a common principal axis system.[12] Moreover, since the weak outer features show precisely the same 16-G spacing and intensity distribution as the center quintet, and have characteristic line shapes, they can be assigned to the $M_1(^{13}C) = +1$ and -1 parallel components, the hyperfine tensors being axially symmetric. The corresponding perpendicular features are obscured by the strong central lines except for the two marked peaks, which are believed to correspond to the extremities of the perpendicular pattern.

A computer simulation of the powder spectrum[13] using the ESR parameters deduced by inspection[14] (Table I) shows a good fit to the experimental spectrum in Figure 2. The only detail that is not faithfully reproduced is the profile of the quartet super-hyperfine substructure from the matrix interaction.[1,15] This varied slightly with sample orientation and shows a greater dependence on the $M_1(^1H)$ value in the experimental spectrum. Nevertheless, this substructure is evident on all the lines and is clearly resolved in the outermost wing features of the parallel pattern, providing further proof that these outer components are related to the strong central quintet.[16]

The stringent requirement that the two ^{13}C hyperfine tensors have a common principal axis system is met by structure **1** since the carbon $2p_z$ orbitals possessing most of the spin density are parallel to each other. On the other hand, the carbon 2p orbitals of the SOMO of structure **2** are not parallel, and computer-simulated ESR spectra for this general case did not give strong central features, as expected.[17] The assignment to the $^2A_2$ state is also consistent with the magnitude of the spin populations in the carbon $2p_z$ orbitals as calculated from the ^{13}C hyperfine anisotropy (Table I). These results show that almost all the spin density resides in these two atomic orbitals, in agreement with structure **1**.[18,19]

(12) Cf.: Iwasaki, M.; *Fluorine Chem. Rev.* **1971**, 5, 1. See pp 14–16 of this review for a comparable example of selective ^{19}F anisotropic broadening in the powder ESR spectrum of the CF_2CONH_2 radical. In the present case, the sharpness of the center lines is accentuated by the nearly isotropic g tensor.

(13) Kasai, P. H.; McLeod, D., Jr.; McBay, H. C. *J. Am. Chem. Soc.* **1974**, 96, 6864. Kasai, P. H. *J. Am. Chem. Soc.* **1972**, 94, 5950.

(14) The ^1H hyperfine anisotropy is much less than that usually associated with α-hydrogens in a rigid structure. This is inconsistent with structure **2**[9] but can be explained in terms of structure **1** if the exo and endo hydrogens rapidly interchange positions since this would greatly reduce the large anisotropy for field directions in the molecular plane. In view of this dynamical model, the residual ^1H anisotropy in Table I is not associated with particular directions in the *xy* plane.

(15) Analysis of this matrix interaction in $CFCl_3$ is complicated by the fact that the precise form of the substructure changes gradually and reversibly with temperature.[1] Although coupling to a single chlorine ($I = {}^3/_2$) nucleus (Table I) seems more consistent with other studies of radical cation–Freon matrix interactions (Snow, L. D.; Williams, F. *Faraday Discuss. Chem. Soc.*, in press) it is, of course, possible that the apparent 1:3:3:1 pattern at 110 K results from coupling to three fortuitously equivalent fluorines, each fluorine ($I = {}^1/_2$) being necessarily in a separate $CFCl_3$ molecule.

(16) ESR studies of the doubly ^{13}C-labeled radical cation in the CF_3CCl_3, CCl_4, and SF_6 matrices also reveal the presence of an intense central ($M_1 = 0$) quintet although the $M_1 = \pm 1$ parallel components are generally not as well resolved as in the $CFCl_3$ matrix. In the CCl_4 matrix at 140 K, the center quintet consists of sharp lines with no substructure giving $A(4 H) = 16.0$ G and $g = 2.0024$, while a tentative analysis of the outer features yields the parameters $A_1(2-^{13}C) = 41.0$ G and $A_\perp(2-^{13}C) = 17.3$ G. Although these latter values differ from those obtained in $CFCl_3$ (Table I) at 110 K, the calculated isotropic values (25.1 ($CFCl_3$) and 25.2 G (CCl_4)) are very similar, suggesting that the reduced ^{13}C anisotropy in CCl_4 at 140 K results from greater motional averaging.

(17) Cf.: Shiotani, M.; Nagata, Y.; Sohma, J. *J. Am. Chem. Soc.* **1984**, 106, 4640.

