Enhancing the Quality of Biologics Patents:
Computational Simulations as Evidence

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Question

What sources of evidence would allow examiners to assess objectively the level of skill and predictability in the art of protein modification?

- Computational simulations could provide objective evidence relating to these issues.
- Such evidence could be provided for every protein in the Protein Data Bank.

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I. Hypothetical Antibody Claim: Issue Spotting

II. Unpredictability of Amino Acid Substitutions

III. Computational Simulations as Evidence

I. A Hypothetical Antibody Claim

An antibody or antibody fragment, comprising V_H and V_L domains,

each **comprising** three complementarity determining regions (**CDRs**) [defined by SEQ ID NO], where

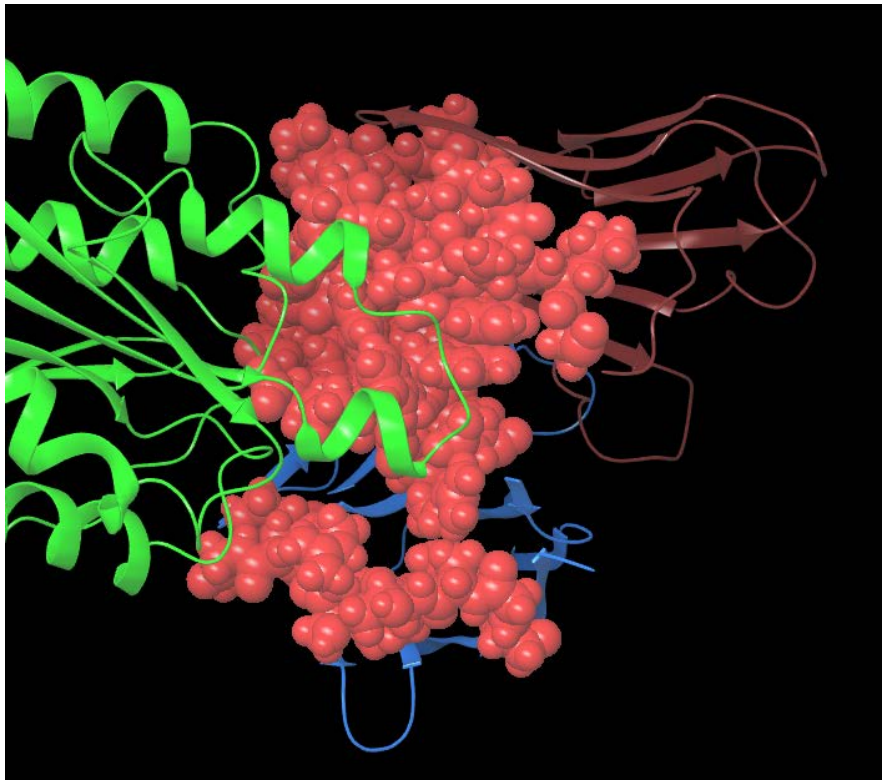
the antibody or antibody fragment **binds** antigen X.

I. Issue Spotting

1) "CDRs"

- Do CDRs provide structures that reasonably correlate with binding?
  - What is the nature of the experimentation required to graft CDRs onto non-native FW regions?

Cf. Sonia Covaceuszach et al., Single Cycle Structure-Based Humanization of an Anti-Nerve Growth Factor Therapeutic Antibody, 7 PLoS ONE e32212 at 8 (2012) ("[A]ntibody humanization by CDR grafting . . . has proved to be an unpredictably daunting and laborious task.").

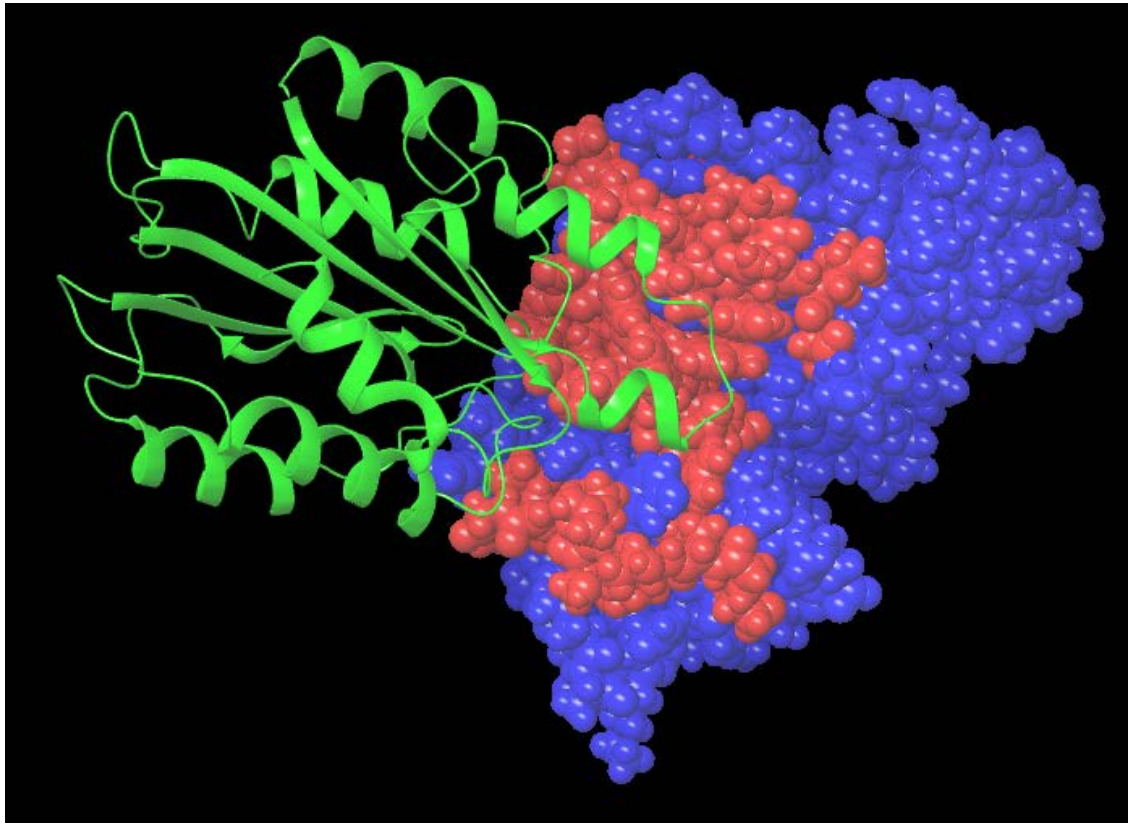


CDR residues of an Fv fragment

I. Issue Spotting

2) "Comprising"

- The "variable domains" of the claim encompass any amount of FW mutations, provided only that the antibody fragment retains the function of "binding."



CDR and **FW** residues of an Fv fragment

I. Issue Spotting

3) When is an example "representative"?

- *AbbVie*: 300 examples were not representative of the claimed genus.
  - What *would* they represent?
  - What information could we obtain from the constructive reduction to practice of an amino acid substitution?

See AbbVie Deutschland GmbH & Co. v. Janssen Biotech, Inc., 759 F.3d 1285 (Fed. Cir. 2014).

I. Issue Spotting

4) When do issues of inoperability and undue experimentation arise?

- "Even if some of the claimed combinations were inoperative, the claims are not necessarily invalid. . . . Of course, if the number of inoperative combinations becomes significant, and in effect forces one of ordinary skill in the art to experiment unduly in order to practice the claimed invention, the claims might indeed be invalid."

