Molecular chaperones act on non-native proteins in the cell to prevent their aggregation, premature folding or misfolding. Different chaperones often exert distinct effects, such as acceleration or delay of folding, on client proteins via mechanisms that are poorly understood. Here we report the solution structure of SecB, a chaperone that exhibits strong antifolding activity, in complex with alkaline phosphatase and maltose-binding protein captured in their unfolded states. SecB uses long hydrophobic grooves that run around its disk-like shape to recognize and bind to multiple hydrophobic segments across the length of non-native proteins. The multivalent binding mode results in proteins wrapping around SecB. This unique complex architecture alters the kinetics of protein binding to SecB and confers strong antifolding activity on the chaperone. The data show how the different architectures of chaperones result in distinct binding modes with non-native proteins that ultimately define the activity of the chaperone.

Molecular chaperones rescue non-native proteins in the cell from aggregation and assist with their folding or unfolding to maintain a functional proteome. Despite common features, different families of chaperones exhibit distinct activity and biological function. Chaperones may exhibit foldase activity, whereby they accelerate folding of client proteins, or antifolding (holdase) activity, whereby they delay folding of client proteins, and the strength of the activity can vary significantly. Molecular chaperones come in different sizes and a great variety of molecular shapes. However, the scarcity of structural data of chaperones in complex with non-native proteins has impeded an understanding of how different chaperones engage these proteins and how distinct chaperone architectures may alter activity.

SecB is a multitasking molecular chaperone in the cytosol that exhibits an unusually strong antifolding activity. SecB is responsible for maintaining secretory proteins in an unfolded, secretion-competent state, as well as for their targeted delivery to the SecA ATPase. SecB also acts as a generalized chaperone in the cell and a SecB null mutation results in severe protein aggregation. Although extensively studied with biochemical and biophysical techniques, the structural and mechanistic details of how SecB recognizes non-native proteins and how it exerts its antifolding activity are unknown. Recent advances in nuclear magnetic resonance (NMR) and isotope labelling approaches have enabled the characterization of large, dynamic protein complexes including molecular chaperones. We have exploited these approaches to determine the solution structure of SecB in complex with client proteins captured in their unfolded state, revealing a unique binding architecture among protein–protein complexes.

**Recognition sites in SecB and client proteins**

SecB exists as a tetramer organized as a dimer of dimers (dissociation constant, $K_d$, of tetramer–dimer equilibrium is ~20 nM) with an overall rectangular disk-like shape. Each subunit consists of 155 residues (17.5 kDa) composed of a simple $\alpha/\beta$ fold. The $^{1}H$-$^{15}N$- and $^{1}H$-$^{13}C$-correlated NMR spectra of the 70 kDa *Escherichia coli* SecB labelled in methyl-bearing (Ala, Ile, Met, Leu, Thr and Val) and aromatic (Phe, Trp and Tyr) residues are of high quality and near-complete assignment has been obtained (Methods and Extended Data Fig. 1a, b). We used maltose-binding protein (MBP) (396 amino acids) and alkaline phosphatase (PhoA) (471 amino acids) as SecB protein substrates. NMR analysis (Extended Data Figs 1c, d and 2a) showed that there are five distinct SecB-recognition sites in PhoA (labelled a–e; Fig. 1b) and seven sites in MBP (labelled a–g; Fig. 1c), with all sites being enriched in hydrophobic and aromatic residues, as shown before.

To determine the client-binding sites in SecB we sought to identify the SecB residues that show intermolecular nuclear Overhauser effects (NOEs) to short fragments of PhoA and MBP encompassing SecB-recognition sites. The SecB residues that interact with the substrates (Fig. 1d, e) collectively form long, continuous hydrophobic grooves that constitute the primary binding sites for non-native proteins (Fig. 1f). Most prominent is a shallow groove running along the surface, formed by helices $\alpha$1 and $\alpha$2, the helix-connecting loop, the crossover loop and strand 32 (Fig. 1a, d–f). This groove, referred to as the primary client-binding site (Fig. 1f), is ~60 Å long and exposes ~1,300 Å$^2$ of hydrophobic surface, per SecB subunit. In addition, a smaller surface (~600 Å$^2$) formed by residues emanating from helix $\alpha$1 and strands 31 and 34 also interacts with the unfolded proteins (Fig. 1e). This small surface, the secondary client-binding site (Fig. 1f), features several bulky non-polar amino acids. All four subunits combined, SecB exposes ~7,600 Å$^2$ of hydrophobic surface that NMR has shown to interact with non-native proteins (Fig. 1f).

**SecB holds proteins in the unfolded state**

We used NMR spectroscopy to monitor at the residue level the effect of SecB on the folding of PhoA and MBP. Urea-treated PhoA and MBP refold quickly to their native state upon removal of urea (Extended Data Fig. 2b, c). Notably, SecB prevents the folding of PhoA or MBP, with both proteins adopting an unfolded conformation when bound to SecB (Extended Data Fig. 2b, c). The NMR data indicate that SecB-bound PhoA and MBP lack a tertiary structure and the regions of the protein substrates in contact with SecB do not form any secondary structure.

**Client proteins wrap around SecB**

To understand how SecB retains bound proteins in the unfolded state, we sought to structurally characterize the complexes of SecB with PhoA and MBP under native conditions. Multi-angle light scattering (MALS), isothermal titration calorimetry (ITC) and NMR all demonstrate that...
SecB forms stoichiometric complexes with PhoA and MBP (Extended Data Fig. 3), as is the case with other large client proteins including OmpA \(^{17,27}\). The structure of the SecB–PhoA complex (~120 kDa) was determined by NMR as detailed in Methods (Extended Data Figs 4 and 5 and Extended Data Table 1) and is shown in Fig. 2. The most remarkable feature is that PhoA wraps around SecB in an overall arrangement that maximizes the interacting surface between the client protein, which is held in an unfolded conformation, and the chaperone. All of the grooves, the primary client-binding sites in SecB, in the four subunits are occupied by specific PhoA sites (a, b, c and e) while the short PhoA site b binds to the smaller, secondary binding site (Fig. 2). The simultaneous engagement of all PhoA sites by SecB results in a significant enhancement in the affinity of the unfolded protein for SecB (Extended Data Fig. 3b, c), although the binding synergy is not strong. This is probably because the linkers tethering the SecB-recognition sites in PhoA are long and flexible (Fig. 1b and Extended Data Fig. 4a), thereby reducing the effective concentration of the sites and the measured avidity\(^{28}\).

