Supplementary Material

Binding of Flexible and Constrained Ligands to the Grb2 SH2 Domain: Structural Effects of Ligand-Preorganization

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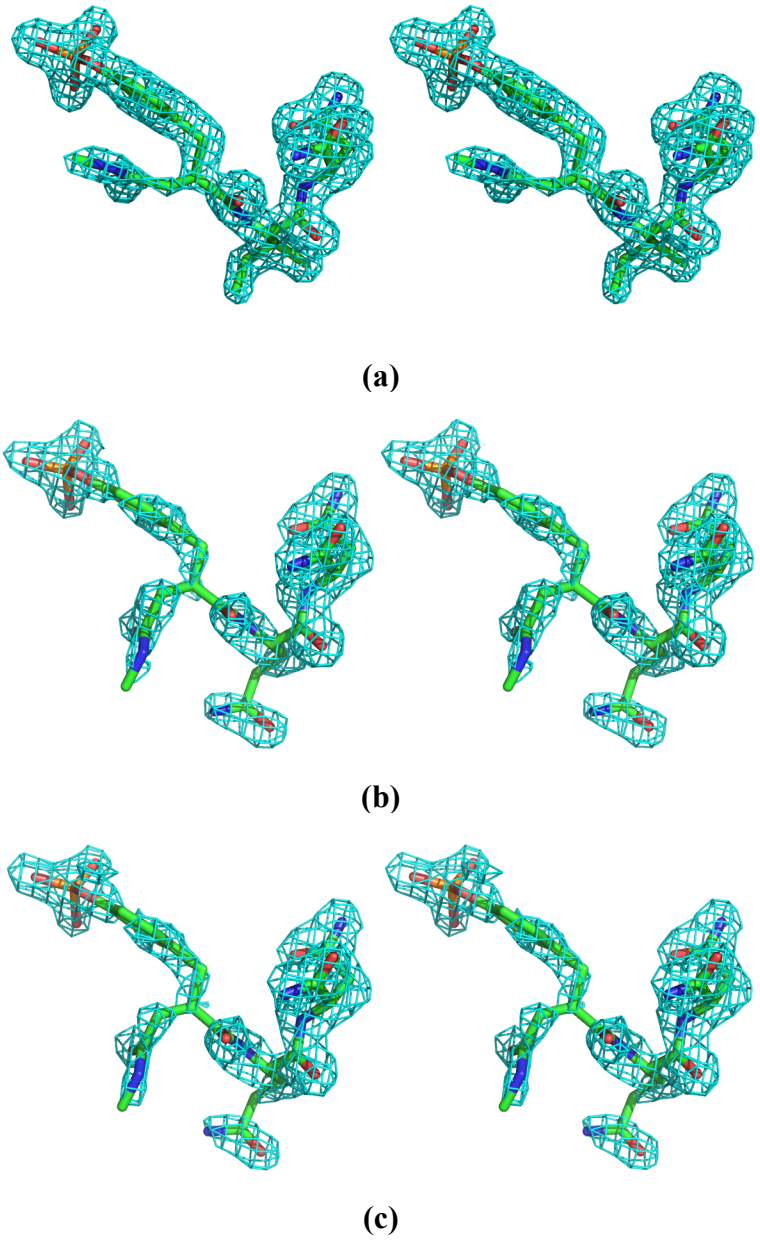


Figure S1. Electron density difference omit maps showing the bound structures of the flexible ligands **3** and **4** in stereo. The maps, indicated by the cyan wire mesh, are unweighted F_o-F_c omit maps contoured at +3 σ, showing only the portion within 1.0–1.5 Å of each ligand atom in the complexes for clarity. a) Complex of the domain with **3**. b) Complex **a** of the domain with **4**. c) Complex **b** of the domain with **4**.

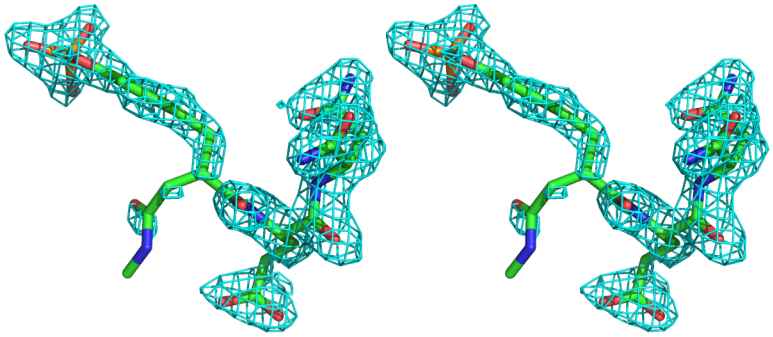
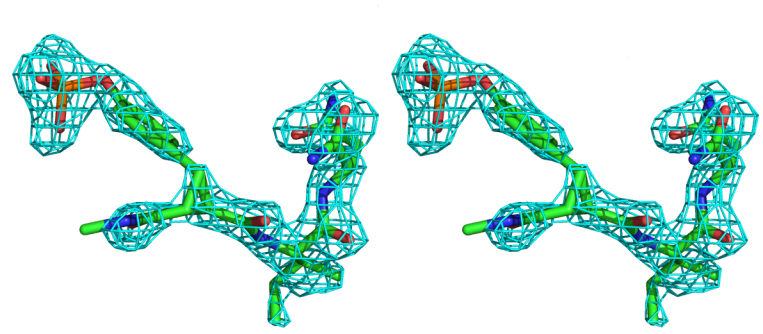
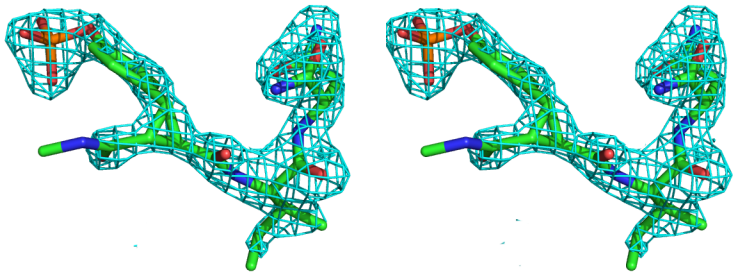


Figure S2. Electron density difference omit maps showing the bound structure of the flexible ligand **5** in stereo. The maps, indicated by the cyan wire mesh, are unweighted F_o-F_c omit maps contoured at +3 σ, showing only the portion within 1.0–1.5 Å of each ligand atom in the complexes for clarity.

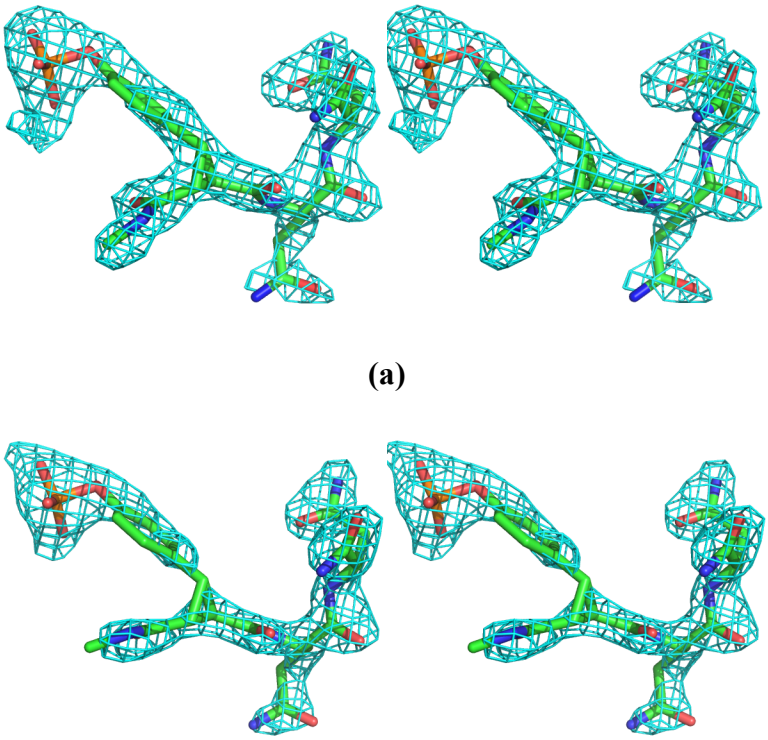


(a)



(b)

Figure S3. Electron density difference omit maps showing the bound structures of the cyclopropyl-constrained ligand **7** in stereo. The maps, indicated by the cyan wire mesh, are unweighted F_o-F_c omit maps contoured at +3 σ, showing only the portion within 1.0–1.5 Å of each ligand atom in the complexes for clarity. a) Complex **a** of the domain with **7**. b) Complex **b** of the domain with **7**.



