permits a defined convection. This method opens new avenues toward an understanding of spatiotemporal pattern formation in electrochemical systems.

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Detection of Creatinine by a Designed Receptor

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An artificial receptor has been designed to bind creatinine with a color change (chromogenic response) caused by proton transfer from one end of the receptor to the other. The receptor was synthesized and found to extract creatinine from water into chlorocarbon solvents. The color change in the organic layer is specific for creatinine relative to other organic solutes, and it is selective for creatinine relative to sodium, potassium, and ammonium ions. The chromogenic mechanism is revealed by x-ray crystal structures of creatinine, the free receptor, and the complex, showing "induced fit" binding resulting from electronic complementarity between host and guest.

Creatinine (*1*) is an end product of nitrogen metabolism. In healthy individuals, it is transported by the kidneys from blood to urine. Blood levels of urea nitrogen and creatinine are key indicators of renal function (*2*), but current creatinine assays have several shortcomings. The Jaffé reaction, in which a colored product is formed from creatinine and picrate in alkaline solution, remains the prevalent method, despite its poor specificity. During the past two decades, several enzymatic methods have appeared, the most promising of which require four or five enzymes (*3*, *4*). Apparently, these approaches are more specific than the Jaffé method, but they suffer the shortcomings of certain interferences, expense, and limited stability. The work reported here demonstrates that supramolecular chemistry (*5*–*7*) can be applied to the design of chromogenic reagents for detection of clinically important molecules.

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The receptor (**1**, Fig. 1) was designed with the use of Cram's principles (*7*) of host-guest complementarity and host preorganization (*5*–*8*). The successful binding of creatinine (**2**) by three hydrogen bonds was expected to cause the transfer of a proton from the OH group of the receptor to its end nitrogen atom. The resulting complex (**1·2**) would then have a dipolar structure; the delocalization of the negative and positive charges was expected to cause a visible color change. In this designed receptor, the chromogenic group is intrinsic to the preorganized binding site and communicates directly with the bound guest (*9*). In contrast, color-producing or fluorescent moieties generally cannot be incorporated into the binding sites of biomolecular receptors, enzymes, or antibodies without altering their desirable binding characteristics.

As shown in Fig. 2, creatinine receptor **1** was synthesized in five steps (*10*) from intermediate **3** (*11*). The phenol ring in **4** was protected from ozonolysis as ester **5**. Partial hydrolysis of the acetate group occurred during isolation of product **6**, so this intermediate was converted to phenol **8** without purification. Nitration of **8** gave the target receptor (**1**), which was difficult to purify because of its acid-base properties and low solubility in most solvents. Chromogenic analytical reagents are used in very small quantities, so the synthesis outlined in Fig. 2 is effective, despite its

