Structural basis for $\sigma_1$ receptor ligand recognition

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The $\sigma_1$ receptor is a poorly understood membrane protein expressed throughout the human body. Ligands targeting the $\sigma_1$ receptor are in clinical trials for treatment of Alzheimer’s disease, ischemic stroke, and neuropathic pain. However, relatively little is known regarding the $\sigma_1$ receptor’s molecular function. Here, we present crystal structures of human $\sigma_1$ receptor bound to the antagonists haloperidol and NE-100, and the agonist (+)-pentazocine, at crystallographic resolutions of 3.1 Å, 2.9 Å, and 3.1 Å, respectively. These structures reveal a unique binding pose for the agonist. The structures and accompanying molecular dynamics (MD) simulations identify agonist-induced structural rearrangements in the receptor. Additionally, we show that ligand binding to $\sigma_1$ is a multistep process that is rate limited by receptor conformational change. We used MD simulations to reconstruct a ligand binding pathway involving two major conformational changes. These data provide a framework for understanding the molecular basis for $\sigma_1$ agonism.

Pharmacological studies of opioid compounds and their chemical analogs in the 1970s led to the identification of several opioid receptor ‘subtypes’, one of which was termed the $\sigma$ receptor. Subsequent pharmacological characterization showed that the $\sigma$ receptor is distinguished from the true opioid receptors by a divergent ligand binding profile. Later pharmacological studies further divided the receptor into $\sigma_1$ and $\sigma_2$ subtypes. Molecular cloning of the $\sigma_1$ receptor and later the $\sigma_2$ receptor showed that these proteins are genetically unrelated to each other and have no similarity to the true opioid receptors. In fact, the $\sigma_1$ receptor subtype shows no sequence similarity to any other human protein.

The $\sigma_1$ receptor continues to be of pharmacological interest because it binds a host of structurally dissimilar pharmacologically active compounds with high affinity (Fig. 1a). These compounds include benzomorphans, antipsychotics, psychosis-inducing drugs, the antifungal agent fenpropimorph, sterols such as progesterone, and numerous other compounds. These molecules contain few shared features, although most include a basic nitrogen atom flanked on two sides by longer hydrophobic moieties (typically phenyl rings), representing a minimal $\sigma_1$-binding pharmacophore (Fig. 1a). The $\sigma_1$ receptor’s nearest homolog is the yeast $\Delta 8$-$\Delta 7$ sterol isomerase, ERG2p; however, the $\sigma_1$ receptor itself has no detectable isomerase activity. Human genetic data have linked point mutations in the $\sigma_1$ receptor to inherited motor neuron diseases, and animal models have implicated the receptor in Parkinson’s disease, addiction, and pain. A $\sigma_1$ receptor antagonist is currently in clinical trials for the treatment of neuropathic pain, and agonists are in clinical trials for Alzheimer’s disease and ischemic stroke.

Despite its potential therapeutic relevance and a wealth of high-affinity ligands, surprisingly little is known about the molecular underpinnings of $\sigma_1$ receptor function. Substantial evidence suggests that the $\sigma_1$ receptor serves as a modulator for other signaling-pathway effectors. Specifically, knockdown or antagonism of the $\sigma_1$ receptor can potentiate signaling by G-protein-coupled receptors, whereas agonists of the $\sigma_1$ receptor result in enhanced IP3 receptor–dependent intracellular calcium flux and inhibition of sodium-channel and potassium-channel currents. The $\sigma_1$ receptor exists in multiple oligomeric states, and reports suggest that agonists cause a shift toward monomeric or low-molecular-weight species, whereas antagonists bias the receptor toward high-molecular-weight species. However, the dominant physiologically relevant oligomeric forms and the precise way in which oligomerization is tied to agonist binding are unknown.

We have recently reported the first structures of the human $\sigma_1$ receptor bound to two different ligands, PD 144418, an antagonist, and 4-IBP, a poorly characterized ligand of ambiguous efficacy class. The receptor crystallized as a trimer, and each protomer shows a fold including a single transmembrane domain and a $\beta$-barrel flanked by $\alpha$ helices (Fig. 1b,c and Supplementary Fig. 1a). Although these initial results have provided initial structural information on $\sigma_1$ receptor, neither ligand is commonly used to study $\sigma_1$ receptor function, and few functional data are available for either ligand.

To understand the molecular basis for agonist activity at $\sigma_1$, we pursued structural studies of three well-characterized classical ligands of the receptor: the antagonists haloperidol and NE-100, and the agonist (+)-pentazocine. Using the lipidic-cubic-phase method, we determined X-ray crystal structures of the receptor in complex with these three compounds at resolutions of 3.1 Å, 2.9 Å, and 3.1 Å, respectively (Table 1 and Supplementary Fig. 2).

Results

Structure of the human $\sigma_1$ receptor bound to antagonists. The structures of the $\sigma_1$ receptor bound to the classical antagonists haloperidol and NE-100 are highly similar to each other and to our previously reported structures of $\sigma_1$ bound to PD 144418 and 4-IBP (Supplementary Fig. 1b–e). Both haloperidol and NE-100 include a shared simple pharmacophore (Fig. 1a), and both adopt similar conformations in the ligand-binding site (Fig. 1d, e). In each case, the ligand’s positively charged nitrogen forms an electrostatic interaction with E172, and the rest of the molecule adopts a linear pose that fits within the space not occluded by the many bulky hydrophobic residues that line the interior of the $\sigma_1$ binding pocket (Fig. 1d,e).

In general, the longer of the two hydrophobic regions occupies the region of the $\beta$-barrel that is proximal to the membrane, near the space between helices $\alpha_4$ and $\alpha_5$ (Fig. 1d,e and Supplementary Fig. 1b–e). In contrast, the shorter hydrophobic region occupies space near the bottom of the $\beta$-barrel, near D126 (Fig. 1d,e and Supplementary Fig. 1b–e).

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Structure of the human $\sigma_1$ receptor bound to an agonist. The structures above reveal the overall pose of ligands in the agonist-bound $\sigma_1$ receptor, confirming a highly conserved binding mode and receptor conformation even for chemically diverse antagonists. Next, we investigated the structure of the receptor bound to (+)-pentazocine at 3.1-Å resolution (Fig. 2, Table 1 and Supplementary Fig. 2d,e). In general, the agonist-bound receptor crystallized similarly to antagonist-bound $\sigma_1$, and the overall conformation of the receptor did not change significantly (Fig. 2a).

