Supplementary Figure Legends

Figure S1. Data processing for Arf6NΔ13Q67L. (**A**) Selected protein and buffer regions used for direct and Guinier optimized buffer subtraction are indicated on the total intensity chromatogram. Guinier R_G values after direct subtraction of Buffer Region #2 are shown in green. (**B**) Weighted Guinier fits after direct subtraction of buffer regions indicated in A. (**C**) R^2 values for Guinier optimization of the U0 coefficient for linear combination following SVD (blue) or scaling constant for buffer subtraction (green). (**D**) Guinier plots for the linear combination using the indicated U0 coefficients. (**E**) Comparison of weighted Guinier plots after direct buffer subtraction, Guinier optimized buffer subtraction, or linear combination of SVD components. (**F**) Fits of experimental protein scattering to the theoretical scattering calculated using CRYSOL for the Arf6NΔ13Q67L-GTPγS structure (PDB ID 2J5X) with or without the His_6 tag from $Grp1_{63\text{-}399}$ (PDB ID 2R09, chain A).

Figure S2. Preliminary data analysis for $Grp1_{63\text{-}399}$. (**A**) Selected protein and buffer regions used for direct and Guinier optimized buffer subtraction are indicated on the total scattering profile. Guinier R_G values after direct subtraction of Buffer Region #3 are shown in green. (**B**) Weighted Guinier fits after direct subtraction of buffer regions indicated in A. (**C**) Columns of **U** corresponding to the rank ordered singular values in E for the entire data matrix in Fig. 3A. (**D**) Columns of **V** multiplied by the corresponding rank ordered singular values in E for the entire data matrix in Fig. 3A. (**E**) Singular values and autocorrelations of the columns of **U** and **V** after SVD of the entire data

matrix in Fig. 3A. (**F**) Singular values from SVD of the multimer region observed for elution volume less than 13 ml in A.

Figure S3. Data correction for Grp1$_{63-399}$. (**A and D**) R^2 values for Guinier optimization of the U0 coefficient for linear combination (blue) or scaling constant for buffer subtraction (green) with data sets for the monomer (A) or dimer (D) regions indicated in Fig. 3B. **(B and E**) Guinier plots of linear combinations of SVD components with the indicated U0 coefficient for monomer (B) or dimer (E) data sets. (**C and F**) Comparison of weighted Guinier plots after direct buffer subtraction and Guinier optimized buffer subtraction or linear combination of SVD components for monomer (C) or dimer (F) data sets.

Figure S4. Requirement for His$_6$ tag in CRYSOL fits. Fits of experimental protein scattering for Grp1$_{63-399}$ to the theoretical scattering calculated with CRYSOL using chain A with or without His$_6$ tag, or Chain B with His$_6$ tag, from the Grp1$_{63-399}$ crystal structure (PDB ID 2R09).

Figure S5. SVD and Guinier optimized reconstruction of monomer and dimer scattering for Cytohesin$_{58-400}$. (**A**) Raw scattering data sets for Cytohesin$_{58-400}$ acquired in line with chromatography on a 24 ml Superdex-200 column. (**B**) Raw and normalized total intensity elution profiles for the data in A. Also shown are the incident (I_0) and transmitted (I_1) beam intensity profiles. (**C, E and G**) Singular values and autocorrelation of the columns of **U** after SVD of the entire data set shown in A (C) or the

data from the monomer (E) or dimer (G) regions indicated in B. (**D, F and H**) Columns of **V** multiplied by the corresponding rank ordered singular values in C, E and G.

Figure S6. Data processing of Cytohesin$_{58\text{-}400}$ SEC-SAXS data. (**A**) Selected protein and buffer regions used for direct and Guinier optimized buffer subtraction are indicated on the total scattering profile. Guinier R_G values after direct subtraction of Buffer Region #3 are shown in green. (**B**) Weighted Guinier analysis after direct subtraction of buffer regions from A. (**C and D**) R^2 values for Guinier optimization of the U0 coefficient for linear combination following SVD (blue) or scaling constant for buffer subtraction (green) for the monomer (C) or dimer (D) data sets as indicated in Fig. S5B. (**E and F**) Comparison of weighted Guinier plots after direct buffer subtraction and Guinier optimized buffer subtraction or linear combination of SVD components for monomer (E) or dimer (F) data sets.

