



# Characterizing the binding of bovine growth hormone with its induced binding proteins

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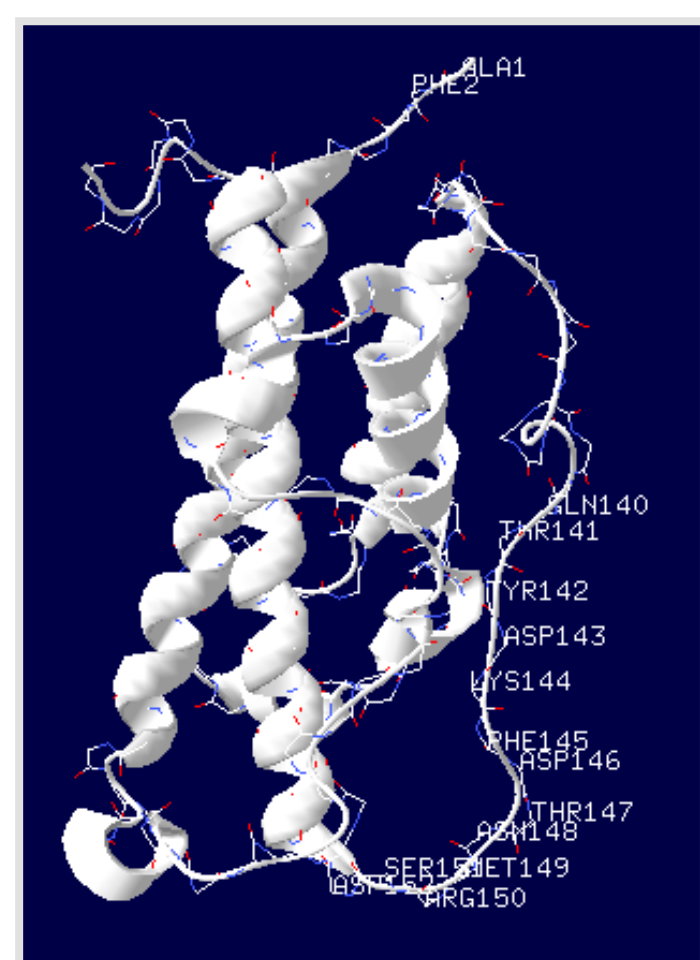
## Background

The recombinant bovine growth hormone (rbGH) is administered to cows to enhance milk production. RbGH is a 191 amino acid long protein and although it differs only 1 amino acid from the endogenous form, in 75% of the dairy cows an effect is triggered by the formation of a binding protein.

## Objective

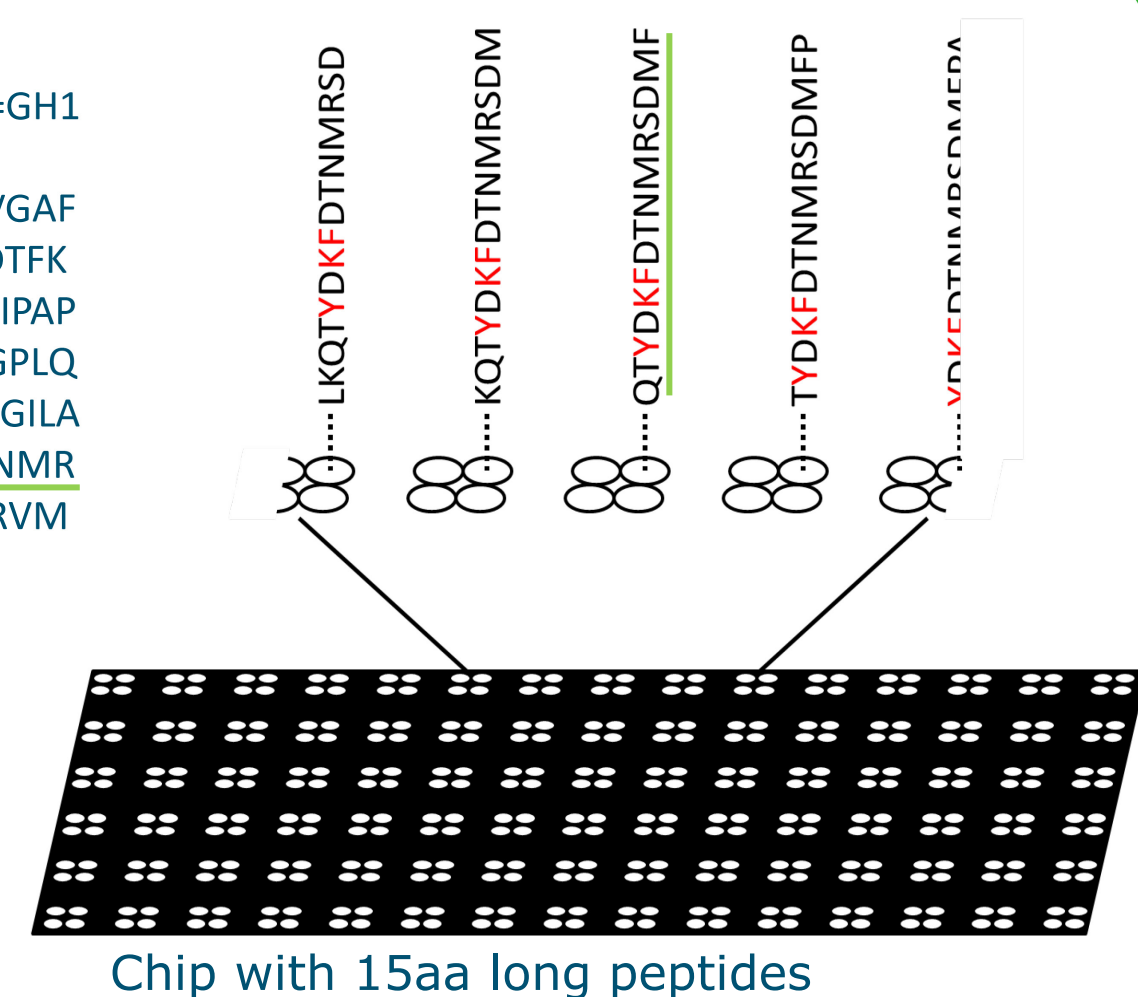
RbGH use is pinpointed by, amongst others, detection of the binding proteins formed after rbGH administration. Characterization of these binding proteins by determination of the epitopes they target, and determination if individual cows react on the same epitope, establishes new opportunities in method development.

