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How virus size and attachment parameters affect the temperature sensitivity of virus binding to host cells: Predictions of a thermodynamic model for arboviruses and HIV

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A R T I C L E   I N F O

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A B S T R A C T

Virus binding to host cells involves specific interactions between viral (glyco)proteins (GP) and host cell surface receptors (Cr) (protein or sialic acid (SA)). The magnitude of the enthalpy of association changes with temperature according to the change in heat capacity (ΔCp) on GP/Cr binding, being little affected for avian influenza virus (AIV) haemagglutinin (HA) binding to SA (ΔCp = 0 kJ/mol/K) but greatly affected for HIV gp120 binding to CD4 receptor (ΔCp = −5.0 kJ/mol/K). A thermodynamic model developed here predicts that values of ΔCp from 0 to −2.0 kJ/mol/K have relatively little impact on the temperature sensitivity of the number of mosquito midgut cells with bound arbovirus, while intermediate values of ΔCp of −3.0 kJ/mol/K give a peak temperature sensitivity of −20 °C as observed experimentally for Western equine encephalitis virus. More negative values of ΔCp greatly decrease arbovirus binding at temperatures below −20 °C. Thus to promote transmission at low temperatures, arboviruses may benefit from ΔCp ~ 0 kJ/mol/K as for HA/SA and it is interesting that bluetongue virus binds to SA in midge midguts. Large negative values of ΔCp as for HIV gp120:CD4 diminish binding at 37 °C. Of greater importance, however, is the decrease in entropy of the whole virus (ΔSa_immob) on its immobilisation on the host cell surface. ΔSa_immob presents a repulsive force which the enthalpy-driven GP/Cr interactions weakened at higher temperatures struggle to overcome. ΔSa_immob is more negative (less favourable) for larger diameter viruses which therefore show diminished binding at higher temperatures than smaller viruses. It is proposed that small size phenotype through a less negative ΔSa_immob is selected for viruses infecting warmer hosts thus explaining the observation that virion volume decreases with increasing host temperature from 0 °C to 40 °C in the case of dsDNA viruses. Compared to arboviruses which also infect warm-blooded vertebrates, HIV is large at 134 nm diameter and thus would have a large negative ΔSa_immob which would diminish its binding at human body temperature. It is proposed that prior non-specific binding of HIV through attachment factors takes much of the entropy loss for ΔSa_immob so enhancing subsequent specific gp120:CD4 binding at 37 °C. This is consistent with the observation that HIV attachment factors are not essential but augment infection. Antiviral therapies should focus on increasing virion size, for example through

Abbreviations: AIV, avian influenza virus; BBF, brush border fragments from midgut; BTV, bluetongue virus; C, heat capacity at constant pressure; ΔCp, change in heat capacity; Cr, host cell receptor; CD4, host cell receptor for HIV; C_num, number of host cells which can bind virus in a given volume of host fluid (midgut or blood); C.Vt, number of host cells with bound virus at temperature T; DENV, Dengue virus; E_a, activation energy; EBOV, Zaire ebolavirus; EM, electron microscopy; Env, HIV gp120 trimer envelope protein which binds to a single CD4 molecule; F_p, fraction of arthropod midgut cells that are infected and that its progeny viruses go on to infect the arthropod midgut cell; ΔHa_virus_T, change in enthalpy for binding of virus to host cell at temperature T; K_a_virus_T, association constant for binding of virus to host cells at temperature T; K_d_receptor_T, dissociation constant for GP from Cr at temperature T; K_d_virus, dissociation constant for virus from host cell; M, molar (moles dm⁻³); n, number of GP/Cr contacts made on virus binding to cell; p_complete, probability of successful infection of the arthropod salivary glands after oral exposure at temperature T; p_transmission, probability a virion has bound to the surface of a midgut cell that that midgut cell becomes infected and that its progeny viruses go on to infect the salivary gland so completing the arthropod infection process within the life time of the arthropod at temperature T; R, ideal gas constant; ΔS_a_receptor_T, change in entropy for binding of virus GP to host Cr receptor; ΔS_a_virus_T, change in entropy for binding of virus to host cell at temperature T; ΔS_a_immob, change in entropy on immobilization of whole virus to cell surface; ΔS_a_non-specific, change in entropy on immobilization of virus to cell surface through non-specific binding; ΔS_a_specific, change in entropy on immobilization of virus to cell surface through specific GP/Cr-driven binding; SA, sialic acid; SIV, simian immunodeficiency virus; V_free, virus not bound to cells; V_total, virus challenge dose in volume of host fluid; WEEV, Western equine encephalitis virus; WNIV, West Nile virus; ZnOT, zinc oxide tetrapod

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1. Introduction

Some viruses infect hosts over a range of temperatures depending on the host species. Most notable are the arboviruses which may infect the arthropod vector over a range of ambient temperatures from ~15 °C to >30 °C (Mullens et al., 2004), and then infect a vertebrate host at temperatures from 37 °C up to 44 °C in the case of West Nile virus (WNV) in infected American crows (Corvus brachyrhynchos) (Kinney et al., 2006). Birds generally have higher body temperatures than mammals (Brault 2009) and the avian influenza virus (AIV) when jumping from birds to humans has to adapt to a temperature drop of 8 °C since the temperature in the human upper respiratory tract is ~33 °C compared to ~41 °C in the avian intestinal tract which is the site of replication of avian viruses (De Graaf and Fouchier, 2014). Bats have higher core body temperatures than primates (up to 42.1 °C for Phyllostomus hastatus which is an omnivorous bat from South America) when flying (O'Shea et al., 2014).

The core body temperature during flight of the insectivorous free-tailed bat (Mops condylurus) which may have been the origin of the 2013/15 severe Zaire ebolavirus (EBOV) outbreak in West Africa (Saez et al., 2015) is 40.5 °C ± 1 °C (O'Shea et al., 2014). Thus EBOV which infects a range of vertebrate species from bats to primates and deer may have to infect mammalian hosts over a range of temperatures. Similarly Nipah virus on jumping from fruit bats to pigs and humans (Daszak et al., 2013) may experience a small fall in temperature. Furthermore, cross-species transmission of viruses to bats (and other mammals) from invertebrates may occur with more regularity than has been appreciated (Bennett et al., 2018; Leendertz 2016), and Bennett et al. (2018) have suggested that arthropods may host many “bat-associated” viruses that have defied detection in bats themselves (e.g. EBOV). Depending on the ambient temperature, viruses in arthropods would experience a 9 °C to 15 °C temperature increase on being ingested by a bat at 41 °C (Gale 2017) and this could affect the binding affinity of the virus to its host cell depending on the thermodynamics of virus binding as is discussed here. In contrast other viruses only infect related host species, and in effect are maintained at similar temperatures. For example, simian immunodeficiency viruses (SIV) infect 36 different nonhuman primate species in sub-Saharan Africa and SIVs from chimpanzees (Pan troglodytes) have crossed species barriers on multiple occasions generating human immunodeficiency virus (HIV) types 1 and 2 (Sharp et al., 2005). Thus the main source of temperature variation experienced by SIV and HIV would be the effect of the circadian rhythm and the slight rise in temperature due to infection of the host which gives a range of 36 °C to 38 °C in the case of Rhesus monkeys (Huitem-Rosendiz et al., 2007).

Viruses bind to host cells through interactions between a virus surface (glyco)protein (GP) and a host cell receptor (Cr). The Cr molecule on the cell surface may be a protein as in the case of HIV (Myszka et al., 2000), EBOV (Yuan et al., 2015), Hendra virus (Xu et al., 2012) and MERS-CoV (Lu et al., 2013) or alternatively is a sialic acid (SA) on a glycan as in the case of AIV (De Graaf and Fouchier, 2014; Fei et al., 2015) and arboviruses such as bluetongue virus (BTV) (Zhang et al., 2010). The thermodynamics of virus binding in terms of the changes in enthalpy (ΔH a receptor T) and entropy (ΔS a receptor T) on the specific interaction of the GP with the Cr receptor at temperature T during binding of virus to the host cell surface has been set out previously (Gale 2018, 2019). The interactions between the HIV GP and its CD4 cellular receptor (Myszka et al., 2000; Dey et al., 2007) and between the AIV haemagglutinin (HA) and its α2,3-SA or α2,6-SA receptors (Fei et al., 2015) are enthalpy driven, i.e. large negative values of ΔH a receptor T overcome unfavourable values of ΔS a receptor T. Also the binding of vesicular stomatitis virus to phospholipid bilayers is enthalpy driven (Carneio et al. 2002) with very large negative values of ΔH a receptor T.

Temperature may impose constraints on viruses’ jumping the species barrier through its effect on the binding affinity of GP to Cr. There may also be constraints on the activities of viral replication proteins such as the AIV polymerase which showed a significantly higher activity at 33 °C than 37 °C (Ngai et al., 2013). Only virus binding is considered here. Thus according to the Van’t Hoff Isochore, the binding affinity for a virus to its host cell at temperature T as represented by the association constant K a_virus_T would be greatly diminished for enthalpy-driven GP/Cr interactions at higher temperatures compared to lower temperatures depending on the magnitude of ΔH a receptor_T (Gale 2019). Indeed, assuming ΔH a receptor_T is constant over the temperature range, the more negative ΔH a receptor_T is in magnitude, the weaker the binding at higher temperatures. This presents a paradox if the K a_virus_T falls to less than ~10^14 M−1 in that higher temperatures would greatly diminish infectivity (Gale 2019).

While the values of ΔH a receptor_T and ΔS a receptor_T may be constant over the biological temperature range for some GP/Cr systems, e.g. AIV haemagglutinin (HA) binding to sialic acid (SA) residues (Fei et al., 2015) they may change considerably in magnitude over the biologically relevant temperature range particularly for protein:protein systems as in the case of HIV gp120 binding to CD4 (Myszka et al., 2000) (Fig. 1). The effect of this on K a_virus_T is considered in this work. The change in ΔH a receptor_T with temperature is defined by the difference in heat capacities (Cp) of the GP/Cr “product” complex and the free GP/free host Cr “reactants” and is represented by ΔCp which is the slope of the lines in Fig. 1 and typically ranges from ~0 kJ/mol/K to ~5.0 kJ/mol/K depending on the system.

