Monolith Protocol MO-P-075

IgG – FcRn

IgG is a significant antibody class found in the blood. In addition to its importance for the immune system, IgG subclasses also display a remarkable persistence with a half-life of 20-23 days, except IgG3 that has a half-life of just 7 days. The extended half-life is due to the large MW of IgGs and to their interactions with the neonatal Fc receptor (FcRn). FcRn is an MHC class I-like molecule that consists of a heavy transmembrane chain (HC) that non-covalently associates with β2-microglobulin. The IgG and FcRn interaction occurs in a strictly pH-dependent manner with negligible binding and release in neutral pH. In this protocol the binding between human IgG1 and the IgG receptor (FcRn) was investigated.

antibody – receptor interaction | IgG | FcRn | pharmacokinetics | IgG half-life

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A1. Target/Fluorescent Molecule

Human Immunoglobulin G1, hIgG1 heavy chain (hIgG1)
uniprot.org/uniprot/P0DOX5

A2. Molecule Class/Organism

Monoclonal antibody
Homo sapiens (Human)

A3. Sequence/Formula

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QVQLQQPGAE LVKPGASVKL SCKASGYTFT SYWMHWVKQR PGRGLEWIGR IDPNSGGTKY NEKFKSKATL TVDKPSSTAY
MQLSSLTSED SAVYYCARYD YYGSSYFDYW GQGTTLTVSS ASTKGPSVFP LAPSSKSTSG GTAALGCLVK DYFPEPVTVS
WNSGALTSGV HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT YICNVNHKPS NTKVDKKVEP KSCDKTHTCP PCPAPELLGG
PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN STYRVVSVLT VLHQDWLNGK
EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPSRDE LTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV
LDSDGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPGK
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A4. Purification Strategy/Source

A vector encoding the hIgG1 HC with specificity for NIP were transiently transfected into HEK293E cells using Lipofectamine 2000 as described by the manufacturer (Life Technologies). Detailed strategy for vector design can be found elsewhere[1]. Production of an anti-NIP hIgG1-IHH variant was done using the J558L murine myeloma cell line stably transfected with pLNOH2-NIPhIgG1-IHH, which also constitutively expresses the anti-NIP mouse λ light chain[2]. Growth medium was harvested and replaced every second day for 2 weeks prior to purification using a CaptureSelectTM pre-packed anti-hIgG-CH1 column (Life Technologies) as described by the manufacturer. The collected proteins were concentrated, and the buffer was exchanged to phosphate-buffered saline (PBS) (Sigma-Aldrich) using Amicon Ultra-15 ml 50K columns (Millipore) prior to the size exclusion chromatography using a Superdex 200 increase 10/300GL column (GE Healthcare) and an ÄKTA FPLC instrument (GE Healthcare). The monomeric fractions were concentrated using Amicon Ultra-0.5 ml 100K columns (Millipore).

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[1] See Norderhaug et al., J Immunol Meth 204(1): 77-87 (1997) for detailed protocol.

[2] See Grevys et al., Nat Commun. 9(1): 621 (2018) for more information.

A5. Stock Concentration/Stock Buffer

6 mg/mL | 41 µM
10 mM PBS, pH 7.4, 140 mM NaCl

A6. Molecular Weight/Extinction Coefficient

146 kDa

A7. Dilution Buffer

6 mM Na_2HPO_4, 94 mM NaH_2PO_4, pH 5.5, 150 mM NaCl, 0.01 % TWEEN® 20

A8. Labeling Strategy

Monolith Protein Labeling Kit RED – NHS (MO-L001, NanoTemper Technologies GmbH)
1* Labeling Buffer NHS | 1* Dye RED-NHS (10 µg) | 1* B-Column

