
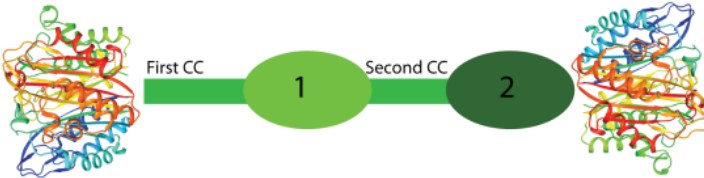


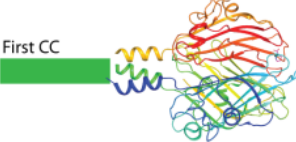

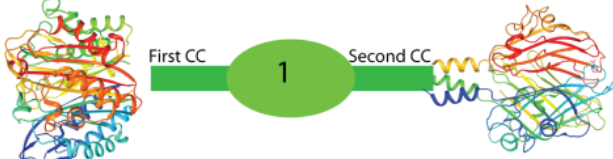
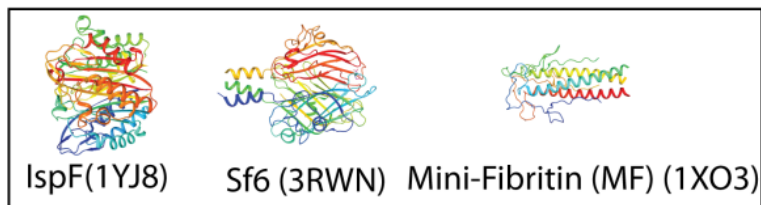


Construct	Spc29 Residues	Spc29 Cartoon
1. Spc29 <sub>asp</sub>	2-253	
2. IspF-Spc29 <sub>asp</sub> -IspF	8-253	
3. Spc29 <sub>asp</sub> -G152-Sf6	2-152	
4. Spc29 <sub>asp</sub> -S160-Sf6	2-160	
5. Spc29 <sub>asp</sub> -K32-Sf6	2-32	
6. MF-R161-Spc29 <sub>asp</sub>	161-253	
7. IspF-26-160-Spc29 <sub>asp</sub> -SF6	26-160	

#### Fusion Proteins



IspF (PDB : 1U3P)  
Mini-Fibrin (PDB : 1OX3)

### 1. Spc29asp (2-253)

GADYSNFGNSASKKFQDDDLNVRKEHEEALKKLREENFDDNDSELGNKKHYRAQERMSSPLHRLSPAGKSDDRKVKDPLDDKLRRQLREGNTRLPPPPFSSYGMPTNRSNLDRIIRRRDDDPVRTDKFADQNVIDDQRLEIKYLERIVYDQGTVIDNLTSRITRLESFILNSISDRGDKNFASLEHSRSDGFPTNKTYGLQMGGLYENDMPYRRSSDNINKEGAREDRDSQIHIEDDEDILKILSDDFHN

MW: 29611.4, as trimer MW: **88834.2**  
SAXS buffer: 500 mM Tris pH 8.0, 5% glycerol

### 2. IspF-Spc29asp-IspF (8-253)

MRIGHGFDVHAFGGEGPIIIGGVRIPIYEKGLLAHSDGDVALHALTDALLGAAALGDIGKLPDTPAFKGADSRELLREAWRRIQAKGYTLGNVDVTIIAQAPKMLPHIPQMRVFAEDLGCHMDDVNVKATTTEKLGFTGRGEGIACEAVALLIKATKSGNSASKKFQDDDLNVRKEHEEALKKLREENFDDNDSELGNKKHYRAQERMSSPLHRLSPAGKSDDRKVKDPLDDKLRRQLREGNTRLPPPPFSSYGMPTNRSNLDRIIRRRDDDPVRTDKFADQNVIDDQRLEIKYLERIVYDQGTVIDNLTSRITRLESFILNSISDRGDKNFASLEHSRSDGFPTNKTYGLQMGGLYENDMPYRRSSDNINKEGAREDRDSQIHIEDDEDILKILSDDFHNHNSAGRIGHGFDVHAFGGEGPIIIGGVRIPIYEKGLLAHSDGDVALHALTDALLGAAALGDIGKLPDTPAFKGADSRELLREAWRRIQAKGYTLGNVDVTIIAQAPKMLPHIPQMRVFAEDLGCHMDDVNVKATTTEKLGFTGRGEGIACEAVALLMKAAGGASHHHHHH

MW: 63869.9, as trimer MW: **191609.7**  
SAXS buffer: 500 mM Tris pH 8.0, 5% glycerol

### 3. Spc29asp-G152-Sf6 (2-152)

GSTADYSNFGNSASKKFQDDDLNVRKEHEEALKKLREENFDDNDSELGNKKHYRAQERMSSPLHRLSPAGKSDDRKVKDPLDDKLRRQLREGNTRLPPPPFSSYGMPTNRSNLDRIIRRRDDDPVRTDKFADQNVIDDQRLEIKYLERIVYDQGTVIDNLTSRIDALEYATTRKKSEVVYSGVSVTIPTAPTNLVSLKLTLPSSGTLAPFFDVTNNKMVVFENKTLFFKLSIVGTWPSGTANRSMQLTFSGSVPTDLVSSRNSATTTDNILLATFFSVDKDGFLATNGSTLTIQSNNGASFTATTIKIIAEQ

MW: 34385.3, as trimer MW: **103155.9**  
SAXS buffer: 50 mM HEPES pH 7.6, 100 mM NaCl 5% glycerol

### 4. Spc29asp-S160-Sf6 (2-160)

GSTADYSNFGNSASKKFQDDTLNVRKEHEEALKKLREENFSSNTSELGNKKHYRAQERMSSPLHRLSPTGKSDDRKVKSPLDLKLRRQLREGNTRLPPPPFSSYGMPTNRSNLDRIIRRTSSPVRTDKFASQNVIDDQRLEIKYLERIVYDQGTVIDNLTSRIDALEYATTRKKSEVVYSGVSVTIPTAPTNLVSLKLTLPSSGTLAPFFDVTNNKMVVFENKTLFFKLSIVGTWPSGTANRSMQLTFSGSVPTDLVSSRNSATTTDNILLATFFSVDKDGFLATNGSTLTIQSNNGASFTATTIKIIAEQ

MW: 34948.2, as trimer MW: **104844.6**  
SAXS buffer: 50 mM HEPES pH 7.6, 100 mM NaCl 5% glycerol

### 5. Spc29asp-K32-Sf6 (2-32)

GSTADYSNFGNSASKKFQDDDLNVRKEHEEALKRIDALEYATTRKKSEVVYSGVSVTIPTAPTNLVSLKLTLPSSGTLAPFFDVTNNKMVVFENKTLFFKLSIVGTWPSGTANRSMQLTFSGSVPTDLVSSRNSATTTDNILLATFFSVDKDGFLATNGSTLTIQSNNGASFTATTIKIIAEQ

MW: 19981.4, as trimer MW: **59944.2**  
SAXS buffer: 50 mM HEPES pH 7.6, 100 mM NaCl 5% glycerol

### 6. MF-R161-Spc29asp (161-253)

GAGSGTDIVLNDLPFVDGPPAEGQSRISWIKNGEELGADTQYGSEGSMNRPTVSVLRNVEVLDKNIGILKTSLETANSDIKTIQERITRLESFILNSISDRGDKNFASLEHSRSDGFPTNKTYGLQMGGLYENDMPYRRSSDNINKEGAREDRDSQIHIEDDEDILKILSDDFHN

MW: 19923.8, as trimer MW: **59771.4**  
SAXS buffer: 50 mM HEPES pH 7.6, 100 mM NaCl 5% glycerol

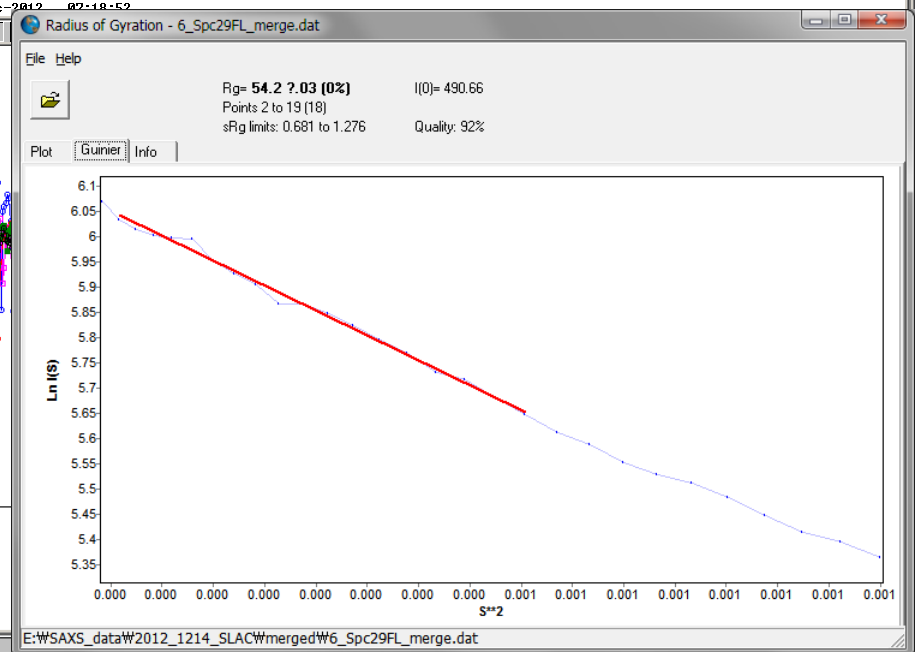
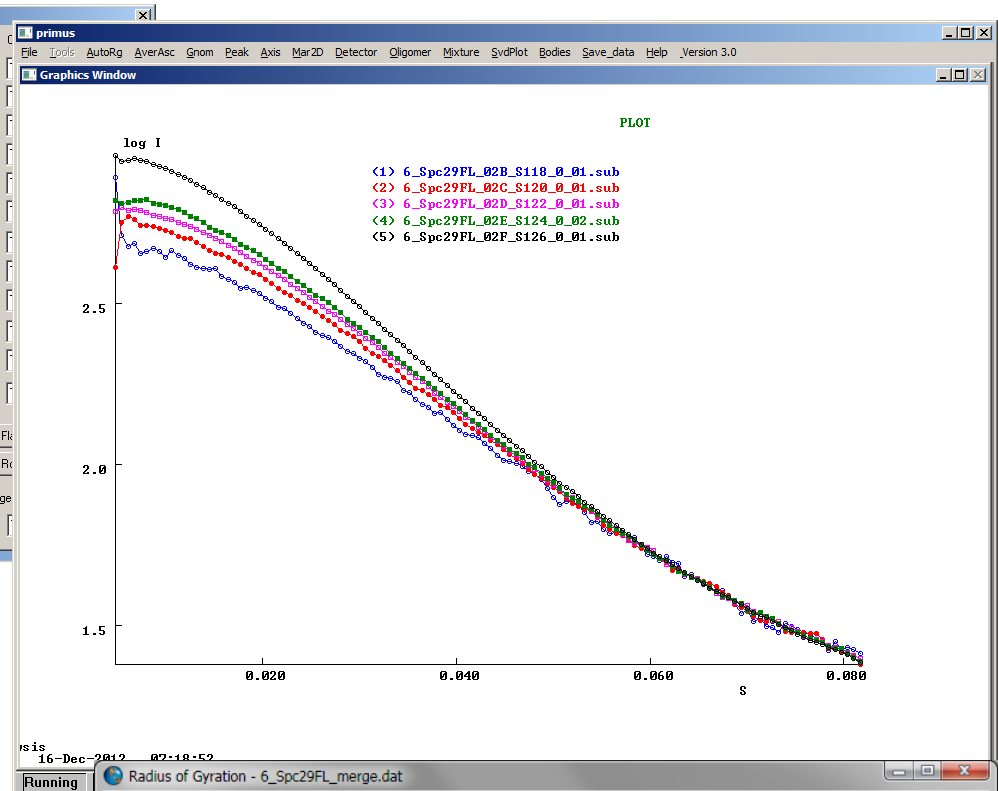
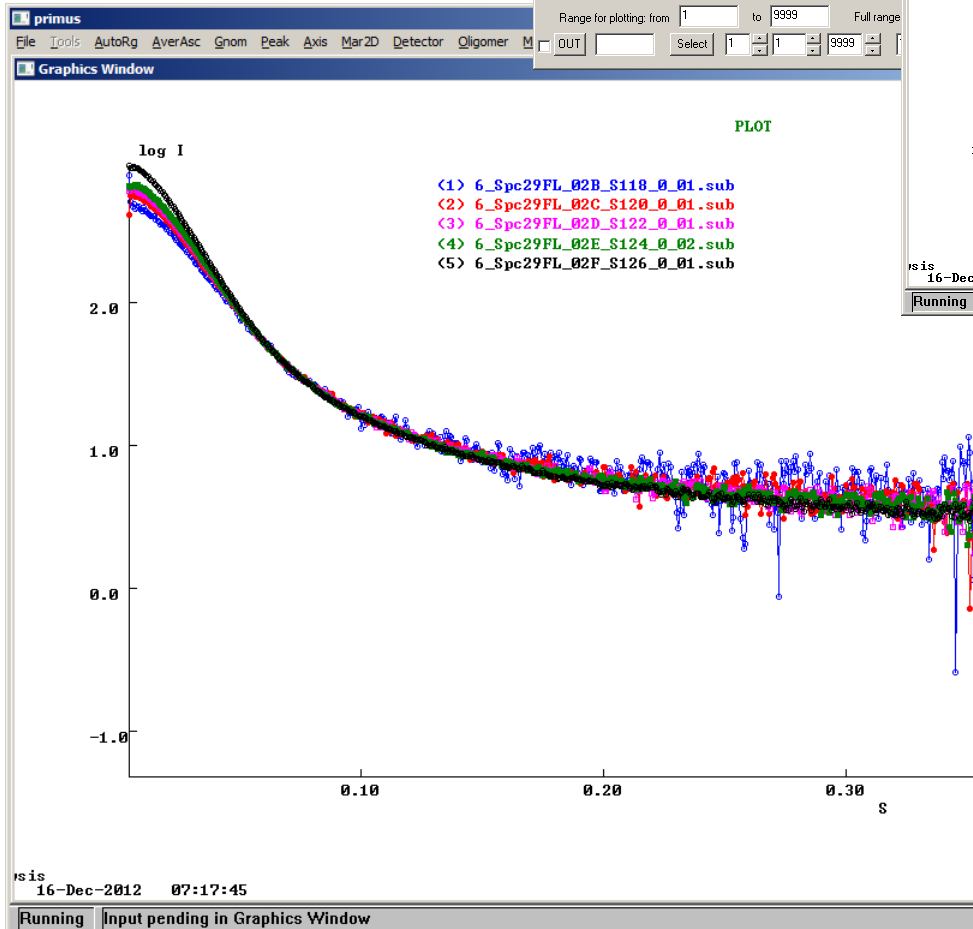
### 7. IspF-26-160-Spc29asp-Sf6 (26-160)

