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Holographic Methods in X-ray Crystallography. III. First Numerical Results

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Abstract

The holographic method for the completion of crystal structures, described in paper II [Szöke (1993). *Acta Cryst.* **A49**, 853–866], is implemented numerically. The purpose of these modest calculations is to show that the holographic method can handle real crystallographic data in easy problems and to test various algorithms for its implementation. Both synthetic and experimental data are used and sources of error are systematically introduced. The numerical experiments support the theory presented in paper II and show that the holographic method may be a potentially viable alternative to conventional methods for the completion of crystal structures.

Introduction

An alternative method for the completion of crystal structures, the holographic method, was described in papers I and II of the series (Szöke, 1992, 1993).

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Paper II gives a detailed derivation of the method and a brief discussion of the algorithms used to solve the holographic equations, together with some of their mathematical properties. This paper presents modest first results obtained using the holographic method on computers. As newcomers to crystallographic data processing, our aim was to place the method on a firm footing and to prepare for more detailed comparisons with established methods. Our computational efforts were directed in two complementary directions.

In the first case, we wanted to demonstrate that the holographic method can handle real crystallographic data, using slight modifications to an existing crystallographic program. For this we chose an 'easy' problem: bovine pancreatic trypsin inhibitor (BPTI), with one of the amino-acid side chains removed. Using two test cases, one employing model data and the other experimental data, our goal was to show that the holographic method could correctly complete the crystal structure by reconstruction of the missing side chain.

In the second case, we performed extensive tests on simple ('toy') models for crystallographic problems. Our

goal was to determine the stability and accuracy of the solutions in the presence of noise, to see how little of the structure can be known before the various algorithms fail and to find out how positivity constraints stabilize the solutions.

In a subsequent paper, a fast algorithm and its use for large crystallographic problems will be described (Goodman, Szöke, Szöke, Somoza & Kim, 1993).

1. Reconstruction of part of a macromolecule

Two parallel numerical experiments were performed to test the ability of the holographic method to complete crystal structures. The first of these experiments used synthetic data and the second used reported experimental data. The system chosen was bovine pancreatic trypsin inhibitor. The Cartesian coordinates and the experimental structure factors were obtained from the Brookhaven Protein Data Bank [PDB; Abola, Bernstein, Bryant, Koetzle & Weng (1987)], entries 5PTI and R5PTISFX (Wlodawer, Walter, Huber & Sjölin, 1984). Since this coordinate set was the result of joint X-ray and neutron data refinement, the H-atom positions were also available. The original refinement used the Hendrickson-Konnert restrained least-squares method (Hendrickson & Konnert, 1980). The X-ray and neutron data sets had resolutions of 1.0 and 1.8 Å, respectively.

We used an existing crystallographic computer program, *X-PLOR* (Brünger, 1990), together with the holographic method. Structure factors were used in their complex Cartesian form. *X-PLOR* uses the standard five Gaussian analytic approximation for the atomic scattering factors and the parameters for hydrogen were obtained from *International Tables for X-ray Crystallography* (1974).

In both reconstructions (using either synthetic or experimental reflections), the 'known' part of the structure was taken to be the reported structure from the PDB entry 5PTI with the side chain of phenylalanine 33 removed. Ordered water and a reported phosphate were also included in the 'known' part. The calculated structure factors, F_{calc} [R of equation (7) of paper II] were generated by *X-PLOR* using the atomic positions, occupancies and Debye-Waller factors (B_j) as reported in the PDB entry 5PTI.

For the synthetic-data reconstruction, the 'measured' structure factors were taken to be the magnitudes of the structure factors calculated by *X-PLOR* for the entire asymmetric unit using the parameters reported above. For the experimental data reconstruction, F_{obs} from the Protein Data Bank file R5PTISFX were used. In each case, indices for the same 3390 reflections were selected. The indices (h, k, l) were selected from the experimental X-ray data set of 17 615 reflections to give a resolution of approximately 2.0 Å. The space group for this unit cell is $P2_12_12_1$, orthorhombic, the occupancy is four and the unit-cell dimensions are $a = 74.10$,

$b = 23.40$ and $c = 28.90$ Å and $\alpha = \beta = \gamma = 90.0^\circ$. The asymmetric unit was obtained by cutting both a and c in half.

