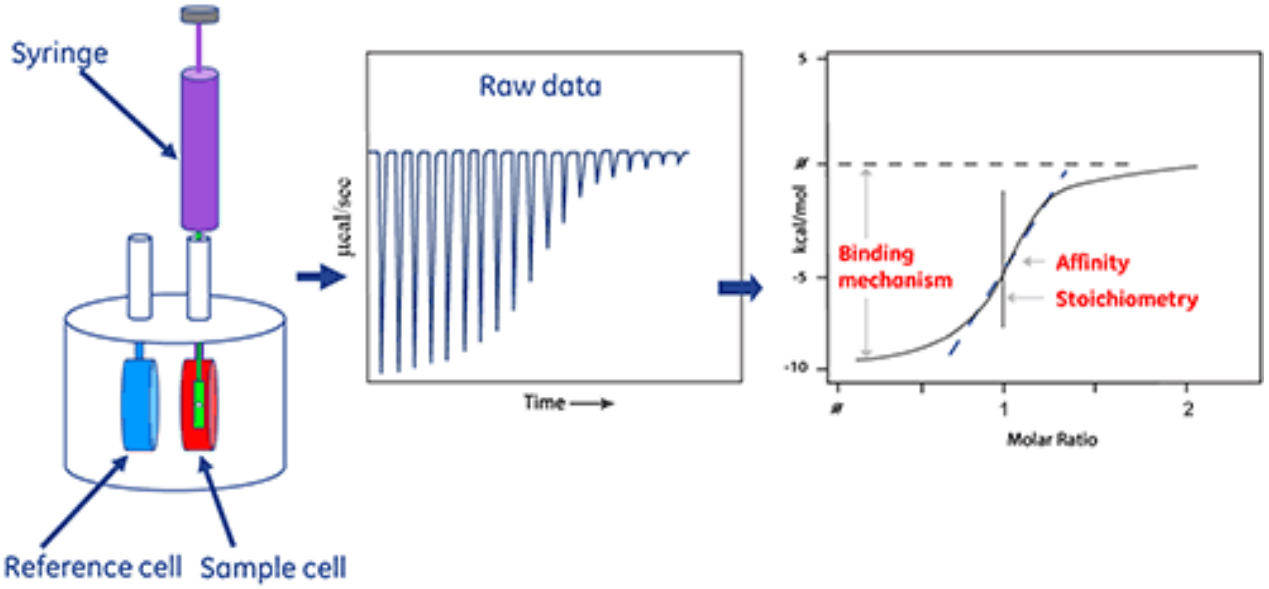
Isothermal titration calorimetry (ITC)



measures heats released/absorbed upon binding: $q = \Delta[PL]V\Delta H^0$

$n, K_d, \Delta H^0$ obtained from fitting the data

$\Delta G^0 = -RTlnK$

$\Delta G^0 = \Delta H^0 - T\Delta S^0$

https://www.malvernpanalytical.com/en/products/technology/microcalorimetry/isothermal-titration-calorimetry/

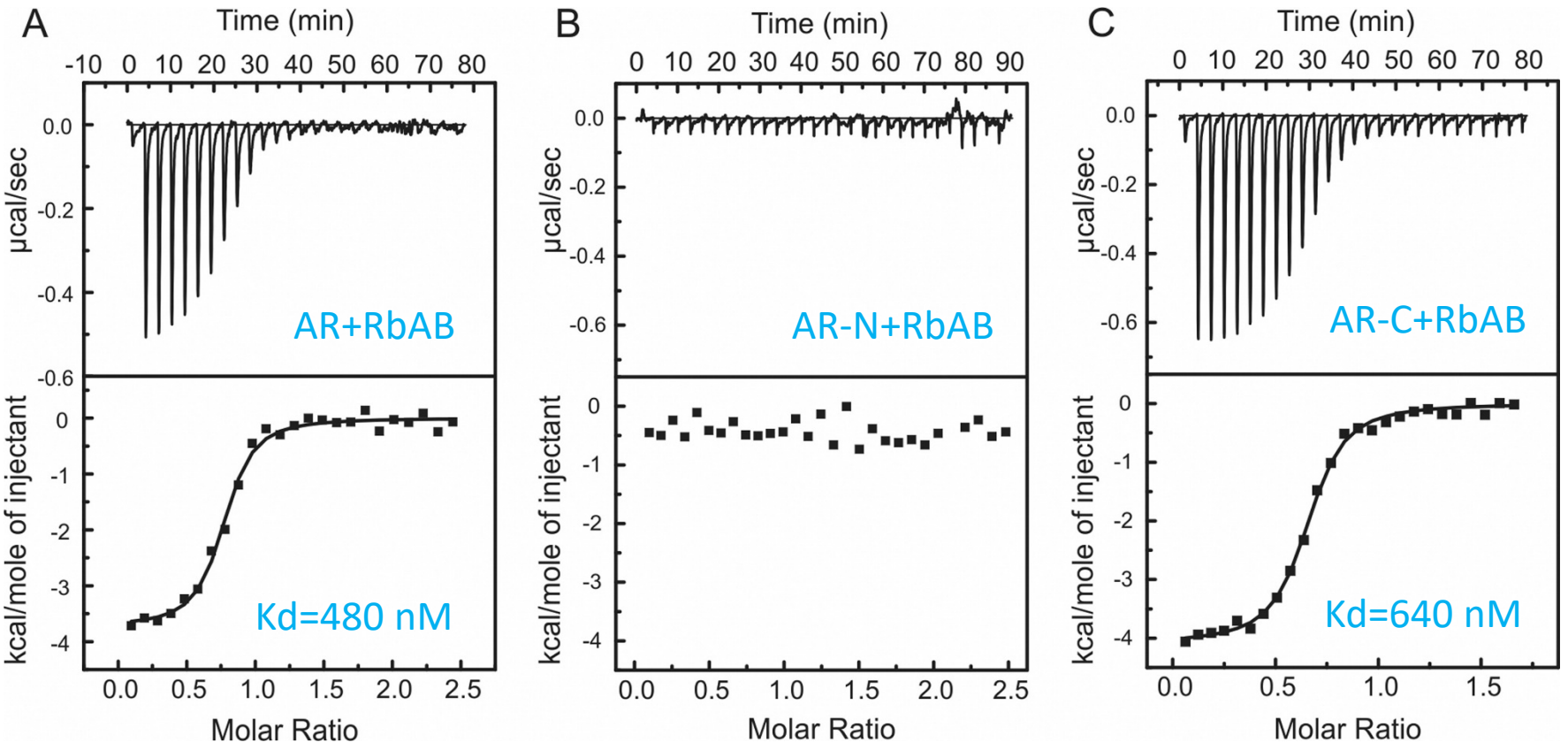
Isothermal titration calorimetry (ITC)

Isothermal titration microcalorimeters measure the heat change that occurs when two molecules interact. Heat is released or absorbed as a result of the redistribution and formation of non-covalent bonds when the interacting molecules go from the free to the bound state. ITC monitors these heat changes by measuring the differential power, applied to the cell heaters, required to maintain zero temperature difference between the reference and sample cells as the binding partners are mixed.
The reference cell usually contains water, while the sample cell contains one of the binding partners (the sample, often but not necessarily a macromolecule) and a stirring syringe which holds the other binding partner (the ligand).
The ligand is injected into the sample cell, typically in 0.5 to 2 µL aliquots, until the ligand concentration is two- to three-fold greater than the sample. Each injection of ligand results in a heat pulse that is integrated with respect to time and normalized for concentration to generate a titration curve of kcal/mol vs molar ratio (ligand/sample). The resulting isotherm is fitted to a binding model to generate the affinity (K_D), stoichiometry (n) and enthalpy of interaction (ΔH).

