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ORIGINAL PAPER

Sensing of cocaine using polarized optical microscopy by exploiting the conformational changes of an aptamer at the water/liquid crystal interface

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Abstract

Liquid crystals (LCs) have the ability to transduce and amplify a molecular stimulus into optical signals due to their elastic and birefringence properties. An aptamer-based LC sensor for cocaine is described here. 3-Morpholinopropanesulfonic acid with amphipathic structure was used to establish recognition sites at a water/LC interface for the detection of cocaine. The cocaine-binding aptamer is formed at the interface. The conformation of the aptamer undergoes a change on binding cocaine, and this triggers the LCs anchoring transition from homeotropic to planar. Binding can also be detected by polarized optical microscopy. The fluorescence spectroscopy and circular dichroism results are used to prove that the conformation of aptamer changed from a hairpin structure to a special three-way junction structure on binding of cocaine at the interface. The assay works in the 1 nM to 10 μM cocaine concentration range and is specific.

Keywords Liquid crystal biosensor · Conformation transition · Optical images · Anchoring transition · Optical signal · Fluorescence spectra · Circular dichroism · Average grayscale value · Urine

Introduction

Cocaine is initially used as a topical anesthetic, but at the same time, it is also one of the most dangerous and illegal drugs. The abuse of cocaine causes instantaneous and overwhelming stimulation on central nervous system and severe side effects on human, such as organ damage and human immunodeficiency [1]. Up to now, many analytical techniques for cocaine detection have been developed, such as colorimetry [2], electrochemical analysis [3, 4], fluorometry [5–7] and surface-enhanced Raman spectroscopy (SERS) [8], and some can even be used in real sample detection, which acquires impressive results. However, these methods always tend to require complicated response substrates or labeled species for the generation of signals. Thus, it is still a compelling goal to achieve real-time and sensitive detection for cocaine.

Liquid crystals (LCs) are sensitive functional soft materials with unique physical properties such as surface anchoring, elasticity and birefringence. These properties make the LCs useful as the responsive substrates in the biochemical analysis field. Furthermore, there is no need for complicated laboratory instruments while the LCs are chosen as substrates, and the changes in optical signals of LCs can be even observed with bare eyes. At the same time, it also shows a potential real-time visualization detecting means.

Some works have been devoted to the research of LC biosensors, and several methods have been developed for the detection of peptides, enzymes, biomolecules, phospholipids, proteins and DNA [9–13]. It is successfully demonstrated that some microscopic chemical signals at the aqueous/LC interface are directly transduced and amplified due to the elasticity of LC molecules. And the orientation transition of LC molecules can be observed by the optical signals owing to its birefringence properties. In our group, some detection methods for DNA have also been developed [14, 15]. In Tan's work, the streptavidin alkaline phosphatase was immobilized on a DNA

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probe and the ascorbic acid was catalyzed by it. A signal-enhanced LC biosensing technique was exploited and even the 0.1 pM target DNA could be successfully detected [14]. In addition, a new DNA detection method based on the formed DNA dendrimers by hybridization chain reaction at aqueous/LC interface was reported to detect p53 mutation gene segment [15]. And it has been concluded that the responsive mechanism of the DNA-mediated aqueous/LC biosensor is mainly related to the change of the electric-dipole coupling between the adsorbed DNA and LC molecules at aqueous/LC interface [16]. This change is caused by the hybridization reaction between the DNA and its corresponding sequences or the conformational change of DNA.

Aptamers are single-stranded nucleic acids with high affinity and specificity for the target molecules and they can fold into defined tertiary structures due to the binding at specific sites. These make them become an indispensable biological recognition element in chemical and biological applications [17]. The special characteristic of aptamers, target-induced conformational change, has been used in many analytical methods. And some detection approaches have been developed for signal amplification in biosensing field base on the characteristic [18–21]. These novel biosensors exhibit high sensitivity and selectivity because their structures are more remarkable flexibility and convenience. Beyond that, the aqueous/LC biosensors based on the significant conformational changes of aptamers have more advantages, such as label-free, inexpensive instruments and visualization.

