FIG. 1

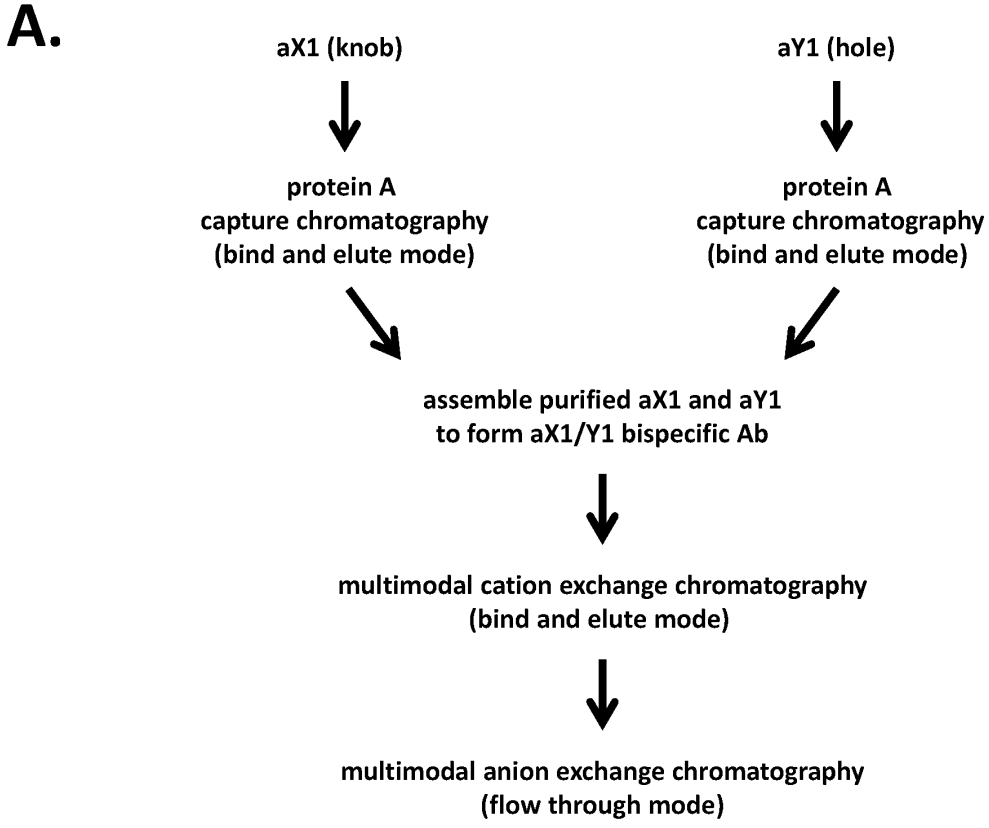

A.
aX1 (knob)
aY1 (hole)
protein A
capture chromatography
(bind and elute mode)
protein A
capture chromatography
(bind and elute mode)
assemble purified aX1 and aY1
to form aX1/Y1 bispecific Ab
multimodal cation exchange chromatography
(bind and elute mode)
multimodal anion exchange chromatography
(flow through mode)

