Bioprocessing India 2024
Oral Presentation

rAbDesFlow: *In silico* engineering of recombinant antibodies for onco-therapeutics

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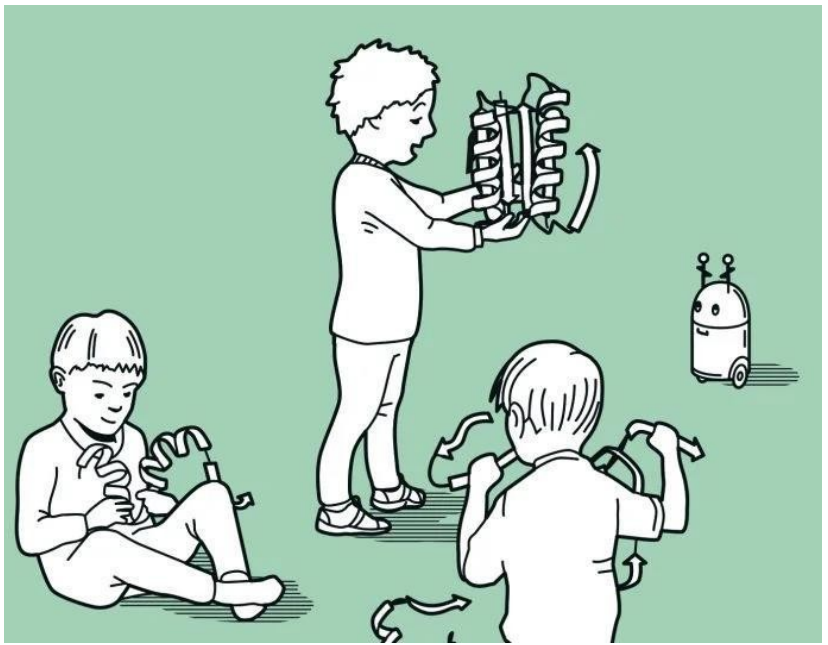
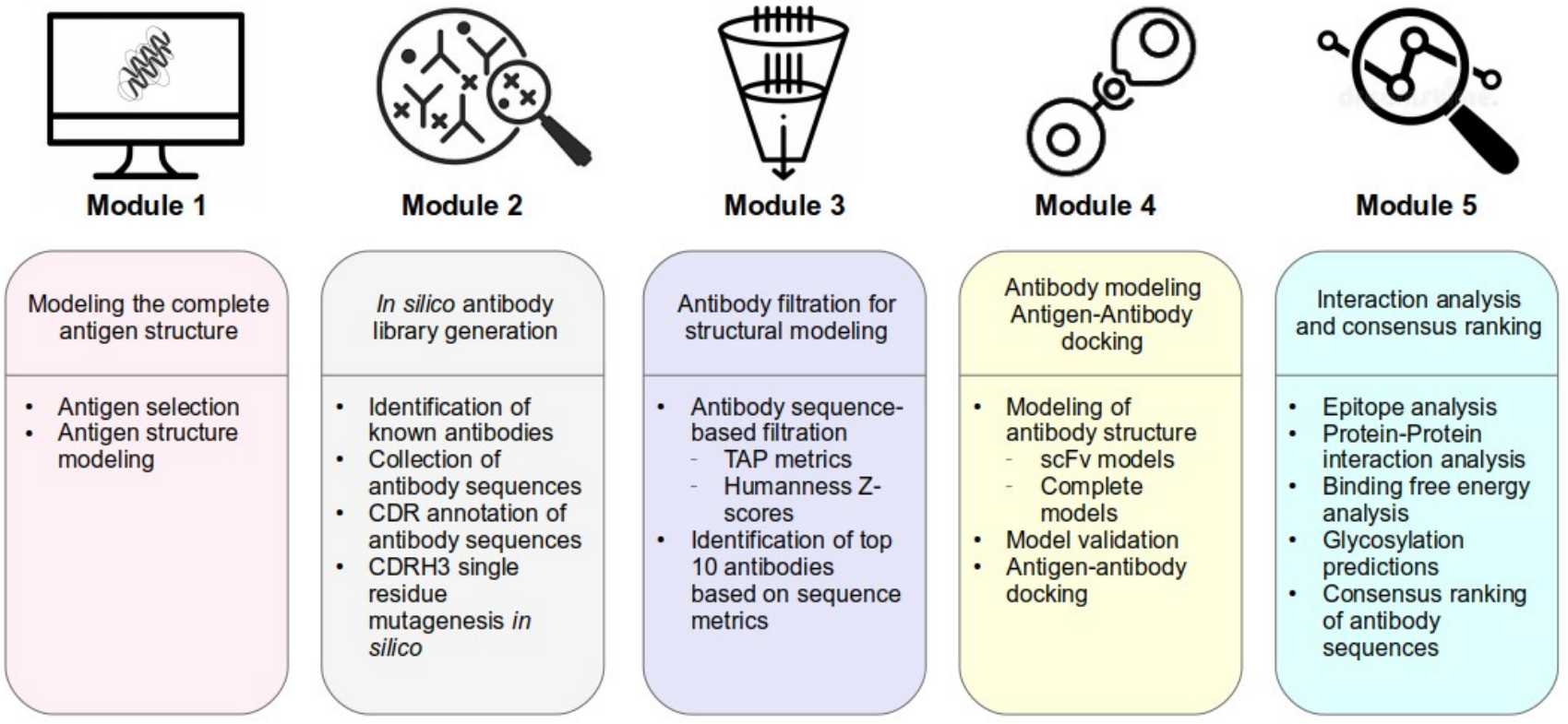
In silico protein engineering

