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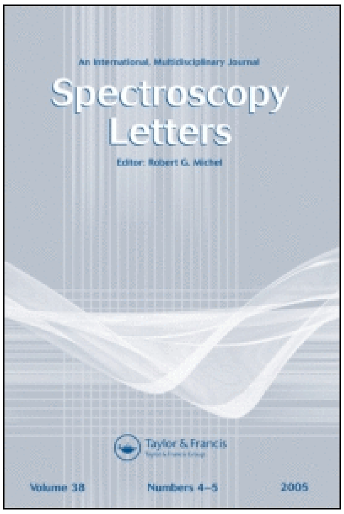

Spectroscopy Letters
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597299

Sers Studies on a Novel Copper Porphyrin and Its Complex with DNA

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To cite this Article Zhou, Guangming(1998) 'Sers Studies on a Novel Copper Porphyrin and Its Complex with DNA', Spectroscopy Letters, 31: 6, 1141 — 1150
To link to this Article: DOI: 10.1080/00387019808003291
URL: http://dx.doi.org/10.1080/00387019808003291

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SPECTROSCOPY LETTERS, 31(6), 1141-1150 (1998)

SERS STUDIES ON A NOVEL COPPER PORPHYRIN AND ITS COMPLEX WITH DNA

Keywords Copper (II) complex of *meso*-tetris (4-N-cyano methyl pyridyl) porphyrin, SERS, Double-stranded DNA, Denatured DNA, Silver sols

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ABSTRACT

The SERS spectra of copper (II) complex of *meso*-tetris (4-N-cyano methyl pyridyl) porphyrin (Cu^{II}NACN) and the complexes with the calf thymus double-stranded and denatured DNA on silver sols obtained by reducing $AgNO_3$ with citrate and the effect of pH and the concentration of Cu^{II}NACN have been investigated. It was pointed out that, pH=5~6, the concentration of Cu^{II}NACN ca 1×10^{-5} mol/L are better for SERS. Cu^{II}NACN acts on Ag sols through the pyridyl N and the porphyrin core together, and is absorbed on Ag sols in the slope-on form which is not influenced by adding the double stranded and the denatured DNA. In the system of Ag sols, Cu^{II}NACN acts on the double stranded DNA at phosphate by electrostatic force, but maybe part-intercalates the single stranded of the denatured DNA accompanied with the electrostatic force similar to the double stranded DNA.

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1 INTRODUCTION

The porphyrins, as colorimetric or fluorescent agents have been studied extensively by analytical chemists. The alkaloids of porphyrins and their complexes with metals play important roles in many biological functions. Moreover they can be used as models of anti-cancer agents[1]. Some porphyrins can restrain HIV-1 virus caused by AIDS[2]. Understanding of the action of porphyrin on biological molecule from molecular structure has been studied in chemistry, medicine, and biology to understand the biological mechanism and based on the results to select the porphyrins of biological activity. Surface-enhanced Raman spectra (SERS) have been proved to be a powerful tool to obtain structural information of fluorescent chromophores at very low concentrations[3]. The very high enhancement (up to 10^7) of the intensity of the Raman spectra of molecules close to (a few angstroms) or adsorbed on a metal surface permits the structural study of small pharmacological probes, even at the femtogram level[4]. It has been used to determine the structure of the adduct of benzo(a)pyrene-DNA[5], ellipticine, 2-N-methylellipticine and their complex with DNA[6], adriamycin, 11-deoxycarminomycin and their complexes with DNA[7]. This paper investigated the SER spectra of a novel kind of porphyrin, copper (II) complex of *meso*-tetris (4-N-cyano methyl pyridyl) porphyrin (Cu^{II}NACN) and its complex with DNA on silver colloidal surface, assigned their wavenumbers, discussed the absorption mode on the silver surface, the effect of pH value and the concentration of the porphyrin on the SERS, and finally analyzed the action model of double stranded DNA and single stranded DNA on the porphyrin.

2 EXPERIMENTAL

This SERS spectra were recorded by a Jobin-Yvon U-1000 double monochromator controlled by a computer, and a photon-controlled system with a thermoelectrically cooled RCA C31034A photomultiplier, An Ar^+ laser device was used as a radiation source, the UV-240 spectrophotometer was used to measure the concentration of DNA.

