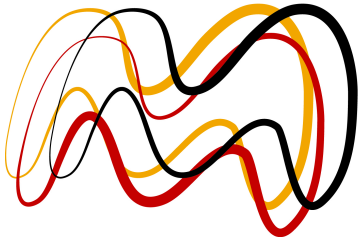

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RESEARCH

Towards Developing a Structural Model of the NISTmAb Reference Material Using Experimental Constraints

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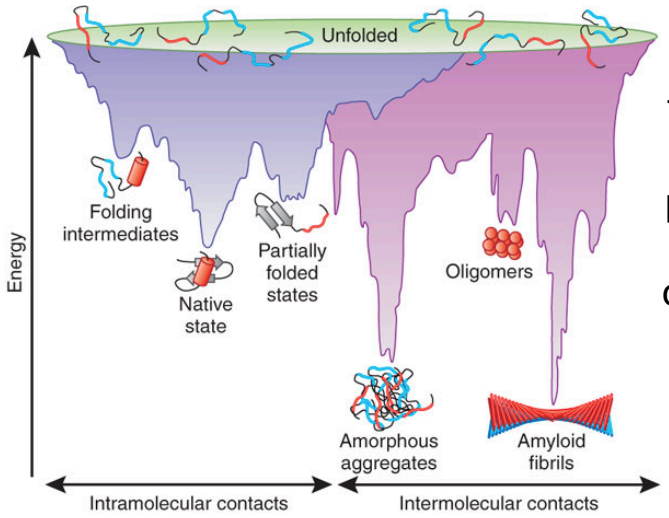
Institute for Bioscience and Biotechnology Research

Higher Order Structure Symposium
Young Scientist Session

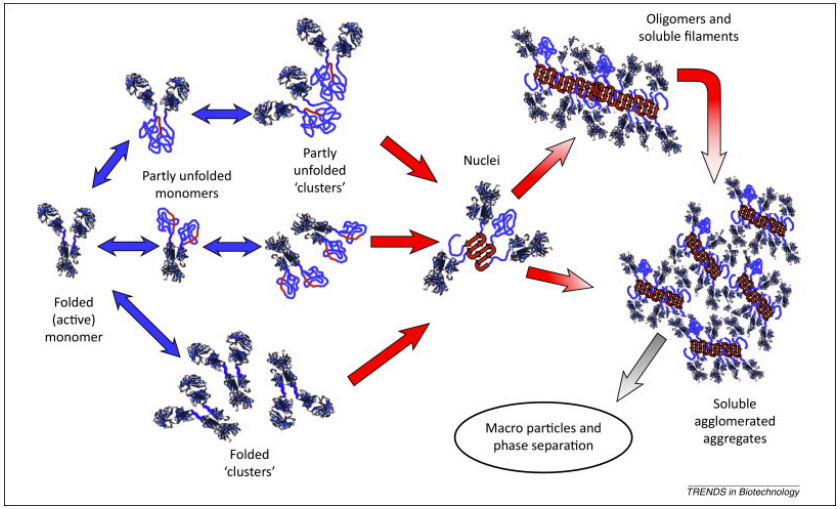
April 4, 2017

Native structure and free-energy landscape

- The three dimensional structure dictates the interactions that stabilize the folded state structure
  - Free energy of native and non-native states
- Funnel-shaped free energy landscape representing ensembles of structures
- Native state is an ensemble of marginally stable structures
- Overlap in configurations that lead to either stable monomers or aggregates
- Many unfolded states are prone to form very stable aggregates



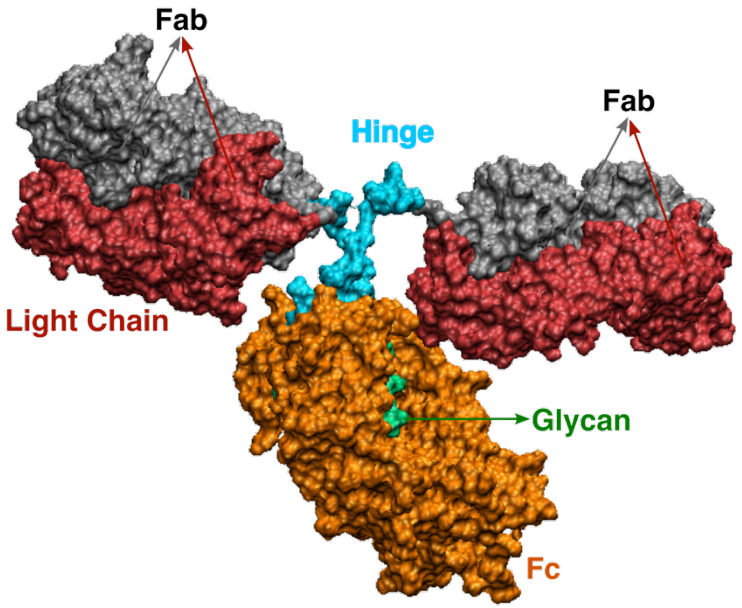
Transitions may occur as proteins explore different configurations in the energy landscape



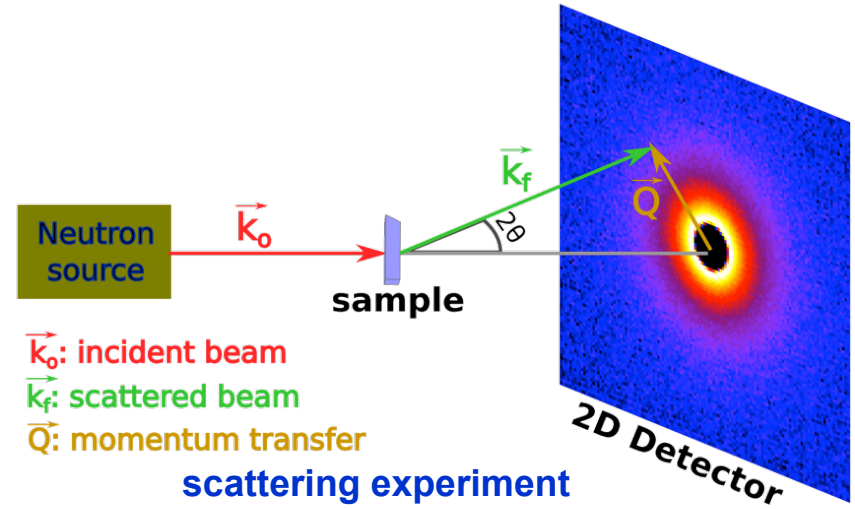
Intermediate states may lead to aggregation

NISTmAb Reference Material

- First of its kind mAb (IgG1) reference material representative of the largest class of biologic drug
- Purposes:
  - Determining that measurement system is working properly
  - Assessing performance of new analytical technologies
- Standard to validate performance of the approximately 20-30 test methods used by industry to assess identity, purity, stability of mAb

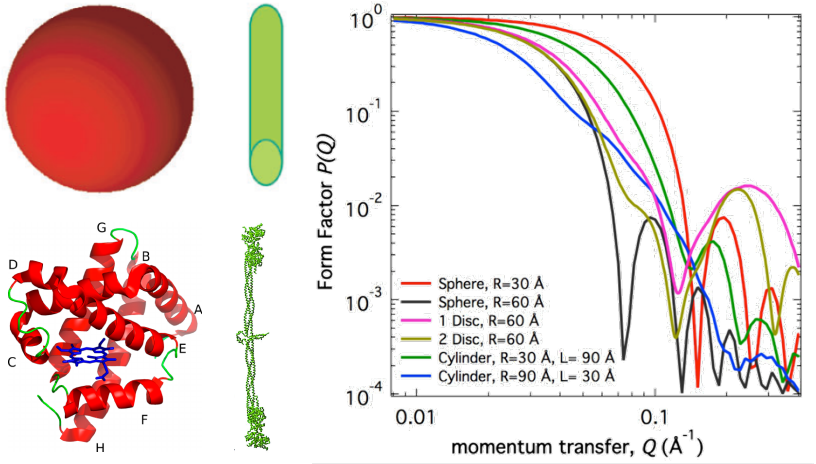


Why using Small-Angle Scattering?



