

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

185,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Technologies for the Use of Protein Kinases into Medical Applications

Yoshiki Katayama
Kyushu University
Japan

1. Introduction

Living systems continuously monitor and respond to the surrounding environment. These processes are made possible by cellular signal transduction systems. When particular information reaches the cell (in many cases to the surface of cells), corresponding molecular networks are activated to process the information. These cascade-type reactions change many enzymes in the cell and ultimately the enzymatic reactions taking place determine the cellular response. Although the system includes a large number of enzymes, protein kinases are the most important group of enzymes and play key roles of signal transduction events. The human genome encodes nearly 500 types of protein kinases (Cohen, 2001). One-third of all cellular proteins act as substrates of protein kinases. Therefore, the monitoring of the activities of protein kinases is a crucial technology not only for understanding life processes, but also for the development of efficient diagnostics or effective drug discovery programs. Modification of certain protein kinase activities will also be an important medical technology for therapy against many diseases. In this context, this chapter will introduce recent technologies that have been developed to monitor protein kinases. In addition, if we use the activity of the protein kinases as a tool for medical engineering, we may be able to control cellular function when needed. In this category, new technologies that use protein kinase activities for controlling transgene regulation will also be introduced.

2. Overview of kinase assays

The activity of protein kinases is easily detected through the incorporation of a radioactive phosphate to a protein or peptide substrate using ^{32}P - or ^{33}P -ATP (Schutkowski et al., 2004; Panse et al., 2004; Diks et al., 2004). Although such assays are highly sensitive and quantitative, there are some important drawbacks such as the requirement of a special facility to handle radioactive materials, production of radioactive waste, the short half-lives (14 days) of the radioactive phosphate, and the potential risk to health. Thus, many types of non-radioactive protein kinase assays have been developed. In these assays, fluorescence-based approaches represent a promising way for high-throughput analysis of protein kinase activities. Alternatively, colorimetry may be easier to handle and cheaper when compared with fluorimetry; however, the sensitivity is generally lower. Mass spectrometry represents another way to monitor protein kinase activity.

When fluorescence techniques are used for the design of protein kinase assays, many useful properties of the fluorescence phenomena can be used such as fluorescence intensity, fluorescence polarization, fluorescence energy transfer and the fluorescence life-time.

What is important in assay design is the ability of the system to distinguish between phosphorylated and non-phosphorylated forms of the substrate. An anti-phospho antibody is a convenient way to recognize phosphorylation of a substrate. However, antibody with sufficient affinity for phosphorylated Ser/Thr is not commercially available. Therefore, other artificial molecules such as metal complexes, polycationic polymers or beads are used for the recognition of phosphor-serine or -threonine. Phos-Tag (Kinoshita et al., 2006; Inamori et al., 2005) or Pro-Q Diamond dye (Steinberg et al., 2003) are typical examples for this category (Fig. 1). Phos-Tag is a zinc complex that was designed by using the alkaline phosphatase structure, and this compound binds to phosphor-amino acids. The molecule also possesses a biotin moiety so that avidin derivatives can also bind tightly. Pro-Q Diamond dye is a gallium complex of a fluorescent molecule. Phosphorylation of a peptide or protein can be detected by agarose gel electrophoresis using this probe. Instead of binding other molecules to the phosphorylation site, phosphorylated amino acid residues may be derivatized to other chemical groups by attaching a marker molecule to detect the phosphorylated substrate (Oda et al., 2001).

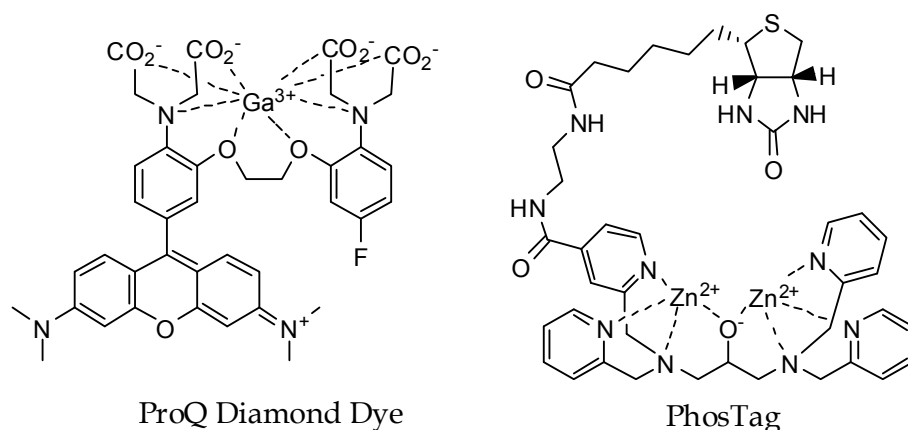


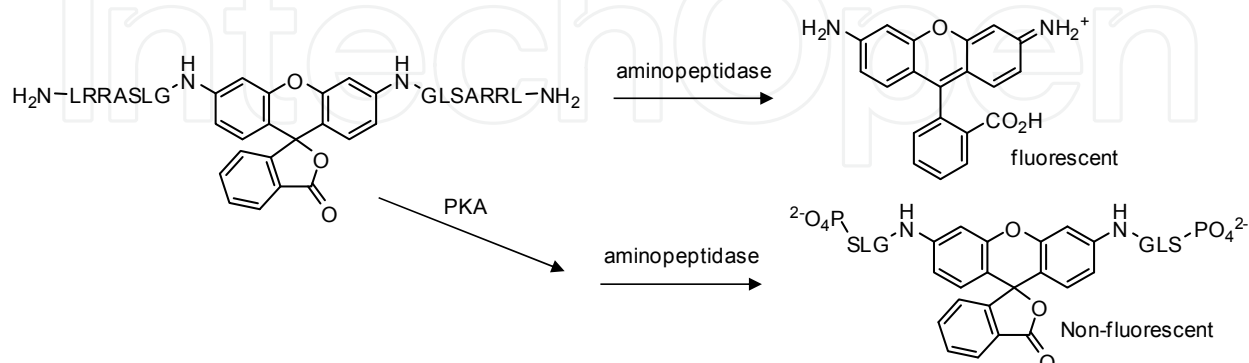
Fig. 1. Chemical structures of ProQ Diamond dye and Phos Tag

3. Protein kinase assay with measurement of fluorescence intensity

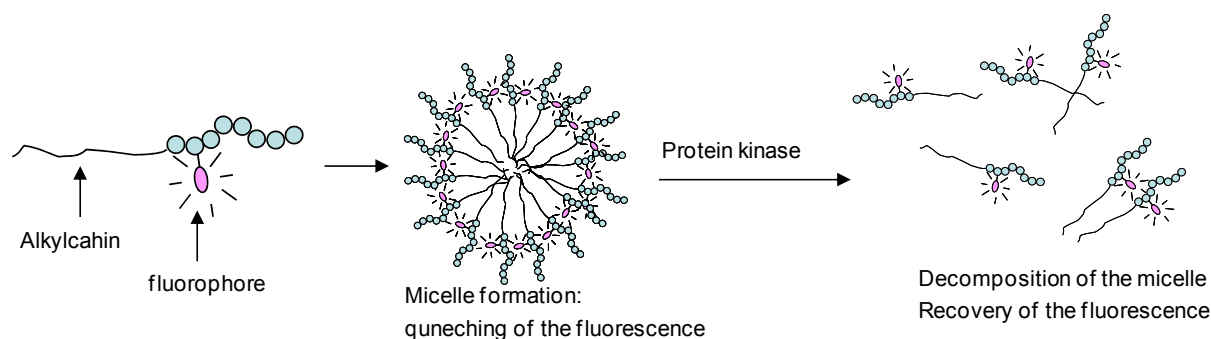
Probably, the simplest way of using fluorimetry in kinase assays is the design of a fluorescent substrate that changes its fluorescence intensity upon phosphorylation. However, it is not so easy to change the fluorescence intensity of a single fluorescent molecule through phosphorylation. The first example was an acrylodan-labeled peptide substrate of protein kinase C (PKC) (McIlroy et al., 1991). The molecule, Acrylodan-CKKKKRFSFKKSLGFSFKKNKK-OH, decreased its fluorescence intensity by 20% upon phosphorylation by PKC. The time course of the fluorescence decrease was found to correlate well with that of [³²P]phosphate incorporation. The assay detected 0.02 nM of PKC. Although the assay cannot be applied to living cells, the PKC activity in a brain homogenate was easily detected. On the other hand, Higashi et al, 1996. reported a cell permeable acrylodan-labeled peptide (syntide 2) for the detection of calcium calmodulin dependent kinase II (CaMKII) activity (Higashi et al., 1996). The probe was used for imaging CaMKII

activity in mice hippocampus slices. However, this type of assay using simply fluorescence-labeled substrates provides only a small change in the fluorescence intensity following phosphorylation. Consequently, the sensitivity of this approach is generally low.