Inspired by these, we have designed a novel aptamer-based LC biosensor based on the structure transformation of aptamer at aqueous/LC interface for the detection of cocaine. The schematic representation of the biosensor is shown in Fig. 1. The aptamer with special hairpin structure is designed to induce ordered orientation of the LC molecules at aqueous/LC interface. In the presence of cocaine, the aptamer will transform to the three-way junction structure owing to the binding with target. This transformation effectively induces the anchoring transition of LCs from homeotropic to planar orientation and further generates a sign-on optical signal from black to bright, and the optical signal can be observed by polarizing optical microscope (POM). The aptamer-based LC biosensor can directly detect cocaine in solution without any labels and realize the highly sensitive determination of analyte. The signal sign-on biosensing strategy based on the aptamer structure transformation and the specific recognition between aptamer and target is used to improve the sensitivity of the method, and it will enrich the application areas of the LC biosensing technique.

Experimental methods

Materials

Premium grade glass slides were obtained from Yanchenfeizhou Glassware Company (http://www.ycfzbl.com). Copper grids of 200-mesh (20 μm in thickness, 100 μm in grid spacing, and 30 μm bar width) were obtained from Zhongjingkeyi Technology Co., Ltd. (http://www.emcn.com.cn). Cocaine, 4-cyano-4′-pentylbiphenyl (5CB), 3-morpholinopropanesulfonic acid (MOPs), sodium nitrate, ascorbic acid, glycine, D(+)-glucose•H_2O, uric acid and N, N-dimethyl-N-aminopropyltrimethoxysilyl chloride (DMOAP) were purchased from Sigma Aldrich (http://www.sigmaaldrich.com). Dopamine, theophylline and tropinone were purchased from Adamas (https://adamas.company.lookchem.cn). Green-DNA Dye (SGI, 10,000) and oligonucleotides were purchased from Sangon Biotech Co., Ltd. (http://www.sangon.com). All regents were of analytical purity and were prepared with ultra-pure water. The urine

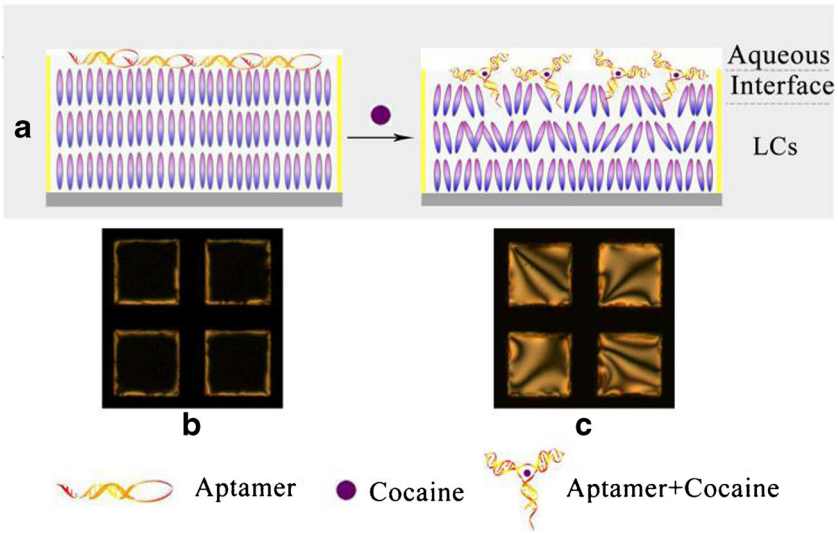

Fig. 1 The schematic diagram of the aptamer-based LC biosensor. After the aptamer binding with cocaine, the conformation of aptamer at the aqueous/LC interface is changed from a hairpin structure to a special three-way junction structure. This triggers the LCs anchoring transition from homeotropic to planar and the sign-on optical signal can be obtained by POM images

sample was obtained from the healthy human morning urine. Ultrapure water (18.25 MΩ•cm) was used throughout the experiment. The oligonucleotide sequence of cocaine aptamer is:

R:5'-CACAGGAGACAAGGAAAATCCTTCAAT GAAGTGGGTCTCCAATCTAACTTGTCTCCTGT GTGTG-3′.