MRIGHGFDVHAFGGEGPIIIGGVRIPIYEKGLLAHSDGDVALHALTDALLGAAALGDIGKLPDTPAFKGADSRELLREAWRRIQAKGYTLGNVDVTIIAQAPKMLPHIPQMRVFAEDLGCHMDDVNVKATTTEKLGFTGRGEGIACEAVALLIKATKSGHEEALKKLREENFDDNDSELGNKKHYRAQERMSSPLHRLSPAGKSDDRKVKDPLDDKLRRQLREGNTRLPPPPFSSYGMPTNRSNLDRIIRRRDDDPVRTDKFADQNVIDDQRL EIKYLERIVYDQGTVIDNLTSRIDALEYATTRKKSEVVYSGVSVTIPTAPTNLVSLKLTLPSSGTLAPFFDVTNNKMVVFENKTLFFKLSIVGTWPSGTANRSMQLTFSGSVPTDLVSSRNSATTTDNILLATFFSVDKDGFLATNGSTLTIQSNNGASFTATTIKIIAEQEGASHHHHHHHH

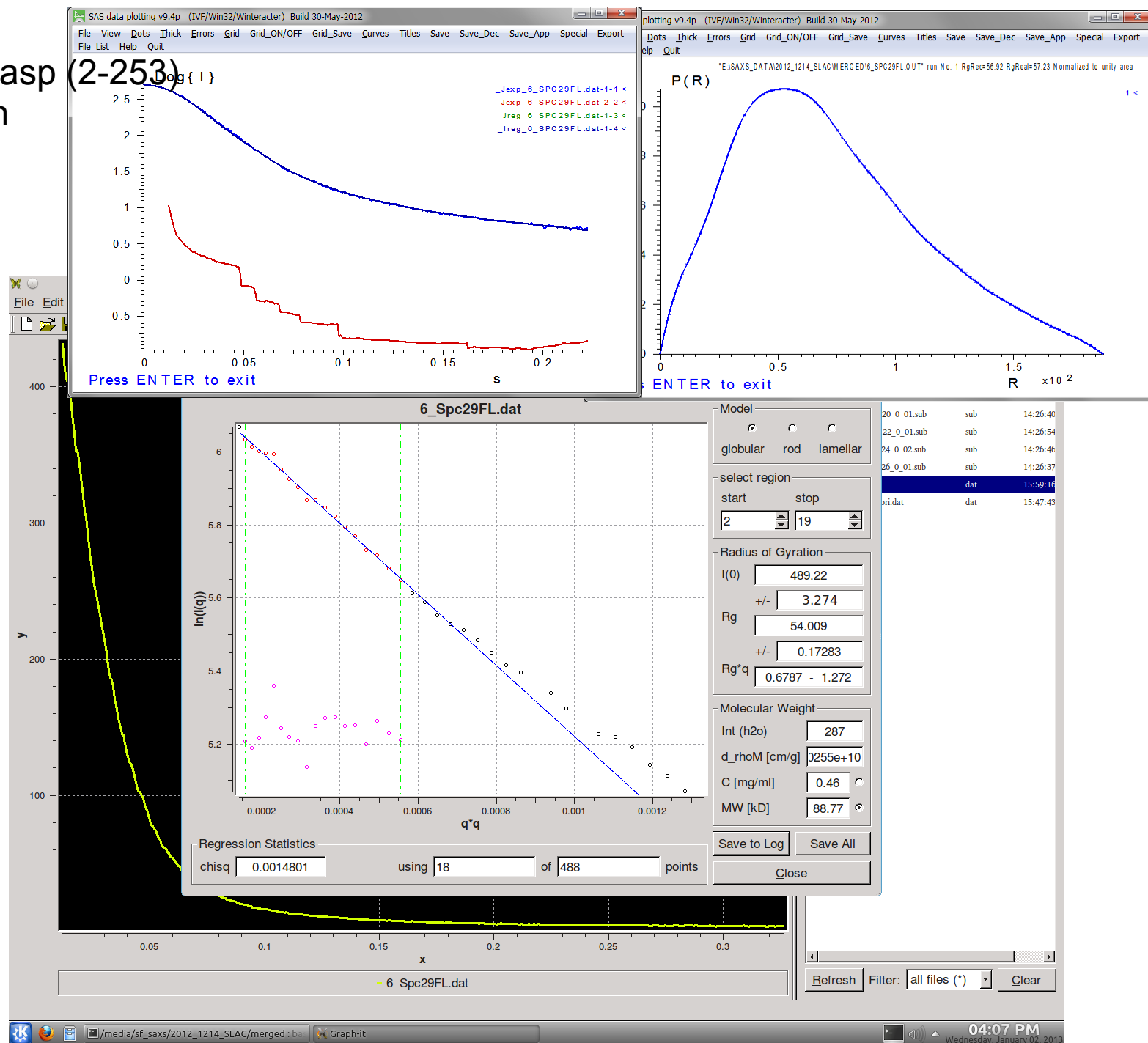
MW: 50388.9, as trimer MW: **151166.7**  
SAXS buffer: 50 mM HEPES pH 7.6, 100 mM NaCl 5% glycerol

(1) Spc29asp (2-253)  
Full length

Strong concentration  
Dependence  
-> highly flexible



(1) Spc29asp (2-253)  
Full length

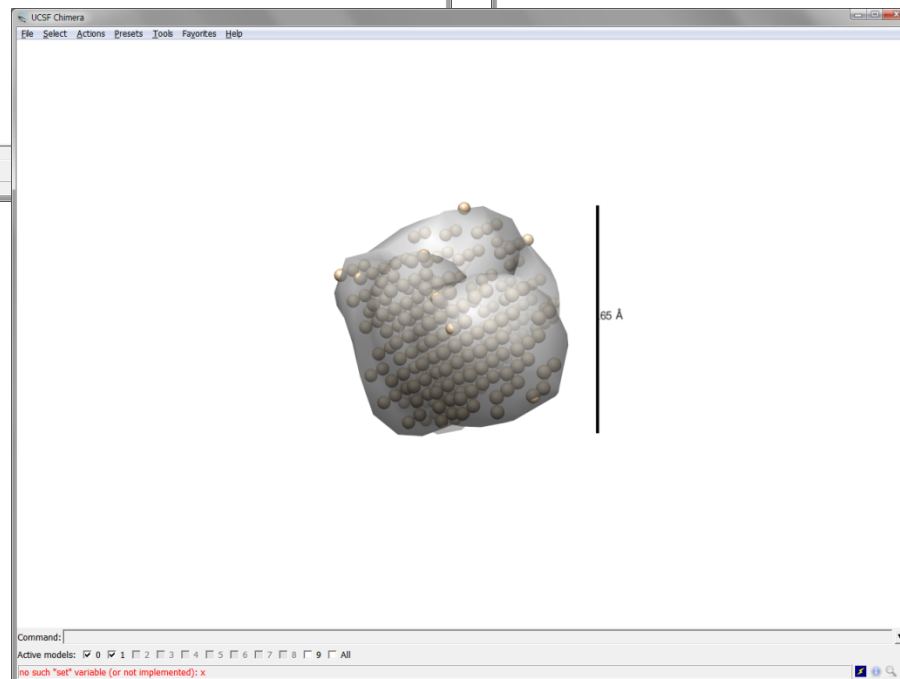
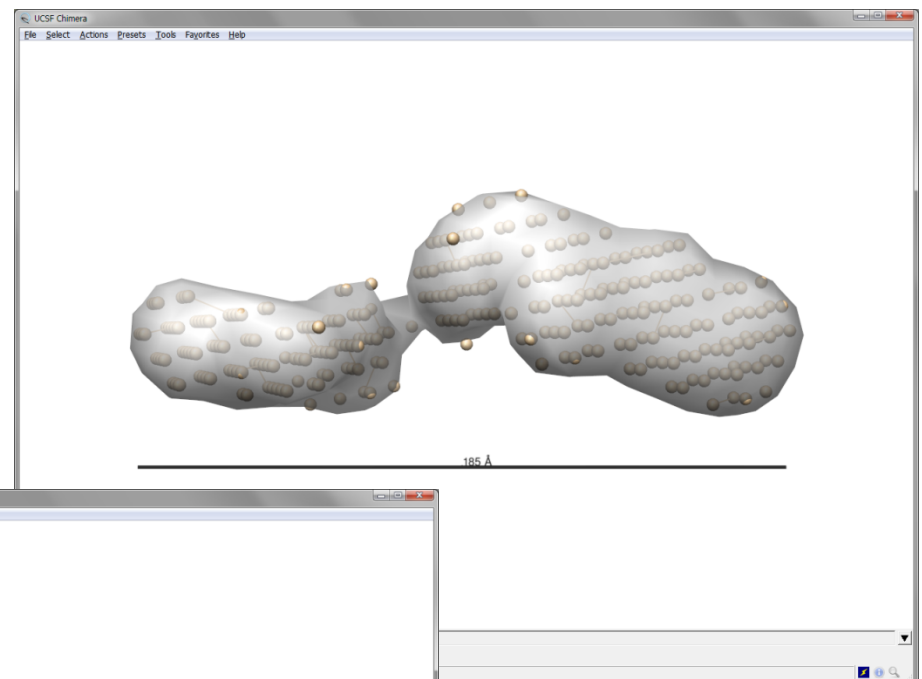
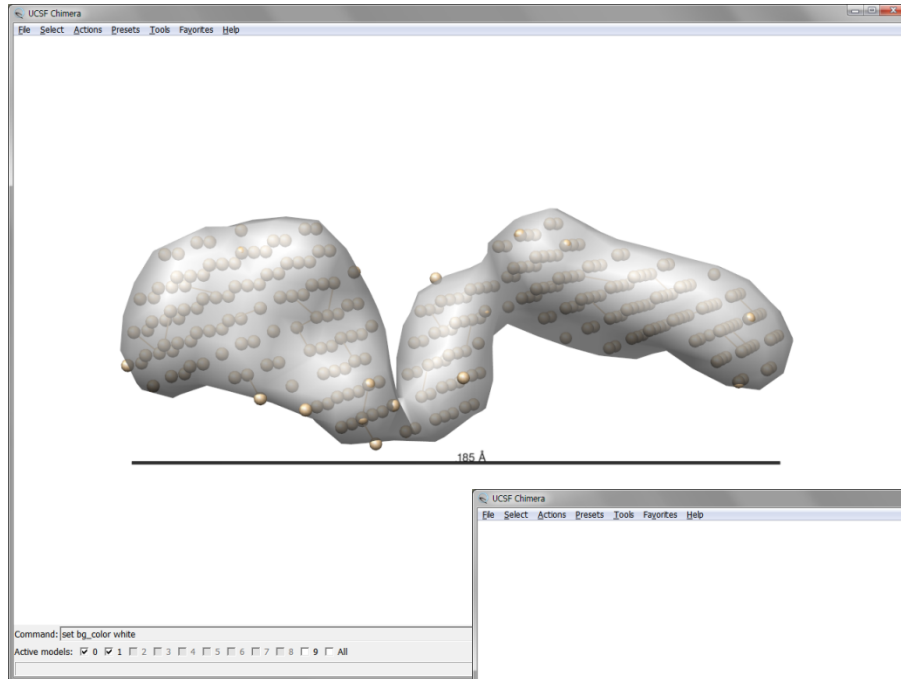