- Proteins can be both therapeutic targets and therapeutics themselves.
- Biologics such as antibodies are proteins that act as selective recognizers of disease-specific antigens to trigger host immune response.
- Emergence of *in silico* protein modelling tools such as AlphaFold and RosettaFold have significantly helped pharma in understanding the sequence-structure-function relationship of proteins.



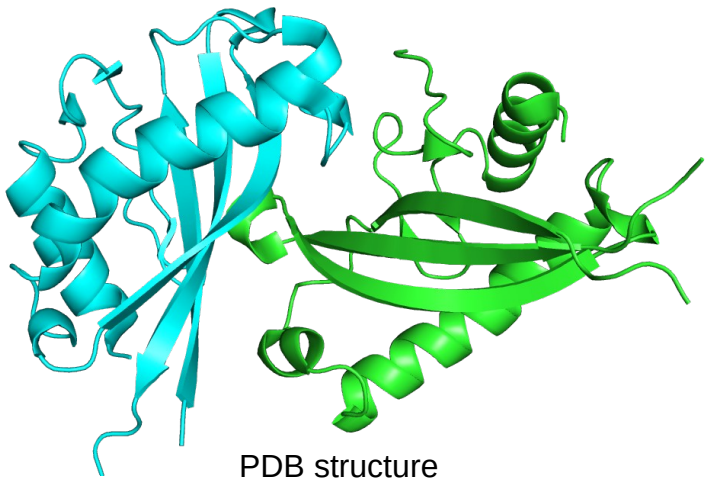
rAbDesFlow pipeline

- Recombinant Antibody Design Workflow (**rAbDesFlow**) is a sequence-based pipeline for designing antibodies for a given antigen.
- Assumes that a few known antibodies with moderate binding affinity to the antigen are already known (not entirely *de novo*).

