



Figure S1. *iASPP-PP-1c* α representative electron density.

Related to Figures 1 and 2.

Shown is representative F_o - F_c difference electron density (green), phased with iASPP₆₂₇₋₈₂₈ and PP-1c α_{1-300} placed by molecular replacement before refinement, and contoured at 2.5 σ . Difference density is shown for the RARL (A), SILK (B) and PxxPxR (C) motifs. In each panel, the omitted motif is shown as beige sticks and residues are labelled.



Figure S2. SEC-SAXS analysis of iASPP-PP-1c and ASPP2-PP-1c.

Related to Figure 3.

(A) SEC-SAXS on ASPP2-PP-1c. The grey trace corresponds to the size exclusion UV_{280} trace, the blue dots give the R_G values calculated by Guinier approximation for each frame, and the orange trace gives the MW as determined by MALLS.

(B) Comparison of the scattering from iASPP-PP-1c with ASPP2-PP-1c, integrated from peak maximum to ½ maximal peak height (pink region in (A)).

(C) and (D) RAW deconvolution of scattering into compact and extended subsets for iASPP-PP-1c (C) and ASPP2-PP-1c (D).

(E) and (F) P(r) plots for RAW deconvoluted scattering for iASPP-PP-1c (E) and ASPP2-PP-1c (F).



Figure S3. MES analysis of ASPP2-PP-1c.

Related to Figure 3.

(A) MES modeling using the 5011 library (based on the open form of the iASPP-PP-1c crystal structure). The three model ensemble is shown superimposed with color coding as shown on the left. The percent of each model, R_G and χ^2 are given.

(B) and (C) Fit of the compact (B) and extended (C) data sets to calculated scattering from the MES models derived from the 5011 library (crystal), the 5001 library, and the combined libraries. (D) and (E) Three model ensembles derived from the combined libraries for the compact data set (D) and the extended data set (E).



Figure S4. *SEC-SAXS analysis of iASPP(621-828) lacking the SILK motif bound to PP-1c.* Related to Figure 3.

(A) SEC-SAXS of iASPP(621-828)-PP-1c. The grey trace corresponds to the size exclusion UV_{280} trace, the blue dots give the R_G values calculated by Guinier approximation for each frame, the orange trace gives the MW as determined by MALLS. The data from the region marked in pink was used for subsequent analyses.

(B) Comparison of scattering from the iASPP(608-828)-PP-1c complex (contains the SILK motif) with scattering from iASPP(621-828)-PP-1c (lacking the SILK motif).

(C) RAW deconvolution of iASPP(621-828)-PP-1c scattering into extended and compact subsets.

(D) P(r) plots for RAW deconvoluted scattering for iASPP(621-828)-PP-1c. For each trace, the D_{max} (left value) and R_G (right value) are listed.



Figure S5. *MES analysis of iASPP(621-828) lacking the SILK motif bound to PP-1c.* Related to Figure 3.

(A) MES modeling against the compact iASPP(621-828)-PP-1c data set using a library based on the open form of the iASPP-PP-1c crystal structure with the SILK motif deleted. The single best fit model is shown with color coding as shown on the left.

(B) and (C) Fit of the compact (B) and extended (C) data sets to calculated scattering from the MES models derived from the crystal structure library, the 7001 library (in which the ASPP and PP-1c domains are defined as independent rigid bodies), and the combined libraries, in which the RVxF and PxxPxR motifs are allowed to release from their respective binding partners.

(D) and (E) Three model ensembles derived from the combined libraries for the compact data set (D) and the extended data set (E). The percent of each model, R_G and χ^2 are given.

