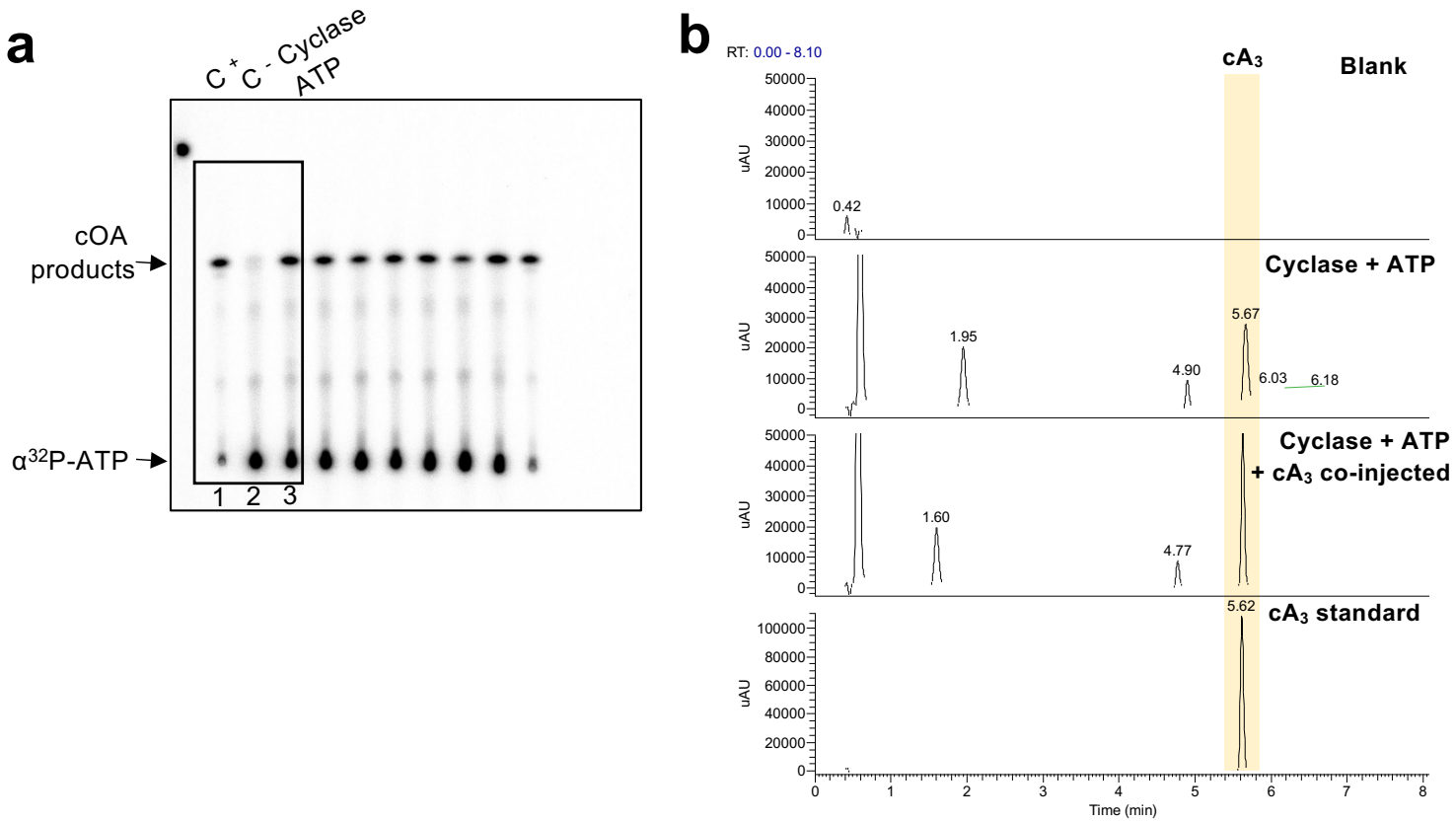

a
C+
C-
Cyclase
ATP
cOA
products
α32P-ATP
1
2
3
b
RT: 0.00 - 8.10
cA3
Blank
Cyclase + ATP
Cyclase + ATP
+ cA3 co-injected
cA3 standard
uAU
0.42
1.95
4.90
5.67
6.03
6.18
1.60
4.77
5.62
Time (min)

