

Fig. 1

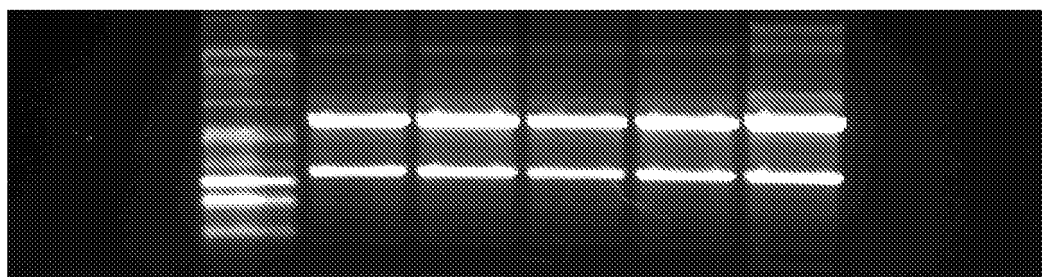


Fig. 2

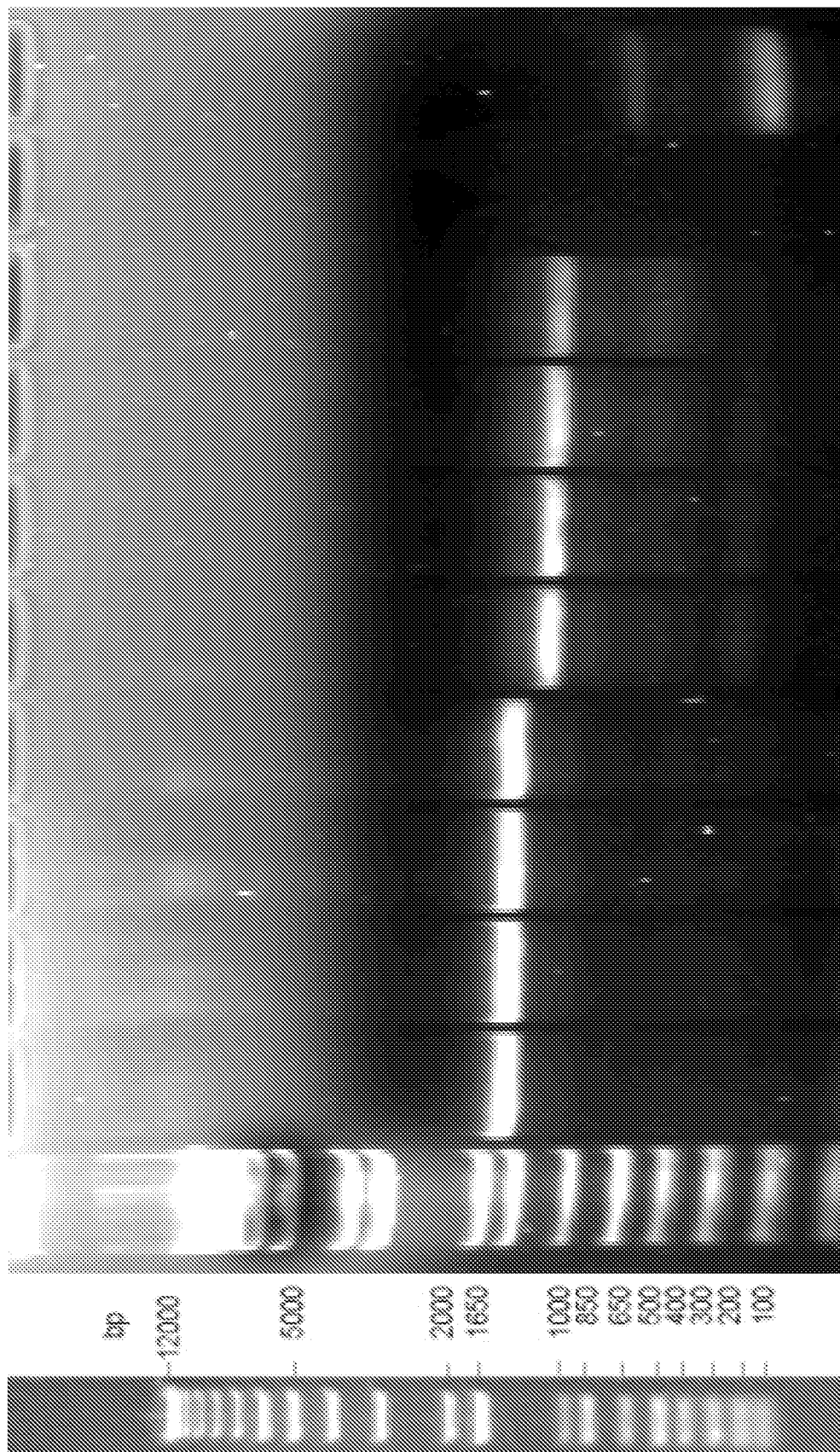


Fig. 3

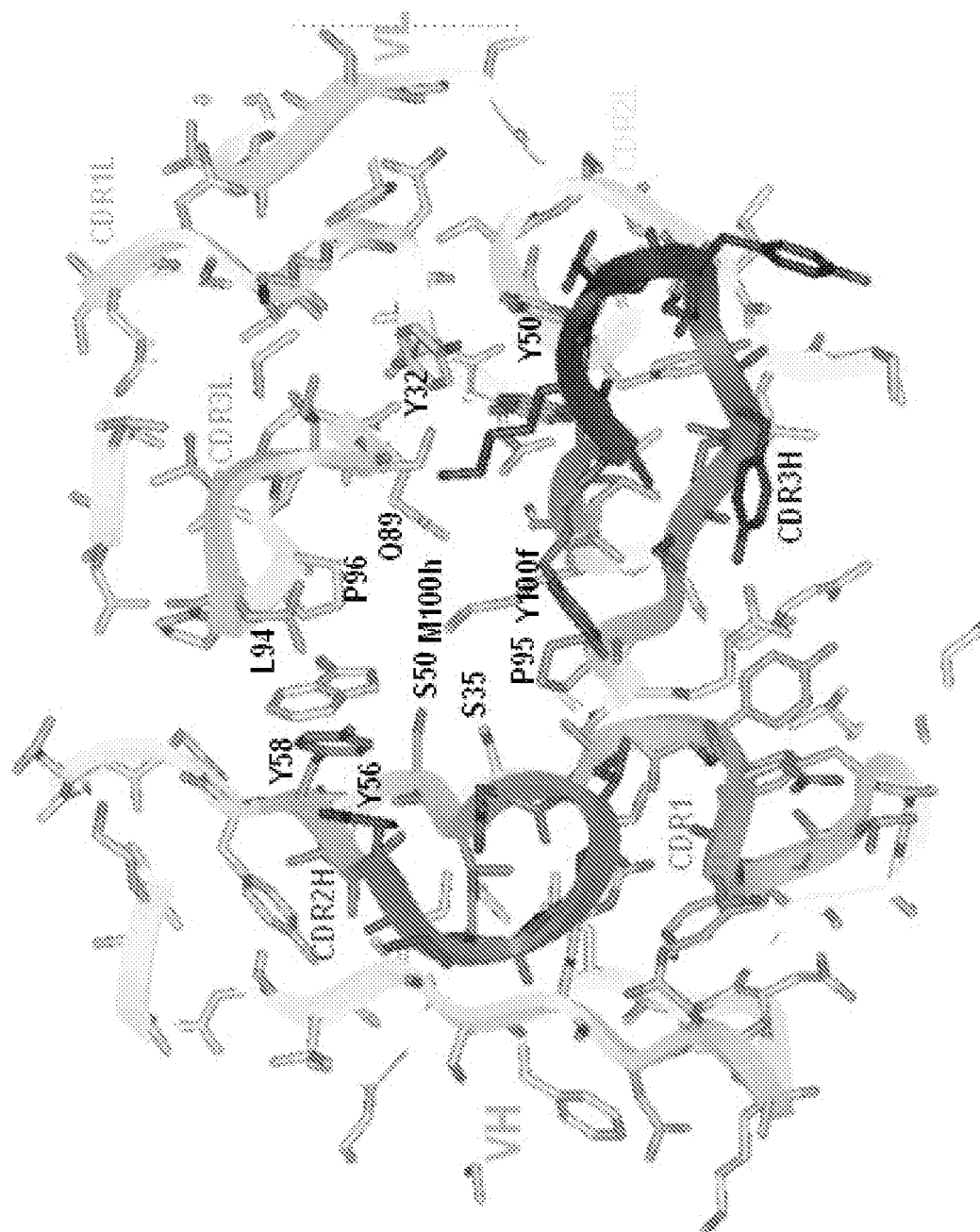


Fig. 4

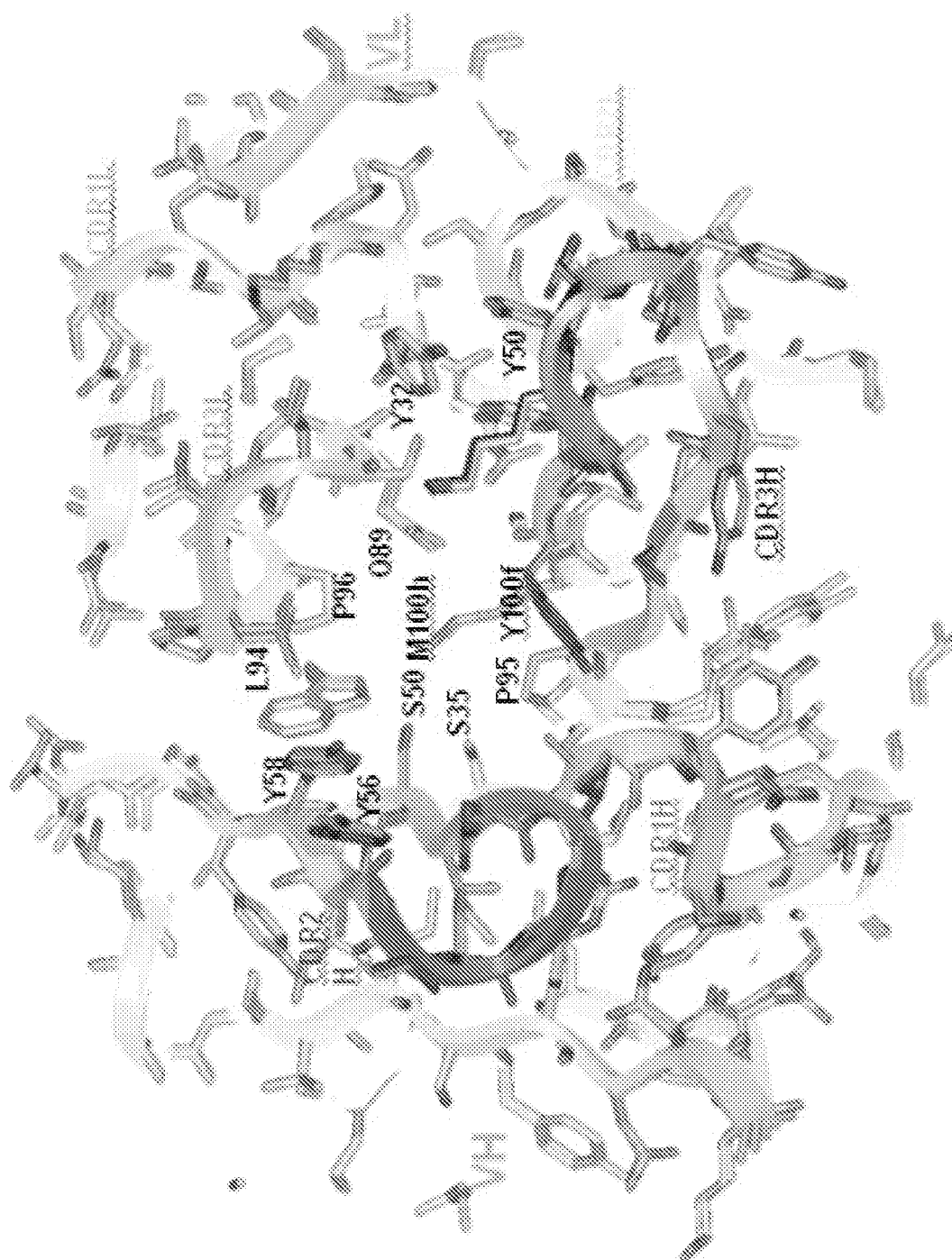


Fig. 5 a)

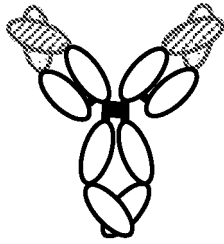


Fig. 5 b)

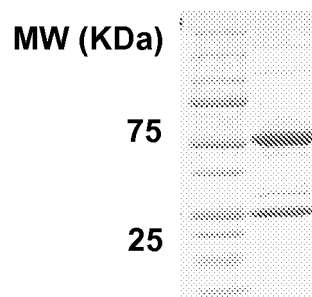


Fig. 5 c)

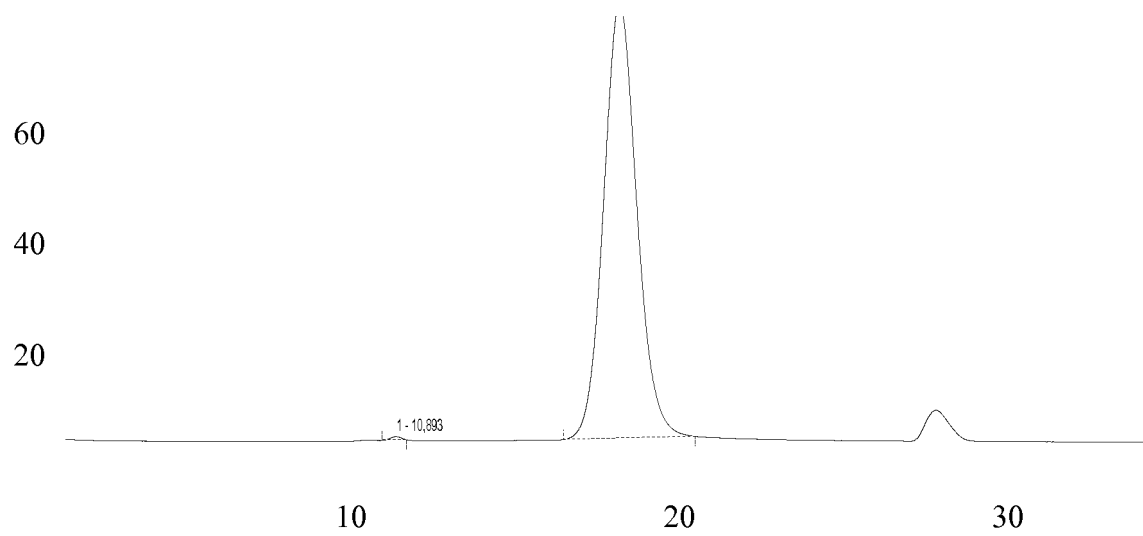


Fig. 6 a)

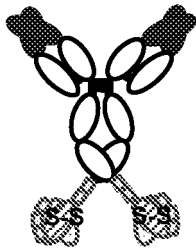


Fig. 6 b)

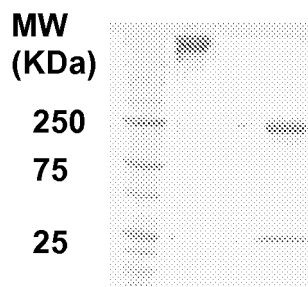


Fig. 6c)

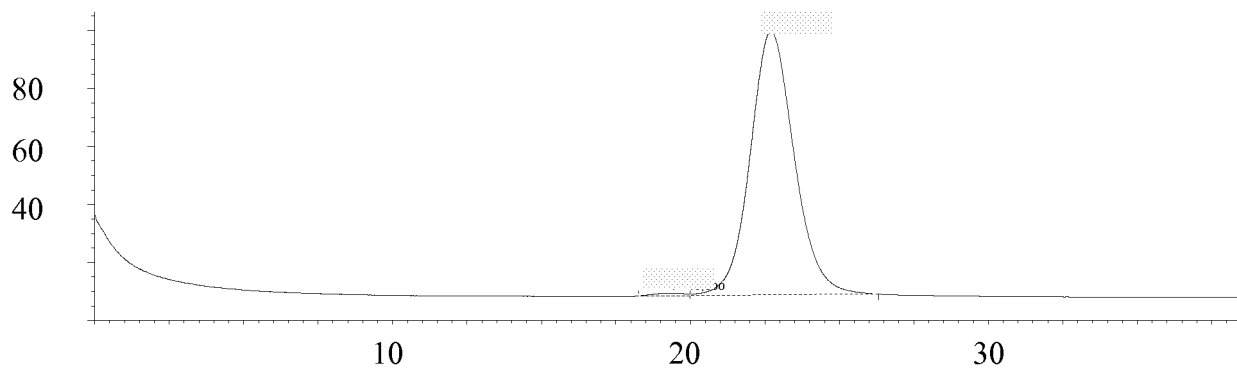


Fig. 7 a)

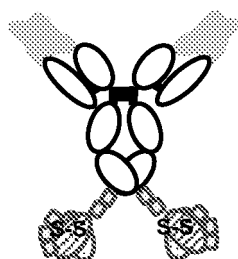


Fig. 7 b)



Fig. 7c)

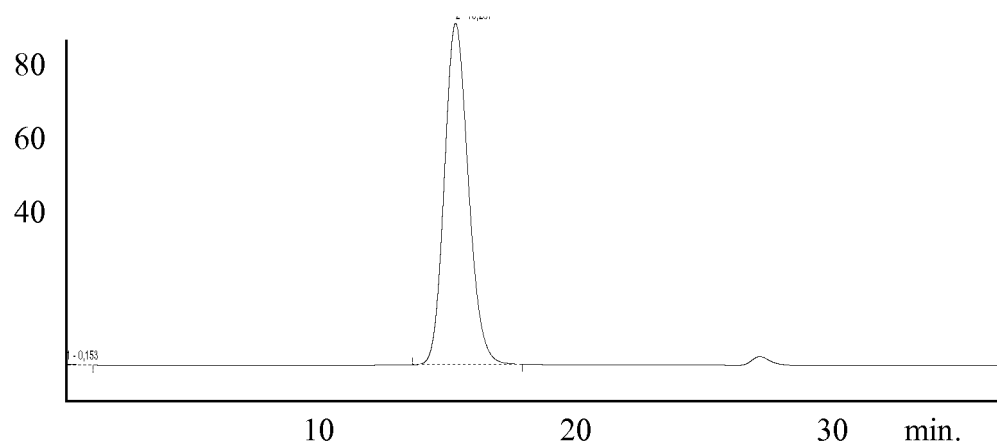


Fig. 8 a)

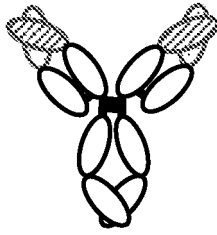


Fig. 8 b)

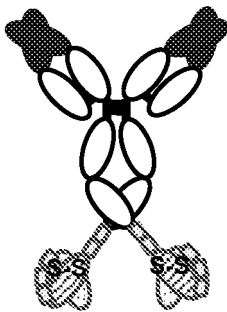


Fig. 8 c)

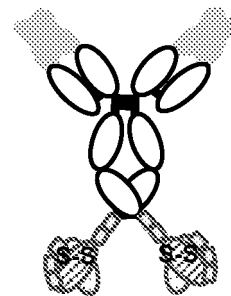


Fig. 8 d)

| Construct Name                                | Expression mg/L |
|-----------------------------------------------|-----------------|
| A – hu<Dig> IgG                               | 18.8            |
| B – <IGF1R-DIG>2321<br>(disulfide stabilized) | 6.4             |
| C – <Her2-DIG>2321<br>(disulfide-stabilized)  | 34.3            |

Fig. 9 a)

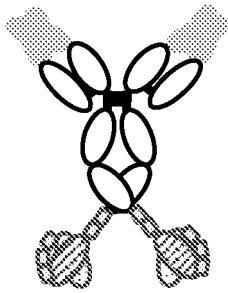


Fig. 9 b)

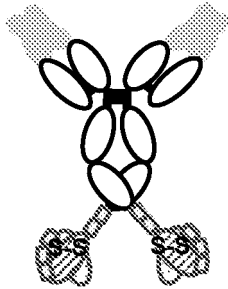


Fig. 9 c)

| Sample          | $\Sigma$ (mg) | Aggregates |
|-----------------|---------------|------------|
| Her2DIGHu2-2320 | 1             | 22 %       |
| Her2DIGHu2-2321 | 31            | 0 %        |

Fig. 10 a)

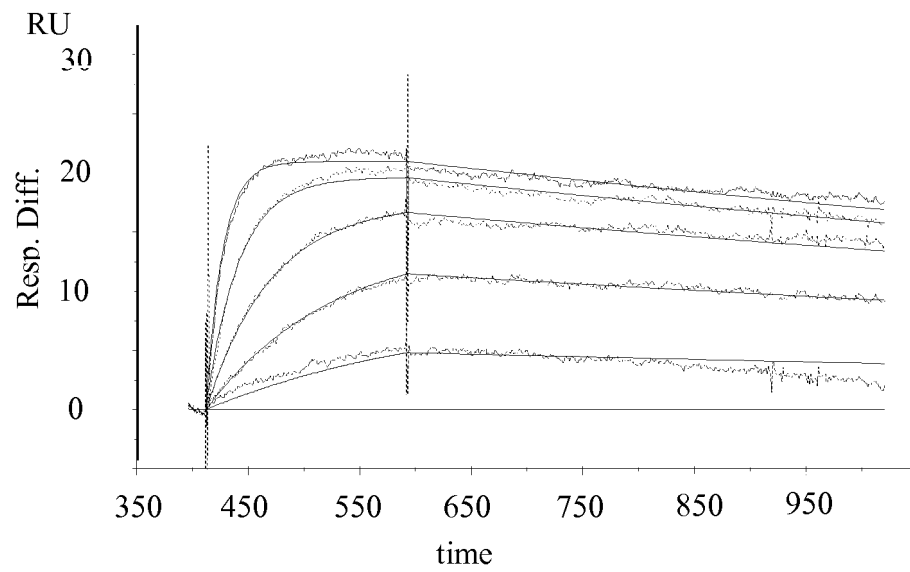


Fig. 10 b)

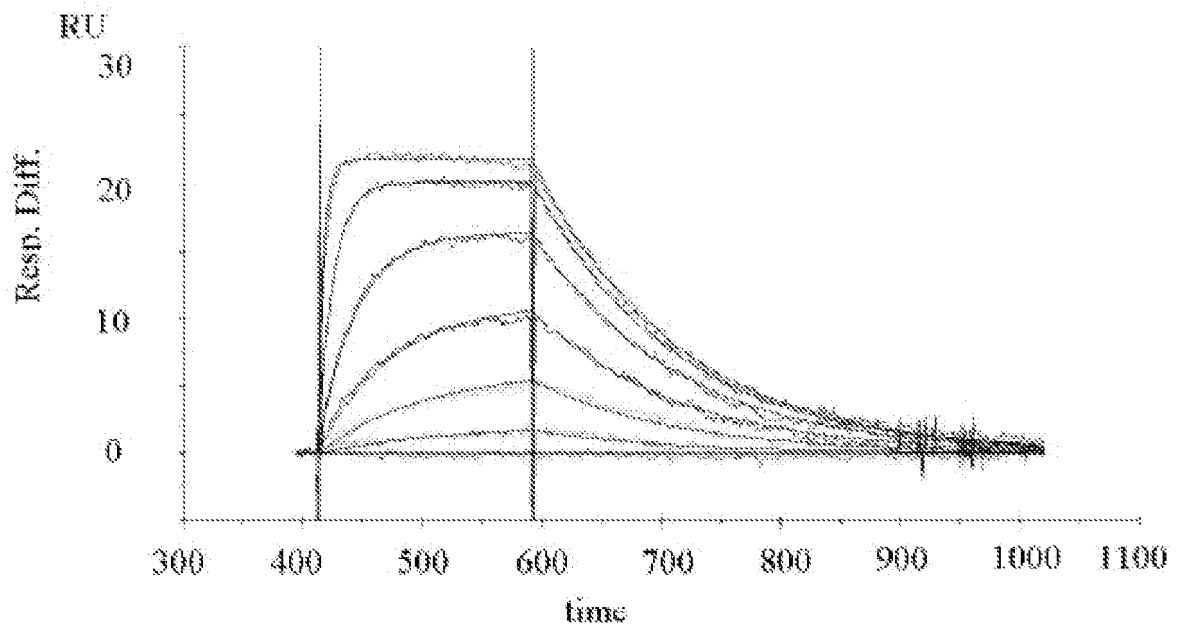


Fig. 10 c)

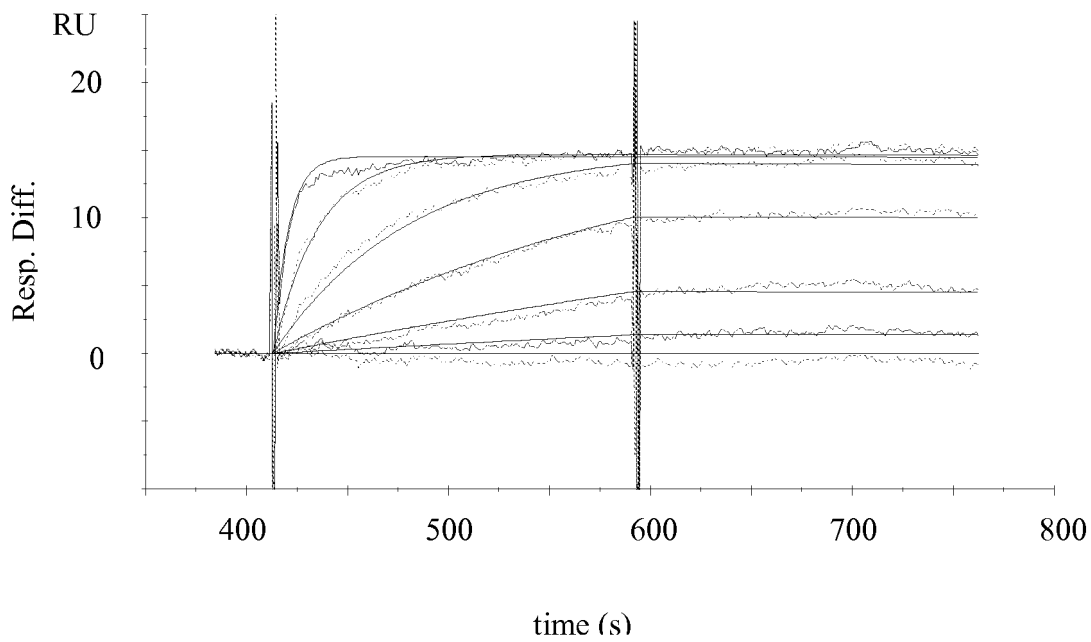


Fig. 10 d)

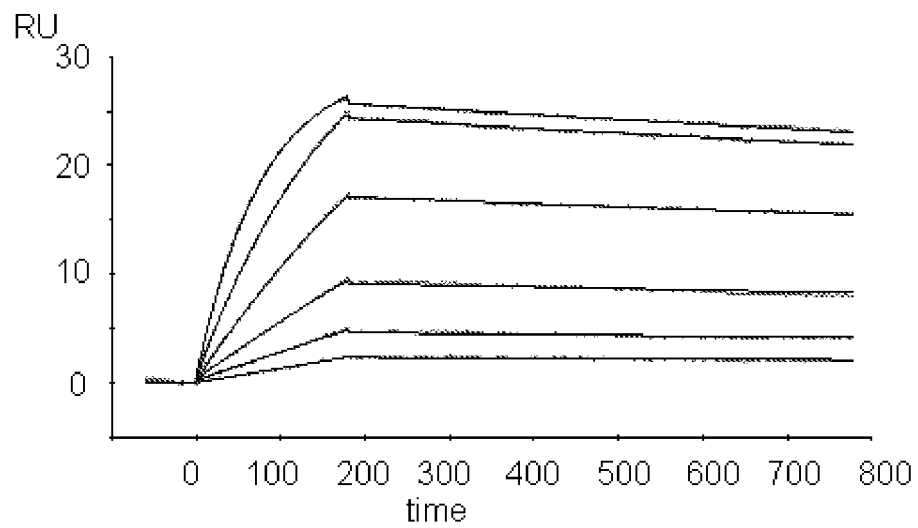


Fig. 10 e)

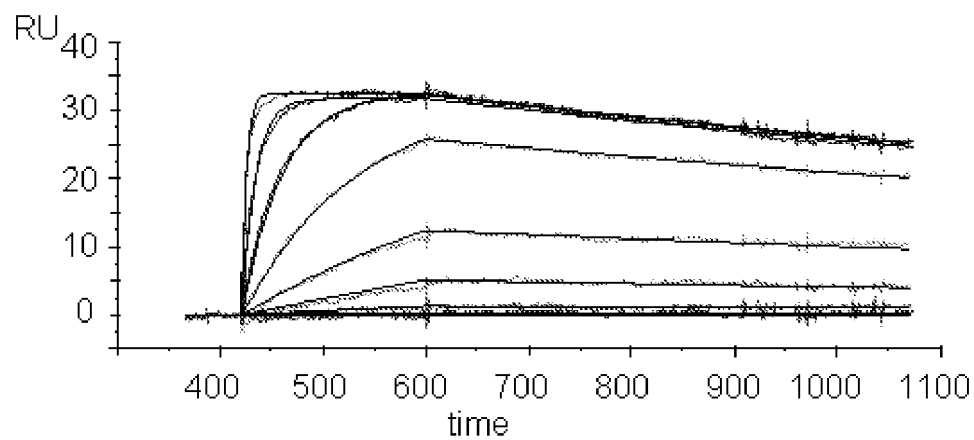


Fig. 10 f)

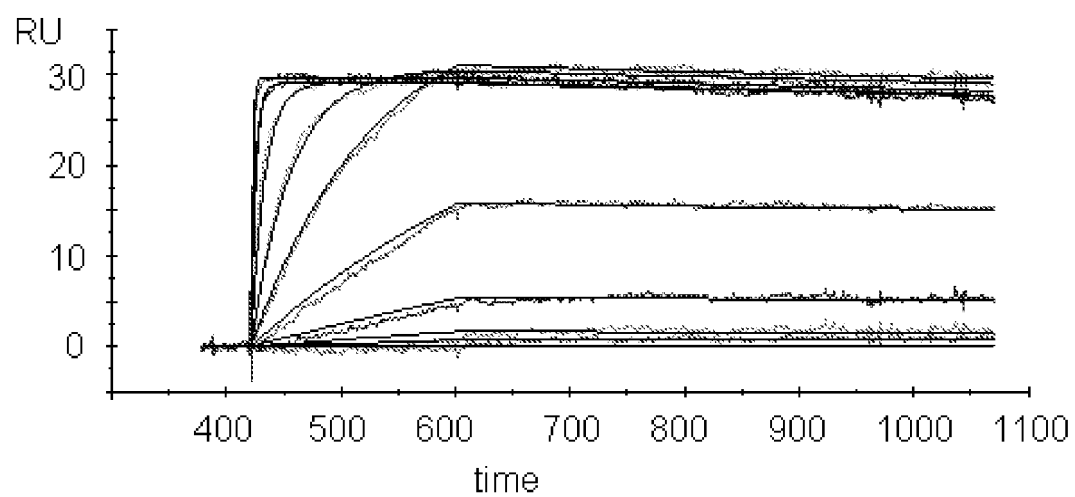


Fig. 11 a)

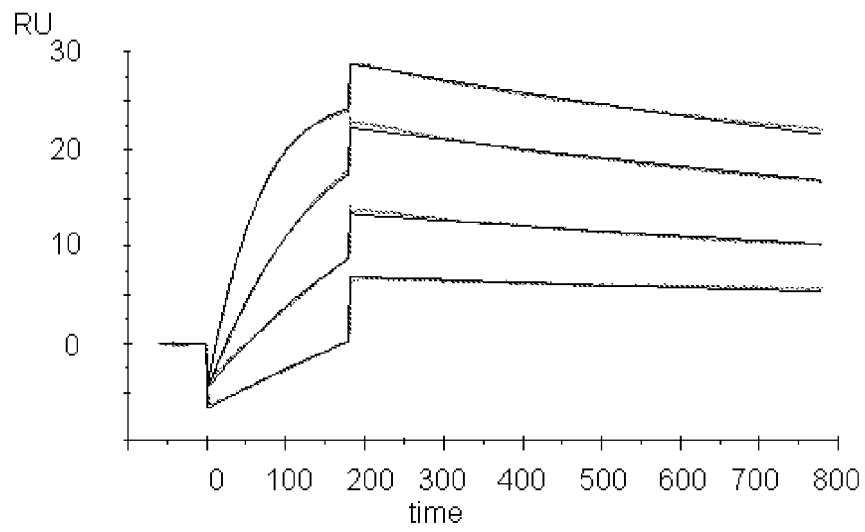


Fig. 11 b)

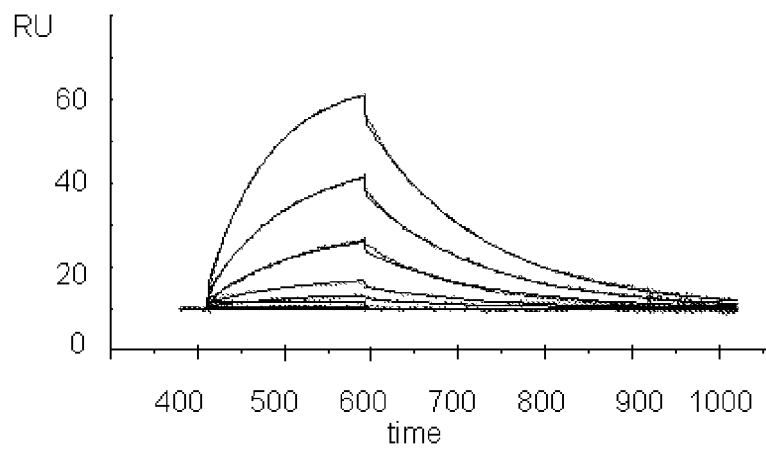


Fig. 11 c)

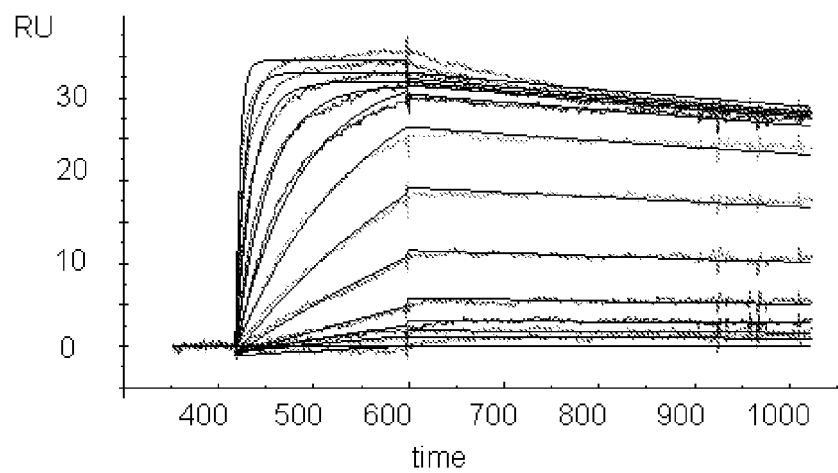


Fig. 12

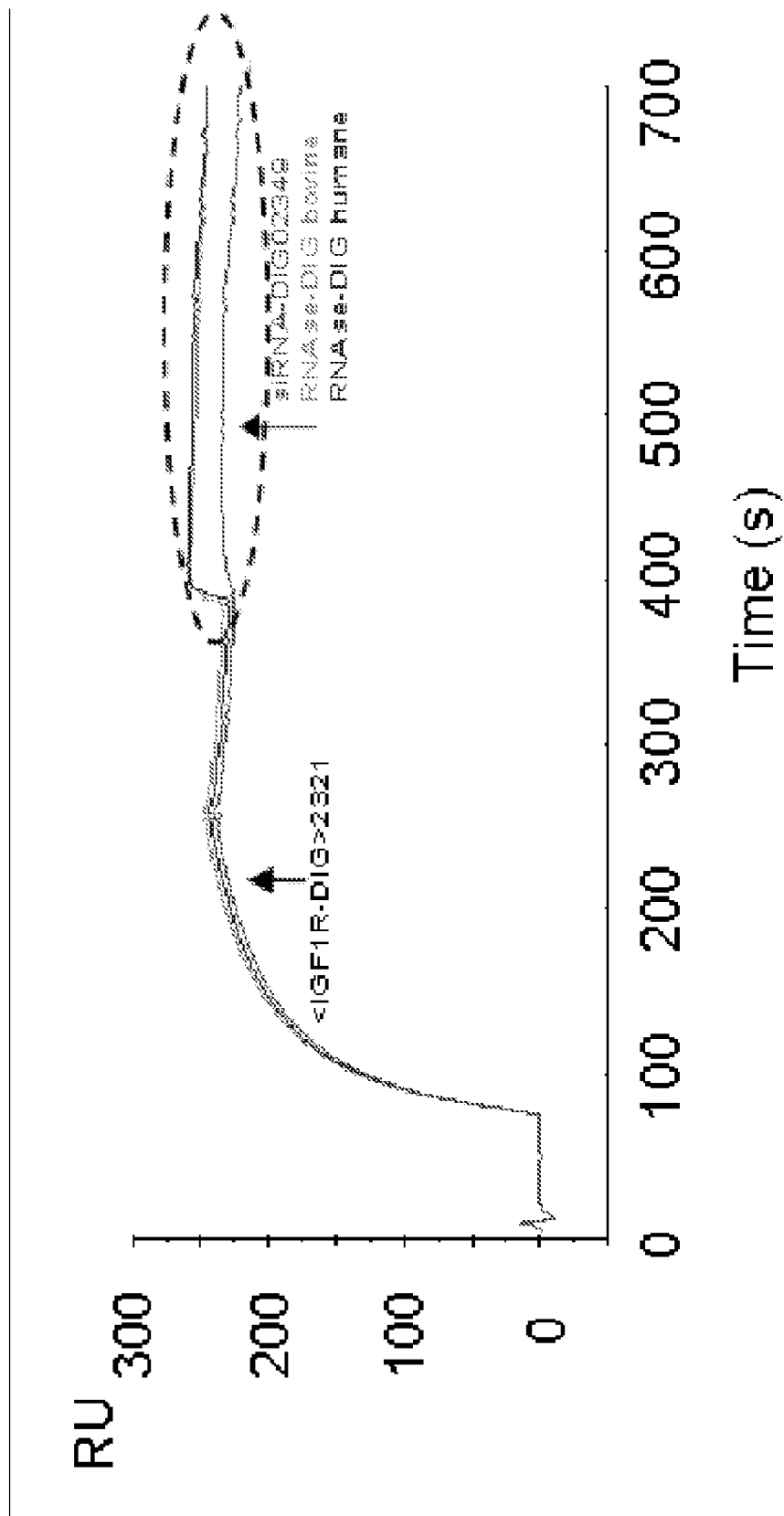


Fig. 13 a)

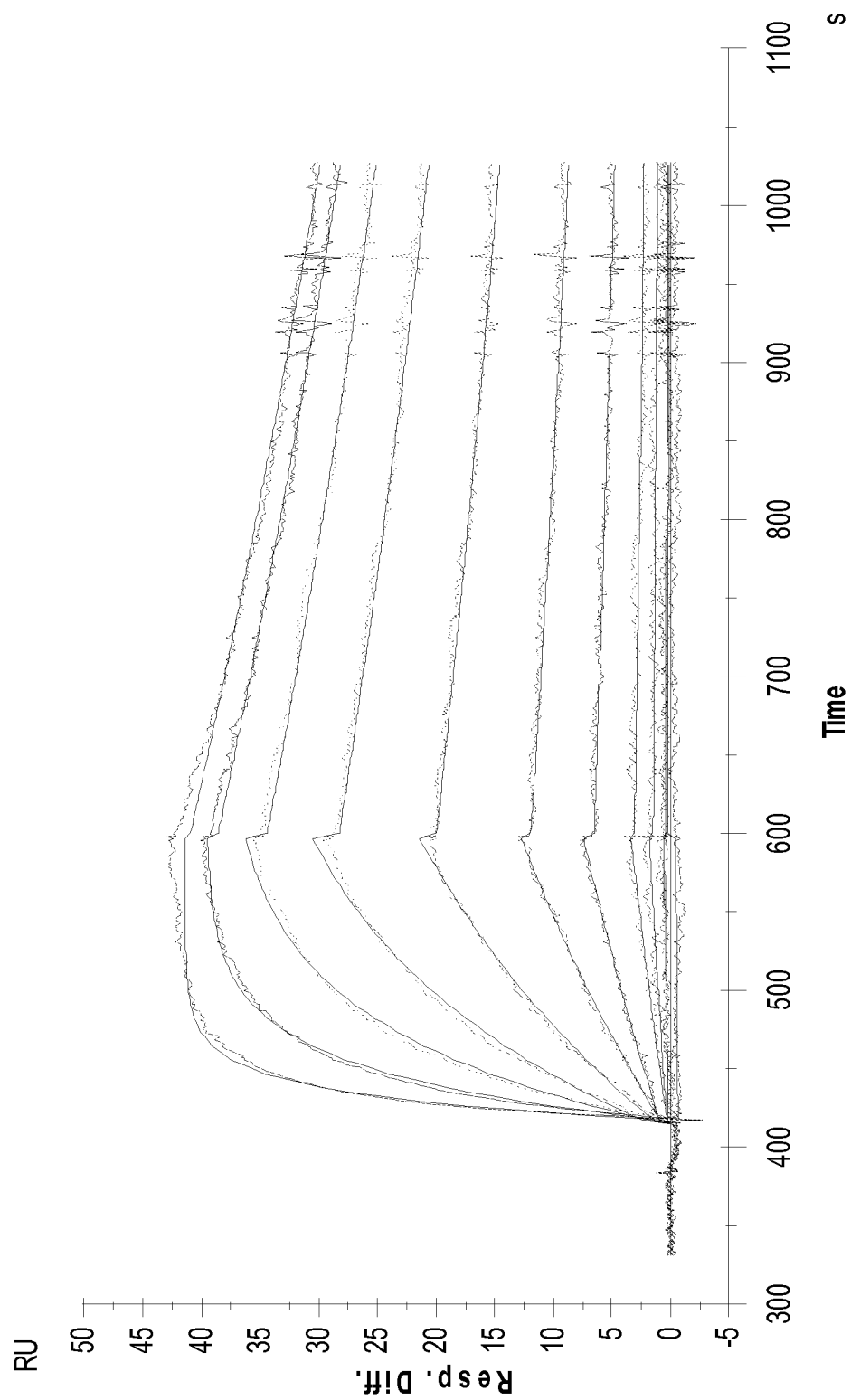


Fig. 13 b)

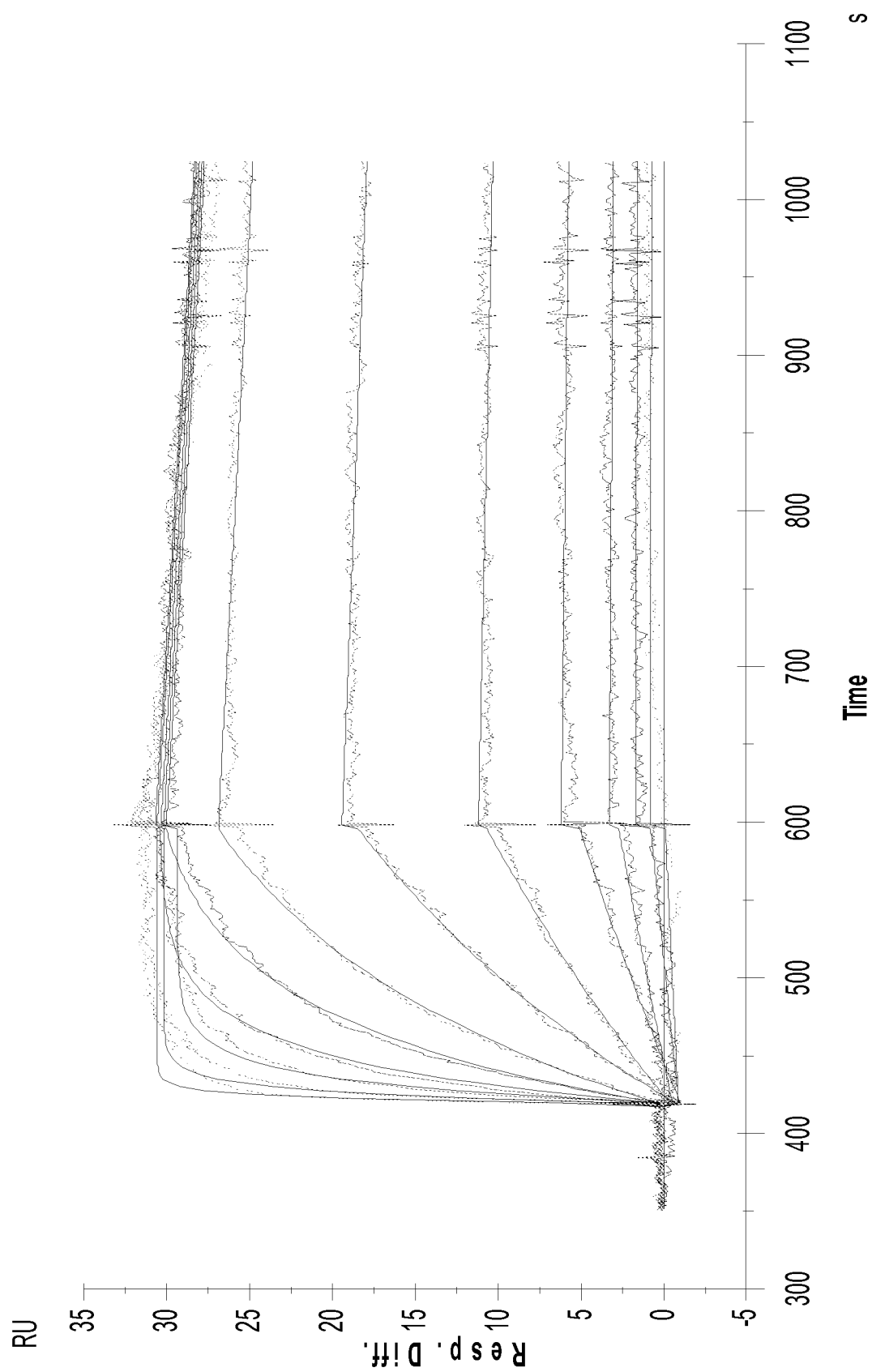


Fig. 14

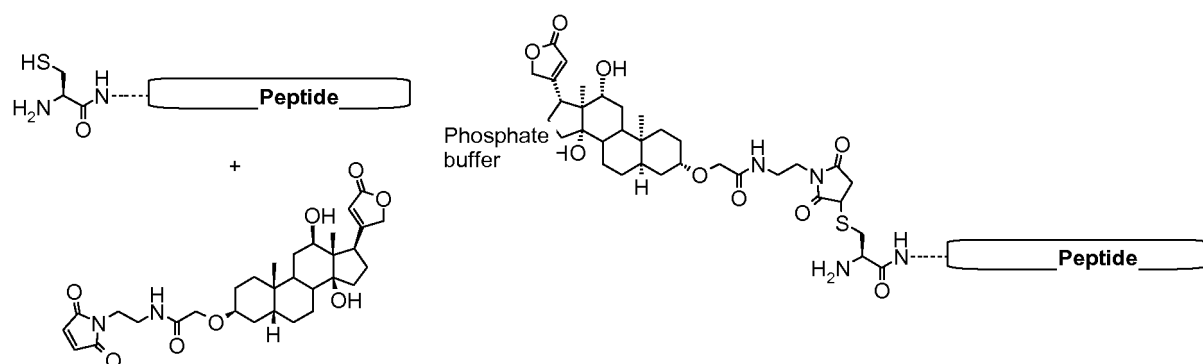


Fig. 15 a)