The exception is a movement of helix $\alpha_4$, which shifts approximately 1.8 Å away from helix $\alpha_5$ in the (+)-pentazocine-bound structure relative to the PD 144418-bound structure (Fig. 2b). This movement appears to be a consequence of the pose adopted by (+)-pentazocine, which occupies a different portion of the receptor binding pocket than the other ligands examined to date (Fig. 2a). This difference in the position of helix $\alpha_4$ was also consistently observed in MD simulations (Fig. 2d). In simulations of ligand-free $\sigma_1$, the helix adopts a position similar to that when an antagonist is bound (Fig. 2d), thus also suggesting that the agonist is responsible for the conformational change. (+)-Pentazocine engages in an electrostatic interaction with E172, and site 2 is positioned similarly to those of the antagonists, but its nonlinear shape forces site 1 to occupy space closer to helix $\alpha_4$ and further from $\alpha_5$ relative to the antagonists. To prevent a steric clash between the aromatic ring of (+)-pentazocine’s benzomorphan group and residue A185 in helix A (Fig. 2c), helix $\alpha_4$ must shift toward the membrane and away from the ligand (Supplementary Fig. 3a). This movement creates a slightly larger gap between helices $\alpha_4$ and $\alpha_5$ in the (+)-pentazocine-bound structure relative to the antagonist-bound structures. As a result, the distance between helix $\alpha_4$ and the adjacent protomer shrinks, and in the interface between chains A and C, an electrostatic bond between Q194 in chain C and the backbone of the $\alpha_4$ helix in chain A is broken (Supplementary Fig. 3b). In the two best-resolved protomers of the (+)-pentazocine-bound structure, chains A and C, two water molecules occupy the space normally occupied by a portion of the antagonist.

A defining feature of $\sigma_1$ receptor pharmacology is high selectivity for (+)-benzomorphans over their (–) enantiomers. It is clear from this structure that (–)-pentazocine would be unable to bind the $\sigma_1$ receptor in the same pose as (+)-pentazocine, because the benzomorphan ring would clash with both Y103 and the receptor backbone (Supplementary Fig. 3c). Therefore, (–)-pentazocine must adopt an alternative, presumably higher-energy, conformation to bind the $\sigma_1$ receptor.

Although (+)-pentazocine and other (+)-benzomorphans are classical $\sigma_1$ agonists, other agonists are structurally unrelated. To determine whether other agonists might also place steric pressure on the $\alpha_4$ helix, we used the Schrödinger Glide package to dock the commonly used agonist PRE-084 into the (+)-pentazocine-bound $\sigma_1$ receptor structure (Supplementary Fig. 3d). In its top-ranked pose, PRE-084 adopted a conformation similar to that seen in molecular-modeling simulations performed by Yano et al., in which one of the carbon rings comes into close contact with the $\alpha_4$ helix. This pose would be sterically prohibited by the $\alpha_4$ helix in the antagonist-bound structure. Therefore, this conformational change might be broadly important for agonist efficacy among $\sigma_1$ receptor ligands.
Kinetic analysis of $\sigma_1$ receptor ligand association and dissociation. As noted above, the ligand-binding site of the $\sigma_1$ receptor is sterically occluded, and so the receptor must undergo a conformational change to allow ligand entry and egress. Previous work has shown that (+)-pentazocine associates with the receptor slowly$^{29,30}$, and rate constants have been determined for association and dissociation at a single concentration in guinea pig membranes$^{30}$. To gain a better understanding of how ligands associate and dissociate with the $\sigma_1$ receptor, we performed an analysis of ligand binding kinetics by using multiple concentrations of $[^3H]$(+)-pentazocine and membranes prepared from Sf9 cells expressing the human $\sigma_1$ receptor.

We began by measuring the off rate at 37 °C and found it to follow a slow exponential decay with a half-life longer than 200 min (Supplementary Fig. 4a). To obtain detailed association kinetics, we used a scintillation proximity assay (SPA), in which purified FLAG-tagged receptor was bound to yttrium silicate SPA beads coated with Protein A and M1 anti-FLAG antibody. In this format, a single tagged receptor was bound to yttrium silicate SPA beads coated with Protein A and M1 anti-FLAG antibody. In this format, a single

| $\sigma_1$ bound to haloperidol (PDB 6DJZ)* | $\sigma_1$ bound to NE-100 (PDB 6DK0)* | $\sigma_1$ bound to (+)-pentazocine (PDB 6DK1)* |
|------------------------------------------|----------------------------------------|-----------------------------------------------|
| Data collection                          |                                        |                                               |
| Space group                              | P2,2,2                                  | P2,2,2                                        |
| Cell dimensions                          |                                        |                                               |
| $a$, $b$, $c$ (Å)                        | 85.1, 126.1, 110.6                      | 85.0, 127.0, 110.0                            |
| Resolution (Å)                           | 50–31 (3.30–3.10)                      | 50–2.9 (3.00–2.90)                             |
| $R_{\text{max}}$                          | 28.1 (291.6)                            | 36.6 (387.7)                                 |
| $I/\sigma(I)$                            | 5.9 (0.4)                               | 5.6 (0.5)                                    |
| $CC_{1/2}$                               | 99.4 (16.4)                             | 99.8 (33.4)                                  |
| Completeness (%)                         | 99.8 (99.6)                             | 95.3 (96.8)                                  |
| Redundancy                               | 5.7 (5.0)                               | 7.2 (7.2)                                    |
| Refinement                               |                                        |                                               |
| Resolution (Å)                           | 10.5–3.1                               | 46.2–2.9                                     |
| No. reflections                          | 21,454 (1,901 in test set)              | 25,243 (2,164 in test set)                    |
| $R_{\text{work}} / R_{\text{out}}$       | 23.9 / 27.6                            | 25.1 / 27.3                                  |
| No. atoms                                | Protein                                 | 5.021                                         |
|                                        | Ligand                                  | 78                                             |
|                                        | Solvent ions/lipid                      | 164                                            |
|                                        | Water                                    | 30                                             |
| $B$ factors                              | Protein                                 | 102.4                                          |
|                                        | Ligand                                  | 112.4                                          |
|                                        | Solvent ions/lipid                      | 127.8                                          |
|                                        | Water                                    | 82.9                                           |
| R.m.s. deviations                       | Bond lengths (Å)                        | 0.003                                          |
|                                        | Bond angles (%)                         | 0.489                                          |

*The haloperidol- and (+)-pentazocine-bound structures were solved from single-crystal datasets; the NE-100 structure was solved with a merged dataset from seven crystals. *Values in parentheses are for the highest-resolution shell.