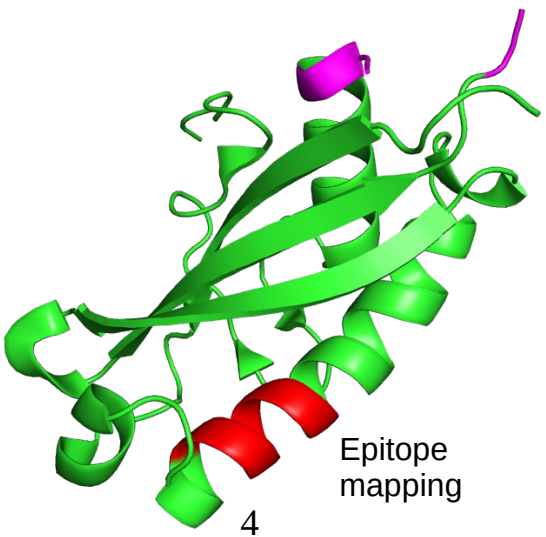


CA125 antigen

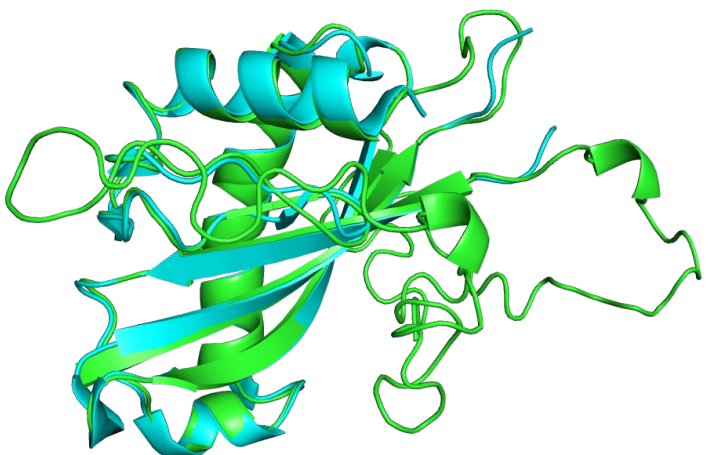
- CA125 – Cancer Antigen 125 or Mucin-16 is an ovarian cancer marker protein (~14,000 residues long)
- Transmembrane protein with a long extracellular region consisting of 60-70 tandem repeats of a SEA (Sperm protein, Enterokinase and Agrin) domain (isoforms vary based on repeat length) made of 196 residues.
- Previously considered SEA domain epitopes for antibody design identified from literature (purple and red) – Examples: OC-125, 5E11, M11 etc.
- UniProt ID: Q8WXI7 (MUC16_HUMAN)
- Human SEA domain partial structure (residues 35 to 160) solved to 1.69 Å resolution (PDB ID: 7SA9)
- Complete structure modelled using ITASSER and AlphaFold-2.0 for antibody design – High RMSD due to long disordered region (Many prolines in N & C terminal regions)



