Optical probes and Sensors as peRSpective tools In epigenetics

Article *in* Bioorganic & medicinal chemistry · January 2017

DOI: 10.1016/j.bmc.2017.01.011

CITATIONS
0

READS
62

9 authors, including:

Robert Kaplánek
University of Chemistry and Technology, Pra…
40 PUBLICATIONS **323** CITATIONS
SEE PROFILE

Tomás Bríza
University of Chemistry and Technology, Pra…
17 PUBLICATIONS **244** CITATIONS
SEE PROFILE

Jarmila Kralova
Institute of Molecular Genetics AS CR
92 PUBLICATIONS **1,351** CITATIONS
SEE PROFILE

Vladimir Kral
University of Chemistry and Technology, Pra…
506 PUBLICATIONS **8,062** CITATIONS
SEE PROFILE

Accepted Manuscript

Optical probes and Sensors as peRSpective tools In epigenetics

Zdeněk Kejík, Robert Kaplánek, Martin Havlík, Tomá š Bř íza, Milan Jakubek, Jarmila Králová, Ivan Mikula, Pavel Martásek, Vladimír Král

PII: S0968-0896(16)30964-6
DOI: http://dx.doi.org/10.1016/j.bmc.2017.01.011
Reference: BMC 13490

To appear in: *Bioorganic & Medicinal Chemistry*

Received Date: 13 October 2016
Revised Date: 13 December 2016
Accepted Date: 11 January 2017

Please cite this article as: Kejík, Z., Kaplánek, R., Havlík, M., Bř íza, T., Jakubek, M., Králová, J., Mikula, I., Martásek, P., Král, V., Optical probes and Sensors as peRSpective tools In epigenetics, *Bioorganic & Medicinal Chemistry* (2017), doi: http://dx.doi.org/10.1016/j.bmc.2017.01.011

OPTICAL PROBES AND SENSORS AS PERSPECTIVE TOOLS IN EPIGENETICS

Zdeněk Kejík, Robert Kaplánek, Martin Havlík, Tomáš Bříza, Milan Jakubek, Jarmila Králová, Ivan Mikula, Pavel Martásek and Vladimír Král*

First Faculty of Medicine, Charles University, Kateřinská 32, 121 08 Prague 2, Czech Republic

*Corresponding author

E-mail: vladimir.kral@lf1.cuni.cz, Tel.: +420 325873004

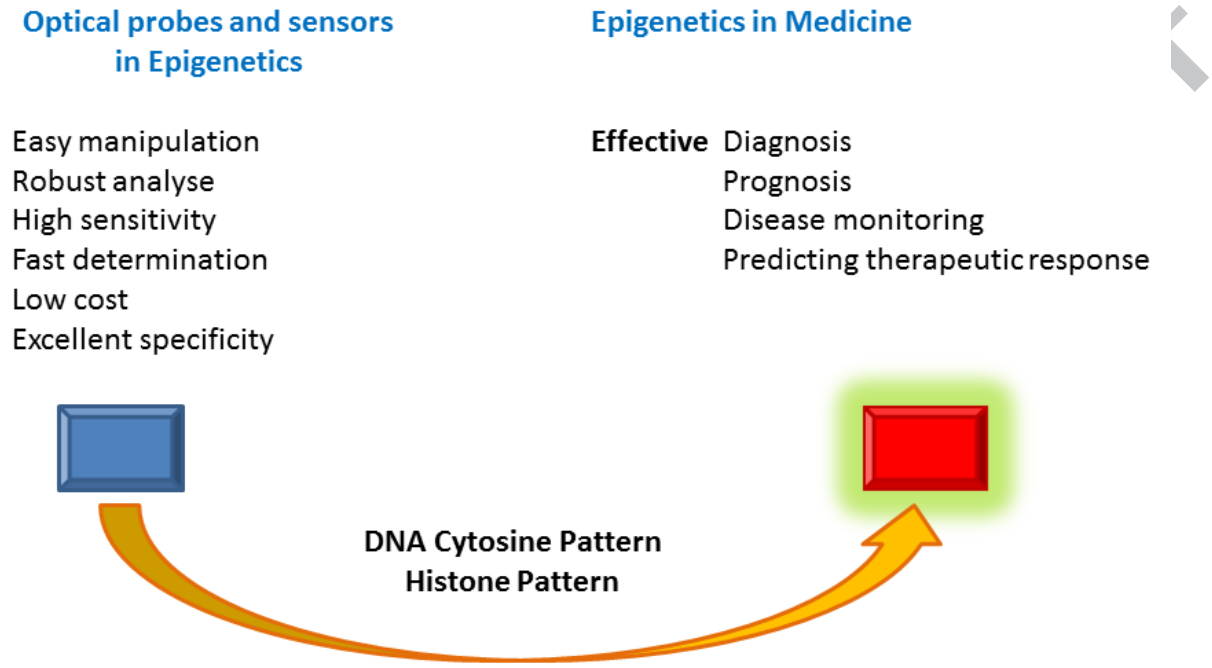
Abstract

Modifications of DNA cytosine bases and histone posttranslational modifications play key roles in the control of gene expression and specification of cell states. Such modifications affect many important biological processes and changes to these important regulation mechanisms can initiate or significantly contribute to the development of many serious pathological states. Therefore, recognition and determination of chromatin modifications is an important goal in basic and clinical research. Two of the most promising tools for this purpose are optical probes and sensors, especially colourimetric and fluorescence devices. The use of optical probes and sensors is simple, without highly expensive instrumentation, and with excellent sensitivity and specificity for target structural motifs. Accordingly, the application of various probes and sensors in the recognition and determination of cytosine modifications and structure of histones and histone posttranslational modifications, are discussed in detail in this review.

Keywords

Bioanalytical methods; DNA modified cytosine bases; Epigenetics; Histone pattern; Optical sensor; Probe

Graphical abstract



1. Introduction

The first part of this review describes the use of optical probes and sensor for determination of modifications to DNA cytosine bases, primarily through cytosine methylation. The second part of the review is devoted to the use of optical probes and sensors for identification of histones and histone posttranslational modification.

Currently, epigenetics is of great interest in the biological sciences. Epigenetic control of chromatin structure involves the complex interplay between genomic and environmental factors. Changes to chromatin can strongly influence important physiological processes, such as genomic imprinting, X-chromosome inactivation, heterochromatin formation, transcriptional regulation, and DNA damage repair [1]. Epigenetic modifications include changes in DNA accessibility (e.g., cytosine methylation) or chromatin structure (i.e., posttranslational modification of histones). Chromatin comprises nuclear DNA bound to histones. The DNA coils around octometric core of histones forming nucleosomes. The relationships among these components are currently being extensively investigated. Changes in the above mentioned mechanisms have been observed in many serious complex diseases, such as heart disease, diabetes, neurological disorders, and cancer. Many research groups have focused on finding unique epigenetic profile “signatures” typical for a studied pathological state. The determination and analysis of such signatures can provide useful information for the diagnosis, prognosis, and monitoring of disease, or for predicting the response to therapy. A number of recent studies demonstrated the potential use of epigenetic profiles for diagnostic applications [2-13].

Cytosine methylation, which occurs at approximately 5 % of total cytosine [14], plays an important role in the regulation of gene expression, imprinting, and X-chromosome inactivation. The addition of methyl group at the fifth position of cytosine in a CpG dinucleotide is the most frequent methylation mechanism and represents one of the most well studied examples of epigenetic regulation in biochemistry and biology. Methylation of CpG islands in gene promoters is associated

with gene silencing. Dysregulation of DNA methylation can have devastating consequences, including oncologic diseases, mental illnesses and neurological disorders [15, 16].

In recent years, many research groups identified DNA modification, especially cytosine modification, as a crucial process but the biological role of such modifications is not yet fully understood. Nevertheless a number of recent works indicated their importance for the control of gene expression [17]. It is assumed that hydroxymethylation in intragenic and proximal regions can lead to increased gene expression [18, 19]. DNA modifications, especially hydroxymethylation, are being explored for their critical roles in epigenetic regulation, as well as participation in other processes such as carcinogenesis, neurological abnormalities etc. [20]. For example, dysregulation of 5-hydroxymethylcytosine has been found to affect the pathogeneses of many serious diseases, including oncologic diseases [21] (gliomas, melanomas, hepatocellular carcinoma and others), and neurodegenerative disorders [22] (e.g., Alzheimer's disease and other dementias). The level of 5-hydroxyemethylcytosine in adult tissues is between 0.03 and 0.69 %, with the highest levels (0.4–0.7 %) found in the central nervous system [23]. In contrast, 5-formylcytosine and 5-carboxylcytosine are 10 to 1,000-fold less abundant than 5-hydroxymethylcytosine. Although the biological function of the molecule is not fully known, it was found that formylcytosine could form relatively stable Schiff base adducts with amine-containing compounds. In addition, thiol can form reversible adducts with formyl groups. It appears that these bases are not only intermediates, but can specifically stabilize interactions between DNA and regulatory proteins and other factors. For example, carboxylcytosine may be recognized by hydrogen bonds between its carboxyl group and the glutamine amid of RNA polymerase [24].

Histones [25] are small, basic proteins (molecular weight 12000–20000 Da) and are attached to the DNA of cell nuclei by ionic linkages. To understand the epigenetic role of histones, it is not sufficient to simply determine histone type and level, but it is also to obtain information about histones structure. The "histone code" refers to the numerous post-translational modifications (e.g., phosphorylation of serine and threonine, acetylation or ubiquitination of lysine, multiple types of

methylation of lysine and arginine, deimination of arginine ("citrullination"), cis–trans isomerization of proline, and others) [26]. The combination of the large variety of modification types and a large number of modification sites leads to millions of possible modification sets for a single histone tail [27, 28]. The most well-known histone modifications are acetylation and methylation [29]. Acetylation of terminal lysine residues is associated with increased gene activity, while deacetylation is associated with gene repression. Histone acetylations typically leads to closer and stronger interactions of histones with DNA and thus to the suppression of gene expression in the associated DNA. Nevertheless, interpretation of histone methylations is complicated. Methylation of histones results in different transcriptional outcomes depending upon the particular residue involved. For example, the addition of three methyl groups to lysine 27 of histone 3 is repressive, whereas methylation of lysine 4 in histone 3 is activating [30]. Some of these modifications, alone or in combination, play important roles in the pathogenesis of various human diseases, e.g. carcinogenesis, neurodevelopmental disorders [31] and autoimmune diseases [32]. Thus, specifications of relevant modifications have very promising potential use for medicinal research and clinical praxes.

Currently, cytosine methylation in DNA is determined using some advanced technique, such as combined bisulphite restriction analysis [33], high-throughput array-based DNA methylation profiling [34], and microarray-based allele-specific extension [35]. The application of these techniques is effective in mapping DNA-methylation patterns and has been used to generate much important knowledge in both basic and clinical research [36]. Identification of DNA methylation using the above techniques is effective as DNA methylation is highly stable, thus enabling detection in a great variety of samples collected using minimally invasive procedures (e.g., collection of saliva, plasma, serum, urine, semen, or stool) [37]. However, widespread use of these advanced techniques is hampered by several inherent disadvantages: they are time consuming, require a large amount of DNA or radioactive labelling, may lead to DNA damage, and result in false-positive results and significant error rates in PCR amplification. In addition, DNA-extraction and bisulphite conversion steps also lead

to unreliable precision [38]. These advanced techniques are also unable to discriminate between 5-methylcytosine and other cytosine modifications such as 5-hydroxymethylcytosine, 5-formylcytosine, and 5-carboxylcytosine [39]. As a result, other techniques, such as optical detection, have been recently intensively studied to overcome these limitations [40].

