Supporting Information

"Characterization of An Extensive Interface on Vitronectin for Binding to PAI-1: Adoption of Structure in an Intrinsically Disordered Region"

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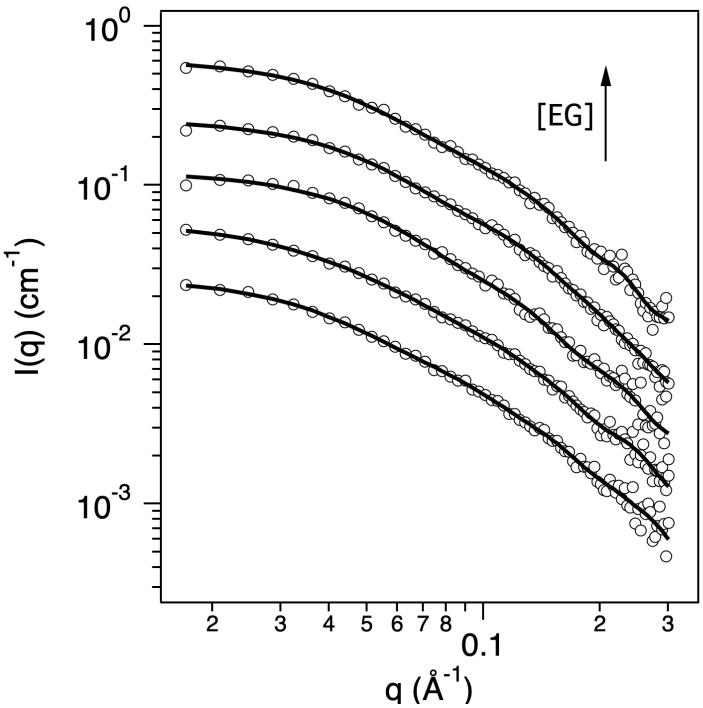
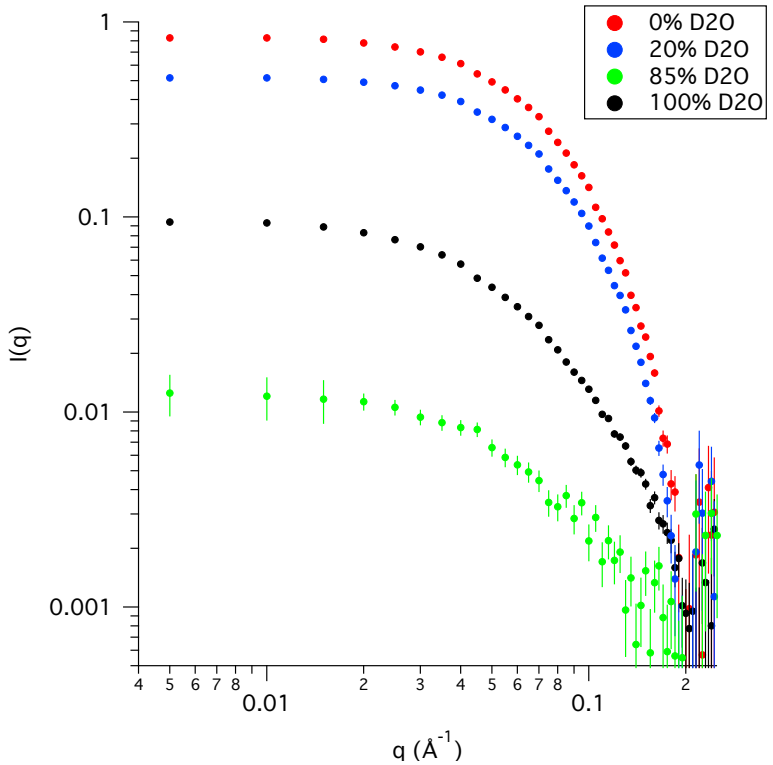
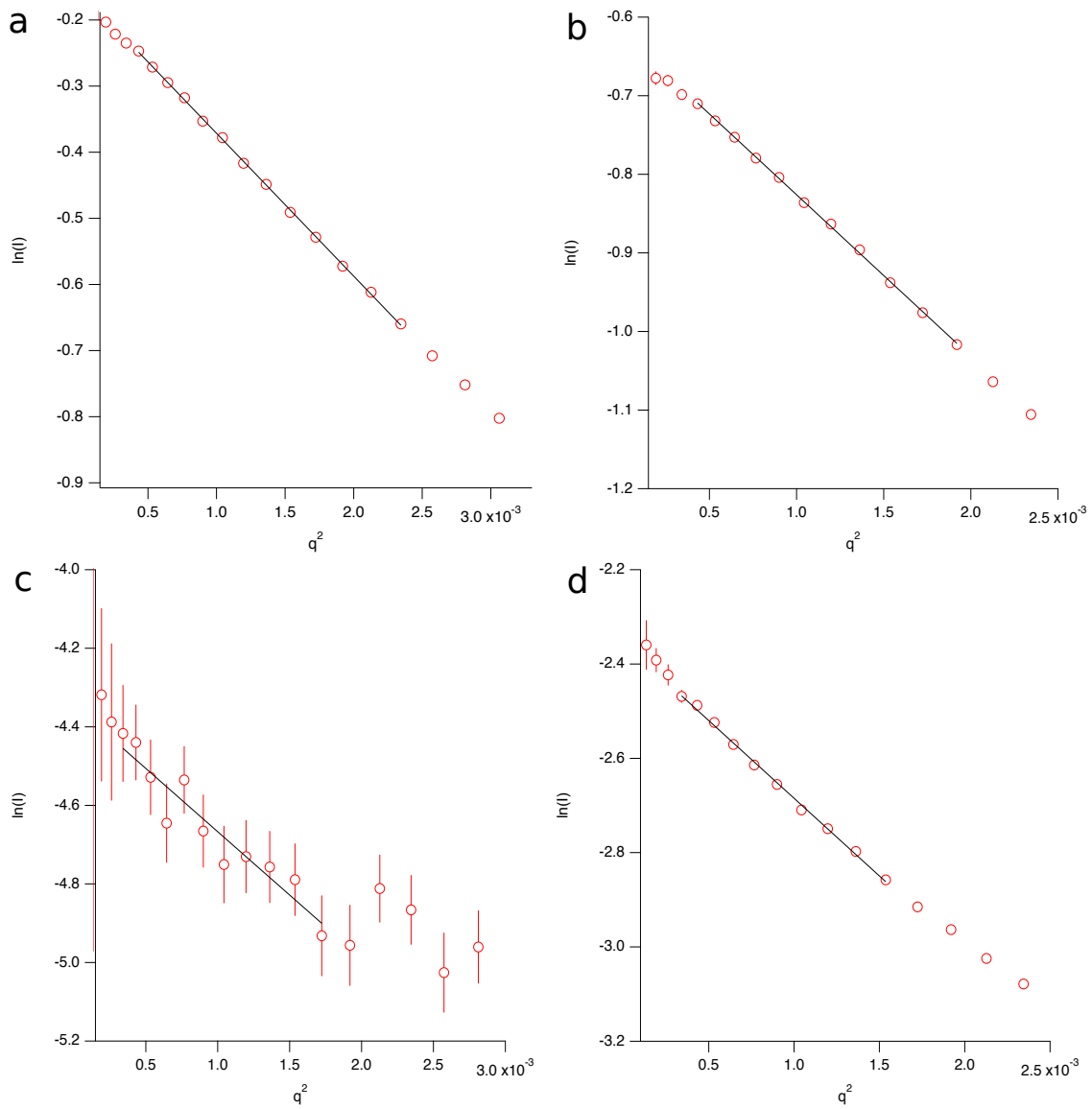
Five figures and three tables are provided as supporting information for this manuscript. These include primary data for SANS experiments, Guinier plots and analysis, scattering length density and match point plots, Stuhrmann analyses, and R_g and center of mass values for best-fit structures for the contrast variation SANS data.