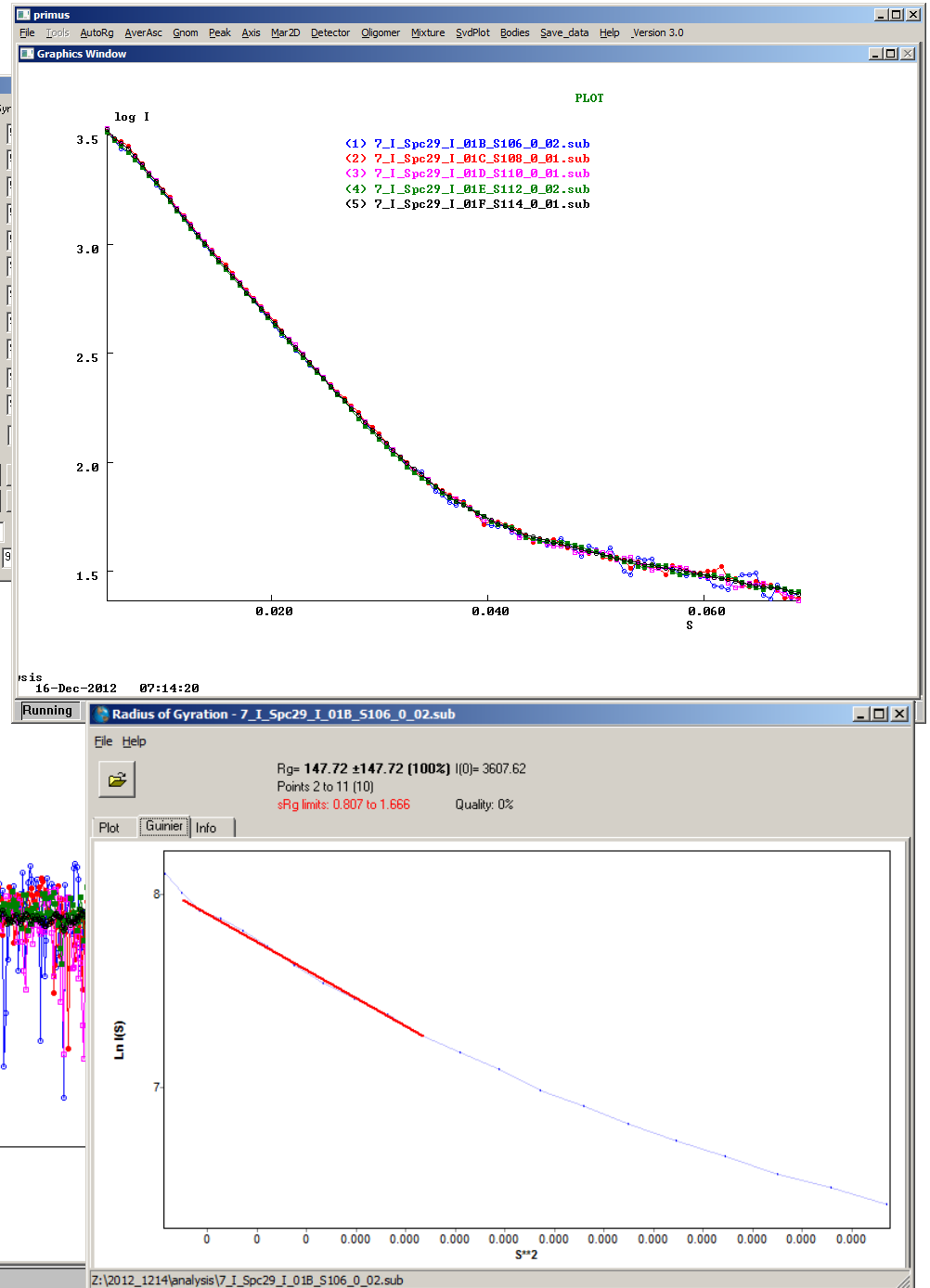
# (1) Spc29asp (2-253) Full length (from multiple viewpoints)

Spc29<sub>asp</sub>

2-253

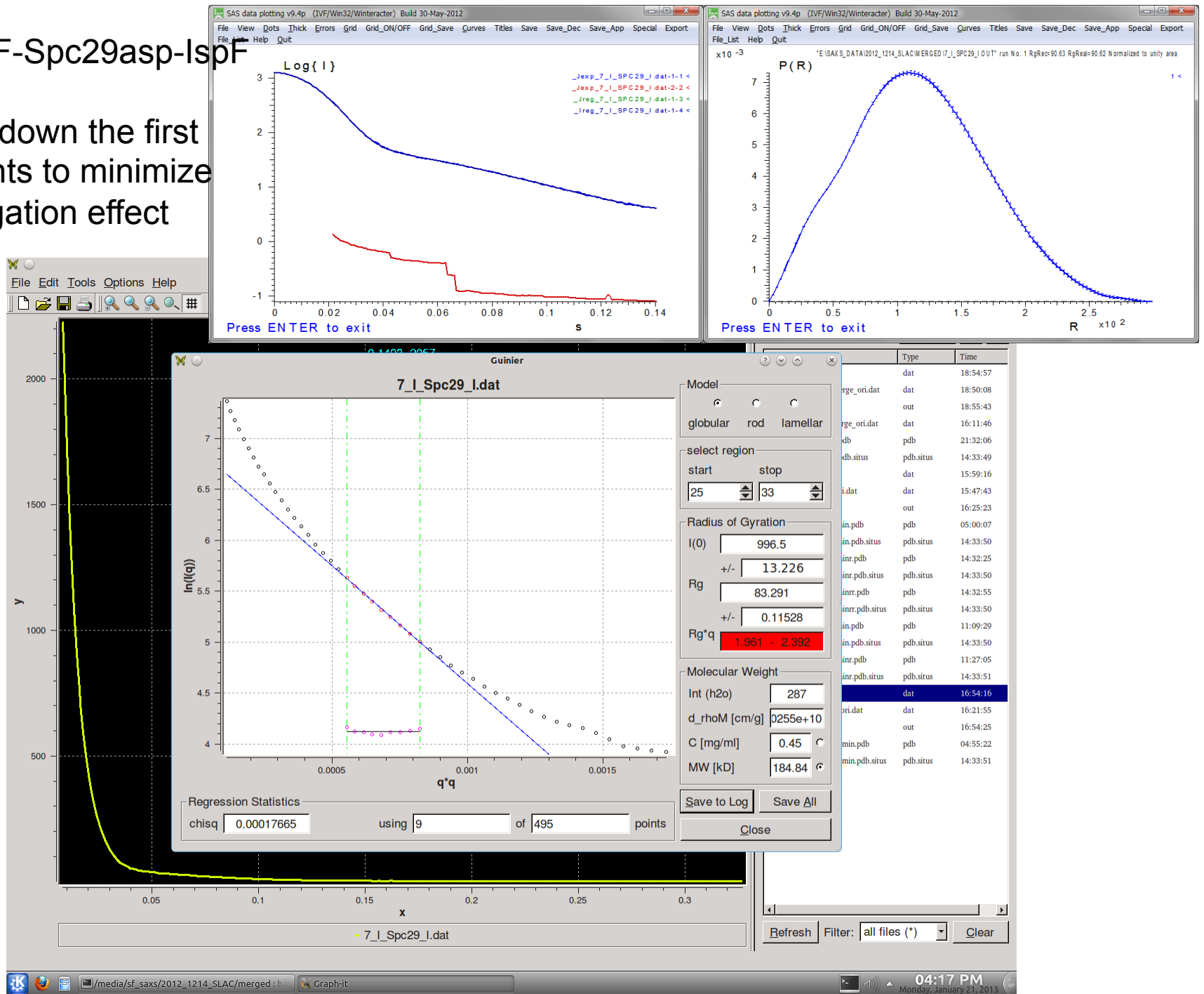


- > Aggregated
- > Mixtures of  $R_g = 145 \text{ \AA}$  &  $R_g = 80 \sim 90 \text{ \AA}$



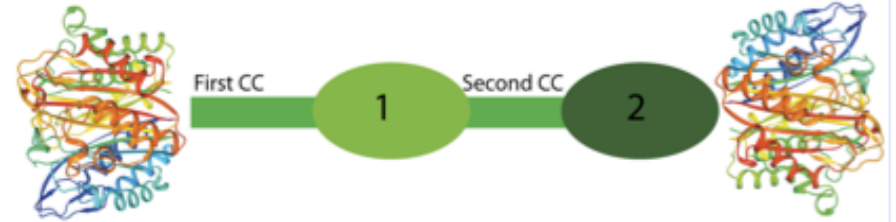
## (2) IspF-Spc29asp-IspF

-> Cut down the first 20 points to minimize Aggregation effect



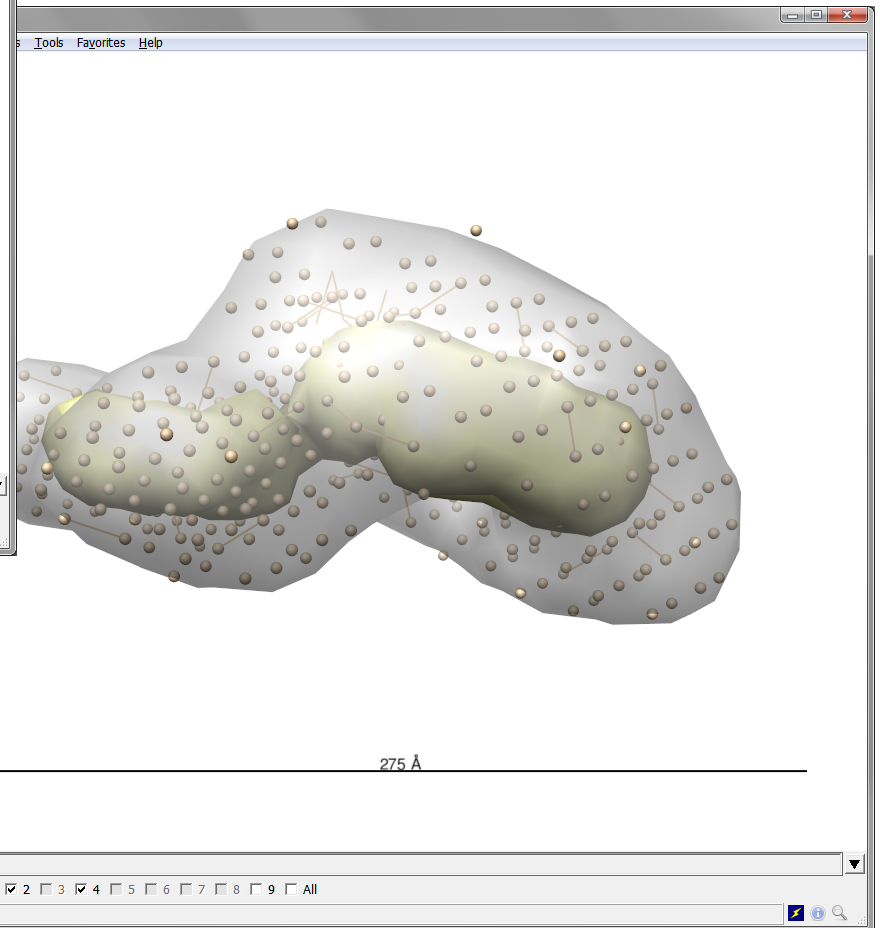
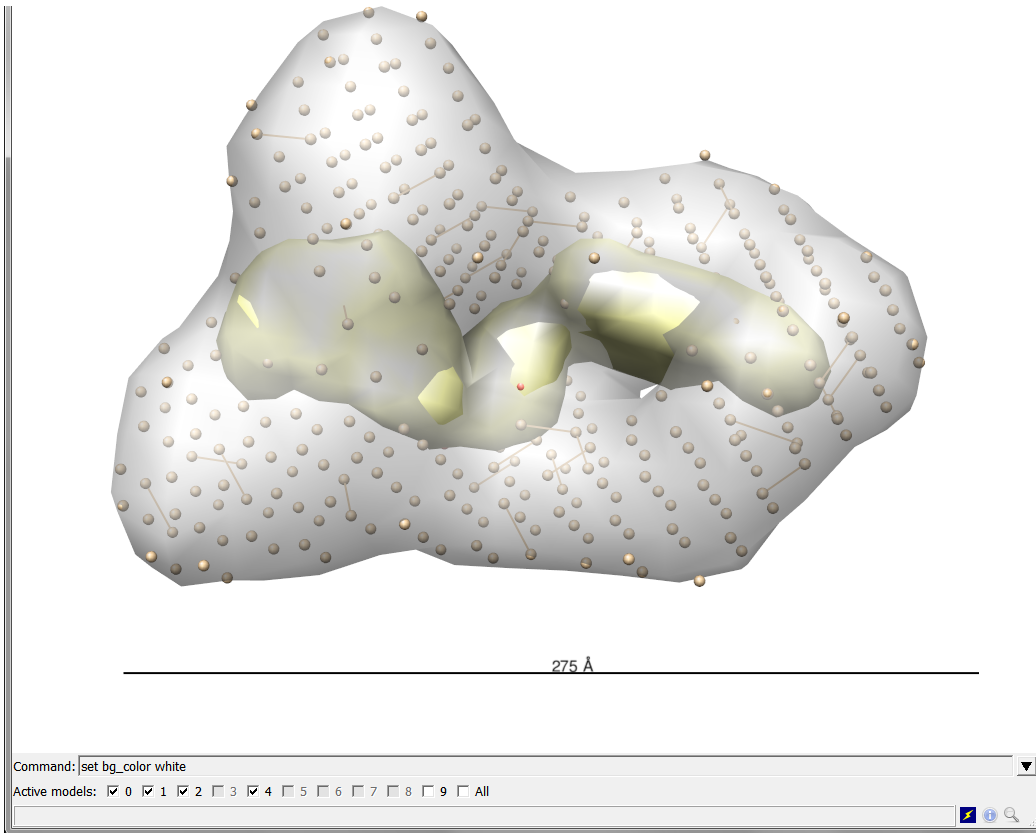
IspF-Spc29<sup>asp</sup>-IspF