Two major proteomic methods can be used for the analysis of histone modifications: peptide and protein array-based assays, and affinity or chemical enrichment coupled with mass spectrometry [41-43]. Microarrays have the advantages of being rapid, flexible, and suitable for high-throughput applications. With modified peptide probes, many different analytes can be rapidly tested, and binding affinities and specificity can be at least semi-quantitatively determined. However, wide-spread application is relative expensive. The mass spectroscopy method provides fast quantitative and qualitative analysis of histones. However, this method is only available in specialized centres with large-scale mass spectrometer machines and trained staff with advanced bioinformatics skills. Therefore, new methods for histone determinations and analysis are being intensively explored. Some high impact studies have demonstrated that suitably designed optical probes and sensors can be applied to overcome the above mentioned obstacles [44, 45].

2. Optical probes and sensors used to determine DNA cytosine modifications

It is very well known, that the optical probes and sensors are one of the most promising and effective tools for the recognition and determination of important bioanalytes [46-55]. The application of optical probes and sensors enables easy and fast detection of DNA modifications without the need for expensive equipment or highly trained staff [56-60]. Some recent works have demonstrated that this approach is a very promising method for real time multi-detection of cytosine modifications (Fig. 1).

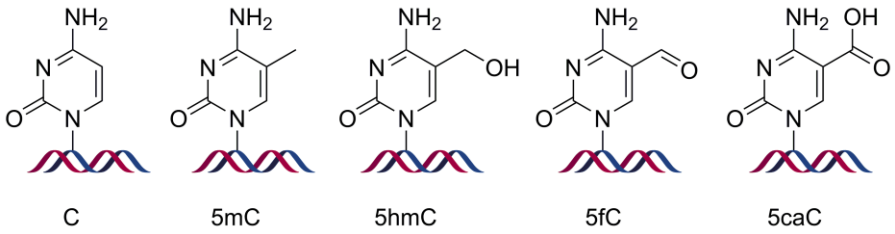


Figure 1. Cytosine bases.

For example, Hawk et al. prepared optical biosensors for the discrimination of cytosine, methylcytosine and hydroxymethylcytosine [58]. Another promising approach was showed by MacConaghy et al. study [61]. Design of their sensors was based on hydrogel matrix functionalised complementary oligonucleotide with incorporated photonic crystal. The binding target sequence was determined by optical diffraction (i.e., a change in the wavelength of peak diffraction). Interaction of methylated DNA sequences with the sensor displayed a smaller red shift relatively to non-methylated sequences. Clinical usability of this approach was demonstrated via the detection of a target methylated sequence from the DNA binding domain of the major tumour suppressor protein p53 at picomole concentrations of the target p53 sequence. Another effective strategy for the optical detection of cancer epigenetic markers, demonstrated by Nguyen et al., was based on the determination of the methylation state of cytosine islands with very low detection limit (~50 fM) [62].

2.1. Probes for labelling DNA cytosine modifications

One method for determining the presence of methylated cytosine in DNA is based on specific oxidation by osmium tetroxide (Fig. 2) [63, 64]. The oxidation leads to the formation of a stable methylcytosine glycol-osmate-bipyridine complex, thus making possible a clear distinction between methylated and unmodified DNA. The fluorescence label in the complex has a significantly lower fluorescence emission than free molecules. Application of this methodology was successfully used by Tanaka et al. to identify methylcytosine in the p53 sequence [65]. One drawback to this method is

that thymine can be sensitive to osmium oxidation resulting in false positive results. Nevertheless, this potential shortcoming can be overcome by suitable design. Oligonucleotide probes with a modified adenine located at the probe centre formed a 5-methylcytosine-selective osmium complex, whereas localization of fluorescence labelled adenine at the strand end led to high reactivity towards thymine as well as 5-methylcytosine. Application of this probe is shown on Fig. 3.

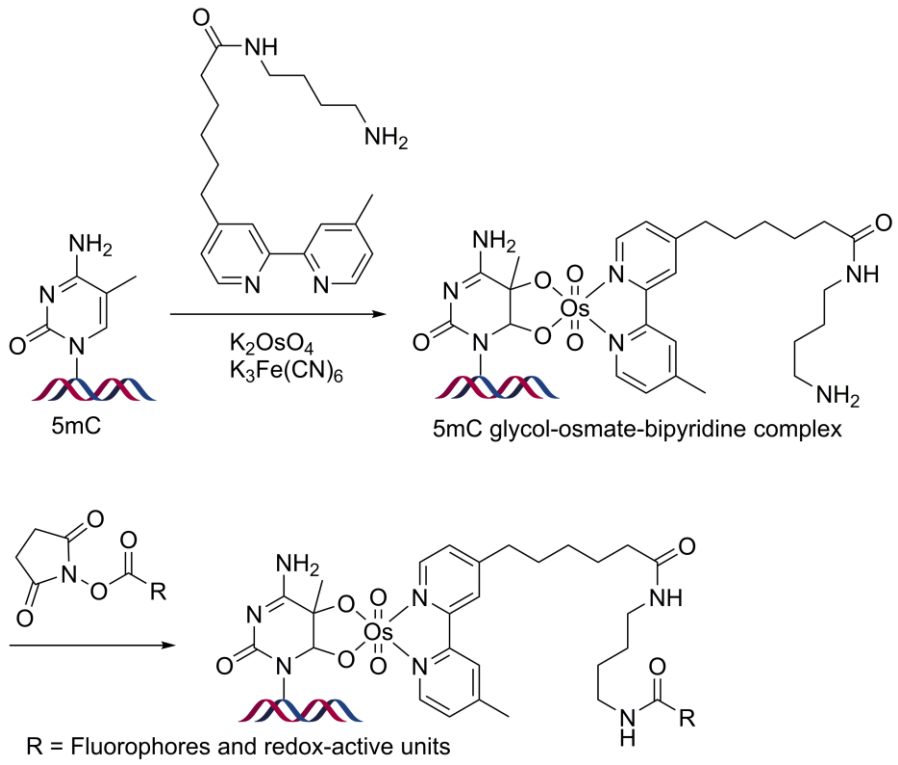


Figure 2. Schematic illustration of the labelling of methylcytosine via osmium complexation.

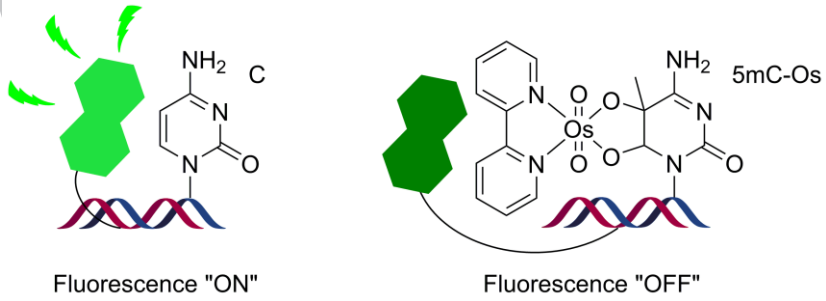


Figure 3. Fluorescence quenching by 5-methylcytosine-selective osmium complexation.

Another method for identification of methylated cytosine was demonstrated by Yamada et al. [66]. The authors designed oligonucleotide probes labelled with fluorescein and DABCYL groups (a quencher of fluorescein emission). This probe hybridizes to target oligonucleotides containing cytosine or methylcytosine. Photo-oxidation and subsequent alkali treatment of the duplex with mC induced exclusive strand cleavage at methylcytosine was used to generate characteristic oligonucleotide fragments. Finally, flap endonuclease-1 cleaved fluorescein labelled nucleotides. This method results in stronger fluorescence in fragments containing methylcytosine, whereas only weak fluorescence was detected from a corresponding nucleotide sequence with cytosine. The method is highly sensitive and capable of detecting methylcytosine in DNA at the subfemtomole level. A similar approach was used by Okamoto et al. to distinguish cytosine and methylcytosine in a DNA duplex [67]. Unlike previous probes, in this study endonuclease (HhaI) was used to cleave fluorescein-labelled oligonucleotides in the presence of unmodified cytosine.

Promising way for the recognition of 5-methylcytosine in DNA was showed by Heimer et al. [68]. Their method is based on the application of biotinylated methyl-CpG-binding protein on DNA chip carrying complementary sequence for the target DNA. This protein binding to DNA duplexes with methylated CpG sequence can be subsequently determinates by labelled streptavidin (known binding partner of biotin). Authors used its conjugates with Cy3 and eosin for the fluorescence and colorimetric detection, respectively. Colorimetric detection is realized by polymeration of added polyethylene glycol diacrylate in the presence of irradiation eosin. Red colour of chip can be observed after two minutes. Detection limits of sensor for the DNA sequence with one and two methylated CpG islands were 1 and 0.3 nmol/l, respectively. Significant advantage of this design based on microarray construction is easy customization to interrogate a target gene, or panel of genes. Beside this study also showed promised potential of this sensor design for the obtaining clinical important data (e.g. methylation of O6-methylguanine DNA methyltransferase).

An effective strategy for fluorescence labelling of 5-hydroxymethylcytosine in DNA was published by Michaeli et al. [69]. Selective functionalization of hydroxymethylcytosine is based on the application of specific T4 β-glucosyltransferase (β-GT) that can attach a glucose moiety onto the hydroxyl group of hydroxymethylcytosine resulting in a glucosylated nucleotide (Fig. 4). In the next step this glucosylated nucleotide is converted to azide, and the final hydroxymethylcytosine is labelled with Alexa Fluor® 647 (Molecular Probes, USA). As described, this method displayed extremely high sensitivity (0.002 % hydroxymethylcytosine).

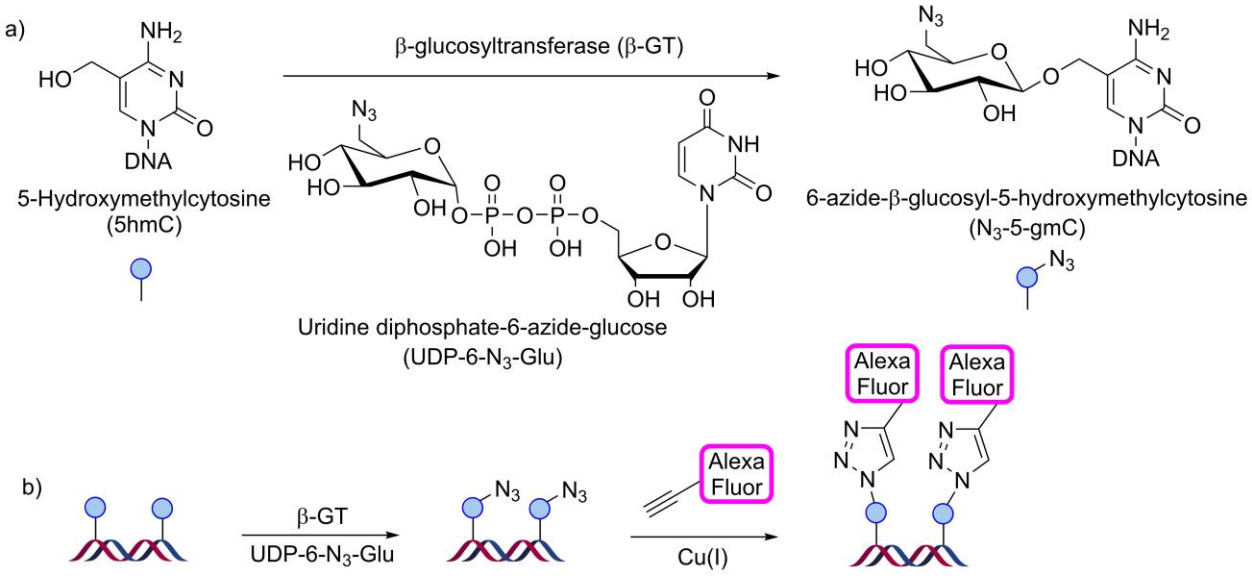


Figure 4. Scheme for selective fluorescent labelling of 5-hydroxymethylcytosine. The hydroxyl group of the hydroxymethylcytosine is glucosylated by β-GT to form 6-azide-β-glucosyl-5-hydroxymethylcytosine using UDP-6-N3-Glu as a substrate (a). A fluoresce alkyne probe is then attached to the azide group by click chemistry via the azide alkyne Huisgen cycloaddition (b).

