Mechanistic Modeling and Simulation for Bispecific Antibodies

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Bispecific mAb target space and formats

One or both targets in solution

Both targets expressed on the same cell surface

Example: CD4/CD70-specific DuetMab binding to cells expressing either $CD4^+$, $CD70^+$ or both at the same time

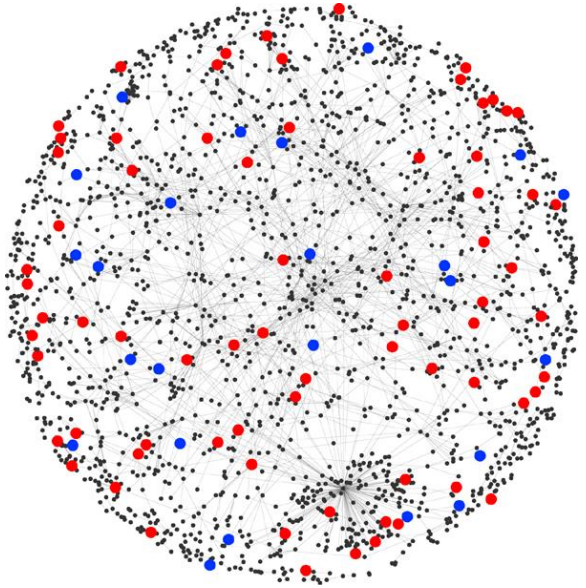
Targets expressed at the surface of different cells

mAb-dAb bispecific construct

Polypharmacology: Not only oncology

- Co-dosing of two or more drugs can be beneficial
  - Infectious: HAART
  - Inflammatory: Antiasthmatics
  - Oncology: NCE and mAbs

- Which target combinations?
  - Experimental insight
  - QSP: *in silico* modelling
  - Combinatorial screening



Xin et al. Molecular Systems Biology (2007) 3, 98

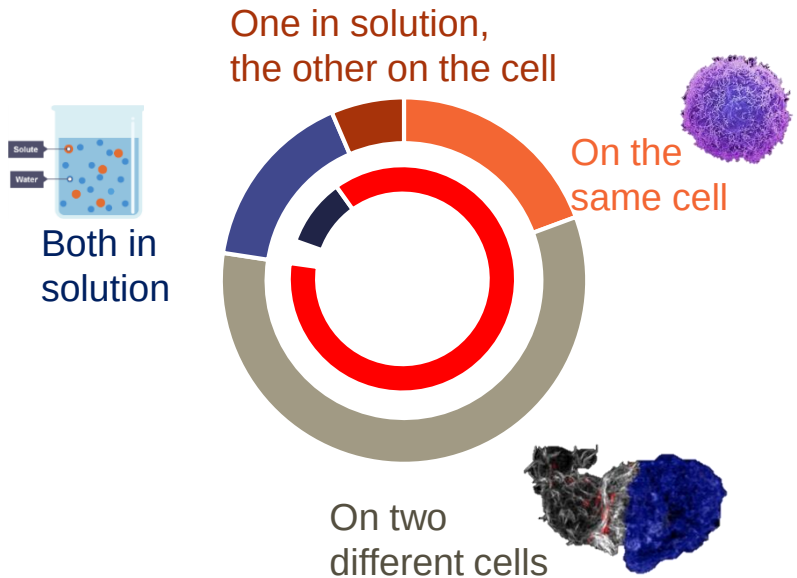
Bispecific mAb target space

In clinical trials

- Oncology (27/31)
  - T cell engagement (15/31)
- Immuno-Inflammation (3/31)

- Target expression
  - Both in solution (5/31)
  - One in solution, the other on a cell (2/31)
  - Both on cell surface
    - Same cell (6/31)
    - Different cells (18/31)