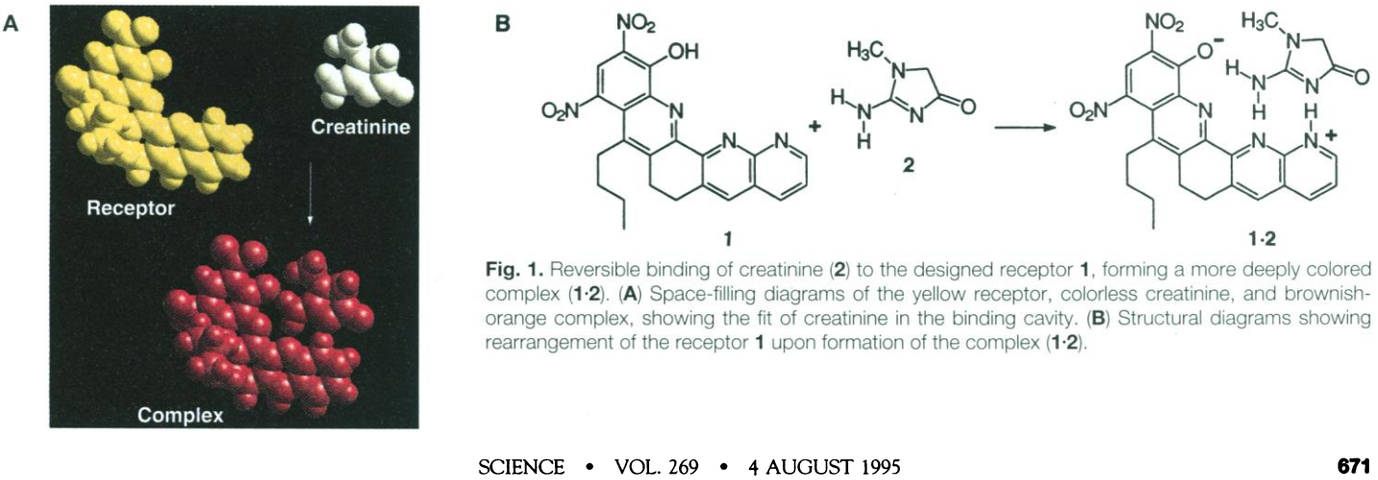

Fig. 1. Reversible binding of creatinine (**2**) to the designed receptor **1**, forming a more deeply colored complex (**1·2**). (**A**) Space-filling diagrams of the yellow receptor, colorless creatinine, and brownish-orange complex, showing the fit of creatinine in the binding cavity. (**B**) Structural diagrams showing rearrangement of the receptor **1** upon formation of the complex (**1·2**).

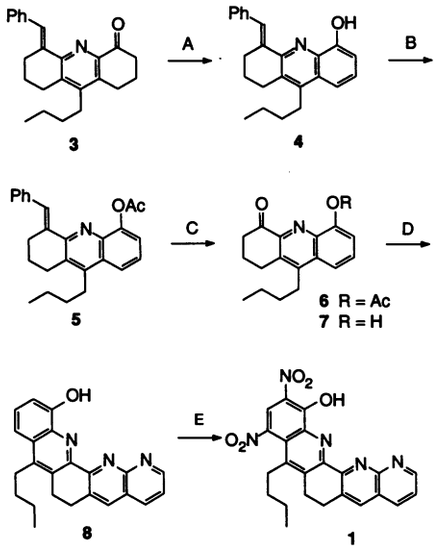

Fig. 2. Synthesis of the designed receptor **1** [for synthesis details, see (*10*)].

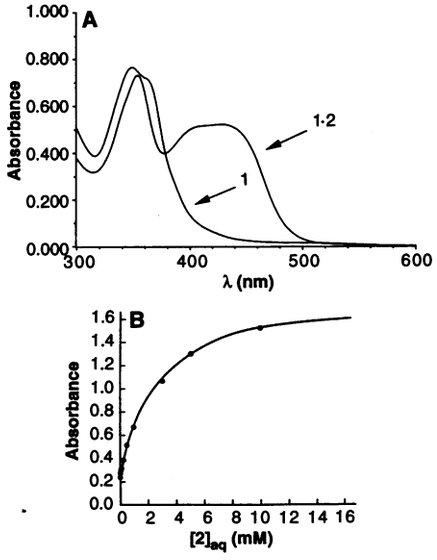

Fig. 3. Chromogenic response of **1** to creatinine (**2**) in water-saturated CH_2Cl_2. (**A**) UV-visible absorption spectra of **1** (51 μM) and **1·2** (51 μM **1** + 43 μM **2**). (**B**) Graph of changes in absorbance at 444 nm for 0.14 mM solutions of **1** in CH_2Cl_2 after shaking with aqueous solutions of creatinine (**2**) (pH 6.0, 0.1 M MES buffer).