Analysis of the SecB–PhoA structure revealed how SecB recognizes PhoA and how it accommodates all five PhoA sites (Fig. 1b) within one SecB molecule (Fig. 3). Most of the PhoA site a residues (Thr5–Ala21) are engaged in non-polar contacts with the SecB residues in the groove, burying a total of ~2,250 Å\(^2\) of surface (~1,900 Å\(^2\) non-polar and ~350 Å\(^2\) polar). Interestingly, helix \(\alpha2\) in SecB, which acts as a lid of the binding groove, swings outwards by ~50° upon PhoA binding (Fig. 4a). Together with an outward displacement of the first two turns of the helix \(\alpha1\), the width of the hydrophobic groove increases significantly to accommodate the large non-polar side chains of the client (Figs 3 and 4a). Moreover, the rearrangement of several side chains lining the SecB groove allows some of the bulky PhoA residues (for example, Leu8, Leu11 and Phe15) to bury their side chains into the groove. Although most of the contacts are hydrophobic, several of the polar groups in PhoA site a are poised to form hydrogen bonds with polar SecB residues lining the groove (Fig. 3). PhoA site a binds to SecB in an extended conformation, which maximizes the interacting surface. Of note, this region of PhoA forms an \(\alpha\)-helix when bound to a hydrophobic groove in the SecA ATPase\(^{29}\). Thus, SecB disfavours the formation of any regular secondary structure of the bound client.

PhoA site c is the longest SecB-recognition site in PhoA consisting of ~50 residues (Fig. 1b). It binds to SecB in an extended conformation spanning a distance of ~100 Å (Figs 2 and 3). The first 33 residues (Phe93–Ala125) of PhoA site c bind exclusively within the groove of one subunit, whereas the remaining PhoA\(^{\prime}\) (the superscript denotes the corresponding site) residues (Ala126–Try138) extend across the surface at the tetramerization interface. The total surface buried by the binding of PhoA site c to SecB is ~5,150 Å\(^2\) (~3,500 Å\(^2\) non-polar and ~1,600 Å\(^2\) polar). PhoA site d encompasses a stretch of 30 residues (Ala271–Thr309) and binds to SecB in an extended conformation, running along the entire groove and spanning a distance of ~70 Å (Figs 2 and 3). The buried surface amounts to a total of ~4,200 Å\(^2\), with ~2,800 Å\(^2\) non-polar and ~1,400 Å\(^2\) polar. PhoA site e (residues Asn450–Lys471) binds to SecB in a very similar manner to PhoA site a. PhoA site e is one of the regions that retains significant \(\alpha\)-helical structure in the unfolded PhoA\(^{20}\). It binds to SecB, however, in an extended conformation, further highlighting the tendency of SecB to disrupt any regular secondary structure.

SecB can adjust the structure of the primary binding grooves to allow longer substrates to fit in the groove. For example, whereas ~25 residues of the PhoA site d fit in the groove in an extended conformation, more than 40 residues of the PhoA site c fit within the same space (Fig. 4b). When the SecB helix \(\alpha2\) swings outwards upon client binding (Fig. 4a), the movement not only widens the binding groove but also exposes additional non-polar and polar surfaces that are available for binding by the unfolded client.

It should be noted that structure determination of isolated PhoA sites (PhoA\(^{\prime}\), PhoA\(^{\prime}\), PhoA\(^{\prime}\) and PhoA\(^{\prime}\); Fig. 1b) in complex with SecB shows...
that multiple molecules of the individual sites can be accommodated within a SecB tetramer, owing to their relatively short length (Extended Data Figs 3c and 5).

NMR structure determination of MBP sites d and e in complex with SecB (SecB−MBP<sup>d</sup> and SecB−MBP<sup>e</sup> complexes; Extended Data Fig. 6 and Extended Data Table 1) showed that MBP binds to SecB in a very similar fashion to PhoA. Thus, non-native proteins share a similar binding mode for SecB. Analysis of the NMR spectra of labelled full-length MBP in complex with SecB demonstrated that all seven binding sites in MBP (Fig. 1c) are engaged by SecB in the SecB−MBP complex (Extended Data Fig. 7a). NMR-driven modelling of the SecB−MBP complex (Methods) shows that MBP, similarly to PhoA, wraps around SecB using the chaperone’s entire binding surface (Extended Data Fig. 7b). Interestingly, the gain in avidity for MBP binding to SecB (K<sub>d</sub>≈0.05 M), compared with the isolated sites, appears to be an order of magnitude stronger than in the case of PhoA (Extended Data Fig. 3c, f). The reasons for the higher avidity are probably the larger interacting interface in the complex with MBP (∼130 PhoA residues compared with ∼240 MBP residues interacting with SecB) and the fact that the SecB-recognition sites in MBP are tethered with linkers that are much shorter in length than in the case of PhoA (Fig. 1b, c). Thermodynamic analysis reveals a large and favourable enthalpy of binding for both SecB−MBP and SecB−PhoA complexes, but with the overall affinity being reduced by unfavourable entropy of binding (Extended Data Fig. 3b, e).

Amino-acid substitutions at the client-binding sites in SecB resulted in a substantial decrease in the affinity for unfolded proteins and a marked decrease of its antifolding activity (Extended Data Fig. 8a−c).

**Figure 2 | Structure of the SecB−PhoA complex.** Lowest-energy structure of the SecB−PhoA complex. SecB is shown as a space-filling model in grey. The five PhoA sites recognized by SecB are shown as space-filling models and coloured per the colour code in the graphic of the PhoA sequence at the top. The flexible regions of PhoA are shown as a pink ribbon. Four views of the complex are shown related by a rotation as indicated by the arrow. One PhoA molecule binds, which wraps around a pink ribbon. Four views of the complex are shown related by a rotation of 90°.