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| Pool (library) ¹ | | _ | Jeconyo | liited Sm | hall ² | Experi | mental I | Data: iAs | SPP-PP-1 | î | Non-de | | Đ l |
|-----------------------------|--------------------------------------------------|------|---------|-----------|-------------------|--------|----------|-----------|----------|------|--------|-----------|-----|
| | R ₆ Range of Pool | | | | | | 28.9 | 9 - 29.7 | | | | | |
| | D _{max} Range of Pool | | | | | | 06 | - 110 | | | | | |
| 5011 | FOXS Chi ² Range of Pool ³ | | 3.2 | - 10.7 | | | 17.8 | 3-41.2 | | | 25. | 3 – 69. | ω |
| | Chi ² of best model/ensemble | | | 3.2 | | | | 17.8 | | | | 25.3 | |
| | Pool ID/R _G /Dmax/% ⁴ | 5011 | 29.7 | 97.2 | 100 | 5011 | 29.7 | 97.2 | 100 | 5011 | 29.7 | 97.2 | |
| | R _G Range of Pool | | | | | | 27. | 1 - 28.3 | | | | | |
| | D _{max} Range of Pool | | | | | | 84 | - 100 | | | | | |
| 5013 | FOXS Chi ² Range of Pool | | 9.3 | - 23.2 | | | 34.0 |) – 70.1 | | | 57.9 |) – 129.3 | |
| | Chi ² of best model/ensemble | | | 9.3 | | | (1) | 34.0 | | | | 57.9 | i i |
| | Pool ID/R _G /Dmax/% | 5013 | 28.3 | 88.5 | 100 | 5013 | 28.3 | 88.5 | 100 | 5013 | 28.3 | 88.5 | |
| | Chi ² of 3 model ensemble | | | 1.0 | | | | 1.4 | | | | 1.4 | |
| Combined Pools | Pool ID/R _G /Dmax/% | 5005 | 31.6 | 120 | 40 | 5007 | 31.6 | 105 | 50 | 5007 | 32.1 | 112 | |
| 5006, 5007 | | 5007 | 32.2 | 113 | 32 | 5005 | 32.6 | 125 | 47 | 5006 | 34.7 | 144 | |
| | | 5006 | 28.6 | 89.7 | 29 | 5007 | 48.9 | 130 | ω | 5007 | 27.8 | 95.4 | |

¹ Identifier for the model library (pool) created using BILBO-MD ² Experimental SAXS data was deconvoluted using RAW ³ Chi² are calculated for the individual models in the pool

⁴ Pool ID: the code representing the BILBO-MD library; R₆: radius of gyration for the selected model; Dmax: maximum pairwise interatomic distance in the selected model; %: fraction of the ensemble represented by the model

