Bulk-solvent correction for use with the CCP4 version of *AMoRe*

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1. Introduction

Low-resolution reflections, being less sensitive to model imperfections, are known to be very useful (Urzhumtsev & Podjarny, 1995a, Fokine & Urzhumtsev, 2002a) for solving the translation problem in molecular replacement (Rossmann, 1972). In protein crystallography, traditional molecular replacement protocols, however, exclude reflections of resolution lower 10 or 15 Å, because they are strongly influenced by the contribution of the bulk solvent in the crystals. As a consequence, at low resolution, comparison of structure factors calculated from the atomic model with experimental values is not reliable. In order to obviate this problem, a bulk solvent correction has to be introduced in the translation search procedure. Two different bulk solvent correction approaches are available (Kostrewa, 1997), the exponential scaling

model (Moews & Kretsinger, 1975) and the mask model (Phillips, 1980; Jiang & Brünger, 1994),-both originally developed for macromolecular refinement purposes-. The exponential scaling model, based on Babinet's principle, is of simple implementation and is now applied to calculate a bulk solvent correction in the molecular replacement programs MOLREP (Vagin & Teplyakov, 1997), QS (Glykos & Kokkinidis, 2000) and BEAST (Read, 2001). The assumptions this approach is based on are, however, only true at resolutions below ~ 15 Å (Urzhumtsev & Podjarny, 1995b) and its performance was shown to be inferior to that of the mask model (Kostrewa, 1997). This latter method involves explicit calculation of an envelope around the protein model, creating a 'solvent mask'. The solvent region delimited by this solvent mask is then filled with bulk solvent electron density, and structure factors for this density are calculated and vectorially added to those derived from the protein model. Fokine and Urzhumtsev (2001) suggested a way to employ the mask method to calculate accurate bulk solvent correction for fast translation searches in molecular replacement. The corresponding program BULK (Fokine, Capitani, Grütter & Urzhumtsev, 2002) can be used with the standalone version of *AMoRe* (Navaza, 1994; Navaza & Vernoslova, 1995), with the CCP4 version of *AMoRe* (CCP4, 1994) and with CNS (Brünger *et al.*, 1998).

2. Description of the BULK program and of its usage

A typical molecular replacement run with *AMoRe* involves three main steps: the calculation of a structure factor (SF) table from the search model (often referred to as 'tabling step'), the cross-rotation function search ('roting step') and the fast translation search ('traing step'). The table below summarizes those steps and their input and output files:

INPUT	STEP	OUTPUT
Search model (PDB file)	Tabling	SF table (‘search tab’) ‘tabbed model’* Tabling step log file
Experimental amplitudes SF table (‘search.tab’)	Roting	List of cross-rotation function peaks
Experimental amplitudes SF table (‘search tab’) List of cross-rotation function peaks	Traing	List of translation function peaks

*see text for the meaning of ‘tabbed model’

The program BULK introduces another step (‘bulking’) in this procedure and calculates a bulk-solvent corrected structure factor table to be used in the ‘traing’ step. The scheme is then modified as follows:

INPUT	STEP	OUTPUT
Search model (PDB file)	Tabling	SF table (‘search tab’) ‘tabbed model’ Tabling step log file
Experimental amplitudes SF table (‘search.tab’)	Roting	List of cross-rotation function peaks
SF table (‘search.tab’) Search model Tabling step log file	Bulking	**Corrected** SF table (**search.tabs**)
Experimental amplitudes **Corrected** SF table (**search.tabs**) List of cross-rotation function peaks	Traing	List of translation function peaks

The ‘bulking’ procedure encompasses various computational tasks, which are to be carried out after the *AmoRe* ‘tabling’ step, where the search model is placed in a large rectangular box (the box dimensions are determined automatically) with its centre of mass at the origin and its principal inertia axes parallel to the box axes. A model rotated and translated in this way can be referred to as a ‘tabbed’ model. Then, structure factors $\mathbf{F}_{\mathrm{mP1}}(\mathbf{h})$ from this model are calculated by *AMoRe*. ‘BULK’ goes then through the following steps:

1) It computes a molecular envelope from the model coordinates, which are placed at the origin exactly as in the *AMoRe* ‘tabling’ step;
2) It calculates $\mathbf{F}_{\mathrm{eP1}}(\mathbf{h})$, the Fourier coefficients for this envelope, using the same box as in the *AMoRe* ‘tabling’ step;
3) It obtains the corresponding solvent structure factors as

$$\mathbf{F}_{\mathrm{sP1}}(\mathbf{h}) = [-\tilde{k}_{sol} \exp(-\tilde{B}_{sol}\, h^2/4)]\, \mathbf{F}_{\mathrm{eP1}}(\mathbf{h}) \qquad (10)$$

   using $\tilde{k}_{sol}$ and $\tilde{B}_{sol}$ values defined in the input file;
4) It carries out the sum $\mathbf{F}_{\mathrm{corrP1}}(\mathbf{h}) = \mathbf{F}_{\mathrm{mP1}}(\mathbf{h}) + \mathbf{F}_{\mathrm{sP1}}(\mathbf{h})$, where $\mathbf{F}_{\mathrm{mP1}}(\mathbf{h})$ are the model structure factors previously calculated by the *AMoRe* ‘tabling’ step. The corrected structure factors $\mathbf{F}_{\mathrm{corrP1}}(\mathbf{h})$ can be then used in the *AMoRe* ‘*traing*’ (and optionally ‘fiting’) step.

The theoretical ground for the above calculations is discussed in (Fokine, Capitani, Grütter & Urzhumtsev, 2002). Here we just point out that the procedure uses constant solvent scaling and B-factor parameters ($\tilde{k}_{sol}, \tilde{B}_{sol}$) as defined by an input file. Mean values for these parameters (0.35 e/Å^3 and 46 Å^2, respectively) were derived by Fokine & Urzhumtsev (2002b) through a statistical analysis of well-refined structures deposited in the Protein Data Bank (Bernstein *et al.*, 1977). If for a certain crystal the buffer density differs markedly from standard values, the user can accordingly change the default $\tilde{k}_{sol}$ and $\tilde{B}_{sol}$ input parameters. Another point worth mentioning is that ‘BULK’ can be used only in cases when one entity in

the asymmetric unit is searched for (this can be also a tetramer or an octamer, provided that a corresponding tetrameric or octameric search model, respectively, is available). On the contrary, if the molecular replacement problem involves, for instance, locating two independent monomers, the current approach cannot be used because it would produce unreliable structure factors (Fokine, Capitani, Grütter & Urzhumtsev, 2002).

To install and run BULK one first has to copy the 'bulk' directory (as extracted from the BULK distribution tar file) into the *AMoRe* working directory, then to compile it with the command:

```
./bulk/make_bulk
```

This creates two executables, prep_bulk and bulking.

The only input file needed, (an example is provided in the distribution), is prep_bulk.inp, with the following contents:

```
search.pdb  | name of the file with model coordinates (the same as used for the
            | 'tabling'step)
search.tab  | name of the file of structure factors from the AmoRe 'tabling' step
tab.log     | name of the log file of  the AmoRe 'tabling' step
search.tabs | name of the file with bulk-solvent-corrected structure factors
            | (this file is created by the 'bulking' procedure)
bulking.inp | name of an intermediate control file to be input to 'bulking';
            | this file is created by running 'prep_bulk'
0.35        | value of k~sol (scaling parameter for solvent electron density, in e/Å^3)
50.0        | value of B~sol (temperature factor for solvent, in Å^2)
1.0         | value of the solvent radius used for the solvent mask calculation
```

To run the procedure, one issues the two following shell commands:

```
prep_bulk < prep_bulk.inp

bulking < bulking.inp
```

'prep_bulk' creates the file 'bulking.inp', containing the information needed by 'bulking' to calculate the actual bulk-solvent correction. The output file 'search.tabs', created by 'bulking', contains the bulk-solvent-corrected structure factors and can be used for the translation search instead of 'search.tab'. Importantly, the corrected structure factor file should not be used in the 'roting' step, since low-resolution reflections contribute rather negatively to the rotation function search results.