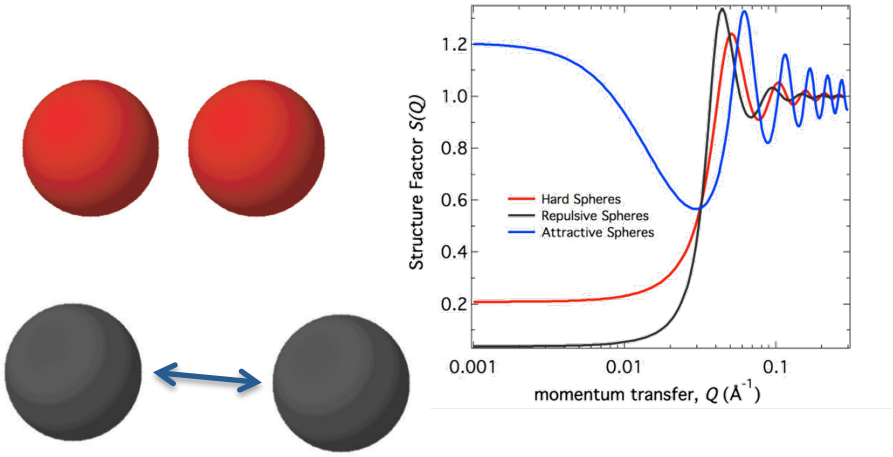
Dilute solution

$$I(Q) \propto P(Q)$$



Concentrated solution

$$I(Q) \propto P(Q)S(Q)$$



SAXS and SANS

Small-angle scattering is sensitive to the **position** of the atoms in the system

Suitable for: protein structure, protein-protein interactions, complexes

Length scales: 1.5 nm – 300 nm

Sample concentrations: 0.1 mg/mL – no upper limit

SANS

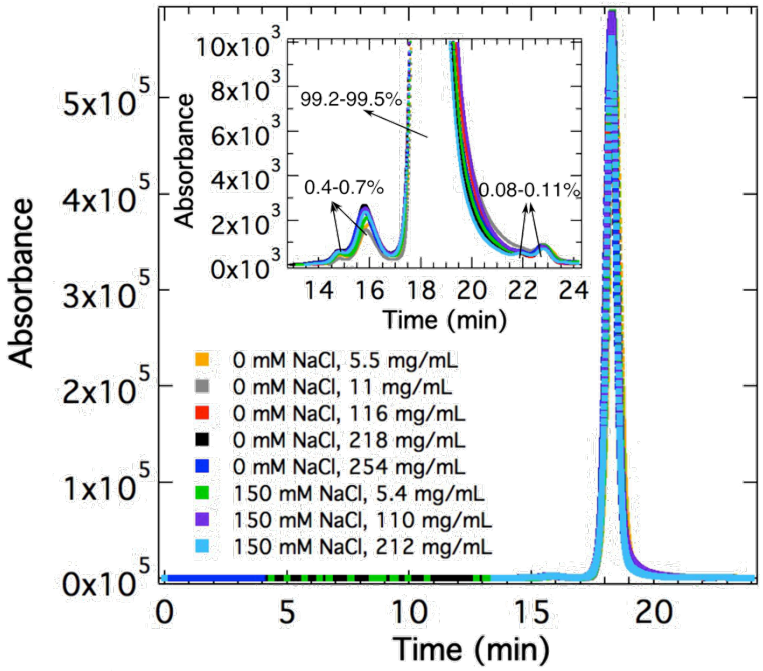
- Neutrons interact with nuclei of atoms. Sensitive to H_2O/D_2O: Contrast matching to study multicomponent systems
- No radiation damage
- Volume ~ 500 µL, sample can be recovered
- Experiments must be done in a neutron scattering facility
- Temperature: few K to 373 K (at NCNR)

SAXS

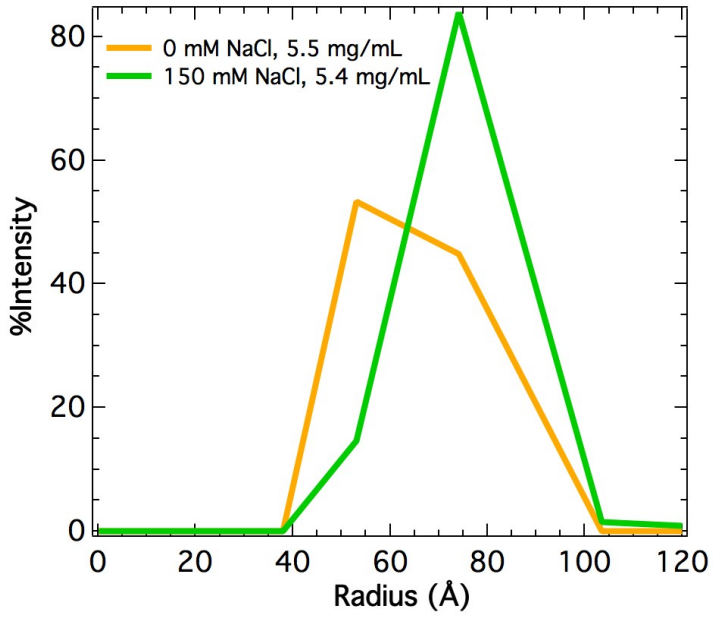
- X-rays interact with electrons of atoms. Not sensitive to isotopic forms of an element
- Radiation damage might occur
- Volume ~ 10-100 µL
- Experiments can be done using a commercial source, or in a synchrotron facility
- Higher throughput than SANS

Sample characterization

SAXS and SANS experiments were performed at various concentrations of NISTmAb in 25 mM Histidine buffer with 0 and 150 mM of added NaCl, pH 6.0



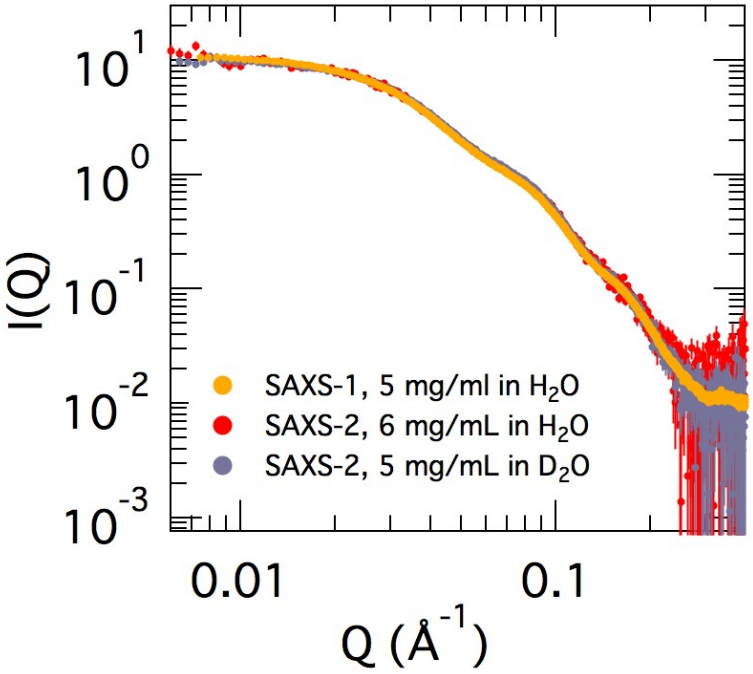
Size-exclusion chromatography (SEC) shows that more than 99.2% of the sample is monomeric