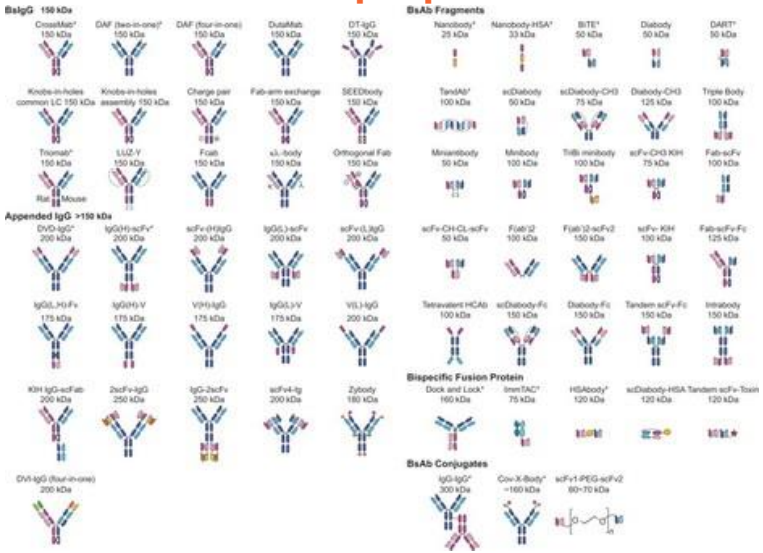
Bispecific antibody targets

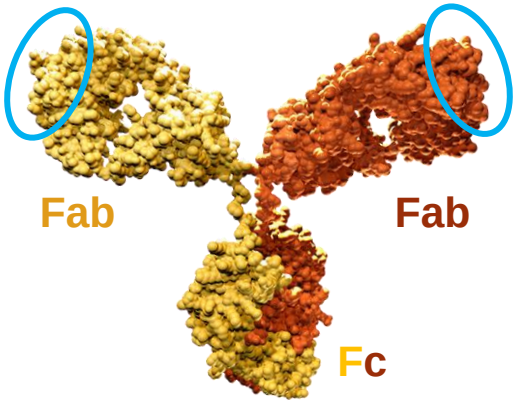


Sheridan, C. (2016). "Despite slow progress, bispecifics generate buzz." Nat Biotech **34**(12): 1215-1217.

Many alternative bispecific formats have been proposed

Many alternative formats for multispecific mAbs have been proposed





Spiess, C., et al. (2015) Mol Immunol 67(2 Pt A): 95-106

Combination therapy vs bispecific? Which format?

The elephant in the room, occasionally

– One bispecific mAb or two monospecific mAbs: what's the difference?

– Is the *additional* time, effort and risk of developing a bispecific mAb justified?

*Datta-Mannan, A., et al. (2016) mAbs 8(5): 969-982.

In silico insight

Experimental data

Mechanistic mathematical model
- Rate equations

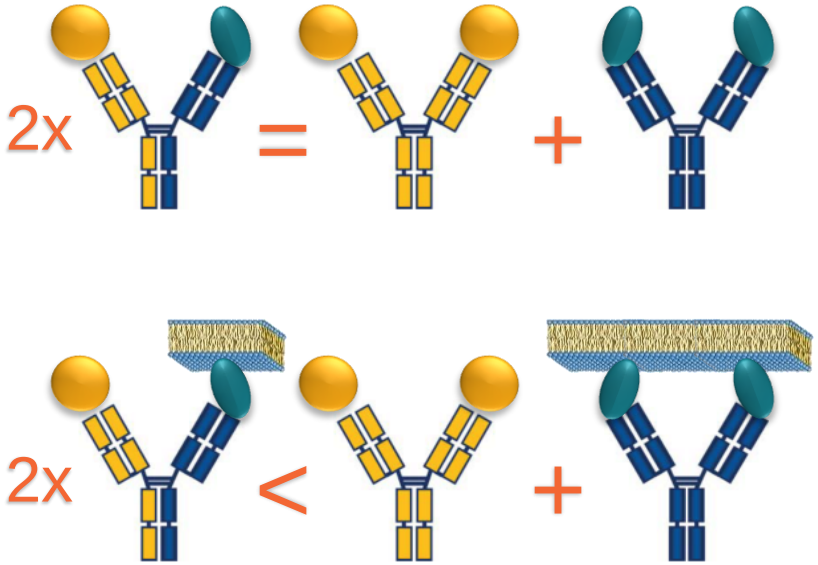
Parameters, measured or fitted
- k_{on}, k_{off}, Kd, D, IC_{50}, EC_{50}...

Predictions
- Alternative drug format
- Affinity and dosing

One or both targets in solution

The target binding at the Fab arms of a mAb are understood to be independent

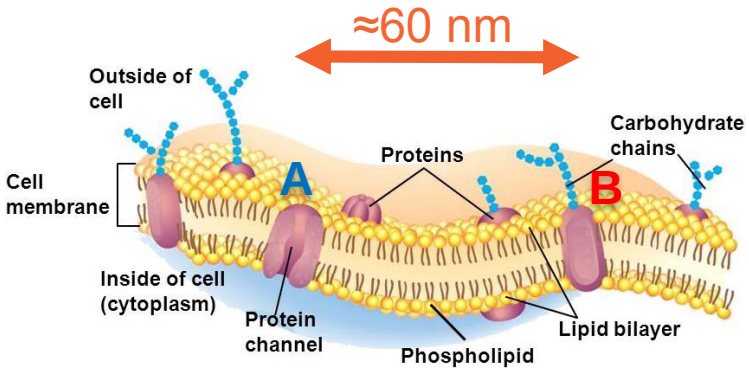
- Both targets are in solution
  - Only mAb binding site concentration matters
  - No difference between a bispecific mAb and combination of monospecific ones is expected
- One of the targets in on cell membrane
  - Monospecific mAb binding to the membrane target benefits from the avidity effect
  - At the same molar dose, the combination can be more efficacious against the membrane target than a bispecific



Both targets on the same cell: avidity from cross-linking

A number of different approaches have been described in literature

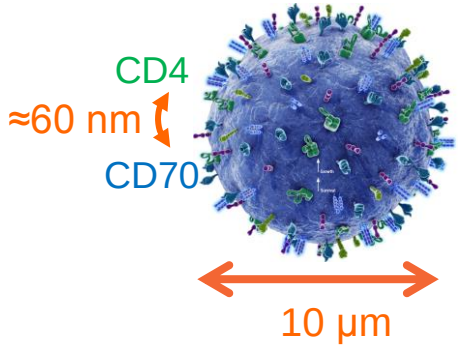
- Assume the targets to behave as if *both* were in solution:
  - How to handle the volume?
- Assume that the targets are *immobile* on membrane
  - How about target cross-linking?
- Membrane proteins are *mobile* in lipid bilayer
  - Lateral diffusion coefficcient is experimentally measurable

