STRUCTURAL CHARACTERIZATION OF PROTEINS USING SMALL-ANGLE X-RAY SOLUTION SCATTERING

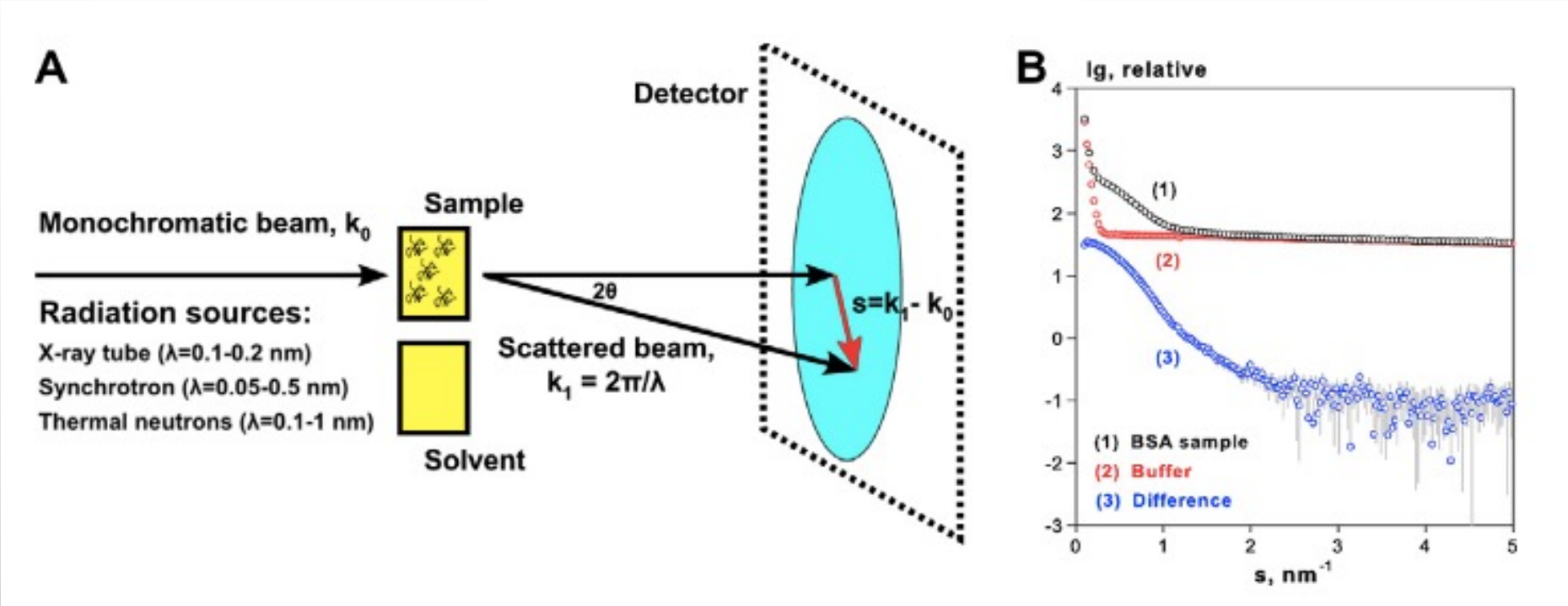
HAYDEN D.T. MERTENS, DMITRI I. SVERGUN

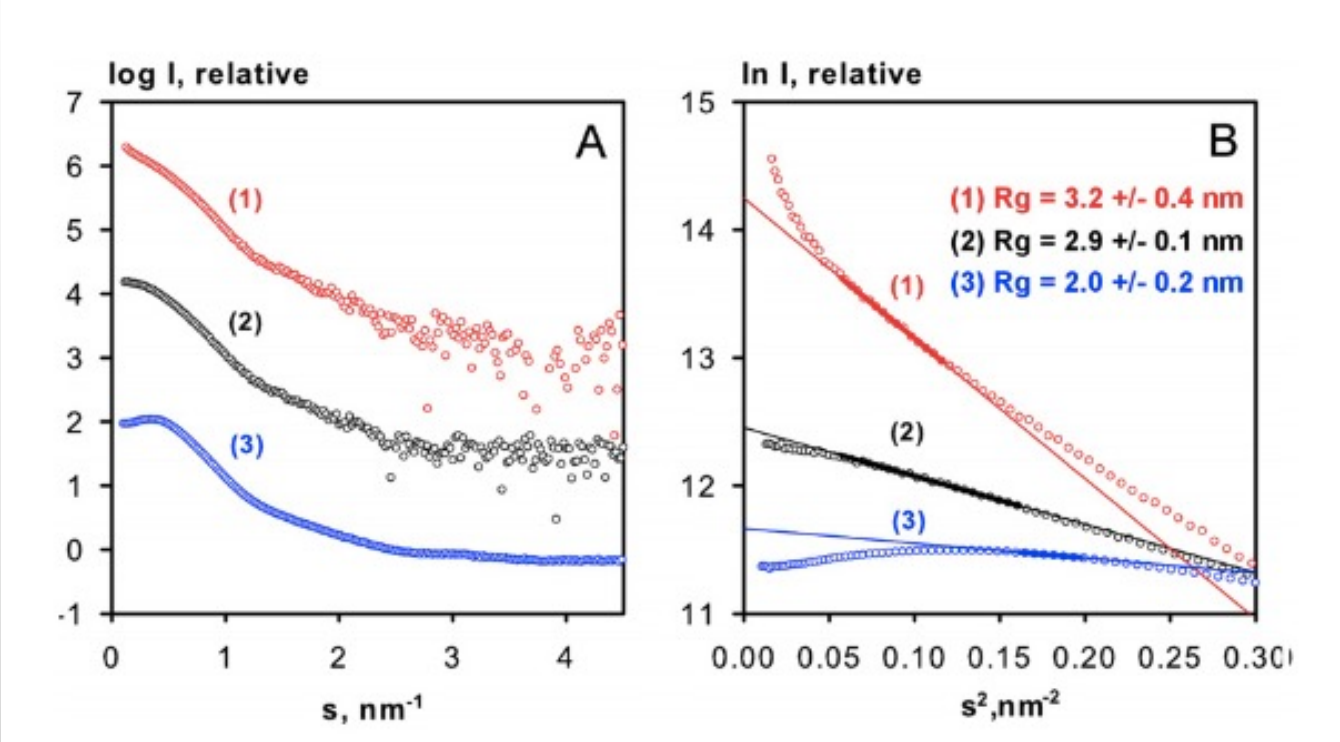
- INTRODUCTION
- DISCUSSION OF PRESENTED TOPICS
- FUTURE DIRECTIONS
- SUMMARY

1. INTRODUCTION

1. SAXS IS USED TO DETERMINE STRUCTURAL INFORMATION FROM NON-CRYSTALLINE SAMPLES
2. ADVANTAGES: REQUIRES ONLY MILLIGRAM AMOUNTS OF PURIFIED PROTEIN, DATA COLLECTION CAN OCCUR IN SECONDS AND IMMEDIATE CHARACTERIZATION CAN THEN BE DONE
3. DISADVANTAGES: DATA ANALYSIS CAN BE COMPLEX, INSTRUMENTATION IS EXPENSIVE/COMPLEX
4. SCATTERING IS DEPENDENT ON THE CONCENTRATION OF BIOMOLECULES IN THE SAMPLE
5. SMALL PROTEINS AND MACROMOLECULAR COMPLEXES (AND LARGE VIRAL PARTICLES) CAN BE MEASURED
6. 1-2 MG OF PROTEIN IS TYPICALLY ALL THAT IS REQUIRED
7. MANY OF THE APPROACHES DESCRIBED FOR SAXS ALSO APPLY TO SANS

