Innovative bioconjugation methods for an improved detection of ovarian cancer at early stage using multiple biomarkers

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Abstract

Ovarian cancer (OC), whose incidence increases with age, is one of the most common cancer types in woman. OC can be successfully treated if detected early but the diagnosis of OC at early stages is difficult since there are no obvious symptoms and no screening test has proven to be effective. Several biomarkers have been identified for the diagnosis and therapy of ovarian carcinomas such as Cancer Antigen 125 (CA125) or Human Epididymis protein 4 (HE4). Furthermore, Secretory leukocyte protease inhibitor (SLPI) and Progranulin (PGRN) are both overexpressed markers related to survival in ovarian cancer. PGRN has been described as a prognostic biomarker for the advanced stages and SLPI has been considered as an early detection marker of OC. However, those biomarkers´ sensitivity is still poor in the early stages of the disease, with an average of 50% for stage I and 90% for the stage II or higher. Since the selectivity and sensitivity of the biomarkers dedicated to OC are still insufficient for the detection at early stages and for monitoring the treatment and the recurrence of the disease, we attempt to develop more efficient biosensor detection strategies based on the bioconjugation between nanobodies and the biomarkers HE4, SLPI and PGRN. Several approaches, by which the introduction of a site-specific and bio-orthogonal functional group can pave the way to a uniform orientation of the nanobodies on the biosensor surface, will be explored. This should lead to an improved sensitivity since all nanobodies will have their active regions accessible for binding the biomarker.