The previous thermodynamic treatment of virus binding (Gale 2019) identified the entropy change (ΔS a_immob) on immobilisation of the whole virus particle on the host cell surface as a key parameter for which there are currently no data. ΔS a_immob would be expected to be large and negative in magnitude due to the decrease in the absolute entropy on immobilisation of a large molecular entity such as a virion. A recent analysis has shown that virion volume (and genome length) for dsDNA viruses decreases by about 55-fold as the temperature of occurrence (i.e. host) increases from 0 to 40 °C (Nifong and Gillooly 2016). The temperature of occurrence in these dsDNA viruses ranges from near zero for those inhabiting polar environments to over

![Fig. 1. Variation in ΔH a receptor_T as a function of temperature as reported for HIV gp120 monomer binding to CD4 (○) (Myszka et al., 2000) and AIV HA monomer binding to soluble α2-6 sialyllectose (■) and α2-3 sialyllectose (□) (Fei et al., 2015). The slopes of the lines represent the ΔCp values which are summarised in Table 3.](image-url)
\[ H_T = H_0 + C_p(T - T_0) \]  
\[ GP + Cr \rightarrow GP.Cr \]  
\[ \Delta H_{a\_receptor\_T} = H_T(\text{GP Cr}) - (H_T(\text{GP}) + H_T(\text{Cr})) \]
\[ \Delta H_{a\_receptor\_T} = \Delta H_{a\_receptor\_T}^{T_0} + \Delta C_p(T - T_0) \]
\[ \Delta C_p = C_p(\text{GP Cr}) - (C_p(\text{GP}) + C_p(\text{Cr})) \]
\[ \Delta S_{a\_receptor\_T} = \Delta S_{a\_receptor\_T}^{T_0} + \Delta C_p \ln \frac{T}{T_0} \]
\[ \Delta H_{a\_virus\_T} = n \times \Delta H_{a\_receptor\_T} \]
\[ \Delta S_{a\_virus\_T} = \Delta S_{a\_virus\_T}^{T_0} - T \Delta S_{a\_virus\_T} \]
\[ \Delta G_{a\_virus\_T} = n \Delta H_{a\_virus\_T} - n T \Delta S_{a\_virus\_T} - T \Delta S_{a\_viro} \]
\[ K_{a\_virus\_T} = \exp\left(\frac{-\Delta G_{a\_virus\_T}}{RT}\right) \]
\[ E_T = \frac{[V_{\text{free}}]}{[V_{\text{total}}]} = K_{a\_virus\_T} \]
\[ P_{\text{complete}} = P_{\text{complete}}^{(\text{CD4})} \times e^{\frac{\Delta S_{a\_viro}}{R}} \]
\[ \Delta S_{a\_immob} \propto -3\ln\left(\frac{3\pi r^3}{2}\right) - 3\ln\left(\frac{4\pi r^3}{3}\right)^{\frac{1}{3}} \]
\[ \Delta S_{a\_specific} = \Delta S_{a\_immob} - \Delta S_{a\_non\_specific} \]

Table 1

| Equations used. See methods for derivation. |
|--------------------------------------------|
| \[ H_T = H_0 + C_p(T - T_0) \] (Eq. 1) |
| \[ GP + Cr \rightarrow GP.Cr \] (Eq. 2) |
| \[ \Delta H_{a\_receptor\_T} = H_T(\text{GP Cr}) - (H_T(\text{GP}) + H_T(\text{Cr})) \] (Eq. 3) |
| \[ \Delta H_{a\_receptor\_T} = \Delta H_{a\_receptor\_T}^{T_0} + \Delta C_p(T - T_0) \] (Eq. 4) |
| \[ \Delta C_p = C_p(\text{GP Cr}) - (C_p(\text{GP}) + C_p(\text{Cr})) \] (Eq. 5) |
| \[ \Delta S_{a\_receptor\_T} = \Delta S_{a\_receptor\_T}^{T_0} + \Delta C_p \ln \frac{T}{T_0} \] (Eq. 6) |
| \[ \Delta H_{a\_virus\_T} = n \times \Delta H_{a\_receptor\_T} \] (Eq. 7) |
| \[ \Delta S_{a\_virus\_T} = \Delta S_{a\_virus\_T}^{T_0} - T \Delta S_{a\_virus\_T} \] (Eq. 8) |
| \[ \Delta G_{a\_virus\_T} = n \Delta H_{a\_virus\_T} - n T \Delta S_{a\_virus\_T} - T \Delta S_{a\_viro} \] (Eq. 9) |
| \[ K_{a\_virus\_T} = \exp\left(\frac{-\Delta G_{a\_virus\_T}}{RT}\right) \] (Eq. 10) |
| \[ E_T = \frac{[V_{\text{free}}]}{[V_{\text{total}}]} = K_{a\_virus\_T} \] (Eq. 11) |
| \[ P_{\text{complete}} = P_{\text{complete}}^{(\text{CD4})} \times e^{\frac{\Delta S_{a\_viro}}{R}} \] (Eq. 12) |
| \[ \Delta S_{a\_immob} \propto -3\ln\left(\frac{3\pi r^3}{2}\right) - 3\ln\left(\frac{4\pi r^3}{3}\right)^{\frac{1}{3}} \] (Eq. 13) |
| \[ \Delta S_{a\_specific} = \Delta S_{a\_immob} - \Delta S_{a\_non\_specific} \] (Eq. 14) |

1. The predicted effect of the magnitude of \( \Delta C_p \) on the temperature dependence of Dengue virus (DENV) transmission by \textit{Aedes albopictus};
2. The predicted effect of the magnitude of \( \Delta C_p \) on the temperature peak observed for the specific binding of Western equine encephalitis virus (WEEV) to susceptible \textit{Culex tarsalis} brush border fragments (BBFs);
3. A large negative \( \Delta C_p \) for a protein:protein GP/Cr system as represented by HIV gp120:CD4 diminishes host cell binding at human body temperature;
4. A large virus diameter as for the HIV virion diminishes host cell binding at the higher temperature of the human body through the large negative \( \Delta S_{a\_immob} \).

5. Non-specific attachment factors may partially overcome the unfavourable \( \Delta S_{a\_immob} \) and hence enhance specific HIV binding through Env:CD4 interactions at human body temperature; and
6. Increasing the number, \( n \), of GP/Cr contacts with temperature to reproduce the data of \textit{Frey et al.} (1995) for binding of HIV Env expressing cells to cells expressing CD4.

It may be thought there is little point in modelling HIV binding with temperature because HIV only infects human hosts at ~37 °C. However, the model here suggests mammalian temperature may be a constraint on HIV binding (due to the large negative \( \Delta C_p \) and \( \Delta S_{a\_immob} \)) in effect exposing a potential weakness in the HIV infection process which could be exploited for antiviral therapy. The implications of making \( \Delta S_{a\_immob} \)
more negative are considered here as a novel approach to developing antiviral therapies with reference to Antoine et al. (2012) using zinc oxide nanoparticles for preventing infection by herpes simplex virus type 2 (HSV-2).

2. Methods

2.1. The change in heat capacity ($\Delta C_p$) on binding of virus GP to Cr receptors on the host cell

The change in enthalpy ($H$) with temperature ($T$) at constant pressure is the heat capacity, $C_p$:

\[
\frac{\Delta H}{\Delta T} = C_p
\]

Integrating with respect to temperature relates the absolute enthalpy ($H_T$) at temperature $T$ to that ($H_{T0}$) at a given reference temperature $T_0$ through $C_p$ according to Eq. (1) (see Table 1). The attachment of a virus to its host cell is driven by the $n$ individual interactions between GP and Cr made on binding as represented by the dynamic equilibrium in Eq. (2). The change in enthalpy for GP binding to Cr at temperature $T$, $\Delta H_{a_receptor_T}$, is given by Eq. (3).

Substituting Eq. (1) into Eq. (3) for the enthalpies for the GP.Cr complex ($H_T(GP,Cr)$), the free GP ($H_T(GP)$) and the free Cr ($H_T(Cr)$) relates the enthalpy change for GP binding to Cr at temperature $T$, $\Delta H_{a_receptor_T}$, to that ($\Delta H_{a_receptor_T0}$) measured experimentally at temperature $T_0$ (Du et al., 2016) according to Eq. (4) where $\Delta C_p$ is the change in heat capacity defined as the difference between the heat capacities of the GP.Cr complex and the sum of the heat capacities of the free GP and Cr molecules as given by Eq. (5). Similarly the entropy change ($\Delta S_{a_receptor_T}$) for GP binding to Cr at temperature $T$ is related to that ($\Delta S_{a_receptor_T0}$) measured experimentally at temperature $T_0$ (Du et al., 2016) according to Eq. (6).

2.2. Modelling the effect of temperature on $\Delta H_{a_virus_T}$ and $\Delta S_{a_virus_T}$ and hence $K_{a_virus_T}$

$\Delta H_{a_virus_T}$ and $\Delta S_{a_virus_T}$ are the changes in enthalpy and entropy respectively on binding of virus to host cell at temperature $T$. Substituting the terms for $\Delta H_{a_virus_T}$ (Eq. (7)) and $\Delta S_{a_virus_T}$ (Eq. (8)) into Eq. (9) expresses $\Delta G_{a_virus_T}$ in terms of $n$, $\Delta S_{a_immob}$, $\Delta H_{a_receptor_T}$ and $\Delta S_{a_receptor_T}$ (Eq. (10)) where $\Delta S_{a_virus_T}$ is the change in Gibbs free energy on association of virus with a host cell at temperature $T$ and $\Delta S_{a_immob}$ is the change in entropy on immobilisation of the whole virus on the cell surface (Gale 2018; 2019).

2.2.1. The change in heat capacity on virus binding to its host cell

For this work it is assumed that the change in heat capacity on binding of a virus to a host cell is determined by the change in heat capacity on interaction of GP with Cr according to Eq. (5) and that $\Delta S_{a_immob}$ in Eq. (10) is unaffected. In effect therefore the heat capacity of the “bulk” (i.e. non-GP/Cr) of the bound virus/host cell is the same as the sum of the heat capacities for the free virus and free host cell. This is acceptable because a change in heat capacity is related to a change in conformation of the molecular components (Myszka et al., 2000) and the conformation of the bulk of the host cell and the virus would be unaffected by attachment. Indeed, $\Delta S_{a_immob}$ is mainly related to changes in rotational and translational mobility of the virus particle as a whole (see below) and does not involve the conformational changes in GP and Cr which are accommodated in the $\Delta S_{a_receptor_T}$ term (Gale 2019). Since the heat capacity of the “bulk” of the bound virus/host cell is the same as the sum of the heat capacities for the free virus and free host cell, $\Delta C_p$ for the bulk equals zero, and according to Eq. (6) therefore, $\Delta S_{a_immob}$ itself at temperature $T$ (calculated as $\Delta S_{a_immob_T} = \Delta S_{a_immob_T0} + \Delta C_p \ln(T/T_0)$) would be unaffected by temperature and hence constant as assumed in the models here.

2.2.2. The effect of the magnitude of $\Delta C_p$ on virus binding affinity at temperature $T$

Substituting $\Delta H_{a_receptor_T}$ and $\Delta S_{a_receptor_T}$ from Eqs. (4) and (6) respectively into Eq. (10) gives Eq. (11) from which $K_{a_virus_T}$ is calculated according to Eq. (12) where $R$ is the ideal gas constant.