INTRODUCTION CONT'D

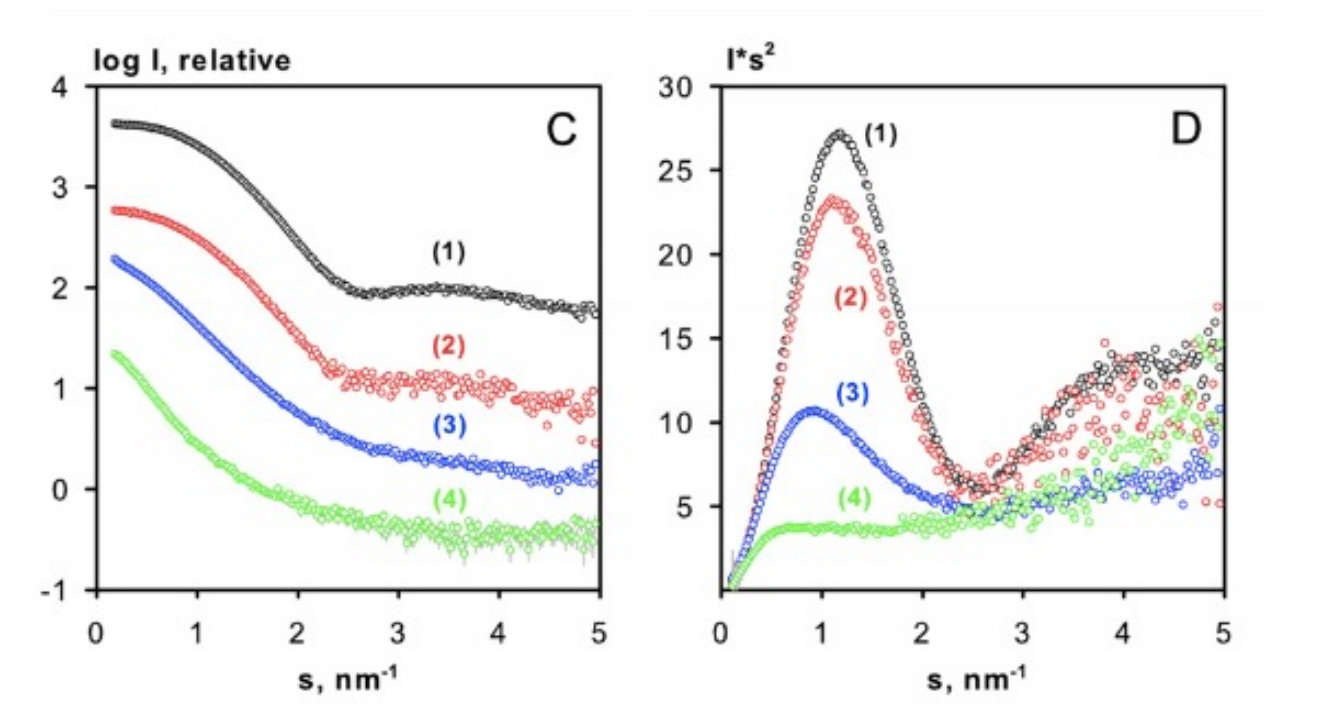


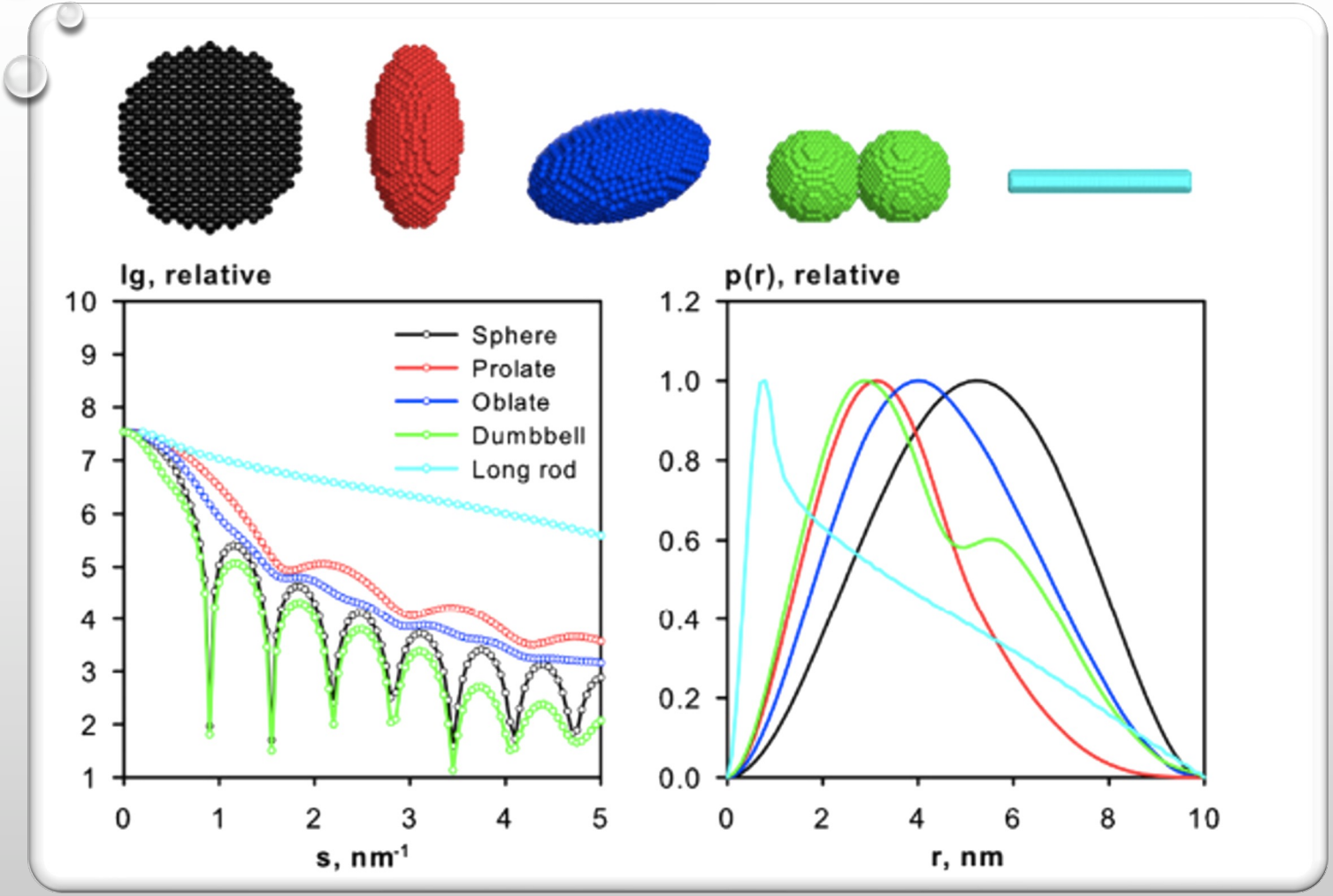


2. OVERALL SAXS PARAMETERS AND RAPID SAMPLE CHARACTERIZATION

1. MANY PARAMETERS CAN BE EXTRACTED FROM THE GUINIER PLOTS; MOLECULAR MASS (MM), RADIUS OF GYRATION (R_G), HYDRATED PARTICLE VOLUME (V_P), AND MAXIMUM PARTICLE DIAMETER (D_{MAX}) AND THE FORWARD SCATTERING INTENSITY (I(O)).
2. NON-LINEAR GUINIER PLOTS ARE INDICATIVE OF POOR SAMPLE QUALITY

1. GUINIER PLOT IS ESSENTIAL FIRST STEP IN SAXS CHARACTERIZATION
2. KRATKY PLOTS ARE HELPFUL TOOLS FOR DETERMINING FOLDED STATE OF PROTEINS
3. KRATKY PLOT OF UNFOLDED PROTEINS SHOULD HAVE A PLATEAU AT HIGH Q (SEEN IN CURVE D4)
4. FOLDED PROTEIN HAS NICE, BELL-SHAPED GAUSSIAN CURVE (D1)
5. FLEXIBLE MULTI-DOMAIN PROTEINS CAN ALSO BE IDENTIFIED