The department of chemistry, Wuhan University, furnished the copper (II) complex of meso-tetris (4-N-cyano methyl pyridyl) porphyrin; The colloidal silver solution was prepared in the manner described in reference 8 and 9;

Calf thymus DNA was purchased from Huamei Biotechnology Corporation and used as received. Bulk solutions were obtained by dissolving the nucleic acid in NaCl solution at 10^{-2} mol/l, stirred under 4 °C for 24 h, and then treated with supersonic vibrations for 20

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min. The concentration was determined (as basic couple) using an extinction coefficient ε_{260} = 13200. The solution of denatured single-stranded DNA was prepared by heating the solution of 1×10^{-3} mol/L double-stranded DNA in 90 °C water bath for 20 min, then cooling in ice-water bath immediately[10].

2.1 Experimental Procedure

Quantities of Cu^{II}NACN or its complexes were mixed with double-stranded DNA and denatured single-stranded DNA together with vary of silver sol, and sealed in a 2-mm inner diameter capillary tube, the tube was placed on the sample holder perpendicularly, Raman spectra were then recorded via 90° set-up. The laser wavelength was 488.0 or 514.5 nm, the emission slit was 400 μm, and the scan rate was $1 cm^{-1}/0.3 s$.

3 RESULTS AND DISCUSSION

3.1 RRS and SER Spectra of Cu^{II}NACN

Figure 1 illustrated the resonance Raman spectra of Cu^{II}NACN at 488.0 nm exciting and the surface-enhanced Raman spectra at 488.0 nm and 514.5 nm exciting wavelength respectively. The Table 1 lists the corresponding shifts and their assignments. It was indicated that although the concentration of Cu^{II}NACN was high, up to 2.5×10^{-4} mol/L, the laser wavelength 488.0 nm and the laser power 400 mW, the resonance Raman scattering intensity was still small. While with 488.0 nm and 514.5 nm exciting at the laser power 100 mW and 60 mW respectively, although the concentration of Cu^{II}NACN was 1×10^{-5} mol/L, the intensities of the SERS peaks were higher than RRS. It indicated that the surface enhancement of the silver sols was obvious, nevertheless the corresponding peaks were different in the extent of enhancement, these differences provided the information of the absorption pattern of the porphyrin on the surface of silver sols[11]. According to the class of the vibration mode of Zn^{II}TMPP(4) by Ok-Keun Song[11], we classified the vibration mode of Cu^{II}NACN into A, B, C and D kinds, and calculated the relative ratio of the SERS intensities to the RRS signals, which are listed in Table 1 with subscripts. Among the characteristic spectra of 225, 408, 670, 749, 803, 904, 1005, 1099, 1203, 1224, 1261, 1358, 1376, 1458, 1563, 1643 cm^{-1}, the bending mode of the pyridine and the in-plane vibration mode of the porphyrin were enhanced larger, and that the stretching mode of pyridine and the out-plane vibration mode of the porphyrin were enhanced smaller. If the molecule of porphyrin were face-on the surface of silver sols, the enhancement of the out-

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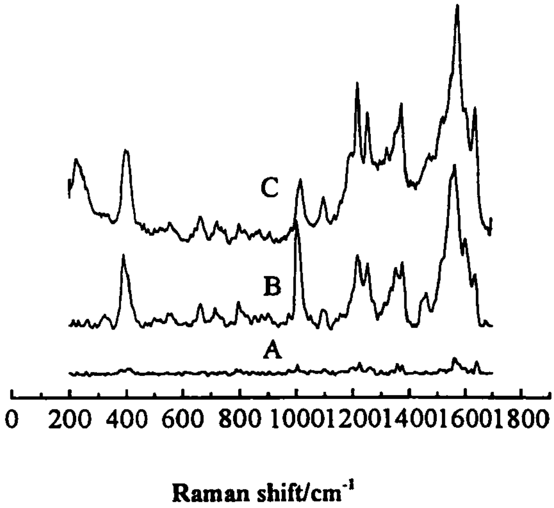