**Figure 3 | Recognition of non-native PhoA by SecB.** Expanded views of the SecB−PhoA complex highlighting the binding details and contacts that mediate recognition of the four PhoA sites (a, c, d and e) by SecB. The colour code of the PhoA sites, shown as ball-and-stick, is as in Fig. 2. SecB in the expanded views is shown as white ribbon and residues contacting PhoA are displayed as blue ball-and-stick.

**Figure 4 | SecB structure adapts to client binding.** a, Superposition of the SecB structures (only subunit A is shown) in the unliganded state (blue) and bound to PhoA (pink). PhoA is not shown for clarity. The SecB helix α<sub>2</sub> swings outward by ∼50° upon PhoA binding. See also Extended Data Fig. 4e. b, Superposition of the structure of SecB subunits in complex with PhoA site c coloured in orange and with PhoA site d coloured in magenta. SecB is shown as a solvent-exposed surface in white.

**Chaperone−client binding mode modulates kinetics**

SecB may prevent the folding of a protein altogether, whereas other chaperones such as trigger factor (TF) cannot typically do so (Fig. 5a). We used surface plasmon resonance (SPR) and bio-layer interferometry (BLI) to measure the kinetics of interaction between unfolded MBP and PhoA with the SecB and TF chaperones (Fig. 5b and Extended Data Figs 3c and 5).

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SecB rescues aggregation-prone folded proteins

To understand how SecB rescues cytosolic proteins and increases the yield of natively folded proteins (Extended Data Fig. 9b), we used the aggregation-prone MBP variant that has a high tendency to aggregate, especially at temperatures higher than 30 °C. Notably, in the presence of SecB, NMR shows that MBP remains folded and soluble even at temperatures as high as 50 °C (Extended Data Fig. 8d, e). At such high temperatures NMR showed that SecB binds to and shields the transiently exposed unfolded state of MBP (0.003 s⁻¹) compared with the k₀ of the pre-form of MBP (0.003 s⁻¹) (Extended Data Fig. 9g, h). Interestingly, an MBP variant with much slower folding rate (k₀ ≈ 0.0008 s⁻¹) allows even TF to delay its folding (Extended Data Fig. 9j), highlighting the importance of the kinetics of client intrinsic folding and binding to the chaperone.

Conclusions

The present data demonstrate how the distinctive binding mode of SecB for non-native proteins (Fig. 2 and Extended Data Fig. 7b) enables the chaperone to prevent folding of bound proteins (Fig. 5a). Compared with TF (Fig. 5c, d), a chaperone for which its structure in complex with full length PhoA is known, the structural data explain how the overall architecture of the chaperone and the way it engages non-native proteins give rise to different chaperone activities (Fig. 5a). Although both SecB and TF prevent aggregation and misfolding, as most molecular chaperones do, SecB has a much stronger antifolding activity than TF. Each TF molecule can accommodate a stretch of up to ~50 interacting residues of an unfolded polypeptide, whereas SecB can accommodate as many as ~250 interacting residues (Fig. 5c, d and Extended Data Fig. 7b). Because SecB recognizes and binds to multiple regions within an unfolded protein, long client proteins wrap around SecB to maximize the binding interface, thereby altering the binding kinetics. The overall binding architecture appears to be unique among known protein–protein complexes. More structural data on complexes of chaperones with proteins are needed to discover the full repertoire of binding architectures and how they influence chaperone activity.

Online Content

Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Author Contributions C.H. and C.G.K. designed the project. C.H. performed protein purification, NMR data collection and analysis, structure determination, and kinetic and thermodynamic assays. P.R. assisted with structure determination. T.S. assisted with NMR analysis and kinetic assays. C.H. and C.G.K. wrote the manuscript.

Author Information Coordinate files for the SecB–PhoA and SecB–MBP complexes have been deposited in the Protein Data Bank under accession numbers 5JTL, 5JTM, 5JTO, 5JTP, 5JTQ and 5JTTR. Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to C.G.K. (ckalodim@umn.edu).

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METHODS

No statistical methods were used to predetermine sample size. The experiments were not randomized. The investigators were not blinded to allocation during experiments and outcome assessment.

Expression and preparation of proteins. The E. coli SecB gene was cloned into the pET-16b vector (Novagen) containing a His6-tag and a tobacco etch virus (TEV) protease cleavage site at the N terminus. Protein samples of E. coli PhoA were produced as described before. All E. coli MBP constructs were cloned into the pET-16b vector containing a His6-tag and a TEV protease cleavage site at the N terminus. The following MBP constructs were prepared in this study (residue numbers of the boundaries are in superscript): MBP1–396, mature MBP27–396, MBP29–99, MBP56–99, MBP57–161, MBP161–201, MBP260–336, MBP331–396, and the MBP variants MBP1G2D/13E3D, MBP2Y2D3D and MBP3G2W2C3D (MBP mutants are numbered on the basis of the amino-acid sequence of the mature form of MBP). All constructs were transformed into BL21(DE3) cells.

Isotopically unlabelled protein samples were produced in cells grown in Luria-Bertani (LB) medium at 37 °C in the presence of ampicillin (100 μg ml−1) to an absorbance at 600 nm (A600nm) of 0.8. Protein induction was induced by the addition of 0.2 mM isopropyl-β-d-1-thiogalactopyranoside (IPTG) and cells were allowed to grow for 16 h at 18 °C. Cells were harvested at A600nm of 1.5 and resuspended in lysis buffer (50 mM Tris-HCl, 500 mM NaCl, pH 8 and 1 mM PMSF). Cells were disrupted by a high-pressure homogenizer and centrifuged at 50,000g. Protein samples were purified using Ni Sepharose 6 Fast Flow resin (GE Healthcare), followed by tag removal by TEV protease at 4 °C (incubation for 16 h) and gel filtration using Superdex 75 16/60 or 200 16/60 columns (GE Healthcare). Protein concentration was determined spectrophotometrically at 280 nm using the corresponding extinction coefficient.