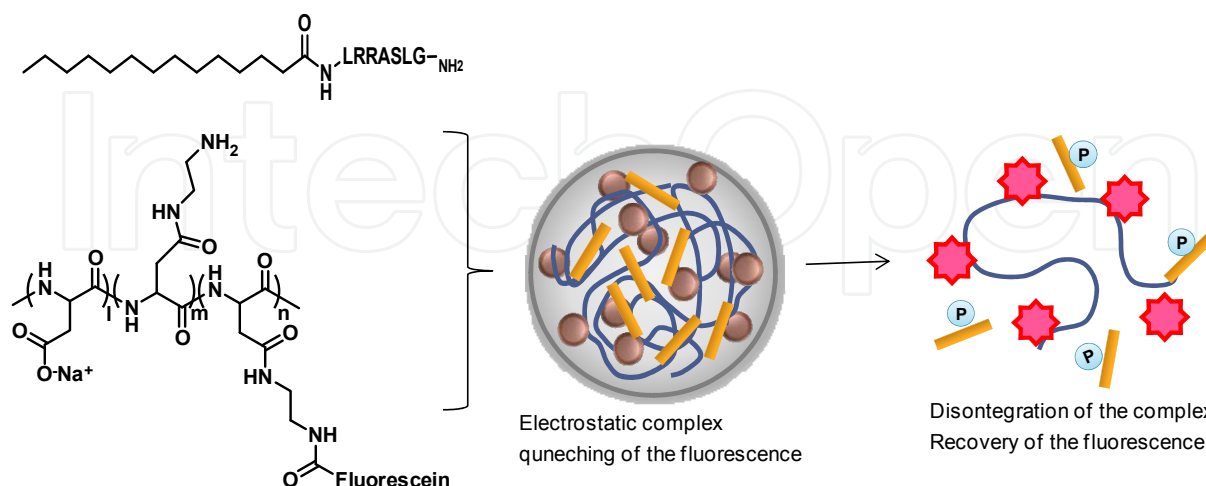
Generally it is difficult to obtain large changes in fluorescence intensity if the fluorophore involves the simple labeling of a peptide. Kupcho et al, 2003. reported a unique fluorescent probe for the detection of protein kinase A (PKA) (Kupcho et al., 2003) (Fig. 2a). In this case,



(a) Molecular probe for PKA monitoring based on rhodamine 110 reported by Kupcho et al.



(b) Micelle-based protein kinase probe for fluorescence monitoring reported by Sun et al.



(c) Electrostatic complex consisting of lipid-type substrate and polyanion for kinase monitoring

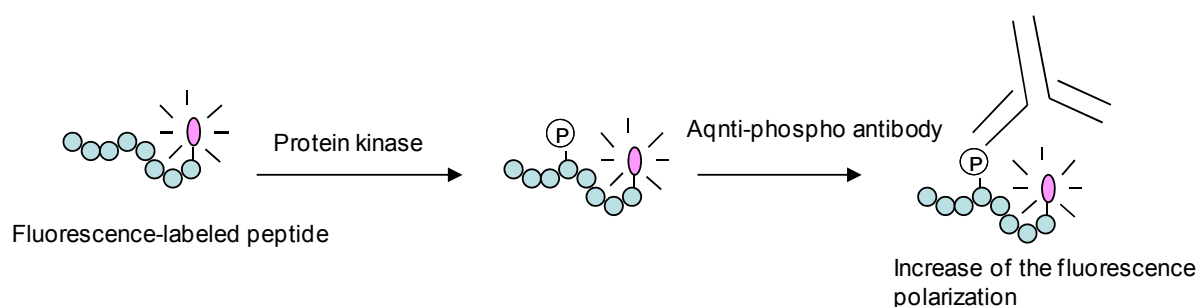
Fig. 2. Protein kinase assay with measurement of fluorescence intensity (for the comment: This is an original figure, although it resembles to that in our paper published in Bioconjugate chemistry, 22, 1526-(2011).)

the substrate peptide of PKA was introduced into the both sides of rhodamine 110 through amide bonds. The molecule is practically non-fluorescent due to the formation of a lactone ring in the rhodamine structure. Peptidase degrades the peptide moieties from their amino termini. When the peptides were completely digested, the fluorescence of the rhodamine 110 recovers. However, if the peptide is phosphorylated with PKA, digestion by peptidase is inhibited at the phosphor-serine such that the fluorescence never recovers. This method can clearly detect PKA activity with an 'on-off' strategy; however, the molecular design is not versatile and therefore not suitable for many kinases. In addition, the method cannot be applied to living cells and *in vivo*. It often requires the design of a complicated probe molecule to obtain large changes in the fluorescence intensity with phosphorylation when using small probe molecules. However, if we use a molecular assembly system such as micelles or polyionic complexes, it becomes easier to obtain larger changes in the fluorescence intensity when probing phosphorylation. Sun et al. 2005 reported a micelle system for the detection of protein kinase activity (Sun et al., 2005) (Fig. 2b). In this system, an aliphatic chain was connected to a peptide substrate of a target kinase that was labeled with a fluorescent molecule. If the length of the hydrocarbon chain is optimized, the material forms a micelle-like assembly. The fluorescence is then quenched due to the concentrating of the fluorophores. However, if the substrate is phosphorylated by a target kinase the fluorescence intensity increases several fold because of the decomposition of the micelle. Phosphorylation of the peptide moiety dramatically changes the hydrophilic-hydrophobic balance of the alkylated peptide substrate. We also developed a polyion complex consisting of an alkylated cationic peptide substrate and a fluorescein-labeled polyaspartic acid for monitoring protein kinase A or protein kinase C α activity (Koga et al. 2011) (Fig. 2c). Such a polyion complex formed a nano-particle with a size of 100-200 nm. In this particle, the fluorescence is quenched because of the high concentration. Phosphorylation of the peptide moiety decreases the cationic net charges of the peptide so that an electrostatic interaction between the lipid-type peptide and the fluorescence-labeled polyanion decreases and this leads to the disintegration of the polyion complex. Such an event leads to an increase in the fluorescence intensity by several fold. In this system, the peptide substrate does not require the direct labeling of a fluorophore, which sometimes affects the ability of the molecule to act as a kinase substrate. This system was successfully applied to validate kinase inhibitors.

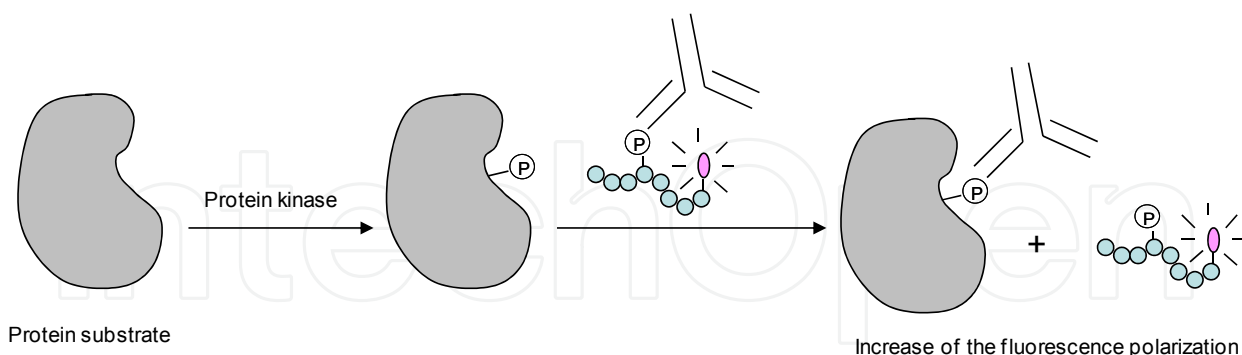
4. Protein kinase assay based on fluorescent polarization

Fluorescence polarization is a technique to detect the rotational property of a target molecule. When a fluorescent molecule is excited with polarized light, the extent of the remaining polarization of the emitted light depends on the rotation of the molecule. If the molecule rotates within the period of its excitation, the emitted light loses the polarization in the plane of excited light. Since the molecular rotation is dependent on the molecular weight of the protein, the technique can detect the binding of a large molecule to the target molecule. This strategy can be applied in the design of protein kinase assays. Seethala et al. 1997 reported the first kinase assay based on a fluorescence polarization experiment (Seethala, 1997). Once a fluorescently labeled peptide substrate is phosphorylated with a protein kinase, an anti-phospho-amino acid antibody binds to the substrate (Fig. 3a). Due to the dramatic increase of the molecular size, the fluorescence polarization signal was observed to increase because of a reduction in the rate of rotation. This direct monitoring of fluorescence polarization is simple, but it usually requires a relatively large amount of anti-phospho antibody. The method also needs a small substrate such as a peptide and the

technique cannot be applied using a protein substrate. On the other hand, if the strategy involves a competition assay, the protein substrate can also be available (Seethala et al., 1998; Kristjansdottir et al., 2003) (Fig. 3b). In this system, after the protein substrate is phosphorylated by the protein kinase, it is added into the complex of the fluorescence-labeled phosphopeptide and anti-phospho antibody to compete for binding to the antibody. Thereafter, we can evaluate the kinase activity by evaluating the decrease in the fluorescence polarization signal. The advantage of the fluorescence polarization assay is that this approach is independent of the concentration or fluorescence intensity of the fluorophore used. However, only an anti-phospho antibody for tyrosine is available. Therefore, another molecule that can bind to phosphoserine or threonine is needed if this type of assay is to be applied to monitor the activity of serine/threonine protein kinases. Polycationic peptides and trivalent cation-containing particles can be used for this purpose (Coffin et al., 2000). However, such compounds have poor specificity. Moreover, polycationic peptides are also limited to the use of neutral substrate peptides and trivalent cation-containing particles sometimes suffer from weak binding to phosphorylated sites. The Phos-Tag may be another practical possibility because of its relatively high specificity and binding constant. Recently, a fluorescent polarization assay was applied to a high-throughput assay for screening inhibitors of a protein kinase (Kumar et al., 2011).