Microcal ITC Systems brochure, Malvern

https://www.malvernpanalytical.com/en/products/technology/microcalorimetry/isothermal-titration-calorimetry/

Isothermal titration calorimetry (ITC)



ITC will only detect binding if $\Delta H^0 \neq 0$!

Sun Y, Stine JM, Atwater DZ, Sharmin A, Ross JB, Briknarová K. Structural and functional characterization of the acidic region from the RIZ tumor suppressor. Biochemistry. 2015 Feb 17;54(6):1390-400.

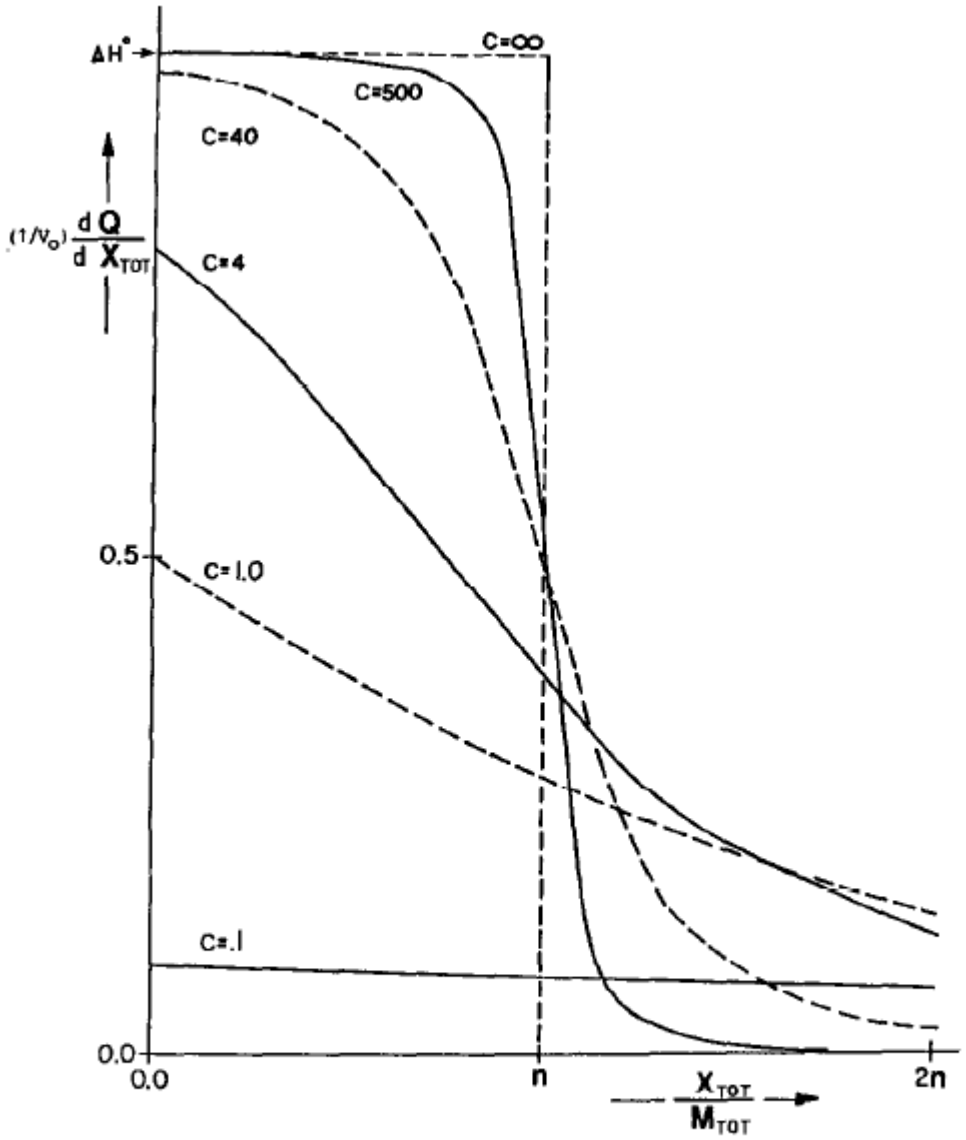


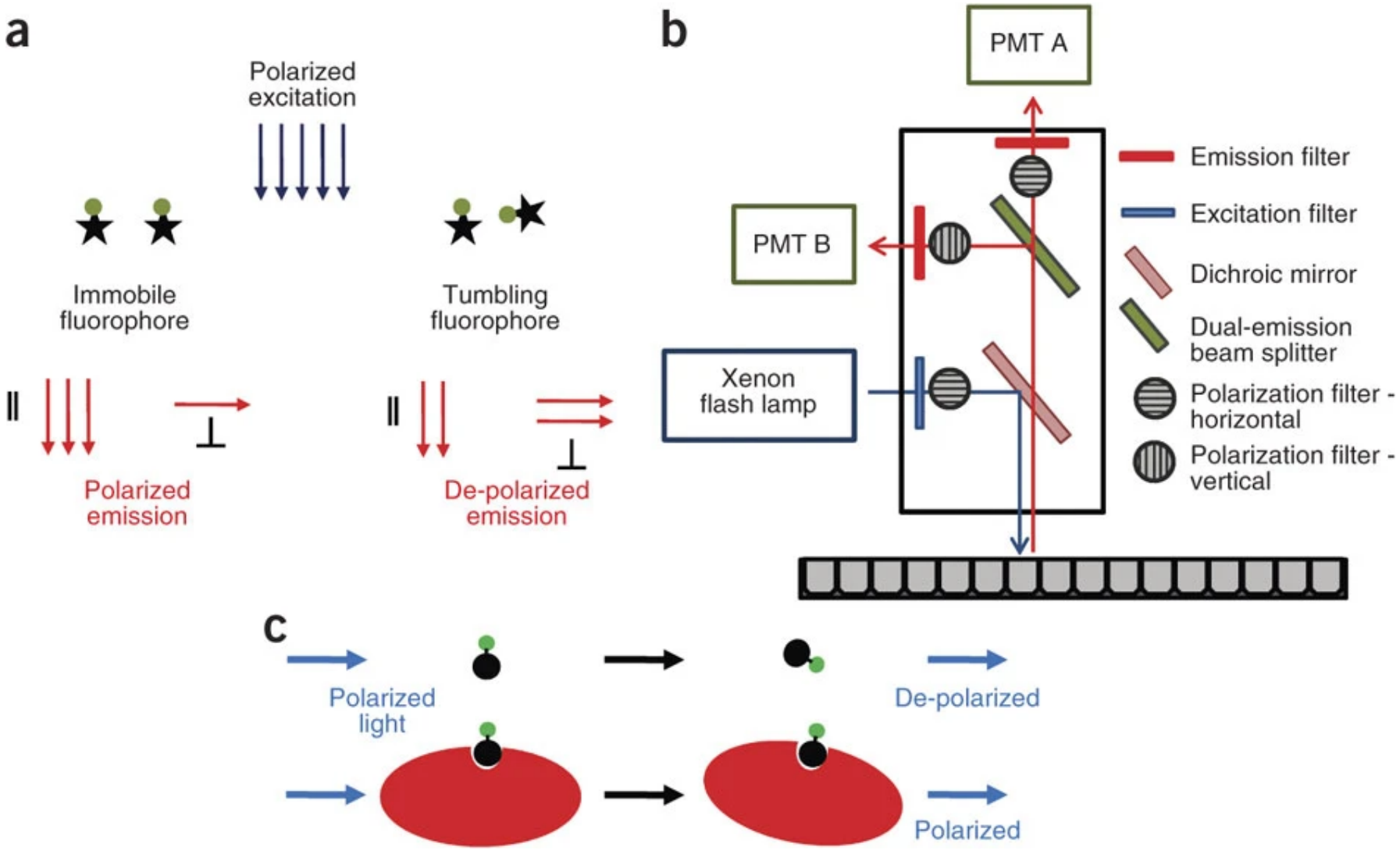
FIG. 3. Simulated binding isotherms for various values of the parameter c (equal to the product of the binding constant times the total macromolecule concentration), presented in derivative format. See text for details.