2.2. Application of intercalators for the recognition and determination of DNA modification

DNA intercalators are promising agents for recognition and determination of other DNA epigenetic markers (i.e., hydroxymethyl-, formyl- and carboxylcytosine). For effective intercalation, the agent's structural motif should include large numbers of aromatic rings, low flexibility, high

molecular weight, and a small number of hydrogen bond acceptors [70]. Methylation, or other DNA modification, can significantly change DNA properties [71]. While cytosine methylation leads to increased melting temperatures (i.e., more closed DNA), hydroxy- and formylmethylation of cytosine results in a lower melting point and thereby easier entry for intercalators. The melting temperature of carboxylcytosine was found to be similar to the temperature of the unmodified DNA oligomer at neutral pH; at an acidic pH, the melting temperature was significantly decreased.

The usability of ethidium bromide (Fig. 5) for the determination of DNA methylation was tested by Bathaie et al. [72]. The authors observed that intercalation into methylated DNA resulted in significantly greater fluorescence emission in comparison with normal DNA.

Another agents based on the homodimeric thiazole orange dye (called TOTO and TOTO11) (Fig. 5) with preferential intercalation into methylated DNA were studied by Bunkenborg et al. [73].

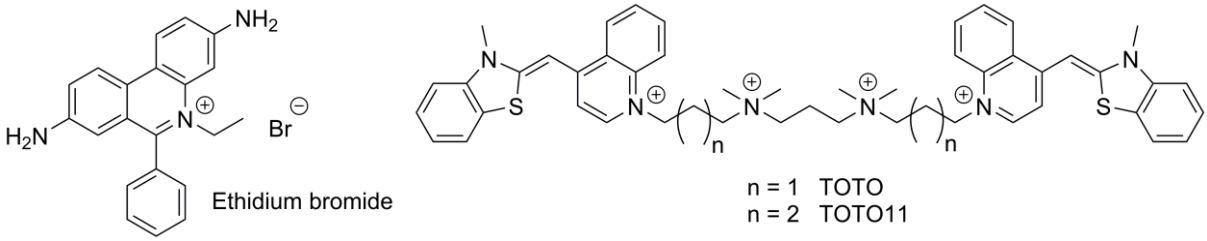


Figure 5. Chemical structure of ethidium bromide and bis-intercalating agents (TOTO and TOTO11)

These works inspired the application of DNA intercalators when designing fluorescent probes. The structural motifs of the probes combine the polyaromatic core of the intercalators and specific binding groups for target modification. For example, Xu et al. [59] prepared and tested conjugates of pyrene with hydrazines (i.e., aldehyde binding groups) for use in identifying 5-formylcytosine (Fig. 6). Authors found that the prepared probes displayed a high fluorescence response for symmetric 5-formylcytosine sites, which are typical for CpG islands in gene promoters [14]. Symmetric sites allowed for the incorporation of two probes, and the resulting dimer displayed

a strong blue fluorescence with a maximum emission intensity approximately 490 nm. Unsymmetrical sites could be determined by UV-Vis spectroscopy.

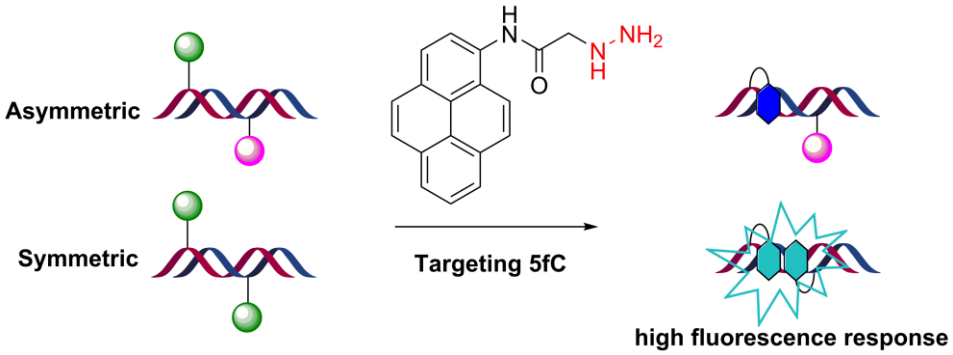


Figure 6. Pyrene–hydrazine probes used to distinguish symmetric formylcytosines from asymmetric formylcytosines.

In a similar approach Xu, et al. [74] identified 5-formylcytosine sites using 9-(hydrazinylmethyl)acridine with green fluorescence emission (Fig. 7). Unlike previously developed probes, 9-(hydrazinylmethyl)acridine binds covalently and is usable for determination of asymmetrical formylcytosine sites.

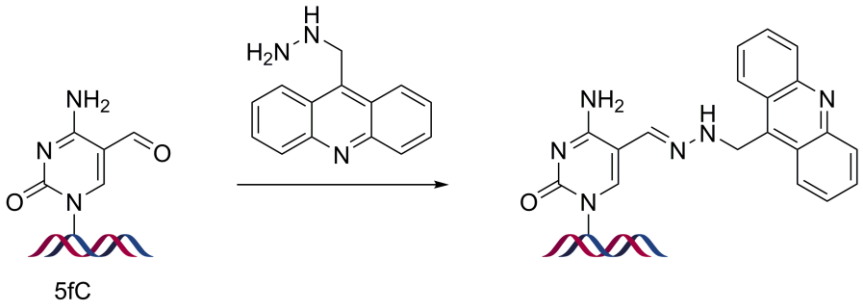


Figure 7. Selective labelling of 5-formylcytosine-containing DNA strand (F-C) by HMA.

2.2.1. Conjugates of oligonucleotides with intercalators for the determination of cytosine modification

One perspective way to determine methylation of DNA involves the combination of oligonucleotides with a suitable fluorophore with the sensitivity and specific fluorescence change necessary for distinguishing for 5-cytosine and 5-methylcytosine (Fig. 8). For example, Duprey et al. [75] developed an anthracene labelled oligonucleotide probe. The probe displayed positive and negative fluorescence changes in the presence of unmodified and methylated oligonucleotides (5'–CATTGAG-**C**-GAGTCCA–3'). This result demonstrated the usability of this method for the recognition and determination of DNA methylation.

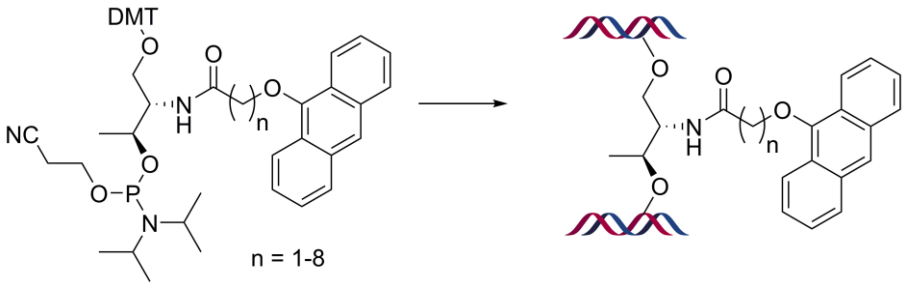


Figure 8. Structures of probes used by Duprey et al.

The full potential of this approach was uncovered in later work by Duprey et al., in which the authors discussed structural motifs used to distinguish cytosine, 5-methylcytosine, and 5-hydroxymethylcytosine in DNA (Fig. 9) [56]. The authors prepared a series of anthracene oligonucleotide probes containing various alkyl linker lengths (n = 3-7). Fluorescence changes, varying from +32 to -34 %, depended on the length of the alkyl probes and the cytosine modification of targeted nucleotide sequences. The sensing system was highly sensitive. Small changes in both linker length and cytosine methylation status having a remarkable effect on the emissivity of the anthracene tag upon duplex formation. Fluorescence emissions of probes with the shortest and longest linkers were decreased for all tested modifications. Other prepared probes (n = 4-6) displayed a positive change for nucleotide sequences with unmodified cytosine and a negative change for hydroxymethylcytosine. Hybridization of nucleotide sequences containing methylcytosine

led to decreased fluorescence emissions for some probes (n = 4) and increases emissions for others (n = 5 and 6).

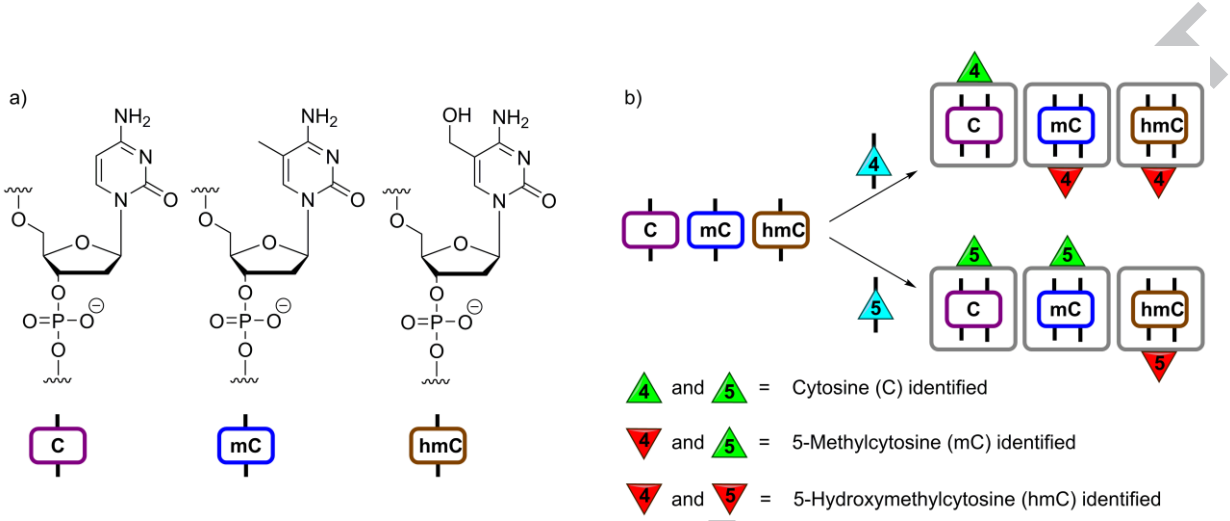


Figure 9. Determination of cytosine, methylcytosine and hydroxymethylcytosine by combination of anthracene labelled oligonucleotide probes. (a) Target nucleobases containing cytosine, 5-methylcytosine, and 5-hydroxymethylcytosine within a DNA strand. (b) Schematic representation of the fluorescent sensing assay, where the coloured boxes denote the different bases to be probed, the blue triangles represent the anthracene tagged probes P-4 and P-5, and the red and green triangles denote increased and decreased fluorescence emissions upon hybridization.

2.3. Metal nanoparticles for the determination of DNA methylation

Simple and universal methods for determination of DNA methylation state can be based on the application of metal nanoparticles, such as gold, or silver. Gold nanoparticles display fascinating optical properties, which are particularly ideal for colorimetric probes and sensors, (e.g., a high extinction coefficient) [76-82]. In addition, the position and absorbance of gold bands are very sensitive to interparticle distances resulting in large differences in spectroscopic properties and colour of aggregated and non-aggregated gold nanoparticles. It was observed that, in the presence some anionic polymers, aggregation of nanoparticles was suppressed and the colour of the solution changed from red and blue [83]. Application of this phenomena for the determination of DNA

methylation in a tumour suppressor gene APC (adenomatous polyposis coli) was shown by Ge at al. [57] (Fig. 10). Another important advantage of this method is the very low detection limit (80 fM).