| | 5004 | 5004 | | | | | 5003 | | | | | | | 5002 | | | | | | | 5001 | | | | | Pool (library) |
|-----------------------------------------|-------------------------------------|--------------------------------|------------------------------|------|------|--------------------------------|-----------------------------------------|-------------------------------------|--------------------------------|------------------------------|------|------|--------------------------------|-----------------------------------------|-------------------------------------|--------------------------------|------------------------------|------|------|--------------------------------|-----------------------------------------|-------------------------------------|--------------------------------|------------------------------|-----------|----------------|
| Chi ² of best model/ensemble | FOXS Chi ² Range of Pool | D _{max} Range of Pool | R _G Range of Pool | | | Pool ID/R _G /Dmax/% | Chi ² of best model/ensemble | FOXS Chi ² Range of Pool | D _{max} Range of Pool | R _G Range of Pool | | | Pool ID/R _G /Dmax/% | Chi ² of best model/ensemble | FOXS Chi ² Range of Pool | D _{max} Range of Pool | R _G Range of Pool | | | Pool ID/R _G /Dmax/% | Chi ² of best model/ensemble | FOXS Chi ² Range of Pool | D _{max} Range of Pool | R _G Range of Pool | | |
| | | | | 5003 | 5003 | 5003 | | | | | 5002 | 5002 | 5002 | | | | | 5001 | 5001 | 5001 | | | | | | |
| | 2.0 | | | 31.2 | 33.0 | 28.9 | | 1.2 | | | 34.8 | 31.3 | 28.9 | | 2.3 | | | 28.5 | 32.2 | 30.2 | | 1.8 | | | Deconvo | |
| 1.0 | - 187 | | | 99.8 | 134 | 91.9 | 1.1 | - 33 | | | 138 | 97.5 | 94.2 | 1.1 | - 31 | | | 89.5 | 120 | 98.1 | 1.7 | 3 - 16 | | | luted Sm | |
| | | | | 24 | 32 | 45 | | | | | 22 | 24 | 54 | | | | | 6 | 20 | 74 | | | | | nall | |
| | | | | 5003 | 5003 | 5003 | | | | | 5002 | 5002 | 5002 | | | | | 5001 | 5001 | 5001 | | | | | _ | Experii |
| 2 | 3.3 | 75 | 25.8 | 28.9 | 31.2 | 33.8 | N | 3.6 | 68 | 28.1 | 28.8 | 31.4 | 34.1 | N | 4.4 | .68 | 27.9 | 30.4 | 32.2 | 32.4 | 4 | 5.6 | .68 | 28.1 | Deconvol | mental D |
| 2.3 | - 320 | - 139 | - 46.4 | 95.7 | 100 | 138.9 | 2.3 | - 68 | - 152 | - 34.7 | 92.1 | 100 | 144 | 2.7 | - 80 | - 185 | - 37.3 | 95.0 | 120 | 115 | 1.8 | - 54 | - 126 | - 33.6 | luted Lar |)ata: iAS |
| | | | | 18 | 34 | 49 | | | | | 17 | 36 | 46 | | | | | 23 | 25 | 53 | | | | | .ge | PP-PP-1 |
| | | | | 5003 | 5003 | 5003 | | | | | 5002 | 5002 | 5002 | | | | | 5001 | 5001 | 5001 | | | | | | 0 |
| | 3.9 | | | 31.2 | 28.9 | 33.8 | | 4.4 | | | 28.9 | 31.3 | 34.1 | | 6.0 | | | 32.4 | 32.2 | 30.4 | _ | 8.0 | | | Non-dec | |
| 2.3 | - 721 | | | 100 | 96 | 139 | 2.4 | - 122 | | | 94.2 | 98.9 | 144 | 2.9 | - 149 | | | 115 | 120 | 95.0 | 6.7 |) - 95 | | | convolut | |
| | | | | 21 | 35 | 44 | | | | | 29 | 30 | 41 | | | | | 15 | 40 | 44 | | | | | éd | |

