Research in the Iverson laboratory focuses on the basis for information encoding in proteins, which may manifest as molecular recognition and cellular communication. We use several model systems to understanding information encoding.

Signaling in mammals. Cells use signal transduction to sense both external and internal stimuli. Much of eukaryotic signal transduction is mediated via membrane-spanning G protein coupled receptors (GPCRs). We are using visual signal transduction as a model system for GPCR signaling [1-10]. We have identified allosteric connections important for signal initiation [2,4-6] and termination [7-10].

Our largest focus in the laboratory involves signaling mediators called arrestins (**GM120569, DA043680**). Arrestins were first discovered for their ability to bind active, phosphorylated GPCRs and suppress G protein-mediated signaling [11]. Subsequent findings suggested that the receptor-bound form was in an activated state [12] and initiates a second, G protein-independent wave of signaling [13]. The conformations of free (basal [14-17]) and active [18-20] arrestins are quite different (**Fig. 1**). Nevertheless, major questions remain on how activated arrestins promote signaling.

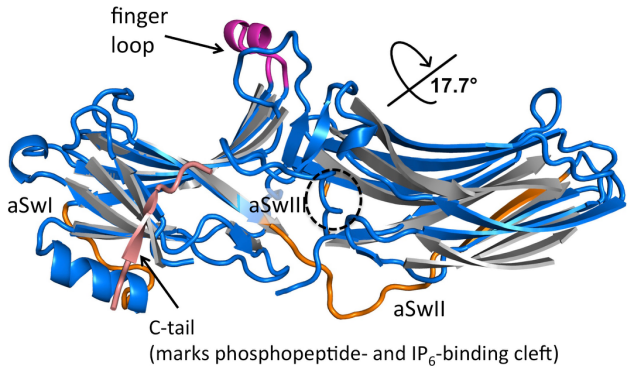

Fig. 1. Arrestin activation. Overlay of the N-domain of IP_6-activated arrestin-3 (*blue*) with the secondary structural elements of basal arrestin-3 (*grey* with a *pink* C-tail) highlights the interdomain rotation. Activating sites include the finger loop, with the active, α-helical conformation in *magenta*, and phosphate binding to the cleft that binds C-tail in the basal conformation. Activation induces previously undescribed conformational changes in the distal aSw regions (*orange*). We hypothesize that this is required for effector activation.

The non-visual arrestins (arrestin-2 and -3) can interact with >100 downstream proteins [21,22], with arrestin-3 uniquely able to scaffold effectors in both a receptor-dependent and a receptor-independent fashion [15,23-29]. These effector proteins include major regulators of cell fate, including mitogen activated protein (MAP) kinases and Src (short for sarcoma) family tyrosine kinases. It is thus perhaps not surprising that arrestin-dependent signaling has been implicated in many central biological processes, such as organ development, cellular remodeling, apoptosis, and learning and memory. Inappropriate arrestin-dependent signaling may contribute to cardiac hypertrophy, parkinsonian dyskinesia, and drug addiction. Exciting recent findings have been in the determination of the structure of the activated form of arrestin-3 (**Fig. 1**). This created a new paradigm for arrestin-mediated signaling [30] that involves “arrestin switch regions”, regions of local conformation change as that are functionally analogous the switch regions of G proteins.

In this context, a major question in the field is how arrestins direct signaling (called signaling bias). Or to put it another way, why do GPCR-bound arrestins promote signaling via the pro-proliferation extracellular receptor kinases (ERKs) and not the pro-apoptotic jun N-terminal kinases (JNKs)? Both of these are MAP kinases and have high sequence identity. One hypothesis in the field is termed the “barcode hypothesis” [31-33], which posits that different phosphorylation patterns of activated GPCR promote different conformations of bound arrestin. Each of those slightly different arrestin conformations then interacts with a different effector. Because arrestins are normally activated only when in complex with a GPCR, the barcode hypothesis has been difficult to validate experimentally as it would require that the arrestin structure is determined when coupled to receptors that have different phosphorylation patterns.