Methods
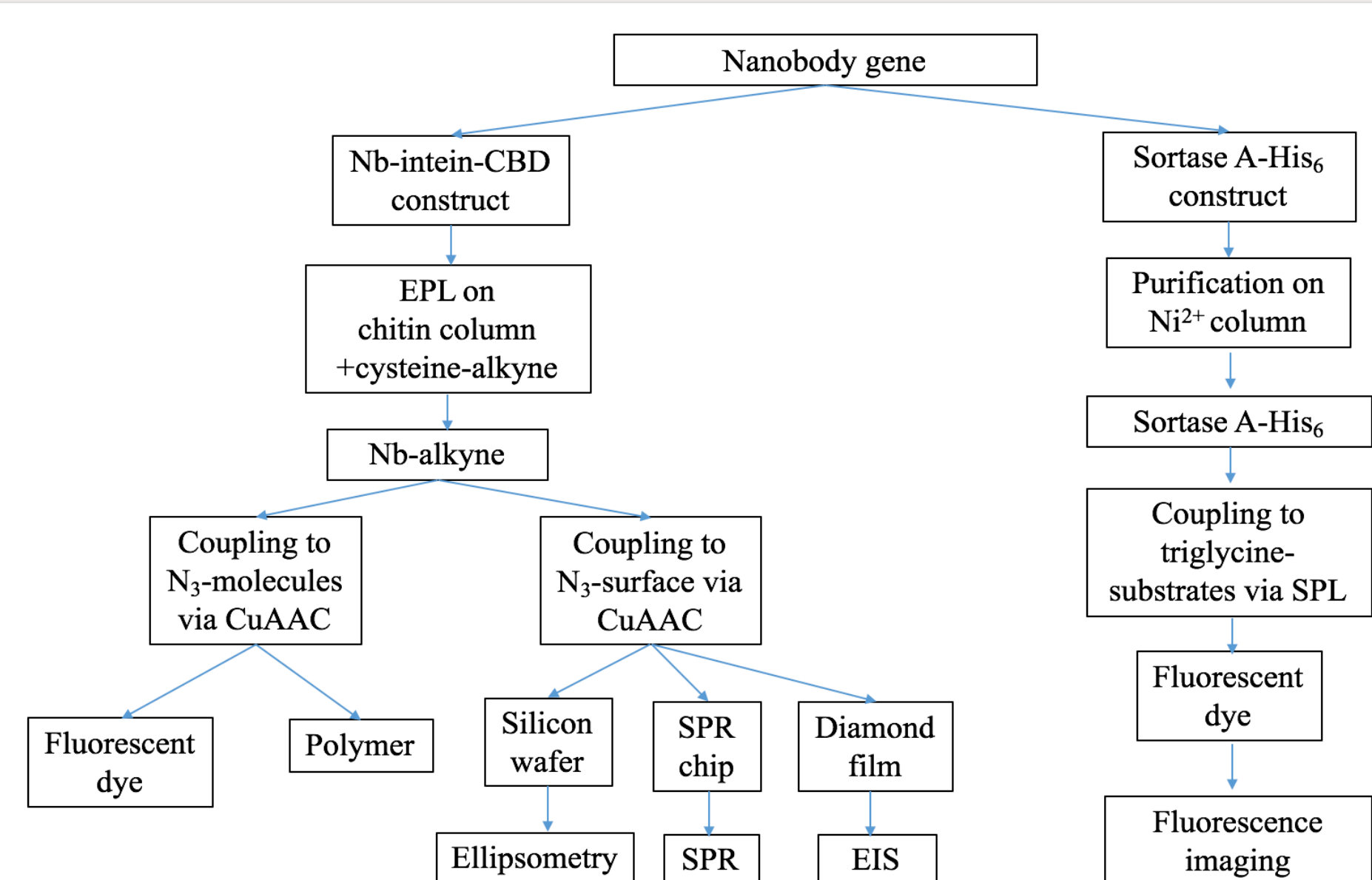
CuAAC: Copper (I)-catalyzed azide-alkyne cycloaddition; **CBD**: Chitin binding domain; **EIS**: Electrochemical