Figure S1

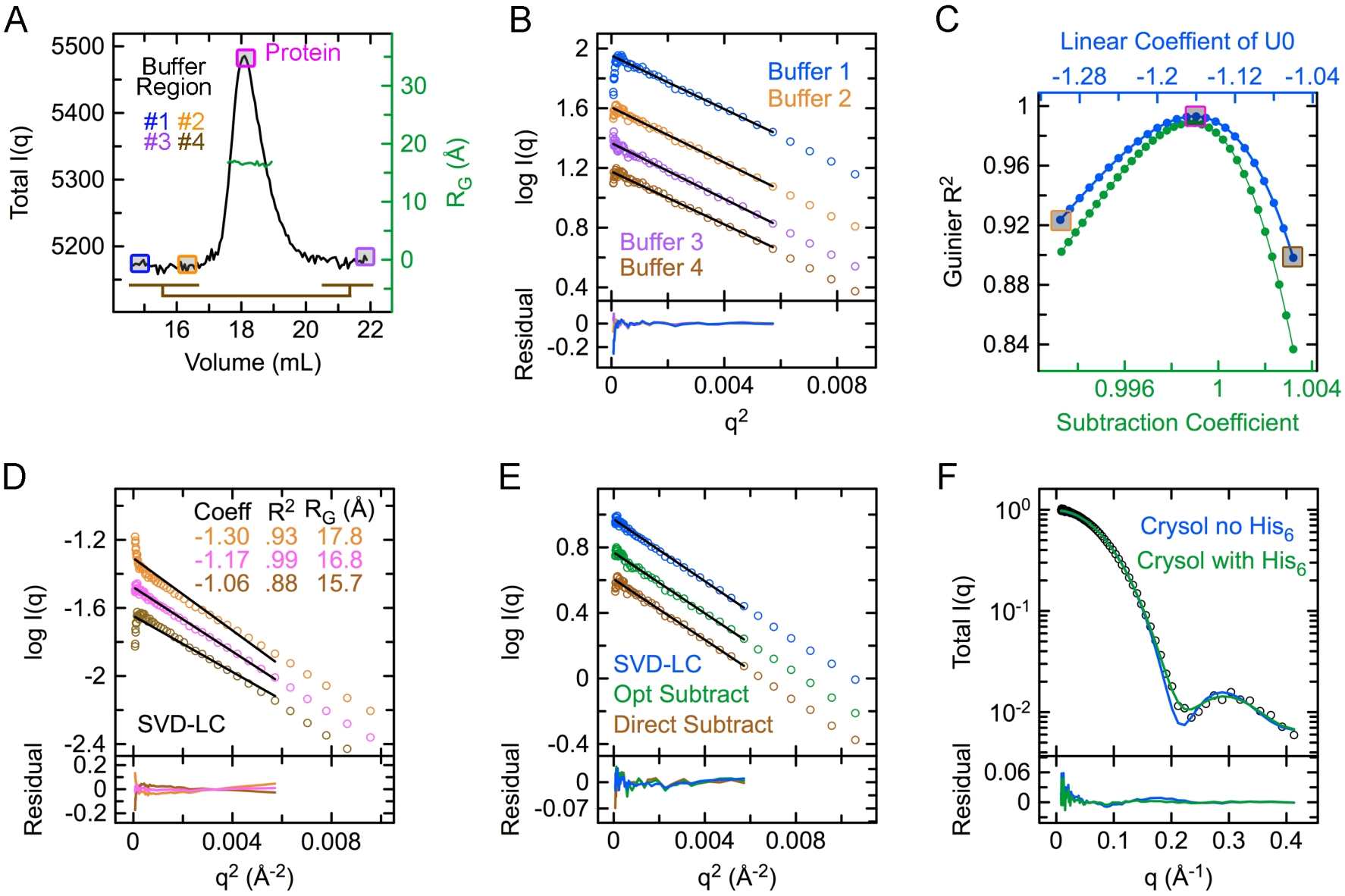

A
Buffer Region
#1 #2
#3 #4
Protein
Total I(q)
R_G (Å)
Volume (mL)
B
Buffer 1
Buffer 2
Buffer 3
Buffer 4
log I(q)
Residual
q^2
C
Linear Coeffient of U0
Guinier R^2
Subtraction Coefficient
D
Coeff R^2 R_G (Å)
-1.30 .93 17.8
-1.17 .99 16.8
-1.06 .88 15.7
SVD-LC
log I(q)
Residual
q^2 (Å^{-2})
E
SVD-LC
Opt Subtract
Direct Subtract
log I(q)
Residual
q^2 (Å^{-2})
F
Crysol no His_6
Crysol with His_6
Total I(q)
Residual
q (Å^{-1})