The 'elementary holograms' [see equation (15) of paper II] were calculated by filling the asymmetric unit with a uniform grid of basis functions, at a spacing ~ 2 Å on a three-dimensional lattice, and the immediate region around the phenylalanine 33 side chain was filled with a grid of spacing ~ 0.5 Å. Each basis function consisted of a single electron and a Debye-Waller factor of 3.96 Å² (average coarse-lattice spacing squared), in essence, an H atom. Basis functions in the 0.5 Å fine grid that duplicated those in the 2 Å coarse grid were removed. The total lattice consisted of 3525 points, composed of 1596 coarse-grid points and 1929 fine-grid points. The predetermined set of 3390 reflections was used and structure factors were generated for each point in the lattice. This resulted in an elementary matrix of size 3390×3525 , corresponding to a set of 3390 linear equations in 3525 variables.

External routines were written to input the structure factors, compute the elementary holograms and the elementary matrix and then solve the linear system of equations. The routine *LLSQF* (IMSL Inc., 1981) was used to perform the QR decomposition. The computations were performed on a Silicon Graphics 4D/480 computer using a single processor. For all reconstructions, an input tolerance of 0.01 was used and pivoting was allowed on all columns. Each calculation took approximately 4 h. The QR decomposition included 105 columns in the basis for the synthetic case and 102 columns for the experimental case. These are essentially the numbers of independent parameters used in the solutions of the problem and show that it is highly overdetermined. The solutions, which represent the number of electrons in the vicinity of each point on the lattice (in each voxel), were smoothed using a 2.0 Å Gaussian spatial filter.

The synthetic and experimental reconstructions are shown in Figs. 1 and 2, respectively. In each diagram, the contours represent the reconstructed electron density [equation (17) of paper II] and the stick figure is the reported X-ray structure of the phenylalanine 33 side

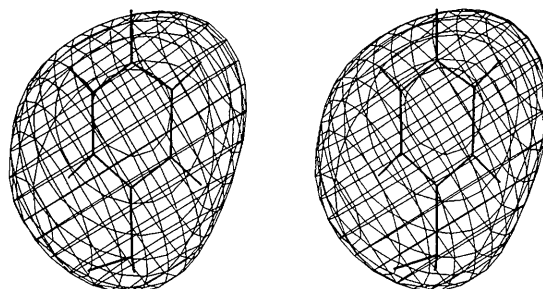


Fig. 1. Electron density of the deleted phenylalanine 33 side chain of BPTI, reconstructed by the holographic method using the squares of structure factors computed from the solved structure. Contours corresponding to $0.75 e \text{ \AA}^{-3}$ are shown.

chain. The data show that the holographic method was able to complete the crystal structure quite well. The synthetic-data reconstruction is clean: there is no negative electron density and only a small amount of noise. The experimental reconstruction has noise bounded between $\pm 0.25 \text{ e} \text{ \AA}^{-3}$ and the maximum electron density is $1.9 \text{ e} \text{ \AA}^{-3}$. Although this is not obvious from the figures, in both the synthetic and experimental cases the center of the reconstructed electron density is offset from the center of the ring in the direction of the β -carbon, as expected.

2. Properties of various reconstruction methods

Two additional methods are described here for the solution of the linearized equations of X-ray crystallography. All the computations described in this section used two simple model crystal structures. The first one was obtained by placing ten C atoms onto a helix. Two helices, placed with $P2_1$ symmetry around the z axis, formed an orthorhombic unit cell. The second model was obtained from the first by moving each of the atoms onto its nearest lattice point. The Cartesian coordinates of the atoms in the asymmetric unit and their B_j values for both structures are shown in Table 1. In all calculations, the absolute values of the structure factors were calculated using equation (2) of paper II for all unique reflections within a resolution range d and thermal factors B_j consistent with the chosen resolution. This served as the simulated diffraction pattern. Various fractions of the molecule were then considered to be known and used as the reference in the holographic reconstruction method. The studies reported in this section did not use standard crystallographic computer programs.

(a) Solution by singular-value decomposition

The singular-value decomposition subroutine was obtained from *LINPACK* (Dongarra, Moler, Bunch & Stewart, 1979). It was used in double precision on a single processor of a Silicon Graphics 4D/240 computer. The solution was obtained using equation (19) of paper II.