(a)

(b)

Figure S4. Electron density difference omit maps showing the bound structures of the cyclopropyl-constrained ligand **8** in stereo. The maps, indicated by the cyan wire mesh, are unweighted F_o-F_c omit maps contoured at +3 σ, showing only the portion within 1.0–1.5 Å of each ligand atom in the complexes for clarity. a) Complex **a** of the domain with **8**. b) Complex **b** of the domain with **8**.

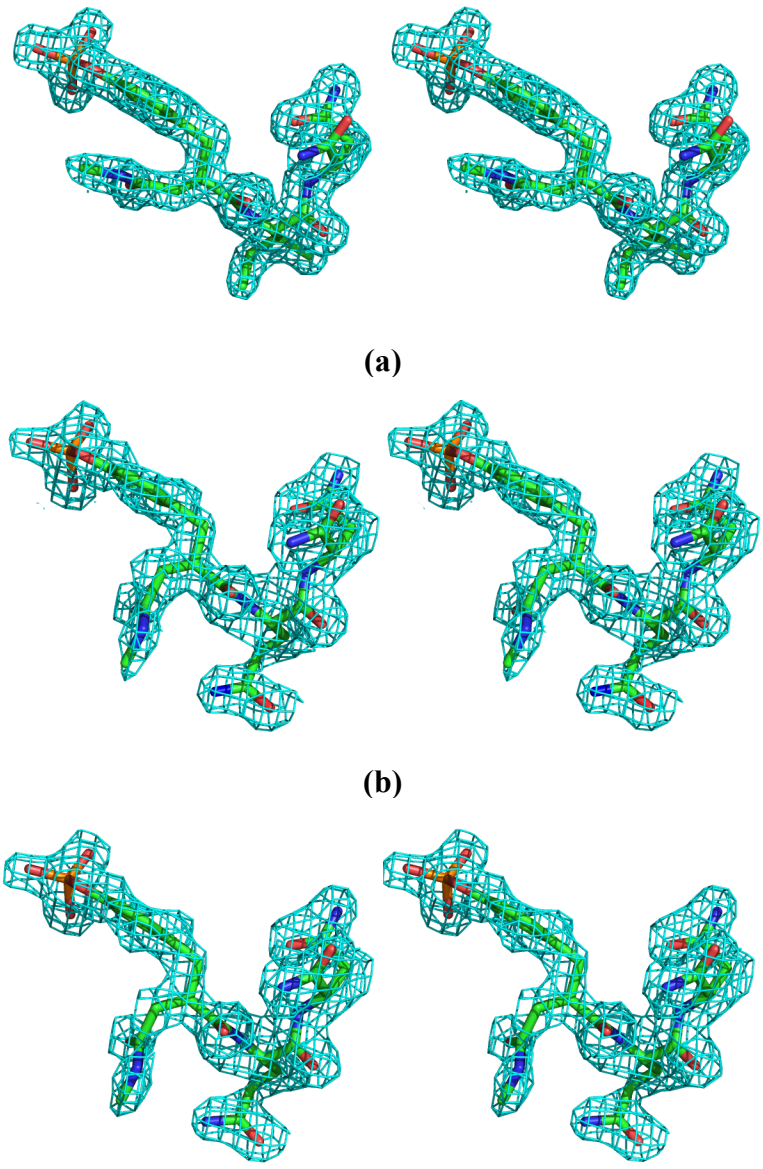


Figure S5. Electron density difference maps showing the bound structures of the flexible ligands **3** and **4** in stereo. The maps, indicated by the cyan wire mesh, are unweighted $2F_o$-F_c maps contoured at +1 σ, showing only the portion within 1.0–1.5 Å of each ligand atom in the complexes for clarity. a) Complex of the domain with **3**. b) Complex **a** of the domain with **4**. c) Complex **b** of the domain with **4**.

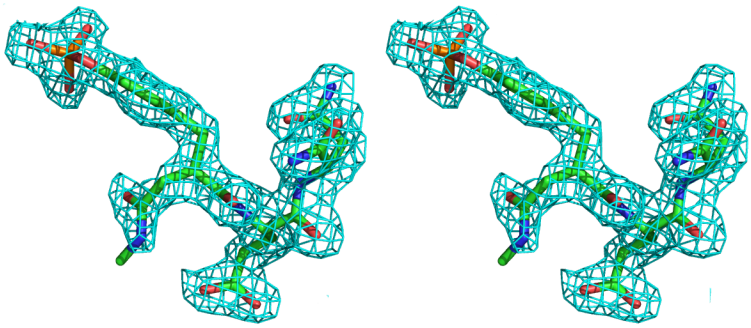


Figure S6. Electron density difference maps showing the bound structure of the flexible ligand **5** in stereo. The maps, indicated by the cyan wire mesh, are unweighted $2F_o$-F_c maps contoured at +1 σ, showing only the portion within 1.0–1.5 Å of each ligand atom in the complexes for clarity.

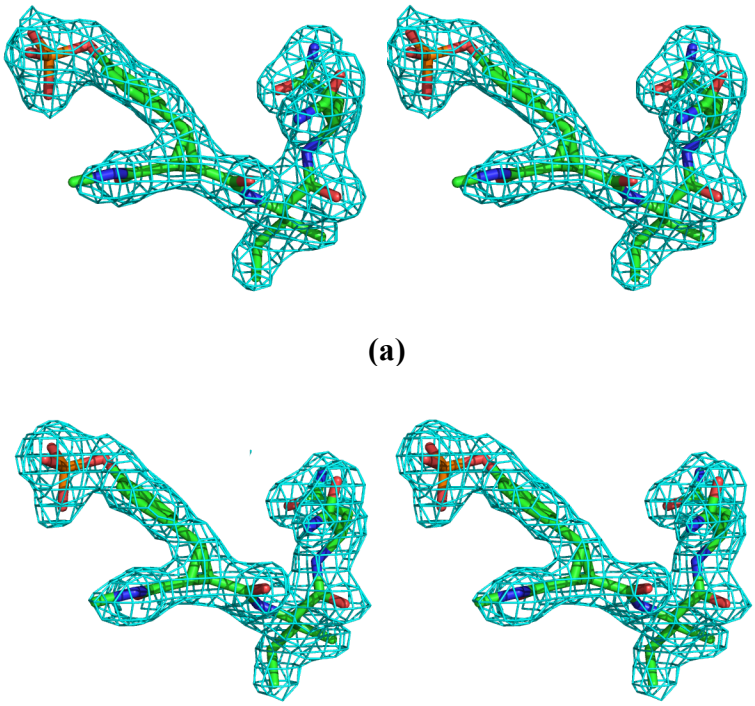
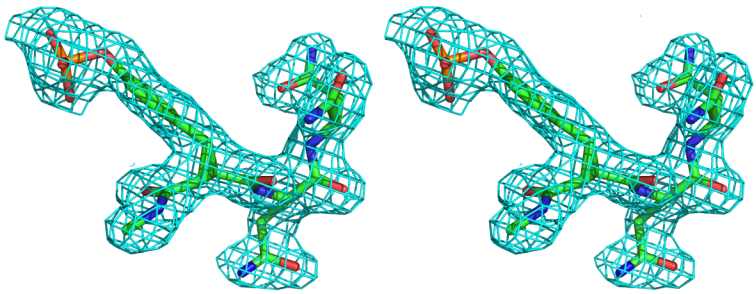
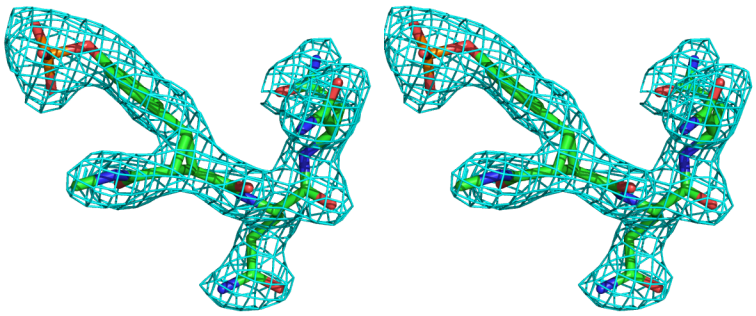


Figure S7. Electron density difference maps showing the bound structures of the cyclopropyl-constrained ligand **7** in stereo. The maps, indicated by the cyan wire mesh, are unweighted $2F_o$-F_c maps contoured at +1 σ, showing only the portion within 1.0–1.5 Å of each ligand atom in the complexes for clarity. a) Complex **a** of the domain with **7**. b) Complex **b** of the domain with **7**.