3. A test case

To evaluate the performance of BULK in a typical CCP4 *AmoRe* run, a test case was carried out based on a structure solved very recently at the University of Zürich. The crystals of that protein (an enzyme involved in apoptosis) diffract well and a good quality dataset (space group $P2_12_12_1$, resolution 15.0-1.8 Å, completeness 96.0%, Rsym 8.1%) could be collected at a synchrotron source. The protein is a heterotetramer composed by two α and two β subunits, and it was solved by molecular replacement with CCP4 *AMoRe* using a related tetrameric protein (PDB code 1CP3) as a search model. For the test case, a more distant search model (PDB code 1F9E) was employed (rmsd 1.6 Å for 144 common Cα atoms considering one α and one β subunit) and its β subunits, corresponding to 37 % of the model, were deleted.

A conventional rotation search was then carried out in the range 8-4 Å and the top 30 rotation function solutions were listed. BULK was then used to calculate a corrected structure factor table. Translation tests were performed in the ranges 10-4 Å and 15-4 Å, both with and without bulk solvent correction. The top 30 solutions in each case, sorted by correlation coefficient, are reported below (α, β, γ are the eulerian rotation angles for each solution, Tx,

Ty, Tz the fractional translations, cc, Rf and cc-I the amplitude-based correlation coefficient, the R-factor and the intensity-based correlation coefficient, respectively):

A) 10-4 Å WITHOUT bulk solvent correction

		α	β	γ	Tx	Ty	Tz	cc	Rf	cc-I
SOLUTIONTF1	1	139.59	56.87	225.89	0.1691	0.4390	0.2361	9.2	55.6	9.5
SOLUTIONTF1	1	134.00	59.87	50.32	0.1338	0.4407	0.4377	6.0	57.2	8.3
SOLUTIONTF1	1	72.06	41.69	133.77	0.3280	0.2580	0.0577	5.4	57.4	6.2
