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On the density modification at very low resolution

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Abstract

The current techniques for *ab initio* macromolecular phasing are applicable at very low resolution and need to be followed by some phase extension procedures in order to improve the obtained image. Density modification methods can be used for this image improvement. Since they are usually applied at medium and high resolution (d < approx. 6 Å), a preliminary analysis of the applicability of these methods at very low resolution becomes necessary and is reported here.

I. Introduction

While direct methods for small molecules have become an usual crystallographic tool, the solution of the phase problem for large macromolecules needs extra information like heavy atom derivatives or anomalous dispersion. During recent years some progress in the development of *ab initio* phasing methods at very low resolution has been reported (see, for example, Lunin, Urzhumtsev and Skovoroda, 1990; Subbiah, 1993; Harris, 1995; Volkman et al., 1995; Lunin et al., 1995, Urzhumtsev & Podjarny, 1995a, Andersson & Hovmöller, 1996; see also the review by Podjarny & Urzhumtsev, 1997). In particular, these methods have obtained the first crystallographic image for the 50S ribosome particle from *Thermus thermophilus* (Volkman et al., 1995; Urzhumtsev, Vernoslova and Podjarny, 1996). However, the resolution which can be obtained today by these methods is not high enough. A possible way to get a higher resolution image is to develop further the methods like FAM (Lunin et al., 1998; its development and application to the T50S ribosome data allowed an increase in the resolution from 100 Å initially to about 30 Å). Another way is to create or to modify existing methods for image improvement. These methods have been developed to be applied at "usual" resolution (2-5 Å) therefore at very low resolution some basic hypotheses may be not fulfilled. A special analysis should be done in order to show how these methods should be updated, if possible, to be applied at very low resolution data.

One of the basic groups of methods for image improvement is density modification (see, for example, Podjarny, Rees and Urzhumtsev, 1997). The usual iteration procedure of modification of the density distribution function, depending either on the density value in a given point or on the coordinates of this point, can be eventually applied at any resolution. The simplest information which can be used is the flat density in the solvent region (Bricogne, 1974). The following basic questions should be analysed :

1. is such flattening applicable at low resolution (i.e., are structure factors recalculated at the same resolution close enough to the correct values) ?

2. can such procedures extend the phases at low resolution (do phases of structure factors at higher resolution have any structural information) ?

3. can the phase information be improved only by refining the envelope border (flat envelope) or are density values important ?

4. if the procedure is applicable, which are the optimal parameters ?

II. Computational experiment

Test calculations have been carried out using model data calculated from a density modulated envelope for the 50S ribosomal particle (Berkovich-Yellin, Wittmann & Yonath, 1990) arbitrarily placed in the experimentally observed unit cell (space group P4$_3$2$_1$2; a = b = 496Å, c = 196Å) as described before in Lunin et al. (1995). Since no molecular model was used in the procedure, the contribution of the disordered solvent (Urzhumtsev & Podjarny, 1995b) was not taken into consideration. No experimental error was introduced.

The following procedure has been applied :

1. calculate a density distribution at a given resolution (60, 40 or 30 Å);

2. define a molecular envelope as a set of unit cell points with the density value above a given threshold; the threshold was defined to leave a given percentage of the unit cell volume above this level;

3. flatten the density distribution below the threshold keeping the density above the threshold ("soft modification"); alternatively, at the test for using flat envelopes, the density above the threshold was also flattened ("hard modification");

4. calculate structure factors form the modified density distribution;

5. compare calculated structure factors with the exact values

We considered that "good" structure factors at the starting resolution indicate the applicability of such flat solvent hypothesis (or flat envelope hypothesis), and that "good" phases at higher resolution indicate the possibility to use such procedure for image improvement. The limit of "goodness of added phases" was taken as 60 degrees (mean cosine value is 0.5) following Lunin & Woolfson (1993). Note that no iterations were done, all numbers are shown for structure factors obtained after a single density modification.

III. Table explanation

The procedure was applied for the maps of the resolution of 60, 40, 30 Å. Tables 1-3 give the results of the comparison of structure factors. Different columns correspond to different cut-off level values varied from 10 to 90% (percentage of the volume above the threshold value).

The amplitude correlation was calculated for F_{obs} (simulated data set) and F_{calc} (after density modification) around their mean values.

