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Supplemental Information

Intrinsically Disordered Protein Exhibits Both Compaction and Expansion under Macromolecular Crowding

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Table S1. Mean R_g values of FlgM from three analysis methods.[a]

Conc. (mg/ml)	BSA			lysozyme			Dextran			Ficoll		
0				28.5 ± 0.5	27.8 ± 0.4	27.8 ± 0.2						
75				27.0 ± 2.2	26.9 ± 0.6	27.6 ± 0.2						
100										30.0 ± 0.6	27.2 ± 0.5	26.3 ± 0.1
120	29.3 ± 0.7	26.0 ± 0.5	25.5 ± 0.1									
130				26.2 ± 1.5	27.5 ± 0.8	28.1 ± 0.2						
150							24.7 ± 1.5	23.1 ± 0.4	23.9 ± 0.7			
190				28.1 ± 1.3	26.9 ± 0.6	27.3 ± 0.4						
220	27.0 ± 1.0	25.8 ± 1.2	25.7 ± 0.3									
250							25.1 ± 0.9	24.4 ± 0.7	25.4 ± 0.7	29.7 ± 1.1	27.6 ± 0.4	27.0 ± 0.1
320	29.1 ± 0.7	28.6 ± 1.0	28.9 ± 1.1							30.9 ± 1.0	28.3 ± 0.6	28.3 ± 0.4
400							28.3 ± 0.7	26.2 ± 0.6	28.0 ± 0.4	48.6 ± 3.0	40.8 ± 0.5	38.4 ±

[a]In each case, mean R_g values and errors in Å are given, from left to right, for fitting to the Debye formula, GNOM, and EOM. Errors for Debye and GNOM are fitting errors, whereas errors for EOM are standard deviations calculated using mean R_g values of 100 lowest-χ^2 subsets of models.

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         10          20          30          40          50
MSIDRTSPLK PVSTVQTRET SDTPVQKTRQ EKTSAATSAS VTLSDAQAKL

         60          70          80          90
MQPGVSDINM ERVEALKTAI RNGELKMDTG KIADSLIREA QSYLQSK
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Figure S1. Amino-acid sequence of FlgM from *Salmonella typhimurium*.