Atlas Powder Co. v. E.I. du Pont de Nemours & Co., 750 F.2d 1569, 1576-77 (Fed. Cir. 1984) (emphasis added).

I. Issue Spotting

5) "Binding": What about embodiments that show low affinity or stability?

- **Substantial utility:** "Courts have used the labels 'practical utility' and 'real world' utility interchangeably in determining whether an invention offers a 'substantial utility.'"

In re Fisher, 421 F.3d 1365, 1371, 1378 (Fed. Cir. 2005); *see also In re Ziegler*, 992 F.2d 1197, 1200 (Fed. Cir. 1993).

I. Issue Spotting

How do we obtain objective evidence pertaining to these issues?

- **Hypothesis:** The capabilities and limitations of computational simulations could be used to define the relevant level skill and predictability in this art.

II. Unpredictability of Amino Acid Substitutions

- What can we deduce from comprehensive public databases that disclose the functional effect of thousands of single amino acid substitutions?

Brian Lathrop and Michael Kinch, *Enhancing the Quality of Patent Claims Directed to Biologics with Biophysical Evidence*, 34 BIOTECHNOLOGY LAW REPORT 213-35 (2015).

E.g., U.S. Published Application No. 2011/0251073 A1. Databases include the level of biological activity obtained in various enzymatic assays, following a single amino acid substitution in a protease, subtilisin DSM7. A total of 5004 substitutions in each of the 275 amino acids of subtilisin DSM7 were reduced to practice. This allows us to investigate the function of embodiments covering 96% of the total sequence space created by a single amino acid substitution.

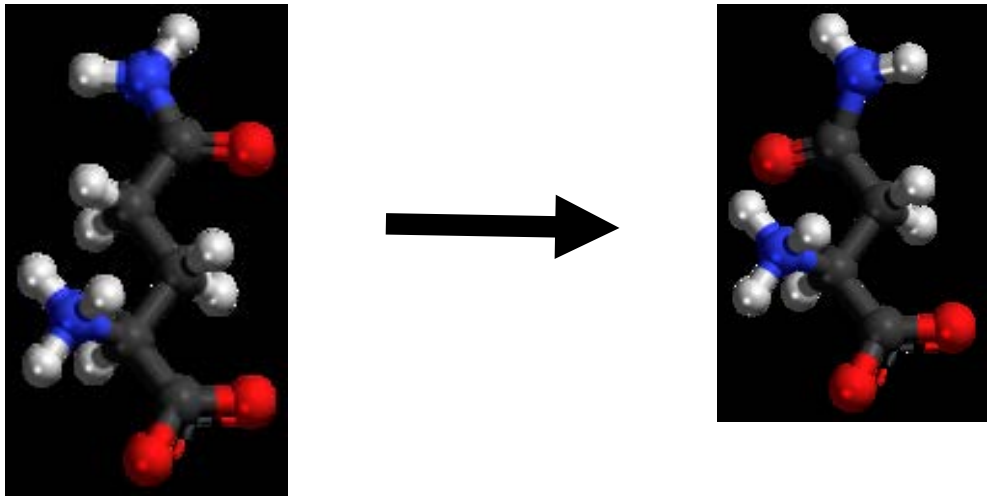
II. Unpredictability of Amino Acid Substitutions

- Protein function is exceptionally sensitive to subtle atomic interactions between amino acid residues.
- Proteins generally become inoperable after a few substitutions are introduced randomly.
- The resulting inoperability issues cannot be easily resolved with non-computational algorithms.

Lathrop and Kinch, *Enhancing the Quality of Patent Claims Directed to Biologics with Biophysical* Evidence, 34 BIOTECHNOLOGY LAW REPORT 213-35 (2015).

II. Unpredictability of Amino Acid Substitutions

Atomic level interactions:



Glutamine (Q) Asparagine (N)

The conservative Q10N substitution removes one $-CH_3$ group yet ***inactivates*** subtilisin.

In what sense would the disclosure of a "Q10N" substitution be "representative"?

Cf. Novozymes A/S v. DuPont Nutrition Biosciences APS, 723 F.3d 1336 (Fed. Cir. 2013) (merely listing hypothetical substitutions did not constitute an adequate written description).

II. Unpredictability of Amino Acid Substitutions

At the residue level:

Mutation	Effect	Mutation	Effect
Q10A	90	Q10L	90
Q10C	90	Q10M	90
Q10D	0	Q10N	0
Q10E	80	Q10P	0
Q10F	110	Q10R	100
Q10G	130	Q10S	110
Q10H	130	Q10T	90
Q10I	80	Q10V	120
Q10K	100	Q10W	100

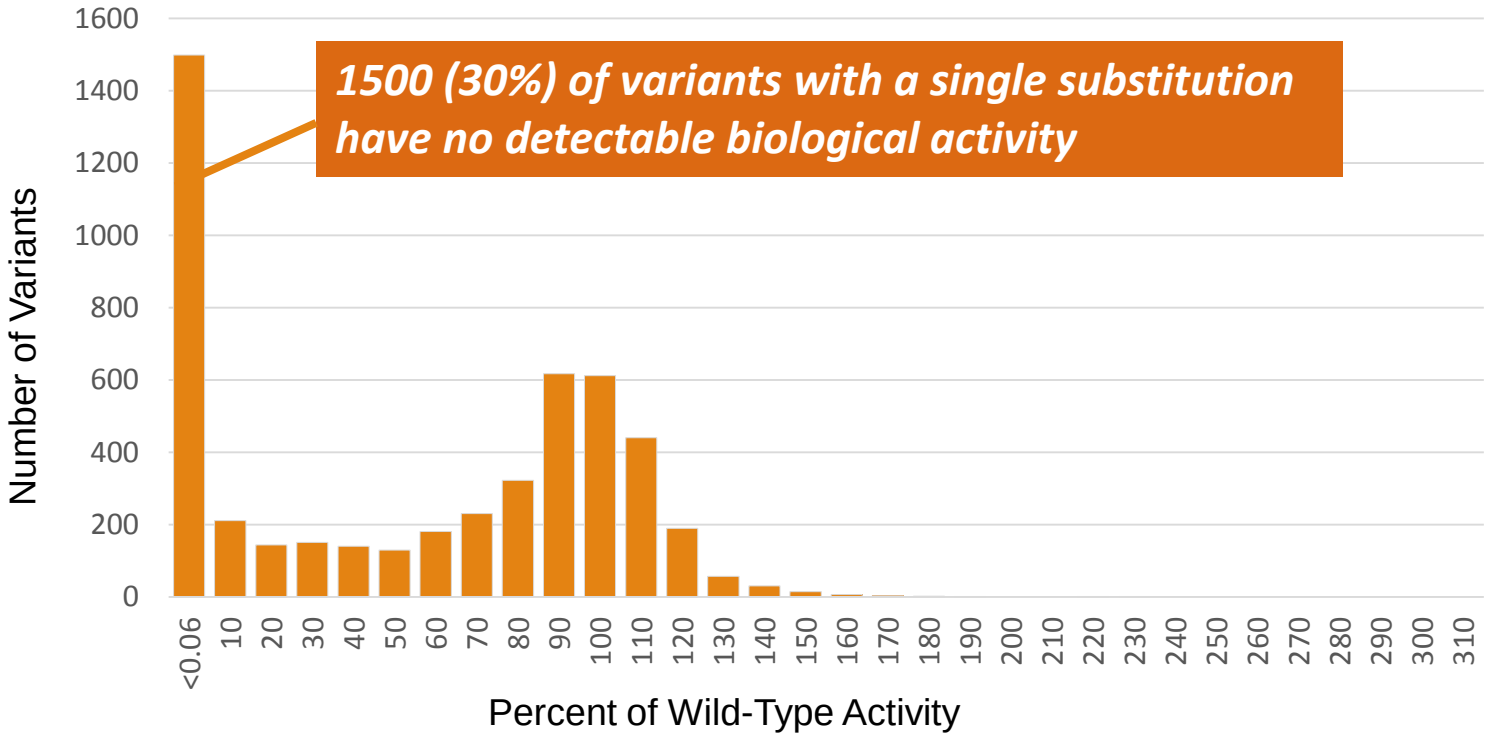
Is any meaningful information conveyed by a disclosing a "Q10" substitution without actual reduction to practice?

