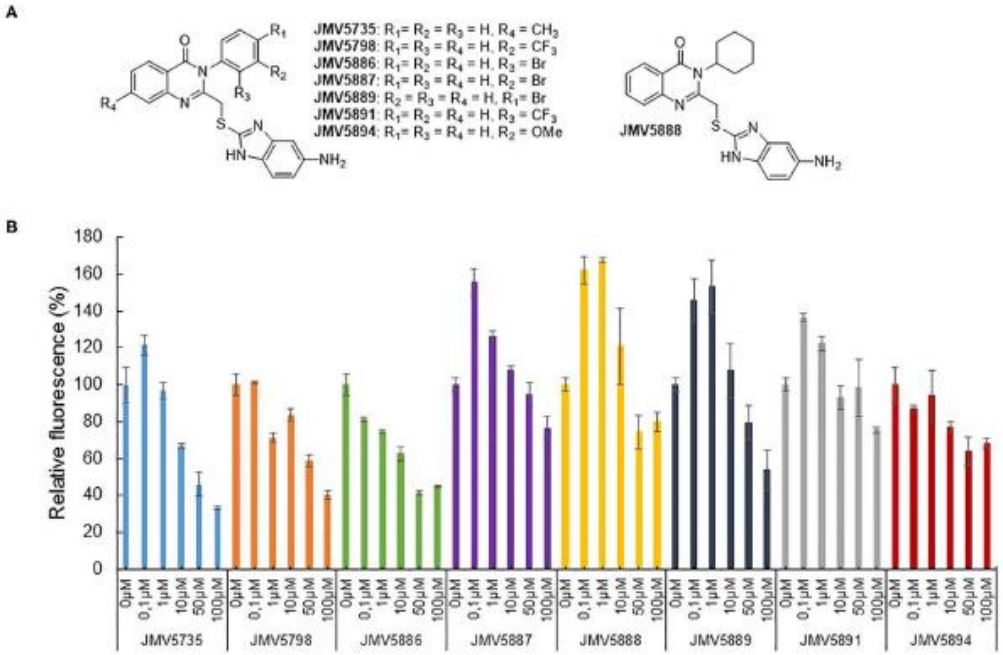
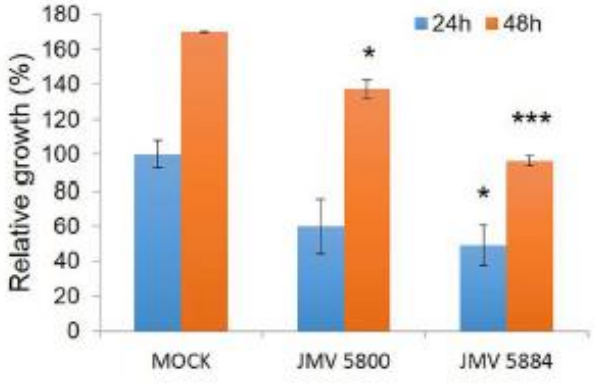
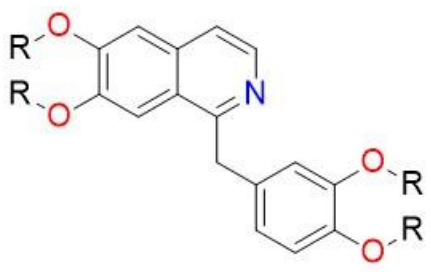


FIGURE 4 | Quinazolinone derivatives inhibit CDK5 activity. **(A)** Quinazolinone derivatives synthesized. **(B)** Concentration-dependent inhibition of CDK5 activity measured with the CDKACT5 biosensor and U87 cell extracts (JMV5735, JMV5798, JMV5886, JMV5887, JMV5888, JMV5889, JMV5891, and JMV5894).