FIG. 1

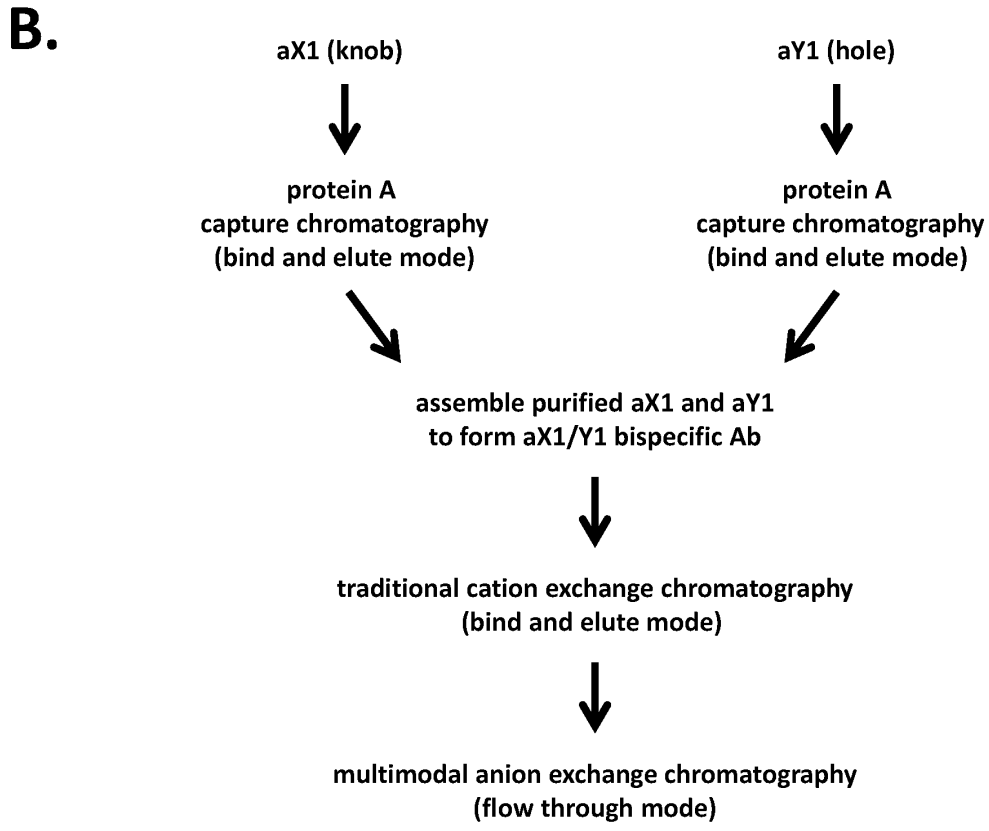

B.
aX1 (knob)
aY1 (hole)
protein A
capture chromatography
(bind and elute mode)
protein A
capture chromatography
(bind and elute mode)
assemble purified aX1 and aY1
to form aX1/Y1 bispecific Ab
traditional cation exchange chromatography
(bind and elute mode)
multimodal anion exchange chromatography
(flow through mode)

FIG. 1

C.

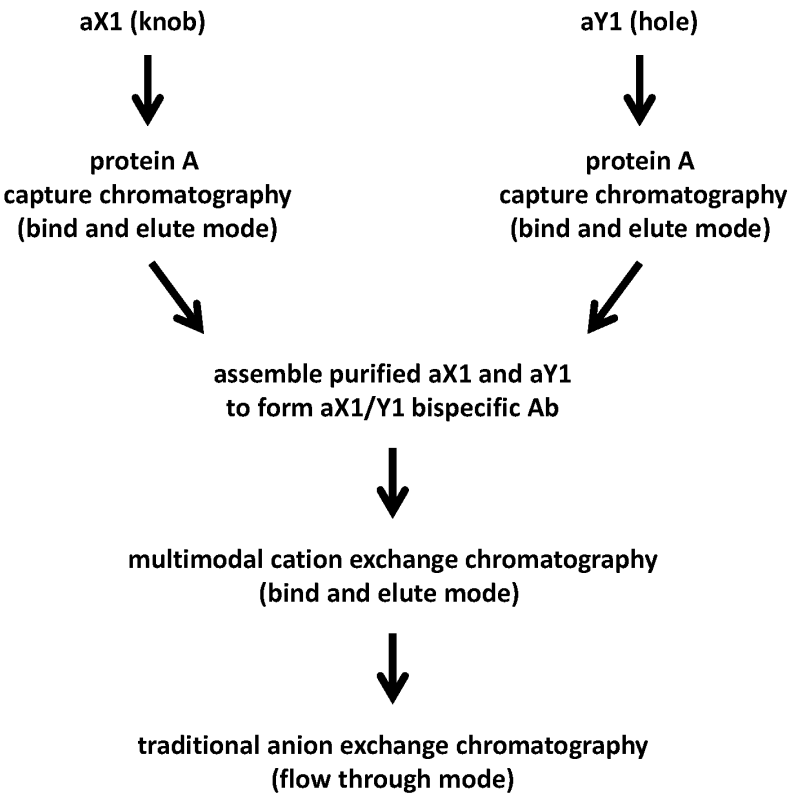

aX1 (knob)
aY1 (hole)
protein A
capture chromatography
(bind and elute mode)
protein A
capture chromatography
(bind and elute mode)
assemble purified aX1 and aY1
to form aX1/Y1 bispecific Ab
multimodal cation exchange chromatography
(bind and elute mode)
traditional anion exchange chromatography
(flow through mode)