PDB structure



Epitope mapping



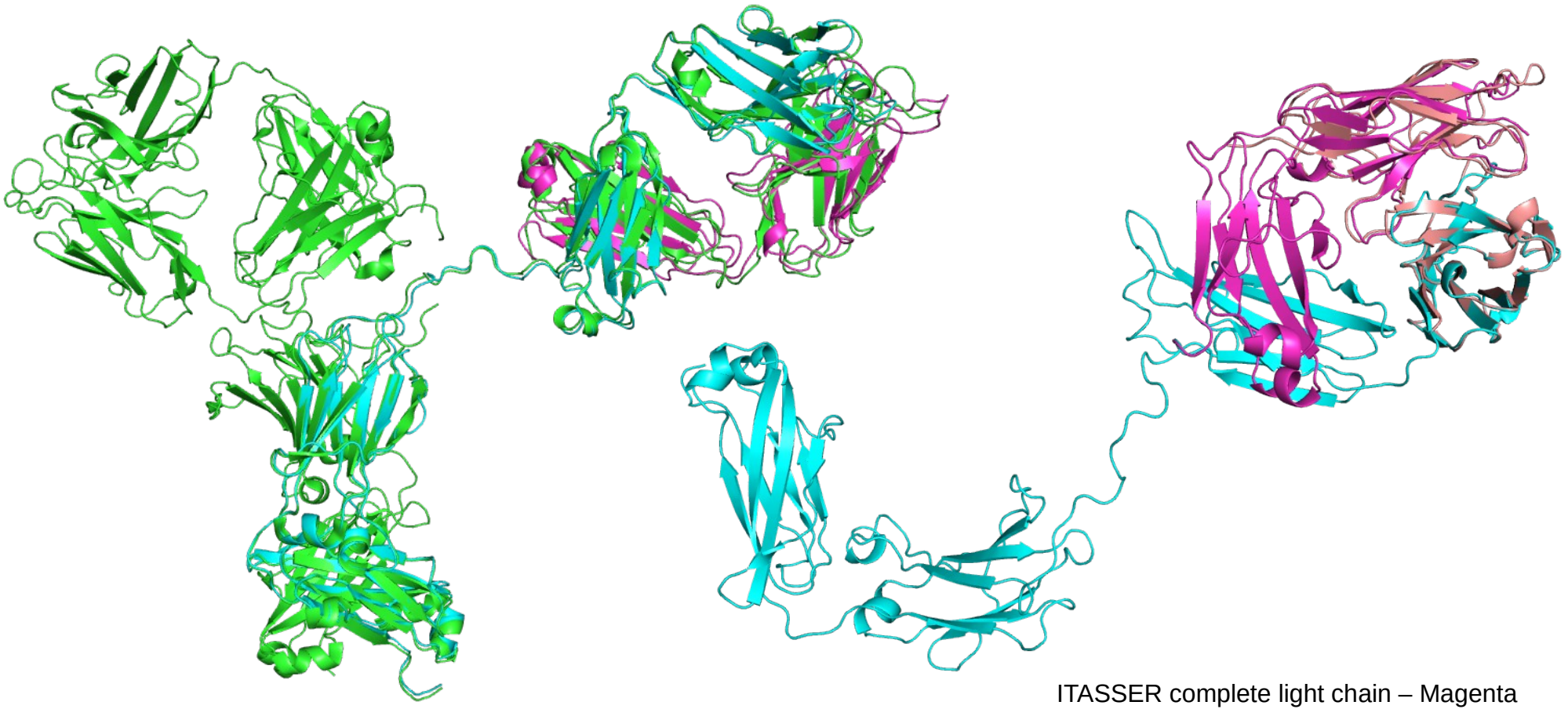
Model overlap

Enumeration of antibody sequences

- The CDR-H3 region (heavy chain variable region 3) – most variable region of existing antibodies – was used to design new antibody sequences.
- Existing CA-125 antibody sequences (Sofituzumab, Abagovomab, anti-CA125-196-14) were collected and CDR regions (L1, L2, L3, H1, H2, H3) were mapped using Kabat numbering scheme using the ANARCI package (Oxford).
- All possible single residue substitutions of the CDR-H3 region sequence was generated using custom program (200 substitutions).
- Therapeutic Antibody Profiler (TAP) metrics from Oxford group were calculated for all 200 substitutions – only antibody sequences passing all 5 metrics (ranges defined for human monoclonal antibodies) were used further (128/200).
- Further the humanness score (H-score) of antibodies calculated from UCL (http://www.bioinf.org.uk/abs/shab/) was calculated.
- Top 10 antibodies with highest heavy chain H-score were considered for detailed analysis.

Information	Value
Final set of mutations	D96G, D96P, D97G, D97P, D97R, D97T, D97Y, Y98G, D99G, M102F
Range of heavy chain H-score	-1.098 to -1.133 (WT = -1.142)
Heavy chain length	118 residues (CDRH3 = SDDYDYGMDY)
Light chain length	112 residues
Fab + Fc region length (1 monomer)	668 residues
Total antibody length	2 monomers = 668*2 = 1336 residues (~150 kDa)