(18) A referee has informed us that Dr. M. Iwasaki and his co-workers have recently reported the observation of a slightly asymmetric form of the planar ring-opened ethylene oxide radical cation[3,4] in the SF_6 matrix at 4–30 K (Muto, H.; et al. *Jpn. Radiat. Chem. Symp. 27th* **1984**, 87), the symmetric structure observed at higher temperatures being attributed to dynamical averaging between the two asymmetric forms.[3,4] We have examined the spectrum of the radical cation in $CFCl_3$ down to 4 K and the symmetric or time-averaged $^2A_2$ structure is retained down to ca. 15 K. Below this temperature the spectrum showed very distinct changes but the pattern is not easily analyzed, possibly on account of complications resulting from the matrix interaction.

(19) In contrast to the symmetric and asymmetric *planar* forms of the ring-opened ethylene oxide radical cation, we have recently observed that the localized and presumably twisted form of this cation is produced in the $CFCl_2CF_2Cl$ matrix at 77 K[20] (cf.: Qin, X.-Z.; Williams, F. *Chem. Phys. Lett.* **1984**, 112, 79. Qin, X.-Z.; Snow, L. D.; Williams, F. *J. Am. Chem. Soc.* **1984**, 106, 7640).

(20) Qin, X.-Z.; Snow, L. D.; Williams, F. *J. Phys. Chem.*, submitted for publication.

Determination of Adsorption Conformation from Surface Resolution Analysis

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The conformation of an adsorbed molecule on a surface is a key parameter which dictates the interactions, chemical and physical, that the molecule can undergo at the surface. This is so because adsorption, unlike dissolution, exposes specific moieties either outward to incoming reagents or inward to a (catalytic) surface and, because the nonsymmetric "one-sided-solvation" by the surface, distorts ground-state conformations of adsorbates, consequently affecting (photo)chemical reaction pathways.[1]

Despite its importance, conformational aspects of adsorbates are still poorly understood. A first-order approach is based on simple chemical arguments, e.g., that adsorbate/adsorbent relative alignment is determined by the availability of hydrogen bonds. Fine tuning of this picture is done by determining the effective cross-sectional area (σ) of the adsorbate by one of several methods:[2] (a) By the use of a molecular model and its projections,[3,4] it is possible to provide a rough estimate of σ for rigid molecules, e.g., aromatic polycyclic hydrocarbons. (b) σ can be calculated from liquid density values, assuming sphericity.[2] This assumption limits the method to short or branched molecules. The two methods are further limited to adsorbates for which conformational distortion by adsorption can be neglected. (c) Methods a and b do not provide information on the *effective* σ, i.e., a value taking into account the envelope of the many conformers of flexible adsorbates and the empty areas, if such exist, between one occupied adsorption site and the next. A common method which tries to overcome this problem indirectly is to divide the surface area from the N_2-BET method[5] by the monolayer value of the investigated molecule.[2,6] This procedure is proper only for smooth surfaces, i.e., for surfaces of low fractal dimension, in which the available surface area is independent of probe size. An unfortunate common error is to apply this procedure to highly irregular surfaces for which surface accessibility is strongly dependent on probe size.[1] Furthermore, it is quite unclear whether values determined for smooth surfaces are applicable to wiggly ones.

Here we wish to report on a new approach in conformational analysis of adsorbates, which seems to be free of the above-mentioned disadvantages. It elucidates σ directly from the adsorption experiment and from resolution analysis of the surface. Resolution (fractal) analysis is a rapidly growing tool in many domains of natural sciences, where geometrical irregularity characterizes the investigated system.[7]

(1) E.g.: Bauer, R. K.; Bornstein, R.; De Mayo, P.; Okada, K.; Rafalska, M.; Ware, W. R.; Wu, K. C. *J. Am. Chem. Soc.* **1982**, 104, 4635.

(2) McClellan, A. L.; Harnsberger, H. F. *J. Colloid Interface Sci.* **1967**, 23, 577.

(3) Amoore, J. E. *Ann. N. Y. Acad. Sci.* **1964**, 116, 457.

(4) Scott, R. P. W,; Simpson, C. F. *Faraday Symp. R. Soc. Chem.* **1980**, 15, 69.