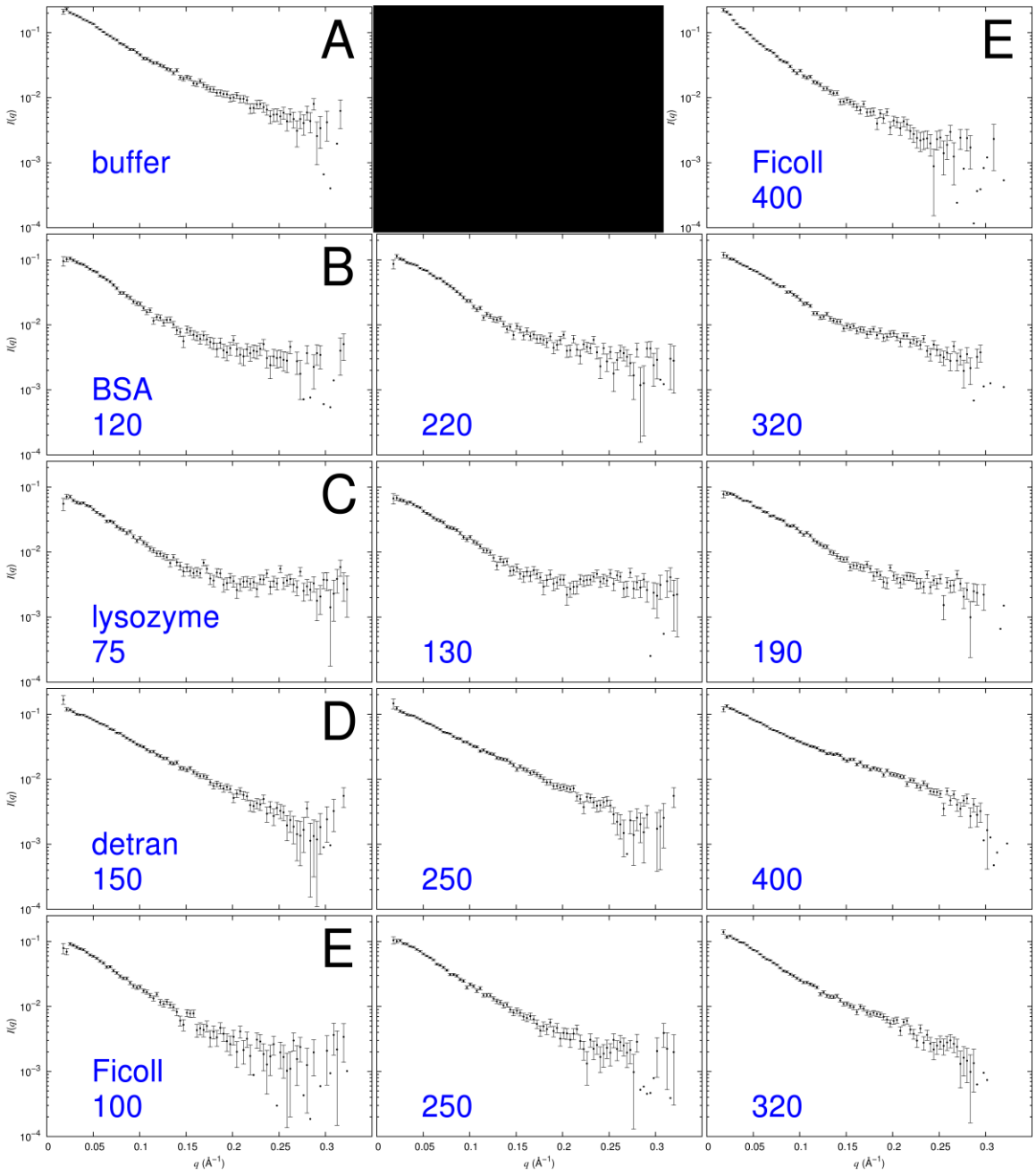


Figure S2. Raw scattering data of FlgM in log-log plot. (A) buffer. (B) BSA. (C) Lysozyme. (D) Dextran. (E) Ficoll. The last panel of (E) is shown at the top right. The crowder concentrations in mg/ml are indicated.

