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Fluorescent biosensors for drug discovery new tools for old targets – Screening for inhibitors of cyclin-dependent kinases

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ABSTRACT

Cyclin-dependent kinases play central roles in regulation of cell cycle progression, transcriptional regulation and other major biological processes such as neuronal differentiation and metabolism. These kinases are hyperactivated in most human cancers and constitute attractive pharmacological targets. A large number of ATP-competitive inhibitors of CDKs have been identified from natural substances, in high throughput screening assays, or through structure-guided approaches. Alternative strategies have been explored to target essential protein/protein interfaces and screen for allosteric inhibitors that trap inactive intermediates or prevent conformational activation. However this remains a major challenge given the highly conserved structural features of these kinases, and calls for new and alternative screening technologies. Fluorescent biosensors constitute powerful tools for the detection of biomolecules in complex biological samples, and are well suited to study dynamic processes and highlight molecular alterations associated with pathological disorders. They further constitute sensitive and selective tools which can be readily implemented to high throughput and high content screens in drug discovery programmes. Our group has developed fluorescent biosensors to probe cyclin-dependent kinases and gain insight into their molecular behaviour in vitro and in living cells. These tools provide a means of monitoring subtle alterations in the abundance and activity of CDK/Cyclins and can respond to compounds that interfere with the conformational dynamics of these kinases. In this review we discuss the different strategies which have been devised to target CDK/Cyclins, and describe the implementation of our CDK/Cyclin biosensors to develop HTS/HCS assays in view of identifying new classes of inhibitors for cancer therapeutics.

1. Introduction

One of the major challenges in medicinal chemistry consists in designing new drugs that interfere with target activity with high specificity and selectivity profiles. In the case of protein kinases, one of the major therapeutic targets for drug discovery programmes, the larger part of efforts made to date have focused on high throughput screening activity-based assays and structure-guided developments to optimize compounds that target ATP pockets. Alternative strategies have been devised to target non-ATP pockets, protein/protein interactions or allosteric sites. However, the implementation of screening assays to identify compounds that interfere with the biological function of targets through non-catalytic sites is not straightforward, and relies on the development of tools that discriminate between ATP-pocket binding and allosteric compounds. In this respect, fluorescent biosensors constitute powerful tools for development of drug discovery programmes and are particularly well suited to high throughput screening formats, due to the inherent sensitivity of fluorescence. Moreover they offer a wealth of opportunities for designing fluorescence-based screening assays aimed at identifying compounds that selectively target enzyme function or conformation.

In particular Cyclin-dependent kinases (CDK/Cyclins), initially identified as key regulators of cell cycle progression and now

Abbreviations: AFP, autofluorescent protein; CAK, CDK-activating kinase; CCL, chronic lymphocytic leukaemia; CDK, cyclin-dependent kinase; CKI, CDK Inhibitor; FLIK, fluorescent labels in kinases; FRET, fluorescence resonance energy transfer; HCS, high content screening; HTS, high throughput screening; MCL, mantle cell lymphoma; NES, nuclear export sequence; NLS, Nuclear localization sequence; NSCLC, non-small cell lung cancer; PAABD, phosphoamino acid binding domain; PPI, protein/protein interaction.

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recognized players of other major biological processes including transcriptional regulation, neuronal differentiation and metabolism [1–4], constitute attractive pharmacological targets for development of anticancer therapeutics. Indeed these kinases have been found to contribute to cancer cell proliferation due to amplification, overexpression or mutation of CDKs and Cyclins in a wide variety of human cancers [28–30].

Several in vitro and cell-based assays have been implemented to screen for inhibitors of these kinases, but these screens have only lead to identification of compounds that bind the ATP-pocket of the CDK and very few allosteric inhibitors have been identified to date. We have developed a family of environmentally-sensitive fluorescent peptide and protein biosensors to probe the relative abundance, activity and conformational status of CDK/Cyclins. These tools provide a means of monitoring subtle alterations in the abundance, activity and conformation of these kinases in response to therapeutics in vitro and in cellulo. As such they constitute highly sensitive probes for drug discovery programmes. Here we will describe the different strategies which have been developed to target cyclin-dependent kinases, and discuss the utility of our CDK/Cyclin biosensors to implement high throughput screening assays.

2. CDK-Cyclins-heterodimeric kinases and therapeutic targets

Cyclin-dependent kinases (CDK/Cyclins) form a family of heterodimeric serine/threonine protein kinases, which were initially identified on the basis of their functions in coordinating cell cycle transitions, and therefore considered as molecular engines driving cell cycle progression [1]. Moreover, after several decades of studies leading to identification of twenty different CDKs and as many Cyclins in mammalian cells, the functional diversity of this class of enzymes has been uncovered, and it is now fully recognized that CDK/Cyclins are involved in a wide variety of important biological processes, including transcriptional regulation, metabolism, neuronal differentiation and development [2–4].

CDK1, 2, 4 and 6 can be considered *bona fide* cell cycle regulators. Cdk4 and Cdk6 associate with cyclin D isoforms (D1, D2, D3) to regulate exit from quiescence and growth factor-stimulated entry into and progression through G1. Cdk2-cyclin E coordinates the G1/S transition, followed by Cdk2-cyclin A, which is involved in regulation of S phase progression and DNA replication. Cdk1 associates sequentially with Cyclin A and B to control entry into and progression through mitosis [5]. CDK7 associates with Cyclin H to form a complex termed CAK, the CDK-Activating Kinase, which promotes activation of several CDK/Cyclins through phosphorylation of the CDK activation loop (or T-loop) [6]. CDK7 is also involved in transcriptional activation of RNA polymerase II. Likewise, CDK8/Cyclin C and CDK9/cyclin T are involved in transcriptional processes [6,7].

Besides these well documented processes, a wealth of non-cell cycle functions has been reported for CDK/cyclin complexes. It is now common knowledge that members of the CDK/Cyclin family participate in a much broader spectrum of biological functions than initially suspected, including transcriptional regulation, epigenetics, DNA damage response and repair, neuronal differentiation, synaptic trafficking and remodelling, glycogen synthesis and lipogenesis, angiogenesis, haematopoiesis, ciliogenesis and spermatogenesis [4] (Fig. 1). Moreover, most CDK/Cyclin complexes have been reported to exert functions in more than one biological process, as exemplified by CDK4/Cyclin D, an established cell cycle regulator of the G1 phase, also known to be essential for regulation of haematopoiesis and lipogenesis, and more recently described for its function in epigenetic regulation [8]. Conversely several Cyclin-dependent kinases may contribute to similar biological processes, such as CDK4/Cyclin D1, CDK5/p35 and CDK8/Cyclin C, which are involved regulation of metabolic functions [9–11] (Fig. 1).

Spatio-temporal patterns of expression and degradation, subcellular localization and sequence determinants dictate the availability of CDKs and Cyclins for formation of heterodimeric complexes with specific functions and substrate selectivity [12]. However, despite the high level of coordination and specificity underlying how, when and where a CDK pairs up with a Cyclin, studies in knockout mouse models have revealed that lack of either of these subunits is more often than not compensated by formation of « illegitimate » complexes which do not normally occur in physiological conditions [13–15].

Monomeric CDKs are catalytically inactive and acquire basal kinase activity through binding of a cyclin partner. Although there are variations on the theme, following their assembly into heterodimeric complexes, cyclin-dependent kinases are generally subject to reversible activating and inhibitory phosphorylations which coordinate the yield of a fully active complex [1,16] (Fig. 2). CDK1/cyclin B for instance is subject to inhibitory phosphorylation of threonine 14 (Thr14) and tyrosine 15 (Tyr15) residues are phosphorylated by Wee1 and Myt1 kinases, which prevent ATP binding and thereby hold the complex in check [17]. These residues are subsequently dephosphorylated by members of the Cdc25 phosphatase family [18], and the fully active kinase complex is generated through phosphorylation of a critical threonine on the activation segment (Thr161 for CDK1) by CDK-activating kinase (CAK) [6,16]. The first structural studies on CDK2/Cyclin A provided

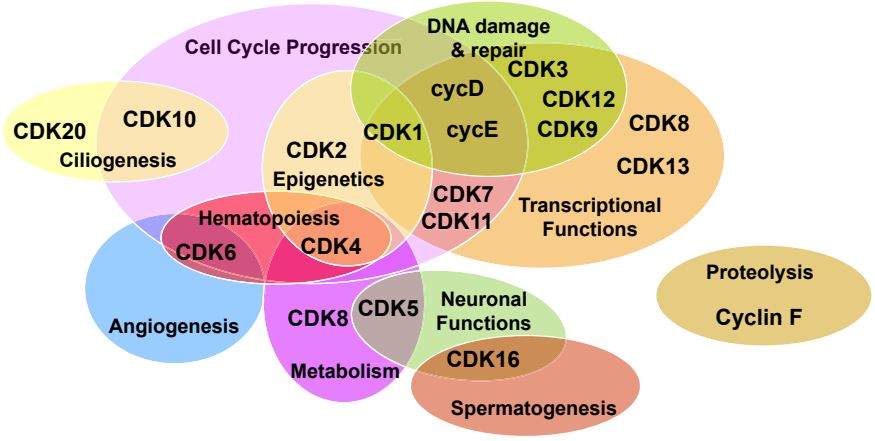

Fig. 1. Functions of Cyclin–dependent kinases schematic representation of the functional diversity of Cyclin-dependent kinases and their converging contributions to subsets of biological processes.

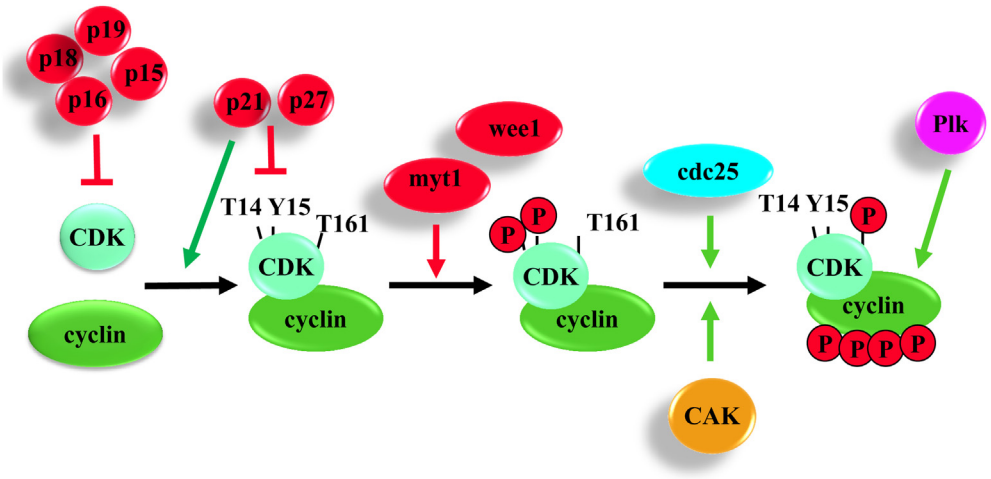

Fig. 2. Molecular Mechanism of Regulation of CDK/Cyclins Schematic representation of the general mechanism of assembly and regulation of Cyclin-dependent kinases. Following interaction of a CDK with a cyclin, heterodimeric CDK/Cyclin complexes, are subject to inhibitory phosphorylation on threonine 14 (Thr14) and tyrosine 15 (Tyr15) residues, for CDK1 and CDK2, by Wee1 and Myt1 kinases, then activated through dephosphorylation of these residues by members of the Cdc25 phosphatase family, together with activating phosphorylation of threonine 161 (Thr161 in CDK1) by CDK-activating Kinase CAK.

the information required to gain insight into the molecular basis of CDK/Cyclin complex activation. These studies revealed that the cyclin played a major role in promoting activation of the CDK by inducing conformational changes required for substrate binding and catalysis [5,19]. Indeed cyclin binding is a two-step process which involves a rapid protein/protein interaction between a well-conserved helix in the N-terminal lobe of the CDK and alpha helix 5 of the cyclin which leads to reorientation of the CDK ATP-binding pocket, aligning it with the catalytic cleft in a position which is appropriate for transfer of phosphate onto the substrate, and a slower isomerization step which promotes a conformational switch of an activating segment termed the T-loop, partially stabilizing it in a position which favours subsequent phosphorylation by CAK [20–24]. Phosphorylation further displaces the T-loop into a position stabilized through interactions between the phosphate group and several residues within the C-lobe of the CDK, thereby generating a fully accessible substrate-binding site. Additional post-translational modifications may occur on the cyclin subunit, in particular phosphorylation by Plk kinases [25]. Aside from regulation of CDK/Cyclins through phosphorylation, their activities are further restricted through interactions with structural inhibitors known as CKIs (CDK Inhibitors). The INK4 family of CKIs (p16, p15, p18 and p19) comprises proteins composed of multiple ankyrin repeats that specifically bind CDK4/6 and either prevent or compete with cyclin binding [26]. The Kip/Cip family of CKIs (p21, p27 and p57) bind and maintain CDK/Cyclin heterodimers in an inactive form [27].

2.1. Cyclin-dependent kinases in disease – pharmacological targets for anticancer drugs

Whereas in healthy cells, CDK/Cyclin kinases are tightly controlled both spatially and temporally, they are frequently over-expressed and/or subject to mutations that directly perturb their function in pathological settings, thereby wreaking havoc in cellular homeostasis and contributing to establish hyper-proliferation [28,29]. In particular, gene amplification, over-expression or mutations leading to exacerbated CDK-cyclin activity have been documented in several cancers and associated with poor prognosis in patients [28,29]. It is now clearly established that deregulation of CDK/Cyclin activities contributes to sustain uncontrolled proliferation, one of the major hallmarks of cancer [30].

CDKs are not frequently subject to mutations that perturb their function, aside from mutations in CDK4 and CDK6 that alter their ability to bind CKIs. Overexpression of CDKs associated with CDK/Cyclin hyperactivity has been reported in a subset of cancer subtypes [28,29,31], but overexpression, amplification or expression of truncated or spliced variants of cyclins have been observed more frequently [32–47], as well as mutations leading to functional inactivation of endogenous inhibitors (CKIs). As such, cyclin-dependent kinases constitute attractive pharmacological targets for the development of anticancer drugs [48,49].