FIG. 2

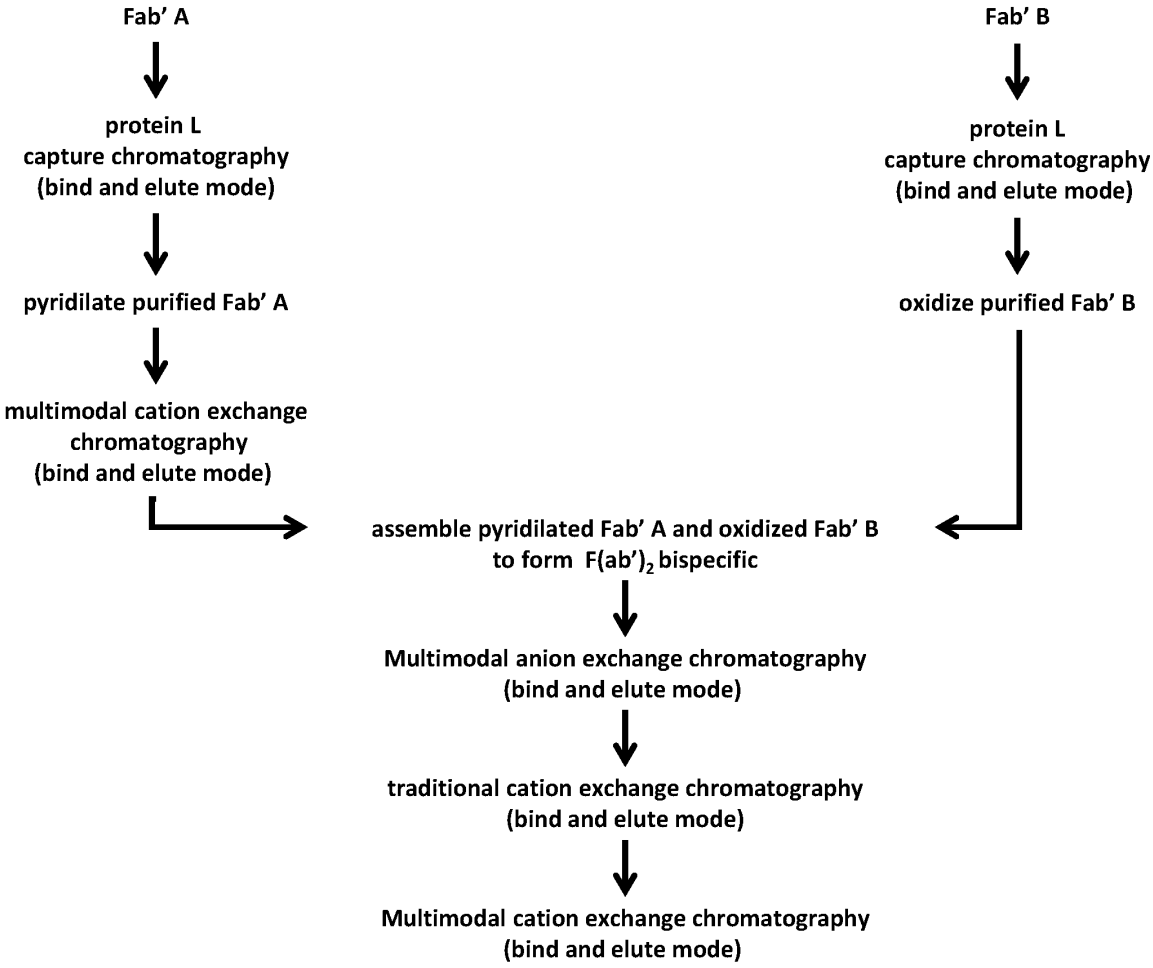

Fab' A
protein L
capture chromatography
(bind and elute mode)
pyridilate purified Fab' A
multimodal cation exchange
chromatography
(bind and elute mode)
Fab' B
protein L
capture chromatography
(bind and elute mode)
oxidize purified Fab' B
assemble pyridilated Fab' A and oxidized Fab' B
to form F(ab')2 bispecific
Multimodal anion exchange chromatography
(bind and elute mode)
traditional cation exchange chromatography
(bind and elute mode)
Multimodal cation exchange chromatography
(bind and elute mode)

FIG. 3

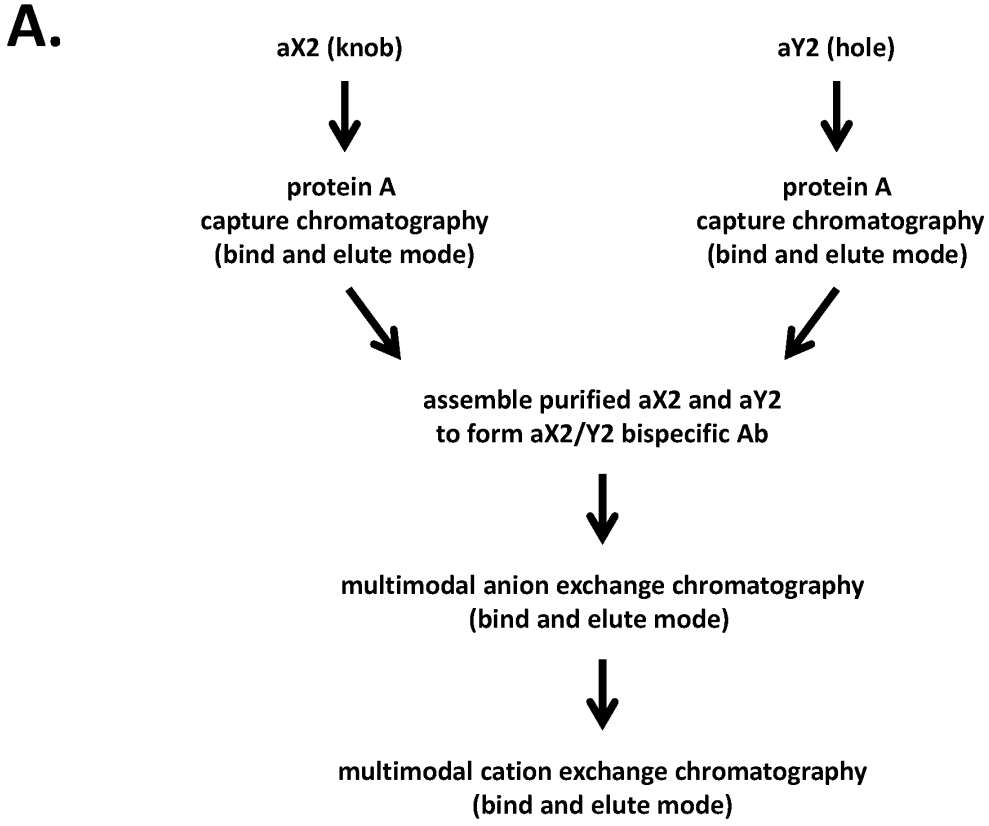

A.
aX2 (knob)
aY2 (hole)
protein A
capture chromatography
(bind and elute mode)
protein A
capture chromatography
(bind and elute mode)
assemble purified aX2 and aY2
to form aX2/Y2 bispecific Ab
multimodal anion exchange chromatography
(bind and elute mode)
multimodal cation exchange chromatography
(bind and elute mode)