The shape of the data is sensitive to the binding constant when c ~ 1-1000

$$c = P_t K_a = \frac{P_t}{K_d}$$

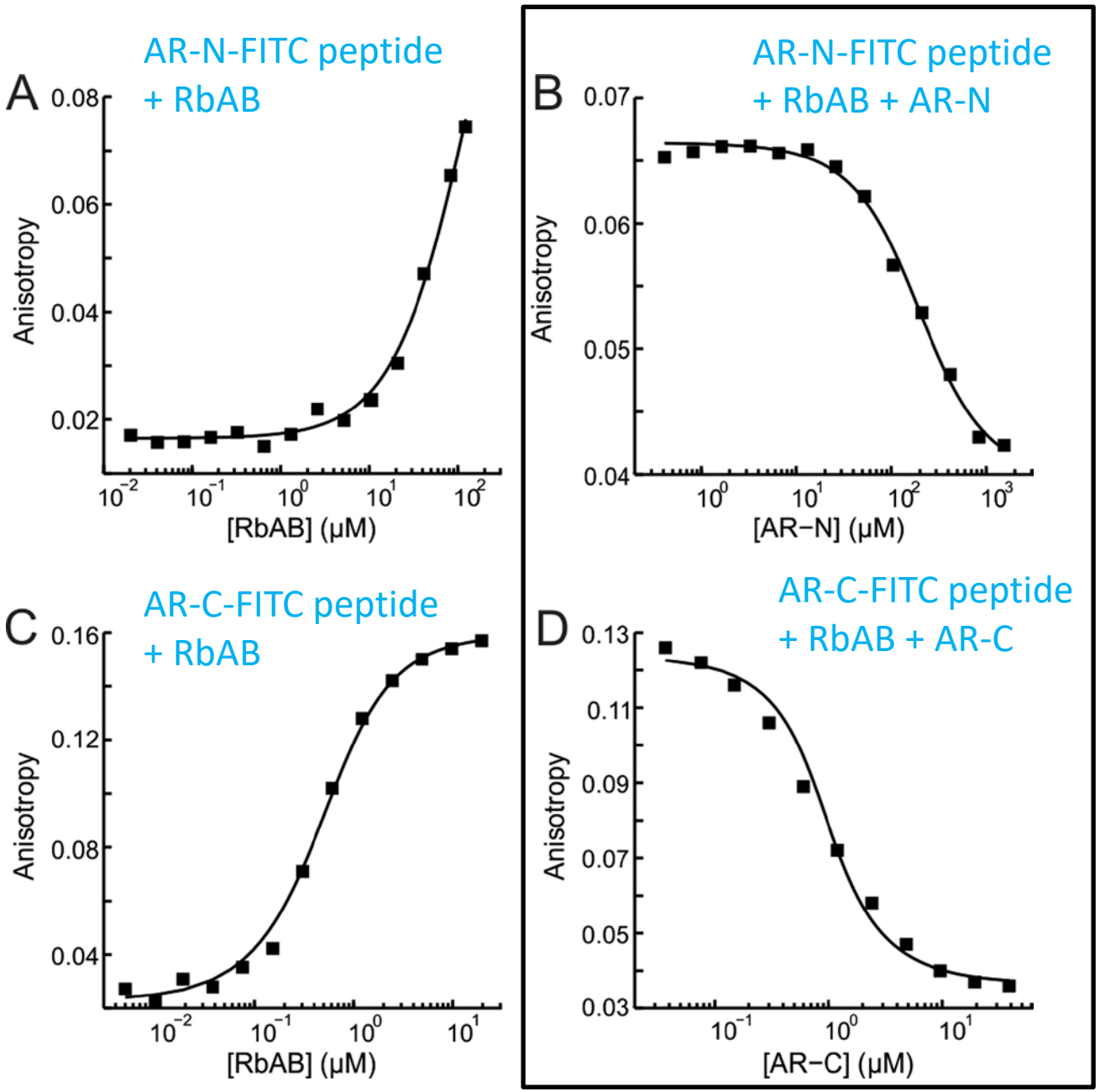
Wiseman T, Williston S, Brandts JF, Lin LN. Rapid measurement of binding constants and heats of binding using a new titration calorimeter. Anal Biochem. 1989 May 15;179(1):131-7.

Fluorescence Anisotropy



Rossi AM, Taylor CW. Analysis of protein-ligand interactions by fluorescence polarization. Nat Protoc. 2011 Mar;6(3):365-87. doi: 10.1038/nprot.2011.305.

Fluorescence Anisotropy



Competition experiments

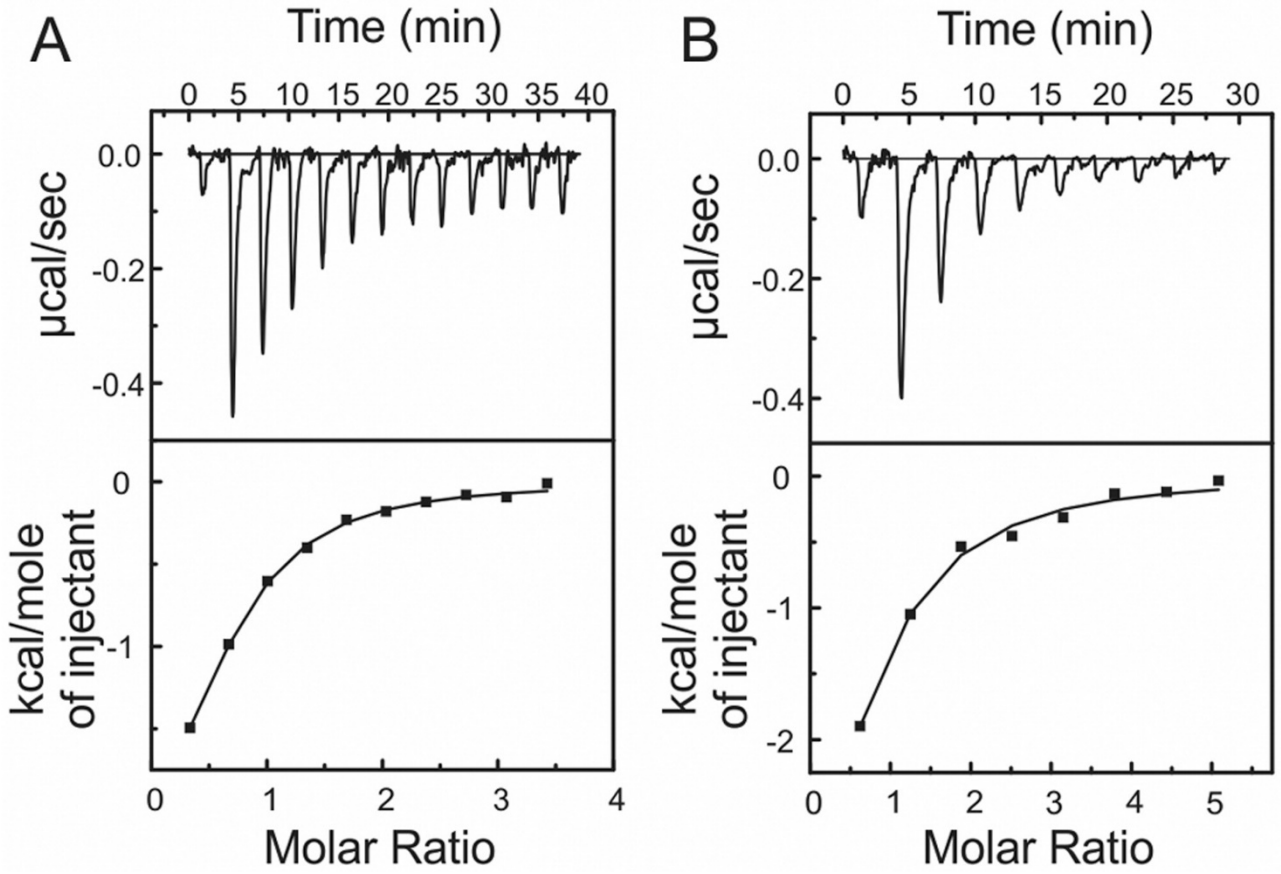
Sun Y, Stine JM, Atwater DZ, Sharmin A, Ross JB, Briknarová K. Structural and functional characterization of the acidic region from the RIZ tumor suppressor. Biochemistry. 2015 Feb 17;54(6):1390-400.