Complex II. Complex II catalyzes the oxidation of succinate to fumarate during the citric acid cycle and passes the two electrons from this reaction to membrane-soluble quinones. Members of the complex II family contain a soluble region consisting of two polypeptide chains (flavoprotein and iron protein), and a poorly-conserved membrane-spanning domain. Quinol-fumarate reductase (QFR) is a complex II homolog that catalyzes the

reduction of fumarate to succinate during anaerobic respiration. I determined this structure (**Fig. 2**), and the architecture both suggests the mechanism of the terminal step of anaerobic fumarate respiration and gives a model for the function of the homologous complex II from mitochondria. The complex exists as a modular enzyme with a linear arrangement of the electron transfer cofactors producing an obvious path between the membrane bound quinone and the active site FAD. My laboratory is using its 15-year expertise on QFR structure and biochemistry [34-43] to extend our knowledge of how bioenergetic proteins function.

We are currently performing several exploratory avenues of research (**GM061606**). The first is how this complicated respiratory protein is assembled, including how the cofactors are inserted. Recent studies have focused on the insertion of the flavin cofactor and the role of newly discovered assembly factors in this process [44-46]. The second avenue of research is how the *E. coli* QFR affects chemotaxis (**Fig. 2**; [34]) Both QFR and its metabolite fumarate are required for cellular locomotion, and QFR controls the onset of tumbling [47]. Our final avenue in this research is focused on the human enzyme, with the long-term goal of understanding how disease-associated mutations affect the biochemical properties of the enzyme. The research on the human enzyme is in the early stages, and we remain focused on methods to express the properly assembled complex.

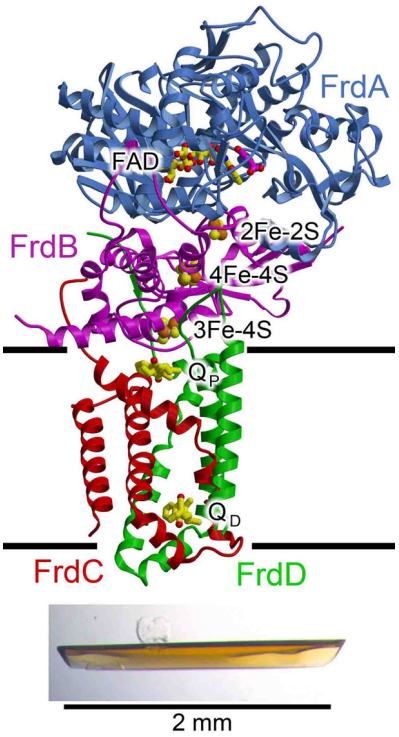

Fig. 2. *E. coli* QFR. The complex contains four polypeptides (FrdABCD). In the figure, the flavoprotein is *cyan*, the iron protein is *red*, and the membrane proteins are colored *green* location of the membrane.

Pathogen-host molecular recognition. Previous investigations in my laboratory focused on how the innate immune system recognizes outer membrane proteins [48-50]. However, our present investigations focus on the interaction between human platelets and the Serine Rich Repeat (SRR) adhesin family (**AI106987**). These proteins are required for the development of infective endocarditis, which is a life threatening infection associated with an in-hospital mortality rate of ~20%, and a 5-year mortality rate of ~40-70%, depending on the causative organism [51]. Indeed, despite prompt therapy, patients of streptococcal endocarditis have a poor prognosis: 50-60% of patients undergo heart failure [52] or progressive valve destruction [53], and ~15% of patients undergo systemic embolization and stroke [54]. Treatment for bacterial endocarditis commonly combines an antibiotic regimen with surgical intervention, with both having shortcomings. Our research investigates the basic mechanisms of bacterial attachment to the host [55-60].