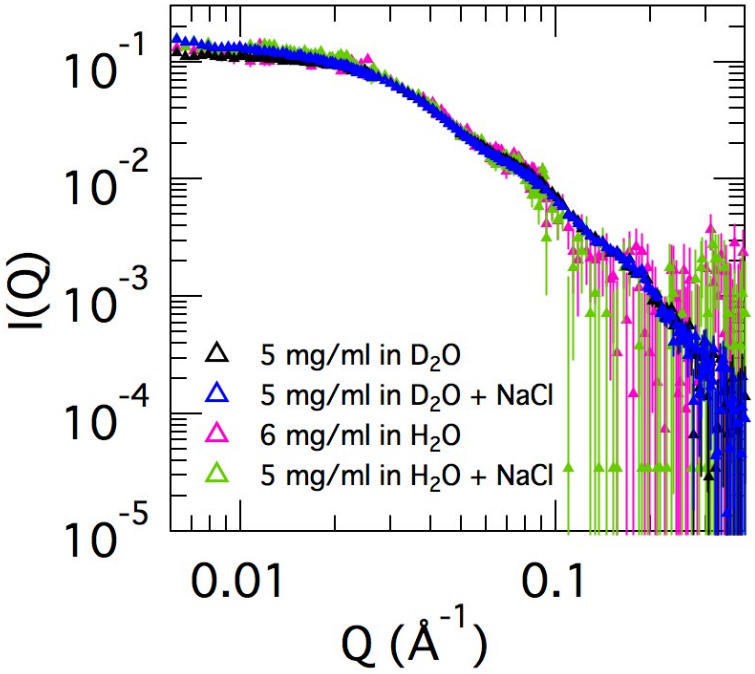
Dynamic light scattering (DLS) shows a slight increase in hydrodynamic size after adding 150 mM NaCl

Scattering profiles of NISTmAb in dilute solutions

SAXS

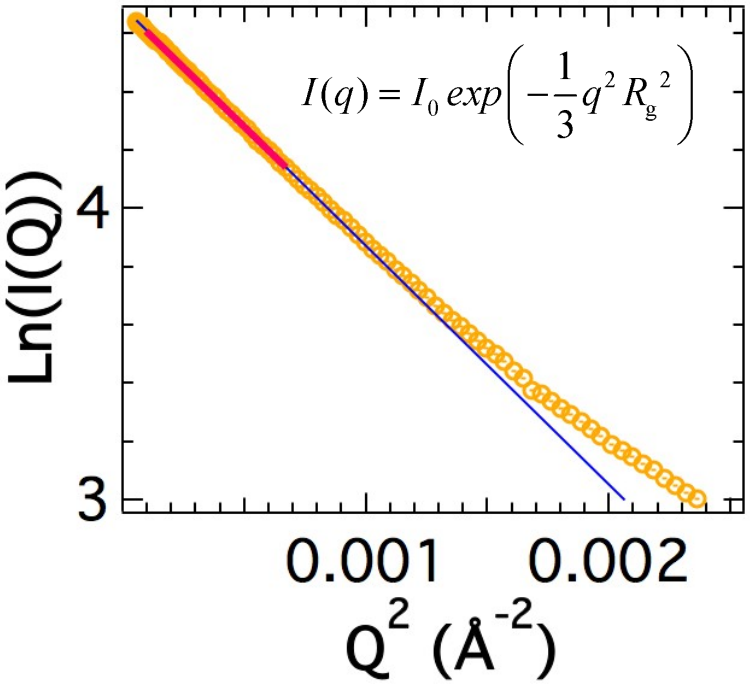


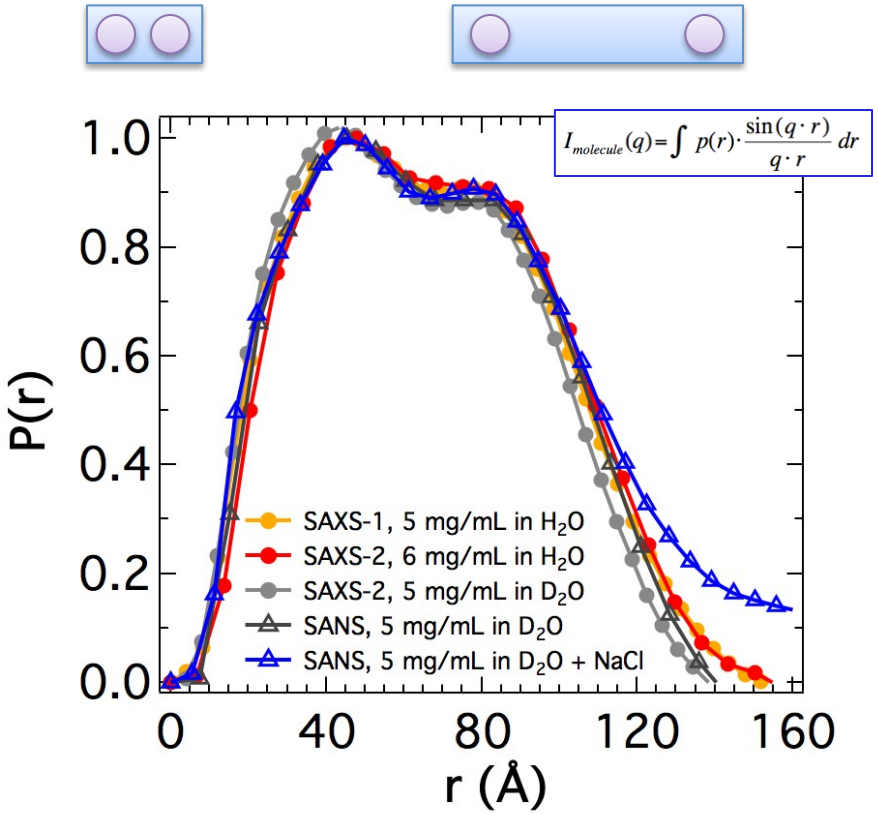
SANS



SAXS data is reproducible in different instruments and facilities. No significant differences detected in the dilute scattering profiles in D_2O, H_2O, after adding 150 mM NaCl

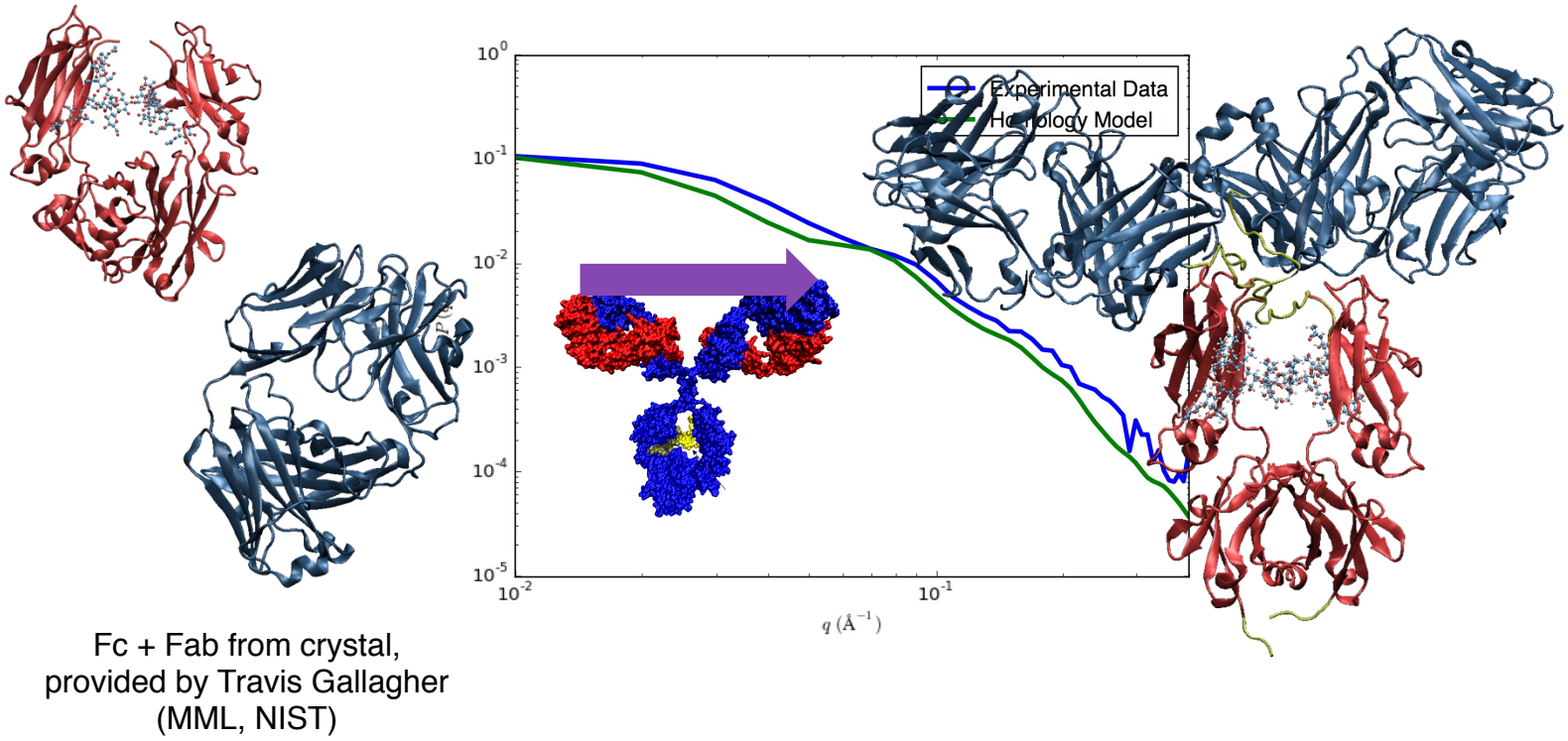
Analysis of scattering profiles of NISTmAb in dilute solution





