

Supporting Information

Investigation of encapsulation of insulin biotemplate within C-methylresorcin[4]arenes

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In this section of supporting information, we report the buffer composition of insulin, BCA analysis of insulin, scattering length densities, full sets of fitting parameters of the Schulz sphere fits and bimodal Schulz sphere fits for insulin, C-methylresorcin[4]arene (RsC₁) and insulin with RsC₁ in D₂O/h6-acetone, D₂O/d6-acetone and D₂O/h6-acetone/d6-acetone solvent systems. Fitted values are compared in a tabulated result summary. All uncertainties reported are one standard deviation of that fitted parameter. Please find below the list of tables and data sets with corresponding explanations.

INSULIN COMPOSITIONS AND DIALYSIS:

The concentration of humalog insulin was estimated to be around 10 mg/ml from the spectroscopic bicinchoninic acid (BCA) protein assay (supporting information). Humalog insulin (A chain 21; B chain 30 amino acids; Mol Wt: 5808 Da) is essentially a human insulin analogue formed by interchanging the amino acids position 28 (Pro) and position 29 (Lys) on the B-chain of insulin. LYSPRO or humalog insulin, obtained commercially, was dialyzed in D₂O buffer to obtain improved scattering statistics in deuterated solvent.

Buffer composition for dialysis experiment:

Buffer composition/100 ml of D₂O: 16 mg glycerin, 1.88 mg dibasic sodium phosphate, 3.15 mg metacresol, zinc oxide content adjusted to provide 0.0197 mg zinc ion, trace amounts of phenol and pH adjusted to 7.0 to 7.8.

SOLVENT COMPOSITIONS:

Solvents systems were composed of (1) 4 equiv. of D₂O with 1 equiv. of h6-acetone ($3.7 \times 10^{-6} \text{ \AA}^{-2}$), (2) 4 equiv. of D₂O with 1 equiv. of d6-acetone (SLD = $6.3 \times 10^{-6} \text{ \AA}^{-2}$), and (3) 4 equiv. of D₂O with 0.5 equiv. of d6-acetone and 0.5 equiv. of h6-acetone ($5.0 \times 10^{-6} \text{ \AA}^{-2}$).

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Table 1. BCA Protein Assay for Humalog insulin

Conc. ($\mu\text{g/ml}$)	Absorbance	$\mu\text{g/ml}$	mg/ml	1:20 dilution	$\mu\text{g/ml}$	mg/ml	1:100 dilution	$\mu\text{g/ml}$	mg/ml
0	0.107								
25	0.145								
125	0.314								
250	0.491								
500	0.815								
750	1.032								
1000	1.389								
1500	1.766								
2000	2.394								
Insulin:H ₂ O DILUTIONS									
1:10 dilution		$\mu\text{g/ml}$	mg/ml	1:20 dilution	$\mu\text{g/ml}$	mg/ml	1:100 dilution	$\mu\text{g/ml}$	mg/ml
1.193	924.1818	924.1818	9.241818	0.744	516	10.32	0.278	92.36364	9.236364
1.281	1004.182	1004.182	10.04182	0.746	517.8182	10.35636	0.274	88.72727	8.872727
1.223	951.4545	951.4545	9.514545	0.747	518.7273	10.37455	0.308	119.6364	11.96364
Insulin: buffer		9.599394	9.599394			10.3503			10.02424
1:10 dilution		$\mu\text{g/ml}$	mg/ml	1:20 dilution	$\mu\text{g/ml}$	mg/ml	1:100 dilution	$\mu\text{g/ml}$	mg/ml
1.347	1064.182	1064.182	10.64182	0.828	592.3636	11.84727	0.288	101.4545	10.14545
1.395	1107.818	1107.818	11.07818	0.833	596.9091	11.93818	0.308	119.6364	11.96364
1.234	961.4545	961.4545	9.614545	0.863	624.1818	12.48364	0.303	115.0909	11.50909
	10.44485	10.44485	10.44485			12.0897			11.20606

Average concentration of humalog insulin in mg/ml= 10.61909

NOTE: BCA (bicinchoninic acid) protein assay of insulin of humalog insulin gave 10.6 mg/ml concentration of insulin.

Supporting information: Small-angle neutron scattering (SANS)

Contrast Variation Method:

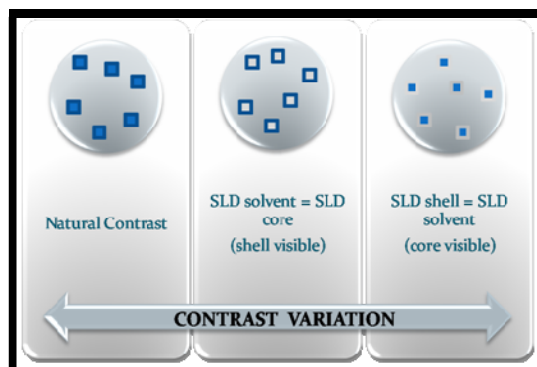


Figure 1. Contrast variation: (left) Natural contrast; (middle) the SLD of solvent is equal to that of solvent that makes shell visible; (right) the SLD of shell is equal to that of solvent that makes core visible

Table 2. Scattering length densities (SLD)