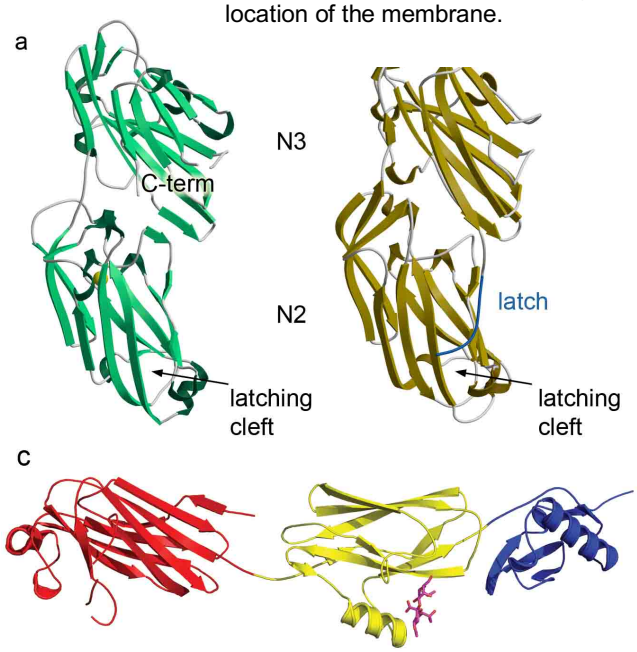

Fig. 3. Structures of SRR adhesins. (**a**) Srr1 from *S. agalactiae*. (**b**) Srr2 from *S. agalactiae*. (**c**) GspB from *S. gordonii* colored by domain.

Summary. Each of these research projects provides complementary information on how proteins encode information into their structures under very different biological settings. This information encoding helps bioenergetics proteins act as primordial signal scaffolds, allows mammalian signaling proteins to use conformations to select between possible outcomes, and allows hosts to recognize pathogens, and vice versa.

Literature cited.

1. Kaya AI, Iverson TM, Hamm HE: **Functional stability of rhodopsin in a bicelle system: Evaluating g protein activation by rhodopsin in bicelles.** *Methods Mol Biol* (2015) **1271**(67-76.

2. Kaya AI, Lokits AD, Gilbert JA, Iverson TM, Meiler J, Hamm HE: **A conserved phenylalanine as a relay between the alpha5 helix and the gdp binding region of heterotrimeric gi protein alpha subunit.** *J Biol Chem* (2014) **289**(35):24475-24487.

3. Kaya AI, Thaker TM, Preininger AM, Iverson TM, Hamm HE: **Coupling efficiency of rhodopsin and transducin in bicelles.** *Biochemistry* (2011) **50**(15):3193-3203.

4. Preininger AM, Funk MA, Oldham WM, Meier SM, Johnston CA, Adhikary S, Kimple AJ, Siderovski DP, Hamm HE, Iverson TM: **Helix dipole movement and conformational variability contribute to allosteric gdp release in galphai subunits.** *Biochemistry* (2009) **48**(12):2630-2642.

5. Thaker TM, Kaya AI, Preininger AM, Hamm HE, Iverson TM: **Allosteric mechanisms of g protein-coupled receptor signaling: A structural perspective.** *Methods Mol Biol* (2012) **796**(133-174.

6. Thaker TM, Sarwar M, Preininger AM, Hamm HE, Iverson TM: **A transient interaction between the p-loop and switch i contributes to the allosteric network between receptor and nucleotide in galphai1.** *J Biol Chem* (2014).

7. Chen Q, Vishnivetskiy SA, Zhuang T, Cho MK, Thaker TM, Sanders CR, Gurevich VV, Iverson TM: **The rhodopsin-arrestin-1 interaction in bicelles.** *Methods Mol Biol* (2015) **1271**(77-95.

8. Vishnivetskiy SA, Chen Q, Palazzo MC, Brooks EK, Altenbach C, Iverson TM, Hubbell WL, Gurevich VV: **Engineering visual arrestin-1 with special functional characteristics.** *J Biol Chem* (2013) **288**(5):3394-3405.

9. Vishnivetskiy SA, Zhan X, Chen Q, Iverson TM, Gurevich VV: **Arrestin expression in e. Coli and purification.** *Curr Protoc Pharmacol* (2014) **67**(Unit 2 11.

10. Zhuang T, Chen Q, Cho MK, Vishnivetskiy SA, Iverson TM, Gurevich VV, Sanders CR: **Involvement of distinct arrestin-1 elements in binding to different functional forms of rhodopsin.** *Proc Natl Acad Sci U S A* (2013) **110**(3):942-947.

11. Carman CV, Benovic JL: **G-protein-coupled receptors: Turn-ons and turn-offs.** *Curr Opin Neurobiol* (1998) **8**(335-344.

12. Gurevich VV, Gurevich EV: **The new face of active receptor bound arrestin attracts new partners.** *Structure* (2003) **11**(9):1037-1042.

13. Gurevich EV, Gurevich VV: **Arrestins are ubiquitous regulators of cellular signaling pathways.** *Genome Biol* (2006) **7**(236.