Figure S2

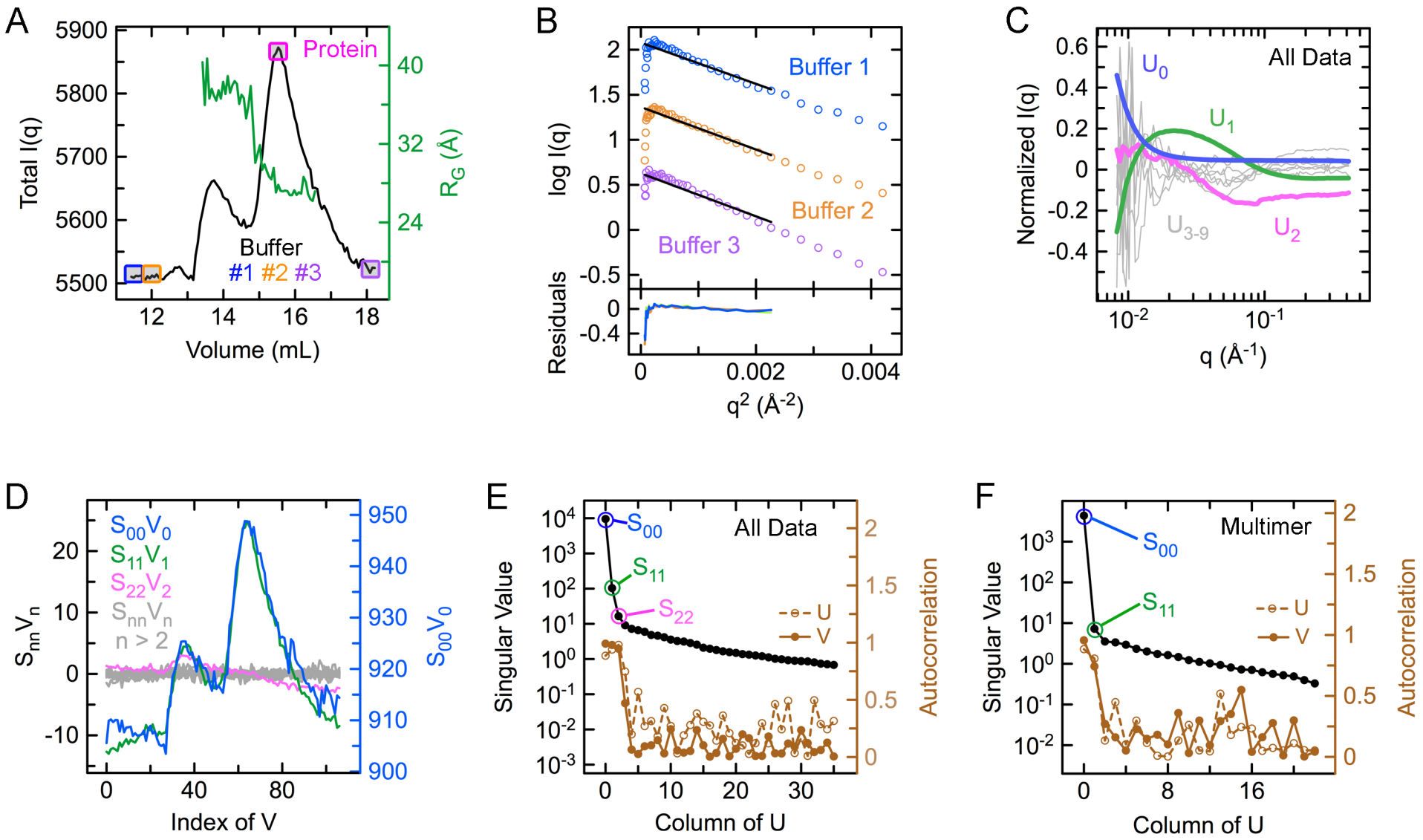

A
Total I(q)
Protein
Buffer
#1 #2 #3
R_G (Å)
Volume (mL)
B
log I(q)
Buffer 1
Buffer 2
Buffer 3
Residuals
q^2 (Å^{-2})
C
All Data
U_0
U_1
U_{3-9}
U_2
Normalized I(q)
q (Å^{-1})
D
$S_{00}V_0$
$S_{11}V_1$
$S_{22}V_2$
$S_{nn}V_n$
n > 2
$S_{nn}V_n$
$S_{00}V_0$
Index of V
E
All Data
S_{00}
S_{11}
S_{22}
U
V
Singular Value
Autocorrelation
Column of U
F
Multimer
S_{00}
S_{11}
U
V
Singular Value
Autocorrelation
Column of U