S.No.	SAMPLE	Formula	Density (g/ml)	λ (Å)	SLD (Å ⁻²)
1	4 D ₂ O : 1 d-acetone	D ₈ O ₄ C ₃ D ₆ O	1.06	6	6.3x10 ⁻⁶
2	4 D ₂ O : 1 h-acetone	D ₈ O ₄ C ₃ D ₆ O	1.06	6	3.7x10 ⁻⁶
3	4 D ₂ O : 1 d-acetone: 1 h-acetone	D ₈ O ₄ C ₃ D ₆ O C ₃ H ₆ O	1.06	6	5.0x10 ⁻⁶
4	RSC ₁ Hexamer	C ₂₄₀ H ₂₄₀ O ₄₈	1.4	6	2.11x10 ⁻⁶
5	Insulin Monomer	C ₂₅₇ H ₃₈₃ N ₆₅ O ₇₇ S ₆	1.105	6	1.54 x10 ⁻⁶
6	Insulin Dimer	(C ₂₅₇ H ₃₈₃ N ₆₅ O ₇₇ S ₆)*2	1.105	6	1.54 x10 ⁻⁶
7	Insulin Hexamer	(C ₂₅₇ H ₃₈₃ N ₆₅ O ₇₇ S ₆)*6	1.105	6	1.54 x10 ⁻⁶
8	RSC ₁ octamer+Insulin Monomer	(C ₄₀ H ₄₀ O ₈) ₈ +C ₂₅₇ H ₃₈₃ N ₆₅ O ₇₇ S ₆ = C ₅₇₇ H ₇₀₃ N ₆₅ O ₁₄₁ S ₆	1.4	6	2.03x10 ⁻⁶
9	24 RSC ₁ +Insulin Monomer	(C ₄₀ H ₄₀ O ₈) ₂₄ +C ₂₅₇ H ₃₈₃ N ₆₅ O ₇₇ S ₆ = C ₁₂₁₇ H ₁₃₄₃ N ₆₅ O ₂₆₉ S ₆	1.4	6	2.08 x10 ⁻⁶

NOTE: The scattering length densities of the three solvent systems (S.No. 1-3), macrocycle (S.No. 4), insulin (S. No. 5-7) and macrocycle with insulin (S. No. 8-9) have been calculated. These scattering length densities are used for data analyses of small-angle neutron scattering data curves. The scattering length densities for insulin monomer, dimer and hexamer do not change with change in molecular mass.

EXPERIMENT DETAILS:

Two sets of experiments were prepared to investigate the reproducibility of results. For the experiment 1, the scattering data for the RsC₁ is fitted to Schulz sphere while that of Insulin and Insulin with RsC₁ is fitted to bimodal Schulz sphere model. For experiment 2, insulin and insulin with RsC₁ samples are fitted to bimodal Schulz sphere model. RsC₁ scattering data is fitted to both bimodal Schulz sphere and Schulz sphere model to investigate the presence of any larger species. The following section has a table summarizing the experimental samples followed by individual data sets fitted to either Schulz sphere or bimodal Schulz sphere models.

EXPERIMENT

Table 3. SANS Result Summary of Insulin and C-methylresorcin[4]arene templation experiment

EXPERIMENT SET 2							
Sample Code	SLD Solvent (Å ⁻²)	R1 (Å)	R2 (Å)	Sqrt(χ^2/N)	SLD R1 (Å ⁻²)	SLD R2 (Å ⁻²)	MODEL
A =RsC ₁	3.70E-06	6.9	10.1	3.63	2.11E-06	2.11E-06	Bimodal Schulz Sphere
A =RsC ₁	3.70E-06	7.0		7.60	2.11E-06		Schulz Sphere
B = RsC ₁	6.30E-06	8.5		2.03	2.11E-06		Schulz Sphere
B = RsC ₁	6.30E-06	7.2	10.2	1.55	2.11E-06	2.11E-06	Bimodal Schulz Sphere
C = RsC ₁	5.00E-06	7.0	10.0	2.84	2.11E-06	2.11E-06	Bimodal Schulz Sphere
C = RsC ₁	5.00E-06	7.3		4.27	2.11E-06		Schulz Sphere
L= D ₂ O hAc	3.70E-06	6.4		2.09	2.11E-06		Schulz Sphere
K= D ₂ O dAc	6.30E-06	7.0		1.18	2.11E-06		Schulz Sphere
M= D ₂ O hdAc	5.00E-06	5.9		1.41	2.11E-06		Schulz Sphere
E = Insulin	6.30E-06	10.7	25	2.99	1.54E-06	1.54E-06	Bimodal Schulz Sphere
E2 = Insulin	5.00E-06	10.1	28	1.48	1.54E-06	1.54E-06	Bimodal Schulz Sphere
E3 = Insulin	3.70E-06	10.9	25	2.18	1.54E-06	1.54E-06	Bimodal Schulz Sphere
G= RsC ₁ +Insulin	3.70E-06	7.0	16.3	1.62	2.11E-06	2.03E-06	Bimodal Schulz Sphere
H= RsC ₁ +Insulin	6.30E-06	6.8	16.4	1.89	2.11E-06	2.03E-06	Bimodal Schulz Sphere
J= RsC ₁ +Insulin	5.00E-06	7.0	16.5	1.43	2.11E-06	2.03E-06	Bimodal Schulz Sphere

NOTE: The results reveal the radius of ~7Å and ~10Å for RsC₁ that corresponds to a dimer and a hexamer respectively. The insulin sample fits to bimodal Schulz sphere model and yields radius of ~10Å & ~24Å that corresponds to an insulin monomer and an insulin trimer. RsC₁ with insulin samples reveals presence of two species of radius ~10 Å and ~16.5 Å. For RsC₁ with insulin sample, the 10Å species corresponds to the radius of either an insulin monomer or RsC₁ hexamer while ~16 Å species corresponds to insulin templated with RsC₁ macrocycle.

1) Bimodal Schulz sphere fit results for ReC₁ in D₂O/h6-acetone solvent

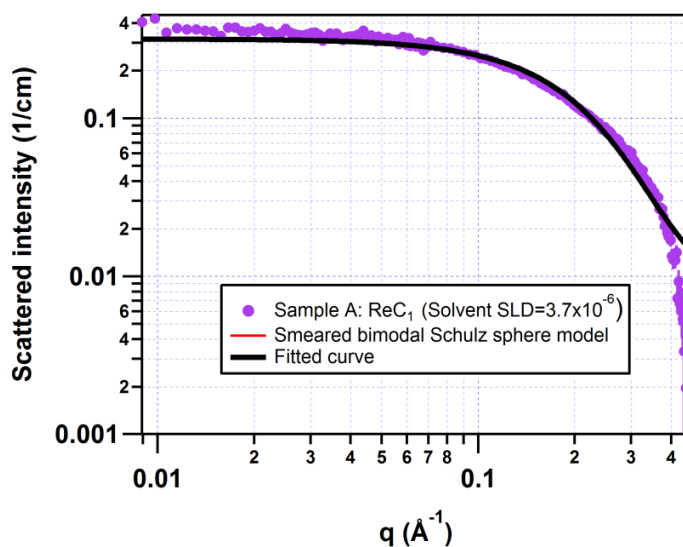


Figure 2. SANS Smeared bimodal Schulz sphere fitting for RsC₁ in a solvent with SLD of $3.7 \times 10^{-6} \text{ \AA}^{-2}$