The weighted phase correlation was calculated as the mean value of cos(delta phi) weighted by Fobs**2, which corresponds to the closeness of the maps calculated with Fobs and with exact or calculated phases.

Cells are coloured accordingly to the criteria value; for the phase difference the limit value is 60 degrees. The shell of reciprocal space corresponding to the resolution slightly higher than the starting one is analysed in more detail and is given at the bottom of every table.

Table 1.1. Starting density at 60 Å; "hard" modification

Table 1.1a. Corr (Fcalc, Fobs), in percentage; resolution *vs* cut-off level

N	Dmin	Dmax	refl	10	20	30	40	50	60	70	80	90
1	80.0	500.	40	91	96	92	87	83				
2	60.	80.	49	83	88	89	84	74				
3	50.	60.	52	29	16	-2	-2	5				
4	45.	50.	48	1	-21	11	20	-3				
5	40.	45.	74	7	-6	-15	0	4				
6	35.	40.	118	- 4	11	-5	-13	-18				
7	30.	35.	211	- 3	-13	-12	-7	3				
8	25.	30.	383	10	5	6	3	7				
9	20.	25.	876	10	14	6	9	7				
3.1	55.	60.	22	36	5	-1	7	1				
3.2	50.	55.	30	-7	0	-5	-5	12				

Table 1.1b. Weighted phase correlation, in percentage; resolution *vs* cut-off level

N	Dmin	Dmax	refl	10	20	30	40	50	60	70	80	90
1	80.0	500.	40	98	99	97	92	90				
2	60.	80.	49	96	97	96	93	85				
2	50.	60.	52	38	55	46	17	-33				
2	45.	50.	48	29	3	-13	-42	-44				
2	40.	45.	74	51	40	11	-17	-46				
2	35.	40.	118	2	23	15	-7	-13				

2	30.	35.	211	27	21	1	-18	-30				
2	25.	30.	383	21	11	-7	-26	-23				
2	20.	25.	876	-5	0	-10	-3	12				
2	55.	60.	22	40	56	55	20	-55				
2	50.	55.	30	16	52	39	16	-16				

Table 1.1c. Mean delta phi, in degrees

N	Dmin	Dmax	refl	10	20	30	40	50	60	70	80	90
1	80.0	500.	40	27	26	20	36	42				
2	60.	80.	49	27	23	31	35	41				
3	50.	60.	52	75	60	65	85	105				
4	45.	50.	48	78	79	88	86	95				
5	40.	45.	74	66	65	76	95	115				
6	35.	40.	118	83	83	83	96	98				
7	30.	35.	211	78	82	94	101	101				
8	25.	30.	383	81	85	94	100	99				
9	20.	25.	876	91	92	96	90	85				
3.1	55.	60.	22	62	43	49	81	121				
3.2	50.	55.	30	85	72	77	87	93				

Table 1.2. Starting density at 60 Å; "soft" modification

Table 1.2a. Corr (F_{calc}, F_{obs}), in percentage; resolution *vs* cut-off level

N	Dmin	Dmax	refl	10	20	30	40	50	60	70	80	90
1	80.0	500.	40	68	87	94	98	99	100	100	100	100
2	60.	80.	49	76	86	91	94	96	98	99	100	100
3	50.	60.	52	-7	4	14	28	33	33	33	31	25

4	45.	50.	48	18	20	15	11	12	15	19	22	22
5	40.	45.	74	-7	-3	5	4	-5	-5	0	1	4
6	35.	40.	118	4	6	3	2	3	1	0	-2	-2
7	30.	35.	211	-11	25	8	27	24	10	27	6	7
8	25.	30.	383	6	23	17	13	1	3	14	18	12
9	20.	25.	876	20	9	16	16	14	14	18	17	12
3.1	55.	60.	22		0	3	10	12	12	12	12	10
3.2	50.	55.	30		7	17	32	38	36	34	34	28

Table 1.2b. Weighted phase correlation, in percentage; resolution *vs* cut-off level

