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Linear Algebraic Analyses of Structures with One Predominant Type of Anomalous Scatterer

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Abstract

Further studies have been made of the information content of the exact linear equations for analyzing anomalous dispersion data in one-wavelength experiments. The case of interest concerns structures containing atoms that essentially do not scatter anomalously and one type of anomalously scattering atoms. For this case, there are three alternative ways of writing the equations. The alternative sets of equations and the transformations for transforming one set into the other are given explicitly. Comparison calculations were made with different sets of equations. Isomorphous replacement information is readily introduced into the calculations and the advantage of doing so is clearly illustrated by the results. Another aspect of the potential of the exact linear algebraic theory is its application to multiple-wavelength experiments. Successful applications of the latter have been made by several collaborative groups of investigators.

Introduction

By means of an algebraic analysis in which the contributions to a structure factor from the real and imaginary anomalous corrections to normal atomic scattering factors are treated separately from that from the normal part, it was possible to develop an exact linear system of simultaneous equations for extracting phase and intensity information from multiwavelength anomalous-dispersion experiments (Karle, 1980). The system of equations applies to any number and types of anomalous scatterers.

It was further indicated that essentially unique values for the phase differences that occur in the

equations can be obtained in a one-wavelength experiment for the case of structures composed of non-anomalous scatterers and one predominant type of anomalous scatterer (Karle, 1985) even though the equations in this case contain a twofold ambiguity. This is achieved by using a least-squares technique for solving the equations in which the process is initiated with statistically reasonable starting values for the unknown quantities. In the one-wavelength calculation, the number of unknown quantities exceeds the number of equations by one. Therefore, one of the starting values obtained from the statistical estimates is held fixed throughout the calculation.

This investigation is concerned with the case of structures composed of non-anomalous scatterers and one predominant type of anomalous scatterer (Karle, 1985). An alternative algebraic description of this case was derived by Hendrickson (1987) in which somewhat different unknown quantities occur. The relationship between the two forms of the equations is described here. In addition, a third form for the equations is presented. The alternatives combined with the original formulation give rise to a new system of equations whose characteristics were investigated.

The purpose of these studies is to examine further the information content of the linear algebraic equations. One-wavelength data are considered here. For one-wavelength data, errors in the unknown phase differences depend on the accuracy of the quantities estimated to make the equations definitive as well as the accuracy of the data and the starting values for the unknown quantities in a least-squares process. The latter values determine whether the more accurate of two possible answers in an ambiguous calculation will be obtained.

With data from two or more wavelengths, the problem is overdetermined and exact data would give unique exact values for the unknown quantities. Errors in the calculated phase differences would be solely dependent on the accuracy of the data, particularly the Bijvoet differences. This is influenced in part by the relative strength of the anomalous signal. Some insight into this matter has been given in several successful structural investigations undertaken quite recently by W. A. Hendrickson and colleagues (Smith, Pahler, Murthy & Hendrickson, 1987). The latter investigations have also involved the development of various technical aspects of the multiple-wavelength anomalous dispersion (MAD) technique.

Theory

The mathematics appropriate to structures composed of non-anomalous scattering atoms and one type of anomalously scattering atoms is now described. It is convenient to make the following definitions:

$$\chi_1 = |F_{1,h}^n|^2 \quad (1)$$

$$\chi_2 = |F_{2,h}^n|^2 \quad (2)$$

$$\chi_3 = |F_{1,h}^n| |F_{2,h}^n| \cos(\varphi_{1,h}^n - \varphi_{2,h}^n) \quad (3)$$

$$\chi_4 = |F_{1,h}^n| |F_{2,h}^n| \sin(\varphi_{1,h}^n - \varphi_{2,h}^n) \quad (4)$$

$$\chi_5 = |F_{i,h}^n|^2 \quad (5)$$

$$\chi_6 = |F_{i,h}^n| |F_{2,h}^n| \cos(\varphi_{i,h}^n - \varphi_{2,h}^n) \quad (6)$$

$$\chi_7 = |F_{i,h}^n| |F_{2,h}^n| \sin(\varphi_{i,h}^n - \varphi_{2,h}^n) \quad (7)$$

$$\chi_8 = |F_{i,h}^n| |F_{1,h}^n| \cos(\varphi_{i,h}^n - \varphi_{1,h}^n) \quad (8)$$

