Contrast Variation Application in Small-Angle Neutron Scattering Experiments.

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Abstract. The mathematical formalism of contrast variation is presented in terms of an expansion of spherical harmonics. Early attempts of contrast variation in X-ray small-angle scattering are compared with the corresponding more versatile techniques of neutron small-angle scattering. Some applications in life sciences illustrate the power of nuclear spin contrast variation.

1. Introduction
In the early times contrast variation aimed at the elimination of intra-particle background scattering in X-ray small-angle scattering [1]. X-ray small-angle scattering from solutions of myoglobin in solvents of different electron density allowed the determination of three basic scattering functions, one of them being due to the scattering from the shape of the protein molecule [2]. As the shape of a molecule is described by much less parameters than its total structure the probability to find a unique molecular shape from its scattering function is greatly enhanced [3].

2. The spherical harmonics as natural co-ordinates in small-angle scattering.
Spherical harmonics can be considered as the natural co-ordinates for the description of the structure of spherical viruses [4]. Another reason for the use of spherical harmonics is the question of uniqueness of structure determination from small-angle scattering (SAS). In order to discuss this point a reminder of some basic equations is mandatory.

We start from an expansion of \( \rho(r) = \rho(r, \omega) \) and of its Fourier transform, the scattering amplitude \( A(Q) = A(Q, \Omega) \), as a series of spherical harmonics, \( Y_{l,m} \).

\[
\rho(r) = \sum_{l=0}^{\infty} \rho_{l,m} (r) Y_{l,m} (\omega) \quad \Leftrightarrow \quad A(Q) = \sum_{l=0}^{\infty} A_{l,m} (Q) Y_{l,m} (\Omega)
\] (1)

\( Q \) is a vector in the Fourier space, and \( Q \) is its modulus. \( \omega \) and \( \Omega \) are unit vectors in the real space and in the Fourier space, respectively. The intensity of small-angle scattering, \( I(Q) \), then is obtained as the sum of absolute squares of the coefficients \( A_{l,m} (Q) \).
\[ I(Q) = 2\pi^2 \sum_{l=0}^{\infty} \sum_{m=-l}^{l} |A_{l,m}(Q)|^2 \equiv 2\pi^2 \sum_{l=0}^{\infty} I_l(Q) \quad (2) \]

The coefficients \( A_{l,m}(Q) \) are the Hankel transforms of \( \rho_{l,m}(r) \). They may be calculated from the polar co-ordinates \((r_n, \theta_n, \varphi_n)\) of the N atoms of a molecule.

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**Fig. 1.** Various density maps giving rise to the scattering function of a cube.
\[ A_{1,m}(Q) = \frac{2}{\pi} l^j \int_{r=0}^{\infty} \rho_{1,m}(r) j_j(Qr) \ r^2 \, dr = \sqrt{2} \frac{l^j}{\pi} \sum_{n=1}^{N} j_j(Qr_n) Y_{l,m}^*(\theta_n, \varphi_n) \]  

(3)

\[ j_j \] are the spherical Bessel functions. \( Y_{l,m}^* = (-1)^m Y_{l,-m} \) is the conjugate complex of \( Y_{l,m} \). Each partial structure \( \rho_{1,m}(r) Y_{l,m}(\theta, \varphi) \) gives rise to a partial scattering function \( |A_{1,m}(Q)|^2 \).

The rotation of a single set of partial structures, \( \sum_{m=-l}^{l} \rho_{1,m}(r) Y_{l,m}(\theta, \varphi) \), by an arbitrary angle with respect to the rest of the structure has no influence on \( I_1(Q) \) defined in (2), while \( \rho(r) \) may change considerably [5]. Some density maps of \( \rho(r) \) giving rise to the scattering function of a cube are shown in Fig. 1. The rotation of the partial structures of the cube by \( l \)-dependent angles blurs the sharp contours of the original structure. Hence, the guess may be allowed that there is only one shape which may be associated with the scattering function of a cube.