N	**Dmin**	**Dmax**	**refl**	**10**	**20**	**30**	**40**	**50**	**60**	**70**	**80**	**90**
1	80.0	500.	40	92	97	99	100	100	100	100	100	100
2	60.	80.	49	88	95	98	99	99	100	100	100	100
3	50.	60.	52	7	24	42	54	59	60	61	61	54
4	45.	50.	48	37	47	52	50	48	47	47	44	40
5	40.	45.	74	-10	20	35	40	33	25	21	20	19
6	35.	40.	118	-3	13	30	32	28	27	25	19	11
7	30.	35.	211	1	25	42	24	15	21	4	5	1
8	25.	30.	383	-5	27	34	18	1	-7	-9	-9	-5
9	20.	25.	876	11	1	-4	-15	-15	-10	-4	-3	2
3.1	55.	60.	22		49	64	69	70	71	71	71	70
3.2	50.	55.	30		4	17	29	35	36	37	37	35

Table 1.2c. Mean delta phi, in degrees

N	Dmin	Dmax	refl	10	20	30	40	50	60	70	80	90
1	80.0	500.	40	27	26	21	20	12	2	1	1	1
2	60.	80.	49	32	25	22	18	16	12	10	3	1
3	50.	60.	52	87	79	67	62	55	53	51	59	72
4	45.	50.	48	80	69	69	69	64	66	69	67	67
5	40.	45.	74	96	79	63	59	68	70	73	79	84
6	35.	40.	118	88	89	82	77	78	79	83	83	85
7	30.	35.	211	90	83	71	91	90	87	90	92	93
8	25.	30.	383	92	78	80	83	88	94	94	94	96
9	20.	25.	876	86	88	96	97	93	91	89	88	89
3.1	55.	60.	22		58	42	38	36	35	34	41	48
3.2	50.	55.	30		94	85	80	69	66	64	70	80

Table 2.1. Starting density at 40 Å; "hard" modification

Table 2.1a. Corr (F_{calc}, F_{obs}), in percentage; resolution *vs* cut-off level

N	Dmin	Dmax	refl	10	20	30	40	50	60	70	80	90
1	80.0	500.	40	91	98	92	89					
2	60.	80.	49	93	89	87	81					
3	50.	60.	52	88	92	79	71					
4	45.	50.	48	63	65	53	55					
5	40.	45.	74	59	72	60	64					
6	35.	40.	118	16	14	5	19					
7	30.	35.	211	10	3	6	17					
8	25.	30.	383	24	2	9	28					
9	20.	25.	876	8	15	15	9					
6.1	37.	40.	66	9	2	15	15					
6.2	35.	37.	52	24	25	-1	23					

Table 2.1b. Weighted phase correlation, in percentage; resolution *vs* cut-off level

N	Dmin	Dmax	refl	10	20	30	40	50	60	70	80	90
1	80.0	500.	40	99	99	96	94					
2	60.	80.	49	98	98	96	95					
3	50.	60.	52	95	96	93	89					
4	45.	50.	48	91	93	90	85					
5	40.	45.	74	86	96	90	85					
6	35.	40.	118	54	44	2	-50					
7	30.	35.	211	49	16	-22	-55					
8	25.	30.	383	29	15	-22	-36					
9	20.	25.	876	9	-18	-30	-21					
6.1	37.	40.	66	48	53	8	-45					
6.2	35.	37.	52	61	32	-8	-55					

Table 2.1c. Mean 

, in degrees

N	Dmin	Dmax	refl	10	20	30	40	50	60	70	80	90
1	80.0	500.	40	23	15	21	43					
2	60.	80.	49	27	22	28	29					
3	50.	60.	52	29	21	22	34					
4	45.	50.	48	30	24	35	42					
5	40.	45.	74	38	26	37	38					
6	35.	40.	118	65	74	88	115					
7	30.	35.	211	65	85	100	114					
8	25.	30.	383	79	84	99	107					
9	20.	25.	876	87	94	106	99					

6.1	37.	40.	66	63	72	81	113					
6.2	35.	37.	52	67	75	96	117					

Table 2.2. Starting density at 40 Å; "soft" modification

Table 2.2a. Corr (Fcalc, Fobs), in percentage; resolution *vs* cut-off level

N	**Dmin**	**Dmax**	**refl**	**10**	**20**	**30**	**40**	**50**	**60**	**70**	**80**	**90**
1	80.0	500.	40	76	91	97	99	99	100	100	100	100
2	60.	80.	49	85	95	98	99	99	100	100	100	100
3	50.	60.	52	72	90	97	99	99	100	100	100	100
4	45.	50.	48	51	77	90	95	96	97	98	99	99
5	40.	45.	74	41	68	83	90	94	96	98	99	99
6	35.	40.	118	34	37	40	40	40	40	39	36	29
7	30.	35.	211	18	24	34	35	34	32	29	28	31
8	25.	30.	383	19	36	42	38	35	34	32	32	34
9	20.	25.	876	20	14	14	14	12	11	11	12	13
6.1	37.	40.	66	26	29	36	39	38	35	31	24	13
6.2	35.	37.	52	42	47	49	49	50	52	53	54	55