8-253

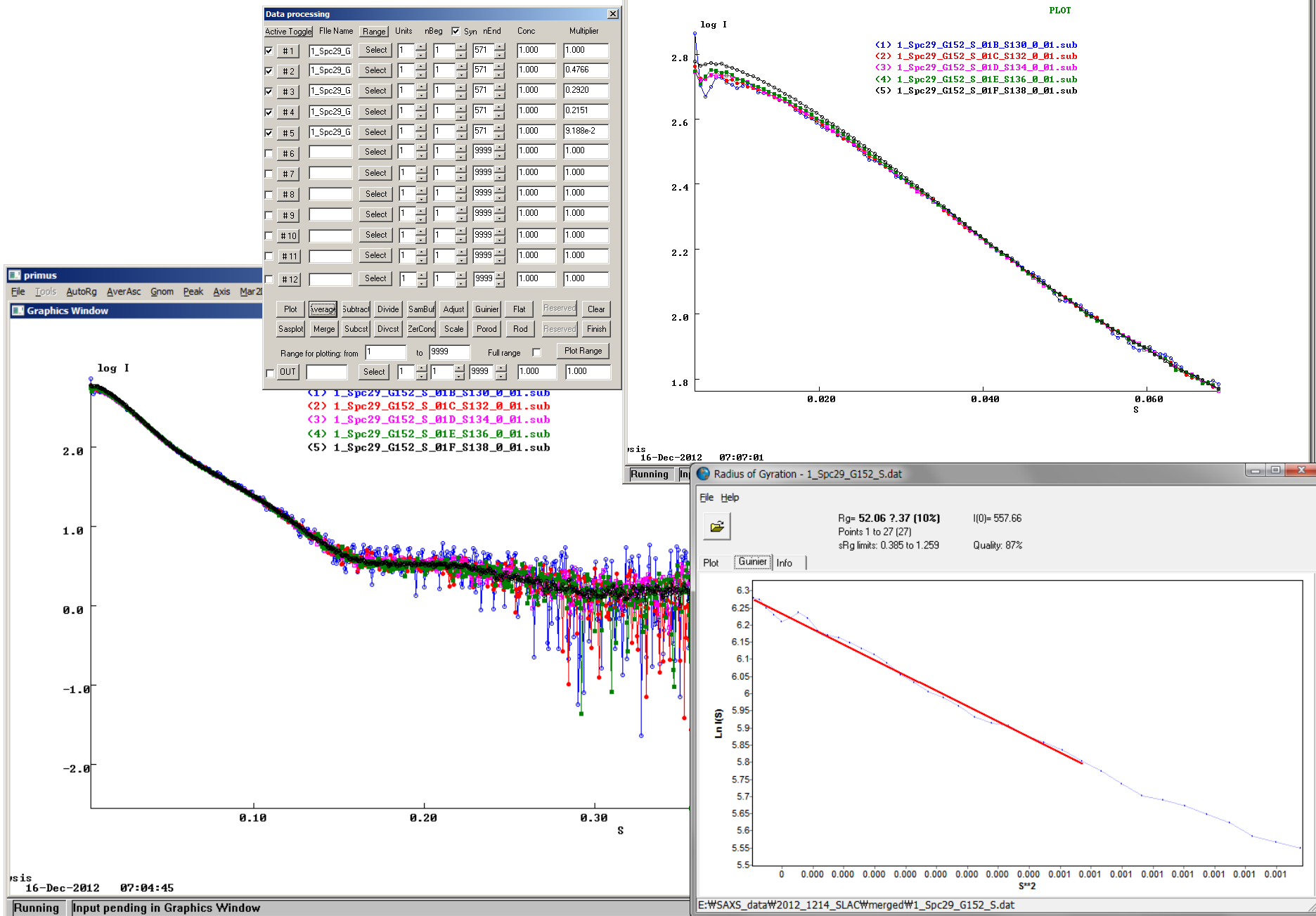


(2) IspF-Spc29<sup>asp</sup>-IspF

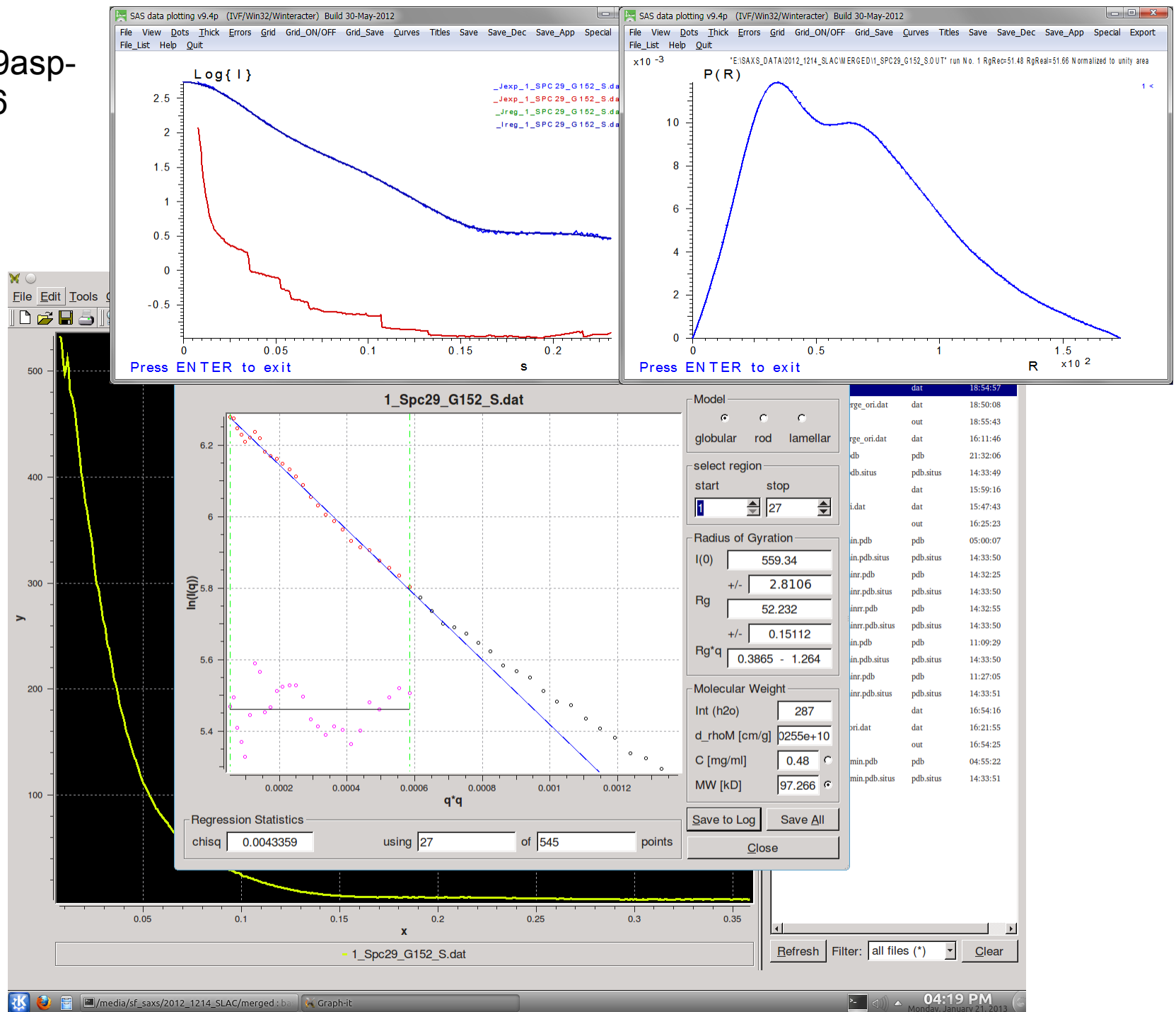
Aggregated



### (3) Spc29asp-G152-Sf6 (2-152)



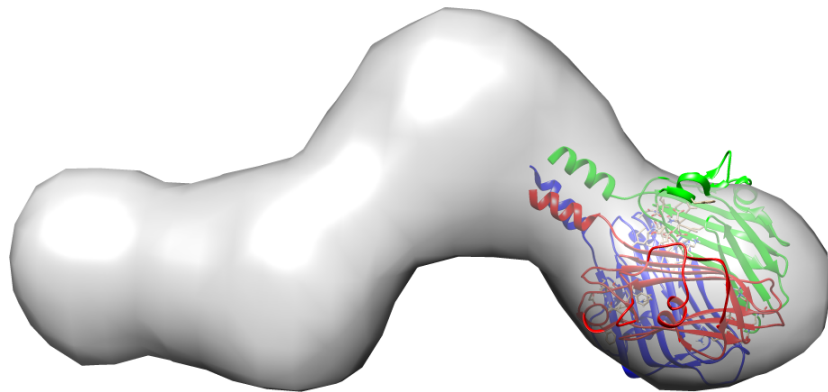
### (3) Spc29asp-G152-Sf6



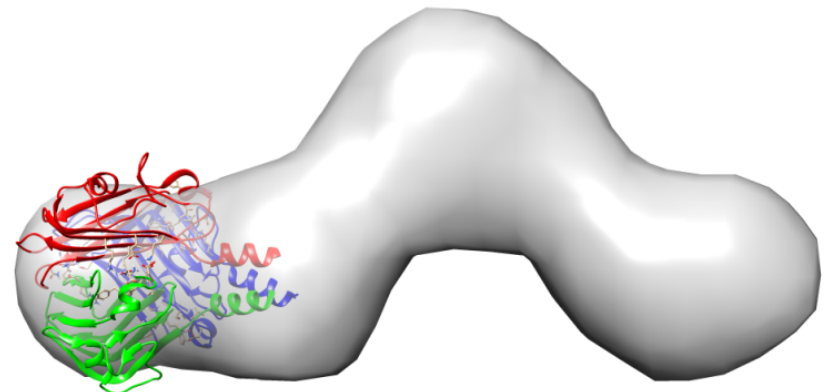