FIG. 3

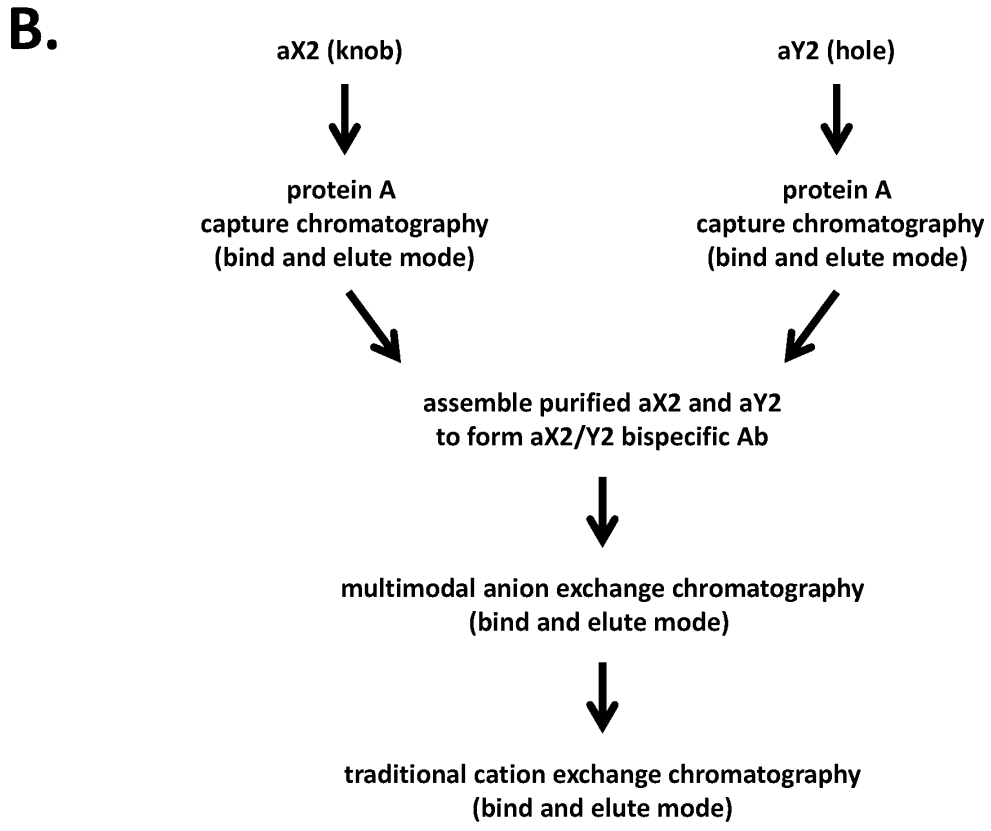

B.
aX2 (knob)
aY2 (hole)
protein A
capture chromatography
(bind and elute mode)
protein A
capture chromatography
(bind and elute mode)
assemble purified aX2 and aY2
to form aX2/Y2 bispecific Ab
multimodal anion exchange chromatography
(bind and elute mode)
traditional cation exchange chromatography
(bind and elute mode)