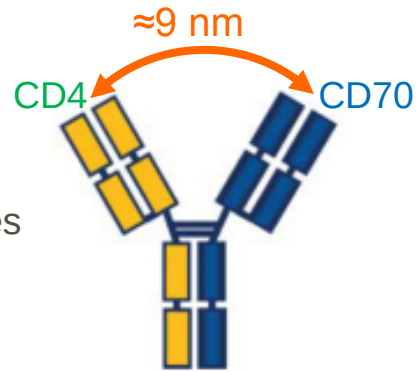


For example: at 50000 receptors **A** and **B** per cell, the *average* distance between them is ≈60 nm

Experimental data: Anti-CD4/CD70 bispecific DuetMab

- T Lymphocytes, targets per cell
  - $CD4^{+}CD70^{+}$ (46000:52000)
  - $CD4^{+}CD70^{-}$ (38000:<100)
  - $CD4^{-}CD70^{+}$ (<100 :≈31000)
- $CD4^{+}CD70^{+}$ cells
  - All bound DuetMab is ib cross-linking complex with CD4 and CD70
  - No DuetMab is attached monovalently, i.e. to CD4 or CD70 only
  - Strong binding even at DuetMab concentrations *below* respective Kd values
- $CD4^{+}CD70^{-}$ and $CD4^{-}CD70^{+}$ cells
  - DuetMab binding is dictated by concentration and Kd

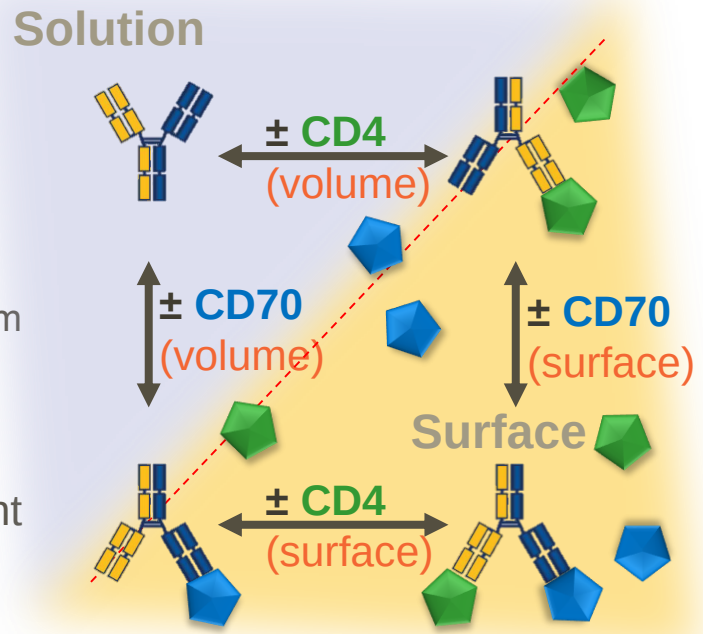




Mazor, Y. et al. (2015) mAbs 7(3): 461-469; Mazor, Y., et al. (2015). mAbs 7(2): 377-389.

Kinetic model of DuetMab binding to $CD4^{+}CD70^{+}$ cells

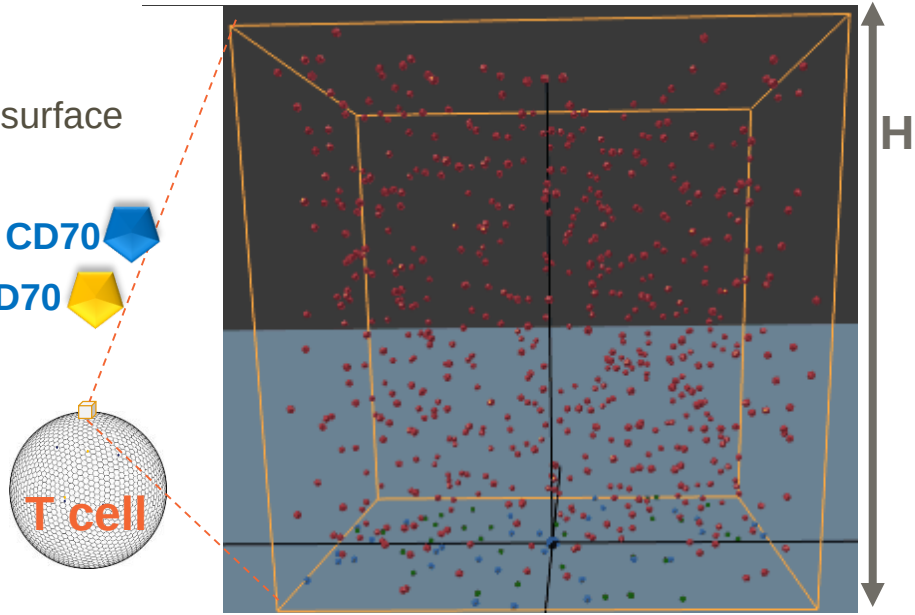
- Sequential binding of DuetMab to CD4 and CD70
  - Trimolecular reactions are very rare
- Lateral diffusion of proteins in cell membrane
  - At typical $D=10^{-10}$ cm^2/s, mean displacement in 1s is ≈200 nm
  - A typical monovalent mAb-target complex dissociation $t_{1/2}$≥2h
- Simulate DuetMab binding and compare with experiment
  - Monte Carlo numerical and ODE analytical