Antibody structure modelling – ScFv region vs complete antibody

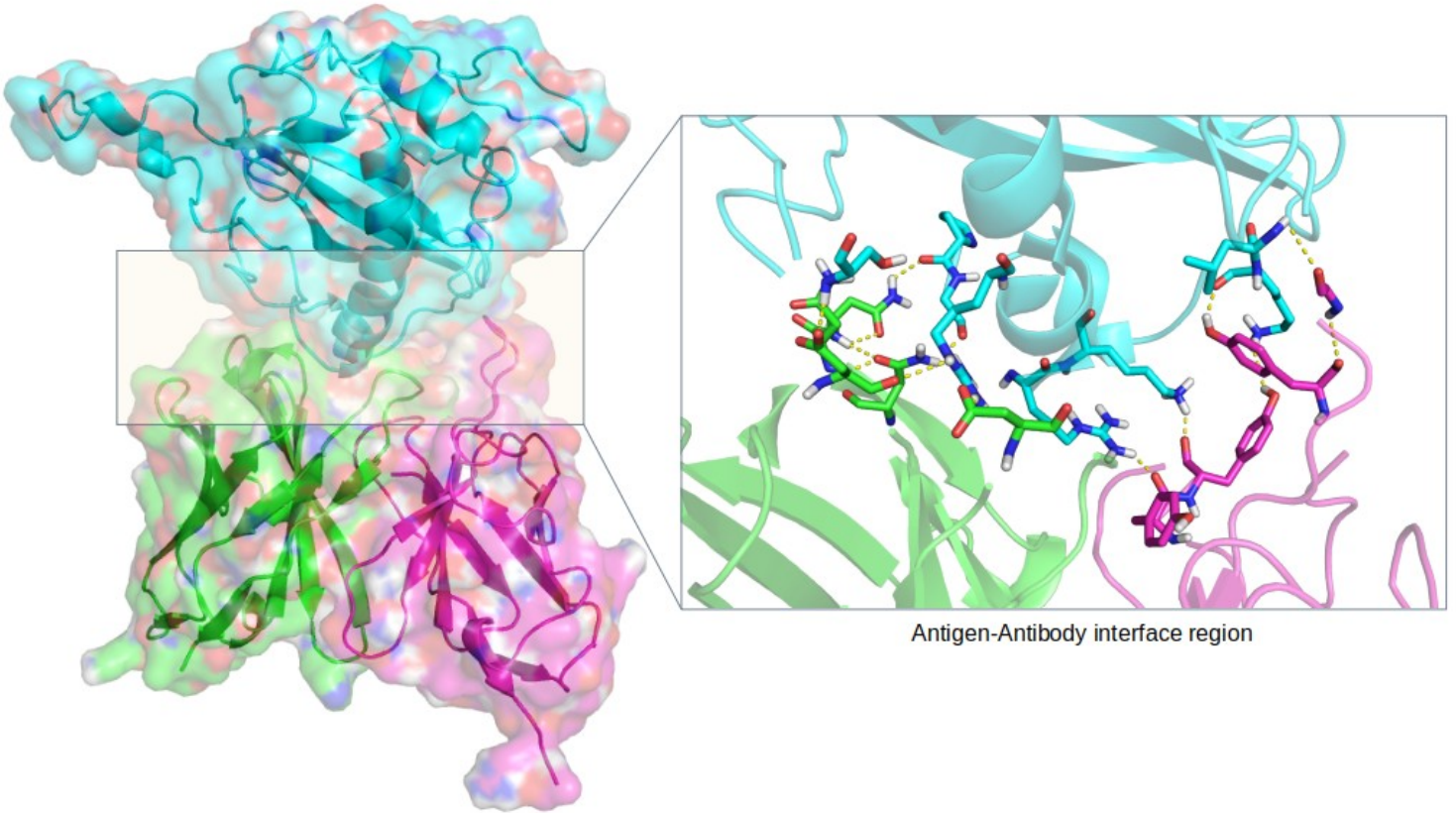


Complete antibody model from ITASSER (Cyan and Magenta)
PDB template antibody structure (Green)

ITASSER complete light chain – Magenta
ITASSER complete heavy chain – Cyan
ROSIE model for ScFv region - Pink

Antigen-Antibody docking with ClusPro

- ClusPro antibody docking mode was used to dock the ScFv model of antibody to SEA domain of CA125 (ITASSER model).
- 30 poses obtained from ClusPro after clustering models.
- Cluster with maximum number of models was identified and cluster representative used as final model.
- Protein-Protein interactions between antigen and antibody analyzed with RING v3.0 standalone tool.
- Interacting residues used to identify epitope and paratope for each antibody.
- Epitopes found to be discontinuous in sequence.