$$\chi_9 = |F_{i,h}^n| |F_{1,h}^n| \sin(\varphi_{i,h}^n - \varphi_{1,h}^n) \quad (9)$$

where the $|F_{1,h}^n|$ are the structure-factor magnitudes for the non-anomalous scattering atoms, the $|F_{2,h}^n|$ those for the anomalously scattering atoms when scattering normally and the $|F_{i,h}^n|$ for all the atoms when scattering normally. The $\varphi_{1,h}^n$, $\varphi_{2,h}^n$ and $\varphi_{i,h}^n$ are the corresponding phase angles, respectively.

Coefficients that will appear in forthcoming equations are defined in terms of the normal atomic scattering factor, f^n , and the real and imaginary corrections for anomalous dispersion, f' and f'' , respectively, as follows:

$$\alpha_h = 1 + 2f'/f_{2,h}^n + (f'^2 + f''^2)/(f_{2,h}^n)^2 \quad (10)$$

$$\beta_h = 2(1 + f'/f_{2,h}^n) \quad (11)$$

$$\gamma_h = 2f''/f_{2,h}^n \quad (12)$$

$$\delta_h = (f'^2 + f''^2)/(f_{2,h}^n)^2 \quad (13)$$

$$\varepsilon_h = 2f'/f_{2,h}^n \quad (14)$$

The values for these coefficients are readily obtained from the tabulated values of the atomic scattering factors and, for K absorptions, the real and imaginary

corrections for anomalous dispersion. The tables of the corrections are not suitable close to the absorption edges. Such information must be obtained experimentally. This also applies, for example, to L absorptions immediately in the vicinity of the edge and otherwise.

The quantities defined in (1)–(14) form the following 14 equations:

$$|F_{\lambda,h}|^2 = \chi_1 + \alpha_h \chi_2 + \beta_h \chi_3 + \gamma_h \chi_4 \quad (15)$$

$$|F_{\lambda,\bar{h}}|^2 = \chi_1 + \alpha_h \chi_2 + \beta_h \chi_3 - \gamma_h \chi_4 \quad (16)$$

$$\chi_3^2 + \chi_4^2 = \chi_1 \chi_2 \quad (17)$$

$$\chi_5 = \chi_1 + \chi_2 + 2\chi_3 \quad (18)$$

$$\chi_2 + \chi_3 = \chi_6 \quad (19)$$

$$\chi_7 = \chi_4 \quad (20)$$

$$|F_{\lambda,h}|^2 = \chi_5 + \delta_h \chi_2 + \varepsilon_h \chi_6 + \gamma_h \chi_7 \quad (21)$$

$$|F_{\lambda,\bar{h}}|^2 = \chi_5 + \delta_h \chi_2 + \varepsilon_h \chi_6 - \gamma_h \chi_7 \quad (22)$$

$$\chi_6^2 + \chi_7^2 = \chi_5 \chi_2 \quad (23)$$

$$\chi_1 + \chi_3 = \chi_8 \quad (24)$$

$$\chi_9 = -\chi_4 \quad (25)$$

$$|F_{\lambda,h}|^2 = \alpha_h \chi_5 + \delta_h \chi_1 - (\varepsilon_h + 2\delta_h) \chi_8 - \gamma_h \chi_9 \quad (26)$$

$$|F_{\lambda,\bar{h}}|^2 = \alpha_h \chi_5 + \delta_h \chi_1 - (\varepsilon_h + 2\delta_h) \chi_8 + \gamma_h \chi_9 \quad (27)$$

$$\chi_8^2 + \chi_9^2 = \chi_5 \chi_1 \quad (28)$$

Some of the equations (15)–(28) originated in previous work and others are new contributions. Equations (15)–(17) were derived earlier (Karle, 1980). The same is true for (21)–(23) (Hendrickson, 1987). The remaining equations were included for several reasons.

The differences between (15)–(17) and (21)–(23) are the replacement of $|F_{1,h}^n|$ and $\varphi_{1,h}^n$ by $|F_{i,h}^n|$ and $\varphi_{i,h}^n$, respectively, and some differences in the coefficients. The two sets of equations are equivalent. It is possible to transform (15)–(17) to (21)–(23) by use of (18)–(20).