2.2.3. The choice of reference temperature, $T_0$

Comparing the predicted effect on $K_{a_virus_T}$ of the magnitude of $\Delta C_p$ in Case studies 1, 2 and 3 requires a reference temperature, $T_0$, to be defined at which the plots of $K_{a_virus_T}$ intercept. Values of $K_{a_virus_T}$ intercept at $T = T_0$ because the $n \Delta C_p (T - T_0 + T \ln(T/T_0))$ term in Eq. (11) equals zero. Therefore for the purpose of demonstrating the effect of $\Delta C_p$ on the temperature sensitivity of $K_{a_virus_T}$, $T_0$ needs to be chosen to be well above or below the temperature range of interest. It should be noted that the choice of reference temperature is artificial and is only used here out of necessity to demonstrate the effect of different $\Delta C_p$ values while fixing the $K_{a_virus_T}$ intercept temperature. Indeed a given GP/Cr system has its own natural $\Delta C_p$ value which cannot be altered and is measured experimentally (Myszka et al., 2000; Fei et al., 2015). The $T_0$ temperature of 37 °C is appropriate to study arthropod vector competence for arbovirus transmission over the 10 to 35 °C temperature range in Case studies 1 and 2 as used previously for the arthropod vector competence model (Gale 2019). However, since 37 °C is the human body temperature at which HIV binds to host cells, 37 °C is not a good choice for $T_0$ in case study 3 for which $T_0$ is therefore set to 4 °C. The reference temperature is 37 °C in Case studies 4, 5 and 6 because the experimentally measured value of $\Delta C_p$, for HIV gp120:CD4 binding (Fig. 1) is used together with $\Delta H_{a_receptor_T0}$ and $\Delta S_{a_receptor_T0}$ values measured experimentally at a temperature $T_0$ of 37 °C (Myszka et al., 2000), i.e. real data are used.

2.3. Case study 1: The predicted effect of the magnitude of $\Delta C_p$ on the temperature dependence of DENV transmission by Aedes albopictus

The approach for modelling arbovirus transmission efficiency by the arthropod vector has been fully described previously (Gale 2019) and is summarised here for the purpose of the models in Figs. 2 and 3.

2.3.1. Modelling $K_{a_virus_T}$ as a function of temperature

The strength of GP/Cr binding at temperature $T$ is often expressed as the dissociation constant, $K_{a_receptor_T}$, for which smaller values indicate stronger binding (Xiong et al., 2013). The thermodynamic model is based on an enthalpy-driven GP/Cr interaction with a $K_{a_receptor_T}$ of $10^{-3}$ M at 37 °C ($T_0$) as used previously (Gale 2019) to represent the interaction between an arbovirus GP and a SA glycan (Cr) on the brush border surface of the epithelial cells lining the arthropod midgut. The parameters $\Delta H_{a_receptor_T0}$ and $\Delta S_{a_receptor_T0}$ at $T_0 = 37$ °C for DENV transmission by Aedes albopictus together with $n$ and $\Delta S_{a_immob}$ are set out in Table 2 and values of $K_{a_virus_T}$ over the temperature range 10–37 °C are calculated for the $\Delta C_p$ values in Table 3 using $\Delta S_{a_virus_T}$ from Eq. (11) in Eq. (12).

2.3.2. Modelling the effect of temperature on the number of cells in the arthropod midgut with bound arboviruses

The arbovirus challenge dose is $10^5$ virions and the midgut volume is $10^{-6}$ dm$^3$ giving a virus concentration $[V_{total}] = 1.7 \times 10^{-13}$ M. The fraction ($F_{CT}$) of arthropod midgut cells with bound arbovirus at temperature $T$ is calculated from $K_{a_virus_T}$ using Eq. (13) assuming the concentration of free virus ($[V_{free}] = [V_{total}]$) the number, $C_{VT}$, of midgut cells with bound arbovirus at temperature $T$ is calculated by multiplying $F_{CT}$ by the total number of midgut cells ($C_{total} = 1000$) (Eq. (14)) as described previously (Gale 2019).
2.3.3. Modelling the effect of temperature on the arbovirus transmission efficiency

The probability, $p_{\text{complete}} T$, of an arthropod midgut cell with bound virus successfully leading to infection of the arthropod salivary glands and completion of virogenesis within the lifetime of the arthropod at temperature $T$ is calculated using the Arrhenius equation (Eq. (15)) where $p_{\text{complete}} 283$ is the probability of this happening at 10 °C (283 K) and $E_A$ is the activation energy of the rate-limiting step in virogenesis (Table 2). The probability, $p_{\text{transmission}} T$, of arbovirus transmission by the arthropod (i.e. successful infection of the arthropod salivary glands after oral exposure) at temperature $T$ given C.V.T midgut cells have bound virus is given by Eq. (16).

2.4. Case study 2: The magnitude of $\Delta C_p$ could explain the temperature peak observed in the specific binding of WEEV to susceptible Culex tarsalis BBFs

The value of $\Delta C_p$ in Eq. (11) with the GP/Cr binding parameters for DENV in Table 2 was optimised so that C.V.T as a function of temperature best approximated the specific binding affinity of WEEV to BBFs from susceptible Culex tarsalis mosquitoes as determined by Houk et al. (1990) and shown in Fig. 3.
### Table 2
Summary of the parameters for the enthalpy-driven GP/Cr binding model for DENV transmission by Aedes albopictus developed previously (Gale 2019).

| Parameter | Value |
|-----------|-------|
| $K_{Dcr,case}$ at $20\degree C$ | $8.0 \times 10^{-7}$ |
| $E_h$ (kJ/mol) | $270.0$ |
| GP/Cr binding parameters | |
| $\Delta H_{\text{receptor},T}$ at $37\degree C$ (kJ/mol) | $-56.545$ |
| $\Delta S_{\text{receptor},T}$ at $37\degree C$ (J/mol/K) | $-125$ |
| $K_{Dcr,case}$ at $37\degree C$ | $10^{-8}$ M |
| Whole virus binding parameters | |
| $\Delta H_{\text{virus},T}$ (kJ/mol) from Eq. (7) | $-226.2$ |
| $\Delta S_{\text{virus},T}$ (J/mol/K) from Eq. (8) | $-500$ |

### Table 3
Summary of some published $\Delta C_p$ values for protein/protein and protein/sialic acid (SA) interactions.

| Parameter | Value |
|-----------|-------|
| $\Delta H_{\text{virus},T}$ (kJ/mol) from Eq. (7) | $-226.2$ |
| $\Delta S_{\text{virus},T}$ (J/mol/K) from Eq. (8) | $-500$ |

### Table 4
Thermodynamic parameters for HIV Env binding to CD4 receptor at different temperatures. In the absence of data, the binding parameters for HIV Env trimer to CD4 as used in the models here are based on those from Myszka et al. (2000) for the full-length gp120 monomer binding to CD4.

| Temperature | $\Delta H_{\text{receptor},T}$ (kJ/mol) | $\Delta S_{\text{receptor},T}$ (J/mol/K) | $K_{Dreceptor}$ (M) |
|-------------|---------------------------------------|---------------------------------------|------------------|
| 37 °C       | $-263.59^a$                          | $-97.9^a$                             | $5.75 \times 10^{-9}$ |
| 4 °C        | $-691.04^b$                          | $-126.01^d$                           | $1.28 \times 10^{-12}$ |
| 25 °C       | $-472.9^f$                           | $-198.2^f$                            | $9.91 \times 10^{-10}$ |

a) From Myszka et al. (2000) for full length gp120 (reported as $-63$ kcal/mol) at $37\degree C$.

b) Calculated from $-T\Delta S = +214.22$ kJ/mol for full length gp120 (reported as $+51.2$ kcal/mol by Myszka et al. (2000)) at $37\degree C$.

c) Calculated from Eq. (4) using $\Delta C_p = -5.02$ kJ/mol/K (Table 3) with $T_0 = 310$ K (37 °C).

d) Calculated from Eq. (6) using $\Delta C_p = -5.02$ kJ/mol/K (Table 3) with $T_0 = 310$ K (37 °C).

e) Estimated from values for $\Delta H_{\text{receptor},T}$ and $-T\Delta S_{\text{receptor},T}$ presented in Fig. 4 of Myszka et al. (2000) for gp120 binding to CD4 at 25 °C.

f) In good agreement with $\Delta S_{\text{receptor},T}$ value at $T = 298$ K (25 °C) of $-492.8$ J/mol/K as calculated from Eq. (6) using $\Delta C_p = -5.02$ kJ/mol/K (Table 3) with $T_0 = 310$ K (37 °C) and $\Delta S_{\text{receptor},T} = -691.04$ J/mol/K (see b).

2.5. Case study 3: A large negative $\Delta C_p$ for a protein:protein GP/Cr system as represented by HIV gp120:CD4 diminishes host cell binding at higher temperatures

2.5.1. Estimation of $n$ for HIV

Yang et al. (2005) using defective HIV gp120 monomers concluded that a single Env trimer is sufficient to mediate the entry of one virion, but that all three gp120 monomers in that trimer must be active. Klasse (2012) has proposed that a realistic model allows both a minimum requirement of two gp120 monomers with an increment in function by the third. This is consistent with the cryo-electron microscopy (EM) structure of the Env glycoprotein trimer (Liu et al., 2017a) showing that in each trimer, one gp120 monomer makes a primary contact with the bound CD4 Cr molecule while a second makes a partial contact (called the quaternary contact reflecting protein structure nomenclature) and the third gp120 monomer makes no direct contact with the CD4 at all. For the purpose of the model here, $n$ represents the number of Env trimers on each HIV virion that interact with a CD4 Cr during binding. Brandenbourg et al. (2015) demonstrate that divergent HIV strains differ in their stoichiometry of entry and require between 1 and 7 Env trimers, with most strains depending on 2 to 3 Env trimers to complete infection. Here it is assumed that $n = 3$ Env:CD4 interactions for HIV binding although the results of Brandenbourg et al. (2015) do not separate cell surface binding itself from the subsequent HIV/cell fusion in the entry process. If it is later found that cell surface binding only requires one Env:CD4 interaction for example, while more are required for the membrane fusion, then the model here for C.V.7 should be re-parameterised with $n = 1$ although the number of Env:CCR5 and non-specific Env interactions with attachment factors (see below) should also be built into the model. It should be noted that as for the arbovirus model, it is assumed that the $n = 3$ Env:CD4 interactions act independently and not co-operatively in binding. This is reflected in Eqs. (7) and (8) where $\Delta H_{\text{receptor},T}$ and $\Delta S_{\text{receptor},T}$ act additively.

