
Squeeze

What to do with disordered solvent?

Disordered solvents and symmetry elements

In general we find increased disorder for co-crystallized solvents:

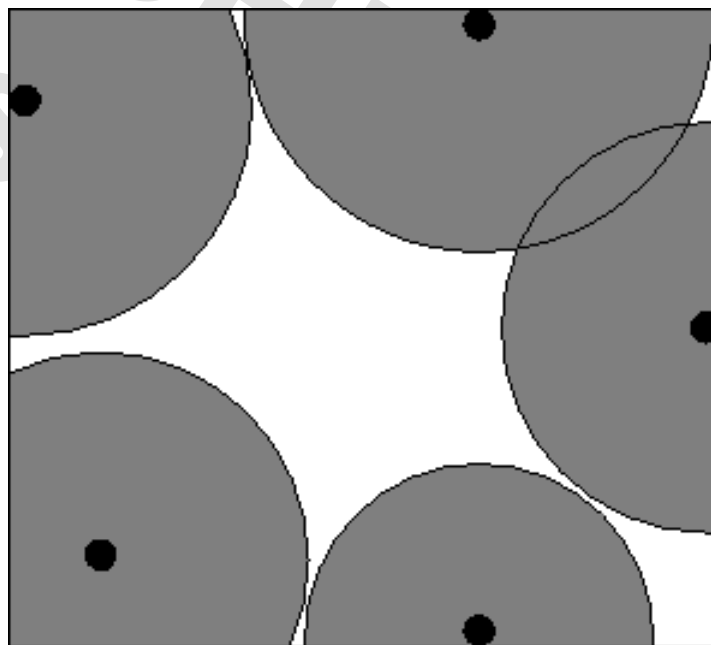
- Localized in a cavity or hole of the structure
- No solvent-solvent and few solvent-molecule contacts
- Often around a symmetry element, which is not part of the point group of the solvent

If the disorder becomes too high to be treated normally we can use the SQUEEZE routine of the PLATON program to remove it artificially..

PLATON and SQUEEZE

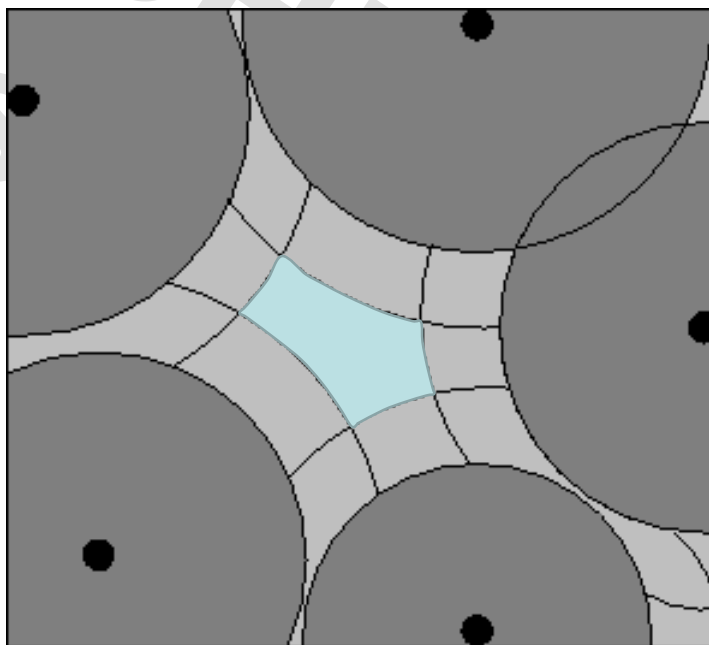
- In a typical structure only 65% of the space is occupied by atoms
- The rest is found in cavities too small to even accommodate a hydrogen atom
- Cavities which are accessible to solvent have a diameter of at least 1.2 Å.

DEFINE SOLVENT ACCESSIBLE VOID



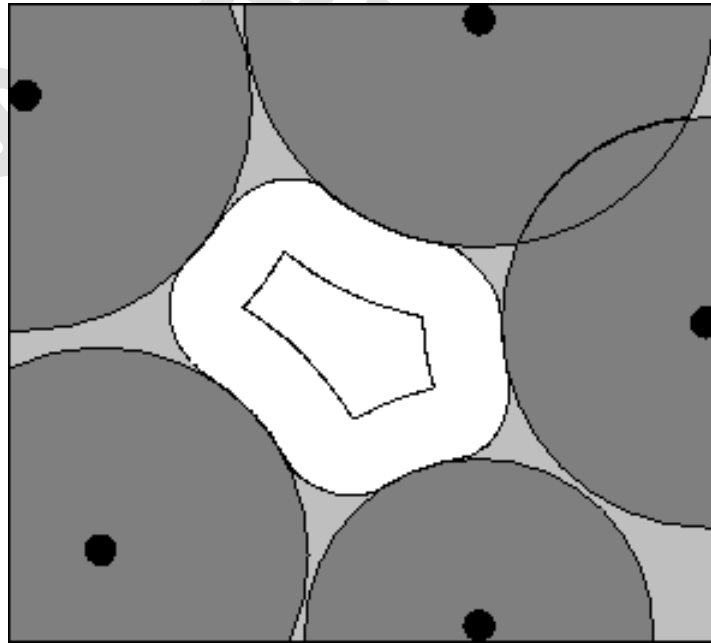
STEP #1 – EXCLUDE VOLUME INSIDE THE
VAN DER WAALS SPHERE

DEFINE SOLVENT ACCESSIBLE VOID



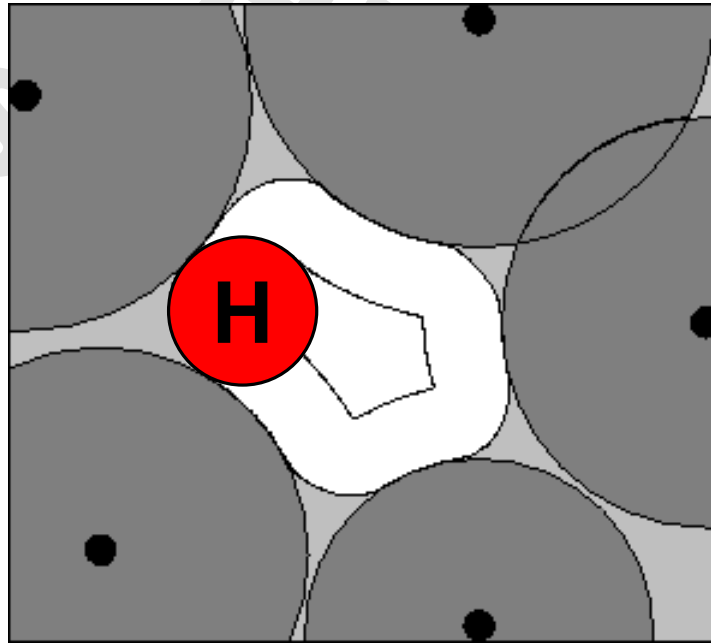
STEP # 2 – EXCLUDE AN ACCESS RADIAL VOLUME
TO FIND THE LOCATION OF ATOMS WITH THEIR
CENTRE AT LEAST 1.2 ANGSTROM AWAY

DEFINE SOLVENT ACCESSIBLE VOID



STEP # 3 – EXTEND INNER VOLUME WITH POINTS WITHIN
1.2 ANGSTROM FROM ITS OUTER BOUNDS

DEFINE SOLVENT ACCESSIBLE VOID



STEP # 3 – EXTEND INNER VOLUME WITH POINTS WITHIN
1.2 ANGSTROM FROM ITS OUTER BOUNDS

Search for and Analysis of Solvent Accessible Voids In the Structure

Area	#Gr	Ld	Pol	Int	VolPerc.	Vol(A	3)	X(av)	Y(av)	Z(av)	El	gen	vector(frac)	Slg(Ang)	
1	20126	[4072]	4	156	[31.6]	0.000	0.184	0.750	1	1.000	-0.003	0.520	1.74
											2	-0.502	-0.002	1.000	1.55
											3	-0.001	-1.000	-0.002	1.35
2	20134	[4072]	4	156	[31.6]	0.500	0.316	0.250	1	1.000	-0.006	0.521	1.74
											2	-0.503	-0.002	1.000	1.55
											3	-0.003	-1.000	-0.001	1.35
3	20125	[4072]	4	156	[31.6]	0.500	0.684	0.750	1	1.000	-0.008	0.522	1.74
											2	-0.504	-0.005	1.000	1.55
											3	-0.003	-1.000	-0.004	1.35
4	20131	[4072]	4	156	[31.6]	0.000	0.816	0.250	1	1.000	-0.003	0.523	1.74
											2	-0.505	-0.002	1.000	1.55
											3	-0.001	-1.000	-0.002	1.35

PLATON MENU

- OptionMenu
- NoMove
- NoDisorder
- Organic
- Round
- Parentheses
- Label-Alias
- R/S-Determ
- Norm-H-bond
- NoSymm
- Join-Expand
- LstARU RCl
- LstCellSymm
- ListAtoms
- ListBonds
- LstFlagRadi
- MinQPeakHgt
- MinQPeakDis
- Q-Peak-Incl
- KeyInstruct
- Prev Next
- SAVE-InstrS
- ENTRY-LIST
- Reset End
- Exit
- MenuActive

INSTRUCTION INPUT via KEYBOARD or LEFT-MOUSE-CLICKS (HELP with RIGHT CLICKS)

Solvent Accessible Void Found (See Listing for Details)
 >> Hit RETURN to Continue

SQUEEZE

- Takes the contribution of disordered solvents into account by back-Fourier transformation of density found in the 'solvent accessible volume' outside the ordered part of the structure.
- Filter: Input shelxl.res & shelxl.hkl
Output: 'solvent free' shelxl.hkl
- Later improvement: no corrected hkl, but a "correction file" *.fab, which SHELXL takes into account.

SQUEEZE PROCEDURE

- Refine structure **including H-atoms**. Finish the refinement as far as possible without including the solvent.

Mode 1 (preferred with SHELXL2014):

- Use *code.cif* and *code.fcf* for the SQUEEZE calculation
- SQUEEZE produces, next to output files, the following files:
- *code_sq.ins*: copy of *code.ins* + instruction “ABIN”.
- *code_sq.hkl*: copy of *code.hkl*
- *code_sq.fab*: contribution of the diffuse electron density, read by ABIN
- *code_sq.sqz*: peak list (for information purposes only)
- Continue normal refinement with *code_sq*. The CIF will be automatically updated.

SQUEEZE PROCEDURE

Mode 2 (only one possible for SHELXL97):

- Use *code.res* and *code.hkl* for the SQUEEZE calculation
- SQUEEZE produces, next to output files, the following files:
- *code_sqd.ins*: copy of *code.res*
- *code_sqd.hkl*: *code.hkl* minus the contribution of the diffuse electron density
- *code_sqd.sqf*: CIF format file with information about the SQUEEZE procedure
- *code_sqd.sqz*: peak list (for information purposes only)
- Continue normal refinement with *code_sqd*.
- At the end of the refinement, run Platon on *code_sqd.ins/.hkl* with the command `calc FCF_SQ` to produce a correct *code_sqd_pl.fcf*. *code_sqd.sqf* wo;; be appended to the CIF to produce and *code_sqd_pl.cif*

SQUEEZE PROCEDURE

In most cases only one SQUEEZE cycle is necessary. In some cases (severe disorder) in the main molecule, it might be advisable to repeat to do several SQUEEZE cycles:

Refinement -> code.cif

SQUEEZE -> code_sq.ins/fab/hkl

Refinement -> code_sq.cif

SQUEEZE -> code_sq_sq.ins/fab/hkl

Refinement -> code_sq_sq.cif

SQUEEZE -> code_sq_sq_sq.ins/fab/hkl

Refinement -> code_sq_sq_sq.cif

The « Bypass » command in Platon allows to do this automatically, but is currently under development and not included in the Windows distribution.

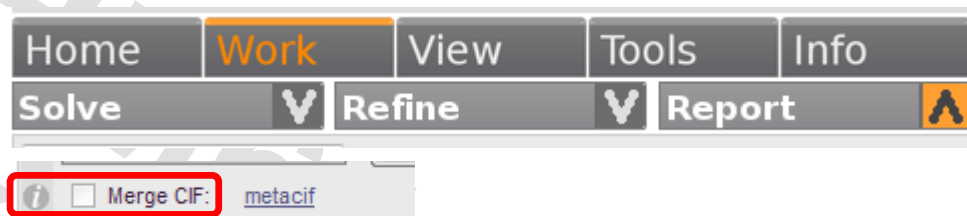
Limitations and requirements

- No residual unresolved density in the discrete model region of the structure (impact in the solvent region).
- The data set should be reasonably complete and with sufficient resolution [i.e. $\sin(\theta)/\lambda > 0.6$].
- No unresolved charge balance issues that might effect the chemistry involved
- The SQUEEZE technique can not handle cases where the solvent region is not constant but varies due to (coupled) disorder in the discrete part of the model.
- The solvent region is assumed not to contain significant anomalous scatterers (Friedels averaged)
- Designed for 'small molecule structures'

SQUEEZE implementation in OLEX

The preferred mode of operation (FAB file) requires a CIF file with an embedded hkl and res file. OLEX2 strips the embedded hkl out of the cif by default if the “MERGE CIF” routine is activated.

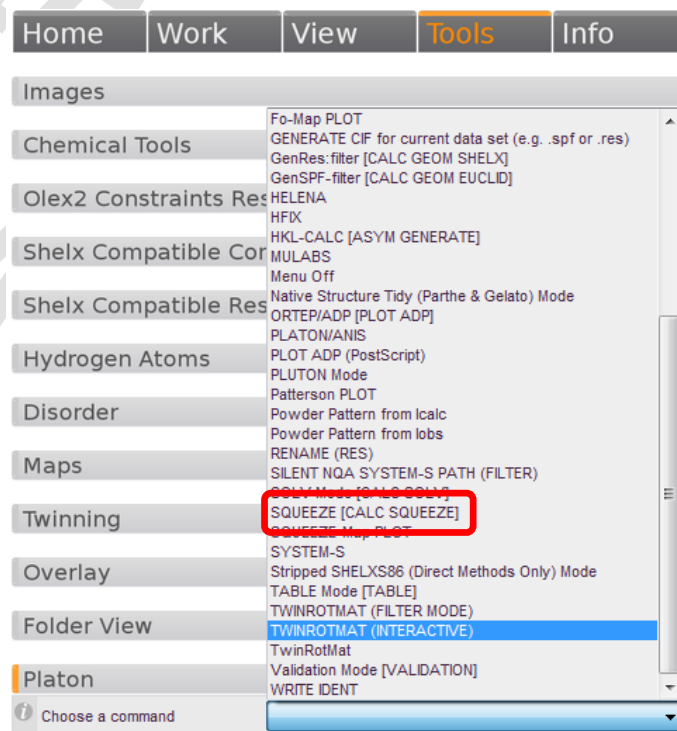
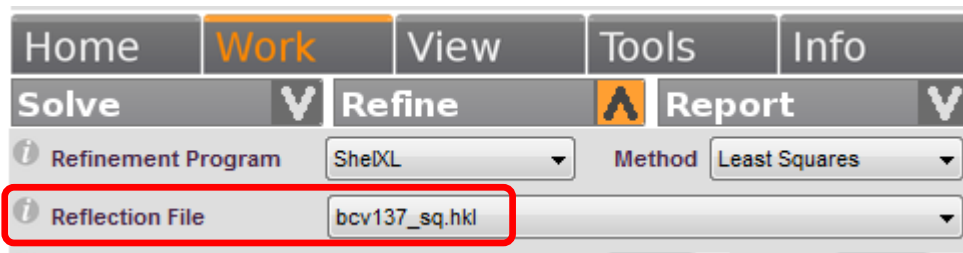
1. Deactivate “Merge CIF”



2. Run Platon – SQUEEZE (CALC) under TOOLS

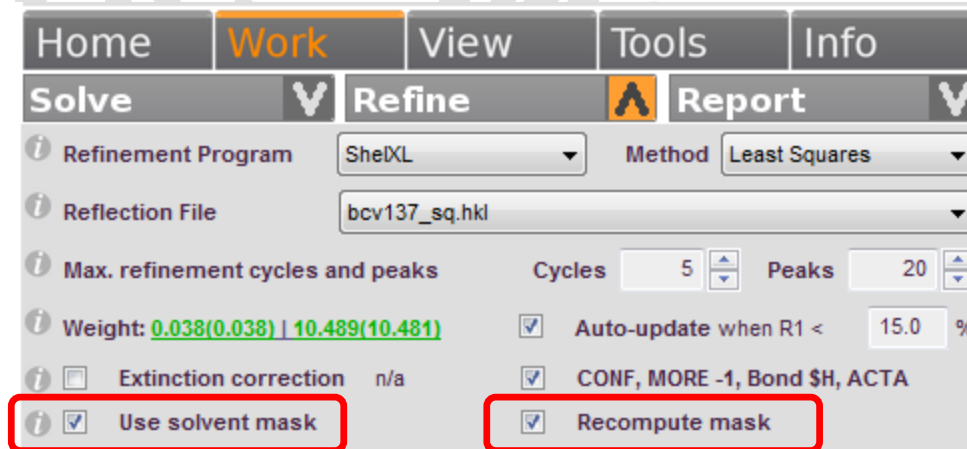
3. Open *code_sq.ins*

4. Verify that the hkl file is correctly chosen as *code_sq.hkl*



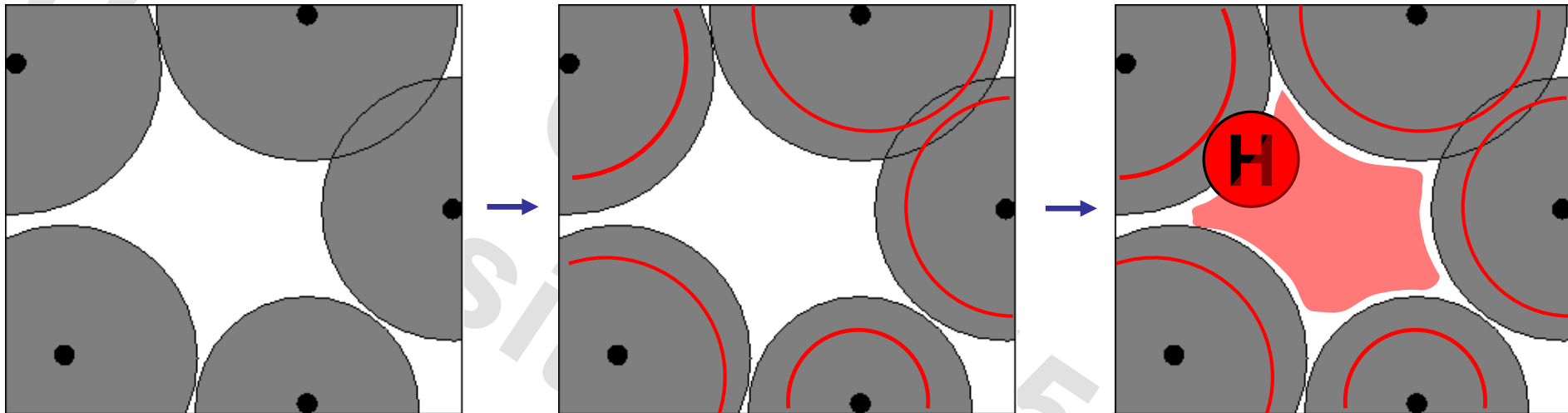
Solvent procedure in OLEX2

- The solvent mask (void determination) in OLEX is based on: Jiang, J.-S.; Brünger, A. T. *J. Molec. Biol.* **1994**, 243, 100.
- The integration of the void is identical to SQUEEZE: van der Sluis, P. Spek, A. L. *Acta Cryst. A* **1990**, A46, 194. (BYPASS algorithm)



- Although the void area is calculated somewhat different, both methods work more or less identical.

Solvent procedure in OLEX2

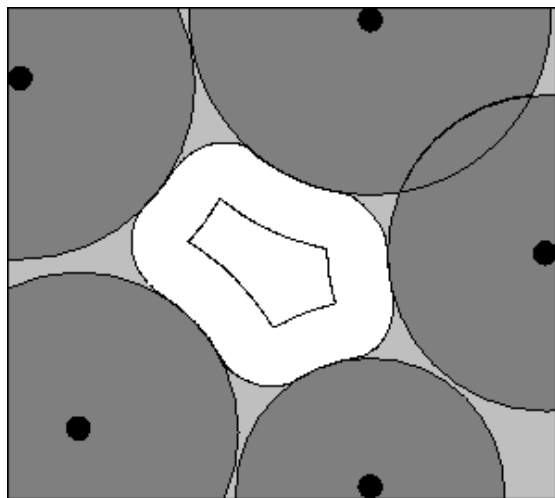


The VdW-surface is reduced by r_{shrink} , typically 1.0 – 1.2 Å.

All volume farther away than r_{probe} (1.0 – 1.2 Å) is part of the solvent volume

SQUEEZE

The procedure is the inverse order of the SQUEEZE masking procedure (where $r_{shrink} = r_{probe}$), which gives a somewhat more reasonable void area.



Solvent procedure in OLEX2

- Although the void area is calculated somewhat different, both methods work more or less identical.
- The only difference is in the treatment of intensities. Olex2-mask produces an internal, solvent-corrected hkl-file (*code-mask.hkl*), which is then passed to SHELXL. (Done by the program in the background.) This resembles the “mode 2” or “SHELX97-style” SQUEEZE procedure.
- Advantages of the OLEX2 procedure:
 - Much easier use (1 click)
 - **Possibility to re-calculate the solvent mask before each refinement.** (Equivalent to multiple Squeeze / refinement cycles.) This allows adaptation of the solvent mask, if major changes are made to the main structure.
- **Disadvantage:** The produced .fcf from OLEX is based on the solvent-corrected hkl-file and thus **does not contain the real experimental intensities.**

SQUEEZE vs. OLEX/mask

hkl

res

observed intensities

solvent-free structure

no solvent treatment

SQUEEZE

OLEX2/mask

hkl

res

hkl

res

fab

hkl

res

I_{obs}

I_{calc}

I_{obs}

I_{calc}

I_{obs}

I_{calc}

loop_
 _refln_index_h
 _refln_index_k
 _refln_index_l
 _refln_F_squared_calc
 _refln_F_squared_meas
 _refln_F_squared_sigma

2	0	0	264642	125748	1230
4	0	0	2490	695	35

I_{calc}

I_{obs}

2	0	0	123714	120180	1176
4	0	0	495	664	33

I_{calc}

I_{obs}

2	0	0	259979	222522	1191
4	0	0	2604	1766	34

I_{calc}

I_{obs}

General guidelines I

- Due to the Fourier transformation, electron density in the cavities might be caused by a bad model. Thus do not use SQUEEZE before the refinement of the rest of your structure including disorders is finished.
- Anion positions contain important structural and chemical information. **Thus never ever use SQUEEZE to suppress your counter-ions!**
- It is **always, always** better to refine a solvent using strong restraints/constraints than to use SQUEEZE! Check the .sqz-file to see if you recognize the solvent.

General guidelines II

- It is under disputation in the community if you should include the disordered solvent in the molecular formula (which effects molecular weight, absorption coefficient, density etc.) Either you report wrong data (e. g., you density is too low) or you include theoretical information about a mystical solvent nobody has seen.

My personal 2cents: Include the solvent, if its amount and nature has been established by other methods (NMR of the crystals). Otherwise, do not include it.

- OLEX/mask produces solvent-corrected I_{obs} , **which are not the experimental data.**

Best procedure: Use OLEX/mask only in early steps of the refinement. If at the end you decide to include a disordered solvent model, de-activate mask and run SQUEEZE. This will include the fab, hkl and res in the cif.

Second-best procedure: Make sure that “Ignore hkl/res” is activated under REPORT to have the observed data included in the CIF.

- And really: It is **always, always** better to refine a solvent using strong restraints/constraints than to use SQUEEZE!

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Fin