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Assessment of Local Heterogeneity of Cryo-EM Maps in Real Space for Type I Pili from *E. coli*

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Abstract. The accuracy of the resolution estimation of an experimental cryo-EM scattering density map has a decisive influence on the accuracy of determining the parameters of atomic position uncertainty (ADP parameters) when refining in direct space. The previously developed method for estimating the local resolution of cryo-EM distribution maps using direct space methods was applied to the study of maps for type I pili from *E. coli*. A study of four maps obtained by three groups of researchers showed that for each of the three standard maps, the resolution can be considered constant and is about 2.6–2.7 Å. At the same time, for the sharpened map, the resolution is higher and is 2.3 Å.

Key words: structure biology, cryo-EM maps, refinement in direct space, local resolution, shell functions

INTRODUCTION

When studying the atomic structure of biological objects by X-ray scattering or cryo-electron microscopy, the atomic coordinates are initially determined by interpreting the scattering density map. The scattering density is the electron distribution density when using X-ray scattering or scattering electrostatic potential in cryo-electron microscopy (cryo-EM). By a density map (cryo-EM map), we further understand a set of values of the scattering density given at the nodes of a regular grid in three-dimensional space, or, as a visual image, a three-dimensional topographic map of the scattering density represented by its isosurfaces. Each map is associated with a three-dimensional set of its complex Fourier coefficients, called structure factors.

For all chemical types of atoms and ions, the density distributions for atoms at rest at the origin are known and collected in tables [1]. The Fourier transforms of these functions are called the atomic scattering factors (or form factors) of these atoms. In practical work, they are usually approximated by the sums of several (from one to five) isotropic Gaussian functions [1–3]. For known atomic coordinates and their form factors, a theoretical scattering density map can be calculated. Comparison of this map with the experimental one and an attempt to achieve the maximum correspondence of these maps by varying the coordinates of atoms is the basis of the procedure for refining the atomic structure in direct space [4–6].

When refining the atomic model in direct space, a significant difficulty is that both the real scattering density distribution and the experimental map contain distortions that must be considered (modeled). One of the main causes for the distortion of the theoretical density is the dynamic and static uncertainty of the positions of atoms in the object under study. In both X-ray and EM studies of the structure, the experimentally obtained distribution is the result of averaging over a huge number of copies of the object (molecules in a crystal or multiple independent EM images) that have small discrepancies in the coordinates of the object's

atoms. Dynamic uncertainty is related to the thermal mobility of atoms. Static uncertainty is associated with the fact that in different copies of the object, some parts of the chain may be in different conformations while the overall structure of the object remains the same. The uncertainty of atomic positions manifests itself as an increase in the width of peaks corresponding to atoms in the theoretical distribution and fusion of peaks of close atoms. The uncertainty of the position of an atom is usually described by a three-dimensional isotropic Gaussian probability distribution and is given by the position uncertainty parameter B_j (Atomic Displacement Parameter, ADP), where j is the ordinal number of the atom in the list of atoms. Parameters $\{B_j\}$, which carry important information about the internal mobility of the object, are determined (along with the coordinates of atoms) in the process of refinement of the structure.

In this paper, we will focus on three parameters that determine the distortions of the experimental map: the background T that appears on the map, the scaling factor Z that brings the theoretical scale of values to the scale of the experimental map, and the resolution value D . The resolution characterizes the detail of the resulting map and is determined by the experimental conditions, such as the sensitivity and resolution of the detector. This value is not an attribute of the object under study, but rather characterizes the experiment. Lowering the map resolution, in addition to increasing the width of peaks on the map, can lead to the appearance of Fourier series termination effects revealed as spherical shells of relatively high amplitude density values alternating in sign.

The similarity of the picture of the manifestation of uncertainty in the positions of atoms and the limited resolution of the experimental map leads to the fact that, when refined in direct space, the found values of parameters B are the sums $B = B^{\text{ADP}} + B^{\text{res}}$, where B^{ADP} describes the true mobility of the object's atoms, and B^{res} is a "parasitic" additive expressed through the map resolution value D [7]. Consequently, an accurate determination of the value of parameter D is a prerequisite for an accurate determination B^{ADP} values. While the values B_j are generally considered as local characteristics (different for different atoms), the values of D , Z , and T are usually considered as global characteristics, identical for all atoms. However, recently several authors have drawn attention to the fact that cryo-EM maps often have a resolution that is visually different for different parts of the map, which led to the development of the concept of local resolution [8–11]. In our calculations, we initially consider the resolution value to be local, different for the neighborhoods of different atoms, and find a set of values $\{B_j\}$ and $\{D_j\}$. Then, based on the results obtained, a decision can be made on the acceptability of a single global value D^* for all atoms.