Construction of the LC cells

The glass slides substrates were cleaned in piranha solution (70% H_2SO_4, 30% H_2O_2) at 80 °C thermostat water bath for 2 h, and were then rinsed three times with copious amounts of ultrapure water, blew dry with nitrogen and drying in thermostat oven at 110 °C for 1.5 h. When the glass slides cooled down, they were dipped in a certain concentration of DMOAP solution for 10 min, rinsed and dried in the same way, and finally heated at 110 °C for 45 min in the oven. The copper grids were put on the silanization-glass substrate, 5CB was then transferred into the copper grids by capillary forces to form LC film, and the LC cells were obtained. The LC cells were immersed in the MOPs buffer when detecting.

Measurements

The LC cells were observed by Nikon Eclipse polarization microscope (Nikon ECLIPS 50i POL) and the optical images were captured by MSX20 digital camera, and images were analyzed by Image J to determine the average grayscale value. The fluorescence spectra photometer (Hitachi F7000) and CD spectrometer (MOS-500) were used to verify the conformational change of aptamer structure. The fluorescent dye SGI is a nucleic acid dye that can specifically identify the double helix DNA and emit strong fluorescence. And the fluorescence intensity of SGI is changed with the conformation change of the aptamer.

Results and discussion

Effects of the MOPs on LC orientation

The aqueous/LC interface biosensing has been studied gradually, and it mostly researches the anchoring transition of LCs at the interface modified by surfactant (such as sodium dodecyl sulfate). However, in previous study, we find that the MOPs can induce the planar-to-homeotropic orientational transition of LCs with the change of pH value [16], the result shows that the effect of MOPs buffer is similar to surfactants at the aqueous/LC interface. When the pH of MOPs buffer is 7.2, the value is favorable for the formation of stable electrical double layer interface between the aqueous and the LC films, and this is also the critical pH of LCs orientation transition. Thus, the MOPs buffer was used and expected to form a sensitive aqueous/LC biosensing interface. (The statement of the MOPs structure has further shown in the Electronic Supporting Material.)

For this aqueous/LC interface sensing system, the ionic strength of the aqueous phase is another important influence factor to the LCs orientation. This is related to the complex LC anchoring mechanisms, which involves a range of noncovalent interactions existing in the LCs and biomolecules, such as electrostatic [22], coordination [23] and steric effects at the interface [24, 25]. The certain ionic strength is conducive to the hybridization of DNA because it can screen the electrostatic repulsion between the DNA chains. So, the suitable condition of the ionic strength is optimized. The average grayscale value of optical images is used to express the level of the LCs anchoring transition. Respective data are given in the Electronic Supporting Material (Fig. S1). The following experimental conditions were found to give best results: (a) The best sample pH value is 7.2; (b) The optimal ionic strength is 10 mM. This state is suitable for the aqueous/LC interface sensing system to induce the reorientation of the LCs.

Effects of the molecular conformation of aptamer on LC orientation

It has been proven that the DNA hybridization can well induce a dynamic LCs orientation transition from planar to homeotropic in the presence of complementary strand at the surfactant-laden aqueous/LC interface [9]. The repulsive force between the hydrophobic nucleobases and the hydrophobic LCs changes with the DNA hybridization reaction, which is essentially relevant to the decreasing of the exposed nucleobases [24]. The reorientation of the LCs also can be changed by the aptamer conformation reconstruction at similar interface condition, which mostly is the formation or change of special secondary structure at aptamer ligand binding events (e.g. G-quadruplex, triplex, i-motif and hairpin structure) [26–28]. So, to verify whether the binding events between the cocaine and its aptamer can directly induce a homeotropic-to-planar anchoring transition of LCs, the optical images of the LC cells are investigated. The aptamer was firstly pretreated in thermostat water bath at 50 °C for 3 h. And a series of cocaine solution with different concentrations were mixed with the same concentration of aptamer solution respectively, then the mixing solution was added into the LC cells for detection.

In Fig. 1, the fully black background POM optical images are observed when the aptamer solution with certain concentration is added into the LC cells, and after the addition of cocaine, the images change from black to bright. As we all know, the designed hairpin structure has a double-stranded

region, in which the formation of hydrogen bonding increases the negative charge density of aptamer. This breaks the balance of the electrostatic interaction between the MOPs and the LCs. And the new electrical double layer between LCs and dsDNA generate an electric field, which induces the homeotropic orientation of the LC molecules. But with the addition of cocaine, the original aptamer structure is changed and forms the three-way junction structure, and the electric field is disturbed by this special spatial structure, which induces the homeotropic-to-planar transition of LC molecules. These results well demonstrate that the aptamer-cocaine binding events successfully induce the LCs anchoring transition at aqueous/LC interface. That is the reorientation along with the orientation coexistence between homeotropic and planar regions in a short time.