Table 2.2b. Weighted phase correlation, in percentage; resolution *vs* cut-off level

N	**Dmin**	**Dmax**	**refl**	**10**	**20**	**30**	**40**	**50**	**60**	**70**	**80**	**90**
1	80.0	500.	40	95	99	100	100	100	100	100	100	100
2	60.	80.	49	95	98	100	100	100	100	100	100	100
3	50.	60.	52	79	94	99	100	100	100	100	100	100
4	45.	50.	48	78	94	98	99	100	100	100	100	100
5	40.	45.	74	69	89	96	98	99	99	100	100	100
6	35.	40.	118	57	69	77	80	81	79	76	72	66
7	30.	35.	211	51	66	70	70	70	68	66	62	56
8	25.	30.	383	31	50	52	46	43	42	40	36	31

9	20.	25.	876	30	28	1	-18	-23	-24	-25	-25	-24
6.1	37.	40.	66	53	68	77	81	81	79	74	69	61
6.2	35.	37.	52	61	70	77	80	80	79	78	76	72

Table 2.2c. Mean delta phi, in degrees

N	**Dmin**	**Dmax**	**refl**	**10**	**20**	**30**	**40**	**50**	**60**	**70**	**80**	**90**
1	80.0	500.	40	34	20	15	7	3	3	1	1	1
2	60.	80.	49	35	21	17	10	9	7	4	3	2
3	50.	60.	52	48	31	18	11	9	8	7	7	1
4	45.	50.	48	48	26	20	10	8	6	5	5	1
5	40.	45.	74	52	38	26	15	12	10	7	5	3
6	35.	40.	118	71	63	58	57	54	53	54	57	66
7	30.	35.	211	64	59	54	52	55	55	57	60	65
8	25.	30.	383	78	72	68	69	74	75	77	78	82
9	20.	25.	876	78	82	93	101	102	102	101	101	100
6.1	37.	40.	66	71	62	59	57	51	49	53	56	73
6.2	35.	37.	52	70	64	56	56	57	58	57	59	58

Table 3.1. Starting density at 30 Å; "soft" modification

Table 3.1a. Corr (F_{calc}, F_{obs}), in percentage; resolution *vs* cut-off level

N	**Dmin**	**Dmax**	**refl**	**5**	**10**	**20**	**30**	**40**	**50**	**60**	**70**	**80**
1	80.0	500.	40	73	85	96	99	100	100	100	100	100
2	60.	80.	49	71	87	97	99	99	100	100	100	100
3	50.	60.	52	49	76	92	97	98	99	99	100	100
4	45.	50.	48	50	69	87	95	96	97	98	99	99
5	40.	45.	74	51	68	89	96	97	98	99	99	99

6	35.	40.	118	67	77	91	96	98	98	99	99	100
7	30.	35.	211	65	76	87	92	95	97	98	99	99
8	25.	30.	383	41	48	56	58	57	57	55	50	40
9	20.	25.	876	25	34	36	31	28	26	25	24	22
8.1	27.	30.	199	45	52	59	63	62	61	58	52	38
8.2	25.	27.	184	31	39	49	48	47	47	48	47	42
9.1	22.	25.	427	21	31	35	29	25	25	25	26	25
9.2	21.	22.	213	14	22	18	14	12	9	6	4	0

Table 3.1b. Weighted phase correlation, in percentage; resolution *vs* cut-off level