On observing that (15)–(17) involve $|F_{1,h}^n|$ and $|F_{2,h}^n|$ and that (21)–(23) involve $|F_{i,h}^n|$ and $|F_{2,h}^n|$, the question arises of whether a third equivalent system occurs that involves the pair $|F_{i,h}^n|$ and $|F_{1,h}^n|$. Such a system is given by (26)–(28). It may be formed from (15)–(17) by use of (18), (24) and (25) as transformation equations.

Equations (15)–(17) are an independent set. Equations (21)–(23) and (26)–(28) also form independent sets. The three sets, however, are not independent of each other. It is seen that, in addition to the linear equations, (17), (23) and (28) are quadratic. They derive from the fact that $\sin^2 \varphi + \cos^2 \varphi = 1$. In general, their use can reduce the number of wavelengths required to generate a definitive number

of independent simultaneous equations and does not interfere with the least-squares procedure that is used to solve for the unknown quantities.

Method of calculation

Solutions for (15)–(28) or subsets of them are obtained by least-squares calculations. Formulas for obtaining starting values for the calculations are now presented.

Initial values for $|F_{i,h}^n|$ and $|F_{1,h}^n|$ may be obtained by use of Wilson (1949) intensity statistics from (Karle, 1984)

$$|F_{i,h}^n| \approx 0.5 W_{\lambda,h} (|F_{\lambda,h}| + |F_{\lambda,\bar{h}}|), \quad (29)$$

where

$$W_{\lambda,h} = \left\{ \frac{\sum_{j=1}^{N_{\text{non}}} f_{jh}^2 + \sum_{j=1}^{N_{\text{ano}}} (f_{jh}^n)^2}{\sum_{j=1}^{N_{\text{non}}} f_{jh}^2 + \sum_{j=1}^{N_{\text{ano}}} [(f_{jh}^n + f_j')^2 + f_j'']^2} \right\}^{1/2}, \quad (30)$$

f_{jh}^n is the normal part of the atomic scattering factor for the j th atom and f_j' and f_j'' are the real and imaginary corrections, respectively. The total atomic scattering factor, f_{jh} , is equal to f_{jh}^n for non-anomalously scattering atoms. N_{non} is the number of non-anomalously scattering atoms and N_{ano} is the number of anomalously scattering atoms in a unit cell. Initial values for $|F_{1,h}^n|$ are obtained from (29) and (30) by omitting the second term in the numerator on the right-hand side of (30).

Starting values for $|F_{2,h}^n|^2$ can be obtained from

$$|F_{2,h}^n|^2 \approx S \{ \left| |F_{\lambda,h}| - |F_{\lambda,\bar{h}}| \right| / [2(f''/f_{2,h}^n)] \}^2, \quad (31)$$

where $f_{2,h}^n$ is the normal atomic scattering factor for the anomalously scattering atoms and S is a scale factor that is equal to 1 when the angles $\varphi_{\lambda,h}$ and $-\varphi_{\lambda,\bar{h}}$ are equal. An estimate of values for S can be based on test examples having the same atomic composition as the substance of interest. For the test examples, a list was made of the differences $\left| |F_{\lambda,h}| - |F_{\lambda,\bar{h}}| \right|$ in order of decreasing magnitude. For successive subsets of these differences, average values of the known functions on both sides of (31) are evaluated, giving an average value for S for each of the subsets. If the number of differences in each of the subsets is sufficiently large to give average values of S for the test examples with small statistical variance, these values can be used for the unknown structure.

Once initial values for $|F_{i,h}^n|^2$, $|F_{1,h}^n|^2$ and $|F_{2,h}^n|^2$ are obtained, as indicated, the remaining unknown quantities χ_3 , χ_4 , χ_6 , χ_7 , χ_8 and χ_9 can be evaluated by taking the sum and difference of (15) and (16) for χ_3 and χ_4 , of (21) and (22) for χ_6 and χ_7 and of (26) and (27) for χ_8 and χ_9 , respectively.

There are also transformation equations that transform (21) and (22) to (26) and (27). They are given by

$$\chi_5 + \chi_1 - \chi_2 - 2\chi_8 = 0 \quad (32)$$

$$\chi_5 - \chi_6 - \chi_8 = 0 \quad (33)$$

$$\chi_7 + \chi_9 = 0. \quad (34)$$

Equations (32)–(34) were not used in the calculations presented in this paper.