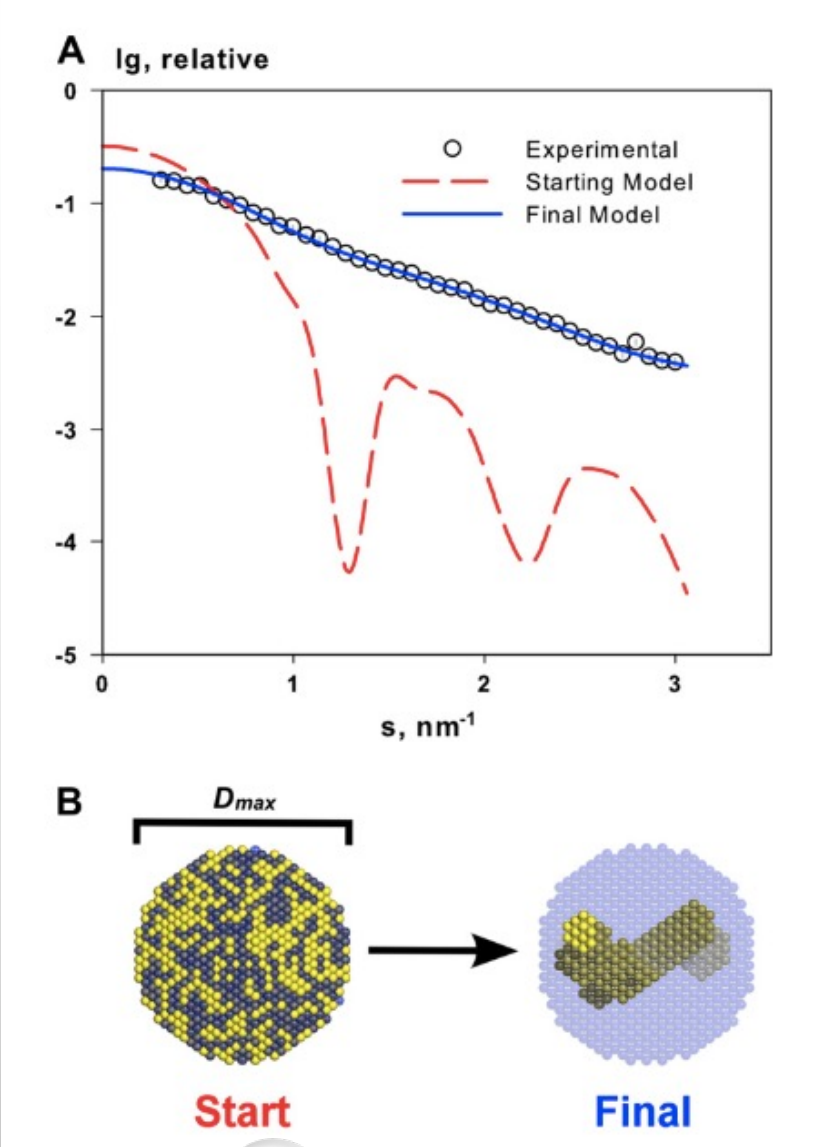




1. GUINIER APPROXIMATION LIMITATIONS LED TO INDIRECT FOURIER TRANSFORM METHODS
2. DISTANCE DISTRIBUTION FUNCTION IS GRAPHICAL DISPLAY OF PARTICLE SHAPE
3. RAPID STRUCTURE CHARACTERIZATION IS MAJOR ADVANTAGE
4. SOME 3-D STRUCTURAL INFORMATION IS ALSO POSSIBLE