Figure S3

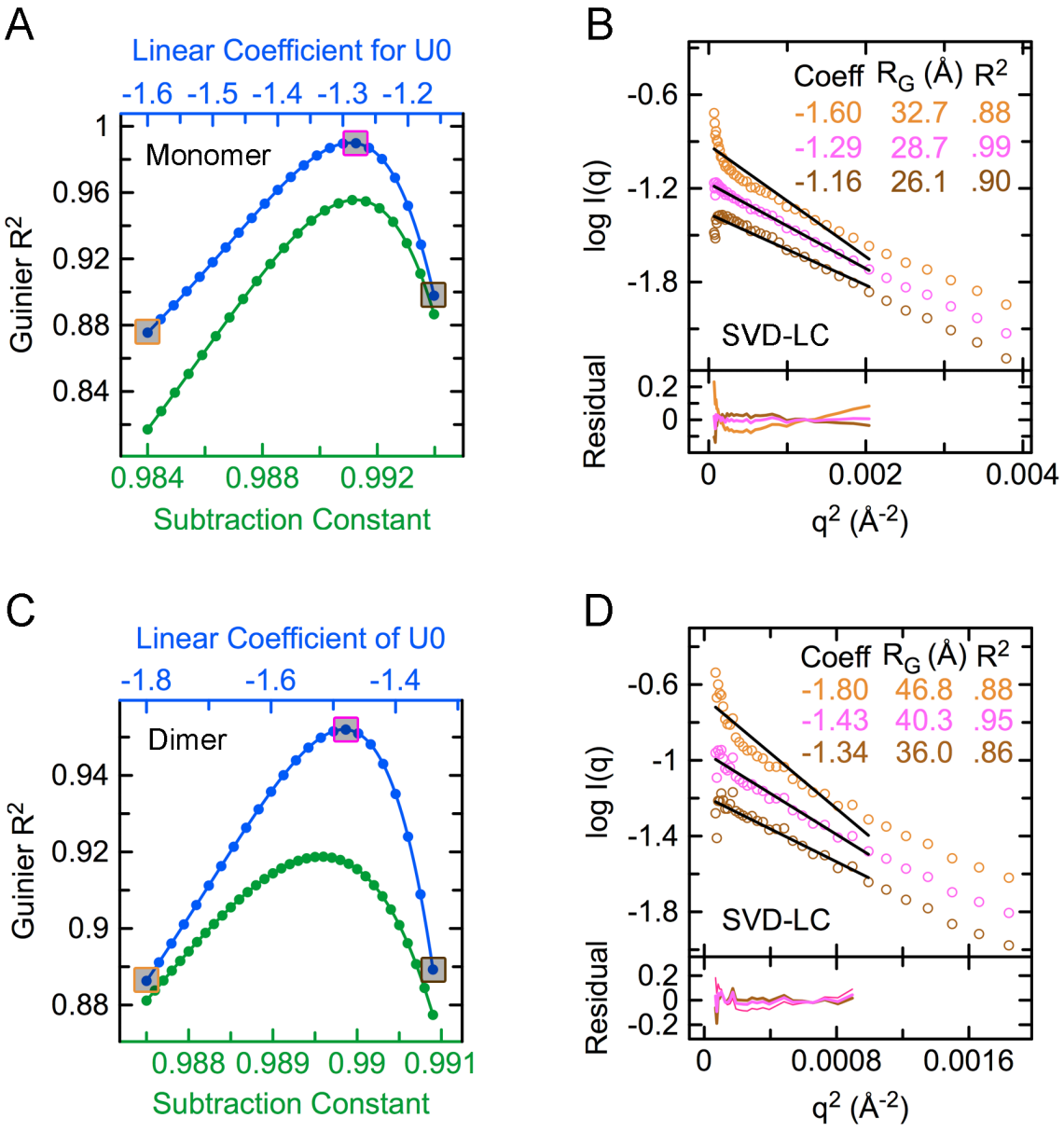

A
Linear Coefficient for U0
-1.6 -1.5 -1.4 -1.3 -1.2
Monomer
Guinier R2
1
0.96
0.92
0.88
0.84
0.984 0.988 0.992
Subtraction Constant
B
Coeff RG (Å) R2
-1.60 32.7 .88
-1.29 28.7 .99
-1.16 26.1 .90
log I(q)
-0.6
-1.2
-1.8
SVD-LC
Residual
0.2
0
0 0.002 0.004
q2 (Å-2)
C
Linear Coefficient of U0
-1.8 -1.6 -1.4
Dimer
Guinier R2
0.94
0.92
0.9
0.88
0.988 0.989 0.99 0.991
Subtraction Constant
D
Coeff RG (Å) R2
-1.80 46.8 .88
-1.43 40.3 .95
-1.34 36.0 .86
log I(q)
-0.6
-1
-1.4
-1.8
SVD-LC
Residual
0.2
0
-0.2
0 0.0008 0.0016
q2 (Å-2)

Figure S4

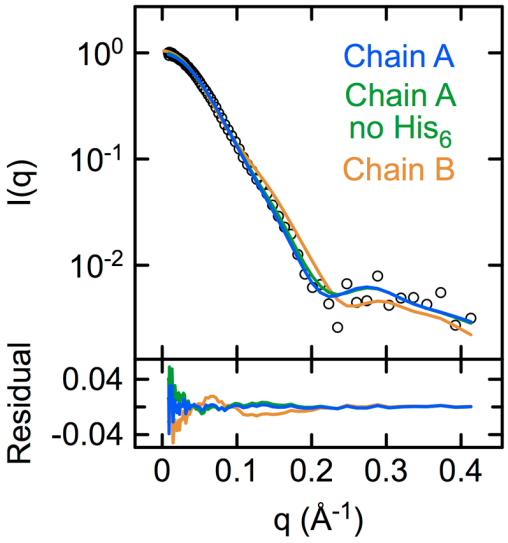

Chain A
Chain A
no His6
Chain B
I(q)
10^0
10^-1
10^-2
Residual
0.04
-0.04
0
0.1
0.2
0.3
0.4
q (Å^-1)