FIG. 1 RRS and SERS spectra of Cu^{II}NACN
A. RRS, 2.5×10^{-5} mol/L, 488.0 nm, 400 mW;
B. SERS, 1.5×10^{-5}mol/L, 488.0 nm, 100 mW;
C. SERS, 1.5×10^{-5}mol/L, 488.0 nm, 100 mW.

plane vibration mode of porphyrin would be larger than the in-plane vibration mode, If the molecule of porphyrin were edge-on the surface of silver sols, the enhancement of the in-plane vibration mode of porphyrin is then lager than the out-plane mode[12]. So it is reasonable to deduce that Cu^{II}NACN is tilted-relative to the surface of silver sols. For the porphyrin with the substituting group of pyridine, the popular thought is that the porphyrin is absorbed on the silver sols by the nitrogen in pyridine. But for Cu^{II}NACN, the -CN radical, the nitrogen in pyridine and the porphyrin core can be the site acting on the surface of silver sols. Because the bending mode involving pyridine and the in-plane mode of porphyrin are enhanced larger as analyzed above, so it is considered that the nitrogen of pyridine and the core of porphyrin are absorbed together on the surface of silver sols. It was clear that the peak of 1018 cm^{-1} in figure 1B shifts to 1005 cm^{-1} in figure 1C, and the peak of 1578 cm^{-1} in figure 1B shifts to 1565 cm^{-1} in figure 1C. This difference can be seen in the following experiment.

3.2The Effect of pH Value on the SERS of $Cu^{II}NACN$

Figure 2 illustrated the effect of pH value on the SERS of $Cu^{II}NACN$ with the exciting wavelength at 488.0 nm. When the pH = 11~12 (figure 2A), the peak of 226 cm^{-1} is weak up to the background, the peak of 399 cm^{-1} is broaden and has two shoulder peaks, the peaks in the range of 665~950 cm^{-1} are not clear, and the mode of 1018 cm^{-1} is weaker. When the pH = 2~3 (figure 2B), the peak of 226 cm^{-1} is still weak and approachs the background, the peak of 399 cm^{-1} is broader and has two shoulder peaks, the peaks in the range of 665~950 cm^{-1} are not clear, and the mode of 1018 cm^{-1} is weaker than those at pH=11~12, the mode of 1377 cm^{-1} is decreased to some extent. While the pH=5~6 (figure 2C), the SERS spectra are very good. The following experiment was carried out at pH=5~6.

3.3 The Effect of the Concentration on the SERS of $Cu^{II}NACN$

Figure 3 shows the SERS of $Cu^{II}NACN$ at different concentrations at pH=5~6. It is seen that the vibration modes of $Cu^{II}NACN$ except 226 cm^{-1} were enhanced gradually with the increasing the concentration from 1.3×10^{-6} mol/L to 1.2×10^{-5} mol/L. When the concentration of $Cu^{II}NACN$ was increased to 5.0×10^{-5} mol/L, the vibration modes all decreased. This indicated that the optimum SERS signals occurred at an optimum concentration, because the optimum SERS signal corresponds to the monolayer concentration of $Cu^{II}NACN$ on the surface of silver sols. 1.2×10^{-5} mol/L of $Cu^{II}NACN$ was shown to be the best.

3.4 The Effect of Double-stranded DNA and Denatured Single-stranded DNA on the SERS of $Cu^{II}NACN$

Figure 4 illustrates the SERS spectra of the complexes of $Cu^{II}NACN$ with the double-stranded DNA and the denatured single-stranded DNA. Figure 4A and figure 4B indicated that no SERS signal was obtained in the system of double-stranded DNA and the denatured single-stranded DNA with silver sols. Therefore the DNA for double-stranded and denatured single-stranded was not absorbed on the silver sols. The similar SERS spectra with $Cu^{II}NACN$ were obtained for the mixture of 100 fold of double-stranded DNA or denatured DNA to $Cu^{II}NACN$ incorporated in some amounts of silver sols. Table 1 gives the ratio of the intensities of SERS spectra of $Cu^{II}NACN$ to the intensities of RRS spectra.