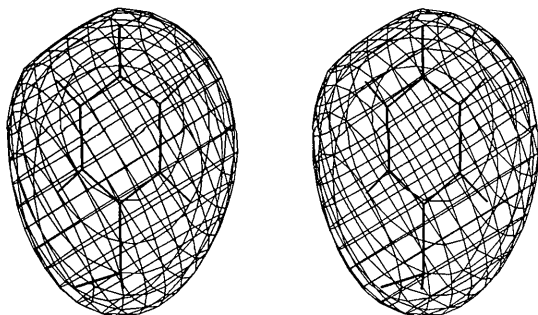


Fig. 2. Electron density of the deleted phenylalanine 33 side chain of BPTI, reconstructed by the holographic method using experimentally measured reflections. Contours corresponding to $0.75 \text{ e} \text{ \AA}^{-3}$ are shown.

Table 1. 'Known' and 'unknown' fractional atom positions for the spiral in one asymmetric unit

The orthorhombic unit cell consisted of a 20 \AA cube containing 20 C atoms with $P2_1$ symmetry around the z axis. The Debye-Waller factor, B_j , was 6.25 \AA^2 . In any particular run, 'unknown' atoms were selected at random. Structure (a) is the original spiral and structure (b) is the spiral in which atoms are moved to the closest grid point.

Atom number	(a) Atoms not on the grid			(b) Atoms on the grid		
	x	y	z	x	y	z
1	0.347	0.695	0.100	0.3125	0.6875	0.0625
2	0.138	0.520	0.137	0.1875	0.5625	0.1875
3	0.185	0.239	0.174	0.1875	0.3125	0.1875
4	0.459	0.116	0.211	0.4375	0.0625	0.1875
5	0.722	0.269	0.247	0.6875	0.3125	0.1875
6	0.747	0.561	0.284	0.6875	0.5625	0.3125
7	0.526	0.730	0.321	0.5625	0.6875	0.3125
8	0.276	0.624	0.358	0.3125	0.6875	0.3125
9	0.242	0.346	0.395	0.1875	0.3125	0.4375
10	0.469	0.156	0.432	0.4375	0.1875	0.4375

The statistics of the singular values obtained when 6 atoms out of 20 were deleted are shown in Fig. 3. About half the singular values are less than 10^{-5} of the largest one. This shows clearly that the equations are ill conditioned for both structures in Table 1. The number of essentially zero singular values agrees with the prediction in §3(a) of paper II. When the atoms are on lattice points [structure (b) in Table 1], the holographic algorithm is capable of an essentially perfect reconstruction of the missing atoms. This is impossible when the atoms are in general positions [structure (a)] but the effect on the distribution of the singular values was minimal, as shown in Fig. 3. The reconstruction was obtained to various values of the cutoff in the weight factor, w_i , in equation (19) of paper II and was compared with the difference Fourier algorithm. Table 2

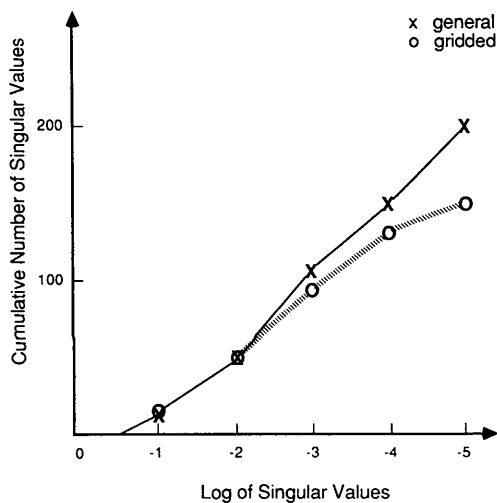


Fig. 3. The cumulative number of singular values in decreasing order for the model system described in Table 1. The largest singular value was normalized to unity. In both the structures of Table 1, half the singular values are essentially zero. This corresponds to a lack of information in the observable diffraction pattern.

