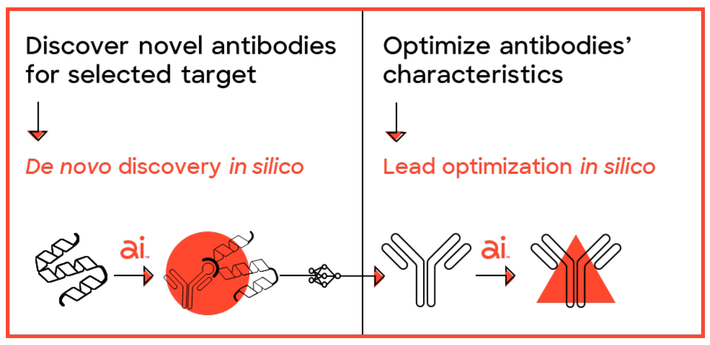
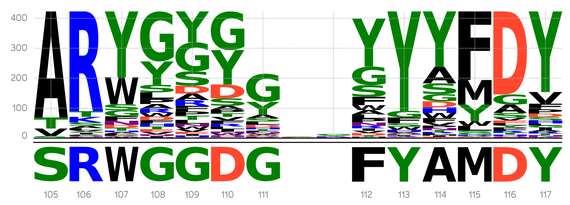
DESIGN OF FUNCTIONAL DEVELOPABLE AND POTENT ANTIBODIES USING GENERATIVE AI

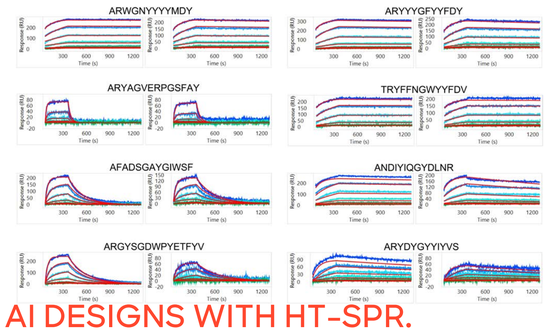
Amir Shanehsazzadeh, Matt McPartlon, George Kasun, Andrea K. Steiger, John M. Sutton, Edriss Yassine, Cailen McCloskey, Robel Haile, Richard Shuai, Julian Alverio, Goran Rakocevic, Simon Levine, Jovan Cejovic, Jahir M. Gutierrez, Alex Morehead, Oleksii Dubrovskyi, Chelsea Chung, Breanna K. Luton, Nicolas Diaz, Christa Kohnert, Rebecca Consbruck, Hayley Carter, Chase LaCombe, Itti Bist, Phetsamay Vilaychack, Zahra Anderson, Lichen Xiu, Paul Bringas, Kimberly Alarcon, Bailey Knight, Macey Radach, Katherine Bateman, Gaelin Kopec-Belliveau, Dalton Chapman, Joshua Bennett, Abigail B. Ventura, Gustavo M. Canales, Muttappa Gowda, Kerianne A. Jackson, Rodante Caguiat, Amber Brown, Douglas Ganini da Silva, Zheyuan Guo, Shaheed Abdulhaqq, Lillian R. Klug, Miles Gander, Engin Yapici, Joshua Meier*, **Sharrol Bachas**