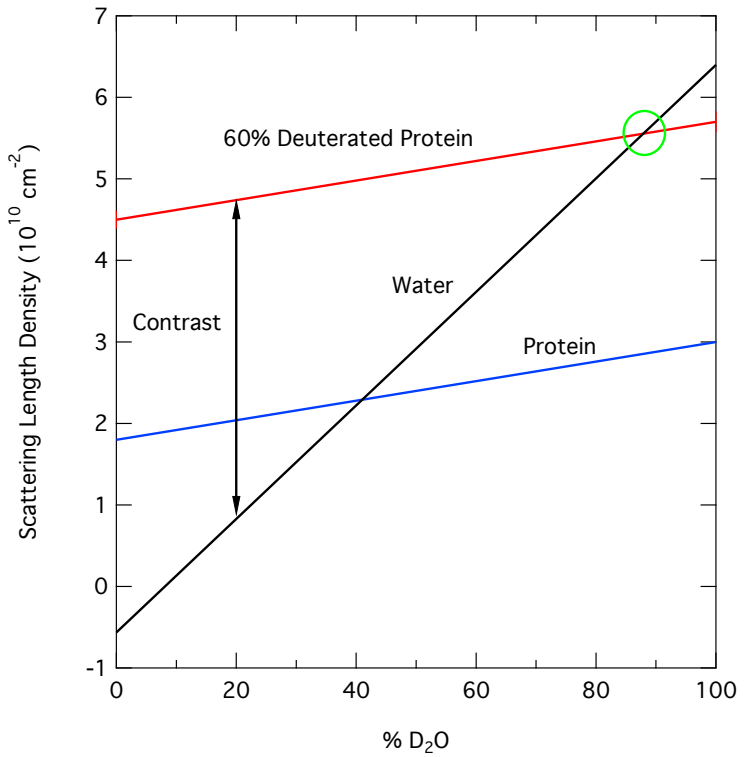
<u>Figure S1</u>. SANS on SMB-IDD with increasing deuterated EG (indicated by arrow; 0, 1.8, 2.9, 4.9, 7.7 osm). The curve with 0 EG is on absolute scale, with all other curves shifted upward for clarity. Solid lines are EOM fits to the data, yielding the *Rg* distribution heat map shown in Fig. 3b. Errors are standard errors of the mean based on the number of pixels used in the data averaging and are smaller than the size of the data points.