### (3) Spc29<sub>asp</sub>-G152-Sf6

Spc29<sub>asp</sub>-G152-Sf6

2-152

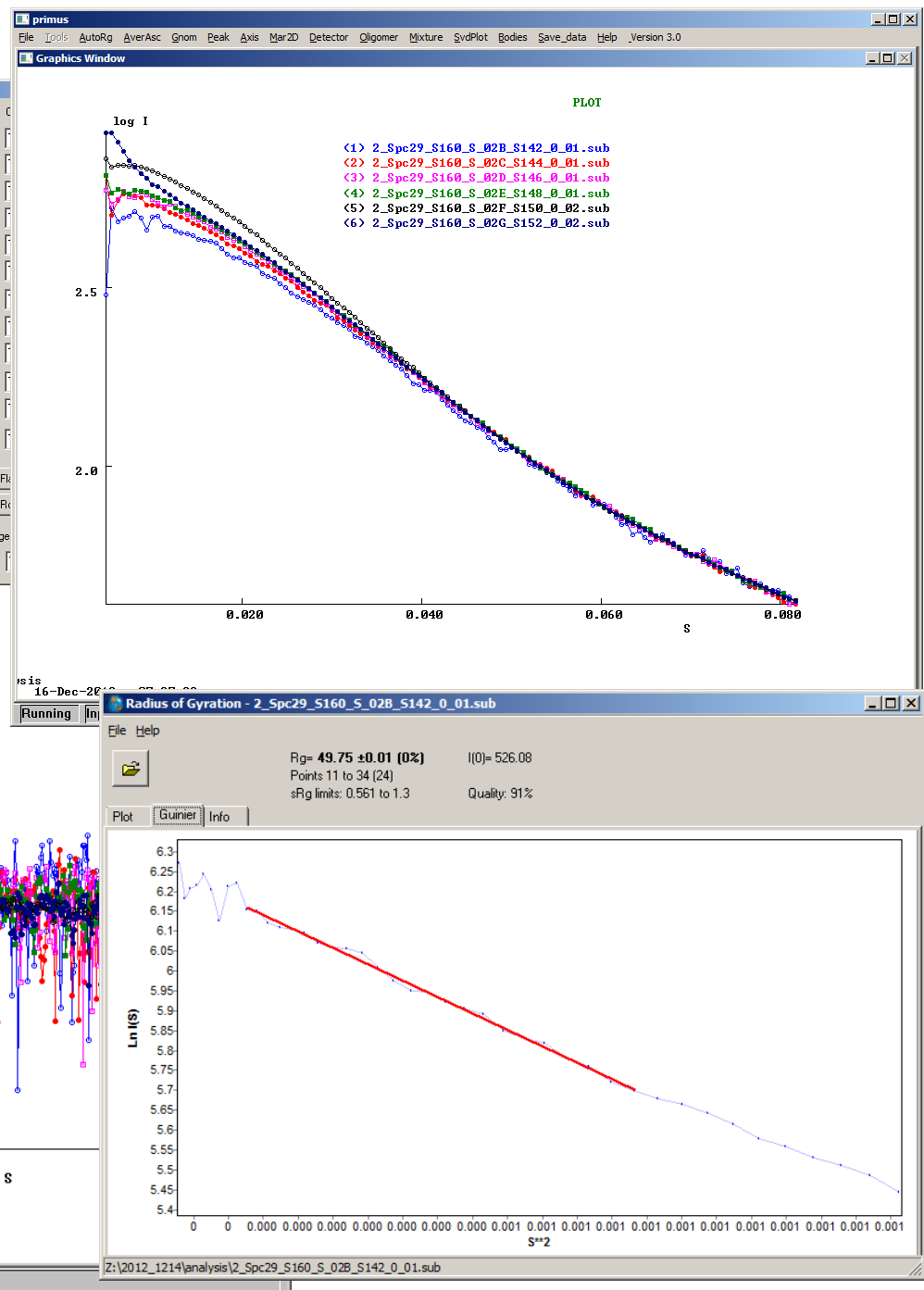


175 Å



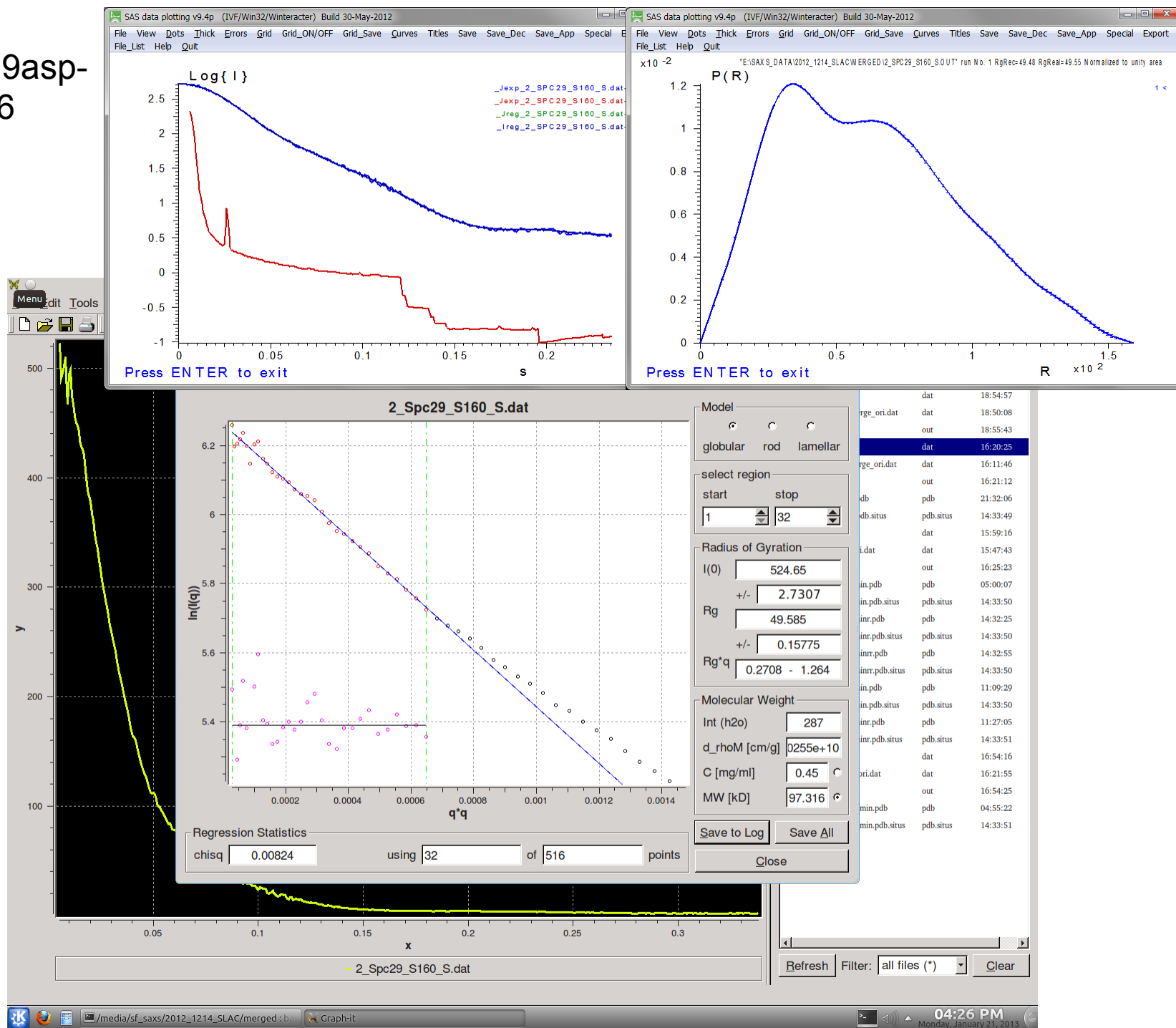
175 Å

G: Unfiltered 2.5mg/ml  
- minor aggregation





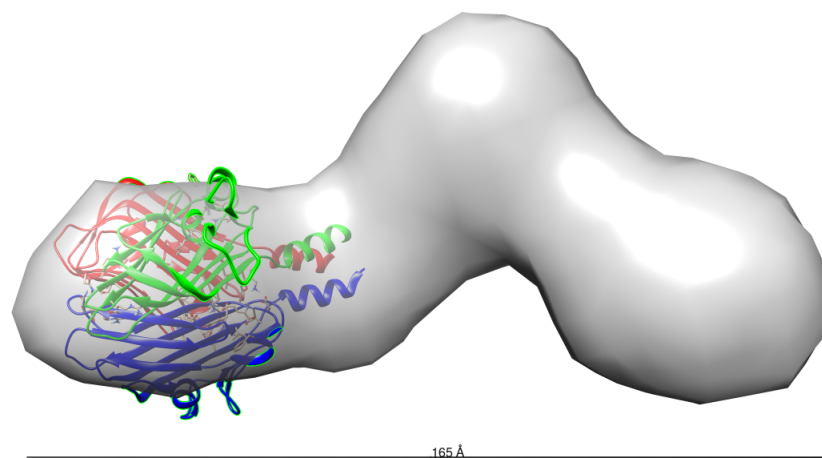
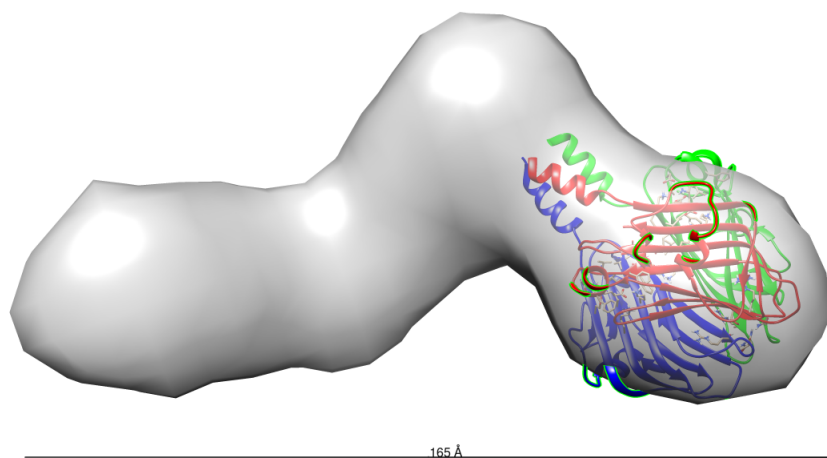
## (4) Spc29asp-S160-Sf6