2.5.2. Thermodynamic parameters for HIV Env:CD4 binding at $T_0$ of $37\degree C$

The Env trimer binds a single soluble CD4 with a $K_{D,\text{receptor}}$ at $25\degree C$ of $1.4 \times 10^{-9}$ M (Chuang et al., 2017) which is 15-fold higher than that for the gp120 monomer:CD4 interaction estimated at $25\degree C$ from the data of Myszka et al. (2000) in Table 4. To the author’s knowledge there are no data for $\Delta H_{\text{receptor},T}$ and $\Delta S_{\text{receptor},T}$ for Env trimer binding to CD4. Therefore the values for $\Delta H_{\text{receptor},T}$ and $\Delta S_{\text{receptor},T}$ for HIV gp120 monomer:CD4 binding at $37\degree C$ from Myszka et al. (2000) are used for Env trimer binding to a single CD4 in the HIV model in Fig. 4a and as set out in Table 4. Dey et al. (2007) reported values at $37\degree C$ of $-287.9$ kJ/mol and $-786.9$ J/mol/K for $\Delta H_{\text{receptor},T}$ and $\Delta S_{\text{receptor},T}$ respectively for the wildtype HIV-1 YU2 gp120 monomer binding to soluble CD4. The more negative $\Delta S_{\text{receptor},T}$ value for gp120 monomer:CD4 binding from Dey et al. (2007) reduces the binding affinity for gp120:CD4 by nine-fold ($K_{D,\text{receptor}} = 4.2 \times 10^{-8}$ M) at $37\degree C$ compared to that of $4.75 \times 10^{-9}$ M from Myszka et al. (2000) (Table 4). This difference may simply reflect the different strains of HIV-1 used, namely YU2 by Dey et al. (2007) and WD61 by Myszka et al. (2000). The HIV gp120 monomer:CD4 binding data of Myszka et al. (2000) are chosen here over those of Dey et al. (2007) because Myszka et al. (2000) present the variation in $\Delta H_{\text{receptor},T}$ with temperature as shown in Fig. 1 together with information on $\Delta C_p$ which Dey et al. (2007) do not consider. It is therefore assumed here that $\Delta C_p = -5.02$ kJ/mol/K for the Env trimer:CD4 interaction as reported by Myszka et al. (2000) for the gp120 monomer:CD4 interaction (Table 3).

Values of $\Delta S_{\text{receptor},T}$ are not documented by Myszka et al. (2000) other than at that $37\degree C$ (Table 4) and values at lower temperatures are therefore calculated from that at $T_0 = 37\degree C$ according to Eq. (6) using $\Delta C_p = -5.02$ kJ/mol/K for HIV gp120:CD4 (Table 3). Some reassurance that Eq. (6) is appropriate is that the value of $\Delta S_{\text{receptor},T}$ of $-493$ J/mol/K predicted at $T = 25\degree C$ by Eq. (6) is very close to that of $-473$ J/mol/K calculated from the $-T\Delta S_{\text{receptor},T}$ value reported by $\Delta C_p$.
that greatly exceeds \( C_{\text{total}} \) and the interpretation applicable to all doses of viruses as there is no cooperation between viruses in this model (Gale 2018). Furthermore, the levels of SIV in Rhesus monkey blood plasma were in the region of \( 2.1 \times 10^8 \) virions per ml (Huitron-Resendiz et al., 2000) at 25 °C (Table 4).

2.5.3. Thermodynamic parameters for HIV Env:CD4 binding at \( T_0 \) of 4 °C

Predicted values for \( \Delta H_{\text{r, receptor}} \) and \( \Delta S_{\text{a, receptor, T}} \) for HIV Env:CD4 binding at 4 °C are set out in Table 4 and are used as \( \Delta H_{\text{r, receptor, T0}} \) and \( \Delta S_{\text{a, receptor, T0}} \) respectively for the HIV model in Figs. 4c and d with the reference temperature, \( T_0 \), set to 4 °C to assess the effect of varying \( \Delta C_p \) on the temperature sensitivity of \( K_{\text{a, virus, T}} \) and \( C.V_T \) up to and above human body temperature.

2.5.4. Model for HIV binding to CD4\(^+\) T cells in blood

The 2.5 to 97.5 percentile range for the number of CD4\(^+\) T cells in humans is 448 to 1611 cells per mm\(^3\) (10\(^{-6}\) dm\(^3\)) of blood with a mean of 919 cells per mm\(^3\) (Thakar et al., 2011). The model here takes 1 mm\(^3\) of blood with \( C_{\text{total}} = 10^5 \) CD4\(^+\) T cells and introduces \( V_{\text{total}} = 10^5 \) HIV virions. Dividing \( 10^5 \) HIV virions in \( 10^{-6} \) dm\(^3\) by the Avogadro number gives \( [V_{\text{total}}] = 1.7 \times 10^{-13} \) M. The advantage of using a high \( V_{\text{total}} \) that greatly exceeds \( C_{\text{total}} \) is that as previously demonstrated by Gale (2018) not only may the fraction, \( F_{\text{CT}} \), of CD4\(^+\) T cells with bound HIV be calculated from Eq. (13) with \( [V_{\text{free}}] \sim [V_{\text{total}}] \) but also any complications of handling stochastic effects at low virus doses are avoided. It is recognised that the HIV concentration at \( 10^5 \) HIV virions per mm\(^3\) used in this model is 10-fold to 100-fold higher than that expected in a recipient person’s blood present in the capillaries lining the skin at the wound site where semen or blood from an infected source were introduced since maximum human plasma and human semen HIV loadings are in the region of 13,000 copies per mm\(^3\) and 1800 copies per mm\(^3\) respectively (Gupta et al., 1997). This is not important, however, because the purpose of the models here in Figs. 4-6 is not to produce a realistic dose-response but to explore the effect of changing \( \Delta C_p \), \( \Delta S_{\text{a, immob}} \), n and temperature on the trend in the predicted number, \( C.V_T \), of host CD4\(^+\) T cells with bound virus. Indeed, the overall conclusions of the model in terms of the effect of temperature on the number of CD4\(^+\) T cells with bound virus will still be representative and the interpretation applicable to all doses of viruses as there is no cooperation between viruses in this model (Gale 2018). Furthermore, the estimate of 8 or 9 HA trimers in Yang et al. (2005) is based on a number of assumptions that may not hold, for example whereas each infection of a cell is a quantal, all-or-nothing event, the infectivity of a virion could span a wide spectrum of propensities and each mathematical model could have different virological interpretations (see Klasse 2012). However, in the absence of any data for \( \Delta S_{\text{a, immob}} \), the value of \( n \) from Yang et al. (2005) at least provides a theoretical approach to estimate it from Eq. (17) for this “proof of concept” model.

2.6. Case study 4: A large virus diameter such as for the HIV virion diminishes host cell binding at the higher temperatures of the human body through the large negative \( \Delta S_{\text{a, immob}} \)

2.6.1. Estimation of \( \Delta S_{\text{a, immob}} \) for a virus

There are currently no data available on the magnitude of \( \Delta S_{\text{a, immob}} \) which was first defined through development of a thermodynamic model (Gale 2019). Xiong et al. (2013) reported a \( K_{d, \text{virus}} \) of \( 10^{-15} \) M for AIV where \( K_{d, \text{virus}} \) is the dissociation constant for virus from the host cell and is the inverse of \( K_{d, \text{virus}} \). According to Yang et al. (2005) HIV virions bearing influenza A virus HA required 8 or 9 HA trimers for cell binding or membrane fusion in Yang et al. (2005). However, if \( n = 24 \) to 27 HA monomers each of \( K_{d, \text{receptor}} = 10^{-3} \) M (Xiong et al., 2013). It is not clear whether the 8 or 9 AIV HA trimers are required for cell binding or membrane fusion in Yang et al. (2005).

2.6.2. Effect of virus size on the magnitude of \( \Delta S_{\text{a, immob}} \)

It was shown previously (Gale 2019) that \( \Delta S_{\text{a, immob}} \) is the sum of the
changes in translational entropy ($\Delta S_{\text{trans}}$) and rotational entropy ($\Delta S_{\text{rot}}$) of the whole virus on binding. According to Mammen et al. (1998) $\Delta S_{\text{trans}} \propto \ln(\text{Mass})$ and $\Delta S_{\text{rot}} \propto \ln(I_x \times I_y \times I_z)$ where $I_x$, $I_y$, and $I_z$ are the moments of inertia about the three principle axes of rotation. Since mass, $M$, is proportional to the volume and $V = M^2/r$ where $r$ is the radius of the virus, it may be shown that the decrease in entropy on immobilisation ($\Delta S_{\text{immob}}$) of a spherical virus can be expressed as Eq. (18).

Examples of diameters for arboviruses are ~50 nm for WNV (Mukhopadhyay et al., 2003) and DENV, 66 nm for Eastern equine encephalitis virus (Hasan et al., 2018) with BTV being slightly larger at 85 nm (Nason et al., 2004). In contrast, HIV is much larger with a mean diameter of 134 nm in the case of mature HIV virions containing a single core (Briggs et al., 2003). The problem with Eq. (18) is that proportionality constants in units of J/mol/K are needed probably based on $r$ as in the Sackur-Tetrode equation and the natural logarithm

The radius of the virus on $\Delta S_{\text{immob}}$ Eq. (18) is used with the virus radius in units of nm. Thus from Eq. (18), the magnitude of $\Delta S_{\text{immob}}$ for a virus of diameter 134 nm is 18.6% more negative than that for a virus of diameter 66 nm. To illustrate the effect of increasing virus size on the number of CD4+ T cells in 1 mm$^3$ of blood with bound virus, $C_{V_T}$ is calculated for the HIV model using values of $\Delta S_{\text{immob}}$ which decrease in steps of 18.6% from $-240$ J/mol/K (i.e. to $-285$ J/mol/K, $-337$ J/mol/K and $-400$ J/mol/K).

2.7. Case study 5: HIV attachment factors may partially overcome the unfavourable $\Delta S_{\text{immob}}$ and enhance specific virus binding through Env/CD4 interactions at human body temperature

2.7.1. Modelling the effect of non-specific binding of HIV to host CD4+ T cells prior to specific Env/CD4 interaction

The term $\Delta S_{\text{immob}}$ represents the decrease in entropy of the virus on going from a freely rotating entity to being immobilised in a specific orientation on the host cell surface through $n = 3$ specific Env/CD4 interactions. Thermodynamically this could be accomplished in two consecutive steps whereby the virus first binds non-specifically to attachment proteins on the host cell surface with an entropy change of $\Delta S_{\text{non-specific}}$ and then rolls into place driven by $n = 3$ specific Env/CD4 interactions resulting in a further entropy loss ($\Delta S_{\text{specific}}$) as the virus particle takes up a specific orientation ready for viral entry. In effect $\Delta S_{\text{immob}}$ is the sum of two components, namely $\Delta S_{\text{non-specific}}$ and $\Delta S_{\text{specific}}$ according to Eq. (19). It should be noted that non-specific binding to HIV attachment factors would not increase $\Delta S_{\text{immob}}$ to zero because there are multiple ways a virus could bind non-specifically compared to the one way in which it binds to the CD4 receptors through a specific interaction between Env and CD4, that is the $\Delta S_{\text{specific}}$ term is always negative i.e. $< 0$ J/mol/K and $\Delta S_{\text{non-specific}}$ is always less negative than $\Delta S_{\text{immob}}$.