Data from U.S. Published Application No. 2011/0251073 A1. "Effect" means the enzymatic activity relative to wild type subtilisin DSM7.

II. Unpredictability of Amino Acid Substitutions

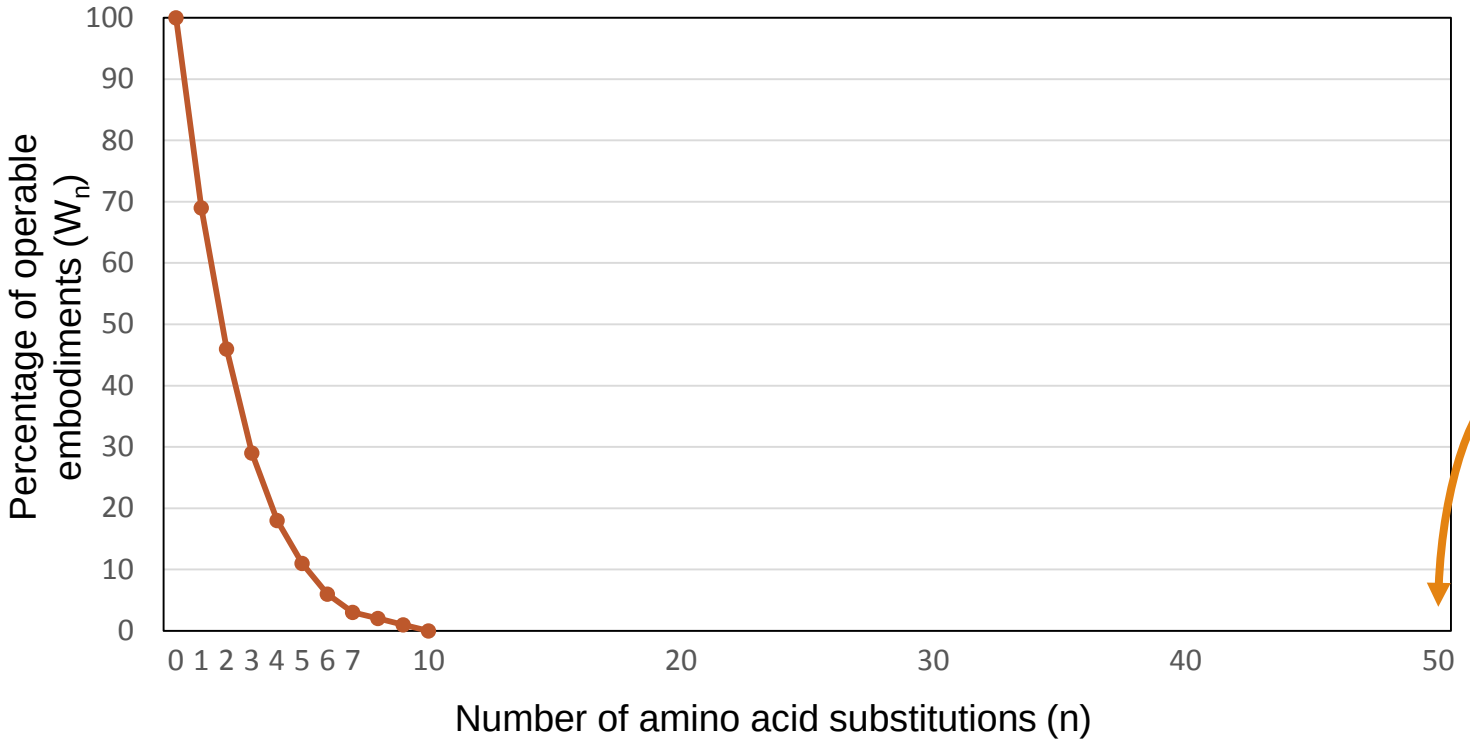
At the protein level:

Are structures essential for function located outside of the active site?



Data compiled from U.S. Published Application No. 2011/0251073 A1. 5004 substitutions were made to all 275 amino acids of subtilisin DSM7. The number of variants is plotted against the relative activity of each variant.