Figure 2 | Crystal structures and MD simulations of the human $\sigma_1$ receptor bound to the classical agonist (+)-pentazocine and antagonists. In a–c, the structure of human $\sigma_1$ receptor bound to (+)-pentazocine (PDB 6DK1) is shown in orange, and the structure of the human $\sigma_1$ receptor bound to PD 144418 (PDB 5HK1) is shown in blue. The ligands (+)-pentazocine and PD 144418 are shown in yellow and cyan, respectively. Wire mesh represents $F_o - F_c$ density, and green mesh corresponds to regions where there is more electron density in the (+)-pentazocine-bound structure than the PD 144418-bound structure, and red mesh is the opposite. a, An alignment of the overall structures of $\sigma_1$ receptor bound to PD 144418 and (+)-pentazocine, with helix $\alpha_4$ highlighted by a red box. b, A close-up view of the helix $\alpha_4$ alignment highlighted in a, shown in a stick representation, with $F_o - F_c$ density contoured at 2.5σ. c, An alignment of the (+)-pentazocine and PD 144418-bound structures in the binding pocket. d, Helix $\alpha_4$ position in simulation for ligand-free (gray), (+)-pentazocine-bound (orange), and haloperidol-bound (blue) conditions. Multiple simulation frames comprising approximately 1 μs of simulation per condition are shown.
reaction can be monitored continuously at room temperature for an extended period (Supplementary Fig. 4b). The measured $K_d$ in SPA experiments (Table 2) was indistinguishable from that measured in membrane-binding experiments, thus suggesting that the receptor–ligand interaction is similar in both lipid membranes and in detergent (Supplementary Fig. 4c). Additionally, the values for these kinetic constants determined by SPA for $[^3H]$(+)-pentazocine association with, and dissociation from, human $\sigma_1$ receptor were highly similar to the $k_{\text{on}}$ (0.021 min$^{-1}$) and $k_{\text{off}}$ (1.46 x 10$^{-3}$ min$^{-1}$) determined by Bowen and colleagues in guinea pig membranes$^{2}$. Unexpectedly, our experiments showed that the association of $[^3H]$(+)-pentazocine with the $\sigma_1$ receptor was not monophasic but was well modeled by a two-step association model, in which a zero-order reaction, represented by the rate constant $k_{\text{on}}$, is followed by a concentration-dependent association step, modeled by the rate constant $k_{\text{int}}$ (Fig. 3a–c and Supplementary Fig. 4d). We also measured ligand dissociation in SPA format. In contrast to the association reaction, the dissociation data fit well to a simple monophasic dissociation curve (Fig. 3d and Supplementary Fig. 4e).

Interestingly, both the apparent $k_{\text{int}}$ and the $k_{\text{a}}$ parameters for $[^3H]$(+)-pentazocine dissociation and association varied non-linearly with concentration, a result indicative of cooperativity in ligand binding (Supplementary Fig. 4f and Table 2). Additionally, the dissociation curves were clearly nonlinear when plotted in a semilog format, another indication of cooperative binding (Fig. 3d). However, the Hill coefficient for ligand binding in equilibrium experiments is indistinguishable from 1 (Supplementary Fig. 4h). Therefore, the binding of (+)-pentazocine to one $\sigma_1$ monomer alters the rate of ligand binding to the next monomer but must equally affect both on and off rates. Additionally, although the association curve for each individual concentration could be fit to a two-step exponential function, a simple two-state model is insufficient to account for the global data. These results suggest that though there are at least two steps to ligand association with the $\sigma_1$ receptor, there are probably additional steps or conformational states that are not accounted for with a simple two-step fit.

Because the rate-limiting step for $[^3H]$(+)-pentazocine association was not dependent on ligand concentration, we suspected that this step might represent a conformational change from a ligand-inaccessible to a ligand-accessible state. To test this hypothesis, we repeated the experiment with $[^3H]$haloperidol. The association of $[^3H]$haloperidol with the $\sigma_1$ receptor was also poorly modeled by a one-step reaction but fit well to a two-step model (Supplementary Fig. 5a,b). Additionally, the rate of the slow step was very similar for both $[^3H]$haloperidol and $[^3H]$(+)-pentazocine (Table 2). Although a $t$ test indicated that the $k_{\text{on}}$ parameter for each ligand was significantly different ($P=0.025$), the 95% confidence intervals overlapped, and the difference in the rate constants was relatively small. Given the similarity of the two $k_{\text{on}}$ estimates, and the ability of oligomerization to cause cooperative effects that could influence the rate of conformational changes in ligand-free protomers, we believe these data are consistent with the conclusion that the slow ligand-association step is a conformational change intrinsic to the receptor that is mostly ligand independent. As seen with $[^3H]$(+)-pentazocine, the dissociation of $[^3H]$haloperidol from the $\sigma_1$ receptor was slow and could be modeled by a monophasic exponential-decay function (Supplementary Fig. 5c,d).

Ligand binding pathway via molecular dynamics simulation. To better characterize the pathway of ligand binding and dissociation, we performed MD simulations of $\sigma_1$ with the goal of characterizing possible conformational rearrangements that could expose the binding pocket. To decrease the computational complexity of the system, we simulated the $\sigma_1$ monomer and used accelerated MD, which applies a boost to dihedral energy minima to speed up the observation of conformational changes$^{11}$. The $\sigma_1$ monomer from the crystal structure was inserted into a hydrated lipid bilayer, with (+)-pentazocine removed from the binding pocket and placed in the water. Using multiple rounds of simulation totaling over 110μs, we were able to assemble a three-step binding pathway: (+)-pentazocine reached a bound state with an r.m.s. deviation <3 Å to the crystallographic pose (Fig. 4a).

This binding pathway requires two major conformational rearrangements for the pocket to become accessible to the ligand. First, the ‘lid’ of the receptor opens, breaking backbone hydrogen bonds between W136 and A161. Next, the $\beta$-barrel structure in the interior of the receptor separates, thereby breaking backbone hydrogen bonds between E123 and R175 and exposing the binding pocket. The ligand enters through this opening near the membrane and assumes a near-crystallographic pose as the protein closes around it (Fig. 4b).