impedance spectroscopy; **EPL**: Expressed protein ligation; **SPL**: Sortase A-mediated protein ligation;
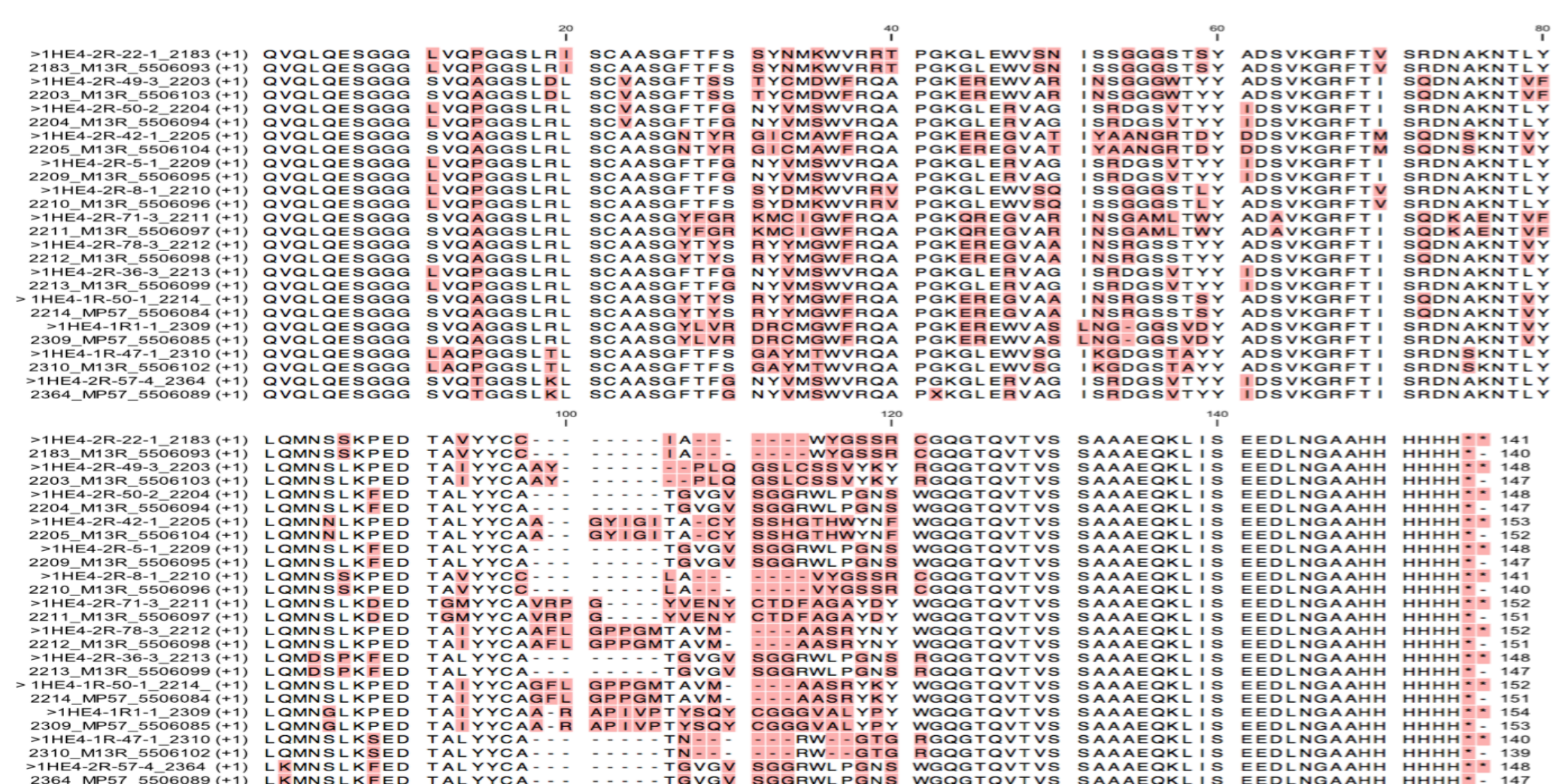
Results