Let us turn to the molecular shape determination as it has been proposed 40 years ago [3]. It was assumed that the surface of the shape could be described by a unique function.

\[ F(\omega) = \sum_{l=0}^{l} \sum_{m=-l}^{l} f_{l,m} Y_{l,m}(\omega) \] . The shape scattering function is developed as a power series of \( Q^2 \) [3].

\[ I_c(Q) = 2 \pi^2 \sum_{k=0}^{\infty} b_k \ Q^{2k} \] where

\[ b_k = \sum_{l=0}^{k} \sum_{p=0}^{k-l} \sum_{m=-l}^{l} \frac{d_{l,p} d_{l,k-p} f_{l,m}^{(l+p+3)} f_{l,m}^{*(2k-l-p+3)}}{(l+p+3)(2k-l+p+3)}, \quad f_{l,m}^{(q)} = \int_{\omega} (F(\omega))^q Y_{l,m}^*(\omega) \, d\omega \]  

(4)

Computer simulations with model bodies indicate that the low resolution shape determination from error-free data is unique, even when very limited ranges are used in the simulated curves [6].

3. Solvent contrast variation Introduction

In a first paper, the idea of contrast variation has been illustrated by a simulated model, consisting of a cube with a non-uniform density [1]. The structure of the model is described by two terms, the shape \( \rho_C(r) \) multiplied by the contrast \( \rho \), and the internal structure \( \rho_S(r) \)

\[ \rho(r) = \rho \rho_C(r) + \rho_S(r) \]  

(5)

The contrast \( \rho \) is the difference between the average scattering density of the dissolved particle and that of the solvent. The shape \( \rho_C(r) \) has the value 1 inside the volume excluded to the solvent. The intensity diffracted by \( \rho(r) \) is the absolute square of the corresponding amplitude, \( |A(Q)|^2 = |\rho A_C(Q) + A_S(Q)|^2 \). For randomly oriented particles we obtain from (2):
Among the three basic scattering functions of solvent contrast variation, $I_s(Q)$ is the only one which in favorable cases can be measured directly at zero contrast, $\rho = 0$. The shape scattering function $I_C(Q)$ is dominant at high contrast. The cross term $I_{CS}(Q)$ reflects the convolution between the shape with the internal structure. As $A^{(S)}_{0,0}(0) = \int \rho_s(r) \, dV = 0$, both $I_{CS}(0)$ and $I_s(0)$ are zero.

Sperm whale myoglobin was the first protein studied by X-ray small-angle scattering using solvent contrast variation. The electron density of the solvent was changed by adding glycerol to water. The seven small-angle scattering curves shown in Fig. 2 were analyzed in term of the basic scattering functions of (6).

![Fig. 2. X-ray small-angle scattering of myoglobin in glycerol/water mixtures. The weight concentration of glycerol varies between 0 (open circles) and 94% (full circles). At large Q small-angle scattering is mainly due to the internal structure $\rho_s(r)$.

The shape scattering function $I_C(Q)$ of myoglobin is developed as a power series of $Q^2$. Up to 9 coefficients of the power series were used for the determination of the shape of the myoglobin molecule. The result from small-angle scattering [5] shown in Fig. 3 is in good agreement with the model from crystallographic studies [8]. More recent examples of protein shape determination have been reviewed by Vachette and Svergun [9].
Fig. 3. The shape function $F(\omega) = F(\cos \theta, \varphi)$ of myoglobin determined from the shape scattering function $I_c(Q)$. The coefficients $f_{l,m}$ up to $l=3$ of $F(\omega)$ have been determined. The distances of the isohypse lines are given in Å.

The internal structure is more complicated than the shape. Its elucidation is often restricted to the analysis of the variation of the apparent radius of gyration with the contrast $\rho$ [10].

$$R^2 = R_c^2 + \frac{\alpha}{\rho} - \frac{\beta}{\rho^2}$$ \hspace{1cm} (7)

The coefficients $\alpha$ and $\beta$ provide an unmistakable feature of the low-resolution of complex particles in solution. Particles with a high density core (e.g. ribosomes, ferritin) will give rise to a negative $\alpha$ whereas particle with a low density core (e.g. nucleosome core particle, low density lipoprotein) give rise to positive $\alpha$, in a spherical approximation. A non-vanishing $\beta$ will be due to a dipolar structure, i.e. the centers of mass of different components do not coincide.