#### (4) Spc29<sup>asp</sup>-S160-Sf6

Spc29<sup>asp</sup>-S160-Sf6

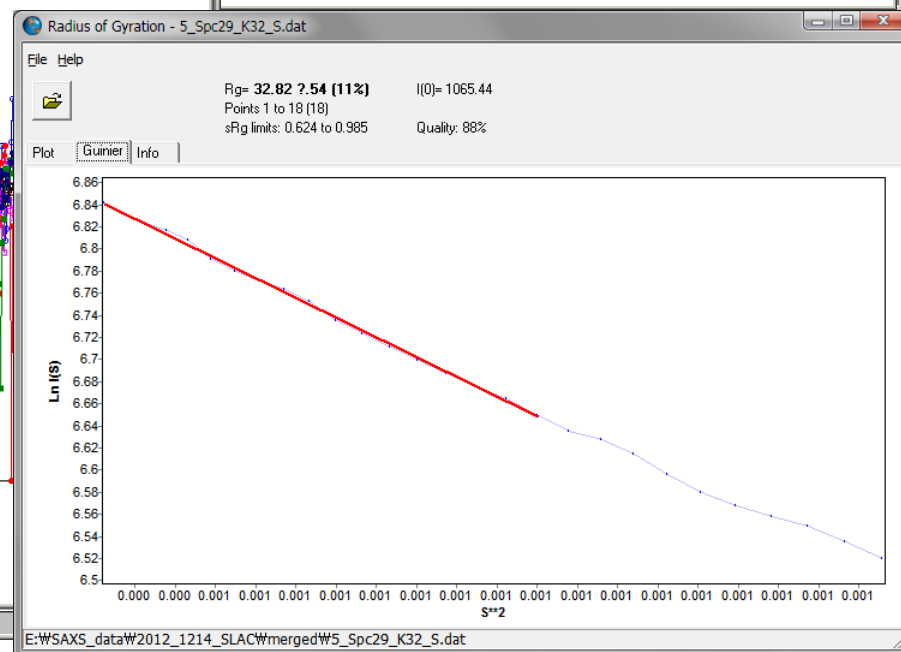
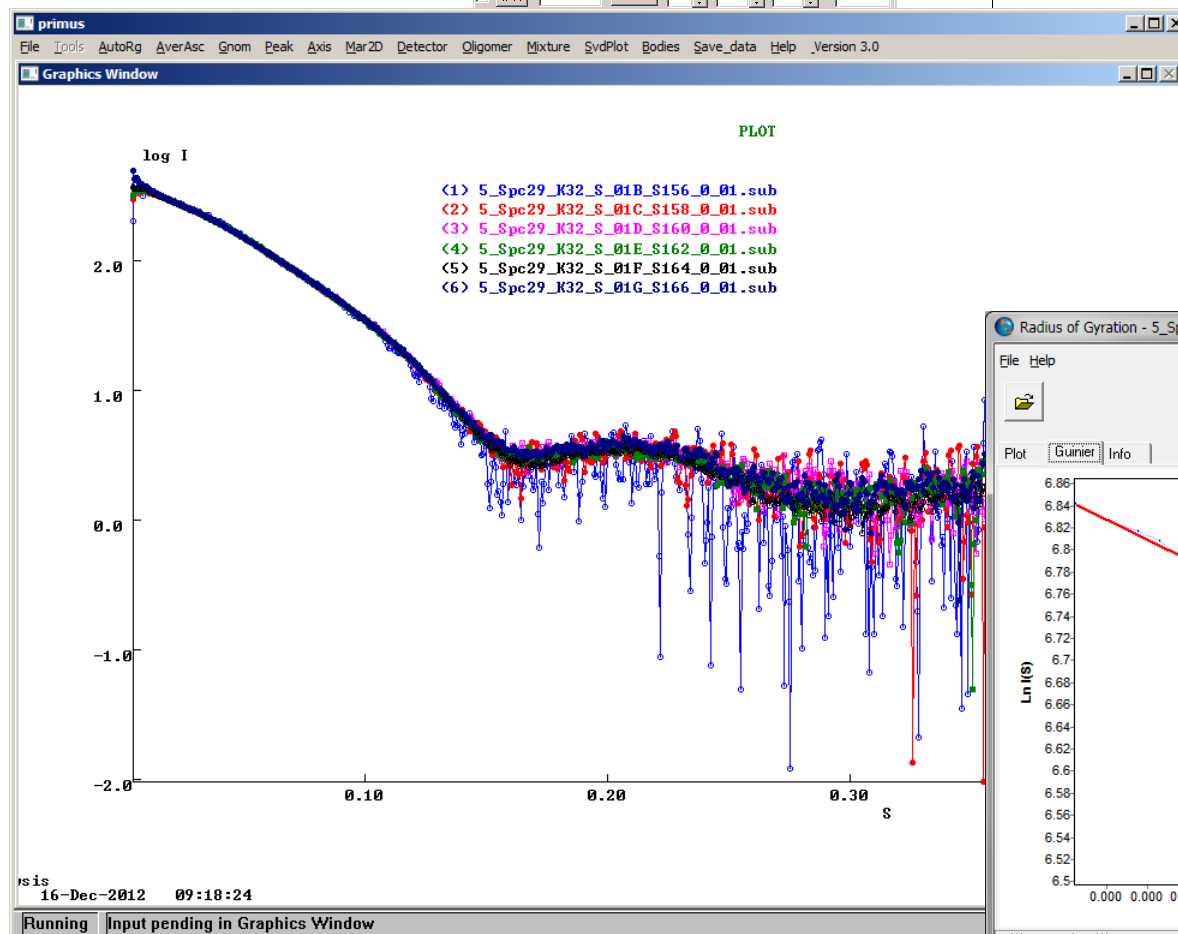
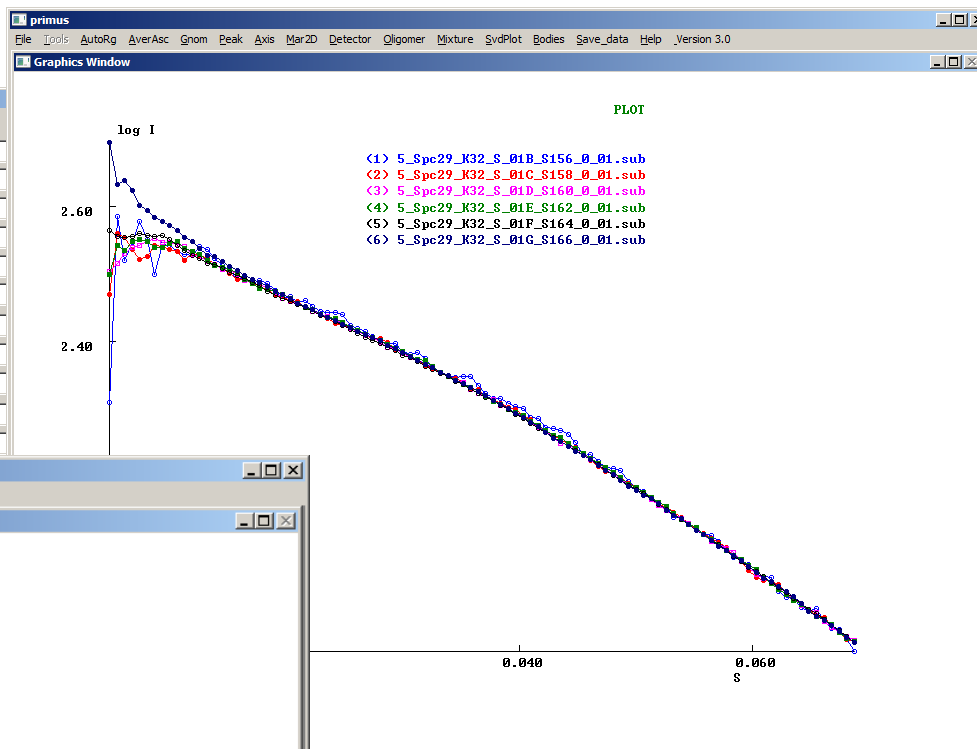
2-160



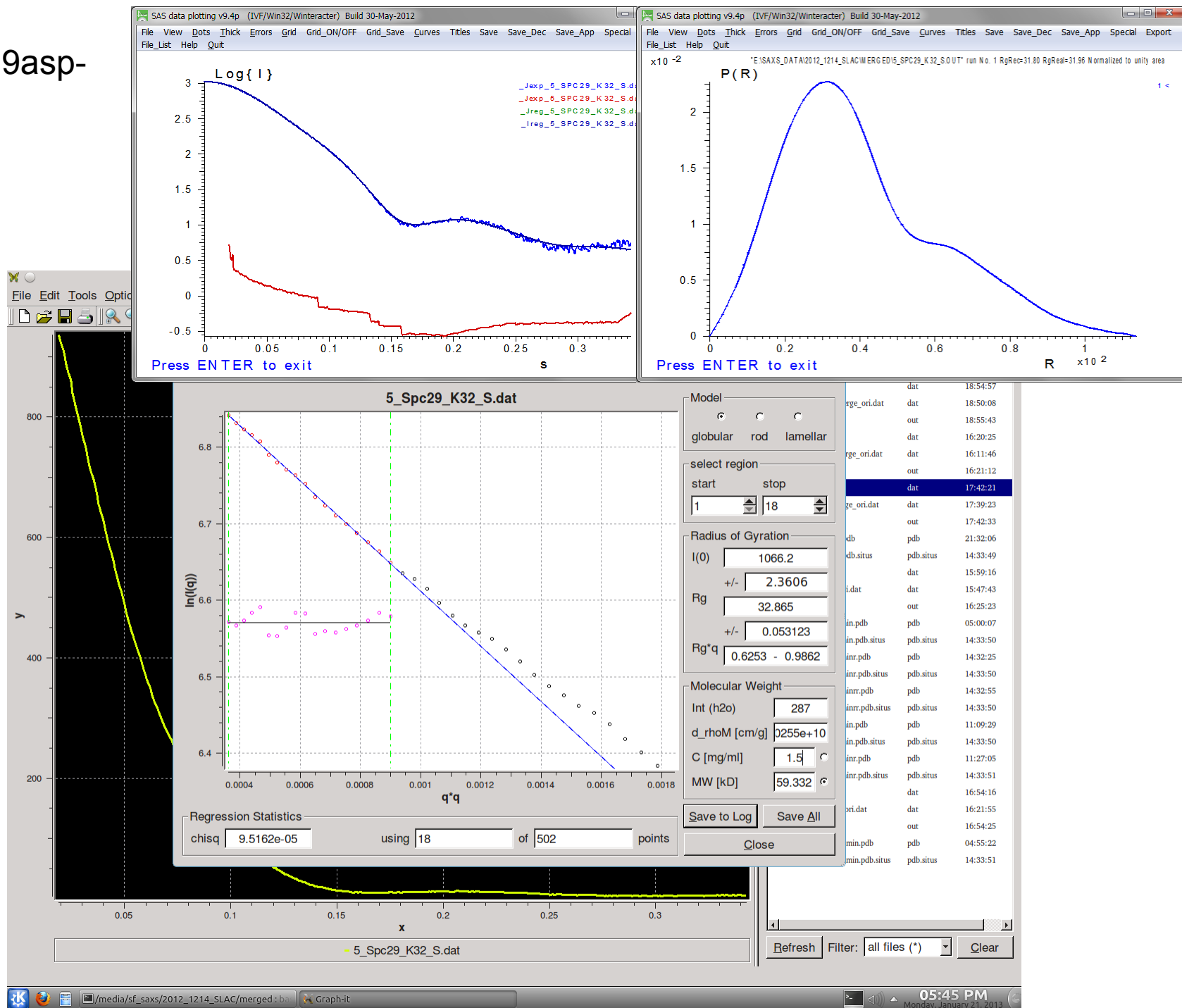
## (5) Spc29asp-K32-Sf6 (2-32)

G: unfiltered 2.5mg/ml  
Minor Aggregation

Data processing						
Active Toggle	File Name	Range	Units	nBeg	✓ Syn	nEnd
✓ #1	5_Spc29_K	Select	1	1	571	1,000
✓ #2	5_Spc29_K	Select	1	1	571	1,000
✓ #3	5_Spc29_K	Select	1	1	571	1,000
✓ #4	5_Spc29_K	Select	1	1	571	1,000
✓ #5	5_Spc29_K	Select	1	1	571	1,000
✓ #6	5_Spc29_K	Select	1	1	571	1,000
✓ #7		Select	1	1	9999	1,000
✓ #8		Select	1	1	9999	1,000
✓ #9		Select	1	1	9999	1,000
✓ #10		Select	1	1	9999	1,000
✓ #11		Select	1	1	9999	1,000



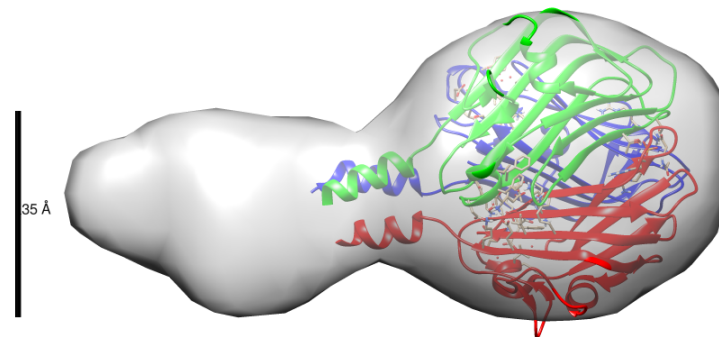
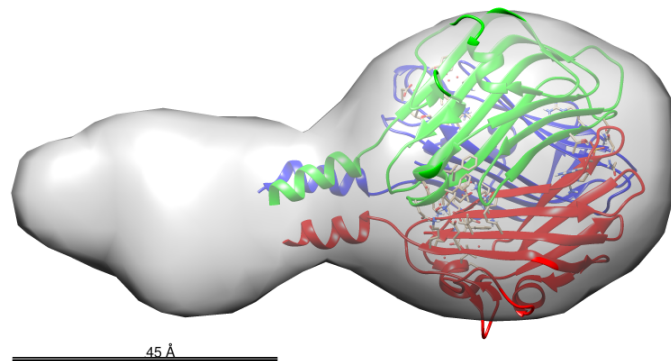
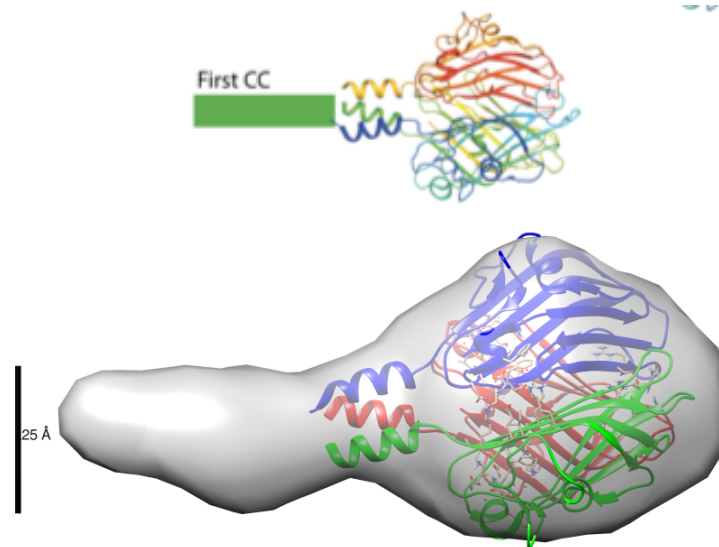
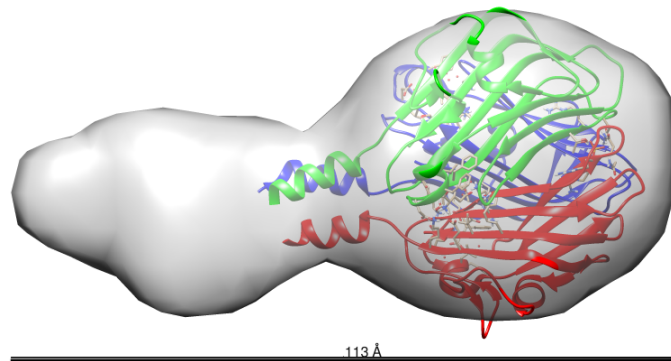
## (5) Spc29asp-K32-Sf6