(a) Basic concept of protein kinase assay base on fluorescence polarization



(b) Protein kinase assay base on fluorescence polarization by using protein substrate

Fig. 3. Protein kinase assay based on fluorescence polarization

5. Use of FRET for protein kinase assay

Fluorescence resonance energy transfer (FRET) is the non-radiation energy transfer between two different fluorophores. If the emission spectrum of the donor fluorophore and the excitation spectrum of the acceptor fluorophore overlap and these two fluorophores exist in close proximity, the excitation light for the donor produces an emitted light derived from

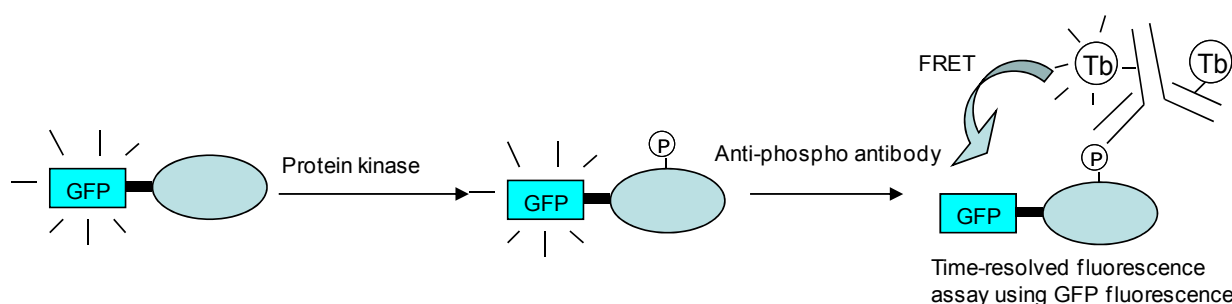
the acceptor. This phenomenon can be observed when the two molecules exist within 10 nm. Consequently, FRET is highly sensitive to distances between donors and acceptors. This methodology can be applied to similar systems characterized by fluorescence polarization-based assays using peptide substrates and an anti-phospho antibody. If the peptide substrate and the antibody are labeled with acceptor and donor molecules, respectively, binding of the antibody to the phosphorylated substrate generates FRET between the donor and acceptor. Therefore, protein kinase activity can be detected by monitoring the ratio of the fluorescence intensity at two emission wavelengths for the donor and acceptor. FRET measurements are sometimes disturbed by background fluorescence derived from other biomaterials and are also affected by direct excitation of the acceptor with excitation light for the donor molecule. To avoid such disturbances, time-resolve FRET is often used. Riddle et al. 2006 reported a FRET system using a GFP-fused peptide substrate and an anti-phosphotyrosine antibody labeled with a terbium ion complex (Riddle et al. 2006 (fig.4a)). The time-resolved FRET technique can be applied because rare earth metal complexes, such as terbium or europium complexes, produce long life-time fluorescences. However, a GFP fusion sometimes disturbs the phosphorylation of the substrate because of its large size. To avoid this effect, small organic fluorophores such as Alexa dyes are also used as acceptors (Zhang et al., 2005). A microplate based-high throughput assay has also been reported using a FRET-based kinase assay (Gratz et al., 2010) (Fig. 4b). In this assay, the substrate peptide of casein kinase 2 was labeled with fluorophore (EDANS) and the quencher (DABSYL) at the C and N terminus, respectively. The fluorescence of EDANS was quenched due to the FRET with DABSYL. Phosphorylation of the peptide by CK2 prohibited the cleavage of the peptide with elastase. Thus, CK2 activity was evaluated by the decrease in fluorescence. This approach was applied to a microplate-based assay and CK2 inhibitors were screened.

Although these systems are applied only to solution samples, if a FRET system can be applied to living cells, it has an advantage of ratiometry, in which, the assay can be performed independent of the thickness of the sample. Phocus is a good example of such a system. In this probe, CFP and YFP are fused with a kinase substrate, linker and phosphor-recognition domain (Sato & Umezawa, 2004) (Fig. 4c). In the free form, FRET between CFP and YFP does not occur because of the long distance between the two molecules. On the other hand, phosphorylation of the substrate domain causes the binding of the phosphor-recognition domain. This moves the two fluorophores into close proximity to cause FRET. Using this probe, activities of c-Jun and Src were observed successfully in living cells. The advantage of this probe is that the probe can be expressed in living cell spontaneously after the transfection of the encoding genes. On the other hand, optimization of the construct is required to design the probe for each kinase, and the large fluorescence moieties, CFP and YFP, may disturb the access of the protein kinase to the substrate domain in some cases. The method is also inconvenient for HTS systems.

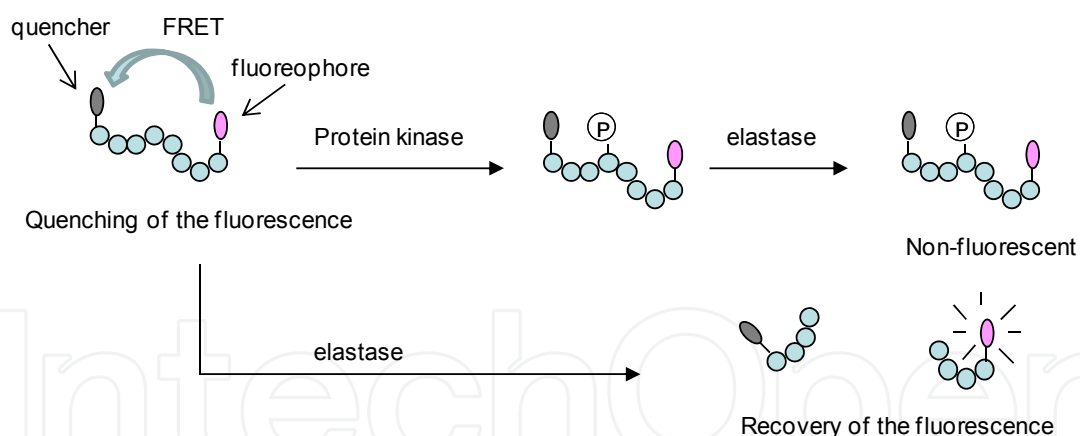
An additional monitoring system of protein kinases uses an alpha-screen assay (Pedro et al., 2010). Alpha-screening is not exactly a FRET system, but the excitation energy of a donor bead transfers to an acceptor bead indirectly via singlet oxygen. Excitation of the donor produces singlet oxygen with a photodynamic effect. Although the lifetime of singlet oxygen is very short, 4 μ sec, if the acceptor exists within 200 nm from the donor, the singlet oxygen can reach the acceptor. The acceptor then produces an emission light with singlet oxygen. Pedro et al. 2010 reported the monitoring of a leucine-rich repeat kinase (LRRK2), which is sometimes active in Parkinson's disease, using the alpha-screen system. Moesin is a

substrate of LRRK2 fused with a GST tag. Donor and acceptor beads were modified with GST and protein A, respectively. An antiphospho-antibody was then introduced onto the acceptor bead through protein A. After the phosphorylation of moecine with LRRK2, the donor and acceptor beads bound the moecine through GST and the phosphorylation site, respectively. In this case, excitation of the donor bead with 680 nm light produced an emission light at 520–600 nm from the acceptor bead through singlet oxygen. The system was applied to HTS analysis using a 384-well plate.

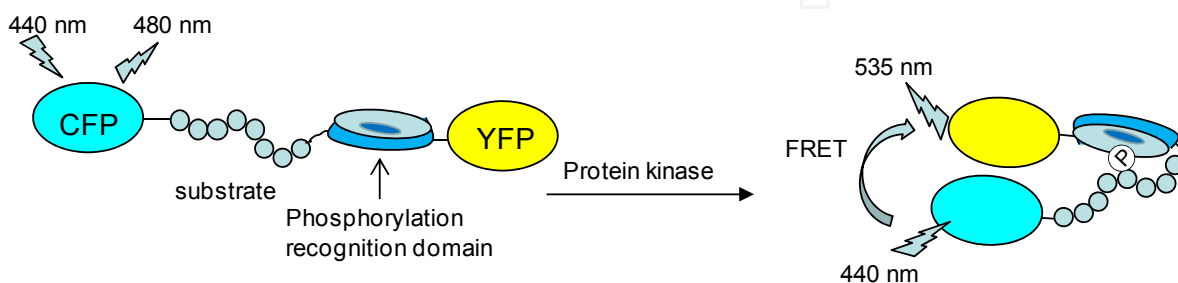
The QTL Light Speed Kinase Activity Assay™ is also a sensitive detection system for detecting protein kinase activity (Moon et al., 2007). This assay uses a highly fluorescent microsphere and quencher-labeled substrate peptide. The peptide can bind to the microsphere if the peptide is phosphorylated by the target kinase, because its surface is modified with a gallium complex. The fluorescence of the microsphere is then quenched with the quencher on the peptide. The system was used in a HTS method involving a microarray.