Having said this, we find ourselves in the field of neutron scattering. In fact, there are only very few examples of contrast variation using X-ray small-angle scattering [2,11]. But there are considerably more applications of contrast variation in neutron scattering.

4. Neutron scattering from hydrogen

Nearly all applications of contrast variation in neutron scattering use the extraordinary properties of the interaction of neutrons with the hydrogen nuclei $^1$H (=H) and $^2$H (=Deuterium). More recent applications use dependence of neutron scattering length $b_{coh}$ of these hydrogen isotopes on their nuclear polarization $P(H)$ and $P(D)$, respectively.
\[ b_{\text{coh}}(H) = \left[ -0.374 + 1.456 \, pP(H) \right] 10^{-12} \, \text{cm} \]

\[ b_{\text{coh}}(D) = \left[ +0.667 + 0.27 \, pP(D) \right] 10^{-12} \, \text{cm} \]

(8)

This equation holds if a completely polarized neutron beam is used. The polarization \( p \) of the neutron beam then will be +1 or -1. The nuclear polarization may assume values between -1 and +1. The methods for achieving neutron and nuclear polarization will be presented in the other paper.

Similarly, there is a strong variation of the cross section of incoherent scattering with polarization.

\[ \sigma_{\text{inc}}(H) = \left[ 105 \left( \frac{3}{4} - \frac{1}{2} \, pP - \frac{1}{4} \, p^2 \right) \right] 10^{-24} \, \text{cm}^2 \]

\[ \sigma_{\text{inc}}(D) = \left[ 1.0 \left( 2 - pP - p^2 \right) \right] 10^{-24} \, \text{cm}^2 \]

(9)

There is no incoherent scattering for \( P=1 \).

5. Solvent contrast variation by isotopic substitution

Most of the applications of neutron small-angle scattering rely on the difference between the lengths of coherent scattering of H and D. This difference is large compared with scattering lengths of other elements. Another fact is also important: hydrogen is abundant in organic matter. The variation of the scattering density of organic solvents due to isotopic substitution in neutron scattering is much larger than equivalent methods in X-ray scattering. Solvent contrast variation by isotopic substitution is almost exclusively used in neutron scattering.

Neutron small-angle scattering in H\(_2\)/D\(_2\)O distinguishes clearly between lipids material protein and RNA/DNA (Fig. 4). Most of the applications aim at the internal structure of complex macromolecules. As an example we cite the studies on the large subunit of E.coli ribosomes, which consists of rRNA by two third of its mass and more than thirty different ribosomal proteins. The variation of the apparent radius of gyration with the D\(_2\)O content of H\(_2\)O/D\(_2\)O mixtures provides a low resolution model, with a high RNA content close to the centre and proteins preferring the surface region [12,13]. Deuteration of the rRNA confirmed this model [14] (Fig. 5). The shape of this ribosomal subunit being asymmetric [15], the elucidation of a more detailed internal structure had to be left to labeling techniques using specific deuteration (see below).

The analysis of the internal structure is greatly simplified with spherical particles, because of the absence of spherical harmonics with \( l > 0 \) in (2).

\[ I(Q) = \left| \int_{r=0}^{\infty} \rho_{0,0}(r) j_0(Qr) r^2 \, dr \right|^2 = \left| \int_{r=0}^{\infty} \rho_{0,0}(r) \frac{\sin Qr}{Qr} r^2 \, dr \right|^2 \]

(10)
Fig. 4. The scattering density of water and of some components of biological macromolecules. The scattering density of H\textsubscript{2}O/D\textsubscript{2}O mixtures varies between \(-0.56 \times 10^{-10}\) (H\textsubscript{2}O) to \(6.4 \times 10^{-10}\) cm\(^{-2}\) (D\textsubscript{2}O) covering the scattering densities of the main components of living cells.

Fig. 5. The variation of the radius of gyration, \(R_g\), of the large subunit of E. coli ribosomes with the contrast \(\rho\). The deuteration of the ribosomal proteins has been varied from 0 (\(\Delta\)) through 40% (\(\Box\)) to about 80% (O). After [14].