A9. Labeling Procedure

1. Prepare 50 µL of a 40 µM IgG solution. Then, add 50 µL of Labeling Buffer NHS to obtain 100 µL of a 20 µM IgG solution.
2. Add 30 µL of DMSO to Dye RED-NHS (10 µg) to obtain a ~470 µM solution. Mix the dye thoroughly by vortexing and make sure that all dye is dissolved.
3. Prepare a new PCR tube and mix 12.8 µL of the ~470 µM dye solution with 87.2 µL of Labeling Buffer NHS to obtain 100 µL of a 60 µM dye solution (3x protein concentration).
4. Mix hIgG1 and dye in a 1:1 volume ratio (200 µL final volume, 6.4% final DMSO concentration).
5. Incubate for 30 minutes at room temperature in the dark.
6. In the meantime, remove the top cap of the B-Column and pour off the storage solution. Remove the bottom cap and place with adapter in a 15 mL tube.
7. Fill the column with dilution buffer and allow it to enter the packed resin bed completely by gravity flow. Discard the flow through collected. Repeat this step 3 more times.
8. Add 200 µL of the labeling reaction from step 4 to the center of the column and let sample enter the bed completely.
9. Add 300 µL of dilution buffer after the sample has entered and discard the flow through.
10. Place column in a new collection tube, add 600 µL of dilution buffer and collect the eluate.
11. Keep the labeled hIgG1 (~2.7 µM) on ice in the dark.

A10. Labeling Efficiency

Measurement of protein concentration and degree of labeling (DOL) using a NanoDrop™:
nanotempertech.com/dol-calculator

Absorbance A_{280}	0.583	Protein concentration	2.7 µM
Absorbance A_{650}	0.643	Degree-of-labeling (DOL)	0.96

B1. Ligand/Non-Fluorescent Binding Partner

IgG Receptor – FcRn, large subunit p51
uniprot.org/uniprot/P55899

B2. Molecule Class/Organism

Membrane receptor
Homo sapiens (Human)

B3. Sequence/Formula

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AESHLSLLYH LTAVSSPAPG TPAFWVSGWL GPQQYLSYNS LRGEAEPSGA WVWENQVSWY WEKETTDLRI KEKLFLEAFK
ALGGKGPYTL QGLLGCELGP DNTSVPTAKF ALNGEEFMNF DLKQGTWGGD WPEALAISQR WQQQDKAANK ELTFLLFSCP
HRLREHLERG RGNLEWKEPP SMRLKARPSS PGFSVLTCSA FSFYPPELQL RFLRNGLAAG TGQGDFGPNS DGSFHASSSL
TVKSGDEHHY SCIVQHAGLA QPLRVEL
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B4. Purification Strategy/Source

Truncated monomeric His-tagged hFcRn was produced using a Baculovirus expression system. Details about the vector design and purification strategies could be found elsewhere[3].

Briefly, 500 ml of high five cells were cultured at 1×10^6 cells/mL at 27°C with gentle agitation (160 rpm). Cells were infected with 1 mL virus stock (Autographica californica nuclear polyhedrosis virus harbouring the pAxUW51 plasmid encoding cDNA of the three extracellular domains of hFcRn HC fused with a His_6-tag and human β2-microglobulin). After 72 h at 24°C post infection, the supernatant was harvested, and the receptor was purified using a HisTrap HP column supplied with Ni^{2+} ions (GE healthcare). After equilibration of the column with PBS containing 0.05% sodium azide, the supernatant was adjusted to pH 7.2 and applied on the column with a flow rate of 5 mL/min. The column was then washed with 200 mL PBS followed by 50 mL PBS containing 25 mM imidazole pH 7.3, and bound hFcRn was eluted with 50 mL PBS containing 250 mM imidazole. Amicon Ultra 10K columns (Millipore) were used for concentration of the purified protein and the buffer was changed to PBS (Sigma-Aldrich) prior to size exclusion chromatography using a HiLoad 26/600 Superdex 200 prep grade column (GE Healthcare) coupled to an ÄKTA FPLC instrument (GE Healthcare). Monomeric receptor was concentrated by Amicon Ultra-0.5 ml 10K columns (Millipore) and stored at 4°C.

B5. Stock Concentration/Stock Buffer

10 mg/mL | 238 µM
PBS, pH 7.4, 140 mM NaCl

B6. Molecular Weight/Extinction Coefficient

42.1 kDa
85,745 $M^{-1}cm^{-1}$ (ε_{280})

[3] See Sand et al., J Biol Chem 289(24): 17228-39 (2014) for more information.