Prior attachment through non-specific binding would realise the $\Delta S_{\text{non-specific}}$ loss before specific Env/CD4 binding so that specific binding involves the $\Delta S_{\text{specific}}$ term alone. The value of $\Delta S_{\text{immob}}$ is estimated to be $-1091$ J/mol/K (see above) and for the purpose of demonstration here $\Delta S_{\text{immob}}$ is replaced by $\Delta S_{\text{specific}}$. In Eq. (11) which is set to values of $-400$, $-337$, $-285$ and $-240$ J/mol/K in effect representing the effect of prior non-specific binding eliminating $\Delta S_{\text{non-specific}}$ values of $-691$, $-754$, $-806$ and $-851$ J/mol/K respectively according to Eq. (19).

2.8. Case study 6: Increasing the number, $n$, of GP/Cr contacts with temperature to reproduce the data of Frey et al. (1995) for binding of HIV Env-expressing cells to cells expressing CD4

Two outputs of the model are demonstrated. The first output builds on the model in Case study 5 with a $\Delta S_{\text{specific}}$ of $-206$ J/mol/K to accommodate prior non-specific binding to attachment factors having eliminated the $\Delta S_{\text{non-specific}}$ of $\sim 900$ J/mol/K. The model assumes $n = 3$ Env/CD4 interactions where $n$ in Eq. (11) is increased from $n = 1$ to $n = 3$ with temperature between 25 °C and 27 °C (Fig. 6a) to represent the increase in contacts reported by Frey et al. (1995).

The second output attempts to include non-specific binding and also CCR5 co-receptor binding and a $\Delta S_{\text{immob}}$ of $-1110$ J/mol/K is used to represent the free virus binding (i.e. prior to non-specific binding). Kuhmann et al. (2000) demonstrated that approximately four to six CCR5 molecules assemble around the HIV virion to form a complex needed for infection. The model here assumes $n = 2$ non-specific Env interactions with attachment factors followed by $n = 4$ Env:CCR5 interactions according to Kuhmann et al. (2000) and finally $n = 3$ Env/CD4 interactions based on Brandenberg et al. (2015) to form a complex for binding with $n = 9$ contacts in total which increase from 1 to 9 with temperature as shown in Fig. 6b. Doranz et al. (1999) estimated the $K_{\text{a, receptor,T}}$ of interaction between CCR5 and HIV gp120 to be $4 \times 10^{-5}$ M. This is remarkably similar to the $K_{\text{a, receptor,T}}$ for the interaction between CD4 and HIV Env of $4.75 \times 10^{-8}$ M at 37 °C (Table 4) and the same values for $\Delta S_{\text{a, immob,T}}$ and $\Delta S_{\text{a, receptor,T}}$ not only for the four HIV Env:CCR5 interactions but also for the two non-specific interactions are therefore used as for the three HIV Env:CD4 interactions in Table 4 with the reference temperature, $T_0$, at 37 °C.

3. Results

3.1. Published data on $\Delta C_p$ for GP/Cr binding and other protein-protein interactions

Published values of $\Delta C_p$ for protein/protein binding and for AIV HA binding to SA glycans vary in magnitude (Table 3). For the binding of HIV gp120 to CD4 which is a protein-protein interaction, the magnitude of $\Delta H_{\text{a, receptor,T}}$ becomes more negative as the temperature increases i.e. $\Delta C_p$ is negative (Fig. 1). Similarly, negative values of $\Delta C_p$ were reported for other protein-protein systems, namely the binding of E3 and E1 proteins, respectively, in the assembly of the enzyme pyruvate dehydrogenase (Jung et al., 2002), although with $\Delta C_p$ values less negative than that for HIV gp120:CD4 (Table 3). In contrast for AIV HA binding to the SA glycan the magnitude of $\Delta C_p$ is not significantly different from zero with $\Delta H_{\text{a, receptor,T}}$ constant over the temperature range 15 °C to 25 °C at least for binding of both o2–3 and o2–6 sialyllactose (Fig. 1).

3.2. Case study 1: The predicted effect of the magnitude of $\Delta C_p$ on the temperature dependence of DENV transmission by Aedes albopictus

Values of $K_{\text{a, virus,T}}$ predicted over the temperature range of 10 °C to 37 °C using different values of $\Delta C_p$ for the intercept at the reference temperature ($T_0$) of 37 °C (Fig. 2a) with $K_{\text{a, virus,T}} = 10^{12}$ M$^{-1}$ as expected for $n = 4$ GP/Cr interactions each of $K_{\text{a, receptor,T}}$ of $10^{-3}$ M (Eq. (17)). With $\Delta C_p = 0$ kJ/mol/K as for AIV HA/SA, $K_{\text{a, virus,T}}$ increases with decreasing temperature according to the Van’t Hoff Isochore. In contrast with $\Delta C_p = -5.02$ kJ/mol/K as for HIV gp120:CD4, $K_{\text{a, virus,T}}$ peaks at 26 °C and decreases rapidly at lower temperatures.

For the simulated DENV/Ae. albopictus model used here, small negative values of $\Delta C_p$ (~2.0 kJ/mol/K to 0 kJ/mol/K) have little effect on the probability of transmission at low temperatures (Fig. 2c,d). This is because in the model here with $\Delta C_p$ in the range of 2.0 kJ/mol/K to 0 kJ/mol/K, $K_{\text{a, virus,T}}$ is high at $>10^{14}$ M$^{-1}$ at low temperatures (Fig. 2a) and thus $C_{V_T}$ is little affected until higher temperatures (Fig. 2b) at which $K_{\text{a, virus,T}}$ falls below $10^{14}$ M$^{-1}$ (Fig. 2a). Large negative values of $\Delta C_p$ (~5.0 kJ/mol/K) cause $C_{V_T}$ to peak rapidly falling at lower temperatures (Fig. 2b). This has a large effect on the predicted probability of transmission at lower temperatures with $p_{\text{transmission,T}}$ 44-fold lower at 10 °C (Fig. 2d). It is interesting to note that $\Delta C_p$ has little effect on $p_{\text{transmission,T}}$ over the range of temperature (23 °C to 32 °C) at which laboratory vector competence experiments (Liu et al.,
2017b) are typically conducted (Fig. 2c).

3.3. Case study 2: The magnitude of $\Delta S_{immob}$ could explain the temperature peak observed in the specific binding of WEEV to susceptible Culex tarsalis BBFs

With a $\Delta C_p = -3.0 \text{ kJ/mol/K}$ the magnitude of $K_{virus,T}$ between 0 and 40 °C is relatively constant compared to those with $\Delta C_p = 0 \text{ kJ/mol/K}$ and $-5.02 \text{ kJ/mol/K}$ (Fig. 3a) varying by only 40-fold and peaking at $1.62 \times 10^{13} \text{ M}^{-1}$ at 18 °C. However, this variation in $K_{virus,T}$ between $10^1$ and $10^{15} \text{ M}^{-1}$ is optimal for affecting the number of midgut cells with bound virus (Fig. 3b). This is because $K_{virus,T}$ is high enough such that a significant proportion of midgut cells have bound virus over the 0 °C to 40 °C temperature range but not high enough to exceed the $10^{14} \text{ M}^{-1}$ above which all the midgut cells are saturated with virus. The variation in $C_VT$ with temperature with $\Delta C_p = -3.0 \text{ kJ/mol/K}$ shows some similarity to the percentage variation in the amount of WEEV bound to BBFs from susceptible Culex tarsalis mosquitoes as measured experimentally by Houk et al. (1990) as represented by the symbols in Fig. 3b. According to Houk et al. (1990) the $K_{virus,T}$ at 20 °C for WEEV binding to BBFs from susceptible Culex tarsalis mosquitoes is $2.2 \times 10^{11} \text{ M}^{-1}$. This is within two orders of magnitude of the value of $1.57 \times 10^{13} \text{ M}^{-1}$ predicted by the model here with $\Delta C_p = -3.0 \text{ kJ/mol/K}$ (Fig. 3a).

3.4. Case study 3: A large negative $\Delta C_p$ for a protein:protein GP/CR system as represented by HIV gp120:CD4 diminishes host cell binding at higher temperature

3.4.1. Reference temperature, $T_0$, equals 37 °C

$K_{virus,T}$ values for HIV binding and $C_VT$ values for the number of CD4+ T cells in 1 mm3 of blood with bound HIV as a function of temperature are shown in Fig. 4a and b respectively for different $\Delta C_p$ values with $T_0 = 37 \text{ °C}$. The $K_{virus,T}$ and $C_VT$ values in Fig. 4a and b respectively assume $n = 3$ Env:CD4 contacts with thermodynamic parameters in Table 4 and $\Delta S_{immob} = -400 \text{ J/mol/K}$. The large negative value of $\Delta C_p = -5.02 \text{ kJ/mol/K}$ reported for gp120:CD4 (Table 3) diminishes binding at higher temperatures compared to $\Delta C_p$ of 0 kJ/mol/K. Thus from Fig. 4b, only half of the 1000 cells have bound virus at 12 °C with $\Delta C_p = -5.02 \text{ kJ/mol/K}$ while with $\Delta C_p = 0 \text{ kJ/mol/K}$ half the cells still have bound virus at a temperature of 6 °C higher (18 °C). With $\Delta C_p = -5.02 \text{ kJ/mol/K}$, $C_VT$ is <1 cell (i.e. no virus bound) at 22 °C while with $\Delta C_p = 0 \text{ kJ/mol/K}$ the slightly higher temperature of 24 °C is required to decrease $C_VT$ to <1 cell (Fig. 4b).