However, the optical images of LC cells are bright when the aptamer with hairpin structure is not pretreated, which is different from the experimental phenomena described above. It is probably considered that the irregular structure of aptamers without pretreatment has some random hydrogen bonds and part single chains, which cannot form an electric field and induce the homeotropic orientation the LC molecules. The phenomenon also signifies that the structure is not completely in conformity with the hairpin structure designed in advance. So, the difference in the aptamers structures with and without pretreatment is explored by the fluorescence spectra and the CD spectra in Fig. S2. And the results show that the hairpin structure uniformly forms after the pretreatment.

Effects of responsive detection of cocaine

The responsiveness of the aptamer-based LC biosensor for cocaine was also examined. The experiment results show that the POM images are uniformly dark in a large concentration range from 1 nM to 10 μM, that is to say, the orientation of the LC molecules is homeotropic in this concentration range. It is supposed that this LC biosensor can be used to detect cocaine in different concentration ranges by using the specific concentration aptamer. The influence of aptamer concentration on the detection capability of this LC biosensor to cocaine was investigated. In Fig. S4, the POM images with different bright textures are distinctly observed and change gradually from dark to bright with the increased cocaine concentration when the aptamer concentration is 10 nM. However, the detection range for cocaine is only from 1 nM to 100 nM and it is narrow for the cocaine analysis. So, the higher aptamer concentration (100 nM and 1 μM) are used to further extend the detection range. It is found that the minimum detectable concentration of the cocaine has no change, but differently, the maximum value increases with the increased aptamer concentration. Specifically, the detection range of cocaine is from 1 nM to 1 μM at the aptamer concentration is 100 nM (Fig. S3), and it can be enlarged to 10 μM when the concentration is 1 μM (Fig. 2a). These results