Monte Carlo numerical simulation for DuetMab** binding

MCell3* model of a cell with 46000 CD4 and 52000 CD70 molecules

- Virtual cuboid 0.1×0.1×**H** µm
  - 31 **CD4** and/or 39 **CD70** diffusing on bottom surface
  - ≈700 **DuetMab** diffusing in volume
  - **DuetMab** monovalent complex with **CD4** **or** **CD70**
  - **DuetMab** cross-linking complex with **CD4** **and** **CD70**
- Reaction-diffusion in volume and on surface
  - Brownian motion
  - 1-100 µs time steps, experimental parameters



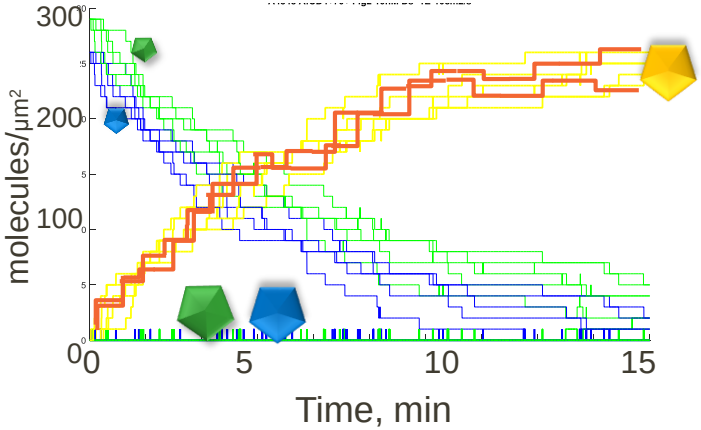
*Kerr, R. A., et al. (2008) SIAM J Sci Comp 30(6): 3126-3149, **Mazor, Y. et al. (2015) mAbs 7(3): 461-469

Monte Carlo simulation predicts DuetMab binding

DuetMab binding to $CD4^+$, $CD70^+$ or $CD4^+CD70^+$ cells

Simulation:

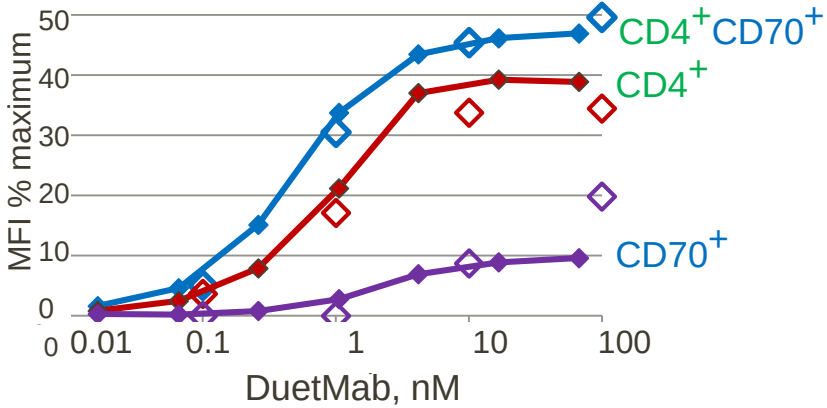
1 nM DuetMab on $CD4^+CD70^+$ cells



Only DuetMab cross-linked complex with $CD4^+$ and $CD70^+$ accumulates

Experiment vs simulation

Total bound DuetMab binding after 1-hour incubation

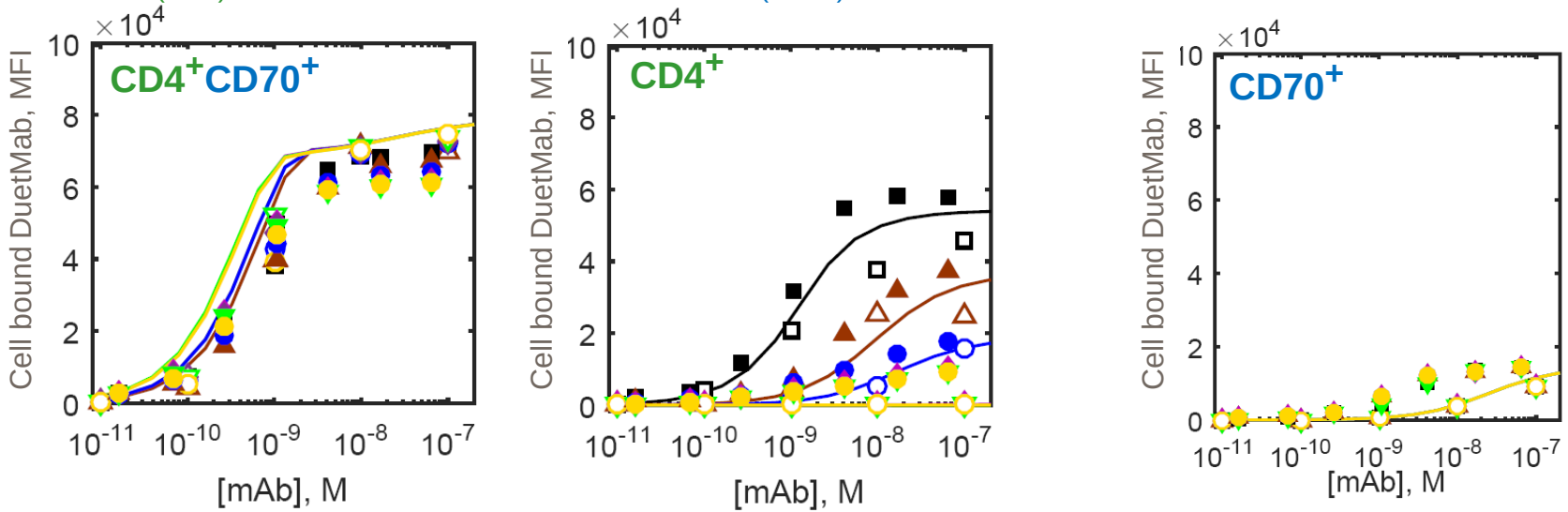


DuetMab binding dose-response curves are predicted for all cell types

Mazor, Y. et al. (2015) mAbs 7(3): 461-469, Sengers, B. G., et al. (2016) mAbs 8(5): 905-915.

ODE simulations confirm Monte Carlo

DuetMab $K_{d(CD4)}$=0.9, 10, 17,42, 63, 69 nM; $K_{d(CD70)}$=25 nM



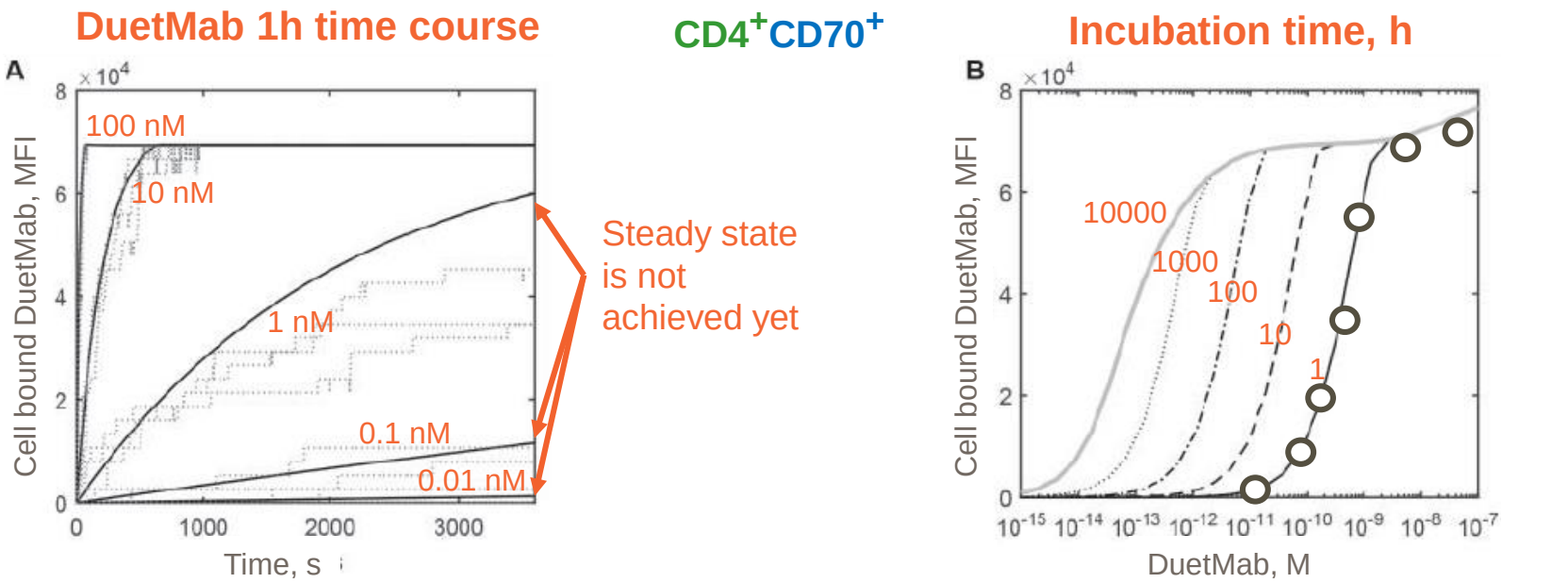
- Surface molecules in surface concentration units
- Surface reaction k_{on} is surface diffusion limited

Good agreement between experimental data and ODE, Monte Carlo predictions is observed

Mazor, Y. et al. (2015) mAbs 7(3): 461-469,

Sengers, B. G., et al. (2016) mAbs 8(5): 905-915.