This paper presents the results of applying the developed technique to estimate the heterogeneity parameters of experimental cryo-EM maps obtained recently [12–14] in studies of type I pili from *E. coli*. Pili are protein filamentous tubular structures that allow bacteria to attach to other cells. Pili can have a diameter of tens to hundreds of angstroms and reach a length of hundreds of thousands of angstroms. In the study mentioned above, atomic models were obtained for fragments of pili with a length of about 130 Å, having an outer diameter of about 70 Å and an inner diameter of about 12 Å (Fig. 1). The main part of pili is formed by helically arranged subunits (chains) of the FimA pilin protein. A single subunit consists of 159 amino acid residues and has a pronounced groove on the surface and a long (19 residues) "hook", through which the previous subunit "clings" to the next one (Fig. 1).

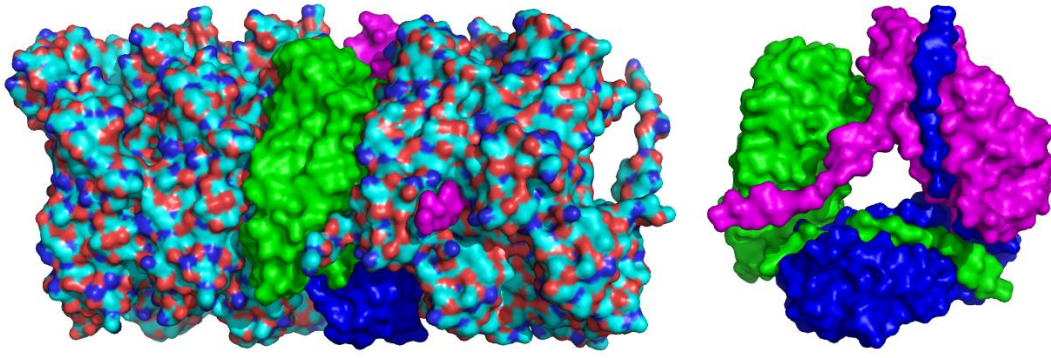


Fig. 1. General view of a fragment of pili consisting of 15 pilin subunits [14], (PDB code 9VKV) and the coupling of individual subunits in the helical structure. The subunits H (green), I (blue), and J (purple) are highlighted in solid color

MAP RESOLUTION ASSESSMENT

There are different approaches to defining the concept of resolution and estimating its value [15, 16]. Currently, the main approach to determining the map resolution in cryo-EM studies is reciprocal space methods based on comparing the structure factors for two maps obtained for the object under study from independent experimental data sets. The comparison of two sets of structure factors is performed by calculating the correlation coefficient of structure factors for different resolution zones (Fourier Shell Correlation, FSC) [17–20]. As an estimate of the map resolution, the value corresponding to the zone for which the FSC graph falls to a certain critical value (usually 0.143) is selected. This approach has some drawbacks. First, the choice of a critical value is rather arbitrary and not generally recognized as correct. Other threshold values are also suggested, for example, 0.5. In addition, schemes are proposed in which structure factors (and, accordingly, the FSC curve) are calculated using modified maps. This leads to the situation when there are many mismatched estimates for the resolution value [14]. Second, this criterion evaluates the level of detail reproducibility on two maps, rather than a quality of the map itself. The found resolution threshold D , at which the FSC value becomes lower than the critical value, indicates that map details that meet a higher resolution than the D value are unreliable. But this does not mean that the map really reaches the resolution D , and does not have an even lower resolution. Third, getting an estimate is much more complicated if a map has a variable local resolution.

