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Summary ~~of the Invention~~

It has been found that for the characterization of antibodies no throughout kinetic characterization is required but furthermore the determination of the association rate constant at 37 °C and the association rate constant at 13 °C is required. The ratio thereof can be used to classify antibodies according to their binding properties.

In a first aspect, there is provided a method for obtaining an antibody comprising the following steps a) providing a multitude of antibodies, b) determining the association rate constant of each antibody to its antigen at 37 °C and the association rate constant of each antibody to its antigen at 13 °C, c) calculating the ratio of the association rate constant at 37 °C to the association rate constant at 13 °C, d) selecting an antibody with a ratio of 10 or less and thereby obtaining an antibody.

In a second aspect, there is provided a method for obtaining an antibody with cross-reactivity for at least two antigens comprising the following steps a) providing a multitude of antibodies, b) determining the association rate constant of each antibody to an antigen at 37 °C and the association rate constant of each antibody to the same antigen at 13 °C, c) calculating the ratio of the association rate constant at 37 °C to the association rate constant at 13 °C, d) selecting an antibody with a ratio of 50 or more and thereby obtaining an antibody with cross-reactivity for at least two antigens.

In a third aspect, there is provided a method for producing an antibody comprising the following steps: a) providing a multitude of antibody producing cells, b) determining the association rate constant for each antibody produced by each antibody producing cell to an antigen at 37 °C and 13 °C, c) calculating the ratio of the association rate constant at 37 °C to the association rate constant at 13 °C, d) selecting an antibody producing cell producing an antibody with a ratio of 10 or less, e) cultivating the selected cell, f) recovering the antibody from the cultivated selected cell or the cultivation medium thereof and thereby producing an antibody.

In a fourth aspect, there is provided a method for obtaining a humanized antibody comprising a) providing a parent antibody, b) providing a set of humanized forms of the parent antibody, c) determining the association rate constant for each humanized antibody form to its antigen at 37 °C and at 13 °C, d) calculating the ratio of the association rate constant at 37 °C to the association rate constant at 13 °C, e) comparing the ratio of the humanized forms with the ratio of the parent antibody, f) selecting a humanized form as humanized antibody with a ratio that is less than twice the ratio of the parent antibody and thereby obtaining a humanized antibody.

The present disclosure relates to ~~The first aspect as reported herein is~~ a method for selecting an antibody comprising the step of selecting an antibody with a velocity factor of 10 or less.

In one embodiment the method for selecting an antibody comprises the following steps

a) providing a set of or a population or a multitude of antibodies,
b) determining temperature-dependent kinetic data,
c) calculating the velocity factor for all provided antibodies,
d) selecting an antibody with a velocity factor of 10 or less.

In one embodiment the velocity factor is of from 0 to 10. In another embodiment the velocity factor is the ratio of the association rate constant at 37 °C to the association rate constant at 13 °C. In still another embodiment the temperature-dependent kinetic data is determined at 13 °C and 37 °C. In another embodiment the determining temperature-dependent kinetic data is with surface plasmon resonance. In a further embodiment the method is a high-throughput method. In still another embodiment the selecting is by the velocity factor and by the $\Delta S°‡ass$

value. In one embodiment in the surface plasmon resonance the antigen is immobilized on the surface plasmon resonance chip. In a further embodiment the temperature-dependent kinetic data is thermodynamic equilibrium data and transition state thermodynamic data. In still another embodiment the temperature-dependent kinetic data is calculated with the linear forms of the van't Hoff and Eyring and Arrhenius equations. In one embodiment an antibody is selected with a $\Delta S°‡ass$ of less than 200 J/mol*K ($\Delta S°‡ass < 200$ J/mol*K). In one embodiment an antibody is selected with a $\Delta S°‡ass$ in the range of from -200 J/mol*K to 200 J/mol*K. In also an embodiment an antibody is selected with a $\Delta S°‡ass$ in the range of from -150 J/mol*K to +100 J/mol*K. In another embodiment the surface plasmon resonance is with the HBS-EP buffer supplemented with 1 mg/ml carboxymethyldextrane. In still another embodiment each of the antibodies is produced by a single hybridoma or B-cell.

The present disclosure also relates to ~~A second aspect as reported herein is~~ a method for obtaining an antibody with cross-reactivity for at least two antigens comprising selecting an antibody with a velocity factor of 50 or more. In one embodiment the velocity factor is of from 50 to 300.

The present disclosure also relates to ~~A further aspect as reported herein is~~ a method for producing an antibody comprising the following steps:

a) providing a set/population/multitude of antibody producing cells,
b) selecting an antibody producing cell producing an antibody with a velocity factor of 10 or less,
c) cultivating the selected cell,
d) recovering the antibody from the cultivated selected cell or the cultivation medium thereof and thereby producing an antibody.

In one embodiment the antibody producing cells are deposited as single cells. In a further embodiment the deposited single cells are cultivated prior to the selecting. In another embodiment the antibody is purified prior to the selecting. In a further embodiment the antibody is purified after the recovering. In a specific embodiment the purifying is by Protein A chromatography.

The present disclosure also relates to ~~Another aspect as reported herein is~~ a method for selecting a humanized form of a parent antibody comprising

a) providing a parent antibody,

b) providing a set/population/multitude of humanized forms of the parent antibody,

c) determining temperature-dependent kinetic data of all provided antibodies,

d) calculating the velocity factor for all provided antibodies,

e) comparing the velocity factors of the humanized forms with the velocity factor of the parent antibody,

f) selecting a humanized form of a parent antibody by selecting an antibody with a velocity factor that is less than twice of the velocity factor of the parent antibody.

In one embodiment the selecting is of a humanized from with a velocity factor that is less than 125% of the velocity factor of the parent antibody, in another embodiment less than 110% of the velocity factor of the parent antibody.

The present disclosure also relates to ~~Still another aspect as reported herein is~~ the use of an antibody selected with a method as reported herein as therapeutic or diagnostic agent. The present disclosure also relates to ~~One aspect of the invention is~~ a pharmaceutical composition comprising an antibody obtained with a method as reported herein.

Detailed Description of the Invention

The methods reported herein are based on the determination of the velocity factor. Based on the velocity factor antibodies can be classified, i.e. antibodies can be characterized according to their binding properties as e.g. entropic or enthalpic antigen binder. It has been found that a velocity factor based classification does not require detailed thermodynamic to be determined and/or calculated, thus, reducing the amount of parameters to be determined and the number of calculations to be performed. The velocity factor is the ratio of the antigen-antibody complex association rate constants (ka) independently determined at 37 °C and at 13 °C. As only two experimental determinations are required to calculate the velocity factor this is a fast and high-throughput suited method.

The velocity factor can be used e.g. to compare a humanized antibody with the parent antibody from which it is derived. It can also be used to evaluate antibodies directly from hybridoma and/or B-cell supernatants. This does not require a preliminary purification as the determination can be made directly with the crude supernatant.