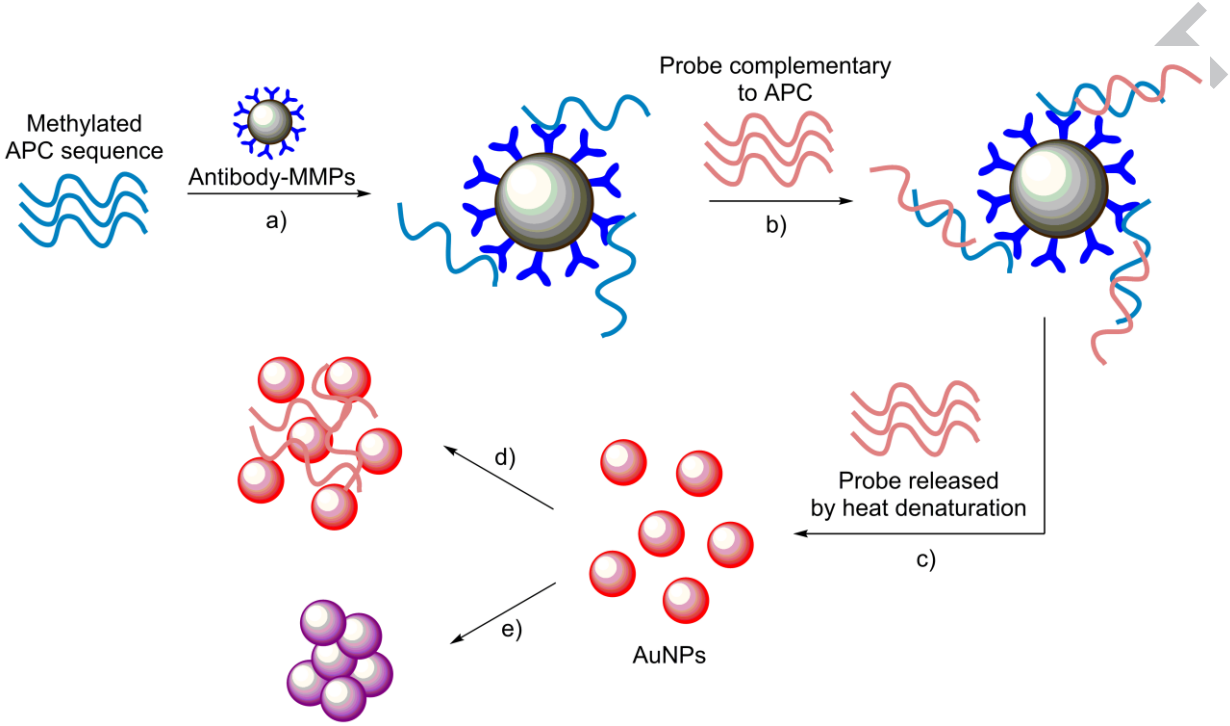


Figure 10. Generalized schematic showing detection of methylated APC sequences: (a) methylated APC (target) was captured by functionalized MMPs; (b) a complementary probe was added to form a partial duplex region with the target; (c) the captured probe was released from hybrid-conjugated microspheres by heat denaturation; (d) the released probe was added into an AuNP solution, and the colour of the solution changed slightly but remained red after the addition of NaCl; (e) unmethylated APC cannot be captured by functionalized MMPs, thus no probe was released into the AuNP solution, and the colour of the solution changed rapidly from red to purple.

Another promising method for the determination of DNA methylation is based on application of silver nanoclusters. This refers to a type of small nanoparticles composed of a few atoms with a size comparable to the Fermi wavelength of an electron that have distinguishing properties due to their quantum confinement [84-88]. Such nanoclusters display excellent optical properties, as strong photoluminescence, long term photostability and high emission rates. Silver nanoclusters also show

an affinity for DNA, with specific spectral responses for various DNA sequences, including cytosine rich DNA strands [86, 89-92]. These facts inspired Dadmehr at al. [93] to use nanoclusters to determine DNA methylation status. The authors observed that the solution of nanoparticles displayed intensive red fluorescence in the presence of unmethylated DNA, while interaction with methylated DNA led to strong fluorescence suppression. The authors found that silver nanoparticles could be used to identify methylated DNA in a wide concentration range (from 2.0×10^{-9} to 6.3×10^{-7} M) and with a low detection limit of 9.4×10^{-10} M. Silver nanoclusters can also be used for colourimetric determination because their solution changes from pink to yellow in the presence of methylated DNA. In addition, Dadmehr et al. demonstrated that nanoparticles could be used to determine DNA methylation status in clinical sample, including in human serum.

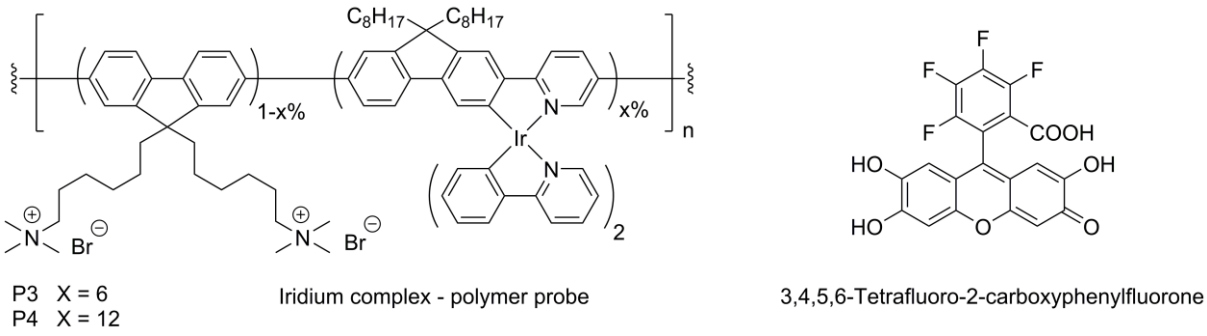
3. Optical probes for identification of histones and histone modifications

The importance of histones in the regulation of gene expression has led to the development of analytical methods, encompassing various types of probes and sensors to identify histone presence and posttranslational modifications [44, 94-97]; these methods have led to very promising results in histone determination and analysis. For example, Sekar et al. successfully tested bioluminescence biosensor for *in vivo* imaging of histone methylation [45].

3.1. Optical probes for the histone determination

Perspective fluorescence probes for histone recognition and determination were prepared by Sun et al. [96]. Structural motif of the probes was based on cationic conjugated polyelectrolytes containing an iridium complex (Fig. 11). The authors clearly showed that this probe displayed high selectivity for histones. Among nine tested proteins (α-fetoprotein, bovine serum albumin, thrombin, glucose oxidase, lysozyme, cytochrome c, haemoglobin and myoglobin), only histones enabled probe disaggregation and suppression of fluorescence resonance energy transfer. As such, the emission colour of the probe solution varied from red to lilac. In addition, this polymer probe displayed

extremely low limits of detection for histones (0.06 μM). The isoectrical points of the control proteins were between 4.7 and 11, while that of histones was 11.7. Disaggregation of probe aggregates is most probably caused by histone hydrophobicity and not by basicity. The probe alone is strongly hydrophobic, and histones also exhibit strong hydrophobicity; thus binding of probes to proteins can suppress FRET [98, 99].



P3 X = 6
P4 X = 12

Iridium complex - polymer probe

3,4,5,6-Tetrafluoro-2-carboxyphenylfluorone

Figure 11. Chemical structures of polymer and carboxyphenylfluorone histone probes.

Another perspective fluorescence probe (manganese complex of 3,4,5,6-tetrafluoro-2-carboxyphenylfluorone; Fig. 11) for determination of histones was studied by Miyachi et al. [100]. The formation of a supramolecular complex with histones was detected by fluorescence quenching of carboxyphenylfluorone. The probe interacted with basic proteins, such as histones, but not with other tested proteins (e.g., albumin, or γ-globulin). In this case, the basic character of histones was involved in the probe interaction. Poly(L-lysine) and poly(L-arginine) showed similar reactivity to histone. Selectivity of this probe for histones was very probably positively influenced by fluorination of the probes carboxyphenyl groups, which increases the affinity of carboxylate to lysine and arginine groups. Fluorine groups can also interact with histones itself. They can create non-covalent bonds [101] such as N-H···F, O-H···F, C-H···F, C–F···F–C and addition of a fluorine atom to π bond [102]. In the case of fluorinated aromates, π-π stacking with aromatic groups of proteins can also be observed. The advantage of this probe is its applicability for other analytic techniques used for histone determination, such as spectrophotometric and filtration methods [103, 104].

3.2. Probes and sensors for determination of histone modification

For the determination of some histone modifications, such as acetylation, a combination of acidic and hydrophobic histone probes could be used. It is well known, that amid de-acetylation leads to increased basicity and hydrophility, which leads to closer and stronger interactions of histones with DNA and resulting suppression of gene expression in the associated DNA. However, determination and interpretation of histone methylations is more complicated.

An elegant solution to this problem of histone analysis was shown by Ge et al. [105]. The authors constructed a sophisticated optical immunosensor using oligonucleotide-functionalized gold nanoparticles (Fig. 12) that combined an anti-histone antibody (histone 3) and antibody for the target histone modification (trimethylation of nine lysine of histone 3). This biosensor was highly sensitive and required minimal time and instrumental to generate. The authors clearly demonstrated that their sensor could be used to visually detect the target histone modification in 20 ng of a histone extract from HeLa cells in 15 min without other instrumentation. In addition, the principle of the enhanced strip biosensor could be applied to detect other types of histone modification.

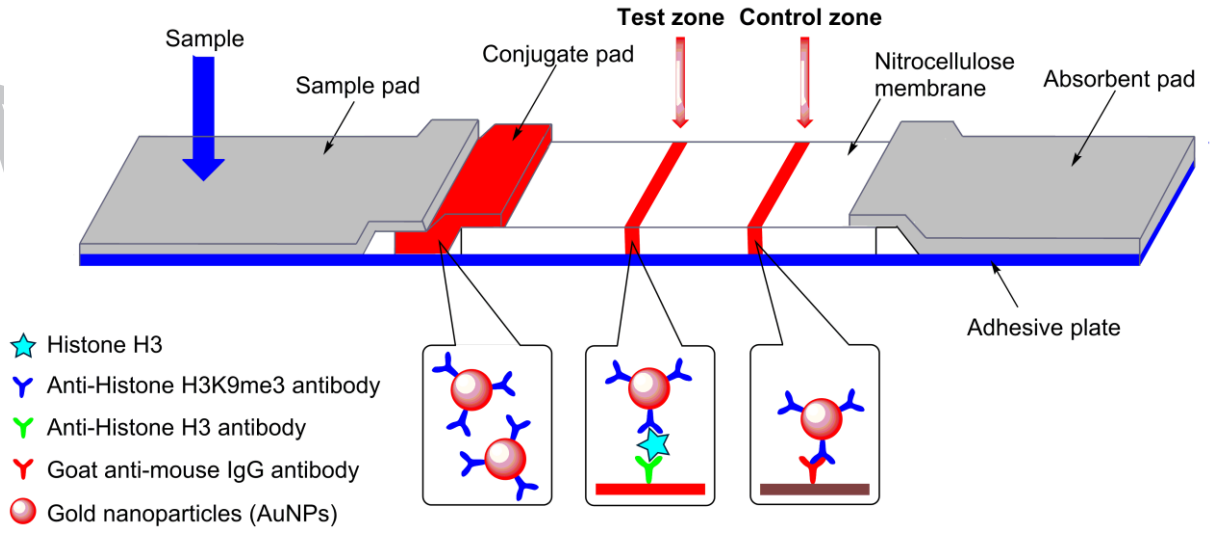


Figure 12. Schematic of an immunosensor used to detect histone methylation.