Hyperactive CDK1 associated with overexpression of Cyclin B1 has been reported in several primary cancers such as breast, colon, prostate, gastric and esophageal squamous cell carcinoma, oral and non small-cell lung cancer lung cancer [37–40]. Since CDK1 constitutes the “master kinase” which is essential for viability [14], and the major partner of cyclin B1, it constitutes an attractive target for therapeutic intervention. CDK1 has been proposed as a target for diffuse large B-cell lymphoma combination therapy [50]. Targeting cyclin B has been demonstrated to block growth of cancer cells and tumours [51–54].

Likewise, CDK2 deregulation accompanied with Cyclin A-E overexpression or Cip/Kip inactivation has been described in breast cancer, lung carcinoma, melanoma, osteosarcoma, ovarian carcinoma [32,43–47]. Since CDK2 is involved in control of DNA replication and S phase progression, it constitutes a major target for development of cancer therapeutics. Moreover overexpression of Cyclin A correlates with tumour relapse of human hepatocellular carcinoma, and hepatitis B integration has been reported in cyclin A gene in hepatocellular carcinoma [55,56].

Misregulation of the pRb/cyclin D/p16(INK4A) pathway is one of the most frequent events in human cancer, which has lead to the suggestion that inhibition of cyclin D-dependent CDK4 and CDK6 kinase activity may have therapeutic value as an anticancer treatment [57–59].

Nuclear cyclin D1 is a recognized oncogenic driver [41]. D-type cyclin overexpression or amplification, polymorphism and aberrant splicing are amongst the most frequent causes of CDK4 and CDK6 hyperactivity, in various types of human cancer [35,57,59]. Overexpression of CDK4 and CDK6 leading to Rb hyperphosphorylation, and a consequent loss of anti-proliferative control have also been reported in different cancer types [58]. Last but not least, CDK4 mutations leading to hyperactivity through loss of ability to bind

p16INK4A have been described in familial melanoma, lung cancer and lymphoma [58–65].

More than 90% of lung cancers contain mutations of CDK4, cyclin D or p16INK4A. In lung cancer, amplification of the cyclin D1 locus is observed in 5–30% of non-small cell lung cancer (NSCLC) and high levels of cyclin D1 protein are found in 18–76% in invasive NSCLC, correlating with a worse outcome [57,60]. CDK4 overexpression in lung cancer may accelerate tumour progression and leads to an overall shorter survival time in lung cancer patients [66]. CDK4 kinase constitutes a therapeutic target in cancer types which express the K-Ras oncogene such as non-small cell lung adenocarcinoma (NSCLC), due to a synthetic lethal interaction between CDK4 ablation/inhibition and K-Ras [64]. Targeting this kinase in NSCLC has been proposed as a therapeutic strategy in KRAS-mutant lung cancer that is resistant to conventional and targeted therapies [64].

CDK4/Cyclin D kinase hyperactivation, associated with mutation of CDK4, amplification of cyclin D (18% melanomas) or loss of $p16^{INK4a}$ inhibitor of CDK4/Cyclin D (deletion of CDK2NA in 50–60 % metastatic tumours), leads to an increase in the risk of developing melanoma [67,68]. This kinase therefore constitutes a key biomarker for diagnostics of melanoma and an emerging pharmacological target in classes of melanoma which present an amplification of cyclin D or CDK4, an R24C mutation of CDK4 which prevents the fixation of an endogenous inhibitor $p16^{INK4a}$, or a mutation of $p16^{INK4a}$ itself [59,68,69].

CDK4 hyperactivity is also implicated in development of lymphomas. Indeed *c-myc*-3′RR/$CDK4^{R24C}$ and *c-myc*-3′RR/$p53^{+/-}$ mice have been reported to spontaneously develop B lymphomas [65,70]. P16 (CDKN2 gene) deletion or CDK4 amplification has been reported in the majority of glioblastomas [71]. CDK4 gene amplification and overexpression has also been reported in sporadic breast carcinomas, in refractory rhabdomyosarcoma and in osteosarcoma [72–75]. CDK6 amplification and overexpression have been reported in human gliomas and in splenic marginal zone lymphoma, respectively [76,77].

CDK5, a cyclin-dependent kinase primarily considered to regulate neuronal functions, has been largely reported to participate in development of neurodegenerative diseases, including Alzheimer's and Parkinson's diseases, as well as amyotrophic lateral sclerosis through hyperphosphorylation of neurofibrillary tangles, Lewy bodies and Lewy body-like inclusions, respectively [78–80]. Moreover, there is well documented evidence that CDK5 is involved in pancreatic cancer, glioblastoma and neuroblastoma, multiple myeloma and breast cancer [81–84]. Inhibitors targeting CDK5 have been developed for neurodegenerative diseases.

2.2. Anticancer therapeutics targeting Cyclin-dependent kinases

Deregulation of protein kinase activities leading to uncontrolled cellular homeostasis is one of the molecular features most frequently associated with disease. Although this class of enzymes is considered one of the major therapeutic targets, they constitute challenging targets and very few inhibitors have been approved for clinical use by the FDA. Notwithstanding, there are currently twenty-three drugs approved for clinical use, including several notorious cancer therapeutics such as Gleevec/Imatinib and second generation inhibitor Nilotinib designed to target Bcr-Abl, as well as Vemurafenib inhibitor of B-Raf. The success of these compounds in oncology has elicited further interest in developing drug discovery programmes to identify drugs targeting protein kinase dysregulation in human cancers. As such there are currently an estimated 260 inhibitors of protein kinases undergoing clinical trials and it is considered that more than 50% of efforts devoted to cancer drug discovery programmes focus on this class of enzymes (for review [85,86]).

Cyclin-dependent kinases have not escaped this trend and a large number of efforts have thus focused on strategies to inhibit these kinases so as to silence their aberrant hyperactivity in human cancers [48,49,87–101]. Different strategies have been applied to identify compounds that target and interfere with the activity of these kinases, including purification of active compounds from natural substances, high-throughput screening of combinatorial libraries of small synthetic molecules, and structure-guided, rational design of inhibitors that target ATP-binding pockets, protein/protein interactions or allosteric patches.

Fig. 3 schematizes the different strategies which have been developed to target CDK/Cyclins and Table 1 summarizes some of the major CDK inhibitors.

2.3. ATP-competitive inhibitors of CDK/Cyclins

The first inhibitors of Cyclin-dependent kinases were isolated from natural sources such as plants, bacteria & fungi [102]. Compounds such as olomoucine, staurosporine, butyrolactone flavopiridol and indirubin all bind the ATP pocket of the CDK, thereby inhibiting kinase activity through competition with ATP [103–108]. Determination of the molecular nature and characterization of the mechanism of action of these compounds revealed that these compounds were essentially purine and pyrimidine analogues, prompting further developments in medicinal chemistry to synthesize derivatives with greater specificity and efficacy [109]. These studies paved the way for chemical and genomic approaches to screen for compounds with similar structural and/or functional features [110,111]. Furthermore, structure-guided approaches enabled the optimization of these classes of inhibitors to yield drugs with superior activity and improved therapeutic index [112, 113]. As such, and to date, the larger part of drugs that inhibit CDK/Cyclin activity are ATP-pocket-binding antagonists. Several of these ATP competitors have entered preclinical studies, and there are currently 16 CDK inhibitors in clinical trials (for review [48, 49, 114]). Some noteworthy examples are described in further detail below and listed in Table 1.

Roscovitine constitutes one of the first CDK inhibitors identified which successfully made it through the drug discovery pipeline to clinical trials in several human cancers, including NSCLC under the name CYC202 or Seliciclib. This purine analogue primarily inhibits CDK2 and CDK5, as well as CDK1, CDK7 and CDK9 in several forms of human cancers. More recent efforts in medicinal chemistry have yielded several potent second generation compounds such as CR8 [115–119].

Flavopiridol was initially found to be a potent inhibitor of CDK1, and later found to be wide spectrum CDK inhibitor that targets a wide variety of CDKs. This flavonoid also known as Alvociclib is effective in various cancers including leukaemia, multiple myeloma, lymphoma, sarcoma and solid tumours, and exhibits significant clinical activity in refractory chronic lymphocytic leukaemia (CCL) [107,120–123].

AT7519 is a pan-CDK inhibitor which was initially identified in a fragment-based screen and developed through a structure-guided approach, which has shown potent antiproliferative activity in several human cancer cell lines. This compound is administered to patients with advanced solid tumours or refractory non-Hodgkin's lymphoma and is in phase 2 trials for multiple myeloma, CLL and MCL [124–128].

Dinaciclib is a potent and selective inhibitor of cyclin-dependent kinases which was selected as a clinical candidate in a functional screen in vivo. This compound potently inhibits CDK2, CDK5, CDK1 and CDK9 activity in vitro, and was found to inhibits cancer growth and induce regression of solid tumours in mouse models

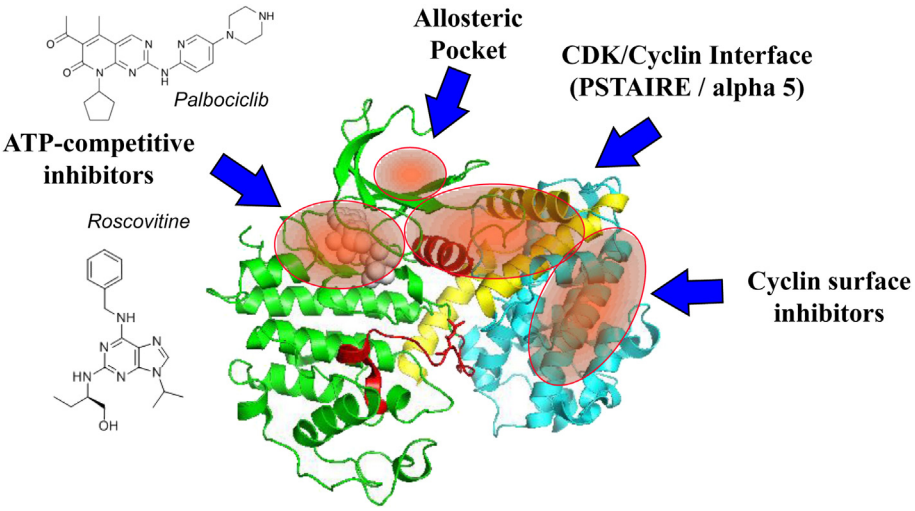

Fig. 3. Strategies for targeting Cyclin–dependent Kinases Cartoon of the different strategies devised to inhibit cyclin-dependent kinases. The structure of CDK2/Cyclin A is represented. CDK2 is in green, with the T-loop and the PSTAIRE helix at the interface with Cyclin A in red; Cyclin A is in cyan; the helices involved in the interaction with CDK2 are highlighted in yellow. ATP-competitive inhibitors bind the hinge region of the CDK subunit and compete with ATP binding; peptide inhibitors of the interface between PSTAIRE alpha helix and alpha5 helix of the cyclin; small molecule inhibitors target an essential interface at the surface of the cyclin; small molecule inhibitors bind an allosteric pocket which is only accessible in an inactive intermediate of the CDK to prevent conformational transitions towards an active form – here the position of the allosteric pocket present in the CDK/Cyclin/p27 is shown. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

[129–131]. Various studies report the anti-proliferative activity of dinaciclib in several human cancer cell lines derived from osteosarcoma, breast and pancreatic cancer, melanoma, chronic lymphocytic and acute leukaemias.

Table 1
CDK inhibitors.

Inhibitor	Target	References
ATP–competitive inhibitors		
Roscovitine (purine)	CDK5, CDK2, CDK1, CDK7, CDK9	[115–119]
Staurosporine (alkaloid)	CDK1, CDK2, CDK4	[103,104]
Olomoucine (purine)	CDK1, CDK2, CDK5	[105]
Butyrolactone I	CDK1 > CDK2	[106]
Flavopiridol	CDK2, CDK4, CDK6, CDK9	[107,120–123]
Indirubin-5 (purine)	CDK1 > CDK2 > CDK5	[108]
PD-0322991 (Palbociclib)	CDK4, CDK6	[132–135]
Dinaciclib	CDK1, CDK2, CDK5, CDK9	[129–131]
AT7519	CDK2, CDK4, CDK5, CDK7, CDK9	[124–128]
Peptide inhibitors targeting protein/protein interfaces		
Interface peptide	CDK2/Cyclin A	[146]
C4 peptide derived from Cyclin A	CDK2/Cyclin A	[147]
Hexapeptide – targets Cyclin A surface pocket	CDK2/Cyclin A	[148]
Spa310 and derivative from p130/pRb spacer domain	CDK2/Cyclin A	[149,150]
CDK4 derived C-terminal hexapeptide	CDK4/Cyclin D	[151]
Interface Peptides derived from p35	CDK5/p35	[152–157]
Small molecule non–ATP competitive/allosteric		
CPD1 3alpha-amino-5alpha androstane	CDK5/p25	[161,162]
Allosteric pocket in CDK2/Cyclin A/p27	CDK2/Cyclin A/p27	[164]
Chrysin-derivative – allosteric site	CDK2 & CDK4/CDK6	[165]

PD-0322991 is a potent and selective inhibitor of CDK4 and CDK6, which has proven efficient in advanced cancer and mantle cell lymphoma (MCL), in multiple myeloma in combination with other drugs (bortezomib and dexamethasone) and oestrogen receptor-positive advanced breast cancer in combination with letrozole. Also known as Palbociclib, this CDK inhibitor is currently considered a blockbuster for the pharmaceutical market/industry which offers promising perspectives for clinical administration [114,132–135].

2.4. Non-ATP-competitive inhibitors of CDK/Cyclins

Whilst ATP-competitive CDK inhibitors offer promising perspectives and have lead to development of second generation drugs intended to increase their specificity, the development of new classes of potent and specific CDK inhibitors remains a major challenge, and one of the major issues remains limited selectivity, which, together with the emergence of acquired drug resistance constitutes an important bottleneck in drug discovery programmes. Indeed, there are 518 protein kinases in the human kinome, which display highly conserved structural features in particular within the ATP-binding pocket. To address this challenge, alternative strategies have been actively explored to identify compounds which inhibit CDK/Cyclins, by targeting sites and pockets which are distinct from the ATP binding pocket, by interfering with substrate recognition, rather than catalytic activity, targeting protein/protein interfaces which are essential for kinase function and/or, transitional intermediates or conformational changes [85,136–138].