MALs experiments. MALs was measured using DAWN HELEOS-II (Wyatt Technology Corporation) downstream of a Shimadzu liquid chromatography system connected to a Superdex 200 10/300 GL (GE Healthcare) gel filtration column. The running buffer for SecB–PhoA complexes was 20 mM KPi (pH 7.0), 100 mM KCl, 4 mM SO42−, and 0.5 mM EDTA, whereas for SecB–MBP complexes was 20 mM HEPES, pH 7, 150 mM KOAc and 0.05% NaN3. Protein samples at a concentration of 0.05–0.2 mM were used. The flow rate was set to 0.5 ml min−1 with an injection volume of 200 μl and the light scattering signal was collected at room temperature (≈23 °C). The data were analysed with ASTRA version 6.0.5 (Wyatt Technology Corporation).

ITC experiments. ITC was performed using an ITCC200 microcalorimeter (GE Healthcare) at temperatures ranging from 4 °C to 25 °C. All protein samples were extensively dialysed against the ITC buffer containing 50 mM KPi (pH 7.0), 50 mM KCl, 0.05% NaN3 and 2 mM tris(2-carboxyethyl)phosphine (TCEP). All solutions were filtered using membrane filters (pore size, 0.45 μm) and thoroughly degassed for 20 min before the titrations. The 40-μl injection syringe was filled with ~0.05–1 mM of SecB solution and the 200-μl cell was filled with ~0.01–0.2 mM PhoA or MBP. To measure the binding affinity of MBP to SecB, the slowly folding MBP1G2D/13E3D variant was used to measure the affinity of MBP for SecB. MBP1G2D/13E3D was unfolded in 8 M urea, 20 mM HEPES, pH 7, 150 mM KOAc and 0.05% NaN3, and diluted 20 times to give a final concentration of 0.2 mM immediately before loading into the cell. The solution containing SecB was precisely adjusted to match the urea concentration. The titrations were performed with a preliminary 0.2-μl injection, followed typically by 15 injections of 2.5 μl each with time intervals of 3 min. The solution was stirred at 1,000 r.p.m. Data for the preliminary injection, which are affected by diffusion of the solution from and into the injection syringe during the initial equilibration period, were discarded. Binding isotherms were generated by plotting heats of reaction against the number of equivalents added.

Protein isotope labelling for NMR studies. Isotopically labelled samples for NMR studies were prepared by growing the cells in minimal (M9) medium. Cells were typically harvested at A600nm of 1.0. U-[13C]N-methyl labelled samples were prepared for the backbone assignment of SecB and large MBP fragments by supplementing the growing medium with 13C4-DCl (1 g l−1) and 13C2-H2-glucose (2 g l−1) in 99.9% 2H2O (CIL and Isotec). The 13C−H−C methyl-labelled samples were prepared as described previously. α-Ketobutyric acid (50 mg l−1) and α-ketoisovaleric acid (85 mg l−1) were added to the culture 1 h before the addition of IPTG. Met-[13C15N] and Ala-[13C15N] labelled samples were produced by supplementing the medium with [13C3]Met (50 mg l−1) and [15N4]Ala (50 mg l−1). For Thr labelling, a Thr-αxitroyclic cell strain was used, and the medium was supplemented with [2H3,13C3]Thr (25 mg l−1). For Phe, Tyr, and Trp labelling, U-[13C]N-methyl labelled amino acids were used. Alternative 13C-labelling of aromatic residues was performed as described.22 All precursors and amino acids were added to the culture 1 h before the addition of IPTG, except Ala, which was added 30 min before induction.

NMR spectroscopy. NMR samples were typically prepared in 50 mM KPi (pH 7.0), 50 mM KCl, 0.05% NaN3, 5 mM l-ME and 7% D2O. NMR experiments were recorded on Bruker 900, 850 and 700 MHz spectrometers. NMR spectra were typically recorded at 10 °C for the isolated PhoA and MBP fragments and at 35 °C for SecB and its complexes. Protein sample concentration ranged from 0.1 to 1.0 mM. All NMR spectra were processed using NMRPipe33 and analysed using NMRView (http://www.orneloscientific.com).

NMR assignment of PhoA and MBP in the unfolded state. We previously described the assignment strategy for unfolded PhoA.22 We followed a similar strategy to assign MBP in the unfolded state by making use of several MBP fragments that remain soluble and unfolded when isolated (Extended Data Table 1c). MBP187–99, MBP201–260, MBP260–336 and MBP331–396. Isolated MBP fragments encompassing the first 26 N-terminal residues (signal sequence) were not stable and this region could only be assigned in complex with SecB. Overlay of the spectra of the MBP fragments with the spectra of full-length MBP in 4 M urea indicated very good resonance correspondence. This is expected because all of the fragments, as well as the MBP, in 4 M urea are unfolded. Resonance assignment obtained for the various fragments was transferred to full-length MBP in urea, and ambiguities were resolved by the use of 3D NMR spectra. It should be noted that although resonance dispersion in unliganded PhoA and MBP is poor, complex formation with SecB alleviates this problem (for the PhoA and MBP residues in the SecB-binding regions) with the spectra being of high resolution (Extended Data Fig. 4c).