Fig. 6 Angular distribution of neutrons scattered by cauliflower mosaic virus. Solution in water (○), solution with 65% D\textsubscript{2}O in the solvent (▲), solution with 42% D\textsubscript{2}O in the solvent (+). The insert shows the radial distribution of DNA (broken line) and of protein (line) [16].
As an example we shall cite the studies on spherical virus, the cauliflower mosaic virus [16]. The virus has a diameter of 50 nm with a molecular weight of 22.8 $10^6$ dalton. Its double stranded DNA has a molecular weight of about 4 $10^6$ dalton. Fig. 6 shows the intensity of neutron small-angle scattering from the virus in H$_2$O, 42% D$_2$O (protein masked), and 65% D$_2$O (DNA masked). The very high intensity of the first side maximum in all buffers is due to the scattering from a hollow sphere indicating that both the protein and the DNA are essentially distributed on shells on the outer part of the virus [16]. A more quantitative analysis is based on a concentric spherical shell model shown in the insert of Fig.6. The solid curves in Fig. 6 are calculated from this model [16].

6. Specific isotopic labeling

Specific deuteration has led to unique applications of neutron scattering. With this technique, single polymer chains can be studied amongst others, which are identical except for their hydrogen isotope content. The determination of the radius of gyration of polymethylmethacrylate in a glassy matrix elucidated the conformation of a polymer in solid state [17]. Similar studies were done with dilute and semidilute solutions of polystyrene [18].

Biological macromolecules often are complexes of several unique components (subunits) of identical or different chemical composition. Sometimes, their dissociation and reconstitution offers an elegant way of selective deuteration. Let $\rho_0 (r)$ be the structure of non-spherical particle. A region $\rho_L (r)$ inside this particle will have an isotopic composition that is different from that of the rest of the particle. The contrast of the label due to the different isotopic composition is $\rho$. The intensity of neutron scattering is written as

$$I(Q) = \sum_{l=0}^{m} \sum_{m=-l}^{l} |A_{l,m}^{(O)}(Q)|^2 + \rho A_{l,m}^{(L)}(Q) + \rho^2 I_L(Q)$$  \hspace{1cm} (11)$$

The scattering intensity of the unlabelled particle being known from a separate measurement, we are left with the sum of a cross term $I_{ol}(Q)$ and the scattering function $I_L(Q)$ of the label. The determination of the latter function requires the preparation of a third sample with an intermediate deuteration of $\rho_L (r)$. The set of three scattering functions is a basis for internal or label contrast variation.

$$I_1(Q) = I_0(Q) \hspace{1cm} no \hspace{0.5cm} labeling$$
$$I_2(Q) = I_0(Q) + \rho I_{ol}(Q) + \rho^2 I_L(Q) \hspace{1cm} (12)$$
$$I_3(Q) = I_0(Q) + \frac{\rho}{2} I_{ol}(Q) + \frac{\rho^2}{4} I_L(Q)$$

The sum of $I_1(Q) + I_2(Q)$ diminished by $2I_3(Q)$ yields the scattering function $\frac{\rho^2}{2} I_L(Q)$ of the label. In practice, the intensity of neutron scattering, $I_{1,2}(Q)$, from a mixture of the solutes giving rise to $I_1(Q)$ and $I_2(Q)$ is measured. The difference $I_{1,2}(Q) - 2I_3(Q)$ provides the scattering function of the label [19]. The latter procedure largely eliminates interparticle scattering and scattering from contamination and aggregates provided these effects are equal for each sample [20].
The determination of the in situ structure of components of a complex is one of the numerous applications of the method of triple isotopic substitution (TIS) [20]. Biological macromolecules are often composed several well-defined constituents. An important approach to determine their architecture starts with the measurement of the spatial correlation function of two selected components. The number of different distinct pair correlation functions is increased to a point where the total structure emerges. The pair of selected regions \( \mathbf{r}_1 \) and \( \mathbf{r}_2 \) is embedded in the total structure \( \mathbf{r}_0 \). The corresponding amplitudes are \( A_1(Q), A_2(Q) \) and \( A_0(Q) \), respectively. Each of the two regions may be labeled, i.e. it may have its isotope \(^1\text{H}\) substituted by \(^2\text{H}\). Each of the labeled region may have an excess spatial scattering length distribution described by \( \sum_{n=-\infty}^{\infty} \delta(r - r_n) \). In fact, four different solutions have to be prepared in order to obtain the inter label scattering function from neutron scattering. 1: the unlabelled particle, 2: \( \rho_{L_1}(\mathbf{r}) \) is labeled, 3: \( \rho_{L_2}(\mathbf{r}) \) is labeled, 4: \( \rho_{L_1}(\mathbf{r}) \) and \( \rho_{L_2}(\mathbf{r}) \) are labeled. Each of these samples gives rise to the following scattering functions.