Table 2. Comparison of the maximum numbers of electrons per voxel recovered with the difference Fourier algorithm (DF) and singular-value decomposition (SVD), with cutoff 10^{-3} , when various numbers of atoms are considered to be unknown in the structures of Table 1

Only the unknown atoms in an asymmetric unit are reported. The maxima listed are at the positions of the unknown atoms or at the nearest grid points. Also listed is the largest number of electrons appearing at incorrect positions in the reconstruction. The correct structure has six electrons at each atom position.

(A) 4 out of 20 unknown atoms

Atom number	(b) Atoms on the grid		(a) Atoms not on the grid	
	DF	SVD	DF	SVD
1	0.66	2.69	0.51, 0.48	2.15, 2.14
2	0.46	2.40	0.47, 0.36	2.51, 1.24
Wrong	0.34	1.25	0.35	1.61

(B) 6 out of 20 unknown atoms

Atom number	(b) Atoms on the grid		(a) Atoms not on the grid	
	DF	SVD	DF	SVD
1	0.49	2.77	0.49	2.39, 1.94
2	0.52	2.84	0.49	1.49
3	0.41	2.67	0.37	2.73, 1.67
Wrong	0.39	1.48	0.47	1.51

lists the maxima of the electron density at the expected positions of the missing atoms and in their close vicinity for the optimum cutoff of 10^{-3} for the singular values. In general, the analysis of paper II was borne out. The reconstruction was quite similar to the difference Fourier method, but in all cases the solution with singular-value decomposition was less diffuse and artifacts were less pronounced. The difference Fourier method and the singular-value decomposition fail at about the same number of missing electrons.

(b) Solution by non-negative least squares

The non-negative-least-squares program was obtained from the SLATEC program library (Scandia National Laboratory, Albuquerque, NM, USA). We used the *DWNNLS* version, with the weights set to unity. The computer program implements the method of Lawson & Hanson (1974). It is based on the QR decomposition of the matrix $M_p(\mathbf{h})$ of equation (17) of paper II. The program starts with an initial guess that can be supplied either by a difference Fourier solution of the crystallographic problem or by an arbitrary solution vector. The program finds the largest negative gradient consistent with the positivity constraint and then finds the minimum along that direction. If none of the components of the solution vector [n_p of equation (17) of paper II] become negative, the program continues with a conjugate-gradient minimization of the square residual between the right- and left-hand sides of equation (17) of paper II,

$$\mathbf{f}_{\text{NNLS}} = \frac{1}{2} \sum_{\mathbf{h}} \left| \sum_{p=1}^P n_p M_p(\mathbf{h}) - H(\mathbf{h}) \right|^2 \quad (1)$$

or, equivalently, equation (18) of paper II with all weights set to one. If, during the search for the minimum of \mathbf{f}_{NNLS} in (1), any one of the components of the solution vector becomes negative, the program sets that component to zero and tries to find another independent vector in the solution space that produces a large negative gradient. The program updates the QR decomposition of the matrix $M_p(\mathbf{h})$ with the new column added and starts a new conjugate-gradient minimization. Generally, the algorithm was used iteratively: the electron density 'recovered' by the algorithm was considered to be 'known'. It was then added to the 'reference' and the procedure was repeated as outlined in § 2 of paper II.

The program is efficient; it scales for our problems approximately as the square of the number of unknowns, P^2 . This is somewhat surprising; naively, one would expect a P^3 scaling. We conjecture that, in simple problems, *i.e.* when the number of unknown atoms is small, the program finds a small subset of the available solution space fairly quickly so it never has to decompose a very large matrix. We found that the solutions obtained were completely insensitive to the first guess. In fact, the number of iterations needed was almost the same for a starting vector of all zeros as for that supplied by the difference Fourier method. Typical running time for the reconstruction was 5 min using a single processor of a Silicon Graphics 4D/240 computer.

We carried out four different sets of studies. In the first set, we chose the same simulated structures as above and deleted an increasing number of atoms. As many as half the atoms could be deleted and a good reconstruction still be obtained. When the atoms were on lattice points [structure (b) of Table 1], a perfect reconstruction was obtained. It is presented in Fig. 4 together with the solution obtained using the difference Fourier method. The comparison is 'unfair' because there are direct methods that could be used to obtain a perfect reconstruction for such a simple problem (Ladd & Palmer, 1985; Stout & Jensen, 1989; Beurskens, 1985). The pictures are intended to show the prominence of the dual image in the difference Fourier method and the ability of the positivity constraint to eliminate it in the holographic method. The computations of the holographic method are completely automatic and the results are quantitatively correct. Also, there is no inherent difficulty in extending the holographic method to large molecules.

