Fabs facilitate single particle cryoEM of small proteins

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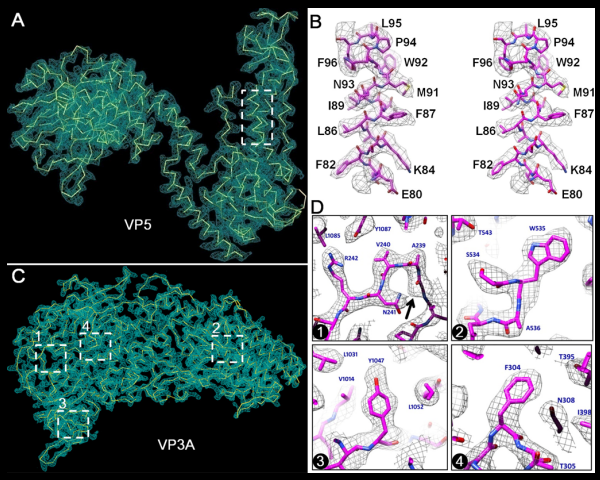
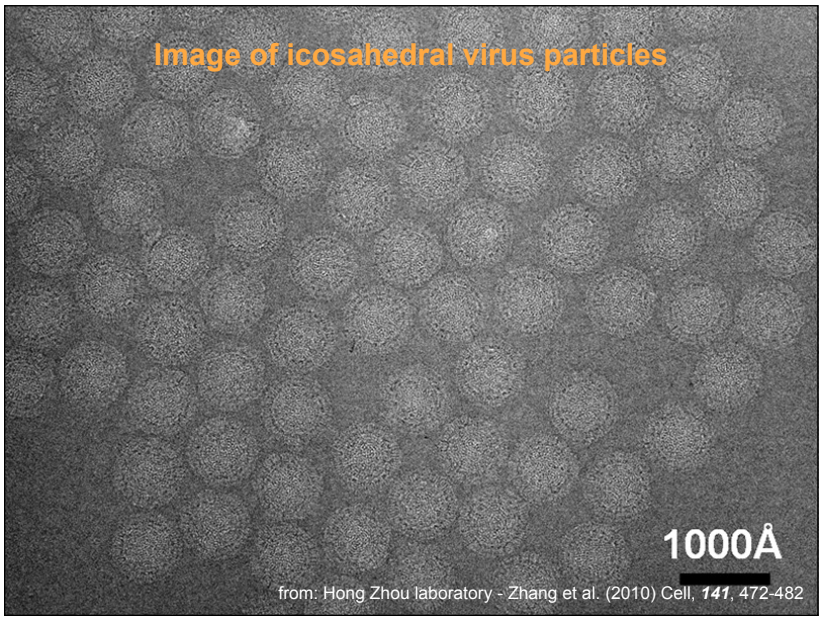
The state-of-the-art of single particle cryoEM: 3.3Å reconstruction of icosahedral virus



from: Hong Zhou laboratory - Zhang et al. (2010) Cell, ***141***, 472-482

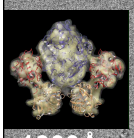
Image of icosahedral virus particles



from: Hong Zhou laboratory - Zhang et al. (2010) Cell, ***141***, 472-482

How to apply single particle cryoEM to study small proteins?

* Images of individual small molecule do not contain sufficient structural information for accurate image alignment;
* 3D reconstruction calculated from images of small molecules are strongly influenced by the initial model and is difficult to be validated.



1000Å

Tfr-Tf: ~300kDa

1000Å

Vglut: ~50kDa

Overall Strategy

Solution: "Below 100,000 molecular weight, some kind of crystal or other geometrically ordered aggregate is necessary to provide a sufficiently high combined molecular weight to allow for the alignment."

- Richard Henderson, Quarterly Reviews of Biophysics, **28** (1995)171-193

Previously tested methods: formation of monolayer crystal, fuse the target protein into an icosahedral virus, etc.