(5) Brunauer, S.; Emmett, P. H.; Teller, E. *J. Am. Chem. Soc.* **1938**, 60, 309.

(6) E.g.: Nay, M. A.; Morrison, J. L. *Can. J. Res., Sect. B* **1949**, 27, 205.

Table I. Monolayer Values[a] of Various Adsorbates on Silica Gel Fractions

av part diam μm	ethanol/ toluene[b]	monolayer values, mmol/g *tert*-amyl alcohol/decalin[b]	naphthalene/ cyclohexane[c]
215			0.49
190	3.04	1.93	0.58
170	3.21	1.85	0.55
150	3.12	1.98	
133	3.09	1.83	
116			0.51
113	3.26	1.95	
90	3.21	1.95	
83			0.59
72	2.89	1.98	
69			0.48
D	3.04 ± 0.05	2.96 ± 0.04	3.03 ± 0.09

[a] From Langmuir equation.[11] Experimental points for analysis were taken from zero up to beginning of the isotherms' plateau. "B-point" analyses[11] yield the same results. [b] Equilibrium concentrations from refraction indices.[13] [c] Equilibrium concentrations from UV absorption.
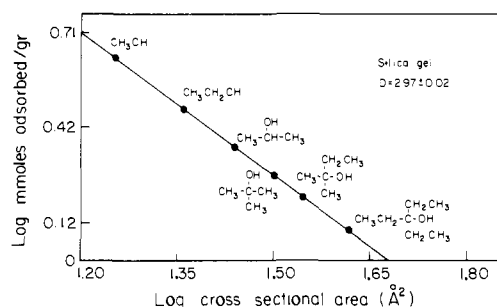

Figure 1. Monolayer values of a series of alcohols as a function of cross-sectional areas. See footnotes a and b in Table I. CH_3OH monolayer was determined by the method of Hoffmann et al.[13]

The idea is based on our finding,[8] corroborated recently in other studies,[9,10] that a large volume of adsorption data obeys the scaling laws

$$n \propto \sigma^{-D/2} \tag{1}$$

$$n \propto R^{D-3} \tag{2}$$

where in eq 1 monolayer values (n, mol/g) are determined as a function of probe-molecule size (σ) and in eq 2 as a function of particle radius for a fixed probe.[8d] The parameter D is the fractal dimension of the surface, and its value ($2 \leqslant D < 3$) is an indication of the degree of surface irregularity. equations 1 and 2 are two forms of resolution analysis: in eq 1 one looks at the surface with different "magnifications" by changing the probe size; in eq 2 the particles are "magnified". In principle, the resolution analysis can be carried out backward: once D is known, and n of the investigated molecule measured, the size of the yardstick (σ) can be calculated (eq 1). We exemplify this approach for the case of linear n-alkanols adsorbed on silica gel. We have chosen

(7) A noncomprehensive list of some recent examples: (a) Paumgartner, D.; Losa, G.; Weibel, E. R. *J. Microsc.* (Oxford) **1981**, *121*, 51. (b) Lovejoy, S. *Science (Washington, D.C.)* **1982**, *216*, 185. (c) Allen, J. P.; Colvin, J. T.; Stinson, D. G.; Flynn, C. P.; Stapleton, H. J. *Biophys. J.* **1982**, *38*, 299. (d) Weitz, D. A.; Oliveria, M. *Phys. Rev. Lett.* **1984**, *52*, 1433. (e) Mandelbrot, B. B.; Passoja, D. E.; Paullay, A. J. *Nature (London)* **1984**, *308*, 721. (f) Brady, R. M.; Ball, R. C. *Nature (London)* **1984**, *309*, 225. (g) Klymko, P. W.; Kopelman, R. *J. Phys. Chem.* **1983**, *87*, 4565. (h) Pfeifer, P.; Welz, U.; Wippermann, H. *Chem. Phys. Lett.*, **1985**, *113*, 535. (i) Farin, D.; Peleg, S.; Yavin, D.; Avnir, D. *Langmuir*, in press.

(8) Some recent references: (a) Avnir, D.; Farin, D.; Pfeifer, P. *Nature (London)* **1984**, *308*, 261. (b) Avnir, D.; Farin, D.; Pfeifer, P. *J. Colloid Interface Sci.* **1985**, *103*, 112. (c) Pfeifer, P.; Avnir, D.; Farin, D. *J. Stat. Phys.* **1984**, *36*, 699. (d) Pfeifer, P.; Avnir, D. *J. Chem. Phys.* **1983**, *79*, 3558; **1984**, *80*, 4573. (e) Avnir, D.; Farin, D.; Pfeifer, P. *J. Chem. Phys.* **1983**, *79*, 3566.

