*Supporting information*

# Regulation of innate immune system by fragmented heparin-conjugated lipid on lipid bilayer membranes *in vitro*

Anna Adler[1], Marlene Fritsch[1,2], Karin Fromell[1], Gero Leneweit[3,4], Kristina N. Ekdahl[1,5], Bo Nilsson[1], and Yuji Teramura[1,6,7*]

[1]Department of Immunology, Genetics and Pathology (IGP), Uppsala University, Dag Hammarskjölds väg 20, SE-751 85, Uppsala, Sweden.

[2]ABNOBA GmbH, Pforzheim, Germany.

[3]Carl Gustav Carus-Institute, Association for the Promotion of Cancer Therapy, Niefern-Öschelbronn, Germany.

[4]Linnaeus Center of Biomaterials Chemistry, Linnaeus University, SE-391 82 Kalmar, Sweden.

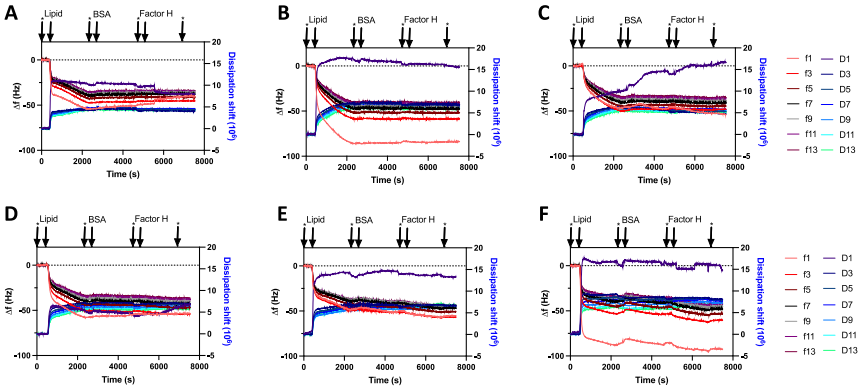
[5]Cellular and Molecular Biotechnology Research Institute (CMB), National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba Central fifth, 1-1-1 Higashi, Tsukuba, Ibaraki 305-8565, Japan.

[6]Master's/Doctoral Program in Life Science Innovation (T-LSI), University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8577, Japan