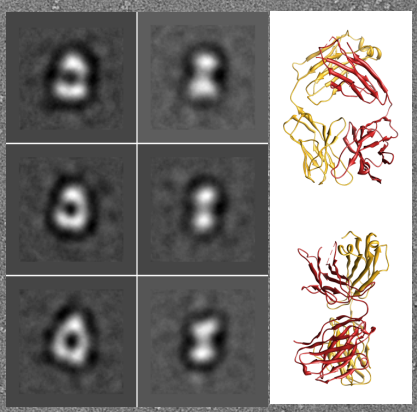
Our strategy: use one or more monoclonal Fab to form a stable and rigid complex with a target protein.

Benefit for single particle cryoEM of small proteins:

- Enlarge the target proteins for better visualization;
- providing fiducial markers for image alignment;
- providing internal control for 3D reconstruction validation;

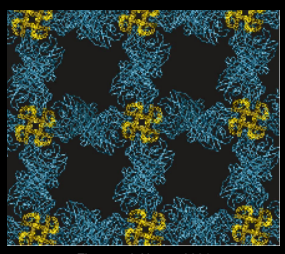
Negative stain EM image of Fab

- Fabs have a well-defined characteristic shape that is easy to be recognized in negative stain EM.

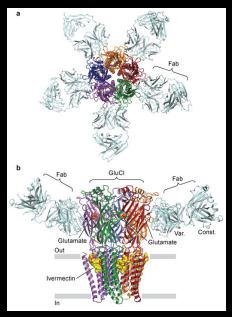


Wu et al., Structure, 2012

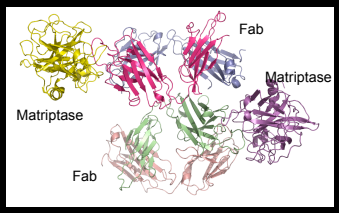
Fab assisted structural analysis



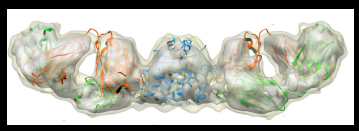
Zhou et al, Nature 2001



Hibbs et al, Nature 2011



Schneider et al, JMB 2012



Wu et al, Structure 2012

What are required to make this approach work for a target protein?

A Fab and a target protein must form a stable and rigid complex:

- good binders: monoclonal Fabs with high binding affinity;
- conformational epitopes instead of linear epitope;
- characterizable in terms of the functionalities of target proteins;

*** Generate Fabs:**

- by phage displayed Fab library technology (our preference);
- by hybridoma technology;

*** Biochemical characterization of high valued Fabs:**

- biochemical assays for rapid biochemical characterization;
- epitope identifications;

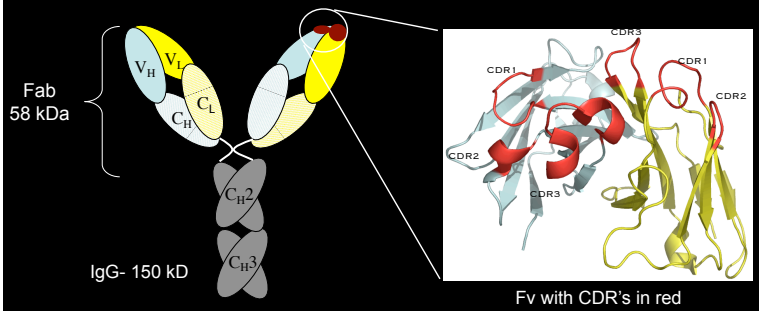
*** Structural characterization of high valued Fabs:**

- by negative stain EM to assess overall homogeneity of the complex;
- by 2D class averages to assess the rigidity of the complex;

*** Single particle cryoEM:**

- will it help to identify the right particles?
- will it help to confirm the correctness of a 3D reconstruction?
- will it work to help to facilitate refinement to high resolution?

