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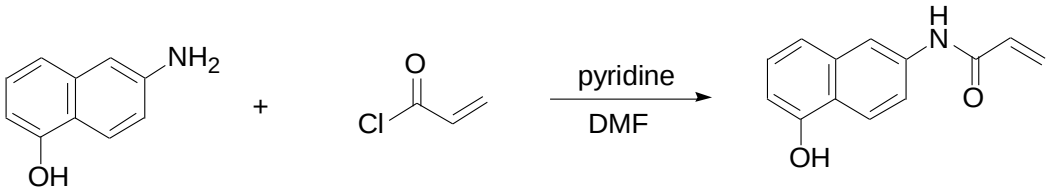
Supporting information for article:

A binding hotspot in *Trypanosoma cruzi* histidyl-tRNA synthetase revealed by fragment-based crystallographic cocktail screens

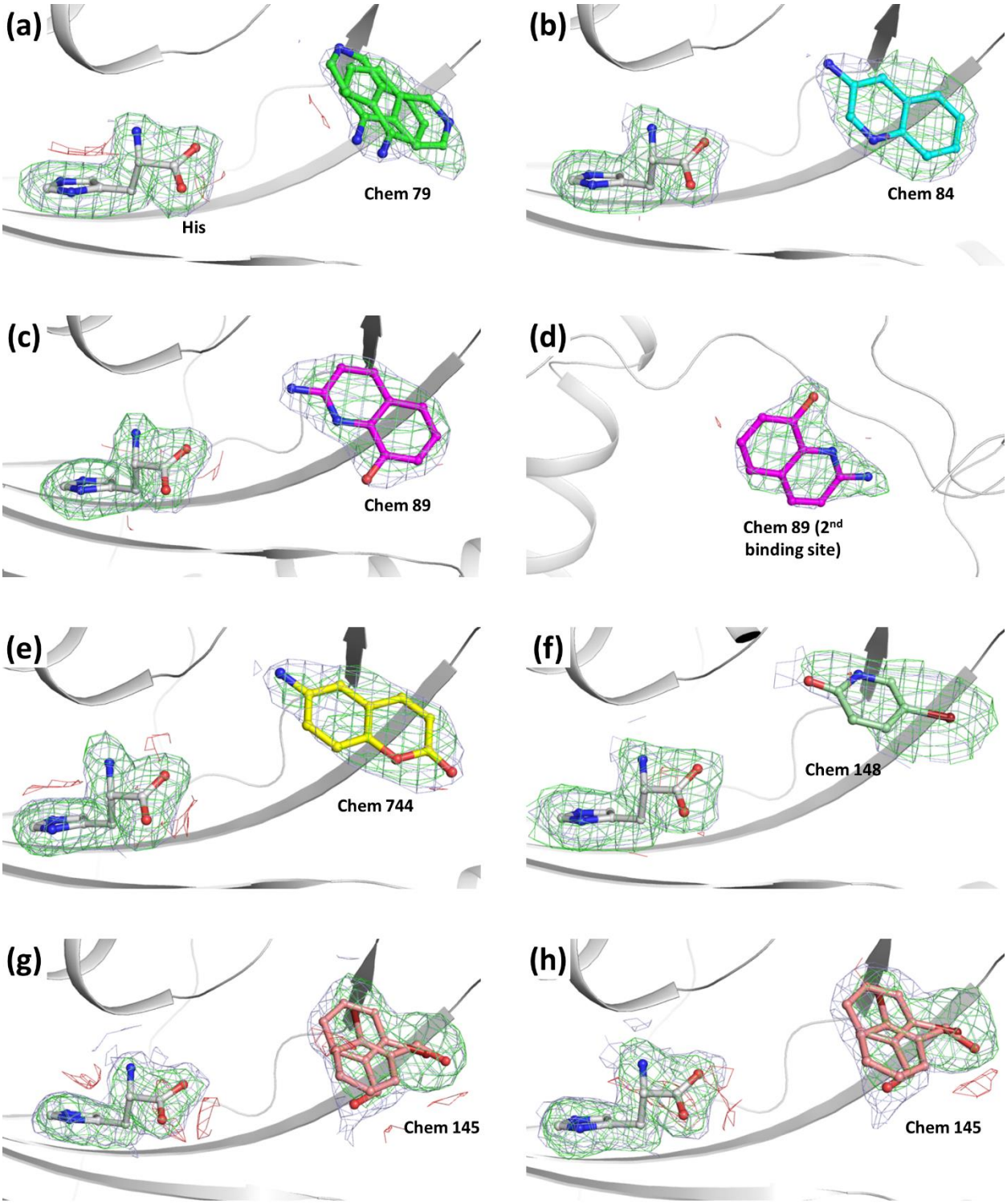
Cho Yeow Koh, Latha Kallur Siddaramaiah, Ranae M. Ranade, Jasmine Nguyen, Tengyue Jian, Zhongsheng Zhang, J. Robert Gillespie, Frederick S. Buckner, Christophe L. M. J. Verlinde, Erkang Fan and Wim G. J. Hol