(a) Conceptual design of Phocus which is FRET based kinase probe for intracellular imaging



(b) FRET assay of protein kinase for high throughput inhibitor screening



(c) Protein kinase assay based on time resolved FRET measurement

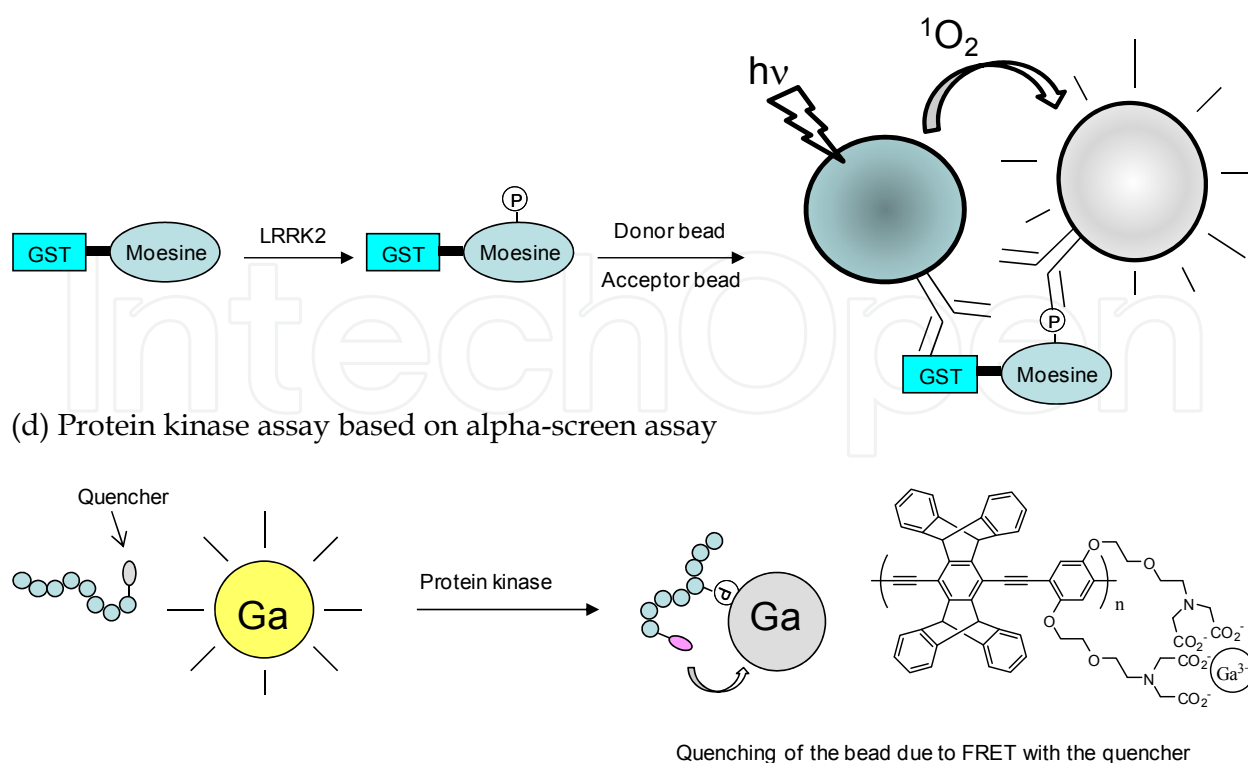


Fig. 4. FRET-based protein kinase assays

6. HTS assay using Gold Nano-Particle (GNP) with colorimetry

Although fluorimetry is sensitive and flexible, it can be affected by factors such as background substances, temperature, pH and the concentration of the fluorophore. Colorimetry, on the other hand, is simple and robust. We reported a label-free kinase assay using gold nano-particle (GNP) (Oishi et al., 2007; Oishi et al., 2008) (Fig. 5). Cationic peptides causes an aggregation of anionic GNP prepared by citrate reducing. This changes the color of the GNP dispersion from red to blue. This aggregation is highly sensitive to the peptide. Cationic peptide aggregates are 1000-fold more effective than inorganic cations that have same cationic charges. However, if the peptide is phosphorylated by the target kinase, the ability to aggregate is reduced dramatically so that the color of the dispersion remains red upon the addition of the peptide. Thus, phosphorylation of the peptide can easily be detected by monitoring the absorbance at 670 nm. The assay has been sufficiently sensitive to detect PKA, PKC α , MAPK, p38 and Src activity in solution, cell lysates and tissue extracts. Detection of PKC α activity in tumor and normal tissues from human patients suggests that the assay can be applied as a diagnostic of breast cancer (Kang et al., 2010). The assay was also used to screen for protein kinase inhibitors using a micro-titer plate format (Oishi et al., 2008; Asami et al., 2011). Using a chemical library containing 3000 chemicals, new PKA inhibitors, which have similar inhibitory activity to current PKA inhibitors, were actually identified using this assay. The aggregation of GNP is affected by the cationic net charges of the peptide and ionic strength. Therefore, the ionic concentration of the detecting solution has to be optimized for each peptide sequence. However, such conditions can be optimized easily, because the conditions of the phosphorylation and detection steps can be

set independently. In addition, this assay does not require any labeling steps to the substrate peptide and is simple, rapid and widely applicable from solution to tissue samples. Since the assay depends on decreasing net charge of the substrate peptide, the original net charge has to be cationic. On the other hand, some protein kinases require anionic peptide sequences as their substrates. This issue can be overcome by the addition of some cationic amino acids at one end of the peptide through a flexible triethylene glycol linker. If gold nano-rods are covered with a cationic surfactant, Cetyltrimethylammonium bromide (CTAB) is used instead of GNP, and an anionic substrate can be used without any addition of cationic amino acids (Kitazaki et al., 2011).