## Experimental



```
>sp|P01246|SOMA_BOVIN  
Somatotropin OS=Bos taurus GN=GH1  
PE=1 SV=1  
MMAAGPRTSLLLAFALLCLPWTQVVGAF  
PAMSLGLFANAVLRAQLHLQAADTFK  
EFERTYIPEGORYSQNTQVAFCSFETIPAP  
TGKNEAQKSDLELLRISLLIQSWLGPLQ  
FLSRVFTNSLVFGTSDRVYEKLDLEEGILA  
LMRELEDGTPRAGQILKQTYDKFDTNMR  
SDDALLKNYLLSCFRKDLHKTETYLRLVM  
KCRRFGEASCAF
```

Sequence of bGH



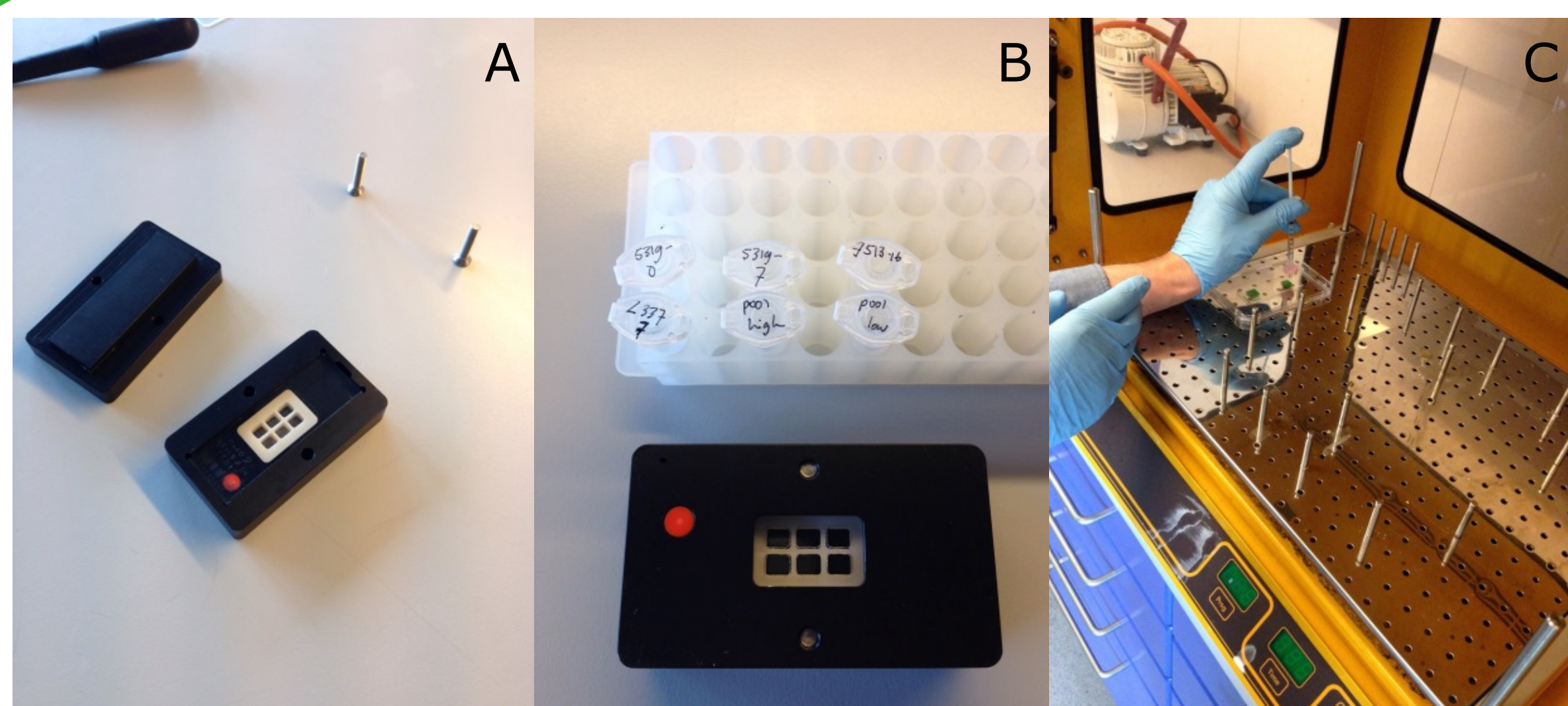
Chip with 15aa long peptides

**Figure 1.** 15 aminoacid long peptides were synthesized in 2x2 mirrors on a chip. The total bST sequence was used for synthesis; for each new 15 aa long peptide, starting point was the next aa compared to the aa used in the former synthesized peptide, until the total bST protein is synthesized.

**Table 1.** 10 cow serum samples were selected on prior binding results obtained with a bead based assay where rbST coated beads were incubated with the cow serum samples.

Before rbGH treatment	After rbGH treatment
Cow 1, 2	Cow 1, 3, 4, 5
Pool* 1, 2	Pool* 1, 2

\* Each pool contains sera of 5 individual cows



**Figure 2.** 6 sector device to clip the glass slide containing the peptide sectors, enabling incubation with the serum samples (A,B) for one hour, followed by half an hour secondary antibody (GAB-CY3) incubation (C). Read out was done by a fluorescence microscope equipped with a digital camera.

## Conclusions

- QTYDKFDTNMRSDMF is the most discriminative epitope as determined manually and with multivariate analyses.
- Tyrosine, lysine and phenylalanine are the most important amino-acids needed for binding to the binding protein.