II. Unpredictability of Amino Acid Substitutions

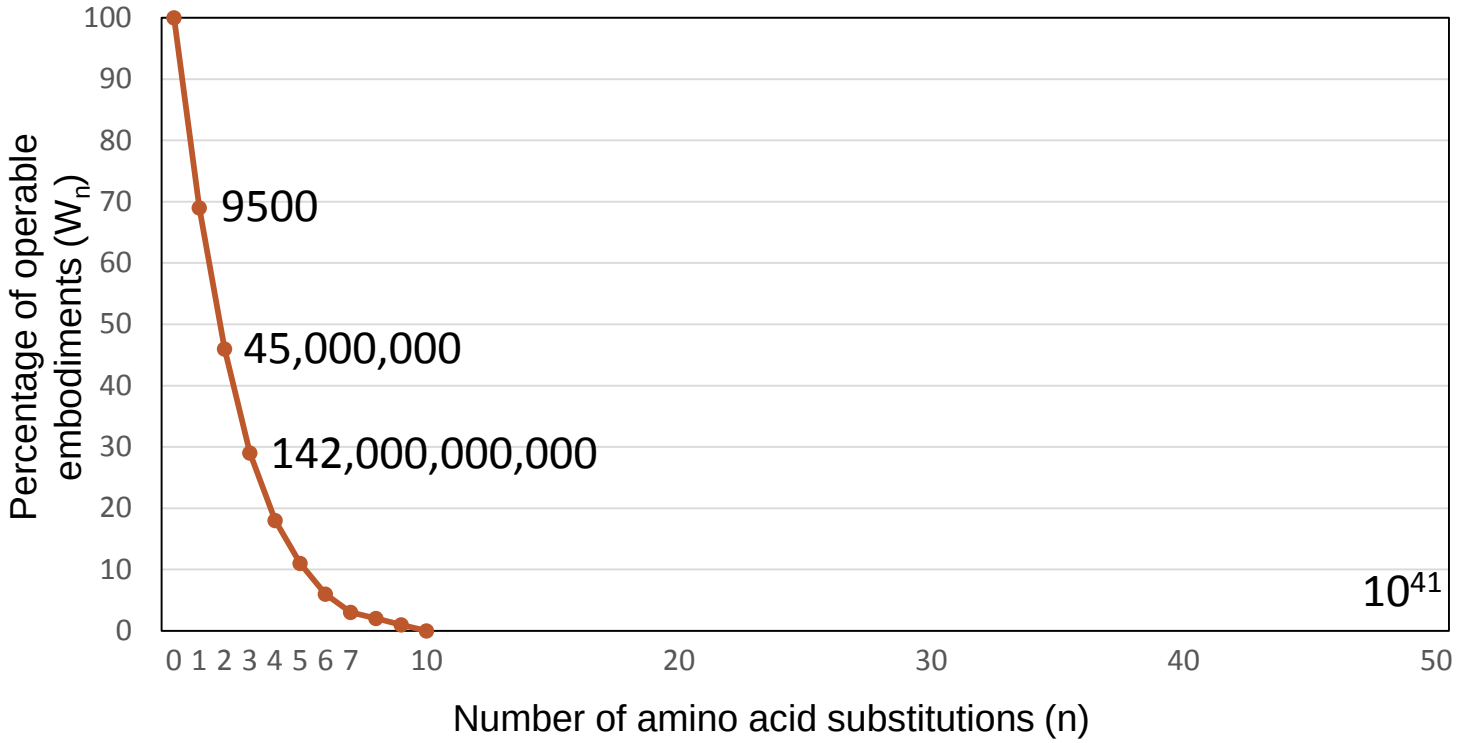


"Kill Curves"

Ex parte Porro: An amino acid sequence is not "representative" of a genus of variants with "at least 90% sequence identity" (e.g., up to 50 substitutions) to the sequence.

Subtilisin data fit to $W_n = \exp(-\alpha n - \beta n^2)$; *see* J.D. Bloom et al., *Thermodynamic Prediction of Protein Neutrality*, 102 PROC. NAT'L ACAD. SCI. USA 606-11 (2005).

II. Unpredictability of Amino Acid Substitutions



"Sequence space"

(the number of possible combinations)

What is the nature of the experimentation to navigate through the vast sequence space created by variants with "at least 99.4% sequence identity" (e.g., three substitutions to a protein containing 500 amino acids)?

II. Unpredictability of Amino Acid Substitutions

This is also true for Fv antibody fragments:

60% of Fv fragments are inactive after a single round of random mutagenesis, and 99% are inactive after five mutations.

Patrick S. Daugherty et al., *Quantitative analysis of the effect of the mutation frequency on the affinity maturation of single chain Fv antibodies*, 97 PROC. NAT'L ACAD. SCI. USA 2029–34 (2000), at FIG. 1; p. 2033.

II. Unpredictability of Amino Acid Substitutions

The relevant level of skill in the art:

The skilled artisan would not randomly mutagenize residues. She would select mutations that were structurally conserved, found in homologues, etc.

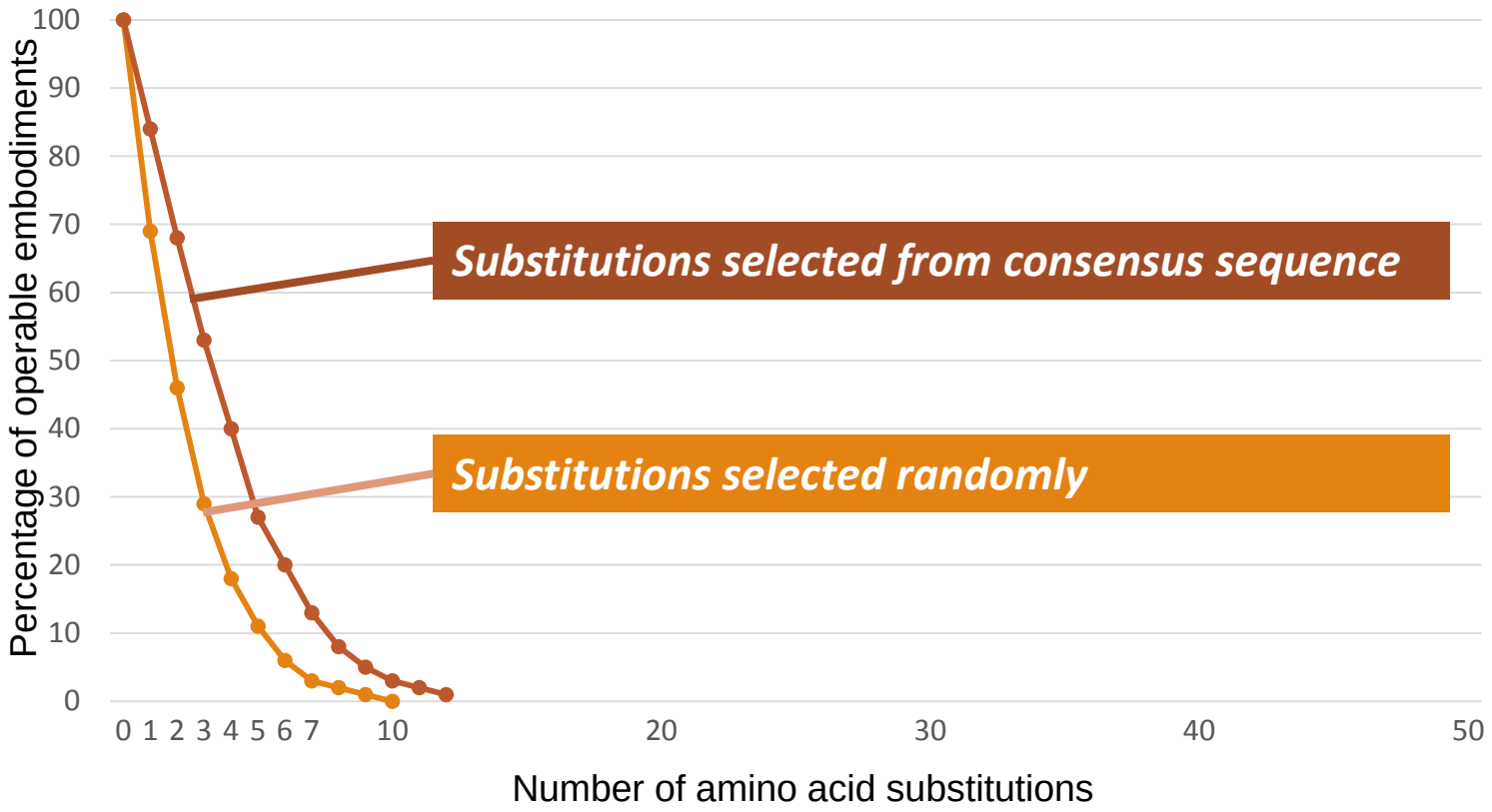
- Thought experiment: what if we selected only substitutions present in subtilisin homologues?