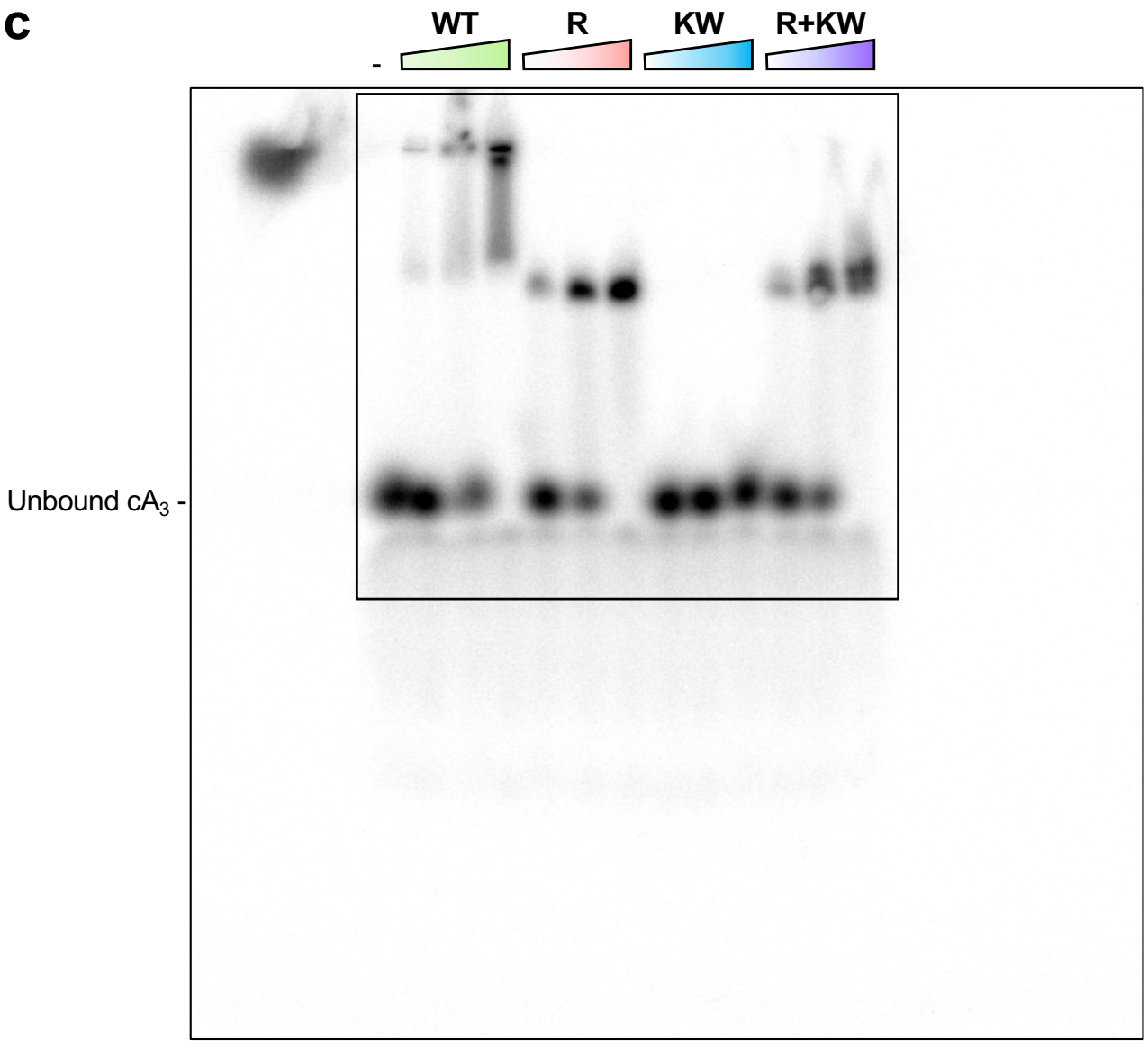

c
-
WT
R
KW
R+KW
Unbound cA3 -