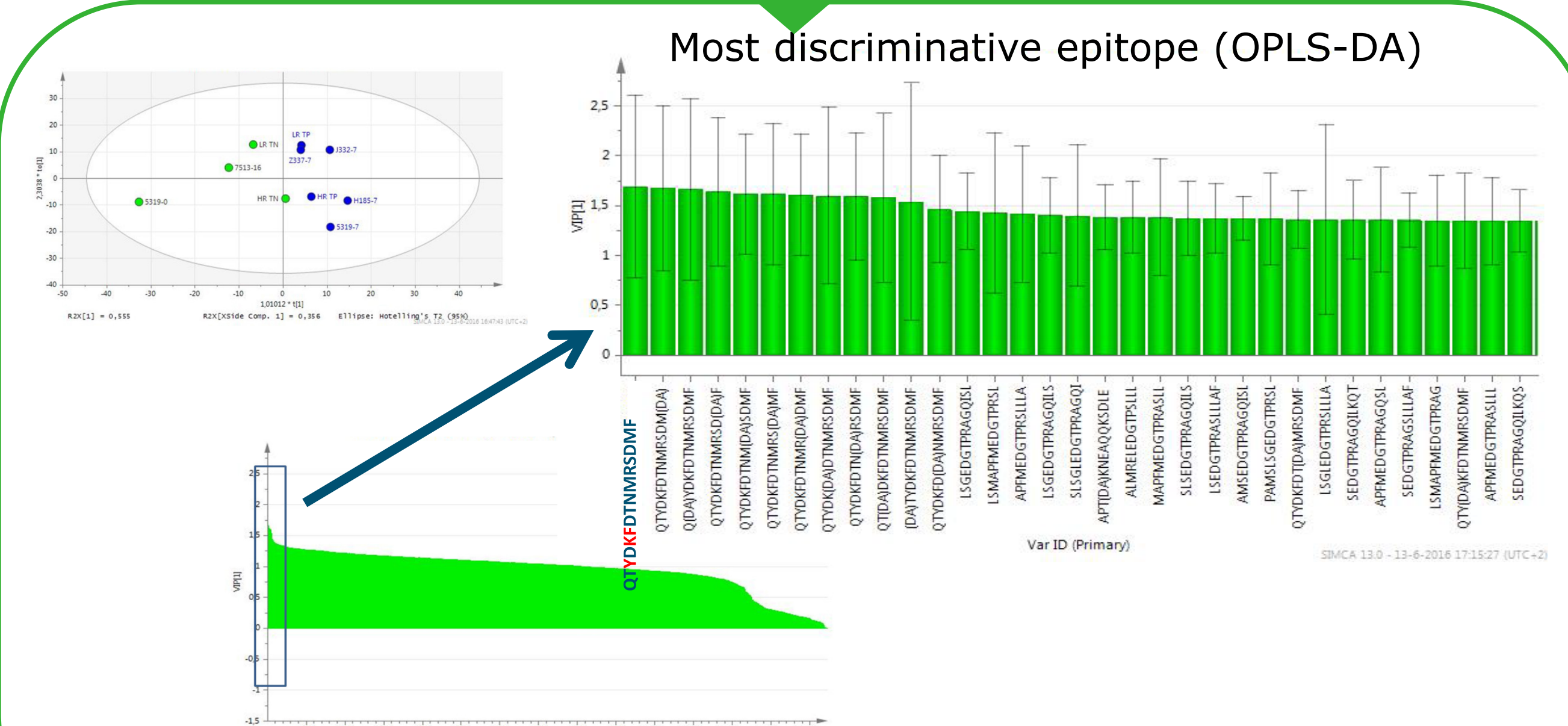
## Outlook

First step was determination of the most discriminative epitope for opportunities in method development; next steps:

- Closer understanding of the interaction of the binding proteins to rbGH and the most discriminative epitope.
- Knowledge on how binding affinities are influenced by the nature of peptide or protein presentation, i.e. in solution or fixed to a platform.

## Results

Signals of each sample for 1500 different peptides were obtained and manually judged. To verify the manual judgement Multi Variate analyses was executed (Figure 3) and similar results were found.



**Figure 3.** VIP score, describes the relative importance of each peptide for the OPLS-DA class separation, most discriminative epitope found: QTYDKFDTNMRSDMF.

The importance of every single amino-acid of the most discriminative epitope QTYDKFDTNMRSDMF is explored by single substitution with D-alanine (Table 2).

**Table 2.** Signals obtained after single substitution of each amino-acid with D-alanine as shown in the epitope sequences mentioned in the left column. Signals > highest signal obtained from samples taken before treatment are highlighted in green. When an important amino-acid is replaced, the signal drops (red).