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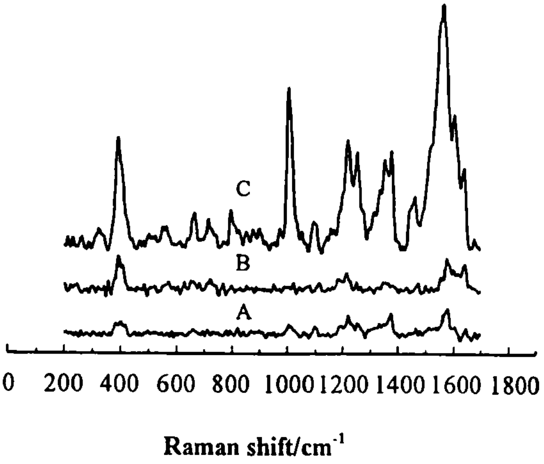

FIG. 2 Effect of pH on SERS of Cu^{II}NACN

A. pH=11~ 12, B. pH=2~ 3, C. pH=6~7

(other conditions as Fig. 1B for A and B).

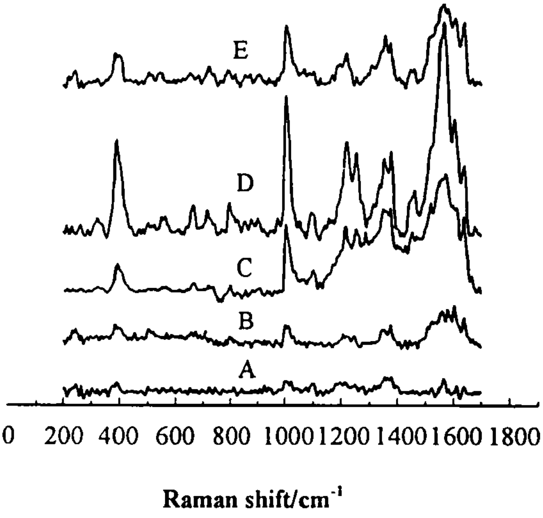

FIG. 3 Effect of the concentration of Cu^{II}NACN

A. 1.3×10^{-6} mol/L; B. 2.0×10^{-6} mol/L;

C. 7.0×10^{-6} mol/L; D. 1.2×10^{-5} mol/L;

E. 5.0×10^{-5} mol/L; other conditions as in Fig. 1C.

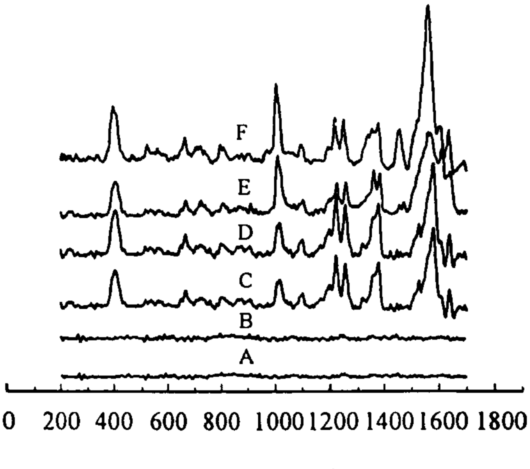

FIG. 4 Effect of double-stranded and denatured DNA on SERS of Cu^{II}NACN

A. SERS of double-stranded DNA. DNA(1×10^{-3} mol/L + Ag sols=1:2, other conditions as in Fig. 1C for A and B; B. SERS of denatured DNA. denatured DNA (1×10^{-3} mol/L)+Ag sols=1:2, other conditions as Fig. 1C; C. denature DNA(1×10^{-3} mol/L+Cu^{II}NACN(1×10^{-5} mol/L)+Ag sols=1:1:2, 488.0 nm, 100mW; D. denatured DNA(1×10^{-3} mol/L + Cu^{II}NACN (1×10^{-5} mol/L)+Ag sols=1:1:2, 514.5 nm, 100mW; E. double-stranded DNA(1×10^{-3} mol/L+Cu^{II}NACN(1×10^{-5} mol/L) + Ag sols = 1:1:2, 488.0 nm, 100mW; F. double-stranded DNA(1×10^{-3} mol/L+Cu^{II}NACN(1×10^{-5} mol/L) + Ag sols = 1:1:2, 514.5 nm, 100mW