The most widely developed strategy has consisted in designing peptide inhibitors to target the interface between CDKs and Cyclins. Targeting protein/protein interactions (PPIs) which are essential for assembly of multimeric complexes constitutes an extremely attractive strategy in drug discovery, since it provides a high level of specificity, given that the sequences and physicochemical features that characterize a given protein/protein interface are unique and contribute to the specificity of molecular recognition between two protein partners [139–141]. Targeting PPIs involves identifying compounds that disrupt or compete with a complementary interface, either through high throughput screening of small molecule

libraries or through structure-guided rational design of mimicks of protein interfaces [99,140–145].

The assembly of cyclin-dependent kinase heterodimers and their activating and inhibitory regulation by cell cycle partners involves essential and specific protein/protein interactions which constitute attractive targets for the design of new generations of inhibitors. Several PPI inhibitors have been designed to inhibit CDK2/Cyclin A, including a peptide that targets a surface pocket of cyclin A that plays a central role in recruitment of substrates to the CDK/Cyclin complex, and a peptide that targets the primary interaction between CDK2 and Cyclin A (PSTAIRE/alpha5 helix) [146–150]. A hexapeptide derived from a Cterminal loop outside the kinase domain of CDK4 and its cyclic derivatives proved to be efficient in killing several cancer cell lines whilst sparing keratinocytes and fibroblasts [151]. A peptide targeting the interface between CDK5 and p35 was developed to Cdk5-p25 hyperactivity and Tau hyperphosphorylation [152–155]. More recently this inhibitor has been shown to reduce neurodegeneration and prevent Alzheimer's disease in mouse models [156,157]. Such peptides may constitute templates for further design of compounds that mimick interfaces of interest or chemical analogues capable of targeting the interaction in a second stage of development [99,158,159].

Small molecule kinase inhibitors targeting essential protein/protein interfaces or allosteric pockets are also actively sought for [140,142,160]. In silico and bioluminescence-based screening strategies have been designed to identify small molecules that target the interface between CDK5 and p35, providing potential leads, such as 3alpha-amino-5alpha-androstane for development of new drugs [161,162].Yet another attractive strategy involves identifying allosteric inhibitors which bind sites remote from the ATP-pocket, which are accessible in intermediate conformations and which stabilize enzymatically inactive conformations by preventing conformational transitions associated with kinase activation. This class of inhibitors can be expected to exhibit superior selectivity profiles, since they bind sites that are not conserved across the kinome [85,136,138,163]. Corsino et al. [164] identified a pocket present only in the inhibited p27KIP-bound form of CDK2-Cyclin A and performed an in silico screen to identify compounds that would bind this pocket, successfully identifying hits that target CDK1, CDK2 and CDK4 and induce cytostatic effects associated with decreased RB phosphorylation and decreased expression of E2F-dependent genes [164]. Liu et al. Identified a chrysin-derivative (compound 69407) which behaves as an efficient ATP noncompetitive inhibitor of CDK2 and CDK4 activities that binds an allosteric pocket of CDK2 [165]. Betzi et al. [166] identified a novel allosteric pocket within CDK2, but their attempts to identify allosteric inhibitors were not rewarded [166,167].

New approaches will have to be designed to meet the demand for allosteric modulators of CDK/Cyclins. Since this class of compounds remains very difficult to design on a rational basis, future efforts will have to rely on the identification of allosteric pockets and on the development of conformation-sensitive assays for high throughput screens. In this respect, conformational biosensors have much to provide (see below).

3. Fluorescent biosensors – attractive tools for drug discovery

Over the last decade, a wide range of imaging probes have been developed to visualize the dynamic behaviour of biomolecules in living cells, tissues and whole organisms [168]. In particular, fluorescent biosensors are sensitive tools that allow to monitor target proteins in real-time and in their native environment in a dynamic and reversible fashion. These molecular probes virtually allow to "find a needle in a haystack" meaning that they can be tailored to identify and report on a specific target or enzymatic activity amongst the myriad of biomolecules and activities present within complex media, such as plasma, urine or even the cellular cytoplasm. They can provide invaluable information on the dynamic behaviour of a target and allow for comparative studies in physiological or pathological conditions [169–174].

Fluorescent biosensors comprise a receptor moiety tailored for specific recognition of the target, which may be associated with one or several fluorescent probes (Fig. 4A). Whilst the fluorescent probes may be synthetic or genetically-encoded autofluorescent proteins (AFPs), the general behaviour of the system follows the same pattern, in that it its designed to undergo sensitive changes in its fluorescent properties (intensity or life-time, spectral shifts, FRET between two probes) upon recognition and/or modification by its target [169–174].Two types of fluorescent biosensors have been developed: non-genetic and genetically-encoded protein, peptide or polymer-based scaffolds. Genetically-encoded biosensors are easy to transfect and to engineer and can be used for HTS assays using stable cell lines, yet face limitations associated with their ectopic expression. Within the genetically-encoded biosensor family, FRET-based biosensors, bearing a donor/acceptor couple of AFPs, have been the most widely developed [169–174]. Non-genetic biosensors allow to bypass the length of time required for ectopic expression of their genetically-encoded counterparts. These peptide, protein or polymeric biosensors are engineered through conjugation of synthetic dyes that can either transfer fluorescence resonance energy between one another (FRET biosensors), or which are sensitive to local changes in their environment which occur upon detection of the target. They are readily applicable in vitro but require microinjection or facilitated delivery for application in living cells and in vivo.

Fluorescent biosensors have been designed to report on changes in abundance, enzymatic activities, conformational changes, protein/protein interactions and posttranslational modifications. In particular, a wide variety of fluorescent biosensors have been designed to probe protein kinase activities in vitro and in living cells [175,176]. They are therefore widely used for fundamental purposes and very well suited for detection of biomarkers in biomedical applications for highlighting molecular alterations associated with pathological disorders, progression of disease and response to therapeutics [177,178]. The high intrinsic sensitivity of fluorescence and tailored selectivity of biosensors makes this technology further attractive for high throughput screening assays and high content cell- and image-based screens [179–187]. Moreover, fluorescent biosensors are well suited to study the kinetics and dynamics of target response to a drug which affects its function, activity or conformation. They can also be used to study kinetic profiles of agonists or antagonists and to study the mechanism of action of a compound. They further provide means of evaluating efforts made in medicinal chemistry to identify the best derivatives of lead compounds for therapeutic administration. Hence fluorescent biosensor technology is useful for every step of the drug discovery pipeline, from initial screening assays to postscreen structure-activity studies and cellular characterization of hit compounds, as well as for preclinical evaluation of leads in living models through fluorescence imaging [179–188].

Last but not least, fluorescent biosensors offer the opportunity to perform multiparametric screens. This may involve screening with several biosensors bearing distinct fluorescent probes, so as to seek for hits with unique specificity profiles for a given target with respect to other related or unrelated targets, in view of limiting off-target effects. Alternatively it may consist in combining a fluorescent biosensor-based assay with phenotypic selection based on cellular morphology, cell cycle status, apoptosis or cytotoxicity profiles [189–191].

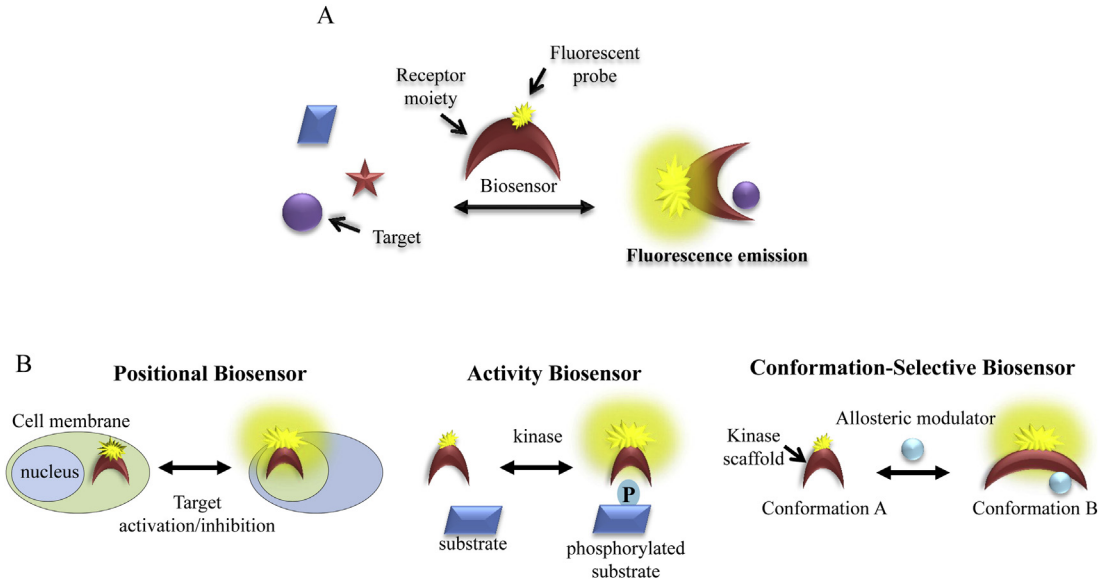

Fig. 4. Fluorescent Biosensors A. Schematic representation of a fluorescent biosensor B. Different examples of fluorescent biosensors: (from left to right) Positional Biosensors, Activity-based biosensors, Kinase-derived conformation-selective sensors.

Different classes of fluorescent biosensors have been developed for screening applications (Fig. 4B). Positional biosensors are genetic fusions of an AFP whose subcellular localization is altered in response to target activity or its inhibition. These reporters generally comprise an NLS or NES subcellular localization sequence, which becomes exposed or remains hidden, depending on target activity. Activity-based biosensors, including FRET-based biosensors and environmentally-sensitive biosensors, have been widely implemented to screen for compounds that inhibit enzymatic activities. Genetically-encoded FRET-based biosensors encode a pair of AFPs together with a substrate sequence which is recognized and modified by the target enzyme of interest (cleavage in the case of a protease, phosphorylation in the case of a kinase, methylation, acetylation etc). This may simply result in cleavage, thereby separating the two AFPs altogether, or in an intramolecular conformational change, in the case of a kinase biosensor for instance, which brings a phosphoamino acid binding domain (PAABD) together with the phosphorylated substrate sequence, thereby bringing the two AFPs close together, allowing the donor to transfer fluorescence resonance energy to the acceptor AFP. Environmentally-sensitive biosensors respond to enzymatic activities thanks to a synthetic fluorescent probe conjugated onto a peptide or protein sequence, generally derived from a substrate recognized by the target enzyme of interest. In their simplest form these biosensors respond directly to the modification imposed by the enzyme of interest (phosphorylation by kinase; cleavage by protease), which is sufficient to affect the spectral properties of the probe. More complex, bipartite biosensors have been designed for probing protein kinase activites, that comprise a PAABD which recognizes and binds a substrate sequence once it is phosphorylated by the kinase, thereby altering the local environment of the fluorescent probe and consequently its spectral properties.

Several examples of these biosensors developed for probing protein kinase activities have been successfully implemented for HTS/HCS and are reviewed in detail by Prével et al. [179]. Noteworthy examples include a positional biosensor of PKA activity designed as a GFP fusion incorporating both an NES and an NLS, the latter being masked upon binding of the catalytic subunit of PKA to an adjacent binding domain, thereby promoting nuclear export of the GFP-tagged biosensor [192]; AKAR biosensors, genetically-encoded FRET biosensors that report on PKA activity [193,194], and a Bcr-Abl kinase FRET biosensor [195]; environmentally-sensitive synthetic peptide biosensors developed by B. Imperiali and D. Lawrence's laboratories [196–203].

One of the bottlenecks in drug discovery concerns the development of inhibitors with high specificity and selectivity together with high bioavailability yet that do not induce emergence of resistance and which exhibit low toxicity profiles. So far, the larger part of strategies employed for screening inhibitors of protein kinases have relied on activity-based assays which have essentially lead to identification of ATP-competitive inhibitors. However, the high conservation of the ATP binding pocket throughout the 518 kinases in the human proteome leads to limited selectivity of this class of inhibitors. To bypass the systematic identification of ATP-pocket binding competitors in kinase activity-based screens, alternative strategies have been devised to search for compounds that target essential protein/protein interfaces or allosteric modulators that bind and stabilize inactive conformational intermediates, and which are thereby thought to be more selective.

Protein kinases undergo significant conformational changes associated with their activation, which can be characterized through structural approaches such as NMR or X-ray crystallography. Although these technologies can be implemented to high throughput screening formats, they rely on heavy and expensive equipment for screens themselves, and require more complex analysis of data than fluorescence-based screens. Development of a sensitive technology, known as FLIK (Fluorescent Labels in Kinases) based on incorporation of one or several environmentally-sensitive fluorescent dyes into a kinase scaffold, at positions which are subject to conformational changes, has been successfully applied to monitor transitions between active and inactive conformations of kinases by fluorescence spectroscopy. Several conformation-selective fluorescent biosensors engineered using this strategy have proven extremely sensitive and potent tools to identify allosteric inhibitors of protein kinases. Their implementation to high throughput screens has lead to the successful identification of small molecule inhibitors of MAPK, Src and Abl kinases, that bind and stabilize the enzymatically incompetent “DFG-out” conformation of these kinases [204–209] (See Fig. 4B).

The successful implementation of fluorescent biosensors to HTS/HCS assays requires their ability to respond in a robust yet sensitive fashion, with high selectivity and reproducibility. The successful outcome of a screen requires essential criteria be met, with respect to the quality of signals and to the signal-to-noise ratio, to differences between average and standard deviation values between positive and negative controls. Ideally, a performance assay should yield robust signals with a high signal-to-noise ratio and reproducible signals, maximal differences between the signals associated with positive and negative controls, and minimal variability between these. The quality, performance and reliability of an assay will be reflected in the Z factor calculated from the screen values lies between 0,5 and 1. The Z factor is defined as follows: $Z = 1 - [3(D^+ + D^-)/(A^+ - A^-)]$, where D^+ and D^- correspond to the standard deviations of positive and negative controls and where A^+ and A^- are the average responses for positive and negative controls, respectively [179,210].

4. Fluorescent biosensors of cyclin-dependent kinases

CDK/cyclin activities are deregulated in several human cancers, and despite the large number of ATP-competitive inhibitors available for laboratory use and for clinical purposes, it is crucial to find new classes of specific and potent inhibitors of these attractive pharmacological targets. However, like most other kinases, cyclin-dependent kinases constitute a challenging class of therapeutic targets due to their highly conserved structural features and to their dependence on ATP binding for kinase activity. So far, several in vitro and cell-based assays have been implemented to screen for inhibitors of these kinases, but these screens have essentially yielded compounds that bind the ATP-binding pocket of the CDK and compete with ATP, which therefore display poor specificity profiles, and very few allosteric inhibitors have been reported (see Section 2 and Table 1). Indeed, the larger part of kinase activity-based assays rely radioactivity and antigenic approaches, but despite their overall efficiency and utility, these assays are laborious, time-consuming, require enzymatically active forms of kinases and can only be used in vitro. These limitations call for alternative strategies which can be readily be adapted to HTS and which will enable to discriminate between ATP-competitive inhibitors and novel classes of kinase inhibitors.