Perspective receptors for the detection of histones, or histone methylation, could be based on the anionic calixarenes (e.g., sulphonated, carboxylated and phosphonated calixarenes) [94, 106-108]. These oligomers are aromatic phenyl macrocycles with binding affinity for a variety of guests depending on the synthetic decorations of the macrocycle. Some recent work demonstrated that these macrocycles displayed significant selectivity for methylated lysine against unmethylated lysine and other amino acids [97, 109-112]. Their combination with rotaxanates can lead to the development of perspective structural motifs used for recognition of histones against other proteins, including basic ones such as lysozyme [94, 113]. A revolutionary approach for the analysis of histones post-translational modification was demonstrated by Minaker et al. [44]. Their idea is based on the application of chemical sensor arrays instead of highly specific probes targeting histone modifications. The principles of their system are depicted in Fig. 13. This design enables the combination of guests (e.g., sulphonatocalixarene) with various histones selectivity. A suitably designed system can produce a pattern of signals that is unique to each histone post-translational modification. The combination of three anionic guests (PSC4, PSC6 and PSC4(Br)) and two reporter dyes (LCG and PSP) was sufficient for the recognition and determination of various scales of histone structural motifs. These motifs included various degrees of lysine methylation with trimethyllysine marks on different peptide sequences and trimethylated groups at various positions in histones 3 and 4 (e.g., H3K4me3, H3K9me3, H3K27me3, H3K36me3, and H4K20me3). The excellent Minaker et al.'s idea was clearly demonstrated by determining individual post-translational modifications of H4R3 (R, RMe H4R3me2-a and H4R3me2-s). Despite their chemical similarity, these sensor arrays allowed for discrimination between these four related analytes, including between H4R3me2-a and H4R3me2-s peptides that differ only by the isomeric transposition of a single methyl group from one nitrogen to another on the same arginine side chain. Currently, a number of anionic calixarenes that bind to

cationic peptides and proteins [106, 109, 111] and a diverse set of dyes that are quenched by such hosts have been identified. This fact convincingly suggests that development of this methodology for the identification and determination of individual histone variants will continue.

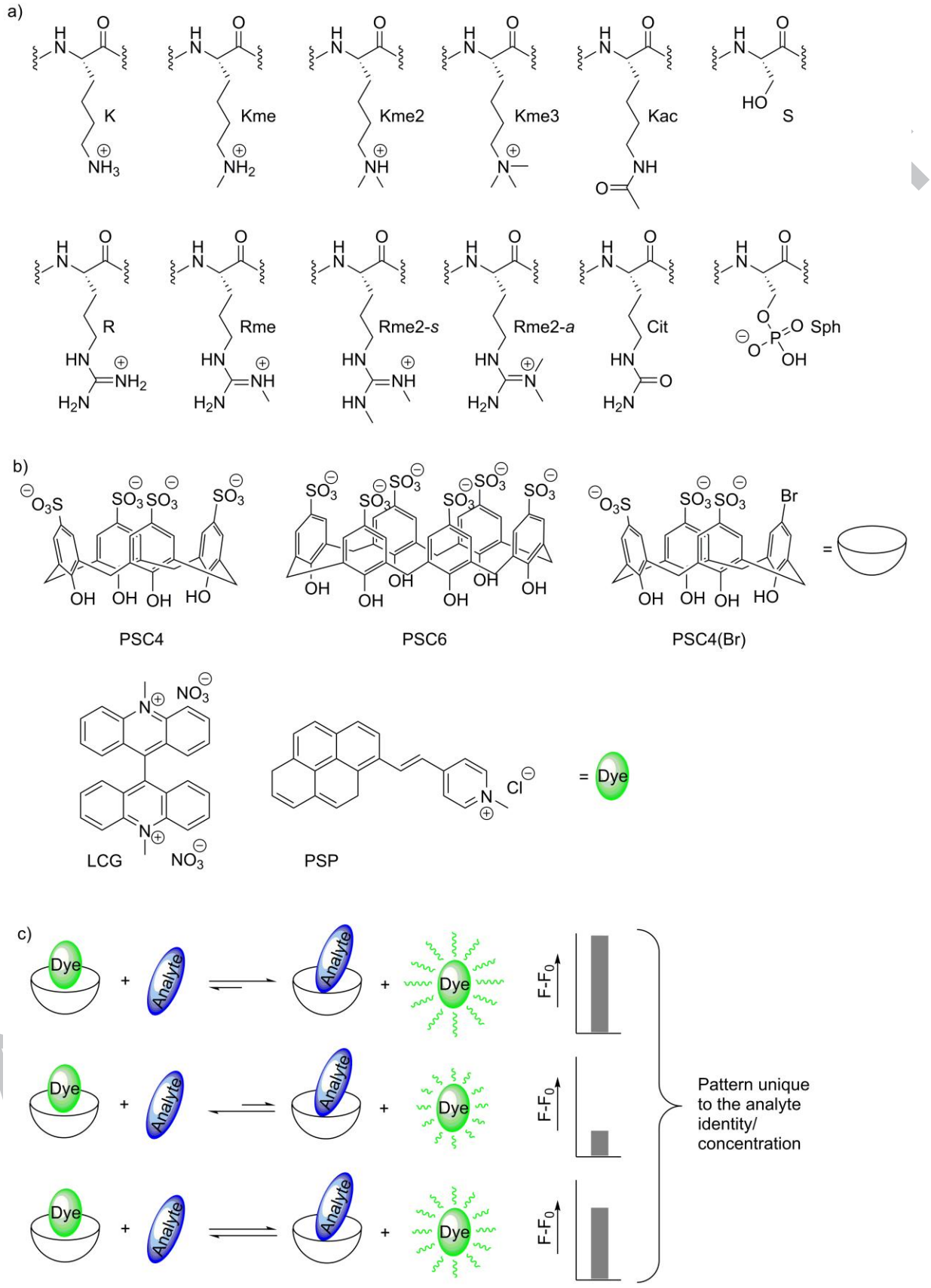


Figure 13. The structure of histone code analytes and basis for the performance of sensor components. (a) Structures of post-translationally modified residues used in this study. (b) Hosts and

fluorescent dyes used in the construction of the sensor arrays. (c) Generalized illustration of the use of data produced by chemical sensor arrays to identify and quantify analytes based on unique patterns.

Another important histone modification involves citrullination [114, 115]. Because citrullination eliminates the positive charges of histones, this modification may weaken the interaction between histones and DNA [116]. Fluorescence probes for labelling citrulline were designed by Wang et al. [117] (Fig. 14).

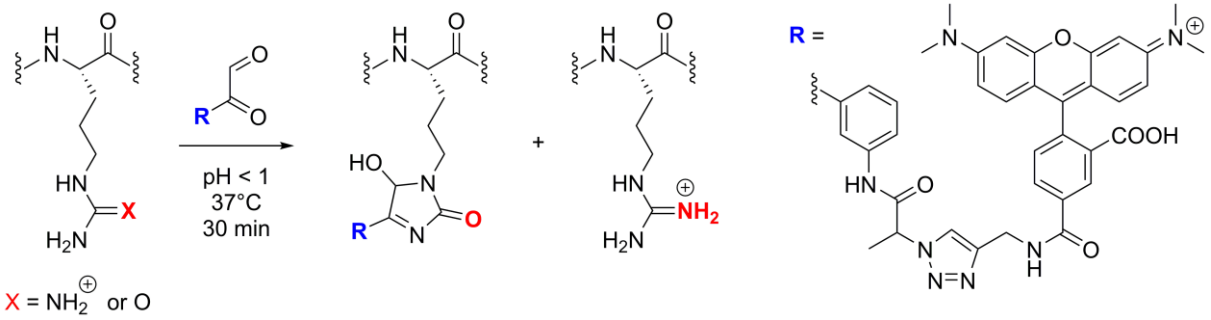


Figure 14. Rhodamine–phenylglyoxal (Rh–PG) probe chemoselectively labels citrulline over arginine at acidic pH.

4. Optical sensors for the determination of DNA and histone modifications in the living systems.

Because above modifications have key influence on the most processes in the living organisms, their determinations give significant data in the basic and medicinal research. However, due to the dynamic nature of these processes, their non-invasive detection directly in the living cells and experimental animals is highly requested. Bioluminescence sensors represent one of the few commonly available tools meet this demanding assignments. Unlike classical fluorescence probes and sensors are not inserted into cells itself, but only gene for their expression. This approach leads

to high sensitivity determination by reduction of the background fluorescence in the unmodified cells and tissues. In addition, because of the source of the signal is continually produced by cells, this method enable itself to repeat imaging of the same subjects or samples. This can be very useful for biological studies, especially imaging signal dynamics and for longitudinal studies. Repeat imaging of the same sample significantly reduces data variability and thereby number of experimental animals necessary for *in vivo* studies. Their design is based on the combination of specific binding domain for target histone, or DNA structure motif with enzyme generating bioluminescence emission such as luciferase [45, 118-120], fluorescence proteins [121].

Luciferase is a enzyme, which product oxyluciferin. It is a highly unstable singlet-excited compound, which emits light upon relaxation to its ground state. Luciferase assays are comparatively cheap and can be performed in a low or high-throughput fashion in a number of different contexts such as in cell lysates, live cells, or even in the living animals. Therefore luciferase biosensors for the detection of DNA modifications (mainly cytosine methylation in the CpG islands) are intensively development [119, 122]. For example, Badran and al. developed luminescence sensors for analysis global methylations [119]. Sensor was expressed in two independent parts, every part of sensor composed of protein binding domain for the methylated CpG island and half luciferase enzyme. After interaction binding domains with DNA sequences with more methylated CpG islands, luciferase strip go together and luminescence is observed. This study showed, that tested sensor can be used for the simple and rapid determination of the global methylation frequency in human native DNA from Hela cells. Authors prove, that sensors is effective tools for monitoring changes in HeLa DNA methylation levels in response to treatment DNA methyltransferase inhibitor with such as a 5-aza-2'-deoxycytidine.

Posttranslation modifications of protein in the living systems are widely study by luciferase bioluminescence sensors [118]. For this purpose can be used fusion proteins, which combine specific binding domain with luciferase splits. Because luciferase is divided on two parts, its enzyme activity is gone and bioluminescence cannot be product. It can be observed when binding domain of protein

sensor interact with target structure motif and protein conformation is change that bring two halves of luciferase together and reconstitute its enzymatic activity. For example Sekar et al. [120] developed luciferase sensors for *in vivo* imaging histone methylations (lysine 9 and 27 of histone 3). Authors clearly demonstrated that, these sensors can be effective used as very perspective tools for developments and study of epigenetic drugs such as inhibitors of histone methyltransferases.

Another strategy for analysis histone modification (acetylation of lysine 12 on histone 4) in living system was described by Kanno et al [121]. For this purpose histone and recognition protein (bromodomain protein) [123] were fused with yellow fluorescent protein and cyan fluorescent proteins, respectively. Own biolumiscence determination is carried out using fluorescence resonance energy transfer. This phenomenon is based on the ability of high-energy donor fluorophore (cyan fluorescent protein) to transfer energy directly to a lower energy acceptor fluorophore (yellow fluorescent protein). Because donor and acceptor molecules must be in close proximity (typically 10–100 Å) it is very useful technic for study protein-protein interactions. Authors clearly demonstrated that methodology can be simple used for mapping histone acetylation.

Similarly bioluminescence technics was described for imaging other protein modifications typical for histones (e. g. phosphorylation [124], ubiquitination [125] and others) in the living systems.

5. Conclusion

Optical probes and sensors are excellent perspective tools for the recognition and determination of DNA cytosine and histone modifications. This review presents and discusses various types of optical probes and sensors, primarily focussing on fluorescence and colorimetric approaches. The number of examples and cited works presented here demonstrate the potential efficacy of these approaches in the field of modern bioanalytical methods.

It is most probably, their implementation into diagnostic methodology will be high impact on the therapeutic efficiency (e.g. inexpensive design of personal therapy, precise prediction of diseases

risk, or more effective control therapy). These ambitious goals give rigorous demands on the future of the bioanalytical methods. It is known, that formation and evolution serious pathological statues such as oncological diseases are coupled with extensive changes in the chromozone structure (DNA cytosine and histone modification). Therefore future diagnostic methods could be analyse whole series of epigenetic markers (e.g. modification of CpG in the various gene promotors, analysis histone modification for individual amino acid). Until today, they were published many panel of genes as markers for number of serious diseases [126]. And in addition to the above, these methods should be fast, easy, robust and inexpensive. It may look like practically impossible. Other hand, successful application of fluorescence probes in the diagnostics and therapy indicated possible feasibility of this ambitious goal [127, 128]. In addition, few authors already published firstly optical probes and sensors, which could be meet the above requirements. For example, Duprey et al. [56] prepared antracene labelled oligonucleotide probes for simultaneous determination of the cytosine, 5-methylcytosine, and 5-hydroxymethylcytosine within a DNA strand. Heimer at al. [68] successful developed fluorescence and colorimetric sensor, which can be simple, customized to interrogate panel of genes. Maybe combination of these approaches could in the future lead to optical sensors for the determination 5-cytonosine modifications in the panel of genes. On the field of analysis of histone posttranslational modification, Minaker et al. [44] already described epoch-making sensor for the analysis of histone posttranslational modification pattern. Another way could be based on the incorporation of spectrally different fluorescence or luminescence nanoparticles such as quantum dots to the Ges methodology[105] for the more detailed analysis of histones posttranslational modification. This approach could be lead to obtaining more detailed information about histone structure thanks possibility simultaneous using of more type histone antibodies.