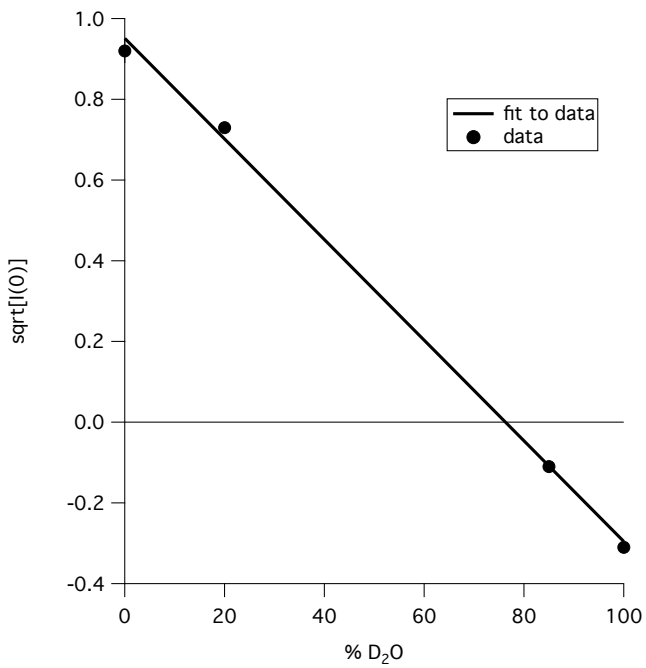
<u>Figure S2</u>. Contrast Variation SANS Experiments on PAI-1:SMB-IDD Complex. SANS on deuterated-PAI-1:SMB-IDD is shown for four different D_2O buffer conditions. Errors are standard errors of the mean based on the number of pixels used in the data averaging.



<u>Figure S3</u>. Guinier Plots for Contrast Variation SANS Experiments on PAI-1:SMB-IDD Complex. Representative Guinier fits (solid lines) to the SANS data (open circles) on deuterated PAI-1:SMB-IDD in a) 0% D_2O, b) 20% D_2O, c) 85% D_2O and d) 100% D_2O. Errors were propagated from the standard error of the mean on the SANS intensities based on the number of pixels used in the data averaging.



<u>Figure S4</u>. Contrast Variation Neutron Scattering Length Density Plot. Plot of neutron scattering length density versus % D_2O in the solvent illustrating the contrast between 60% deuterated PAI-1 and water in 20% D_2O by the two-sided arrow between the black and red lines. Also shown is the match point of 60% deuterated PAI-1 at 85% D_2O buffer (green circle), where the scattering length density of the 60% deuterated protein and water are the same. Similarly, for protiated SMB-IDD, the match point is at 40% D_2O and the contrast between protein and water in 20% D_2O would be drawn between the black and blue lines. Thus, the scattering is dominated by PAI-1 in 20% D_2O buffer, since the contrast between the black (water) and blue lines (protiated protein) is less than that between the black (water) and red (60% deuterated protein) lines. Similarly, the scattering is dominated by PAI-1 in 0% D_2O, whereas it is dominated by SMB-IDD in 85% D_2O.