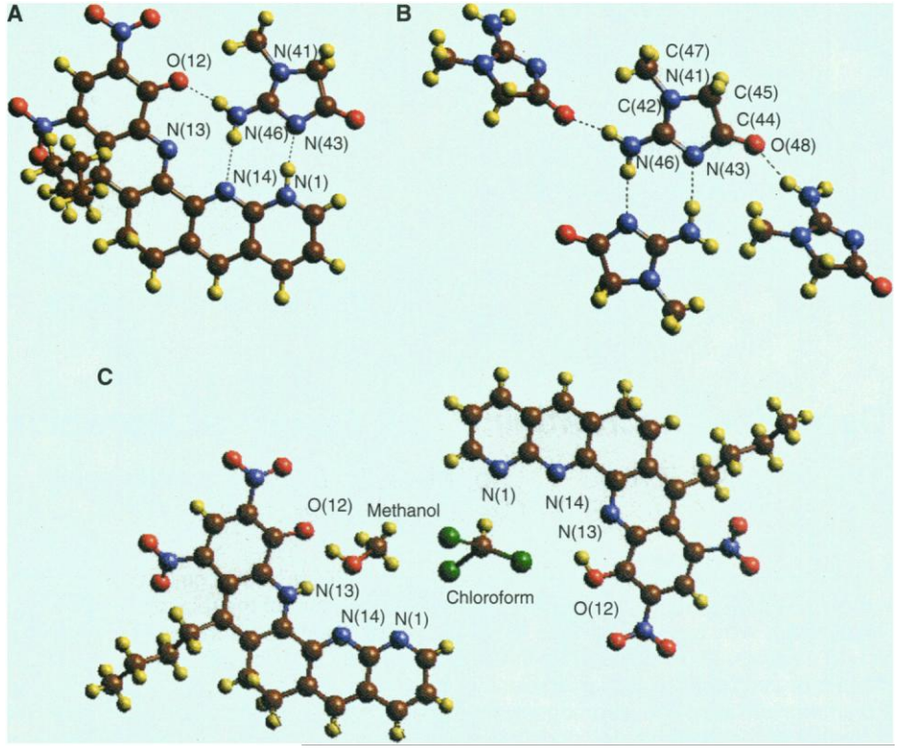

Fig. 4. Ball-and-stick representation of the x-ray structures [see (*13*)] of complex **1·2** (**A**), creatinine (**B**), and receptor **1** (**C**). Color scheme: carbon, brown; hydrogen, yellow; nitrogen, blue; oxygen, red; and chlorine, green.

length and modest overall yield (20%).

Creatinine receptor **1** is an orange solid that is insoluble in water but slightly soluble in methanol, dimethyl sulfoxide, dichloromethane, and chloroform. Creatinine (**2**) is soluble in water and has very low solubility in chlorocarbon solvents; it is extracted from water into dichloromethane solutions of **1**. Solutions of **1** in dichloromethane turn from yellow to brownish-orange upon binding creatinine, forming the colored complex (**1·2**). Figure 3 shows that this color change corresponds to the appearance of a broad band in the ultraviolet (UV)–visible absorption spectrum (400 to 500 nm). The intensity of absorbance of this band is clearly correlated with the concentration of **2** in the buffered aqueous layer (Fig. 3B), proving that the color change is caused by formation of a creatinine complex, not by acid-base chemistry. The chromogenic response is selective for creatinine; no color change was detected in experiments with histidine, proline, uric acid, urea, or creatine. The dissociation constant of complex **1·2** in water-saturated chloroform was calculated to be 0.5 μM from the results of extraction and titration experiments. Weak responses to cationic analytes were observed, but the intensities of the optical responses to ammonium, potassium, and sodium were weaker by factors of 790, 3200, and 80, respectively. We conclude that this new analytical method is sufficiently sensitive for measurement of creatinine at normal blood serum levels [40 to 130 μM (*3*)], with correction for background levels of Na^+ [130 to 150 mM (*12*)].

The details of the molecular recognition process are revealed by the crystal structures of receptor **1**, creatinine (**2**), and their complex (**1·2**) (Fig. 4) (*13*, *14*). The structure of the creatinine complex (Fig. 4A) shows that the hydrogen atoms are located in the positions indicated in the diagram of **1·2** (Fig. 1). A second crystal of **1·2** grown from chloroform and methanol contained a molecule of chloroform (**1·2**·$CHCl_3$). The structure of the **1·2** complex in this crystal was almost identical to that shown in Fig. 4A, with the chloroform molecules packing between the complexes. The positions of the hydrogen atoms in the three hydrogen bonds were located unequivocally in the crystal structures.