Using scattering, we can obtain the radius of gyration (R_g = 48.8 +1.2 Å) and the distribution of atoms in the molecule

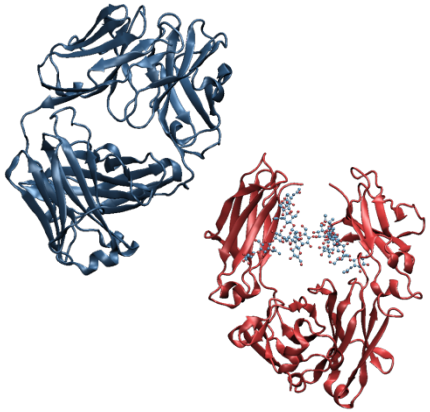
Solution Structure of the NISTmAb



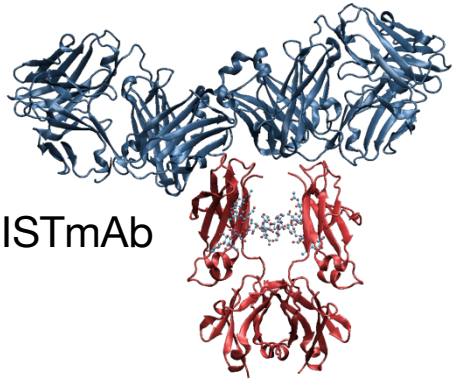
Fc + Fab from crystal, provided by Travis Gallagher (MML, NIST)

Using the crystallographic coordinates of Fc and Fab, a model of the intact NISTmAb in solution is developed using atomistic simulations and experimental scattering constraints

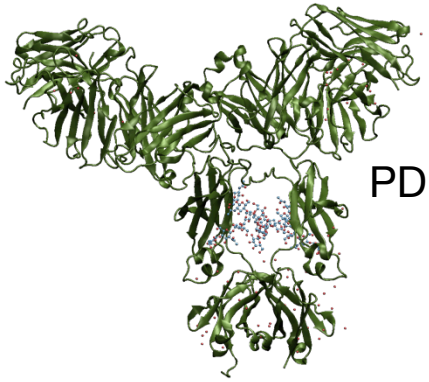
Building a starting structure for the NISTmAb



Fc + Fab from crystal,
provided by Travis Gallagher

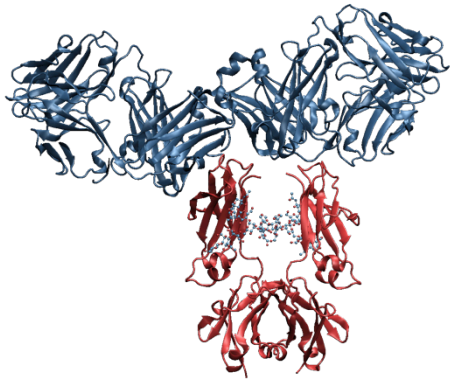


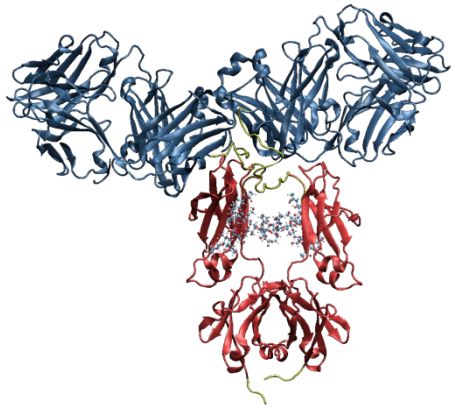
NISTmAb



PDB: 1HZH

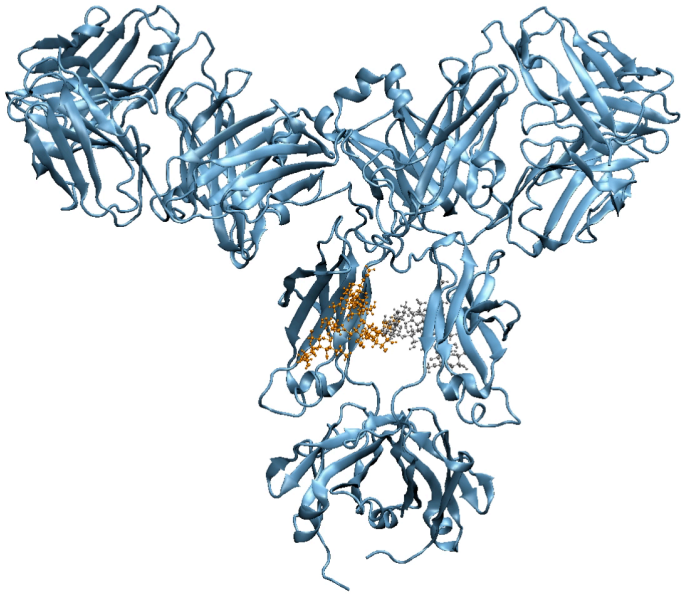
Alignment of Fc + 2Fabs using a structure of an intact antibody