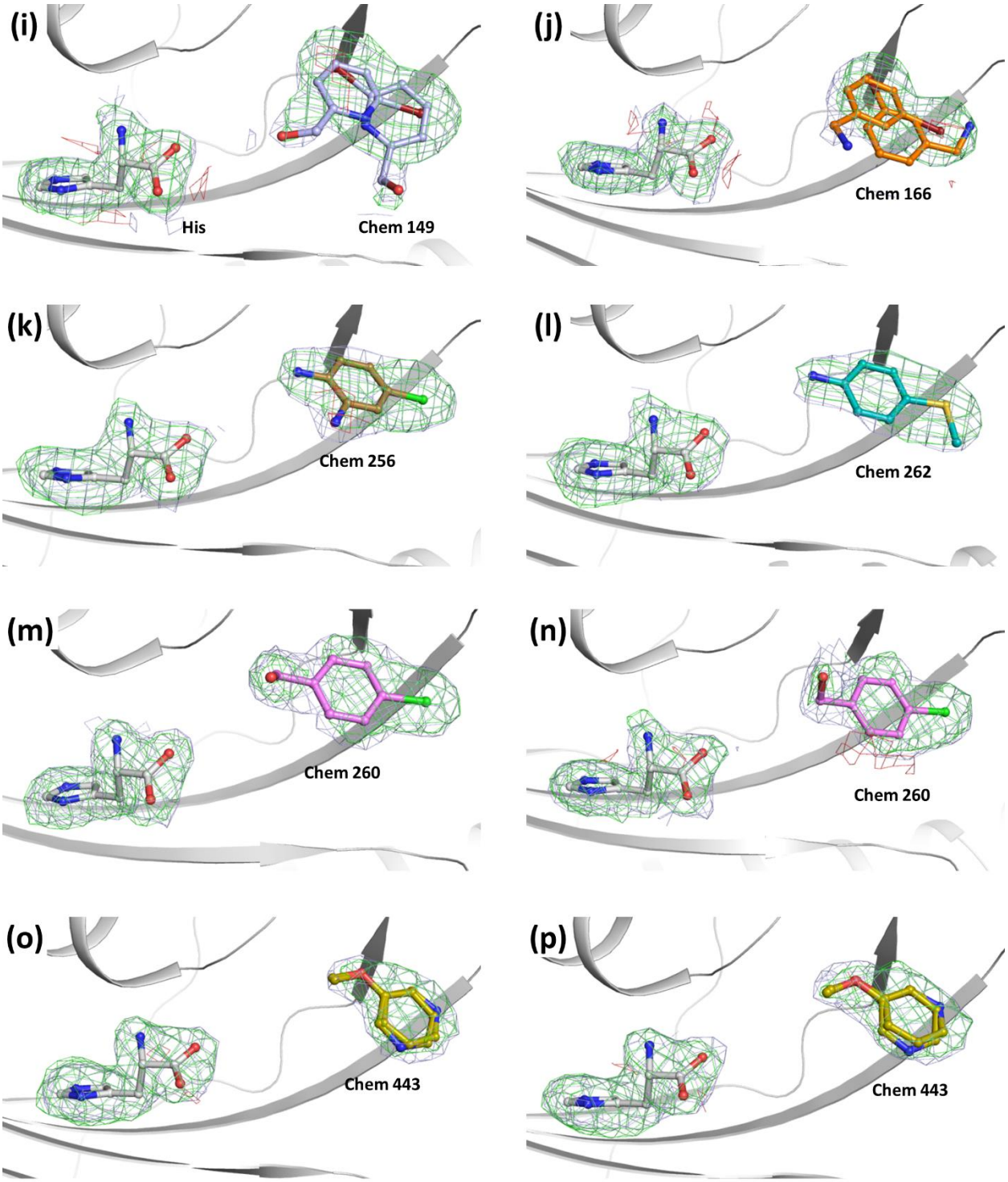
S1. Chemical Synthesis



ChemID **1781**

Synthesis of Chem **1781**: To a stirred solution of 6-amino-1-naphthol (159 mg, 1.0 mmol) in DMF (5 mL) and pyridine (0.15 mL) was added acrylic chloride (81 µl, 1.0 mmol) at 0°C. The resulting mixture was allowed to warm to room temperature and stirred further overnight. After the solvent was removed, the residue was dissolved in ethyl acetate. The organic mixture was washed with water twice, brine and dried over Na_2SO_4. After the solvent was removed, the residue was purified via flash chromatography with ethyl acetate/hexane elution to obtain a brown solid, yield 90 mg, 42 %. ^{1}H NMR (500 MHz, CD_3OD), δ = 8.23 (d, *J* = 1.8 Hz, 1H), 8.16 (d, *J* = 9.0 Hz, 1H), 7.54 (dd, *J* = 9.0, 2.0 Hz, 1H), 7.29-7.23 (m, 2H), 6.76 (dd, *J* = 5.4, 3.1 Hz, 1H), 6.51 (m, 1H), 6.41 (m, 1H), 5.81 (dd, *J* =10.0, 1.7 Hz, 1H); MS(ESI) $(M+H)^+$ = 214.5.


(a)
Chem 79
His
(b)
Chem 84
(c)
Chem 89
(d)
Chem 89 (2nd binding site)
(e)
Chem 744
(f)
Chem 148
(g)
Chem 145
(h)
Chem 145


(i)
His
Chem 149
(j)
Chem 166
(k)
Chem 256
(l)
Chem 262
(m)
Chem 260
(n)
Chem 260
(o)
Chem 443
(p)
Chem 443