Structure determination of SecB–PhoA and SecB–MBP complexes. Assignment of the resonances in SecB–PhoA was accomplished by first assigning the complexes between SecB and the individual PhoA sites (SecB–PhoAa, SecB–PhoAb, SecB–PhoAc, SecB–PhoAd, SecB–PhoAe). We used U-[13C]N-labelled samples that contained specifically protonated methyl groups of Ala, Val, Leu, Met, Thr and Ile (41) and protonated aromatic residues Phe, Tyr and Trp in an otherwise deuterated background. The high sensitivity and resolution of the methyl region, combined with the high abundance of these nine amino acids in SecB (Extended Data Fig. 1a) and in the SecB-binding sites of PhoA and MBP, provided a large number of intermolecular NOEs for SecB–PhoA and SecB–MBP complexes (Extended Data Table 1). Because PhoA in complex with SecB provided higher quality spectra than the spectra of MBP in complex with SecB, we determined first the structure of the SecB–PhoA complex (~120 kDa) by NMR. We initially characterized the structure of the each PhoA site (a–e) individually in complex with SecB (Extended Data Fig. 5). The structures of SecB–PhoAa, SecB–PhoAb, SecB–PhoAc, and SecB–PhoAd, were determined by NMR and are presented in Extended Data Fig. 5. A large number of intermolecular NOEs were collected for each one of the complexes (Extended Data Table 1). Because of the relatively short length of the polypeptides encompassing the individual PhoA sites, multiple PhoA molecules were used to SecB, as shown in Extended Data Fig. 5. We also note that we detected the presence of a small number of intermolecular NOEs that were suggestive of alternative conformational preferences of the PhoA sites bound to SecB. However, the intensity of these sets of NOEs was much weaker, indicating that the population of such alternative complexes is low. To solve the structure of the SecB–PhoA complex, we sought to determine how each one of the PhoA sites binds to SecB in the context of the full length PhoA. To circumvent the signal overlap in this large complex, we used samples where the two proteins were isotopically labelled in different amino acids. For example, in one of these samples SecB was labelled in the methyls of Leu, Val, and Met, whereas PhoA in the methyls of Ile amino acids. Because of the distinct chemical shifts of 1H and 13C resonances of the methyls and the isotope labelling scheme, it was possible to measure specific intermolecular NOEs between SecB and PhoA (Extended Data Fig. 4b). Several of these samples were used to determine as many intermolecular NOEs as possible. As expected, the NOEs were compatible with the structure of each PhoA site in complex with SecB, with the
crucial difference that only one PhoA molecule could be accommodated in SecB. Owing to its short length, the isolated PhoA site b (PhoA-b) binds to almost all of the exposed hydrophobic surface of SecB, as determined by NMR. In the SecB–PhoA complex with SecB, PhoA site b can only bind to the secondary binding site, as determined by NOEs. To further corroborate the structure of the SecB–PhoA complex we used PRE data (see below). The PRE-derived distances were fully compatible with the NOE data collected on SecB–PhoA. The structure of the SecB–PhoA complex was determined using the set of intermolecular NOE connectivities from the complex and further refined using the intermolecular NOE collected for the corresponding isolated PhoA sites in complex with SecB. It should be noted that because of the symmetry in SecB, the various PhoA sites may bind to any of the four SecB subunits. The final arrangement will be dictated by the length of the linkers tethering the SecB-recognition sites (as shown in Fig. 2), namely how far nearby recognition sites can bind from each other, and thus alternative routes of the polypeptide bound to SecB may be present. The only conceivable difference among the various conformations is the relative disposition of the PhoA sites. In all cases all of the SecB-recognition sites in PhoA are engaged by SecB in the complex and PhoA wraps around SecB. The NMR-driven structural model of the SecB–MBP complex (Extended Data Fig. 7b) was determined as follows: NMR analysis demonstrated that all seven recognition sites in MBP (labelled a–g) are bound to SecB in the SecB–MBP complex (Extended Data Fig. 7a). We have determined the high-resolution structure of MBP<sup>e</sup> and MBP<sup>f</sup> in complex with SecB (Extended Data Fig. 6). Because of their length and the short linker tethering the two sites, d and e sites most probably bind to the same side of SecB. MBP site f is the longest one, consisting of ~90 residues, and is thus entirely accommodated on the other side of SecB. With sites d, e and f occupying the primary binding sites, the other recognition sites (a, b, c and g), being much shorter, can be accommodated within the secondary client-binding sites on SecB. The structure of MBP<sup>e</sup>–SecB complex was further confirmed by the experimental intermolecular NOE data. The hydrophobic residues of the sites a, b, c, f, and g, showing the strongest effect upon SecB binding as determined by differential line broadening, were used to drive the docking of these sites to non-polar residues on SecB. The modelled structure shows that the entire MBP sequence can be accommodated within one SecB molecule. The structures of SecB in complex with PhoA and MBP were calculated with CYANA 3.97 (ref. 34), using NOE peak lists from 3D (<sup>1</sup>H–<sup>15</sup>N HSQC–NOESY–<sup>1</sup>H–<sup>13</sup>C HSQC), 3D (1H–1H HSQC–NOESY–<sup>1</sup>H–<sup>13</sup>C HSQC, 3D (1H–13C HSQC–NOESY–<sup>1</sup>H–15N HSQC), 15N-edited NOESY–HSQC, and 13C-edited NOESY–HSQC, and 15N- and 13C-chemical shifts served as input for the TALOS+ program<sup>33</sup> to extract dihedral angles (\( \phi \) and \( \psi \)). The side chains of SecB residues within or nearby the PhoA and MBP binding sites were set flexible and their conformation was determined using intermolecular NOEs collected for each one of the complexes. The SecB regions remote to the binding sites were set rigid using the crystal structure coordinates for <i>E. coli</i> SecB<sup>36</sup>. The 20 lowest-energy structures were refined by restrained molecular dynamics in explicit water with CNS<sup>39</sup>. The percentage of residues falling in favoured and disallowed regions, respectively, of the Ramachandran plot is as follows: 99.4% and 0.6% for SecB–PhoA; 99.4% and 0.4% for SecB–PhoA<sup>e</sup>; 99.3% and 0.7% for SecB–PhoA<sup>f</sup>; 99.2% and 0.8% for SecB–PhoA<sup>b</sup>; 98.3% and 0.6% for SecB–PhoA<sup>c</sup>; 98.4% and 0.6% for SecB–MBP<sup>e</sup>, and 99.4% and 0.6% for SecB–MBP<sup>f</sup>, and 99.4% and 0.6% for SecB–MBP<sup>e</sup>. PRE experiments. PRE experiments were used to confirm the position of each individual PhoA binding site in the SecB–PhoA complex. First, a ‘Cys-free’ variant of PhoA was prepared by mutating the four naturally occurring Cys residues in PhoA to Ser. We then introduced a Cys label was removed by extensive buffer exchange using Centricon Centrifugal Filter with a MWCO of 10,000 (Millipore) at 4°C using a buffer containing 20 mM HEPES (pH 7.4), 150 mM KCl and 0.05% NaN<sub>3</sub>. The association and dissociation time for data collection was set at 90s and 120s, respectively. After urea was removed, MBP remained in the unfolded conformation for sufficient time to interact with SecB. This was confirmed by monitoring the refolding behaviour of MBP using an Infinite 200 PRO microplate reader (Tecan) at the temperature range of the experiments. All SPR experiments were repeated three times and highly reproducible data were obtained. The sensorgrams obtained from the assay channel were subtracted by the buffer control, and data were fitted using the Biacore T200 evaluation software (version 1.0). Bio-layer interferometry (BLI). BLI experiments were performed using an Octet system (forteBIO) at room temperature (~23°C). MBP was biotinylated using the biotination kit EZ-Link NHS-PEG4-Biotin (Thermo Fisher Scientific). Biotin label was removed by extensive buffer exchange using Centricon Centrifugal Filter with a MWCO of 10,000 (Millipore) at 4°C using a buffer containing 20 mM HEPES (pH 7), 150 mM KCl and 0.05% NaN<sub>3</sub>. The association and dissociation time for data collection was set at 90s and 120s, respectively. After urea was removed, MBP remained in the unfolded conformation for sufficient time to interact with SecB. This was confirmed by monitoring the refolding behaviour of MBP using an Infinite 200 PRO microplate reader (Tecan) at the temperature range of the experiments. All SPR experiments were repeated three times and highly reproducible data were obtained. The sensorgrams obtained from the assay channel were subtracted by the buffer control, and data were fitted using the Biacore T200 evaluation software (version 1.0).
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Extended Data Figure 1 | NMR characterization of SecB and unfolded MBP. a, SecB is enriched in hydrophobic amino acids, such as methyl-bearing (Ala, Ile, Leu, Met, Thr and Val) and aromatic (Phe and Tyr). b, $^1$H–$^{13}$N TROSY HSQC (left) and $^1$H–$^{13}$C methyl HMQC (right) spectra of [U-2H; Ala-13CH3; Met-13CH3; Ile-$\delta$1-13CH3; Leu,Val-13CH3/13CH3; Thr-13CH3]-labelled SecB. SecB packing gives rise to two pairs of spectroscopically equivalent subunits: one pair is formed by subunits A and D, and the other pair by subunits B and C. Select assignment is included in the methyl spectrum with the asterisk indicating the other pair. c, $^1$H–$^{15}$N HSQC spectra of select MBP fragments spanning the entire sequence of MBP. d, Secondary structure propensity (SSP) values of unfolded MBP (extracted collectively from the fragments) plotted as a function of the amino-acid sequence. A SSP score at a given residue of 1 or −1 reflects a fully formed α-helical or β-structure (extended), respectively, whereas a score of, for example, 0.5 indicates that 50% of the conformers in the native-state ensemble of the protein are helical at that position. The data show that several of the secondary structure elements in the folded MBP retain some transient secondary structure in the unfolded MBP fragments.
Extended Data Figure 2 | NMR characterization of PhoA and MBP binding to SecB. a, To determine the SecB-recognition sites within PhoA and MBP, $^{15}$N-labelled PhoA and MBP fragments were titrated with unlabelled SecB. Owing to the labelling scheme and the size of SecB, the intensity of the PhoA and MBP residues that are bound by SecB decreases dramatically or disappears. Several titration points were recorded but here only the spectra for the SecB:PhoA and SecB:MBP 1:1 are shown for two select fragments. The $^1$H–$^{15}$N HSQC spectra of PhoA or MBP are shown in the absence (blue) and presence (red) of SecB. b, c, PhoA (b) and MBP (c) refolding in the presence and absence of SecB monitored by $^1$H–$^{15}$N HSQC spectra. Spectra of the ‘refolded’ state were recorded after rapid dilution of urea-treated MBP/PhoA in native buffer. Spectra of the ‘unfolded’ state were recorded in urea. MBP and PhoA refolded in their native structure in the absence of SecB but were retained in the unfolded state in the presence of SecB.
Extended Data Figure 3  |  Energetics of SecB interaction with PhoA and MBP.  