With half the atoms unknown, we studied the effect of displacing atoms from the lattice points. When the unknown atoms were left on the lattice points, the reconstruction succeeded to machine precision whether or not the known atoms were on the lattice points. When the unknown atoms were off the lattice points, the reconstruction still succeeded but was not perfect. The crystallographic *R* factor was reduced only to 24–27%, yet the atoms were clearly recognizable. The number of electrons per atom varied between four and seven, in contrast to the correct six, but the centers of gravity

of the atoms were correct to 0.4 Å. When 12 out of the 20 atoms were considered to be unknown, the reconstruction did not converge.

In the rest of the studies described below, all the atoms were placed on grid points, so perfect recovery of them was, in principle, possible. In the second set of studies, 10 atoms out of 20 were still considered to be unknown, but half the reflections were deleted. As expected, the $P2_1$ symmetry was initially lost in the reconstruction but eventually the reconstruction converged perfectly and the symmetry was regained. In the third set, all the reflections were retained but Gaussian noise was added to the 'observed' reflections. The added noise was proportional to the intensity of each reflection. As much as 30% noise could be added and a good recovery still be obtained. The results are shown in Table 3. The standard deviation of the calculated and observed $|F^2|$ and the standard crystallographic R factor are both linearly proportional to the noise added. At 30% noise,

Table 3. Reconstruction of 10 atoms out of 20 in structure (b) of Table 1 with Gaussian noise added to the 'observed' structure factors

The atoms were on grid points. The non-negative-least-squares algorithm was iterated to convergence. In all cases, the reconstructed electron densities were centered at the correct positions.

Fractional noise (%)	Final standard deviation	Final R factor (%)
0	1.0×10^{-5}	6.0×10^{-6}
3	0.82	1.4
10	2.66	4.8
20	5.13	9.8
30	7.55	14.1

the R factor is 14%. In the fourth set, 12 atoms were deleted but the Z of one of the known atoms was increased. The reconstruction succeeded as long as the total Z of the unknown atoms stayed below 55% of the total Z in the unit cell.