14. Han M, Gurevich VV, Vishnivetskiy SA, Sigler PB, Schubert C: **Crystal structure of beta-arrestin at 1.9 a: Possible mechanism of receptor binding and membrane translocation.** *Structure* (2001) **9**(9):869-880.

15. Zhan X, Gimenez LE, Gurevich VV, Spiller BW: **Crystal structure of arrestin-3 reveals the basis of the difference in receptor binding between two non-visual subtypes.** *Journal of molecular biology* (2011) **406**(3):467-478.

16. Hirsch JA, Schubert C, Gurevich VV, Sigler PB: **The 2.8 a crystal structure of visual arrestin: A model for arrestin's regulation.** *Cell* (1999) **97**(2):257-269.

17. Sutton RB, Vishnivetskiy SA, Robert J, Hanson SM, Raman D, Knox BE, Kono M, Navarro J, Gurevich VV: **Crystal structure of cone arrestin at 2.3a: Evolution of receptor specificity.** *Journal of molecular biology* (2005) **354**(5):1069-1080.

18. Kim YJ, Hofmann KP, Ernst OP, Scheerer P, Choe HW, Sommer ME: **Crystal structure of pre-activated arrestin p44.** *Nature* (2013) **497**(7447):142-146.

19. Shukla AK, Manglik A, Kruse AC, Xiao K, Reis RI, Tseng WC, Staus DP, Hilger D, Uysal S, Huang LY, Paduch M *et al*: **Structure of active beta-arrestin-1 bound to a g-protein-coupled receptor phosphopeptide.** *Nature* (2013) **497**(7447):137-141.

20. Kang Y, Zhou XE, Gao X, He Y, Liu W, Ishchenko A, Barty A, White TA, Yefanov O, Han GW, Xu Q *et al*: **Crystal structure of rhodopsin bound to arrestin determined by femtosecond x-ray laser.** *Nature* (2015) **523**(7562):561-567.

21. Gurevich VV, Gurevich EV: **Overview of different mechanisms of arrestin-mediated signaling.** *Curr Protoc Pharmacol* (2014) **67**(Unit 2 10.

22. Xiao K, McClatchy DB, Shukla AK, Zhao Y, Chen M, Shenoy SK, Yates JR, 3rd, Lefkowitz RJ: **Functional specialization of beta-arrestin interactions revealed by proteomic analysis.** *Proceedings of the National Academy of Sciences of the United States of America* (2007) **104**(29):12011-12016.

23. McDonald PH, Chow CW, Miller WE, Laporte SA, Field ME, Lin FT, Davis RJ, Lefkowitz RJ: **Beta-arrestin 2: A receptor-regulated mapk scaffold for the activation of jnk3.** *Science* (2000) **290**(5496):1574-1577.

24. Miller WE, McDonald PH, Cai SF, Field ME, Davis RJ, Lefkowitz RJ: **Identification of a motif in the carboxyl terminus of beta -arrestin2 responsible for activation of jnk3.** *The Journal of biological chemistry* (2001) **276**(30):27770-27777.

25. Breitman M, Kook S, Gimenez LE, Lizama BN, Palazzo MC, Gurevich EV, Gurevich VV: **Silent scaffolds: Inhibition of c-jun n-terminal kinase 3 activity in cell by dominant-negative arrestin-3 mutant.** *The Journal of biological chemistry* (2012) **287**(23):19653-19664.

26. Zhan X, Kook S, Gurevich EV, Gurevich VV: **Arrestin-dependent activation of jnk family kinases.** *Handbook of experimental pharmacology* (2014) **219**(259-280.

27. Palczewski K, Pulvermuller A, Buczylko J, Hofmann KP: **Phosphorylated rhodopsin and heparin induce similar conformational changes in arrestin.** *The Journal of biological chemistry* (1991) **266**(28):18649-18654.

28. Gurevich VV, Chen CY, Kim CM, Benovic JL: **Visual arrestin binding to rhodopsin. Intramolecular interaction between the basic n terminus and acidic c terminus of arrestin may regulate binding selectivity.** *The Journal of biological chemistry* (1994) **269**(12):8721-8727.

29. Zhuang T, Vishnivetskiy SA, Gurevich VV, Sanders CR: **Elucidation of inositol hexaphosphate and heparin interaction sites and conformational changes in arrestin-1 by solution nuclear magnetic resonance.** *Biochemistry* (2010) **49**(49):10473-10485.