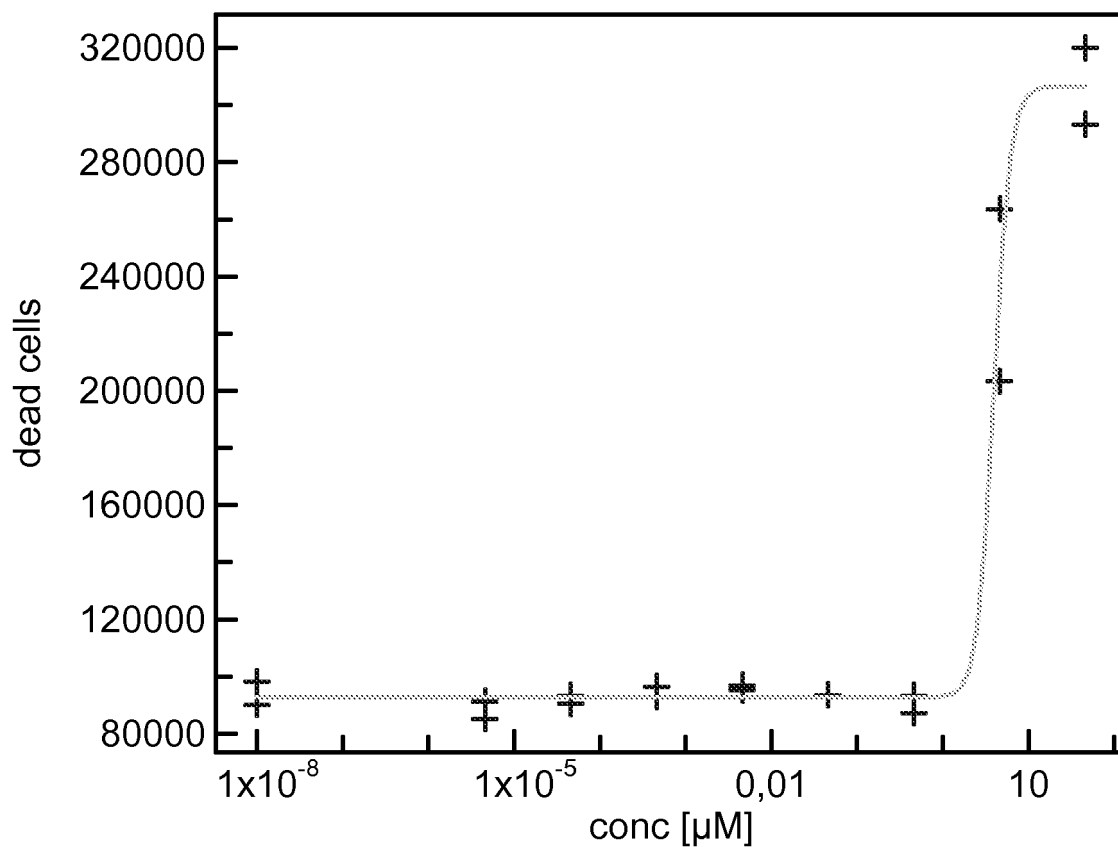


Fig. 15 b)

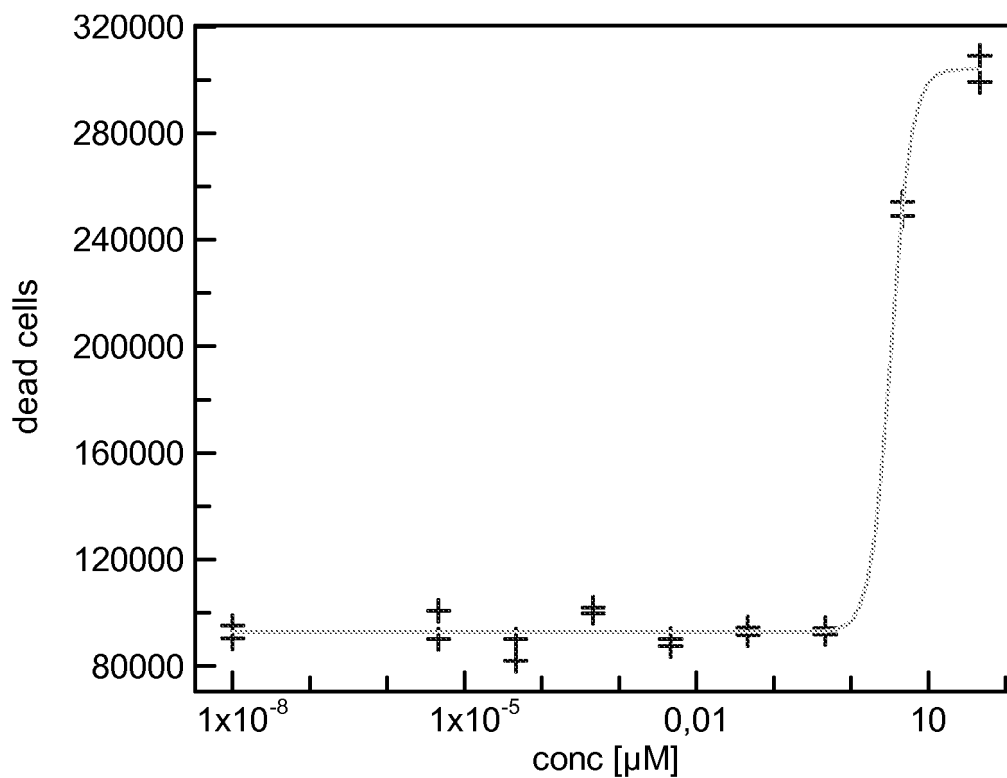


Fig. 15 c)

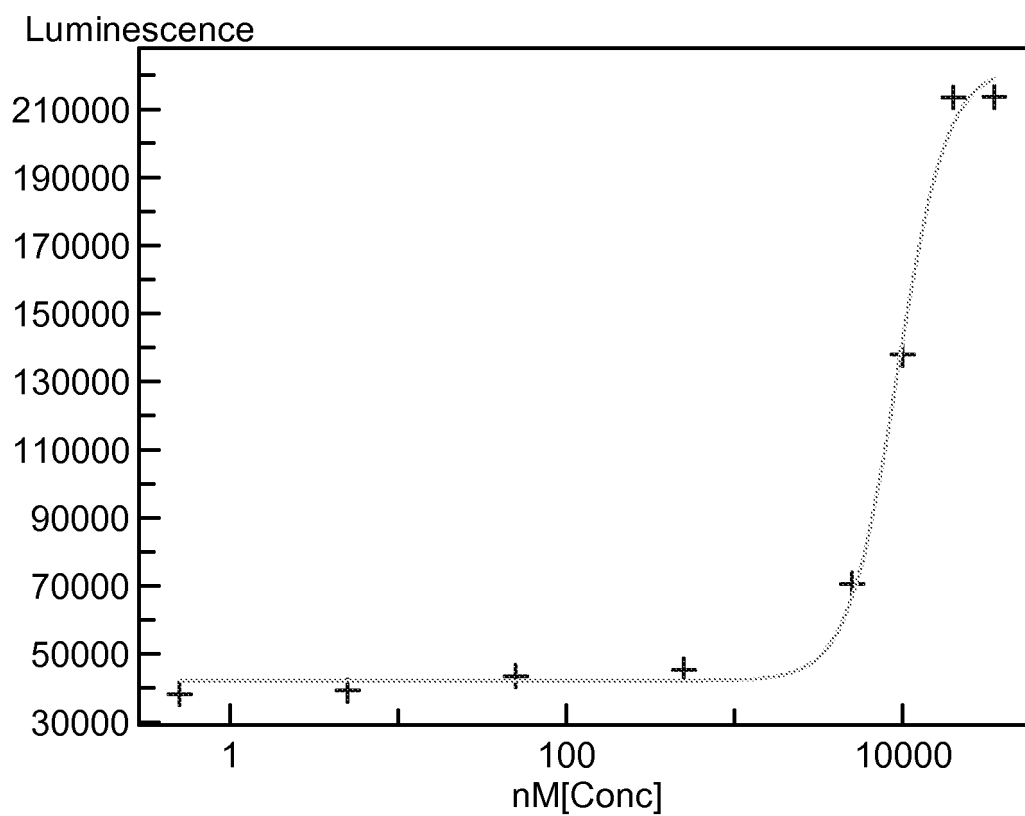


Fig. 15 d)

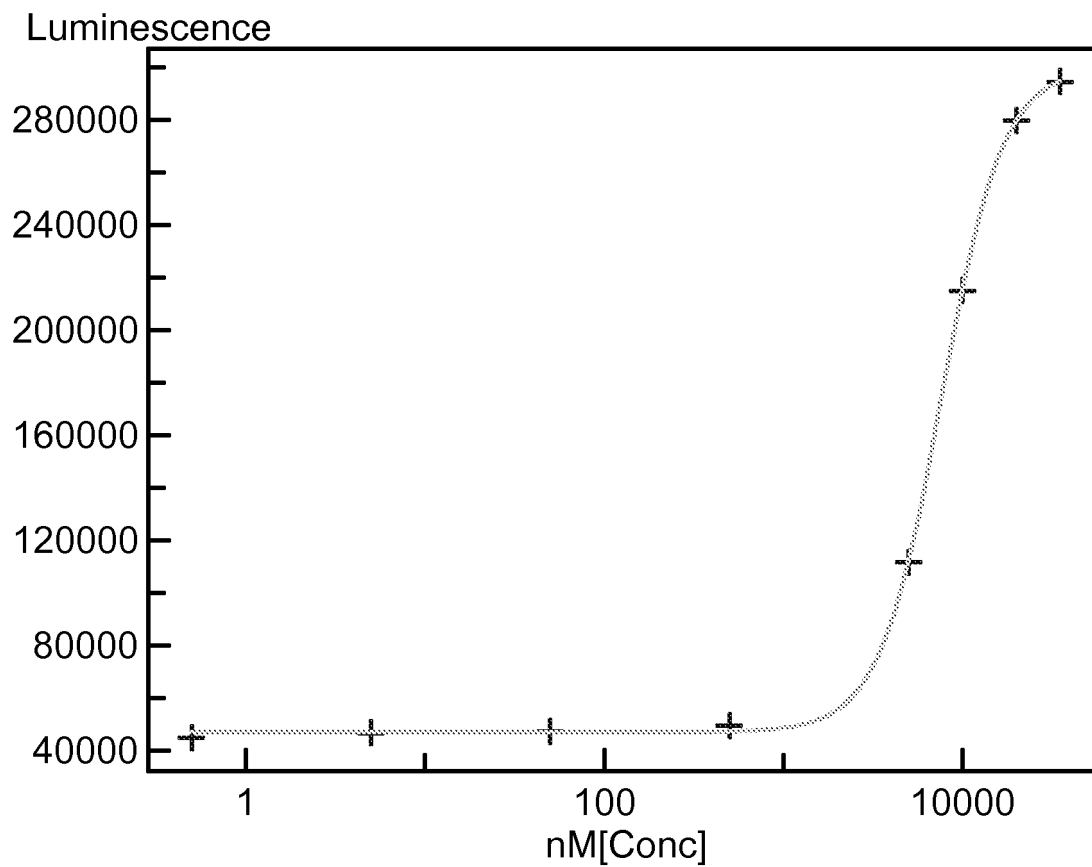


Fig. 15 e)

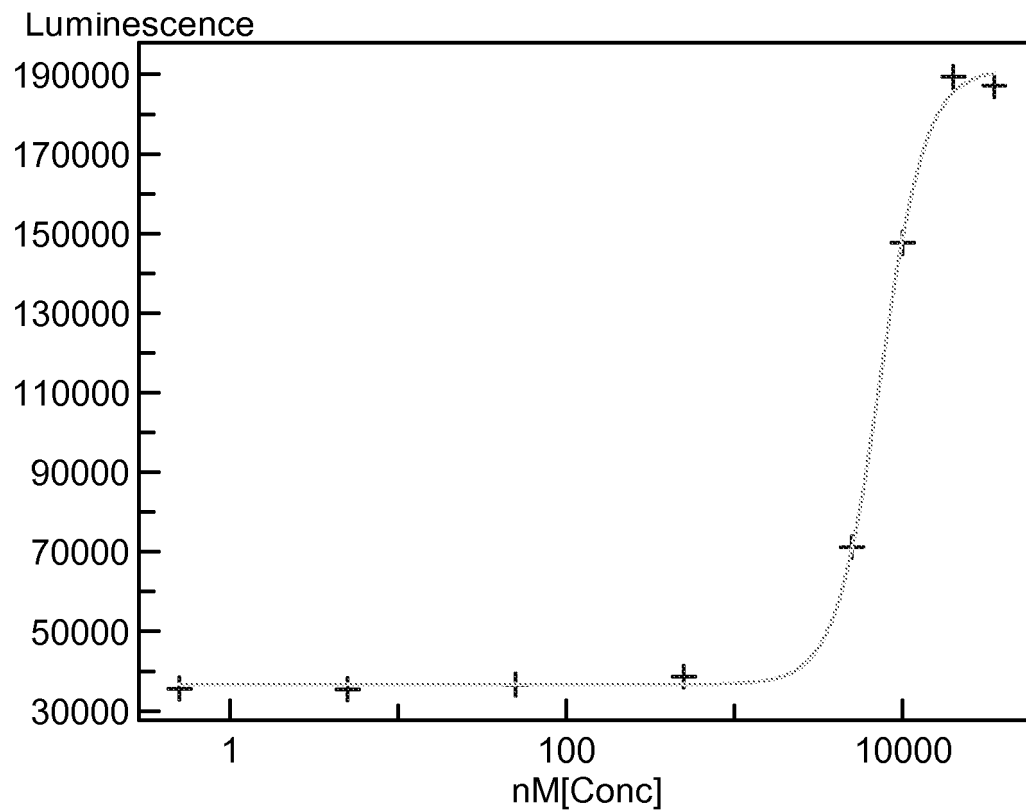


Fig. 15 f)

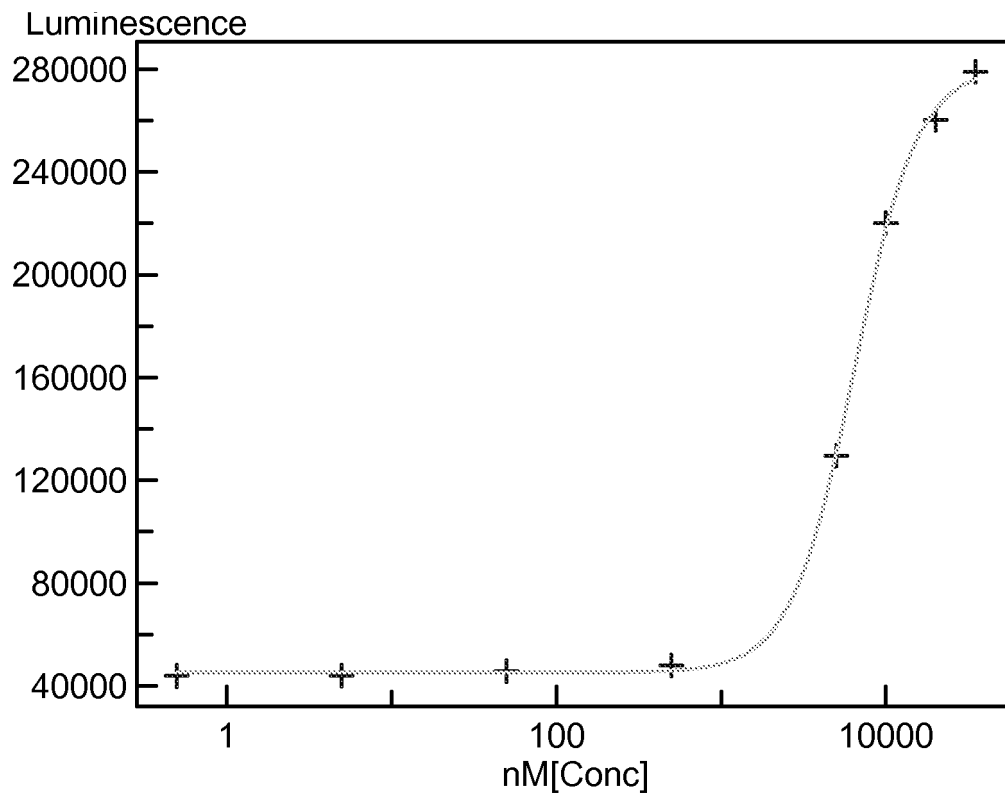
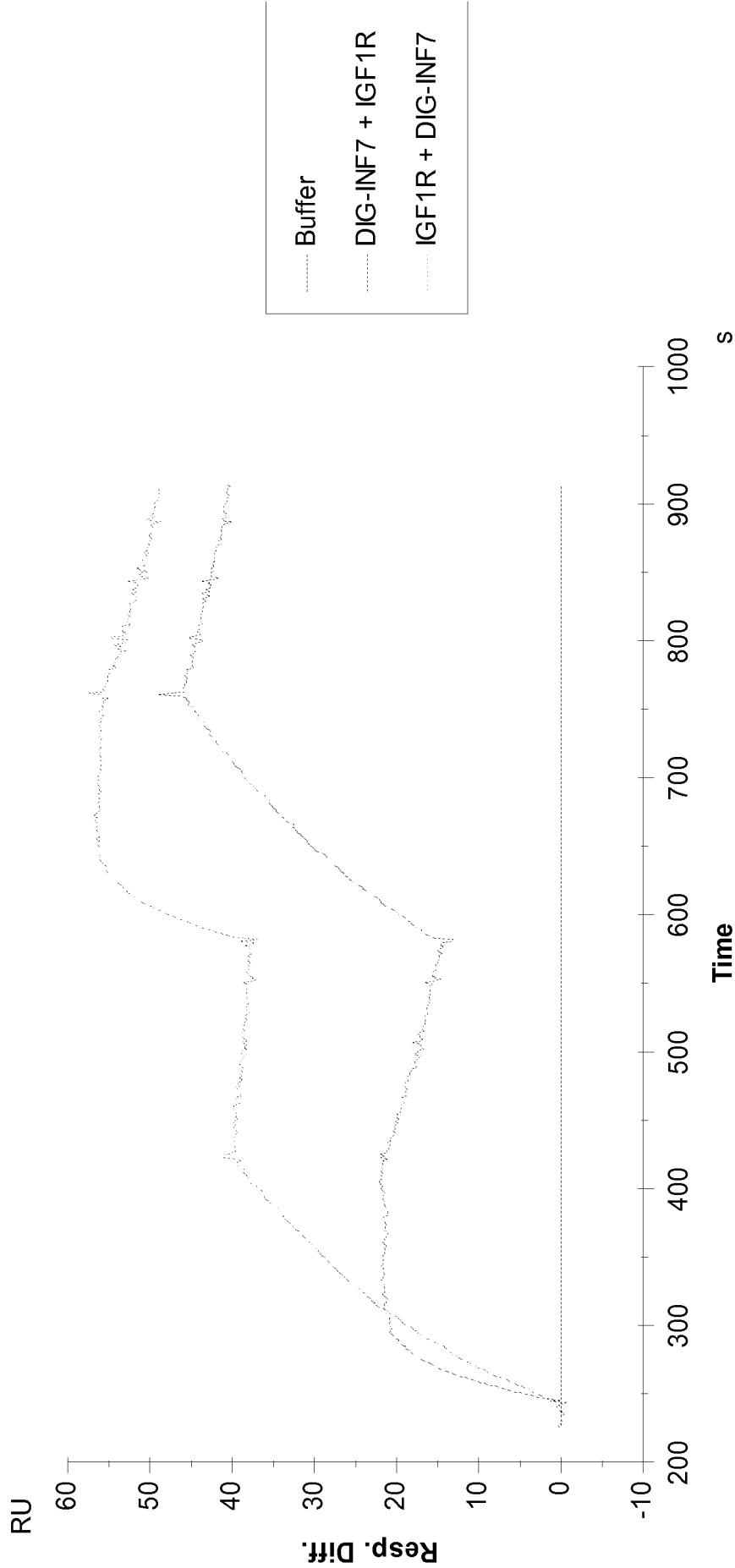


Fig. 16 a)



16 b)

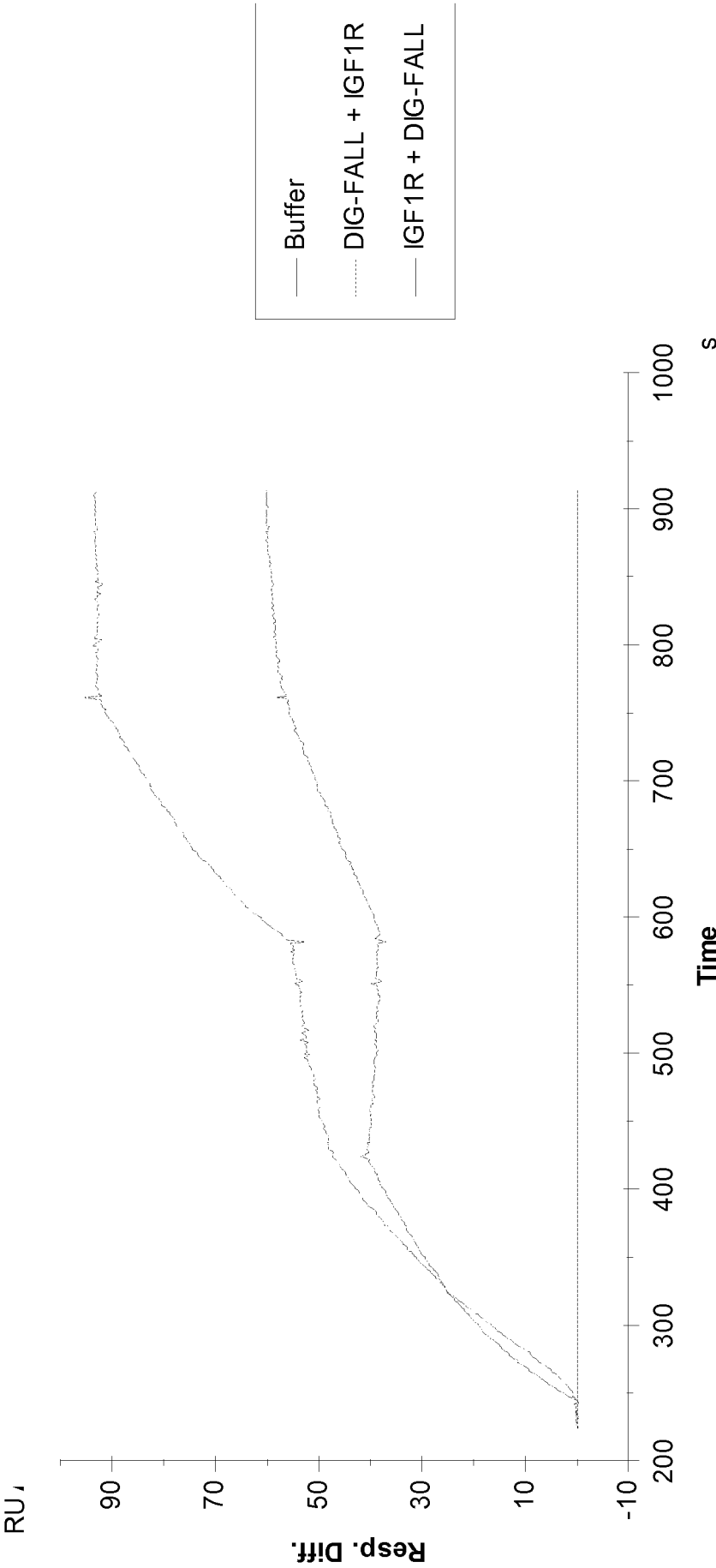


Fig. 17 a)

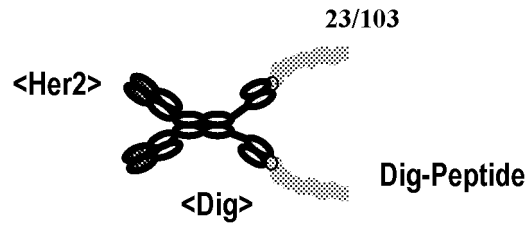


Fig. 17 b)

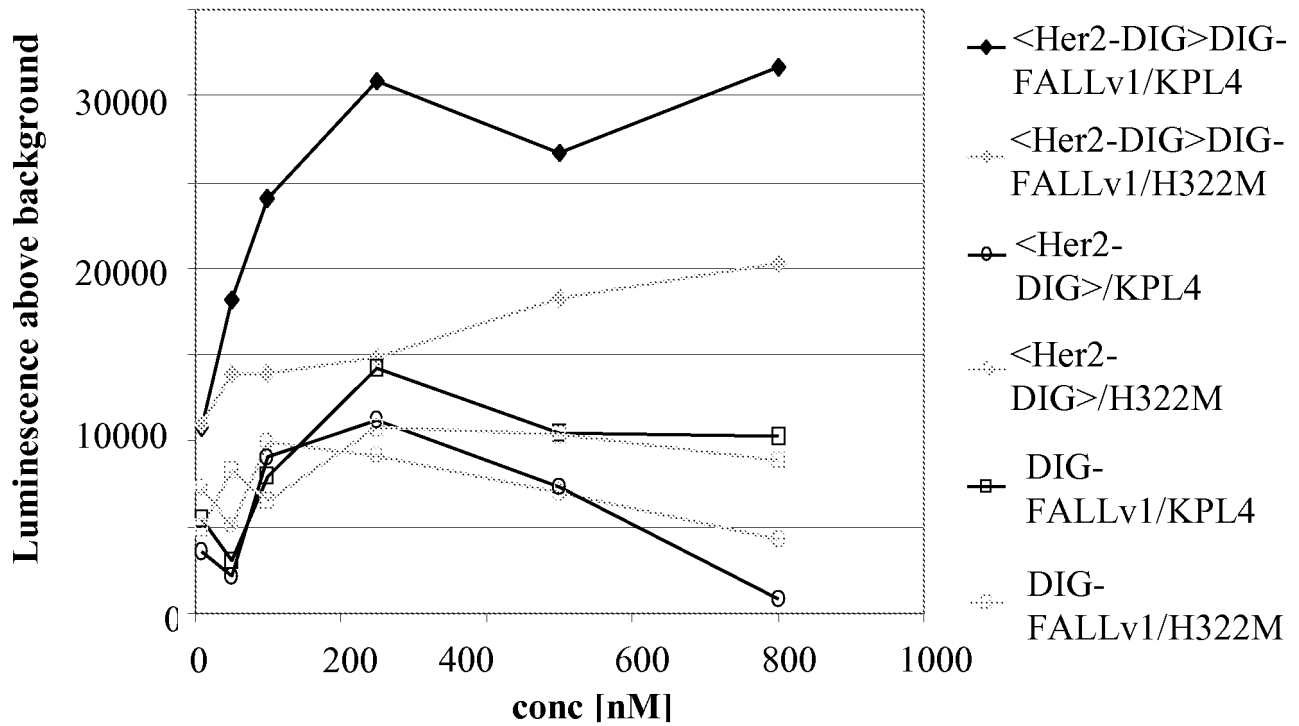


Fig. 17 c)

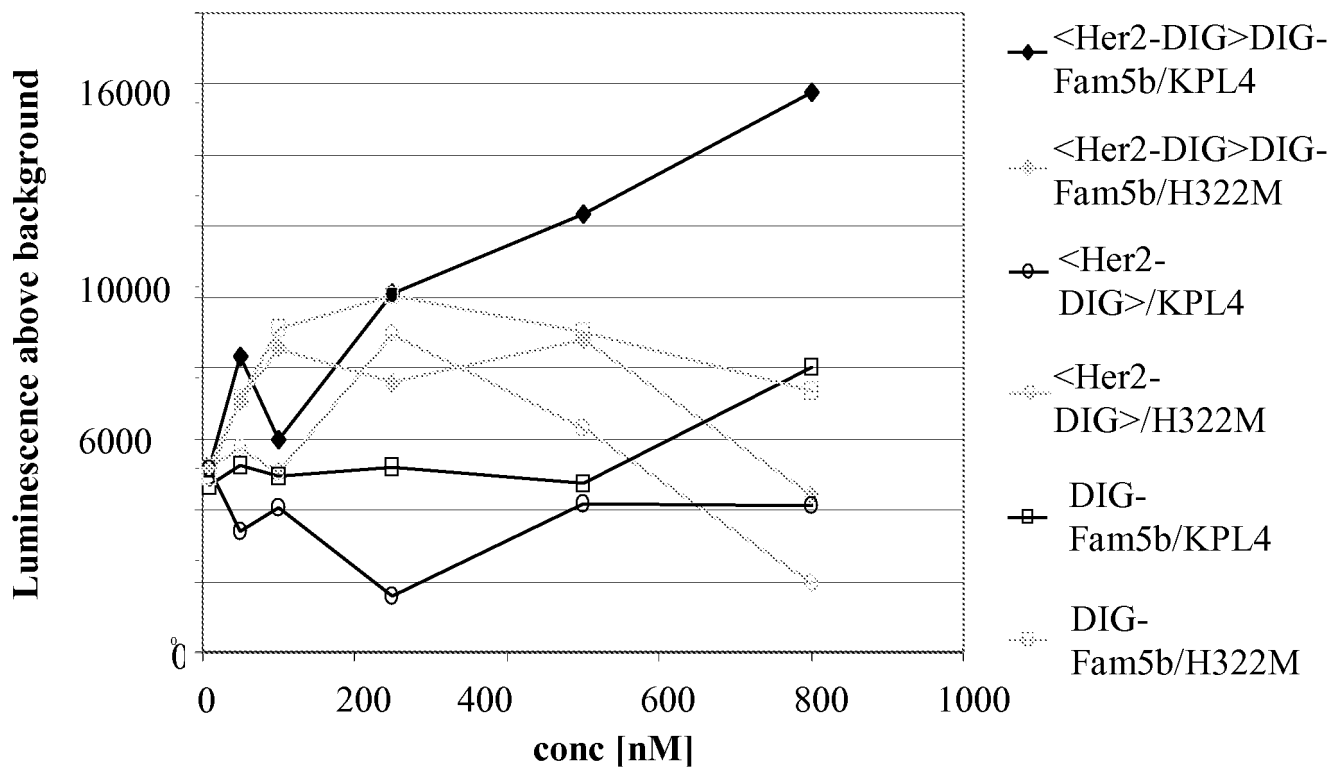


Fig. 18

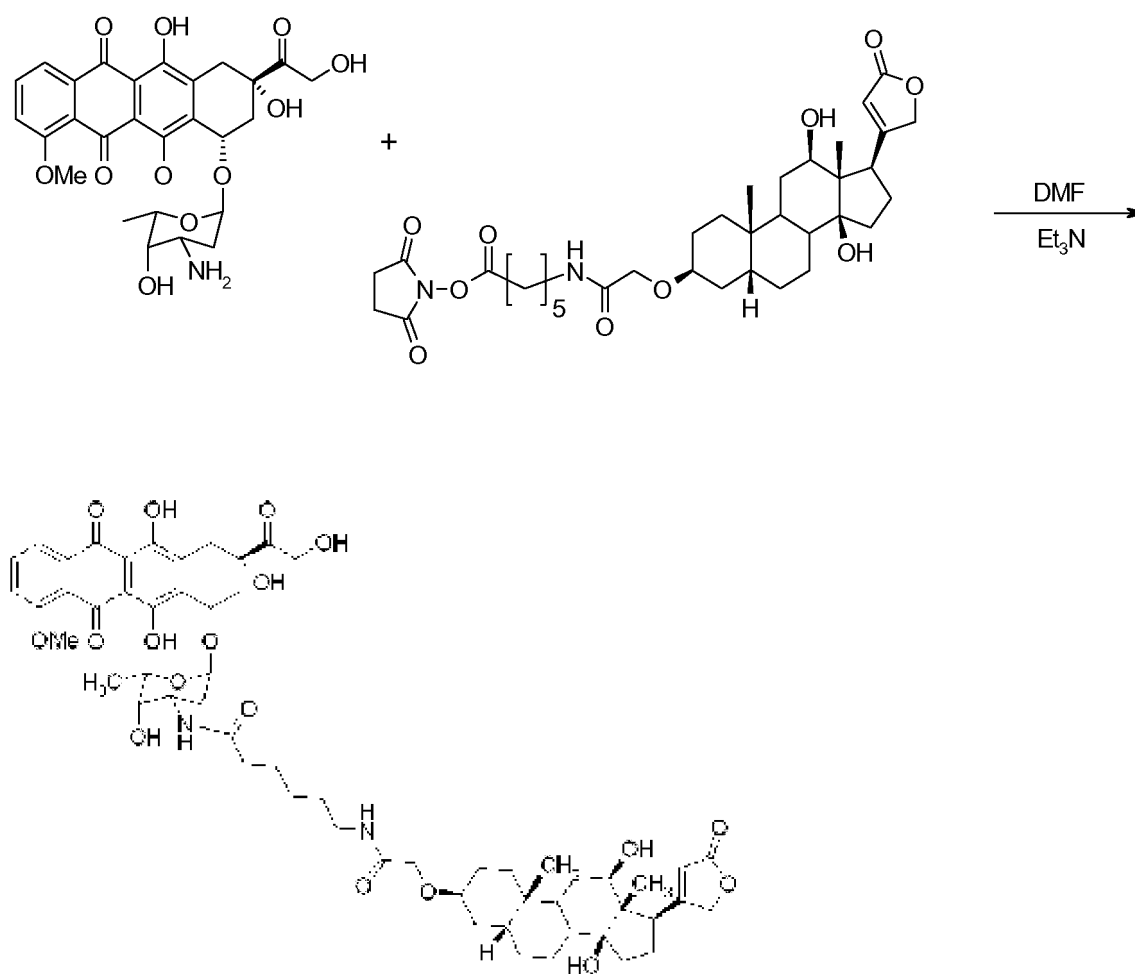


Fig. 19 a)

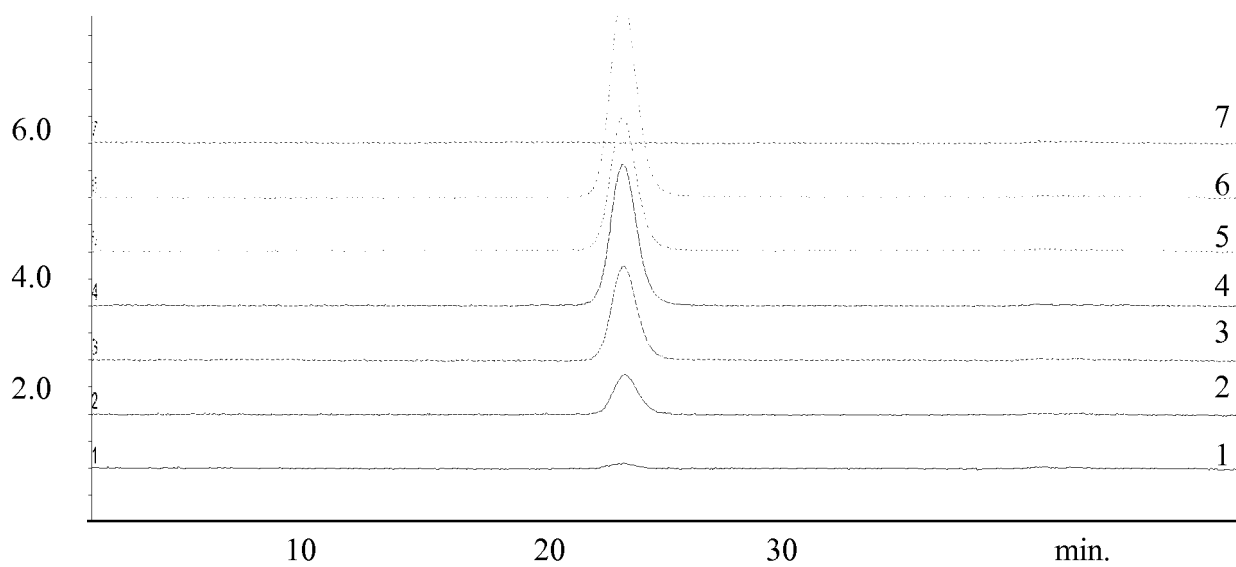


Fig. 19 b)

| ratio<br><Her2-<br>Dig>:Doxo | Area<br>(Ex: 488 nm / Em: 540<br>nm)<br>[mAU*min] |
|------------------------------|---------------------------------------------------|
| 1:0                          | 0                                                 |
| 1:0,5                        | 1,1                                               |
| 1:1                          | 2,4                                               |
| 1:2                          | 3,4                                               |
| 1:3                          | 3,3                                               |
| 1:5                          | 4,9                                               |
| 0:1                          | 0                                                 |

Fig. 20 a)

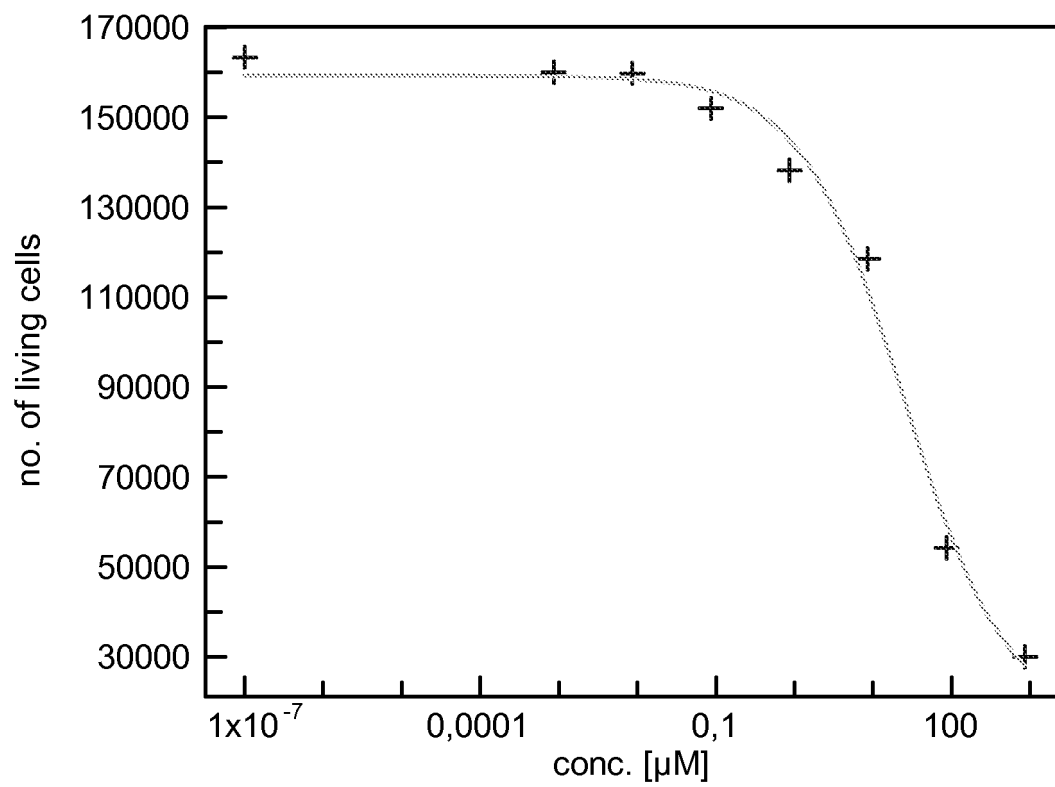


Fig. 20 b)

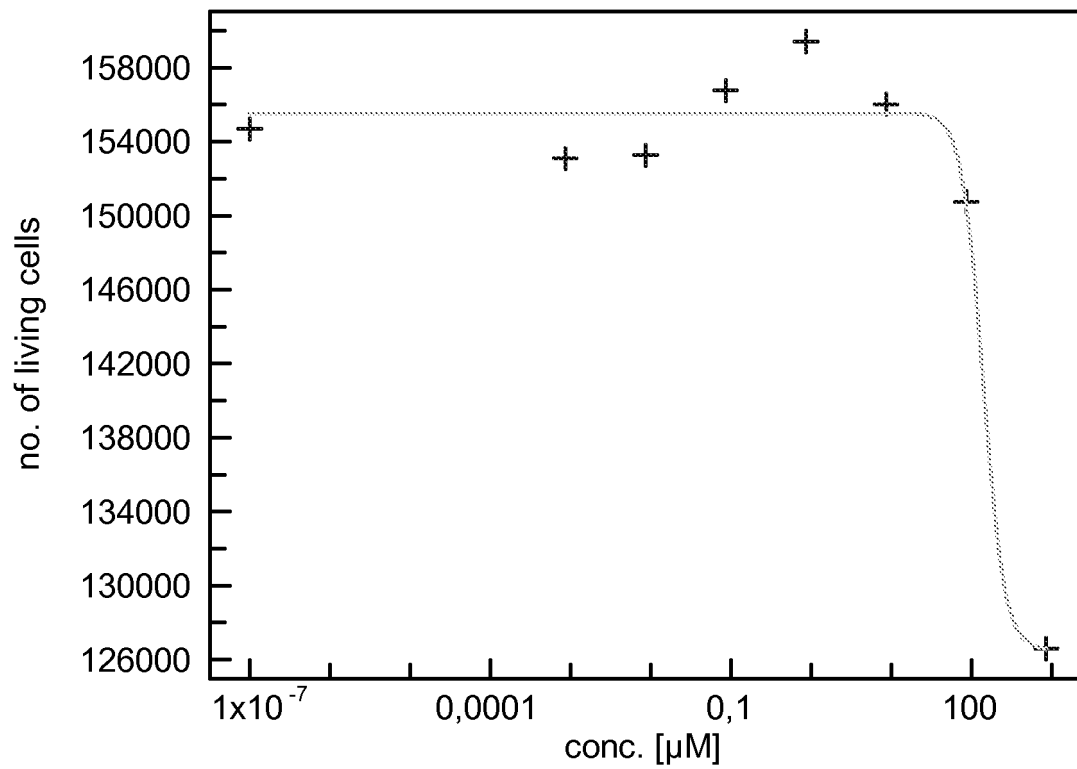


Fig. 20 c)