a, MALS of SecB—PhoA complex showing a stoichiometry of 1:1.  
b, ITC of SecB binding to PhoA and the energetics of binding.  
c, $K_d$ values for complexes between select PhoA fragments encompassing the five (a–c) SecB-recognition sites and SecB.  
d, MALS of SecB—MBP complex showing a stoichiometry of 1:1.  
e, ITC of SecB binding to MBP and the energetics of binding.  
f, $K_d$ values for complexes between select MBP fragments encompassing the seven (a–g) SecB-recognition sites and SecB.  
More than one of the smaller PhoA or MBP fragments (for example, PhoA$^a–c$, PhoA$^d–e$, MBP$^c–d$) can be accommodated within SecB.  
Of note is the large favourable enthalpy of binding for the interaction of MBP and PhoA with SecB reflecting the large interacting surface.  
However, a large but unfavourable entropy diminishes the overall binding.
Extended Data Figure 4 | See next page for caption.
Extended Data Figure 4 | NMR characterization of the SecB-PhoA complex. 

**a.** ^1^H–^1^5^N^ TROSY HSQC spectra of PhoA in the unfolded state (light blue) and in complex with SecB (grey). The unfolded state was induced by the addition of reducing agent^2^0^0^ or urea and assigned and characterized by NMR as shown before^2^0. Select resonance assignment of SecB-recognition sites in PhoA is included (the colour is per the colour code for each SecB-recognition site within PhoA; see Fig. 1b). There is an excellent correspondence between the PhoA residues identified to bind to SecB using the various PhoA fragments (Extended Data Fig. 2a) and the residues of full-length PhoA that are bound to SecB in the SecB−PhoA complex. All five SecB-recognition sites in PhoA (a–e) are engaged by SecB in the SecB−PhoA complex. The PhoA regions that are not bound to SecB (they retain their intensity in the complex) are all in an unfolded conformation as suggested by their essentially identical chemical shifts to the unfolded PhoA.

**b.** Select strips from 13^C^-edited NOESY experiments highlighting intermolecular NOEs in the SecB−PhoA complex. Owing to severe resonance overlap in the 120 kDa SecB−PhoA complex, to identify specific intermolecular NOEs we prepared samples wherein the two protein partners are labelled in different methyl-bearing type of amino acids. In this example, SecB was labelled in Leu, Met and Val residues and PhoA in Ile residues. Thus, all NOEs detected between Leu/Val/Met and Ile methyls are intermolecular. c, ^1^H–^1^3^C^ methyl HMQC spectra of SecB in complex with PhoA fragments carrying the individual PhoA sites: PhoA^a^ (green), PhoA^c^ (orange), PhoA^d^ (magenta) and PhoA^e^ (red). Both SecB and PhoA fragments are [U−2^H^; Ala−1^3^CH3; Met−1^3^CH3; Ile−δ1−1^3^CH3; Leu,Val−1^3^CH3/1^3^CH3; Thr−1^3^CH3]−labelled.