\[
I_1 = A_0^2 \\
I_2 = (A_0 + A_1)^2 = A_0^2 + 2A_0A_1 + A_1^2 \\
I_3 = (A_0 + A_2)^2 = A_0^2 + 2A_0A_2 + A_2^2 \\
I_4 = (A_0 + A_1 + A_2)^2 = A_0^2 + A_1^2 + A_2^2 + 2A_0A_1 + 2A_0A_2 + 2A_1A_2
\]  \( (13) \)

The samples giving rise \( I_1 \) and \( I_4 \) are mixed and so are the samples giving rise to \( I_2 \) and \( I_3 \). From the difference between \( I_1 + I_4 \) and \( I_2 + I_3 \) one obtains the cross term \( 2A_1A_2 \). This method of mixing the samples has the great advantage that it eliminates interparticle scattering. Using (3) and the development of \( \frac{\sin Q|\mathbf{r} - \mathbf{r}'|}{|\mathbf{r} - \mathbf{r}'|} \) as a series of spherical harmonics we obtain

\[
A_1A_2 = \sum_{l=0}^{\infty} \sum_{m=-l}^{l} A_{1,m}^{(1)}(Q) A_{2,m}^{(2)}(Q) \\
\approx \sum_{l=0}^{\infty} \sum_{m=-l}^{l} \sum_{n=1}^{N} j_l(Qr_n^{(1)}) j_l(Qr_n^{(2)}) Y_{l,m}(\varphi_n^{(1)}) Y_{l,m}^*(\varphi_n^{(2)}) = \sum_{n=1}^{N} \sum_{l=0}^{\infty} \sum_{m=-l}^{l} \frac{\sin Q|\mathbf{r}_n^{(1)} - \mathbf{r}_n^{(2)}|}{Q|\mathbf{r}_n^{(1)} - \mathbf{r}_n^{(2)}|}
\]  \( (14) \)

The super scripts (1) and (2) refer to the hydrogen atoms of the labels \( \rho_{L_1}(\mathbf{r}) \) and \( \rho_{L_2}(\mathbf{r}) \), respectively. Representing the labels by their center of gravity, one obtains.

\[
I_X(Q) = [I_1(Q) + I_4(Q)] - [I_2(Q) + I_3(Q)] \approx \frac{\sin Qd}{Qd}
\]  \( (15) \)

\( I_X(Q) \) images the spatial relationship of the pair of components in question [21]. The Fourier transform of \( I_X(Q) \) is the distribution of lengths of all possible vectors connecting the non-exchangeable hydrogen sites in the two labeled regions. The second moment of the length distribution related to a pair of labels is equal to the sum of the squares of their radii of gyration in situ plus the
square of the separation between their centers [22]. A table of such distances yields the spatial arrangement of the components. In total, \( n(n-1)/2 \) distances exist between \( n \) components. In order to reconstruct their spatial arrangement by triangulation at least \( 4n - 10 \) distance values must be known for \( n \geq 4 \) [23].

The three-dimensional model of the 21 proteins of the small ribosomal subunit is shown in Fig. 7. It has been constructed by triangulation from 105 distances, appreciably more than the minimum of 78 required for a 21 protein structure [23].

![Fig. 7. Two views of the neutron map of the small ribosomal subunit from Escherichia coli. Each protein is represented by a sphere whose volume is the same as that of the protein. The maximum linear dimension of the array is about 190 Å. The two views are related to each other by a 180° rotation about an axis oriented vertically in the plane of the figure. After [23].](image)

7. Nuclear spin contrast variation

In contrast to the methods of isotopic substitution, which require the preparation of several samples, a single sample will suffice for contrast variation by nuclear spin polarization. Using a completely polarized neutron beam, the variation of the length of coherent scattering and of the intensity of coherent scattering with the polarization of the hydrogen isotopes \(^1\)H and \(^2\)H is given by (11) and (12), respectively.