Isothermal titration calorimetry (ITC)

Competition ITC experiments detect binding with $\Delta H^0 = 0$

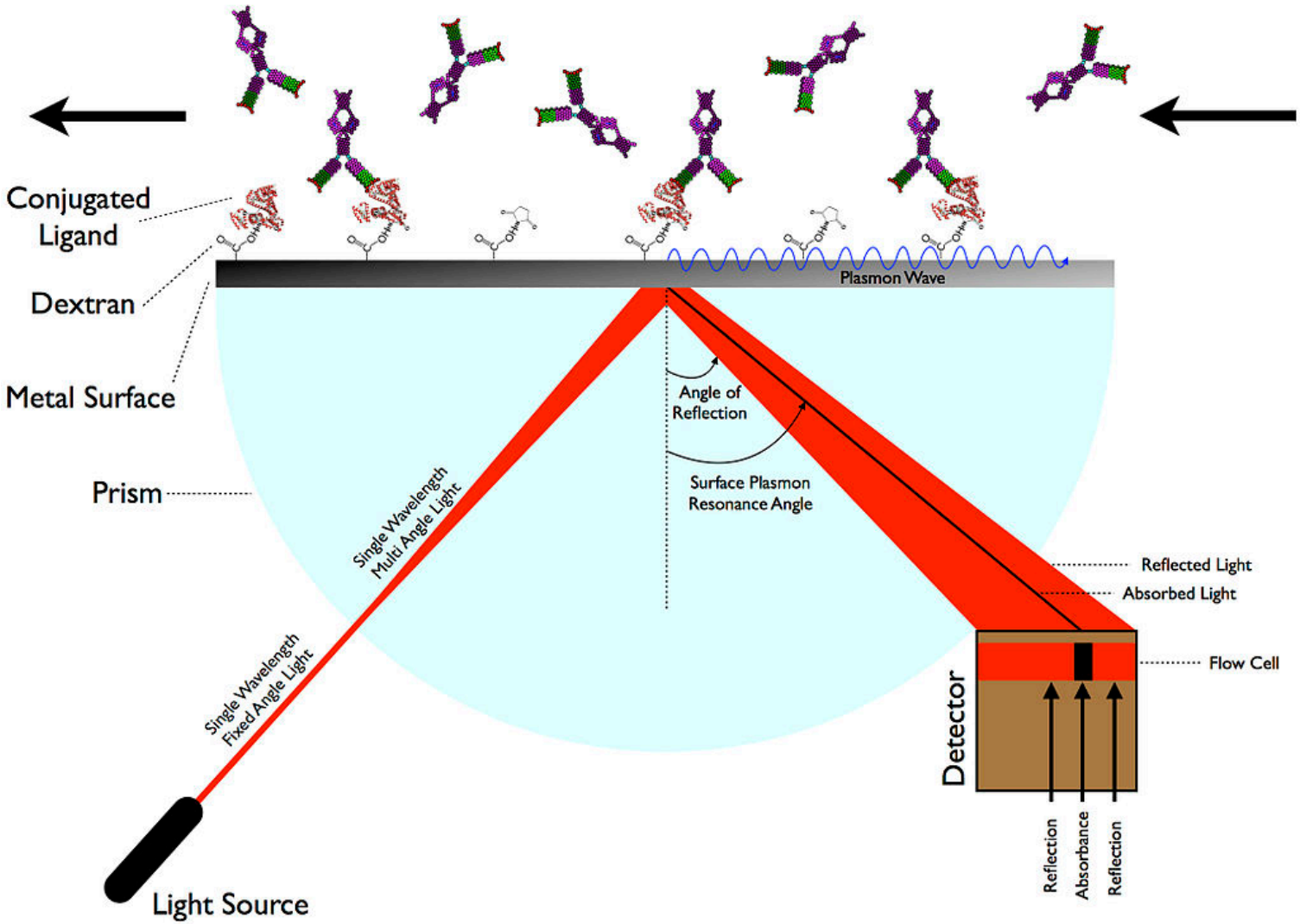
RbAB + AR-N peptide + AR-C

RbAB + AR-N + AR-C



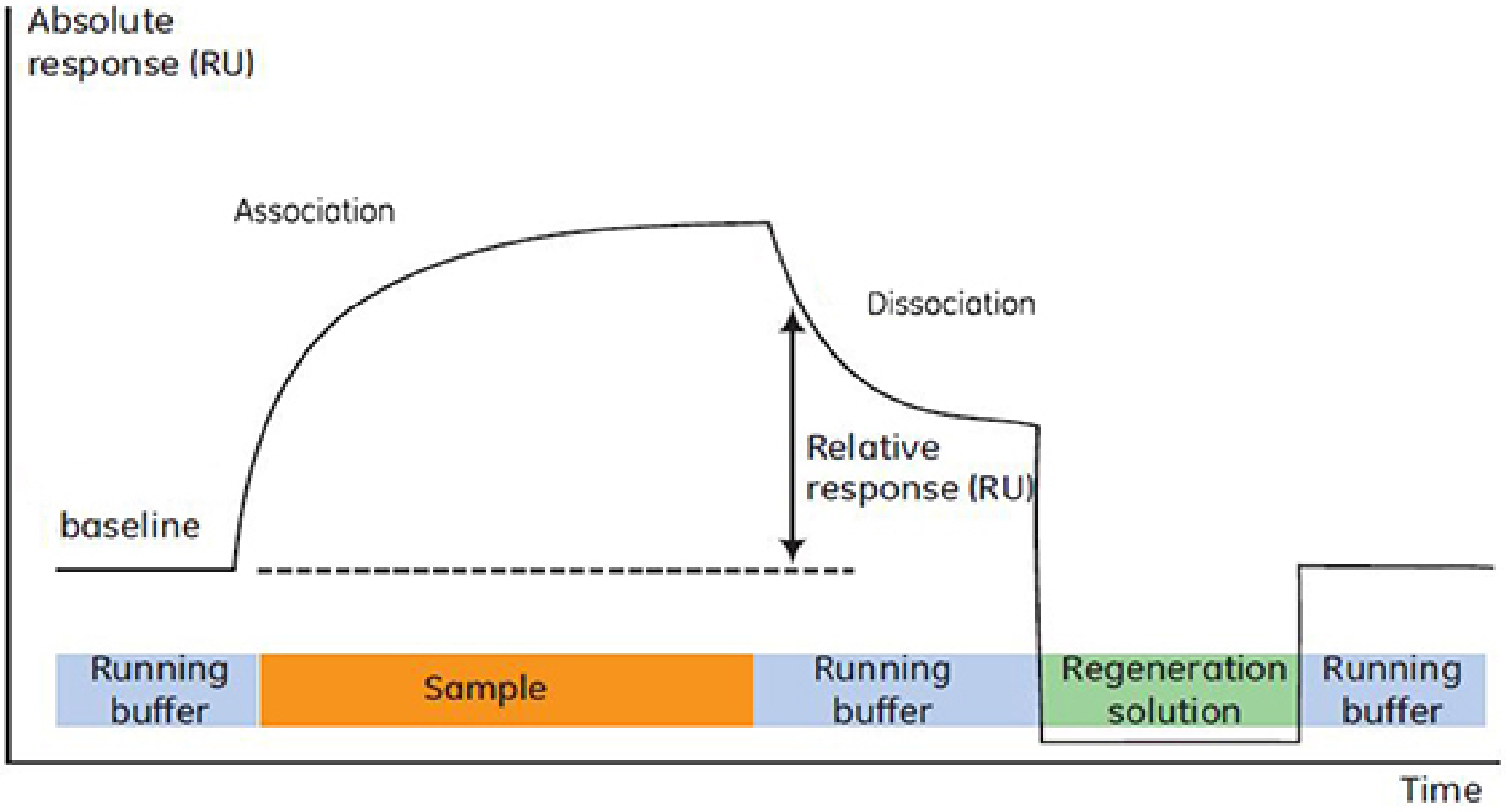
Sun Y, Stine JM, Atwater DZ, Sharmin A, Ross JB, Briknarová K. Structural and functional characterization of the acidic region from the RIZ tumor suppressor. Biochemistry. 2015 Feb 17;54(6):1390-400.