(a)



(b)

Figure S8. Electron density difference maps showing the bound structures of the cyclopropyl-constrained ligand **8** in stereo. The maps, indicated by the cyan wire mesh, are unweighted $2F_o$-F_c maps contoured at +1 σ, showing only the portion within 1.0–1.5 Å of each ligand atom in the complexes for clarity. a) Complex **a** of the domain with **8**. b) Complex **b** of the domain with **8**.

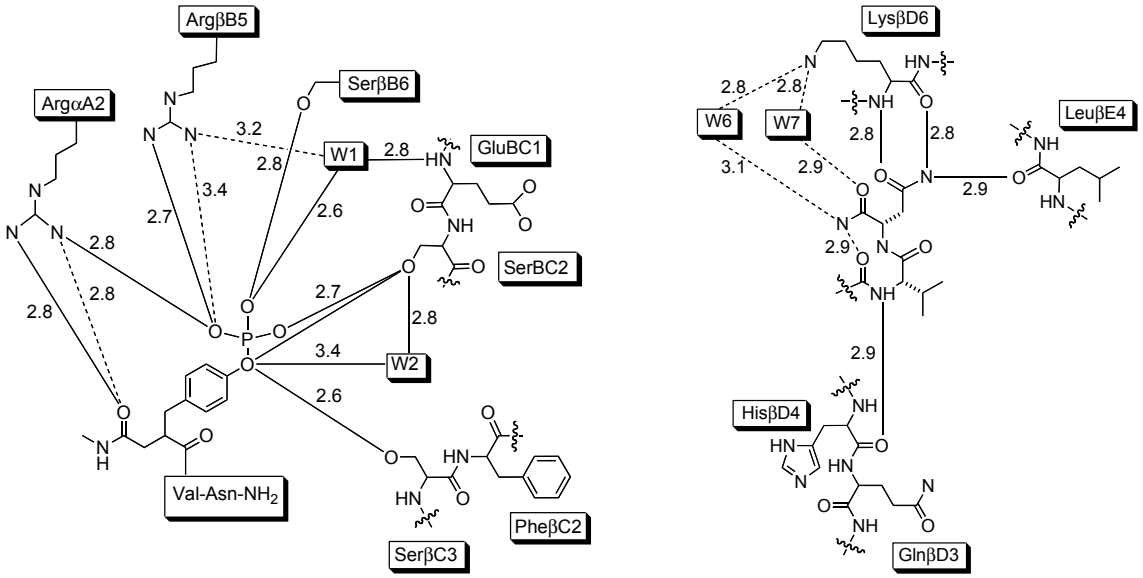
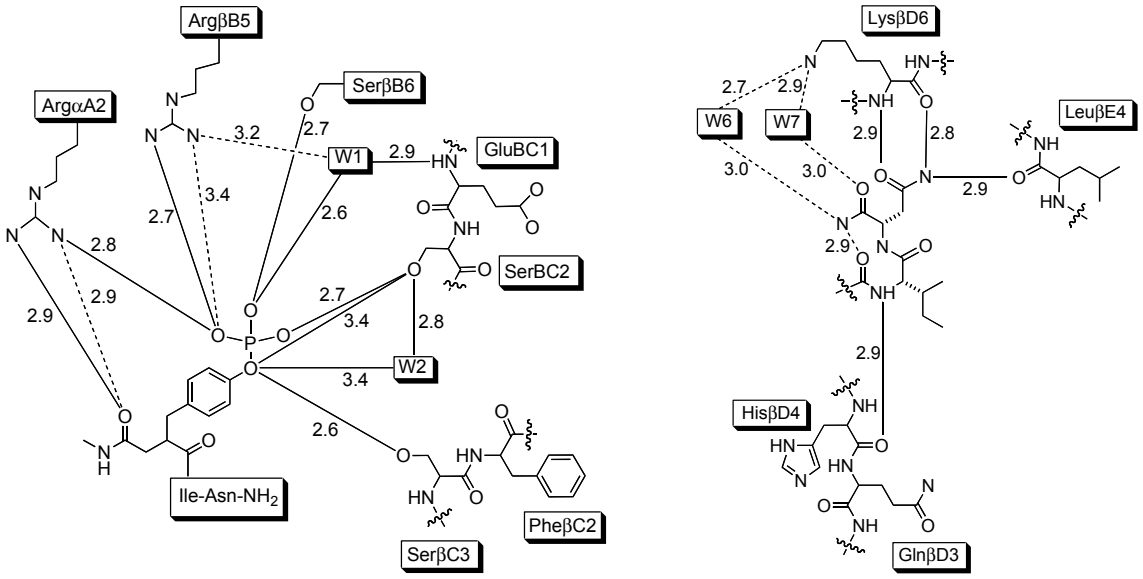


Figure S9. Polar interactions in the range of 2.5–3.4 Å in the complex of **2** with the Grb2 SH2 domain. All labile hydrogen atoms have been omitted for clarity except those on protein backbone nitrogen atoms. Only those ordered water molecules that mediate a single protein-ligand interaction are shown, and these are numbered so that water molecules that are conserved in at least two complexes have the same number. Solid lines indicate those polar contacts that are conserved for all complexes. (a) Interactions between the domain and the Ac–pY replacement. (b) Interactions between the domain and the X–N region of the ligand.



(a) **(b)**

Figure S10. Polar interactions in the range of 2.5–3.4 Å in the complex of **3** with the Grb2 SH2 domain. All labile hydrogen atoms have been omitted for clarity except those on protein backbone nitrogen atoms. Only those ordered water molecules that mediate a single protein-ligand interaction are shown, and these are numbered so that water molecules that are conserved in at least two complexes have the same number. Solid lines indicate those polar contacts that are conserved for all complexes. (a) Interactions between the domain and the Ac–pY replacement. (b) Interactions between the domain and the X–N region of the ligand.