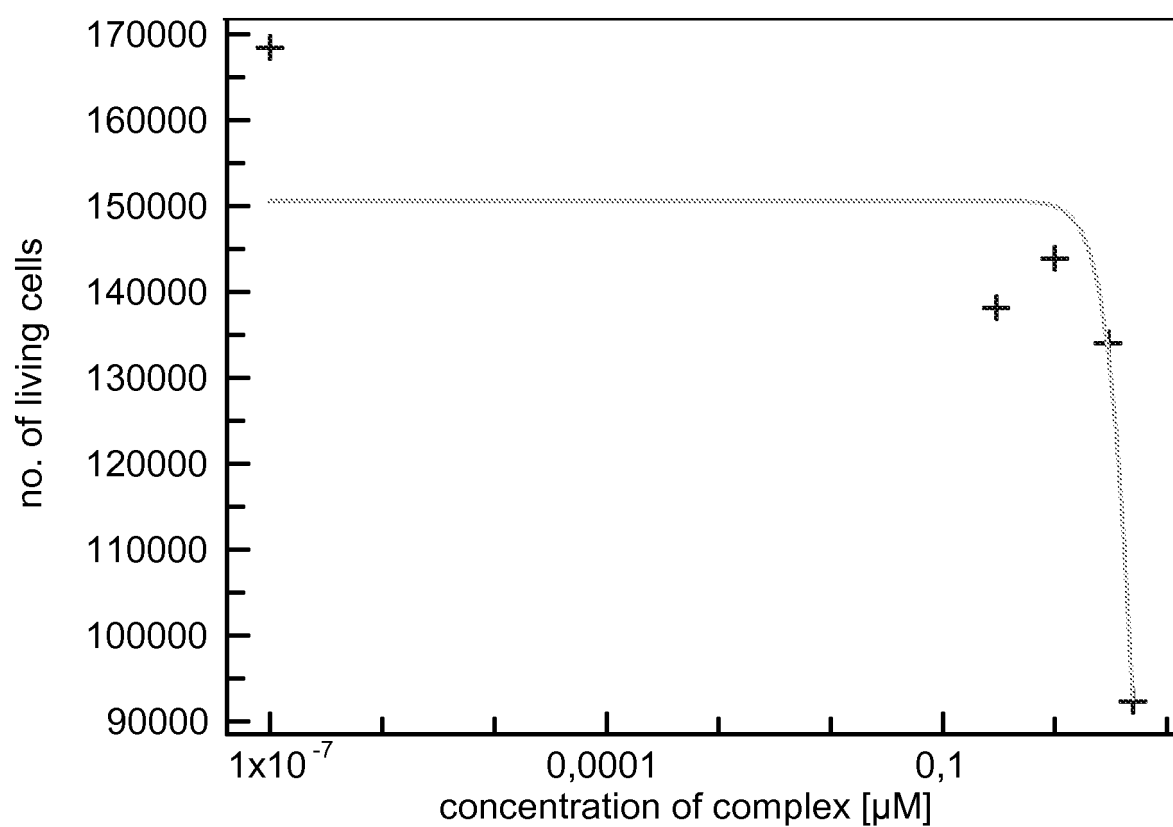


Fig. 21 a)

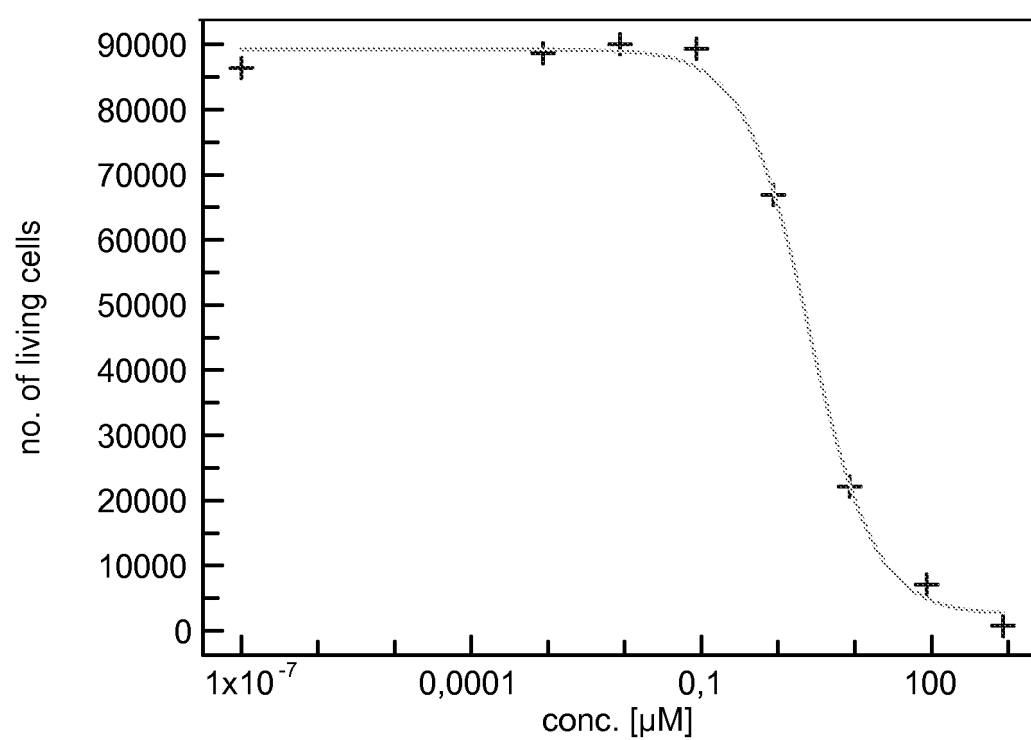


Fig. 21 b)

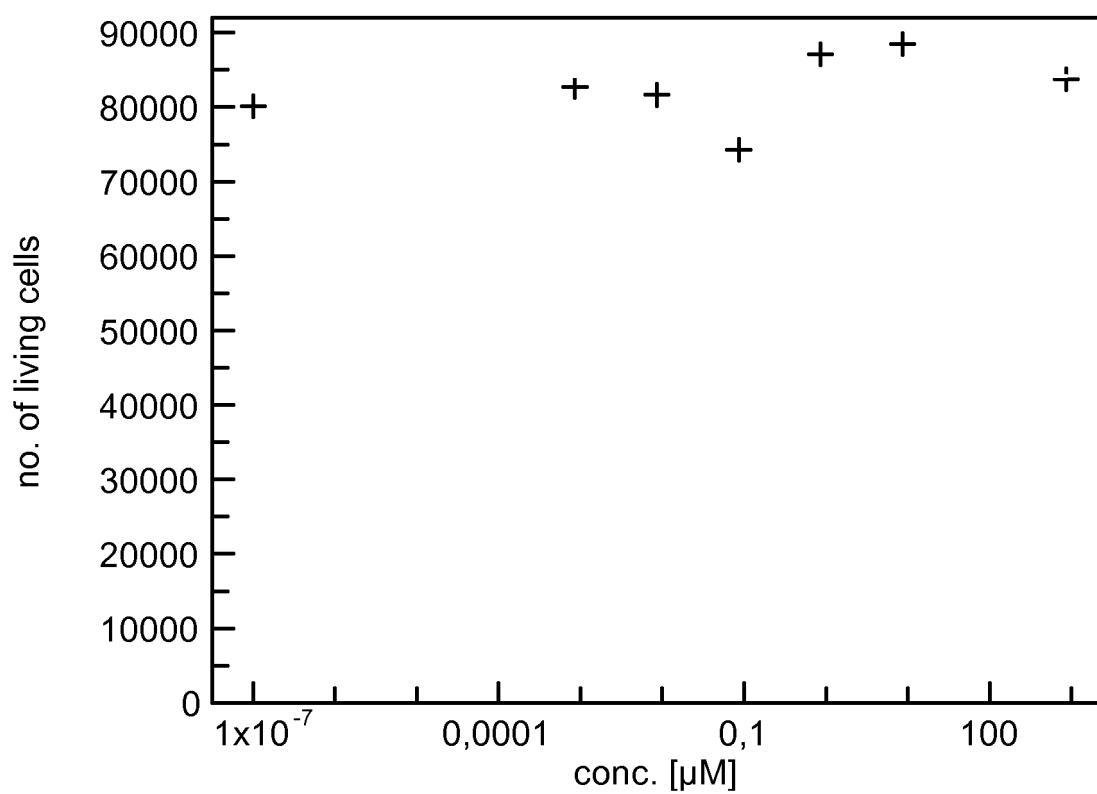


Fig. 21 c)

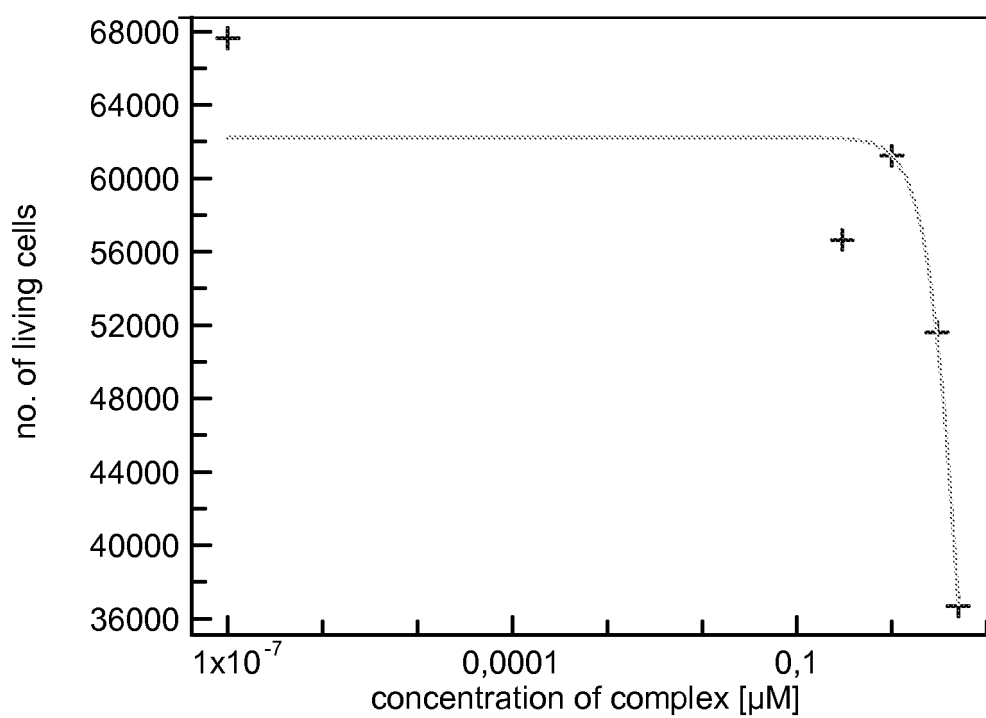


Fig. 22 a)

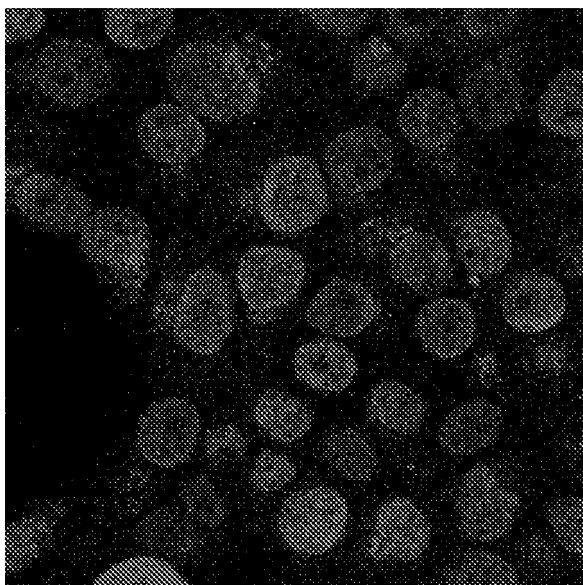


Fig. 22b)

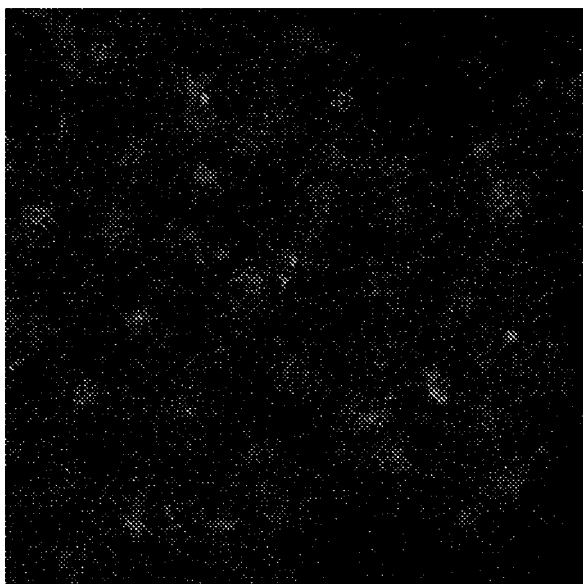


Fig. 22 c)

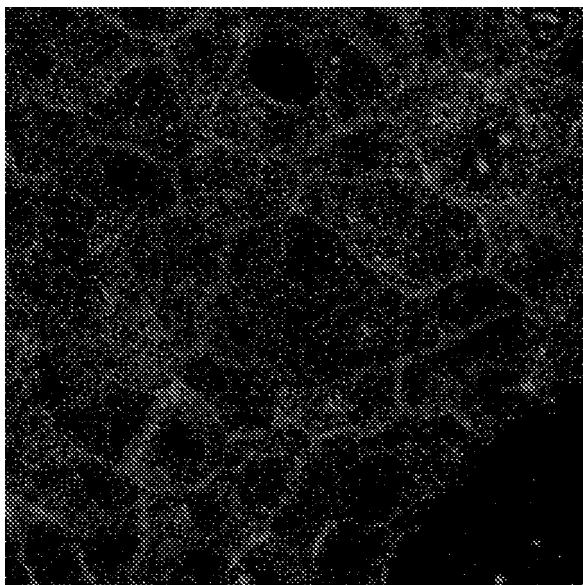


Fig. 23

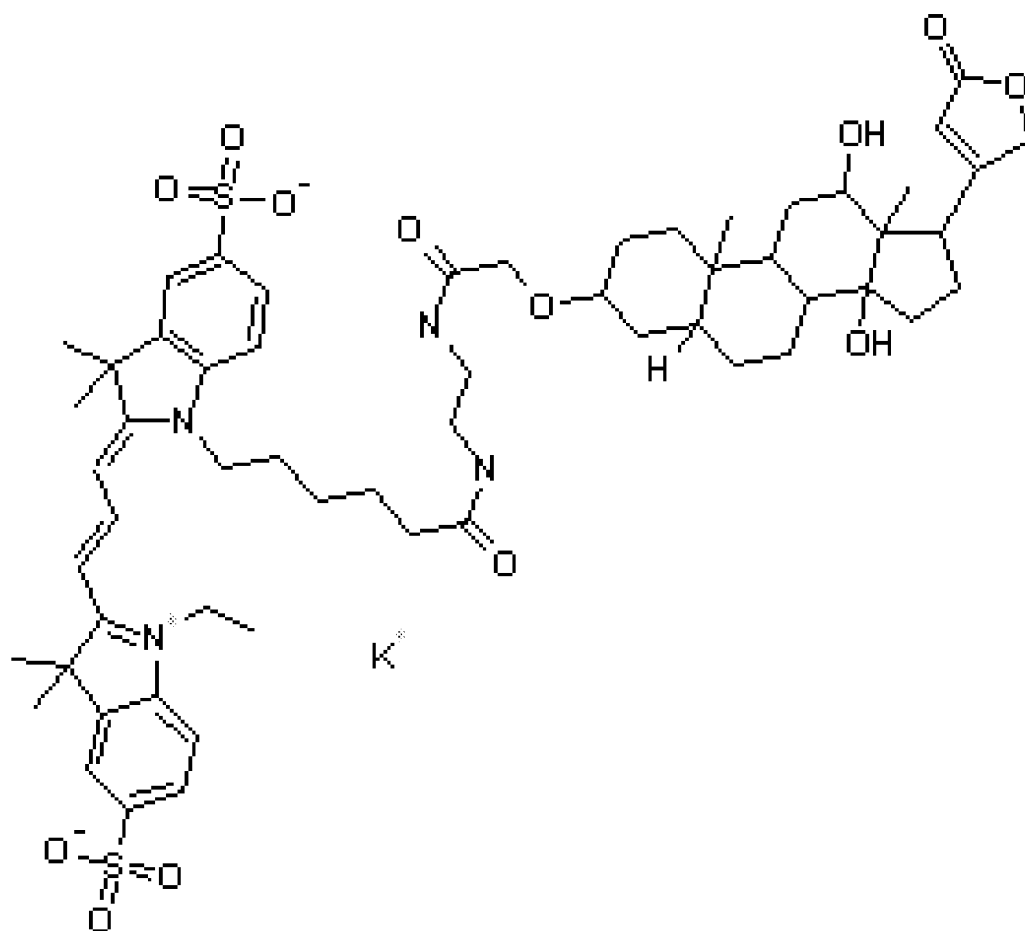


Fig. 24 a)

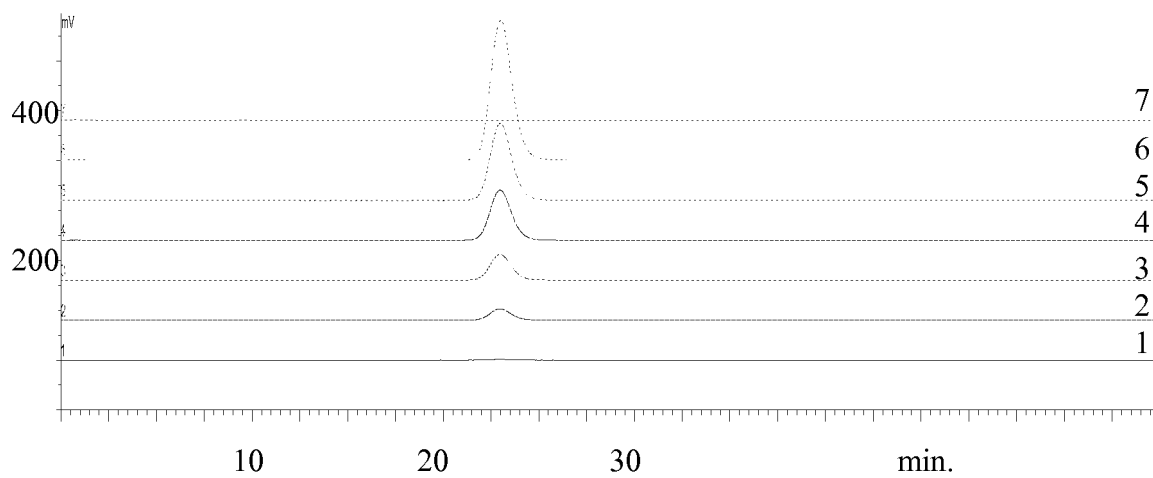


Fig. 24 b)

| ratio<br><Her2-<br>Dig>:Dig-<br>Cy5 | Area<br>(Ex: 650 nm<br>Em: 667<br>nm)<br>[mAU*min] |
|-------------------------------------|----------------------------------------------------|
| 1:0                                 | 2                                                  |
| 1:0,5                               | 30                                                 |
| 1:1                                 | 68                                                 |
| 1:2                                 | 135                                                |
| 1:3                                 | 209                                                |
| 1:5                                 | 381                                                |
| 0:1                                 | 0                                                  |

Fig. 25 a)

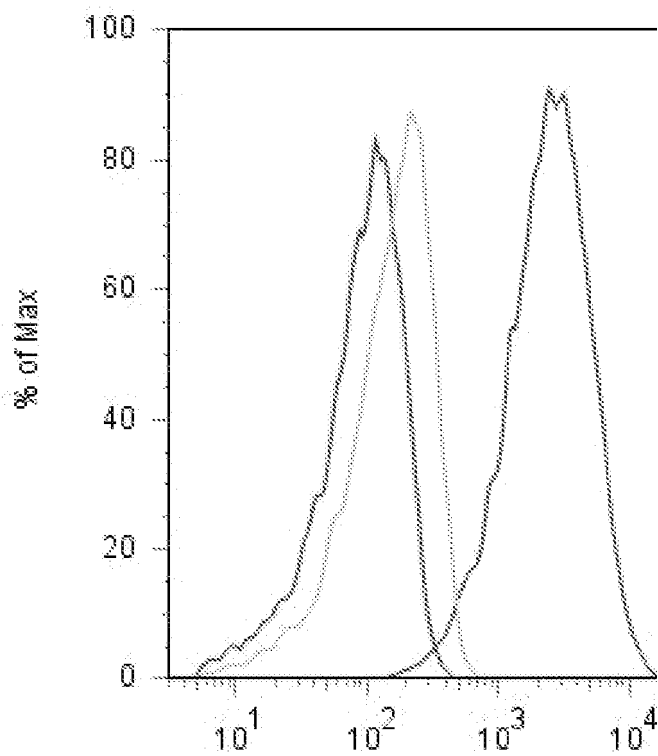


Fig. 25 b)

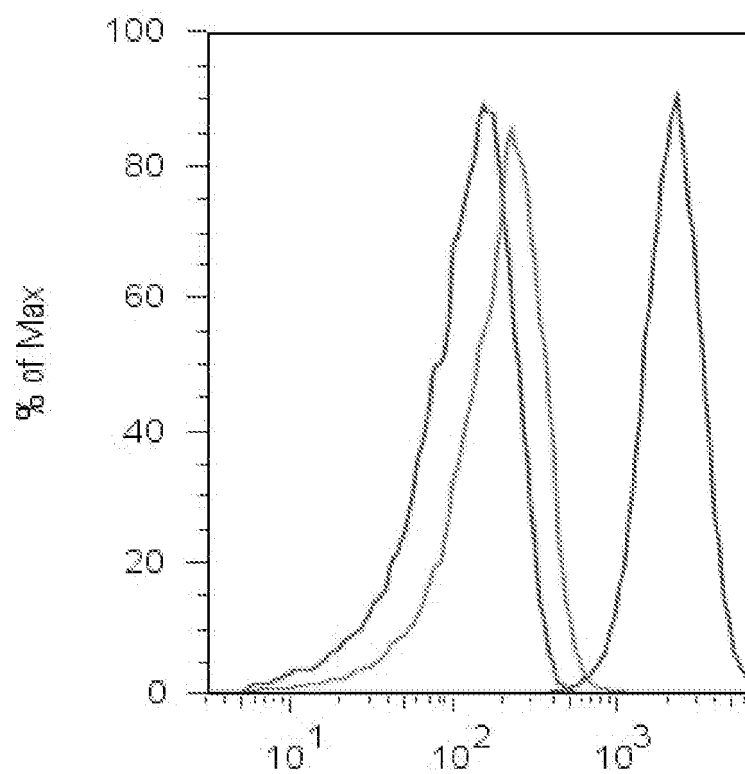


Fig. 26 a)

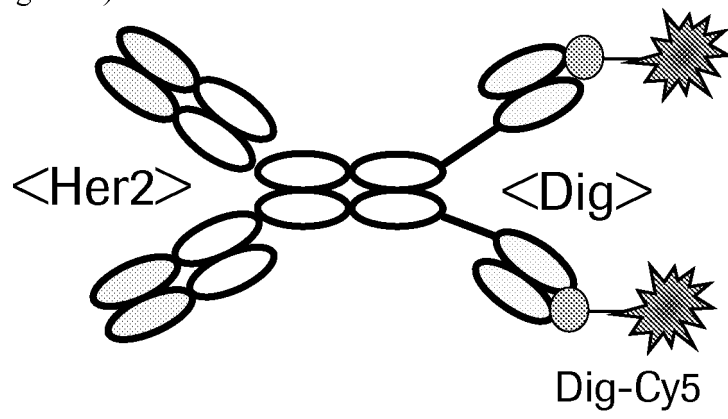


Fig. 26 b)

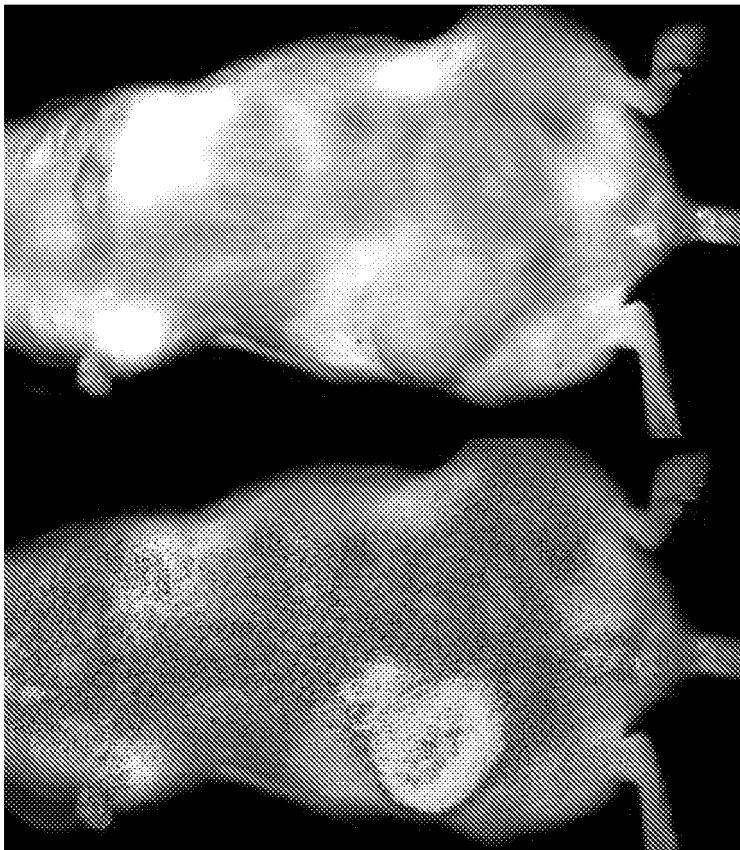


Fig. 27 a)

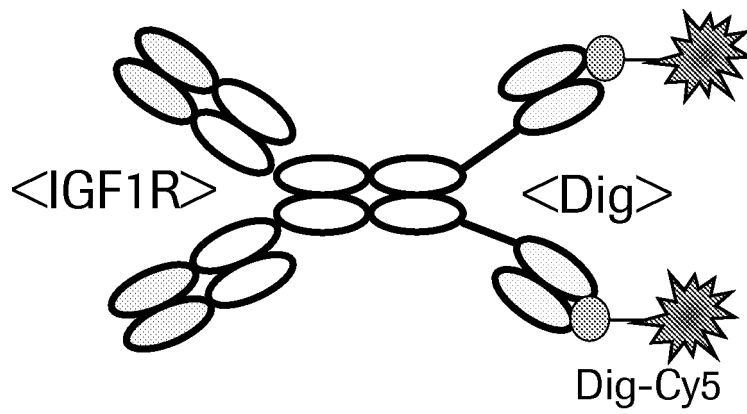


Fig 27 b)



Fig 27 c)



Fig. 28 a)

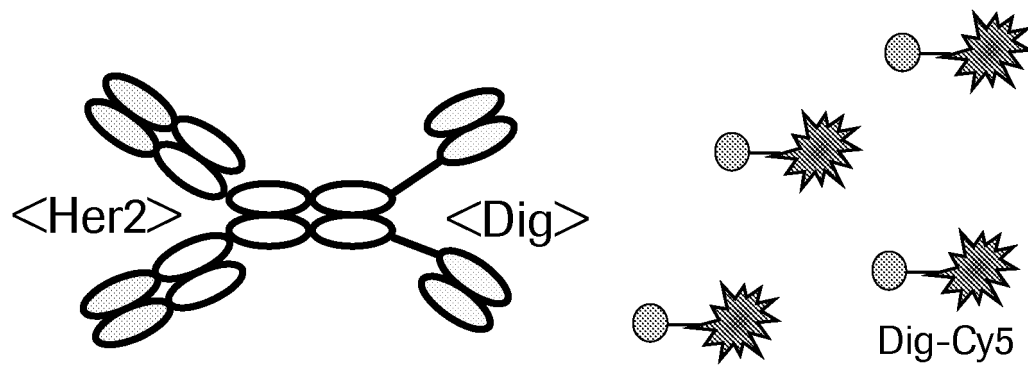


Fig. 28 b)

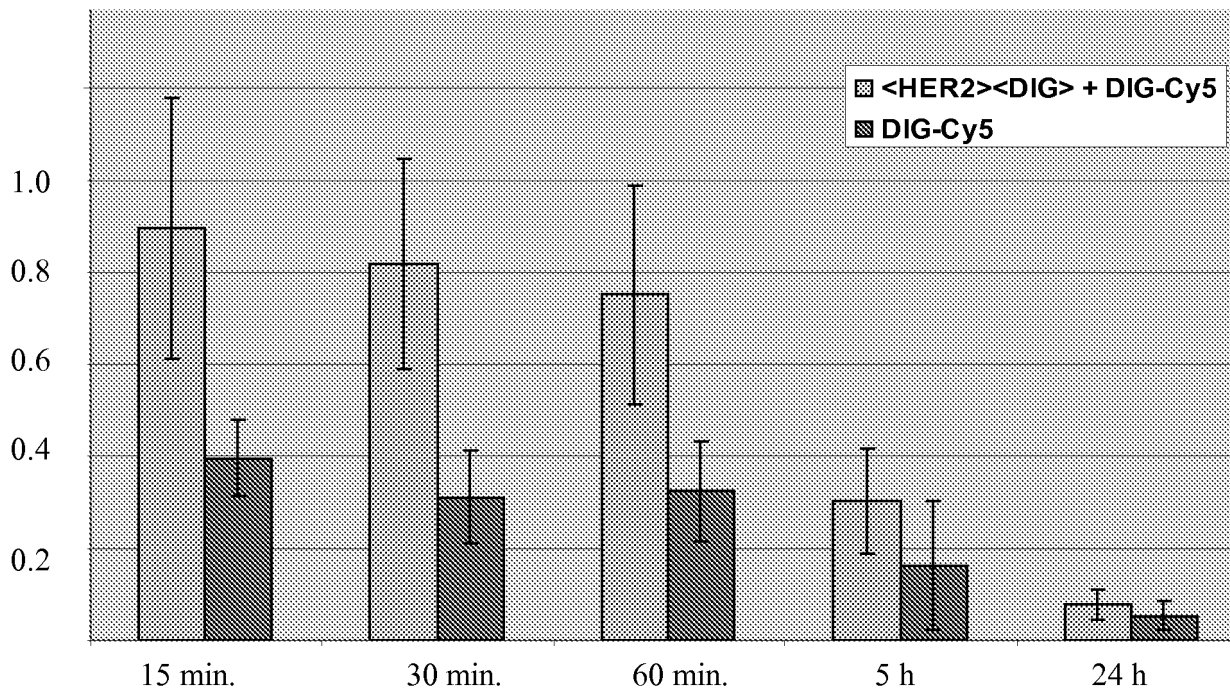


Fig. 29

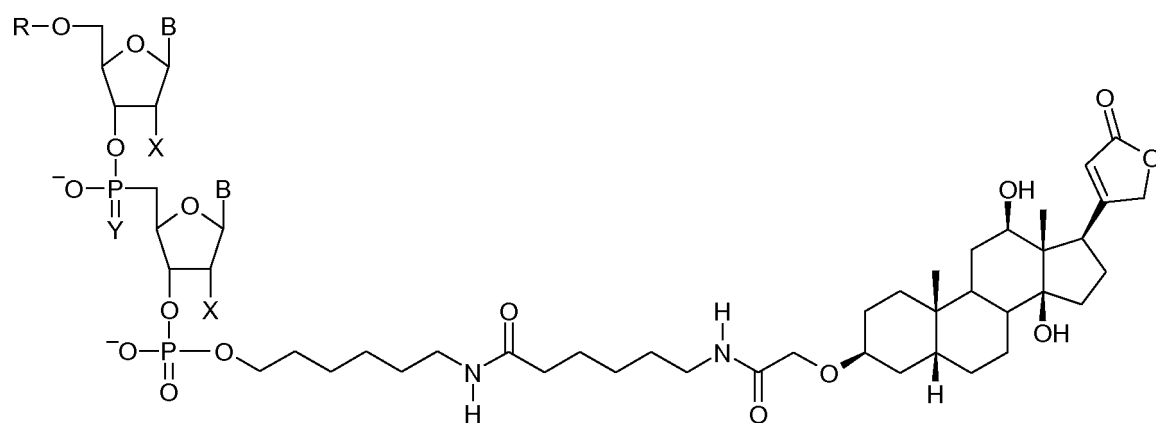


Fig. 30 a)

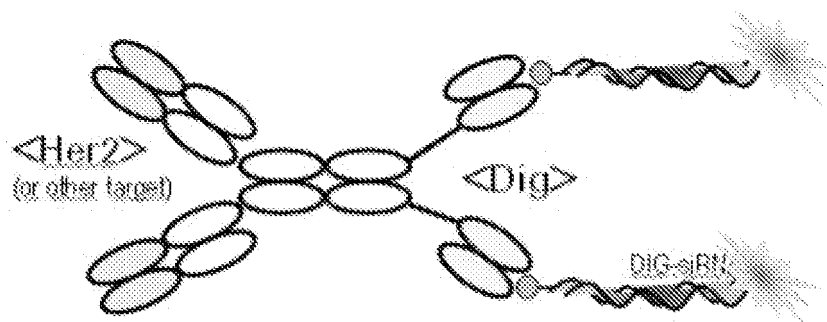


Fig. 30 b)

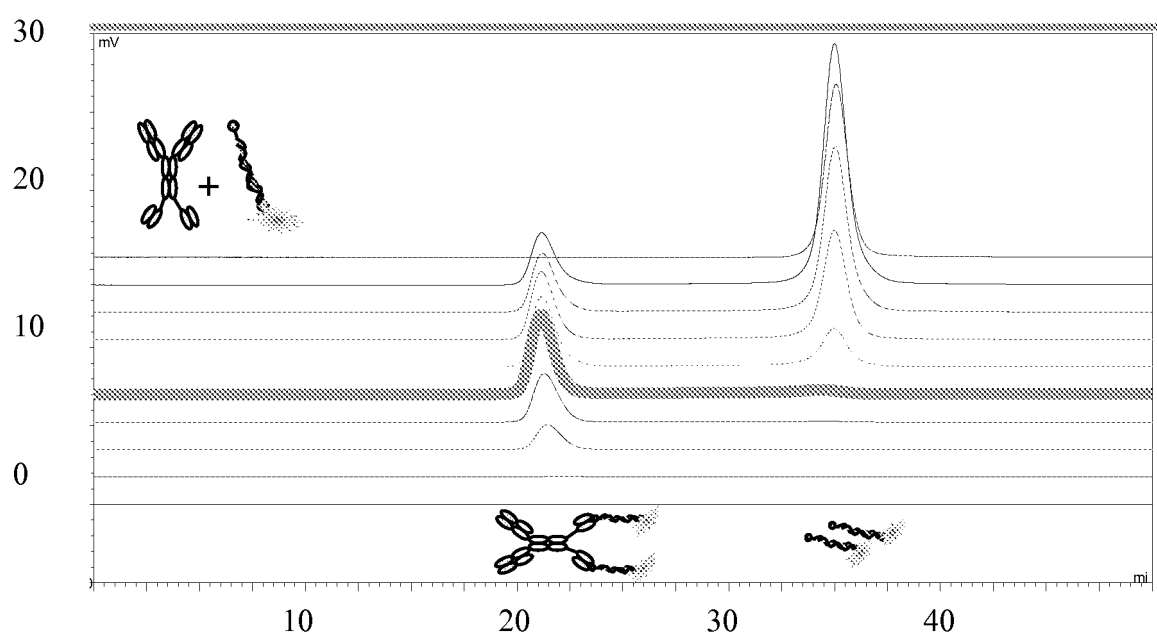


Fig. 31 a)

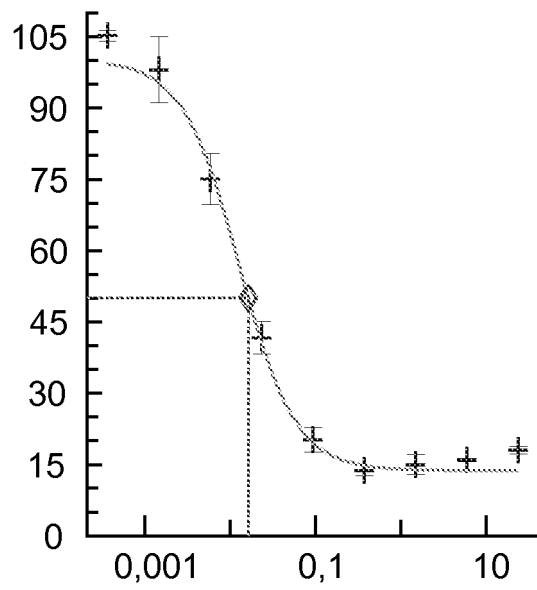


Fig. 31 b)

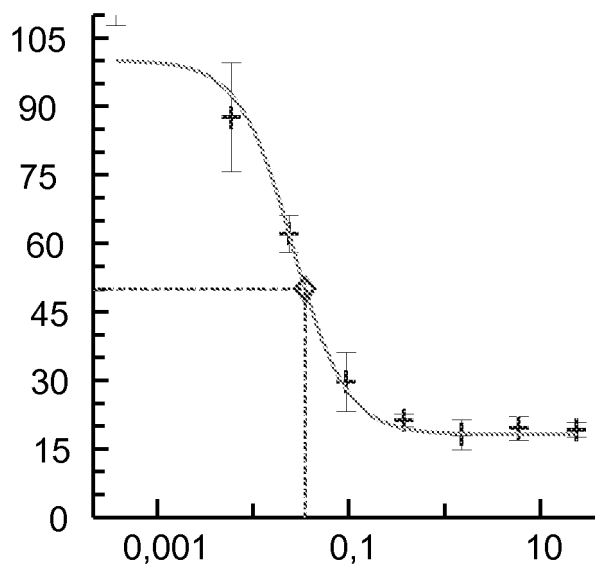


Fig. 31 c)

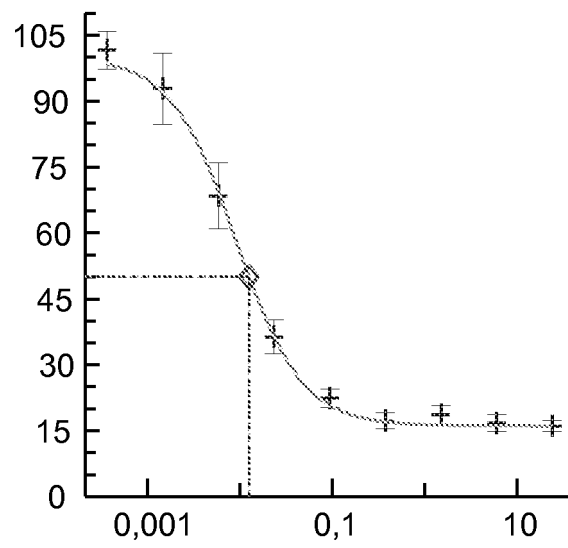


Fig. 31 d)

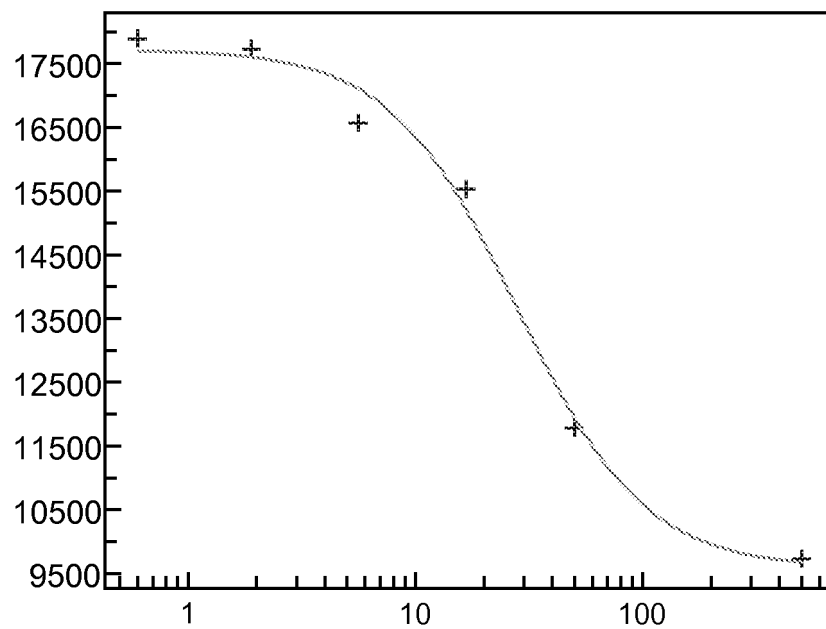


Fig. 31 e)

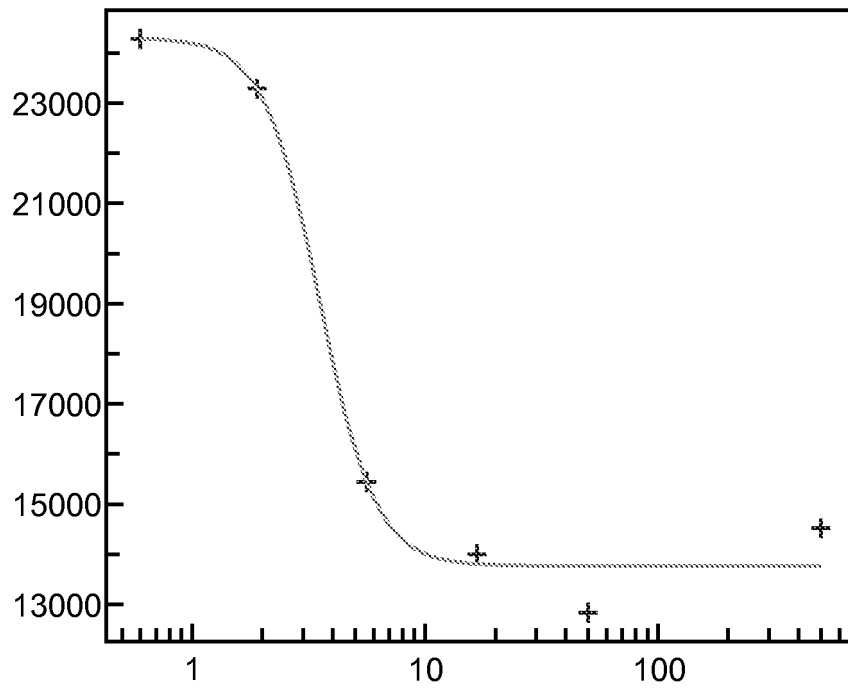


Fig. 31 f)

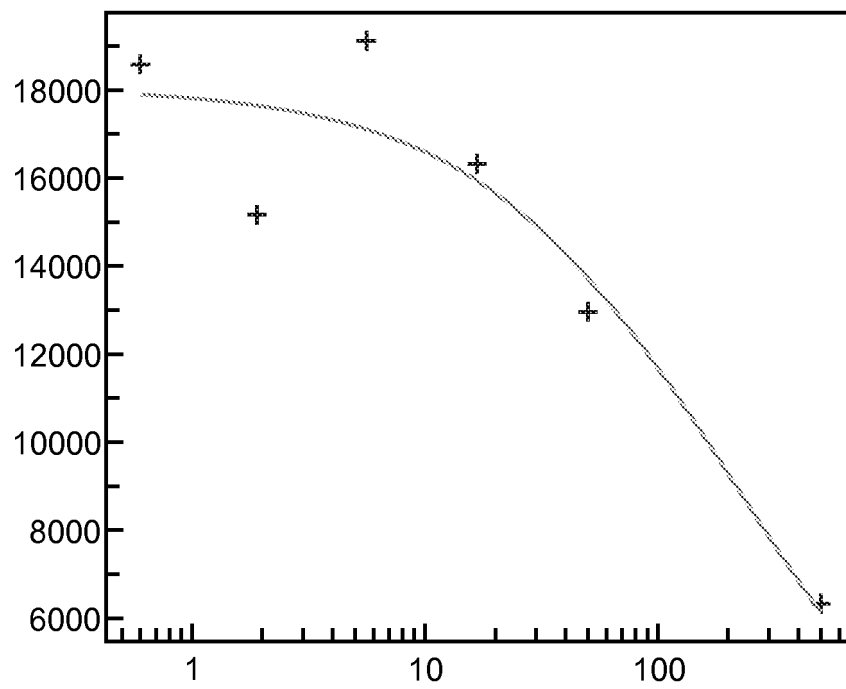


Fig. 31 g)

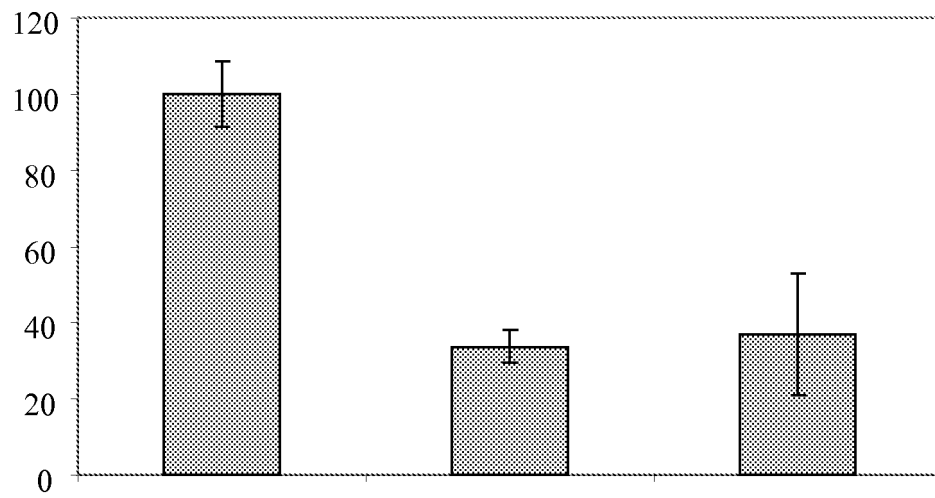


Fig. 32

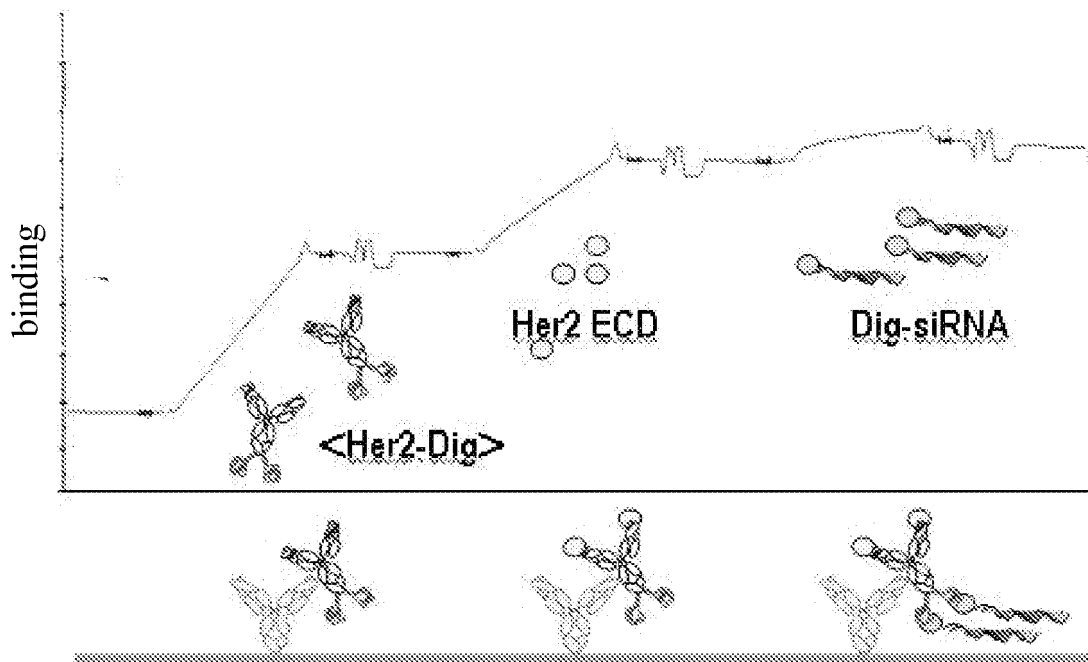


Fig. 33 a)

