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[13] The cycloaddition reaction of 1,3-butadien-2-ylboronic ester with acrolein and methyl vinyl ketone was shown to yield regioselectively the 1,4-disubstituted products; see reference [10a].

[14] Epimerization of the Diels–Alder adducts with NaOMe (1.2 equiv) in THF:MeOH (3:1) at room temperature afforded quantitatively the β epimers; this allowed identification of one of the minor isomers.

[15] The LiCl or iPr$_2$NH$_2$Cl resulting from the preparation of the intermediate alkynylboronate **28** was removed by filtration through Whatman glass fiber filters before the metathesis reaction.

A Light-Modulated Sequence-Specific DNA-Binding Peptide**

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In eukaryotes, gene expression is primarily regulated at the transcription level through the action of a host of sequence-specific DNA-binding proteins known as transcription factors.[1] At any given time, most transcription factors are present in the cell in an inactive form and they become activated upon sensing and responding to specific external signals.[2] In a number of cases, it has been shown that activation involves the transcription factor undergoing a stimulus-induced conformational change that converts it from a non DNA binding protein into a form capable of recognizing the appropriate binding site at the promotor.[3] We reasoned that molecules mimicking this natural activation mechanism, which are capable of binding to specific DNA sequences with high affinity only after receiving an external stimulus, might offer a promising potential for applications in cell biology and molecular medicine[4] and could provide new insights into the mechanisms underlying DNA recognition. As a first step towards this goal, we have designed a peptide whose sequence-specific DNA-binding affinity can be modulated by light.[5]

Our design is inspired by the well-known ability of artificially dimerized basic regions (BRs) of bZIP proteins to recognize DNA sites.[6, 7] We envisaged that appropriate linking of these BRs through a rigid photoresponsive device such as an azobenzene moiety, capable of undergoing a substantial geometrical change upon irradiation,[8] might allow modulation of the site-specific DNA affinity of the resulting dimer. In designing the system we sought to favor the DNA-binding ability of the *cis* over the *trans* isomer by taking advantage of the considerably shorter distance between the benzylic carbon atoms of the *cis*-azobenzene template and the favorable preorientation of its recognition arms to grip the major groove of the DNA (Scheme 1). Molecular modeling corroborated this idea for hybrid **3** (Scheme 2); each of the peptide segments of **3** consists of amino acids 226–248 from the BR of the yeast transcription

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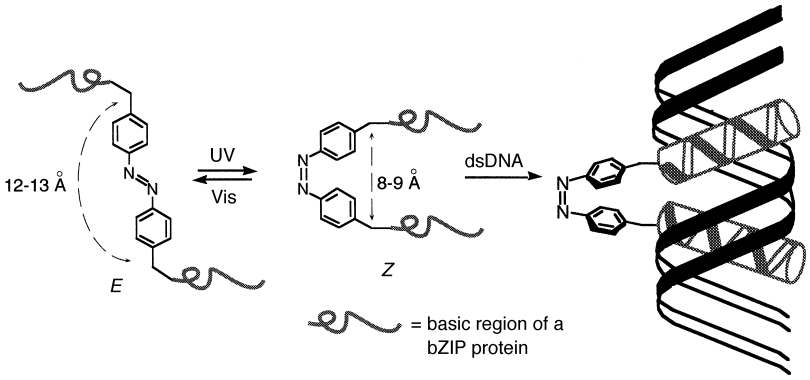
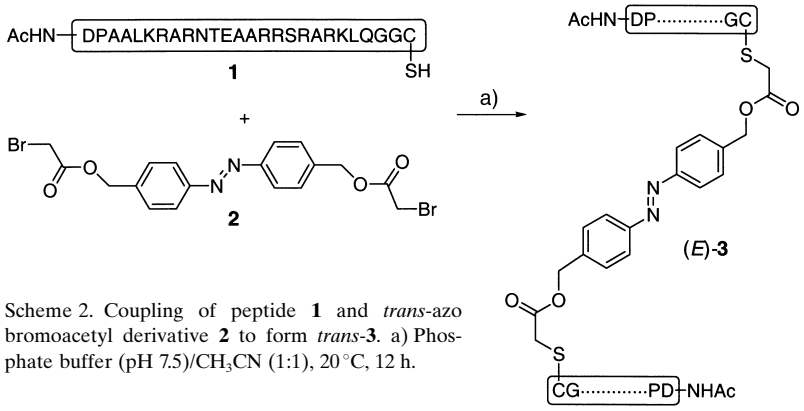