Table S1: MES analysis of iASPP-PP-1c SAXS data, Related to Figure 3

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| Pool (library) | | | | | | Experii | nental E |)ata: iAS | pp-pp-1 | 0 | | | |
|----------------|-----------------------------------------|------|------|--------|----|---------|----------|-----------|---------|------|------|--------|----|
| | Pool ID/R _G /Dmax/% | 5004 | 31.7 | 111 | 65 | 5004 | 31.8 | 108 | 46 | 5004 | 31.0 | 91.5 | 66 |
| | | 5004 | 27.6 | 81.9 | 32 | 5004 | 31.0 | 91.5 | 45 | 5004 | 40.6 | 128 | 17 |
| | | 5004 | 43.5 | 126 | ω | 5004 | 43.4 | 124 | 9 | 5004 | 27.4 | 91.4 | 17 |
| | R _G Range of Pool | | | | | | 26.1 | - 54.3 | | | | | |
| | D _{max} Range of Pool | | | | | | 73 | - 225 | | | | | |
| | FOXS Chi ² Range of Pool | | 1.3 | - 306 | | | 1.9 | - 518 | | | 2.7 | - 1162 | |
| 5005 | Chi ² of best model/ensemble | | | 1.0 | | | | L.7 | | | | 1.6 | |
| | Pool ID/R _G /Dmax/% | 5005 | 32.3 | 117 | 35 | 5005 | 32.3 | 117 | 37 | 5005 | 32.3 | 117 | 59 |
| | | 5005 | 31.3 | 120 | 35 | 5005 | 32.6 | 129 | 36 | 5005 | 32.7 | 128 | 29 |
| | | 5005 | 28.9 | 94.2 | 29 | 5005 | 32.0 | 125 | 27 | 5005 | 26.7 | 68 | 12 |
| | R ₆ Range of Pool | | | | | | 27.7 | - 38.0 | | | | | |
| | D _{max} Range of Pool | | | | | | 89.1 | 208 | | | | | |
| | FOXS Chi ² Range of Pool | | 1.9 | 9 - 57 | | | 2.9 | - 94 | | | 3.0 | - 178 | |
| 5006 | Chi ² of best model/ensemble | | | 1.1 | | | | 2.0 | | | | 2.0 | |
| | Pool ID/R _G /Dmax/% | 5006 | 28.6 | 90.2 | 44 | 5006 | 34.6 | 134 | 53 | 5006 | 34.6 | 134 | 45 |
| | | 5006 | 31.5 | 98.3 | 34 | 5006 | 28.8 | 91.9 | 24 | 5006 | 28.8 | 91.9 | 34 |
| | | 5006 | 34.3 | 132 | 22 | 5006 | 31.8 | 98.3 | 23 | 5006 | 31.8 | 98.3 | 21 |
| | R ₆ Range of Pool | | | | | | 24.3 | - 56.1 | | | | | |
| | D _{max} Range of Pool | | | | | | 71 | - 202 | | | | | |
| | FOXS Chi ² Range of Pool | | 1.4 | - 378 | | | 2.5 | - 676 | | | 5.0 | - 1525 | |
| 5007 | Chi ² of best model/ensemble | | | 1.0 | | | Ц | | | | | 1.3 | |
| | Pool ID/R _G /Dmax/% | 5007 | 31.6 | 106 | 50 | 5007 | 31.6 | 105 | 62 | 5007 | 31.6 | 105 | 44 |
| | | 5007 | 28.6 | 91 | 41 | 5007 | 38.1 | 121 | 22 | 5007 | 28.1 | 90.3 | 29 |
| | | 5007 | 37.8 | 118 | 9 | 5007 | 28.0 | 92.9 | 16 | 5007 | 36.9 | 135 | 27 |

Table S1: MES analysis of iASPP-PP-1c SAXS data, Related to Figure 3

5001, 5002, 5003, 5004, 5005, **Combined Pools** Pool (library)¹ 5011 5013 Pool ID/R₆/Dmax/% Chi² of 3 model ensemble Pool ID/R_G/Dmax/% Chi² of best model/ensemble FOXS Chi² Range of Pool D_{max} Range of Pool R_G Range of Pool Pool ID/R_G/Dmax/%⁴ Chi² of best model/ensemble FOXS Chi² Range of Pool³ D_{max} Range of Pool R_G Range of Pool 5007 5013 5011 5011 5011 Deconvoluted Small² 29.1 28.3 29.7 29.6 29.5 1.3 - 4.83.9 - 123.9 1.2 1.3 89.7 98.0 94.2 88.5 105 36 66 9 100 25 5013 Experimental Data: ASPP2-PP-1c 5005 5011 Deconvoluted Large² 32.4 28.3 29.7 27.4 - 28.3 28.9 - 29.7 45 – 99 90 - 110 84 - 100 22 – 56 1.3 \$ 22 97.2 120 88.5 57 100 100 5013 5011 5007 Non-deconvoluted² 32.1 29.7 28.3 57 21 – 7 - 143 1.3 57 21 97.2 115 88.5 2 100 100 ω

² Experimental SAXS data was deconvoluted using RAW ¹ Identifier for the model library (pool) created using BILBO-MD

5006, 5007

5001 5005

28.9 31.3

90.0 109

28

5007

71.8

N 40

5003 5005

28.6

94.9

22 25

36

5007

31.6 24.6

105

32.2

122

³ Chi2 are calculated for the individual models in the pool

model; %: fraction of the ensemble represented by the model ⁴ Pool ID: the code representing the BILBO-MD library; R_G: radius of gyration for the selected model; Dmax: maximum pairwise interatomic distance in the selected