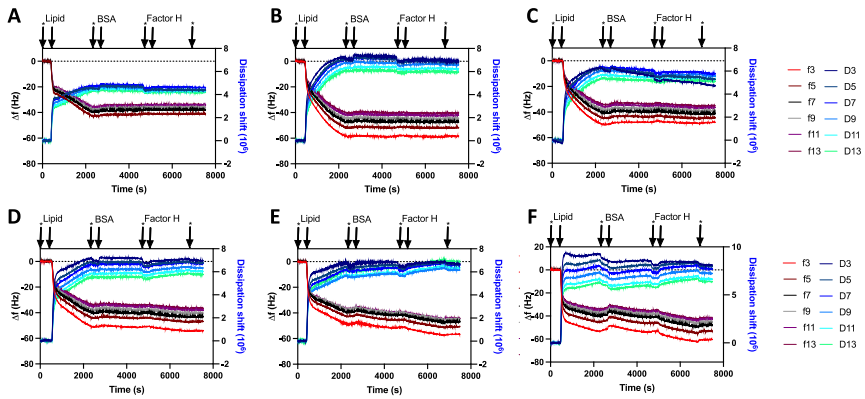
[*]**Corresponding Author**

Yuji TERAMURA
Tel: +81(0)29-861-6582

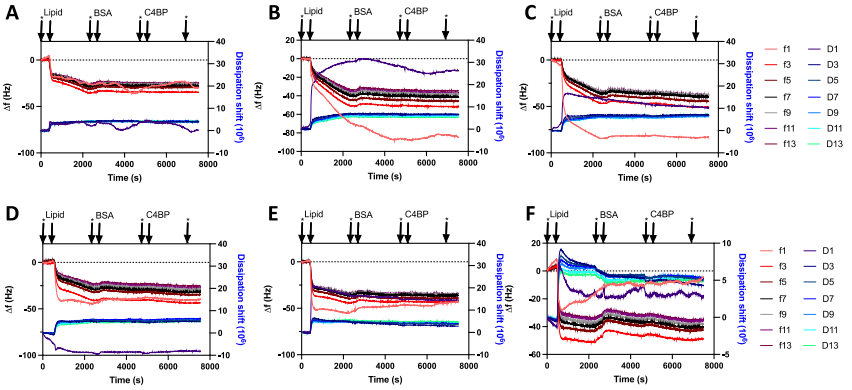
E-mail: y.teramura@aist.go.jp



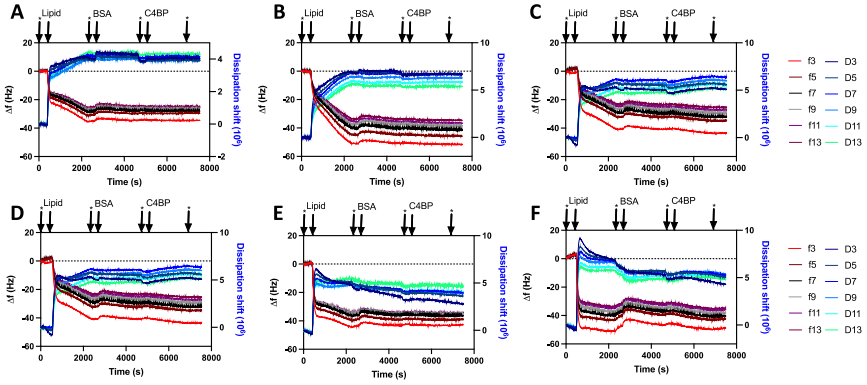

**Supplement Fig. S1.** QCM-D analysis of interaction between MeO-PEG-lipid or fragmented-heparin conjugated lipids (fHep-lipids) with 50 µg/mL complement regulator factor H. BSA (1 mg/mL) was used to block for unspecific binding to the sensors. Representative complete sensorgrams of: **A.** MeO-PEG-lipid, **B.** fHep-C-lipid, **C.** fHep-K1C-lipid, **D.** fHep-K2C-lipid, **E.** fHep-K4C-lipid, and **F.** fHep-K8C-lipid. The washing steps with PBS are indicated with an asterisk (*). For all experiments n = 3.



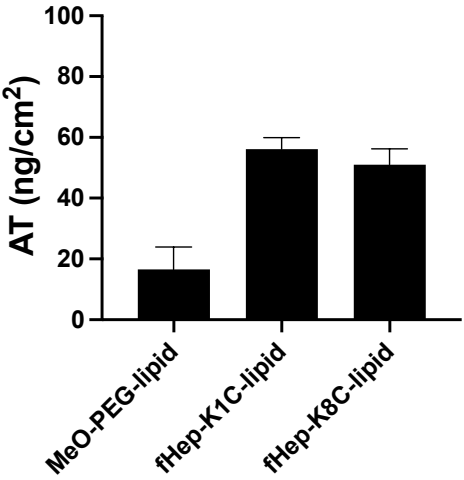

**Supplement Fig. S2.** QCM-D analysis of interaction between MeO-PEG-lipid or fragmented-heparin conjugated lipids (fHep-lipids) with 50 µg/mL complement regulator factor H. BSA (1 mg/mL) was used to block for unspecific binding to the sensors. Representative complete sensorgrams of: **A.** MeO-PEG-lipid, **B.** fHep-C-lipid, **C.** fHep-K1C-lipid, **D.** fHep-K2C-lipid, **E.** fHep-K4C-lipid, and **F.** fHep-K8C-lipid. The washing steps with PBS are indicated with an asterisk (*). For all experiments n = 3. **Note: the overtone for frequency 1 and dissipation 1 is removed, due to a defect in overtone 1 observed in all runs, see Fig. S1A-F.**