Our group has focused on the development of fluorescent peptide and protein biosensors to study the molecular dynamics of cyclin-dependent kinases and gain insight into their behaviour in physiological and pathological settings [211–213]. This sensing technology is based on the rational design of receptor moieties derived from specific interactors or substrates of CDK/cyclins, onto which environmentally-sensitive fluorescent dyes are coupled, which respond to changes in the polarity of their environment through significant changes in their fluorescence emission, upon detection of their target. These fluorescent biosensors are useful laboratory tools for in vitro applications, but can also be used to report on CDK/Cyclins in cell extracts or in living cells following facilitated delivery with cell-penetrating peptide complexes, through fluorescence imaging and ratiometric quantification. As such, they provide sensitive means of monitoring subtle alterations in the abundance, activity or conformational dynamics of these kinases in vitro, in healthy or in cancer cells, following activation or in response to targeted therapeutics. Moreover they constitute attractive tools for drug discovery programmes which we have implemented to develop high throughput screening assays in view of identifying novel inhibitors of cancer cell proliferation.

4.1. CDKSENS biosensor – screening for modulators of kinase abundance

CDKSENS is biligand peptide that bears both a CDK-binding sequence and a cyclin-binding sequence (Fig. 5A). This design allows for recognition of both CDK and Cyclin subunits and preferential binding of heterodimeric CDK/cyclin complexes in vitro and in living cells [211]. We have previously shown that CDKSENS could be used to report on differences in CDK/Cyclin levels between different cell types, or in response to ectopic expression or downregulation of a CDK or Cyclin subunit through changes in fluorescence intensity of an environmentally-sensitive dye [211]. Since this biosensor is well suited to identify modulators of CDK/Cyclin levels, we developed a RFP-fusion which we expressed in *Escherichia coli* and purified by FPLC chromatography, then labelled with Cy5 on the cysteine residue between the CDK and Cyclin-binding moieties. RFP-CDKSENS was then introduced into living cells using the cell-penetrating peptide Pep1 [214], so as to establish an assay which could be applied to an automated high content screen on a Cellomics ArrayScan Robot. The RFP moiety served as a fluorescent internal standard for ratiometric quantification of cyanine fluorescence intensity in different cell types. In order to determine the potential and sensitivity of this system to monitor differences in CDK/Cyclin levels, we first examined the differences between HS68, HeLa and U20S cell types, as distribution profiles of fluorescence ratio intervals from the average Cy5/RFP value in a population of 3000–5000 cells (Fig. 5B). In agreement with data published previously, we found that the maximum of the fluorescent ratio distribution profiles shifted by 20% between HS68 and HeLa cells (Fig. 5C). We therefore further treated cells with Nocodazole, an inhibitor of mitotic spindle assembly which leads to accumulation of cells at the G2/M transition. In this assay, we found that the fluorescence distribution profiles were shifted to the left, indicating an overall decrease of total CDK/Cyclin levels (Fig. 5D). Finally we treated cells with a formulation of p27 inhibitor of CDK2 kinase (Pep1/p27 protein) at two different concentrations, and found a dose-dependent response, with two fluorescent populations at 1ug/ml and a net 40% shift towards the second population at 1.4ug/ml), inferring that p27 inhibitor leads to an increase in overall CDK/Cyclin concentration (Fig. 5D). Hence this approach is clearly well suited for a high content screen on mammalian cells, and is further applicable to develop a multiparametric assay in which cytotoxicity, cell cycle analysis and CDK/Cyclin levels can be assessed simultaneously.

4.2. CDKACT biosensor – screening for modulators of kinase activity

CDKACT is a modular protein biosensor designed to report on CDK/cyclin activity. This non-genetic biosensor is constituted of a CDK-specific peptide substrate and a phosphoamino acid binding domain that recognizes the substrate sequence following phosphorylation by an active CDK/Cyclin kinase, thereby leading to fluorescence enhancement of an environmentally-sensitive probe conjugated proximal to the phosphorylation site [212]. (Fig. 6A). We have previously reported the use of CDKACT biosensor to probe CDK/Cyclin activities in vitro using recombinant kinases, and have further applied this technology to report on CDK/Cyclin activities directly using cell extracts, as well as in living cells. Since CDKACT offers a means of probing the kinetics of CDK/Cyclin activities in a sensitive and fully reversible fashion, we implemented it to design a fluorescence-based assay for high throughput screening of CDK/Cyclin inhibitors within cell extracts. Assay conditions were standardized using 25 nM CDKACT biosensor and 20ug HeLa cell

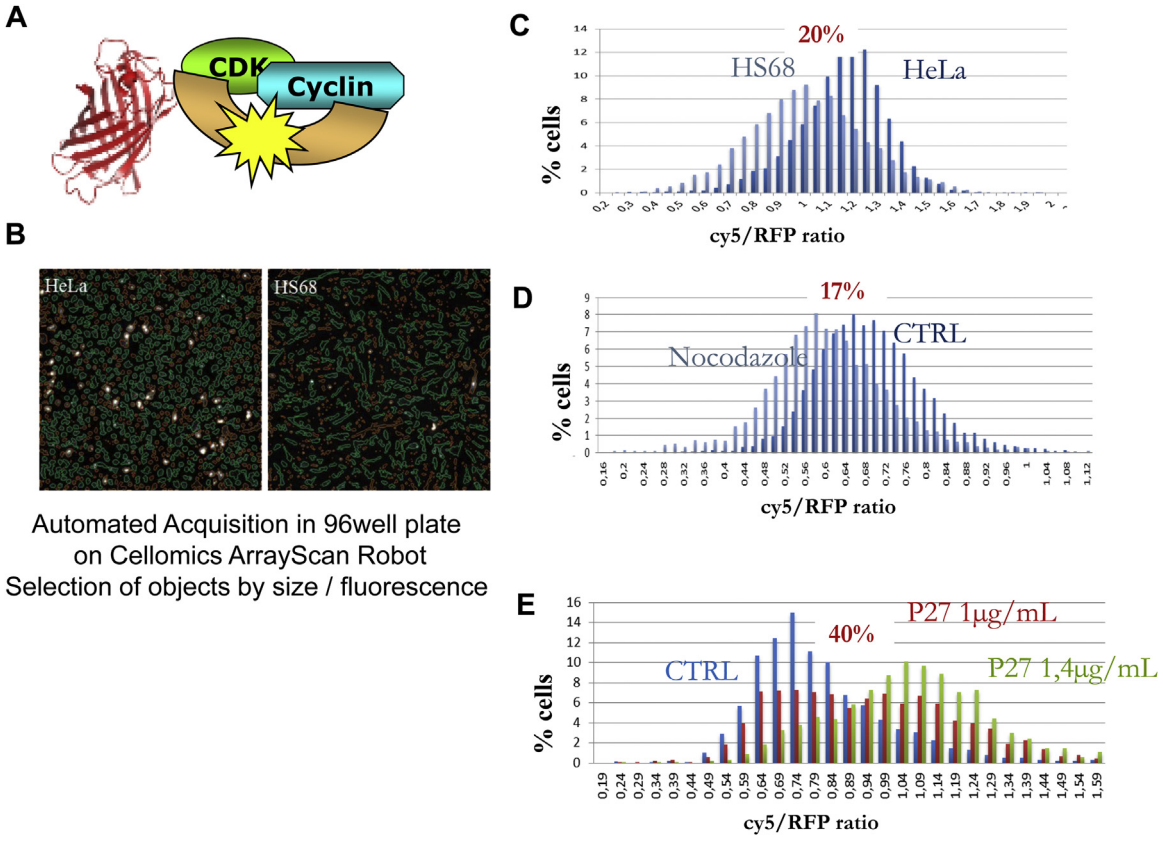

Fig. 5. CDKSENS technology & application to HTS A. Schematic representation of RFP-CDKSENS Biosensor B. CDKSENS-based fluorescence-based assay for high content screening (for experimental details see Supplementary information) C. Fluorescence ratio distribution profiles of CDKSENS-Cy5/RFP in HeLa versus HS68 cells. D. Fluorescence ratio distribution profiles of CDKSENS-Cy5/RFP in untreated versus HeLa cells treated with Nocodazole. E. Fluorescence ratio distribution profiles of CDKSENS-Cy5/RFP in HeLa cells treated with 1 mg/ml or 1.4 mg/ml p27 inhibitor.

extracts in PBS supplemented with 5 mM ATP 20 mM MgCl2 at 37 °C for 60 min. The CDK inhibitor Roscovitine was used as a control inhibitor at 20uM and H89 as a negative control (Fig. 6B). This assay is well suited to identify drugs that inhibit CDK/Cyclin activity in cell extracts, which offers a closer reflection of reality than assays performed with purified recombinant kinases, yet is easier to implement than a cell-based assay.

4.3. CDKCONF biosensors – fluorescent labels in kinases to probe conformational changes

Cyclin-dependent kinases undergo major conformational changes associated with their activation, upon association with their cyclin partners. The activation loop of CDKs, or T-loop, behaves like a drawbridge, that undergoes a positional switch upon

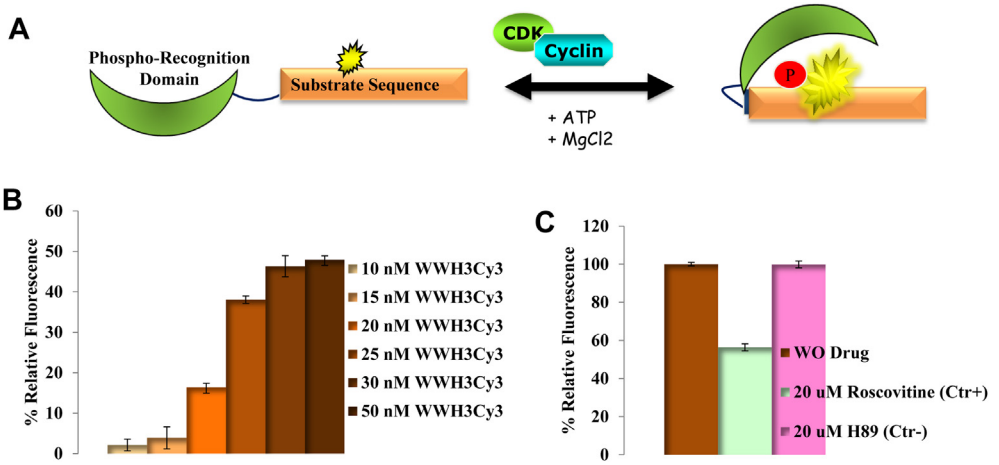

Fig. 6. CDKACT technology & application to HTS A. Schematic representation of CDKACT Biosensor B. Dose-dependent response of 25 nM CDKACT-Cy3 biosensor to 20 µg HeLa cell extracts (for experimental details see Supplementary information) C. Optimization of the assay conditions for application of CDKACT to HTS using cell extracts as a source of CDK/Cyclins. Positive Control: 20 µM Roscovitine; Negative Control: 20 µM H89.

cyclin binding, thereby contributing to CDK activation by stabilizing the loop in a conformation that enables complete access to the substrate. Compounds capable of interfering with this critical conformational change would provide an efficient and selective means of inhibiting CDK/Cyclin activation. In an attempt to identify inhibitors that would target the positional switch of the T-loop, we developed CDKCONF biosensors by introducing synthetic fluorophores into the CDK kinase scaffold, where they serve as molecular hinges in reporting on conformational dynamics of the Tloop [213] (Fig. 7A). We showed that CDKCONF responds to Cyclin binding in a sensitive fashion, but does not respond to compounds that bind the ATP-binding pocket [213]. As such we designed CDKCONF biosensors for CDK2 and for CDK4 and developed a fluorescence-based assay allowing their implementation to high throughput screens in view of identifying allosteric modulators specific to each kinase. Optimization of CDKCONF biosensors for a standardized robust and reproducible assay involved downscaling and kinetic assays in the presence of partner or substrate proteins versus ATP or ATP-competitive inhibitors (Roscovitine or PD-0332991, respectively for CDK2 and CDK4) (Fig. 7B). CDKCONF2 and CDKCONF4 were successfully applied to screen the French National Library of chemical compounds (personal communication). These screens lead to identification of new and different classes of compounds, revealing the specificity of the assay for each kinase (personal communication). Our studies reveal that CDKCONF biosensors constitute exquisitely selective tools for discriminating between ATP-competitive inhibitors and conformational modulators, allosteric inhibitors of CDK/cyclin complexes in high throughput screening formats.

4.4. Pros and Cons of fluorescent biosensors for HTS/HCS

The fluorescent biosensors designed to probe specific and characteristic features of CDK/Cyclins constitute a promising technology for implementation of high throughput screening approaches to identify novel classes of inhibitors, in particular compounds that interfere with conformational changes or critical protein/protein interactions.