Figure S1

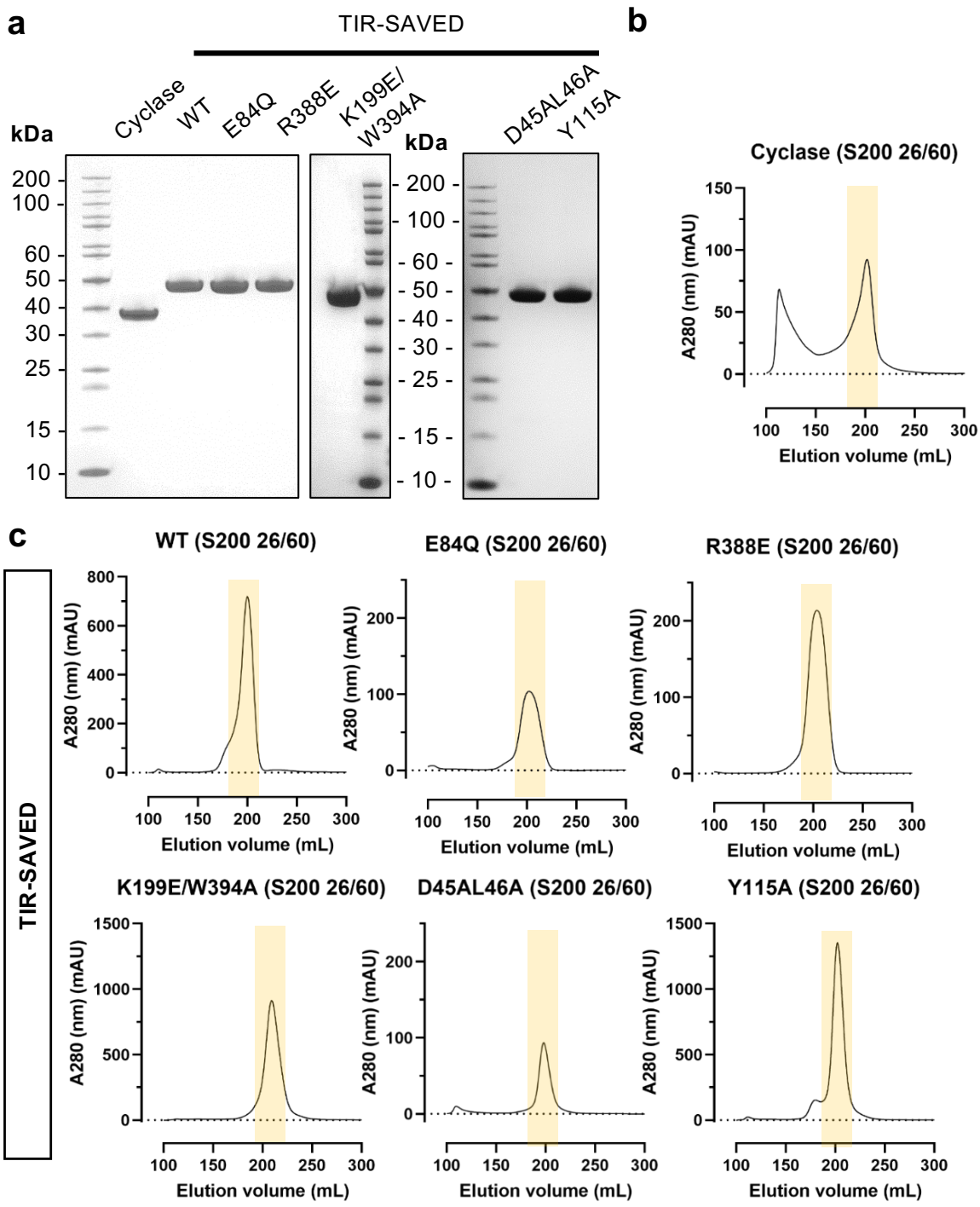
