Rational design of therapeutic mAbs against aggregation through protein engineering and incorporation of glycosylation motifs applied to bevacizumab

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Supplementary material

$$SAP_{Atom} = \frac{1}{n_{\substack{Simulation\\Frames}}} \sum_{\substack{Simulation\\Average}} \left[\sum_{\substack{Residues\\Within\ R}} \left(\frac{\substack{SAA\ of\ side\ chain\ atoms\\within\ radius\ R}}{\substack{SAA\ of\ side\ chain\ atoms\\of\ fully\ exposed\ residue}} \times Residue\ Hydrophobicity \right) \right]$$

$$SAP_{residue,j} = \frac{1}{n_{atoms}} \sum_{\substack{all\ atoms\ in\\residue\ j}} SAP_{atom,i}$$

$$\phi_{eff,residue\ j} = \frac{SAA\ of\ side\ chain\ atoms}{\substack{SAA\ of\ side\ chain\ atoms\\of\ fully\ exposed\ residue}} \times Residue\ Hydrophobicity$$

S1. SAP calculation

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                                  <-----------FWR1--------><CDR1><------FWR2-----><2><---------------FWR3-------------->
                  Query_1      1  DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQ-YSTVP  95
V  87.4% (83/95)  IGKV1D-33*01  1  .....................Q.....................L...DA.N.ET.............F.........I.......-.DNL.  95
V  87.4% (83/95)  IGKV1-33*01    1  .....................Q.....................L...DA.N.ET.............F.........I.......-.DNL.  95
V  90.6% (87/96)  IGKV1-39*01    1  .....................R...S..S.............L...AA...Q.................................S...P.  96
                                                                                    ★

                                  <----------FWR1--------><--CDR1><------FWR2-----><--CDR2><----------------FWR3-------------->
                  Query_1      1  EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTYAADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAK  98
V  72.4% (71/98)  IGHV7-4-1*02  1  Q....Q..SE.KK..A.VKV..K.......S.A.........Q....M.....N..N....QG.TG..V......V......IS..K..........R  98
V  72.4% (71/98)  IGHV7-4-1*01  1  Q....Q..SE.KK..A.VKV..K.......S.A.........Q....M.....N..N....QG.TG..V......V......IC..K..........R  98
V  71.4% (70/98)  IGHV7-4-1*04  1  Q....Q..SE.KK..A.VKV..K.......S.A.........Q....M.....N..N....QG.TG..V......V.M....IS..K..........R  98
                                     ★
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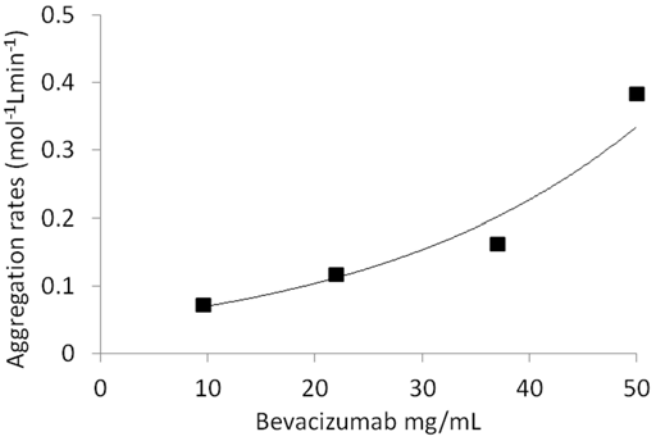
S2. Alignment of bevacizumab with germline sequences. High SAP residues present in the variable regions are indicated with a star, i.e., F50 in the light chain and V5 in the heavy chain.



S3. Aggregation rates of bevacizumab in histidine buffer (pH 6.0) as a function of mAb concentrations. Aggregation rates were measured by SEC-HPLC by following the amount of monomer loss over a 48-h incubation period at 52°C and fitted to a second order equation.

(A)

	LC-WT	F50D	V110K	L154K	L154D	L201K	HC-WT	V5K	L180K
Score	-4.561	-4.653	-4.811	-4.760	-4.528	-4.760	-2.409	-2.658	-2.607

(B)

Residues	V5	V5K	L180	L180K	F50	F50D	V110	V110K	L154	L154K	L154D	L201	L201K
Score	1.11	1.045	1.114	1.069	1.084	1.052	1.041	0.976	0.98	0.934	0.925	1.047	1.002

S4. Immunogenicity predictions. The risk of immunogenicity of bevacizumab WT and all the variants was predicted using the free online tool from IEDB (http://tools.immuneepitope.org/main/). (A) T Cell Epitopes - Immunogenicity Prediction. The tool was applied to the light chain of bevacizumab WT (LC-WT) and variants, and to part of the heavy chain, residues 1 to 222 (HC-WT). The higher score indicates a greater probability of eliciting an immune response. (B) Kolaskar and Tongaonkar antigenicity scale (parameters: center position: 4, window size: 7, threshold: 1) was used to predict linear B cell epitopes. The calculated scores are listed for each high SAP residue of bevacizumab WT and for their corresponding variants. The larger the score is the higher the probability that the residue is part of an epitope. Both T-cell and B-cell epitopes predictions show that the single point mutations did not increase the potential immunogenicity of the mAbs.