Scheme 1. It is known that sequence-specific DNA binding by bZIP proteins or synthetic minimized versions of these proteins is accompanied by folding to an α helix.[6, 7]



Scheme 2. Coupling of peptide **1** and *trans*-azo bromoacetyl derivative **2** to form *trans*-**3**. a) Phosphate buffer (pH 7.5)/CH_3CN (1:1), 20 °C, 12 h.

factor GCN4 together with an additional Gly-Gly-Cys linker at the C terminus.[9]

The azo compound **3** was synthesized by coupling peptide **1** with the *trans*-azo bromoacetyl derivative **2**.[10] Irradiation of an aqueous solution of *trans*-**3** at 365 nm[11] led to a mixture containing a 95:5 ratio of *cis*:*trans* isomers, with photostationary equilibrium being reached after approximately 60 min.[12] As expected, the isomerization process was completely reversed by irradiating the previous *cis*:*trans* mixture at 430 nm,[13] or by thermal activation.[14]

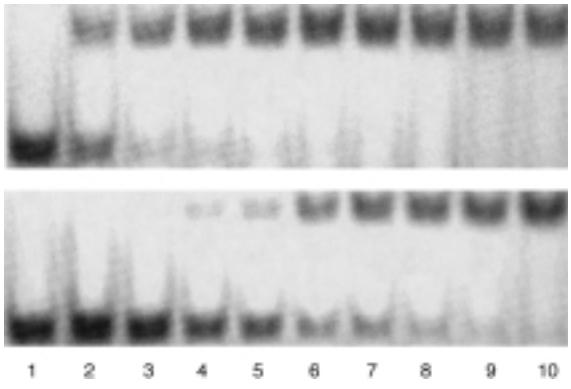


Figure 1. Autoradiograms showing the titration of ^{32}P end-labelled CREB20 with increasing amounts of *cis*-**3** (top) and *trans*-**3** (bottom). Peptide concentrations in lanes 1–10, respectively, were: 0, 1, 5, 10, 30, 60, 100, 200, 500, and 1000 nM. Binding reactions were performed over 45 min at 4 °C using labeled CRE20 (< 1 nM) in a binding mixture (20 μL) containing Tris (20 mM, pH 7.5), KCl (100 mM), $MgCl_2$ (2 mM), EDTA (2 mM), glycerol (10 %), BSA (0.3 mg mL^{-1}), and NP-40 (82 %). Products were resolved by polyacrylamide gel electrophoresis using a 10 % nondenaturing acrylamide gel and 0.5X TBE buffer. Duplex oligonucleotide CREB20 (containing a consensus ATF/CREB site):

5′-d(TGGAG*ATGACGTCAT*CTCGT)-3′
3′-d(ACCTCTACTGCAGTAGAGCA)-5′

The DNA affinities of *trans*-**3** and *cis*-**3** were assessed by gel mobility shift analysis, by titrating a 20 base-pair duplex DNA containing a consensus ATF/CREB recognition site (CREB20) with increasing concentrations of the peptides.[15] As is shown in Figure 1, *cis*-**3** bound this DNA with very high affinity, and approximately 60–70 times more efficiently than the *trans* isomer. The difference in affinity can be easily visualized by observing that, at a peptide concentration of 5 nM (lane 3) the *cis* isomer was completely bound while the *trans* isomer remained unbound. Thus, as hypothesized, the geometric alteration that occurs upon irradiation brings about a substantial change in the sequence-specific DNA affinity of the azobenzene–peptide hybrid.[16]