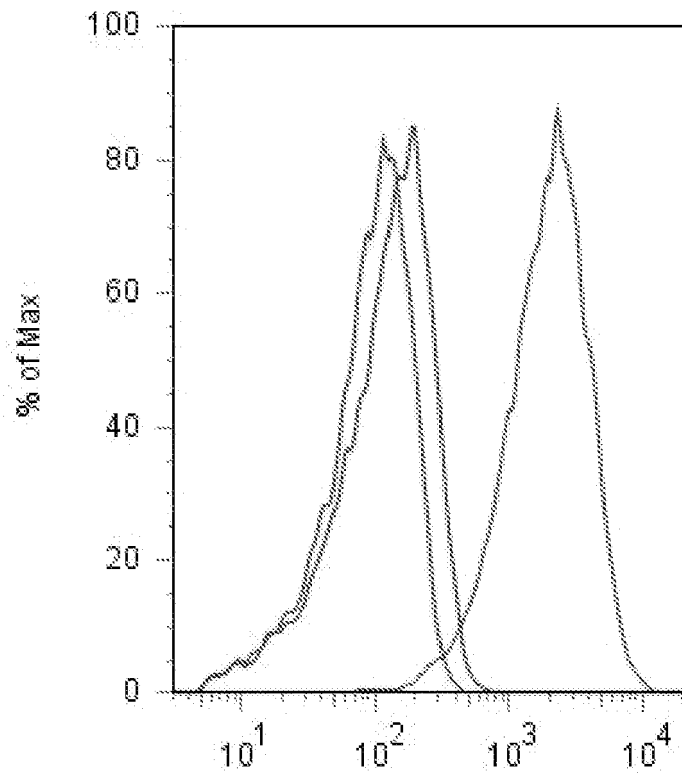


Fig. 33 b)

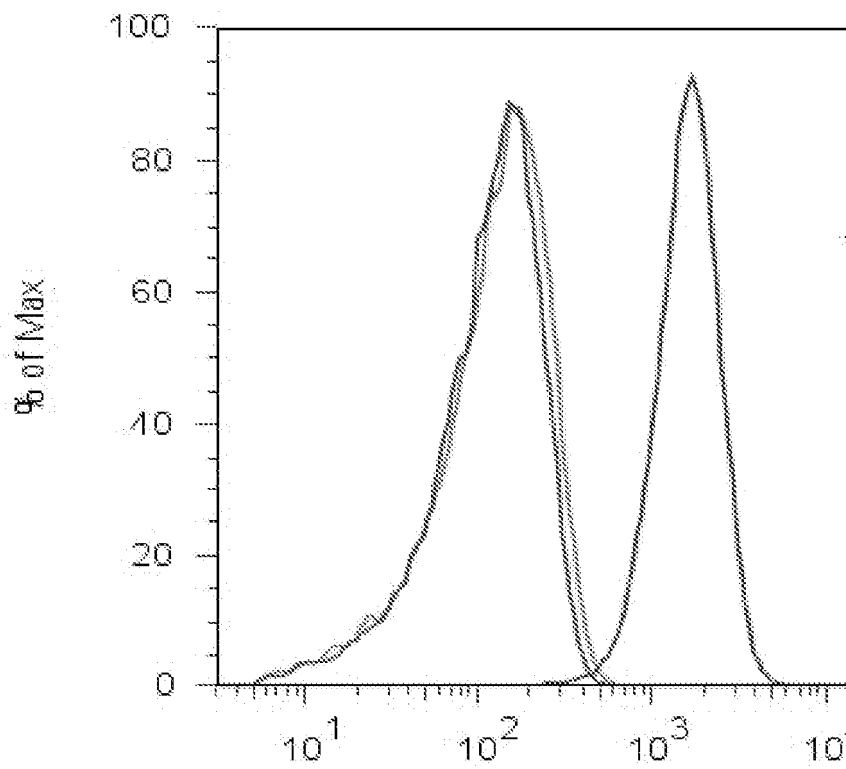


Fig. 34 a)

Fig. 34 b)

Fig. 34 c)

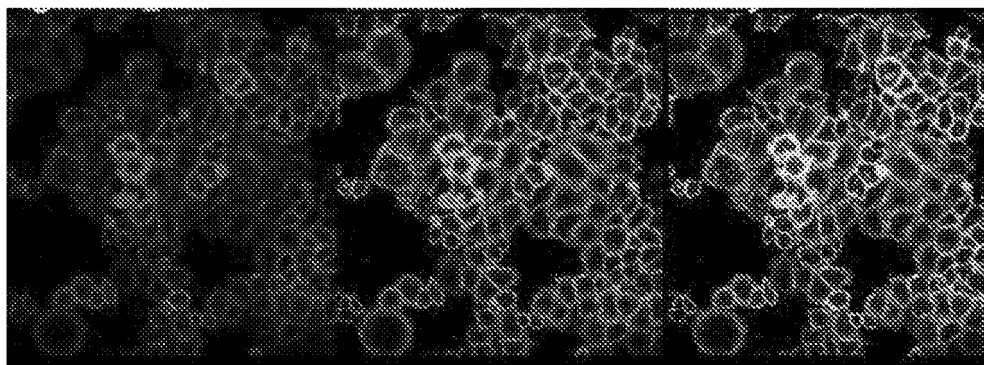


Fig. 34 d)

Fig 34 e)

Fig. 34 f)

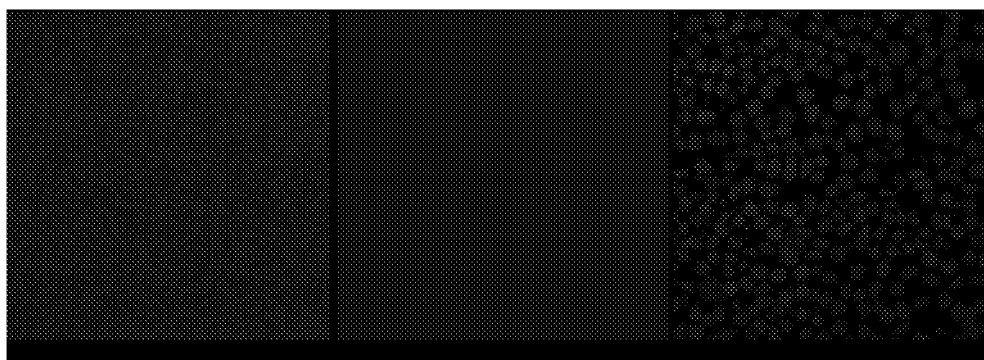


Fig. 34 g)

Fig 34 h)



Fig. 34 i)

Fig 34 j)

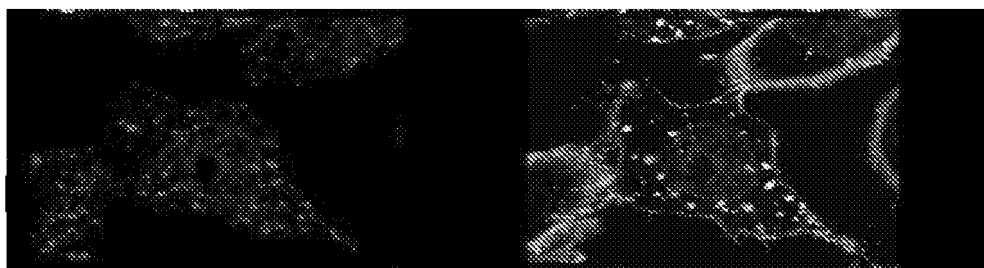


Fig. 35 a)

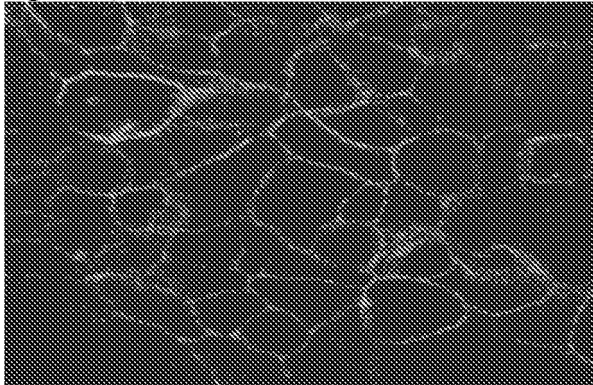


Fig. 35 b)

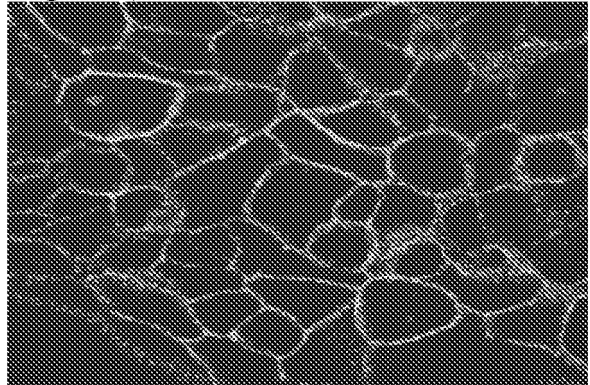


Fig. 35 c)

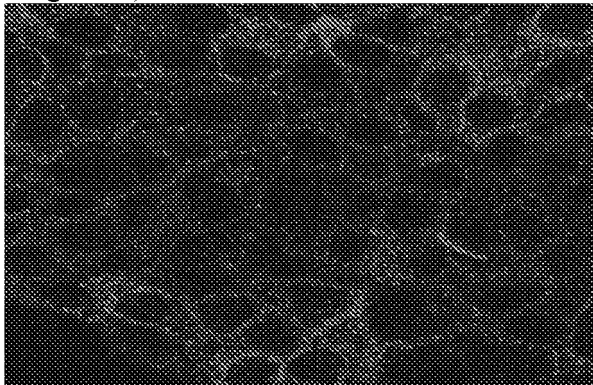


Fig 35 d)

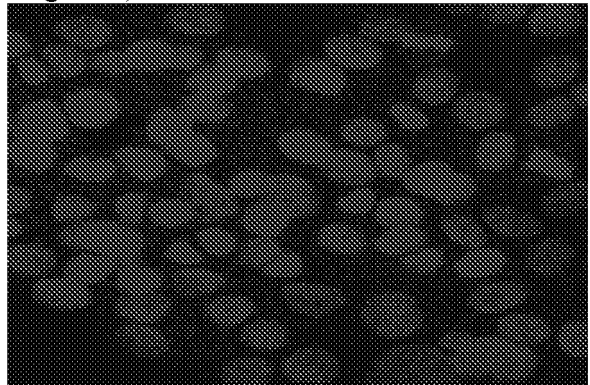


Fig. 35 e)

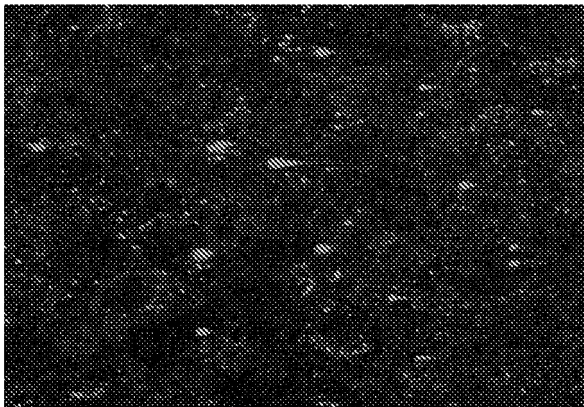


Fig 35 f)

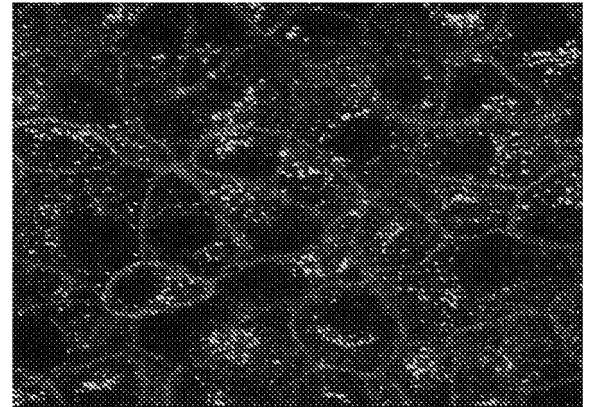


Fig. 35 g)

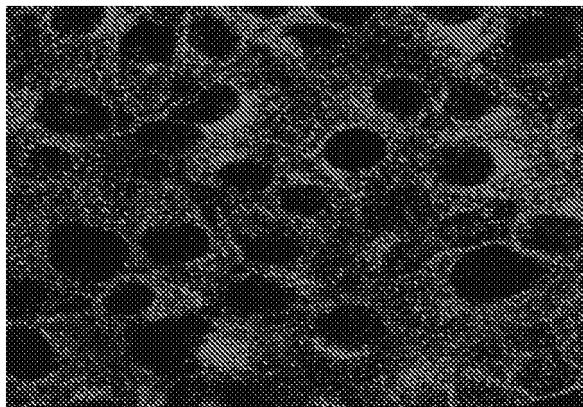


Fig 35 h)

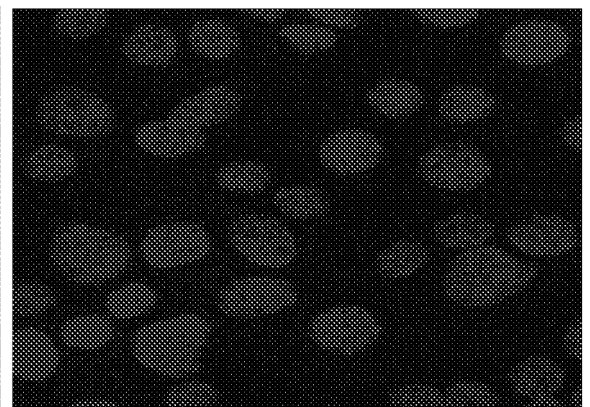


Fig. 36 a)

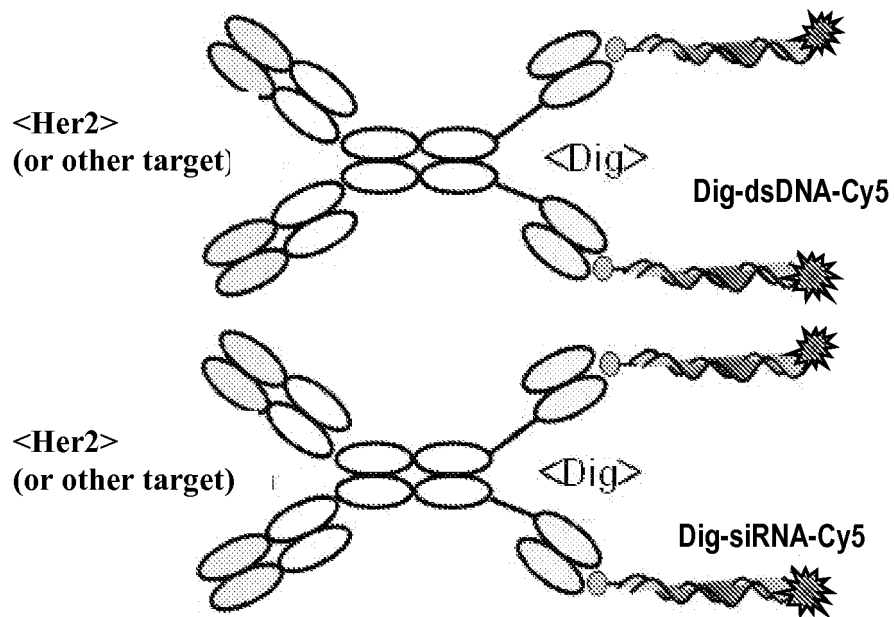


Fig. 36 b)

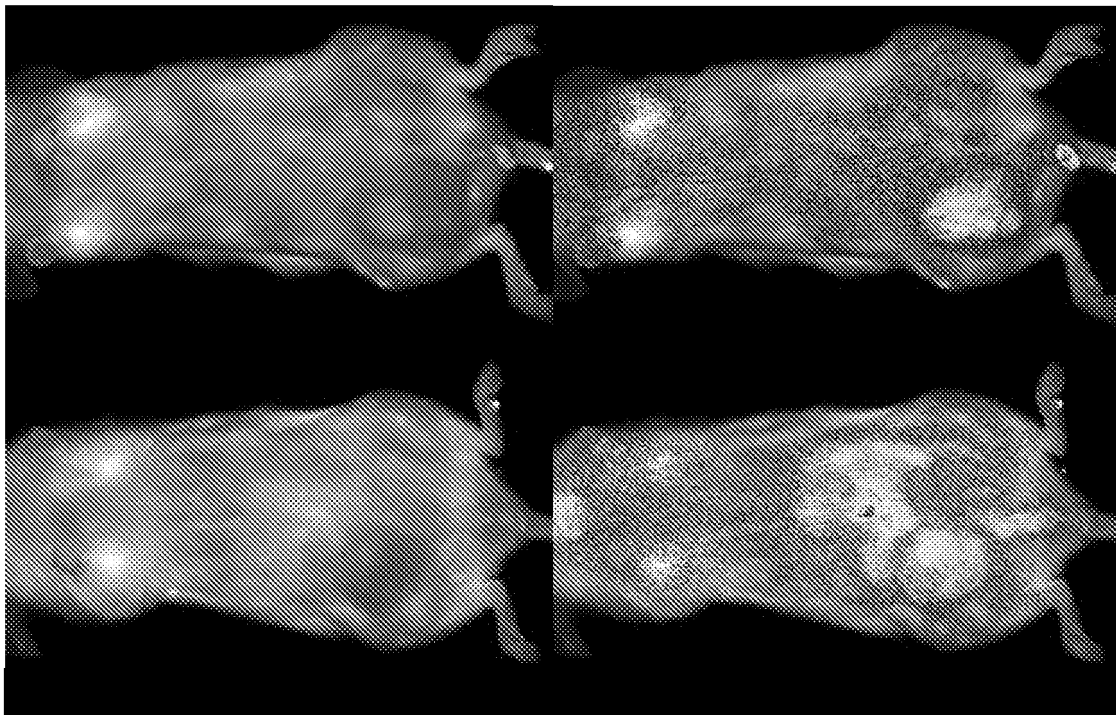


Fig. 37 a)

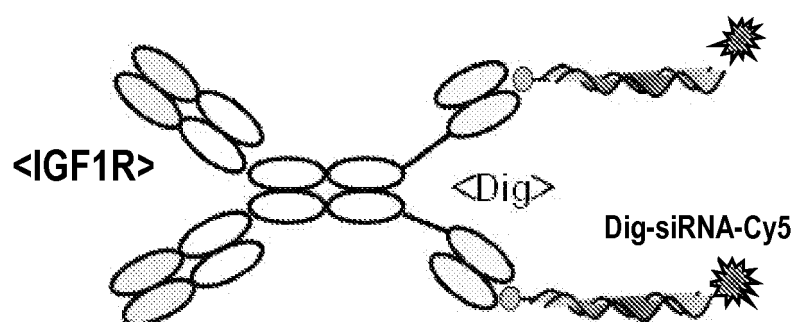


Fig. 37 b)



Fig. 38 a)

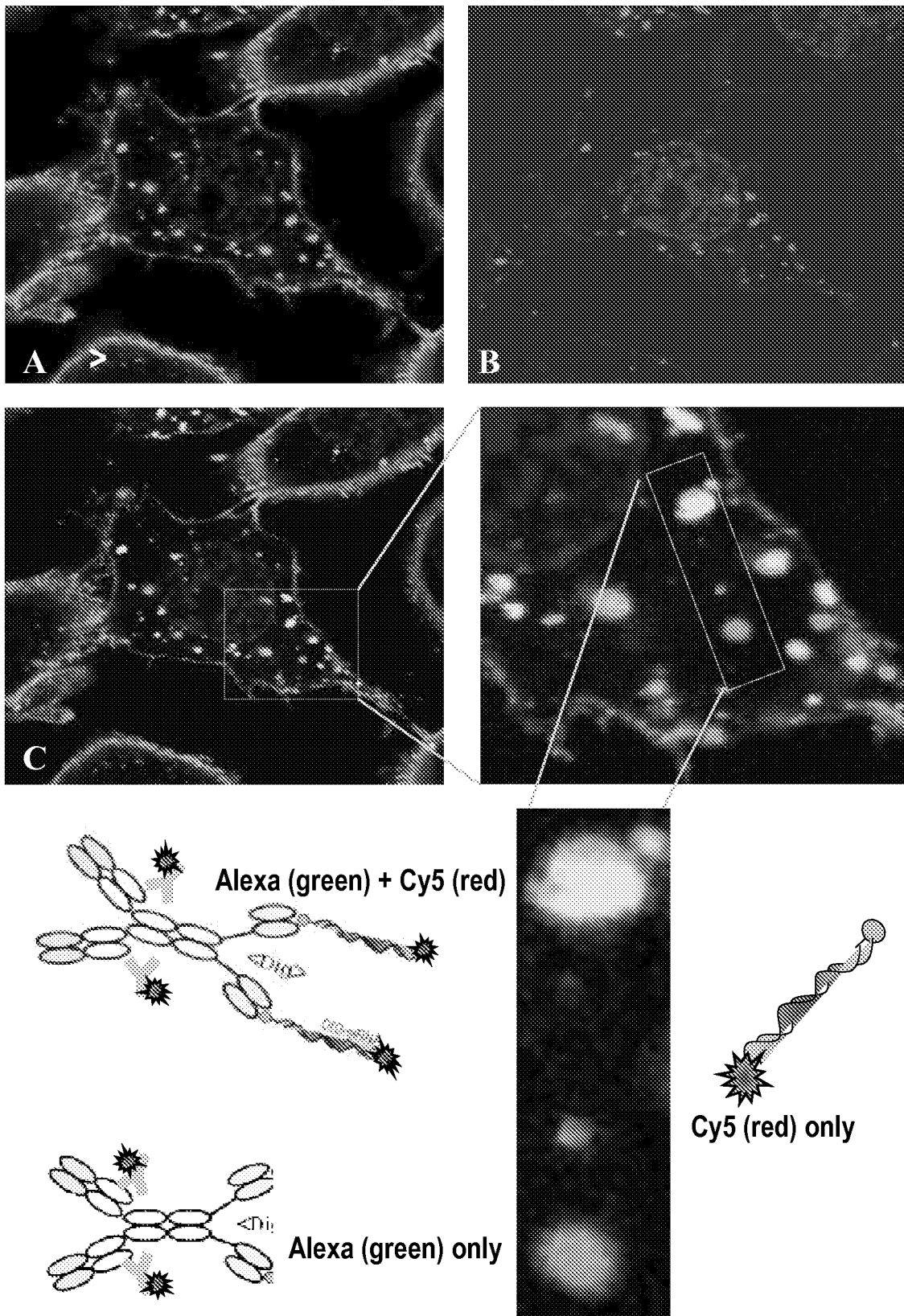


Fig. 38 b)

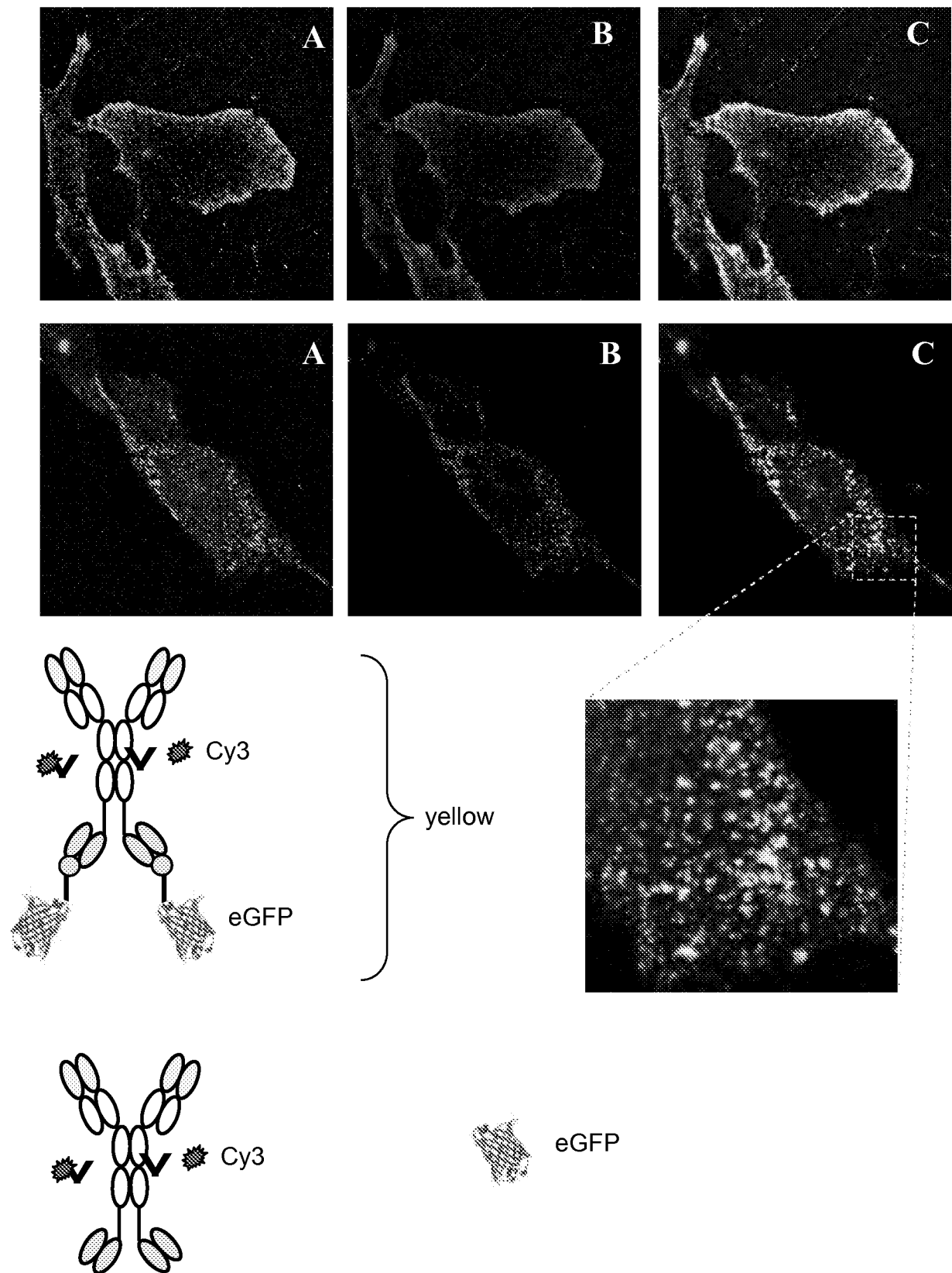


Fig. 38 c)

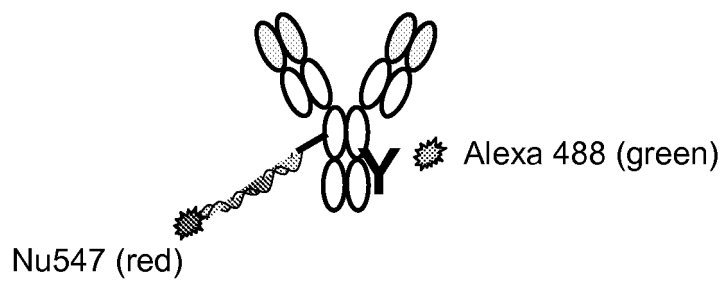
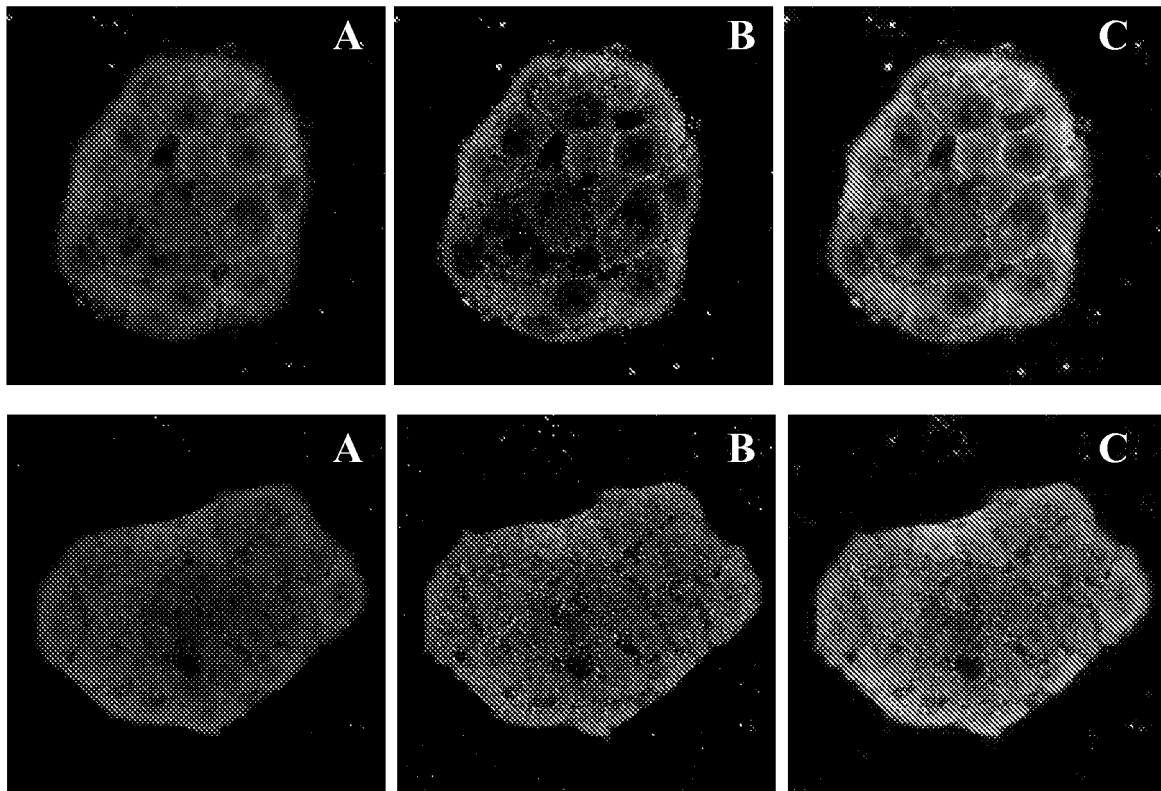


Fig. 38 d)

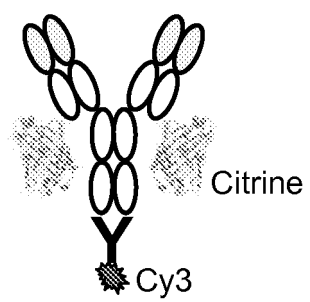
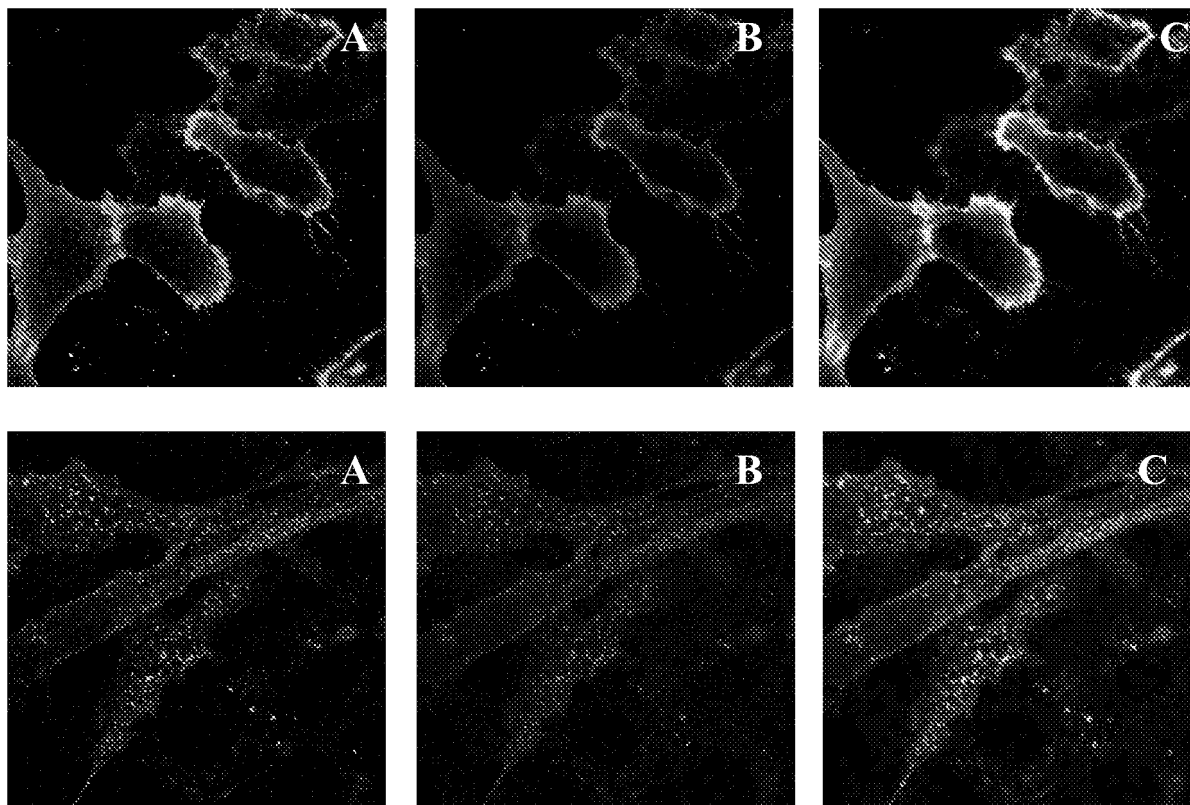


Fig. 39

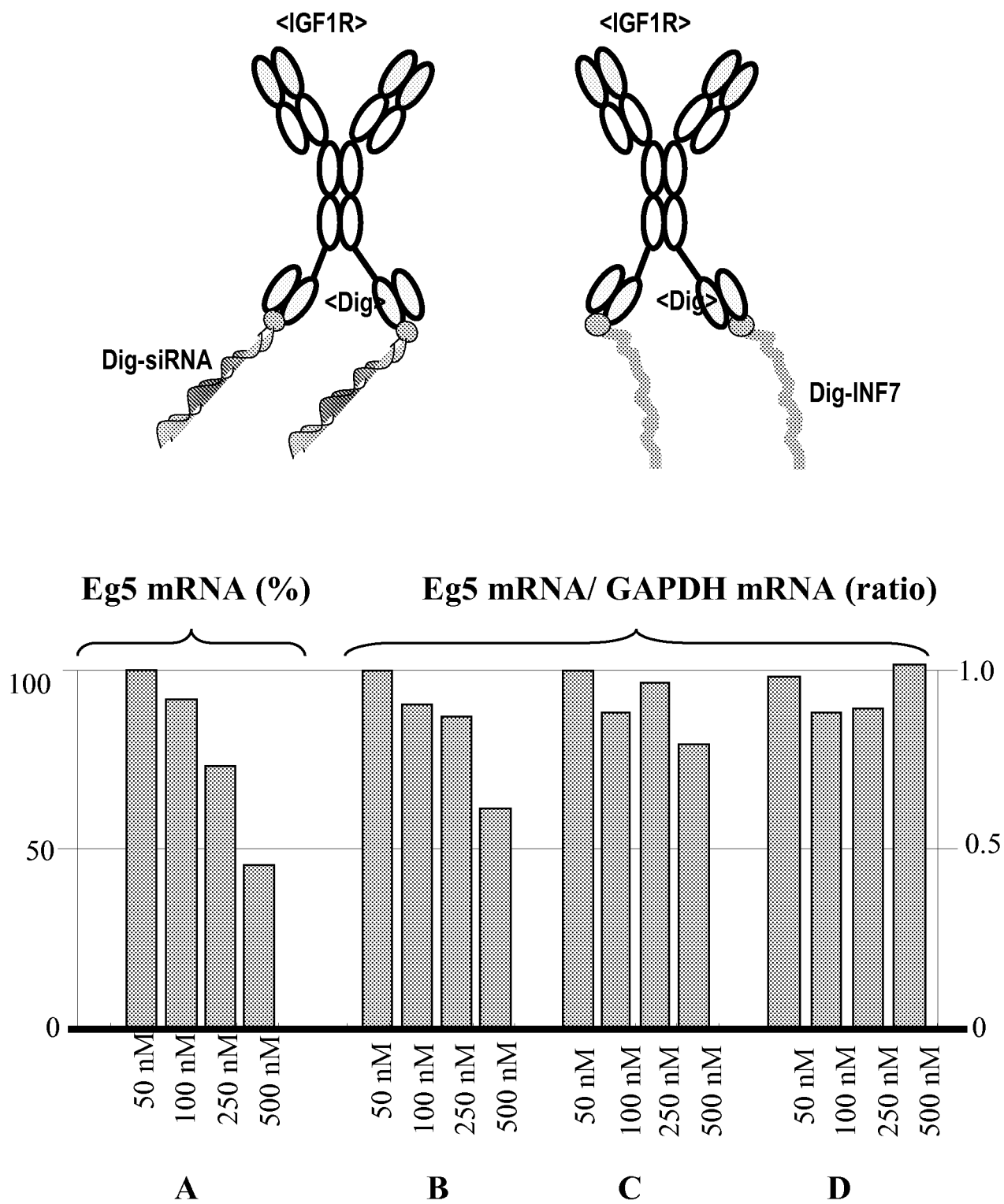


Fig. 40 a)

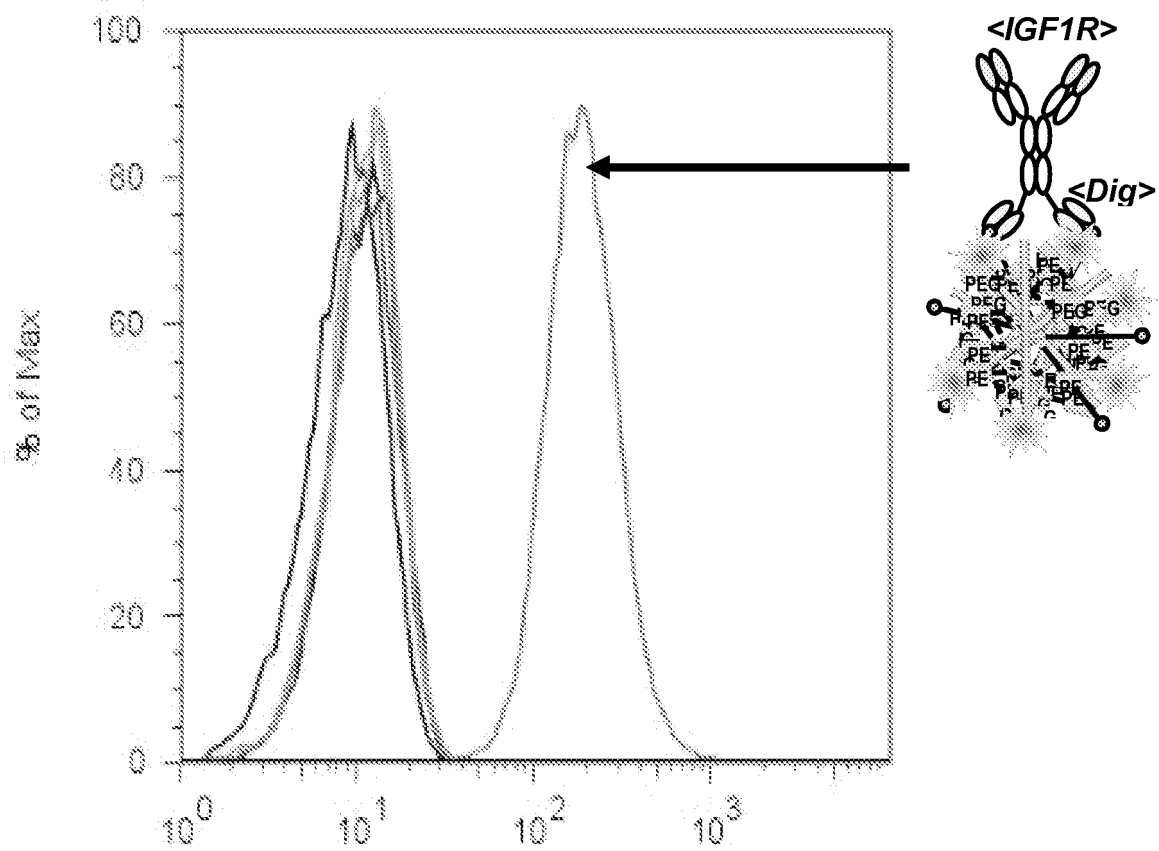


Fig. 40 b)