Figure S5

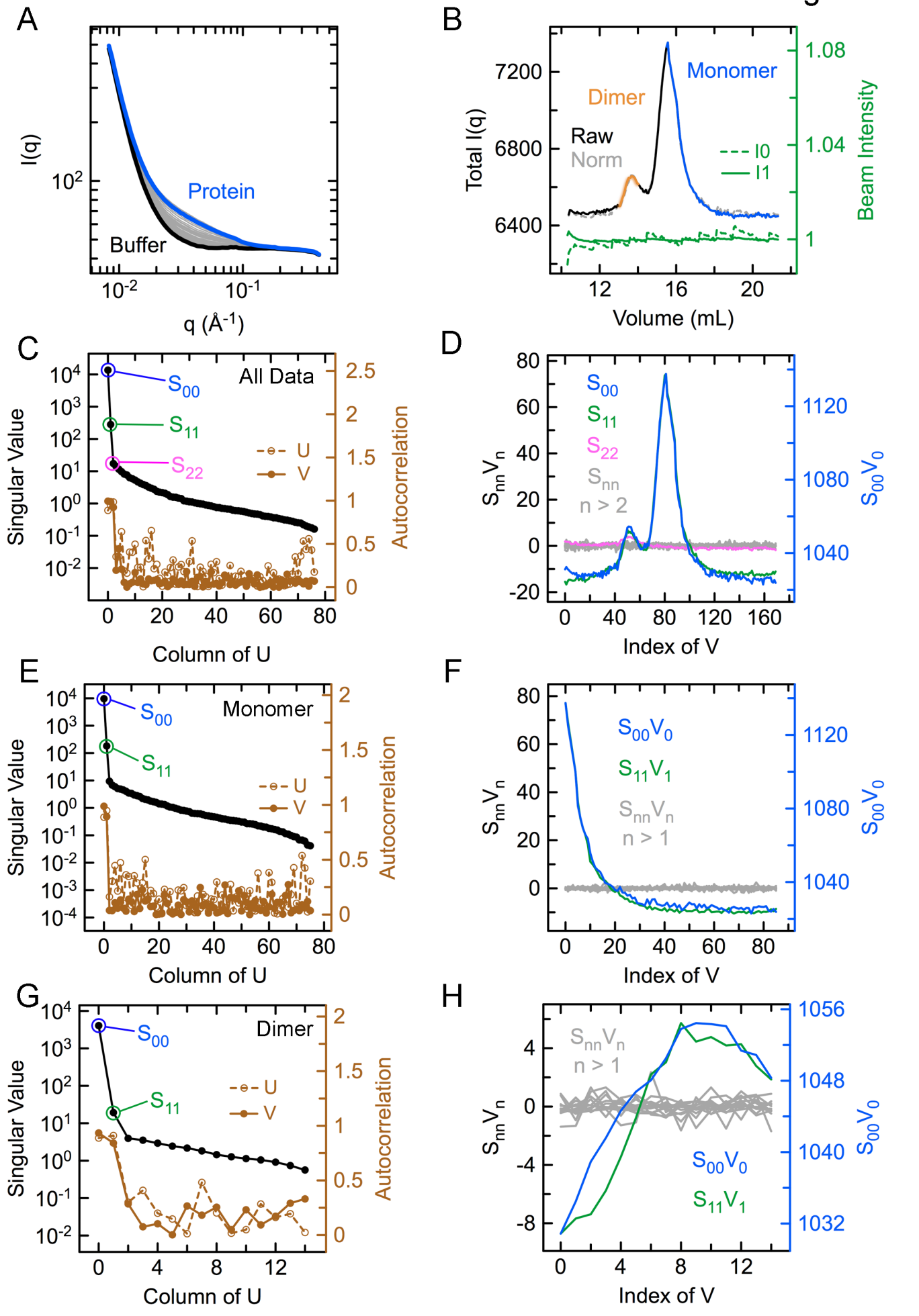