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It was demonstrated that the pyridine stretching modes and the in-plane modes of Cu^{II}NACN were greatly enhanced for the systems of Cu^{II}NACN with double-stranded DNA and denatured DNA, thus the double-stranded DNA and denatured DNA did not change the tilted-orientation of CuIINACN on the surface of the silver sols. It was noted that the double-stranded DNA and denatured DNA caused the intensities of SERS of Cu^{II}NACN to decrease using laser 488.0 nm and 514.5 nm. The DNA at higher concentration masked the Raman scattering light of Cu^{II}NACN. The changed value of the shift before and after Cu^{II}NACN added (see table 2) indicated that different excitation

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TABLE 1 The Frequency Shifts and Assignment in RRS and SERS of Cu^{II}NACN

		RRS	SERS^{1B}	SERS^{1C}	SERS^{4E}	SERS^{4F}	SERS^{4C}	SERS^{4D}	assignment
	D	225	$226_{5.43}$	$232_{2.50}$	$234_{7.81}$		$230_{0.99}$	$242_{6.34}$	pyrrole tilting[11]
	B	408	$399_{8.25}$	$391_{6.98}$	$401_{8.00}$	$396_{8.92}$	$402_{8.76}$	$397_{4.99}$	Cu-N[13]
	A	670	$665_{5.47}$	$665_{4.77}$	$665_{6.59}$	$664_{7.93}$	$666_{8.27}$	$665_{4.63}$	δ(pyr)+δ(C-N$^+$CH$_2$-)[15]
	D	749	$723_{5.19}$	$717_{4.89}$	$729_{5.19}$	$722_{8.68}$	$723_{5.98}$	$724_{5.49}$	pyrrole X[11]
	C	803	$799_{3.53}$	$797_{4.82}$	$799_{4.18}$	$796_{7.29}$	$798_{4.58}$	$807_{3.55}$	pyr ν(C-C)+ν(N$^+$-CH$_2$-)[11]
	B	904	$905_{2.47}$	$901_{3.35}$	$906_{2.34}$	$906_{5.24}$	$908_{3.91}$	$912_{4.49}$	δ_s(por)[15]
I	B	1005	$1018_{5.38}$	$1005_{10.12}$	$1017_{4.20}$	$1005_{15.07}$	$1018_{5.25}$	$1011_{8.92}$	$\nu(C_\alpha\text{-}C_m)$[15]
II	B	1099	$1098_{5.15}$	$1094_{4.32}$	$1097_{4.03}$	$1095_{7.18}$	$1101_{5.13}$	$1105_{4.32}$	$\nu_{5a}(C_\beta\text{-}H)$[11]
III	A	1203	$1198_{6.72}$	$1198_{4.31}$	$1193_{4.60}$	$1195_{8.10}$	$1197_{6.19}$	$1199_{4.88}$	δ(pyr)+ν(N$^+$-CH$_2$-)[11]
IV	A	1224	$1220_{8.77}$	$1220_{5.48}$	$1219_{6.37}$	$1217_{9.64}$	$1221_{7.78}$	$1225_{5.37}$	C-pyridyl δ(pyr)[11]
V	A	1261	$1255_{8.70}$	$1253_{6.32}$	$1255_{6.96}$	$1249_{13.24}$	$1255_{9.00}$	$1258_{7.24}$	$\nu(C_m\text{-pyr})$
		1342	1323	1337	1330				
		1358		1353	1357	1353	1356	1361	
VI	B	1376	$1377_{8.05}$	$1378_{5.82}$	$1377_{5.56}$	$1377_{11.94}$	$1377_{7.82}$	$1383_{7.13}$	$\nu_4(C_\alpha\text{-}N)$[11]
VII	B	1458	$1473_{7.20}$	$1462_{5.11}$	$1471_{3.98}$	$1456_{15.86}$	$1471_{7.25}$	$1471_{6.46}$	$\nu_s(C_\alpha\text{-}C_\beta)$[10]
	B	1527	1523	1522			1526		$\nu_{20}\nu_{as}(C_\alpha\text{-}C_m)$[11]
VIII	B	1563	$1578_{8.15}$	$1565_{6.94}$	$1577_{6.40}$	$1560_{17.60}$	$1580_{8.27}$	$1561_{6.61}$	$\nu_2\nu_s(C_\beta\text{-}C_\beta)$[11]
	A	1623	$1606_{7.24}$	$1602_{6.67}$	$1607_{4.93}$	$1608_{14.37}$	$1608_{6.88}$	$1612_{8.24}$	δ(pyr)[15]
IX	C	1643	$1641_{5.22}$	$1638_{3.05}$	$1640_{3.74}$	$1637_{7.96}$	$1640_{5.10}$	$1641_{4.02}$	$[\nu\ (C\text{-}C)+\nu\ (C\text{-}N)]_{pyr}$[15]