Recently, we proposed an alternative approach based on working in a direct space [21]. The approach aims to determine the local parameters B , D , Z , and T . It is based on a recently developed method for fast calculation of the image $\rho^{\text{img}}(\mathbf{r}; B, D)$ of an atom placed at the origin for given values of parameters B and D . The calculation is performed using analytically specified approximations [11, 22]. For the neighborhood of the j -th atom, the optimal values of the parameters B^{opt} , D^{opt} , Z^{opt} , T^{opt} are determined by minimizing the local criterion for matching the experimental and calculated maps:

$$Q_j(B, D, Z, T) = \frac{\sum_{\mathbf{u}: |\mathbf{u} - \mathbf{r}_j| \leq R_{\text{vic}}} \left(\rho^{\text{obs}}(\mathbf{u}) - Z \left(T + \sum_{\substack{k: \\ |\mathbf{u} - \mathbf{r}_k| \leq R_{\text{atom}}}} \rho^{\text{img}}(\mathbf{u} - \mathbf{r}_k; B, D) \right) \right)^2}{\sum_{\mathbf{u}: |\mathbf{u} - \mathbf{r}_j| \leq R_{\text{vic}}} (\rho^{\text{obs}}(\mathbf{u}))^2}.$$

Here R_{vic} is a given radius of the neighborhood, R_{atom} is a given radius of the atom, summation in $\mathbf{u}$ goes along the grid nodes that are in the neighborhood of the j -th atom, summation in k

goes along the atoms that are located near the grid point $\mathbf{u}$, $\mathbf{r}_k$ are the coordinates of the k -th atom, $\rho^{\text{obs}}(\mathbf{u})$ is an experimental map.

Minimization of the Q criterion is performed by search over the values of B and D with a given step in given intervals of parameters. It is assumed that all values of parameters B and D are the same within the considered neighborhood. Since the criterion is a quadratic function with respect to Z and BT , the optimal values $Z^{\text{opt}}(B, D)$ и $T^{\text{opt}}(B, D)$ corresponding to the current trial values B and D may be found using formulas for solving a system of two linear equations [21].

ESTIMATION OF LOCAL INHOMOGENEITY PARAMETERS OF CRYO-EM MAPS FOR TYPE I PILI FROM *E. coli*

Analysis of map J171

The scattering density map J171 for the uro-pathogenic bacterium *E. coli* was obtained on a $256 \times 256 \times 256$ grid in a cubic virtual cell with an edge length of $214.016 \text{ \AA}$ [14]. The model constructed from the map was refined in direct space by the program *Phenix* [23, 24]. The PDB and EMDB codes of the deposited model and map are 9VKV and EMD-65144, respectively. Local heterogeneity analysis was performed for subunit H. When calculating images of atoms, Four-Gaussian approximations of form factors were used [2]. The radius of the neighborhood R_{vic} for calculating criterion Q was chosen to be $4.0 \text{ \AA}$, the radius of the atom R_{atom} depended on the value D of the tested resolution was defined as $R_{\text{atom}} = 3D$. The values of parameters B and D were scanned with steps of $1.0^2 \text{ \AA}^2$ and $0.025 \text{ \AA}$, respectively.

The first stage of the work was devoted to determining the values of global (for the entire molecule) scaling parameters Z^* and T^* . To do this, at the first stage of the work, these parameters were temporarily considered as variable local parameters, and individual optimal $\{B_j^{\text{opt}}, D_j^{\text{opt}}, Z_j^{\text{opt}}, T_j^{\text{opt}}\}$ values were found for each atom. Figure 2 shows the found values of local parameters $\{Z_j^{\text{opt}}\}$ and $\{T_j^{\text{opt}}\}$ for the main chain atoms ($-\text{N}-\text{C}_\alpha-\text{C}-$). The global values $Z^* = 1.1$ and $T^* = 0.053$ were defined as the median values $\{Z_j^{\text{opt}}\}$ and $\{T_j^{\text{opt}}\}$, respectively.

At the second stage, the work was carried out with the values of the scaling parameters $Z^* = 1.1$ and $T^* = 0.053$ fixed for all atoms. Figure 3 shows the distribution of local resolution values D for all atoms and atoms of the main chain. The graphs show that, in general, these values are close and are grouped around the median value of $D = 2.7 \text{ \AA}$. Areas of reduced map resolution are mainly associated with chains of side groups on the outer surface of the saw facing the solvent, for which there is no visible density on the map. At the same time, for groups lying deep in the groove, the resolution may be slightly higher than the median. In general, for the J171 map, the resolution can be considered constant, equal to $2.7 \text{ \AA}$.