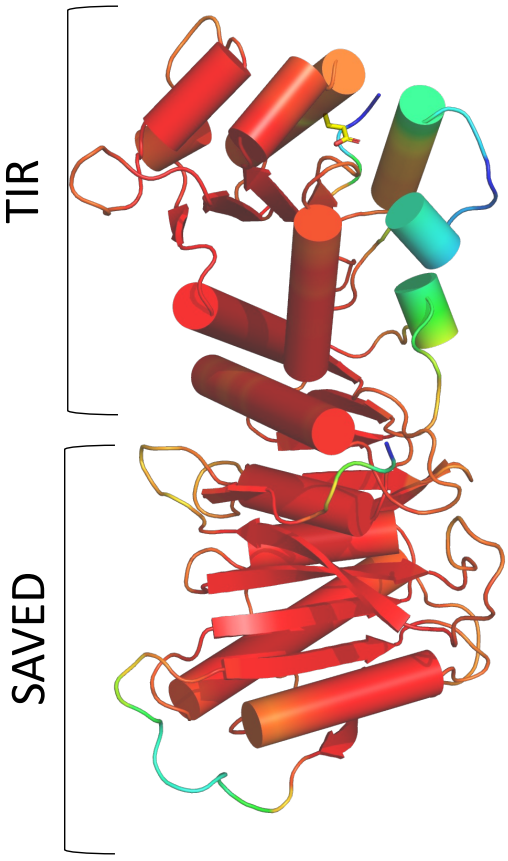
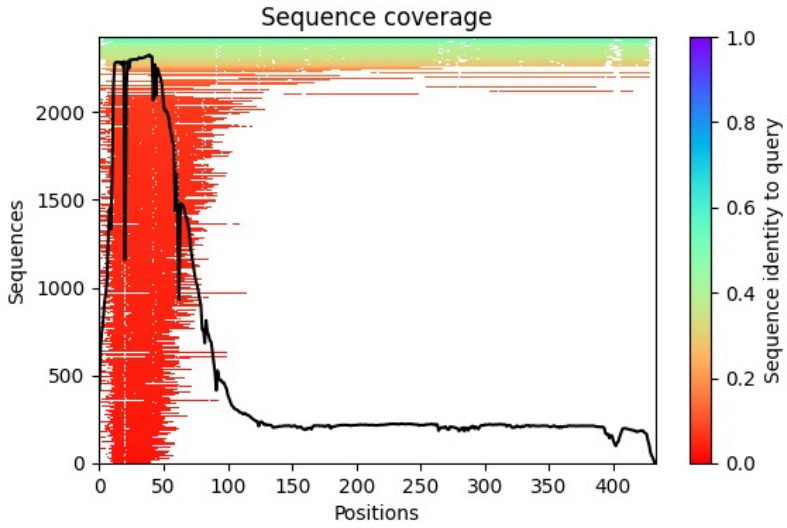