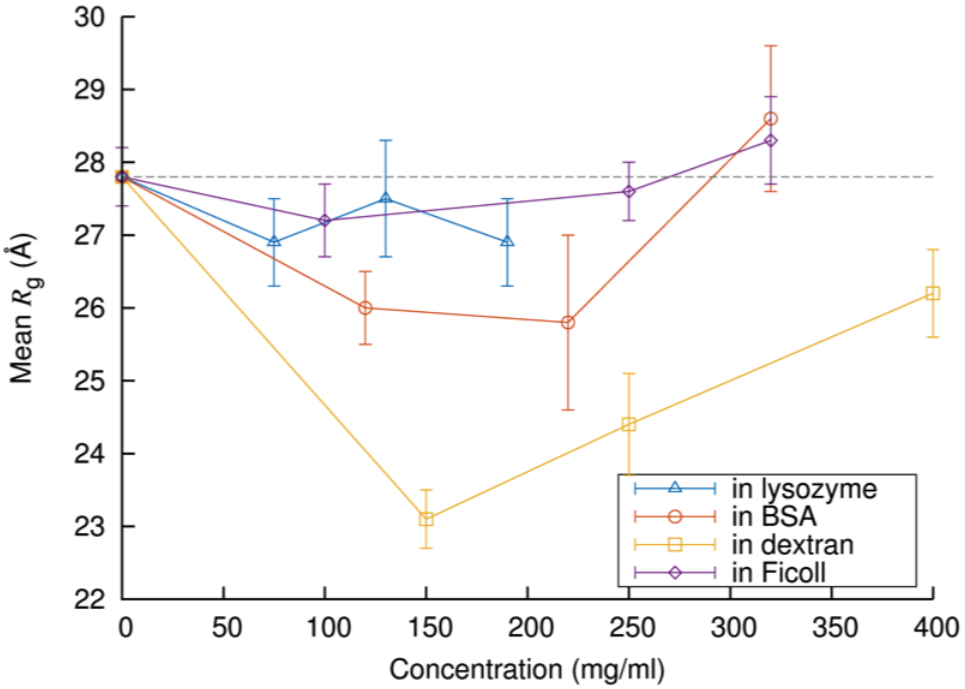
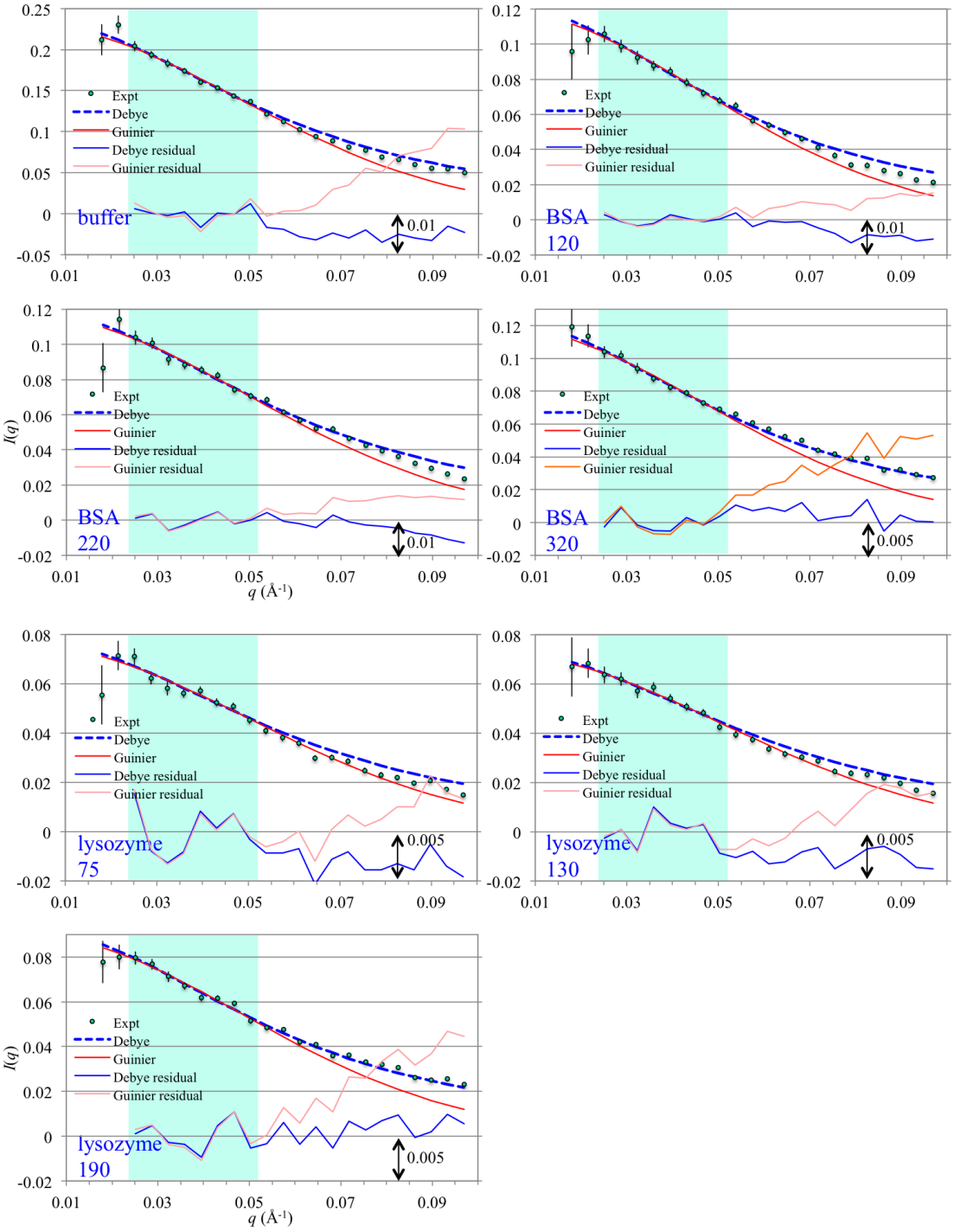


Figure S3. Mean R_g values in buffer and in the four crowders at concentrations up to 400 mg/ml, obtained by GNOM.