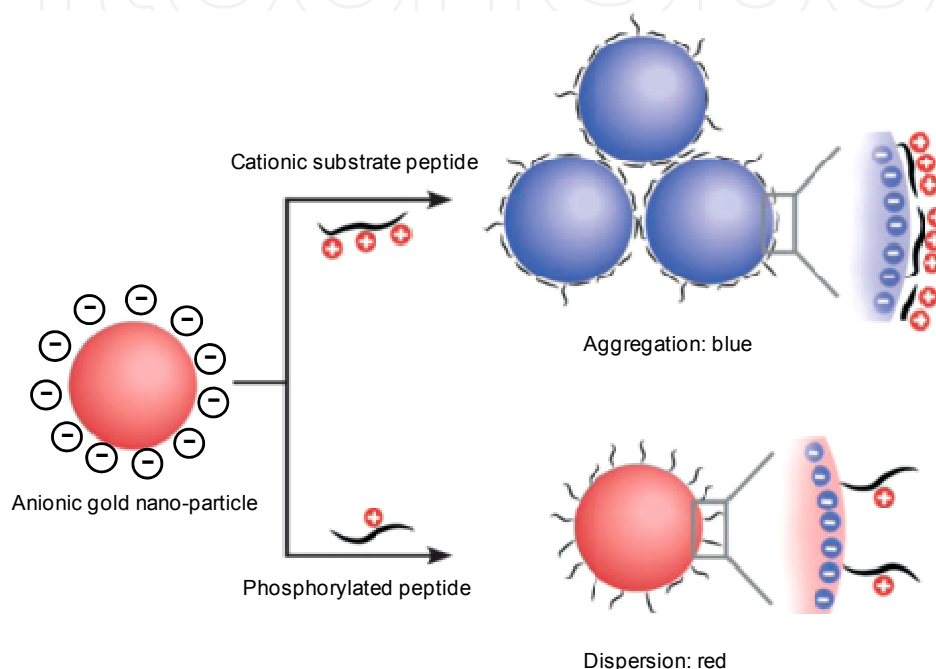


Fig. 5. Concept of colorimetric assay of protein kinase using gold nano-particle

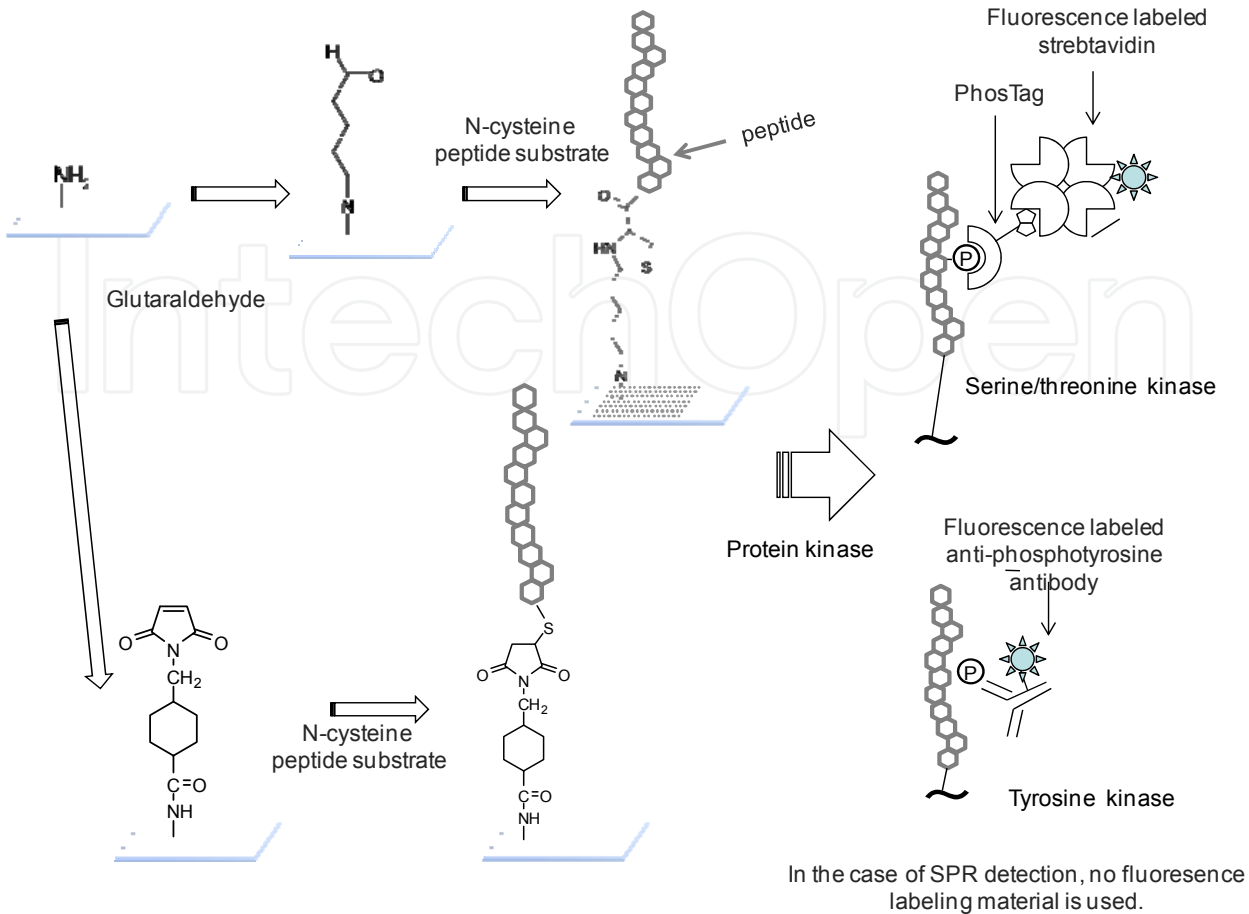
7. Peptide array

Genomic and post-genomic research has enabled us to understand life at the molecular level. Many aspects of molecular processes have been elucidated. As a result, evolutionary changes have been made in methodologies in drug discovery, diagnostics and other medical technologies. Developments of molecular targeted drugs that primarily target tyrosine kinases for cancer therapy represent a typical example. However, life was not constructed through a simple combination of such pieces, but involves many pieces that interact with each other to generate a complicated network system. Therefore, diseases are not simply treated by inhibiting a single target molecule. For example, cancer cells often acquire a tolerance against molecular targeted drugs during their applications, even though the drugs continue to inhibit the target protein kinases. Therefore, we have to clarify the condition of entire signal network to know the cellular condition exactly. From research using gene chip technology, it has been clarified that a major part of the transcriptome is necessary to maintain the basic functions of living cells, and only a part of the transcriptome relates to each cell-specific function. Tiny fluctuations of the transcriptome sometimes cause a significant change in the enzymatic network determining cell function (Irish et al., 2004).

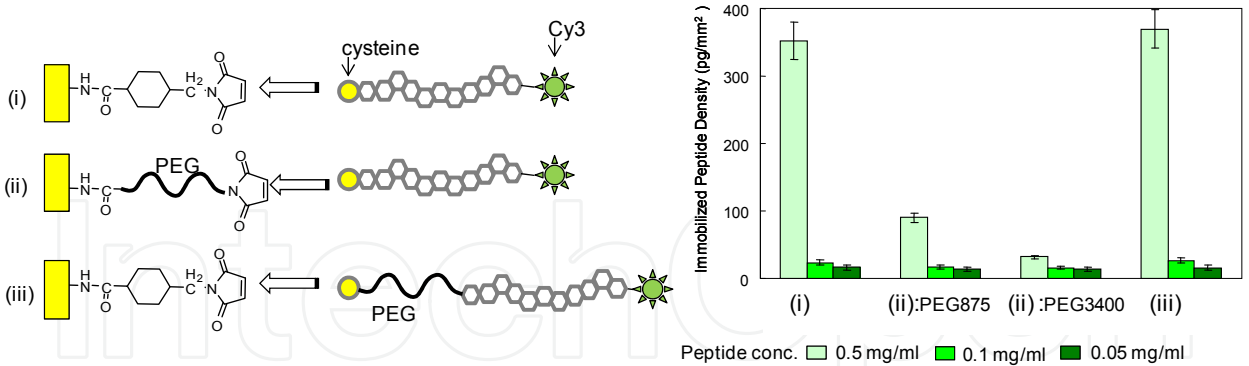
This indicates that monitoring enzymatic activities in cellular signal transduction events must be efficient and effective for precisely evaluating cell conditions. Kinome, the entire profile of protein kinases in cells, is a new concept presented in this issue. The most practical format of the kinome analysis is peptide array. Peptide array involves chip technology, in which many peptide substrates of protein kinases are immobilized on a solid support. Some peptide array systems are now commercially available. The PepScan array is probably the first example in which kinome analysis was carried out using a peptide array (Diks et al., 2004). In this array, many peptide substrates are immobilized onto a glass slide and phosphorylation is detected with the incorporation of [^{33}P]-phosphate from RI-labeled ATP. Using the array immobilizing 192 peptides, changes in the activities in particular kinases after LPS stimulation was analyzed. Following this report, kinome in Barrett's esophagus, endothelial cells and also c-Met activity in colon cancer after the inhibition of cyclooxygenase-2 were monitored using more than 1000 peptides (van Baal et al., 2011). Although the array seems to give reasonable evaluations in activation of particular kinases, the system assumes that one peptide is phosphorylated by a single kinase; however, such short peptides may be phosphorylated by plural protein kinases. The CelluSpot system is another similar peptide array format (Olaussen et al., 2009). In this array, peptide substrates that were synthesized on a nitrocellulose membrane are cleaved and set on a solid support. The effect of tyrosine kinase inhibitors on the kinome in a carcinoma cell line was evaluated using this system with 144 peptides. Phosphorylation of the peptides was detected using a fluorescence-labeled anti-phosphotyrosine antibody. Although this system is simple and does not use radio-active material, the size of each spot is large (1 mm) such that a relatively large amount of sample is required.

Enzymatic reactions are sometimes inconvenient to perform on solid surfaces. The Pamchip is an array to address this issue (Jinnin et al., 2008; Maat et al., 2009). In this system, peptides are immobilized in a well consisting of porous material. The porous material is 60 μm thick and has long branched interconnected capillaries with a diameter of 200 nm. It results in a 500-fold increase in the reactive surface and the reaction and washing steps can also be performed by pulsing back and forth through the porous material many times. The profile of tyrosine kinase activities in a pediatric brain tumor was evaluated and compared with kinomes from other various cancer cell lines in 144 peptides (Sikkema et al., 2009). A group of peptides that were phosphorylated by all cancer cell lines and by particular cell lines were identified. However, it is relatively difficult to identify each kinase from such profiles. Activation of vascular endothelial growth factor receptor type2 (VEGFR2) and Src signaling were also confirmed in infantile hemangioma and melanoma cells, respectively, with this system.

The above mentioned peptide arrays are useful for kinome analysis. However, it is unclear whether they can be used for quantitative analysis. For monitoring kinase activity, it is important to know how much activity has changed. In many cases, cellular function will be influenced by the degree of activation of particular kinases. Recently we developed a peptide array in which peptide substrates are immobilized onto gold or glass support for surface Plasmon resonance (SPR) and fluorescence detection, respectively (Shigaki et al., 2007; Inamori et al. 2005) (Fig. 6a). By careful optimization of the surface chemistry, these arrays secured quantitative data describing the detection of the phosphorylation ratio in each peptide (Han et al., 2008; Shimomura et al., 2011). For example, the efficiency of peptide immobilization influenced the results from the methodology (Inamori et al., 2008) (Fig. 6b).



(a) Schematic illustration of peptide immobilization and detection in quantitative peptide array



(b) Correlation between immobilized peptide density and immobilization protocol

Fig. 6. Quantitative peptide array: concept and immobilization method of peptide substrate

Figure 6b shows the efficiency of peptides immobilized onto a gold chip. Although the polyethylene glycol (PEG) moiety is effective in suppressing non-specific adsorption of bio-substances, peptide immobilization was also suppressed if the PEG moiety was modified on the chip in advance. On the other hand, when the PEG-linked peptide was reacted with a rigid group on the chip, the amount of the immobilized peptide increased dramatically. In our system, phosphorylation of the peptide was achieved by using an anti-phosphotyrosine antibody or PhosTag molecules followed by the addition of streptavidin. Changes in the

activities of particular protein kinases were detected using the SPR chip in solution and a cell lysate after the cell was stimulated with NGF (Han et al., 2009). An advantage of the SPR detection system is that it does not require any labeling for detection. However, this advantage contains a risk that binding of any other substances gives rise to a detectable signal at the same time. In this case, fluorescence detection will be more practical. In the quantitative detection of the kinome on a peptide array, peptides that have cysteines at the amino terminus were immobilized through a formyl group or maleimide. In the former case, high-density amino-modified glass surfaces were treated with glutaraldehyde, and then the peptide was linked to the formyl group at the thiol group of the cysteine residue by forming a thiazoline ring (Mori et al., 2009). The surface of the chip was then blocked from unspecific adsorption with Blocking One-P, which is a commercial cocktail. We recently used a plastic plate that was modified, called the 'S-Bio' system, to avoid the adsorption of biomacromolecules. In this case, cysteine-containing peptides were immobilized on the chip using maleimide chemistry. Detection of phosphorylation was achieved using a Cy-3 labeled anti-phosphotyrosine antibody or a PhosTag and Alexa647-labeled streptavidin. After the optimization of the conditions of immobilization, the obtained chip provides quantitative phosphorylation ratios of the peptide. This quantitative analysis is sufficient for measuring peptide phosphorylation immobilized on a single chip. However, an internal standard is required for the inter-plate comparison. Alexa647 labeled peptide is used for this purpose. Using this array, Src activity in various cancer cell lines and mouse tissues was successfully monitored. Changes in the kinome profile with drug stimulation such as NGF or Iressa was also obtained (Han et al., 2010). Such arrays were also applied to screen kinase inhibitors (Inamori et al., 2009).

Kinome analysis using peptide array has not been a well-establish technology. Especially, bio-informatics technique which converts obtained phosphorylation profile into actual signal network of protein kinases. Reproducibility of peptide array has also to be improved.