a
TIR-SAVED
Cyclase
WT
E84Q
R388E
K199E/
W394A
D45AL46A
Y115A
kDa
200
100
60
50
40
30
25
15
10
b
Cyclase (S200 26/60)
A280 (nm) (mAU)
Elution volume (mL)
c
TIR-SAVED
WT (S200 26/60)
E84Q (S200 26/60)
R388E (S200 26/60)
K199E/W394A (S200 26/60)
D45AL46A (S200 26/60)
Y115A (S200 26/60)

Figure S2


TIR
SAVED


Sequence coverage
Sequences
Positions
Sequence identity to query
0
500
1000
1500
2000
0
50
100
150
200
250
300
350
400
0.0
0.2
0.4
0.6
0.8
1.0

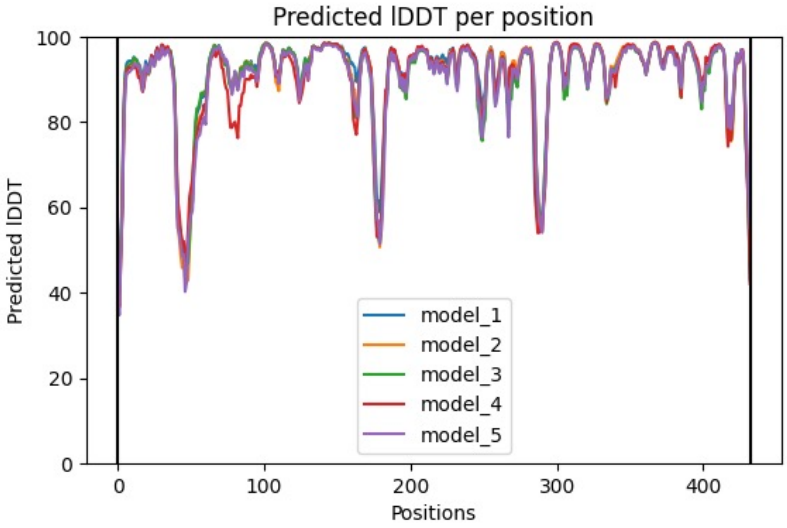
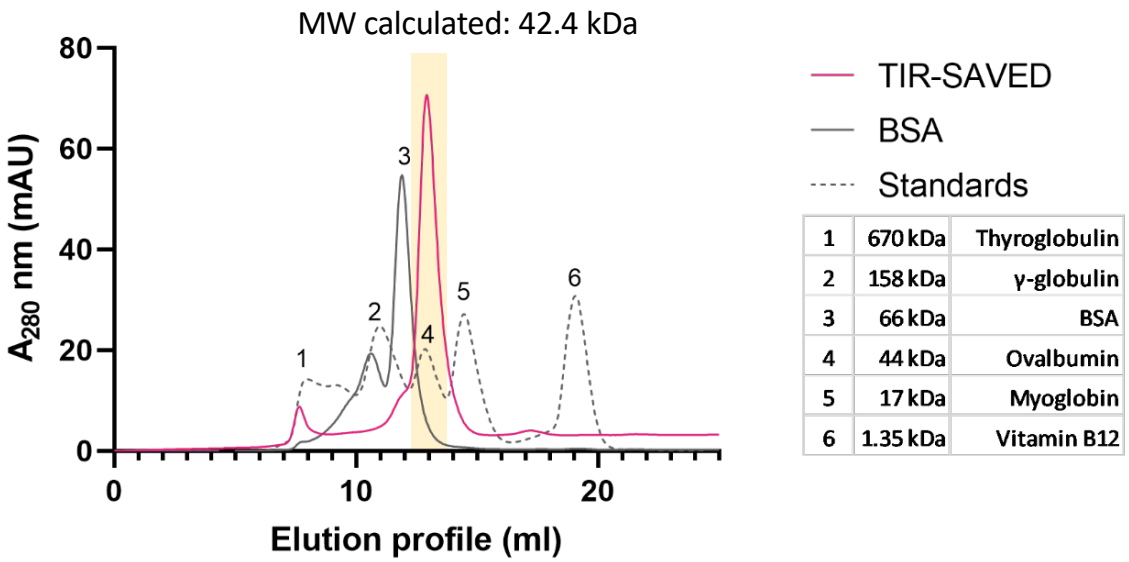

Predicted lDDT per position
Predicted lDDT
Positions
model_1
model_2
model_3
model_4
model_5
0
20
40
60
80
100
0
100
200
300
400

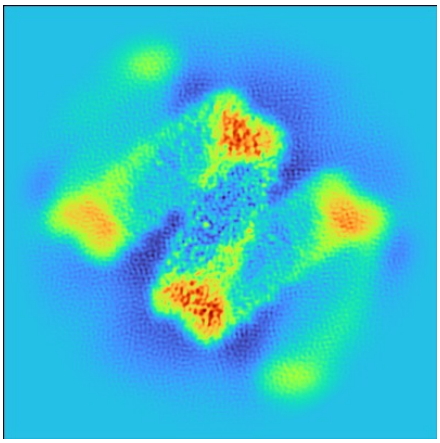
Figure S3



1	670 kDa	Thyroglobulin
2	158 kDa	γ-globulin
3	66 kDa	BSA
4	44 kDa	Ovalbumin
5	17 kDa	Myoglobin
6	1.35 kDa	Vitamin B12