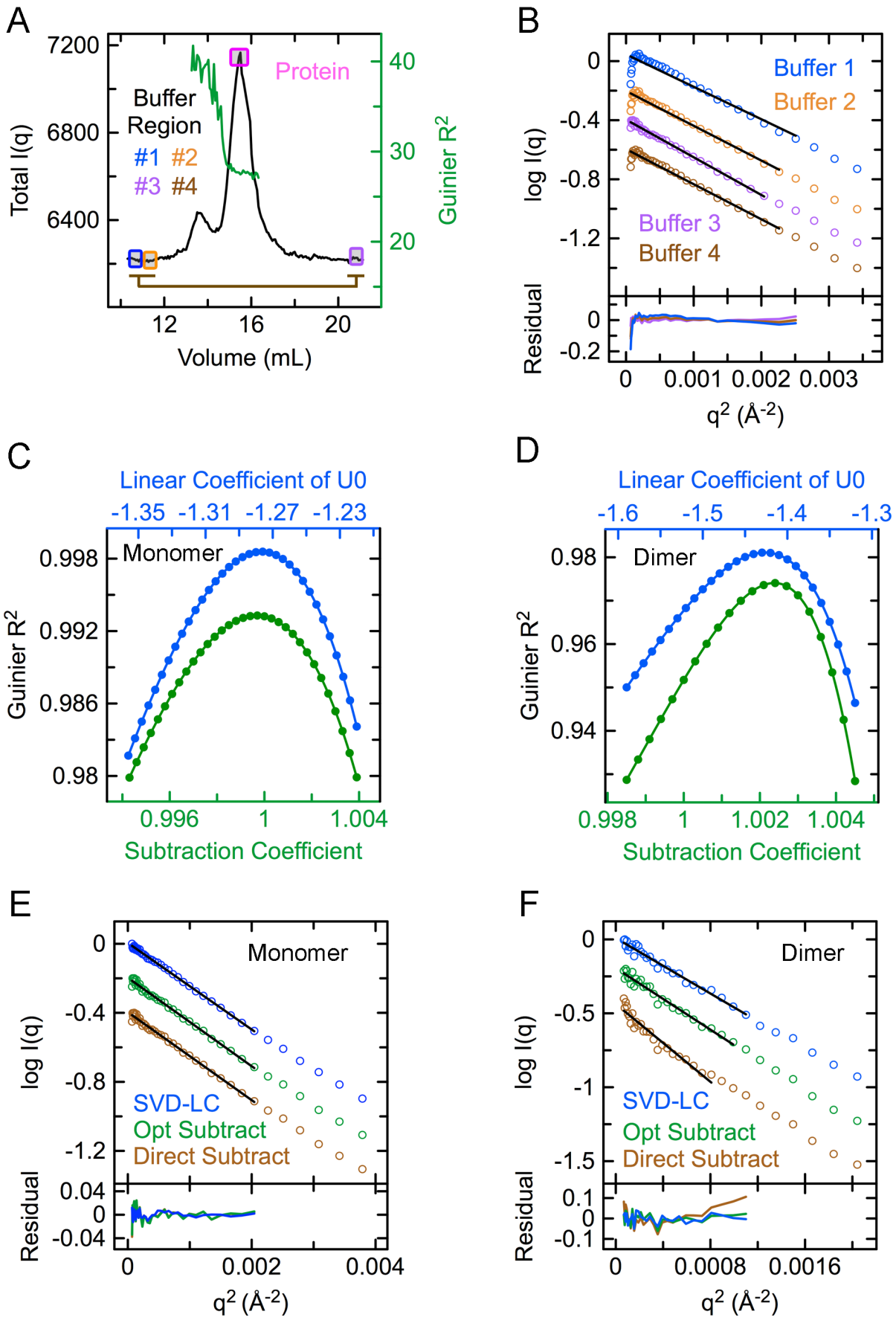

