Supplement 1:

Current usage of the “buffering” paradigm outside acid-base chemistry

Buffering of electrolytes

Similar to H⁺ ions, several other electrolytes are stabilized or “buffered” in their respective body fluid compartments, including cytoplasmic calcium [1] and magnesium [2-4]. The definitions of buffering, cast originally in acid-base terminology, were adapted readily to these two ion species. Another important ion associated with buffering is potassium [5-7], even though the physico-chemical processes involved differ profoundly from H⁺, Ca⁺⁺ and Mg⁺⁺ buffering, and their elucidation remains a challenge to researchers. Further biologically relevant ions that are buffered include phosphate [8], molybdate [9], iron [10], and ADP/ATP [8,11]. Interestingly, buffering also seems to participate in shaping the spatial and temporal concentration profile of signalling molecules, e.g. those of inositol-1,4,5-trisphosphate [12], or those of neurotransmitters by binding to “decoy receptors” [13-15]. The buffering of ions is also an important aspect in aquatic, atmospheric and geochemistry. Besides H⁺, buffering in these fields was studied for Ca⁺⁺, and Mg⁺⁺ ions [16], sulfide [17], or phosphate [18].

Buffering of non-electrolyte solutes

An example for an uncharged molecule for which buffering plays an important physiological role is oxygen, with hemoglobin and myoglobin as its major buffers within red blood cells and myocytes, respectively. The fundamental importance of hemoglobin in oxygen transport is well known, including the remarkable affinity switch between source and sink compartments that enhances net transport. The star-nosed mole provides a further example: This small diving mammal exploits oxygen buffering and aerobic metabolism for diving, rather than relying on H⁺ buffering and anaerobic glycolysis [19]. An important role for oxygen buffering by myoglobin was also found in a modeling study on capillary networks of aerobic muscles: its contribution to oxygen transport and oxygenation on the tissue level outstripped by far that of vasomotion [20]. Buffering against the cholesterol-raising action of dietary cholesterol could be attributed to a hepatic enzyme, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase [21]. Buffering against the influx of fat by healthy adipose tissue, but not by
adipose tissue in insulin-resistance was claimed to prevent excessive exposure of other tissues to this influx [22].

**Buffering of thermodynamic and hydraulic quantities**

A step away from simple solute concentrations, the terms “redox buffering” or “oxidant buffering” were used in biochemistry, pathology, and geochemistry to denote the relative stability of the redox potentials in the face of added reducing or oxidizing equivalents [23-27]. In the context of metabolic control, the thermodynamic potential of intermediates in energy metabolisms was found to be stabilized by “thermodynamic-buffer enzymes” [28], by “energy buffers” that could be deliberately introduced into transgenic plants [29], or by phosphagen systems conveying a “metabolic capacitance” [8].

The term buffering is also associated frequently with hydraulic phenomena such as “blood pressure buffering”. By some, this term is used more or less synonymously with “autoregulation” for the stabilization of organ blood flow [30,31]. By others, “blood pressure buffering” is employed in the sense of “blood pressure variability buffering” in the face of external or internal disturbances; this has become a popular paradigm in cardiovascular physiology [31-37].

In synovia surrounding joints, hyaluronan chains in the interstitial spaces were found to modify the pressure-flow relationship in a way described as “outflow buffering” of synovial fluid [38,39]. In the plant kingdom, polysaccharide hydrocolloids in leaves were reported to “buffer” leaf water status against environmental fluctuations, due to their high water-binding capacity and ability to act as hydraulic capacitors [40].

**Systems level buffering**

In evolutionary biology, a recent concept is “phenotypic” or “genetic buffering”: The effect of genetic variations on phenotype – and thus on fitness and selection - is minimized or completely intercepted by mechanisms such as redundancy or negative feedback; limitedness of genetic buffering capacity gives rise to threshold behavior and complex patterns of evolutionary stasis and change [41-43]. In the social sciences, “social buffering”, “cognitive buffering”, or similar hypothetical mechanisms were proposed and studied. According to these hypotheses, the harmful effects of various forms of stress on mental or physical health may be attenuated by social support, cognitive processes, or other factors [44-47].

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