volume fraction(1)	0.224834	±	0.00361554
Radius (1) (Å)	6.91071±		0.00322399
polydispersity(1)	0.200847	±	8.40673e-05
SLD(1) (Å ⁻²)	2.11e-06	±	0
volume fraction(2)	0.110196	±	0.00128328
Radius (2)	10.1481±		0.0104009
polydispersity(2)	0.198482	±	0.000216823
SLD(2)	2.11e-06	±	0
SLD (solvent)	3.7e-06 ±		0
background (cm ⁻¹ sr ⁻¹)	0.00906032	±	0.00039851

chisq = 2485.75

Npnts = 188

Sqrt(χ^2/N) = 3.63622

Fitted range = [0,187] = $0.008936 < Q < 0.4578$

NOTE: The SANS data for RsC₁ for D₂O with h6-acetone (SLD $3.7 \times 10^{-6} \text{ \AA}^{-2}$) fits well to the bimodal Schulz sphere model with $\chi^2 = 3.6$. The χ^2 value is reasonable with small error bars on the parameters and gives a reasonable representation of the data.

2) Schulz sphere fit results for RsC₁ in D₂O/h6-acetone solvent

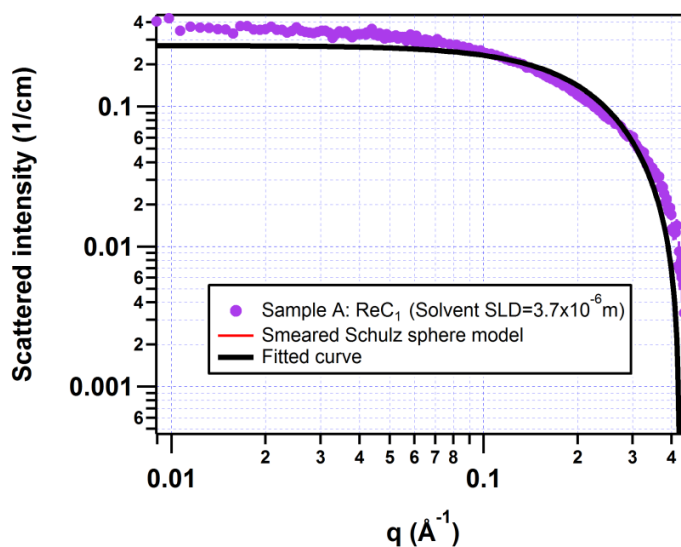


Figure 3. SANS Smearred Schulz sphere fitting for RsC₁ in a solvent with SLD of $3.7 \times 10^{-6} \text{ \AA}^{-2}$

Volume Fraction (scale)	0.520961	±	0.00103244
mean radius (Å)	7.03388	±	0.00108475
polydisp (sig/avg)	0.194957	±	1.73937e-05
SLD sphere (Å ⁻²)	2.11e-06	±	0
SLD solvent (Å ⁻²)	3.7e-06	±	0
bkg (cm ⁻¹ sr ⁻¹)	-0.0162878	±	0.000270899

chisq = 10865.5

Npnts = 188

Sqrt(χ^2/N) = 7.60233

Fitted range = [0,187] = $0.008936 < Q < 0.4578$

NOTE: The SANS data for RsC₁ in D₂O with h6-acetone (SLD $3.7 \times 10^{-6} \text{ \AA}^{-2}$) fits poor to the Schulz sphere model with $\chi^2 = 7.6$. The χ^2 value is high and visually the model does not give a good representation of the data.

3) Schulz sphere fit results for RsC₁ in D₂O/d6-acetone solvent

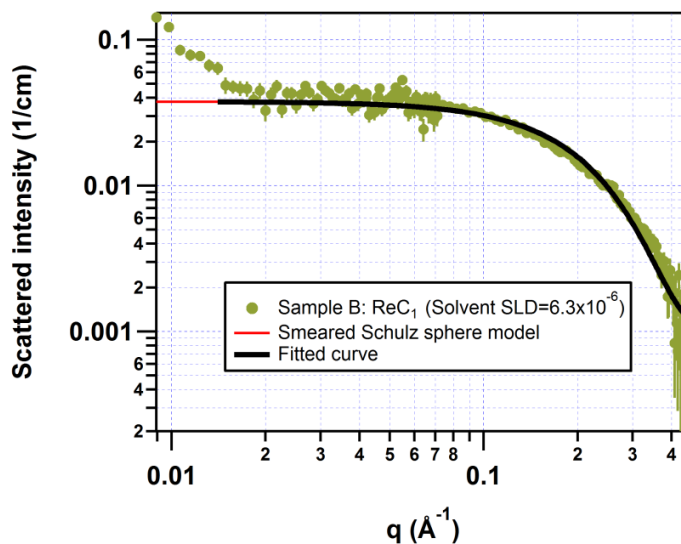


Figure 4. SANS Smeared Schulz sphere fitting for RsC₁ in a solvent with SLD of $6.3 \times 10^{-6} \text{ \AA}^{-2}$

Volume Fraction (scale)	0.00540242	±	3.66138e-05
mean radius (\AA)	8.49774	±	0.0147914
polydisp (sig/avg)	0.193473	±	0.000581531
SLD sphere (\AA^{-2})	2.11e-06	±	0
SLD solvent (\AA^{-2})	6.3e-06	±	0
bkg (cm ⁻¹ sr ⁻¹)	0.000803747	±	7.59234e-05

chisq = 751.6

Npnts = 182

Sqrt(X^2/N) = 2.03216

Fitted range = [6,187] = $0.01404 < Q < 0.4578$

NOTE: The SANS data for RsC₁ in D₂O with d6-acetone (SLD $6.3 \times 10^{-6} \text{ \AA}^{-2}$) fits to the Schulz sphere model with $\chi^2 = 2.0$. The χ^2 value is low but the polydispersity is a little higher. However, the error bars on the parameters are low.