In the crystalline creatinine-free receptor (Fig. 4C) we found the hydrogen atom in the binding site to be disordered over two positions, oxygen atom O(12) and nitrogen atom N(13). The crystal therefore contains two structures corresponding to diagrams **1a** and **1b** in Fig. 5. We located two superimposed solvent molecules (chloroform and methanol), each with approximately 50% occupancy (Fig. 4C). In the structure, a chloroform complex of **1a** is opposite a methanol complex of **1b**. These receptor structures agree with the color responses observed for receptor **1**. Neutral form **1a** predominates in the light yellow dichloromethane solutions of **1**, whereas the reddish color of methanol solutions indicates the presence of a dipolar tautomer such as **1b**. The receptor in complex **1·2** also has a dye-like structure that can be drawn either in the aromatic dipolar form shown in Fig. 1 or in a neutral nonaromatic form.

Creatinine can exist in two tautomeric forms (Fig. 5); the C=N double bond can be outside or inside the five-membered ring (**2a**

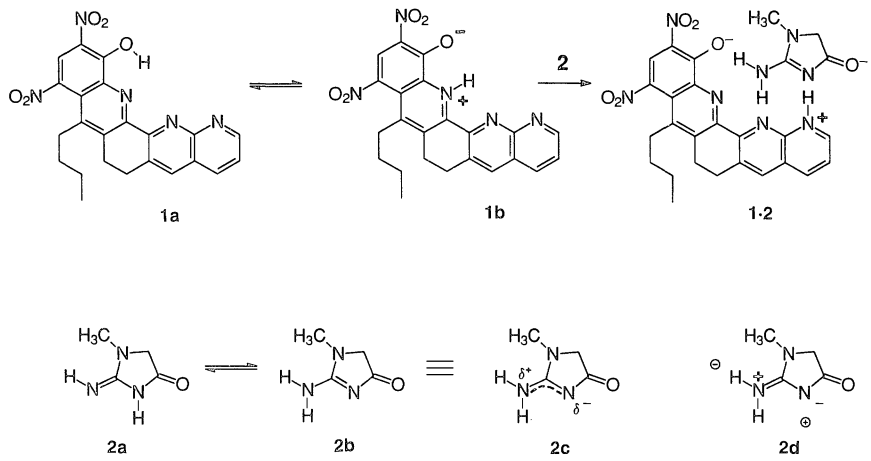

Fig. 5. Structural diagrams of possible forms of receptor **1** and creatinine (**2**) in the free and complexed states. Form **1a** occurs in crystals of receptor **1** and is proposed to be the major form in dichloromethane solution; **1b** also occurs in crystals of **1** and may be present in methanol solution. Tautomer **2a** is theoretically the most stable form of creatinine (**2**), but **2b** is found in the solid state and is the major form in solution. Diagram **2c** represents the bond distances and electron distribution in crystalline **2** (**2b**); **2d** represents the bond distances and electron distribution upon polarization of **2** in the complex (**1·2**).