Expt
Debye
Guinier
Debye residual
Guinier residual
buffer
BSA 120
BSA 220
BSA 320
lysozyme 75
lysozyme 130
lysozyme 190
0.01
0.005
I(q)
q (Å-1)

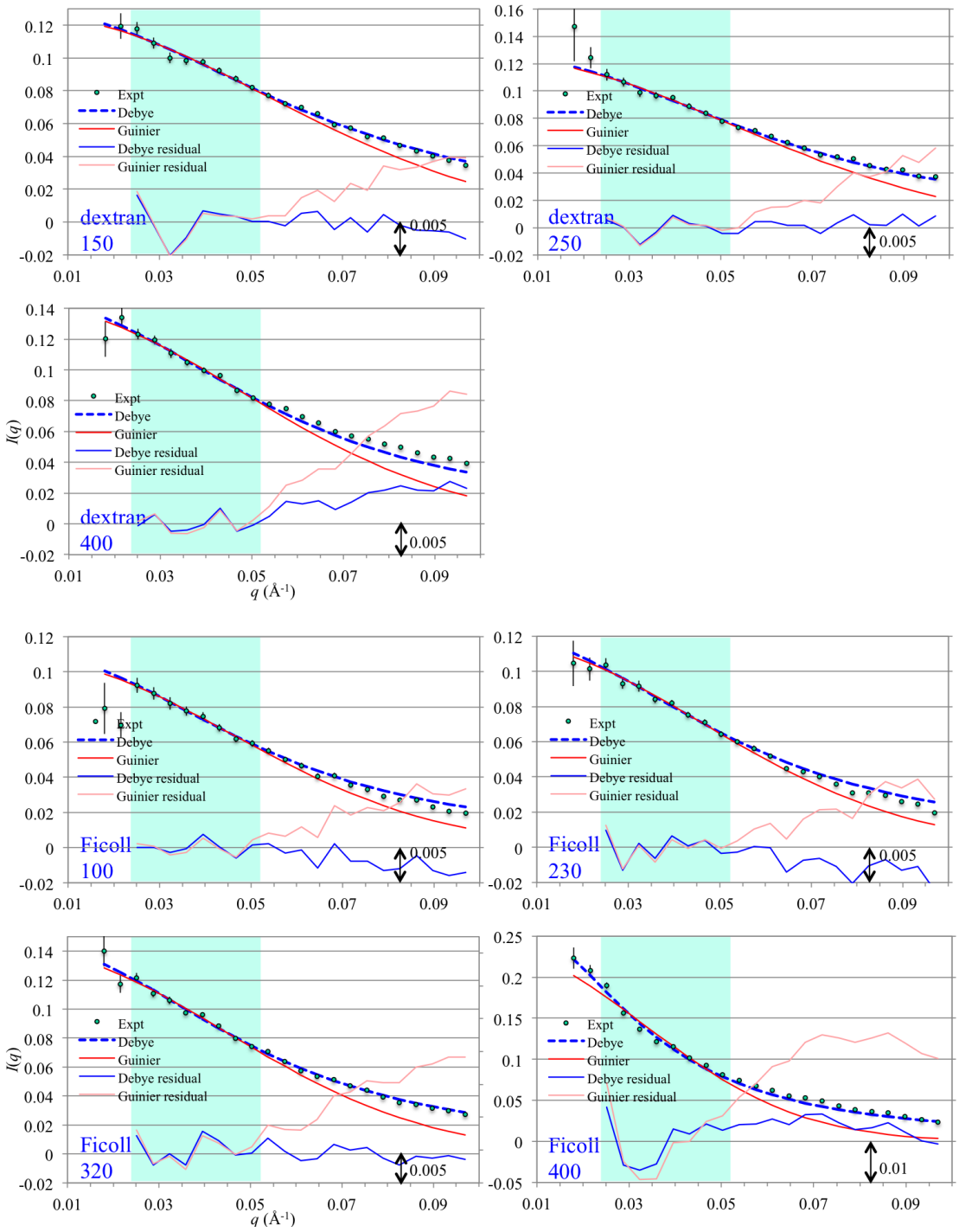
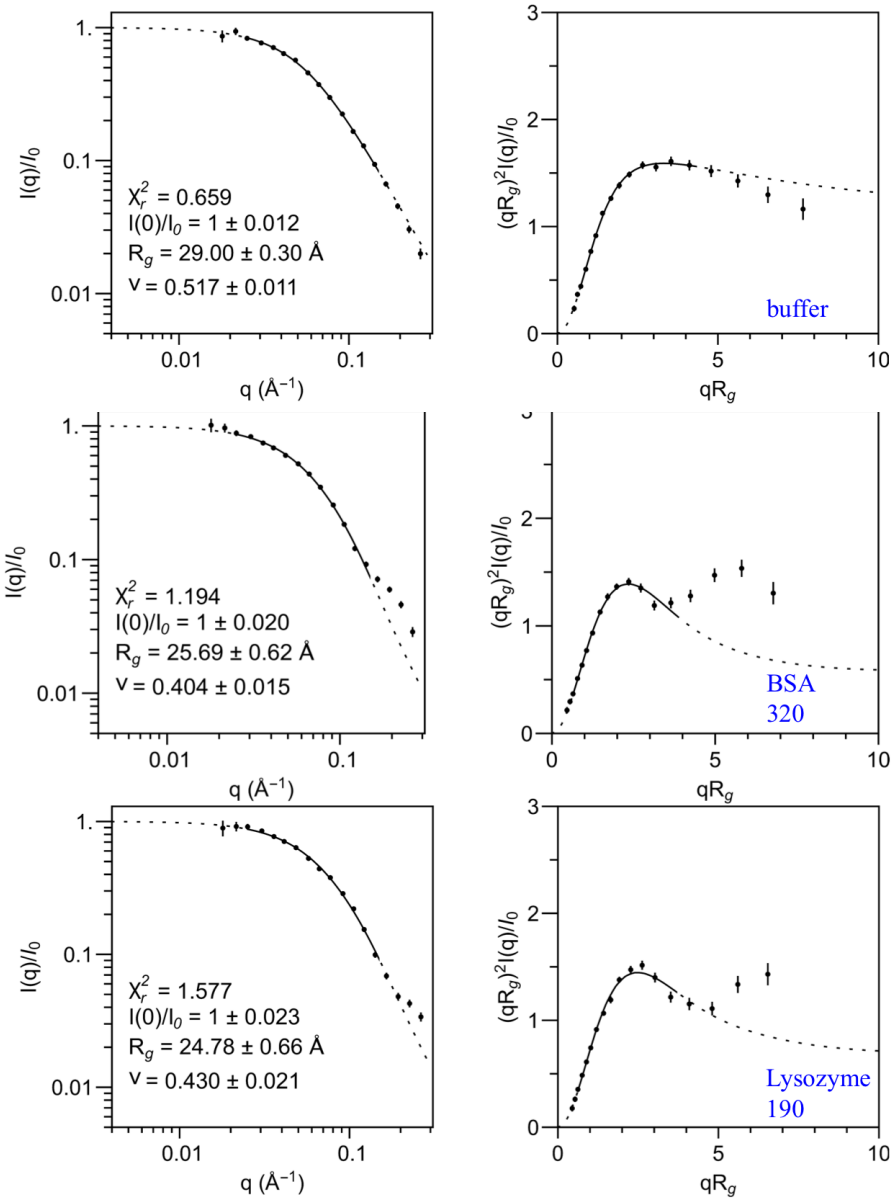