4) Bimodal Schulz sphere fit results for RsC₁ in D₂O/d6-acetone solvent

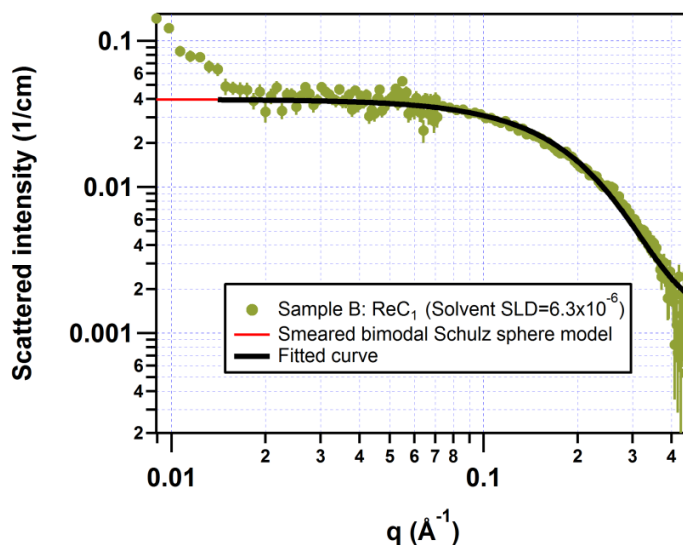


Figure 5. SANS Smeared bimodal Schulz sphere fitting for RsC₁ in a solvent with SLD of $6.3 \times 10^{-6} \text{ \AA}^{-2}$

volume fraction(1)	0.00317392	±	0.00017386
Radius (1) (Å)	7.16258	±	0.028233
polydispersity(1)	0.2	±	0.000772398
SLD(1) (Å ⁻²)	2.11e-06	±	0
volume fraction(2)	0.00211357	±	7.91294e-05
Radius (2)	10.2	±	0.0564399
polydispersity(2)	0.2	±	0.00203875
SLD(2)	2.11e-06	±	0
SLD (solvent)	6.3e-06	±	0
background (cm ⁻¹ sr ⁻¹)	0.00123961	±	0.000121532

chisq = 438.747

Npnts = 182

Sqrt(χ^2/N) = 1.55264

Fitted range = [6,187] = 0.01404 < Q < 0.4578

NOTE: The SANS data for RsC₁ in D₂O with d6-acetone (SLD $6.3 \times 10^{-6} \text{ \AA}^{-2}$) fits well to the Schulz sphere model with $\chi^2 = 1.6$. The χ^2 value is small with small the error bars on the parameters and give a good representation of the data.

5) Bimodal Schulz sphere fit results for RsC₁ in D₂O/d6-acetone/h6-acetone solvent

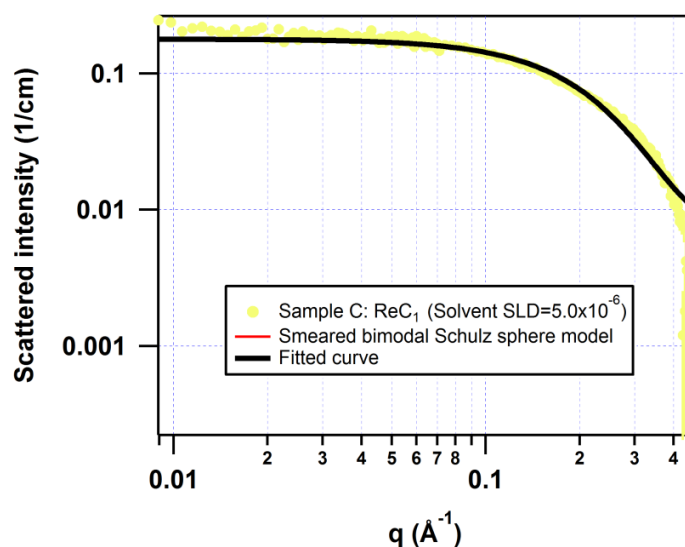


Figure 6. SANS Smeared bimodal Schulz sphere fitting for RsC₁ in a solvent with SLD of $5.0 \times 10^{-6} \text{ \AA}^{-2}$

volume fraction(1)	0.0169962	±	0.000325926
Radius (1) (Å)	9.9855	±	0.00535286
polydispersity(1)	0.192959	±	7.65902e-05
SLD(1) (Å ⁻²)	2.11e-06	±	0
volume fraction(2)	0.0438748	±	0.000867433
Radius (2)	6.99957	±	0.00610695
polydispersity(2)	0.198993	±	0.000135316
SLD(2)	2.11e-06	±	0
SLD (solvent)	5e-06	±	0
background (cm ⁻¹ sr ⁻¹)	0.00719799	±	0.000305478

chisq = 1516.38

Npnts = 188 Sqrt(χ^2/N) = 2.84004

Fitted range = [0,187] = $0.008936 < Q < 0.4578$

NOTE: The SANS data for RsC₁ for D₂O withd6/ h6-acetone (SLD $5.0 \times 10^{-6} \text{ \AA}^{-2}$) fits well to the bimodal Schulz sphere model with $\chi^2 = 2.8$. The χ^2 value is fine with small error bars on the parameters and gives a reasonable representation of the data.

6) Schulz sphere fit results for RsC_1 in $\text{D}_2\text{O}/\text{d}_6\text{-acetone}/\text{h}_6\text{-acetone}$ solvent

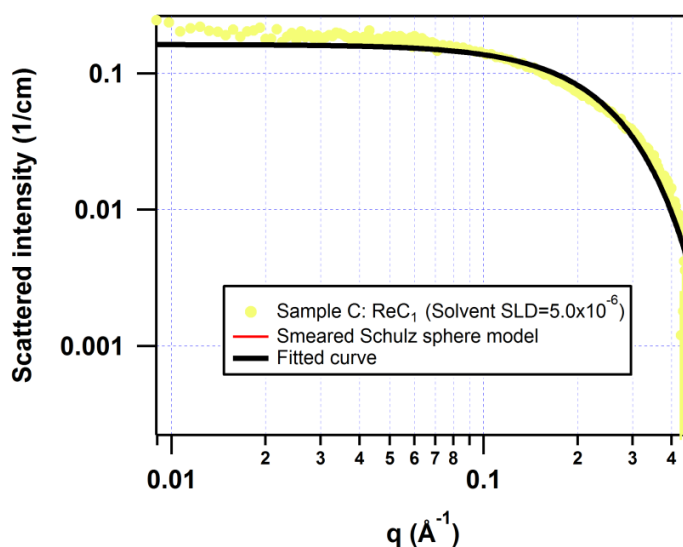


Figure 7. SANS Smeared Schulz sphere fitting for RsC_1 in a solvent with SLD of $5.0 \times 10^{-6} \text{ \AA}^{-2}$

