

**Supporting information for manuscript:**

**“Engineering camel single-domain antibodies and immobilization chemistry for human prostate-specific antigen sensing”**

Dirk Saerens<sup>1,\*,#</sup>, Filip Frederix<sup>2,4,#</sup>, Gunter Reekmans<sup>2</sup>, Katja Conrath<sup>1</sup>, Karolien Jans<sup>2,4</sup>, Lea Brys<sup>1</sup>, Lieven Huang<sup>1,2</sup>, Eugène Bosmans<sup>3</sup>, Guido Maes<sup>4</sup>, Gustaaf Borghs<sup>2</sup>, Serge Muyldermans<sup>1</sup>.

<sup>1</sup> Laboratory of Cellular and Molecular Immunology, Department of Molecular and Cellular Interactions, Vlaams Interuniversitair Instituut voor Biotechnologie, Vrije Universiteit Brussel, Pleinlaan 2, 1050 Brussels, Belgium. <sup>2</sup> Interuniversity MicroElectronics Center (IMEC), MCP-ART, Kapeldreef 75, 3001 Leuven, Belgium. <sup>3</sup> Epsilon-Biotech, Beverzakkbroekweg 91, 3520 Zonhoven, Belgium. <sup>4</sup> Quantum Chemistry and Physical Chemistry Section, Department of Chemistry, Katholieke Universiteit Leuven, Celestijnenlaan 200F, 3001 Leuven, Belgium.

# Authors contributed equally to the work

\* Corresponding author: Dirk Saerens

Tel.: +32-2-629.19.77

Fax: +32-2-629.19.81

Email: [dsaerens@vub.ac.be](mailto:dsaerens@vub.ac.be)

**Table of contents**

Page S-3: Table S-1 containing the details of antibodies and antibody constructs

Page S-4: Table S-2 containing the LOD values for assay development

Table S-1 Details of antibodies and antibody constructs

Different antibody constructs are given with their attached tag. The expression yield is given in  $\text{mg l}^{-1}$ . The equilibrium dissociation constant ( $K_D$ ) is represented in  $10^{-9}$  M according to the presence of the epitope on  $\text{hPSA}_{\text{free}}$  and/or  $\text{hPSA-ACT}$  (ND = Not Determined).

Antibody	tag	MW	$K_D$ ( $10^{-9}$ M)	epitope	expression yield ( $\text{mg l}^{-1}$ )
cAbPSA-N50	His	15.000	12	$\text{hPSA}_{\text{free}}$	1
cAbPSA-N50	Fc	90.000	ND	$\text{hPSA}_{\text{free}}$	3
cAbPSA-N7	His	15.000	0.09 / 0.18	$\text{hPSA}_{\text{free}}$ / $\text{hPSA-ACT}$	0.2
cAbPSA-N7	BAD	17.000	ND	$\text{hPSA}_{\text{free}}$ / $\text{hPSA-ACT}$	0.25
cAbPSA-N7	StrepII	15.000	ND	$\text{hPSA}_{\text{free}}$ / $\text{hPSA-ACT}$	0.3
cAbPSA-N7	Fc	90.000	ND	$\text{hPSA}_{\text{free}}$ / $\text{hPSA-ACT}$	2
mAb-3E6	Fc	150.000	ND	$\text{hPSA}_{\text{free}}$ / $\text{hPSA-ACT}$	ND
scFv-3E6	His	30.000	0.15 / 0.9	$\text{hPSA}_{\text{free}}$ / $\text{hPSA-ACT}$	0.1

Table S-2 LOD values for hPSA<sub>free</sub> versus hPSA<sub>total</sub> assay development. Both cAbPSA-N50 and cAbPSA-N7 were immobilized onto CM5 chip. As stated in Figure 5 different hPSA isoforms were detected directly or in a sandwich assay (ND = Not Done). Corresponding LOD values are given in ng ml<sup>-1</sup>.

Immobilized VHH	Detected antigen	Sandwich VHH-Fc	LOD (ng ml <sup>-1</sup> )
cAbPSA-N50	hPSA <sub>free</sub> (1)	ND	1.80 ± 0.09
cAbPSA-N50	hPSA <sub>free</sub> (2)	cAbPSA-N7-Fc	1.30 ± 0.06
cAbPSA-N7	hPSA <sub>total</sub>	ND	0.200 ± 0.002
cAbPSA-N7	hPSA <sub>free</sub> (3)	cAbPSA-N50-Fc	0.30 ± 0.02