**d.** Representative strips from 13^C^-edited NOESY−HSQC and HMQC−NOESY−HMQC NMR experiments. The NOE cross-peaks between SecB and residues of PhoA fragments are designated by a dashed-line red circle.

**e.** Characteristic NOEs showing that the primary binding groove in SecA is enlarged by the displacement of helix α2 as shown in Fig. 4a. For example, the NOE between SecB residues Ala95 and Phe137 is consistent with the closed conformation observed in apo SecB. This NOE is not present in the SecB−PhoA complex because the two SecB residues have moved apart as a result of the displacement of the helix α2.

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Extended Data Figure 5 | Strategy for the structure determination of the SecB – PhoA complex. The three main steps are briefly described here. More details can be found in Methods. The lowest-energy NMR structures of the SecB complexes with the individual PhoA sites a, c, d and e are shown. The structural and NMR statistics for each structure are shown in Extended Data Table 1 and Methods.
Extended Data Figure 6 | Structures of SecB with MBP sites. **a,** Lowest-energy structure of SecB in complex with a MBP fragment encompassing site d (MBP, residues 105–152). **b,** Lowest-energy structure of SecB in complex with a MBP fragment encompassing site e (MBP, residues 165–210). SecB is shown as grey solvent-accessible surface (left) or as white cartoon (right). Expanded views (right) of the contacts between SecB and MBP. The SecB residues mediating contacts with MBP are shown as blue ball-and-stick. In both complexes an additional MBP molecule binds symmetrically to the opposite face of SecB but are not shown for clarity.
Extended Data Figure 7 | See next page for caption.
Extended Data Figure 7 | NMR-driven model structure of SecB—MBP complex. a, ¹H–¹⁵N TROSY HSQC spectra of MBP fragments (grey), MBP fragments in complex with SecB (blue) and full-length MBP in complex with SecB (magenta). The Gly (left) and Trp Nε (right) regions are shown as examples because of the excellent dispersion and lack of severe resonance overlap. The various MBP fragments covering the entire MBP sequence (Extended Data Fig. 1c) are coloured grey and if they are located within a SecB-recognition site it is denoted in the superscript. The MBP residues that do not interact with SecB retain their intensity. These are residues located in regions that are not SecB-recognition sites (Fig. 1c). When these spectra are compared with the spectra of full-length MBP in complex with SecB (in magenta) a very good resonance correspondence is observed. Thus, two important observations can be made: first, all seven SecB-recognition sites (a–g) in MBP are engaged by SecB in the SecB–MBP complex; and, second, the MBP regions that do not interact with SecB in the SecB–MBP complex remain in an unfolded state. The Trp spectra (right) provide direct evidence in support of these observations: all Trp residues, with the exception of Trp155, are located in SecB-recognition sites and they all interact with SecB in the SecB–MBP complex. In contrast, Trp155 does not bind to SecB when the corresponding MBP fragment was used, and this was also the case for MBP. b, Modelled structure of the SecB–MBP complex. SecB is shown as a solvent-exposed surface and MBP as a pink ribbon. The seven MBP sites recognized by SecB are shown as side-chain surface and coloured per the colour code in the graphic of the MBP sequence at the top. The structure of the complex was modelled as detailed in Methods. Briefly, as mentioned above, NMR analysis demonstrated that all seven recognition sites in MBP (labelled a–g) are bound to SecB in the SecB–MBP complex. We have determined the high-resolution structure of MBP and MBP in complex with SecB (Extended Data Fig. 6). Because of their length and the short linker tethering the two sites, d and e, most probably bind to the same side of SecB. MBP site f is the longest one, consisting of ~90 residues, and is thus entirely accommodated on the other side of SecB. With sites d, e and f occupying the primary binding sites, the other recognition sites (a, b, c and g), being much shorter, can be accommodated within the secondary client-binding sites on SecB. The structure of MBP sites d and e in complex with SecB was determined using the experimental intermolecular NOE data. The hydrophobic residues of the sites a, b, c, f and g showing the strongest effect upon SecB binding, as determined by differential line broadening, were used to drive the docking of these sites to non-polar residues on SecB. The modelled structure shows that the entire MBP sequence can be accommodated within one SecB molecule.
Extended Data Figure 8 | Anti-aggregation activity of SecB. a, A triple amino-acid substitution in the SecB (V40A/L42A/L44A) client-binding site was prepared and is referred to as the triple mutant SecB (SecB\textsuperscript{TM}). ITC profile of the binding of PhoA to SecB\textsuperscript{TM} to be compared with PhoA binding to wild-type SecB (Extended Data Fig. 3b). The triple substitution causes a 40-fold reduction in the affinity of SecB for PhoA. b, Fluorescence-monitored MBP folding in the absence of SecB (blue), in the presence of wild-type SecB (green) and in the presence of SecB\textsuperscript{TM} (red). The triple mutant diminishes significantly the antifolding activity of SecB. c, $^1$H–$^{15}$N TROSY HSQC spectra of MBP refolded in the absence (blue) and presence of SecB\textsuperscript{TM} (red). In contrast to wild-type SecB (Extended Data Fig. 2c), SecB\textsuperscript{TM} cannot hold MBP in the unfolded state. d, $^1$H–$^{13}$C methyl HMQC spectra of MBP\textsuperscript{mut} (blue) and in the presence of SecB (red) recorded at 22 °C. The MBP mutant (MBP\textsuperscript{mut}) carries two amino-acid substitutions (G32D/I33P) that renders the protein prone to aggregation\textsuperscript{41}, especially at temperatures above 30 °C. No NMR signal of MBP\textsuperscript{mut} can be detected at temperatures above 30 °C and the protein precipitates in the NMR tube. At 22 °C, MBP\textsuperscript{mut} is folded, as evidenced by the resonance dispersion in the NMR spectra, and does not interact with SecB. e, $^1$H–$^{13}$C methyl HMQC spectrum of MBP\textsuperscript{mut} in the presence of SecB recorded at 50 °C. MBP\textsuperscript{mut} suffers heavy precipitation and aggregation at temperatures higher than 30 °C, but in the presence of SecB it is stable and folded even at temperatures as high as 50 °C. f, $^1$H–$^{15}$N TROSY HSQC spectra of SecB (blue) and in the presence of MBP\textsuperscript{mut} (orange) at 42 °C, indicating binding. Because of the elevated temperature, a significant unfolded population of MBP\textsuperscript{mut} is present, which binds to SecB (see main text). g, Mapping of the sites (orange) used by SecB to interact with MBP\textsuperscript{mut}, on the basis of the chemical shift perturbation data from the spectra in f.
Extended Data Figure 9 | Kinetics of PhoA and MBP interaction with SecB and TF. a–c, SPR analysis of the interaction of SecB with PhoA (a) and MBP at 20 °C (b) and 30 °C (c). Single-cycle and multiple-cycle procedures were used for the SPR analysis of SecB with PhoA and MBP, respectively. d–f, BLI analysis of the binding of MBP to SecB (d), SecB\textsuperscript{TMS} (e) and TF (f). His-tagged PhoA or MBP (for SPR) or biotinylated MBP (for BLI) experiments was immobilized on an NTA chip (SPR) or streptavidin biosensor (BLI) and interactions were examined at different SecB or TF concentrations as indicated. Binding is reported in response units (RU) for SPR and wavelength shift (nanometers) for BLI as a function of time. g, h, Effect of SecB on the kinetics of MBP folding. g, Fluorescence-monitored folding of MBP (pre form) and mature MBP (h) in the absence (blue) and presence of one- (green) and fourfold (purple) excess of SecB. SecB does not appreciably delay folding of mature MBP. In fact, SecB excess appears to increase the yield of soluble, folded mature MBP (purple). i, \textsuperscript{1}H–\textsuperscript{15}N TROSY HSQC spectra of mature MBP refolded in the absence (blue) and presence of SecB (red). SecB cannot retain the mature MBP unfolded. j, Fluorescence-monitored folding of the slowly folding MBP Y283D variant in the absence (blue), and presence of one- (green) and fivefold (orange) TF. As elaborated in the main text, TF does not delay folding of pre-MBP (Fig. 5a). However, it does delay folding of an inherently slowly folding MBP mutant (MBPY283D), thus highlighting the importance of the intrinsic folding of the client protein and its association rate to the chaperone.
Extended Data Table 1 | NMR and refinement statistics for the SecB complexes with PhoA and MBP