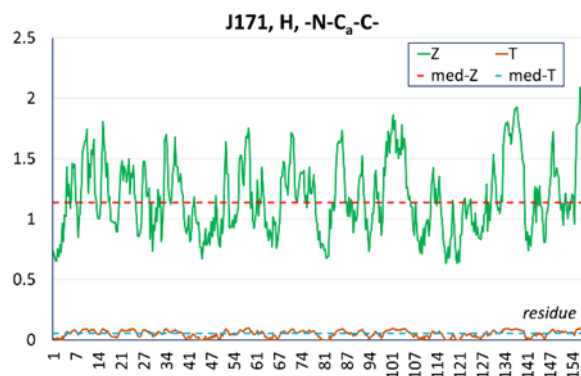


Fig. 2. Distribution of local values of scaling coefficients Z and T along the main chain of subunit H and their median values

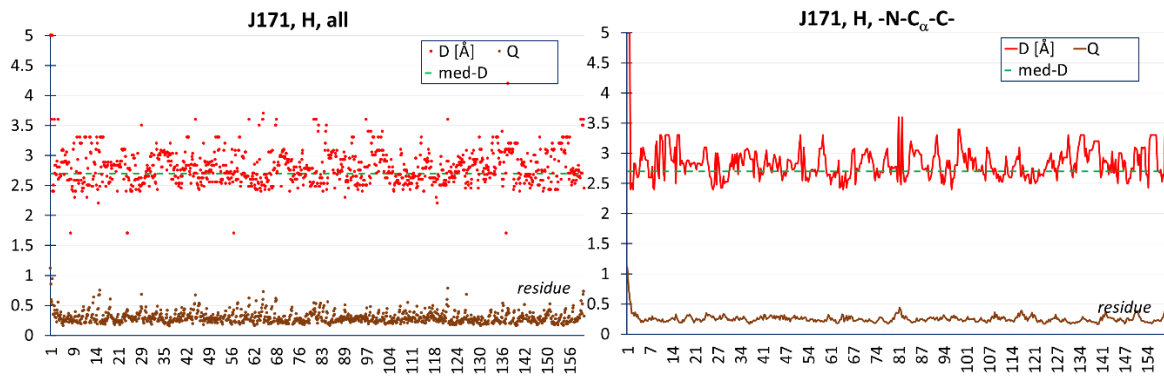


Fig. 3. Local resolution and Q criterion values for all atoms of subunit H and of the main chain. The median resolution value $D^* = 2.7 \text{ \AA}$ is shown

Analysis of the "sharpened" map J167

The next object of analysis was map J167, obtained from map J171 by the sharpening procedure [25]. This procedure consists in calculating the values of structure factors from the original map, multiplying these values by a function growing at infinity, and calculating the inverse Fourier transform. The purpose of this procedure is to approximate the images of individual atoms on the map by images of the delta function, *i.e.*, to reduce the width of the peaks on the map. A side effect of this procedure is an increase in the noise level on the map, which, in some cases, may exceed the signal level. The use of a sharpened map made it possible to increase the visual resolution of the map and to add water molecules in the model [14].

The local resolution assessment for this map was carried out in the same way as it was done for J171 map. Initially, the search for optimal parameters was carried out with varying all parameters of the model and map, and then the scaling parameters were fixed at the median values $Z^* = 1.76$ and $T^* = 0.09$, and the values $\{B_j^{\text{opt}}, D_j^{\text{opt}}\}$ were searched with the values of scaling parameters fixed for all atoms.