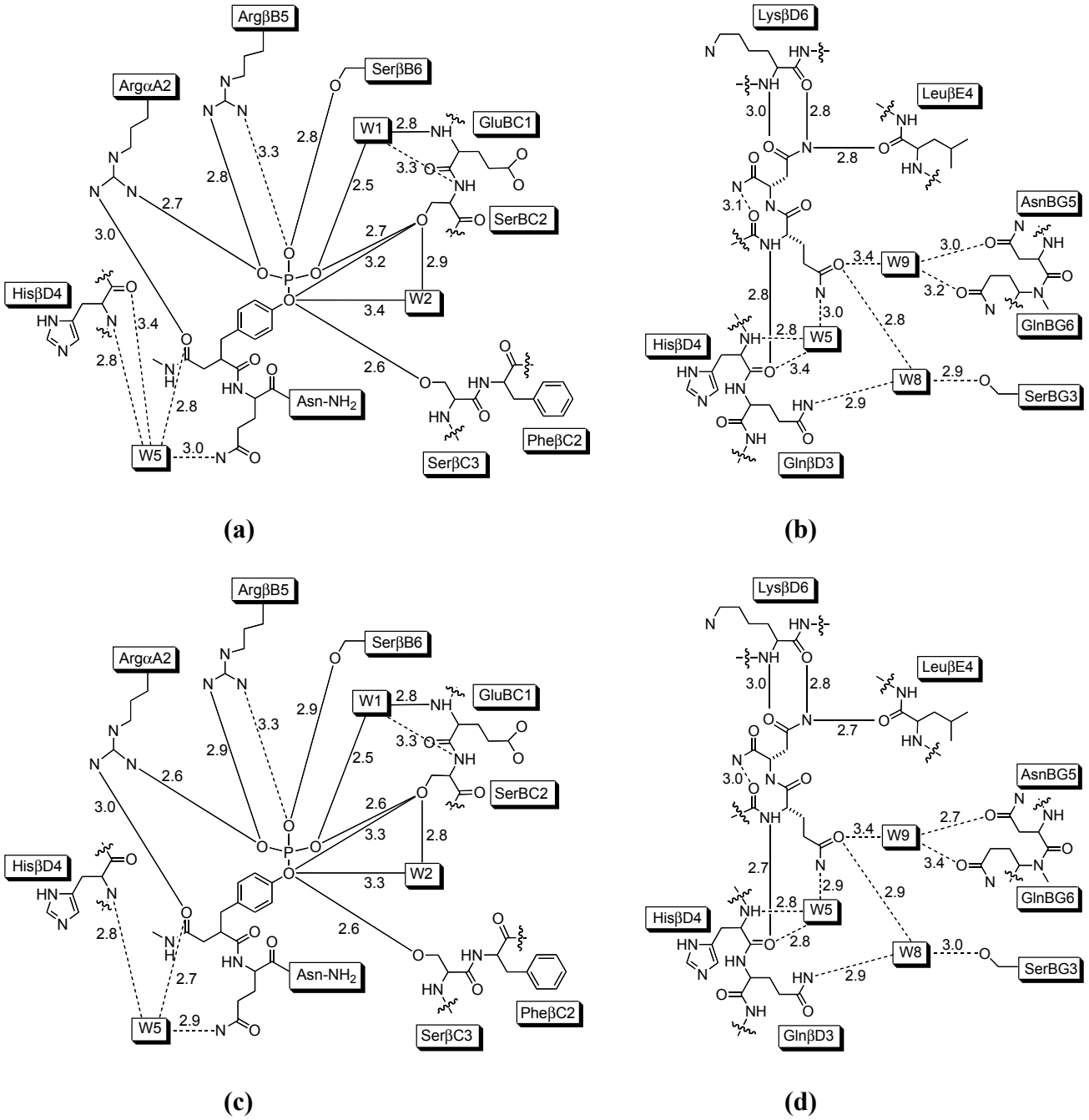


Figure S11. Polar interactions in the range of 2.5–3.4 Å in the complex of **4** with the Grb2 SH2 domain. All labile hydrogen atoms have been omitted for clarity except those on protein backbone nitrogen atoms. Only those ordered water molecules that mediate a single protein-ligand interaction are shown, and these are numbered so that water molecules that are conserved in at least two complexes have the same number. Solid lines indicate those polar contacts that are conserved for all complexes. (a, c) Interactions between the domain and the Ac–pY replacement in each of the two complexes in the asymmetric unit. (b, d) Interactions between the domain and the X–N region of the ligand in each of the two complexes in the asymmetric unit.

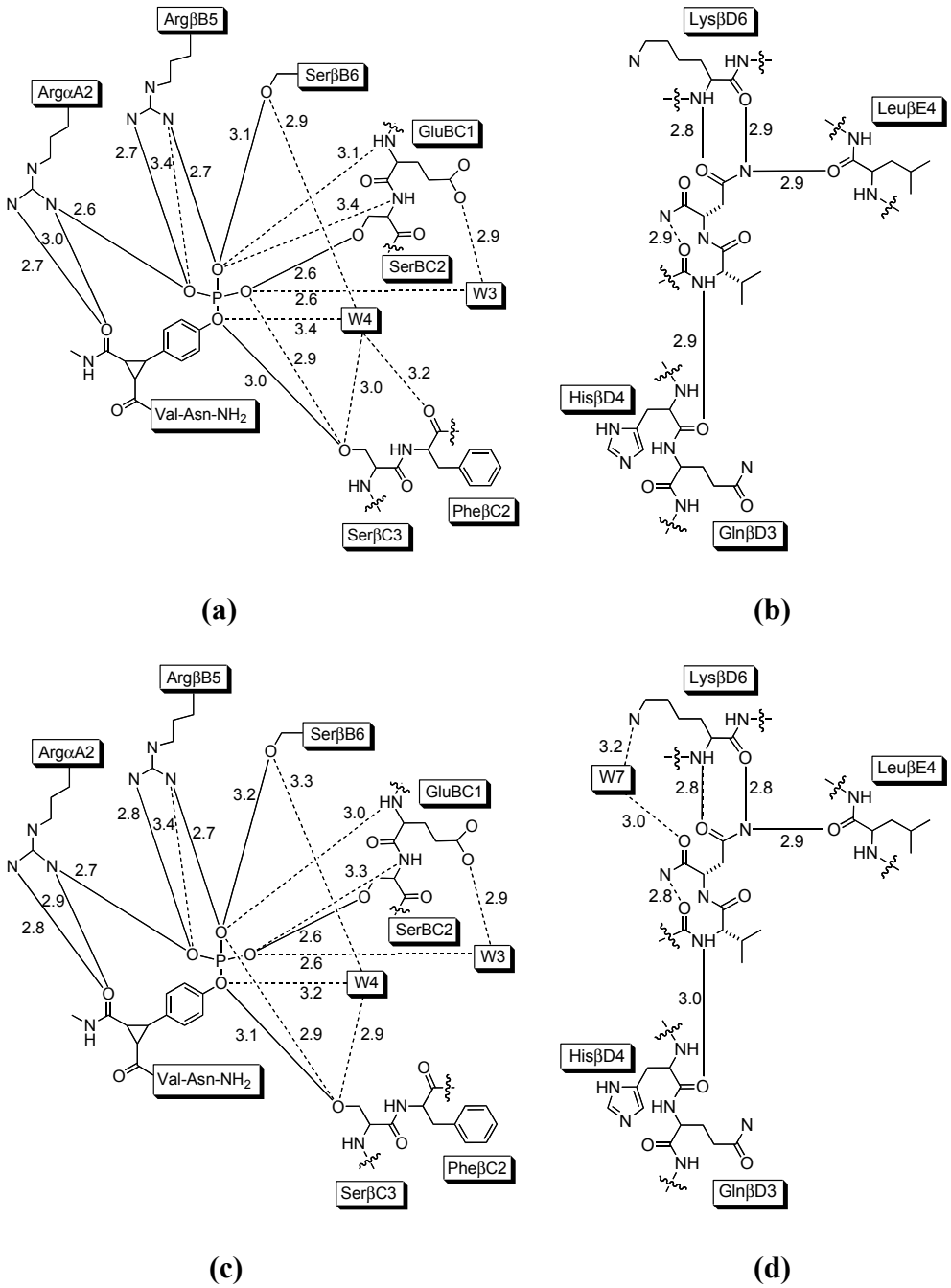


Figure S12. Polar interactions in the range of 2.5–3.4 Å in the complex of **6** with the Grb2 SH2 domain. All labile hydrogen atoms have been omitted for clarity except those on protein backbone nitrogen atoms. Only those ordered water molecules that mediate a single protein-ligand interaction are shown, and these are numbered so that water molecules that are conserved in at least two complexes have the same number. Solid lines indicate those polar contacts that are conserved for all complexes. (a, c) Interactions between the domain and the Ac–pY replacement in each of the two complexes in the asymmetric unit. (b, d) Interactions between the domain and the X–N region of the ligand in each of the two complexes in the asymmetric unit.