SOLUTIONTF1	1	145.89	45.72	44.62	0.4242	0.1225	0.0964	5.2	57.3	5.1
SOLUTIONTF1	1	88.83	90.00	60.70	0.2029	0.1829	0.1960	5.2	58.8	5.4
SOLUTIONTF1	1	68.57	36.76	316.05	0.1218	0.4297	0.3087	5.2	57.6	4.5
SOLUTIONTF1	1	60.70	42.44	244.79	0.3353	0.3195	0.1074	4.6	56.8	5.0
SOLUTIONTF1	1	111.50	36.91	56.00	0.2603	0.3188	0.1500	4.5	56.9	5.1
SOLUTIONTF1	1	113.50	46.00	58.30	0.2170	0.0728	0.2099	4.2	57.7	5.1
SOLUTIONTF1	1	86.00	90.00	18.47	0.4114	0.9958	0.0000	4.2	61.3	3.9
SOLUTIONTF1	1	95.24	90.00	69.66	0.4683	0.2917	0.1845	4.0	57.8	3.6
SOLUTIONTF1	1	43.81	38.01	48.64	0.2352	0.2299	0.0202	4.0	56.9	3.0
SOLUTIONTF1	1	116.65	35.64	233.53	0.0059	0.0018	0.4482	3.9	57.3	5.4
SOLUTIONTF1	1	87.08	26.20	306.42	0.3940	0.3887	0.1388	3.9	57.8	4.6
SOLUTIONTF1	1	56.96	81.80	194.07	0.1389	0.0040	0.1650	3.9	57.4	3.3
SOLUTIONTF1	1	54.21	51.00	39.16	0.3014	0.3577	0.3644	3.8	57.4	2.7
SOLUTIONTF1	1	15.39	70.84	128.24	0.0188	0.0999	0.4365	3.7	58.2	3.5
SOLUTIONTF1	1	158.40	86.17	268.98	0.4258	0.3079	0.0769	3.5	57.8	2.7
SOLUTIONTF1	1	91.00	29.96	121.50	0.3952	0.3952	0.1324	3.5	57.7	4.5
SOLUTIONTF1	1	83.68	80.82	311.33	0.1649	0.1792	0.0221	3.5	57.6	4.9
SOLUTIONTF1	1	76.30	36.00	311.71	0.3069	0.2979	0.0116	3.5	58.0	4.1
SOLUTIONTF1	1	57.79	87.04	284.00	0.3811	0.2135	0.4233	3.5	57.6	2.7
SOLUTIONTF1	1	94.96	70.55	251.19	0.1450	0.1805	0.2657	3.4	57.5	3.6
SOLUTIONTF1	1	80.54	34.85	309.13	0.0546	0.0800	0.2310	3.4	58.0	4.0
SOLUTIONTF1	1	51.00	35.70	221.50	0.2660	0.1106	0.0859	3.2	57.5	2.8
SOLUTIONTF1	1	92.23	90.00	197.89	0.3074	0.0060	0.0000	3.1	63.3	3.5
SOLUTIONTF1	1	11.86	64.00	129.40	0.3105	0.4471	0.4815	3.1	57.2	3.7
SOLUTIONTF1	1	151.00	41.61	314.00	0.3379	0.4513	0.2173	2.9	58.2	2.6
SOLUTIONTF1	1	43.60	81.50	13.16	0.1041	0.3664	0.3687	2.8	57.7	3.3
SOLUTIONTF1	1	86.40	86.41	247.14	0.2704	0.4661	0.2848	2.5	57.5	2.7