(9) Van Damme, H.; Fripiat, J. J. *J. Chem. Phys.*, **1985**, *82*, 2785.

(10) Drake, M.; Klafter, J. *J. Lumin.*, **1984**, *31–32*, 642.

Table II. Adsorption Conformation of Linear 1-Alkanols

alcohol	n, mmol/g[a]	σ, Å^{2b}	ellipsoid axes short axis[c] (s), Å	long axis[d] (l), Å	l/s
1-butanol	2.32	27.2	2.9	7.1	2.4
1-hexanol	1.95	30.5	3.1	9.6	3.1
1-octanol	1.68	33.8	3.2	12.1	3.8
1-decanol	1.55	35.6	3.4	14.6	4.3
1-dodecanol	1.34	39.3	3.5	17.1	4.9

[a] Silica/toluene interface. See footnotes a and b in Table I. [b] From eq 1 with the parameters of Figure 1: log n = 2.50 − 2.97/2 log σ. [c] From σ. [d] Rod-conformer length.
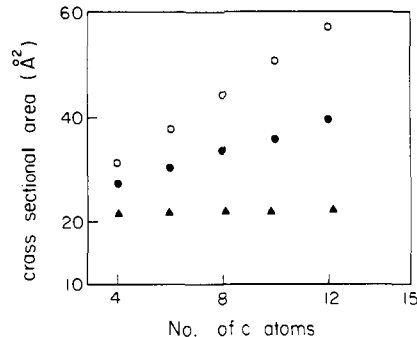

Figure 2. Cross-sectional areas of adsorbed linear alkanols on silica: (O) from liquid density,[2] (●) this work, (▲) rod-shaped[11] conformer.

n-alkanols because it seemed to us that the currently used conformational assumptions for linear molecules, i.e., rodlike with fixed σ[4,11] and a spherical envelope of conformers,[2] may be oversimplified.

Silica gel has a D value close to the limit 3 (i.e., its surface is so wiggly that its points are almost space filling), as indicated by a number of independent measurements: (a) Silica gel[12] was sieved to fractions (Table I) and monolayer values determined by adsorption of *tert*-amyl alcohol from decalin. From eq 2, D = 2.96 ± 0.04. Similar D values were obtained by adsorption of naphthalene from cyclohexane and by adsorption of ethanol from toluene (Table I). (b) For a series of alcohols, for which the liquid density method for σ determination (see above) seems adequate, monolayer values for silica/toluene interface were determined. Equation 1 is nicely obeyed (Figure 1) with D = 2.97 ± 0.02. (c) Analysis of published adsorption data[13] for silica yielded D = 2.94 ± 0.04.[14] (d) Woelm reports that on its silica surface area is independent of particle size.[15] Indeed, for D = 3, eq 2 becomes n = constant. (e) Small angle X-ray scattering experiments[16] performed by Bale, Xie, and Schmidt on a sample of our silica[17] suggest, again, D = 3.

Conformational analysis of the n-alkanols was done by determining monolayer values on silica (Table II) and then applying eq 1 with D = 2.97. The σ values thus obtained (Table II) indicate (Figure 2) that the conformational envelope of the linear alkanols is neither linear nor spherical (see above) but in between, i.e., prolate. The experimental points follow an empirical relation between σ and the number of carbons (C_n):

$$\sigma = 1.46(\pm 0.08)C_n + 21.6(\pm 0.7) \tag{3}$$

$$C_n = 4\text{-}12, \qquad \text{correlation coefficient } 0.996$$

Taking the longest axis of the ellipsoid to be the rod conformer,

(11) Kipling, J. J. "Adsorption from Solution of Non-Electrolytes"; Academic Press: New York, 1965.

(12) Silica, Woelm, No. 04662; Lot No. 90 754/B; particle size 63–200 μm; N$_2$-BET surface area 500–600 m^2/g; average pore diameter 60 Å.

(13) Hoffmann, R. L.; McConnell, D. G.; List, G. R.; Evans, C. D. *Science (Washington, D.C.)* **1967**, *157*, 550.