Surface plasmon resonance (Biacore)



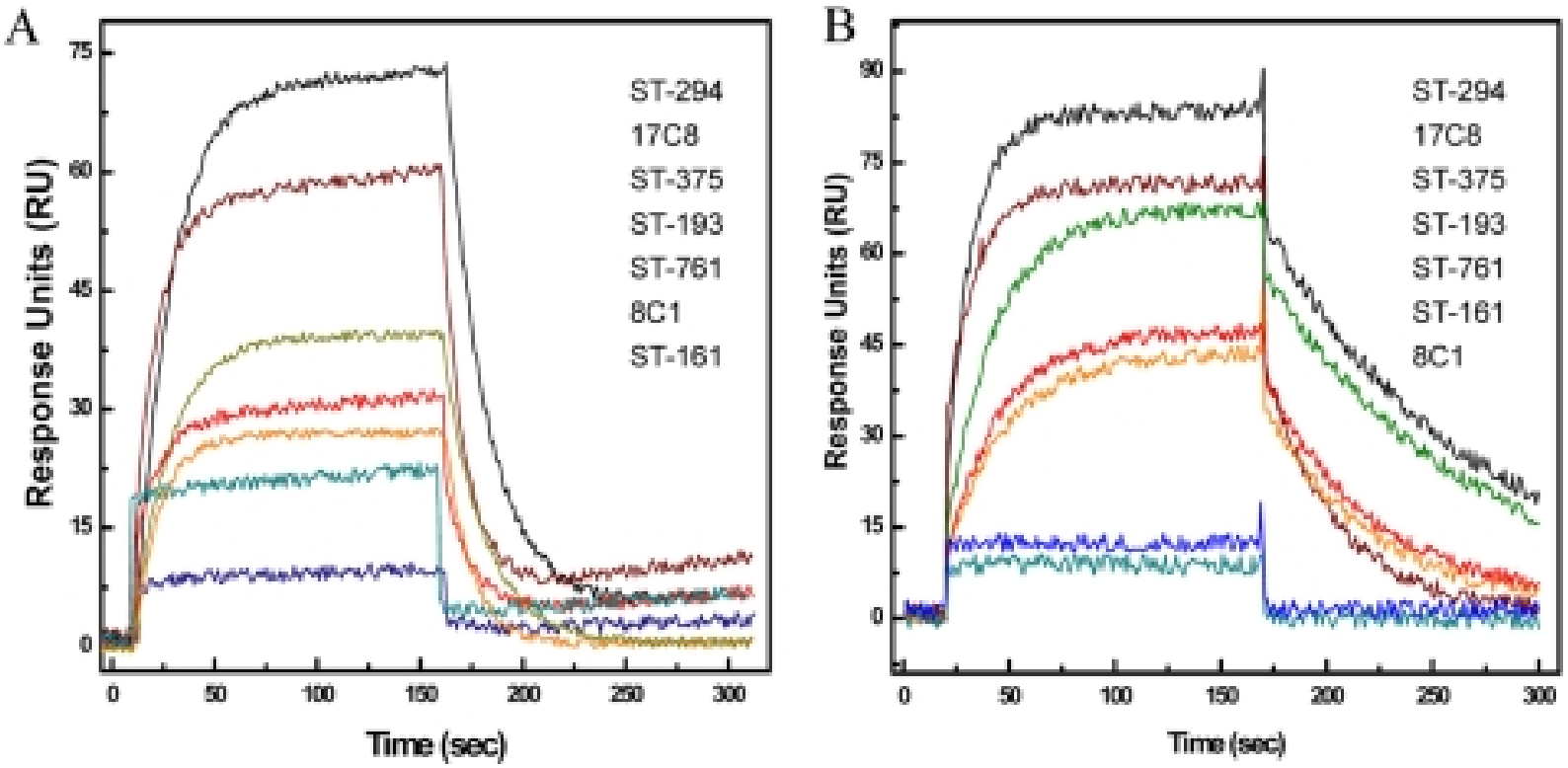
https://en.wikipedia.org/wiki/Surface_plasmon_resonance

Surface plasmon resonance (Biacore)