II. Unpredictability of Amino Acid Substitutions

Subtilisin homologues with >90% identity:

```
YNGTSMASPHVAGAAALILSKHPNWTNTQVRSSLENTTTKLGDSFYYGKGLINVQAAAQ Subtilisin DSM7
YSGTSMASPHVAGAAALILSKHPNWTNTQVRSSLENTTTKLGDSFYYGKGLINVQAAAQ Homologues
KSGTSMASPHVAGAAALILSKHPNWTNTQVRSSLENTTTKLGDSFYYGKGLINVQAAAQ
YNGTSMASPHVAGAAALILSKHPNWTNTQVRSSLENTTTKLGDAFYYGKGLINVQAAAQ
YNGTSMASPHVAGAAALILFKHPNWTNTQVRSSLENTTTKLGDAFYYGKGLINVQAAAH
YNGTSMASPHVAGAAALILSKHPNWTNTQVRSSLENTTTKLGDAFYYGKGLINVQAAAQ
YSGTSMASPHVAGAAALILSKHPNWTNTQVRSSLENTTTYLGDSFYYGKGLINVQAAAQ
KSGTSMASPHVAGAAALILSKHPNWTNTQVRSSLENTTTKLGDSFYYGKGLINVQAAAQ
KSGTSMASPHVAGAAALILSKHPNWTNTQVRSSLENTTTKLGDSFYYGKGLINVQAAAQ
YNGTCMASPHVAGAAALILSKHPNWTNTQVRSSLENTTTKLGDAFYYGKGLINVQAAAQ
YNGTSMASPHVAGAAALILSKHPNWTNTQVRSSLQNTTTKLGDSFYYGKGLINVQAAAQ
KSGTAMASPHVAGAAALILSKHPNWTNTQVRSSLENTTTKLGDSFYYGKGLINVEAAAQ
KSGTAMASPHVAGAAALILSKHPNWTNTQVRSSLENTTTKLGDSFYYGKGLINVQAAAQ
KSGTXMASPHVAGAAALILSKHPNWTNTQVRSSLENTTTKLGDSFYYGKGLINVEAAAQ
YNGTSMASPHVAGAAALILSKHPNWTNTQVRSSLENTTTKLGDSFYYGKGLINVQAAAQ Consensus Sequence
KS  C             F             Q   Y   A         E   H
    A
```

II. Unpredictability of Amino Acid Substitutions



How "representative" are 26 homologues with at least 90% sequence identity?

16% of 55 mutations present in homologues of subtilisin DSM7 would be fatal, if selected as substitutions.

III. Computational Simulations

- To what extent can computational simulations replace or supplement non-computational algorithms as predictive tools?
  - Predict structure/function relationships?
  - Identify representative examples?

III. Computational Simulations

Computational output:

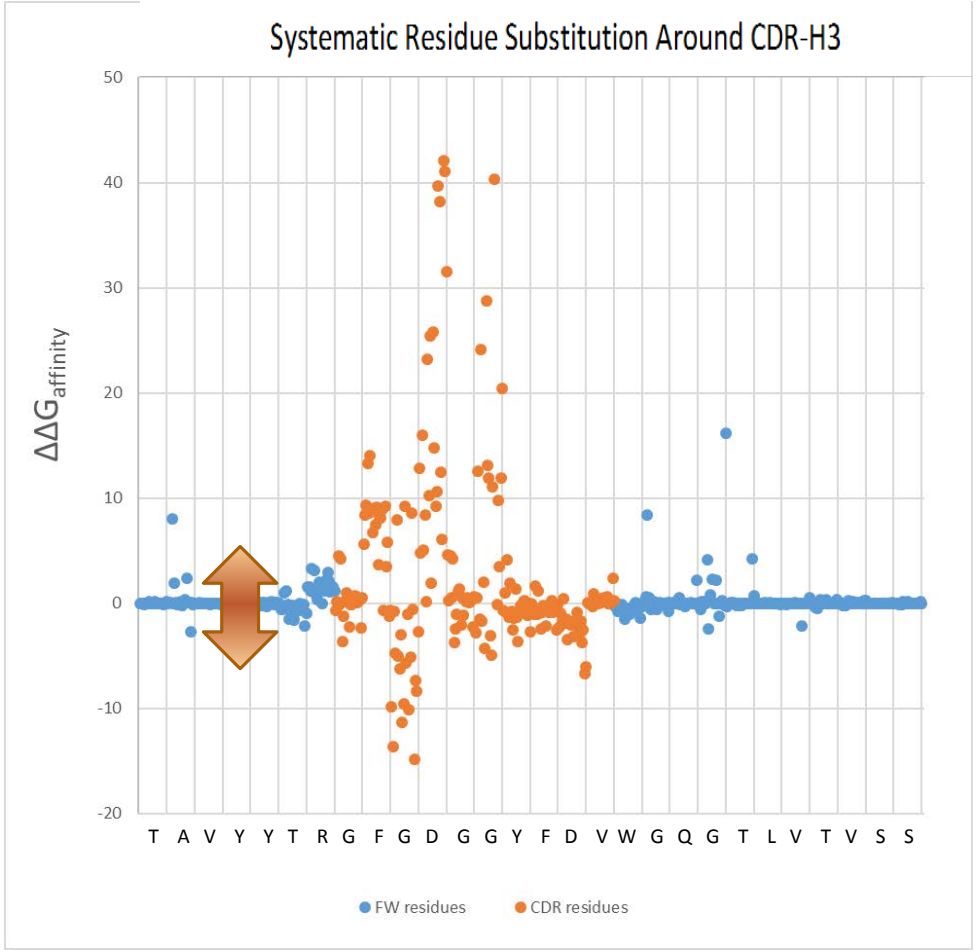
- BioLuminate™ calculates the energetic effect of structural modifications on affinity and stability, "$\Delta\Delta G_{affinity}$" and "$\Delta\Delta G_{stability}$", respectively.
- How do the $\Delta\Delta G$ for affinity and stability relate to biological activity?

III. Computational Simulations

Assuming 3 units ≈ 1 kcal/mol:

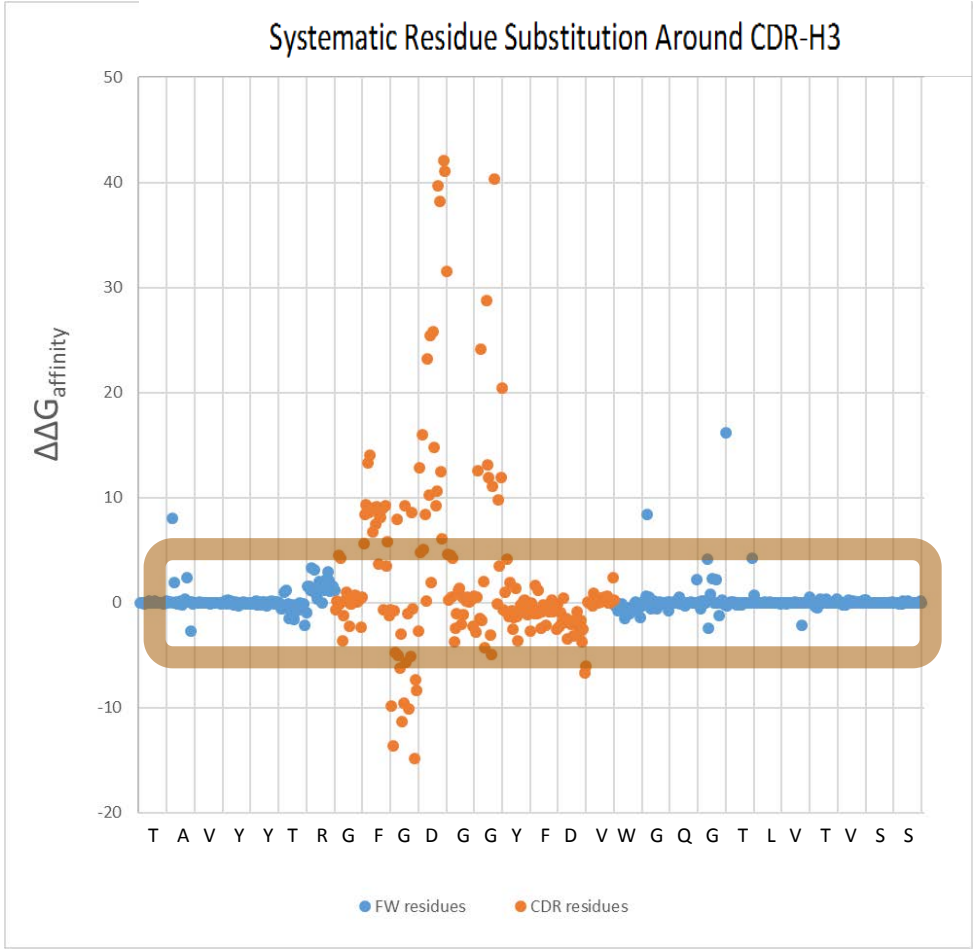
- Binding affinity accuracy is "a few kcal/mol" using the generation of algorithms evaluated here.