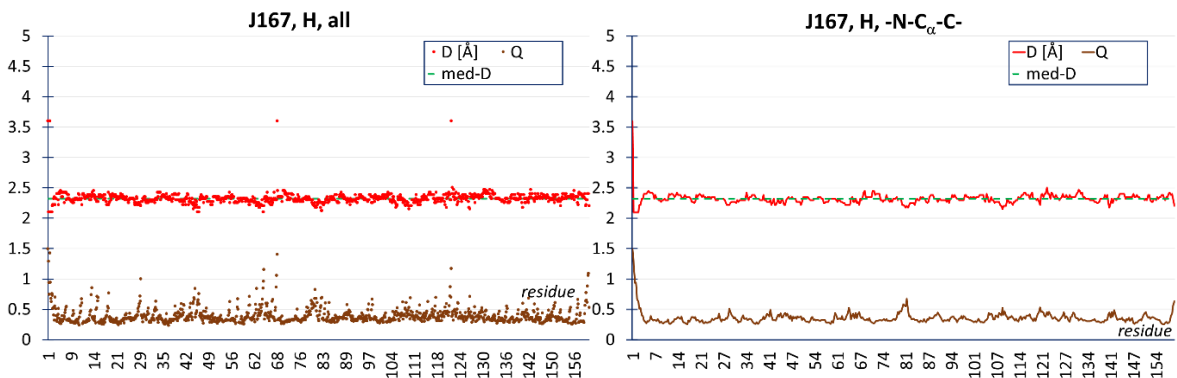


Fig. 4. Local resolution value D and Q criterion value for all atoms of subunit H (left) and main chain atoms (right) for map J167. The median resolution value $D^* = 2.3 \text{ \AA}$ is shown

Figure 4 shows the local resolution values found for J167 map. Here, the spread of the found values D_j^{opt} relative to the median value is significantly smaller than for the map J171, and the median value $D^* = 2.32 \text{ \AA}$ is less than the value $2.7 \text{ \AA}$ calculated for the map J171. This confirms the earlier conclusion that the J167 map has a higher resolution [14], based on visual analysis and the use of the *phenix.mtriage* procedure [24]. On the other hand, for the sharpened map, the match with it, calculated using the atomic map model, is slightly worse

than it was for the original J171 map: the median value is 0.35 of the Q criterion value compared to 0.27. This is due to the mentioned property of sharpened maps to have a higher noise level.

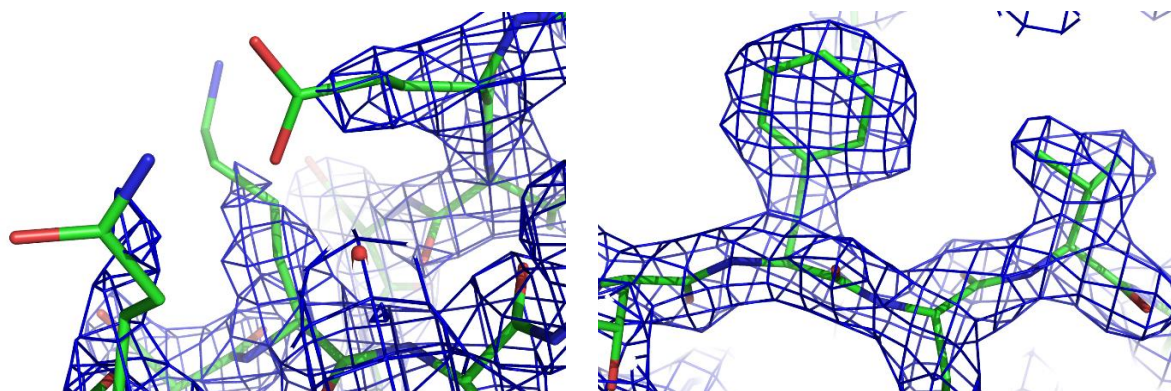


Fig. 5. Fragments of J167 scattering density map for poorly visible residues (left) and a section of the map with a well-defined density (right)