This first case represents a ‘classical’ resolution range for a translation search run. Sorting by correlation coefficient identifies one slightly emerging solution but the signal is weak. Further analysis, including comparison with the newly solved structure, shows that the top-ranking solution (framed) is correct, whereas the second is wrong.

B) 10-4 Å WITH bulk solvent correction

		α	β	γ	Tx	Ty	Tz	cc	Rf	cc-I
SOLUTIONTF1	1	139.59	56.87	225.89	0.1701	0.4397	0.2365	15.5	53.1	16.5
SOLUTIONTF1	1	134.00	59.87	50.32	0.1824	0.4440	0.2365	11.2	54.9	13.4
SOLUTIONTF1	1	145.89	45.72	44.62	0.1943	0.4757	0.2176	9.9	54.5	9.7
SOLUTIONTF1	1	72.06	41.69	133.77	0.2079	0.1879	0.4616	9.1	55.3	9.2
SOLUTIONTF1	1	113.50	46.00	58.30	0.2201	0.0987	0.2040	8.5	55.4	9.8
SOLUTIONTF1	1	111.50	36.91	56.00	0.4681	0.3272	0.3476	8.3	55.0	8.1
SOLUTIONTF1	1	88.83	90.00	60.70	0.2028	0.1860	0.1941	8.3	57.2	8.1
SOLUTIONTF1	1	68.57	36.76	316.05	0.2626	0.0711	0.4542	8.1	55.3	8.5
SOLUTIONTF1	1	57.79	87.04	284.00	0.3830	0.2146	0.4251	7.8	55.3	6.9
SOLUTIONTF1	1	15.39	70.84	128.24	0.3441	0.9989	0.4286	7.8	55.4	7.5
SOLUTIONTF1	1	76.30	36.00	311.71	0.3643	0.4896	0.2877	7.7	55.5	8.8
SOLUTIONTF1	1	158.40	86.17	268.98	0.1451	0.4607	0.0658	7.5	55.5	7.7
SOLUTIONTF1	1	95.24	90.00	69.66	0.3852	0.0560	0.3756	7.4	55.2	7.0
SOLUTIONTF1	1	116.65	35.64	233.53	0.0096	0.4709	0.4455	7.3	55.1	8.0
SOLUTIONTF1	1	80.54	34.85	309.13	0.0566	0.0790	0.2314	7.3	55.9	8.8
SOLUTIONTF1	1	56.96	81.80	194.07	0.4703	0.3603	0.1054	7.3	55.1	6.9
SOLUTIONTF1	1	94.96	70.55	251.19	0.1271	0.4514	0.3930	7.1	55.6	6.8
SOLUTIONTF1	1	60.70	42.44	244.79	0.4287	0.3201	0.0630	7.1	54.6	7.4
SOLUTIONTF1	1	54.21	51.00	39.16	0.3420	0.2334	0.0730	7.1	55.5	7.3
SOLUTIONTF1	1	87.08	26.20	306.42	0.2921	0.0411	0.4443	6.9	55.5	7.4
SOLUTIONTF1	1	83.68	80.82	311.33	0.2528	0.4874	0.1895	6.9	56.3	9.3
SOLUTIONTF1	1	11.86	64.00	129.40	0.3803	0.4433	0.1825	6.9	55.7	8.0
SOLUTIONTF1	1	86.00	90.00	18.47	0.4117	0.9946	0.0000	6.8	59.9	5.9
SOLUTIONTF1	1	43.81	38.01	48.64	0.2616	0.0488	0.3946	6.7	55.4	6.0
SOLUTIONTF1	1	51.00	35.70	221.50	0.4401	0.1680	0.0470	6.4	55.4	6.2
SOLUTIONTF1	1	92.23	90.00	197.89	0.4567	0.0409	0.2660	6.3	58.8	5.5
SOLUTIONTF1	1	86.40	86.41	247.14	0.1486	0.4503	0.2280	6.3	56.2	7.4
SOLUTIONTF1	1	43.60	81.50	13.16	0.1480	0.4897	0.2492	6.3	56.3	6.4
SOLUTIONTF1	1	91.00	29.96	121.50	0.2939	0.2384	0.4492	6.1	55.8	6.3
SOLUTIONTF1	1	151.00	41.61	314.00	0.0900	0.2623	0.3265	5.7	56.2	6.2

In this run the resolution range is the same as before, but the first solution (also same as before) now exhibits a much higher correlation coefficient. By comparing the first to the second ranking solution (both framed), it appears that they are both correct and equivalent (same translations and rotations, except for a ~ 180 º difference in γ, which is readily explained by the two-fold symmetry of the search model. Interestingly, also in run A) a

similar situation is observed, but the value for Tz in the second solution differs from that of the first and turns out to be wrong by further analysis. Comparison of runs A) and B) shows then that using the bulk solvent correction was instrumental for obtaining correct translation parameters for the second solution.