FIG. 4

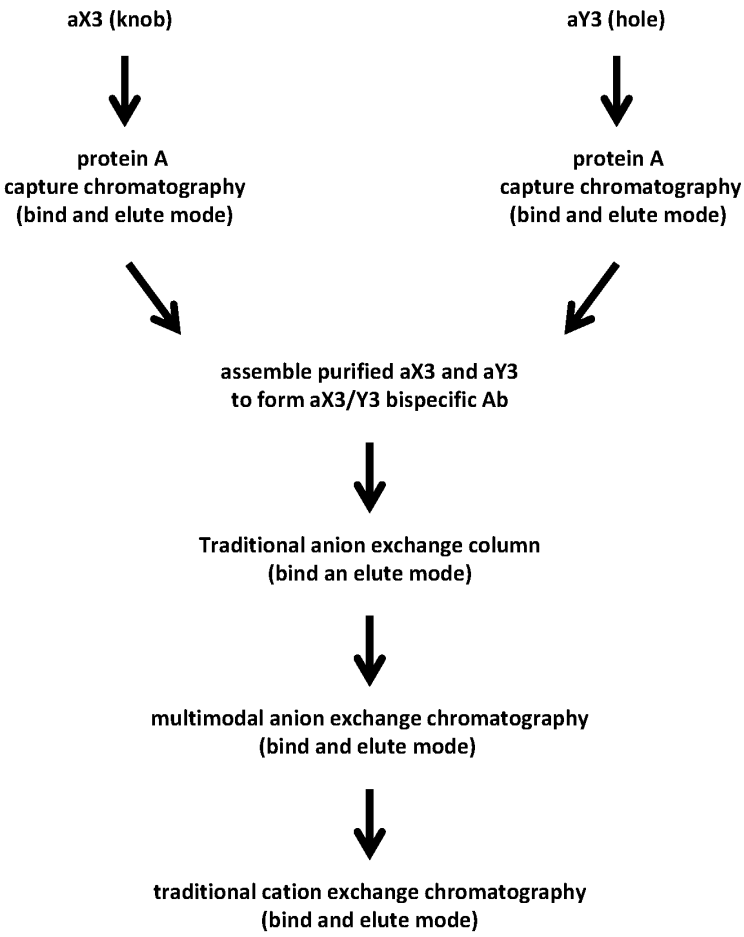

aX3 (knob)
aY3 (hole)
protein A
capture chromatography
(bind and elute mode)
protein A
capture chromatography
(bind and elute mode)
assemble purified aX3 and aY3
to form aX3/Y3 bispecific Ab
Traditional anion exchange column
(bind an elute mode)
multimodal anion exchange chromatography
(bind and elute mode)
traditional cation exchange chromatography
(bind and elute mode)

FIG. 5

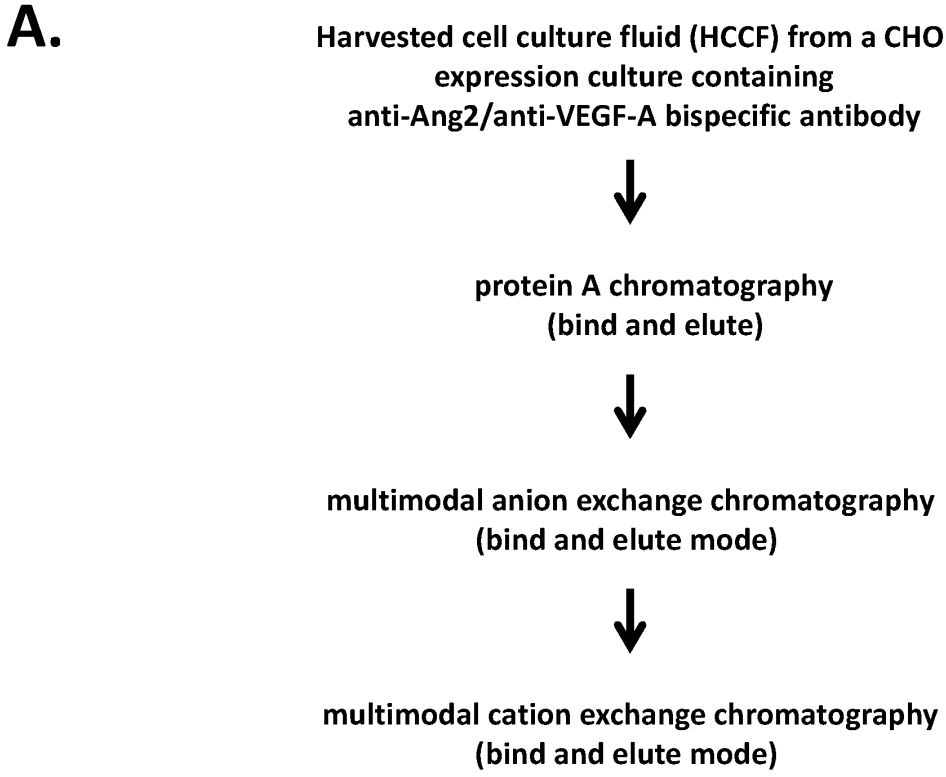

A.
Harvested cell culture fluid (HCCF) from a CHO expression culture containing anti-Ang2/anti-VEGF-A bispecific antibody
protein A chromatography (bind and elute)
multimodal anion exchange chromatography (bind and elute mode)
multimodal cation exchange chromatography (bind and elute mode)