Figure S4. Debye and Guinier fits to data at low *q*. The shaded region shows the range of *q* used for fitting. The residuals of the fits (experimental – predicted) are also shown, with scales indicated by double-headed arrows. The crowder concentrations in mg/ml are indicated.


I(q)/I0
q (Å−1)
X2r = 0.659
I(0)/I0 = 1 ± 0.012
Rg = 29.00 ± 0.30 Å
ν = 0.517 ± 0.011
(qRg)2I(q)/I0
qRg
buffer
X2r = 1.194
I(0)/I0 = 1 ± 0.020
Rg = 25.69 ± 0.62 Å
ν = 0.404 ± 0.015
BSA
320
X2r = 1.577
I(0)/I0 = 1 ± 0.023
Rg = 24.78 ± 0.66 Å
ν = 0.430 ± 0.021
Lysozyme
190

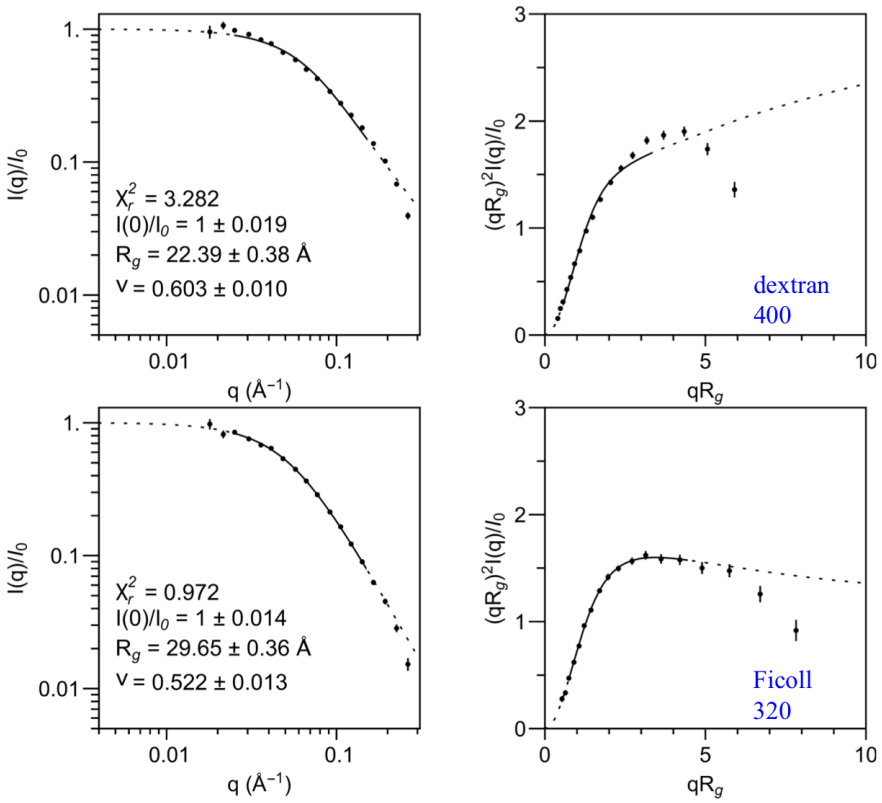


Figure S5. Fits of the FlgM data to the model of Riback et al., shown as log-log plot (left) and Kratky plot (right). The fitted curves are shown as solid in range of *q* (0.021 to 0.15 $Å^{-1}$) used for fitting. The crowder concentrations in mg/ml are indicated.