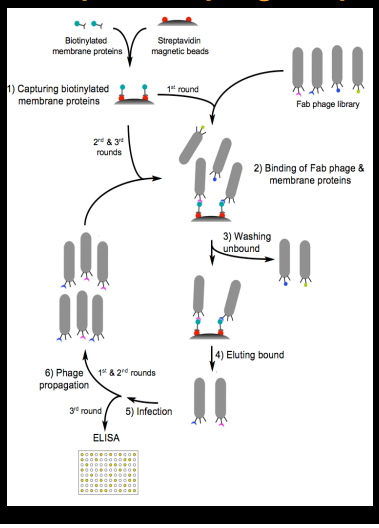
Anatomy of an Antibody



Fv with CDR's in red

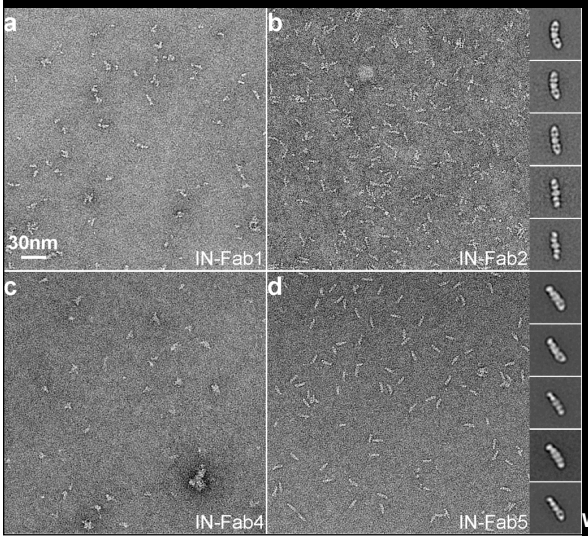
* Fab phage library: Human origin, unbiased B-cells, with approximate diversity of 4 x 10^{10}, which was prepared in Charly Craik Lab at UCSF in 2008; and successfully amplified in 2011: Duriseti et al, JBC, 285, 26878-88 (2010);
*

Optimized phage display panning procedure



Kim et al. "Rapid identification of recombinant Fabs that binds to membrane proteins" Methods (2011) **55**, 303-309.

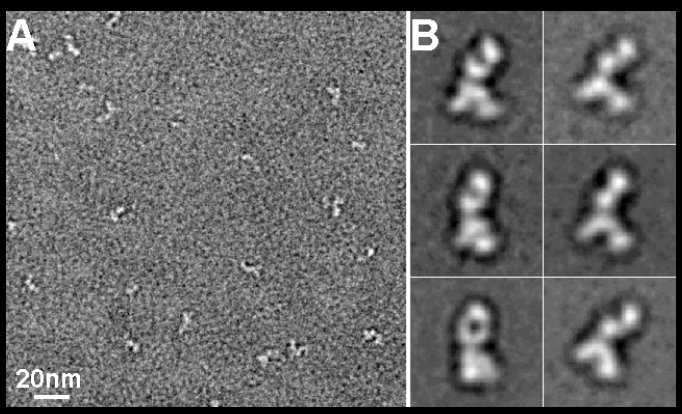
Fabs in complexes with dimeric HIV integrase



IN: 4 Fabs were identified biochemically as good binders.

Wu et al., Structure, 2012

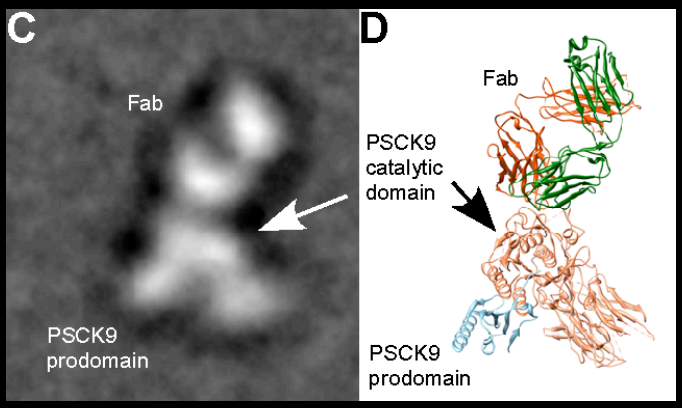
PCSK9-J16 complex



Fab generated from hydridoma technology (Pfizer Inc.)