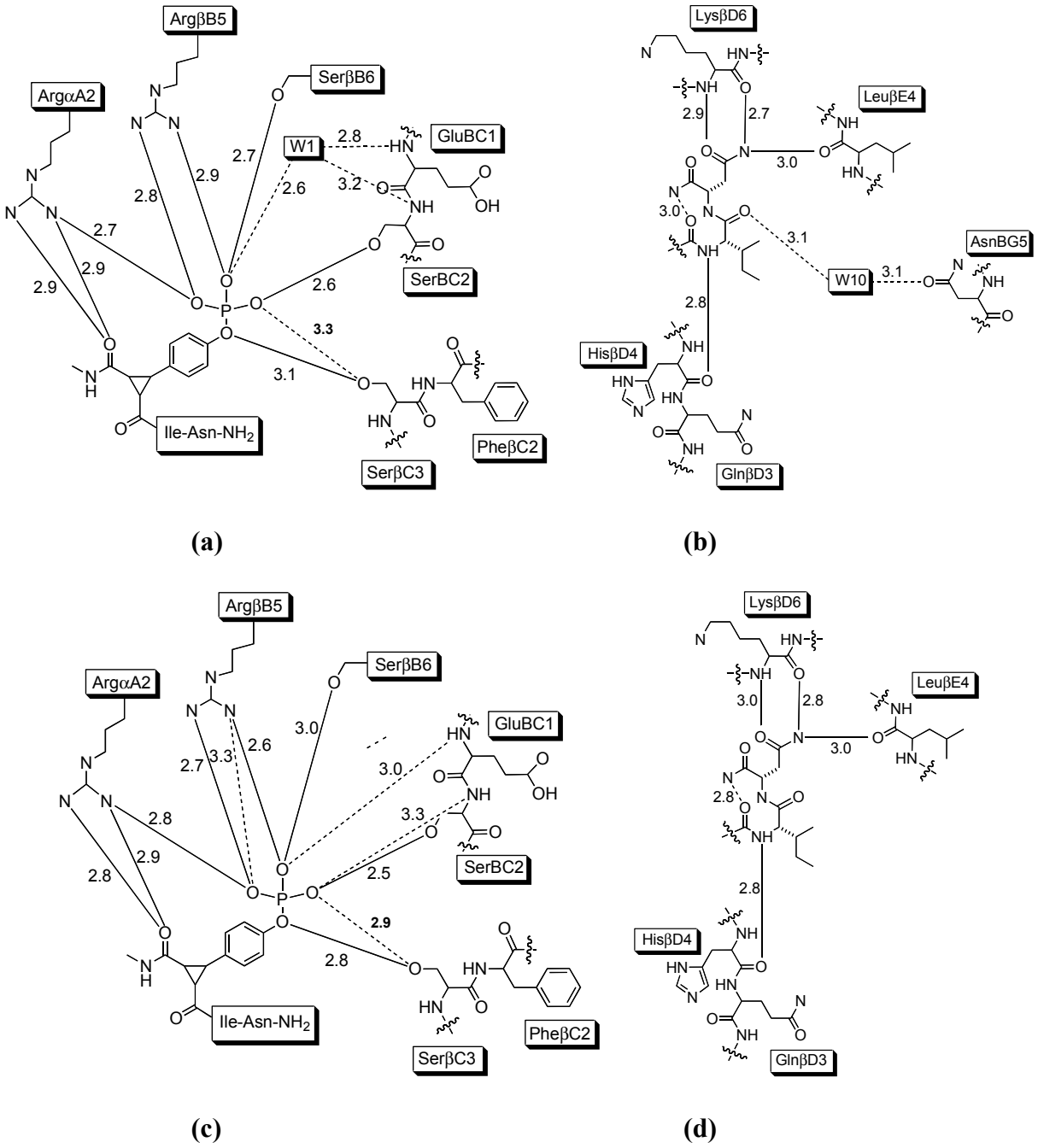


Figure S13. Polar interactions in the range of 2.5–3.4 Å in the complex of **7** with the Grb2 SH2 domain. All labile hydrogen atoms have been omitted for clarity except those on protein backbone nitrogen atoms. Only those ordered water molecules that mediate a single protein-ligand interaction are shown, and these are numbered so that water molecules that are conserved in at least two complexes have the same number. Solid lines indicate those polar contacts that are conserved for all complexes. (a, c) Interactions between the domain and the Ac–pY replacement in each of the two complexes in the asymmetric unit. (b, d) Interactions between the domain and the X–N region of the ligand in each of the two complexes in the asymmetric unit.

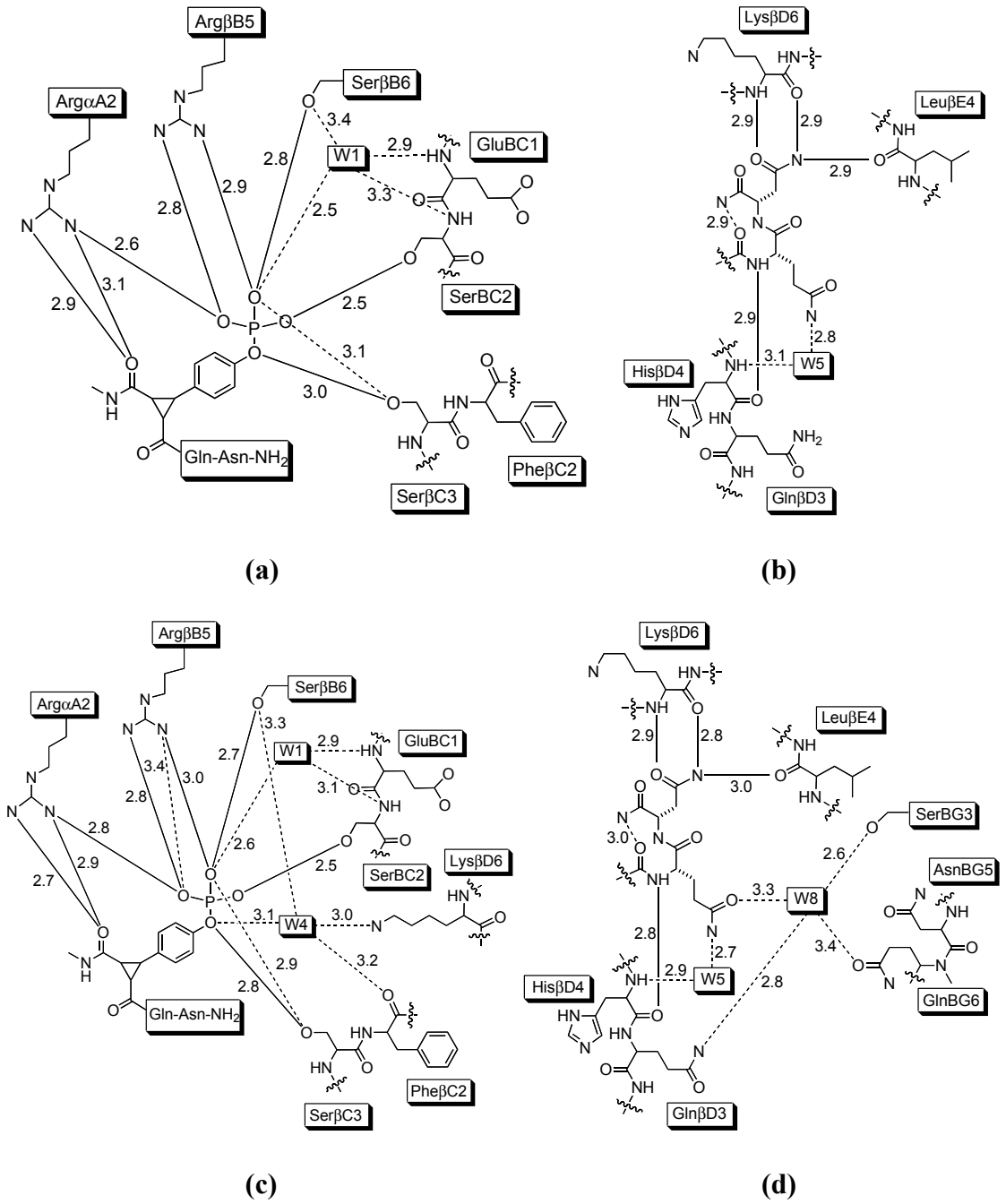


Figure S14. Polar interactions in the range of 2.5–3.4 Å in the complex of **8** with the Grb2 SH2 domain. All labile hydrogen atoms have been omitted for clarity except those on protein backbone nitrogen atoms. Only those ordered water molecules that mediate a single protein-ligand interaction are shown, and these are numbered so that water molecules that are conserved in at least two complexes have the same number. Solid lines indicate those polar contacts that are conserved for all complexes. (a, c) Interactions between the domain and the Ac–pY replacement in each of the two complexes in the asymmetric unit. (b, d) Interactions between the domain and the X–N region of the ligand in each of the two complexes in the asymmetric unit.

Table S1. Direct and single water-mediated polar protein-ligand contacts in the complexes of **2**–**8** as determined by x-ray crystallography.