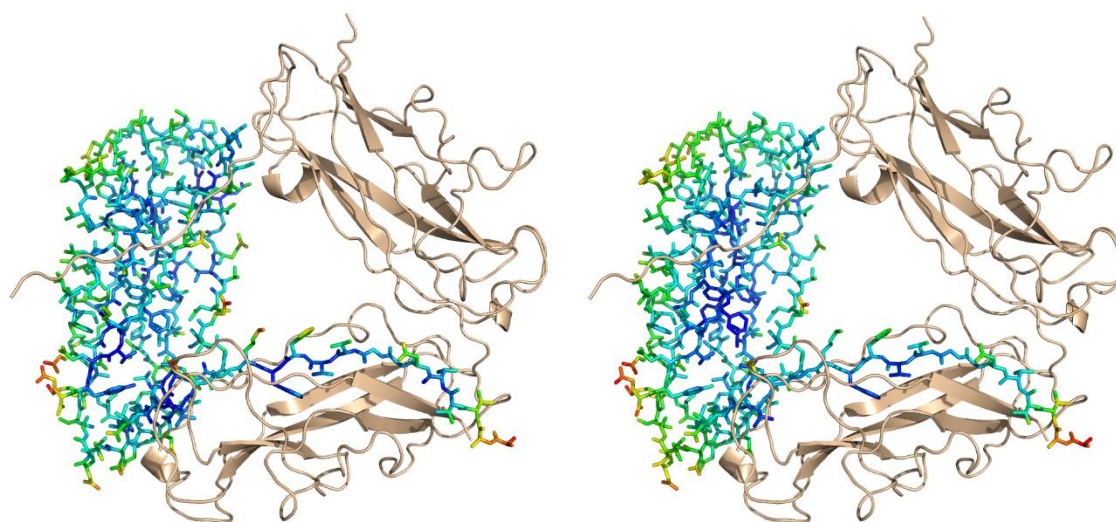


Fig. 6. Left: the value of the quality criterion Q for atoms of subunit H is shown in color (from dark blue for small values of Q to red for high values). Right: values of the uncertainty B of atomic positions. Chains H, I, and J are shown in the direction of the pili axis

The outliers present in the diagram, which relate to Lis68, Glu121, and Ala1 residues, correspond to poorly defined areas of the map (Figure 5).

Figure 6 shows the distribution in space of the values of the Q criterion for the H subunit. The best match of the calculated and experimental maps occurs for the more stable parts of the molecule located inside the pilin subunit or in the contact areas between the subunits. For parts of the molecule exposed to the outer side or to the inner cavity of the saw, the criterion values are slightly higher. A similar distribution of values in space takes place for other parameters of the model (B) and map (Z , T).

Analysis of EMD_17878 and EMD_60903 maps

A similar analysis was performed for maps obtained by other groups of researchers.

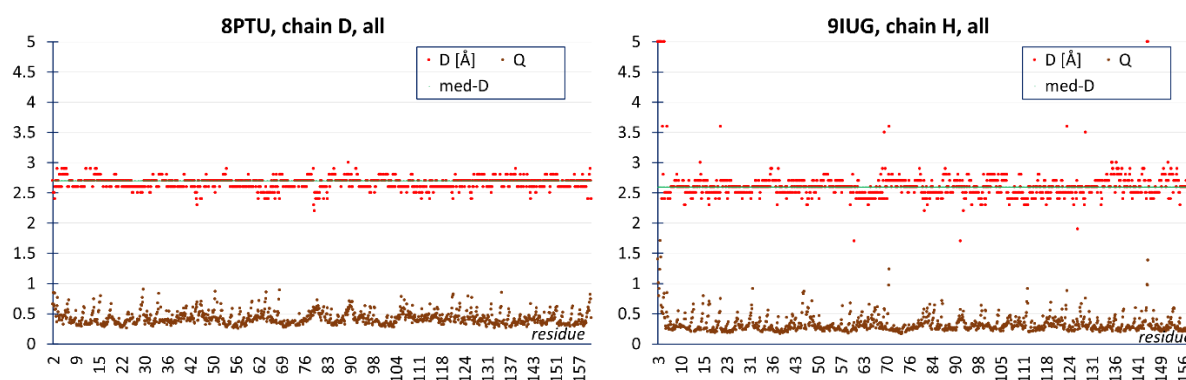


Fig. 7. The value of local resolution for the atoms of the studied chain and the value of the Q criterion for the maps EMD_17878(left) and EMD_60903(right)