Test calculations and discussion

Test calculations were made on cytochrome c550.PtCl₄²⁻ (Timkovich & Dickerson, 1976) and two elastases (Shotton & Watson, 1970; Watson, Shotton, Cox & Muirhead, 1970), one with an Hg substitution and a second with a U substitution. In making the calculations, it was assumed that there was one Pt atom per molecule of cytochrome and one Hg atom or one U atom per molecule of elastase. The radiation was assumed to be Cu K α and no thermal factors entered since it was assumed that overall correction for positional disorder would be made by use, for example, of Wilson statistics. In these tests, the number of equations did not exceed the number of unknown quantities.

As seen in Table 1, single-wavelength calculations of averages of the magnitudes of error for the phase differences, $\varphi_{1,h}^n - \varphi_{2,h}^n$, for cytochrome c550.PtCl₄²⁻, with only Pt treated as an anomalous scatterer, gave results that parallel those obtained in an earlier publication (Karle, 1985; Table 1). The calculations were performed here in a somewhat different fashion from those in the earlier paper. Instead of using three different starting values for $|F_{1,h}^n|^2$, only one value, obtained from (29) and an appropriately modified (30) as described above, was used. Estimates for $|F_{2,h}^n|^2$ were obtained from (31) and when using the 14 equations, (15)–(28), initial values for $|F_{i,h}^n|^2$ were obtained from (29) and (30). Errors in the phase differences occur from errors in the estimated $|F_{2,h}^n|^2$ which are held fixed in the calculations, errors in the starting values of $|F_{1,h}^n|^2$ and $|F_{i,h}^n|^2$, errors in the starting values of the cosines and sines of the phase differences and errors introduced into $|F_{\lambda,h}| - |F_{\lambda,\bar{h}}|$. Errors in the starting estimations of $|F_{1,h}^n|^2$ and $|F_{i,h}^n|^2$ and the cosines and sines of phase differences can cause, on occasion, a convergence of the least-squares calculation to one of the two alternative minima which is farthest from the correct answer. It is seen from Table 1 that there is little difference between the use of the 3 or 14 equations except perhaps for the case of U elastase with a 1.50 error factor, where use of 14 equations has produced in some cases about 0.15 rad increase in error. The errors in the magnitudes of the phase differences, $\varphi_{1,h}^n - \varphi_{2,h}^n$, as shown in Table 1, are comparable except for an increase manifested for U elastase, particularly for an error factor of 0.50.

Further calculations were made to ascertain the improvement to be expected from obtaining values for $|F_{1,h}^n|^2$ from isomorphous replacement rather than

Table 1. Averages of the magnitudes of error for the phase differences, $\varphi_{1,h}^n - \varphi_{2,h}^n$, as a function of sampling level and errors in the data for the three test structures

Sampling level is a point at some fraction of the total data set, listed in decreasing values of $||F_{\lambda,h}| - |F_{\lambda,h}||$, and the average magnitudes of error in the phase differences, $\varphi_{1,h}^n - \varphi_{2,h}^n$, are averages of several hundred determinations taken in the vicinity of the sampling level. The total number of independent non-centric data for cytochrome was 3250 and for elastase 8243, both at 2.5 Å resolution. The factor 1.50 means that an error was introduced into the correct difference $|F_{\lambda,h}| - |F_{\lambda,h}|$ by making it 1.50 larger. The factor 1.00 signifies that no error was introduced into the difference and 0.50 that the correct difference was reduced by a factor of 2. As in a previous case (Karle, 1985; Table 1), a value of $|F_{2,h}^n|^2$ was estimated by use of (31) and held fixed. Unlike the previous case, only one initial value was used for $|F_{1,h}^n|^2$ and, when appropriate, for $|F_{2,h}^n|^2$, obtained from (29) and (30) modified as required to define $|F_{1,h}^n|^2$. The results obtained from the use of three equations, (15)–(17), are to the left of the column headings and those from 14 equations, (15)–(28), are to the right.