B7. Serial Dilution Preparation

1. Prepare a PCR-rack with 16 PCR tubes. Transfer 20 µL of a 120 µM hFcRn solution into tube **1**. Then, transfer 10 µL of dilution buffer into tubes **2** to **16**.
2. Prepare a 1:1 serial dilution by transferring 10 µL from tube to tube. Mix carefully by pipetting up and down. Remember to discard 10 µL from tube **16** to get an equal volume of 10 µL for all samples.
3. Mix 3 µL of labeled hIgG1 (~2.7 µM) with 197 µL of dilution buffer to obtain 200 µL of ~40 nM hIgG1.
4. Add 10 µL of labeled hIgG1 (~40 nM) to each tube from **16** to **1** and mix by pipetting[4].
5. Incubate for 10 minutes before loading capillaries.

D1. MST System/Capillaries

Monolith NT.115 Red (NanoTemper Technologies GmbH)
Premium Capillaries Monolith NT.115 (MO-K025, NanoTemper Technologies GmbH)

D2. MST Software

MO.Control v1.6 (NanoTemper Technologies GmbH)
nanotempertech.com/monolith-mo-control-software

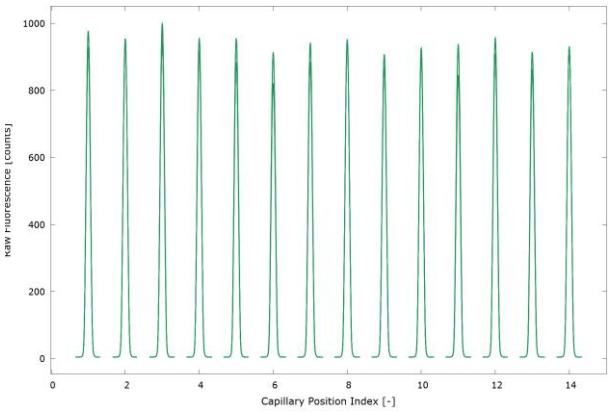
D3. MST Experiment (Assay Buffer/Concentrations/Temperature/MST Power/Excitation Power)

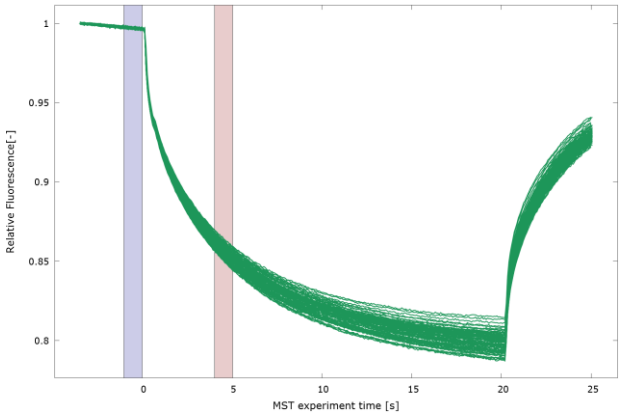
6 mM Na_2HPO_4, 94 mM NaH_2PO_4, pH 5.5, 150 mM NaCl, 0.01 % TWEEN® 20
20 nM hIgG1 | 60 µM – 0.3 nM hFcRn | 25°C | medium MST power | 40% excitation power

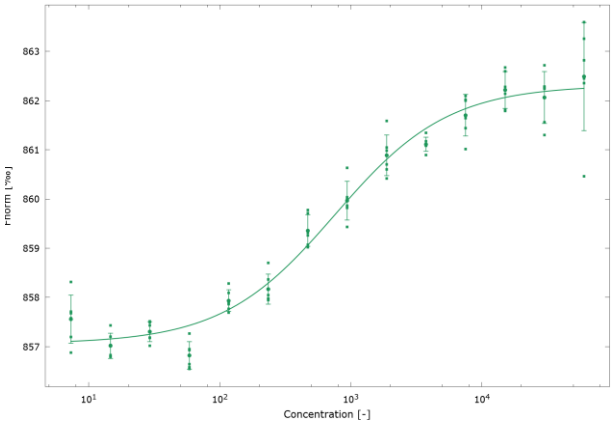
[4] To have a homogeneous buffer in the experiments after mixing FcRn with IgG1, gel filtration has been chosen as a buffer exchange method (see A4). This was particular important when using the acidic buffer.

D4. MST Results (Capillary Scan/Time Traces/Dose Response)

K_d = 964 ± 128 nM







D5. Reference Results/Supporting Results

K_d = 800 nM Surface Plasmon Resonance (SPR)
Borrok et al., J. Biol. Chem. 290, 4282–4290 (2015)

E. Contributors

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