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Reply to: Limitations of the iterative electron density reconstruction algorithm from solution scattering data

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REPLYING TO P. V. Konarev & D. I. Svergun *Nature Methods* https://doi.org/10.1038/s41592-021-01082-x (2021)

In their Matters Arising[1], Konarev and Svergun claim that several statements made in the original article[2] are not accurate, namely that DENSS (DENsity from Solution Scattering) cannot reconstruct multiple-density particles from one-dimensional solution scattering data and that the averaging procedure results in a type of low-pass filter effect that negates the accuracy of the averaged density map. Here I demonstrate that, while three-dimensional (3D) ab initio reconstructions from DENSS must not be overinterpreted, DENSS can indeed reconstruct multi-density particles provided that sufficient contrast differences exist between components, and that the averaging procedure is an effective way to improve the accuracy of the reconstructions.

The authors raise some valid points highlighting constraints on DENSS that users of the algorithm should take into account. Small-angle X-ray scattering (SAXS) is not only a low-resolution technique, but also a low-information one, leading to ambiguity in reconstructions of 3D objects ab initio. Conventional shape restoration algorithms cannot generate higher-resolution reconstructions for two primary reasons: low information content and implicit assumptions of uniform density. The iterative structure factor retrieval algorithm implemented in DENSS removes the assumption of uniform density. However, it does not remove the more fundamental barrier of limited information content. Thus, it is important for researchers to understand that DENSS is still subject to the same ambiguity and low resolution limiting conventional algorithms, which correspond to a fundamental limitation of SAXS.

DENSS is an entirely new approach to the problem of 3D reconstruction, using iterative structure factor retrieval, and this fact should not be lost on the reader. DENSS is capable of creating reconstructions of similar quality to those of traditional shape reconstruction methods while removing one of the primary restraints, demonstrating the power of the approach and its utility for researchers. It is of course always advantageous to achieve similar or better solutions with fewer restraints. The added advantage of DENSS over conventional shape reconstruction is its ability to visualize density within the object envelope. However, owing to the low information content in SAXS, the density difference between two regions must be sufficiently large to be distinguishable at low resolution.

Konarev and Svergun claim that the protein–micelle complex is an unsuitable example of density difference, as bead modeling can also reconstruct hollow spheres in a dumbbell shape. It is true that the coenzyme A (CoA) micelles are reasonably well approximated by hollow spheres at low resolution, so it is valuable to also use an example where this approximation is no longer valid. Some common use cases include SAXS or small-angle neutron scattering (SANS) experiments on membrane proteins in micelles, nanodiscs, detergents, etc., as well as non-biological targets such as block copolymers, nanoparticles and other soft matter[3–6]. In the case of some micelles, such as those with DDM detergent, the density of the hydrophobic core is often so low as to exhibit substantial negative contrast in the interior[3,7]. In such cases, the default restraint of positivity can be disabled when running DENSS to allow for negative contrast; the algorithm is then able to successfully reconstruct the multi-density particle showing large negative density in the interior (Fig. 1a), which is not possible with bead modeling, where uniform density is assumed. Multi-phase bead modeling algorithms can theoretically produce a viable bead model with a contrast series of multiple scattering curves, although this does not appear to be possible with only one scattering curve[7], as can be done with DENSS. The ability to model negative contrast is indeed a unique advantage of DENSS.

Konarev and Svergun next claim that protein–nucleic acid complexes would be more appropriate examples to demonstrate that DENSS can distinguish the nucleic acid in the reconstruction, as nucleic acid has twice the contrast of protein. However, this assumption is an oversimplification of the actual problem. It is true that nucleic acid has higher contrast than protein owing to the high proportion of phosphates. However, the density of a biological molecule is not a single value, but rather is a broad distribution of values, and the distribution of nucleic acid densities largely overlaps that of protein[8]. Even for intermediate-resolution cryo-electron microscopy (cryo-EM) reconstructions, nucleic acid cannot be distinguished from protein using density alone; rather, advanced algorithms are required to discern it[8]. The assumption of higher density for nucleic acid does not take atomic packing into consideration. Proteins are often much more compact than nucleic acids, as RNA and DNA mostly form helical or double-helical structures containing large voids in the major and minor grooves. At low resolution, such packing must be accounted for when estimating density. Figure 1b shows low-resolution density calculated from atomic coordinates using EMAN2 (ref. [9]) for the given examples. In each case, it is clear that the nucleic acid cannot be distinguished from the protein on the basis of higher density, and thus the nucleic acid is also not expected to be distinguishable in DENSS reconstructions.