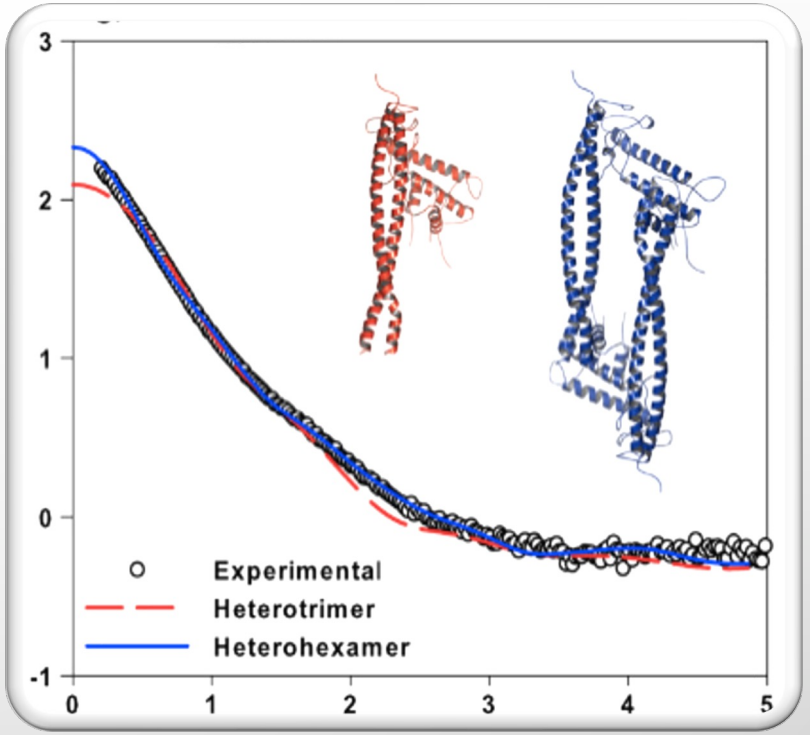
3. *AB INITIO* METHODS

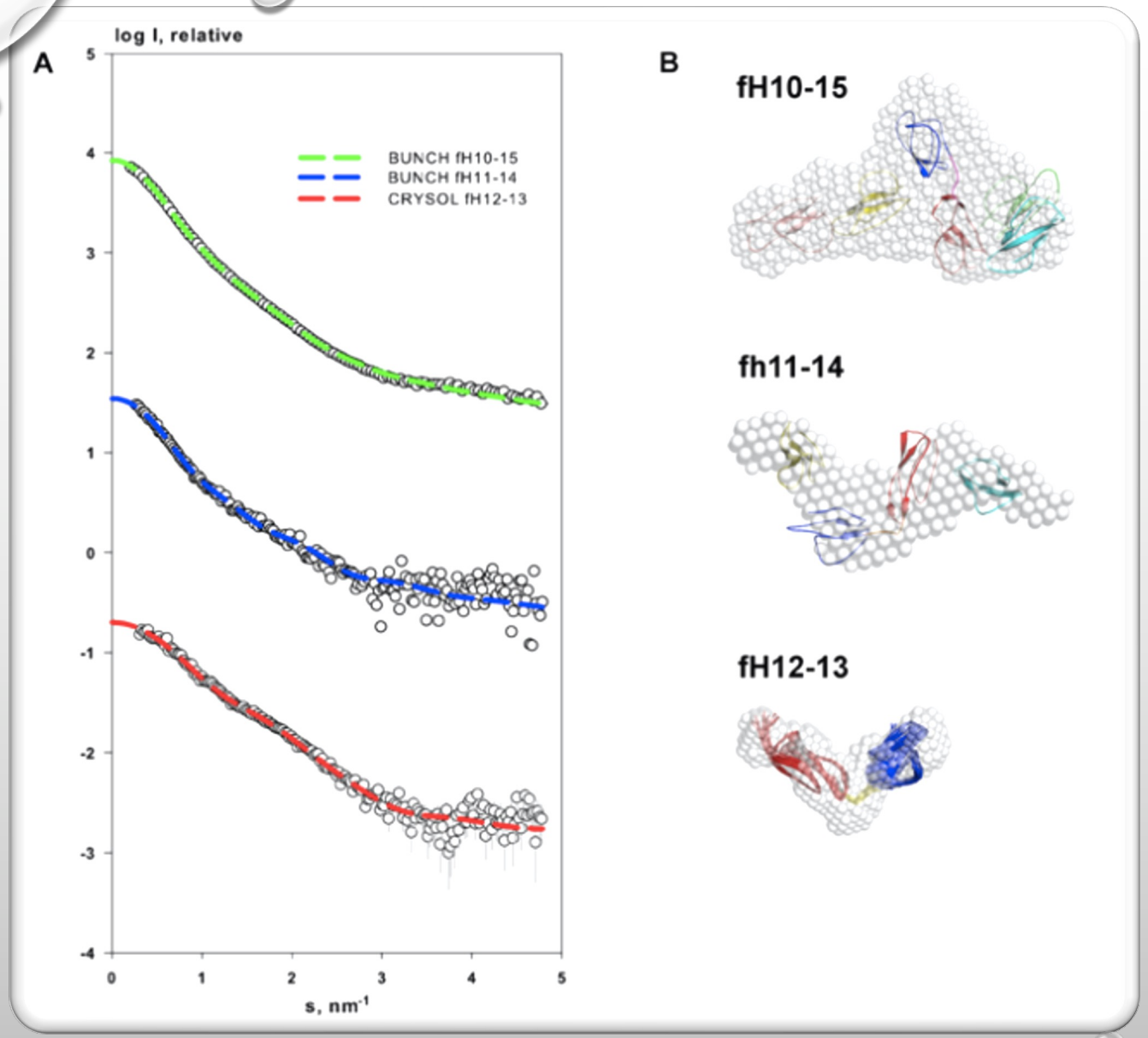
1. PARTICLE SHAPES ARE ESTIMATED FROM SAS DATA USING SPHERICAL HARMONICS AND BEAD MODELING
2. PROGRAMS HAVE BEEN DEVELOPED FOR THESE LOW-RESOLUTION MODELS
3. THE RESOLUTION OF SHAPE DETERMINATION WITH THESE METHODS IS LIMITED
4. NEW APPROACH IS TO REPRESENT PROTEINS AS DUMMY RESIDUES (DR) INSTEAD OF BEADS