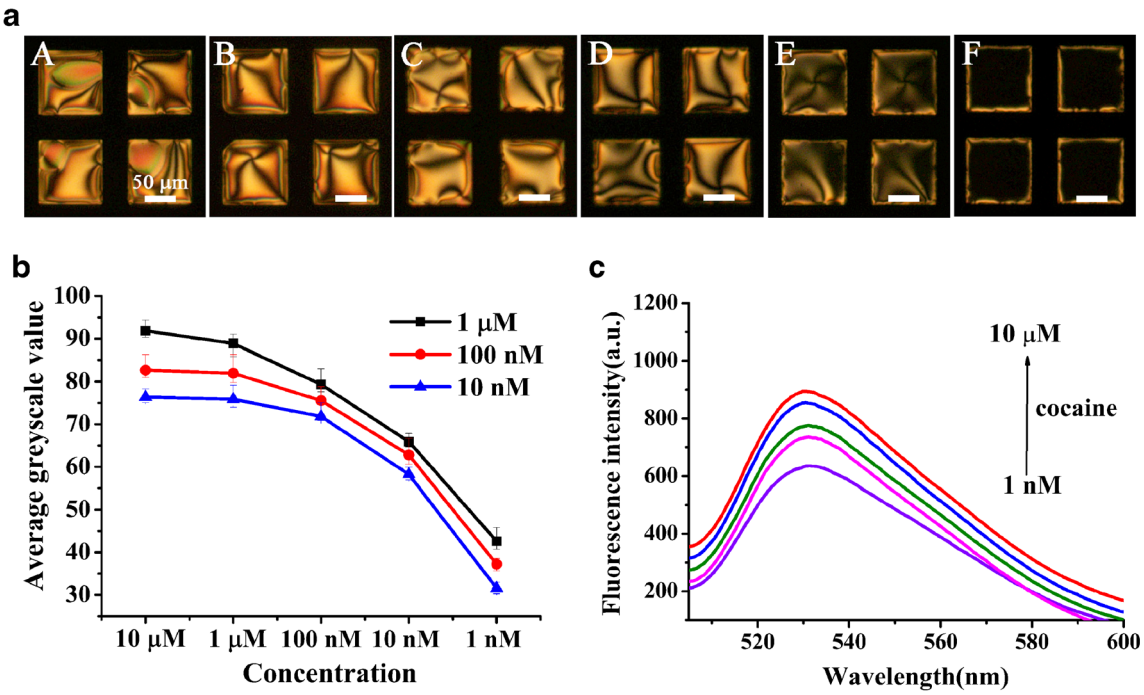

Fig. 2 **a** The POM images of LC cells at different cocaine concentration when the aptamer concentration is 1 μM. The concentration of cocaine is respectively 10 μM, 1 μM, 100 nM, 10 nM, 1 nM and 0 nM from A to F. **b** The average grayscale value of POM images at different cocaine concentration while the aptamer concentration is respectively 1 μM (square), 100 nM (circle) and 10 nM (triangle). **c** The fluorescence spectra of different cocaine concentrations when the aptamer concentration is 1 μM. The cocaine concentration is respectively 1 nM, 10 nM, 100 nM, 1 μM and 10 μM

indicate that the response range of cocaine can be enlarged by increasing the aptamer concentration and it reaches the largest when the aptamer concentration is 1 μM, which is further verified by the average grey value and the fluorescence spectra (Fig. 2b-c). And it is also found that the response concentration of cocaine has no further changes when the higher aptamer concentration is used, such as, 10 μM.

In Fig. 2b, the average grayscale value of POM images decreases with the reduced cocaine concentration and a small platform appears at the beginning of line when the aptamer concentration is 10 nM and 100 nM. This is because the reaction between the cocaine and the aptamer reaches saturation and will not further induce the LC molecules transition. Additionally, under the same cocaine concentration, the average grayscale value increases with the increasing aptamer concentration, indicating that the increased aptamer concentration has certain amplified effects on the responsiveness of the LC biosensor. The fluorescence spectra data are also shown in Fig. 2c. The obvious distinction can be observed that the fluorescence intensity decreases with the reduced concentration of cocaine, and this change tendency is well matched with the POM images and the average greyscale value. The lowest limit of detection for the biosensor is 1 nM. Comparing with the results reported by some literatures (Table S1 in Supporting information), this value is superior to most available cocaine optical biosensors. It can be concluded that the sensitive detection of cocaine in different range becomes possible by this method.

Selectivity of the aptamer biosensor

The selectivity is a vital parameter for the application of the designed aptamer-based LC biosensor. The main interfering substances in urine detection (eg. uric acid, glucose, glycine, ascorbic acid and urea) and similar chemical structures as cocaine (eg. edopamine, theophylline and tropinone) were used to investigate the selectivity of this biosensor. In Fig. 3a, it is clearly distinguished that the POM images are bright when cocaine existed and they are black in other solution. The results show that these interfering substances not notably induce the reorientation of LCs and the average greyscale value also verifies it. In Fig. 3b, the grey intensity is about 80 in the presence of the cocaine and it is obviously higher than those of the other analytic substances, which is corresponding with the POM optical images. All these data show that the aptamer-based LC biosensor displays a remarkable specificity for cocaine due to the inherent selectivity of aptamers for the target molecule.

Sample detection of the aptamer biosensor

The cocaine detection in urine is of great significance, and it also used as the initial screening for drug abusers. After a stabilizer was added to the collected human urine, it was diluted 100 times using MOPs buffer, and the cocaine solutions with different concentrations (1 nM-10 μM) were detected by the standard addition method. In Fig. 4a, it can be obviously observed that the POM images gradually change from the black to bright with the increased cocaine concentration from 1 nM to 10 μM when the aptamer concentration is 1 μM, which is similar with the data detected in the buffer. And the average greyscale value also gradually decreases with the reduced cocaine concentration of cocaine in Fig. 4b. The detection concentration range is in the range of the cocaine concentration typically found in urine, i.e. 0.25–10 μg/mL (825 nM - 33 μM) after consumption, and concentrations below 0.15 μg/mL (495 nM) are reported as negative, as set by the National Institute of Drug Abuse [29]. And the lowest concentration of detection is 1 nM. These results clearly demonstrated the great potential of this biosensor for the detection of cocaine in real solution.