See Schrödinger, Inc., Knowledge Base, Article ID: 573, *Can I calculate binding affinities with Schrödinger software?*, at http://www.schrodinger.com/kb/573 (last modified July 15, 2011); *see also See* Hege Beard et al., *Applying Physics-Based Scoring to Calculate Free Energies of Binding for Single Amino Acid Mutations in Protein-Protein Complexes*, 8(12) PLoS ONE: e82849.doi:10.1371/journal.pone.0082849 (2013).



III. Computational Simulations

- The error is roughly equivalent to an order of magnitude change in the binding constant, e.g., 1 nM to 10 nM (+1.4 kcal/mol).

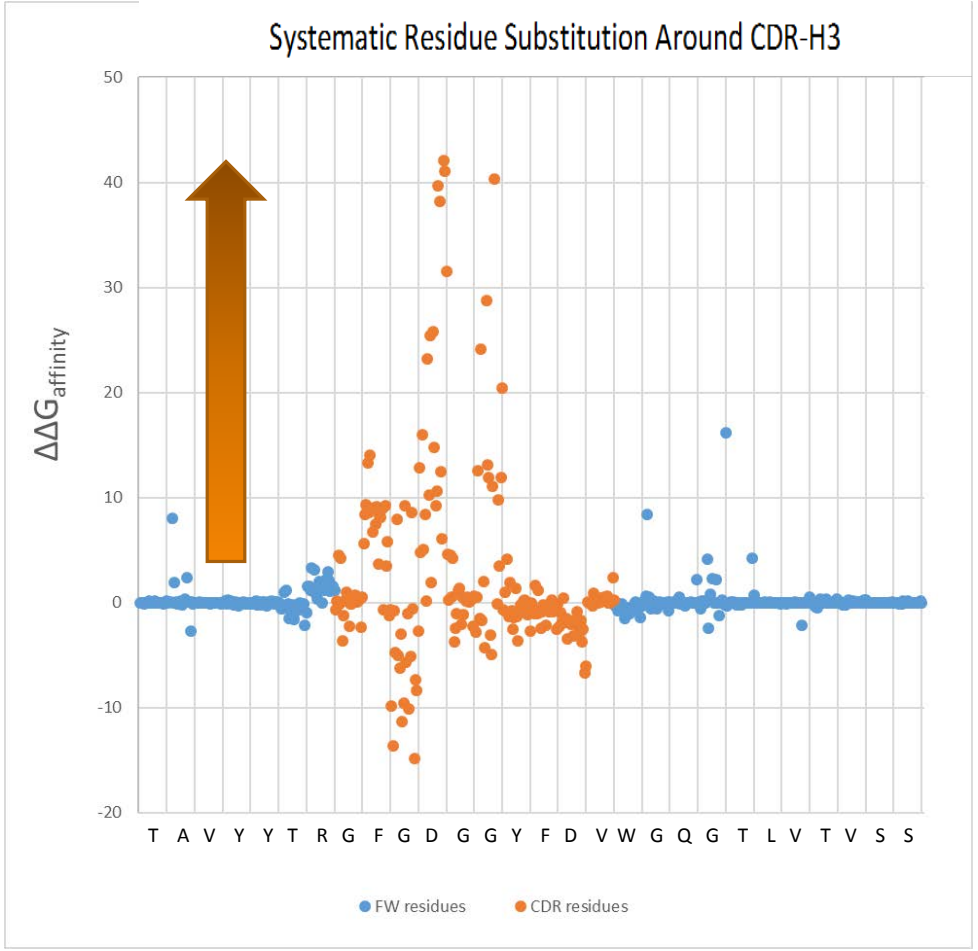


Cf. Louis A. Clark et al., Affinity Enhancement of an In Vivo Matured Therapeutic Antibody Using Structure-Based Computational Design, 15 PROTEIN SCIENCE 949-60 (2006).

III. Computational Simulations

- "Hotspot" identification: defined as a calculated ΔΔG > 1 kcal/mol. Accuracy varies from protein to protein; confidence increases with higher ΔΔG values.

See Hege Beard et al., *Applying Physics-Based Scoring to Calculate Free Energies of Binding for Single Amino Acid Mutations in Protein-Protein Complexes*, 8(12) PLoS ONE: e82849.doi:10.1371/journal.pone.0082849 (2013).



III. Computational Simulations

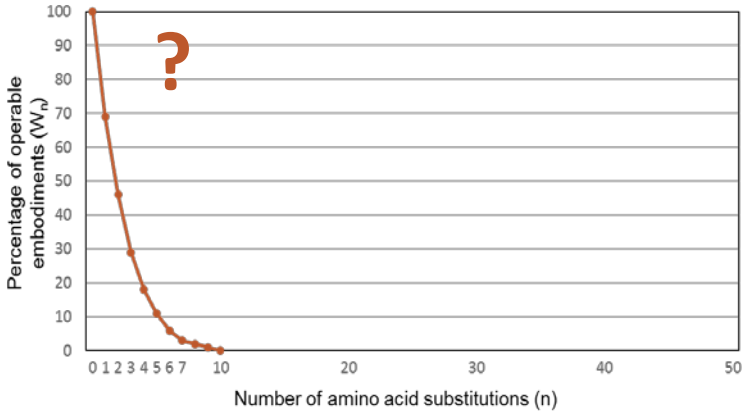
Predicted substitutions as "representative":

- Computational simulations generally are performed substitution by substitution. Would one calculation be "representative" of another?
- Modeling instead provides representative examples of a genus of mutational "hotspots."

III. Computational Simulations

Unanswered questions:

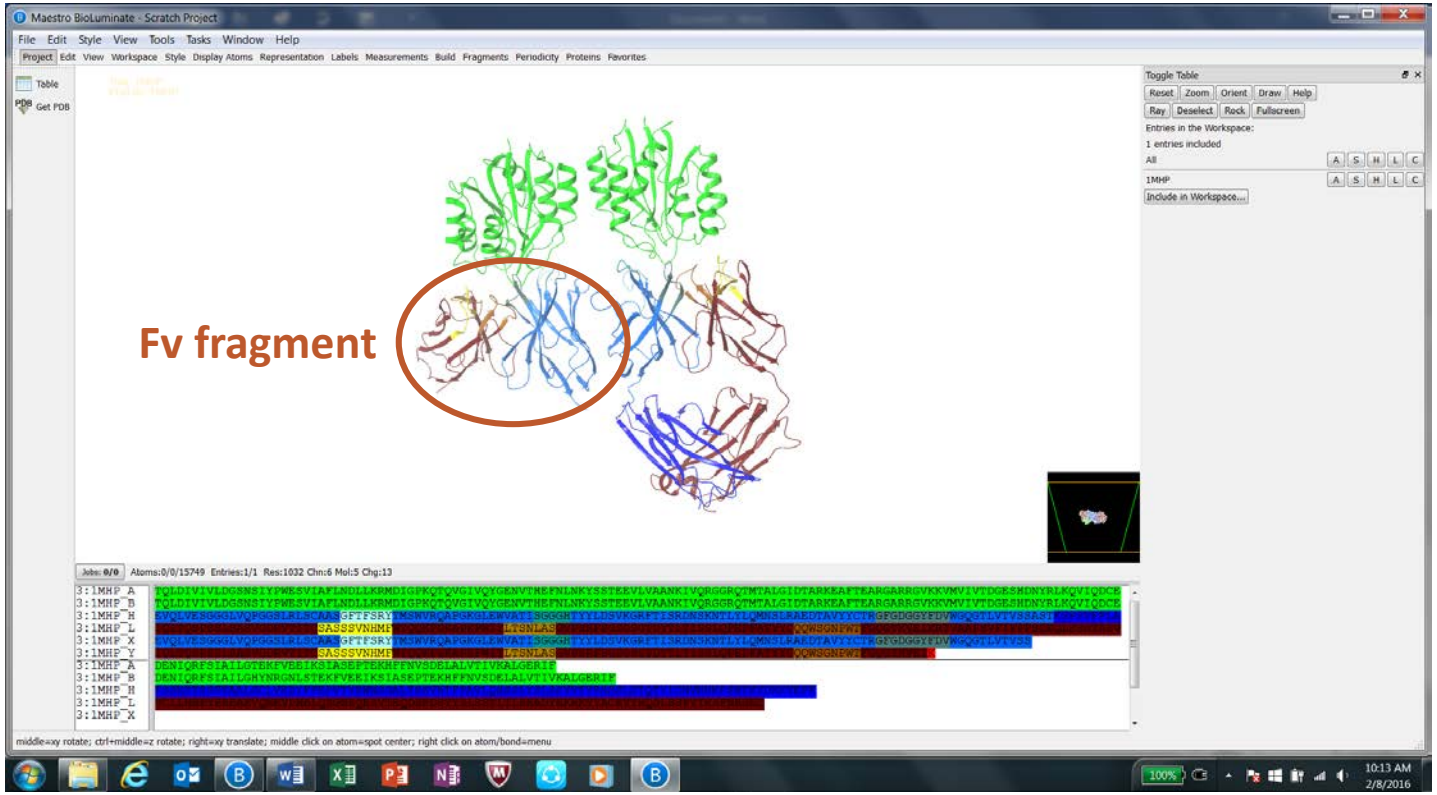
- Assisted by computational simulations, how far could the artisan navigate through large sequence spaces?



III. Computational Simulations

Modeling the 1MHP complex:

See Louis A. Clark et al., Affinity Enhancement of an *In Vivo* Matured Therapeutic Antibody Using Structure-Based Computational Design, 15 PROTEIN SCIENCE 949-60 (2006).

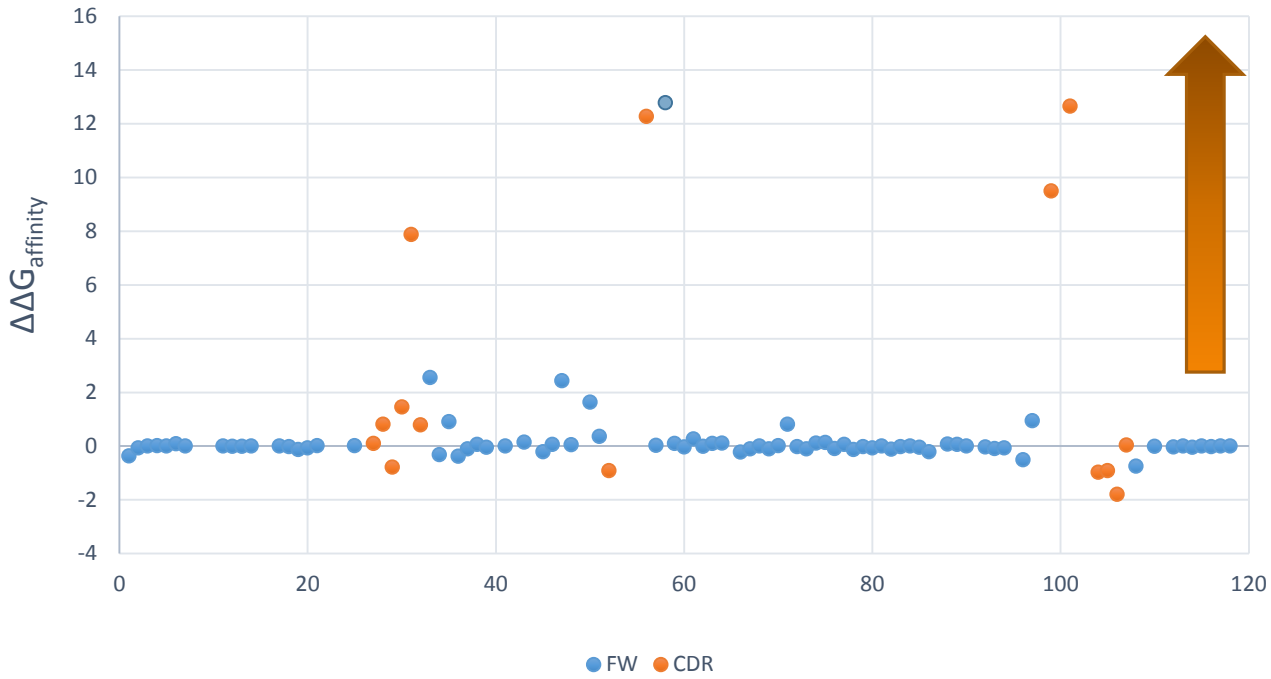


III. Computational Simulations

Using simulations to identify affinity hotspots in the 1MHP complex:

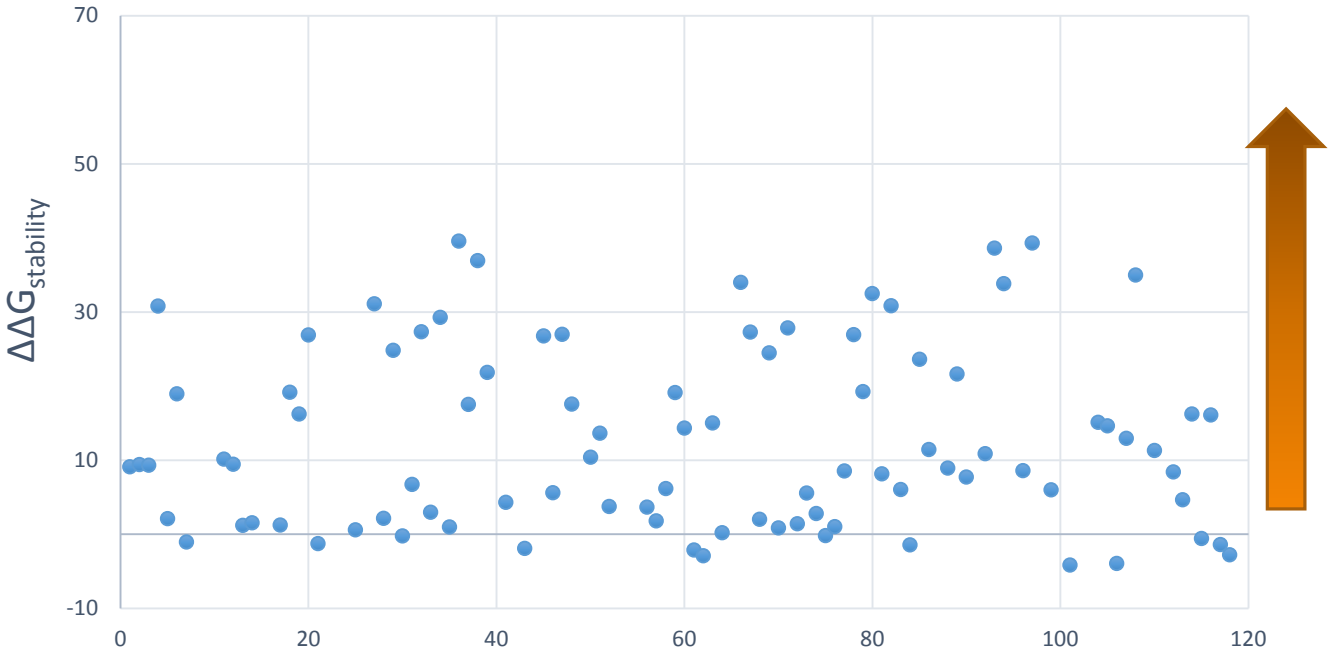
Affinity "hotspots" with a $\Delta\Delta G_{affinity}$ (y-axis) cutoff at ≈ 3 relative units.