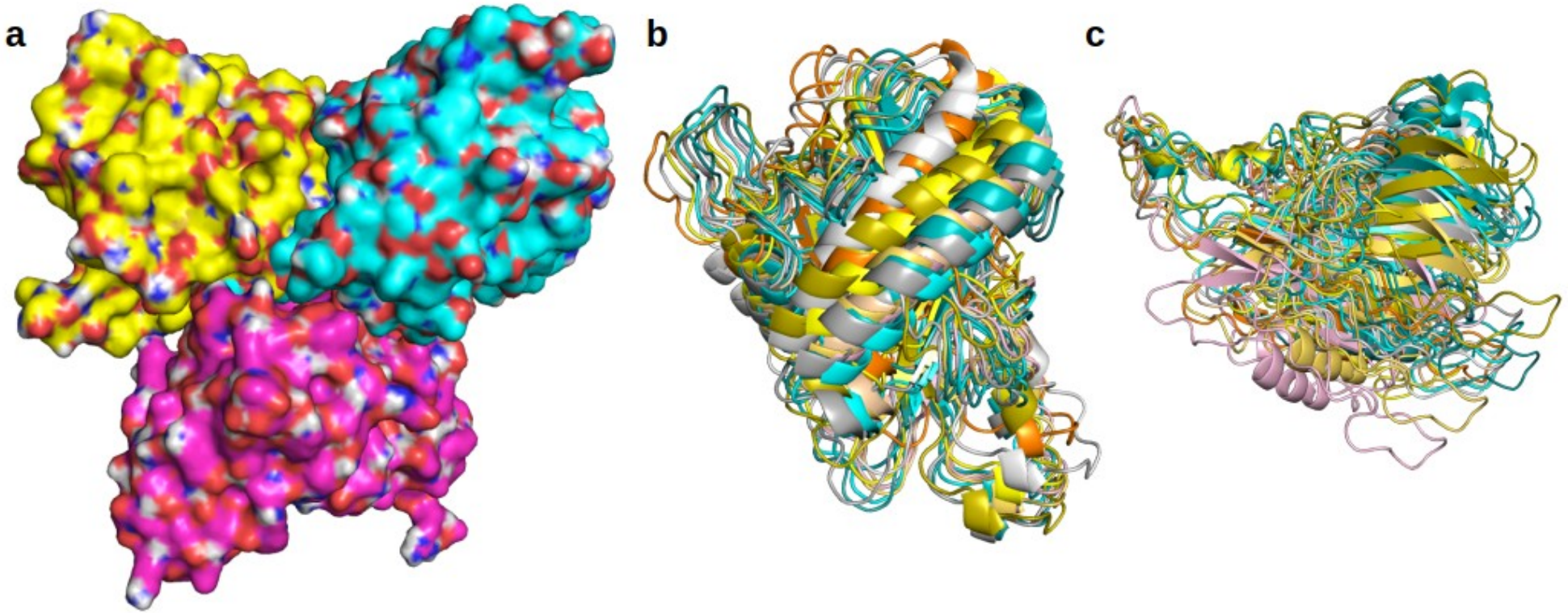


Antigen-Antibody interface region

Wild-type antigen-antibody complex obtained from ClusPro (Antigen in cyan, Antibody light chain in green, Antibody heavy chain in magenta) – Visualization generated using PyMOL

Antigen-Antibody orientations possible

- Two predominant orientations of the Antigen and Antibody were identified by aligning all 30 poses from ClusPro for each antigen-antibody complex and further alignment of antigen orientations between the different antibodies.
- The two predominant antigen orientations among all docking poses compared are highlighted below (b and c).



PPI analysis results – Possible orientation 1

Antibody	Mutation	Hydrogen bonds			Ionic	Cation-Pi	Pi-Pi stacking	Disulfide bridges	van der Waals
		MC-MC	MC-SC	SC-SC					
WT	-	0	11	9	1	0	2	0	13
ab1_26	D96G	0	4	**14**	**2**	0	2	0	13
ab1_33	D96P	0	6	9	1	0	0	0	**19**
ab1_46	D97G	0	11	**10**	0	0	2	0	**21**
ab1_53	D97P	0	5	**11**	1	0	**3**	0	**18**
ab1_55	D97R	0	5	8	0	0	**3**	0	**14**
ab1_57	D97T	0	5	9	0	0	2	0	11
ab1_60	D97Y	0	5	4	1	0	0	0	10
ab1_66	Y98G	0	4	6	0	0	0	0	12
ab1_86	D99G	0	9	**11**	0	0	**3**	0	**19**
ab1_145	M102F	0	7	7	1	0	2	0	**19**

*MC – Mainchain; SC – Sidechain

PPI analysis results – Possible orientation 2

Antibody	Mutation	Hydrogen bonds			Ionic	Cation-Pi	Pi-Pi stacking	Disulfide bridges	van der Waals
		MC-MC	MC-SC	SC-SC					
WT	-	1	7	11	3	0	0	0	17
ab1_26	D96G	0	5	9	3	0	0	0	17
ab1_33	D96P	0	**8**	7	1	0	0	0	**18**
ab1_46	D97G	0	6	**12**	1	0	0	0	**19**
ab1_53	D97P	0	2	6	1	0	0	0	12
ab1_55	D97R	0	4	7	2	0	0	0	14
ab1_57	D97T	1	**9**	**12**	2	0	0	0	**22**
ab1_60	D97Y	0	7	6	2	0	0	0	**19**
ab1_66	Y98G	0	2	9	2	0	0	0	16
ab1_86	D99G	0	4	8	2	0	0	0	15
ab1_145	M102F	0	8	**12**	2	0	0	0	**21**

*MC – Mainchain; SC – Sidechain

Antibody prioritization (Possible orientation 1)

- Binding free energy and Kd of antigen-antibody complex structures were predicted using the PRODIGY server (Bonvin lab).
- **Antibody sequence 46 (D97G) was found to be the best** in comparison witht the wild-type antibody.
- Overall, substitutions involving replacement of aspartic acids (D96, D97 and D99) with G, P, R, T and Y were found to be favorable (8/10).
- Replacement with Glycine is most frequent among the top 10 antibodies considered (4/10).
- Replacement of D97 leads to maximum improvement in antigen-antibody interaction profile among the other residues considered in the CDRH3 region (5/10).
- Top 3 ranked sequences match the previous results obtained.