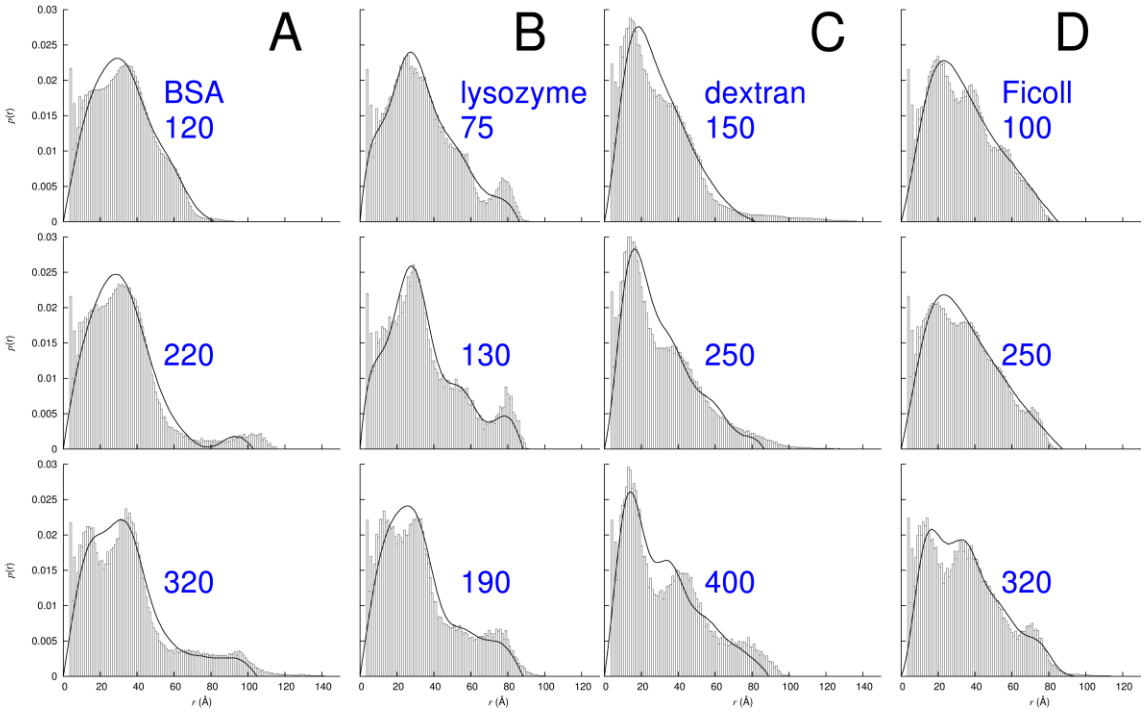


Figure S6. Comparison of distance distribution functions calculated from EOM structural models and generated by GNOM. *r* refers to Cα–Cα distance; *p*(*r*) is the probability density. (A) BSA. (B) Lysozyme. (C) Dextran. (D) Ficoll. The crowder concentrations in mg/ml are indicated.

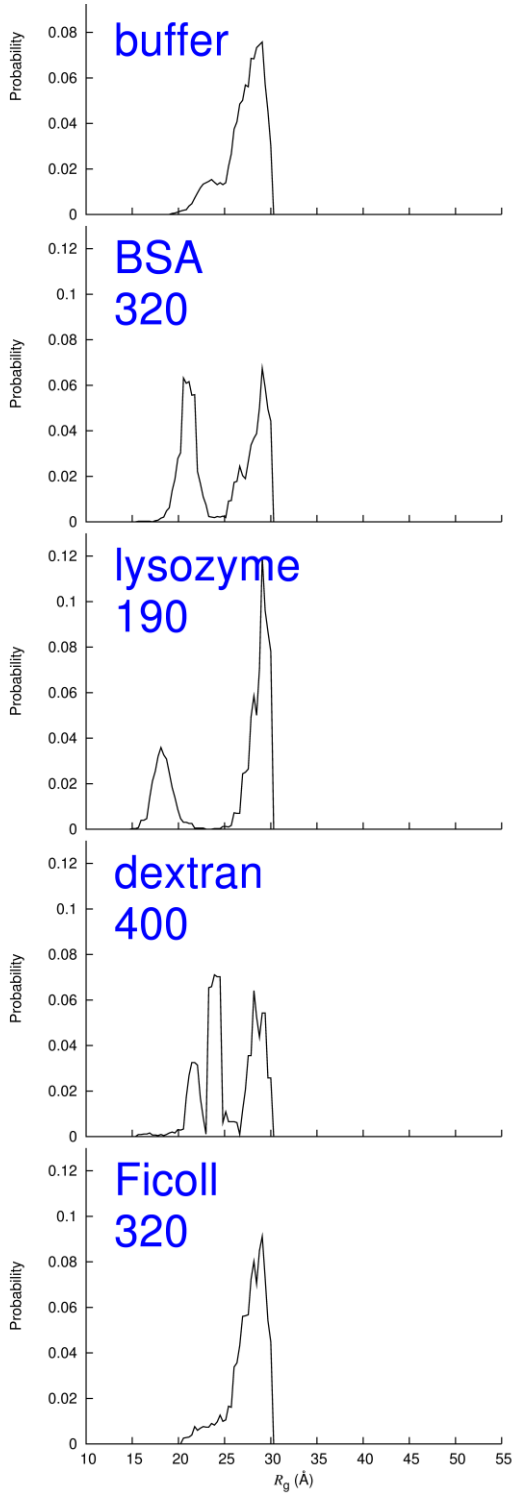


Figure S7. R_g distributions in buffer and under crowding, determined by EOM while removing from the initial pool all structural models with R_g > 30 Å. The crowder concentrations in mg/ml are indicated.