Artificial intelligence (AI) has the potential to greatly increase the speed, quality and controllability of drug creation. Here, we showcase such potential on two antibody drug creation tasks: *de novo* design and lead optimization. For the first task, we utilize generative AI models for "zero shot" design of antibody CDRs, were we screen over 1 million antibody variants designed for binding to human epidermal growth factor receptor 2 (HER2) using our high-throughput wet lab capabilities. Our models successfully design all CDRs in the heavy chain of the antibody and compute likelihoods that are calibrated with binding. We achieve binding rates that are several multiples higher than HCDR3s and HCDR123s randomly sampled from the Observed Antibody Space (OAS). We further characterize 421 AI-designed binders using surface plasmon resonance (SPR), finding three that bind tighter than the therapeutic antibody trastuzumab. The binders are highly diverse, have low sequence identity to known antibodies, and adopt variable structural conformations. For the second task, we show that our high-throughput screening assay can generate quantitative binding affinity scores for hundreds of thousands of antibody variants. After validating these scores with SPR, we use this data to train large language models that accurately predict binding affinities for unseen antibody variants. Next, we use this data to train large language models that accurately predict binding affinities for unseen antibody variants. We demonstrate the performance of our model by designing variants of trastuzumab with up to seven mutations and with over ten-fold increase in binding affinity. Furthermore, these models can be used to co-optimize multiple antibody properties, demonstrated by the identification of pH sensitive trastuzumab binders (> 1 log differential KD) and introducing broad antigen reactivity to SARS-CoV-2 targeting casirivimab. Combined, these approaches promise to accelerate and improve antibody drug creation, and may increase the success rates in developing novel antibodies and related drug candidates.



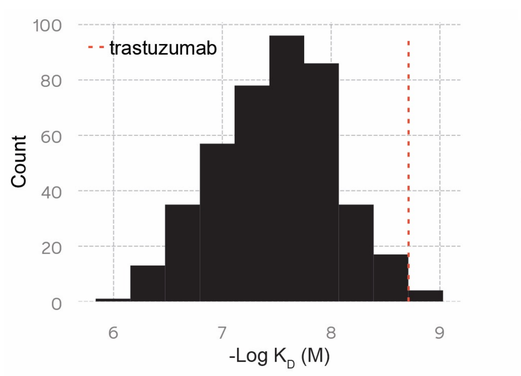
THE LEADING AI PLATFORM FOR BIOLOGICS DRUG CREATION

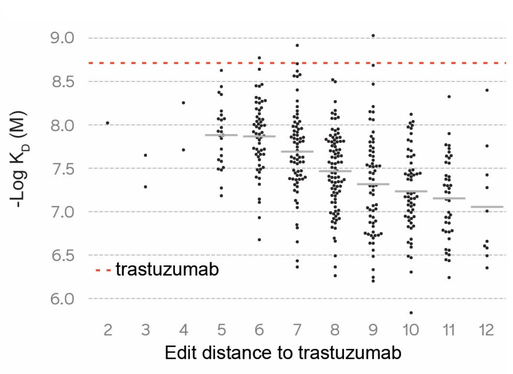




ACCURATE ASSESMENT OF AI DESIGNS WITH HT-SPR.

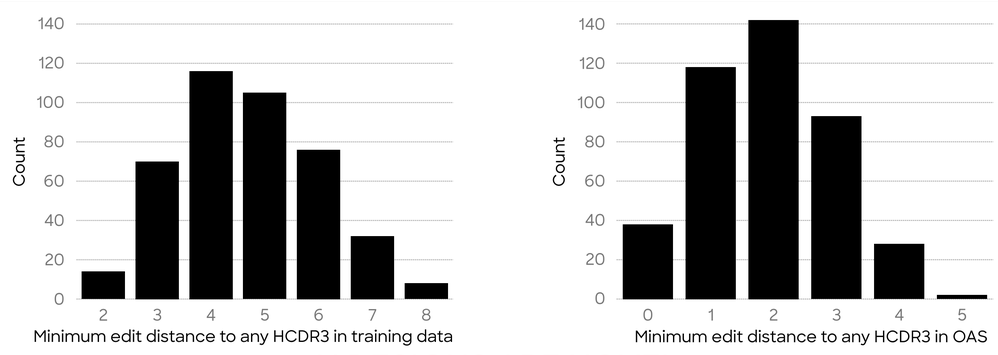
AI designs are screened using high-throughput surface plasmon resonance to obtain accurate representation of sequence performance





