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Expression and Characterization of 20 Bi- and Trispecific Antibody Formats

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Background

With the development of so-called next-generation protein therapeutics, a new class of drugs is coming into use which, through their specific design, can specifically attack and effectively combat diseases. These next-generation protein therapeutics include bi- and trispecific antibodies, molecules which are capable of binding to two (bispecific) or three (trispecific) different targets and combating disease by inhibition, activation or neutralization. In collaboration with a biotech company, 20 different antibody formats (Ab formats, Figure 1) have been expressed and characterized.

Method

In this study, 8 bi- and 12 trispecific Ab formats were cloned, expressed in HEK cells, and purified by affinity chromatography. Characterization was performed by SE- and RP-HPLC, mass spectrometry, and surface plasmon resonance (SPR) spectroscopy. The Ab formats are Fab fragments fused with one or two single-chain Fv fragments (scFv) at the N- or C-terminus.

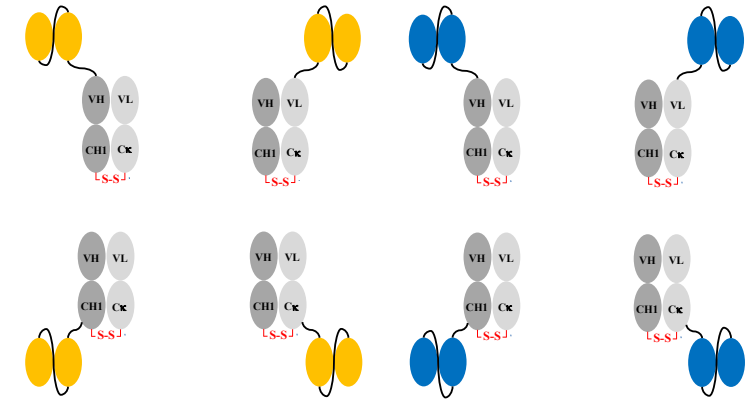
Results

All the Ab formats were expressed and purified by affinity chromatography, and yields of 0.17 - 6.7 mg were obtained. The expression level of the individual Ab-formats was estimated using RP-HPLC measurements of cell culture supernatants. In addition, RP-HPLC was used to determine the purity of the pools and dialysates from the affinity chromatography; this was between 96% and 100%. The monomer content was determined by SE-HPLC, and was between 60 and 99%. The identity of the proteins was verified by the masses in intact and reduced state, and after tryptic digestion by mass spectrometry (ESI-Q-TOF). The affinity of the bi- and tri-specific Ab formats was tested using SPR. A scFv or the Fab fragment, specific to the corresponding target molecule, was used as a reference. With the exception of three Ab formats, all K_D values for the corresponding two or three antigens were only a factor of 3 higher than the K_D values of the monospecific reference scFv or Fab fragments.

Conclusion

The bi- and trispecific Ab formats were all successfully expressed, but with different yields.
The affinity to the target molecules was not significantly altered for most formats. This shows that, in the multispecific formats examined, accessibility for binding of target molecules is not limited.

Bispecific Ab formats



Trispecific Ab formats

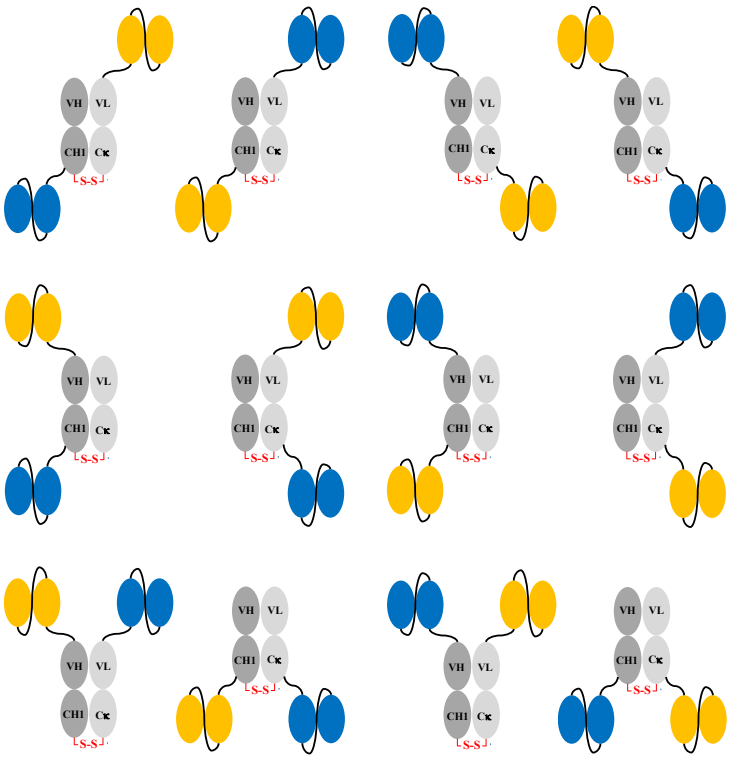


Fig. 1 The 20 different antibody formats are composed of one Fab (grey) and one or two different scFv (yellow and blue). The scFv are fused at either the C- or N- terminus of the light or heavy chain of the Fab.