30. Chen Q, Perry NA, Vishnivetskiy SA, Berndt S, Gilbert NC, Zhuo Y, Singh PK, Tholen J, Ohi MD, Gurevich EV, Brautigam CA *et al*: **Structural basis of arrestin-3 activation and signaling.** *Nature communications* (2017) **8**(1):1427.

31. Nobles KN, Xiao K, Ahn S, Shukla AK, Lam CM, Rajagopal S, Strachan RT, Huang TY, Bressler EA, Hara MR, Shenoy SK *et al*: **Distinct phosphorylation sites on the beta(2)-adrenergic receptor establish a barcode that encodes differential functions of beta-arrestin.** *Science signaling* (2011) **4**(185):ra51.

32. Tobin AB, Butcher AJ, Kong KC: **Location, location, location...Site-specific gpcr phosphorylation offers a mechanism for cell-type-specific signalling.** *Trends in pharmacological sciences* (2008) **29**(8):413-420.

33. Tobin AB: **G-protein-coupled receptor phosphorylation: Where, when and by whom.** *British journal of pharmacology* (2008) **153 Suppl 1**(S167-176.

34. Iverson TM, Luna-Chavez C, Cecchini G, Rees DC: **Structure of the escherichia coli fumarate reductase respiratory complex.** *Science* (1999) **284**(5422):1961-1966.

35. Iverson TM, Luna-Chavez C, Croal LR, Cecchini G, Rees DC: **Crystallographic studies of the escherichia coli quinol-fumarate reductase with inhibitors bound to the quinol-binding site.** *J Biol Chem* (2002) **277**(18):16124-16130.

36. Iverson TM, Luna-Chavez C, Schroder I, Cecchini G, Rees DC: **Analyzing your complexes: Structure of the quinol-fumarate reductase respiratory complex.** *Curr Opin Struct Biol* (2000) **10**(4):448-455.

37. Iverson TM, Maklashina E, Cecchini G: **Structural basis for malfunction in complex ii.** *J Biol Chem* (2012) **287**(42):35430-35438.

38. Luna-Chavez C, Iverson TM, Rees DC, Cecchini G: **Overexpression, purification, and crystallization of the membrane-bound fumarate reductase from escherichia coli.** *Protein Expr Purif* (2000) **19**(1):188-196.

39. Maklashina E, Iverson TM, Sher Y, Kotlyar V, Andrell J, Mirza O, Hudson JM, Armstrong FA, Rothery RA, Weiner JH, Cecchini G: **Fumarate reductase and succinate oxidase activity of escherichia coli complex ii homologs are perturbed differently by mutation of the flavin binding domain.** *J Biol Chem* (2006) **281**(16):11357-11365.

40. Singh PK, Sarwar M, Maklashina E, Kotlyar V, Rajagukguk S, Tomasiak TM, Cecchini G, Iverson TM: **Plasticity of the quinone-binding site of the complex ii homolog quinol:Fumarate reductase.** *J Biol Chem* (2013) **288**(34):24293-24301.

41. Tomasiak TM, Archuleta TL, Andrell J, Luna-Chavez C, Davis TA, Sarwar M, Ham AJ, McDonald WH, Yankovskaya V, Stern HA, Johnston JN *et al*: **Geometric restraint drives on- and off-pathway catalysis by the escherichia coli menaquinol:Fumarate reductase.** *J Biol Chem* (2011) **286**(4):3047-3056.

42. Tomasiak TM, Maklashina E, Cecchini G, Iverson TM: **A threonine on the active site loop controls transition state formation in escherichia coli respiratory complex ii.** *J Biol Chem* (2008) **283**(22):15460-15468.

43. Iverson TM: **Catalytic mechanisms of complex ii enzymes: A structural perspective.** *Biochim Biophys Acta* (2013) **1827**(5):648-657.

44. Maklashina E, Rajagukguk S, Starbird CA, McDonald WH, Koganitsky A, Eisenbach M, Iverson TM, Cecchini G: **Binding of the covalent flavin assembly factor to the flavoprotein subunit of complex ii.** *The Journal of biological chemistry* (2016) **291**(6):2904-2916.

45. Starbird CA, Tomasiak TM, Singh PK, Yankovskaya V, Maklashina E, Eisenbach M, Cecchini G, Iverson TM: **New crystal forms of the integral membrane escherichia coli quinol:Fumarate reductase suggest that ligands control domain movement.** *Journal of structural biology* (2018) **202**(1):100-104.