Table S2: MES analysis of ASPP2-PP-1c SAXS data, Related to Figure 3

| | 5004 | 6002 | | | | | 5003 | | | | | | | 5002 | | | | | | | 5001 | | | | | Pool (library) |
|-----------------------------------------|-------------------------------------|--------------------------------|------------------------------|------|------|--------------------------------|-----------------------------------------|-------------------------------------|--------------------------------|------------------------------|------|------|--------------------------------|-----------------------------------------|-------------------------------------|--------------------------------|------------------------------|------|------|--------------------------------|-----------------------------------------|-------------------------------------|--------------------------------|------------------------------|----------|----------------|
| Chi ² of best model/ensemble | FOXS Chi ² Range of Pool | D _{max} Range of Pool | R ₆ Range of Pool | | | Pool ID/R _G /Dmax/% | Chi ² of best model/ensemble | FOXS Chi ² Range of Pool | D _{max} Range of Pool | R _G Range of Pool | | | Pool ID/R _G /Dmax/% | Chi ² of best model/ensemble | FOXS Chi ² Range of Pool | D _{max} Range of Pool | R _G Range of Pool | | | Pool ID/R _G /Dmax/% | Chi ² of best model/ensemble | FOXS Chi ² Range of Pool | D _{max} Range of Pool | R _G Range of Pool | | |
| | | | | 5003 | 5003 | 5003 | | | | | 5002 | 5002 | 5002 | | | | | 5001 | 5001 | 5001 | | | | | _ | |
| | 1.8 | | | 28.9 | 28.9 | 30.8 | | 1.4 | | | 28.6 | 31.3 | 28.7 | | 1.7 | | | 28.4 | 29.0 | 30.2 | | 2.3 | | | Deconvo | |
| L.2 | - 183 | | | 94.0 | 90.5 | 116.3 | L.2 | - 43 | | | 91.5 | 98.4 | 91.3 | L.2 | - 38 | | | 91.7 | 91.6 | 98.1 | 1.6 | - 22 | | | luted Sm | |
| | | | | 24 | 33 | 44 | | | | | 21 | 37 | 42 | | | | | 4 | 38 | 58 | | | | | all | |
| | | | | 5003 | 5003 | 5003 | | | | | 5002 | 5002 | 5002 | | | | | 5001 | 5001 | 5001 | | | | | | Experin |
| N | 2.7 | 75.4 | 25.8 | 28.9 | 31.2 | 33.8 | | 3.4 | 89.1 | 28.1 | 28.8 | 34.3 | 31.8 | N | 4.4 | .68 | 27.9 | 32.2 | 30.4 | 32.4 | 4 | 5.8 | 89.2 | 28.3 | econvol | nental D |
| .2 | - 511 | - 139 | - 46.4 | 95.7 | 100 | 119 | .9 | - 97 | - 152 | - 34.7 | 92.1 | 144 | 105 | 8 | - 114 | - 185 | - 37.3 | 120 | 95.0 | 115 | .9 | - 76 | - 126 | - 33.6 | uted Lar | ata: ASP |
| | | | | 18 | 40 | 42 | | | | | 25 | 29 | 46 | | | | | 25 | 29 | 46 | | | | | ge | P2-PP-1 |
| | | | | 5003 | 5003 | 5003 | | | | | 5002 | 5002 | 5002 | | | | | 5001 | 5001 | 5001 | | | | | | C |
| | 3.2 | | | 31.2 | 33.8 | 28.9 | | 3.2 | | | 34.1 | 32.0 | 28.9 | | 6.9 | | | 30.2 | 30.4 | 32.2 | | 7.3 | | | Non-dec | |
| 1.9 | - 1029 | | | 100 | 119 | 93.4 | 1.7 | - 138 | | | 144 | 103 | 94.2 | 2.8 | - 169 | | | 98.1 | 95.0 | 120 | 5.7 | - 104 | | | convolut | |
| | | | | 22 | 37 | 41 | | | | | 18 | 38 | 44 | | | | | 16 | 40 | 44 | | | | | ed | |