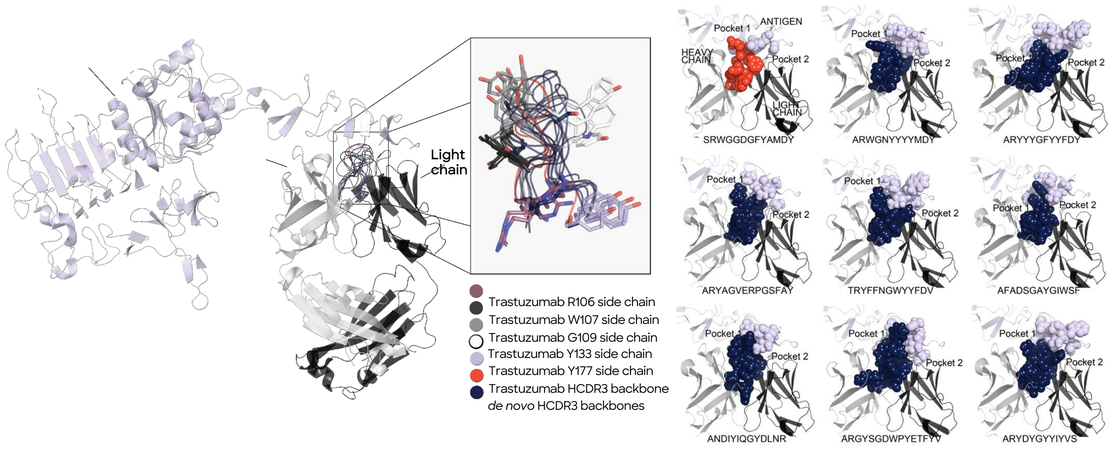
HUNDREDS OF DIVERSE BINDERS CREATED USING GENERATIVE AI

We characterize over 400 binders that span a broad affinity range. We can seamlessly design HCDR3 that reproduce wildtype affinities that are up to 12 mutations away from the wildtype CDR.



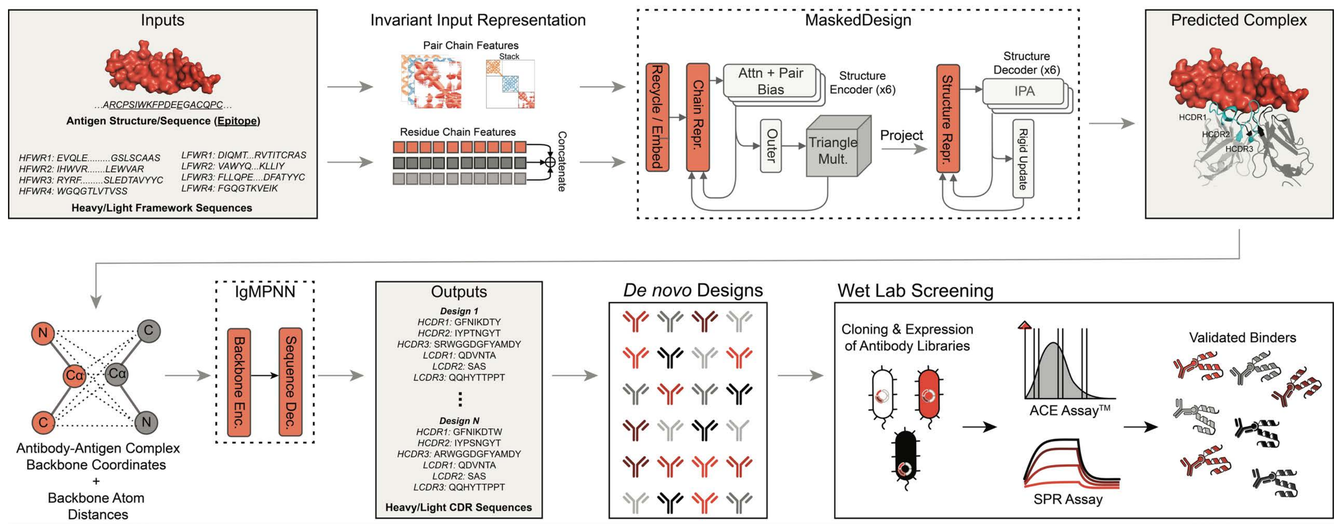
AI DESIGNS ARE NOVEL

Our HCDR3 designs are unlike any sequence in the training set or sequences in found in large public databases.