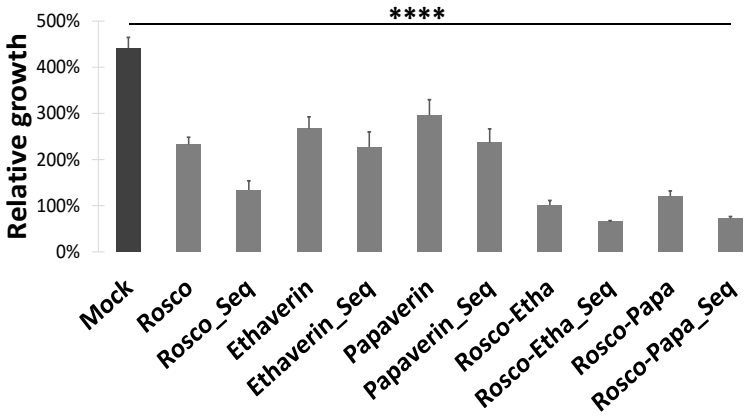


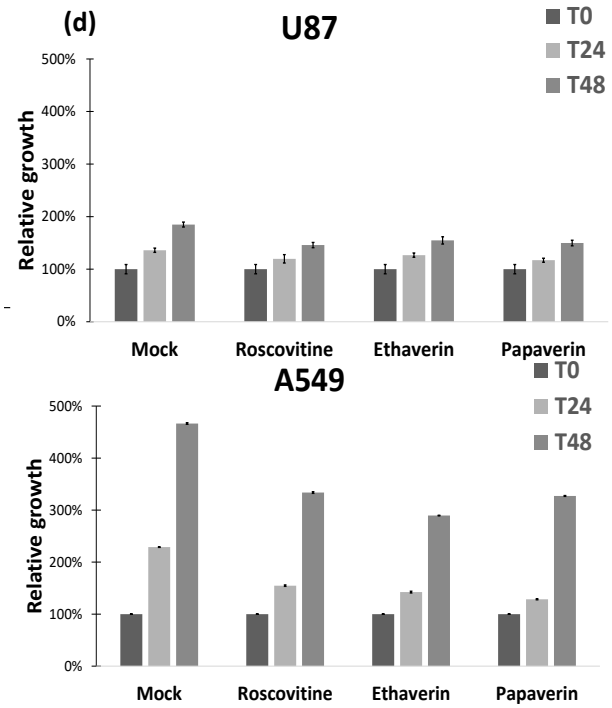
Proliferation assays in U87 cells

CDK5 INHIBITORS FOR LUNG CANCER

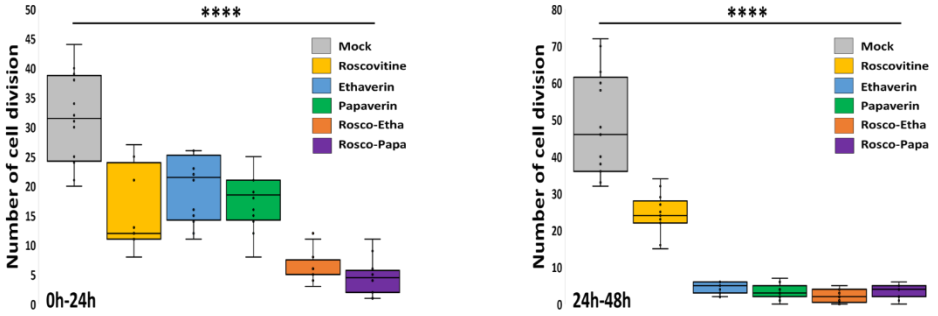


Papaverine, R = Me
Ethaverine, R = Et

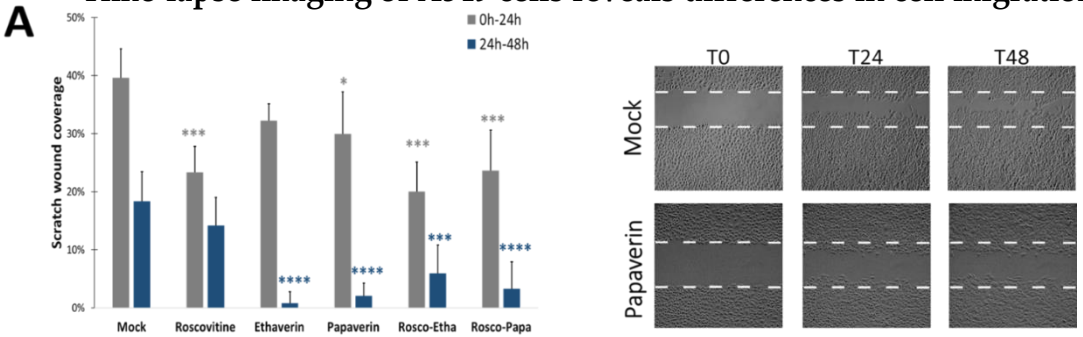




Time-lapse imaging of A549 cells reveals differences in cell division



Time-lapse imaging of A549 cells reveals differences in cell migration



Ethaverine and Papaverine target CDK5 and inhibit lung cancer cell proliferation and migration

Arthur Laure et al. *ACS Pharmacol Trans Sci 2024*

CONCLUDING REMARKS

Fluorescent Biosensors for Drug Discovery

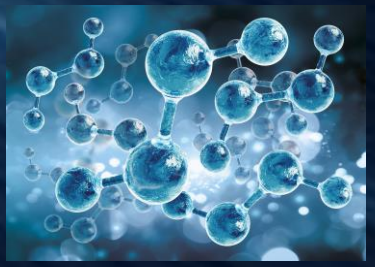
ACS Medicinal Chemistry Letters
Viewpoint
pubs.acs.org/acsmedchemlett

Spotlight on Fluorescent Biosensors—Tools for Diagnostics and Drug Discovery

May C. Morris*

Institut des Biomolécules Max Mousseron IBMM- UMR 5247, 15 Av. Charles Flahault, 34093 Montpellier, France

- **Sensitive & Selective Tools for HTS**
- **Hit identification**
- **Optimization of druggable leads**
- **Conformational Biosensors: selective identification of allosterics**
- **Discrimination against ATP comp. drugs**