Complex	Direct Contacts of Ac–pY	Single Water-Mediated Contacts of Ac–pY	Direct Contacts of pY+1–N	Single Water-Mediated Contacts of pY+1–N	Total Direct Contacts	Total Single Water-Mediated Contacts	Total Contacts
2	9	2	4	2	13	4	17
6	12	2	4	0	16	2	18
6	12	2	4	1	16	3	19
3	9	2	4	2	13	4	17
7	9	1	4	1	13	2	15
7	12	0	4	0	16	0	16
4	8	3[a]	4	3[a]	12	6[a]	18
4	8	3[a]	4	3[a]	12	6[a]	18
8	9	1	4	1	13	2	15
8	10	2	4	2	14	4	18
5	9	2[a]	4	3	13	5[a]	18

[a] One water molecule makes a contact with the backbone nitrogen atom of HisβD4 and two contacts with the ligand, one with the *N*-terminal amide nitrogen atom of the Ac–pY replacement and the other with the pY+1 Gln/Glu side chain; each are counted independently.

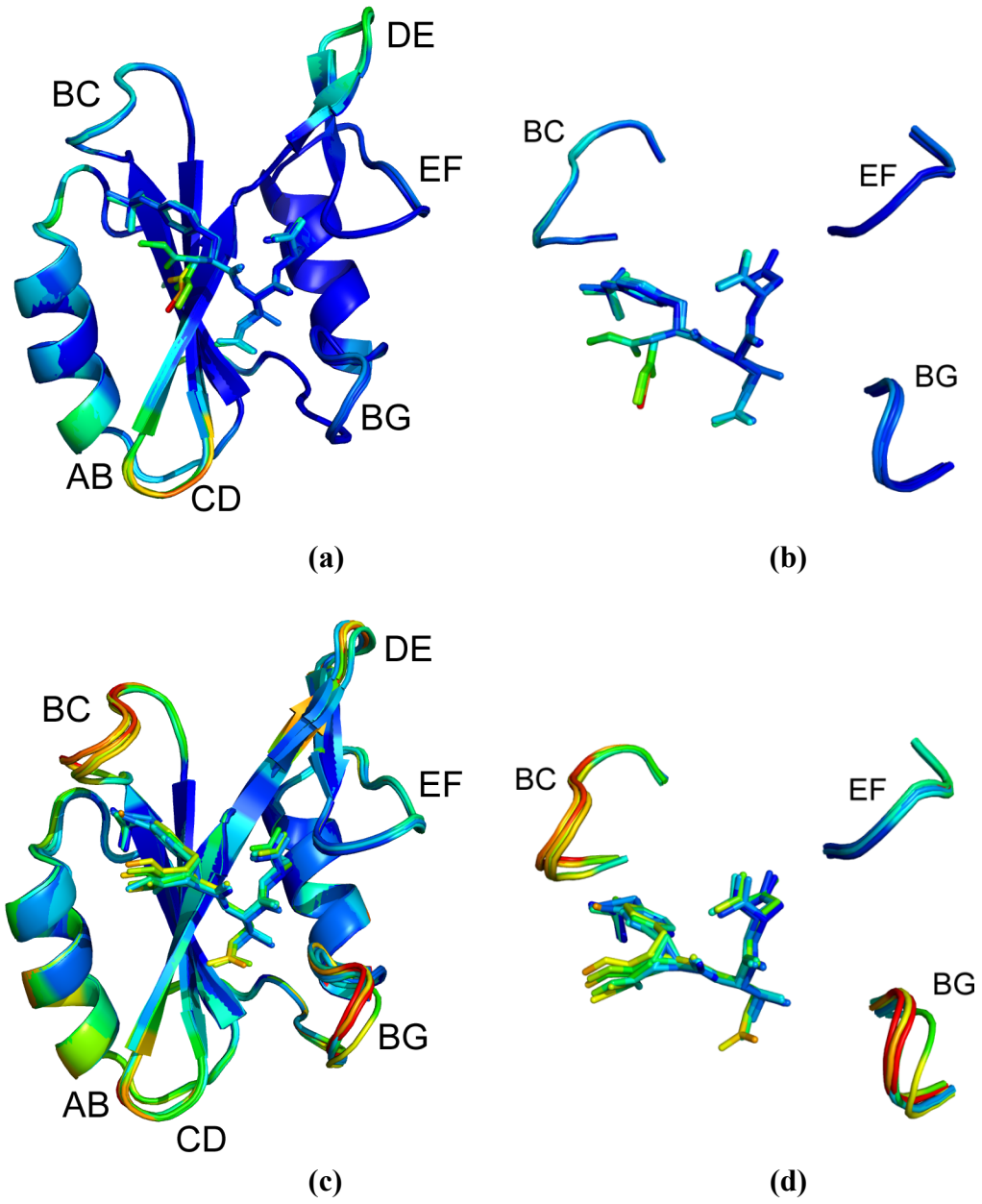


Figure S15. Overlay of Grb2 SH2 complexed with flexible ligands **2–5** (a, b) and constrained ligands **6–8** (c, d). The domain (a, c) and BC, EF, and BG loop regions (b, d) are color-coded according to the adjusted atomic B-factors. The domain and loop regions are represented as ribbons and the ligands as sticks. Atomic B-factors are color-coded based on a linear spectrum from blue (≤ 20 Å^2) to green (30 Å^2) to red (≥ 40 Å^2).

Ramachandran-Type Plots. Plots of phi-psi coordinates for each residue for which sufficient electron density was observed in the crystal structure are shown in Figs. S16–S22. The boundaries employed were determined by Morris *et al*., 1992 based on an analysis of phi-psi coordinates for all structures released to the Brookhaven Protein Structure Databank (Bernstein *et al*., 1977) as of October, 1990. All 10° x 10° phi/psi regions of conformational space enclosing more than 100 residue data points were defined as "core" regions. All regions enclosing 8-100 data points were defined as "allowed" regions. A "generous" region was defined as those 10° x 10° phi/psi regions extending outward 20° from the allowed region. Lastly, all remaining 10° x 10° phi/psi regions were defined as "disallowed" regions. The percentage populations of all residues in the core, allowed, generous, and disallowed regions was found to be 81.9, 14.8, 2.0, and 1.3%, respectively.

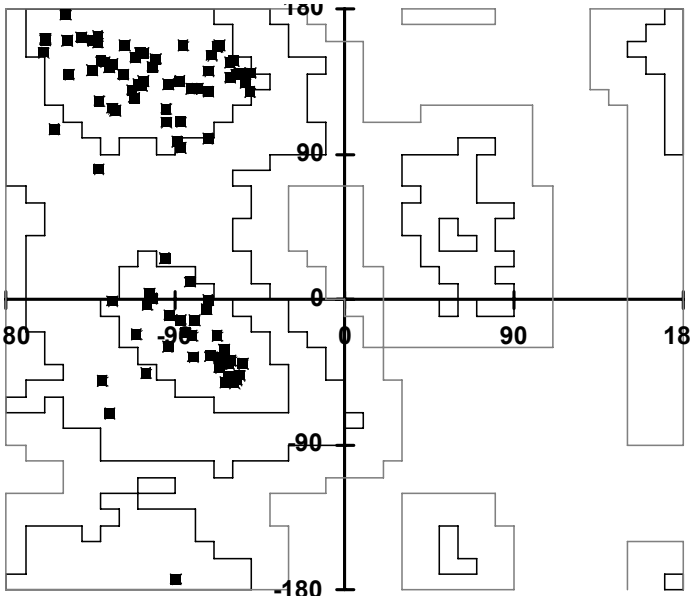


Figure S16. Ramachandran-type plot of the complex of the Grb2 SH2 domain with **2**. All phi/psi combinations within the boundaries delineated by the heavy black border, thin black border, and gray border represent core, allowed, and generous regions, respectively. All regions outside these borders are considered to be disallowed. The phi/psi combinations for every residue in the structure having sufficient electron density (black squares) are shown.