Sampling level of test set	Error factor	Average error (rad) of $\varphi_{1,h}^n - \varphi_{2,h}^n$					
		Cytochrome c550.PtCl ₄ ²⁻		Hg elastase		U elastase	
0.10	1.50	0.27	0.34	0.26	0.36	0.31	0.47
0.25	1.50	0.35	0.41	0.30	0.36	0.37	0.51
0.50	1.50	0.51	0.56	0.43	0.46	0.46	0.56
0.10	1.00	0.28	0.30	0.27	0.29	0.31	0.36
0.25	1.00	0.43	0.43	0.36	0.41	0.40	0.44
0.50	1.00	0.57	0.57	0.49	0.50	0.49	0.52
0.10	0.50	0.59	0.59	0.56	0.54	0.80	0.79
0.25	0.50	0.69	0.69	0.60	0.59	0.84	0.84
0.50	0.50	0.73	0.73	0.67	0.67	0.83	0.83

from (29) and appropriately modified (30). To simulate isomorphous replacement, a random average error of 10% was introduced into the values of $|F_{1,h}^n|^2$. These values were held fixed in the calculations. Initial values of $|F_{2,h}^n|^2$ were obtained from (31), but were not held fixed in the calculations. As seen in Table 2, there is apparently no significant distinction among the results for cytochrome c550.PtCl₄²⁻ and Hg and U elastase. It is also seen that there is little difference in the average errors for the phase differences when the error factors are 1.50 and 1.00. The average errors are at least twice as large for the error factor of 0.50. A key feature shown by Table 2 is the virtue of holding the average error in $|F_{1,h}^n|^2$ to 10%, as may be possible in an isomorphous-replacement experiment. The average errors in Table 2 are much less than those in Table 1. No significant differences were found in the results whether three equations, (15)–(17), or 14 equations, (15)–(28), were used.

There may be circumstances in which use of the 14 equations, (15)–(28), may be superior to the use of the three, (15)–(17). An extreme example is given in Table 3. For cytochrome c550.PtCl₄²⁻, an initial value of $|F_{2,h}^n|^2$ was overestimated by a factor of about 55, by altering S in (31), for 100 reflections in the sequential range 1701–1800, based on decreasing magnitudes for $||F_{\lambda,h}| - |F_{\lambda,h}||$. A random average error

Table 2. Average of the magnitudes of error for the phase differences, $\varphi_{1,h}^n - \varphi_{2,h}^n$, as a function of sampling level and errors in the data for the three test structures

Sampling level, error factor and averages of the magnitudes of error in $(\varphi_{1,h}^n - \varphi_{2,h}^n)$ mean the same as in Table 1. The total number of independent non-centric data for cytochrome was 3250 (2.5 Å resolution) and for elastase 15800 (2.0 Å resolution). A random average error of 10% was introduced into the values of $|F_{1,h}^n|^2$. These values were held fixed in the calculations to simulate the combination of isomorphous replacement and anomalous dispersion. Essentially the same results were obtained from the use of three equations, (15)–(17), or 14 equations, (15)–(28).

Sampling level of total set	Error factor	Average error (rad) of $\varphi_{1,h}^n - \varphi_{2,h}^n$			
		Cytochrome c550.PtCl ₄ ²⁻	Hg elastase	U elastase	U elastase
0.10	1.50	0.19	0.19	0.20	0.20
0.25	1.50	0.22	0.26	0.26	0.26
0.50	1.50	0.27	0.29	0.29	0.26
0.10	1.00	0.16	0.17	0.15	0.15
0.25	1.00	0.18	0.23	0.19	0.19
0.50	1.00	0.21	0.24	0.21	0.21
0.10	0.50	0.39	0.44	0.43	0.43
0.25	0.50	0.45	0.48	0.46	0.46
0.50	0.50	0.44	0.42	0.42	0.42

Table 3. The effect of an improper start for the least-squares calculation on the averages of the magnitudes of error for the phase differences, $\varphi_{1,h}^n - \varphi_{2,h}^n$

Different results were obtained depending upon whether three equations, (15)–(17), or 14 equations, (15)–(18), were used. For cytochrome c550.PtCl₄²⁻, an initial value of $|F_{2,h}^n|^2$ was overestimated by a factor of about 55, by altering S in (31), for 100 reflections in the sequential range 1701–1800, based on decreasing magnitudes for $||F_{\lambda,h}| - |F_{\lambda,h}||$. The data were of 2.5 Å resolution. The error factor has the same meaning as in Table 1. A random average error of 10% was introduced into the values of $|F_{1,h}^n|^2$ which were held fixed in the calculations. With the improper start, considerably improved results were obtained with the use of 14 equations instead of three.