Each of these rare conformational changes or binding events was observed multiple times in simulation. Interestingly, the $\beta$-barrel separation that exposes the binding site was observed only in simulations in which the receptor lid had already opened, thus suggesting that two sequential conformational changes may be necessary before the ligand can bind. The lid opening may be a prerequisite for further conformational change, because it may perturb the internal hydrogen-bond network of $\sigma_1$ so that larger rearrangements may occur. These conformational changes occur spontaneously and also spontaneously revert in some simulations, thus suggesting that the $\sigma_1$ receptor exists in a conformational ensemble of open, ligand-accessible states combined with closed states resembling the crystallographic pose.

Discussion
Antagonism or genetic ablation of the $\sigma_1$ receptor has analgesic effects in animals and humans at the whole-body level$^{14,15,32}$, and potentiates signaling by G-protein–coupled receptors at the cellular level$^{16,17}$. In contrast, $\sigma_1$ receptor agonists are usually defined by their ability to oppose the effects of antagonists and have been associated with cytotoxic effects$^{33–35}$. Currently, the biochemical basis
for agonism or antagonism at the σ₁ receptor is largely unknown, thereby complicating the unambiguous assignment of efficacy class for σ₁ ligands. The best-documented biochemical difference between the two ligand classes is that agonists increase the receptor’s oligomeric state, whereas agonists decrease the oligomeric state of σ₁ receptors. The structural data presented here show that these ligands occupy a different region of the binding pocket (Fig. 2c,d). Antagonists adopt a more linear pose, with the primary hydrophobic region of the molecule pointing toward the space between helices α₄ and α₅, whereas (+)-pentazocine’s primary hydrophobic site points toward helix α₄ (Fig. 2c,d). Presumably, structurally similar agonists such as (+)-SKF-10,047 adopt a similar pose, thus accounting for their shared biological activities. Indeed, computational docking (Supplementary Fig. 3d,e) and modeling suggest that other agonists such as (+)-SKF-10,047 adopt a similar pose, thus explaining the shared biological activities of agonists at the σ₁ receptor in at least two steps, and MD simulation antibody-fragment stabilizers

We also showed by kinetic analysis that (+)-pentazocine associates with the σ₁ receptor in at least two steps, and MD simulation suggested a three-step process requiring two substantial conformational changes to the receptor. Ligand association and dissociation at the σ₁ receptor is very slow, and the rate-limiting step is independent of ligand concentration. Although many groups have analyzed the effects of σ₁ receptor ligands in cells, there is no standard incubation time for observing σ₁-dependent effects of σ₁ ligands. A brief survey of the literature revealed that when σ₁ receptor ligands are used in cellular or biochemical assays, incubation times and temperatures vary from room temperature for 20 min (ref. 37) to 37 °C for up to 72 h (ref. 35). Ligand concentrations are sometimes nearly 10,000-fold greater than the Kᵣ. Our data indicate that 1.5 h or more may be required to reach saturation at 37 °C (Supplementary Fig. 5f), and at room temperature, saturation can take nearly a day (Fig. 3a). Furthermore, because the rate-limiting step is concentration independent, high ligand concentrations cannot overcome the receptor’s slow binding kinetics. However, previous reports have shown that σ₁ receptor–dependent effects can be observed in as little as 10 min with 100 nM (+)-pentazocine at 37 °C, although the effect sizes observed were quite small (ref. 37). This finding is consistent with our data demonstrating that approximately 50% of the

![Fig. 3](https://www.nature.com/nsmb/figures/3/figs/3a.png) | Kinetic analysis of ligand binding to the σ₁ receptor. a. Association of [³H](+)-pentazocine with the σ₁ receptor, measured in SPA format at 23 °C. The six ligand concentrations assayed are 300 nM (red), 100 nM (orange), 30 nM (yellow), 10 nM (green), 3 nM (blue), and 1 nM (violet). The best-fit monophasic curve for each concentration is shown as a dotted black line, and the best-fit biphasic curve for each concentration is shown as solid black lines. Data shown are representative of three independent experiments performed in duplicate; error bars are s.e.m. from the technical duplicates. b,c. Residual plots for the monophasic (b) and biphasic (c) curves and the individual data points shown in a. Colors indicate the concentration of [³H](+)-pentazocine used, and are as in a. d. Dissociation of [³H](+)-pentazocine from the σ₁ receptor in SPA format at 23 °C. Colors indicate initial [³H](+)-pentazocine concentrations, as denoted in a. Solid black lines represent the best-fit monophasic exponential-decay curve. Data shown are representative of two independent experiments performed in duplicate; error bars are s.e.m. from the technical duplicates.

Importantly, crystallographic studies by necessity favor conformationally stable, low-energy states, and so the structures shown here may not represent a fully activated state of the receptor. Indeed, studies of G-protein-coupled receptors bound to agonists often show inactive-state structures in the absence of G proteins or antibody-fragment stabilizers.
Fig. 4 | Molecular dynamics simulation reveals a putative binding pathway for (+)-pentazocine. a, Binding pathway of (+)-pentazocine, with the lid region shown in red and the β-strands that separate shown in purple. From left to right, simulation begins with a ligand-free receptor whose lid region then opens. Next, the interior of the receptor opens, and the ligand enters and binds through this opening. b, A simulation frame after the ligand has entered the binding pocket (tan), compared with a frame initiated from the crystal structure with the ligand bound (blue). Protein backbone is shown for the binding simulation in gray. Helix α4 is located at the top of the rendering.

receptor population should be occupied under these conditions (Supplementary Fig. 5f). Therefore, when ascribing the effects of σ1 receptor ligands to the σ1 receptor, researchers must allow sufficient time for the ligands to engage with the receptor, and the signal-to-noise ratio may be improved by longer incubation times. If effects are observed too quickly and are observed only at ligand concentrations vastly higher than the Kd, then it is unlikely that the effects are σ1 mediated.

We showed that the agonist (+)-pentazocine adopts a binding pose in the σ1 receptor–binding pocket that is different from that of antagonists, which tend to bind similarly to one another despite their chemical diversity. We also demonstrated that ligands associate with the σ1 receptor very slowly and in multiple steps. Our simulations suggest that ligands enter the binding pocket through a dynamic opening that would be challenging to predict on the basis of a single crystal structure.

The precise details of σ1 signaling in cells have yet to be determined. Although myriad binding partners for the σ1 receptor have been proposed, the critical effects of σ1 receptor signaling still remain to be unambiguously established. Future work will need to focus on these functional questions to fully understand the function of the σ1 receptor and its potential as a therapeutic target.