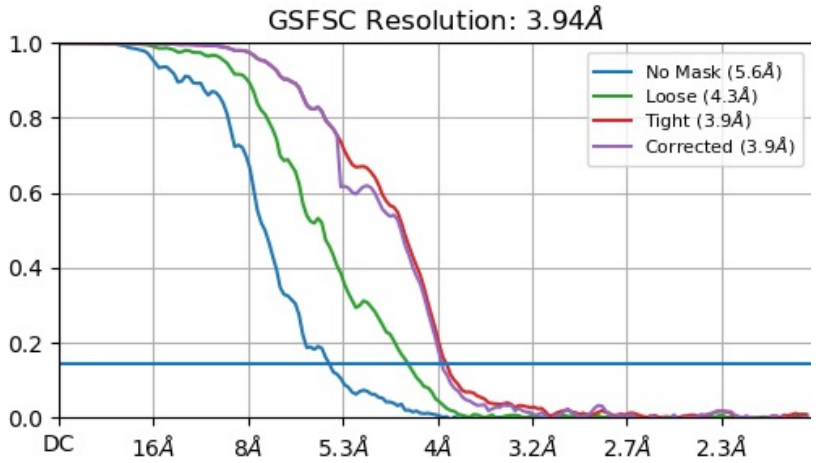
Figure S4

a

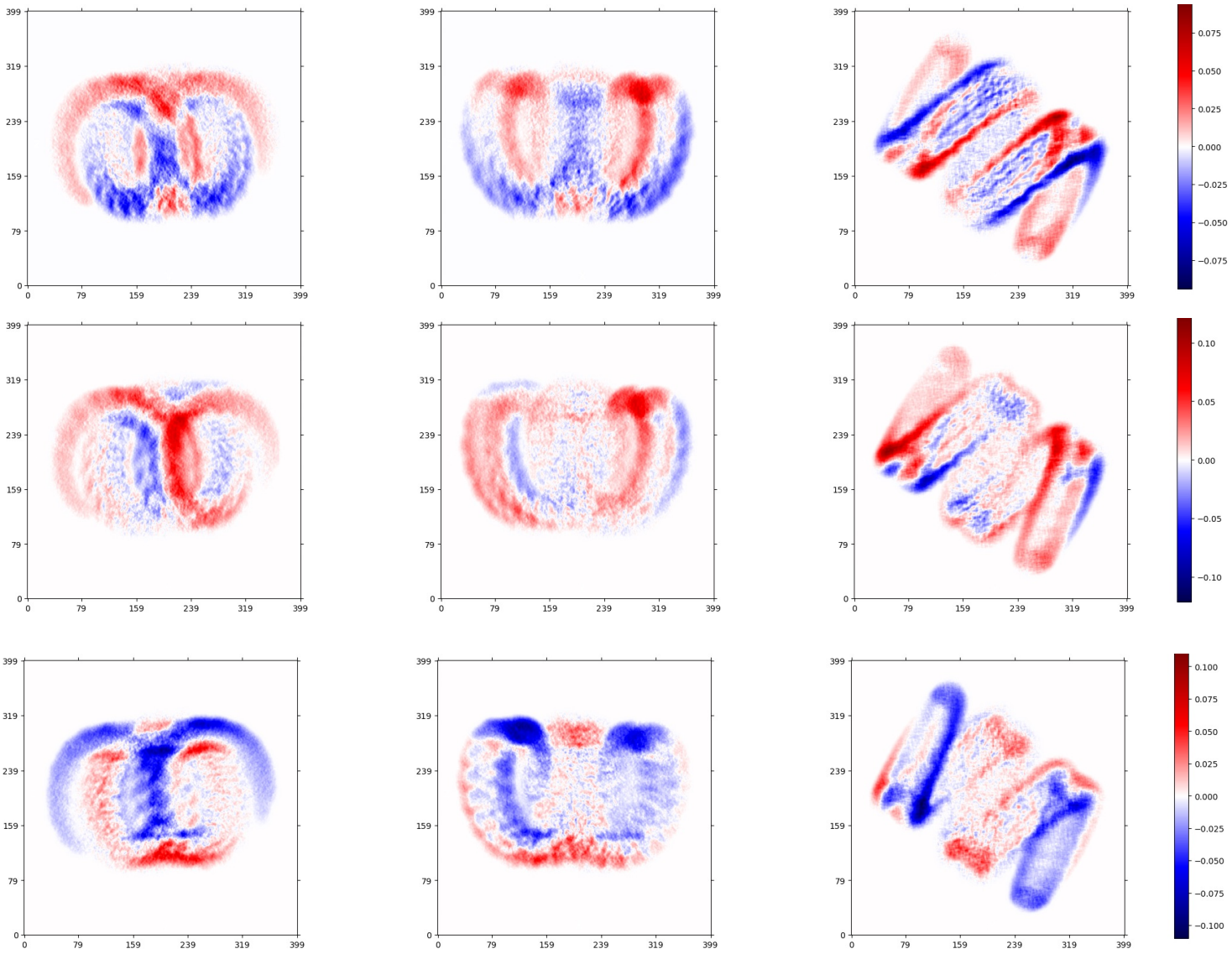


Two-tiered filament
(dynamic masking)

