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Supplementary Material

Bioprocessing method is a critical factor for IgM oligomerization

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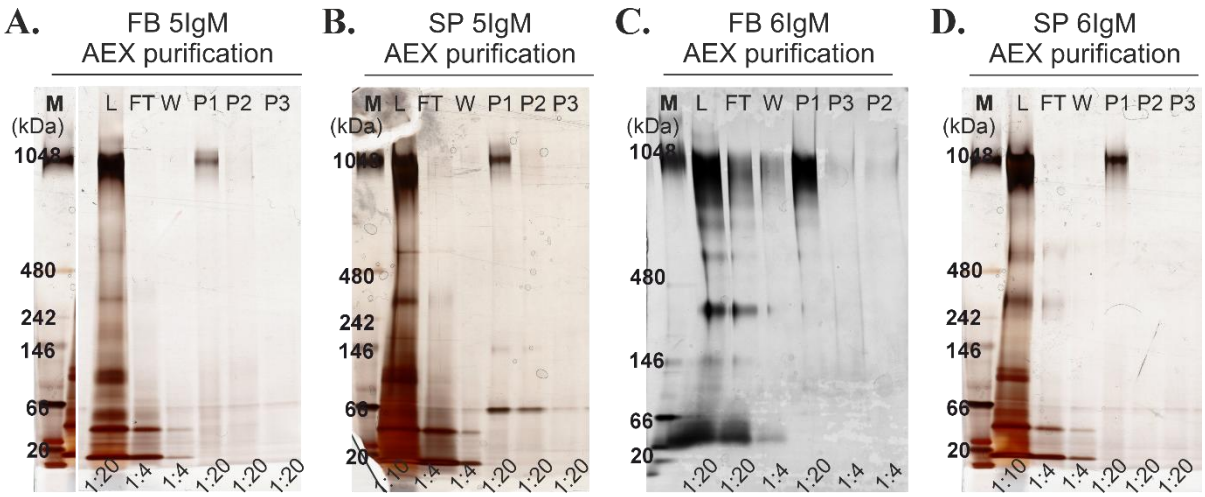

Fig. S1. Supplementary 3-12% NativePAGE gels for Fig. 4. Silver-stained AEX purification summary gels are shown for FB-5IgM (**A.**), for SP-5IgM (**B.**), for FB-6IgM (**C.**) and for SP-6IgM (**D.**). Abbreviations used indicate the following samples, M: protein marker in kDa, L: load, FT: flow-through, W: wash, P1: pool 1, P2: pool 2 and P3: pool 3. Dilution factors used for sample preparations are indicated below the lanes, adjusted according to protein and/or salt content.