Volume Fraction (scale)	0.0762006	±	0.000205149
mean radius (Å)	7.3307	±	0.00227844
polydisp (sig/avg)	0.200824	±	0.000120436
SLD sphere (Å ⁻²)	2.11e-06	±	0
SLD solvent (Å ⁻²)	5e-06	±	0
bkg (cm ⁻¹ sr ⁻¹)	-0.000389152	±	0.000196865

chisq = 3429.76

Npnts = 188 Sqrt(χ^2/N) = 4.27123

Fitted range = [0,187] = $0.008936 < Q < 0.4578$

NOTE: The SANS data for RsC_1 for D_2O with $\text{d}_6/\text{h}_6\text{-acetone}$ (SLD $5.0 \times 10^{-6} \text{ \AA}^{-2}$) fits to the Schulz sphere model with $\chi^2 = 4.3$. The χ^2 value is higher than the bimodal Schulz sphere fit. Therefore, the bimodal fit gives a better representation of the data.

7) Schulz sphere fit results for D₂O/h6-acetone solvent

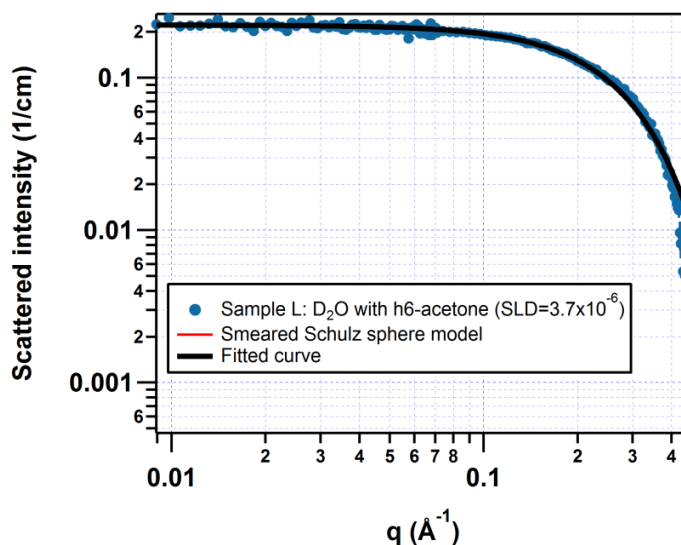


Figure 8. SANS Smearred Schulz sphere fitting for solvent with SLD of $3.7 \times 10^{-6} \text{ \AA}^{-2}$

Volume Fraction (scale)	0.514398	±	0.0011891
mean radius (Å)	6.4098	±	0.00119736
polydisp (sig/avg)	0.201238	±	3.60556e-05
SLD sphere (Å ⁻²)	2.11e-06	±	0
SLD solvent (Å ⁻²)	3.7e-06	±	0
bkg (cm ⁻¹ sr ⁻¹)	-0.00192676	±	0.000265317

chisq = 826.648

Npnts = 188

Sqrt(X^2/N) = 2.09692

Fitted range = [0,187] = $0.008936 < Q < 0.4578$

NOTE: The SANS data for D₂O with h6-acetone (SLD $3.7 \times 10^{-6} \text{ \AA}^{-2}$) fits well to the Schulz sphere model with $\chi^2 = 2.1$. The χ^2 value is fine with small error bars on the parameters and gives a reasonable representation of the data.

8) Schulz sphere fit results for D₂O/d6-acetone solvent

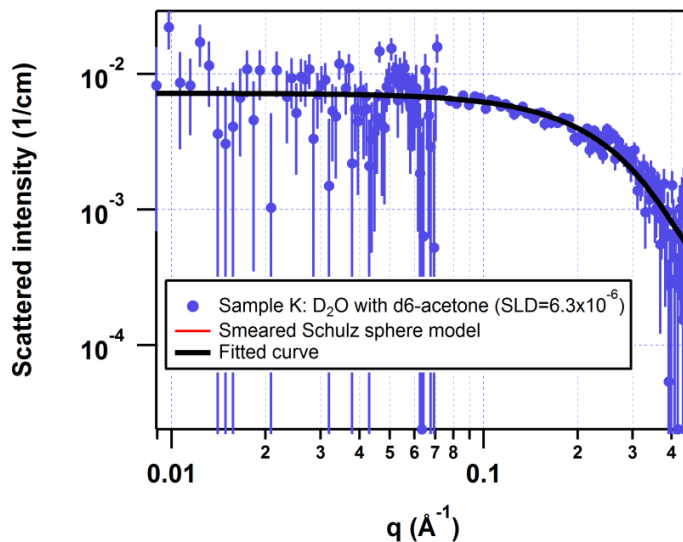


Figure 9. SANS Smeared Schulz sphere fitting for solvent with SLD of $6.3 \times 10^{-6} \text{ \AA}^{-2}$

Volume Fraction (scale)	0.00179017	±	3.81739e-05
mean radius (Å)	6.98313	±	0.0150534
polydisp (sig/avg)	0.199408	±	0.000459761
SLD sphere (Å ⁻²)	2.11e-06	±	0
SLD solvent (Å ⁻²)	6.3e-06	±	0
bkg (cm ⁻¹ sr ⁻¹)	0.000253573	±	7.0095e-05

chisq = 252.689

Npnts = 188

Sqrt(X^2/N) = 1.15935

Fitted range = [0,187] = $0.008936 < Q < 0.4578$

NOTE: The SANS data for D₂O with d6-acetone (SLD $3.7 \times 10^{-6} \text{ \AA}^{-2}$) fits well to the Schulz sphere model with $\chi^2 = 1.2$. The χ^2 value is good with small error bars on the parameters and gives a reasonable representation of the data.