FIG. 5

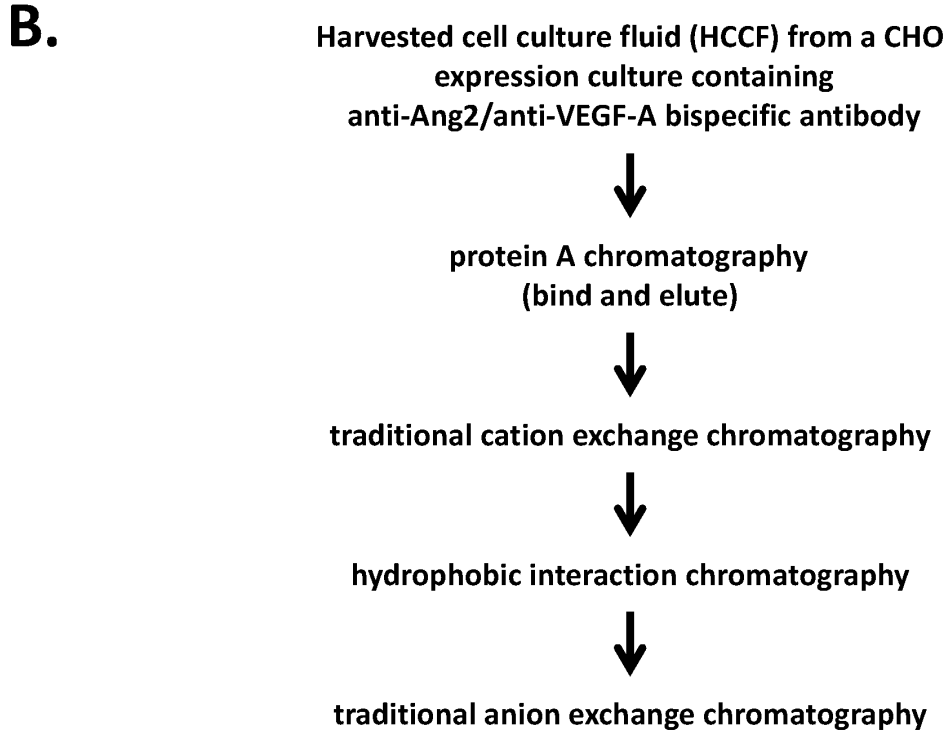

B.
Harvested cell culture fluid (HCCF) from a CHO expression culture containing anti-Ang2/anti-VEGF-A bispecific antibody
protein A chromatography (bind and elute)
traditional cation exchange chromatography
hydrophobic interaction chromatography
traditional anion exchange chromatography

FIG. 6

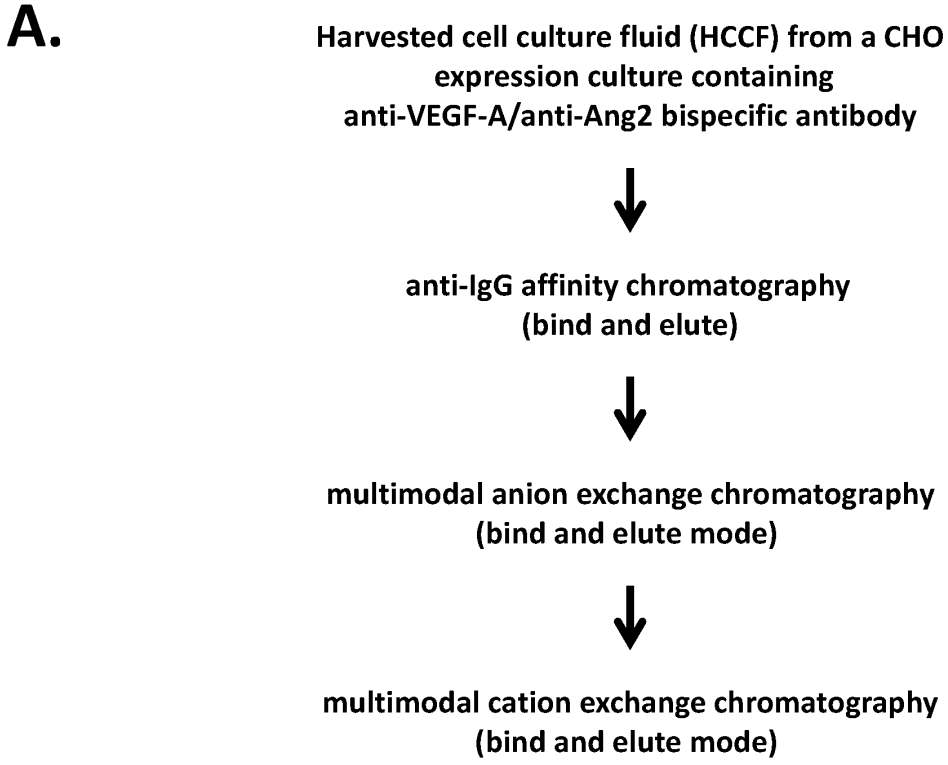

A.
Harvested cell culture fluid (HCCF) from a CHO expression culture containing anti-VEGF-A/anti-Ang2 bispecific antibody
anti-IgG affinity chromatography (bind and elute)
multimodal anion exchange chromatography (bind and elute mode)
multimodal cation exchange chromatography (bind and elute mode)