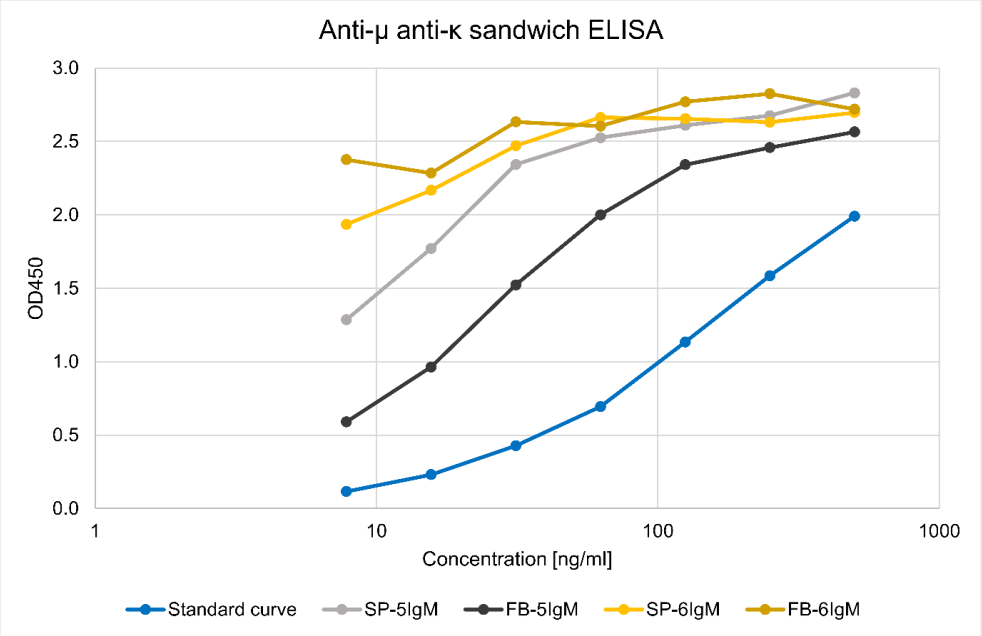

Fig. S2. Sandwich ELISA to quantify purified 5IgM and 6IgM from FB and SP cultivations. All samples were prediluted 1:1000. Unknown sample concentrations could only be calculated from the data points which were within the range of the standard curve (0-500 ng/ml).

Table S1. Lost IgM amounts in downstream purification steps determined by sandwich ELISA and BLI[§].

	FB-5IgM		**SP-5IgM**		**FB-6IgM**		**SP-6IgM**	
	[µg/ml]	[µg]	[µg/ml]	[µg]	[µg/ml]	[µg]	[µg/ml]	[µg]
FT	21.0	235	45.2	669	580.0[§]	7888	50.9[§]	790
W	2.1	32	5.8	87	-	-	14.6	220
P2	7.6	82	19.2	208	86.7	937	16.9	182
P3	0.3	3	0.7	8	-	-	-	-
Permeate	0.8	9	0	0	3.1	22	3.3	23
Total loss [µg]	361		972		8847*		1215*	

FT: flow-through, W: wash, P2: pool 2, P3: pool 3 belong to the purification step. Permeate fraction is collected post-purification and refers to the permeate during concentration and dialysis of the corresponding pool 1 fraction. -: not measured. *: indicates the minimum calculated lost IgM due to incomplete quantification of other fractions. Quantification was performed with sandwich ELISA, unless specified otherwise. [§]: marks the quantification measurements performed by BLI.

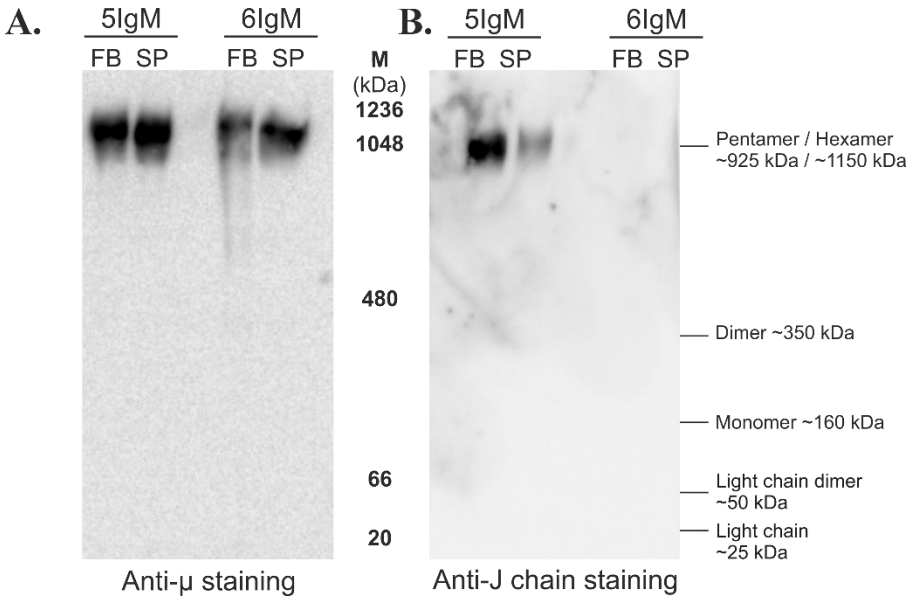

Fig. S3. The protein marker (in kDa) is aligned for both blot images and is indicated by M. **A.** Supplementary western blot image of a 3-12% NativePAGE gel loaded with ~500 ng of purified 5IgM and 6IgM. The membrane was initially stained with anti-κ-HRP antibody as shown in Fig. 4D. Same membrane was stripped and re-stained with anti-μ-HRP. **B.** Supplementary western blot for J-chain staining. 3-12% NativePAGE of ~500 ng of purified 5IgM and 6IgM were blotted on a membrane and stained with anti J-chain rabbit IgG and anti-rabbit HRP-labeled antibody.