3.4.2. Effect of changing the reference temperature, $T_0$, from 37 °C to 4 °C

The three lines representing $K_{virus,T}$ for different values of $\Delta C_p$, in Fig. 4a intercept at 37 °C ($T_0$) and would do for $C_VT$ in Fig. 4b if extrapolated to 37 °C. To demonstrate how the magnitude of $\Delta C_p$ affects $K_{virus,T}$ at temperatures of 37 °C representing mammalian body temperature, the reference temperature is set to 4 °C such that the three plots for $K_{virus,T}$ intercept at 4 °C in Fig. 4c with much lower $K_{virus,T}$ values predicted at 37 °C with a large negative $\Delta C_p$ than for a system with $\Delta C_p = 0 \text{ kJ/mol/K}$. The effect of $\Delta C_p$ on the number of cells with bound virus at higher temperatures is considerable with $C_VT$ reduced to 1 cell at 22 °C with $\Delta C_p = -5.02 \text{ kJ/mol/K}$ compared to 31 °C with $\Delta C_p = 0 \text{ kJ/mol/K}$ (Fig. 4d).

It should be noted that the dashed lines in Fig. 4c and d are the same as those in Fig. 4a and b respectively being based on experimental data for HIV gp120:CD4 (Fig. 1). The purpose of the dotted and solid lines is merely to illustrate the effect of less negative $\Delta C_p$ increasing the binding at higher temperatures for the purpose of understanding.

3.5. Case study 4: A large virus diameter as for the HIV virion diminishes host cell binding at the higher temperature of the human body through the large negative $\Delta S_{immob}$

From Eq. (18), $\Delta S_{immob}$ becomes more negative as the diameter of the virus increases. The value of $K_{virus,T}$ decreases with increasing temperature in Fig. 5a while more negative values of $\Delta S_{immob}$ merely shift each parallel curve to lower $K_{virus,T}$ values as expected from Eq. (17) such that $K_{virus,T}$ falls below $10^{-4} \text{ M}^{-1}$ at progressively lower temperatures. Values of $C_VT$ with $\Delta S_{immob}$ decreasing from -240 J/mol/K to -400 J/mol/K in steps of 18.6% are plotted in Fig. 5b and show that the number of cells with bound virus falls to <1 at progressively lower temperature as $\Delta S_{immob}$ becomes more negative through increasing the virus diameter. The temperature at which $C_VT$ falls to <1 is predicted to increase from 22 °C to 43 °C as $\Delta S_{immob}$ increases in magnitude from -400 J/mol/K to -240 J/mol/K. The conclusion from Fig. 5a and b is that smaller viruses with less negative $\Delta S_{immob}$ bind more strongly at higher temperatures than larger viruses. Only with $\Delta S_{immob}$ as high as -240 J/mol/K representing smaller viruses do more than 30% of the 1000 cells have bound virus at the human body temperature of 37 °C according to the model in Fig. 5b. This is shown in Fig. 5c where nearly all the cells have bound virus at 10 °C irrespective of $\Delta S_{immob}$. While at higher temperatures fewer cells have bound virus as $\Delta S_{immob}$ becomes more negative.

3.6. Case study 5: HIV attachment factors may partially overcome the unfavourable $\Delta S_{immob}$ and enhance specific virus binding through Env/CD4 interactions at human body temperature

Fig. 5 can also be interpreted in terms of the effect of prior non-specific binding of HIV to attachment factors eliminating $\Delta S_{immob}$ values of -691, -754, -806 and -851 J/mol/K according to Eq. (19) thus increasing $\Delta S_{specific}$ for specific Env/CD4 binding (as $\Delta S_{immob}$ in Eq. (11)) from -400 J/mol/K to -240 J/mol/K. Fig. 5b shows that the number of CD4+ T cells in a 1 mm3 vol of blood with bound HIV falls from 1000 to <1 over a temperature range of ~10 °C. The temperature at which fewer than half the T cells in the 1 mm3 vol of blood have bound HIV virus increases with increasing $\Delta S_{specific}$ from 12.5 °C for $\Delta S_{specific} = -400 \text{ J/mol/K}$ to 36.5 °C for $\Delta S_{specific} = -240 \text{ J/mol/K}$ (Fig. 5b). Indeed once the $\Delta S_{specific}$ is >-200 J/mol/K, 98% of the 1000 T cells have bound HIV at 37 °C (not shown). With $\Delta S_{specific} <$ -285 J/mol/K the virus is not specifically bound to any of the 1000 T cells at temperatures above 37 °C. It is concluded that the effect of making $\Delta S_{immob}$ less unfavourable through prior non-specific binding taking some of the entropy loss enhances subsequent specific binding of HIV through Env/CD4 interactions at higher temperatures. Fig. 5c shows that at 37 °C increasing $\Delta S_{specific}$ from -400 to -240 J/mol/K increases the number of CD4+ T cells with HIV bound through specific Env/CD4 interactions in a 1 mm3 vol of blood from $2 \times 10^{-6}$ to 0.07. It should be noted that $2 \times 10^{-6}$ cells in 1 mm3 is equivalent to 1 cell in 500 cm3 of blood.

3.7. Case study 6: Increasing the number, $n$, of GP/CR contacts with temperature to reproduce the data of Frey et al. (1995) for binding of HIV Env-expressing cells to cells expressing CD4

Cells expressing HIVEnv on their surface can bind and fuse with cells expressing CD4 Cr (Frey et al., 1995). Frey et al. (1995) reported a three to four fold increase in cell binding at temperatures above 25 °C with a substantial decrease in binding at temperatures above 37 °C (Fig. 6), the latter being predicted by the thermodynamic model in Fig. 5b. Frey et al. (1995) attribute the increase at 25 °C to the additional adhesion molecules which are brought into contact to increase the avidity of binding at higher temperatures. The thermodynamic model for HIV binding with $\Delta S_{immob}$ of -206 J/mol/K reproduces the increase in predicted $C_VT$ in Fig. 6a by increasing n from 1 to 3 over the
temperature range 25 °C and 27 °C to reflect the effect of temperature facilitating multiple HIV adhesion/binding sites due to increased membrane fluidity (Frey et al., 1995; Harada et al., 2004). Increasing n from 1 to 9 over the temperature range 19 °C and 27 °C gives a sharp increase in C.VT at 26 °C (Fig. 6b) with ΔS a_immob of −1110 J/mol/K. The subsequent sharp fall in C.VT at temperatures above 37 °C is due to the large negative magnitude of ΔS a_immob (−206 J/mol/K in Fig. 6a and -1110 J/mol/K in Fig. 6b) relative to n such that the diminished GP/Cr affinity at higher temperatures is not sufficient to maintain binding even with n = 9 GP/Cr interactions in Fig. 6b. The decrease in GP/Cr binding affinity with temperature for HIV Env:CD4 is apparent from the K a_virus_T values in Fig. 5a and the increase in the values of Kd_receptor_T for the temperatures 4 °C, 25 °C and 37 °C in Table 4.

4. Discussion

Previously a thermodynamic model for the effect of temperature on arthropod vector competence was developed (Gale 2019) based on the parameters ΔH a_virus_T, ΔS a_virus_T, ΔS a_immob and n, together with the kinetic parameters, p_total and EA (Table 2). This paper furthers that model by allowing the change in heat capacity, ΔC p, on GP/Cr binding to be included. This is important because it defines the effect of temperature on ΔH a_virus_T (Eq. (4)) and ΔS a_virus_T (Eq. (6)) and hence K a_virus_T. Of potentially greater importance, however, is the parameter ΔS a_immob first formally identified in the thermodynamic analysis of virus binding (Gale 2019). Thus it is proposed here that the value of ΔS a_immob is not only important in explaining the observation that dsDNA viruses infecting warmer hosts are generally smaller in volume than those infecting colder hosts (Nifong and Gillooly 2016) but also may contribute to explaining the need for attachment factors to allow binding of a large virus such as HIV at the relatively high human body temperature of 37 °C. This has implications for the design of novel antiviral therapies. The mechanistic model developed here for HIV goes as far as cell binding and viral membrane fusion (Harrison 2015) could be added as the next stage to complete the cell entry process.

4.1. Justification of assumptions for ΔS a_immob

Calculations using Eq. (17) on the basis of limited available data suggest ΔS a_immob ~ −1100 J/mol/K for HIV although it is acknowledged above that there is considerable uncertainty in the absence of definitive experimental data. It is possible that statistical thermodynamics approaches analogous to the Sackur-Tetrode equation for the entropy of a gas may be developed to estimate ΔS a_immob theoretically. The large size of the HIV virion (134 nm mean diameter) compared to a typical arbovirus (50 to 70 nm diameter) is consistent with a more negative value of ΔS a_immob for the HIV models in Figs. 4–6 compared to that used for the arbovirus models in Figs. 2 and 3.

4.2. Interpretation of figures

Figs. 2–5 show the variation of K a_virus_T and C.VT with temperature as predicted by models assuming C host = 10^3 host cells in a 10^-6 dm^3 volume (i.e. mosquito midgut or human blood sample) challenged with V total = 10^5 virions. Previously it was shown that if K a_virus_T > ~10^{14} M^-1 then nearly all the host cells have bound virus at such a high virus dose (Gale 2018, 2019). Thus the K a_virus_T plots and corresponding C.VT...
4.3. The importance of negligible $\Delta C_p$ for low temperature arbovirus transmission

For the arbovirus models in Figs. 2 and 3, the main conclusion regarding the change in heat capacity on GP/Cr binding is that as $\Delta C_p$ becomes more negative so a peak temperature for $K_{a_virus,T}$ becomes apparent such that binding affinity falls at lower and higher temperatures, directly affecting C.V T when $K_{a_virus,T}$ falls below $10^{14}$ M$^{-1}$. Values of $\Delta C_p$ from 0 to $\sim -2.0$ kJ/mol/K have relatively little impact on the temperature sensitivity of the number, C.Vp, of mosquito midgut cells with bound virus (Fig. 2b), while intermediate values of $\Delta C_p$ of $\sim -3.0$ kJ/mol/K give a peak binding at a temperature of $\sim 20$ °C as observed experimentally for WEEV binding to BBFs from susceptible mosquito midguts in Fig. 3b. More negative values of $\Delta C_p$ at $\sim -5.0$ kJ/mol/K greatly diminish arbovirus binding affinity at temperatures below $\sim 20$ °C (Fig. 2b) which in turn diminishes the transmission efficiency of the arbovirus at temperatures below $20$ °C compared to GP/Cr systems with $\Delta C_p$ in the range of $\sim 2.0$ to $0$ kJ/mol/K (Fig. 2d). In evolutionary terms, to optimise arbovirus infectivity with $n = 4$ GP/Cr interactions over a range of temperatures, GP/Cr interactions with small $\Delta C_p$ would be selected as for AIV HA/SA binding (Table 3). It is proposed here that to maintain some transmission efficiency at lower temperatures, arboviruses may use SA binding interactions as shown for the BTV coat protein VP2 (Zhang et al., 2010) for which $\Delta C_p$ may approximate 0 kJ/mol/K as in the case of AIV HA/SA (Table 3). This would facilitate arboviruses’ expanding their range into temperate regions of the world with climate change.