4. COMPUTATION OF SCATTERING FROM HIGH-RESOLUTION MODELS

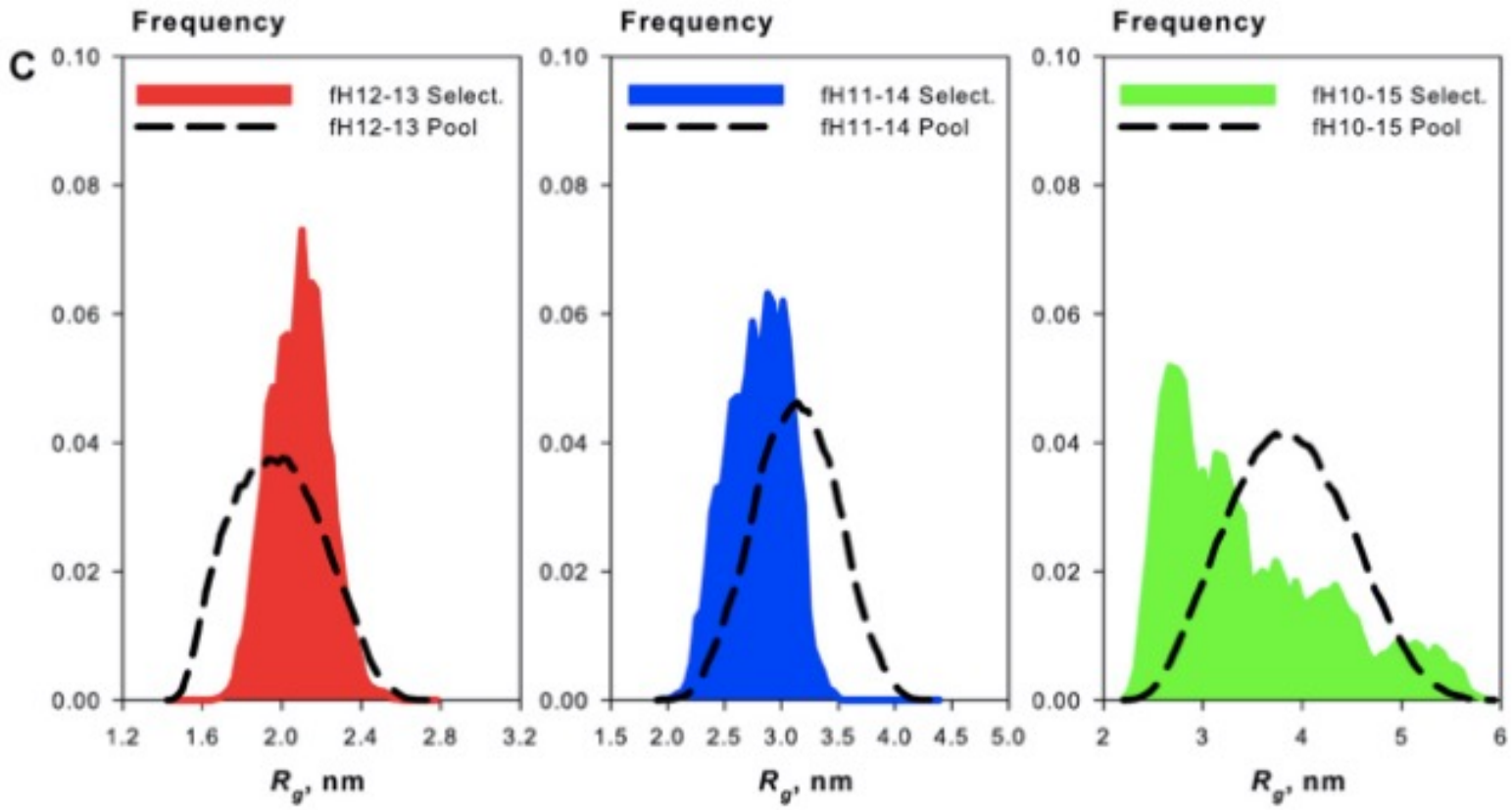
1. DESCRIBES THE THEORETICAL SCATTERING CURVE GENERATED FROM A HIGH-RESOLUTION STRUCTURAL MODEL
2. GLOBBIC APPROXIMATIONS USED FOR FASTER COMPUTATION USING DEBYE FORMULA – GIVES GAUSSIAN SPHERE APPROXIMATION OF EXCLUDED VOLUME
3. HEXAMER OF CDT 1 AND GEMININ CRYSTALLIZED; SAXS MODEL VALIDATED NEW DATA ON THIS PROTEIN COMPLEX