8. New technology using protein kinase activity in artificial bio-regulation

As mentioned above, many technologies for monitoring protein kinase activity have been developed. On the other hand, any technology that uses protein kinase activity should also be useful for medicine, because protein kinases play key roles in determining cellular functions. Abnormal activation of particular kinases is often observed in many diseases. For example, hyper-activation of EGFR, c-MET, bcl-Abl, PKC α , or Src has been reported in many types of tumors. In myocardial infarction, over expression of Rho kinase is also reported. Activation of I- κ -kinase is a key signal to initiate inflammation. Therefore, abnormal activity of such kinases can be markers to distinguish disease cells. In this context, if these signals can be converted to other information artificially, such signal conversion should identify disease cells specifically. Such artificial signal converters will offer a new strategy for cell-specific medicines. We recently reported some artificial gene regulators that activated transgene expression in response to target protein kinase activity (Oishi et al., 2006; Sonoda et al., 2005; Kawamura et al., 2005) (Fig.7). The regulators consist of polymer backbone and some cationic peptide side-chains. The peptide is also designed as a specific substrate of a target protein kinase. Since the polymer-peptide conjugates are polycationic, they can form electrostatic complexes with DNA such as an expression vector. In the complex, this type of conjugate suppresses the gene transcription much more efficiently

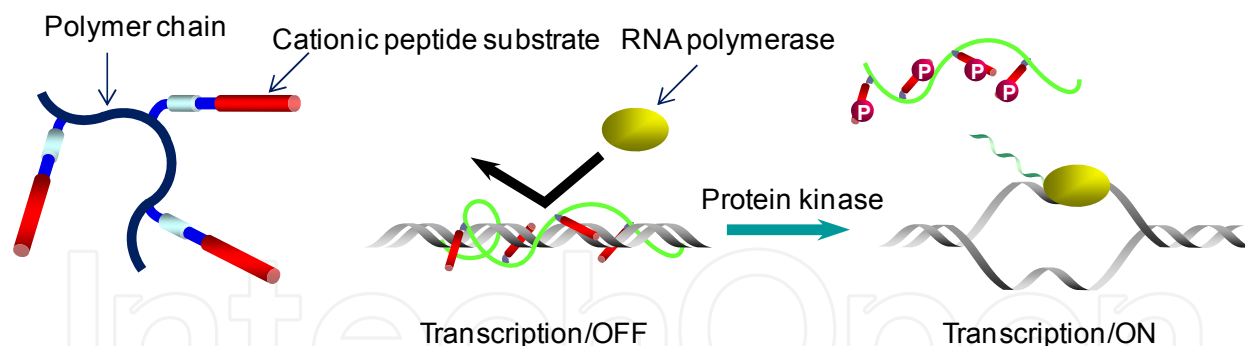


Fig. 7. Structure of artificial gene regulator (left) and concept of its gene regulation in response to protein kinase

than ordinary polycations such as polyethyleneimine, or poly-L-lysine. When the complex is taken up by target disease cells in which target protein kinases are hyper-activated, the peptide side-chains are phosphorylated. This introduction of anionic charges decreases the net cationic charges of the conjugate, and the electrostatic interaction between the conjugate and DNA is attenuated. As a result, the gene can be expressed due to the disintegration of the complex. This system is the first strategy for cell-specific gene therapy using kinase activity as a marker of cellular identification. Using this strategy, various gene regulators have been developed for Src, PKC α , I- κ -kinase, Rho kinase and PKA as target signals (Sato et al., 2010; Kang et al., 2010; Asai et al., 2009; Tsuchiya et al., 2011; Oishi et al., 2006). These materials realize highly cell-specific gene expression. Figure 8b indicates examples of such signal-responsive gene expression in I- κ -kinase and PKC α -responsive systems (Asai et al., 2009). The I- κ -kinase responsive system activated expression of a GFP encoding gene only following stimulation of NHI 3T3 cells with LPS or TNF- α , thereby initiating inflammation. However, if the serine residue in the peptide side-chain, which is a phosphorylation site, was replaced with alanine, such gene expression was not observed even following stimulation by LPS or TNF- α . PLC α is another important kinase for the proliferation of many types of cancer cells (Kang et al., 2009). Therefore, transfection of GFP encoding plasmid as a complex with the PKC α responsive regulator gave massive expression of GFP in various cancer cell lines (Asai et al., 2009). Conversely, a negative control-regulator, in which the serine residue was replaced with alanine, did not show any expression in such cell lines. In addition, no activation of GFP expression was observed when such cells were pre-treated with an inhibitor of PKC. These results clearly indicate that such systems regulated gene expression in response to target kinases (Toita et al., 2009). In particular, the PKC α responsive system worked also in tumor bearing mice (Kang et al., 2008; Toita et al., 2009; Kang et al., 2010) (fig. 8c). When a complex between a PKC α -responsive conjugate and the luciferase encoding gene was injected into a tumor directly, expression of luciferase was observed successfully. On the other hand, injection of the complex in normal subcutaneous tissue or injection of the complex using a negative control conjugate into a tumor did not show any expression of luciferase. The obtained image of luciferase indicates a proliferation activity, because the enzymatic activity regulates cancer proliferation directly and is closely related to the cancer malignancy. Thus, this system will be useful for cancer imaging, because this is the first functional imaging of cancer in contrast to ordinary imaging techniques of cancer that mainly visualize the existence of a tumor. Such functional images should provide much more sharpshooting information for prognosis than currently used

to satisfy medical or pharmaceutical demands. Detecting dysfunctional activity of particular kinases and relating this to a disease condition will require the development of simple and rapid assays. Fluorescence polarization, bead techniques using fluorimetry and colorimetric assays offer a way to reach this goal. Mass spectrometry is also a promising approach (Kang et al., 2008; Kang et al., 2007; Shigaki et al., 2006). Peptide arrays are another promising technology for detail evaluation of cellular conditions. Since cellular function is determined by a network of signaling reactions governed by enzymes including protein kinases, the exact state of living cells in various diseases cannot be evaluated by a single protein kinase assay. In this context, kinome analysis will be crucial for providing a detailed diagnosis before medication, prognosis after medication and validation of drugs in pharmaceutical testing. However, problems with the current peptide array systems, including their low reproducibility of obtaining similar phosphorylation profiles on chips, are hampering progress towards fully accurate kinome analysis. Relatively low specificity of peptide substrates is another issue. It is difficult to convert the obtained phosphorylation profile into a profile that represents the actual protein kinase activity. Bioinformatics and mathematical technology should be combined with array technologies in the future.

Protein kinases are also attractive as a marker to distinguish between disease cells and normal cells. Our gene regulation system that responds to target protein kinase activity is the first artificial system that uses intracellular signaling as a trigger to output another biological signal. Such signal engineering represents a new cell-specific medicine approach. We need such a new technological field, termed “Cell Signalomics”, to link basic biological findings to clinical approaches. Protein kinases will be one of the most important elements for such a technology.

10. References

- Asai, D., Kang, J-H., Toita, R., Tsuchiya, A., Niidome, T., Nakashima, H. & Katayama, Y., (2009). Regulation of transgene expression in tumor cells by exploiting endogenous intracellular signals, *Nanoscale Res. Lett.* Vol. 4, 229-233.
- Asai, D., Tsuchiya, A., Kang, J-H., Kawamura, K., Oishi, J., Mori, T., Niidome, T., Shoji, Y., Nakashima, H. & Katayama, Y., (2009). Inflammatory cell-specific gene regulation system responding to Ikappa-B kinase beta activation, *J. Gene Med.*, Vol. 11, 624-632.
- Asami, Y., Oishi, J., Kitazaki, H., Kamimoto, J., Kang, J-H., Niidome, T., Mori, T. & Katayama, Y., (2011). A simple set-and-mix assay for screening of protein kinase inhibitors in cell lysates, *Anal. Biochem.*, Vol. 418, 44-49.
- Coffin, J., Latev, M., Bi, X. & Nikiforov, T. T. (2000). Detection of phosphopeptides by fluorescence polarization in the presence of cationic polyamino acids: Application to kinase assays, *Anal. Biochem.*, Vol. 278, 206-212.
- Cohen, P. (2001). The role of protein phosphorylation in human health and disease, *Eur. J. Biochem.* Vol. 268: 5001-5010.
- Diks, S. H., Kok, K., Toole, T. O., Hommes, D. W., van Dijken, P., Joore, J. & Peppelenbosch, M. P., (2004). Kinome profiling for studying lipopolysaccharide signal transduction in human peripheral blood mononuclear cells, *J. Biol. Chem.*, Vol. 279 (No. 47), 49206-49213.
- Diks, S.H., Kok, K., Toole, T., Hommes, D.W., Dijken, P., Joore, J. & Peppelenbosch, M.P. (2004). Kinome profiling for studying lipopolysaccharide signal transduction in