DNA binding was further characterized by circular dichroism, a technique that is particularly useful for studying DNA–bZIP interactions owing to the well-established folding transition of the bZIP BR from random coil to α helix upon specific DNA binding.[6, 7] Addition of CREB20 to *cis*-**3** at 4 °C brought about a significant increase in the magnitude of the signal at 222 nm (Figure 2 A, spectra a and e), whereas for *trans*-**3** the ellipticity increase was smaller (Figure 2 A, spectra c and d). To check whether this relatively small change of ellipticity might result from *trans*-**3** binding specifically with just one of its BR arms, with the other arm remaining unstructured and non-specifically bound, this peptide was incubated with duplex oligonucleotides containing just half of the dimer recognition site. Since no significant helicity changes were observed (Figure 2 B),[17] it can be inferred that *trans*-**3** binds specifically CREB20 with both BR arms.

Interestingly, the *trans* → *cis* isomerization can be carried out in the presence of DNA, although it is about eight times slower than in the absence of DNA and leads to a slightly lower proportion of the *cis* isomer at the equilibrium (inset to Figure 2 A, curve 1). This rate decrease can be attributed to partial stabilization of the *trans* conformation by nonspecific electrostatic peptide–DNA interactions. Indeed, increasing the ionic strength of the medium by adding $MgCl_2$ slightly accelerates the isomerization process and restores the *cis*:*trans* ratio to the value it has in the absence of DNA (inset to Figure 2 A, curve 2). Remarkably, the stability of the DNA–*cis*-**3** complex completely inhibited the *cis* → *trans* reverse process, at least under the present photoreaction conditions.[18]

In summary, linking two bZIP basic regions through an azobenzene group produces a peptide whose sequence-

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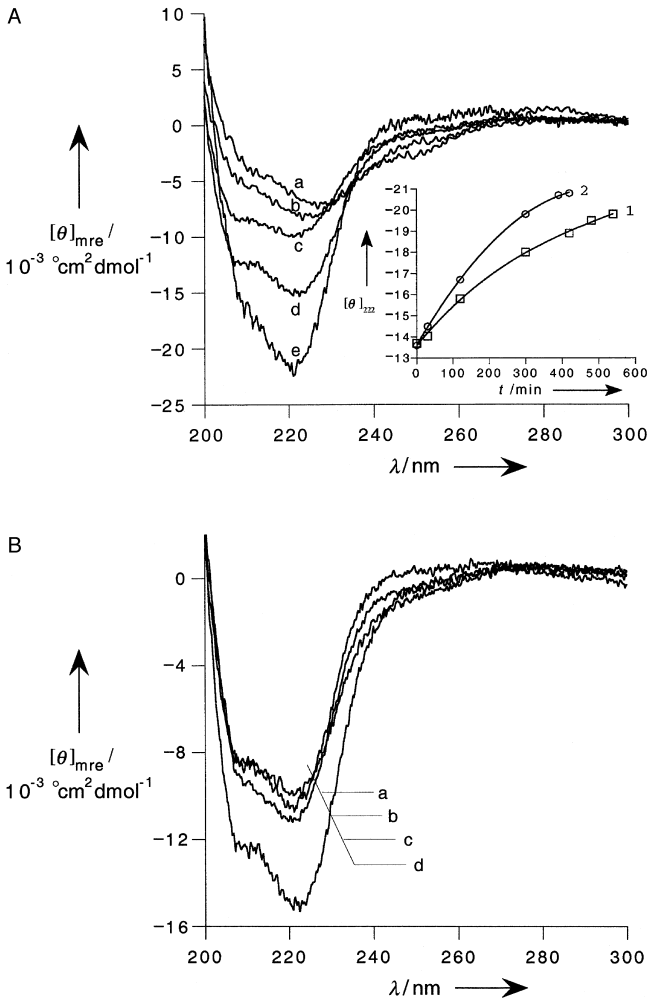