|                         | SecB-PhoA | SecB-PhoA<sup>a</sup> | SecB-PhoA<sup>b</sup> | SecB-PhoA<sup>c</sup> | SecB-PhoA<sup>d</sup> | SecB-MBP<sup>d</sup> | SecB-MBP<sup>e</sup> |
|-------------------------|-----------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|
| **NMR distance and dihedral constraints** |           |                       |                       |                       |                       |                       |                       |
| **Distance restraints** |           |                       |                       |                       |                       |                       |                       |
| Total NOE               | 1362      | 1636                  | 2151                  | 1338                  | 1043                  | 1320                  | 1446                  |
| Inter-residue           |           |                       |                       |                       |                       |                       |                       |
| Sequential (|i-j| = 1) | 343       | 402                   | 435                   | 376                   | 151                   | 371                   | 362                   |
| Non-sequential (|i-j| > 1) | 1019      | 1234                  | 1716                  | 962                   | 892                   | 949                   | 1084                  |
| Inter-molecule          | 171       | 33                    | 52                    | 54                    | 27                    | 25                    | 22                    |
| Total dihedral angle restraints | 1169 | 1004                  | 996                   | 1012                  | 1012                  | 1006                  | 976                   |
| phi                     | 583       | 502                   | 498                   | 506                   | 506                   | 503                   | 488                   |
| psi                     | 586       | 502                   | 498                   | 506                   | 506                   | 503                   | 488                   |
| **Structure statistics** |           |                       |                       |                       |                       |                       |                       |
| Violations (mean and s.d.) |           |                       |                       |                       |                       |                       |                       |
| Distance constraints (Å) | 0.012     | 0.015                 | 0.015                 | 0.016                 | 0.018                 | 0.013                 | 0.016                 |
| ±0.047                  | ±0.052    | ±0.052                | ±0.052                | ±0.056                | ±0.056                | ±0.045                | ±0.054                |
| Dihedral angle constraints (°) | 0.42  | 0.26                  | 0.26                  | 0.28                  | 0.23                  | 0.027                 | 0.31                  |
| ±1.4                    | ±0.85     | ±0.85                 | ±0.89                 | ±0.79                 | ±0.85                 | ±0.96                 |
| Max. dihedral angle violation (°) | 26.8 | 9.3                   | 8.7                   | 9.9                   | 9.7                   | 9.5                   | 9.9                   |
| Max. distance constraint violation (Å) | 1.11 | 0.86                  | 0.89                  | 0.80                  | 0.83                  | 0.95                  | 0.95                  |
| Average pairwise r.m.s.d. (Å) |           |                       |                       |                       |                       |                       |                       |
| Heavy                   | 4.4       | 2.1                   | 2.9                   | 2.4                   | 2.5                   | 3.6                   | 2.9                   |
| Backbone                | 4.0       | 1.5                   | 2.1                   | 1.7                   | 1.7                   | 2.8                   | 2.1                   |

Statistics for each structure were computed for the ensembles of 20 deposited structures. Ordered residue ranges (S(ϕ) + S(ψ) > 1.8), 10–141 (of SecB subunits A, B, C and D); backbone (heavy atom) root mean squared deviation (r.m.s.d.) was ~1.0 (1.3) Å within the specified range for all complexes. Additionally, the r.m.s.d. within the PhoA fragments is reported for each structure. Average distance constraint violations were calculated with PDBStat<sup>42</sup>.