Antibody	Mutation in CDRH3 region	PRODIGY predicted K_d (M) at 25°C	PRODIGY energy (kcal/mol)	Overall rank
WT	-	2.2E-09	-11.8	4
ab1_26	D96G	2.0E-09	**-11.9**	**3**
ab1_33	D96P	3.7E-09	-11.5	5
ab1_46	D97G	3.9E-10	**-12.8**	**1**
ab1_53	D97P	1.4E-08	-10.7	7
ab1_55	D97R	1.9E-08	-10.5	9
ab1_57	D97T	1.1E-08	-10.8	6
ab1_60	D97Y	5.8E-08	-9.9	11
ab1_66	Y98G	3.4E-08	-10.2	10
ab1_86	D99G	1.1E-09	**-12.2**	**2**
ab1_145	M102F	1.3E-08	-10.7	8

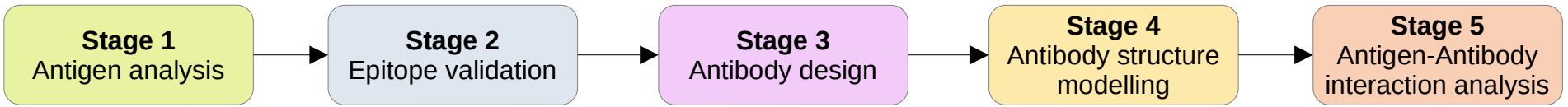
Antibody prioritization (Possible orientation 2)

- Binding free energy and Kd of antigen-antibody complex structures were predicted using the PRODIGY server (Bonvin lab).
- **Antibody sequence 33 (D96P) was found to be the best** in comparison witht the wild-type antibody.
- Overall, substitutions involving replacement of aspartic acids (D96, D97 and D99) with G, P, R, T and Y were found to be favorable (8/10).
- Replacement with Glycine is most frequent among the top 10 antibodies considered (4/10).
- Replacement of D97 leads to maximum improvement in antigen-antibody interaction profile among the other residues considered in the CDRH3 region (5/10).
- Ranking is reshuffled significantly compared to the last results.

Antibody	Mutation in CDRH3 region	PRODIGY predicted K_d (M) at 25°C	PRODIGY energy (kcal/mol)	Overall rank
WT	-	6.8E-11	-13.9	4
ab1_26	D96G	1.7E-09	-11.9	9
ab1_33	D96P	2.8E-11	**-14.4**	**3**
ab1_46	D97G	1.3E-11	**-14.9**	**1**
ab1_53	D97P	2.6E-08	-10.4	11
ab1_55	D97R	1.1E-08	-10.9	10
ab1_57	D97T	1.8E-10	-13.3	5
ab1_60	D97Y	8.3E-10	-12.4	7
ab1_66	Y98G	8.5E-10	-12.4	8
ab1_86	D99G	3.0E-10	-13.0	6
ab1_145	M102F	1.5E-11	**-14.8**	**2**

Phase 2 of rAbDesFlow: Adaptation to under-explored antigens

- Two more cancer-specific human antigens namely, Adipsin and IGFBP-2 are also to be tested with targeted antibodies. However, neither of these antigens have any known antibodies reported in literature.
- Hence, a modified workflow is to be considered to derive the antibody sequences.



- Antigen structure modelling
- B-cell epitope prediction
- Solvent accessibility prediction
- Glycosylation prediction
- Finalization of epitopes

- Gathering PPIs involving antigen and other human proteins (STRING)
- Modelling of interacting proteins
- PPI interface extraction
- Overlap analysis between PPI interface and identified epitopes

- Development of a generative model using Antigen+epitope as input
- Inference of antigen-specific antibodies from (pre-)trained model

- IgFold-derived scFv modelling of designed sequences
- CDR-H3 loop optimization using ROSIE server

- Antigen-antibody complex structure prediction through docking programs
- Interaction analysis
- Binding affinity prediction
- Prioritization of top-scoring antibodies for experimental validation

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JOURNAL ARTICLE

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