C) 15-4 Å WITH bulk solvent correction

		α	β	γ	Tx	Ty	Tz	cc	Rf	cc-I
SOLUTIONTF1	1	139.59	56.87	225.89	0.1716	0.4378	0.2365	16.4	53.5	16.8
SOLUTIONTF1	1	134.00	59.87	50.32	0.1846	0.4415	0.2346	12.7	54.8	14.2
SOLUTIONTF1	1	145.89	45.72	44.62	0.1950	0.4740	0.2176	11.1	54.7	9.7
SOLUTIONTF1	1	113.50	46.00	58.30	0.3360	0.3380	0.3054	9.0	55.9	9.7
SOLUTIONTF1	1	68.57	36.76	316.05	0.3924	0.4734	0.2250	8.9	56.4	9.0
SOLUTIONTF1	1	111.50	36.91	56.00	0.0206	0.0148	0.4481	8.8	55.6	8.9
SOLUTIONTF1	1	88.83	90.00	60.70	0.2027	0.1848	0.1937	8.8	57.6	8.3
SOLUTIONTF1	1	72.06	41.69	133.77	0.1529	0.3820	0.4211	8.8	55.9	9.9
SOLUTIONTF1	1	158.40	86.17	268.98	0.4053	0.4080	0.0285	8.4	55.6	6.8
SOLUTIONTF1	1	60.70	42.44	244.79	0.3510	0.2223	0.1524	7.9	55.4	6.7
SOLUTIONTF1	1	76.30	36.00	311.71	0.3651	0.4889	0.2886	7.8	56.2	7.4
SOLUTIONTF1	1	116.65	35.64	233.53	0.0059	0.0035	0.4482	7.7	55.9	8.7
SOLUTIONTF1	1	94.96	70.55	251.19	0.0268	0.2212	0.2433	7.6	55.8	6.5
SOLUTIONTF1	1	83.68	80.82	311.33	0.4264	0.0023	0.3584	7.2	56.7	8.0
SOLUTIONTF1	1	80.54	34.85	309.13	0.0563	0.0787	0.2313	7.2	56.4	7.3
SOLUTIONTF1	1	15.39	70.84	128.24	0.2261	0.1199	0.4634	7.2	56.2	6.8
SOLUTIONTF1	1	11.86	64.00	129.40	0.1529	0.1712	0.1759	7.0	55.5	6.6
SOLUTIONTF1	1	43.81	38.01	48.64	0.4538	0.1625	0.0477	6.9	56.1	6.5
SOLUTIONTF1	1	56.96	81.80	194.07	0.0170	0.3098	0.1236	6.7	55.9	5.8
SOLUTIONTF1	1	43.60	81.50	13.16	0.1484	0.4888	0.2491	6.7	56.8	6.1
SOLUTIONTF1	1	54.21	51.00	39.16	0.4464	0.3624	0.2688	6.6	55.8	4.6
SOLUTIONTF1	1	95.24	90.00	69.66	0.3267	0.4105	0.0458	6.5	56.4	6.6
SOLUTIONTF1	1	87.08	26.20	306.42	0.2932	0.0408	0.4435	6.5	56.2	6.5
SOLUTIONTF1	1	86.00	90.00	18.47	0.4130	0.9969	0.2583	6.5	58.3	5.7
SOLUTIONTF1	1	57.79	87.04	284.00	0.2481	0.2693	0.4858	6.3	56.0	6.3
SOLUTIONTF1	1	51.00	35.70	221.50	0.4421	0.1697	0.0471	6.3	55.9	5.9
SOLUTIONTF1	1	86.40	86.41	247.14	0.1754	0.2091	0.4515	6.1	56.4	6.6
SOLUTIONTF1	1	151.00	41.61	314.00	0.0314	0.2624	0.1190	5.8	56.4	5.7
SOLUTIONTF1	1	92.23	90.00	197.89	0.3987	1.0000	0.2551	5.8	59.5	4.8
SOLUTIONTF1	1	91.00	29.96	121.50	0.0376	0.3296	0.3309	5.7	56.6	6.7

In run C), using the full available resolution range was used with bulk solvent correction, the results are similar to those of run B), but with higher correlation coefficients (also cc-I) for the first- and second-ranking solution (both framed).

D) 15-4 Å WITHOUT bulk solvent correction

		α	β	γ	Tx	Ty	Tz	cc	Rf	cc-I
SOLUTIONTF1	1	139.59	56.87	225.89	0.1678	0.4373	0.2365	9.7	58.3	8.3
SOLUTIONTF1	1	134.00	59.87	50.32	0.1305	0.4409	0.4365	7.3	59.4	7.1
SOLUTIONTF1	1	145.89	45.72	44.62	0.2279	0.2650	0.3167	6.2	59.8	5.7
SOLUTIONTF1	1	111.50	36.91	56.00	0.3655	0.2451	0.0275	6.1	59.9	6.1
SOLUTIONTF1	1	113.50	46.00	58.30	0.1324	0.2200	0.0288	5.7	59.7	6.0
SOLUTIONTF1	1	68.57	36.76	316.05	0.4704	0.4331	0.3752	5.6	60.0	5.1
SOLUTIONTF1	1	94.96	70.55	251.19	0.0260	0.2183	0.4526	5.3	59.9	4.7
SOLUTIONTF1	1	72.06	41.69	133.77	0.4068	0.4374	0.1118	5.0	59.9	5.5
SOLUTIONTF1	1	158.40	86.17	268.98	0.4706	0.1000	0.3846	4.9	60.3	4.8
SOLUTIONTF1	1	88.83	90.00	60.70	0.3382	0.3900	0.1923	4.8	62.2	4.1
SOLUTIONTF1	1	60.70	42.44	244.79	0.0529	0.2115	0.3682	4.7	60.2	5.4
SOLUTIONTF1	1	56.96	81.80	194.07	0.1636	0.0649	0.4190	4.6	59.4	3.9
SOLUTIONTF1	1	87.08	26.20	306.42	0.2059	0.2900	0.2788	4.3	60.4	3.5
SOLUTIONTF1	1	83.68	80.82	311.33	0.4454	0.1092	0.0475	4.3	60.6	4.3
SOLUTIONTF1	1	43.81	38.01	48.64	0.4706	0.2500	0.2596	4.3	59.5	3.1
SOLUTIONTF1	1	80.54	34.85	309.13	0.2072	0.2827	0.3633	4.0	60.9	3.8
SOLUTIONTF1	1	76.30	36.00	311.71	0.0452	0.1494	0.0303	4.0	60.7	5.2
SOLUTIONTF1	1	116.65	35.64	233.53	0.2206	0.2600	0.1058	3.9	60.3	4.5
SOLUTIONTF1	1	43.60	81.50	13.16	0.1034	0.3654	0.3704	3.8	60.0	4.0
SOLUTIONTF1	1	11.86	64.00	129.40	0.2546	0.1097	0.2098	3.7	60.4	3.2
SOLUTIONTF1	1	95.24	90.00	69.66	0.3196	0.2313	0.4455	3.6	60.6	2.9
SOLUTIONTF1	1	92.23	90.00	197.89	0.0335	0.3088	0.2327	3.6	63.4	3.0
SOLUTIONTF1	1	15.39	70.84	128.24	0.0600	0.0896	0.1717	3.6	60.8	3.3
SOLUTIONTF1	1	91.00	29.96	121.50	0.2794	0.3400	0.4038	3.5	60.8	3.6
SOLUTIONTF1	1	86.40	86.41	247.14	0.2928	0.2094	0.4518	3.4	60.6	2.8
SOLUTIONTF1	1	51.00	35.70	221.50	0.3235	0.2900	0.3269	3.4	60.5	2.9
SOLUTIONTF1	1	86.00	90.00	18.47	0.1664	0.3998	0.0288	3.2	62.2	3.0
SOLUTIONTF1	1	57.79	87.04	284.00	0.1324	0.2700	0.4904	3.1	60.2	2.8
SOLUTIONTF1	1	54.21	51.00	39.16	0.4559	0.3600	0.3654	2.9	60.3	1.7
SOLUTIONTF1	1	151.00	41.61	314.00	0.0987	0.3222	0.0750	2.5	60.2	3.3

Run D) shows the importance of applying the bulk solvent correction when using low-resolution reflections down to 15 Å: the results are in fact much poorer compared to those of

run C) and similar to those of run A). Also in this case the Tz translation is wrong for the second-ranking solution (framed).

4. Future developments

BULK has been tested with CCP4 up to version 4.2.1. It is at the moment distributed upon request by the authors at the following addresses: fokine@lcm3b.uhp-nancy.fr, sacha@lcm3b.uhp-nancy.fr and capitani@bioc.unizh.ch. Future developments will involve the integration of BULK into the CCP4 distribution.

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