1B, 1C, 4C, 4D, 4E and 4F are the SERS frequency shifts of Fig. 1B, 1C, 4C, 4D, 4E and 4F; I...IX: type of bands; A: bending mode of pyridyl; B: in-plane mode of porphyrin; C: stretching mode of pyridyl; D: out-plane mode of porphyrin; pyr: N-cyano-methyl pyridyl; por: core of porphyrin; ν: stretching; δ: bending or deforming; X: folding; s: symmetric; as: asymmetric; subscript: relative ratio of SERS intensity to RRS intensity.

TABLE 2 The Frequency Shift Changes in the SERS of Cu^{II}NACN with Double-strand DNA or Denatured DNA versus the SERS of Cu^{II}NACN

	I*	II	III	IV	V	VI	VII	VIII	IX
dDNA, 514.5nm**	0	+1	-3	-3	-4	-1	-6	-5	-1
dDNA, 488.0nm	-1	-1	-5	-1	0	0	-2	-1	-1
sDNA, 514.5nm	+6	+11	+1	+5	+5	+5	+9	-4	+3
sDNA, 488.0nm	0	+3	-1	+1	0	0	-2	+2	+1

Note: *I...IX are the types of the bands, **is the type of DNA, dDNA is double-stranded wavelength coincided with the different values. Laser at 488.0nm brought about the value smaller than 514.5 nm, so the inferior interaction exist between Cu^{II}NACN and DNA, the stronger the laser, the bigger the decrease of the weak interaction, the smaller the changed value. For the bands of II, V, IX, the changed values made by double-stranded DNA are +1(-1), 4(0) and -1(-1) cm^{-1}, while denatured single-stranded DNA are +11(+3), +5(0) and +3(+1) cm^{-1}. It was considered that Cu^{II}NACN did not intercalate the double-stranded DNA[16]. But electrostatic interaction exists between the -PO_4 radical in the double-stranded DNA and the pyridine nitrogen in Cu^{II}NACN. It corresponded to the conclusions of with RRS by Zhao[17]. It was believed that Cu^{II}NACN partly intercalated the denatured single-stranded DNA, the electrostatic interaction exists the same as double-stranded DNA.

DNA, sDNA is denatured single-stranded DNA, the numbers in the table are the frequency shifts changed.

4 CONCLUSION

The RRS and SERS spectra of Cu^{II}NACN with silver sols as the active carrier, the effect of pH value and the concentration of Cu^{II}NACN on the SERS of Cu^{II}NACN, the SERS of the complexes of Cu^{II}NACN with calf thymus double-stranded and denatured single-stranded DNA were investigated. It was shown that the silver sols enhanced the Raman scattering of Cu^{II}NACN at low levels. The Cu^{II}NACN was absorbed tilted-on the surface of the silver sols. The optimum pH value was 5~6, and the best suitable concentration of Cu^{II}NACN was about 1×10^{-5} mol/L. The pattern of tilted-on absorption of Cu^{II}NACN on the surface of silver sols was not changed by the addition of the double-stranded and denatured DNA. The radical of -PO_4 interacts with Cu^{II}NACN weakly by electrostatic force, Cu^{II}NACN may partially intercalate the denatured single-stranded DNA, and interacts with it by electrostatic force. The surface-enhanced Raman scattering technology can effectively used to obtain useful structural signals of the molecular complex of biological activity, and is a powerful tool to understand the biological mechanism of biological active species from molecular level.

ACKNOWLEDGMENT. This work was supported by the grant of the National Natural Science Foundation in China. We thank Prof. Suqu Huang for the generous gift of sample of Cu^{II}NACN.

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Date Received: December 8,1997
Date Accepted: May 20, 1998

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