9) Schulz sphere fit results for D₂O/d6-acetone/h6-acetone solvent

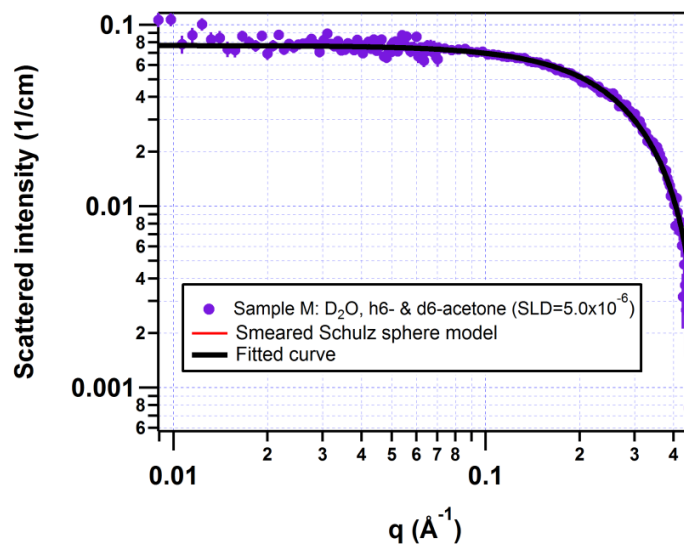


Figure 10. SANS Smearred Schulz sphere fitting for solvent with SLD of $5.0 \times 10^{-6} \text{ \AA}^{-2}$

Volume Fraction (scale)	0.0985031	±	0.00037264
mean radius (Å)	5.89853	±	0.000611643
polydisp (sig/avg)	0.129994	±	9.86534e-06
SLD sphere (Å ⁻²)	2.11e-06	±	0
SLD solvent (Å ⁻²)	5e-06	±	0
bkg (cm ⁻¹ sr ⁻¹)	-0.00895711	±	0.000198135

chisq = 375.4

Npnts = 188

Sqrt(X^2/N) = 1.41308

Fitted range = [0,187] = $0.008936 < Q < 0.4578$

NOTE: The SANS data for D₂O with d6/h6-acetone (SLD $5.0 \times 10^{-6} \text{ \AA}^{-2}$) fits well to the Schulz sphere model with $\chi^2 = 1.40$. The χ^2 value is good with small error bars on the parameters and gives a reasonable representation of the data.

10) Bimodal Schulz sphere fit results for insulin in D₂O/d6-acetone solvent

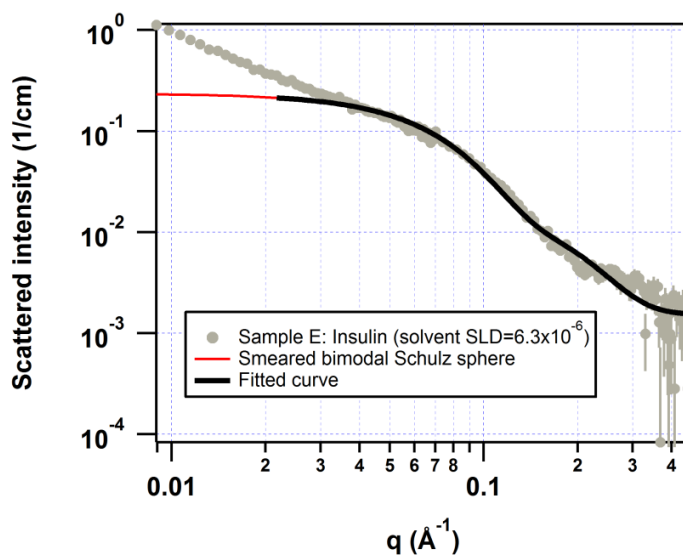


Figure 11. SANS Smearred bimodal Schulz sphere fitting for insulin in a solvent with SLD of $6.3 \times 10^{-6} \text{ \AA}^{-2}$

volume fraction(1)	0.000800676	±	6.31943e-05
Radius (1) (Å)	10.6801±		0.909249
polydispersity(1)	0.2	±	0.0477876
SLD(1) (Å ⁻²)	1.54e-06	±	0
volume fraction(2)	0.000897045	±	4.43761e-05
Radius (2)	25.0012±		0.928568
polydispersity(2)	0.200078	±	0.0193765
SLD(2)	1.54e-06	±	0
SLD (solvent)	6.3e-06 ±		0
background (cm ⁻¹ sr ⁻¹)	0.00165025	±	0.000126041

chisq = 1519.05

Npnts = 169 Sqrt(χ^2/N) = 2.99808

Fitted range = [19,187] = 0.0251 < Q < 0.4578

NOTE: The SANS data for insulin in D₂O with d6-acetone (SLD $6.3 \times 10^{-6} \text{ \AA}^{-2}$) fits well to the bimodal Schulz sphere model with $\chi^2 = 3.0$. The χ^2 value is fine with small error bars on the parameters and gives a reasonable representation of the data.

11) Bimodal Schulz sphere fit results for insulin in D₂O/d₆-acetone/h₆-acetone solvent

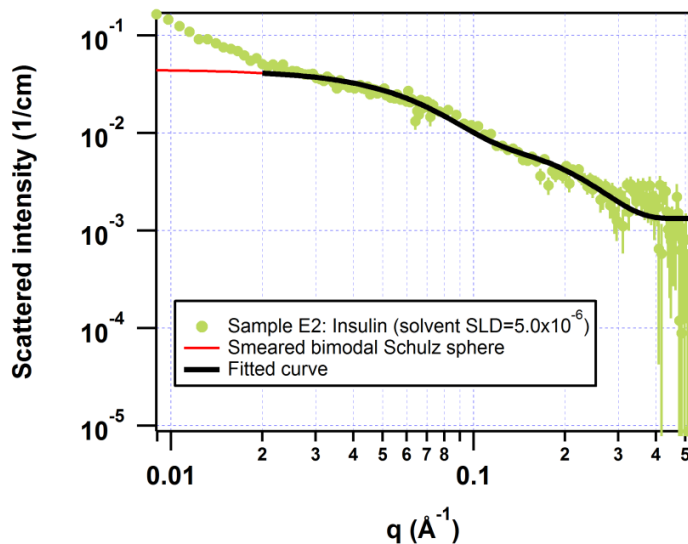


Figure 12. SANS Smeared bimodal Schulz sphere fitting for insulin in a solvent with SLD of $5.0 \times 10^{-6} \text{ \AA}^{-2}$