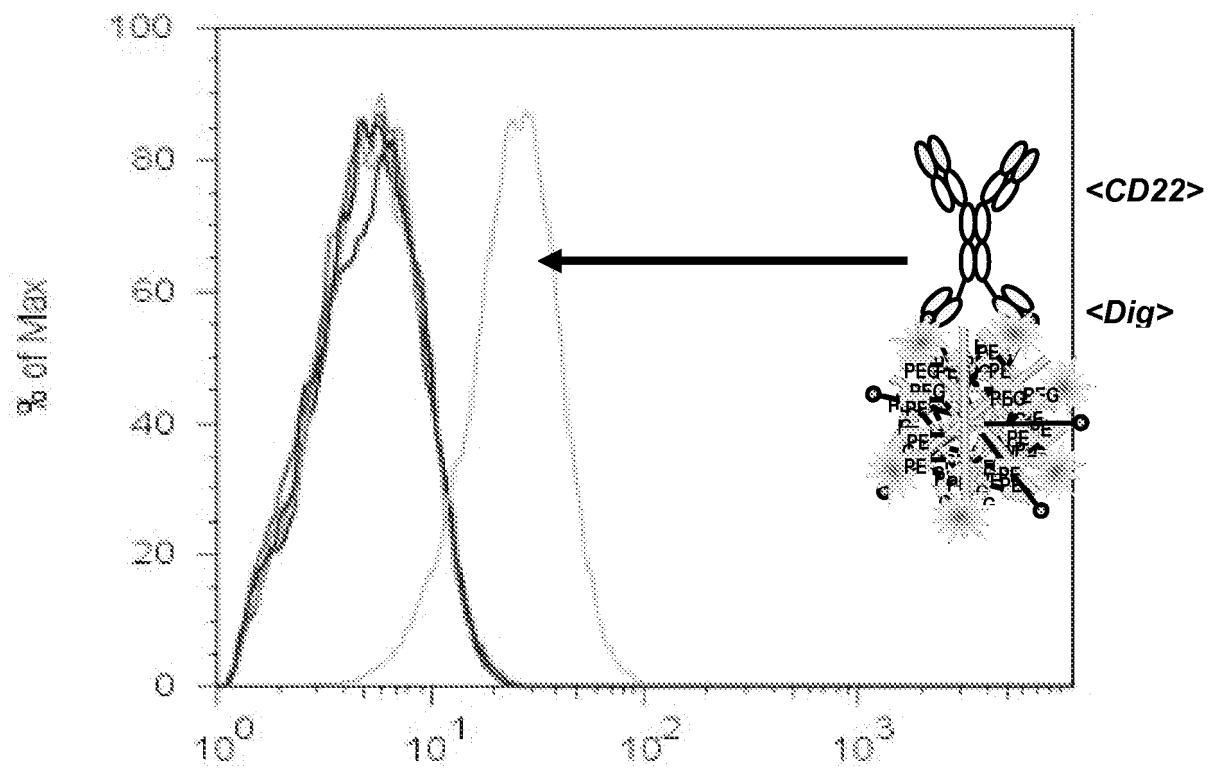


Fig. 41

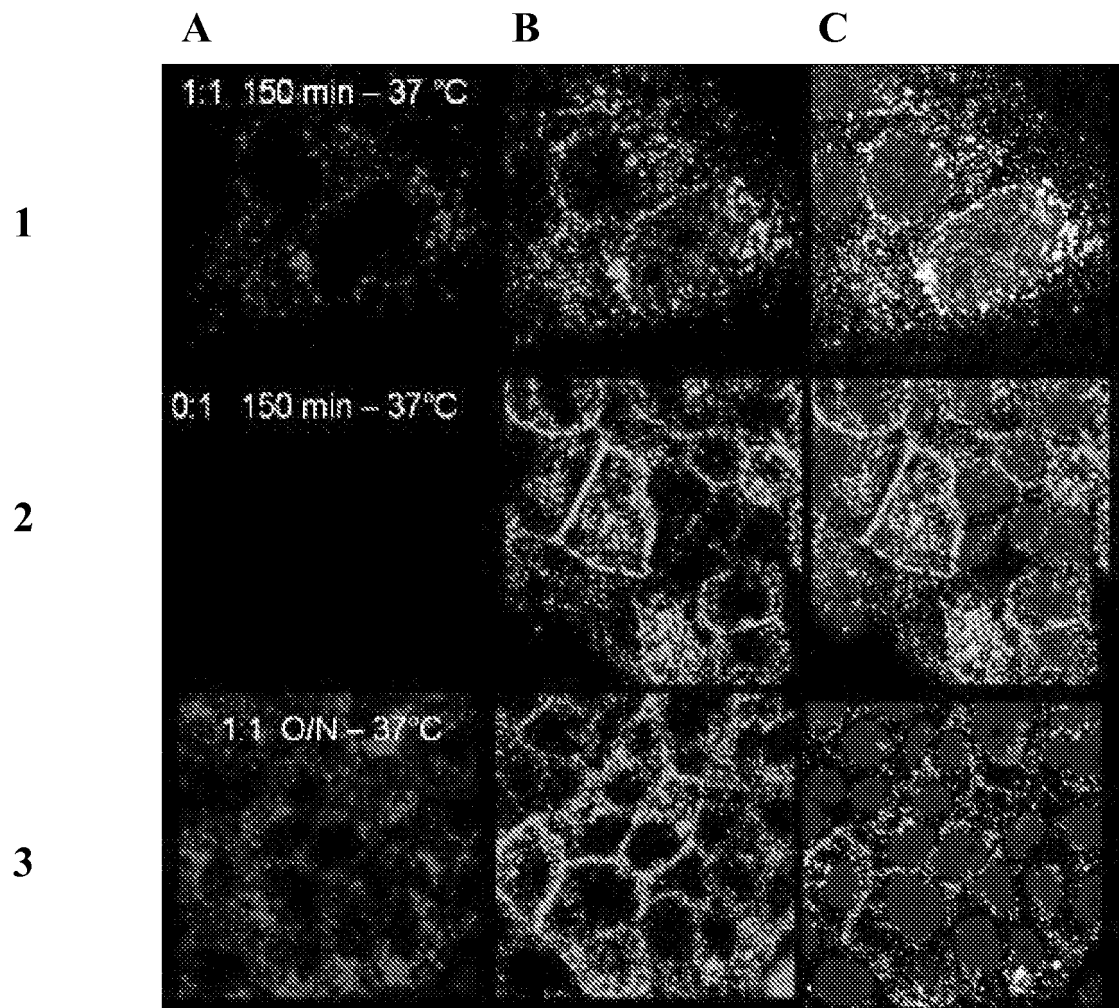


Fig. 42

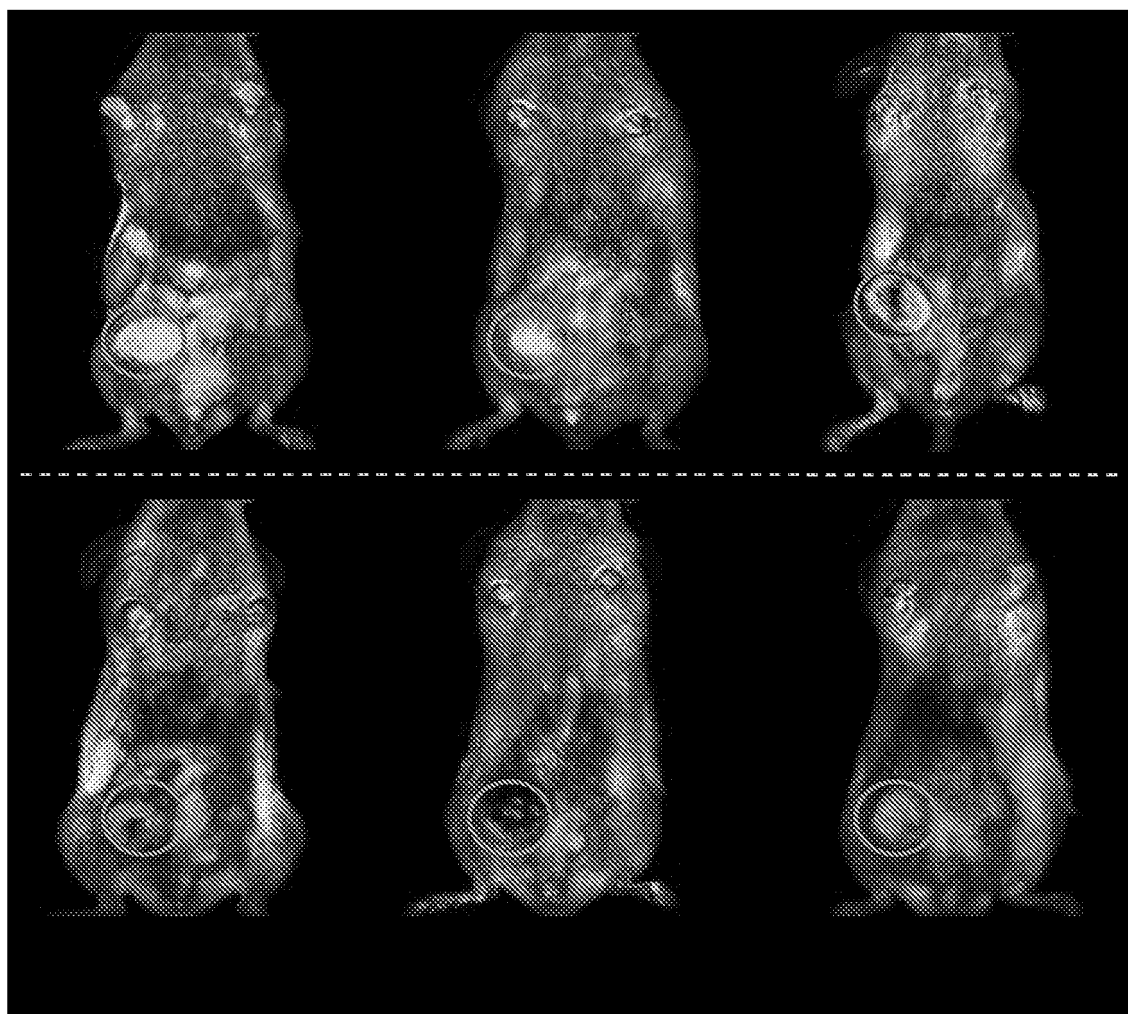


Fig. 43 a)

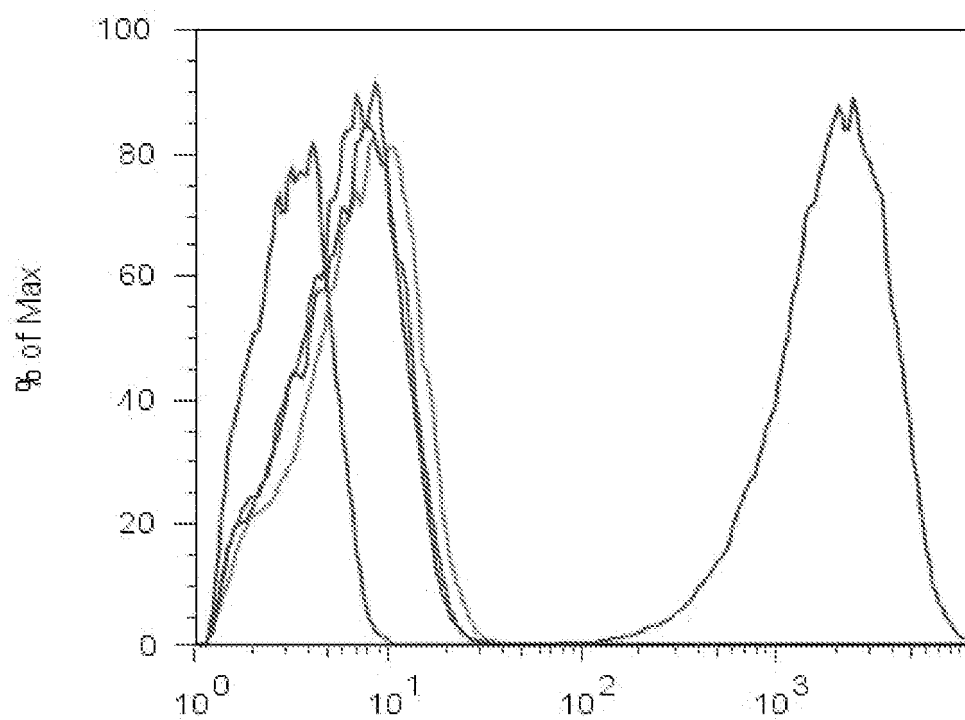


Fig. 43 b)

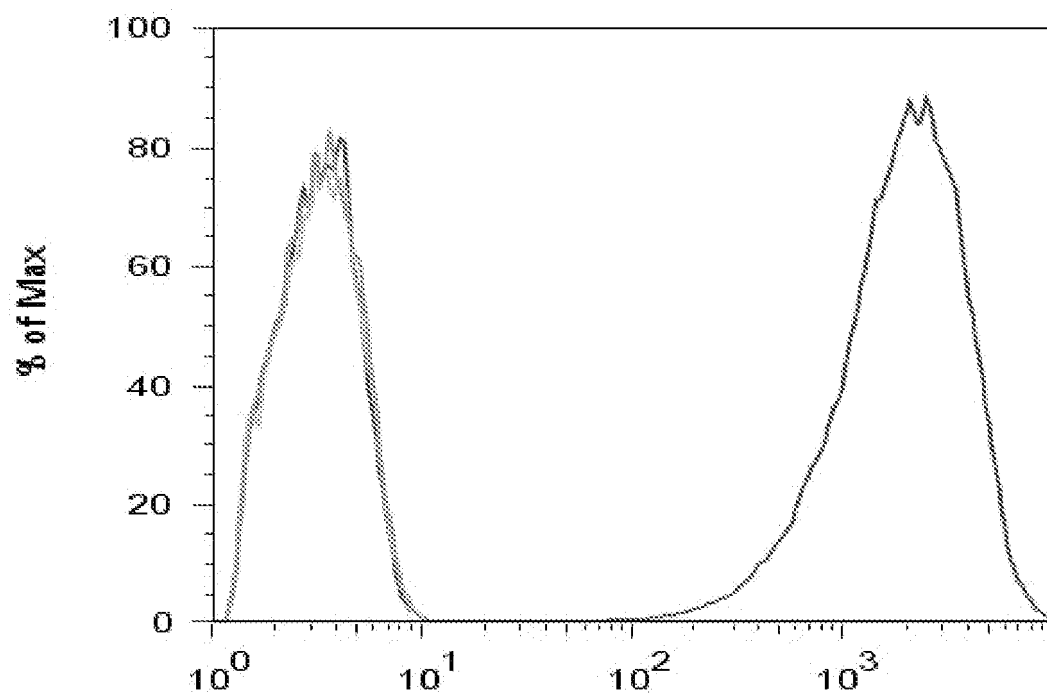


Fig. 44 a)

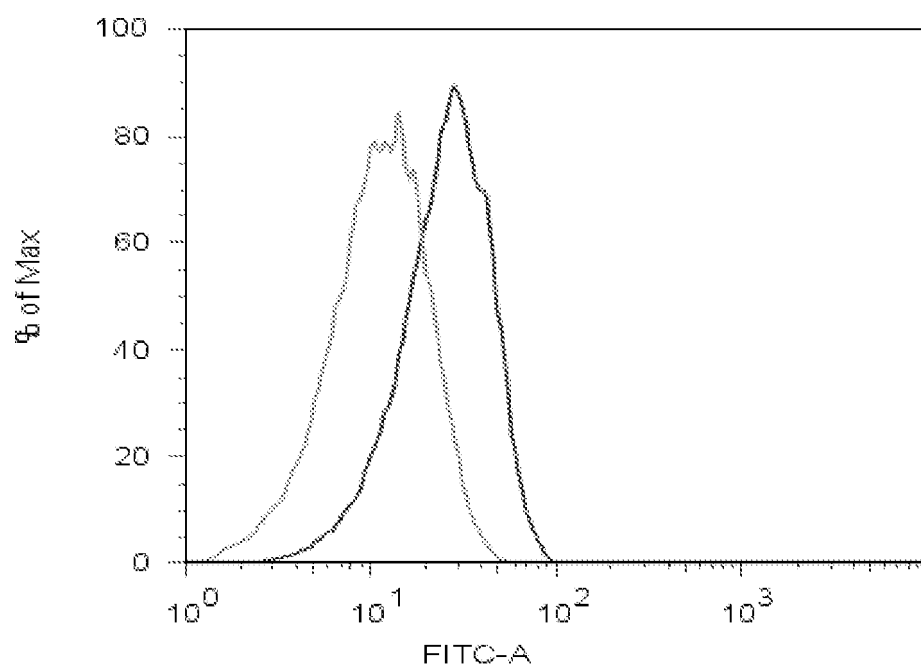


Fig. 44 b)

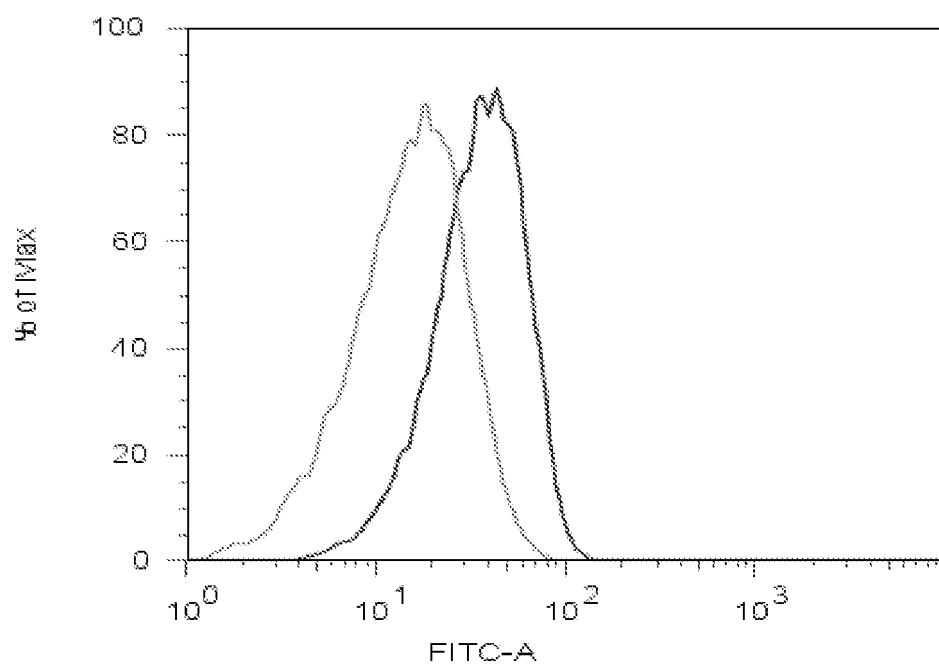


Fig 45 a)

|                                   |                                  |
|-----------------------------------|----------------------------------|
|                                   | complex<br>murine Fab<br>DIG-Cy5 |
| <b>Data collection</b>            |                                  |
| Beamline                          | X06SA (SLS)                      |
| Space group                       | P 42 21 2                        |
| Cell dimensions                   |                                  |
| a, b, c (Å)                       | 138.103 138.103 123.696          |
| $\alpha$ , $\beta$ , $\gamma$ (°) | 90.00 90.00 90.00                |
| Wavelength                        | 0.9795                           |
| Resolution (Å)                    | 50.0-2.80                        |
| Rsym                              | 10.1 (32.1)                      |
| I / $\sigma$                      | 26.38 (9.11)                     |
| Completeness (%)                  | 98.1 (95.0)                      |
| Redundancy                        | 14.7                             |
| <b>Refinement</b>                 |                                  |
| Resolution (Å)                    | 46.040 – 2.798                   |
| No. reflections (test)            | 29999 (1534)                     |
| Rwork / Rfree                     | 20.340 / 23.12                   |
| <b>No. atoms</b>                  |                                  |
| Protein                           | 6660                             |
| Ligand                            | 2x28                             |
| Water                             | 348                              |
| <b>B-factor (Å<sup>2</sup>)</b>   |                                  |
| Protein                           | 23.65                            |
| Ligand                            | 16.00                            |
| Water                             | 32.29                            |
| <b>R.m.s deviations</b>           |                                  |
| Bonds (Å)                         | 0.006                            |
| Angles (°)                        | 1.292                            |
| <b>Ramachandran</b>               |                                  |
| Core (%)                          | 87.4                             |
| Disallowed (%)                    | 0.4                              |

Fig 45 b)

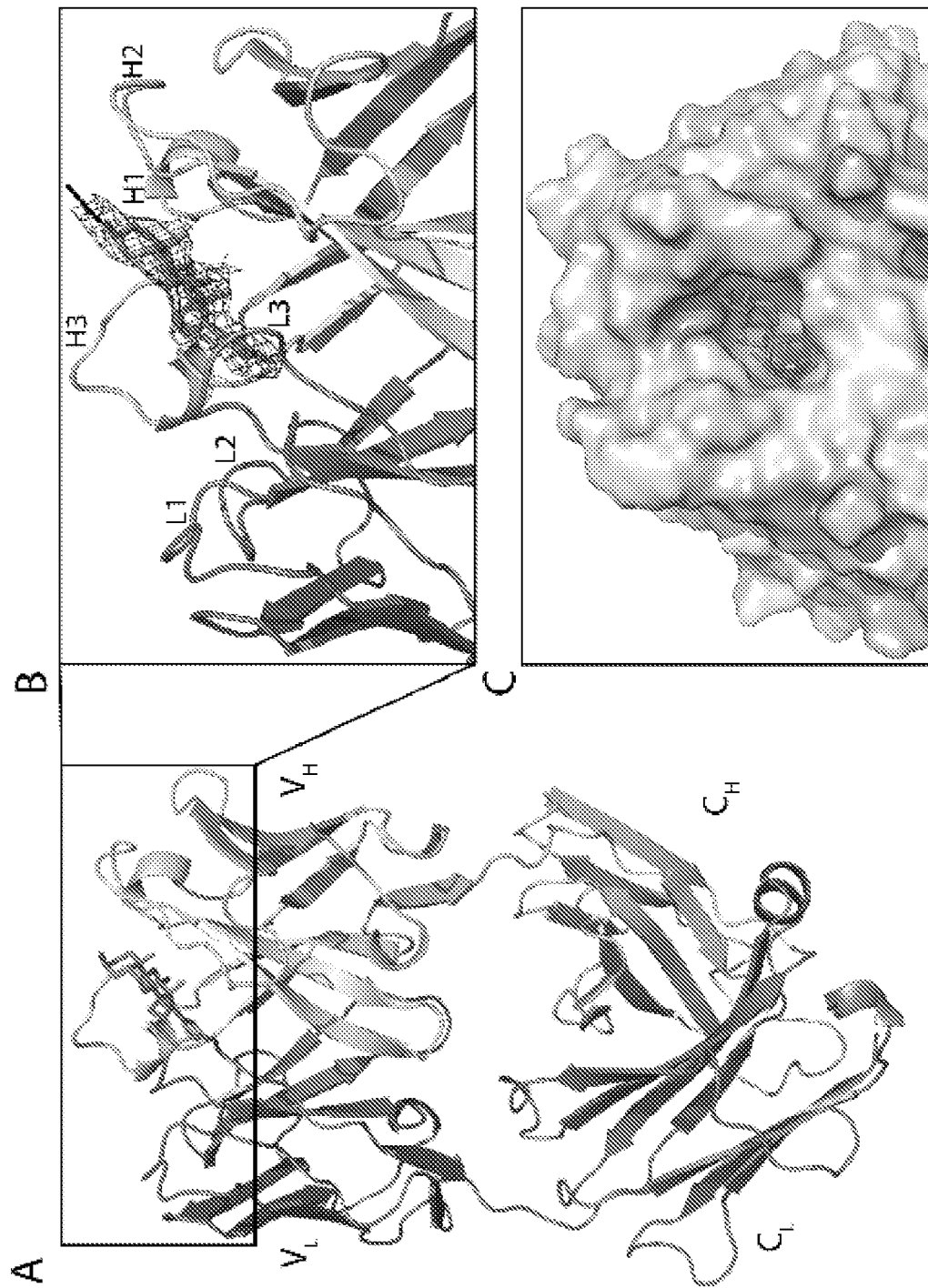


Fig. 45 c)

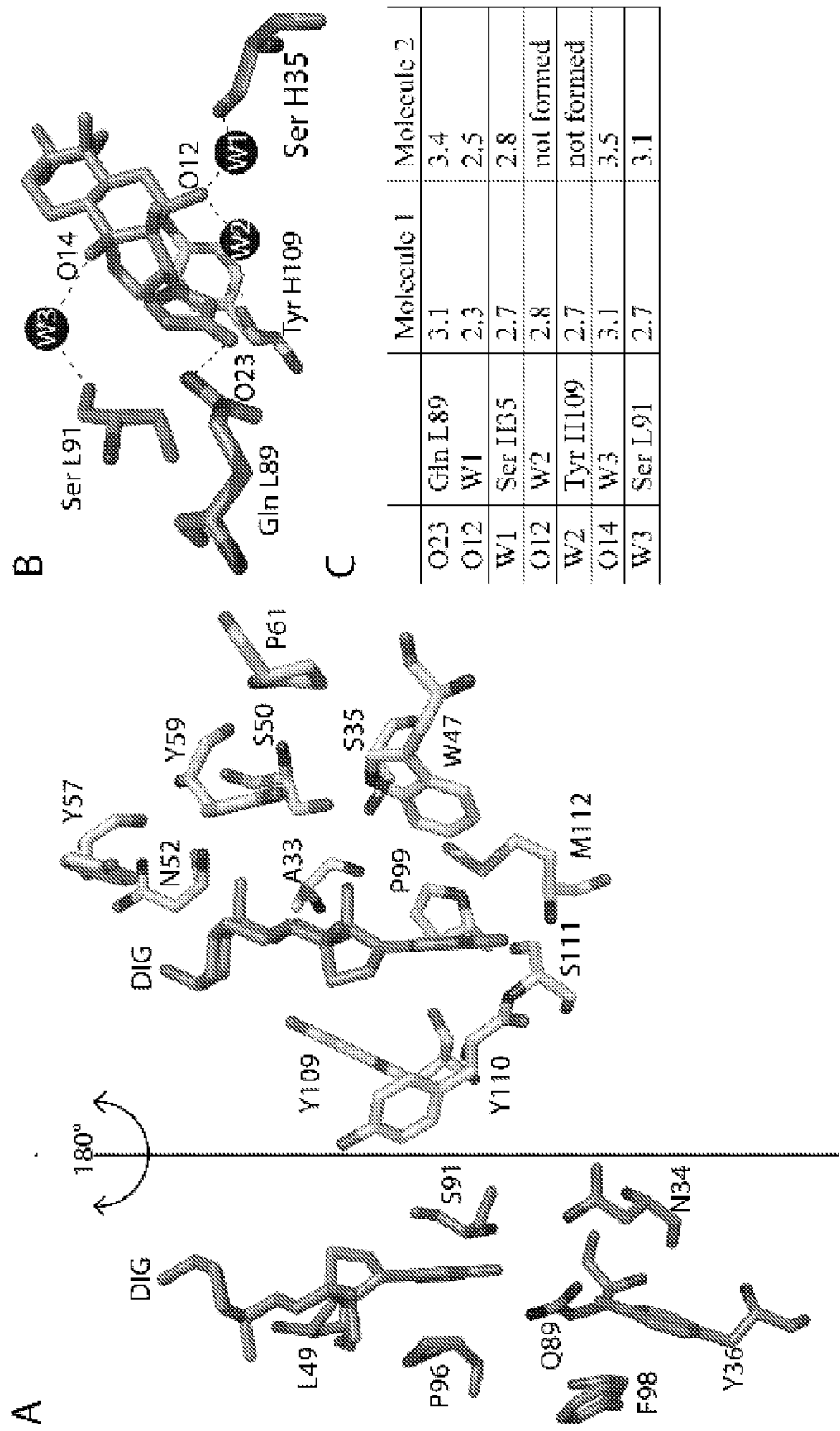


Fig. 46 a)

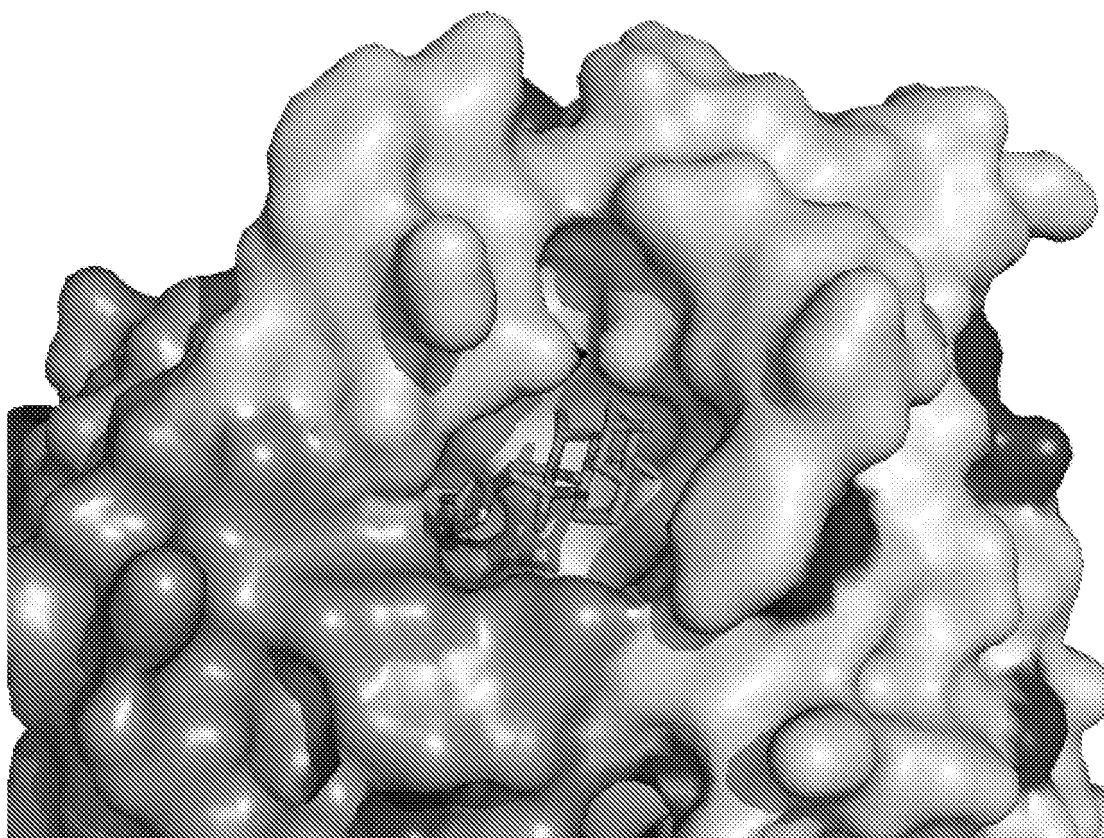


Fig. 46 b)

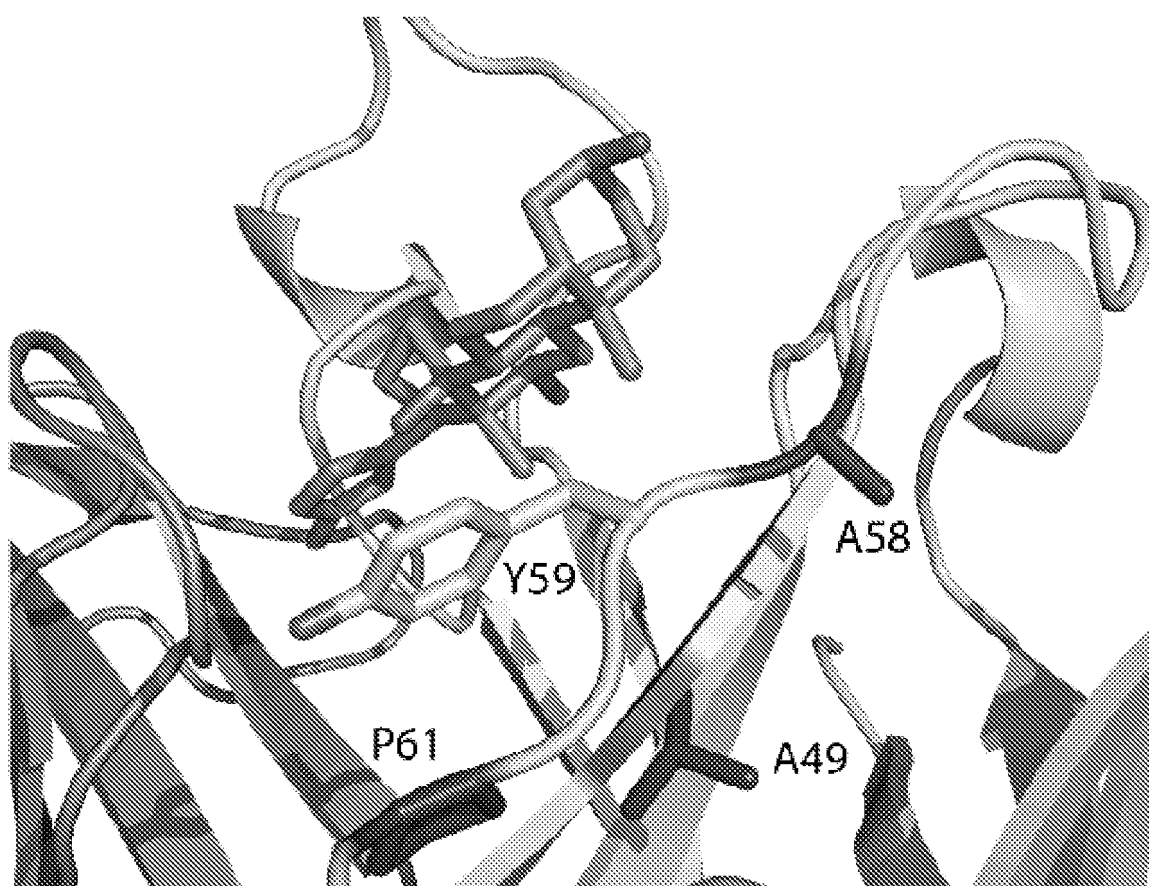


Fig. 46 c)

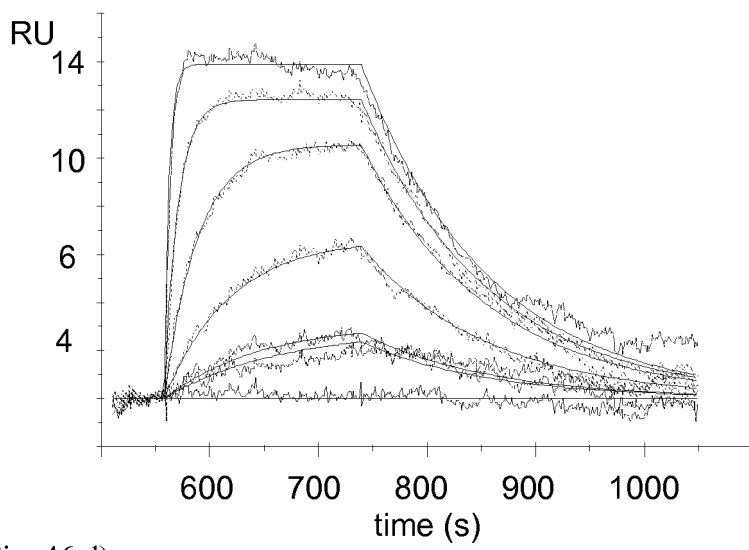


Fig. 46 d)

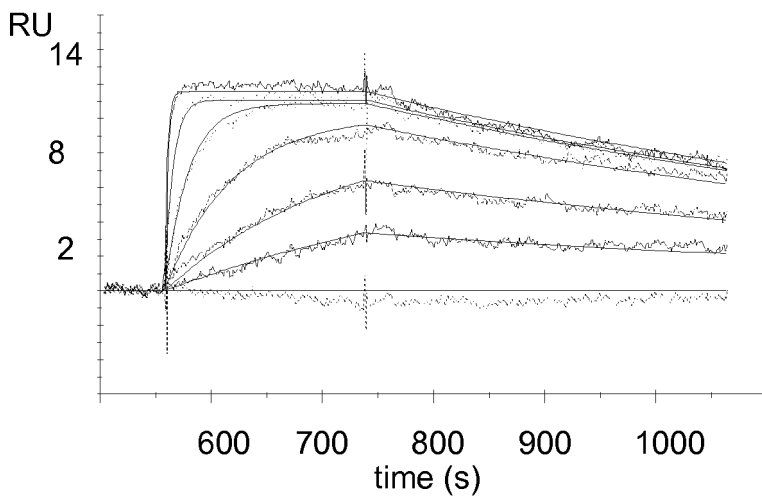


Fig. 47 a)

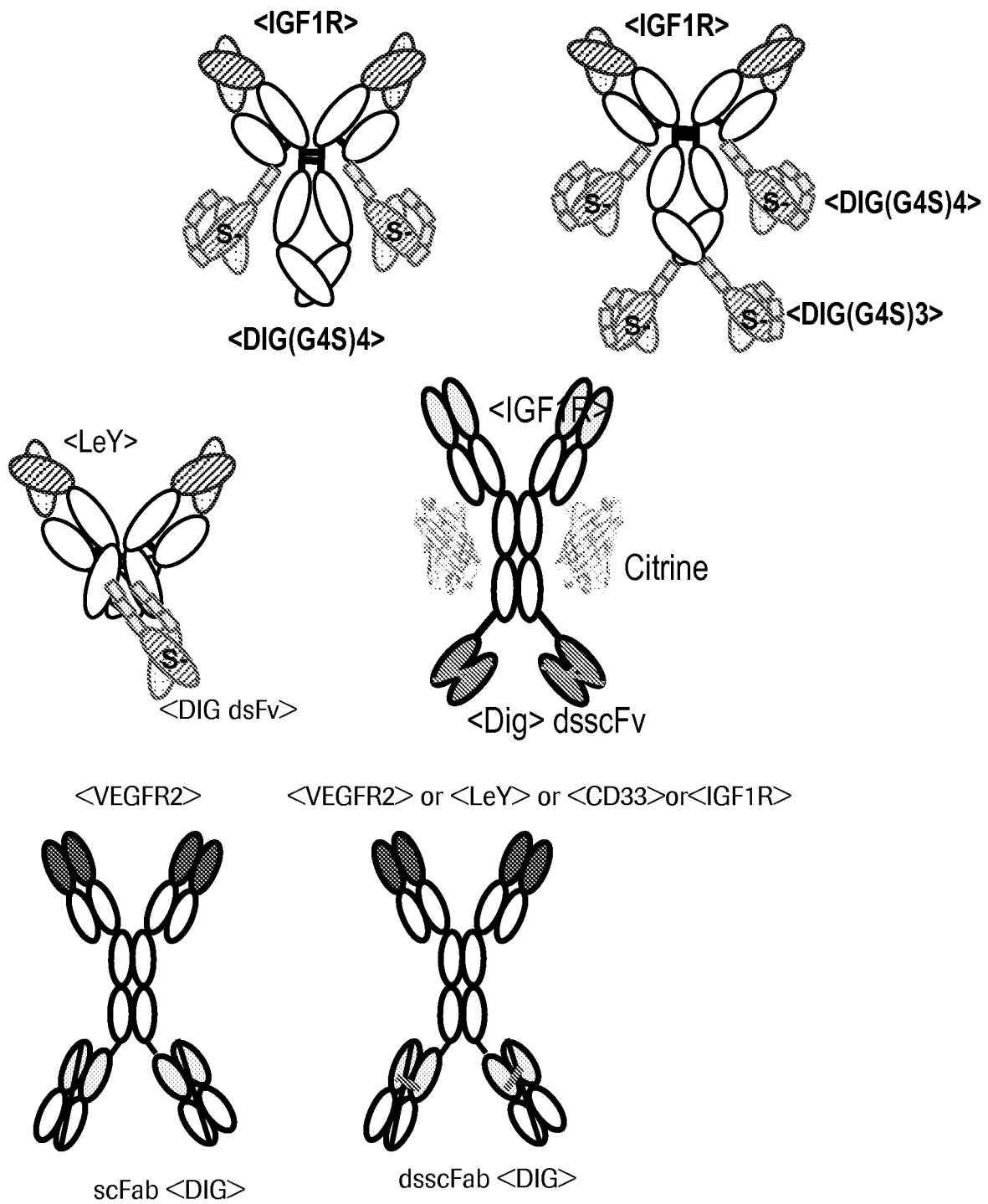


Fig 47 b)

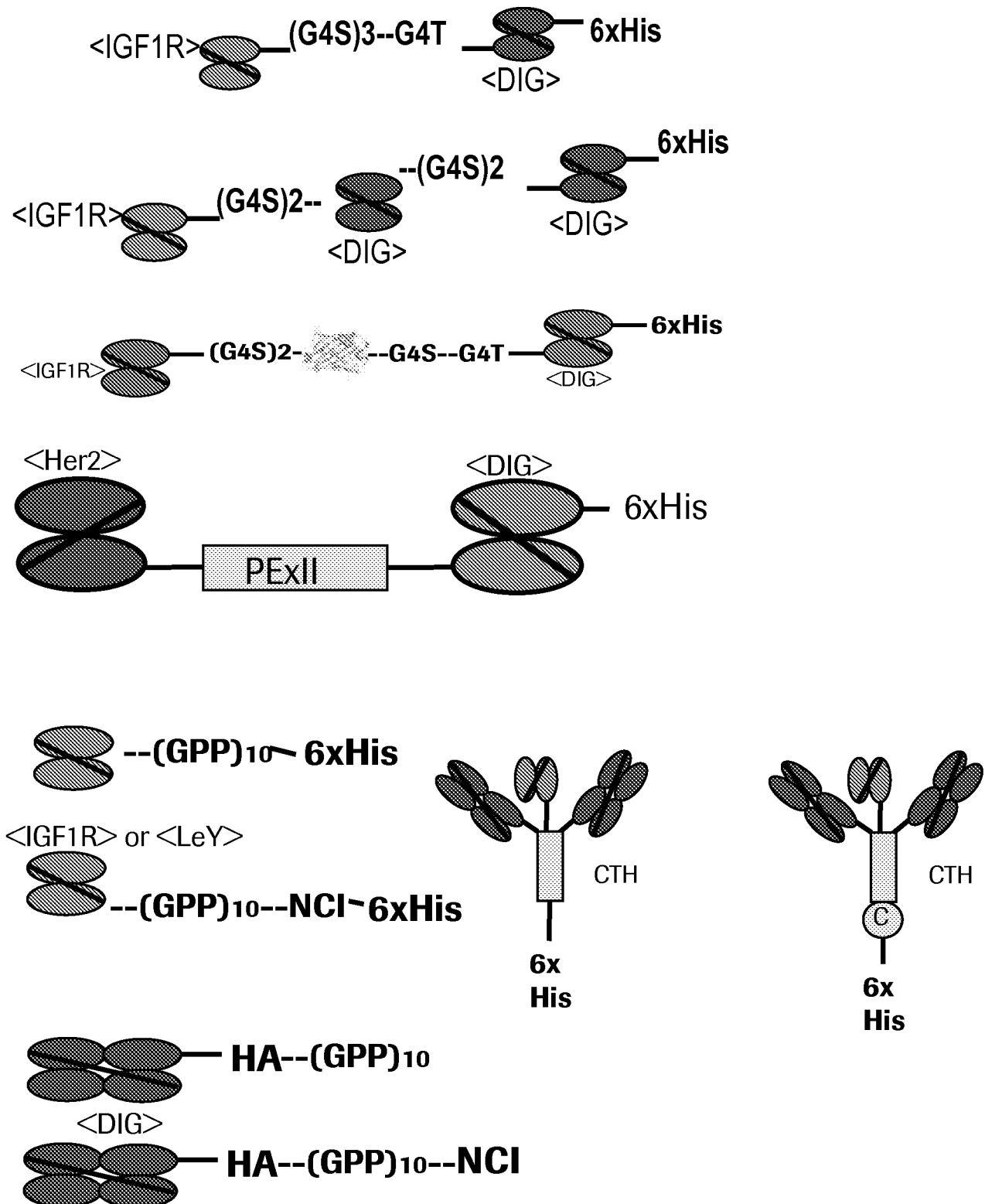


Fig. 47 c)

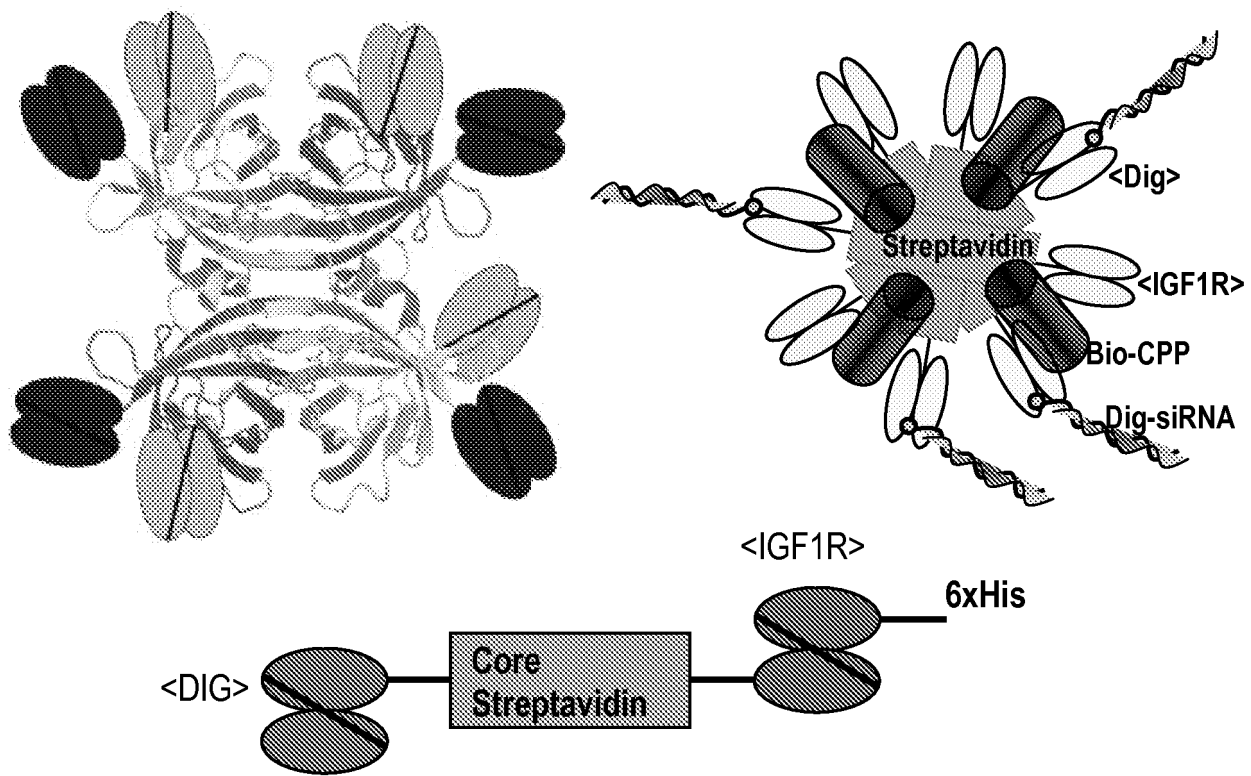


Fig. 47 d)

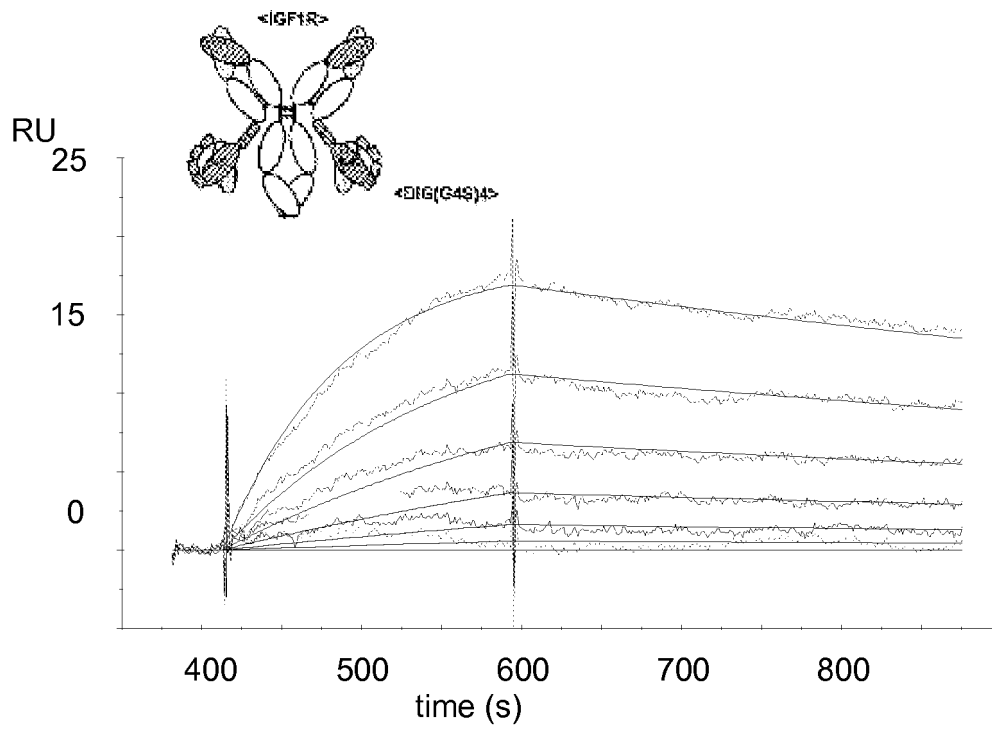


Fig. 47 e)