In silico insight: antibody binding can be kinetically limited



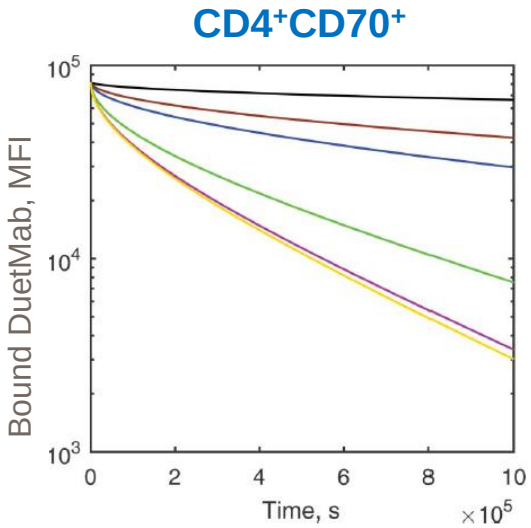
– Bimolecular reactions can be very slow at low concentrations

Sengers, B. G., et al. (2016) mAbs 8(5): 905-915.

In silico insight: cross-linking and dissociation rate

ODE model: DuetMab K_d(CD4)=0.9, 10, 17,42, 63, 69 nM; K_d(CD70)=25 nM

- Simulated dissociation from pre-formed complex
  - Monovalent
    - $t_{½}$(CD4)=19 s to 45 min
    - $t_{½}$(CD70)=2.5 min
  - Bivalent cross-linking is effectively irreversible:
    - Terminal half-life: $t_{½}$=83 h to 16 months
    - Target internalization is likely to be *much* faster
- The net result is up to 10^4-fold more stable binding of DuetMab to $CD4^+CD70^+$ cells through avidity effect

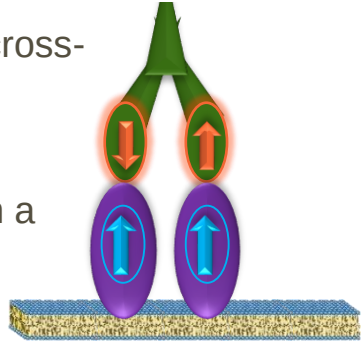


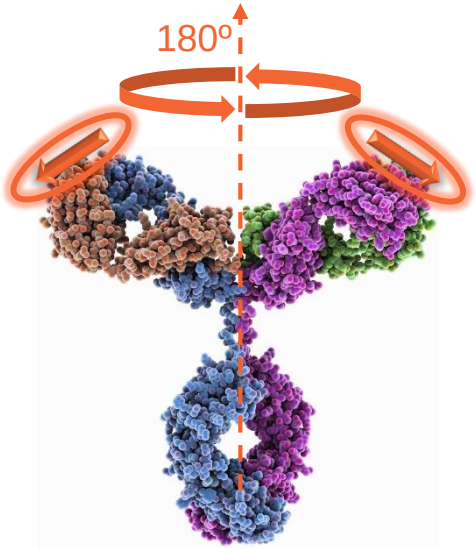
Sengers, B. G., et al. (2016) mAbs 8(5): 905-915

Biparatopic mAbs and dimeric targets

Avidity effect can be lower for a normal mAb

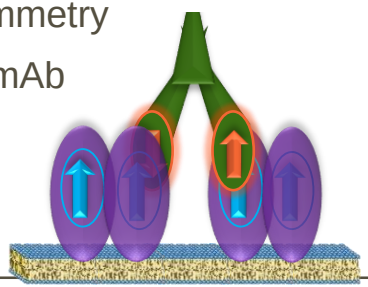
- Fab rotation around the hinge may be required for cross-linking, or…
- Membrane distortion or target conformation change
- A suitable combination of epitopes and paratopes in a bispecific format may alleviate these constraints in biparatopic format





Dimeric receptors

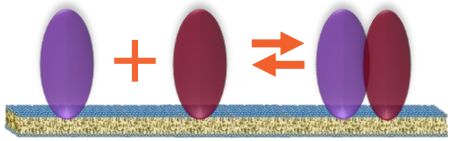
- Homodimeric receptor subject to 180º rotational symmetry
- Epitopes are unlikely to be accessible *to the same* mAb
- Cross-linking of two dimers is more likely
- Linear oligomers on cell surface could form



Bispecifics in cell-cell interaction

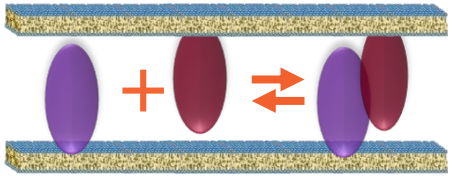
Reactions on cell surface follow 2D kinetics

- DuetMab on CD4/CD70 cells
- EGF
- INFγ…



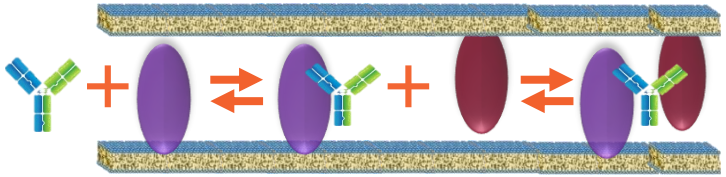
Cellular synapse can be considered a 2D space