46. Sharma P, Maklashina E, Cecchini G, Iverson TM: **Crystal structure of an assembly intermediate of respiratory complex ii.** *Nature communications* (2018) **9**(1):274.

47. Cohen-Ben-Lulu GN, Francis NR, Shimoni E, Noy D, Davidov Y, Prasad K, Sagi Y, Cecchini G, Johnstone RM, Eisenbach M: **The bacterial flagellar switch complex is getting more complex.** *Embo J* (2008) **27**(7):1134-1144.

48. Tanabe M, Iverson TM: **Expression, purification and preliminary x-ray analysis of the neisseria meningitidis outer membrane protein porb.** *Acta Crystallogr Sect F Struct Biol Cryst Commun* (2009) **65**(Pt 10):996-1000.

49. Tanabe M, Iverson TM: **A practical guide to x-ray crystallography of β-barrel membrane proteins: Expression, purification, detergent selection & crystallization.** In: *Current topics in membranes.* 63. DeLucas L (Ed) Academic Press, San Diego, CA (2009):229-267.

50. Tanabe M, Nimigean CM, Iverson TM: **Structural basis for solute transport, nucleotide regulation, and immunological recognition of neisseria meningitidis porb.** *Proc Natl Acad Sci U S A* (2010) **107**(15):6811-6816.

51. Hoen B, Duval X: **Clinical practice. Infective endocarditis.** *The New England journal of medicine* (2013) **368**(15):1425-1433.

52. Bashore TM, Cabell C, Fowler V, Jr.: **Update on infective endocarditis.** *Current problems in cardiology* (2006) **31**(4):274-352.

53. Tornos P, Almirante B, Olona M, Permanyer G, Gonzalez T, Carballo J, Pahissa A, Soler-Soler J: **Clinical outcome and long-term prognosis of late prosthetic valve endocarditis: A 20-year experience.** *Clin Infect Dis* (1997) **24**(3):381-386.

54. Jones HR, Jr., Siekert RG: **Neurological manifestations of infective endocarditis. Review of clinical and therapeutic challenges.** *Brain : a journal of neurology* (1989) **112 (Pt 5)**(1295-1315.

55. Bensing BA, Khedri Z, Deng L, Yu H, Prakobphol A, Fisher SJ, Chen X, Iverson TM, Varki A, Sullam PM: **Novel aspects of sialoglycan recognition by the siglec-like domains of streptococcal srr glycoproteins.** *Glycobiology* (2016) **26**(11):1222-1234.

56. Bensing BA, Loukachevitch LV, McCulloch KM, Yu H, Vann KR, Wawrzak Z, Anderson S, Chen X, Sullam PM, Iverson TM: **Structural basis for sialoglycan binding by the streptococcus sanguinis srpa adhesin.** *The Journal of biological chemistry* (2016) **291**(14):7230-7240.

57. Loukachevitch LV, Bensing BA, Yu H, Zeng J, Chen X, Sullam PM, Iverson TM: **Structures of the streptococcus sanguinis srpa binding region with human sialoglycans suggest features of the physiological ligand.** *Biochemistry* (2016).

58. Pyburn TM, Bensing BA, Xiong YQ, Melancon BJ, Tomasiak TM, Ward NJ, Yankovskaya V, Oliver KM, Cecchini G, Sulikowski GA, Tyska MJ *et al*: **A structural model for binding of the serine-rich repeat adhesin gspb to host carbohydrate receptors.** *PLoS pathogens* (2011) **7**(7):e1002112.

59. Pyburn TM, Yankovskaya V, Bensing BA, Cecchini G, Sullam PM, Iverson TM: **Purification, crystallization and preliminary x-ray diffraction analysis of the carbohydrate-binding region of the streptococcus gordonii adhesin gspb.** *Acta crystallographica Section F, Structural biology and crystallization communications* (2010) **66**(Pt 11):1503-1507.

60. Seo HS, Minasov G, Seepersaud R, Doran KS, Dubrovska I, Shuvalova L, Anderson WF, Iverson TM, Sullam PM: **Characterization of fibrinogen binding by glycoproteins srr1 and srr2 of streptococcus agalactiae.** *The Journal of biological chemistry* (2013) **288**(50):35982-35996.