**SUPPLEMENTARY INFORMATION:**

**Implementation of the equilibrium constants fitting procedure in Excel**

Spreadsheet cells whose values have to be modified before the fitting procedure are highlighted in yellow (rows 1 to 10).

Cells B1 and B2 should be initialized with the estimated dissociation equilibrium constant values *K*d1 and *K*d2 in nanomolar units. After fitting, the optimized *K*d1 and *K*d2 values will be returned here.

Cells B12 and B13 show the association equilibrium constant values calculated from the *K*d1 and *K*d2 according to the formulas: *K*a1=(*K*d1)-1, *K*a2=(*K*d2)-1.

The total concentrations of the antibody and antigen in nanomolar units should be entered into cells D2 and E2, respectively. If several measurements at different concentrations were performed, additional values can be entered in rows 3 to 10 for a global fit. For each set of concentration values entered in columns D and E, three concentration fractions obtained by normalization of the mass distribution peak heights (Protocol step 4.3) should be entered into the adjacent cells. The concentration fraction values of the free antibody, single bound antibody, and double bound antibody are entered in columns F, G, and H, respectively.

Values entered into the yellow-highlighted cells (D2:H10) are copied into cells in columns A to E, rows 21 to 29. Columns G to AB in those rows contain intermediate values used to solve the binding equation as follows:

After substituting *K*a1 and *K*a2 for *f*Ab·Ag/*f*Ab and *f*Ab·Ag2/*f*Ab, respectively, equation (2) can be rearranged to obtain a cubic equation of *c*Ag,:

with coefficients defined as:

For the given values of *K*d1, *K*d2, the total concentration of the Antibody (*cAb*)*tot* and the total concentration of the Antigen (*cAg*)*tot*, the coefficients a, b, c and d are calculated in columns G, H, I and J respectively.

Values of *p* and *q*, defined as:

and:

are calculated in columns L and M, respectively, and the cubic formula discriminant is calculated in column O.

According to Cardano’s formula, when , the real root of the cubic equation is given by:

The value of *c*Ag is calculated in column T.

When , three real roots for the cubic equation exist:

where and . Values of *r* and ** are calculated in columns Q and R, respectively, and values of roots *c*Ag,1, *c*Ag,2, and *c*Ag,3 are calculated in columns U, V, and W, respectively. Any root calculations in columns T, U, V and W that result in an invalid value are replaced by 0. If the root values in columns T through W are in the range from 0 to (*cAg*)*tot,* they are copied to a corresponding cell in column Y to AB. Values outside this range are set to 0. That way, the final value of *c*Ag can be calculated as the sum of the cells in columns Y to AB. The final *c*Ag value for the first sample is returned in cell A31. For a global fit, root values for additional data points are displayed in rows 32 to 39 (rows marked in blue). The values of *c*Ag are used to obtain the concentrations of other reaction components according to the formulas:

The values of *c*Ab, *c*Ab·Ag, and *c*Ab·Ag2 are calculated from row 31 to 39 in columns B, C and D, respectively. Column F contains the sum of *c*Ab, *c*Ab·Ag, and *c*Ab·Ag2, which is the total antibody concentration and, for the first sample, should be identical to the value in cell A21. To avoid Excel calculation errors, very small numbers (1x10-30) are entered into cells in column F for rows not used to fit experimental sample data.

The fractions *f*Ab, *f*Ab·Ag, and *f*Ab·Ag2—based on the estimated *K*d values—are calculated in row 41 in columns C, D, and E, respectively. If data obtained for several samples are utilized in the global fit, additional values will be calculated in rows 42 to 49 (rows marked in gray).

To obtain the sum of squared errors (SSE), the squares of differences between the fit values (rows 41 to 49, marked in gray) and the experimental values (rows 21 to 29, marked in green) are calculated in rows 51 to 59 (rows marked in orange). All of those values are added together, and the final SSE is displayed in cell B15.

The Solver function in Excel is used to minimize the SSE value in cell B15 by adjusting the values of *K*d1 and *K*d2 in cells B1 and B2. Once the minimum value of the SSE is found, the corresponding *K*d1 and *K*d2 values represent the best fit equilibrium constants determined from the MP data.