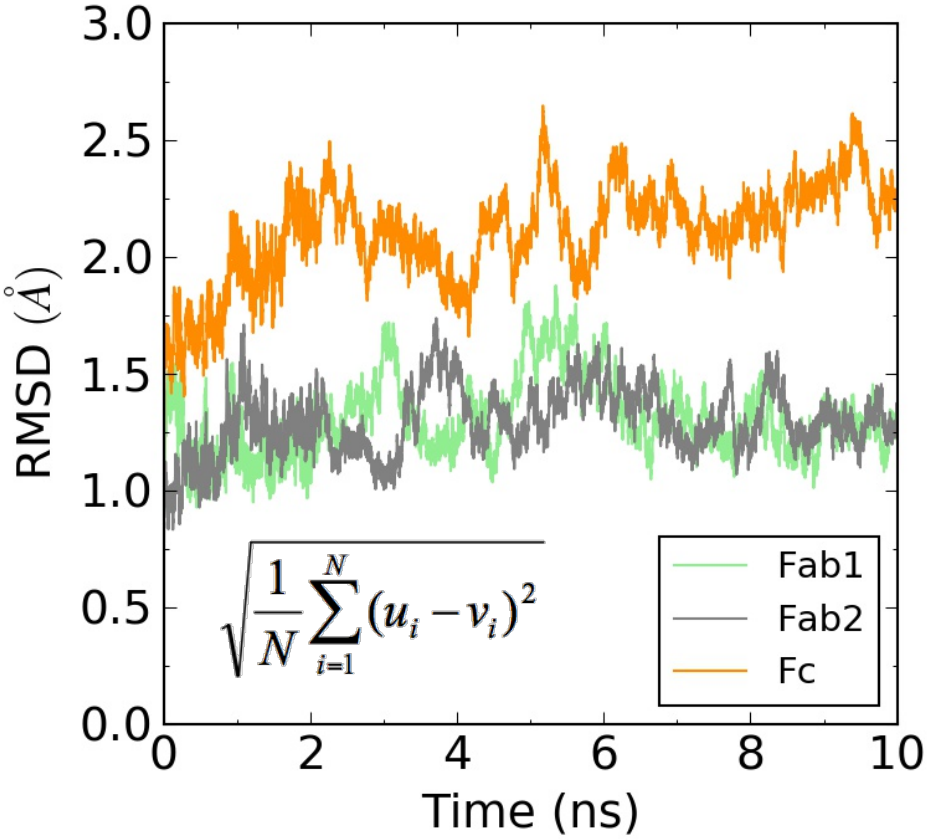




Add missing residues using psfgen:
hinge region + C-terminal in heavy chain + hydrogens

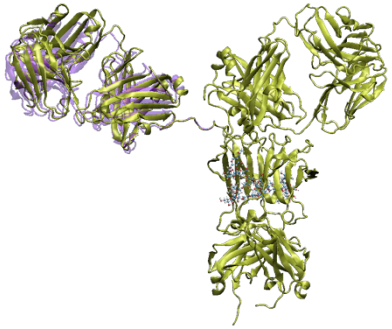
Dilute solutions – MD simulations

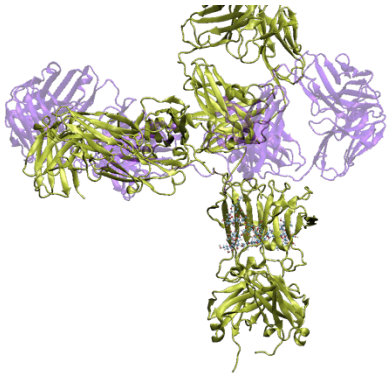


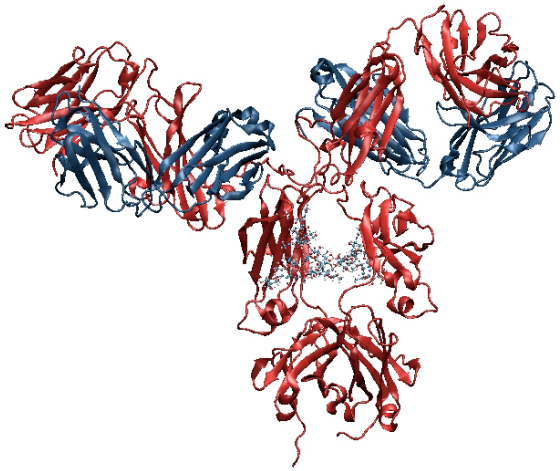


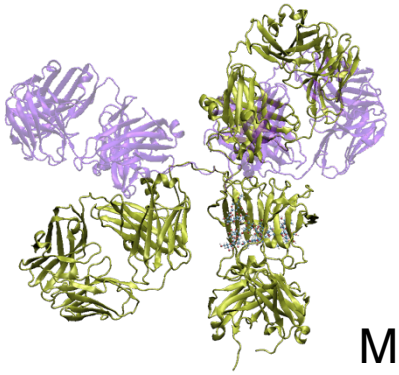
- 10 ns MD simulations in explicit water used to equilibrate the initial structure
- Steady RMSD values of the fragments are obtained throughout the simulation

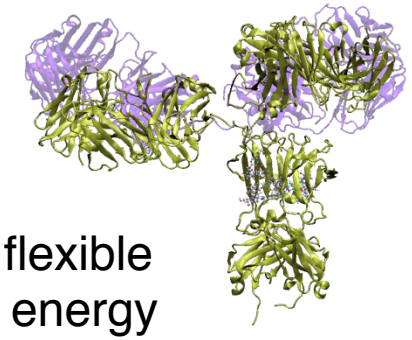
Torsion-angle MC simulations of NIST mAb





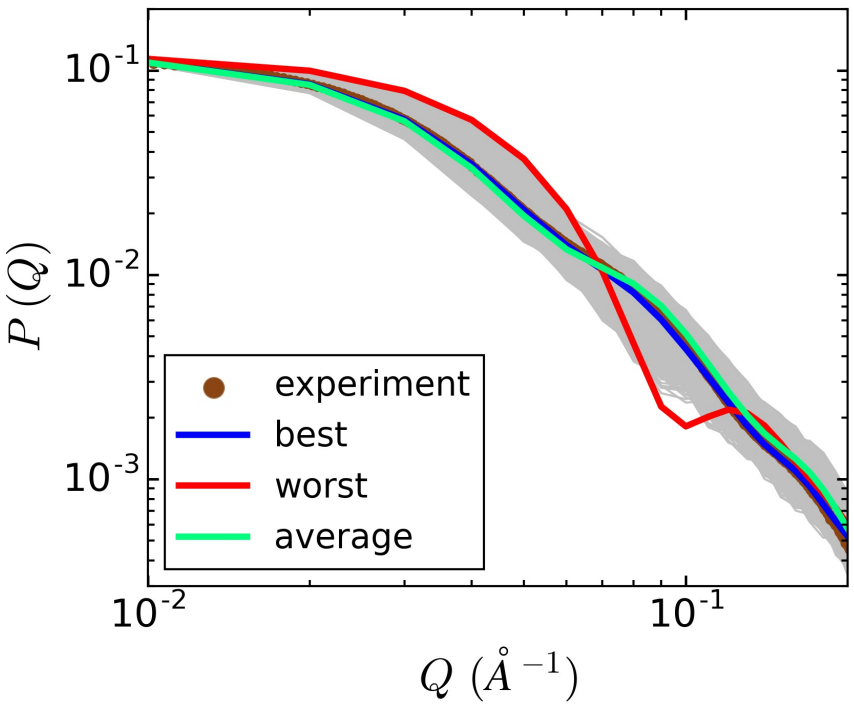


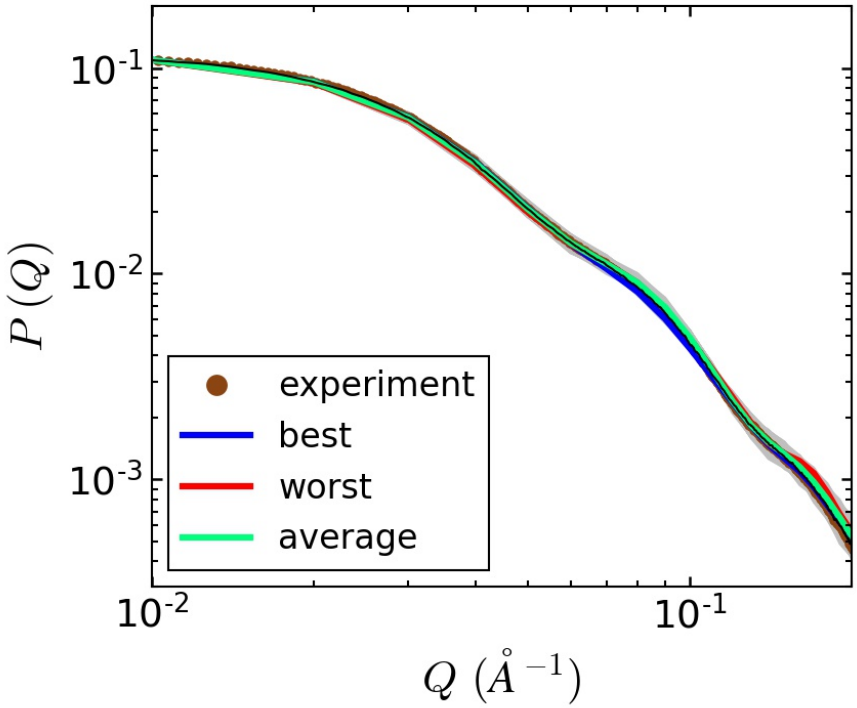




Monte Carlo simulations to generate ensemble of flexible structures in SASSIE. The resulting structures are energy minimized before calculating scattering profiles