Online content
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References
1. Martin, W. R., Eades, C. G., Thompson, J. A., Huppler, R. E. & Gilbert, P. E. The effects of morphine- and nalorphine-like drugs in the nondependent and morphine-dependent chronic spinal dog. J. Pharmacol. Exp. Ther. 197, 517–532 (1976).
2. Tam, S. W. & Cook, L. Sigma opiates and certain antipsychotic drugs mutually inhibit (+)-[3H]SKF 10,047 and (+)-haloperidol binding in guinea pig brain membranes. Proc. Natl. Acad. Sci. U.S.A. 81, 5618–5621 (1984).
3. Su, T. P. Evidence for sigma opioid receptor: binding of [3H]SKF-10047 to etorphine-inaccessible sites in guinea-pig brain. J. Pharmacol. Exp. Ther. 223, 284–290 (1982).
4. Hellewell, S. B. & Bowen, W. D. A sigma-like binding site in rat pheochromocytoma (PC12) cells decreased affinity for (+)-benzomorphan and lower molecular weight suggest a different sigma receptor form than that of guinea pig brain. Brain Res. 527, 244–253 (1990).
5. Hannan, M. et al. Purification, molecular cloning, and expression of the mammalian sigma1-binding site. Proc. Natl. Acad. Sci. U.S.A. 93, 8072–8077 (1996).
6. Alon, A. et al. Identification of the gene that codes for the σ1 receptor. Proc. Natl. Acad. Sci. U.S.A. 114, 7160–7165 (2017).
7. Walker, I. M. et al. Sigma receptors: biology and function. Pharmacol. Rev. 42, 355–402 (1990).
8. Glennon, R. A. et al. Structural features important for sigma 1 receptor binding. J. Med. Chem. 37, 1214–1219 (1994).
9. Ullah, M. I. et al. In silico analysis of SIGMAR1 variant (rs4879809) segregating in a consanguineous Pakistani family showing amyotrophic lateral sclerosis without frontotemporal lobar dementia. Neurogenetics 16, 299–306 (2015).
10. Wong, A. Y. et al. Aberrant subcellular dynamics of Sigma-1 receptor mutants underlying neuromuscular diseases. Mol. Pharmacol. 90, 238–253 (2016).
11. Greggianni, E. et al. Loss-of-function mutations in the SIGMAR1 gene cause distal hereditary motor neuropathy by impairing ER-mitochondria tethering and Ca2+ signalling. Hum. Mol. Genet. 25, 3741–3753 (2016).
12. Hong, J. et al. Sigma-1 receptor deficiency reduces MPTP-induced parkinsonism and death of dopaminergic neurons. Cell Death Dis. 6, e1832 (2015).
13. Katz, J. L., Hong, W. C., Hirana, T. & Su, T. P. A role for sigma receptors in stimulant self-administration and addiction. Behav. Pharmacol. 27, 100–115 (2016).
14. Castany, S., Gris, G., Vela, J. M., Verdú, E. & Roadas-Vaello, P. Critical role of sigma-1 receptors in central neuropathic pain-related behaviours after mild spinal cord injury in mice. Sci. Rep. 8, 8373 (2018).
15. Bruna, J. et al. Efficacy of a novel sigma-1 receptor antagonist for oxaliplatin-induced neuropathy: a randomized, double-blind, placebo-controlled phase IIa clinical trial. Neurotherapeutics 15, 178–189 (2018).
16. An extension study of ANAVEX2–73 in patients with mild to moderate Alzheimer’s disease (report no. NCT02756858) https://clinicaltrials.gov/ct2/show/NCT02756858 (Anave Life Sciences Corp., 2018).
17. Ufer, R. et al. Phase II trial of the Sigma-1 receptor agonist cutameistine (SA4503) for recovery enhancement after acute ischemic stroke. Stroke 45, 3304–3310 (2014).
18. Kim, F. J. et al. Sigma 1 receptor modulation of G-protein-coupled receptor signaling: potentiation of opioid transduction independent from receptor binding. Mol. Pharmacol. 77, 695–703 (2010).
19. Navarro, G. et al. Direct involvement of sigma-1 receptors in the dopamine D1 receptor-mediated effects of cocaine. Proc. Natl. Acad. Sci. U.S.A. 107, 18676–18681 (2010).
20. Maurice, T. & Su, T. P. The pharmacology of sigma-1 receptors. Pharmacol. Rev. 62, 195–206 (2009).
21. Hayashi, T. & Su, T. P. Sigma-1 receptor chaperones at the ER-mitochondrion interface regulate Ca2+ signaling and cell survival. Cell 131, 596–610 (2007).
22. Aydar, E., Palmer, C. P., Klyachko, V. A. & Jackson, M. B. The sigma receptor as a ligand-regulated auxiliary potassium channel subunit. Neuron 34, 399–410 (2002).
23. Kourrich, S. et al. Dynamic interaction between sigma-1 receptor and Kv1.2 shapes neuronal and behavioral responses to cocaine. Cell 152, 236–247 (2013).
24. Hong, W. C. et al. The sigma-1 receptor modulates dopamine transporter expression and cocaine binding and may thereby potentiate cocaine self-administration in rats. J. Biol. Chem. 292, 11260–11261 (2017).
25. Mishra, A. K. et al. The sigma-1 receptors are present in monomeric and oligomeric forms in living cells in the presence and absence of ligands. Biochem. J. 466, 263–271 (2015).
26. Gromek, K. A. et al. The oligomeric states of the purified sigma-1 receptor are stabilized by ligands. J. Biol. Chem. 289, 20333–20344 (2014).
27. Yano, H. et al. Pharmacological profiling of sigma 1 receptor ligands by novel receptor homomer assays. *Neuropharmacology* **133**, 264–275 (2018).

28. Schmidt, H. R. et al. Crystal structure of the human σ1 receptor. *Nature* **532**, 527–530 (2016).

29. Kovács, K. J. & Larson, A. A. Discrepancies in characterization of sigma sites in the mouse central nervous system. *Eur. J. Pharmacol.* **285**, 127–134 (1995).

30. Bowen, W. D., de Costa, B. R., Hellewell, S. B., Walker, J. M. & Rice, K. C. [3H]+-Pentazocine: a potent and highly selective benzomorphan-based probe for sigma-1 receptors. *Mol. Neuropharmacol.* **3**, 117–126 (1993).