- human peripheral blood mononuclear cells, *J. Biol. Chem.*, Vol.279 (No. 47), 49206-49213.
- Gratz, A., Gotz, C. & Jose, J. (2004). A FRET-based microplate assay for human protein kinase CK2, a target in neoplastic disease, *J. Enzyme Inhibit.Med. Chem.*, Vol. 25(No. 2): 234-239.
- Han, X., Shigaki, S., Yamaji, T., Yamanouchi, G., Mori, T., Niidome, T. & Katayama, Y., (2008). A quantitative peptide array for evaluation of protein kinase activity, *Anal. Biochem.*, Vol. 372, 106-115.
- Han, X., Sonoda, T., Mori, T., Yamanouchi, G., Yamaji, T., Shigaki, S., Niidome, Y. & Katayama, Y., (2010). Protein kinase substrate profiling with a high-density peptide microarray, *Comb. Chem. High Throughput Screen.* Vol. 13, 777-789.
- Han, X., Yamanouchi, G., Mori, T., Kang, J-H., Niidome, T. & Katayama, Y., (2009). Monitoring protein kinase activity in cell lysates using a high density peptide microarray, *J. Biomol. Screen.* Vol. 14, 256-262.
- Higashi, H., Sato, K., Omori, A., Sekiguchi, M., Ohtake, A. & Kudo, Y. (1996) Imaging of Ca^{2+} /calmodulin-dependent protein kinase II activity in hippocampus neurons, *Neuroreport* Vol. 7(No. 15-17), 2695-2700.
- Inamori, K., Kyo, M., Matsukawa, K., Inoue, Y., Sonoda, T., Mori, T., Niidome, T. & Katayama, Y., (2009). Establishment of screening system toward discovery of kinase inhibitors using label-free on-chip phosphorylation assays, *BioSystems*, Vol. 97, 179-185.
- Inamori, K., Kyo, M., Matsukawa, K., Inoue, Y., Sonoda, T., Tatematsu, K., Tanizawa, K., Mori, T. & Katayama, Y., (2008). Optimal surface chemistry for peptide immobilization in on-chip phosphorylation analysis, *Anal. Chem.*, Vol. 80, 643-650.
- Inamori, K., Kyo, M., Nishiya, Y., Inoue, Y., Sonoda, T., Kinoshita, E., Koike, T. & Katayama, Y., (2005). Detection and quantification of on-chip phosphorylated peptides by surface plasmon resonance imaging techniques using a phosphate capture molecule, *Anal. Chem.*, Vol. 77, 3979-3985.
- Inamori, K., Kyo, M., Nishiya, Y., Inoue, Y., Sonoda, T., Kinoshita, E., Koike, T. & Katayama, Y. (2005). Detection and quantification of on-chip phosphorylated peptides by surface Plasmon resonance imaging techniques using a phosphate capture molecule, *Anal. Chem.*, Vol. 77, 3979-3985.
- Irish, J. M., Hovland, R., Krutzik, P. O., Perez, O. D., Bruserud, Ø., Gjertsen, B. T. & Nolan, G. P., (2004). Single cell profiling of potentiated phospho-protein networks in cancer cells, *Cell*, Vol. 118, (No. 2) 217-228.
- Jinnin, M., Medici, D., Park, L., Limaye, N., Liu, Y., Boscolo, E., Bischoff, J., Vikkula, M., Boye, E. & Olsen, B. R., (2008), Suppressed NFAT-dependent VEGFR1 expression and constitutive VEGFR2 signaling in infantile hemangioma, *Nat. Med.*, Vol. 14 (No. 11), 1236-1246.
- Kang, J-H., Asai, D., Kim, J-H., Mori, T., Toita, R., Tomiyama, T., Asami, Y., Oishi, J. Sato, Y. T., Niidome, T., Jun, B., Nakashima, H. & Katayama, Y., (2008). Design of polymeric carriers for cancer-specific gene targeting: Utilization of abnormal protein kinase C α activation in cancer cells, *J. Am. Chem. Soc.*, Vol. 130, 14906-14907.
- Kang, J-H., Asai, D., Toita, R., Kitazaki, H. & Katayama, Y., (2009). Plasma protein kinase C (PKC) α as a biomarker for the diagnosis of cancers, *Carcinogenesis*, Vol. 30, 1927-1931.

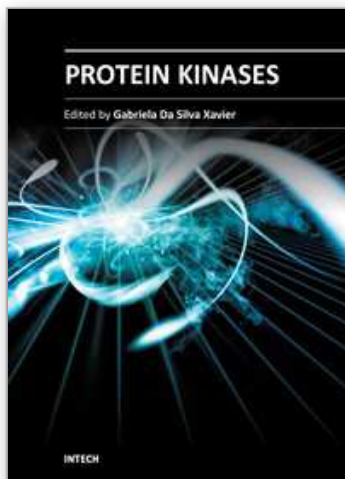
- Kang, J-H., Asami, Y., Murata, M., Kitazaki, H., Sadanaga, N., Tokunaga, E., Shiotani, S., Okada, S., Maehara, Y., Niidome, T., Hashizume, M., Mori, T. & Katayama, Y., (2010). Gold nanoparticle-based colorimetric assay for cancer diagnosis, *Biosens. Bioelectron.*, Vol. 25, 1869-1874.
- Kang, J-H., Han, A., Shigaki, S., Oishi, S., Kawamura, K., Toita, R., Han, X., Mori, T., Niidome, T. & Katayama, Y., (2007). Mass-tag technology responding to intracellular signals as a novel assay system for the diagnosis of tumor, *J. Am. Soc. Mass Spectr.* Vol. 18, 106-112.
- Kang, J-H., Kuramoto, M., Tsuchiya, A., Toita, R., Asai, D., Sato, Y. T., Mori, T., Niidome, T. & Katayama, Y., (2008). Correlation between phosphorylation ratios by MALDI-TOF MS analysis and enzyme kinetics, *Eur. J. Mass Spectrom.*, Vol. 14, 261-265.
- Kang, J-H., Oishi, J., Kim, J-H., Ijuin, M., Toita, R., Jun, B., Asai, D., Mori, T., Niidome, T., Tanizawa, K., Kuroda, S. & Katayama, Y., (2010). Hepatoma-targeted gene delivery using a tumor cell-specific gene regulation system combined with a human liver cell-specific bionanocapsule, *Nanomedicine* Vol. 6, 583-589.
- Kang, J-H., Toita, R. & Katayama, Y., (2010). Bio and nanotechnological strategies for tumor-targeted gene therapy, *Biotechnol. Adv.* Vol. 28, 757-763.
- Kawamura, K., Oishi, J., Kang, J-H., Kodama, K., Sonoda, T., Murata, M., Niidome, T. & Katayama, Y., (2005). Intracellular Signal-Responsive Gene Carrier for Cell-Specific Gene Expression, *Biomacromolecules*. Vol. 6, 908-913.
- Kinoshita, E., Kinoshita-Kikuta, E., Takiyama, K. & Koike T. (2006). Phosphate-binding tag: A new tool to visualize phosphorylated proteins, *Molecular & Cellular Proteomics*, Vol. 5, 749-757.
- Kitazaki, H., Mori, T., Kang, J-H., Niidome, T., Murata, M., Hashizume, M. & Katayama, Y., (2011). A colorimetric assay of protein kinase activity based on peptide-induced coagulation of gold nanorods, *Coll. Surf. B*, in press.
- Koga, H., Toita, R., Mori, T., Tomiyama, T., Kang, J-H., Niidome, T. & Katayama Y., (2011). Fluorescent nanoparticles consisting of lipopeptides and fluorescein-modified polyanions for monitoring of protein kinase activity, *Bioconjugate Chem.* Vol. 22, 1526-1534.
- Kristjansdottir K. & Rudolph J. (2003). A fluorescence polarization assay for native protein substrates, *Anal. Biochem.*, Vol. 316, 41-49.
- Kumar, E. A., Charvet, C. D., Lokesh, G.L. & Natarajan, A. (2011). High-throughput fluorescence polarization assay to identify inhibitors of Cbl(TKB)-protein tyrosine kinase interactions, *Anal. Biochem.*, Vol. 411, 254-260.
- Kupcho, K., Somberg, R., Bullent, B., Goueli, S.A. (2003). A homogeneous, nonradioactive high-throughput fluorogenic protein kinase assay, *Anal. Biochem.* Vol. 317, 210-217.
- Maat, W., el Filali, M., Dirks-Mulder, A., Luyten, G. P. M., Gruis, N. A., Desjardins, L., Boender, P., Jager, M. J. & van der Velden, P. A., (2009). Episodic Src activation in uveal melanoma revealed by kinase activity profiling, *Br. J. Cancer*, Vol. 101, 312-319.
- McIlroy, B. K., Walters, J. D. & Johnson, J. D. (1991). A Continuous Fluorescence Assay for Protein Kinase *Anal. Biochem.* Vol. 195, 148-152.
- Moon, J. H., MacLean, P., McDaniel W. & Hancock, L. F., (2007). Conjugated polymer nanoparticles for biochemical protein kinase assay, *Chem. Commun.*, 4910-4912