or **2b**, respectively). Quantum mechanical calculations indicate that form **2a** is slightly more stable than **2b** (*15*), but spectroscopic studies show that **2b** predominates in solution (*15*, *16*). Hydrogen atoms were not located in a crystal structure of creatinine determined in 1955 (*12*), but form **2a** was inferred later on the basis of bond lengths (*15*). To clarify this situation, we redetermined the crystal structure of creatinine and clearly located the two hydrogen atoms on N(46) outside the five-membered ring, identifying form **2b**. The hydrogen bond pattern is shown in Fig. 4B. The C(42)–N(46) bond [1.320(3) Å] is slightly shorter than the C(42)–N(43) bond [1.349(3) Å, where the number in parentheses is the error in the last digit], leading to the previous erroneous assignment of the structure as **2a** (*15*). Conjugation in **2** apparently produces an electron-delocalized structure best represented by diagram **2c** in Fig. 5 (*17*). Electron delocalization reverses the usual relation between bond length and nitrogen valence; the C–NH_2 bond is shorter than the C=NH bond. Reversal of bond order is further exaggerated in the complex **1·2**; here the C(42)–N(46) and C(42)–N(43) bond lengths are 1.290(9) and 1.409(10) Å, respectively. In the complex **1·2**, the positively and negatively charged positions of the creatinine dipole (**2c**, Fig. 5) pair with the receptor dipole's negatively and positively charged positions, respectively; electrostatic host-guest attraction apparently deforms the structure and alters the charge distribution of creatinine, as indicated by diagram **2d** (Fig. 5). We have reproduced this effect qualitatively with quantum mechanical calculations using the MOPAC93 program (*18*), placing positive and negative charges in the positions shown (**2d** in Fig. 5).

Rearrangement of receptor **1**, signaling complexation of creatinine, is reminiscent of the "induced fit" mechanism proposed for enzyme-substrate binding (*5*, *19*). Moreover, the structural deformation of creatinine induced by binding to the rigid dipolar cleft suggests that designed receptors might be used as artificial enzymes, operating by the principle of transition-state stabilization (*20*). The molecular recognition demonstrated in this study is characterized by preorganization of hydrogen-bonding groups in the polycyclic receptor and electronic complementarity producing mutual polarization of host and guest in the complex. These features explain why receptor **1** is able to extract creatinine from water into organic solvents, whereas neutral receptors previously designed to bind creatinine form weaker complexes (*21*). This illustrates the possibility of designing chromogenic reagents for quantification of important organic analytes by application of the principles of supramolecular chemistry.