Scattering profile of model structures compared to experimental data

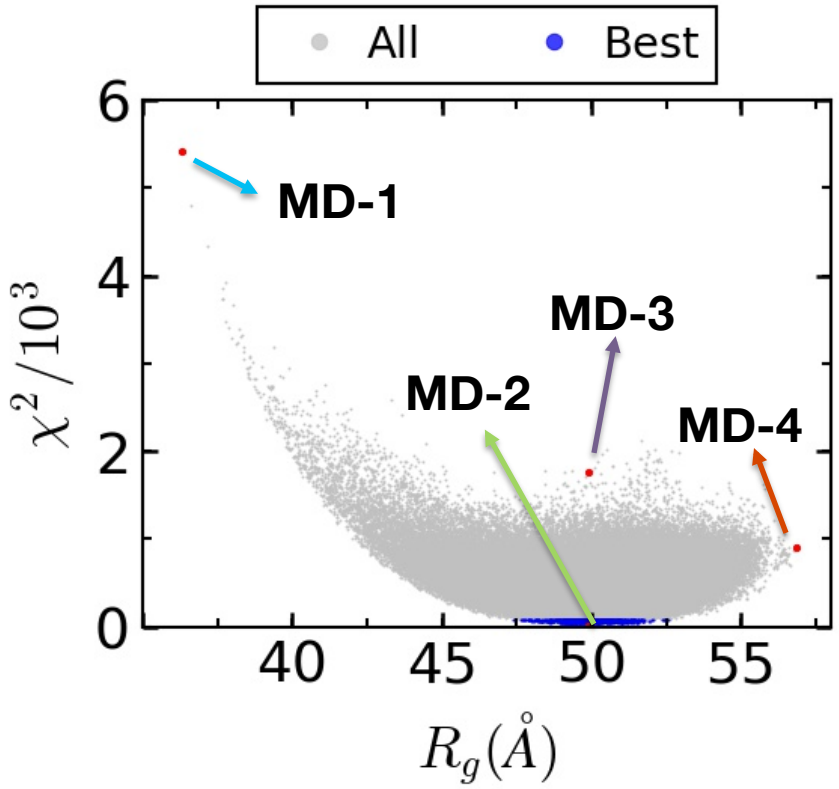




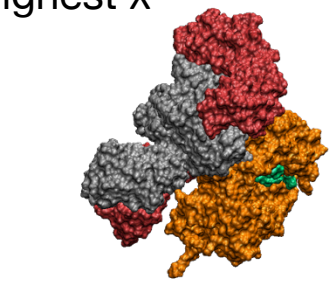
Scattering profiles of ensemble of simulated structures compare well with experimental SAXS data. A subset of the structures that best describe the experimental data are obtained

A closer look at the ensemble of all structures

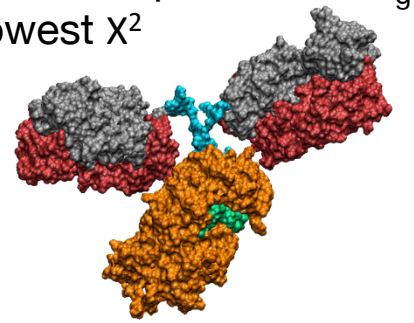
$$\chi^2 = \frac{1}{(N-1)} \sum_{Q_i} \frac{(I_{\exp}(Q_i) - I_{calculated}(Q_i))^2}{\sigma_{\exp}(Q_i)^2}$$