Other hot topic issue in the epigenetics potentially useful in the medicine are *in vitro* and *in vivo* imaging histone and DNA modifications. For this purpose, few research groups [45, 120, 121] successfully developed bioluminescence sensors based fluorescence proteins and luciferase assay. Another possible way could be based on the application of suitable designed nanoparticles systems.

In the present time, some authors such as Ma et al. [129] published works indicating high potential of this approach.

Interested topic with possible potential for diagnostic applications can be also development and study of tools and methods for the determination modifications of RNA bases [130, 131], mainly N^6-methyladenosine (most abundant modified base in the mammalian RNA). Because above discussed methodology is focused on the analysis 5-cytosine modification in the double-stranded nucleic acid, especially in the CpG islands. Transferability of these methods may not be simple. Nevertheless colorimetric method described by Ge at al. [57] could be relative simple applied for this purpose by using respectively antibody.

Based on the above we can expected that, in the near future, optical probes and sensors will be establish as standard tools for analytical methods in the epigenetics. We believe, that this trend lead to not only to development of perspective diagnostic methods, but even significantly participate to obtain new the knowledge in the biological sciences.

Acknowledgements

This work was supported by the Ministry of Education, Youth and Sports of the Czech Republic within the National Sustainability Program II (Project BIOCEV-FAR, LQ1604), under the projects ‘‘BIOCEV’’ (CZ.1.05/1.1.00/02.0109) and LH 14008. This work was further supported by Charles University in Prague (UNCE 204011/2012 and PRVOUK P24/LF1/3). The work was also supported by institutional projects of the Ministry of Agriculture of the Czech Republic by project QJ1610515.

References

1. A. Sadakierska-Chudy; R.M. Kostrzewa; M. Filip, A Comprehensive View of the Epigenetic Landscape Part I: DNA Methylation, Passive and Active DNA Demethylation Pathways and Histone Variants. Neurotoxicity Research 27 (2015) 84-97.
2. P.J. Couto; R.M. Millis, PET Imaging of Epigenetic Influences on Alzheimer's Disease. Int J Alzheimers Dis 2015 (2015) 575078.

3. A. Cribbs; M. Feldmann; U. Oppermann, Towards an understanding of the role of DNA methylation in rheumatoid arthritis: therapeutic and diagnostic implications. Therapeutic Advances in Musculoskeletal Disease 7 (2015) 206-219.
4. Y. Dong; H. Zhao; H. Li; X. Li; S. Yang, DNA methylation as an early diagnostic marker of cancer (Review). Biomed Rep 2 (2014) 326-330.
5. H. Heyn; M. Esteller, DNA methylation profiling in the clinic: applications and challenges. Nat. Rev. Genet. 13 (2012) 679-692.
6. H. Heyn; J. Mendez-Gonzalez; M. Esteller, Epigenetic profiling joins personalized cancer medicine. Expert Review of Molecular Diagnostics 13 (2013) 473-479.
7. D. Li; J.J. Shi; Y.H. Du; K.M. Chen; Z.P. Liu; B. Li; J. Li; F. Tao; H. Gu; C.Z. Jiang; J.M. Fang, Profiling Analysis of Histone Modifications and Gene Expression in Lewis Lung Carcinoma Murine Cells Resistant to Anti-VEGF Treatment. PLoS One 11 (2016) e0158214.
8. M. Prelog; D. Hilligardt; C.A. Schmidt; G.K. Przybylski; J. Leierer; G. Almanzar; N. El Hajj; K.P. Lesch; V. Arolt; P. Zwanzger; T. Haaf; K. Domschke, Hypermethylation of FOXP3 Promoter and Premature Aging of the Immune System in Female Patients with Panic Disorder? PLoS One 11 (2016) e0157930.
9. D. Gao; J.G. Herman; M. Guo, The clinical value of aberrant epigenetic changes of DNA damage repair genes in human cancer. Oncotarget 7 (2016) 37331-37346.
10. H. Shi; X. Chen; C. Lu; C.M. Gu; H.W. Jiang; R.W. Meng; X. Niu; Y.X. Huang; M.X. Lu, Association between P16(INK4a) Promoter Methylation and HNSCC: A Meta-Analysis of 21 Published Studies. PLoS One 10 (2015) e0122302.
11. R.G. Wallace; L.C. Twomey; M.A. Custaud; N. Moyna; P.M. Cummins; M. Mangone; R.P. Murphy, Potential Diagnostic and Prognostic Biomarkers of Epigenetic Drift within the Cardiovascular Compartment. Biomed Research International 2016 (2016) ID2465763.
12. L. Wang; Y. Cui; L. Zhang; J. Sheng; Y. Yang; G. Kuang; Y. Fan; Q. Zhang; J. Jin, The Silencing of CCND2 by Promoter Aberrant Methylation in Renal Cell Cancer and Analysis of the Correlation between CCND2 Methylation Status and Clinical Features. PLoS One 11 (2016) e0161859.
13. F.C. Zhou; M. Resendiz; C.L. Lo; Y. Chen, Cell-Wide DNA De-Methylation and Re-Methylation of Purkinje Neurons in the Developing Cerebellum. PLoS One 11 (2016) e0162063.
14. C.X. Song; C.Q. Yi; C. He, Mapping recently identified nucleotide variants in the genome and transcriptome. Nat. Biotechnol. 30 (2012) 1107-1116.
15. B. Sadikovic; K. Al-Romaih; J.A. Squire; M. Zielenska, Cause and consequences of genetic and epigenetic alterations in human cancer. Curr. Genomics 9 (2008) 394-408.
16. Y.L. Weng; R. An; J. Shin; H. Song; G.L. Ming, DNA Modifications and Neurological Disorders. Neurotherapeutics 10 (2013) 556-567.
17. C.X. Song; C. He, Potential functional roles of DNA demethylation intermediates. Trends Biochem. Sci. 38 (2013) 480-484.
18. M. Mellen; P. Ayata; S. Dewell; S. Kriaucionis; N. Heintz, MeCP2 Binds to 5hmC Enriched within Active Genes and Accessible Chromatin in the Nervous System. Cell 151 (2012) 1417-1430.
19. M.A. Hahn; R.X. Qiu; X.W. Wu; A.X. Li; H.Y. Zhang; J. Wang; J. Jui; S.G. Jin; Y. Jiang; G.P. Pfeifer; Q. Lu, Dynamics of 5-Hydroxymethylcytosine and Chromatin Marks in Mammalian Neurogenesis. Cell Reports 3 (2013) 291-300.
20. J. Liang; F. Yang; L. Zhao; C. Bi; B. Cai, Physiological and pathological implications of 5-hydroxymethylcytosine in diseases. Oncotarget (2016) doi: 10.18632/oncotarget.9281.
21. A. Vasanthakumar; L.A. Godley, 5-hydroxymethylcytosine in cancer: significance in diagnosis and therapy. Cancer Genetics 208 (2015) 167-177.

22. D.L.A. van den Hove; L. Chouliaras; B.P.F. Rutten, The Role of 5-Hydroxymethylcytosine in Aging and Alzheimer's Disease: Current Status and Prospects for Future Studies. Curr. Alzheimer Res. 9 (2012) 545-549.
23. D. Globisch; M. Munzel; M. Muller; S. Michalakis; M. Wagner; S. Koch; T. Bruckl; M. Biel; T. Carell, Tissue Distribution of 5-Hydroxymethylcytosine and Search for Active Demethylation Intermediates. PLoS One 5 (2010) e15367.
24. L.F. Wang; Y. Zhou; L. Xu; R. Xiao; X.Y. Lu; L. Chen; J. Chong; H.R. Li; C. He; X.D. Fu; D. Wang, Molecular basis for 5-carboxycytosine recognition by RNA polymerase II elongation complex. Nature 523 (2015) 621-625.
25. J.C. Harr; A. Gonzalez-Sandoval; S.M. Gasser, Histones and histone modifications in perinuclear chromatin anchoring: from yeast to man. EMBO Rep. 17 (2016) 139-155.
26. A. Sadakierska-Chudy; M. Filip, A Comprehensive View of the Epigenetic Landscape. Part II: Histone Post-translational Modification, Nucleosome Level, and Chromatin Regulation by ncRNAs. Neurotoxicity Research 27 (2015) 172-197.
27. K.E. Gardner; C.D. Allis; B.D. Strahl, OPERating ON Chromatin, a Colorful Language where Context Matters. J. Mol. Biol. 409 (2011) 36-46.
28. Z. Wang; C. Zang; J.A. Rosenfeld; D.E. Schones; A. Barski; S. Cuddapah; K. Cui; T.Y. Roh; W. Peng; M.Q. Zhang; K. Zhao, Combinatorial patterns of histone acetylations and methylations in the human genome. Nat. Genet. 40 (2008) 897-903.
29. A. Galvani; C. Thiriet, Nucleosome Dancing at the Tempo of Histone Tail Acetylation. Genes 6 (2015) 607-621.
30. Y. Kondo; L. Shen; A.S. Cheng; S. Ahmed; Y. Boumber; C. Charo; T. Yamochi; T. Urano; K. Furukawa; B. Kwabi-Addo; D.L. Gold; Y. Sekido; T.H.M. Huang; J.P.J. Issa, Gene silencing in cancer by histone H3 lysine 27 trimethylation independent of promoter DNA methylation. Nat. Genet. 40 (2008) 741-750.
31. C.N. Vallianatos; S. Iwase, Disrupted intricacy of histone H3K4 methylation in neurodevelopmental disorders. Epigenomics 7 (2015) 503-518.
32. M.A. Jeffries; A.H. Sawalha, Autoimmune disease in the epigenetic era: how has epigenetics changed our understanding of disease and how can we expect the field to evolve? Expert Rev Clin Immunol 11 (2015) 45-58.
33. Z.G. Xiong; P.W. Laird, COBRA: A sensitive and quantitative DNA methylation assay. Nucleic Acids Res. 25 (1997) 2532-2534.
34. R.A. Irizarry; C. Ladd-Acosta; B. Carvalho; H. Wu; S.A. Brandenburg; J.A. Jeddeloh; B. Wen; A.P. Feinberg, Comprehensive high-throughput arrays for relative methylation (CHARM). Genome Res. 18 (2008) 780-790.
35. A.E.K. Ibrahim; N.P. Thorne; K. Baird; N.L. Barbosa-Morais; S. Tavare; V.P. Collins; A.H. Wyllie; M.J. Arends; J.D. Brenton, MMASS: an optimized array-based method for assessing CpG island methylation. Nucleic Acids Res. 34 (2006) e136.
36. S. Kurdyukov; M. Bullock, DNA Methylation Analysis: Choosing the Right Method. Biology (Basel) 5 (2016) 3.
37. J. Paluszczak; W. Baer-Dubowska, Epigenetic diagnostics of cancer - the application of DNA methylation markers. Journal of Applied Genetics 47 (2006) 365-375.
38. E. Olkhov-Mitsel; B. Bapat, Strategies for discovery and validation of methylated and hydroxymethylated DNA biomarkers. Cancer Medicine 1 (2012) 237-260.