31. Pierce, L. C., Salomon-Ferrer, R., Augusto F de Oliveira, C., McCammon, J. A. & Walker, R. C. Routine access to millisecond time scale events with accelerated molecular dynamics. *J. Chem. Theory. Comput.* **8**, 2997–3002 (2012).

32. Romero, L., Merlos, M. & Vela, J. M. Antinociception by sigma-1 receptor antagonists: central and peripheral effects. *Adv. Pharmacol.* **75**, 179–215 (2016).

33. Mancuso, R. et al. Sigma-1R agonist improves motor function and motoneuron survival in ALS mice. *Neurotherapeutics* **9**, 814–826 (2012).

34. Maher, C. M. et al. Small-molecule sigma1 modulator induces autophagic degradation of PD-L1. *Mol. Cancer Res.* **16**, 243–255 (2018).

35. Li, D. et al. Sigma-1 receptor agonist increases axon outgrowth of hippocampal neurons via voltage-gated calcium ions channels. *CNS. Neurosci. Ther.* **23**, 930–939 (2017).

36. Rosenbaum, D. M. et al. Structure and function of an irreversible agonist-β2 adrenoceptor complex. *Nature* **469**, 236–240 (2011).

37. Goguadze, N., Zhuravliova, E., Morin, D., Mikeladze, D. & Maurice, T. Sigma-1 receptor agonists induce oxidative stress in mitochondria and enhance complex I activity in physiological condition but protect against pathological oxidative stress. *Neurotox. Res.* https://doi.org/10.1007/s12640-017-9838-2 (2017).

38. Zhu, J. et al. Involvement of the delayed rectifier outward potassium channel Kv2.1 in methamphetamine-induced neuronal apoptosis via the p38 mitogen-activated protein kinase signaling pathway. *J. Appl. Toxicol.* **38**, 696–704 (2018).

39. Ornith-Renteria, M. et al. TRPV1 channels and the progesterone receptor Sig-1R interact to regulate pain. *Proc. Natl. Acad. Sci. U.S.A.* **115**, E1657–E1666 (2018).

40. Kim, F. J., Schrock, J. M., Spino, C. M., Marino, J. C. & Pasternak, G. W. Inhibition of tumor cell growth by Sigma1 ligand mediated translational repression. *Biochem. Biophys. Res. Commun.* **426**, 177–182 (2012).

41. Hayashi, T., Maurice, T. & Su, T. P. Ca2+ signaling via sigma(1)-receptors: novel regulatory mechanism affecting intracellular Ca2+ concentration. *J. Pharmacol. Exp. Ther.* **293**, 788–798 (2000).

42. Hayashi, T. & Su, T. P. Regulating ankyrin dynamics: roles of sigma-1 receptors. *Proc. Natl. Acad. Sci. U.S.A.* **98**, 491–496 (2001).

43. Kourrich, S., Su, T. P., Fujimoto, M. & Bonci, A. The sigma-1 receptor: roles in neuronal plasticity and disease. *Trends Neurosci.* **35**, 762–771 (2012).

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**Author contributions**

H.R.S. and A.C.K. designed crystallographic and pharmacological experiments. H.R.S. expressed, purified, and crystallized protein, solved crystal structures, performed radioligand binding assays, and performed Glide docking with supervision from A.C.K. R.M.B. designed, performed, and analyzed MD simulations with supervision from A.C.K. All authors interpreted results and contributed to writing of the manuscript.

**Competing interests**

The authors declare no competing interests.

**Additional information**

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Methods

Protein expression and purification. Human σ1 receptor was expressed and purified in Sf9 cells in a manner similar to that described previously28. In brief, the receptor was cloned into pFastBac1 with an N-terminal hemagglutinin signal sequence followed by a FLAG epitope tag and a 3 C protease-cleavage site. The receptor was expressed in Sf9 cells (Expression Systems) with the FastBac baculovirus system (Thermo Fisher). Cells were grown in a shaker at 27 °C and infected when they reached a density of 4 × 10^6 cells/mL. After infection, the cells were allowed to grow for 48–52 h, at which point they were harvested for centrifugation. Pellets were stored at −80 °C until use.

For samples used in crystallography, 1 μM of haloperidol, (+)-pentazocine, or NE-100 was added to all purification steps. Haloperidol and NE-100 were purchased from Tocris Biosciences, and (+)-pentazocine was kindly provided by F. Kim. For samples used in SPA binding experiments, no ligand was added. Cell pellets were thawed and lysed by osmotic shock in 20 mM HEPS, pH 7.5, 2 mM MgCl₂, and 1:200,000 (vol/vol) benzazone nucleoside (Sigma Aldrich). The lysate was then spun at 48,000 g for 20 min at 4 °C. The supernatant was discarded, and the pellets were solubilized with a glass Dounce tissue homogenizer in a buffer containing 1% (wt/vol) lauryl maltose neopentyl glycol (LMNG, Anatrace), 20 mM HEPS, pH 7.5, 250 mM NaCl, and 20% glycerol. For samples used in the crystallization of σ1 receptor with haloperidol and (+)-pentazocine, the solubilization buffer also contained 0.1% (wt/vol) cholerato hemicinnsulate (CHS; Steroloids). However, samples used in the crystallization of σ1 receptor with NE-100 and in SPA experiments were not solubilized with CHS, because doing so was found to have no effect on protein quality or yield. After homogenization, samples were stirred at 4 °C overnight before centrifugation. The supernatant was filtered over glass microfiber filters, and 2 mM CaCl₂ was added to the solution. The sample was then run over 4 mL of anti-FLAG affinity resin. After the sample was washed on the resin, it was washed once with 50 mL of buffer containing 0.1% LMNG, 20 mM HEPS, pH 7.5, 150 mM NaCl, 2% glycerol, and 2 mM CaCl₂, then again with a buffer containing 0.01% LMNG, 20 mM HEPS, pH 7.5, 150 mM NaCl, 0.2% glycerol, and 2 mM CaCl₂. After these wash steps, the protein was eluted in an identical buffer that lacked CaCl₂, but was supplemented with 0.2 mg/mL FLAG peptide and 5 mM EDTA. Samples used for crystallography were incubated with 3 C protease at 4 °C overnight to remove the FLAG tag. Samples used for SPA experiments were also left at 4 °C overnight but were not digested.