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10. Reactions were conducted under an atmosphere of anhydrous N_2 as follows: Reaction A: A mixture of 2.24 g (6.5 mmol) of **3** (*11*), 1.63 g (7.2 mmol) of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, and 130 ml of anhydrous toluene was heated with stirring at 55° to 60°C for 20 min, then 0.9 ml of triethylamine was added and heating was continued for 3.5 hours. The cooled mixture was filtered through neutral alumina, washing the residue and alumina with 200 ml of hexane/ethyl acetate (4:1, v/v). Evaporation of solvents, followed by trituration with cold methanol, gave 1.37 g (62%) of **4** as an off-white solid, melting point (mp) 83° to 84°C. Reaction B: A solution of 3.2 g (9 mmol) of **4** and 7.1 ml (5.15 g, 51 mmol) of triethylamine in 100 ml of CH_2Cl_2 was stirred for 40 min, then 3.6 ml (4.0 g, 51 mmol) of acetyl chloride was added drop by drop. The mixture was stirred for 17 hours, diluted with 50 ml of CH_2Cl_2, and extracted with water (2 × 70 ml). The organic layer was dried over Na_2SO_4 and evaporated; the residue was recrystallized from hexane to yield 3.6 g (100%) of **5** as an off-white solid, mp 122° to 123°C. Reaction C: Ozone was bubbled through a −78°C solution of 2.19 g (5.7 mmol) of **5** in 200 ml of CH_2Cl_2/methanol (1:1, v/v) until the color of the solution changed to light blue, then dimethyl sulfide (0.63 ml, 0.53 g, 6.8 mmol) was added and the solution was allowed to warm to room temperature over 14 hours. The solvents were evaporated and the crude **6** was stirred with 0.1 ml of pyridine in 60 ml of methanol at 40°C for 13 hours. Evaporation gave a residue, which was triturated with 5 ml of methanol, yielding 0.64 g (41%) of **7** as an off-white solid. Reaction D: A mixture of 0.17 g (0.55 mmol) of crude **6**, 75 mg (0.61 mmol) of 2-aminonicotinaldehyde [T. G. Majewicz and P. Caluwe, *J. Org. Chem.* **39**, 720 (1974)], 0.1 ml of 1,8-diazabicyclo[5.4.0]undec-7-ene, and 16 ml of methanol was heated under reflux for 20 hours, then cooled to room temperature and filtered. The yellow solid was washed with methanol and dried under vacuum, yielding 0.16 g of **8** (75% from **5**). Reaction E: A mixture of 0.45 g (1.2 mmol) of **8** and 9.0 ml of 70% aqueous HNO_3 was stirred for 12 hours at 0°C, then poured into 150 ml of ice-cold water. The reddish-brown precipitate was collected by filtration and shaken with 120 ml of CH_2Cl_2/pyridine (10:1, v/v). The resulting solution was washed with water (2 × 100 ml) and evaporated. The residue was washed with 30 ml of hot $CHCl_3$, yielding 0.24 g (45%) of **1** as an orange solid after filtration and drying (60°C, 0.1 mm Hg).
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13. Data for all three crystals were collected by a variable-width ω scan on an Enraf-Nonius diffractometer by using CuKα radiation at room temperature. To obtain **1·2** equimolar amounts of **1** and **2** were dissolved in a hot 1:1 (v/v) mixture of methanol and chloroform; reddish-orange crystals [$C_{23}H_{19}N_5O_5$, formula weight (FW) 276.25] were deposited from the solution at 0°C. A total of 4734 independent reflections were measured. The structure was solved by direct methods [*Shelxl, Program for Crystal Structure Refinement* (1993); G. M. Sheldrick, University of Göttingen]; all nonhydrogen atoms were refined anisotropically. Hydrogen atoms bonded to carbon were placed in calculated positions, whereas hydrogen atoms on N(1) and N(46) were located from a difference Fourier map and successfully refined. The final *R* factor was 0.083. A crystal of creatinine (**2**) was selected from a commercial sample ($C_4H_7N_3O$, FW 113.12). A total of 691 independent reflections above background were collected, and the structure was refined with the use of the TEXSAN suite of programs, initially using the published coordinates (*14*). Hydrogen atoms were located on a difference Fourier map, and the structure was refined with the use of anisotropic thermal parameters for all nonhydrogen atoms. The hydrogen atom positions were allowed to refine independently. The final *R* factor was 0.048. A brown crystal (**1**·½$CHCl_3$·½CH_3OH, $C_{24}H_{23.5}N_5O_{5.5}Cl_{1.5}$, FW = 288.26) of the receptor (**1**) was grown by slow evaporation of a solution of receptor **1** in 3:1 (v/v) chloroform/methanol. A total of 2070 independent reflections were collected.

The structure was determined by direct methods; hydrogen atoms bonded to carbon were placed in calculated positions. The solvent molecules were disordered over a center of symmetry and refined to 50% occupancy with the carbon of methanol and a chlorine of chloroform occupying the same site. Hydrogen atoms on N(13) and O(12) were located from a difference Fourier map and refined at 50% occupancy with constrained bond lengths and thermal parameters. All nonhydrogen atoms were refined anisotropically, whereas solvent atoms and hydrogen atoms were refined isotropically to a final R value of 0.078. The coordinates and other crystallographic details for all three structures have been deposited with the Cambridge Crystallographic Database.

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Molecular Diffuse Interstellar Band Carriers in the Red Rectangle

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High-resolution optical spectroscopic observations of unidentified emission bands from the unusual biconical nebula known as the Red Rectangle are reported. The peak wavelengths and the widths of prominent bands near 5799, 5853, and 6616 angstroms decrease with increasing offset from the central A0-type star HD 44179 and, in the limit of large distance from the star, are shown to converge toward the known values for some of the narrower diffuse interstellar absorption bands at 5797, 5850, and 6614 angstroms. The same carriers give rise to both Red Rectangle emission and corresponding diffuse interstellar absorption bands, and these particular bands arise from electronic transitions in gas-phase molecules.