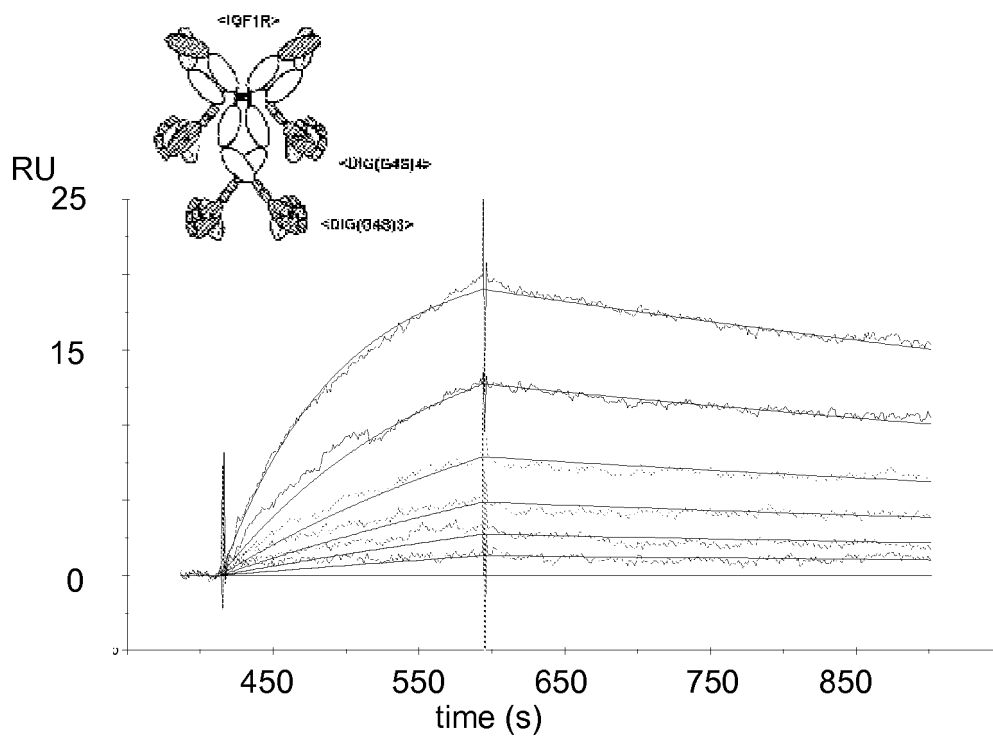


Fig. 47 f)

| <b>Affinity to siRNA-DIG</b> | <b>kd (1/s)</b> | <b>KD (nM)</b> |
|------------------------------|-----------------|----------------|
| <IGF1R-DIG> Dig on CH3       | 9,81E-03        | 20             |
| <IGF1R-DIG> Dig on Ckappa    | 9,71E-03        | 23             |
| <IGF1R-DIG-DIG> hexavalent   | 9,17E-03        | 24             |
| <b>Affinity to IGF-1R</b>    | <b>kd (1/s)</b> | <b>KD (nM)</b> |
| <IGF1R-DIG> Dig on CH3       | 1,09E-03        | 5              |
| <IGF1R-DIG> Dig on Ckappa    | 7,99E-04        | 3              |
| <IGF1R-DIG-DIG> hexavalent   | 7,64E-04        | 3              |

| <b>Affinity for DIG-siRNA</b> |         | <b>kd (1/s)</b> | <b>KD (nM)</b> |
|-------------------------------|---------|-----------------|----------------|
| <IGF1R-DIG> 2321              | 5,03E+0 | 1,17E-02        | 23             |
| <IGF1R-scFab-DIG> 2321        | 8,32E+0 | 1,12E-02        | 14             |
| <IGF1R-dsFab-DIG> 2321        | 5,79E+0 | 7,82E-02        | 14             |

## Affinity for DIG-siRNA

| Capture         | Antibody                 | ka (1/Ms) | kd (1/s) | KD (M)   |
|-----------------|--------------------------|-----------|----------|----------|
| human VEGFR2-Fc | <VEGFR2(D1F7)-DIG>_001   | 3.44E+06  | 6.54E-03 | 1.90E-09 |
|                 | <VEGFR2(D1F7)-scFab-DIG> | 4.98E+06  | 6.70E-03 | 1.35E-09 |
|                 | <VEGFR2(D1F7)-dsFab-DIG> | 2.49E+06  | 9.68E-03 | 3.89E-09 |
| mouse VEGFR2-Fc | <VEGFR2(D1F7)-DIG>_001   | 3.89E+06  | 6.56E-03 | 1.69E-09 |
|                 | <VEGFR2(D1F7)-scFab-DIG> | 3.21E+06  | 8.92E-03 | 2.78E-09 |
|                 | <VEGFR2(D1F7)-dsFab-DIG> | 2.01E+06  | 1.14E-02 | 5.65E-09 |

Fig. 47 g)

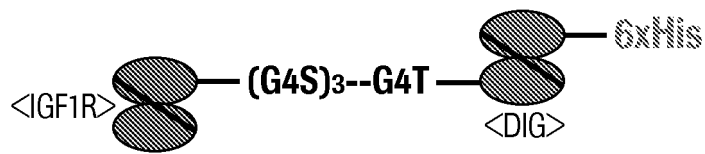


Fig. 47 h)

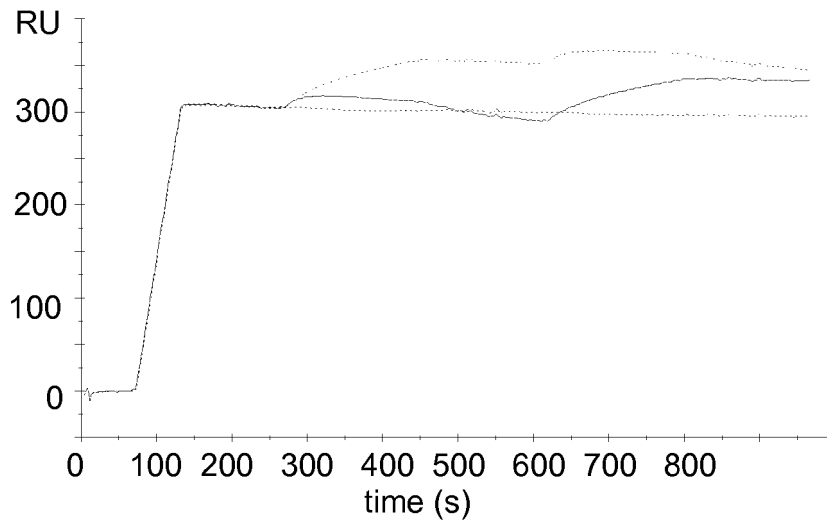


Fig. 47 i)

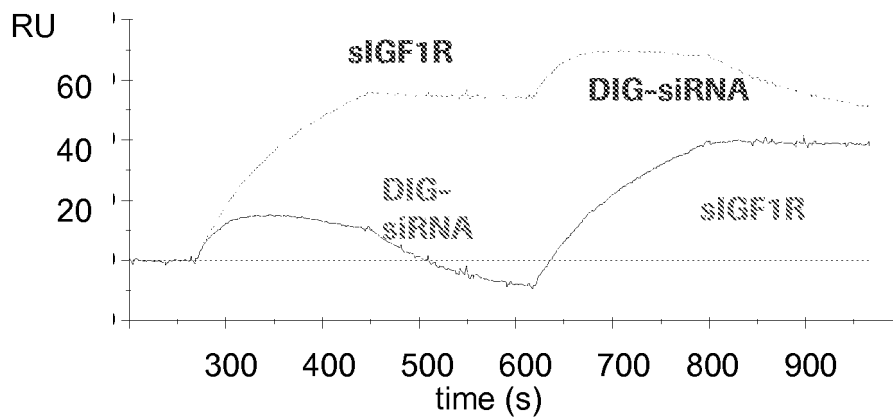


Fig. 47 j)

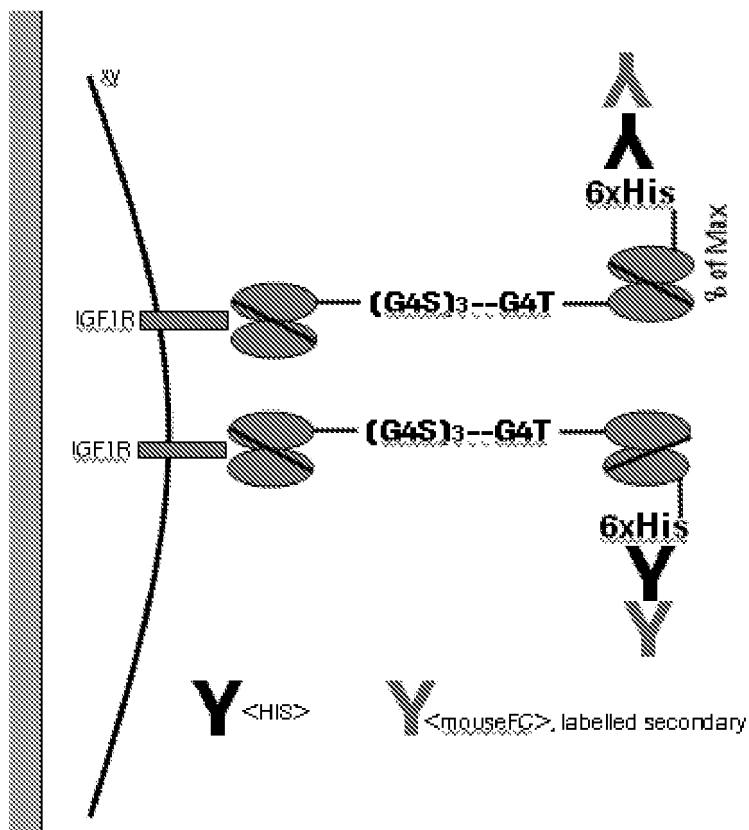
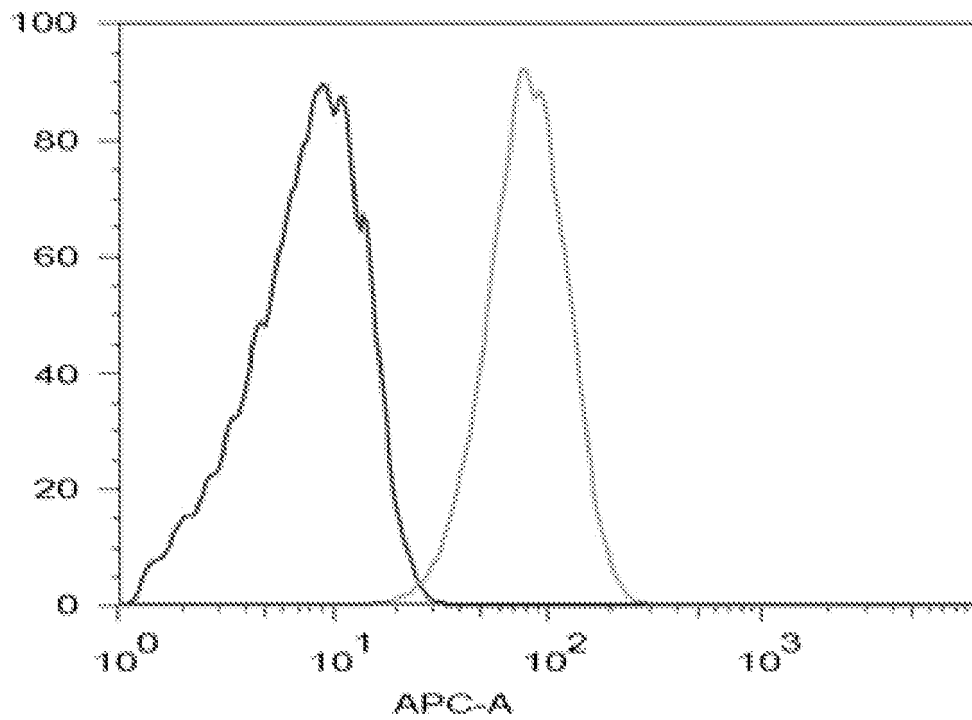


Fig 47 k)

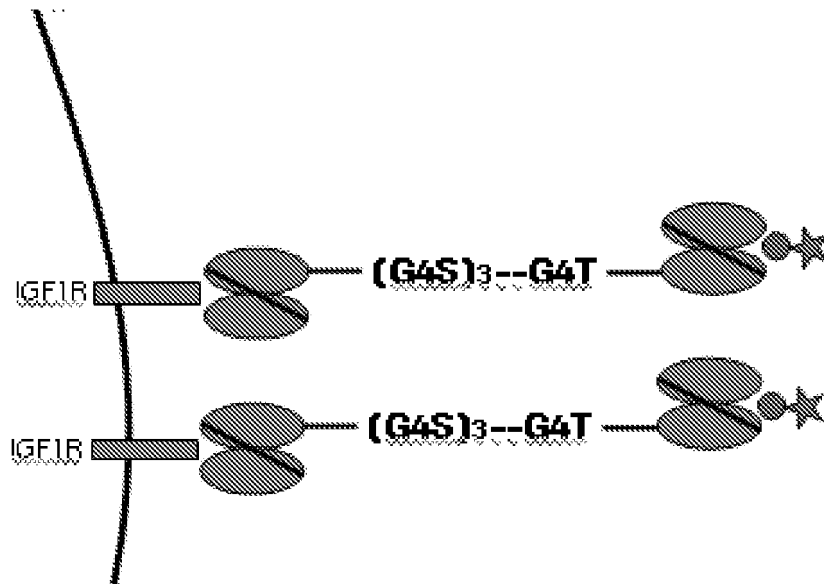
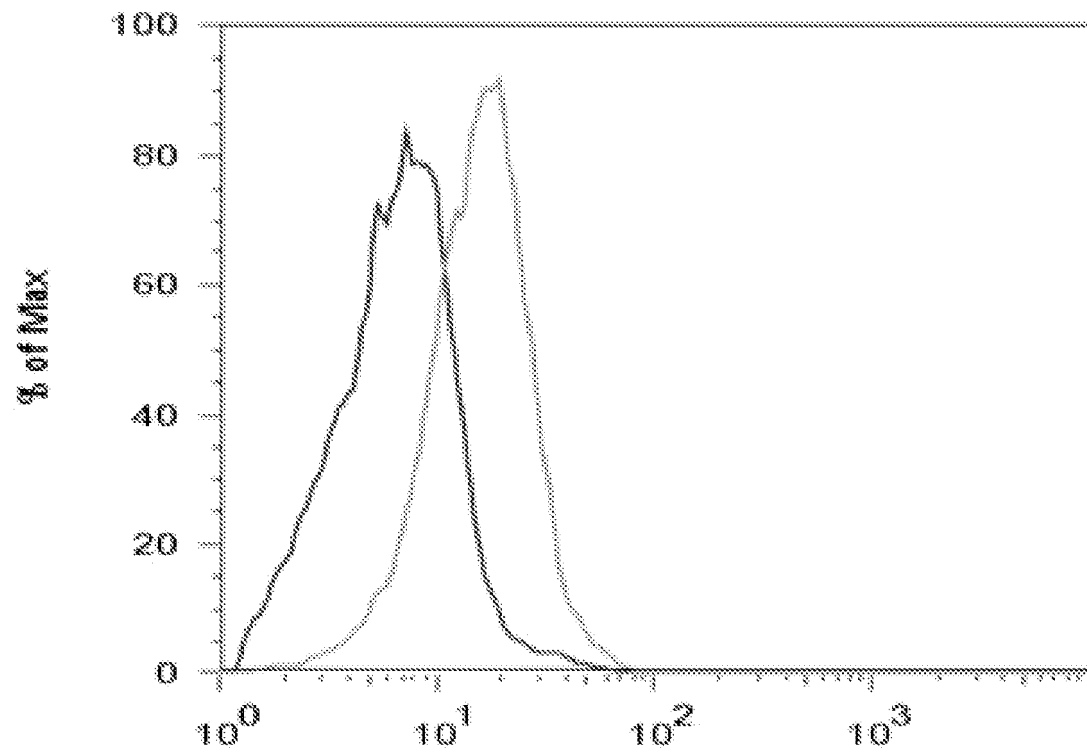


Fig. 47 l)

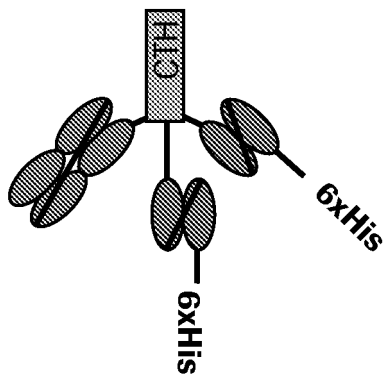


Fig. 47 n)

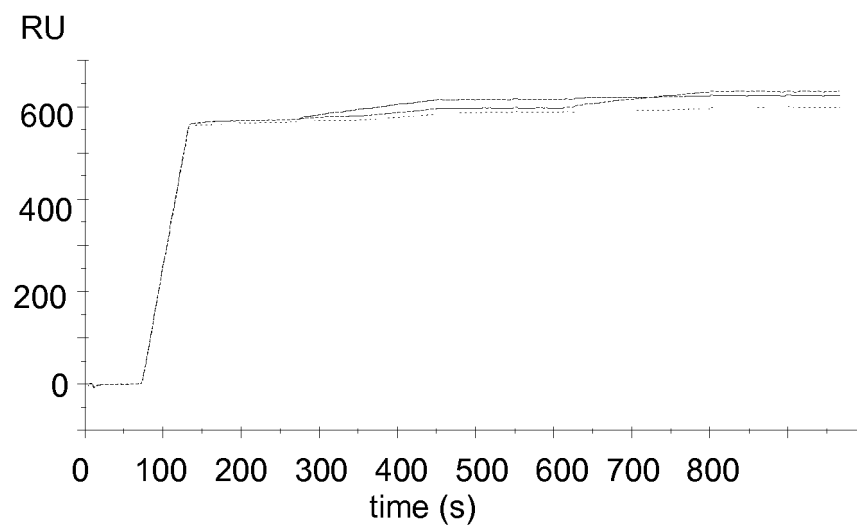


Fig. 47 m)

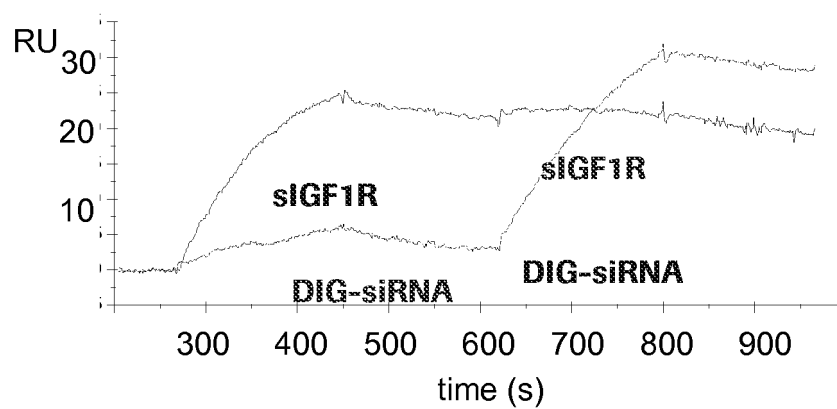


Fig. 47 o)

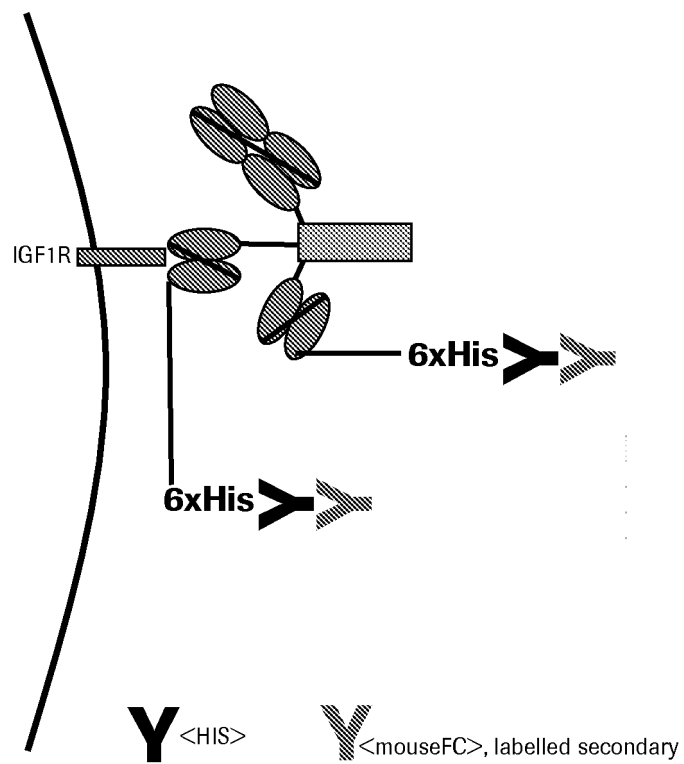
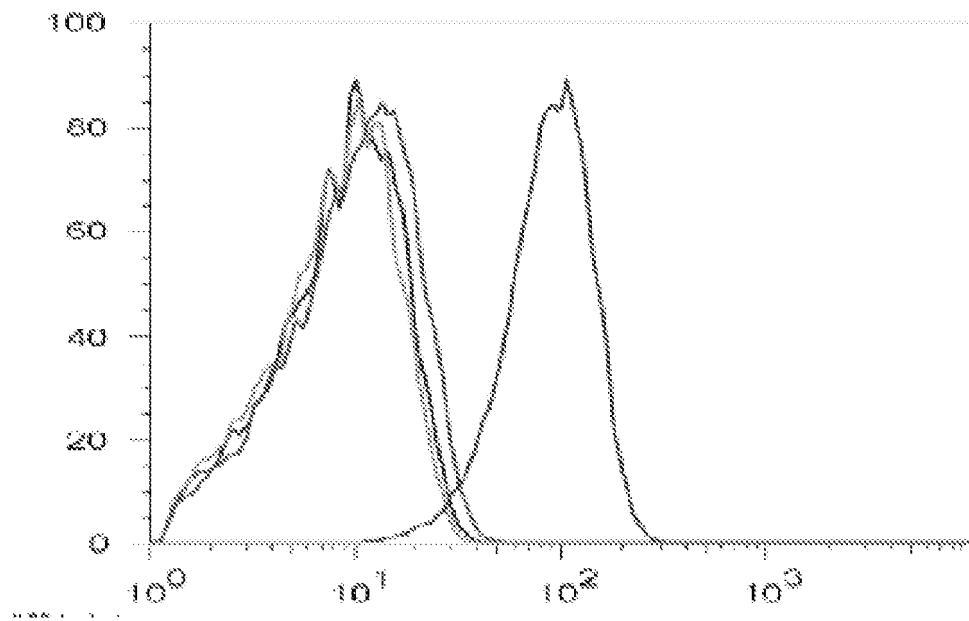


Fig. 47 p)

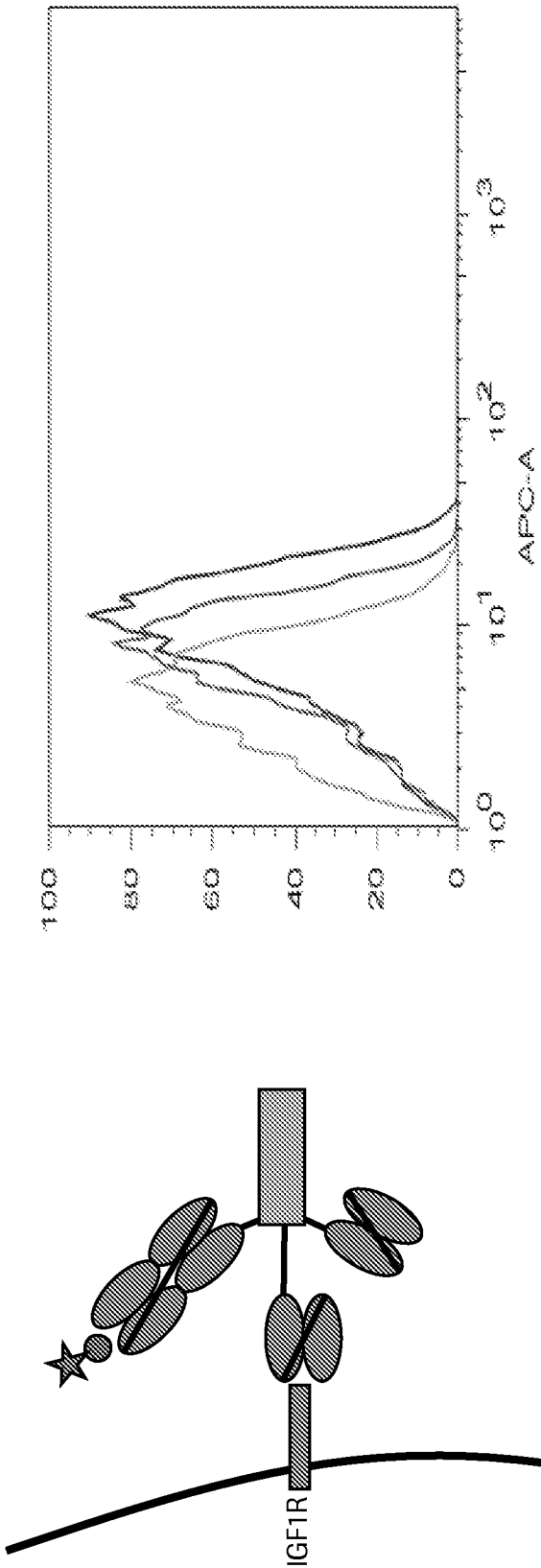


Fig. 47 q)



Fig. 47 r)

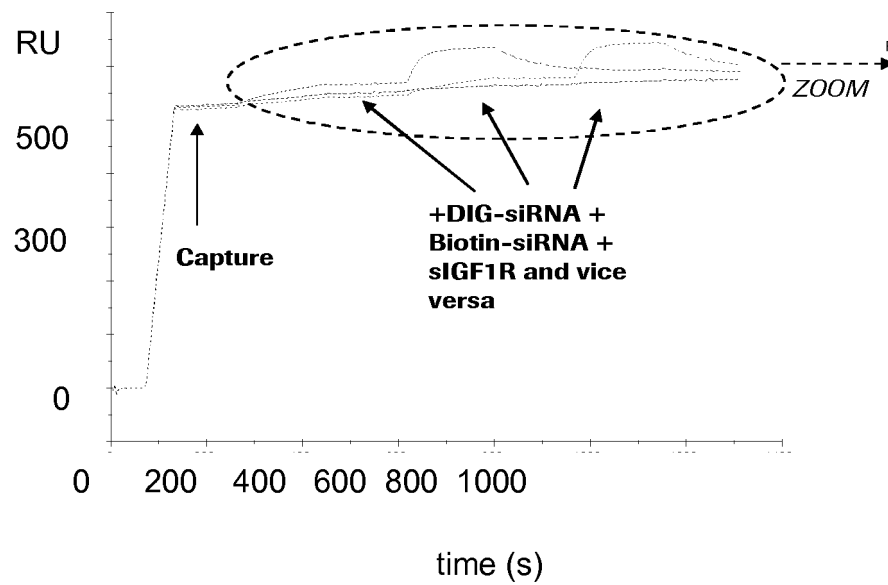


Fig. 47 s)

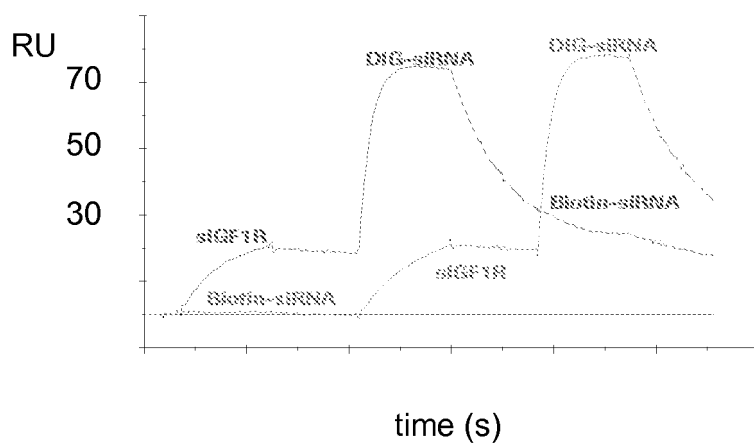


Fig 47 t)

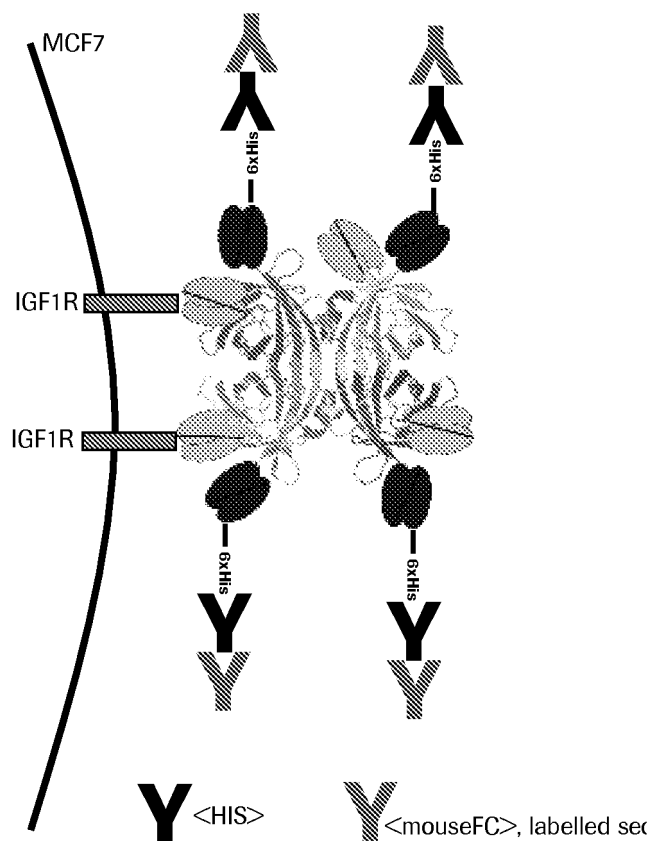
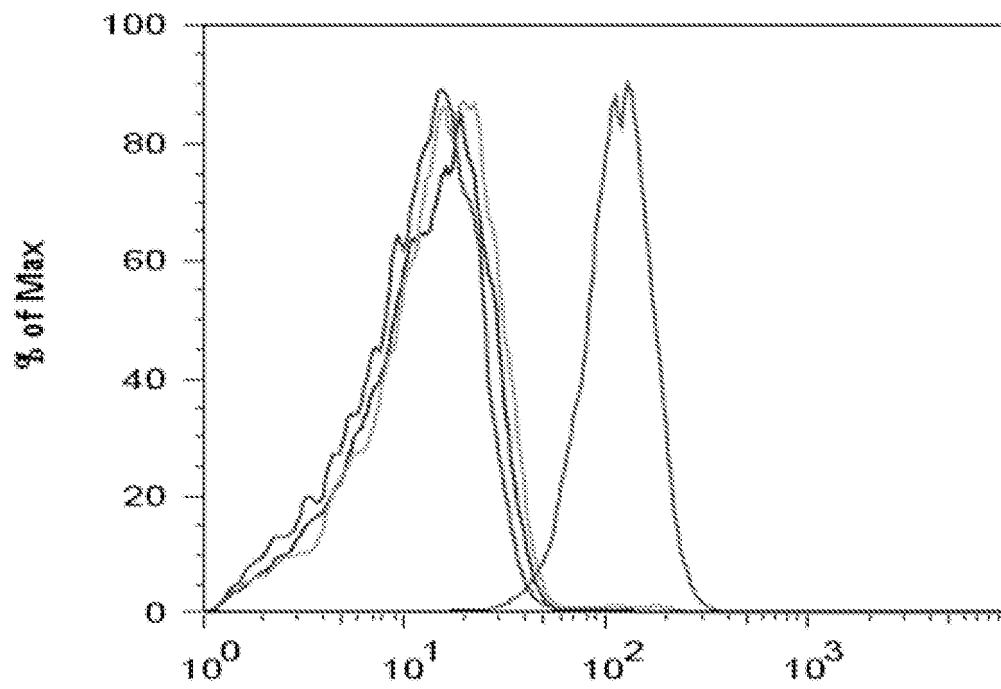


Fig. 47 u)

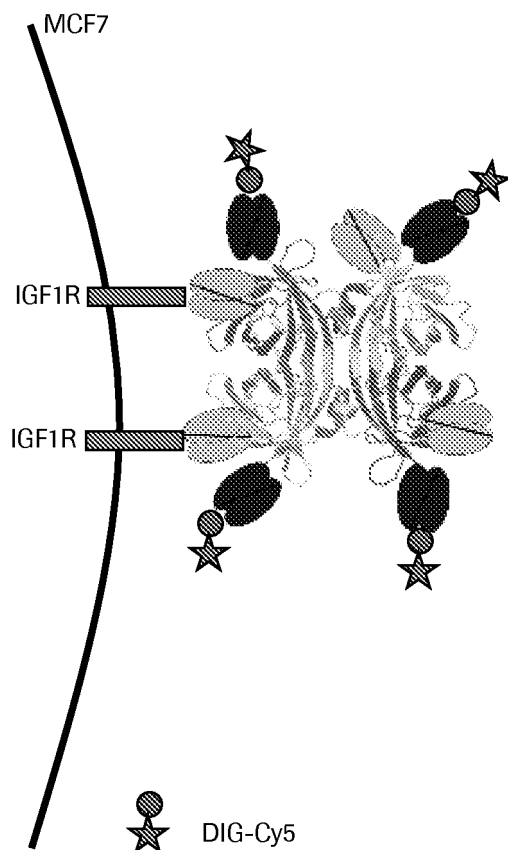
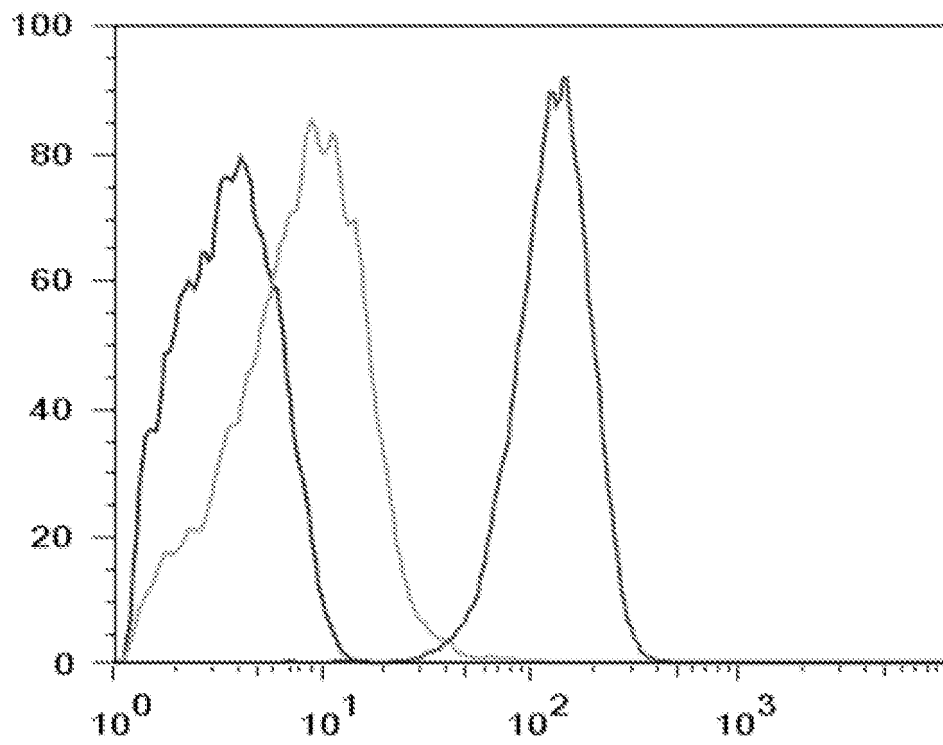


Fig. 47 v)

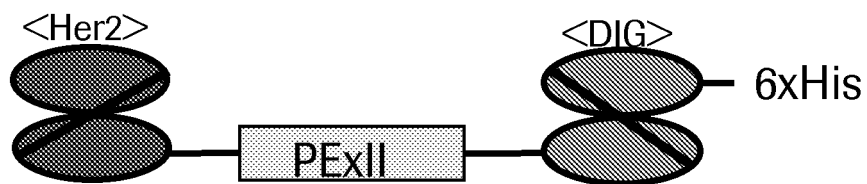


Fig. 47 w)

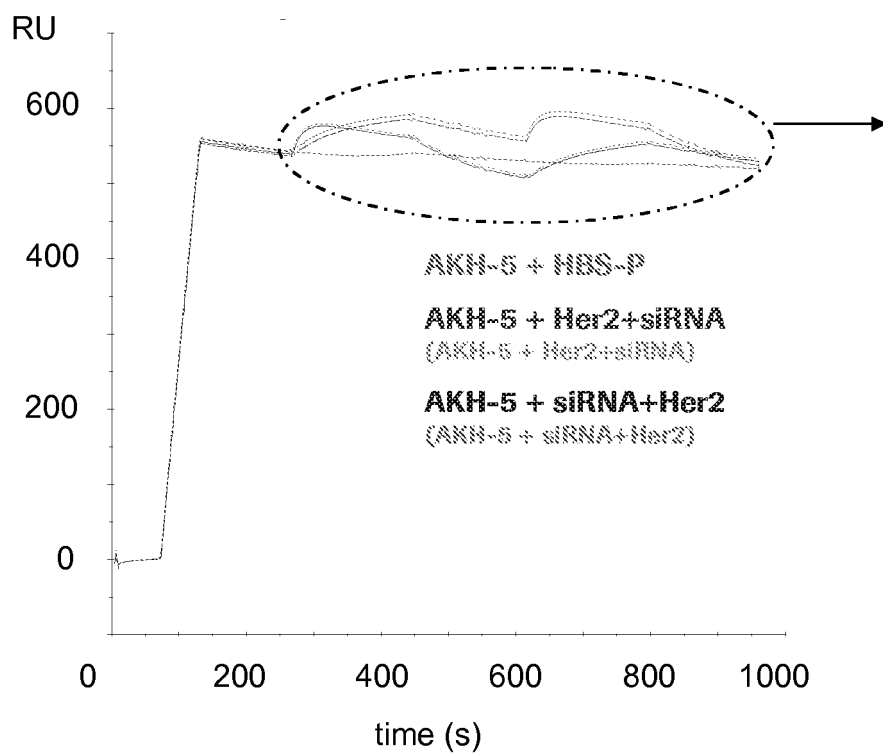


Fig. 47 x)

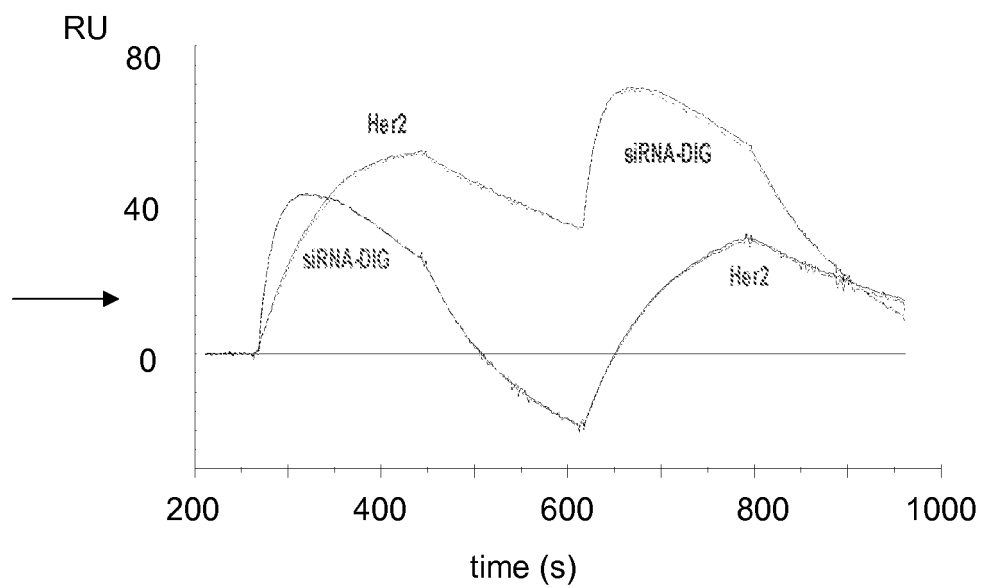


Fig. 48 a)

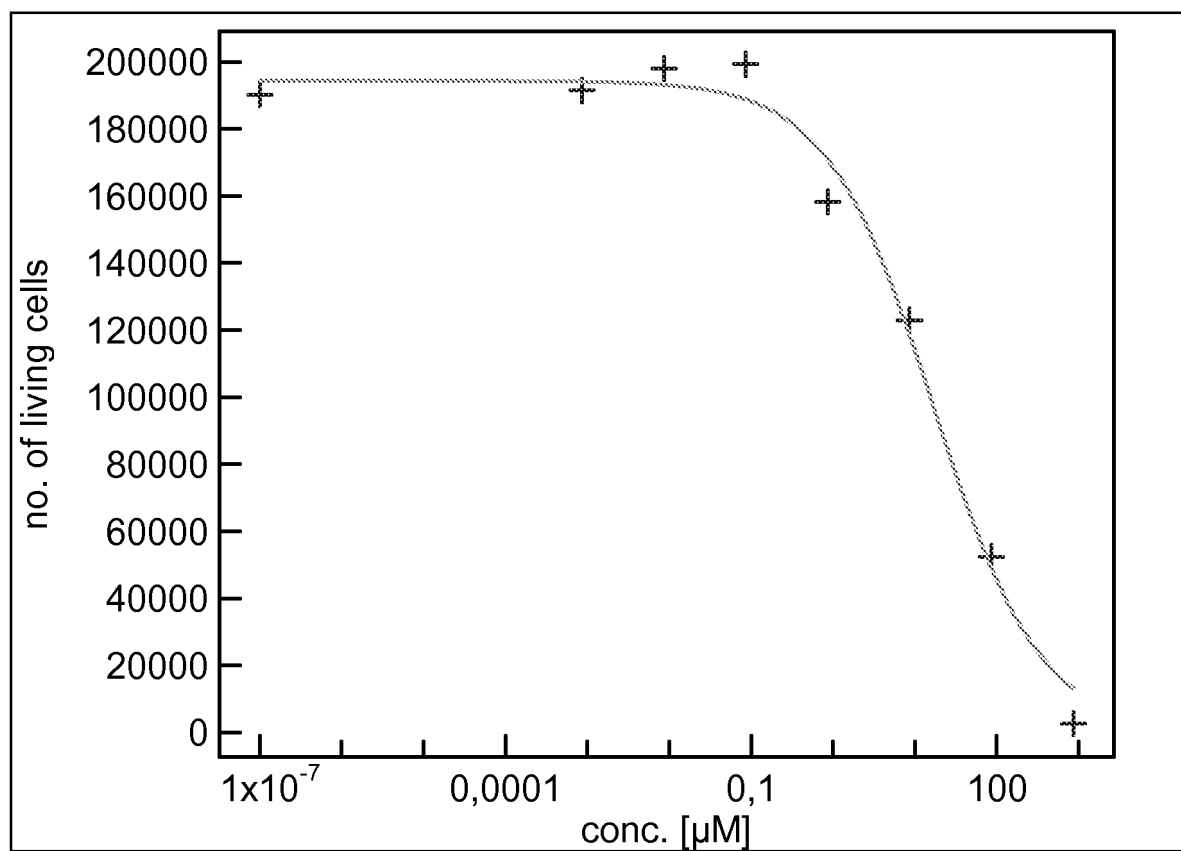


Fig. 48 b)