The next day, samples were further purified by SEC on a Sephadex 500 column (GE Healthcare). The buffer for SEC contained 0.01% LMNG, 20 mM HEPS, pH 7.5, and 150 mM NaCl. For samples used for crystallography, the buffer also contained 1 μM of the desired ligand. After SEC, samples intended for crystallization were concentrated to 25–35 mg/mL and quickly frozen in liquid nitrogen in aliquots of 8–9 μL. Samples intended for SPA experiments were concentrated to 300–400 μM and diluted to 200 μM in SEC buffer supplemented with 20% glycerol. The SPA samples were divided into 6 μL aliquots and flash frozen in liquid nitrogen. All samples were stored at −80 °C and were never frozen again after thawing.

Crystallography and data collection. Purified σ1 receptor was reconstituted into lipidic cubic phase, as described previously44,45. The cubic phase was dispersed in 30-nL drops onto a hanging-drop cover and overlaid with 600 nl of precipitant solution with a Gryphon LCP robot (Art Robbins Instruments). The crystal that provided the (haloperidol-bound structure) was grown in 240 μL of 0.25 mg/mL protein and the indicated concentration of [3H](+)-pentazocine. To assay nonspecific binding, equivalent reactions containing 2 μM haloperidol were performed in parallel. Samples were then centrifuged at 37 °C for 90 min. After centrifugation, the supernatant was removed, and 500 μg of haloperidol (Tocris Biosciences) was added to a set of wells in triplicate for a particular time point. This procedure was repeated for a total of eight time points over the course of 24 h. After completion of the time course in either the association or dissociation experiment, the reaction was terminated by massive dilution in ice-cold water and filtration over a glass microfiber filter with a Brandel harvester. Filters were soaked with 0.3% polyethyleneimine for at least 30 min before use. Radioactivity was measured in liquid scintillation counting.

Measurement of ligand dissociation in S9 membranes. Membrane samples prepared as described above were thawed, syringe homogenized, and diluted in 50 mM Tris, pH 8.0, to a final concentration of 0.05 mg/mL in a 96-well plate with a final volume of 100 μL per well. For association experiments, samples were incubated with 1 nM, 10 nM, or 100 nM [3H](+)-pentazocine for 5 min to 6 h, and all points were performed in triplicate. For dissociation experiments, samples were first incubated with 10 nM [3H](+)-pentazocine (Perkin Elmer) for 90 min at 37 °C to reach equilibrium. After equilibration, 1 μL of 500 μM haloperidol (Tocris Biosciences) was added to a set of wells in triplicate with a particular time point. This procedure was repeated for a total of eight time points over the course of 24 h. After completion of the time course in either the association or dissociation experiment, the reaction was terminated by massive dilution in ice-cold water and filtration over a glass microfiber filter with a Brandel harvester. Filters were soaked in 0.3% polyethyleneimine for at least 30 min before use. Radioactivity was quantified by liquid scintillation counting. Data analysis was performed in GraphPad Prism.

Scintillation proximity assay. All SPA experiments were performed with Protein A–coated YSI scintillation proximity beads (PerkinElmer, RPN143). Beads were coupled with M1 anti-FLAG antibody and stored in HBS at 4 °C until use in 5 μg aliquots. Immediately before use, 4–6 μg of beads was spun down twice in a cold centrifuge and resuspended every time in HBS with 0.01% LMNG and 2 mM CaCl₂. The beads were incubated with 50 mM σ1 receptor purified as described above for 30 min at 4 °C. After coupling of the receptor, the beads were again centrifuged and resuspended twice in HBS with 0.01% LMNG and 2 mM CaCl₂. To start the reaction, 40 μL containing 0.2 mg of receptor-linked beads was added to a solution containing the desired concentration of radioligand in a total volume of 360 μL, for a total reaction volume of 400 μL. To assay nonspecific binding, quan equivalent reactions were performed that also contained 5 μM nonradioactive haloperidol ([3H](+)-pentazocine binding) or nonradioactive NE-100 ([3H] haloperidol binding) at a concentration of 5 μM. After association measurements were completed, 5 μM haloperidol ([3H](+)-pentazocine binding) or NE-100 ([3H] haloperidol binding) was added to each vial to begin the dissociation measurements. Samples were measured in duplicate at room temperature with a Beckman Coulter LS 6500 multipurpose scintillation counter.

To average duplicate points, both the signal in c.p.m. and the time at which the two different vials were measured were averaged. The values for each kinetic constant were first determined for each independent experiment with averaged duplicate time points. Thereafter, the constants from each independent experiment were averaged to obtain a final value with associated errors. s.d. and 95% confidence intervals were determined with only the differences in constants between independent experiments, and did not include error estimates from technical replicates, because the duplicate measurements were averaged into single points before calculation of each constant. For all SPA association experiments, the final constants were obtained that also contained 5 μM of haloperidol. Data analysis was performed in GraphPad Prism.

Ramachandran statistics. The SGB Grid Consortium supported all crystallographic data processing, refinement, and analysis software19.
Glide docking. Molecular docking into the σ1 receptor was performed in the manner of previous work56–58 with Glide 5.6 extra precision (XP) Maestro 11. Preparing PDB 6DK1 revealed 18 Å of hydrogen bonds and minimization of energy for the OPLS force field. The grid used for docking was centered on the location of the cocrystallized ligand, and was 20 Å in the x, y, and z dimensions. Poses were ranked according to glide score.

**Statistics.** To compare the k_dissociation values from SPA association measurements of [H](+)pentazocine and [H]haloperidol association, we used a two-tailed t test. This t test had four degrees of freedom and a t value of 3.502, and yielded a P value of P = 0.0249.

To test for potential k_dissociation differences between concentrations of [H](+)pentazocine, we performed a one-way ANOVA test, which had an F value of 6.535 and 17 degrees of freedom. The P value was 0.1078, thus indicating that the k_dissociation values were not significantly different.

**Molecular dynamics simulations.** MD simulation setup. Simulations of the σ1 receptor were based on either the (+)-pentazocine- or the haloperidol-bound crystal structures described in this manuscript. The receptor was simulated in four different conditions: (i) the (+)-pentazocine-bound structure, (ii) the haloperidol-bound structure, (iii) the (+)-pentazocine-bound structure with ligand removed, and (iv) the (+)-pentazocine-bound structure with the ligand removed from its binding pocket and the ligand replaced in solvent. All simulations were of a σ1 monomer.