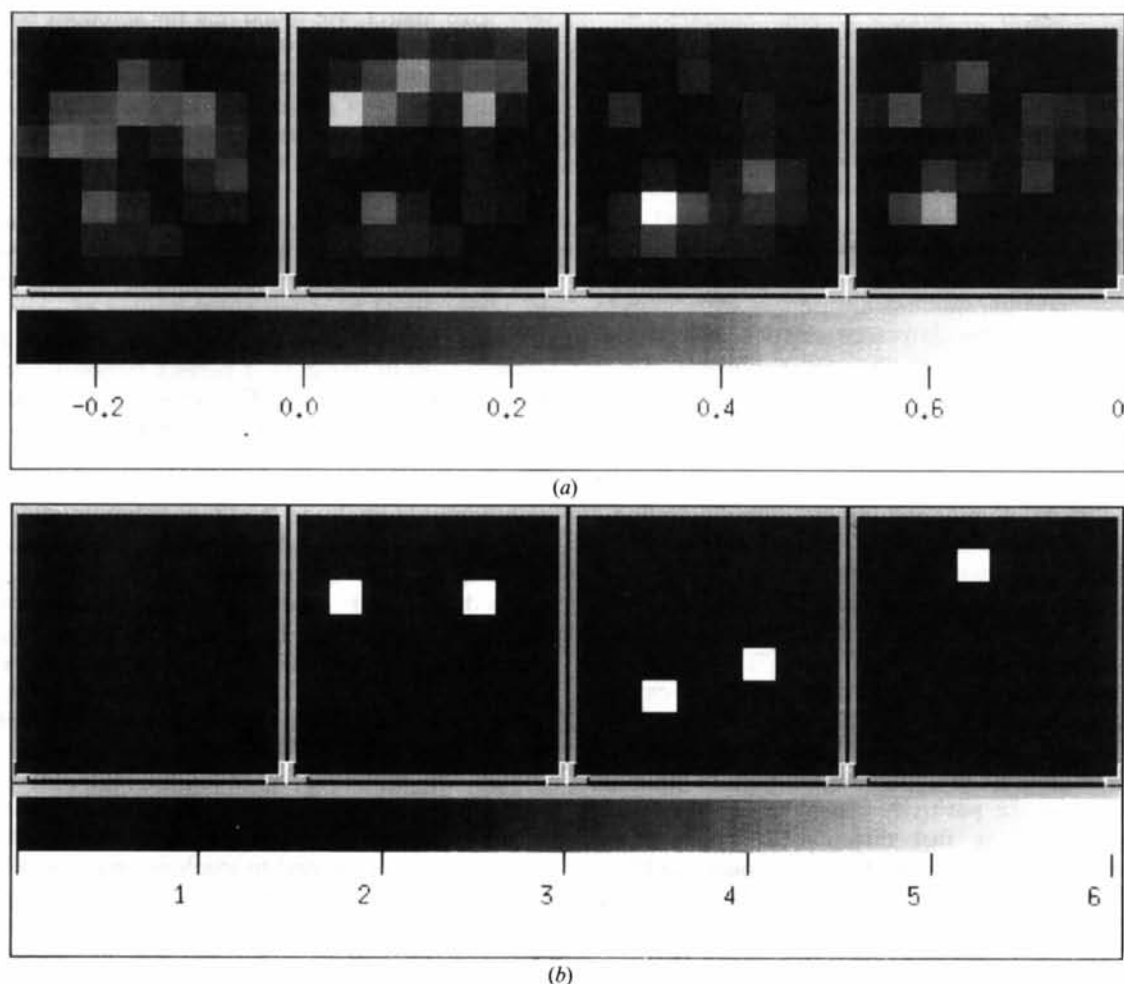


Fig. 4. Comparison of the electron density map recovered by (a) the difference Fourier algorithm and (b) the holographic method for the structure presented in Table 1 with half the atoms deleted. The figures show the electron density in successive layers of the unit cell. The holographic method was iterated to convergence. A non-negative-least-squares algorithm was used and the unknown atoms were on grid points. The reconstruction was done on a $4 \times 8 \times 8$ grid spanning the asymmetric unit of an orthorhombic unit cell with $P2_1$ symmetry.

3. Summary

Computer calculations have been carried out using the holographic method in order to complete the interpretation of X-ray crystal structures. There were two complementary efforts. In the first one we used the experimental diffraction intensities of bovine pancreatic trypsin inhibitor, deleted one of the amino-acid side chains from the solved structure and considered the rest of the solved structure to be known. We used a QR decomposition algorithm to solve the linearized holographic equations in order to obtain the unknown part of the structure. The reconstructed electron density is very good. The conclusion from this study is that the holographic reconstruction algorithm may become a viable alternative for the completion of crystal structures. In simple cases, it can be used by modifying existing crystallographic computer programs. The second prong of our computational effort was to find alternative algorithms, to establish some of the capabilities of the holographic method and to include some external information. In this paper, we have discussed the solution of the holographic reconstruction equations using singular-value decomposition and non-negative least squares. We have established that the equations are indeed ill conditioned and that the number of practically independent equations is in accordance with the theoretical discussion of paper II. We have also found that without additional information, *i.e.* use of the singular-value decomposition, the reconstructed electron density is quite similar to that obtained using the difference Fourier method; however, it is better concentrated at the positions of the missing atoms and has fewer artifacts. Both methods fail at about the same fraction of unknown atoms. The addition of non-negativity constraints improves the reconstruction dramatically. When the non-negative-least-squares algorithm was used, as many as 55% of the electrons could be retrieved. We also found the retrieval to be quite insensitive to missing reflections and Gaussian noise.

The computational experiments reported in this paper are very promising and are in good agreement with the theoretical discussions of paper II. However, the algo-

rithm used for the non-negative-least-squares solution does not scale well with the complexity of the crystals: its storage requirements are proportional to $\sim P^2$ and its running time is proportional to $\sim P^2$ or even $\sim P^3$, where P is the number of resolution elements in the unit cell. In a forthcoming paper, a fast algorithm will be described that can be used to solve model problems of magnitudes comparable to those of problems of current interest in macromolecular crystallography (Goodman, Szöke, Szöke, Somoza & Kim, 1993).

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