Figure 2. CD spectra of peptides in the the presence or absence of various double-stranded (ds) oligonucleotides. CD measurements were performed at 4 °C on a JASCO J-715 apparatus in a 2 mm cell. Samples contained phosphate buffer (10 mM, pH 7.0), NaCl (100 mM), peptide (2 μM), and oligonucleotide (2 μM) when present. The spectra are averages of 10 scans and were neither smoothed nor noise reduced. Spectra of the peptides in the presence of DNA were calculated as the difference between the spectra of the peptide–DNA mixture and the spectrum of free DNA. It should be noted that *cis*-**3** is contaminated by a small proportion of the *trans* isomer (5%).

NON20 (random): 5′-d(TGGAGTATGCGTCGATTCGT)-3′
3′-d(ACCTCATACGCAGCTAAGCA)-5′

CREB20-half1: 5′-d(TGGAG*ATGAC*GTTGTCTCGT)-3′
3′-d(ACCTCTACTGCAACAGAGCA)-5′

CREB18-half2: 5′-d(AGGATTTT*ATGAC*GTTCG)-3′
3′-d(TCCTAAAATACTGCAAGC)-5′

A) Curve a: *cis*-**3** without DNA; curve b: *cis*-**3** with NON20; curve c: *trans*-**3** without DNA; curve d: *trans*-**3** with CREB20; curve e: *cis*-**3** with CREB20. B) Spectra of *trans*-**3**: Curve a: without DNA; curve b: with CREB18-half2; curve c: with CREB20-half1; curve d: with CREB20. At 20 °C, the ellipticity of all the complexes with CREB20 is about 12% less. Inset: Time evolution of the CD difference spectrum of a mixture of duplex CREB20 and *trans*-**3** upon irradiation at 365 nm. Curve 1: in a buffer containing NaCl (100 mM); HPLC analysis of the final mixture showed a 85:15 *cis*:*trans* ratio. Curve 2: in a buffer containing $MgCl_2$ (10 mM) in addition to the NaCl (100 mM); HPLC analysis of the final mixture indicated a 95:5 *cis*:*trans* ratio.

specificDNA-binding affinity can be modulated by light. Although further development is needed, in particular to allow external induction of the dissociation of the DNA–peptide complex, we envisage that this kind of externally regulatable peptide system will eventually prove invaluable for in situ control of gene expression.

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[10] The syntheses of **1** and **2** are described in the Supporting Information. The coupling reaction required the use of an excess of peptide **1** (approximately 3 equivalents). Azopeptide *trans*-**3** was isolated by RP-HPLC and identified by ES-MS: *m*/*z* [*M*+H$^+$] found: 6110.2; calculated: 6110.3.

[11] Irradiation at 365 nm was performed with a 8 W UV lamp, at a concentration of 2 μM.

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[12] The isomer ratio was calculated by integration of the HPLC peaks corrected by the extinction coefficients. Remarkably, the equilibrium *cis*:*trans* ratio is slightly higher than for the azobenzene precursor **2** (90:10).
[13] Irradiation was performed in a spectrofluorometer (Fluromer-2; light source: 150 W xenon lamp; 5 nm path). At a concentration of 2 μM, isomer *trans*-**3** was obtained after 15 min.
[14] In the absence of visible light, the isomerization of *cis*-**3** to *trans*-**3** at 35 °C had a half-life of approximately 65 h.
[15] It should be borne in mind that *cis*-**3** is contaminated by 5% of the *trans* isomer.
[16] An azobenzene–peptide hybrid related to **3** but lacking the two glycines at the C terminus is unable to bind to the CREB site in either the *cis* or *trans* form (results not shown).
[17] The absence of specific binding was also corroborated by PAGE using ^{32}P end-labelled CREB18-half2 as the DNA probe.
[18] The UV spectrum of the peptide–DNA complex after 4 h irradiation with visible light is identical to that obtained before irradiation, suggesting that the *cis*-azobenzene unit was unable to switch to the *trans* form (see the Supporting Information).