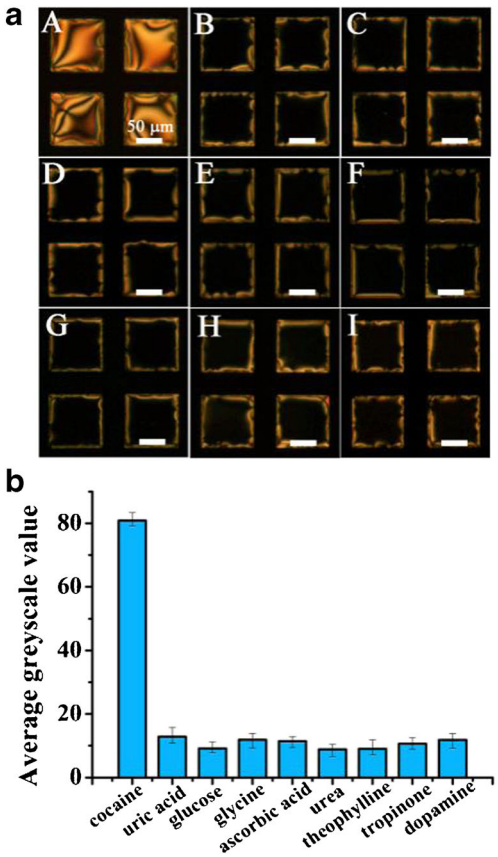

Fig. 3 The specificity of the aptamer-based LCs biosensor for cocaine. **a** The POM images of the LC cells when the aptamer concentration is 1 μM. The cocaine concentration is 500 nM and other interfering substances concentration are both 200 μM (B-I respectively represents uric acid, glucose, glycine, ascorbic acid, urea, theophylline, tropinone and dopamine). **b** The average greyscale value of the POM images for interfering substances

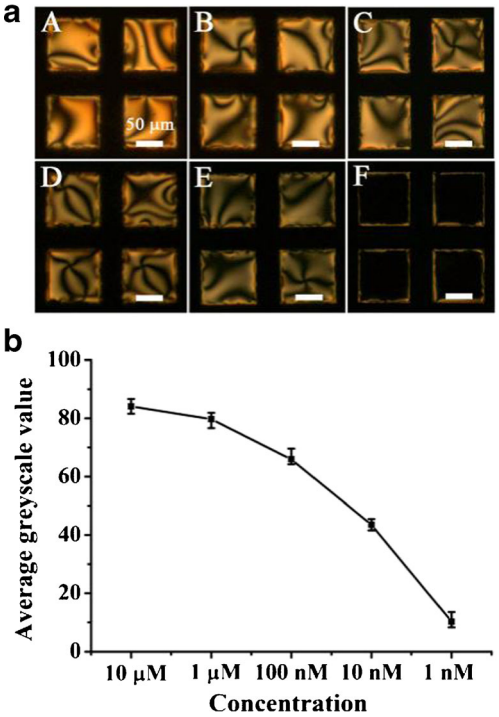

Fig. 4 The detection in urine sample of the LCs biosensor when the aptamer concentration is 1 μM. **a** The concentration of cocaine is respectively 10 μM, 1 μM, 100 nM, 10 nM, 1 nM and 0 nM from A to F. **b** The average greyscale value of POM images at different cocaine concentration

Conclusions

We have reported a novel aptamer-based LC biosensor for cocaine detection at aqueous/LC interface using the sensitivity of LC substrates. The MOPs buffer was used in the LC biosensing system and the aqueous/LC sensing interface was easily broken by the biomolecular interaction. The lowest detection concentration of cocaine is 1 nM. Additionally, the LCs biosensor was able to detect cocaine in human urine solution with the detection concentration as low as 1 nM. The aptamer-based LC biosensor shows good sensitivity and specificity on the detection of cocaine. However, there is a challenge of the method that it is not easy to provide an accurate quantitative equation to describe the relationship between the average greyscale value and the concentration of cocaine. After solving this problem, the aptamer-based LC biosensor platform has great prospects for the detection of cocaine in the future.

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Compliance with ethical standards

Competing interests The author(s) declare that they have no competing interests.

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