FIG. 6

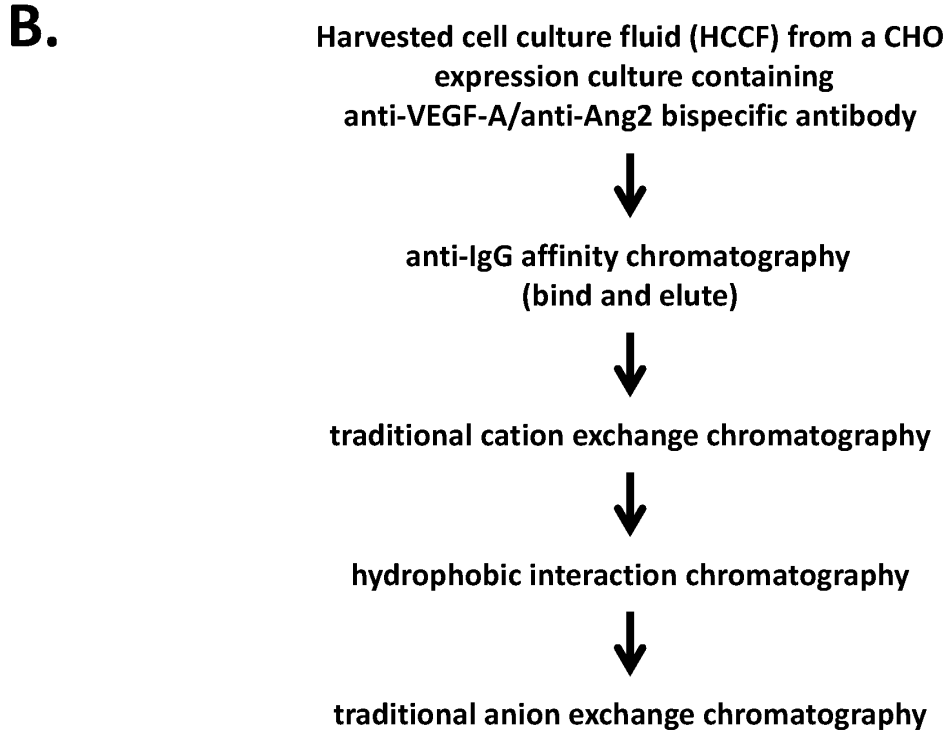

B.
Harvested cell culture fluid (HCCF) from a CHO expression culture containing anti-VEGF-A/anti-Ang2 bispecific antibody
anti-IgG affinity chromatography (bind and elute)
traditional cation exchange chromatography
hydrophobic interaction chromatography
traditional anion exchange chromatography

FIG. 7

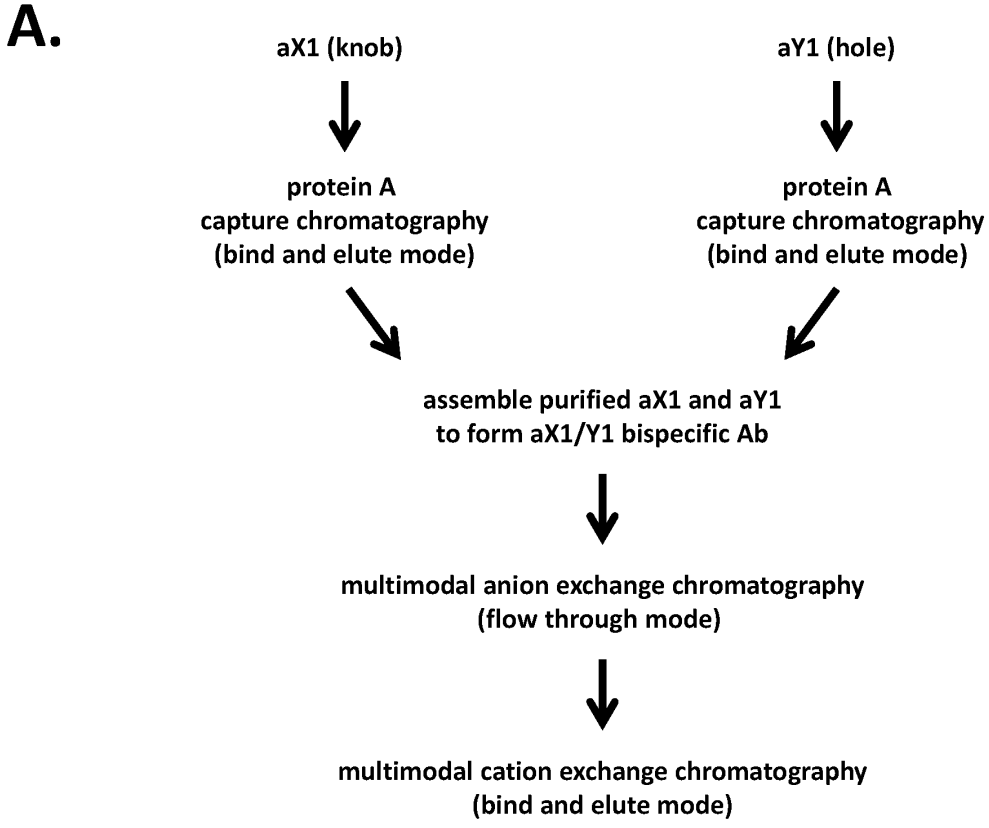

A.
aX1 (knob)
aY1 (hole)
protein A
capture chromatography
(bind and elute mode)
protein A
capture chromatography
(bind and elute mode)
assemble purified aX1 and aY1
to form aX1/Y1 bispecific Ab
multimodal anion exchange chromatography
(flow through mode)
multimodal cation exchange chromatography
(bind and elute mode)