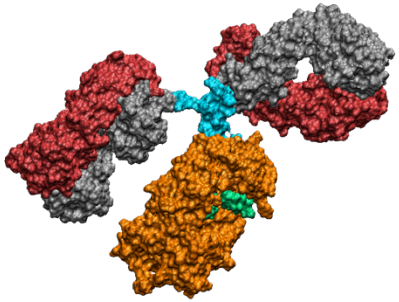


MD-1: Lowest R_g, highest χ^2

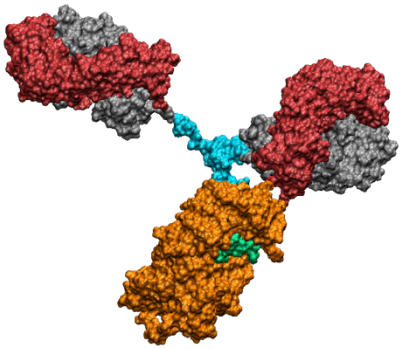


MD-2: Experimental R_g, lowest χ^2





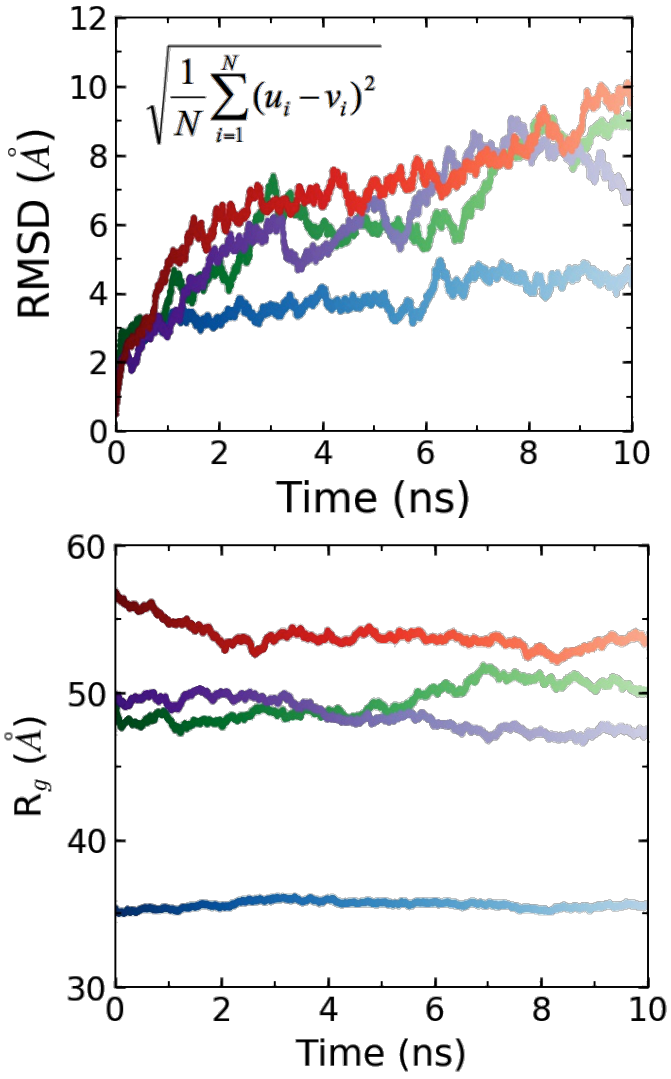
MD-3: Experimental R_g, high χ^2

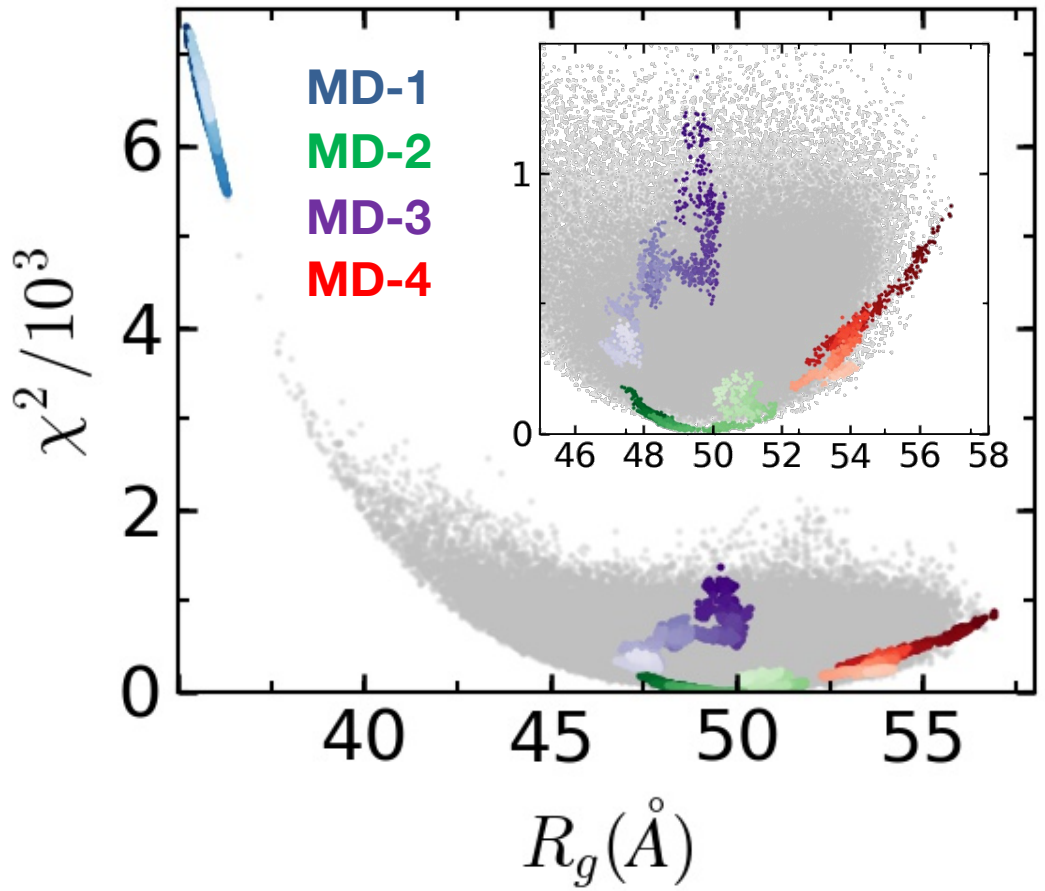


MD-4: Highest R_g

Four structures of the entire ensemble with different R_g and X^2 were chosen for further analysis

MD Simulations for four different configurations

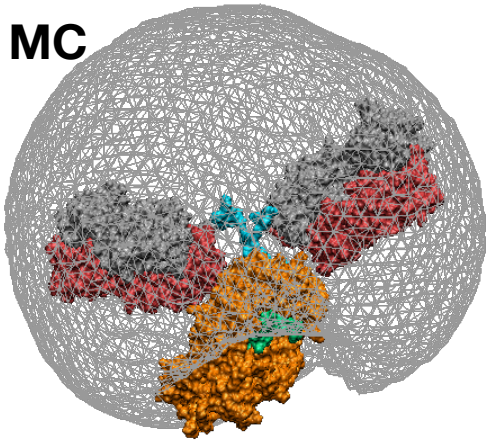




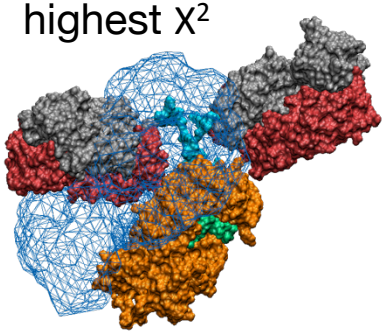
After 10 ns of MD simulations, only small changes in structure are observed

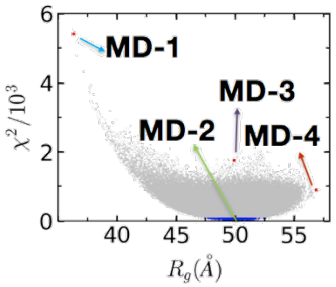
Configurational space sampled by different ensembles

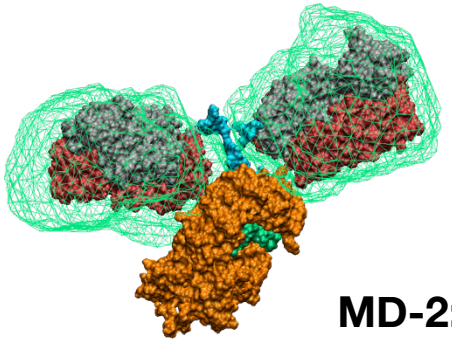
All MC



MD-1: Lowest R_g, highest χ^2

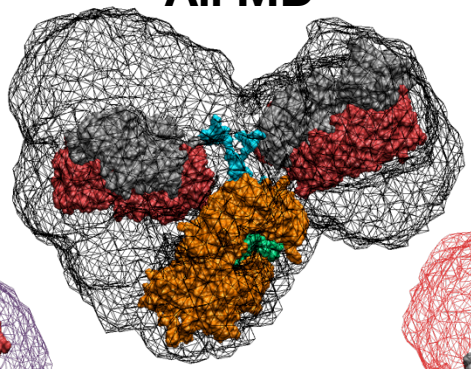




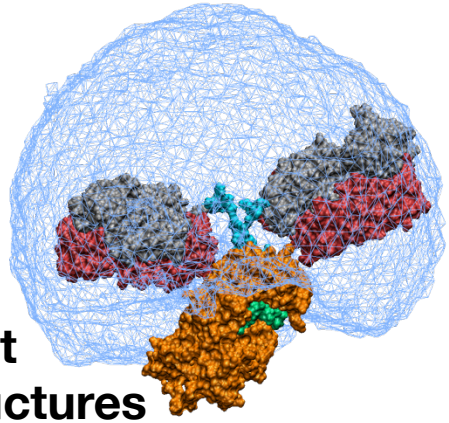


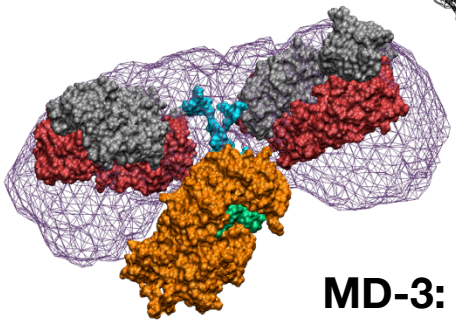
MD-2: Experimental R_g, lowest χ^2

All MD

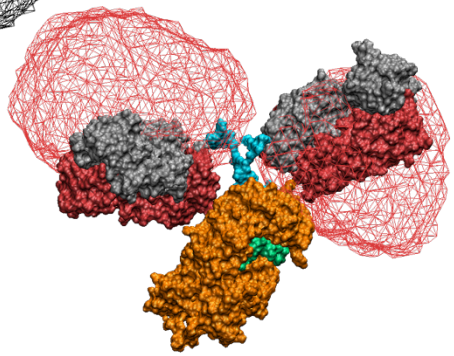


Best structures





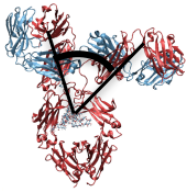
MD-3: Experimental R_g, high χ^2

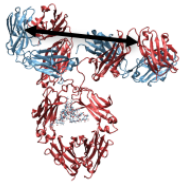


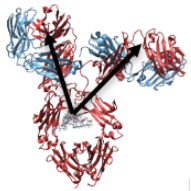
MD-4: Highest R_g

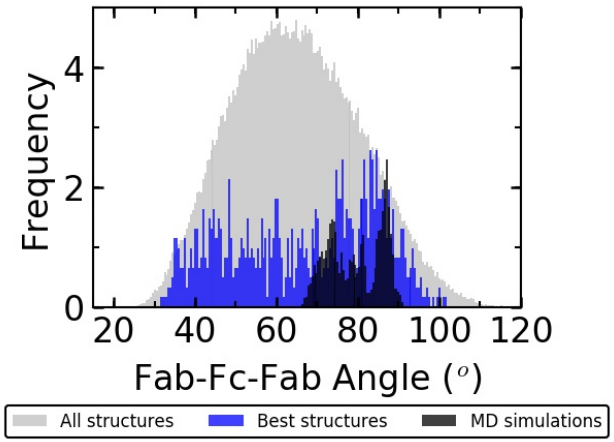
Wide range of configurations are obtained in each ensemble. Ensemble of structures that best describes experimental data is highly flexible