Several other strategies have been reported for high throughput screening of chemical libraries with the aim of identifying allosteric kinase inhibitors. For instance, an NMR-based strategy developed in R. Amstutz's laboratory, based on displacement measurements of a spin-labeled adenine analogue bound to the ATP-binding site of the kinase, was reported to identify potential inhibitors binding a neighbouring allosteric site [215]. Klüter and co-workers developed a luminescence displacement assay using a labeled probe consisting of a type III inhibitor, which binds the allosteric site of the inactive kinase, chemically conjugated to a small enzyme donor peptide fragment of β-galactosidase [216]. Lebakken and co-workers designed a time-resolved FRET displacement assay thanks to a fluorescently-labelled ATP-competitive small-molecule kinase inhibitor, binding of which to a tagged kinase (GST or polyhistidine) is detected thanks to a europium-labelled antitag antibody [217]. However these approaches all rely on displacement measurements, which require the use of compounds that are known binders of the target kinases, either of ATP-binding pocket or of an already identified allosteric pocket. Moreover these assays are an indirect reflection of inhibitor binding, as compared to assays performed with fluorescent biosensors that directly report on kinase activity or conformation. It is important to emphasize that structure-based

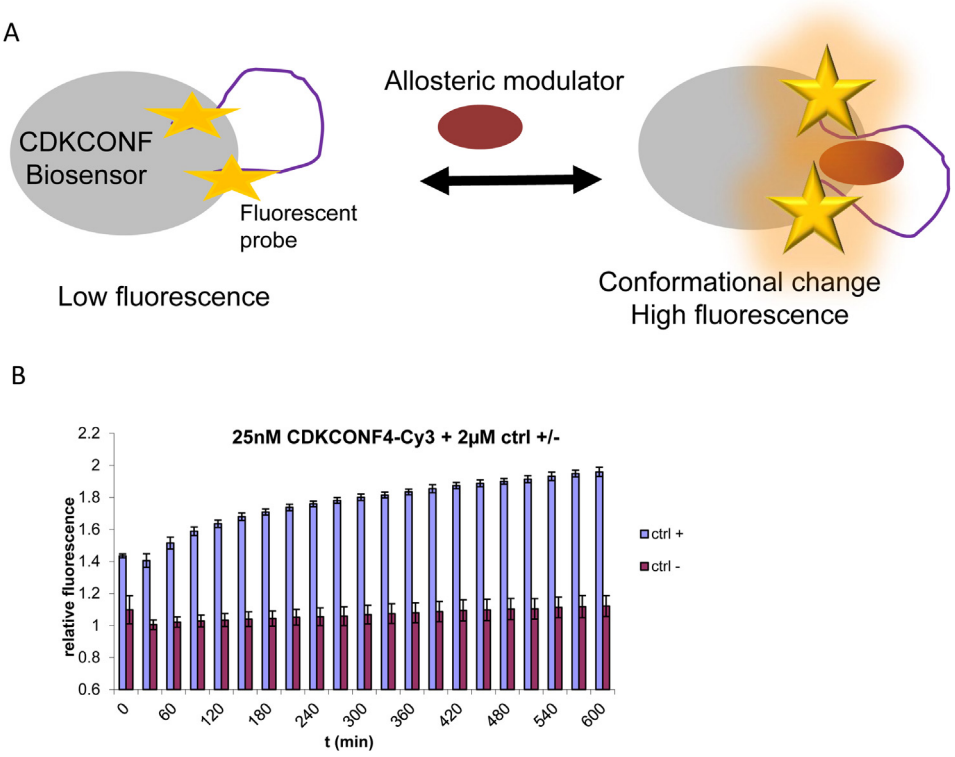

Fig. 7. CDKCONF technology & application to HTS A. Schematic representation of CDKCONF Biosensors – principle of conformational biosensor B. Optimization of the assay conditions for application of CDKCONF-based fluorescence assay to HTS – miniaturization and determination of concentration and kinetics of the assay for optimal differences between positive and negative controls (for experimental details see Supplementary information). Positive control: 2 µM retinoblastoma protein; negative control: peptide derived from PISTVRE helix of CDK4.

assays (NMR, X-ray cristallography) implemented to high-throughput screening are not readily applicable to kinetic analyses, whereas fluorescent biosensors are in essence dynamic and reversible, and therefore allow for kinetic and comparative profiling of inhibitors throughout the screen, and in postscreen studies, providing further insights into their mechanism of action. Moreover, fluorescent biosensors can be implemented to in vitro high-throughput screens using purified recombinant kinase, as well as to assays in which the kinase is in more complex environments, and to highcontent cell-based assays, enabling acquisition of changes in fluorescence signals together with cell images. Last but not least, given the simplicity of their application and of signal readout, these biosensors offer means of developing multi-parametric screens, for instance to monitor information regarding phenotype, cell cycle status or cytotoxicity.

5. Concluding remarks

Cyclin-dependent kinases play central roles in regulation of cell cycle progression, transcriptional regulation and other major biological processes such as neuronal differentiation, development and metabolism. Despite their central functions, they have frequently been reported to be deregulated, hyperactivated in a wide variety of human cancers through amplification, overexpression or mutation. These kinases therefore constitute established pharmacological targets, and several inhibitors have been developed to target their activity.

Although a large number of ATP-competitive inhibitors of CDKs have been identified from natural substances, and further optimized through structure-guided approaches and medicinal chemistry to yield potent anticancer drugs, they face limitations with respect to selectivity. Alternative strategies have therefore been explored to target essential protein/protein interfaces and screen for allosteric inhibitors. However it remains extremely challenging to design allosteric inhibitors through rational approaches, and calls for sensitive and tailored technologies which can be adapted to HTS/HCS.

Fluorescent biosensors provide alternatives to studying target enzyme activities with a high level of sensitivity and specificity when tailored to meet specific demands, in buffer or in complex biological samples. These tools are well suited to study dynamic processes and highlight molecular alterations associated with pathological disorders. They constitute powerful tools for drug discovery purposes, from the early stage establishment of primary HTS/HCS, for validation and postscreen characterization of hits in structure/activity studies, for studies of their mechanism of action, and for kinetic profiling of candidate inhibitor behaviour. Furthermore they are useful tools for optimization of lead compounds, and preclinical evaluation of drug candidates in vivo.

We have developed three families of fluorescent biosensors to probe cyclin-dependent kinases abundance, activity and conformational dynamics. While these tools allow to gain insight into CDK/Cyclin molecular behaviour, they also provide a means of monitoring subtle alterations in the abundance and activity of CDK/Cyclins.

In this review we discuss the different classes of inhibitors which have been synthesized and/or identified so far with particular focus on allosteric inhibitors. We further discuss issues related to fluorescent biosensors in drug discovery; and provide more specific examples concerning the implementation of our CDK/Cyclin biosensors to establish sensitive assays for HTS/HCS.

Conflict-of-Interest-Statement

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.10.003.

References

[1] D.O. Morgan, Cyclin-dependent kinases: engines, clocks, and microprocessors, Annu. Rev. Cell Dev. Biol. 13 (1997) 261–291.
[2] M. Malumbres, M. Barbacid, Cell cycle, CDKs and cancer: a changing paradigm, Nat. Rev. Cancer 9 (2009) 153–166.
[3] M. Malumbres, Physiological relevance of cell cycle kinases, Physiol. Rev. 91 (2011) 973–1007.
[4] S. Lim, P. Kaldis, Cdks, cyclins and CKIs: roles beyond cell cycle regulation, Development 140 (2013) 3079–3093.
[5] M. Dorée, T. Hunt, From Cdc2 to Cdk1: when did the cell cycle kinase join its cyclin partner? J. Cell Sci. 115 (2002) 2461–2464.
[6] P. Kaldis, The cdk-activating kinase (CAK): from yeast to mammals, Cell Mol. Life Sci. 55 (1999) 284–296.
[7] R.P. Fisher, Secrets of a double agent: CDK7 in cell-cycle control and transcription, J. Cell Sci. 118 (2005) 5171–5180.
[8] P. Aggarwal, L.P. Vaites, J.K. Kim, H. Mellert, B. Gurung, H. Nakagawa, et al., Nuclear cyclin D1/CDK4 kinase regulates CUL4 expression and triggers neoplastic growth via activation of the PRMT5 methyltransferase, Cancer Cell 18 (2010) 329–340.
[9] S.J. Tudhope, C.-C. Wang, J.L. Petrie, L. Potts, F. Malcomson, J. Kieswich, et al., A novel mechanism for regulating hepatic glycogen synthesis involving serotonin and cyclin-dependent kinase-5, Diabetes 61 (2012) 49–60.
[10] X. Zhao, D. Feng, Q. Wang, A. Abdulla, X.-J. Xie, J. Zhou, et al., Regulation of lipogenesis by cyclin-dependent kinase 8-mediated control of SREBP-1, J. Clin. Invest. 122 (2012) 2417–2427.
[11] E.A. Hanse, D.G. Mashek, J.R. Becker, A.D. Solmonson, L.K. Mullany, M.T. Mashek, et al., Cyclin D1 inhibits hepatic lipogenesis via repression of carbohydrate response element binding protein and hepatocyte nuclear factor 4α, Cell Cycle 11 (2012) 2681–2690.
[12] F. Heitz, M.C. Morris, D. Fesquet, J.C. Cavadore, M. Dorée, G. Divita, Interactions of cyclins with cyclin-dependent kinases: a common interactive mechanism, Biochemistry 36 (1997) 4995–5003.
[13] M. Malumbres, R. Sotillo, D. Santamaría, J. Galán, A. Cerezo, S. Ortega, et al., Mammalian cells cycle without the D-type cyclin-dependent kinases Cdk4 and Cdk6, Cell 118 (2004) 493–504.
[14] D. Santamaría, C. Barrière, A. Cerqueira, S. Hunt, C. Tardy, K. Newton, et al., Cdk1 is sufficient to drive the mammalian cell cycle, Nature 448 (2007) 811–815.
[15] C.J. Sherr, J.M. Roberts, Living with or without cyclins and cyclin-dependent kinases, Genes. Dev. 18 (2004) 2699–2711.
[16] D.O. Morgan, Principles of CDK regulation, Nature 374 (1995) 131–134.
[17] T.R. Coleman, W.G. Dunphy, Cdc2 regulatory factors, Curr. Opin. Cell Biol. 6 (1994) 877–882.
[18] R. Boutros, C. Dozier, B. Ducommun, The when and wheres of CDC25 phosphatases, Curr. Opin. Cell Biol. 18 (2006) 185–191.
[19] T. Hunt, Cyclins and their partners: from a simple idea to complicated reality, Semin. Cell Biol. 2 (1991) 213–222.
[20] P.D. Jeffrey, A.A. Russo, K. Polyak, E. Gibbs, J. Hurwitz, J. Massagué, et al., Mechanism of CDK activation revealed by the structure of a cyclinA-CDK2 complex, Nature 376 (1995) 313–320.
[21] A.A. Russo, P.D. Jeffrey, N.P. Pavletich, Structural basis of cyclin-dependent kinase activation by phosphorylation, Nat. Struct. Biol. 3 (1996) 696–700.
[22] N.R. Brown, M.E. Noble, A.M. Lawrie, M.C. Morris, P. Tunnah, G. Divita, et al., Effects of phosphorylation of threonine 160 on cyclin-dependent kinase 2 structure and activity, J. Biol. Chem. 274 (1999) 8746–8756.
[23] J.C. Hagopian, M.P. Kirtley, L.M. Stevenson, R.M. Gergis, A.A. Russo, N.P. Pavletich, et al., Kinetic basis for activation of CDK2/cyclin A by phosphorylation, J. Biol. Chem. 276 (2001) 275–280.
[24] M.C. Morris, C. Gondeau, J.A. Tainer, G. Divita, Kinetic mechanism of activation of the Cdk2/cyclin A complex. Key role of the C-lobe of the Cdk, J. Biol. Chem. 277 (2002) 23847–23853.