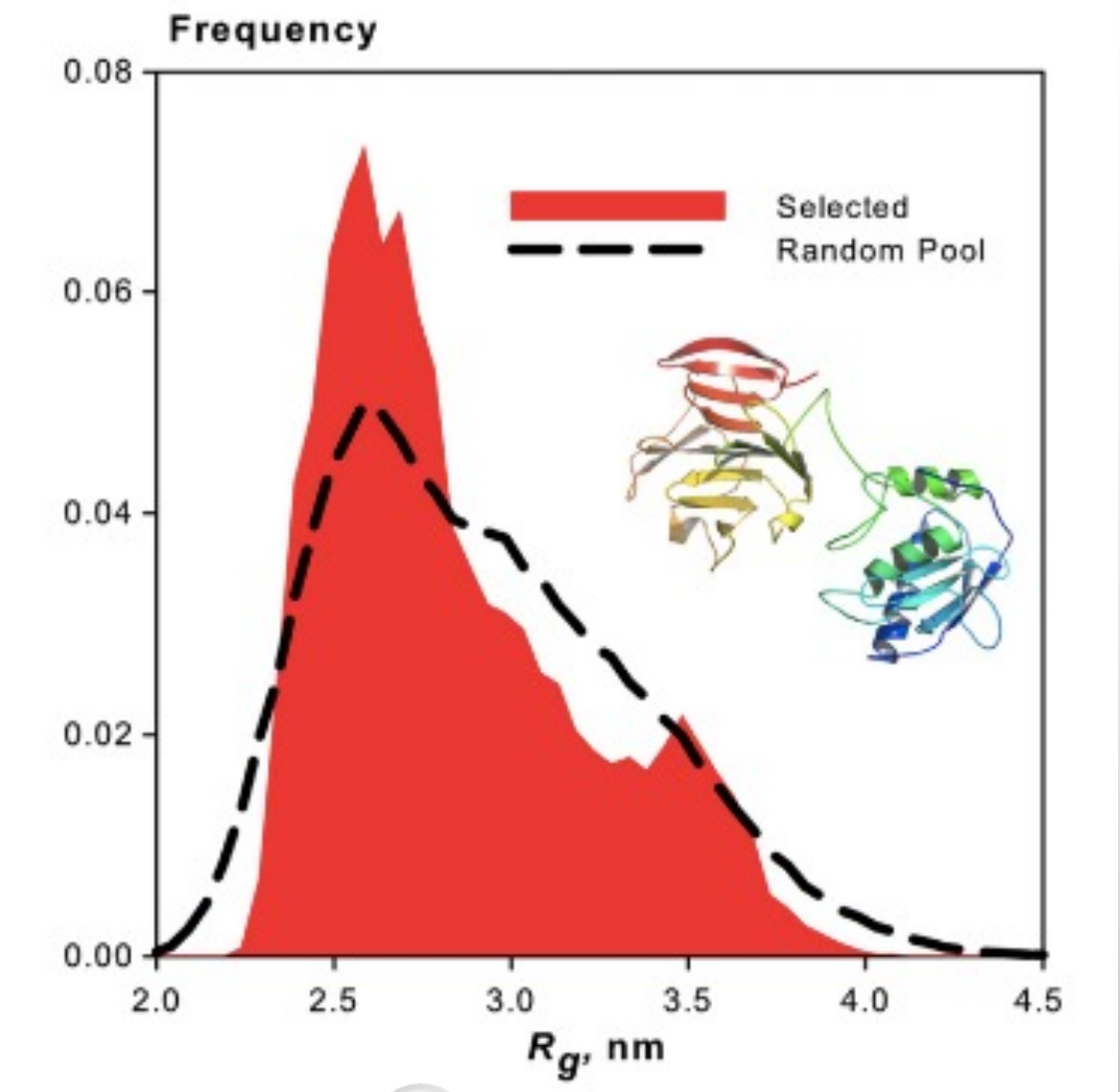
5. RIGID BODY MODELING

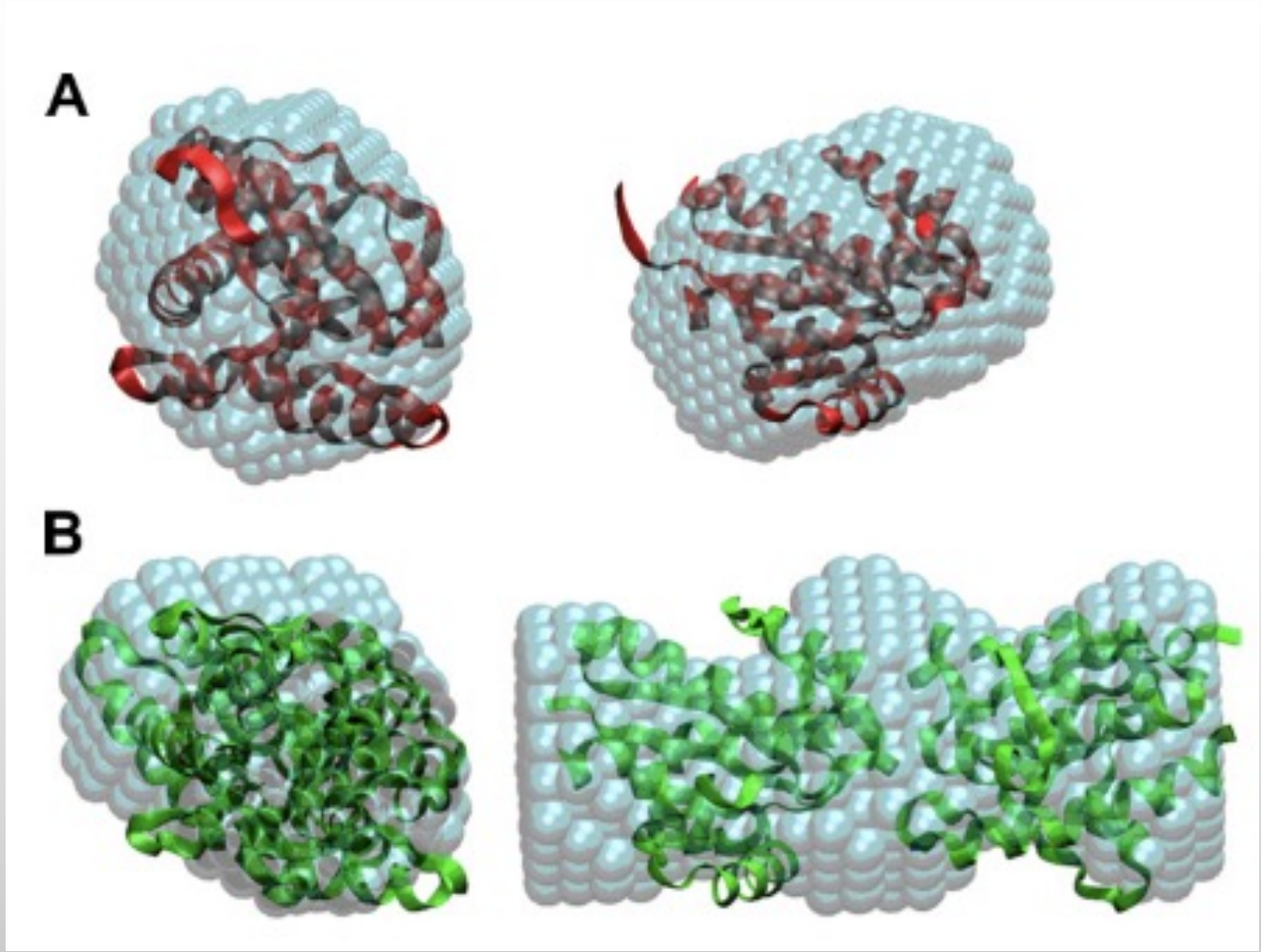
1. THEORETICAL SCATTERING FROM COMPLEXES CAN BE CALCULATED FROM OTHER, HIGH RESOLUTION MODELS
2. SCATTERING INFORMATION CAN BE SEPARATELY OBTAINED FROM INDIVIDUAL COMPONENTS OF COMPLEXES
3. RIGID BODY MODELING TO DISCERN COMPACT STRUCTURE OF CENTRAL PORTION OF HUMAN COMPLEMENT FACTOR H (FH)
4. THREE CONSTRUCTS WERE DETERMINED TO BE MONOMERIC AND SAXS DATA CONFIRMED THAT THE CORE OF FH IS COMPACT


