Evaluating Crystal Structure Papers: Protein

- What was crystallized? Was it full-length? A domain? Tagged?
- Were complexes preformed or soaked?
- Is the molecule functional in solution?

Evaluating Crystal Structure Papers: Phases

- How were phases obtained? MIR? MAD? Molecular Replacement?
- How was the structure validated? Omit maps?

Evaluating Crystal Structure Papers: Data

- What is the quality of the diffraction data? Generally want $I/\sigma_I > 2\text{-}3$ in the last resolution bin, $R_{sym} < 0.40$, and 75% completion.

Evaluating Crystal Structure Papers: Model

- What is the quality of the model? Any Ramachandran outliers for proteins? Small deviations in bond lengths (~0.02 Å) and bond angles (~2.5 degrees)? $R_{free} < 0.30$ and close to R_{crys}?
- Was a systematic search for problems undertaken? Each residue subjected to omit maps? A thorough geometry checking program run like PROCHECK?
- Have appropriate restraints been applied? E.g. group B-factors at low resolution?
- Is the solvent appropriate? No more than 1-2 water molecules per residue? Hydrogen bonds checked?

Evaluating Crystal Structure Papers: Example

Fallon & Quiocho, A Closed Compact Structure of Native Ca^{2+}-Calmodulin, *Structure,* 11: 1303-7, (2003)

PDB:1PRW

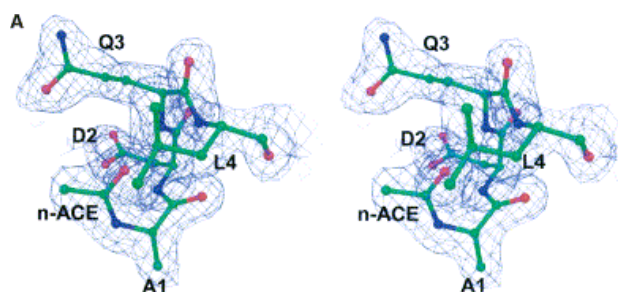
Crystallization Crystals were initially obtained from bovine brain CaM (Sigma) dissolved in 10 mM $CaCl_2$ to 10 mg/ml; 20 l of this solution was mixed with 10 l well solution and set up as hanging drops with wells of 40% PEG 6000 (Fluka), 50 mM sodium acetate, and 10 mM $CaCl_2$ (pH 5.6) (23°C). Crystals for the final structure refinement were obtained in the same way except bovine brain CaM (Calbiochem) was dissolved in 2 mM EGTA (pH 6.0) to 10 mg/ml, exhaustively dialyzed against 2 mM EGTA (pH 6.0) to remove bound calcium, along with any potential bound endogenous ligand, and then against distilled deionized water until the protein precipitated. The protein was reconstituted by dialysis against 10 mM $CaCl_2$ and crystallized as described above, with the addition of 10% glycerol in the well solution prior to drop mixing as a cryoprotectant. Microseeding greatly assisted crystal production.

Fallon & Quiocho, *Structure,* 11: 1303-7, (2003)

Diffraction: Initial diffraction data to 1.7 Å for the C2 crystals were collected on a MACScience DIP2030k with a Rigaku RU220HB CuK source and reduced with DENZO-SCALEPACK [Otwinowski and Minor 1997]. The structure was solved by the SIRAS method as applied in the PHASES software package (Furey, W.) using data from a single Pb derivative obtained by a 24 hr soak in 1 mM $Pb(NO_3)_2$. The native dataset for the final refinement was collected to 1.7 Å at the HHMI NSLS Beamline X4A and reduced with DENZO-SCALEPACK. The structure was refitted and refined using CHAIN [Sack and Quiocho 1997] and CNS [Brünger et al. 1998] for iterative model improvement. Final refinement statistics are shown in Table 1.

Fallon & Quiocho, *Structure,* 11: 1303-7, (2003)

Figure 1 : Electron Density Map



Fallon & Quiocho, *Structure,* 11: 1303-7, (2003)

Data Collection	Statistics
Space group	C2
Unit cell parameters	
—axis (Å)	a = 63.32, B = 35.75, C = 68.03
—β angle (deg.)	117.19
Resolution (Å)	1.7
Molecule per asymmetric unit	1
Number of unique reflections ($F > 3.0\ \sigma F$)	12598
R-merge[a] (%) (highest shell)	6.2 (32.2)
Completeness (%) (highest shell)	92.4 (90.7)
$I/\sigma(I)$[b] (highest shell)	14.9 (4.8)
Refinement	
Resolution (Å)	1.7
Reflections (working/test)	11318/1280
R-cryst[c]/R-free	0.188 /0.235
Mean B-factor ($Å^2$)	18.4
Number of atoms	
—Protein/Ca^{2+}/water	1367/4/178
R.m.s. deviation	
—Bonds (Å)	0.005
—Angles (deg.)	1.01
Ramachandran analysis (%)	
—Most favored	94.7
—Allowed	5.3

Table 1. Data Collection and Refinement Statistics

Fallon & Quiocho, *Structure,* 11: 1303-7, (2003)