A
I(q)
Protein
Buffer
q (Å-1)
B
Total I(q)
Beam Intensity
Monomer
Dimer
Raw
Norm
I0
I1
Volume (mL)
C
Singular Value
Autocorrelation
All Data
S00
S11
S22
U
V
Column of U
D
SnnVn
S00V0
S00
S11
S22
Snn
n > 2
Index of V
E
Singular Value
Autocorrelation
Monomer
S00
S11
U
V
Column of U
F
SnnVn
S00V0
S00V0
S11V1
SnnVn
n > 1
Index of V
G
Singular Value
Autocorrelation
Dimer
S00
S11
U
V
Column of U
H
SnnVn
S00V0
SnnVn
n > 1
S00V0
S11V1
Index of V

Figure S6


A
Total I(q)
Guinier R²
Protein
Buffer Region
#1 #2
#3 #4
Volume (mL)
B
log I(q)
Buffer 1
Buffer 2
Buffer 3
Buffer 4
Residual
q² (Å⁻²)
C
Linear Coefficient of U0
Monomer
Guinier R²
Subtraction Coefficient
D
Linear Coefficient of U0
Dimer
Guinier R²
Subtraction Coefficient
E
Monomer
log I(q)
SVD-LC
Opt Subtract
Direct Subtract
Residual
q² (Å⁻²)
F
Dimer
log I(q)
SVD-LC
Opt Subtract
Direct Subtract
Residual
q² (Å⁻²)