C
Frequency
fH12-13 Select.
fH12-13 Pool
0.10
0.08
0.06
0.04
0.02
0.00
1.2 1.6 2.0 2.4 2.8 3.2
R_g, nm
Frequency
fH11-14 Select.
fH11-14 Pool
1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0
R_g, nm
Frequency
fH10-15 Select.
fH10-15 Pool
2 3 4 5 6
R_g, nm

6. FLEXIBLE SYSTEMS

1. RIGID BODY MODELING ASSUMES NO FLEXIBILITY IN SOLUTION – OFTEN NOT THE CASE
2. REPRESENTING PROTEINS AS ENSEMBLES OF STRUCTURES THAT ARE SELECTED FROM A POOL AND FITTING THESE TO THE SCATTERING DATA HELPS WITH STRUCTURES OF INTRINSICALLY UNFOLDED PROTEINS OR DOMAINS THAT CONTAIN FLEXIBLE LINKERS
3. EOM DATA OF MMP-1, CONFIRMED WITH NMR, SHOWS MAJORITY OF COMPACT CONFORMATIONS





7. ANALYSIS OF MIXTURES

1. SAXS IS USEFUL TO QUANTITATIVELY CHARACTERIZE SOLUTIONS OF PROTEIN MIXTURES
2. MULTIVARIATE CURVE RESOLUTION METHODS (MCR-ALS) WERE USED TO CHARACTERIZE TO MONOMER-DIMER EQUILIBRIUM OF LMWPTP – GENERATED MODELS EXTRACTED FROM SAXS DATA APPEARED IN AGREEMENT WITH CORRESPONDING CRYSTAL STRUCTURES
3. TIME RESOLVED SAXS (TR-SAXS) CAN ALSO BE UTILIZED TO ANALYZE MIXTURES OF COMPONENTS

8. COMBINING NMR, CRYSTALLOGRAPHY AND SAXS

1. X-RAY CRYSTALLOGRAPHY AND SAXS HAVE BEEN WELL ESTABLISHED AS COMPLIMENTARY SYSTEMS
2. SAXS CAN ALSO BE APPLIED AS A TOOL FOR MOLECULAR REPLACEMENT IN CRYSTALLOGRAPHY
3. PROTEINS AND COMPLEXES LARGER THAN 30 KDA ARE DIFFICULT TO STRUCTURALLY ANALYZE WITH NMR
4. SAXS DATA INTRODUCED INTO STRUCTURAL CALCULATIONS OF NMR STUDIES OF LARGE PROTEINS OR COMPLEXES CAN HELP REDUCE THE LIMITATIONS
5. DETERMINATION OF DNA AND RNA STRUCTURES BY NMR IN CONJUNCTION WITH SAXS HAVE ALSO PROVEN USEFUL

9. FUTURE DIRECTIONS

1. SAXS HAS BECOME FAIRLY STRAIGHTFORWARD AND CAN EASILY BE APPLIED TO MANY STUDIES
2. IT IS IMPORTANT FOR CONTINUED DEVELOPMENT OF SAXS TECHNIQUES WHICH PUSH THE BOUNDARIES OF THIS ANALYTICAL TOOL
3. NEWER INSTRUMENTATION ALLOWS FOR BETTER, HIGH-BRILLIANCE BEAMLINES, AND AUTOMATED SAMPLE CHANGERS – SOME COMBINED WITH ON-LINE HPLC PURIFICATION AND UV-VIS ABSORPTION MONITORING
4. SAXS IS BEST EMPLOYED IN CONJUNCTION WITH OTHER BIOCHEMICAL AND STRUCTURAL TECHNIQUES SUCH AS NMR AND X-RAY CRYSTALLOGRAPHY

SUMMARY

1. GUINIER PLOTS ARE GOOD FOR DETECTING AGGREGATION/INTER-PARTICLE REPULSION – BETTER AT SHOWING POOR DATA
2. KRATKY PLOTS GIVE INFORMATION ABOUT FLEXIBILITY OR DEGREE OF FOLDING OR UNFOLDING OF A PROTEIN
3. *AB INITIO* METHODS GENERATE LOW-RESOLUTION STRUCTURAL INFORMATION THAT CAN BE COMPARED TO HIGH-RESOLUTION TECHNIQUES AS CONFIRMATION OF ACCURACY
4. COMPUTING SAXS DATA FROM HIGH-RESOLUTION STRUCTURES CAN ALSO CONFIRM INFORMATION ABOUT MACROMOLECULAR COMPLEXES
5. RESOLUTION OF SAXS DERIVED SHAPES IS LOW, SO IT IS BETTER TO MODEL STRUCTURES AGAINST MORE RELIABLE METHODS AND CONFIRM WITH SAXS DATA
6. CAREFUL SAMPLE CHARACTERIZATION SHOULD BE DONE BEFORE EMPLOYING ANY OF THESE SAXS TECHNIQUES
7. TR-SAXS MAY PROVIDE INSIGHT INTO KINETIC PROCESSES LINKING BIOLOGICAL FUNCTION TO STRUCTURE
8. SAXS DATA CAN BE USED TO COMPLIMENT MANY STUDIES, AND HAVE PROVEN USEFUL IN SOLVING THE PHASE PROBLEM IN X-RAY CRYSTALLOGRAPHY