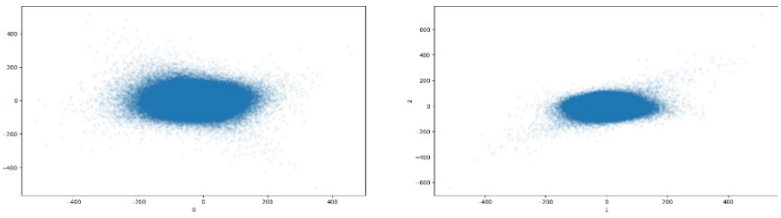
b



c



d



e

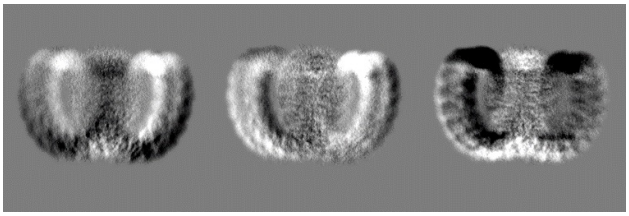
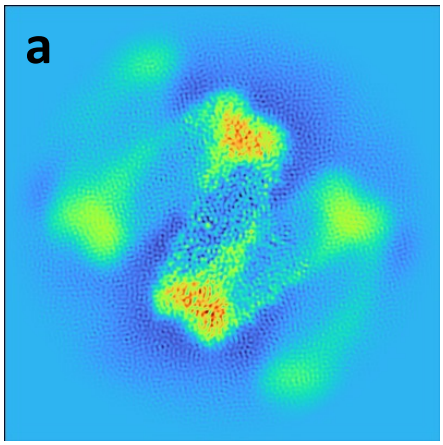
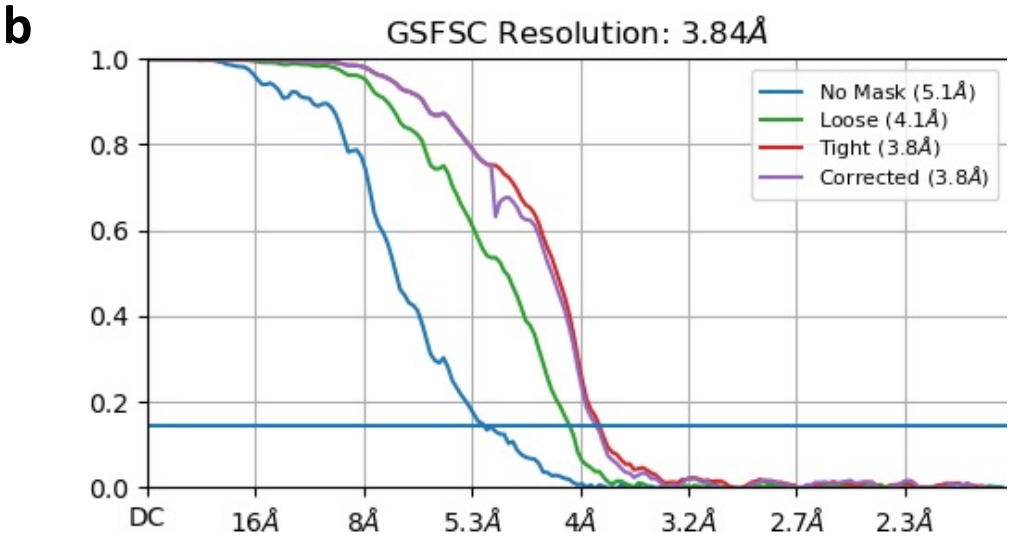
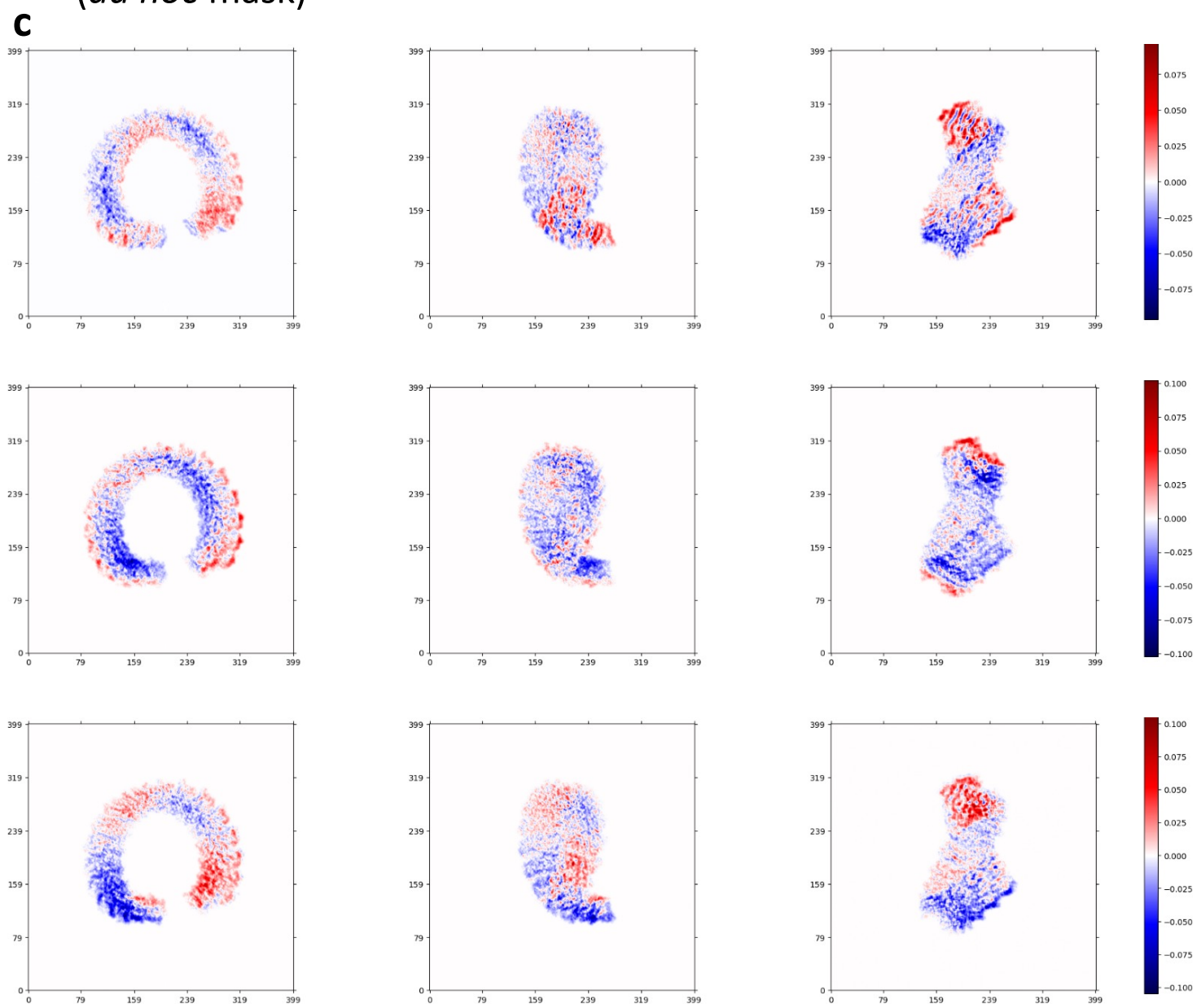


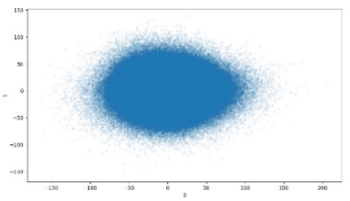
Figure S5

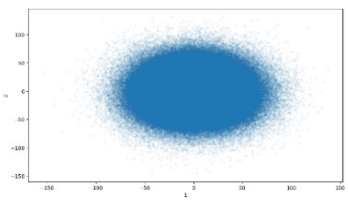




One-tiered filament
(*ad hoc* mask)







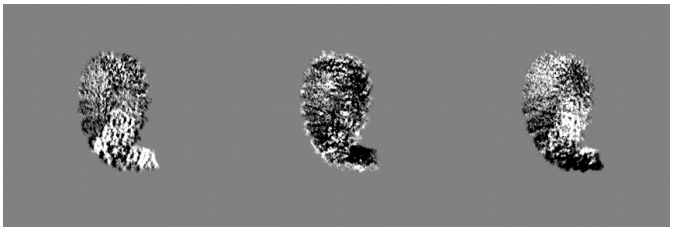


Figure S6