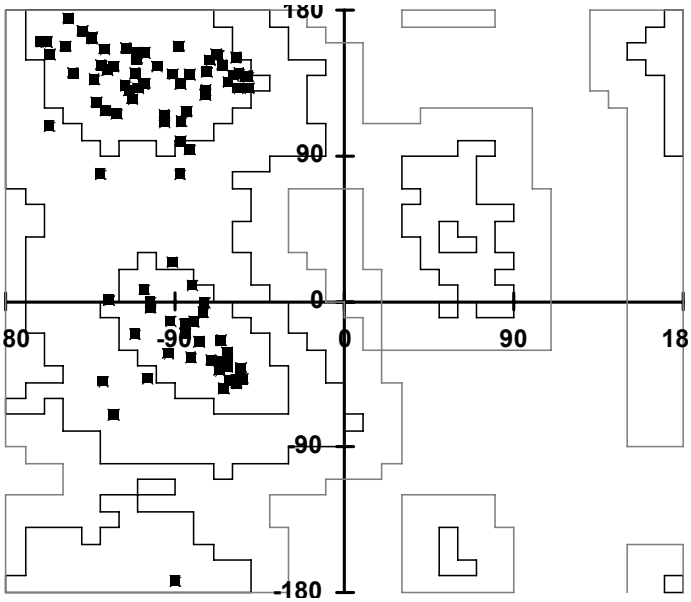
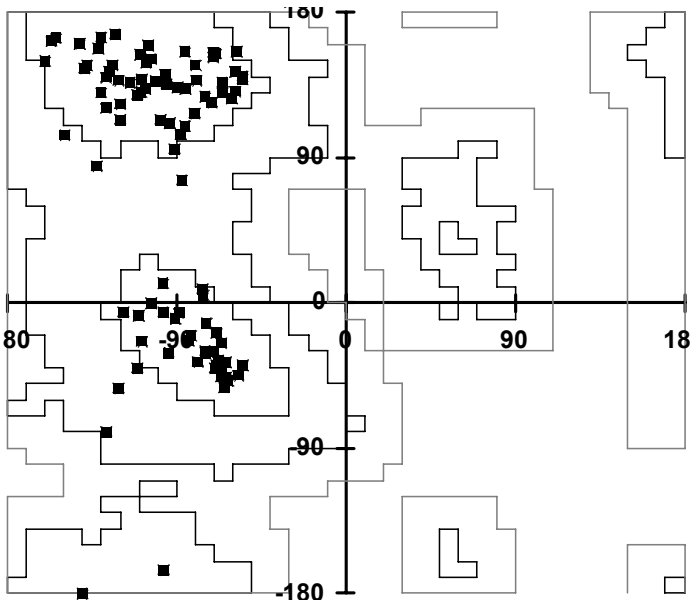
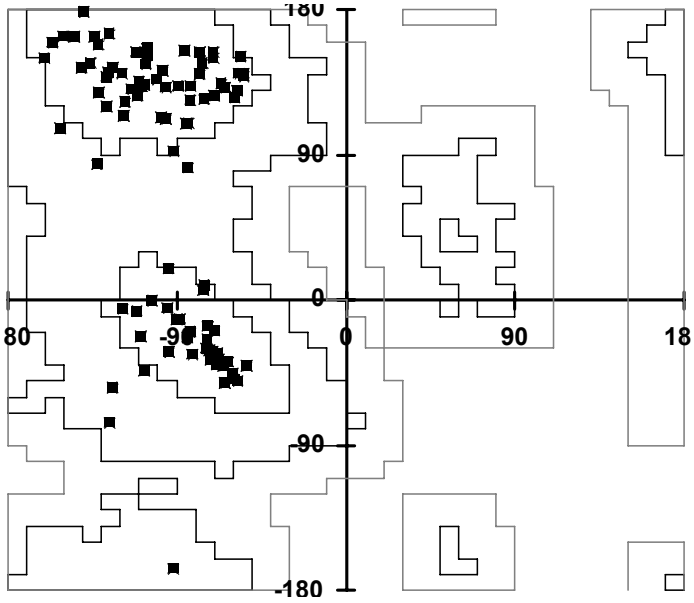


Figure S17. Ramachandran-type plot of the complex of the Grb2 SH2 domain with **3**. All phi/psi combinations within the boundaries delineated by the heavy black border, thin black border, and gray border represent core, allowed, and generous regions, respectively. All regions outside these borders are considered to be disallowed. The phi/psi combinations for every residue in the structure having sufficient electron density (black squares) are shown.



(a)



(b)

Figure S18. Ramachandran-type plots of the complex of the Grb2 SH2 domain with **4**. All phi/psi combinations within the boundaries delineated by the heavy black border, thin black border, and gray border represent core, allowed, and generous regions, respectively. All regions outside these borders are considered to be disallowed. The phi/psi combinations for every residue in the structure having sufficient electron density (black squares) are shown for each of the two complexes **a** and **b** that comprise the asymmetric unit.

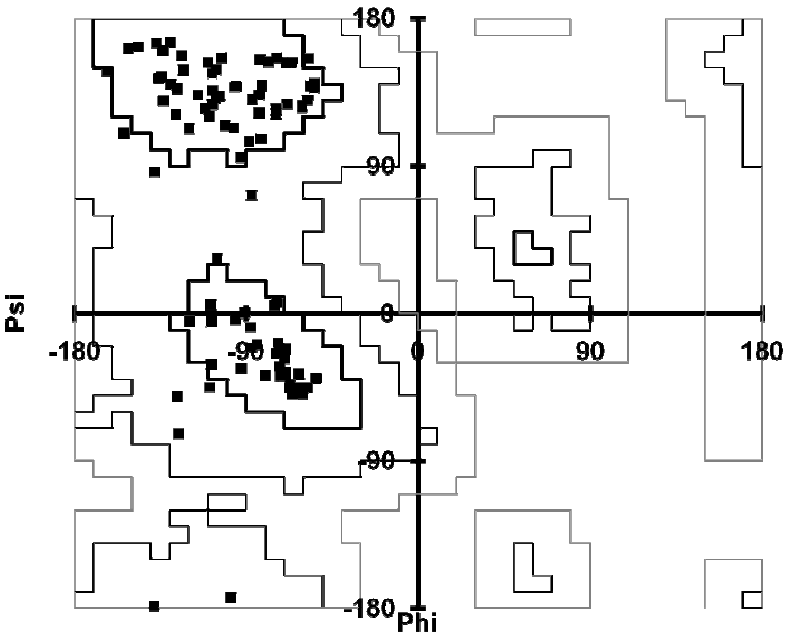
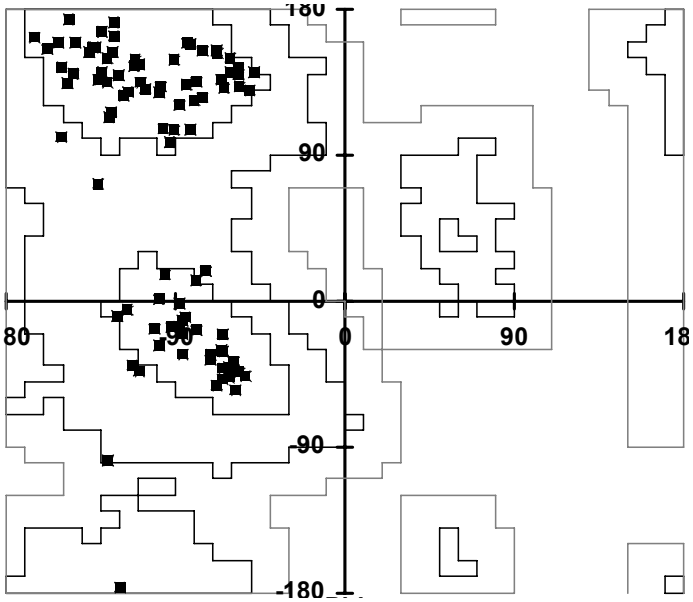
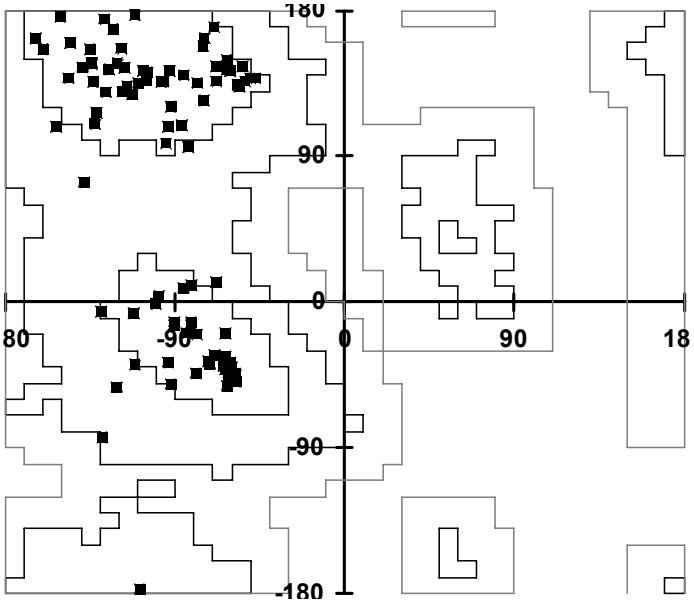


Figure S19. Ramachandran-type plot of the complex of the Grb2 SH2 domain with **5**. All phi/psi combinations within the boundaries delineated by the heavy black border, thin black border, and gray border represent core, allowed, and generous regions, respectively. All regions outside these borders are considered to be disallowed. The phi/psi combinations for every residue in the structure having sufficient electron density (black squares) are shown.

