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Difference between conformations of immunoglobulin M and human rheumatoid factor based on small-angle X-ray solution scattering data

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Radial distribution functions calculated from molecular models point to the difference between conformations of immunoglobulin M and human rheumatoid factor, based on small-angle X-ray solution scattering data.

Previously,[1] it was shown that the shapes of molecules of rheumatoid factors IgMRF calculated from X-ray small-angle solution X-ray scattering (SAXS) data differ from immunoglobulin IgM in peripheral Fab regions that are more flocculent, which point to a higher flexibility of Fab-RF domains. This conclusion was based on the analysis of structural models obtained from SAXS data. An important question is the reliability of the results, *i.e.*, what is the degree of ambiguity and instability of shape restoration from 1D SAXS data. Because the problem of 3D particle shape restoration is mathematically not only ill-posed but ambiguous,[2] numerical experiments for investigation of solution stability should be performed using model scattering data calculated from expected theoretical structures. In this work, the 2RCJ[3] structure of IgM was used as an 'unknown' model from which SAXS intensity was calculated with the help of CRYSOL program.[4] This intensity was employed in a series of shape restorations using the dummy-atom modeling program DAMMIN.[5] This program, which was modified in some details, searches for a spatial configuration of small spherical elements by fitting experimental data $I_{exp}(s)$ using the target function

$$\min\{\Phi(B) = R^2 + w_D P_D + w_L P_L + w_C P_C + w_G P_G\}, \quad (1)$$

$$R^2 = \frac{\sum_{i=1}^{N} \{[I_{exp}(s_i) - \xi I(s_i)] W(s_i)\}^2}{\sum_{i=1}^{N} [I_{exp}^2(s_i) W^2(s_i)]}, \quad \xi = I_{exp} I / \|I\|^2,$$

where $s = 4\pi \sin\theta/\lambda$ is the modulus of the scattering vector, 2θ is the scattering angle, N is the number of points in $I(s)$ and $W(s)$ is the weighting function which reduces the dynamic range of the intensities according to the value of n which may be chosen from 1 to 4:

$$W(s) = \begin{cases} s^n, & \text{if } s > s|_{\max[I_{exp}(s)s^n]} \\ 0.5\left(s^n + \dfrac{\max[I_{exp}(s)s^n]}{I_{exp}(s)}\right), & \text{if } s \leqslant s|_{\max[I_{exp}(s)s^n]}. \end{cases} \quad (2)$$

A set of small spherical elements (beads) representing the particle are arranged in the close packed knots inside the search area of the diameter D_{max} obtained from the pair distribution function calculated from the experimental data using the GNOM program.[6] The current set of beads is considered a trial body. The theoretical scattering intensity $I(s)$ was calculated from it as described elsewhere.[5] In the target function, w_D, w_L, w_C, and w_G are weighting coefficients for the corresponding penalty terms. P_D and P_L are the penalties for disrupting the trial structure into disconnected pieces and for looseness of it.[5] The minimization procedure is based on the random search global minimization procedure known as the Metropolis algorithm. The first modification of DAMMIN was in the new variants of penalties for the shift of the body center from the origin (P_C) and in a separate penalty for the deflection of the gyration radius from that preliminary calculated by the GNOM program (P_G). These penalties are calculated during the search for the structure parts consisting only of contacting beads in contrast to the basic version of DAMMIN. Another modification was in the new search mode: after reaching a compact body (the relative number of lone atoms does not exceed 1%) or if the number of successful steps at the iteration becomes less than a half of the threshold value specified by the user, the program started to randomly modify only spatial knots that are occupied by the particle beads and their neighbours occupied by the solvent. These modifications led to increasing the relative number of successful steps during the search from 6–10 to 20–25% and, at the same time, to an increased number of successful trial models.

In this study, as well as in previous papers,[1,7] the structure models of IgM and IgMRF were searched inside a disk area, which allows one to reduce the relative bead diameter and to use the same number of knots in the search area as in the default spherical region. This allows one to increase the spatial resolution of the structure model if required. The relevance of the use of a disk area is demonstrated in Figure 1, where a search was performed inside a spherical area. As found previously,[2] it is

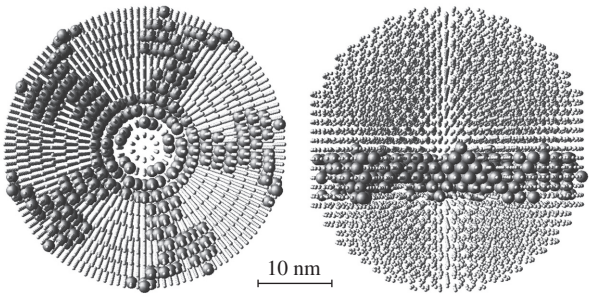

Figure 1 Restoration of the shape of model 2RCJ from artificial scattering data in the default spherical area. The structure is shown in two perpendicular orientations. The spherical search area knots are displayed by small beads.