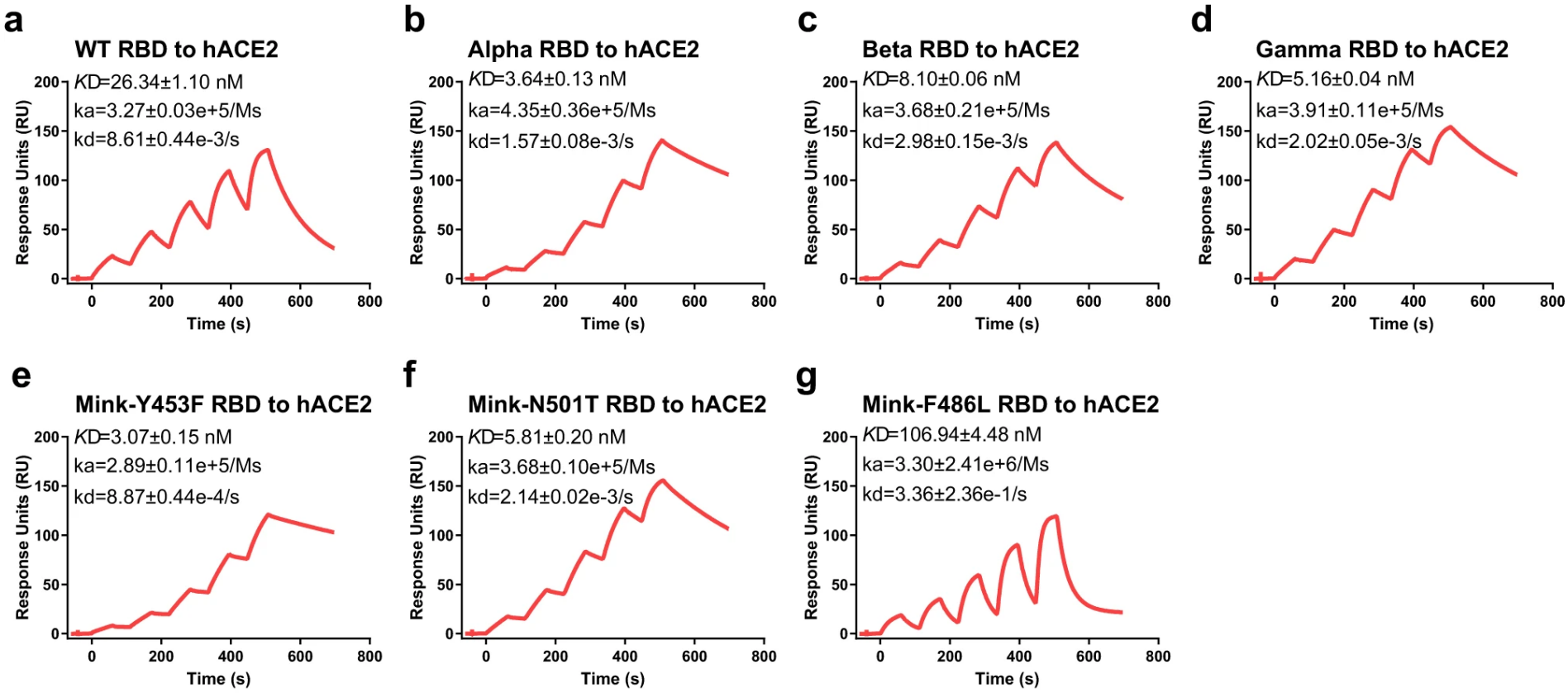
https://www.cytivalifesciences.com/en/us/solutions/protein-research/knowledge-center/surface-plasmon-resonance/surface-plasmon-resonance

Surface plasmon resonance (Biacore)



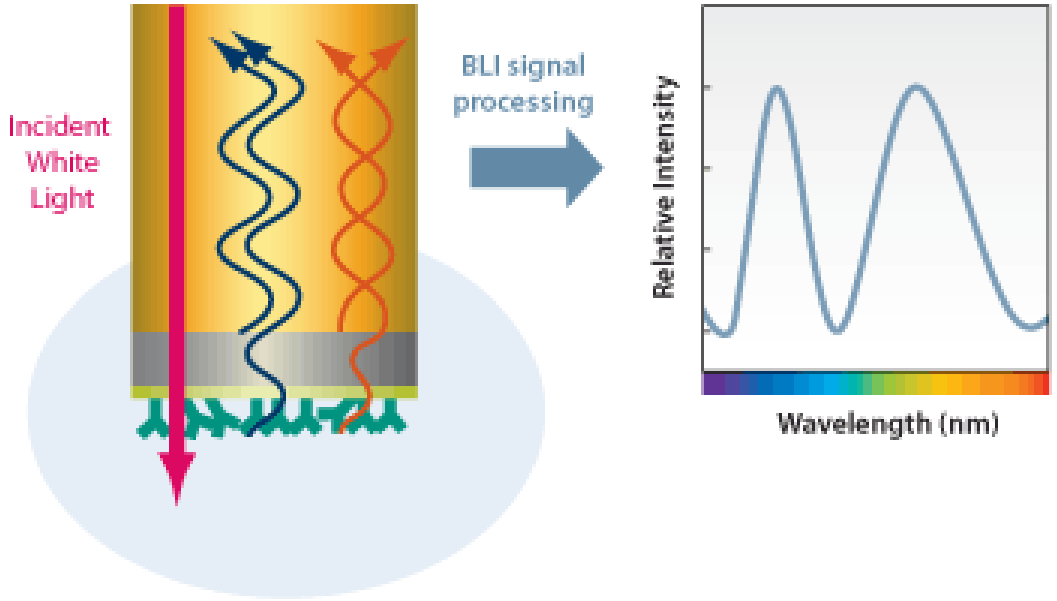
Thomas CJ, Casquilho-Gray HE, York J, DeCamp DL, Dai D, Petrilli EB, Boger DL, Slayden RA, Amberg SM, Sprang SR, Nunberg JH. A specific interaction of small molecule entry inhibitors with the envelope glycoprotein complex of the Junín hemorrhagic fever arenavirus. J Biol Chem. 2011 Feb 25;286(8):6192-200.

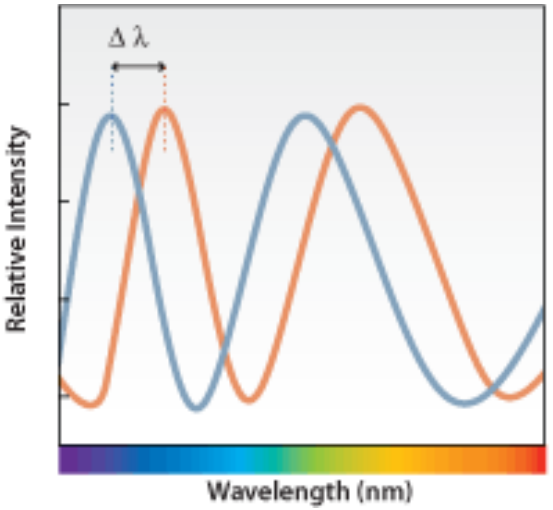
Surface plasmon resonance (Biacore)



Han P, Su C, Zhang Y, Bai C, Zheng A, Qiao C, Wang Q, Niu S, Chen Q, Zhang Y, Li W, Liao H, Li J, Zhang Z, Cho H, Yang M, Rong X, Hu Y, Huang N, Yan J, Wang Q, Zhao X, Gao GF, Qi J. Molecular insights into receptor binding of recent emerging SARS-CoV-2 variants. Nat Commun. 2021 Oct 20;12(1):6103.