What is the significance of numerous unpredictable atomic interactions between FW and CDR residues having a $\Delta\Delta G < 3$ relative units?



In silico alanine scanning of the 1MHP complex across V_H domain residues using BioLuminate™ (residue number from N-terminus to C-terminus on the x-axis).

III. Computational Simulations



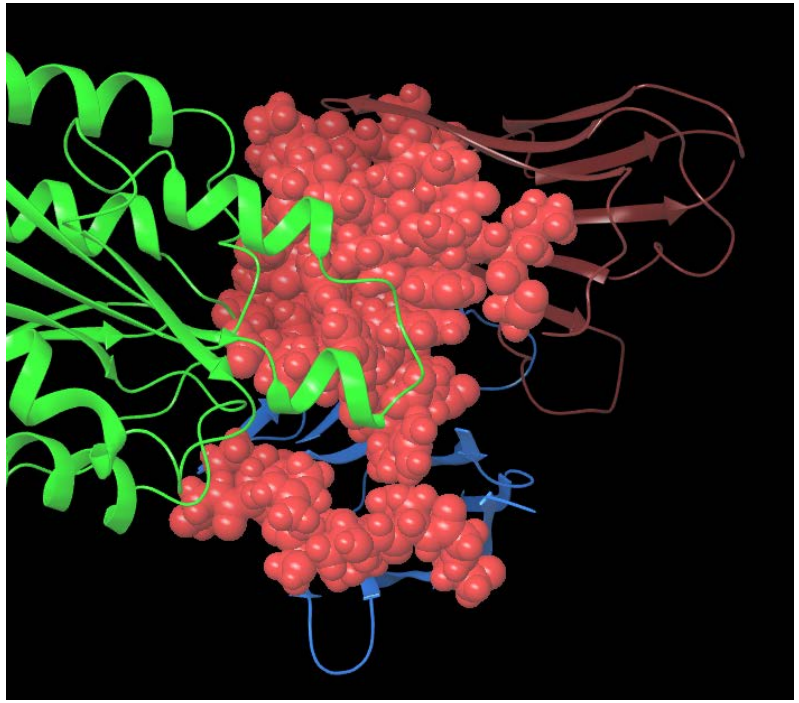
In silico alanine scanning of the 1MHP complex across V_H domain residues using BioLuminate™ (residue number from N-terminus to C-terminus on the x-axis). $\Delta\Delta G_{stability}$ on the y-axis in relative units.

Using simulations to identify stability hotspots in the 1MHP complex:

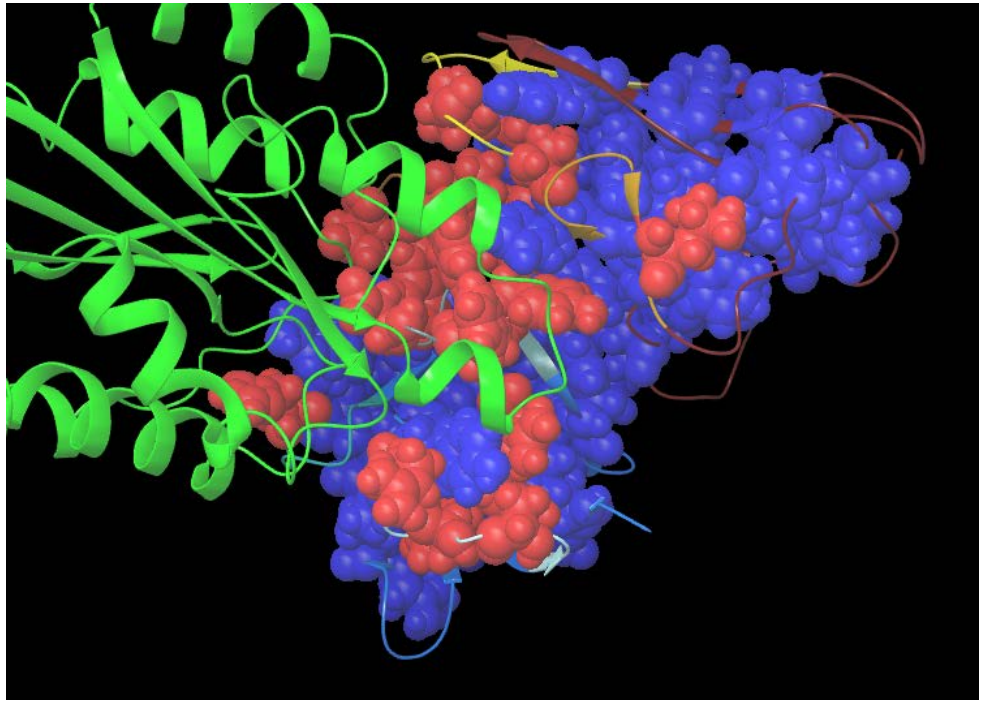
Where should the cutoff be? Should the cutoff be ≈ 3 units (i.e., "hotspots"), or should it be higher, reflecting a larger destabilizing effect (i.e., "essential for function")?

III. Computational Simulations

Which model is more consistent with the evidence?



Left: The hypothetical claim presumes that only **CDR** residues are essential.



Right: BioLuminate™ predicts 79 (35%) **CDR** and **FW** residues are essential **(Assume ΔΔG >15 units = essential for stability)**.

Conclusion

- Computational simulations open up a whole new source of evidence that would assist the USPTO in fairly and accurately enforcing the *quid pro quo* for patent protection.
  - This may mean narrower, but stronger, claims.
- The evidence could be submitted with a third party protest under 35 U.S.C. § 122.

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For More Detail

- Brian Lathrop and Michael Kinch, *Enhancing the Quality of Patent Claims Directed to Biologics with Biophysical Evidence*, 34 BIOTECHNOLOGY LAW REPORT 213-35 (2015).
- Brian Lathrop, *Using Computer Simulations to Evaluate a Hypothetical Patent Claim Directed to a Recombinantly Engineered Antibodies* (manuscript in preparation).

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