(14) Avnir, D.; Pfifer, P. *Nouv. J. Chim.* **1983**, *7*, 71.

(15) Woelm-Technical Brochure No. Al-20.

(16) Bale, H. D.; Schmidt, P. W. *Phys. Rev. Lett.* **1984**, *53*, 596; Proceedings of Symposium P (Fractal Aspects of Materials), Materials Research Society Meeting, Boston, 1984.

(17) Schmidt, P. W., private communication.

eq 3 indicates that the prolate conformation envelope becomes more elongated as C_n grows (Table II), i.e., that the relative weight of trans–rich conformers increases with C_n.

We have briefly described the application of surface resolution analysis to a basic problem in heterogeneous chemistry and demonstrated it by obtaining what seems to us a more realistic picture of the adsorption of monofunctional linear molecules. Other conformational aspects of adsorbates on inert and on catalytic surfaces are currently being explored by the new tool described here.

Ultraviolet Resonance Raman Spectra of Hemoglobin Excited at 200 and 218 nm: Tertiary and Quaternary Structure Differences

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We report resonance Raman (RR) spectra excited at 218 and 200 nm (Figures 1 and 2), using an H_2 Raman-shifted quadrupled YAG laser, for fluoromethemoglobin (metHb(F⁻)), with and without the effector molecule inositol hexaphosphate (IHP). Selectively enhanced modes of tryptophan (218 nm) and tyrosine (200 nm) show clear intensity differences among these species, which are interpretable in terms of changes in the environment of aromatic residues at the subunit interfaces. Hemoglobin is a tetrameric protein which adopts alternative quaternary structures, T and R, associated respectively with the deoxy and ligated protein.[1] It is generally accepted that the switch between the two accounts for the main features of cooperativity in ligand binding by hemoglobin,[1,2] although the importance of intermediate structures remains a matter of controversy. Met forms of hemoglobin, which contain Fe^{III} and bound H_2O or added exogenous ligands, are in the R quaternary state; but in the case of metHb(F⁻), addition of IHP switches the quaternary structure to T, as judged by a comparison of the crystal structure of IHP-bound metHb(F⁻) from horse with that of human deoxy-hemoglobin[3] and by NMR[4] and optical[5] studies.

Ultraviolet laser excitation has recently been shown to produce strong and characteristic enhancement patterns for the vibrational Raman modes of benzene[6] and its derivatives[7] and of protein aromatic side chains.[8-11] Asher[11] has shown myoglobin Raman

(1) (a) Perutz, M. F. *Br. Med. Bull.* **1976,** *32,* 195–208. (b) Dickerson, R. E.; Geis, I. "Hemoglobin"; Benjamin/Cummings Publishing Co., Inc.: Menlo Park, CA, 1983. (c) Perutz, M. F. *Annu. Rev. Biochem.* **1979,** *48,* 327–386.
(2) Hopfield, J. J.; Shulman, R. G.; Ogawa, S. *J. Mol. Biol.* **1971,** *61,* 425–443.
(3) Fermi, G.; Perutz, M. F. *J. Mol. Biol.* **1977,** *114,* 421–431.
(4) Perutz, M. F.; Sanders, J. K. M.; Chenergy, D. H.; Nobel, R. W.; Pennelly, R. R.; Fung, L. W.-M.; Ho, C.; Gianniani, I.; Porschke, D.; Winkler, H. *Biochemistry* **1978,** *17,* 3640.
(5) Perutz, M. F.; Fersht, A. R.; Simon, S. A.; Roberts, G. C. K. *Biochemistry* **1974,** *13,* 2174–2186.
(6) Ziegler, L. D.; Hudson, B. *J. Chem. Phys.* **1981,** *74,* 982.
(7) Ziegler, L. D.; Hudson, B. *J. Chem. Phys.* **1983,** *79,* 1134.
(8) Rava, R. P.; Spiro, T. G. *J. Am. Chem. Soc.* **1984,** *106,* 4062.
(9) Rava, R. P.; Spiro, T. G. *Biochemistry,* in press.
(10) Rava, R. P.; Spiro, T. G. *J. Chem. Phys.,* in press.
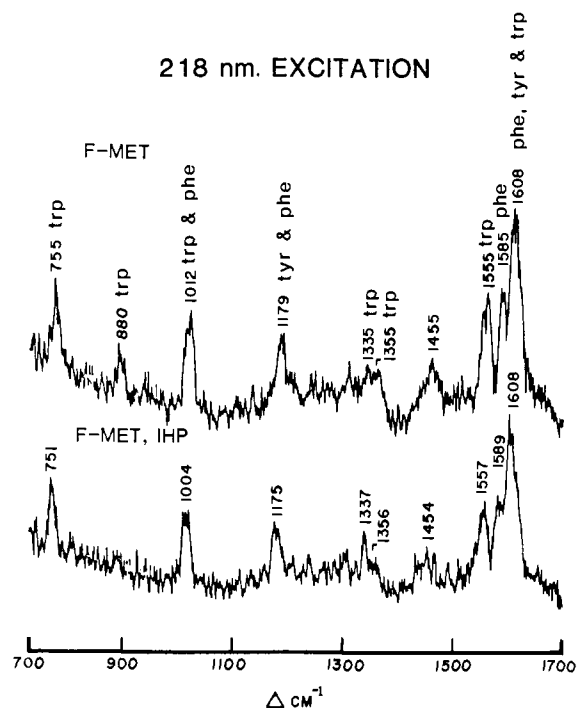