The identification of the carriers of the diffuse interstellar absorption bands is one of the most challenging astrophysical problems of this century. A large number of bands have been discovered (*1*), and their relative strengths, their widths, and in a few cases their shapes (*2*) have been characterized. Evidence in favor of free molecules as carriers has grown in recent years, but there is as yet no proof as to whether the bands originate in dust grains or molecules. There has also been a lack of any spectroscopic patterns that might guide laboratory-based experiments. A step forward was made with the recognition (*3*, *4*) that some of the more prominent emission bands observed from the Red Rectangle appear to arise from the same carriers as a subset of the diffuse band carriers (*5*). Similar emission bands have also recently been detected in the carbon-rich R CrB star V854 Cen during minimum light (*6*). We report here high-resolution and high signal-to-noise optical spectra of the Red Rectangle that show conclusively that the Red Rectangle emission and complementary diffuse interstellar absorption bands arise from the same carriers and that these particular diffuse bands arise from electronic transitions in free, but as yet unidentified, gas-phase molecules. In addition, the results provide clear support for the idea of "families" of diffuse bands, demonstrate that some of the diffuse band carriers fluoresce, and open up new opportunities for laboratory study through the search for molecular emission spectra.

The Red Rectangle is a biconical nebula that is seen in both reflection and emission. The reflected component arises from light from the central star HD 44179 scattered by dust grains in the extended nebular medium. The optical emission consists of a broad red component [extended red emission (ERE)] on which is superimposed a remarkable spectrum of unidentified emission bands that are also excited by light from the central star (*7*–*9*), probably through a resonance fluorescence mechanism such as occurs in comets. Recent observations confirm that HD 44179 is a binary system (*10*), and a plausible scenario is that one component acts as the illumination source for material emanating from one star or possibly from both stars.

Earlier studies of the ultraviolet-visible and infrared (IR) spectra of the nebula have revealed a range of unusual aspects, including emission lines of Na D, Ca II H and K, Hα (*8*), and the CO (*11*) and CH^+ (*12*) molecules; there is also strong ERE (*13*) and a prominent set of "unidentified" IR bands (*14*), both of which probably arise from polycyclic aromatic hydrocarbon (PAH) material or molecules (*15*). Very striking, however, is the set of strong optical emission bands, the most prominent of which fall near 5799, 5853, and 6616 Å, that lie close in wavelength to those of some strong, relatively narrow diffuse absorption bands (λ=5797, λ=5850, and λ=6614, where λ indicates the nominal wavelength); these diffuse bands have been linked into a "family" (*16*) on the basis of their common relative absorption strengths along various lines of sight.

Our data covering the region from 5100 to 6900 Å were recorded with the RGO spectrograph on the 3.9-m Anglo-Australian telescope at a resolution of 0.64 Å and with the slit of width 150 μm aligned along the northwest-southeast whisker at position angle 162°. A comparison between the 5800 Å region of the Red Rectangle at intermediate offset (centered at 7.5 arc sec) and the diffuse interstellar band absorption spectrum along the line of sight toward the heavily reddened star HD 183143 is shown in Fig. 1. The narrow λ=5797 and λ=5850 diffuse interstellar bands lie on the short-wavelength edge of the corresponding broader Red Rectangle fea-

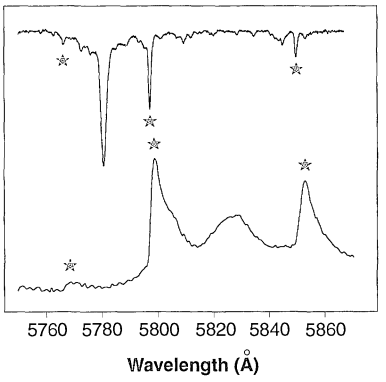

Fig. 1. Comparison between the diffuse absorption band spectrum toward HD 183143 (upper curve) (*25*) and the Red Rectangle spectrum (lower curve) in the 5800 Å region. Note the correspondence between the diffuse band and Red Rectangle features near 5769, 5799, and 5853 Å (stars), and the absence of Red Rectangle emission in the region of the λ=5780 diffuse band, which falls in a different family (*16*). The Red Rectangle data are the summed spectra for the region 5 to 10 arc sec offset from HD 44179 recorded with an integration time of 7200 s.

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