- TCR-pMHC complex
- *Perhaps all PPIs across cellular synapse*



A bispecific for cell-cell cross-linking

- A bispecific mAb or fragment bound to a receptor cross-links to another on the other cell



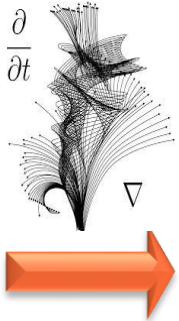
Integration into PBPK for exposure and dose prediction

Physiologically based pharmacokinetics

- Bispecific vs monospecific target engagement in all organs

Cross-species

- Mouse-rat-cyno-human



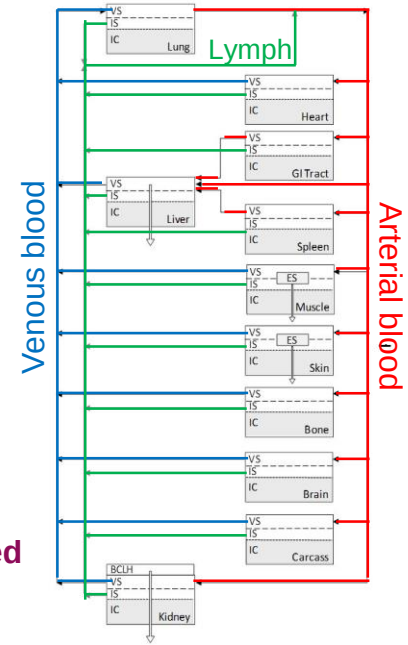
Parameters:

Experimental

- Organ volumes
- Blood flow rates
- Glomerular filtration

Empirically estimated

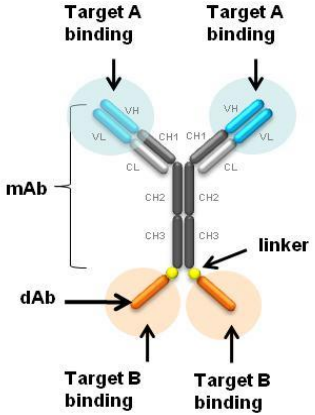
- Lymph flow rates



GSK Bispecific Antibody: mAb-dAb

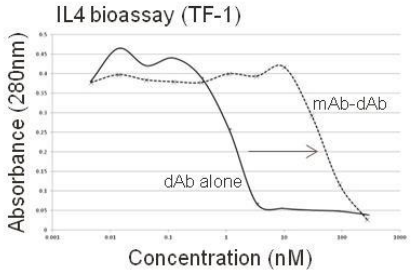
VH or VL domain antibody (dAb) C-terminal fusion to a mAb heavy chain

- dAb is from phage display
- bivalent target binding/neutralisation
- Fc effector function and FcRn binding for longer serum half-life
- mammalian expression/protein A capture



Design know-how

- Linker optimization
- Final format based screening for early identification of higher potency dAb leads
- Enhanced potency for dimeric targets (IL-5, VEGF) and monomeric ones too (IL-4)



Scott, M. J., J. A. Lee, et al. (2017). "'In-Format' screening reveals significant potency improvements relative to unformatted molecules." mAbs 9(1): 85-93.

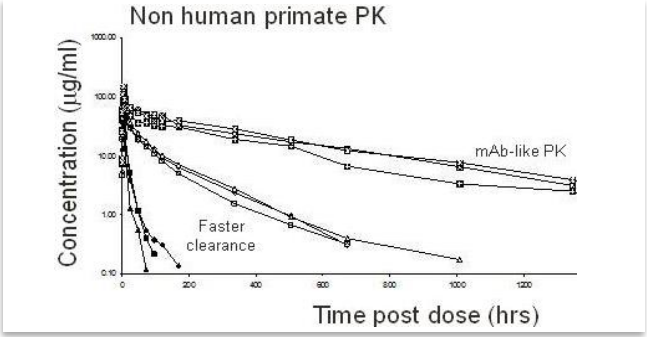
mAbdAb challenges

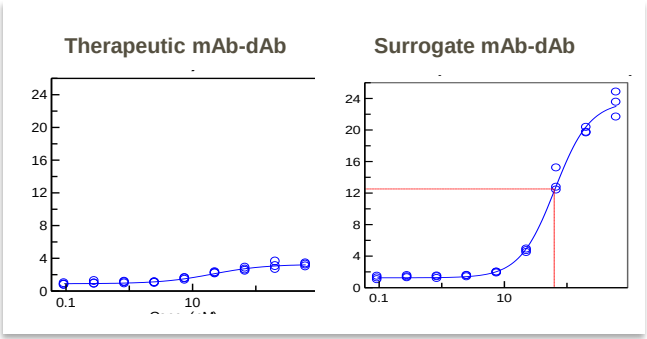
The Caveats

- Good stability, solubility and biophysical properties can be achieved but PK remains unpredictable
  - The best may be mAb-like
  - The worst can be significantly compromised
    - No proteolytic degradation was observed

- mAb moiety can affect the dAb activity
  - Same dAb fused to two different mAb molecules may have different potencies

"All animal studies were ethically reviewed and carried out in accordance with Animals (Scientific Procedures) Act 1986 and the GSK Policy on the Care, Welfare and Treatment of Animals."