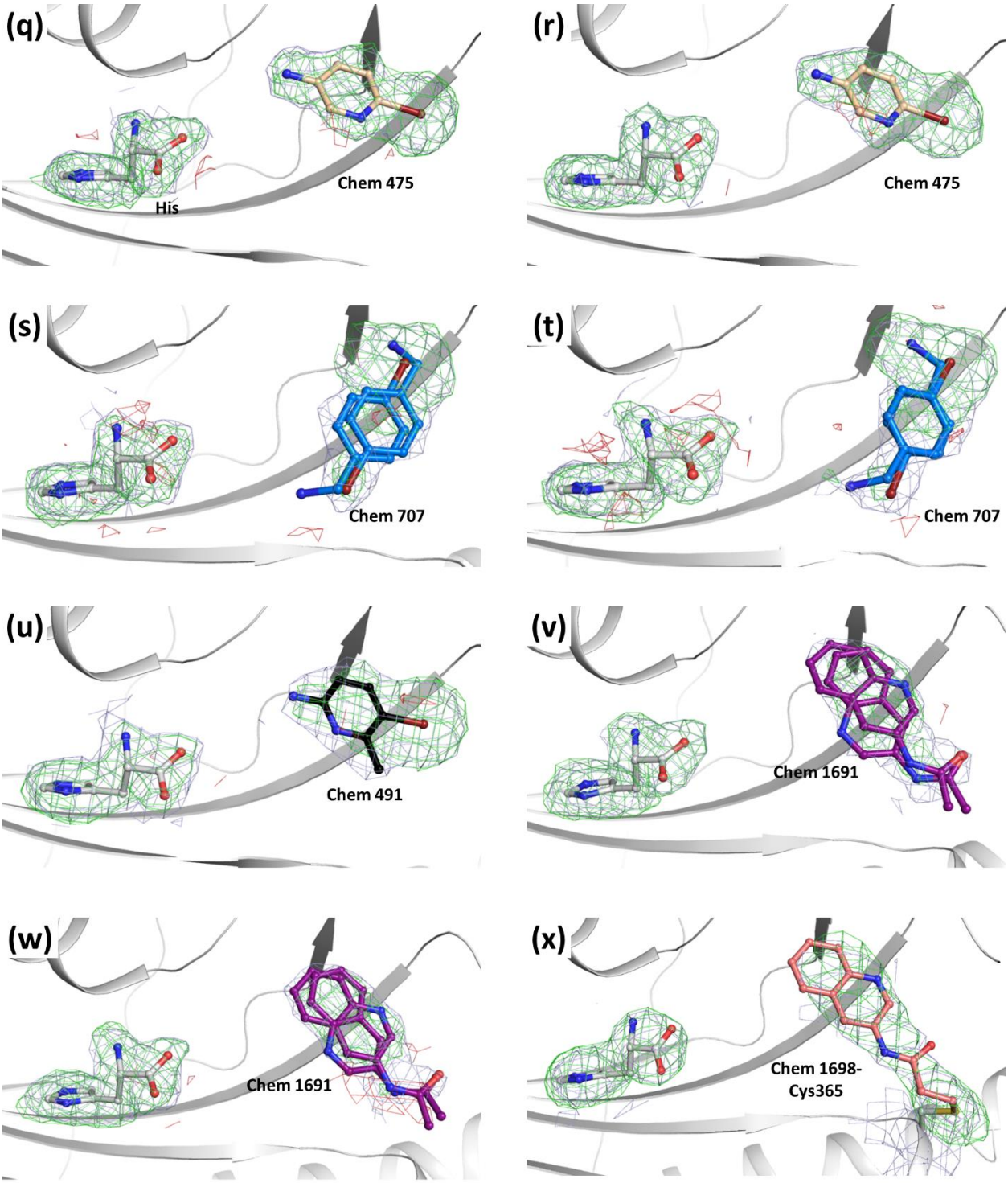

(q)
His
Chem 475
(r)
Chem 475
(s)
Chem 707
(t)
Chem 707
(u)
Chem 491
(v)
Chem 1691
(w)
Chem 1691
(x)
Chem 1698-
Cys365

Figure S1 Electron densities of fragments and histidine bound to *Tc*HisRS. σA-weighted $F_{obs} - F_{calc}$ electron densities calculated by omitting histidine and fragments are shown in green (positive) and red (negative) counters at the 3σ level, and σA-weighted $2F_{obs} - F_{calc}$ electron densities in light blue at the 1.0 σ level. Histidine and fragments are depicted in ball and stick models while *Tc*HisRS is shown in ribbon representation. The bound fragments presented in this figure are from structures determined with individual soaks of: (a) **Chem 79** (two alternative binding modes), (b) **Chem 84**, (c) **Chem 89** (typical fragment-binding site), (d) **Chem 89** (second binding site), (e) **Chem 744**, (f) **Chem 148**, (g) **Chem 145** (alternative binding modes in *Tc*HisRS subunit A), (h) **Chem 145** (alternative binding modes in *Tc*HisRS subunit B). (i) **Chem 149** (two alternative binding modes), (j) **Chem 166** (two alternative binding modes), (k) **Chem 256**, (l) **Chem 262**, (m) **Chem 260** (in *Tc*HisRS subunit A), (n) **Chem 260** (in *Tc*HisRS subunit B), (o) **Chem 443** (two alternative binding modes in *Tc*HisRS subunit A), (p) **Chem 443** (two alternative binding modes in *Tc*HisRS subunit B), (q) **Chem 475** (in *Tc*HisRS subunit A), (r) **Chem 475** (in *Tc*HisRS subunit B), (s) **Chem 707** (two alternative binding modes in *Tc*HisRS subunit A), (t) **Chem 707** (two alternative binding modes in *Tc*HisRS subunit B), (u) **Chem 491**, (v) **Chem 1691** (two alternative binding modes in *Tc*HisRS subunit A), (w) **Chem 1691** (two alternative binding modes in *Tc*HisRS subunit B), (x) **Chem 1698** (covalently linked to Cys365). For electron densities for **Chem 1781** see Figure 4c and 4d in the main text. For data collection and refinement statistics see Table 1, and for chemical formulae see Tables 2 and 3 in the main text.