N	**Dmin**	**Dmax**	**refl**	**5**	**10**	**20**	**30**	**40**	**50**	**60**	**70**	**80**
1	80.0	500.	40	93	97	100	100	100	100	100	100	100
2	60.	80.	49	87	95	99	100	100	100	100	100	100
3	50.	60.	52	78	90	98	100	100	100	100	100	100
4	45.	50.	48	72	88	97	99	100	100	100	100	100
5	40.	45.	74	84	89	96	99	99	100	100	100	100
6	35.	40.	118	85	91	97	99	99	100	100	100	100
7	30.	35.	211	87	93	97	98	99	100	100	100	100
8	25.	30.	383	67	73	81	85	85	84	84	83	81
9	20.	25.	876	53	64	67	60	54	50	47	43	40
8.1	27.	30.	199	71	77	83	87	87	87	86	85	84
8.2	25.	27.	184	58	64	76	79	79	78	77	76	73
9.1	22.	25.	427	56	67	74	72	68	67	66	64	62
9.2	21.	22.	213	48	58	53	35	27	21	15	10	6

Table 3.1c. Mean | delta | phi |, in degrees

N	Dmin	Dmax	refl	5	10	20	30	40	50	60	70	80
1	80.0	500.	40	35	25	20	7	1	1	1	1	1
2	60.	80.	49	44	35	25	7	5	2	2	2	1
3	50.	60.	52	48	40	24	10	8	7	7	4	4
4	45.	50.	48	51	39	24	13	10	9	8	8	5
5	40.	45.	74	43	33	24	15	10	8	7	6	4
6	35.	40.	118	50	36	21	11	9	8	7	5	1
7	30.	35.	211	35	38	21	17	14	10	9	6	4
8	25.	30.	383	57	53	44	43	43	43	44	46	49
9	20.	25.	876	66	60	60	66	69	71	72	73	73
8.1	27.	30.	199	53	52	42	42	42	42	43	45	48
8.2	25.	27.	184	62	55	46	43	43	44	46	47	50
9.1	22.	25.	427	63	55	50	53	55	58	59	60	60
9.2	21.	22.	213	67	63	65	76	81	82	83	85	86

IV. Results and discussion

The following observations can be made :

1. The structure factors, calculated at the same resolution as the starting synthesis, are close enough to the correct values, which means that the hypothesis on the flat solvent can be used at such resolution.

2. For the structure factors calculated in higher resolution shells, the calculated amplitudes are not so good. However, in many cases the phases in the closest resolution shell have sufficiently high quality to be used to improve the starting image.

3. The results obtained by the "soft" modification, i.e. when the highest density distribution values are not changed (keeping the density "inside the envelope") are much less sensitive to the threshold level. Note that a similar observation was found for the molecular replacement searches with an envelope (Urzhumtsev & Podjarny, 1995a).

4. The hard modification is significantly less useful at 40 Å than at 60 Å. It means that at such (and higher) resolution the phase extension cannot be achieved by a simple refinement of the envelope border and needs the knowledge of density distribution values.

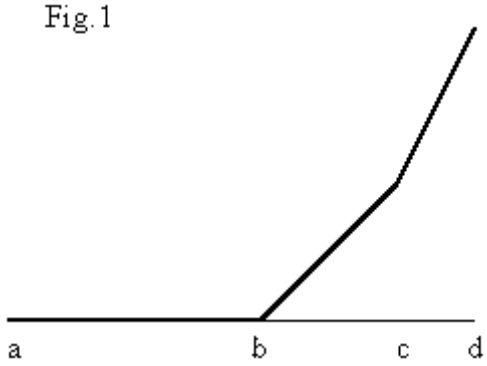
5. It is interesting to note that in several cases (for example, Tables 2.2) a "wave effect" can be observed : when starting from the 40Å -resolution map, the amplitudes and the phases in

the resolution shell 35-37 Å are better than those in the resolution shell 37-40 Å. However, it could be a purely statistical effect.

6. The density modification became more efficient when increasing the resolution of the starting synthesis.

7. Optimal cut-off level does not necessarily correspond to the correct molecular volume (in this case it was about 35 percent).

Fig.1



As was discussed by Lunin (1988), the basic idea which is behind most of density modification procedures and which defines the form of the density modification function is the closeness of the electron density histograms. In our case, the corresponding histograms have been calculated at different resolution from 20 to 90 Å. We have no possibility to discuss here the details of this analysis and can only mention that the density modification function corresponding to the optimal density modification from 90 to 20 Å resolution maps can be nicely approximated by a function schematically presented in Figure 1 : at the interval (a,b) it corresponds to the solvent flattening, at the interval (b,c) it "keeps" the density values and the interval (c,d) the function needs to sharpen the highest density values. In general, this function supports the idea of "soft" modification. The application of histogram-fitted density modification will be discussed elsewhere.

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