Wu et al., Structure, 2012

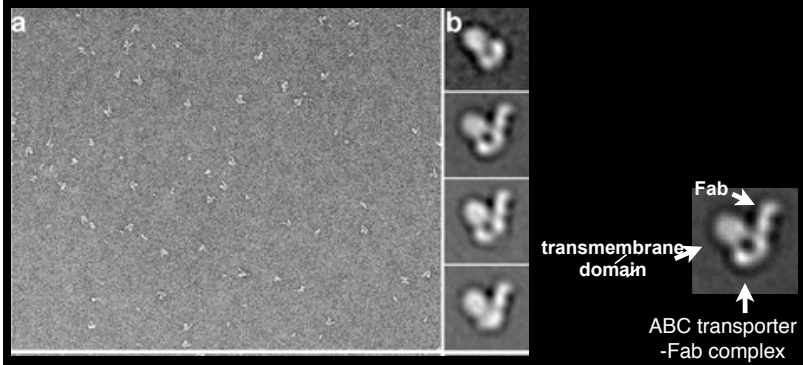
PCSK9-J16 complex



J16 binds to PCSK9 in the catalytic domain and such binding blocks interaction between PCSK9 and LDL-R.

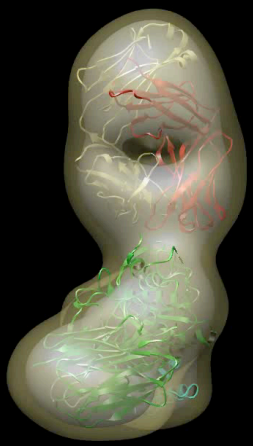
Wu et al., Structure, 2012

2D class averages of ABC-AH5 complex



Wu et al., Structure, 2012

Negative stain 3D reconstruction of PCSK9-J16

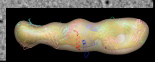


PCSK9: ~70kDa
Fab: ~50kDa

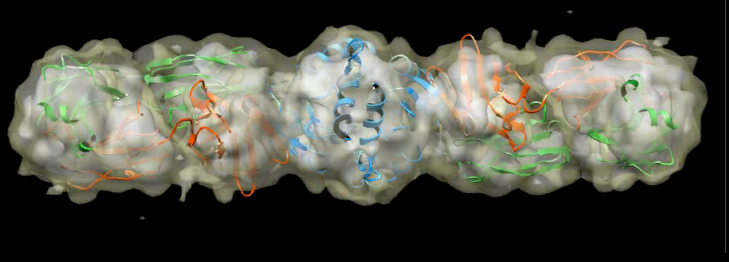
Agustin Avila-Sakar

cryoEM of HIV-1 IN-Fab complex

Sample: IN-Fab complex: ~165kDa; Quantifoil grid, Vitrobot;
Microscope: TF20 operated at 200kV; TVIPS 8K CMOS camera;
Image condition: 70um objective aperture; Defocus 2 ~ 5um;~25e/A2 dose;
Image processing: Negative stain reconstruction as initial model; CTFFIND; Frealign on GPU (GeFrealign);



3D reconstruction of IN-Fab5 complex



Shenping Wu

Conclusions

* We propose a method of using Fab to enable single particle cryoEM of small proteins;
* Some Fabs are sufficiently rigid to be used as fiducial markers for image alignment;
* Phage displayed Fab library is an efficient method to generate conformational epitope Fab;
* 2D class averages can be used as criteria for Fab selection;
* We demonstrate one example of IN-Fab complex by single particle cryoEM;
* Potential application in epitope mapping of pharmacological Fabs;

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