Supporting information for:

Determination of Rigidity of Protein Bound Au_{144} Clusters by Electron Cryomicroscopy

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Hydrodynamic radius of the Au_{144}/scFv/neuraminidase complex.

To confirm expected binding activity of the Au_{144}/scFv conjugate against the neuraminidase tetramer, to which it should bind with 4:1 stoichiometry, we performed a set of gel filtration chromatography experiments which served to both prepare a stoichiometric complex for electron microscopy and also to confirm expected binding activity of the Au_{144}/scFv bioconjugate.

The Superdex 200 gel filtration column, was calibrated with the standards shown in table S.1. A calibration curve based upon the information in table 1 is presented in figure S.1.

Standard Name	Molecular Wt (kDa)	Hydrodynamic Radius (nm)	Elution Volume (ml)
Chymotrysinogen	25	2.4	1.723
Ovalbumin	43	3.0	1.542
Aldolase	157	4.8	1.382
Catalase	232	5.2	1.361
Ferritin	476	8.2	1.195
Thyroglobylin	669	10.1	1.058

Table S.1. Standards for calibration of Superdex 200 gel filtration column.

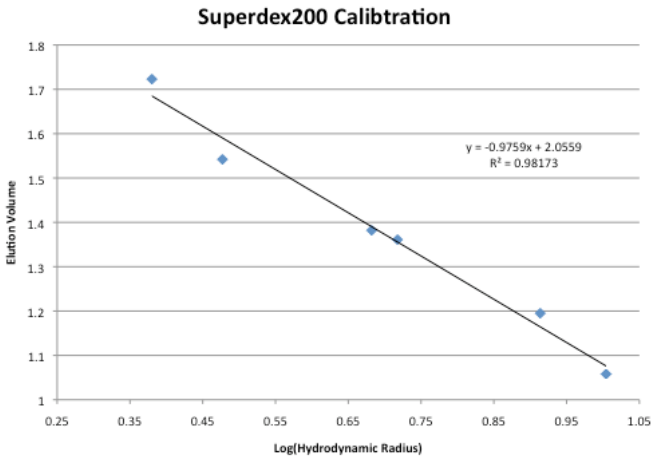

Figure S.1. Calibration curve for Superdex 200 column.

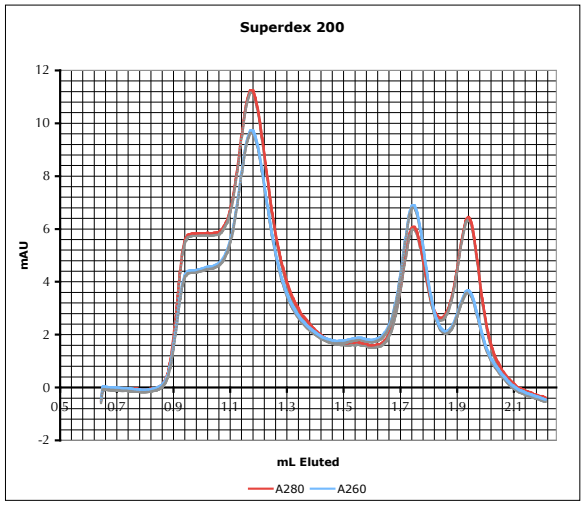

Figure S.2. Purification of Au_{144}/scFv/neuraminidase complex.

The calibration curve of Figure S.1. allows the assignment of hydrodynamic radius values to the experimental peaks of figure S.2. as shown in table S.2. Table S.2 includes for reference the elution volumes of relevant proteins that are not shown in figure S.2, such as Neuraminidase and Neuraminidase/scFv complexes, noted in table S.2. by their N9 and NC10 designations.

Sample Name	Measured Elution Vol	Calculated Hydrodynamic Radius (nm)
Au144	1.76	2.01
scFv (NC10)	1.665	2.52
scFv-Au144	1.559	3.23
Neuraminidase (N9)	1.365	5.10
N9/NC10	1.185	7.81
N9/NC10-Au144	1.169	8.11

Table S.2. Hydrodynamic radii of tested samples.

From this analysis, it is clear that the Au_{144} label increases the apparent hydrodynamic radius of scFv component by ~0.7nm and the overall complex by ~0.3nm. This small value of overall hydrodynamic radius increase may be due to the overall concave shape of the complex (see figure 3 in the main text) or possibly a substoichiometric decoration of the neuraminidase with gold labeled antibody. The problem of substoichiometry in this step is considered unlikely because we detect an 'free Au_{144}/scFv' peak at 1.559mL elution volume. Even if the 'free Au_{144}/scFv peak' represents inactive or unbound scFv from equilibrium affinity consideration, we can correct for substoichiometric complexes in the electron microscopy data processing by selecting only stoichiometric complexes for data processing.

To account for whether the small increase in hydrodynamic radius is due to substoichiometry or is a realistic assessment of the hydrodynamic radius of the Au_{144} labeled complex, we constructed an all-atom model shown in figure 3 and used the Hydropro(1) algorithm to assess the change in calculated hydrodynamic radius for labeled and unlabeled complexes.

Hydropro calculations for the all-atom model of figure 3 indicate that the addition of 4 Au_{144}(*p*-mercaptobenzoic acid)$_{60}$ clusters alters the hydrodynamic radius by about 6%. This compares well to the 4% increase in hydrodynamic radius observed by gel filtration, from 7.81nm to 8.11nm. The small scFv-Au_{144} peak observed at 1.559 mL elution volume implies that the purified complex is saturated with scFv

The all atom model of figure 3 implies secondary contacts between the ligand layer of the cluster and the scFv surface. This orientation is consistent with the electron cryomicroscopy data and hydrodynamic radius data. For instance, cluster orientation is changed so that the ligand layer of the cluster does not interact at all with the scFv, making the linker fully extended increases calculated hydrodynamic radius by 12% to 8.74nm. This is inconsistent with the increase in hydrodynamic radius measured by gel filtration.

We argue that the preponderance of data suggests that the peak at 1.169mL elution volume represents stoichiometric Au_{144}/scFv/neuraminidase complex. We cannot conclusively rule out, though, the possibility that this peak represents a substochiomentric complex.

1. Garcia De La Torre J, Huertas ML, Carrasco B. 2000. *Biophysical Journal* 78: 719-30