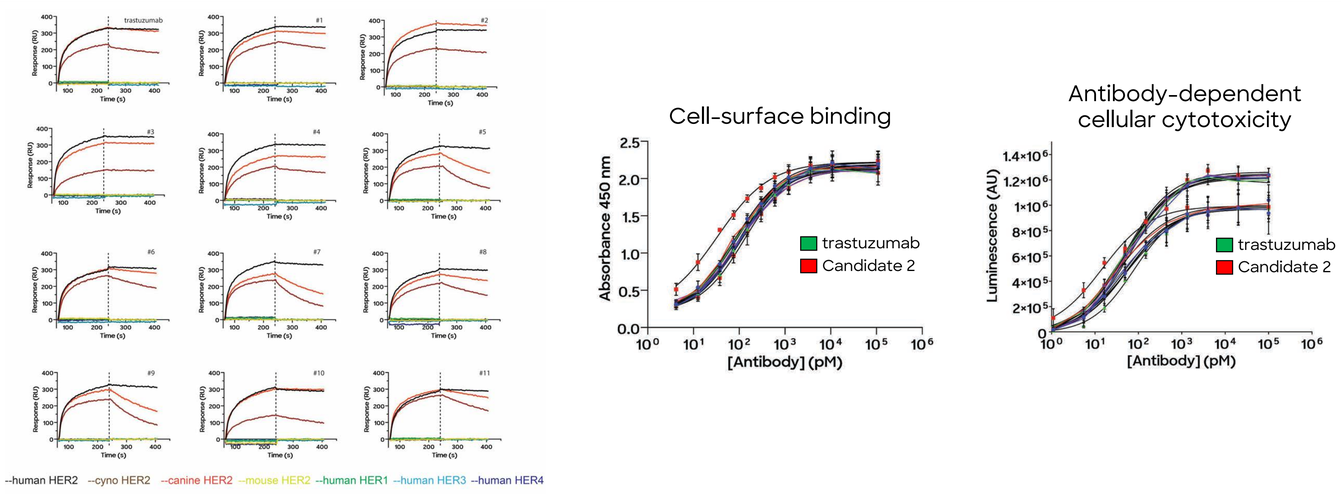
HCDR3 (Length) [ED]		$-\log_{10}(K_D)$	Interface (Å²)	RMSD (Å)	Hydropathy
SRWGGDGFYAMDY	(13) [0]	8.71	771	0.000	-0.81
ARWGNYYYYMDY	(12) [6]	8.77	739	2.435	-1.30
ARYYYGFYYFDY	(12) [7]	8.92	819	2.832	-0.73
ARYAGVERPGSFAY	(14) [11]	6.24	764	1.107	-0.42
TRYFFNGWYYFDV	(13) [9]	9.03	843	1.974	-0.37
AFADSGAYGIWSF	(13) [12]	7.00	824	5.738	0.57
ANDIYIQGYDLNR	(13) [12]	8.40	833	5.506	-0.80
ARGYSGDWPYETFYV	(15) [10]	7.01	863	6.767	-0.76
ARYDYGYYIYVS	(12) [10]	8.02	718	3.032	-0.43