TEM Studies of Platinum Nanowires Fabricated in Mesoporous Silica MCM-41**

Zheng Liu, Yasuhiro Sakamoto, Tetsu Ohsuna, Kenji Hiraga, Osamu Terasaki,* Chang Hyun Ko, Hyun June Shin, and Ryong Ryoo

Porous materials attract much attention as catalysts, catalyst supports, and shape/size-selective adsorbents, as well as containers for making new materials. According to the definition of IUPAC,[1] porous materials can be divided into three types according to their pore diameters: microporous (<2 nm), mesoporous (2–50 nm), and macroporous (>50 nm). Although zeolites, which belong to the microporous group, provide excellent catalytic properties as a result of their crystalline aluminosilicate networks, their applications are limited by the relatively small internal spaces and pore openings. Therefore, an expansion of the spaces is one of the main targets in the synthesis of porous materials.

In 1992, researchers at Mobil Corporation used a liquid crystal template to synthesize a new family of mesoporous silicates and aluminosilicates known as the M41S family.[2, 3] These ordered mesoporous materials have since attracted much attention. Silca MCM-41 is a member of the M41S family with a hexagonal array of one-dimensional pores. The diameter of the pores can be varied from 1.6 to 10 nm by using surfactants of different chain lengths or organic molecules for expanding the surfactant micelles.[4–11] Numerous studies have been reported on the synthesis of high-quality MCM-41, especially with pore diameters below 4.5 nm.[2–5, 12–14] Recently, the synthesis of very high quality MCM-41 was achieved by controlling the micelle packing parameter with two kinds of surfactant.[15] These MCM-41 molecular sieves attract considerable and growing attention because of their remarkable properties. The spaces of the mesoporous materials can be used as containers or templates for the synthesis of other new materials. Here we report on the structural characterization by transmission electron microscopy (TEM) and thermal stability of Pt nanowires as synthesized in the MCM-41 channels and of Pt nanowires removed from the silica template.

We synthesized MCM-41 silica by following a previously reported procedure;[15] platinum nanowires were prepared in the MCM-41 channels in a manner similar to that reported previously;[16] and unsupported Pt nanowires were obtained by removing the silica framework from the Pt/MCM-41 sample with an aqueous solution of hydrofluoric acid (see Experimental Section).

Figures 1 a and 1 b show high-resolution electron micoscopy (HREM) images of the calcined MCM-41 sample. Figure 1 a shows a uniform hexagonal arrangement of bright dots corresponding to the straight channels of MCM-41. The diffraction pattern in the inset of Figure 1 a clearly shows that the incident beam is along the [001] direction. The brightness and the shape of the channels seem to differ slightly from

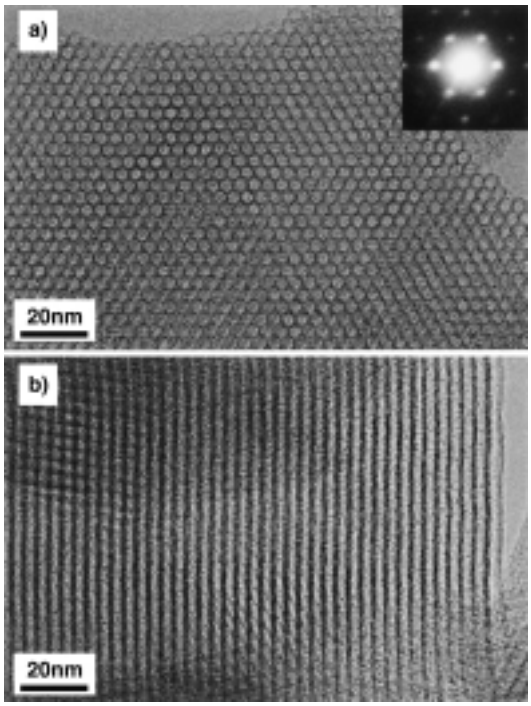

Figure 1. TEM images of calcined mesoporous MCM-41 along the channel direction (*c* axis; a) and perpendicular to it (b).

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