Table S2: MES analysis of ASPP2-PP-1c SAXS data, Related to Figure 3

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| | | | 5007 | | | | | | | 5006 | | | | | | | 5005 | | | | | | | Pool (library) |
|------|------|--------------------------------|-----------------------------------------|-------------------------------------|--------------------------------|------------------------------|------|------|--------------------------------|-----------------------------------------|-------------------------------------|--------------------------------|------------------------------|------|------|--------------------------------|-----------------------------------------|-------------------------------------|--------------------------------|------------------------------|------|------|--------------------------------|----------------|
| | | Pool ID/R _G /Dmax/% | Chi ² of best model/ensemble | FOXS Chi ² Range of Pool | D _{max} Range of Pool | R _G Range of Pool | | | Pool ID/R _G /Dmax/% | Chi ² of best model/ensemble | FOXS Chi ² Range of Pool | D _{max} Range of Pool | R _G Range of Pool | | | Pool ID/R _G /Dmax/% | Chi ² of best model/ensemble | FOXS Chi ² Range of Pool | D _{max} Range of Pool | R ₆ Range of Pool | | | Pool ID/R _G /Dmax/% | |
| 5007 | 5007 | 5007 | | | | | 5006 | 5006 | 5006 | | | | | 5005 | 5005 | 5005 | | | | | 5004 | 5004 | 5004 | |
| 31.6 | 28.0 | 30.3 | | 1.5 | | | 28.6 | 28.7 | 31.6 | | 1.4 | | | 31.6 | 28.4 | 29.5 | | 2.4 | | | 28.9 | 27.2 | 31.7 | |
| 103 | 85.9 | 95.7 | 1.2 | - 373 | | | 89.7 | 91.5 | 114 | 1.2 | - 65 | | | 103 | 87.7 | 95.3 | 1.2 | - 300 | | | 92.0 | 83.4 | 105 | |
| 23 | 34 | 43 | | | | | 26 | 34 | 41 | | | | | 28 | 31 | 40 | | | | | 28 | 31 | 41 | |
| 5007 | 5007 | 5007 | | | | | 5006 | 5006 | 5006 | | | | | 5005 | 5005 | 5005 | | | | | 5004 | 5004 | 5004 | Experii |
| 27.8 | 37.8 | 31.4 | | 2.5 | 71 | 24.3 | 28.7 | 31.8 | 34.5 | | 2.6 | .68 | 27.7 | 26.9 | 32.3 | 32.3 | | 1.8 | 73.1 | 26.1 | 41.9 | 30.9 | 31.5 | mental E |
| 89.1 | 118 | 101 | 1.2 | - 1057 | - 202 | 3 - 56.1 | 94.7 | 102 | 132 | 2.0 | - 137 | 1 - 208 | - 38.0 | 90.4 | 117 | 119 | 1.4 | - 815 | 1 - 225 | - 54.3 | 120 | 88.5 | 105 |)ata: ASI |
| 9 | 18 | 72 | | | | | 23 | 36 | 41 | | | | | 10 | 42 | 47 | | | | | 7 | 24 | 68 | PP2-PP-1 |
| 5007 | 5007 | 5007 | | | - | | 5006 | 5006 | 5006 | | | | | 5005 | 5005 | 5005 | | | | | 5004 | 5004 | 5004 | Ĉ |
| 37.8 | 27.7 | 32.1 | | 3.9 | | | 34.3 | 28.7 | 31.6 | | 3. 3. | | | 26.8 | 31.8 | 32.3 | | 2.3 | | | 27.0 | 34.5 | 31.5 | |
| 123 | 95.3 | 114 | 1.4 | - 2156 | | | 132 | 94.7 | 98.4 | 1.9 | - 249 | | | 89.1 | 110 | 117 | 1.5 | - 1652 | | | 77.0 | 112 | 106 | |
| л | 21 | 75 | | | | | 28 | 31 | 41 | | | | | 20 | 28 | 52 | | | | | 21 | 22 | 58 | |

Table S2: MES analysis of ASPP2-PP-1c SAXS data, Related to Figure 3

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