Figure 1. Sequencing Alignment of 13 strains of HE4

HE4-2R22.1(2183); HE4-2R49.3(2203); HE4-2R50.2(2204); HE4-2R42.1 (2205); HE4-2R5.1(2209); HE4-2R8.1(2210); HE4-2R71.3(2211); HE4-2R78.3 (2212); HE4-2R36.3(2213); HE4-1R50.1(2214); 1R1.1(2309); HE4-1R47.1(2310); HE4-2R57.4(2364)

Figure 2. Affinity of HE4 Nanobodies by ELISA
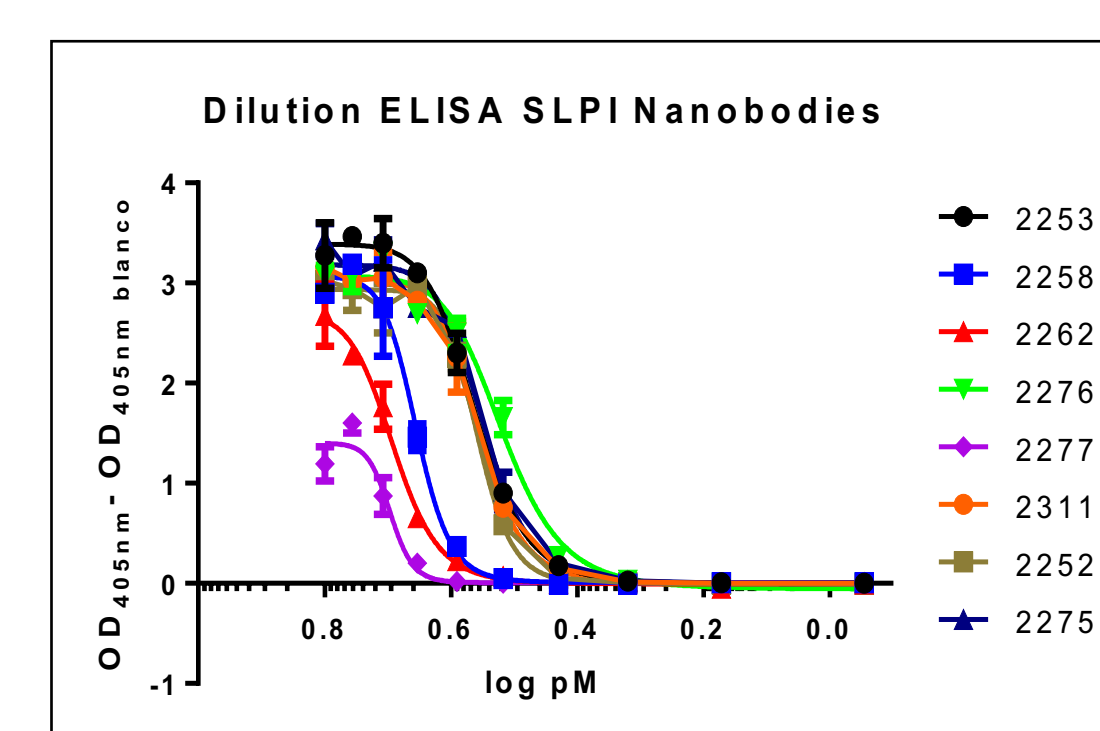
Figure 3. Affinity of SLPI Nanobodies by ELISA
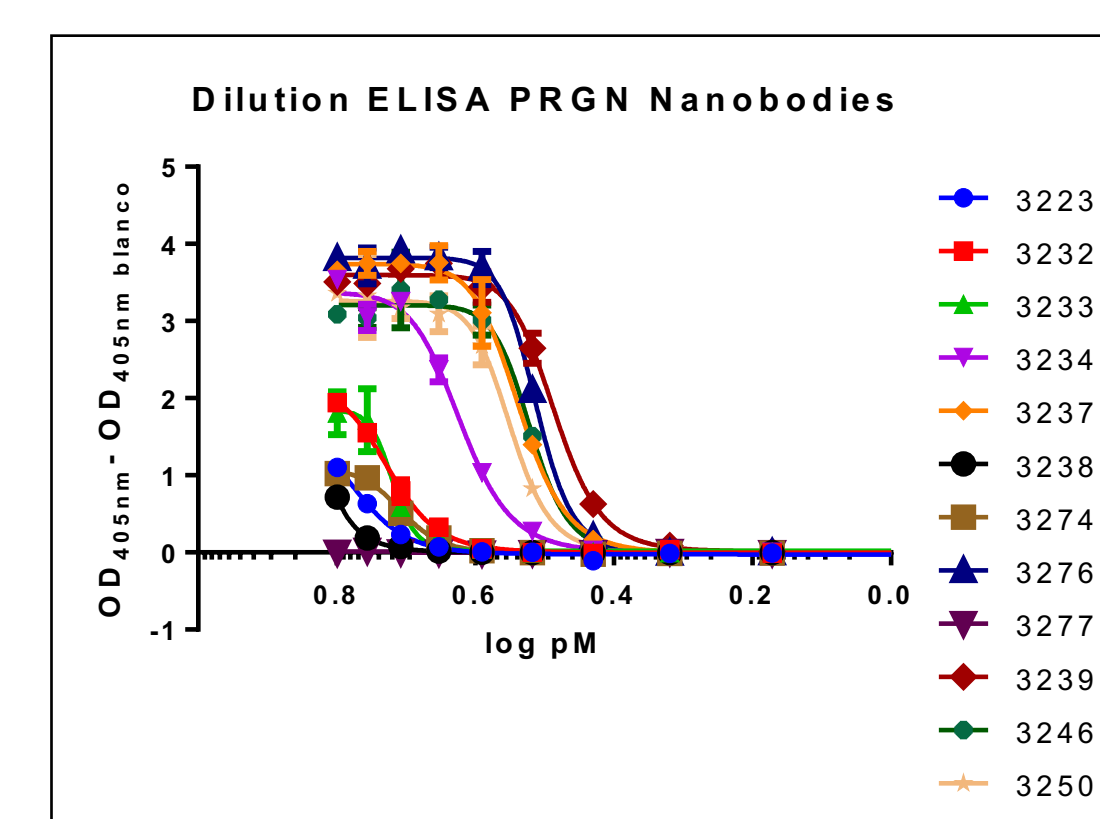
Figure 4. Affinity of PGRN Nanobodies by ELISA

	HE4			SLPI		PGRN		
Clone	2183	2205	2210	2252	2276	3237	3239	3276
Expression	++	+	+++	+++	+/-	+++	+	+++
ELISA	+++	+++	+++	+++	+++	+++	+++	+++
Biacore k_{on}	5.2E+6	5.1E+6	3.2E+6	3.2E+6	2.1E+6	1.0E+5	1.1E+6	5.2E+5
Biacore k_{off}	1.9E-3	3.1E-3	8.9E-3	5.6E-2	3.5E-3	3.3E-3	7.9E-4	2.2E-3
Epitope mapping	- independent of 2211 & 2212 - interferes with 2205 & 2309	- competitive with 2211; 2212& 2309 - interferes with 2183	Not measured but same behavior expected as 2183	- independent of 2276 - interferes with 2253; 2258 & 2311	- independent of 2252; 2258 - interferes with 2253; 2258 & 2311	- independent of 3239; 3246 & 3250 - competitive with 3276	- independent of 3237; 3246 & 3250 - competitive with 3250	- independent of 3239; 3246 & 3250 - competitive with 3237

Figure 5. Characterization of best clones of HE4; SLPI and PGRN Nanobodies

References

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