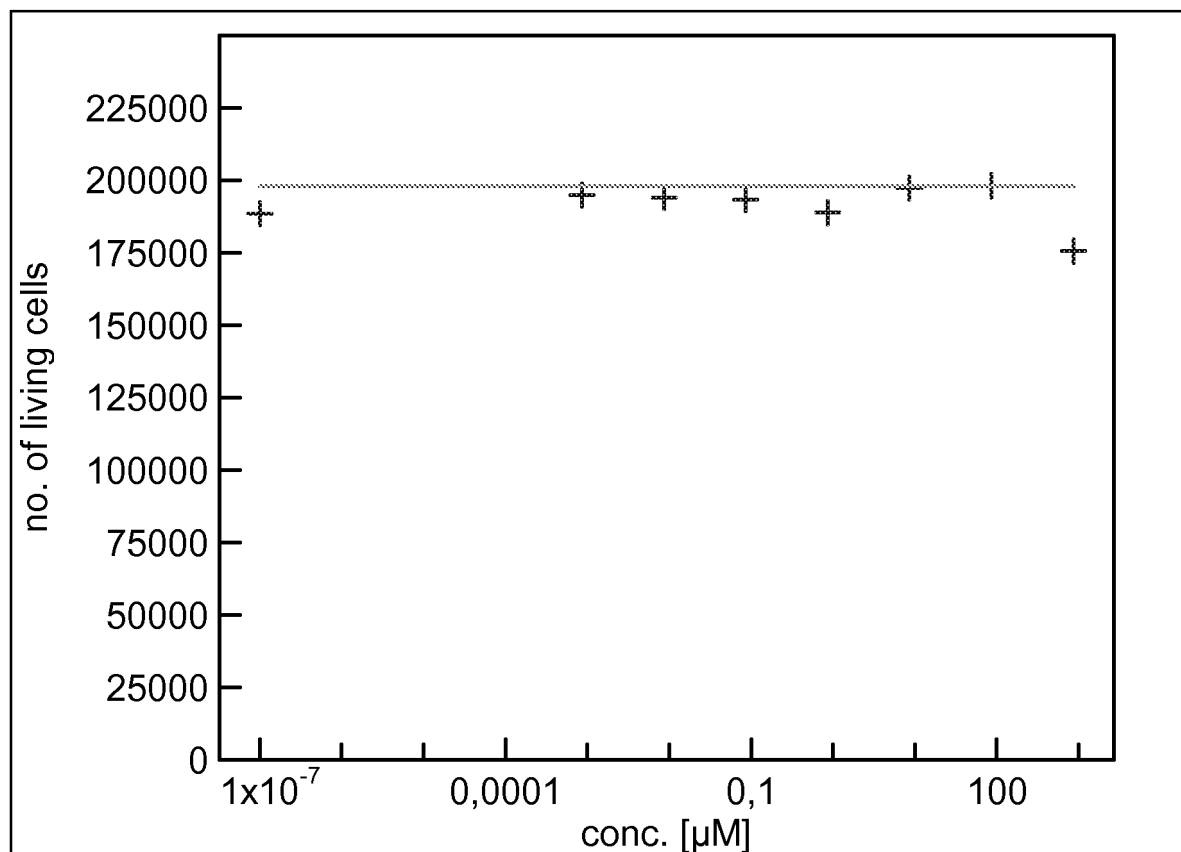


Fig. 48 c)

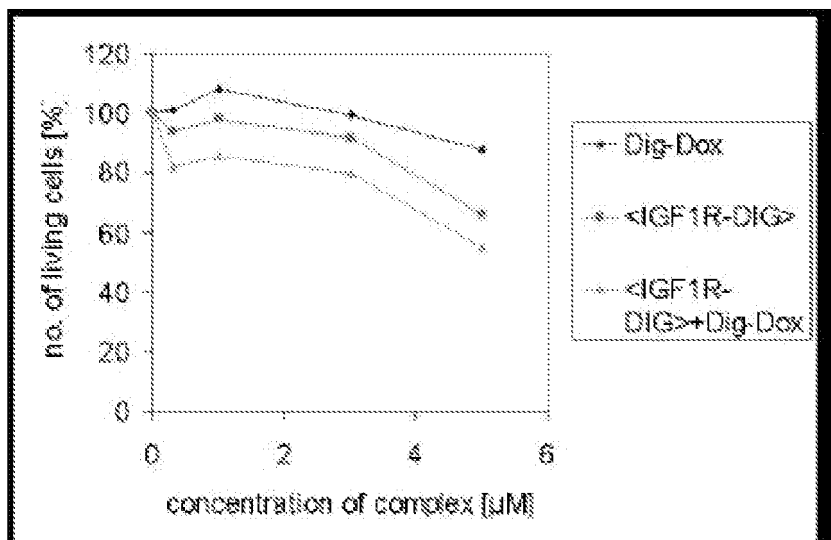


Fig. 48 d)

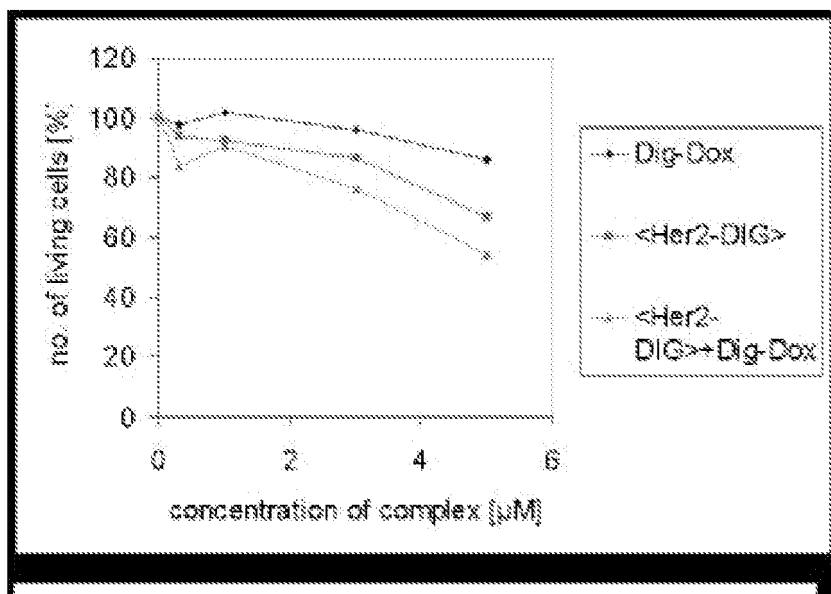


Fig. 48 e)

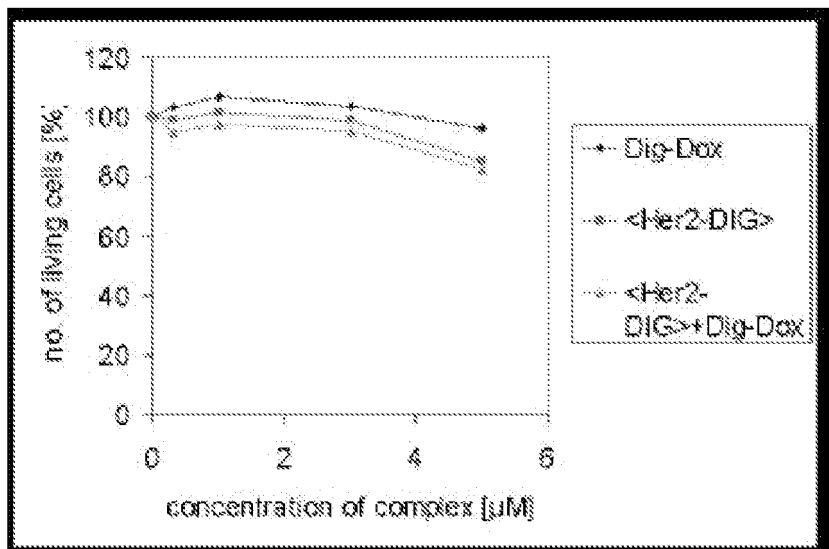


Fig. 49 a)

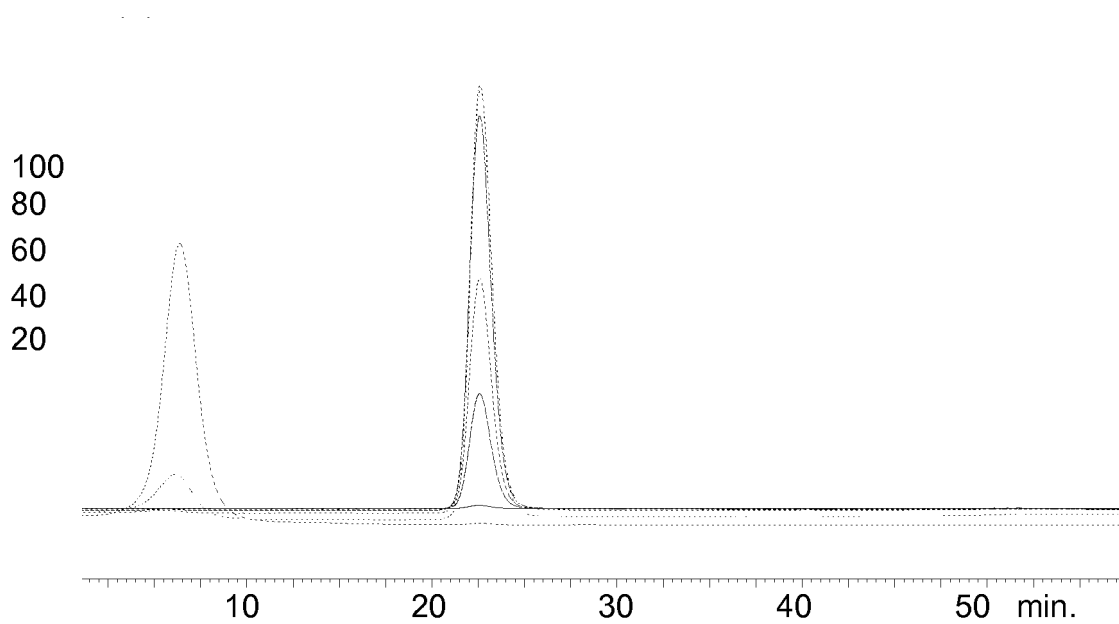


Fig. 49 b)

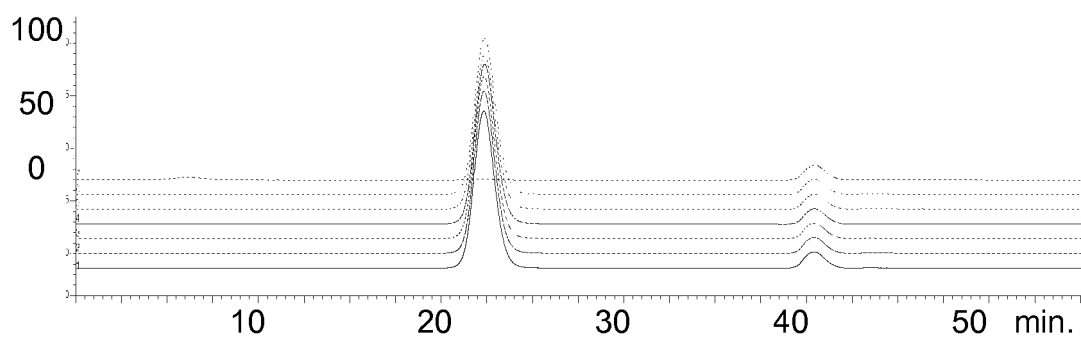


Fig. 49 c)

| Molar ratio<br>AK: DIG-Cy5 | Area<br>(Ex. 650 nm / Em. 667 nm)<br>[mAU*min] |          |            |
|----------------------------|------------------------------------------------|----------|------------|
|                            | MAb Her2-DIG                                   | MAb Her2 | MAb IGF-1R |
| 1:0                        | 2                                              | 2        | 1          |
| 1:0,5                      | 46                                             | 2        | 1          |
| 1:1                        | 94                                             | 2        | 1          |
| 1:2                        | 159                                            | 2        | 1          |
| 1:3                        | 175                                            | 2        | 1          |
| 1:5                        | 177                                            | 2        | 1          |
| 0:1                        | 1                                              | 0        | 0          |

Fig. 49 d)

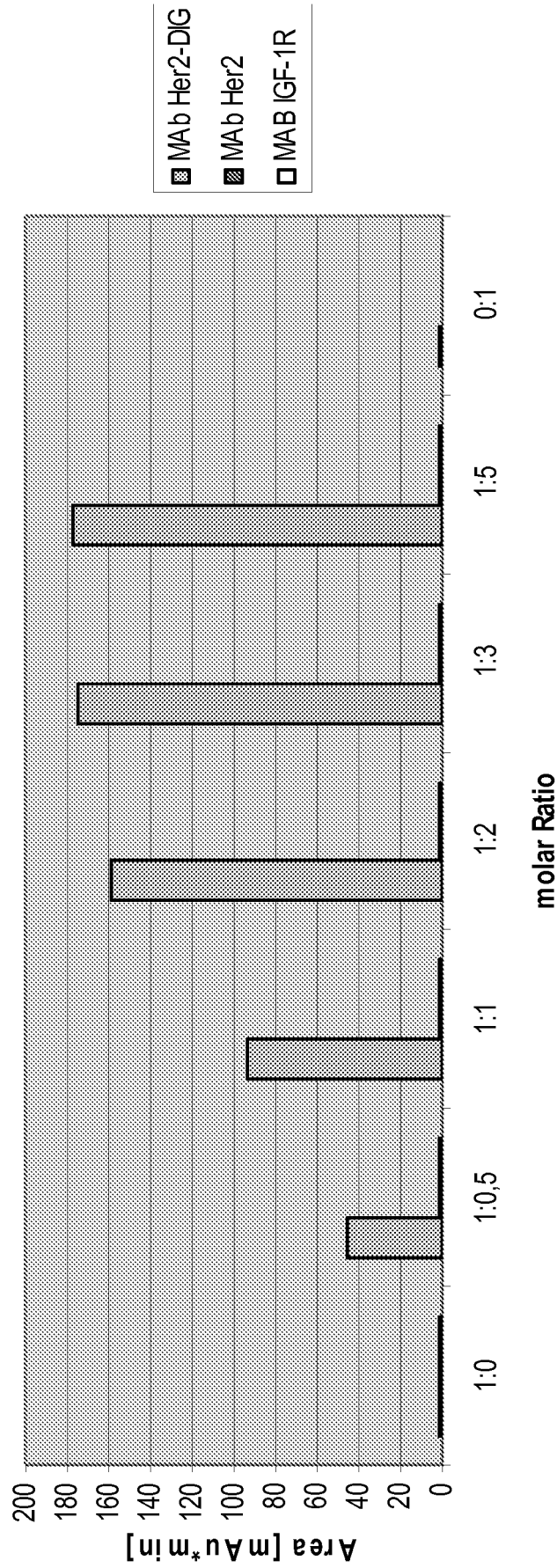


Fig. 50 a) (replacement sheet)

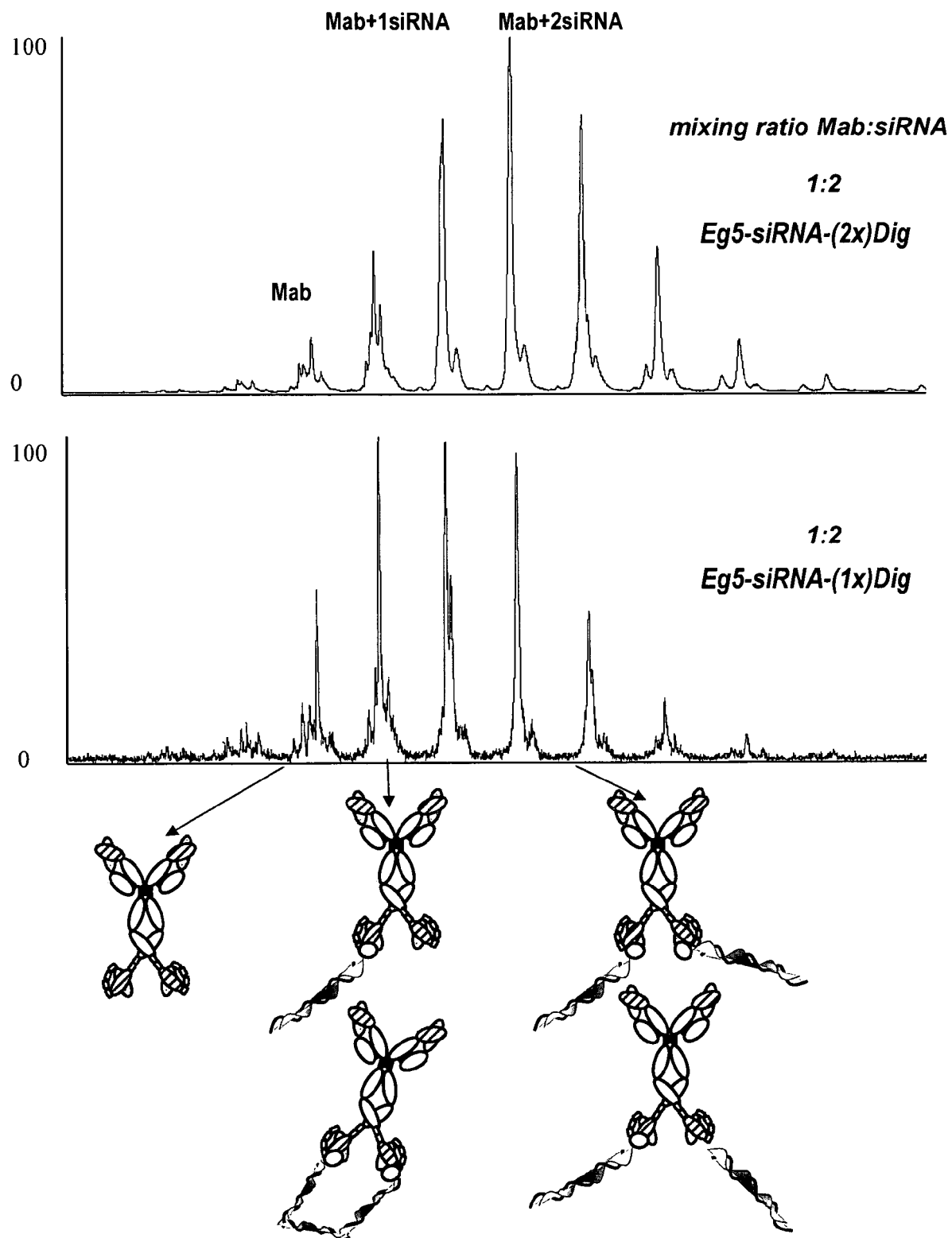


Fig. 50 b)

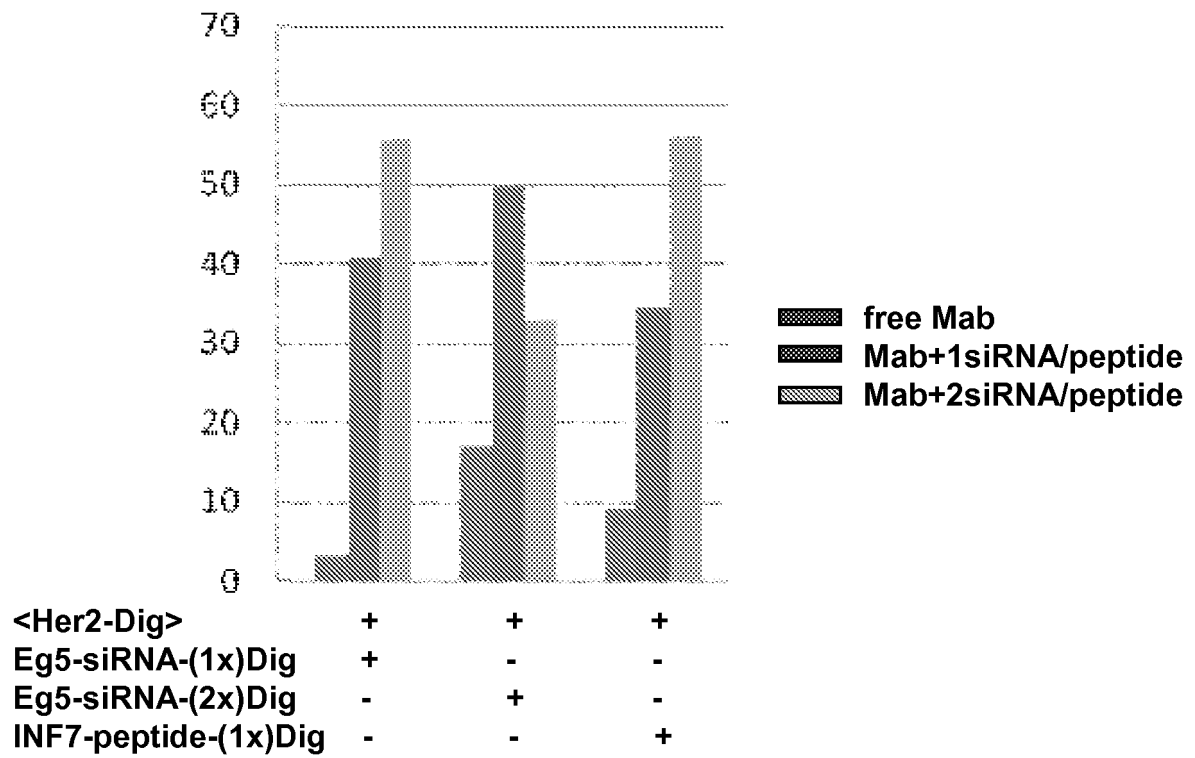


Fig. 51 a)

| cell surface antigen | molecule         | format                    |
|----------------------|------------------|---------------------------|
| IGF1-Receptor        | <IGF1R-DIG> Fv   | scFv/dsscFv; Dig - CH3    |
| IGF1-Receptor        | <IGF1R-DIG> Fv   | scFv/dsscFv; Dig - Ckappa |
| IGF1-Receptor        | <IGF1R-DIG> Fv   | dsscFv; Dig- CH3+Ckappa   |
| IGF1-Receptor        | <IGF1R-DIG> Fab  | scFab; Dig- CH3           |
| IGF1-Receptor        | <IGF1R-DIG> Fab  | dsscFab; Dig- CH3         |
| VEGF-Receptor 2      | <VEGFR2-DIG> Fv  | scFv/dsscFv; Dig - CH3    |
| VEGF-Receptor 2      | <VEGFR2-DIG> Fab | scFab; Dig- CH3           |
| VEGF-Receptor 2      | <VEGFR2-DIG> Fab | dsscFab; Dig- CH3         |
| CD33                 | <CD33-DIG> Fab   | dsscFab; Dig- CH3         |
| CD33                 | <CD33-DIG> Fv    | scFv/dsscFv; Dig - CH3    |
| LeY carbohydrate     | <LeY-DIG> Fab    | dsscFab; Dig- CH3         |
| LeY carbohydrate     | <LeY-DIG> Fv     | scFv/dsscFv; Dig - CH3    |
| CD22                 | <CD22-DIG> Fv    | dsscFv; Dig - CH3         |
| Her2                 | <Her2-DIG> Fv    | dsscFv; Dig - CH3         |
| CDCP1                | <CDCP1-DIG> Fv   | dsscFv; Dig - CH3         |

Fig. 51 b)

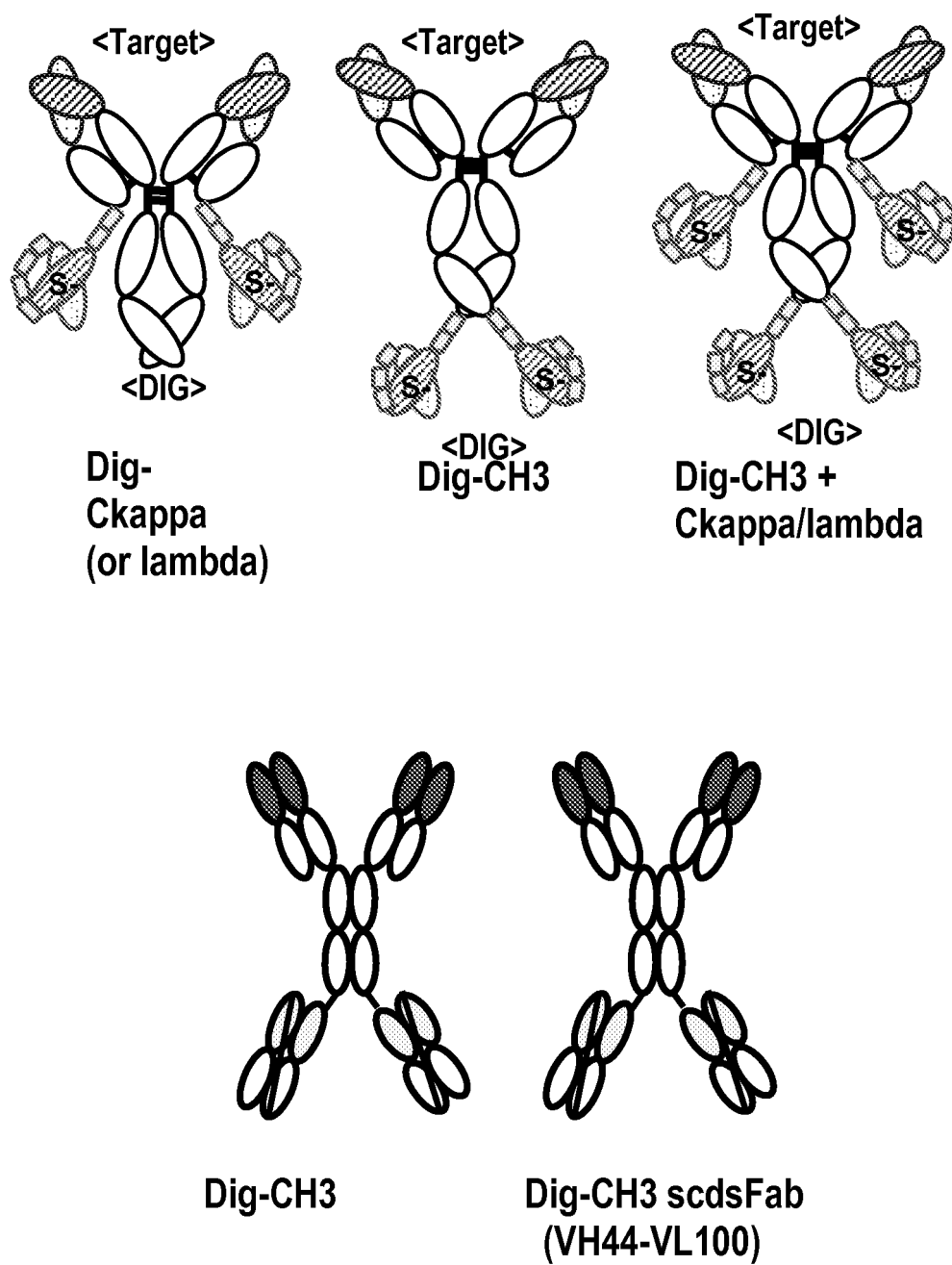


Fig. 51 c)

| Capture         | Antibody                 | ka (1/Ms) | kd (1/s) | KD (M)   |
|-----------------|--------------------------|-----------|----------|----------|
| human VEGFR2-Fc | <VEGFR2(D1F7)-DIG>_001   | 3.44E+06  | 6.54E-03 | 1.90E-09 |
|                 | <VEGFR2(D1F7)-scFab-DIG> | 4.98E+06  | 6.70E-03 | 1.35E-09 |
|                 | <VEGFR2(D1F7)-dsFab-DIG> | 2.49E+06  | 9.68E-03 | 3.89E-09 |
| mouse VEGFR2-Fc | <VEGFR2(D1F7)-DIG>_001   | 3.89E+06  | 6.56E-03 | 1.69E-09 |
|                 | <VEGFR2(D1F7)-scFab-DIG> | 3.21E+06  | 8.92E-03 | 2.78E-09 |
|                 | <VEGFR2(D1F7)-dsFab-DIG> | 2.01E+06  | 1.14E-02 | 5.65E-09 |

Figure 51 d)

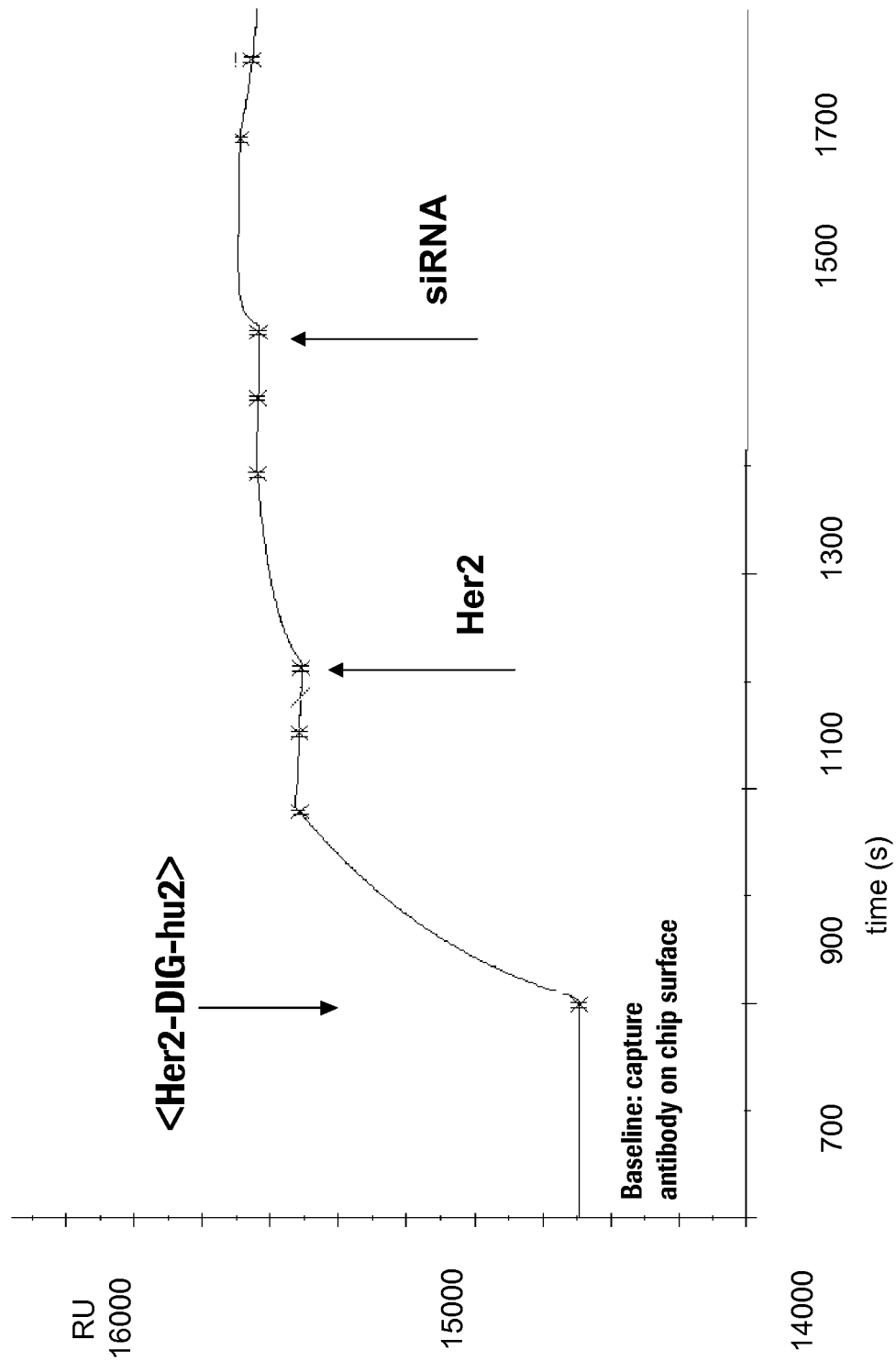


Fig. 51 e)

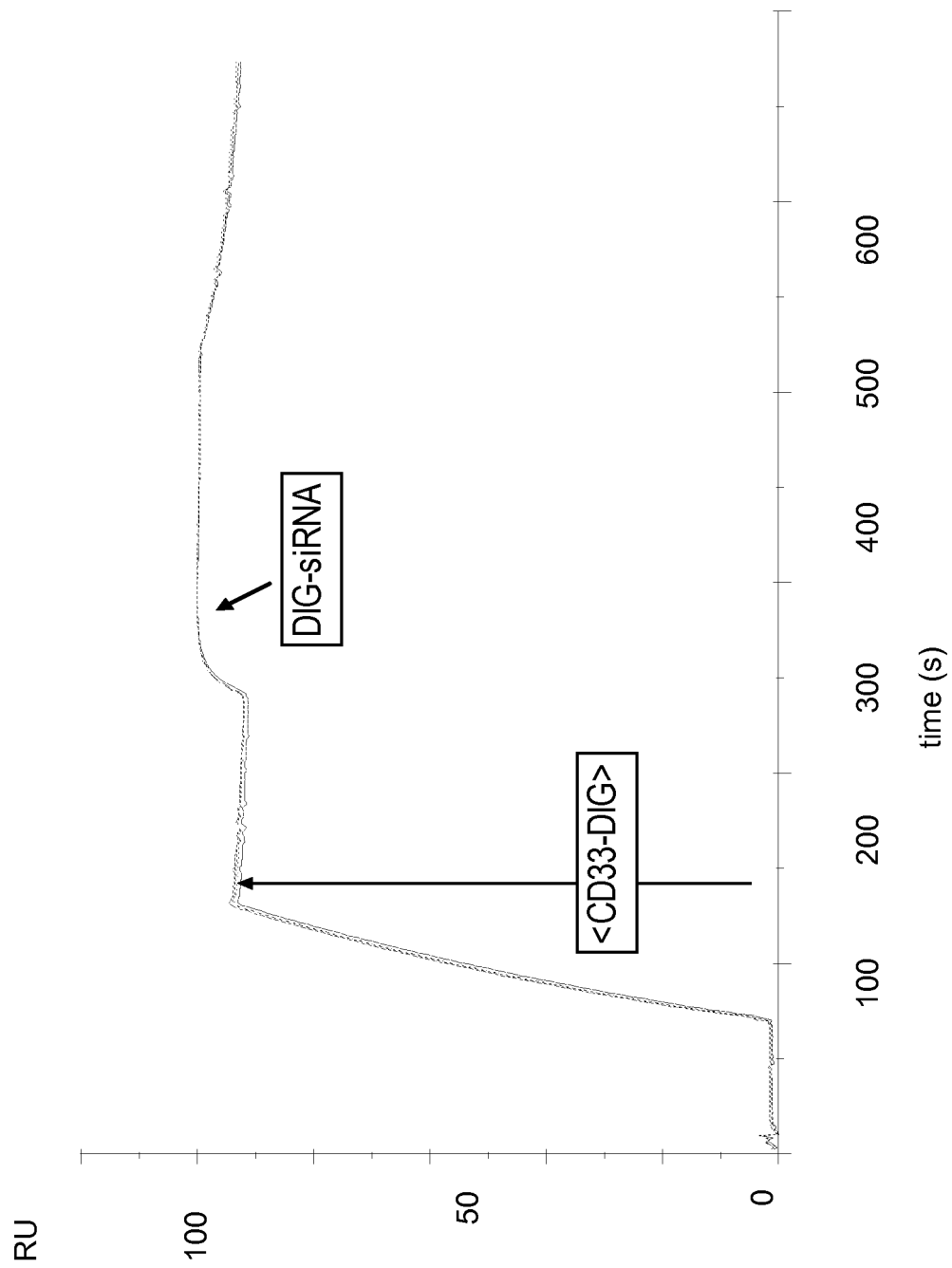


Fig. 51 f)

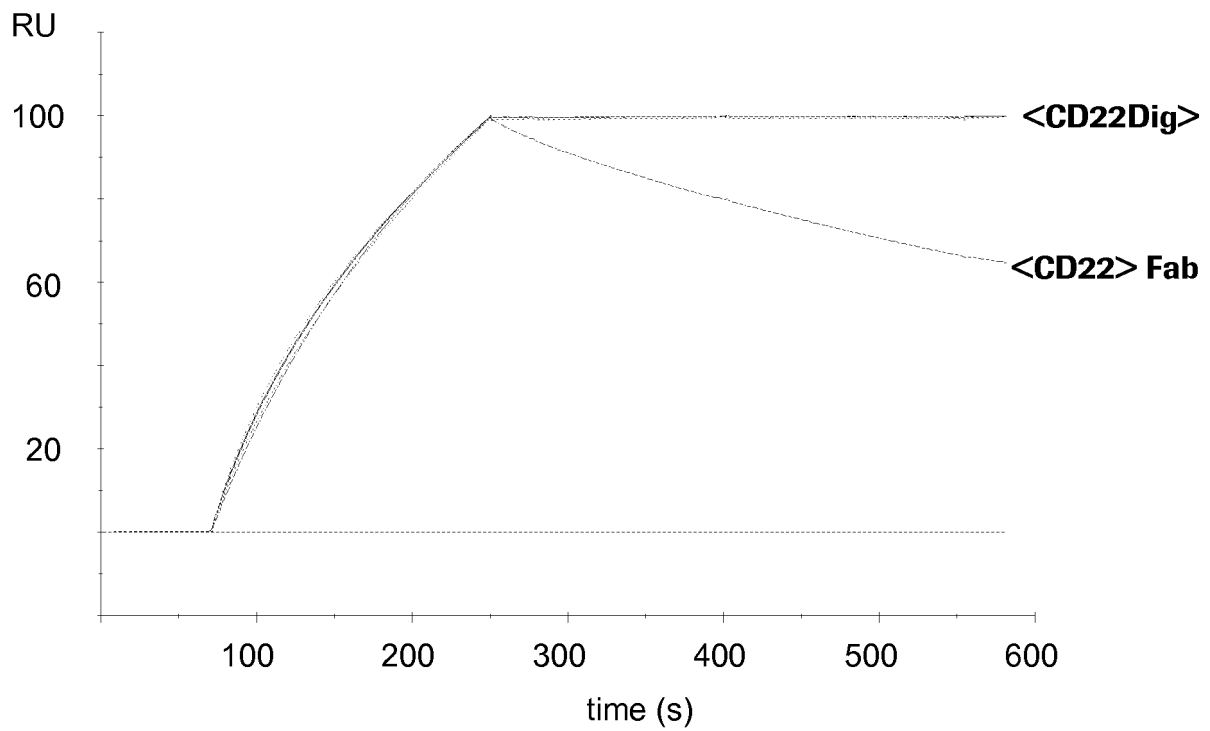


Fig. 51 g)

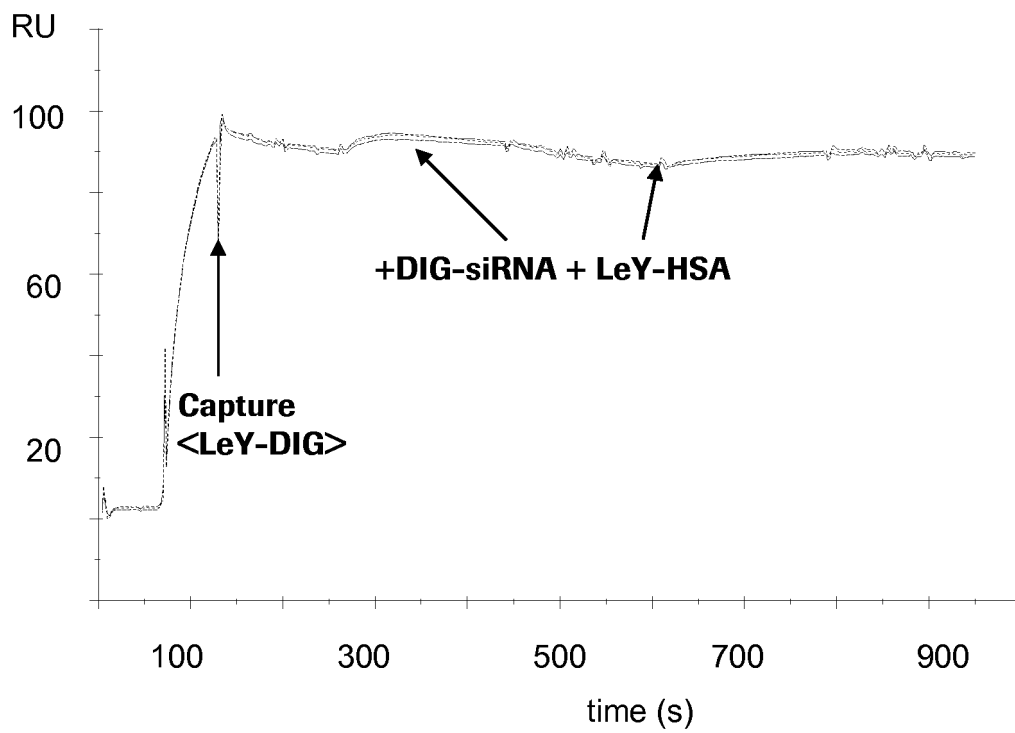


Fig. 51 h)

|                  | <b>ka (1/Ms)</b> | <b>kd (1/s)</b> | <b>KD (nM)</b> |
|------------------|------------------|-----------------|----------------|
| <b>CDCP1-ECD</b> | 2.02E+05         | 1.32E-03        | 7              |
|                  | 2.02E+05         | 1.60E-03        | 8              |
| <b>DIG-siRNA</b> | 4.37E+05         | 0.0097          | 22             |
|                  | 5.54E+05         | 0.011           | 20             |

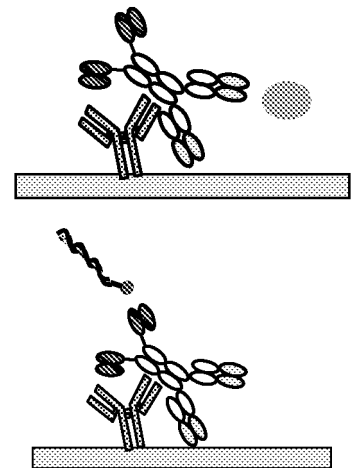


Fig. 52 a)

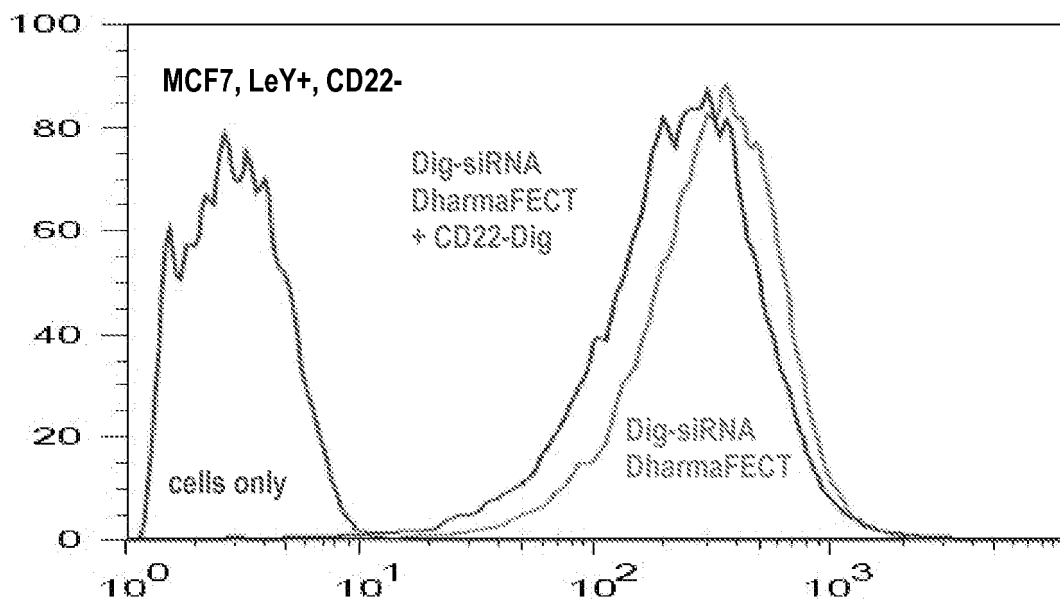


Fig. 52 b)

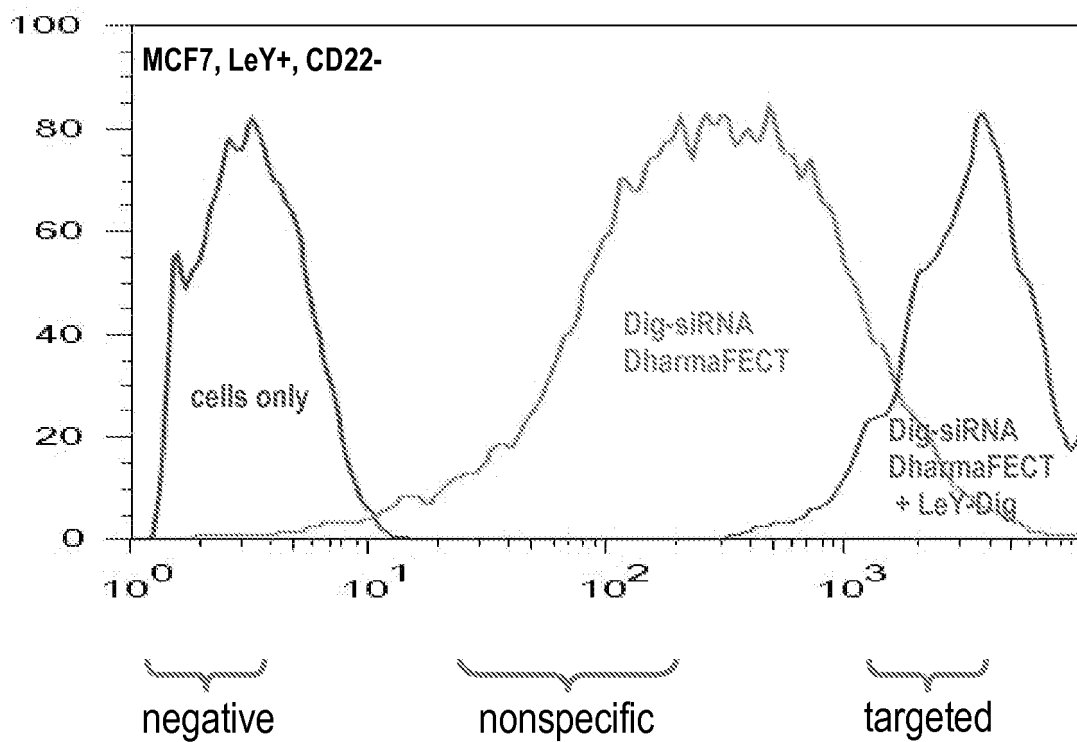


Fig. 52 c)