STRUCTURAL ALIGNMENT SHOWS BINDERS ADOPT VARIABLE BINDING MECHANISMS



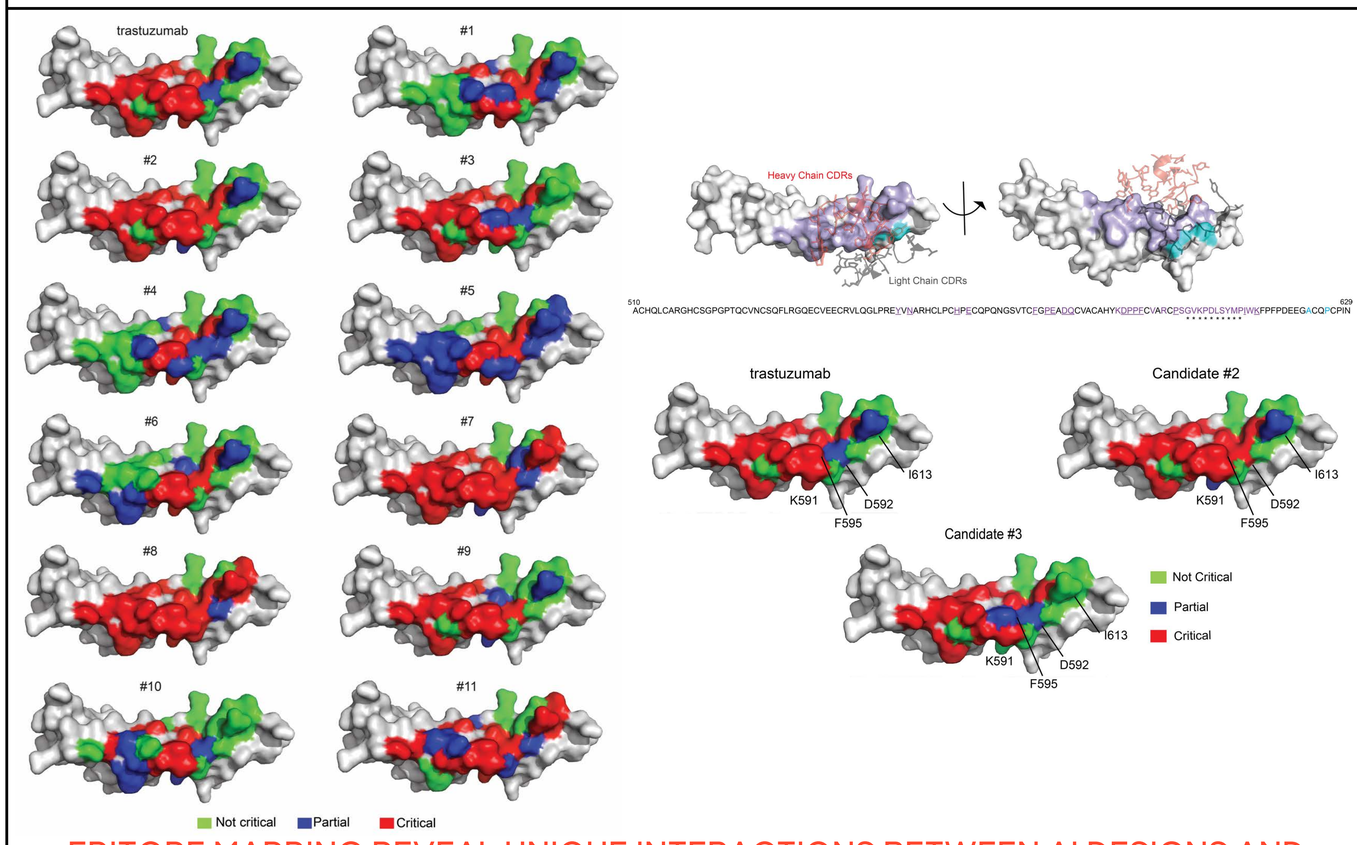
GENERATIVE AI MODEL FOR *DE NOVO* ANTIBODY DESIGN

Deep learning models trained on antibody-antigen interactions combined with high-throughput wet-lab experimentation enabled the design of binders to an antigen never-before-seen by the models without further affinity maturation or lead optimization. Model architectures are depicted in dashed boxes. Model inputs and outputs are depicted with gray boxes in the background. Inputs to the model consisted of target antigen structure and sequence, target epitope region, and antibody framework sequences. None of the CDR sequences were provided to the models as input. Inputs are processed into the invariant input representation and passed into the MaskedDesign model which predicts a docked antibody-antigen complex structure. The predicted complex is passed to IgMPNN which designs CDRs. *De novo* designed HCDRs are ordered as a library and are screened *in vitro* for binding