[25] F. Toyoshima-Morimoto, E. Taniguchi, N. Shinya, A. Iwamatsu, E. Nishida, Polo-like kinase 1 phosphorylates cyclin B1 and targets it to the nucleus during prophase, Nature 410 (2001) 215–220.
[26] P.D. Jeffrey, L. Tong, N.P. Pavletich, Structural basis of inhibition of CDK-cyclin complexes by INK4 inhibitors, Genes. Dev. 14 (2000) 3115–3125.
[27] C.J. Sherr, J.M. Roberts, CDK inhibitors: positive and negative regulators of G1-phase progression, Genes. Dev. 13 (1999) 1501–1512.
[28] M. Malumbres, M. Barbacid, To cycle or not to cycle: a critical decision in cancer, Nat. Rev. Cancer 1 (2001) 222–231.
[29] M. Malumbres, M. Barbacid, Cell cycle kinases in cancer, Curr. Opin. Genet. Dev. 17 (2007) 60–65.
[30] D. Hanahan, R.A. Weinberg, The hallmarks of cancer, Cell 100 (2000) 57–70.
[31] M. Hall, G. Peters, Genetic alterations of cyclins, cyclin-dependent kinases, and Cdk inhibitors in human cancer, Adv. Cancer Res. 68 (1996) 67–108.
[32] A. Husdal, G. Bukholm, I.R.K. Bukholm, The prognostic value and over-expression of cyclin A is correlated with gene amplification of both cyclin A and cyclin E in breast cancer patient, Cell Oncol. 28 (2006) 107–116.
[33] A. Nar, O. Ozen, N.B. Tutuncu, B. Demirhan, Cyclin A and cyclin B1 over-expression in differentiated thyroid carcinoma, Med. Oncol. 29 (2012) 294–300.
[34] A. Wang, N. Yoshimi, M. Suzui, A. Yamauchi, M. Tarao, H. Mori, Different expression patterns of cyclins A, D1 and E in human colorectal cancer, J. Cancer Res. Clin. Oncol. 122 (1996) 122–126.
[35] K.E. Knudsen, J.A. Diehl, C.A. Haiman, E.S. Knudsen, Cyclin D1: polymorphism, aberrant splicing and cancer risk, Oncogene 25 (2006) 1620–1628.
[36] T. Suzuki, T. Urano, Y. Miki, T. Moriya, J. Akahira, T. Ishida, et al., Nuclear cyclin B1 in human breast carcinoma as a potent prognostic factor, Cancer Sci. 98 (2007) 644–651.
[37] K. Aaltonen, R.M. Amini, P. Heikkila, K. Aittomaki, A. Tamminen, H. Nevanlinna, C. Blomqvist, High cyclin B1 expression is associated with poor survival in breast cancer, Br. J. Cancer 100 (2009) 1055–1060.
[38] M.D. Begnami, J.H.T.G. Fregnani, S. Nonogaki, F.A. Soares, Evaluation of cell cycle protein expression in gastric cancer: cyclin B1 expression and its prognostic implication, Hum. Pathol. 41 (2010) 1120–1127.
[39] H. Murakami, M. Furihata, Y. Ohtsuki, S. Ogoshi, Determination of the prognostic significance of cyclin B1 overexpression in patients with esophageal squamous cell carcinoma, Virchows Arch. 434 (1999) 153–158.
[40] J.-C. Soria, S.J. Jang, F.R. Khuri, K. Hassan, D. Liu, W.K. Hong, L. Mao, Over-expression of cyclin B1 in early-stage non-small cell lung cancer and its clinical implication, Cancer Res. 60 (2000) 4000–4004.
[41] J.K. Kim, J.A. Diehl, Nuclear cyclin D1: an oncogenic driver in human cancer, J. Cell Physiol. 220 (2009) 292–296.
[42] R. Van Dross, P.J. Browning, J.C. Pelling, Do truncated cyclins contribute to aberrant cyclin expression in cancer? Cell Cycle 5 (2006) 472–477.
[43] J. Ekberg, C. Holm, S. Jalili, J. Richter, L. Anagnostaki, G. Landberg, et al., Expression of cyclin A1 and cell cycle proteins in hematopoietic cells and acute myeloid leukemia and links to patient outcome, Eur. J. Haematol. 75 (2005) 106–115.
[44] R.M. Harwell, B.B. Mull, D.C. Porter, K. Keyomarsi, Activation of cyclin-dependent kinase 2 by full length and low molecular weight forms of cyclin E in breast cancer cells, J. Biol. Chem. 279 (2004) 12695–12705.
[45] R. Donnellan, R. Chetty, Cyclin E in human cancers, FASEB J. 13 (1999) 773–780.
[46] A.M. Karst, P.M. Jones, N. Vena, A.H. Ligon, J.F. Liu, M.S. Hirsch, D. Etemadmoghadam, D.D.L. Bowtell, R. Drapkin, Cyclin E1 deregulation occurs early in secretory cell transformation to promote formation of fallopian tube – derived high-grade serous ovarian cancers, Cancer Res. (2013).
[47] R.L. Huuhtanen, C.P. Blomqvist, T.O. Böhling, T.A. Wiklund, E.J. Tukiainen, M. Virolainen, B. Tribukait, L.C. Andersson, Expression of cyclin a in soft tissue sarcomas correlates with tumor aggressiveness, Cancer Res. 59 (1999) 2885–2890.
[48] S. Lapenna, A. Giordano, Cell cycle kinases as therapeutic targets for cancer, Nat. Rev. Drug Discov. 8 (2009) 547–566.
[49] C. Bruyère, L. Meijer, Targeting cyclin-dependent kinases in anti-neoplastic therapy, Curr. Opin. Cell Biol. 25 (2013) 772–779.
[50] M.Y. Zhao, A. Auerbach, A.M. D'Costa, A.P. Rapoport, A.M. Burger, E.A. Sausville, S.A. Stass, F. Jiang, A.M. Sands, N. Aguilera, X.F. Zhao, Phospho-p70S6K/p85S6K and cdc2/cdk1 are novel targets for diffuse large b-cell lymphoma combination therapy, Clin. Cancer Res. 15 (2009) 1708–1720.
[51] J. Yuan, A. Krämer, Y. Matthess, R. Yan, B. Spänkuch, R. Gätje, et al., Stable gene silencing of cyclin B1 in tumor cells increases susceptibility to taxol and leads to growth arrest in vivo, Oncogene 25 (2006) 1753–1762.
[52] M.C. Morris, E. Gros, G. Aldrian-Herrada, M. Choob, J. Archdeacon, F. Heitz, et al., A non-covalent peptide-based carrier for in vivo delivery of DNA mimics, Nucleic Acids Res. 35 (2007) e49.
[53] I. Androic, A. Krämer, R. Yan, F. Rödel, R. Gätje, M. Kaufmann, et al., Targeting cyclin B1 inhibits proliferation and sensitizes breast cancer cells to taxol, BMC Cancer 8 (2008) 391.
[54] L. Crombez, M.C. Morris, S. Dufort, G. Aldrian-Herrada, Q. Nguyen, G. Mc Master, et al., Targeting cyclin B1 through peptide-based delivery of siRNA prevents tumour growth, Nucleic Acids Res. 37 (2009) 4559–4569.
[55] Y. Chao, Y.L. Shih, J.H. Chiu, G.Y. Chau, W.Y. Lui, W.K. Yang, S.D. Lee, T.S. Huang, Overexpression of cyclin A but not Skp 2 correlates with the tumor relapse of human hepatocellular carcinoma, Cancer Res. 58 (1998) 985–990.
[56] J. Wang, X. Chenivesse, B. Henglein, C. Brechot, Hepatitis B virus integration in a cyclin A gene in a hepatocellular carcinoma, Nature 343 (1990) 555–557.
[57] E.A. Musgrove, C.E. Caldon, J. Barraclough, A. Stone, R.L. Sutherland, Cyclin D as a therapeutic target in cancer, Nat. Rev. Cancer 11 (2011) 558–572.
[58] S. Ortega, M. Malumbres, M. Barbacid, Cyclin D-dependent kinases, INK4 inhibitors and cancer, Biochim. Biophys. Acta 1602 (2002) 73–87.
[59] K.E. Sheppard, G.A. McArthur, The cell-cycle regulator CDK4: an emerging therapeutic target in melanoma, Clin. Cancer Res. 19 (2013) 5320–5328.
[60] O. Gautschi, D. Ratschiller, M. Gugger, D.C. Betticher, J. Heighway, Cyclin D1 in non-small cell lung cancer: a key driver of malignant transformation, Lung Cancer 55 (2007) 1–14.
[61] R. Sotillo, P. Dubus, J. Martín, E. de la Cueva, S. Ortega, M. Malumbres, et al., Wide spectrum of tumors in knock-in mice carrying a Cdk4 protein insensitive to INK4 inhibitors, EMBO J. 20 (2001) 6637–6647.
[62] R. Sotillo, O. Renner, P. Dubus, J. Ruiz-Cabello, J. Martín-Caballero, M. Barbacid, et al., Cooperation between Cdk4 and p27kip1 in tumor development: a preclinical model to evaluate cell cycle inhibitors with therapeutic activity, Cancer Res. 65 (2005) 3846–3852.
[63] R. Chawla, J.A. Procknow, R.V. Tantravahi, J.S. Khurana, J. Litvin, E.P. Reddy, Cooperativity of Cdk4R24C and Ras in melanoma development, Cell Cycle 9 (2010) 3305–3314.
[64] M. Puyol, A. Martín, P. Dubus, F. Mulero, P. Pizcueta, G. Khan, et al., A synthetic lethal interaction between K-Ras oncogenes and Cdk4 unveils a therapeutic strategy for non-small cell lung carcinoma, Cancer Cell 18 (2010) 63–73.
[65] C. Vincent-Fabert, R. Fiancette, P. Rouaud, C. Baudet, V. Truffinet, V. Magnone, et al., A defect of the INK4-Cdk4 checkpoint and Myc collaborate in blastoid mantle cell lymphoma-like lymphoma formation in mice, Am. J. Pathol. 180 (2012) 1688–1701.
[66] Y. Dobashi, A. Goto, M. Fukayama, A. Abe, A. Ooi, Overexpression of cdk4/cyclin D1, a possible mediator of apoptosis and an indicator of prognosis in human primary lung carcinoma, Int. J. Cancer 110 (2004) 532–541.
[67] C. Abdullah, X. Wang, D. Becker, Expression analysis and molecular targeting of cyclin-dependent kinases in advanced melanoma, Cell Cycle 10 (2011) 977–988.
[68] H. Tsao, L. Chin, L.A. Garraway, D.E. Fisher, Melanoma: from mutations to medicine, Genes. Dev. 26 (2012) 1131–1155.
[69] S.J. Vidwans, K.T. Flaherty, D.E. Fisher, J.M. Tenenbaum, M.D. Travers, J. Shrager, A melanoma molecular disease model, PLoS One 6 (2011) e18257.
[70] R. Fiancette, P. Rouaud, C. Vincent-Fabert, B. Laffleur, V. Magnone, M. Cogné, et al., A p53 defect sensitizes various stages of B cell development to lymphomagenesis in mice carrying an IgH 3′ regulatory region-driven c-myc transgene, J. Immunol. 187 (2011) 5772–5782.
[71] E.E. Schmidt, K. Ichimura, G. Reifenberger, V.P. Collins, CDKN2 (p16/MTS1) gene deletion or CDK4 amplification occurs in the majority of glioblastomas, Cancer Res. 54 (1994) 6321–6324.
[72] H.X. An, M.W. Beckmann, G. Reifenberger, H.G. Bender, D. Niederacher, Gene amplification and overexpression of CDK4 in sporadic breast carcinomas is associated with high tumor cell proliferation, Am. J. Pathol. 154 (1999) 113–118.
[73] S. Park, J. Lee, I.G. Do, J. Jang, K. Rho, S. Ahn, L. Maruja, S.J. Kim, K.M. Kim, M. Mao, E. Oh, Y.J. Kim, J. Kim, Y.L. Choi, Aberrant CDK4 amplification in refractory rhabdomyosarcoma as identified by genomic profiling, Sci. Rep. 4 (2014).
[74] G. Wei, F. Lonardo, T. Ueda, T. Kim, A.G. Huvos, J.H. Healey, M. Ladanyi, CDK4 gene amplification in osteosarcoma: reciprocal relationship with INK4A gene alterations and mapping of 12q13 amplicons, Int. J. Cancer 80 (1999) 199–204.
[75] J.S. Wunder, K. Eppert, S.R. Burrow, N. Gokgoz, R.S. Bell, I.L. Andrulis, N. Gogkoz, Co-amplification and overexpression of CDK4, SAS and MDM2 occurs frequently in human parosteal osteosarcomas, Oncogene 18 (1999) 783–788.
[76] M.M. Corcoran, S.J. Mould, J.A. Orchard, R.E. Ibbotson, R.M. Chapman, A.P. Boright, C. Platt, L.C. Tsui, S.W. Scherer, D.G. Oscier, Dysregulation of cyclin dependent kinase 6 expression in splenic marginal zone lymphoma through chromosome 7q translocations, Oncogene 18 (1999) 6271–6277.
[77] J.F. Costello, C. Plass, W. Arap, V.M. Chapman, W.A. Held, M.S. Berger, H.J.S. Huang, W.K. Cavenee, Cyclin-dependent kinase 6 (CDK6) amplification in human gliomas identified using two-dimensional separation of genomic DNA, Cancer Res. 57 (1997) 1250–1254.
[78] J.P. Brion, A.M. Couck, Cortical and brainstem-type Lewy bodies are immunoreactive for the cyclin-dependent kinase 5, Am. J. Pathol. 147 (1995) 1465–1476.
[79] S. Nakamura, Y. Kawamoto, S. Nakano, I. Akiguchi, J. Kimura, p35nck5a and cyclin-dependent kinase 5 colocalize in Lewy bodies of brains with Parkinson's disease, Acta Neuropathol. 94 (1997) 153–157.
[80] H.K. Paudel, J. Lew, Z. Ali, J.H. Wang, Brain proline-directed protein kinase phosphorylates tau on sites that are abnormally phosphorylated in tau associated with Alzheimer's paired helical filaments, J. Biol. Chem. 268 (1993) 23512–23518.
[81] J.P. Eggers, P.M. Grandgenett, E.C. Collisson, M.E. Lewallen, J. Tremayne, P.K. Singh, et al., Cyclin-dependent kinase 5 is amplified and overexpressed

