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PII: S0021-9673(15)01675-1
DOI: http://dx.doi.org/doi:10.1016/j.chroma.2015.11.047
Reference: CHROMA 357061

To appear in: *Journal of Chromatography A*

Received date: 8-10-2015
Revised date: 12-11-2015
Accepted date: 12-11-2015

Please cite this article as: H.S. Karkov, J. Woo, B.O. Krogh, H. Ahmadian, S.M. Cramer, Evaluation of selectivity in homologous multimodal chromatographic systems using *in silico* designed antibody fragment libraries, *Journal of Chromatography A* (2015), http://dx.doi.org/10.1016/j.chroma.2015.11.047

Evaluation of selectivity in homologous multimodal chromatographic 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 chromatographic evaluations of a diverse set of antibody Fab fragment variants. Based on previous findings, we hypothesized that the complementarity-determining regions (CDRs) constitute important binding sites for multimodal chromatographic ligands. Given that antibodies are highly diversified molecules and in particular the CDRs, we set out to examine the generality of this result. 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 libraries were subsequently generated by site-directed mutagenesis and produced by recombinant expression and affinity purification to enable examination of their chromatographic retention behavior. The effects of geometric re-arrangement of the functional moieties on the multimodal resin ligands were also investigated with respect to Fab variant retention profiles by comparing two commercially available multimodal cation-exchange ligands, Capto MMC and Nuvia cPrime, and two novel multimodal ligand prototypes. Interestingly, the chromatographic data demonstrated distinct selectivity trends between the four Fab variant libraries. For three of the Fab libraries, the CDR regions appeared as major binding sites for all multimodal ligands. In contrast, the fourth Fab library displayed a distinctly different chromatographic behavior, where Nuvia cPrime and related multimodal ligand prototypes provided markedly improved selectivity over Capto MMC. Clearly, the results illustrate that the discriminating power of multimodal ligands differ between different Fab fragments. The results are promising indications that multimodal chromatography using the