volume fraction(1)	0.00126557	±	7.71984e-05
Radius (1) (Å)	10.1235±		0.176015
polydispersity(1)	0.113143	±	0.0018584
SLD(1) (Å ⁻²)	1.54e-06	±	0
volume fraction(2)	0.000208089	±	2.35004e-05
Radius (2)	28.0404±		3.51929
polydispersity(2)	0.2	±	0.0724347
SLD(2)	1.54e-06	±	0
SLD (solvent)	5e-06	±	0
background (cm ⁻¹ sr ⁻¹)	0.00129761	±	8.77663e-05

chisq = 356.65

Npnts = 162

Sqrt(χ^2/N) = 1.48376

Fitted range = [13,174] = $0.019997 < Q < 0.51793$

NOTE: The SANS data for insulin in D₂O with d₆-acetone/h₆-acetone (SLD $5.0 \times 10^{-6} \text{ \AA}^{-2}$) fits well to the bimodal Schulz sphere model with $\chi^2 = 1.5$. The χ^2 value is fine with small error bars on the parameters and gives a reasonable representation of the data. The radius value of $28.0 \text{ \AA} \pm 3 \text{ \AA}$ agrees well with 25 Å species of pure deuterated insulin sample E.

12) Bimodal Schulz sphere fit results for insulin in D₂O/h6-acetone solvent

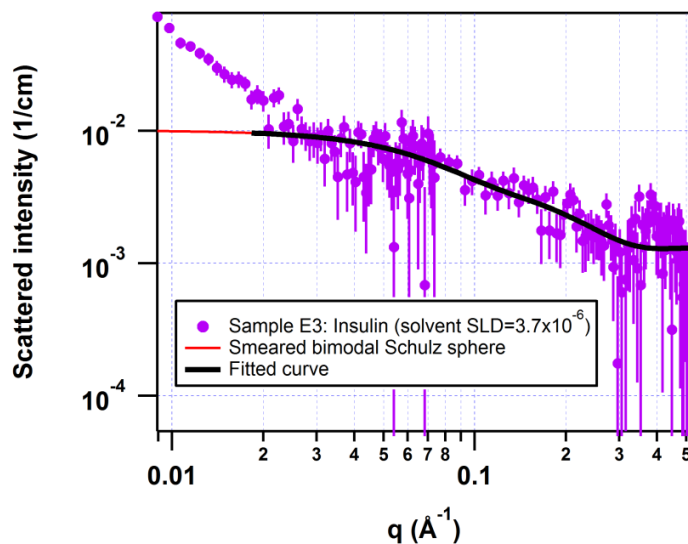


Figure 13. SANS Smeared bimodal Schulz sphere fitting for insulin in a solvent with SLD of $3.7 \times 10^{-6} \text{ \AA}^{-2}$

volume fraction(1)	0.00123037	±	0.00024338
Radius (1) (Å)	10.9502±		0.0201098
polydispersity(1)	0.0593514	±	7.99332e-05
SLD(1) (Å ⁻²)	1.54e-06	±	0
volume fraction(2)	0.000246417	±	5.81943e-05
Radius (2)	24.9505±		2.01593
polydispersity(2)	0.190133	±	0.00747531
SLD(2)	1.54e-06	±	0
SLD (solvent)	3.37e-06	±	0
background (cm ⁻¹ sr ⁻¹)	0.00137975	±	0.000100033

chisq = 832.729

Npnts = 175

Sqrt(X²/N) = 2.18139

Fitted range = [0,174] = 0.0089374 < Q < 0.51793

NOTE: The SANS data for insulin in D₂O with d6-acetone (SLD $3.7 \times 10^{-6} \text{ \AA}^{-2}$) fits well to the bimodal Schulz sphere model with $\chi^2 = 2.2$. The χ^2 value is fine with small error bars on the parameters and gives a reasonable representation of the data. The radius value of $24.9 \text{ \AA} \pm 2 \text{ \AA}$ agrees well with 25 \AA species of pure deuterated insulin sample E.

13) Bimodal Schulz sphere fit results for RsC₁ with insulin in D₂O/h6-acetone solvent

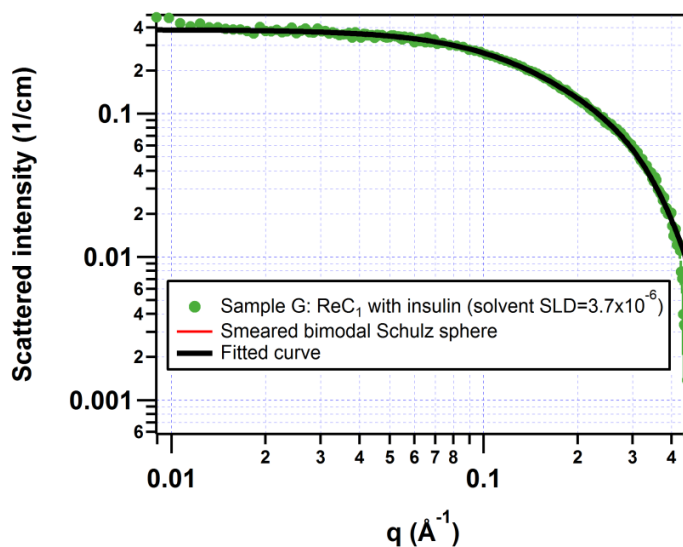


Figure 14. SANS Smearred bimodal Schulz sphere fitting for RsC₁ with insulin in a solvent with SLD of $3.7 \times 10^{-6} \text{ \AA}^{-2}$

volume fraction(1)	0.410847	±	0.00191969
Radius (1) (Å)	7.0008	±	0.00126996
polydispersity(1)	0.199932	±	3.13124e-05
SLD(1) (Å ⁻²)	2.11e-06	±	0
volume fraction(2)	0.0201735	±	0.000400964
Radius (2)	16.2904±		0.114009
polydispersity(2)	0.198779	±	0.00129361
SLD(2)	2.03e-06	±	0
SLD (solvent)	3.7e-06 ±		0
background (cm ⁻¹ sr ⁻¹)	-0.000627838	±	0.000297076

chisq = 491.705

Npnts = 188

Sqrt(χ^2/N) = 1.61724

Fitted range = [0,187] = $0.008936 < Q < 0.4578$

NOTE: The SANS data for insulin+octameric RsC₁ in D₂O with h6-acetone (SLD $3.7 \times 10^{-6} \text{ \AA}^{-2}$) fits well to the bimodal Schulz sphere model with $\chi^2 = 1.6$. The χ^2 value is good with small error bars on the parameters and gives a reasonable representation of the data.