39. S. Ito; L. Shen; Q. Dai; S.C. Wu; L.B. Collins; J.A. Swenberg; C. He; Y. Zhang, Tet Proteins Can Convert 5-Methylcytosine to 5-Formylcytosine and 5-Carboxylcytosine. Science 333 (2011) 1300-1303.
40. C.X. Song; K.E. Szulwach; Y. Fu; Q. Dai; C.Q. Yi; X.K. Li; Y.J. Li; C.H. Chen; W. Zhang; X. Jian; J. Wang; L. Zhang; T.J. Looney; B.C. Zhang; L.A. Godley; L.M. Hicks; B.T. Lahn; P. Jin; C. He, Selective chemical labeling reveals the genome-wide distribution of 5-hydroxymethylcytosine. Nat. Biotechnol. 29 (2011) 68-72.
41. K.E. Moore; O. Gozani, An unexpected journey: Lysine methylation across the proteome. Biochimica Et Biophysica Acta-Gene Regulatory Mechanisms 1839 (2014) 1395-1403.
42. M. Amaya; A. Baer; K. Voss; C. Campbell; C. Mueller; C. Bailey; K. Kehn-Hall; E. Petricoin; A. Narayanan, Proteomic strategies for the discovery of novel diagnostic and therapeutic targets for infectious diseases. Pathogens and Disease 71 (2014) 175-187.
43. Y.K. Chae; M. Gonzalez-Angulo, Implications of Functional Proteomics in Breast Cancer. Oncologist 19 (2014) 328-335.
44. S.A. Minaker; K.D. Daze; M.C.F. Ma; F. Hof, Antibody-Free Reading of the Histone Code Using a Simple Chemical Sensor Array. J. Am. Chem. Soc. 134 (2012) 11674-11680.
45. T.V. Sekar; K. Foygel; R. Devulapally; R. Paulmurugan, Degron Protease Blockade Sensor to Image Epigenetic Histone Protein Methylation in Cells and Living Animals. ACS Chem. Biol. 10 (2015) 165-174.
46. C.I.L. Justino; T.A. Rocha-Santos; A.C. Duarte; T.A. Rocha-Santos, Review of analytical figures of merit of sensors and biosensors in clinical applications. TrAC Trends in Analytical Chemistry 29 (2010) 1172-1183.
47. T. Briza; Z. Kejik; I. Cisarova; J. Kralova; P. Martasek; V. Kral, Optical sensing of sulfate by polymethinium salt receptors: colorimetric sensor for heparin. Chem. Commun. (Cambridge, U. K.) (2008) 1901-1903.
48. Z. Kejik; T. Briza; J. Kralova; P. Martasek; V. Kral, Selective recognition of a saccharide-type tumor marker with natural and synthetic ligands: a new trend in cancer diagnosis. Anal. Bioanal. Chem. 398 (2010) 1865-1870.
49. L. Rodriguez-Lorenzo; L. Fabris; R.A. Alvarez-Puebla, Multiplex optical sensing with surface-enhanced Raman scattering: A critical review. Anal. Chim. Acta 745 (2012) 10-23.
50. Z. Kejik; K. Zaruba; D. Michalik; J. Sebek; J. Dian; S. Pataridis; K. Volka; V. Kral, Optical sensing system for ATP using porphyrin-alkaloid conjugates. Chem. Commun. (Cambridge, U. K.) (2006) 1533-1535.
51. O. Alpturk; O. Rusin; S.O. Fakayode; W.H. Wang; J.O. Escobedo; I.M. Warner; W.E. Crowe; V. Kral; J.M. Pruet; R.M. Strongin, Lanthanide complexes as fluorescent indicators for neutral sugars and cancer biomarkers. Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 9756-9760.
52. J. Kralova; J. Koivukorpi; Z. Kejik; P. Pouckova; E. Sievanen; E. Kolehmainen; V. Kral, Porphyrin-bile acid conjugates: from saccharide recognition in the solution to the selective cancer cell fluorescence detection. Org. Biomol. Chem. 6 (2008) 1548-1552.
53. Z. Kejik; T. Briza; J. Kralova; I. Mikula; P. Pouckova; P. Martasek; V. Kral, New method for recognition of sterol signalling molecules: methinium salts as receptors for sulphated steroids. Steroids 94 (2015) 15-20.
54. J.L. Sessler; V. Kral; T.V. Shishkanova; P.A. Gale, Cytosine substituted calix[4]pyrroles: Neutral receptors for 5 '-guanosine monophosphate. Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 4848-4853.

55. Z. Kejík; T. Bříza; M. Havlík; B. Dolenský; R. Kaplánek; J. Králová; I. Mikula; P. Martásek; V. Král, Specific ligands based on Tröger's base derivatives for the recognition of glycosaminoglycans. Dyes Pigm. 134 (2016) 212-218.
56. J.L.H.A. Duprey; G.A. Bullen; Z.Y. Zhao; D.M. Bassani; A.F.A. Peacock; J. Wilkie; J.H.R. Tucker, Single Site Discrimination of Cytosine, 5-Methylcytosine, and 5-Hydroxymethylcytosine in Target DNA Using Anthracene-Tagged Fluorescent Probes. ACS Chem. Biol. 11 (2016) 717-721.
57. C.C. Ge; Z.Y. Fang; J.H. Chen; J. Liu; X.W. Lu; L.W. Zeng, A simple colorimetric detection of DNA methylation. Analyst 137 (2012) 2032-2035.
58. R.M. Hawk; A.M. Armani, Label free detection of 5'hydroxymethylcytosine within CpG islands using optical sensors. Biosens. Bioelectron. 65C (2014) 198-203.
59. L.N. Zhou; Y. Liao; P.Y. Yin; Z.D. Zeng; J. Li; X. Lu; L.M. Zheng; G.W. Xu, Metabolic profiling study of early and late recurrence of hepatocellular carcinoma based on liquid chromatography-mass spectrometry. Journal of Chromatography B-Analytical Technologies in the Biomedical and Life Sciences 966 (2014) 163-170.
60. J. Yoon; M.K. Park; T.Y. Lee; Y.J. Yoon; Y. Shin, LoMA-B: a simple and versatile lab-on-a-chip system based on single-channel bisulfite conversion for DNA methylation analysis. Lab Chip 15 (2015) 3530-3539.
61. K.I. MacConaghy; D.M. Chadly; M.P. Stoykovich; J.L. Kaar, Label-free detection of missense mutations and methylation differences in the p53 gene using optically diffracting hydrogels. Analyst 140 (2015) 6354-6362.
62. A.H. Nguyen; S.J. Sim, Nanoplasmonic biosensor: Detection and amplification of dual bio-signatures of circulating tumor DNA. Biosens. Bioelectron. 67 (2015) 443-449.
63. K. Tanaka; K. Tainaka; T. Kamei; A. Okamoto, Direct labeling of 5-methylcytosine and its applications. J. Am. Chem. Soc. 129 (2007) 5612-5620.
64. K. Tanaka; K. Tainaka; A. Okamoto, Fluorescence quenching by methylcytosine-metal complexation. Nucleic Acids Symp Ser (Oxf) (2006) 139-140.
65. K. Tanaka; K. Tainaka; A. Okamoto, Methylcytosine-selective fluorescence quenching by osmium complexation. Bioorg. Med. Chem. 15 (2007) 1615-1621.
66. H. Yamada; K. Tanabe; S.I. Nishimoto, Fluorometric identification of 5-methylcytosine modification in DNA: Combination of photosensitized oxidation and invasive cleavage. Bioconjugate Chem. 19 (2008) 20-23.
67. A. Okamoto; K. Tanabe; I. Saito, Site-specific discrimination of cytosine and 5-methylcytosine in duplex DNA by peptide nucleic acids. J. Am. Chem. Soc. 124 (2002) 10262-10263.
68. B.W. Heimer; T.A. Shatova; J.K. Lee; K. Kaastrup; H.D. Sikes, Evaluating the sensitivity of hybridization-based epigenotyping using a methyl binding domain protein. Analyst 139 (2014) 3695-3701.
69. Y. Michaeli; T. Shahal; D. Torchinsky; A. Grunwald; R. Hoch; Y. Ebenstein, Optical detection of epigenetic marks: sensitive quantification and direct imaging of individual hydroxymethylcytosine bases. Chem. Commun. (Cambridge, U. K.) 49 (2013) 8599-8601.
70. Y. Gilad; H. Senderowitz, Docking Studies on DNA Intercalators. J. Chem. Inf. Model. 54 (2014) 96-107.
71. Q. Dai; P.J. Sanstead; C.S. Peng; D.L. Han; C. He; A. Tokmakoff, Weakened N3 Hydrogen Bonding by 5-Formylcytosine and 5-Carboxylcytosine Reduces Their Base-Pairing Stability. ACS Chem. Biol. 11 (2016) 470-477.

72. S.Z. Bathaie; F. Sedghgoo; A. Jafarnejad; B. Farzami; M. Khayatian, Spectroscopic studies of STZ-induced methylated-DNA in both in vivo and in vitro conditions. Spectrochimica Acta Part a-Molecular and Biomolecular Spectroscopy 71 (2008) 803-808.
73. J. Bunkenborg; M.M. Stidsen; J.P. Jacobsen, On the sequence selective bis-intercalation of a homodimeric thiazole orange dye in DNA. Bioconjugate Chem. 10 (1999) 824-831.
74. L. Xu; Y.C. Chen; S. Nakajima; J. Chong; L.F. Wang; L. Lan; C. Zhang; D. Wang, A chemical probe targets DNA 5-formylcytosine sites and inhibits TDG excision, polymerases bypass, and gene expression. Chemical Science 5 (2014) 567-574.
75. J.L.H.A. Duprey; Z.Y. Zhao; D.M. Bassani; J. Manchester; J.S. Vyle; J.H.R. Tucker, Detection of DNA base variation and cytosine methylation at a single nucleotide site using a highly sensitive fluorescent probe. Chem. Commun. (Cambridge, U. K.) 47 (2011) 6629-6631.
76. N.L. Rosi; C.A. Mirkin, Nanostructures in biodiagnostics. Chem. Rev. 105 (2005) 1547-1562.
77. P.K. Jain; K.S. Lee; I.H. El-Sayed; M.A. El-Sayed, Calculated absorption and scattering properties of gold nanoparticles of different size, shape, and composition: Applications in biological imaging and biomedicine. J. Phys. Chem. B 110 (2006) 7238-7248.
78. K.S. Lee; M.A. El-Sayed, Gold and silver nanoparticles in sensing and imaging: Sensitivity of plasmon response to size, shape, and metal composition. J. Phys. Chem. B 110 (2006) 19220-19225.
79. V. Kral; J. Sotola; P. Neuwirth; Z. Kejik; K. Zaruba; P. Martasek, Nanomedicine - Current status and perspectives: A big potential or just a catchword? Chem. Listy 100 (2006) 4-9.
80. P. Rezanka; K. Zaruba; V. Kral, Application of modified nanoparticles in analytical chemistry. Chem. Listy 101 (2007) 881-885.
81. J.J. Giner-Casares; M. Henriksen-Lacey; M. Coronado-Puchau; L.M. Liz-Marzán, Inorganic nanoparticles for biomedicine: where materials scientists meet medical research. Materials Today 19 (2016) 19-28.
82. D. Ghosh; N. Chattopadhyay, Gold and silver nanoparticles based superquenching of fluorescence: A review. J. Lumin. 160 (2015) 223-232.
83. B.K. Jena; C.R. Raj, Optical sensing of biomedically important polyionic drugs using nano-sized gold particles. Biosens. Bioelectron. 23 (2008) 1285-1290.
84. I. Diez; M. Pusa; S. Kulmala; H. Jiang; A. Walther; A.S. Goldmann; A.H.E. Muller; O. Ikkala; R.H.A. Ras, Color Tunability and Electrochemiluminescence of Silver Nanoclusters. Angewandte Chemie-International Edition 48 (2009) 2122-2125.
85. Z. Shen; H.W. Duan; H. Frey, Water-soluble fluorescent Ag nanoclusters obtained from multiarm star poly(acrylic acid) as "molecular hydrogel" templates. Adv. Mater. (Weinheim, Ger.) 19 (2007) 349-352.
86. T. Vosch; Y. Antoku; J.C. Hsiang; C.I. Richards; J.I. Gonzalez; R.M. Dickson, Strongly emissive individual DNA-encapsulated Ag nanoclusters as single-molecule fluorophores. Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 12616-12621.
87. J.G. Zhang; S.Q. Xu; E. Kumacheva, Photogeneration of fluorescent silver nanoclusters in polymer microgels. Adv. Mater. (Weinheim, Ger.) 17 (2005) 2336-2340.
88. L.B. Zhang; E.K. Wang, Metal nanoclusters: New fluorescent probes for sensors and bioimaging. Nano Today 9 (2014) 132-157.
89. C.I. Richards; S. Choi; J.C. Hsiang; Y. Antoku; T. Vosch; A. Bongiorno; Y.L. Tzeng; R.M. Dickson, Oligonucleotide-stabilized Ag nanocluster fluorophores. J. Am. Chem. Soc. 130 (2008) 5038-5039.
90. J.T. Petty; J. Zheng; N.V. Hud; R.M. Dickson, DNA-templated Ag nanocluster formation. J. Am. Chem. Soc. 126 (2004) 5207-5212.