Number of equations	Error factor	Average error (rad) of $\varphi_{1,h}^n - \varphi_{2,h}^n$	
		Improper start	Proper start
3	1.50	1.40	0.30
14	1.50	0.87	0.30
3	1.00	1.35	0.23
14	1.00	0.49	0.22
3	0.50	0.85	0.44
14	0.50	0.44	0.44

of 10% was introduced into the values of $|F_{1,h}^n|^2$ which were held fixed in the calculations. With the improper start, it is seen that much smaller average errors were obtained with use of the 14 equations. The calculations with an error factor of 0.50 were the least affected. With the use of 14 equations instead of three, additional quantities are introduced, e.g. $|F_{i,h}^n|^2$. Apparently here they play the role of helping to select the more nearly correct ambiguous alternatives.

$|F_{i,h}^n|^2$ is computed rather accurately from (29) and (30) and such a calculation has been suggested as a

basis for relatively scaling multiple-wavelength data (Karle, 1984). In the calculations made here, the average error was less than 2% and it was thought that this would have a beneficial effect on the accuracy of the computed phase differences, $(\varphi_{1,n}^n - \varphi_{2,n}^n)$, as compared with calculations based solely on estimates of the $|F_{2,n}^n|^2$ made from (31), in which the estimated values were held constant, e.g. Table 1. This did not turn out to be the case.

Concluding remarks

The mathematical analysis of anomalous-dispersion data that forms the basis for the calculations presented in these studies has been intended for multiple-wavelength experiments. Nonetheless, this investigation and a previous one (Karle, 1985) have shown that even a one-wavelength experiment offers the potential for phase determination. If sufficiently accurate anomalous-dispersion data are available, it may not be necessary to collect data at more than one wavelength. This is a matter for future consideration. Evidently the availability of isomorphous-replacement data, which can be readily incorporated into the analysis, can be very helpful. If the anomalous-dispersion data are of limited accuracy because, for example, the anomalous signal is relatively weak, phase determination may be facilitated by collecting multiple-wavelength data.

Several multiple-wavelength investigations that have made use of exact linear equations (21) and (22) in the analysis of anomalous-dispersion data have been undertaken by W. A. Hendrickson and colleagues. An initial test was made on lamprey hemoglobin (Hendrickson, Smith, Phizackerley & Merritt, 1988). Investigations on new structures were carried out on ferredoxin from *Clostridium acidi-urici* (Murthy, Hendrickson, Orme-Johnson, Merritt & Phizackerley, 1989) and streptavidin from *Streptomyces avidinii* (Hendrickson, Pähler, Smith, Satow, Merritt & Phizackerley, 1988). For the lamprey hemoglobin data set that was 87% complete out to 3 Å spacings, the average of the magnitudes of the discrepancies between the phases from multiple-wavelength anomalous dispersion and those from the least-squares refined atomic model was 50.5°. The analysis of ferredoxin was limited because of weak data to 5 Å resolution. The phase values obtained however for these data could be shown to be comparable to those of a previously determined related ferredoxin. For streptavidin, three-wavelength data measured at the Photon Factory from a single crystal of the selenobiotinyl complex produced a very fine map at 3.3 Å resolution. An atomic model fitted to

this map has been refined to $R = 0.17$ with 2 Å data collected with Cu $K\alpha$ radiation (Pähler, Hendrickson & Satow, 1988). Hendrickson anticipates the possibility of developing general techniques associated with multiple-wavelength anomalous dispersion in which methionine is systematically replaced by selenomethionine in proteins and bromination is applied to nucleic acids.

Another application of the multiple-wavelength anomalous-dispersion technique has been reported by several collaborative groups (Guss *et al.*, 1988) in which the technique has been used to solve the structure of cucumber basic protein.

In a somewhat different context, Chapuis, Templeton & Templeton (1985) investigated the multiple-wavelength method with the use of a form of (15)–(17). Studies were made with crystals of NaHo(edta).8H₂O and NaSm(edta).8H₂O. Both were successful and particularly high accuracy was obtained with the Sm compound and synchrotron radiation.

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