However, if the density of the nucleic acid were substantially greater, DENSS should be able to distinguish the two components in

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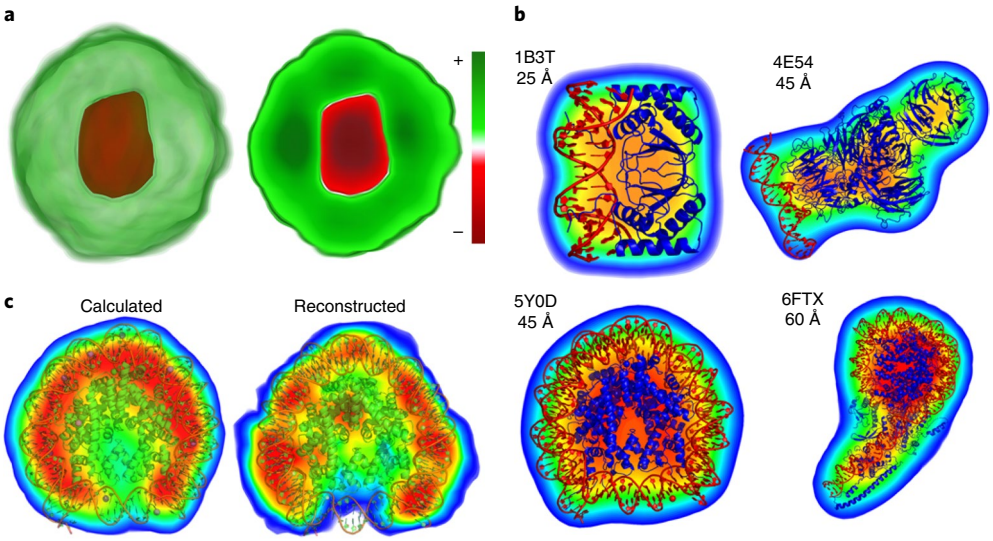

Fig. 1 | Calculated or reconstructed low-resolution density of DDM micelles and protein–nucleic acid complexes. a, Reconstructions from SAXS data of DDM micelles with large interior negative contrast. The average of 20 DENSS reconstructions is shown as a 3D transparent volume (left) and a cross-section (right). Density is colored in green (positive density) and red (negative density). **b**, Calculated density from atomic coordinates at low resolution using EMAN2 (ref. [9]) for the protein–nucleic acid complexes shown by Konarev and Svergun. Maps were calculated at the resolution shown in the figure as estimated by DENSS reconstructions. Maps are colored from low density (blue) to high density (red). **c**, Calculated and reconstructed electron density maps of the modified protein–DNA complex for PDB ID 5Y0D with the same coloring as in **b**.

such a scenario. To test this with one example, I have modified the structure for DNA-wrapped histone (PDB ID 5Y0D), duplicating the DNA molecule to artificially double its contribution to the scattering. This increases the contrast to the point where low-resolution density calculated from the atomic coordinates shows an expected maximum where the DNA is located (Fig. 1c). DENSS reconstructions demonstrate that the final averaged density does indeed reflect the higher expected density at the outer edge of the histone disc where the DNA is located (Fig. 1c). Thus, if given sufficient contrast, DENSS is capable of reconstructing multi-density systems. As noted by Konarev and Svergun, in some circumstances where the density difference between two regions is sufficient to be distinguished by DENSS, bead modeling can approximate the shape as having hollow regions where there is low relative density. However, in such cases, DENSS reproduces actual density where it exists, even if it is low (for example, the protein in this simulation and the hydrophobic core in the DDM micelle), and is thus a more accurate representation of the true object.

