Fig. 1

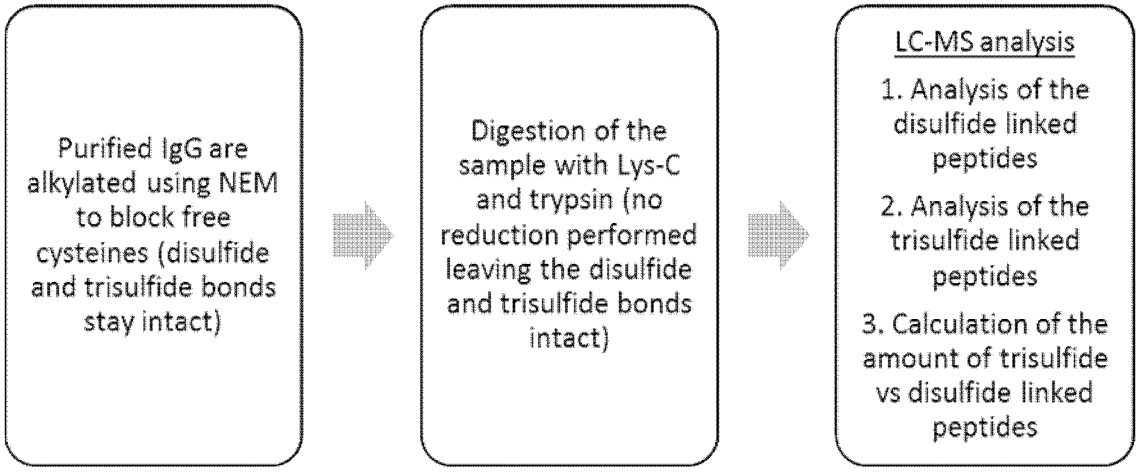

Purified IgG are alkylated using NEM to block free cysteines (disulfide and trisulfide bonds stay intact)
Digestion of the sample with Lys-C and trypsin (no reduction performed leaving the disulfide and trisulfide bonds intact)
LC-MS analysis
1. Analysis of the disulfide linked peptides
2. Analysis of the trisulfide linked peptides
3. Calculation of the amount of trisulfide vs disulfide linked peptides

Fig. 2

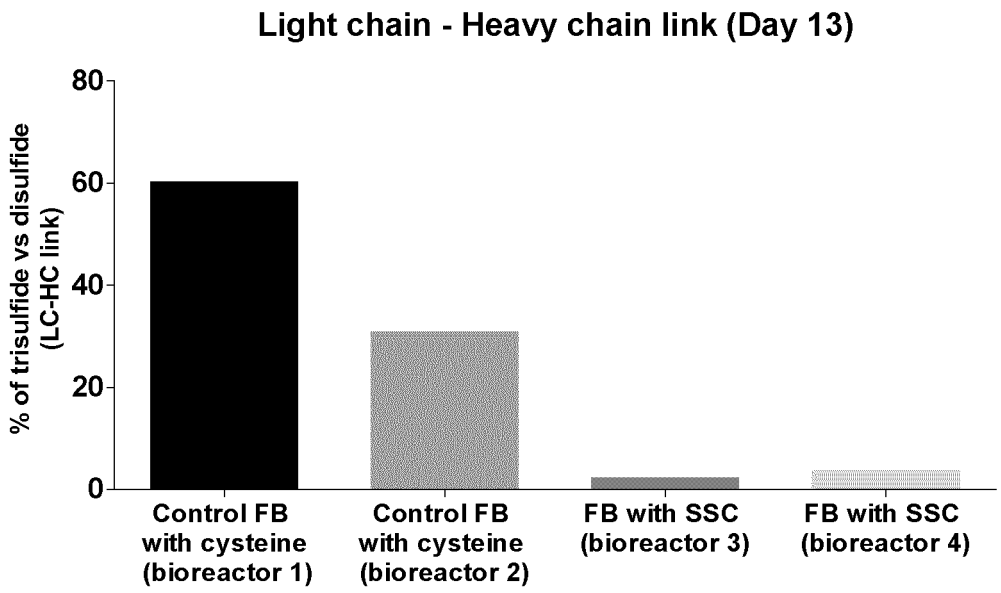

Light chain - Heavy chain link (Day 13)
% of trisulfide vs disulfide (LC-HC link)
80
60
40
20
0
Control FB with cysteine (bioreactor 1)
Control FB with cysteine (bioreactor 2)
FB with SSC (bioreactor 3)
FB with SSC (bioreactor 4)