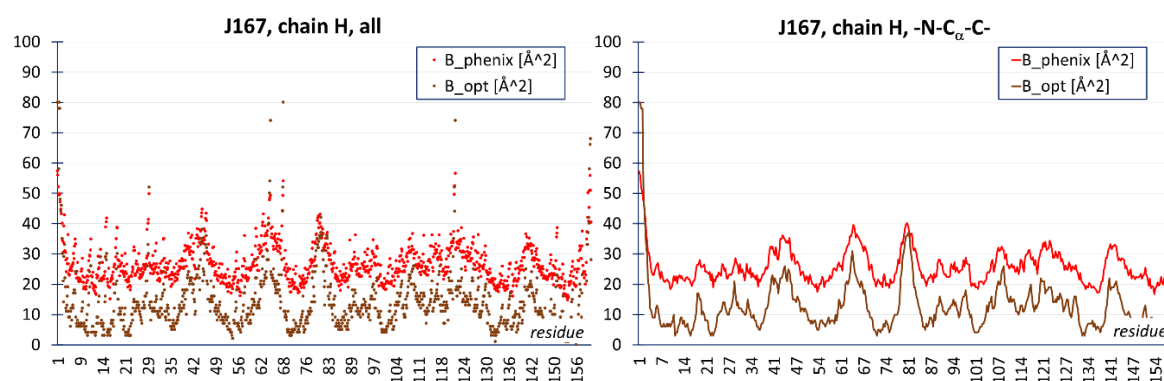


Fig. 8. The values of the B_{opt} parameters found in the process of minimizing the residual Q , and the values of B_{phenix} determined in the process of refinement of the 9VKV model by *Phenix* program

The EMD_17878 scattering density map for pili from *E. coli* was obtained on a $256 \times 256 \times 256$ grid in a cubic virtual cell with an edge length of 270.08 Å [12]. The model built on the map was updated using *Phenix* program. The PDB and EMDB codes of the deposited model and map are 8PTU and EMD-17878, respectively. Local heterogeneity analysis was performed for chain D. When calculating the map matching criterion Q the neighborhood radius Q was chosen to be 4.0 Å. The values of parameters B and D were scanned with steps of 2.0 Å² and 0.1 Å, respectively.

The EMD_60903 scattering density map for pili from enterotoxigenic *E. coli* was obtained on a $400 \times 400 \times 400$ grid in a cubic virtual cell with an edge length of 300.8 Å [13]. The model built on the map was updated by *Phenix* program. The PDB and EMDB codes of the deposited model and map are: 9IUG and EMD-60903, respectively. Local heterogeneity analysis was performed for chain H. The neighborhood radius for calculating the map matching criterion Q was chosen to be 4.0 Å. The values of parameters B and D were scanned with steps of 2.0 Å² and 0.1 Å, respectively.

As before, the search for optimal parameters was initially carried out with varying all parameters of the model and map, and then the scaling parameters were fixed at the median values $Z^* = 0.423$, $T^* = 0.08$, for 9PTU and $Z^* = 0.181$, $T^* = 0.063$ for 9IUG, and the values $\{B_j^{opt}, D_j^{opt}\}$ were searched with the fixed values of scaling parameters for all atoms. Figure 7 shows the results of determining the local resolution for these two maps. As before, the

resolution can be considered constant for the entire map and is estimated by the median value $D = 2.7 \text{ \AA}$ for EMD_17878 map and $D = 2.6 \text{ \AA}$ for EMD_60903 map.

Determination of the uncertainty parameters values of the atomic positions

In addition to the local resolution values, local values of B parameters, which characterize the uncertainty of atomic positions (ADP), were also determined during the scanning of heterogeneity parameters. Figure 8 shows the values B_j^{opt} obtained for the J167 map and the 9VKV atomic model. The distribution of parameter B in space is shown in the Figure 6. The vibrations seen in Figure 8 correspond to the output of the polypeptide chain on the surface of the pili exposed to the solvent. For comparison, Figure 8 also shows the ADP parameter values obtained during refinement of the 9VKV model by *Phenix* program. The graphs show that these values are highly correlated and differ in the overall shift of the graph. The main reason for this difference is that the constant values of atomic form factors corresponding to point atoms (delta functions) were used in calculations using *Phenix* program. For atoms that have a Gaussian density distribution with the B^{atom} parameter, this technique increases the resulting B_{phenix} value by B^{atom} [7]. This effect is shown in Figure 9. Replacing four-Gaussian form factors with constant form factors in the process of minimizing Q criterion leads to B_{delta} values that fairly accurately reproduce the values obtained by *Phenix* program. The remaining difference can be caused both by the actual replacement of the four-Gaussian approximation of the form factor by a single-Gaussian one, and by the fact that when working with *Phenix* program, a constant local resolution value $D = 2.09 \text{ \AA}$ was used for all atoms.