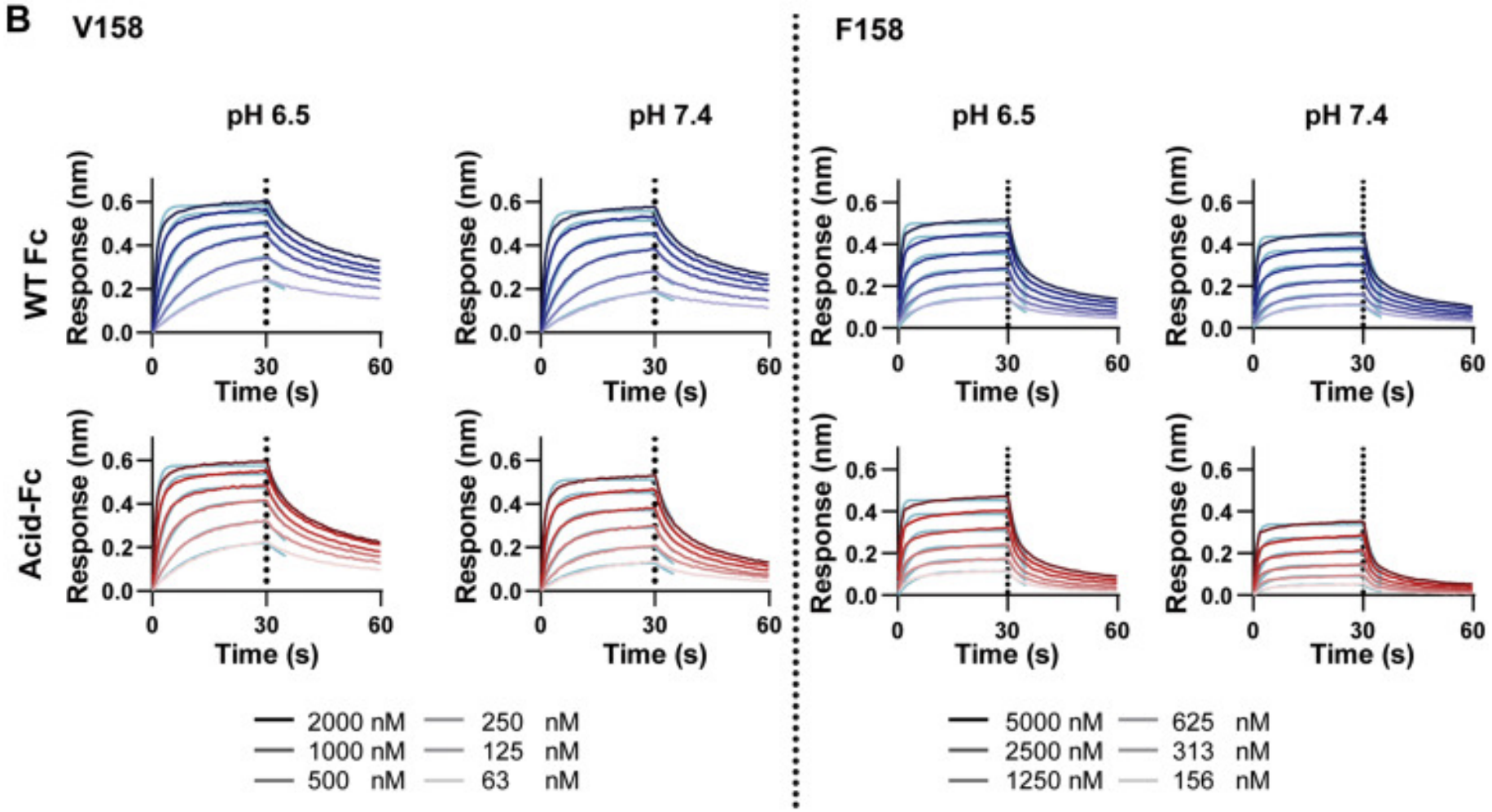
Biolayer interferometry (Octet)





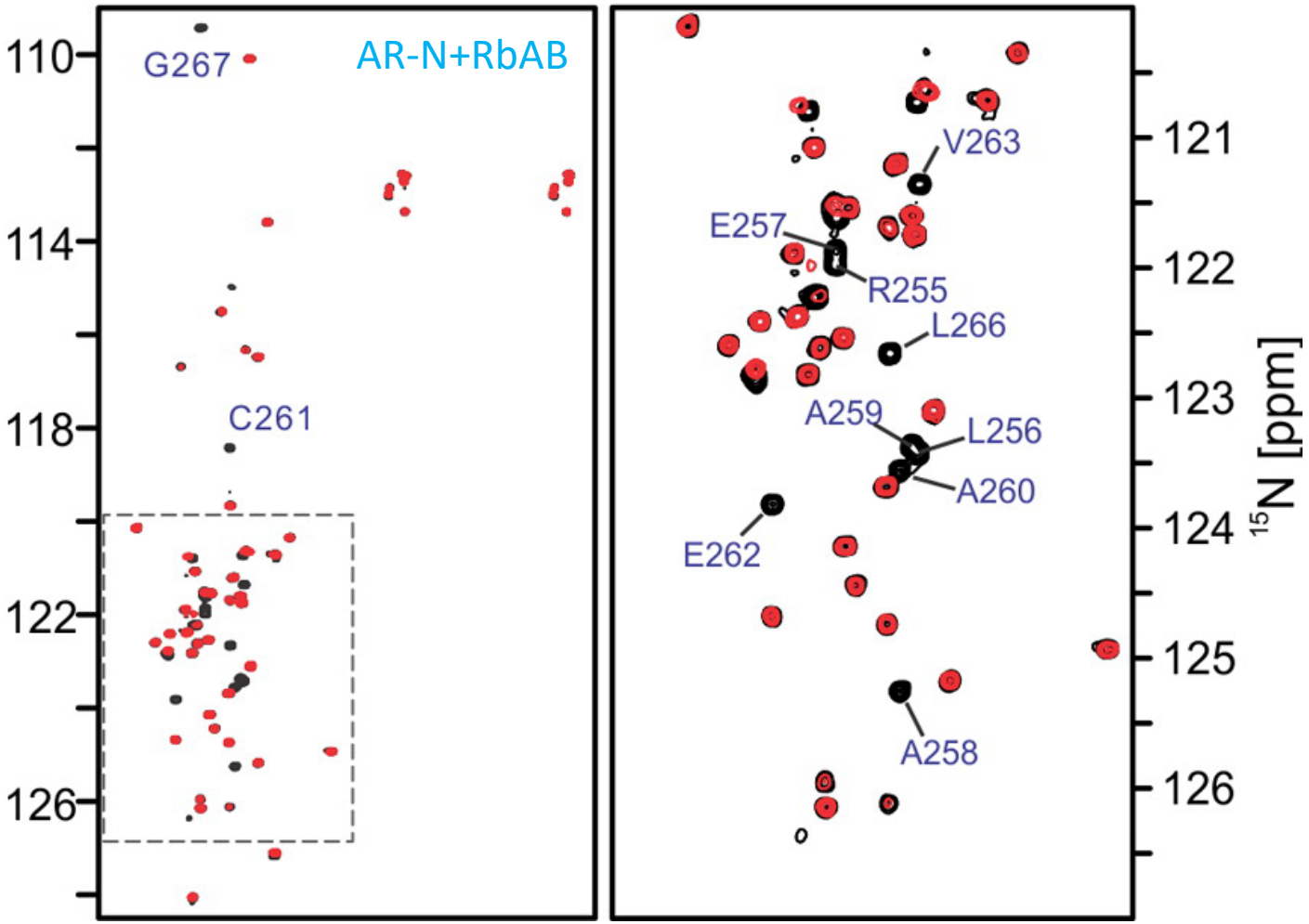
https://en.wikipedia.org/wiki/Bio-layer_interferometry

Biolayer interferometry (Octet)



Liu Y, Lee AG, Nguyen AW, Maynard JA. An antibody Fc engineered for conditional antibody-dependent cellular cytotoxicity at the low tumor microenvironment pH. J Biol Chem. 2022 Apr;298(4):101798. doi: 10.1016/j.jbc.2022.101798.

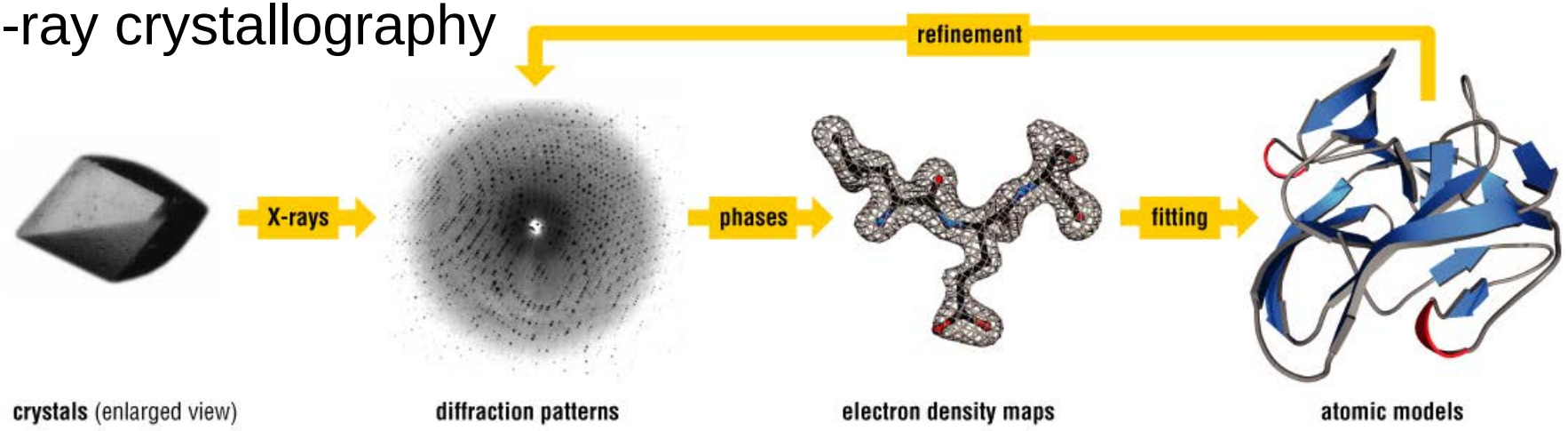
Nuclear magnetic resonance (NMR) spectroscopy



