

Abstract

Optimization of therapeutic antibody candidates for desired binding properties is a critical step in the development of antibody therapeutics. Optimization is achieved by synthesizing many antibody variants via mutagenesis, and screening these variants to identify those which display optimal binding properties. Antibody variants produced in bacteria are usually used for the optimization process. Although, mammalian cell display is preferable because it utilizes the human IgG form, as opposed to the non-native antibody forms produced by a bacterial phage display (Fab, Sc-Fv). To achieve clinical significance, these bacterial antibodies will usually have to undergo humanization; a difficult process which may result in a loss of the desired binding properties. Optimization for specific binding properties of antibodies produced by mammalian cell transfections can be an extremely time consuming limitation in the development of therapeutics. We hypothesized that it would be possible to expedite this process by developing a high through-put method for expression of antibody variants, and for screening their kinetic binding properties directly from conditioned medium (as opposed to purified protein) via Octet kinetic assays. Through this method of mammalian cell transfection we were able to synthesize 1075 "Antibody X" mutants, and screened these mutants for their kinetic binding properties in a significantly reduced time frame. This method of optimization could dramatically decrease the time frame needed to develop an ideal therapeutic antibody candidate.

Objectives

- Develop a high throughput method for synthesizing 1075 combinatorial mutants of "Antibody X" through mammalian cell transfection
- Screen these mutants for their kinetic binding properties in under 60 days via high through-put Octet kinetic assays.

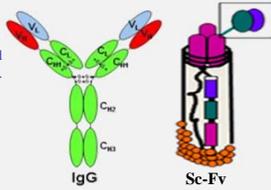


Figure 1. Comparison of human IgG produced in mammalian cells and bacterial ScFv antibodies



Figure 2. A flow chart demonstrating the synthesis of therapeutic antibodies via bacterial phage display.

Background and Introduction

Antibody therapy, or treatment of disease with antibodies (Fig. 3, 4), is a technology with nearly infinite utilities due to the specificity of antibody-target binding, and the massive spectra of epitopes that antibodies can specifically bind to. In order to maximize the efficiency of drug development for this growing market (>\$50 billion), optimization of kinetic antibody binding properties is a necessity. This step is usually done by creating many mutants, or modified versions, of an antibody, then screening these mutants to identify variants which exhibit a desired kinetic binding property. Antibody variants are usually synthesized in bacteria for an optimization, because it is a relatively fast and easy process for creating and screening many antibody variants. However, the antibodies produced by this bacterial phage display may have different binding properties in the Fab or Sc-Fv form than the human IgG form produced by mammalian cells. Production of human IgG antibodies through mammalian cell transfection eliminates the risks and complications of antibody production in bacteria (caused by the inability of bacterial cells to properly fold the native structure of human IgG). Optimization of an antibody's kinetic binding properties (association-rate, dissociation-rate, etc.) via mammalian cell transfections is typically a process which can take many months or years. The Octet (ForteBio) is an efficient device for testing the kinetic binding properties of antibodies, which does not require antibody purification or antibody/antigen labelling. In order to accelerate optimization with human IgG, we developed a high throughput method for producing antibody variants via mammalian cells, and for screening their kinetic binding properties. It was hypothesized that by performing transfections on a small scale (6-well format), and testing their kinetic properties in a high-throughput Octet screening or conditioned medium, it would be possible to perform optimization of antibodies produced in mammalian cells in a substantially shortened time span: within 2 months. The antibody we optimized, Antibody X, is a therapeutic antibody which is currently used to treat disease in humans.