Konarev and Svergun also simulate an ellipsoid that is cut in half in different orientations, assigning different contrasts to each half. DENSS reconstructions from simulated data do not discriminate between the different orientations in this case. This is a valid criticism and shows opportunities for future improvements to DENSS. This is likely due to the sharp cutoffs of uniform density in the simulated system, as such unnatural, sharp cutoffs are known to result in aliasing and striping artifacts in image reconstruction, resulting in stagnation of the phase retrieval procedure and falling into local minima. Various advanced algorithms for dealing with such artifacts have been developed[10,11] and could be incorporated into DENSS. In such cases, default parameters may not be appropriate to yield reliable reconstructions and may need to be adjusted for adequate performance. It is noteworthy that the three distinct ellipsoid models have highly similar scattering profiles, as seen in Supplementary Fig. 2 of the critique, and thus likely represent an example of a fundamental inability of SAXS to distinguish between such similar objects.

Konarev and Svergun's final criticism is that the averaging procedure used for DENSS reconstructions results in a type of low-pass filter on the maps, producing averages that do not fit the data and thus are unreliable reconstructions. It is true that the averaged maps do not fit the data. However, the assumption that an averaged map is unreliable because it does not fit the data is not correct. Supplementary Fig. 1 shows a plot of the Fourier shell correlation (FSC) between 20 individual reconstructions and the known structure along with the FSC between the averaged map and the known structure (using PDB ID 4FE9 as the example[12], chosen for its highly ambiguous shape as estimated by AMBIMETER[13]). One can clearly see that the averaged density has a better correlation to the actual structure than any of the individual reconstructions, despite the poorer fit of the scattering profile. Additionally, the poor fit of the average to the data is a common occurrence in ab initio SAXS reconstructions and is similarly seen when generating an average of multiple bead models. Similarly to how bead modeling programs offer refinement for such purposes (for example, the DAMSTART program[14]), DENSS also provides a refinement script for a similar reason (denss.refine.py), although in my tests this does not appear to improve the FSC resolution. Future improvements to DENSS may be realized by incorporating goodness of fit into the averaging procedure, to aid generation of averaged maps that simultaneously fit the data.

The primary aim of the original DENSS publication was to describe a new approach to solving the phase problem for experiments that do not yield full 3D Fourier amplitudes. This approach is not limited to SAXS but can theoretically be used for other experiments where the information content is substantially greater, for example, in fiber diffraction. I used one-dimensional solution scattering as the example as it provided a low-information limiting case showing the power of the technique. I have provided the DENSS software open source so that others may take advantage of it and investigate the algorithm themselves. There are likely cases where DENSS and traditional shape reconstruction methods perform equivalently, cases where DENSS excels and cases where traditional methods excel. Researchers should use the tool most appropriate for their needs.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41592-021-01083-w.

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Methods

The DDM micelle shown in Fig. 1a was reconstructed using the MEMBRANE mode of DENSS (v1.6.0) to allow for negative contrast and using the default 20 reconstructions for averaging. The simulated maps in Fig. 1b were calculated using the EMAN2 script e2pdb2mrc.py, and the resolution was set to the resolution of the ab initio reconstruction calculated by DENSS. The scattering profile of the modified DNA-wrapped histone structure was calculated using FoXS[15], and the reconstruction shown in Fig. 1c was performed using the default SLOW mode of DENSS, with 100 reconstructions for averaging. The scattering profile for PDB ID 4FE9 was calculated using FoXS and reconstructed using the default SLOW mode of DENSS with 20 reconstructions for averaging. EMAN2 was used to align the maps to the atomic coordinates and to calculate the FSC curves shown in Supplementary Fig. 1.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

The experimental DDM micelle data were kindly provided by F. Gabel and are available as described in ref. [7].

Code availability

DENSS is available open source on GitHub at https://github.com/tdgrant1/denss.git, and instructions for downloading, installing and running DENSS can be found on the GitHub page and at https://www.tdgrant.com/denss/.

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Competing interests

The author declares no competing interests.

Additional information

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41592-021-01083-w.

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Data collection	SAXS data for the DDM micelle was provided by Dr. Frank Gabel and previously published in Reference 5.
Data analysis	DENSS v1.6.0 was used for generating the DDM micelle density, using the "MEMBRANE" mode option of DENSS to disable the positivity restraint. 20 reconstructions were averaged together using denss.all.py.

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