FIG. 7

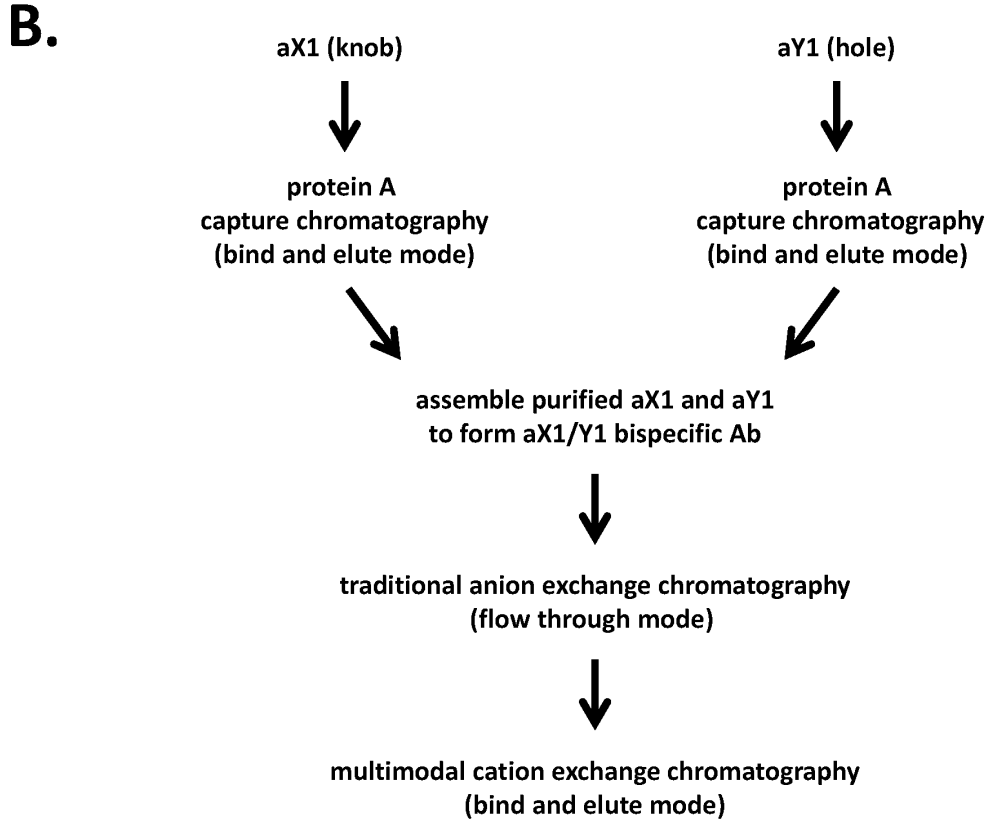
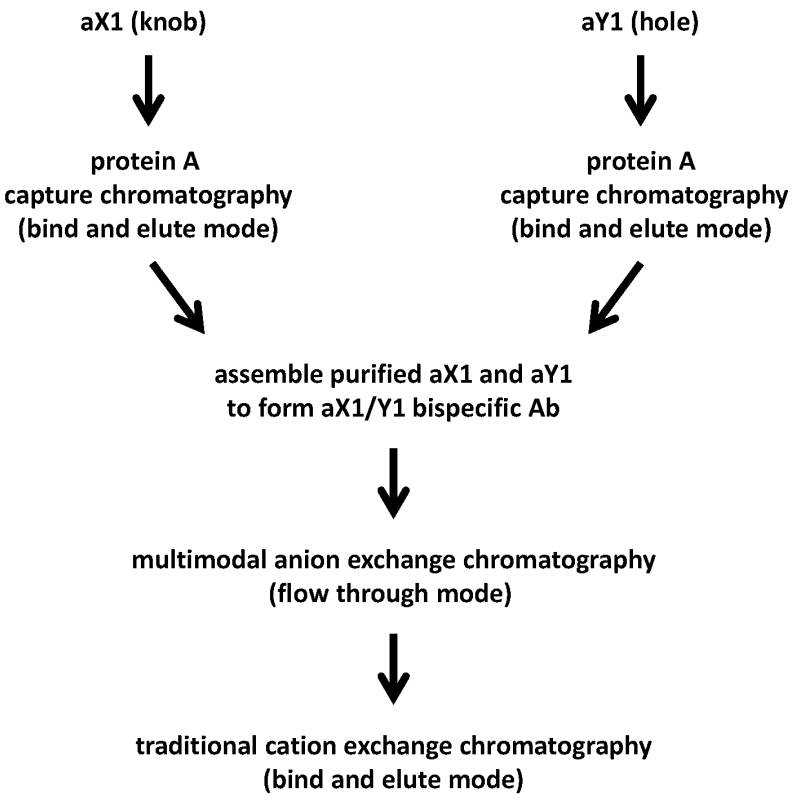

B.
aX1 (knob)
aY1 (hole)
protein A
capture chromatography
(bind and elute mode)
protein A
capture chromatography
(bind and elute mode)
assemble purified aX1 and aY1
to form aX1/Y1 bispecific Ab
traditional anion exchange chromatography
(flow through mode)
multimodal cation exchange chromatography
(bind and elute mode)

FIG. 7

C.


aX1 (knob)
aY1 (hole)
protein A
capture chromatography
(bind and elute mode)
protein A
capture chromatography
(bind and elute mode)
assemble purified aX1 and aY1
to form aX1/Y1 bispecific Ab
multimodal anion exchange chromatography
(flow through mode)
traditional cation exchange chromatography
(bind and elute mode)