AI DESIGNS SHOW IMPROVED FUNCTIONAL PROPERTIES

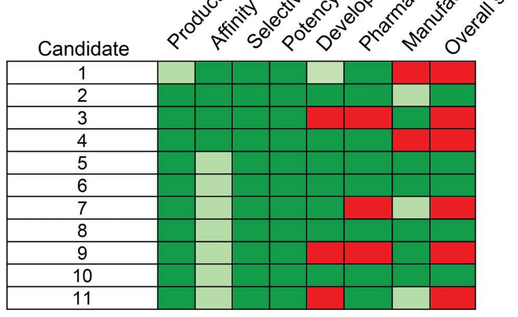
11 AI designs with K_D similar to trastuzumab were tested for species cross-reactivity by SPR, cell surface binding in cell-based ELISA and for activation of antibody-dependent cellular cytotoxicity (ADCC). Individual designs perform similar as trastuzumab in all functional assays with two individual variants showing superior cross-reactivity and cellular function. These results demonstrate the efficiency of AI at enhancing antibody design for improved biological function.



EPITOPE MAPPING REVEAL UNIQUE INTERACTIONS BETWEEN AI DESIGNS AND HER2 AND ILLUMINATE HOTSPOT CONTACTS THAT MODULATE FUNCTION

Epitope mapping was used to determine residues in domain IV of HER2 that are important for each high affinity candidate. Epitope maps show that most candidate has a unique epitope signature that was different from trastuzumab. High potency and most cross-reactive designs interact with unique contact sites that explain gain in function.

Candidate #	HCDR3 sequence	Cell surface binding (EC50, pM)	ADCC (EC50, pM)	AC-SINS (nm shift)	HIC (Relative RT)	Polyspecificity		FcRn chromatography (RT, min)
						Insulin (score)	DNA (score)	
1	TRYFFNGWYYFDV	87.4	53.8	2.4	1.21	**0.517**	0.175	28.13
2	ARYYYGFYYFDY	**33.2**	**14.6**	3.1	1.11	0.176	0.127	26.98
3	ARWGNYYYYMDY	122.2	77.9	**9.1**	1.27	0.205	0.132	**29.79**
4	ANDIYIQGYDLNR	105.7	58.7	1.8	1.14	0.168	0.101	27.35
5	ARYYGYGGYYFDY	107.4	46.5	2.4	1.09	0.250	0.122	27.31
6	ARWGGDFYAMDY	78.1	34.8	0.4	1.05	0.187	0.122	26.74
7	ARWYGYGGYYFDY	87.1	57.7	4.4	1.16	0.175	0.133	**29.56**
8	ARYGYAPGFYYMDV	103.4	62.8	3.1	1.14	0.187	0.126	27.50
9	TRWGGYYYFDY	104.8	50.2	**9.1**	1.21	0.200	0.121	**29.10**
10	APYGPGYWYGV	99.8	50.1	-0.9	1.16	0.138	0.125	28.06
11	ARYYYDYYYYFDY	128.1	48.8	**5.1**	**1.56**	0.170	0.124	27.61
trastuzumab	SRWGGDGFYAMDY	110.7	57.0	0.4	1.01	0.195	0.121	26.74



HIGH AFFINITY AI DESIGNS DISPLAY FAVORABLE THERAPEUTIC PROPERTIES

Candidates were studies in a panel of assays to compare developability to trastuzumab. Most high affinity AI designs showed comparable developability properties to trastuzumab. Overall score shows a subset of designs show favorable therapeutic properties that are well-suited for clinical development.

absci®