in pancreatic cancer and activated by mutant K-Ras, Clin. Cancer Res. 17 (2011) 6140–6150.
[82] Z.H. Cheung, N.Y. Ip, Cdk5: a multifaceted kinase in neurodegenerative diseases, Trends Cell Biol. 22 (2012) 169–175.
[83] Z. Levaque, J.L. Rosales, K.Y. Lee, Level of Cdk5 expression predicts the survival of relapsed multiple myeloma patients, Cell Cycle 11 (2012) 4093–4095.
[84] Q. Liang, et al., Cdk5 is essential for TGF-b1-indiced epithelial-mesenchymal transition and breast cancer progression, Sci. Rep. 3 (2013) 1–12.
[85] P. Cohen, D.R. Alessi, Kinase drug discovery–what's next in the field? ACS Chem. Biol. 8 (2013) 96–104.
[86] H. Galons, Introduction for the special issue on kinase inhibitors, Eur. J. Med. Chem. 61 (2013) 1.
[87] M.D. Garrett, A. Fattaey, CDK inhibition and cancer therapy, Curr. Opin. Genet. Dev. 9 (1999) 104–111.
[88] P.M. Fischer, D.P. Lane, Inhibitors of cyclin-dependent kinases as anti-cancer therapeutics, Curr. Med. Chem. 7 (2000) 1213–1245.
[89] A.M. Senderowicz, E.A. Sausville, Preclinical and clinical development of cyclin-dependent kinase modulators, J. Natl. Cancer Inst. 92 (2000) 376–387.
[90] M. Knockaert, P. Greengard, L. Meijer, Pharmacological inhibitors of cyclin-dependent kinases, Trends Pharmacol. Sci. 23 (2002) 417–425.
[91] P.M. Fischer, J. Endicott, L. Meijer, Cyclin-dependent kinase inhibitors, Prog. Cell Cycle Res. 5 (2003) 235–248.
[92] A. Huwe, R. Mazitschek, A. Giannis, Small molecules as inhibitors of cyclin-dependent kinases, Angew. Chem. Int. Ed. Engl. 42 (2003) 2122–2138.
[93] K. Vermeulen, D.R. Van Bockstaele, Z.N. Berneman, The cell cycle: a review of regulation, deregulation and therapeutic targets in cancer, Cell Prolif. 36 (2003) 131–149.
[94] C. Benson, S. Kaye, P. Workman, M. Garrett, M. Walton, J. de Bono, Clinical anticancer drug development: targeting the cyclin-dependent kinases, Br. J. Cancer 92 (2005) 7–12.
[95] G.I. Shapiro, Cyclin-dependent kinase pathways as targets for cancer treatment, J. Clin. Oncol. 24 (2006) 1770–1783.
[96] G.R. Rosania, Y.-T. Chang, Targeting hyperproliferative disorders with cyclin dependent kinase inhibitors, Expert Opin. Ther. Pat. 10 (2000) 215–230.
[97] M.A. Dickson, G.K. Schwartz, Development of cell-cycle inhibitors for cancer therapy, Curr. Oncol. 16 (2009) 36–43.
[98] J. Cicenas, M. Valius, The CDK inhibitors in cancer research and therapy, J. Cancer Res. Clin. Oncol. 137 (2011) 1409–1418.
[99] D. Cirillo, F. Pentimalli, A. Giordano, Peptides or small molecules? Different approaches to develop more effective CDK inhibitors, Curr. Med. Chem. 18 (2011) 2854–2866.
[100] A.A. Abate, F. Pentimalli, L. Esposito, A. Giordano, ATP-noncompetitive CDK inhibitors for cancer therapy: an overview, Expert Opin. Investig. Drugs 22 (2013) 895–906.
[101] L. Esposito, P. Indovina, F. Magnotti, D. Conti, A. Giordano, Anticancer therapeutic strategies based on CDK inhibitors, Curr. Pharm. Des. 19 (2013) 5327–5332.
[102] J. Mann, Natural products in cancer chemotherapy: past, present and future, Nat. Rev. Cancer 2 (2002) 143–148.
[103] D.M. Gadbois, J.R. Hamaguchi, R.A. Swank, E.M. Bradbury, Staurosporine is a potent inhibitor of p34cdc2 and p34cdc2-like kinases, Biochem. Biophys. Res. Commun. 184 (1992) 80–85.
[104] T. Tamaoki, H. Nomoto, I. Takahashi, Y. Kato, M. Morimoto, F. Tomita, Staurosporine, a potent inhibitor of phospholipid/Ca++ dependent protein kinase, Biochem. Biophys. Res. Commun. 135 (1986) 397–402.
[105] N. Glab, B. Labidi, L. X Qin, C. Trehin, C. Bergounioux, L. Meijer, Olomoucine, an inhibitor of the cdc2/cdk2 kinases activity, blocks plant cells at the G1 to S and G2 to M cell cycle transitions, FEBS Lett. 353 (1994) 207–211.
[106] M. Kitagawa, T. Okabe, H. Ogino, H. Matsumoto, I. Suzuki-Takahashi, T. Kokubo, et al., Butyrolactone I, a selective inhibitor of cdk2 and cdc2 kinase, Oncogene 8 (1993) 2425–2432.
[107] M.D. Losiewicz, B.A. Carlson, G. Kaur, E.A. Sausville, P.J. Worland, Potent inhibition of CDC2 kinase activity by the flavonoid L86-8275, Biochem. Biophys. Res. Commun. 201 (1994) 589–595.
[108] R. Hoessel, S. Leclerc, J.A. Endicott, M.E. Nobel, A. Lawrie, P. Tunnah, et al., Indirubin, the active constituent of a Chinese antileukaemia medicine, inhibits cyclin-dependent kinases, Nat. Cell Biol. 1 (1999) 60–67.
[109] C.E. Arris, F.T. Boyle, A.H. Calvert, N.J. Curtin, J.A. Endicott, E.F. Garman, A.E. Gibson, et al., Identification of novel purine and pyrimidine cyclin-dependent kinase inhibitors with distinct molecular interactions and tumor cell growth inhibition profiles, J. Med. Chem. 43 (2000) 2797–2804.
[110] N.S. Gray, L. Wodicka, A.M. Thunnissen, T.C. Norman, S. Kwon, F.H. Espinoza, et al., Exploiting chemical libraries, structure, and genomics in the search for kinase inhibitors, Science 281 (1998) 533–538.
[111] Z.A. Knight, K.M. Shokat, Chemical genetics: where genetics and pharmacology meet, Cell 128 (2007) 425–430.
[112] M. Noble, P. Barrett, J. Endicott, L. Johnson, J. McDonnell, G. Robertson, et al., Exploiting structural principles to design cyclin-dependent kinase inhibitors, Biochim. Biophys. Acta 1754 (2005) 58–64.
[113] C. Zhang, M.S. Lopez, A.C. Dar, E. Ladow, S. Finkbeiner, C.-H. Yun, et al., Structure-guided inhibitor design expands the scope of analog-sensitive kinase technology, ACS Chem. Biol. 8 (2013) 1931–1938.
[114] M.A. Dickson, Molecular pathways: CDK4 inhibitors for Cancer therapy, Clin. Cancer Res. 20 (2014) 3379–3383.
[115] W.F. De Azevedo, S. Leclerc, L. Meijer, L. Havlicek, M. Strnad, S.H. Kim, Inhibition of cyclin-dependent kinases by purine analogues: crystal structure of human cdk2 complexed with roscovitine, Eur. J. Biochem. 243 (1997) 518–526.
[116] Y.T. Chang, N.S. Gray, G.R. Rosania, D.P. Sutherlin, S. Kwon, T.C. Norman, et al., Synthesis and application of functionally diverse 2,6,9-trisubstituted purine libraries as CDK inhibitors, Chem. Biol. 6 (1999) 361–375.
[117] S. Bach, M. Knockaert, J. Reinhardt, O. Lozach, S. Schmitt, B. Baratte, et al., Roscovitine targets, protein kinases and pyridoxal kinase, J. Biol. Chem. 280 (2005) 31208–31219.
[118] L. Demange, F.N. Abdellah, O. Lozach, Y. Ferandin, N. Gresh, L. Meijer, et al., Potent inhibitors of CDK5 derived from roscovitine: synthesis, biological evaluation and molecular modelling, Bioorg. Med. Chem. Lett. 23 (2013) 125–131.
[119] K. Bettayeb, N. Oumata, A. Echalier, Y. Ferandin, J.A. Endicott, H. Galons, et al., CR8, a potent and selective, roscovitine-derived inhibitor of cyclin-dependent kinases, Oncogene 27 (2008) 5797–5807.
[120] V. Patel, A.M. Senderowicz, D. Pinto, T. Igishi, M. Raffeld, L. Quintanilla-Martinez, et al., Flavopiridol, a novel cyclin-dependent kinase inhibitor, suppresses the growth of head and neck squamous cell carcinomas by inducing apoptosis, J. Clin. Invest 102 (1998) 1674–1681.
[121] A. König, G. K Schwartz, R. M Mohammad, A. Al-Katib, J. L Gabrilove, The novel cyclin-dependent kinase inhibitor flavopiridol downregulates Bcl-2 and induces growth arrest and apoptosis in chronic b-cell leukemia lines, Blood 90 (1997) 4307–4312.
[122] T.S. Lin, A.S. Ruppert, A.J. Johnson, B. Fischer, N.A. Heerema, L.A. Andritsos, et al., Phase II study of flavopiridol in relapsed chronic lymphocytic leukemia demonstrating high response rates in genetically high-risk disease, J. Clin. Oncol. 27 (2009) 6012–6018.
[123] B.A. Christian, M.R. Grever, J.C. Byrd, T.S. Lin, Flavopiridol in the treatment of chronic lymphocytic leukemia, Curr. Opin. Oncol. 19 (2007) 573–578.
[124] P.G. Wyatt, A.J. Woodhead, V. Berdini, J.A. Boulstridge, G.G. Carr, D.M. Cross, D.J. Davis, et al., Identification of N-(4-piperidinyl)-4-(2,6-dichlorobenzoylamino)-1H-pyrazole-3-carboxamide (AT7519), a novel cyclin dependent kinase inhibitor using fragment-based X-Ray crystallography and structure based drug design, J. Med. Chem. 51 (2008) 4986–4999.
[125] M.S. Squires, R.E. Feltell, N.G. Wallis, E.J. Lewis, D.-M. Smith, D.M. Cross, et al., Biological characterization of AT7519, a small-molecule inhibitor of cyclin-dependent kinases, in human tumor cell lines, Mol. Cancer Ther. 8 (2009) 324–332.
[126] M.S. Squires, L. Cooke, V. Lock, W. Qi, E.J. Lewis, N.T. Thompson, J.F. Lyons, D. Mahadevan, AT7519, a cyclin-dependent kinase inhibitor, exerts its effects by transcriptional inhibition in leukemia cell lines and patient samples, Mol. Cancer Ther. 9 (2010) 920–928.
[127] L. Santo, S. Vallet, T. Hideshima, D. Cirstea, H. Ikeda, S. Pozzi, K. Patel, Y. Okawa, G. Gorgun, G. Perrone, E. Calabrese, M. Yule, M. Squires, M. Ladetto, M. Boccadoro, P.G. Richardson, N.C. Munshi, K.C. Anderson, N. Raje, AT7519, A novel small molecule multi-cyclin-dependent kinase inhibitor, induces apoptosis in multiple myeloma via GSK-3beta activation and RNA polymerase II inhibition, Oncogene 29 (2010) 2325–2336.
[128] D. Mahadevan, R. Plummer, M.S. Squires, D. Rensvold, S. Kurtin, C. Pretzinger, T. Dragovich, J. Adams, V. Lock, D.M. Smith, D. Von Hoff, H. Calvert, A phase I pharmacokinetic and pharmacodynamic study of AT7519, a cyclin-dependent kinase inhibitor in patients with refractory solid tumors, Ann. Oncol. 22 (2011) 2137–2143.
[129] K. Paruch, M.P. Dwyer, C. Alvarez, C. Brown, T.-Y. Chan, R.J. Doll, et al., Discovery of dinaciclib (SCH 727965): a potent and selective inhibitor of cyclin-dependent kinases, ACS Med. Chem. Lett. 1 (2010) 204–208.
[130] D. Parry, T. Guzi, F. Shanahan, N. Davis, D. Prabhavalkar, D. Wiswell, et al., Dinaciclib (SCH 727965), a novel and potent cyclin-dependent kinase inhibitor, Mol. Cancer Ther. 9 (2010) 2344–2353.
[131] G. Feldmann, A. Mishra, S. Bisht, C. Karikari, I. Garrido-Laguna, Z. Rasheed, et al., Cyclin-dependent kinase inhibitor Dinaciclib (SCH727965) inhibits pancreatic cancer growth and progression in murine xenograft models, Cancer Biol. Ther. 12 (2011) 598–609.
[132] D.W. Fry, P.J. Harvey, P.R. Keller, W.L. Elliott, M. Meade, E. Trachet, M. Albassam, X. Zheng, W.R. Leopold, N.K. Pryer, P.L. Toogood, Specific inhibition of cyclin-dependent kinase 4/6 by PD 0332991 and associated antitumor activity in human tumor xenografts, Mol. Cancer Ther. 11 (2004) 1427–1438.
[133] P.L. Toogood, P.J. Harvey, J.T. Repine, D.J. Sheehan, S.N. VanderWel, H. Zhou, et al., Discovery of a potent and selective inhibitor of cyclin-dependent kinase 4/6, J. Med. Chem. 48 (2005) 2388–2406.
[134] L. Cen, B.L. Carlson, M.A. Schroeder, J.L. Ostrem, G.J. Kitange, A.C. Mladek, S.R. Fink, et al., p16-Cdk4-Rb axis controls sensitivity to a cyclin-dependent kinase inhibitor PD0332991 in glioblastoma xenograft cells, Neuro Oncol. 14 (2012) 870–881.
[135] M. Guha, Blockbuster dreams for Pfizer's CDK inhibitor, Nat. Biotechnol. 31 (2013) 187.
[136] Z. Fang, C. Grütter, D. Rauh, Strategies for the selective regulation of kinases with allosteric modulators: exploiting exclusive structural features, ACS Chem. Biol. 8 (2013) 58–70.
[137] Y. Pommier, J. Cherfils, Interfacial inhibition of macromolecular interactions: nature's paradigm for drug discovery, Trends Pharmacol. Sci. 26 (2005) 138–145.