A high polarization of protons or deuterons is obtained by the method of dynamic nuclear spin polarization (DNP). Without entering into details of this method, we mention that we use the method proposed by Abragam, which works in insulators [24][25]. In this case the nuclear spins are polarized in the presence of a small amount of paramagnetic centers. The temperature is kept between 0.1 and 1 K, and a high magnetic field is used. Microwave irradiation at a frequency slightly below or above the paramagnetic resonance will polarize the nuclear spins in positive or negative direction with respect to the external magnetic field direction, respectively. With solutions of biomolecules in a mixture of deuterated glycerol and heavy water the final values of nuclear polarization will be around 70% (or -70%) for protons and 20% (or -20%) for deuterons after several hours of microwave irradiation. At a temperature of 0.1 K, the polarization of the protons and deuterons remains nearly constant for days.
A proton spin polarized sample is obtained by destroying the deuteron polarization. A deuteron spin sample is obtained by destroying the proton polarization. At a temperature of 0.1 K, differently polarized nuclear spin systems will coexist for many hours without significant change of polarization.

A typical experiment of polarized neutron scattering takes 5 days [26]. Loading the frozen sample and cooling the refrigerator to 1 K takes 6 hours. After calibration of the proton NMR signal and neutron scattering from the sample at P=0, microwave irradiation will polarize the nuclear spins of the sample at a temperature well below 1 K. The microwaves are switched off and the temperature of the sample will drop to 0.1 K. The deuterons are depolarized. Neutron scattering from the proton spin polarized sample is measured for two days. The direction of the neutron spin polarization is changed each ten minutes. Then the same procedure is repeated for the deuteron spin polarized sample for another two days. The sample is unloaded on a Friday afternoon. The data set consists of five spectra of neutron small-angle scattering. The spectra of the sample at P=0, and the spectra of the proton polarized target and deuteron polarized target at two neutron beam polarizations, in direction and opposite to the external field [26].

Nuclear spin contrast variation is mainly used as a method of internal contrast variation.

\[
I_{(\pm)}(Q) = \sum_{l=0}^{\infty} \sum_{m=-l}^{l} U_{l,m}(Q) + p P V_{l,m}(Q) \left( I_{(\pm)}(Q) + p P I_{UV}(Q) + P^2 I_{V}(Q) \right)^2
\]

\[
I_{(\pm)}(Q) \quad \text{and} \quad I_{(-)}(Q) \quad \text{are obtained with positive and negative polarization p of the neutron beam, respectively. The nuclear polarization P stands for the proton polarization P(H) or deuteron polarization P(D).} \]

\[
U(Q) \quad \text{is the amplitude of the sample at P=0. With deuterated solvents U(0) will be negative. With increasing proton spin polarization P(H) the amplitude P(H)V(0) will decrease the intensity of forward scattering (Fig. 8). At P(H) = 0.65 the nuclear spin contrast matches the native}
\]
contrast of the protein. The matching point changes hardly with RNA or DNA as solute. This means that nuclear spin contrast is an excellent amplifier of an existing contrast, but unlike the method of isotopic substitution it is not suitable for the distinction between chemically different components.

Assuming that the extreme polarizations $P$ are equal, the basic scattering functions are easily obtained from $I_{(+)}(Q)$ and $I_{(-)}(Q)$.

$$
I_{uv}(Q) = \frac{1}{2P} \left[ I_{(+)}(Q) - I_{(-)}(Q) \right]
$$

$$
I_{y}(Q) = \frac{\left[ I_{(+)}(Q) + I_{(-)}(Q) \right] - 2I_{u}}{2P^2}
$$

(17)