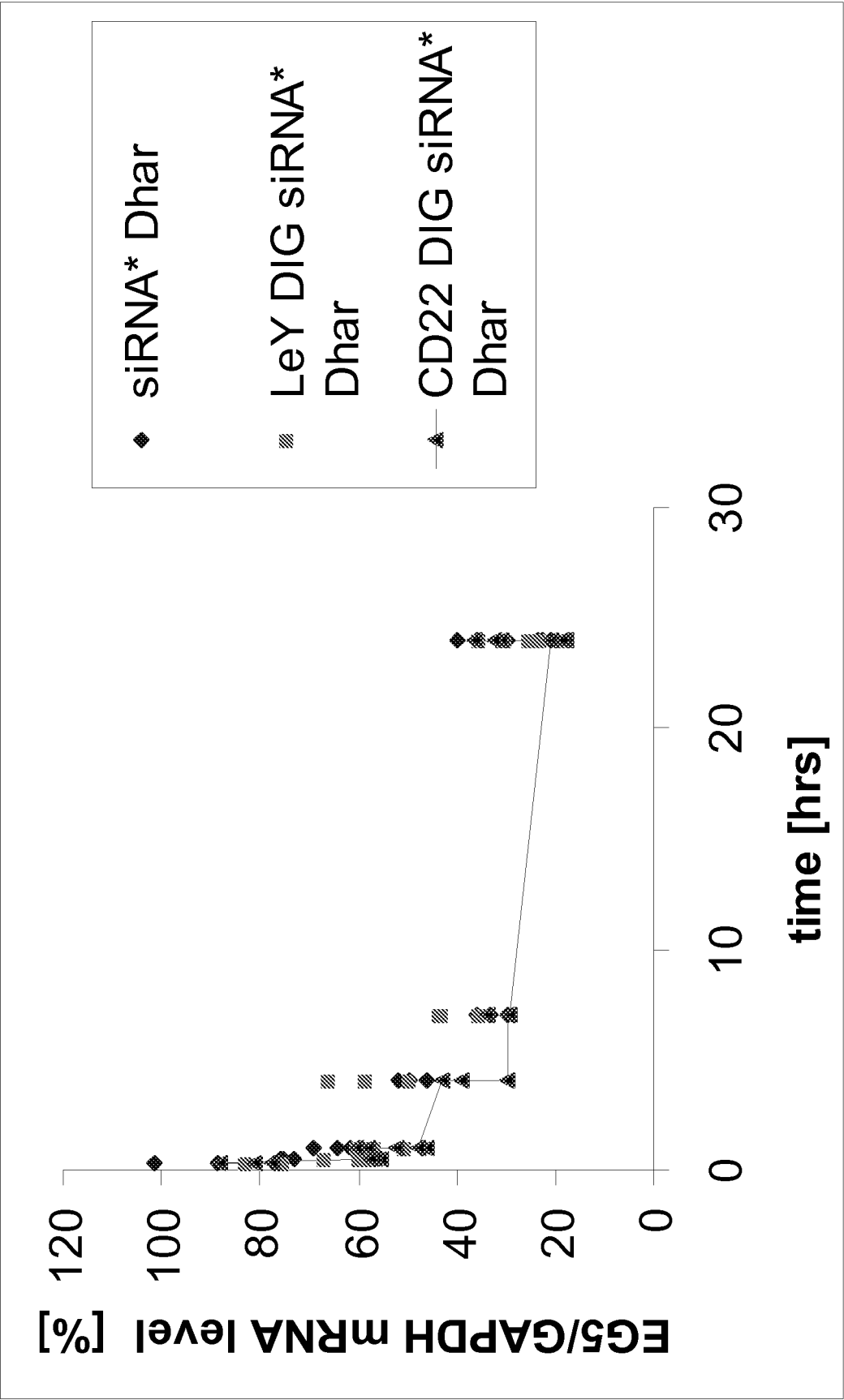


Fig. 53 a)

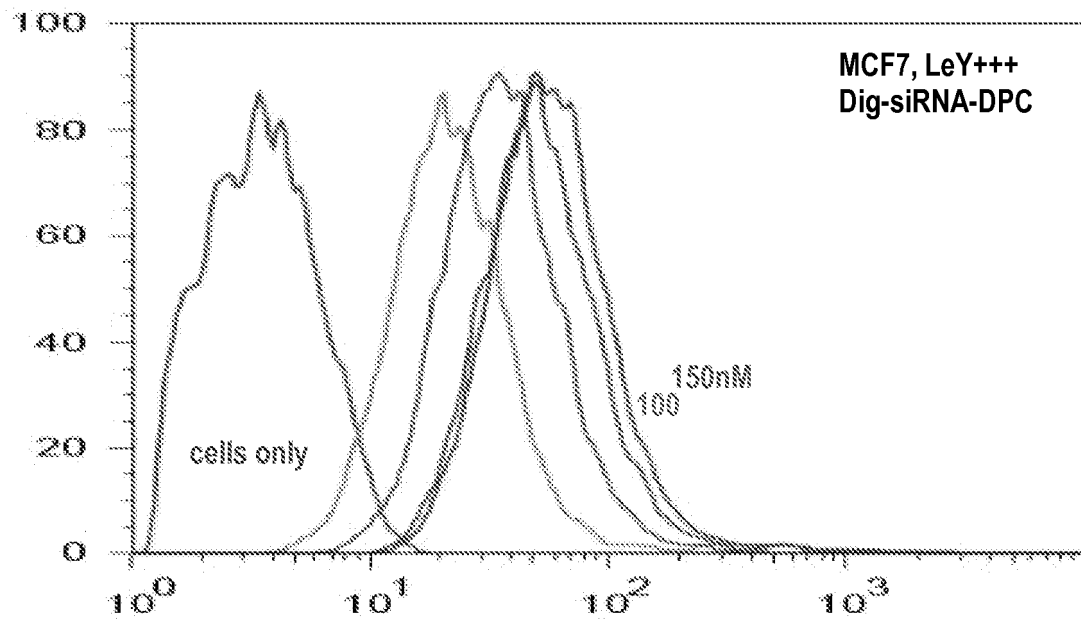


Fig. 53 b)

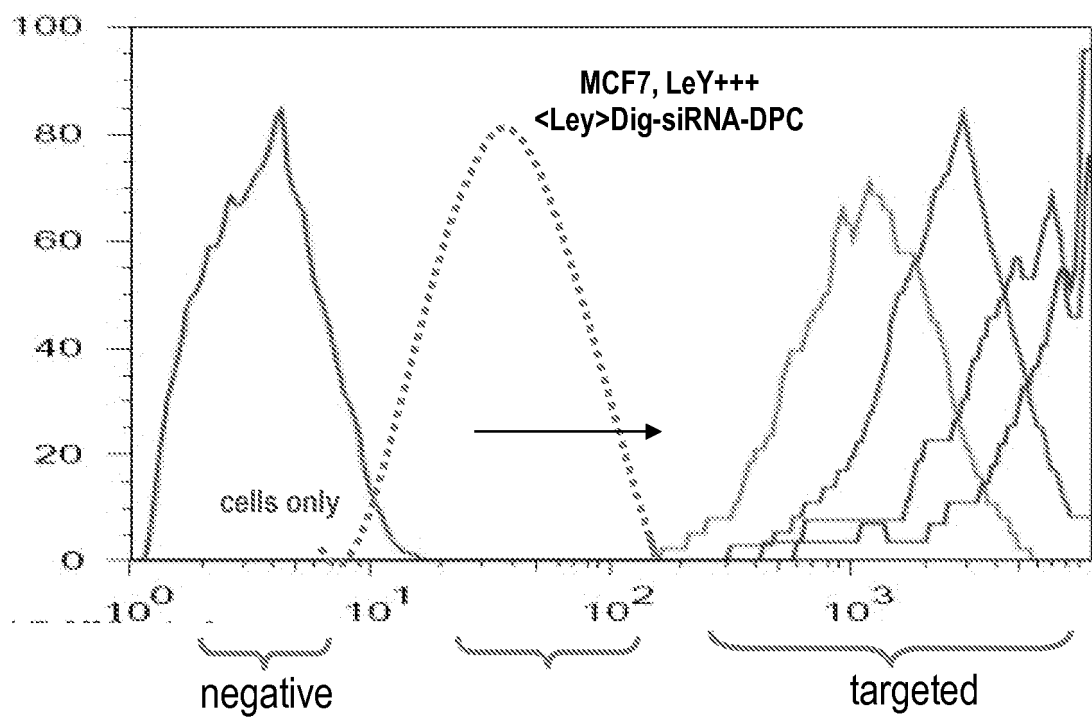


Fig. 53 c)

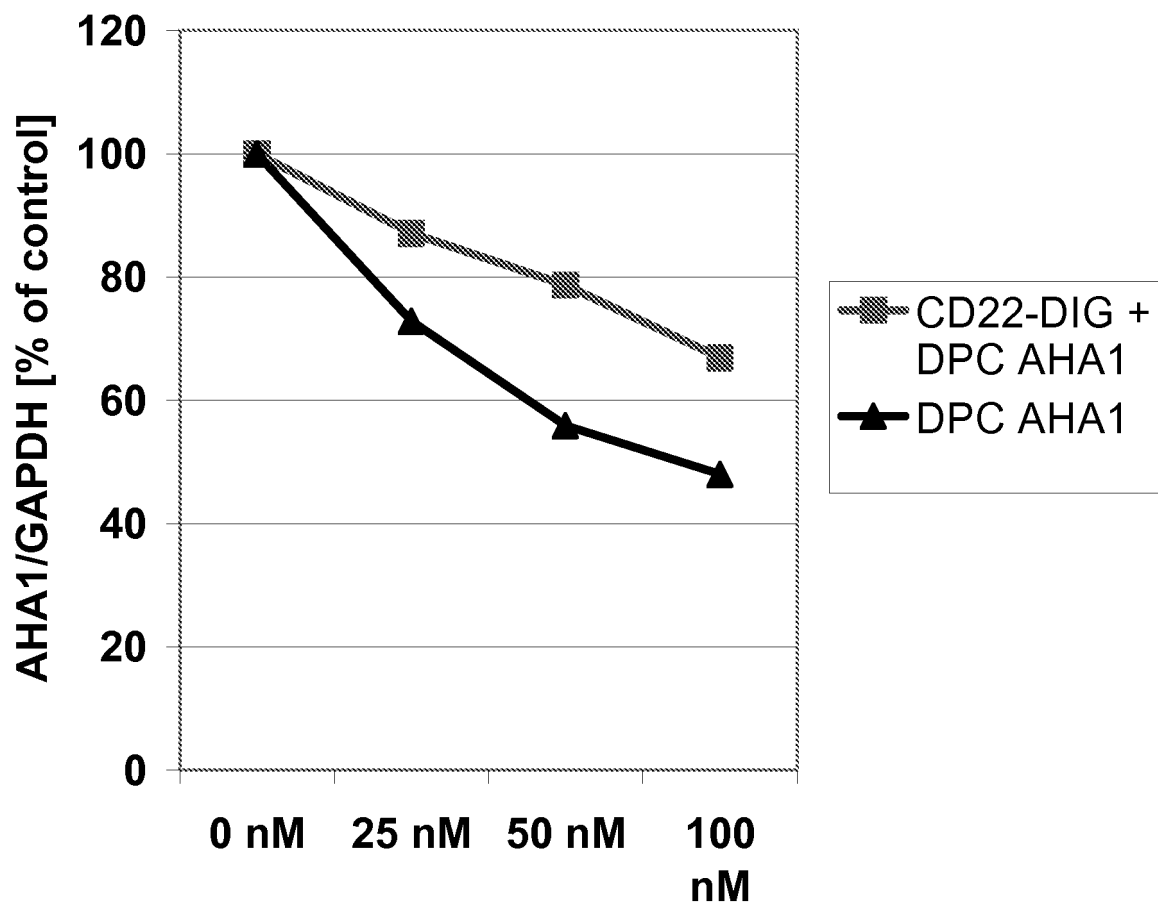


Fig. 53 d)

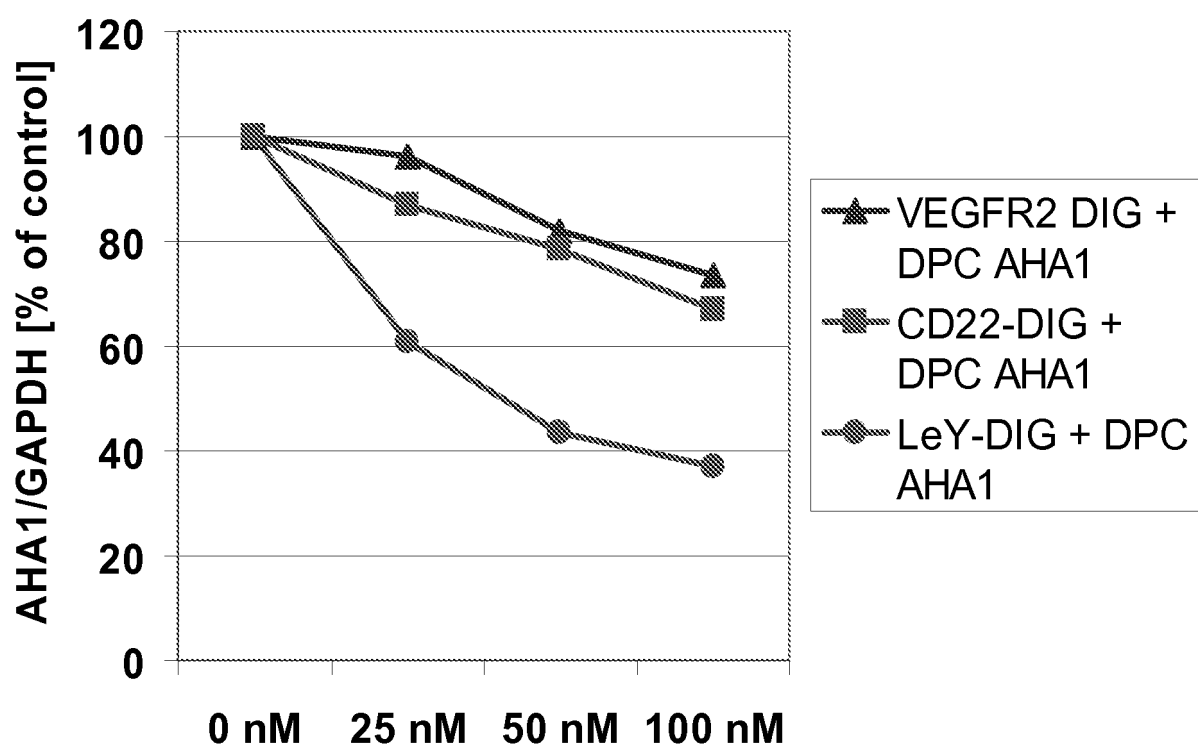


Fig. 53 e)

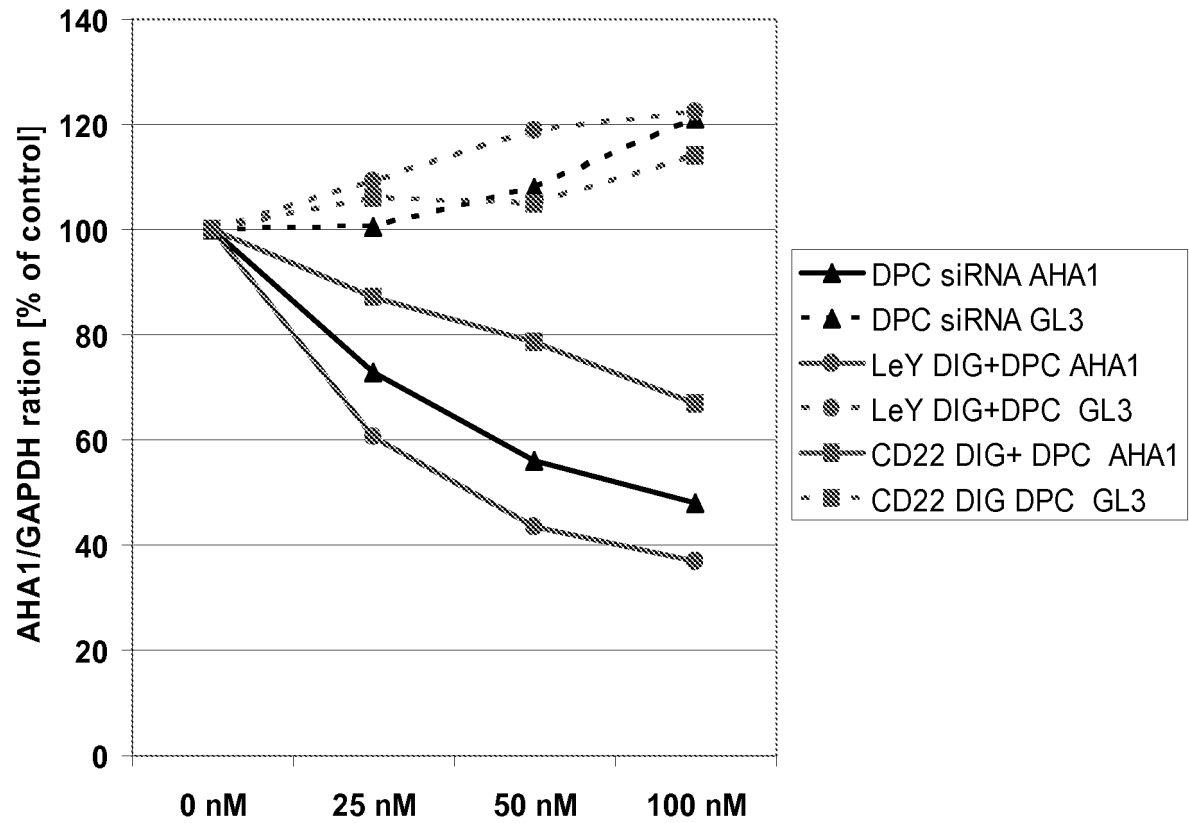


Fig. 53 f)

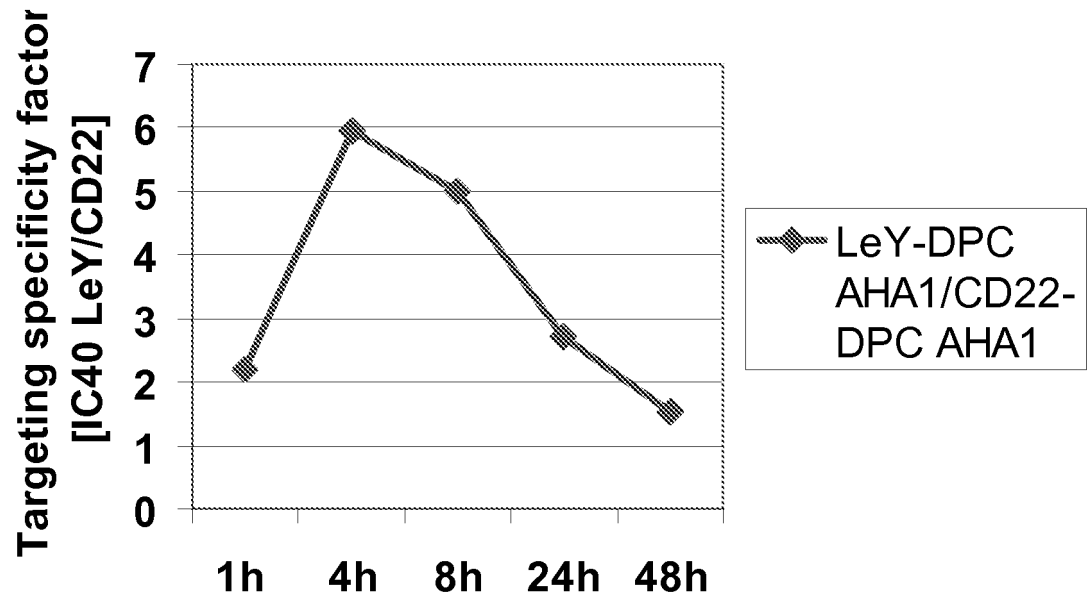


Fig. 54 a)

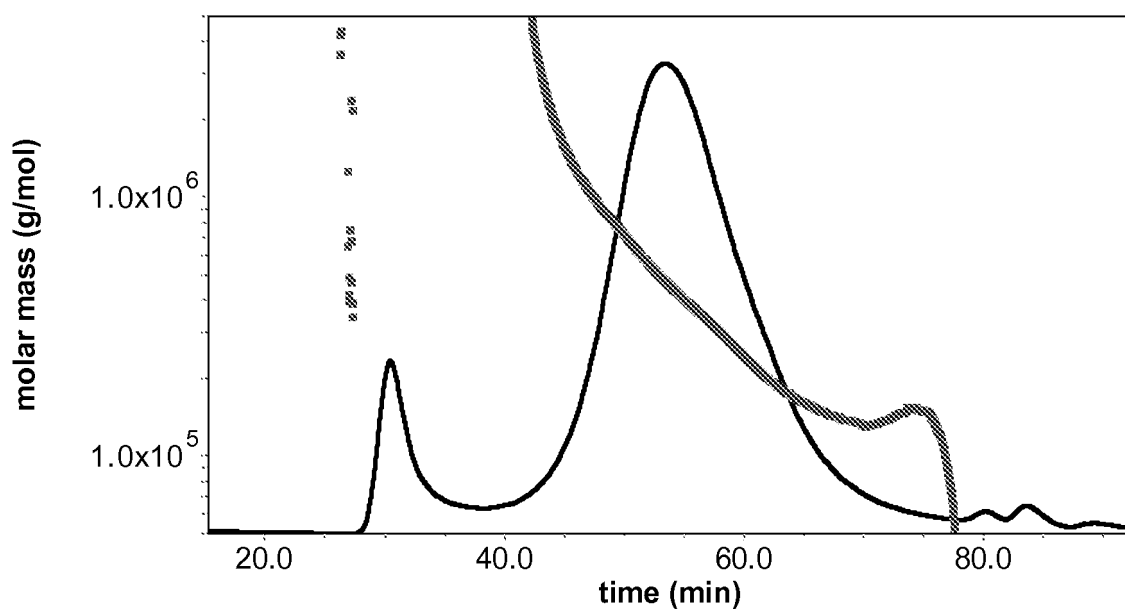


Fig. 54 b)

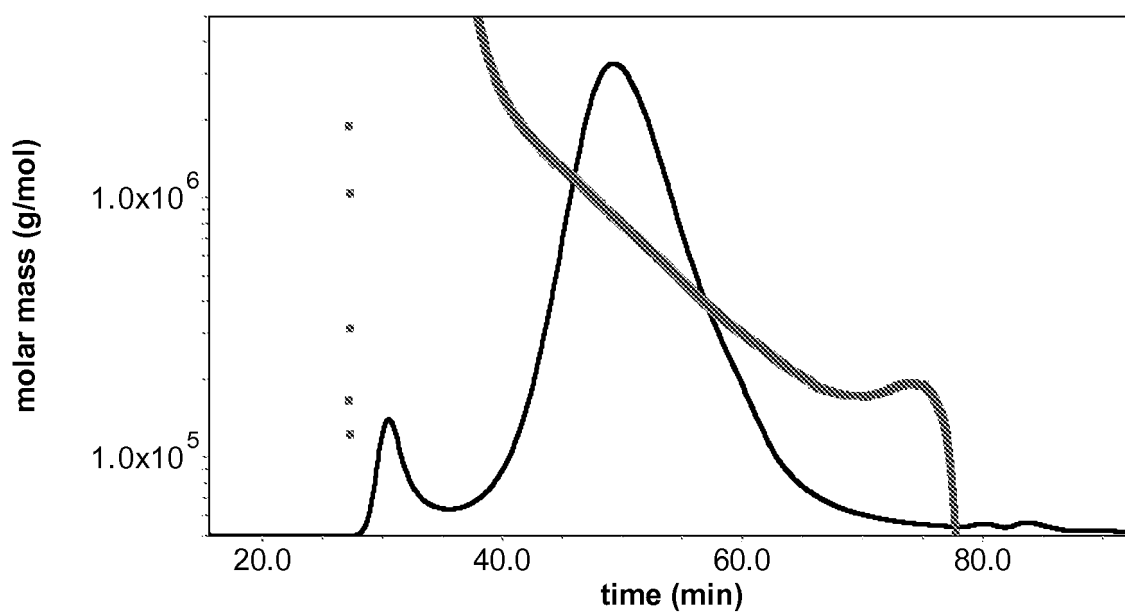


Fig. 54 c)

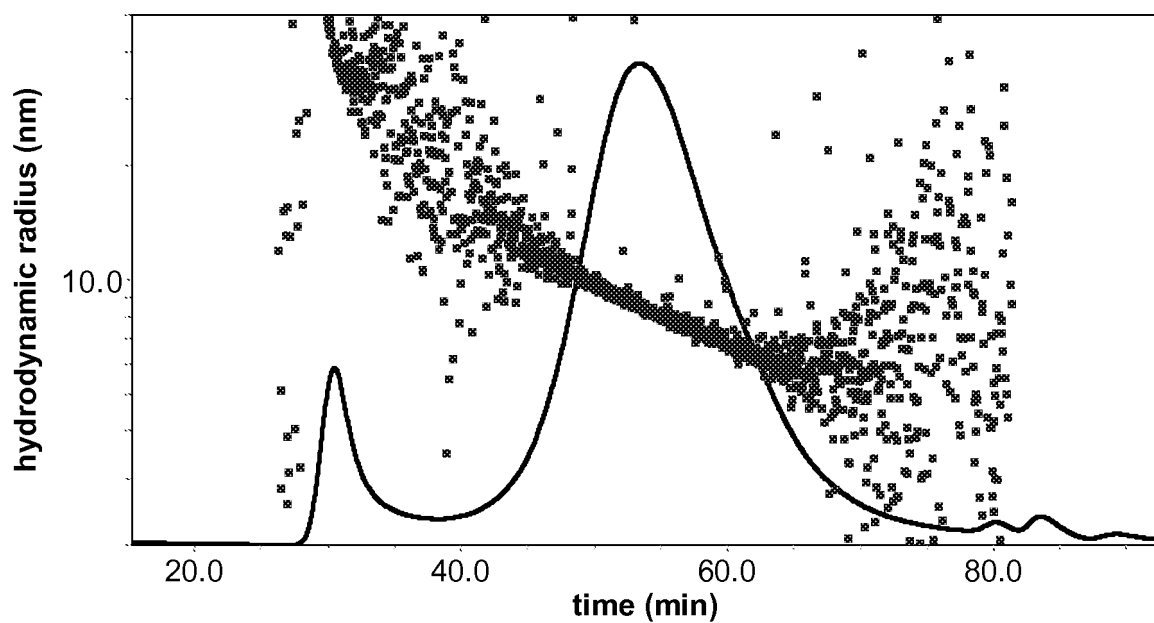


Fig. 54 d)

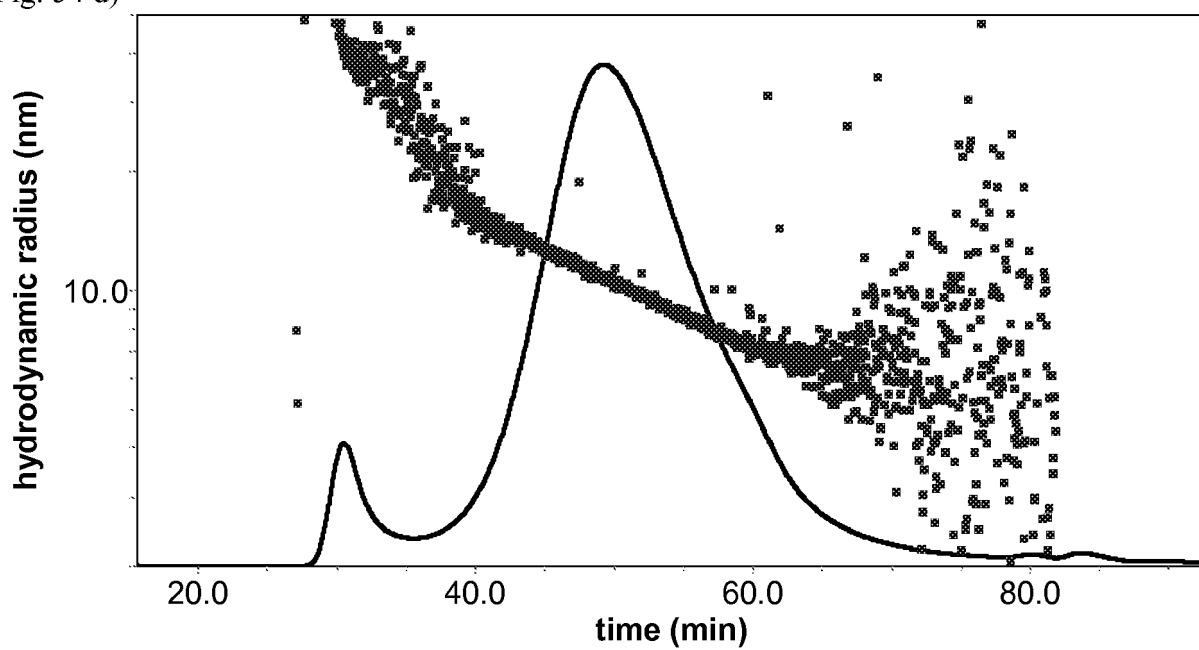


Fig. 55 a)

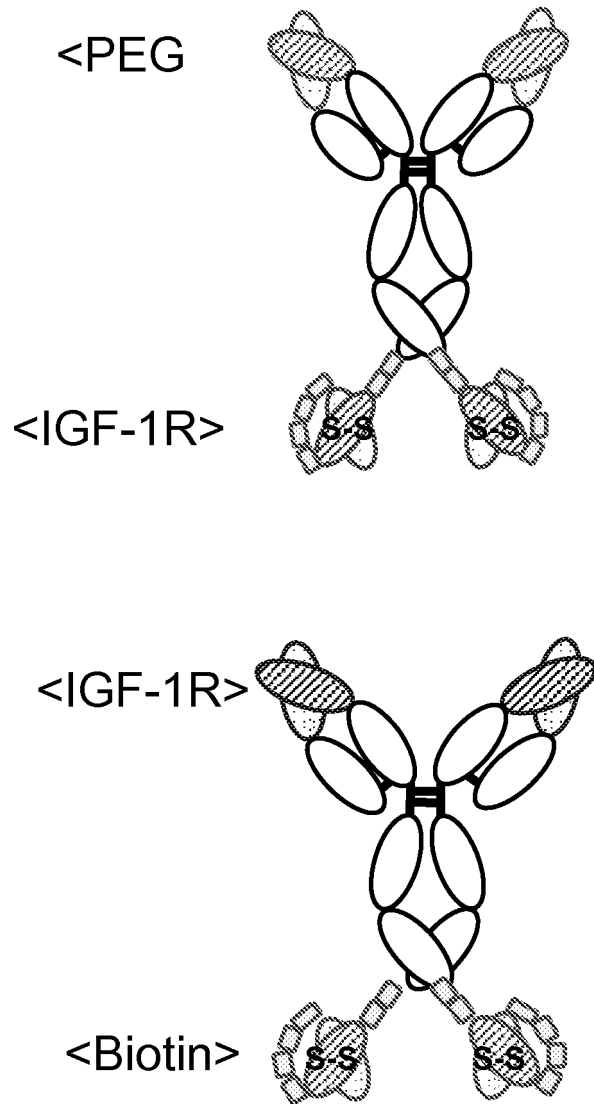


Fig. 55 b)

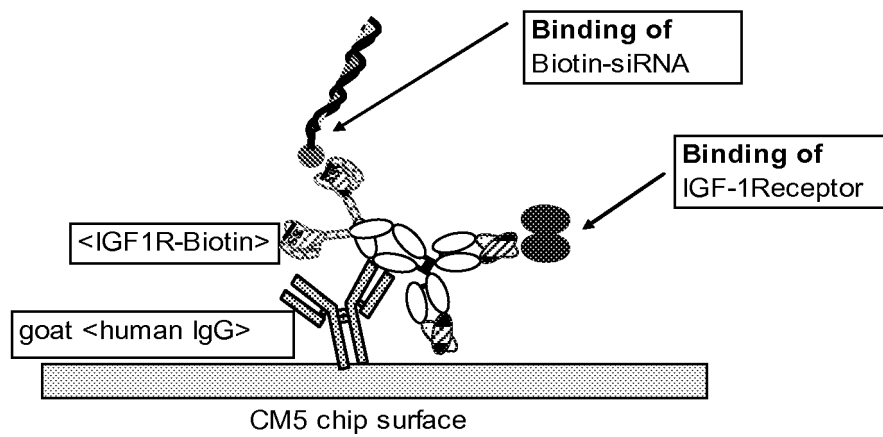


Fig. 55 c)

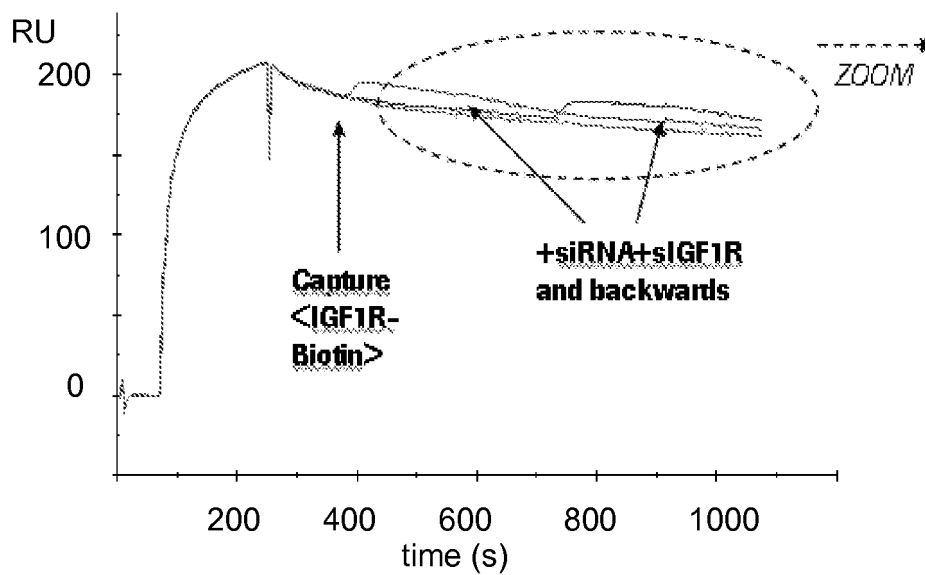


Fig. 55 d)

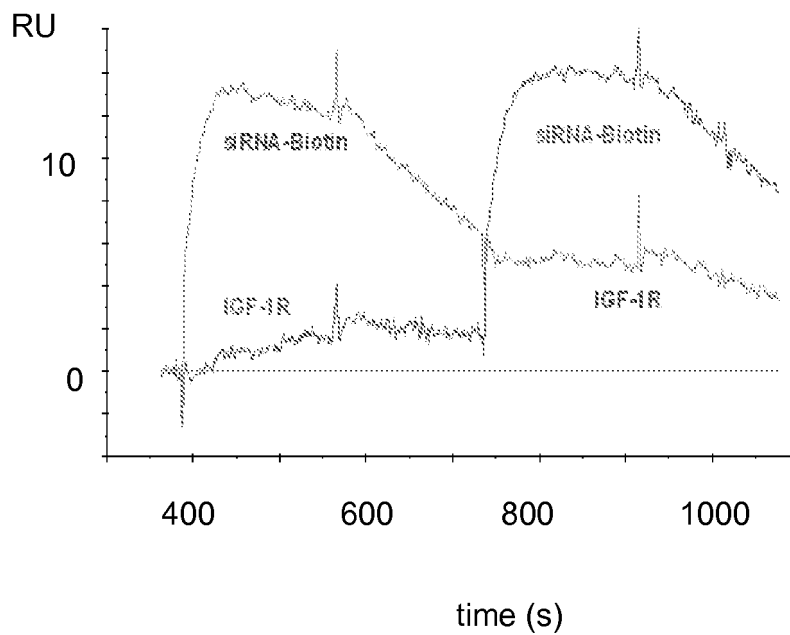


Fig. 56 a)

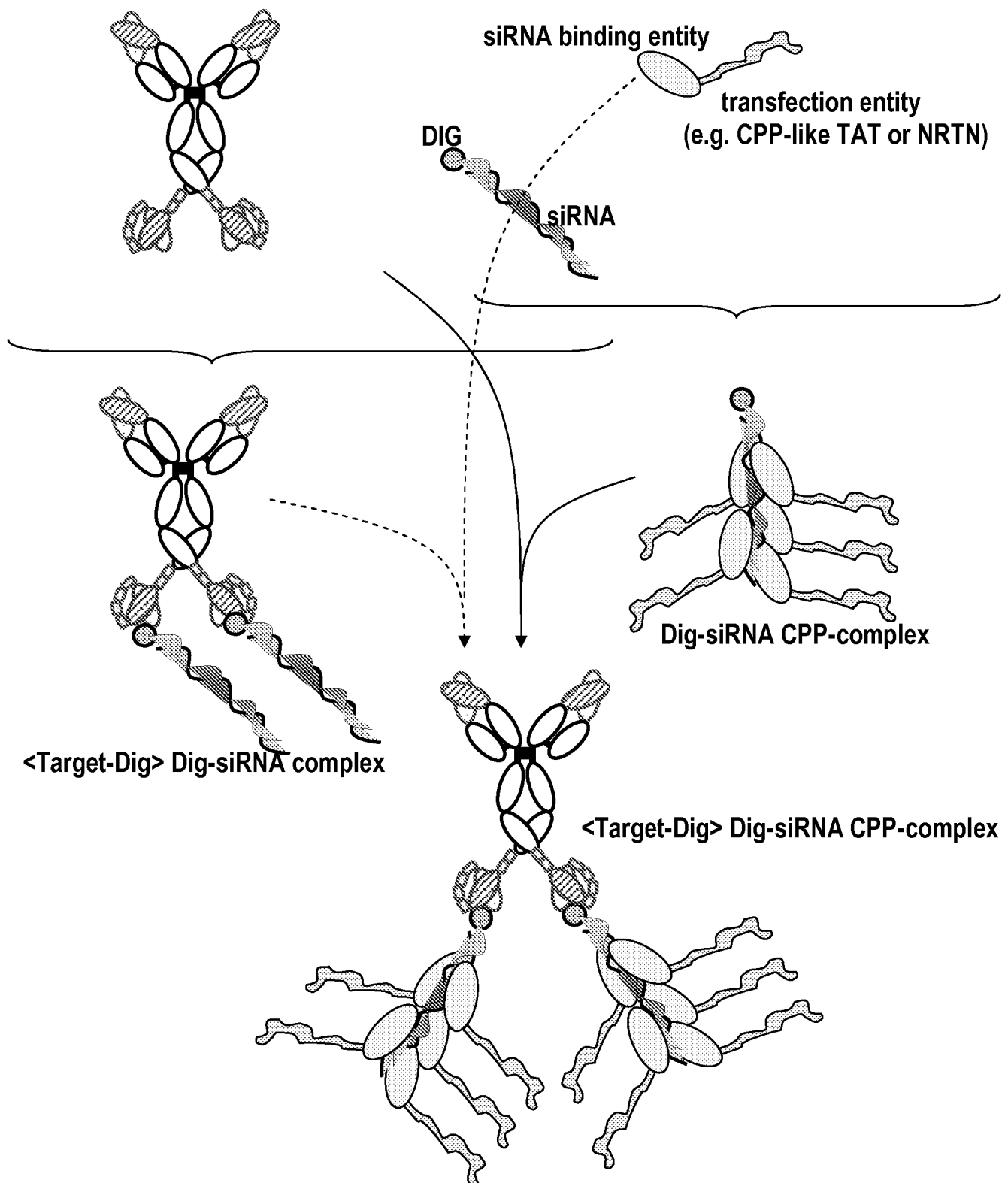


Fig. 56 b)

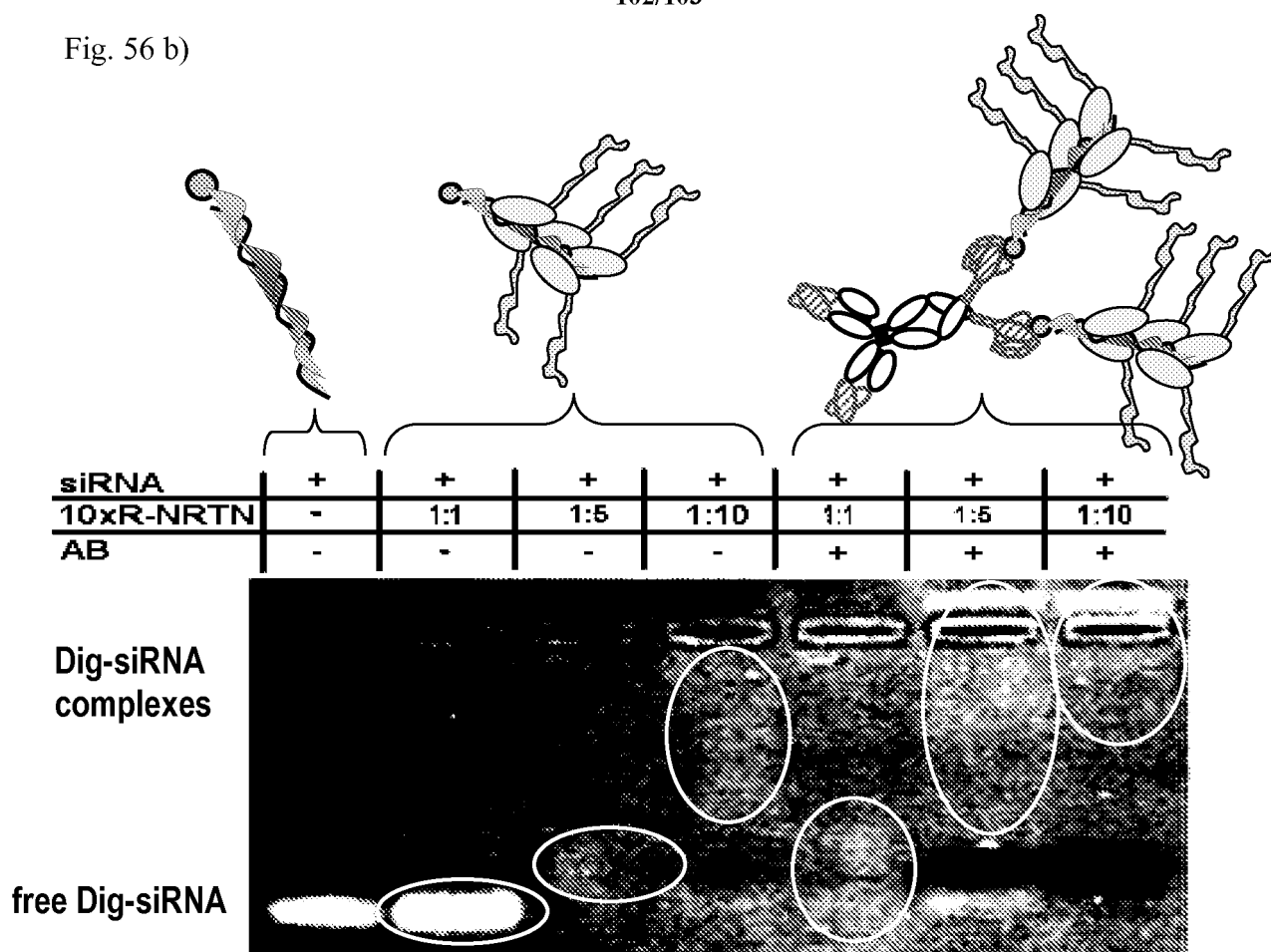


Fig. 56 c)

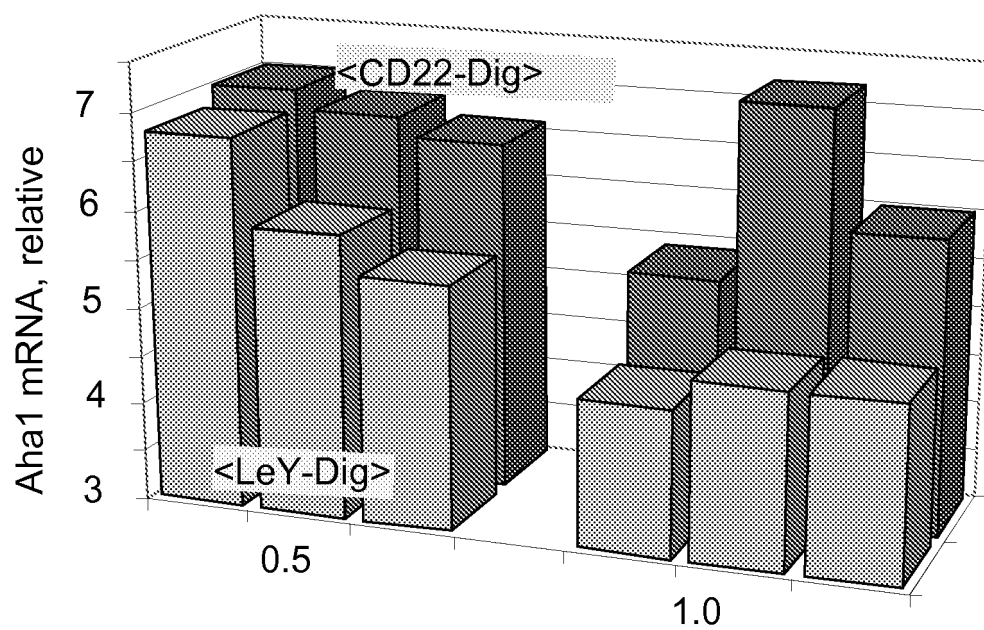


Fig. 57

