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Evaluation of selectivity in homologous multimodal chromatographic

2 systems using in silico designed antibody fragment libraries

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Abstract

This study describes the in silico design, surface property analyses, production and 15 chromatographic evaluations of a diverse set of antibody Fab fragment variants. Based on previous 16 17 findings, we hypothesized that the complementarity-determining regions (CDRs) constitute 18 important binding sites for multimodal chromatographic ligands. Given that antibodies are highly 19 diversified molecules and in particular the CDRs, we set out to examine the generality of this result. 20 For this purpose, four different Fab fragments with different CDRs and/or framework regions of the variable domains were identified and related variants were designed in silico. The four Fab variant 21 22 libraries were subsequently generated by site-directed mutagenesis and produced by recombinant 23 expression and affinity purification to enable examination of their chromatographic retention 24 behavior. The effects of geometric re-arrangement of the functional moieties on the multimodal 25 resin ligands were also investigated with respect to Fab variant retention profiles by comparing two 26 commercially available multimodal cation-exchange ligands, Capto MMC and Nuvia cPrime, and 27 two novel multimodal ligand prototypes. Interestingly, the chromatographic data demonstrated 28 distinct selectivity trends between the four Fab variant libraries. For three of the Fab libraries, the 29 CDR regions appeared as major binding sites for all multimodal ligands. In contrast, the fourth Fab 30 library displayed a distinctly different chromatographic behavior, where Nuvia cPrime and related 31 multimodal ligand prototypes provided markedly improved selectivity over Capto MMC. Clearly, 32 the results illustrate that the discriminating power of multimodal ligands differ between different 33 Fab fragments. The results are promising indications that multimodal chromatography using the

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