Fig. 3

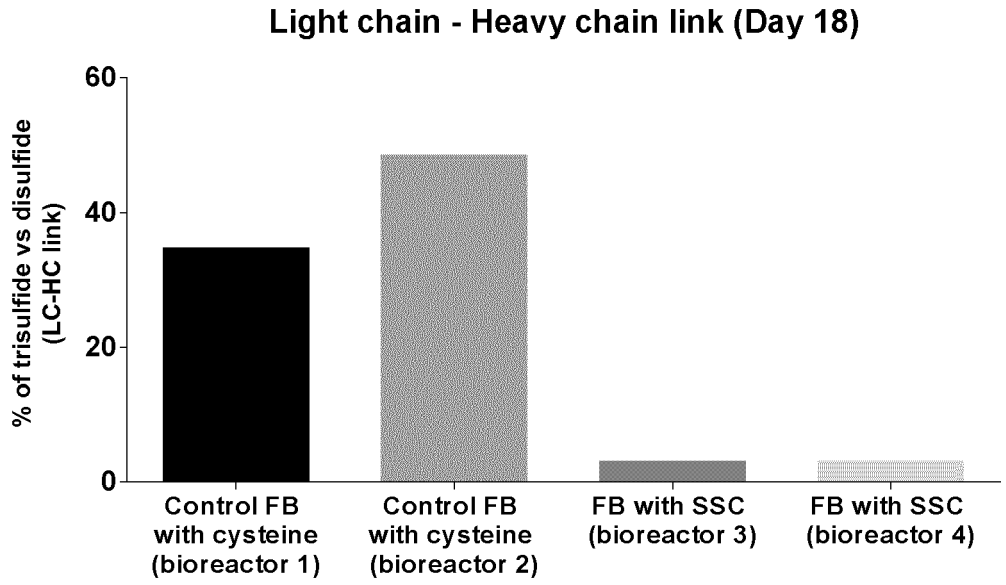

Light chain - Heavy chain link (Day 18)
% of trisulfide vs disulfide (LC-HC link)
60
40
20
0
Control FB with cysteine (bioreactor 1)
Control FB with cysteine (bioreactor 2)
FB with SSC (bioreactor 3)
FB with SSC (bioreactor 4)

Fig. 4

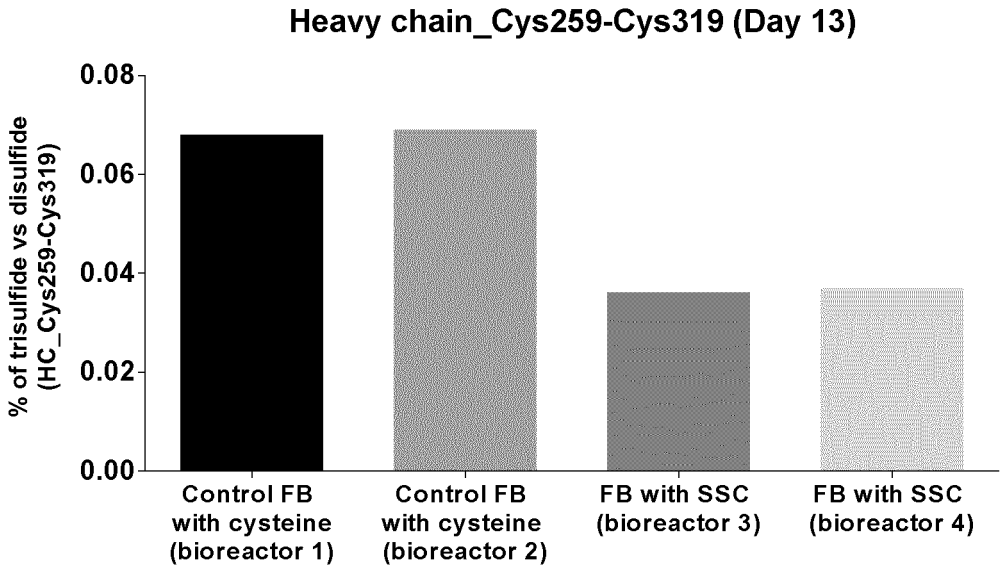

Heavy chain_Cys259-Cys319 (Day 13)
% of trisulfide vs disulfide (HC_Cys259-Cys319)
0.08
0.06
0.04
0.02
0.00
Control FB with cysteine (bioreactor 1)
Control FB with cysteine (bioreactor 2)
FB with SSC (bioreactor 3)
FB with SSC (bioreactor 4)