4.3.1. The value of $\Delta C_p$ does not affect arbovirus binding to arthropod midguts at higher temperatures

With the reference temperature of $T_0 = 37$ °C, C.V T falls rapidly at temperatures above $20$ °C in the arbovirus model here with $n = 4$ GP/Cr interactions irrespective of the magnitude of $\Delta C_p$ (Fig. 2b) as the $K_{a_virus,T}$ values fall below $10^{14}$ M$^{-1}$ and converge at $10^{12}$ M$^{-1}$ at $37$ °C (Fig. 2a). This fall is observed experimentally as the temperature is increased from $20$ °C to $40$ °C (Fig. 3b) for WEEV binding to BBFs from susceptible mosquito midguts as reported by Houk et al. (1990).

4.4. The large negative $\Delta C_p$ for HIV gp120:CD4 diminishes binding at human body temperature in the HIV model

The $\Delta C_p$ for HIV gp120:CD4 binding is well documented unlike that for arbovirus binding and the values of $\Delta H_{a_receptor,T0}$ and $\Delta S_{a_receptor,T0}$ at temperature in a) from 1 to 3 between 25 and 27 °C; and in b) from 1 to 9 number, n, of GP/Cr contacts increasing according to the dotted line with $\Delta H_{a_non_specific} = -206$ J/mol/K to represent free virus binding.

plots are best interpreted in terms of the temperature ranges over which $K_{a_virus,T} < \sim 10^{14}$ M$^{-1}$.

4.5. $\Delta S_{a_immob}$ as a repulsive force in virus binding

A negative value of $\Delta S_{a_immob}$ presents a repulsive force between the virus and the host cell in effect preventing virus binding and with $n = 0$ GP/Cr contacts, $K_{a_virus,T}$ is $< 1$ M$^{-1}$ according to Eq. (17) (Gale 2019). Increasing the value of $n$ is one way for the virus to overcome a large negative $\Delta S_{a_immob}$ for HIV Env:CD4 binding is that cryo-EM demonstrates considerable conformational shifts (Liu et al., 2017a).

4.6. A less negative $\Delta S_{a_immob}$ maintains virus binding at higher temperatures

Even for $\Delta S_{a_immob} = 0$ J/mol/K as in Figs. 2a and 3a, the magnitude of $K_{a_virus,T}$ decreases with increasing temperature (albeit after a peak in the case of $\Delta C_p < -2.0$ kJ/mol/K). This is due to the reduced affinity of the enthalpy-driven GP/Cr interactions at higher temperatures. Making $\Delta S_{a_immob}$ more negative shifts the $K_{a_virus,T}$ curve to lower values (Fig. 5a) thus exacerbating the effect of higher temperature on reducing binding. As $K_{a_virus,T}$ falls below $10^{14}$ M$^{-1}$ at higher temperatures, so C.V T falls (Fig. 5b). The magnitude of $\Delta S_{a_immob}$ merely positions the C.V T curve relative to the temperature scale with less negative values of $\Delta S_{a_immob}$ shifting the curve to higher temperatures (Fig. 5b) such that high C.V T values are maintained at progressively higher temperatures as $\Delta S_{a_immob}$ becomes less negative (Fig. 5c).

4.7. Balancing opposing constraints at mammalian host temperatures

The thermodynamic model here suggests that a large negative $\Delta C_p$ for HIV Env:CD4 diminishes binding at higher temperatures. However,
there may be molecular constraints such that a large negative $\Delta C_p$ value is a direct consequence of a molecular mechanism involving a large conformational change that serves a purpose for cell entry/fusion (Harrison 2015) such that a negligible $\Delta C_p$ value may not be achievable. Furthermore, a large negative $\Delta S_{a_{\text{immob}}}$ imposes additional constraints on virus binding at higher temperatures exacerbating the reduced strength of the enthalpy-driven GP/Cr interactions at higher temperatures. Therefore other mechanisms may be needed by the virus to achieve binding at 37 °C. These could include more GP/Cr contacts at higher temperature (Frey et al., 1995; Harada et al., 2004). An alternative strategy evolved by viruses could be to make $\Delta S_{a_{\text{immob}}}$ itself less negative such that virus binding is maintained at the higher body temperatures of mammals and birds. Two possible evolutionary strategies are suggested here which could make $\Delta S_{a_{\text{immob}}}$ less negative. The first is decreasing the size of the virus and the second is the use of non-specific attachment factors to randomly trap the virus on the cell surface prior to specific GP/Cr binding.

4.8. Small virus size makes $\Delta S_{a_{\text{immob}}}$ less negative thus maintaining binding at higher temperatures

Decreasing the size of a virus makes $\Delta S_{a_{\text{immob}}}$ less negative (Eq. (18)) such that smaller viruses bind more strongly at the higher temperatures of mammals and birds (Fig. 5). It is suggested that binding to cells at the higher temperatures of avian and mammalian hosts selects for viruses of smaller diameter through a less negative $\Delta S_{a_{\text{immob}}}$. This could explain the decrease in volume of viruses with increasing host/environment temperature as reported by Nifong and Gillooly (2016) for dsDNA viruses. Thus the average diameters of dsDNA viruses decreased from 182 nm at a host/environment temperature of 10 °C down to 74 nm at 37 °C according to the best fit line of Nifong and Gillooly (2016) suggesting the HIV virion at 134 nm mean diameter is almost double the size for a virus infecting at avian/mammalian host temperature. It is interesting to note that thermophilic bacteria adapted to high temperature may be subject to selection favouring smaller cell size. This may aid attachment to biofilms which provide some protection against temperature stress.

4.9. The importance of prior non-specific binding in taking some of the entropy loss in $\Delta S_{a_{\text{immob}}}$

It is proposed here that by taking the entropy loss $\Delta S_{a_{\text{non-specific}}}$ in $\Delta S_{a_{\text{immob}}}$ that non-specific binding to attachment factors facilitates subsequent specific HIV Env binding to CD4 receptors on the host CD4+ T cell at human body temperature. Thus the shift in the $K_{a_{\text{virus,T}}}$ curve to higher values with a less negative $\Delta S_{a_{\text{immob}}}$ in Fig. 5a is consistent with HIV attachment factors augmenting infection (Wilen et al., 2012). Indeed with $n = 3$ Env:CD4 contacts, significant binding of HIV to host cells is only achieved at 37 °C if $\Delta S_{a_{\text{immob}}}$ is increased to $> -280$ J/mol/K (Fig. 5c). $\Delta S_{a_{\text{immob}}}$ is estimated here to be $\sim 1091$ J/mol/K for HIV. Thus the prior non-specific binding of HIV would need to realise a $\Delta S_{a_{\text{non-specific}}}$ of $\sim 810$ J/mol/K for subsequent effective Env:CD4 binding at human body temperature according to the model such that $\Delta S_{a_{\text{non-specific}}}$ in Eq. (19) takes ~75% of the entropy loss in $\Delta S_{a_{\text{immob}}}$ - Wilen et al. (2012) also report that HIV attachment factors are not essential. Again this is consistent with the model here when it is noted that the C.V.T value of $< 1$ CD4 + T cell with bound virus in Fig. 5b is not a threshold and merely represents 1 mm$^3$ of blood. Thus with $\Delta S_{a_{\text{immob}}}$ = $-348$ J/mol/K, C.V.T = $1 \times 10^{-3}$ at 37 °C (Fig. 5c). This represents one CD4+ T cell with bound HIV in 1000 × 1 mm$^3$ vol of blood, i.e. in 1 cm$^3$ of blood. Decreasing $\Delta S_{a_{\text{immob}}}$ further to $< 405$ J/mol/K gives C.V.T = $1 \times 10^{-6}$ at 37 °C representing one CD4+ T cell with bound HIV in 1 litre of blood.

4.10. Making $\Delta S_{a_{\text{immob}}}$ more negative as a strategy for development of antiviral therapeutics

The model here suggests human body temperature may be a constraint on HIV binding (due to the large negative values of both $\Delta C_p$ and $\Delta S_{a_{\text{immob}}}$) in effect exposing a potential weakness in the HIV infection strategy which could be exploited for antiviral therapy. Figure 9 of Antoine et al. (2012) shows a herpes simplex type 2 (HSV-2) bound to a tetrahedral nanoparticle of zinc oxide (ZnO) called a zinc oxide tetrapod (ZnOT). The mechanism behind the ability of ZnOTs to prevent, neutralize or reduce HSV-2 infection relies on their ability to bind the HSV-2 virions (Antoine et al., 2012). The ZnOT being some 40 μm in size is much larger than the attached virion which could still attach to a host cell surface in terms of spatial considerations. However, the magnitude of $\Delta S_{a_{\text{immob}}}$ for an HSV-2 virion bound to a ZnOT nanoparticle would be very large and negative, indeed much more negative than for $\Delta S_{a_{\text{immob}}}$ for a free HIV-2 virion. Thus according to Fig. 5c, binding of HSV-2/ZnOT to host cells would be greatly diminished particularly at the human body temperature due to the very large negative $\Delta S_{a_{\text{immob}}}$—Although $\Delta S_{a_{\text{immob}}}$ changes logarithmically (i.e. slowly) with virus size according to Eq. (18), the number, C.V.T, of cells with bound virus is very sensitive to changes in $\Delta S_{a_{\text{immob}}}$ at 37 °C (Fig. 5c). It is proposed that antiviral research should take into account and even focus on making the magnitude of $\Delta S_{a_{\text{immob}}}$ more negative in addition to looking at conventional approaches to block the GP/Cr interaction. The advantage of focusing on $\Delta S_{a_{\text{immob}}}$ is that only one ZnOT nanoparticle has to bind to a virus to markedly affect $\Delta S_{a_{\text{immob}}}$ while a large proportion of the GP molecules on the virus have to be bound with inhibitors, including neutralising antibodies, to block virus binding (Klasse 2012) and hence eliminate $\Delta H_{a_{\text{receptor,T}}}$ in Eq. (11). The temperature dependence of virus diffusion in mucus (Erickson et al., 2015) could also be considered not only in mechanistic dose-response models for viral infection at mucosal epithelial membranes in the intestine (Gale 2018) but also in terms of therapeutics when combined with the thermodynamic approach developed here. Normal, acidic cervicovaginal mucus greatly hinders the movement of virions of HIV and HIV, whereas mucus that is neutralized by semen provides a much less effective barrier against the same virions (Erickson et al., 2015). Thus binding of the HSV or HIV virion to a ZnOT particle may not only decrease its diffusion coefficient in mucus but also make the magnitude of $\Delta S_{a_{\text{immob}}}$ more negative hence reducing the binding affinity of those virions which do make it through the mucus to the epithelial cell surface at human body temperature.