Figure 1. Ultraviolet resonance Raman spectra of 200 μM human hemoglobin in the metHb(F⁻) form without (top) and with (bottom) IHP, with 218-nm excitation. Each spectrum is the sum of 10 scans collected with 0.05 Å/s accumulation time. All other conditions were as in ref 8. MetHb(F⁻) was prepared using the method described by Asher et al.[17] IHP was added, in solid form, to 3 mol per mol of tetramer as described in ref 4. All solutions were buffered with 50 mM phosphate (pH 6.8); 50 mM tris buffer (pH 6.8) gave identical results.

spectra excited at ~230 nm with no discernable contributions from the heme, which dominates Raman scattering when visible laser excitation is used.[12]

The 218-nm spectra (Figure 1) of metHb(F⁻) show several tryptophan (Trp) ring modes, as well as some contributions from tyrosine (Tyr) and phenylalanine (Phe).[9] The following changes in the Trp modes are seen upon IHP addition: (1) a 4-cm⁻¹ downshift of the 755-cm⁻¹ band; (2) dramatic intensity decreases for the bands at 880 and 1012 cm⁻¹ (the latter produces an apparent downshift of the composite Trp and Phe band to 1004 cm⁻¹, close to the Phe frequency); (3) a shift in intensity from the 1355- to the 1335-cm⁻¹ band. A 1355- → 1335-cm⁻¹ intensity shift was previously observed[9] upon partial unfolding of the protein α-lactalbumin at low pH, but in that case the 876-cm⁻¹ band moved up 4 cm⁻¹, without intensity lowering, while the 759-cm⁻¹ band did not shift. The Hb A tetramer contains three pairs of symmetry-related tryptophans.[1,13] Two of these (α14 and β15) occur on the surface of the protein and are unaffected by changes in quaternary structure. The third pair (β37), however, occurs in the "flexible joint" region[13] near the interfaces between the βC and αFG helices. In the T structure, Trp β37 stacks against Tyr α 42, and this interaction is lost in the R structure; we attribute the RR alternations to this disruption. Perutz and co-workers[5] have shown that the T–R absorption difference spectrum for metHb(F⁻) has a hyperchromic contribution at 294 and 302 nm attributable to Trp β37. We were unable to obtain satisfactory RR spectra near this wavelength (266 nm), but the 218-nm RR spectra show clear *hypo*chromism for the 880- and 1012-cm⁻¹ Trp modes. We speculate that the Trp β37-Tyr α42 interaction produces a shift in Trp electronic oscillator strength from the 220-

(11) Johnson, C. R.; Ludwig, M.; O'Donnell, S.; Asher, S. A. *J. Am. Chem. Soc.* **1984,** *106,* 5008–5010.
(12) Spiro, T. G. In "The Porphyrins"; Lever, A. B. P., Gray, H. B., Eds.; Addison-Wesley: Reading, MA, 1983; Part II, pp89–159.
(13) Perutz, M. F.; Fermi, G. "Haemoglobin and Myoglobin, Atlas of Molecular Structures in Biology"; Oxford University Press: New York, 1981; Vol. 2.