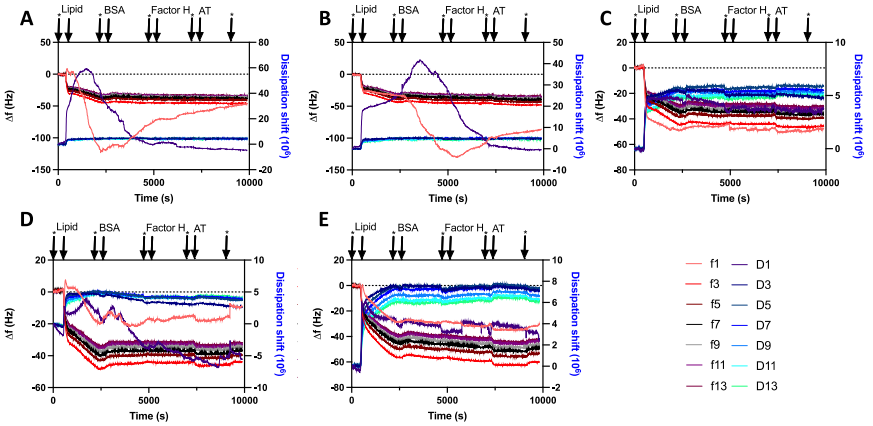

**Supplement Fig. S3.** QCM-D analysis of interaction between 0.1 mg/mL MeO-PEG-lipid or fHep-lipids and 10 µg/mL complement regulator C4b-binding protein (C4BP). Representative complete sensorgrams of: **A.** MeO-PEG-lipid, **B.** fHep-C-lipid, **C.** fHep-K1C-lipid, **D.** fHep-K2C-lipid, **E.** fHep-K4C-lipid, and **F.** fHep-K8C-lipid. The washing steps with PBS are indicated with an asterisk (*). For all experiments n = 3.



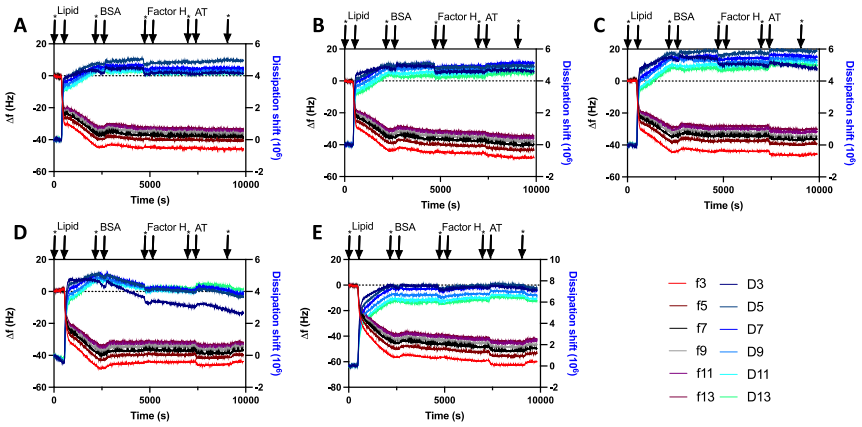

**Supplement Fig. S4.** QCM-D analysis of interaction between 0.1 mg/mL MeO-PEG-lipid or fHep-lipids and 10 µg/mL complement regulator C4b-binding protein (C4BP). Representative complete sensorgrams of: **A.** MeO-PEG-lipid, **B.** fHep-C-lipid, **C.** fHep-K1C-lipid, **D.** fHep-K2C-lipid, **E.** fHep-K4C-lipid, and **F.** fHep-K8C-lipid. The washing steps with PBS are indicated with an asterisk (*). For all experiments n = 3. **Note: the overtone for frequency 1 and dissipation 1 is removed, due to a defect in overtone 1 in observed all runs, see Fig S3A-F.**