<u>Figure S5</u>. Match Point Analysis for Contrast Variation SANS Experiments on PAI-1:SMB-IDD Complex. The points are the $\sqrt{I(0)}$ values obtained as a function of % D_2O from the SANS data. Errors are propagated from the I(0) values obtained from the Guinier fits and are smaller than the data points. The solid black line is the weighted linear fit to the data. The sample concentration was 7.7 mg/mL in all cases. The x-intercept is the match point of the complex, which was calculated to be 76.29 ± 0.03 % D_2O. This value agrees well with the value of 77 % D_2O calculated from the amino acid sequences of PAI-1 and SMB-IDD assuming that PAI-1 is 60% deuterated.

% D_2O	Guinier R_g (Å)	Guinier I(0) (cm^{-1})	qR_g
0	25.45 ± 0.07	0.856 ± 0.001	0.53 – 1.23
20	24.95 ± 0.09	0.539 ± 0.001	0.52 – 1.21
85	28 ± 3[#]	0.012 ± 0.001[#]	0.53 – 1.23[#] 0.64 – 1.27
100	31.3 ± 0.4	0.095 ± 0.001	0.58 – 1.23

Errors are standard errors from the linear fit to the Guinier equation.
[#]Average taken from two different Guinier ranges due to noisy data. Error is the standard error of the mean.

Table S1. Parameters from Guinier Analysis of SANS Data from the PAI-1:SMB-IDD Complex. Radius of gyration, *Rg* and forward scattering, I(0), obtained from Guinier analysis of the SANS contrast variation data. The sample concentration was 7.7 mg/mL in all cases.

	Stuhrmann Analysis*	Parallel Axis Analysis
PAI-1 *Rg* (Å)	23.64 ± 0.12	23.58 ± 0.13
SMB-IDD Rg (Å)	30.31 ± 0.28	30.32 ± 0.29
Center of Mass Distance (Å)	17.92 ± 0.96	18.43 ± 0.99

*α = -249 ± 15 and β = 72 ± 8
Errors are standard deviations.

Table S2. Stuhrmann and Parallel Axis Analysis of SANS Contrast Variation Data for PAI-1:SMB-IDD Complex. The Stuhrmann analysis α parameter, is negative, indicating that the component with the lower scattering length density (SMB-IDD) lies toward the periphery of the complex. The non-zero β parameter indicates that the centers of mass of PAI-1 and SMB-IDD are not concentric. The 100% D_2O data were not included in this analysis.

Structure Number	CM Distance (Å)	R_g of PAI-1 (Å)	R_g of SMB-IDD (Å)
1	25.43	21.86	35.07
2	30.62	21.86	28.96
3	35.14	21.86	30.80
4	33.15	21.86	33.19
5	31.37	21.86	30.14
6	29.13	21.86	30.46
7	22.85	21.86	35.18
8	28.74	21.86	34.14
9	28.91	21.86	31.07
10	28.58	21.86	32.21
11	33.66	21.86	29.68
12	29.11	21.86	33.54
13	29.61	21.86	33.75
14	32.16	21.86	33.15
15	30.85	21.86	33.32
16	27.54	21.86	30.99
17	33.78	21.86	29.64
18	29.19	21.86	34.53
19	33.91	21.86	29.56
20	29.39	21.86	29.76
21	28.83	21.86	30.00
22	32.87	21.86	28.54
23	30.01	21.86	29.68
24	32.50	21.86	28.82
25	33.26	21.86	32.27
26	28.88	21.86	37.80
27	30.41	21.86	32.46
28	31.66	21.86	29.46
29	31.71	21.86	33.56
30	20.65	21.86	35.38

Table S3. *Rg* and Center of Mass Distance Values for the 30 Best-Fit Structures at all Contrasts. The center of mass (CM) distance between the PAI-1 and SMB-IDD components, as well as the radii of gyration for each component are shown as a function of structure number. Structure number 16 corresponds to the best-fit structure at all contrasts, with a χ^2 value of 1.29 (85% D_2O). Structure number 13 has the highest χ^2 value of 3.95. All R_g values for SMB-IDD agree well with the value of 30.31 ± 0.28 Å calculated from the Stuhrmann analysis (Table S2). The *Rg* of PAI-1, at a fixed value of 21.86 Å, is a little smaller the value of 23.64 ± 0.12 Å from the Stuhrmann analysis. The CM distances of the best-fit structures are significantly larger than the value of 18 ± 1 Å from the Stuhrmann analysis in almost all cases. It may be that the CM distance was not determined accurately from the Stuhrmann analysis since only one contrast in which SMB-IDD was dominant was used (85% D_2O). Thus, the analysis was perhaps biased toward contrasts in which PAI-1 was dominant. However, the CM distances from

the best-fit structures are still indicative of a compact complex where IDD is in close proximity to PAI-1 and SMB.