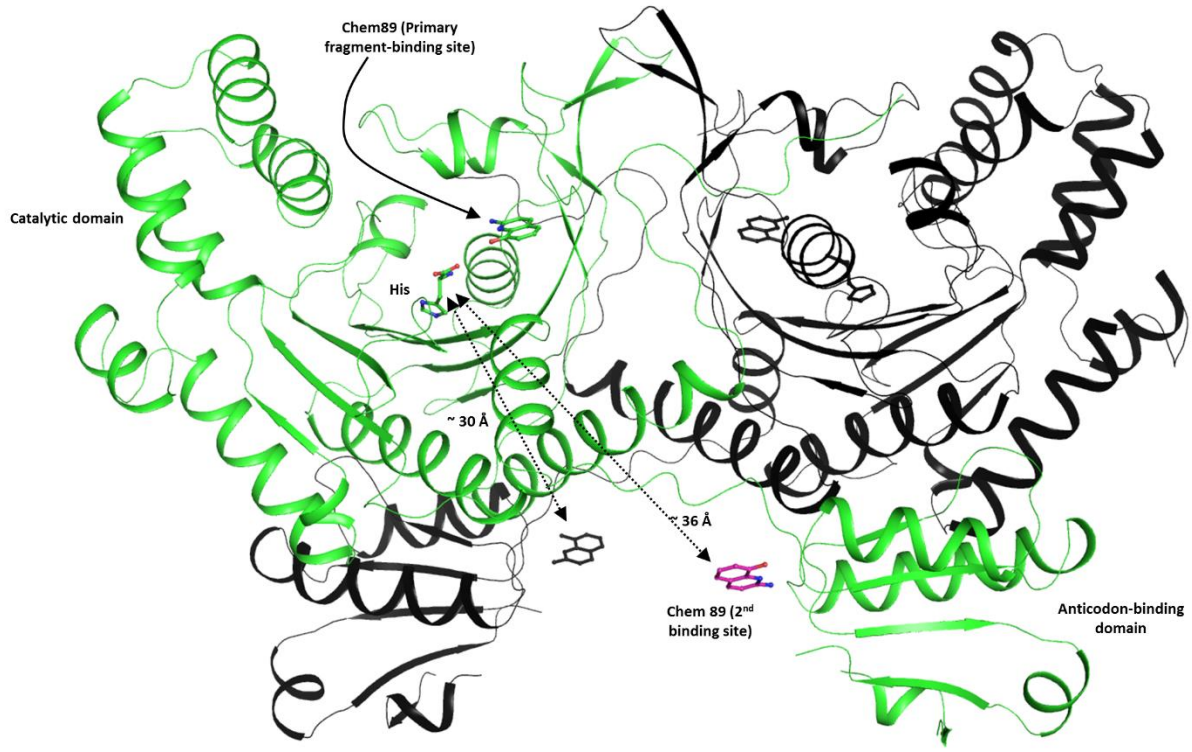


Figure S2 In addition to the primary fragment-binding site, **Chem 89** also occupy a second, likely lower affinity, site (magenta). The binding site is in close proximity to the peptide linker between the catalytic domain and the anticodon-binding domain of the *Tc*HisRS•His structure. This secondary binding site is approximately 36 Å away from the active site when measured within a *Tc*HisRS monomer (green), and 30 Å away from the active site when measured between two different monomers (green against black) that form the biological-relevant dimer. This second binding site is not only far away from the active sites in the dimer, but it is also not at the HisRS dimer interface. Hence, this second binding site of **Chem 89** is unlikely to be relevant for inhibitor design purposes