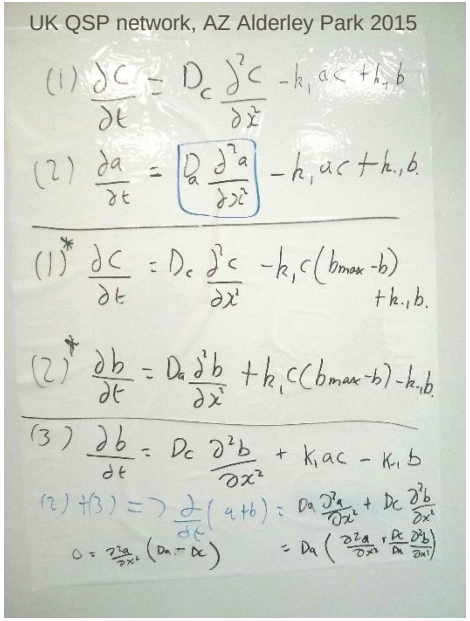




Conclusion: Bispecific mAbs can be precision medicines

Bispecific mAbs can be designed to bind predominantly cells simultaneously expressing two different antigens only

- Lateral diffusion allows rapid ternary complex formation on cell surface
- The cross-linked complexes are very stable
- First principles modelling and simulation was possible and can guide
  - No empirical fitting involved, all parameters are measurable
  - Volume reaction constants by SPR
  - Surface association reaction constant calculated from diffusion coefficient



The challenges we have met

- Experimental: protein engineering, linkers, stability, PK
- Modeling: Mechanistic framework for cell-cell cross-linking scenario

Wish-list: FDA-approved set of physiological parameters for biologics PBPK….

Sengers, B. G., et al. (2016) mAbs 8(5): 905-915.

Acknowledgements

GSK

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Maryam Argungu (Imperial)

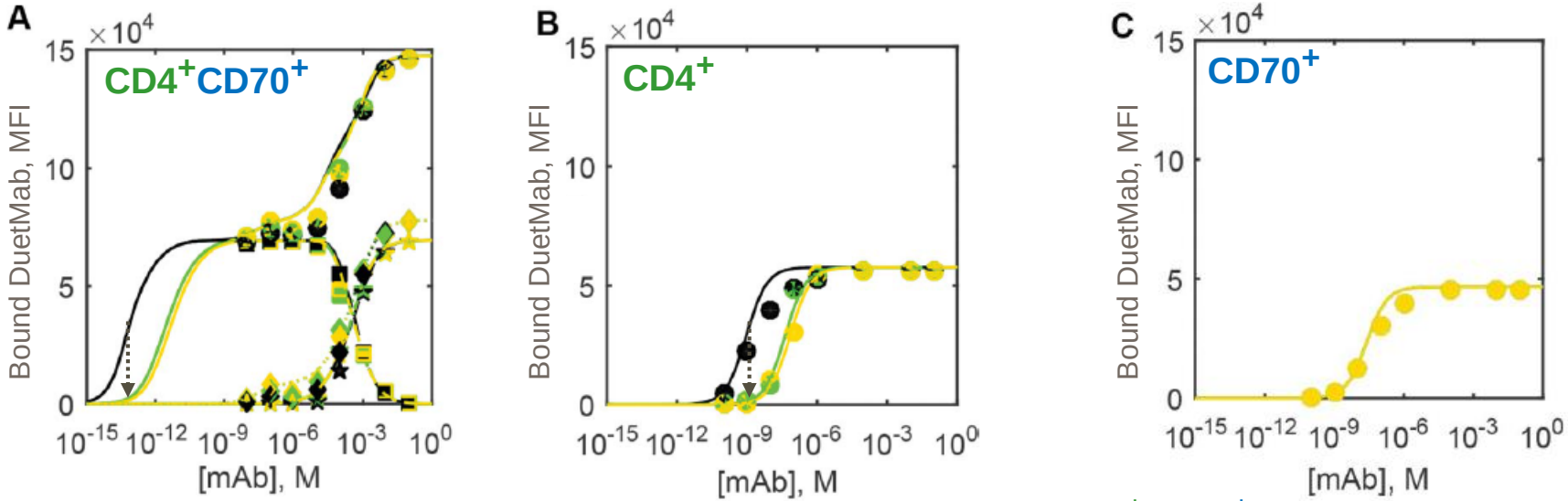
Emma Hawkins (U Surrey)

Aymen Hadji (U Badji-Mokhtar)


Thank you

In silico insight: avidity effect and competition

DuetMab: $K_{d(CD4)}$=0.9, 42 and 69 nM, $K_{d(CD70)}$=25 nM



- Target cross-linking boosts effective affinity ≈10000-fold for DuetMab on $CD4^+CD70^+$ cells
- Unrealistic concentrations would be required for DuetMab *monovalent* binding to $CD4^+CD70^+$ cells