## (5) Spc29asp-K32-Sf6

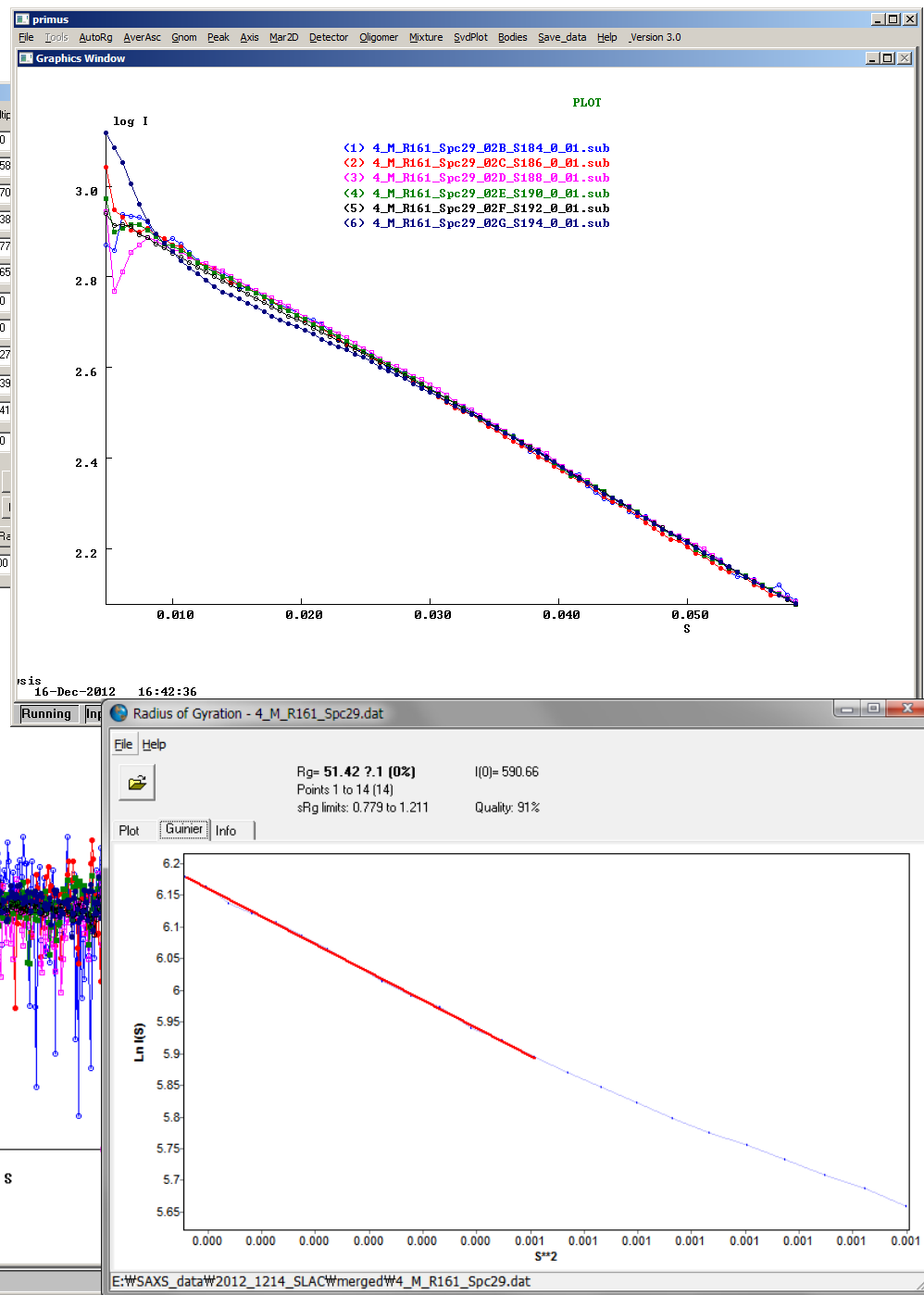
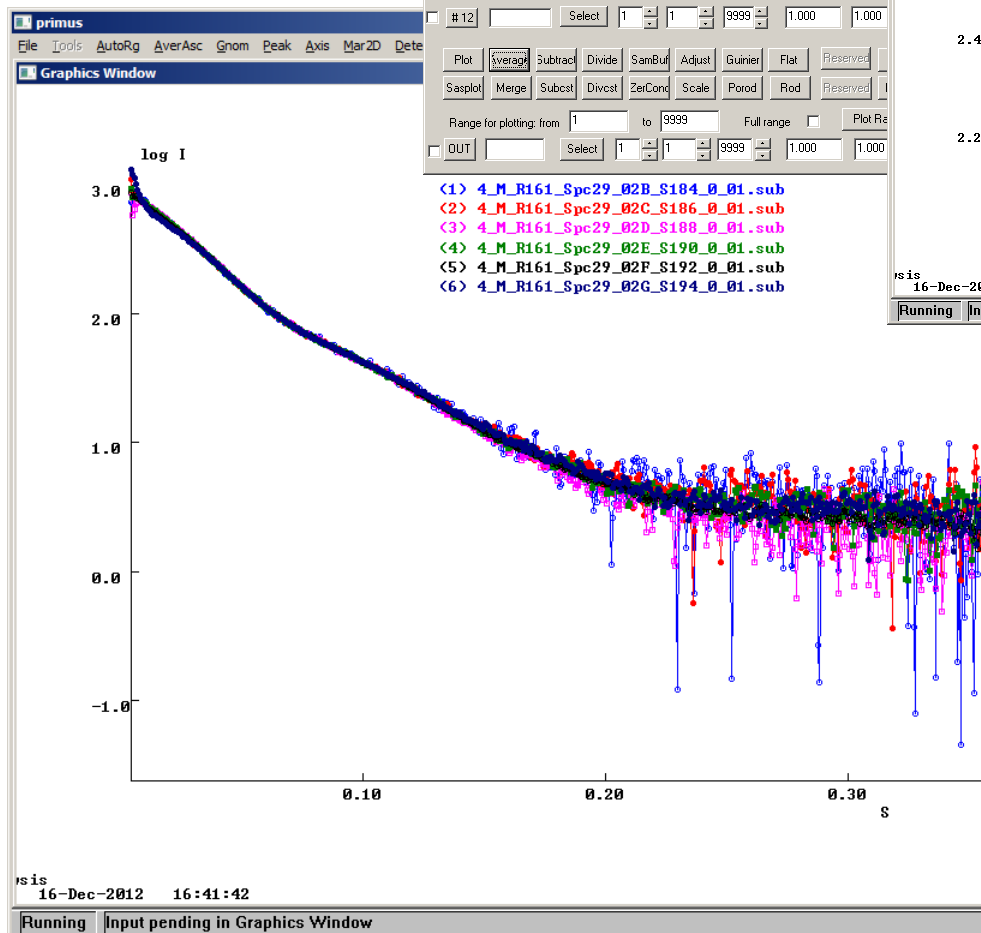
Spc29asp-K32-Sf6

2-32

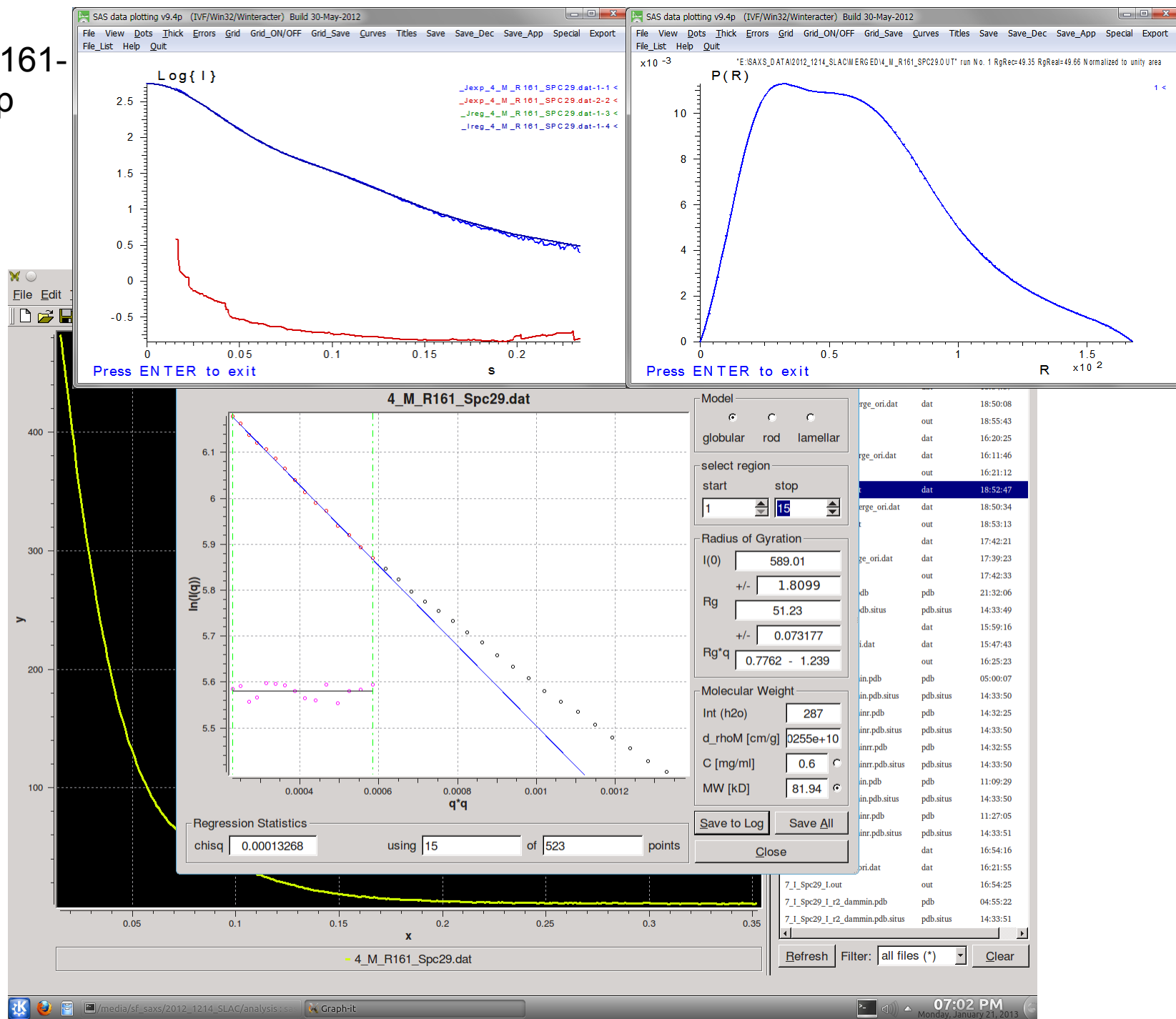


## (6) MF-R161-Spc29asp (161-253)

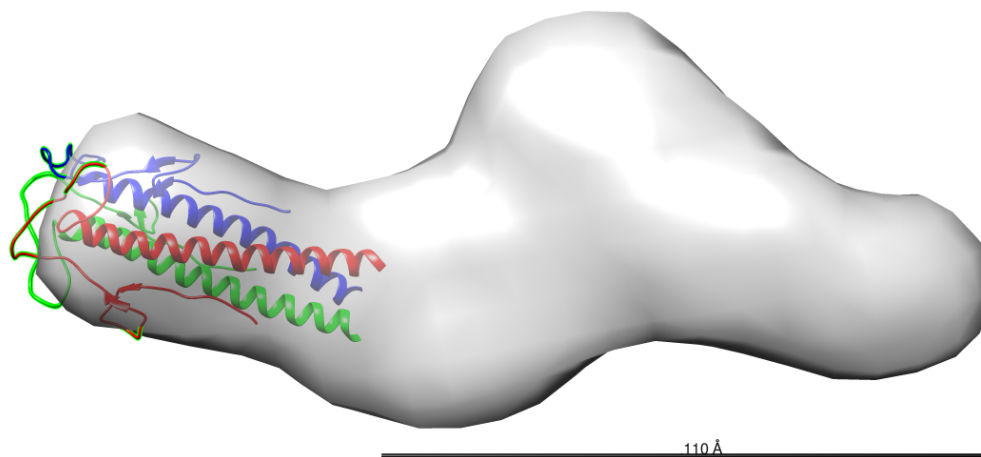
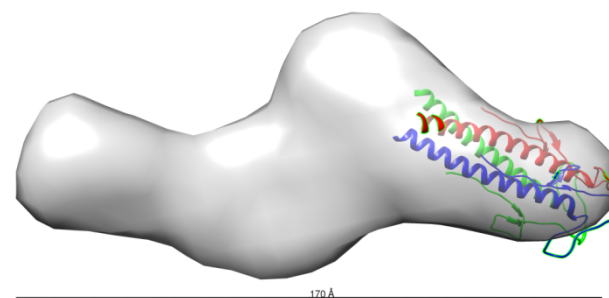
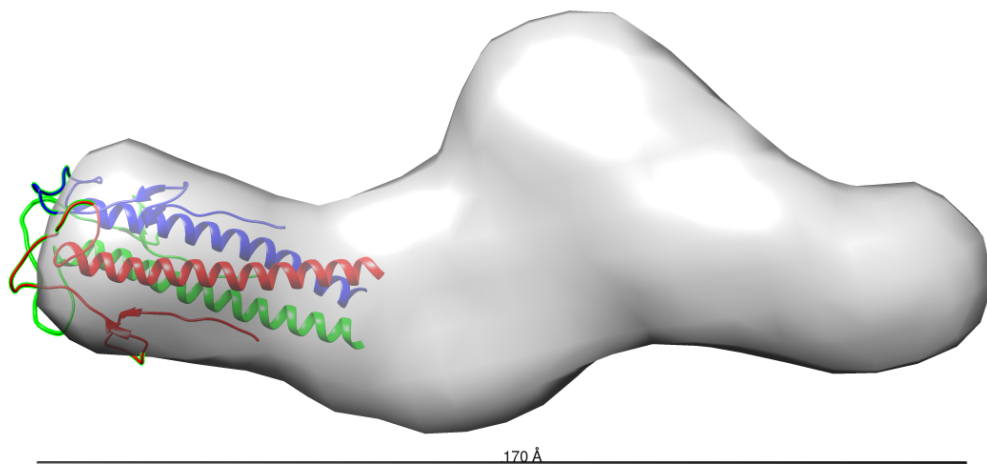
G: unfiltered 2.5mg/ml  
Minor Aggregation



