APTAMER INFORMATION
CMVpp65_G2PVJ:1035:858_Oligo #757

Description:

- *Identifiers*: G2PVJ:1035:858 (oligo #757)
- *Number of DNA nucleotides*: 32 bases
- *Molecular weight (includes 5'-bioTEG): 10,387* g/mol
- *Target for selection:* **CMV pp65 protein**

Aptamer was selected from a randomized 32-mer library against CMV pp65 protein. Proprietary methods were then used to select the aptamer.

Aptamer folding instruction before use:

Once the aptamer is in its working concentration, it needs to be heated to 85-90 degC for 2 minutes, and then cooled to room temperature before use.

1. Validation data with CMVpp65 protein:

- *Immobilized Ligand:* Biotinylated CMVpp65 aptamer #757
- *Analyte:* CMVpp65 protein
- *Random aptamer as negative control:*
  Random aptamer with same length, similar molecular weight and same 3'modifications (i.e 5'bioTEG) like CMVpp65 aptamer#757

Kinetics Screening Assay using Streptavidin Biosensors :

By two types of reference methods, we validate the binding data.

- Single reference data: All curves are referenced to a sensor dipped in buffer alone (no protein) (see Figures 1, 2 and Table 1).

- Double reference data: For even more stringent validation of aptamer binding, we "double-reference" all data to both protein-free buffer (as above) as well as to a sensor with a control or "sham" aptamer (see Figures 1, 3 and Table 2). This random ~32-mer controls for any non-specific charge-only interactions.
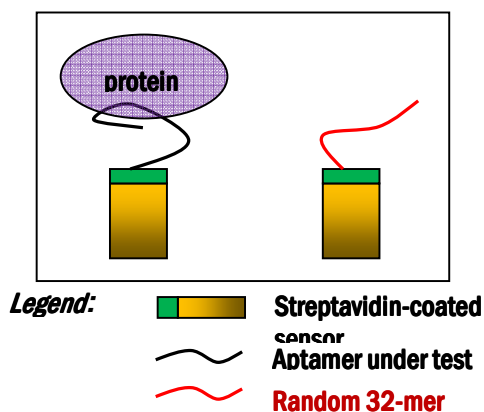

Legend:

Streptavidin-coated sensor

Aptamer under test

Random 32-mer

Figure1: Double-referencing for highly stringent validation of aptamer: protein binding.
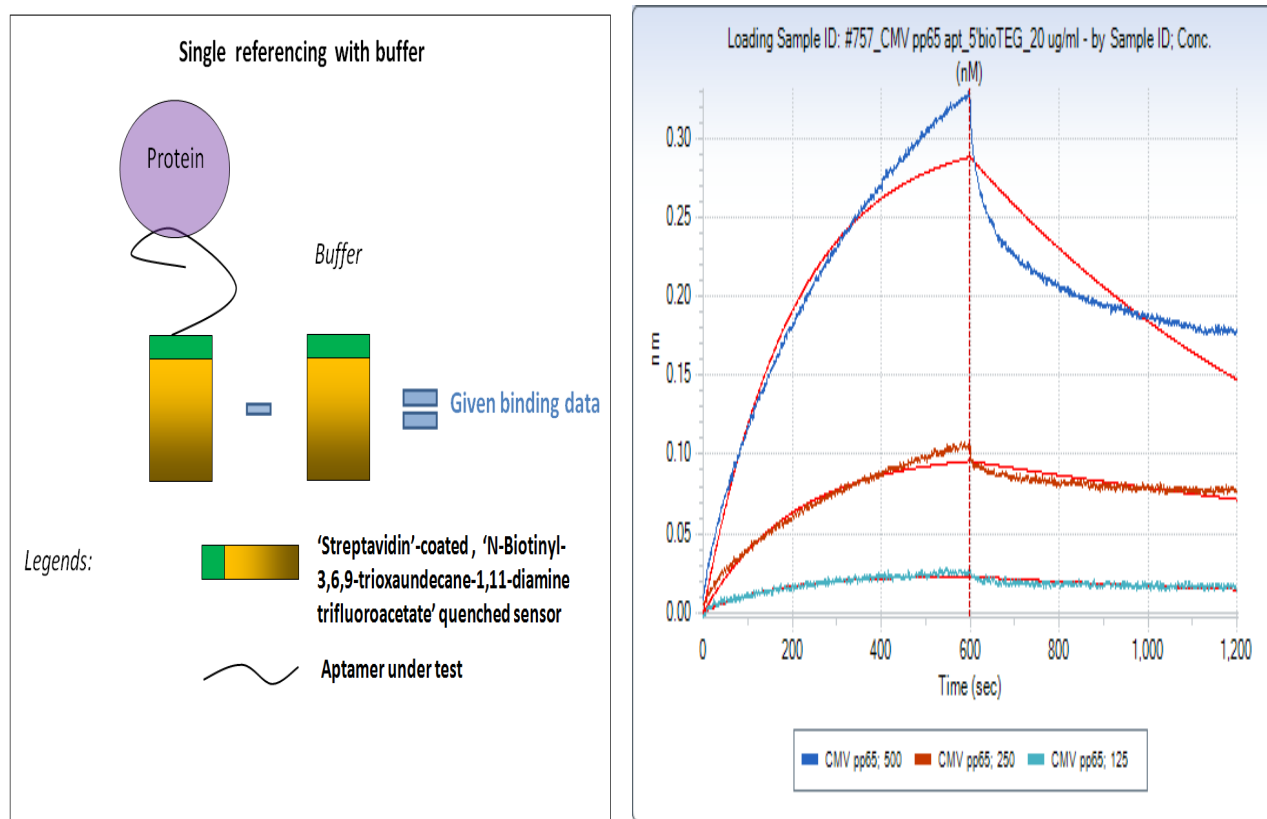
(i) Single reference data:

Figure 2. Association and dissociation graph of 1:1 fitting model of CMVpp65 aptamer # 757 (biotinylated) to CMVpp65 protein concentrations 500, 250 and 125 nM, by single reference method.
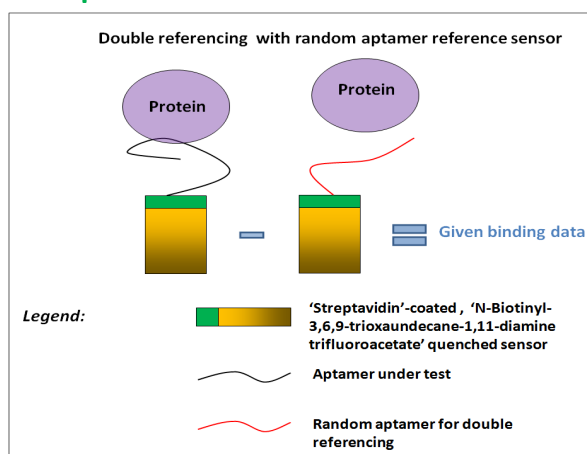
Table 1: Kd, R^2 and Chi^2 values by Local fitting for single reference method. Avg Kd = 64 nM						
Immobilized Aptamer	**Analyte**	**Conc. (nM)**	**Response**	**KD (M)**	**Full X^2**	**Full R^2**
#757_CMV pp65 apt_5'bioTEG_20 ug/ml	CMV pp65	500	0.3262	1.47E-07	0.414949	0.914212
#757_CMV pp65 apt_5'bioTEG_20 ug/ml	CMV pp65	250	0.1053	2.80E-08	0.021597	0.955026

#757_CMV pp65 apt_5'bioTEG_20 ug/ml	CMV pp65	125	0.0252	1.73E-08	0.003901	0.859368

(ii) Double reference data with 'random aptamer immobilized sensors as reference sensors':

Double referencing with random aptamer reference sensor

Legend:
- 'Streptavidin'-coated, 'N-Biotinyl-3,6,9-trioxaundecane-1,11-diamine trifluoroacetate' quenched sensor
- Aptamer under test
- Random aptamer for double referencing

a) Highest conc. excluded in double reference data   **b) Highest conc. included in double reference data**
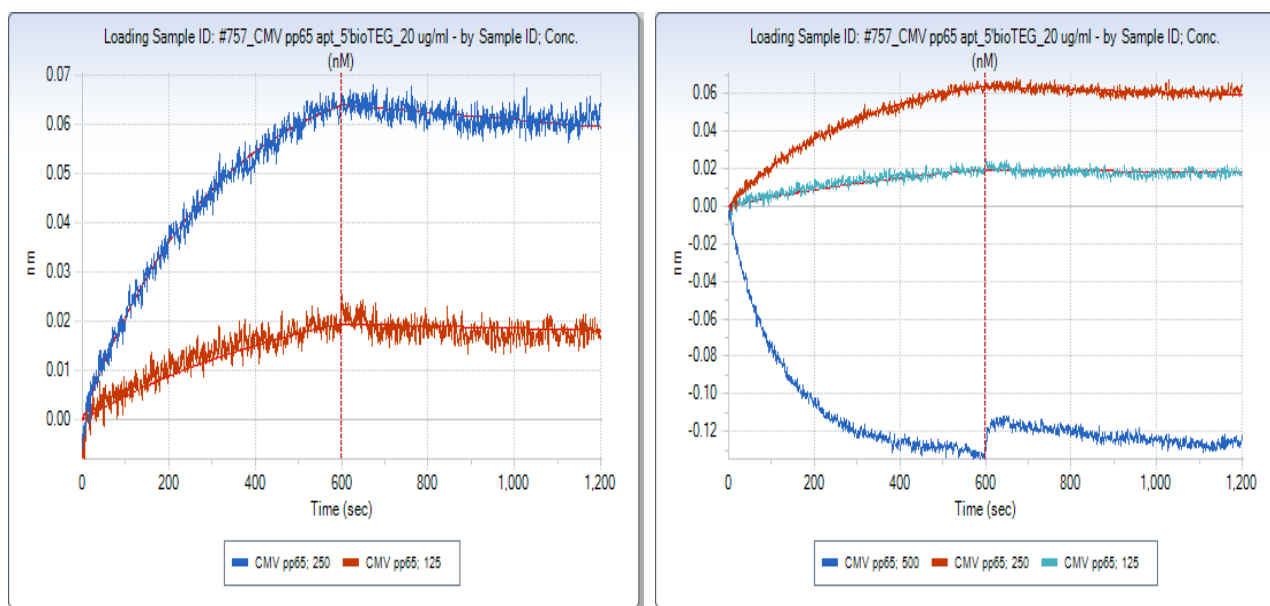
Figure 3. Association and dissociation graph of 1:1 fitting model of CMVpp65 aptamer # 757 (biotinylated) to CMVpp65 protein concentrations 500, 250 and 125 nM, by double reference method with 'random aptamer immobilized and sensors as reference sensors'.

Table 2: Kd, R^2 and Chi^2 values by Global fitting for double reference method with 'random aptamer immobilized and sensors as reference sensors'. **Kd = 9.31 nM**						
Immobilized Aptamer	**Analyte**	**Conc. (nM)**	**Response**	**KD (M)**	**Full X^2**	**Full R^2**
#757_CMV pp65 apt_5'bioTEG_20 ug/ml	CMV pp65	250	0.0628	9.31E-09	0.008206	0.992869
#757_CMV pp65 apt_5'bioTEG_20 ug/ml	CMV pp65	125	0.0192	9.31E-09	0.008206	0.992869