The gain in contrast due to nuclear polarization considerably extends the application of contrast variation. The neutron scattering studies on ribosomes at the end of the last century may serve as an illustration. While the small 30S subunit of the E.coli ribosome has been studied in great detail (Fig. 7), a similar approach for the large 50S subunit of the ribosome seemed to be prohibitively difficult. Attempts were made to reduce the incoherent scattering by massive deuteration of the solvent and of the solute. The idea of the contrast free ‘glassy ribosome’ should facilitate the in situ structure determination of the labeled (protiated) component [27]. However, the interference of any residual internal contrast between deuterated rRNA and the deuterated proteins with the strong contrast of the label could not be entirely excluded. It was at that time when first experiments of nuclear spin contrast variation were started. It happened that the optimal composition of samples for this kind of study were those developed for the ‘glassy ribosome’. In fact, the scattering density of a completely deuterated ribosome is almost equal to that of a mixture of deuterated glycerol and D$_2$O. The first experiments of proton spin contrast variation on the deuterated 50S subunit with one of its proteins left protiated showed that the forward scattering from the high contrast of the label and the weak contrast of the deuterated ribosome were comparable in size. Thus the analysis of the data had to take into account the shape and internal structure in order to determine the site (and if possible) the shape the labeled ribosomal component [26].

The four additional basic scattering functions from nuclear spin contrast variation of (20) increased considerably the structural information. Clearly, the basic scattering functions from proton spin contrast variation, and in particular the cross term $I_{uv}(Q)$, were most important. Valuable additional structural information came from electron microscopy [28]. A number of ribosomal proteins of the large subunit have been studied by this method. We point out to the fact that the results concerning the co-ordinates of the label with respect to the large ribosomal subunit depend on the knowledge of the low resolution structure of the ribosome known at that time [29].

The study of labeled components in the functional E. coli ribosome with its two protiated tRNAs and a protiated mRNA fragment in a completely deuterated environment was a still larger challenge for two reasons:

1: The contrast of RNA in a deuterated solvent is lower by a factor 1.5 with respect to proteins due to the lower hydrogen content. The same holds for the proton spin added contrast.

2: the occupation density of the ribosomal binding sites by tRNAs does not exceed 40%.
Fig. 9. The spin dependent scattering function $I_{uv}(Q)$ of protiated tRNA bound to the functional complex of the deuterated E.coli ribosome. The polarizations of the proton spins and deuteron spins were 0.7 and 0.15, respectively. For the intensity scale: a solution of the protiated ribosome in the same deuterated solvent would have an $I(0) = 8 \times 10^6$, concentrations being equal. After [30].

Fig. 10. The site and the orientation of two tRNAs in the E. coli ribosome. The tRNAs are given in the low resolution description of triangles. The anticodon (AC) of the tRNA is close to the neck of the small subunit whereas the aminoacyl group (AA) of the tRNA approaches the central protuberance of the large subunit. After [30].
Nevertheless, polarized neutron scattering from the nuclear spin polarized sample clearly revealed the basic scattering functions of proton and deuteron spin contrast variation [30]. The change of the neutron scattering intensity with the polarization direction of the incident neutron beam is shown in Fig. 9 for both the proton spin polarized and the deuteron spin polarized sample.

The cross terms shown in Fig. 9 differ in the sign at very small scattering angles, and so do the contrasts induced by the polarization of protons and deuterons, respectively. As the density of deuterons in the solvent was higher than in the solute, the deuteron spin contrast was negative. Hence the proton spin contrast was positive and so was \( U(0) \). At \( Q > 0.05 \), the intensity is mainly due to the amplitude of the protiated tRNAs.

The determination of the in situ structure of the two tRNAs was done in two steps. In a first step the centre of gravity of the \((tRNA)_2\)-mRNA complex was determined. In a next step, the orientation of this complex with respect to the ribosome had to be found. Two facts were helpful: (i) the structure of the tRNA is known to atomic resolution, (ii) the extremes of the structure of tRNA must be rather close to each other. There is also an estimation of the angle between the planes of the tRNAs, which may be as large as 90° [31]. The orientation of the tRNA complex was varied over the whole range of Eulerian angles while the center was kept. The best fit of the experimental data was obtained with orientation of the tRNAs shown in Fig. 10.

8. Conclusion

Contrast variation techniques have found many applications in neutron small-angle scattering both in life sciences and in condensed matter research in general. As the structural aspect prevails in biological applications, we have developed the concept and the mathematical formalism of contrast variation in this frame. Isotopic substitution is still the mostly used technique. It discriminates between chemically different components of complex structures. Nuclear spin contrast variation remains an excellent amplifier of an existing contrast.

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