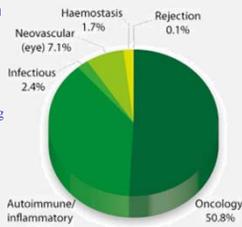


Figure 3. Segmentation of the >\$50 billion market for antibody therapeutics

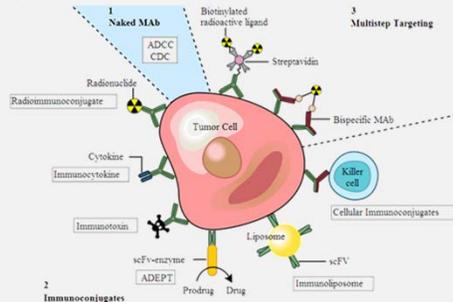


Figure 3. Methods of antibody therapy for treatment of disease

Methods

- Synthesize 1075 variants of Antibody X through small scale mammalian cell (COS-1) transfections in 6-well plates
- Screen conditioned media directly for yield and neutral pH binding via Octet quantitation and enzyme linked immunoassays (ELISA)
- Perform high through-put kinetic screening of conditioned medium via Octet kinetics at pH A and pH B
- Scale-up transfections for mutants of interest to be used for stability testing, cytotoxicity testing, cell based ELISA's, and other functional tests.

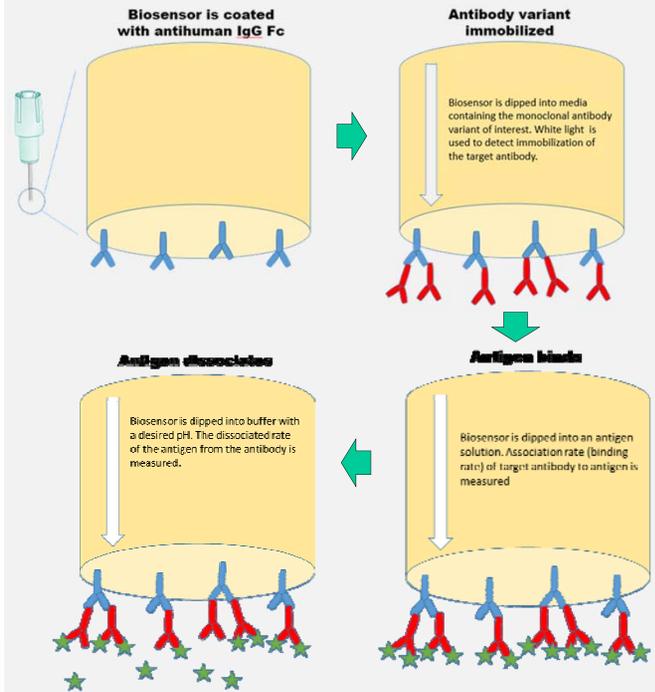


Figure 4. Illustration of the steps in an Octet kinetic assay for the binding properties of antibody variants

Results

	1	2	3	4	5	6	7	8	9	10	11	12
A	63.4	2.64	40.1	49.9	63.6	77.2	48.3	23.4	79.6	47.3	53.1	58.7
B	47.5	1.42	37.1	17.7	55.3	58.8	33	40.2	33.2	45.7	43.4	67.6
C	55.5	6.6341	22.1	28.1	52.2	53	51	58	48.7	50.2	30.2	48.5
D	46.1	3.19	35.5	41.7	40.4	60.3	44.6	45.3	33.4	52	43.4	74.4
E	3.47	L	1.48	5.19	5.63	19.3	4.11	4.23	3.45	2.44	1.24	5.6
F	46.4	L	17.5	37	47.9	60.7	42.8	26.2	32.5	40.8	20.6	47.2
G	32.3	2.27	35.8	48.8	43.8	37.2	35.7	13.5	21.7	38.3	38.1	16
H	172	L	1.04	1.47	1.72	1.53	1.45	1.63	1.61	1.48	6.6276	1.19

Figure 2. Representative plate of Octet Quantitation results containing estimates of Antibody X mutant concentrations (ug/mL) in conditioned medium. Each column and row represents a mutation in combinatorial mutants of Antibody X; each well is a different antibody variant. Highlighted rows and columns represent mutations which diminish expression of Antibody X.