## (6) MF-R161-Spc29asp



(6) MF-R161-Spc29asp



MF-R161-Spc29<sub>asp</sub>

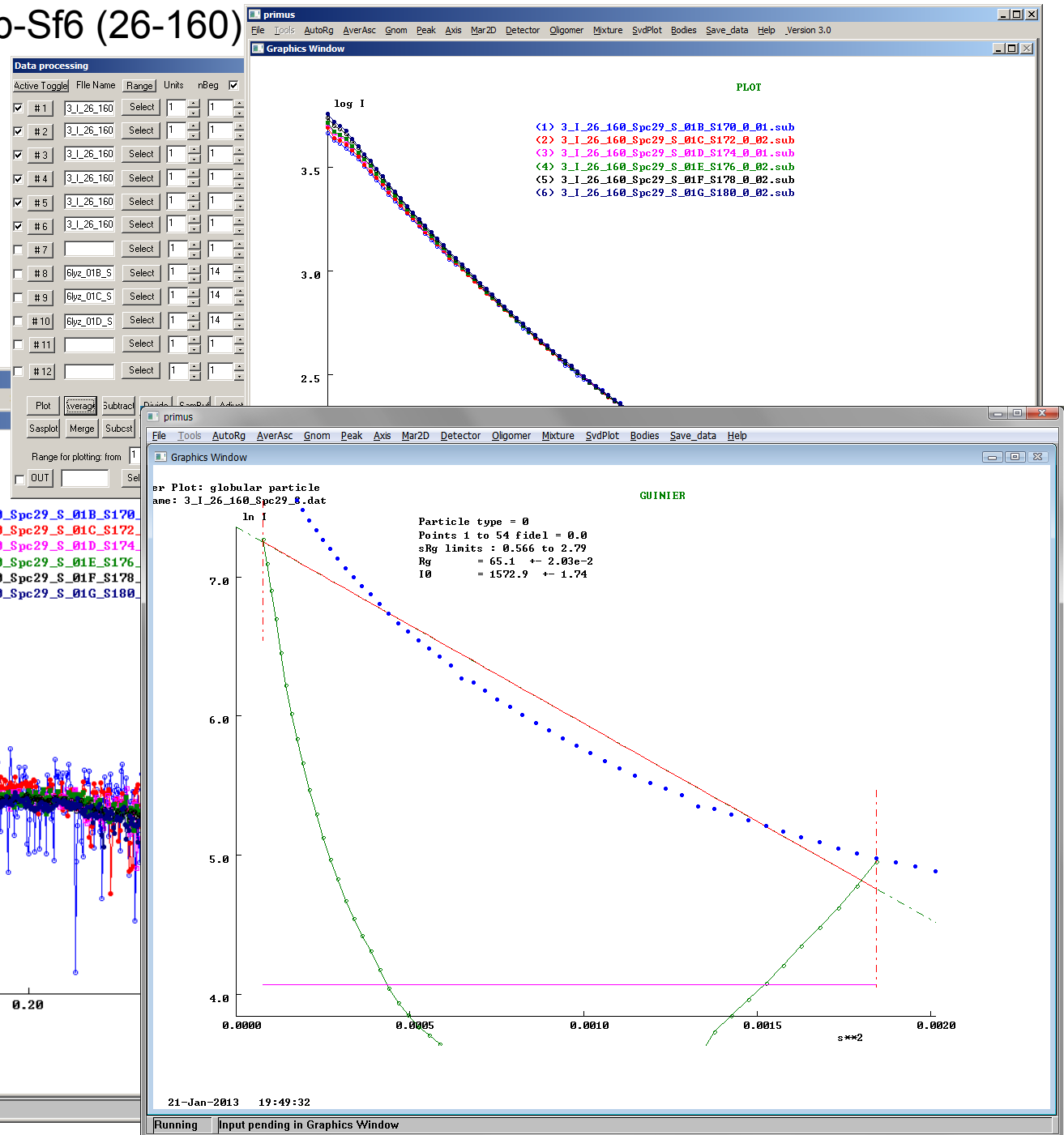
161-253



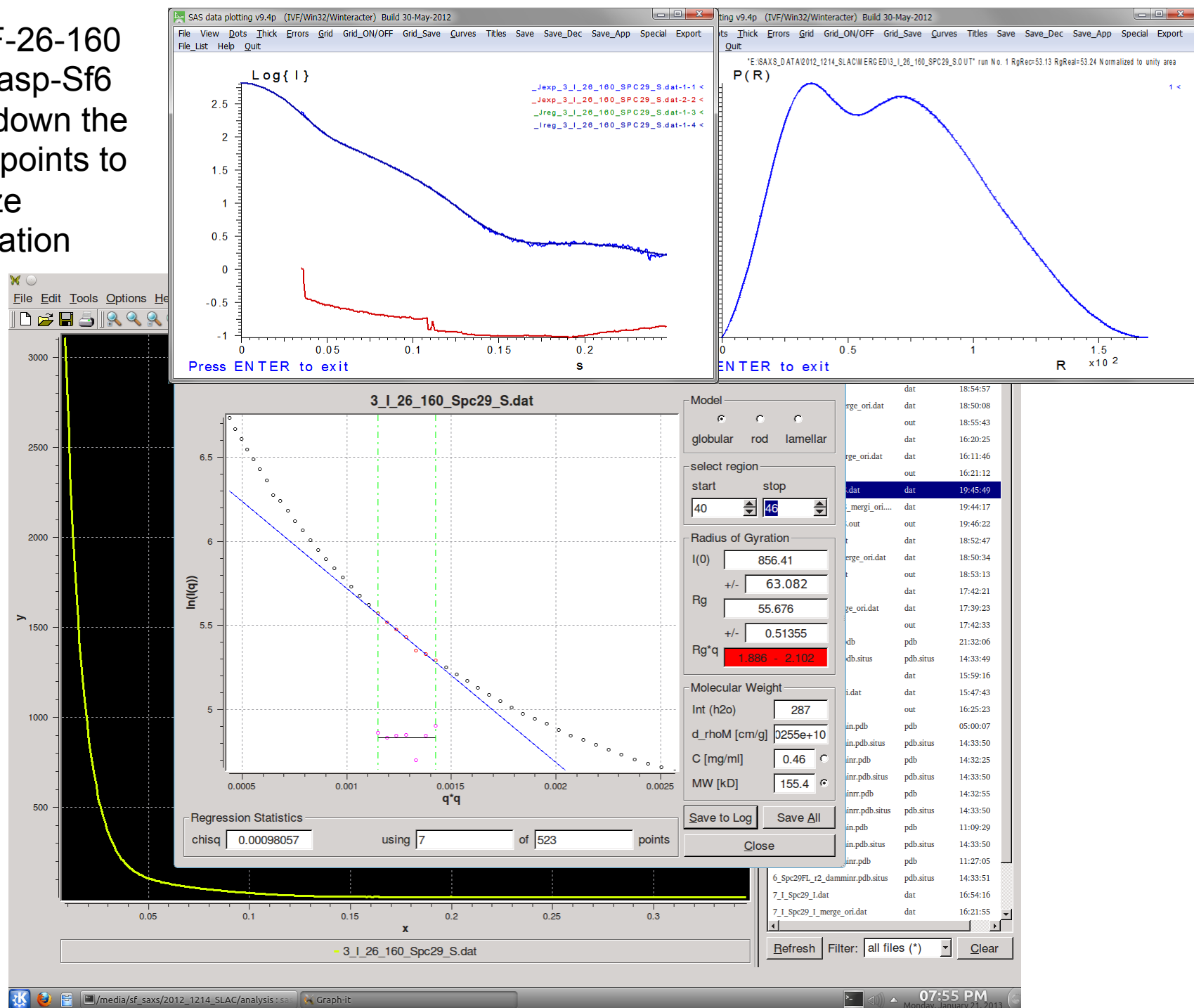


## (7) IspF-26-160-Spc29asp-Sf6 (26-160)

-> Aggregation  
 -> Mixtures of  $R_g \sim 110 \text{ \AA}$   
 &  $R_g = 60 \sim 70 \text{ \AA}$

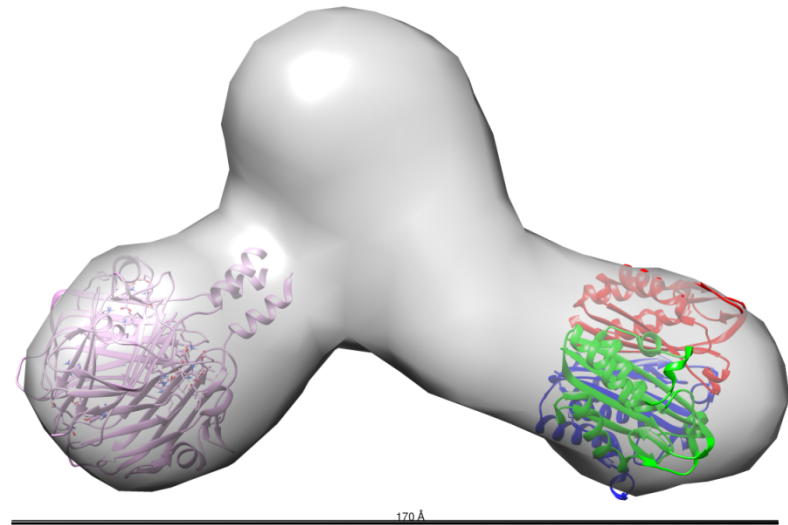
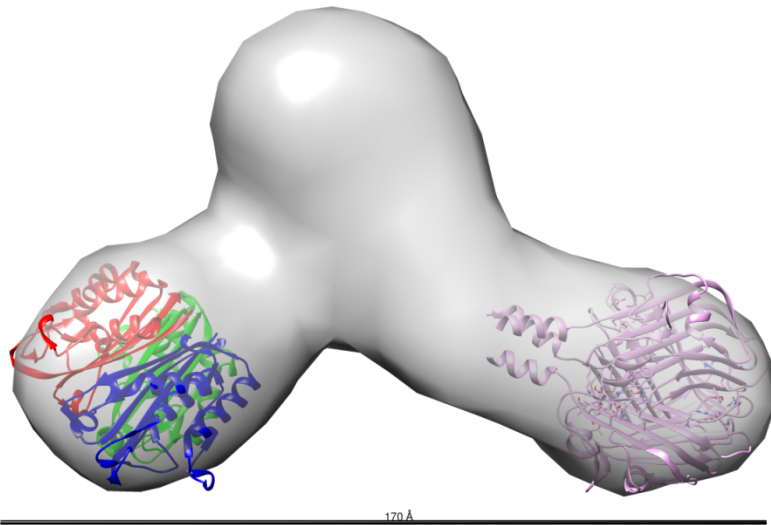
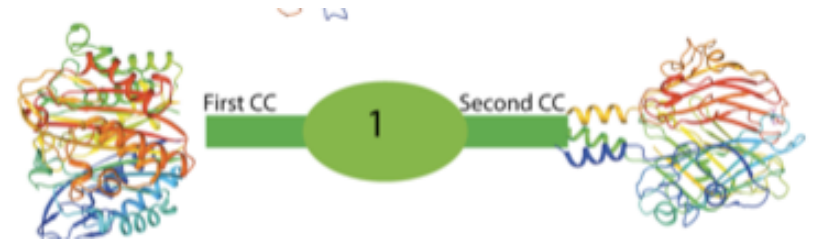


(7) IspF-26-160  
-Spc29asp-Sf6  
-> Cut down the  
first 40 points to  
minimize  
Aggregation  
effect



(7) IspF-26-160-Spc29asp-Sf6

IspF-26-160-Spc29<sub>asp</sub>-SF6      26-160



-> Not reliable due to Aggregation