[138] Y. Liu, N.S. Gray, Rational design of inhibitors that bind to inactive kinase conformations, Nat. Chem. Biol. 2 (2006) 358–364.
[139] L. Pagliaro, J. Felding, K. Audouze, S.J. Nielsen, R.B. Terry, C. Krog-Jensen, et al., Emerging classes of protein-protein interaction inhibitors and new tools for their development, Curr. Opin. Chem. Biol. 8 (2004) 442–449.
[140] M.R. Arkin, J.A. Wells, Small-molecule inhibitors of protein-protein interactions: progressing towards the dream, Nat. Rev. Drug Discov. 3 (2004) 301–317.
[141] B.O. Villoutreix, C.M. Labbé, D. Lagorce, G. Laconde, O. Sperandio, A leap into the chemical space of protein-protein interaction inhibitors, Curr. Pharm. Des. 18 (2012) 4648–4667.
[142] M.R. Arkin, M. Randal, W.L. DeLano, J. Hyde, T.N. Luong, J.D. Oslob, et al., Binding of small molecules to an adaptive protein-protein interface, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 1603–1608.
[143] X. Morelli, R. Bourgeas, P. Roche, Chemical and structural lessons from recent successes in protein-protein interaction inhibition (2P2I), Curr. Opin. Chem. Biol. 15 (2011) 475–481.
[144] M.A. Bogoyevitch, R.K. Barr, A.J. Ketterman, Peptide inhibitors of protein kinases-discovery, characterisation and use, Biochim. Biophys. Acta 1754 (2005) 79–99.
[145] S.K. Sharma, T.M. Ramsey, K.W. Bair, Protein-protein interactions: lessons learned, Curr. Med. Chem. Anticancer Agents 2 (2002) 311–330.
[146] N. Mendoza, S. Fong, J. Marsters, H. Koeppen, R. Schwall, D. Wickramasinghe, Selective cyclin-dependent kinase 2/cyclin A antagonists that differ from ATP site inhibitors block tumor growth, Cancer Res. 63 (2003) 1020–1024.
[147] C. Gondeau, S. Gerbal-Chaloin, P. Bello, G. Aldrian-Herrada, M.C. Morris, G. Divita, Design of a novel class of peptide inhibitors of cyclin-dependent kinase/cyclin activation, J. Biol. Chem. 280 (2005) 13793–13800.
[148] N. Canela, M. Orzáez, R. Fucho, F. Mateo, R. Gutierrez, A. Pineda-Lucena, et al., Identification of an hexapeptide that binds to a surface pocket in cyclin A and inhibits the catalytic activity of the complex cyclin-dependent kinase 2-cyclin A, J. Biol. Chem. 281 (2006) 35942–35953.
[149] L. Bagella, A. Sun, T. Tonini, G. Abbadessa, G. Cottone, M.G. Paggi, et al., A small molecule based on the pRb2/p130 spacer domain leads to inhibition of cdk2 activity, cell cycle arrest and tumor growth reduction in vivo, Oncogene 26 (2007) 1829–1839.
[150] A. Giordano, E. Bellacchio, L. Bagella, M.G. Paggi, Interaction between the Cdk2/cyclin A complex and a small molecule derived from the pRb2/p130 spacer domain: a theoretical model, Cell Cycle 6 (2007) 2591–2593.
[151] H.M. Warenius, J.D. Kilburn, J.W. Essex, R.I. Maurer, J.P. Blaydes, U. Agarwala, et al., Selective anticancer activity of a hexapeptide with sequence homology to a non-kinase domain of cyclin dependent kinase 4, Mol. Cancer 10 (2011) 72.
[152] K.T. Chin, S.Y. Ohki, D. Tang, H.C. Cheng, J.H. Wang, M. Zhang, Identification and structure characterization of a Cdk inhibitory peptide derived from neuronal-specific Cdk5 activator, J. Biol. Chem. 274 (1999) 7120–7127.
[153] Y.-L. Zheng, B.-S. Li, N.D. Amin, W. Albers, H.C. Pant, A peptide derived from cyclin-dependent kinase activator (p35) specifically inhibits Cdk5 activity and phosphorylation of tau protein in transfected cells, Eur. J. Biochem. 269 (2002) 4427–4434.
[154] Y.-L. Zheng, S. Kesavapany, M. Gravell, R.S. Hamilton, M. Schubert, N. Amin, et al., A Cdk5 inhibitory peptide reduces tau hyperphosphorylation and apoptosis in neurons, EMBO J. 24 (2005) 209–220.
[155] Y.-L. Zheng, N.D. Amin, Y.-F. Hu, P. Rudrabhatla, V. Shukla, J. Kanungo, et al., A 24-residue peptide (p5), derived from p35, the Cdk5 neuronal activator, specifically inhibits Cdk5-p25 hyperactivity and tau hyperphosphorylation, J. Biol. Chem. 285 (2010) 34202–34212.
[156] V. Shukla, Y.-L. Zheng, S.K. Mishra, N.D. Amin, J. Steiner, P. Grant, et al., A truncated peptide from p35, a Cdk5 activator, prevents Alzheimer's disease phenotypes in model mice, FASEB J. 27 (2013) 174–186.
[157] J.R. Sundaram, C.P. Poore, N.H.B. Sulaimee, T. Pareek, A.B.M.A. Asad, R. Rajkumar, et al., Specific inhibition of p25/Cdk5 activity by the Cdk5 inhibitory peptide reduces neurodegeneration in vivo, J. Neurosci. 33 (2013) 334–343.
[158] P.W. Latham, Therapeutic peptides revisited, Nat. Biotechnol. 17 (1999) 755–757.
[159] I.C. Baines, P. Colas, Peptide aptamers as guides for small-molecule drug discovery, Drug Discov. Today 11 (2006) 334–341.
[160] J. Zhang, P.L. Yang, N.S. Gray, Targeting cancer with small molecule kinase inhibitors, Nat. Rev. Cancer 9 (2009) 28–39.
[161] B. Zhang, C. Corbel, F. Guéritte, C. Couturier, S. Bach, V.B.C. Tan, An in silico approach for the discovery of CDK5/p25 interaction inhibitors, Biotechnol. J. 6 (2011) 871–881.
[162] C. Corbel, Q. Wang, H. Bousserouel, A. Hamdi, B. Zhang, O. Lozach, et al., First BRET-based screening assay performed in budding yeast leads to the discovery of CDK5/p25 interaction inhibitors, Biotechnol. J. 6 (2011) 860–870.
[163] R.M. Eglen, T. Reisine, Human kinome drug discovery and the emerging importance of atypical allosteric inhibitors, Expert Opin. Drug Discov. 5 (2010) 277–290.
[164] P. Corsino, N. Horenstein, D. Ostrov, T. Rowe, M. Law, A. Barrett, et al., A novel class of cyclin-dependent kinase inhibitors identified by molecular docking act through a unique mechanism, J. Biol. Chem. 284 (2009) 29945–29955.
[165] H. Liu, K. Liu, Z. Huang, C.M. Park, N.R. Thimmegowda, J.H. Jang, I.J. Ryoo, L. He, S.O. Kim, N. Oi, K.W. Lee, N.K. Soung, A.M. Bode, Y. Yang, X. Zhou, R.L. Erikson, J.S. Ahn, J. Hwang, K.E. Kim, Z. Dong, B.Y. Kim, A chrysin derivative suppresses skin cancer growth by inhibiting cyclin-dependent kinases, J. Biol. Chem. 288 (2013) 25924–25937.
[166] S. Betzi, R. Alam, M. Martin, D.J. Lubbers, H. Han, S.R. Jakkaraj, et al., Discovery of a potential allosteric ligand binding site in CDK2, ACS Chem. Biol. 6 (2011) 492–501.
[167] M.P. Martin, R. Alam, S. Betzi, D.J. Ingles, J.-Y. Zhu, E. Schönbrunn, A novel approach to the discovery of small-molecule ligands of CDK2, Chembiochem 13 (2012) 2128–2136.
[168] R. Weissleder, M.J. Pittet, Imaging in the era of molecular oncology, Nature 452 (2008) 580–589.
[169] J. Zhang, R.E. Campbell, A.Y. Ting, R.Y. Tsien, Creating new fluorescent probes for cell biology, Nat. Rev. Mol. Cell Biol. 3 (2002) 906–918.
[170] A. Ibraheem, R.E. Campbell, Designs and applications of fluorescent protein-based biosensors, Curr. Opin. Chem. Biol. 14 (2010) 30–36.
[171] A.E. Palmer, Y. Qin, J.G. Park, J.E. McCombs, Design and application of genetically encoded biosensors, Trends Biotechnol. 29 (2011) 144–152.
[172] H. Wang, E. Nakata, I. Hamachi, Recent progress in strategies for the creation of protein-based fluorescent biosensors, Chembiochem 10 (2009) 2560–2577.
[173] E. Pazos, O. Vázquez, J.L. Mascareñas, M.E. Vázquez, Peptide-based fluorescent biosensors, Chem. Soc. Rev. 38 (2009) 3348–3359.
[174] M.C. Morris, Fluorescent biosensors of intracellular targets from genetically encoded reporters to modular polypeptide probes, Cell Biochem. Biophys. 56 (2010) 19–37.
[175] T.N.N. Van, M.C. Morris, Fluorescent sensors of protein kinases: from basics to biomedical applications, Prog. Mol. Biol. Transl. Sci. 113 (2013) 217–274.
[176] J.A. Gonzalez-Vera, Probing the kinome in real time with fluorescent peptides, Chem. Soc. Rev. 41 (2012) 1652–1664.
[177] M.C. Morris, Fluorescent biosensors for Cancer cell imaging and diagnostics, in: V. Preedy, J. Hunter (Eds.), Biosensors and Cancer, CRC Press, 2012, ISBN 978-1-57808-734-1.
[178] M.C. Morris, Fluorescent biosensors - probing protein kinase function in Cancer and drug discovery, Biochim. Biophys. Acta 1834 (2013) 1387–1395.
[179] C. Prével, M. Pellerano, T.N.N. Van, M.C. Morris, Fluorescent biosensors for high throughput screening of protein kinase inhibitors, Biotechnol. J. 9 (2014) 253–265.
[180] K.A. Giuliano, D.L. Taylor, Fluorescent-protein biosensors: new tools for drug discovery, Trends Biotechnol. 16 (1998) 135–140.
[181] M. Wolff, J. Wiedenmann, G.U. Nienhaus, M. Valler, et al., Novel fluorescent proteins for high-content screening, Drug Discov. Today 11 (2006) 1054–1060.
[182] G.J. Zaman, A. Garritsen, T. de Boer, C.A. van Boeckel, Fluorescence assays for high-throughput screening of protein kinases, Comb. Chem. High. Throughput Screen 6 (2003) 313–320.
[183] O. von Ahsen, U. Bömer, High throughput screening for kinase inhibitors, ChemBioChem 6 (2005) 481–490.
[184] G.T. Hanson, B.J. Hanson, Fluorescent probes for cellular assays, Comb. Chem. High. Throughput Screen 11 (2008) 505–513.
[185] P. Lang, K. Yeow, A. Nichols, A. Scheer, Cellular imaging in drug discovery, Nat. Rev. Drug Discov. 5 (2006) 343–356.
[186] A.E. Carpenter, Image-based chemical screening, Nat. Chem. Biol. 3 (2007) 461–465.
[187] F. Zanella, J.B. Lorens, W. Link, High content screening: seeing is believing, Trends Biotechnol. 28 (2010) 237–245.
[188] M. Nobis, N.O. Carragher, E.J. McGhee, J.P. Morton, et al., Intravital FLIM-FRET imaging reveals dasatinib-induced spatial control of src in pancreatic cancer, Cancer Res. 73 (2013) 4674–4686.
[189] A. Piljic, C. Schultz, Simultaneous recording of multiple cellular events by FRET, ACS Chem. Biol. 3 (2008) 156–160.
[190] H.J. Carlson, R.E. Campbell, Genetically encoded FRET-based biosensors for multiparameter fluorescence imaging, Curr. Opin. Biotechnol. 20 (2009) 19–27.
[191] C.M. Welch, H. Elliott, G. Danuser, K.M. Hahn, Imaging the coordination of multiple signalling activities in living cells, Nat. Rev. Mol. Cell Biol. 12 (2011) 749–756.
[192] K.A. Giuliano, Y.T. Chen, J.R. Haskins, Positional biosensors: a new tool for high content screening, Mod. Drug Discov. 6 (2003) 33–37.
[193] M.D. Allen, L.M. DiPilato, M. Rahdar, Y.R. Ren, et al., Reading dynamic kinase activity in living cells for high-throughput screening, ACS Chem. Biol. 1 (2006) 371–376.
[194] J. Zhang, M.D. Allen, FRET-based biosensors for protein kinases: illuminating the kinome, Mol. Biosyst. 3 (2007) 759–765.
[195] T. Mizutani, T. Kondo, S. Darmanin, M. Tsuda, et al., A novel FRET-based biosensor for the measurement of BCR-ABL activity and its response to drugs in living cells, Clin. Cancer Res. 16 (2010) 3964–3975.
[196] M.D. Shults, K.A. Janes, D.A. Lauffenburger, B. Imperiali, A multiplexed homogeneous fluorescence-based assay for protein kinase activity in cell lysates, Nat. Methods 2 (2005) 277–283.
[197] M.D. Shults, D. Carrico-Moniz, B. Imperiali, Optimal Sox-based fluorescent chemosensor design for serine/threonine protein kinases, Anal. Biochem. 352 (2006) 198–207.
[198] E. Luković, E. Vogel Taylor, B. Imperiali, Monitoring protein kinases in cellular media with highly selective chimeric reporters, Angew. Chem. Int. Ed. Engl. 48 (2009) 6828–6831.

[199] C.I. Stains, E. Luković, B. Imperiali, A p38α-selective chemosensor for use in unfractionated cell lysates, ACS Chem. Biol. 6 (2011) 101–105.
[200] C.A. Chen, R.H. Yeh, X. Yan, D.S. Lawrence, Biosensors of protein kinase action: from in vitro assays to living cells, Biochim. Biophys. Acta 1697 (2004) 39–51.
[201] D.S. Lawrence, Q. Wang, Seeing is believing: peptide-based fluorescent sensors of protein tyrosine kinase activity, Chembiochem 8 (2007) 373–378.
[202] V. Sharma, Q. Wang, D.S. Lawrence, Peptide-based fluorescent sensors of protein kinase activity: design and applications, Biochim. Biophys. Acta 1784 (2008) 94–99.
[203] Q. Wang, E.I. Zimmerman, A. Toutchkine, T.D. Martin, et al., Multicolor monitoring of dysregulated protein kinases in chronic myelogenous leukemia, ACS Chem. Biol. 5 (2010) 887–895.
[204] J.R. Simard, M. Getlik, C. Grütter, V. Pawar, et al., Development of a Fluorescent-tagged kinase assay system for detection and characterization of allosteric kinase inhibitors, JACS 131 (2009) 13286–13296.
[205] J.R. Simard, M. Getlik, C. Grütter, R. Schneider, et al., Fluorophore labelling of the glycine-rich loop as a method of identifying inhibitors that bind to active and inactive kinase conformations, JACS 132 (2010) 4152–4160.
[206] J.R. Simard, S. Klüter, C. Grütter, M. Getlik, et al., A new screening assay for allosteric inhibitors of cSrc, Nat. Chem. Biol. 5 (2009) 394–396.
[207] M. Getlik, J.R. Simard, M. Termathe, C. Grütter, et al., Fluorophore labelled kinase detects ligands that bind within the MAPK insert of p38a kinase, PLoS One 7 (2012) e39713.
[208] J. Zhang, F.J. Adrian, W. Jahnke, S.W. Cowan-Jacob, et al., Targeting Bcr-Abl by combining allosteric with ATP-binding-site inhibitors, Nature 463 (2010) 501–506.
[209] R. Schneider, R. Becker, J.R. Simard, M. Getlik, et al., Direct binding assay for the detection of type IV allosteric inhibitors of abl, JACS 134 (2012) 9138–9141.
[210] J.H. Zhang, T.D. Chung, K.R. Oldenburg, A simple statistical parameter for use in evaluation and validation of high throughput screening assays, J. Biomol. Screen 4 (1999) 67–73.
[211] L. Kurzawa, M. Pellerano, J.B. Coppolani, M.C. Morris, Fluorescent peptide biosensor for probing the relative abundance of cyclin-dependent kinases in living cells, PlosOne 6 (2011) e26555.
[212] T.N.N. Van, M. Pellerano, S. LyKaSo, M.C. Morris, Fluorescent protein biosensor for probing CDK/cyclin activity in vitro and in living cells, ChemBioChem (2014), http://dx.doi.org/10.1002/cbic.201402318 (Epub ahead of print).
[213] S. Tcherniuk, C. Perals, M. Pellerano, T.N.N. Van, E. Garcin, F. Mahuteau-Betzer, M.P. Teulade-Fichou, M.C. Morris, Allosteric inhibitors of CDK2 identified by high throughput screening with a conformation-sensitive biosensor, ACS Chem. Biol. (2014) (in revision).
[214] M.C. Morris, J. Depollier, J. Mery, F. Heitz, G. Divita, A peptide carrier for the delivery of biologically active proteins into mammalian cells, Nat. Biotechnol. 19 (2001) 1173–1176.
[215] W. Jahnke, M.J. Blommers, C. Fernández, C. Zwingelstein, R. Amstutz, Strategies for the NMR-based identification and optimization of allosteric protein kinase inhibitors, Chembiochem 6 (2005) 1607–1610.
[216] S. Klüter, C. Grütter, T. Naqvi, M. Rabiller, J.R. Simard, V. Pawar, M. Getlik, D. Rauh, Displacement assay for the detection of stabilizers of inactive kinase conformations, J. Med. Chem. 53 (2010) 357–367.
[217] C.S. Lebakken, L.J. Reichling, J.M. Ellefson, S.M. Riddle, Detection of allosteric kinase inhibitors by displacement of active site probes, J. Biomol. Screen 17 (2012) 813–821.