4.11. Validation of the HIV model

It is assumed for the purpose of the “proof of concept” model here that the thermodynamic parameters for the HIV gp120 monomer:CD4 interaction in Myszka et al. (2000) can be used for HIV Env trimer binding to a single CD4 in the absence of data. Although the $K_{a_{\text{receptor,T}}}$ value for the Env trimer:CD4 interaction $(1.4 \times 10^{-9}$ M, Chuang et al., 2017) is 15-fold higher than that for the gp120 monomer:CD4 interaction $(0.9 \times 10^{-10}$ M, Table 4) at 25 °C, this is close enough to support using the gp120 monomer:CD4 data, particularly as $K_{a_{\text{receptor,T}}}$ values for the gp120 monomer:CD4 interaction vary nine-fold between two HIV strains (see above).

The predicted values of $K_{a_{\text{virus,T}}}$ increase with decreasing temperature according the HIV model in Fig. 5a. This is unexpected on the basis of the $\Delta H_{a_{\text{receptor,T}}}$ values reported by Myszka et al. (2000) for gp120:CD4 which become less negative with decreasing temperature (Fig. 1). However, according to Eq. (6) the predicted $\Delta S_{a_{\text{receptor,T}}}$ term becomes more favourable (less negative) with decreasing temperature and it is therefore the entropy term that facilitates the tighter binding of gp120 to CD4 at lower temperatures. Unfortunately Myszka et al. (2000) only give the full range of values for $\Delta H_{a_{\text{receptor,T}}}$ (Fig. 1) with no data for $\Delta S_{a_{\text{receptor,T}}}$ over the range of temperature. However,
Myszka et al. (2000) do give some graphical data from which $\Delta S_{\text{a, receptor, T}}$ at 25 °C can be estimated and it is reassuring this $\Delta S_{\text{a, receptor, T}}$ is much less negative at $-473$ J/mol/K than that of $-691$ J/mol/K measured at 37 °C and furthermore is actually less negative than the value of $-492.8$ J/mol/K predicted for 25 °C by Eq. (6) (Table 4) and used in the model in Fig. 5. This is strong experimental evidence that warming to 37 °C and above does indeed give a significant reduction in the binding affinity of Env to CD4 as borne out by the 52-fold increase in the $K_{\text{s, receptor, T}}$ value for gp120:CD4 on warming from 25 °C to 37 °C as calculated from the actual data of Myszka et al. (2000) (Table 4). Furthermore it suggests that the actual increase in $K_{\text{s, virus, T}}$ on cooling from 37 °C down to 25 °C may be greater than that predicted by the model in Fig. 5a. The decrease in binding affinity with increasing temperature for HIV Env:CD4 is also supported by experimental data of Moore and Klasse (1992) which showed that soluble CD4 dissociated from HIV virions at temperatures above 35 °C. Similarly Doranz et al. (1999) demonstrated that gp120 bound to CD4$^+$ T cells began to dissociate at temperatures above 45 °C. The data of Frey et al. (1995) in Fig. 6 offer further validation of the model in that the binding of HIV Env-expressing cells to cells expressing CD4 decreases substantially at 37 °C as predicted by the model. The Env trimer is stable up to at least 60 °C (Chuang et al., 2017) suggesting it is not denaturation of the Env trimer itself that is responsible for the decreased binding at higher temperatures. However, care has to be taken in using Eqs. (4) and (6) to predict $\Delta H_{\text{a, receptor, T}}$ and $\Delta S_{\text{a, receptor, T}}$ respectively down to very low temperatures. Thus although the predicted value of $K_{\text{s, receptor, T}}$ for gp120:CD4 at 4 °C is 3700-fold lower than that at 37 °C (Table 4), Frey et al. (1995) cite some studies which suggested weaker binding of soluble CD4 to HIV virions at 4 °C compared to 37 °C. Without actual binding data for HIV over the full temperature range, it cannot be ruled out that $K_{\text{s, virus, T}}$ does not exhibit a peak with temperature as for the WEEV binding in Fig. 3b. Indeed, binding of $^{125}$I-labelled gp120 to T cells peaked at 16 °C with binding at 4 °C much lower than that at 37 °C (Frey et al., 1995). The observed increase between 4 °C and 16 °C is unlikely to be due to increased fluidity of the membrane allowing recruitment of more CD4 molecules because unlike the virus, each gp120 only binds a single Cr molecule. Dimitrov et al. (1992) showed that the activation energy for binding of soluble CD4 to cells expressing HIV Env changed at 18 °C suggesting 18 °C is a transition temperature. This could explain the peak in binding of gp120 at 16 °C reported by Frey et al. (1995). Thus extrapolation of $\Delta H_{\text{s, receptor, T}}$ and $\Delta S_{\text{s, receptor, T}}$ through Eqs. (4) and (6), respectively to temperatures below 18 °C may not be justified in the case of HIV binding.

It should be remembered that HIV only infects humans at 37 °C and therefore the lower temperature predictions are more of academic interest, although temperature trends may give an insight into weaknesses in the HIV infection strategy as suggested here. It is only the 37 °C line in Fig. 5c which is important for HIV and it is reassuring to note that this is based on actual experimental data for gp120 mono-mer:CD4 binding measured at that temperature. The lower temperature lines in Fig. 5c are of more general interest, for example in explaining the observed relationship (Nifong and Gillooly 2016) between larger virus volume and lower host temperature.

### 4.12. Variation between virions in an exposure dose

Individual virions of the same virus may vary both in size (Briggs et al., 2003) and in the case of some RNA viruses in the actual base sequence of the genome giving a spectrum of mutants. The infection process may represent a bottleneck such that only one or two viral variants in the exposure dose successfully establish infection (Bull et al., 2011). Thus in the case of norovirus, only minor variants at frequencies as low as 0.01% were successfully transmitted to establish a new infection (Bull et al., 2012). The work here suggests that the actual size of the virion itself may present an additional constraint particularly at mammalian body temperatures. Thus although the average diameter for HIV virions with a single core is 134 nm, individual virions display a broad range of diameters extending from 120 to 200 nm with a skewed distribution (Briggs et al., 2003). Furthermore while the majority of HIV virions contained a single core, almost a third contained two or more cores. The mean diameter of the HIV virions containing a single core (134 ± 11 nm, $n = 89$) was significantly smaller than the mean diameter of virions containing two cores (158 ± 16 nm, $n = 43$) (Briggs et al., 2003). Thus the magnitude of $\Delta S_{\text{s, immob}}$ may vary between individual HIV virions within the same exposure dose. The key conclusion from Fig. 5c is that the number of CD4$^+$ T cells with bound virus is highly sensitive to the magnitude of $\Delta S_{\text{s, immob}}$ with just a 4.5% increase in the magnitude of $\Delta S_{\text{s, immob}}$ from −290 to −277 J/mol/K for example increasing C.V.T by five-fold from 1 to 5 CD4$^+$ T cells per mm$^3$ of blood at 37 °C. Thus smaller diameter viruses in a challenge does may have an advantage in binding and hence initiating infection at mammalian body temperatures. This also raises the question of the magnitude of $\Delta S_{\text{s, immob}}$ for an aggregate containing several virions of the same virus and in the case of EBOV, for the long filaments linking multiple genome copies (Beniac et al., 2012). It has previously been proposed that fusing multiple genome copies into the same filament in the case of EBOV takes some of the entropy loss for $\Delta S_{\text{s, immob}}$ prior to binding to the cell surface (Gale 2017) such that aggregation can be viewed as a cooperative effect in the infection process. The effect of aggregation and virus size on the magnitude of $\Delta S_{\text{s, immob}}$ is important to further the work here.

### 5. Conclusion

From a thermodynamic perspective, the affinity of virus binding to a host cell is greatly decreased at the higher body temperatures of mammals and birds such that temperature can be considered a constraint to be overcome. The relationship between binding affinity and temperature can be markedly affected by the change in heat capacity ($\Delta C_p$) on virus binding through its effect on the enthalpy and entropy of the GP/Cr interaction. Specifically large negative values of $\Delta C_p$ reduce binding at both low and high temperatures giving a peak binding at a medium temperature as has been observed experimentally in binding of WEEV to mosquito midguts. Very large negative values of $\Delta C_p$ for protein/protein GP/Cr interactions are predicted to diminish arbovirus transmission at low temperatures compared to the HA/SA glycan interaction for which $\Delta C_p$ is negligible. Thus selecting for SA glycan interactions as reported for BTV binding may be a mechanism to allow transmission at the lower temperatures experienced by arboviruses infecting arthropods in temperate regions of the world.

The magnitude of $\Delta S_{\text{s, immob}}$ is currently unknown but is predicted to be large and negative and to be more negative for larger viruses such as HIV (mean diameter 134 nm) compared to the smaller arboviruses (diameter 50 to 70 nm). Small virion diameter through a less negative $\Delta S_{\text{s, immob}}$ favours virus binding at higher temperatures giving smaller viruses a selective advantage for infecting hosts at the higher body temperatures of mammals and birds. This is in agreement with the published observational findings that virus volume decreases with increasing host temperature in the case of dsDNA viruses.

In the case of HIV, the unfavourable effect at human body temperature of the large negative $\Delta S_{\text{s, immob}}$ value due to the large size of the virus may be overcome by non-specific binding. It is proposed here that non-specific binding of HIV as the virus first randomly attaches to a host CD4$^+$ T cell surface takes a large proportion (represented by $\Delta S_{\text{s, non-specific}}$) of the entropy loss of $\Delta S_{\text{s, immob}}$ such that HIV can then bind specifically through Env:CD4 interactions at the relatively high temperature of the human body. The randomly attached virus “rolls into place” driven by $n = 3$ specific Env:CD4 interactions resulting in a further entropy loss ($\Delta S_{\text{s, specific}}$) as the virus particle takes up a specific orientation ready for viral entry. Non-specific attachment factors are all the more important for a GP/Cr system such as the HIV Env:CD4 interaction for which $\Delta C_p$ is large and negative and diminishes binding at
higher temperatures. It is concluded that experimental data on ΔSA_immob should be obtained and antiviral therapeutic strategies, for example using zinc oxide nanoparticles for herpes simplex virus or neutralising antibodies to block virus binding, should focus not only on blocking the Cr-binding sites on the GP molecules themselves but also on targeting ΔSA_immob, by increasing the size of the virus itself and so minimising host cell binding affinity at human body temperatures according to the model here. Bacterial cells are larger than viruses and ΔSA_immob would be expected to be more negative. Targeting ΔSA_immob may also have application as an alternative approach to antibiotics for bacterial pathogens when bacterial pathogenesis depends on attachment to cells. However, it should be noted that ΔSA_immob decreases logarithmically (i.e. slowly) with increasing size which may limit the practicability to bacteria.

Declaration of Competing Interest
None declared.

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Disclaimer
The views expressed in this paper are those of the author and not necessarily those of any organisations.

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