91. C.M. Ritchie; S.T. Gibbs; J.R. Kiser; R.M. Dickson; J.T. Petty, PHYS 512-DNA templated Ag nanocluster formation. Abstr. Pap. Am. Chem. Soc. 232 (2006) 512-PHYS.
92. E.G. Gwinn; P. O'Neill; A.J. Guerrero; D. Bouwmeester; D.K. Fygenson, Sequence-dependent fluorescence of DNA-hosted silver nanoclusters. Adv. Mater. (Weinheim, Ger.) 20 (2008) 279-283.
93. M. Dadmehr; M. Hosseini; S. Hosseinkhani; M.R. Ganjali; R. Sheikhnejad, Label free colorimetric and fluorimetric direct detection of methylated DNA based on silver nanoclusters for cancer early diagnosis. Biosens. Bioelectron. 73 (2015) 108-113.
94. O. Hayashida; N. Ogawa; M. Uchiyama, Surface recognition and fluorescence sensing of histone by dansyl-appended cyclophane-based resorcinarene trimer. J. Am. Chem. Soc. 129 (2007) 13698-13705.
95. O.F. Sanchez; D. Williamson; L.T. Cai; C.L. Yuan, A sensitive protein-based sensor for quantifying histone acetylation levels. Talanta 140 (2015) 212-218.
96. P.F. Sun; X.M. Lu; Q.L. Fan; Z.Y. Zhang; W.L. Song; B. Li; L. Huang; J.W. Peng; W. Huang, Water-Soluble Iridium(III)-Containing Conjugated Polyelectrolytes with Weakened Energy Transfer Properties for Multicolor Protein Sensing Applications. Macromolecules 44 (2011) 8763-8770.
97. S. Tabet; S.F. Douglas; K.D. Daze; G.A.E. Garnett; K.J.H. Allen; E.M.M. Abrioux; T.T.H. Quon; J.E. Wulff; F. Hof, Synthetic trimethyllysine receptors that bind histone 3, trimethyllysine 27 (H3K27me3) and disrupt its interaction with the epigenetic reader protein CBX7. Bioorg. Med. Chem. 21 (2013) 7004-7010.
98. I.B. Kim; A. Dunkhorst; U.H.F. Bunz, Nonspecific interactions of a carboxylate-substituted PPE with proteins. A cautionary tale for biosensor applications. Langmuir 21 (2005) 7985-7989.
99. B.D. Silverman, The hydrophobicity of the H3 histone fold differs from the hydrophobicity of the other three folds. J. Mol. Evol. 60 (2005) 354-364.
100. K. Miyachi; M. Hoshino; H. Kadobayashi; K. Moriyama; M. Asano; T. Yamaguchi; Y. Fujita, Fluorophotometric Determination of Histone with 3,4,5,6-Tetrafluoro-2-carboxyphenylfluorone-Manganese(II) Complex and Its Characterization. Chem. Pharm. Bull. 61 (2013) 379-383.
101. J.C. Biffinger; H.W. Kim; S.G. DiMagno, The polar hydrophobicity of fluorinated compounds. ChemBioChem 5 (2004) 622-627.
102. D. Chopra, Is Organic Fluorine Really "Not" Polarizable? Cryst. Growth Des. 12 (2012) 541-546.
103. H. Kadobayashi; T. Nakamori; T. Yamaguchi; Y. Fujita, Spectrophotometric determination of DNA binding protein, histone, with 3,4,5,6-tetrafluoro-2-carboxyphenylfluorone and manganese(II). Chem. Lett. 33 (2004) 610-611.
104. H. Tominaga; H. Kadobayashi; T. Yamaguchi; S. Kamino; M. Nishimura; Y. Fujita, Determination of histone by membrane filter collection with 3,4,5,6-tetrafluoro-2-carboxyphenylfluorone and manganese(II). Bunseki Kagaku 55 (2006) 127-131.
105. C.C. Ge; L.X. Yu; Z.Y. Fang; L.W. Zeng, An Enhanced Strip Biosensor for Rapid and Sensitive Detection of Histone Methylation. Anal. Chem. 85 (2013) 9343-9349.
106. R. Ludwig, Calixarenes for biochemical recognition and separation. Microchim. Acta 152 (2005) 1-19.
107. S. Friedman; S. Kolusheva; R. Volinsky; L. Zeiri; T. Schrader; R. Jelinek, Lipid/polydiacetylene films for colorimetric protein surface-charge analysis. Anal. Chem. 80 (2008) 7804-7811.
108. R. Zadmard; T. Schrader, Nanomolar protein sensing with embedded receptor molecules. J. Am. Chem. Soc. 127 (2005) 904-915.

109. K.D. Daze; M.C.F. Ma; F. Pineux; F. Hof, Synthesis of New Trisulfonated Calix[4]arenes Functionalized at the Upper Rim, and Their Complexation with the Trimethyllysine Epigenetic Mark. Org. Lett. 14 (2012) 1512-1515.
110. R.E. McGovern; B.D. Snarr; J.A. Lyons; J. McFarlane; A.L. Whiting; I. Paci; F. Hof; P.B. Crowley, Structural study of a small molecule receptor bound to dimethyllysine in lysozyme. Chemical Science 6 (2015) 442-449.
111. K.D. Daze; T. Pinter; C.S. Beshara; A. Ibraheem; S.A. Minaker; M.C.F. Ma; R.J.M. Courtemanche; R.E. Campbell; F. Hof, Supramolecular hosts that recognize methyllysines and disrupt the interaction between a modified histone tail and its epigenetic reader protein. Chemical Science 3 (2012) 2695-2699.
112. K.D. Daze; F. Hof, The Cation-pi Interaction at Protein-Protein Interaction Interfaces: Developing and Learning from Synthetic Mimics of Proteins That Bind Methylated Lysines. Acc. Chem. Res. 46 (2013) 937-945.
113. O. Hayashida; M. Uchiyama, Rotaxane-type resorcinarene tetramers as histone-sensing fluorescent receptors. Org. Biomol. Chem. 6 (2008) 3166-3170.
114. S. Wang; Y. Wang, Peptidylarginine deiminases in citrullination, gene regulation, health and pathogenesis. Biochim. Biophys. Acta 1829 (2013) 1126-1135.
115. C. Anzilotti; F. Pratesi; C. Tommasi; P. Migliorini, Peptidylarginine deiminase 4 and citrullination in health and disease. Autoimmun Rev 9 (2010) 158-160.
116. M. Leshner; S. Wang; C. Lewis; H. Zheng; X.A. Chen; L. Santy; Y. Wang, PAD4 mediated histone hypercitrullination induces heterochromatin decondensation and chromatin unfolding to form neutrophil extracellular trap-like structures. Front Immunol 3 (2012) 307.
117. K.L. Bicker; V. Subramanian; A.A. Chumanevich; L.J. Hofseth; P.R. Thompson, Seeing Citrulline: Development of a Phenylglyoxal-Based Probe To Visualize Protein Citrullination. J. Am. Chem. Soc. 134 (2012) 17015-17018.
118. M.C. Lake; E.O. Aboagye, Luciferase fragment complementation imaging in preclinical cancer studies. Oncoscience 1 (2014) 310-325.
119. A.H. Badran; J.L. Furman; A.S. Ma; T.J. Comi; J.R. Porter; I. Ghosh, Evaluating the global CpG methylation status of native DNA utilizing a bipartite split-luciferase sensor. Anal. Chem. 83 (2011) 7151-7157.
120. T.V. Sekar; K. Foygel; J.G. Gelovani; R. Paulmurugan, Genetically Encoded Molecular Biosensors To Image Histone Methylation in Living Animals. Anal. Chem. 87 (2015) 892-899.
121. T. Kanno; Y. Kanno; R.M. Siegel; M.K. Jang; M.J. Lenardo; K. Ozato, Selective recognition of acetylated histones by bromodomain proteins visualized in living cells. Mol. Cell 13 (2004) 33-43.
122. X. Huang; R. Narayanaswamy; K. Fenn; S. Szpakowski; C. Sasaki; J. Costa; P. Blancafort; P.M. Lizardi, Sequence-specific biosensors report drug-induced changes in epigenetic silencing in living cells. DNA Cell Biol. 31 Suppl 1 (2012) S2-10.
123. O. Matangkasombut; S. Buratowski, Different sensitivities of bromodomain factors 1 and 2 to histone H4 acetylation. Mol. Cell 11 (2003) 353-363.
124. J.M. Spotts; R.E. Dolmetscht; M.E. Greenberg, Time-lapse imaging of a dynamic phosphorylation dependent protein-protein interaction in mammalian cells. Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 15142-15147.
125. Z.L. Chen; Y.W. Zhong; Y. Wang; S. Xu; Z. Liu; I.V. Baskakov; M.J. Monteiro; M. Karbowski; Y.X. Shen; S.Y. Fang, Ubiquitination-Induced Fluorescence Complementation (UiFC) for Detection of K48 Ubiquitin Chains In Vitro and in Live Cells. PLoS One 8 (2013.

126. H. Akhavan-Niaki; A.A. Samadani, DNA Methylation and Cancer Development: Molecular Mechanism. Cell Biochem. Biophys. 67 (2013) 501-513.
127. H. Kobayashi; M. Ogawa; R. Alford; P.L. Choyke; Y. Urano, New Strategies for Fluorescent Probe Design in Medical Diagnostic Imaging. Chem. Rev. 110 (2010) 2620-2640.
128. N.J. Harlaar; W. Kelder; A. Sarantopoulos; J. Bart; G. Themelis; G.M. van Dam; V. Ntziachristos, Real-time near infrared fluorescence (NIRF) intra-operative imaging in ovarian cancer using an alpha(v)beta(3-)integrin targeted agent. Gynecol. Oncol. 128 (2013) 590-595.
129. Y. Ma; Y. Bai; H. Mao; Q. Hong; D. Yang; H. Zhang; F. Liu; Z. Wu; Q. Jin; H. Zhou; J. Cao; J. Zhao; X. Zhong; H. Mao, A panel of promoter methylation markers for invasive and noninvasive early detection of NSCLC using a quantum dots-based FRET approach. Biosens. Bioelectron. 85 (2016) 641-648.
130. W.V. Gilbert; T.A. Bell; C. Schaening, Messenger RNA modifications: Form, distribution, and function. Science 352 (2016) 1408-12.
131. R.F. Wu; D.H. Jiang; Y.Z. Wang; X.X. Wang, N (6)-Methyladenosine (m(6)A) Methylation in mRNA with A Dynamic and Reversible Epigenetic Modification. Mol. Biotechnol. 58 (2016) 450-459.

Highlights

- Optical probes and sensor for determination of modifications to DNA cytosine bases
- Optical probes for identification of histones and histone modification
- Epigenetic modifications include changes in DNA accessibility
- Determination of DNA methylation by sensors
- Use of epigenetic profiles for diagnostic applications