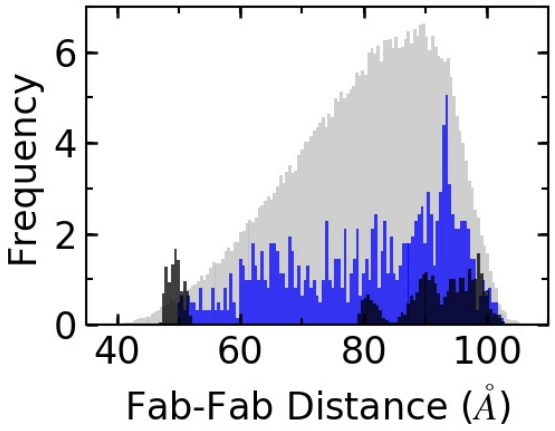
Distribution of domains in different ensembles

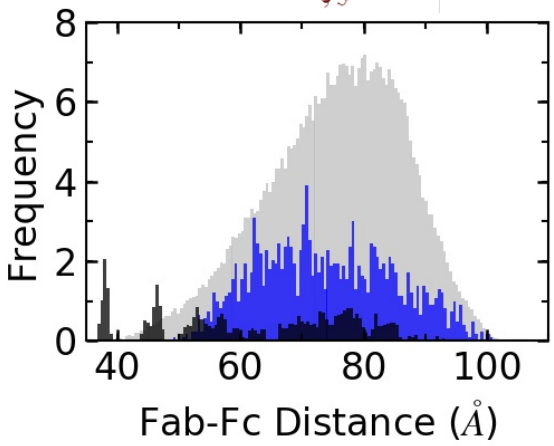


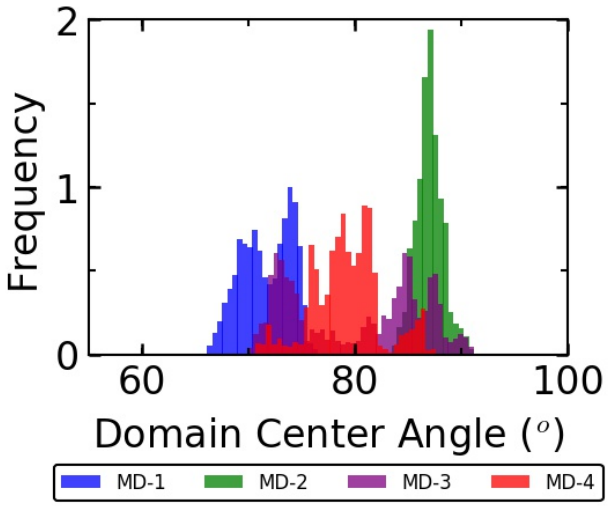


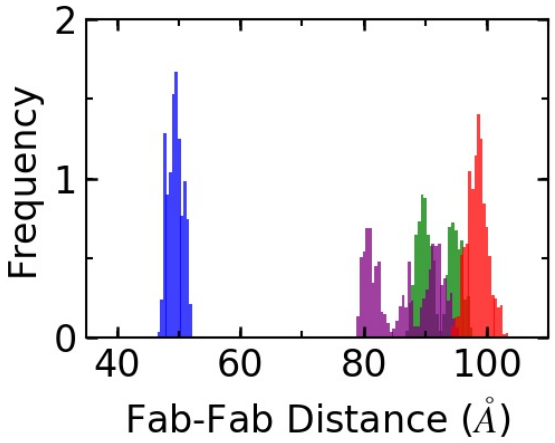


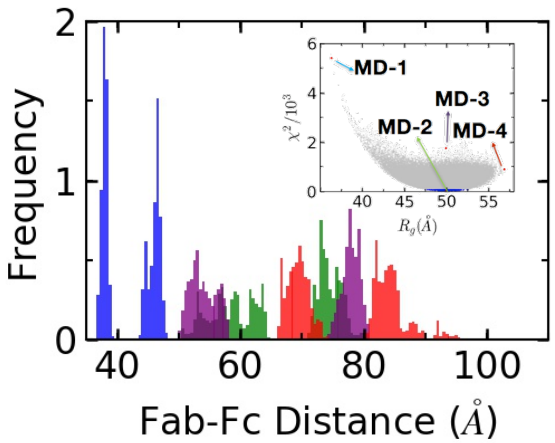






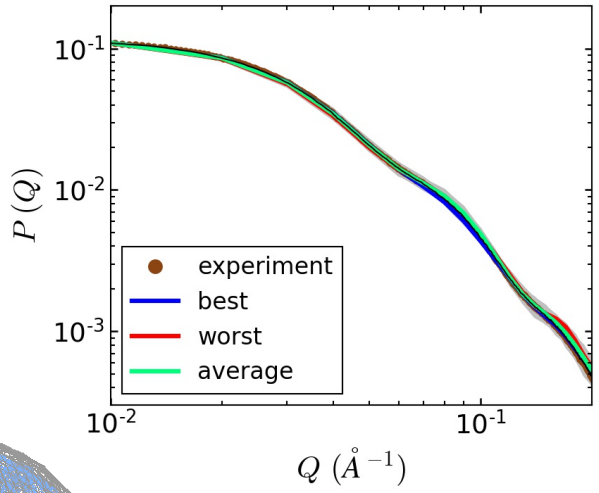


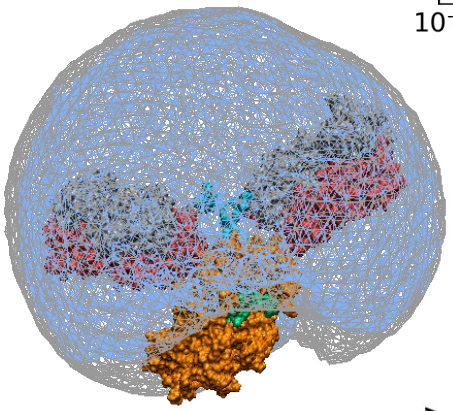


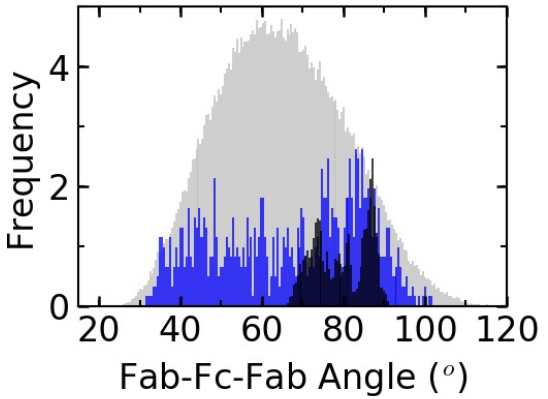


Conclusions

- Scattering was combined with molecular simulations to provide unique information about the structure of mAbs in solution
  - ✓ Tools to analyze SAS data with atomistic simulations are freely available in SASSIE https://sassie-web.chem.utk.edu/sassie2/
- The ensemble of structures that best describe the experimental data sample a wide configurational space; a range of Fab-Fc and Fab-Fab distributions are likely to occur
- Despite better computational resources, the wide range of configurations sampled by the NIST mAb cannot be solely obtained by MD simulations







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