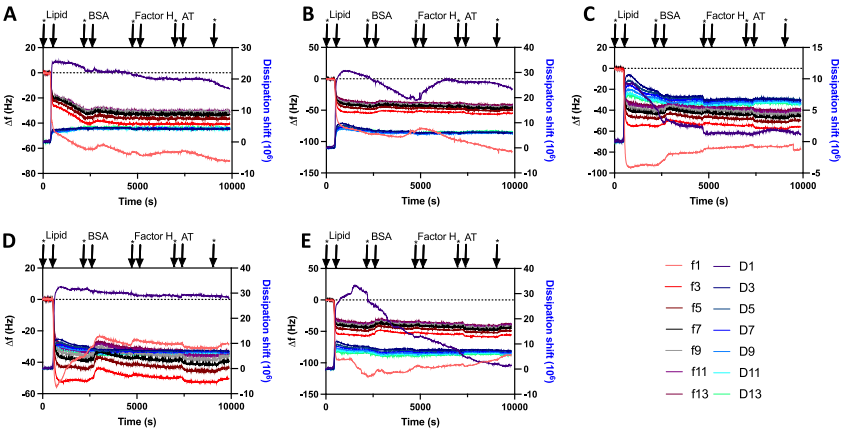

**Supplement Fig. S5.** QCM-D analysis of interaction between MeO-PEG-lipid or fragmented-heparin conjugated lipids (fHep-lipids) fHep-K1C-lipid or fHep-K8C-lipid with 25 µg/mL human antithrombin (AT). Quantification of amount of bound AT to each of the lipids (n = 3).



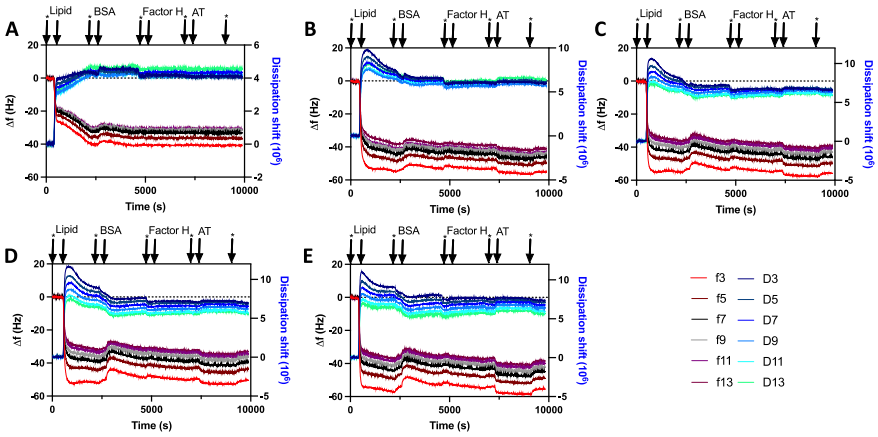

**Supplement Fig. S6.** QCM-D analysis of different ratios of fragmented-heparin conjugated lipids (fHep-lipids):MeO-PEG-lipids (0:100, 25:75, 50:50, 75:25 and 100:0) followed by flowing of 25 µg/mL factor H and then 25 µg/mL antithrombin (AT). Representative complete sensorgrams of: **A.** 0:100 molar ratio fHep-K1C-lipid:MeO-PEG-lipid, **B.** 25:75 molar ratio fHep-K1C-lipid:MeO-PEG-lipid, **C.** 50:50 molar ratio fHep-K1C-lipid:MeO-PEG-lipid, **D.** 75:25 molar ratio fHep-K1C-lipid:MeO-PEG-lipid, and **E.** 100:0 molar ratio fHep-K1C-lipid:MeO-PEG-lipid. The washing steps with PBS are indicated with an asterisk (*). For all experiments n = 3.