Epitope sequence	Before rbST treatment				After rbST treatment					
	Cow 1	Cow 2	Pool 1	Pool 2	Cow 1	Cow 3	Cow 4	Cow 5	Pool 1	Pool 2
QTYDKFDTNMRSDMF	19	29	53	24	98	134	104	113	128	45
(DA)TYDKFDTNMRSDMF	43	41	62	34	138	136	150	114	135	62
Q(DA)YDKFDTNMRSDMF	18	29	49	23	81	118	95	106	93	50
QT(DA)DKFDTNMRSDMF	14	17	40	17	71	77	57	77	68	29
QTY(DA)KFDTNMRSDMF	23	37	57	28	98	114	87	73	97	39
QTYD(DA)FDTNMRSDMF	19	28	47	23	65	68	47	69	59	32
QTYDK(DA)DTNMRSDMF	15	19	41	19	74	77	64	75	80	34
QTYDKF(DA)TNMRSDMF	21	34	51	27	105	100	66	42	75	41
QTYDKFD(DA)NMRSDMF	24	31	54	26	104	151	78	68	97	45
QTYDKFDT(DA)MRSDMF	18	28	45	24	84	118	71	40	80	38
QTYDKFDTN(DA)RSDMF	19	26	46	23	82	133	90	56	99	48
QTYDKFDTNM(DA)SDMF	16	26	47	20	85	131	80	56	100	46
QTYDKFDTNMRS(DA)DMF	19	25	54	23	96	137	102	60	118	45
QTYDKFDTNMRS(DA)MF	20	34	54	27	123	145	103	89	113	47
QTYDKFDTNMRSD(DA)F	20	31	54	26	101	139	106	84	120	51
QTYDKFDTNMRSDM(DA)	18	29	53	25	103	133	93	102	121	47

## Acknowledgements

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