- **Original Mechanisms of Action**

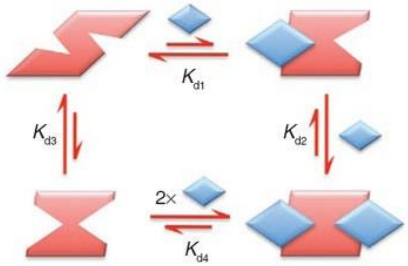


TAKE-HOME MESSAGES

New inhibitors are required to overcome limitations of ATP-pocket inhibitors targeting essential protein / protein interactions or conformational transitions

New and Smart Strategies are required to identify allosteric inhibitors : structure-based rational design or screening with conformational biosensors

New and unexpected Pathways and Functions can be uncovered thanks to allosteric modulators



Allosteric inhibitors can be combined with Orthosteric Inhibitors to achieve cooperative inhibition

« THE KINASE BIOSENSOR & INHIBITOR GROUP»

Chloé Royet
Yacine Djellal
Arthur Laure
Ameni Hadj Mohamed
Marion Peyressatre
Morgan Pellerano
JA Gonzalez Vera
Sébastien Diot
Sabrina Bedja
Camille Prével
Sergey Tcherniuk
Carmen Tilmaciu
Laetitia Kurzawa
T.N. Ngoc Van

Contact : may.morris@umontpellier.fr

COLLABORATORS

F. Bihel, LIT, Strasbourg
E. Garcin, Marseille
F. Mahuteau-Betzer, Institut Curie Orsay
N. Masurier, IBMM, Montpellier
M.Tramier, IGDR, Rennes

PROJET COFINANCÉ PAR LE FONDS EUROPÉEN DE DÉVELOPPEMENT RÉGIONAL