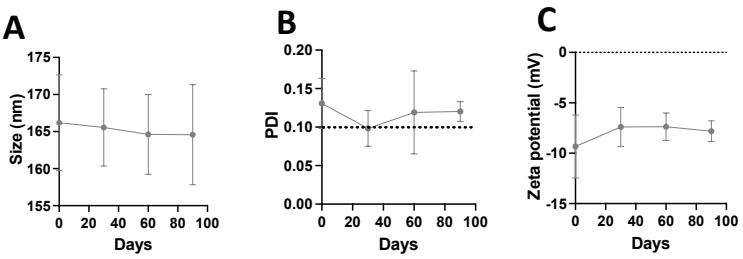

**Supplement Fig. S7.** QCM-D analysis of different ratios of fragmented-heparin conjugated lipids (fHep-lipids):MeO-PEG-lipids (0:100, 25:75, 50:50, 75:25 and 100:0) followed by flowing of 25 µg/mL factor H and then 25 µg/mL antithrombin (AT). Representative complete sensorgrams of: **A.** 0:100 molar ratio fHep-K1C-lipid:MeO-PEG-lipid, **B.** 25:75 molar ratio fHep-K1C-lipid:MeO-PEG-lipid, **C.** 50:50 molar ratio fHep-K1C-lipid:MeO-PEG-lipid, **D.** 75:25 molar ratio fHep-K1C-lipid:MeO-PEG-lipid, and **E.** 100:0 molar ratio fHep-K1C-lipid:MeO-PEG-lipid. The washing steps with PBS are indicated with an asterisk (*). For all experiments n = 3. **Note: the overtone for frequency 1 and dissipation 1 are removed, due to a defect in overtone 1 observed in all runs, see Fig. S6A-F.**




**Supplement Fig. S8.** QCM-D analysis of different ratios of fragmented-heparin conjugated lipids (fHep-lipids):MeO-PEG-lipids (0:100, 25:75, 50:50, 75:25 and 100:0) followed by flowing of 25 µg/mL factor H and then 25 µg/mL antithrombin (AT). Representative sensorgrams of: **A.** 0:100 molar ratio fHep-K8C-lipid:MeO-PEG-lipid, **B.** 25:75 molar ratio fHep-K8C-lipid:MeO-PEG-lipid, **C.** 50:50 molar ratio fHep-K8C-lipid:MeO-PEG-lipid, **D.** 75:25 molar ratio fHep-K8C-lipid:MeO-PEG-lipid, and **E.** 100:0 molar ratio fHep-K8C-lipid:MeO-PEG-lipid. The washing steps with PBS are indicated with an asterisk (*). For all experiments n = 3.




**Supplement Fig. S9.** QCM-D analysis of different ratios of fragmented-heparin conjugated lipids (fHep-lipids):MeO-PEG-lipids (0:100, 25:75, 50:50, 75:25 and 100:0) followed by flowing of 25 µg/mL factor H and then 25 µg/mL antithrombin (AT). Representative complete sensorgrams of: **A.** 0:100 molar ratio fHep-K8C-lipid:MeO-PEG-lipid, **B.** 25:75 molar ratio fHep-K8C-lipid:MeO-PEG-lipid, **C.** 50:50 molar ratio fHep-K8C-lipid:MeO-PEG-lipid, **D.** 75:25 molar ratio fHep-K8C-lipid:MeO-PEG-lipid, and **E.** 100:0 molar ratio fHep-K8C-lipid:MeO-PEG-lipid. The washing steps with PBS are indicated with an asterisk (*). For all experiments n = 3. **Note: the overtone for frequency 1 and dissipation 1 are removed, due to a defect in overtone 1 observed in all runs, see Fig S8A-F.**




**Supplement Fig. S10.** Long-term stability test (90 days) by DLS analysis of non-modified 60:40 mol% DPPC:cholesterol liposomes stored in MQ-$H_2O$ at 4 °C. The liposomes were diluted 1/100 in 1 mM NaCl aqueous solution **A.** Size, **B.**, PDI and **C.** zeta potential (n = 4).