14) Bimodal Schulz sphere fit results for RsC₁ with insulin in D₂O/d6-acetone solvent

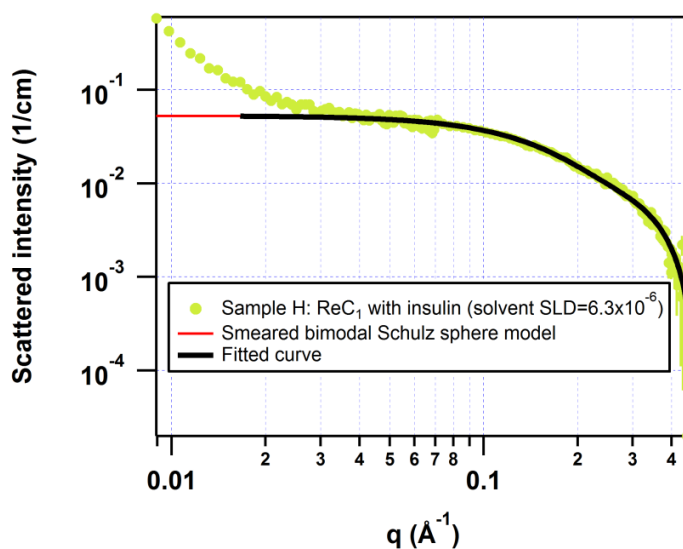


Figure 15. SANS Smeared bimodal Schulz sphere fitting for RsC₁ with insulin in a solvent with SLD of $6.3 \times 10^{-6} \text{ \AA}^{-2}$

volume fraction(1)	0.00831521	±	9.80527e-05
Radius (1) (Å)	6.84996±		0.00698206
polydispersity(1)	0.101857	±	0.000117753
SLD(1) (Å ⁻²)	2.11e-06	±	0
volume fraction(2)	0.000959888	±	9.73064e-06
Radius (2)	16.3505±		0.0169858
polydispersity(2)	0.0098698	±	6.49497e-06
SLD(2)	2.03e-06	±	0
SLD (solvent)	6.3e-06 ±		0
background (cm ⁻¹ sr ⁻¹)	-0.00150767	±	0.000107212

chisq = 629.482

Npnts = 175

Sqrt(X²/N) = 1.89659

Fitted range = [13,187] = 0.02 < Q < 0.4578

NOTE: The SANS data for insulin + octameric RsC₁ for D₂O with d6-acetone (SLD $6.3 \times 10^{-6} \text{ \AA}^{-2}$) fits well to the bimodal Schulz sphere model with $\chi^2 = 1.9$. The χ^2 value is good with small error bars on the parameters and gives a reasonable representation of the data.

15) Bimodal Schulz sphere fit results for RsC₁ with insulin in D₂O/h6-acetone/d6-acetone solvent

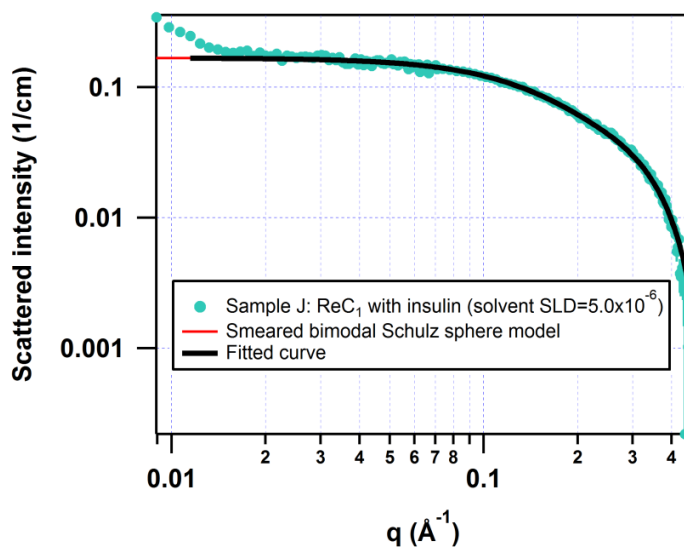


Figure 16. SANS Smeared bimodal Schulz sphere fitting for RsC₁ with insulin in a solvent with SLD of $5.0 \times 10^{-6} \text{ \AA}^{-2}$

volume fraction(1)	0.071979	±	0.000441769
Radius (1) (Å)	6.99879±		0.000449895
polydispersity(1)	0.100019	±	4.4619e-06
SLD(1) (Å ⁻²)	2.11e-06	±	0
volume fraction(2)	0.00400791	±	6.18437e-05
Radius (2)	16.4673±		0.0713023
polydispersity(2)	0.10205±		0.000454781
SLD(2)	2.03e-06	±	0
SLD (solvent)	5e-06	±	0
background (cm ⁻¹ sr ⁻¹)	-0.00401367	±	0.000227834

chisq = 380.063

Npnts = 185

Sqrt(χ^2/N) = 1.43332

Fitted range = [3,187] = $0.01149 < Q < 0.4578$

NOTE: The SANS data for insulin + octameric RsC₁ in D₂O withd6/ h6-acetone (SLD $5.0 \times 10^{-6} \text{ \AA}^{-2}$) fits well to the bimodal Schulz sphere model with $\chi^2 = 1.4$. The χ^2 value is good with small error bars on the parameters and gives a reasonable representation of the data.