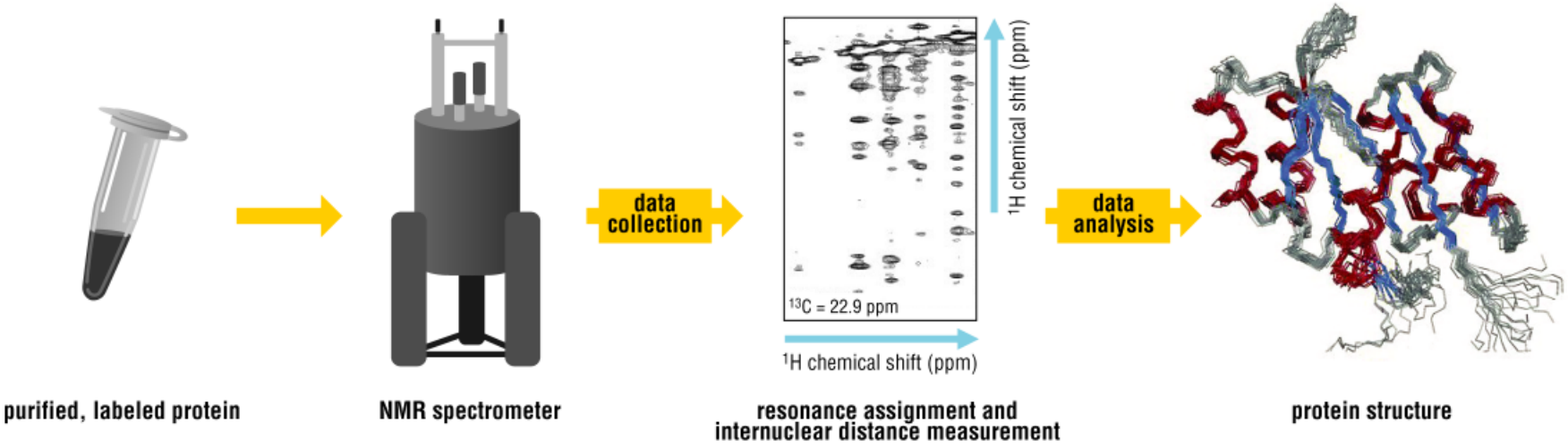
Sun Y, Stine JM, Atwater DZ, Sharmin A, Ross JB, Briknarová K. Structural and functional characterization of the acidic region from the RIZ tumor suppressor. Biochemistry. 2015 Feb 17;54(6):1390-400.

Methods to determine 3D structures of proteins

X-ray crystallography

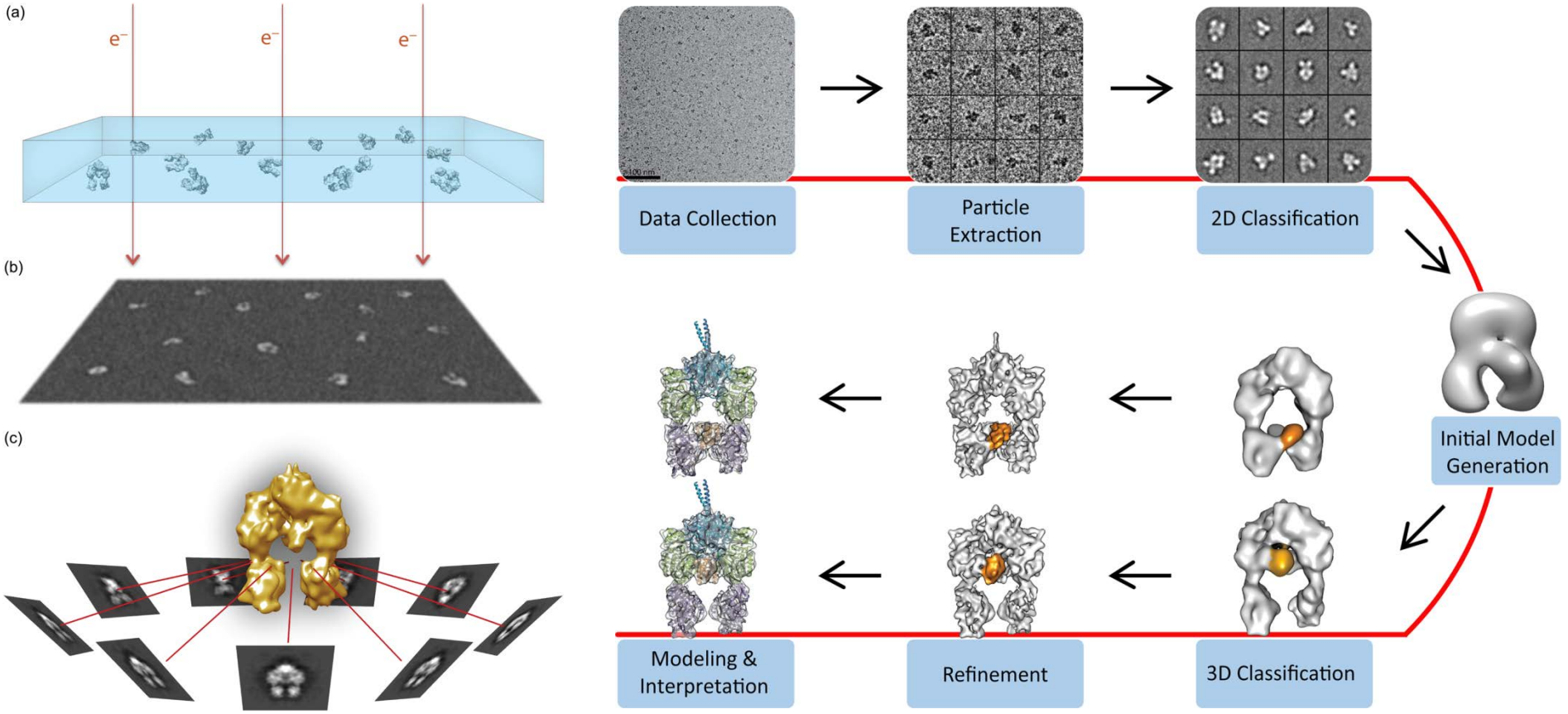


Nuclear magnetic resonance (NMR) spectroscopy



Petsko, G. and Ringe, D. Protein Structure and Function, OUP, 2003

Single-particle cryo-electron microscopy



Skiniotis G, Southworth DR. Single-particle cryo-electron microscopy of macromolecular complexes. Microscopy (Oxf). 2016 Feb;65(1):9-22.