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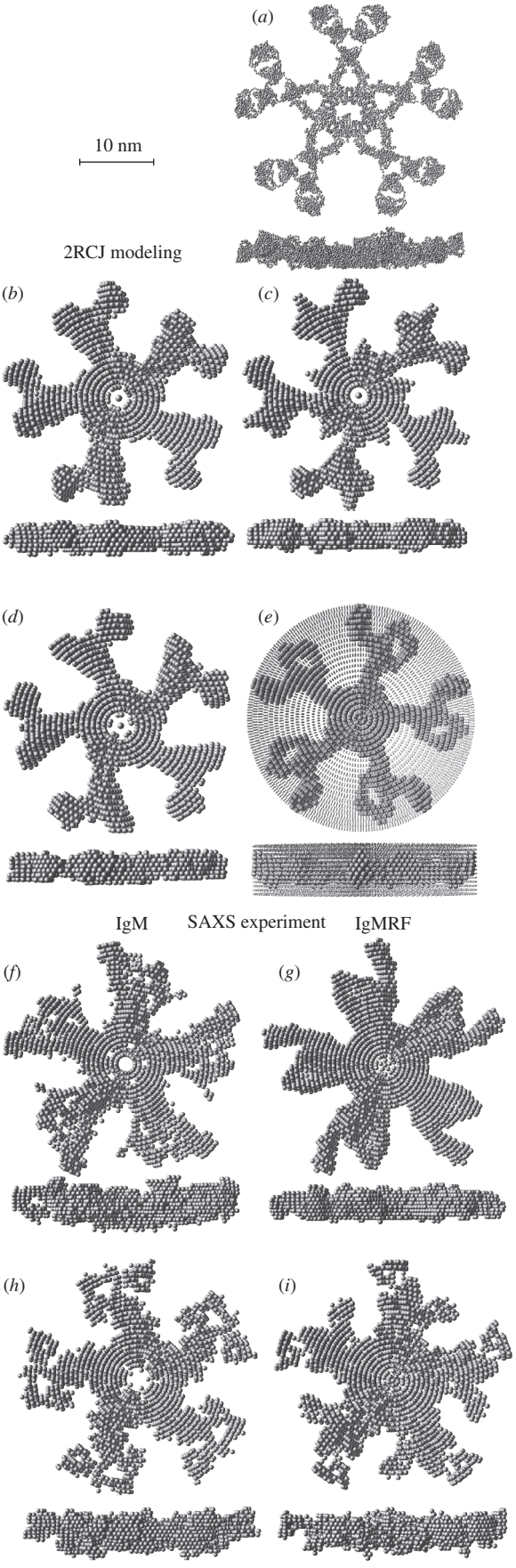

Figure 2 (*a*) The shape of 2RCJ.PDB, (*b*)–(*e*) restoration of the shape from artificial scattering data. (*f*), (*h*) Typical solutions obtained from SAXS data measured for IgM samples; (*g*), (*i*) results for IgMRF samples. (*b*), (*c*), (*f*), (*g*) Typical solutions obtained with the weight $W(s)$ at $n = 1$ [see equation (2)], (*d*), (*e*), (*h*), (*i*) at $n = 2$. The structures are shown in two perpendicular orientations. Structure (*e*) is shown inside the flat search area which knots are displayed by small beads.

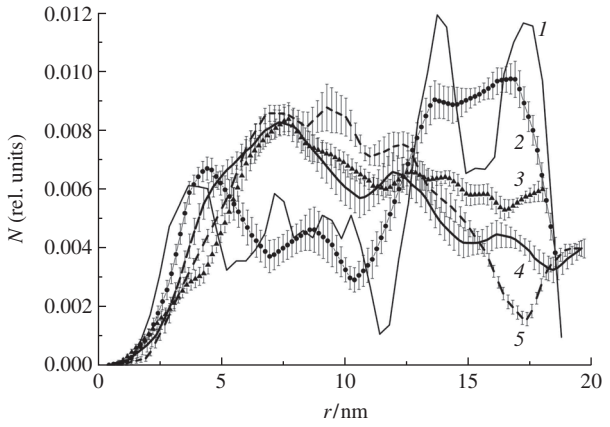

Figure 3 Radial distribution functions for the structure models: (*1*) 2RCJ,[3] (*2*) over solutions partially shown in Figure 2; (*3*) over models obtained from SAXS data of IgM solutions; (*4*) over models of IgMRF and (*5*) for IgMRFz. Scattering bars on the distribution curves were calculated using 20–30 models for each sample and reflect the degree of their ambiguity.

impossible to obtain a reasonably stable solution for a flat particle such as 2RCJ without imposing restrictions on its shape. Because of this, all the shape restorations were performed as described elsewhere[1,7] on the assumption that the shapes of IgM and IgMRF have a five-order axis symmetry.

The results of the restoration of 2RCJ structure from artificial data are shown in Figure 2. In spite of variations in high-resolution details, all structures are close to the initial model in general. This picture shows what kind of unstability may be present in the restored models.

Using the modified software, a series of calculations of the shapes of IgM and IgMRF was made for published experimental data.[1] The results were close to those reported earlier. To clarify the difference between IgM and IgMRF shapes and to check the main conclusion about less density of peripheral parts of IgMRF, the radial distribution functions $W(r)$ were calculated for all structures as the numbers of dummy atoms N_r in the structure model, which are at the distance r from the center of mass. Such a procedure is relevant in this case because all of the used structures have five-order axial symmetry and may be considered quasi-centrosymmetric (Figure 3).

Thus, the simulation revealed a reasonable degree of stability in the reconstruction of the complex shapes of the particles from the scattering data with respect to the structure of IgM, and the differences in the shapes of the radial distributions confirm the main conclusion that the molecules of rheumatoid factors have in average a ‘loose’ occupancy at the periphery.

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