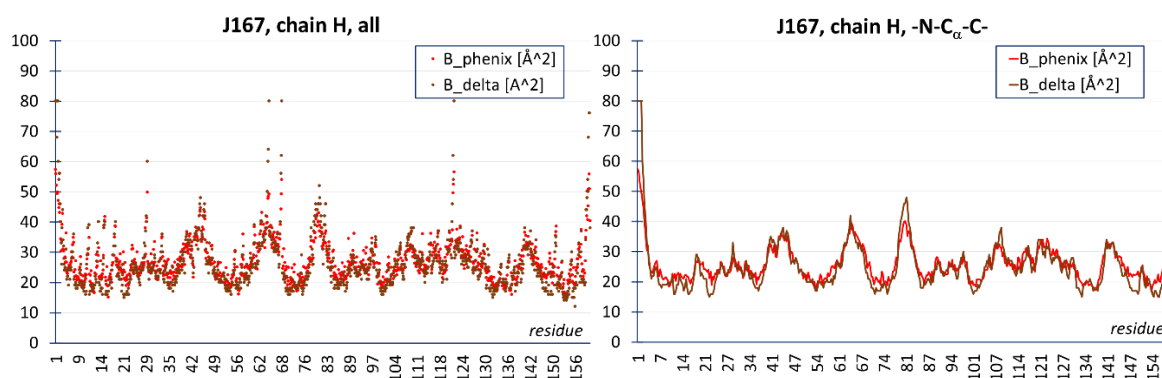


Fig. 9. B_{phenix} values determined for atoms in the process of refining the 9VKV model by the *Phenix* program and B_{delta} parameters obtained in the process of the minimization of the residual Q using constant form factors

It should be noted that the described substitution of form factors does not lead to a significant change in the determined values of the local resolution (Figure 10), but, as demonstrated above, it leads to distorted values of the B parameters.

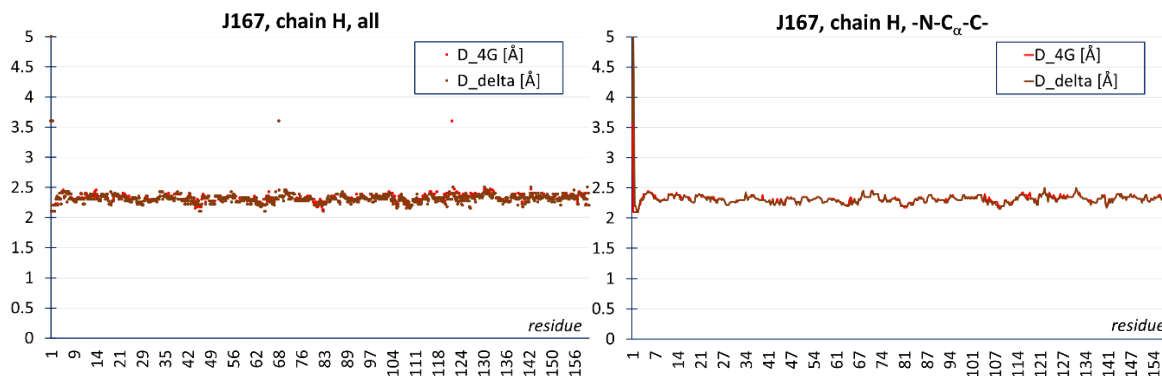


Fig. 10. Local resolution values D_{4G} obtained using four-Gaussian approximation of form factors, and local resolution values D_{δ} obtained using constant form factors for all atoms of subunit H (left) and atoms of the main chain (right)

CONCLUSION

This paper describes the study of the local heterogeneity of four cryo-EM maps for *E. coli* pili by direct space methods. The analysis showed that all maps can be considered as maps with constant local resolution. The three raw maps (obtained by different authors) have approximately the same resolution of 2.6–2.7 Å, while the sharpened map has a higher resolution of 2.3 Å. These results are consistent with the results of visual analysis of EM maps. The obtained resolution estimates can be used to improve the reliability of determining the values of Atomic Displacement Parameters (ADP) in the studied objects and to select the map that is most promising for constructing the most detailed model of the object, including a network of water molecules.

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Received 14.02.2026.

Published 09.04.2026.