Coordinates were prepared by initial removal of chains B and C to obtain a monomer, and removal of crystallographic ligands. For conditions iii and iv, the ligand was also removed from the binding pocket, and for condition iv, it was replaced at least 10 Å from the protein. Prime (Schrödinger) was used to model a monomer, and removal of crystallographic ligands. For conditions iii and iv, neutral groups acetyl and methylamide.

Neutral groups acetyl and methylamide were added, and the protein and ligand were further refined by assignment of hydrogen bonds and minimization of energy for the OPLS force field. The grid used for docking was centered on the location of the cocrystallized ligand, and was 20 Å in the x, y, and z dimensions. Poses were ranked according to glide score.

Accelerated molecular dynamics (aMD) was used to boost dihedral potential energies, with parameters E_restraint_x = 10.427 kcal mol_–1, E_restraint_y = 1 Å, and E_restraint_z = 170 kcal mol_–1. For condition iii, the ensemble of simulations was periodically visualized for major conformational change in the protein, and new sets of 5–10 simulation replicates were initialized from restart files corresponding to rare events, with velocities either retained or equilibration performed once more (Supplementary Table 1).

**MD simulation analysis protocols.** Trajectory snapshots were saved every 200 ps during production simulations. Trajectory analysis was performed with VMD and CFPTRAJ, and visualization was performed in VMD.

**Reporting Summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability**

Atomic coordinates and crystallographic structure factors have been deposited in the Protein Data Bank under accession codes PDB 6DJZ (σ1 receptor–haloperidol complex), PDB 6DK0 (σ1 receptor–NE-100 complex), and PDB 6DK1 (σ1 receptor–(+)-pentazocine complex). All other data that support the findings of this study are available from the corresponding author upon reasonable request.

**References**

44. Caffrey, M. & Cherezov, V. Crystalizing membrane proteins using lipidic micelles. Nat. Protoc. 4, 706–731 (2009).
45. Kabsch, W. Xds. Acta Crystallogr. D. Biol. Crystallogr. 66, 125–132 (2010).
46. Cesareni, G. & Murshudov, Y. N. How good are my data and what is the resolution? Acta Crystallogr. D. Biol. Crystallogr. 69, 1204–1214 (2013).
47. Emsley, P. & Cowtan, K. Coot: model-building tools for molecular graphics. Acta Crystallogr. D. Biol. Crystallogr. 64, 2126–2132 (2004).
48. Afonine, P. V. et al. Towards automated crystallographic structure refinement with phenix.refine. Acta Crystallogr. D. Biol. Crystallogr. 68, 352–367 (2012).
49. Chen, V. B. et al. MolProbity: all-atom structure validation for macromolecular crystallography. Acta Crystallogr. D. Biol. Crystallogr. 66, 21–22 (2010).
50. The PyMOL Molecular Graphics System, Version 1.3r1 (Schrödinger, LLC, 2010).
51. Mornin, A. et al. Collaboration gets the most out of software. eLife 2, e01456 (2013).
52. Squazzini, E., Schmidt, H. R., Iyer, K. A., Kruse, A. C. & Dukat, M. Reevaluation of fenpropimorph as a σ receptor ligand: structure-affinity relationship studies at human, receptors. Bioorg. Med. Chem. Lett. 27, 2912–2919 (2017).
53. Willard, B. J., John, C. S. & Bowen, W. D. Sigma-1 and sigma-2 receptors are expressed in a wide variety of human and rodent tumor cell lines. Cancer Res. 55, 408–413 (1995).
54. Chu, U. B. & Ruoho, A. E. Biochemical pharmacology of the sigma-1 receptor. Mol. Pharmacol. 89, 142–153 (2016).
55. Linkens, K., Schmidt, H. R., Sano, J. K., Kruse, A. C. & Martin, S. F. Investigating isooindoline, tetrahydroisouquinoline, and tetrahydrobenzazepine scaffolds for their sigma receptor binding properties. Eur. J. Med. Chem. 151, 557–567 (2018).
56. Friesner, R. A. et al. Extra precision glide: docking and scoring incorporating a model of hydrophobic enclosure for protein-ligand complexes. J. Med. Chem. 49, 6177–6196 (2006).
57. Lomize, M. A., Lomize, A. L., Pogozhova, I. D. & Mosberg, H. I. OPM: orientations of proteins in membranes database. Bioinformatics 22, 623–625 (2006).
58. DOWSER program (Department of Biochemistry and Biophysics, School of Medicine, University of North Carolina, Chapel Hill, 2003).
59. Betz, R. M. Dabble (version v.2.6.3) https://doi.org/10.5281/zenodo.836913 (2017).
60. Huang, I. & MacKerell, A. D. Jr. CHARMM36 all-atom additive protein force field: validation based on comparison to NMR data. J. Comput. Chem. 34, 2135–2145 (2013).
61. Best, R. B., Mittal, J., Feig, M. & MacKerell, A. D. Jr. Inclusion of many-body effects in the additive CHARMM protein CMAP potential results in enhanced cooperativity of α-helix and β-hairpin formation. Biophys. J. 103, 1045–1051 (2012).
62. Best, R. B. et al. Optimization of the additive CHARMM all-atom protein force field targeting improved sampling of the backbone ϕ, ψ, and side-chain χ(1) and χ(2) dihedral angles. J. Chem. Theory. Comput. 8, 3257–3273 (2012).
63. Klaua, J. B. et al. Update of the CHARMM all-atom additive force field for lipids: validation on six lipid types. J. Phys. Chem. B 114, 7830–7843 (2010).
64. Vanommeslaeghe, K. & MacKerell, A. D. Jr. Automation of the CHARMM General Force Field (CGenFF) II: assignment of bonded parameters and partial atomic charges. J. Chem. Inf. Model. 52, 3155–3168 (2012).
65. AMBER (University of California, San Francisco, 2015).
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|-------------|-------------|
| Data collection | Data collection was performed at APS beamlines 23-ID-B and 23-ID-D. All programs typically used at these beamlines were used, including Adxv and JBlulce |
| Data analysis | Data were processed using XDS. Scaling was done with either XSCALE or Aimless. Model building was done with Coot, and refinement was performed in phenix.refine. Following refinement, structures were evaluated with MolProbity, and figures were prepared with PyMOL. |

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Antibodies used M1 anti-FLAG antibody from ATCC hybridoma 4E11
Validation The antibody is a commonly used monoclonal antibody that we purified from a validated hybridoma using peptide affinity chromatography.

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Cell line source(s) Sf9 cells were purchased from Expression Systems (Davis, CA).
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