Fig. 5

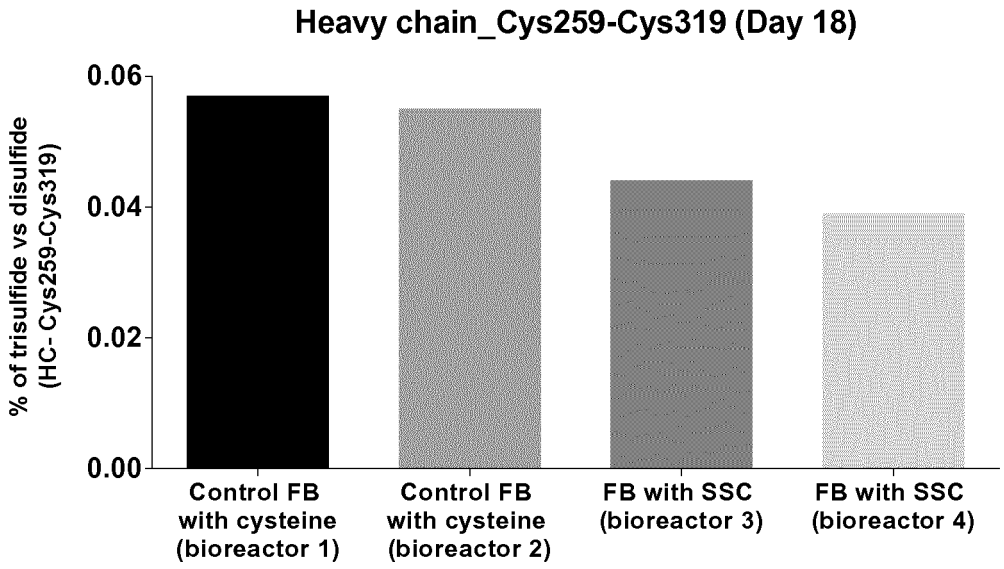
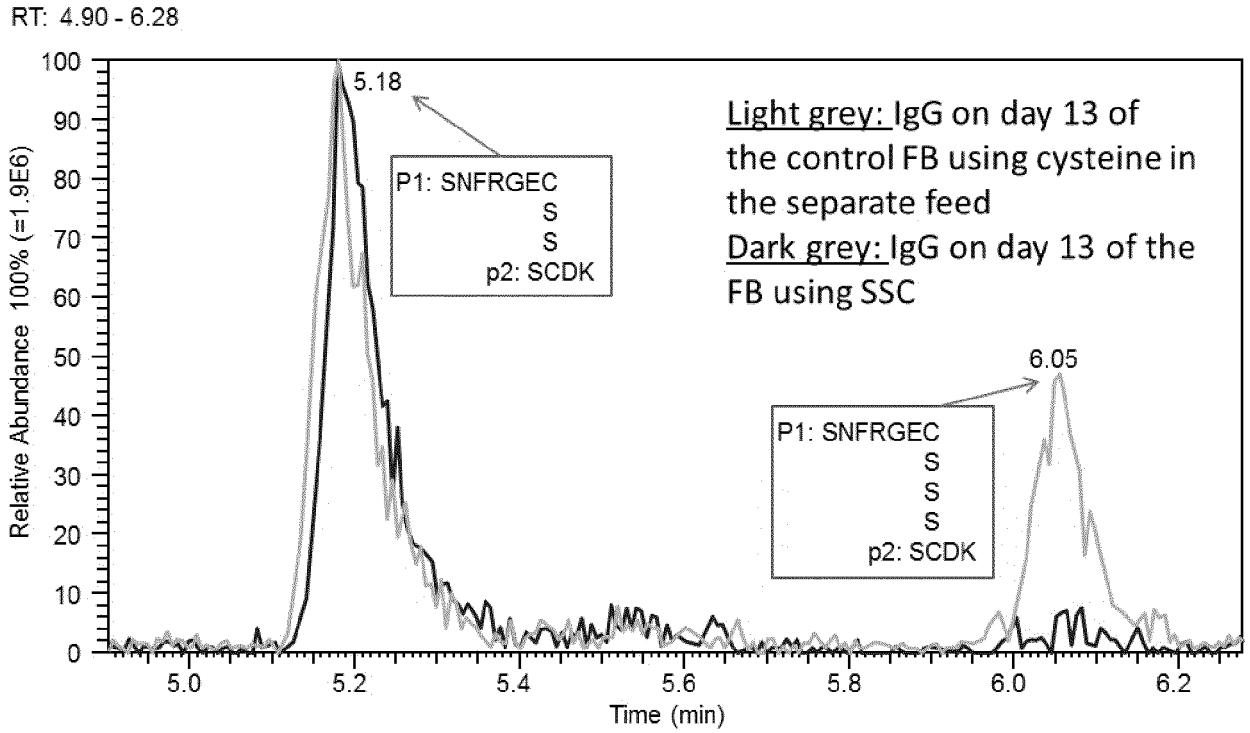

Heavy chain_Cys259-Cys319 (Day 18)
% of trisulfide vs disulfide (HC- Cys259-Cys319)
0.06
0.04
0.02
0.00
Control FB with cysteine (bioreactor 1)
Control FB with cysteine (bioreactor 2)
FB with SSC (bioreactor 3)
FB with SSC (bioreactor 4)

Fig. 6


RT: 4.90 - 6.28
Relative Abundance 100% (=1.9E6)
Time (min)
5.18
P1: SNFRGEC
S
S
p2: SCDK
6.05
P1: SNFRGEC
S
S
S
p2: SCDK
Light grey: IgG on day 13 of the control FB using cysteine in the separate feed
Dark grey: IgG on day 13 of the FB using SSC