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.855	0.945	0.791	1.296	1.153	0.829	1.288	1.164	0.946	1.077	1.519	0.815
B	2.533	2.358	1.932	2.344	2.659	2.676	2.717	2.557	2.672	2.372	2.855	2.628
C	2.881	2.743	2.116	2.835	2.757	2.914	2.825	2.856	2.859	2.949	3.18	2.845
D	2.795	2.867	2.204	2.641	2.977	2.904	2.853	2.931	2.831	2.906	3.146	2.863
E	2.945	3.08	2.945	3.098	3.161	3.121	3.13	3.119	3.104	3.086	3.148	2.971
F	2.241	2.166	2.451	2.44	2.682	2.448	2.414	2.537	2.453	2.582	2.473	2.751
G	2.437	2.617	1.905	2.854	2.974	2.946	2.908	2.964	2.883	2.921	2.879	2.945
H	2.699	2.908	0.799	2.502	2.878	2.972	2.922	2.889	2.899	2.904	2.981	3.007

Table 1. Representative absorbances in a capture ELISA for Antibody X mutants using plate coated with target "Receptor Y" to test neutral pH binding of antibody to antigen. Each column and row represents a mutation in combinatorial mutants of Antibody X. The mutation in column 3 killed expression (confirmed by Octet quantitation). The mutation in row "A" is expressed well, although it results in significantly lower binding at neutral pH.

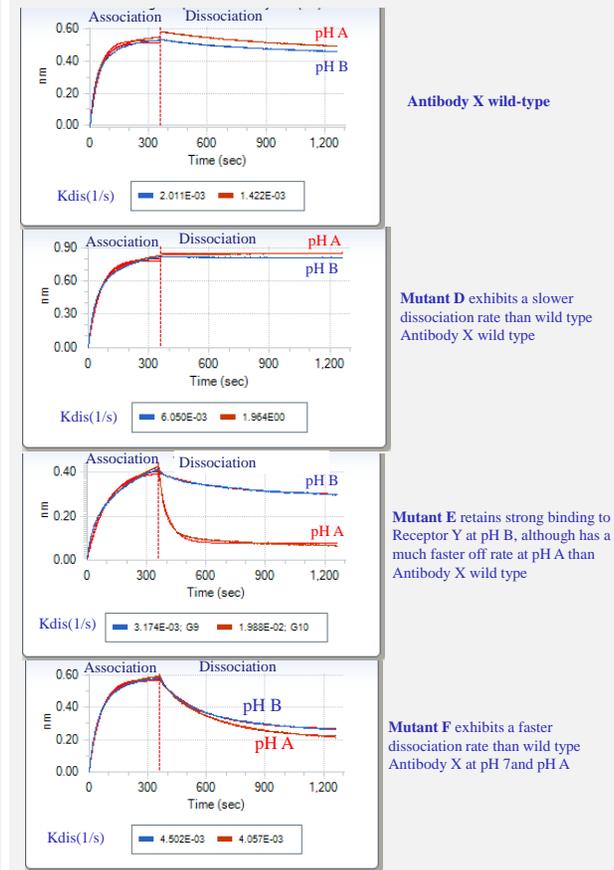


Figure 3. Octet kinetic assays for Antibody X wild-type and representative mutants, with dissociation in PBS pH 7.4 and PBS pH A. Association quantifies antibody binding to antigen, dissociation quantifies the off-rate of antigen from antibody when the biosensor is dipped in buffer.

Conclusions

- In under 60 days, 1075 human IgG mutants of Antibody X were synthesized by mammalian cell transfection and screened for their yield and kinetic binding properties
- Utilizing small scale high throughput mammalian cell transfections, and performing high throughput Octet kinetic assays, it was possible to optimize for specific kinetic binding properties of Antibody X in a significantly reduced time

References

Protein Engineering, Design and Selection (2009) 22 (3): 169-174. doi: 10.1093/protein/gzn068 First published online: November 20, 2008