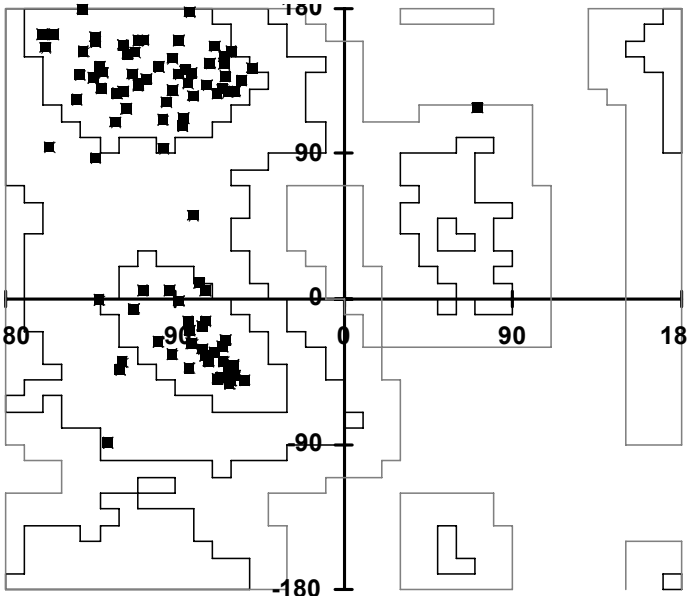


(a)

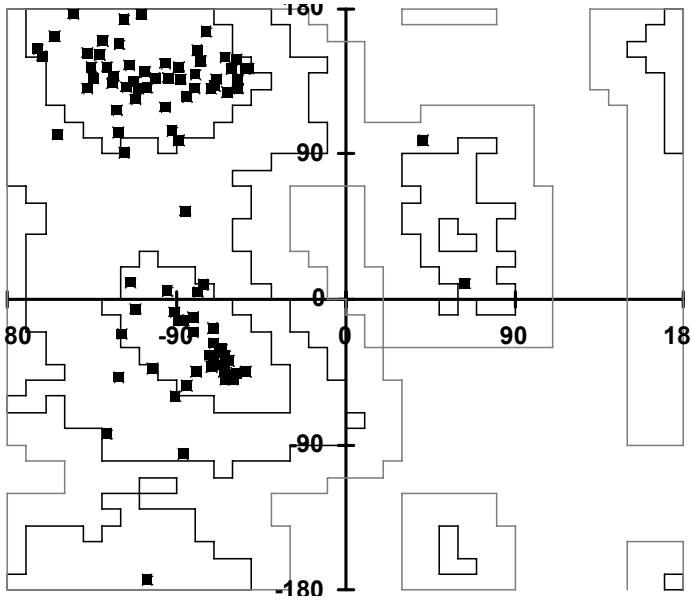


(b)

Figure S20. Ramachandran-type plots of the complex of the Grb2 SH2 domain with **6**. All phi/psi combinations within the boundaries delineated by the heavy black border, thin black border, and gray border represent core, allowed, and generous regions, respectively. All regions outside these borders are considered to be disallowed. The phi/psi combinations for every residue in the structure having sufficient electron density (black squares) are shown for each of the two complexes **a** and **b** that comprise the asymmetric unit.

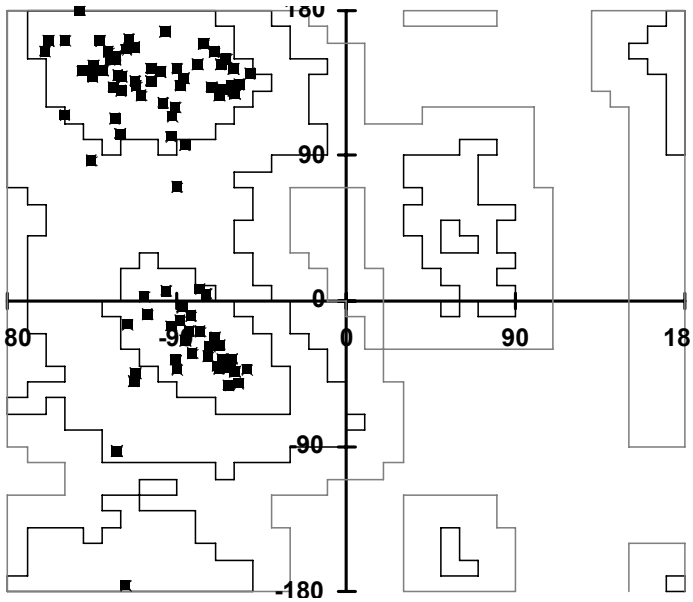


(a)

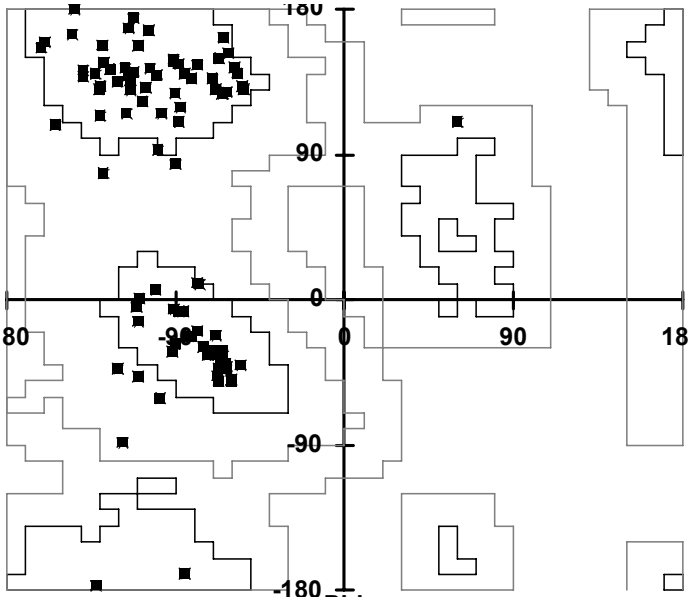


(b)

Figure S21. Ramachandran-type plots of complex of the Grb2 SH2 domain with **7**. All phi/psi combinations within the boundaries delineated by the heavy black border, thin black border, and gray border represent core, allowed, and generous regions, respectively. All regions outside these borders are considered to be disallowed. The phi/psi combinations for every residue in the structure having sufficient electron density (black squares) are shown for each of the two complexes **a** and **b** that comprise the asymmetric unit.



(a)



(b)

Figure S22. Ramachandran-type plots of complex of the Grb2 SH2 domain with **8**. All phi/psi combinations within the boundaries delineated by the heavy black border, thin black border, and gray border represent core, allowed, and generous regions, respectively. All regions outside these borders are considered to be disallowed. The phi/psi combinations for every residue in the structure having sufficient electron density (black squares) are shown for each of the two complexes **a** and **b** that comprise the asymmetric unit.

References

Morris, A. L., MacArthur, M. W., Hutchinson, E. G., Thornton, J. M. (1992). *Proteins* **12**, 345-364.
Bernstein, F. C., Koetzle, T. F., Williams, G. J. B., Meyer, E. F., Jr., Brice, M. D., Rodgers, J. R., Kennard, O., Shimanouchi, T., Tasumi, M. (1977). *J. Mol. Biol*. **122**, 535-542.