- Mori, M., Yamanouchi, G., Han, X., Inoue, Y., Shigaki, S., Yamaji, T., Sonoda, T., Yasui, K., Hayashi, H., Niidome, T. & Katayama, Y., (2009). Signal-to-noise ratio improvement of peptide microarrays by using hyperbranched-polymer materials, *J. Appl. Phys.*, Vol. 105, 102020.
- Oda, Y., Nagasu, T. & Chait, B. T. (2001). Enrichment analysis of phosphorylated proteins as a tool for probing the phosphoproteome Vol. 19 (No. 4) 379-382.
- Oishi, J., Asami, Y., Mori, T., Kang, J-H., Tanabe, M., Niidome, T. & Katayama, Y., (2007). Measurement of homogeneous kinase activity for cell lysates based on the aggregation of gold nanoparticles, *ChemBioChem*, Vol. 8, 875-879.
- Oishi, J., Asami, Y., Mori, T., Kang, J-H., Tanabe, M., Niidome, T. & Katayama, Y., (2008). Colorimetric enzymatic activity assay based on "non-crosslinking aggregation" of gold nanoparticles induced by adsorption of substrate peptides, *Biomacromolecules*, Vol. 9, 2301-2308.
- Oishi, J., Han, X., Kang, J-H., Asami, Y., Mori, T., Niidome, T. & Katayama, Y., (2008). High-throughput colorimetric detection of tyrosine kinase inhibitors based on the aggregation of gold nanoparticles, *Anal. Biochem.*, Vol. 373, 161-163.
- Oishi, J., Ijuin, M., Sonoda, T., Kang, J-H., Kawamura, K., Mori, T., Niidome, T. & Katayama, Y., (2006). A protein kinase signal-responsive gene carrier modified RGD peptide, *Bioorg. Med. Chem. Lett.* Vol. 16, 5740-5743.
- Oishi, J., Jung, J., Tsuchiya, A., Toita, R., Kang, J-H., Mori, T., Niidome, T., Tanizawa, K., Kuroda, S. & Katayama, Y., (2010). A gene delivery system specific for hepatoma cells and an intracellular kinase signal based on human liver-specific bionanocapsules and signal-responsive artificial polymer, *Int. J. Pharm.*, Vol. 396, 174-178.
- Oishi, J., Kawamura, K., Kang, J-H., Kodama, K., Sonoda, T., Murata, M., Niidome, T. & Katayama, Y., (2006). An intracellular kinase signal-responsible gene carrier for disordered cell-specific gene therapy, *J. Controlled Release* Vol. 110, 431-436.
- Olaussen, K. A., Commo, F., Tailler, M., Lacrix, L., Vitale, I., Raza, S. Q., Richon, C., Dessen, P., Lazar, V., Sorita, J-C. & Kroemer, G., (2009). Synergistic proapoptotic effects of the two tyrosine kinase inhibitors pazopanib and lapatinib on multiple carcinoma cell lines, *Oncogene*, Vol. 28, 4249-4260.
- Panse, S., Dong, L., Burian, A., Carus, R., Schutkowski, M., Reimer, U. & Schneider-Mergener, J. (2004). Profiling of generic antiphosphopeptide antibodies and kinases with peptide microarrays using radioactive and fluorescence-based assays, *Mol. Divers.*, Vol. 8(No. 3), 291-299.
- Pedro, R., Padros, J., Beaudet, L., Schubert, H-D., Gillardon, F. & Dahan, S., (2010). Development of a high-throughput AlphaScreen assay measuring full-length LRRK2(G2019S) kinase activity using moesin protein substrate, *Anal. Biochem.*, Vol. 404, 45-51.
- Riddle, S. M., Vedvik, K. L., Hanson, G. T. & Vogel, K. W. (2006). Time-resolved fluorescence resonance energy transfer kinase assays using physiological protein substrates: Applications of terbium-fluorescein and terbium-green fluorescent protein fluorescence resonance energy transfer pairs, *Anal. Biochem.* Vol. 356, 108-116.
- Sato, M. & Umezawa, Y., (2004). Imaging protein phosphorylation by fluorescence in single living cells, *Methods*, Vol. 32, 451-455.

- Sato, Y., Kawamura, K., Niidome, T. & Katayama, Y., (2010). Characterization of gene expression regulation using D-RECS polymer by enzymatic reaction for an effective design of enzyme-responsive gene regulator, *J. Controlled Release*, Vol. 143, 344-349.
- Schutkowski, M., Reimer, U., Panse, S., Dong, L., Lizacano, J.M., Alessi, D. R. & Schneider-Mergener, J. (2004). High-content peptide microarrays for deciphering kinase specificity and biology, *Anew. Chem. Int. Ed.*, Vol. 43(No. 20), 2671-2674.
- Seethala, R. & Menzel, R. (1997). A homogeneous, fluorescence polarization assay for Src-family tyrosine kinases, *Anal. Biochem.*, Vol. 253, 210-218.
- Seethala, R. & Menzel, R. (1998). A Fluorescence polarization competition immunoassay, *Anal. Biochem.*, Vol. 255, 257-262.
- Shigaki, S., Sonoda, T., Nagashima, T., Okitsu, O., Kita, Y., Niidome, T. & Katayama, Y., (2006). A new method for evaluation of intracellular protein kinase signals using mass spectrometry, *Science and Technology of Advanced Materials* Vol. 7, 699-704.
- Shigaki, S., Yamaji, T., Han, X., Yamanouchi, G., Sonoda, T., Okitsu, O., Mori, T., Niidome, T. & Katayama, Y., (2007). A peptide microarray for the detection of protein kinase activity in cell lysate, *Anal. Sci.*, Vol. 23, 271-275.
- Shimomura, T., Han, X., Hata, A., Niidome, T., Mori, T. & Katayama, Y., (2011). Optimization of peptide density on microarray surface for quantitative phosphoproteome, *Anal. Sci.*, Vol. 27(No. 11), 13-17.
- Sikkema, A. H., Diks, S. H., den Dunnen, F. A., ter Elst, A., Scherpen, F. J. G., Hoving, E. W., Ruijtenbeek, R., Boender, P. J., de Wijn, R., Kamps, W. A., Peppelenbosch, M. P. & de Bont, S. J. M., (2009). Kinome profiling in pediatric brain tumors as a new approach for target discovery, *Cancer Res.*, Vol. 69 (No. 14), 5987-5995.
- Sonoda, T., Niidome, T. & Katayama, Y., (2005). Controlled gene delivery responding to cell signals using peptide-polymer conjugates, *Recent. Res. Devl. Bioconjugate Chem.* Vol. 2, 145-158.
- Steinberg, T. H., Agnew, B. J., Gee, K. R., Leung, W. Y., Goodman, T., Schulenberg, B., Hendrickson, J., Beechem, J. M., Haugland, R. P. & Patton, W. F. (2003). Global quantitative phosphoprotein analysis using multiplexed proteomics technology. *Proteomics* Vol. 3, 1128-1144.
- Sun, H., Low, K.E., Woo, S., Noble, R.L., Graham, R.J., Connaughton, S.S., Gee, M.A. & Lee, L-G. (2005). Real-time protein kinase assay, *Anal. Chem.* Vol. 77 (No. 7), 2043-2049.
- Toita, R., Kang, J-H., Kim, J-H., Tomiyama, T., Mori, T., Niidome, T., Jun, B. & Katayama, Y., (2009). Protein kinase C α -specific peptide substrate graft-type copolymer for cancer cell-specific gene regulation systems, *J. Controlled Release*, Vol. 139, 133-139.
- Tomiyama, T., Kang, J-H., Toita, R., Niidome, T. & Katayama, Y., (2009). Protein kinase C α -responsive polymeric carrier: Its application for gene delivery into human cancers, *Cancer Sci.*, Vol. 100, 1532-1536.
- Tomiyama, T., Toita, R., Kang, J-H., Asai, D., Shiosaki, S., Mori, T., Niidome, T. & Katayama, Y., (2010). Tumor therapy by gene regulation system responding to cellular signal, *J. Controlled Release*, Vol. 148, 101-105.
- Tomiyama, T., Toita, R., Kang, J-H., Koga, H., Shiosaki, H., Mori, T., Niidome, T. & Katayama, Y., (2011). Effect of the introduction of chondroitin sulfate into polymer-peptide conjugate responding to intracellular signals, *Nanoscale Res. Lett.*, Vol. 6, 532.

- Tsuchiya, A., Kang, J-H., Asai, D., Mori, T., Niidome, T. & Katayama, Y., (2011). Transgene regulation system responding to Rho associated coiled-coil kinase (ROCK) activation, *J. Controlled Release*, Vol. 155, 40-46.
- van Baal, J. W. P., Diks, S. H., Wanders, R. J. A., Rygiel, A. M., Milano, F., Joore, J., Bergman, J. G. H. M., Peppelembosch, M. P. & Krishnadath, K., (2011). Comparison of kinome profile of Barrett's esophagus with normal squamous esophagus and normal gastric cardia, *Cancer Res.*, Vol. 66 (No. 24), 11605-11612.
- Zhang, W. X., Wang, R., Wisniewski, D., Marcy, A. I., LoGrasso, P., Lisnock, J-M., Cummings, R. T. & Thompson, J. E. (2005). Time-resolved Forster resonance energy transfer assays for the binding of nucleotide and protein substrates to p38_ protein kinase, *Anal. Biochem.* Vol. 343, 76-83.

IntechOpen



Protein Kinases

Edited by Dr. Gabriela Da Silva Xavier

ISBN 978-953-51-0640-1

Hard cover, 484 pages

Publisher InTech

Published online 05, June, 2012

Published in print edition June, 2012

Proteins are the work horses of the cell. As regulators of protein function, protein kinases are involved in the control of cellular functions via intricate signalling pathways, allowing for fine tuning of physiological functions. This book is a collaborative effort, with contribution from experts in their respective fields, reflecting the spirit of collaboration - across disciplines and borders - that exists in modern science. Here, we review the existing literature and, on occasions, provide novel data on the function of protein kinases in various systems. We also discuss the implications of these findings in the context of disease, treatment, and drug development.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Yoshiki Katayama (2012). Technologies for the Use of Protein Kinases into Medical Applications, Protein Kinases, Dr. Gabriela Da Silva Xavier (Ed.), ISBN: 978-953-51-0640-1, InTech, Available from: <http://www.intechopen.com/books/protein-kinases/technologies-for-the-use-of-protein-kinases-into-medical-applications>

INTECH
open science | open minds

InTech Europe

University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China

Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2012 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](https://creativecommons.org/licenses/by/3.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen