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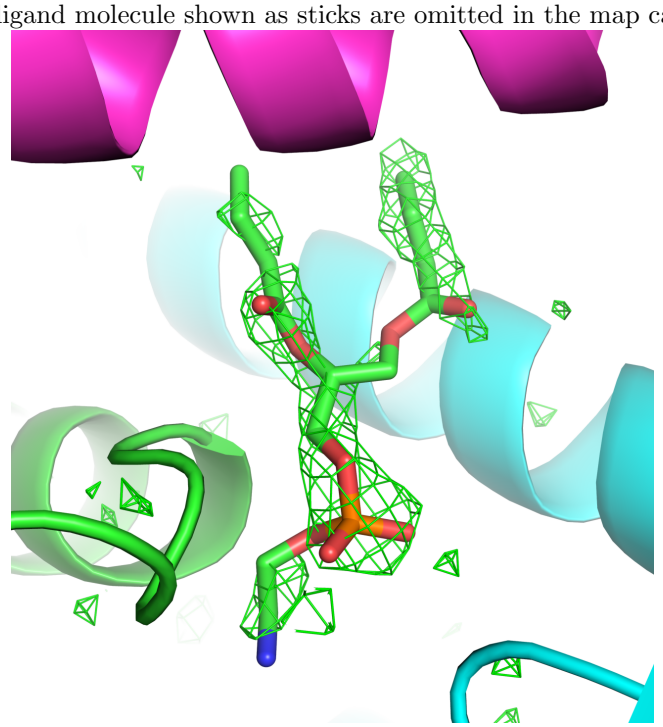
Supporting information for article:

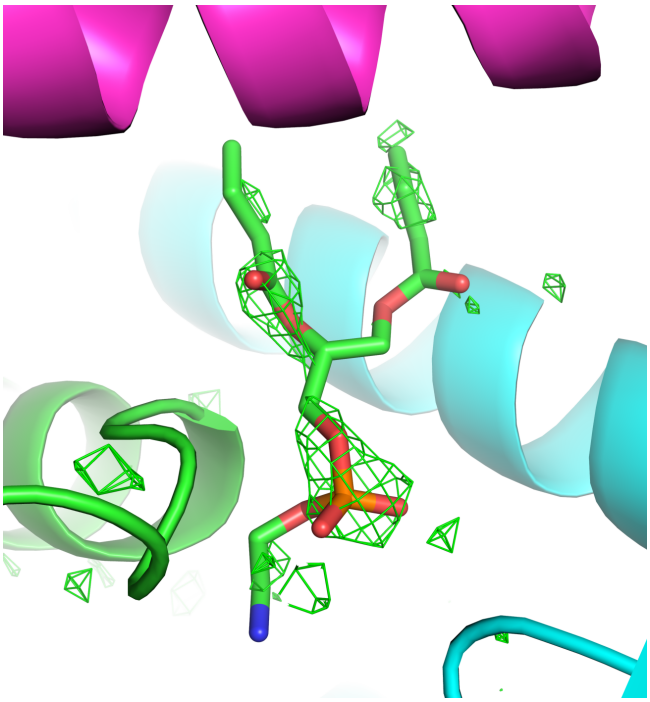
Cryo-EM single-particle structure refinement and map calculation using *Servalcat*

Keitaro Yamashita, Colin M. Palmer, Tom Burnley and Garib N. Murshudov

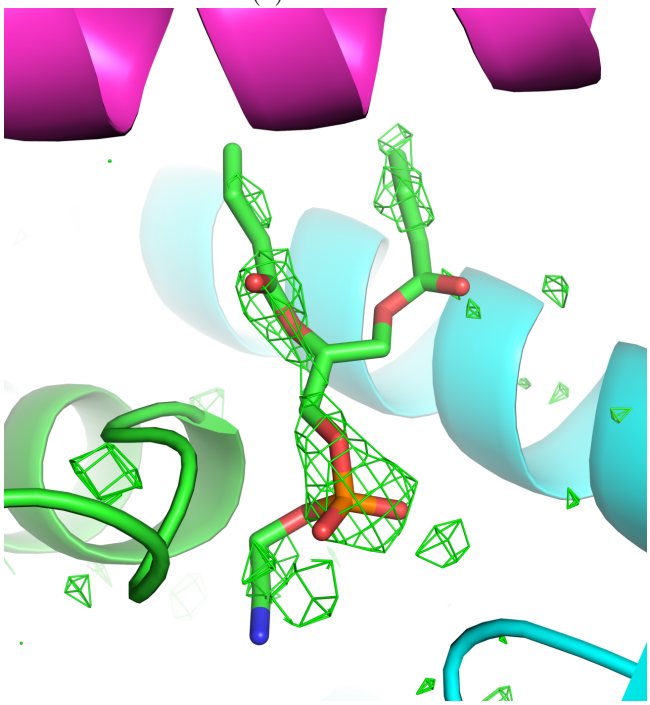
Fig. S1: Comparison of weighting and sharpening scheme for Fig. 2a (PDB 7kjr/EMD-22898 at 2.08 Å). (a) Weighted and sharpened map using (17). (b) No FSC-based weights, and sharpening by B value determined by PostProcess in RELION; $(F_o - DF_c)/e^{-B|s|^2/4}$ with $B = -34.5$ Å^2. (c) Using postprocess.mrc; $\sqrt{\text{FSC}_{\text{full}}}(F_o - DF_c)/e^{-B|s|^2/4}$ with the same B. The $F_o - F_c$ omit maps are contoured at 3σ (scaled within the mask). The ligand molecule shown as sticks are omitted in the map calculation.



(a) *Servalcat*

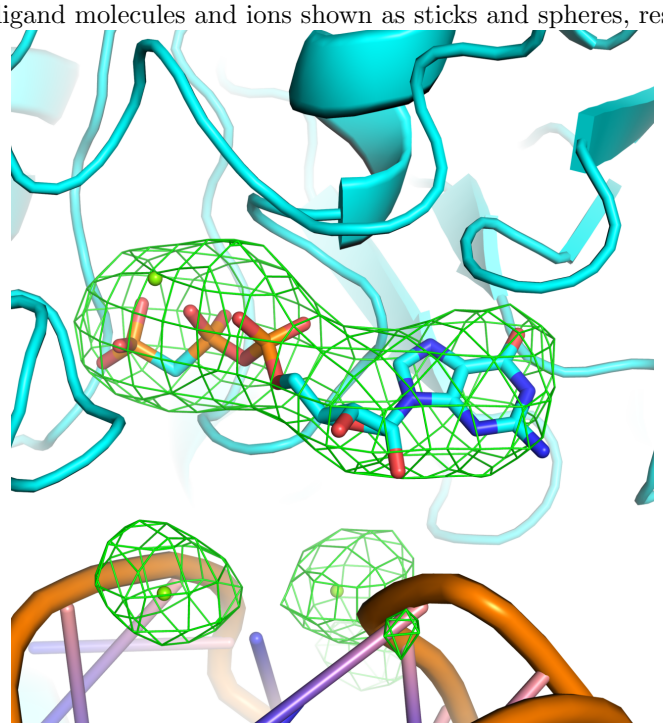


(b) sharpened by $B_{\text{PostProcess}}$ (no weights)



(c) FSC weighted, sharpened by $B_{\text{PostProcess}}$

Fig. S2: Comparison of weighting and sharpening scheme for Fig. 2b (PDB 5it7/EMD-8123 at 3.6 Å). (a) Weighted and sharpened map using (17). (b) No FSC-based weights, and sharpening by B value determined by PostProcess in RELION; $(F_o - DF_c)/e^{-B|s|^2/4}$ with $B = -150.1$ Å^2. (c) Using postprocess.mrc; $\sqrt{\text{FSC}_{\text{full}}}(F_o - DF_c)/e^{-B|s|^2/4}$ with the same B. The $F_o - F_c$ omit maps are contoured at 3σ (scaled within the mask). The ligand molecules and ions shown as sticks and spheres, respectively, are omitted in the map calculation.



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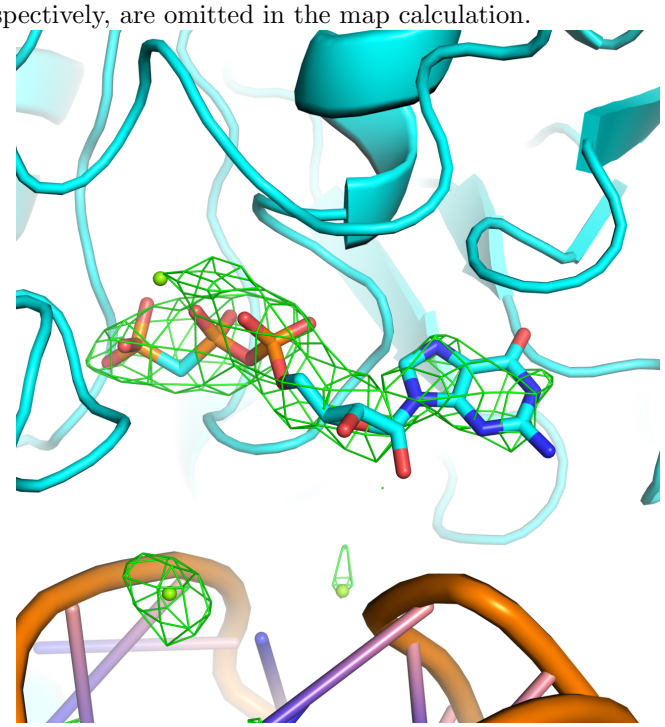
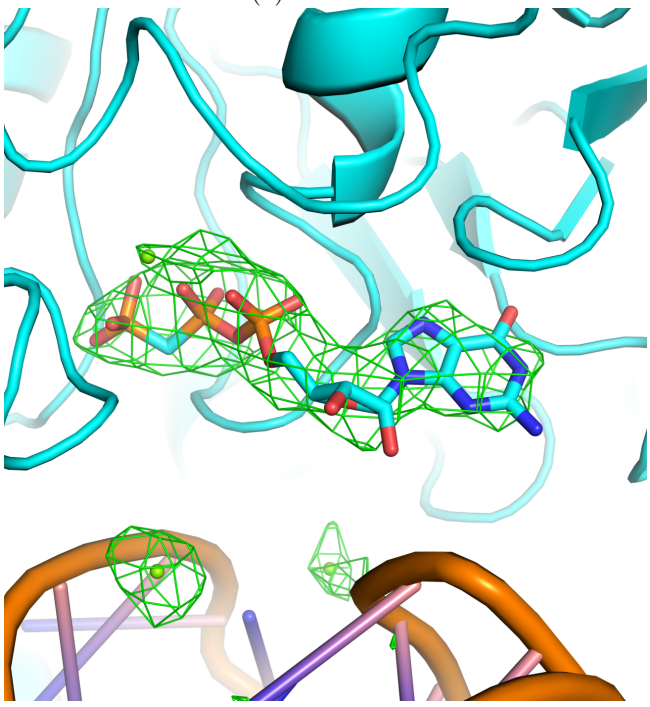
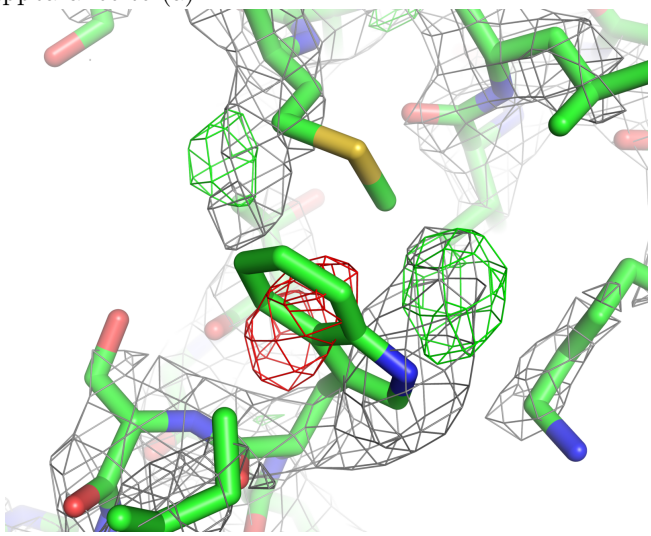


(b) sharpened by $B_{\text{PostProcess}}$ (no weights)

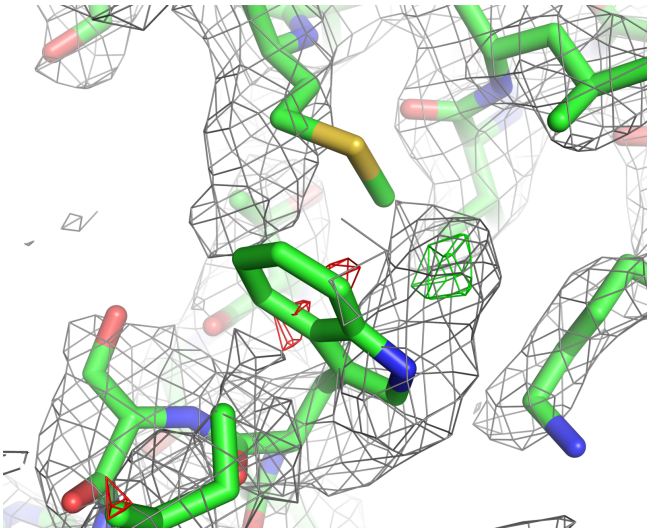


(c) FSC weighted, sharpened by $B_{\text{PostProcess}}$

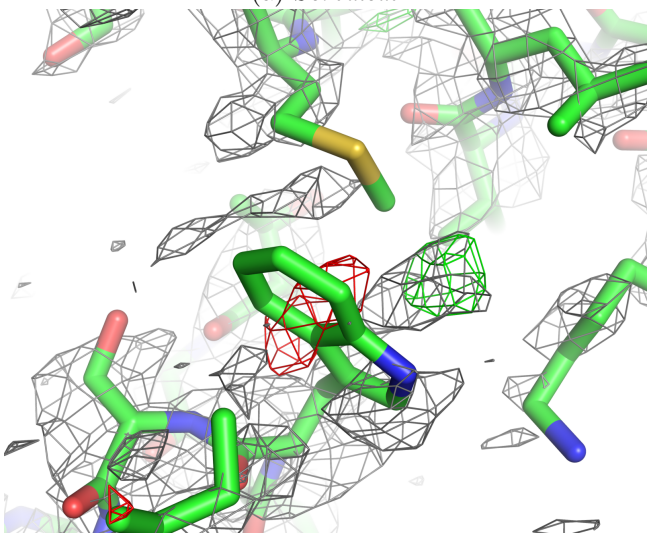
Fig. S3: Comparison of weighting and sharpening scheme for Fig. 3 (PDB 6lmt/EMD-0919 at 2.66 Å). (a) Weighted and sharpened maps using (18) and (17). (b) No FSC-based weights, and sharpening by B value determined by PostProcess in RELION; $F_{\rm o}/e^{-B|s|^2/4}$ and $(F_{\rm o}-DF_{\rm c})/e^{-B|s|^2/4}$ with $B=-74.0$ Å^2. (c) Using postprocess.mrc; $\sqrt{\rm FSC_{full}}F_{\rm o}/e^{-B|s|^2/4}$ and $\sqrt{\rm FSC_{full}}(F_{\rm o}-DF_{\rm c})/e^{-B|s|^2/4}$ with the same B. The $F_{\rm o}-F_{\rm c}$ maps are contoured at $\pm4\sigma$ (scaled within the mask). The contouring levels of $F_{\rm o}$ maps are adjusted to give similar appearance to (a).



(a) *Servalcat*

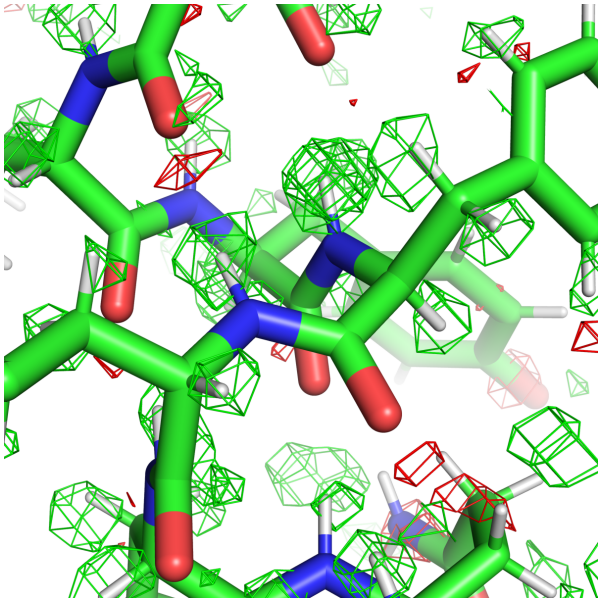


(b) sharpened by $B_{\rm PostProcess}$ (no weights)

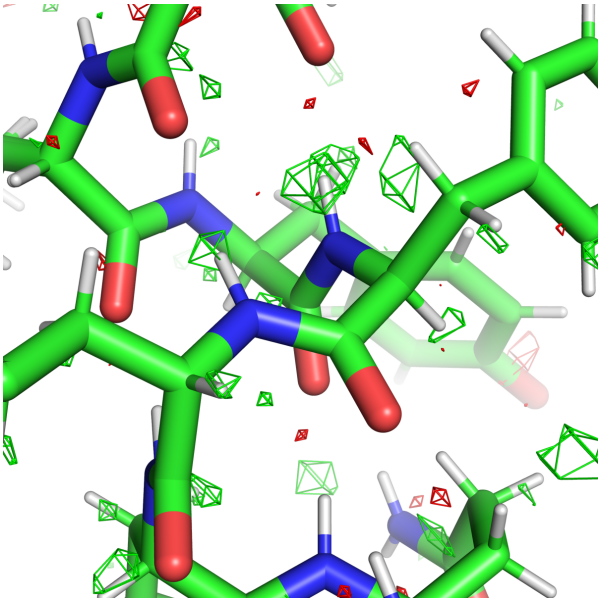


(c) FSC weighted, sharpened by $B_{\rm PostProcess}$

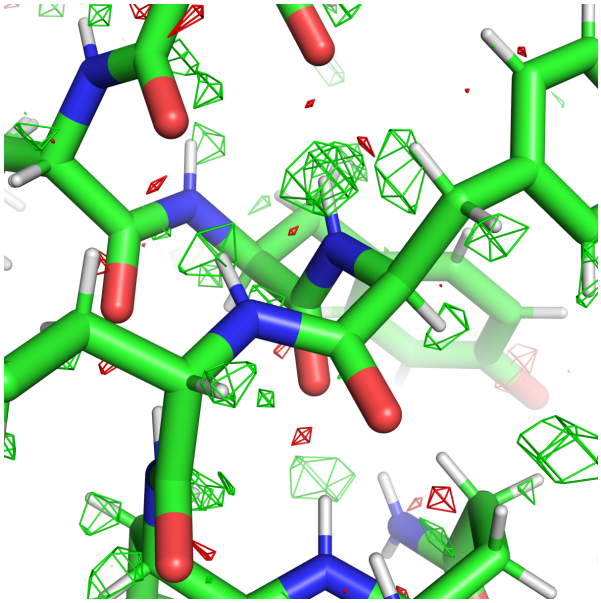
Fig. S4: Comparison of weighting and sharpening scheme for Fig. 5a (PDB 6z6u/EMD-11103 at 1.25 Å). (a) Weighted and sharpened map using (17). (b) No FSC-based weights, and sharpening by B value determined by PostProcess in RELION; $(F_\mathrm{o} - DF_\mathrm{c})/e^{-B|s|^2/4}$ with $B = -33.4$ Å^2. (c) Using postprocess.mrc; $\sqrt{\mathrm{FSC}_\mathrm{full}}(F_\mathrm{o} - DF_\mathrm{c})/e^{-B|s|^2/4}$ with the same B. The $F_\mathrm{o} - F_\mathrm{c}$ maps are contoured at $\pm 3\sigma$ (scaled within the mask).



(a) *Servalcat*

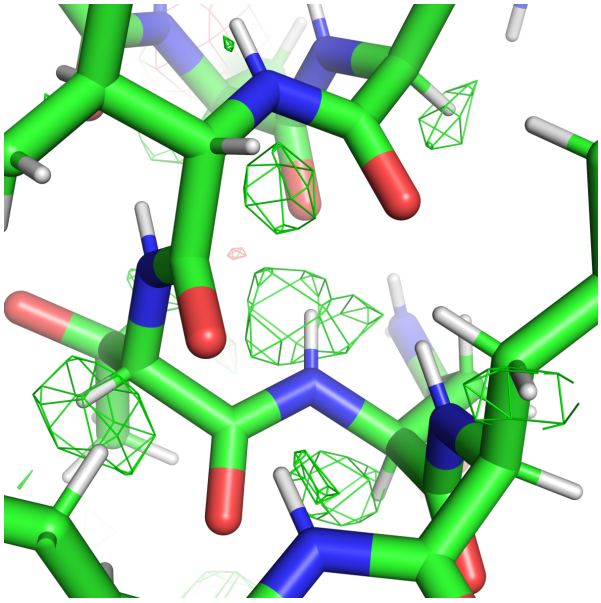


(b) sharpened by $B_\mathrm{PostProcess}$ (no weights)

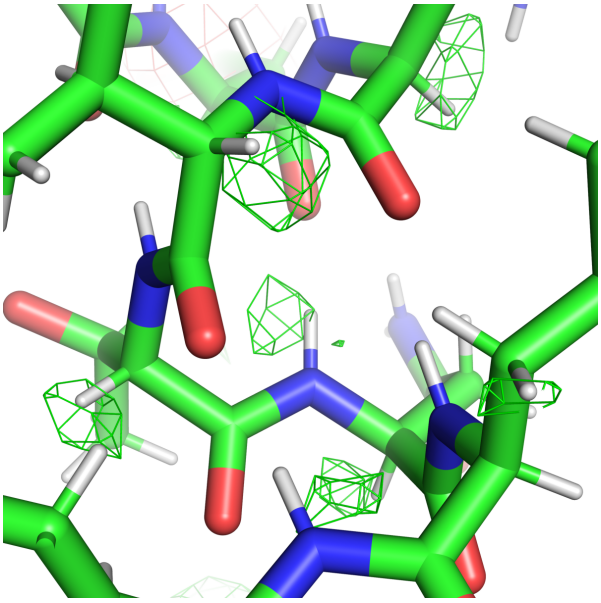


(c) FSC weighted, sharpened by $B_\mathrm{PostProcess}$

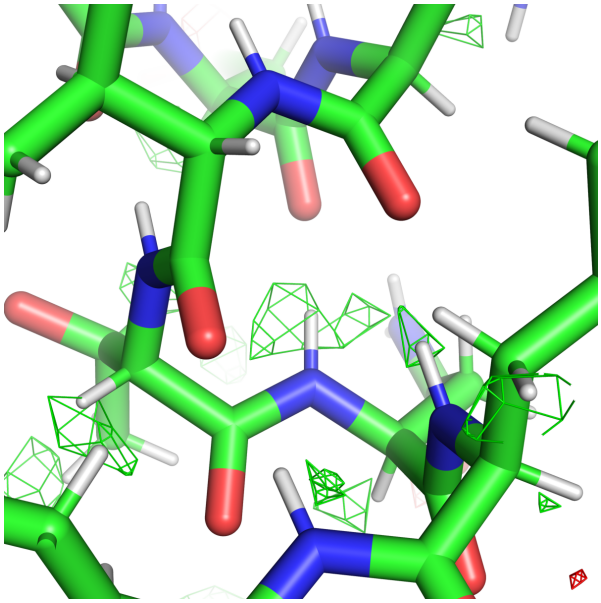
Fig. S5: Comparison of weighting and sharpening scheme for Fig. 5b (PDB 6s61/EMD-10101 at 1.84 Å). (a) Weighted and sharpened map using (17). (b) No FSC-based weights, and sharpening by B value determined by PostProcess in RELION; $(F_\mathrm{o} - DF_\mathrm{c})/e^{-B|s|^2/4}$ with $B = -70.9$ Å^2. (c) Using postprocess.mrc; $\sqrt{\mathrm{FSC}_\mathrm{full}}(F_\mathrm{o} - DF_\mathrm{c})/e^{-B|s|^2/4}$ with the same B. The $F_\mathrm{o} - F_\mathrm{c}$ maps are contoured at $\pm 3\sigma$ (scaled within the mask).



(a) *Servalcat*

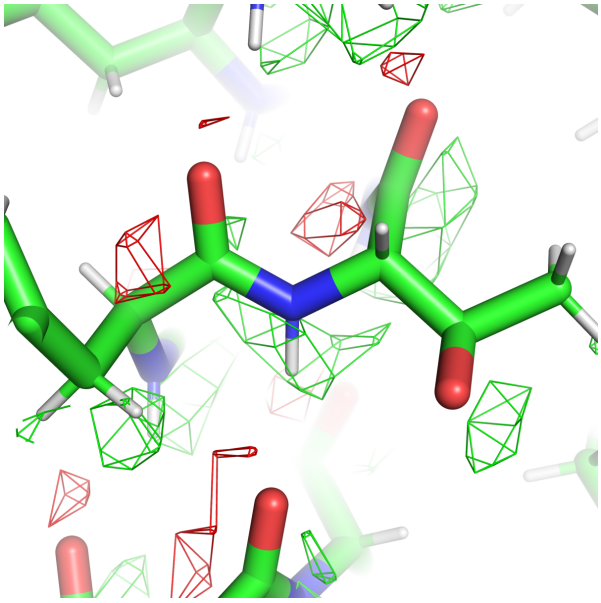


(b) sharpened by $B_\mathrm{PostProcess}$ (no weights)

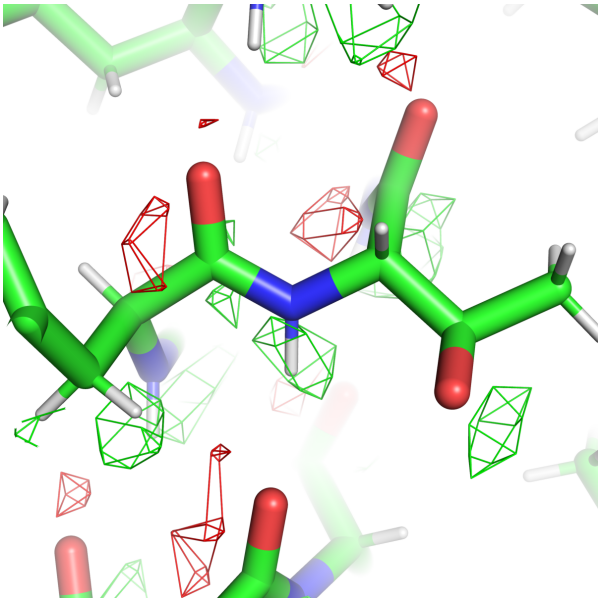


(c) FSC weighted, sharpened by $B_\mathrm{PostProcess}$

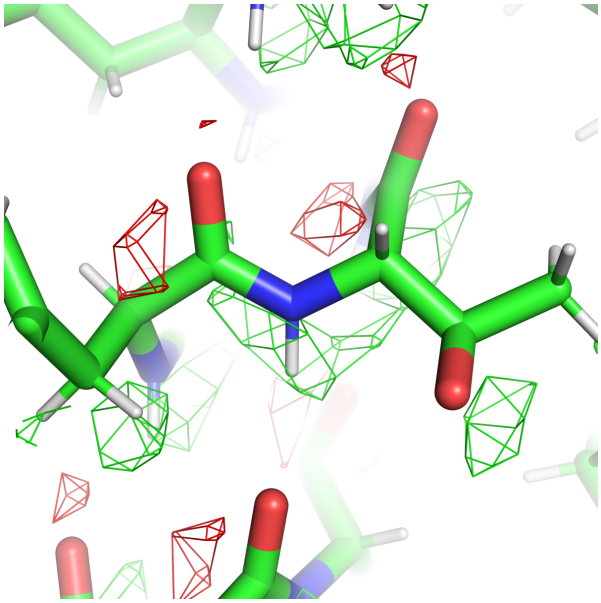
Fig. S6: Comparison of weighting and sharpening scheme for Fig. 5c (PDB 6wx6/EMD-21951 at 2.00 Å). (a) Weighted and sharpened map using (17). (b) No FSC-based weights, and sharpening by B value determined by PostProcess in RELION; $(F_\mathrm{o} - DF_\mathrm{c})/e^{-B|s|^2/4}$ with $B = -98.6$ Å^2. (c) Using postprocess.mrc; $\sqrt{\mathrm{FSC}_\mathrm{full}}(F_\mathrm{o} - DF_\mathrm{c})/e^{-B|s|^2/4}$ with the same B. The $F_\mathrm{o} - F_\mathrm{c}$ maps are contoured at $\pm 3\sigma$ (scaled within the mask).



(a) *Servalcat*



(b) sharpened by $B_\mathrm{PostProcess}$ (no weights)



(c) FSC weighted, sharpened by $B_\mathrm{PostProcess}$